

# BESA®

## Statistics 2.0

# Manual

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## 1. Notes

This user manual is a guide to the research product BESA Statistics – a stand-alone software program.

The manual introduces the features, workflows, and the Main Windows and dialogs of the BESA Statistics software. A help function is provided within the software. Press **F1** on the keyboard or select menu item **Help → BESA Statistics Help** for help.

Before starting to work with BESA Statistics, please read this user manual carefully.

Please report any suggestions and errors found in this manual via email to [feedback@besa.de](mailto:feedback@besa.de).

BESA GmbH provides support to all customers who have purchased BESA Statistics directly from our sales office ([sales@besa.de](mailto:sales@besa.de)) or through our distributors.

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**Support:** <http://besa.de/contact/support/form.php>

## 2. Indications for Use

BESA Statistics is a software-only product compatible with personal computers running under a Windows operating system or an emulator thereof. The software is intended (a) to find reliable differences between EEG / MEG data of two or more groups / conditions, and (b) to look for reliable correlations between EEG / MEG data and an external factor using a cluster-permutation approach.

It is possible to compute cross-subject statistics of event-related potentials / fields (ERP / ERF), source waveforms, images, time-frequency data, coherence values and (inter-trial) phase-locking values.

The statistical method is permutation testing on the basis of t-tests, F-tests, or correlations. If applicable, permutation tests run on temporal, frequency and / or spatial clusters, thus enabling the best-possible control of multiple comparisons without restricting statistical power unnecessarily.

BESA Statistics integrates optimally with data that were analyzed in BESA Research, but it can also process data from other software packages as long as they conform to the BESA Statistics file format.

The intended user is a neuroscience professional with a basic understanding of statistical methods applied to experiments in neurophysiology.

It is not allowed to use the BESA Statistics software directly or indirectly for medical diagnosis and/or treatment of humans. BESA GmbH is not liable for the use of the software beyond the intended research purpose.

BESA Statistics software is licensed for research use only.

### 3. Product Classification and Certification

#### 3.1. Classification

According to **MDD 93/42/EEC**, Annex IX, rule 10, BESA Statistics is a **Class I** software product for the statistical analysis of EEG / MEG data across subjects.

According to safety classifications in **DIN/EN IEC 62304:2006**, BESA Statistics is a **Class A** product (class A: no injury or damage to health is possible).

The product is noninvasive since it is stand-alone software and does not control any equipment physically connected to a patient. It is designed purely for data analysis. No physical devices are controlled or driven by BESA Statistics, nor is any active feedback provided to the patient. Any direct physical damage to the patient, for example, during the recording of data, can therefore be excluded.

According to **DIN EN IEC 60601-1:2005**, BESA Statistics does not affect the possibility of using the computer within a patient environment. BESA Statistics does not interfere with other programs or hardware connected to the computer (third-party equipment)

BESA Statistics is classified with the following codes:

GMDN collective term: CT112 (Software, application program)

#### 3.2. Certification



The CE marking certifies that this product fulfills the basic requirements of the Medical Devices Directive **MDD 93/42/EEC**.

### 4. System Requirements

- Operating system: Windows® 8.1 (Touch not supported)  
Windows® 7 – 32bit and 64bit systems
- CPU: Minimum 2 GHz
- RAM: Minimum 2 GB
- Display resolution: Minimum 1280x800 pixels
- Graphics card: OpenGL 1.1 with 16 MB RAM or more

### 5. Disposal Information

The BESA dongle and the BESA Statistics CD must be disposed of according to the national guidelines on environmental protection.

## 6. Installation and Initial Setup

The installation and initial setup of BESA Statistics must be carried out by an administrator or an experienced technician.

### 6.1. Before Setup

**BESA Statistics is available as a 32 bit or a 64 bit program.** The latter version prevents memory problems caused by a 32 bit restriction. Therefore, **we recommend using the 64 bit version** if your Windows system supports it. Please make sure to check this before the installation. The installation and initial setup of BESA Statistics must be carried out by an administrator or experienced technical staff.



### 6.2. Installation

1. Start Windows and insert the installation disk into the CD-ROM drive.
2. If the PC permits auto start from the CD drive, the installation starts automatically after a short time. If the PC does not permit auto start, please run **Setup.exe** from the CD. The file is located in the **Setup** folder.
3. Please follow the on-screen instructions.
4. Once the installation is complete, proceed with the initial setup as explained in the following chapter.

### 6.3. Initial setup

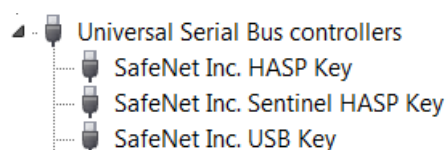
The following section explains the steps for the initial setup of BESA Statistics. These steps have to be performed only once per installation.

#### 6.3.1. Connect the BESA Dongle

BESA Statistics is protected by a licensing scheme developed by SafeNet Inc. During the installation the device drivers for the BESA dongles will be installed automatically.

Connect the BESA dongle to your personal computer or a server machine and proceed with the next step. If required, check for correct installation as described below.

If the dongle is installed correctly, the Windows Device Manager reports three items in the "Universal Serial Bus controllers" section: SafeNet Inc. HASP Key, SafeNet Inc. Sentinel HASP Key and SafeNet Inc. USB Key

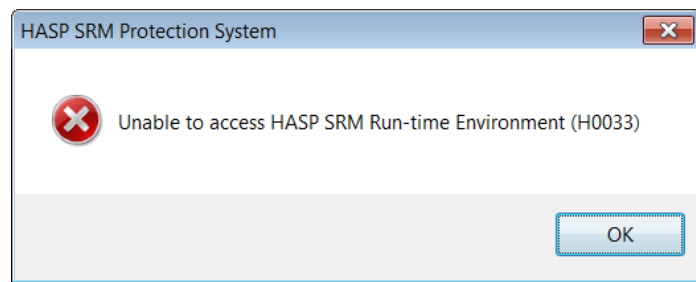
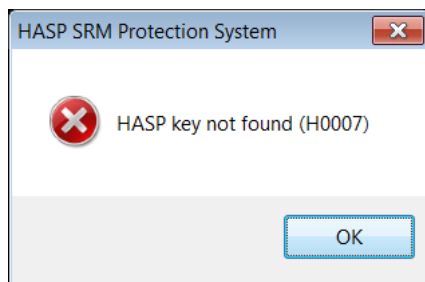


The SafeNet Inc. HASP key installer includes the HASP License Manager application: **C:\Windows\system32\hasplms.exe**

When this application is running, you should be able to load the Sentinel Admin Control Center by typing **http://localhost:1947** in your internet browser. The connected dongle should be displayed there. If you do not see the dongle, please wait a few seconds.



If the dongle is not installed correctly, one of the following message boxes is displayed when the program is started.



The message box on the left appears for one of the following reasons:

- The BESA dongle is not connected.

Solution: Ensure that the key is accessible in order to run the program.

- USB port is defective.

Solution: Check if the LED is lit on the HASP Protection Key. If not, remove and re-insert. Wait for the operating system to detect the device. If it still does not light up, try another USB port.

- Faulty dongle.

Solution: On rare occasions, the HASP Protection Keys may be faulty. The dongle does not light up in this case and is not detected in the Device Manager. Request a replacement dongle.

The above right message box comes up for one of the following reasons:

- **C:\Windows\system32\hasplms.exe** is blocked by a firewall or an anti-virus application.

Solution: Add **C:\Windows\system32\hasplms.exe** in the exception list of the anti-virus and firewall applications.

- Port 1947 is blocked by a firewall application.

Solution: Add port 1947 to the exception list of the firewall application.

- HASP License Manager Service is stopped.

Solution: Restart the Sentinel HASP License Manager Service ([Control Panel\System and Security\Administrative Tools → Services](#))

### 6.3.2. Start the Program



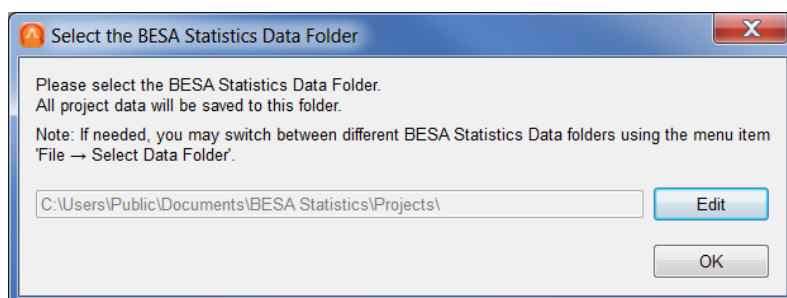
Start BESA Statistics using the shortcut created on the desktop or run **BESA Statistics.exe** from the [All Programs → BESA → Statistics 2.0](#) folder in Startup menu.

First, the Welcome Screen appears. Press **Accept** to start BESA Statistics.

### 6.3.3. Specify the BESA Statistics Data Folder

As initial configuration step, the BESA Statistics Data Folder must be specified. This is the folder where BESA Statistics stores all project and result data to and reads from.

When starting the program for the first time, the following dialog box appears:

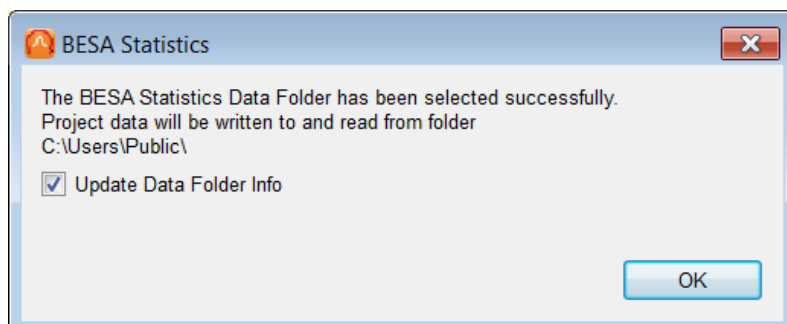


The BESA Statistics Data Folder is preselected to All Users on the local PC (shown above for Windows 7).

Press the **OK** button if you want to use the preselected local folder in All Users.

Press the **Edit** button to create your BESA Statistics Data Folder elsewhere, e.g. on a server.

When the selection is accepted, the following confirmation dialog appears:



Press the **OK** button.

BESA Statistics is now configured to start projects.

#### **6.3.3.1. Notes**

1. The procedure to define the shared BESA Statistics Data folder on the server needs to be carried out only once at each PC where you install BESA Statistics, i.e. when starting the program for the first time.
2. When switching between BESA Statistics Data folders, it is recommended to leave the tick mark on to update all existing projects in the new BESA Statistics Data folder.

## 7. Introduction

### 7.1. Features of BESA Statistics

BESA Statistics provides optimized, user-guided workflows for cross-subject analysis of EEG / MEG data. The statistical method used is parameter-free permutation testing on the basis of t-tests, F-tests (ANOVA / ANCOVA), or correlations. The program is maximally user-friendly. All analyses are computed automatically with required user-interaction reduced to a minimum. Statistical values computed in BESA Statistics can be directly used for scientific reports. No further analysis in other programs is needed. All results are visualized and can be directly used for publications.

BESA Statistics integrates optimally with data that were analyzed in BESA Research, but it can also process data from other software packages as long as they conform to the BESA Statistics file format. The native format of the BrainVision Analyzer 2 software is also supported.

BESA Statistics will automatically identify clusters in time, and if applicable frequency and space where data of the input groups / conditions are not interchangeable, i.e. where the null-hypothesis that all groups / conditions are equal must be rejected. Results are considered corrected for multiple comparisons as only those clusters will be identified that have higher cluster values than 95% of all clusters derived by random permutation of data (8.2). Thus, results obtained by BESA Statistics are objective and robust.

There are four (for ANOVA / ANCOVA five) worksteps in BESA Statistics:

1. Selecting the statistical test and starting a project (see 12.3)
  - t-test
  - ANOVA / ANCOVA (F-test)
  - Correlation
2. Loading data and selection of data type (see 12.4)
  - ERP /ERF data
  - Source waveforms
  - Time-frequency data
  - Coherence values
  - (Inter-trial) phase-locking values
  - Image data
3. Preliminary parametric statistics (see 12.8)

An initial parametric test according to the choice made in the first workstep will be computed to retrieve a preliminary statistical comparison between groups / conditions, or a correlation between a covariate of interest and EEG / MEG data. The preliminary results are used for cluster-building and subsequent permutation testing. At this step, it is possible to define time-windows and / or frequency windows of interest that will be passed on to permutation testing. The methods used for preliminary parametric testing is described in chapter 8.1.

#### 4. Non-parametric Permutation testing (12.9)

A permutation test will run on the time and / or frequency period as defined in 3). The generated results will show clusters in time, frequency and / or space where the null-hypothesis that assumes equality or exchangeability of all groups / conditions is rejected, i.e. where groups / conditions are not exchangeable and thus significantly different from each other. The non-parametric permutation test is described in more detail in chapter 8.2.

#### 5. Non-parametric Post-Hoc testing (for ANOVA / ANCOVA only, 12.10)

If an ANOVA / ANCOVA was used as the input for permutation testing, a post-hoc Scheffe's test will be computed to determine, which pairwise comparison(s) were responsible for the group / condition main effect. Post-hoc testing is described in detail in chapter 8.3.

## 8. Statistical Methods

### 8.1. Preliminary Tests and Subsequent Permutation

BESA Statistics offers three statistical tests for analyzing EEG / MEG data. A **t-test** is applicable if the exchangeability of two groups / conditions is to be tested (8.1.1). If the exchangeability of more than two groups / conditions is to be tested, a **one-way ANOVA (F-test)** can be chosen (8.1.2). If the relationship between a continuous factor (e.g. cortisol level) and EEG / MEG data is of interest, BESA Statistics offers a **correlation** workflow (8.1.3). These tests are considered preliminary in BESA Statistics, as they are calculated sample-wise and are subject to a multiple comparisons error, i.e. they can wrongly suggest significance. Therefore, BESA statistics runs a permutation test on data clusters derived from the preliminary test statistic. The resulting parameter-free permutation statistics are robust and not subject to the multiple comparisons problem. The following chapters describe the preliminary statistical tests as well as the permutation test in more detail.

#### 8.1.1. Preliminary t-test - parametric

Student's t-test (Hays 1988) 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 an interdependency 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 interdependency 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.

#### 8.1.2. Preliminary One-Way Analysis of (Co)Variance (ANOVA / ANCOVA) – parametric (F-test)

##### 8.1.2.1. ANOVA

A one-way analysis of variance ANOVA investigates the influence of an independent variable with two or more factor levels on a dependent variable. The null-hypothesis assumes that the means of all groups / conditions are equal. It gets rejected as soon as there are at least two groups / conditions with significantly different means, i.e. it is not required that all groups / conditions must differ from each

other. An ANOVA is associated with an F-value and a corresponding p-value indicating the significance of an effect. As outlined in the previous chapter (8.1.1.) p-values smaller than 0.05 are generally considered significant. The p-value becomes smaller if the F-value becomes larger. F-values are influenced by the size of the difference between the group / condition means and the size of the variance in the groups / conditions and the degrees of freedom. For more details on ANOVAs, see (Bortz 2005).

ANOVAs can be computed between-group or within-group (repeated measures). Between-group means that there is no interdependency between the factor levels of the independent variable. This is usually the case if different groups are compared, i.e. groups of subjects from different populations. For example, it might be a research question whether the P300 amplitude is significantly different between control subjects, depressed patients and anxiety patients. As different subjects populate the groups, they are considered independent. Within-group (repeated measures) means that there is a relationship between the factor levels of the independent variable. This is usually the case if different conditions within the same set of subjects are compared. For example, the N100 amplitude might differ when the same set of subjects listen to 100dB, 80dB, and 60dB versions of the same sound. An alternative scenario for a within-group (repeated measures) ANOVA is that the dependent variable is investigated in the same set of subjects more than once, e.g. one week before a treatment, immediately after a treatment and at one month follow-up. Therefore, BESA Statistics considers the independent variable to be "Group" for between-group analyses and "Condition" for within-group analyses.

#### 8.1.2.2. Preliminary One-Way Analysis of Covariance (ANCOVA) - parametric

It is possible to add a covariate of no interest in the one-way ANOVA computation altering it to a one-way analysis of covariance (ANCOVA). "Covariate of no interest" denotes a factor that might influence the dependent variable in its own right, thus interacting with the independent variable of interest in an unwanted fashion. By computing an ANCOVA, the dependent variable is corrected for the influence of the covariate of no interest. It is required that the covariate of no interest correlates with the dependent variable, that the direction of the correlation is the same in all factor levels of the independent variable and also that the factor levels of the independent variable should not differ significantly in the covariate of no interest (see important note below).

A covariate of no interest must have numerical values (e.g. 5.9, 4.2, etc.), it cannot be categorical (e.g. male, female). One value must be present per subject. Here is an example for a between-group ANCOVA: A research question might be, whether the ERN component is different in control subjects, depressed patients and patients with anxiety disorder. It is suspected that the cortisol blood level can also influence the ERN. As the main question refers to group differences and not the influence of cortisol on the ERN, "cortisol level" is considered a covariate of no interest. Thus, it is entered in the ANCOVA *for each group* to correct the ERN data for the influence of cortisol level. If there is *no dependency* between the influence of blood cortisol level and the influence of the independent variable (e.g. group status) on ERN amplitude, a potential group main effect for ERN amplitude will become more significant after controlling for the covariate of no interest.

It is also possible to run a within-group (repeated measures) ANCOVA: For example, it might be of interest, if the ERN is influenced by an error monitoring training. Thus, anxiety patients were tested 1 week before the training, immediately after the training and at one month follow-up. "Cortisol level" is the covariate of no interest as it might change from measurement to measurement and have an unwanted influence on the ERN. Thus, cortisol levels for each measurement are entered in the ANCOVA.

### Important note:

If groups / conditions differ significantly in the covariate of no interest (this can be checked by an independent ANOVA), results of the ANCOVA must be interpreted with caution as there is a systematic relationship between the independent variable and the covariate of no interest. Let us revisit the between-group ANOVA example from above and assume that there was a significant difference in ERN amplitude between control subjects and patients with depression and patients with anxiety. At the same time, the three groups differ significantly in their blood cortisol level. When entering cortisol level in the ANCOVA to control for the influence of blood cortisol levels, the ERN group-effect becomes non-significant. The conclusion that after “controlling for” blood cortisol level the groups do not differ in ERN amplitude is wrong. It is very likely that blood cortisol level is a *descriptor* of the three patient groups, e.g. a low blood cortisol level is likely to predict that a person is a control subject, whereas a high cortisol level likely predicts that a subject is a patient with depression. Taking out the effect of cortisol blood level in the ANCOVA – in this scenario – means that an important factor that predicts the affiliation with the three group factors (independent variables) gets eliminated. The correct interpretation would be that the groups do differ in ERN amplitude, but that this effect is likely to be related to differing blood cortisol levels, which are inherently related to group status. If however the group difference in ERN amplitude remains intact even after using blood cortisol level as covariate of no interest, it is a very strong argument that it is indeed the independent variable (i.e. group membership) that is associated with different ERN amplitudes. ERN amplitude does not seem to be influenced by cortisol levels, despite the groups differing significantly in this factor. For a very good discussion on ANCOVA analyses and their interpretations see (Miller and Chapman 2001).

### 8.1.2.3. Post-Hoc Testing

When a main effect in a one-way ANOVA / ANCOVA with more than two groups / conditions becomes significant, it is not clear, which groups / conditions differ from each other. As described in chapter 8.1.2., it is sufficient that at least two groups / conditions differ from each other for the F-test to become significant. In order to find out, which / groups conditions are responsible for the main effect, post hoc testing needs to be performed. In principle, post-hoc testing could be done with t-tests for all available group / condition pairings. This is not recommended, however, as a t-test would over-estimate the statistical significance of mean differences as it does not take into account that more than two sample sets were drawn from the population in the original F-test. This can lead to data clusters outside the cluster boundaries originally identified with the F-test. In order to prevent this from happening, BESA Statistics uses Scheffe’s test (Winer, Brown et al. 1991) for post-hoc testing. It specifically takes into account that more than two sample sets were drawn from the population and guarantees that no clusters can appear outside the boundaries set by the F-test. The application of Scheffe’s test in the permutation setting is described in chapter 8.3.

### 8.1.3. Preliminary Correlation

In contrast to the ANCOVA, where the influence of a covariate on the dependent variable is unwanted and of no interest, the opposite is the case for correlation. Correlation analysis investigates the relationship between two variables. It is dependent on the covariance of the two variables, i.e. the estimate of how much the two variables are changing together, the standard deviation of the two variables and the degrees of freedom. Correlation analysis yields an r-value that can be positive or negative, and associated t-values and p-values. An r-value of 1 means that there is a perfect positive relationship between the two variables. The direction of the relationship is the same, i.e. if values in one variables increase, values in the other variable also increase. An r-value of -1 means that there is a perfect negative relationship between the two variables. If the values in one variable increase, the values in the other variable decrease. An r-value of 0 means that there is no relationship at all. Besides the r-value it is also possible to depict the relationship of the two variables with a regression line. For more details on correlation analysis see (Pearson 1896).

