BESA®

Research 5.3.9

Tutorials

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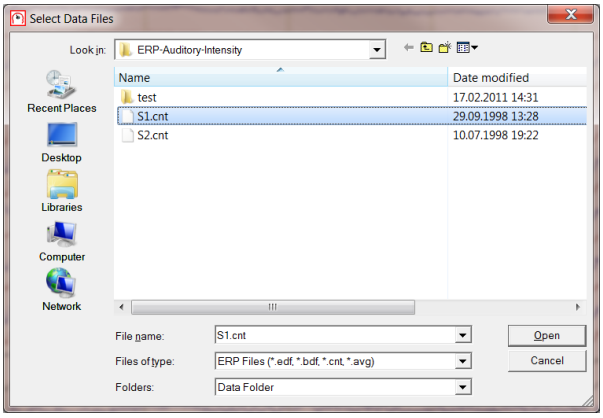
This Tutorial is created to guide you through a complete data analysis from preprocessing of individual datasets to cross-subject statistics of source analysis, time-frequency analysis or source coherence results. We will work with simulated datasets that help to understand basic mechanisms. Further 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_5_3\Examples\ERP-Auditory-Intensity). In this experiment 10 subjects listened to tones (1000 Hz) of different intensity (60, 70, 80, 90, 100dB).

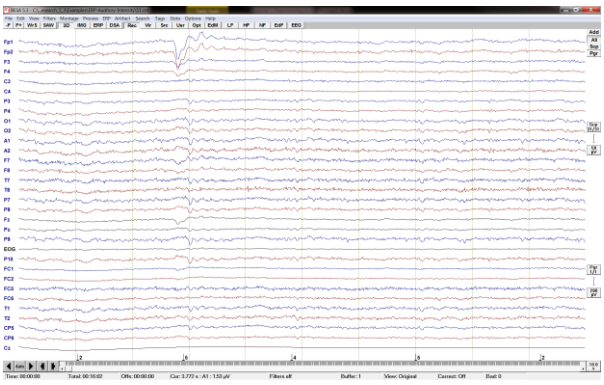
- ✓ Paging through your data screen by screen or jumping to selected time-points / events
- ✓ Adjusting signal amplitude and time-scale
- ✓ Interpolation of bad channels, changing the channel status
- ✓ Marking of Artifacts
- ✓ 3-D mapping of the topography at the selected cursor position
- ✓ Remontaging 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

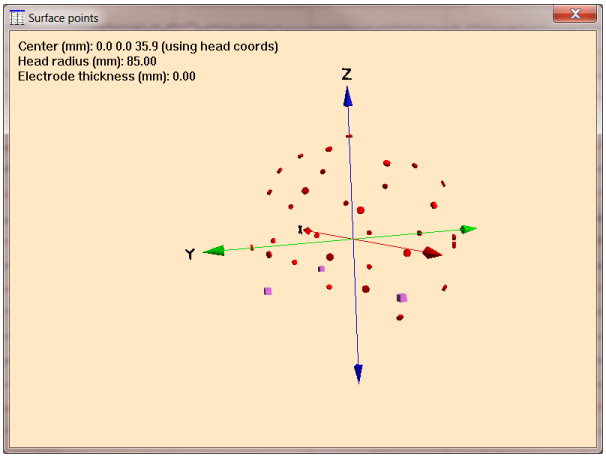
1. **Start BESA Research.** From the **File** menu, choose **Open**. In the 'Select Data Files' box, select folder **ERP-Auditory-Intensity** in the **Examples** folder. Change the file type to 'ERP files' (*.edf, *.bdf, *.cnt, *.avg)' and open file **S1.cnt**.



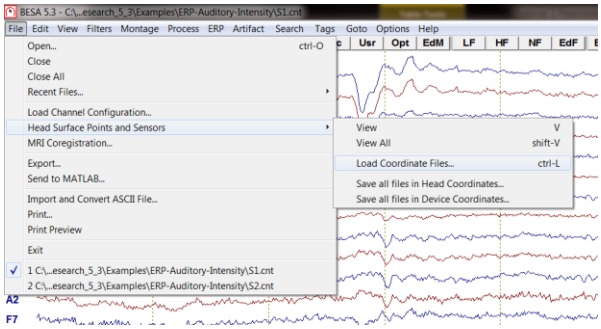
2. This is a dataset recorded from a 32-channel electrode cap with Cz as reference and one EOG channel. Blue electrodes are over the left side of the head, red electrodes over the right side, black electrodes are over the midline. Triggers are coded as vertical black bars at the bottom of the screen.



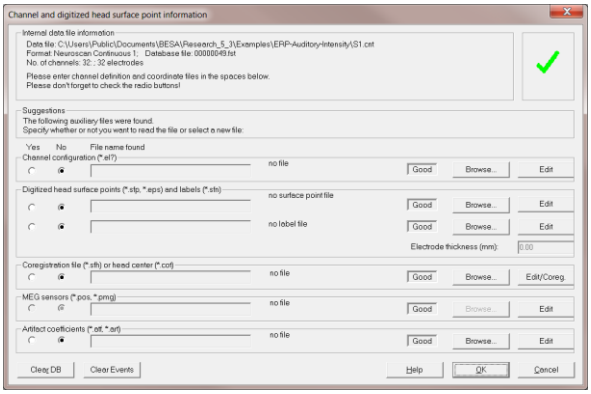
3. To see the 3D-coordinates of the electrodes and additional head surface points, select **File / Head Surface Points and Sensors / View**. (Alternatively, you can type the short cut 'V' on the keyboard.) Fiducials are displayed as pink cubicles, electrodes as red discs. If you click on an electrode 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.



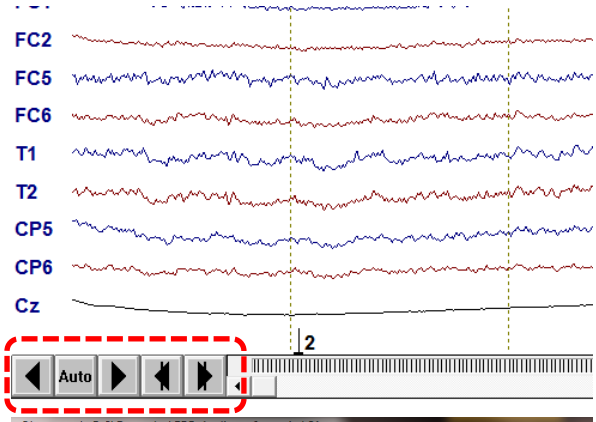
4. Select **File / Head Surface Points and Sensors / Load Coordinate Files…** (alternatively: ctrl-L) A window opens showing all auxiliary information that is associated with the data file.



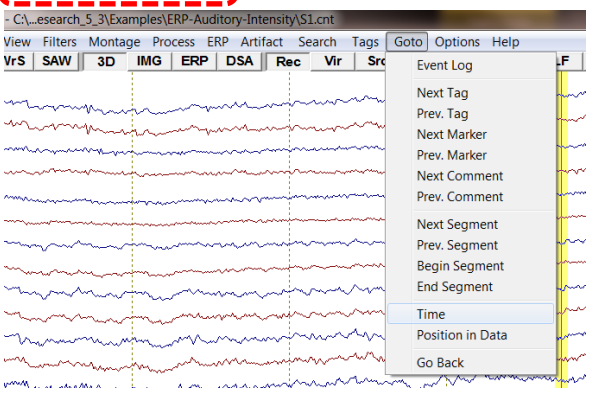
5. This dialog window allows to specify additional information on channel configuration (*.ela), digitized head surface points (*.sfp, *.eps) and labels (*.sfn) and more. Here, no additional files need to be loaded as all the relevant information is stored within the data file. If BESA finds auxiliary files with the same basename as the data file, they will be automatically loaded. Close the window.



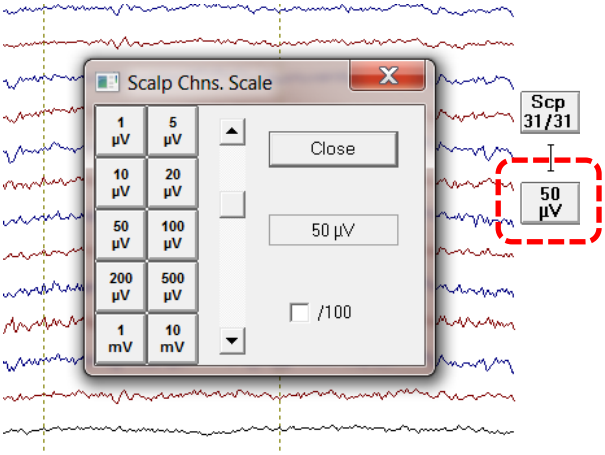
6. In order to page through your data you can either hit the space bar or use the arrows in the bottom left corner of the main window. The arrows with the vertical bar allow you to move half a page forward or backward.



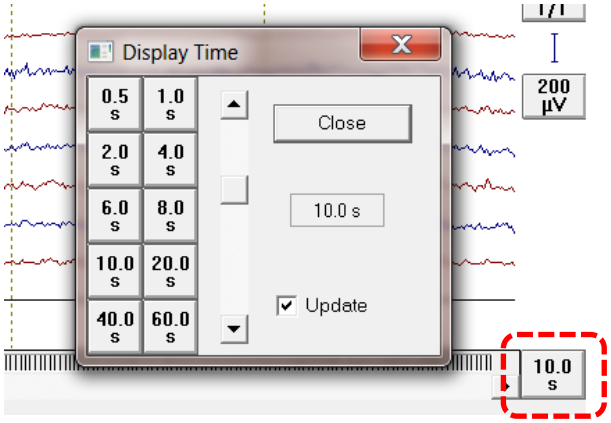
7. You can also jump directly to a certain time-point by pressing **Goto / Time…**
You might also add markers or comments to your data by right-clicking into the data and selecting 'Marker' or 'Comment'. You can then jump directly to these events via the 'Goto' menu as well.



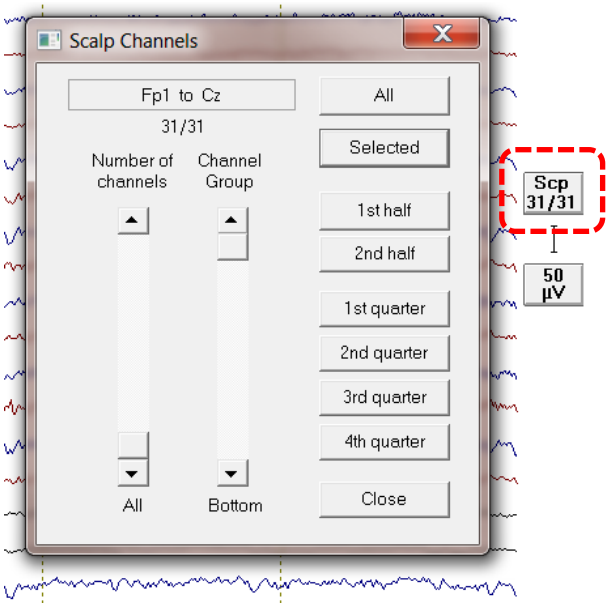
8. To rescale your data use the **amplitude scaling button** on the right side of the main window (just below the button 'Scp'). In the present case the scaling of 50 µV is appropriate.



9. In order to change the length of the data window that is displayed at a time use the **time-scaling button** at the bottom right of the main window. In the present case 10.0 s is an appropriate viewing window.

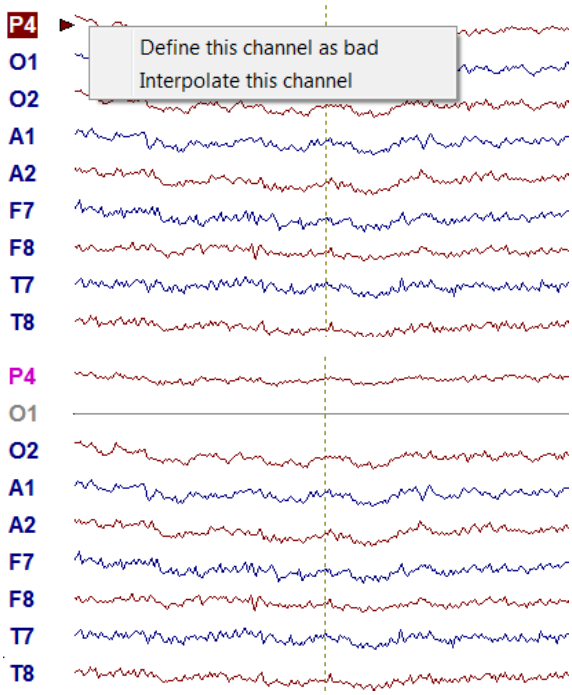


10. If you only want to view a sub-set of your electrodes use the button **'Scp'** on the right of the main window. You can either choose the first / second half or the 1st to 4th quarter of the recorded channels. You may also adjust the number of electrodes you want to have displayed by using the sliders.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

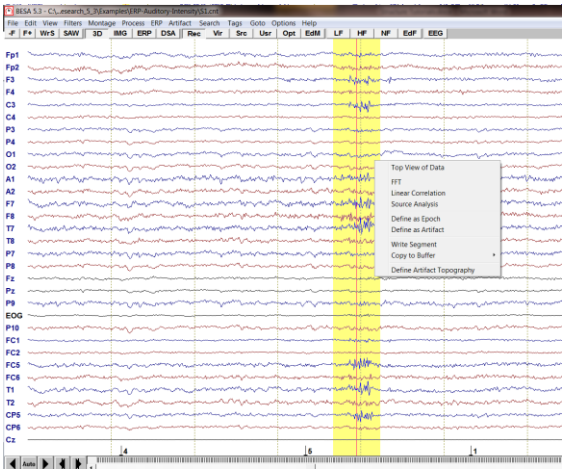


B: Interpolation, Marking of Artifacts, 3-D Mapping, Remontaging

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 'Define this channel as bad' or 'Interpolate this channel'[1]. 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.*

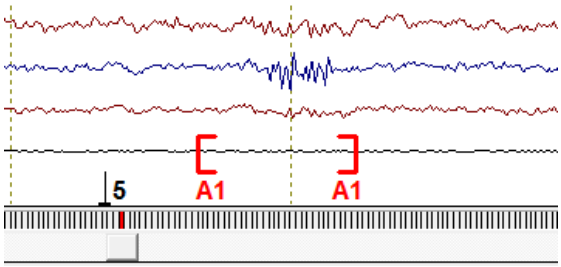


2. BESA uses amplitude, gradient and low-signal thresholds when scanning for artifacts (see Tutorial 2). Still, you might want to specify artifacts in your data by hand. This can be useful for e.g. muscle artifacts that are high-frequent and small in amplitude. In the current dataset **Goto Time 00:04:28**. 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

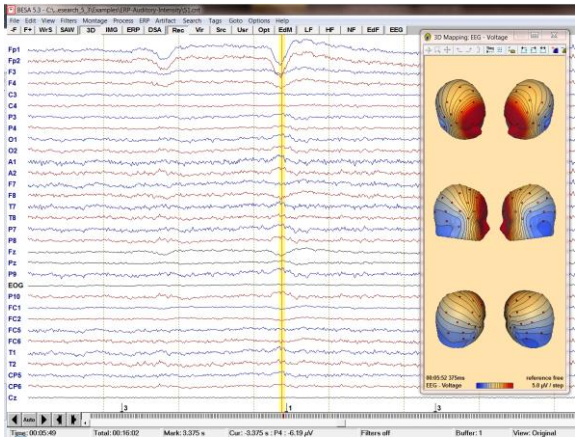


[1] Important note: in case a channel is at located at the rim of the electrode cap it is recommended to set it as bad rather than interpolate it as interpolation might be inaccurate. The position of an electrode can be checked following step A3 of this tutorial.

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.

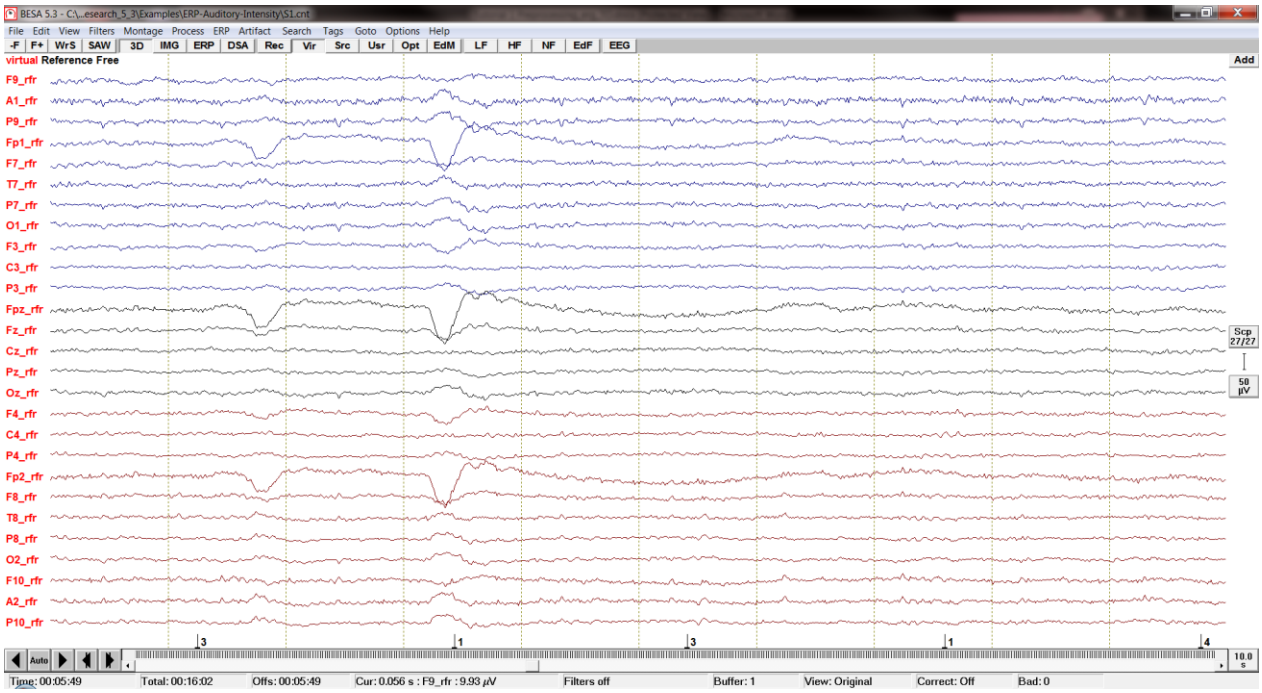


3. **Goto Time 00:05:49**. You will see an eyeblink that is mainly picked up by electrodes Fp1 and Fp2. **Double-click** on it to open the **3D-Mapping** window. You will see a strong frontal positivity that is typical for eye-blinks in EEG data. We will not deal with eyeblinks at this stage.

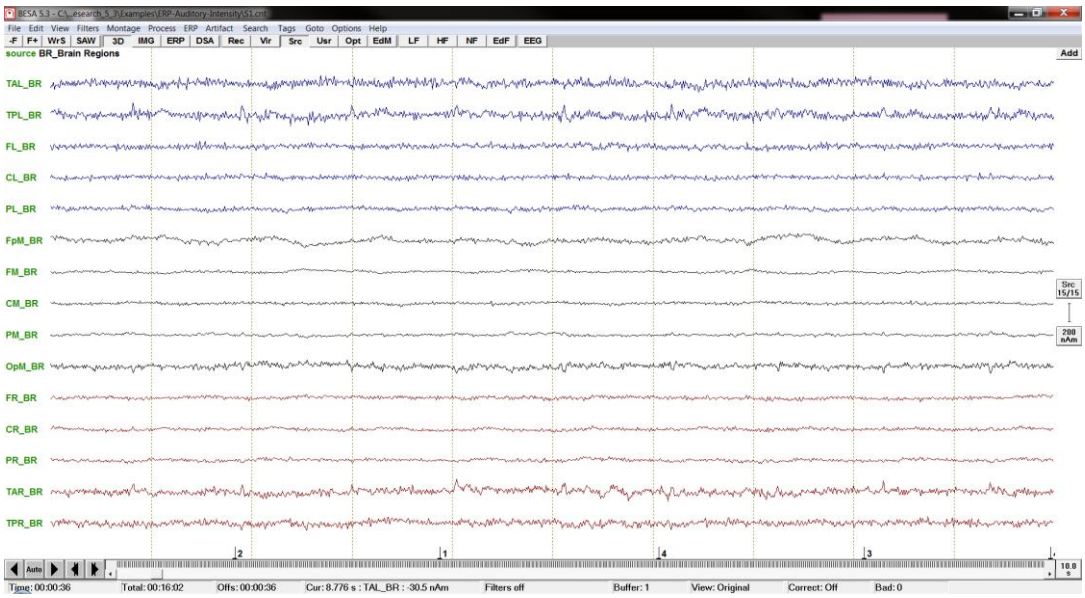


4. It is possible in BESA Research to remontage the recorded data using virtual standard electrode caps (**VIR-Button**) (international 10-10-system) or source montages. In the former case you can view the data as if they had been recorded using a standard electrode cap. In the latter case data are translated into source space using source montages provided by BESA (**Src-Button**) or created by the user (**USR-Button**, see Tutorial 7) so that you can get an immediate display of activity in the respective sources in the continuous recording.

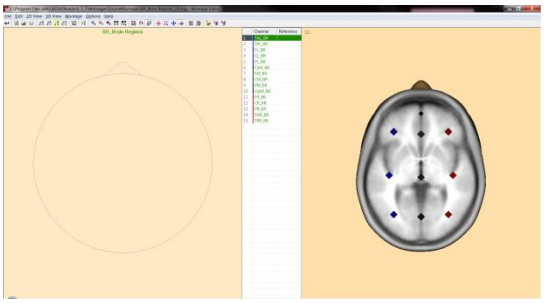
a) Select **'Vir' Reference Free**. This virtual montage consists of 27 standard EEG channels that have been average-referenced. Note that the Cz electrode carries signal in this montage. Also note that the signal becomes cleaner as the noise that is common to all electrodes gets subtracted by average referencing.

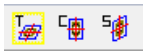


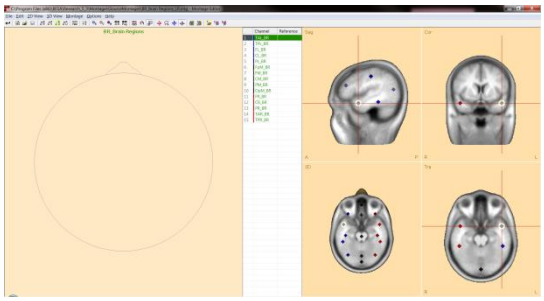
b) Select 'Src' BR_Brain Regions. Paging through the data you can immediately see that the left and right temporal sources carry more signal than the other sources.



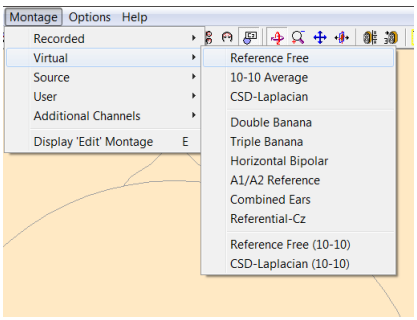
5. It is possible to view the current montage by pressing the **'EdM' button** (alternatively you can press **Edit / Montage**). In the current case we see a source montage that consists of 15 sources covering the whole brain. If you

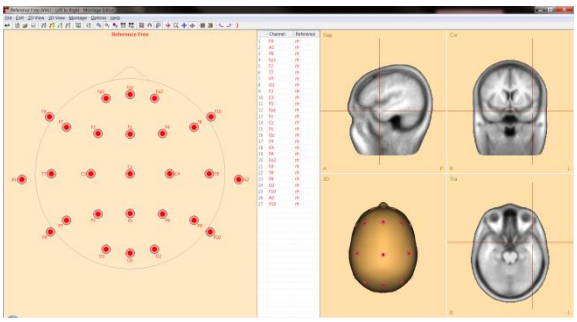


click on a source label in the middle panel BESA will slice the standard MRI to the position of the source. You may change to a coronal or saggital view by pressing the according buttons  . To see different views at the same time, press the button .



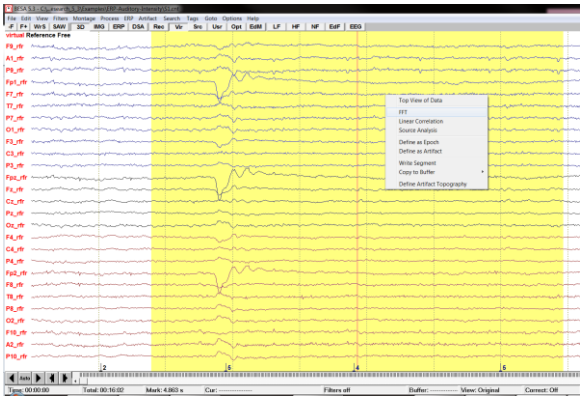
6. In the montage editor press '**Montage / Virtual / Reference Free**' to change back to sensor level. Now you see the position of the 27 standard electordes. Note that you can create your own montages or re-reference your electrodes in the montage editor as well. Return to the raw data using the current montage by pressing the button located in the top left corner of the montage window.





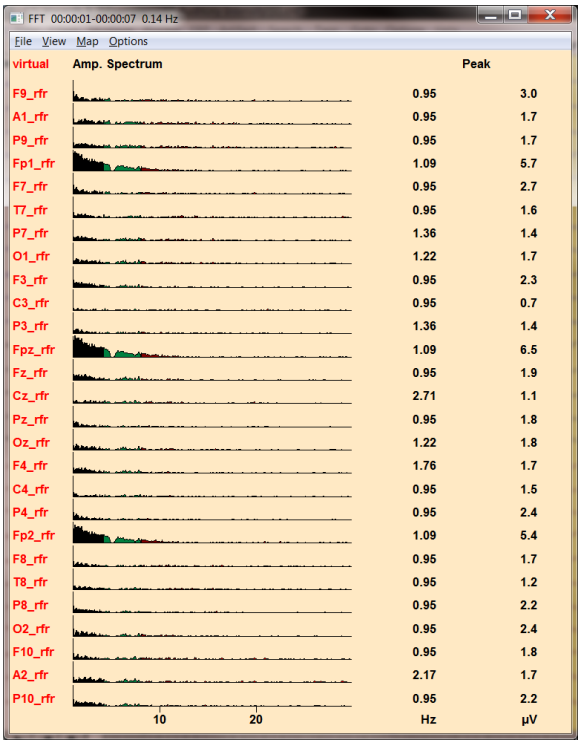
C: FFT and DSA

1. BESA allows you to calculate an FFT on a marked datablock[2] to get a quick idea of the amplitude or power in different frequency ranges. Start the FFT by **left-dragging 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.*

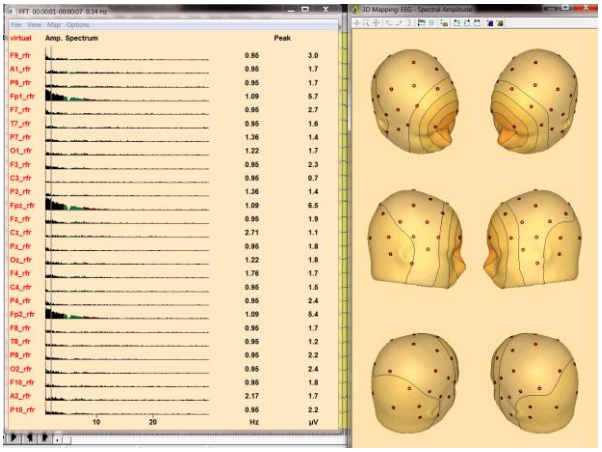


[2] In case you want to look at longer data periods or the whole dataset it is recommended you use the option **Process / Mean FFT-Spectrum**

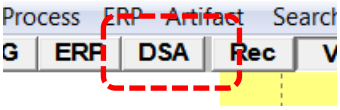
2. In the FFT window the electrodes of the current montage are listed along with their amplitude spectrum from 1 to 30 Hz (default). Beside every electrode the frequency is displayed that carries the peak amplitude in the specified time-range. The current view shoes that the frontal electrodes are characterised by high-amplitude low frequencies. **Click** into the low-frequent activity to open the 3-D Mapping window.



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.

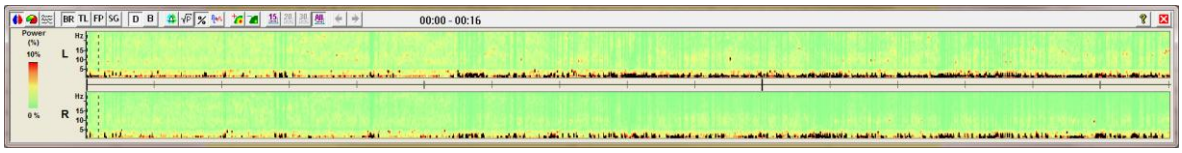


4. The DSA view is also based on an FFT and allows you to quickly get a feeling for the whole dataset. To start it press the **'DSA'** button in the main window.



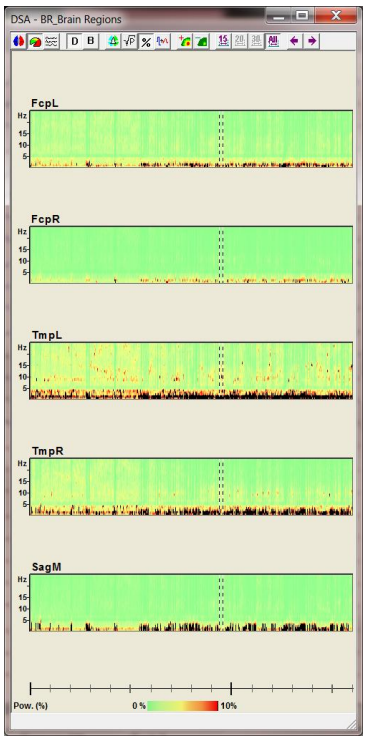
5. The default DSA window is divided into left and right hemisphere. It shows a condensed FFT view of the whole dataset subdivided into blocks of 2 seconds. If you click into an

event in the DSA window, BESA will jump to the according timepoint in the data in the main window.



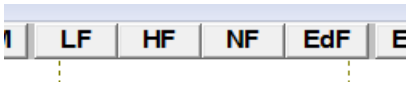
6. Change the view of the DSA window to multiple brain regions by pressing the button in the top left corner of the DSA window. In this view it is possible to see that the left and right temporal regions show activity in the alpha / low beta band that is not present in the other brain regions.

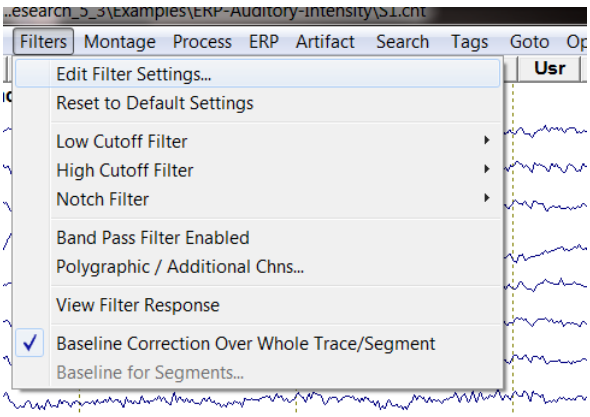
   The DSA view is valuable to quickly identify time-windows in the dataset that are characterised by high signal power. This can be very useful for e.g. epilepsy research to identify seizure periods and get a rough idea of the origin of the seizure. Close the DSA view.



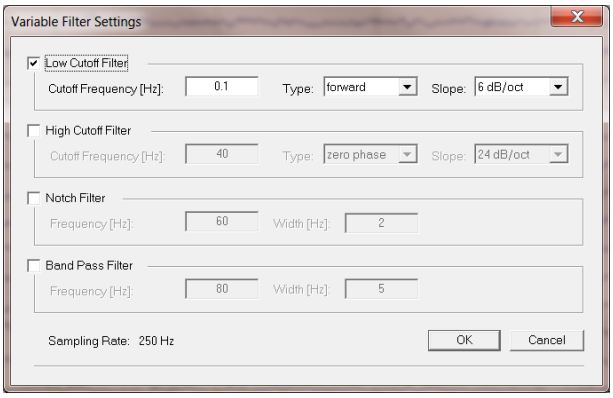
D: Filtering

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

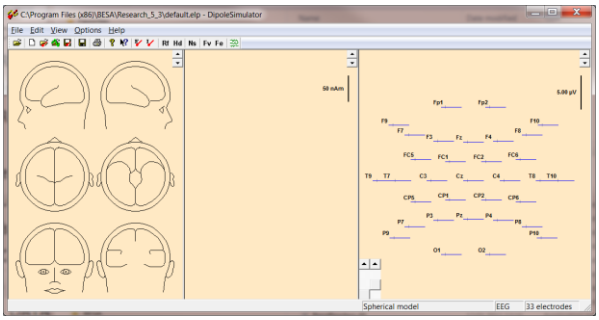




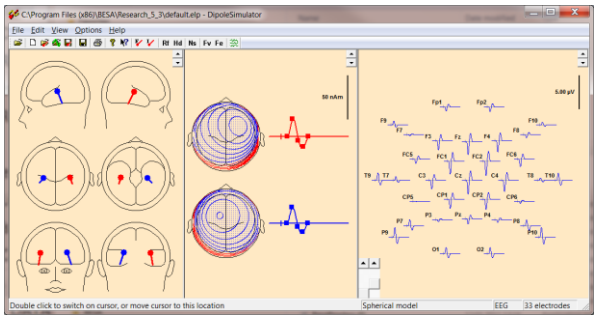
2. For Low and High Cutoff filters the filter type can be set to forward, zero-phase, or backward. The slope of the filter can also be edited. In order to understand the properties of filters better we will now switch to the program “DipoleSimulator”. *Please close the filter menu without setting any filters.*



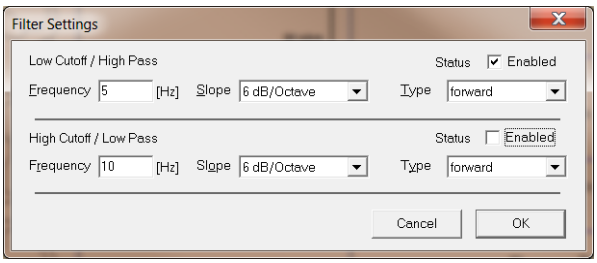
3. Open the program “**DipoleSimulator**” DipoleSimulator.exe located in your BESA installation folder (C:\Program Files (x86)\BESA\Research_5_3). On the left of the window you see the BESA head schemes, on the right you see an electrode layout.



4. Press **File / Load Model File** and select the model “**AC-2D-biphasic-synchronous.mod**” located in the Examples Folder “Learn by Simulations” (C:\Users\Public\Documents\BESA\Research_5_3\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.

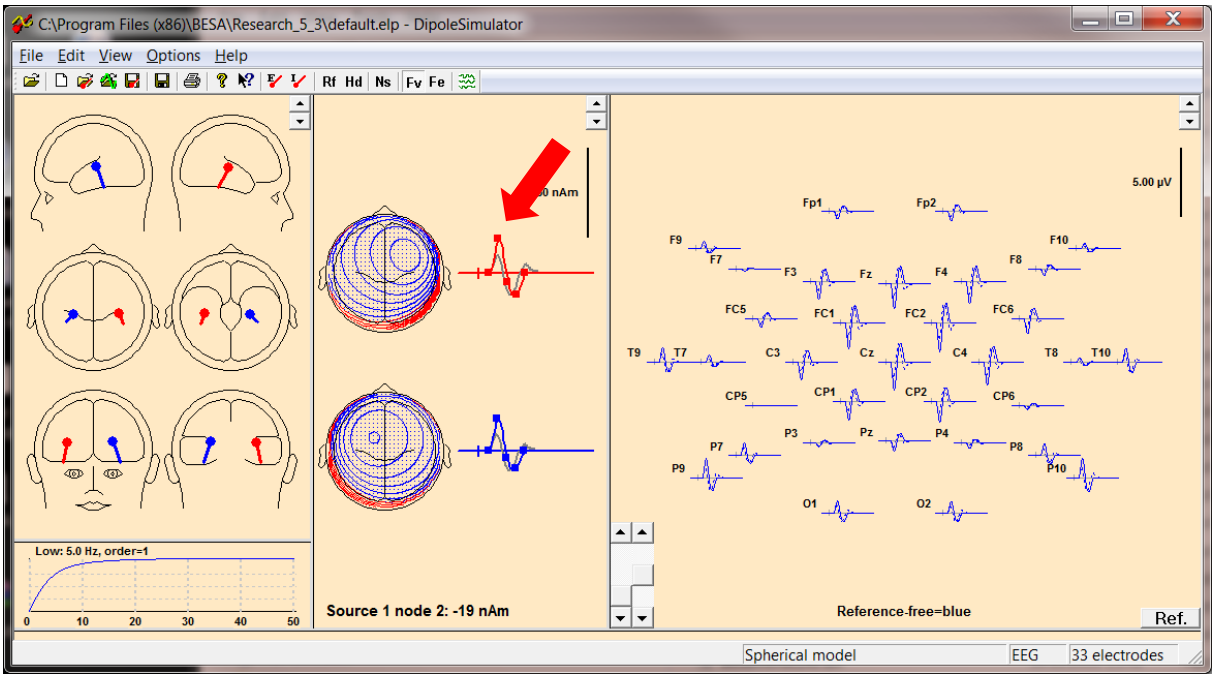


Press the button ‘Fe’ to open the filter dialog. Ns Fv Fe Specify a **Low Cutoff filter of 5 Hz, 6dB/Octave, forward** and make sure that the box **‘Enabled’** is only ticked for the Low Cutoff filter.

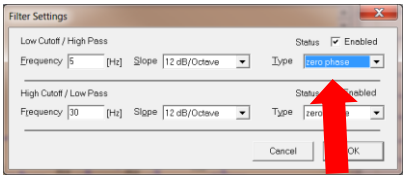


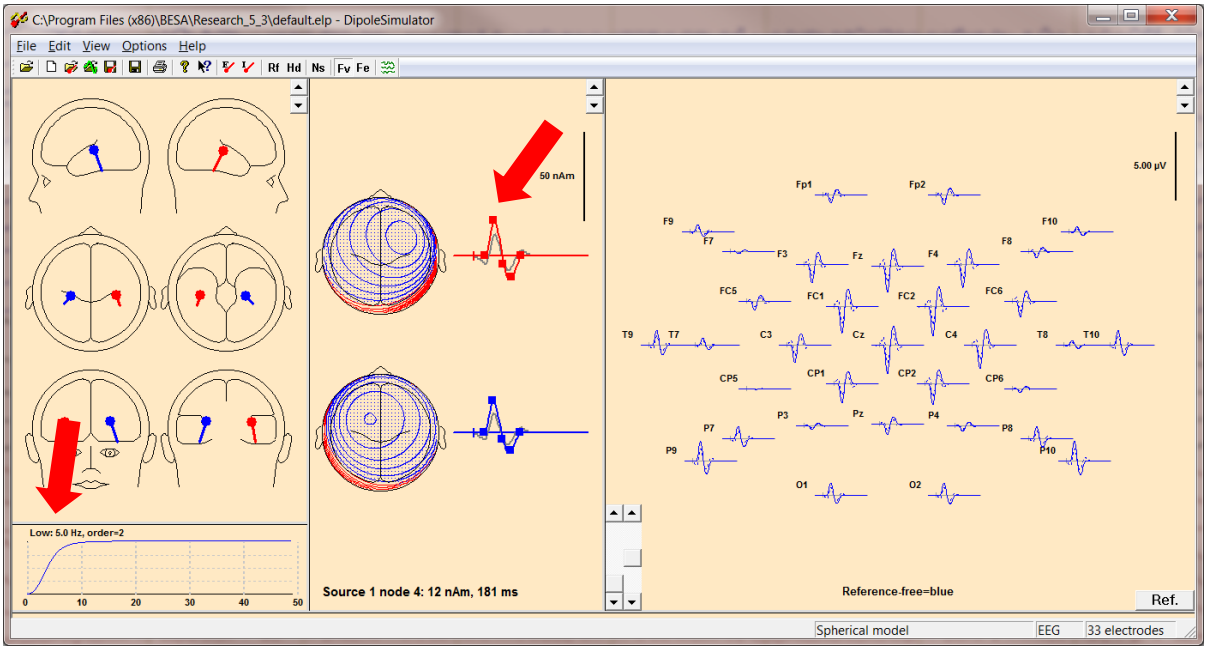
5. Leave the filter settings dialog open and move it next to the DipoleSimulator main window in order to view both the filter settings and the data. The grey waveforms show the effect of the filter. In the bottom left corner the filter response is displayed. You can see that the

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.

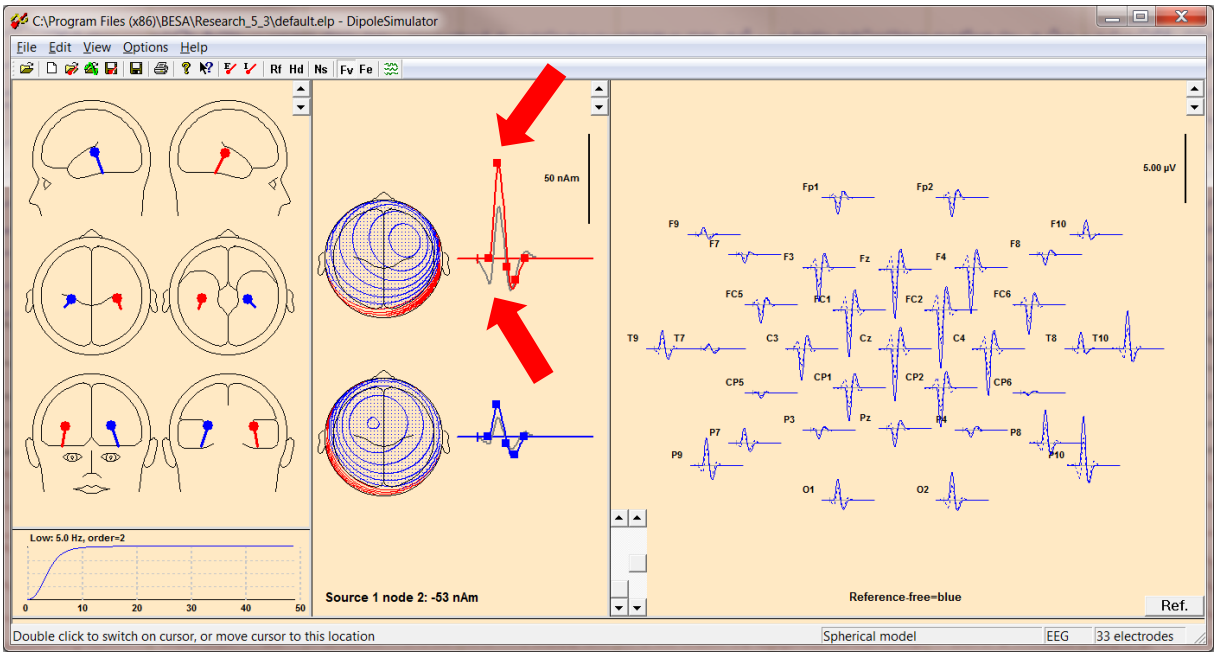


6. Change the filter type to **zero-phase**. Note that the filter response changed as well as the filtered waveforms. While the amplitude of the filtered waveform is still diminished, the peaks of the filtered waveforms are the same as in the unfiltered data. However, a small new "component" was caused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

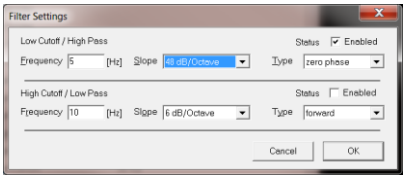


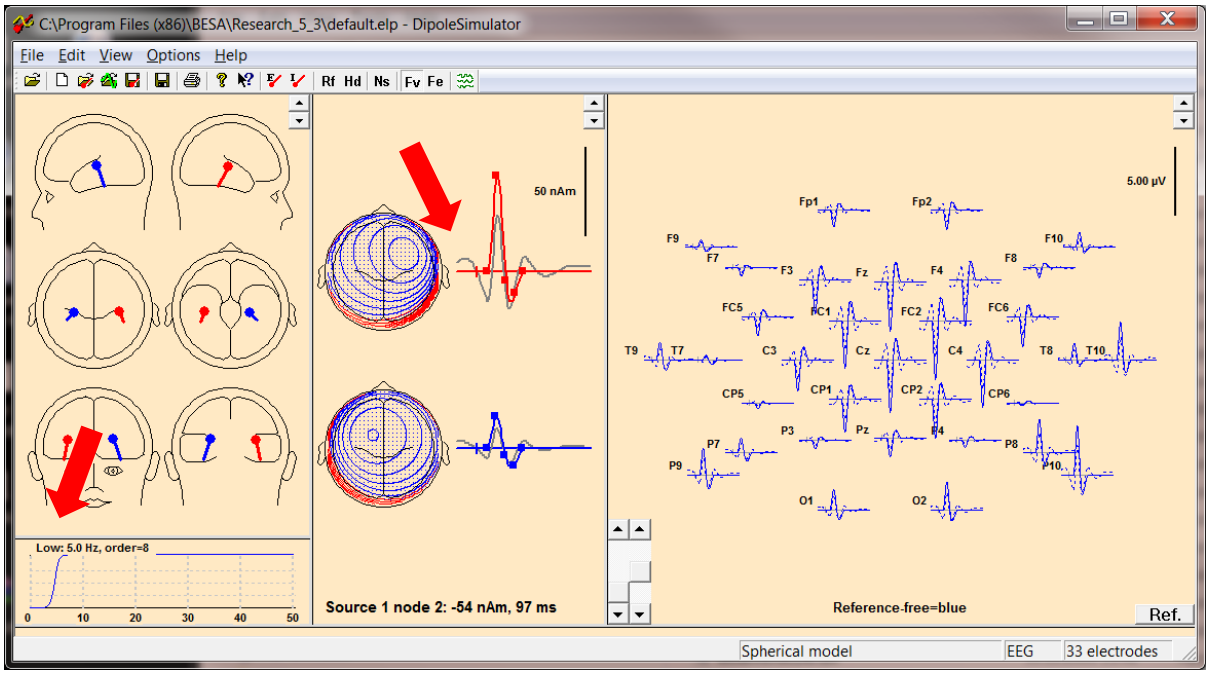


7. Increase the amplitude of the red source waveform by dragging up the second node with the mouse. Note how the “component” at waveform onset also increases.

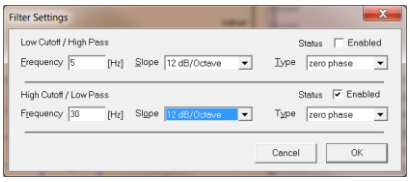


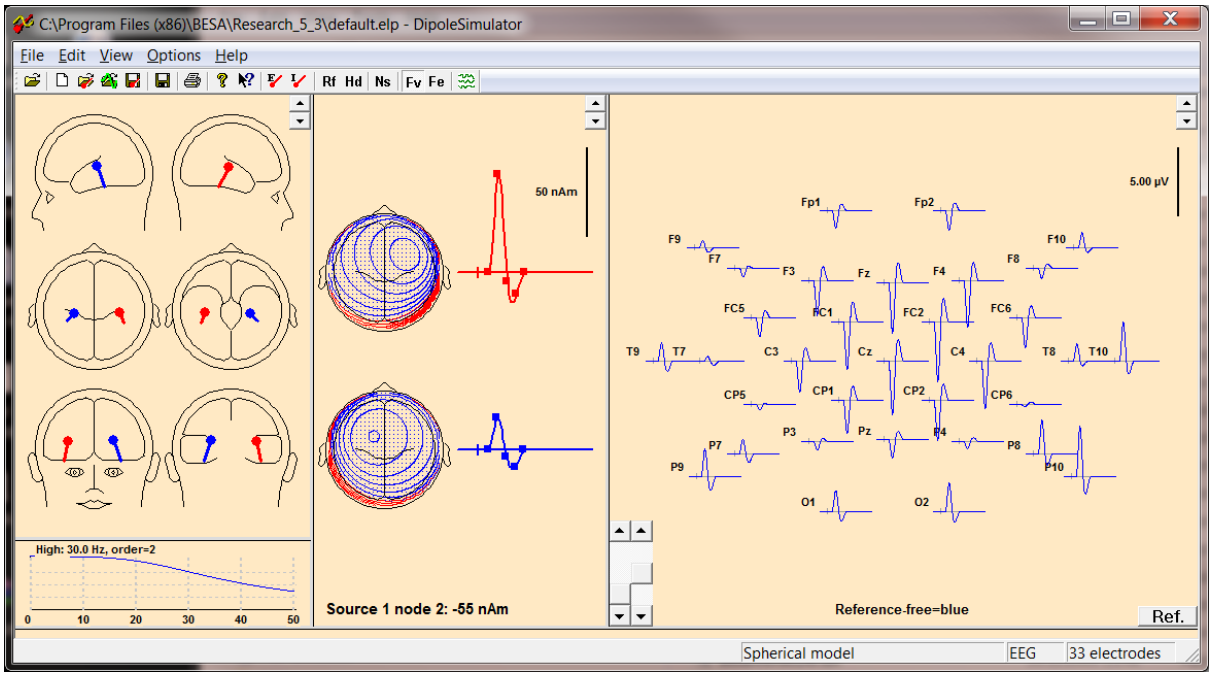
8. Change the filter slope to **48 dB/Octave**. Note that the filter response suggests better filtering properties, i.e. it filters out frequencies below 5 Hz more effectively. The downside is that this creates greater artifacts, distorting the waveform even further.



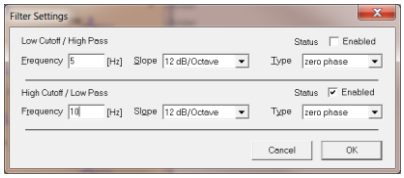


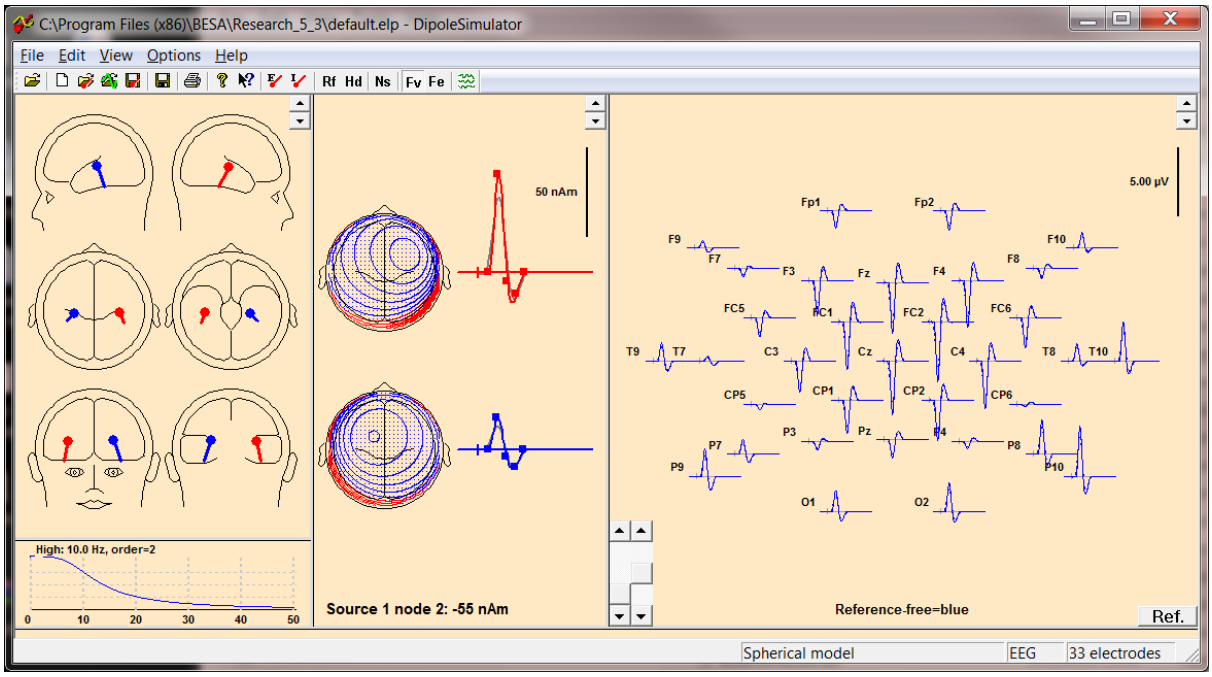
9. Disable the Low Cutoff filter and enable a **High Cutoff** filter of **30 Hz, 12 dB/Octave, zero phase**. Note that the original waveform was not changed by the filter. This is the case because evoked potentials are generally lower in frequency.



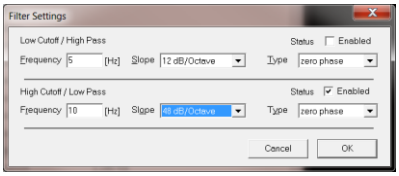


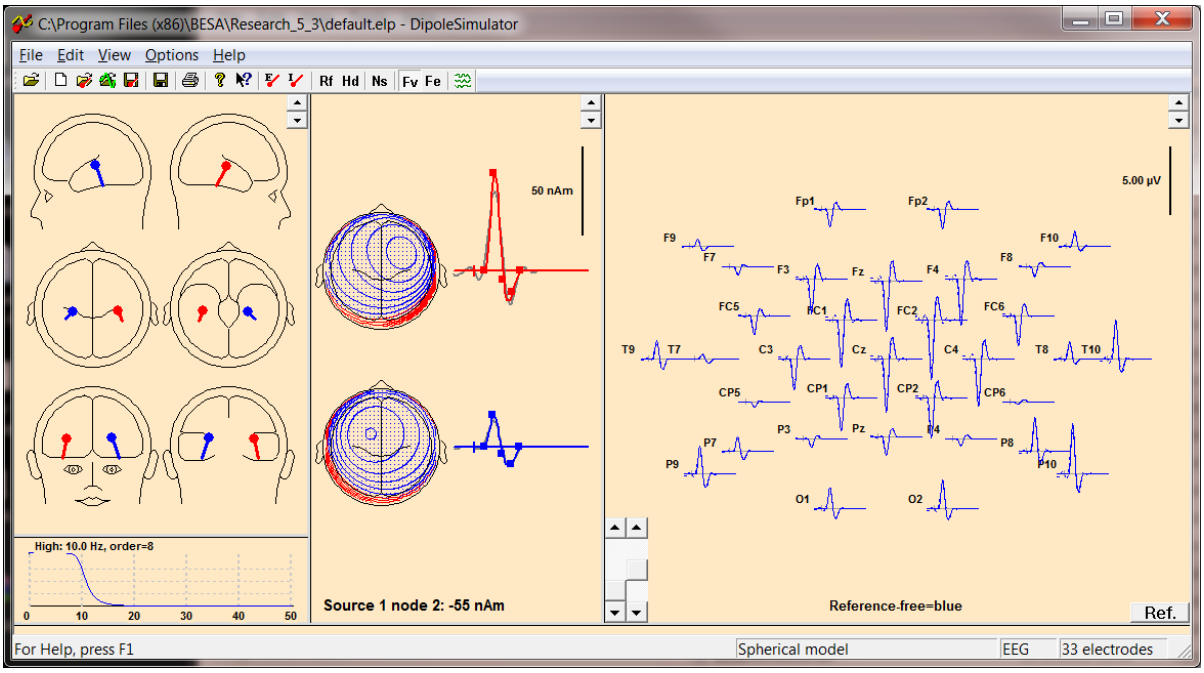
10. Change the High Cutoff filter to **10 Hz, 12** dB/Octave, zero phase. The amplitude of the waveforms get decreased, while the shape of the waveform is not as distorted as in the example of the Low Cutoff filter.





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.





Things to keep in mind when filtering:

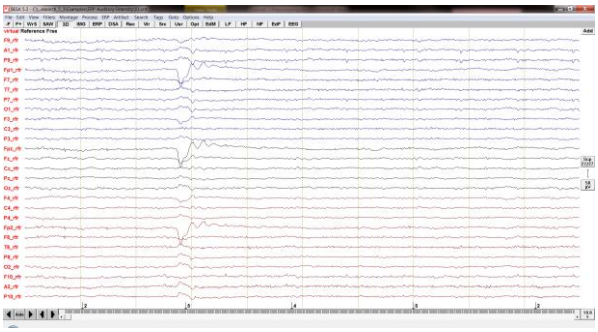
- The closer the frequency of the filters to your signal of interest, the greater the effect of the filter on your signal of interest
- Filters affect both amplitude and shape of your waveforms
- Low Cutoff filters usually have a greater impact on evoked potentials than High Cutoff filters as evoked 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

What does BESA Research provide?

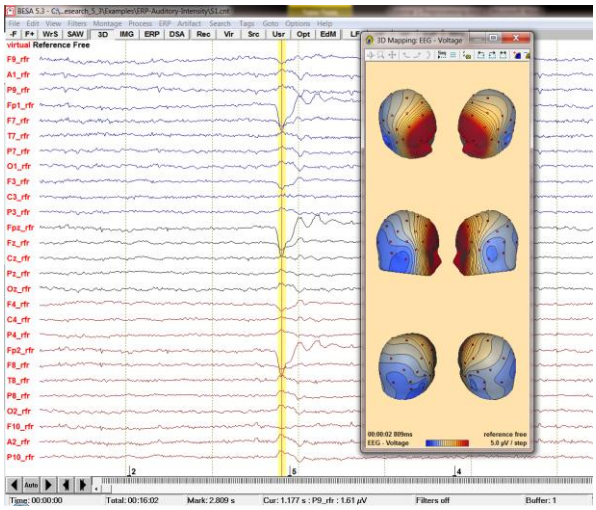
- ✓ Automatic and user-defined artifact correction
- ✓ Definition of conditions based on trigger values, names and attributes
- ✓ Artifact rejection based on amplitude, gradient and low-signal criteria
- ✓ Averaging
- ✓ Classic ERP analysis

A: Artifact Correction

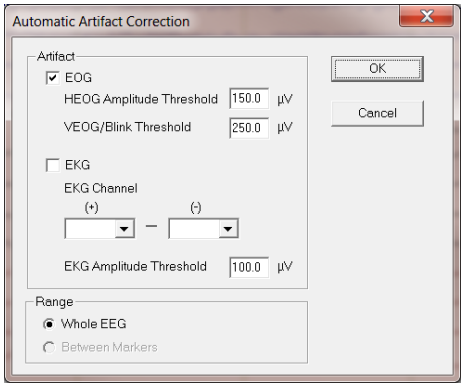
1. In case you have closed the data file as used in Tutorial 1, reopen file '**S1.cnt**' located in the **Examples** folder '**ERP-Auditory-Intensity**' and switch on the virtual Reference-Free view (VIR button → Reference Free).



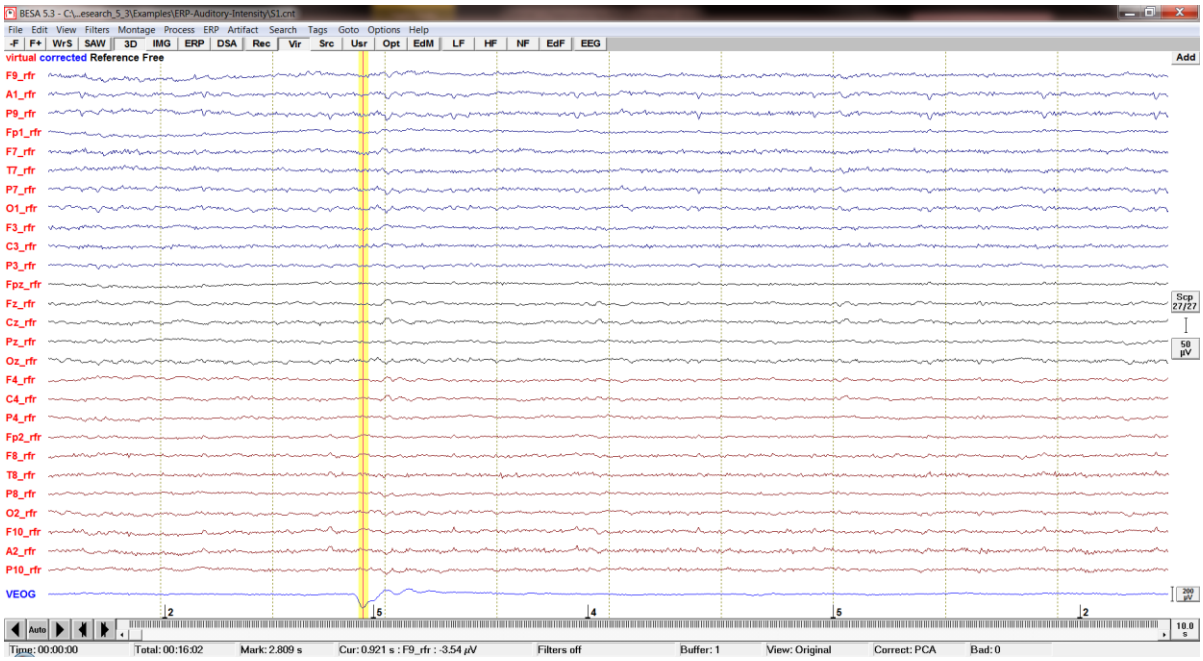
2. On the first screen an eye-blink dominates the data. Double click on it to open the 3D Mapping window and view the associated typical strong frontal positivity.



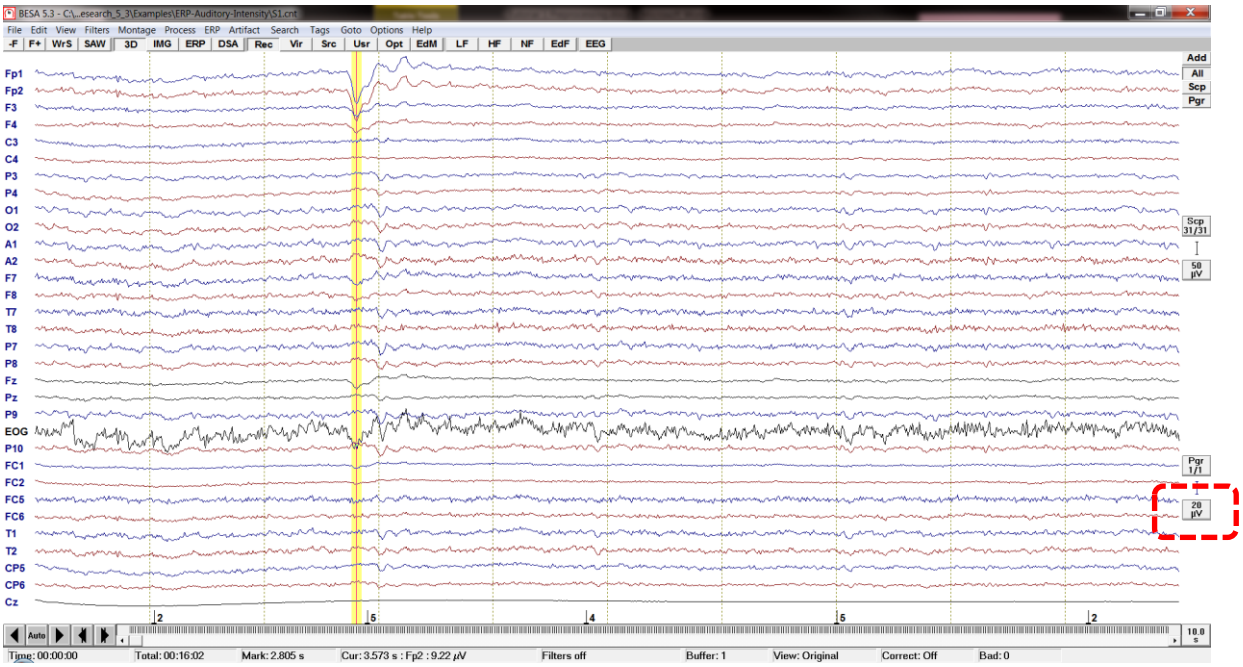
3. Close the mapping window and select '**Artifact / Automatic**'. In the dialog box make sure that only the option "EOG" is ticked and leave the settings at default. After pressing '**OK**' BESA will now automatically scan the data for eye movements using an internal model of artifact topographies.



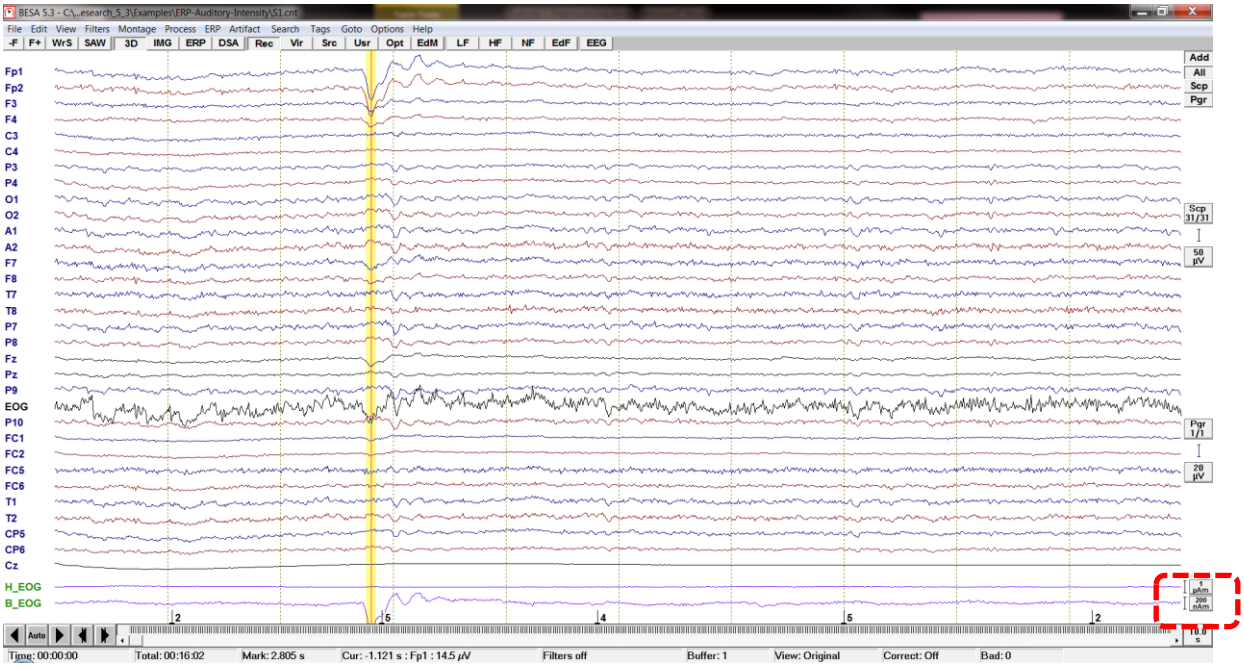
4. No horizontal eye movements were found so only a virtual VEOG channel was created by BESA. The eyeblink that was clearly visible in the data before correction is prepresented in the virtual VEOG channel. Note that “corrected” is displayed in the top left corner of the main window when artifact correction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



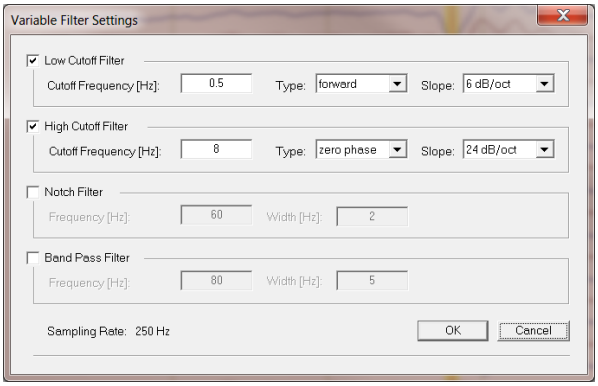
5. Next we will do the eyeblink correction by hand. For this purpose, switch off the automatic artifact correction and hide the virtual VEOG channel by pressing **Artifact / View**. Switch back to the original data by pressing the ‘**Rec**’ button and selecting ‘**Original Recording**’. You can see that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



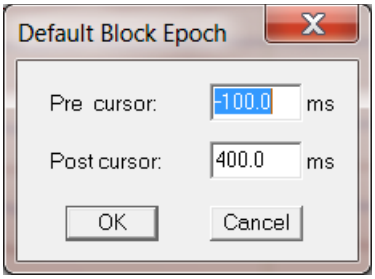
6. Use the button '**Add**' in the top right corner of the main window and select **EOG-HB**. This will add virtual electrodes for picking up horizontal eye movements (H_EOG) and eyeblinks (B_EOG). Scale up the **B_EOG** channel to **200nAM** by using the according amplitude scaling button. Note that 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.



