BESA®

BESA Research Tutorial

BESA

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Research 7.1

Tutorial

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BESA[®] Research 7.1 Introduction

Introduction

This Tutorial is created to guide you through a complete data analysis from preprocessing of individual datasets to cross-subject statistics of source analysis, time-frequency analysis or source coherence results. We will work with simulated datasets that help to understand basic mechanisms. Further we will be working with a real dataset of an auditory intensity experiment located in the BESA Examples folder

C:\Users\Public\Documents\BESA\Research_7_1\Examples\ERP-Auditory-Intensity. In this experiment 10 subjects listened to tones (1000 Hz) of different intensity (60, 70, 80, 90, and 100 dB).

BESA[®] Research 7.1 Introduction

Tutorial 1 – Preprocessing

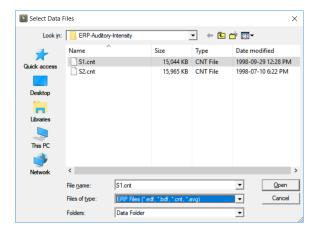
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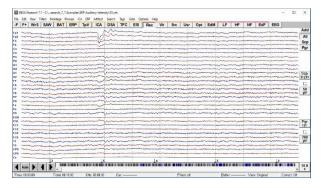
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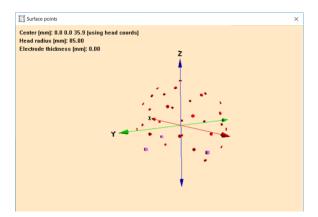
- Paging through your data screen by screen or jumping to selected time-points / events
- ✓ Adjusting signal amplitude and time-scale
- ✓ Interpolation of bad channels, changing the channel status
- ✓ Marking of Artifacts
- ✓ 3-D mapping of the topography at the selected cursor position
- ✓ Remontaging in sensor and source space
- ✓ Fast-Fourier-Transform (FFT) of the selected data block
- ✓ Density Spectral Array (DSA) view of the whole data set
- ✓ Filtering

A. Data Review

- Start BESA Research. From the File menu, choose Open. In the Select Data Files box, select folder ERP-Auditory-Intensity in the Examples folder. Change the file type to ERP files (*.edf, *.bdf, *.cnt, *.avg) and open file S1.cnt.
- 2. This is a dataset recorded from a 32channel electrode cap with Cz as reference and one EOG channel. Blue electrodes are over the left side of the head, red electrodes over the right side, black electrodes are over the midline. Triggers are coded as vertical black bars at the bottom of the screen.
- 3. To see the 3D-coordinates of the electrodes and additional head surface points, select File / Head Surface Points and Sensors / View (or press V on the keyboard). Fiducials are displayed as pink cubicles, electrodes as red discs. If you click on an electrode its label will be displayed. If you select an electrode in the main window, it will be highlighted in the surface points view. Close the surface points window.

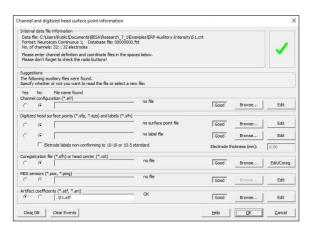


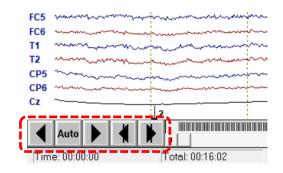




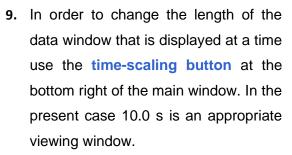
- Select File / Head Surface Points and Sensors / Load Coordinate Files... (alternatively: ctrl-L) A window opens showing all auxiliary information that is associated with the data file.
- 5. This dialog window allows to specify additional information on channel configuration (*.ela), digitized head surface points (*.sfp, *.eps) and labels (*.sfn) and more. Here, no additional files need to be loaded as all the relevant information is stored within the data file. If BESA finds auxiliary files with the same basename as the data file, they will be automatically loaded. Close the window.
- 6. In order to page through your data, you can either hit the space bar or use the arrows in the bottom left corner of the main window. The arrows with the vertical bar allow you to move half a page forward or backward.

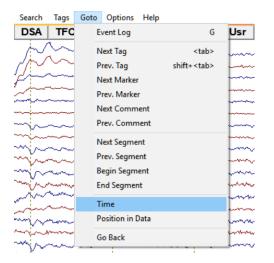


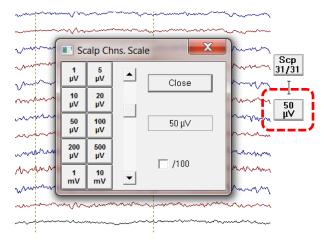


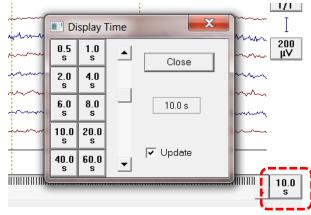


- 7. You can also jump directly to a certain time-point by pressing Goto / Time... You might also add markers or comments to your data by right-clicking into the data and selecting Marker or Comment. You can then jump directly to these events via the Goto menu as well.
- 8. To rescale your data, use the amplitude scaling button on the right side of the main window (just below the button Scp). In the present case, the scaling of 50 μV is appropriate.

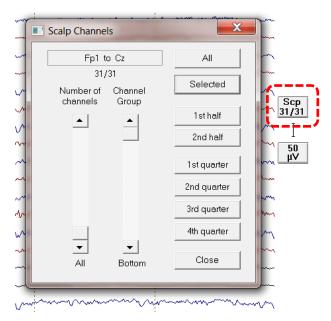








10. If you only want to view a sub-set of your electrodes use the button Scp on the right of the main window. You can either choose the first / second half or the 1st to 4th quarter of the recorded channels. You may also adjust the number of electrodes you want to have displayed by using the sliders. In the current case, change to the all-channel view.



B. Interpolation, Marking of Artifacts, 3-D Mapping, Remontaging

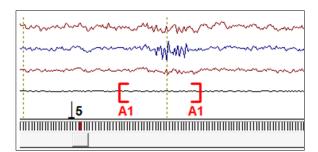
1. Electrodes that display a faulty recording can either interpolate or set as bad. Interpolation is based on spherical splines, i.e. the information of all other electrodes is used to compute the interpolated signal. If a channel is set as bad it will be ignored. To interpolate or set a channel as bad right-click onto the according channel label and select according channel as bad or Interpolate this channel¹. Interpolated channels will be displayed in pink, bad channels in grey. In the current dataset, there are no bad channels and no interpolation is needed.

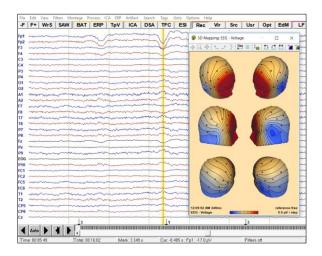
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¹ Important note: in case a channel is at located at the rim of the electrode cap it is recommended to set it as bad rather than interpolate it as interpolation might be inaccurate. The position of an electrode can be checked following step A3 of this tutorial.

- 2. BESA uses amplitude, gradient and lowsignal thresholds when scanning for artifacts (see Tutorial 2). Still, you might want to specify artifacts in your data by hand. This can be useful for e.g. muscle artifacts that are high-frequent and small in amplitude. In the current dataset Goto Time 00:04:28. You will see a muscle artifact. Left-drag a window across it, right-click into it and select Define as Artifact. A red bracket will appear at the bottom of the main window marking the artifact. It will be ignored for further analysis. The artifact marker can be deleted by right-clicking on a bracket and choosing Delete. Please delete the artifact marker in the current dataset.
- 3. Goto Time 00:05:49. You will see an eyeblink that is mainly picked up by electrodes Fp1 and Fp2. Double-click on it to open the 3D-Mapping window. You will see a strong frontal positivity that is typical for eye-blinks in EEG data. We will not deal with eyeblinks at this stage.

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4. It is possible in BESA Research to remontage the recorded data using virtual standard electrode caps (Vir-Button) (international 10-10-system) or source montages. In the former case, you can view the data as if they had been recorded using a standard electrode cap. In the latter case, data are translated into source space using source montages provided by BESA (Src-Button) or created by the user (Usr-Button, see Tutorial 9) so that you can get an immediate display of activity in the respective sources in the continuous recording.

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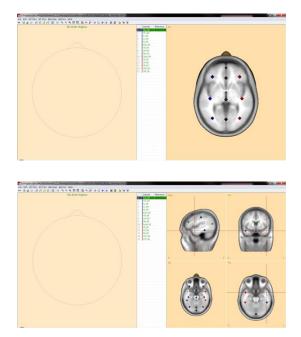
a) Select Vir / Reference Free. This virtual montage consists of 27 standard EEG channels. The reference was computed as the average signal of an interpolation over the complete head surface (approximated by a sphere). Since the physical head model assumes a zero integral over the head surface, this type of referencing removes the bias of the reference electrode. Note that the Cz electrode carries signal in this montage. Also note that the signal becomes cleaner as the noise that is common to all electrodes gets subtracted by average referencing.

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b) Select Src / BR_Brain Regions. Paging through the data you can immediately see that the left and right temporal sources carry more signal than the other sources.

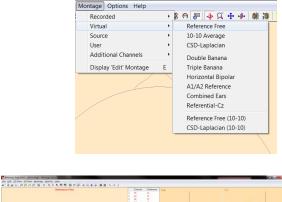
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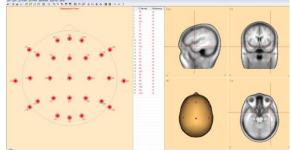
5. It is possible to view the current montage by pressing the EdM button (alternatively you can press Edit / Montage). In the current case we see a source montage that consists of 15 sources covering the whole brain. If you click on a source label in the middle panel BESA will slice the standard MRI to the position of the source. You may change to a coronal or saggital view by pressing the according buttons is the same time, press the button is a source of the source.



6. In the montage editor press Montage / Virtual / Reference Free to change back to sensor level. Now you see the position of the 27 standard electordes. Note that you can create your own montages or rereference your electrodes in the montage editor as well. Return to the raw data using

the current montage by pressing the button located in the top left corner of the montage window.





C. FFT and DSA

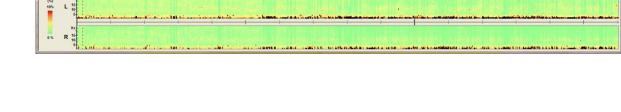
- BESA allows you to calculate an FFT on a marked datablock² to get a quick idea of the amplitude or power in different frequency ranges. Start the FFT by leftdragging a window in your raw data, right-clicking into it and selecting FFT. Please mark a block in the first data window that covers the eyeblink.
- 2. In the FFT window the electrodes of the current montage are listed along with their amplitude spectrum from 1 to 30 Hz (default). Beside every electrode the frequency is displayed that carries the peak amplitude in the specified time-range. The current view shoes that the frontal electrodes are characterised by high-amplitude low frequencies. Click into the low-frequent activity to open the 3-D Mapping window.

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² In case you want to look at longer data periods or the whole dataset it is recommended you use the option **Process / Mean FFT-Spectrum** 

- 3. Scale up the the voltage map by using the upper arrow-key on your keyboard or using the button . It becomes clear that the high low-frequent amplitudes are caused by an eye-blink in the current example. Note that you can change the frequency-range you want to view or change from amplitude to power spectrum in the View menu. In the Map menu you can choose the frequency band you want see a voltage topography of in the 3D-Mapping window. Close both the FFT and Mapping window.
- The DSA view is also based on an FFT and allows you to quickly get a feeling for the whole dataset. To start it press the DSA button in the main window.
- 5. The default DSA window is divided into left and right hemisphere. It shows a condensed FFT view of the whole dataset subdivided into blocks of 2 seconds. If you click into an event in the DSA window, BESA Research will jump to the according timepoint in the data in the main window.



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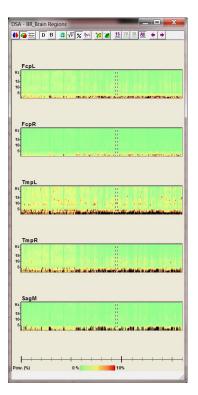


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6. Change the view of the DSA window to multiple brain regions by pressing the button in the top left corner of the DSA window. In this view it is possible to see that the left and right temporal regions show activity in the alpha / low beta band that is not present in the other brain regions.

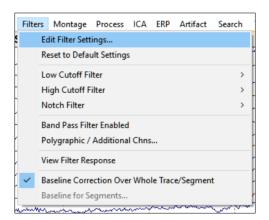
The DSA view is valuable to quickly identify time-windows in the dataset that are characterised by high signal power. This can be very useful for e.g. epilepsy research to identify seizure periods and get a rough idea of the origin of the seizure. Close the DSA view.



#### **D.** Filtering

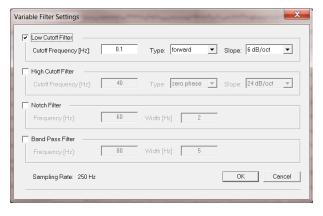
 It is possible to specify Low Cutoff, High Cutoff Notch and Bandpass filters in BESA. This can be done via Filters / Edit Filter Settings (also: EdF button) or the buttons LF, HF and NF.

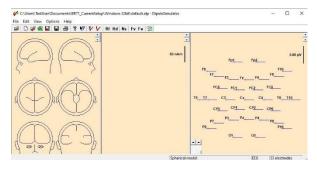


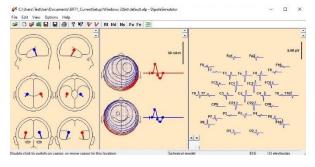


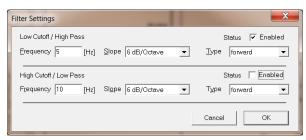
- 2. For Low and High Cutoff filters the filter type can be set to forward, zero-phase, or backward. The slope of the filter can also be edited. In order to understand the properties of filters better we will now switch to the program "DipoleSimulator". *Please close the filter menu without setting any filters.*
- 3. Open the program DipoleSimulator ³ DipoleSimulator.exe</sup> located in your BESA installation folder (C:\Program Files (x86)\BESA\Research_7_1). On the left of the window you see the BESA head schemes, on the right you see an electrode layout.
- 4. Press File / Load Model File and select the model AC-2D-biphasicsynchronous.mod located in the Examples Folder Learn by Simulations (C:\Users\Public\Documents\BESA\Re search_7_1\Examples\Learn-by-

Simulations). You will see two dipoles representing activity in the left and right hemisphere along with their source waveforms, i.e. their activity over time in the middle panel. Press the button Fe to open the filter dialog. Ns Fv Fe Second Specify a Low Cutoff filter of 5 Hz, 6 dB/Octave, forward and make sure that the box Enabled is only ticked for the Low Cutoff filter.

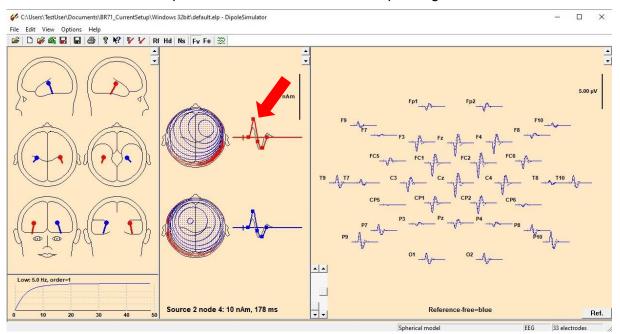






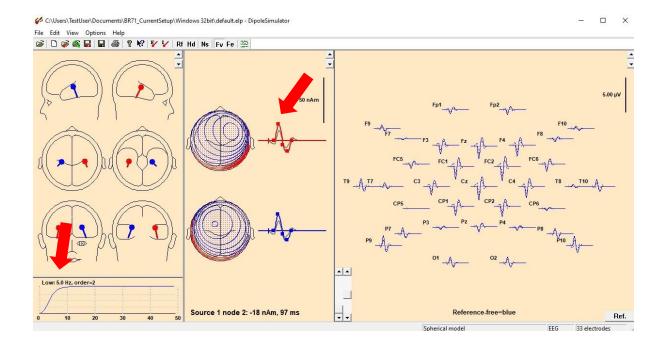


5. Leave the filter settings dialog open and move it next to the DipoleSimulator main window in order to view both the filter settings and the data. The grey waveforms show the effect of the filter. In the bottom left corner the filter response is displayed. You can see that the Low Cutoff filter as specified in step D4 has an effect on both the amplitude and the shape of the waveforms. Amplitudes become smaller and the peaks get shifted forward.

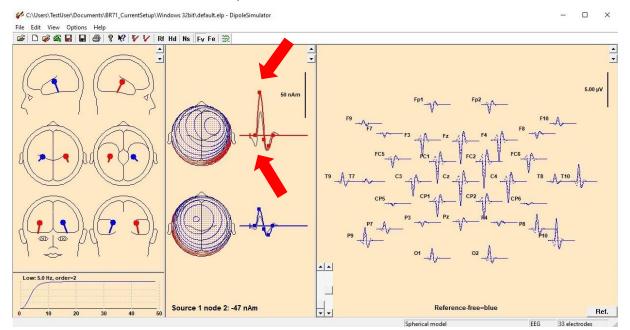


6. Change the filter type to zero-phase. Note that the filter response changed as well as the filtered waveforms. While the amplitude of the filtered waveform is still diminished, the peaks of the filtered waveforms are closer to the ones in the unfiltered data. However, a small new "component" was caused by the filter at waveform onset and another one at the waveform offset.



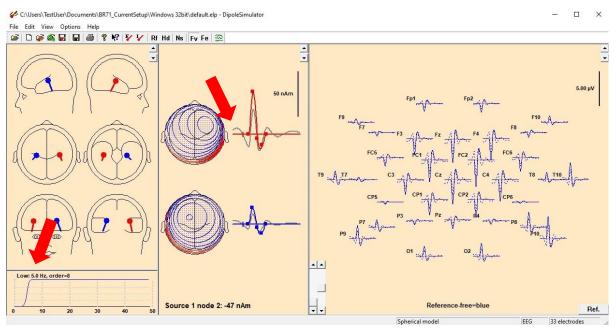


**7.** Increase the amplitude of the red source waveform by dragging up the second node with the mouse. Note how the "component" at waveform onset also increases.



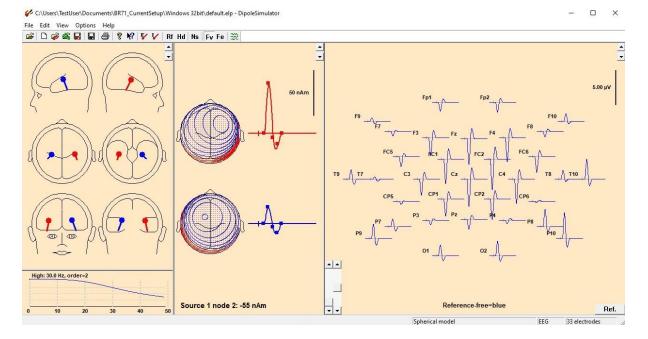
8. Change the filter slope to 48 dB/Octave. Note that the filter response suggests better filtering properties, i.e. it filters out frequencies below 5 Hz more effectively. The downside is that this creates greater artifacts, distorting the waveform even further.

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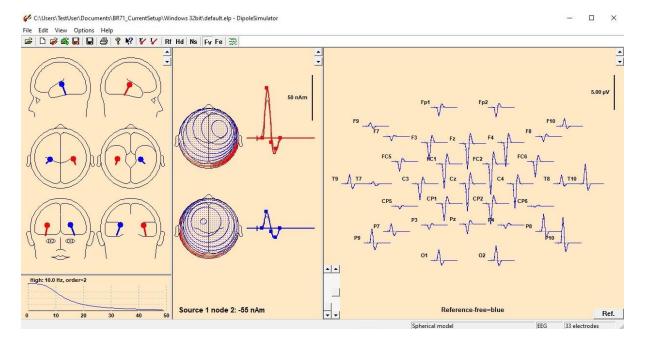
9. Disable the Low Cutoff filter and enable a High Cutoff filter of 30 Hz, 12 dB/Octave, zero phase. Note that the original waveform was not changed by the filter. This is the case because evoked potentials are generally lower in frequency.

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10. Change the High Cutoff filter to 10 Hz, 12 dB/Octave, zero phase. The amplitude of the waveforms get decreased, while the shape of the waveform is not as distorted as in the example of the Low Cutoff filter.

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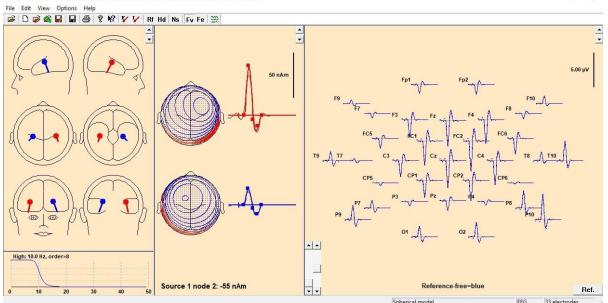
BESA Research 7.1 Tutorial Copyright © 2020 BESA GmbH, Freihamer Str. 18, 82166 Graefelfing, Germany

C:\Users\TestUser\Documents\BR71_CurrentSetup\Windows 32bit\default.elp - DipoleSimulator

11. Increase the slope to 48 dB/Octave. Note that the filter response again suggests better filtering properties suppressing activity > 10 Hz more effectively. The consequence is a slightly distorted waveform. Still, the effect is not as extreme as in the Low Cutoff examples.

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#### Things to keep in mind when filtering

- The closer the frequency of the filters to your signal of interest, the greater the effect of the filter on your signal of interest
- Filters affect both amplitude and shape of your waveforms
- Low Cutoff filters usually have a greater impact on evoked potentials than High Cutoff filters as evoked potentials are relatively low in frequency
- The application of a filter can lead to the introduction of false "components", the effect being the greater, the greater the signal amplitude of your waveforms
- If you are interested in early components (< 100 ms) you should not use Low Cutoff filters that are zero-phase, as the signal of later components will be projected onto the earlier components

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

#### Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

#### What does BESA Research provide?

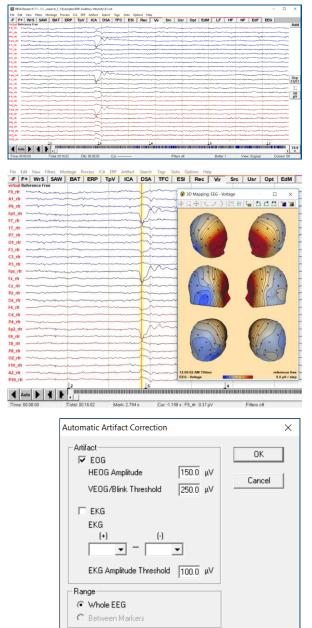
- ✓ Automatic and user-defined artifact correction
- ✓ Definition of conditions based on trigger values, names and attributes
- ✓ Artifact rejection based on amplitude, gradient and low-signal criteria
- ✓ Averaging
- ✓ Classic ERP analysis

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

#### A. Basic Artifact Correction

- In case you have closed the data file as used in Tutorial 1, reopen file S1.cnt located in the Examples folder ERP-Auditory-Intensity and switch on the virtual Reference-Free view (Vir button → Reference Free).
- On the first screen, an eye-blink dominates the data. Double click on it to open the 3D Mapping window and view the associated typical strong frontal positivity.

3. Close the mapping window and select Artifact / Automatic. In the dialog box make sure that only the option EOG is ticked and leave the settings at default. After pressing OK, BESA will now automatically scan the data for eye movements using an internal model of artifact topographies.



Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

4. No horizontal eye movements were found so only a virtual VEOG channel was created by BESA Research. The eyeblink that was clearly visible in the data before correction is prepresented in the virtual VEOG channel. Note that "corrected" is displayed in the top left corner of the main window when artifact correction is switched on. You may switch artifact correction on and off by pressing ctrl+E or by pressing Artifact / Correct.

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5. Next we will do the eyeblink correction by hand. For this purpose, switch off the automatic artifact correction and hide the virtual VEOG channel by pressing Artifact / View. Switch back to the original data by pressing the Rec button and selecting Original Recording. You can see that an EOG electrode was used in this experiment. Scale it up to 20 μV using the according amplitude scaling button.

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

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6. Use the button Add in the top right corner of the main window and select EOG-HB. This will add virtual electrodes for picking up horizontal eye movements (H_EOG) and eyeblinks (B_EOG). Scale up the B_EOG channel to 200 nAM by using the according amplitude scaling button. Note that the eyeblink is reflected in the EOG electrode as well as the virtual B_EOG electrode. Thus, virtual electrodes can be very useful for the identification of eyeblinks if an EOG was not specifically recorded.

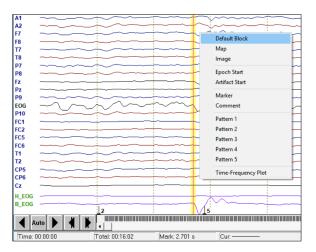
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Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

- Now we will apply filter settings that are optimal for the identification of blinks. Press the EdF button to open the filtering dialog. Choose a Low Cutoff filter of 0.5 Hz, forward, 6 dB and a High Cutoff filter of 8 Hz, zero-phase, 12 dB.
- Before marking an eyeblink, we need to define a default block epoch that will later be used for the pattern search. To do so press Edit / Default Block Epoch and choose the settings Pre cursor: -100 ms and Post Cursor: 400 ms. This time window is usually sufficient to cover the whole extension of the blink signal.
- Right-click on the onset of the blink (you may use the virtual B_EOG channel as a reference) and select Default Block.

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✓ High Cutoff Filter Cutoff Frequency [Hz]: 8 Type: zero phase ▼ Slope: 12 dB/oct ▼	
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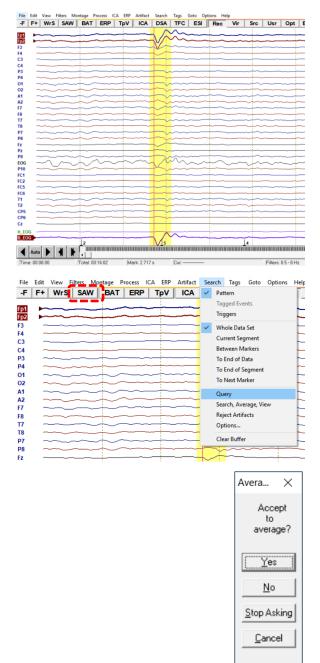


Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

- 10. You may select the channels that pick up the eyeblink most clearly by holding down the ctrl-key and left-clicking on the according electrode labels. Select Fp1, Fp2 and the virtual B_EOG channel. BESA Research will only use the selected channels for the pattern search. However, when data are filtered it is often not necessary to specify channels, the search algorithm may be run using all channels.
- 11. Before we start the pattern search, we will tell BESA Research to stop everytime a pattern is classified as a blink. To do so press Search / Query.

Next press the button **SAW** (Search – Average – Write) to start the pattern search.

12. Whenever BESA Research finds a pattern that correlates high with the blink we have just defined, it will ask you if you want to accept it to average. Click Yes to move on to the next pattern. If you are confident that the pattern search works well you may select Stop Asking. BESA Research will now automatically scan through the rest of the data.³



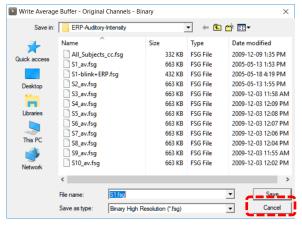
³ If your data are contaminated by many other artifacts, it is recommended to only accept blinks that do not coincide with another artifact event in order to receive a clean eyeblink topography.

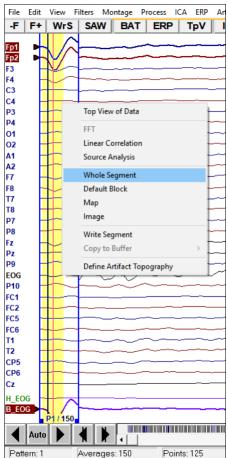
Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

13. When prompted whether to save the average buffer, choose **cancel**.

14. On the left of the main window, an average segment consisting of all blink events that were accepted during the pattern search is displayed with a baseline of -100 ms and a window length of 400 ms as specified by the default block epoch. Left-drag a window in it, right-click and select Whole Segment.

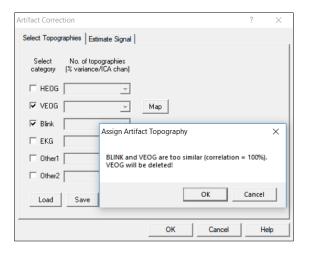
Right click again and specify **Define Artifact Topography**. (If you click next to the buffer window, it disapears. It can be displayed again by pressing View / Average Buffers.)

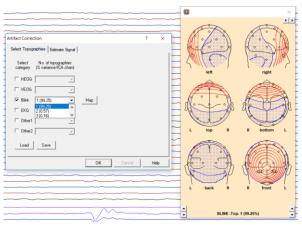




Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

- 15. Note that VEOG is already ticked in the artifact correction window. The reason is that BESA Research still remembered the automatic artifact correction from before. Select Blink. An error message will appear notifying you that the blink and VEOG topographies are too similar and that VEOG will be deleted. Confirm with OK.
- **16.** A 2D-mapping window opens that displays the topography of the first PCA component (principal component analysis) that was computed on the PCA average blink signal. The decomposes the blink signal into independent topographies. Generally the first component explains > 99% of the variance, so it is sufficient to only subtract the first component from the data⁴. Open the drop-down menu to view the other PCA components. Make sure that only the first component is selected and press OK.





⁴ It is recommended to only select the first PCA component for artifact correction in order to prevent distortion of the data.

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

17. Note that another virtual blink channel was created. If you scale it up to 100 μV using the its amplitude scaling button you will see that it is almost identical to the virtual blink channel that was created automatically using the Add button. Switch off H_EOG and B_EOG by pressing Add and selecting Switch off. Also make sure to swich off artifact correction for now by pressing ctrl+E.

E+ I	WrS	SAW	BAT	ERP	Tp!	v 1	ICA	DSA	TEC	ESI	Rec	Vir	Src	Usr	Opt	EdM	LE	HF	NF	EdF	EEG	
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B. Definition of conditions based on trigger values, names and attributes

 We will now use the trigger information in the file to specify conditions that will later be used for averaging. Press ERP / Edit Paradigm.

ICA	ERP	Artifact	Search	Tags	Goto	Options	Help	
T		Open Para	digm					ir
		Edit Paradi	igm					Γ
-		Save Parad	ligm As					F
		Average						F.
_		Combine (Condition	s, Chanı	nels, Fin	d Peaks		E.
-+		Coherence	2					F
		Top View o	of Data					È.
		Edit Trigge	rs					F.
_		Insert Trigg	gers					
-+		Create Trig	gers Fron	n EMG/E	EG			F
		Open Even	t File					F
		Save Event	s As					Ľ
		Delete Imp	orted Eve	nts				┢

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

 Switch to the Trigger tab. 5 triggers are detected in the dataset. They correspond to stimuli that were presented at 60dB, 70dB, 80dB, 90dB and 100dB. Enter 60dB in the field under name and press Add to List. Do the same with the rest of the labels.

digm						? ×
Trigger Cont	dition Epoch	Filter Artifa	ict Average	Coherence		
Attributes		((6	()	-
code	name	Attribute1	Attribute2	Attribute3	Attribute4	Define
Attribute ⊻alues						
1 2	100dB					Add to List
3 4 5	60dB 70dB 80dB 90dB					Bemove from List
Current Assignm	100dB ent	-				Set
1 4 1 5						Load
						Save As
					ок	Cancel Help

3. We will add further attributes as they will make the definition of conditions easier later on. Change the name Attribute1 to modality and press Define. In the field below write auditory and Add to List. Change the name Attribute2 to side and press Define. In the field below write bilateral and Add to List. Change the name Attribute3 to frequency and press Define. In the field below write 1000_Hz and Add to List. Change the name Attribute4 to intensity and press Define. In the field below again add 60dB, 70dB, 80dB, 90dB and 100dB to the list. Finally, select Attribute Values/1, name/60dB, modality/auditory, side/bilateral, frequency/1000_Hz, intensity/60dB ad press Set. Proceed with Attribute value/2, name/70dB, modality/auditory, ...

code	name	modality	side	frequency	intensity	<u>D</u> efine
Attribute <u>V</u> alu	es					
1 2	60dB	auditory	bilateral	1000_Hz	60dB	Add to List
3	60dB	auditory	bilateral	1000_Hz	60dB	Remove from List
4 5	70dB 80dB 90dB				70dB 80dB 90dB	
	100dB				100dB	
Current Assig	nment					
code	name	modality	side	frequency	intensity	S <u>e</u> t
T ¹	60dB	auditory	bilateral	1000_Hz	60dB	
$\overline{\mathbf{T}}^2$	70dB	auditory	bilateral	1000_Hz	70dB	
T 3	80dB	auditory	bilateral	1000_Hz	80dB	
ζ4	90dB	auditory	bilateral	1000_Hz	90dB	_ <u>F</u> ile
T 5	100dB	auditory	bilateral	1000_Hz	100dB	Load
	10040	auditory	bilateral	1000_Hz	100dB	

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

4. Switch to the Condition tab. First we want to specify our individual conditions. Enter the Name 60dB. Then select Qualifier Current, Attribute intensity, Operator Is, Value 60dB and press Insert. Do the same for Names 60dB to 100dB.

Next we want to specify the condition **Low**. Enter the name Low and again select **Current intensity Is 60dB** and press **Insert**. We want the Low condition to also contain 70dB stimuli. Thus, additionally specify the condition Low to be **Current intensity Is 70dB** and press **OR** instead of Insert. Now, the Low condition contains 60dB and 70dB stimuli. Specify condition **High** to contain 90dB and 100dB stimuli.

Finally we will specify the condition **All** by specifying **Current modality Is auditory**. (Make sure your condition definition looks like in the screenshot below.)

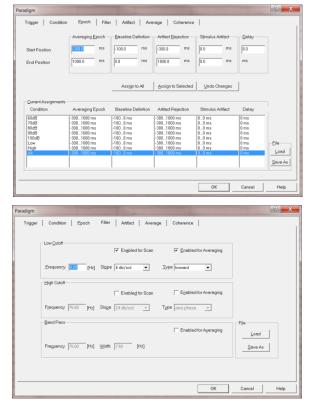
Paradigm					? ×
Trigger Condition E	poch Filter	Artifact Avera	age Coherence		
<u>N</u> ame	<u>Q</u> ualifier	Aţtribute	Operator	⊻alue	
All <u>C</u> ondition Count	Previous Current Next	code name modality side frequency intensity interval	Is Is Not	auditory	Insert Replace
60dB 96 70dB 93 80dB 104 90dB 85 100dB 89 Low 189 High 174 All 467	80dB 90dB 100dB Low	Current intensit, Current intensit, Current intensit, Current intensit, or Current intensit,	y Is "90dB" y Is "100dB" y Is "60dB" y Is "70dB"		AND <u>OR</u> {}
	High All	Current intensity or Current intensity Current modalit	y Is "100dB"	_	File Load Save As
				ОК	Cancel Help

Note that it is also possible to specify other logic combinations using the Qualifier Previous and Next as well as the Operator Is Not. It is also possible to define an interval during which a previous or next event can occur under Attribute.

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

- 5. Move to the Epoch tab. Set Averaging Epoch to -300 : 1000, Baseline Definition to -100 : 0, Artifact Rejection to -300 : 1000 and choose Assign to All. Important note: We recommend using long pre- and post-stimulus times to allow for optimum Low Cutoff filtering.
- 6. Move to the Filter tab. Specify a Low Cutoff filter of 0.2 Hz, 6 dB/Octave, forward. Enable it for Scan and Averaging (i.e. the Low Cutoff filter will be switched on for the artifact scan and averaging). This prevents us from having to filter the averaged data later on and we can avoid the negative effect Low Cutoff filters can have on relatively short data epochs. We will not use a High Cutoff filter here as we can apply it later without distorting the data.

You can now save the paradigm definition by pressing **Save As**. Choose any name and add **-test** to it in order to not over-write any predefined files.

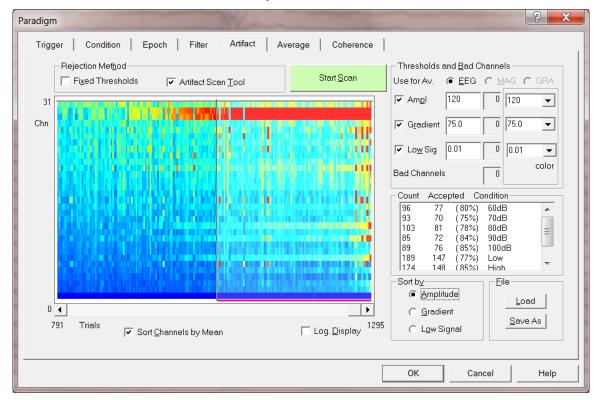


C. Artifact rejection based on amplitude, gradient and low-signal criteria

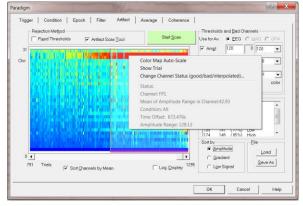
1. To make sure you have the identical paradigm settings as described here, please load the pre-defined Paradigm-file AEP_Intensity.PDG located in the Paradigm directory under Auditory. Move to the Artifact tab and press Start Scan. You will see a 2-dimensional diagram showing the channels (rows) and trials (columns) having the most noise. By default, values based on maximum amplitudes within the predefined artifact

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

epochs are shown. Epochs that exceed the amplitude threshold (default: $120 \mu V$) will be excluded from further processing. You can view the number of accepted trials per condition in the Count box. As artifact correction is switched off at the moment, a substantial number of trials will be rejected.



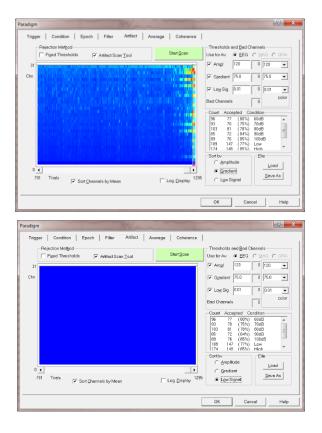
2. Right-click on the red channels to see their labels. The two channels that contain most bad epochs are FP1 and FP2, two frontal electrodes that pick up eyeblinks most strongly. Note that it is also possible at this stage to change a channel status to good, bad or interpolated by choosing the according option in the dialog box or by dragging the horizontal bar up or down. We will not do this at the moment, however.



Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

- 3. It is also possible to view the result of sorting the data after a Gradient criterion (default: 75 μV), i.e. epochs will be marked as bad that contain amplitude jumps of > 75 μV between two sampling points. Choose Sort by Gradient to view this.
- 4. The Low Signal criterion can be useful to identify data periods where an electrode drops off during recording. Epochs will be marked as bad during which the signal is smaller than the threshold criterion (default: 0.01 μV). Choose Sort by Low Signal to view this. In the present case, no epochs fall under this criterion. Change back to the Amplitude sorting criterion and press OK to close the Paradigm window.
- Switch on Artifact correction by pressing ctrl+E. Open the paradigm window again by pressing ERP / Edit Paradigm and move to the Artifact tab. Start the artifact scan again (now with switched on artifact correction) by pressing Start Scan.

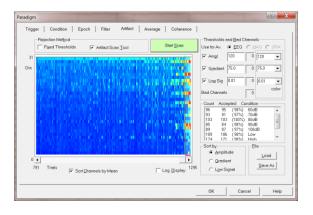
A warning message will appear that a scan has already been performed. Confirm with **Yes** that you want to scan anyway.



Scan Exists
A scan has already been performed. Assignments of bad channels which were done in this editing session will be lost if you scan again. Do you want to scan again?
Yes No

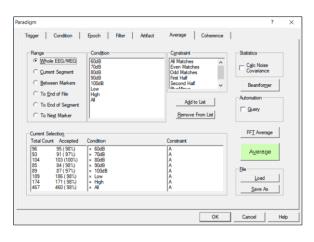
Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

6. Note that a greater number of epochs got accepted and that the two bad electrodes (FP1 and FP2) that were contaminated with eyeblinks are not conspicuous any longer. Some epochs still will be rejected as they exceed the threshold criteria. These epochs are contaminated by other, non-eyeblink related artifacts. It is possible to view the corresponding epoch in the main window by right-clicking into a red pixel and selecting Show Trial.



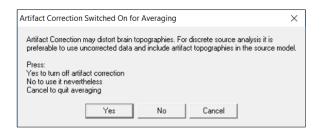
D. Averaging and Classic ERP Analysis

 Move to the Average tab. It is possible to restrict the number of conditions for averaging by removing unwanted conditions from the condition list. Additionally, it is possible to use constraints, e.g. to only average epochs of the first or second half of the experiment. This can be useful to make a split-half comparison. We will now average all conditions by not changing any settings and pressing Average.



Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

- 2. A warning message will appear that artifact correction is switched on, which can be problematic for later source analysis⁵. We recommend to switch off artifact correction for averaging and to load the artifact topographies again at later stages. Press Yes to confirm artifact correction to be turned off. Note that all epochs will still be averaged that were selected with artifact correction being switched on!
- When prompted to save the *.fsg file containing averaged epochs of the conditions defined in the paradigm, add a -test to the suggested filename in order to not over-write any predefined files (i.e. choose the name S1_avtest.fsg).
- 4. You will be prompted to specify names for the averaged epochs. By default, BESA will suggest the names as defined in the paradigm. Unless you want to alter them, choose Stop Asking and the original names will be maintained.



Write Averag	e Buffer - Original Channels - I	Binary		×
Save in:	ERP-Auditory-Intensity	-	- + 1	➡ ■ •
<u>المج</u>	Name	Size	Туре	Date modified
Quick access	All_Subjects_cc.fsg	332 KB	FSG File	2009-12-09 1:35 PM
QUICK access	S1_av.fsg	663 KB	FSG File	2005-05-13 1:53 PM
	S1-blink+ERP.fsg	432 KB	FSG File	2005-05-18 4:19 PM
Desktop	S2_av.fsg	663 KB	FSG File	2005-05-13 1:55 PM
-	S3_av.fsg	663 KB	FSG File	2009-12-03 11:58 AM
	S4_av.fsg	663 KB	FSG File	2009-12-03 12:09 PM
Libraries	S5_av.fsg	663 KB	FSG File	2009-12-03 12:08 PM
	S6_av.fsg	663 KB	FSG File	2009-12-03 12:07 PM
	S7_av.fsg	663 KB	FSG File	2009-12-03 12:06 PM
This PC	S8_av.fsg	663 KB	FSG File	2009-12-03 12:04 PM
1	S9_av.fsg	663 KB	FSG File	2009-12-03 11:55 AM
Network	S10_av.fsg	663 KB	FSG File	2009-12-03 12:02 PM
	<			>
	File name: S1_av-test.	fsg		▼ <u>S</u> ave
	Save as type: Binary High	Resolution (*.fsg)		✓ Cancel

Please Enter a Seg	Please Enter a Segment Comment								
60dB									
60dB									
·									
Ok	Stop Asking	Cancel							

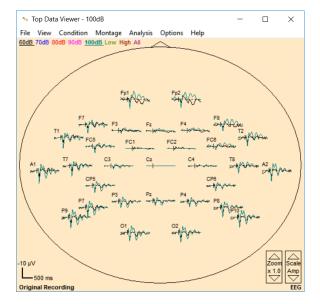
⁵ See the effect of switched on artifact correction during averaging in Tutorial 9: Source Montages.

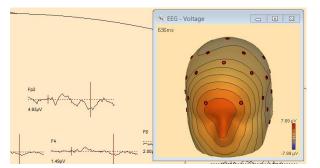
Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

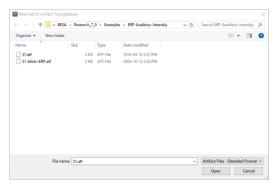
 The Top Data Viewer will automatically open displaying the averaging results. You may choose two conditions at once to be overlaid by holding down the ctrlkey and left-clicking on the conditions of interest.

It is possible to change the viewing properties (color of waveforms, labels, etc.) by selecting **Options** / **Preferences** or right-clicking in the background and pressing **Viewing Preferences**. The viewing epoch may be changed by selecting **View** / **Epoch** or right-clicking in the background and selecting **Viewing Epoch**.

- 6. Select condition 60 dB and double click in the waveforms at 636 ms. At this time, strong activity in electrodes Fp1 and Fp2 can be seen. The 3-D mapping window will open. Again, we clearly see the frontal positivity that is typical for eyeblinks.
- 7. Close the TopViewer to return to the main window. After averaging, BESA Research automatically opens the newly created average file. Therefore, we see a segmented file containing segments of the conditions as defined in the paradigm. We are now going to load the artifact topographies we defined



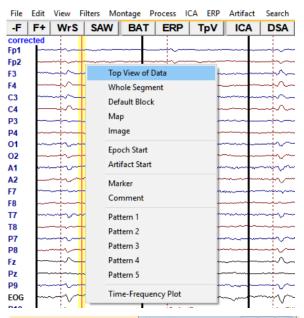


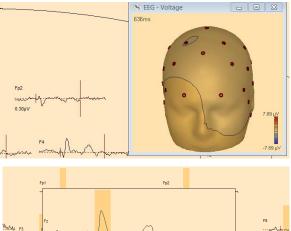


earlier. To do so press Artifact / Load... and select file S1.atf. This file was automatically written by BESA when we defined the blink topography. Press Open.

Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

- 8. Loading the *.atf file will lead to BESA Research subtracting the eyeblink topography. Note that corrected appears in the top-left corner and a virtual EOG channel is displayed at the bottom. Switch off the filters by pressing the EdF button and deselecting all filters. Open the TopViewer again by right-clicking in the data and choosing Top View of Data.
- 9. Return to the 60 dB condition and note that the large activity around 636 ms has disappeared. If you now doubleclick to bring up the 3D-mapping window you will see that the blink topography is gone.
- Zoom electrode Fz by right-clicking on it and selecting Zoom Waveform. Leftdrag a window over the most prominent peak. Right-click again and select Find and Mark Peak.





Find and Mark Peak

Unzoom Waveform

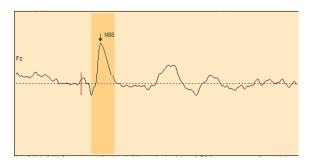


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Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

- 11. The Find Peaks window will open and automatically display the search results for the marked data-block in the specified electrode as well as all other electrodes. A time of the large negative peak is 88 ms. BESA also outputs the mean amplitude in the specified timerange as well as the Area under the signal. These results can be saved using the ASCII Export option. Press OK.
- **12.** Back in the TopViewer an arrow will now mark the peak position.

Search Channel	fro <u>m</u> 48 ms	to 156 ms	Eind	
C <u>u</u> rrent Peak	Delete Peak		ASCII E	cport
Channel	Ampl at pos Peak	Ampl at neg Peak	Mean Amgl	Area [signal units * ms]
FC2 FC1	1.45 1.34 1.19	-5.10 -4.56 -4.14	-2.18 -1.83 -1.72	268.51 × 230.71 209.70 =
F2 C2 F4 C4 F3	1.03 1.43 1.18 1.00	-3.89 -3.59 -3.40	-1.63 -1.54 -1.31	205.78 192.70 193.43
F-3 FC6 C3 FC5	1.00 1.20 1.12 0.36	-2.97 -2.62 -2.34 -0.79	-1.16 -0.81 -0.84 -0.32	145.03 152.88 132.57 54.52 +
Display Options Mark Selected	C Mark All	Positive Peak L 48.0		ak Latency
Peak Label	Show Label	All Amplitud Same Later	lesat v DetectAl ncy Maxima a	osolute and Minima
1.00		Advanced >>	ОК	Cancel



Tutorial 2 – Basic Artifact Correction, Triggers, Averaging

Tutorial 3 – Batch Processing, Combine Conditions

Tutorial 3 – Batch Processing, Combine Conditions

What does BESA Research provide?

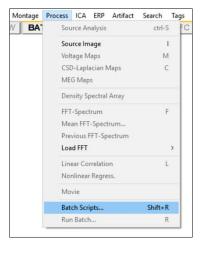
- ✓ Batch processing
- ✓ Creating grand averages
- ✓ Creating new conditions from combinations of existing conditions
- ✓ Averaging of channels

Tutorial 3 – Batch Processing, Combine Conditions

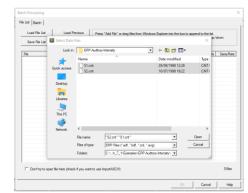
A. Batch Processing

In the following we will create a batch script that performs the same preprocessing, artifact treatment and averaging in all individual datasets.

 In BESA Research, select File / Close All. Now select Process / Batch Scripts. This will bring up the Batch Processing dialog.



Press Add File and select both available
 *.cnt files (S1.cnt and S2.cnt). Press
 Open.



3. The file-list now contains the raw data of subjects S1 and S2. For display purposes only two datasets were selected, in principle, an arbitrary number of files can be selected. We can see that both files contain data of 32 electrodes sampled at 250 Hz. It is possible to save or reload a file list at this stage by pressing the according buttons.

Batch Processing			×
File List Batch			
Load File List Load Previous Press "Add File" or drag files from Windows Explorer int Right Click on file names for further options. CDe's delet moves marked items.	to the box to ap tes marked item	end to the list. . Ctrl+Cursor up/o	lown
File		Channels	SampRate
C. Vuleni Yubic Documenta BESA/Research_7_IVExamples ERP Auditory-Intensity (SI ont C: Users Yubic: Documenta BESA/Research_7_IVExamples (ERP Auditory-Intensity (S2 ont Documenta BESA/Research_7_IVExamples (S2 ont		32 32	250.0 250.0
Don't try to open file here (check if you want to use importASCIII)			2 files
	OK	Cancel	Help

Tutorial 3 – Batch Processing, Combine Conditions

4. Move to the Batch tab and press Add command. This will bring up the Select Command window that is sub-divided into 4 command sections corresponding to the main program, source analysis, imaging, time-frequency analysis and general commands.

eneral commands:	Main Program:	Source Analysis:	Source Analysis Imaging
BatchError Comment MATLABcommand MATLABwaitForVariable Pause WindowPosition	ArtifactAutoCorrect ArtifactMethod ArtifactOn/off ArtifactScan AuxiliaryFiles Average Baseline EdtDefaultEpoch	AddSource ChannelTypeForFit ConverSource Delete DisplayMRI Exit Fit FitConstraint	Beamformer CLARA Clip Export GotoMax Import LAURA LORETA
 Apply to all files 	EventRead EventWrite Export FFTsave FileOpen Filter	FitInterval MinimumNorm NewSolution OpenSolution PCA Regularization	SetCrosshair sLoreta Smooth SSLOFO UserDefined
 Apply at beginning of batch Apply at end of batch 	ImportASCII MarkBlock Montage Paradigm PatternToTrigger SendToMATLAB	SaveBitmap SaveModelWaveforms SaveResidualWaveforms SaveRVandGFPWaveforms SaveSolution SaveSolution	Time-Frequency Analysis Display
Current Selection	TriggerSelect	SaveSourceWaveforms SendToMATLAB SetCursor SetDefaultSourceType SetOrActivateSource SetOrientation SwitchCandition	Image Save SendToMATLAB StartTFAnalysis

5. First, we want to apply the predefined paradigm file (cf. Chapter A) to each data set, therefore select Paradigm from the list of commands in the Main Program group to load a defined paradigm file to each data set. Hit OK. The Load Paradigm window opens. Select Auditory / AEP_Intensity.PDG, press Open and confirm the paradigm selection by pressing the OK button.

General commands:	Main Program:	Source Analysis:	Source Analysis Imaging:	Load Paradigm Task	• • 🖻 🖆 🖽 •
Betchlimer Comment MATLABoommand MATLABoomforVariable Pause WindowPosition	ArtifectMutoCorrect ArtifactMethod ArtifactDr(eff ArtifactScan AudioryFiles Average Bassline EddDefeultEpoch EverpRead	AddSource ChannelTypeForFit ConvertSource DisplayARI Bott Fit Fit FitConstraint FitConstraint FitConstraint	Beamformer CLARA Clip Export GotoMax Import LAURA LORETA SetCrosshar	Instructions Press Browseto select a paradigm file name. %basenews?will be replaced by the base name of the current file.	Date modified 23.11.2004 15:19 17.12.2009 21:34 23.11.2004 15:20
Apply to all files Apply at beginning of batch Apply at end of batch Apply at end of batch Current Selection MAIDFirmsdigm OK Cancel	EventWrite Daport IPFTare FileOgen Filter ImportASCII MartBlock Mantage Partifyin Pattern10 rigger SandToMATLAB TriggerSelect	Meimumiterm Inavidation PCA Regularization Sawelfang Saw	slareta Simoth SSLOPD UserOPfinad Time-Frequency Analysis: Simoga Sang Sang Sang Sang Sang Sang Sang Sa	File name [%basename%.pdg BrowseOK Cancel File damage decision File damage damage decision File damage	v Qoen Cancel v

Tutorial 3 – Batch Processing, Combine Conditions

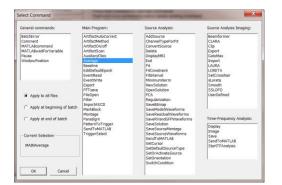
6. Next, instruct BESA Research to perform an artifact scan on each data set. Press Add Command, select Artifact Scan from the list of commands in the Main Program group and hit OK. The Artifact Scan Task window opens. You may have artifact rejection run fully automatically. However, it is recommendable to visually inspect the results of the artifact scan in each subject, because e.g. in some data sets in might be necessary to exclude a bad channel manually. Therefore, make sure the option Wait after scan is selected, which will prompt for an OK after each artifact scan and allows making manual corrections. Press OK.

General commands:	Main Program:	Source Analysis:	Source Analysis Imaging:	
BatchError Comment MATLABootimend MATLABootimend Pause WindowPosition	ArtifectAutsCorrect ArtifectDetHod ArtifectDryfeff ArtifectDryfeff AustlaryFiles Awer ope Baseline EditDefeulEpoch EventWrite	AddSource ChannelTypeForFit ConvertSource Delete DisplayMRJ Exit Fit FitConstraint FitConstraint FitConstraint Meinammärerm	Beamformer CLARA Clip Export GotoMax Import LUNKA LORETA SetCrossbar storeta	Instructions Check "Wait after scan" if you want to adjust thresholds and check bad channels after each
 Apply to all files Apply at beginning of batch 	Export FFTsave FileOpen Filter ImportASCII MarkBlock	NewSolution OpenSolution PCA Regularization Save8/imap Save8/imap	stareta Smooth SSLOFO UserDefined	✓ (Wait after scan
C Apply at end of batch	Montage Paradigm PatternToTrigger SandToMATLAB TriggerSelect	SaveResidualWaveforms SaveRVsndGCPWaveforms SaveSourceMortage SaveSourceWortage SaveSourceWaveforms SendToMATLAB	Time-Frequency Analysis: Droplay Image Sare SendToMATLAB	
MADU/utfactScan		SetCurtor SetDefaultSourceType SetOrActivetSource SetOvientation SwitchCondition	StartTFAnalysis	

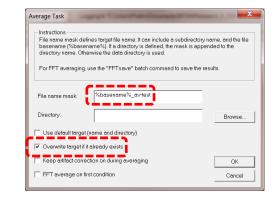
7. Finally, press Add command again, select Average from the Main Program group and press OK. In the Average Task window, BESA Research allows to specify a file name for the averaged individual data. Uncheck the option Use default target and change the File name mask to %basename%_av-test. This will create BESA binary files (*.fsg) with a basename derived from the corresponding raw data set. The averaged segments of file S2.cnt will be written to file S2_av-test.fsg, for example.

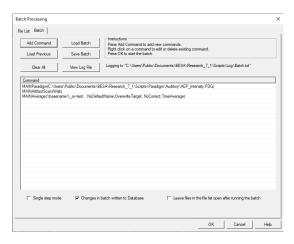
Make sure **Overwrite target if it already exists** is checked. You can also specify whether artifact correction should be left active during averaging. This is relevant only if one is working with artifact corrected data (using either the semi-automatic artifact correction options described in the Tutorial on Artifact Correction or the automatic artifact correction that can be executed using the **ArtifactCorrect** command in the command list). Here, however, for the sake of simplicity, we will reject all artifacts from further processing rather than performing an individual blink correction. For the grand averaged data, enough artifact-free epochs will be available even after artifact rejection. Press **OK**.

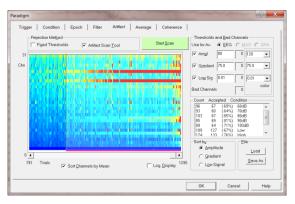
Tutorial 3 – Batch Processing, Combine Conditions



- 8. The three commands have been added to the command list with all specified options. You may save this batch and reload it for further analyses using the Save Batch and Load Batch buttons. Press OK to start the batch processing with the first file (S1.cnt).
- 9. BESA Research stops after the artifact scan has been performed. You can now manually adjust the number of rejected trials and channels. In file S1.cnt, many blinks can be observed, primarily in channels Fp1 and Fp2. The preset amplitude threshold is not low enough to exclude all blink-contaminated trials. Drag the vertical red bar to the left to exclude more trials or set the amplitude threshold manually to 80 μV. This will reject more epochs but we can be certain that no artifacts are retained in the data.







Tutorial 3 – Batch Processing, Combine Conditions

- Select Sort by Gradient. Place the scrollbar to the right end of the display to see the trials with large gradient artifacts. The gradient threshold should be lowered (i.e. the vertical red bar in the 2D display should be moved to the left) to exclude all artifact-contaminated trials. Set the gradient threshold to 30 μV.
- 11. Select Sort by Low Signal to see whether there are any channels that dropped out during the measurement. This is not the case.
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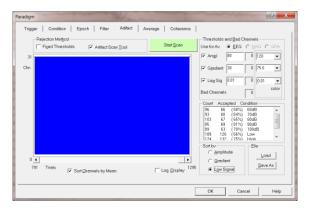
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12. Press OK to finish artifact rejection in the first data file. BESA Research now averages all specified conditions and proceeds with the next data file. In file S2.cnt, adjust the artifact thresholds to 100 μV (amplitude criterion) and 50 μV (gradient criterion). After processing the batch on all data files, BESA Research gives a notification and offers to view the automatically generated log file (View Log button). Press OK.

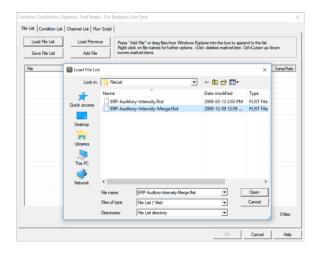
Batch Co	mpleted
File:	2: C:\Us\BESA\Research_5_3\Examples\ERP-Auditory-Intensity\S2.cnt
Task:	Batch Completed
	View Log Cancel

Tutorial 3 – Batch Processing, Combine Conditions

B. Creating Grand Averages

As a next step we will create a grand average across subjects using individual average files. Grand averages are useful for later source analysis and also for documenting differences between conditions across all subjects in classic ERP analysis. Individual average files (*.fsg) of 10 subjects (S1 to S10) are provided in the BESA Research examples folder Auditory Intensity.

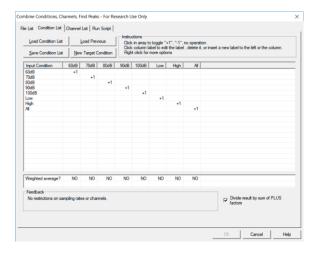
- In the BESA Research main window, select ERP / Combine Conditions to open the according dialog window. Press Load File List and select the pre-defined file ERP-Auditory-Intensity-Merge.flist and press Open. This will load *.fsg-files containing average data of subjects S1 to S10.
- 2. The number of channels, electrodes, epochs, conditions, and the sampling rate is displayed for each file. You may increase or reduce the width of the columns in the table by dragging the column border in the title bar.



Load File List	Load Previous	Press "Add File" or drag file Right click on file names fo					
Save File List	Add File	moves marked items.	r runner options	< Del> deletes n	larked item. C	un+cursor up/r	aown
File			Channels	Bectrodes	Epochs	Conditions	SampRat
		uditory-Intensity\S1_av.fsg	32	31	8	8	250.
C:\UserSA\Researc	h_7_1\Examples\ERP-A	uditory-Intensity\S2_av.fsg	32	31	8	8	250.
		uditory-Intensity\S3_av.fsg	32	31	8	8	250.
C:\UserSA\Researc	h_7_1\Examples\ERP-A	uditory-Intensity\S4_av.fsg	32	31	8	8	250.
		uditory-Intensity\S5_av.fsg	32	31	8	8	250.
		uditory-Intensity\S6_av.fsg	32	31	8	8	250.
		uditory-Intensity\S7_av.fsg	32	31	8	8	250.
		uditory-Intensity\S8_av.fsg	32	31	8	8	250.
C:\UserSA\Researc	h_7_1\Examples\ERP-A	uditory-Intensity\S9_av.fsg ditory-Intensity\S10_av.fsg	32	31	8	8	250.
					ок 1	Cancel	10 files Help

Tutorial 3 – Batch Processing, Combine Conditions

- 3. Switch to the Condition List tab. Here it can be specified how the input conditions (the conditions available in the loaded data files) are to be combined to new target conditions. In the left column, the available input conditions are listed - in our case the eight conditions that were defined in the paradigm file (Tutorial 2, step B) and averaged using the batch script used in the present tutorial under step A. Conditions having the same segment comment in the different files are grouped into one entry.
- 4. Right-click onto the input condition low in the left column. A window opens displaying information about the condition. The bottom line indicates that a low condition with the specified parameters is present in all ten input files. Press OK to close this window.



Condition information	\times
Condition label: Low Samples: 325 Zero sample: 75 Time Range: -300 to 1000 ms Sampling rate: 250.0 Occurs in 10 files	
ОК	

5. BESA Research automatically defined new target conditions (columns 2 – 9) with the same name as the input conditions. The numbers in the table indicate how the input conditions are to be combined to form the new target conditions. The default entry +1 in the column of the 60dB condition, for example, will generate an average of all input conditions labeled 60dB. Hence, the default definitions define grand averages of all input conditions.

Tutorial 3 – Batch Processing, Combine Conditions

6. We will modify the default definitions. Instead of creating a grand average of the All condition over subjects, we will create a new condition that contains the grand averaged *difference* between the High and the Low condition. Left-click onto the label of the target condition All in the title bar of the table. This allows to create new target conditions by inserting new columns, or to rename an existing target condition. Change the label of target condition All to Difference and press OK.

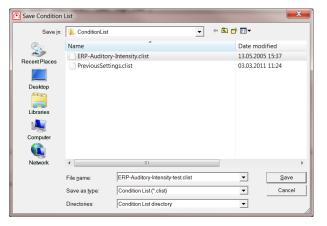
Load Condition List		oad Prev	ious	Old	ctions in array to column la	toggle "-	1", "-1", i the label	no operatio	m. or insert a new label to the left or the column.
Save Condition List	New	/ Target C	ondition	Righ	t click for r	more optic	ns		
Input Condition	60dB	70dB	80dB	90dB	100dB	Low	High	AI	
60dB	+1								
70dB		+1							
80dB 90dB			+1	- 1					
100dB			Tarc	et Cond	ition 8				
Low									-
High			TR	le of targ	et condition	π.		OK	1
Al									
				Differenc	d			Cancel	
				□ Insert	new cond new cond e this cond e all condi	ition to th lition	e right	his column	
Weighted average?	NO	NO	NO	NO	NO	NO	NO	NO	-
Feedback									
No restrictions on sar	mpling rate	es or chan	nels.						Divide result by sum of PLUS factors

7. The target condition has been renamed to Difference. Left-click twice onto the +1 entry that links this condition to the All input condition. This will remove the link and leave the field blank. To define the Difference condition as the average of *High minus Low* over subjects, left-click once into the field linking input **High** with target **Difference** to generate an entry +1 in this field. Left-click twice into the field linking input Low with target **Difference** to generate an entry -1. Note that arbitrary weights of conditions apart from +1 and -1 can be assigned by a right-click into the corresponding field. This is not necessary in our case. The bottom line (Weighted average?) allows to weigh each input condition with the number of averages it contains. We will leave the default settings to create non-weighted grand averages. Un-ticking the option Divide result by PLUS factors allows creating a target file containing the sum rather than the average of input conditions. We want to create a grand average, so we will not remove the tick-mark for Divide result by PLUS factors.