A covariate of no interest must have numerical values (e.g. 5.9, 4.2, etc.), it cannot be categorical (e.g. male, female). One value must be present per subject. As an example, it might be of interest, if there is a relationship between a subject’s cortisol blood level and the ERN component in the EEG. A positive correlation would indicate that higher cortisol levels are associated with higher ERNs. A negative correlation would indicate that higher cortisol levels are associated with lower ERNs and vice versa.

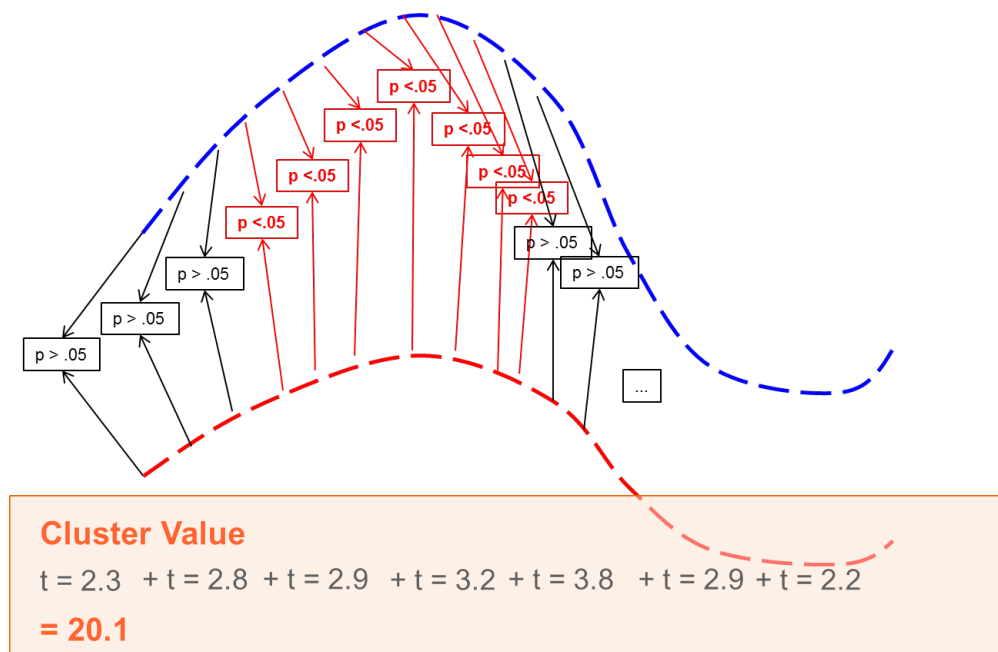
## 8.2. Permutation Test (non-parametric)

When computing a large number of tests, the probability of obtaining a significant result by chance is high. Typically, an  $\alpha$ -error of 5% ( $p$ -value = 0.05) is assumed when calculating statistical tests. Thus, if a significant result is achieved, there is a 5% chance that this result is considered significant although in truth it is not. The more tests are computed, the higher the probability that some statistically significant results are obtained by chance.

Statistically analyzing EEG / MEG data usually involves computing a large number of tests, as two or more conditions are compared for a large number of electrodes / sensors 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), where 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. Bonferroni correction treats all data samples as independent, which is not a correct assumption in the case of EEG / MEG data.

BESA Statistics uses an alternative approach to deal with the multiple comparisons problem: data clustering in combination with permutation testing. 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 pronounced effects in the preliminary parametric tests. For each cluster, a cluster value can be derived consisting of the sum of all  $t$ -values /  $F$ -values of all data points in the cluster.




**Figure 8.2a** Example for how a cluster value is derived.

For  $t$ -test and correlation (not for ANOVA / ANCOVA), a statistical effect can have a direction (i.e. condition 1 can have larger or smaller values than condition 2 or the correlation can be positive or negative), **positive and negative clusters can be found**, i.e. the cluster values can also be positive or negative. The initial clustering based on the preliminary parametric tests is followed by permutation of the data.


**The null-hypothesis behind any kind of permutation test is the exchangeability of data (see also: [http://www.fielddriptoolbox.org/faq/how\\_not\\_to\\_interpret\\_results\\_from\\_a\\_cluster-based\\_permutation\\_test](http://www.fielddriptoolbox.org/faq/how_not_to_interpret_results_from_a_cluster-based_permutation_test)).** For t-test and ANOVA / ANCOVA, the null-hypothesis assumes that the data of the input groups / conditions are exchangeable. For correlation it is assumed that the assignment of the covariate per person is random and exchangeable. In other words, the null-hypothesis in permutation testing assumes that the initial cluster values derived by the preliminary parametric test in the recorded data are equally likely as any other cluster values derived in each permutation step.

Permutation means that the data of subjects (for unpaired t-tests, between-group ANOVA / ANCOVAs and correlations) or conditions (for paired t-tests or within-group ANOVA / ANCOVAs) get systematically interchanged.


Original data		Permutation 1		Permutation 2	
Condition 1	Condition 2	Condition 1	Condition 2	Condition 1	Condition 2
S1_C1	S1_C2	S1_C2	S1_C1	S1_C1	S1_C2
S2_C1	S2_C2	S2_C1	S2_C2	S2_C2	S2_C1
S3_C1	S3_C2	S3_C1	S3_C2	S3_C1	S3_C2
...	...	...	...	...	...



Original cluster values



Derive cluster values  
for permutation 1



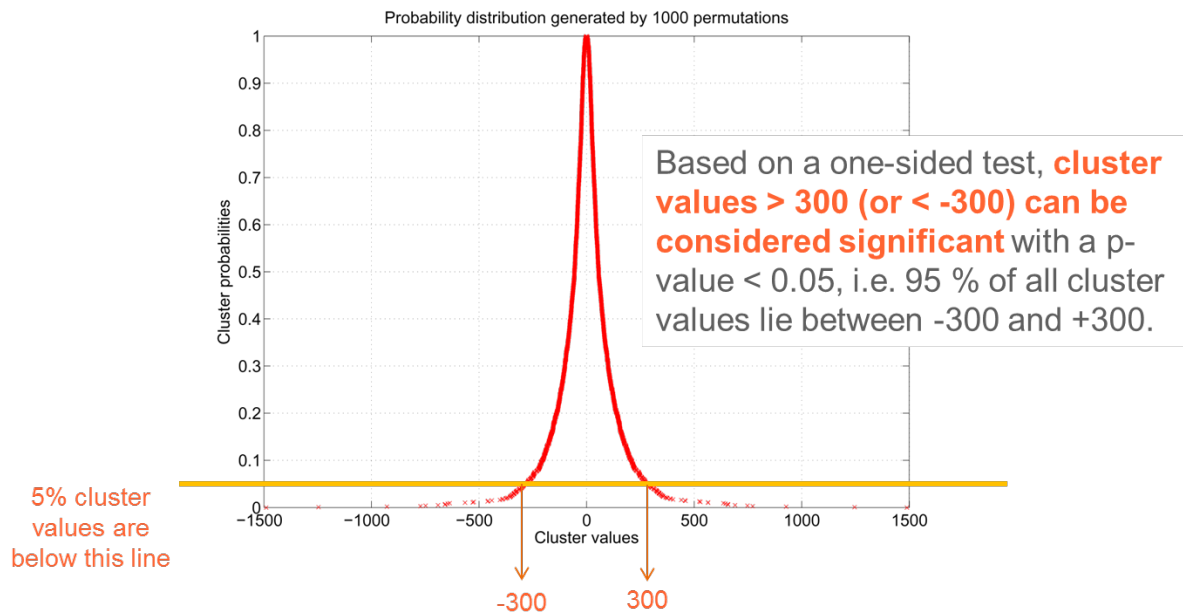
Derive cluster values  
for permutation 2

**Figure 8.2b** Example for how data are exchanged during permutation testing when a paired t-test is used as the input test.

Depending on the number of subjects per group / condition and the type of test, a certain number of permutations are possible. The following example is for t-tests: If two conditions are compared in 10 subjects using a paired t-test, 1024 ( $2^n$ , 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; please note that in this example, the groups are the same size!) permutations are possible. For each of the calculated permutations (default: 1000<sup>1</sup>), a new t- test is computed per data-point. For each permutation, new clusters are determined and the according cluster values are derived for each cluster. Thus, a distribution of cluster-values can be established across all permutations. Based on this new distribution, the  $\alpha$ -error of the initial cluster value can be directly determined. For example, if only 2% of all cluster values are larger than the initial cluster value, the initial cluster has a 2% chance that the null-hypothesis was falsely rejected, i.e. the data of groups / conditions are considered un-exchangeable although in truth they are exchangeable. Conversely, the data of groups / conditions are not interchangeable with a chance of 98%. Therefore, the cluster is associated with the p-value 0.02 (one-sided test). Thus, based on the computed cluster-value distribution, the probability of each initial cluster can be directly determined (see Figure 8.2c).

The same permutation logic as for the above t-test example also applies for F-tests (ANOVA / ANCOVAs) and correlations. As the preliminary test is only used for clustering, the type of input test does not matter. Depending on experimental design, it is still important to pick the appropriate preliminary test.

<sup>1</sup> 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.



**Figure 8.2c** A realistic distribution of cluster values based on 1000 permutations is displayed. The figure indicates that a cluster value of 300 is associated with a probability value of  $p=0.05$  (one-sided t-test), meaning that only 5% of all clusters have absolute values larger than 300.

Since in permutation testing the distribution of cluster values is computed from the input data, and probability 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). For permutation testing in the case of correlation, see (Freedman and Lane 1983; O’Gorman 2012).

#### Important Note on the Interpretation of Clusters:

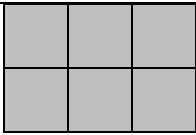
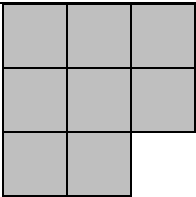
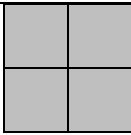
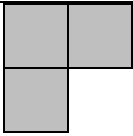
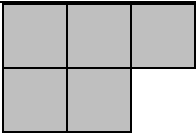


The size of a cluster depends on various factors. On the one hand it is directly influenced by the cluster alpha value (see 12.8.2.) on the other hand it depends on factors such as signal to noise ratio, recording modality, and analysis type. Particular caution is advised when interpreting clusters that were derived from sensor space data. For example, sources in the auditory cortex will lead to dipolar fields over temporal areas with a positive and a negative pole in MEG and a fronto-central negativity in EEG. In the MEG case, it would not make sense to interpret two clusters, one in the positive and one in the negative pole of the same dipolar field separately, as they both reflect the same underlying activity, i.e. the auditory cortex source. In the EEG case, it does not make sense to interpret the extent of a cluster in fronto-central sensors as again the extremely wide-spread fronto-central activity reflects potentially very focal activity in auditory cortex that is subject to the poor conductivity of the skull resulting in a widely distributed field potential. In the case of image data, i.e. results from distributed source analysis like LORETA, the extent of a cluster should also be interpreted with caution as the extent of the activity is extremely dependent on the input method. For example, an iterative approach will lead to less wide-spread activity and in consequence clusters compared to non-iterative methods, although they reflect the same underlying activity. Regarding the extent of a cluster in time, it should be taken into consideration that the temporal extent of an event-related potential / field is dependent on other factors besides the underlying activity in a cortical patch. For example, a brain response can be more or less time-locked to a stimulus, leading to very sharp (highly time-locked) or more wide-spread (more jitter) ERPs / ERFs, although the underlying activity might in fact be the same. Likewise, identifying two clusters in the same ERP / ERF or source waveform cannot necessarily be interpreted as two separate activities. They might well reflect the same underlying brain activity.

### 8.3. Post-Hoc Permutation Test (non-parametric) – Scheffe’s Test

As mentioned in chapter 8.1.2.3., it is necessary to run a post-hoc test when a main effect in a one-way ANOVA / ANCOVA with more than two groups / conditions becomes significant. BESA Statistics uses Scheffe’s test for this purpose. Scheffe’s test computes F-values for all pairwise comparisons of groups / conditions and determines a new critical F-value indicating, which individual pairwise comparisons of groups / conditions are significant (for more details about Scheffe’s test theory see (Bortz 2005). In our case, this yields new clusters that will always be sub-sets of the ANOVA / ANCOVA clusters. As each permutation is associated with different clusters, and in consequence different cluster F-values, Scheffe’s test must also be computed for each permutation. A new distribution of Scheffe’s test F-values is built and p-values are derived directly from the computed distribution as described in chapter 8.2.

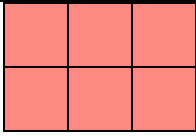
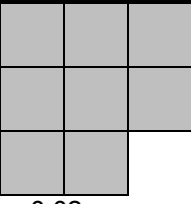
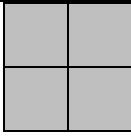
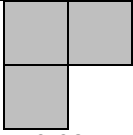
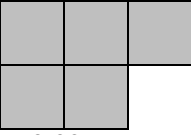


As Scheffe’s test runs pairwise comparisons, the permutation test based on Scheffe’s F-values is repeated several times ( $\frac{n!}{2}$ ). As an example, if 3 groups / conditions are compared, 3 permutation tests need to be computed ((group / cond. 1 vs group / cond. 2), (group / cond. 2 vs group / cond. 3), (group / cond. 2 vs group / cond. 3)). In the case of 4 groups / conditions, 6 permutation tests must be run. Thus, we are yet again faced with a multiple comparison problem. In order to correct for this, BESA Statistics uses the Bonferroni Holm method. It is less conservative than the Bonferroni correction, but less liberal than using no correction at all.

The following example delineates the application of the Bonferroni-Holm correction in a cluster-permutation setting. Let us assume we ran Scheffe’s test in 3 groups, and that the following cluster structure was revealed for the individual pairwise comparisons:

Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3
 p=0.01	 p=0.02	 p=0.04
 p=0.02	 p=0.03	 p=0.07– n.s.
 p=0.08		

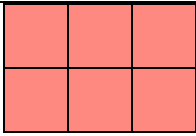
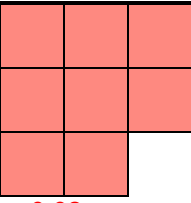

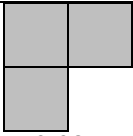
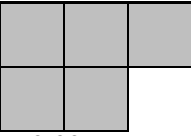


**Figure 8.3a** Clusters for pair-wise comparisons as derived by Scheffe’s test before Bonferroni-Holm correction

The **first step** of the Bonferroni-Holm method is to calculate the corrected p-value following Bonferroni. As we are running 3 pairwise comparisons, we need to divide our p-value by 3  $\rightarrow 0.05 / 3 == 0.0167$ . We then identify the pairwise test that contains the cluster with the smallest p-value, in the present example it is the comparison Group 1 vs. Group 2. We determine, if this comparison contains a cluster that survives the Bonferroni correction. In the above example, the first cluster (with 6 boxes) does ( $0.01 < 0.0167$ , see fig. 8.3b below). The other two clusters within this pairwise comparison do not survive the Bonferroni correction and must be discarded.

Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3
 <p>p=0.01</p>	 <p>p=0.02</p>	 <p>p=0.04</p>
 <p>p=0.02 – n.s.</p>	 <p>p=0.03</p>	 <p>p=0.07</p>
 <p>p=0.08 – n.s.</p>		

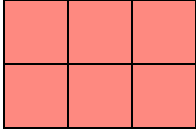
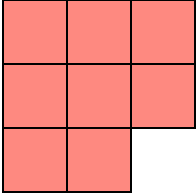
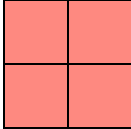
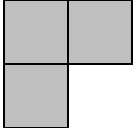
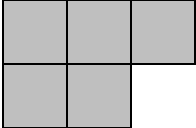


**Figure 8.3b** Step 1 of Bonferroni-Holm correction

The **second step** of the Bonferroni-Holm method takes into account that the first comparison contained at least one cluster that survived Bonferroni correction. Therefore, the p-value is not divided by 3 anymore, but only **by 2** (3 minus 1)  $\rightarrow 0.05 / 2 == 0.025$ . Then we identify the pairwise test with the **second-most** significant cluster: in the above example this is the comparison Group 1 vs. Group 3. We determine, if this comparison contains a cluster that survives the adjusted Bonferroni correction (p-value 0.025). In the present example, the first cluster (with 8 boxes) does ( $0.02 < 0.025$ , see fig. 8.3c below). The other cluster does not survive the Bonferroni correction.

Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3
 <p>p=0.01</p>	 <p>p=0.02</p>	 <p>p=0.04</p>
 <p>p=0.02 – n.s.</p>	 <p>p=0.03 – n.s.</p>	 <p>p=0.07</p>
 <p>p=0.08 – n.s.</p>		

**Figure 8.3c** Step 2 of Bonferroni-Holm correction

The **third and final step** of the Bonferroni-Holm method in the present example takes into account that two comparisons contained at least one cluster that survived the (adjusted) Bonferroni correction. Therefore, the p-value is not divided by 3 anymore, but only **by 1** (3 minus 2)  $\rightarrow 0.05 / 1 == 0.05$ . Then we check, if our last pairwise test contains clusters with an associated p-value  $< p=0.05$ . In the present example, this applies to the first cluster (with 2 boxes) (see fig. 8.3d below). The other cluster must be discarded.

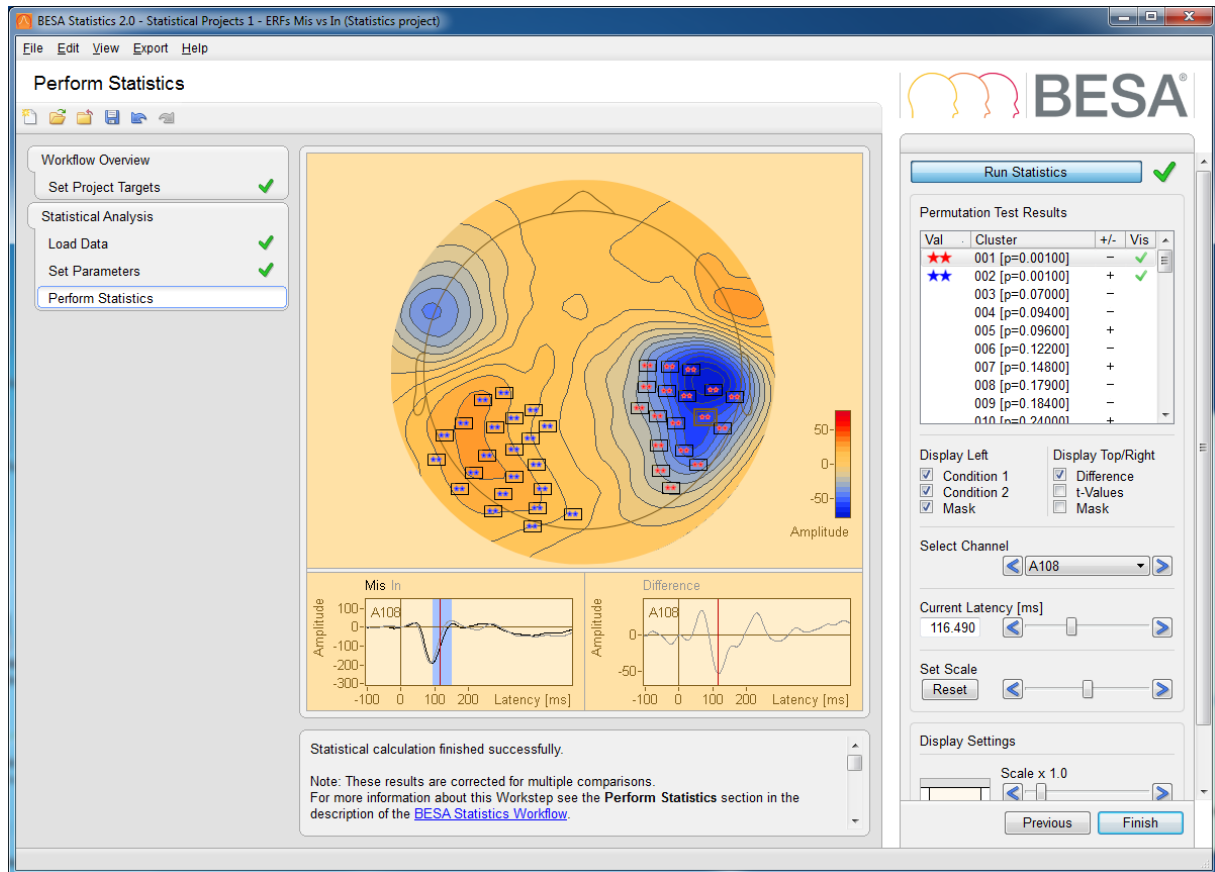
Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3
 <p>p=0.01</p>	 <p>p=0.02</p>	 <p>p=0.04</p>
 <p>p=0.02 – n.s.</p>	 <p>p=0.03 – n.s.</p>	 <p>p=0.07 – n.s.</p>
 <p>p=0.08 – n.s.</p>		

**Figure 8.3d** Step 3 of Bonferroni-Holm correction

The above example demonstrates that the Bonferroni-Holm correction reduced the number of clusters as derived by the permutation test based on Scheffe's test. While the first pairwise test (Group 1 vs. Group 2) originally yielded 3 clusters, now only 1 cluster remains. The second pairwise-test (Group 1 vs. Group 3) originally yielded 2 clusters: however, after the Bonferroni-Holm correction only 1 cluster remains. In the third pairwise-test (Group 2 vs. Group 3), only the first cluster remains. This example also shows that the Bonferroni-Holm method is less conservative than the Bonferroni correction, as this would only have yielded one cluster in the first pairwise comparison.