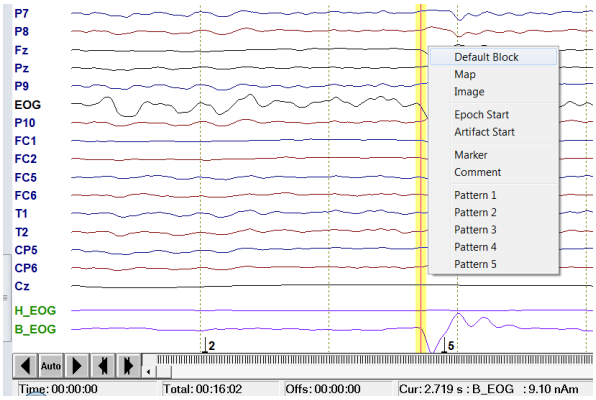
7. Now we will apply filter settings that are optimal for the identification of blinks. Press the '**EdF**' button to open the filtering dialog. Choose a **Low Cutoff** filter of 0.5Hz, forward, 6dB and a **High Cutoff** filter of **8Hz, zero-phase, 12dB**.



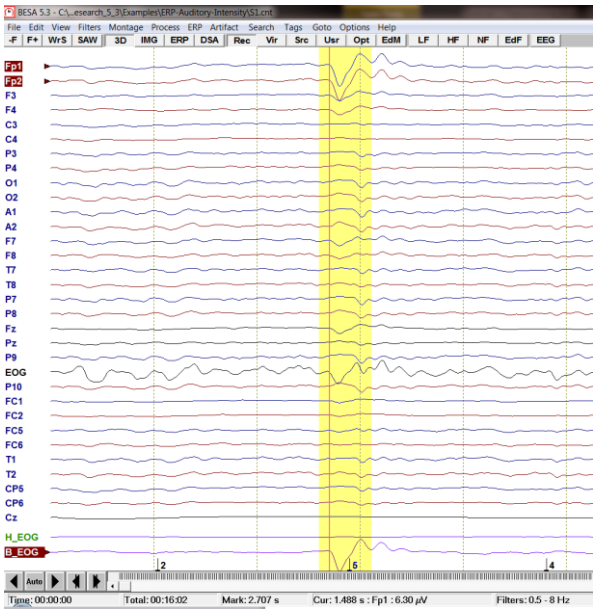
8. Before marking an eyeblink, we need to define a default block epoch that will later be used for the pattern search. To do so press **Edit / Default Block Epoch** and choose the settings **Pre cursor: -100ms** and **Post Cursor: 400ms**. This time window is usually sufficient to cover the whole extension of the blink signal.



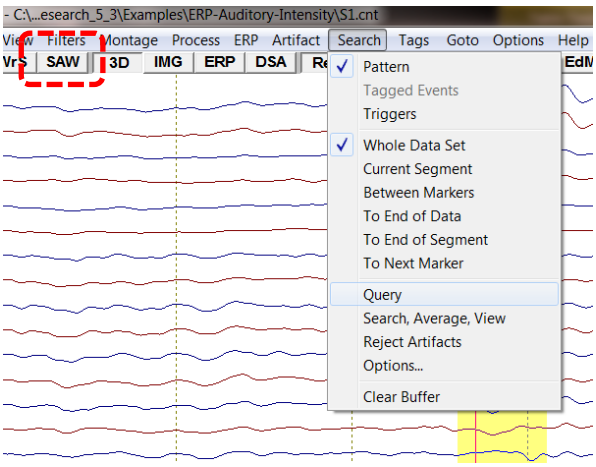
9. **Right-click** on the onset of the blink (you may use the virtual B_EOG channel as a reference) and select '**Default Block**'.



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 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.

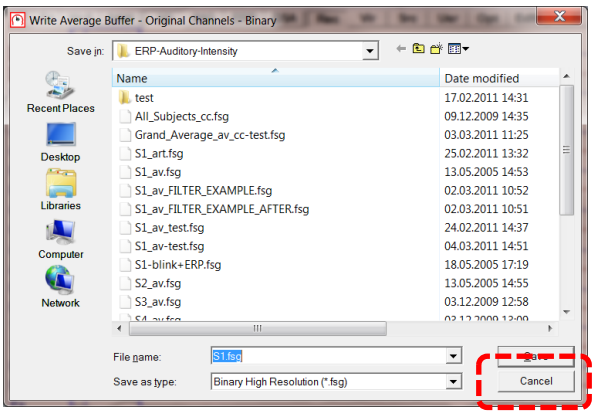


11. Before we start the pattern search, we will tell BESA to stop everytime a pattern is classified as a blink. To do so press **Search / Query**. Next press the button '**SAW**' (Search – Average – Write) to start the pattern search.

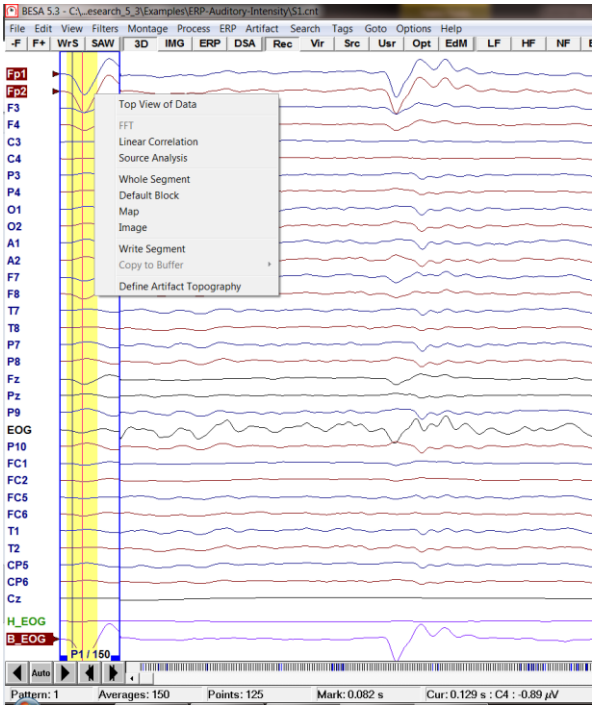


12. Whenever BESA finds a pattern that correlates high with the blink we have just defined it will ask you if you want to accept it to average. Click '**Yes**' to move on to the next pattern. If you are confident that the pattern search works well you may select '**Stop Asking**'. BESA will now automatically scan through the rest of the data.[1]

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

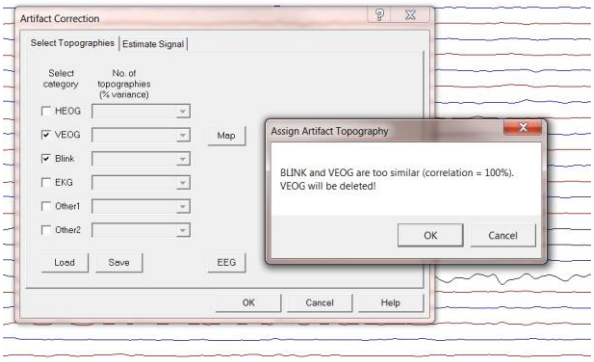


14. On the left of the main window an average segment consisting of all blink events that were accepted during the pattern search is displayed with a baseline of -100ms and a window length of 400ms as specified by the default block epoch. **Left-drag** a window in it, **right-click** and select '**Whole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

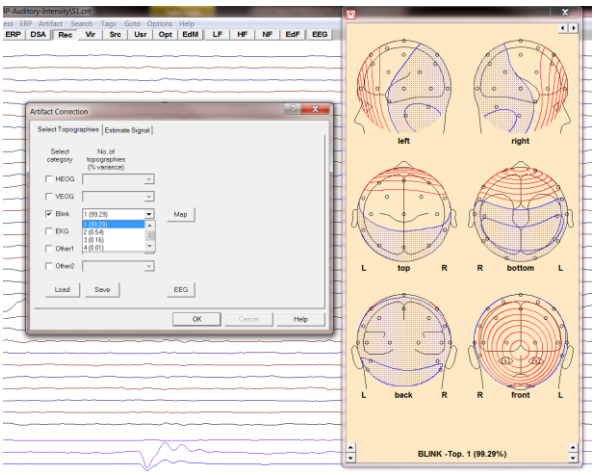


[1] If you data are contaminated by many other artifacts, it is recommended to only accept blinks that do not coincide with another artifact event in order to receive a clean eyeblink topography.

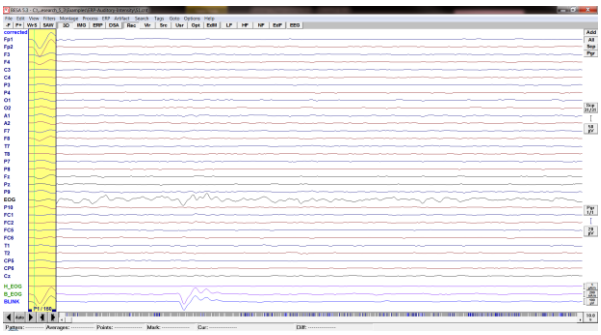
15. Note that VEOG is already ticked in the artifact correction window. The reason is that BESA still remembered the automatic artifact correction from before. Select '**Blink**'. An error message will appear notifying you that the blink and VEOG topographies are too similar and that VEOG will be deleted. 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 average blink signal. The PCA 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 data[2]. Open the drop-down menu to view the other PCA components. Make sure that only the first component is selected and press '**OK**'.



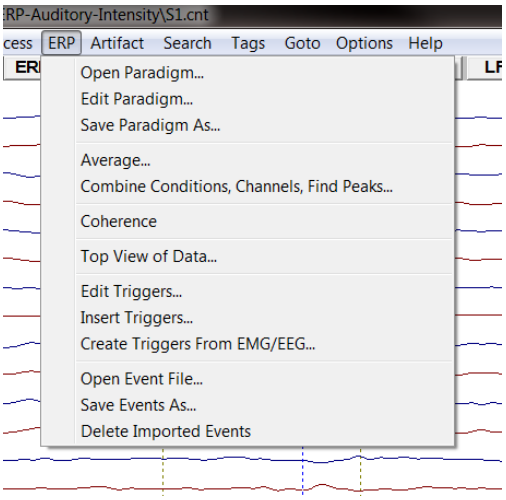
17. Note that another virtual blink channel was created. If you scale it up to **100µV** using the its amplitude scaling button you will see that it is almost identical to the virtual blink channel that was created automatically using the 'Add' button. Switch off H_EOG and B_EOG by pressing '**Add**'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



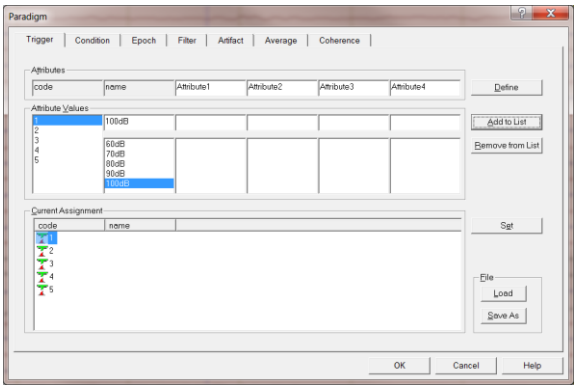
[2] It is recommended to only select the first PCA component for artifact correction in order to prevent distortion of the data.

B: Definition of conditions based on trigger values, names and attributes

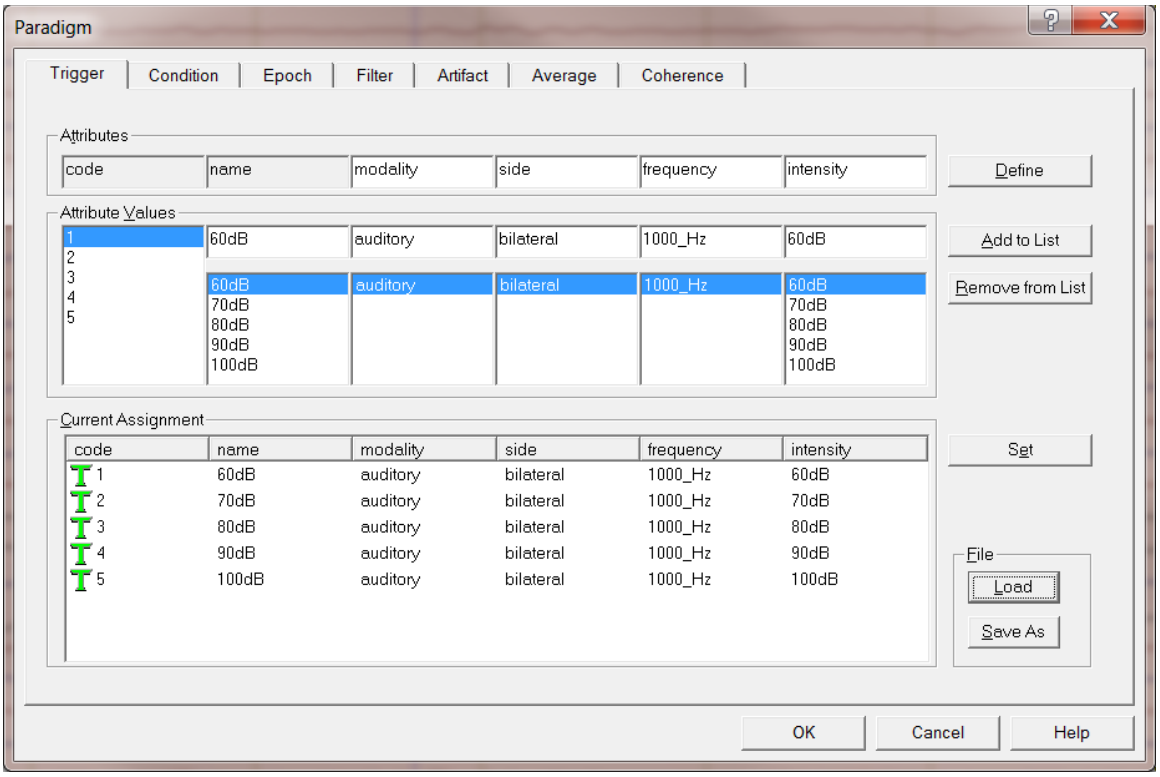
1. We will now use the trigger information in the file to specify conditions that will later be used for averaging. Press **ERP / Edit Paradigm**.



2. 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.



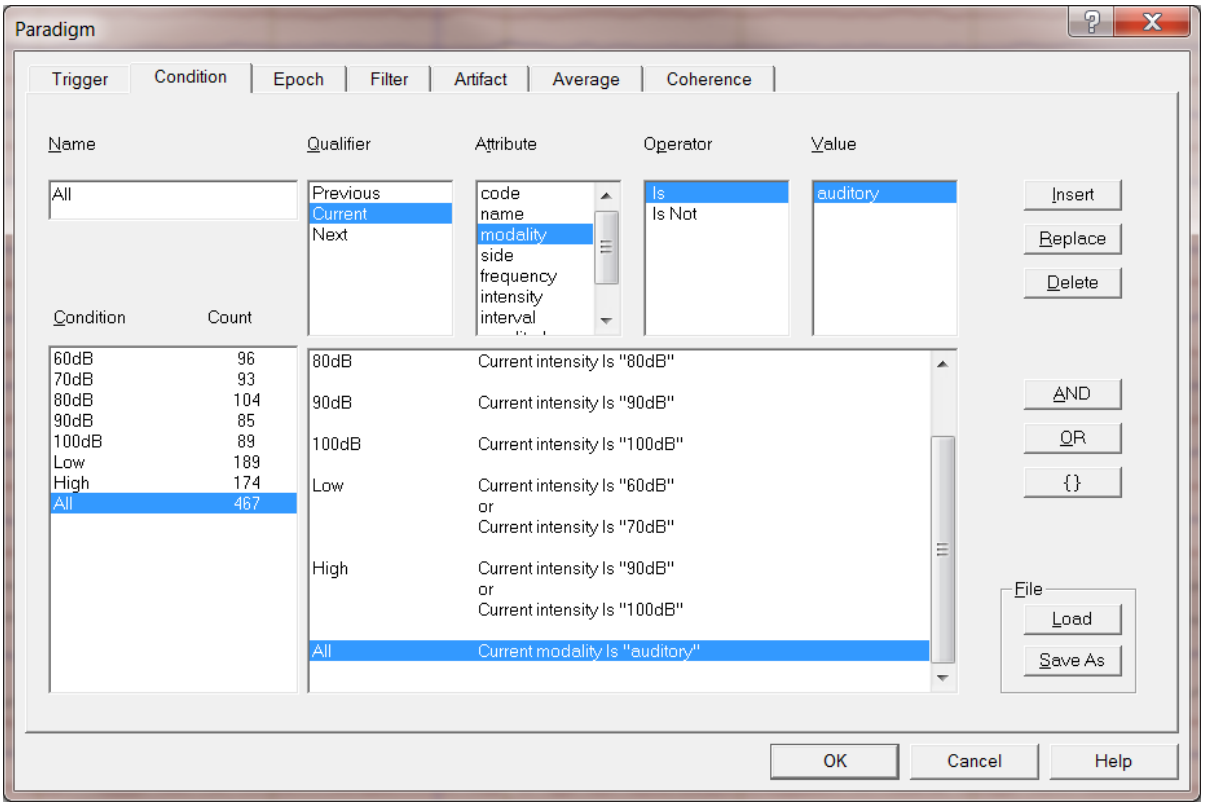
3. We will add further attributes as they will make the definition of conditions easier later on. Change the name ‘Attribute1’ to ‘modality’ and press ‘**Define**’. In the field below write ‘auditory’ and ‘**Add to List**’. Change the name ‘Attribute2’ to ‘side’ and press ‘Define’. In the field below 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, …



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.

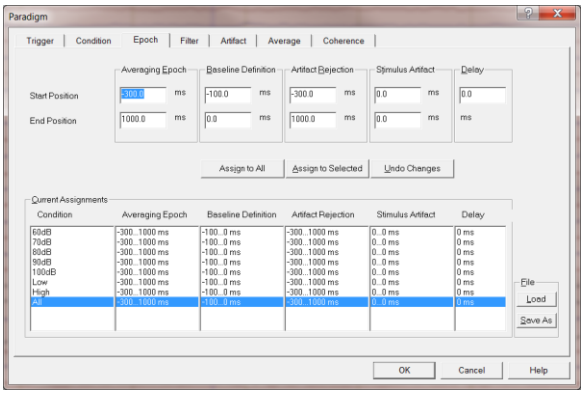
   Next we want to specify the condition '**Low**'. Enter the name 'Low' and again select '**Current**' '**intensity**' '**Is**' '**60dB**' and press '**Insert**'. We want the 'Low' condition to also contain 70dB stimuli. Thus, additionally specify the condition 'Low' to be '**Current**' '**intensity**' '**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.)

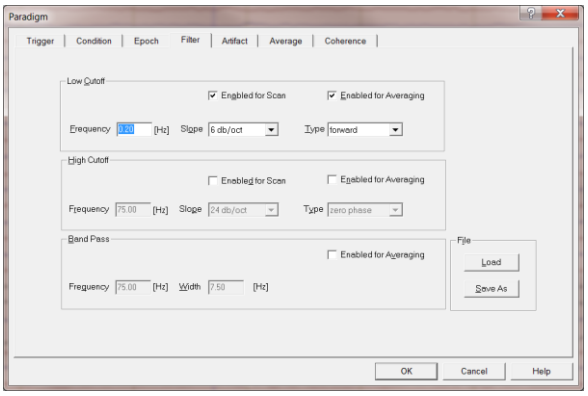


   Note that it is also possible to specify other logic combinations using the Qualifier 'Previous' and 'Next' as well as the Operator 'Is Not'. It is also possible to define an interval during which a previous or next event can occur under 'Attribute'.

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 to use long pre- and post-stimulus times to allow for optimum Low Cutoff filtering.

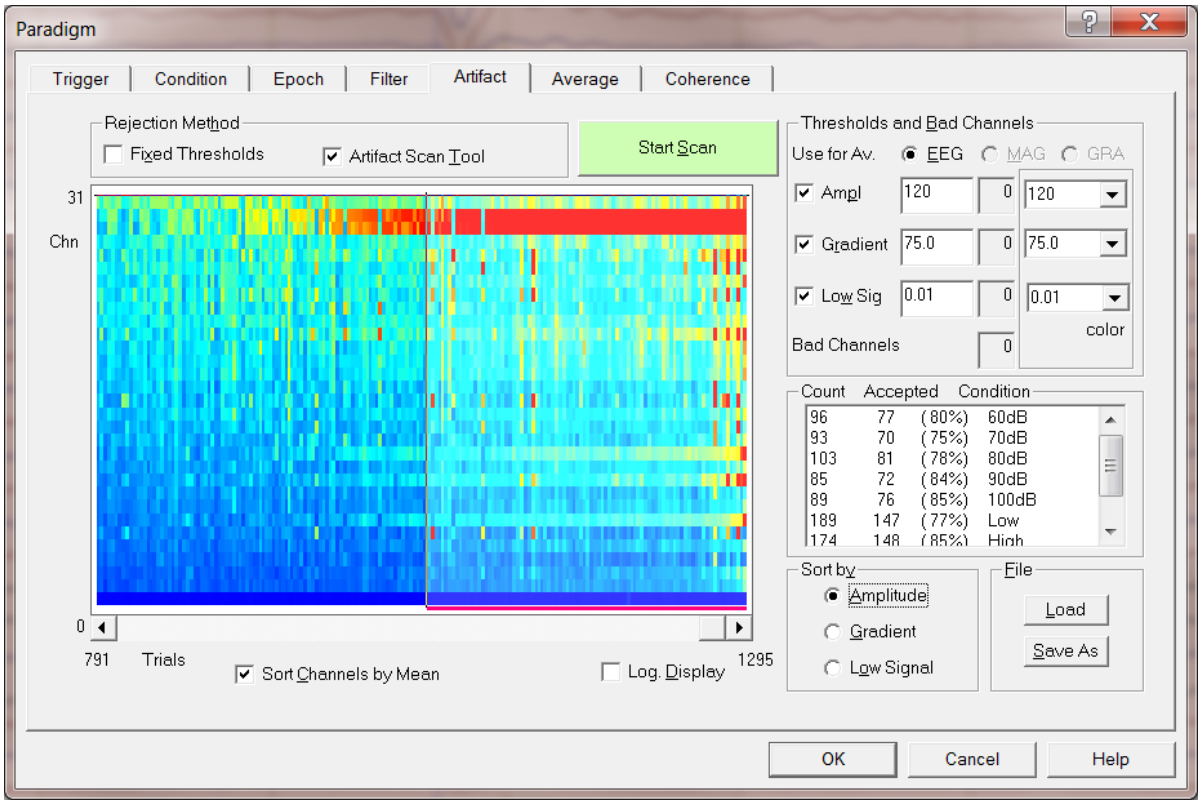


6. Move to the **Filter** tab. Specify a Low Cutoff filter of **0.2Hz, 6dB/Octave, forward**. Enable it for Scan and Averaging. I.e. the Low Cutoff filter will be switched on for the artifact scan and averaging. This prevents us from having to filter the averaged data later on and we can avoid the negative 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.

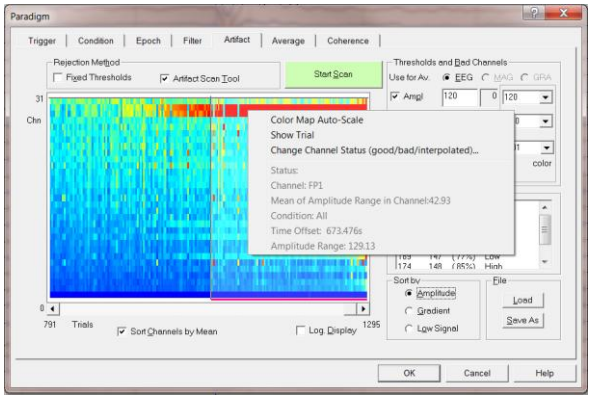


C: Artifact rejection based on amplitude, gradient and low-signal criteria.

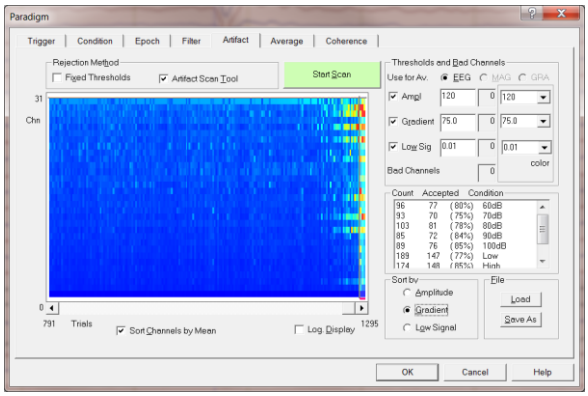
1. To make sure you have the identical paradigm settings as described here, please load the pre-defined Paradigm-file '**AEP_Intensity.PDG**' located in the **Paradigm directory** under '**Auditory**'. Move to the Artifact tab and press '**Start Scan**'. You will see a 2-dimensional diagram showing the channels (rows) and trials (columns) having the most noise. By default values based on maximum amplitudes within the predefined artifact epochs are shown. Epochs that exceed the amplitude threshold (default: 120µV) 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.



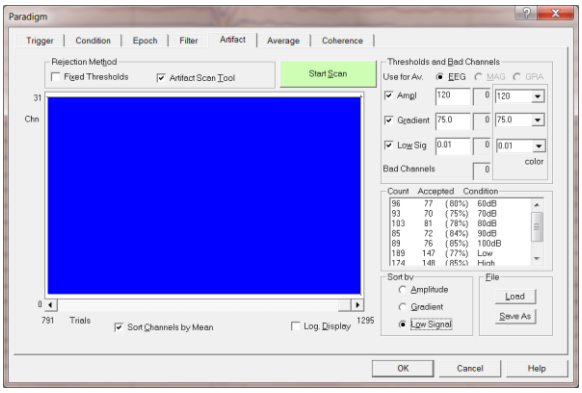
2. Right-click on the red channels to see their labels. The two channels that contain most bad epochs are FP1 and FP2, two frontal electrodes that pick up eyeblinks most strongly. Note that it is also possible at this stage to change a channel status to good, bad or interpolated by choosing the according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



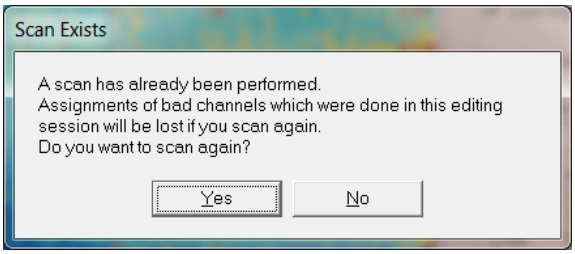
3. It is also possible to view the result of sorting the data after a Gradient criterion (default: 75µV), i.e. epochs will be marked as bad that contain amplitude jumps of >75µV between two sampling points. Choose '**Sort by Gradient**' to view this.



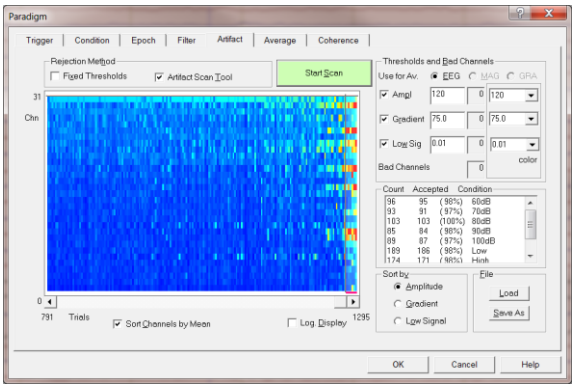
4. The Low Signal criterion can be useful to identify data periods where an electrode drops off during recording. Epochs will be marked as bad during which the signal is smaller than the threshold criterion (default: 0.01µV). Choose '**Sort by Low Signal**' to view this. In the present case, no epochs fall under this criterion. Change back to the Amplitude sorting criterion and press '**OK**' to close the Paradigm window.



5. 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.

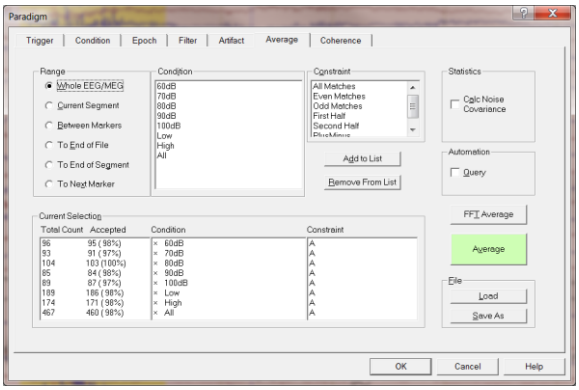


6. Note that a greater number of epochs got accepted and that the two bad electrodes (FP1 and FP2) that were contaminated with eyeblinks are not conspicuous any longer. Some epochs still will be rejected as they exceed the threshold criteria. These epochs are contaminated by other, non-eyeblink related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

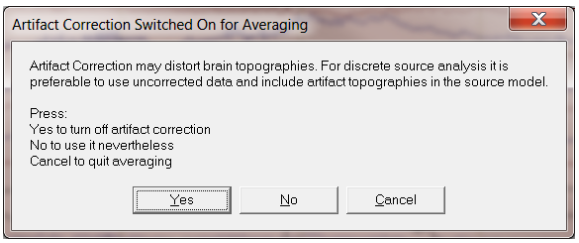


D: Averaging and Classic ERP Analysis

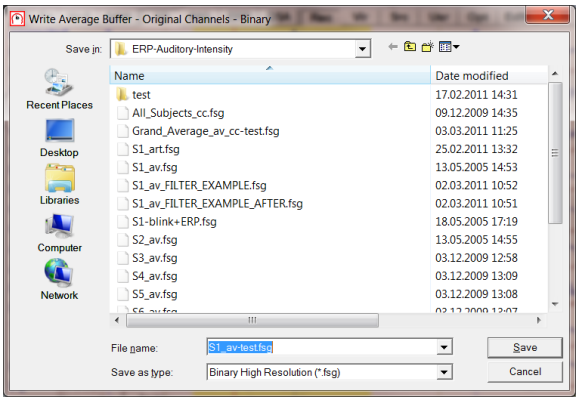
1. Move to the **Average** tab. It is possible to restrict the number of conditions for averaging by removing unwanted conditions from the condition list. Additionally, it is possible to use constraints, e.g. to only average epochs of the first or second half of the experiment. This can be useful to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



2. A warning message will appear that artifact correction is switched on, which can be problematic for later source analysis[3]. 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!

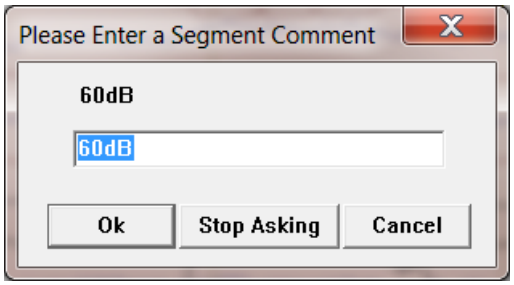


3. When prompted to save the *.fsg file containing averaged epochs of the conditions defined in the paradigm, add a '**-test**' to the suggested filename in order to not over-write any predefined files (i.e. choose the name **S1_av-test.fsg**).

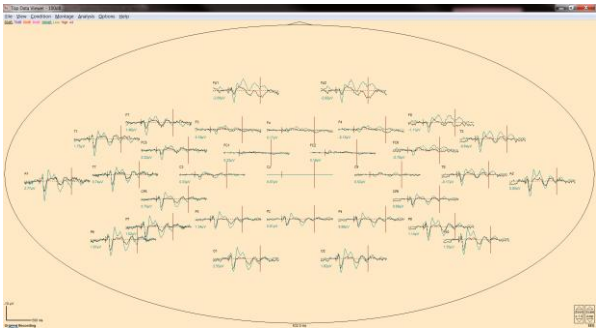


[3] See the effect of switched on artifact correction during averaging in Tutorial 7: Source Montages.

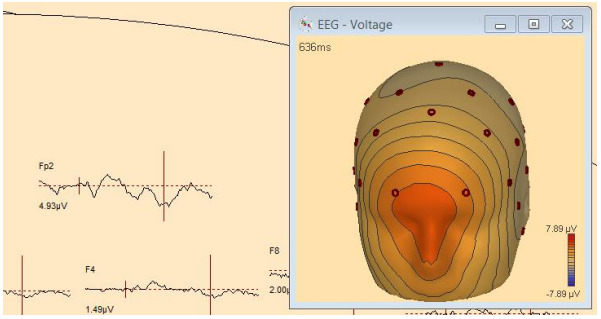
4. You will be prompted to specify names for the averaged epochs. By default, BESA will suggest the names as defined in the paradigm. Unless you want to alter them, choose '**Stop Asking**' and the original names will be maintained.



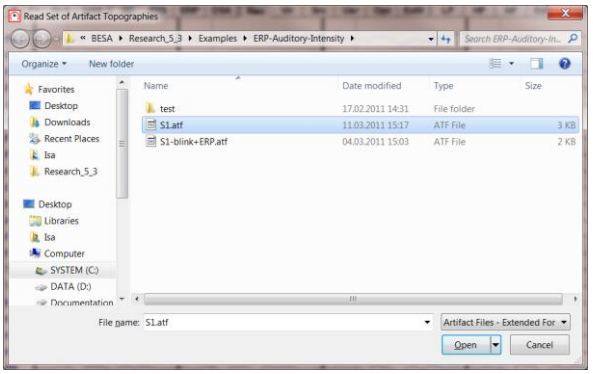
5. The Top Data Viewer will automatically open displaying the averaging results. You may choose two conditions at once to be overlayed by holding down the ctrl-key and left-clicking on the conditions of interest. It is possible to change the viewing properties (color of waveforms, labels, etc.) by selecting '**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 '60dB' and double click in the waveforms at 636ms. 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.

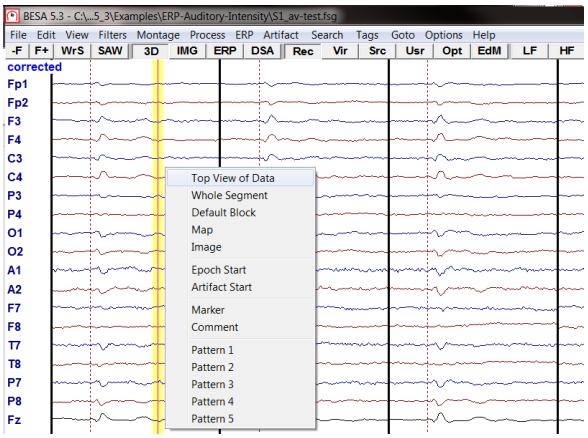


7. Close the TopViewer to return to the main window. After averaging, BESA automatically opens the newly created average file. Therefore, we see a segmented file containing segments of the conditions as defined in the paradigm. We are now going to load the artifact topographies we defined earlier. To do so press '**Artifact / Load**' and

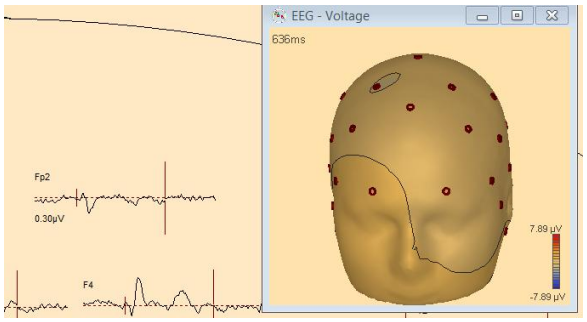


select file '**S1.atf**'. This file was automatically written by BESA when we defined the blink topography. Press '**Open**'.

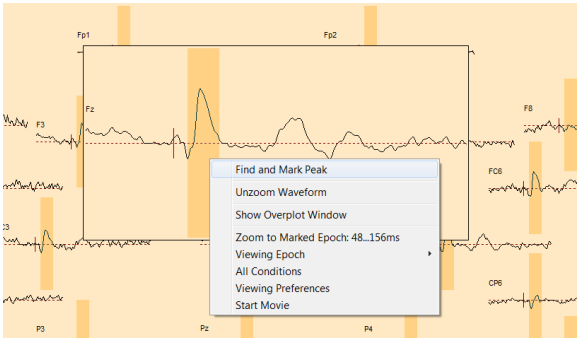
8. Loading the *.atf file will lead to BESA subtracting the eyeblink topography. Note that '**corrected**' appears in the top-left corner and a virtual EOG channel is displayed at the bottom. Switch off the filters by pressing the **EdF button** and deselecting all filters. Open the TopViewer again by right-clicking in the data and choosing '**Top View of Data**'.



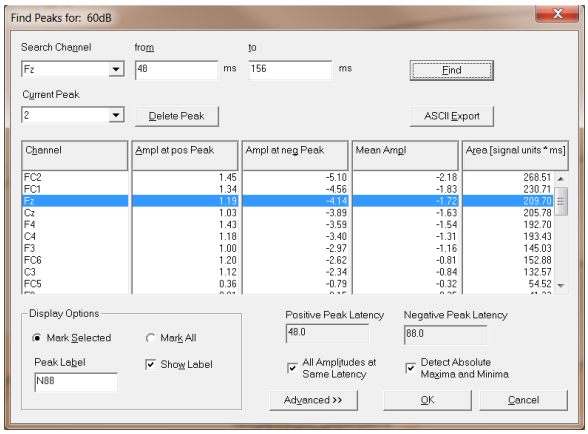
9. Return to the 60dB condition and note that the large activity around 636ms has disappeared. If you now double-click to bring up the 3D-mapping window you will see that the blink topography is gone.



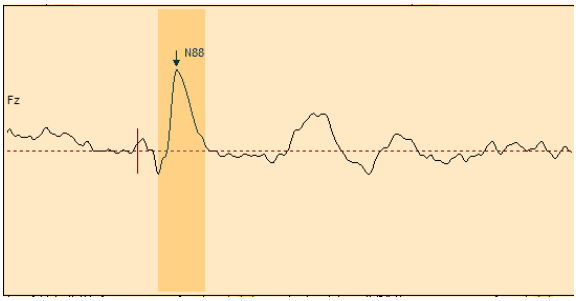
10. Zoom electrode Fz by right-clicking on it and selecting '**Zoom Waveform**'. Left-drag a window over the most prominent peak. Right-click again and select 'Find and Mark Peak'.



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 88ms. BESA also outputs the mean amplitude in the specified time-range 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.



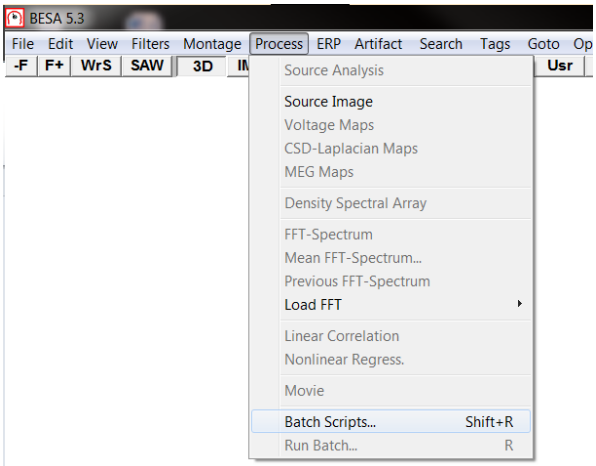
What does BESA Research provide?

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

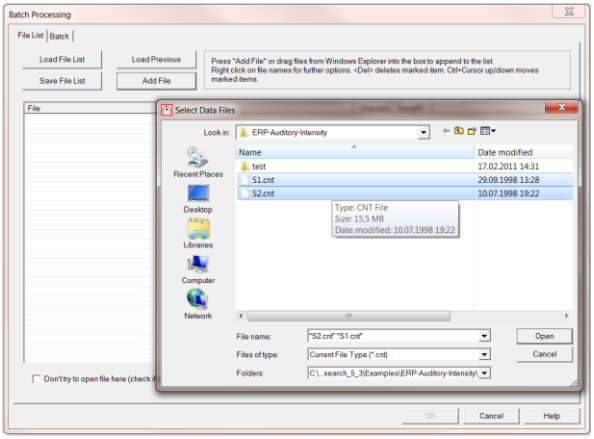
A: Batch Processing

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

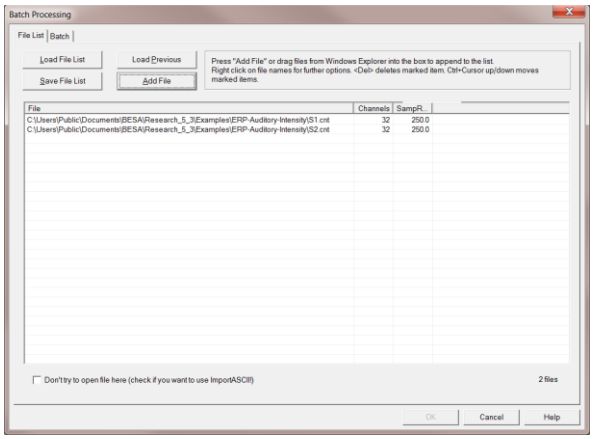
1. In BESA Research select File / Close All. Now select Process / Batch Scripts. This will bring up the Batch Processing dialog.



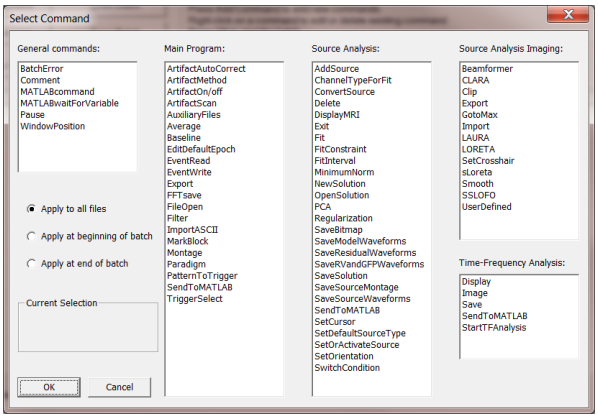
2. Press '**Add File**' and select both available *.cnt-files (S1.cnt and S2.cnt). Press '**Open**'.



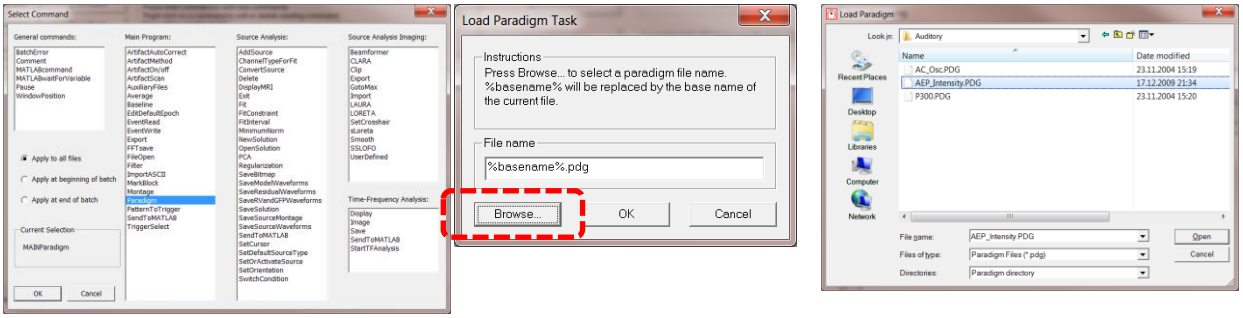
3. The file-list now contains the raw data of subjects S1 and S2. For display purposes only two datasets were selected, in principle, an arbitrary number of files can be selected. We can see that both files contain data of 32 electrodes sampled at 250Hz. It is possible to save or reload a file list at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



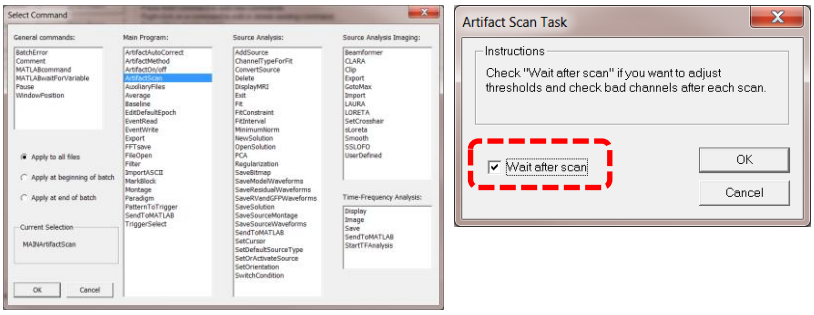
4. Move to the **Batch** tab and press '**Add command**'. This will bring up the 'Select Command' window that is sub-divided into 4 command sections corresponding to the main program, source analysis, imaging, time-frequency analysis and general commands.



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.



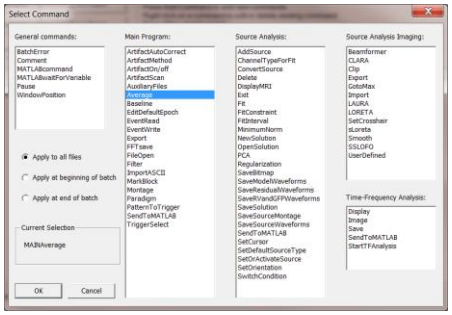
6. Next, instruct BESA Research to perform an artifact scan on each data set. Press '**Add Command'**, select '**Artifact Scan**' from the list of commands in the Main Program group and hit '**OK**'. The '**Artifact Scan Task**' window opens. You may have artifact rejection run fully automatically. However, 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 to make manual corrections. Press '**OK**'.

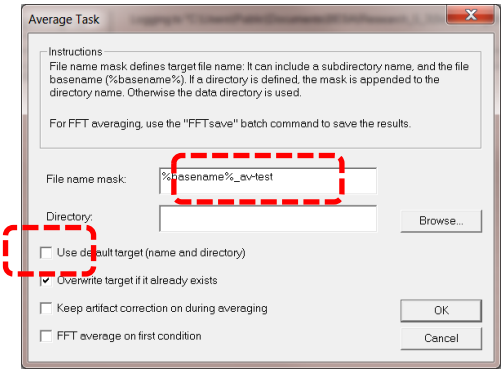


7. Finally, press '**Add command**' again, select '**Average**' from the Main Program group and press '**OK**'. In the '**Average Task**' window, BESA Research allows to specify a file name

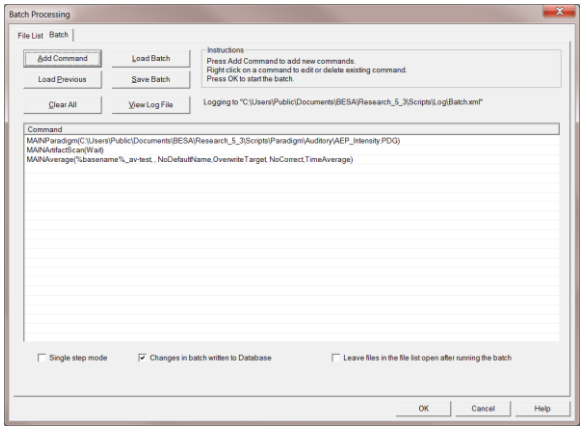
for the averaged individual data. Uncheck the option ‘Use default target’ and change the File name mask to ‘**%basename%_av-test**’. This will create BESA binary files (*.fsg) with a basename derived from the corresponding raw data set. The averaged segments of file ‘S2.cnt’ will be written to file ‘S2_av-test.fsg’, 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’.

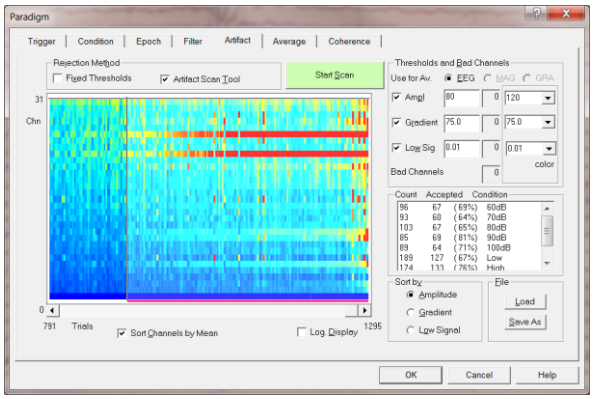




8. The three commands have been added to the command list with all specified options. You may save this batch and reload it for further analyses using the ‘Save Batch’ and ‘Load Batch’ buttons. Press ‘OK’ to start the batch processing with the first file (S1.cnt).

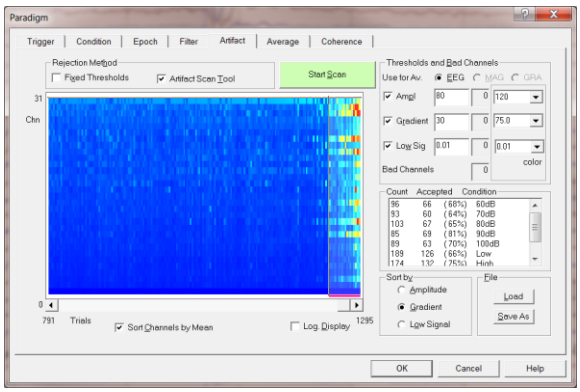


9. BESA Research stops after the artifact scan has been performed. You can now manually adjust the number of rejected trials and channels. In file S1.cnt, many blinks can be observed, primarily in channels Fp1 and Fp2. The preset amplitude threshold is not low enough to exclude all blink-contaminated trials. Drag

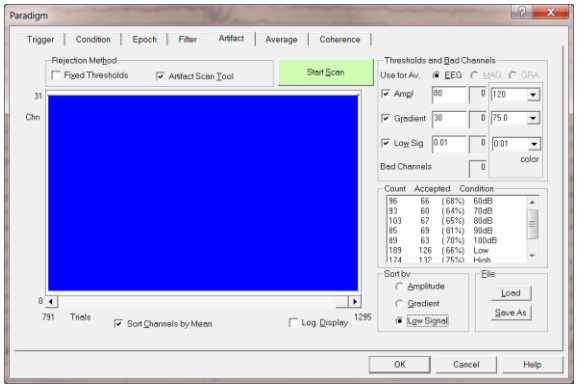


the vertical red bar to the left to exclude more trials or **set the amplitude threshold manually to 80µV**. This will reject more epochs but we can be certain that no artifacts are retained in the data.

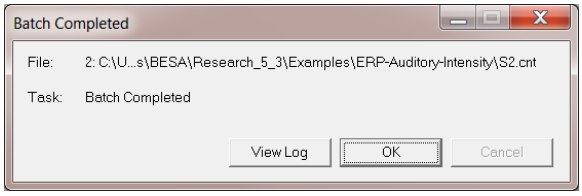
10. Select '**Sort by Gradient**'. Place the scrollbar to the right end of the display to see the trials with large gradient artifacts. The gradient threshold should be lowered (i.e. the vertical red bar in the 2D display should be moved to the left) to exclude all artifact-contaminated trials. **Set the gradient threshold to 30µV**.



11. Select '**Sort by Low Signal**' to see whether there are any channels that dropped out during the measurement. This is not the case.



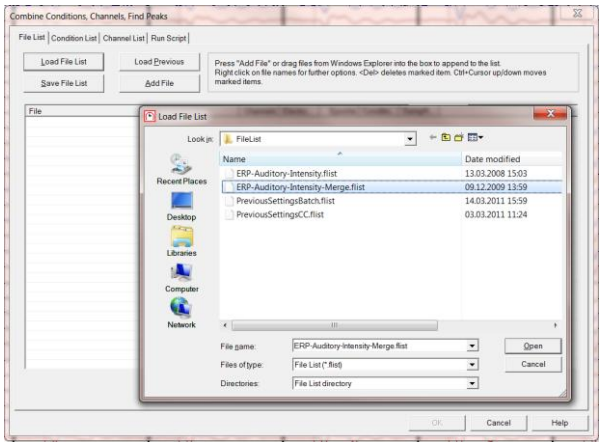
12. Hit '**OK**' to finish artifact rejection in the first data file. BESA Research now averages all specified conditions and proceeds with the next data file. In file **S2.cnt**, adjust the artifact thresholds to **100µV** (amplitude criterion) and **50µV** (gradient criterion). After processing the batch on all data files, BESA Research gives a notification and offers to view the automatically generated log file (**View Log** button). Press **OK**.



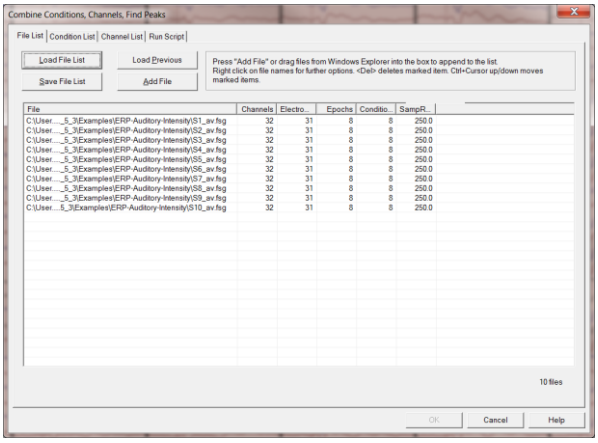
B: Creating Grand Averages

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**'.

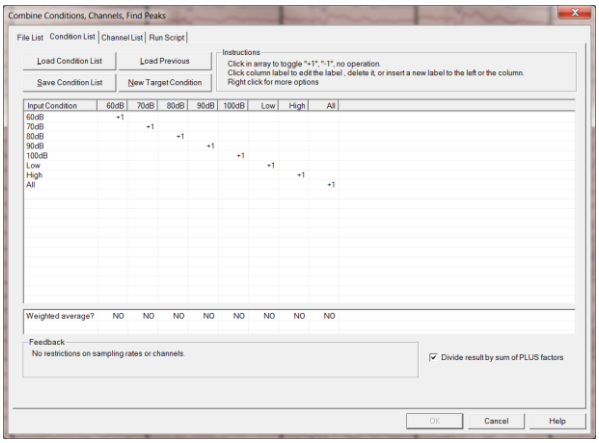
1. In the BESA main window select **ERP / Combine Conditions** to open the according dialog window. Press 'Load File List' and select the pre-defined file '**ERP-Auditory-Intensity-Merge.flist**' and press '**Open**'. This will load *.fsg-files containing average data of subjects S1 to S10.



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.

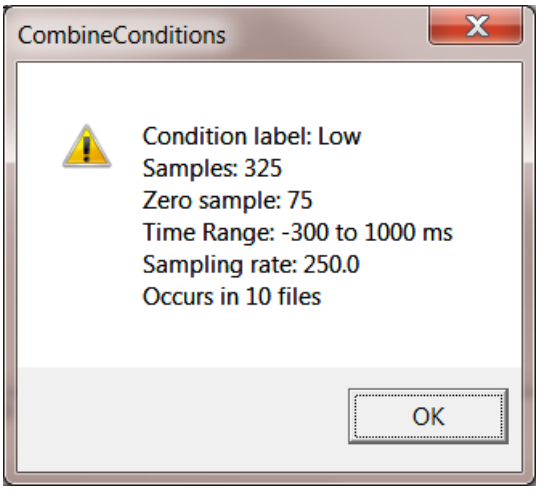


3. Switch to the **Condition List** tab. Here it can be specified how the input conditions (the conditions available in the loaded data files) are to be combined to new target conditions. In the left column, the available input conditions are listed - in our case the eight conditions that were defined in the paradigm file (Tutorial 2, step B) and averaged using the batch script used in the present tutorial under step



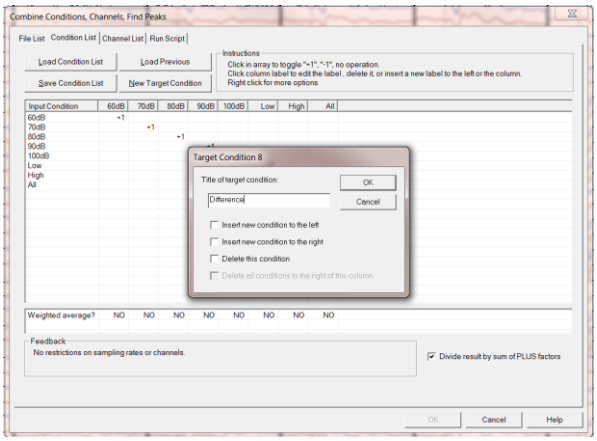
A. Conditions having the same segment comment in the different files are grouped into one entry.

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.



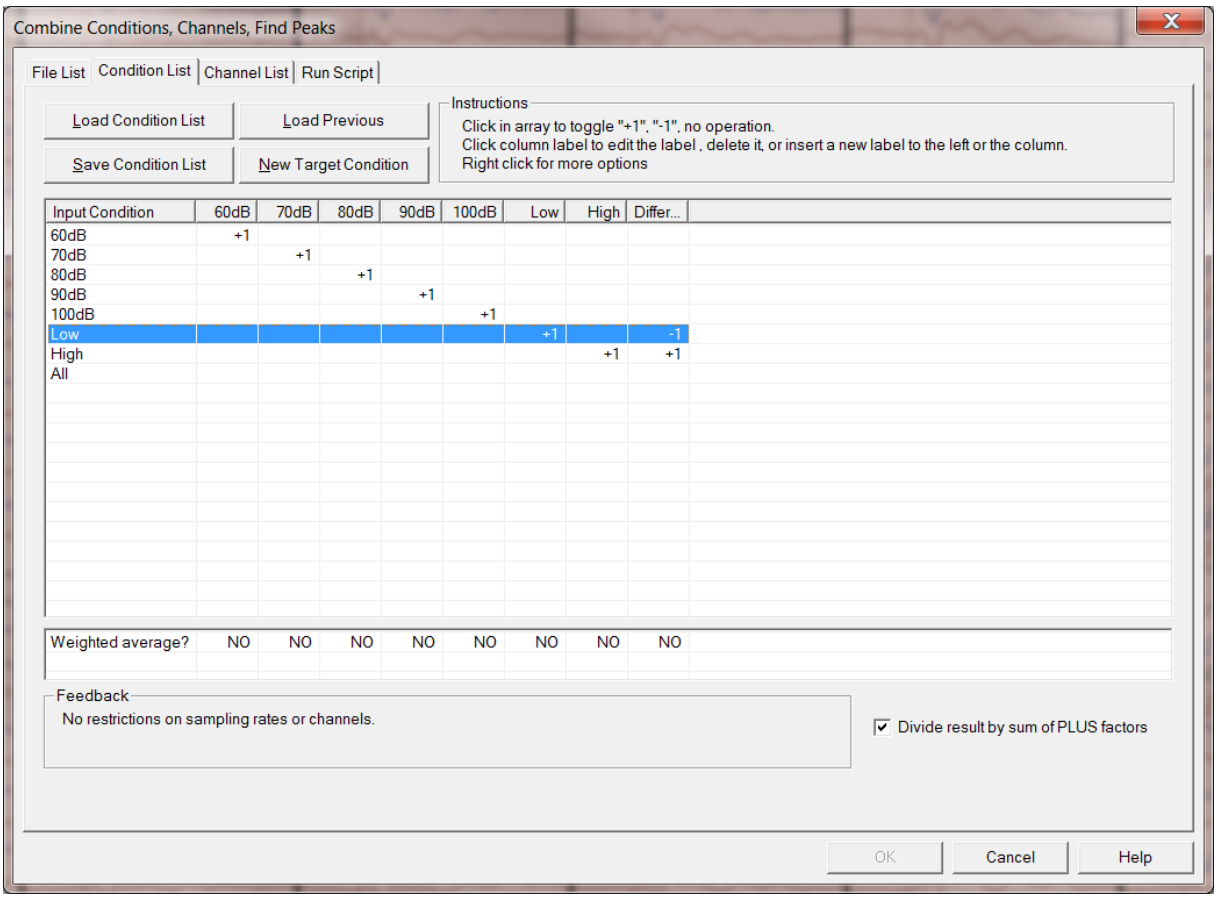
5. BESA Research automatically defined new target conditions (columns 2 – 9) with the same name as the input conditions. The numbers in the table indicate how the input conditions are to be combined to form the new target conditions. The default entry '+1' in the column of the '60dB' condition, for example,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

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**'.

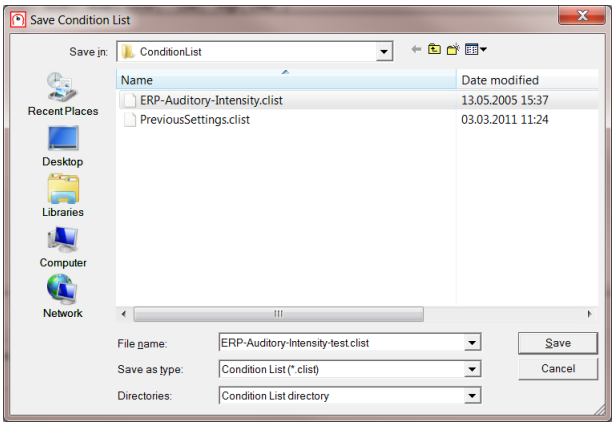


7. The target condition has been renamed to 'Difference'. **Left-click twice** onto the '+1' entry that links this condition to the 'All' input condition. This will remove the link and leave the field blank. To define the 'Difference' condition as the average of *'High' minus*

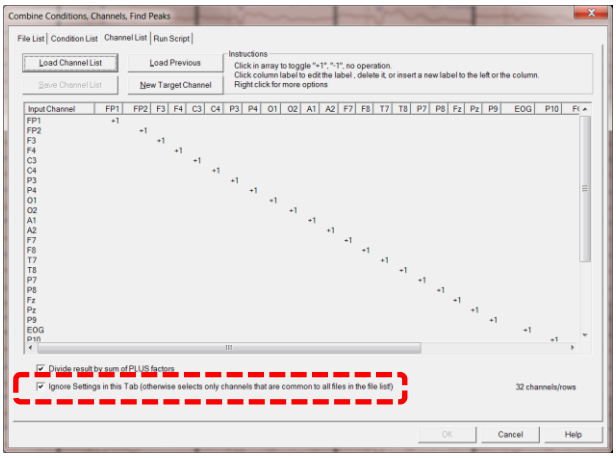
'Low' over subjects, **left-click once** into the field linking input '**High**' with target '**Difference**' to generate an entry '**+1**' in this field. **Left-click twice** into the field linking input '**Low**' with target **'Difference'** to generate an entry '**-1**'. Note that arbitrary weights 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 to create 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'.



8. 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'**.

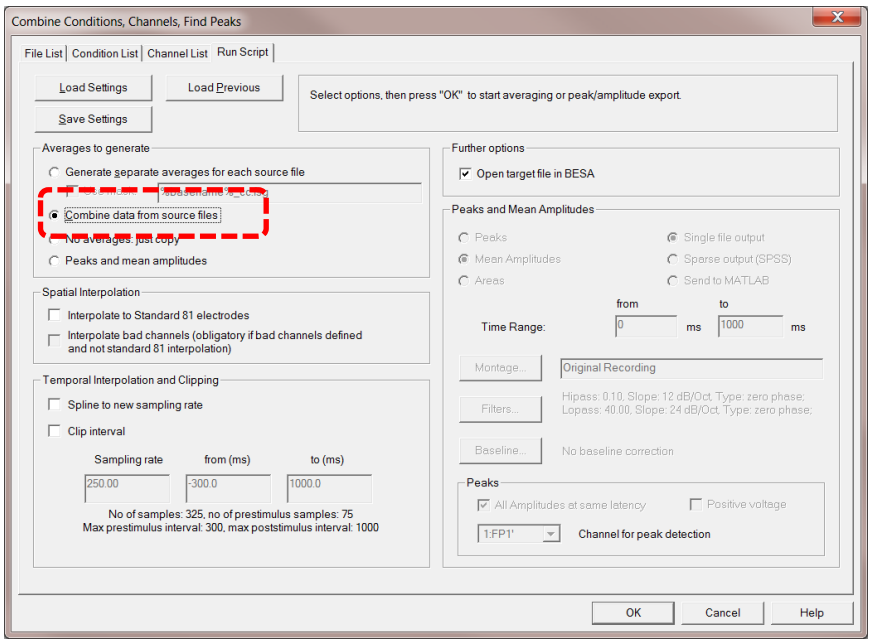


9. Proceed to tab **Channel List**. By default, settings in this tab are ignored (checkmark at the bottom left). It allows combining data of different channels. To create our grand average, this is not necessary, therefore we leave the settings in this tab unchanged.

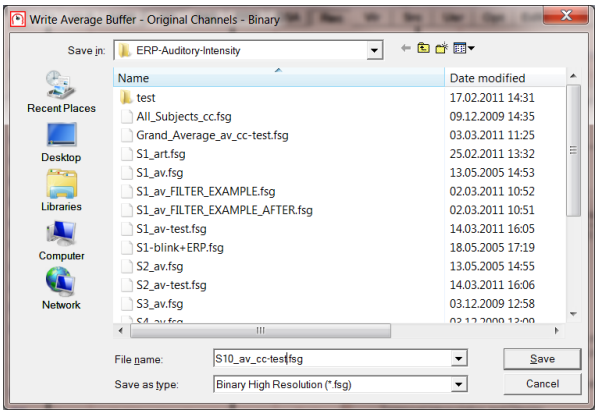


10. Switch to the **Run Script** tab. Global output options can be specified here. Make sure '**Combine data from source files**' is selected in the box 'Averages to generate', as we want to create a file containing averages over subjects. The options under 'Spatial Interpolation' allow to handle differences 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.

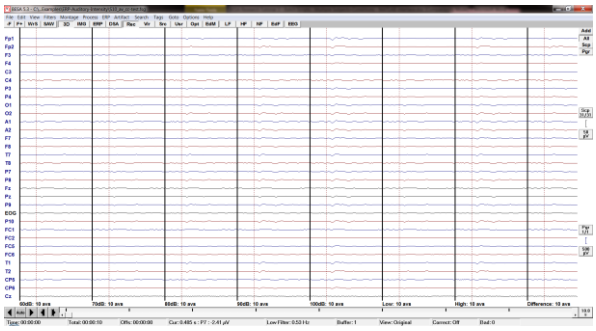
Note that the 'Run Scripts' tab also allows to **analyze peaks and mean amplitudes** in multiple data files. This is achieved by selecting 'Peaks and mean amplitudes', which activates the bottom right segment of the window. Traditional peak analysis can be performed quickly in an automated fashion, producing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



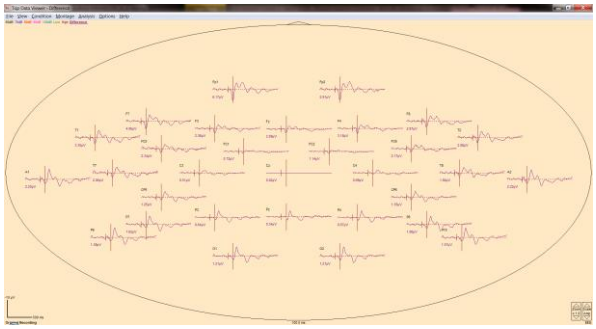
11. Click ‘**OK**’ to start the batch. BESA Research computes the specified grand averages and prompts for a file name. Enter ‚**All_Subjects_cc-Test.fsg**‘ and press ‚**Save**‘.