Tutorial 3 – Batch Processing, Combine Conditions

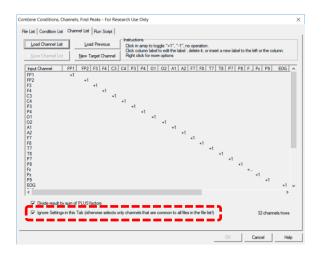
Input Condition 60dB 70dB 80dB 90dB 100dB Low High Difference 60dB +1 - <td< th=""><th>Load Condition List</th><th></th><th>_oad Prev ⁄Target C</th><th></th><th>Click</th><th>in array to</th><th>cel to edit</th><th>the label</th><th>no operation , delete it, or</th><th>i. rinsert a new label to the left or the column.</th></td<>	Load Condition List		_oad Prev ⁄Target C		Click	in array to	cel to edit	the label	no operation , delete it, or	i. rinsert a new label to the left or the column.
70dB +1	Input Condition	60dB	70dB	80dB	90dB	100dB	Low	High	Difference	
30dB +1 0dB +1 100dB +1		+1								
900B +1 1000B +1 Low +1 High +1 All			+1							
100dB +1 Low +1 -1 High +1 +1 All - Indicating the second sec				+1						
Low +1 -1 High +1 +1 All					+1					
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	Neighted average?	NO	NO	NO	NO	NO	NO	NO	NO	
Feedback	Foodback									
No restrictions on sampling rates or channels.		npling rate	es or chan	nels.						Divide result by sum of PLUS
	No restrictions on san									tactors

 Press Save Condition List to store the defined conditions to disk. Enter ERP-Auditory-Intensity-test.clist as file name and press Save. You may press Load Condition List to load the predefined condition list ERP-Auditory-Intensity.clist.



Tutorial 3 – Batch Processing, Combine Conditions

9. Proceed to tab Channel List. By default, settings in this tab are ignored (checkmark at the bottom left). It allows combining data of different channels. To create our grand average, this is not necessary, therefore we leave the settings in this tab unchanged.



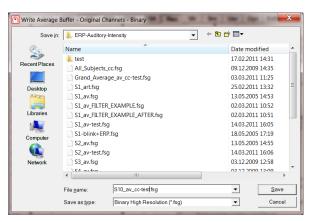
10. Switch to the Run Script tab. Global output options can be specified here. Make sure Combine data from source files is selected in the box Averages to generate, as we want to create a file containing averages over subjects. The options under Spatial Interpolation allow to handle differences in the electrode configurations of the different source files. In our example, all subjects have the same electrode configuration and no bad channels have been defined. Therefore, no spatial interpolation is required. The same is true for Temporal Interpolation and Clipping: All input conditions have the same latency range and sampling rate, therefore no temporal interpolation of the data is necessary.

Note that the Run Scripts tab also allows analyzing peaks and mean **amplitudes** in multiple data files. This is achieved by selecting Peaks and mean amplitudes, which activates the bottom right segment of the window. Traditional peak analysis can be performed quickly in an automated fashion, producing ASCII output files containing the selected peak parameters (latency, amplitude, area).

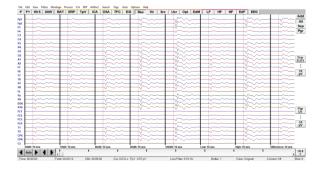
Tutorial 3 – Batch Processing, Combine Conditions

	en press "OK" to start averaging or peak/amplitude export.					
werages to generate	ESULTS FOR DIAGNOSTIC PURPOSES!					
C Generate separate averages for each source file	✓ Open target file in BESA					
Use mask: %basename%, ccfsg Combine data from source files]	Peaks and Mean Amplitudes					
C No averages: just copy	C Peaks C Single file output					
C Peaks and mean amplitudes	Mean Amplitudes C Statistical sparse output					
	C Areas C Send to MATLAB					
Spatial Interpolation Interpolate to Standard 81 electrodes Interpolate bad channels (obligatory if bad channels	from to Time Range: 0 ms 1000 ms					
defined and not standard 81 interpolation)	Montage Original Recording					
Temporal Interpolation and Clipping	Montage Original Recording					
Spline to new sampling rate	Filters					
Clip interval Sampling rate from (ms) to (ms)	Baseline No baseline correction					
250.00 -300.0 1000.0	- Peaks					
No of samples: 325, no of prestimulus samples: 75 Max prestimulus interval: 300, max poststimulus interval: 1000	All Amplitudes at same latency Positive voltage					
max presumulus interval. 500, max postsumulus interval. 1000	1:Fp1 Channel for peak detection					

 Click OK to start the batch. BESA Research computes the specified grand averages and prompts for a file name. Enter All_Subjects_cc-Test.fsg and press Save.

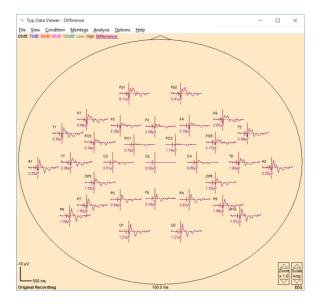


12. The generated grand average file contains the specified target conditions 60dB to 100dB, Low, High and the Difference condition. The number of source conditions is shown at the bottom next to the condition label, in our case 10, one for each subject.



Tutorial 3 – Batch Processing, Combine Conditions

13. Right-click into the data and select Top View of Data. Let's have a look at the condition Low, High and Difference. It becomes very clear that there is a difference in brain activity when listening to low and high-frequent tones. On the one hand, we can see an amplitude modulation; on the other hand, we can see a change of waveform morphology from low to high. Close the TopViewer.



Tutorial 3 – Batch Processing, Combine Conditions

Tutorial 4 – Discrete Source Analysis

Tutorial 4 – Discrete Source Analysis

What does BESA Research provide?

- ✓ Fitting of single dipoles and regional sources
- ✓ Using PCA
- ✓ Using regularization

This tutorial is designed to teach the basic concepts of multiple source analysis. At the same time, it can be used to get familiar with the user interface of the source analysis window in BESA Research.

The first chapter provides the basic theoretical foundation of the concept of discrete source analysis. The following hands-on examples introduce the source analysis interface of BESA Research and apply the provided principles to simulated EEG data sets. A concluding chapter summarizes the obtained results and lists some guidelines for source analysis.

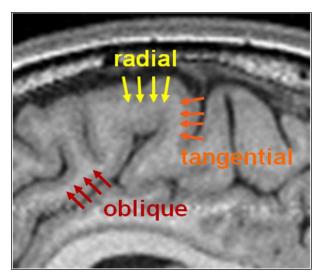
A. Theoretical introduction

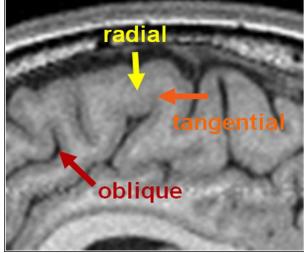
Equivalent current dipoles

Neuronal current in the cortex flows predominantly perpendicular to the cortical surface for two reasons: First, the pyramidal cells in the cortical columns are aligned perpendicular to the cortical surface. Second, the dendritic trees that are parallel to the cortical surface have near-rotational symmetry and the electric fields of the related intracellular currents cancel to a large degree.

Activity in multiple brain regions = postsynaptic current flow in pyramidal cells

Discrete equivalent current dipoles as model for the activity in each brain region





The intracellular postsynaptic current vectors of nearby cortical columns sum linearly and can be represented very accurately by an equivalent, compound dipole current vector. Areas with up to 3 cm in diameter can be very accurately (> 99%) modeled by a single equivalent dipole.

Currents at the cortical convexity have a predominantly radial orientation; currents in cortical fissures have predominantly tangential orientation. Generally, a patch of activated cortex in a sensory, motor or spiking area will have an oblique orientation depending on the net orientation of the activated cortex.

Thus, an equivalent current dipole is specified by its location (the equivalent center of the modeled gray matter patch) and its orientation (the net direction of the modeled postsynaptic neuronal current, perpendicular to the surface of the modeled gray matter patch). The orientation of a dipole therefore indicates the local orientation of the pyramidal cells in the gray matter – it is not to be confused with a direction of signal propagation across the brain! The orientation of a dipole is usually symbolized by an arrow or a short line.

The third parameter of an equivalent current dipole is its strength or amplitude, reflecting the modeled net postsynaptic current flow. Its units are that of a dipole moment, i.e. nAm (nano-Ampere × meter). It can be thought of as the product of the total postsynaptic current flow (in nA) and the length over which this current is flowing (on the order of the length of a pyramidal cell in meters). The temporal evolution of the dipole moment is called the source waveform and is in many respects the most important outcome of source analysis.

Volume conduction and the principle of linear superposition

An ideal patch of superficial cortex creates a net radial current flow that can be very accurately modeled by an equivalent dipole near its center.

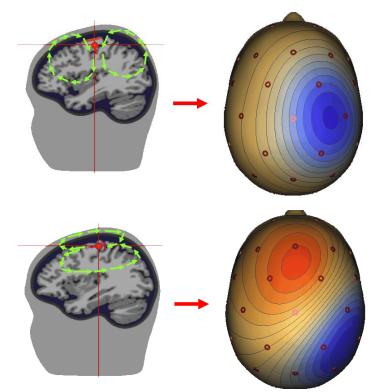
Current loops in a conductive medium like the head are closed. Therefore, the intracellular currents resulting from action and post-synaptic potentials are accompanied by secondary return currents in the head volume. Since the brain and scalp have a higher electrical conductivity as compared to the cranium, most currents return within the extracellular brain space. Only a very small fraction flows out through the poorly conducting cranium and along the scalp before returning to the brain.

Inside the head:

Primary (postsynamtic, neuronal) current in red, secondary (volume) in green

Signal on the head surface:

Generated potential changes (= EEG maps / topographies). Blue: negativity, Red: positivity



Top: Radial primary current flow at the cortical convexity in the right central cortex.

Bottom: Tangential primary current flow in a cortical fissure.

Although the location of the active brain region is nearly the same, the different orientation of the patch surfaces and the associated different flow of the secondary volume currents lead to a completely different potential distribution. Maximum EEG activity is not necessarily generated directly on top of the active brain region.

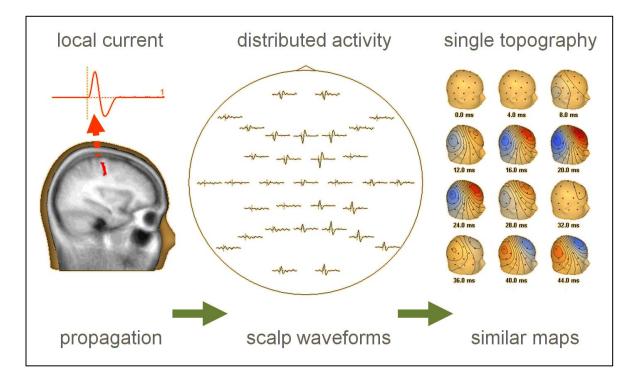
The propagation of the volume currents to the scalp is described by the so-called head model. The head model, or forward model, predicts the voltage at any electrode due to an equivalent dipole with a given location and orientation within the brain.

The volume conduction results in a widespread, smeared voltage topography over the whole scalp with a maximum over the activated cortical sheet. A corresponding activity of opposite polarity appears on the other side of the head. By the laws of physics, the integral of the potential over the whole head is zero. Therefore, any negativity has a corresponding positivity somewhere else over the head. The voltage map (topography) displayed on the top right of the figure above is typical for focal radial activities at the cortical surface. The shown maps illustrate the limited spatial resolution of the EEG. The precise orientation of the map, and the underlying equivalent dipole, can only be determined if inferior electrodes are present to help define the location of the positivity on the other side of the head.

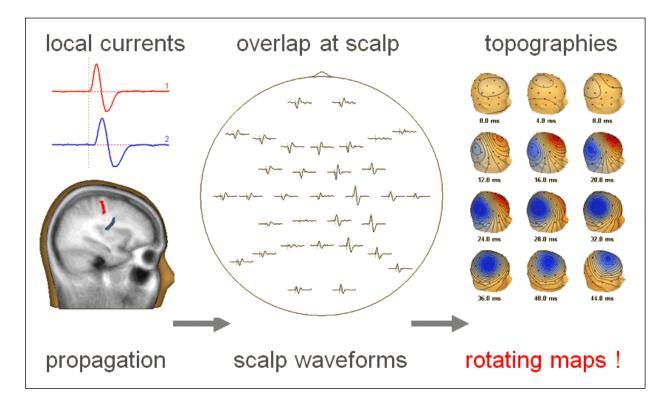
A cortical patch in a fissure generates a tangentially oriented dipole field (bottom of the figure above). The return currents in the scalp create a dipole map with symmetric positive and negative poles aligned in the direction of the dipole. The potential change directly over the source is zero, but the gradients are maximal. The source is below the site of the densest equipotential lines. These lines and the whole shape of the topography carry more information on the location of the underlying generators than the colorful peaks.

An EEG electrode does not record the potential at a certain location on the head surface. Rather, it records a voltage (= potential difference), i.e. an EEG signal is always the difference between the potential at a certain electrode location on the head surface and the potential at another electrode - the reference electrode - or the average of several electrodes. Thus, the choice of the reference electrode(s) determines the recorded signals, but the underlying potential distribution (the potential map) is independent of this and solely determined by the underlying brain processes.

Using a simulated example, we will now learn how to discriminate the scalp waveforms and topographies due to a focal activity of one brain region and temporally overlapping activity of two brain regions.

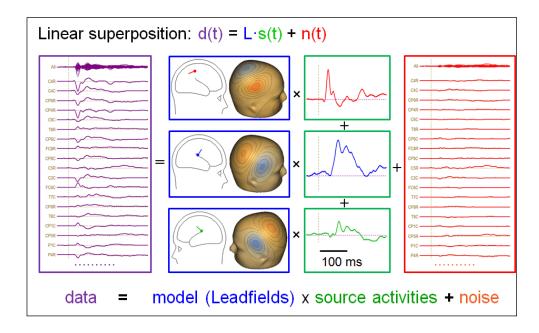


An assumed activity in the right central sulcus produces a near-tangential dipole field with a positive peak (shown downwards in the EEG top view in the middle) over the mid-frontal region (Fz and FC2) and a negative more widespread peak over the right inferior parietal regions (max. at P4). The patch is synchronously activated and there is no propagation. Accordingly, the net orientation remains the same. The waveforms at the different electrodes have different magnitudes but the same evolution over time. The topographic maps change only in magnitude but not in shape. Map polarity simply reverses in the second phase following the initial activity.



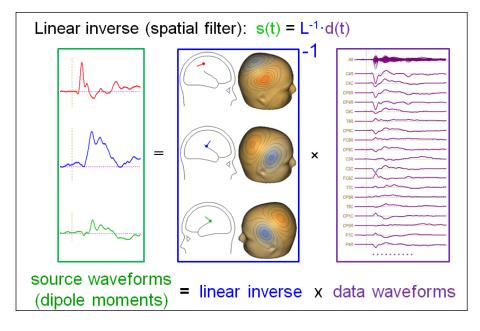
Now consider the situation of two brain regions separated by about 3 cm and activated within a few milliseconds. Each of the areas has a biphasic pattern with onset, peak, and polarity reversal. The two patches have different orientations. This is the main cause for their very different scalp topographies. Due to the time difference in activation their maps overlap with continuously changing magnitudes according to the instantaneous strength of the 2 compound currents. This results in an apparent rotation of the maps over time, and it becomes difficult to identify and separate the two sources by mere visual inspection.

Tutorial 4 – Discrete Source Analysis



Using the laws of basic physics, we can now formulate the principle of linear superposition. This is illustrated here for 3 equivalent dipoles, but it would be the same for 10000 cortical current elements: The measured data (in this example MEG sensor signals, left, generated by tactile stimulation of the left index finger) are the sum over the contributions of all sources. In source analysis, each active brain region is modeled by one equivalent dipole source. Each source is fixed to the cortical patch or region it represents, and changes its total current strength over time according to the local physiology. In 1986, this was named a source waveform (Scherg and von Cramon, Electroenceph. Clin. Neurophysiol. 65:344). With a given volume conductor model, e.g. a spherical head model, a boundary element model (BEM), or a finite element model (FEM), it is now possible to predict the leadfields, i.e. the magnitude of the signal each source will contribute to each sensor. Because the model is an approximation, both in terms of the volume conductor and the simplification of using equivalent dipoles at the centers of activity, there is a residual. Ideally, if we have a good model, this residue should be small and consist only of sensor noise and brain background activity not related to the tactile stimulus.

Tutorial 4 – Discrete Source Analysis

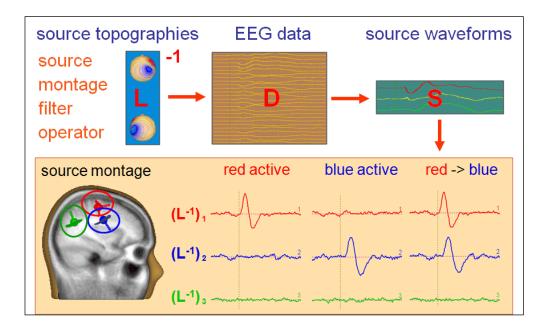


The inverse operator: Reconstructing brain activities

In our superposition, equation the source waveforms are the unknown, given we know the centers (and orientations) of source activity in the brain. In this case, the source activities can by calculated directly by inverting the leadfield matrix and multiplying from the left onto the superposition equation. In the illustrated case, the leadfield matrix consists of the three sensor topographies which have to be inverted. The noise contribution is neglected in this process. Accordingly, unmodeled noise will be projected onto the calculated source waveforms.

The linear inverse operator acts like a spatial filter that deblurs the measured waveform to unfold and separate the underlying source waveforms (Scherg and Picton, EEG Suppl. 42, 1991). In other words, source waveforms are calculated by combining the temporal information in all channels giving specific weights to each signal. Each row in the matrix L⁻¹ is a linear operator reconstructing one source waveform.

Tutorial 4 – Discrete Source Analysis



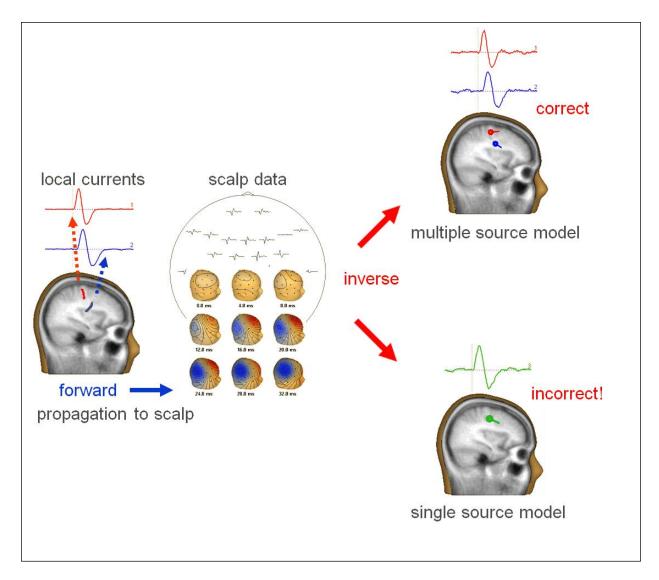
The above example demonstrates the full separation of the source activities 1 & 2 in three simulated cases and illustrates the absence of activity in source area 3 since its source waveform shows only EEG background signal.

Thus, multiple sources can mutually contrast and separate the activities of the brain areas that they represent.

The displayed circles on the left illustrate that separation of the activity from several brain areas is principally possible, if they are sufficiently remote from each other (> 3 cm). However, precise localization within each region is not possible in typical data because of the EEG background noise.

In the overdetermined case, i.e. if there are less sources than measured channels, the linear inverse operator L⁻¹ is constructed to fully separate the different source activities. The vector operator for source 1 will fully recover source activity 1, but will not be sensitive to any activity at sources 2, 3... and vice versa. This sharp separation has a drawback, if some of the sources have a high spatial correlation in the sensor space. Then the inverse operator will have large entries and the noise will be amplified accordingly. However, this problem is easily handled by modest regularization (default in BESA Research is 1%) when calculating the inverse of the topography matrix. How regularization effects the source waveforms will be demonstrated in Chapter B (Step 16).

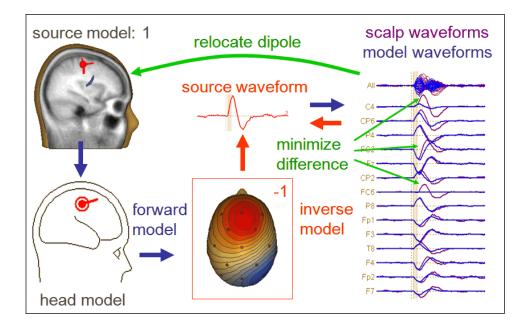
If we apply a two-dipole model to our simulated data set, they separate the two activities in their source waveforms provided that appropriate equivalent locations and orientations had been selected. If we try to model the data with a single dipole only, we obtain an incorrect localization intermediate between both sources. The source waveform combines both underlying activities into a broader pattern which has a latency intermediate between the original activities. Therefore, care must be taken to create a multiple source model that is appropriate for the current data set. In the following we will see how this can be achieved. The following section shows how a source model can be created by fitting discrete sources to the EEG or MEG data.



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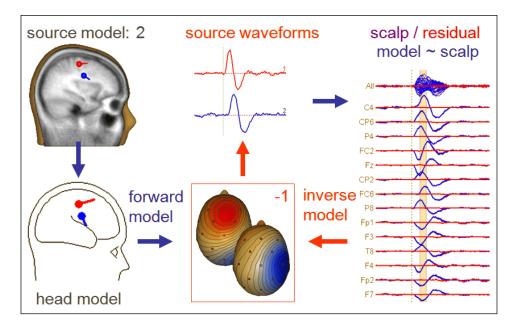
The fit procedure

We assume that a single dipole will explain the early onset phase (i.e. initial source model hypothesis is a single equivalent dipole). Using the head model, the forward model topography (= leadfield) is estimated. The inverse of this leadfield matrix is applied to the data to estimate the source waveform. The source waveform is projected back to the scalp using the forward coefficients of the map to estimate the model signals (blue curves in the figure below). Measured and modeled data are subtracted to estimate the residual waves. In an interactive process, dipole location and orientation is adjusted and the calculation process is repeated until the residual difference between scalp and model waveforms is minimized. The equivalent dipole locates in or near the active cortex if the hypothesis, head model, and data are sufficiently accurate.



Fitting strategy for multiple activities – step 1:

Use the 3D maps to define the fit interval from the time when a clear dipole field emerges until it starts changing. Perform a principal components analysis (PCA) over this interval: The PCA decomposition should show one dominant component. The percentage of variance it explains should decrease, if the interval is extended further. Fit the first dipole over this interval.

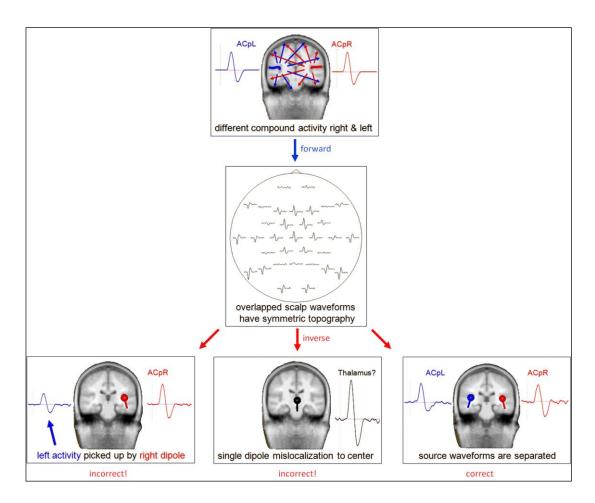


Fitting strategy for multiple sources – step 2:

Display the residual waves and maps. Perform a PCA on the residual waves and repeat the same procedure to mark the next onset interval in the residual data. Fit a second dipole to this interval while keeping the first dipole fixed in location and orientation. In the simulated example with good signal-to-noise, this results in the separation of the underlying active areas and their source waveforms.

Finally, we should check the homologous brain region in the other hemisphere for a potential spread of activity using a probe source at the mirror location of dipoles 1 and 2.

Tutorial 4 – Discrete Source Analysis



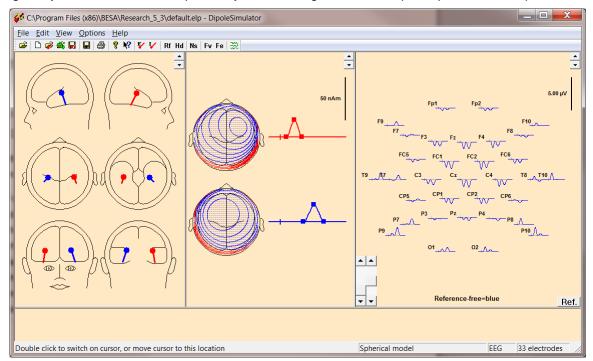
The above figure shows a simulated example that illustrates the need to create an appropriate source model in order to obtain correct source waveforms. We assume a situation where both auditory cortices are active. Their topographies on the scalp surface overlap, and the recorded signal at each electrode is a superposition of the contributions of the two sources. If we apply a source model with one dipole in the right auditory cortex only, its source waveform will not only pick up the right hemispheric activity. It will rather contain some contribution from the left hemisphere as well, which is not properly modeled. An attempt to fit a single equivalent dipole to this data results in a mislocalization in the head center close to the thalamus. The reason is that the ACpL and ACpR sources generate a symmetric topography on the scalp surface with largest signals over the scalp midline. The obtained source waveform is a linear superposition of the activities of both brain regions. The correct source activities are reconstructed only when two equivalent current dipoles are used to model the data. Only with this two-dipolar model, the spatial filter can separate the activities of the two brain regions.

Tutorial 4 – Discrete Source Analysis

B. Single Dipole Fitting with Simulated Data

Before we will create a source model for the auditory intensity data we have been working on so far, we will work with three simulated datasets to understand the principles of dipole fitting.

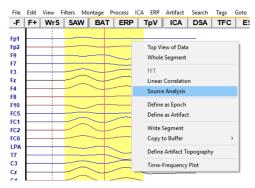
1st simulated data set: In the first example, non-overlapping (asynchronous) activity in both auditory cortices is simulated. Current flowing in the depth of the Sylvian fissure is oriented orthogonal to the temporal plane, because pyramidal cells are perpendicular to the cortical surface. Therefore, the net effective dipole is oriented vertically and orthogonal to the fissure. This source is nearly tangential to the scalp surface. Because the current flows into the cortex, the dipole is pointing downward. The resulting voltage topography shows a fronto-central negativity and an associated positivity over the right infero-temporal posterior scalp.



Tutorial 4 – Discrete Source Analysis

- In the main window, select File / Open and browse to the Learn-by-Simulations subfolder of the Examples folder to load the simulated data set AC-2D-nooverlap.avr. You might need to change the file-type to Averaged Files (*.a??, *.m??, *.fsg) to see it. Press Open.
- 2. We want to perform source analysis on this data now to see if we can recover the brain activity that is underlying the recorded signals. Source analysis is performed in a separate window of BESA Research. To start source analysis, leftdrag over the data to mark a block. Then rightclick into the block. From the popup menu select Source Analysis.
- A dialog window appears which allows specifying the time range of the data to be sent into the source analysis window. Select Whole Segment. It is also possible to specify temporal filters to be applied before

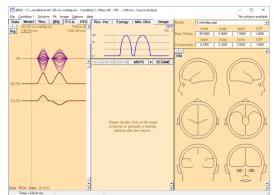
Select Data Files X Look in: Leam-by-Simula - 🗧 🖻 🖛 Name Date modified Туре AC-Coherence 2018-03-21 11:08 ... File fol Quick access 2018-03-21 11:08 ... CTE-MEG143 File fol 2018-03-21 11:08 ... ModelData File fol AC-2D-biphasic-synchronous.avr 2002-03-29 11:29 ... AVR Fil Desktop AC-2D-biphasic-synchronous+noise.avi 2002-03-29 11:30 . AVR Fil 2002-02-12 12:28 ... AVR Fil AC-2D-no-overlap.avr Libraries AC-2D-no-overlap+noise.avr 2002-02-12 12:31 AVR Fil RegionalSource2D.avr 2002-02-08 5:55 PM AVR Fil Unknown.avr 2002-02-07 6:12 PM AVR Fil This PC Unknown-no-noise.avr 2002-02-07 6:10 PM AVR Fil AC-2D-biphasic-synchronous.mod 2002-03-29 11:29 ... MODE AC-2D-no-overlap.av • Open File name Averaged Files (*.a??, *.m??, *fsq) Files of type • Cancel Folders Examples Folder -



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All Conditions	Source Analysis	Set <u>B</u> lock	<u>C</u> ancel

source analysis to remove slow drifts and noise from the data. For our simulated data sets we don't need to apply filters. Press **Source Analysis** to open the source analysis window with the specified data segment.

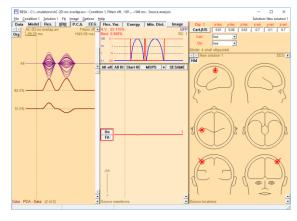
4. On the left of the source analysis window, the recorded channels are displayed in average reference: The top row shows a butterfly plot of all channels; below that, the PCA decomposition is shown by default, indicating the number of components that can be expected in the data. In the top middle box, the global field power (GFP; the sum of

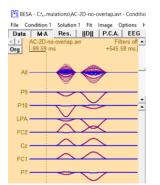


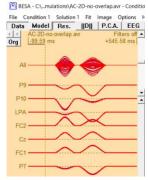
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squares of all channels, normalized to 100%) is displayed in a logarithmic scale. The central and right-most sections of the window are dedicated to the model that describes the data; as there is currently no model defined yet, they are empty at this point. On the right, the head sketches are shown analogously to the DipoleSimulator program. The parameters of the applied head model are shown at the upper right. **Double-click** into any of the 6 heads to insert a first dipole.

- 5. This defines a first (still inappropriate) source model. The source waveform in the middle box is calculated from the recorded data using the head model (= model for the distribution of the electrical conductivity inside the head) and the source model on the right as a hypothesis. Source waveforms are dependent variables. Source locations and orientation are independent variables.
- 6. Based on the source waveforms and the source leadfields (as defined by source location, orientation, and head model), the model waveforms can be computed, i.e. the data that would be generated by the current source model. First of all, by un-checking the P.C.A. button above the waveform display, you can visualize the measured data for each channel (sorted by variance). Now you can visualize the model waveforms (blue) by hitting the Model button in the upper left corner. The difference recorded between (purple) and modeled (blue) waveforms is the residual. It can be displayed in red by hitting the **Res.** button.

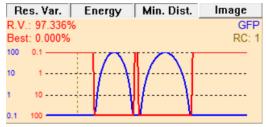






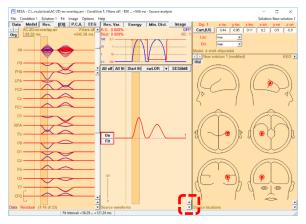
Tutorial 4 – Discrete Source Analysis

7. The normalized sum of squares over channels of this residual activity is the *residual variance* (RV), i.e. the unexplained fraction of the data variance. It is displayed in the top middle box (red) together with the GFP. Note the inverted logarithmic scale for the RV. The goal of the



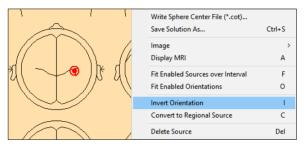
source fitting process is to find a source model that minimizes this residual variance.

8. Left-drag over the data approximately from the onset of the first EEG activity to the first peak. Then hit the Start Fit button in the waveform box to determine the optimum location and orientation for our first dipole in order to explain the data in the marked fit interval. The result is independent of the initial dipole position and confirms the simulated generator of

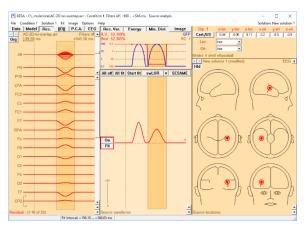


the brain activity in this time range in the right auditory cortex. Use the scaling buttons at the bottom right of the middle panel to increase the source waveform scaling.

9. Note that it is arbitrary if the dipole is represented with its flag pointing in one direction or in the opposite direction. If you wish, you can invert the source orientation by right-clicking onto the source and selecting Invert Orientation.

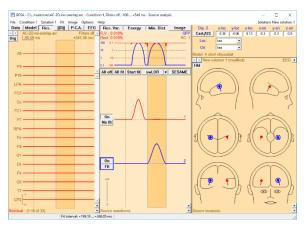


10. Release the Data button in the upper left corner to show the residual activity only. In our marked initial fit interval, the dipole in the right auditory cortex explains the recorded data without any remaining residual. However, its source waveform is not correct – it shows activity also during the later time range of simulated activity in the left hemisphere. This is so because



the required source accounting for this activity is not included in our source model. This is also reflected in the high residual variance in this latency range. Therefore, left-drag to mark a second fit interval across this later time range.

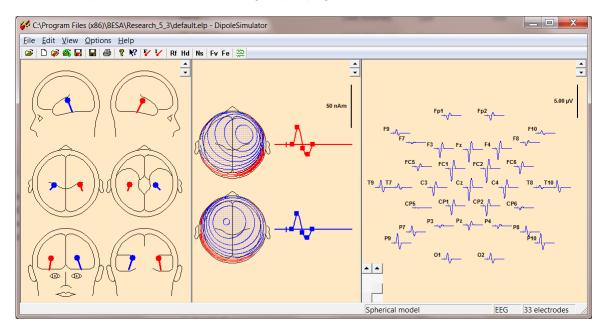
11. Double-click into the head to insert a second dipole. The button next to its source waveform will automatically show Fit to indicate that its location and orientation will be optimized in the next fitting step; in contrast, the button of the first dipole says No Fit, because it will held fixed – its location and orientation has already been determined by the first fitting



step. Press **Start Fit** to start the fitting process. As expected from our simulation, the second dipole localizes in the left auditory cortex. The flat residuum indicates that this two-source model perfectly explains variance in the data.

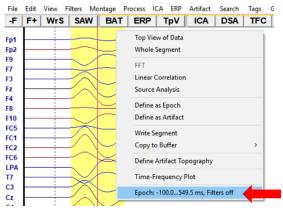
The fit results in a complete separation of the activities of the right and left AC. The addition of the left dipole has changed the calculation of the right source waveform. It is now blind to activity from the left source, and does not show any activity in the later interval when the left source is active. The analogous statement is true for the source in left AC. Therefore, an appropriate source model that should reconstruct the correct source activities must contain dipoles representing at least all active brain areas. Otherwise, activity in unmodeled brain areas will be projected onto the source waveform of the modeled sources.

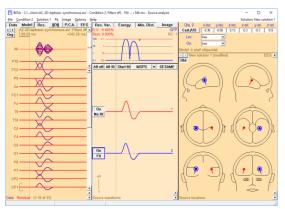
2nd simulated data set: Severe overlap and high synchrony between hemispheres is what we must expect in auditory evoked potentials. Furthermore, cortical source activities are more complex and often biphasic. Therefore, the next simulated data set, **AC-2D-biphasic-synchronous.avr**, contains biphasic source waveforms which are synchronous in both auditory cortices. Source waveforms pointing upwards (positive) represents postsynaptic current flow into the direction of the flag of the dipole symbol; downwards deflections (negative source waveforms) reflect current flow in the opposite direction. Remember that the source waveforms reflect the local current flow within the modeled gray matter patch – it does not represent the direction of signal propagation within the brain!

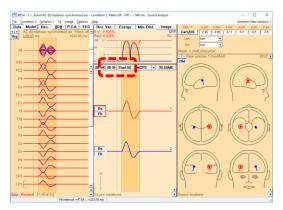


Tutorial 4 – Discrete Source Analysis

- 12. Minimize the source analysis window and select File / Open in the main window to load the second simulated data set AC-2D- biphasic-synchronous.avr. Left-drag to mark a block and right-click into it. BESA Research remembers the previous settings used for source analysis that you can quickly re-apply by selecting it at the bottom of the popup menu.
- 13. The source analysis window appears with the new data set, the previous dipole model still applied. Press the Data button to show the biphasic EEG signals of this data set. Since the data set has been simulated with the same active brain regions, the source model is correct for this data, the residual variance being nearly zero. The source waveforms are now computed from the new data and therefore reflect the synchronous bilateral biphasic activity.
- 14. We could have obtained this source model by fitting both dipoles simultaneously to the data. To see that, left-drag to mark a fit interval over the time range of EEG activity. Then hit the All fit button this turns both sources into the *Fit* state. When pressing the Start Fit button now, location and orientation of both sources will be optimized. The result does not differ from the previous model.

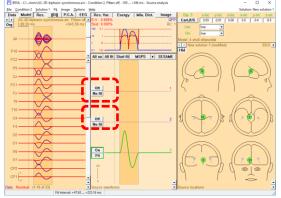


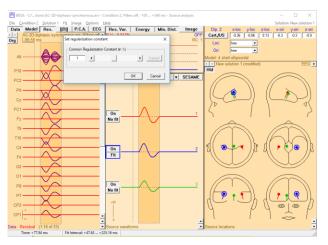




Tutorial 4 – Discrete Source Analysis

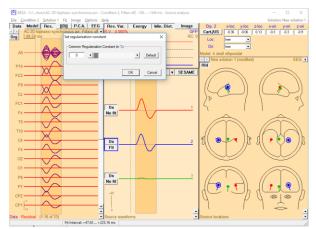
- 15. Let's see what happens if we had tried to model this data (incorrectly) with a single dipole only. Deactivate the two dipoles by hitting their On buttons. This effectively removes them from the source model. Double-click to add a new dipole and press Start Fit. This results in a dipole in the middle of the head, compromising to explain the left-and right-hemispheric activities. Although the result is incorrect, the residual variance is less than 1% (as displayed as *R.V.* at the top)!
- 16. How can one decide whether the one-source or the two-source model is correct? This can be done by contrasting the two source models.
 Switch back On the two first dipoles. Now the three source waveforms should be able to contrast and mutually separate the activities in the three modeled brain regions. The reason for the still non-vanishing source activity of





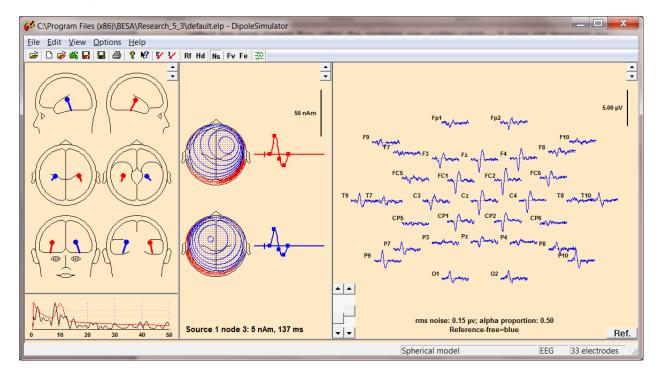
the third dipole is a parameter called **regularization constant**. Select the corresponding entry from the **Options** menu.

17. Reduce the regularization constant to zero. With this setting, the separation capability of the source waveforms is optimal, and the flat source waveform of the third dipole indicates that there is no brain activity in the middle of the head. Rather, the two-dipoles in the bilateral auditory cortices correctly represent the truly active brain regions. So if



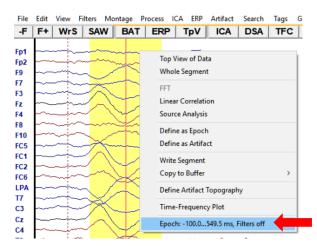
regularization blurs the spatial filter and increases crosstalk between sources, what are the benefits of it? We will see the answer in the next simulation. Click **OK** to close the regularization dialog.

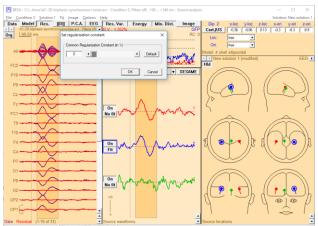
3rd **simulated data set:** Finally, the third simulated data set has been designed to be even more realistic by adding background noise to the same simulated brain activity as before (AC-2D-biphasic-synchronous+noise.avr).

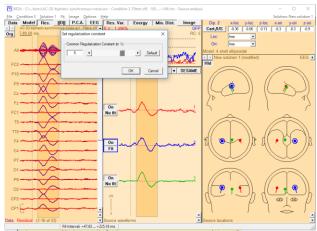


Tutorial 4 – Discrete Source Analysis

- 18. Minimize the source analysis window. In the main window select File / Open to load the file AC-2D-biphasic-synchronous+noise.avr, the same simulation as the previous one, but with some small background noise added to the data. Again, send the data into the source analysis window: left-drag to mark a block, right-click into it and select the bottommost entry.
- 19. The previous solution is now applied to the noisy data. The channel noise is strongly projected onto the source waveforms when the regularization is set to zero. Select Options / Regularization constant again and see how increasing the regularization constant affects the reconstructed source waveforms.
- 20. As regularization is increased, crosstalk increases as in the previous example, i.e. the third source waveform reflects activity that is truly generated in the brain regions represented by the other two sources. However, as a positive effect, the source waveforms are substantially cleaned from noise. This positive effect in noisy data implicitly stabilizes source fitting results in



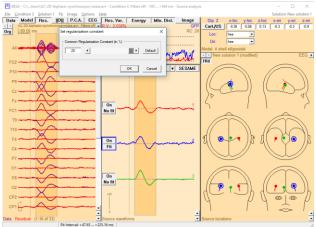




Tutorial 4 – Discrete Source Analysis

the presence of noise. Therefore, a modest regularization is recommended in real data sets. The default regularization of 1% is usually an appropriate compromise and does not need to be changed. Crosstalk between source waveforms is usually smaller than in the current simulation, in which the correlation between the leadfields of the modeled sources is especially high.

21. Set back the regularization to 1% and hit OK. Then close the source analysis window (confirm with OK that the developed source model does not need to be saved to disk).



Set regularization constant	×						
Common Regularization Constant (in %)							
	Default						
ОК	Cancel						

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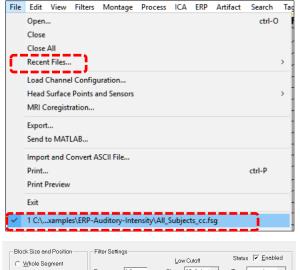
Tutorial 4 – Discrete Source Analysis

C. Single Dipole Fitting in Real Data

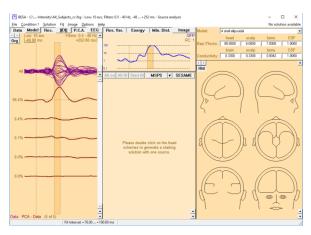
 Back in the main BESA window the file All_Subjects_cc-Test.fsg should still be available at the bottom of the File menu. If it is not, please select it pressing File / Recent Files /

Left-drag a block in condition Low, rightclick to send it into Source Analysis with custom definition settings –50 to 250 ms, a Low Cutoff filter of 0.5 Hz, 12 dB/oct, zero-phase shift, and a High Cutoff filter of 40 Hz (keep the default parameters 24 dB/oct, zero-phase). The low cutoff filter will create an improved baseline and reduce the overlapping slow activity.

2. It is not possible to fit a good source model for the P50 as its signal-to-noise ratio is poor. Therefore, we will focus on the N100, which has the largest amplitude in our signal. Mark a time-window from the beginning to the maximum of the auditory N100 (76 to 100 ms). As we are dealing with auditory data, we know that we need to fit at least 2 dipoles to obtain a correct source model. Double-click in the heads twice to place 2 initial dipoles. Press All fit and Start fit.

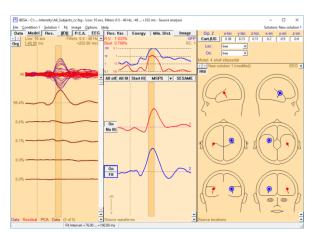


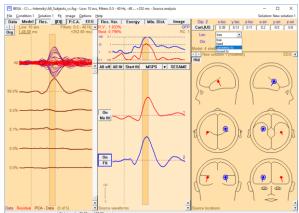
<u>Whole Segment</u> Custom Definition	Erequency 0.5 [Hz]	Low Cutoff Slope 12 db/oct ▼	Status 🔽 Enabled Type zero phase 💌
Image: Previous Settings -50 (ms) pre-Eyent [250] (ms) post-Event	Frequency 40.0 [Hz]	High Cutoff Sloge 24 db/oct 💌	Status 🔽 Enabled
All Conditions	Source Analysis	Set <u>B</u> lock	Cancel

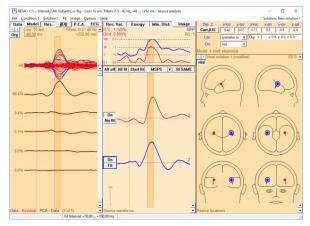


 Both sources will fit in auditory areas, the right source being located further anterior than the left source. The right source seems located slightly too medial.

- 4. The fit can be improved by introducing a symmetry constraint. This can be very useful for stabilizing a fit, particularly when data are noisy. To do so click on one of the sources and select symmetric to in the Loc.: drop-down menu. Press All fit and Start fit again.
- 5. Both dipoles are located symmetrically in the auditory cortex. Note that the residual variance is slightly bigger using the symmetry constraint. However, the solution with the smallest residual variance is not always the correct anatomical solution. You should keep in mind that deeper dipoles explain more variance. Therefore, in noisy data dipoles will have a bias to localize deeper to explain more variance.

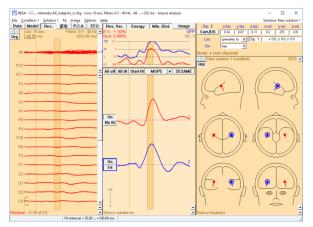


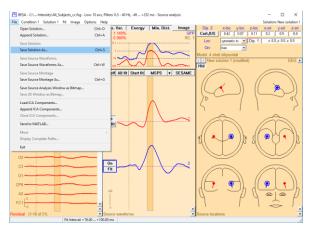




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- 6. Release the Data button and the PCA button to only display the residual variance (if it is not activated, click on the Res. Button). Note that our source model explains the data very well for the given time-range. Keep in mind for later that the source model does not explain the P50 or the N150 sufficiently. This can be seen by the higher residual variance in the according time-ranges. You may click on the ||D|| button to change channel sorting.
- Save the current source solution for later by pressing File / Save Solution As... under the name LowIntensity_2SDtest.bsa. Close the source analysis window.





Tutorial 4 – Discrete Source Analysis

D. Fitting Regional Sources in Simulated Data

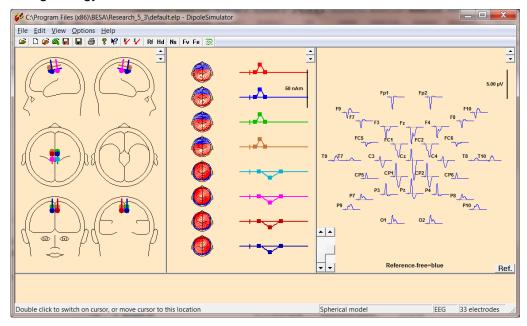
In order to understand the concept and advantages of regional sources we will again work with a simulated dataset.

Simulated dataset: Two extended square surfaces (~10-15 cm²) of planar cortex are modeled, each represented by four equivalent dipoles. The dipoles are approximately 2 cm apart. Each dipole can be thought to represent a smaller square surface (2×2 cm).

The first simulated brain region is thought to represent **supplementary motor area** (SMA). The four dipoles are located symmetrical to the interhemispheric cleft with **nearly tangential orientation**, reflecting a planar fissure to both sides of the interhemispheric cleft. The modeled time-course is the same in each dipole, representing synchronous monophasic activity in the SMA (dipoles 1-4 in the above figure).

Similarly, four **nearly radially oriented** dipoles are simulated, representing **cingulate gyrus** activity (dipoles 5-8).

Thus, the two modeled extended brain regions are in **close proximity to each other**, but **differ from each other by the direction of the neuronal current flow** (i.e. the orientation of the active gray matter surfaces). The time courses of the two activities are simulated to be partly overlapping, with the activity of the SMA starting earlier than that of the cingulate gyrus.

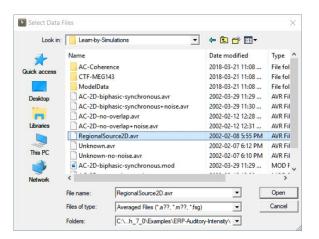


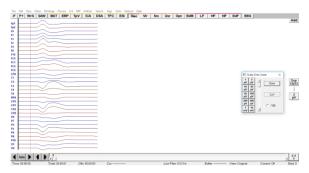
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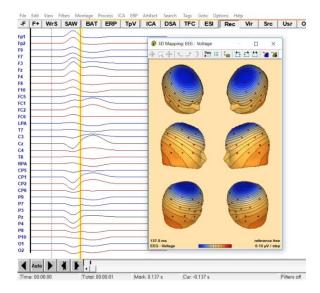
Tutorial 4 – Discrete Source Analysis

 Load this data set in BESA Research: Select File / Open and open RegionalSource2D.avr.

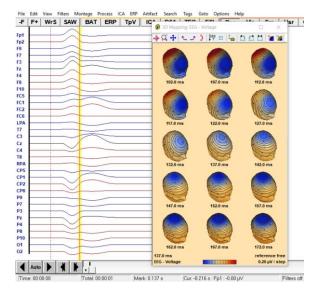
- You may increase the amplitude scaling by pressing the amplitude scaling button at the right of the window. Change the scaling to 2 μV. It is evident that due to the effect of volume conduction, each electrode records signals from both modeled brain regions to some extent.
- 3. The overall distribution of the EEG activity at the scalp surface can best be observed by viewing a 3D whole-head map. Open the map window by double-clicking into the data approximately where a polarity reversal is observed in electrode Cz. A cursor is set and the map window appears.



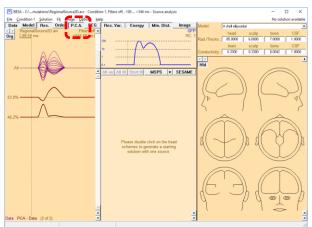




- 4. To obtain a time sequence of maps, **click** onto one of the heads, e.g. the view of the right side (left head in the middle row). The display changes, and maps from 35 ms before to 35 ms after the cursor are shown in steps of 5 ms. The map seems to rotate from an early tangential map (activity of the tangentially oriented SMA) to a later nearly radial map (cingulate gyrus activity). Note that this apparent rotation is not generated by the rotation of one active brain region, but by the differential activity of multiple fixed gray matter patches with different orientations.
- In order to model the underlying activity, we will perform source analysis again: Left-drag to mark a block, right-click into it and send the whole segment into the source analysis window.
- 6. The PCA button should already be pressed. This means that a principle component analysis of the data is computed. A PCA decomposes the data into contributions of mutually orthogonal topographies (i.e. topographies that are maximally dissimilar to each other in a mathematical sense). In this process, the first PCA topography is selected to explain the most data variance, the second topography the largest part of the



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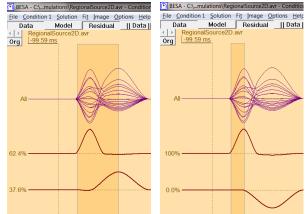
remaining variance etc. The curves at the left indicate the associated time courses.

The variance explained by the corresponding component is displayed next to it. It becomes obvious from the time courses, that a **PCA does not provide a separation of the multiple brain activities** (they were simulated to be monophasic, but the PCA time courses show two peaks each). However, the PCA provides a good estimate for the **minimum number of brain regions** that are contributing to the EEG data: A single brain region cannot generate EEG data that contains contributions of two mutually orthogonal topographies. We can therefore immediately conclude that we are dealing at least with two active brain regions in the current data set. Since **synchronously active brain regions cannot be separated by a PCA**, we only obtain a lower, but no upper bound on the number of sources required to model the data.

Note that in real data, there are as many PCA components as there are recording channels, most of them solely representing noise activity. The ones relevant for our considerations are those with a substantial contribution to the data variance and a time-course that indicates activity that clearly emerges from the background noise.

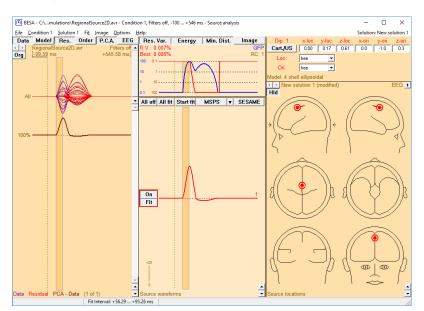
Next, we will see how we can also employ the PCA to estimate the appropriate duration of a fit interval.

7. If a fit interval is marked, the PCA decomposition is computed for the marked data segment only. Mark a fit interval that starts at the onset of observable EEG activity and vary its duration. Note that every time you change the length or position of the fit interval, the PCA waveforms and explained variances change.



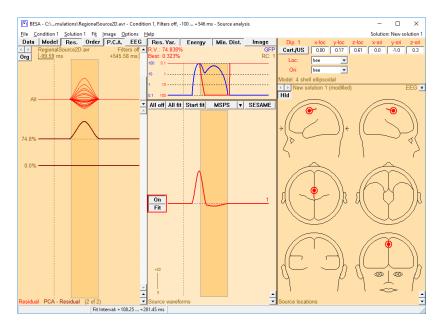
8. We want to apply a *sequential fitting strategy*, i.e. first fit a dipole to represent the earliest brain activity. For that purpose, we should make the fit interval short enough not to include any brain activity from other brain regions that starts at higher latencies. Therefore, the fit interval should be chosen such that **one topography dominates the data**, i.e. one PCA

topography should explain the majority of the variance. If this is not the case, this would indicate that at least two brain regions contribute to its generation. We would get incorrect fitting results if we tried to fit a single dipole to the data. On the other hand, the fit interval should not be shorter than necessary either, so that the effect of channel noise on the fit result gets reduced. In our example, an appropriate first fit interval is e.g. approximately **from the onset of EEG activity to the first peak** – in that time range, the SMA region is active, whereas there is no cingulate gyrus activity yet (PCA topographies show 100% and 0% contribution, respectively). Then **double-click** into the heads to create one dipole and hit **Start Fit**. The dipole localizes in the center of the simulated SMA activity. Note that the SMA region was modeled with 4 dipoles to represent activity of a relatively large cortical patch. Still it is possible to reconstruct its activity with only 1 dipole with near perfect explanation of the variance.

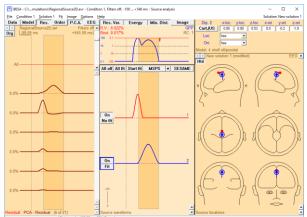


9. Release the Data button in the upper left corner to display the residual (i.e. unexplained) activity only. Now mark a second fit interval to include the left-over residual variance. Because the data is not displayed anymore, the PCA is computed for the residual activity only. For the second and all additional fitting steps, this is our criterion for the selection of the fit intervals. In this simulated example, there is only one additional brain activity, i.e.

no matter how long you choose the fit interval, it will result in one additional PCA component with substantial contribution⁶.



10. After deciding on a fit interval, add a second dipole by double-clicking into the heads. Hit Start Fit to localize it into the cingulate gyrus area. Note the vertical orientation of this source, in agreement with our simulation. The two-dipole model now separates the activities of the two brain regions. The source waveforms reconstruct the correct time courses, indicating the earlier activation of the SMA as compared to the cingulate gyrus.



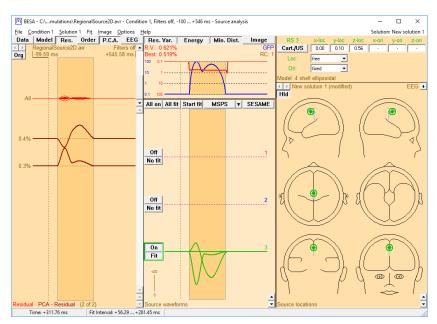
⁶ The PCA variances computed for the residual do not sum up to 100%, but rather to the total residual variance in the marked block

Tutorial 4 – Discrete Source Analysis

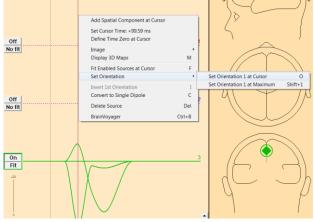
11. Open the Options menu. Currently the default source type is set to Dipole (2nd row). Please click onto this entry. When you open the Options menu again, the default source type has been set to Regional Source. With this setting, a double-click into the heads will automatically add a regional source as opposed to a dipole.

<u>O</u> pt	ions <u>H</u> elp					
	Regularization Constant: 1 %					
	Default Source Type: Reg. Src.					
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12. A regional source has a location and three (MEG: two) orthogonal orientations, in order to model all activity that stems from a particular brain region, no matter in what direction the current flows. To model the two simulated brain regions with a single regional source, switch off the two single dipoles in the model. Double-click into the heads to insert a regional source. Because it consists of three orthogonal dipoles, the corresponding source waveform consists of three curves, each representing the source waveform of one underlying dipole. Mark a fit interval over the whole time range of activity and hit Start Fit. The fitted source location compromises between explaining the SMA and the cingulate gyrus activity. The obtained residual variance is less than 1%, indicating that this source models the data very accurately.



13. The three mutually orthogonal dipoles of a regional source models current flow in any arbitrary direction in a certain brain region. Mathematically speaking, we will get exactly the same representation of activity if we rotate the axes from the initial x-, y- and z-direction to new orientations such that the 1st dipole of the regional source represents the initial peak of activity. To do that,



double-click onto the source waveform to set a cursor at the first peak (around 100ms). Rightclick and select Set Orientation / Set Orientation 1 at Cursor.

14. This rotates the regional source so that the first of its dipoles points into the direction of current flow at the cursor latency. Note that as a consequence its source waveform picks up all activity at the cursor latency; the other two source waveforms show zero activity at the cursor. The second and third dipole components are rotated with it so that they remain mutually orthogonal. The second dipolar component is



automatically set to model the largest current flow perpendicular to the first source. As a consequence, the source waveform of the third component is flat in this simulated example.

This means that we can apply regional sources for the purpose of modelling the activity of a specific brain region, and, by orienting the components, also for the purpose of separating activities with different orientations of cortical patches. Note, however, that a full separation of differently oriented components is only possible if their orientations are indeed orthogonal to each other, like in this simulated example. We will see the strengths and drawbacks of regional sources again shortly when we apply them to real data sets.

Tutorial 4 – Discrete Source Analysis

- 15. With the cursor still set at the first peak, you can hit the M key on the keyboard (or right-click and select Display 3D Maps) to view the voltage topography at that latency. It reflects the tangential dipolar topography of the SMA region.
- Voltage map Data

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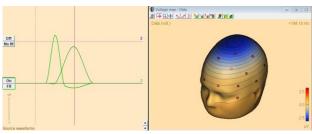
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- 16. Use the arrow keys or the mouse to drag the cursor to the second peak and observe the radial EEG map generated by the cingulate gyrus with the large central negativity. Close the source analysis window without saving the solution.



E. Fitting Regional Sources in Real Data

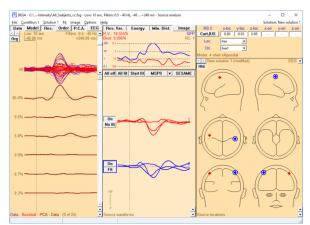
We will now use regional sources instead of single dipoles to reconstruct activity in our real auditory intensity dataset.

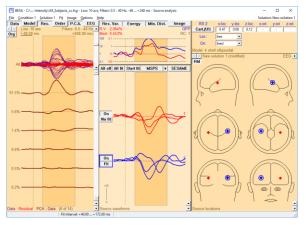
 We will now return to the dataset All_Subjects_cc-Test.fsg, which should still be available at the bottom of the File menu. If it is not, please select it pressing File / Recent Files / Left-drag a block in condition Low, right-click to send it into Source Analysis with settings –50 to 250 ms, a Low Cutoff filter of 0.5 Hz, 12 dB/oct, zero-phase shift, and a High Cutoff filter of 40 Hz, 24 dB/oct, zerophase.

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Tutorial 4 – Discrete Source Analysis

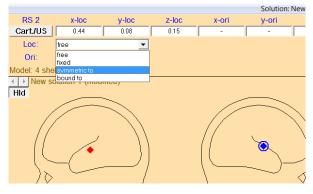
- With pressed P.C.A. button, a principal component decomposition of the data is displayed. Condition Low is dominated by one large PCA topography, mainly representing the N100 activity. Create an initial source configuration with two sources by double clicking onto the right and left head scheme.
- 3. As regional sources can explain activity with up to three brain orientations at the same time we can expect the two sources to model the three main components in the auditory cortex area (P50, N100 and N150). Therefore, we will not set a short fit interval comprising only one component, but rather mark a fit interval over the activity range of these three AEP





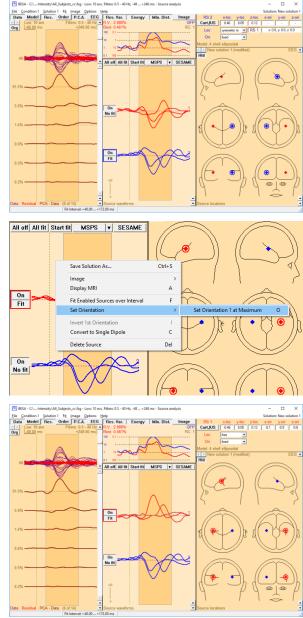
components. **Drag** the cursor over the source waveforms to mark the interval from **40 ms to 172 ms**. You may set the interval by **right-clicking** and selecting **Set fit interval**. Press **All Fit** and **Start fit** to fit both sources simultaneously. Use the arrow buttons in the source waveform box to **adjust waveform scaling**.

4. The sources are quite symmetric with a slightly more anterior source in the right hemisphere. As stability might be much worse in individual data, we will impose a symmetry constraint on the source location again. Activate the second source by clicking onto its waveform. In the upper right box, select symmetric to from the drop-down menu next to Loc.



 Press All Fit and Start fit again. Note that the residual variance (Res. button needs to be pressed to view this) and the source waveforms only change marginally.

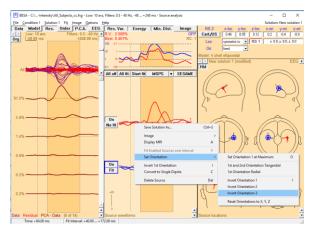
6. We want to identify the direction of current flow of the N100 component. To do so, activate source one by clicking onto the source. Right-click and select Set orientation / Set orientation 1 at Maximum. This rotates the source such that the peak around 100 ms is now fully accounted for by the first component (source waveforms for orientations 2 and 3 are now zero at this latency).

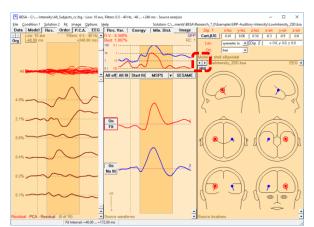


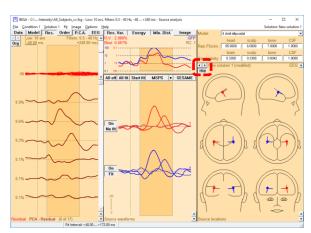
BESA Research not only sets the first orientation, but also automatically adjusts the second orientation of the regional source to explain the maximum activity perpendicular to orientation 1 in the whole time interval. Thus, the second orientation accounts for the N150 component (second largest component). The orientation of N150 is close to radial and reflects current flow at the lateral surface of the supratemporal gyrus. Alternatively, the second component of a regional source could be set at a specified latency by double-clicking to set a cursor, right-clicking and selecting **Set orientation / Set orientation 2 at Cursor**.

Tutorial 4 – Discrete Source Analysis

- 7. Next, we will orient the second source as described in step D6. If necessary, Right-click onto its waveform and select Set Orientation / Invert Orientation 3 to make orientations of source one and two consistent. (This 2-source model has been saved in file LowIntensity_2RS.bsa. You may load this file from the menu entry File / Open Solution.)
- 8. Let us compare the solution using regional sources with the solution using single dipoles we saved earlier on. Please press File / Open Solution and select LowIntensity_2SD-test.bsa. Release the Data button to only display the residual variance. You can toggle between the two solutions using the arrow buttons in the top left corner of the head scheme field. Note again that the single dipole solution does not explain the P50 and N150 components, while the regional source solution explains all activity from the temporal region very well.