## 9. The Workflow Concept

BESA Statistics uses a modern workflow concept that guides you through all the worksteps needed to complete a project, suggests and explains necessary inputs and user actions, and performs many worksteps automatically. At any time, you may interrupt and inspect earlier worksteps or redo them with modified input parameters if required.



A workflow consists of a series of worksteps required to finalize a project. Each workstep contains a set of user interactions needed to achieve the workstep-specific result. Only few user interactions are needed.

When pressing the **Next** button or **Space**, a workstep is completed and the next workstep is loaded. Thus, the workflow of a project sequentially proceeds from workstep to workstep.

The layout of the BESA Statistics screen is optimized for this workflow concept. The workflow menu - as shown on the left in the **Workflow Window** - gives an overview over all the worksteps to be completed in the selected project (see 10.1). The workflow menu can be used to switch between the worksteps. For example, you may redo an earlier step with different or advanced parameters and continue with automatic processing of all subsequent steps as defined before.

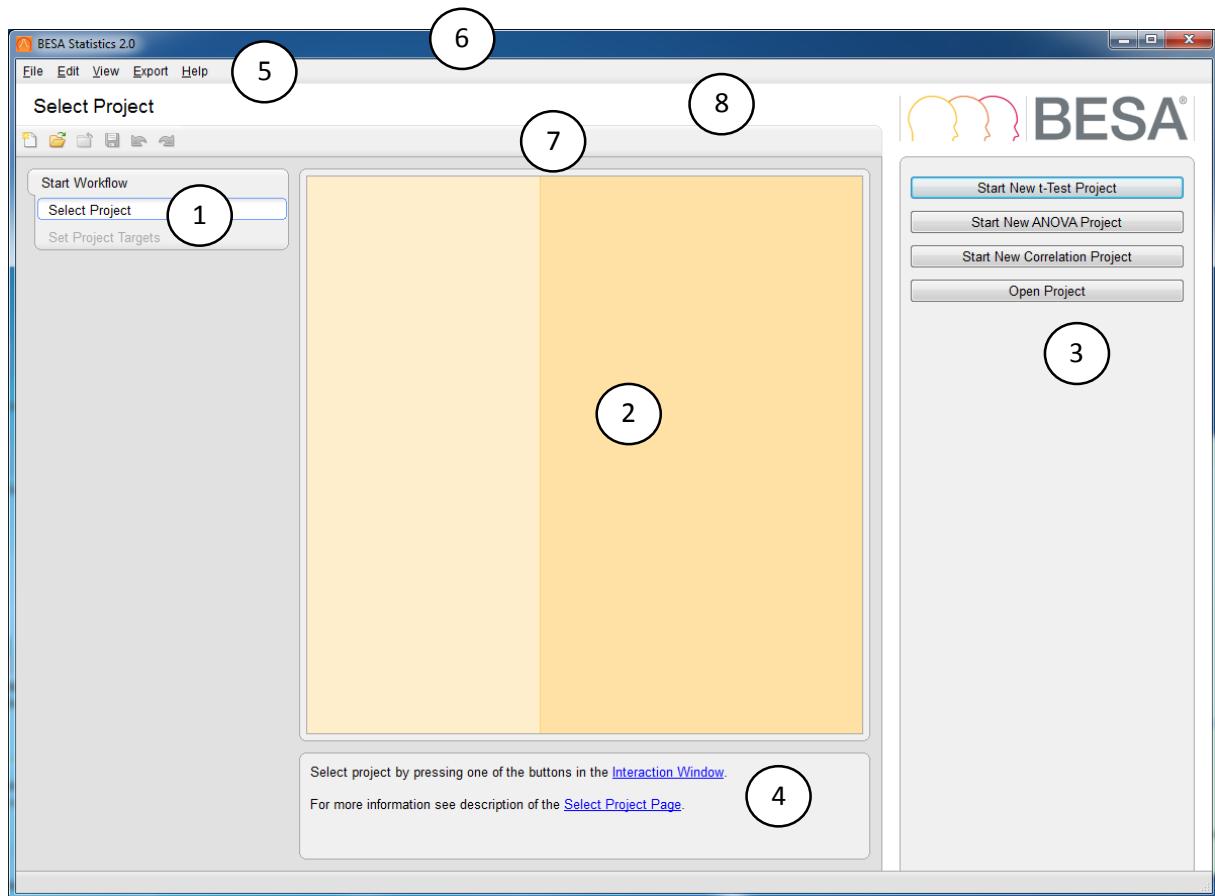
The current results during a workstep are shown in the **Main Window** in the center of the screen (see 10.2). The **Information Window** below (see 10.4) provides advice and help information for the current workstep.

During each workstep a specific dialog is shown in the **Interaction Window** on the right to allow for optimized user interaction relevant for the current workstep (see 10.3). After completing all interactions, press the **Next** button at the bottom or **Space** to proceed to the next workstep. Press the **Previous** button to go back one workstep. After completing the last workstep of the workflow, press the **Finish** button at the bottom of the Interaction Window to save all data created during the project. This saving step enables reloading the full workflow of a project with all worksteps at a later time.

A project is defined by a specific workflow together with the stored individual input and output data. Stored projects can be viewed by selecting the menu item **File → Open Project**, by using the open button in the toolbar, or by pressing the **Open Project** buttons on the start screen. After opening a stored completed project, the final workflow result is displayed.

## 10. Elements of the BESA Statistics Screen

The layout of BESA Statistics screen is optimized for the workflow concept as described in chapter 0. The figure below shows the BESA Statistics screen with all its elements:



- 1 Workflow Window (see chapter 10.1)
- 2 Main Window (see chapter 10.2)
- 3 Interaction Window (see chapter 10.3)
- 4 Information Window (see chapter 10.4)
- 5 Menu Bar (see chapter 11.1)
- 6 Title Bar (see chapter 11.2)
- 7 Toolbar (see chapter 11.3)
- 8 Caption (see chapter 11.4)

## 10.1. Workflow Window

The Workflow Window on the left displays the workflow menu. The workflow menu gives an overview over the sequential steps needed to finish the current project. Steps already completed are marked with a green OK sign. ✓

The current workstep is highlighted. This step is also shown in the caption.

The workflow menu can be used like any other menu to jump to another workstep provided that the previous worksteps have already been completed.

## 10.2. Main Window

The Main Window area displays the results of the current workstep. A detailed description how to use the elements shown in this window is given in chapter 12.8.

## 10.3. Interaction Window

The Interaction Window provides the user interaction for the current workstep.

The **upper interaction elements** are used to set parameters needed in the workstep by using standard elements like buttons, edit boxes, and sliders. The effect of changing parameters is seen in the Main Window left of the Interaction Window.

In some worksteps, additional interaction elements are shown at the bottom of the Interaction Window to enable changing visualization parameters.

Current parameters are accepted by typing **Space**, or by pressing the **Next** button at the bottom prompting the workflow to proceed to the next workstep.

The **Previous** button is used to jump back to the previous workstep.

### 10.3.1. Notes

1. You can also undo or redo a workstep using the menu items **Edit → Undo** and **Edit → Redo** or by typing **Ctrl-Z** or **Ctrl-Y**.
2. Moving on to the next workstep is fastest by typing **Space** or **Enter**.

## 10.4. Information Window

The information window displays a comprehensive help text related to the current workstep. The help texts provide additional information, e.g. which file has been loaded. They suggest and explain the next user interaction to be done in the current workstep.

Underlined text in blue provides a hyperlink. Click on such a link to open the BESA Statistics help at the indicated topic.

Use the check boxes in [Interaction Window](#) to select the data type to be analyzed and the statistical test to be used for the analysis. The [Main Window](#) lists the targets to be achieved by the workflow. To continue the workflow, you can always type **Space** or **Enter** or press the **Next** button.

For more information see description of the [BESA Statistics Workflow](#).

## 11. Program Bars

### 11.1. Menu Bar

The Menu Bar provides the menus listed below. Click on a menu to select and choose from the menu items that pop up.

#### 11.1.1. File Menu

The **File** menu contains the following items:

File menu item	Description
<b>Create New Project</b>	Start a new project.
<b>Open Project</b>	Load an existing project. A choice can be made between project types t-test, ANOVA, and correlation.
<b>Close Project</b>	Close currently active project. Show the Select Project Page.
<b>Save</b>	Save the currently active project.
<b>Save As...</b>	Specify a filename for currently active project and save it.
<b>Delete Projects...</b>	Select one or multiple projects that shall be.
<b>Select Data Folder</b>	Select the folder to be used as BESA Statistics Data Folder (see 6.3.3).
<b>Update Data Folder Info</b>	Update the information file in the BESA Statistics Data Folder (see 6.3.3).
<b>Exit</b>	Terminate the BESA Statistics program.

#### 11.1.2. Edit Menu

The **Edit** menu contains the following items:

Edit menu item	Description
<b>Undo</b>	Undo the last step completed in the current workstep.
<b>Redo</b>	Redo the last step that was undone in the current workstep.

#### 11.1.3. View Menu

The **View** menu can be used to change display settings for BESA Statistics. It contains the following entries:

View menu item	Description
<b>Show Workflow Window</b>	By selecting or deselecting this menu item the Workflow Menu is displayed or hidden (see 10.1).

<b>Show Information Window</b>	By selecting or deselecting this menu item the Information Window is displayed or hidden (see 10.4).
<b>Standard Font Size,</b>	
<b>Large Font Size,</b>	Select one of these menu items to specify the size of the font to be used in BESA Statistics.
<b>Extra Large Font Size</b>	

#### 11.1.4. Export Menu

The **Export** menu can be used for exporting vector graphics (\*.eps) or bitmap images (\*.png) of the main window, of the detail windows or of a combination of all windows. It also allows exporting the statistical results of the permutation test either as a summary (Cluster and p-values, cluster boundaries) or as detailed means per subject / cluster. For a more detailed description of the exporting options please see chapter 17.

Export menu item	Description
<b>Image</b>	Allows exporting images of the main window, the left detail window, the right details window or all windows. In the saving dialogue, the format (*.eps or *.png) can be specified. Images can also be exported by right-clicking in the according window.
<b>Statistical Results</b>	Allows exporting the summary of cluster statistics and detailed results per cluster. Results are saved as comma-separated values.

#### 11.1.5. Help Menu

Use the **Help** menu to open the program help or to get information about BESA Statistics.

Help menu item	Description
<b>BESA Statistics Help</b>	Open the program help.
<b>About...</b>	Get information about the current program version.

### 11.2. Title Bar

The title bar of BESA Statistics displays the program name and version number. In addition, it shows information on the current project:

- Group name
- Project name

### 11.3. Toolbar

The Toolbar provides direct access to the most important menu items:

 **File → Create New Project**

 **File → Open Project**

 **File → Close Project**

 **File → Save Project**

 **Edit → Undo**

 **Edit → Redo**

## 11.4. Caption

The caption is located between the Menu Bar and the Toolbar and specifies the current workstep.

## 12. How to use BESA Statistics

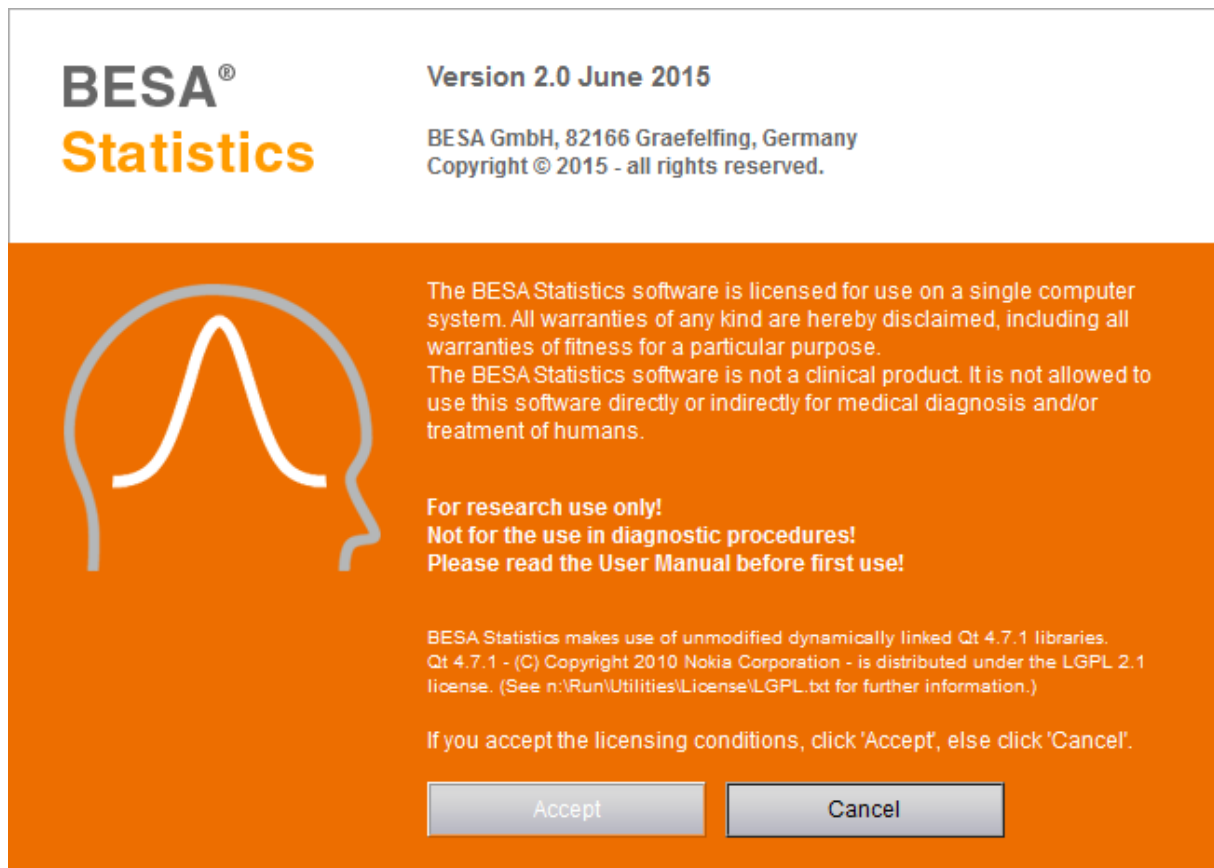
Before working with BESA Statistics, please consult chapter 10 on the elements of the BESA Statistics screen.

### 12.1. Starting BESA Statistics

#### 12.1.1. Welcome Screen

Start BESA Statistics from its icon on the desktop.

When starting BESA Statistics, a welcome screen appears. It provides some information on the BESA Statistics program including the current version number and date:



Press **Accept** to start BESA Statistics.

## The Workflow of BESA Statistics

### 12.2. The Workflow

Three different workflows (or projects) can be chosen: **t-test**, **ANOVA (incl. ANCOVA)**, and **Correlation**. Each workflow consists of specifying the experimental design, loading EEG / MEG data, the calculation of a preliminary parametric test used for cluster-building and optionally further specification of data regions of interest, and finally the calculation of a cluster permutation test. If an ANOVA project is started, there is an additional workstep for post-hoc testing. The worksteps are described in the following chapters.

### 12.3. Select Project – Parametric Test

In order to start a workflow for statistical analysis, a selection of the statistical project must be made:

- **Start New t-test Project**
- **Start New ANOVA Project**
- **Start New Correlation Project**
- **Open Project**

For a more detailed description of the statistical tests, please see the previous chapters 8.1.

#### 12.3.1. Notes

Projects can also be started or opened at any time by selecting the menu items [File → Create New Project](#) or [File → Open Project](#).

### 12.4. Set Project Targets: Data Type

After selecting a statistical project, the type of data that will be analyzed needs to be specified:

- **TFC (Time-Frequency and Coherence)**: time-frequency analysis or coherence results in source space (TFC > SWF) or sensor space (TFC > ERP / ERF)
- **Image**: data derived from distributed source analysis(e.g. LORETA) **in a volume**<sup>2</sup>
- **SWF (Source waveforms)**: data derived from discrete source analysis using single dipoles or regional sources
- **ERP / ERF**: event-related potential /field data (sensor-level) obtained by averaging across trials

The required file format of the different data types is described in chapter 19.

After selecting the input data type, further specifications need to be made for the chosen statistical test. Press [Start](#) to initialize the selected workflow or return to the workflow selection with [Previous](#).

---

<sup>2</sup> Please note that Minimum Norm solutions are calculated on a standard cortex in BESA Research. Statistical analysis of cortex-based solutions is currently not yet supported.

## 12.5. Set Project Targets for Parametric Test

### 12.5.1. t-test

The type of t-test that will be used as the base for permutation testing needs to be set (see 8.1.1):

- **Paired t-test:** In case two conditions are compared within the same subjects, a paired t-test should be selected. E.g. The EEG of 10 subjects was measured while they were listening to tones of high and low frequency. The brain response to tones of high frequency will be compared to the brain response to tones of low frequency within the same 10 subjects.
- **Unpaired t-test:** In case two groups of subjects are compared for the same condition, an unpaired-t-test should be selected. E.g. the EEG of a control group and a patient group is measured while subjects are viewing pleasant pictures. The brain response to pleasant pictures will be compared between control subjects and patients.
- **Two-tailed, one-tailed left, one-tailed right:** Generally speaking, the likelihood to obtain significant results is higher if a specific hypothesis about the data is tested. In case there is no specific hypothesis as to whether one group/condition has higher / lower values than the other group/condition, a two-tailed t-test should be selected. In case 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 selected. In case 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 selected.

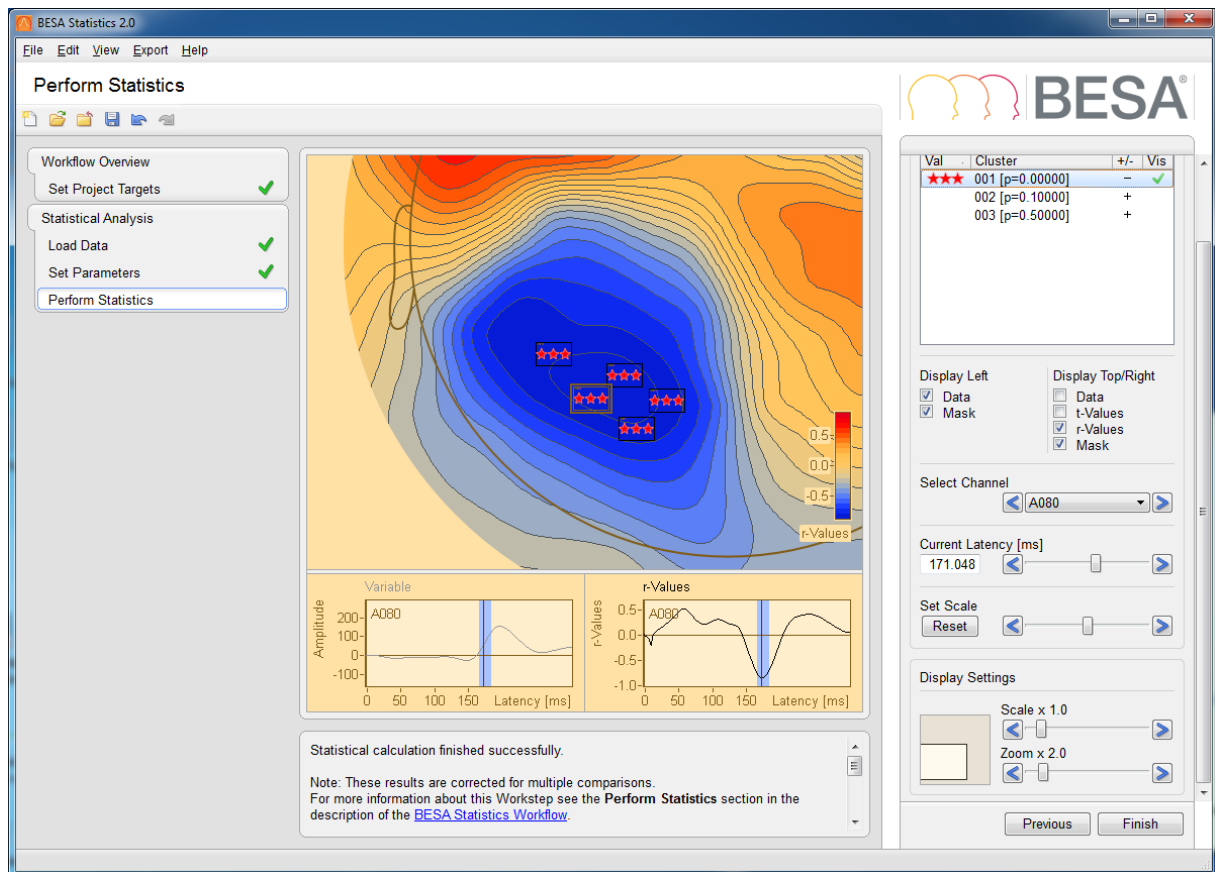
### 12.5.2. ANOVA

The type of ANOVA that will be used as the base for permutation testing needs to be selected (see 8.1.2):

- **Univariate ANOVA Within-Group:** In case more than two conditions are compared within the same subjects, a univariate within-group ANOVA should be selected. E.g. the EEG of the same 10 anxiety patients was measured before, directly after and 1 month after an anti-anxiety training. The research question is, which, if any, brain regions, EEG / MEG sensor measurements, and / or time-frequency regions are sensitive to the influence of the training.
  - **With Covariate of no Interest (ANCOVA):** In case there is a variable that also influences the dependent variable and co-varies with the independent variable in an undesired fashion, a covariate of no interest can be entered in the ANOVA. E.g. the blood cortisol level was measured **per person at each of the three time-points** in the above experiment, and may have an influence on the subject's response value, which we have no interest in within the framework of our research question. Blood cortisol level can then be used as the covariate of no interest.
- **Univariate ANOVA Between-Group:** In case one condition shall be compared between more than two groups of different subjects, a univariate between-group ANOVA should be selected. E.g. The EEG was measured in 10 control subjects, 15 patients with depression, and 12 patients with schizophrenia while they were presented words with affective content. The research question is, which brain regions, EEG / MEG sensor measurements, and / or time-frequency regions differentiate between controls, depressed and schizophrenic patients in emotion processing.
  - **With Covariate of no Interest (ANCOVA):** In case there is a variable that also influences the dependent variable and co-varies with the independent variable in an undesired fashion, a covariate of no interest can be entered in the ANOVA. E.g. the blood cortisol level was measured **per person for each of the three groups** in the above experiment, and may have an influence on the subject's response value, which we have no interest in within the framework of our research question. Blood cortisol level is then the covariate of no interest.