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.



13. **Right-click** into the data an 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.



What does BESA Research provide?

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

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.

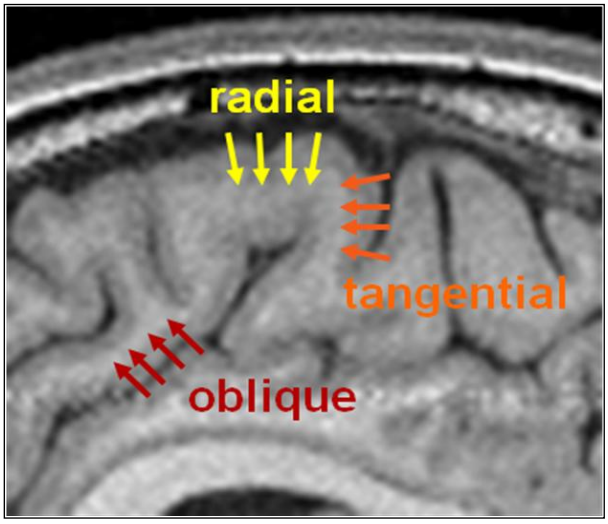
A: Theoretical introduction

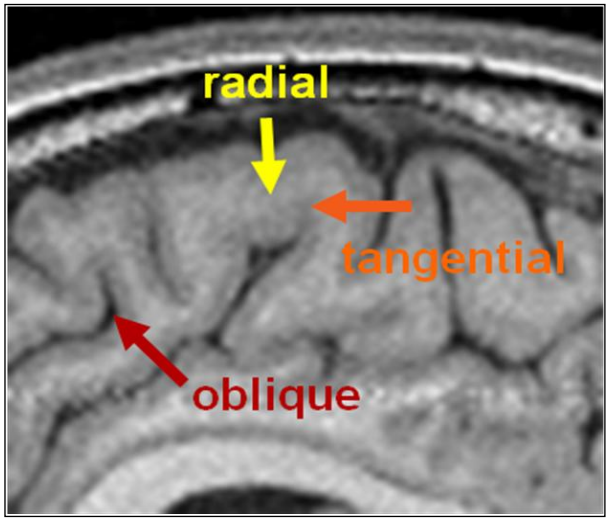
Equivalent current dipoles

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 x 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.

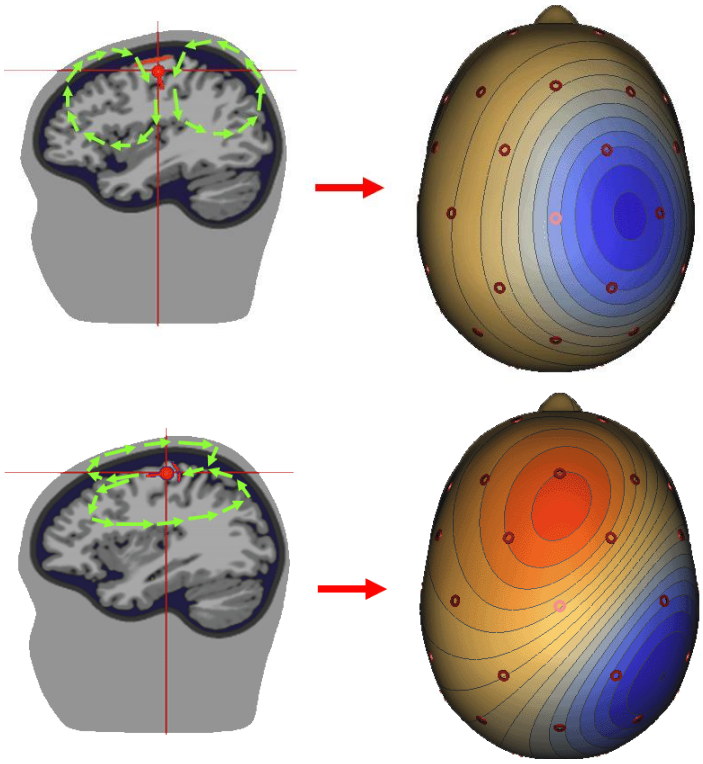
Volume conduction and the principle of linear superposition

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 (postsynaptic, neuronal) current in red, secondary (volume) currents 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.

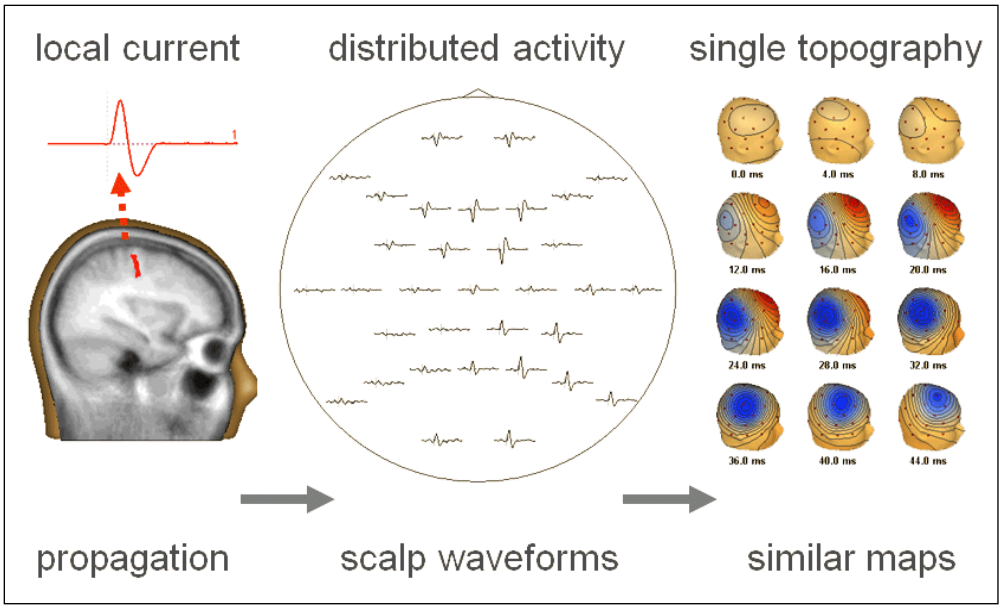
The propagation of the volume currents to the scalp is described by the so-called ‘head model'. The head model, or forward model, predicts the voltage at any electrode due to an equivalent dipole with a given location and orientation within the brain.

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.

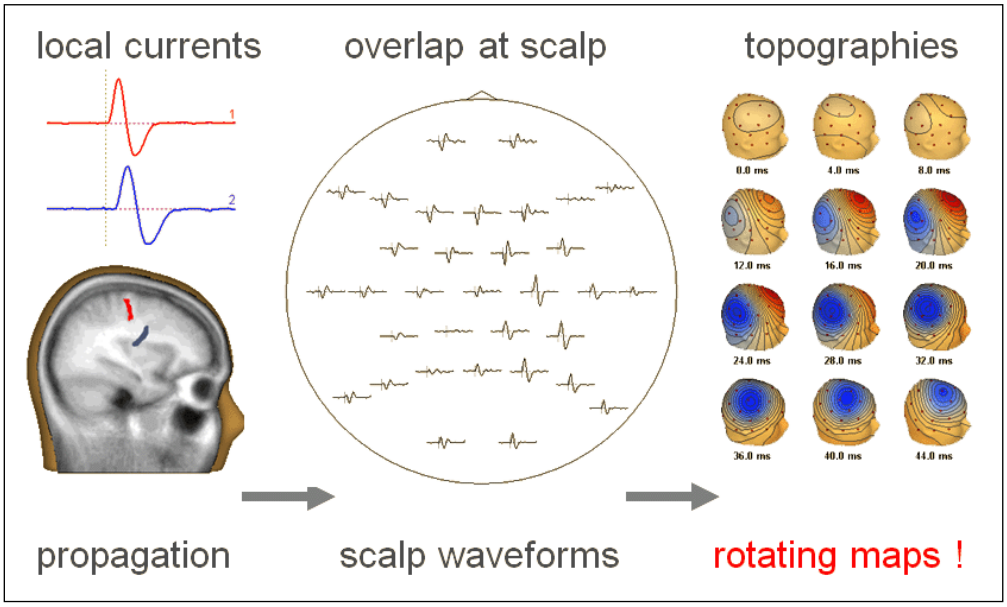
A cortical patch in a fissure generates a tangentially oriented dipole field (bottom of the figure above). The return currents in the scalp create a dipole map with symmetric positive and negative poles aligned in the direction of the dipole. The potential change directly over the source is zero, but 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.

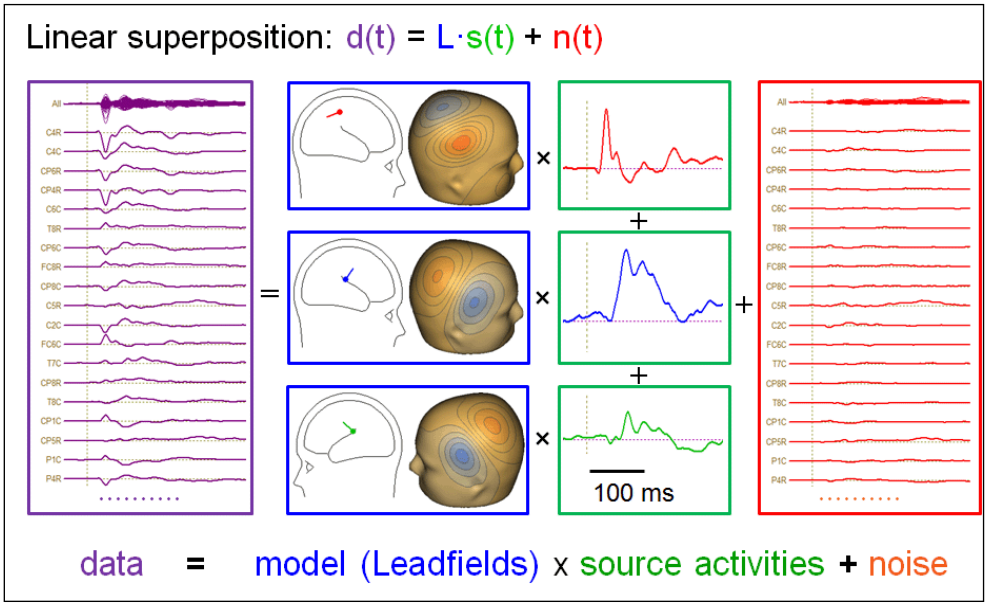


An assumed activity in the right central sulcus produces a near-tangential dipole field with a positive peak (shown downwards in the EEG top view in the middle) over the mid-frontal region (Fz and FC2) and a negative more widespread peak over the right inferior parietal regions (max. at P4). The patch 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.



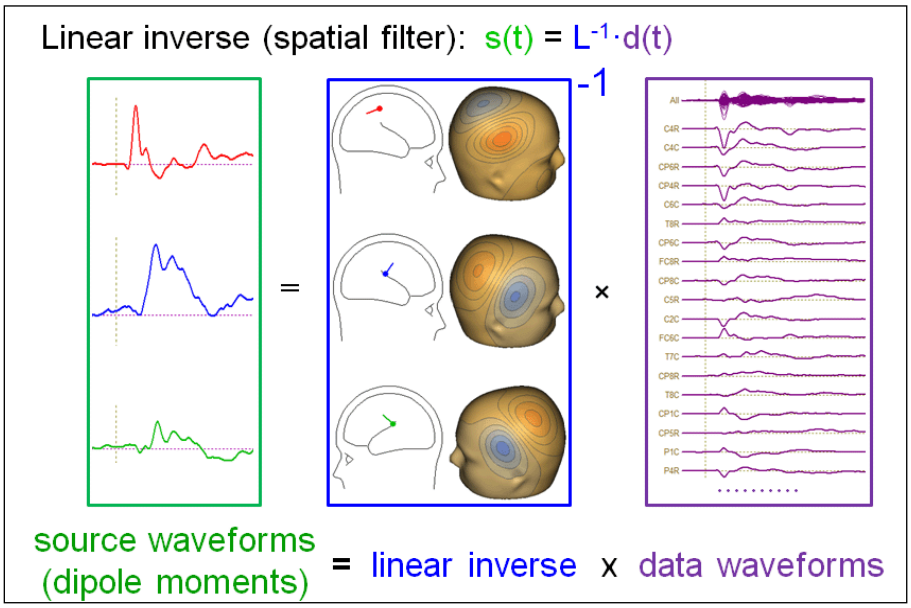
Now consider the situation of two brain regions separated by about 3 cm and activated within a few milliseconds. Each of the areas has a biphasic pattern with onset, peak, and polarity reversal. The two patches have different orientations. This is the main cause for their very different scalp topographies. 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.



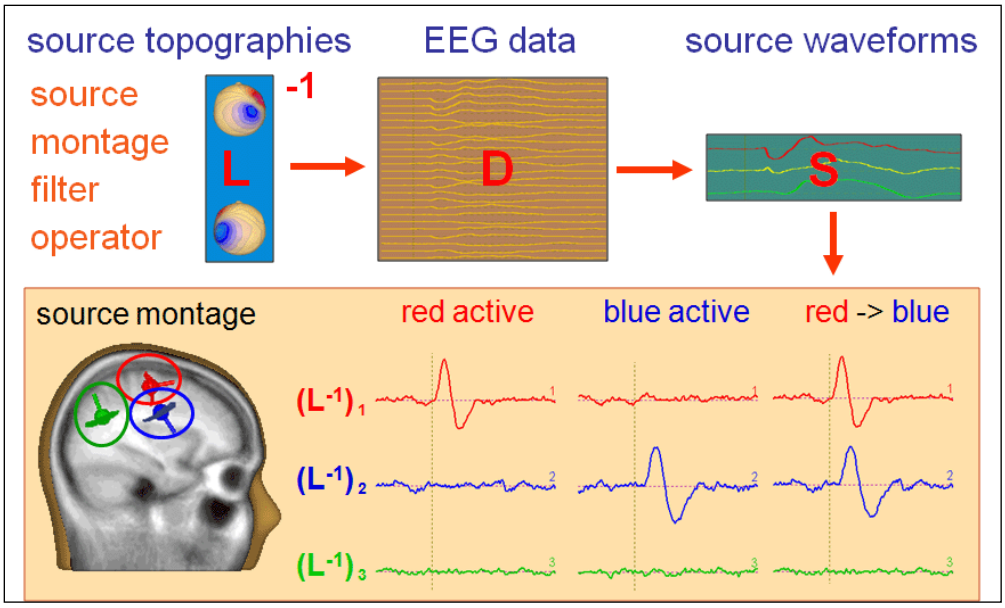
Using the laws of basic physics, we can now formulate the principle of linear superposition. This is illustrated here for 3 equivalent dipoles, but it would be the same for 10000 cortical current elements: The measured data (in this example MEG sensor signals, left, generated by tactile stimulation of 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). If we have a volume conductor model, for example a spherical head model, a boundary element model (BEM) or a finite element model (FEM), we can now 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.

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.



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.

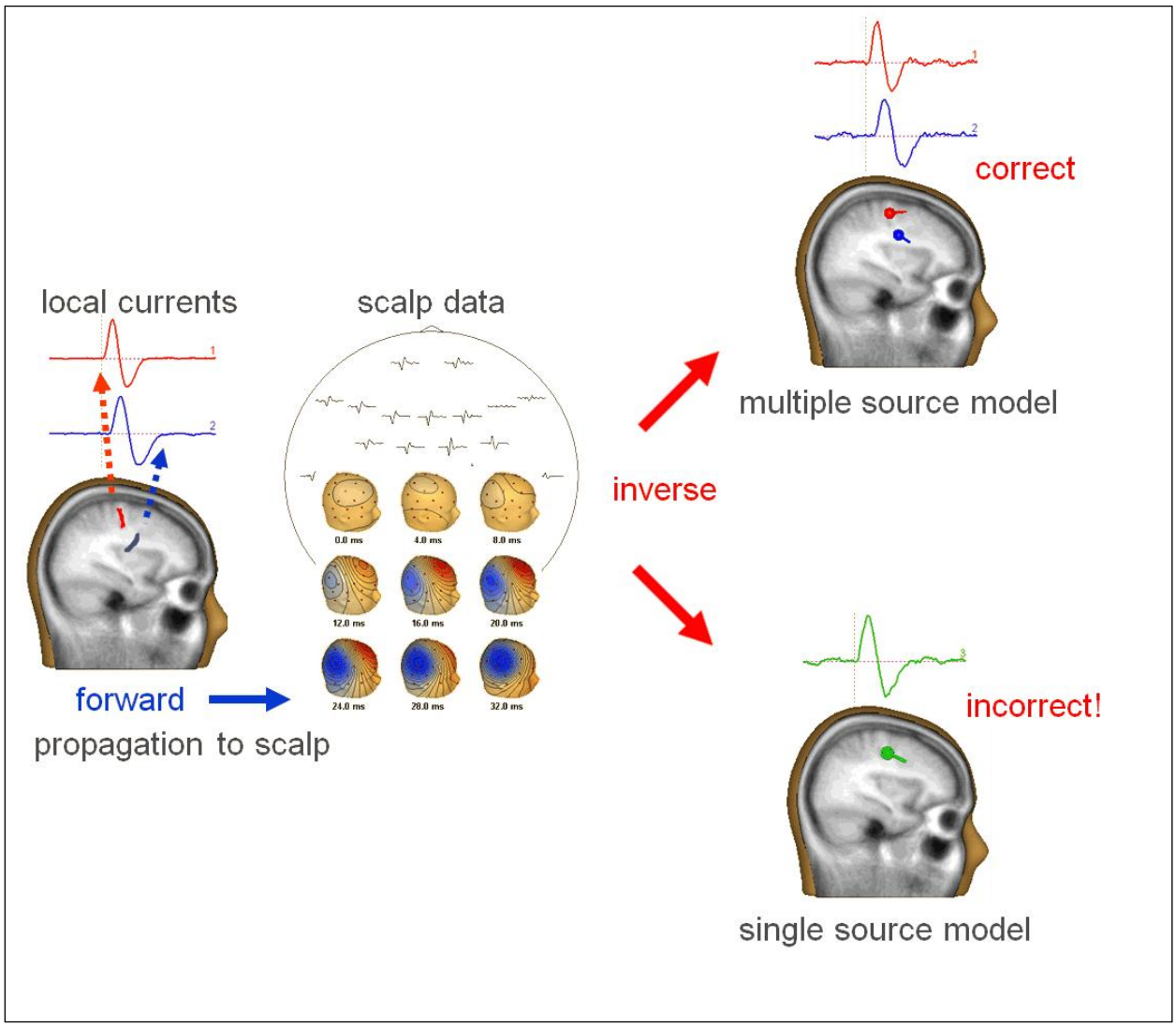
Thus, multiple sources can mutually contrast and separate the activities of the brain areas that they represent.

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).

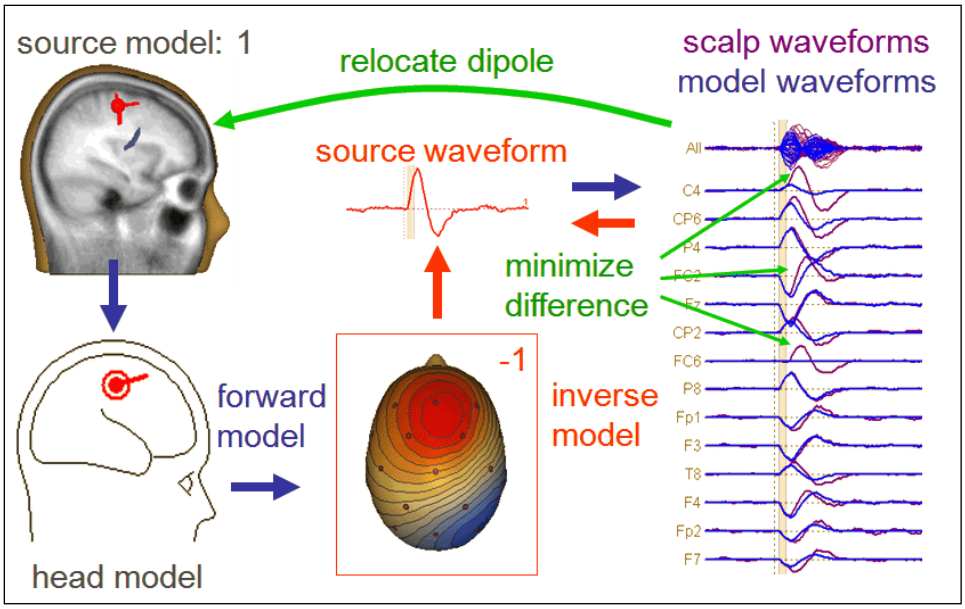
If we apply a two dipole model to our simulated data set, they separate the two activities in their source waveforms provided that appropriate equivalent locations and orientations had been selected. If we try to model the data with a single dipole only, we obtain an incorrect localization intermediate 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.



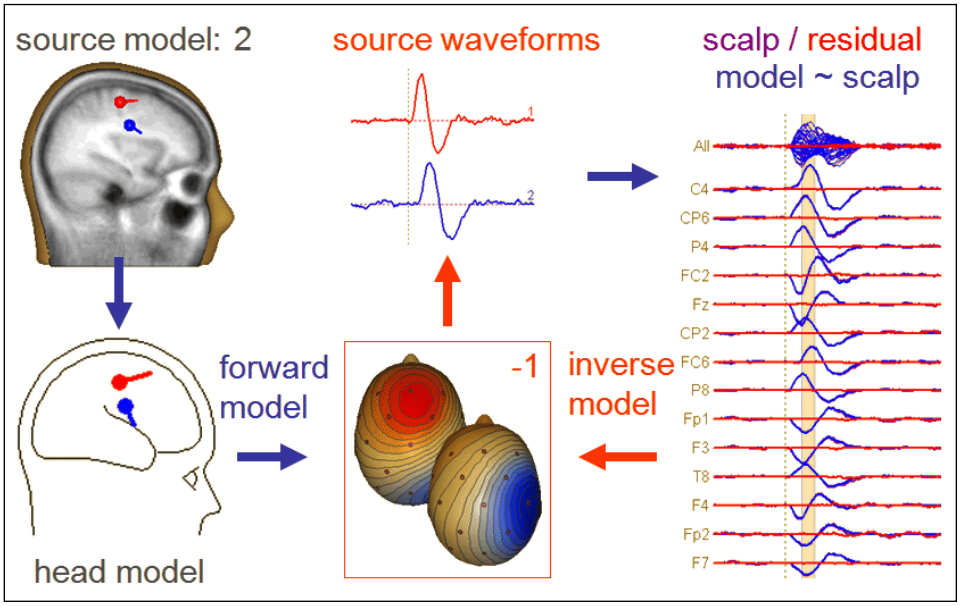
The fit procedure

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:

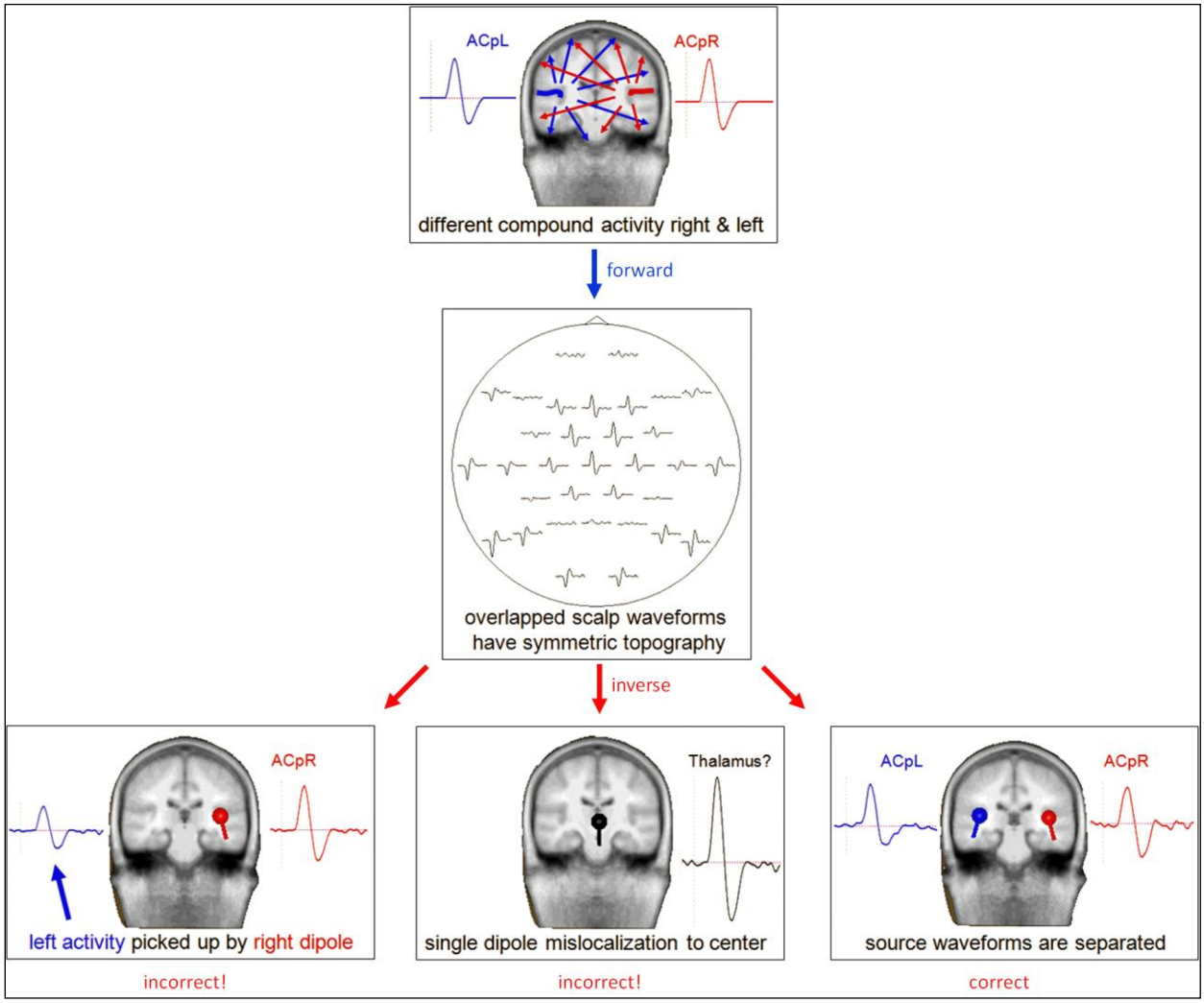
Use the 3D maps to define the fit interval from the time when a clear dipole field emerges until it starts changing. Perform a principal components analysis (PCA) over this interval: The PCA decomposition should show one dominant component. The percentage of variance it explains should decrease, if the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

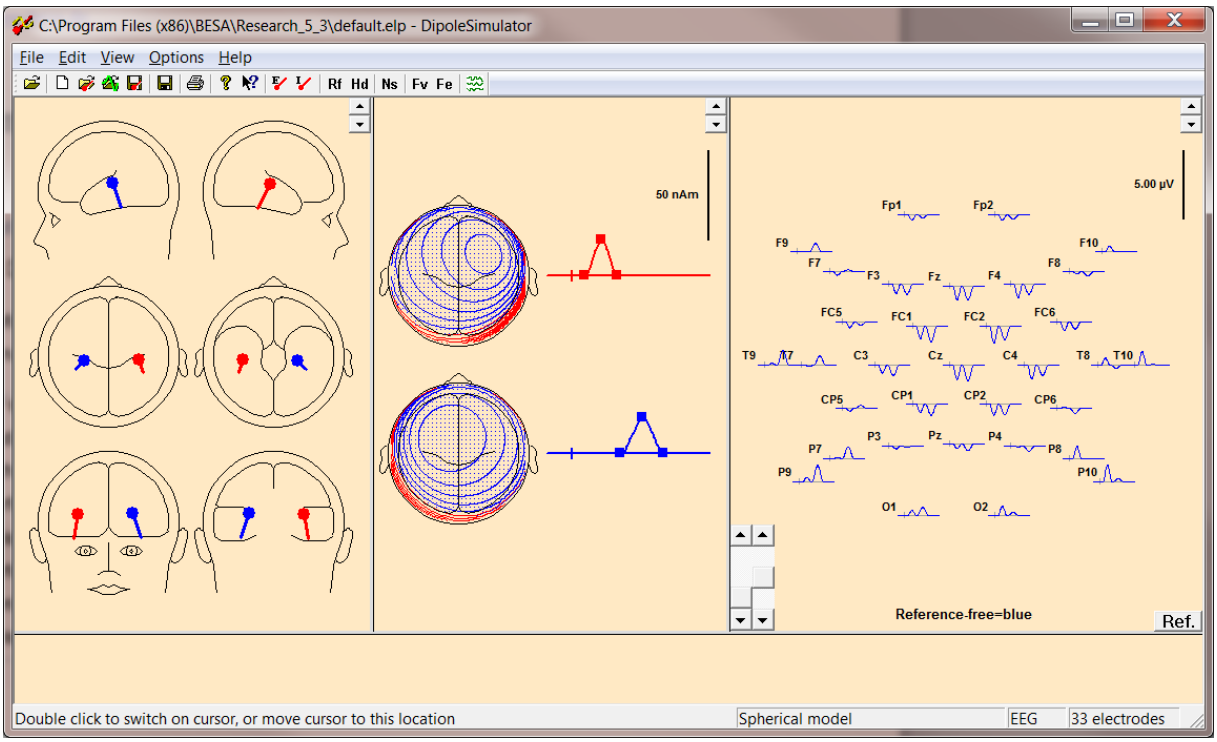


The above figure shows a simulated example that illustrates the need to create an appropriate source model in order to obtain correct source waveforms. We assume a situation where both auditory cortices are active. Their topographies on the scalp surface overlap, and the recorded signal at each electrode 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.

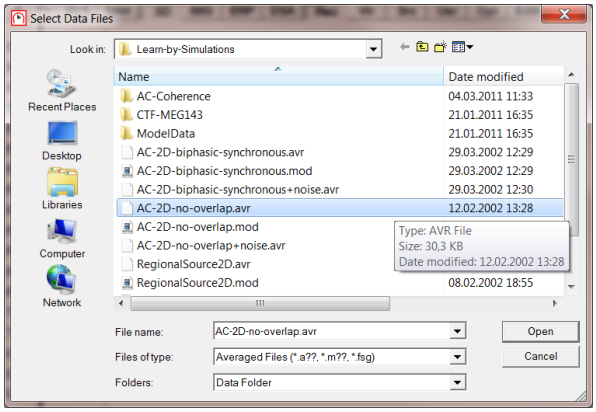
B: Single Dipole Fitting with Simulated Data

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.

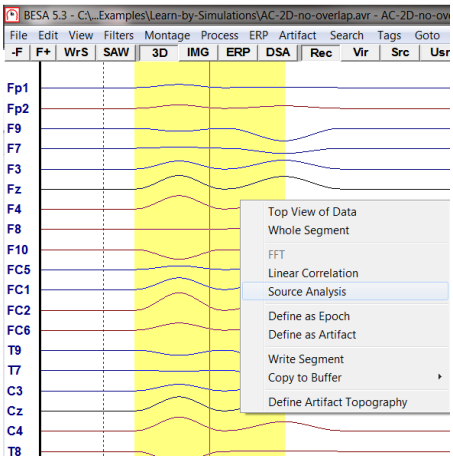
1st simulated data set: In the first example, non-overlapping (asynchronous) activity in both auditory cortices is simulated. Current flowing in the depth of the Sylvian fissure is oriented orthogonal to the temporal plane, because pyramidal cells are perpendicular to the cortical surface. Therefore, 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.



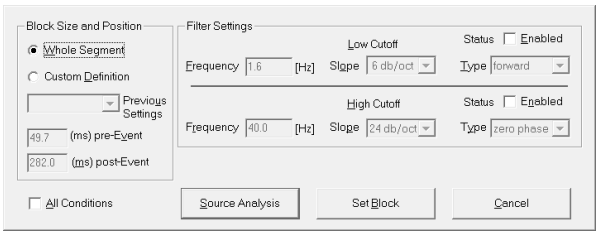
1. In the main window, select '**File / Open**' and browse to the '**Learn-by-Simulations**' subfolder of the Examples folder to load the simulated data set '**AC-2D-no-overlap.avr**'. You might need to change the file-type to 'Averaged Files (*.a??, *.m??, *.fsg)' to see it. Press '**Open**'.



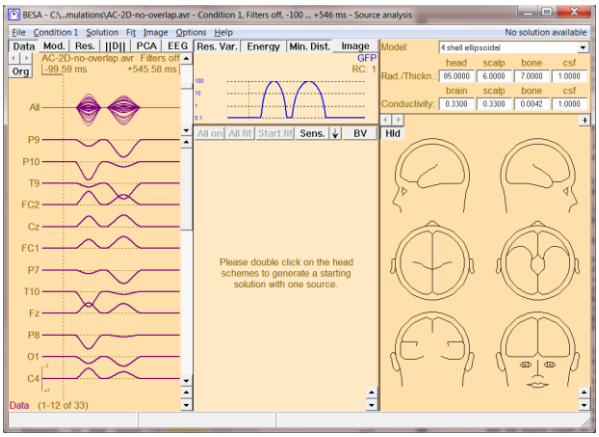
2. We want to perform source analysis on this data now to see if we can recover the brain activity that is underlying the recorded signals. Source analysis is performed in a separate window of BESA Research. To start source analysis, **left- drag over the data** to mark a block. Then **right-click** into the block. From the popup menu select '**Source Analysis**'.



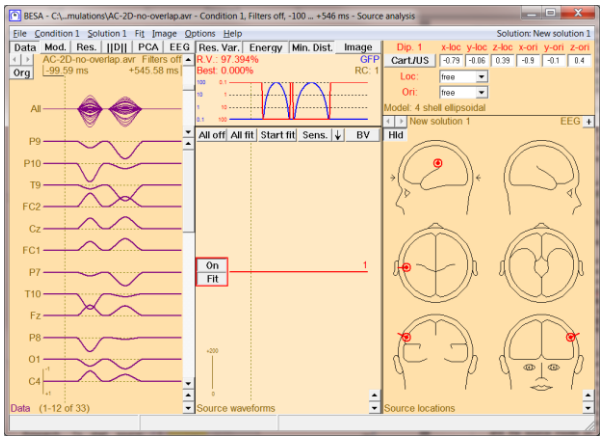
3. A dialog window appears which allows specifying the time range of the data to be sent into the source analysis window. Select '**Whole Segment**'. It is also possible to specify temporal filters to be applied before source analysis to remove slow drifts and noise from the data. For our simulated data sets we don't need to apply filters. Hit '**Source Analysis**' to open the source analysis window with the specified data segment.



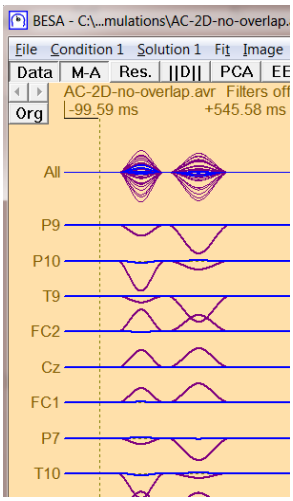
4. On the left of the source analysis window, the recorded channels are displayed in average reference. In the top middle box, the global field power (GFP; the sum of squares of all channels, normalized to 100%) is displayed in a logarithmic scale. On the right, the head sketches are shown analogously 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.

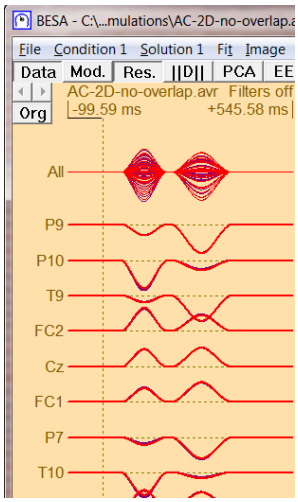


5. This defines a first (still inappropriate) source model. The source waveform in the middle box is calculated from the recorded data using the head model (= model for the distribution of the electrical conductivity inside the head) and the source model on the right as a hypothesis. Source waveforms are dependent variables. Source locations and orientation are independent variables.

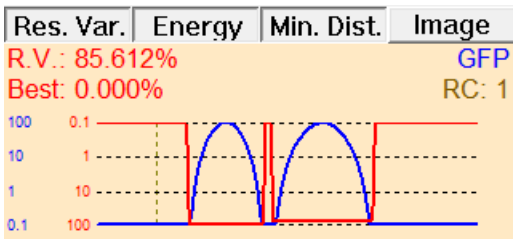


6. Based on the source waveforms and the sources' leadfields (as defined by source location, orientation, and head model), the model waveforms can be computed, i.e. the data that would be generated by the current source model. You can visualize the model waveforms (blue) by hitting the **Model** button in the upper left corner. The difference between recorded (purple) and modeled (blue) waveforms is the *residual*. It can be displayed in red by hitting the **Res.** button.

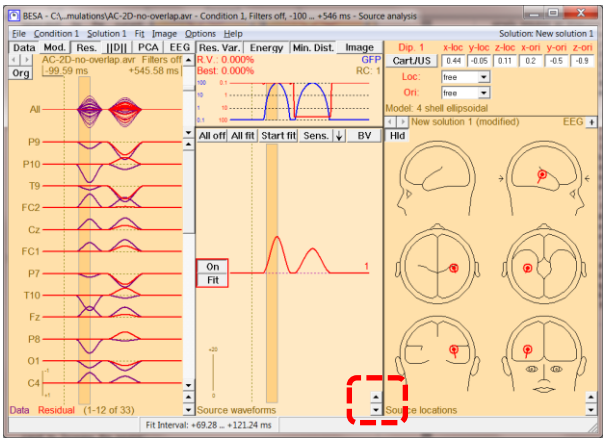




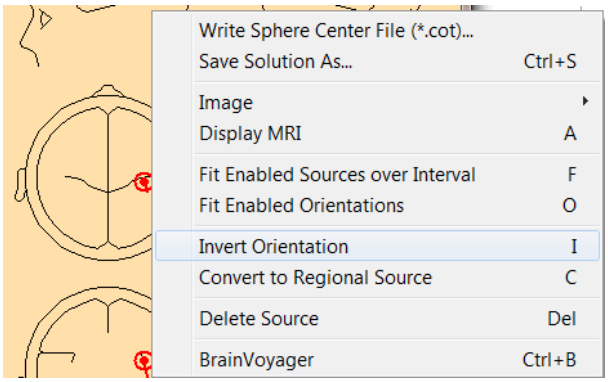
7. The normalized sum of squares over channels of this residual activity, is the *residual variance* (RV), i.e. the unexplained fraction of the data variance. It is displayed in the top middle box (red) together with the GFP. Note the inverted logarithmic scale for the RV. The goal of the source fitting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



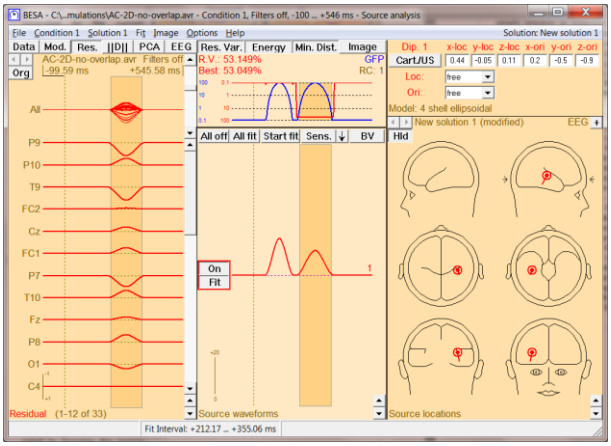
8. **Left-drag** over the data approximately from the onset of the first EEG activity to the first peak. Then hit the '**Start Fit**' button in the waveform box to determine the optimum location and orientation for our first dipole in order to explain the data in the marked fit interval. The result is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

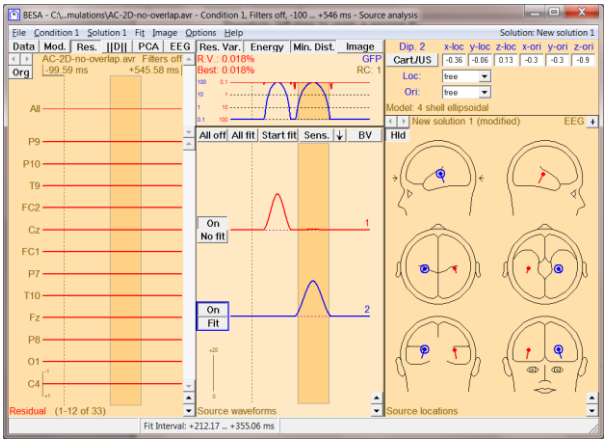


10. Release the **Data** button in the upper left corner to show the residual activity only. In our marked initial fit interval, the dipole in the right auditory cortex explains the recorded data without any remaining residual. However, its source waveform is not correct – it shows activity also during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



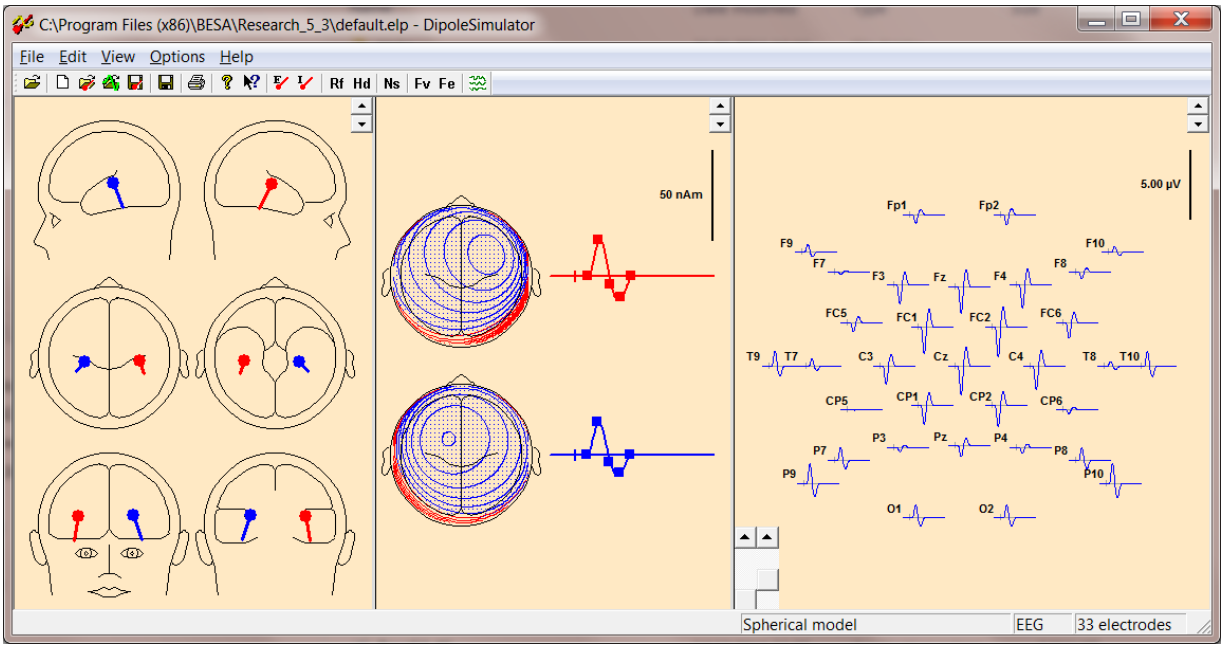
residual variance in this latency range. Therefore, left-drag to mark a second fit interval across this later time range.

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.

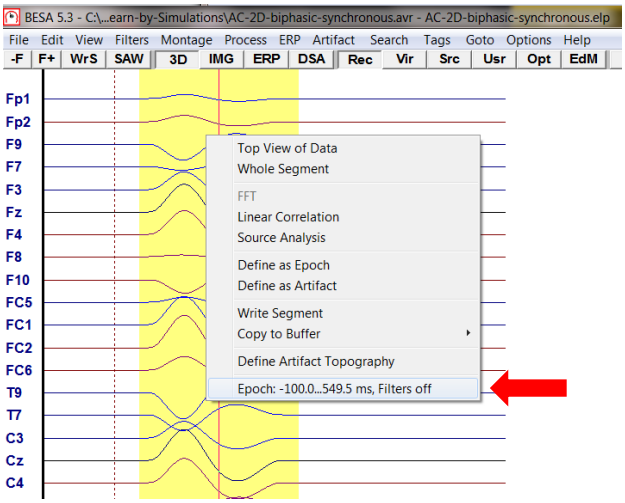


The fit results in a complete separation of the activities of the right and left AC. The addition of the left dipole has changed the calculation of the right source waveform. It is now 'blind' to activity from the left source, and does not show any activity in the later interval when the left source 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.

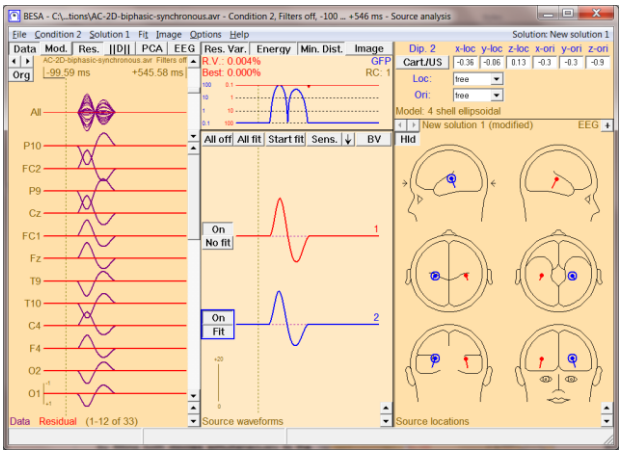
2nd simulated data set: Severe overlap and high synchrony between hemispheres is what we must expect in auditory evoked potentials. Furthermore, cortical source activities are more complex and often biphasic. Therefore, the next simulated data set, **AC-2D-biphasic-synchronous.avr**, contains biphasic 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!



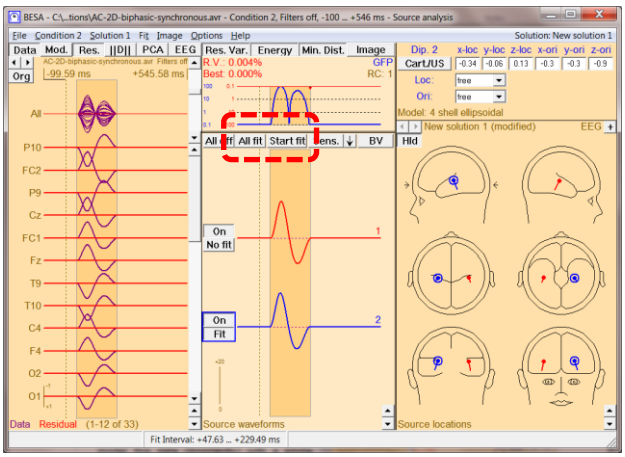
12. Minimize the source analysis window and select '**File / Open**' in the main window to load the second simulated data set '***AC-2D-biphasic-synchronous.avr***'. **Left-drag** to mark a block and **right-click** into it. BESA Research remembers the previous settings used for source analysis that you can quickly re-apply by selecting it at the bottom of the popup menu.



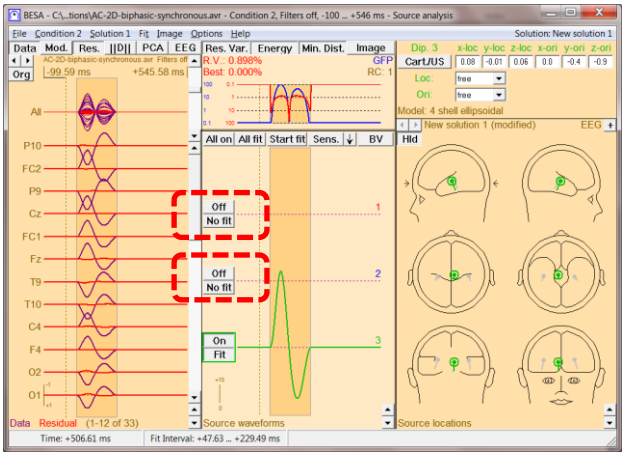
13. The source analysis window appears with the new data set, the previous dipole model still applied. Press the **Data** button to show the biphasic EEG signals of this data set. Since the data set has been simulated with the same active brain regions, the source model is correct for this data, the residual variance being nearly zero. The source waveforms are now computed from the new data and therefore reflect the synchronous bilateral biphasic activity.



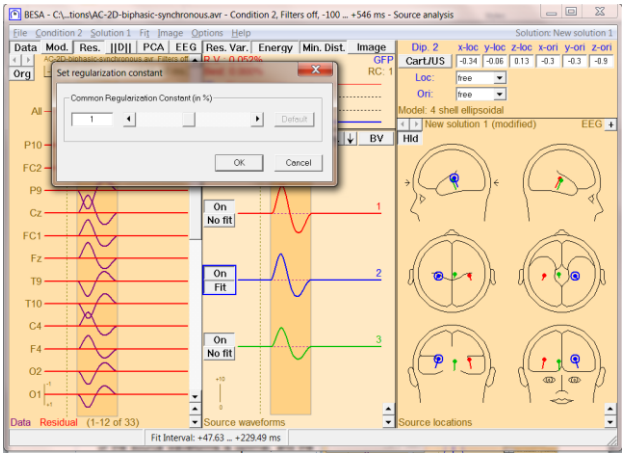
14. We could have obtained this source model by fitting both dipoles simultaneously to the data. To see that, **left-drag** to mark a fit interval over the time range of EEG activity. Then hit the **All fit** button – this turns both sources into the *Fit* state. When pressing the **Start Fit** button now, location and orientation of both sources will be optimized. The result does not differ from the previous model.



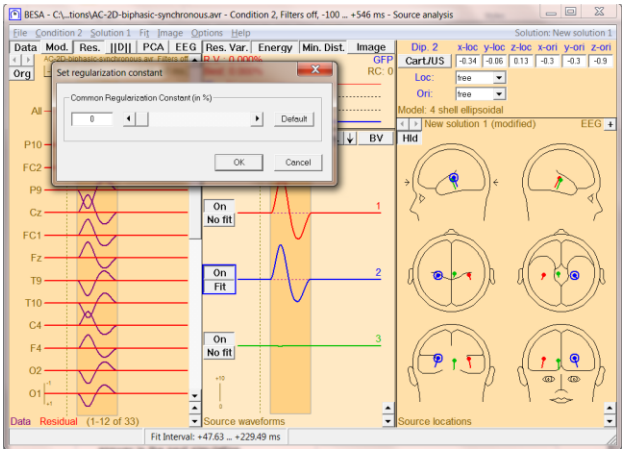
15. Let's see what happens if we had tried to model this data (incorrectly) with a single dipole only. the two dipoles by hitting their **On** buttons. This effectively removes them from the source model. **Double-click** to add a new dipole and press '**Start Fit**'. This results in a dipole in the 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)!



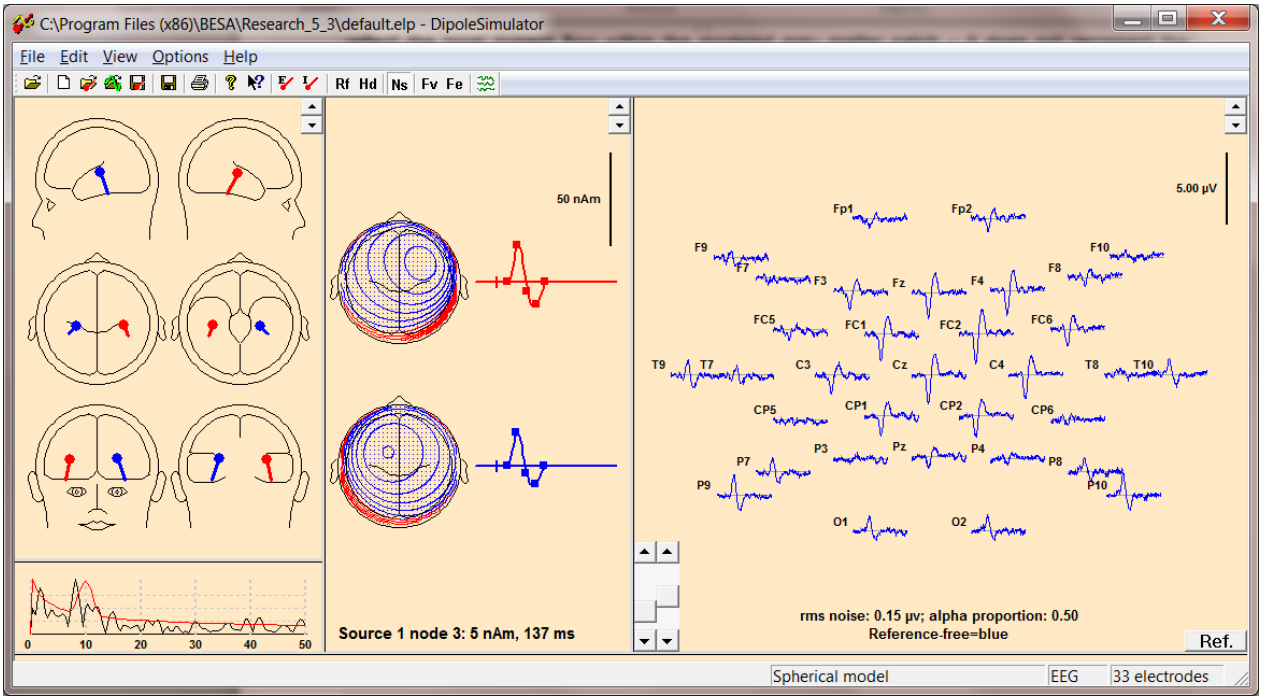
16. How can one decide whether the one-source or the two-source model is correct? This can be done by contrasting the two source models. **Switch back** '**On**' the two first dipoles. Now the three source waveforms should be able to contrast and mutually separate the activities in the three modeled 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.



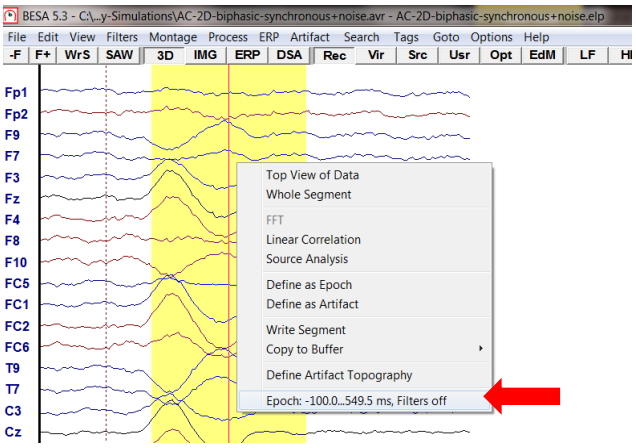
17. Reduce the regularization constant to **zero**. With this setting, the separation capabilitiy of the source waveforms is optimal, and the flat source waveform of the third dipole indicates that there is no brain activity in the middle of the head. Rather, the two-dipoles in the bilateral auditory 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.



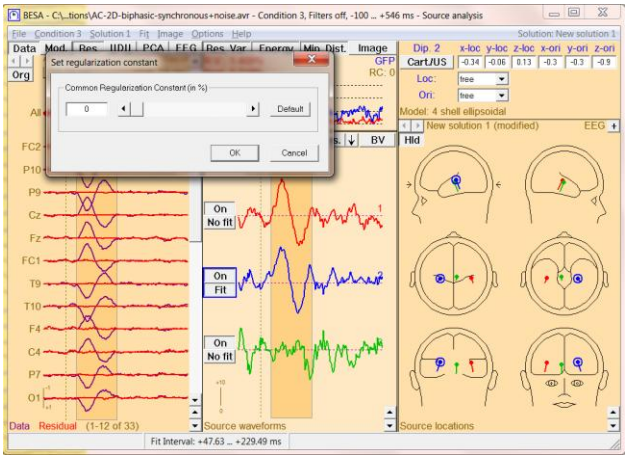
3rd simulated data set: Finally, the third simulated data set has been designed to be even more realistic by adding background noise to the same simulated brain activity as before (**AC-2D-biphasic-synchronous+noise.avr**).



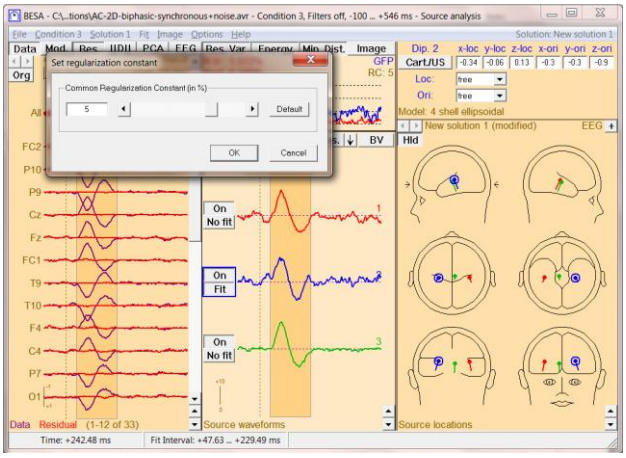
18. Minimize the source analysis window. In the main window select '**File / Open**' to load the file '**AC-2D-biphasic-synchronous+noise.avr**', the same simulation as the previous one, but with some small background noise added to the data. Again, send the data into the source analysis window: left-drag to **mark a block**, right-click into it and **select the bottom-most entry**.

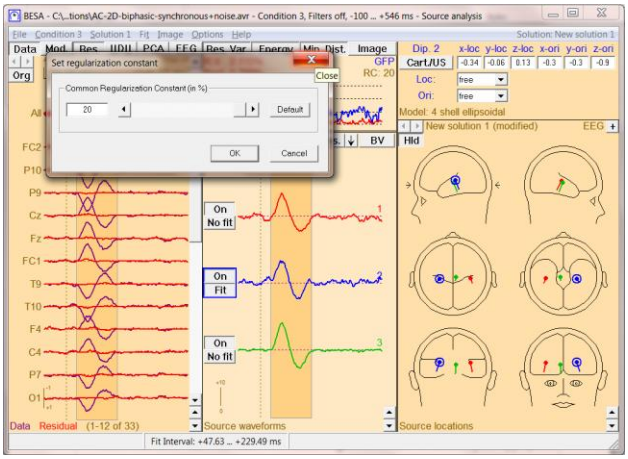


19. The previous solution is now applied to the noisy data. The channel noise is strongly projected onto the source waveforms when the regularization is set to zero. Select '**Options / Regularization constant**' again and see how increasing the regularization constant affects the reconstructed source waveforms.

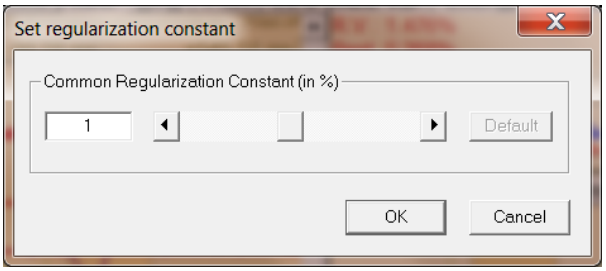


20. As regularization is increased, crosstalk increases as in the previous example, i.e. the third source waveform reflects activity that is truly generated in the brain regions represented by the other two sources. However, as a positive effect, the source waveforms are substantially cleaned from noise. This positive effect in noisy data implicitly stabilizes source fitting results in 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.



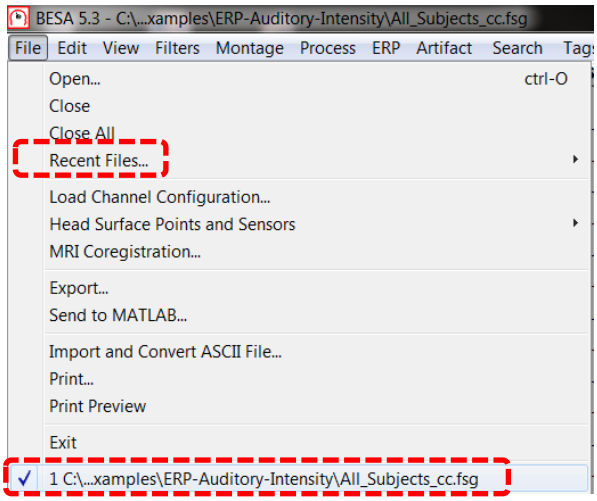


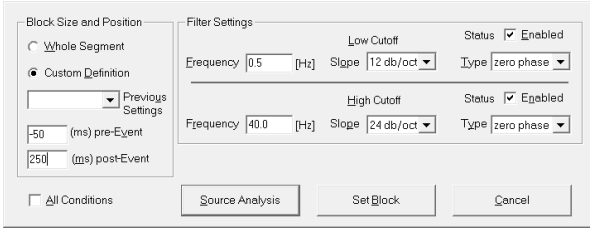
21. Set back the regularization to 1% and hit *OK*. Then **close the source analysis window** (confirm with '**OK**' that the developed source model does not need to be saved to disk).



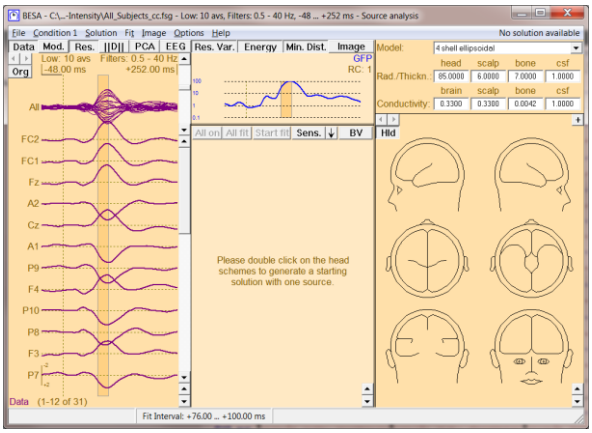
C: Single Dipole Fitting in Real Data

1. 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**', **right-click** 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.

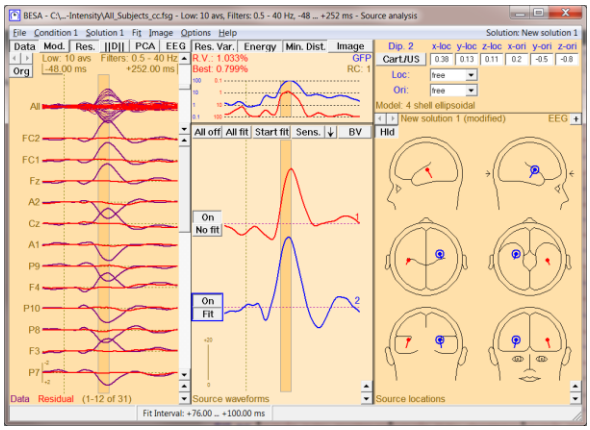




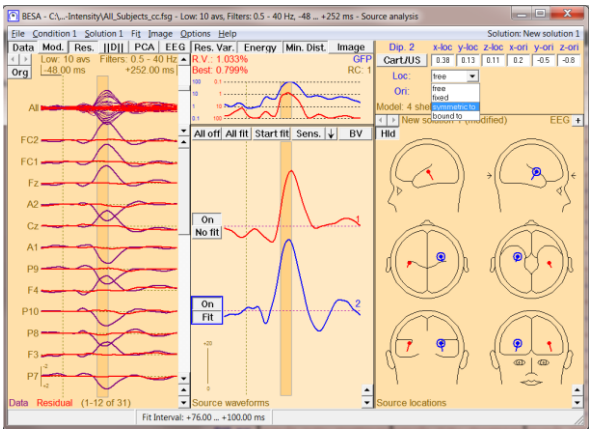
2. It is not possible to fit a good source model for the P50 as its signal-to-noise ratio is poor. Therefore, will focus on the N100, which has the largest amplitude in our signal. Mark a time-window from the beginning to the maximum of the auditory N100 (76 to 100 ms). As we are dealing with auditory 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**'.



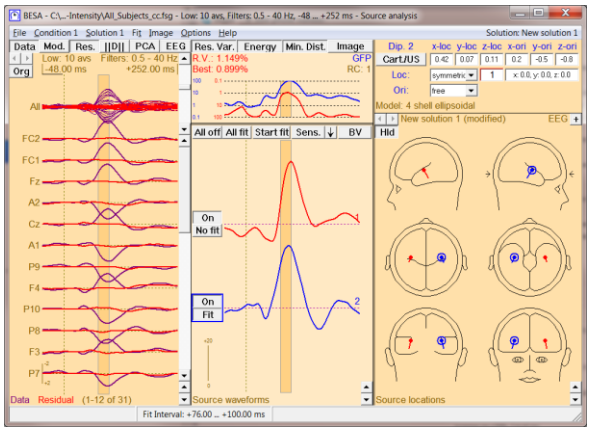
3. Both sources will fit in auditory areas, the right source being located further anterior than the left source. The right source seems located slightly too medial.



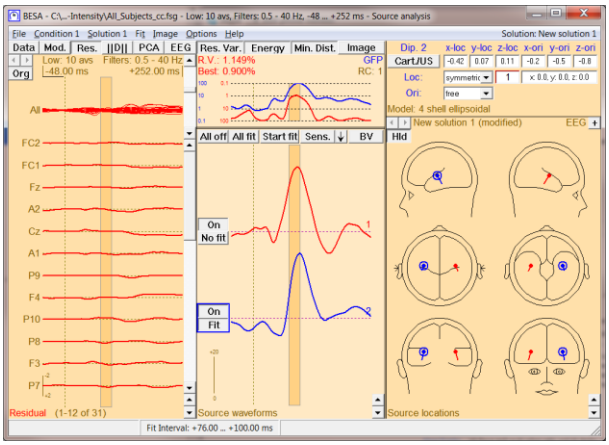
4. The fit can be improved by introducing a symmetry constraint. This can be very useful for stabilizing a fit, particularly when data are noisy. To do so click on one of the sources and select '**symmetric to**' in the '**Loc.:**' drop-down menu. Press '**All fit**' and '**Start fit**' again.



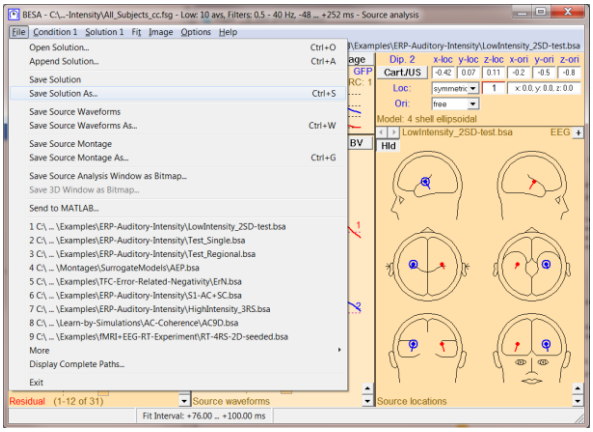
5. Both dipoles are located symmetrically in the auditory cortex. Note that the residual variance is slightly bigger using the symmetry constraint. However, the solution with the smallest residual variance is not always the correct anatomical solution. You should keep in mind that deeper dipoles explain more variance. Therefore, in noisy data dipoles will have a bias to localize deeper to explain more variance.