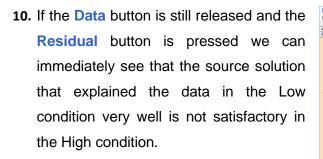




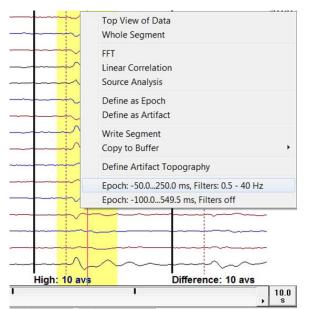


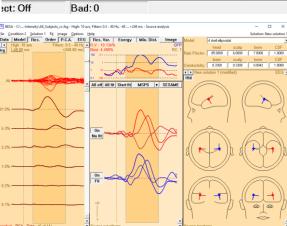
Tutorial 4 – Discrete Source Analysis

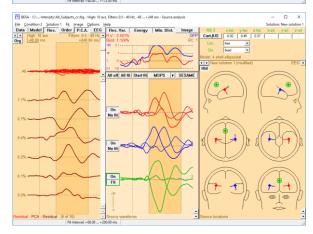
9. Make sure you switch back to the regional source solution and minimize the source analysis window (don't close it) to return to the average data in the main window. Select condition High and send it to source analysis with the same settings as the Low condition by left-dragging a block, right-clicking and selecting the according entry at the bottom of the dialog.



 Mark a fit interval from 68 to 200 ms that covers the unexplained activity. Doubleclick into the head schemes to place a third regional source. Press Start Fit.

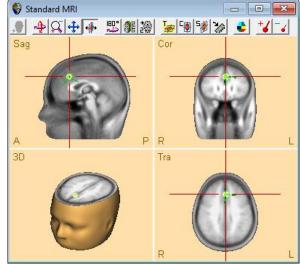


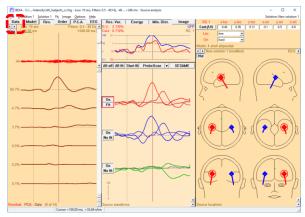




Tutorial 4 – Discrete Source Analysis

- 12. Right-click on source waveform 3 to set orientation 1 at the maximum or hit the O key, the short cut for this action. Scale down the source waveforms if necessary by using the arrow buttons in the bottom right corner of the source waveform field. Right-click on the source waveform and select Display MRI (or press the A key). Press the B button to change to multiple view. The third source localizes frontally, representing activation in the cingulate gyrus or frontal cortex. Close the standard MRI window.
- **13.** Switch back to condition **Low** by using the arrow buttons in the top left corner of the channel field. Note that the ACC/frontal source is silent in the Low condition. Its presence does not alter the source waveforms of the auditory sources. You can probe this by switching the frontal source on and off. Close the source analysis window and save the current solution as HighIntensity_3RS-test.bsa. (This source solution has already been saved as HighIntensity_3RS.bsa.)





Tutorial 4 – Discrete Source Analysis

F. Conclusions

General guidelines for source analysis

We can conclude this tutorial with the following general results and guidelines:

- The idea behind source analysis is to place or fit sources into the brain at all regions contributing to the data. Localization/fitting is hypothesis testing: The computed source waveforms separate the modeled brain activities and answer the question if and when activity takes place in the modeled brain region. Thus, BESA Research reconstructs the brain activity with high temporal resolution.
- 2. Synchronous activity over several square cm can be modeled by a single equivalent source.
- 3. Sources that are close together, have the same orientation and synchronous activity are indistinguishable.
- 4. Sources that are close together but have different orientations and non-synchronous activity can well be separated by a discrete source model.
- 5. Source waveforms are relatively insensitive to variations in dipole location. Therefore, a single regional source can accurately model activity of multiple gray matter patches in its vicinity. Regional sources should have a mutual distance of approximately 3 cm or more between each other to prevent crosstalk of their source waveforms.
- Regional sources tend to provide more reliable solutions in noisy data as compared to single dipoles, because during a fit of a regional source only its location needs to be determined (no orientation has to be fitted).
- 7. Very often the brain responds to a stimulus with bilateral symmetrical activation. Therefore, a pair of symmetrical regional sources is often a good initial source configuration. The resulting source waveforms will then indicate if the activity is really bilateral.
- 8. The criterion for a good source model is not solely the residual variance. Rather, the following criteria should be met:
 - The source model should be in agreement with proven knowledge about the underlying brain activity (e.g. bilateral activation of the auditory cortex after an auditory stimulus as opposed to a single central brain area).

Tutorial 4 – Discrete Source Analysis

- The source waveforms are an important indicator for the quality of a source model: When the time course of the obtained source waveforms for a (pair of) source(s) are distinctly different from those of other sources, this indicates that the corresponding source correctly models distinct brain activity. On the contrary, when two source waveforms show nearly the same time course, it should be checked if they pick up activity of a brain region that is not part of the model, rather than truly modeling brain activity generated at their source locations.
- PCA can be helpful in determining the minimum number of sources required to adequately model the data.

What does BESA Research provide?

Although source analysis is the core of the program, BESA Research provides comprehensive and excellent tools for complete data analysis. This includes among others:

- Data review and mapping
- Preprocessing: Paradigm definition, artifact detection and correction
- Averaging
- Traditional ERP analysis
- Interactive source analysis and source imaging
- Coregistration with MRI/fMRI
- Time-Frequency and coherence analysis

It is recommended to perform preprocessing of your raw data with BESA Research, to secure that your averaged files match the quality requirements for source analysis. This is not guaranteed if you import averages from other systems.

What should you provide?

Independent of the analysis capability of the software, good analysis results also depend on the experimenter. Make sure to:

Tutorial 4 – Discrete Source Analysis

- Define the goals of your experiment/measurement
- Acquire data of appropriate quality
- Understand the basic principles of source imaging
- Utilize knowledge on brain anatomy and function
- Appreciate the limits of source localization. For example, in data with low signal-tonoise ratio, source fitting might not provide reliable results. In that case, it is preferable not to fit dipoles at all. Rather, the source model can be created by seeding dipoles or regional sources into the brain based on knowledge e.g. from anatomy or fMRI.
- Do not try to determine parameters if they cannot be estimated reliably (e.g. dipole localization in depth)

Tutorial 5 – Source Montages

Tutorial 5 – Source Montages

What does BESA Research provide?

- ✓ Pre-defined source montages
- ✓ Resting state montages
- ✓ User-defined source montages

BESA[®] Research 7.1 Tutorial 5 – Source Montages

The present chapter will introduce the concept of source montages. In BESA Research, source montages can be created by the user, e.g. by a fitting process or by placing sources in regions of interest. Alternatively, it is possible to use pre-defined source montages.

Source montages (Scherg et al., 2002) are especially useful for reviewing high density data. They are provided to facilitate the detection of focal source processes in the brain by reversing the overlap from different brain regions seen at the scalp. If brain activity is focal, i.e. represented predominantly in one source waveform, a large amount of the recorded scalp activity reflected in this trace will originate in the related brain region. If source activity is distributed over several traces, the origin is likely to be more widespread or in areas not precisely modeled by the combination of sources in the selected source montage. Each channel in a brain source montage can be viewed as a 'gross virtual electrode' placed onto a particular brain region. Using either pre-defined or user defined source montages allows to evaluate data at the source level, rather than sensor level. This transition allows for in example allows for faster and more precise epilepsy assessment (Beniczky et al., 2016; Rodin et al., 2004). It may allow for observation of brain physiology that is not clearly visible with the traditional approach and might be helpful to monitor activity in deep brain structures where it is not possible to use classical montages (Scherg et al., 2002). Source montages are highly beneficial for connectivity studies as well (see Tutorial 12 - Time-frequency Analysis, Connectivity Analysis and Beamforming).

The resting state montages are a special case of pre-defined source montages. Resting state network nodes are well-established in fMRI studies (Damoiseaux et al., 2006) and have been related to functional patterns in many publications over the last years. In BESA Research the resting state montages contain regional sources in brain regions associated with the Default Mode Network – DMN (Raichle et al., 2001), fronto-parietal task control system as well as the dorsal attention system and the ventral attention system (Power et al., 2011). They allow investigating resting state EEG and MEG data in a swift and straightforward manner. They can be also used to compare EEG data directly with fMRI results (Rusiniak et al., 2018).

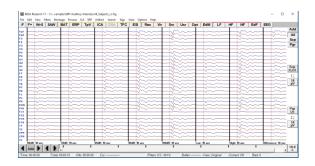
More details about how these pre-defined montages were created can be found in the BESA Research help documentation.

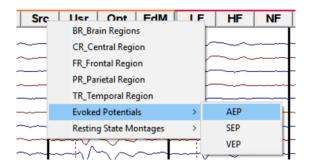
A. Pre-defined Source Montages

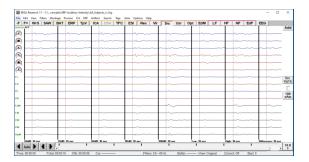
- The file All_Subjects_cc.fsg should still be open. If it isn't, open it from your recent file list: File / Recent Files...
- Make sure the button Rec is pressed to display the original recorded data.
- 3. From what we see on the screen at the moment, we cannot infer which brain regions might be responsible for creating the evoked potentials that can be seen across all electrodes.
- We will now load a source montage that is part of the BESA installation and was created for auditory evoked potentials. Press the Scr button and select Evoked Potentials / AEP.
- **5.** Now the sensor-space data are automatically translated on the sources in the source montage. As discrete sources have good separation properties we can immediately see that activation is not distributed over all channels any longer but mainly reflected by the temporal sources. You might notice that some sources are displayed with a head scheme, while others only

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have a label. We want to look at the montage in more detail to find out why.

- Press Edit / Montage to open the montage editor window. The current montage will automatically be loaded.
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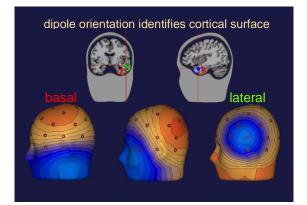
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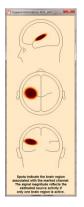
IC/

- In the montage editor window press the button to display a multiple head view. The sources are listed in the middle panel. Have a look at the different sources by clicking on the label.
- 8. You will note that the first 6 sources are single dipoles with an orientation while the other sources are regional sources that have not been oriented. The idea behind this montage is that it was optimized to model auditory data. Therefore, the auditory cortex sources have an orientation to reflect activity in the different major temporal lobe surfaces (see graphic), while the regional sources were placed to increase the sensitivity of the auditory cortex sources. Thus, any activity that does not originate in the temporal lobe will be projected on the regional sources.





9. Close the montage editor window and right-click on the first of the head schemes on the left of the main window. A window will pop up that displays the cortical area that is covered by the according source. You can view the associated brain regions of all sources displayed on the left of the main window.

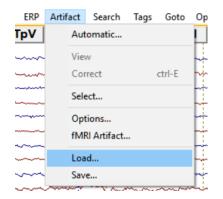


- 10. Note that BESA Research provides predefined source montages for auditory, somatosensory and visually evoked potentials as well as montages for central, frontal, parietal and temporal brain regions. The according brain regions are modeled by oriented dipoles, while the regions that are not of interest are modeled by un-oriented regional sources. The source montage BR_Brain Regions does not contain any oriented dipoles and was designed to get a quick overview about activity in the whole brain. Note that source montages can not only be applied on average data to get a quick impression about the active sources. Their particular strength is that they can be applied on raw data. We will do this in the next step.
- 11. We will return to the dataset S1.cnt of the auditory intensity experiment that should still be available in your recent file list: File / Recent Files...
- Next, we will filter to the data by pressing the EdF button or (Filter / Edit Filter Settings). Define a Low Cutoff filter of 0.1 Hz, forward, 6 dB/oct and a notch filter of 50 Hz, Width: 2.

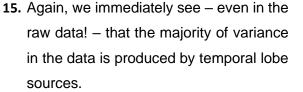
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Sampling Rate: 250 Hz	OK Cancel

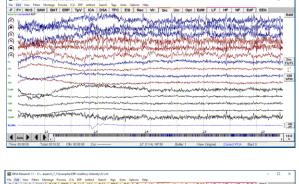
- 13. Now we will load the artifact definition file that contains the blink information we created earlier. Press Artifact / Load and select the file S1.atf. Make sure the artifact correction is switched on (a blue corrected should be displayed in the top left corner of the main window).
- 14. We will now apply the source montage AEP again by pressing the button Src and selecting Evoked Potentials / AEP.

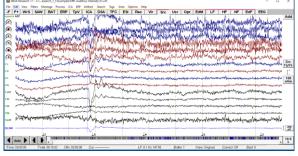


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16. Make sure you are in the first screen and switch off artifact correction by pressing ctrl-E. Note that a large signal now appears in the frontal sources (mainly FpM). Also note, how the eyeblink has an effect on auditory cortex sources. This demonstrates the necessity to control eye artifacts by either rejecting them or correcting them. Switch





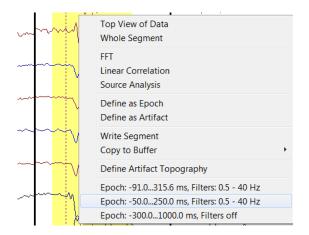
Tutorial 5 – Source Montages

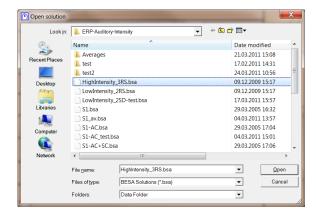
correction back on by pressing **ctrl-E** again.

B. Creating a source montage

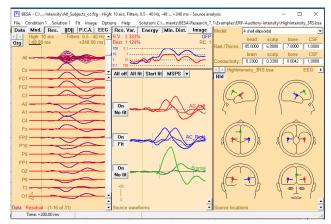
- We will now create our own source montage based on the source model we created using the grand average data of the auditory intensity experiment. Return to the dataset All_Subjects_cc.fsg that should be available in the file menu. If it isn't, open it from your recent file list: File / Recent Files...
- Select condition High, right-click and send it to source analysis with settings -50 to 250 ms, a Low Cutoff filter of 0.5 Hz, forward, zero-phase, 12 dB/oct. and a High Cutoff filter of 40 Hz, zerophase, 24 dB/oct.
- Open the source solution we created earlier on in step D of Tutorial 4. Press File / Open Solution and select the file HighIntensity_3RS.bsa.

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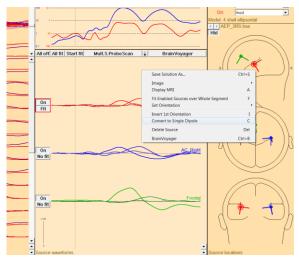


4. We will use this source model as a source montage for our raw data. In the present case, this source model is sufficient, but when data are very noisy, it is advisable to add probe sources to the model.

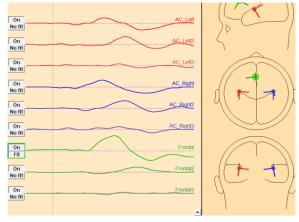


Remember that activity in any brain region that is not modeled by a regional source will be projected on the sources of interest. I.e. any noise or unsystematic brain activity that is not related to the stimulation would be projected on our three sources.

5. As we are interested in auditory cortex activity and activity in the frontal area that becomes active in the high intensity condition, we need to make sure that BESA uses oriented sources in the source montage. In order to make the orientations of the auditory cortex and frontal sources available for the source montage, we need to convert the according sources to single dipoles. Select source AC_Left in the head schemes, right-click and press Convert to Single Dipole.



 You will now see three individual traces for AC_Left, AC_Left2 and AC_Left3. They correspond to orientation 1 to 3 of the regional source. Repeat the previous step for source AC_Right and Frontal.

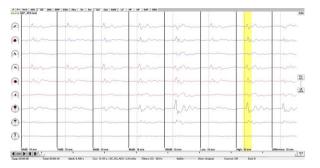


7. Save the source montage by pressing File
/ Save Source Montage As... and specify the name HighIntensity_3RS-test.mtg.
Close the source analysis window. Press OK to terminate without saving the source solution.

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Now we have created our own source montage for the auditory intensity condition that includes the fitted sources along with probe sources. We will now apply this montage on our grand average data and the raw data of subject 1.

8. Return to the main window. The newly generated source montage was already applied to the grand average data. Please note that the activity increase from 60 to 100 dB in the frontal source can clearly be observed using this montage.



BESA[®] Research 7.1

Tutorial 5 – Source Montages

- We will now return to the raw data of subject 1 (S1.cnt). It should still be available in your recent file list: File / Recent Files...
- Press the Usr button and select our source montage HighIntensity_3RStest. We will use this source montage later on for coherence analysis.

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### **BESA[®] Research 7.1**

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

### Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

### What does BESA Research provide?

- Automated MRI segmentation (in BESA MRI) using T1 and optionally T2 sequence data:
  - Loading MRIs in DICOM, NIFTI and ANALYZE format
  - Guided definition of landmarks for AC/PC and Talairach transformation
  - Automatic inhomogeneity correction
  - Automatic segmentation of skin and cortex
  - Automatic setup of 4-layer FEM model
- ✓ Co-registration with and without individually digitized electrode positions
- ✓ Display of individual cortex and skin in source analysis window
- ✓ Source Localization with Individual FEM Model

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

### **A.** Introduction

Co-registration of EEG and MRI data allows mapping a source position to an actual cortical area. Without co-registration it can only be roughly estimated, which cortical area a certain x-y-z position corresponds with. Further, individual anatomy can be used for seeding sources or verifying source localizations.

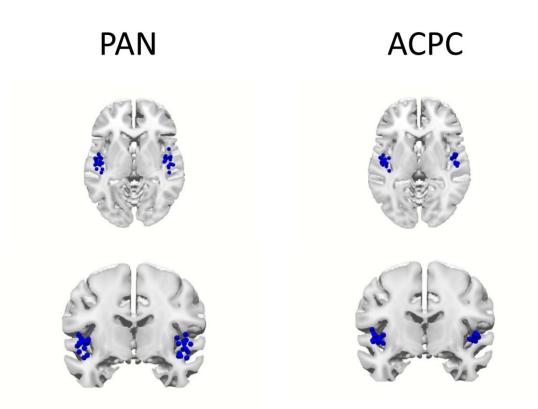
When EEG and MRI data are co-registered using BESA Research and BESA MRI, data are transformed into a coordinate system, which is based on internal landmarks of the individual subject. First, a transformation into the AC/PC (anterior / posterior commissure) coordinate system is performed. Data are then transformed into standard Talairach space. This ensures that the same standard coordinate space based on individual anatomical landmarks is used when different subjects are compared.

Using the same standard coordinate space based on **internal landmarks** is *essential* when source localizations are to be compared across several subjects:

- a. between two groups of subjects (e.g. patients vs. control subjects)
  - Anatomy can vary considerably between subjects and a different x-y-z localization in two people can still be in the same anatomical region. Not taking individual anatomy into account when constructing the coordinate system would disregard this fact.
- b. at two measurement time-points within the same group of subjects (e.g. pre vs. post treatment, test vs. retest)
  - If an EEG (and more so MEG) is measured at two instances in the same subject, electrodes (MEG sensors) are likely positioned slightly differently at the two instances. Here, not taking individual anatomy into account when constructing the coordinate system can lead to higher variance in source localization within the same subject.

### BESA[®] Research 7.1

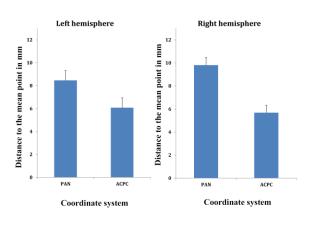
Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling



The above image illustrates the variance of Heschl's gyrus positions across 15 subjects. The PAN (PreAuricular points + Nasion) coordinate system is based on the fiducials only without using any internal landmarks. The same (!) positions are displayed in the AC/PC coordinate system on the right. It is apparent that the variance across subjects is greatly reduced merely by using internal landmarks when defining the coordinate system.

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

The reduction in variance using the AC/PC coordinate system compared to the PAN is statistically significant (see image on the right). The effect is particularly prominent in the right hemisphere (p<0.0001), but also apparent in the left hemisphere (p<0.05). This illustrates that it is important to use the individual anatomy for defining the coordinate system whenever possible.



Usually, good co-registration depends on digitized electrode positions in order to precisely fit the measured electrodes to the skin of the subject (as segmented from the MRI). Using BESA Research and BESA MRI it is possible to co-register EEG with MRI data even if no individual electrode positions were digitized as long as there were some electrodes that coincide with positions of the standard 10-10 system. The idea is that as long as some standard electrode positions are known in a subject, positions of other electrodes of the 10-10 system are also known and can be used for co-registration purposes only (not for any signal analysis). This procedure makes co-registration much more precise than only relying on fiducial positions.

Before starting the co-registration MRI data has to be segmented. The segmentation in BESA MRI is fully automatic after setting up anatomical landmarks. Entire procedure is described below.

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

### **B. MRI segmentation.**

 Start the BESA MRI program and press Accept on the welcome screen. Press the Start New Segmentation button in the interaction window



 In the next screen additional features can be added, like Cortex Inflation or Volume Conductor Segmentation. Also, a T2 sequence can be added to improve the quality of segmentation. Please read the BESA MRI manual for further details. Press Next button to proceed to the next screen.

# BESA

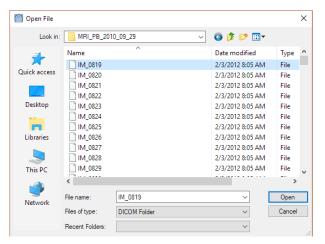
### Steps required to process MRI data

- Load T1 MRI data
- Load T2 MRI data
- Set ACPC Orientation
- M Set Talairach Transformation
- Run Inhomogeneity Correction
- Run Scalp Segmentation
- Run Cortex Segmentation
- Run Cortex Inflation
- Run Volume Conductor Segmentation (input for FEM leadfield)

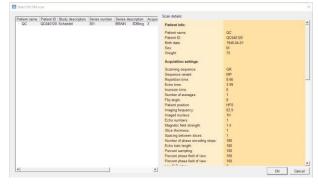
### **BESA[®] Research 7.1**

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

3. Press Load T1 Data in the interaction window. A new dialog box will be opened. Navigate to folder MRI_PB_2017_09_297. Select any file from the folder and press Open



- 4. In the next displayed window the MRI series detected in the folder will be presented. For this particular case only the sequence named **BRAIN 3DBerg** is available. Please choose it. On the right part of the screen you will see the series details. Press the **OK** button to advance.
- 5. Now the main window of BESA MRI is filled with MRI acquisition details that were read from the DICOM header. Note that there is a green checkmark beside the button Load T1 data. Press the OK button to proceed to the next step.

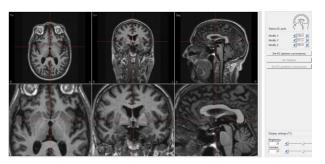




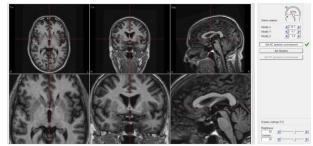
⁷ The folder will be provided by the presenter during the tutorial. The zipped folder can be downloaded from ftp://h1772544.stratoserver.net/public/Data/MRI/ **BESA Research 7.1 Tutorial** Page 119 of 397 Copyright © 2020 BESA GmbH, Freihamer Str. 18, 82166 Graefelfing, Germany www.besa.de

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

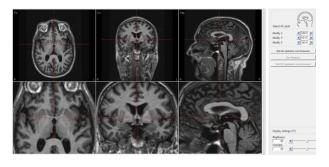
6. Mark the anterior commissure (AC) point on the MRI slices using the crosshair. The bottom views provide higher detail for easier navigation to the correct location. Note the head schematic image in the interaction window that shows the rough location of AC. Press Next to continue.



7. Set rotation of MRI slices by placing the mouse in the bottom views, and dragging the cursor to the desired position while the left mouse button is pressed. Start with the left view (coronal) to align left and right hemisphere. The rotation should be set such that axial and coronal view are symmetric; in the sagittal view, the rotation is set such that commissures are aligned horizontally (both visible in the coronal view). Press Next to continue.

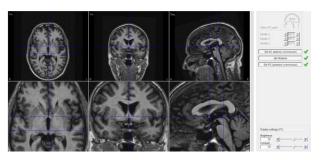


 Mark the posterior commissure (PC) point on the MRI slice using the crosshair. Press Next to continue.

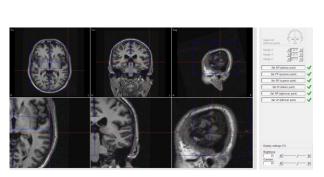


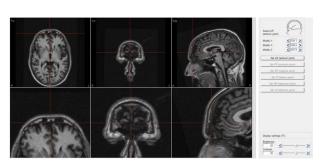
Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

 Review if all landmarks definitions (shown as blue lines) are correct, and press Next to continue if so. Press Previous if you need to make any adjustments.



- 10. Mark the anterior point (AP) on the MRI slice using crosshair. Note the head schematic image in the interaction window that shows roughly where the AC is located. Press Next to continue.
- 11. Repeat step 10 for posterior point (PP), superior point (SP), inferior point (IP), rightmost point (RP), left most point (LP)Mark anterior point (AP) on the MRI slice using the crosshair. Note the head schematic image in the interaction window that shows roughly where each landmark is located. Press Next to continue.
- 12. When all points are defined you can again review them (note green checkmarks beside the buttons). The brain should be inscribed in a blue box. Press Next to continue.





Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

 Mark the nasion on the sagittal slice as indicated in the head scheme. Press Next to continue.

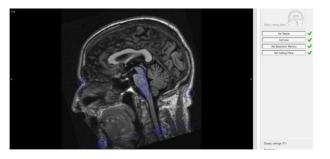


 Mark the inion on sagittal slice as indicated in the head scheme. Press Next to continue.



- 15. Mark 3-5 Brainstem markers on the sagittal slice as indicated in the head scheme. The markers have to be located within the brainstem, but there is no rule about exactly where to place them. Press Next to continue.
- 16. When all points are defined you can again review them (note green checkmarks beside the buttons). Press Next to continue.





Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

17. Finally set cutting plane on the sagittal slice as indicated in the head scheme. Dragging the left arrowed circle moves the cutting plane up and down, dragging the right one changes the angle. Set the cutting plane such that it includes visible MRI data; the inion needs to be above the cutting plane. Press Next to continue.

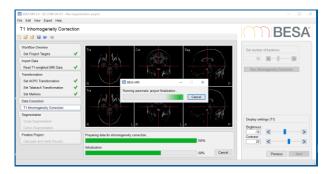


- **18.** A new window will pop up. Here you can decide whether you want to
  - a. Start automatic segmentation
  - b. Perform step by step segmentation
  - c. Quit to prepare another subject
  - d. Finalize all unfished projects

By default, the **first option** is selected. Press **OK** to start segmentation.

Note: This process may take a while. During segmentation, the current step and overall progress is presented on the screen.

# Image: Second secon



Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

 When segmentation is finished the results can be reviewed by pressing buttons in the interaction window at the right. Press the Finish button to save it.



20. A popup window for saving will be displayed. Press the Save button to save with default settings. After saving the segmentation workflow will be closed. The segmentation results will now be available for co-registration.

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### C. Co-registration of EEG and MRI data using BESA Research and BESA MRI

In the following, we will co-register the EEG data of subject S1 (Auditory Intensity Experiment) with an MRI image using **standard electrodes**, as we do **not** have individually digitized electrodes available.

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- 21. In BESA Research please open dataset S1_av.fsg (located here: C:\Users\Public\Documents\BESA\Rese arch_7_1\Examples\ERP-Auditory-Intensity). Press File / Head Surface Points and Sensors / Load Coordinate Files (or press ctrl-L).
- 22. In the section Digitized head surface points press Browse.

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hannel and digitized head surface point information			<b>— X</b>
Internal data file information Data file: C.(UJarsi/Public)Counsents/UBESA/Research_5_3/Ex Format EESA 56.0002: Debabase file: 00000014 fat No of channels 32; 31 electrodes: 1 polygraphic Information should be complete for this data file: No auxiliary files it a=alo file defines EEG, you must use 10=10/10-20 labels or supp.	required.	sg	-
Suggestions Please enter electrode thickness.			
Yes No File name found Channel configuration (* el?)	- no file	Good Browse	Edit
Digitized head surface points (*.stp, *.eps) and labels (*.stn)	3 points in data file	Good Browse	Edit
C @	no label file	Good Browse Electrode thickness (mr	
		Electrode trickness (mr	u): June
Coregistration file (".sth) or head center (".cot)	no file	Good Browse	Edit/Coreg.
MEG sensors (*.pos. *.pmg)	- no file	Good Browse	Edit
Artifact coefficients (*.art)	— no file	Good Browse	Edit
Clear Events		Help	Çancel

23. Under Directories please select the option Standard electrode folder.

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LOOK IN:	StandardEle		• • • • •	. 60 .		
(Pa)	Name	×		Date mod	ified	
	BESA-MRI-	BESA-MRI-Standard-33-Electrodes.sfp				
Recent Places		Standard-Electrodes.sfp	14.10.2010	) 14:54		
Desktop						
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	File name:			<b>•</b>	Open	
					Cancel	
	Files of type:	Surface points (*.sfp)		<b>•</b>	Cancel	
	Directories:	Standard electrode folder		-		
		Channel directory				
		Standard electrode folder Data directory		bod	Browse	
		C:\5_3\Montages\Channels	StandardElectrode	c)	-	

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

Select the file **BESA-MRI-Standard-Electrodes.sfp** and press **Open**. It contains 96 standard electrode positions. Some of the S1's electrode names match the labels in this standard electrode file so BESA can match them.

load Surface Pc	pints					×
Look jn:	StandardElectro	odes	•	(= 🗈 🖻	*	
Recent Places Mecont Places Desktop Libraries Libraries Computer		ndard-33-Electrodes.sfp indard-Electrodes.sfp	)		Date modified 14.12.2010 18:44 14.10.2010 14:54	
Network	<ul> <li>✓</li> <li>File <u>n</u>ame:</li> <li>Files of type:</li> <li>Directories:</li> </ul>	III BESA-MRI-Standard-Ele Surface points (*.sfp) Standard electrode folde				► Open Cancel

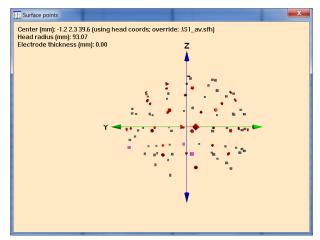
The other file (**BESA_MRI_Standard-33-Electrodes.sfp**) contains 33 standard electrode positions. It is not suitable for us as none of the electrodes that were measured in subject S1 have names that coincide with the 33 standard electrode names. Thus, BESA cannot match them.

**24.** Press **OK** to close the dialogue.

Internal	data file i	nformation				
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		06.0002; Database file: 00000014.tst				1
		32:; 31 electrodes; 1 polygraphic				· · · ·
		uld be complete for this data file. No auxiliary files requines EEG, you must use 10-10/10-20 labels or supply o				
1 Gel	a me uen	nes c.c.d, you musi use 10-10/10-20 idules of supply i	agnized coordinates:			
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Please	enter ele	ctrode thickness.				
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Diaitize	d head su	rface points (".stp." eps) and labels (".stn)				
-			96 locs; fiducials found; electrode locs found; units; millimeters	Good	Browse	Edit
(	C	C:\Users\Public\Documents\BESA\Research	iound; units: millimeters	J G000	BIOWSe	Lui
C			no label file	Good	Browse	Edit
		,				
				Electrode f	hickness (mm):	0.00
Coregis	tration file	(".sth) or head center (".cot)	no file			
C	(		no tile	Good	Browse	Edit/Coreg.
MEG ##	neore (* r	oos. * pmg)				
C	a		no file	Good	Browse	Edit
		1		,		
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C				Good	Browse	Edit
Clear	DB	Clear Events		Help	OK	Cancel

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- 25. Press V on the keyboard to bring up the surface points window. The 96 standard electrodes are now displayed. Those standard electrodes whose labels coincide with S1's electrode labels are marked red. Please note that the grey standard electrode positions are merely used for better fitting the electrodes to the subject's skin. They do not carry any signal. Close the surface points window.
- 26. Press File / MRI Coregistration.



File	Edit	View	Filters	Montage	Process	ICA	ERP	Artifact	Search		
	Open.								ctrl-0		
	Close										
	Close	All									
	Recen	t Files							>		
	Load	Channe	l Config	uration							
	Head	Surface	Points a	nd Sensors					>		
	MRI C	MRI Coregistration									
	Export	t									
	Send t	to MAT	LAB								
	Impor	t and C	onvert A	SCII File							
	Print.							c	trl-P		
	Print P	Preview									
	Exit										
~	1 C:\	.arch_7	_0\Exam	ples\ERP-Au	iditory-Int	ensity\	S1_av.	fsg			

27. You will be prompted to create and save an *.sfh file, which will be used to store all coregistration information necessary for the interaction between BESA Research and BESA MRI. BESA will automatically suggest the filename of the current subject plus the extension .sfh. Confirm the suggested name by pressing Save.

Organize • New	folde	er					• 11	
Sk Recent Places	*	Name	A		Date modified	Type	Size	
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File name:	\$1_a	/.sth						
Save as type:	Sfh Fi	les (*.sfh)						

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

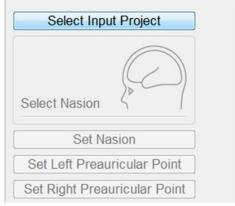
- 28. BESA MRI will open. Press Accept in the welcome screen. Important note: do <u>not</u> close BESA Research!
  Please make sure that the data folder is set to the BESA MRI Example projects folder (File / Select Data Folder).
- 29. New coregistration project is started automatically. Press Next on the bottom right (or hit the space bar).



BESA MRI 3.0 - New Coregistration project			- 0 ×
File Edit View Export Help			
Set Project Targets			BESA
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Works Ooker Gerback Dark Darke Per Cognition Darke Per Cognition Ser Racent Fri Ender Pilote	Completenties Project Targets Completenties AMIC and the second second second second second second second second second Calculate traditions for the grant second.	ys press the Space bar, the Enter key or the Next button.	Bergs für comparisation 

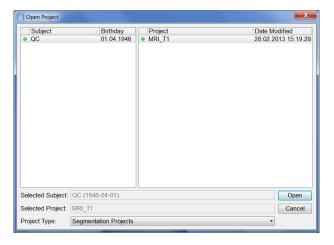
**30.** Press **Select Input Project** in the interaction window. Please note that the segmentation of the subject's MRI has already been performed and pre-saved in order to save time.





Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

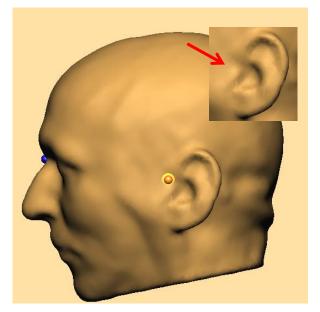
**31.** Choose the project MRI_T1 of subject QC. Press **Open**.



32. Mark Nasion on the segmented skin surfaces as indicated in the head scheme.Press Next to continue.



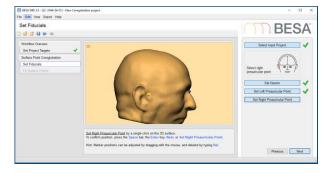
**33. Mark LPA** (Left preauricular point) as indicated in the head scheme in the interaction window. In this subject LPA position can be identified quite well by the shadow that is cast in LPA's dent. Press Next to continue.



Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

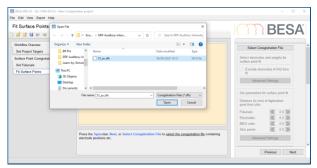
34. Mark RPA (Right preauricular point) and press Next to continue.

**35.** All fiducials are now positioned. Press **Next** to continue.

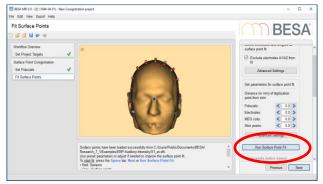




36. Press Select Coregistration File to load the S1-av.fsh file we created in step C7. It is located in the Examples" folder under Auditory Intensity. Press Open.

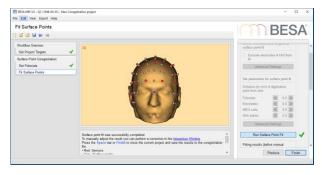


37. We can see the standard electrodes we loaded in step C3. They do not optimally fit the head surface, as the initial fit solely attempts to match the fiducials we defined in steps C12-C14 and the fiducial information in the sfh-file. Press Run Surface Point Fit to optimally match the standard electrodes to the segmented skin.



Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

38. Now we achieved a good fit between the standard electrode positions and the segmented skin. Press Finish.



**39.** Please **save** the coregistration project under the suggested name.

Save Project As			×
Subjects		Projects	
P300-Auditory (1	946-04-01)		
Selected Subject:	P300-Auditory (1946-04-01	)	Save
Selected Project:	CORP300-Auditory (194	6-04-01)_MRI_T1S1_av	Cancel
Project Type:	Coregistration Projects	Ţ	

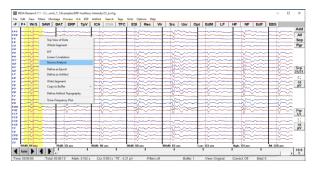
### D. Utilizing the individual anatomy in BESA Research

 Return to BESA Research. All coregistration information containing surfaces and transformations have automatically been sent to BESA Research. A green arrow indicates that all necessary information is available. Press OK.

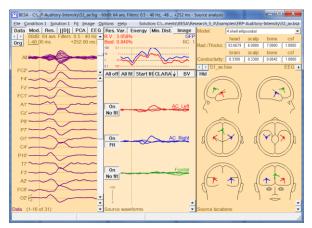
Coregistration files		
No coregistration file (*.sh) defined		Besa MRI
Surface coregistration file (*.sth. *.bhm):	<b>_</b>	Select MRI prog
\S1_av.sfh		Browse
Talairach coordinates (*tal)		
	Read from sth file	Browse
Talairach-transformed MRI file (*.vmr)		
D:\BESAMRI Demo\BESAMRI Shared\P300-Auditory_19460401\MRIFiles'	ОК	Browse
Talairach-transformed head surface mesh (*.srf)		
D:\BESA MRI Demo\BESA MRI Shared\P300-Auditory_19460401\MRIFiles'	ОК	Browse
Talairach-transformed brain surface mesh (*.srf)		
D:\BESA MRI Demo\BESA MRI Shared\P300-Auditory_19460401\MRIFiles ¹	OK	Browse
Coordinate information		01
Talairach coordinates defined		ОК
	Help	Cancel

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

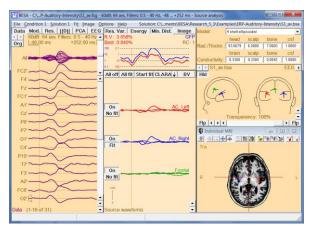
- Let us now send the condition 60dB to source analysis by left-dragging a block, right-clicking and selecting Source Analysis.
- Select the custom definition -50 to 250 ms, Low Cutoff Filter 0.5 Hz, 12 dB/Oct, Zero Phase, High Cutoff Filter 40 Hz, 24 dB/Oct, zero phase. Press Source Analysis.
- Our source model consisting of 3 regional sources will automatically open as we saved it per person in the batch created for tutorial 6.



Block Size and Position	Filter Settings
C Whole Segment Custom Definition	Erequency 0.5 [Hz] Signe 12 db/oct      Type zero phase
▼ Previo <u>u</u> s Settings	High Cutoff Status V Enabled
-50 (ms) pre-E⊻ent	Frequency 40.0 [Hz] Sloge 24 db/oct  Type zero phase
250 (ms) post-Event	
All Conditions	Source Analysis Set Block Cancel

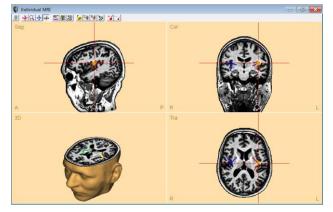


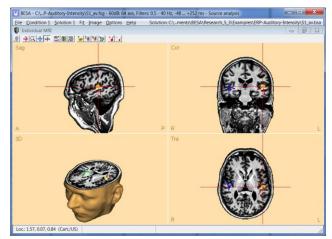
5. Press A on the keyboard to bring up the individual anatomy of the present subject. Since we coregistered the EEG and MRI data, the individual MRI is now available. Without coregistration, only a standard MRI can be displayed.

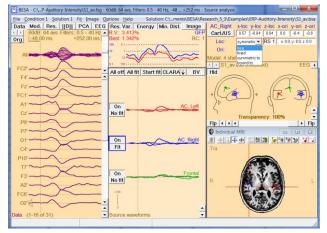


Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- 6. Select the first source by clicking on its source waveforms in the middle pane and enlarge the MRI window to get a better view. It can be seen that the source model we created using the grand average is located in the auditory cortex of subject S1. The model can still be optimized, however.
- 7. Let us first drag the red regional source to left Heschl's gyrus. You will notice that the position of the right auditory source (blue) also automatically changes position, as we imposed a symmetry constraint. We will now change this constraint back to free.
- 8. Minimize the MRI window and select the blue source by clicking on its source waveform. From the dropdown menu under Loc at the top right of the window please change symmetric to free. Maximize the MRI window again.

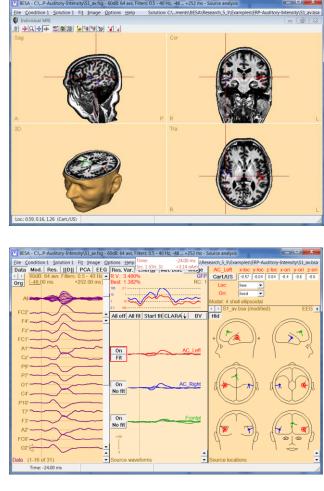






Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- 9. Now let us move the blue auditory cortex source to right Heschl's gyrus. We now see a slight asymmetry in with this subject the right hemispheric source (blue) located more anterior than the lefthemispheric source (red). Please close the MRI window.
- 10. We have now optimized the positions of the auditory cortex sources based on the subject's individual anatomy. What we still need to do is to refit the orientations of the regional sources based on the new positions. The blue source is still selected, so simply **press O** on the keyboard to set the first orientation at the maximum of the activity. **Repeat** for the red source.



We are now finished optimizing our source model based on the individual anatomy of the subject.

### E. Individual FEM Model for Source Analysis - Introduction

Localization precision can further be increased by using a **volume conductor** model that is also based on the individual subject's anatomy.

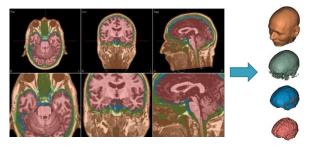
When is localization precision critical? For example:

- When the precise position of a source is the leading research question
- Localization of epileptiform activity

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- Comparison between patients and control subjects regarding the brain region involved in a particular cognitive task
- Investigation of treatment effects on source localization

In BESA MRI, T1 (and optionally T2) images can be used for creating individual, realistic head models using the finite element method (FEM). The model will consist of four compartments, scalp, skull, brain and CSF.



Each compartment is associated with a specific conductivity value (scalp 0.33 S/m, skull 0.0042 S/m, brain 0.33 S/m, CSF 1.79 S/m).

BESA MRI creates a 4-shell FEM model also containing a CSF compartment. Modeling CSF is critical for source localization precision as it is yet another layer with a specific conductivity in addition to brain, skull and scalp (Baumann et al. 1997). Ignoring CSF leads to wrong assumptions about the origin of the current (Ramon et al. 2003, Wendel et al. 2008).

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

When current flows through a volume, it will change direction whenever it passes a new layer with differing conductivity (see image on the right).

In principle, the head model can consist of even more than four layers. The skull, for example contains at least two different tissues with differing conductivities. However, this would mean that head model generation becomes more complex, less automated and more heavy on computation. Additionally, MRI data quality needs to be exceptionally good to model all the different tissue types and the combined use of T1, T2- weighted MRIs as well as CT data is vital. We will see in the following that a four-layer model leads to very good results.

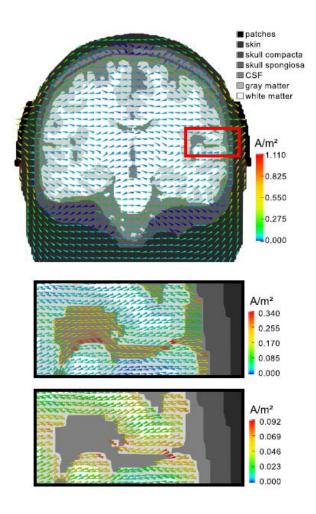
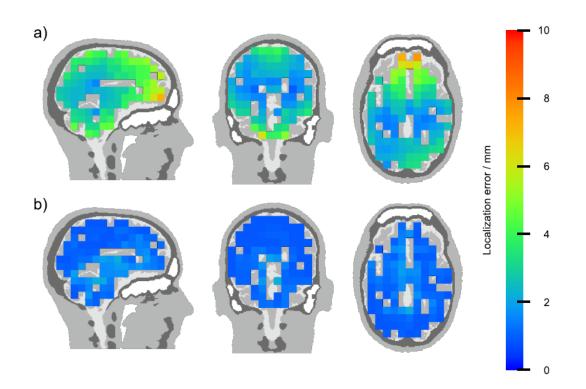


Image taken from Neuling et al., 2012, Neuroimage

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling



The above image displays source localization errors when using a 3-shell model (scalp, skull, brain) **neglecting** CSF (a) and when using a 4-shell model **including** the outer CSF layer between cortex and inner skull (b). It is apparent that including CSF in the forward computation drastically reduces localization errors. Optimally, the CSF-filled ventricles should also be modeled, yet BESA Research requires a homogeneous volume without gaps for source analysis. This is why only the outermost CSF-layer is taken into account⁸. As the outer CSF layer is the most critical for source analysis precision, localization errors caused by un-modeled ventricles are negligible.

Once the FEM model is built, a new leadfield table needs to be computed for the individual forward model and the current electrode layout. This is done during the last step of the BESA MRI coregistration dialogue. This means that the magnitude of the signal any source inside the head will contribute to each sensor must be calculated anew based on the individual FEM model. The computation can take several minutes, depending on the number of electrodes in the electrode layout. A grid resolution of 2 mm is assumed.

⁸ BESA MRI models all CSF, BESA Research only uses the layer between cortex and inner skull.

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

Individual FEM models that were created in BESA MRI are available in the head model drop-down menu of the BESA Research source analysis module after coregistration.

	_
Individual FEM	•
4 shell ellipsoidal	
Individual FEM	
Realistic Approximation (adult, cr 80)	
Realistic Approximation (age 16-20, cr70)	
Realistic Approximation (age 14-16, cr60)	
Realistic Approximation (age 12-14, cr50)	
Realistic Approximation (age 10-12, cr40)	
Realistic Approximation (age 8-10, cr30)	
Realistic Approximation (age 6-8, cr20)	
Realistic Approximation (age 4-6, cr15)	
Realistic Approximation (age 3-4, cr10)	
Polynomial 4 shells	
3 shell Ary approximation	
Homogeneous sphere	

By using a FEM model, the forward model for source analysis is tailored to the individual subject and in consequence the inverse solution can become more precise (Vanrumste et al. 2002, Roth et al. 1993, Cuffin 1996). This is especially true (a) for sources in brain regions that are not described well by a spherical head model (e.g. basal temporal lobe); (b) when individual heads show deviations from the norm (e.g. lesions). Thus, if the research target is to achieve maximal localization precision, an individual realistic head model is strongly recommended.

The segmentation algorithm and FEM model creation were developed in cooperation with the research group around Dr. Carsten Wolters (University of Münster).

### F. Coregistration with Individual MRI and use of FEM Model for Source Analysis

In the following, we will coregister an individual subject's MRI with their EEG data that were recorded in an auditory oddball experiment. We will see the improvements in source localization precision when using an individual FEM volume conductor in contrast to a 4-shell ellipsoidal model.

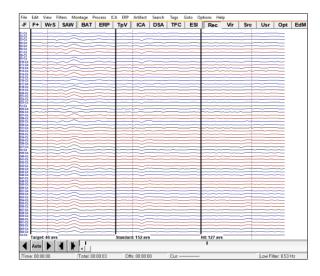
The data we will be working with are located in the BESA Research examples folder "ERP-P300-Auditory". During the oddball experiment, standard and rare tones were presented that differed in frequency. 160 standard tones were presented at 1200 Hz, 40 rare tones were interspersed at 800 Hz. The subject pressed a button with the left index finger whenever he heard a standard tone.

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

1. In BESA Research, open File RareFrequentResponseLeft.fsg located here: C:\Users\Public\Documents\BESA\Re search_7_1\Examples\ERP-P300-Auditory.

Select Data Files						×
Look jn:	ERP-P300-Audi	tory	•	🗢 🗈 🖆	-	
Recent Places Peskop Libraries Libraries Computer	Name RareFrequent	ResponseLeft.fsg			Date mod 26.07.2010	
Network	<ul> <li>✓</li> <li>File <u>n</u>ame:</li> <li>Files of type:</li> <li>Folders:</li> </ul>	III RareFrequentRespons BESA Binary Files (*.foo Examples Folder	-		• •	► Qpen Cancel

 The file contains 3 segments containing average data of conditions Target, Standard, and Hit. We will be working with condition Target, as the condition only involved listening to tones without any button pressing.



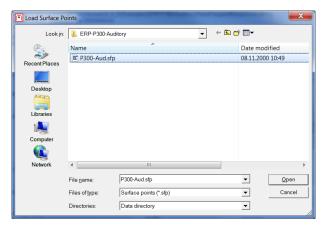
3. Press File / Head Surface Points and Sensors / Load Coordinate Files.

File	Edit	View	Filters	Montage	Process	ICA	ERP	Artifact	Search	Т	ags	Goto	Op	otions	Help				
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	Close	All													-	_			
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	Exit											_			-	-	-	÷	
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		arch_7_	0\Examp	oles\ERP-Au	ditory-Inte	nsity\	\$1_av.f	sg						=	3				-

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- Press the second Browse button (for Digitized Head Surface Points) and choose file P300-Aud.sfp. This file contains individual digitization points of electrode positions. Press Open.
- Press the first Browse button (for Channel Configuration) and choose the file P300-Aud.ela containing the electrode labels and channel specifications. Press Open.
- 6. Instead of standard electrode positions, we are now going to use the digitized electrode positions of the subject. A green arrow should be present indicating that all necessary channel information is available.

In the same dialogue, press button **Edit/Coreg.** to start coregistration of the EEG data with the individual MRI.



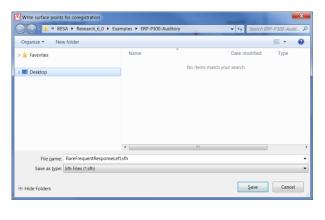
Load Channel C	onfiguration					x
Look <u>i</u> n:	ERP-P300-Audi	tory	•	+ 🗈 🗂	•	
e.	Name	*			Date mod	lified
Recent Places	P300-Aud.ela				04.05.2000	0 11:12
Desktop						
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	File name:				•	<u>O</u> pen
	Files of type:	Channel definitions (*.ela, *.elp)			-	Cancel
	Directories:	Data directory			•	

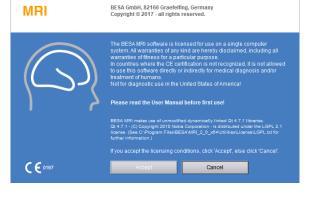
Internal data file information				
Internatioada tile intoimation Data fille: CUSers/Fubic/Documents/BESA/Research_6_ Format: BESA/2009.40.0000; Database file: 00000000 ist No. of channels: 64: 54 electrodes Information should be complete for this data file. No auxiliary If a*ela file defines EEG, you must use 10-10/10-20 labels o	rfiles required.	kesponseLeft.fr	59	~
Suggestions	w file:			
Yes No File name found - Channel configuration (* el?) (* C \V300-Aud.ela	64 chans: EEQ:85 REFion	Good	Browse	Edit
Digitized head surface points (".sfp, "eps) and labels (".sfn) C [\P300-Aud.sfp	68 locs; fiducials found; electrode loc found; units: centimeters no label file	Good	Brawse	Edit
		,	thickness (mm):	8.00
Corregistration file (*.sth) or head center (*.cot) C	no file	Good	Browse	Edit/Coreg.
MEG sensors (*.pos. *.pmg) C @	no file	Good	Browse	Edit
Artifact coefficients (*.art)	na file	Good	Browse	Edit
Clear DB Clear Events		Help	QK	Gancel

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

**BESA**[®]

- You will be prompted to save an sfh-file that will store information about the coregistration later on. You can use the suggested name RareFrequentResponseLeft.sfh.
   Press Save.
- 8. BESA MRI will open. Press Accept.





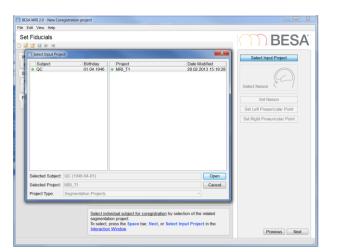
Version 2.0 January 2017

BESA MRI will automatically display the coregistration workflow. Make sure to select the option Generate FEM leadfield. Press Next or hit the space bar to continue with the workflow.

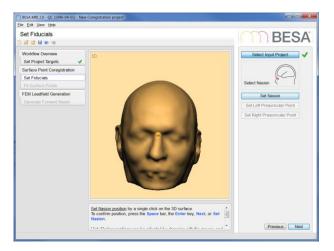
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Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

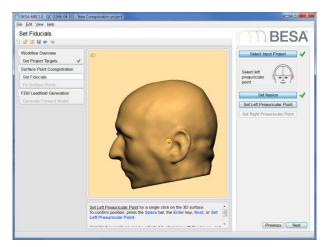
 Press Select Input Project and choose project MRI_T1 of subject QC. Press Open.



11. Select Nasion and press Next.

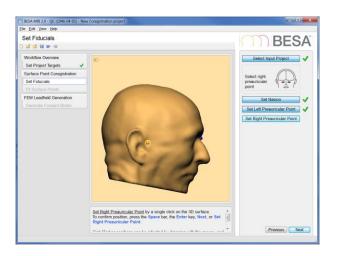


12. Select LPA and press Next.



Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

13. Select RPA and press Next.



14. The fiducial positions will be used as a first reference for a first coregistration of the electrodes with the scalp surface. Press Next to continue.

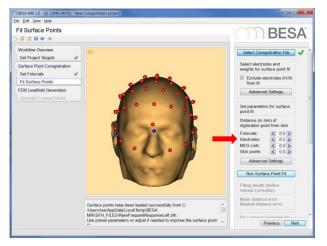


 Press Select Coregistration File and choose RareFrequentResponseLeft.sfh. Press Open.



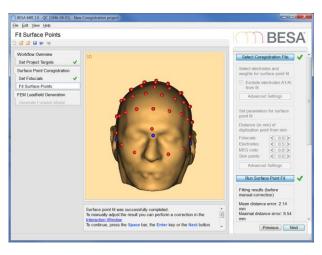
Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

16. In the field Electrodes, enter 0.5 cm to indicate the distance of the digitization points from the scalp. Then press Run Surface Point Fit.

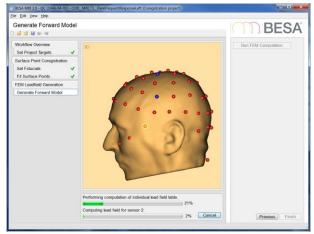


 Once the surface point fit has run and the result is satisfactory, press Next to continue.

**Note**: In case the surface point fit appears inadequate, you might consider revisiting the definition of the fiducials.

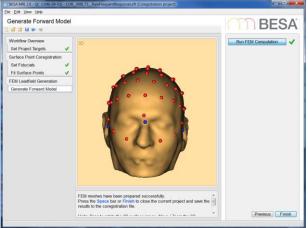


18. Press Run FEM Computation to start leadfield calculation with the given FEM model and electrode layout. This will take several minutes as the forward signal for each electrode needs to be computed under the assumption of activity in each brain voxel. The electrode that is currently being processed is highlighted in orange, the electrodes that are already finished are displayed in blue.



Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

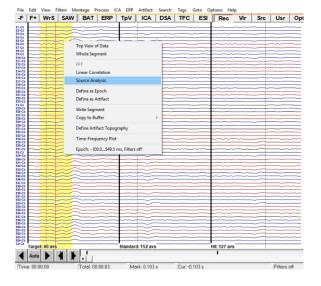
19. Once the FEM computation is done, press Finish and save the project under the suggested name. Close BESA MRI and return to BESA Research.



20. In the window Coregistration with MRI you should now see a green tick mark indicating that all information regarding the coregistration and FEM model are available to BESA Research. Close the window by pressing OK.

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Talairach-transformed brain surface mesh (*.srf)		
D:\lsa\Desktop\BESAWorkshop Examples\BESAMRI_projects\QC_19460	OK	Browse
-FEM Individual FEM defined!		
Coordinate information		
Talairach coordinates defined		OK

21. Left-drag a block in the condition Target, right-click and select Source Analysis.



Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- 22. Select a custom definition of -100 to 300 ms, Low Cutoff Filter of 0.5 Hz, 12 dB/Oct., zero-phase and a High cutoff Filter of 30 Hz, 24 dB/Oct., zero-phase. Press Source Analysis.
- 23. In the SA-window, please left-drag a block from 80 to 110 ms over the EEG signal. You can easily select the time-range by right-clicking into the marked segment and choosing Set Fit Interval.

24. Make sure that the default source type is set to Regional Source by pressing Options. In case the default source type is Dipole. Change it to Regional Source.

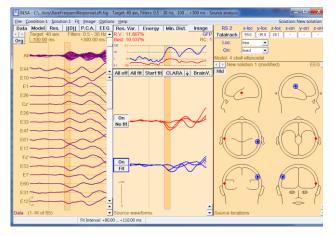
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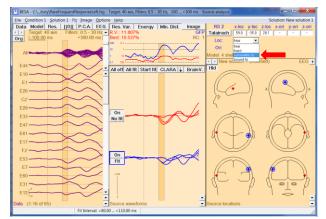
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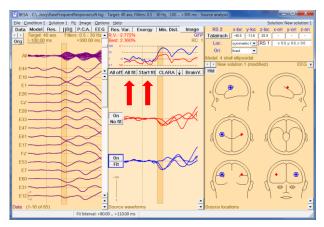
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Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- 25. We will first try to fit the auditory N100 with two regional sources using the default head model 4-shell ellipsoidal. Please double-click into the head schemes twice to place two regional sources, one in the left hemisphere, one in the right hemisphere.
- 26. We will add a symmetry constraint as we are working with bilateral individual activity that is heavily overlapping. Open the drop-down menu next to Loc.: and change the constraint of the second source to Symmetric to.
- 27. Press All Fit and Start Fit to start source localization.

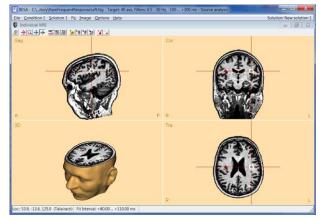


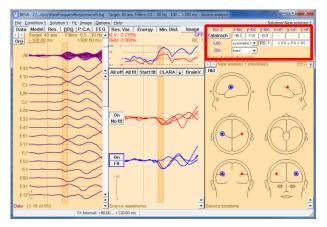


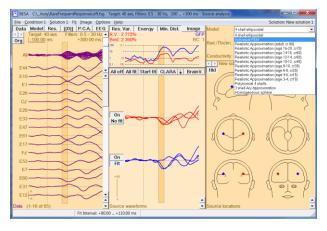


Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

- 28. Press A on the keyboard to bring up the anatomical view. We will see that the two regional sources are located in bilateral auditory areas. We do notice, however, that the sources are not located in Heschl's gyrus as expected, but too superior.
- 29. Close the window displaying the individual MRI. Click into the empty area in the box in the upper right corner that is now displaying the source position. This will switch the contents to the head model selection.
- 30. Open the head model drop-down menu and select Individual FEM. It might take a couple of seconds to load the FEM model.

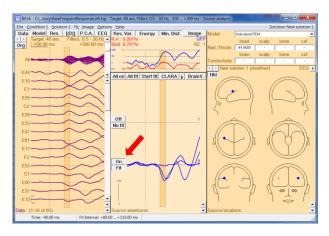




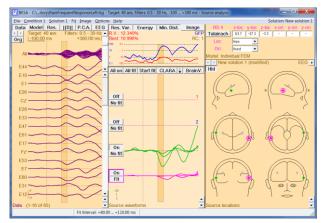


Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

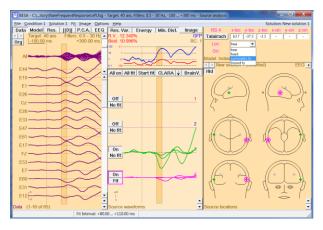
31. Switch off both regional sources off by clicking on the On button next to the source waveforms.



32. Insert two new regional sources by double-clicking in the head schemes, one source in the left, one source in the right hemisphere.

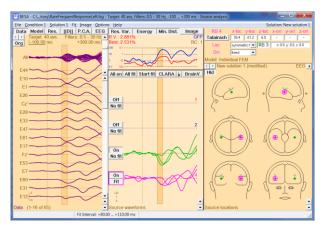


33. Add a symmetry constraint again by opening the drop-down menu next to Loc.: and changing the constraint of the fourth source to Symmetric to.

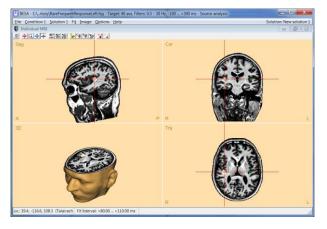


Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

34. Press All Fit and Start Fit to start source localization. It becomes apparent that source localization with the individual FEM model leads to more inferior solutions compared to the 4-shell ellipsoidal model.



35. Bring up the individual MRI by pressingA on your keyboard. We now see that both sources are located in Heschl's gyrus.



This example illustrates the superiority of an individual realistic forward model for source localization precision. The temporal lobe is a structure that is described particularly badly by a spherical model, so individual anatomical information will lead to obvious advantages.

Tutorial 6 – MRI Segmentation, Coregistration and FEM Modeling

Tutorial 7 – Distributed Sources I: Volume Methods

### **Tutorial 7 – Distributed Sources I: Volume Methods**

### What does BESA Research provide?

- ✓ LAURA
- ✓ LORETA
- ✓ sLORETA
- ✓ swLORETA
- ✓ CLARA
- ✓ sSLOFO
- ✓ Minimum Norm
- ✓ User-defined
- ✓ Export of images per time-point or for time-series

Tutorial 7 – Distributed Sources I: Volume Methods

### **Distributed Source Imaging: Volume Methods**

This tutorial introduces the principles of distributed source imaging. A short theoretical introduction is presented, followed by hands-on examples that demonstrate the properties of the different methods and the important effects of regularization on the obtained results.

### A. Short theory of distributed source images

#### Discrete versus distributed source analysis

Both discrete and distributed source models use dipoles or regional sources as their basic elements to model brain activity. However, the relationship between the number of sources and the number of recording sensors differs between those two approaches. This has a number of consequences that are listed schematically in the table on the next page.

The following abbreviations are used:

D: The recorded EEG or MEG data. The dimension of this matrix is [Number of sensors × Number of time points].

Each row of D contains the signal of one sensor as a function of time. Each column of D contains the recorded topography at one time point.

S: The source waveforms. The dimension of this matrix is [Number of sources x Number of time points].

Each row of S contains the activity (in units of dipole moment, nAm) of one source as a function of time.

L: The leadfield matrix. The dimension of this matrix is [Number of sensors × Number of sources].