### 12.5.3. Correlation

Correlation can be computed between data of one group / condition and one covariate of interest (see 8.1.3). The t-value associated with the correlation coefficient will then be used as the base for permutation testing. For example an MEG component in 15 subjects can be correlated with the results of an attention test.



## 12.6. Define Experimental Design

In this workstep, the experimental design must be specified further in case an ANOVA or correlation project was started. **This step is not required for t-test.** The interaction window is sub-divided into 3 boxes. The top box gives an overview of the selected data type and statistical test. The middle box includes the experimental design, the bottom box includes the factor level names.

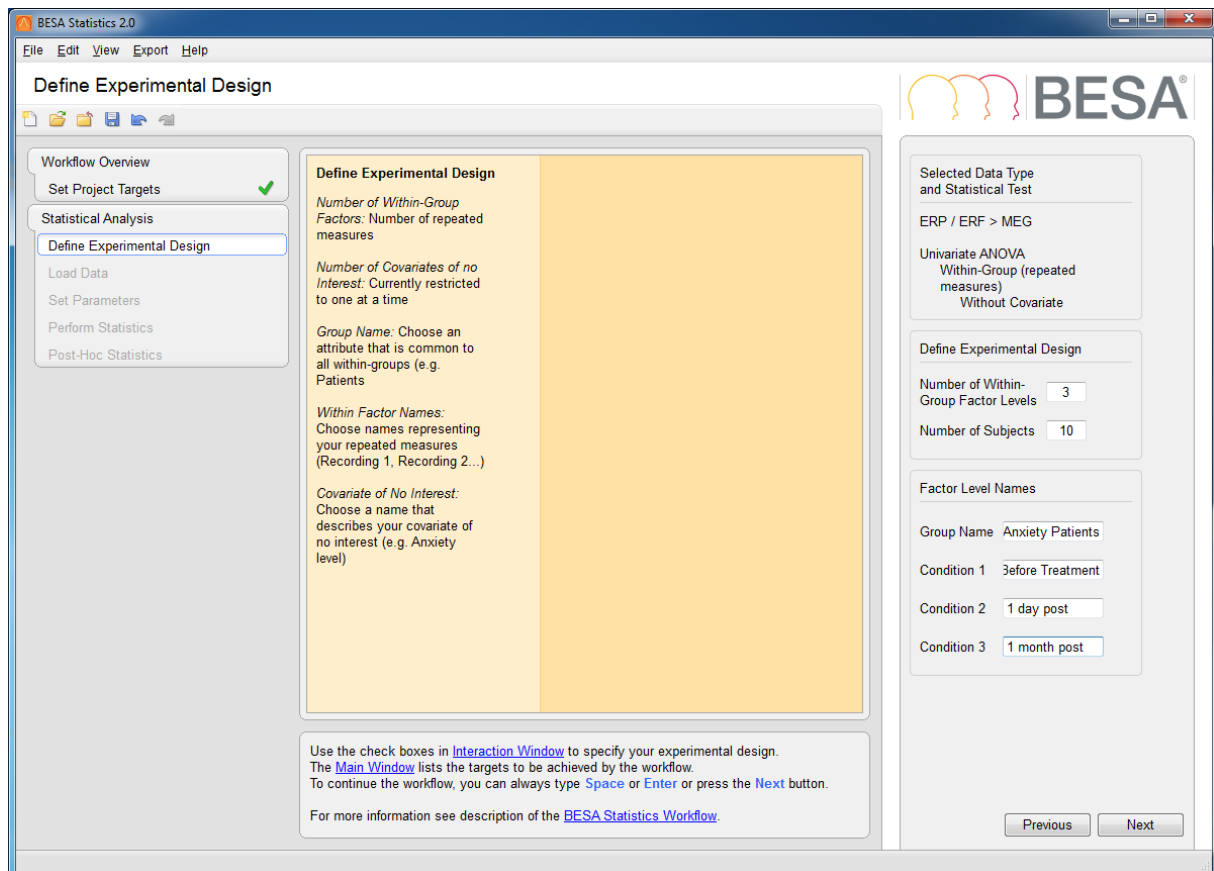
### 12.6.1. Within-Group ANOVA

For **Experimental Design**, the following input has to be provided:

- **Number of Within-Group Factor Levels:** The number of conditions that shall be compared within the same set of subjects is provided here. E.g. The EEG of the same 10 anxiety patients was measured before, directly after and 1 month after an anti-anxiety training. In this case, the number of factor levels is 3.
- **Number of Subjects:** The number of subjects, i.e. datasets per factor level, is specified here. Only one number can be entered, i.e. the **number of subjects must be identical** for all factor levels / conditions and the **subjects have to be the same**.

For **Factor Level Names**, the following input is expected:

- **Group Name:** A name should be entered that describes the subjects best. E.g. the group name could be "Anxiety Patients".
- **Condition 1 through n:** Names should be provided for all factor levels / conditions. E.g. Condition 1: "Before treatment"; Condition 2: "1 day post-treatment"; Condition 3: "1 month post-treatment"



**Define Experimental Design**

Workflow Overview  
Set Project Targets ✓  
Statistical Analysis  
Define Experimental Design  
Load Data  
Set Parameters  
Perform Statistics  
Post-Hoc Statistics

**Define Experimental Design**

Number of Within-Group Factors: Number of repeated measures  
3

Number of Covariates of no Interest: Currently restricted to one at a time

Group Name: Choose an attribute that is common to all within-groups (e.g. Patients)  
Anxiety Patients

Within Factor Names: Choose names representing your repeated measures (Recording 1, Recording 2...)  
Before Treatment, 1 day post, 1 month post

Covariate of No Interest: Choose a name that describes your covariate of no interest (e.g. Anxiety level)

Use the check boxes in [Interaction Window](#) to specify your experimental design. The [Main Window](#) lists the targets to be achieved by the workflow. To continue the workflow, you can always type [Space](#) or [Enter](#) or press the [Next](#) button.

For more information see description of the [BESA Statistics Workflow](#).

Selected Data Type and Statistical Test  
ERP / ERF > MEG  
Univariate ANOVA  
Within-Group (repeated measures)  
Without Covariate

**Define Experimental Design**

Number of Within-Group Factor Levels: 3  
Number of Subjects: 10

**Factor Level Names**

Group Name: Anxiety Patients  
Condition 1: Before Treatment  
Condition 2: 1 day post  
Condition 3: 1 month post

Previous Next

### 12.6.2. Within-Group ANCOVA

The same input has to be provided as for Within-Group ANOVAs (12.6.1). Additionally, the following information is entered for **Experimental Design**:

- **Number of Covariates of no Interest**: Currently restricted to 1.

For **Factor Level Names**, the following input is expected:

- **Covariate of no Interest**: A name should be provided that describes the covariate whose influence will be subtracted from the data. E.g. "Blood cortisol level"

### 12.6.3. Between-Group ANOVA

For **Experimental Design**, the following input has to be provided:

- **Number of Between-Group Factor Levels**: The number of groups that shall be compared for the same dependent variable is provided here. E.g. The EEG of anxiety patients, depressed patients and control subjects was measured while reading emotionally charged words. In this case, the number of factor levels is 3.
- **Number of Subjects per Group**: The number of subjects per group, i.e. datasets per factor level, is specified here. The **number of subjects can be different** for all factor levels / conditions and the **subjects cannot be the same** per group.

For **Factor Level Names**, the following input is expected:

- **Study Name**: A name should be entered that describes the experiment best. E.g. the study name could be "Affective processing".
- **Between Group 1 through n**: Names should be provided for all factor levels / groups. E.g. Group 1: "Depression; Group 2: "Anxiety"; Group 3: "Control"

### 12.6.4. Between-Group ANCOVA

The same input has to be provided as for Between-Group ANOVAs (12.6.3). Additionally, the following information must be entered for **Experimental Design**:

- **Number of Covariates of no Interest**: Currently restricted to 1.

For **Factor Level Names**, the following input must be expected:

- **Covariate of no Interest**: A name should be provided that describes the covariate whose influence will be subtracted from the data. E.g. "Blood cortisol level"

### 12.6.5. Correlation

For **Experimental Design**, the following input has to be provided:

- **Number of Subjects**: The number of subjects, i.e. datasets, is specified here.

For **Factor Level Names**, the following input is expected:

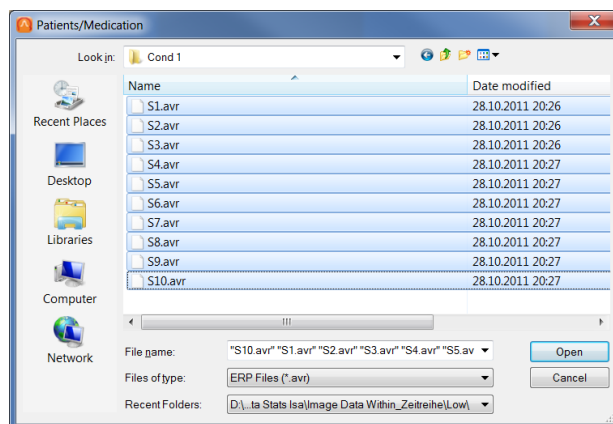
- **Covariate of Interest**: A name should be provided that describes the covariate that will be used for the correlation analysis. E.g. "Number of correct trials"

## 12.7. Loading Data

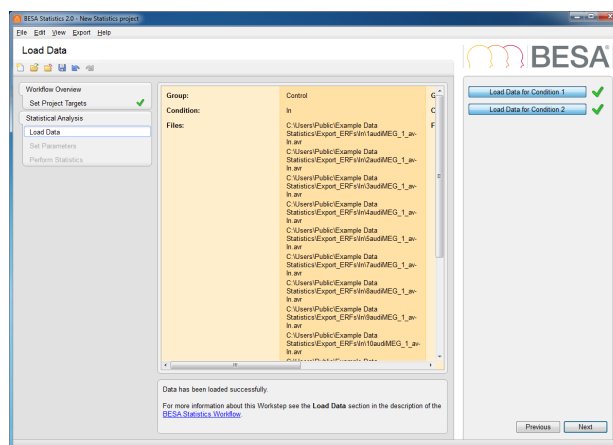
It is recommended that the data that are to be analyzed by BESA Statistics are organized **into separate folders**, one folder per condition / group containing as many files as there are subjects. **One of the data folders needs to contain 1 copy of the additional file required for ERP / ERF, TFC and SWF data.** (see 12.7.4).



Pressing the first **Load Data...** button will bring up a file selector box. A group and a condition name need to be specified before loading the data of the first group / condition.



The file names will be listed in the Main Window along with the specified group and condition name. A green tick-mark will be displayed next to the Load Data button in case the data were successfully loaded. Type **Space** or press **Next**, or the next **Load Data** button to continue the workflow. In case a paired t-test was selected previously only the second condition name can be entered in the dialogue, the group name will remain fixed. If an unpaired t-test was selected previously only the second group name can be entered, the condition name will remain fixed. After loading all required data, the Loading Data workstep is complete.



### 12.7.1. Loading paired t-test data

In case a **paired t-test** project was started, data files for condition 1 will be loaded in the first step, and data files for condition 2 will be loaded in the second step. There needs to be one data file per person per condition. The number and order of files in both conditions needs to be the same (see 12.7.a). In case an **unpaired t-test** project was started, the order and the number of files do not have to be the same for both groups of data, but need to originate from different subjects.

It is crucial for **paired T-tests** that the **same number of files** is loaded for both conditions and that the **order of the files** is the same for both conditions!

### Correct

Data Cond. 1	Data Cond. 2
Subject 1	Subject 1
Subject 2	Subject 2
Subject 3	Subject 3
Subject 4	Subject 4
Subject n	Subject n

### Incorrect

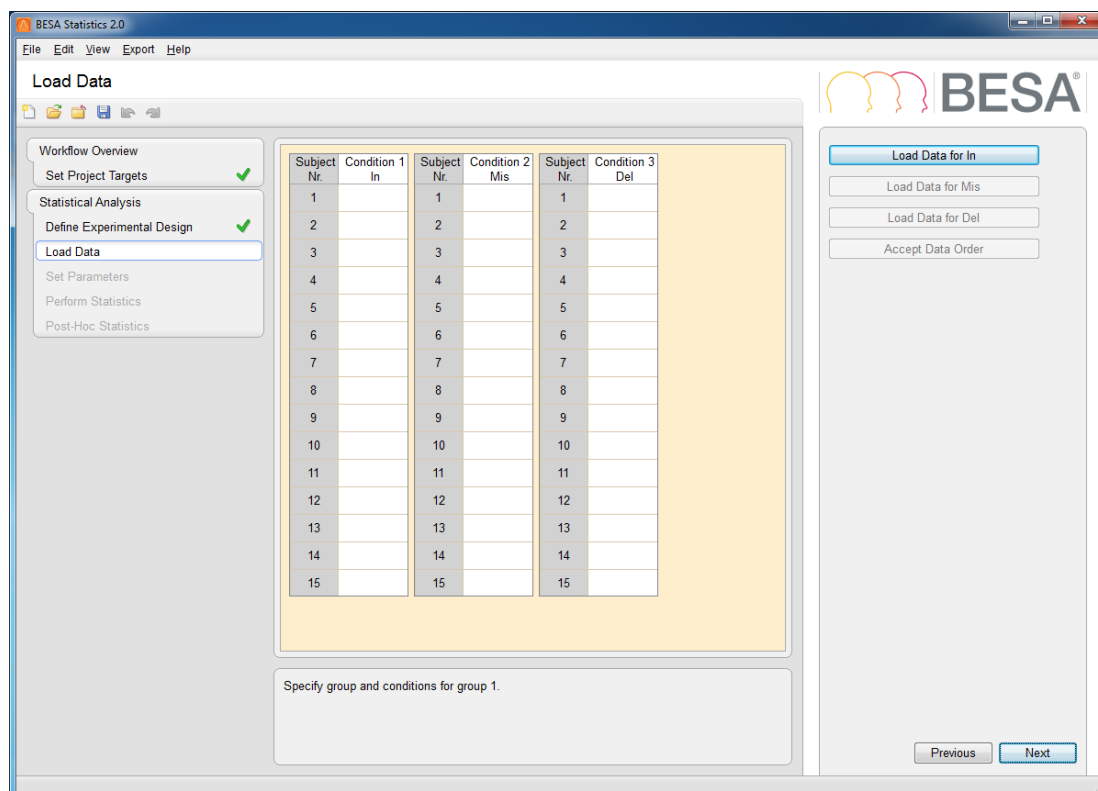
Data Cond. 1	Data Cond. 2
Subject 1	Subject 3
Subject 2	Subject 1
Subject 3	Subject 2
Subject 4	Subject 4
Subject n	



**Box 12.7.a** Data Organization for paired t-tests

## 12.7.2. ANOVA / ANCOVA

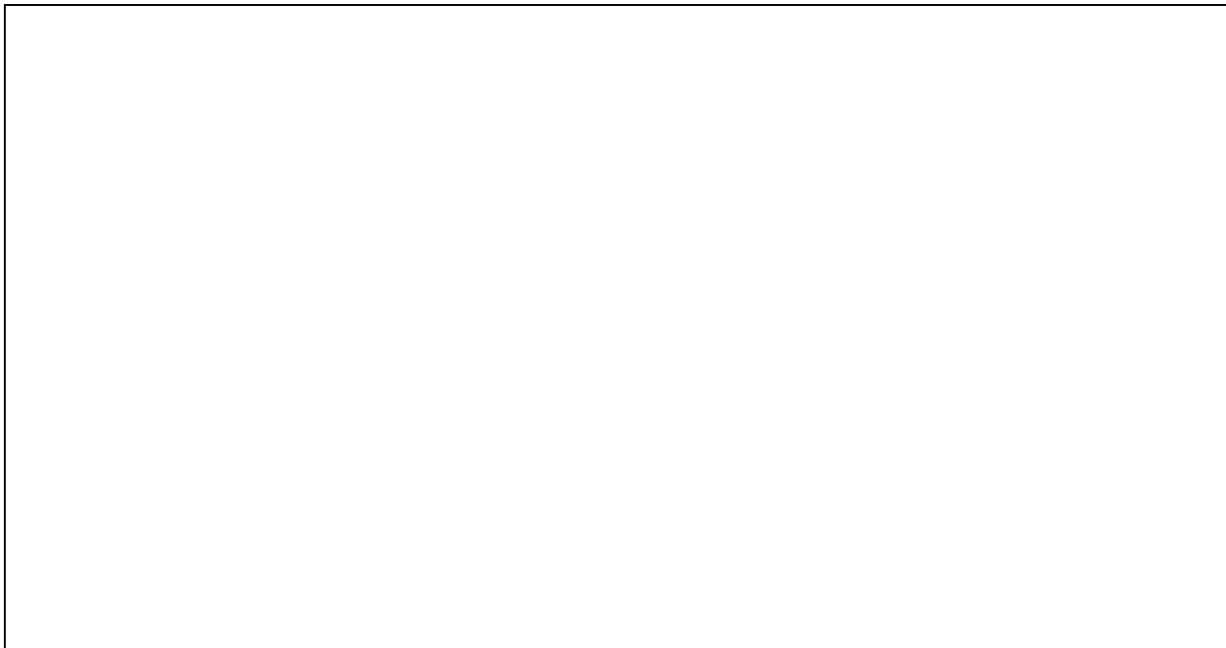
If an ANOVA was selected, the **Load Data...** buttons will already be named in line with the user input in the previous step **Design Experimental Paradigm**. The number of **Load Data...** buttons depends on the number of factor levels.



### 12.7.2.1. Loading within-group ANOVA data

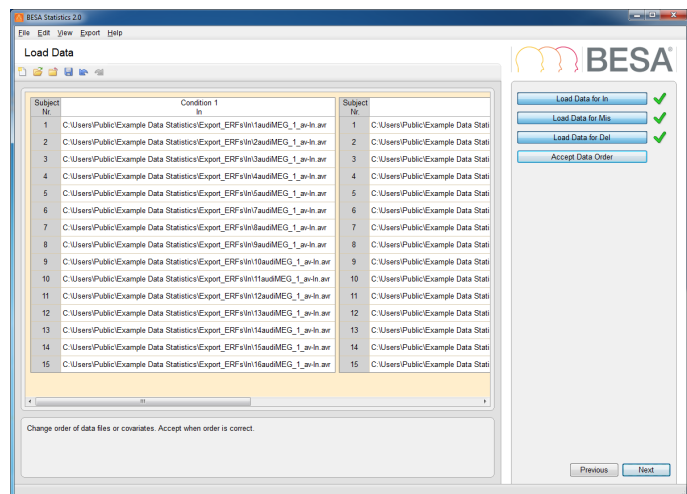
In case a **within-group ANOVA project** was started, data files for condition 1 will be loaded in the first step, data files for condition 2 will be loaded in the second step, and data files for condition n will be loaded in the n<sup>th</sup> step. There needs to be one data file per person per condition. The number and order of files in all conditions needs to be the same (see Box 12.7.b). In case a **between-group ANOVA**

project was selected, the order and the number of files do not have to be the same for the different groups of data.

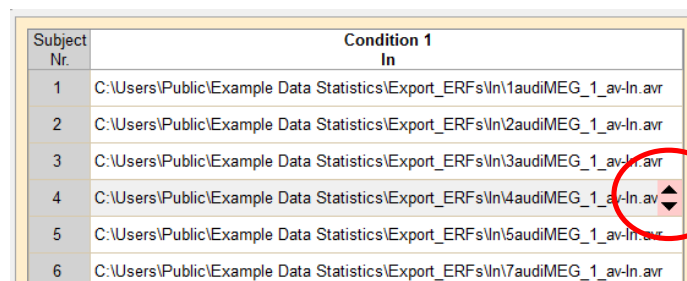


### Box 12.7.b Data Organization for a within-group ANOVA with 3 factor levels

After loading the data, the table in the Main Window will be filled with the file names. The table allows a quick check if the file order is correct or needs adjustment.