6. Release the **Data** 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 nor the N150 sufficiently. This can be seen by the higher residual variance in the according time-ranges.



7. Save the current source solution for later by pressing '**File / Save Solution As…**' under the name '**LowIntensity_2SD-test.bsa**'. Close the source analysis window.



D: Fitting Regional Sources in Simulated Data

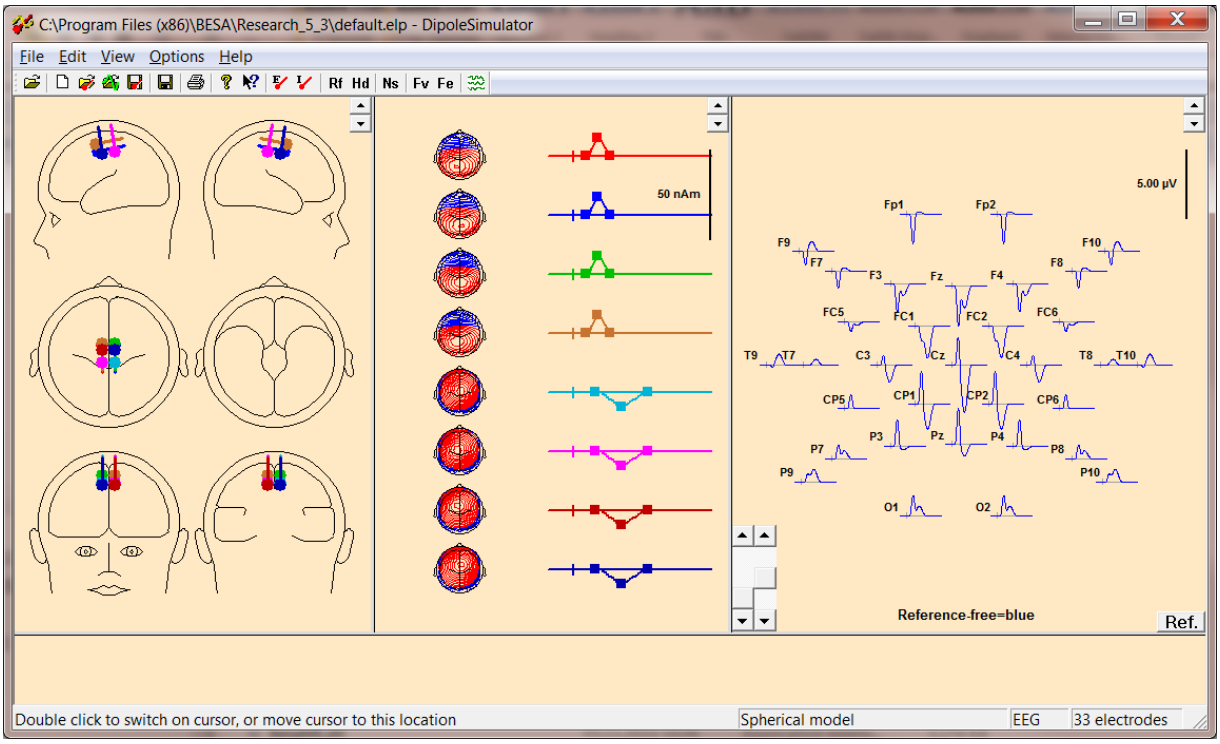
In order to understand the concept and advantages of regional sources we will again work with a simulated dataset.

Simulated dataset: Two extended square surfaces (~10-15 cm^2) 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).

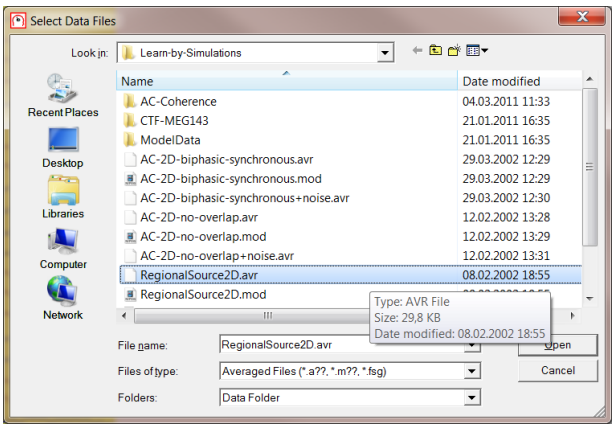
The first simulated brain region is thought to represent **supplementary motor area** (SMA). The four dipoles are located symmetrical to the interhemispheric cleft with **nearly tangential orientation**, reflecting a planar fissure to both sides of the interhemispheric cleft. The modeled time-course 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).

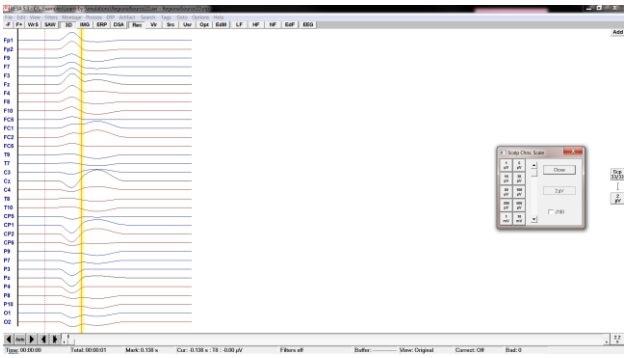
Thus the two modeled extended brain regions are in **close proximity to each other**, but **differ from each other by the direction of the neuronal current flow** (i.e. the orientation of the active gray matter surfaces). The time courses of the two activities are simulated to be partly overlapping,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



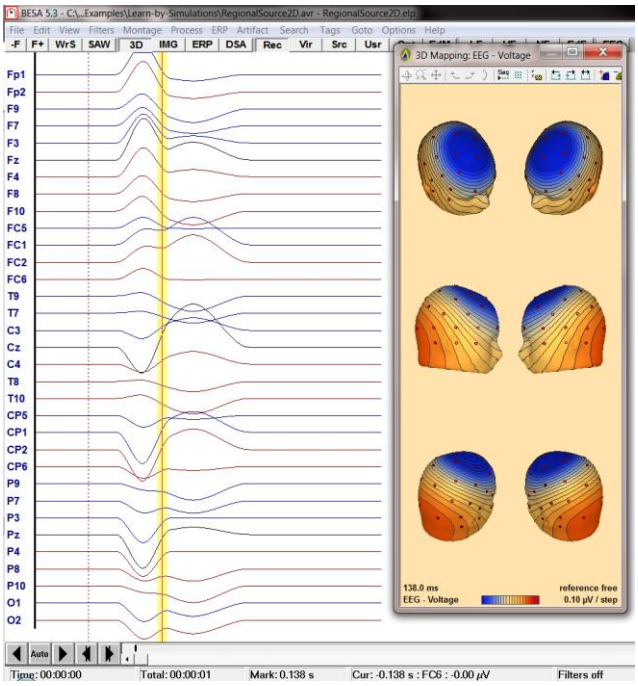
1. Load this data set in BESA Research: Select '**File / Open**' and open '**RegionalSource2D.avr**'.



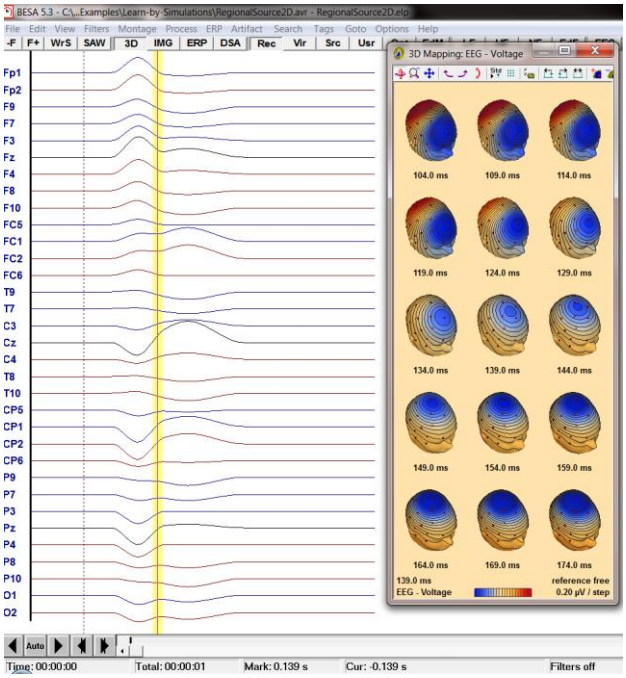
2. You may increase the amplitude scaling by pressing the **amplitude scaling button** at the right of the window. Change the scaling to **2µV**. It is evident that due to the effect of volume conduction, each electrode records signals from both modeled brain regions to some extent.



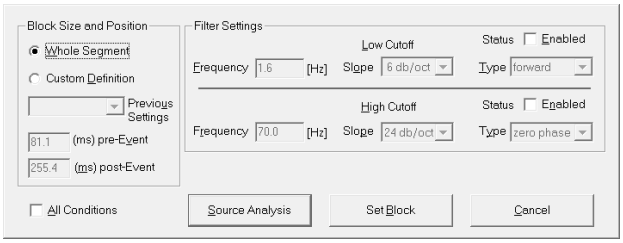
3. The overall distribution of the EEG activity at the scalp surface can best be observed by viewing a 3D whole-head map. Open the **map window** by **double-clicking** into the data approximately where a polarity reversal is observed in electrode Cz. A cursor is set and the map window appears.



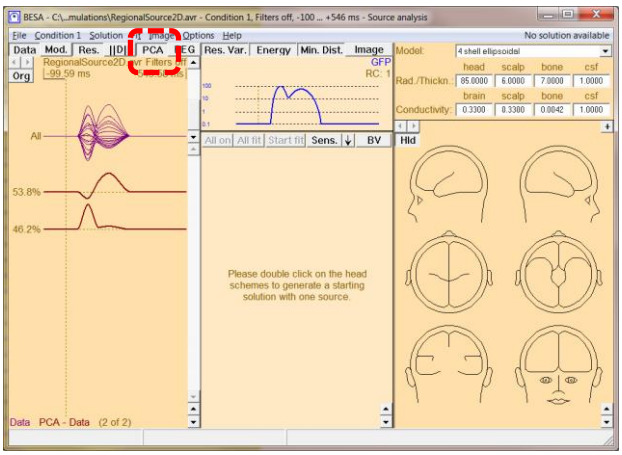
4. To obtain a **time sequence** of maps, **click** onto one of the heads, e.g. the view of the right side (left head in the middle row). The display changes, and maps from 35 ms before to 35 ms after the cursor are shown in steps of 5 ms. The map seems to rotate from an early tangential map (activity 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.



5. 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. Press the **PCA** button to compute a principle component analysis of the data. 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 remaining variance etc. The curves at the left indicate the associated time courses.



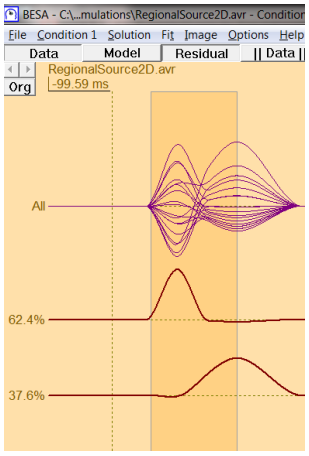
The variance explained by the corresponding component is displayed next to it. It becomes obvious from the time courses, that a **PCA does not provide a separation of the multiple brain activities** (they were simulated to be monophasic, but the PCA time courses show two peaks each). However, the PCA provides a good estimate for the **minimum number of**

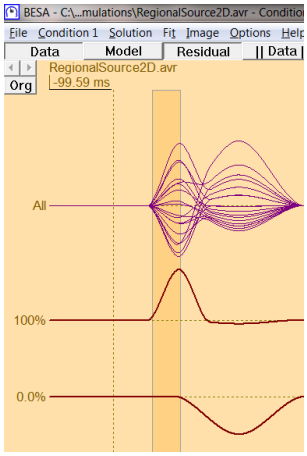
brain regions that are contributing to the EEG data: A single brain region cannot generate EEG data that contains contributions of two mutually orthogonal topographies. We can therefore immediately conclude that we are dealing at least with two active brain regions in the current data set. Since **synchronously 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.

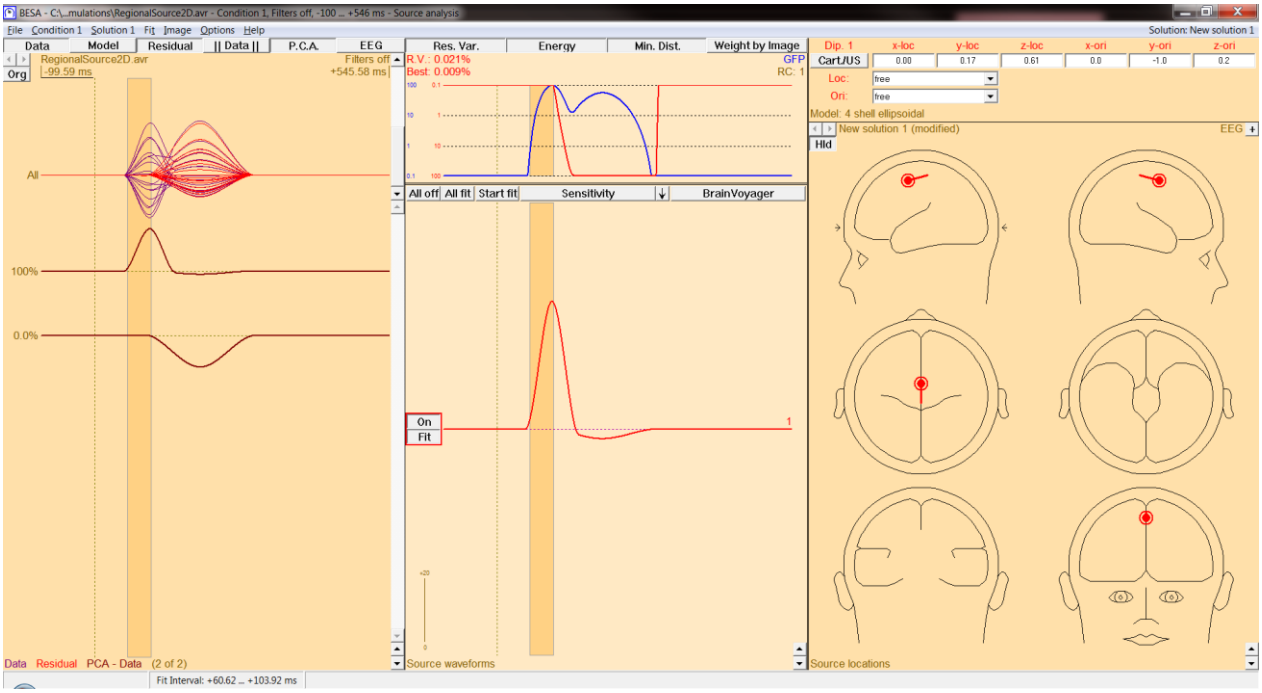
7. If a fit interval is marked, the PCA decomposition is computed for the marked data segment only. **Mark a fit interval** that starts at the onset of observable EEG activity and vary its duration. Note that every time you change the length or position of the fit interval, the PCA waveforms and explained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





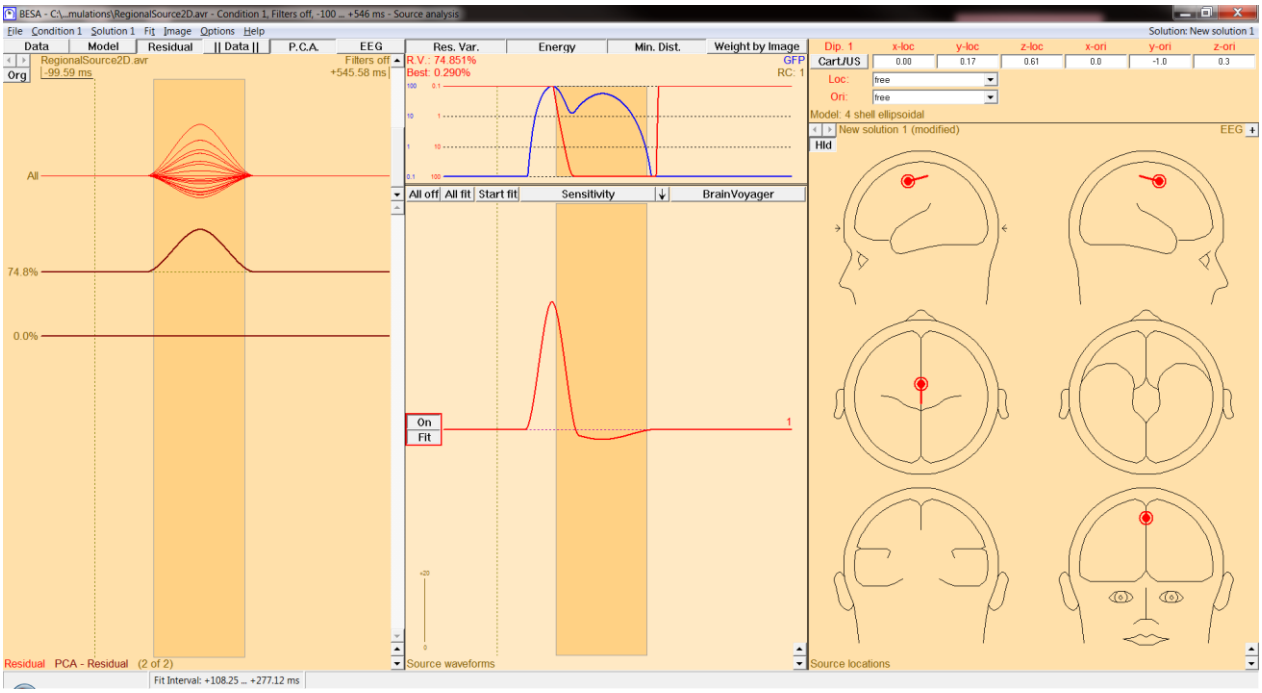
8. We want to apply a *sequential fitting strategy*, i.e. first fit a dipole to represent the earliest brain activity. For that purpose, we should make the fit interval short enough not to include any brain activity from other brain regions that starts at higher latencies. Therefore, the fit interval should be chosen such that **1 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.

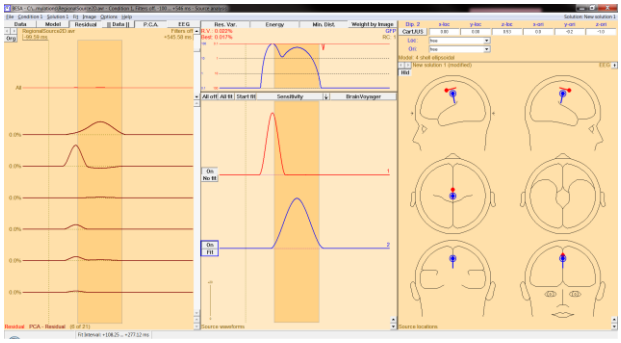


9. Release the **Data** button in the upper left corner to display the residual (i.e. unexplained) activity only. Now **mark a second fit interval** to include the left-over residual variance. Because the data is not displayed anymore, the PCA is computed for the residual activity only. For the second 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 contribution[1].

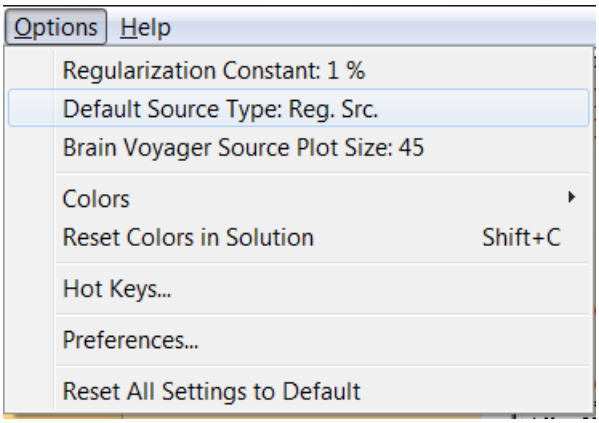
[1] The PCA variances computed for the residual do not sum up to 100%, but rather to the total residual variance in the marked block



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 that. The source waveforms reconstruct the correct time courses, indicating the earlier activation of the SMA as compared to the cingulate gyrus.

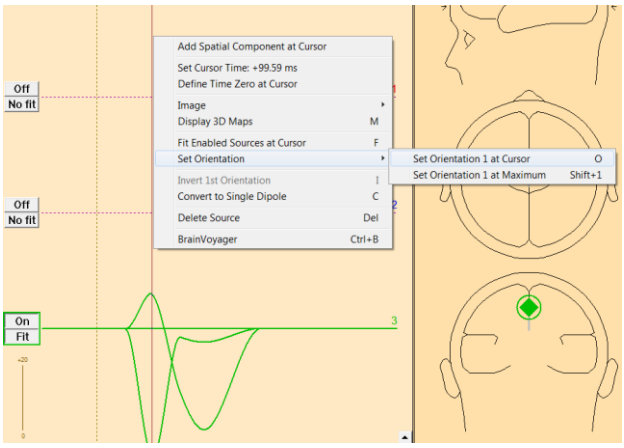


11. Open the **Options** menu. Currently the default source type is set to Dipole (2nd row). Please **click** onto this entry. When you open the Options menu again, the default source type has been set to **Regional Source**. With this setting, a double-click into the heads will automatically add a regional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

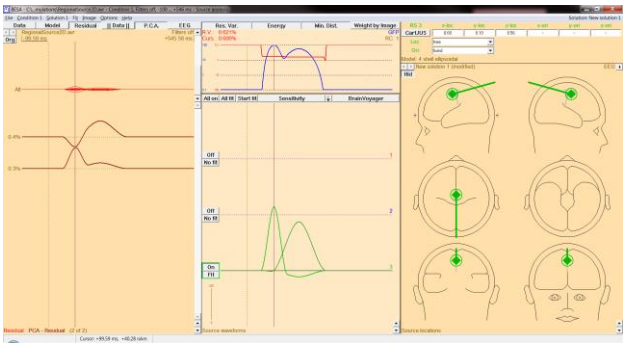


12. To model the two simulated brain regions with a single regional source, **switch off** the two single dipoles in the model. **Double-click** into the heads to insert a regional source. Because it consists of three orthogonal dipoles, the corresponding source waveform consists of three curves, each 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.

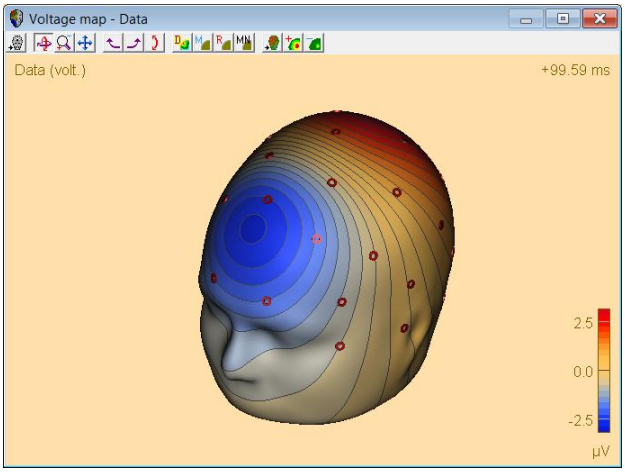
13. The three mutually orthogonal dipoles of a regional source models current flow in any arbitrary direction in a certain brain region. We get exactly the same representation of activity if we rotate the axes from the initial x-, y- and z-direction to new orientations such that the 1st dipole of the 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). **Right-click** and select '**Set Orientation / Set Orientation 1 at Cursor**'.



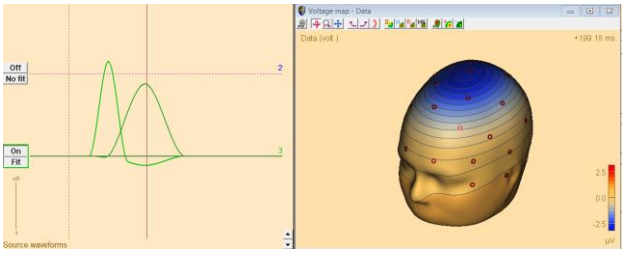
14. This rotates the regional source so that the first of its dipoles points into the direction of current flow at the cursor latency. Note that as a consequence its source waveform picks up all activity at the cursor latency, the other two source waveforms show zero activity at the cursor. The second and third dipole component 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.



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.



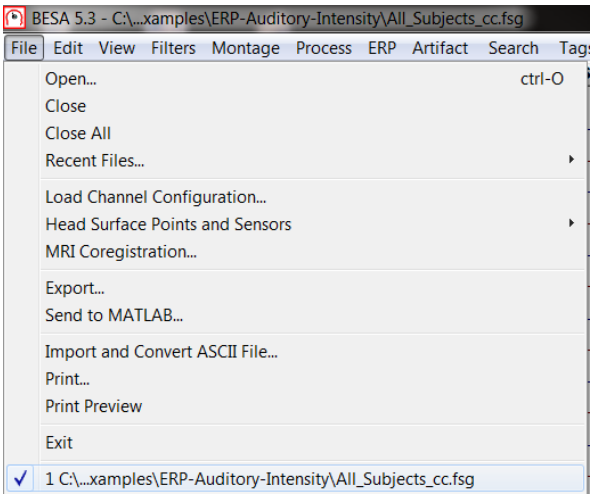
16. Use the **arrow keys** or the mouse to **drag the cursor to the second peak** and observe the radial EEG map generated by the cingulate gyrus with the large central negativity. **Close** the source analysis window without saving the solution.



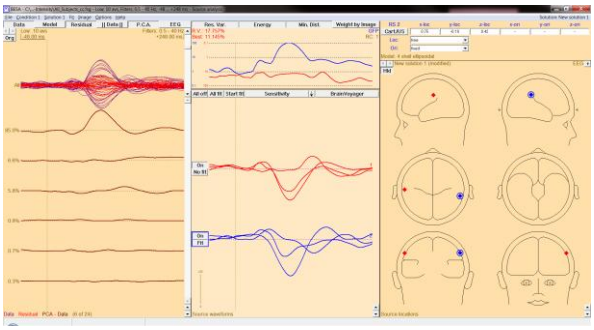
E: Fitting Regional Sources in Real Data

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

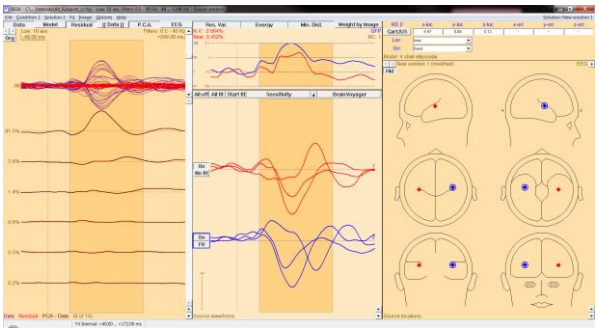
1. We will now return to the dataset '**All_Subjects_cc-Test.fsg**', which should still be available at the bottom of the File menu. If it is not, please select it pressing '**File / Recent Files / …**'. **Left-drag** a block in condition '**Low**', **right-click** to send it into **Source Analysis** 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, zero-phase**.



2. Press the **PCA** button to display a principal component decomposition of the data. 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.

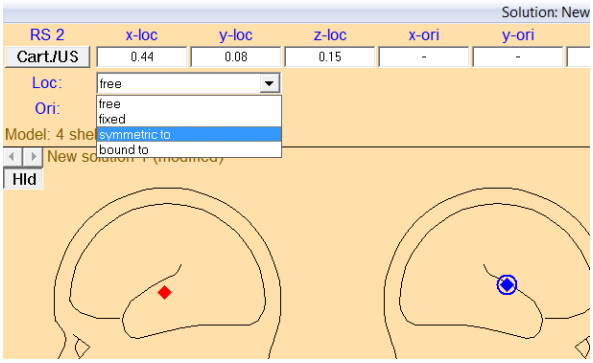


3. As regional sources can explain activity in up to three brain regions at the same time we can expect the two sources to model the three main components in the auditory cortex area (P50, N100 and N150). Therefore, we will not set a short fit interval comprising only one component, but rather mark a fit interval over the activity range of these three AEP components. **Drag** the cursor over the source waveforms to mark the

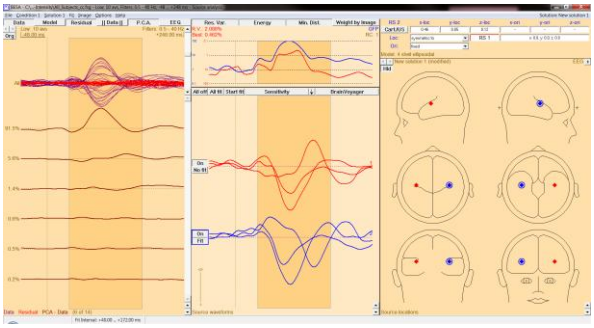


interval from **40 ms to 172 ms**. You may set the interval by **right clicking** and selecting '**Set fit interval**'. Press '**All Fit**' and '**Start fit**' to fit both sources simultaneously. Use the arrow buttons in the source waveform box to **adjust waveform scaling**.

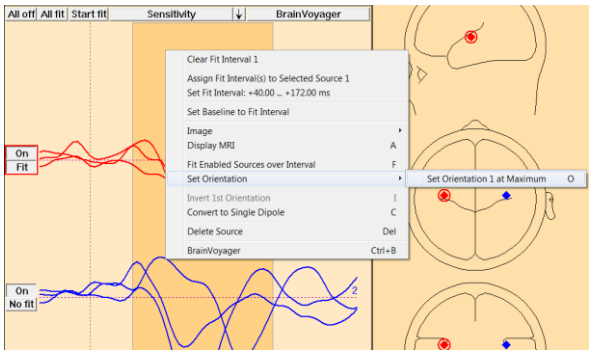
4. The sources are quite symmetric with a slightly more anterior source in the right hemisphere. As stability might be much worse in individual data, we will impose a symmetry constraint on the source location again. Activate the second source by clicking onto its waveform. In the upper right box, select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



5. 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.



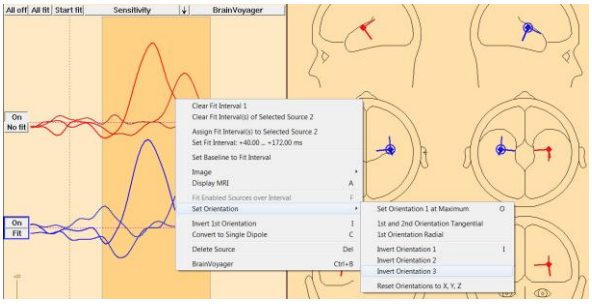
6. We want to identify the direction of current flow of the N100 component. To do so, activate source one by clicking onto the source. **Right click** and select '**Set orientation / Set orientation 1 at Maximum**'. This rotates the source such that the peak around 100 ms is now fully



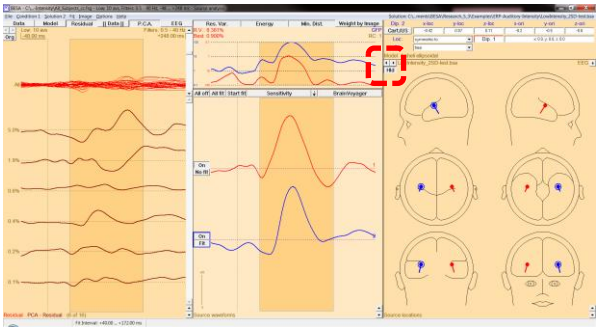
accounted for by the first component (source waveforms for orientations 2 and 3 are now zero at this latency).

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**'.

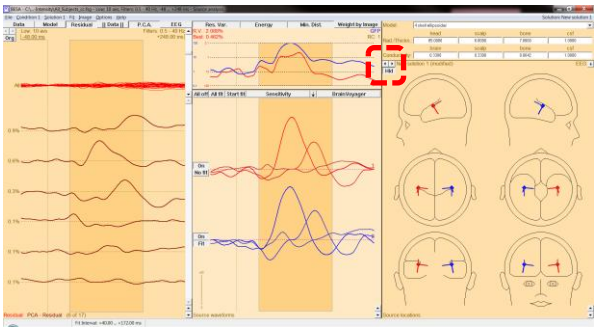
7. Next we will orient the second source as described in step D6. **Right click** onto its waveform and select '**Set Orientation / Invert Orientation 3**' to make orientations of source one and two consistent. (This 2-source model has been saved in file 'LowIntensity_2RS.bsa'. You may load this file from the menu entry '**File / Open Solution**'.)



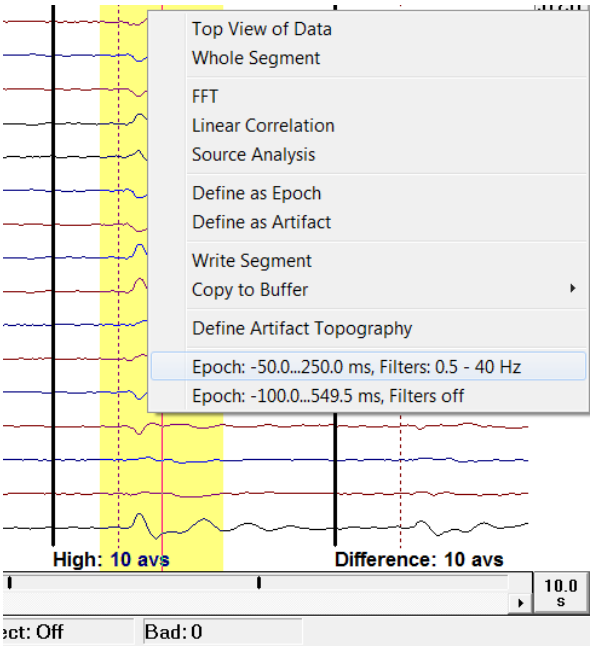
8. Let us compare the solution using regional sources with the solution using single dipoles we saved earlier on. Please press '**File / Open Solution**' and select '**LowIntensity_2SD-test.bsa**'. Release the **Data** button to only display the residual variance. You can toggle



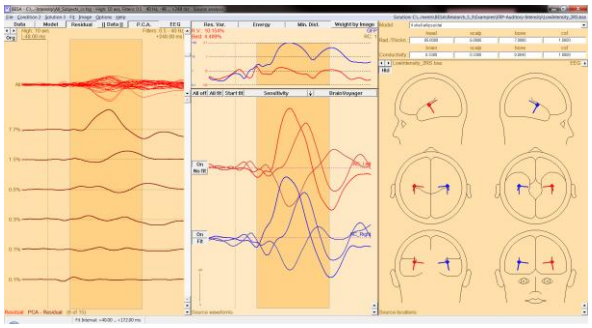
between the two solutions using the arrow buttons in the top left corner of the head scheme field. Note again that the single dipole solution does not explain the P50 and N150 components, while the regional source solution explains all activity from the temporal region very well.



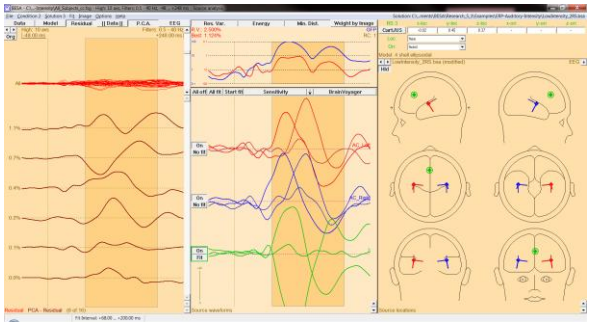
9. Make sure you switch back to the regional source solution and minimize the source analysis window (don't close it) to return to the average data in the main window. Select condition '**High**' and send it to source analysis with the same settings as the 'Low' condition by **left-dragging a block**,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



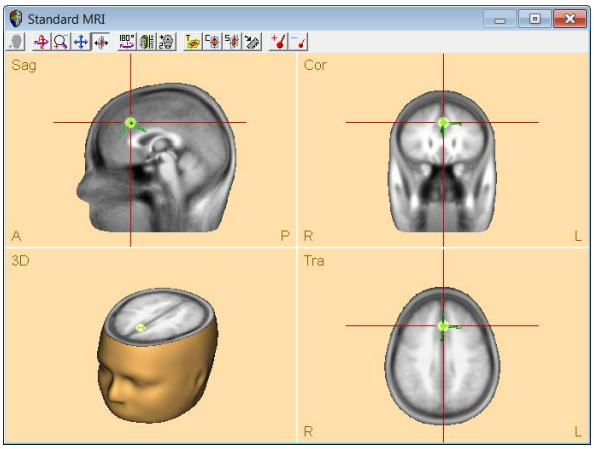
10. If the **Data** button is still released and the **Residual** button is pressed we can immediately see that the source solution that explained the data in the Low condition very well is not satisfactory in the High condition.



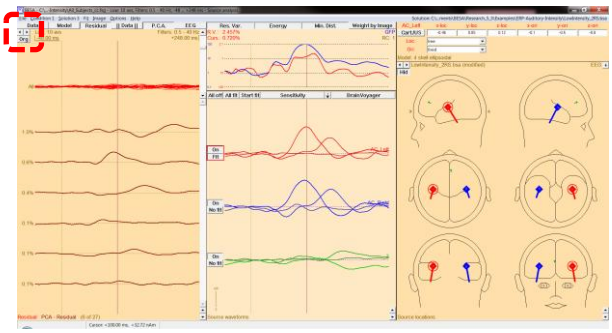
11. Mark a fit interval from 68 to 200ms that covers the unexplained activity. **Double-click** into the head schemes to place a third regional source. Press '**Start Fit**'.



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 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'.)



F: Conclusions

General guidelines for source analysis

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

1. 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 evolution.

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 3cm between each other to prevent crosstalk of their source waveforms.
6. 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).
    - 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.
    - The 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:

- 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-to-noise 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)

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
  - Image Data
  - Time-Frequency / Coherence Results

In the previous step, 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.

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 of the 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)

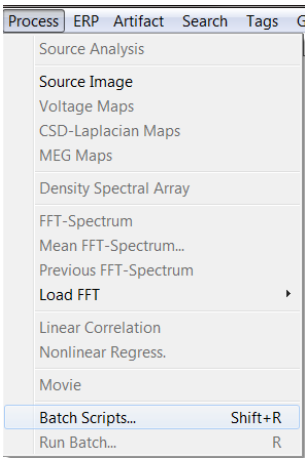
The orientation of the different components (N100, N150 and frontal source) differs considerably between subjects according to individual gyral anatomy and functional representation. Since orientation differences have a larger effect on the EEG scalp topography than small changes in location (in the 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).

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.

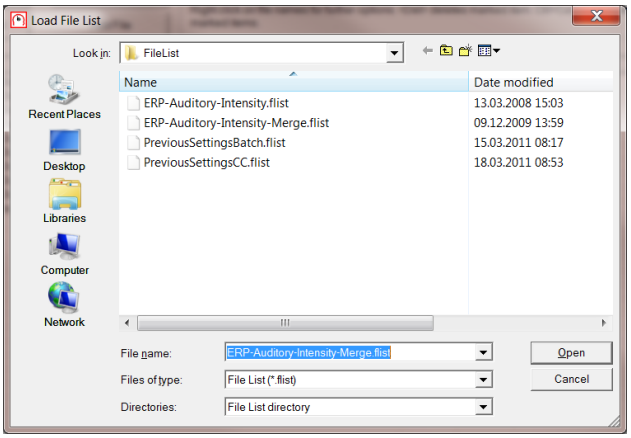
We could apply all required steps manually to each individual data file. This is not necessary, however, and it saves a lot of time to use the source analysis batch functions. In the following, we will create a script that performs all steps automatically.

We will then use BESA Statistics to quickly and conveniently analyze our source waveform results across all 10 subjects.

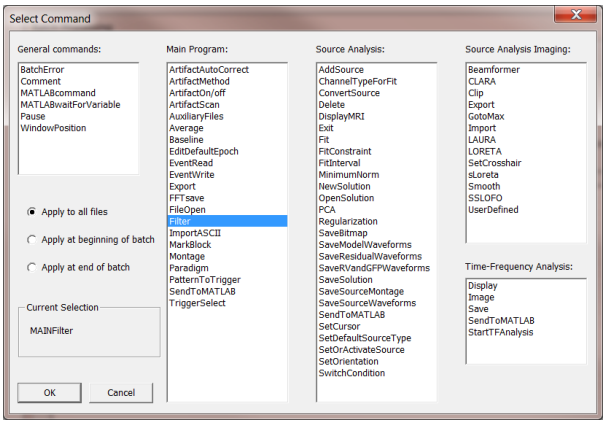
1. Select '**Process / Batch Scripts**' in the main window to open the Batch processing window. We will now create a batch that applies the grand average master model to all individual datasets, orients the regional sources individually and exports the individual source waveforms for further statistical analysis.

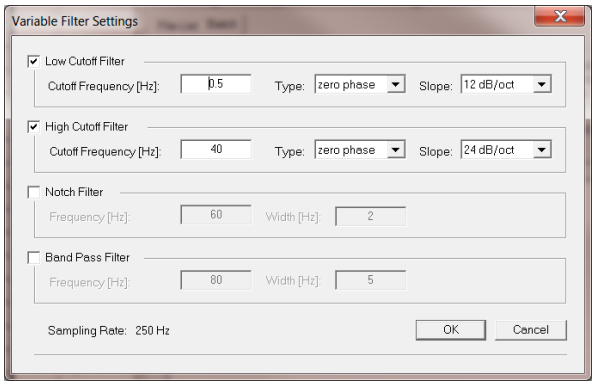


2. In the '**File List**' tab, click on '**Load File List**', select '**ERP-Auditory-Intensity-Merge.flist**' and press '**Open'**. This file list has already been used in Tutorial 3 Step B to create the grand average. It specifies individual average files of all subjects.

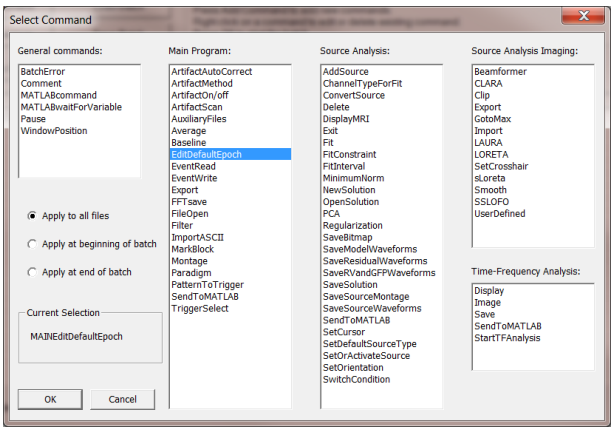


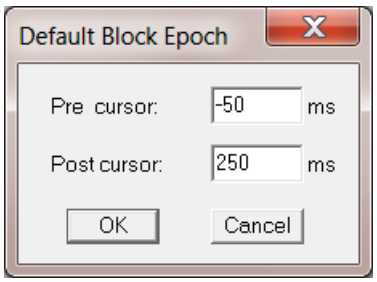
3. Switch to the '**Batch**' tab. As a first command in this batch, apply appropriate filters to each data set. Press **Add Command** and select **Filter** (Main Programs group)**.** Select a **low cutoff** filter of **0.5 Hz, zero-phase, 12 dB/oct**, and a high cutoff filter of **40 Hz, zero-phase, 24 dB/oct**. Click '**OK**' twice to confirm these filter settings.



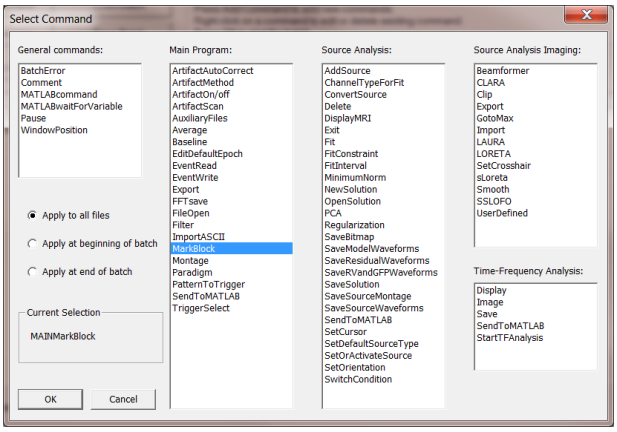


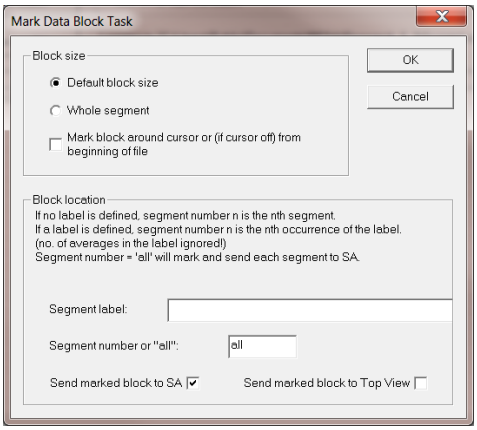
4. As a next command, specify the epoch that will be sent into the source analysis window to extract the source waveforms. Press '**Add Command**' and select '**EditDefaultEpoch**' (Main Programs group)**.** Specify an interval from **-50 to 250 ms**.



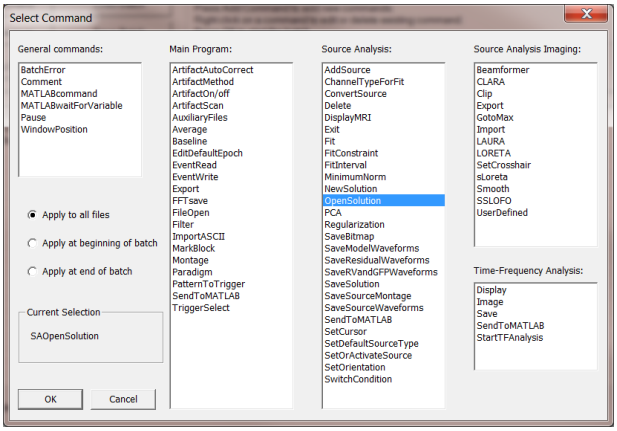


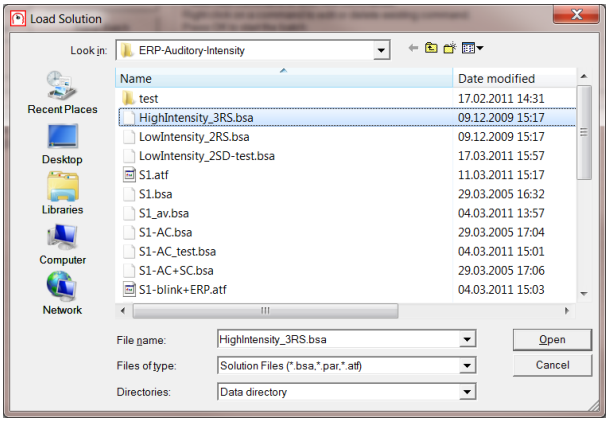
5. The next command marks the default block in all 8 conditions, and sends these data blocks into the source analysis window. Press '**Add Command**' and select '**MarkBlock**' (Main Programs group). In the upcoming window, select **Default block size.** Instead of specifying a single segment label or 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.



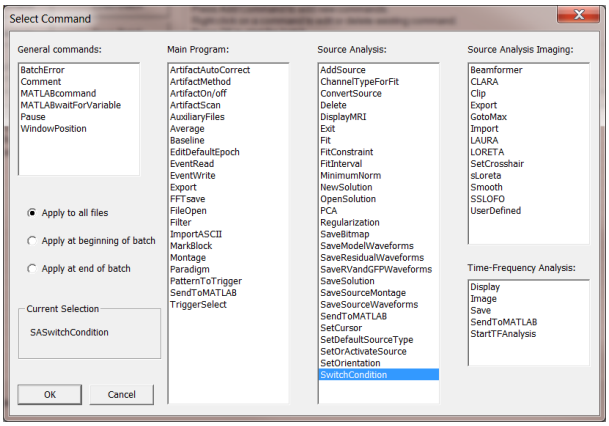


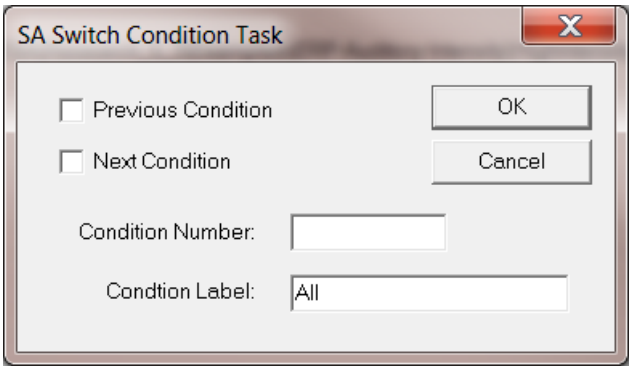
6. Now apply the master model that was generated from the grand average. Press '**Add Command**' and select '**SAopenSolution**' (Source Analysis group). Select file '**HighIntensity_3RS.bsa**' that contains the two regional sources in the auditory cortex and the frontal source. Press '**Open**' and '**OK**'.



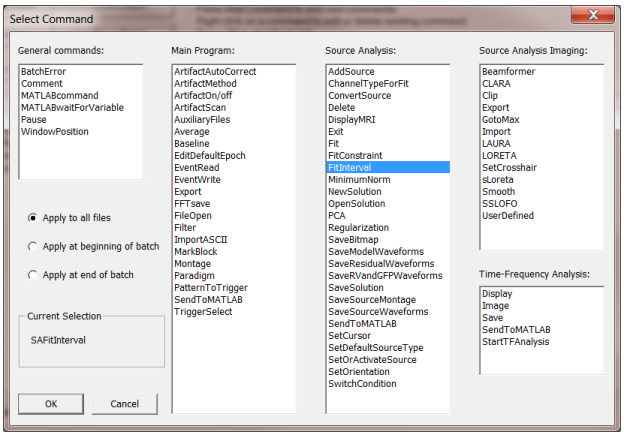


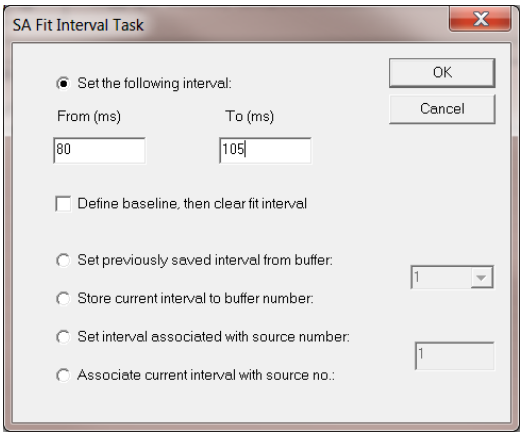
7. To obtain correct source waveforms for each individual subject, adjust the master model to each subject's cortical folding by adjusting the orientations of the regional sources. First, we will reorient the auditory cortex sources in condition 'All' that contains the average of all five conditions. Press '**Add Command**' and select '**SAswitchCondition**' (Source Analysis group). As Condition Label, enter '**All'**. Press '**OK**'.





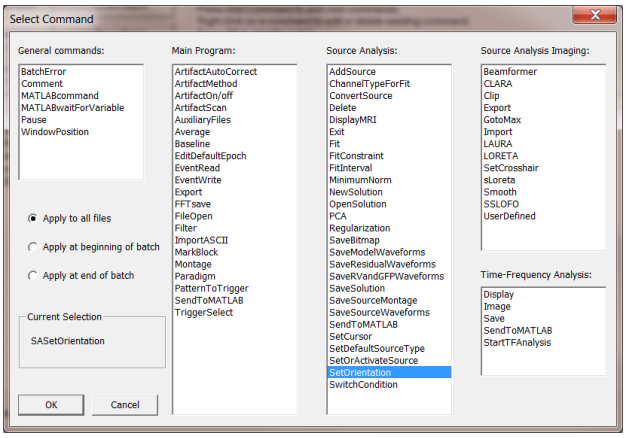
8. To adjust the N100 orientation, we need to specify the corresponding latency. Since N100 latencies will differ slightly from subject to subject, we define a time range rather than a fixed latency. Press '**Add Command**' and select '**FitInterval**' (Source Analysis group). Specify a latency range of **80 to 105 ms.** Leave all other options unchanged and hit '**OK**'.

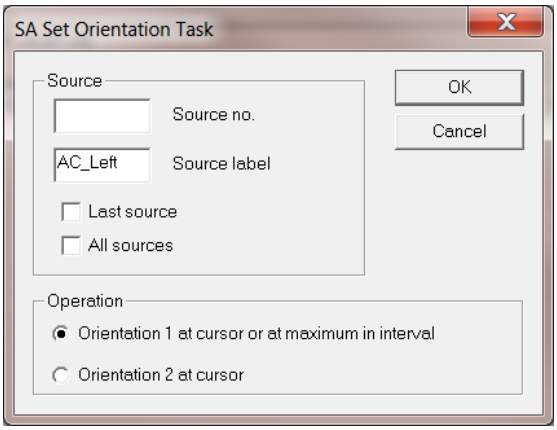




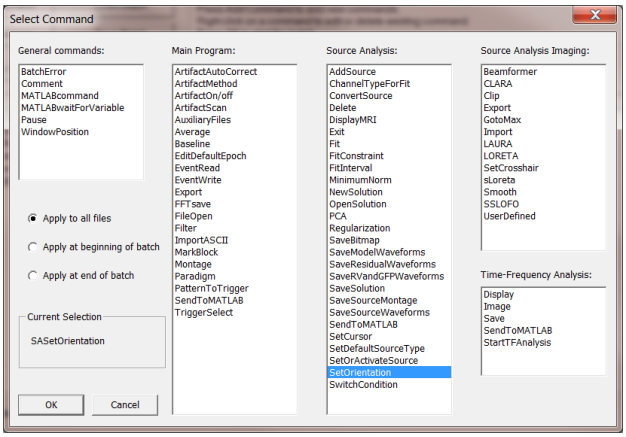
Note: At this point in the batch, we could insert a Pause command (General group). It would cause the batch to interrupt at this stage and allow us to modify the marked fit interval to match each subject's data before it continues on our OK. In this tutorial we pass this step, but generally it is recommended to keep an eye on what the batch is doing when working with your own data.

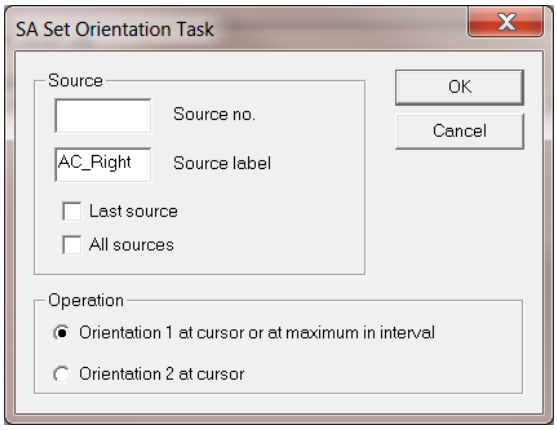
9. Now the first and second regional source in the model (representing the bilateral auditory cortices) can be oriented. '**Add Command**' and select '**SAsetOrientation**' (Source Analysis group). In the upcoming parameter window, specify '**Source label**' **'AC_Left**'. As operation select '**Orientation 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**'.



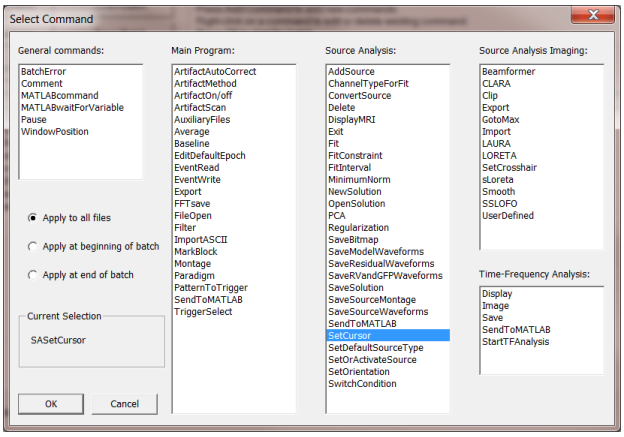


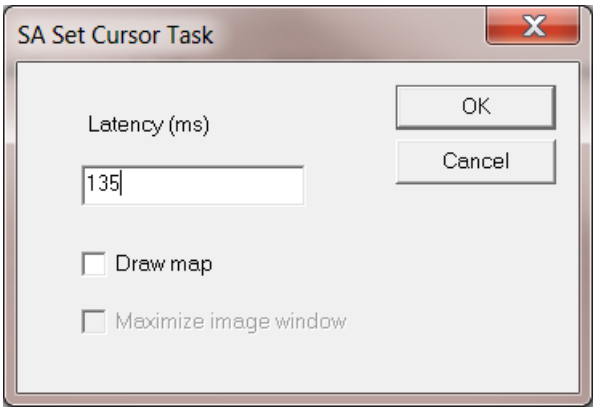
10. Repeat the previous command '**SAsetOrientation**' with '**Source label**' '**AC_Right**', the auditory cortex source in the other hemisphere.





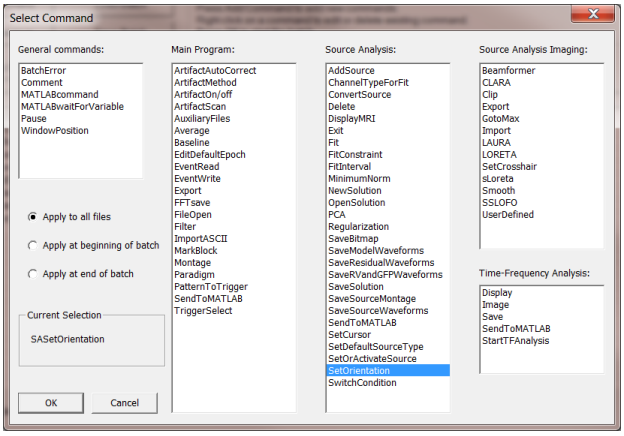
11. To set the second orientation to account for the N140 component, press '**Add Command**', select '**SAsetCursor**' (Source Analysis group) and specify a latency of **135 ms**.

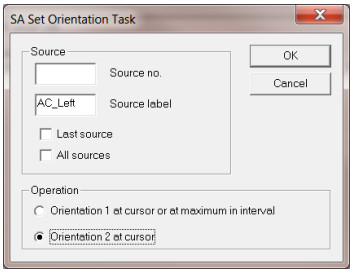


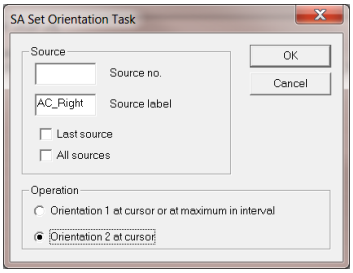


Note: Again, at this stage it could be advisable to insert a Pause command (General group) to adjust the cursor position to the individual N140 latency, if required, before the batch continues.

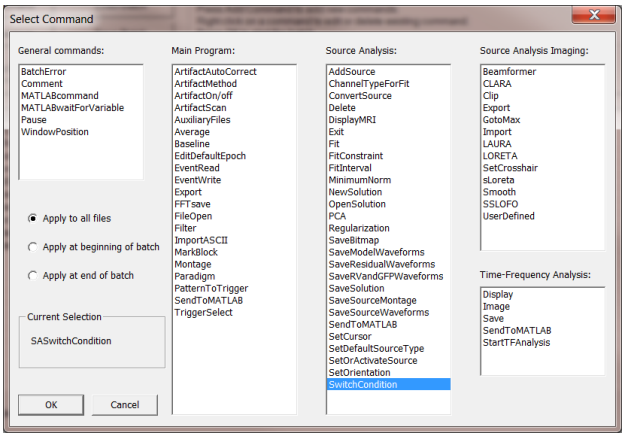
12. As in steps 10 and 11, add the command '**SAsetOrienation**' twice. First, specify **Source** '**AC_Left**' and select '**Orientation 2 at cursor**' as operation. Then repeat the command and specify **Source** '**AC_Right**', again with operation '**Orientation 2 at cursor**'.

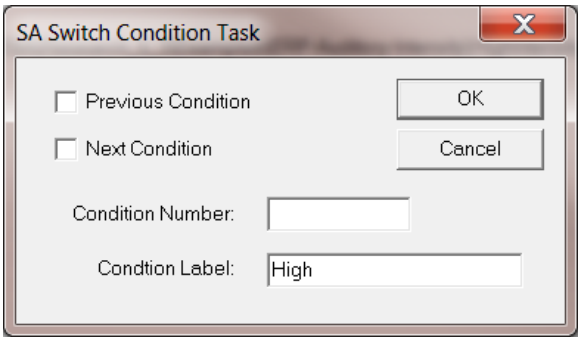




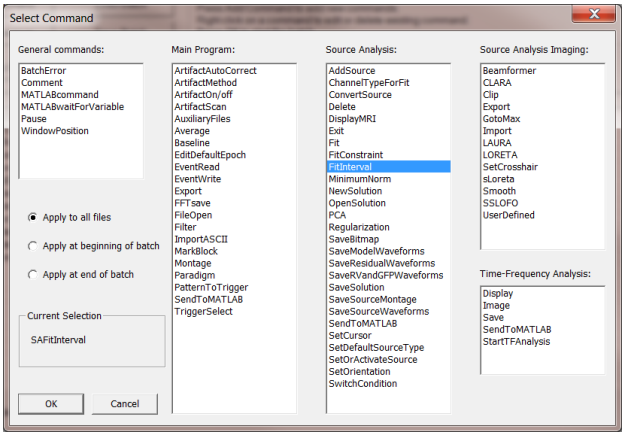


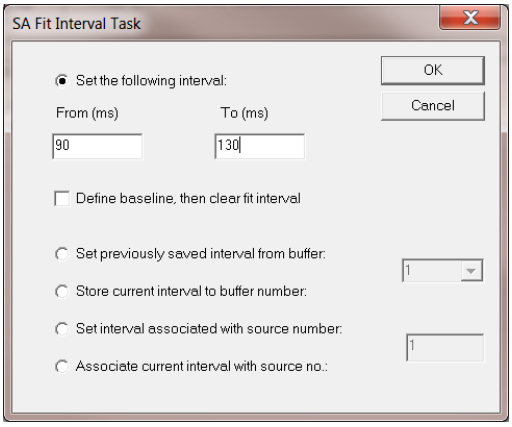
13. Finally we need to set the orientation of the frontal source. Its amplitude is largest in condition 'High'. Therefore, add a command '**SAswitchCondition**' (Source Analysis group) and specify condition label '**High**'.



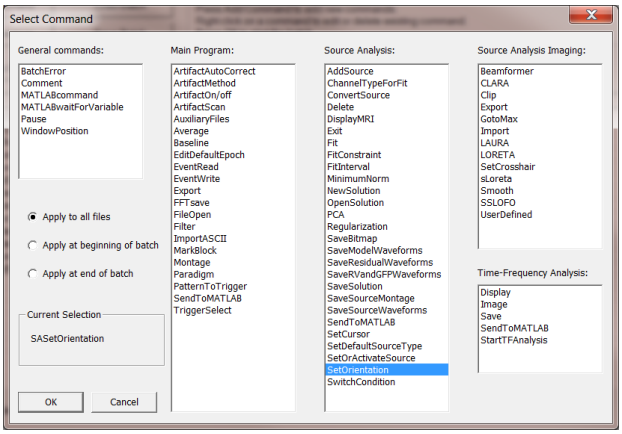


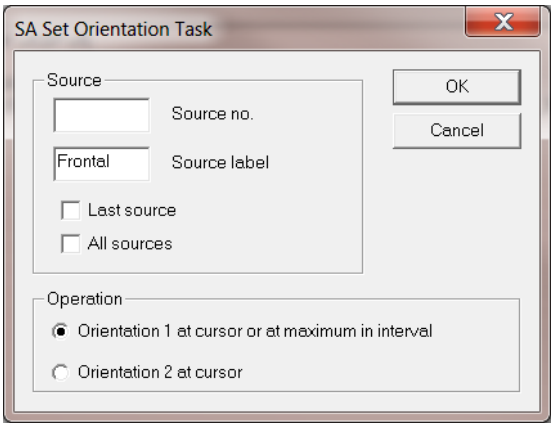
14. To orient the frontal source, we will again mark a fit interval that includes its maximum activity. Add command '**FitInterval**' (Source Analysis group). Specify a latency range of **90 to 130 ms.** Leave all other options unchanged and hit **OK**.



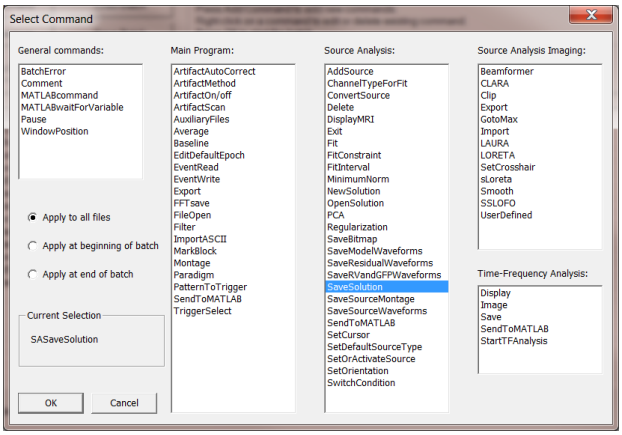


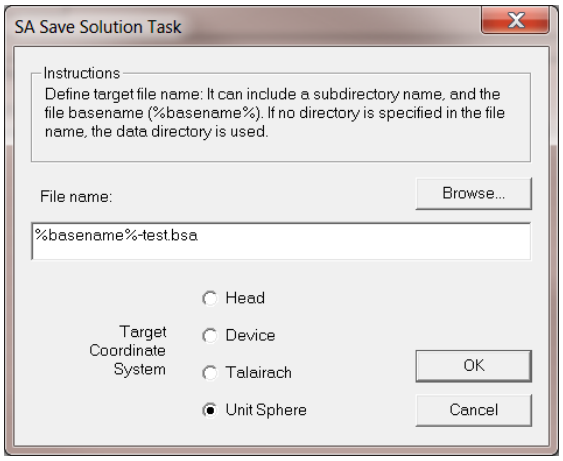
15. Add the command '**SAsetOrienation**'. This time specify source '**Frontal**' and '**Orientation 1 at cursor or at maximum in interval**'. Because no cursor is set anymore, BESA Research orients the source at the latency of maximum source power. This completes the adjustment of the master model to the individual subject's brain gyration.





16. We will now save this model for later re-use. Add the command '**SAsaveSolution**'. 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.