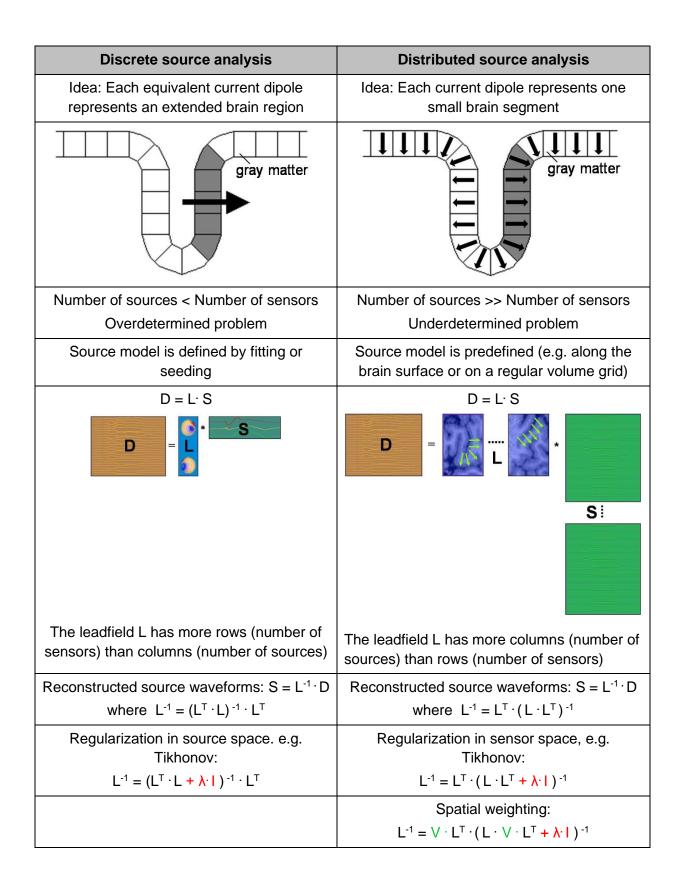
Each column of L contains the topography (the signal pattern) of one source, i.e. the signals that normalized activity of this source generates on the different EEG/MEG sensors. L contains all information about the source model (the multiple dipoles or regional sources) and the head model (the assumed electrical conductivity distribution inside the head).

L^T: The transposed leadfield matrix, i.e. rows and columns are swapped. Accordingly, the dimension of this matrix is [Number of sources × Number of sensors].

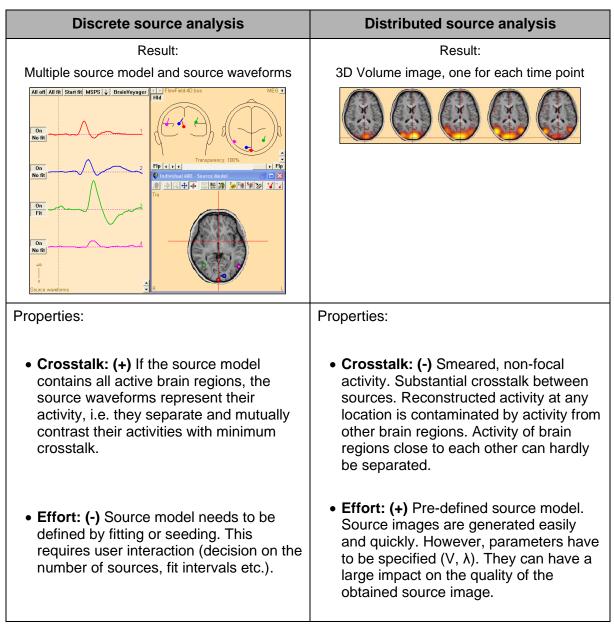
### Tutorial 7 – Distributed Sources I: Volume Methods

- λ: A regularization parameter. The formula in the table holds for Tikhonov regularization, which is just one way of regularizing a matrix.
- I: The identity matrix, containing ones in the diagonal elements and zeroes in the off-diagonal elements.
- V: A spatial weighting matrix on source level. The dimension is [Number of sources × Number of sources].

Tutorial 7 – Distributed Sources I: Volume Methods



### Tutorial 7 – Distributed Sources I: Volume Methods



In BESA Research, distributed source models consist of regional sources at each source location. In the 3D volume images demonstrated in this tutorial, the sources are located on a regular cubic grid spanning the whole brain volume. The grid spacing in Talairach units can be specified by the user.

### Spatial weighting: Minimum norm, LORETA and LAURA

Because distributed source models contain more sources than recording sensors, there are many different source current distributions that perfectly model the recorded data. Therefore, side constraints need to be defined that allow the selection of the optimum solution. These side
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Page 156 of 397

Tutorial 7 – Distributed Sources I: Volume Methods

constraints are represented in the spatial weighting matrix V (see above). Different methods have been published in literature. The following is an overview of the imaging methods implemented as standards in BESA; variations and combinations of them can be specified as a user-defined image (see the corresponding chapter below). More detailed descriptions are given in the BESA Help menu.

- Minimum norm (Hämäläinen & Ilmoniemi 1984): In its simplest form, V is set to the identity matrix. This results in the image with the smallest overall energy (the sum of squares of all sources magnitudes). Usually, depth weighting is used by applying a diagonal matrix V, whose entries are inversely proportional to some measure for the magnitude of the respective sources lead field vector (i.e. deep sources get a larger apriori weight than superficial sources).
- LORETA ("Low Resolution Electromagnetic Tomography", R.D. Pascual-Marqui, International Journal of Psychophysiology. 1994, 18:49-65): Here, V is non-diagonal and contains both a depth weighting term and a representation of the 3D Laplacian matrix. This leads to the image with the smoothest distribution of activity across the source space.
- LAURA ("Local Auto Regressive Average", R. Grave de Peralta Menendez 2001, Brain Topography 14(2), 131-137): In LAURA, V is non-diagonal and contains both a depth weighting term and a representation of a local autoregressive function.

### Standardization with the resolution matrix: sLORETA and swLORETA

All distributed source images suffer from smearing and crosstalk, leading to the following effects:

- Even in data generated by focal brain activity, the reconstructed 3D image is blurred and non-focal. Activity of the active brain region gets projected onto neighboring sources.
- Consequently, the reconstructed activity at one source location is representing not only brain activity at the modeled location, but is also reflecting activity of other brain regions.

These effects are captured mathematically by the resolution matrix R, defined as:

 $R = L^{-1} \cdot L$ 

Tutorial 7 – Distributed Sources I: Volume Methods

The diagonal elements of R are a measure for the percentage of the reconstructed activity of the respective source that is due to brain activity at the modeled location. The idea behind sLORETA and swLORETA is to divide (standardize) the reconstructed minimum norm activity of each source with this measure. This serves as a compensation for the non-uniform depth sensitivity of the minimum norm approach.

 sLORETA ("standardized LORETA", R.D. Pascual-Marqui, Methods & Findings in Experimental & Clinical Pharmacology 2002, 24D:5-12): Unlike the name suggests, this method is not a standardization of LORETA, but rather of the unweighted minimum norm image. The sLORETA activity at location r: is estimated from the minimum norm source estimate S_{MN,r} as:

 $S_{sLORETA,r} = R_{rr}^{-1/2} \cdot S_{MN,r}$ 

In simulated data sets with only one focal activity and no noise, the sLORETA image has its maximum exactly at the simulated location. However, this property does not hold anymore in the realistic case of noisy data and multiple simultaneously active brain regions.

• **swLORETA** (E. Palmero-Soler et al 2007 Phys. Med. Biol. 52 1783-1800) is a standardized version of the depth-weighted minimum norm.

### Iterative approaches: CLARA and sSLOFO

One approach to make distributed source images more focal is to apply them iteratively. The spatial weighting matrix V in each iteration step contains contributions that reflect the image obtained in the previous iteration step. CLARA and sSLOFO are such approaches:

- CLARA ("Classical LORETA Analysis Recursively Applied") is an iterative application of the LORETA algorithm with an implicit reduction of the source space in each iteration. First a regularized LORETA image is computed as initialization. Then in each iteration step the following actions are carried out:
  - 1. The obtained image is spatially smoothed (this step is left out in the first iteration).
  - 2. All grid points with amplitudes below a threshold of 1% of the maximum activity are set to zero, thus being effectively eliminated from the source space in the following step.

Tutorial 7 – Distributed Sources I: Volume Methods

3. The resulting image defines a spatial weighting term (for each voxel the corresponding image amplitude). A LORETA image is computed with this additional spatial weighting term for each voxel. By the default settings in BESA Research, the regularization values used in the iteration steps are slightly higher than that of the initialization LORETA image.

After a specified number of iterations (default: 2), the computation stops and the image computed in the last iteration is displayed.

- sSLOFO ("standardized shrinking LORETA-FOCUSS", H. Liu et al. 2005, IEEE Transactions on Biomedical Engineering 52(10), 1681-1691).) is an iterative application of weighted distributed source images with a reduced source space in each iteration. As initialization step, an sLORETA image is computed. Then in each iteration step the following operations are performed:
  - 1. The obtained image is spatially smoothed (this step is left out in the first iteration).
  - 2. All grid points with amplitudes below a threshold of 1% of the maximum activity are set to zero, thus being effectively eliminated from the source space in the following step.
  - 3. A depth-weighted, standardized minimum norm is being computed, where V has additional diagonal weights proportional to the image amplitudes in the previous iteration steps.

After a specified number of iterations (default: 3), the computation stops and the image computed in the last iteration is displayed.

An overview of these methods is provided in the next page.

### Tutorial 7 – Distributed Sources I: Volume Methods

Method name	Type	Regularization	Spatial weighting	Operator	Optimization / specific features	Pros	Cons
Multiple dipoles / Regional sources	discrete	optional	ę	$s = (L^T L + \lambda I)^{-1} L^T * D$	Provided that all br Adjust <i>L</i> regions are modeled minimize    <i>D</i> model - <i>D</i> measured    cross talk and good separation of sourc	Provided that all brain regions are modeled, little cross talk and good separation of source	Takes skill to model and sequentially fit sources; user-dependent
Minimum norm (L ₂ ) distributed	distributed	mandatory	optional (diagonal only)	$s = L^{T} (LL^{T} + \lambda I)^{-1} * D$ $s = VL^{T} (LVL^{T} + \lambda I)^{-1} * D$	With spatial weighting V : diagonal matrix with depth weighting	No user interaction necessary after definition of <i>λ</i> , and <i>V</i>	cross talk, smearing; activities of nearby brain regions cannot be separated
LORETA	distributed	mandatory	yes (with off- diagonal elements)	$s = VL^{T}(LVL^{T} + \lambda I)^{-1} * D$	V holds depth weighting (diagonal) and Laplace operators (off-diagonal)	No user interaction necessary after definition of $\lambda$ and $V$	Very smeared signal
LAURA	distributed	mandatory	yes (with off- diagonal elements)	$s = VL^{T}(LVL^{T} + \lambda I)^{-1} * D$	V holds depth weighting (diagonal) and a local autoregressive function (off- diagonal)	No user interaction necessary after definition of λ. and V	Very smeared signal
sLORETA	distributed	mandatory	yes (diagonal only)	$\begin{split} \mathbf{S}_r &= R_{rr}^{-1/2} L^T (L L^T + \lambda I)^{-1} * D, \\ \text{where } R &= L^T (L L^T + \lambda I)^{-1} \mathbf{I} \end{split}$	R is a measure of the percentage of the reconstructed activity at a source location that is due to the brain activity at	cf. Minimum norm	cf. Minimum norm
swLORETA	distributed	mandatory	yes (diagonal only)	yes (diagonal only) like Minimum norm	With spatial weighting V : diagonal matrix with standardized depth weighting	cf. Minimum norm	cf. Minimum norm
CLARA	distributed	mandatory	yes (with off- diagonal elements)	like LORETA	Iterative approach, reduce source space and use image as spatial weighting term	No user interaction necessary after definition of parameters (no of iterations,)	some smearing
sslofo	distributed	mandatory	yes (diagonal only)	yes (diagonal only) like sLORETA / Minimum norm	lterative approach, reduce source space and use image as spatial weighting term. Initial solution uses LORETA,	No user interaction necessary after definition of parameters (no of iterations,)	some smearing

Tutorial 7 – Distributed Sources I: Volume Methods

### Regularization

Distributed source images require the inversion of a term of the form L·V·L^T. This term is generally regularized before its inversion. In BESA, selection can be made between two different regularization approaches

- Tikhonov regularization: In Tikhonov regularization, the term L·V·L^T is inverted as (L·V·L^T+λ·I)⁻¹. λ is the regularization constant, and I is the identity matrix.
- Truncated singular value decomposition (TSVD): Here, an SVD decomposition of L·V·L^T is computed as L·V·L^T = U·X·U^T, where the diagonal matrix S contains the singular values. All singular values smaller than the specified percentage of the maximum singular values are set to zero. The inverse is computed as U·X⁻¹·U^T, where the diagonal elements of X⁻¹ are the inverse of the corresponding non-zero diagonal elements of X.

As we will see, regularization has a critical effect on the obtained distributed source images. The results may vary substantially with the choice of the regularization parameter (see examples below). Therefore, it is important to evaluate the generated image critically with respect to the regularization constant, and to keep in mind the uncertainties resulting from this fact when interpreting the results.

### **B.** Comparison of different distributed source imaging methods

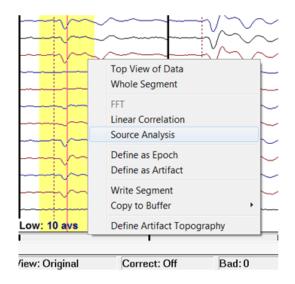
We will now return to the grand average data of the auditory intensity experiment to compare distributed source analysis methods with one another as well as with the regional source model we fitted earlier on.

 Select the file All_Subjects_cc.fsg from File / Recent Files.

File	Edit	View	Filters	Montage	Process	ICA	ERP	Artifact	Search	Tags	Gote	0 0	ptions	Hel	р				
	Open.								ctrl-0	FC	: E	ESI	R	ec	Vir	Src	Usr	Opt	E
	Close											Т		~			~	<u> </u>	_
	Close	All								I.		L		~	$\sim$	. 1		$\sim$	$\sim$
	Recen	t Files								•	1. C:\	xan	nples\E	RP-A	uditory-l	ntensity\/	II_Subject	s_cc.fsg	
	Load	Channe	l Config	uration							2. C:\	les\	ERP-P	300-A	uditory\	RareFrequ	entRespon	seLeft.fsg	
Head Surface Points and Sensors							<ol> <li>C:\arch_7_0\Examples\ERP-Auditory-Intensity\S1_av.fsg</li> </ol>												
	MRI C	oregist	ration								4. C:\	Exa	imples\	Learn	-by-Sim	ulations\R	egionalSo	urce2D.av	r
5. C:\y-Simulations\AC-2D-biphasic-synchrone						nchronous	+noise.av	r											
		to MAT									6. C:\	ean	n-by-Si	imula	tions\AC	-2D-biph	isic-synch	ronous.av	т
	Send	to MAT	LAD								7. C:\	Exa	mples\	Learn	-by-Sim	ulations\/	C-2D-no-	overlap.av	r

Tutorial 7 – Distributed Sources I: Volume Methods

 Select condition Low, left-drag a block and send it to source analysis by right-clicking and pressing Source Analysis.



- Select a time range from -50 to 250 ms, and specify a Low Cutoff filter of 0.5 Hz, 12dB/oct, zero-phase and a High Cutoff filter of 40 Hz, 24 db/oct, zero-phase. Press Source Analysis to open the specified data in the source analysis window.
- As a reference and for comparison, load the regional source model we created in Tutorial 4, steps D1-7. Press File / Open Solution... and load file LowIntensity_2RS.bsa.

Block Size and Position	Filter Settings	Low Cutoff Slope 12 db/oct ▼	Status 🔽 Enabled
Custom Definition     Previous     Settings     -50     (ms) pre-Event	Frequency 40.0 [Hz]	High Cutoff Sloge 24 db/oct 💌	Status 🔽 Enabled
250 (ms) post-Event	Source Analysis	Set <u>B</u> lock	Cancel

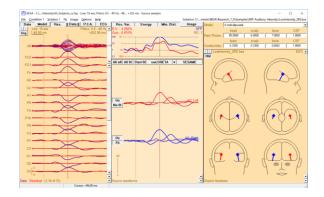
Look in:	ERP-Auditory-Intens	sity		- + 🗈 (	* 📰 •
<u>_</u>	Name	Size		Туре	Date modified
	HighIntensity_3R	5.bsa	1 KB	BSA File	2009-12-09 2:17 PM
Quick access	LowIntensity_2RS	.bsa	1 KB	BSA File	2009-12-09 2:17 PM
	LowIntensity_2SD	.bsa	1 KB	BSA File	2012-02-27 4:14 PM
Desktop	S1.bsa		1 KB	BSA File	2005-03-29 3:32 PM
	S1-AC.bsa		2 KB	BSA File	2005-03-29 4:04 PM
	S1-AC+SC.bsa		2 KB	BSA File	2005-03-29 4:06 PM
Libraries	S2.bsa		1 KB	BSA File	2005-03-29 3:35 PM
This PC					
<b>S</b>					
Network	<				
	File name: Low	Intensity_2RS.bsa			▼ Open
	Files of type: BES	A Solutions (*.bsa	)		▼ Cancel
	Folders: Dat	a Folder			- -

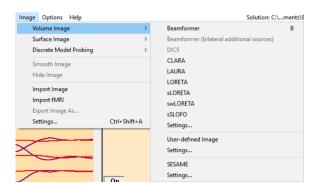
Tutorial 7 – Distributed Sources I: Volume Methods

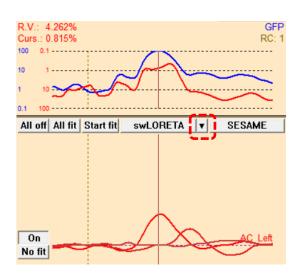
 Set the cursor at 96 ms, at the peak of the N100 component.

6. Distributed 3D volume images are generated with a single click in BESA. They can be generated by selecting the corresponding method from menu entry Image / Volume Image, or from the dropdown menu of the image selector button (the arrow left of the SESAME button in the middle of the source analysis window).

In this chapter, we will use the default parameters for all 3D images. The importance and potentially large influence of these parameters on the obtained result will be illustrated in the next chapter.





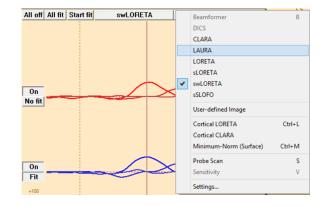


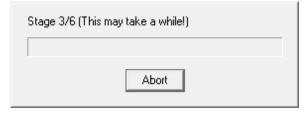
Tutorial 7 – Distributed Sources I: Volume Methods

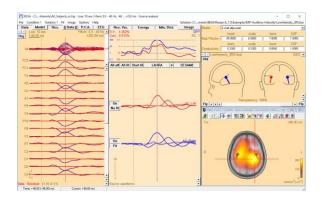
 First, compute a LAURA image at the cursor latency. Simply select LAURA from the Image selector button dropdown menu.

- 8. The computation of a LAURA (and also LORETA) image is divided into 6 steps. A dialog window informs you about the processing stage. When computing a LAURA image for the first time for a data set, steps 2 and 3 can take some time to complete (depending, among others, on the selected grid spacing, see below). The results of this intermediate steps are stored to disk, however, so that these steps will be omitted in all future LAURA images. This speeds up the image calculation.
- 9. The result is displayed superimposed to the anatomical MR image in the 3D window in the lower right corner of the source analysis window. Because for this subject, no individual MRI is available, BESA automatically uses a standard MRI. The regional source model is also shown and superimposed on the LAURA image. Press

the button in the 3D window to get a 4head view. (You may also maximize the 3D window to get a larger view of the obtained LAURA image.)





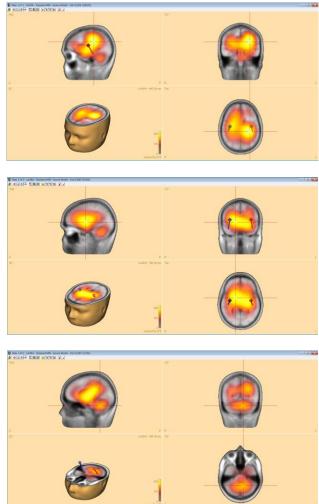


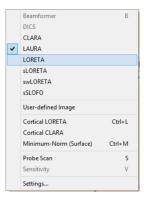
Tutorial 7 – Distributed Sources I: Volume Methods

10. The units of the displayed LAURA images are nAm/cm³. The image correctly shows activity in both hemispheres. However, the focal activity is substantially blurred – a negative effect inherent in all distributed source images. It becomes evident from this example that this does not allow to draw any conclusion on the extent of the active brain regions.

The LAURA image detected 3 maxima. The largest maximum is in the left hemisphere, roughly in the temporal lobe. Compared to the regional source it is located more medial, posterior and superior. You can jump to the next maximum using the **Button (4th** button from the right in the 3D window). The second maximum is roughly in the right-temporal lobe, and located more medial inferior and anterior to the corresponding regional source. LAURA also detected a third maximum in the left cerebellum. The regional source model does not suggest activity there.

11. Compare the LAURA method with LORETA: Select LORETA from the image selector button dropdown menu. Like LAURA, LORETA includes a spatial crossvoxel weighting term, which increases the computational load, so that the first LORETA image computed for a data set



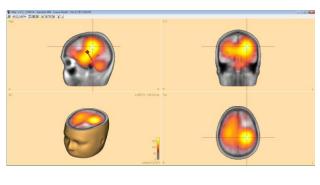


### **Tutorial 7 – Distributed Sources I: Volume Methods**

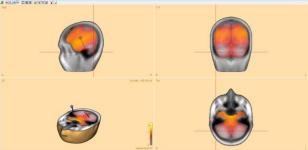
takes a longer time to complete than sLORETA or swLORETA. LORETA and LAURA often produce similar results: The first two maxima are located roughly in the temporal lobes, a third maximum is located in the cerebellum.

12. Compare the LORETA method with sLORETA: Select sLORETA from the image selector button dropdown menu. The result is an image (units: standardized nAm/cm3) with two maxima. The first maximum is located roughly in the middle of the head, the second maximum is located in the right cerebellum. There is no convergence between the discrete source solution and the sLORETA image.

swLORETA additionally uses depth weighting, but will come up with a very similar result; we will not pursue this method now.







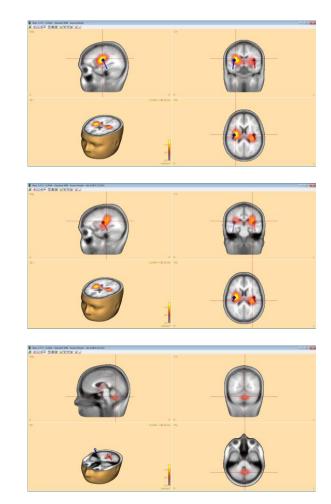
13. Iterative methods aim at combining advantages of discrete and distributed source images. Implemented as default methods in BESA Research are sSLOFO and the new CLARA method. Both methods iteratively apply distributed source images with a successive shrinking of the source space. They differ from each other in the type of distributed source images applied in this procedure.

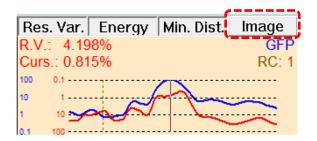
**Tutorial 7 – Distributed Sources I: Volume Methods** 

14. Let us try out the CLARA method, an iterative application of weighted LORETA images. Select CLARA from the image selector button dropdown menu, and observe that the result is much more focal than the previous methods, shrinking the activity substantially, and indicating bilateral activation of the supra-temporal lobes. The first two maxima are very close to the discrete source solution. CLARA detected a third maximum, again located in the cerebellum.

The other iterative method **sSLOFO** has a similar approach to CLARA, but uses sLORETA during the iterative refinement of the initial result. The result is similar to CLARA, however the two main maxima do not coincide quite as closely with the regional sources. You can try it at your own discretion.

15. Next, we want to re-fit our discrete sources using Image weighting. Press the Image button in the top-right corner of the GFP and residual variance field. By default, only buttons for criteria Residual Variance,





### Tutorial 7 – Distributed Sources I: Volume Methods

Minimum Distance Energy and are pressed. Right-click on the Image button and click on Weighting Factors to bring up the Preferences dialog. You can see that by default weighting factors Residual Variance, Energy, Minimum Distance and Image Weighting are treated equally (all are set to 1). This can be altered if desired. We will leave the default settings and press OK.

- Preferences ? × Display Boxes 3D Window File Head model Constraints Fit Genetic Algorithm R(AP)-Music Regularization constant [%] Default Default Minimum distance criterion [mm] 8.0 Hide advanced options Advanced options: Smoothing constant for electrode interpolation: 1E- 7 🕂 Default Weighting factors of fit criteria 1.00 Residual variance criterion: Energy criterion: 1.00 Default 1.00 Minimum distance criterion: 1.00 Image weighting criterion OK Cancel Apply
- Click on the right-hemispheric source and change the symmetry constraint to free in the Loc drop-down menu.

17. Press All Fit and Start Fit. The regional sources are now fitted again with additional weighting by the current image. Maximize the MRI view to observe the new fitting-results. Close the source analysis window without saving anything.





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This first glance at the different imaging methods on our example dataset illustrates both the advantages and disadvantages of distributed source images: While they allow to get a very quick result at a certain latency, they all suffer from severe blurring (least in CLARA and sSLOFO), which can make it impossible to separate brain regions that are located close to each other. The different methods do not necessarily agree in their image maxima. It is not straightforward to determine which method is the most appropriate right away – the answer may be different in different data sets. Therefore, generally, a comparison of different methods is advisable.

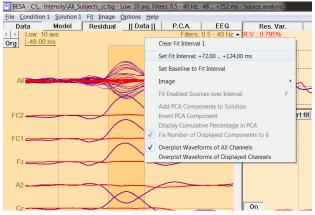
To be able to interpret distributed source images, it is essential to understand their sensitivity towards the parameters that are applied during the computation. Especially regularization has a crucial effect on the result; this is demonstrated in the following.

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### C. Settings for distributed source images: The effect of regularization

Distributed source images apparently have a big advantage over discrete multiple source models: They are quickly generated by a single mouse click, and the experimenter does not have to decide on the number of sources and the respective fit intervals. However, there is an important parameter also for distributed source images that has to be decided on: the regularization parameter, which can have a large impact on the quality of the obtained source images. This is illustrated in the following.

1. As an example, we will compare LORETA images with different regularization settings. We could use images computed for the cursor time only, as we did in the previous chapter. Alternatively, we can mark an extended latency interval. By default, BESA research will then compute a mean image over that interval. This is of advantage in noisy data, because it reduces the noise influence. Left-drag to mark a fit interval from 72 to 124 ms to include the time range around the N100 peak. Note that you can drag the edges of the interval, or right-click into it and select Set Fit Interval to adjust the time range.



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 Regularization and other imaging parameters can be specified in the Image Settings dialog window. Open it by pressing Image / Settings or from the bottom of the image selector button dropdown menu.

3. The Image Settings dialog window for volume imaging consists of three tabs: The Standard Volume tab lists all adjustable parameters of the standard distributed volume images. We will focus here on the effect of the regularization parameter, which has a critical influence on all distributed source images. Parameters can be specified separately for each imaging method (and differently for EEG and MEG). The window displays the parameters of one method at a time only. From the upper dropdown menu, select LORETA to adjust the LORETA parameters only.

	Beamformer	В
	DICS	
~	CLARA	
	LAURA	
	LORETA	
	sLORETA	
	swLORETA	
	sSLOFO	
	User-defined Image	
	Cortical LORETA	Ctrl+L
	Cortical CLARA	
	Minimum-Norm (Surface)	Ctrl+M
	Probe Scan	S
	Sensitivity	v
	Settings	

	?
eneral Standard Volume User-D	Jefined Volume SESAME
Image weighting	ctor: 1.00 Default
Specify settings for:	
Regularization	DICS CLARA © EEG © MEG
C CVE (Tikhonov)	
C User-defined (Tikhonov):	C swLORETA
SVD cutoff:	0.0050 %
C Minimum regularization	
Regularization for Iterations	
C CVE (Tikhonov)	C Use data in marked block/at cursor 1.00 C Use non-baseline data epoch
C User-defined (Tikhonov):	0.01 % CVE smoothing factor
SVD cutoff:	0.0100 %
C Minimum regularization	
Iterations (CLARA/sSLOFO)	
Iterations (CLARA/sSLOFO) No. of iterations 2	ze iterations           1.00         Image smoothing half-width (voxels)           10         Amount to clip from image (percent)
No. of iterations	ze iterations

### Tutorial 7 – Distributed Sources I: Volume Methods

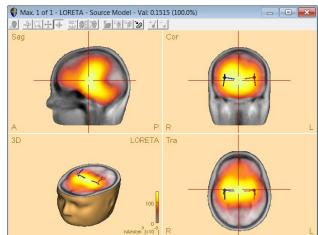
- We will now compute and compare images with different regularization constants. The default is an SVD cutoff of 0.005%. First observe the effect of too high regularization: Increase the SVD cutoff to 0.1%.
- 5. Press Go in the Image Settings dialog window to apply the current settings and compute a LORETA image with too high regularization: In contrast to the result of the multiple dipole model, the LORETA image suggests a single midline generator of the recorded signals. This is a typical result of a too strong regularization: This tends to lead to deep, widely distributed image maxima. In our example, the bilateral activity of the left and right auditory cortex are combined and mis-localized in the middle of the head.
- Regularization

   EEG
   MEG

   Regularization for Simple Images

   Use data in marked block/at cursor
   CVE (Tikhonov)
   Use non-baseline data epoch
   User-defined (Tikhonov):
   0.01
   %
   CVE smoothing factor
   SVD cutoff:
   0.1
   %
   Minimum regularization



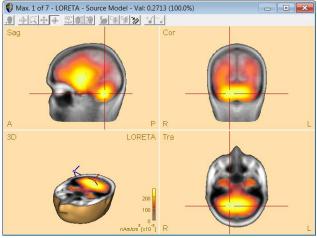


 Now compare too high with too low regularization: Select Minimum regularization to use only the minimum regularization required for numerical reasons.

- Regularization			
		EEG	O MEG
Regularization for Simple Image	s		
	C Use data in marked block/at curso	r [1	.00
C CVE (Tikhonov)	C Use non-baseline data epoch		
C User-defined (Tikhonov):	0.01 %	0.5	 noothing factor
() Oser-delined (Tikiloliov).	0.01	UVE SI	nootning tactor
C SVD cutoff:	0.1000 %		
	0.1000		
Minimum regularization			

### Tutorial 7 – Distributed Sources I: Volume Methods

7. Press Go again to recompute the LORETA image. In contrast to a too high regularization constant we now obtain a solution with many maxima. This is a typical effect of too low regularization.



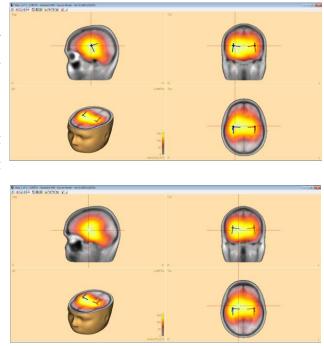
The above examples were computed with SVD regularization, but the same principle holds for Tikhonov regularization as well. You may test this by selecting the user-defined Tikhonov regularization in the Image Settings dialog window and compute LORETA images with different parameters.

How to determine the optimum 8. regularization parameter for a given data set and imaging method is still an unsolved problem. Minimization of the generalized cross validation (CVE) is an attempt to achieve this. However, this method is not guaranteed to work properly in every data set and with every method. We will compute a LORETA image with the regularization determined automatically by CVE minimization. Select CVE and have the regularization optimized to the Data in marked block. Press Go.

F	legularization Regularization for Simple Images			● EEG	C MEG
	<ul> <li>CVE (Tikhonov)</li> </ul>	Use data in marked block/at cursor O Use non-baseline data epoch		1.	00
	<ul> <li>User-defined (Tikhonov):</li> </ul>	0.01	%	CVE sm	ioothing factor
	○ SVD cutoff:	0.0050	%		
	<ul> <li>Minimum regularization</li> </ul>				
	Regularization for Iterations				

### Tutorial 7 – Distributed Sources I: Volume Methods

9. The resulting automatically chosen regularization appears to be slightly higher than the default value of 0.005% (the image contains 2 rather than 4 maxima). The second maximum corresponds well with the regional source in the right hemisphere, but the *first* maximum mislocalizes to a right medial temporal area.



BESA uses predefined default regularization constants specific for each method and channel type. These default values are appropriate in many situations. However, the optimum regularization depends on the signal-to-noise ratio of the data, the number of channels and other properties of the specific data at hand. There is no strict objective criterion for the choice of the optimum or correct regularization parameter. However, it is generally recommended to adjust the regularization parameter such that the obtained image neither shows too many, superficial maxima (too low regularization) nor only one or two very broad midline maxima (too high regularization).

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### **D.** Other image settings

 The Image Settings dialog window not only allows to modify parameters of the predefined imaging methods. Select the General tab. Here the grid spacing of the source grid used for the 3D volume imaging methods can be specified. The default setting is a spacing of 7 mm, which is appropriate for the rather distributed images in most situations.

Another setting allows to specify which kind of image to display when a fit interval is marked. BESA Research computes a separate image for each latency within the fit interval. With the default setting, the **mean image** across the different latencies in the fit interval is computed and displayed. Alternatively, you can have BESA Research to automatically set a cursor at the latency of **maximum global field power** and display the corresponding image.

Image Settings	?	×
General Standard Volume User-Defined Volume SESAME		
OK Cancel	App	ly l

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- It is also possible to create a user-defined volume imaging method: Switch to the User-Defined Volume tab. Here, a wide range of parameters can be specified.
  - In box Resolution Matrix, specify whether you would like to standardize your image with the resolution matrix entries as in sLORETA.
  - In box Spatial Weighting, different types of depth weighting can be selected. Cross-voxel weighting as in LORETA (3D Laplacian) and LAURA (local autoregressive function) can be applied. In addition, an additional spatial weighting can be imposed by the previously generated or imported 3D

mage Settings	?	х
		~
General Standard Volume User-Defined Volume SESAME	_	1
LORETA LAURA SLORETA SwLORETA Min-Norm CLARA SSLOFO	1	
Resolution matrix		
Spatial weighting		
Depth     C 1st eigenvalue of RS leadfield     Help		
Mean norm of RS leadfield     Each component separately		
Cross-voxel O LORETA, O LAURA		
By previous image.         Factor:         1.00		
Regularization (for iterations, or if no iterations, for this image)	7	
C CVE (Tikhonov) C Use non-baseline data epoch		
C User-defined (Tikhonov): 0.01 % CVE smoothing factor		
C Minimum regularization		
Iterations (Specify settings for initialization method in Standard Volume Tab)     No. of iterations Initialization method	7	
1.00     Image smoothing half-width (voxels)     Image smoothing half-width (voxels)		
Regularize iterations		
OK Cancel	A	pply

image (e.g. an imported fMRI image). The strength of the image weighting can be adjusted by the Factor sliding bar.

- The **Regularization** parameters specified in this tab are in analogy to those in the Standard Volume tab.
- The Iterations box allows to define iterative methods in which the source space is shrunk in each step, leading to more focal images. An initialization image can be selected, defining the initial voxel weight (this image is regularized as specified in the Standard Volume tab). In each iteration step, the image is then smoothed and the source space clipped with the parameters specified here.
- The **Default** buttons in the upper row set the parameters to those of the respective predefined imaging method so that you can understand the details of those methods and can easily modify them if you wish to.

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 After specifying the settings, you can compute a user-defined image by selecting the corresponding entry from the list of images.

	Beamformer	В
	DICS	
	CLARA	
	LAURA	
	LORETA	
	sLORETA	
	swLORETA	
	sSLOFO	
~	User-defined Image	
	Cortical LORETA	Ctrl+L
	Cortical CLARA	
	Minimum-Norm (Surface)	Ctrl+M
	Probe Scan	S
	Sensitivity	v
	Settings	

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#### E. Brain atlases

A brain atlas can be overlaid on discrete or distributed sources in the 3D window. Please remember that brain atlases are created using either averaged data or single-subject post mortem data, and might not fully correspond to your subject's individual MRI data. For details about how each atlas is created please refer to the author's publication. If you use a brain atlas in your publication, please cite the corresponding work as reference. In BESA Research, the following atlases are available:

- **Brainnetome** (Fan, Lingzhong, Hai Li, Junjie Zhuo, Yu Zhang, Jiaojian Wang, Liangfu Chen, Zhengyi Yang, et al. 2016. The Human Brainnetome Atlas: A New Brain Atlas Based on Connectional Architecture. Cerebral Cortex 26 (8): 3508–26)
- AAL (Tzourio-Mazoyer, N., B. Landeau, D. Papathanassiou, F. Crivello, O. Etard, N. Delcroix, B. Mazoyer, and M. Joliot. 2002. Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. NeuroImage 15 (1): 273–89)
- **Brodmann** (Damasio, Hanna, and Antonio R. Damasio. 1989. Lesion Analysis in Neuropsychology. Oxford University Press, USA)
- AAL2015 (Rolls, Edmund T., Marc Joliot, and Nathalie Tzourio-Mazoyer. 2015. Implementation of a New Parcellation of the Orbitofrontal Cortex in the Automated Anatomical Labeling Atlas. NeuroImage 122 (November): 1–5)
- **Talairach** (Talairach, J, and P Tournoux. 1988. Co-Planar Stereotaxic Atlas of the Human Brain. 3-Dimensional Proportional System: An Approach to Cerebral Imaging. Thieme)

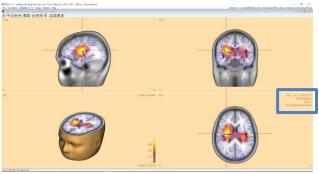
Below it is illustrated how to turn on the brain atlas and how to change settings.

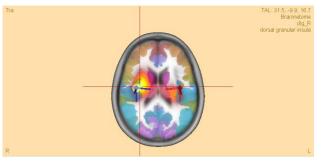
Tutorial 7 – Distributed Sources I: Volume Methods

 Use the dataset and fitting interval in source analysis settings from chapter C of this tutorial (72 to 124 ms). Go to Image / Volume Image /Settings... and switch to settings for CLARA, then press the Default button and Go button afterwards. Close window by pressing OK button. The CLARA results are displayed alongside the discrete solution (two regional sources).

Press on the toolbar (or Shift-A) to turn on brain atlas overlay. A Brainnetome atlas will be displayed using contour blending mode. Note that the crosshair position in Talairach coordinates along with the region name is provided.

 Right click on the empty area in the 3D window and from the popup menu select Atlas Blending Mode / brainCOLOR.





#### Tutorial 7 – Distributed Sources I: Volume Methods

3. Right click on the empty area in the 3D window again and from the popup menu select Options.... Here more settings for brain atlas can be adjusted. From the brain atlas drop-down menu, different brain atlases can be selected. With the slider opacity the opaqueness of the brain atlas overlay over the MRI image can be adjusted. Finally, the visualization mode can be changed here by selection of the mode from the drop-down menu.

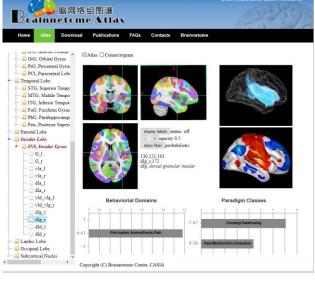
Constraints Fit Genetic Algorithm R(AP)-Music										
File Display Boxes 3D Window Head model										
Show crosshairs in 3D view										
Scale source size with source strength at cursor      Default      Start new session with last 3D head view										
Automatically reopen 3D window at new session										
✓ Interpolate EEG and MEG maps on scalp surface										
Transparent brain surface										
Brain Atlas Brainnetome										
Opacity: 50 % Atlas visualization mode: brainCOLOR										
Display advanced options										

 The Brainnetome atlas project provides additional details about parcellated regions.
 Please visit:

#### http://atlas.brainnetome.org/bnatlas.html

In the online version of the atlas the following information can be found about every brain region (indicated in BESA Research):

- Anatomical connections to the other brain regions (white matter)
- Behavioral domains
- Paradigm classes
- Connectogram



Tutorial 7 – Distributed Sources I: Volume Methods

#### F. Export and import options

All images obtained in BESA Research can be exported for further analysis (e.g. for averaging and statistics in BrainVoyager or MATLAB). Images can also be imported into BESA Research. These will then be displayed superimposed to the available MR image (the BESA standard MRI or the individual MRI of the corresponding subject). In addition, images can be used to define an initial spatial weighting for the different brain regions – both for distributed 3D images (see above) and for a discrete multiple source fit, as will be demonstrated in the following.

- Image ASCII Files (*.dat), BrainVoyager VMP Files (*.vmp) and ANALYZE files (*.hdr): Selection of one of these three formats leads to the export of exactly the currently displayed 3D volume image. The resulting image file contains one image intensity for each grid location (as specified in the Volume Grid Spacing tab of the Image Settings dialog window. The ASCII format contains the image in its original resolution (as specified in the Volume Grid Spacing tab of the Image Settings window). ANALYZE files will be interpolated to a 1x1x1mm³ resolution. The BrainVoyager format allows for both options.
- Same as above, but type all latencies: These options not only export the currently displayed image at the cursor latency, but rather multiple images (one for each latency in the available data set). In the present example, selecting one of these file types would export LORETA images for each latency into one file. This file then contains the image intensities for each location at each latency. This option is not available for iteratively generated source images.
- Image Ascii Voxel Time Series (*.vts): Exports the image intensity of the voxel at the current crosshair position at all latencies.
- Voxel values for each dipole orientation (*.dat): This generates an ASCII file that not only contains the image intensities (i.e. the magnitude of the regional source activity at each grid location), but rather exports the activity of all 3 components of each regional source separately. This results in 3 activity values for each grid location.

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

#### Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

#### What does BESA Research provide?

- ✓ SESAME
- ✓ Cortical LORETA
- ✓ Cortical CLARA
- ✓ Minimum Norm
- ✓ Age-appropriate template head models

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

# Distributed Sources: Bayesian Source Imaging, Surface Methods and Age-Appropriate Template Head Models

This tutorial will explore the different methods available for surface imaging, and also introduce the age-appropriate template models as an alternative to a realistic head model.

#### A. Background

#### Volume imaging using Bayesian methods

Bayesian source imaging uses the idea of sampling the posterior probability distribution using assumptions about prior distributions. BESA Research implements the method **Se**quential **S**emi-**A**nalytic **M**onte-Carlo **E**stimation (SESAME) of sources (Sommariva and Sorrentino 2014, Sorrentino et al. 2014). It allows to automatically estimate simultaneously the number of dipoles, their locations and time courses requiring virtually no user input.

The algorithm is divided in two blocks:

The first block consists of a Monte Carlo sampling algorithm that produces, with an adaptive number of iterations, a set of samples representing the posterior distribution for the number of dipoles and the dipole locations.

The second block estimates the source time courses, given the number of dipoles and the dipole locations.

The Monte Carlo algorithm in the first block works by letting a set of weighted samples evolve with each iteration. At each iteration, the sample (a multi-dipole state) approximates the n-th element of a sequence of distributions  $p_1, ..., p_N$ , that reaches the desired posterior distribution  $(p_N = p(x|y))$ . The sequence is built as  $p_n = p(x).p(y|x) \alpha$  (n), such that  $\alpha$  (1)=0,  $\alpha$  (N) = 1. The actual sequence of values of alpha is determined online. Dipole moments are estimated after the number of dipoles and the dipole locations have been estimated with the Monte Carlo procedure. This continues until a steady state is reached.

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

Prior distribution assumptions are:

- Poisson distribution for the number of sources
- Uniform distribution for the source locations
- Gaussian distribution for the dipole moments

Dipole locations are constrained to pre-defined grid points. Perturbation of the dipole locations exploits a neighbours matrix. The output is a posterior probability for number of dipoles, and a 3D probability map for dipole locations.

#### References:

Sommariva S and Sorrentino A: Sequential Monte Carlo Samplers for Semi-Linear Inverse Problems and Application to Magnetoencephalography. Inverse Problems 30(11):114020, 2014

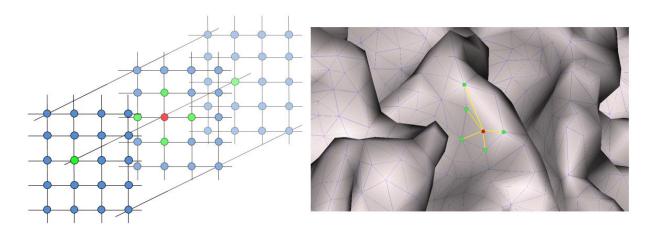
Sorrentino A, Luria G, Aramini R: Bayesian Multi-Dipole Modeling of Single MEG Topographies by Adaptive Sequential Monte-Carlo Samplers. Inverse Problems 30(4):045010, 2014

#### Surface imaging methods

Volume imaging methods typically work on a regular grid that spans the brain, which serves as source space for potentially active sources. There are no particular constraints as to where in this source space a source is allowed to be, or how it should be oriented.

Surface imaging methods use a mesh which reconstructs the brain surface. The source space is then usually constrained to this cortex region. In some cases, orientations are also constrained to be perpendicular to the surface, or allowed to vary in a cone around the perpendicular orientation.

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models



**Left**: In a volume image, neighborhood is defined on a grid. **Right**: On the surface, the neighborhood relationship depends on the mesh.

If spatial weighting terms are used, then the neighborhood comes into play. Considering the formulation of the inverse problem introduced in the precious tutorial on Tutorial 7 – Distributed Sources I: Volume Methods, the spatial weighting matrix V has diagonal terms, and off-diagonal terms. The diagonal terms can be used for e.g. depth weighting. Off-diagonal terms describe the influence of neighboring nodes in the grid or mesh.

The cortical methods discussed here in more detail, Cortical LORETA and Cortical CLARA, use a surface Laplacian operator. The Minimum Norm method uses no neighborhood information – only diagonal weights are applied in the V matrix.

Cortical CLARA is a method which iteratively applies a Cortical LORETA to the data set. After each iteration, the source space is reduced by eliminating the sources with the smallest signal. Also, the result of the current iteration will be used as an additional spatial weighting term for the computation of the next iteration. After several iterations, the solution approaches a focal solution, which, if a large number of iterations are applied, may result in a focus of activity on few individual mesh nodes, which makes it comparable to a solution using discrete sources.

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

#### **B. Source imaging with SESAME**

Let us apply the Bayesian approach to the auditory data example.

 Select the file All_Subjects_cc.fsg from the folder ERP-Auditory-Intensity.

 Select condition Low, left-drag a block and send it to source analysis by right-clicking and pressing Source Analysis. 
 Open...
 ctrl-0

 Close
 Close

 Close All
 *

 Recent Files...
 *

 Load Channel Configuration...
 *

 Head Suffice Points and Sensors
 *

 MRI Corregistration...
 *

 Export...
 *

 Send to MATLAB...
 *

 Import and Convert ASCII File...
 print...

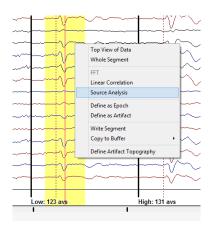
 Print...
 ctrl-P

 Print.
 ctrl-P

 Print.
 ctrl-P

 Ext
 Ext

File Edit View Filters Montage Process ICA ERP Artifact Search Tag



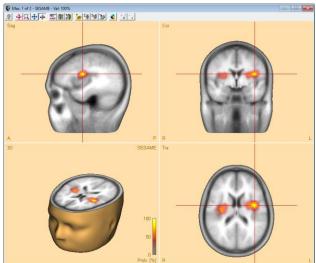
 Select a time range from -100 to 250 ms, and specify a Low Cutoff filter of 0.5 Hz, 12dB/oct, zero-phase and a High Cutoff filter of 40 Hz, 24 db/oct, zero-phase. Press Source Analysis to open the specified data in the source analysis window.

Block Size and Position <u>W</u> hole Segment Custom Definition	Filter Settings	Low Cutoff Slope 12 db/or ▼	Status 🔽 Enabled Type zero phase 💌
Previous Settings -100 (ms) pre-Event 250 (ms) post-Event	Frequency 40.0 [Hz]	High Cutoff Sloge 24 db/oc	Status 🔽 Enabled Type zero phase 💌
	Source Analysis	Set <u>B</u> lock	Cancel

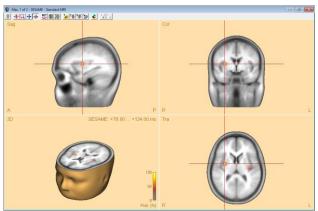
4. Drag a fit interval from 76 ms to 124 ms over the data. Then press the SESAME button in the center of the screen.

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

5. During computation, press the button in the toolbar of the 3D window to follow the live update (you may have to press it twice to enforce the action). Note how the number of maxima indicated in the title bar of the 3D window changes over time.

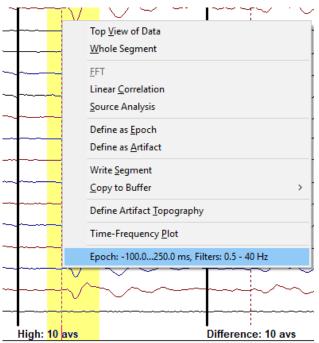


6. Toggle through the maxima using the Maxima button in the toolbar. Note that after the end of computation, there are only two maxima; they appear in lateralized areas, one slightly anterior and medial, the other one very close to where the auditory cortex is expected.

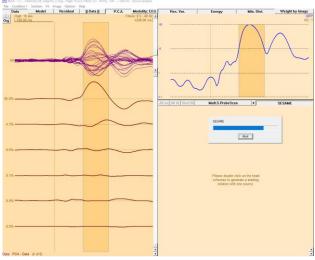


Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

 Minimize the Source Analysis window. Now drag over the condition High, right-click and select the last entry in the popup menu. This will send the High condition to the Source Analysis window.



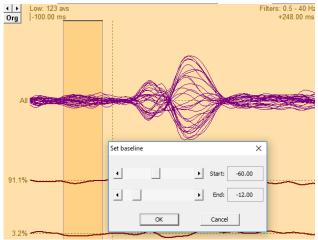
8. In the Source Analysis window, drag a block in the global field power display to mark an interval from 72 ms to 136 ms, which covers the interval where global field power exceeds 10%, and the main activity in the butterfly plot. Then press the SESAME button again.



Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

- 9. The first peak shows the cingulate activity that we modelled in the discrete source analysis earlier. Toggle through the other maxima: The next two represent the auditory activity; the last one is insignificant. Note that this result was obtained without the need for sequential dipole fitting, in a completely automated fashion.
- 10. Open the Image Settings (available from the dropdown arrow in the centre of the screen). When you browse to the SESAME tab, you can see that the noise variance computed over the baseline interval is shown at the bottom. There are two parameters that can be adjusted: Noise Scale Factor, and Source Scale Factor. Close the dialog box.
- 11. We have seen a very good solution obtained with a fully automated approach. For the sake of argument, let us assume that the baseline interval was not well defined, or not defined at all (possible e.g. if you used pattern search for finding peaks of activity in the data, and averaged these). In this case, you can change the baseline interval definition:
- 12. Above the butterfly plot, click on the left arrow at the top left of the window to switch back to the previous condition. Then click on the baseline indicator at the top left





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above the butterfly plot (or use the menu entry **Condition / Baseline interval**) to open the dialog for setting the baseline. Adjust the baseline to **-48 ms** ... **-12 ms** using the sliders. Press OK. Note that the horizontal line showing the baseline has changed.

13. Open the Image settings again and browse to the SESAME tab. Note that the noise variance has changed from 0.12 to 0.08. This reduced noise variance estimate would now potentially result in an image with less regularization, which could lead to more maxima. Similarly, increasing the values for Noise Scale Factor, and Source Scale Factor would have the effect of more regularization. Leave with OK and close Source Analysis.

#### Notes for using SESAME:

The noise variance is a very important parameter for the SESAME method. It should be ensured that the data set contains a large enough baseline interval. You can manually adjust the baseline interval by clicking on the baseline indicator at the top left. This is especially important when pattern search was used to create an average.

Computation time rises non-linearly with the number of channels used. One measure to reduce the computation time is to increase the spacing between grid points, e.g. to 10 mm.

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

#### C. Comparison of different surface imaging methods

We will now compare the different surface imaging methods using the auditory intensity experiment data. When investigating surface data, we often want to use the individual anatomy, or a representation that is as close as possible. We will first look at one subject from our auditory intensity pool.

м

 Open the file S1-av.fsg in the folder ERP-Auditory-Intensity.

 Press CTRL-L on the keyboard and ensure that the additional files we loaded in a previous tutorial will not be used to begin with – we will use these again later. So, please make sure that the rows for Digitized Head Surface Points and Coregistration file are set to "No". Leave with OK.

S	end to MATLAB			
P	nport and Convert ASCII File rint rint Preview			ctrl-P
E	át			
hannel and digitized head surface point information				×
Internal data file information				
Data file: (C) Users (PUblic) Cournents (BESA Research _7_1)Ex. Format: BESA 5.06.0002; Database file: 00000002, fist No. of channels: 32; 33 electrodes; 1 polygraphic Information should be complete for this data file. No auxiliary If a *,ela file defines EEG, you must use 10-5/10-10/10-20 lab	files required.			~
Suggestions Please enter electrode thickness.				
Yes No File name found				
Channel configuration (*.el?)	no file	Good	Browse	Edit
Digitized head surface points (*.sfp, *.eps) and labels (*.sfn)				
C C:\Users\Public\Documents\BESA\Researd	3 points in data file	Good	Browse	Edit
с с	no label file	Good	Browse	Edit
Eletrode labels non-conforming to 10+10 or 10-5 s	tandard	Electrode thi	ckness (mm):	0.00
Coregistration file (*.sfh) or head center (*.cot)	File will be ignored!	Bad	Browse	Edit/Coreg.
MEG sensors (*.pos, *.pmg)	no file	Good	Browse	Edit
Artifact coefficients (*.atf, *.art)	no file	Good	Browse	Edit
Clear DB Clear Events		Help	QK	Çancel

File Edit View Filters Montage Process ICA ERP Artifact

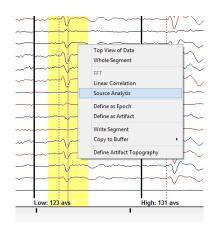
Open

Close

Close All Recent Files... Load Channel Configuration... Head Surface Points and Sensors MRI Coregistration... Export... Ta

ctrl-O

 Select condition Low, left-drag a block and send it to source analysis by right-clicking and pressing Source Analysis.



Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

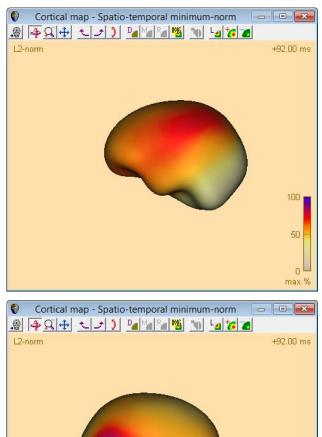
- 4. Select a time range from -50 to 250 ms, and specify a Low Cutoff filter of 0.5 Hz, 12dB/oct, zero-phase and a High Cutoff filter of 40 Hz, 24 db/oct, zero-phase. Press Source Analysis to open the specified data in the source analysis window.
- 5. Ensure that the head model is set to "4shell ellipsoidal". Then use the arrow button in the centre of the Source Analysis window to select Minimum Norm from the popup menu.

Block Size and Position	−Filter Settings Low Cutoff Status 🔽 Enable	ed
○ Whole Segment	Erequency 0.5 [Hz] Slope 12 db/oct - Type zero phase	<b>-</b>
Custom Definition		_
✓ Previous Settings	High Cutoff Status V Enable	ed
-50 (ms) pre-E⊻ent	Frequency 40.0 [Hz] Sloge 24 db/oct  Type zero phase	•
250 (ms) post-Event		
All Conditions	Seurce Analysis Set Block Cancel	

	Beamformer	В
	DICS	
	CLARA	
	LAURA	
	LORETA	
	sLORETA	
	swLORETA	
	sSLOFO	
	User-defined Image	
	Cortical LORETA	Ctrl+L
	Cortical CLARA	
~	Minimum-Norm (Surface)	Ctrl+M
	Probe Scan	S
	Sensitivity	V
	Settings	

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

6. A cursor is automatically set at the peak of the global field power at 92 ms. A minimum norm estimate is computed with the default settings and is displayed on the standardized smoothed surface reconstructed from an average of 50 brains. The result is a broadly smeared distribution in the left hemisphere. Use the toolbar buttons to rotate the brain. A more focal maximum is visible in the right hemisphere, posterior to the Sylvian fissure where we would have expected the bulk of activity.



 Use the arrow in the centre of the screen again to select Cortical LORETA, or select the entry from the menu Image and the sub-menu Surface Image.

	Co	ndition 1 Solution Fit	Image	Options Help	
1		Volume Image	+	der P.C.A. EEG	Res. Va
2		Surface Image	•	Cortical LORETA	Ctrl+L
1		Discrete Model Probing	•	Cortical CLARA	
		Smooth Image		Settings	
	Hide Image			Minimum-Norm (Surface) C	trl+M
	Import Image			Settings	
		Export Image As			
		Settings			~

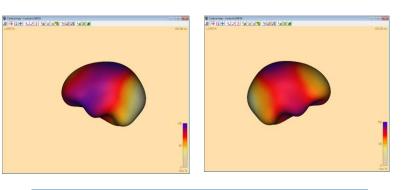
100

50

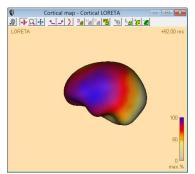
max.

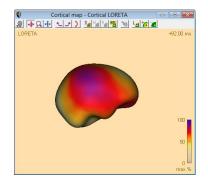
Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

- The display changes and now shows more activity in lower brain regions, but still a maximum in a central location.
- 9. We will slightly adapt regularization to get a better separation: Click the down-arrow I in the centre of the screen to open the popup menu, and select the bottom menu entry Settings. In the dialog, change the regularization to 0.004%. Then press OK.
- 10. The display changes to show two maxima which are now more frontal, closer to the Sylvian fissure.



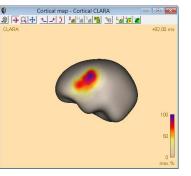
Cortical Imaging	?	$\times$
Surface Minimum Norm Cortical Image		
Specify settings for LORETA	EG C MEG	

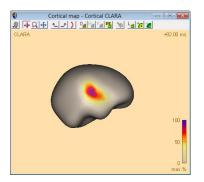




Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

 Select Cortical CLARA from the popup menu or from the menu Image / Surface Image.





The display now shows two clear maxima, one for each hemisphere, which are much more focal than with the previous methods. In the right hemisphere, the location looks approximately correct; however, in the left hemisphere the maximum is too anterior.

We now want to use the individual anatomy of this subject for the estimation of activity on the cortex. We follow the same steps as earlier in the Tutorial on **Coregistration and FEM Modeling, part B**.

Close the Source Analysis module. Press
 CTRL-L and in the dialog box that appears, in the row for Digitized Head Surface
 Points, press Browse. Then select the
 Standard Electrode folder in the File
 Open box, choose BESA-MRI-Standard Electrodes.sfp, and open it.

		Load Surface Points						
Look in:	🔒 StandardElect	rodes 💌	⇔ 🗈 💣 📰▼					
(Ha	Name	<u>^</u>	Date modified	Туре				
Recent places	BESA-MR	I-Standard-33-Electrodes.sfp	14/12/2010 18:40	SFP File				
Necerii piaces	🗹 📝 BESA-MR	l-Standard-Electrodes.sfp	09/04/2014 13:59	SFP File				
Desktop	📔 BESA-Std	_20-24yrs.sfp	05/08/2015 11:47	SFP File				
Libraries								
This PC								
Network	<			>				
	File <u>n</u> ame:	BESA-MRI-Standard-Electrodes.sfp	•	Open				
	Files of type:	Surface points (*.sfp)	-	Cancel				
	Directories:	Standard electrode folder	-					
		Channel directory		///				

- Close the Channel and digitized head surface point information dialog with OK and select File / MRI Coregistration.
- **14.** If you had previously coregistered the file, you will be taken again to the dialog you just

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

closed. Now you simply have to check the box for using the already existing coregistration file. Leave with OK. In case you are asked to write a surface point file, press Cancel. Select File / MRI Coregistration again. All information should be there. Leave with OK.

If there is no previous coregistration, then you will be prompted to save an *.sfh file. BESA MRI will start. In BESA MRI, click the button Start New Coregistration.

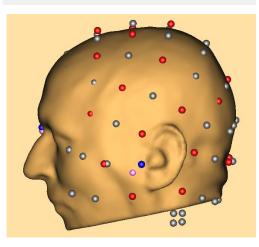
In **Steps for coregistration**, do not check anything.

Press **Next**. Select the input project that we used in the tutorial on **Coregistration and FEM Modeling**. Press **Next**.

Press Select Coregistration File and open the file that you just saved from BESA Research. Then simply press Next and Finish to complete the Coregistration. Save the project and return to BESA Research. If you click into one of the fields in the dialog box, a green tick mark should appear to indicate that coregistration was successful. Leave the MRI Coregistration dialog with OK.

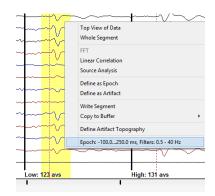


Generate FEM MEG leadfield



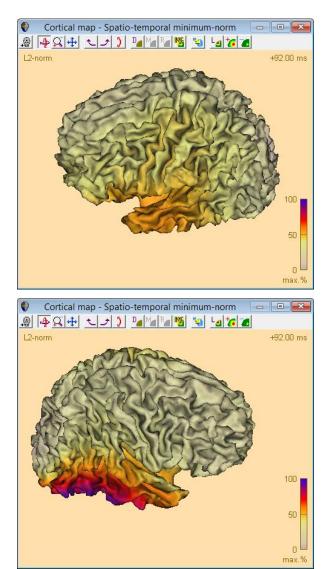
Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

 Left-drag across condition Low again to mark a block, right-click and select the previous entry from the popup menu to start Source Analysis with the previous parameters.



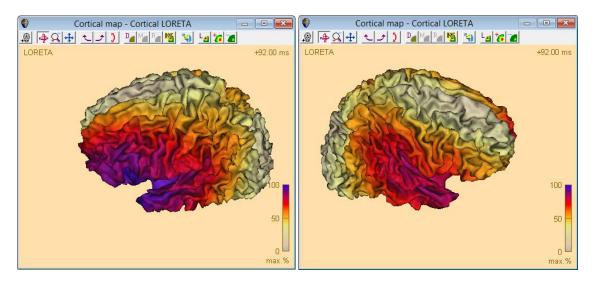
 Now press the arrow button in the centre of the window again and start Minimum Norm.

It now shows the individual anatomy, and the activity is estimated lower than before. The Minimum Norm solution places the main activity in the temporal lobe.

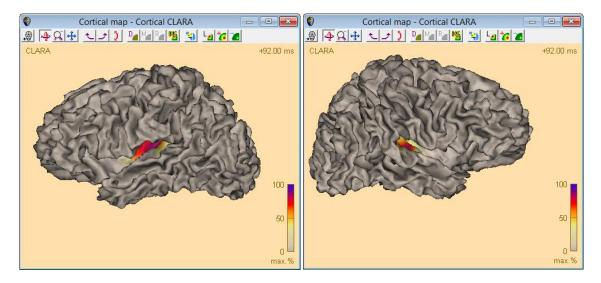


Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

**17.** Use the **arrow button** again and select **Cortical LORETA**. The activity now moves closer to the Auditory Cortex.

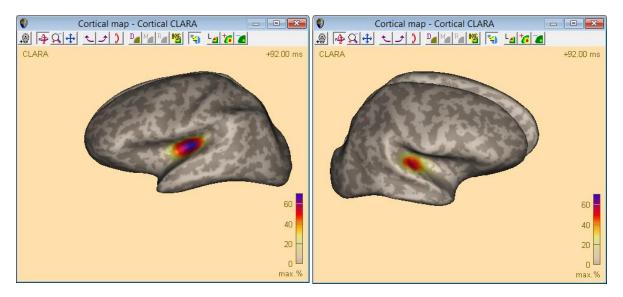


Use the arrow button again and select Cortical CLARA. The activity now shows in the area of the Heschl's gyrus. It is actually not easy to detect – you may need to rotate and zoom the display.



Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

**19.** Press the inflation icon icon on the toolbar. On the inflated brain, it is clearly visible that the reconstructed activity is almost exactly on Heschl's gyrus. You may want to scale up once to see the focal spot more clearly.



#### D. Settings for surface imaging methods

So far, we have worked with the default settings for the Cortical Imaging methods. We will now explore some of the settings that can be changed, and see the effect on the source images.

 Select the menu entry Image / Surface Image and then the first Settings... entry.