In case the file order is incorrect, it is possible to move individual files within the according column by clicking on the file name and using the arrow buttons to move the content up or down.



### 12.7.2.2. Loading ANCOVA data

If an ANCOVA project was selected, additional **Load Data...** buttons will be present for the covariate of no interest. In the first step, data for the first covariate of no interest are loaded. In the second step data for the first factor level are loaded. In the third step data for the second covariate of no interest are loaded, in the fourth step data for the second factor level are loaded, etc.

Please note that for both, within and between-group ANCOVAs there need to be **separate text files for each factor level**. The file containing the covariate of no interest needs to contain 1 column with 1 numerical value per line, i.e. one value per person (see Box 12.7.c). If a between-group ANCOVA is to be computed for 3 groups, there need to be 3 text-files containing the covariate of no interest, one file for each group. Likewise, if a within-group ANCOVA is to be computed for 3 conditions, a separate text-file containing the covariate of no interest must be available for each of the 3 conditions. The individual files containing the covariate of no interest must have different content. As an example, if depressed patients, anxiety patients and control subjects are to be compared before, immediately after and 1 month follow up of a treatment, it is not possible to use “age (in years)” as the covariate of no interest, as it would be the same for all 3 factor levels. If “blood cortisol level” was measured separately per test time-point, it could be used as the covariate of no interest (see also the chapter on ANCOVA 8.1.2).

The order of the files for a factor level and the order of the entries of the text-file containing the covariate of no interest have to be the same (see Box 12.7.c).

<b>Correct</b>		<b>Incorrect</b>	
<b>Data Factor Level n</b>	<b>Data Covariate of no Interest n</b>	<b>Data Factor Level n</b>	<b>Data Covariate of no Interest n</b>
Subject 1	Subject 1	Subject 1	Subject 3
Subject 2	Subject 2	Subject 2	Subject 1
Subject 3	Subject 3	Subject 3	Subject 2
Subject 4	Subject 4	Subject 4	Subject 4
Subject n	Subject n	Subject n	

**Box 12.7.c** Data Organization for ANCOVAs

After loading the data, the table in the Main Window will be filled with the file names and the entries of the covariate files (see 12.7.2.1). The table allows a quick check if the file order is correct, or whether it needs adjustment. In case the file order is incorrect, it is possible to move individual files or covariate entries within the according column by clicking on the file name and using the arrow buttons to move the content up or down (see 12.7.2.1).

### 12.7.3. Loading Correlation Data

If a correlation project was selected, the **Load data...** buttons will already be named in line with the user input in the previous step. In the first step data for the Covariate of Interest will be loaded, in the second step EEG / MEG data for all subjects will be loaded.

The order of the files containing the EEG / MEG data and the order of the entries of the text-file containing the covariate of interest have to be the same (see Box 12.7.d).

<b>Correct</b>		<b>Incorrect</b>	
EEG / MEG Data	Data Covariate of Interest	EEG / MEG Data	Data Covariate of Interest
Subject 1	Subject 1	Subject 1	Subject 3
Subject 2	Subject 2	Subject 2	Subject 1
Subject 3	Subject 3	Subject 3	Subject 2
Subject 4	Subject 4	Subject 4	Subject 4
Subject n	Subject n	Subject n	

**Box 12.7.d** Data Organization for Correlation

After loading the data, the table in the Main Window will be filled with the file names and the entries of the covariate file (see 12.7.2.1). The table allows a quick check if the file order is correct, or whether it needs adjustment. In case the file order is incorrect, it is possible to move individual files or covariate entries within the according column by clicking on the file name and using the arrow buttons to move the content up or down (see 12.7.2.1).

#### 12.7.4. Loading Data that were created in BESA Research

BESA Statistics expects the loaded data to conform to a certain format. Data that were created in BESA Research automatically follow the format requirements if they are exported the following way:

Data Type	Extension	Description	Data export from BESA Research
<b>TFC ERP / ERF</b>	*.tfc	File containing results from TFC analysis <b>on sensor level</b>	TFC Window: File / Export to ASCII File → Time Frequency Data Files
<b>Additional File</b>	*.elp	Electrode positions	Main Window: File / Head Surface Points and Sensors / Save all Files in Head Coordinates <sup>3</sup>
<b>TFC SWF</b>	*.tfc	File containing results from TFC analysis <b>on source level</b>	TFC Window: File / Export to ASCII File → Time Frequency Data Files
<b>Additional File</b>	*.bsa	Source positions	SA Window: File / Save Solution As → BESA Solution – Unit Sphere <sup>3</sup>
<b>Image</b>	*.dat	File containing solution from distributed source	SA Window: Image / Export Image As →

<sup>3</sup> The sensor / source positions only need to be saved once per experiment, assuming that the same positions are used for all participants. Source orientations may differ across subjects as BESA Statistics does not display source orientations. BESA Statistics will use the first elp / bsa-file detected in the specified data folders.

		analysis	Image ASCII Files (optional: - all latencies)
<b>SWF</b>	*.swf	File containing solution from discrete source analysis	SA Window: File / Save Source Waveforms As → BESA Source Waveforms Files
<b>Additional File</b>	*.bsa	Source positions	SA Window: File / Save Solution As → BESA Solution – Unit Sphere <sup>4</sup>
<b>ERP / ERF</b>	*.avr	File containing event-related potential / field data	Main Window, marked segment: File / Export → Marked segment, ASCII Vectorized <sup>5</sup>
<b>Additional File</b>	*.elp	Electrode positions	Automatically exported with ASCII Vectorized data <sup>6</sup>
<b>Covariate</b>	*.txt	File containing 1 column with 1 numerical value per line (=subject)	No export from BESA Research.

### 12.7.5. Loading data that were created in other software

Data exported from Brain Vision Analyzer 2 (ERP and time-frequency data with the extension \*.vhdr, \*.vmrk, \*.dat) can be read directly by BESA Statistics.

In case BESA Statistics is used for analyzing data created in other software, it is important to ensure that files are prepared to meet file format expectations. Please see chapter 19 for a detailed file format description.

## 12.8. Set Parameters, Preliminary Test

After successful loading of the data, the next workstep is Set Parameters. In order to access the parameter settings, the **Preliminary Test** (8.1) as defined in Project Targets (12.5) has to be run. Please note that results of the parametric preliminary test **should not be interpreted in any way**. The preliminary probability distribution is required for cluster-building. Parameters for clustering that will be used in the subsequent permutation test can be set in this workstep. They are to some degree dependent on the input data type (ERP / ERF, Image, SWF, TFC ERP / ERF or TFC SWF). In the following section, the elements of the Interaction Window will be described.

<sup>4</sup> Coordinates must be in unit sphere. Talairach coordinates are not yet supported.

<sup>5</sup> Only segmented data are supported, continuous data cannot be analyzed in BESA Statistics.

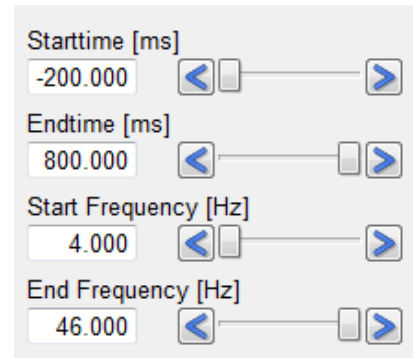
<sup>6</sup> In case bad channels were defined, data were re-referenced or in case a montage was used that only contained a subset of channels, please refer to chapter 18.1

### 12.8.1. Starttime, Endtime (Start Frequency, End Frequency)

Editing the **Starttime** and **Endtime** and if applicable **Start Frequency** and **End Frequency**, the original data epoch will be adjusted accordingly.

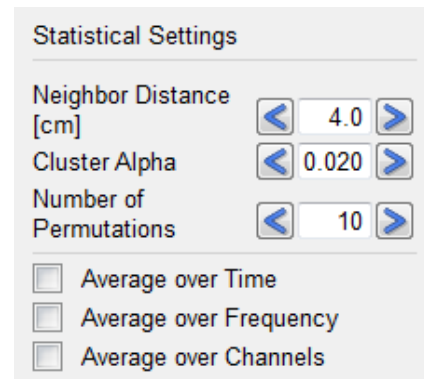
Defining a region of interest can increase the chance that a cluster will be significant after permutation. It will not alter the significance of the target cluster per se unless the selected starttime (frequency) / endtime (frequency) cut the cluster boundaries. Instead, the cluster value of the target cluster will have a different relative position in the cluster value distribution as derived by permutation (see 8.2) when a smaller region of interest is used.

If averages over time are computed, the starttime and endtime selection will impact on the permutation test result.



### 12.8.2. Statistical Settings

The **Neighbor Distance** (circle arc length between two points, **only available for sensor space data**, i.e. ERP / ERF and TFC ERP / ERF) should be set to reflect the spacing between electrodes / MEG sensors. If the value is set correctly, BESA Statistics will only treat those electrodes / sensors as neighbors that are directly neighboring. If the value is set too low, no spatial clustering will be possible. If the value is set too high, clusters might become larger as also more distant electrodes / sensors are treated as neighbors. For **image data**, the neighborhood structure is given for a volume image consisting of adjacent voxels. I.e. the **neighborhood information is automatically retrieved by BESA Statistics!** For **SWF data**, **no neighbor distance can be specified**. The reason is that single dipoles and regional sources are discrete entities, i.e. they represent separate brain regions differing from each other in their activity pattern. Clustering them in space would destroy the advantageous property of discrete sources to separate brain regions.



**Cluster Alpha** determines the significance level for building clusters in time and / or space / frequency (see below). As the preliminary test is run point-wise, every data point will be associated with a p-value. BESA Statistics looks for data clusters where the null-hypothesis can be rejected, i.e. only those data points are eligible to be part of a cluster, which fall below the Cluster Alpha value. **Please note that the Cluster Alpha value is not the significance level for the permutation test!** It is only used for clustering. The larger the Cluster Alpha value, the larger the clusters.

**Number of Permutations** determines, how many of all possible permutations (see 8.2) will be computed. If the number is smaller than the number of possible permutations, permutations are determined randomly, no repetitions allowed. If the number is larger than the number of possible permutations, BESA Statistics automatically corrects the value down to the maximum amount of possible permutations.

It is possible to **Average over Time** and / or **Frequency**

/ **Channels** if desired. If selected, BESA Statistics will average across all available data-points of the selected dimension.

**Display Left** defines what will be displayed in the left Detail Window. **Display Top/Right** defines what will be displayed in the Main Window and the right Detail Window (10.2).

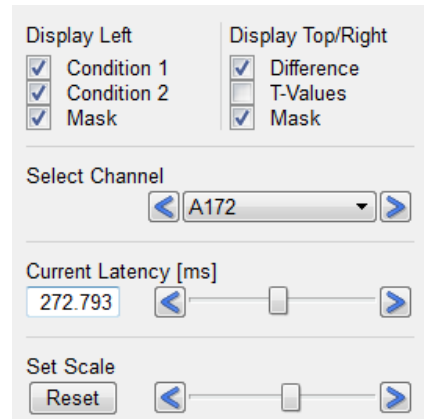
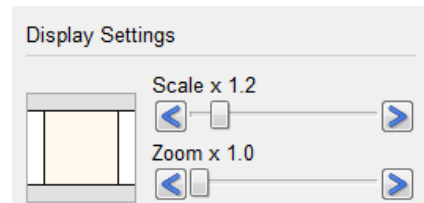
**Select Channel** allows the selection of a desired channel (TFC (ERP / ERF)) / source ((TFC) SWF) or maximum (Image) to be displayed in the Detail Windows via a drop-down menu. Alternatively, the channel / source / maximum of choice can be selected in the Main Window by a left-click. The arrow buttons left and right of the channel selector box can be used to quickly browse through all channels / sources / maxima.

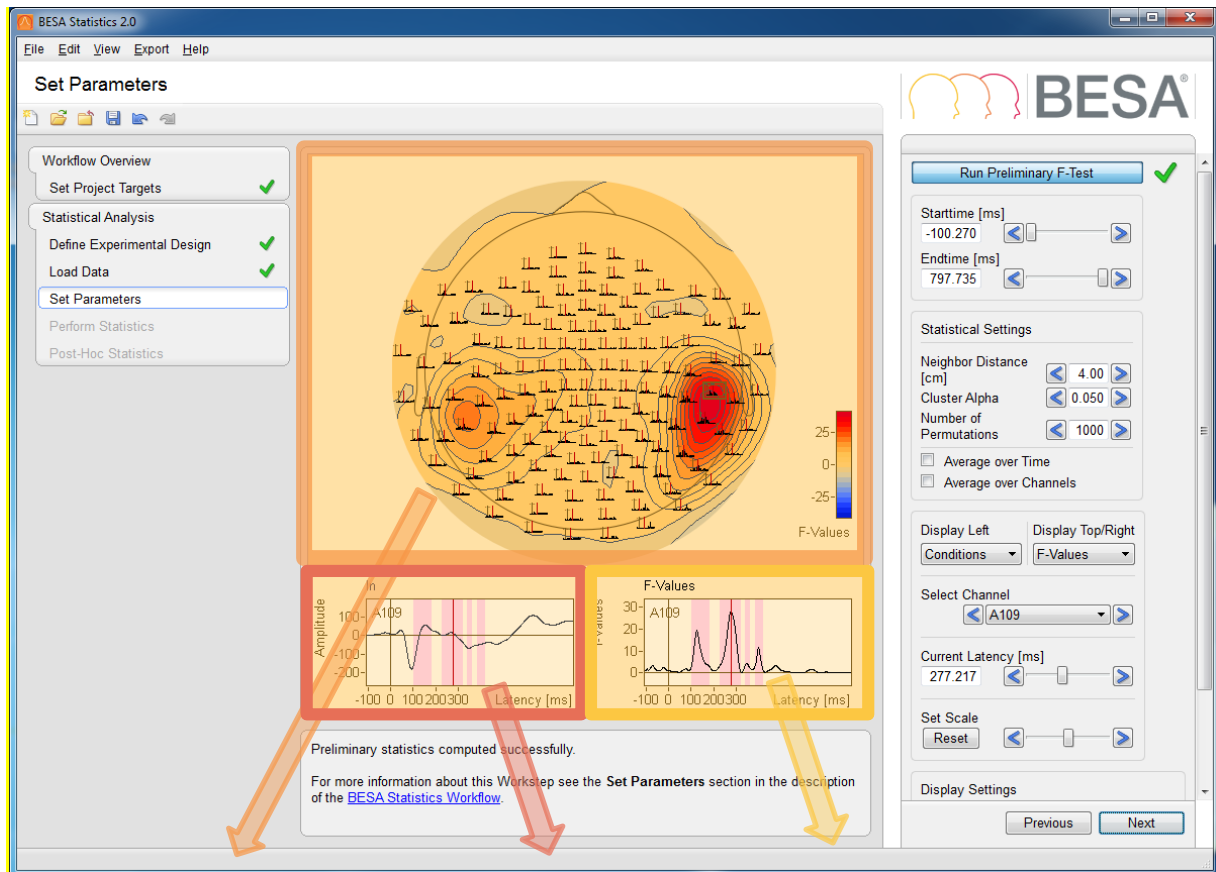
**Current Latency** allows setting a cursor at a particular latency (if TFC data are available, a **Current Frequency** can be selected additionally). The slider can be dragged to the point of interest or the arrow buttons at the left and right of the slider bar can be used to adjust the position.

**Set Scale** (only ERP / ERF) allows adjusting the scaling of the topographic map in the Main Window. For **t-test**, the map corresponds to either the difference between conditions or the corresponding t-values, depending on the selection under Display Top/Right. For **ANOVA / ANCOVA**, the map corresponds to either F-values or the average of the input groups / conditions. For **Correlation**, the map corresponds to either r-values or the average of the input condition. A **Reset** button is available to return to default settings.

**Scale** allows altering the scaling of the data. The arrow buttons at the left and right of the slider bar can be used to scale the data up or down.

**Zoom** allows viewing the Main Window contents in greater detail. The arrow buttons at the left and right of the slider bar can be used to adjust the zoom factor. The black frame at the left of the Zoom slider displays the position of the currently displayed window fraction relative to the complete window. The frame can be left-dragged to another relative position. Alternatively, it is possible to left-drag the Main Window while holding down the control key.



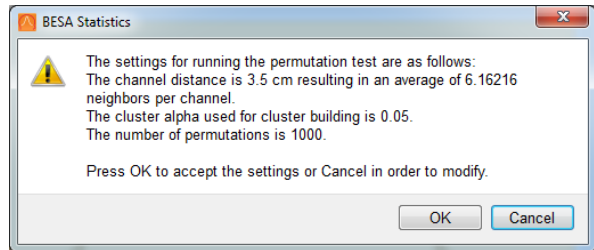
The Main Window shows the result of the statistical analysis (t-values, F-values, r-values) per time-point and channel (ERP / ERF), time-point and voxel (image), time-frequency point and channel / source (TFC ERP /ERF or SWF) or the source positions if source waveforms are analyzed.

The left Detail Window displays an overplot of groups / conditions of the selected channel (ERP / ERF), voxel (image), source (SWF). For TFC data, only one group / condition can be displayed at a time. If selected, masks indicating significant clusters determined from the preliminary test will be overplotted additionally.

The right Detail Window displays either the difference between conditions or the corresponding t-values (t-test), F-values or the average of the input groups / conditions (ANOVA / ANCOVA). For Correlation, the map corresponds to either the r-values or the average of the input condition.

## 12.9. Perform Statistics

Pressing **Run Statistics** opens a window with a summary of the Statistical Settings. If applicable, it displays the number of neighbors found with the given neighbor distance (12.8.2). Further, the cluster alpha value is displayed, as well as the number of permutation. If these values need changing, it is possible to return to the Set Parameters workstep by pressing **Cancel** and the **Previous** button. Confirming the summary window with **OK** will start the permutation test. **Please note that depending on the number of subjects, electrodes / sensors and data points, the computation might take several minutes.**



Results of the permutation test will be displayed optimized for the data type in the Main Window and the Detail Windows.

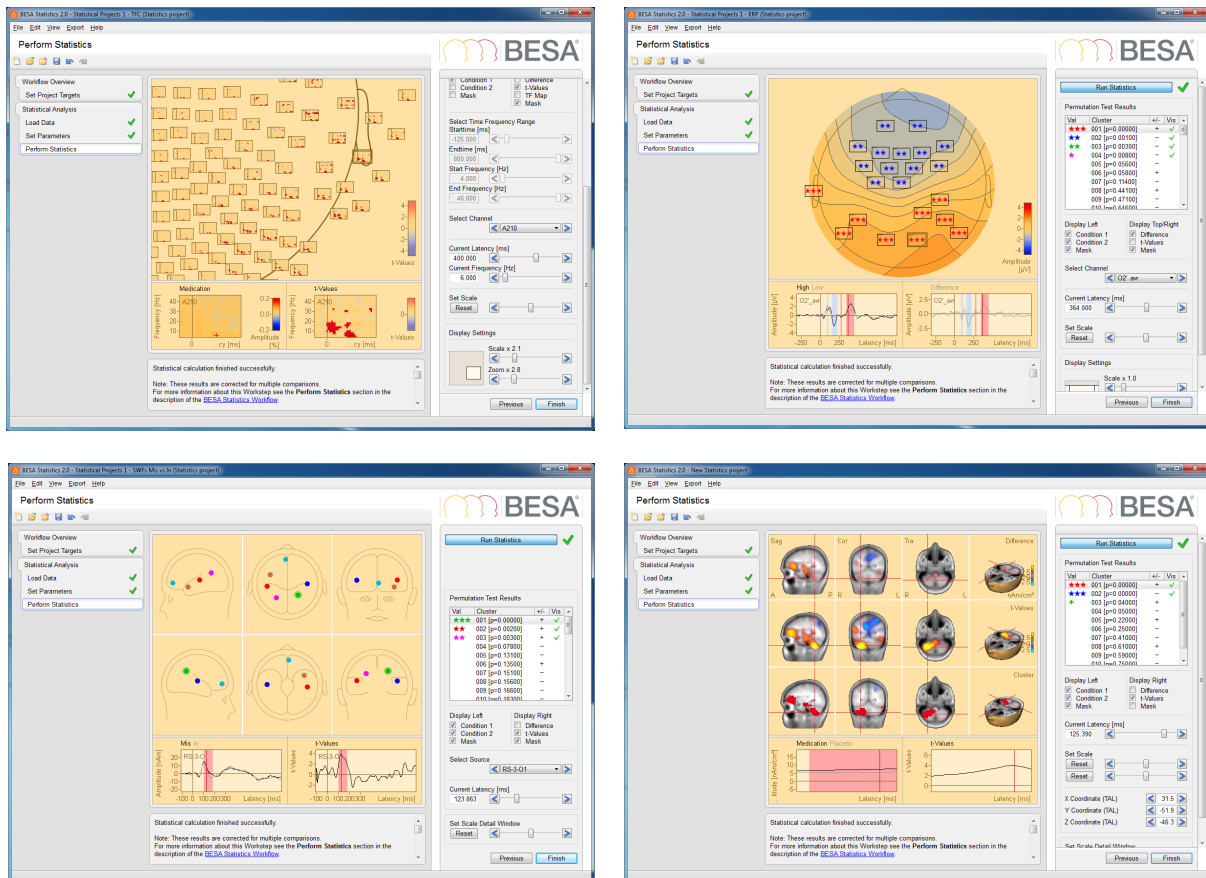


Figure 12.9.1 Visualization of significant data clusters obtained by permutation statistics

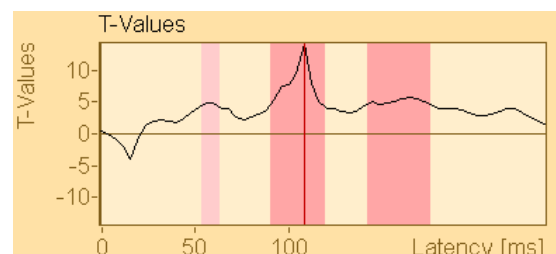
Permutation results are data clusters that are now associated with a specific p-value. Up to 100 data clusters are listed. Significant clusters are labeled with stars in the first column of the permutation results:

Val	Cluster	+/-	Vis
★★★	001 [p=0.00000]	+	✓
★	002 [p=0.01000]	+	✓
★	003 [p=0.01000]	+	✓
★	004 [p=0.01000]	+	✓
★	005 [p=0.02000]	+	✓
+	006 [p=0.03000]	+	
	007 [p=0.06000]	+	
	008 [p=0.06000]	+	
	009 [p=0.09000]	+	
	010 [p=0.20000]	-	

One-tailed t-test, ANOVA / ANCOVA, Correlation	Two-tailed t-test
★★★: p-values < 0.001	★★★: p-values < 0.0005
★★: p-values < 0.01 and ≥ 0.001	★★: p-values < 0.005 and ≥ 0.0005
★: p-values < 0.05 and ≥ 0.01	★: p-values < 0.025 and ≥ 0.005
+: p-values < 0.1 and ≥ 0.05	+: p-values < 0.05 and ≥ 0.025

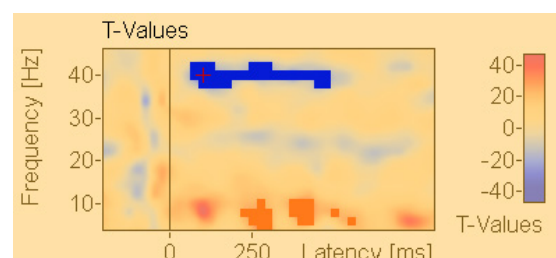
If there is more than one cluster in the same category, clusters will have a different color to make them distinguishable.