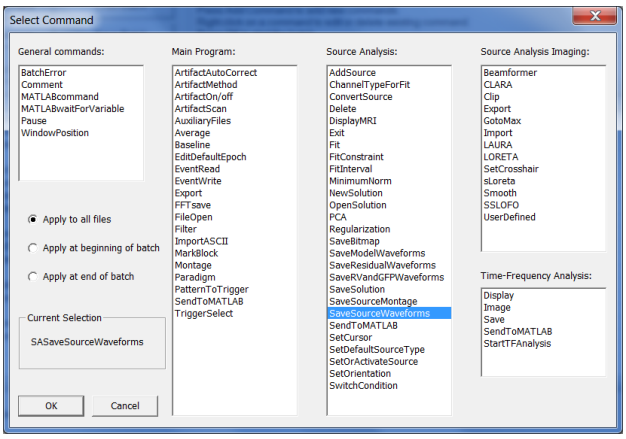


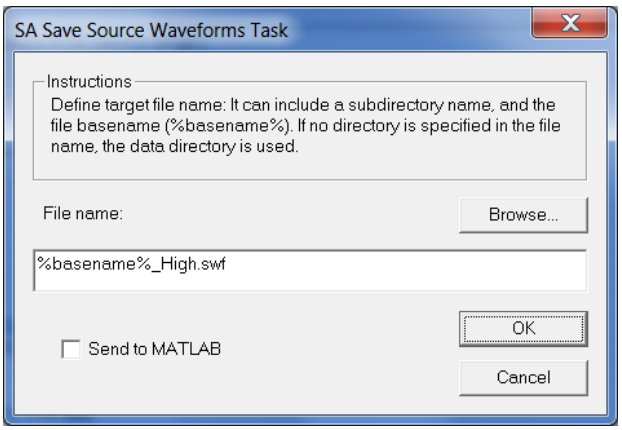


B: Exporting individual source waveforms via batch scripting

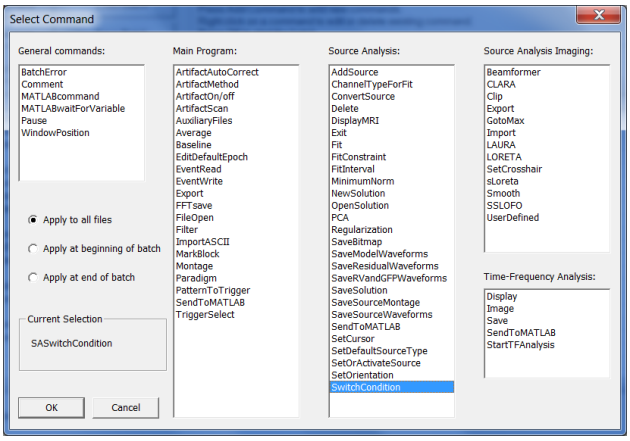
Next we want to save the individual source waveforms of conditions High and Low, which we will later load in BESA Statistics.

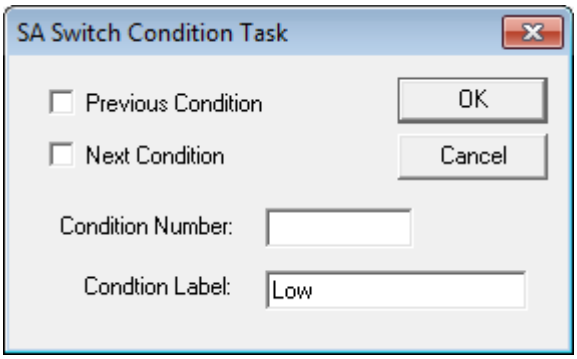
1. Add the command **SaveSourcewaveforms** (Source Analysis group) to save the source waveforms to an ASCII file (*.swf). In the file name include **%basename%** as a placeholder for the datafile, and add the exension “High” in order to be able to associate each *.swf file with the corresponding subject and condition.





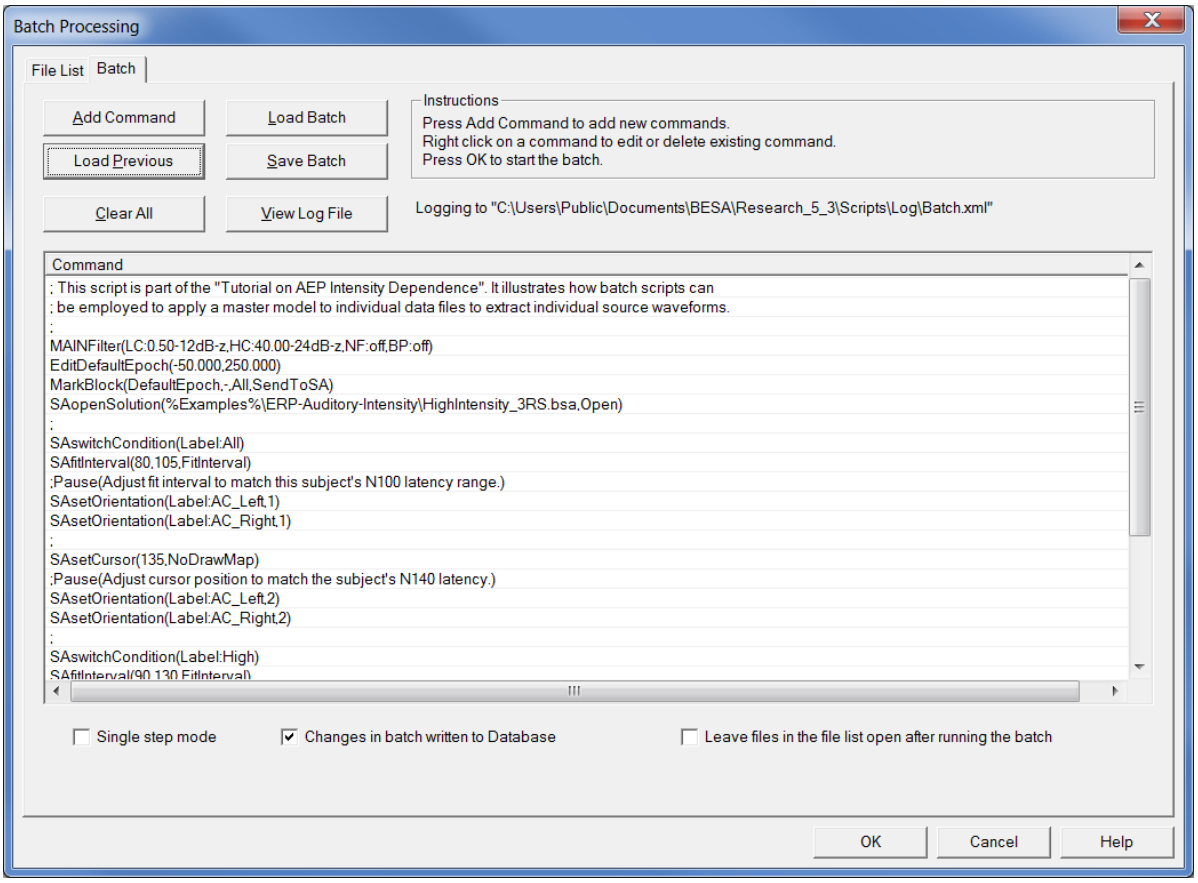
2. Add a command **SAswitchCondition** (Source Analysis group). As Condition Label, enter **Low**. Hit **OK**.





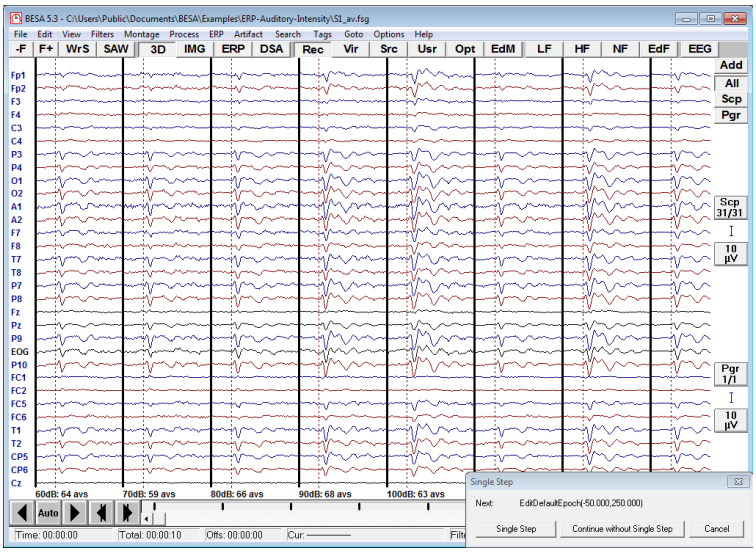
3. Repeat Step B1 for condition “Low”.

4. 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-IndividualWaveforms-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.

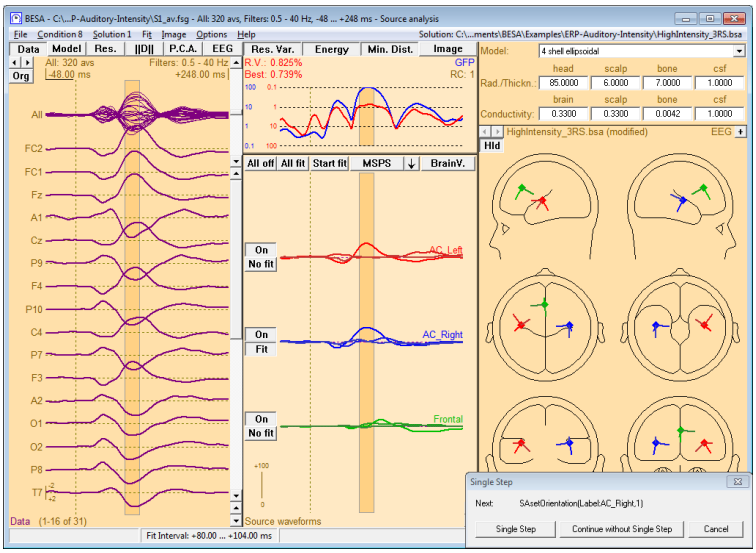


5. Select the option **Single step mode** at the bottom right – this will cause the batch to pause after every single command. This helps us in this tutorial to visually follow the processing steps and understand how the batch works. Press **OK** to start the batch.

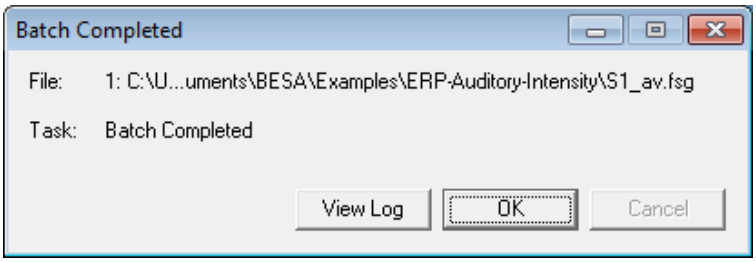
6. BESA Research starts applying the batch commands to the first data file in the file list (S1_av.fsg). It loads the file and applies the specified filters. At the bottom right a small window appears that indicates the next batch command to be executed. Proceed step by step by hitting the **Single Step** button and observe how BESA Research processes the different commands



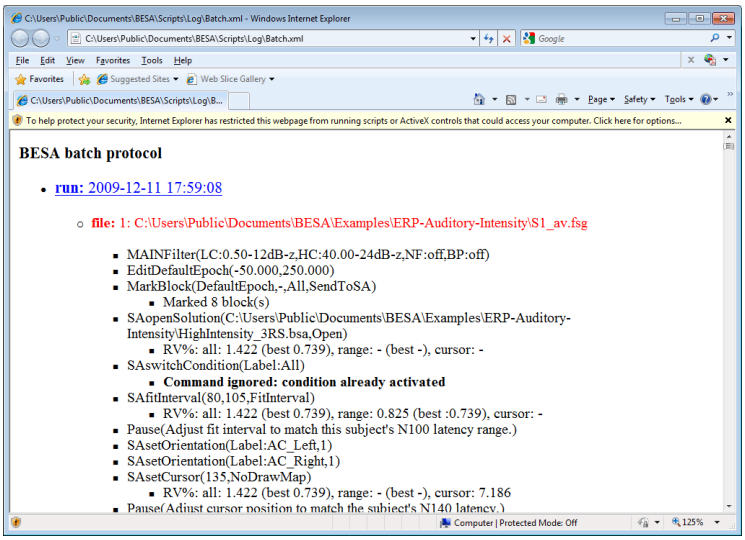
7. Note for example how the orientation of the N100 components (the first components of AC_Left and AC_Right) takes place in the marked fit interval. If the marked fit interval would not include this subject's N100 latency, we could manually adjust the fit interval while tha batch pauses before continuing. 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.



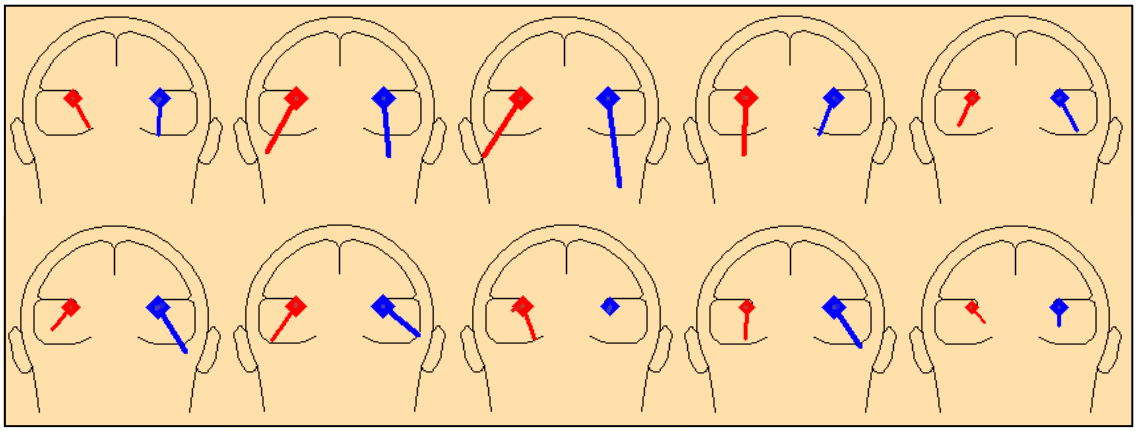
8. When the batch processing has completed, a notification window will appear.



9. 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 inter-subject 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.

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 t-Test (parametric)

As a first step, BESA Statistics calculates a preliminary Student's 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 second group /condition has higher values than the first group / condition, a one-tailed-left t-test should be calculated.

Permutation Test (non-parametric)

When computing a large number of t-tests, the probability of obtaining a significant result by chance is high. Typically, an α-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 (if unpaired t-tests were used) or conditions (if paired t-tests were used) get systematically interchanged. Depending on the number of subjects per group / condition and the type of t-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: 10001), a new t-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 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 C1 C1).

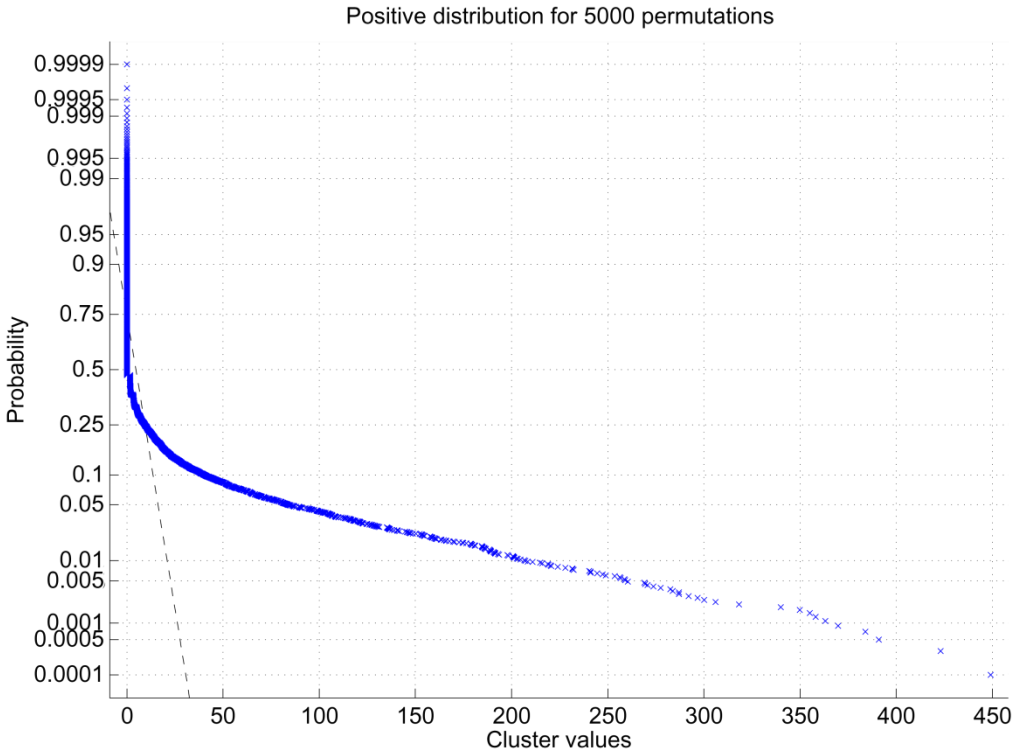


Figure C1: A realistic distribution of cluster values based on 5000 permutations is displayed. The figure indicates that a cluster value of 200 is associated with a probability value of p=0.01, meaning that only 1% of all clusters have values larger than 200.

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

[1] 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.

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.

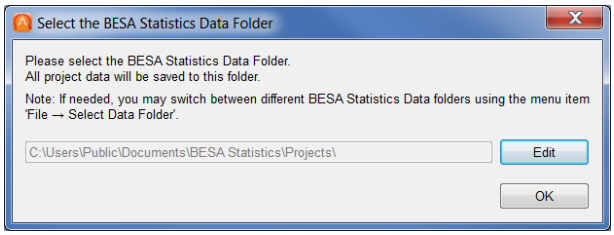
For more details on the implementation of the permutation test as implemented in BESA Statistics please refer to the following publications: (Bullmore, Suckling et al. 1999; Maris and Oostenveld 2007; Ernst 2004)

D: Analyzing source waveforms statistically using BESA Statistics

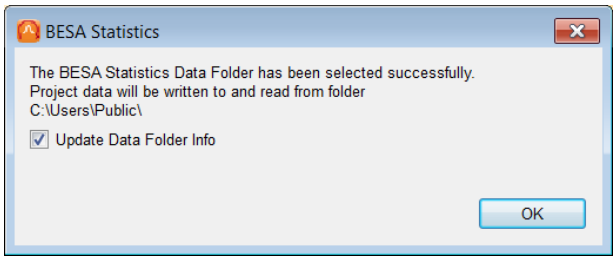
In the following, we will load the source waveforms we created in BESA Research and run a cluster permutation test in order to identify time-regions and sources, which show reliable differences between conditions High and Low across all subjects.

1. Start BESA Statistics by double-clicking its icon on your desktop.

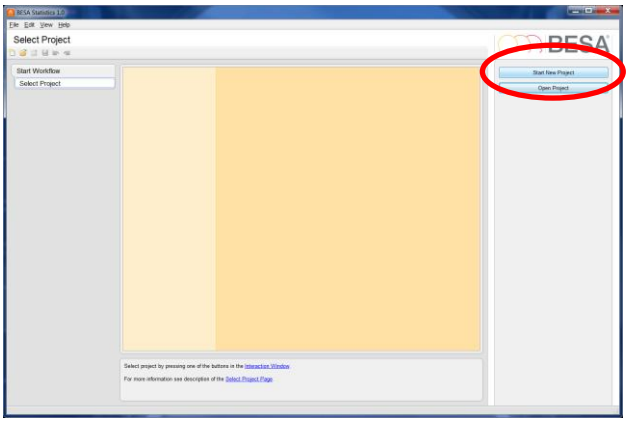
2. When BESA Statistics is opened for the first time, the project folder needs to be defined. This is the folder where BESA Statistics stores and reads all project and result data. A dialog box appears. Press **OK** to confirm the pre-selected folder.



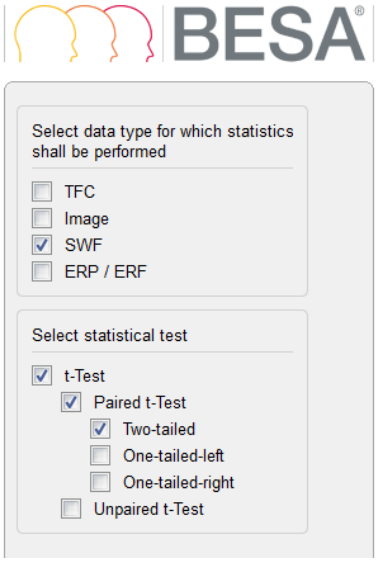
3. Please close the notification box by pressing **OK**. We can now start with setting our project targets.



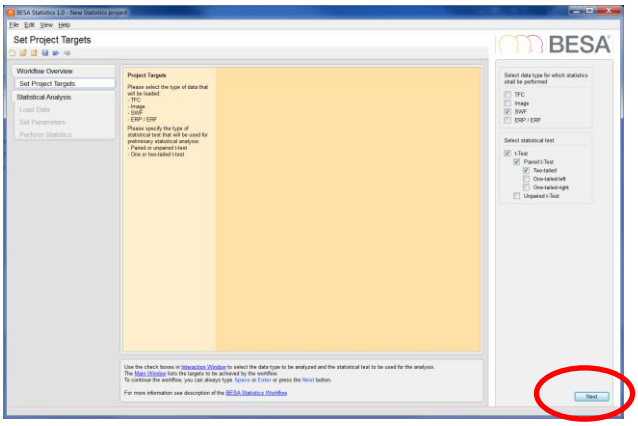
4. Press **Start New Project** in the interaction window on the right-hand side.



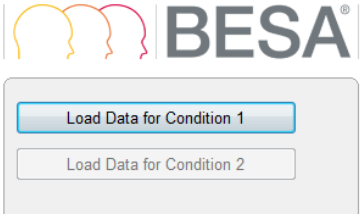
5. As we want to analyze the source waveforms we created in the previous steps, please select SWF in the interaction window. We will use a paired t-test for preliminary statistics as we compare two dependent samples. i.e. two conditions within the same set of subjects. We will use a two-tailed t-test as we do not want to test a specific hypothesis.



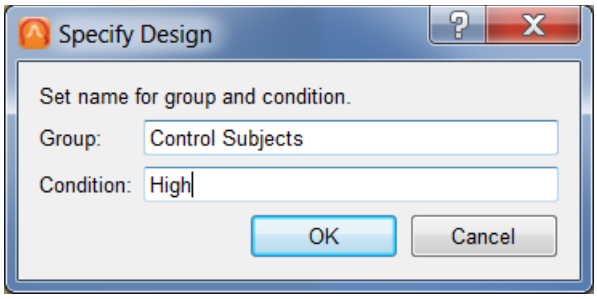
6. Press next or hit the space bar to continue to the next workstep.



7. Press **Load Data for Condition 1** in the interaction window. (Please note: Had we selected a paired t-test, we would be prompted to load data for Group 1.)

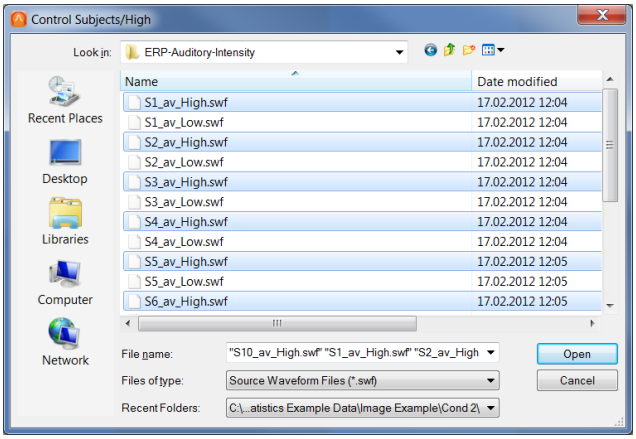


8. Specify the Group name to be “Control Subjects” and the Condition name to be “High” and press **OK**.

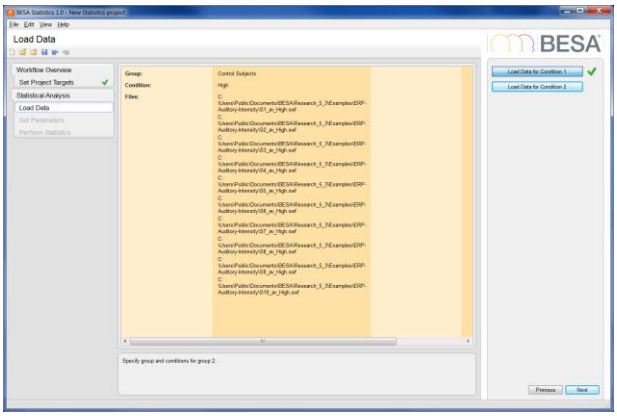


9. Please browse to the folder C:\Users\Public\Documents\BESA\Research_5_3\Examples\ERP-Auditory-Intensity and select the 10 swf-files we previously created for condition “High”. Press **Open**.

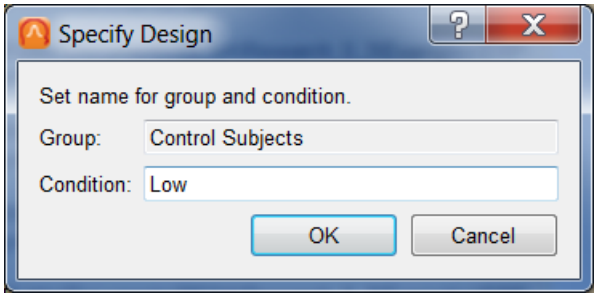
   Note: Depending on the data selection under Set Project Targets, Files of Type will be automatically set to the expected file format.



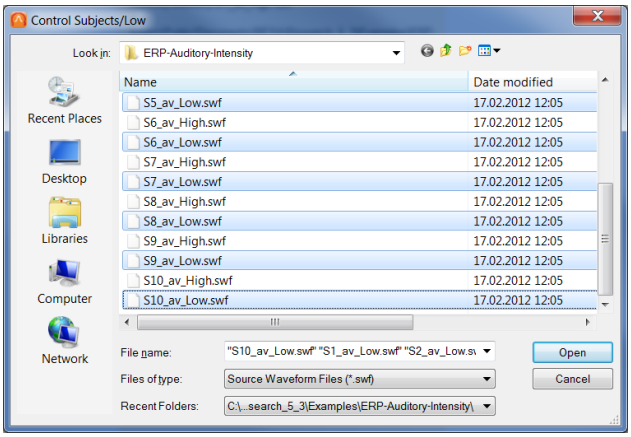
10. A summary of the files we selected will be displayed in the main window and a green tick-mark will appear next to the first load-button. Press **Load Data for condition 2** (or press Next or hit the space bar).



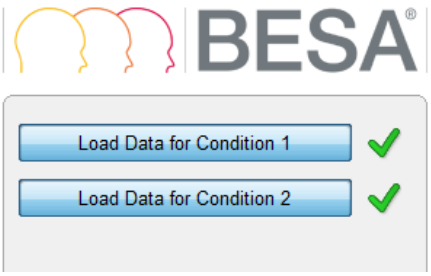
11. Specify the second Condition name to be "Low". Note that Group cannot be edited anymore, as the paired t-test expects two different conditions the **same** set of subjects. Press **OK**.



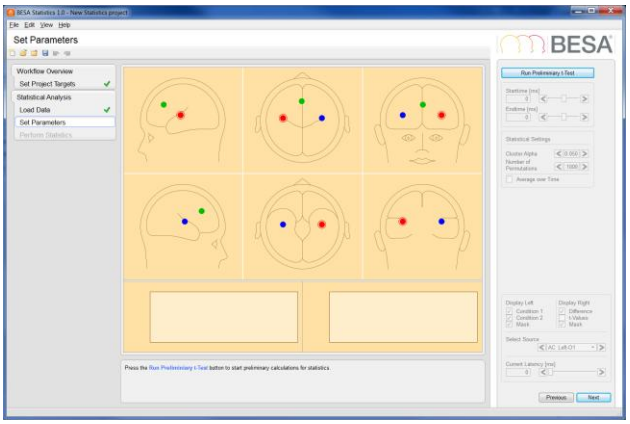
12. Now select all 10 swf-files with the extension "_Low" and press **Open**. **Important note:** If a paired t-test was selected under Set Project Targets, the number of files loaded in both conditions needs to be **identical**. This is not the case for paired t-tests.

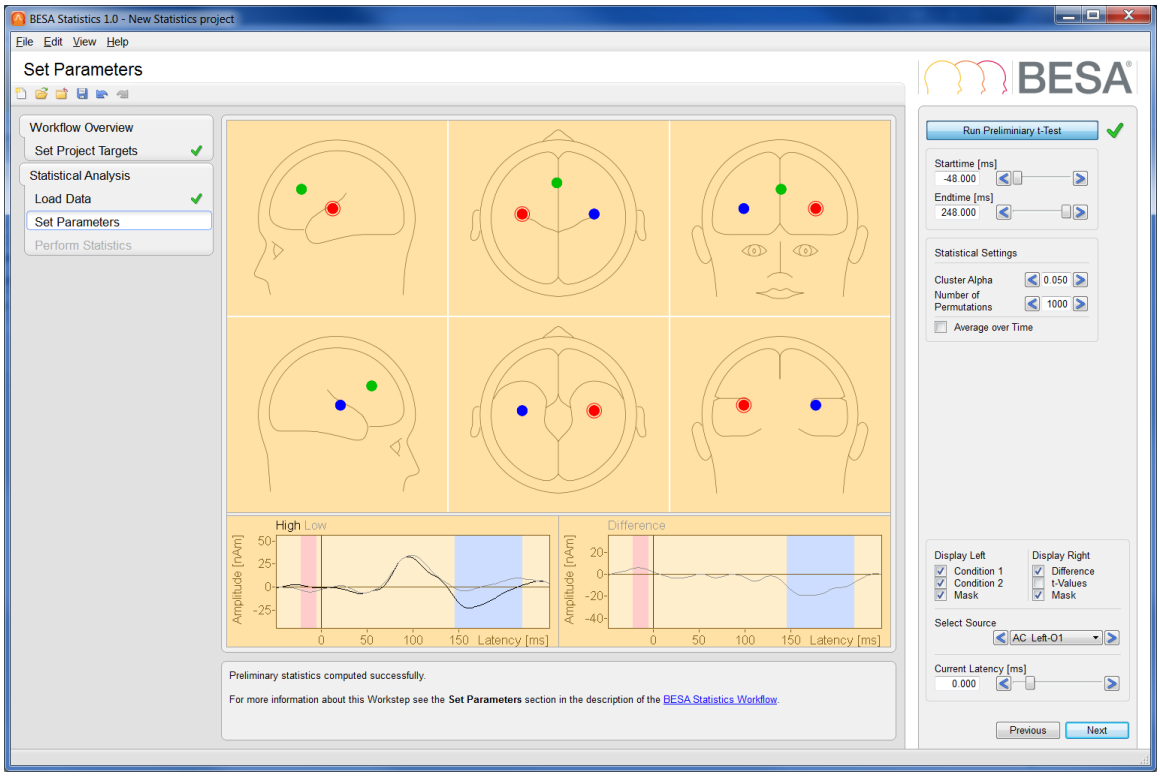


13. Again, a summary of all files loaded for the second condition will be displayed in the main window and a green tick-mark will appear next to the second load-button. Press **Next** or hit the space bar to continue.



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 Statistics** or **Next** to see point-wise t-test results.

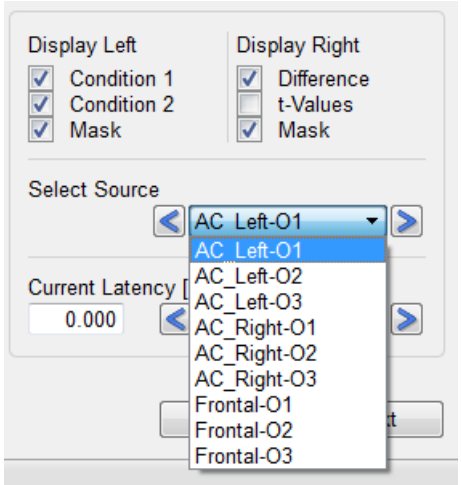




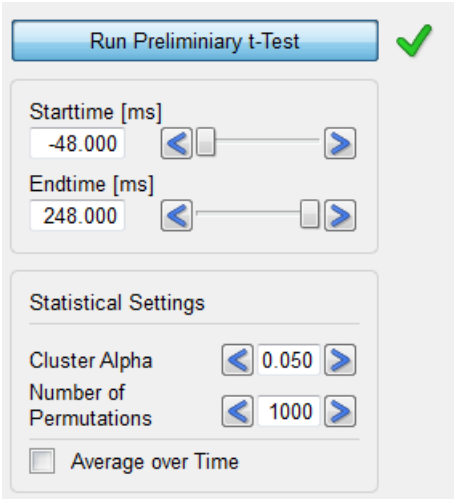
15. Results for the first orientation of the first source in the source model (AC_Left) are displayed in the detail windows. It appears that “Low” has smaller amplitudes than “High” in the baseline, and “High” has smaller amplitudes then “Low” over an extended time-range starting roughly at 150 ms.

Important note: These are preliminary results that are not corrected for multiple comparisons! They should not be interpreted. The only serve as an initial orientation for what’s in the data and for defining regions of interest.

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 t-values (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.



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 data-points. For example, one might only include the post-stimulus period in the calculations. We will not restrict the time-window now but see what happens to the cluster in the baseline after permutation.

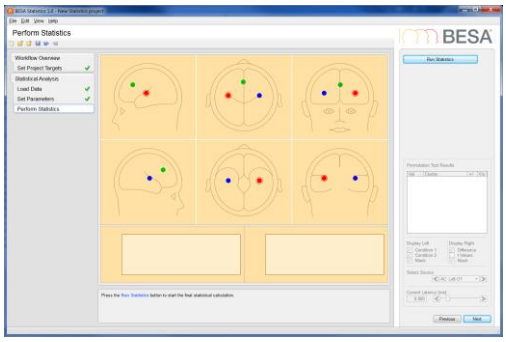


Under the section „Statistical Settings“, a Cluster Alpha value needs to be set. This value refers to the significance level that is the threshold for a data-point to enter a cluster. The default value – which we will use now – is p=0.05. However, it can also be a value larger or smaller than 0.05, larger p-values leading to larger clusters. **Please note that the Cluster Alpha value does not equal the significance level of the permutation statistic!**

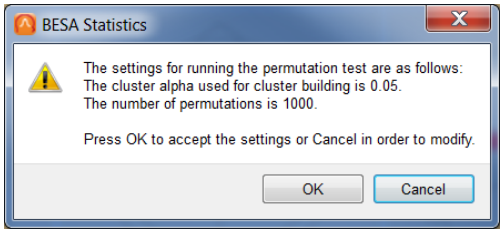
The number of permutations also needs to be set. The default level – which we will be using – is 1000 in order to get a reliable estimate for a significance level of p=0.05, i.e. if the test is run multiple times, the same clusters will stay significant. The possible number of permutations is restricted by the number of subjects and the type of preliminary t-test (paired 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.

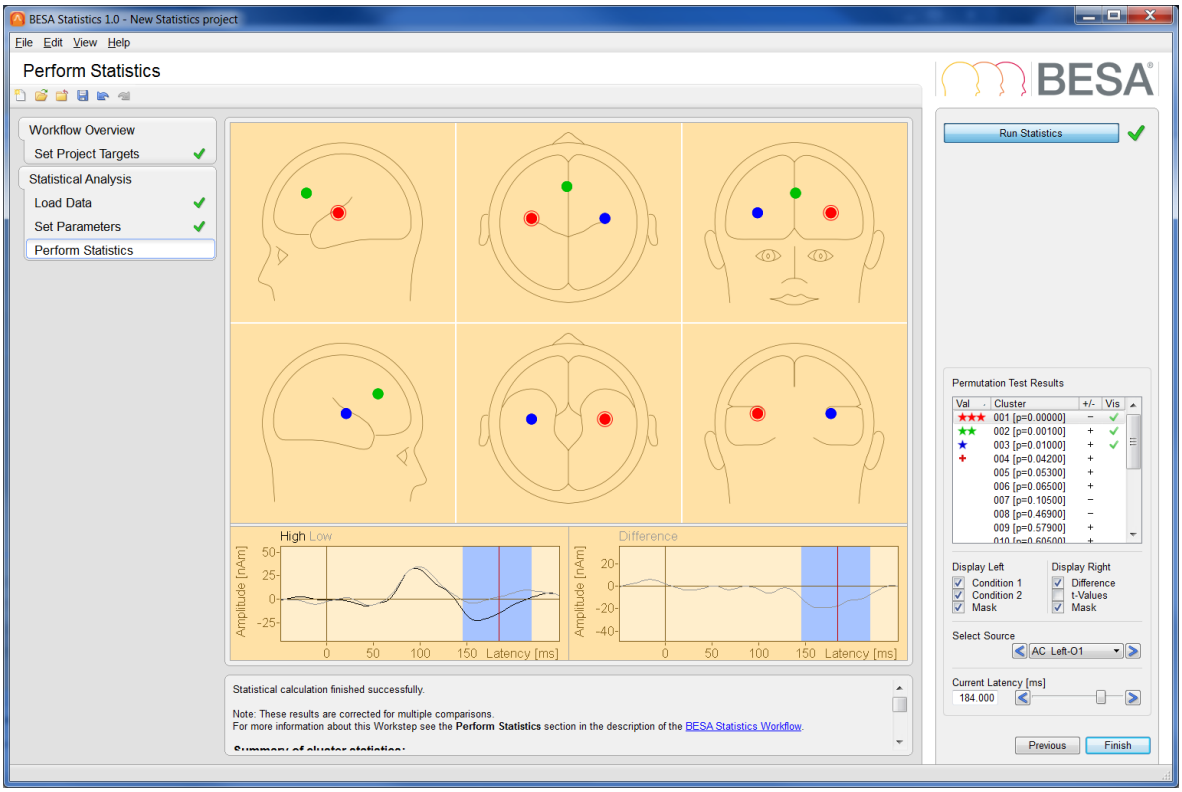
Press **Next** or hit the space bar to continue.

18. Press **Run Statistics** to start the permutation process and all according calculations necessary for determining the probability of our initial clusters.



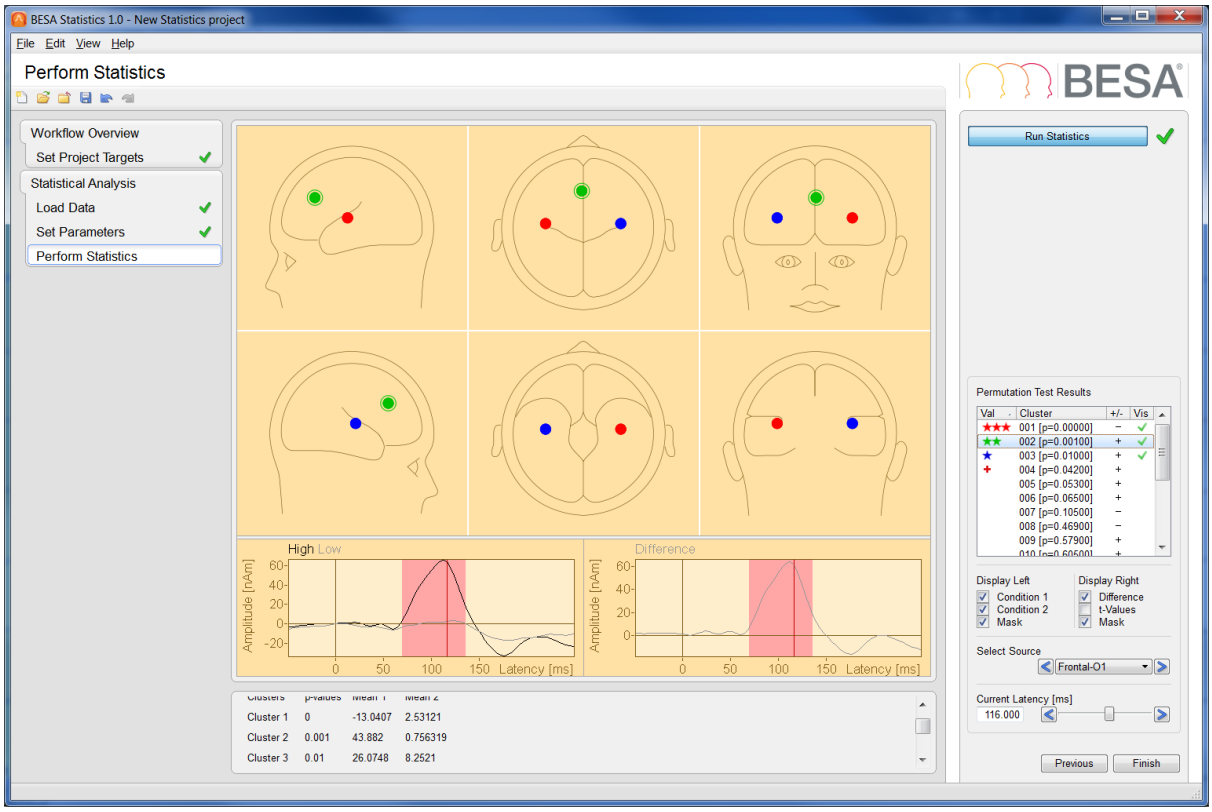
19. An information window appears summarizing the settings for the cluster permutation test. Press **OK** to continue. Please note that the following calculations can be time-consuming depending on the type of data and the number of data-points.



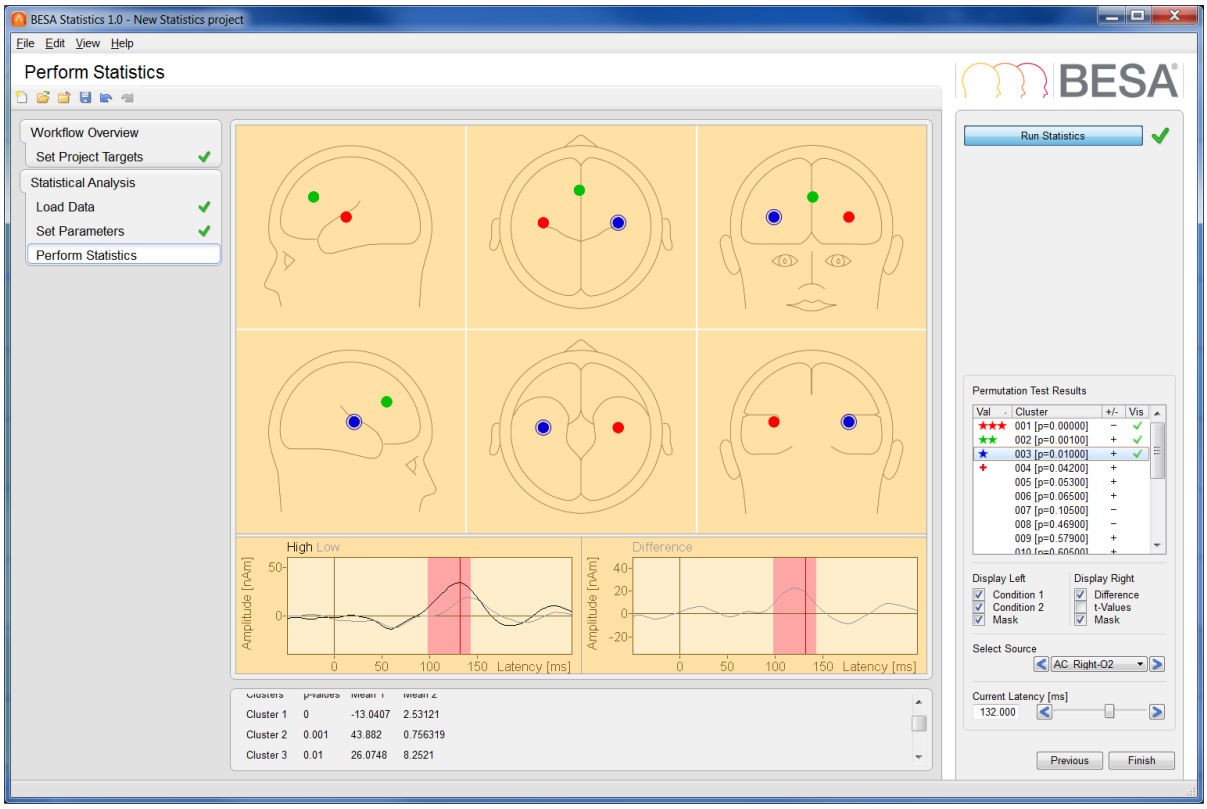


20. Once permutation statistics are computed, the cluster with the largest cluster value (and smallest p-value) is automatically selected and displayed. Up to 100 clusters are displayed in the interaction window and significant clusters are marked with stars according to their significance level. Clusters 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!

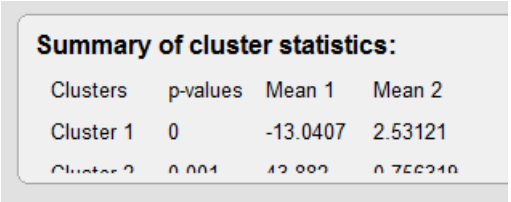


21. The second significant cluster is found in the first orientation of the frontal source. “High” leads to larger amplitudes than “Low”. This effect starts at 68 ms and ends at 136 ms.



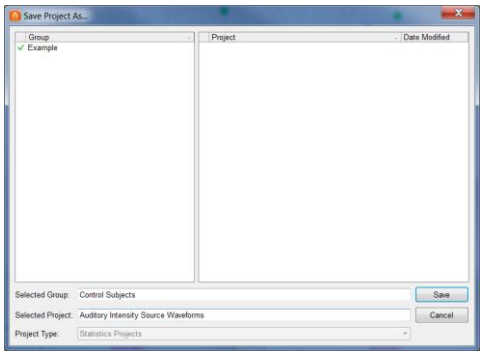
22. The last significant cluster can be found in the second orientation of AC_Right, i.e. the auditory cortex region associated with radial activity. Again, “High” has larger amplitudes than “Low” from 100 ms to 144 ms.

23. A summary of cluster statistics containing the cluster p-values and the condition means are displayed[2] in the information window below the main window.



[2] With the release of BESA Statistics 1.1 in spring 2012 it will be possible to export all statistical results as well as group/condition and individual means as csv-files. Likewise, it will be possible to export all images as png or eps-files.

24. Press **Finish** to end and save the current project. Save it under an appropriate name.



What does BESA Research provide?

- ✓ Coregistration with and without individually digitized electrode positions
- ✓ Direct integration with BESA MRI
  - Loading MRIs in DICOM[1] format
  - Guided definition of landmarks for AC/PC and Talairach transformation
  - Automatic inhomogeneity correction
  - Automatic segmentation of skin and cortex
- ✓ Display of individual cortex and skin in source analysis window

[1] The next release of BESA MRI in May 2012 will also allow Nifti and Analyze data format. A major new feature of BESA MRI 2.0 will be the creation of individual head models with FEM that can be used as volume conductors for source analysis.

A: Introduction

Coregistration of EEG and MRI data allows mapping a source position to an actual cortical area. Without coregistration 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 coregistered 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 commisure) 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.

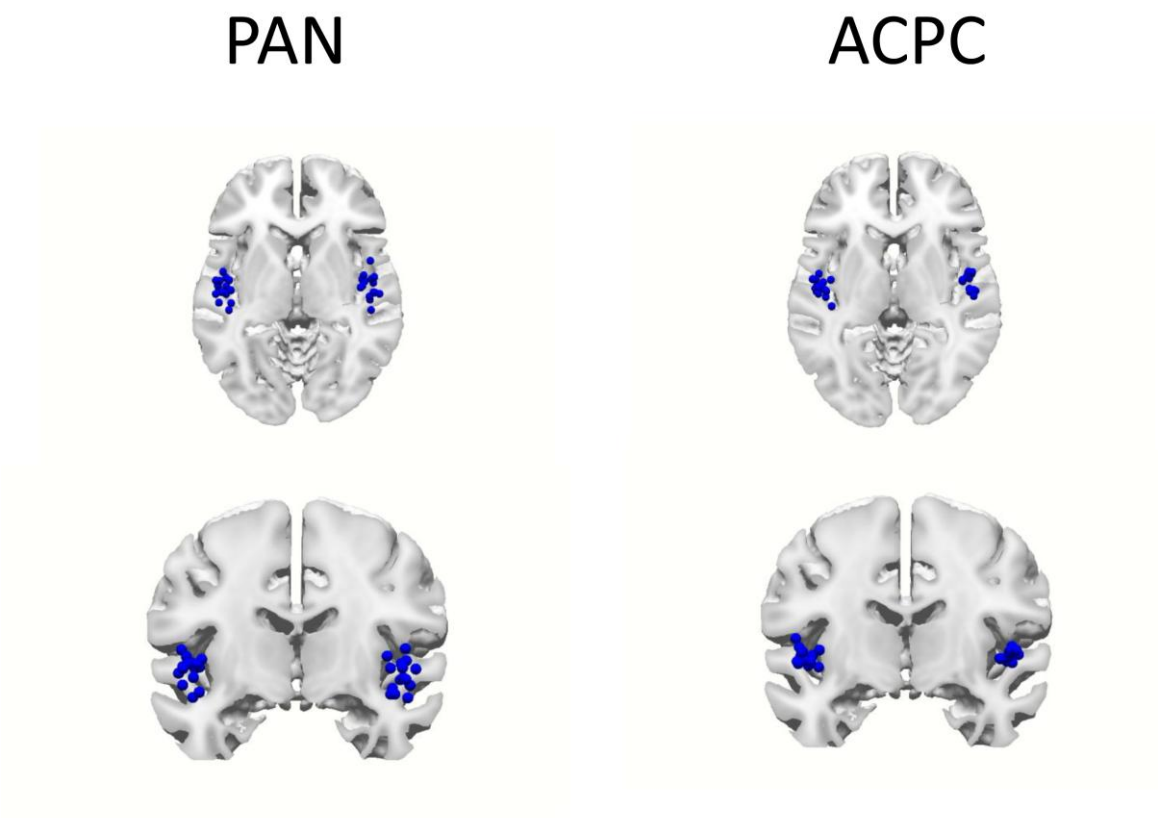
Using the same standard coordinate space is *essential* when source localizations are to be compared across several subjects

a. between two groups of subjects (e.g. patients vs. control subjects).

b. at two measurement time-points within the same group of subjects (e.g. pre vs. post treatment, test vs. retest).

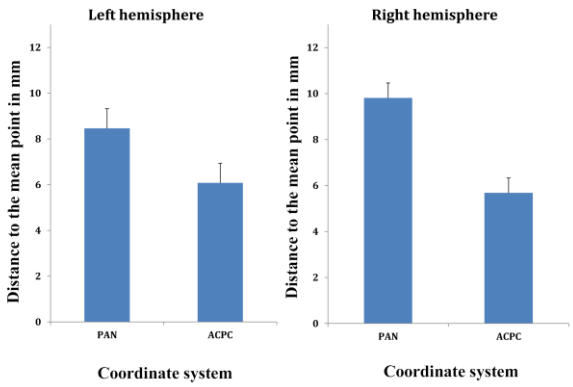
Ad a. Anatomy can vary considerably between subjects and a different x-y-z localization in two people can still be in the same anatomical region. Not taking individual anatomy into account when constructing the coordinate system would disregard this fact.

Ad b. 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.



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.

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.

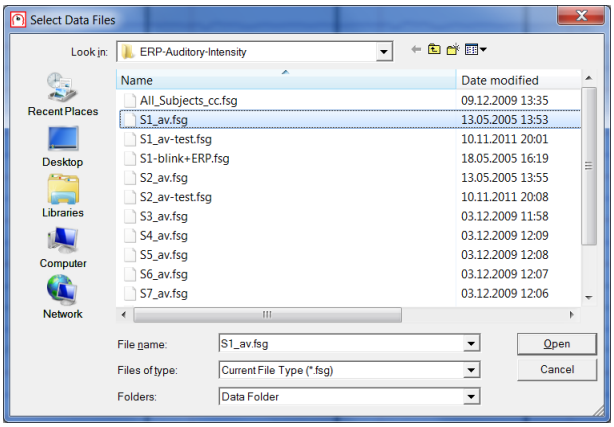


Usually, good coregistration depends on digitized electrode positions in order to precisely fit the measured electrodes to the skin of the subject (as segmented from the MRI). Using BESA Research and BESA MRI it is possible to coregister EEG with MRI data even if no individual electrode positions were 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 coregistration purposes only (not for any signal analysis). This procedure makes coregistration much more precise than only relying on fiducial positions.

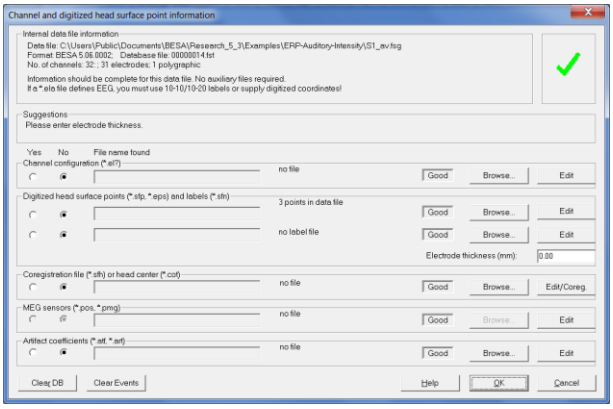
B: Starting the coregistration of EEG and MRI data using BESA Research and BESA MRI

In the following, we will coregister 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.

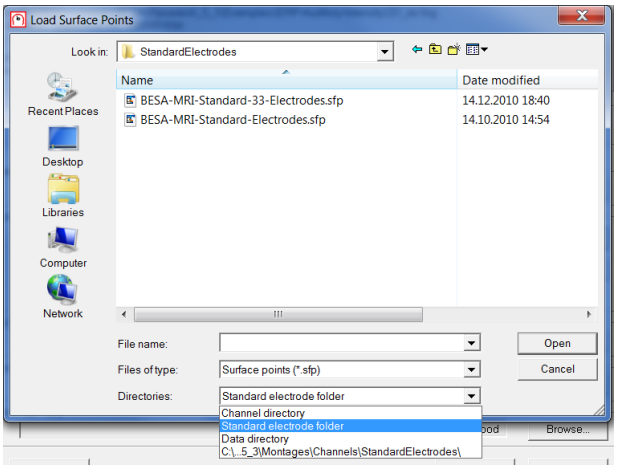
1. In BESA Research please **open** dataset **S1_av.fsg** (located here: C:\Users\Public\Documents\BESA\Research_5_3\Examples\ERP-Auditory-Intensity). Press **File / Head Surface Points and Sensors / Load Coordinate Files** (or press ctrl-L).



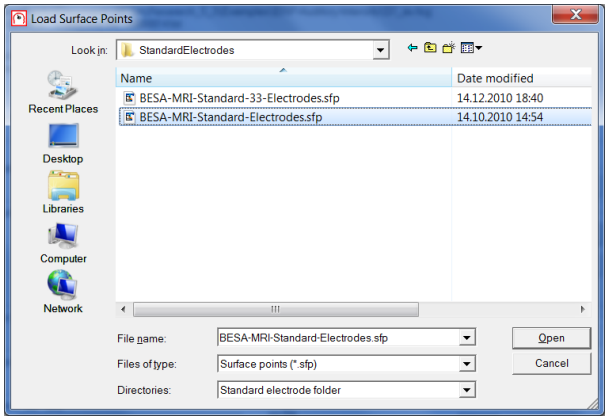
2. In the section "Digitized head surface points" press **Browse**.



3. Under “Directories” please select the option **Standard electrode folder**.

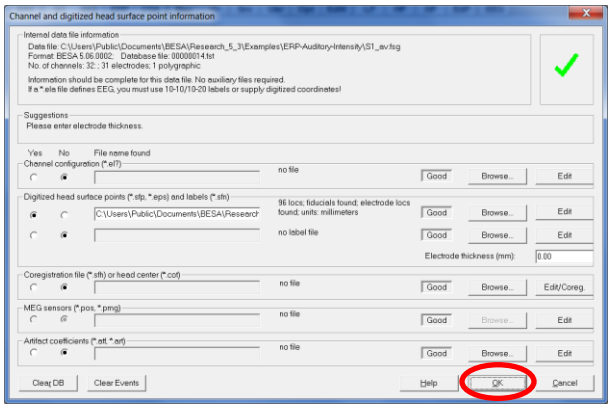


Select the file **BESA-MRI-Standard-Electrodes.sfp** and press **Open**. It contains 96 standard electrode positions. Some of the S1’s electrode names match the labels in this standard electrode file so BESA can match them.

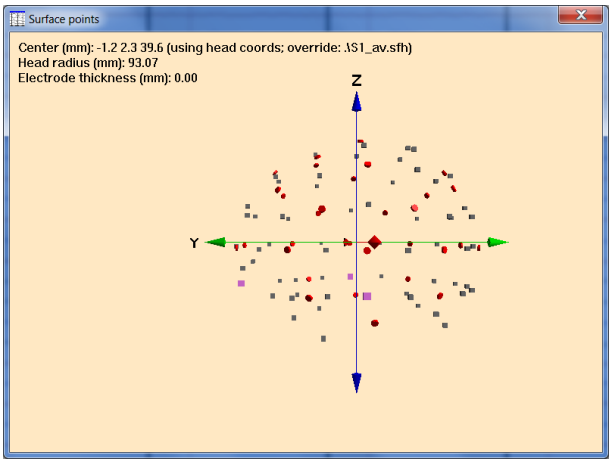


The other file (BESA_MRI_Standard-33-Electrodes.sfp) contains 33 standard electrode positions. It is not suitable for us as none of the electrodes that were measured in subject S1 have names that coincide with the 33 standard electrode names. Thus BESA cannot match them.

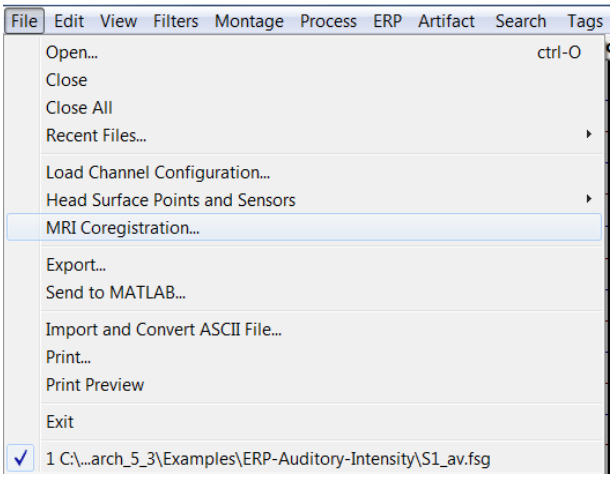
4. Press **OK** to close the dialogue.



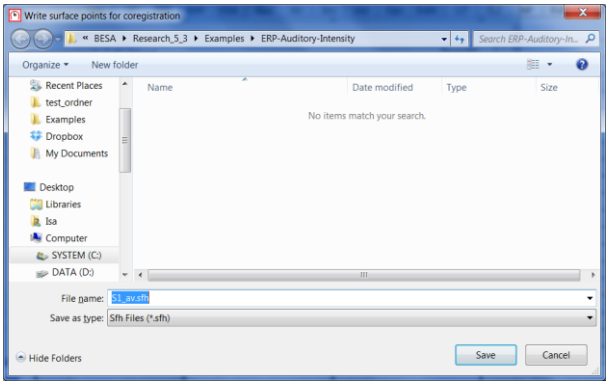
5. Press "**v**" on the keyboard to bring up the surface points window. The 96 standard electrodes are now displayed. Those standard electrodes whose labels coincide with S1's electrode labels are marked red. Please note that the grey standard electrode positions are merely used for better fitting the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



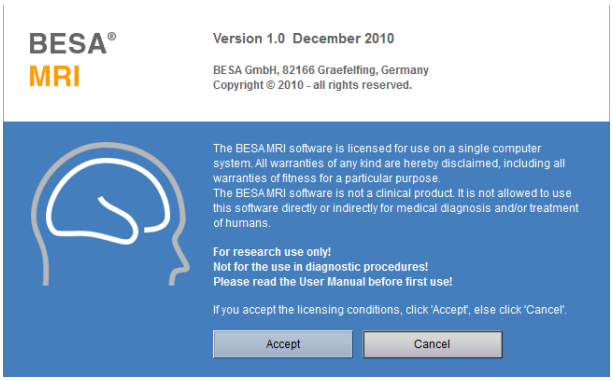
6. Press **File / MRI Coregistration**.



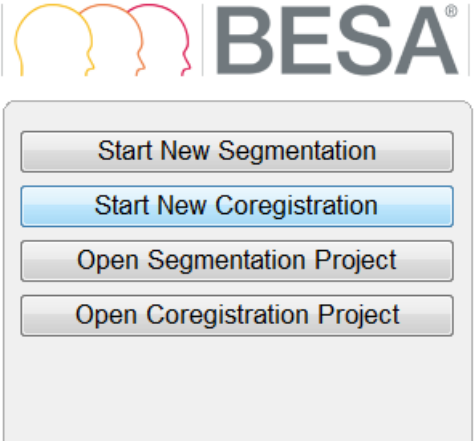
7. You will be prompted to create and save a "*.sfh" file, which will be used to store all coregistration information necessary for the interaction between BESA Research and BESA MRI. BESA will automatically suggest the filename of the current subject plus the extension ".fsh". Confirm the suggested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



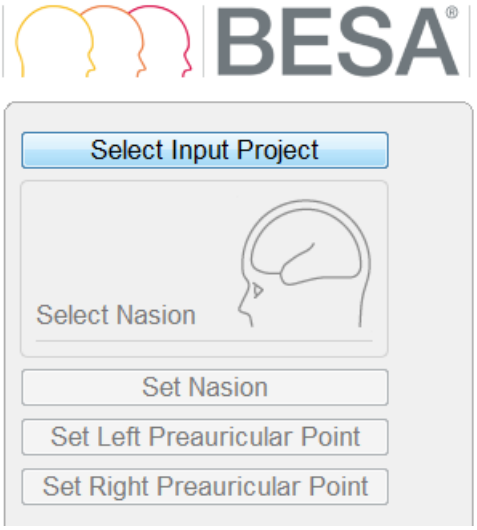
8. BESA MRI will open. Press **Accept** in the welcome screen. Important note: **do not close BESA Research**!



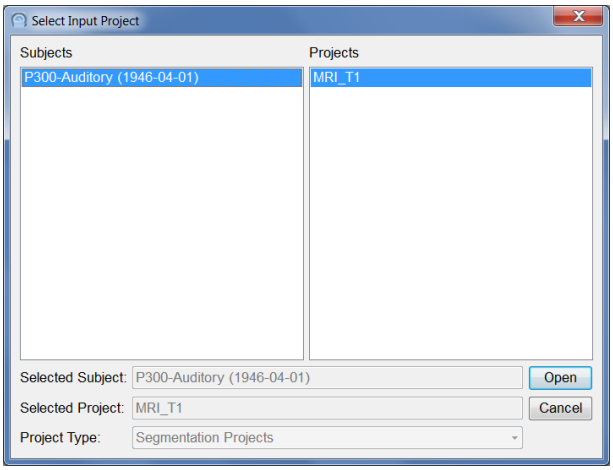
9. Press **Start New Coregistration** in the interaction window on the right and press **Next** on the bottom right (or hit the space bar).



10. 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.

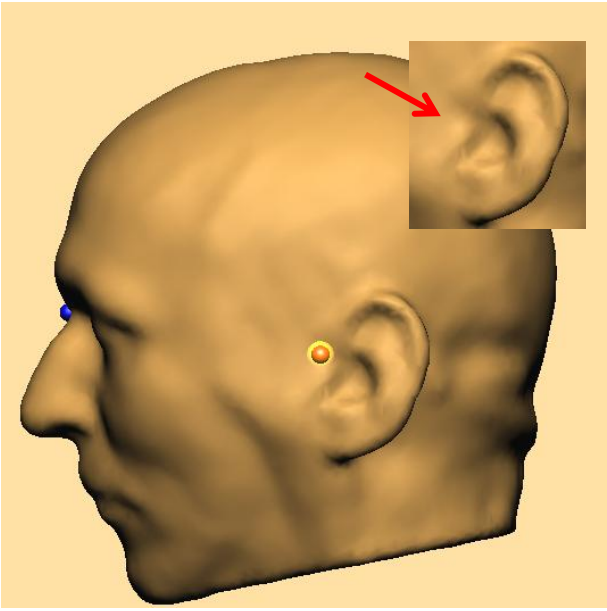


11. Choose the project P300-Auditory and the associated MRI. Press **Open**.

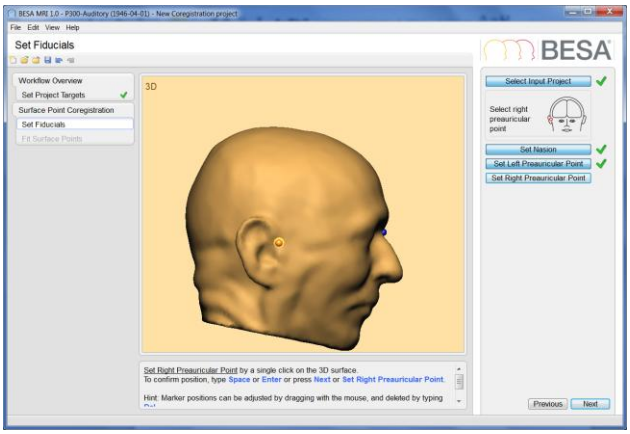


12. Mark Nasion on the segmented skin surfaces as indicated in the head scheme. Press **Next** to continue.

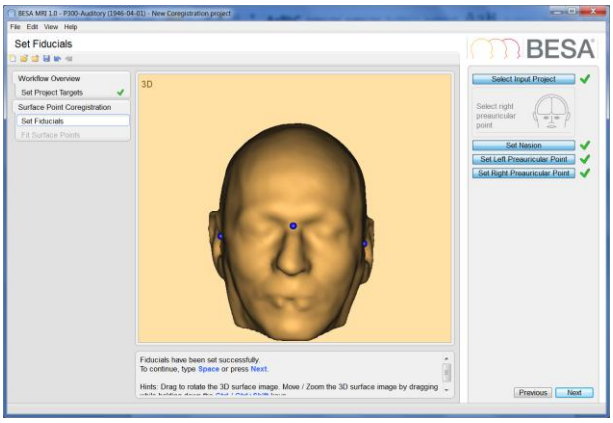
13. Mark LPA (Left preauricular point) as indicated in the head scheme in the interaction window. In this subject LPA position can be identified quite well by the shadow that is cast in LPA's dent. Press **Next** to continue.



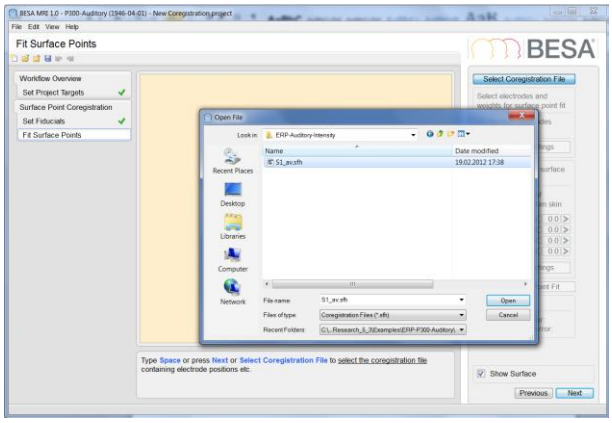
14. **Mark RPA** (Right preauricular point) and press **Next** to continue.



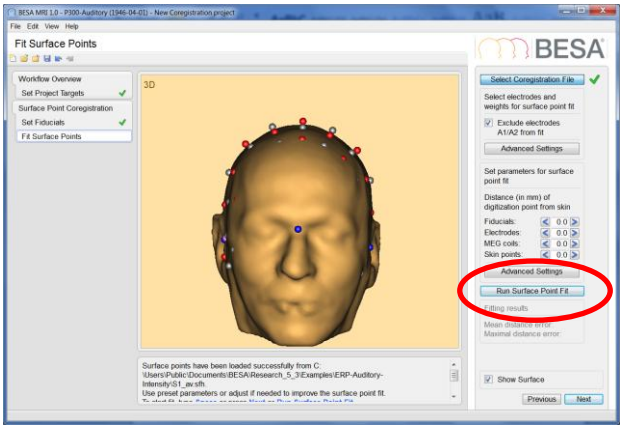
15. All fiducials are now positioned. Press **Next** to continue.