				BESA - C:\RI-Visual-Motio	n\l
<u>F</u> ile	Co	ndition 1 Solution Fit	<u>I</u> mage	<u>Options</u> <u>H</u> elp	
D		Volume Image	+	P.C.A. MEG Res. Var.	
1		Surface Image	×	Cortical LORETA Ctrl+L	
		Discrete Model Probing	•	Cortical CLARA	
		Smooth Image		Settings	
T		Hide Image		Minimum-Norm (Surface) Ctrl+M	
T.		Import Image		Settings	
		Export Image As			
		Settings		λ.	$\sim$
	_				

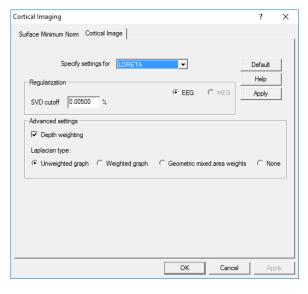
Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

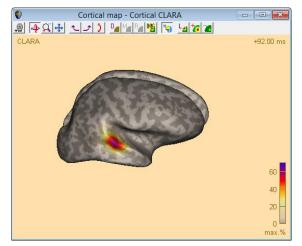
- Note that the dialog that appears contains two tabs – one for Minimum Norm, and the other one for Cortical Images. In the Cortical Image part, LORETA is selected by default. You can adjust the following parameters:
  - SVD cutoff
  - Depth weighting on or off
  - Type of Laplacian

Note that SVD cutoff can be adjusted separately for EEG and MEG.

Since Cortical CLARA is an iterative application of the Cortical LORETA solution, these parameters for Cortical LORETA also affect the Cortical CLARA settings.

 Un-tick the Depth Weighting. Press the OK button. The dialog box closes and Cortical CLARA is re-computed. Note that the activity patch has moved.





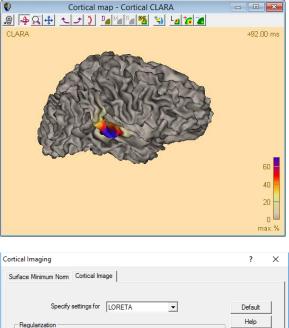
Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

 Press the inflation toolbar button to show the cortex again. It is evident that the activity has moved towards more superficial locations on the cortex.

 Use the arrow button in the center of the window to select the last entry Settings. It will automatically open the settings for Cortical Imaging again since the last selected image was a cortical map.

Now change the **Laplacian** from **Unweighted graph** to **Weighted graph**. Press **OK**.

 6. This Laplacian applies weights to the Laplacian operator terms depending on the distance to the respective neighbor. The result here is that the activity moves to another gyrus in the left hemisphere.



C Unweighted graph . • Weighted graph C Geometric mixed area weights

SVD cutoff 0.00500 %

Depth weighting
 Laplacian type:

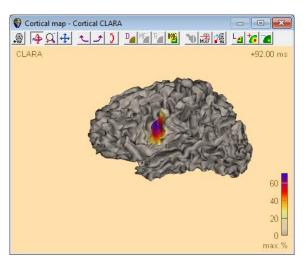
• EEG C MEG

ОК

Cancel

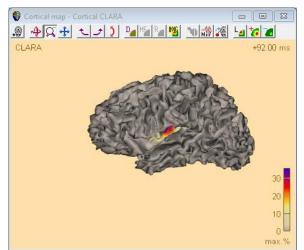
Apply

C None



Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

 Change the settings again to re-activate depth weighting. Activity moves back to the Heschl's gyrus.



 Now select the third option for the Laplacian: Geometric mixed area weights. This changes the result again slightly.

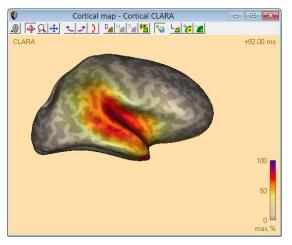
Open the **Settings** once more, and now select **CLARA** from the dropdown list. Three new sets of settings appear.

Change the Laplacian type back to Unweighted graph, and change the number of iterations to 2. Leave the other settings unchanged. Press OK.

Cortical Imaging	?	Х
Surface Minimum Norm Cortical Image		
Specify settings for CLARA  Regularization  C EEG C MEG  SVD cutoff 0.00500 %	Default Help Apply	
Advanced settings ↓ Depth weighting Laplacian type: ← Unweighted graph ← Weighted graph ← Geometric mixed area weig	hts C Nor	ne
CLARA  No of iterations  Automatic  SVD cutoff  0.0100  %  10.0  Amount to cli	ip from img (%)	
OK Cancel	App	ply

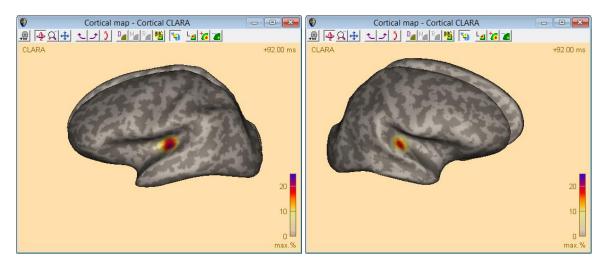
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9. Change scaling back to 100 and press the inflation button on the toolbar again. It is evident that the solution now resembles the Cortical Loreta solution more closely.



 Now try a larger number of Cortical CLARA iterations: Open the Settings again and now push the lever for No of iterations to the maximum value of 25. Press OK.

The maximum is now smaller in amplitude, and you will need to scale the image up a few times to see it clearly. A very focal maximum is visible on each of the two hemispheres, in a location which closely approximates that of Heschl's gyrus.



11. Close the Source Analysis module.

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

#### E. Head models

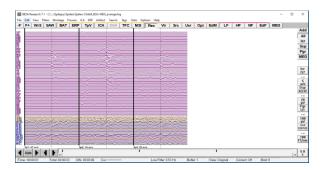
BESA Research offers several age-appropriate template head models, which were kindly provided by Dr. John E. Richards, University of South Carolina, USA⁹. We will look into the effect of applying an appropriate head model for fitting dipole sources to an averaged epileptic spike.

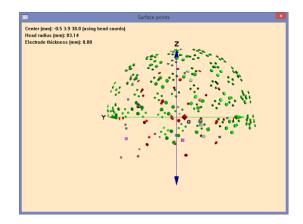
 Select File / Open and browse to the folder Examples/Epilepsy/Spikes.

> Open the file Spikes-Child4_EEG+MEG_average.fsg.

The file is a combined EEG-MEG recording, where occurrences of three different types of spikes in a 12-year old child were averaged.

 Press Shift-V on the keyboard to view the sensor configuration. The red circles denote the electrodes. Coordinates were digitized. Grey donuts denote coils which were placed on the head to coregister the electrode cloud with the MEG sensor positions.





- Richards, J.E., & Xie, W. (2015). Brains for all the ages: Structural neurodevelopment in infants and children from a life-span perspective. In J. Bensen (Ed.), Advances in Child Development and Behavior (Vol 48, Chapter 1, pps 1-52)
- Richards, J.E., Sanchez, C., Phillips-Meek, M., & Xie, W. (2015). A database of age-appropriate average MRI templates. Neuroimage, doi:10.1016/j.neuroimage.2015.04.055.

⁹ If you would like to publish results obtained with the use of the age-specific models, please reference the publications below:

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

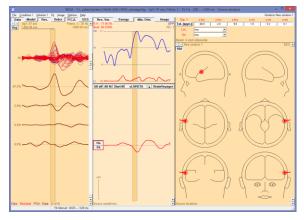
- Press the Scp button on the right to show only EEG channels. You can see activity on most channels. Drag over the first segment which has the most averages of spike events. Rightclick and select Source Analysis.
- 4. Define the interval and filters as follows: -200 ms .. 200 ms, 3.0 Hz forward with 6dB slope, and 35 Hz zero phase with 12 dB. The reason for using a forward filter for the high pass is that in epilepsy, the initial (crucial) activity is often smaller than the later activity, and can be compromised by signal from later components bleeding back into the early components if a zero phase shift filter is used.

Press the Source Analysis button.

5. Check that the selected model is the 4-shell ellipsoidal model. Doubleclick into the head schemes to create a dipole. Click the P.C.A. button and mark a fit interval over the onset period from -30 ms to -5 ms, such that the first PCA component explains more than 97% of the overall variance in that interval.

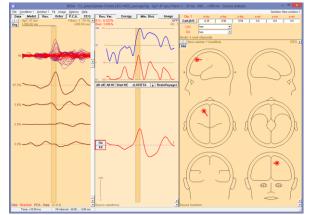
File	Edit	View	Filters	Monta	ge Pro	ocess I	CA ERP	Artifact	Search	Tags	Goto C	
-F	F+	WrS	SA	WE	BAT	ERP	TpV	ICA	DSA	TFC	ESI	
Fp1'-F Fp2'-F F7'-Pz	'z'	~~~~	~~				ew of Data Segment	3	i		Ę	
F3'-Pz Fz'-Pz F4'-Pz			~	$ \rightarrow $			Correlatio Analysis	'n			f	
F8'-P2 T7'-P2	e e	~~~~	$\sim$			Define	as Epoch	•			-	
C3'-P2 Cz'-P2 C4'-P2	e e		~	$\rightarrow$		Write S	egment o Buffer				 	
T8'-P7 P7'-P7 P3'-P7	r i	~~~~						opography	,		-	
P4'-P					_	Time-	requency	Plot			-	

Block Size and Position C Whole Segment C Custom Definition	Filter Settings Low Cutoff Status ♥ Enabled Frequency 3.0 [Hz] Slope 6 db/oc ▼ Type forward ▼	
✓ Previous Settings     ·200 (ms) pre-Event     200 (ms) post-Event	High Cutoff     Status ▼ Enabled       Frequency 35.0     [Hz]     Slope     12 db/oc ▼     Type [zero phase ▼	
All Conditions	Source Analysis Set Block Cancel	

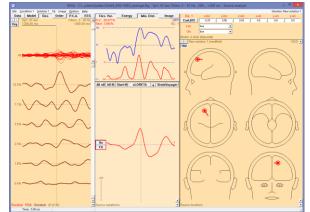


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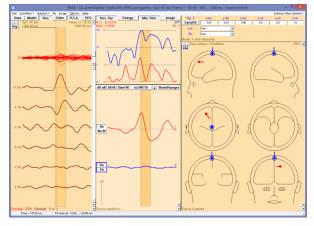
6. Start the fit. The dipole fits to a left frontal location.



 Untick the Data button and click outside the fit interval to remove the fit interval. The residual variance PCA plot is shown on the left. The largest component holds 12.5%.



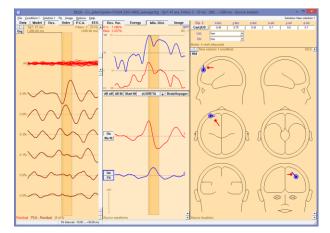
 Double-click into the head schemes again to set a second dipole, and mark a fit interval over the PCA component from -10 ms to +50 ms.

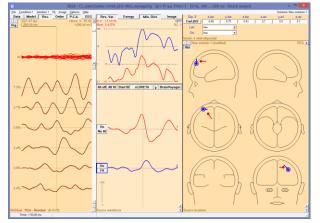


Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

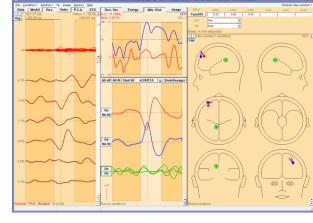
Press the Start fit button. The dipole localizes to a peripheral location.

 Unmark the fit interval again. The largest unexplained component now is a rhythmic one. We will try and explain this component by a regional source.



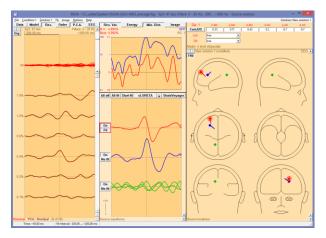


11. Double-click in the head schemes and press C on the keyboard to convert the source to a regional source. Mark a fit interval from -200 ms to -40 ms, where the two sources are not yet active. Press the CTRL key and drag from +80 ms until the end. The fit interval is now split into two. Fit the regional source. It moves to a posterior location.



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12. Note that we fitted the first two sources not accounting for the rhythmic activity that is present also during the time when the two dipoles are active. Mark the complete interval and press the buttons All fit and Start fit. Then invert the first source by selecting its waveform and pressing the I key on the keyboard. We now have a solution with clearly separated waveforms and a small residual variance.

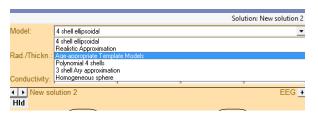


This solution was achieved using the standard 4-shell head model. However, a 12-year old child has vastly different conductivities from an adult. We will now take this into account for an alternative model.

 Save the current model as Spikes-Child4_EEG+MEG_average-4shell.bsa.

> Click into the box at the top right to display the model, and use the **dropdown list** to select Age**appropriate Template Models**. When the warning concerning the source location appears, click **OK**.

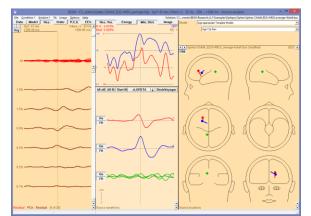
 A new dropdown list appears below the first one. Select Age 12y from this list.

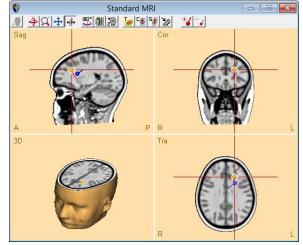


Model: Ag	e-appropriate Template Models
	Age 20 to 24y Omo 💌
	Age 20 to 24y Omo
	Age 18y Omo Age 16y Omo
	Age 14y Omo Age 12y Omo
▲ New solut	Age 10y Omo
HId	Age 8y Omo - Age 6y Omo
	Age 4y Omo
1/	Age 2y Omo Age 0y 18mo

Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

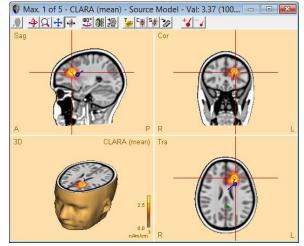
- 15. Now press the buttons All fit and Start fit again. The two sources move deeper into the brain volume. Press CTRL-Z and CTRL-Y to compare the situation before and after the fit. The source locations differ between 2 and 3 cm.
- 16. Press the A key on the keyboard to show the sources in the anatomy.
  Use the button on the toolbar to show the different views. Select the first source to slice at that position.





17. Now mark the interval where the first source becomes active, from -40 ms to -10 ms. Start CLARA to compute the average CLARA image during that interval.

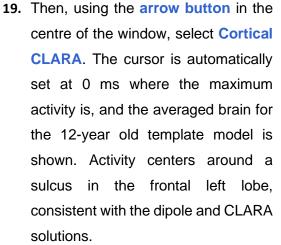
CLARA coincides very well with the location of the first dipole source.



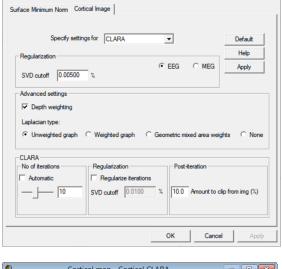
Tutorial 8 – Distributed Sources II: Bayesian Source Imaging, Surface Methods and Template Models

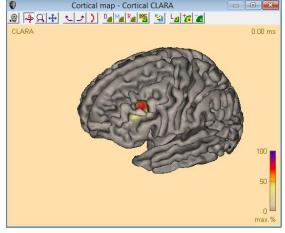
Cortical Imaging

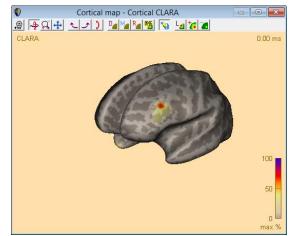
 We will now try Cortical CLARA for this example as well. Select the menu entry Image / Surface Image / Settings. In the dialog box for Cortical Imaging, select CLARA and re-set all settings to Default using the button. Close the dialog box with OK.



20. Press the inflation button on the toolbar to check for other maxima. There is a small activity visible at the tip of the right temporal lobe, but it is much smaller in amplitude.





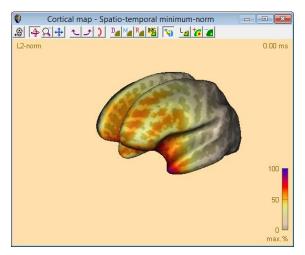


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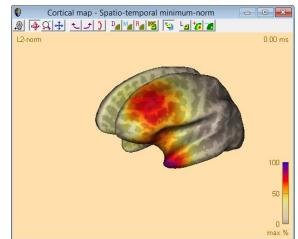
21. For comparison, select Minimum-Norm (Surface) button from the arrow drop list. It shows a widespread activity, with a maximum on the of the left temporal lobe.

> Note that this type of maximum can be an indicator of noise modelling. We will try and change the noise estimator for the minimum norm.

- 22. Select Settings from the arrow drop list. The dialog box for Minimum Norm settings comes up. The current noise estimator is set to Use baseline. However, this is not useful when dealing with epileptic spike averages, as there is no proper prestimulus baseline. Select Use 15% lowest values instead and change the noise scale factor to 0.5 to reduce regularization. Press OK.
- 23. Minimum Norm is recomputed, and the result shows an improvement. However, there is still a larger spread of activity compared to the Cortical CLARA method.



Cortical Imaging					
Surface Minimum Nom Cortical Image					
Weighting           Image: Depth weighting           Default           Image: Default					
<ul> <li>Subspace correlation after single source scan [p²] Dimension: 6 → </li> <li>C Dale &amp; Sereno 1993 Estimate dimension from data </li> </ul>					
Noise					
Estimation: Mean [ µV ] : noise signal					
C Use baseline 2.42 3.99	1				
← Use 15% lowest values     0.97     3.31 <u>Help</u>					
Weighting:					
Individual channels Noise scale factor: 0.50     Default					
Selected mean poise and SNB: 0.48 6.84					
Selected filed intoise and SMIT.					
Estimate mean noise from data 🗹					
OK Cancel Apply					

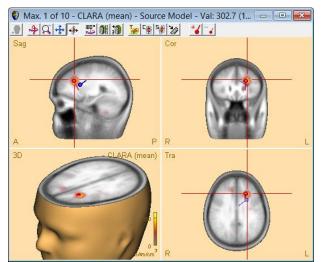


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24. Finally, we want to have a look at the MEG modality. Press the button EEG to toggle the measurement data and switch to MEG. Then mark an interval from -30 ms to -5 ms again as before and switch on the Data button to review the P.C.A. We can see that the variance explained by the first component is reduced to 88.6%.

Run **CLARA** over this interval. The result is almost the same as for EEG using the age-appropriate template model.



We have seen the influence of the head model onto the solution. In a different research project, the dataset used here was investigated with the patient's MRI and using a realistic head model, and it was shown that indeed the age-appropriate head model template resulted in the closest match to the location found with a realistic FEM, and to the anatomical lesion seen in the MRI (Lanfer et al., 2015)¹⁰.

#### F. Batch processing and export of cortical results

#### **Batch processing**

The methods for Cortical Imaging can also be used in batch processing. For these methods, the following commands are available in the list of batch commands:

¹⁰ Lanfer B, Spangler R, Richards JE, and Paul-Jordanov IP (2015) Age-specific Template Head Models for EEG Source Analysis. OHBM 2015. <u>https://www.besa.de/wp-</u> <u>content/uploads/2015/07/Lanfer_OHBM2015_Poster.pdf</u>

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- CorticalClara
- CorticalLoreta
- MinimumNorm

The parameters can be entered after selecting one of the commands.

	Selec	t Command	
General commands:	Main Program:	Source Analysis:	Source Analysis Imaging:
BatchError	ArtifactAutoCorrect	AddSource	Beamformer
Comment	ArtifactMethod	ChannelTypeForFit	CLARA
EndFor	ArtifactOn/off	ConvertSource	Clip
For	ArtifactScan	CorticalClara	DICS
MATLABcommand	AuxiliaryFiles	CorticalLoreta	Export
MATLABwaitForVariable	Average	Delete	GotoMax
Pause	Baseline	DisplayMRI	Import
RunProcess	EditDefaultEpoch	Exit	LAURA
WindowPosition	EventRead	Fit	LORETA
	EventWrite	FitConstraint	SetCrosshair
	Export	FitInterval	sLoreta
	FFT	MinimumNorm	Smooth
Apply to all files	FFTmean	NewSolution	SSLOFO
() (pp)) to an mes	FFTsave	OpenSolution	UserDefined
	FileOpen	PCA	
Apply at beginning of batch	Filter	Regularization	
	GoTo	SaveBitmap	
Apply at end of batch	ICA	SaveLeadfields	
	ICAsave	SaveModelWaveforms	
	ICAselect	SaveResidualWaveforms	
Current Selection	ImportASCII	SaveRVandGFPWaveform	Time-Frequency Analysis:
Current selection	MarkBlock	SaveSolution	
SACorticalClara	Montage	SaveSourceMontage	Display
SACUITICAICIALA	Paradigm	SaveSourceWaveforms	Image
	PatternToTrigger	SendToMATLAB	Save
	SendToMATLAB	SetCursor	SendToMATLAB
	TriggerDelete	SetDefaultSourceType	StartTFAnalysis
	TriggerSelect	SetOrActivateSource	
OK Cancel		SetOrientation V	

#### Export of results

Cortical images can be exported as ASCII files. There is a choice between current latency, or all latencies.

Each mesh node location is provided with x, y, and z coordinates, followed by the value for either the current latency, or for all latencies. In the case of all latencies, BESA Research calculates the values for each latency of the current condition during the export process.

Tutorial 9 – Beamforming and virtual sensor montages

#### **Tutorial 9 – Beamforming and virtual sensor montages**

#### What does BESA Research provide?

- ✓ Time-domain beamforming tools
- ✓ Several beamformer types
- ✓ Graphical user interfaces for using the tools
- ✓ Virtual sensor montages

#### Tutorial 9 – Beamforming and virtual sensor montages

#### A. Principles of beamforming

Beamforming (Van Veen et al., 1997) is a technique for the reconstruction of source activity that differs from other discrete or distributed modelling approaches:

- A spatial filter is used like in the other approaches, but for each brain voxel, a different spatial filter is computed.
- The measured data are directly taken into account for computing the beamformer result.
- Activity is contrasted against either a reference interval, or a reference condition.

Several methods for computing beamformers exist. We will explain them and their (sometimes subtle) differences, and work with them in the tutorial.

Beamformer ingredients are:

- Data covariance matrix
- Leadfield matrix derived from the head model and sensor configuration
- Spatial filters computed from the leadfield matrix and data covariance matrix

Let us start by looking at the "standard" beamformer (single-source beamformer):

If  $C_r^{-1}$  is the inverse of the regularized data covariance matrix C in the time range of interest; L(**r**) is the leadfield matrix of the model containing a regional source at target location **r** and, optionally, additional sources, whose interference with the target source is to be minimized; tr[ ] is the trace of the [3×3] (MEG: [2×2]) submatrix of the bracketed expression that corresponds to the source at location **r**; then P(**r**) = tr[L^T(**r**)C_r^{-1}L(**r**)]^{-1} is the power at location **r**.

Beamformers can suppress noise sources that are correlated across sensors. However, uncorrelated noise will be amplified in a spatially non-uniform manner, with increasing distortion with increasing distance from the sensors (Van Veen et al., 1997). For this reason, estimated source power should be normalized by a noise power. In BESA Research, the output power P(r) is normalized by the output power in a reference interval  $P_{ref}(r)$ , defining a value

q(r):

Tutorial 9 – Beamforming and virtual sensor montages

$$q(\mathbf{r}) = \begin{cases} \sqrt{\frac{P(\mathbf{r})}{P_{ref}(\mathbf{r})}} - 1 = \sqrt{\frac{tr[L^{T}(\mathbf{r}) \cdot C_{r}^{-1} \cdot L(\mathbf{r})]^{-1}}{tr[L^{T}(\mathbf{r}) \cdot C_{ref,r}^{-1} \cdot L(\mathbf{r})]^{-1}}} - 1 & \text{for } P(\mathbf{r}) \ge P_{ref}(\mathbf{r}) \\ 1 - \sqrt{\frac{P_{ref}(\mathbf{r})}{P(\mathbf{r})}} = 1 - \sqrt{\frac{tr[L^{T}(\mathbf{r}) \cdot C_{ref,r}^{-1} \cdot L(\mathbf{r})]^{-1}}{tr[L^{T}(\mathbf{r}) \cdot C_{r}^{-1} \cdot L(\mathbf{r})]^{-1}}} & \text{for } P(\mathbf{r}) < P_{ref}(\mathbf{r}) \end{cases}$$

#### Multiple source beamformer vs single source beamformer

Traditional single-source beamformers are known to mislocalize activity if several brain regions have highly correlated activity (Van Veen et al., 1997). The BESA multiple source beamformer tries to overcome this problem by extending the traditional single-source beamformer. The multiple-source beamformer can implicitly account for activity from possibly correlated brain regions (This is also called suppression beamformer (Dalal et al., 2006) or nulling beamformer (Hui et al., 2010)). This is achieved by using a multiple-source beamformer calculation that contains not only the leadfields of the source at the location of interest  $\mathbf{r}$ , but also those of possibly interfering sources.

BESA Research uses a bilateral beamformer, where specifically contributions from the homologue source in the opposite hemisphere are taken into account (the leadfield matrix  $L(\mathbf{r})$  thus being of dimension  $N \times 6$  for EEG and  $N \times 4$  for MEG, respectively, where N is the number of sensors). This allows for imaging of highly correlated bilateral activity in the two hemispheres that commonly occurs during processing of external stimuli.

#### Vector beamformer vs scalar beamformer

A beamformer source at position  $\mathbf{r}$  can have 3 orientations for EEG or 2 orientations for MEG. The beamformer can either optimize the orientation, "scalar beamformer" (Robinson and Vrba, 1998; Sekihara et al., 2004), or compute the mean amplitude over all orientations, "vector beamformer" (Van Veen et al., 1997).

### Tutorial 9 – Beamforming and virtual sensor montages

#### References

Dalal, S. S., Sekihara, K., & Nagarajan, S. S. (2006). Modified Beamformers for Coherent Source Region Suppression. IEEE Transactions on Biomedical Engineering, 53(7), 1357–1363.

Hui, H. B., Pantazis, D., Bressler, S. L., & Leahy, R. M. (2010). Identifying true cortical interactions in MEG using the nulling beamformer. NeuroImage, 49(4), 3161–3174.

Robinson, S. E., & Vrba, J. (1998). Functional neuroimaging by synthetic aperture magnetometry (SAM). In: Yoshimoto T, Kotani M, Kuriki S, Karibe H,Nakasato N, editors. Recent Advances in Biomagnetism. Sendai: Tohoku University Press, 302–305.

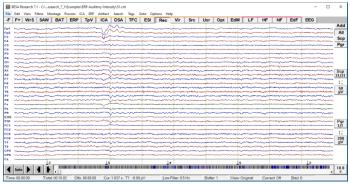
Sekihara, K., Nagarajan, S. S., Poeppel, D., & Marantz, A. (2004). Asymptotic SNR of scalar and vector minimum-variance beamformers for neuromagnetic source reconstruction. IEEE Transactions on Biomedical Engineering, 51(10), 1726–1734.

Van Veen, B. D., Van Drongelen, W., Yuchtman, M., & Suzuki, A. (1997). Localization of brain electrical activity via linearly constrained minimum variance spatial filtering. IEEE Transactions on Biomedical Engineering, 44(9), 867–880.

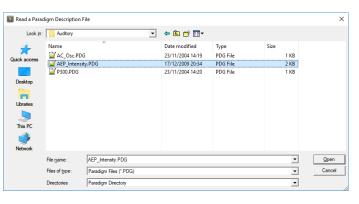
Tutorial 9 – Beamforming and virtual sensor montages

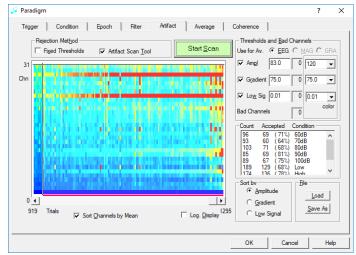
#### **B.** Time-domain beamforming in EEG data

 Open data set s1.cnt from the folder /Examples/ERP-Auditory-Intensity/. It contains 32 EEG channels and one polygraphic channel.



- Click the ERP pushbutton. If the paradigm file for the data file was not loaded before, a dialog box opens, and you can browse to the sub-folder Auditory and open file AEP-Intensity.pdg. 8 conditions are visible. Switch to the Artifact tab and start the artifact scan.
- **3.** Move the vertical slider to the left until all eye blinks are excluded.

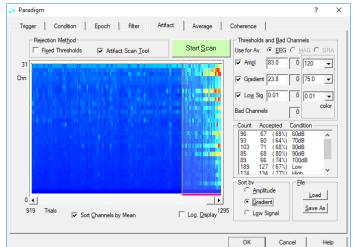




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#### Tutorial 9 – Beamforming and virtual sensor montages

 Click the Gradient radio button and exclude some muscular activity by moving the slider to the left.



 Move to the Average tab. In the list of conditions, click once on the Low condition to select it.

aradigm				? >
Trigger	Condition	Epoch Filter	Artifact Average Coher	rence
С <u>С</u> ит С <u>В</u> еtv СТој	ole EEG/MEG ent Segment ween Markers End of File End of Segment	Condition 60dB 70dB 80dB 90dB 100dB Low High All	Constraint Al Matches Even Matches Odd Matches First Haf Second Haif De statement Agd to List	Statistics Calc Noise Covariance Beanformer Automation Query
Current S	Ne <u>x</u> t Marker electio <u>n</u> unt Accepted	Condition	<u>R</u> emove From List	FF <u>T</u> Average
96 93 104 85 89	67 ( 69%) 60 ( 64%) 71 ( 68%) 68 ( 80%) 66 ( 74%)	× 60dB × 70dB × 80dB × 90dB × 100dB	A A A A A	Average
189 174 467	127 ( 67%) 134 ( 77%) 332 ( 71%)	× Low × High × All	A A A	Load Save As

#### Tutorial 9 – Beamforming and virtual sensor montages

 Click the Beamformer button. An additional dialog box appears for choosing the beamformer intervals for condition Low.

- Click Butterfly plot. The butterfly plot shows the average signal for all channels, and the currently defined intervals. Three intervals can be defined:
  - Baseline
  - Signal
  - Common

Set	beamf	ormer interv	/als				×
	Targe	t condition:	Low				
	Beam	former interv	al —				
		Baseline		Signal		Common	
	Start	-100	ms	0	ms	-300	ms
	End	-70	ms	30	ms	1000	ms
						mage, the nu	
	Therefo		mended			should be eq tion to the Ba	
	Butter	fly plot			ОК	Can	cel

Set	beamfo	ormer inter	/als				×
	Targe	t condition:	Low				
	Beamf	former interv	al				
		Baseline		Signal		Common	
	Start	-100	ms	0	ms	-300	ms
	End	-70	ms	30	ms	1000	ms
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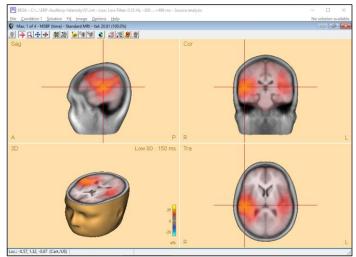
### Tutorial 9 – Beamforming and virtual sensor montages

 Using the Edit boxes, set signal to [80...150] to cover the main activity of the N100. Set baseline to [-120...-50] (matching duration). Common can be set to [-120...150] to cover the complete duration.

Set beamf	ormer interv	vals				×
Targe	t condition:	Low				
Beam	former interv Baseline	al —	Signal		Common	
Start	-120	ms	80	ms	-120	ms
End	-50	ms	150	ms	150	ms
						***
of samp Therefo	les in the Ba	seline an mended	reliable beam Id the Signal ii to assign equ	ntervals	should be eq	ual.
Butter	fly plot			ОК	Can	cel

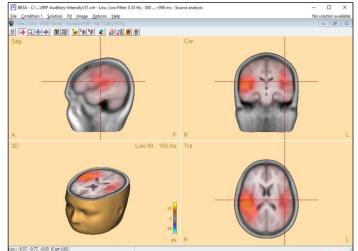
 Click OK to start the beamformer computation. The Source Analysis module opens. Scale the beamformer result up several times to visualize the activation pattern using the scaling

toolbar buttons

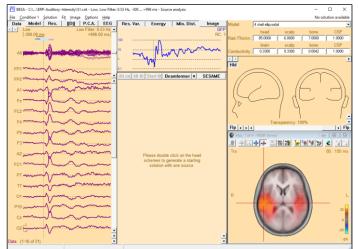


#### Tutorial 9 – Beamforming and virtual sensor montages

10. Click the Maxima toolbar button to switch to the second maximum.Both first and second maximum localize close to auditory areas.



11. Click the Maxima button two more times. The other two maxima are much weaker. Click again to return to the first maximum. Then minimize the 3D window using the button at the top right, to visualize the data and global field power. You can see the beamformer intervals plotted above the butterfly plot on the left.

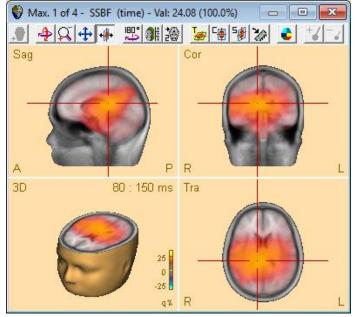


#### Tutorial 9 – Beamforming and virtual sensor montages

- 12. Click the arrow in the center of the display to open a popup menu. Then select Settings. The dialog box for defining beamformer settings opens. Alternatively, you can use the menu and select Image / Settings.
- The dialog contains settings for regularization, and the beamformer options. We can see that by default, the Multipole-source beamformer is selected, and a Vector type is used.

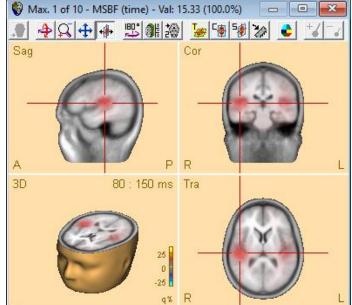
nage Settings ?	×
General Standard Volume User-Defined Volume SESAME	
Image weighting	
Specify settings for:  Beamformer  Apply	
- Regularization Beamformer (Time-domain)	
C No covariance regularization	
User-defined (Tikhonov) [%]:     5	
C SVD cutoff (Data [SVD abs.]): -1	
C SVD cutoff (Data [SVD %]): 0.0001	
Beamformer Options	
Beamformer type: Compute beamformer image:	
Multiple-source beamformer     Use only weights of the signal interval     Single-source beamformer     Use common unside	
Source orientation:     Use common weights     Source orientation:     Use noise normalized weights	
Vector (LCMV) % of uncorrelated noise variance: 5	
C Scalar	
herations (CLARA/sSLOFO)	
Regularization Probe Scan	
OK Cancel	Apply

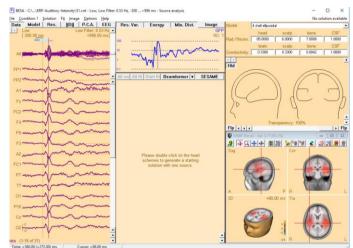
14. We will first try the single-source beamformer. Select Single-source beamformer and click the Go button at the top right of the dialog. The result changes. To check on what the new result means, leave the dialog box with OK, and in the toolbar of the 3D window, activate the 2x2-view with the button . Then click the Maxima button to jump to the first maximum. The first one is now much more central and explains both auditory activations.



Tutorial 9 – Beamforming and virtual sensor montages

- 15. Next, in the Settings, select Use noise normalized weights. А Gaussian noise distribution is assumed, and the contribution of uncorrelated noise variance can be adjusted manually. Use the default value of 5, then press OK and the Beamformer button again. The result resembles the one where the signal interval was used. Again, the first two beamformer maxima with the highest power percentage cover the auditory areas.
- 16. In the Global field power display at the top center of the screen, doubleclick into the plot to set a cursor. Scale the 3D window intensity up by pressing the scaling toolbar button several times, and then move the cursor around using the cursor keys on the keyboard. You can see that the intensity display is now updated with the cursor latency.



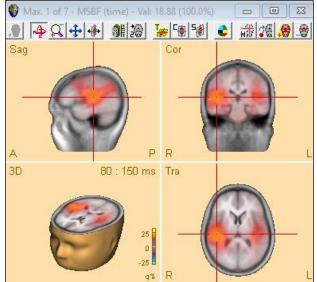


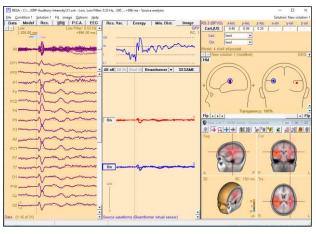
#### Tutorial 9 – Beamforming and virtual sensor montages

#### C. Virtual sensor montages

We will now look at further uses of beamformer imaging. One powerful application is the ability to create virtual sensors in a brain voxel of interest. The spatial filter that was computed for estimating its power can be applied not just to the covariance matrix, but also to the raw data.

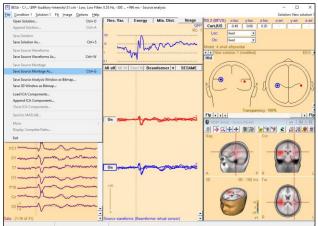
- 1. Let us reconsider the good result we achieved with the bilateral vector beamformer. Once more, open the Settings dialog on the Standard Volume tab and select the Multiple-source beamformer with the check box Use common weights. Press Go and OK, and then the Maxima button to slice at the first maximum of the newly computed beamformer.
- 2. In the toolbar, press the Insert button to insert a source at this particular location in the brain. Press the Maxima button to move to the second maximum. Then press the Insert button again. You have now created a solution of two virtual sensors for this data set.





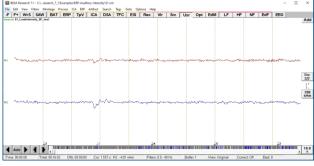
#### Tutorial 9 – Beamforming and virtual sensor montages

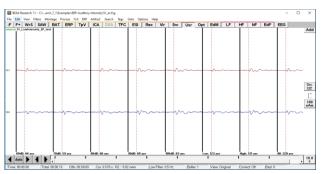
 This solution can be applied to the raw data.
 From the File menu, select Save Source Montage As... In the dialog, save it with the name S1_LowIntensity_BF_test.mtg.

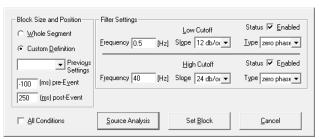


- 4. Minimize the Source Analysis Window. The source montage is applied to the raw data. Two channels are visible. These show the orientation-optimized source waveforms of the two beamformer sources, which consist in reality of 3 different orthogonal orientations.
- 5. Open the File s1-av.fsg. Then press the Usr push button and select the montage from the dropdown menu that appears. You can now see how the source channels reconstruct the auditory activity in the averaged conditions.
- 6. How does this compare with a standard source montage? Let us assume that we had defined a source montage with just two regional sources, fitted to the auditory cortex, for comparison:

Left-drag over the condition Low, then right-click and select Source Analysis



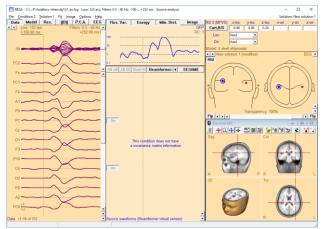




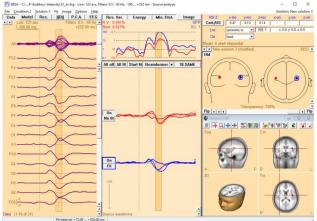
#### Tutorial 9 – Beamforming and virtual sensor montages

from the popup menu. In the dialog that appears, use an interval of **-100 to 250 ms**, and **0.5 Hz**, zero-phase, **12 db/Oct**, and **40** Hz, zero-phase, **24db/Oct**. Press the Source Analysis button.

 The beamformer virtual source condition cannot be applied to these data since no covariance matrix is present.



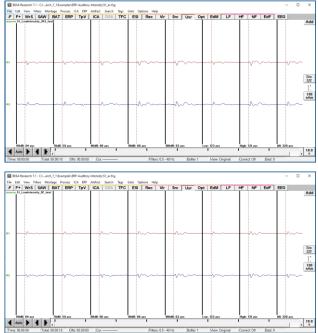
8. Create a new solution by pressing the N key on the keyboard, and insert two regional sources. If dipoles are inserted instead, then convert the sources to regional sources by selecting each of them, then using the key C on the keyboard to convert. Apply a symmetry constraint to one of the sources by selecting it, then using the drop-down menu beside Loc at the top right. Then mark an interval from 72–100 ms and press the All fit and Start fit buttons. The sources fit into an area close to the auditory cortex.



#### Tutorial 9 – Beamforming and virtual sensor montages

9. Use the menu File / Save Source Montage As... to save this as a source montage with the file name S1_LowIntensity_2RS_test.mtg. Then close the Source Analysis window. You can see that the auditory activation is also picked up by these sources as shown in the upper screen shot on the right; however, if you switch back to the beamformer virtual sensors using the Usr button, you will see the difference: The beamformer virtual sensors pick up much less background activity (lower screen shot).

This is due to the fact that in classical source montages, any brain activity needs to be modelled; otherwise, background activity that is spatially correlated with the activity of interest will be projected onto the source of interest. In the beamformer case, the spatial filter is already optimized for each source, independent of the presence of other sources in the model.

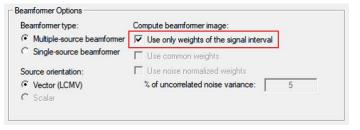


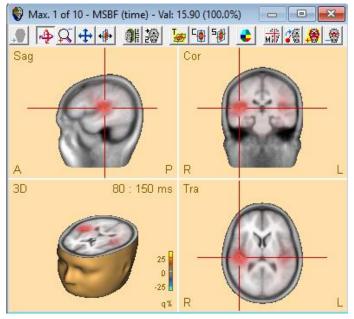
Tutorial 9 – Beamforming and virtual sensor montages

#### D. (Optional section) More settings in the time-domain beamforming

The options to reduce the influence of different spatial leakage profiles of the beamformer spatial weights for baseline and signal intervals.

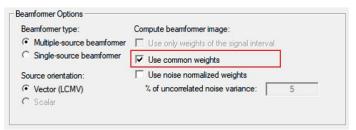
- Use only weights of the signal interval: Beamformer spatial weights for *signal interval* is only used when a beamformer image is computed.
- Use common weights: Beamformer spatial weights for the *common interval* is only used when a beamformer image is computed.
- Open the Image Settings dialog again (hotkey: Ctrl + Shift + A). Select the Multiple-source beamformer, and check the box for Use only weights of the signal interval.
- Press Go and then OK. Click on the Maxima button. The first two cover again the auditory activation. They are more focal than the initial ones.

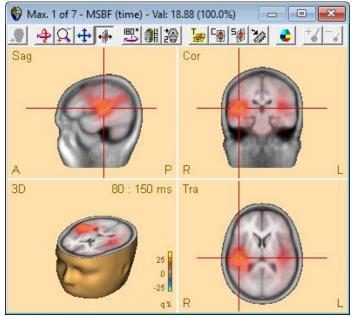




#### Tutorial 9 – Beamforming and virtual sensor montages

- In the Image Settings dialog, uncheck the previous box and check the Common weights box instead.
- Press OK, then press the Beamformer button in the center of the screen. The beamformer is recomputed and automatically slices the MRI at the first maximum.





#### Scalar beamformer

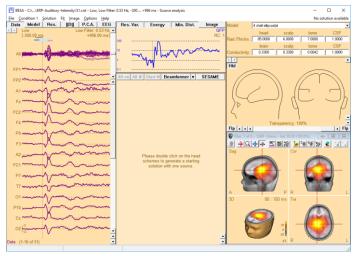
When vector beamformer is used, a beamformer source at position **r** can have 3 orientations for EEG or 2 orientations for MEG. A single optimal orientation that gives the maximum power output at each location is considered in scalar beamformer (Robinson and Vrba, 1999; Sekihara et al., 2004). Therefore, single source waveform is computed for a virtual sensor of the scalar beamformer, while three waveforms (MEG: two waveforms) are estimated for a virtual sensor of the vector beamformer.

Note that this option cannot be used with "Multiple-source beamformer" or "Use noise normalized weights" option.

#### Tutorial 9 – Beamforming and virtual sensor montages

- We will have a look at the Scalar beamformer. We go back to the Settings dialog, and now select Single-source beamformer, with the option Use common weights. With these options, the Scalar beamformer becomes available. Select this option and press OK, then click the Beamformer button.
- Slice the display at the first maximum using the Maxima button on the toolbar. Like in the vector beamformer case with single source, the maximum moves to a more medial location. The other maxima localize outside of auditory areas.
- You can try to go back to the Settings and check Common weights and noise-normalized weights at the same time. This will not substantially change the result, however.

eamformer type:	Compute beamformer image:	
Multiple-source beamformer	🔲 🔲 Use only weights of the signal interval	
Single-source beamformer	✓ Use common weights	
ource orientation:	Use noise normalized weights	
Vector (LCMV)	% of uncorrelated noise variance:	5
Scalar		



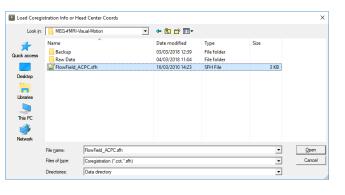
Tutorial 9 – Beamforming and virtual sensor montages

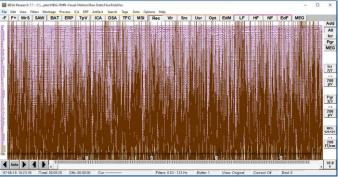
#### E. (Optional section) Time-domain beamforming in MEG data

We will now use the beamformer to analyze MEG data, and also try to find out whether propagation of activity over time can be modelled via the beamformer.

- Select File / Open and browse to the folder ./Examples/MEG+fMRI-Visual-Motion/Raw Data. Open the data set FlowField.foc (you may have to change the extension filter at the bottom of the dialog box to see the file in the list). The Channel and digitized head surface information dialog appears.
- In the section Coregistration, click the Browse button. In the dialog that appears, go up one folder, and select file FlowField_ACPC.sfh.
- 3. Press OK to close the Channel and digitized head surface information dialog. The file opens. Display of MEG waveforms is obscured by the large signal of the stimulus channels, which have been assigned to channel type ICR.

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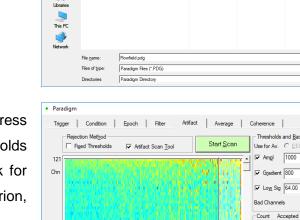




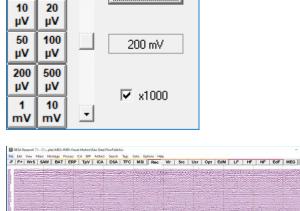
Tutorial 9 – Beamforming and virtual sensor montages

- Click the scaling button of the lcr channel type at the right side of the screen and check the x1000 check box to change scaling to 200 mV.
- Now the MEG is visible. Trigger codes 1 and 2 were applied.

- Press the ERP push button. Then, in sub-folder Visual, open the paradigm file Flowfield.pdg.
- Move to the Artifact tab and press Start Scan. The current thresholds are appropriate. You can check for gradient and Low Signal criterion, too.



Sort Channels by Mean



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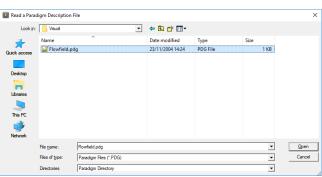
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### Tutorial 9 – Beamforming and virtual sensor montages

Move to the Average Tab. Select the first condition Motion and press the Beamformer button. In the beamformer intervals dialog, press the Butterfly plot button. MEG channels are shown in a dark purple colour.

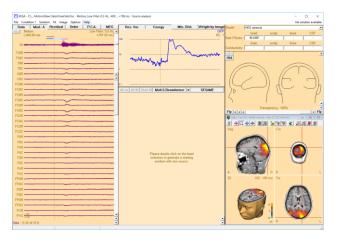
t beamfo	ormer interv	/als				×
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9. From tutorial 7, we know that the activity related to Motion happens later than the initial visual evoked activity that follows the stimulus. Let us mark a signal interval from 100 to 190 ms. Accordingly, set the baseline interval from -100 to -10 ms, and the Common interval from - 100 to 190 ms.

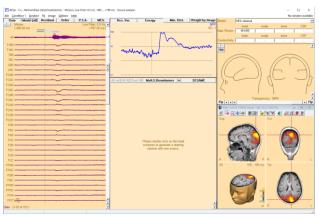
Set	beamfo	ormer interv	vals				$\times$	
	Targe	t condition:	Motion					
[	Beamf	ormer interv	al					
		Baseline		Signal		Common		
	Start	-100	ms	100	ms	-100	ms	
	End	-10	ms	190	ms	190	ms	
					2.0	and the factor	hall-out	
				10 Auroson		an a		
1	I Important note: To obtain a reliable beamformer image, the number of samples in the Baseline and the Signal intervals should be equal. Therefore it is recommended to assign equal duration to the Baseline and the Signal intervals.							
[	Butterf	ly plot			ОК	Can	cel	

### Tutorial 9 – Beamforming and virtual sensor montages

 Press the OK button to start the beamformer computation. The Source Analysis module opens.

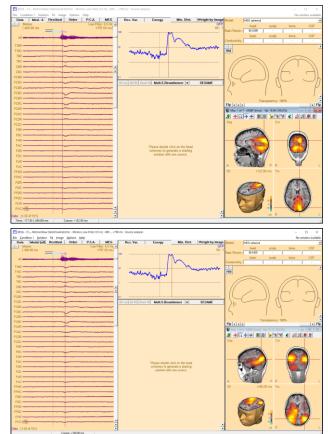


11. Click the arrow in the center of the display to open a popup menu. Then select Settings. The dialog box for defining beamformer settings opens. Select Multiple-source beamformer and Vector (LCMV) options, and check the boxes for Use common weights and Use noise normalized weights. Then, click the Go button. The results change.



Tutorial 9 – Beamforming and virtual sensor montages

12. In the Global field power display at the top center of the screen, doubleclick into the plot to set a cursor. Then move the cursor around using the cursor keys on the keyboard. You can see that the beamformer image is now updated with the cursor latency. Check the beamformer image at 122.50 ms and 160 ms.



Tutorial 9 – Beamforming and virtual sensor montages

Tutorial 10 – Advanced Artifact Correction

#### **Tutorial 10 – Advanced Artifact Correction**

#### What does BESA Research provide?

- ✓ Artifact correction based on surrogate models,
- ✓ Artifact correction based on adaptive artifact correction,
- ✓ ICA based artifact spatial filtration and artifact free data generation

**Tutorial 10 – Advanced Artifact Correction** 

#### **A.** Artifact Correction

In the following we will learn about the background of artifact correction. Artifact correction always aims at extracting unwanted signals like EOG, EKG or external noise from the data, while leaving all brain activity of interest as undisturbed as possible. To achieve this, artifact and brain topographies must be separated. Depending on whether one is dealing with spontaneous or evoked activity, different approaches for artifact correction are appropriate. These will be discussed and demonstrated in this tutorial.

#### Principles of artifact correction

For artifact correction, artifact and brain activities must be identified and separated. In general, artifact and brain topographies will be spatially correlated. Hence, a simple regression or the projection of the data onto the subspace orthogonal to the artifact topographies will severely distort the data (see Subspace Projection approach below).

For a correction without distortion, it is not sufficient to define the artifact topographies (to be removed), but it is equally necessary to create a model or a spatial description of the brain topographies (to be retained). The first two of the following three methods also create a model for the brain activity:

 Adaptive artifact correction: (Ille N, Berg P, Scherg M. Artifact correction of the ongoing EEG using spatial filters based on artifact and brain signal topographies. J. of Clin. Neurophysiol. 19:113-24, 2002.)

This method estimates the brain activity from the data currently displayed on the screen. The data is scanned in specified time intervals. Those segments are considered to represent brain activity where 1) the correlation between data and artifact topography does not exceed a certain threshold and 2) the signal amplitudes are below a specified threshold. Of the remaining segments a principal component analysis (PCA) is performed. All PCA components explaining more than the minimum variance specified in the box Adaptive Model: PCA Topography are maintained. They span the brain signal subspace.

In a next step, the recorded data is decomposed using all topographies into a linear combination of brain and artifact activities. Thus, the estimated artifact signals are much

#### **Tutorial 10 – Advanced Artifact Correction**

less overlapped with brain activity and can be subtracted from the original signals without much distortion.

This approach is recommended, in particular, for the review of continuous EEG or MEG data.

2) Surrogate Model approach: (Berg P, Scherg M. A multiple source approach to the correction of eye artifacts. Electroencephal. Clin. Neurophysiol. 90:229-41, 1994.)

Here, brain activity is modeled by a model consisting of multiple equivalent current dipoles. The artifact topographies are added to this model and the combined model is then applied to the recorded data. Again, the estimated inverse signals separate the brain activity associated with the surrogate sources from the artifacts to a high degree. Thus, the artifact signals can be subtracted without considerable distortion of the activities originating in the modeled regions. This approach considers the activity in the modeled brain regions while the on-going EEG is not modeled accurately.

Therefore, the surrogate method is especially recommended for the correction of data to be averaged if the average signal is smaller than the EEG or MEG background. In this case a model cannot be estimated from the on-going data. Therefore, a-priori knowledge of the involved brain regions should be employed to create an appropriate surrogate model.

3) Subspace projection (SSP, regression): This approach has been commonly applied in the literature. SSP does not contrast artifacts and brain activity. Rather, the complete subspace spanned by the artifact topographies is projected away from the recorded data. This leads to undistorted data only in the highly unlikely case when artifact and brain activity have exactly orthogonal topographies. This is generally not the case in real data. In the likely event that evoked brain activity has a topography correlated with the artifact, this method removes the correlated fraction of the brain activity. As one of the negative consequences, maps of the corrected brain activity will be severely distorted after SSP correction, as we will see below.

Therefore, this method is not recommended.

The following chapters demonstrate the practical application of these different approaches.

Tutorial 10 – Advanced Artifact Correction

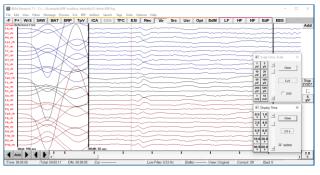
#### **B.** The Effect of Artifact Correction on Averaged ERP data

For a traditional ERP analysis (e.g. analysis of peak channel amplitudes and latencies), artifact correction can be applied to the averaged ERP data. This chapter demonstrates how the different correction methods affect the obtained result.

 Please open the file S1-blink+ERP.fsg located in the ERP-Auditory Intensity folder. This file contains the average of conditions 60 to 100 dB, Low, High and All along with an averaged eyeblink. Change to the virtual reference-free montage by pressing the Vir button and selecting the entry Reference Free.

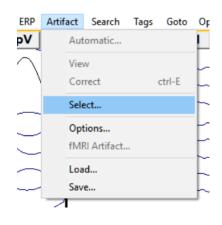
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We will demonstrate the effect of artifact correction with focus on the 60dB condition. Use the time scaling button at the bottom right of the window to set the time scaling to 2.0 s. Press the amplitude scaling button under the Scp button to increase the displayed scalp channel amplitude to 5 μV.



**Tutorial 10 – Advanced Artifact Correction** 

3. From the Artifact menu, choose Select....



4. Note that the blink artifact topography that we defined in the raw data has been assigned automatically to the averaged file as well, but is not selected by default. Set the checkmark next to the Blink category.

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Select Topog	raphies Estimate	Signal			
Select category	No. of topographies (% variance)				
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 Switch to the Estimate Signal tab. We will compare the three different approaches for artifact correction offered by BESA Research. First, select None (SSP-Regression) in the Model of Brain Activity box to correct the data by subspace projection. Press OK.

Select Topographies Estimate Signal	
Model of Brain Activity C Adaptive C Surrogate ( <u>None (SSP-Regression</u> ) Surrogate dipole model (*bsa)	Brain Activity: View Waveforms
xamples\ERP-Auditory-Intensity\S1-AC.bsa Browse	

#### Tutorial 10 – Advanced Artifact Correction

 Make sure that artifact correction and view is selected in the Artifact menu.

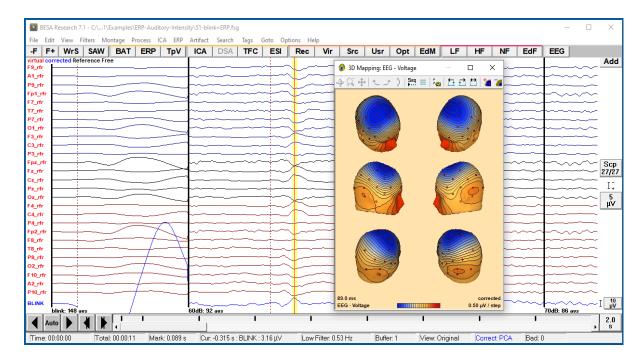
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7. Press the scaling button for the Blink channel to scale it up to 10 μV. The blink activity has been largely corrected. Remaining activity in the first segment is due to the 0.6% of variance that is not explained by the first PCA component. Including a second or third PCA component in the Select Topographies tab would improve the correction. However, the more artifact topographies are being defined, the more likely the brain activities become distorted.

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#### **Tutorial 10 – Advanced Artifact Correction**

8. Double-click in the second segment at the latency of the N100 component (ca. 90 ms) to obtain a 3D whole head map of the corrected data at this latency. The map is severely distorted by the SSP correction: Instead of the N100 topography, a strong frontal positivity is observed that is typical for the blink topography. The reason is that SSP is not able to contrast the artifact topographies with the brain signals. Since the N100 topography is correlated with the blink topography, the correlated fraction of the N100 contribution is removed from the data by the subspace projection. The correlation can be seen in the bottom trace which depicts the blink signal estimated using its topography throughout the data set. At the time of the N100, it shows an upward deflection. Hence, SSP subtracts this part weighted with the blink map. Thus, a more inferior frontal positivity is introduced. The net resulting map, however, has been made orthogonal to the blink topography over all electrodes.

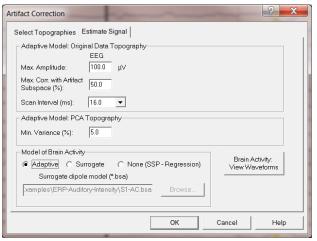


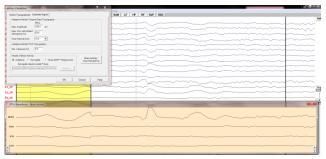
9. To see how the adaptive method improves the result, select Artifact / Options.

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#### **Tutorial 10 – Advanced Artifact Correction**

- 10. As Model of Brain Activity select Adaptive. The Minimum Variance threshold determines the number of topographies that are considered brain activity which is contrasted against the artifact topography. Set the threshold to 5%. To view the time course of the brain topographies, press Brain Activity: View Waveforms.
- 11. Three PCA topographies serve as model for the brain activity. In their time courses, the auditory N100 component is mainly represented by the first component. Note that the 4 displayed components have topographies that are orthogonal to each other, but they are not orthogonal to the artifact topography. Accordingly, this approach will retain brain activity even when it is spatially correlated to the artifact topography.
- 12. To see this, close the PCA Waveforms and Estimate Signals windows. If the cursor at ca. 90 ms is still set in the second condition, select Process / Voltage Maps or type the hot key M on the keyboard to obtain the 3D map of the adaptively corrected data.

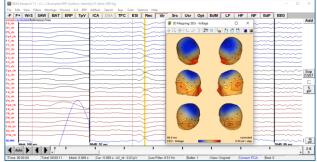




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#### **Tutorial 10 – Advanced Artifact Correction**

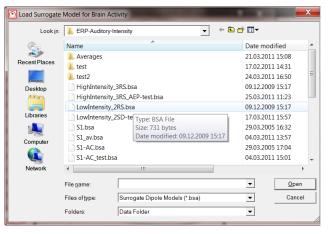
- 13. The maps are nearly undistorted by the artifact correction and show the typical N100 topography. As a correlate, note that the blink waveform does not show any interaction with the auditory evoked components. This indicates that blink and brain activities have been very well separated by the adaptive method.
- 14. Press Artifact / Options and check Surrogate in the Model of Brain Activity box. The surrogate model approach is useful if evoked activity is to be contrasted against eye movement artifacts and if the evoked activity is small as compared to the background EEG / MEG. In this case, the adaptive model does not well model the evoked activity and artifact correction is likely to create distortion. Therefore, a surrogate source model should be established a) on the basis of a priori functional-anatomical knowledge, or b) by using a multiple source model or spatial components defined by a PCA from an average with all artifact epochs rejected and fewer trials.

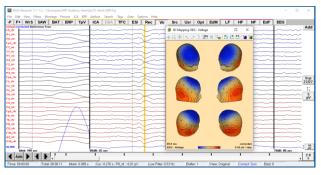


Artifact Correction
Select Topographies Estimate Signal
Surrogate Model: Regularization
Artifact (%): 0.0
Brain (%):
Surrogate Model: PCA threshold
Min. Variance (%): 1.0
Model of Brain Activity
C Adaptive  Surrogate C None (SSP - Regression) Brain Activity: View Waveforms
Surrogate dipole model (*.bsa)
xamples\ERP-Auditory-Intensity\S1-AC.bsa Browse
OK Cancel Help

#### **Tutorial 10 – Advanced Artifact Correction**

- 15. Press the Browse button to load the solution LowIntensity_2RS.bsa that was created earlier on, consisting of a symmetrical pair of regional sources in the auditory cortex. The stored two regional sources model the bilateral brain activity in supratemporal region the and are combined with the defined artifact topography to separate artifact and brain signals¹¹.
- 16. Press OK and press M to view the 3D map of the surrogate-corrected data at the cursor latency. Similar to the adaptive method applied to the averaged data, the brain topographies are nearly undistorted. The blink waveform does not show any interaction with the auditory evoked components.





#### C. Source analysis of artifact-corrected data (not recommended)

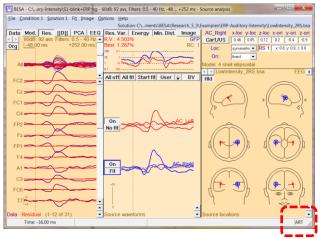
Accurate source analysis of artifact-corrected data requires knowledge of the artifact topographies that were removed from the data. Otherwise, source activities will also be distorted. If an (averaged) data segment has been created (and/or stored as binary segment) by the program, BESA Research knows the artifact coefficients as indicated by corrected at the upper left. Otherwise, an ASCII file (*.art) with the artifact topographies must be provided.

¹¹ It is not necessary to create a surrogate source model. Predefined surrogate models are provided by BESA Research.

#### **Tutorial 10 – Advanced Artifact Correction**

- Left-drag over the second segment to mark a block. Right-click and send it to source analysis with settings -50 to 250 ms, Low Cutoff filter of 0.5 Hz, 6 db/oct, forward and High Cutoff Filter of 40 Hz, 24 db/oct, zero-phase.
- 2. Load the source model LowIntensity_2RS.bsa by pressing File / Open Solution. The letters ART in the status bar at the bottom right corner of the window indicate that this data is artifactcorrected and that BESA Research knows the topographies of the corrected artifacts.





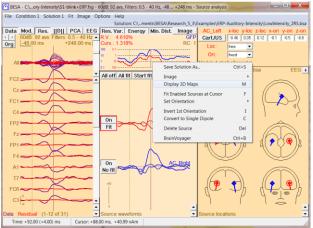
Depending on the correction method, a fraction (adaptive, surrogate) or the complete (SSP) dimension of the artifact subspace is missing. Despite this fact, the source analysis window ensures correct localization and correct source waveforms (S) of fitted sources. This is achieved by a SSP method that projects both the data (D) and the source topographies (L) onto the subspace orthogonal to the subspace that is spanned by the artifact topographies. Accordingly, the display shows the SSP-corrected data, independent of the correction method used in the main window. The fact that the source waveforms are recovered correctly by this approach can be seen from the following equations:

Uncorrected data:	D = L * S
	Ļ
Corrected data:	(P*D) = (P*L) * S

Here, P is the operator that projects into the subspace orthogonal to the subspace spanned by the artifact topographies.

#### **Tutorial 10 – Advanced Artifact Correction**

Double-click in the waveform box to set a cursor at the N100 latency. Right-click and select Display 3D Maps or type the hot key M on the keyboard.