Clusters can be positive or negative (see 8.2) and are accordingly labeled “+” or “-” in the third column of the permutation results. **For ANOVA / ANCOVA, clusters can only be positive as there are only positive F-values.**



Clusters can be selected to be **visualized** in the Main Window by setting a **green tick-mark** in the fourth column of the permutation results. Only selected clusters will be displayed.

Positive clusters (see 8.2) are marked by light red bars (ERP /ERF, image, SWF) or voxel masks (TFC). Negative clusters are marked by light blue bars or voxel masks (TFC) in the Detail Windows. The **currently selected** cluster is colored dark red or dark blue.

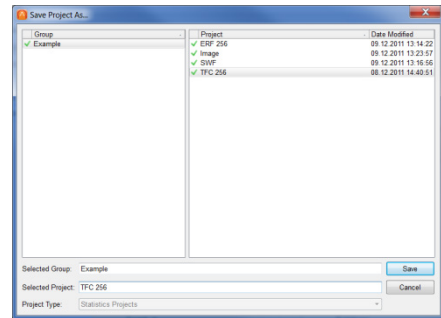


The remaining settings in the Interaction Window are analogue to the Set Parameters worksteps (12.8) with the difference that “Mask” now only refers to selected clusters to be visualized.

Pressing **Finish** saves the current project.

After specifying a group and project name, projects including all completed workflows steps will be saved and marked with a green arrow. Completed, but not finished projects are marked by a green dot. Incomplete projects are marked by a red dot.

A project can be re-opened at any time by pressing **File / Open Project**

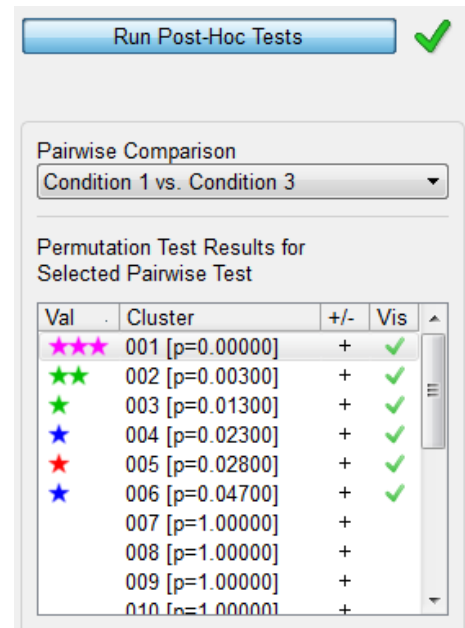
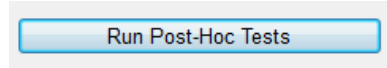
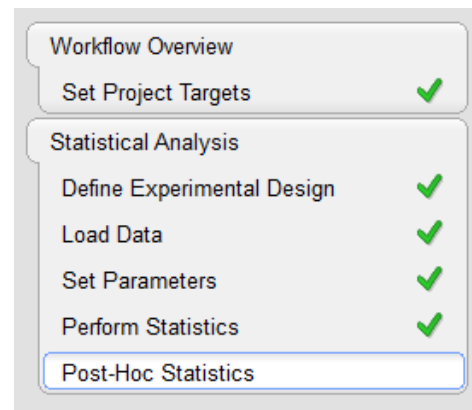


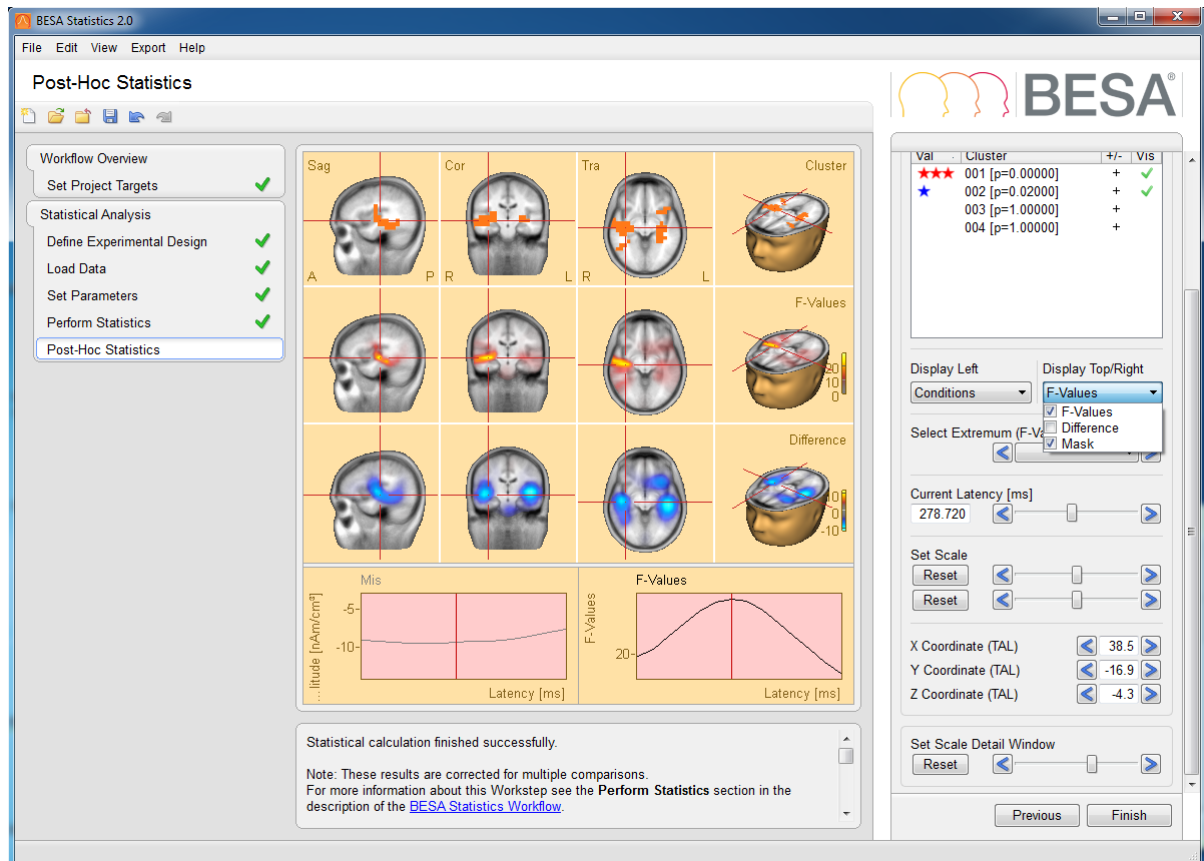
## 12.10. Post-Hoc Testing – ANOVA / ANCOVA only

After finishing the Perform Statistics workstep in the ANOVA / ANCOVA workflow and (only) if clusters were identified that reject the null-hypothesis of exchangeability of groups / conditions, i.e. that show a significant main effect in the ANOVA / ANCOVA, it is necessary to run post-hoc tests in order to find out, which pairwise comparisons were responsible for the main effect (8.1.2).

Pressing **Run Post-Hoc Tests** will initialize the computation of pairwise comparisons with Scheffe's test (8.3). Post-hoc testing also follows the cluster-permutation logic (8.2). Therefore, a permutation test is computed for each pairwise comparison, the significance level being adjusted following Bonferroni-Holm (8.3). Please note that computation might take a while, depending on the number of pairwise comparison and data size.

Results for the Post-Hoc tests can be displayed for each pairwise comparison following the same logic as described in the Perform Statistics workstep (12.9).





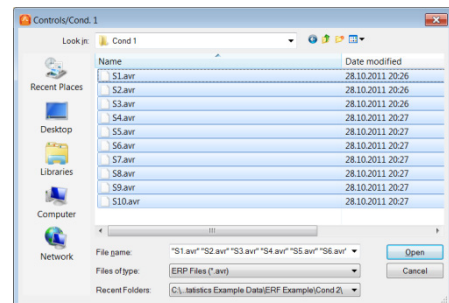
In the drop-down menu **Display Top/Right**, it is now possible to either select **F-values** for the current pair-wise comparison or **Difference** in order to display the mean difference between the input groups / condition of the current pair-wise comparison.

## 13. Specific Issues when Analyzing ERP / ERF data

### 13.7. Load Data

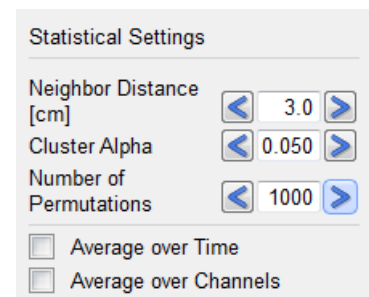
When ERP / ERF data are loaded, BESA Statistics expects the file format \*.avr (see 12.7.4) or the format \*.vhdr for data created in BrainVision Analyzer 2.

**Please note that in case EEG or MEG data data in the \*.avr format are analyzed, one of the folders must contain a channel position file (\*.elp, see 12.7.4).**

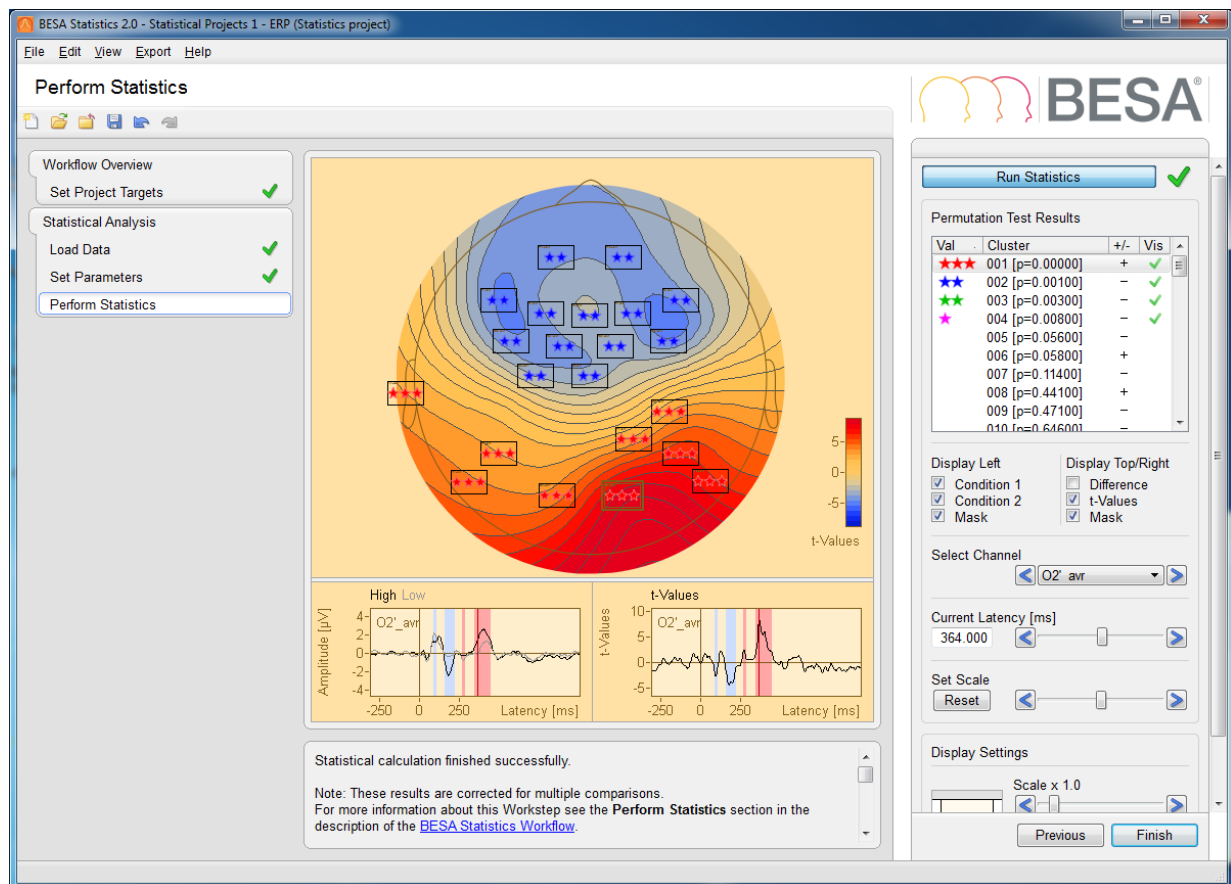


### 13.8. Set Parameters

The **Neighbor Distance** should be specified according to the current channel layout. As different EEG caps will have a different number of electrodes with different mean distances, each electrode cap will require a particular Neighbor Distance. The same is true for MEG systems. The summary window in the Run Statistics worstep allows inspecting the correctness of the Neighbor Distance.



## 13.9. Perform Statistics



When analyzing sensor space data, the permutation test can reveal several clusters where the null-hypothesis must be rejected, i.e. the groups / conditions / subjects are not interchangeable. Please note that it is possible that different clusters may reflect the same underlying effect, e.g. the positive and negative field resulting from underlying dipolar activity of the same generator inside the brain. **These clusters should not be interpreted separately (see Important note on the Interpretation of Clusters in chapter 8.2).**

**Permutation Test Results**

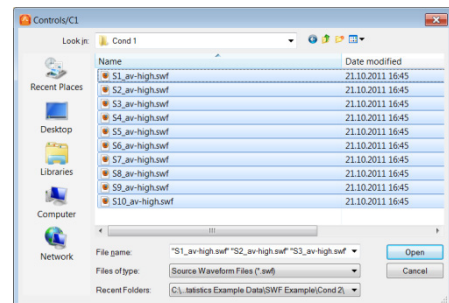
Val	Cluster	+/-	Vis
★★★	001 [p=0.00000]	+	✓
★★	002 [p=0.00100]	-	✓
★★	003 [p=0.00300]	-	✓
★	004 [p=0.00800]	-	✓
	005 [p=0.05600]	-	
	006 [p=0.05800]	+	
	007 [p=0.11400]	-	
	008 [p=0.44100]	+	
	009 [p=0.47100]	-	
	010 [p=0.64600]	-	

## 14. Specific Issues when Analyzing Source Waveforms

### 14.1. Load Data

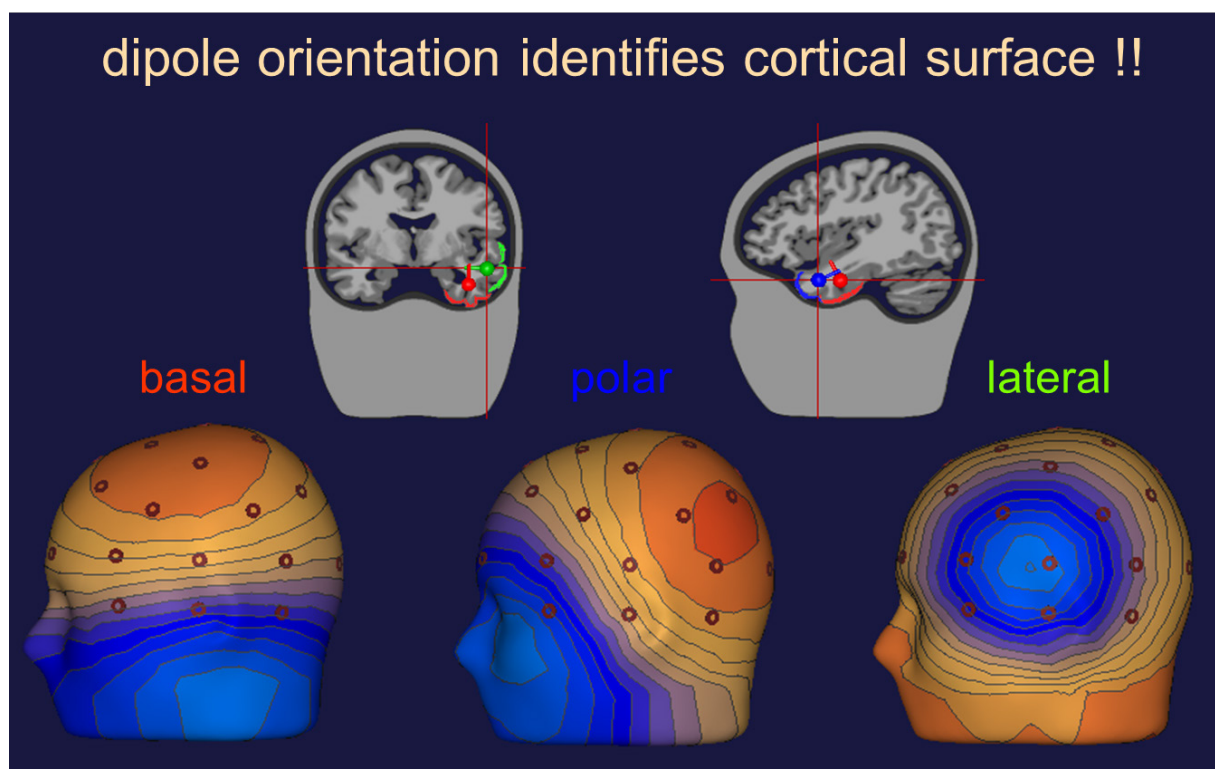
When source waveform data are loaded, BESA Statistics expects the file format \*.swf (see 12.7.4).

Please note that when SWF data are analyzed, one of the folders must contain a source position file (\*.bsa, see 12.7.4).