16. Press **select** Coregistration File to load the S1-av.fsh file we created in step C7. It is loacated in the “Examples” folder under “Auditory Intensity”. Press **Open**.

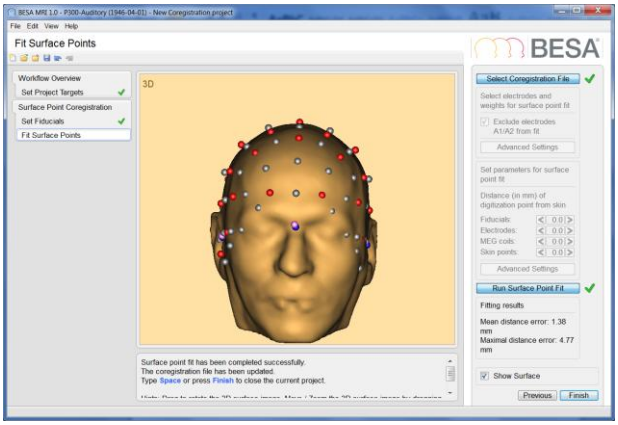


17. We can see the standard electrodes we loaded in step C3. They do not optimally fit the head surface, as the initial fit solely attempts to match the fiducials we defined in steps C12-C14 and the fiducial information in the sfh-file. Press **Run Surface Point Fit** to optimally match the standard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

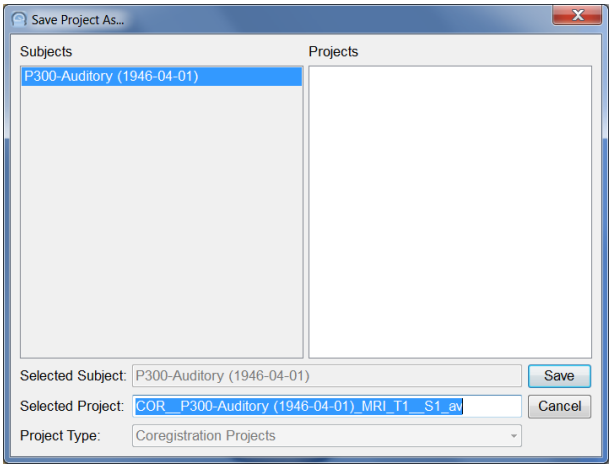


skin.

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

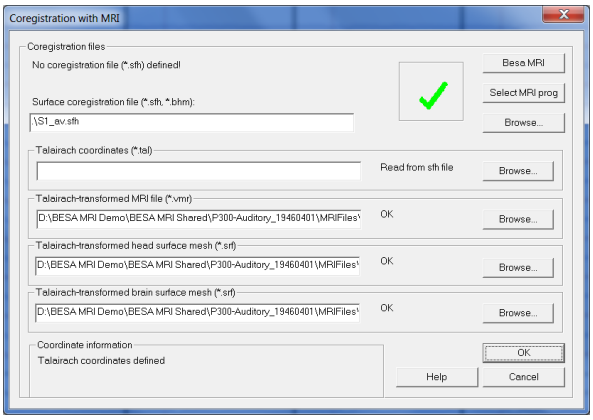


19. Please **save** the coregistration project under the suggested name.

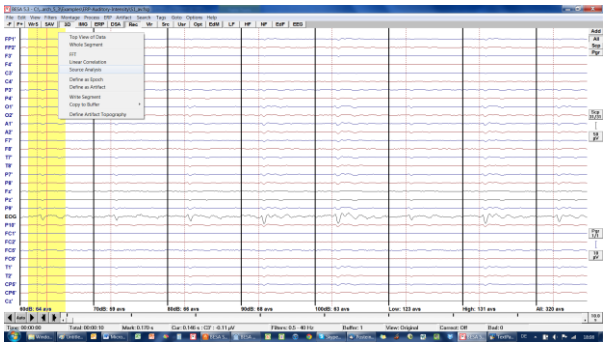


C: Utilizing the individual anatomy in BESA Research

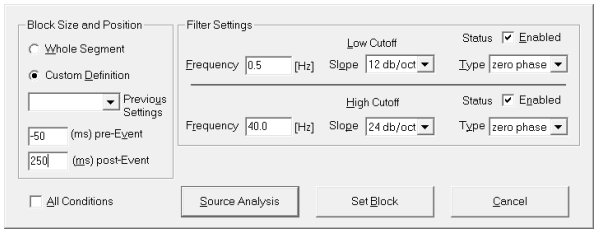
1. Return to BESA Research. All coregistration information containing surfaces and transformations have automatically been sent to BESA Research. A green arrow indicates that all necessary information is available. Press **OK**.



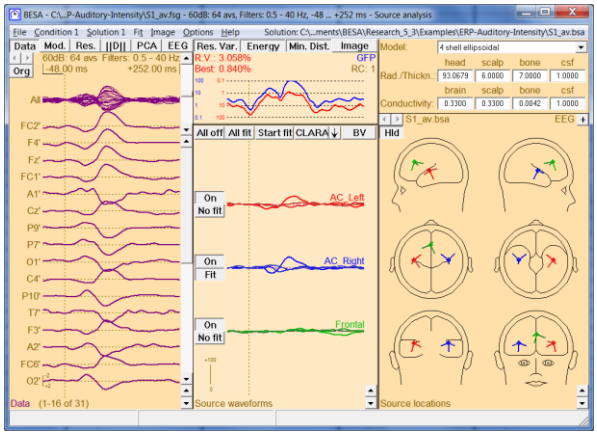
2. Let us now send the condition 60 dB t source analysis by **left-dragging** a block, **right-clicking** and selecting **Source Analysis**.



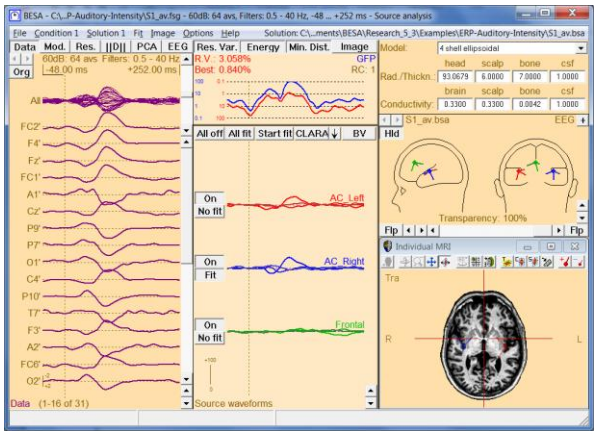
3. Select the custom definition -50 to 250 ms, Low Cutoff Filter 0.5 Hz, 12 dB/Oct, Zero Phase, High Cutoff Filter 40 Hz, 24 dB/Oct, zero phase. Press **Source Analysis**.



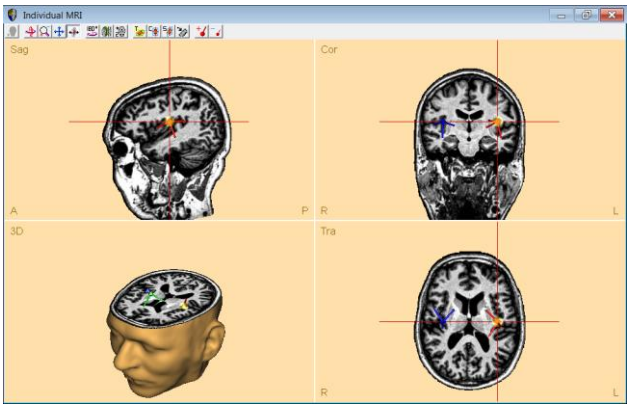
4. Our source model consisting of 3 regional sources will automatically open as we saved if per person in the batch created for tutorial 6.



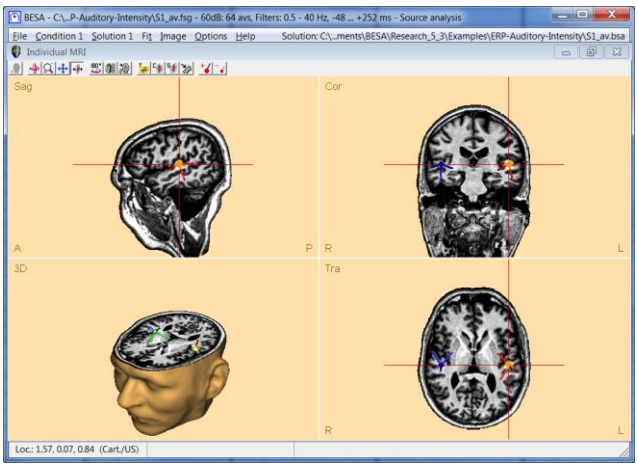
5. Press “a” on the keyboard to bring up the individual anatomy of the present subject. Since we coregistered the EEG and MRI data, the individual MRI is now available. Without coregistration, only a standard MRI can be displayed.



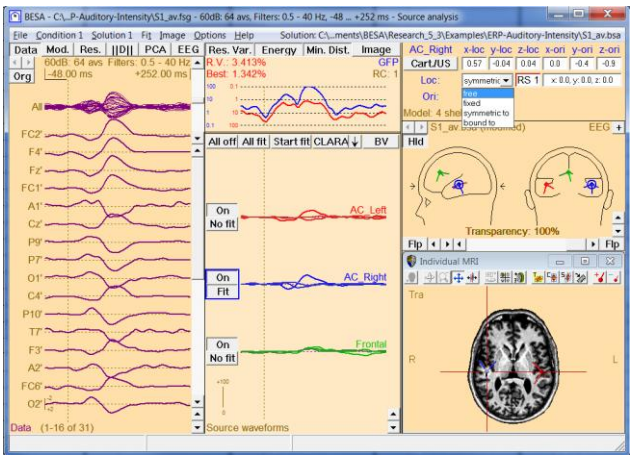
6. Select the first source by clicking on its source waveforms in the middle pane and enlarge the MRI window to get a better view. It can be seen that the source model we created using the grand average is located in the auditory cortex of subject S1. The model can still be optimized, however.



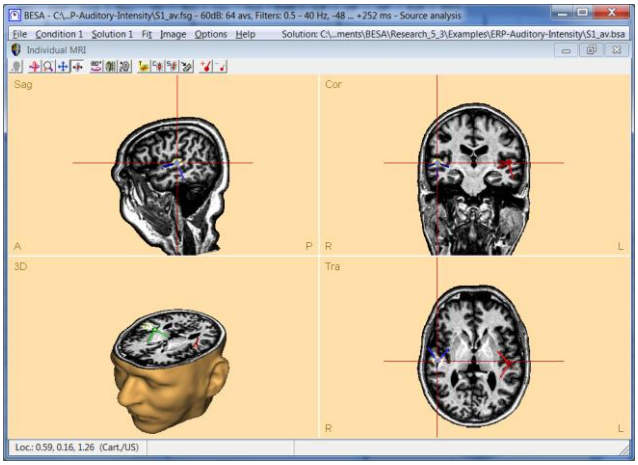
7. Let us first drag the red regional source to left Heschl's gyrus. You will notice that the position of the right auditory source (blue) also automatically changes position, as we imposed a symmetry constraint. We will now change this constraint back to "free".



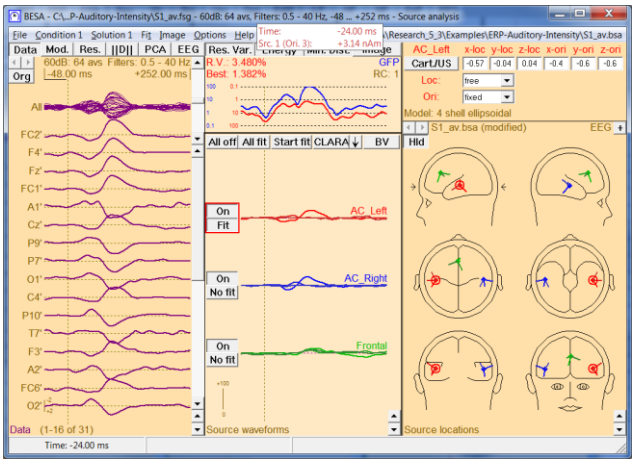
8. Minimize the MRI window and select the blue source by clicking on its source waveform. From the drop-down menu under "Loc" at the top right of the window please change "symmetric" to "**free**". Maximize the MRI window again.



9. Now let us move the blue auditory cortex source to right Heschl's gyrus. We now see a slight asymmetry in this subject with the right hemispheric source (blue) located more anterior than the left-hemispheric 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.

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

Distributed Source Imaging

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 x 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 x Number of sources].

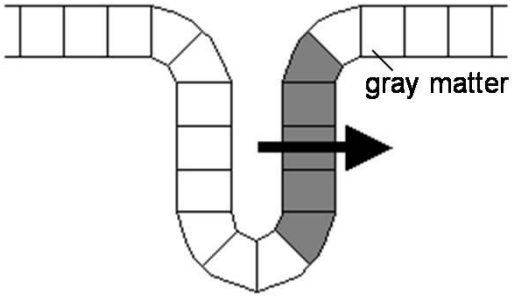
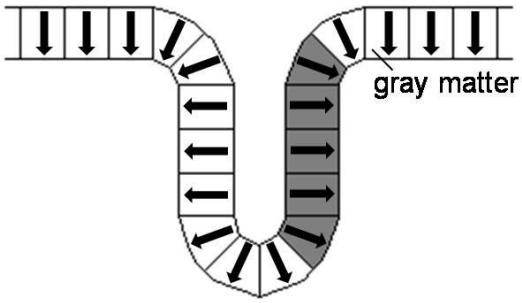
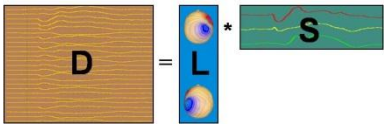
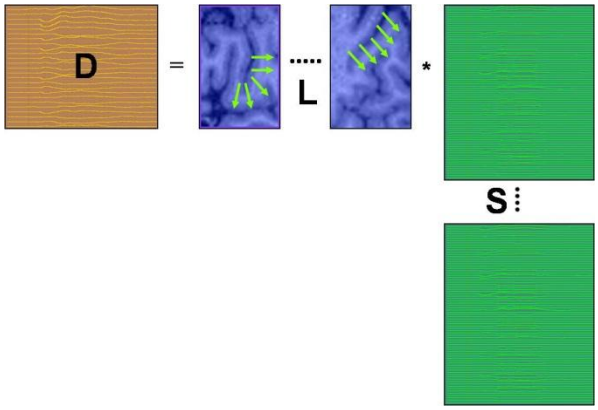
Each column of L contains the topography (the 'signal pattern') of one source, i.e. the signals that normalized activity of this source generates on the different EEG/MEG sensors. L contains all information about the source model (the multiple dipoles or regional sources) and the head model (the assumed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

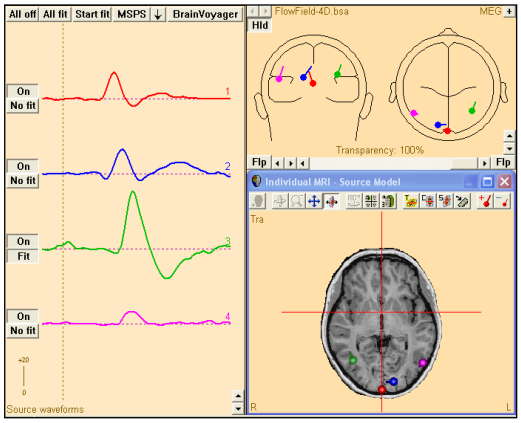
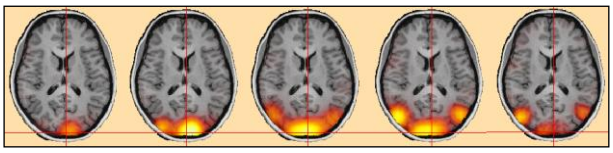
L^T: The transposed leadfield matrix, i.e. rows and columns are swapped. Accordingly, the dimension of this matrix is [Number of sources x Number of sensors].

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 x Number of sources].

Discrete source analysis	Distributed source analysis
Idea: Each equivalent current dipole represents an extended brain region	Idea: Each current dipole represents one small brain segment
	
Number of sources < Number of sensors Overdetermined problem	Number of sources >> Number of sensors Underdetermined problem
Source model is defined by fitting or seeding	Source model is predefined (e.g. along the brain surface or on a regular volume grid)
$D = L \cdot S$  The leadfield L has more rows (number of sensors) than columns (number of sources)	$D = L \cdot S$  The leadfield L has more columns (number of sources) than rows (number of sensors)
Reconstructed source waveforms: $S = L^{-1} \cdot D$ where $L^{-1} = (L^T \cdot L)^{-1} \cdot L^T$	Reconstructed source waveforms: $S = L^{-1} \cdot D$ where $L^{-1} = L^T \cdot (L \cdot L^T)^{-1}$
Regularization in source space. e.g. Tikhonov: $L^{-1} = (L^T \cdot L + \lambda \cdot I)^{-1} \cdot L^T$	Regularization in sensor space, e.g. Tikhonov: $L^{-1} = L^T \cdot (L \cdot L^T + \lambda \cdot I)^{-1}$
	Spatial weighting: $L^{-1} = V \cdot L^T \cdot (L \cdot V \cdot L^T + \lambda \cdot I)^{-1}$

Discrete source analysis	Distributed source analysis
Result: Multiple source model and source waveforms  	Result: 3D Volume image, one for each time point 
Properties: • **Crosstalk: (+)** If the source model contains all active brain regions, the source waveforms represent their activity, i.e. they separate and mutually contrast their activities with minimum crosstalk • **Effort: (-)** Source model needs to be defined by fitting or seeding. This requires user interaction (decision on the number of sources, fit intervals etc.)	Properties: • **Crosstalk: (-)** Smeared, non-focal activity. Substantial crosstalk between sources. Reconstructed activity at any location is contaminated by activity from other brain regions. Activity of brain regions close to each other can hardly be separated. • **Effort: (+)** Pre-defined source model. Source images are generated easily and quickly. But: parameters have to be specified (V, λ). They can have a large impact on the quality of the obtained source image.

In BESA, 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 & 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 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 ist 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 source's lead field vector (i.e. deep sources get a larger a-priori 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:

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

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 (**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.
  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.

Regularization

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

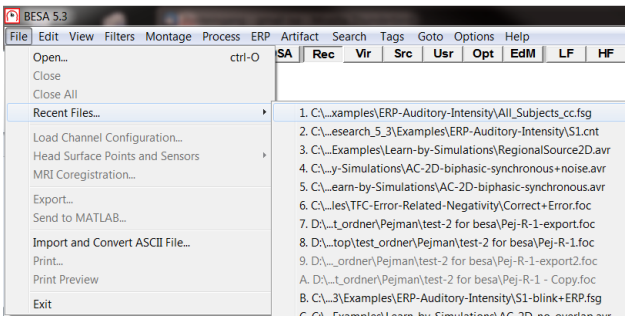
- **Tikhonov regularization:** In Tikhonov regularization, the term $L \cdot V \cdot L^T$ is inverted as $(L \cdot V \cdot L^T + \lambda \cdot I)^{-1}$. λ is the regularization constant, and I is the identity matrix.
- **Truncated singular value decomposition (TSVD):** Here, an SVD decomposition of $L \cdot V \cdot L^T$ is computed as $L \cdot V \cdot L^T = U \cdot X \cdot U^T$, 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 $U \cdot X^{-1} \cdot U^T$, 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.

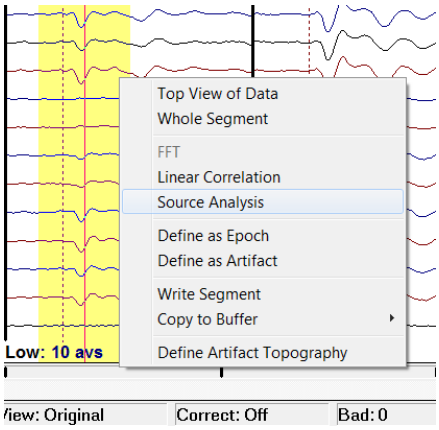
B. Comparison of different distributed source imaging methods

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.

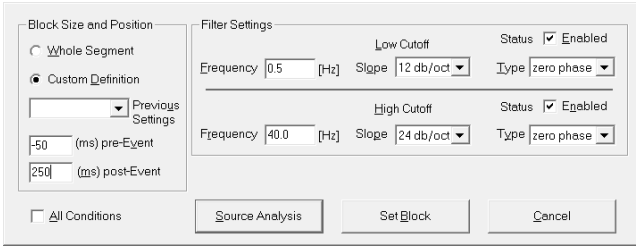
1. Select the file '**All_Subjects_cc.fsg**' from '**File / Recent Files**'.



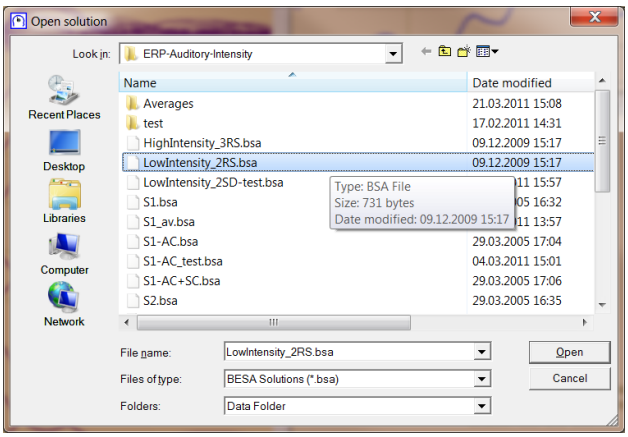
2. Select condition '**Low**', left-drag a block and send it to source analysis by right-clicking and pressing '**Source Analysis**'.



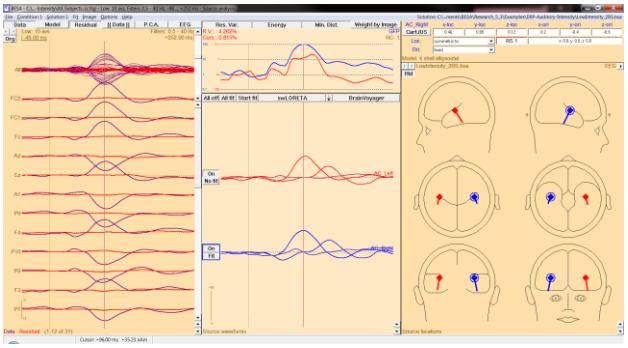
3. Select a time range from **-50 to 250 ms**, and specify a **Low Cutoff filter of 0.5 Hz, 12dB/oct, zero-phase** and a **High Cutoff filter of 40 Hz, 24 db/oct, zero-phase**. Press '**Source Analysis**' to open the specified data in the source analysis window.



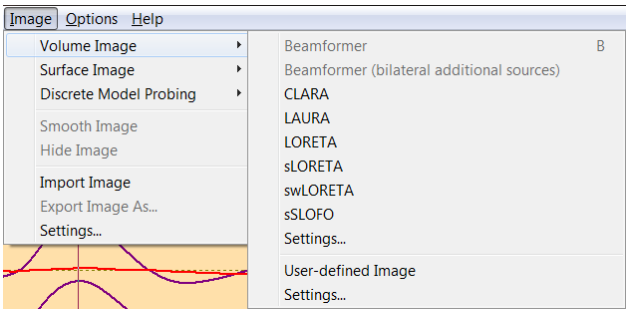
4. As a reference and for comparison, load the regional source model we created in Tutorial 4, steps D1-7. Press '**File / Open Solution**' and load file '**LowIntensity_2RS**'.



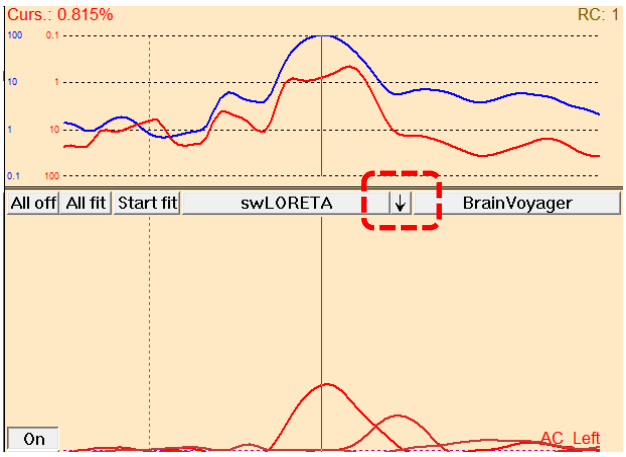
5. Set the cursor at 96 ms, at the peak of the N100 component.



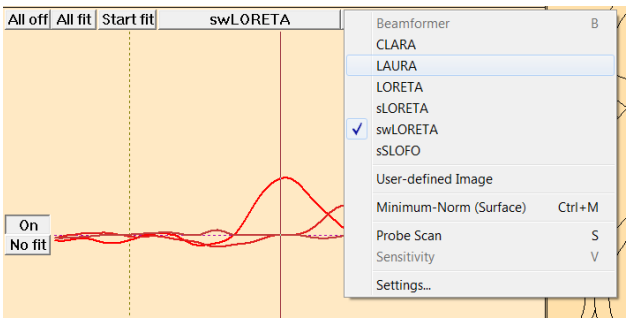
6. Distributed 3D volume images are generated with a single click in BESA. They can be generated by selecting the corresponding method from menu entry '**Image / Volume Image**', or from the dropdown menu of the image selector button (the arrow left of the BrainVoyager button in the middle of the source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

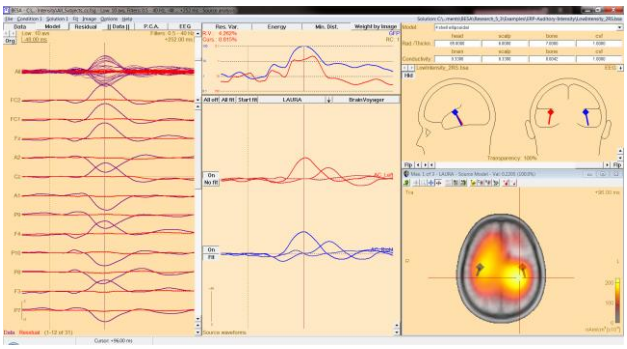


7. First, compute a LAURA image at the cursor latency. Simply select '**LAURA**' from the Image selector button dropdown menu.

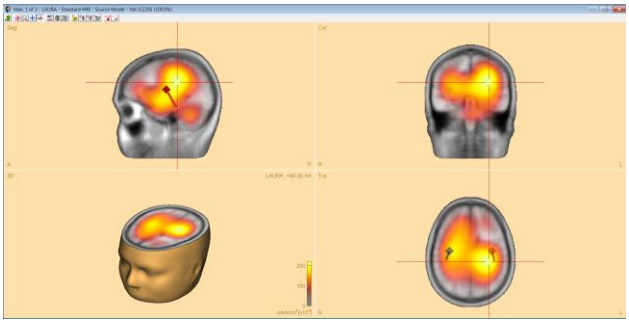


8. The computation of a LAURA (and also LORETA) image is divided into 6 steps. A dialog window informs you about the processing stage. When computing a LAURA image for the first time for a data set, steps 2 and 3 can take some time to complete (depending, among others, on the selected grid spacing, see 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.

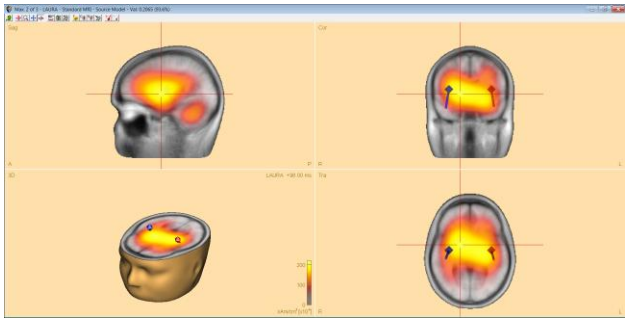
9. The result is displayed superimposed to the anatomical MR image in the 3D window in the lower right corner of the source analysis window. Because for this subject, no individual MRI is available, BESA automatically uses a standard MRI. The regional source model is also shown and superimposed on the LAURA image. Press the **button** in the 3D window to get a 4-head view. (You may also maximize the 3D window to get a larger view of the obtained LAURA image.)

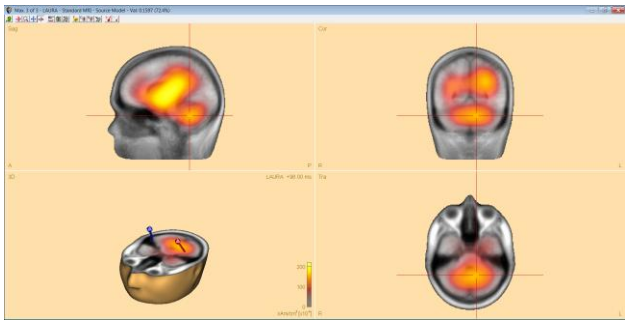


10. The units of the displayed LAURA images are nAm/cm^3. The image correctly shows activity in both hemispheres. However, the focal activity is substantially blurred – a negative effect inherent in all distributed source images. It becomes evident from this example that this does not allow to draw any conclusion on the extent of the active brain regions.

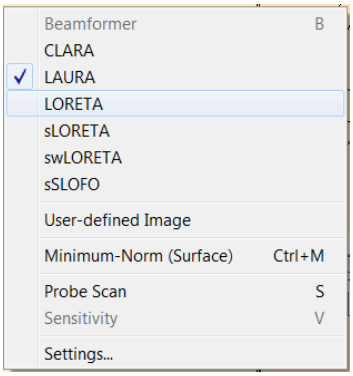


The LAURA image detected 3 maxima, The largest maximum is in the left hemisphere, roughly in the temporal lobe. Compared to the regional source it is located more medial, posterior and superior. You can jump to the next maximum using the **Button (**4th button from the right in the 3D window). The second 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.

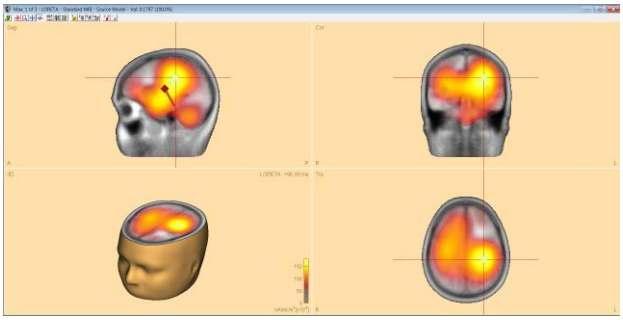




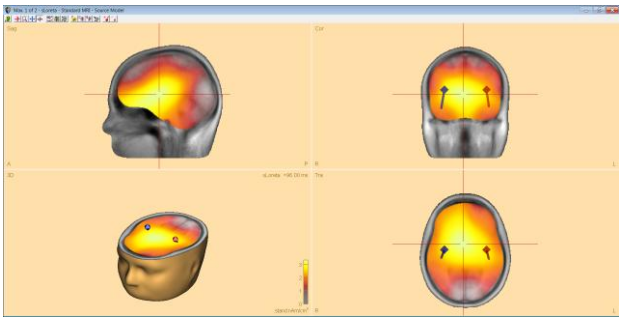
11. Compare the LAURA method with LORETA: Select **LORETA** from the image selector button dropdown menu. Like LAURA, LORETA includes a spatial cross-voxel weighting term, which increases the computational load, so that the first LORETA image computed for a data set

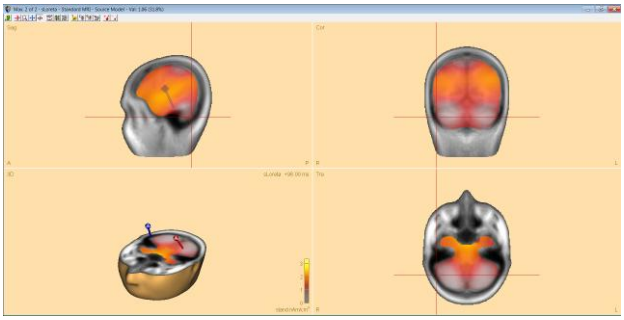


takes a longer time to complete than sLORETA or swLORETA. LORETA and LAURA often produce similar results: The first two maxima are located roughly in the temporal lobes, a third maximum is located in the cerebellum.

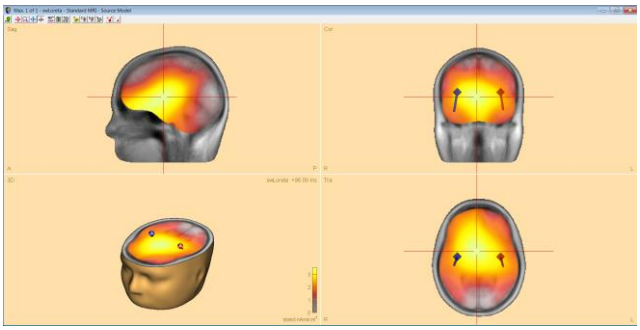


12. Compare the LORETA method with **sLORETA**: Select sLORETA from the image selector button dropdown menu. The result is an image (units: standardized nAm/cm3) with two maxima. The first maximum is located roughly in the middle of the head, the second maximum is located in the right cerebellum. There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



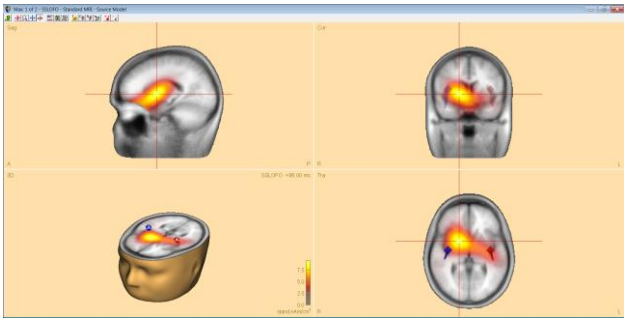


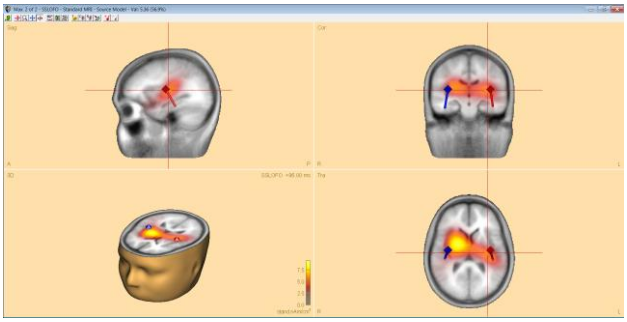
13. Compare the sLORETA method with **swLORETA**: Select swLORETA from the image selector button dropdown menu. swLORETA differs from sLORETA only in an additional depth weighting factor. Properties of swLORETA and sLORETA images are very similar. Here, swLORETA found 1 maximum in the middle of the head that corresponds to the first maximum of the sLORETA solution.



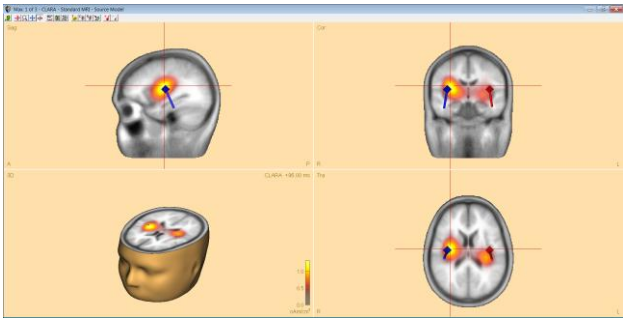
14. Iterative methods aim at combining advantages of discrete and distributed source images. Implemented as default methods in BESA Research are sSLOFO and the new CLARA method. Both methods iteratively apply distributed source images with a successive shrinking of the source space. They differ from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

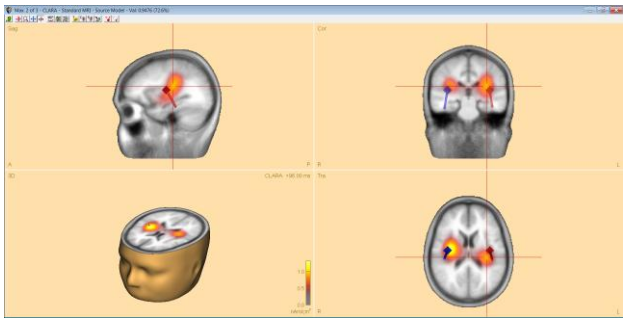
15. Let's first compute an **sSLOFO** image. Select sSLOFO from the image selector button dropdown menu and observe that the result is much more focal than the previous methods, shrinking the activity substantially, indicating bilateral activation of the temporal lobes. The first maximum in the right hemisphere is located more medial than the regional source, but the left hemispheric maximum nearly coincides with the regional source.

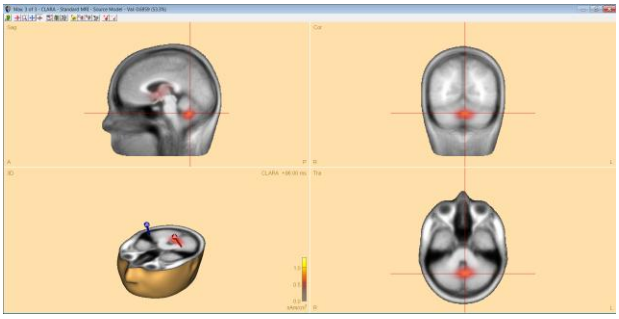




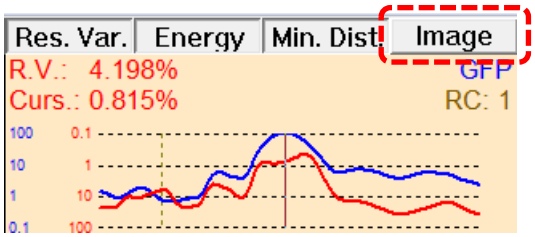
16. Now compare this with the **CLARA** method, an iterative application of weighted LORETA images. Select CLARA from the image selector button dropdown menu. Like sSLOFO, the resulting image is quite focal. The first two maxima are very close to the discrete source solution. CLARA detected a third maximum, again located in the cerebellum.

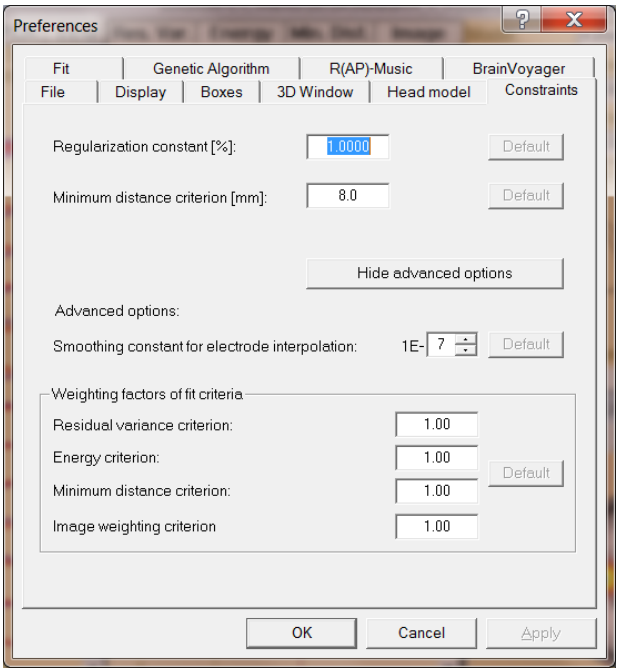




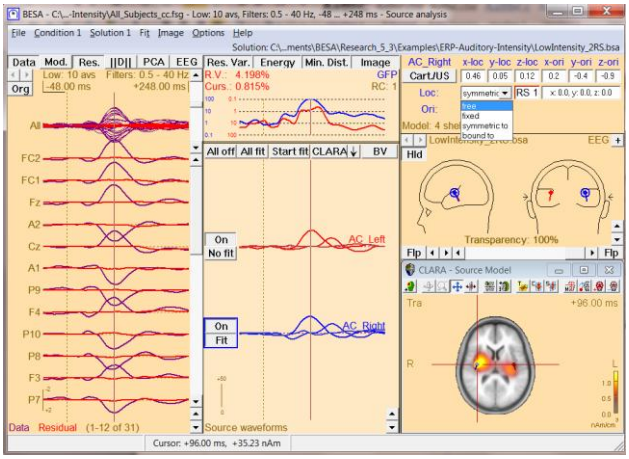


17. Next we want to re-fit our discrete sources using Image weighting. Press the '**Image**' button in the top-right corner of the GFP and residual variance field. By default, only buttons for criteria 'Residual Variance', 'Energy' and 'Minimum Distance' are pressed. **Right-click** on the '**Image**' 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**'.

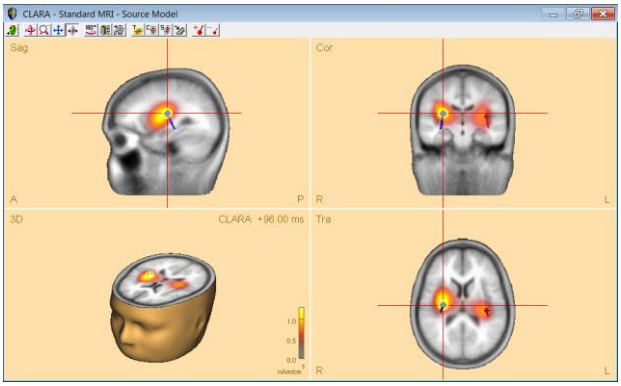




18. Click on the right-hemispheric source and change the symmetry constraint to '**free**' in the '**Loc**' drop-down menu.



19. 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.



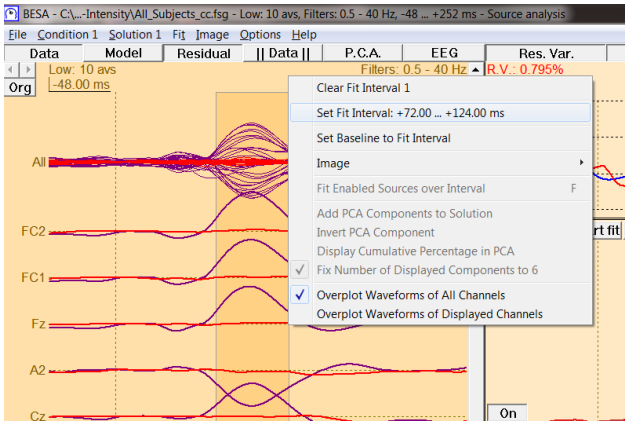
This first glance at the different imaging methods on our example dataset illustrates both the advantages and disadvantages of distributed source images: While they allow to get a very quick result at a certain latency, they all suffer from severe blurring (least in CLARA and sSLOFO), which can make 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.

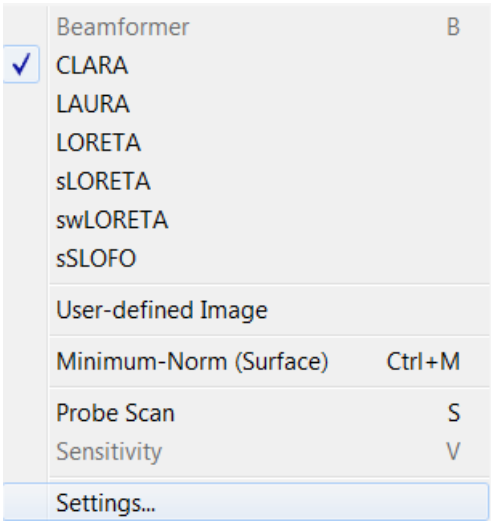
C. Settings for distributed source images: The effect of regularization

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.

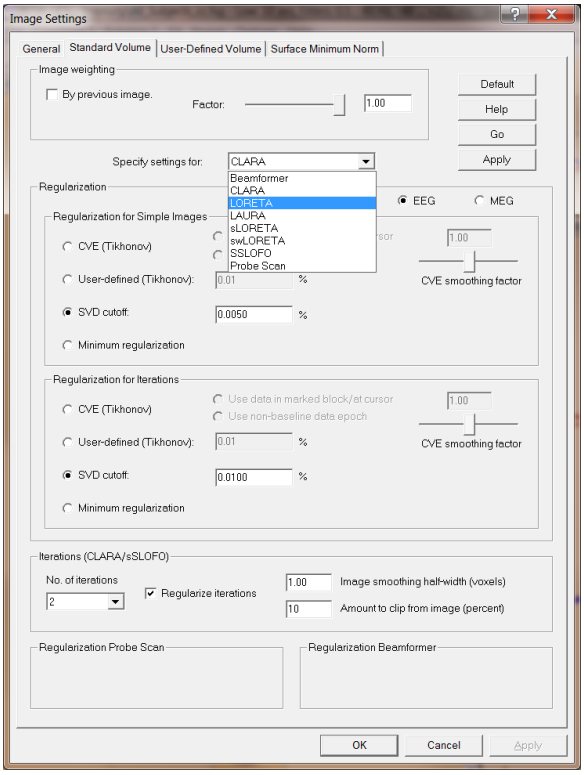
1. As an example, we will compare **LORETA** images with different regularization settings. We could use images computed for the cursor time only, as we did in the previous chapter. Alternatively, we can mark an extended latency interval. By default, BESA research will then compute a mean image over 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.



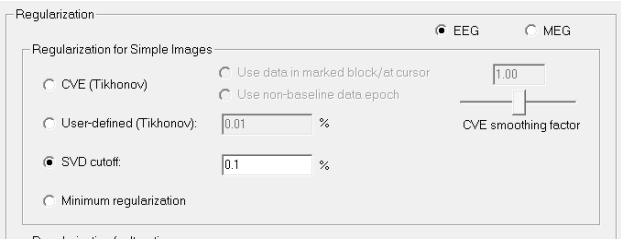
2. Regularization and other imaging parameters can be specified in the '**Image Settings'** dialog window. Open it by pressing '**Image / Settings**' or from the bottom of the image selector button dropdown menu.



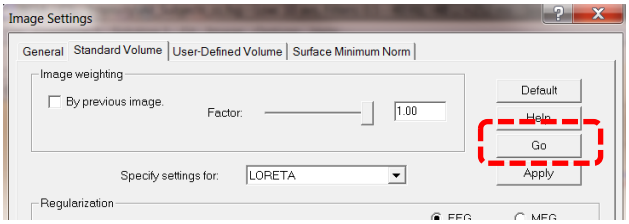
3. The '**Image Settings'** dialog window consists of four tabs: The 'Standard Volume' tab lists all adjustable parameters of the standard distributed volume images. We will focus here on the effect of the **regularization** parameter, which has a critical influence on all distributed source images. 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.



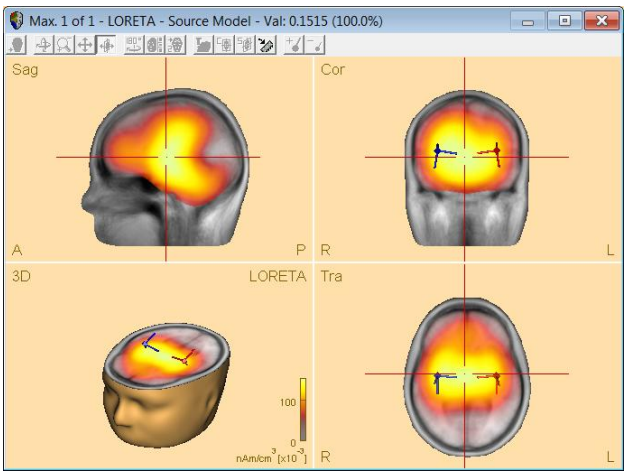
4. We will now compute and compare images with different regularization constants. The default is an SVD cutoff of 0.005%. First observe the effect of too high regularization: Increase the SVD cutoff to **0.1%.**



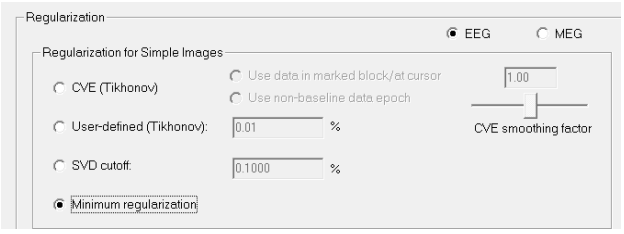
5. Press '**Go'** in the Image Settings dialog window to apply the current settings and compute a LORETA image with too high regularization: In contrast to the result of the multiple dipole model, the LORETA image suggests a single midline generator



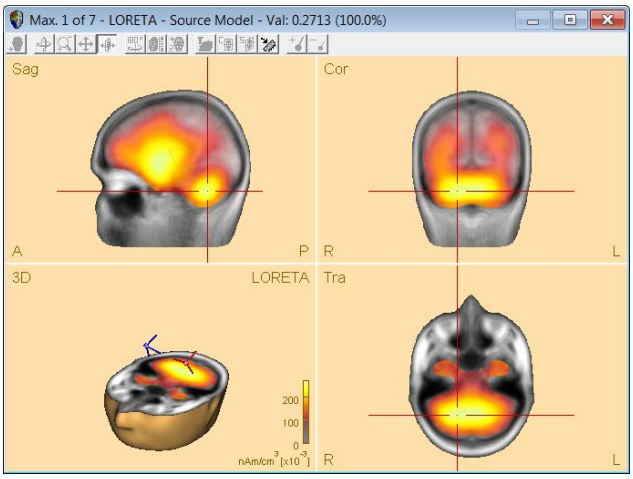
of the recorded signals. This is a typical result of a too strong regularization: This tends to lead to deep, widely distributed image maxima. In our example, the bilateral activity of the left and right auditory cortex are combined and mislocalized in the middle of the head.



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

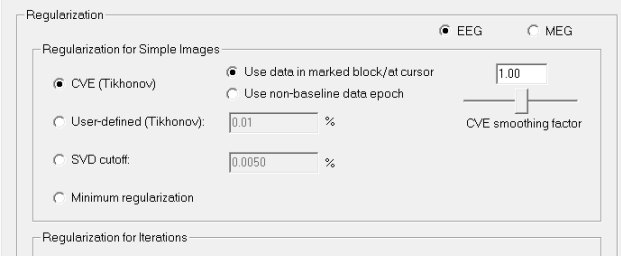


7. Press '**Go**' again to recompute the LORETA image. In contrast to a too high regularization constant we now obtain a solution with many maxima. This is a typical effect of too low regularization.

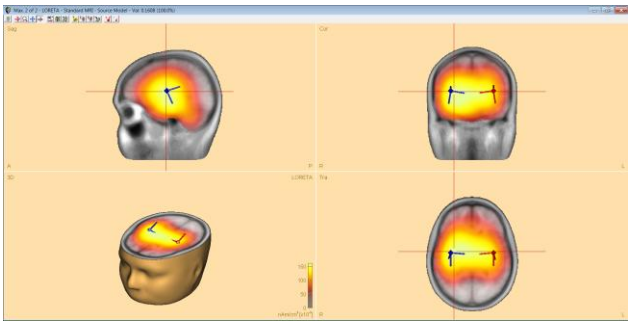


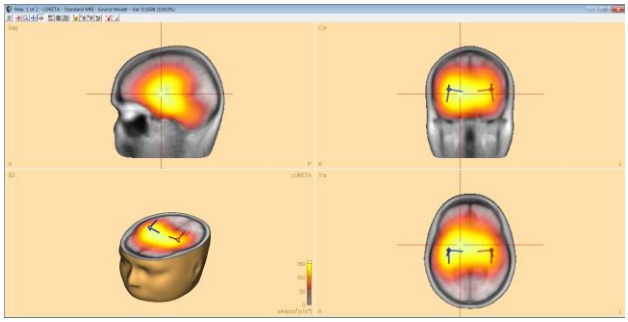
The above examples were computed with SVD regularization, but the same principle holds for Tikhonov regularization as well. You may test this by selecting the user-defined Tikhonov regularization in the 'Image Settings' dialog window and compute LORETA images with different parameters.

8. How to determine the 'optimum' 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**'.



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.





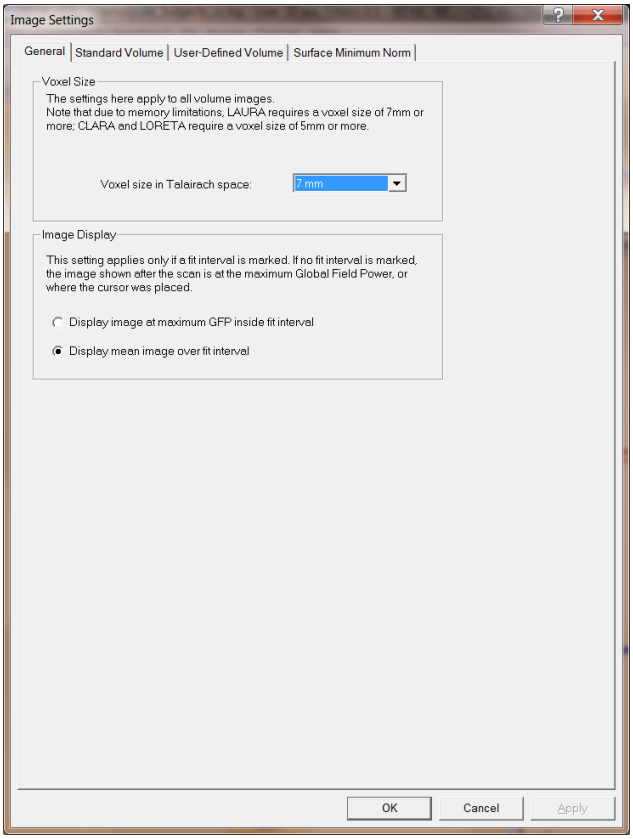
BESA uses predefined default regularization constants specific for each method and channel type. These default values are appropriate in many situations. However, the optimum regularization depends on the signal-to-noise ratio of the data, the number of channels and other properties of the specific data 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).

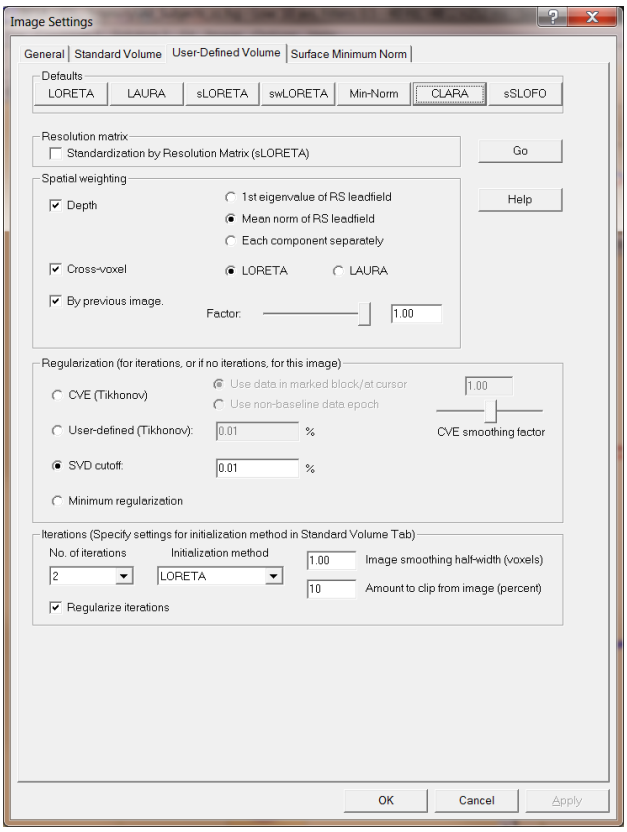
D: Other image settings

1. The ‘Image Settings’ dialog window not only allows to modify parameters of the pre-defined 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 7mm, 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.

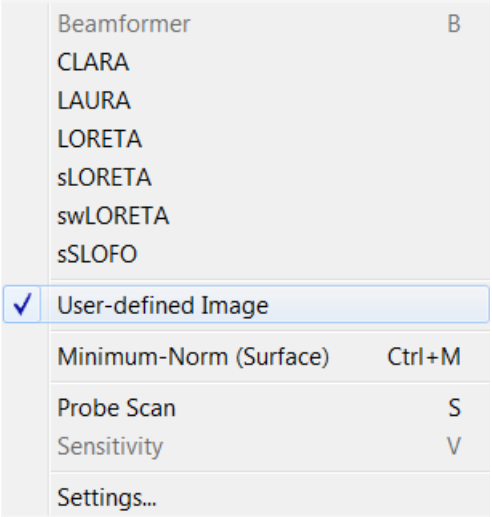


2. It is also possible to create a user-defined volume imaging method: Switch to the **User-Defined Volume** tab. Here, a wide range of parameters can be specified.



- 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 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 pre-defined imaging method so that you can understand the details of those methods and can easily modify them if you wish to.

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



E: 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 1x1x1mm^3 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.

What does BESA Research provide?

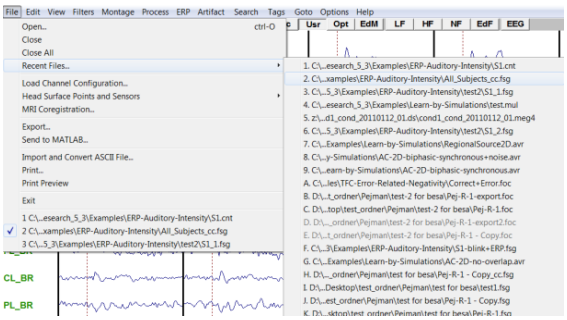
- ✓ Pre-defined source montages
- ✓ User-defined source montages
- ✓ Artifact correction based on surrogate models, adaptive artifact correction

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. Alternatively it is possible to use pre-defined source montages. In the following we will learn about the advantages and the use of source montages.

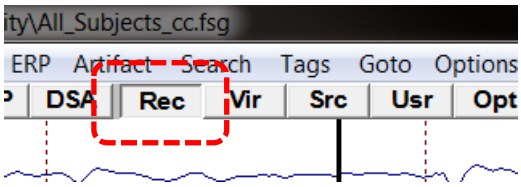
Based on the knowledge about source montages, we will learn about the background of artifact correction.

A: Pre-defined Source Montages

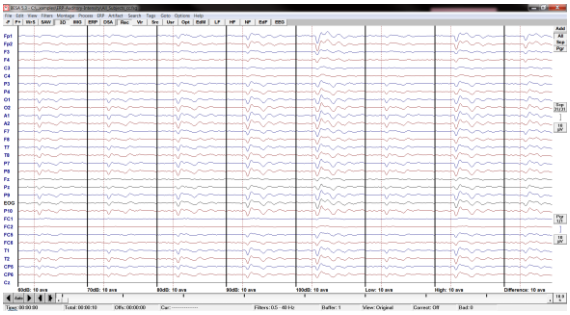
1. The file '**All_Subjects_cc.fsg**' should still be open. If it isn't, open it from your recent file list: '**File / Recent Files…**'



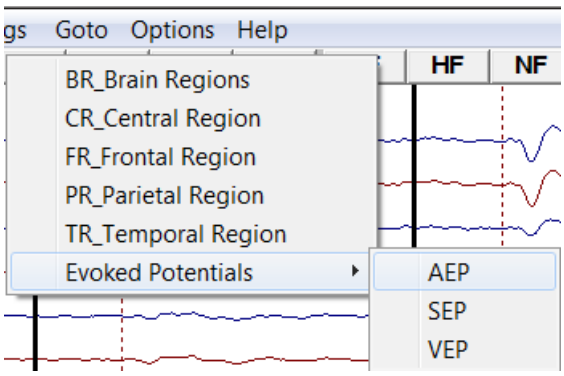
2. Make sure the button **Rec** is pressed to display the original recorded data.



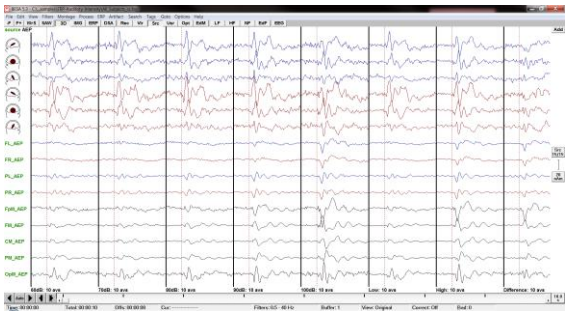
3. From what we see on the screen at the moment, we cannot infer which brain regions might be responsible for creating the evoked potentials that can be seen across all electrodes.



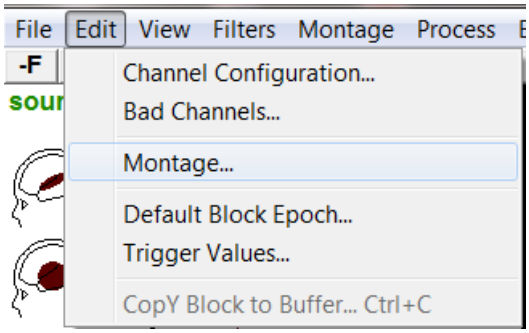
4. We will now load a source montage that is part of the BESA installation and was created for auditory evoked potentials. Press the **Scr** button and select '**Evoked Potentials / AEP**'.



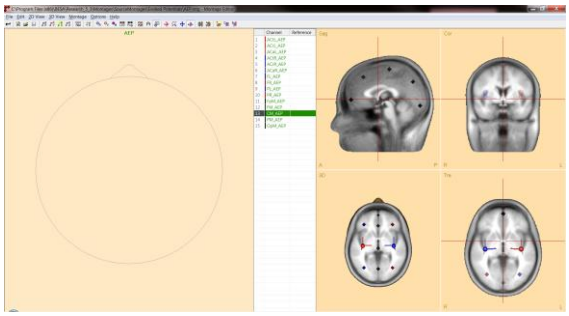
5. Now the sensor-space data are automatically translated on the sources in the source montage. As discrete sources have good separation properties we can immediately see that activation is not distributed over all channels any longer but mainly reflected by the temporal sources. You might notice that some sources are displayed with a head scheme, while others only have a label. We want to look at the montage in more detail to find out why.



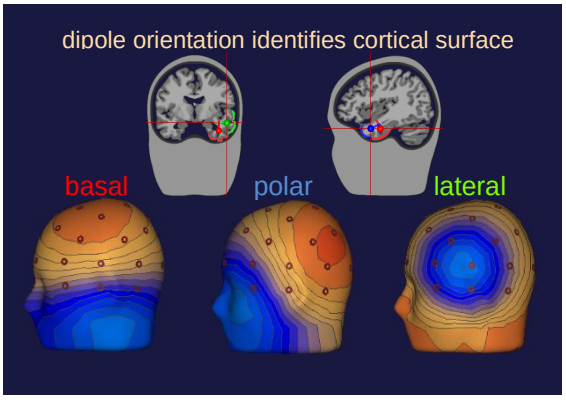
6. Press 'Edit / Montage' to open the montage editor window. The current montage will automatically loaded.



7. 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.

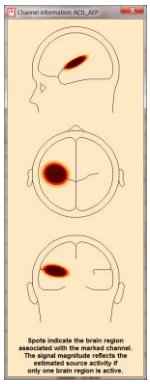


8. You will note that the first 6 sources are single dipoles with an orientation while the other sources are regional sources that have not been oriented. The idea behind this montage is that it was optimized to model auditory data. Therefore, the auditory cortex sources have an orientation to reflect 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.

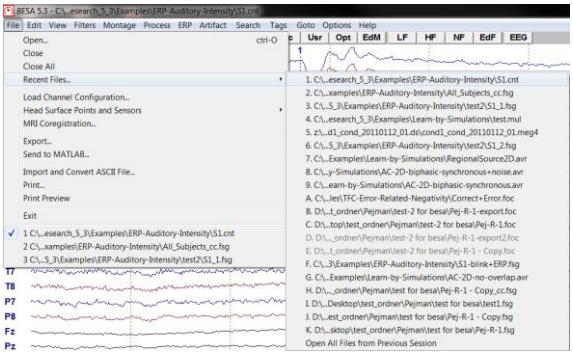
9. Close the montage editor window and **right-click** on the first of the head schemes on the left of the main window. A window will pop up that displays the cortical area that is covered by the according source. You can view the associated brain regions of all sources displayed on the left of the main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



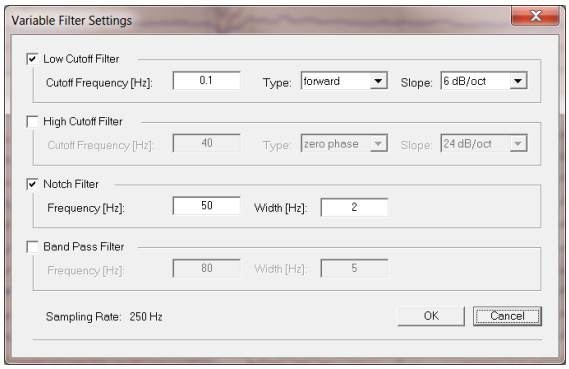
10. Note that BESA Research provides predefined source montages for auditory, somatosensory and visually evoked potentials as well as montages for central, frontal, parietal and temporal brain regions. The according brain regions are modeled by oriented dipoles, while the regions that are not of interest 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.

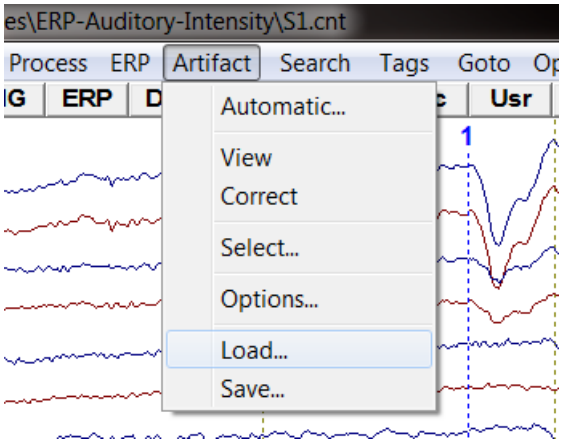
11. We will return to the dataset '**S1.cnt**' of the auditory intensity experiment that should still be available in your recent file list: '**File / Recent Files…**'



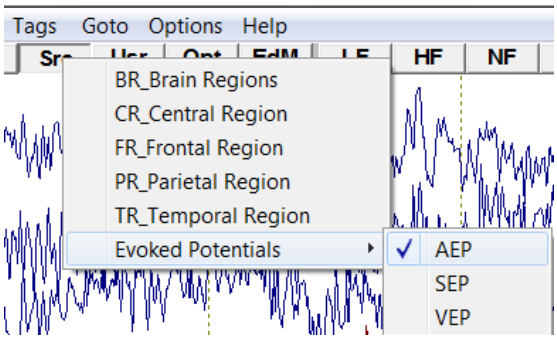
12. Next we will filters to the data by pressing the **EdF** button or ('**Filter / Edit Filter Settings**'). Define a Low Cutoff filter of **0.1 Hz, forward, 6dB/oct** and a notch filter of **50 Hz, Width: 2**.



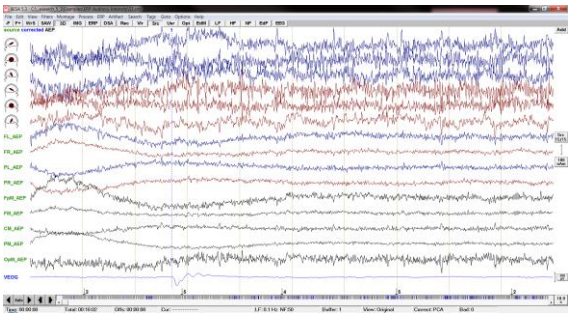
13. Now we will load the artifact definition file that contains the blink information we created earlier. Press '**Artifact / Load**' and select the file '**S1.atf**'. Make sure the artifact correction is switched on (a blue 'corrected' should be displayed in the top left corner of the main window).