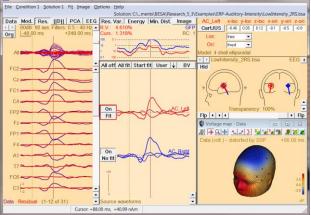
- BESA Research displays a warning message. It explains why the following map will be distorted. Press OK to confirm.
- 5. The topography is severely distorted by the subspace projection that was applied to the data. Although the source waveforms obtained by modeling the corrected data are undistorted, the data displayed in the channel box and in the global field power (GFP, blue line in the upper middle box) are distorted. As a consequence, *loading corrected data into the source analysis window is less recommended.* Close the source analysis window.

The SSP removes the artifact topography from the data. Due to this fact, the 3D maps in the Source Analysis module will be distorted.
This will be indicated by the additional text "distorted by SSP" in the header line of the map window.

 Image: State of the state of th

This is an artifact-corrected data set. In order to ensure correct source localization, BESA performs a subspace projection (SSP) on the data and the source leadfields.

Voltage map - Data



#### **Tutorial 10 – Advanced Artifact Correction**

# D. Contrasting artifact and brain topographies – the Optimizing method (recommended)

In order to obtain correct source localization, the presence of blink artifacts in the averaged segments must be taken into account. This is done by including the artifact topography in the multiple source model during source analysis.

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- Switch off artifact correction by pressing ctrl-E. You can confirm it is switched off as corrected is no longer displayed in the top left corner of the main window.
- Left-drag over the second segment to mark a block. Right-click and send it to source analysis with settings -50 to 250 ms, Low Cutoff filter of 0.5 Hz, 6 db/oct, forward and High Cutoff Filter of 40 Hz, 24 db/oct, zero-phase.
- Top View of Data

   Whole Segment

   FFT

   Linear Correlation

   Source Analysis

   Define as Epoch

   Define as Artifact

   Write Segment

   Copy to Buffer

   Define Artifact Topography

   Epoch: -50.0..250.0 ms, Filters: 0.5 40 Hz

2.0

 Load the source model LowIntensity_2RS.bsa by pressing File / Open Solution.

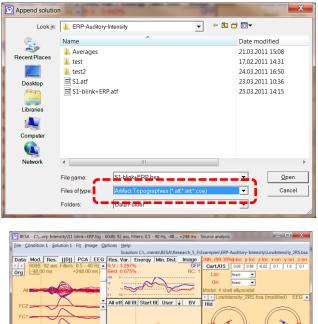


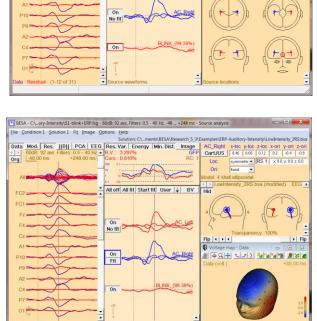
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#### **Tutorial 10 – Advanced Artifact Correction**

- **4.** Next, we will load the artifact topography into the source analysis window. This will create a spatial component explaining the blink topography.
- 5. Press File / Append Solution.... In the drop-down menu Files of type: select Artifact Topographies (*.atf;*.art;*.coe). Select S1-blink+ERP.atf and press Open.

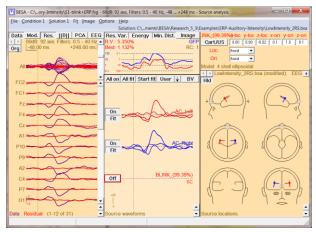
- 6. The topography of the main PCA component of the blink artifact is now appended to the current solution. The corresponding waveform and the equivalent location are shown in the source analysis window. This is the component explaining 99.4% of the artifact variance that was selected during the artifact definition.
- 7. Again, double-click in the waveform box to set a cursor at the N100 latency. Rightclick and select Display 3D Maps or type the hot key M on the keyboard. Note that the map is not distorted but shows the typical N100 topography.





#### **Tutorial 10 – Advanced Artifact Correction**

8. Switch off the spatial blink component by pressing its On button and close the 3D mapping window. Press All Fit and Start Fit to see how the fit changes if we don't take eyeblink activity into account. Note that the auditory sources locate more anterior and medial than before. Switch the spatial blink component back on and repeat the fit for comparison. Close the source analysis window without saving anything.



#### E. Artifact Correction of raw data using ICA – spatial filtration

Independent Component Analysis (ICA) allows decomposing EEG/MEG data into independent components. Independent components (IC) can be used for artifact correction, as spatial components in source analysis or for creating ICA-reconstructed data only containing signal from specified IC. ICA is performed on the current screen and can be started from the ICA entry in the menu bar or by pressing the ICA button located in the button menu. The amount of data available in the current screen can be manipulated by using the time scaling button in the bottom right corner of the main window (max. 1200 s).

The method behind ICA can be chosen between the extended Infomax algorithm (Lee TW et al., Independent component analysis using an extended infomax algorithm for mixed sub-Gaussian and super-Gaussian sources. Neural Computation 11(2), 1999, 409-433) or Second-order blind identification – SOBI (Belouchrani A et al. A blind source separation technique using second-order statistics. IEEE Transactions on Signal Processing, 45(2), 1997, 434-444). Before the ICA is calculated, the dimensionality of data is optionally reduced by PCA. By default, all PCA components are ignored that explain less than 1% variance. The use of PCA can be switched off or the variance cutoff can be altered by pressing ICA / Options.

While ICA is being computed, the current analysis step is displayed in a dialog box along with the changing weights. The first ICA step may take longer for many samples than the subsequent steps. ICA analysis stops when the change of weights from 1 step to the next is

#### **Tutorial 10 – Advanced Artifact Correction**

smaller than 1.0e-6 or after max. 500 steps. ICA analysis can be stopped by pressing the **Abort** button in the dialog box.

In case EEG and MEG data are both available, ICA analysis will only run on the data type that is selected by the EEG/MEG button. Running ICA on combined gradiometer/magnetometer data is not possible as it can only run on one channel type at a time.

- Open file S1.cnt from the ERP-Auditory-Intensity Examples folder. Make sure, the Rec button is pressed, that you are displaying the first screen and that artifact correction and all filters are switched off.
- Please select ICA / Current Screen to start ICA analysis.

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3. The results of the ICA analysis are displayed like a montage. The units of the waveforms are nAm since ICA waveforms can be described as source activity of a spatial component localized at the center of gravity of the corresponding map. ICA waveforms have the labels ICA1 – ICAx. The menu item ICA / Current screen is ticked and the ICA button is pressed. ICA components are sorted in descending order of their explained variance. The displayed ICA montage is automatically recomputed whenever ICA options change or the data change, e.g. when filtering, changing the time-range to display, etc.

Here, ICA decomposition delivers 21 independent components. It appears that the second ICA component represents an eyeblink and the fourth ICA component represents a cardiac artifact.

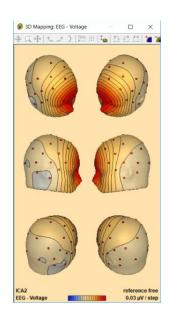
### **Tutorial 10 – Advanced Artifact Correction**

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4. Right-click on the second ICA component and select Map Topography.

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ICA6	r	Export ICA Reconstructed Data Without Selected Components: Whole File	

**5.** The map indicates that the second ICA component indeed reflects an eyeblink.



#### **Tutorial 10 – Advanced Artifact Correction**

- We can now use this topography for artifact correction. Close the mapping window, right-click on the second ICA component label and choose Define As Artifact Topography.
- The Artifact Correction window opens.
   Please check the box next to Blink.
   Close the mapping window and press OK.
- ICA1
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   Define As Artifact Topography

   ICA3

   Send Topography To Source Analysis

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   Save Topography

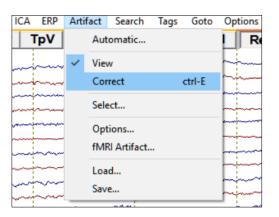
   ICA5

   Export ICA Reconstructed Data Without Selected Components: Current Screen

   ICA6

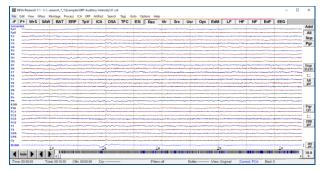
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 Return to the original recording by pressing the <u>Rec</u> button. Now press <u>Artifact / Correct</u> to switch on artifact correction.



9. Note that the eyeblink has now disappeared. The topography of the second ICA component is now subtracted from the data.

We will learn more about the background and other methods of artifact correction throughout the tutorial.



#### **Tutorial 10 – Advanced Artifact Correction**

 Please switch off artifact correction again by pressing Artifact / Correct.

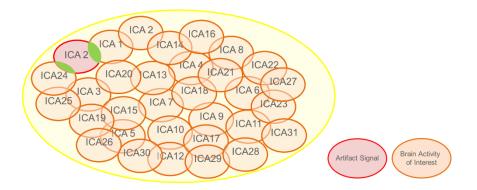
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#### F. Artifact Correction of raw data using ICA – data reconstruction

We already saw that it is possible to use ICA components for artifact correction. However, we only used ICA to determine the artifact topography and proceeded with artifact correction also using the adaptive or surrogate method. Thus, we used ICA decomposition as a different technique to estimate the artifact topography (in contrast to averaging the artifact and decomposing it using PCA). There is an alternative way to use ICA decomposition for artifact correction by creating ICA-reconstructed data, i.e. creating a "new" dataset only consisting of non-artifact-related ICA components.

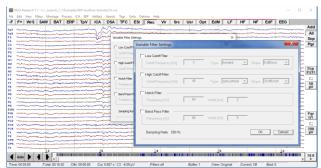
EEG/MEG data can be considered as a summation of topographies that are differentially active at each sampling point. Subtracting an artifact *topography* reduces the data by one dimension. This can potentially lead to severe distortion of brain activity of interest (as previously demonstrated by the SSP correction method) if the brain activity of interest is not modeled. Creating ICA-reconstructed data leaving out artifact topographies poses the same problem. However, ICA decomposition has the advantage that ICA components must be as independent as possible, but they may overlap to some degree. This means that if the artifact topography is correlated with a topography representing brain activity of interest, some other ICA components will also most likely be correlated with brain activity of interest.

#### **Tutorial 10 – Advanced Artifact Correction**



Thus, subtracting an ICA component will not cause distortion as pronounced as SSP correction does, as the correlated part of the signal will still be represented by the remaining ICA components at least to some degree. Nevertheless, there will be some distortion as it cannot be fully prevented. The distortion effect gets the more reduced, the more electrodes are available, as the number of electrodes determines the number of ICA components. The more ICA components are available that represent brain activity of interest, the less likely data will be distorted after subtracting an artifact topography.

- Make sure that S1.cnt file is opened and, the Rec button is pressed. Press CTRL-E to switch off artifact correction. Check if you are displaying the first screen and that artifact correction and all filters are switched off.
- 2. Press ICA / Options and select PCA off. Switching PCA off will lead to ICA decomposition running on all data. PCA on, Minimum Variance of 1% (default) will reduce the data by all PCA components explaining less than 1% variance before running ICA. PCA on, Nr. of Components 12 (default) will reduce the data by the smallest 12 components before running ICA.



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Tutorial 10 – Advanced Artifact Correction

3. Press ICA / Current Screen to start ICA decomposition.

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4. ICA will output 31 ICA components, the same number as EEG channels are present on the current screen. Some of the channels will represent brain activity, and some will represent artifact signal. Each ICA component is associated with a topography, and a source waveform. Source waveforms can be estimated by assuming a spatial component at the center of gravity of each ICA topography. Multiplying the data with the pseudo-inverse of the matrix containing the ICA topographies will yield the ICA source waveforms.

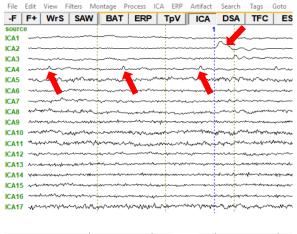
Tutorial 10 – Advanced Artifact Correction

 ICA2 seems to represent an eyeblink and ICA4 seems to represent cardiac activity, which we had not noticed previously.

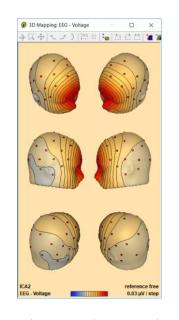
Note: it is possible that in your case the ICA components representing the blink/EKG are not ICA2 and ICA4. If this is the case, locate the respective ICA components and use their according label in the following!

- Right-click on the label ICA2 and select Map Topography.
- 7. The map confirms that ICA2 does indeed represent blink activity. In the following, we want to neglect ICA2 and create a new dataset containing all ICA components but ICA2. Close the mapping window.

 Make sure to left-click on ICA2 only in order to select it. Right-click and choose Export ICA Reconstructed Data Without Selected Components: Whole File. BESA Research will now create a new file containing ICA-reconstructed data.



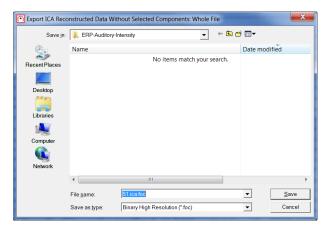
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-	Export ICA Reconstructed Data Without Selected Components: Whole File

Tutorial 10 – Advanced Artifact Correction

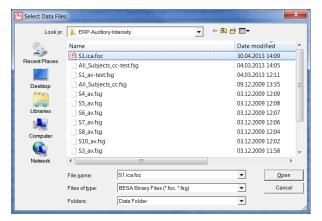
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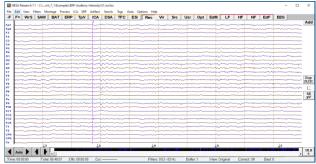
10. As segment comment, enter **without blink** and press **OK**.

Please Enter a Segment Comment
Segment Comment
without blink
Ok Cancel

 Press File / Open. Change Files of type to BESA Binary Files. Select S1.ica.foc and hit Open.



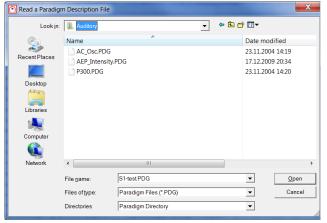
12. We can immediately see that the eyeblink that was present at the position of the tag is no longer visible. Browsing through the data will confirm that eyeblinks are no longer present anywhere in the data. The question remains, if creating ICAreconstructed data without the eyeblink topography distorted our N100 topography.



Tutorial 10 – Advanced Artifact Correction

To investigate, we will average the data again to look at evoked potentials.

 Press ERP / Open Paradigm. Browse to the Auditory folder and select AEP_Intensity.PDG. Press Open.



Move to the Artifact tab and press Start
 Scan to exclude artifacts from averaging.

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 Move to the Average tab and press Average.

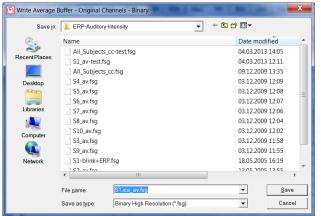
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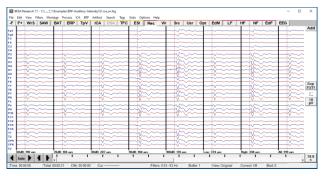
Tutorial 10 – Advanced Artifact Correction

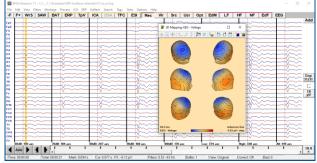
16. Save the average file under the suggested name S1.ica_av.fsg. When prompted to specify a segment comment, press Stop Asking to use the suggested names from the paradigm.

The new average file will be automatically opened along with the TopView window.
 Close the TopViewer.

18. Double-click at the N100 peak in condition
60dB to bring up the 3D window. Make sure to move to 94 ms using the left and right arrow keys. Scale up the topographic view to 0.63 µV / step. The N100 map appears nearly undistorted.







G. Applying the Optimizing method when using ICA-reconstructed data for source analysis

Despite the apparent lack of distortion of the N100 component in the example of ICA-reconstructed data in section F, it is recommended to account for the artifact topographies that were subtracted during source analysis. Whenever artifact correction is performed, a spatial dimension of the data (the topography) is changed. This can distort the data. To prevent this distortion from affecting source analysis, the same distortion should be applied to the leadfields. Alternatively, the spatial dimension can be projected out of the leadfields. As BESA Research does not save any

Tutorial 10 – Advanced Artifact Correction

information about the topographies that were subtracted when creating ICA-reconstructed data, the artifact topographies need to be saved manually and later loaded in source analysis.

- Follow steps 1 to 4 of section F in the present tutorial to run ICA decomposition on the first screen of S1.cnt. Locate the ICA component reflecting the blink (usually ICA2). Right-click on the label and select Save Topography.
- 2. Save the topography under the suggested name S1.ica.

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 Open dataset S1.ica_av.fsg we created in section G of the present tutorial. It should be located in the Auditory Intensity examples folder.

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e.	Name	Date modified	-
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	All_Subjects_cc.fsg	09.12.2009 13:35	
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	S6_av.fsg	03.12.2009 12:07	
Computer	S7_av.fsg	03.12.2009 12:06	
Computer	S8_av.fsg	03.12.2009 12:04	
	S10_av.fsg	03.12.2009 12:02	-
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	Files of type: BESA Binary Files (*.foc, *.fsg)	▼ Cancel	
	Folders: Data Folder		

Tutorial 10 – Advanced Artifact Correction

- 4. Send condition Low to source analysis by left-dragging a block, right-clicking and selecting Source Analysis. Use a custom definition of -50 to 250 ms, a Low Cutoff Filter of 0.5 Hz, 12 dB/Oct, zero-phase and a High Cutoff Filter of 40 Hz, 24 dB/Oct, zero-phase. Press Source Analysis.
- In the Source Analysis Window press File / Open Solution and select our source model LowIntensity_2RS.bsa. Press Open.

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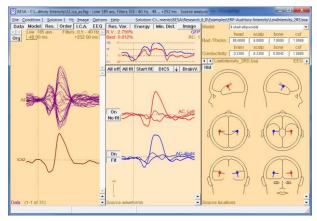
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 Now we will append the ICA blink component to the current solution. Press File / Append ICA Components. Change Folders to Data Folder and select S1.ica that we created in step 2. Press Open.

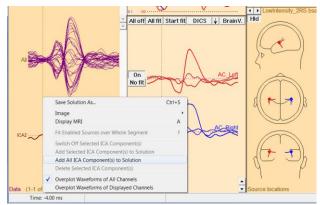
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### **Tutorial 10 – Advanced Artifact Correction**

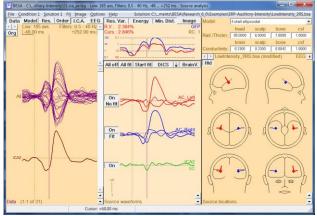
7. You will see the waveform associated with the blink topography plotted beneath the EEG butterfly plot. We want to utilize it in our source model.



Right-click on the ICA waveform and press
 Add All ICA Components to Solution.

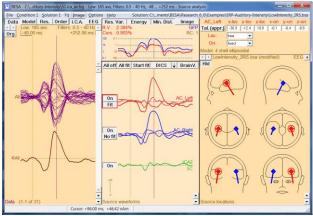


9. We will now see that the blink was added to our source model as a spatial component. Its activity pattern for the current data can be seen in the middle panel.

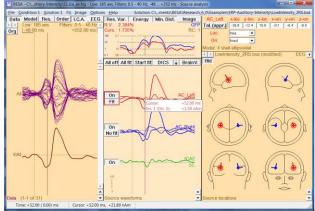


### **Tutorial 10 – Advanced Artifact Correction**

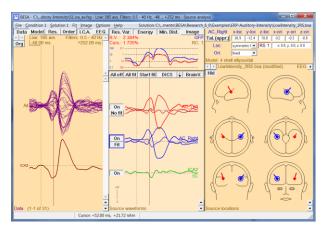
Double-click at the N100 peak at around
 96 ms. The green source waveform is nearly 0 at this point, suggesting that there was indeed no blink activity at the N100 peak.



11. Double-click in the red source waveform (AC_Left) and move the cursor to 52 ms using the arrow keys on your keyboard.
Press O on the keyboard to orient the first regional source approximately at the P50 peak.

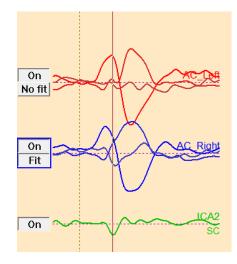


 Repeat for the blue source (AC_Right). Now both regional sources are oriented so that the first orientation optimally reflects the P50 peak.



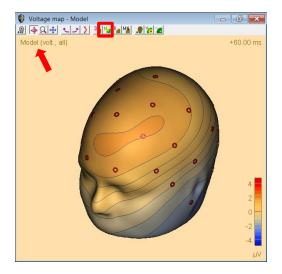
#### **Tutorial 10 – Advanced Artifact Correction**

13. Use the arrow keys on your keyboard to move the cursor to the peak of AC_Left at 60 ms. This coincides with the peak of the blink component!



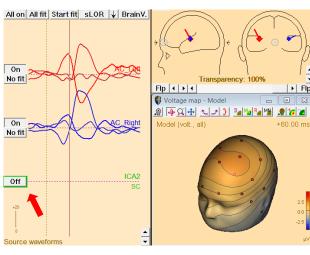
When we created ICA-reconstructed data without the blink topography, we subtracted an ICA component that was not correlated with the N100 topography. Thus, if we now tried to localize the N100 activity in the present example, we would not make an error. However, the ICA component we subtracted did correlate with the P50 topography! Thus, we subtracted part of the P50 topography when creating ICA-reconstructed data. If we now tried to localize the P50 activity, we would end up with a localization error caused by artifact correction with ICA. Therefore, it is vital to use the subtracted ICA component in the source analysis window in order to prevent this error. Adding the blink as a spatial component corrects the distortion of the P50 topography and allows correct reconstruction of the underlying sources.

Press M on the keyboard to bring up 3D mapping. Press the 6th button from the right labeled M until the 3D window is labeled Model (volt, all).

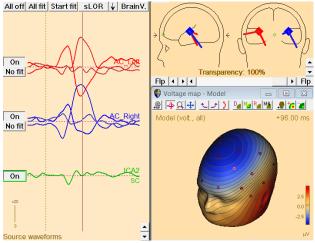


### **Tutorial 10 – Advanced Artifact Correction**

15. Switch off the ICA component in the middle panel by clicking on the On button. Note how the map changes if the blink component is left out of the model. This difference corresponds to the distortion imposed on the P50 map by subtracting the blink topography.



 Move the cursor to the N100 peak at 96 ms by using the arrow keys on your keyboard.
 Toggle between on and off for the ICA component. Note that the N100 map is barely influenced by the blink topography.



The above steps illustrate that creating ICA-reconstructed data will lead to some distortion of the data. Therefore, the recommended strategy for performing source analysis on ICA-reconstructed data is to use the subtracted topographies as spatial components in the source model.

Tutorial 11 – Simultaneous EEG-fMRI

### Tutorial 11 – Simultaneous EEG-fMRI

#### What does BESA Research provide?

- ✓ fMRI artifact gradient removal
- ✓ Ballistocardiograph (BCG) artifact reduction
- ✓ fMRI-informed source analysis

#### Tutorial 11 – Simultaneous EEG-fMRI

**Note:** Currently data collected during simultaneous EEG-fMRI is not intended to be used in any diagnostic procedures. Following this guideline, <u>fMRI artifact removal implemented in</u> <u>BESA Research may also not be used for any diagnostic procedures</u>. This tool was developed for research purposes only.

For acquisition of simultaneous EEG-fMRI, please use only EEG hardware that is certified for MRI! Remember that bringing any metal or other (para-)magnetic parts into the magnetic field area is dangerous.

When EEG data is recorded during an fMRI session there are plenty problems waiting to be solved before being able to analyze the real brain signal. Here we going to investigate the following:

- removal of fMRI gradient artifact
- reduction of heart beat-induced ballistocardiogram (BCG) and blinking artifacts
- performing source analysis of EEG-fMRI data

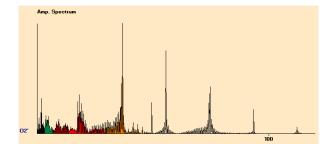
#### A. Removal of fMRI gradient artifact

The fMRI gradient artifact results from fast switching of MRI gradient coils which is a part of volume acquisition in the Echo-Planar sequence used for fMRI imaging. This switching introduces variable magnetic fields that in turn induce variable electrical fields in the EEG recording system (electrodes and wires namely), as described by Maxwell's second law. The induced artifact is typically very large in amplitude (i.e. approximately 16 mV in a 3T MR scanner). Also, the frequency range of the artifact overlaps with the frequency band of physiological EEG signal.

The artifact frequency and its harmonics can be easily assessed using the following equation (Rusiniak et al., 2013b):

Tutorial 11 – Simultaneous EEG-fMRI

$$f_x = \frac{S(x+1)}{TR}$$



where  $f_x$  is the harmonic frequency, x is the harmonic number, *NS* represents the number of slices, and *TR* stands for Repetition Time of fMRI volume acquisition. As will be explained further, each fMRI gradient removal algorithm has its limitations, so using this equation allows to set up fMRI acquisition in a way that minimalizes frequency coupling with the band of interest. For example, consider that we want to investigate the alpha frequency range (7-13 Hz). Using 37 slices and TR equal to 2 s ensures proper spectral separation since the first frequency peak of fMRI gradient artifact will be at 18.5 Hz.

One of the known methods to deal with the fMRI gradient induced artifact is based on moving average subtraction, first introduced by Allen and colleagues (2000). This type of method works only satisfactorily when the following assumptions are true:

#### • Each artifact occurrence is identical

Each fMRI volume acquisition is identical (gradient switching does not alter between volumes). This assumption is true when we take into consideration only fMRI acquisition. However, if the subject moves during the acquisition the artifact changes since electrode positions in relation to the local magnetic field have altered.

• EEG signal is stationary

In principle this assumption is true for the EEG signal. The signal stationarity can be affected by the paradigm, therefore jitter between trigger events is strongly advised.

#### • Each start point of artifacts has to be precisely indicated

This can be achieved either by hardware clock synchronization between EEG system and MRI machine, or by a very high sampling rate (> 10 kHz). All modern EEG hardware units designed for simultaneous EEG-fMRI registration are capable of clock synchronization.

#### **Filtration Methods**

#### Allen et al., 2000

This method is a starting point for two other methods as well. In all methods available in BESA Research, a moving average artifact subtraction algorithm is used and the difference between methods can be noted in the template creation matrix.

To obtain artifact clean data C, at epoch n, the artifact template B has to be subtracted from artifact contaminated data A. The mathematical principle for creating the artifact template at the n-th epoch ( $B_n$ ) is:

$$B_{n} = \frac{1}{k} \sum_{i=n-\frac{k-1}{2}}^{n+\frac{k-1}{2}} A_{i}$$

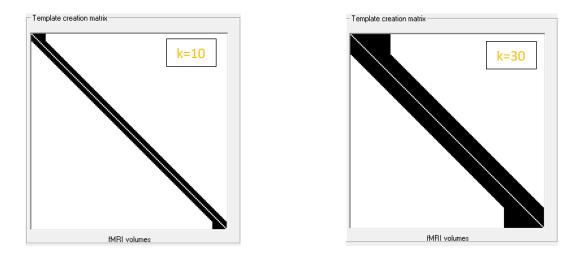
$$B_{n} = \frac{1}{k} \sum_{i=1}^{k} A_{i} \text{ for } n \le (k-1)/2$$

$$B_{n} = \frac{1}{k} \sum_{i=m-k+1}^{m} A_{i} \text{ for } n \ge m - \frac{k-1}{2} + 1$$

,where m is the number of fMRI volumes recorded, k is the number of artefact volumes used for template creation (averaging). Once an artifact template was created for every epoch, the final subtraction can be performed:

$$C_n = A_n - B_n$$

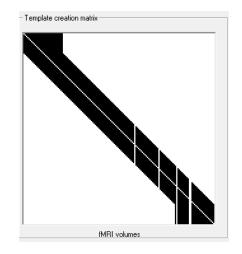
For example, to clean the epoch indicated by the green shaded box in the image above, the surrounding epochs – indicated by yellow color – have to be averaged first (here the averaging constant is  $\mathbf{k} = 10$ ). After the template is created, it is subtracted from the recorded data (at each channel independently) to obtain artefact free data. Next this procedure is repeated for all epochs related to fMRI acquisition as indicated in the template creation matrix on the left:



Note how the template creation matrix on the right changes when the number of artifact occurrence used for averaging is increased to k = 30.

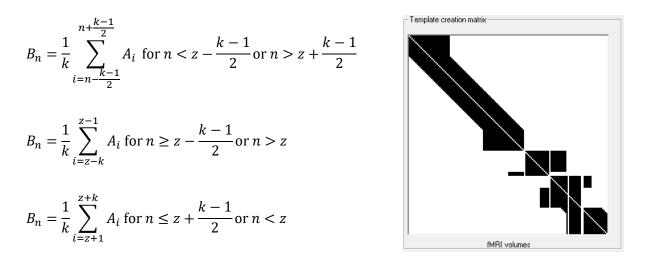
#### Allen et al., 2000 Modified

This method is available only if a matching realignment file is provided. Also, the changes in filtration will be visible only if there is significant movement in the data. In the modified Allen method, the epochs where subject's movement exceeds the threshold (a recommended value for the movement threshold is 0.3 mm) are not taken into account during artefact template creation, as shown in the template creation matrix at the right. Note the vertical white lines, which indicate the moments were movement exceeded the threshold. As a results, the data will be filtered but less artifact occurrences will be used for template creation in the (temporal) vicinity of a movement area.



#### Moosmann et al., 2009

This method (Moosmann et al., 2009) is also available only if a matching realignment file is provided. The adjustment of the template creation matrix is much more advanced than in the modified Allen method and can be explained by following equation:



, where all parameters are defined as above and z means an epoch number when movement exceeds the threshold. The result of this operation is shown in the template creation matrix above.

Note that this operation results in a different shape of the template create matrix. Importantly there are now also horizontal white lines. This indicates that there was more than one epoch affected by movement. In such a situation the matrix is transformed to fit to the largest movements and smaller movements are treated as in the Allen et al., 2000 Modified method.

#### **B.** Reduction of heart beat-induced ballistocardiogram (BCG)

This artifact is one of the most challenging artefacts in the EEG when acquired simultaneously with fMRI. The artifact is related to following phenomena:

#### • Head movement due to blood ejection by the heart

Each heartbeat results in a very slight movement of the whole body due to its inertia. In most of the cases we are not able to notice this. This tiny movement results in

#### Tutorial 11 – Simultaneous EEG-fMRI

movement of the electrodes, which in turn results in local magnetic field changes. Considering the fact that in an MR scanner the static magnetic field is usually 3T this normally negligible effect induces an artifact of amplitude larger than 100  $\mu$ V.

#### Arterial pulsation

The blood flow induced by the heart beat can introduce an additional factor of the artifact. If some blood vessels are close to the skin surface the changes in blood volume result in skin pulsation. This pulsation also moves electrodes up and down in the rhythm of the heart beat.

#### Hall effect

Blood by itself is an electrically conductive fluid, therefore the changes in blood flow velocity related to the heartbeat add another factor altering the artefact.

All aforementioned factors contribute to the ballistocardiographic artefact and make it particularly difficult to determine and reduce. It is worth mentioning that this artifact, as related to the heartbeat, cannot be controlled and varies during the time of experiment. A different approach to fMRI gradient removal has to be used to reduce its influence on data. In the literature many different approaches (both software and hardware) can be identified, please refer to Abreu and colleagues work (2018) for more details. Here we focus on spatial filtration where individual artifact topographies are estimated using the PCA method. Also, we recommend to use a surrogate model to separate brain signal from artifact subspace. More details about this method can be found in the Besa Research Tutorial 9.

#### C. Example data

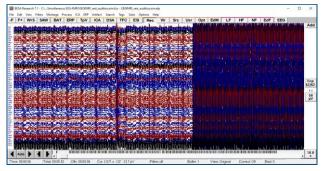
The example data set provided with BESA Research installation is a mixture of real and simulated data. We used a 64-channel EEG data recorded during a simultaneous EEG-fMRI session. It was a 5-minute resting state session with eyes open. We added simulated ERP data to this recording. It consists of two bilateral dipolar sources simulating auditory cortex activity. The dipoles positions in brain were set as described by Gutschalk and colleagues (2002). Waveforms were replicated from the *Low* condition ERP in the Auditory Intensity experiment also available in the example directory. The ERPs were added to the resting state

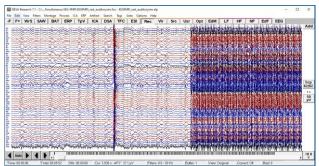
#### Tutorial 11 – Simultaneous EEG-fMRI

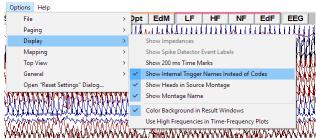
EEG, thus simulating an ERP experiment in fMRI. The first ERP is present after the start of the fMRI session, the inter-stimulus interval was 1.5 s with a jitter of ±200 ms.

Consider the fact that in a real ERP experiment, the ERP signal would be a pure brain signal independent of MRI scanning. Therefore, using the provided data example, we can see what the influence of the MRI scanner environment on the data is and assess filtration results by comparing the filtration outcome with the pure stimulation signal.

- From the folder /Examples/Simultaneous EEG-fMRI/ open data set EEGfMRI_rest_auditorysim.foc. It contains 64 EEG channels. Data is very noisy from the beginning, but from around 12 seconds the data is not readable – this is the time when the fMRI sequence starts.
- Press the EDF button and specify Low Cutoff filter of 0.5 Hz, 6 dB/Octave, forward and High Cutoff filter of 30 Hz, 12 dB/Octave, zero-phase shift. Note that the data is still not interpretable because of the fMRI gradient artifact.
- 3. Goto Time 00:00:12. For this data set internal trigger names are not available, so select Options / Display / Show Internal Trigger Names Instead of Codes to uncheck this option if checked. Now you will see two trigger codes 8015 and 100. The first indicates the start of fMRI volume acquisition, the other one the simulated ERPs.

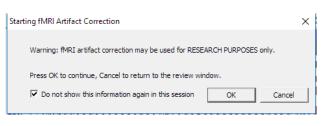




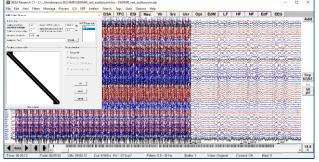


#### Tutorial 11 – Simultaneous EEG-fMRI

- 4. Select Artifact / fMRI Artifact... to open the dialog where all the settings for fMRI artifact filtration can be set. If you work with the international version of BESA Research, you will need to acknowledge that this can be used for Research purposes only before you are able to use this feature. Please check the checkbox and press OK on the displayed notification window.
- 5. You will see the fMRI Artifact Removal dialog. Note that fMRI Trigger code and length of fMRI volume was automatically detected from the data. The automatic detection of length works only for continuous data. If you use so-called *sparse* fMRI acquisition you have to adjust this value manually. The Number of artifact occurrence averages is set to a default value. Switch Removal method to Allen et al., 2000.
- 6. The template creation matrix is filled instantaneously. Press Apply to perform artifact removal. The first six seconds of the artifact are not removed. This fMRI sequence starts with dummy scans that do not have associated triggers. The fMRI images are not collected for these, so no stimulation should be performed either. Press OK to close dialog box.

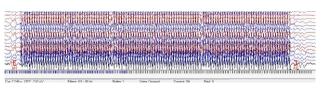


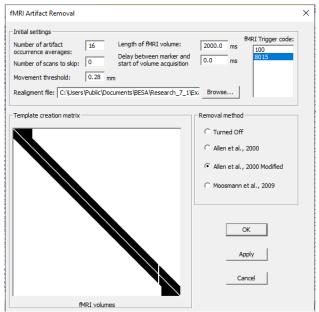
MRI Artifact Removal	-	
Initial settings Number of artifact occurance averages: Number of scans to skip: Movement threshold: Realigment file:	I6         Length of fMRI volume:           0         Delay between marker and start of volume acquisition           1.00         mm	2000.0         ms         IMRI Trigger code:           100         100           0.0         ms           Browse
Template creation matrix-		Removal method  C Turned Off  Allen et al., 2000  Allen et al., 2000 Modified  Moosmann et al., 2009  OK  Apply  Cancel
ſ	MRI volumes	



#### Tutorial 11 – Simultaneous EEG-fMRI

- 7. While keeping the left mouse button pressed, drag the mouse over this noisy part of signal, to highlight it. Then right click within the yellow marked area and select the Define as artifact option from the popup menu.
- 8. Go to 00:04:44. There is a slight movement starting at 00:04:49. Press Artifact / fMRI Artifact... In the displayed window change the value of Movement Threshold to 0.28 mm. Press Browse... button to select Realignment file. In the dialog box that comes up, choose EEGfMRI_rest_auditorysim.txt and press Open. Now two additional methods are available: Allen et al., 2000 Modified and Moosmann et al., 2009.
- Change the method to Moosmann et al.
   2009 and press Apply. Note that the data is again recomputed using the new algorithm. Press OK to close the window.

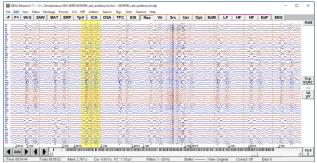




fMRI Artifact Removal	×
Initial settings         Number of artifact       16         Length of fMRI volume:         occurrence averages:         Number of scans to skip:       0         Belay between marker and start of volume acquisition         Movement threshold:       0.28         Realigment file:       C:\Users\Public\Pocuments\BESA\Research_7_1\	2000.0         ms         fMRI Trigger code:           100         100         8015           0.0         ms         8015           Exi         Browse         100
Template creation matrix	Removal method         C       Turned Off         C       Allen et al., 2000         C       Allen et al., 2000 Modified         Image: Moosmann et al., 2009         OK         Apply         Cancel
, fMRI volumes	

#### Tutorial 11 – Simultaneous EEG-fMRI

10. Now we will reduce the influence of the ballistocardiogram (BCG). First press the EdF button to adjust filters to the BCG frequency band. Specify a Low Cutoff filter of 1 Hz, 12 dB/Octave, zero-phase shift and High Cutoff filter of 20 Hz, 24 dB/Octave, zero-phase shift. For the provided dataset the Fpz channel (last in the list) is also highly contaminated with noise, right click on the channel label and select Interpolate this channel from the popup menu. The BCG is now clearly visible.



#### Tutorial 11 – Simultaneous EEG-fMRI

11. Select Search / Search, Average, View to turn off automatic saving of averaged buffer. Note that the 4th button in the control ribbon changed from SAW to SAV. Highlight one of the BCG occurrences and press the SAV button. Select a radio button to use All channels, Current filter, Current Buffer Width. Leave Threshold on the default value of 60% and Search Range on Whole File. In Options you may uncheck Selected View after Search and Selected view 300.0% width. Press the OK button to start searching.

Search Average View	×
Select Tag Buffer	1
© 1 C 2 C 3 C 4 C 5 Cancel	I.
	-
Channels	
C MZ' C Selected • All	
Filters	
C Default: 2 - 35 Hz Current: 1 - 20 Hz	
Buffer Width	1
C -250 : 150 ms	
Threshold	
▼ 60.00 %	
Detection for SNR > 2.5	
- Search Range	
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Marked Event     C Averaged Tags	
Options	-
Query     Selected View after Search	
Restore filters after search 🗌 Selected view 300.0 % width	
☐ Zero template baseline 🔽 Zero mean	

12. After the search is finished an averaged buffer with averaged BCG is shown on the left side of the screen. Right click on it and select whole segment. The whole buffer will be highlighted. Right click again on the highlighted area and select Define Artifact Topography.



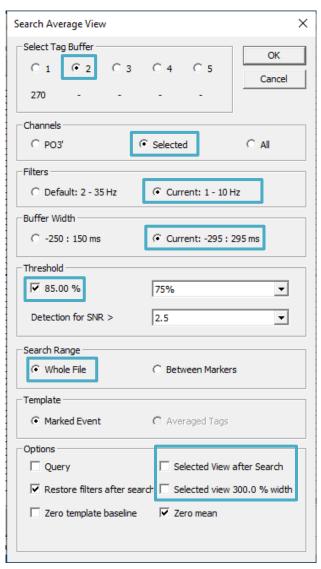
#### Tutorial 11 – Simultaneous EEG-fMRI

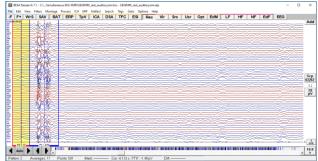
13. The Artifact Correction window will be displayed. Check the EKG check box. When a warning is displayed, check **Do not** show this message again and press OK. Change the No of topographies from the drop-down menu adjacent to EKG. The number of topographies has to be selected with caution, however here select a number with value close to **1%** (i.e. 5 components). Note that there will be a warning that using too many coefficients can lead to remove non-artifact signal. The BCG is a very strong and complex artifact and it is ok to use a slightly stronger filtration; thus press the **Yes button** to continue. After this operation the data should look much better. Press **OK** to close the dialog. Increase scaling using the up arrow on the keyboard. Verify if data is BCG free; if not the number of used coefficients used during artifact correction can be adjusted.

Artifact Correction	?	$\times$
Select Topographies Estimate Signal		
Select No. of topographies category (% variance/ICA chan)		
VEOG 📃		
🗖 Blink 🚽		
🔽 EKG 5 (0.69) 💌 Map		
🗖 Other1 📃		
🗆 Other2 📃		
Load Save		
OK Cancel	He	lp

Warning		×
Note that using too many coefficients non-artifact signals from the data! Plea Do you want to continue?		
	Yes	No

- 14. Now you can see that the F2 channel does not carry a correct signal (the cable was broken). Right click on the channel label and select Interpolate this channel from the popup menu. Press the EdF button to adjust filters to the blink frequency band: Low Cutoff filter of 1 Hz, 6 dB/Octave, forward and High Cutoff filter of 10 Hz, 12 dB/Octave, zero-phase shift. Go to 00:01:30. You will see some clear blinks. Left Click on the Fp1 label to select this channel and highlight data contaminated with one of the blinks. Press the SAV button again. All the settings should be used as previously, except for Channel selection, where Selected should now be checked. Note that the **second buffer** will be used and that for single channel based searching the default threshold is 85.00%. Press **OK** to start searching.
- 15. After the search is finished average buffers with averaged BCG and blink are shown on the left side of the screen. Right click on the first displayed buffer indicated with red color and select whole segment. The whole buffer will be highlighted. Right click again on the highlighted area and select Define Artifact Topography.





Tutorial 11 – Simultaneous EEG-fMRI

- In the displayed Artifact Correction dialog box check the Blink checkbox, leave No of topographies at 1, and press OK. Data can be now considered artifact free.
- 17. Press the ERP button, select EEGfMRI_rest_auditorysim.PDG file and press OK. Switch to the Artifact tab and press Start Scan. The threshold values may be left at default values.

- 18. Switch to the Average tab and press Average. Press the Yes button on the display dialog to turn off the artifact correction during averaging process. We will perform artifact correction in the next step.
- **19.** Save the result of averaging using the file name

### EEGfMRI_rest_auditorysim_av2.fsg.

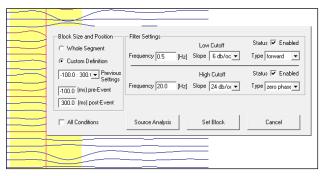
Press **OK** on the displayed dialog.

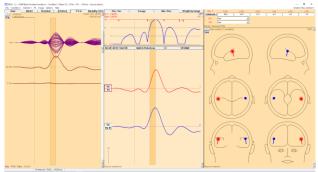
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Artifact Correction Switched On for Averaging	×
Artifact Correction may distort brain topographies. For discrete source analysis it is preferable to use uncorrected data and include artifact topographies in the source n	nodel.
Press: Yes to turn off artifact correction No to use it nevertheless Cancel to quit averaging	
Yes No Cancel	

#### Tutorial 11 – Simultaneous EEG-fMRI

- 20. Open the Besa Simulator \ model.avr file. This is a modeled ERP data which was used during simulation. Press CTRI-L on the keyboard. In the displayed dialog, press Browse... in the section Coregistration Select file (*.sfh). file EEGfMRI_rest_auditorysim.sfh file from the experiment's main folder. Highlight a block of data and right-click on it. Select Source Analysis from popup menu. Select an interval from -100 to 300 ms and set filters as specified: low cutoff filter of 0.5 Hz, 6 dB/Octave, forward and high Cutoff filter of 20 Hz, 24 dB/Octave, zero-phase shift.
- 21. In the Source Analysis window, insert two dipolar sources by double clicking twice into the head schemes in the right area of the window. Select a fitting interval of 70 ms to 95 ms. Select Individual FEM as head model. Press the All fit button and then the Start fit button. Dipoles are located perfectly in the auditory cortex and the residual variance is almost equal to zero.





## Tutorial 11 – Simultaneous EEG-fMRI

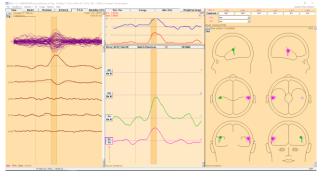
- 22. Minimize the Source Analysis window and go back to the main BESA Research Window. Press the -F button to go back to the averaged file: EEGfMRI_rest_auditorysim_av2.fsg.
- 23. You will note that ERPs are highly contaminated with artifacts. Press Artifact
  / Load... and select the previously automatically created file:
  EEGfMRI_rest_auditorysim.atf. Press Artifact / Options... and switch Model of Brain Activity to Surrogate (use the default model: BR_Brain Regions_LR.bsa). Press OK to close the dialog.
- 24. Increase the High cutoff filter to 20Hz, 24dB/Octave, zero-phase shift. Highlight a block of data by left mouse-drag and right-click on it. Select Source Analysis from the popup menu. Select an interval from -100 to 300 ms. Make sure that the following filters are specified: low cutoff filter of 0.5Hz, 6dB/Octave, forward and high Cutoff filter of 20Hz, 24dB/Octave, zero-phase shift.

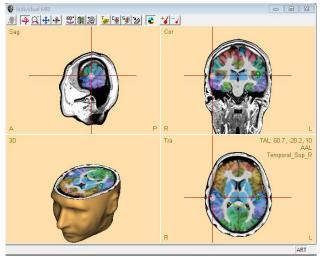
Artifact Correction	?	$\times$
Select Topographies Estimate Signal		
Surrogate Model: Regularization		
Artifact (%): 0.0 Brain (%): 2.0		
Surrogate Model: PCA threshold Min. Variance (%): 1.0		
Model of Brain Activity C Adaptive I Surrogate C None (SSP - Regression) Surrogate dipole model (*.bsa) TogateModels\BR_Brain Regions_LR.bsa Browse		
OK Cancel	Help	

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Tutorial 11 – Simultaneous EEG-fMRI

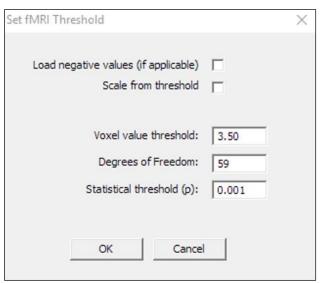
- 25. Note that the previous model does not explain all of the signal. Disable both dipoles from the previous model by clicking on the **On button** beside each dipole waveform to switch to Off. Insert two new dipolar sources by double clicking twice in the head schemes in the right window area. Select a fitting interval from 70 ms to 95 ms by left mouse-drag over the waveforms. Make sure that Individual FEM is selected as head model at the top right. Press the All fit button and the Start fit button. Dipoles are located in the auditory cortex, but in slightly different positions. Residual variance is smaller than before but still not close to zero due to noise in the data.
- 26. Press A on the keyboard to show the anatomy. Press on the toolbar to switch to multiple view. Press Shift-A to display the brain atlas. Right-click on the 3D area and select Options... from the popup menu. Change Brain atlas to AAL, visualization mode to brainCOLOR and press OK. Now you can see that one of the sources is in the Rolandic operculum, and the second one in the Superior Temporal Gyrus.



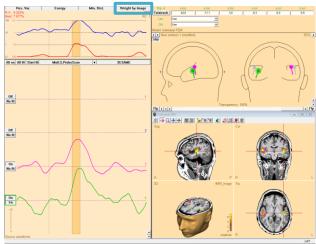


Tutorial 11 – Simultaneous EEG-fMRI

27. Press Image / Import fMRI. Change filter to ANALYZE/NIfTI files and select sound vs silence.nii file. On the next dialog box uncheck Load negative values, set Degrees of Freedom to 59 and statistical threshold to 0.001. The voxel value threshold will adjust automatically. Press OK.



28. Press Shift-A to turn off the atlas. Note that dipoles are not matched perfectly with fMRI results, however they indicate the same region. In the button row above the Global Field Power section (top center part of the window) press the Weight by Image button. Again, press the All Fit button and the Start Fit button. Turn the brain atlas off again (Shift-A) and note that both sources are now located in the superior temporal lobe.



D. Good practice

Please keep in mind that simultaneous EEG-fMRI recording is a difficult yet powerful technique. The following rules could help one to perform a successful experiment:

• For an ERP experiment remember to introduce temporal **jitter between trials** (e.g. a random value in the range of ±200 ms). Also, applying a pure EEG or a pure fMRI paradigm will probably have the effect that one of the modalities will not show satisfying results. Proper paradigm preparation is essential for success. Some further guidelines can be found here: (Rusiniak et al., 2013a).

Tutorial 11 – Simultaneous EEG-fMRI

- Inform your subject how important it is not to move.
- Keep electrode to skin impedance as low as possible.
- The EEG-fMRI recording session should be long enough to allow for proper artifact creation. Usually the experiment should last at least 6 minutes.
- At the same time try to **limit the time of experiment to a minimum** and preferably perform EEG-fMRI registration before other sequences to limit movement due to an inconvenient supine position.
- From the standard position, move the **subject about 4 cm towards caudal direction** to reduce artifacts: The MRI laser crosshair should be not in the Nasion position but in the middle of forehead (Mullinger et al., 2011).
- Especially for the first few registrations repeat the experiment outside of the MR bore to compare results.

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Tutorial 11 – Simultaneous EEG-fMRI

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Rusiniak, M., Wolak, T., Lewandowska, M., Cieśla, K., Skarzynski, H., 2013b. The relation between EPI sequence parameters and electroencephalographic data during simultaneus EEG-fMRI registration: an initial report., in: ESMRMB 2013 Congress, Book of Abstracts, Saturday. Presented at the ESMRMB, Springer, Toulouse, p. 661. https://doi.org/10.1007/s10334-013-0384-5

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

What does BESA Research provide?

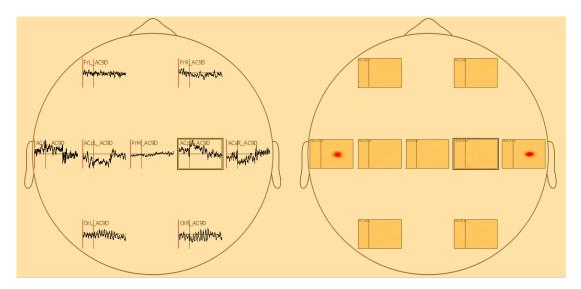
- ✓ Time-Frequency Analysis
 - Complex Demodulation
 - Wavelets (Morlet / Mexican Hat)
- ✓ Connectivity Analysis
 - o Coherence
 - o Imaginary Part of Coherency
 - Phase Locking Value
 - Non-parametric Granger Causality
 - o Non-parametric Partial Directed Coherence
 - Non-parametric Directed Transfer Function
- ✓ Multiple Source Beamforming
- ✓ Dynamic Imaging of Coherent Sources (DICS)

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

A. Introduction to Time-Frequency Analysis

Recently, an increasing number of papers on oscillatory coupling between brain regions and on time-frequency analysis of human EEG and MEG data has been published. BESA Connectivity provides several tools for fast and user-friendly time-frequency analysis.

Due to the fact that oscillatory components in EEG or MEG signals are not necessarily timelocked to an event, these components will be cancelled out when averaging across trials and therefore, will not be represented in an ERP / ERF analysis. Time-frequency analysis is able to reveal signal that are related to a stimulus, but not perfectly time-locked. It reveals which frequencies have the most power at specific latencies and how their phase synchronizes across time. The decomposition of a time-series into complex numbers for specified latencies and frequencies provide the magnitude and phase angles of the oscillations. To extract this information, a sliding time-window is used that compares the EEG / MEG signal to a reference template at multiple frequencies. This multiplication of a time-series with a windowed transformation function is a mathematical operation called convolution. Common templates are Morlet and Mexican Hat wavelets, as well as sine and cosine waves.



BESA Connectivity uses TSE (temporal-spectral evolution) plots to visualize changes in power or amplitude over time normalized to the mean power or amplitude of the baseline epoch for the respective frequency (see figure above).

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

B. Introduction to Connectivity Analysis

Brain connectivity is based on two central principles that form the functional organization of the brain. These principles are characterized by segregation and integration of information being processed. Modes of brain connectivity of large-scale brain networks are divided up into three separate, but related aspects: structural connectivity, functional connectivity and effective connectivity.

- Structural connectivity, also called anatomical connectivity refers to the anatomical structure of the brain.
- Functional connectivity is defined as the temporal correlation of spatially separated brain areas.
- Effective connectivity combines anatomical and functional connectivity, as it provides a measure for the directional influence of one neural system over another.

BESA Connectivity provides the following methods to estimate functional or effective connectivity collected from EEG and MEG recordings:

- Coherence (Rosenberg et al., 1989)
- Imaginary Part of Coherency (Nolte et al., 2004)
- Phase Locking Value (Lachaux et al., 1999)
- Non-parametric Granger Causality (in the frequency domain; Granger, 1969; Geweke, 1982)
- Non-parametric Directed Transfer Function (Kaminski and Blinowska, 1991)
- Non-parametric Partial Directed Coherence (Baccala and Sameshima, 2001)

Non-parametric Granger causality, Directed Transfer Function and Partial Directed Coherence are based on a non-parametric estimation of the transfer matrix (Wilson, 1972; Dhamala et al., 2008).

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

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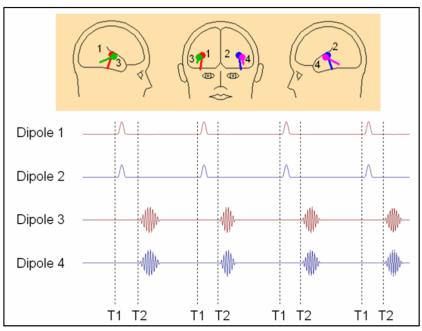
Rosenberg, J., Amjad, A., Brillinger, D., Halliday, D., 1989. The Fourier Approach To The Identification Of Functional Coupling Between Neuronal Spike Trains.

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Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

C. Simulation of evoked activity and oscillations in the auditory cortex

The simulated data sets can be found in subfolder Learn-by-Simulations\AC-Coherence of the BESA Research Examples folder. The data have been generated by superimposing a real



continuous 27-channel EEG-recording with the simulated activity of two pairs of bilateral sources in the auditory cortex (AC). The purpose of this combination was to understand the sensitivity of time-frequency analysis to reveal oscillatory activity and coupling in a realistic ongoing EEG. The figure below displays the underlying source configuration. Sources 1 & 2 were simulated as simultaneous evoked monophasic activity with a time-locked onset of 50 ms after a hypothetic auditory stimulus (trigger T1). The duration of this monophasic activity was 150 ms, and the amplitude was chosen to generate a signal of 10 μ V at Cz. Dipoles 3 & 4 simulate a more antero-lateral secondary area of AC with an orientation differing by about 30° from dipoles 1 & 2. They reflect oscillatory activity (trigger T2) that jitters in latency (300-550 after T1), duration (300-450 ms) and frequency (20-28 Hz). The envelope of this oscillatory activity had the same amplitude in all 200 simulated trials. The amplitude generated at electrode Fz was 5 μ V (file AC_Osc5.foc) and 20 μ V (file AC_Osc20.foc), respectively. The oscillation of dipole 4 follows that of dipole 3 with a constant delay of 5 ms.

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

D. Prepare Data for Time-Frequency Analysis

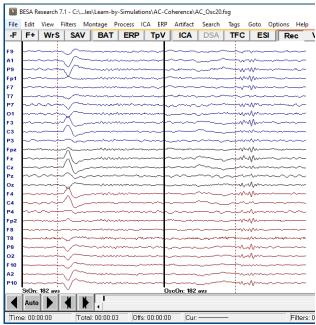
- Open file Learn-by-Simulations\AC-Coherence\AC_Osc20.foc in the BESA Research Examples folder. Note that e.g. at electrode C3 the evoked monophasic response locked to trigger 1 is just visible on top of the background noise. The oscillatory activity following trigger 2 can be observed as well.
- In order to better understand the properties of the simulated activity, we load the averaged evoked response file. Open file AC-Coherence\AC_Osc20.fsg.

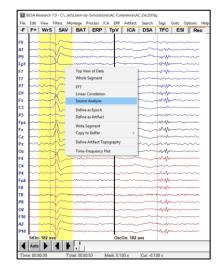
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# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 3. The first segment contains the average triggered by the stimulus onset, the second averaged segment is time-locked to the onset of the jittering oscillations. Relative to stimulus onset, the induced oscillations cancel out in the average. The jitter in the frequency range of the oscillation (20–28 Hz) also reduced the signal average relative to the onset of the oscillation. Note that the wave shape of the monophasic activity is altered by low cutoff filtering applied to the average to reduce slow drifts in the data.
- 4. To see how the averaged surface activity is transformed into brain space, we use the source analysis module. Left-drag over the first segment. Right-click and select Source Analysis. For this segment, we use an interval from -50 to 1000 ms and choose a Low Cutoff filter of 1.6 Hz, 12 dB/Oct zero-phase and set the High Cutoff filter to 40 Hz, 24 dB/Oct, zero-phase.



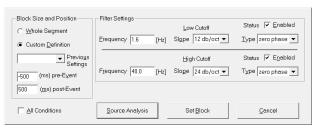


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All Conditions	Source Analysis Set Block Qancel

# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

 The source analysis window opens. Minimize this window to load the second segment as well, but now select an epoch from -500 to +500 ms relative to the onset of the oscillation.

6.	Select File / Open Solution to load the
	predefined solution AC9D.bsa.



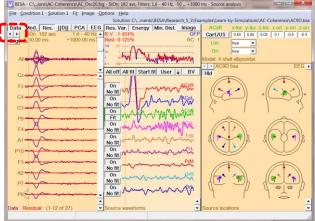
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7. The model contains the two pairs of dipoles that were used for the simulation. In addition, five probe sources are included that pick up background EEG activation. In condition OscOn (containing the oscillationlocked averages), dipoles 3 and 4 show small oscillatory activities. The evoked activation in sources 1 and 2 is barely visible, because of its large onset jitter with respect to trigger 2.

		Solution: C:\ments\BESA\Research_5_3\Examples\Learn-by-Simulations\AC-Coherence\AC9D.bs
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# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 8. Use the arrow buttons in the upper left corner to switch to condition 1. The timelocked evoked activity appears only in the related sources 1 and 2. The induced oscillations in source waveforms 3 and 4 cancel out almost completely. The five probe sources show only small background noise activity. Note the almost complete separation of the activities in the different brain regions as compared to their wide distribution in the scalp waveforms.
- 9. We want to use our nine-dipole model as a source montage for the continuous data. Create a source montage from the displayed multiple source model by selecting File / Save Source Montage As.... Note that the source montage not only contains the source coordinates, but also stores the selected regularization constant (set to 1 by default).



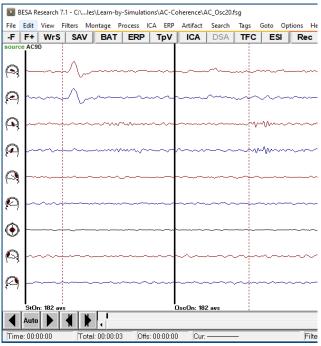
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# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

10. When prompted for a name for the montage to be saved, enter AC9D. Press Save. This will create the new source montage and automatically apply it to the current file in the main window.

Save source	montage			×
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11. Close the source analysis window. The display now shows the averaged data segments transformed into brain space by the generated source montage. Note the advantage of the source montage: instead of the distributed overlap of the different activities at the surface channels, we now obtain largely separated activities of the modeled brain regions with the evoked time-locked activity in source 1 & 2 and the induced oscillatory activity in sources 3 & 4.



# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

12. Next, we want to apply this source montage to transform the continuous data. Press the -F button to switch back to file AC_Osc20.foc. Press the USR button in the push button bar to show all user-montages that are available for this file. Select the predefined montage AC9D.

- 13. The continuous 27-channel EEG data has been transformed to nine source waveforms. We want to use the advantage of having contrasted the activities of the different waveforms to apply timefrequency analysis to the nine source waveforms and analyze the connectivity between them.
- Select ERP / Open Paradigm to load the predefined settings in file Auditory\AC_Osc.pdg in the Paradigm directory.

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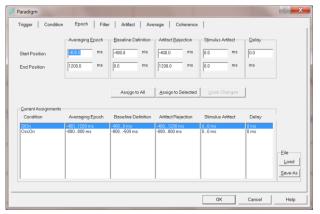
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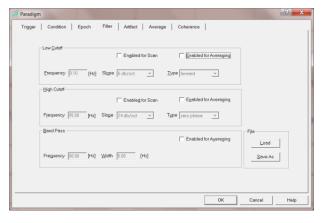
# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

15. Conditions StOn and OscOn were predefined to represent triggers 1 and 2.200 events are detected for stimulus onset and oscillation onset.

Trigger	Condition	poch Filter	Artifact Averag	e Coherence		
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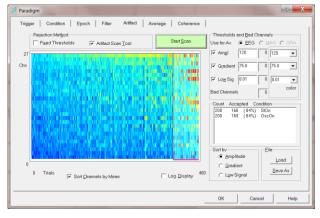
- 16. View the Epoch tab. The epoch settings have been predefined to span a sufficiently long epoch including a baseline of 400 ms for TSE. Note the different settings for both conditions. The later oscillatory onset requires an earlier baseline prior to stimulus onset.
- 17. In the Filter tab no filters are selected, in order to obtain undistorted results of the time-frequency analysis. In the presence of strong low frequency activity, it can be useful to set a low cutoff of 0.2, 0.5, 1, or 1.6 Hz.



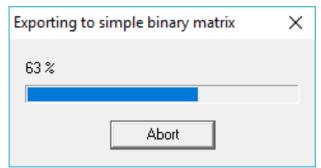


Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 18. Select the Artifact tab and press Start Scan. Artifact epochs are automatically identified and excluded according to the default thresholds displayed in the upper right corner. Sort by amplitude, gradient and low signal by checking the corresponding item in the Sort by box. The suggested threshold settings do not have to be modified for the current file.
- 19. Select the Coherence tab. The Condition drop down menu allows choosing one condition for event-related time-frequency analysis. The time-frequency sampling settings in the Settings box are only relevant if you want to use the built-in Source Coherence module. We can ignore them for the moment.
- 20. Press the button Start Connectivity. Data is exported for BESA Connectivity.

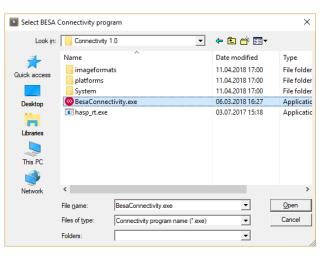


	1	Coherence
gger Condition Epoch Filter	Artifact Average	Contenence
Important note: Time-Frequency Analysis is for	research use only. Results may not l	be used for diagnostic purposes!
<ul> <li>Time Frequency Analysis</li> </ul>	Target Condition	Use Control Condition
	StOn	<b></b>
C-111	,	Destand Courses
Settings		Regional Sources
Frequency and Time Sampling	2.0 Hz, 25 ms 💌	Radial <u>Orientation</u>
Lower Frequency Cutoff	4.0 Hz	C All Traces
Higher Frequency Cutoff	50.0 Hz	C First Orientation
Scan for <u>A</u> rtifacts	Start Connectivity	Start Time-Frequency Analysis



# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

21. Subsequently, a file dialog is opened to select the BESA Connectivity program. Please select the file BESAConnectivity .exe in the installation directory of BESA Connectivity (default: C:\Program Files\BESA\Connectivity 1.0\) and press Open. Note that this step only needs to be performed once, when starting BESA Connectivity for the first time. (To change this assignment at a later stage, hold down the Shift key when clicking the Start Connectivity button).