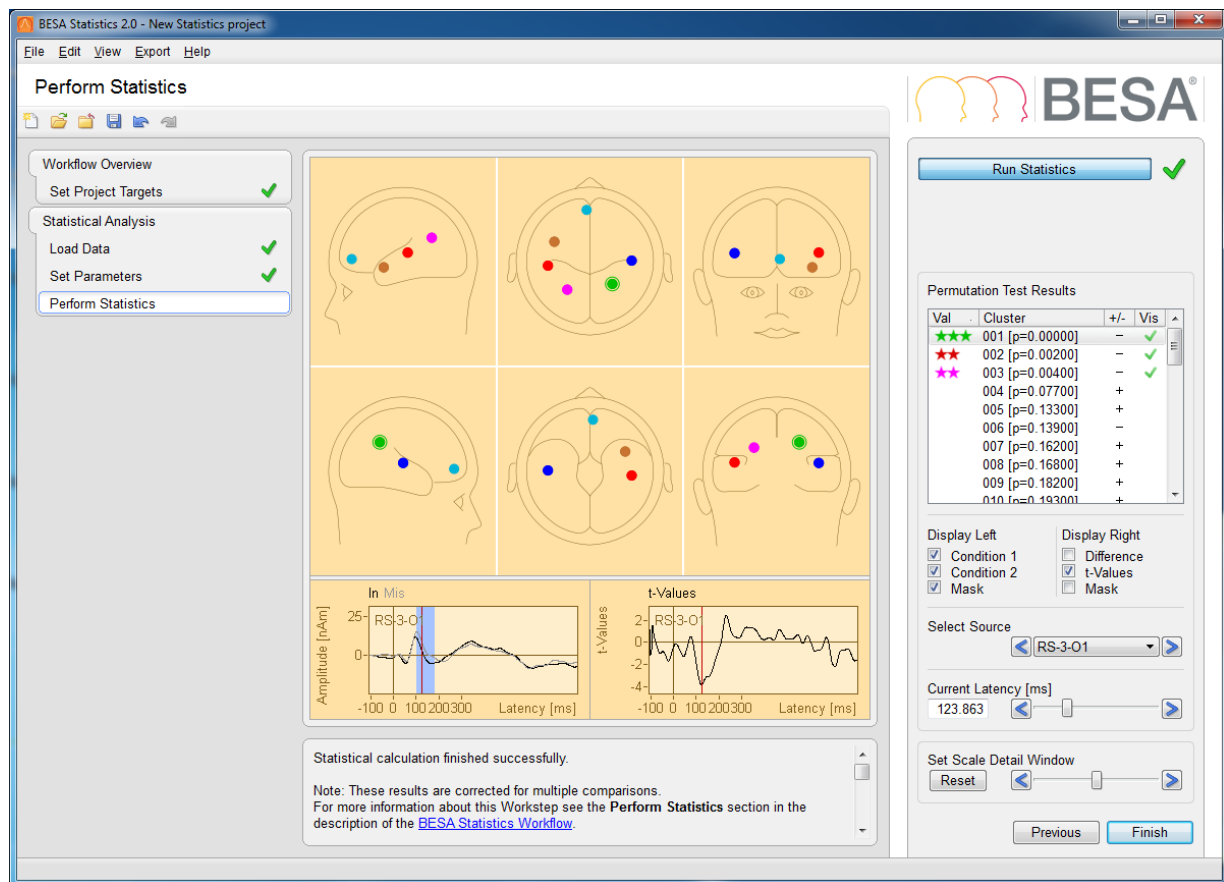


### 14.2. Set Parameters

**No Neighbor Distance** can be set as individual sources are thought to reflect activity in discrete cortical patches. Grouping discrete sources would lead to misleading results. Thus, clusters will only be built within each individual source. Please note that each orientation of a regional source is considered as a separate source, as each orientation represents a different cortical patch as illustrated in the example below.



### 14.3. Perform Statistics



Permutation test results will be clusters where the null-hypothesis is rejected, i.e. data are not interchangeable. By default, BESA Statistics automatically selects the cluster and the corresponding source / orientation with the largest T-/F-/R- value for visualization in the Detail Windows.

## 15. Specific Issues when Analyzing Time Frequency Data

Start BESA Statistics and press [Start new Project](#).

### 15.1. Set Project Targets

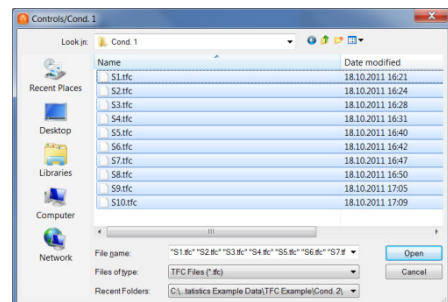
It is possible to analyze TFC results based on source montages (SWF) or based on channels in sensor space (ERP / ERF). If the latter is selected, it is necessary to choose between EEG, MEG, intra-cranial (ICR) and polygraphic data (POL).

Select data type for which statistics shall be performed

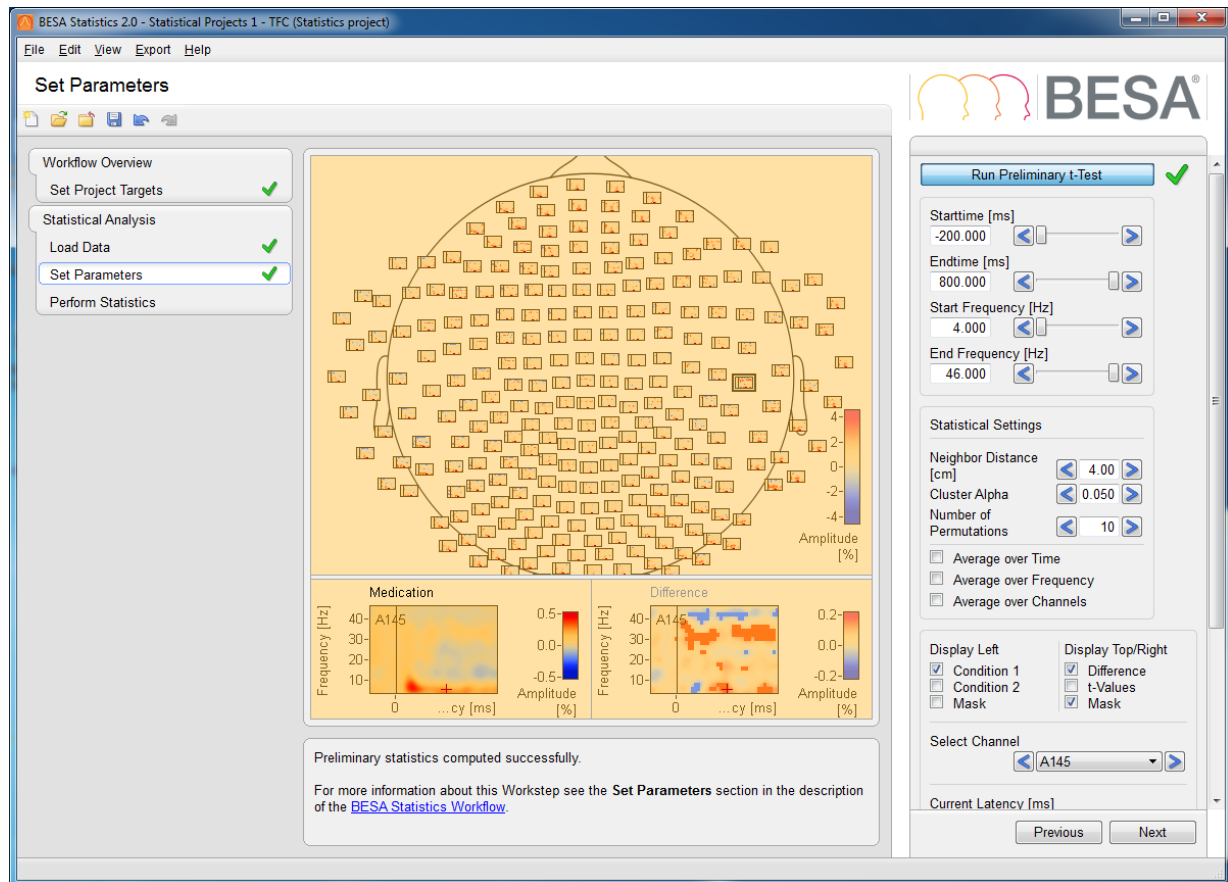
- ☒ TFC
  - ☐ SWF
  - ☒ ERP / ERF
    - ☐ EEG
    - ☒ MEG
    - ☐ ICR
    - ☐ POL
- ☐ Image
- ☐ SWF
- ☐ ERP / ERF

### 15.2. Load Data

If TFC analysis is performed on ERP / ERF EEG and MEG data files that were created by BESA, the data should be associated with a channel position file (\*.elp, see 12.7.1) so that BESA Statistics can display the channels at their correct position and also retrieve the correct neighborhood information for spatial clustering. ICR and POL data are **not** associated with a channel position file and no spatial clustering will be computed. **TFC analysis based on source montages (SWF) should be associated with a source position file (\*.bsa, see 12.7.4)** so that BESA Statistics can display the sources at their correct position.



### 15.3. Set Parameters



Once preliminary statistics are computed, an electrode / sensor of interest can be selected by either left-clicking on it in the Main Window or by using the **Select Channel** drop down box in the Interaction Window. The selected channel is marked by a frame. Please note that you can quickly browse through the channels using the arrow buttons to the left and right of the Select Channel drop-down box. The selected channel will be displayed in the left and right Detail Windows.

A crosshair can be placed at a time-frequency point of interest by entering a latency (in ms) / frequency (in Hz), dragging the **Current Latency** / **Current Frequency** slider or using the left and right arrow buttons next to the sliders in the Interaction Window.

**Display Left** in the Interaction Window defines what is being displayed in the left Detail Window. By default, **Group / Condition 1** and **Group / Condition 2** will be displayed along with a **Mask** indicating significant time periods. Time-frequency ranges, during which group / condition 1 has smaller values than condition 2 are displayed in blue. Time-frequency ranges, during which group / condition 1 has larger values than condition 2 are displayed in red. If desired, the mask as well as each of the groups / conditions can be switched off by un-checking the tick-mark in the according selector boxes.

**Display Top/Right** in the Interaction Window determines what will be displayed in the right Detail Window and the Main Window. A selection can be made between **Difference**, i.e. the difference between groups / conditions or the according **T-/F-/R-Values**. It is also possible to display the **Mask** of significant time-periods in the right Detail Window analogue to the left Detail Window.

In case TFC data based on EEG / MEG channels are analyzed, the **Neighbor Distance** should be specified according to the current channel layout. As different EEG caps will have a different number of electrodes with different mean distances, each electrode cap will require a particular Neighbor Distance. The same is true for MEG systems. The summary window in the Run Statistics workstep allows inspecting the correctness of the Neighbor Distance.

If desired, the **Starttime / Start Frequency** and **Endtime / End Frequency** can be adjusted.

Starttime [ms]  
-200.000

Endtime [ms]  
800.000

Start Frequency [Hz]  
4.000

End Frequency [Hz]  
46.000

When analyzing TFC data it is possible to **Average over Time**, **Average over Channels** and **Average over Frequency**

Statistical Settings

Neighbor Distance [cm] 3.0

Cluster Alpha 0.050

Number of Permutations 1000

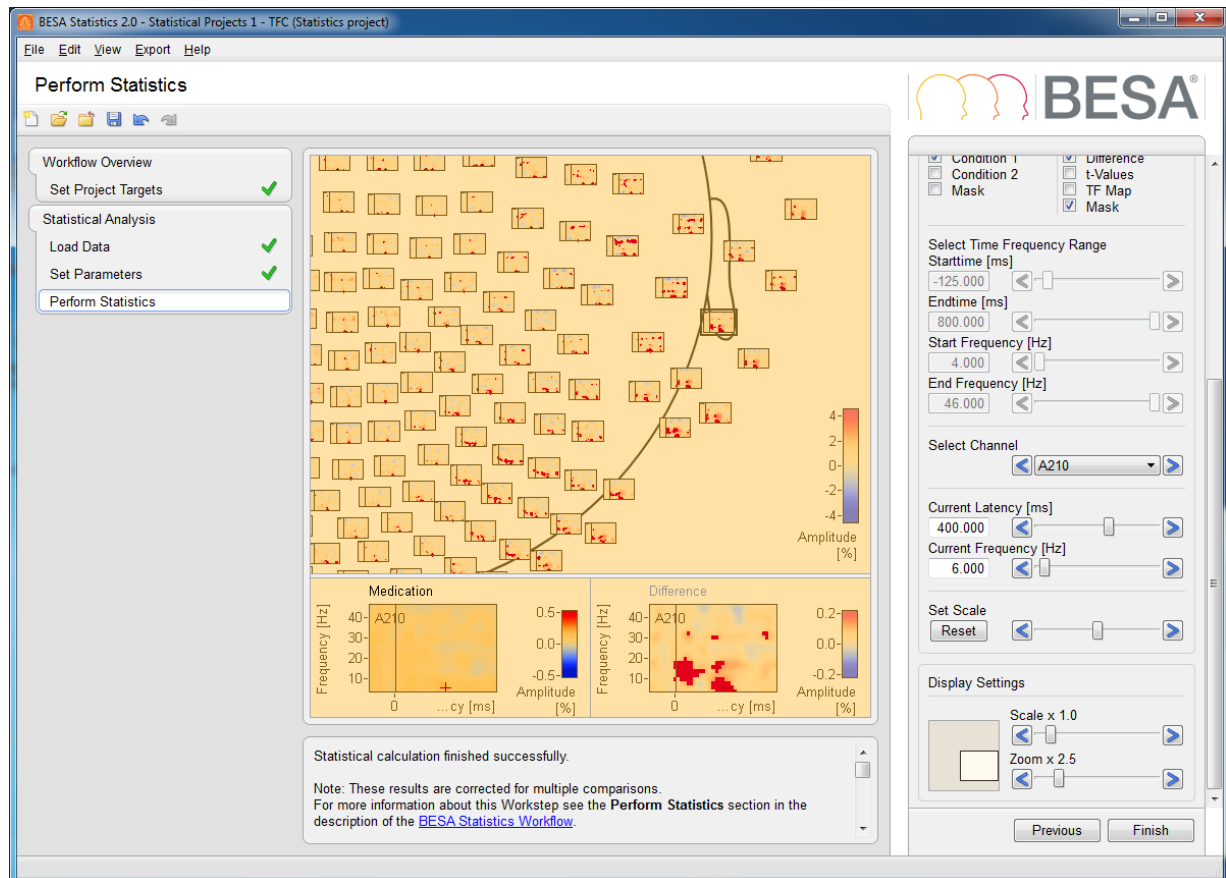
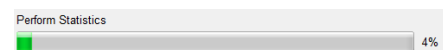
☐ Average over Time

☐ Average over Frequency

☐ Average over Channels

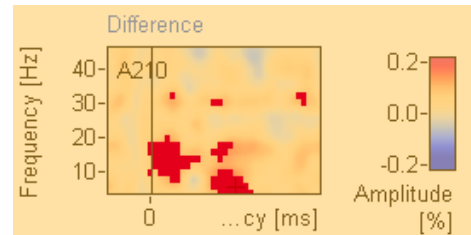
## 15.4. Perform Statistics

Please note that running the permutation test on TFC data can take several minutes depending on the number of subjects, channels, and time-frequency bins.



Permutation test results will be clusters where the null-hypothesis is rejected, i.e. data are not interchangeable. By default, BESA Statistics automatically selects the cluster and the corresponding source / orientation with the largest T-/F-/R- value for visualization in the Detail Windows.

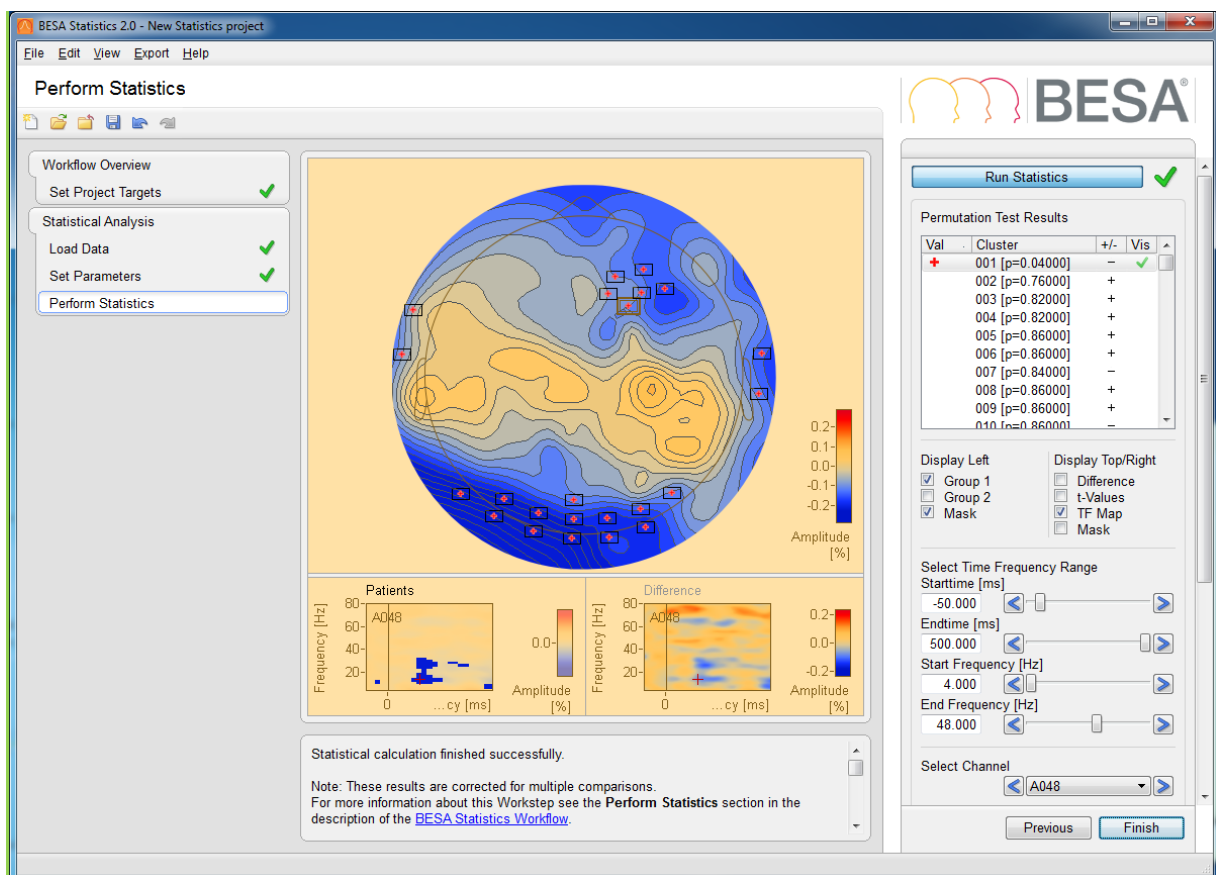
All channels belonging to a cluster are overlaid with the according cluster mask, if the mask is displayed.



### 15.4.1. Time-Frequency Mapping in TFC ERP / ERF Data

If a t-test or a correlation project was chosen and permutation testing was run on sensor space TFC data (TFC ERP / ERF), it is possible to display a topographic map of the mean difference between input groups / conditions (t-test) or the mean of the input group / condition (correlation) for TFC clusters. This feature can be helpful in order to investigate, what the distribution of the original sensor space data was that led to a significant effect in the time-frequency domain for t-testing or correlation.

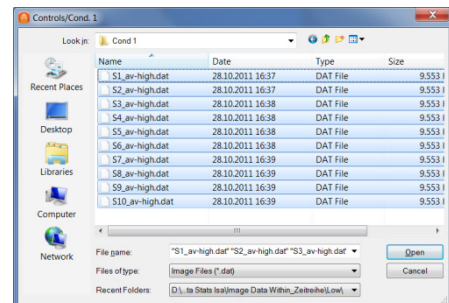
By default, the mean values displayed in the TF map are based on the time and frequency range of the selected cluster. Starttime / Endtime, as well as Start Frequency / End Frequency are automatically set to these boundaries when a cluster is selected, and can be changed within the boundaries set by the cluster.



## 16. Specific Issues when Analyzing Image Data

### 16.1. Load Data

When analyzing Image data, BESA Statistics expects \*.dat files (12.7.4). Each file can contain voxel information on 1 time-point or a time-series. **No additional files are required.**



### 16.2. Set Parameters



Depending on the parametric test chosen, the main window displays activity in the input groups / conditions, the activation difference between groups / conditions (t-test only), and statistical values (t-values, F-values, r-values), based on the selection under **Display Image**.

If an ANOVA / ANCOVA is calculated, the main window displays the preliminary F-values in the first row, the second and third row display groups / conditions as selected by the user in the **Display Image** drop-down menu. Please note that a maximum of two groups / conditions can be selected at a time. If the group / condition selection is altered by the user, the previous selection must first be undone by un-ticking the according group / condition.

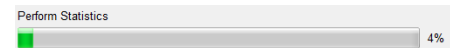
Once preliminary statistics are computed, it is possible to select a voxel of interest by using the **Select Extremum** drop down box in the Interaction Window. The selected voxel is marked by a crosshair.

Please note that you can quickly browse through the extrema using the arrow buttons left and right of the Select Extremum drop-down box. The activation time-course of the selected extremum are displayed in the left and right Detail Windows. Additionally, Talairach coordinates of the selected extremum are displayed at the bottom of the Interaction Window.

Please note that it is not possible to specify a **Neighbor Distance** for image data. The reason is that the neighborhood structure is given for a volume image consisting of adjacent voxels. I.e. the **neighborhood information is automatically retrieved by BESA Statistics!**

### 16.3. Perform Statistics

Please note that running the permutation test on Image data can take several minutes depending on the number of subjects and time-points.