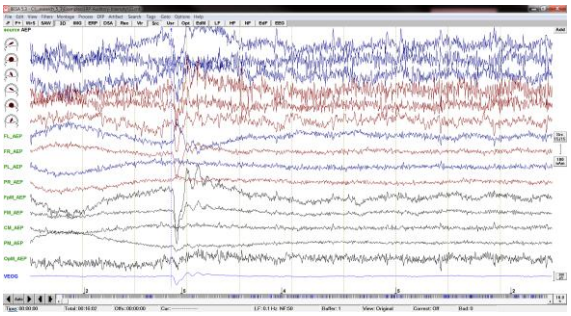
14. We will now apply the source montage '**AEP**' again by pressing the button Src and selecting 'Evoked Potentials / AEP'.



15. Again, we immediately see – even in the raw data! – that the majority of variance in the data is produced by temporal lobe sources.

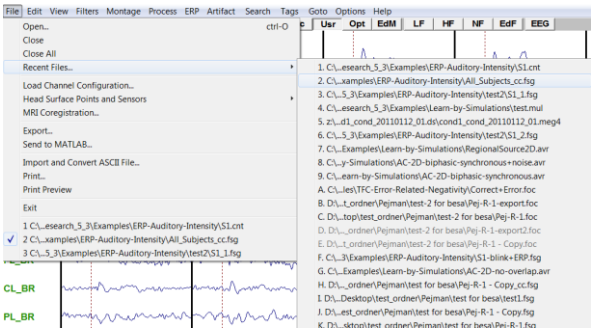


16. Make sure you are in the first screen and switch off artifact correction by pressing '**ctrl-E**'. Note that a large signal now appears in the frontal sources (mainly FpM). Also note, how the eyeblink has an effect on auditory cortex sources. This demonstrates the necessity to control eye artifacts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

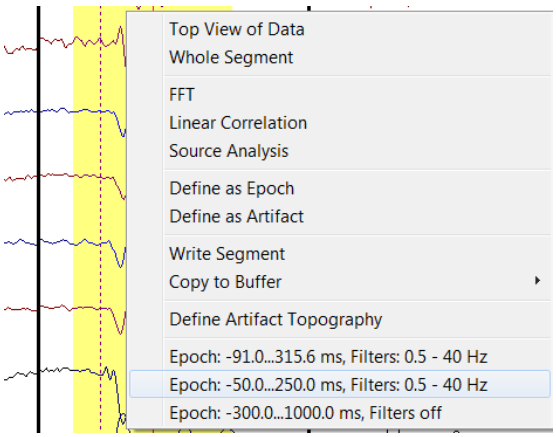


B: Creating a source montage

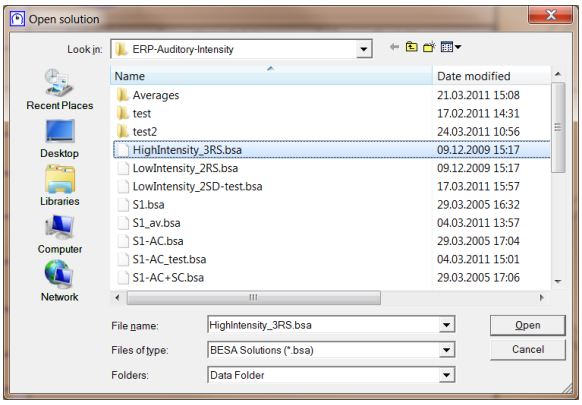
1. We will now create our own source montage based on the source model we created using the grand average data of the auditory intensity experiment. Return to the dataset '**All_Subjects_cc.fsg**' that should be available in the file menu. If it isn't, open it from your recent file list: '**File / Recent Files…**'



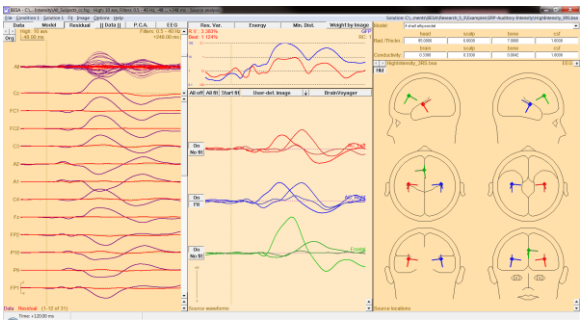
2. Select condition 'High', right-click and send it to source analysis with settings -50 to 250 ms, a Low Cutoff filter of 0.5 Hz, forward, 12 dB/oct. and a High Cutoff filter of 40 Hz, zero-phase, 24 dB/oct.



3. 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**'.



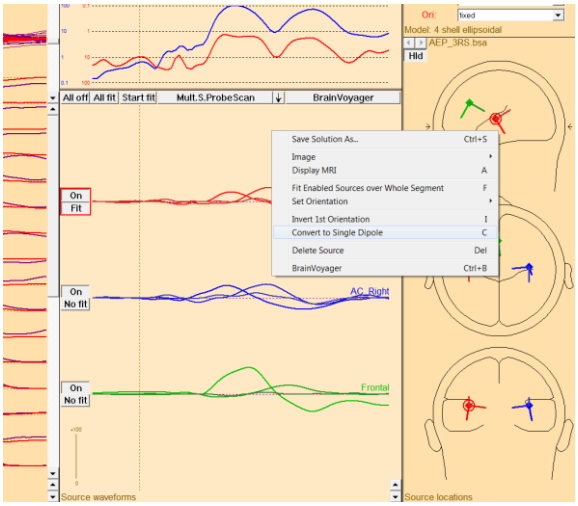
4. We will use this source model as a source montage for our raw data. In the present case this source model is sufficient, but when data are very noisy, it is advisable to add probe sources to the model.



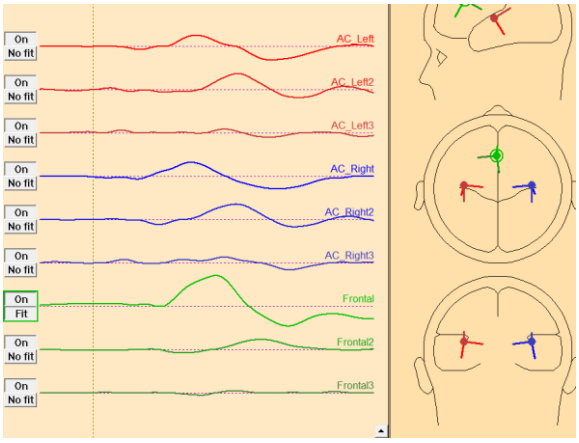
Remember that activity in any brain region that is not modeled by a regional source will be projected on the sources of interest. I.e. any noise or unsystematic brain activity that is

not related to the stimulation would be projected on our three sources.

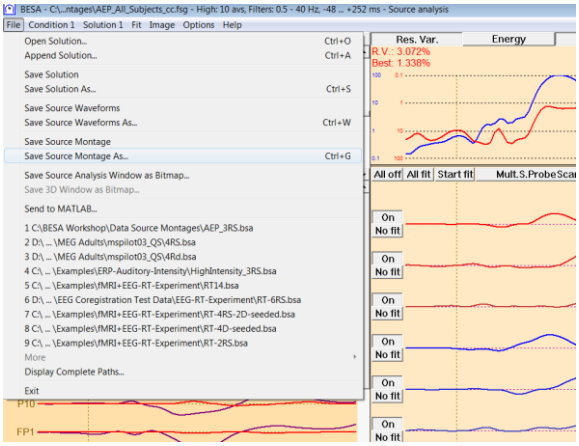
5. As we are interested in auditory cortex activity and activity in the frontal area that becomes active in the high intensity condition, we need to make sure that BESA uses oriented sources in the source montage. In order to make the orientations of the auditory cortex and frontal sources available 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**'.



6. 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**'.

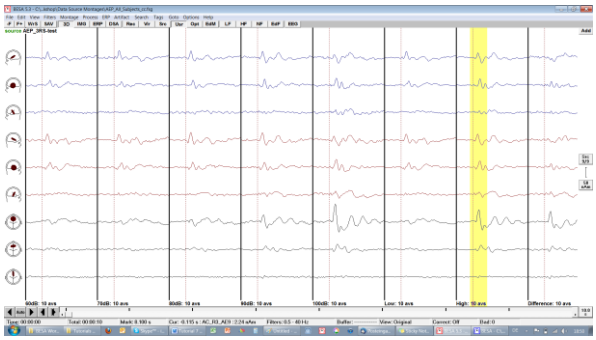


7. Save the source montage by pressing '**File / Save Source Montage As…**' and specify the name '**HighIntensity_3RS-test.mtg**'. Close the source analysis window. Press '**OK**' to terminate without saving the source solution.

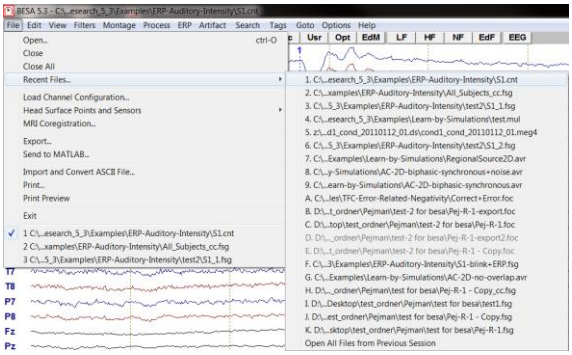


Now we have created our own source montage for the auditory intensity condition that includes the fitted sources along with probe sources. We will now apply this montage on our grand average data and the raw data of subject 1.

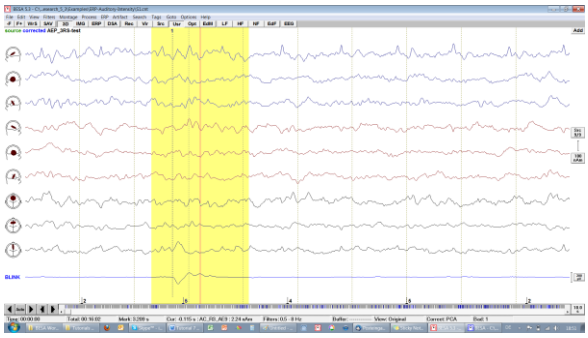
8. Return to the main window. The newly generated source montage was already applied to the grand average data. Please note that the activity increase from 60 to 100 dB in the frontal source can clearly be observed using this montage.



9. 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…**'



10. Press the **Usr** button and select our source montage '**HighIntensity_3RS-test**'. We will use this source montage later on for coherence analysis.



C: 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:

1) **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 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.

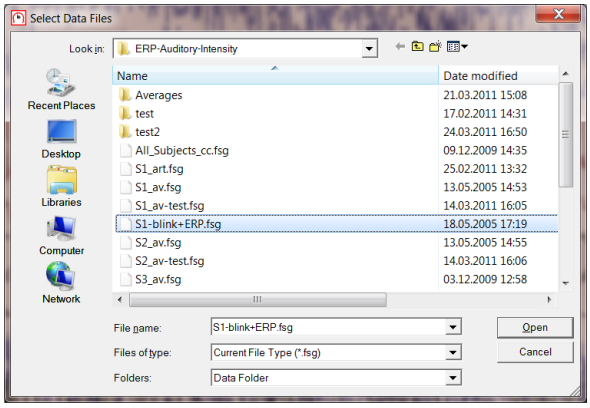
   Therefore, this method is not recommended.

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

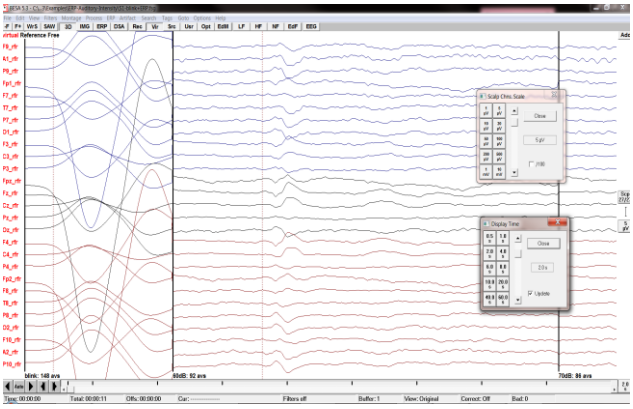
D: Artifact Correction of averaged ERP data

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.

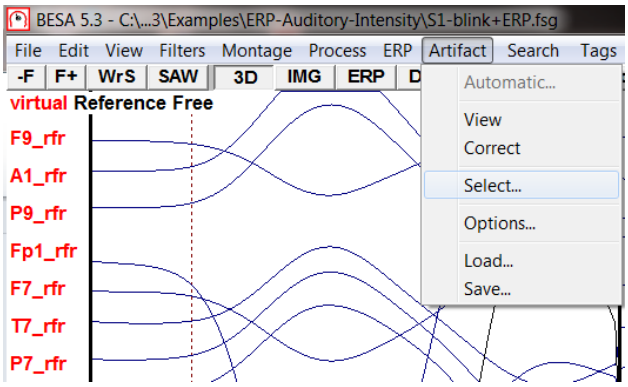
1. Please open the file 'S1-blink+ERP.fsg' located in the 'ERP-Auditory Intensity folder'. This file contains the average of conditions 60 to 100 dB, Low, High and All along with an averaged eyeblink. Change to the virtual reference-free montage by pressing the **Vir** button and selecting the entry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



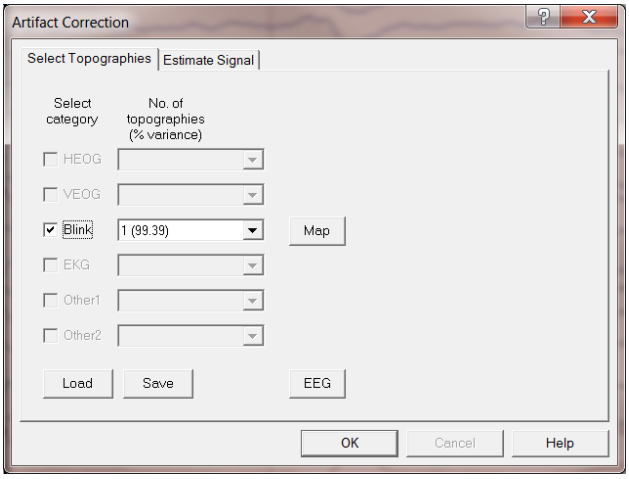
2. We will demonstrate the effect of artifact correction with focus on the 60 dB condition. Use the **time scaling button** at the bottom right of the window to set the time scaling to **2.0 s**. Press the **amplitude scaling button** under the **Scp** button to increase the displayed scalp channel amplitude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



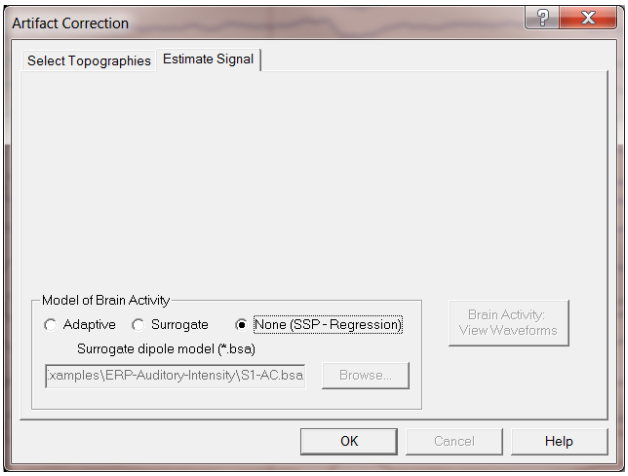
3. From the **'Artifact'** menu, choose **'Select…'**.



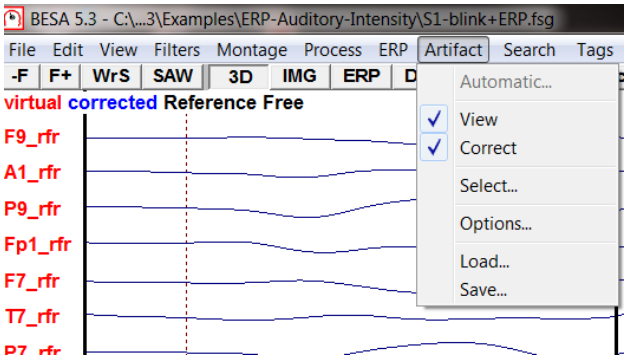
4. Note that the blink artifact topography that we defined in the raw data has been assigned automatically to the averaged file as well, but is not selected by default. **Set the checkmark** next to the '**Blink**' category.



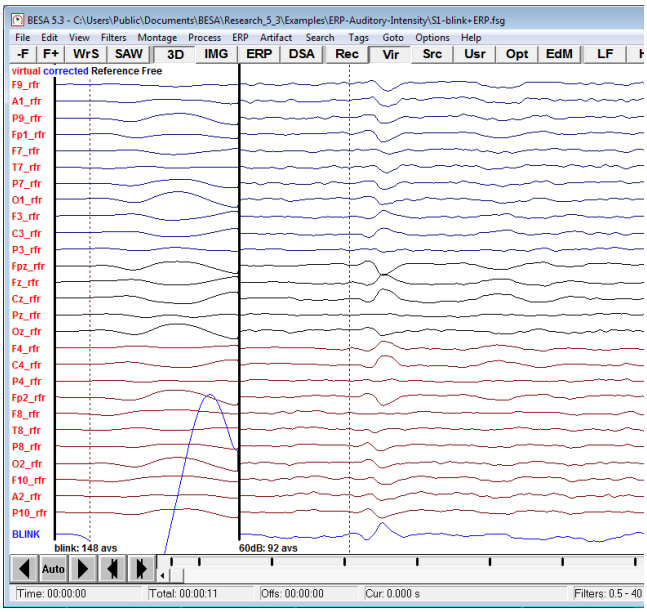
5. Switch to the **'Estimate Signal'** tab. We will compare the three different approaches for artifact correction offered by BESA Research. First, select **'None (SSP-Regression)'** in the '**Model of Brain Activity**' box to correct the data by subspace projection. Press **'OK'.**



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

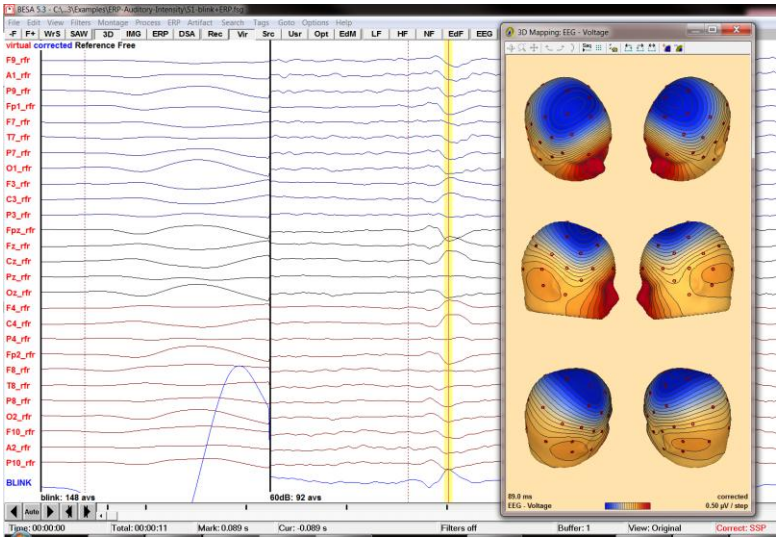


7. Press the **scaling button** for the Blink channel to scale it up to **10 µV**. The blink activity has been largely corrected. Remaining activity in the first segment is due to the 0.6 % of variance that is not explained by the first PCA component. Including a second or third PCA component in the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

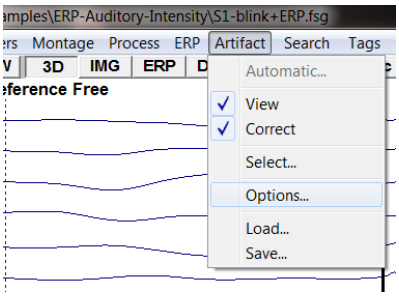


8. **Double-click** in the second segment at the latency of the N100 component (ca. **90 ms**) to obtain a 3D whole head map of the corrected data at this latency. The map is severely distorted by the SSP correction: Instead of the N100 topography, a strong frontal positivity is observed that is typical 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

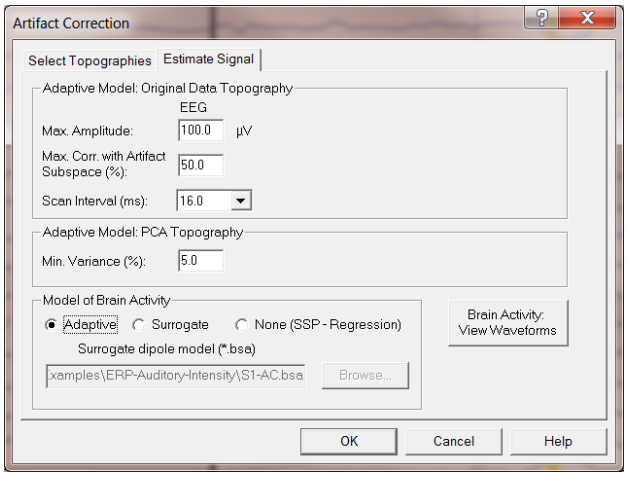
removed from the data by the subspace projection. The correlation can be seen in the bottom trace which depicts the blink signal estimated using its topography throughout the data set. At the time of the N100, it shows an upward deflection. Hence, SSP subtracts this part weighted with the blink map. Thus, a more inferior frontal positivity is introduced. The net resulting map, however, has been made orthogonal to the blink topography over all electrodes.



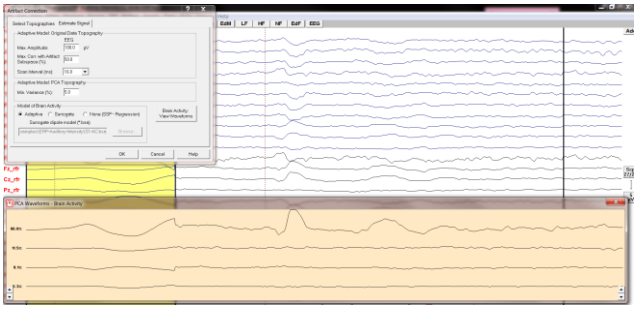
9. To see how the adaptive method improves the result, **select 'Artifact/ Options'.**



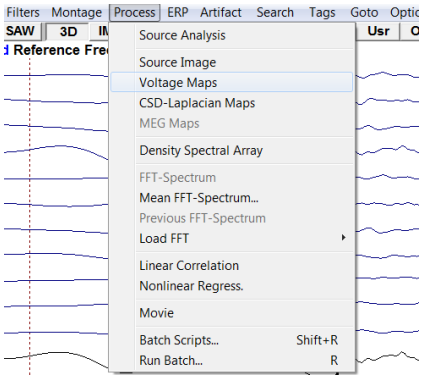
10. As 'Model of Brain Activity' select **'Adaptive'**. The '**Minimum Variance**' threshold determines the number of topographies that are considered brain activity which is contrasted against the artifact topography. Set the threshold to **5%.** To view the time course of the brain topographies, press **'Brain Activity: View Waveforms'.**



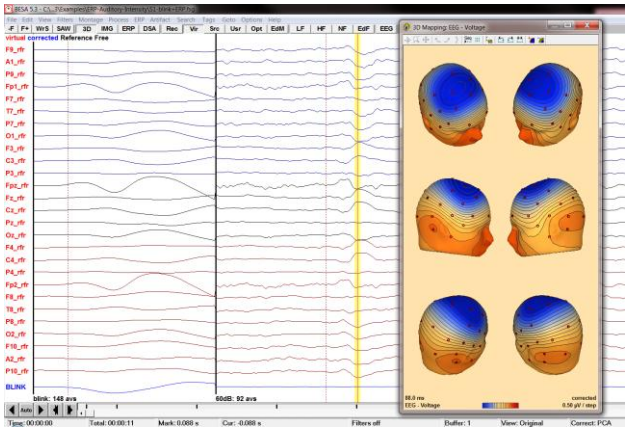
11. Three PCA topographies serve as model for the brain activity. In their time courses, the auditory N100 component is mainly represented by the first component. Note that the 4 displayed components have topographies that are orthogonal to each other, but they are not orthogonal to the artifact topography. Accordingly, this approach will retain brain activity even when it is spatially correlated to the artifact topography.



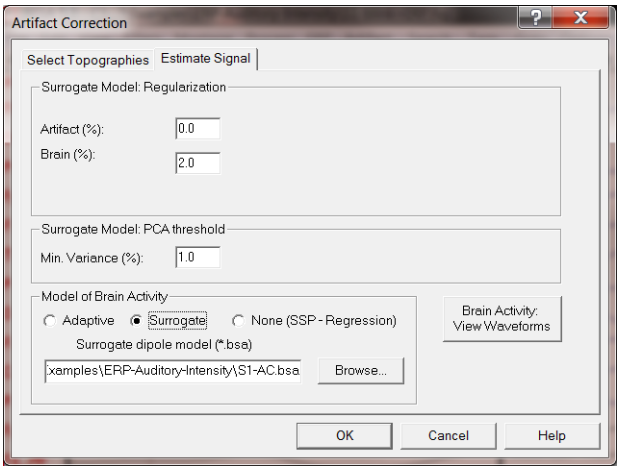
12. To see this, close the 'PCA Waveforms' and 'Estimate Signals' windows. If the cursor at ca. **90 ms** is still set in the second condition, select '**Process/Voltage Maps**' or type the hot key '**M**' on the key board to obtain the 3D map of the adaptively corrected data.



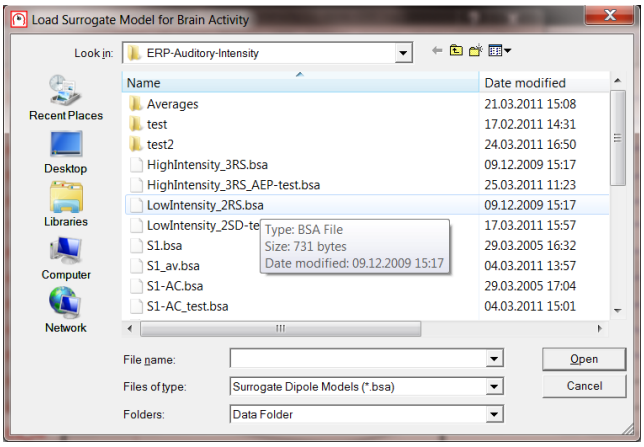
13. The maps are nearly undistorted by the artifact correction and show the typical N100 topography. As a correlate, note that the blink waveform does not show any interaction with the auditory evoked components. This indicates that blink and brain activities have been very well separated by the adaptive 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.

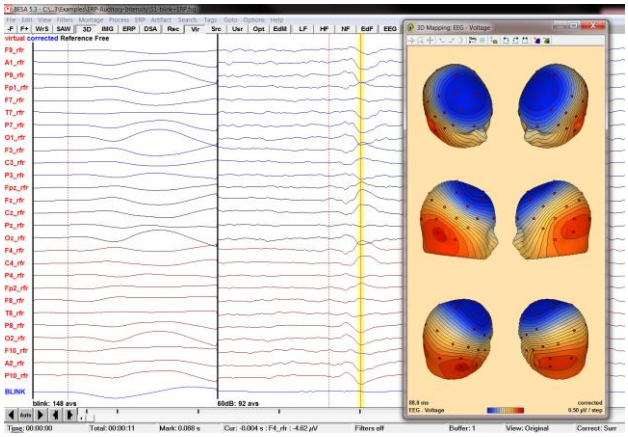


15. Press the '**Browse**' button to load the solution '**LowIntensity_2RS.bsa**' that was created earlier on, consisting of a symmetrical pair of regional sources in the auditory cortex. The stored two regional sources model the bilateral brain activity in the supratemporal region and are combined with the defined artifact topography to separate artifact and brain signals[1].



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

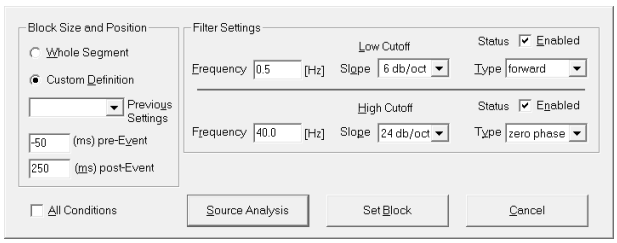
16. Press '**OK**' and press '**M**' to view the 3D map of the surrogate-corrected data at the cursor latency. Similar to the adaptive method applied to the averaged data, the brain topographies are nearly undistorted. The blink waveform does not show any interaction with the auditory evoked components.



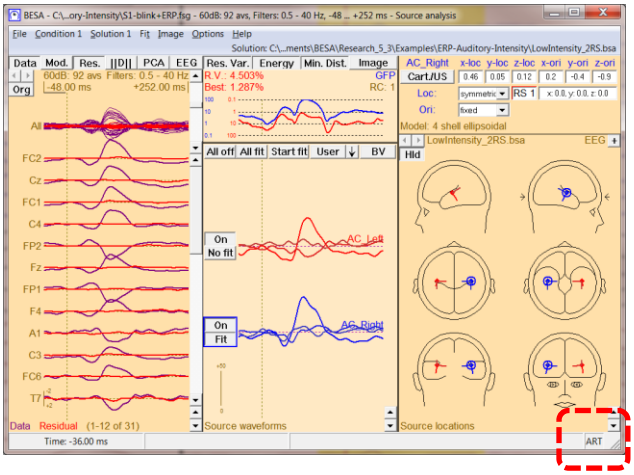
E: Source analysis of artifact-corrected data (not recommended)

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.

1. **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 artifact-corrected 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:

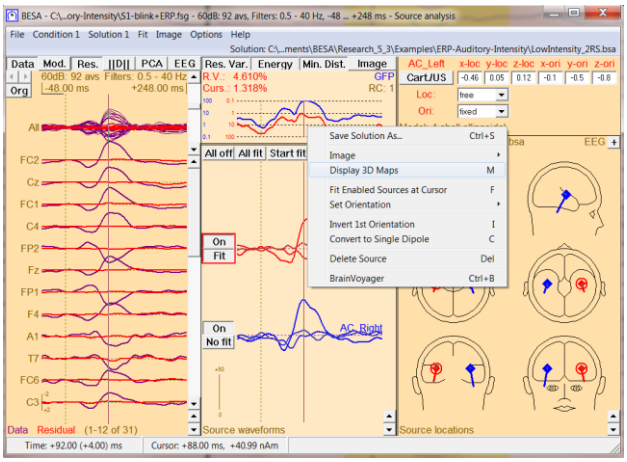
Uncorrected data: $D = L * S$

$$\Downarrow$$

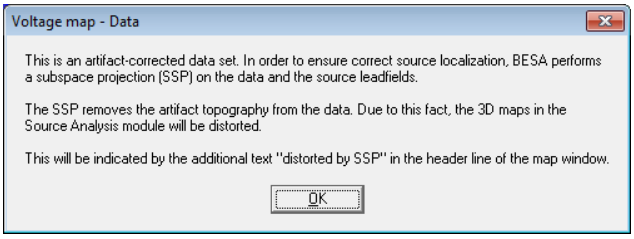
Corrected data: $(P*D) = (P*L) * S$

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

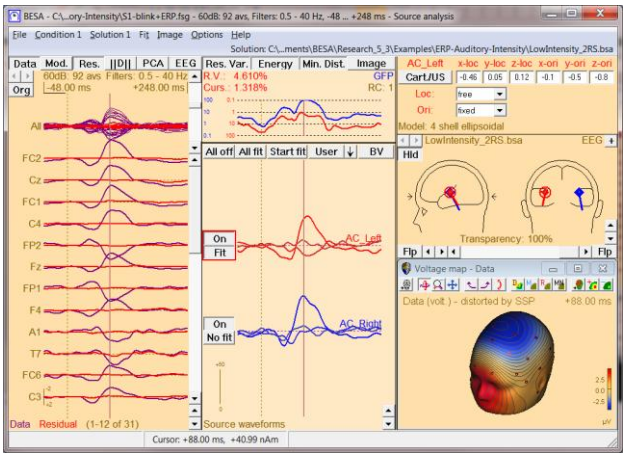
3. **Double-click** in the waveform box to set a cursor at the N100 latency. **Right-click** and select **'Display 3D Maps'** or type the hot key '**M**' on the keyboard.



4. BESA Research displays a warning message. It explains why the following map will be distorted. Press **'OK'** to confirm.



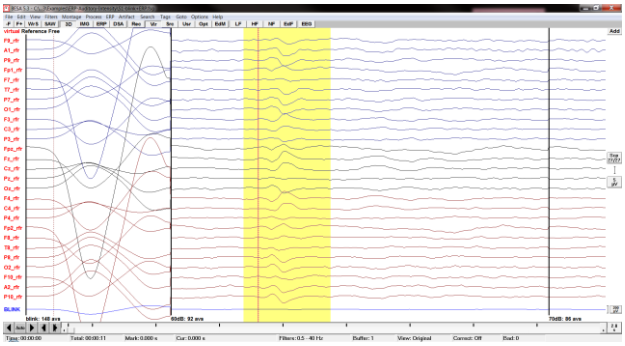
5. The topography is severely distorted by the subspace projection that was applied to the data. Although the source waveforms obtained by modeling the corrected data are undistorted, the data displayed in the channel box and in the global field power (GFP, blue line in the upper middle box) are distorted. As a consequence, *loading corrected data into the source analysis window is less recommended*. Close the source analysis window.



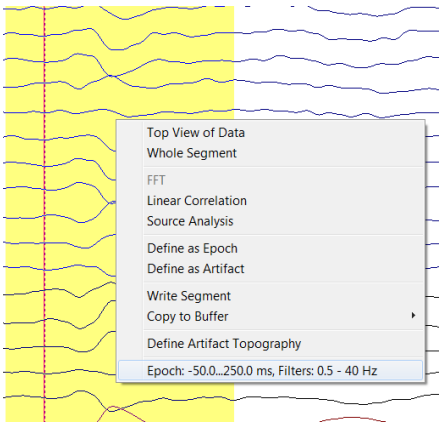
F: Contrasting artifact and brain topographies – the 'Optimizing method' (recommended)

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.

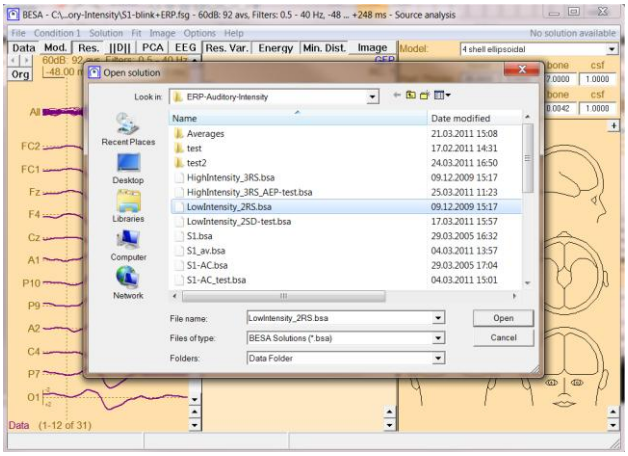
1. **Switch off artifact correction** by pressing '**ctrl-E**'. You can confirm it is switched off as 'corrected' is no longer displayed in the top left corner of the main window.



2. **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**.

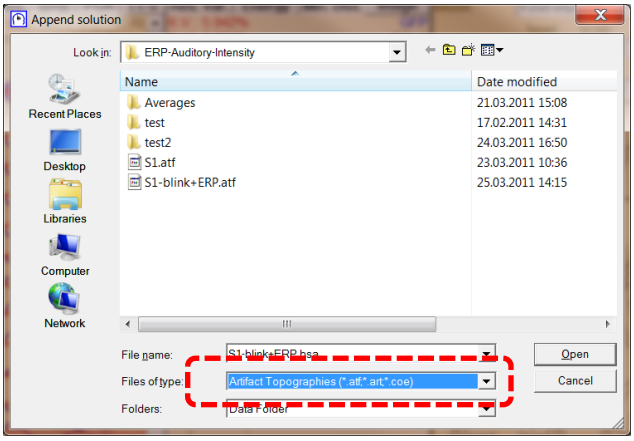


3. Load the source model '**LowIntensity_2RS.bsa**' by pressing '**File / Open Solution**'.

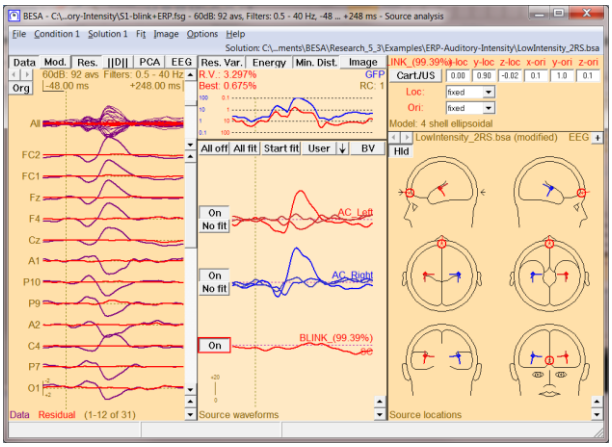


4. Next we will load the artifact topography into the source analysis window. This will create a spatial component explaining the blink topography.

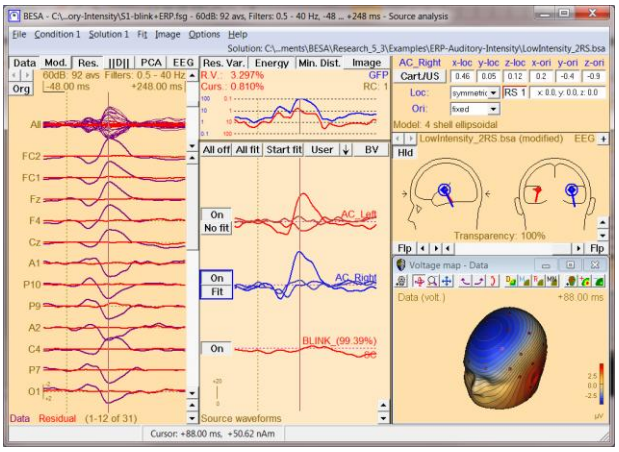
5. Press '**File/Append Solution…**'. In the drop-down menu **'Files of type:'** select **'Artifact Topographies (*.atf;*.art;*.coe)'.** Select '**S1-blink+ERP.atf**' and press '**Open**'.



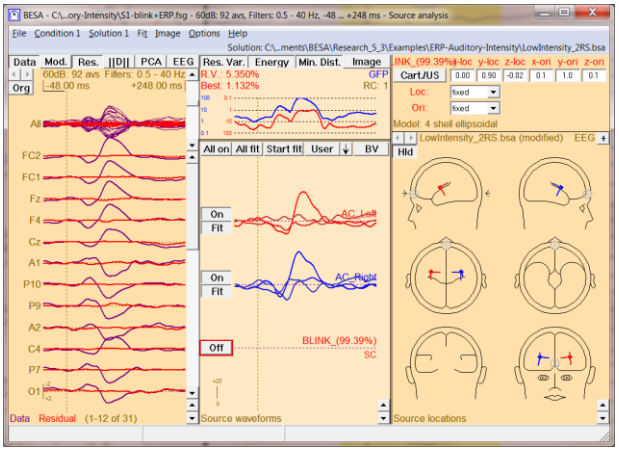
6. The topography of the main PCA component of the blink artifact is now appended to the current solution. The corresponding waveform and the equivalent location are shown in the source analysis window. This is the component explaining 99.4% of the artifact variance that was selected during the artifact definition.



7. Again **double-click** in the waveform box to set a cursor at the N100 latency. **Right-click** 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.



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 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.



What does BESA Research provide?

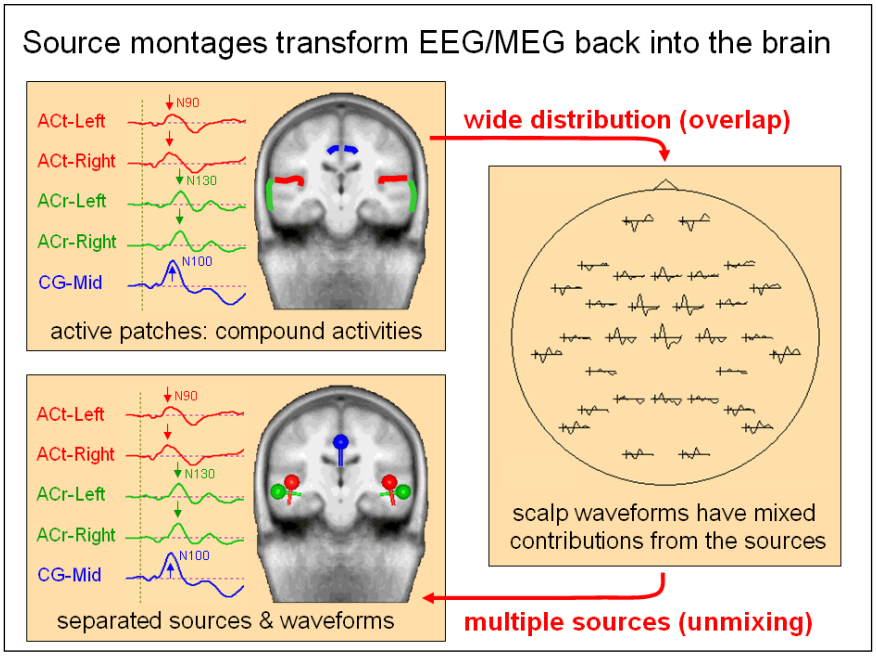
- ✓ Time-Frequency Analysis (Complex Demodulation)
- ✓ Scalp Coherence
- ✓ Source Coherence
- ✓ Single-subject statistics (Permutation Test)
- ✓ Multiple Source Beamforming

A: Principles of scalp and source coherence

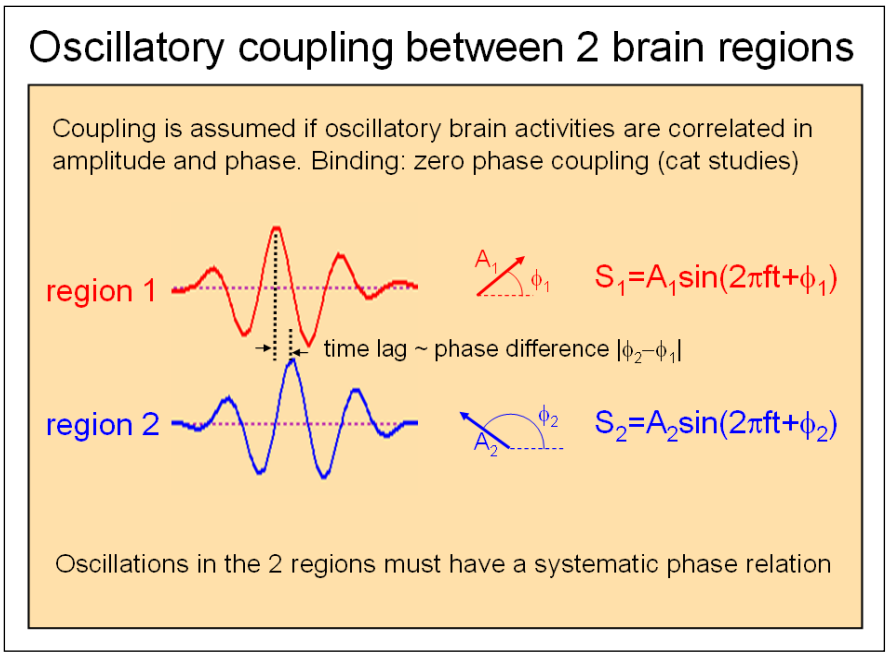
Recently, an increasing number of papers on oscillatory coupling between brain regions in animal studies and on time-frequency analysis of human EEG and MEG data has been published. BESA Research features several tools for fast and user-friendly time-frequency analysis including source and scalp coherence.

First, let us introduce some terminology for clarification of the concepts:

- Surface waveform: a time signal recorded from EEG-electrodes or MEG sensors
- Source waveform: a time signal calculated for a specified brain region or cortical surface
- Source montage: transformation of the on-going EEG/MEG into the estimated contributions or source waveforms of a set of brain regions



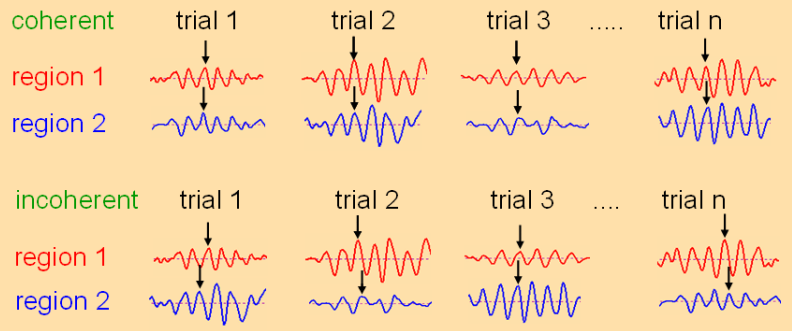
- Time-frequency analysis: analysis or display of the event-related time-locked or induced activity in the time-frequency domain
- Time-locked activity: event-related signal with similar waveshape over trials
- Induced activity: oscillatory activity occurring in a certain event-related time window with varying time lag and phase
- Oscillatory activity: activity occurring with several oscillations in a narrow frequency band; can be time-locked and/or induced



- TSE: temporal spectral evolution, change in power or amplitude over time
- ERD/ERS: event-related (de)-synchronization, change in power or amplitude over time, used in equivalence to TSE
- ERSP: event-related spectral perturbations, change in power or amplitude over time. This is the more general term and comprises ERD/ERS/TSE that are related to baseline.
- Correlation: correlation between two time signals, i.e. scalar product of normalized time signals in time domain

Coherence measures correlation over trials

- same frequency in coupled regions within each trial
- possible variation of frequency and duration across trials
- similar phase difference across trials
- similar amplitude fluctuations across trials



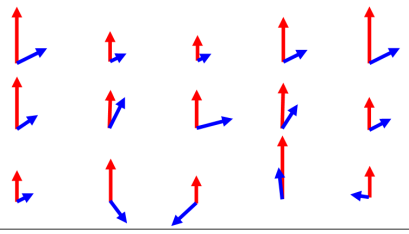
- Spectral-temporal density function S(f,t): amplitude of a signal at a certain frequency f and interval t relative to an event
- Coherence: correlation of two spectral density functions $S_1(f,t)$ and $S_2(f,t)$ over trials, i.e. squared scalar product of $S_1(f,t)$ and $S_2(f,t)$ over trials, normalized across all trials
- Phase locking value (PLV): correlation of two normalized spectral density functions $S_1(f,t)$ and $S_2(f,t)$ over trials, i.e. mean of the scalar product of the normalized $S_1(f,t)$ and $S_2(f,t)$ over trials. Amplitudes are neglected, only phase relationships between two oscilllations are considered.

Coherence: correlation in frequency domain

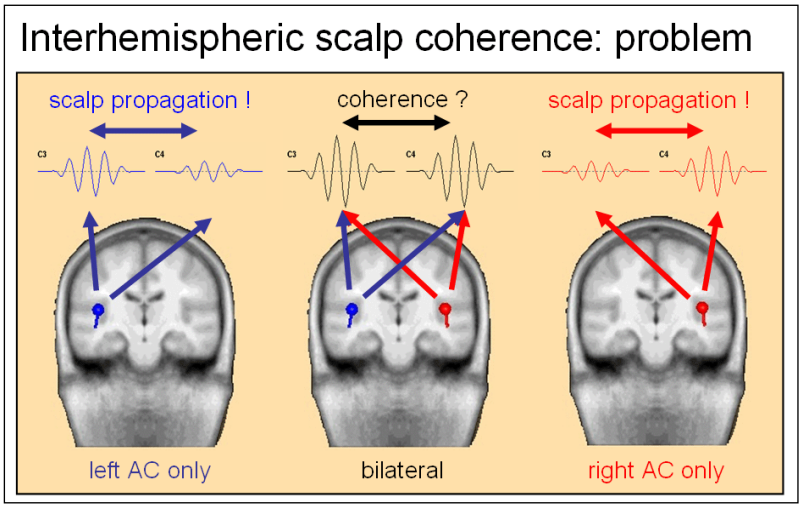
Time-frequency signal: $S(f,t) = A(f,t) \cdot e^{i \cdot \phi(f,t)}$

Coherence: $$\mathrm{Coh}(f,t) = \frac{\left|\sum_n S_{1,n} \cdot S_{2,n}^*\right|^2}{\sum_n |S_{1,n}|^2 \cdot \sum_n |S_{2,n}|^2}$$

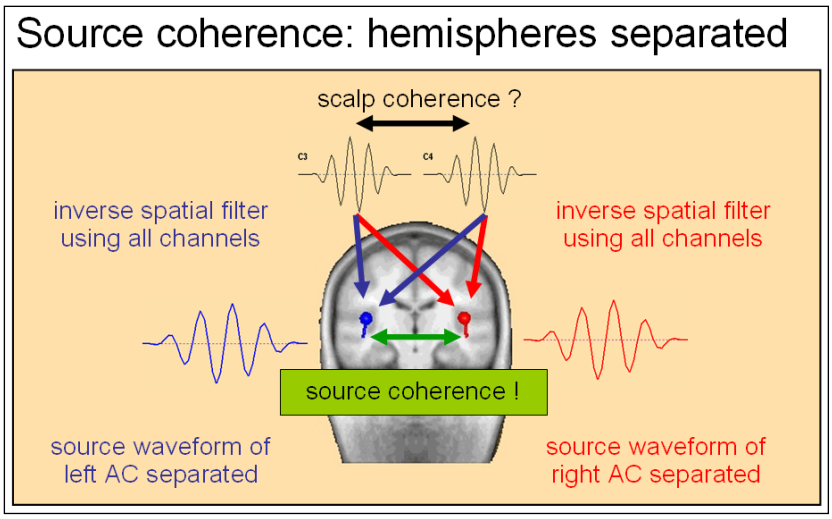
Phase locking value: $$\mathrm{PLV}(f,t) = \left|\frac{1}{N}\sum_n e^{i(\phi_{1,n}-\phi_{2,n})}\right|$$

t_1	t_2	t_3	t_4	t_5	Amp.	Phase	Coh
					ideal	ideal	1
					corr.	corr.	<1
					rand.	rand.	0

When coherence is calculated at the surface between 2 EEG channels or 2 MEG sensors, activity from various brain regions is picked up in each of the surface channels. Oscillatory activity in one brain region can already lead to a strong coherence between 2 surface channels because of the wide distribution of focal brain activity at the surface. This is due to the nature of the dipole fields when recording remotely and due to the smearing effect of the volume conduction in EEG. As a consequence, a coherence measure between surface channels cannot distinguish between coherence due to propagation and real coherence between the oscillatory activities in two coupled brain regions.



Whether true coherence can be detected at the scalp, depends mainly on the relative orientation of the source currents in the underlying brain regions and, to a lesser extent, on their distance in location. In the case of the auditory cortex, for example, both the left and right temporal planes produce vertical activity with strong bilateral contributions centrally (e.g. at C3/C4 and F3/F4). The spatial correlation of the radial activities at the lateral surfaces of the superior temporal gyrus is also very large between right and left scalp electrodes, e.g. at T3/T4. A current-source density montage (CSD or Laplacian) can reduce the effects of propagation on scalp coherence to a certain extent, because it enhances the radial current from the underlying cortex relative to more remote sources to some extent.

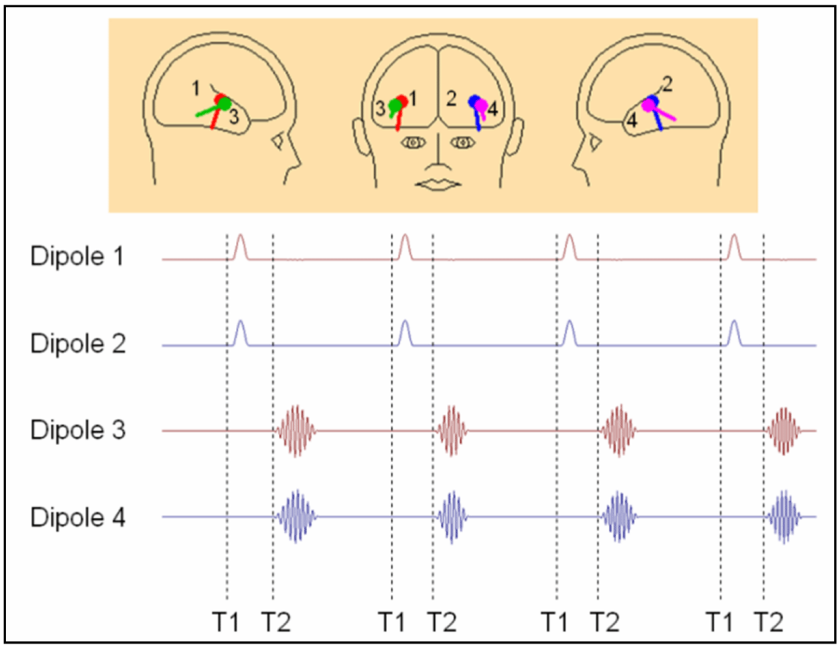


An optimal separation is obtain by a source montage derived from a multiple source model. The model is used to create an inverse spatial filter, i.e. a source montage, that separates the different brain activities. Activities that are not accounted for by the model, e.g. from background noise or EEG, are distributed amongst the sources and may therefore lead to 'noise coherence' between source channels. This 'noise coherence' can be large for sources which have very similar spatial topographies. It can be reduced by increasing the regularization constant of the inverse at the expense of a larger cross-talk between the sources or by including specific sources accounting for the 'noise'.

The principal steps to calculate a time-frequency diagram and source coherence are illustrated in the figure below for the real data example of ERN. A multiple source model is created from averaged ERP data and/or sources in brain regions known to contribute from fMRI/PET studies using a similar task. The source model is then used to calculate a source montage and the source waveforms of the single trials. Next, each single trial is transformed into the time-frequency domain by selecting a certain temporal resolution using complex demodulation (a principle similar to FM radio). From the single trials, time-frequency displays are generated by averaging spectral density amplitude or power over trials. Source coherence is calculated by averaging the cross-spectral density of one reference channel with all other channels over trials and normalizing by the averaged auto-spectral densities (cf. illustration above).

B: Simulation of evoked and induced activity 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 on-going 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 µV 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 induced 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 µV (file AC_Osc5.foc) and 20 µV (file AC_Osc20.foc), respectively. The oscillation of dipole 4 follows that of dipole 3 with a constant delay of 5 ms.

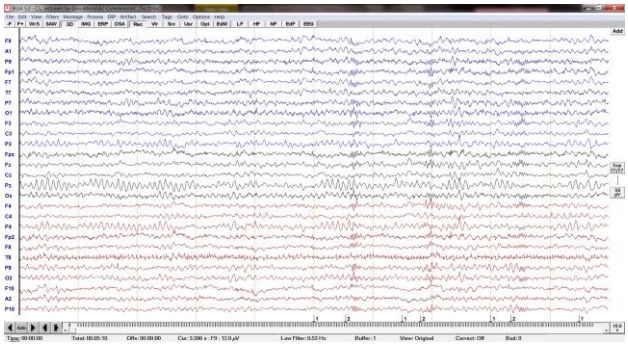


We will now learn how to process these data to extract both the evoked time-locked and the induced random-phase oscillatory activity. Next we start with the data set which has a high signal-to-noise ratio to learn the processing steps (AC_Osc20.foc with induced activity of 20

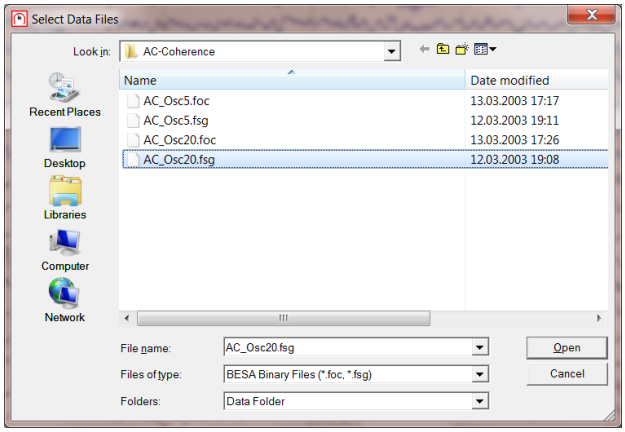
µV at Fz, corresponding in size approximately to the background EEG amplitude). Then we will explore the sensitivity in the data set with the smaller induced activity of 5 µV at Fz.

C: Inspecting and understanding the simulation with real EEG background

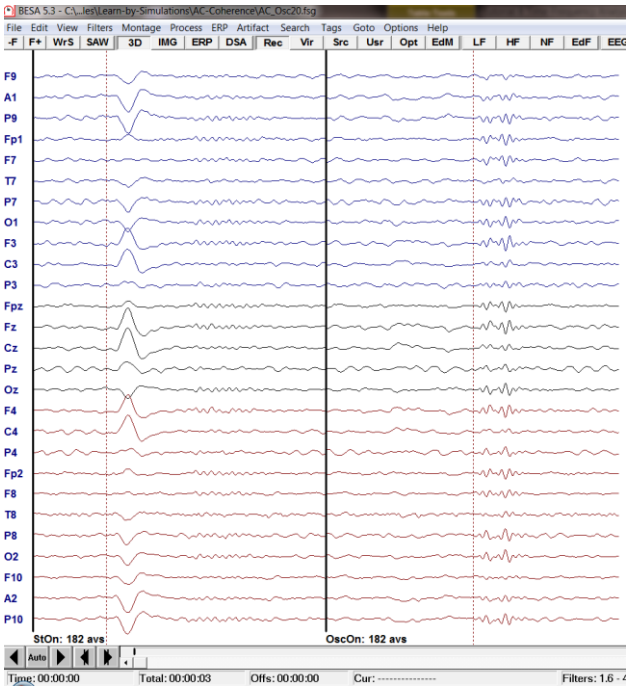
1. Open file '**Learn-by-Simulations\ AC-Coherence\AC_Osc20.foc**' in the BESA Research Examples folder. Note that e.g. at electrode C3 the evoked monophasic response locked to trigger 1 is just visible on top of the background noise. The induced oscillatory activity following trigger 2 can be observed as well.



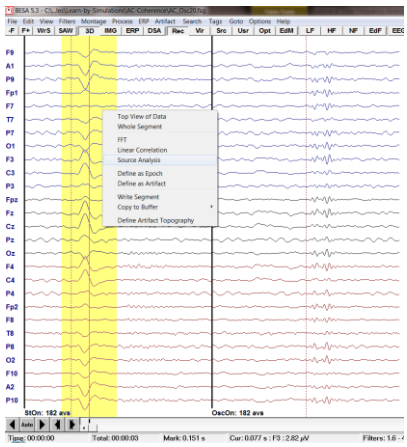
2. In order to better understand the properties of the simulated activity, we load the averaged evoked response file. Open file '**AC-Coherence\AC_Osc20.fsg**'.

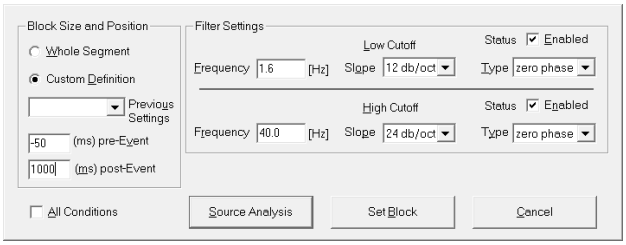


3. The first segment contains the average triggered by the stimulus onset, the second averaged segment is time-locked to the onset of the jittering oscillations. Relative to stimulus onset, the induced oscillations cancel out in the average. The jitter in the frequency range of the oscillation (20-28 Hz) also reduced the signal average relative to the onset of the oscillation. Note that the waveshape of the monophasic activity is altered by low cutoff filtering applied to the average to reduce slow drifts in the data.

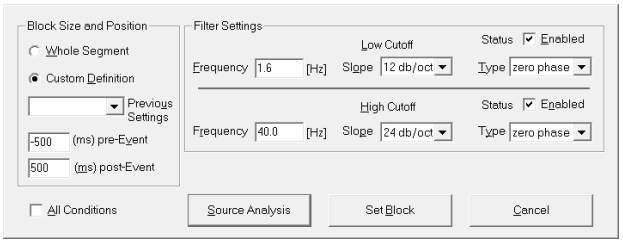


4. To see how the averaged surface activity is transformed into brain space, we use the source analysis module. **Left drag** over the first segment. **Right click** and select **'Source Analysis'**. For this segment we use an interval from **-50 to 1000 ms** and choose a **Low Cutoff** filter of **1.6 Hz, 12dB/Oct zero-phase** and set the **High Cutoff** filter to **40 Hz, 24 dB/Oct, zero-phase**.

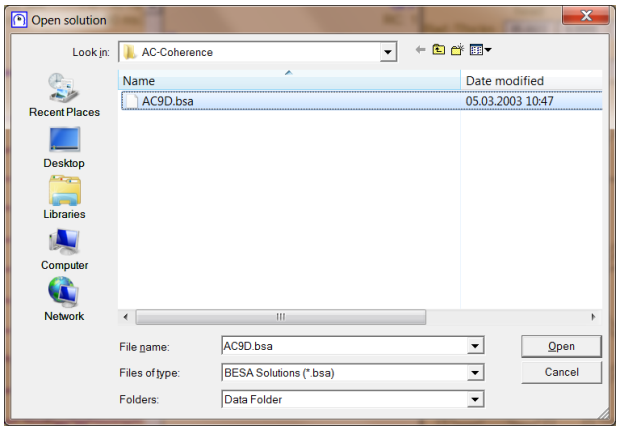




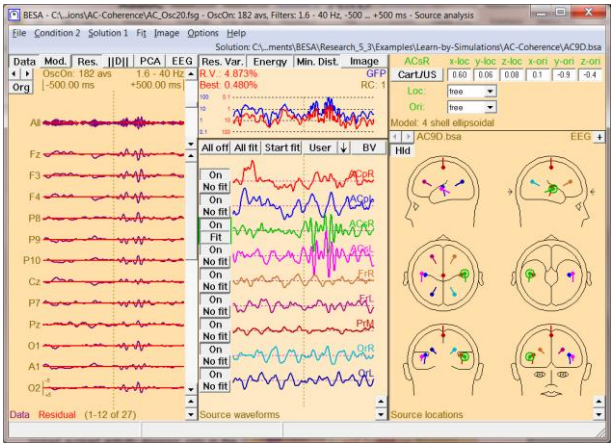
5. The source analysis window opens. Minimize this window to load the **second segment** as well, but now select an epoch from **-500 to +500 ms** relative to the onset of the oscillation.



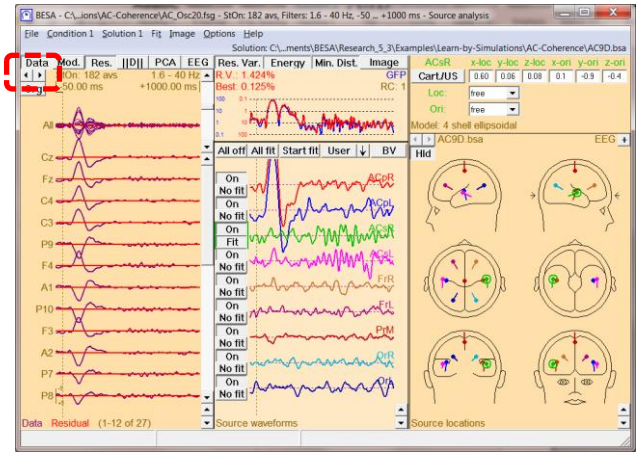
6. Select '**File / Open Solution'** to load the predefined solution '**AC9D.bsa**'.



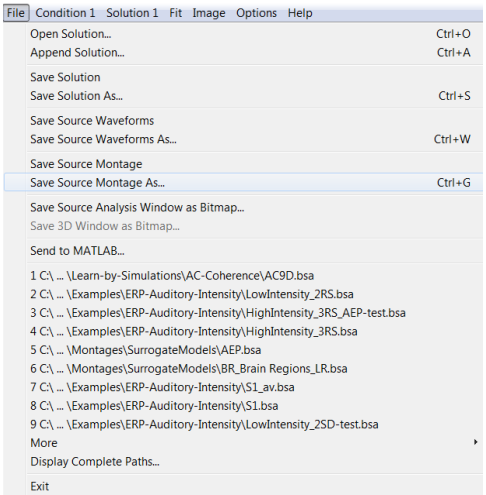
7. The model contains the two pairs of dipoles that were used for the simulation. In addition, five probe sources are included that pick up background EEG activation. In condition ‘OscOn’ (containing the oscillation-locked averages), dipoles 3 and 4 show small oscillatory activities. The evoked activation in sources 1 and 2 is barely visible, because of its large onset jitter with respect to trigger 2.



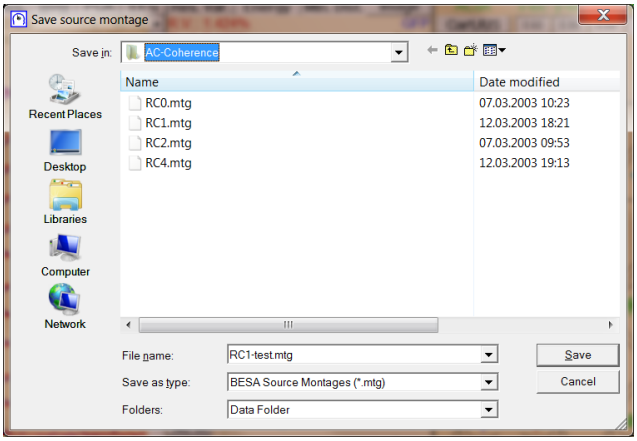
8. Use the **arrow buttons** in the upper left corner to switch to condition 1. The time-locked evoked activity appears only in the related sources 1 and 2. The induced oscillations in source waveforms 3 and 4 cancel out almost completely. The five probe sources show only small background noise activity. Note the almost complete separation of the activities in the different brain regions as compared to their wide distribution in the scalp waveforms.



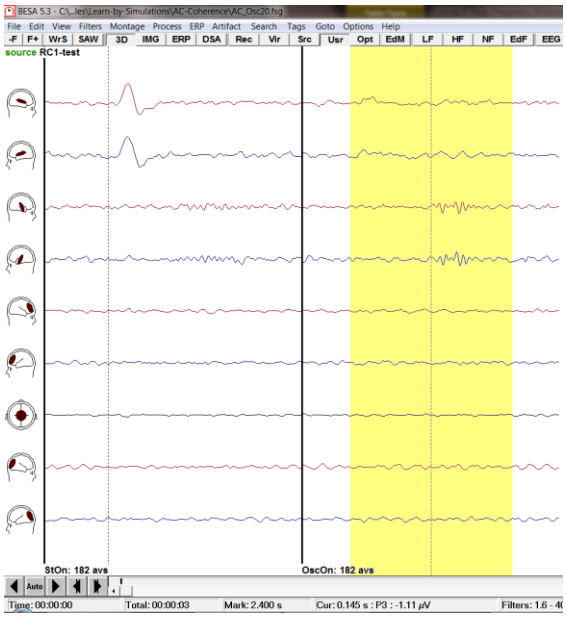
9. We want to use our nine-dipole model as a source montage for the continuous data. Create a source montage from the displayed multiple source model by selecting **‘File –> Save Source Montage As…’**. Note that the source montage not only contains the source coordinates, but also stores the selected regularization constant (set to 1 by default).