#### E. Time-Frequency Analysis with Simulated Data

 BESA Connectivity will open. Press Accept in the welcome screen.



 When BESA Connectivity is opened for the first time, the project folder needs to be defined. This is the folder where BESA Connectivity stores and reads all project and result data. A dialog box appears. Press OK to confirm the pre-selected folder.

 Select the BESA Connectivity Data Folder
 ×

 Please select the BESA Connectivity Data Folder.
 All project data will be saved to this folder.

 Note: If needed, you may switch between different BESA Connectivity Data folders using the menu item 'File → Select Data Folder'.
 Edit

 C:\Users\Public\Documents\BESA Connectivity\Projects\
 Edit

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

3. A new time-frequency project is started automatically. To compare results from different time-frequency decompositions, also enable Wavelet Transform using Morlet wavelets. Press Next on the bottom right (or hit the space bar).

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Press Load Data for StOn in the interaction window.

5. Please browse to the folder C:\Users\Robert\Documents\BESA\Res earch_7_1\Export and select the AC_Osc20_StOn_AC9D.generic file we previously exported for condition "StOn". Press Open.

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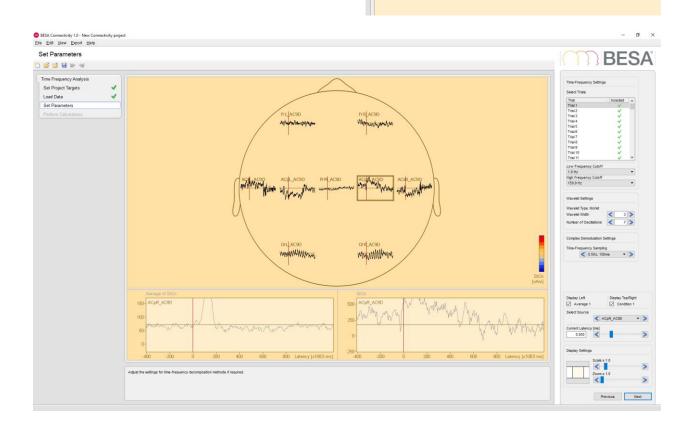
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Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

6. A summary of the loaded file will be displayed in the main window and a green tick-mark will appear next to the Load button. Press Next on the bottom right (or hit the space bar) to go to the next work step.

Condition name (from file):	StOn
Filename:	C:/Users/Robert/Documents/BESA/ Research_7_0/Export/ AC_Osc20_StOn_AC9D.generic
Number of trials:	168
Number of channels:	9
Sampling rate:	320 samples/s
Epoch start:	-400 ms
Epoch end:	1200 ms
Epoch length:	1600 ms
Pre-stimulus interval:	400 ms
Baseline start:	-400 ms
Baseline end:	0 ms
Padding:	2000 ms



Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

7. Waveforms of the currently loaded dataset are visualized in this work step. The signals of all sources for the currently selected trial are displayed in the main window. An average over all trials for the selected source channel is displayed in the left detail window. The right detail window shows the waveform of the current trial for the selected source.

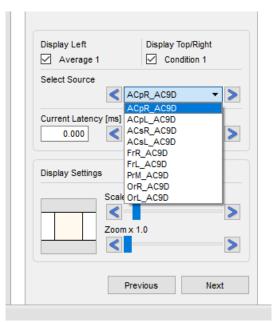
8. Trials containing artifacts and/or noisy signals can be excluded from further analysis by removing the green checkmark icon to the right of the corresponding trial with a left-click. However, this is not necessary for this dataset since artifact rejection was already performed in BESA Research.

After removing unwanted trials, the average is recomputed and displayed in the left detail window for the currently selected source channel.

Select Trials		
Trial	· Included	^
Trial 1	✓	
Trial 2		
Trial 3	✓	
Trial 4		
Trial 5		
Trial 6		
Trial 7		
Trial 8	<ul> <li>Image: A second s</li></ul>	
Trial 9	~	
Trial 10	×	
Trial 11	<u> </u>	¥

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

 Different source channels can be selected in the drop-down menu of the interaction window.



10. The Time-Frequency Settings section provides drop-down boxes to define the frequency interval. We will use the default frequency range of 2.0 Hz to 150.0 Hz.

Low Frequency Cutoff				
2.0 Hz 🔻				
High Frequency Cutoff				
150.0 Hz	•			
Wavelet Settings				
Wavelet Type: Morlet				
Wavelet Width	3 >			
Number of Oscillations	< 7 >			
Complex Demodulation S	Settings			
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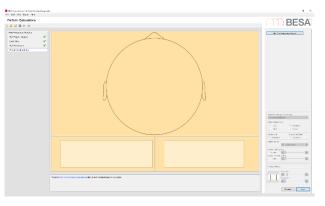
Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

11. If Wavelet Transform was selected in the first work step, the Wavelet Settings section is displayed. The parameter Wavelet Width determines the length of the used wavelets in standard deviations of the implicit Gaussian kernel. The parameter Number of Oscillations defines the width of the wavelets in number of cycles. 'Shorter' wavelets will provide a finer temporal resolution at the expense of a coarser frequency resolution and vice versa.

The **Complex Demodulation Settings** section is displayed if the method was selected in the Set Project Targets work step. For the time-frequency sampling, a compromise has to be made between high frequency and low temporal resolution or low frequency and high temporal resolution. Default time-frequency sampling is intermediate with a frequency sampling of **1.0 Hz** and a time sampling of **50 ms**.

Press Next or hit the space bar to continue.

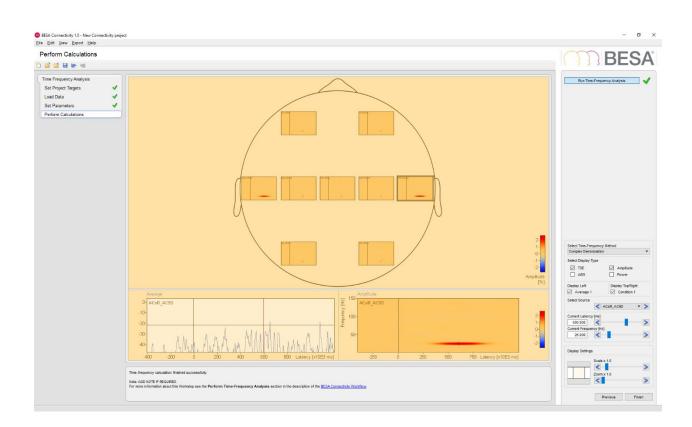
 Press Run Time-Frequency Analysis to start the time-frequency decomposition using the selected methods and defined settings.



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13. An information window appears summarizing the settings for the time-frequency methods. Press OK to continue. Please note that the following calculations can be time-consuming depending on the selected methods, settings, type of data and the data length.

🛞 BESA	Connectivity X
	The settings for running the time-frequency analysis are as follows: Wavelet settings: Use Moriet wavelets. Wavelets width: 3 Wavelet oscillations: 7 Complex Demodulation settings: Time-frequency sampling: 0.5Hz, 100ms
	OK Cancel



Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

14. After all calculations are completed, the Temporal-Spectral Evolution (TSE) visualization for the selected time-frequency method are displayed in the main window. TSE shows the power/amplitude for each time-frequency bin, normalized to the mean power/amplitude of the baseline epoch for that frequency. Whether amplitude or power is used for visualization can be chosen in the Select Display Type section of the interaction window. Absolute power or amplitude values can be displayed when selecting the ABS option.

The left detail window shows the averaged waveform of the currently selected source, whereas the TSE/ABS visualization for the currently selected source is displayed in the right detail window.

For Complex Demodulation, induced activity around 25 Hz appears in channels ACsL and ACsR in the latency range of 400 ms to 800 ms. The short mono-phasic time-locked activity of the sources ACpL and ACpR is revealed in the latency range of 50 ms – 200 ms. Wavelet Transform shows induced activity in a narrower time window from 550 ms to 740 ms.

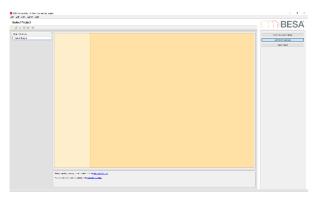
 Press Finish to save and close this timefrequency project. Please enter StOn AC9D as Selected Project name and press Save to store the project and go back to the start-up screen.

Save Project	As		
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Time-Fre	quency Projects		
elected Group:	Time-Frequency Projects	11	Save
elected Project:	StOn AC9D		Cancel
oject Type:	Time-Frequency Projects		Υ.

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

## F. Connectivity Analysis with Simulated Data

 Press Connectivity Analysis to start a new connectivity analysis workflow.



2. The first work step provides an overview of all connectivity methods that are available. selected folder. Well-established methods like Coherence, Imaginary Part of Coherency and Phase Locking Value are supported, as well as state-of-the-art methods like Granger Causality, Partial Directed Coherence and Directed Transfer Function. Please select all methods to enable a comprehensive comparison of all options.

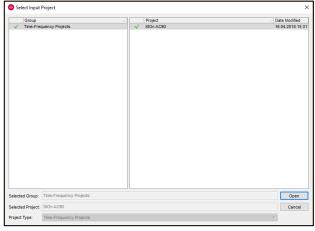
Press **Next** on the bottom right (or hit the space bar) to get to the next work step.

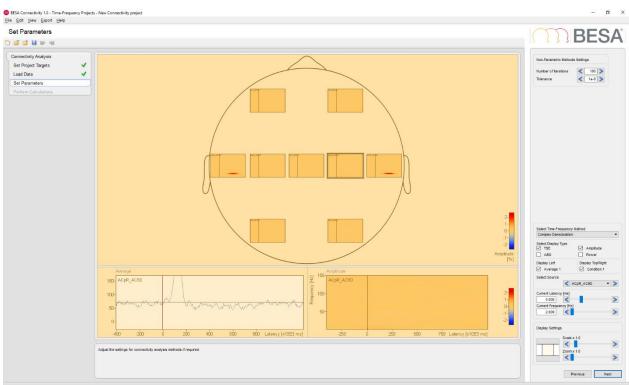
# Select Connectivity Methods ✓ Coherence (Rosenberg et al., 1989) ✓ Imaginary Part of Coherence (Nolte et al., 2008) ✓ Phase Locking Value (Lachaux et al., 1999) ✓ Granger Causality (Granger, 1969) ✓ Partial Directed Coherence (Baccala and Sameshima, 2001) ✓ Directed Transfer Function (Kaminski and Blinowska, 1991)

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

 Press Select Time-Frequency Input Project to load the currently saved timefrequency results for the StOn condition. A summary of the loaded data file is shown in the main window.

Press Next or hit the space bar to continue.





 Results from the selected time-frequency project are loaded and displayed in the Set Parameters work step.

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5. The Non-Parametric Methods Settings section provides parameters for the calculation of the non-parametric methods (Granger Causality, Partial Directed Coherence or Directed Transfer) and is only available if one of them was selected.

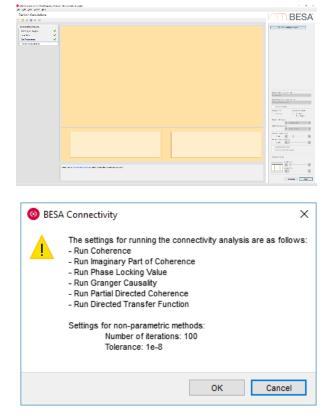
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Number of Iterations	
Tolerance	< 1e-8 >

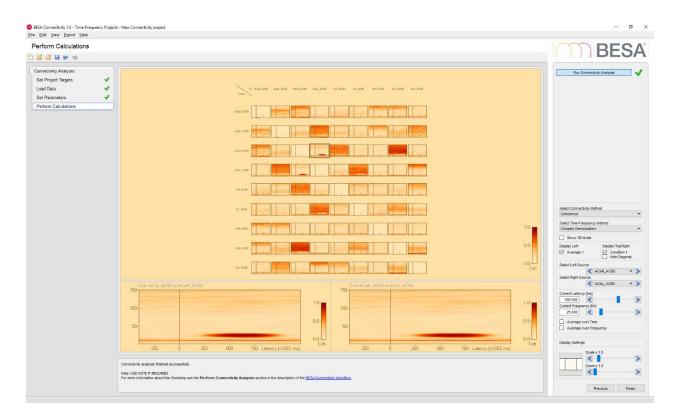
The parameters **Number of Iterations** and **Tolerance** are required during the factorization of the cross-spectral density matrix. For every iteration, the estimation error of the spectral factors is recomputed. If the estimation error is lower than the **Tolerance** threshold, the computation is terminated.

Press Next or hit the space bar to continue.

 Press Run Connectivity Analysis to start the connectivity analysis using the selected methods and defined settings.

7. An information window appears summarizing the selected connectivity methods as well as the specific settings. Press OK to continue. Please note that the following calculations can be time-consuming, particularly if one or more non-parametric methods have been selected.





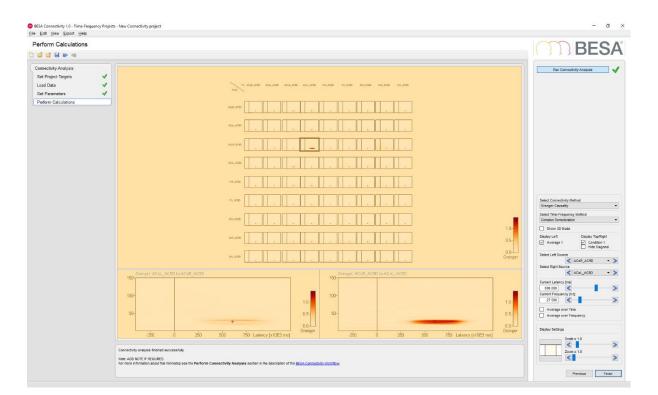
8. After all calculations are completed, the results of Coherence based on Complex Demodulation are displayed as a matrix view. Normalized power spectral densities are displayed on the diagonal of the matrix.

The detail windows show the connectivity between the selected pair of sources. Since Coherence is a symmetric measure, it does not provide information regarding the direction of information flow between any pair of signals. Therefore, the displayed matrix is also symmetric.

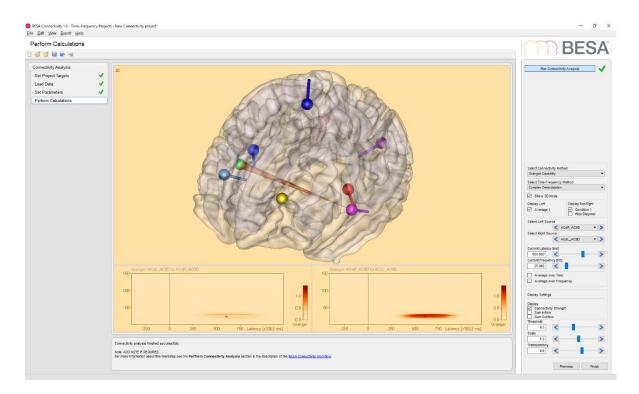
Choose ACsR in the **Select Left Source** drop-down box and ACsL in the **Select Right Source** drop-down box. As expected from the simulation, high correlation between the two channels is shown from 400 ms to 800 ms at a frequency of 25 Hz. There is practically no coherence with all other channels.



**9.** Select Imaginary Part of Coherency in the **Selected Connectivity Methods** drop-down box. This method is able to determine the direction of information flow and returns positive values to indicate information is flowing from source ACsR to ACsL.



10. Select Granger Causality in the Selected Connectivity Methods drop-down box. Granger Causality is rather insensitive to noisy signals and will provide stable and reliable results even for poor signal-to-noise ratios. Furthermore, Granger Causality results show less spurious connectivity among all sources.



- 11. Select Show 3D Mode in the interaction window. The main window will switch to a 3D view of a transparent brain with all sources and arrows between connected sources. Select a current latency of 600ms and current frequency of 25 Hz to visualize the Granger Causality for the simulated induced activity. To increase the size of the arrows, please use the Scale slider in the Display Settings sections. There is only one prominent arrow indicating the information flow from source ACsR (green dipole) to source ACsL (pink dipole).
- **12.** Press **Finish** to end and save the current project. Save it under an appropriate name.

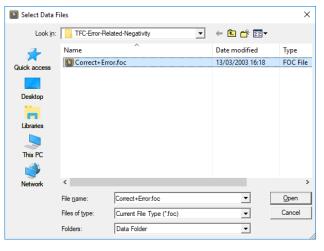
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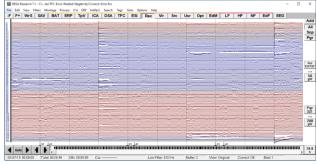
Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

#### G. Time-Frequency Analysis with Error-Related-Negativity Data

We will now move to a real data set of an experiment on error-related negativity. It consists of a 128-channel EEG recording where bilateral visual stimuli were presented to the subject with a specific discrimination task. The subject was supposed to respond with a mouse click as fast as possible. Right and left hand response trials were mixed to obtain a large number of ERN trials (cf. Luu P, Tucker DM. Clin. Neurophysiol. 2001, 112:1295-1306).

- Go back to BESA Research and open the data set Correct+Error.foc in subfolder TFC-Error-Related-Negativity of the Examples folder. This data set was converted from concatenated ASCII segments with intervals of 1s before and 2s after each visual stimulus.
- 2. The first block of the file contains correct responses (stimulus trigger 31, response trigger 41), the second block the erroneous responses (triggers 32 and 42). If you don't see these trigger numbers, then go to Options/Display and switch off Show Internal Trigger Names Instead of Codes. The concatenation of different segments without segment markers causes jumps and trailing artifacts from the boundaries when filtering the data. We will adjust this filter in the paradigm definition.





# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 3. Press the Usr button in the control ribbon and select the source montage ErN. Data are transformed to 11 sources, which model the following brain areas:
- Visual cortex (Vi) 3 sources
- Basal temporal lobes (Tb) 2 sources
- Motor cortices (MC) 2 sources
- Cingulate gyrus (Cg) 2 sources
- In addition, two dipoles were modeled in the left and right eye to account for blink artifacts in the data.
- Select ERP/ Open Paradigm and open the predefined paradigm definition file Cognitive\ERN.pdg. This will open the Condition tab of the ERP menu.

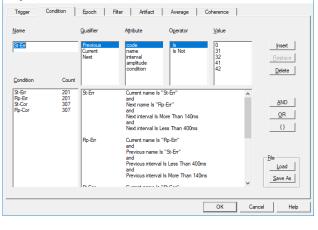
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	Directories	Paradigm Directory	•	

# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

Paradigm

- 5. The Condition tab shows four predefined conditions: correct and erroneous responses (Rp-Cor & Rp-Err, mouse clicks), and the preceding visual stimuli (St-Cor & St-Err). By appropriate logical expressions, contingent stimuli and responses have been selected which have a reaction time window of 140 to 400 ms. 201 error trials and 307 correct trials fulfill these conditions.
- 6. Go to the Filter tab. The slow background activity and these artfacts can be sufficiently reduced by switching on a zero phase low-cutoff filter of 1.6 Hz. As we are interested in activity > 4 Hz, the slow frequency artifacts will not cause a problem in downstream analysis.
- 7. Switch to the 'Coherence' tab. In the 'Condition' drop down menu, select St-Err if not yet preset. Check the box for Use Control Condition and select St- Corr for this condition. Thus, we will analyse the correct responses as well as the erroneous responses. Press the Start Connectivity button.



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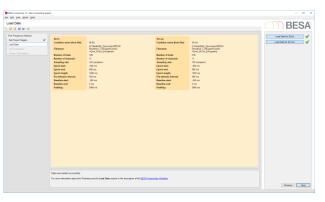
Paradigm ? × Condition Epoch Filter Artifact Average Coherence Enabled for Averaging Finabled for Scan Frequency [1.60 [Hz] Slope 12 db/oct High Cutor Enabled for Averaging Enabled for Scan Frequency 40.00 [Hz] Sloge 24 db/oct -Type zero phase 👻 Enabled for Averaging Load Freguency 40.00 [Hz] Width 4.00 [Hz] Save As OK Cancel Help Paradigm Coherence Trigger Condition Epoch Filter Artifact Average

<ul> <li>Time Frequency Analysis</li> </ul>	Target Condition	See Control Condition
	St-Err 💌	St-Cor 🗸
Settings		Regional Sources
Frequency and Time Sampling	2.0 Hz, 25 ms 💌	Radial <u>Orientation</u>
Lower Frequency Cutoff	4.0 Hz	C All Traces
Higher Frequency Cutoff	50.0 Hz	C First Orientation
Scan for <u>A</u> rtifacts	Start Connectivity	Start Time-Frequency Analysis

# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

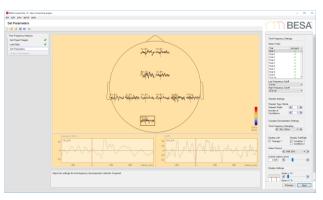
- BESA Connectivity starts if not already open, and automatically launches the Time-Frequency workflow. Check the boxes for Complex Demodulation and Wavelet (Morlet) and define the condition names St-Err and St-Corr. Then press Next.
- Now load the data for St-Err (filename Correct+Error_St-Err_ErN.generic) and St-Corr (filename Correct+Error_St-Cor_ErN.generic). The information for both data sets is shown.





 Condition 1 data is shown. Adjust the high frequency cutoff to 50 Hz to remove effects of the mains frequency at 60 Hz. Change number of oscillations to 5.

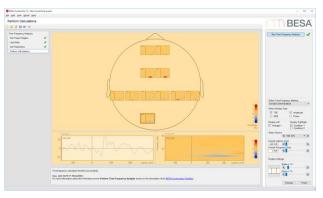
Note: You can click into the Selected Trials field and view individual trials. If you select a trial in the lower half of the display, and then click one below the current trial, you can quickly toggle through all without having to adjust the mouse position. No major artifacts can be seen as we have removed them from analysis.



#### Press Next.

# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

**11.** Run time-frequency analysis. The TSE display of the first condition (error condition) is shown.



 Scale up the display to 1.5. You can notice the strong desynchronization in the visual cortex. Select channel CgP. This has the largest ERN amplitude at low frequencies.



13. Switch condition. The ERN is much less pronounced in the correct response condition. Switch from Complex Demodulation to Wavelet and select channel MCR (right motor cortex). There is synchronization at 6.5 Hz, and a desynchronization at 11-12 Hz. A similar pattern is found in the right visual cortex channel ViR.



Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

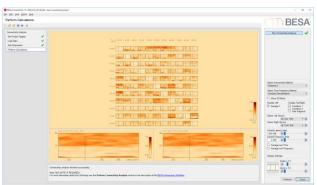
14. Press the Finish button and save the project: Create a group ERN and save the project under the name ERN_2cnd_CD_Morlet

#### H. Connectivity Analysis with Error-Related-Negativity Data

 Start the Connectivity workflow. Select the following connectivity methods: Coherence, Imaginary Part of Coherency, Granger Causality, Partial Directed Coherence. Press Next.

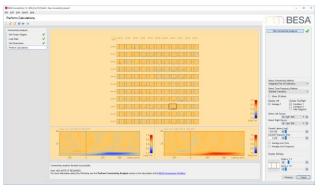


2. In the next work step, select the project ERN_2cnd_CD_Morlet that you stored a minute ago (it should be automatically suggested). It will display the TSE display of the first condition (error condition) again. Press Next and then run the calculation with the default parameters. After finishing the calculations, it will show the matrix plot for coherence of the first condition, using complex demodulation. We can see a lot of pre-stimulus alpha-band coherence.



Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

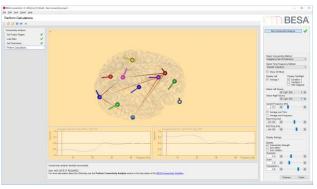
- 3. Switch to Imaginary Part of Coherency. Switch to Wavelet transform. Then click on the channel CgA to show its connectivity with CgP. There is a strong theta band coherence between these channels, from anterior to posterior cingulate. Double-click into the right display to set the cursor at the maximum around 5 Hz and 350 ms.
- 4. Start the 3D mode by checking the Show 3D Mode box. Using the sliders at the bottom, change the threshold to 0.4, the scale to 3, and the transparency to 0.9. Adjust current latency and current frequency to 356 ms and 4.8 Hz. (Note that with wavelets, the frequency steps are nonlinear - the resolution is finer for low frequencies than for higher ones.) You can see varying network associations when changing the latencies and frequencies, but a very strong coupling between the cingulate sources is predominant, as well as connections to the temporal and visual areas. Rotate the brain using left mouse drag; use Shift-Ctrl plus mouse drag to zoom the brain in or out.

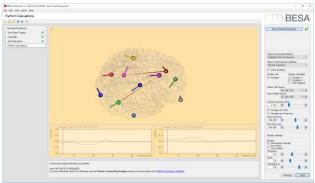




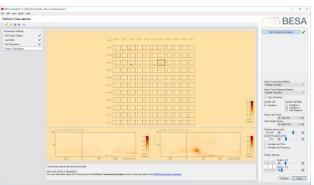
# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 5. Now we want to average over the time interval of interest. Check the Average over Time box. Two new sliders appear. Adjust the time interval for averaging from 296 ms to 440 ms. Now, for 4.1 Hz, the network shows very clearly the connectivity between cingulate, temporal, and visual areas. The motor cortices are not involved at these frequencies.
- Switch to Condition 2. For this condition with correct responses, only very little cingulate coupling is visible.





7. Un-check the Show 3D Mode and the Average over Time boxes again. Then change Connectivity Method to Granger Causality. Peaks are less prominent now. Scale up to 2.0, and check the box Hide Diagonal to hide the auto-spectrum. There is some higher frequency coupling that now becomes apparent, but not in the cingulate areas. Select the channel ViR where it connects with MCR.

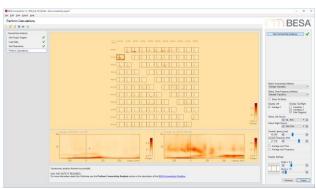


# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 8. The selection window shows a peak from ViR to MCR at approximately 13-14 Hz around 150ms after the stimulus. Switch to condition 1 – interestingly, in the error condition, this connection is almost nonexistent. Switch back to condition 2. Double-click on the peak in the right selection window, then switch on 3D mode to visualize this connection. Lower the threshold to 0.2 and scale up to 6.0. The Granger causality connection only shows this one link; also, the ViR area clearly drives the motor cortex (indicated by the cone that is wider at the ViR end).
- 9. To summarize the activity in the time range where the Granger causality appears, check the box for Average over Time again, and select a time window of 104 to 188 ms. Lower the threshold to 0.1 Again, at 13.4 Hz, the connection from ViR to MCR is apparent, as is some coupling within the visual areas (ViR, ViM, ViL).
- 10. Quit 3D mode again and click on the first channel in the second row to show Granger Causality between ViL and ViM. Scale up to 3.0 It is apparent that there is beta and low gamma band activity before and shortly after the stimulus, flowing from ViL to ViM.

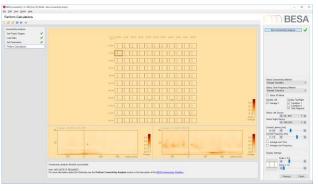




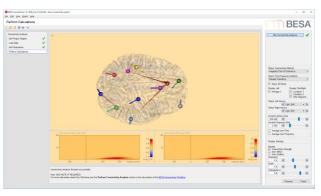


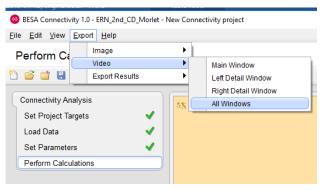
# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

 Switch back to condition 1. Again, the high frequent activity is less pronounced in this condition.



- 12. We now want to export the connectivity data for the error condition in a video. Change the method back to Imaginary Part of Coherency and enable the 3D Mode. Select a frequency of 4.1 Hz, set the threshold to 0.4, scale to 5.0, and transparency to 0.8.
- From the menu, select Export / Video / All Windows.





# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

14. In the dialog, adjust parameters as follows:

Choose HD resolution and select a time interval for the recorded video from 56ms to 656ms. Decrease the duration per latency step to 0.3s.

🞯 Video Export	? ×
Video Settings	Preview
Resolution         HD: (1280 x 720 px)         Start Time [ms]         200.000         End Time [ms]         560.000	
Duration Per Latency Step [s]	
Video Length [s] Rotation [deg] 🗘	
Background Color	
Recording	
	Save Cancel

- 15. Press the **Recording** button. Then press **Save** to store the video to a file on the desktop.Open the video to play in the Media player.
- Back in BESA Connectivity, finish the project and save with the name ERN_2cnd_4Methods.

Save Project	A3			
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roject Type:	Connectivity Projects			~

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

#### I. Multiple Source Beamforming in time-frequency domain

In discrete multiple source analysis, the best source model (i.e. source amplitudes, locations and orientations) is one that minimizes a cost function (basically the residual variance).

Beamforming takes a different approach to image brain activity: Here, the whole brain is scanned point by point. The brain activity at each voxel is estimated by applying a spatial filter to the data. This spatial filter is designed to be fully sensitive to activity from the target voxel, while being as insensitive as possible to activity from other brain regions. This is achieved by constructing the spatial filter in an adaptive way, i.e. it takes into account the recorded data.

The BESA beamformer is a modified version of the linearly constrained minimum variance vector beamformer in the time-frequency domain as described in Gross et al., "Dynamic imaging of coherent sources: Studying neural interactions in the human brain", PNAS 98, 696-699, 2001. The beamformer operator is computed using the cross spectral density matrix (the time-frequency equivalent of the data covariance matrix) computed from the single-trial data. This allows to image evoked as well as induced oscillatory activity in a user-defined time-frequency range, where time is taken relative to a triggered event.

The output power P(r) of the beamformer for a specific brain region at location r is then computed by the following equation:

$$P(\boldsymbol{r}) = tr'[L^{T}(\boldsymbol{r}) \cdot C_{r}^{-1} \cdot L(\boldsymbol{r})]^{-1}$$

Here,  $C_r^{-1}$  is the inverse of the regularized cross spectral density matrix C in the time-frequency range of interest; L is the leadfield matrix of the model containing a regional source at target location r and, optionally, additional sources, whose interference with the target source is to be minimized; tr'[] is the trace of the [3×3] (MEG:[2×2]) submatrix of the bracketed expression that corresponds to the source at location r.

In BESA Research, the output power P(r) is normalized by the output power in a reference time-frequency interval  $P_{ref}(r)$ :

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

$$q(\mathbf{r}) = \begin{cases} \sqrt{\frac{P(\mathbf{r})}{P_{ref}(\mathbf{r})}} - 1 = \sqrt{\frac{tr'[L^{T}(\mathbf{r}) \cdot C_{r}^{-1} \cdot L(\mathbf{r})]^{-1}}{tr'[L^{T}(\mathbf{r}) \cdot C_{ref,r}^{-1} \cdot L(\mathbf{r})]^{-1}}} - 1 & \text{for } P(\mathbf{r}) \ge P_{ref}(\mathbf{r}) \\ 1 - \sqrt{\frac{P_{ref}(\mathbf{r})}{P(\mathbf{r})}} = 1 - \sqrt{\frac{tr'[L^{T}(\mathbf{r}) \cdot C_{ref,r}^{-1} \cdot L(\mathbf{r})]^{-1}}{tr'[L^{T}(\mathbf{r}) \cdot C_{r}^{-1} \cdot L(\mathbf{r})]^{-1}}} & \text{for } P(\mathbf{r}) < P_{ref}(\mathbf{r}) \end{cases}$$

P_{ref} can be computed either from the corresponding frequency range in the baseline of the same condition (signal-to-noise ratio) or from the corresponding time-frequency range in a control condition. The beamformer image is constructed from values q(r) computed for all locations on a specified grid.

Traditional single-source beamformers are known to mislocalize activity if several brain regions have highly correlated activity. The BESA beamformer tries to overcome this problem by extending the traditional single-source beamformer. The BESA beamformer can implicitly account for activity from possibly correlated brain regions. This is achieved by using a multiple source beamformer calculation that contains not only the leadfields of the source at the location of interest **r**, but also those of possibly interfering sources. As a default, BESA Research uses a bilateral beamformer, where specifically contributions from the homologue source in the opposite hemisphere are taken into account (the matrix L thus being of dimension N×6 for EEG and N×4 for MEG, respectively, where N is the number of sensors). This allows for imaging of highly correlated bilateral activity in the two hemispheres that commonly occurs during processing of external stimuli.

In addition, the beamformer computation can be performed taking into account possibly correlated sources at other specified locations by including them in the leadfield matrix L.

The following chapters illustrate the different properties and application examples of the BESA beamformer.

# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

 Open file S1.cnt in subfolder ERP-Auditory-Intensity of the Examples folders.

2. Press the EdF toolbar button to edit the filter settings. Disable all but the notch filter that should be set at 50 Hz to reduce mains interference.

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	Files of type:	ERP Files (*	edf, *.bdf, *.cnt, *.a	avg)	•	Cancel
	Folders:	Data Folder			-	

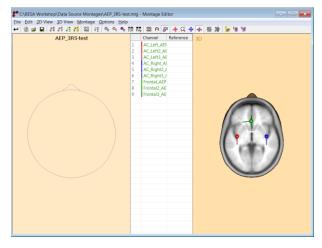
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High Cutoff Filter Cutoff Frequency [Hz]: 40 Type: Zero phase V Slope: 24 dB/oct V	]
Frequency [Hz]:         50         Width [Hz]:         2	
Band Pass Filter Frequency [Hz]: 80 Width [Hz]: 5	
Sampling Rate: 250 Hz OK Cance	el 🔤

 Press the Usr button. Select montage HighIntensity_3RS-test from the list of predefined user-montages that are available for this file. Press the EdM button to remind us of the details about the current montage again.