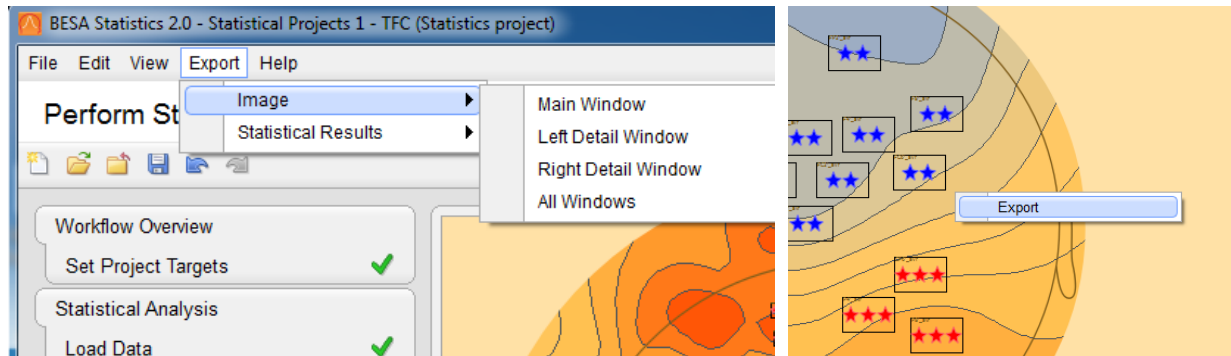
Permutation test results will be clusters where the null-hypothesis is rejected, i.e. data are not interchangeable. By default, BESA Statistics automatically selects the cluster and the corresponding source / orientation with the largest T-/F-/R- value for visualization in the Detail Windows.

If an ANOVA / ANCOVA is calculated, the main window displays the clusters in the first row, the F-values in the second and third row display groups / conditions as selected by the user in the **Display Image** drop-down menu. Please note that only one group / condition can be selected at a time. If the group / condition selection is altered by the user, the previous selection must first be un-done by un-ticking the according group / condition.

## 17. Exporting Images and Results

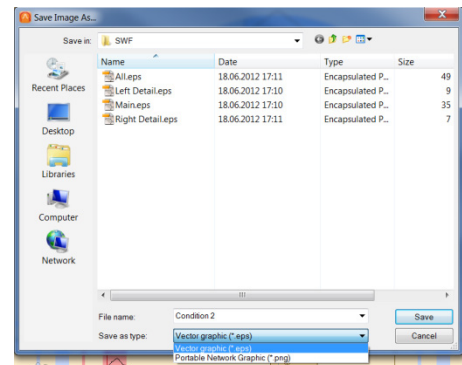
### 17.1. Exporting Images

After running preliminary statistics or after the Perform Statistics workstep is finalized it is possible to export resulting images in the main window and the detail windows. This can be achieved via the menu bar (see 11.1.4) or via right-clicking in the corresponding window.



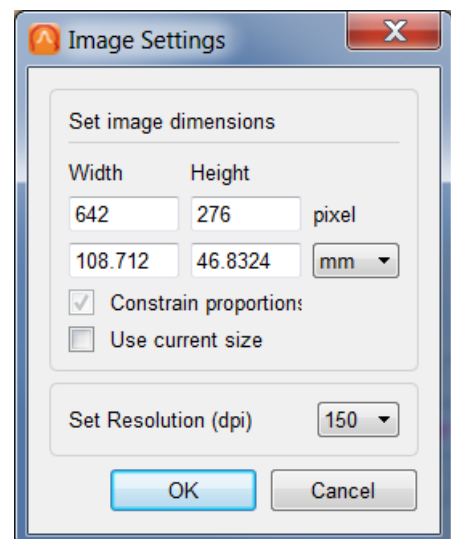
Images can be saved as **portable network graphics (\*.png)** or **vector graphics (\*.eps)**.

The selection can be made in the saving dialogue under **Save as type:**



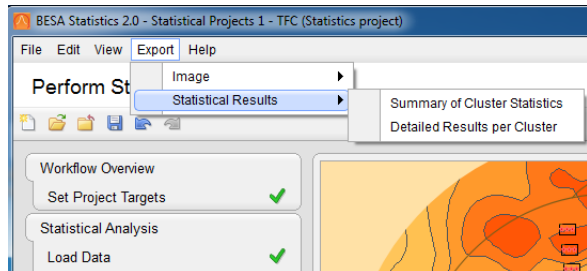
When saving \*.png files, the dimension and resolution of the resulting image can be defined. By default, the current size of the image on the screen is used. Width and height can be altered with or without constraining proportions using mm or inch as units. The resolution can be set to 150, 300 or 600 dots per inch.

When saving \*.eps files the image size cannot be changed as vector graphics are by definition fully resizable.



## 17.3. Exporting Statistical Results

After the Perform Statistics workstep is finalized it is possible to export statistical results via the menu bar (see 11.1.4). A choice can be made between “Summary of Cluster Statistics” and “Detailed Results per Cluster”. The resulting files are comma-separated.



**Summary of Cluster Statistics** will output the cluster value, p-value, cluster boundaries and means for condition/group 1 and 2 for each cluster found. For ERP/ ERF and TFC data, labels of the channels that are associated with a cluster are also exported.

	A	B	C	D	E	F	G	H	I
1	Cluster ID	p-value	Cluster value	Mean for High	Mean for Low	Start Time	End Time	Start Frequency	Frequency
2	Cluster 1	0	1968.64	142.438	0.308131	144	484	0	0
3	Cluster 2	0.001	-1505.43	-143.998	-0.351573	252	548	0	0
4	Cluster 3	0.003	-537.501	-138.388	0.0501521	152	240	0	0
5	Cluster 4	0.009	-394.209	-245.404	-109.723	72	132	0	0
6	Cluster 5	0.056	214.41	209.692	-0.208071	68	128	0	0
7	Cluster 6	0.057	-212.52	-0.93748	-0.110508	572	644	0	0

...

**Detailed Results per Cluster** will output mean values per person and group/condition for each cluster.

File Name	Group/Condition	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	...
S1_av-exp High		171.953	-151.161	-194.226	-263.472	0.801743	-0.507554	
S2_av-exp High		169.847	-181.393	-136.974	-505.065	341.797	-157.004	
S3_av-exp High		145.465	-160.939	-0.763417	-308.214	271.868	-0.869147	
S4_av-exp High		208.697	-200.994	-282.052	-168.923	220.373	-0.962667	
S5_av-exp High		0.97476	-116.565	-0.769849	-0.856455	0.872301	-18.556	
S6_av-exp High		191.059	-198.049	-141.889	-389.266	424.777	-0.356282	
S7_av-exp High		110.585	-132.591	-105.209	-131.805	-0.285907	-0.332387	
S8_av-exp High		116.152	-108.109	-0.81392	-0.738287	0.0227702	-0.72513	
S9_av-exp High		0.445815	-0.88784	0.176864	-233.962	240.692	-0.845003	
S10_av-exp High		168.562	-101.396	-306.497	-293.862	460.878	-135.099	
S1_av-exp Low		0.643345	-0.626427	-0.36212	-128.735	-0.628347	-0.282522	
S2_av-exp Low		0.59677	-0.681179	-0.1748	-271.552	0.938874	-0.371693	
S3_av-exp Low		0.0202673	-0.110512	0.431863	-106.496	-0.256526	-0.231328	
S4_av-exp Low		0.721863	-0.556209	-0.448149	-0.711987	-0.436034	0.275218	
S5_av-exp Low		0.161173	-0.47262	0.152608	-0.349198	0.217433	-0.421711	
S6_av-exp Low		0.469015	-0.533854	0.0798142	-191.912	0.542202	0.0566957	
S7_av-exp Low		-0.155377	-0.149824	0.434543	-0.605937	-0.473177	0.934194	
S8_av-exp Low		0.408926	-0.377373	0.168674	-0.268427	-115.001	-0.418365	
S9_av-exp Low		0.0253412	-0.16751	0.595101	-0.932934	-0.485215	0.112018	
S10_av-exp Low		0.240665	0.159776	-0.376014	-111.691	-0.349912	-0.757587	

### Important Note on mean values derived from ANOVAs / ANCOVAs

When ANOVA / ANCOVA is used as preliminary test, clusters are derived based on F-values. F-values can only be positive, irrespective of the underlying data having positive or negative values. For example, an F-test cluster can be identified in MEG sensors picking up activity of dipolar fields, i.e. some sensors will be associated with positive and some with negative values. Exporting mean values for the cluster can then result in values around 0. Therefore, mean values based on F-test clusters should be interpreted with caution.

## 18. Appendix

### 18.1. How to deal with bad channels when analyzing ERP / ERF data

By default, BESA Statistics only utilizes information about EEG / MEG channels configurations as available in the elp-file (see 12.7.4 and 19). The elp-file does not contain information on channel status, e.g. channels that were excluded from analysis in BESA Research by setting them as bad. Thus, all channels that were previously defined as bad will still be used by BESA Statistics. This might not be desirable, as the channels might contain flawed data.

On the other hand, it can be a problem for cross-subject statistics if bad channels are defined and shall be excluded. If different bad channels are defined for different subjects, the number of channels does not match across subjects. This is a requirement for cross-subject statistics as it is implemented in BESA Statistics, however. There are two ways to deal with this problem:

#### 18.1.1. Interpolating bad channels in BESA Research

The easiest solution might be to interpolate all bad channels in BESA Research by right-clicking on the channel label and choosing “Interpolate this channel” rather than “Define channel as bad”. Alternatively, you can press “Edit / Bad Channels” and double-click on all channel labels in the right panel. This will also lead to bad channels being interpolated. After interpolation you should export (“File / Export”) the data with the setting “Current Montage”. This will lead to BESA Research outputting the interpolated EEG / MEG channel traces rather than the flawed data of the bad channels.

#### 18.1.2. Creating a channel montage only containing good channels in BESA Research

If you want to omit bad channels from statistical analysis, you should create a new channel montage in BESA Research. With a concerned dataset open, press “Edit / Montage” to open the montage editor. In the middle pane you can now delete all channels you want to omit from statistical analysis by right-clicking and selecting “Delete montage channel ...”. Save the newly created montage by pressing “File / Save Montage as”. Please specify a name of your choice. This user-montage can now be applied to the datasets of all subjects that should enter statistical analysis by pressing the USR button and selecting the according montage for each dataset.

The data can now be exported for statistical analysis (“File / Export”) with the settings “ASCII vectorized” and “Current Montage”. This will make sure that all subjects are associated with the same number of channels.

What you will need to do additionally is to export a new elp-file containing only information about the channels in the montage. To do so, make sure that the montage you want to use is applied on the data. Next, press “**File / Head Surface Points and Sensors / Save Electrode File for Current Montage**”. This will make sure that the number of channels and the labels of the used channels are identical in the exported data and the elp-file.

### 18.2. How to deal with re-montaged or re-referenced data

In case you have already created a channel montage only containing channels of interest or if you are dealing with re-referenced data, you should follow the same procedure as outlined in chapter 18.1.2., second and third paragraph.

## 19. File Formats

In case BESA Statistics is used for data created in other software, files need to be prepared to meet file format expectations.

In the following we describe the requirements which data created using other software should meet to be compatible with the BESA Statistics data formats described in the table of section 12.7.4. All BESA Statistics data formats are in ASCII format to facilitate human readability. All floating point numbers are represented in scientific notation with up to eight significant digits. An exception to this rule are the BrainVision Analyzer 2 data formats that are supported (\*.vhdr files; for a format description please consult the manual of the BrainVision Analyzer 2 software).

### 19.1. The BESA Statistics Time-Frequency File Format (\*.tfc)

The header line contains the following data descriptors (13 descriptors displayed in **bold**, the values shown are only examples and comments are in *italic*):

1. **VersionNumber**=\_\_v\_5.1: *Version number of the current software.*
2. **DataType**=COHERENCE\_SQUARED: *Data type contained of the file. Possibilities are:*
  - COHERENCE\_SQUARED: *coherence values squared.*
  - ERDERS\_AMP: *ERD(event-related desynchronization) and ERS(event-related synchronization) amplitudes.*
  - ERDERS\_POWER: *power.*
  - ERDERS\_AMP\_P\_VALUE *(not allowed for BESA Statistics)*
  - ITPL: *Inter-trial phase locking is a measure for the phase consistency of the oscillatory response of a single surface or source channel across multiple trials.*
  - PHASE\_COHERENCE: *phase coherence.*
  - ERDERS\_AMP\_P\_VALUE *(not allowed for BESA Statistics)*
  - TIME\_FREQUENCY\_AMP: *time frequency amplitude.*
  - TIME\_FREQUENCY\_POWER: *power.*
3. **ConditionName**=s: *Condition name. Usually indicated by experiment. E.g. "high-" or " low intensity".*
4. **NumberTrials**=250: *Number of trials.*
5. **NumberTimeSamples**=25: *Number of time samples.*
6. **TimeStartInMS**=-100.0: *Start time in [ms].*
7. **IntervallInMS**=25.0: *Interval length in [ms].*
8. **NumberFrequencies**=24: *Number of frequency bins.*
9. **FreqStartInHz**=4.0: *Starting frequency in [Hz].*
10. **FreqIntervallInHz**=2.0: *Frequency interval in [Hz].*

11. **NumberChannels=127:** *Number of channels in the data set.*
12. **StatisticsCorrection=Off:** *Statistics correction flag.*
13. **EvokedSignalSubtraction=Off:** *Flag to signal evoked signal subtraction (On if evoked signal is subtracted, Off otherwise).*

The second row of the header contains the labels of the channels. Their labeling can be chosen freely provided only letters and number are used and the amount of characters per entry does not exceed eight (e.g. E1 E2 ... for electrodes).

The remaining lines contain **NumberChannels** blocks starting from the first channel. Each block is separated from the next one by an empty line. Each block contains **NumberFrequencies**-rows and **NumberTimeSamples**-columns. The values for each time-frequency entry in the blocks are floating point numbers and should be separated by a tab.

## 19.2. The BESA Statistics Channel Definition File Format (\*.elp)

Each line of the channel definition file contains one label, an identifier and 2D coordinates. The format is: **Identifier Label Theta-coordinate Phi-coordinate**.

Identifiers can be:

- **EEG** -- scalp electrode (use also instead of SCP).
- **POL** -- polygraphic channel (use also instead of PGR).
- **ICR** -- intracranial electrode.
- **MEG** -- MEG sensor.
- **REF** -- reference electrode. This can only occur once.

Labels should have at most 8 characters and be constituted only by letters and numbers, but can otherwise be chosen freely. Note though that the corresponding \*.avr files (see 19.6) have to use the same convention for the label naming.

Coordinates are represented as floating point numbers in [deg] in the unit sphere coordinate system. This coordinate system is defined by reference points on the head known as fiducials. The reference points are normally the nasion (NAS), the left preauricular point (LPA), and the right preauricular point (RPA). The x axis is defined by the line joining LPA and RPA, positive towards RPA. The y axis is defined by the line through NAS that is perpendicular to the x axis (positive towards NAS). The z axis is perpendicular to the x and y axes, and goes up out of the upper part of the head (e.g. Cz). The Theta-coordinate (azimuth) is zero at the z axis, 90 deg at the x axis and -90 deg at the negative x axis. The Phi-coordinate instead goes from [-90 deg, 90 deg] from the negative y axis to the positive y axis for positive theta (i.e. for the right hand side of the scalp), while it goes from [90 deg, -90 deg] from the negative y axis to the positive y axis for negative theta (i.e. for the left hand side of the scalp).

### 19.3. The BESA Statistics Solution File Format (\*.bsa)

The file contains the position and orientation of dipoles/sources.

The first line of the file contains a dummy identifier **BSA\_1.04.19990715**.

The second line of the file contains the labels: **Type**, **x-loc**, **y-loc**, **z-loc** (the position of the dipole/source in unit sphere Cartesian coordinates), **x-ori**, **y-ori**, **z-ori** (the unit vector orientation of the dipole/source).

This coordinate system is defined by reference points on the head known as fiducials. The reference points are normally the nasion (NAS), the left preauricular point (LPA), and the right preauricular point (RPA). The x axis is defined by the line joining LPA and RPA, positive towards RPA. The y axis is defined by the line through NAS that is perpendicular to the x axis (positive towards NAS). The z axis is perpendicular to the x and y axes, and goes up out of the upper part of the head (e.g. Cz).

Type can assume the values:

1. **SngDip** - single dipole.
2. **RegSrc** - regional source.

## 19.4. The BESA Statistics Image File Format (\*.dat)

Source analysis files are used to store a number of possible scenarios:

- **Image ASCII Files:** Image amplitudes of all grid points in the currently displayed image in the current image resolution.
- **Image ASCII Files - all latencies:** Image amplitudes of all grid points in the current image resolution for all time points.

The header looks like the following example (required labels are in **bold**, comments are in *italic* and all other text serves as sample input):

**BESA\_SA\_IMAGE:2.0**

**Data file:** C:\ExampleDirectory\exampleFile.foc: *Meta information.*

**Condition:** s Low Filter: 0.5 Hz: *Condition name, usually specified by the experiment.*

**Method:** Standard LORETA: *Used method. See below.*

**Regularization:** First: SVD 0.0050Iterations: SVD 0.0050;: *Meta information.*

**nAm/cm<sup>3</sup>**

**Grid dimensions ([min] [max] [nr of locations]):**

**X:** -73.500000 73.500000 22: *Min, max and number of points along the X axis.*

**Y:** -107.930000 74.070000 27: *Min, max and number of points along the Y axis.*

**Z:** -60.340000 79.660004 21: *Min, max and number of points along the Z axis.*

=====

**Method** can assume the following values:

- MSBF
- CLARA
- Standard LAURA
- Standard LORETA
- Standard sLORETA
- Standard swLORETA
- Standard SSLOFO: 3 iterations, clip 10, smooth 1.0;

The data section following the header is composed of matrixes of **nr of locations** - of **X** columns and **Y** rows with values for each voxel at this slice (where the slice is indexed by **Z**). There are **nr of locations** (specified in the header for **Z**) slices and the index starts at zero. Slices are separated from each other by an empty line.

The data section for **Image ASCII Files** will look like e.g.:

```
.
.

Z: 12

0.0000000000 0.0000000000 0.0000000000 0.0000000000 ... nr of locations of X entries.

0.0000000000 0.0000000000 0.0000000000 0.0000000000
0.0000000000 0.0000000000 0.0000000000 0.0000000000
0.0000000000 0.0000000000 0.0000000000 0.0000000000
0.0000000000 0.0000000000 0.0000000000 0.0000000000
0.0000000000 0.0000000000 0.0000000000 0.0000000000
0.0000000000 0.0000000000 0.0000000000 0.0000000000
0.0000000000 0.0000000000 0.0000000000 1.1316120625
0.0000000000 0.0000000000 0.0000000000 1.6725080013
0.0000000000 0.0000000000 1.0726528168 2.2005338669
0.0000000000 0.0000000000 1.5812959671 2.8961703777
0.0000000000 0.0000000000 2.1061606407 3.6263871193
0.0000000000 0.0000000000 2.6111714840 4.3519711494
0.0000000000 0.0000000000 2.8889267445 4.7082519531
0.0000000000 0.0000000000 2.8630757332 4.6019945145
0.0000000000 0.0000000000 2.5799186230 4.1517233849
0.0000000000 0.0000000000 2.1543741226 3.4361450672

.
.

... nr of locations of Y entries.

.
```

For each time sample the corresponding data block starts with the label **Sample** and the number of the current sample e.g. 1, and after a comma separator follows the latency in milliseconds followed by the unit label "ms". Furthermore the amount of equal signs ('=') to limit each latency sample is 94.

The data section for **Image ASCII Files - all latencies** - all latencies will look like e.g.:

```
.
.
.
```

Sample 1, 0.00 ms

Z: 0

0.0000000000 0.0000000000 0.0000000000 0.0000000000 ... **nr of locations of X entries.**

.

.

... **nr of locations of Y entries.**

Z: 1

.

.

.

=====

Sample 2, 4.00 ms

Z: 0

.

.

.

## 19.5. The BESA Statistics Source Waveforms File Format (\*.swf)

The header line contains the following data descriptors (8 descriptors displayed in **bold**, separated by blank spaces, the values shown are only examples. Comments are in *italic*.):

1. **Npts=** 200: *number of sampled points in each channel.*
2. **TSB=** -500: *time sweep begin [ms]. Time of first data point relative to zero of epoch.*
3. **DI=** 5: *digitization or sampling interval [ms].*
4. **SB=** 1: *default scaling bins/nAm in file.*
5. **SC=** 200: *default scaling calibration.*
6. **CondFile=** "OrigFile.fsg": *Name of the original bin file.*
7. **CondName=** "S1: 100 avs": *Condition name, usually specified by the experiment.*
8. **Filter=** "Filters off": *status of the filters.*

Each remaining line begins with the name of the source followed by floating point values for all **Npts** time points.

In the case of regional sources which were oriented before the export, we obtain three rows for each source (one for each orientation).

## 19.6. The BESA Statistics ERP / ERF ASCII Vectorized File Format (\*.avr)

The first of two header lines contains the following data descriptors (7 descriptors, the values shown are only examples):

1. **Npts=** 200: *number of sampled points in each channel.*
2. **TSB=** -500: *time sweep begin [ms]. Time of first data point relative to zero of epoch.*
3. **DI=** 5: *digitization or sampling interval [ms].*
4. **SB=** 1: *default scaling bins/nAm in file.*
5. **SC=** 200: *default scaling calibration.*
6. **Nchan=** 27: *number of channels.*
7. **SegmentName=** 60dB : *an optional label describing the data.*

The second line of the header contains the labels for each channel separated by a blank space. They can be chosen freely, however they have to correspond to whatever convention was used in the corresponding (see 19.2).

Each of the subsequent **Nchan** lines of the file contains floating point values for all **Npts** time points.

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