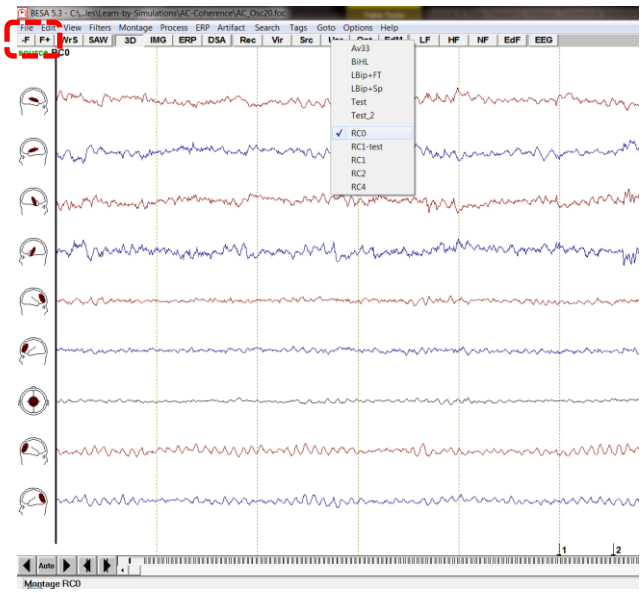
10. When prompted for a name for the montage to be saved, enter '**RC1-test**', to indicate that a regularization constant of 1% was used. Press **'Save'**. This will create the new source montage and automatically apply it to the current file in the main window.



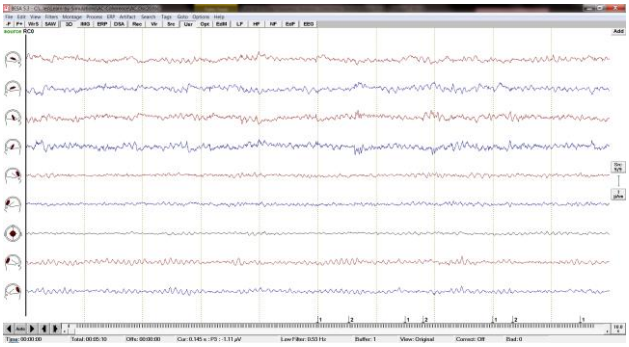
11. Close the source analysis window. The display now shows the averaged data segments transformed into brain space by the generated source montage. Note the advantage of the source montage: instead of the distributed overlap of the different activities at the surface channels, we now obtain largely 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.



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 '**RC0**'.

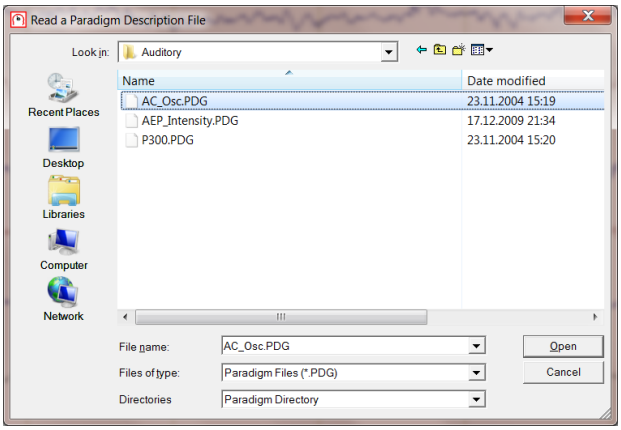


13. The continuous 27-channel EEG data has been transformed to nine source waveforms. We want to use the advantage of having contrasted the activities of the different waveforms to apply time-frequency analysis to the nine source waveforms and analyze the coherence between them.

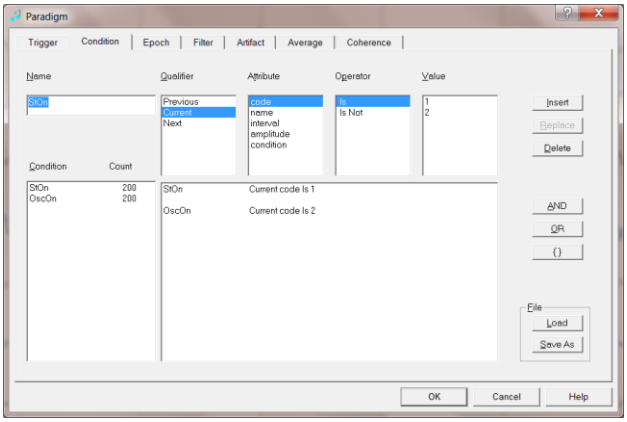


D: Time-frequency analysis

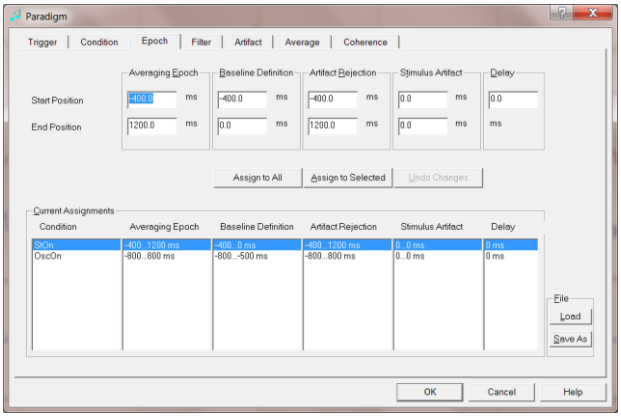
1. Select '**ERP / Open Paradigm**' to load the predefined settings in file '**Auditory\AC_Osc.pdg'** in the Paradigm directory.



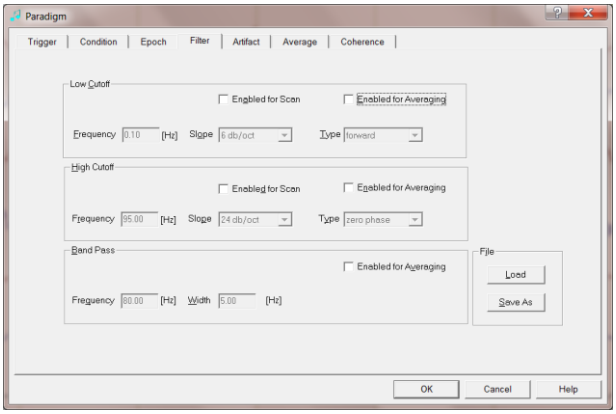
2. The conditions 'StOn' and 'OscOn' have been predefined to represent triggers 1 and 2. 200 events are detected for stimulus onset and oscillation onset.



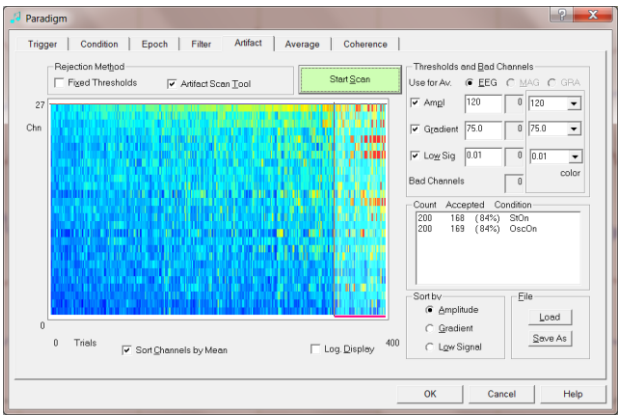
3. View the **Epoch** tab. The epoch settings have been predefined to span a *sufficiently long epoch* including a baseline of 400 ms for TSE. Note the different settings for both conditions. The later oscillatory onset requires an earlier baseline prior to stimulus onset.



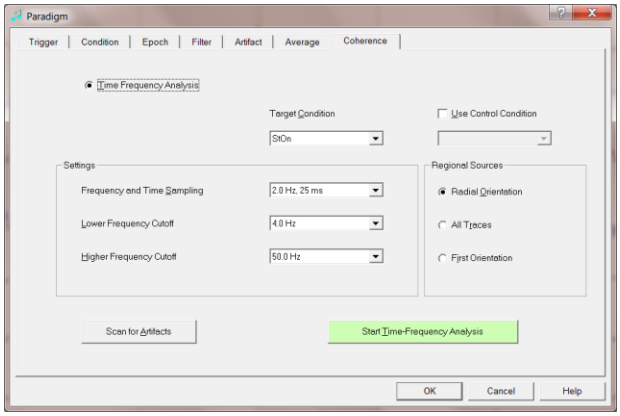
4. In the **Filter** tab no filters are selected, in order to obtain undistorted results of the time-frequency analysis. In the presence of strong low frequency activity, it can be useful to set a low cutoff of 0.2, 0.5, 1, or 1.6 Hz.



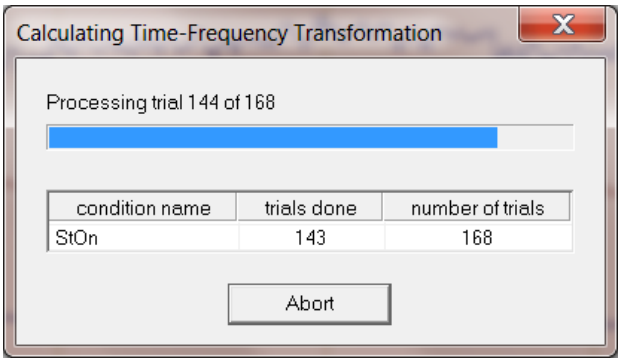
5. Select the **Artifact** tab and press '**Start Scan**'. Artifact epochs are automatically identified and excluded according to the default thresholds displayed in the upper right corner. Sort by amplitude, gradient and low signal by checking the corresponding item in the 'Sort by' box. The suggested threshold settings do not have to be modified for the current file.



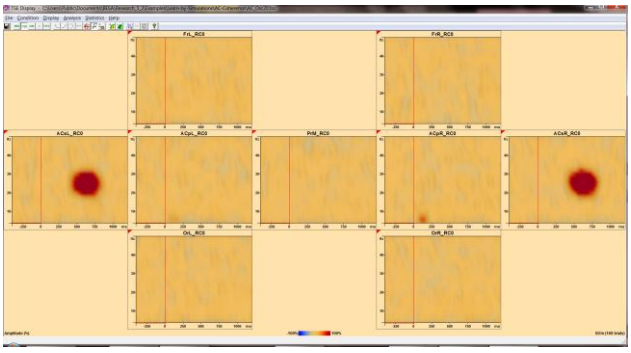
6. Select the **Coherence** tab. The '**Condition**' drop down menu allows choosing one condition for event-related time-frequency analysis. Time-frequency sampling is set in the 'Settings' box. A compromise has to be made between high frequency and low temporal resolution or low frequency and high temporal resolution. Default time-frequency sampling is intermediate with a frequency sampling of **2 Hz** and a time sampling of **25 ms**. Leave this and set the frequency range to **4-50 Hz**.



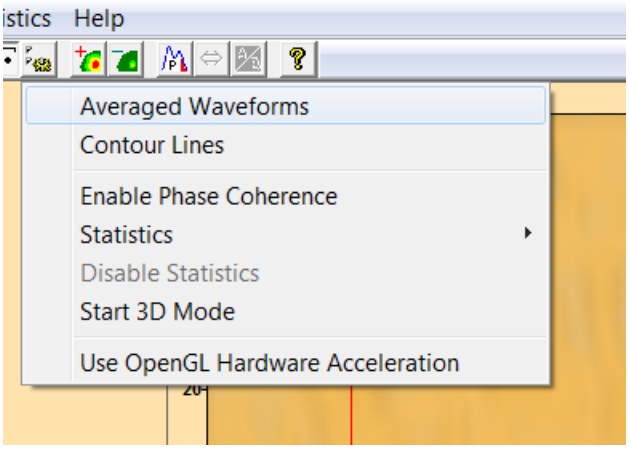
7. Press the button '**Start Time-Frequency Analysis**'. Time-frequency analysis starts and complex demodulation is performed for each trial and each montage channel.



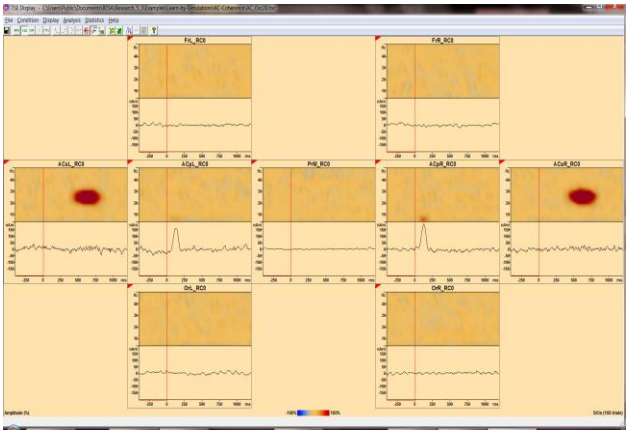
8. After calculation is completed, the time-frequency display window pops up with the TSE view. Each graph plots the spectral amplitude density of one montage channel over time (x) and frequency (y) normalized relative to the baseline for each frequency. Channel and montage labels are displayed on top of each diagram. Induced activity around 25 Hz appears in channels ACsL and ACsR in the latency range of 400 to 800 ms. The short mono-phasic time-locked activity of the sources ACpL and ACpR is revealed in the latency range of 50-200 ms.



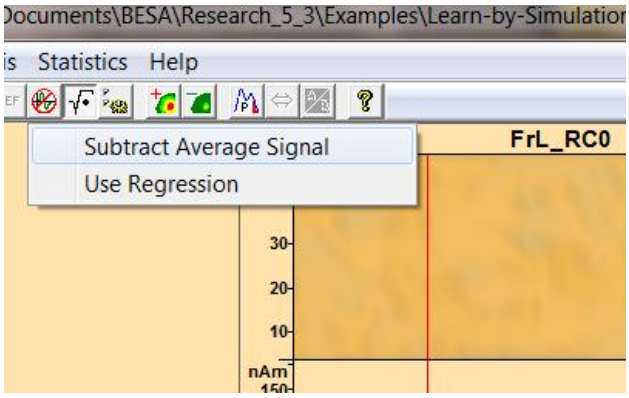
9. Press the **Options toolbar button** and select '**Averaged Waveforms**'. You may also right click into any of the panels to select this option from the popup menu.



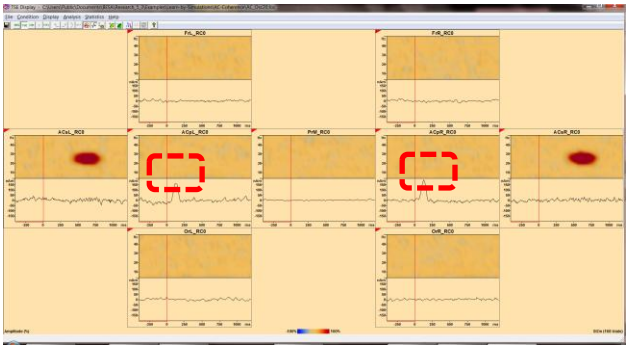
10. The display now shows the averaged evoked source waveform of each of the nine sources below the time-frequency diagram. Only the monophasic evoked potentials of channels ACpL and ACpR are seen after averaging. The oscillatory induced activities are barely visible in the average, but they dominate the TSE display. This is because the magnitudes of the spectral density over the single trials have been averaged irrespective of phase differences.



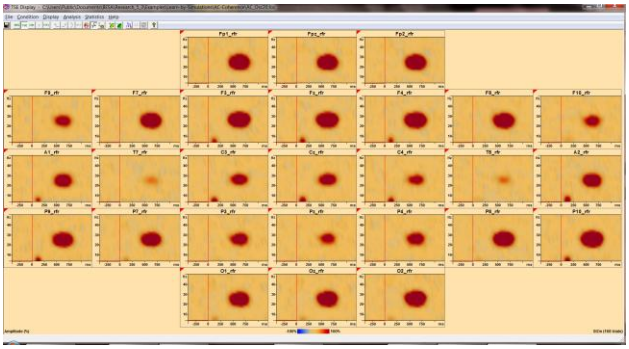
11. It is possible to remove the averaged evoked response signal from the single trial time series before computation of the TSE. Press the button. The button menu allows to subtract the averaged signal in each trial or to use regression analysis to account for amplitude fluctuations between trials before subtraction of the average. Select ‚**Subtract Average Signal**'.



Note that the evoked low-frequency amplitude increase in source channels ACpL and ACpR vanished, whereas the induced oscillatory activity is unaffected. Close the time frequency window.

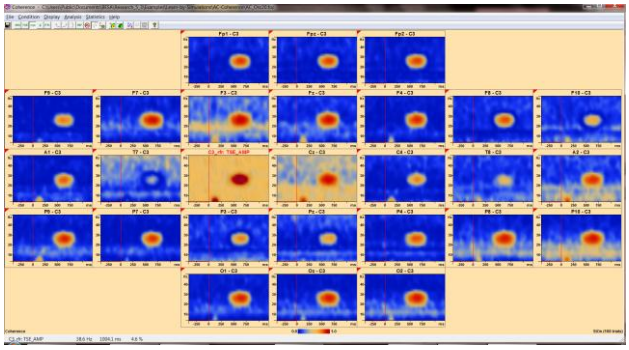


12. Next, we want to look at the time frequency diagrams at the scalp for comparison. Use the **Vir** button in the main window to change to the **reference free** montage with 27 standard electrode channels. In the **ERP** menu select '**Coherence**' and press '**Start Time-Frequency Analysis**'. Implicitly the same artifact-free selection of epochs will be used. Rescanning for artifacts is not necessary. Both evoked and induced activities appear very widespread over the scalp in contrast to the good separation in the source montage.



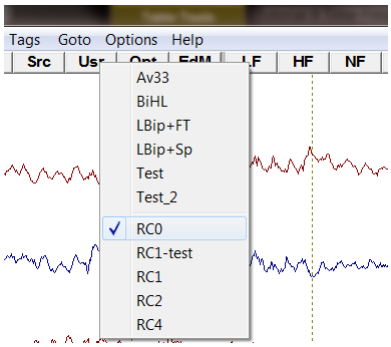
E: Coherence

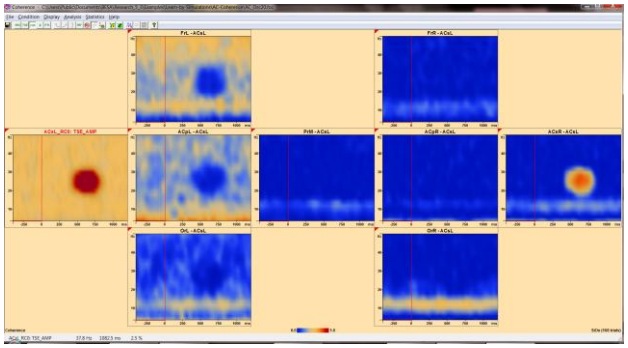
1. To obtain the coherence display, press the **Coh** button . This will display coherence relative to the first channel in the montage. **Double click** onto the channel display of C3 to obtain the coherence diagrams relative to C3. Due to the wide propagation on the scalp, coherence of the oscillatory activity can be seen almost between all channels. A similar widespread coherence can be seen for the low-frequency monophasic activity.



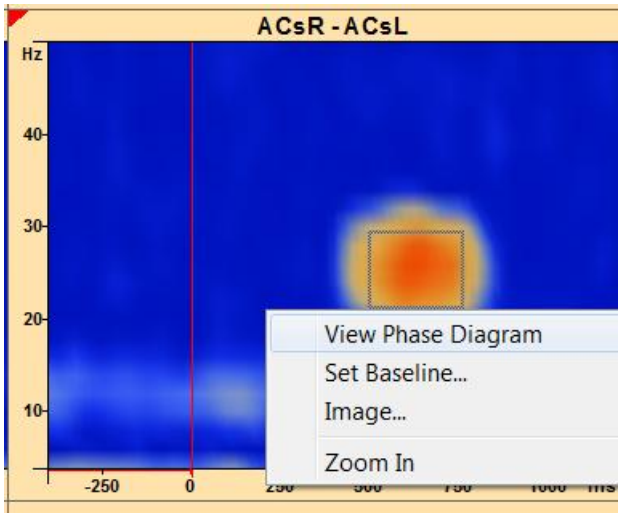
The ring-shaped coherence effect that can be observed in channel T7, for example, is due to the interference of the background noise and the oscillatory activity. Phases become more random across trials while the spectral amplitude of the reference channels becomes larger. This leads to a drop of coherence in the ring zone. In the regions where one signal dominates coherence is larger, i.e. in the noise only zones outside and in the core zone of the oscillatory activity. Close the time frequency window.

2. In the main window, press the **Usr** button to select the user defined source montage '**RC0**'. In the **ERP** menu select '**Coherence**' and press '**Start Time-Frequency Analysis**'. In the TSE diagram **double click** on the panel of channel **ACsL** to calculate the coherence with this channel**.** As expected from the simulation, ACsL is highly coherent with the corresponding right-hemispheric oscillatory source ACsR. There is practically no coherence with all other channels in contrast to the scalp.

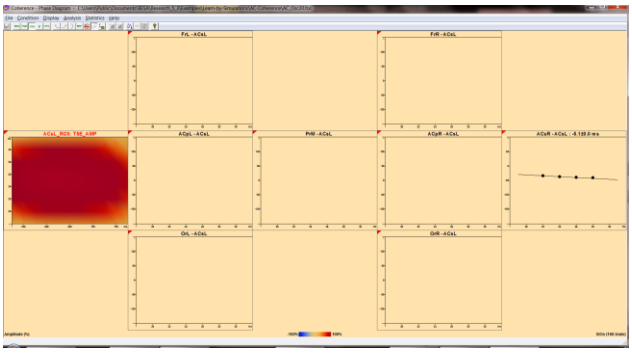




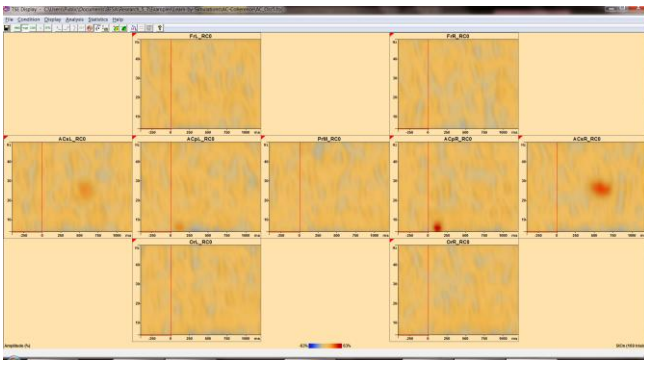
3. BESA Research can analyze the phase difference between coherent sources and compute the time delay between coherent oscillations. **Left-drag** to draw a rectangle around the time-frequency range of high coherence in source ACsR. Release the mouse button and select **'View Phase Diagram'**.



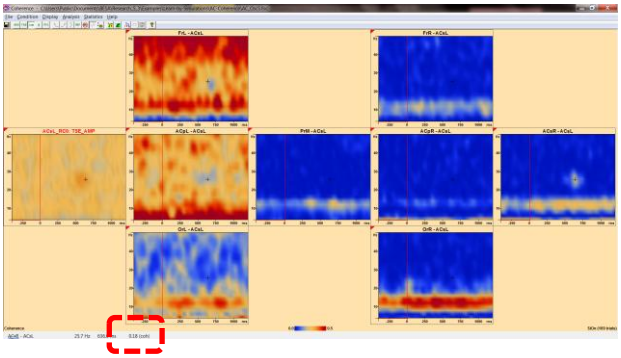
4. The panel of source ACsR now shows the phase difference relative to ACsL over the selected frequencies. A constant time delay between the oscillations results in a linear phase-frequency-relationship. From the slope of a straight line fitted to these data, the time-difference between the two sources is computed and displayed on top of the graph. In our example, a delay of -5.1 ± 0.1 ms between ACsR and ACsL is obtained (simulated: 5.0 ms).



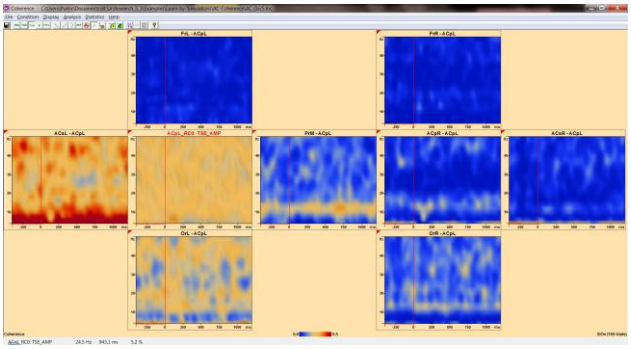
5. Next, we want to see if the coherence of the oscillatory activity in the left and right secondary AC areas ACsL & ACsR can also be extracted if the amplitude of the oscillation is smaller. **Close** the time frequency window and **open file** '**AC_Osc5.foc**', set **Usr** source montage '**RC0**' and load the file '\Paradigm\Auditory\AC_Osc.pdg' from the **ERP** menu. Run the **Artifact Scan** and start the **Coherence** analysis with the preset parameters. **Scale up** the TSE display to **+/- 63%** using the button.



6. **Double click** onto the panel of '**ACsL**'. Coherence at ACsR is now weaker but clearly emanates from the background. Use the **scale buttons** to increase the coherence scale to **0.5** and **place the cursor on top of the peak** of the oscillatory activity in **ACsR**. You can read a peak coherence of *0.18 around 630 ms* and 26 Hz in the status bar. Note the high noise correlation between ACsL and the other left hemispheric sources. Also, since the alpha rhythm leads to high correlation amongst sources because the occipital sources in the model were not optimized to account for the alpha activity.

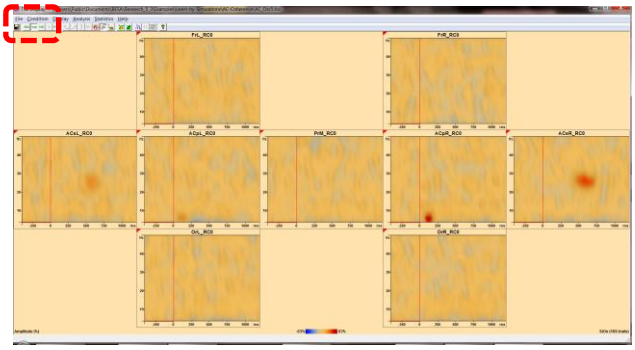


7. **Double click** onto the left primary auditory cortex '**ACpL**'. Some low frequency coherence with ACpR can be seen early post-stimulus when the monophasic time-locked activity occurs synchronously in ACpL and ACpR. Note again that there is no cross-talk and coherence with other channels except for the noise coherence with near and similarly oriented channels.

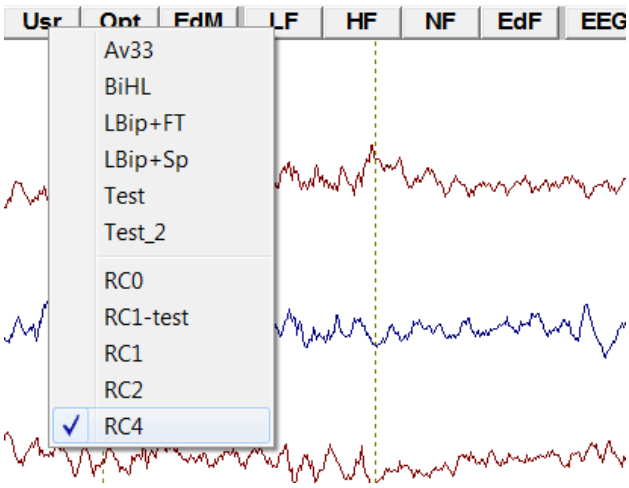


F: Regularization effects

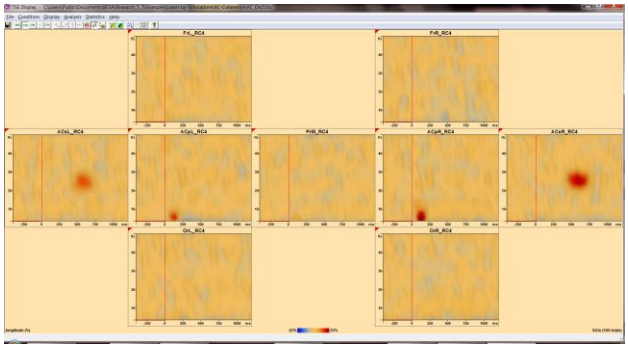
8. Click on the **TSE** button to switch back to TSE view. Observe that both the time-locked low-frequency and the induced 25 Hz activity emanate from the spectral background (oscillatory peak in ACsR 47.4% above baseline). Again there is no cross-talk between the channels as opposed to the TSE display of the scalp activity (see above). This is due to the tuning of the inverse spatial filter to full contrast between the 9 sources by setting the *regularization constant to 0* when constructing the source montage '**RC0**'.



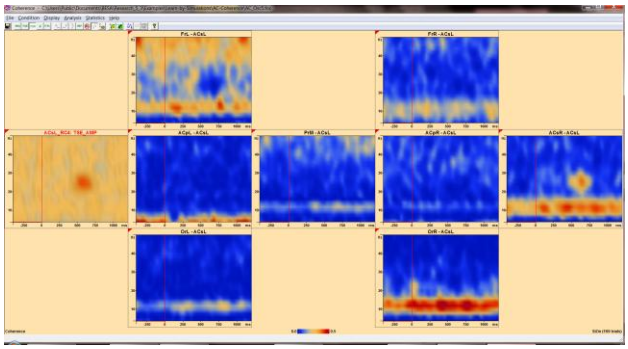
9. To see how a change of this regularization constant affects the emergence of TSE & coherence and the influence of the background noise, close the time-frequency window and press the **Usr** button to switch to montage '**RC4**'. This montage uses the same source channels as 'RC0', but was constructed with a regularization constant set to 4%.



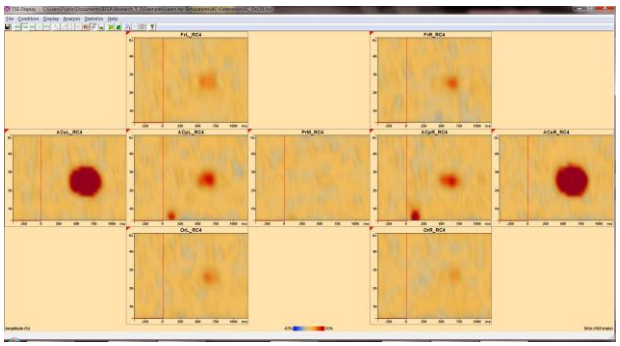
10. From the **ERP** menu, select '**Coherence**' again, press '**Start Time-Frequency Analysis**' and scale up to **+/-63%**. The stronger regularization leads to an increased emergence of the oscillatory activity (66.5% above baseline at ACsR peak around 576 ms as opposed to 47.4% without regularization). In this case regularization resulted in a *further reduction of the background and baseline noise*. At the same time the signal was weak enough that the cross-talk with other channels remained below the noise level.



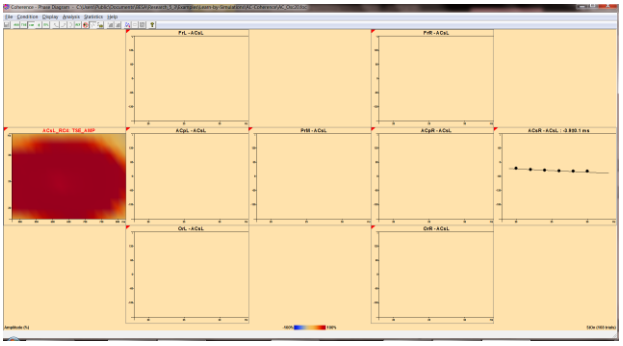
11. **Double click** on channel '**ACsL**' to recalculate coherence using a regularization constant of 4%. **Scale up** the display to **0.5**. Note that the 26 Hz *coherence peak around 630 ms is now enhanced to 0.36.* The noise coherence in the left hemisphere is reduced. However, the stronger smoothing effect of the underlying inverse filter has also enhanced the noise background in the ACsR-ACsL panel.



12. Close the time frequency window and switch back to the file '**AC_Osc20.foc**' with the stronger oscillatory activity, load the user montage '**Usr / RC4**', and recalculate **TSE**. Scale up to **+/-63%**. Now the signal is well above the noise and the *cross-talk between the sources* due to the stronger regularization is becoming more apparent. However, it is still much less as compared to the scalp.

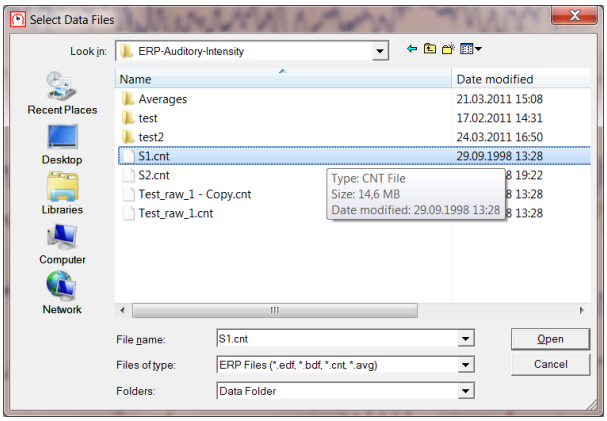


13. Compute the **coherence with ACsL** as reference channel and **display the phase diagram** to analyze the time delay between **ACsR** and ACsL. Now a delay of -3.90 ms is computed instead of the simulated delay of -5.0 ms. The reason is the cross-talk due to the regularized spatial filter. It causes a fraction of the activity of source ACsL to be mapped onto the activity of ACsR and vice versa. This leads to an assimilation of the oscillations and an apparent reduction of the time delay.

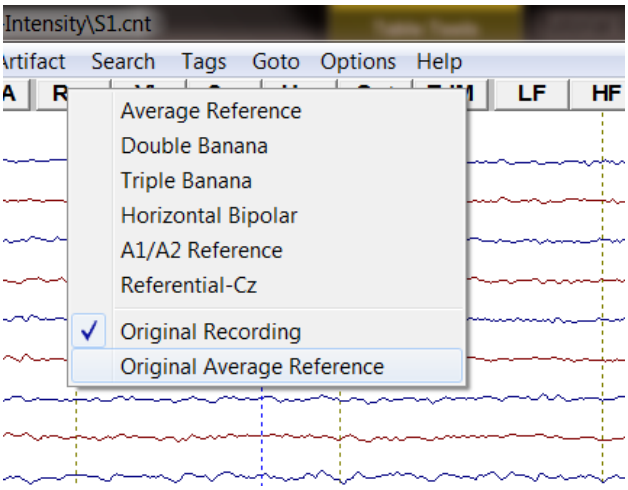


G: Scalp Coherence in the Auditory Intensity Experiment

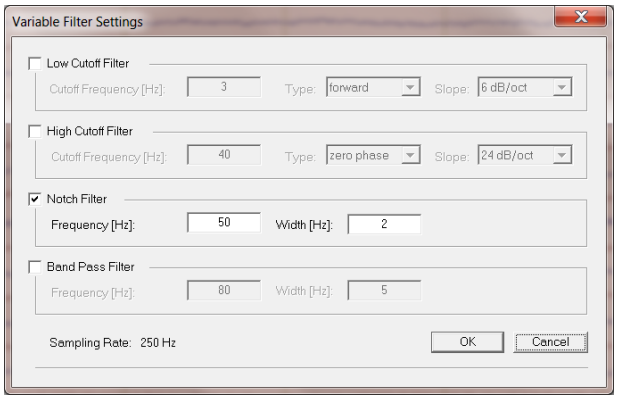
1. **Open** file 'S1.cnt' in subfolder 'ERP-Auditory-Intensity' of the Examples folder.



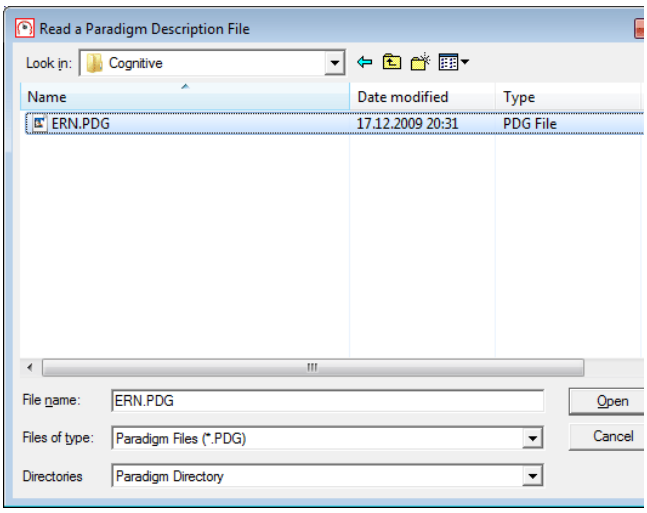
2. Press the **Rec** button and select '**Original Average Reference**'. Make sure the artifact correction is switched on.



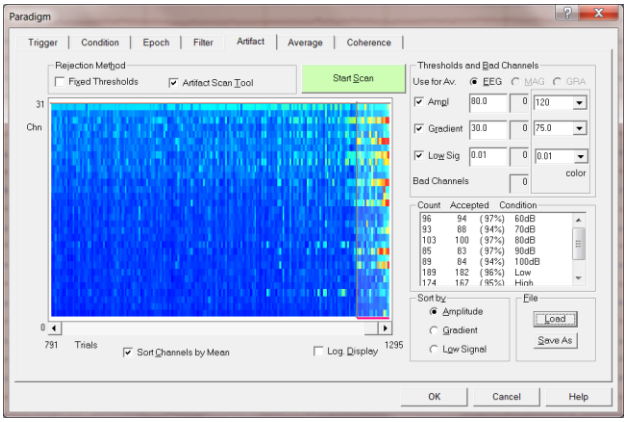
3. Press the EdF toolbar button to edit the filter settings. Disable all but the notch filter that should be set at 50 Hz to reduce mains interference.



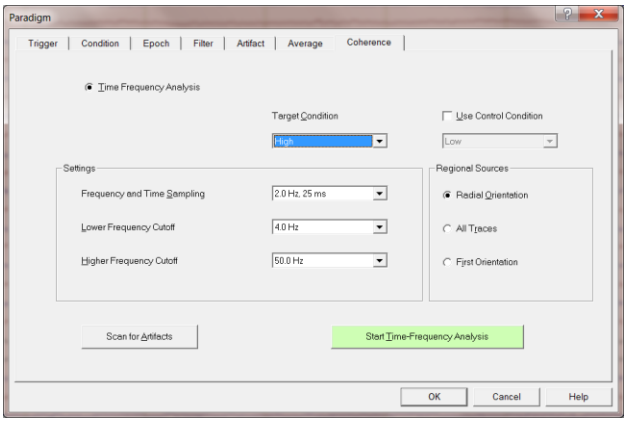
4. In a first step, we will perform time-frequency coherence analysis between surface channels. Press **'ERP / Edit Paradigm'**. The paradigm-file 'AEP_Intensity.PDG' should still be available.



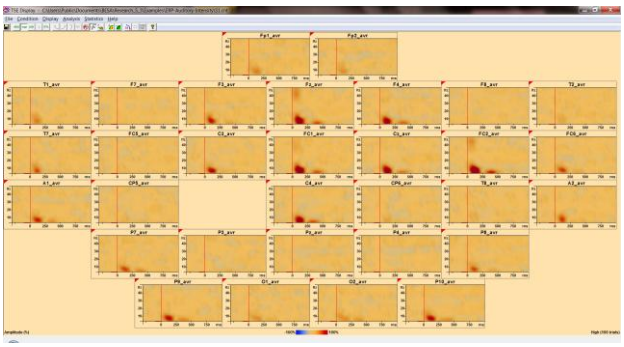
5. Move to the **'Artifact'** tab and press **'Start Scan'** to re-run the automatic artifact scan with artifact correction switched on.



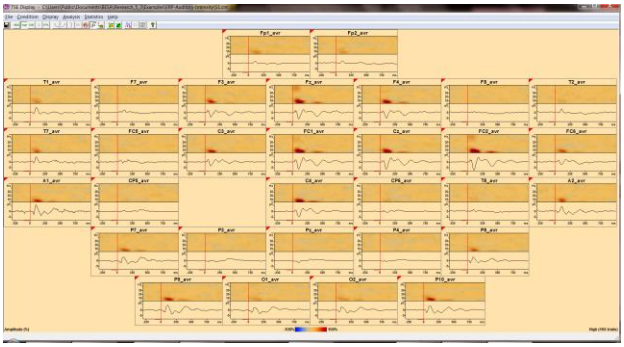
6. Switch to the **'Coherence'** tab. In the **'Condition'** drop down menu, select '**High**'. To start time-frequency analysis with the default sampling of 2.0 Hz, 25 ms in the frequency range of 4-50 Hz, press the **'Start Time-Frequency Analysis'** button**.**



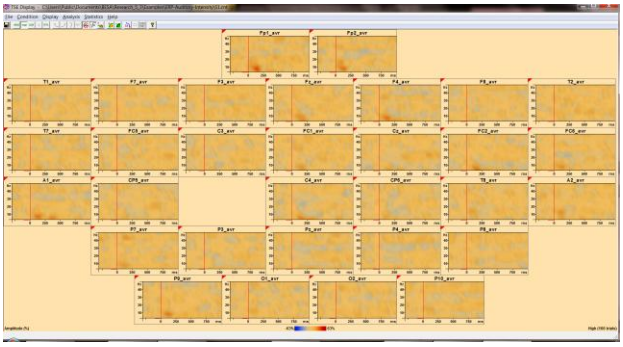
7. As expected we observe a similar activation pattern across many scalp channels.



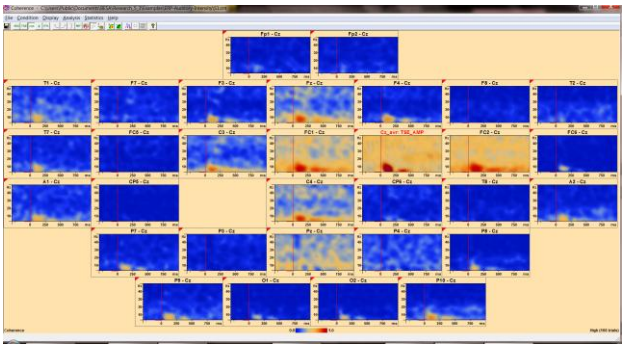
8. Display the averaged waveforms by **right-clicking** on a channel and selecting '**Averaged Waveforms**' to see which activity corresponds to the evoked signal. It seems that the majority of the wide-spread low-frequent activity is evoked.



9. Subtract the evoked signal by pressing the button and selecting '**Subtract Average Signal**'. Switch off the display of 'Averaged Waveforms' and scale up to **+/- 63%**. Apart from some portion of the evoked activity that is less-stimulus-locked and thus present in the "induced" display, no other particularly strong induced activity can be seen.



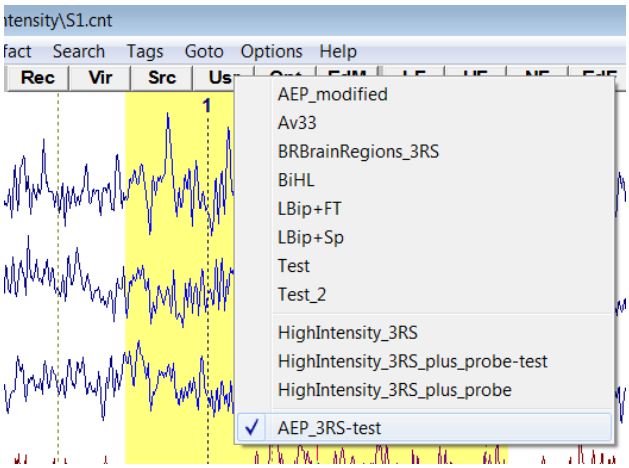
10. Undo the subtraction of the evoked signal by selecting '**Subtract Average Signal**' again and **double-click** on channel Cz_avr to display the coherence of all channels relative to Cz. Note the high coherence between Cz and almost all other channels at lower frequencies.



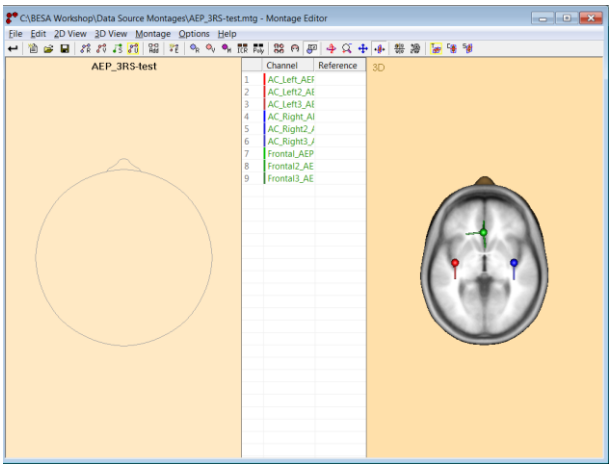
Whether these observed coherences are due to coherent activation of different brain regions or to the wide-spread surface distribution of the activity of focal brain regions cannot be concluded from the surface montage. Therefore, we will now transform the surface signals into brain activation using our previously created source montage and repeat the time-frequency analysis. **Close** the time-frequency window.

H: Source Coherence in the Auditory Intensity Experiment

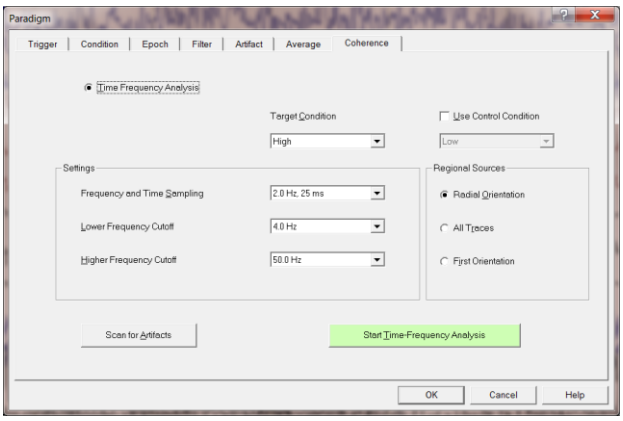
1. Press the Usr button. Select montage '**HighIntensity_3RS-test'** from the list of predefined user-montages that are available for this file. Press the EdM button to remind us of the details about the current montage again.



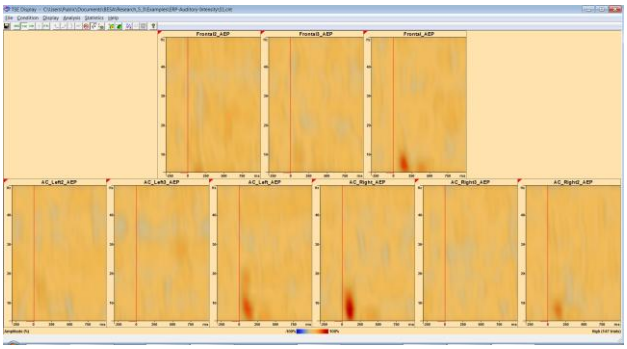
2. The montage consists of the sources we fitted earlier on: 2 auditory regional sources and a frontal source, which have been converted to single dipoles. Close the montage editor.



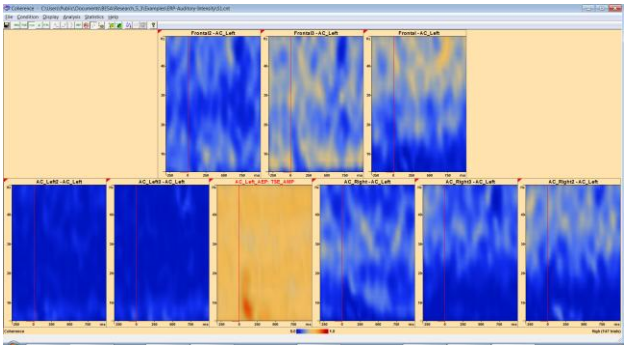
3. Select **'ERP / Coherence'** and press **'Start Time-Frequency Analysis'**.



4. We can see now that the source montage was constructed well as we see the main activity in the first frontal and in the first and second auditory components. The source montage has very good separation properties and will thus be a good model for investigating coherence between our sources of interest.

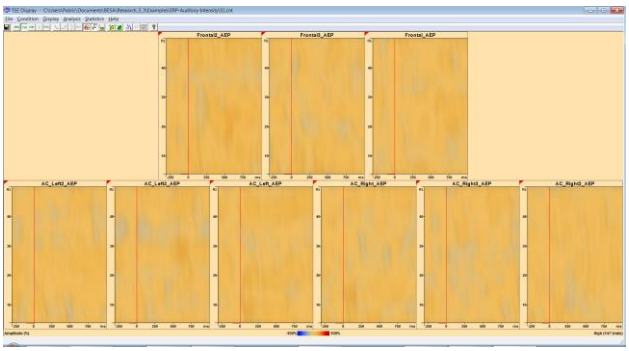


5. Double-click on source AC_Left. It seems that the low-frequent activity around 100 ms in the left and right hemisphere is not related.

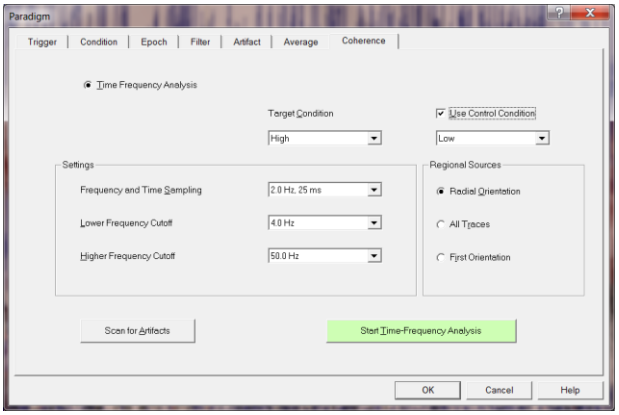


There is also some coherence at higher frequencies that can be observed in several channels. It is very likely that this is the effect of noise that was not modeled specifically by a source and is thus projected onto several sources in the montage. As a rule of thumb: it is very unlikely that similar coherence across a large number of sources is meaningful. It is rather the consequence of unmodeled activity/noise in the data.

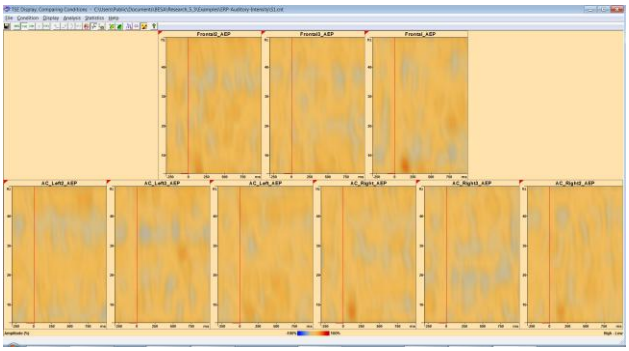
6. Return to the TSE view and subtract the evoked signal by pressing the button and selecting '**Subtract Average Signal**'. There does not seem to be strong induced activity. Close the Time-Frequency window.



7. Next we want to compare activity in the 'High' and 'Low' condition. Press '**ERP / Coherence**' again. Tick the box 'Use Control Condition' and select condition '**Low**'. Press **'Start Time-Frequency Analysis'**.



8. Press the button to display the difference 'High-Low'. The TSE display confirms that the 'High' condition has a stronger synchronization in the alpha-range between roughly 50 and 150 ms post stimulus that is present in both auditory sources and the frontal source.



I: Multiple Source Beamforming

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 of the beamformer for a specific brain region at location r is then computed by the following equation:

$$P(\mathbf{r}) = \mathrm{tr}'\left[L^T(\mathbf{r}) \cdot C_r^{-1} \cdot L(\mathbf{r})\right]^{-1}$$

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 [3x3] (MEG:[2x2]) 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 $P_{ref}(r)$:

$$q(\mathbf{r}) = \begin{cases} \sqrt{\dfrac{P(\mathbf{r})}{P_{ref}(\mathbf{r})}} - 1 = \sqrt{\dfrac{\mathrm{tr}'\left[L^T(\mathbf{r}) \cdot C_r^{-1} \cdot L(\mathbf{r})\right]^{-1}}{\mathrm{tr}'\left[L^T(\mathbf{r}) \cdot C_{ref,\,r}^{-1} \cdot L(\mathbf{r})\right]^{-1}}} - 1 & \text{for } P(\mathbf{r}) \geq P_{ref}(\mathbf{r}) \\ 1 - \sqrt{\dfrac{P_{ref}(\mathbf{r})}{P(\mathbf{r})}} = 1 - \sqrt{\dfrac{\mathrm{tr}'\left[L^T(\mathbf{r}) \cdot C_{ref,\,r}^{-1} \cdot L(\mathbf{r})\right]^{-1}}{\mathrm{tr}'\left[L^T(\mathbf{r}) \cdot C_r^{-1} \cdot L(\mathbf{r})\right]^{-1}}} & \text{for } P(\mathbf{r}) < P_{ref}(\mathbf{r}) \end{cases}$$

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.

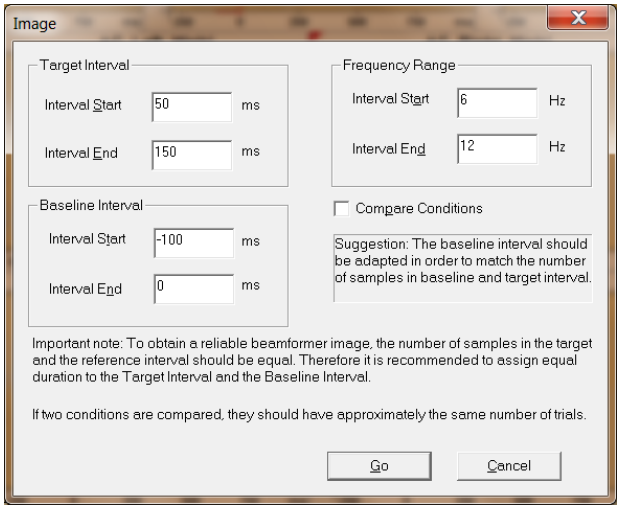
Traditional single-source beamformers are known to mislocalize activity if several brain regions have highly correlated activity. The BESA beamformer tries to overcome this problem by extending the traditional single-source beamformer. The BESA beamformer can implicitly account for activity from possibly 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 Nx6 for EEG and Nx4 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.

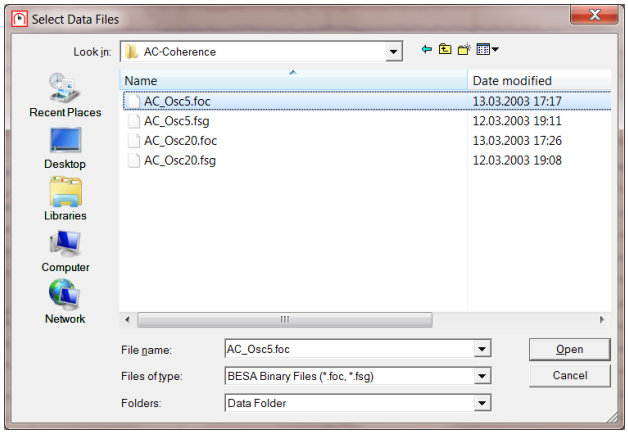
1. As a next step we will fit the prominent activity in the 'High' condition. Release the button to display 'High'-condition. Mark a window around the strongest activity in the left hemispheric auditory source. When releasing the left mouse-button choose the option '**Image**'. If necessary change the 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 interval[1]. Otherwise, Beamforming results can be unstable. Press '**Go**'.



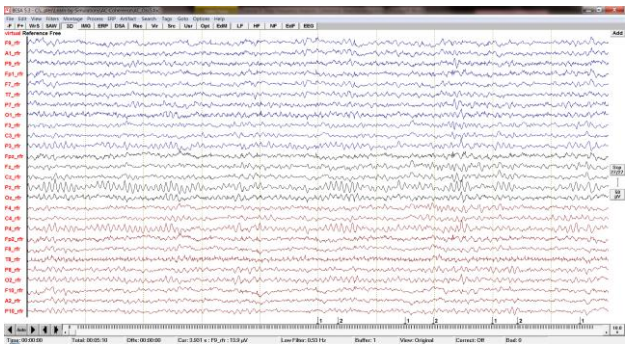
[1] It is also possible to compute beamforming images of the difference between conditions. Tick the box 'Compare Conditions' for this purpose. Instead of using the baseline as reference interval, the same time-frequency range as specified in the target interval will be used in the control condition as a reference.

2. 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.

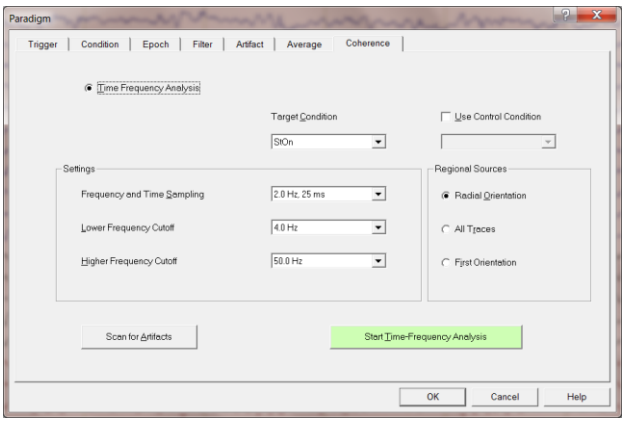
3. BESA Research uses a multiple source beamformer by default. It is also possible to choose a classic single-source beamformer. We will have a look at our model coherence data to see the difference between the multiple source and the single beamformer. Back in the main window open file '**AC_Osc5.foc'** which is located in the examples subfolder '**Learn-by-Simulations**'.



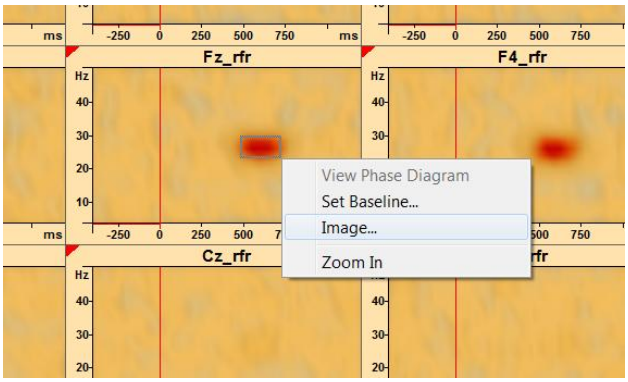
4. Change to the the **virtual reference-free** display using the **Vir** button and selecting the top-most option.



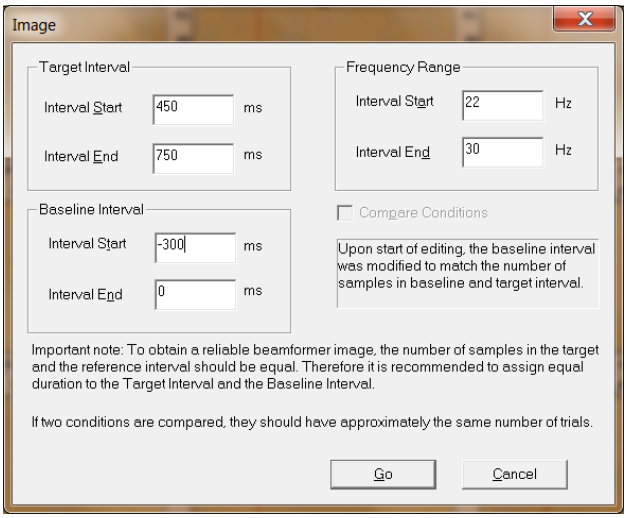
5. Press ‘ERP / Coherence’ to ‘Start Time-Frequency Analysis’ of condition ‘StOn’ using the default settings.



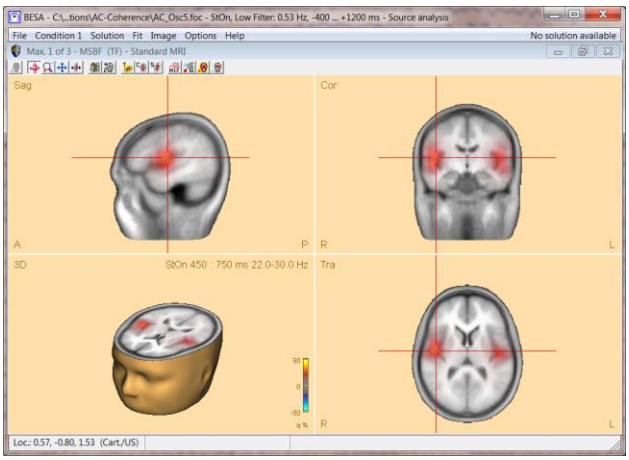
6. Subtract the evoked signal by pressing the button and selecting ‘**Subtract Average Signal**’. Now we only see the induced activity that is not present in the evoked potential. We want to fit this induced activity by marking a window arounf the most prominent activity.



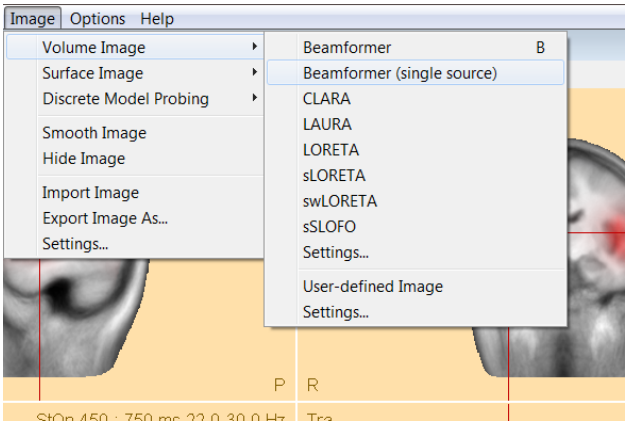
7. In the Image setting dialog box specify a time-range of **450 to 750 ms**, a baseline interval from **-300 to 0 ms** and a frequency range from **22 to 30 Hz**. Press ‘**Go**’.



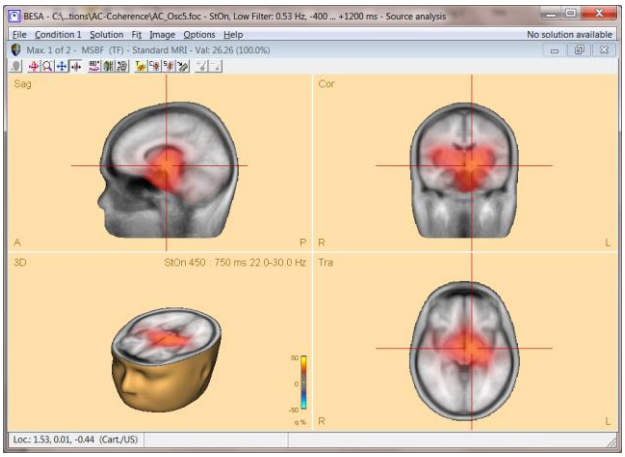
8. Scale up the image to **+/- 25%**. Observe the bilateral auditory activity. We know the maxima are at the correct location from the simulation.



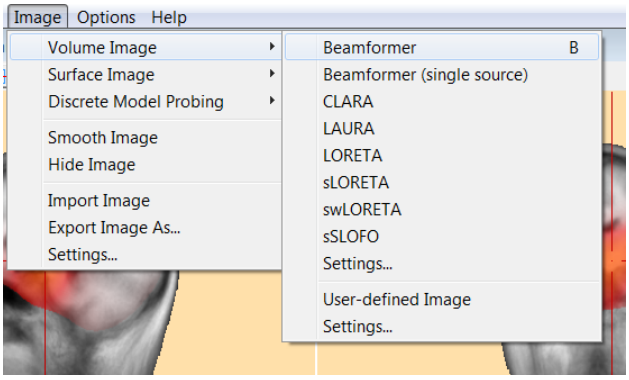
9. Change the type of beamformer by pressing '**Image / Volume Image**' and select '**Beamformer (single source)**'. The beamformer image is calculated again.



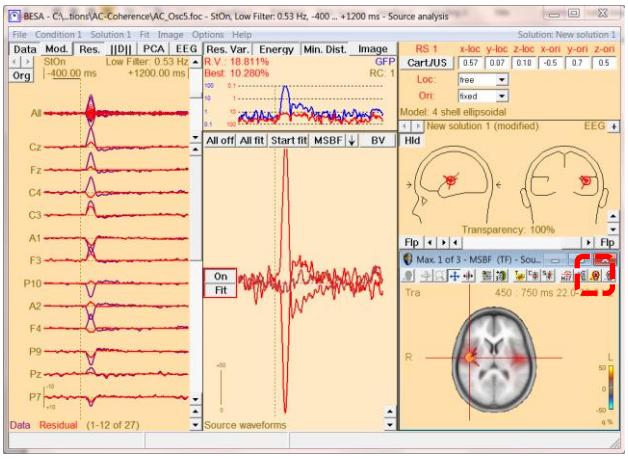
10. The single source beamformer mis-localizes the bilateral auditory activity to the middle of the head. The reason is that single-source beamformers produces faulty results if activation in two brain regions is highly correlated.



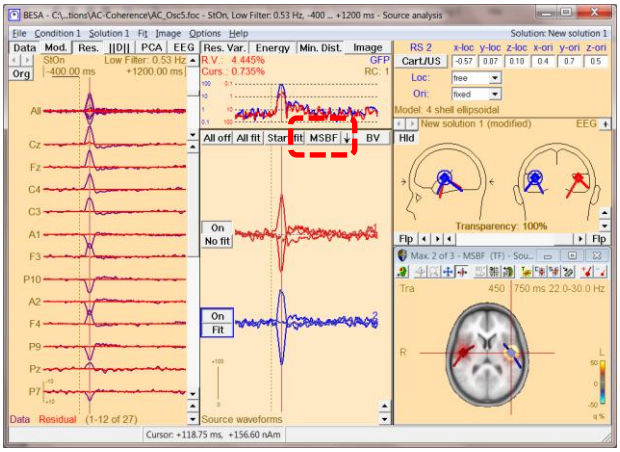
11. Return to the multiple source beamformer by pressing '**Image / Volume Image / Beamformer**'.



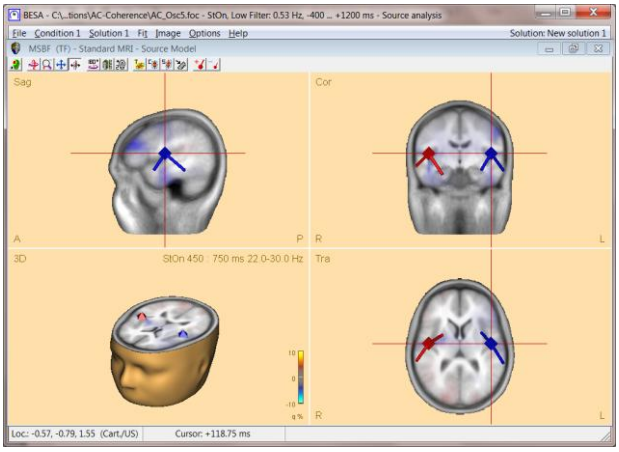
12. Minimize the MRI view by pressing the button in the top-right corner. We now want to use the beamformer image to seed dipoles. Select the first maximum and press the button. Move to the next maximum and place another source.



13. 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 re-compute the beamformer on the residual activity.



14. We need to scale up the image to **+/- 10%** to start seeing some patchy left-over desyncronization that is not modeled by the regional sources. This activity is very weak, so we can neglect it.



15. Next we want to re-fit our sources using image-weighting.