Usr	Opt EdM LF	HF
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	LBip+FT	
~~~~~	LBip+Sp	
	LBip	
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	HighIntensity_3RS	from
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Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

4. The montage consists of the sources we fitted earlier on: 2 auditory regional sources and a frontal source, which have been converted to single dipoles. Close the montage editor.

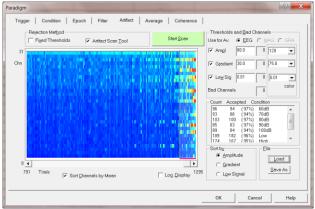


 In a first step, we will perform timefrequency analysis for all sources of the selected montage.

Press **ERP** / Edit Paradigm. The paradigm-file AEP_Intensity.PDG should still be available.

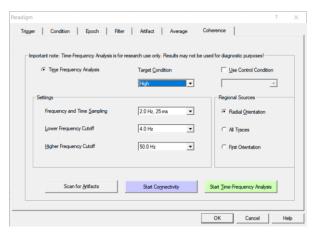
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Move to the Artifact tab and press Start
 Scan to re-run the automatic artifact scan with artifact correction switched on.

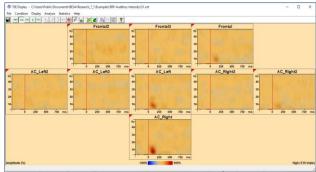


Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

7. Switch to the Coherence tab. In the Target Condition drop down menu, select condition High. To start time-frequency analysis with the default sampling of 2.0 Hz, 25 ms in the frequency range of 4–50 Hz, press the Start Time-Frequency Analysis button.



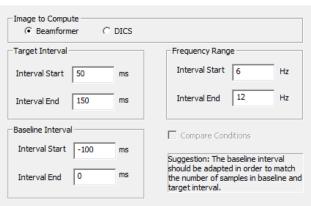
8. After the calculations of the time-frequency decomposition is finished, the temporal-spectral evolution for all sources of the montage are displayed. The main activity in the first frontal and in the first and second auditory components are most prominent.



# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

Image

- 9. As a next step, we will fit the prominent activity in the High condition. Left-drag a window around the strongest activity in the left-hemispheric auditory source. When releasing the left mouse-button choose the option Image. If necessary change the Target interval to 50 to 150 ms and the Frequency Range to 6 to 12 Hz. It is very important to choose approximately the same length between the Target and the Baseline interval¹². Otherwise, Beamforming results can be unstable. Press Go.
- 10. Scale up the image to +/- 25%. Use the button to view the different maxima. The first maximum localizes in the right auditory cortex, the second and third maxima indicate activity in bilateral frontal areas, while the fourth maximum shows activity in the left supra-temporal area. Close the source analysis window.



Important note: To obtain a reliable beamformer image, the number of samples in the target and the reference interval should be equal. Therefore it is recommended to assign equal duration to the Target Interval and the Baseline Interval.

If two conditions are compared, they should have approximately the same number of trials.



×

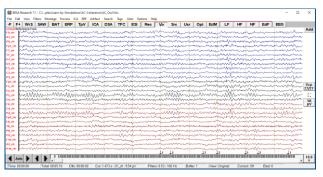


¹² It is also possible to compute beamforming images of the difference between conditions. Tick the box 'Compare Conditions' for this purpose. Instead of using the baseline as reference interval, the same time-frequency range as specified in the target interval will be used in the control condition as a reference.

# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 11. BESA Research uses a multiple source beamformer by default. It is also possible to choose а classic single-source beamformer. We will have a look at our model coherence data to see the difference between the multiple source and the single beamformer. Back in the main window open file AC Osc5.foc which is located in the examples subfolder Learn-by-Simulations.
- Change to the virtual reference-free display using the Vir button and selecting the top-most option.

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QUICK access	AC_Osc5.	fsg	2003-03-12 6:11 PM	A FSG File
	AC_Osc20	foc	2003-03-13 4:26 PM	A FOC File
Desktop	AC_Osc20	fsg	2003-03-12 6:08 PM	A FSG File
This PC	¢			
	File name:	AC_Osc5.foc	-	Open
	Files of type:	BESA Binary Files (*.foc, *	fsg) 💌	Cancel



 Press ERP / Coherence to Start Time-Frequency Analysis of condition StOn using the default settings.

Important note: Time-Frequency Analysis in	s for research use only. Results may not b	e used for diagnostic purposes!
<ul> <li>Time Frequency Analysis</li> </ul>	Target Condition	Use Control Condition
	StOn 🗸	Ψ
Settings		Regional Sources
Frequency and Time Sampling	2.0 Hz, 25 ms	Radial Orientation
Lower Frequency Cutoff	4.0 Hz	C All Traces
Higher Frequency Cutoff	50.0 Hz	C First Orientation
Scan for Artifacts	Start Connectivity	Start Time-Frequency Analysis

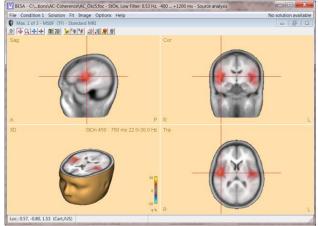
# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 14. Subtract the evoked signal by pressing the button and selecting Subtract Average Signal. Now we only see the induced activity that is not present in the evoked potential. We want to fit this induced activity by marking a window around the most prominent activity.
- In the Image setting dialog box specify a time-range of 450 to 750 ms, a baseline interval from -300 to 0 ms and a frequency range from 22 to 30 Hz. Press Go.

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Interval <u>E</u> nd	750	ms		Interval En <u>d</u>	30	Hz	
Baseline Interval							
Interval S <u>t</u> art	-300	ms		Upon start of editi			
Interval E <u>n</u> d	0	ms	was modified to match the number of samples in baseline and target interval.				
Important note: To obtain a reliable beamformer image, the number of samples in the target and the reference interval should be equal. Therefore it is recommended to assign equal duration to the Target Interval and the Baseline Interval.							
If two conditions a	are compared,	they shou	uld have	e approximately the	e same number	r of trials.	
				Go	Cancel		

16. Scale up the image to +/- 25%. Observe the bilateral auditory activity. We know the maxima are at the correct location from the simulation.

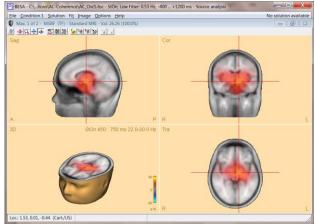


# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

17. Change the type of beamformer by pressing Image / Volume Image and select Beamformer (single source). The beamformer image is re-calculated.

Image Options Help				
Volume Image	•	Beamformer	В	
Surface Image	•	Beamformer (single source)		
Discrete Model Probing	•	CLARA		
Smooth Image Hide Image		LAURA LORETA SLORETA		
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		User-defined Image Settings		
	Ρ	R		6.0
StOp 450 - 750 mc 22.0.30	о H-	Tra		

18. The single source beamformer mislocalizes the bilateral auditory activity to the middle of the head. The reason is that single-source beamformers produces faulty results if activation in two brain regions is highly correlated.

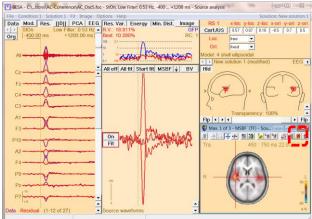


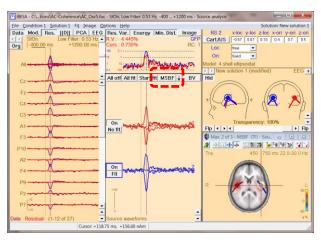
 Return to the multiple source beamformer by pressing Image / Volume Image / Beamformer.

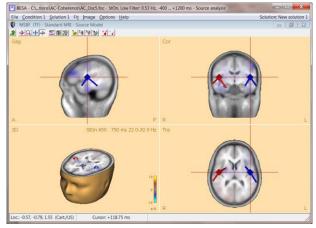
Image Options Help			
Volume Image	•	Beamformer	В
Surface Image Discrete Model Probing	) }	Beamformer (single source) CLARA	
Smooth Image Hide Image		LAURA LORETA SLORETA	
Import Image Export Image As Settings		suloreta swLORETA sSLOFO Settings	
		User-defined Image Settings	

# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- 20. Minimize the MRI view by pressing the button in the top-right corner. We now want to use the beamformer image to seed dipoles. Select the first maximum and press the button. Move to the next maximum and place another source.
- 21. We want to use the beamformer to see, whether there is still some unexplained activity if we model the auditory activity with two regional sources. Press MSBF to recompute the beamformer on the residual activity.
- 22. We need to scale up the image to +/- 10% to start seeing some patchy left-over desynchronization that is not modeled by the regional sources. This activity is very weak, so we can neglect it.







Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

#### J. Dynamic Imaging of Coherent Sources (DICS) – a short introduction

Dynamic Imaging of Coherent Sources (DICS) is a sophisticated method for imaging corticocortical coherence in the brain or coherence between an external reference (e.g. EMG channel) and cortical structures. DICS can be applied to localize evoked as well as induced coherent cortical activity in a user-defined time-frequency range.

DICS was implemented in BESA Research closely following Gross et al., "Dynamic imaging of coherent sources: Studying neural interactions in the human brain", PNAS 98, 696-699, 2001.

The computation is based on a transformation of each channels single trial data from the time domain into the frequency domain. This transformation is performed by the BESA Research Coherence module and results in the complex spectral density matrix that is used for the construction of the spatial filter similar to beamforming.

DICS computation yields a 3-D image, each voxel being assigned a coherence value. Coherence values can be described as a **neural activity index** and do not have a unit. The neural activity index contrasts coherence in a target time-frequency bin with coherence of the same time-frequency bin in a baseline.

#### DICS for cortico-cortical coherence is computed as follows:

Let L(r) be the lead field in voxel r in the brain and C the complex cross-spectral density matrix. The spatial filter W(r) for the voxel r in the head is defined as follows:

$$W(r) = (L^{T}(r) \cdot C^{-1} \cdot L(r))^{-1} \cdot L^{T}(r) \cdot C^{-1}$$

The cross-spectrum between two locations (voxels)  $r_1$  and  $r_2$  in the head are calculated with the following equation:

$$C_s(r_1, r_2) = W(r_1) \cdot C \cdot W^{*T}(r_2)$$

where  $T^{T}$  means the transposed complex conjugate of a matrix. The cross-spectral density can then be calculated from the cross spectrum as follows:

$$c_s(r_1, r_2) = \lambda_1 \{ C_s(r_1, r_2) \},\$$

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# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

where  $\lambda_1$  indicates the largest singular value of the cross spectrum. Once the cross spectral density is estimated, the connectivity¹³ (CON) between the two brain regions  $r_1$  and  $r_2$  are calculated as follows:

$$CON(r_1, r_2) = \frac{c_s^{sig}(r_1, r_2) - c_s^{bl}(r_1, r_2)}{c_s^{sig}(r_1, r_2) + c_s^{bl}(r_1, r_2)},$$

where  $c_s^{sig}$  is the cross-spectral density for the signal of interest between the two brain regions r1 and r2, and  $c_s^{bl}$  is the corresponding cross spectral density for the baseline or the control condition, respectively. In the case DICS is computed with a cortical reference, r1 is the reference region (voxel) and remains constant while r2 scans all the grid points within the brain sequentially. In that way the connectivity between the reference brain region and all other brain regions is estimated. The value of  $CON(r_1, r_2)$  falls in the interval [-1 1]. If the cross-spectral density for the baseline is 0 the connectivity value will be 1. If the cross-spectral density for the signal is 0 the connectivity value will be -1.

#### DICS for cortico-muscular coherence is computed as follows:

When using an external reference, the equation for coherence calculation is slightly different compared to the equation for cortico-cortical coherence. First of all, the cross-spectral density matrix is not only computed for the MEG/EEG channels, but the external reference channel is added. This resulting matrix is  $C_{all}$ . In this case, the cross-spectral density between the reference signal and all other MEG/EEG channels is called  $c_{ref}$ . It is only one column of  $C_{all}$ . Hence, the cross-spectrum in voxel r is calculated with the following equation:

$$C_s(r) = W(r). c_{ref}$$

and the corresponding cross-spectral density is calculated as the sum of squares of Cs:

$$c_s(r) = \sum_{i=1}^n C_s(r)_{i,j}^2$$

¹³ Here, the term *connectivity* is used rather than *coherence*, as strictly speaking the coherence equation is defined slightly differently. For simplicity reasons the rest of the tutorial uses the term *coherence*.

## Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

where n is 2 for MEG and 3 for EEG. This equation can also be described as the squared Euclidean norm of the cross-spectrum:

$$c_s(r) = \|C_s\|^2$$
.

The power in the voxel r is calculated like in the cortico-cortical case:

$$p(r) = \lambda_1 \{ C_s(r, r) \}.$$

At last, coherence between the external reference and the cortical activity is calculated with the equation:

$$CON(r) = \frac{c_s(r)}{p(r) \cdot C_{all}(k,k)},$$

where  $C_{all}(k, k)$  is the (k,k)-th diagonal element of the matrix  $C_{all}$ .

DICS is particularly useful, if coherence is to be calculated without an a-priory source model (in contrast to source coherence based on pre-defined source montages). However, the recommended analysis strategy for DICS is to use a brain source as a starting point for coherence calculation that is known to contribute to the EEG/MEG signal of interest. For example, one might first run a beamformer on the time-frequency range of interest and use the voxel with the strongest oscillatory activity as a starting point for DICS. The resulting coherence image will again lead to several maxima (ordered by magnitude), which in turn can serve as starting points for DICS calculation. This way, it is possible to detect even weak sources that show coherent activity in the given time-frequency range.

The other significant application for DICS is estimating coherence between an external source and voxels in the brain. For example, an external source can be muscle activity recoded by an electrode placed over the according peripheral region. This way, the direct relationship between muscle activity and brain activation can be measured.

In the following, we will first examine cortico-cortical coherence in the model dataset AC_Osc5.foc in the subfolder **Learn-by-Simulations\AC-Coherence** of the BESA Research Examples folder. For a full description of the model data see chapter B of the present tutorial.

Second, we will estimate cortico-muscular coherence in an MEG dataset kindly provided by Dr. Jan-Mathjis Schoffelen and Dr. Robert Oostenveld (Donders Institute). The dataset is also

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

available in the fieldtrip tutorial (<u>http://www.fieldtriptoolbox.org/tutorial/coherence</u>), the results of the experiment were published in Science in 2005 (Schoffelen, Oostenveld, Fries; Science 2005). This is the description of the experiment:

The dataset used in this example has been recorded in an experiment in which the subject had to lift her hand and exert a constant force against a lever. The force was monitored by strain gauges on the lever. The subject performed two blocks of 20 trials in which either the left or the right wrist was extended for about 10 seconds. A trial started as soon as the subject managed to get his force output within a specified range from 1 to 2 N. If the force was not kept constant during the course of a trial, the trial was terminated prematurely.

The bipolar EMG signal was recorded from the right extensor carpi radialis longus muscle in the lower arm. MEG signals were recorded with a 151 sensor CTF Omega System (Port Coquitlam, Canada). In addition, the EOG was recorded to later discard trials contaminated by eye movements and blinks. The ongoing MEG and EOG signals were lowpass filtered at 300 Hz, digitized at 1200 Hz and stored for off-line analysis. To measure the head position with respect to the sensors, three coils were placed at anatomical landmarks of the head (nasion, left and right ear canal). While the subjects were seated under the MEG helmet, the positions of the coils were determined before and after the experiment by measuring the magnetic signals produced by currents passed through the coils. Magnetic resonance images (MRIs) were obtained from a 1.5 T Siemens system. During the MRI scan, ear molds containing small containers filled with vitamin E marked the same landmarks. This allows us, together with the anatomical landmarks, to align source estimates of the MEG with the MRI.

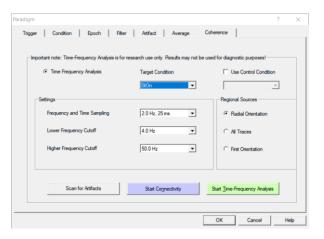
Our data example (Cortico-Muscular_DICS.foc) consists of the data for the left EMG condition, exported and compressed to a BESA binary (*.foc) file. Triggers with code 99 were added to the data at 1 s intervals. A paradigm file (Cortico-Muscular_DICS.pdg) defines 1 s epochs around these triggers. An artifact scan using an amplitude threshold of 3400 fT leaves 115 out of 192 epochs for analysis.

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

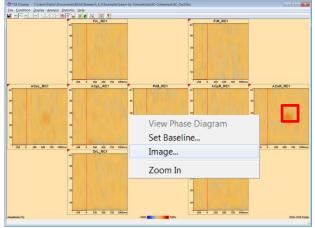
#### K. Cortico-cortical coherence with DICS

- Please open file Learn-by-Simulations\ AC-Coherence\AC_Osc5.foc in the BESA Research Examples folder. Please press the Usr button from the button menu and select the source montage RC1.
- Run time-frequency analysis by pressing ERP / Coherence. Leave all settings at default and press Start Time-Frequency Analysis.

IBSA Research 7.1 - C\plesLteam-by-Simulations\AC-Coherence\AC_Osc5.foc	- 0	×
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me: 00.00.00 Total 00.0510 [Offs: 00.00:00 [Cur: 2:477 s: OrR: 371.6 nAm ]Low Filter: 0.53 Hz Buffer: 1 [View: Original [Correct: Off ]Bad. 0		



 Left-drag a window over the induced activity in the source ACsR_RC1, release the left mouse-button and select Image.

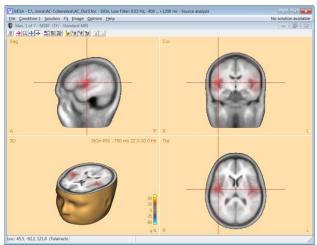


Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

 Select the Image to Compute to Beamformer. Set the Target Interval to 450 to 750 ms, the Baseline Interval to -300 to 0 ms and the Frequency Range to 22 to 30 Hz. Press Go.

mage	ute	<b></b>
e Beamforr	mer C DICS	
Target Interval-		Frequency Range
Interval <u>S</u> tart	450 ms	Interval Start 22 Hz
Interval <u>E</u> nd	750 ms	Interval En <u>d</u> 30 Hz
-Baseline Interve	d	Compare Conditions
Interval Start	-300 ms	Upon start of editing, the baseline interval
Interval E <u>n</u> d	0 ms	was modified to match the number of samples in baseline and target interval.
and the reference duration to the Ta	e interval should be equ arget Interval and the Ba	」 mformer image, the number of samples in the target al. Therefore it is recommended to assign equal seline Interval. uld have approximately the same number of trials.
		<u>G</u> o <u>C</u> ancel

5. Scale up the resulting image by pressing the button several times. The first maximum of the beamformer is located in the right auditory cortex. We will use this maximum (voxel) to probe coherence with all other voxels in the brain.



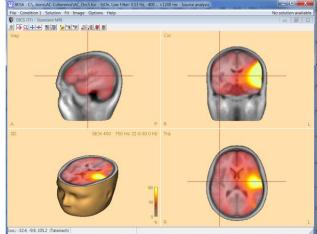
# Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

- In the middle panel of the source analysis window, press the arrow key and select DICS.
- В Beamformer DICS CLARA LAURA LORETA **sLORETA swLORETA sSLOFO** User-defined Image Minimum-Norm (Surface) Ctrl+M Probe Scan S Sensitivity V Settings...
- 7. In the Set Reference dialogue check the option Use current cross-hair position. Please note that it is also possible to select a dipole or regional source as the reference in case one is working with a discrete source model. Press OK.

Set Reference for DICS		
External reference channel		
C Reference is external channel		
Reference channel:		
Brain source		
Reference is brain source		
C Use location of currently selected source		
Use current cross-hair position		
OK Cancel		

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

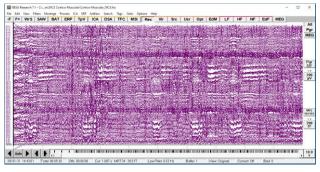
8. The resulting image suggests strong coherence between the right and left auditory cortices. You can use the button to jump through the DICS maxima and use them as new starting points for further DICS calculations.

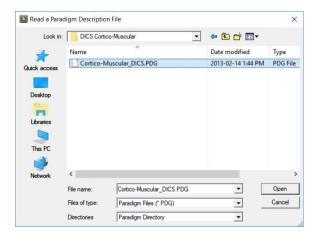


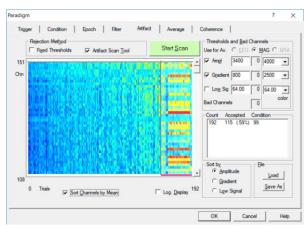
Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

#### L. Cortico-muscular coherence with DICS

- Please open file Examples\DICS Cortico-Muscular\Cortico-Muscular_DICS.foc in the BESA Research Examples folder. This dataset contains 151 axial gradiometers and 2 polygraphic channels used for recording the bilateral EMG signal.
- Select ERP / Edit Paradigm to auto-load the file Cortico-Muscular_DICS.PDG from the data folder. The paradigm file specifies a condition containing the triggers with code 99 that were added to the data at 1 s intervals. Epochs are defined to last 1 s epochs around these triggers.
- Move to the Artifact tab and run the artifact scan by pressing Start Scan. Using an amplitude threshold of 3400 fT and a gradient threshold of 800 fT leaves 115 out of 192 epochs for analysis.

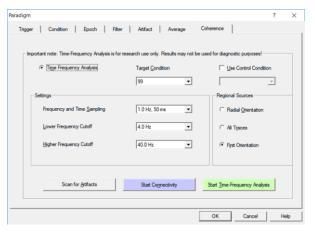




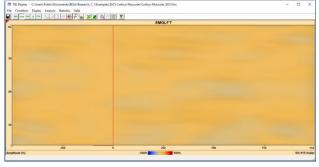


Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

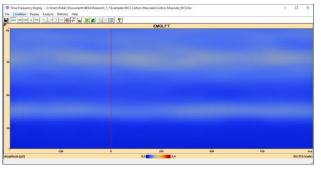
4. Move to the Coherence tab and make sure the Frequency and Time Sampling to be 1.0 Hz, 50 ms, the Low Frequency Cutoff to be 4.0 Hz and the High Frequency Cutoff to be 40.0 Hz. Start Time-Frequency Analysis.

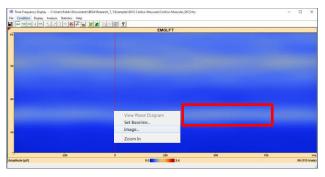


 In the resulting TFC window, please locate the EMGLFT channel, right-click and specify Show only this channel (this might take a few seconds!)



- 6. Press the button to display the absolute amplitude in the given time-frequency range. Scale up the image by pressing the button until the scale ranges from 0.0 to 2.4 μV. It becomes apparent that there is an increase in amplitude around 15 Hz.
- Left-drag a window over the 15 Hz activity, and select Image from the popup menu.





Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

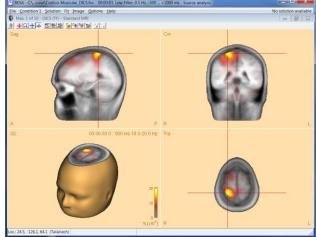
- 8. Select DICS under the section Image to Compute, a Target Interval from 0 to 500 ms, a Baseline Interval from -500 to 0 ms and a Frequency Range from 10 to 20 Hz. Press Go. Please note that the baseline interval is relevant only for cortico-cortico DICS computations. It is not used in the cortico-muscular computations, as the one shown in the following points.
- 9. After calculating the cross-spectral density matrix in the given time-frequency range, the Set Reference for DICS window pops up. Select the option Reference is an external channel and choose channel EMGLFT to calculate coherence between the left EMG channels and all brain voxels. Press OK.

Image	X			
Image to Compute C Beamformer				
Target Interval	Frequency Range			
Interval <u>S</u> tart 0 ms	Interval St <u>a</u> rt 10 Hz			
Interval <u>E</u> nd 500 ms	Interval En <u>d</u> 20 Hz			
Baseline Interval	Compare Conditions			
Interval Start -500 ms	Suggestion: The baseline interval should			
Interval End 0 ms	be adapted in order to match the number of samples in baseline and target interval.			
Important note: To obtain a reliable beamformer image, the number of samples in the target and the reference interval should be equal. Therefore it is recommended to assign equal duration to the Target Interval and the Baseline Interval. If two conditions are compared, they should have approximately the same number of trials.				
	<u>G</u> o <u>C</u> ancel			

Set Reference for DICS		
External reference channel		
Reference channel: EMGLFT		
Brain source		
O Reference is brain source		
<ul> <li>O Use location of currently selected source</li> <li>O Use current cross-hair position</li> </ul>		
OK Cancel		

Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

10. The resulting image indicates the maximum coherence between the left EMG channel and the right somato-motor area. This is to be expected as the subject was pressing a lever with the left hand, which is represented in the right motor/somatosensory area.



Tutorial 12 – Time-frequency Analysis, Connectivity Analysis and Beamforming

Tutorial 13 – Cross-subject Statistics

#### **Tutorial 13 – Cross-subject Statistics**

#### What does BESA Research provide?

- ✓ Export of individual results from BESA Research via batch scripting
- ✓ Cross-subject statistics using BESA Statistics
  - ERP / ERF data
  - Source Waveforms
  - o Image Data
  - o Time-Frequency / Coherence Results

Tutorial 13 – Cross-subject Statistics

In a previous step (Tutorial 4), we created a source model for conditions high and low based on the grand average data. We now want to compare conditions **high** and **low** statistically. I.e. we want to know, whether a particular source is more or less active in the high compared to the low condition. For this purpose, we will first create a batch that applies our source model to the individual datasets and extracts the source waveforms per person. We will then export the individual source waveforms and analyze them statistically with BESA Statistics.

We will also investigate the differences between more than two conditions in the same experiment, and apply statistics to a different experiment where we look at correlations between measured MEG data and an external covariate of interest.

#### A. Applying a source model to individual datasets using batch scripting

The grand average model explains the data variance in all conditions. Therefore, it can serve as a master model to calculate the source activity of each individual subject in all 5 conditions. The hypothesis is that the mean source location of the grand average provides a sufficiently good model for each individual data set since a change in source location of 1-2 cm has little effect on the temporal course of the source waveforms. Generally, fitting in individual data results in a large uncertainty of source location, especially in depth, due to poorer signal-to-noise ratio. We therefore want to stabilize the individual solutions by using

- a) a model with fixed sources and individual orientations (locations from grand average or mean Talairach coordinates, cf. Hoechstetter et al., Neuroreport 11: 2461, 2000)
- b) a model with fixed sources and orientations (same as grand average model)
- c) a model with fixed regional sources (same as grand average model, calculating mean amplitude of each regional source as an orientation-independent measure (cf. Weisser et al., Neuroreport 12:3303, 2002)

The orientation of the different components (N100, N150 and frontal source) differs considerably between subjects according to individual gyral anatomy and functional representation. Since orientation differences have a larger effect on the EEG scalp topography than small changes in location (in the order of 1-2 cm) we should try to define the individual orientation of each component to obtain robust source waveforms (cf. Scherg, M. and von Cramon, D. Evoked dipole source potentials of the human auditory cortex. Electroenceph. Clin. Neurophysiol. 65: 344-360, 1986).

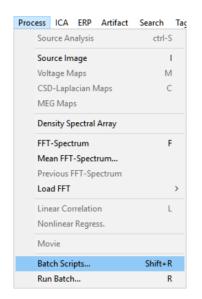
**Tutorial 13 – Cross-subject Statistics** 

Therefore, we will use strategy a) with the mean sources defined by the grand average model and set the orientations of the 3 regional sources individually for each subject in order to obtain appropriate source waveforms. These individual source waveforms will then be used to perform cross-subject statistics. Strategies b) and c) are alternatives for more noisy data.

We could apply all required steps manually to each individual data file. This is not necessary, however, and it saves a lot of time to use the source analysis batch functions. In the following, we will create a script that performs all steps automatically.

We will then use BESA Statistics to quickly and conveniently analyze our source waveform results across all 10 subjects.

 Select Process / Batch Scripts in the main window to open the Batch processing window. We will now create a batch that applies the grand average master model to all individual datasets, orients the regional sources individually and exports the individual source waveforms for further statistical analysis.

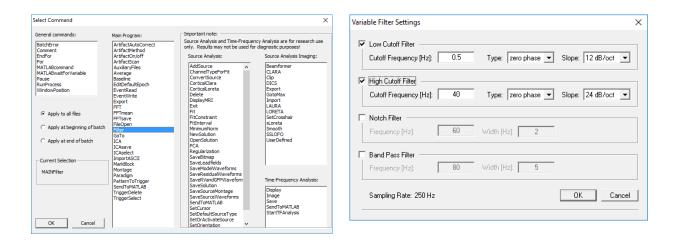


 In the File List tab, click on Load File List, select ERP-Auditory-Intensity-Merge.flist and press Open. This file list has already been used in Tutorial 3 Step B to create the grand average. It specifies individual average files of all subjects.

📔 Load File List	:			×
Look in:	FileList	•	+ 🗈 💣 📰 ◄	
Quick access Desktop Libraries This PC	ERP-Audi	^ tory-Intensity-Merge.flist ettingsBatch.flist ettingsCC.flist	Date modified 3/13/2008 2:03 PM 12/9/2009 12:59 PM 5/24/2017 4:09 PM 3/20/2017 4:43 PM	Type FLIST File FLIST File FLIST File FLIST File
Network	<			>
	File <u>n</u> ame: Files of <u>type</u> : Directories:	ERP-Auditory-Intensity-Merge flist File List (* flist) File List directory	• •	Open Cancel

**Tutorial 13 – Cross-subject Statistics** 

Switch to the Batch tab. As a first command in this batch, apply appropriate filters to each data set. Press Add Command and select Filter (Main Programs group). Select a low cutoff filter of 0.5 Hz, zero-phase, 12 dB/oct, and a high cutoff filter of 40 Hz, zero-phase, 24 dB/oct. Click OK twice to confirm these filter settings.



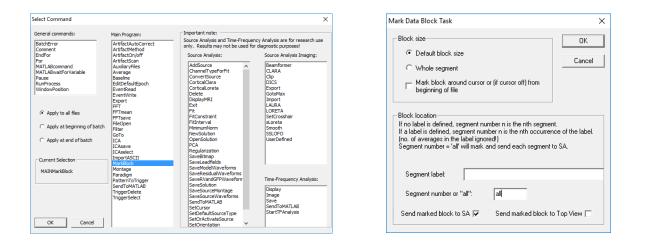
 As a next command, specify the epoch that will be sent into the source analysis window to extract the source waveforms. Press Add Command and select EditDefaultEpoch (Main Programs group). Specify an interval from -50 to 250 ms.

Seneral commands:	Main Program:	Important note:	
BatchError	ArtifactAutoCorrect	<ul> <li>Source Analysis and Time-Frequen</li> </ul>	
Comment	ArtifactMethod	only. Results may not be used for	diagnostic purposes!
ForFor	ArtifactOn/off	Source Analysis:	Source Analysis Imaging:
For	ArtifactScan		
MATI ABcommand	AuxiliaryFiles	AddSource	Beamformer
MATI ABwaitForVariable	Average	ChannelTypeForFit	CLARA
Pause	Baseline	ConvertSource	Clip
RunProcess	EditDefaultEpoch	CorticalClara	DICS
WindowPosition	EventRead	CorticalLoreta	Export
WHICOMPOSICION	EventWrite	Delete	GotoMax
	Export	DisplayMRI	Import
	EXPORT	Exit	LAURA
	FETmean	Fit	LORETA
<ul> <li>Apply to all files</li> </ul>		FitConstraint	SetCrosshair
	FFTsave	FitInterval	sloreta
C Apply at beginning of batch	FileOpen	MinimumNorm	Smooth
in the second second second	Filter	NewSolution	ISSI OFO
	GoTo	OpenSolution	UserDefined
<ul> <li>Apply at end of batch</li> </ul>	ICA	PCA	Userbeimed
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	MarkBlock	SaveLeadfields	1
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- marked abered tepoer	Paradigm	SaveResidualWaveforms	
	PatternToTrigger	SaveRVandGFPWaveform	Time-Frequency Analysis:
	SendToMATLAB	SaveSolution	Display
	TriggerDelete	SaveSourceMontage	
	TriggerSelect	SaveSourceWaveforms	Image
		SendToMATLAB	Save
		SetCursor	SendToMATLAB
		SetDefaultSourceType	StartTFAnalysis
		SetOrActivateSource	
OK Cancel		SetOrientation Y	

Default Block Epoch 🛛 🗙		
Pre cursor:	-50 ms	
Post cursor:	250.0 ms	
OK	Cancel	

Tutorial 13 – Cross-subject Statistics

5. The next command marks the default block in all 8 conditions, and sends these data blocks into the source analysis window. Press Add Command and select MarkBlock (Main Programs group). In the upcoming window, select Default block size. Instead of specifying a single segment label or segment number, write all in the entry Segment number. Note that this will automatically set the checkmark behind Send marked block to SA at the bottom of the window. Thus, all conditions from 60dB to All will be loaded in the source analysis window simultaneously.



6. Now apply the master model that was generated from the grand average. Press Add Command and select OpenSolution (Source Analysis group). Select file HighIntensity_3RS.bsa that contains the two regional sources in the auditory cortex and the frontal source. Press Open and OK.

Select Command			×	Load Solutio	n			×
General commands: BatchError Comment	Main Program: ArtifactAutoCorrect ArtifactMethod	Important note: Source Analysis and Time-Frequence only. Results may not be used for		Look in:	ERP-Auditory	y-Intensity	← 🗈 💣 📰▼	
Configure For MATLABounderforVariable Pause RumProcess WindowPosition C Apply to all files C Apply at beginning of batch C Apply at beginning of batch C Apply at end of batch Current Selection SAOpenSolution	Artifaction)eff Artifaction AuxilaryFiles Average Baseline ExterNatification EventRicad EventRicad EventRicad EventRicad FFT FFT FFT FFT FFT FFT FFT FFT FFT Goto ICA aver Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen Filespen 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7. To obtain correct source waveforms for each individual subject, adjust the master model to each subject's cortical folding by adjusting the orientations of the regional sources. First, we will reorient the auditory cortex sources in condition All that contains the average of all five conditions. Press Add Command and select SwitchCondition (Source Analysis group). As Condition Label, enter All. Press OK.

Select Command			×	SA Switch Condition Task	~
General commands:	Main Program:	Important note: Source Analysis and Time-Frequen	cy Analysis are for research use	SA Switch Condition Task	~
BatchError Comment EndFor For MATLABcommand MATLABcommand	ArtifactAutoCorrect ArtifactMethod ArtifactOn/off ArtifactScan AuxilaryFiles Average	only. Results may not be used for Source Analysis: ConvertSource	diagnostic purposes! Source Analysis Imaging: Beamformer CLARA	Previous Condition	ОК
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<ul> <li>Apply to all files</li> <li>Apply at beginning of batch</li> <li>Apply at end of batch</li> </ul>	FFT FFTmean FFTsave FileOpen Filter GoTo ICA ICAswe	FitConstraint FitInterval MinimumNorm NewSolution OpenSolution PCA Regularization SaveBitmap	LAURA LORETA SetCrosshair skoreta Smooth SSLOFO UserDefined	Condition Number:	
Current Selection	ICAselect ImportASCII MarkBlock Montage	SaveLeadfields SaveModelWaveforms SaveResidualWaveforms SaveRVandGFPWaveform SaveSolution		Condtion Label: All	
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OK Cancel		SwitchCondition V			

8. To adjust the N100 orientation, we need to specify the corresponding latency. Since N100 latencies will differ slightly from subject to subject, we define a time range rather than a fixed latency. Press Add Command and select FitInterval (Source Analysis group). Specify a latency range of 80 to 105 ms. Leave all other options unchanged and hit OK.

Select Command			×	SA Fit Interval Task	×
General commands: BatchError EndFor For MATLABoonmand MATLABoontForVariable Pause RurPhocess (* Apply to all files (* Apply at beginning of batch (* Apply at beginning of batch (* Apply at end of batch SAFitInterval	Man Program: ArtifactAutocret ArtifactAutocret ArtifactAutocret ArtifactOnyloff ArtifactOnyloff ArtifactOnyloff ArtifactOnyloff ArtifactOnyloff ArtifactOnyloff ArtifactOnyloff ArtifactOnyloff Export EventWrite Export FFT FFTmeen FFT FFTmeen FFT FFTmeen FFT FFTmeen FFT FFTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FFT FTTmeen FTT FTTmeen FTT FTTmeen FTT FTTmeen FTT FTTmeen FTT FTTmeen FTT FTT FTT FTT FTT FTT FTT FTT FTT FT	Source Analysis and Time-Frequency source Analysis Addisource Analysis Addisource Analysis Addisource Analysis Contraction Contract Analysis Contract Aneta Delete Display/MRI Exit Exit Contract Aneta Delete Display/MRI Exit FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint FitConstraint Fi		<ul> <li>Set the following interval:</li> <li>From (ms) To (ms)</li> <li>80 105</li> <li>Define baseline, then clear fit interval</li> <li>Set previously saved interval from buff</li> <li>Store current interval to buffer number:</li> <li>Set interval associated with source num</li> <li>Associate current interval with source of</li> </ul>	mber:

**Note:** At this point in the batch, we could insert a Pause command (General group). It would cause the batch to interrupt at this stage and allow us to modify the marked fit interval to match each subject's data before it continues on our OK. In this tutorial we pass this step, but generally it is recommended to keep an eye on what the batch is doing when working with your own data.

9. Now the first and second regional source in the model (representing the bilateral auditory cortices) can be oriented. Add Command and select SetOrientation (Source Analysis group). In the upcoming parameter window, specify Source label AC_Left. As operation select Orientation 1 at cursor or at maximum in interval. BESA will automatically determine the latency within the fit interval at which the total power of the regional source is maximal and perform source orientation at this latency. Confirm with OK.

Select Command	)	SA Set Orientation Task X
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OK Cancel	v	

**10.** Repeat the previous command SetOrientation with Source label AC_Right, the auditory cortex source in the other hemisphere.

Select Command			×	SA Set Orientat	ion Task	×
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 To set the second orientation to account for the N140 component, press Add Command, select SetCursor (Source Analysis group) and specify a latency of 135 ms.

Select Command			×	SA Set Cursor Task	
General commands:	Main Program:	Important note:		SA SEL CUISOF TASK	
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SASetCursor	MarkBlock Montage Paradigm PattenToTrigger SendToMATLAB TriggerDelete TriggerDelete	SaveModelWaveforms SaveResidualWaveform SaveSavadGPWaveform SaveSolution SaveSourceMontage SaveSourceWaveforms SendToMATLAB SendTomATLAB	Time-Frequency Analysis: Display Image Save SendToMATLAB StartTFAnalysis	Maximize image window	

**Note:** Again, at this stage it could be advisable to insert a Pause command (General group) to adjust the cursor position to the individual N140 latency, if required, before the batch continues.

12. As in steps 10 and 11, add the command SetOrienation twice. First, specify Source
 AC_Left and select Orientation 2 at cursor as operation. Then repeat the command and specify Source AC_Right, again with operation Orientation 2 at cursor.

General commands:	Main Program:	Important note:	
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For	ArtifactScan		
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RunProcess	EditDefaultEpoch	Delete	DICS
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	EventWrite	Exit	GotoMax
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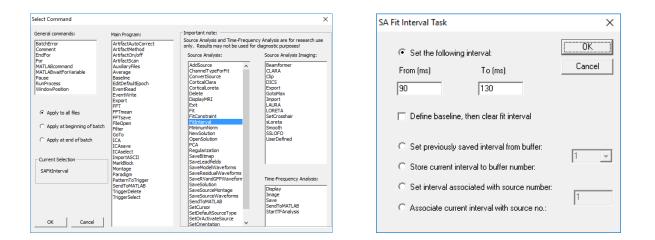
SA Set Orientatio	n Task	×					
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Operation C Drientation 1 at cursor or at maximum in interval C Drientation 2 at cursor							

SA Set Orientation Task	×
Source Source no. AC_Right Source label Last source All sources	OK Cancel
Operation     Orientation 1 at cursor or at max     Orientation 2 at cursor	ximum in interval

 Finally, we need to set the orientation of the frontal source. Its amplitude is largest in condition High. Therefore, add a command switchCondition (Source Analysis group) and specify condition label High.

Select Command			×	SA	Switch Condition T	ack	~
General commands:	Main Program:	Important note:		34	Switch Condition	ask	~
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OK Cancel		~					

14. To orient the frontal source, we will again mark a fit interval that includes its maximum activity. Add command FitInterval (Source Analysis group). Specify a latency range of 90 to 130 ms. Leave all other options unchanged and hit OK.



15. Add the command SetOrienation (Source Analysis group). This time specify source Frontal and Orientation 1 at cursor or at maximum in interval. Because no cursor is set anymore, BESA Research orients the source at the latency of maximum source power. This completes the adjustment of the master model to the individual subjects' brain gyration.

Select Command			×	SA Set Orientat	ion Task	×
General commands: DathError Ender Ender For For MATLABcommand MATLABcommand MATLABcommand MATLABcommand MATLABcommand MATLABcommand MATLABcommand For For For For For For For For	Man Program: PrifactAutoret ArtifactAutoret ArtifactOn/off ArtifactOn/off ArtifactOn/off ArtifactOn/off ArtifactOn/off ArtifactOn/off ArtifactOn Baseline Eventive Export Firit Firite Firite Firite Firite Firite Firite Firite Case Con ICAave ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAeve ICCAEve ICCAEve	Jinportant note:     Source Analysis and Time-Frequence     source Analysis:     ConvertSource     ContractCara     ContractCaraa     Contractara     ContractCara     ContractCaraa     ContractCaraa     Co		Source Frontal Last source		Cancel
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**Tutorial 13 – Cross-subject Statistics** 

16. We will now save this model for later re-use. Add the command SaveSolution (Source Analysis group). As a file name, specify %basename%-test. The string %basename% will be replaced by the basename of the individual data file. This makes sure that the solution is saved under different names for each subject and is not overwritten during the repeated execution of this command with different datasets. Note that you can specify the coordinate system in which the solution is to be saved. If the solution is opened with the same data set later on, the coordinate system is irrelevant. However, for comparison of different solutions across subjects, a standardized coordinate system like Talairach or Unit Sphere is recommended.

Select Command			×	SA Save Solution Tas	k	×
General commands: BatchError Comment EndFor For MATLABcommand MATLABrowtForVariable Pause RumProcess WindowPosition	Main Program: ArtifactAutoCorrect ArtifactWethod ArtifactOn/off ArtifactOn/off AutifactScan AuxilaryFiles Average Baseline EdIDefaultEpoch EventRead	Important note: Source Analysis and Time-Frequenc only. Results may not be used for or Source Analysis: AddSource ChannelTypeForFit ConvertSource CorticalCara CorticalCara	diagnostic purposes! Source Analysis Imaging: Beamformer CLARA Clip DICS Export	the file basename (	ame: It can include a subdir %basename%). If no directo lata directory is used.	
Apply to all files     Apply at beginning of batch     Apply at end of batch	EventWrite Export FFT FFTmean FFTsave FileOpen Filter GoTo ICA	Delete DisplayMRI Exit FitConstraint FitConstraint MinimumNorm NewSolution OrenSolution	GotoMax Import LAURA LORETA SetCrosshair sLoreta Smooth SSLOFO LiserDefined	File name: %basename%-test.b	\$a	Browse
Current Selection SASaveSolution OK Cancel	ICAsave ICAselect ImoortASCII MarkBlook Montage Paradigm PatternToTrigger SendToMATLAB TriggerSelect TriggerSelect	PCA Regularization SaveLeadRelds SaveLeadRelds SaveReadJWaveforms SaveReadJWaveforms SaveReadJWaveforms SaveReadJWaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveRviaveforms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveForms SaveSaveF	Time-Frequency Analysis: Display Image Save SendToMATLAB StartTFAnalysis	Target Coordinate System	C Head C Device C Talairach ☞ Unit Sphere	Cancel

#### B. Exporting individual source waveforms via batch scripting

Next, we want to save the individual source waveforms of conditions High and Low, which we will later load in BESA Statistics.

 Add the command SaveSourcewaveforms (Source Analysis group) to save the source waveforms to an ASCII file (*.swf). In the file name include %basename% as a placeholder for the datafile, and add the exension "High" in order to be able to associate each *.swf file with the corresponding subject and condition.

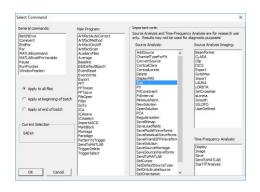
Select Command			×		SA Save Source Waveforms Task	×
General commands:	Main Program:	Important note:		1		
BatchError Comment Endfor For MATLABcommand MATLABwaltForVariable Pause RunProcess WindowPosition	ArtifactAutoCorrect ArtifactMethod ArtifactOn/off ArtifactOn/off AuxilaryFiles Average Baseline EditDefaultEpoch EventWrite	Source Analysis and Time-Frequenc only. Results may not be used for of Source Analysis: AddSource A ChannelTypeForFit ConvertSource CorticalCara CorticalCara Delete	iagnostic purposes! Source Analysis Imaging: Beamformer CLARA Clip DICS Export GotoMax		Instructions Define target file name: It can inc the file basename (%basename% the file name, the data directory is	). If no directory is specified in
	Export	DisplayMRI	Import			
Apply to all files     Apply at beginning of batch     Apply at end of batch	FFT FFTmean FFTsave FileOpen Filter GoTo ICA ICAsave ICAselect	Exit Fit FitConstraint FitInterval MinimumNorm NewSolution OpenSolution PCA Regularization	LAIRA LORETA SetCrosshair st.oreta Smooth SSLOFO UserDefined		File name: %basename% High.swf	Browse
Current Selection	ImportASCII	SaveBitmap SaveLeadfields				
SASaveSourceWaveforms	MarkBock Montage Paradign PatternToTrigger SendToMATLAB TriggerDelete TriggerSelect	SeverSedRevEst SaveRedGWaveforms SaveResdualWaveforms SaveResdualWaveforms SaveSourceMontage SaveSourceMontage SeveSourceMontage SeveSourceMontage SetOrEnduISourceType SetOrEnduISourceType SetOrActivateSource SetOrEnduISourceType	Time-Frequency Analysis: Display Image Save SendToMATLAB StartTFAnalysis		Send to MATLAB	Cancel

 Add a command SwitchCondition (Source Analysis group). As Condition Label, enter Low. Hit OK.

General commands:	Main Program:	Important note:	
BatchError Comment EndFor	ArtifactAutoCorrect ArtifactMethod ArtifactOn/off	<ul> <li>Source Analysis and Time-Frequen only. Results may not be used for Source Analysis:</li> </ul>	
For MATLABcommand MATLABwaitForVariable Pause RunProcess WindowPosition	ArtifactScan AuxilaryFiles Average Baseline EditDefaultEpoch EventRead	ConvertSource CorticalClara CorticalLoreta Delete DisplayMRI Exit	Beamformer CLARA Clip DICS Export GotoMax
Apply to all files     Apply at beginning of batch     Apply at end of batch     Ourrent Selection	EventWite Export FFT FFTmean FFTsave FileOpen Fileor GoTo ICA ICAselect InportASCII MarkBlock	Fit FitConstraint FitCinterval MinimumNorm NexSolution OperSolution PCA Regularization SaveBitmap SaveEcatifields SaveBitdelWaveForms SaveRodelWaveForms	Gotoriax Import LAIRA LORETA SetCrosshair st.oreta Smooth SSLOPO UserDefined
SASwitchCondition	Montage Paradigm PatternToTrigger SendToMATLAB TriggerDelete TriggerSelect	SaveRVandGPIVAveform SaveSourceMontage SaveSourceWaveforms SendToMATLA8 SetCursor SetDefaulSourceType SetOrActivateSource SetOrientation SetOrientation	Time-Frequency Analysis: Display Image Save SendToMATLAB StartTFAnalysis

SA Switch Condition Task	×
Previous Condition	OK
Next Condition	Cancel
Condition Number:	]

- **3.** Repeat Step B1 for condition "Low".
- Add command Exit (Source Analysis Group). This will close the source analysis window after each subject to prevent data remaining when processing the next subject.



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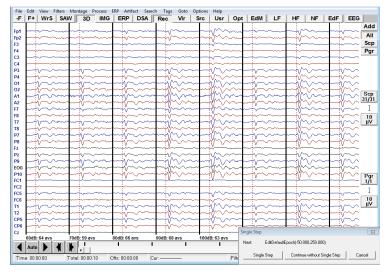
Tutorial 13 – Cross-subject Statistics

5. The script is now ready to be applied to all data files in the file list. You may save the script to disc for later reuse by pressing the Save Batch button. At this point you may open the predefined batch script AudIntensity-SWFs-for-BESA-Statistics.bbat using the button Load Batch (then navigate to folder Auditory). This script contains all commands as described above. In addition, a script comment has been added in the first lines using the command Comment. These lines (preceded by a semicolon) are not evaluated during script execution. Note that the batch scripts are stored in ASCII files (*.bbat) that can also be edited with a standard text editor.

atch Processing		×			
File List Batch					
Add Command	Load Batch	Instructions Press Add Command to add new commands. Right click on a command to edit or delete existing command.			
Load Previous	Save Batch	Press OK to start the batch.			
Clear All	View Log File	Logging to "C:\Users\Public\Documents\BESA\Research_7_1\Scripts\Log\Batch.txt"			
Command					
; This script is part of th	e "Tutorial on AEP Inter	nsity Dependence". It illustrates how batch scripts can			
; be employed to apply	a master model to individ	dual data files to extract individual source waveforms.			
	IB-z,HC:40.00-24dB-z,NI	F:off,BP:off)			
EditDefaultEpoch(-50.0					
MarkBlock(DefaultEpo					
		tensity\HighIntensity_3RS.bsa,Open)			
SAswitchCondition(Lab					
SAfitInterval(80,105,Fit					
SAsetOrientation(Label					
SAsetOrientation(Label:AC_Right,1)					
SAsetCursor(135,NoDr					
SAsetOrientation(Label					
SAsetOrientation(Label					
SAswitchCondition(Lab					
SAfitInterval(90,130,Fit					
SAsetOrientation(Label					
	name%.bsa,Unit Sphere				
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SAswitchCondition(Lab					
SAsaveSourceWavefo SAexit()	rms(%basename%_Low.	swf)			
SAexit()	e 🔽 Changes in	batch written to Database 🛛 Leave files in the file list open after running the batch			
		OK Cancel Help			

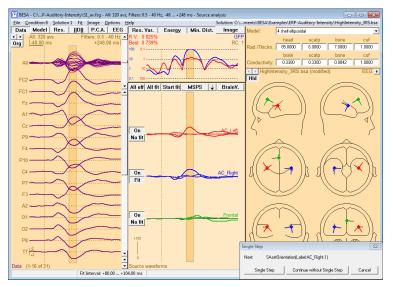
**Tutorial 13 – Cross-subject Statistics** 

- 6. Select the option Single step mode at the bottom right this will cause the batch to pause after every single command. This helps us in this tutorial to visually follow the processing steps and understand how the batch works. Press OK to start the batch.
- 7. BESA Research starts applying the batch commands to the first data file in the file list (S1_av.fsg). It loads the file and applies the specified filters. At the bottom right, a small window appears that indicates the next batch command to be executed. Proceed step by step by hitting the Single Step button and observe how BESA Research the different processes commands



**Tutorial 13 – Cross-subject Statistics** 

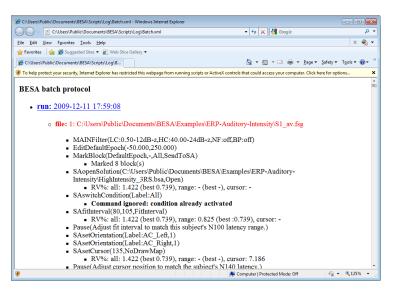
- 8. Note for example how the N100 orientation of the (the components first components of AC_Left and AC_Right) takes place in the marked fit interval. If the marked fit interval would not include this subjects N100 latency, we could manually adjust the fit interval while the batch pauses before continuing. For the data in this tutorial, the fit intervals have been defined appropriately for each subject, therefore you can hit Continue without single step at any time to make the batch proceed automatically without interruption.
- When the batch processing has completed, a notification window will appear.



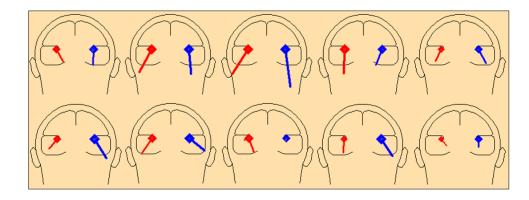
Batch C	ompleted
File:	1: C:\Uuments\BESA\Examples\ERP-Auditory-Intensity\S1_av.fsg
Task:	Batch Completed
	View Log OK Cancel

### Tutorial 13 – Cross-subject Statistics

10. BESA Research keeps track of all steps that have been carried out and documents them in a log file. After completion of the batch, you can press View Log in the Batch Completed window to view this log file. Then press OK to close the Batch Completed message window.



Batch scripts like the one shown above allow for simple and fast analysis of a complete EEG or MEG study. Output of our example script are individually adjusted source model files (*.bsa) and individual source waveforms for each condition, containing the brain responses to the different auditory stimuli in each subject.



The plot above illustrates the necessity to model the orientations individually. Shown are the ten different orientations obtained for the N100 component as stored in the individual solution files (*.bsa). The large variation of the N100 orientation between subjects is a correlate of the intersubject variability in the orientation of the auditory cortex. Without adjusting the orientations individually, the first components of **AC_Left** and **AC_Right** would not optimally reflect the individual N100 component.

**Tutorial 13 – Cross-subject Statistics** 

#### **C. Introduction Cluster Permutation Statistics**

Before we get started with BESA Statistics, it is important to understand the background of cluster permutation statistics. The following paragraphs will give a brief overview.

#### Preliminary parametric test

The preliminary parametric test is the first step in the analysis of data in BESA Statistics. The type of test that is applied depends on the experimental design that was chosen.

#### t-Test: Comparing two groups or conditions

For the comparison of two groups or two conditions, as a first step, BESA Statistics calculates a preliminary Students t-test (Hays 1988) between groups / conditions per data point. A t-test is computed to determine whether there is a significant difference between the mean of two groups / conditions. A t-test is associated with a t-value and a corresponding p-value which indicates the significance of an effect. P-values smaller than 0.05 are generally considered significant. The p-value becomes smaller if the t-value becomes larger. t-values are influenced by the size of the difference between the group / condition means and the size of the variance in both groups / conditions. Generally speaking, the difference between two group / condition means can be small for the test to become significant if the variance in both groups is also small. On the other hand, a large difference between two group / condition means does not automatically imply a significant effect. It is also required that the variance in both groups / conditions is not too large. Apart from the t-value, the p-value is influenced by the degrees of freedom (DF) for the specific test computed. DF are dependent on the number of subjects. The more subjects constitute the groups / conditions, the larger the DF. Larger DF are more likely to be associated with a significant test result, as variance can be estimated more precisely with a larger number of subjects.

A t-test can be paired or unpaired. A paired t-test is appropriate when there is a dependency between the two conditions that are compared. This is generally the case if two conditions in the same set of subjects are compared (e.g. subjects listening to high intensity vs. low intensity tones) or if the same subject is measured twice for the same task (e.g. listening to 1000 Hz tones before and after a training period). An unpaired t-test is appropriate when there is no dependency between the two groups that are being compared. This is generally the case if two different groups of subjects are compared for the same condition (e.g. motor response in left-handed

subjects vs. right-handed subjects). The selection of the correct type of t-test is important: in a paired t-test smaller differences between conditions are necessary for the test to become significant in comparison to an unpaired t-test.

Generally speaking, the likelihood of obtaining significant results is higher if a specific hypothesis about the data is tested. When there is no specific hypothesis as to whether one group / condition has higher or lower values than the other group /condition, a two-tailed t-test should be selected. When there is the hypothesis that the first group / condition has higher values than the second group / condition, a one-tailed-right t-test should be calculated. When there is the hypothesis that the first group be calculated. When there is the hypothesis that the second group / condition has higher values than the first group / condition, a one-tailed-right t-test should be calculated. When there is the hypothesis that the first group / condition, a one-tailed-left t-test should be calculated.

#### F-Test: Comparing more than two groups or conditions

If more than two groups or conditions need to be compared, the t-test is no longer feasible. For this scenario, the preliminary test of choice is the F-test. Here, an F-value is computed that corresponds to a p-value. The F distribution is not symmetric – there are no negative F values - and so there is no scope for choosing a one-tailed or two-tailed test as in the t-test scenario. Similarly to the t-test, the p-value is a measure for the likelihood that all groups / conditions were drawn from the same underlying distribution, and are thus interchangeable. In other words, a significant p-value (e.g. p < 0.05) points to a high probability that at least one of the groups / conditions differs from at least one of the other ones. It is, of course, also possible, that more than one pair of conditions differs from each other.

#### Permutation Test (non-parametric)

When computing a large number of t-tests or F-tests, the probability of obtaining a significant result by chance is high. Typically, an  $\alpha$ -error of 5% (p-value = 0.05) is assumed when calculating statistical tests. Thus, if a significant result is achieved, there is a 5% chance that this result is considered significant although in truth it is not. The more tests are computed, the higher the probability those statistically significant results are obtained by chance.

Statistically analyzing EEG / MEG data usually involves computing a large number of tests, as two conditions are compared for a large number of electrodes over a time interval containing

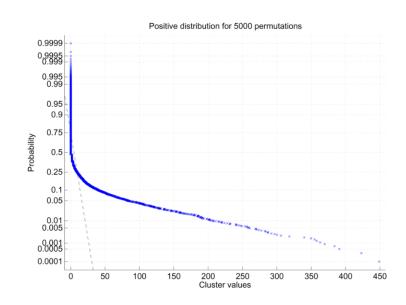
many data samples. Therefore, the multiple comparisons problem is particularly prominent when running statistics on EEG / MEG data.

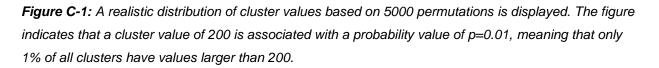
There are different ways to address the multiple comparisons problem. The easiest is to apply the Bonferroni correction (Abdi 2007), in which the significance level (e.g. p = 0.05) is divided by the number of tests computed and only those tests are assumed to be significant with p-values smaller than the corrected value. For example, if 100 tests are computed, the corrected p-value would be 0.05 / 100 = 0.0005. This approach is very conservative and the chance is high that significant results are wrongfully rejected.

BESA Statistics uses an alternative approach to deal with the multiple comparisons problem: Permutation testing in combination with data clustering. The main idea is that if a statistical effect is found over an extended time period in several neighboring channels, it is unlikely that this effect occurred by chance. Thus, the initial step is to define data clusters that show a significant effect between groups / conditions. For each cluster, a cluster value can be derived consisting of the sum of all t-values of all data points in the cluster. Then it is tested if the initial data clusters survive permutation. Permutation means that the data of subjects (i.e. unpaired tests were used for t-test, or between-group measures were taken for F-test) or conditions (i.e. paired t-tests, or repeated measures within the same group were used) get systematically interchanged. Depending on the number of subjects per group / condition and the type of test, a certain number of permutations are possible. For example, if 10 subjects are compared for two conditions using a paired t-test, 1024 (2ⁿ, where n is the number of subjects) permutations are possible. If 10 subjects are compared with another set of 10 subjects for the same condition using an unpaired t-test, 184756 ( $\binom{n+k}{k}$ ), where n is the number of subjects in the larger group, and k is the number of subjects of the smaller group; NB: in this example, the groups are the same size!) permutations are possible. For each of the calculated permutations (default: 1000¹⁴), a new test is computed and a new cluster value is derived for each of the initial clusters. Thus, a new distribution of cluster-values is determined for each of the initial clusters. Based on this new distribution, the significance of the initial cluster value can be determined. For example, if only 2% of all cluster values are larger than the initial cluster value, the initial cluster can be considered significant with

¹⁴ Drawn randomly without repetitions from all possible permutations. If there are not enough subjects to select 1000 permutations, the number is automatically adjusted to the maximum possible number of permutations.

a p-value of 0.02. Thus, based on the computed cluster-value distributions, the significance of each initial cluster can be determined directly (see the following Figure C-1).



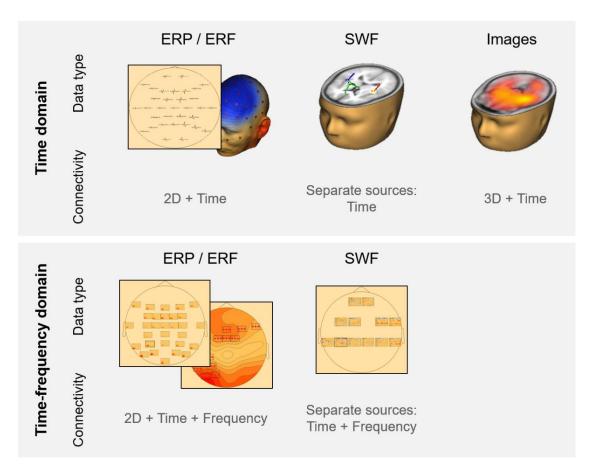


Depending on the direction of a statistical effect (i.e. condition 1 can have larger or smaller values that condition 2), negative and positive clusters can be found. If a negative cluster is tested for significance, it will survive permutation testing if the initial cluster value is more negative than 95% of all cluster values generated by permutation.

Since in permutation testing the distribution of cluster values is computed from the input data, and p-values are derived directly from the computed distribution, permutation testing is considered non-parametric, or parameter-free. This is a great advantage, since it is not required that data are normally distributed, as would be the case if classic parametric tests were used.

For more details on the implementation of the permutation test as implemented in BESA Statistics please refer to the following publications: (Bullmore, Suckling et al. 1999; Maris and Oostenveld 2007; Ernst 2004).

Cluster neighborhoods depend on the type of data: The figure below shows an overview of neighborhood connectivity with the data types supported by BESA Statistics.



#### **Post-hoc testing**

In the case of F-tests used in univariate ANOVA, it is highly desirable to investigate which pairs of conditions / groups were responsible for the significant effect. For this purpose, a post-hoc test is available. BESA Statistics uses a Scheffe test with subsequent Bonferroni-Holmes correction - after all, the problem of multiple testing applies for post-hoc tests as well: If, for example, 4 conditions are part of the test, 6 different combinations of pairwise comparisons need to be made. The Scheffe test ensures that the pairwise clusters do not grow outside the boundaries of the previously established F-test clusters. The Bonferroni-Holmes correction is less conservative than the pure Bonferroni correction but still provides a valid correction for multiple comparisons.

Tutorial 13 – Cross-subject Statistics

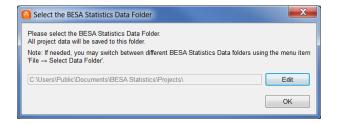
#### D. Analyzing source waveforms statistically using BESA Statistics

In the following, we will load the source waveforms we created in BESA Research and run a cluster permutation test in order to identify time-regions and sources, which show reliable differences between conditions High and Low across all subjects.

1. Start BESA Statistics by double-clicking its icon on your desktop.



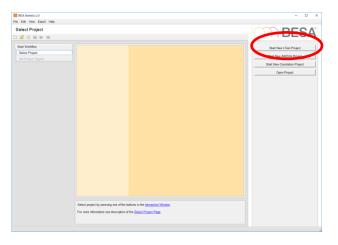
- When BESA Statistics is opened for the first time, the project folder needs to be defined. This is the folder where BESA Statistics stores and reads all project and result data. A dialog box appears. Press OK to confirm the pre-selected folder.
- Please close the notification box by pressing OK. We can now start with setting our project targets.





Tutorial 13 – Cross-subject Statistics

 Press Start New t-test Project in the interaction window on the right-hand side.



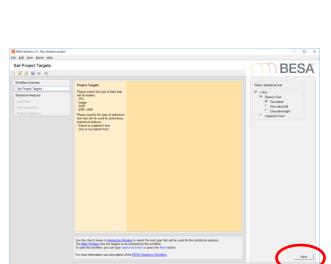
BESA

Select data type for which statistics shall be performed

TFC

□ Image ▼ SWF

- 5. As we want to analyze the source waveforms we created in the previous steps, please select SWF in the interaction window. Press Next. We will use a **paired t-test** for preliminary statistics as we compare two dependent samples, i.e. two conditions within the same set of subjects. We will use a twotailed t-test as we do not want to test a specific hypothesis.
- 6. Press Next or hit the space bar to continue to the next workstep.



BESA

Select statistical test

Paired t-Test

One-tailed-left

One-tailed-right
Unpaired t-Test

₩ t-Test

Tutorial 13 – Cross-subject Statistics

- Press Load Data for Condition 1 in the interaction window. (Please note: Had we selected an un-paired t-test, we would be prompted to load data for Group 1.)
- Specify the Group name to be "Control Subjects" and the Condition name to be "High" and press OK.



🙆 Specify Design				
Set name f	or group and condition.			
Group:	Control Subjects			
Condition:	High			
	OK Cancel			

9. Please browse to the folder C:\Users\Public\Documents\BESA\Res earch_7_1\Examples\ERP-Auditory-Intensity and select the 10 swf-files we previously created for condition "High". Press Open.

**Note:** Depending on the data selection under Set Project Targets, Files of Type will be automatically set to the expected file format.

Ontrol Subject	s/High					X	
Look <u>i</u> n:	👢 ERP-Auditory-In	tensity 👻	G 🤌 📁	• •			
e.	Name	A		Date modif	ied	•	
	S1_av_High.sw	f		17.02.2012	12:04		
Recent Places	S1_av_Low.swt			17.02.2012	12:04		
	S2_av_High.sw	f		17.02.2012	12:04	=	
	S2_av_Low.swf			17.02.2012	12:04		
Desktop	S3_av_High.sw	f		17.02.2012	12:04		
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	S4_av_High.sw	f		17.02.2012	12:04		
Libraries	S4_av_Low.swf	S4_av_Low.swf			17.02.2012 12:04		
	S5_av_High.sw	f		17.02.2012	12:05		
	S5_av_Low.swf			17.02.2012 12:05			
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	Recent Folders:	C:\atistics Example Data\Image Exa	mple\Cond 2	<u>n</u> •		.41	

### Tutorial 13 – Cross-subject Statistics

 A summary of the files we selected will be displayed in the main window and a green tick-mark will appear next to the first loadbutton. Press Load Data for condition 2 (or press Next or hit the space bar).

Load Data			BESA
Cell De la de la de la de la deservación deservación deservación deservación deservación deservación deservación deserva	Grage Contribut Filter	Creater bayes: For The Construction of the Construction of the	
	4		
	Specify group and condition	ves for group 2	Prevents

- Specify the second Condition name to be "Low". Note that Group cannot be edited anymore, as the paired t-test expects two different conditions the same set of subjects. Press OK.
- 12. Now select all 10 swf-files with the extension "_Low" and press Open. Important note: If a paired t-test was selected under Set Project Targets, the number of files loaded in both conditions needs to be identical. This is not the case for un-paired t-tests.

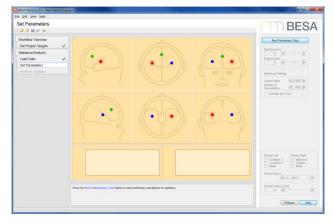
🙆 Specify Design					
Set name for group and condition.					
Group:	Control Subjects				
Condition:	Low				
	OK Cancel				

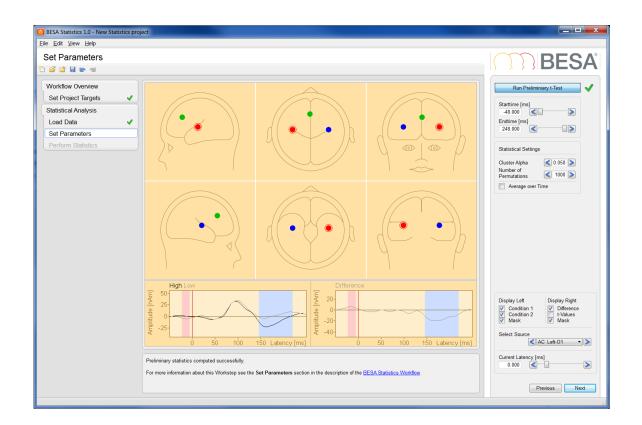
Look <u>i</u> n	ERP-Auditory	r-Intensity	▼ ③ ∅ ▷ □ ▼	
P	Name	·	Date m	odified
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	S6_av_Low.s	wf	17.02.20	012 12:05
·	S7_av_High.	swf	17.02.2	012 12:05
Desktop	S7_av_Low.s	wf	17.02.20	012 12:05
	S8_av_High.swf		17.02.2	012 12:05
	S8_av_Low.s	wf	17.02.20	012 12:05
Libraries	S9_av_High.	swf	17.02.2	012 12:05
	S9_av_Low.s	wf	17.02.2	012 12:05
	S10_av_High	n.swf	17.02.2	012 12:05
Computer	S10_av_Low	.swf	17.02.2	012 12:05
	•	III		Þ
Network	File <u>n</u> ame:	"S10_av_Low.swf" "S1_av	_Low.swf" "S2_av_Low.sv 🔻	Open
	Files of type:	Source Waveform Files (*.	swf) 🔻	Cancel

### Tutorial 13 – Cross-subject Statistics

- 13. Again, a summary of all files loaded for the second condition will be displayed in the main window and a green tick-mark will appear next to the second load-button. Press Next or hit the space bar to continue.
- 14. The three sources that are part of our source model are now displayed in the main window. They have the same colors as in the source analysis window in BESA Research. Press Run Preliminary t-Test or Next to see point-wise t-test results.







**15.** Results for the first orientation of the first source in the source model (AC_Left) are displayed in the detail windows. It appears that "Low" has smaller amplitudes than "High" in the baseline, and "High" has smaller amplitudes then "Low" over an extended time-range starting roughly at 150 ms.

**Important note:** These are preliminary results that are not corrected for multiple comparisons! They should not be interpreted. They only serve as an initial orientation for what is in the data and for defining regions of interest.

#### **Tutorial 13 – Cross-subject Statistics**

- 16. Different sources and orientations can be selected in the drop-down menu of the interaction window. Under "Display Right" a selection can be made between Difference (i.e. in this case "High" minus "Low") or tvalues (point-wise). The according selection will be displayed in the right detail window. A mask indicating significant time-periods can be switched on or off for both detail windows.
- Display Left Display Right J Condition 1 Difference Condition 2 t-Values 1 Mask J Mask Select Source AC Left-O1 > • AC Left-O2 Current Latency [ AC_Left-O3 0.000 AC_Right-O1 AC_Right-O2 AC_Right-O3 Frontal-O1 Frontal-O2 Frontal-O3
- 17. It is possible to restrict further analysis to a region of interest by changing the Starttime and Endtime. This can increase the chance that a cluster survives permutation as the distribution of cluster values is estimated based on a restricted sub-set of datapoints. For example, one might only include the poststimulus period in the calculations. We will not restrict the time-window now but see what happens to the cluster in the baseline after permutation.

Run Preliminiary t-Test	<b>√</b>
Starttime [ms] -48.000  Endtime [ms] 248.000  Starttine [ms]	
Statistical Settings	
Cluster Alpha Number of Permutations 1000 Average over Time	

Under the section "Statistical Settings", a Cluster Alpha value needs to be set. This value refers to the significance level that is the threshold for a data-point to enter a cluster. The default value – which we will use now – is p = 0.05. However, it can also be a value larger or smaller than 0.05, larger p-values leading to larger clusters. Please note that the Cluster Alpha value does not equal the significance level of the permutation statistic!

The number of permutations also needs to be set. The default level – which we will be using – is **1000** in order to get a reliable estimate for a significance level of p = 0.05, i.e. if the test is run multiple times, the same clusters will stay significant. The possible number of permutations is restricted by the number of subjects and the type of preliminary t-test (paired

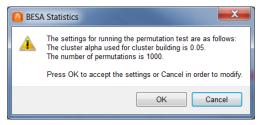
Tutorial 13 – Cross-subject Statistics

or unpaired, see above). If 1000 exceeds the number of possible permutations, BESA Statistics will automatically reduce the value to the number of possible permutations.

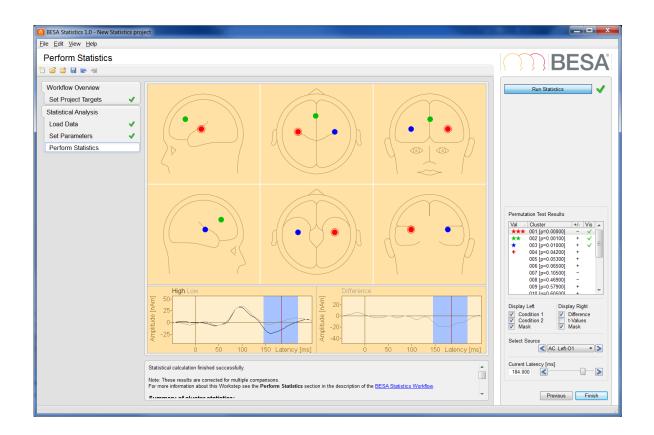
Press Next or hit the space bar to continue.

- Press Run Statistics to start the permutation process and all according calculations necessary for determining the probability of our initial clusters.
- 19. An information window appears summarizing the settings for the cluster permutation test. Press
  OK to continue. Please note that the following calculations can be time-consuming depending on the type of data and the number of datapoints.



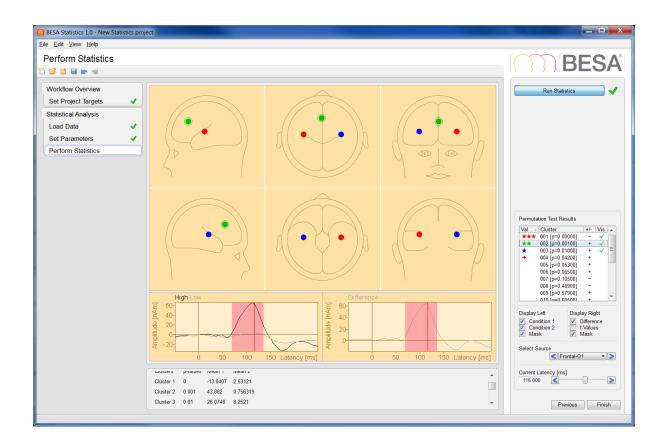


Tutorial 13 – Cross-subject Statistics

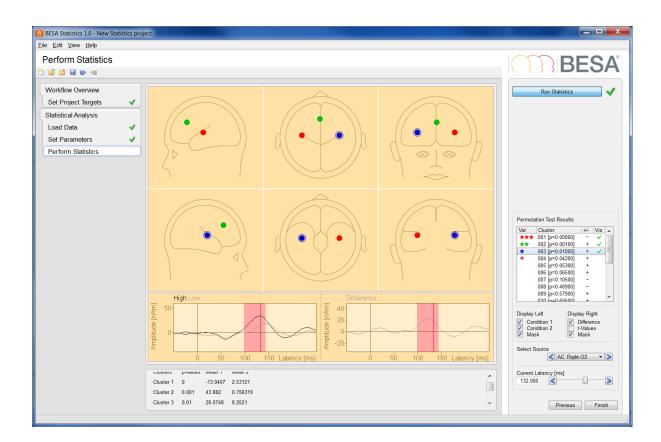


**20.** Once permutation statistics are computed, the cluster with the largest cluster value (and smallest p-value) is automatically selected and displayed. Up to 100 clusters are displayed in the interaction window and significant clusters are marked with stars according to their significance level. Clusters that do not reach p = 0.05 but are smaller than p = 0.1 are marked with a "+".

The biggest cluster is the one we saw previously (see step C 15) in source AC_Left in the first orientation. We can now be certain that "High" does indeed lead to a more negative amplitude than "Low" in an area of the left auditory cortex associated with tangential activity. This effect lasts from 144 ms to 216 ms. It is noteworthy that the preliminary cluster that could be seen in the baseline did not survive permutation!



21. The second significant cluster is found in the first orientation of the frontal source. "High" leads to larger amplitudes than "Low". This effect starts at 68 ms and ends at 136 ms.



- **22.** The last significant cluster can be found in the second orientation of AC_Right, i.e. the auditory cortex region associated with radial activity. Again, "High" has larger amplitudes than "Low" from 100 ms to 144 ms.
- 23. A summary of cluster statistics containing the cluster p-values and the condition means are displayed¹⁵ in the information window below the main window.

Summary of cluster statistics:					
Clusters	p-values	Mean 1	Mean 2		
Cluster 1	0	-13.0407	2.53121		
Cluster 2	0.004	10 000	0 756940		

¹⁵ It is also possible to export all statistical results as well as group/condition and individual means as csv-files. Likewise, it is possible to export all images as png or eps-files.

Tutorial 13 – Cross-subject Statistics

24. Press Finish to end and save the current project. Save it under an appropriate name.

Group		Project	<ul> <li>Date Modified</li> </ul>
✓ Example			
Selected Group: Selected Project:	Control Subjects Auditory Intensity Source Waveforms		Save
Project Type:	Statistics Projects		*

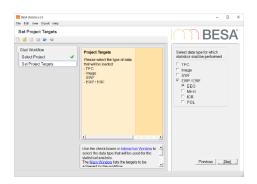
#### E. Comparing more than two conditions or groups with BESA Statistics

In the following, we will explore the possibility to compare more than two conditions or groups using the ANOVA workflow. We will continue using our auditory intensity data set, however this time we will work with scalp data and will be asking if there is a significant difference between the conditions: 60dB, 70dB, 90dB and 100dB. Additionally, we will consider a variable of no interest, in this case the number of trials per average per condition per subject, and will thus be using the ANCOVA workflow.

 Start BESA Statistics by double-clicking its icon on your desktop. Then start a new ANOVA project by selecting the Start ANOVA project button.



 Please select the data type to be analyzed. In this case it will be an ERP/ERF experiment and we will be looking at EEG data directly. Press Start to proceed to the next step.



**Tutorial 13 – Cross-subject Statistics** 

- 3. As we are dealing with different conditions within one group of subjects we select the Within Group (repeated measures) option. We wish to consider the number of trials per condition per subject as a covariate of no interest and thus select the option With Covariate of no Interest (ANCOVA). Please press Next.
- 4. Our example auditory intensity experiment has 10 subjects and we are considering 4 conditions (60dB, 70dB, 90dB and 100dB) and our covariate of no interest is the number of trials. Please enter the values in the respective fields and press Next.





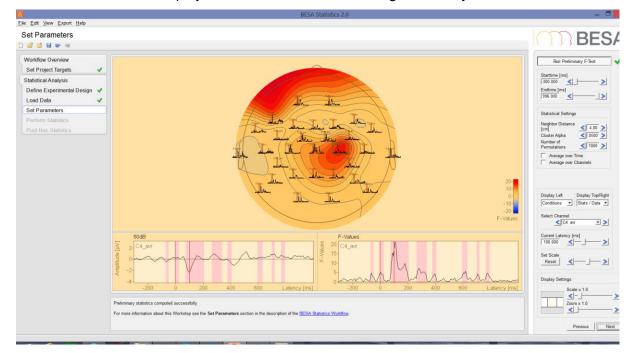
5. Load all covariates of no interest and conditions for all subjects. The respective files are located in the BESA examples folder:

..\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\... Please note that as we will be permuting over the conditions (whose difference we are probing) the order of the subject files must be the same for all conditions. If this was an in-between group case instead, the order of the subject files would not matter. Check that the order is indeed the same. In case that it is not, the order can be changed by clicking into the respective cell and using the arrow button that appears. When

	ata		()) BES
ế 📫	8 🖻 🕯		
Subject	Covariate	Condition 1	Load Data for number of trials 60dB
Nr.	number of trials 60dB	60dB	Load Data for 60dB
1	64	D:\BESA Statistics 2.0 Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S1 av-60dB.avr	
2	84	D:\BESA Statistics 2.0 Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S2 av-60dB.avr	Load Data for number of trials 70dB
3	110	D:\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S3_av-60dB.av-	
4	66	D:\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S4_av-60dB.av	Load Data for 70dB
5	70	D:\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S5_av-60dB.avr	Load Data for number of trials 90dB
6	94	D:\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S6_av-60dB.avr	
7	88	D:\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S7_av-60dB.avr	Load Data for 90dB
8	54	D:\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S8_av-60dB.avr	
9	97	D:\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S9_av-60dB.avr	Load Data for number of trials 100dB
10	51	D:\BESA_Statistics_2.0_Examples\Examples\ANOVA ANCOVA Correlation Example Data\ERP 4 Conditions\60dB\S10_av-60dB.av	Load Data for 100dB
			Accept Data Order
		•	
a were	loaded successfu	lly.	

#### you have confirmed the order please select Accept Data Order and press Next.

6. Once you pressed Run Preliminary F-Test you can recognize several regions where significant results were found. Change the Current Latency to 100 ms. Using the map of F-values you can pick the channel with the peak of the map (C4). Click on this channel in the Top View to see the F value and amplitude distribution displayed in the selection window. You can choose to visualize each of the conditions via the Display Top/Right drop down menu. Note the maximum around the C4_avr channel that corresponds to a preliminary cluster as is evidenced by the red shading in the right display window. In the left detail window, the amplitude plot for the 60dB condition is shown. The Display Left drop down



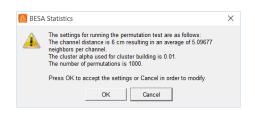
menu can be used to display the other conditions including an overlay of several conditions.

7. In the menu Statistical Settings, we should choose a neighborhood relation for the channels. Please select 6 cm for neighbor distance. Larger values would produce more spatially extended clusters. The cluster alpha should be set to 0.01. Please note that, as previously mentioned, the cluster alpha is not the significance level of the permutation test. Instead, it determines the threshold for which points will be part of the same cluster in each permutation. The number of permutations should be set to 1000.

Neighbor Distance	
[cm]	< 6.00 >
Cluster Alpha	< 0.01 >
Number of	
Permutations	< 1000 >
Average over T	imo

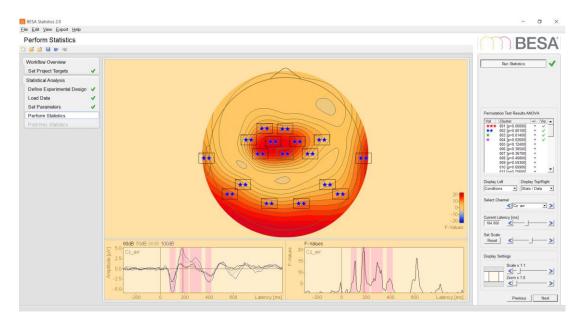
Tutorial 13 – Cross-subject Statistics

 Please click on Next and on the ensuing screen press Run Statistics. A dialog will pop up requesting to confirm the statistical settings. Press Ok to proceed with the computations.



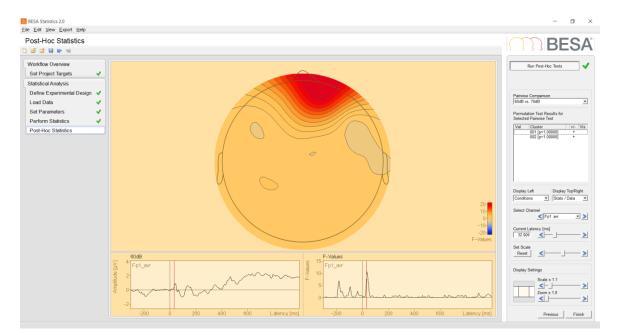
#### 9. On the Permutation Test Results ANOVA list the

clusters, that now also have a spatial dimension, are shown in order of smallest p-value first. The clusters marked with stars or plus signs (the latter is not present in the screenshot) have p-values smaller than 0.05. Note that contrary to our previous source waveform example the interpretation of the clusters of scalp recordings might be more challenging. Different clusters might actually be due to the same physiological effect and as such should be interpreted together.

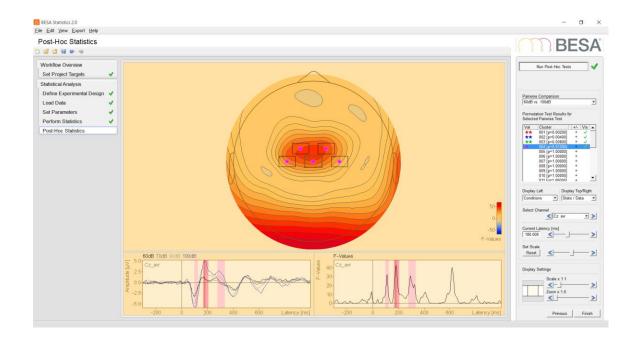


- 10. While browsing through the clusters we might already recognize that the signal from the green source we analyzed earlier in section D is part of one of the star marked clusters. Press Next to proceed to the post hoc evaluation.
- 11. In the post hoc workstep we can determine which pairs of conditions actually differ from each other. Select the Run Post-Hoc Tests button to start the computations. In the Pairwise Comparison menu choose which two conditons are to be compared. By default we start with the first conditions 60dB vs 70dB. The Permutation Test Results for

**Selected Pairwise Test** menu shows that all clusters were discarded by the post-hoc test. Hence we conclude that the conditions 60dB and 70dB are interchangeable. Note that all clusters that were discarded by the post-hoc test are set to p-value = 1.0.



12. When comparing 60dB to 100dB instead a number of clusters survive the post-hoc test. These conditions are therefore significantly different. This goes in line with our earlier findings with the source space data set where we find a frontal activity at high intensities to be significantly different from that at low intensities. The cluster highlighted below as well as the third cluster suggests the same interpretation for early latencies. The clusters 1 and 2 are active at later latency ranges.



### F. Analyzing the correlation between physiological response and a covariate of interest

The data we will use for the following example were kindly provided by Peter Schneider, University of Heidelberg, Germany. Part of the data was used in a publication by Schneider et al. (2005) in Nature Neuroscience¹⁶.

The experiment involved measuring MEG data while playing tones (instrumental sounds) to professional musicians. Subjects listened passively. Between 500 and 1000 stimuli were recorded and averaged for each subject.

For each subject, a musicality index was also established using the AMMA test for musical aptitude (Advanced Measurement of Music Audiation by E.E. Gordon, 1989). The tonal score was used for this experiment. It can lie in a range between approx. 20 points (random guessing) and 40 points.

¹⁶ P. Schneider, V. Sluming, N. Roberts, M. Scherg, R. Goebel, H.J. Specht, H. G. Dosch, S. Bleeck, C. Stippich & A. Rupp (2005). Structural and functional asymmetry of lateral Heschl's gyrus reflects pitch perception preference. Nature Neuroscience 8:9, 1241-1247 **BESA Research 7.1 Tutorial** Page 390 of 397 Copyright © 2020 BESA GmbH, Freihamer Str. 18, 82166 Graefelfing, Germany

### Tutorial 13 – Cross-subject Statistics

We want to find out whether there is a correlation between the strength of source activation following the musical stimulus and the musical aptitude.

- Start BESA Statistics by double-clicking its icon on your desktop. Then start a new Correlation project by selecting the Start New Correlation Project button.
- Select type SWF. Press the Start button.
- Enter the Number of Subjects: 18, and names for the covariate of interest and data (AMMA tonal and Musicians). Then press the Next button.

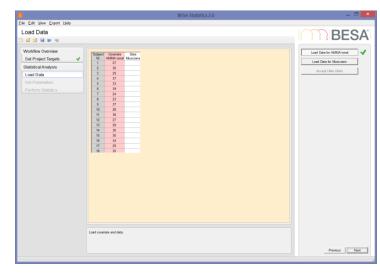
_ ī <mark>×</mark>
BESA
Start New t-Test Project
Start New ANOVA Project
Start New Correlation Project
Open Project

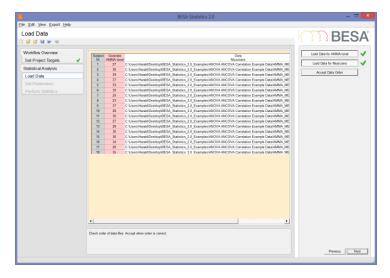
	ct data type for which statistics I be performed
- -	TFC Image SWF ERP / ERF

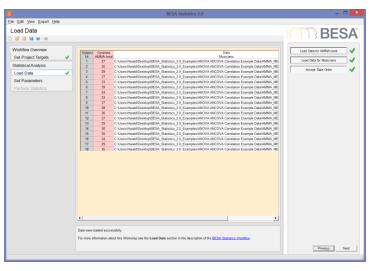
Selected Da and Statistic	
SWF	
Correlation	
Dofino Evpo	rimental Design
Denne Cybe	innental Design
	Subjects 18
	Subjects 18
Number of S	Subjects 18

### Tutorial 13 – Cross-subject Statistics

- 4. The next step is loading the data for the covariate. Press the Space key on the keyboard or the button Load Data for AMMA tonal. Then browse to the file AMMA_MusAlphabetical.txt in the folder AMMA_MEG that should be on your desktop. Open the file.
- Now load the MEG data.
   Press the second button or the Space key on the keyboard. Browse to the folder Mus_SWF300 and select all files in the dialog box. Then press Open.
- 6. Check that the files are sorted alphabetically (the AMMA scores were given in alphabetical order). If not, click into a row to re-sort using the arrows that appear. Once finished, press the button Accept Data Order. Then press Next.

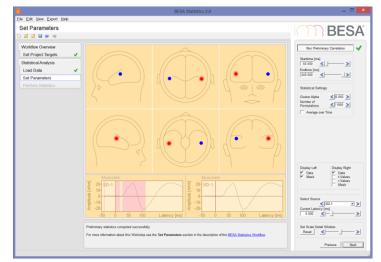


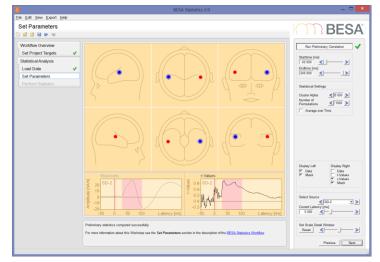


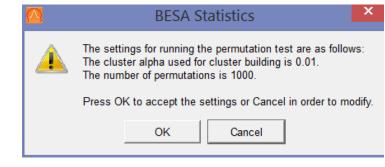


### Tutorial 13 – Cross-subject Statistics

- The plot shows the approximate position of the dipoles. Press the button Run Preliminary Correlation. The widgets below the head symbols show the zones where an initial correlation was found for source SD-1 in the right auditory cortex.
- 8. Select the source SD-2 and change the display of the right widget to show r-Values and the Mask. The oscillations of r values in the baseline and early post-stimulus and a more steady behavior at later latencies are apparent. Note that for SD-1, there is some significant correlation in the baseline, too.
- Change the cluster alpha to 0.01. Leave the number of permutations and the time interval unchanged. Press Next, then Run Statistics. Press OK when the dialog box appears.





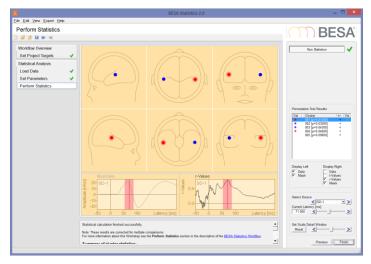


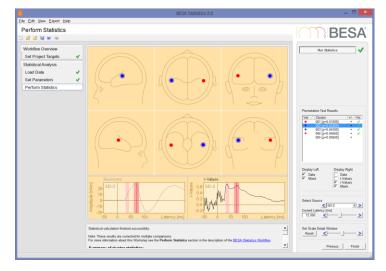
### Tutorial 13 – Cross-subject Statistics

10. The Correlation test finds one significant cluster in SD-1, and three trends. (Note that the trends have p-values < 0.05, but are not deemed significant since we can have positive or negative correlation, i.e. we are running a two-sided test).</li>

The right-hemispheric cluster starts just after 50ms poststimulus, and extends to about 85 ms. It ends well before the N100 response takes place.

- Select cluster 2. This cluster is in the left hemisphere, and is in a similar interval between approximately 60 and 80 ms.
- **12.** The other trends also appear in early processing intervals.
- Press Finish and save the project.





This experiment shows that for professional musicians, there is a (positive) correlation between the auditory response and the musical aptitude, which manifests itself in the early auditory processing stages between 50 and 90 ms after a tonal stimulus. There is no significant correlation at later stages of the processing (N100, N150).

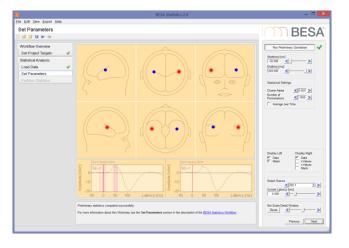
Now let us see if we can get similar results for non-musicians. We will follow the same steps.

14. Start a new Correlation project by selecting the Start Correlation
Project button. Then select SWF as data type. Press Start. In the step
Set Project Targets, set Number of Subjects to 15, and name the factors as AMMA tonal and Nonmusicians. Press Next.

Selected Da and Statistic		
SWF		
Correlation		
Define Expe	rimental Design	
Number of S	ubjects 15	
Factor Name	es	
Covariate of Interest	AMMA tonal	
Data	Non-musicians	

 Load the data. The covariate is in the text file
 AMMA_NonmusAlphabetical.txt.

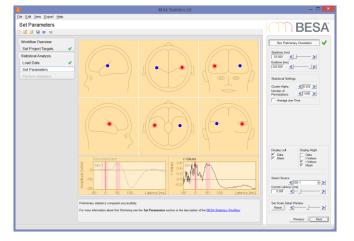
The SWF data are in folder NonMus_SWF300. Select all files and open. Then accept the data order and press Next. On the Set Parameters workstep, press the button Run Preliminary Correlation. The initial significance plot appears.



### Tutorial 13 – Cross-subject Statistics

- 16. Switch to r-Values for the right widget and activate the mask.
  Toggle between the two sources.
  We can see that there is some preliminary correlation around the P50 latency similar to the previous example, and also around the stimulus latency.
- 17. Let us proceed as before. Set the cluster alpha value to 0.01. Then press Next and Run Statistics on the next screen. Accept the settings in the dialog.
  - A message box appears to inform that no clusters emerged. The result is a question mark beside the **Run Statistics** button, and a notification at the bottom that the calculation failed.
- 18. The reason for this is the problem that not a single cluster could be built with the stronger condition of a 0.01 alpha value.

Press the **Previous** button to go back to the workstep **Set Parameters**. Increase the alpha

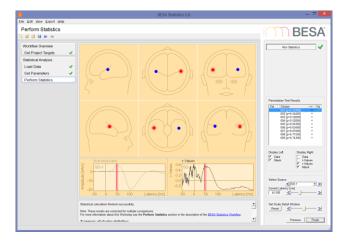




Tutorial 13 – Cross-subject Statistics

value back to the original of 0.05. Then advance and re-run the Statistics.

19. With this setting, clusters are found. However, none of them is significant or shows even a trend.



We can conclude that contrary to professional musicians, for non-musicians, there is no significant correlation between the tonal musical aptitude and the amplitude of the auditory response to musical tones in the primary auditory cortex.

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