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About this Document

Preface

Welcome to the BrainVoyager Getting Started Guide! This guide is used as the basis for BrainVoyager training courses and offers an overview of the essential basic features of the software. This guide can also be used as a self-study tutorial. Note that you do not have to proceed sequentially through the steps of the Getting Started Guide, but that files saved at an earlier step are needed at later stages for data analysis. BrainVoyager is a 64-bit program available for several computer platforms currently supporting Windows 7/8/10, Mac OS 10.10 or higher, and Linux (e.g. Ubuntu, SUSE, Fedora).

If you would like to learn how to use BrainVoyager, we have a free educational version of the software available, BrainVoyager EDU, that works only with predefined training data sets, as the Getting Started Guide data, but provides the same functionality as the full version.

This guide has been prepared with the Windows version of BrainVoyager EDU, but it can also be used for learning the program with the full version and on any of the other supported operating systems. Take your time going through the steps. If the steps you are performing are not making sense to you, consider repeating some previous steps. In the beginning all actions will be explicitly written out, in later steps, acquired knowledge is needed to complete the steps.

Data

You can find the PDF version of this guide in the “GettingStartedGuides” folder of your BrainVoyager installation directory, e.g. C:\Program Files\BrainVoyagerEDU\GettingStartedGuides.

The respective data for the example dataset can be found on our website:
http://download.brainvoyager.com/bv/GSGData.zip
Please extract all files to the following folder (replace <USER> with your login name):

Windows: “C:/Users/<USER>/Documents/BVSampleData/GSGData”
Mac OS X: “/Users/<USER>/Documents/BVSampleData/GSGData”
Linux: “/home/<USER>/Documents/BVSampleData/GSGData”

How to use the Getting Started Guide

Bold

Bold body text indicates that you should perform an action.

“File” -> “New Document”

This style indicates that you should use the respective menu entries (here File and New Document).

Information or fields that require special attention will be marked by red boxes

We hope you will enjoy working with BrainVoyager as much as we do. We are grateful to many BV users for helping us improving this guide.

If you have any questions, don’t hesitate to contact us at: support@BrainVoyager.com.

Sincerely yours,
Judith Eck, Armin Heinecke, Caroline Benjamins, Henk Jansma, Hester Breman and Rainer Goebel
The “Faces-Houses-Left-Right-Center” Tutorial

This tutorial gives an introduction into essential data processing steps with BrainVoyager. The focus is on getting you started in using the program quickly and easily. The tutorial uses NIfTI data from a simple block design which was acquired in a multi-run and multi-session experiment. The acquisition of the ‘Faces-Houses-Left-Right-Center’ data was followed by another functional run which is not used in the present guide. After this second functional run, 5 volumes with reversed phase-encoding direction and otherwise identical acquisition parameters have been acquired for the option to apply distortion correction in the functional preprocessing pipeline (see https://support.brainvoyager.com/brainvoyager/available-tools/86-available-plugins/62-epi-distortion-correction-cope-plugin for more details). This was followed by the intra-session anatomical 3D scan (T1, MPRAGE). The data has been organized following version 1.1.1 of the Brain Imaging Data Structure (BIDS) (see http://bids.neuroimaging.io/ and https://doi.org/10.1038/sdata.2016.44). The “anat” folder contains the intrasession structural scan (sub-01_ses-04_acq-nondistorted_T1w*). The functional data is located in the “func” folder (sub-01_ses-04_task-blocked_run-1_bold*), which also contains the onset, duration (in seconds) and trial type definition of the experimental stimulation blocks (sub-01_ses-04_task-blocked_run-1_events.tsv).

```
│ ├── BVSampleData
│ │   └── GSGData
│ │       └── dataset_description.json
│ │       └── sub-01
│ │           └── ses-04
│ │               └── anat
│ │                   └── sub-01_ses-04_acq-nondistorted_T1w.json
│ │                   └── sub-01_ses-04_acq-nondistorted_T1w.nii.gz
│ │               └── func
│ │                   └── sub-01_ses-04_task-blocked_run-1_bold.json
│ │                   └── sub-01_ses-04_task-blocked_run-1_bold.nii.gz
│ │                   └── sub-01_ses-04_task-blocked_run-1_events.tsv
```

The experimental block design is described in the next section.

The Faces-Houses-Left-Right-Center Experiment

Black and white images of faces and houses subtending 4.7° by 4.7° were presented alternately in the left visual field, the right visual field or in the center of the visual field (see figure), resulting in six different experimental conditions. Each stimulation block lasted 16 seconds and was repeated three times within a run. Stimulation blocks were separated by “fixation blocks” of equal length. In a fixation block, only a cross was shown, which the subject had to fixate.
### Scanning Session Information

**Experiment:** Faces Houses in LVF, CVF, RVF  
**Session:** ses-04  
**Subject:** sub-01

**Conditions:**  
- Fixation  
- Faces in LVF (Left Visual Field)  
- Faces in RVF (Right Visual Field)  
- Faces in CVF (Central Visual Field)  
- Houses in LVF  
- Houses in RVF  
- Houses in CVF

**Acquisition Parameters:**  
- **NrOfSlices:** 64 (multiband sequence, factor: 2)  
- **SliceThickness:** 2 mm  
- **FoV:** 200 mm x 200 mm  
- **Matrix:** 100 x 100  
- **NrOfVolumes:** 291  
- **TR:** 2000 ms

**Intra-session structural scan (T1)**  
- **NrOfSlices:** 192

<table>
<thead>
<tr>
<th>Volume nr</th>
<th>Condition name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 3</td>
<td>Fixation</td>
</tr>
<tr>
<td>4 - 11</td>
<td>Faces_LVF</td>
</tr>
<tr>
<td>12 - 19</td>
<td>Fixation</td>
</tr>
<tr>
<td>20 - 27</td>
<td>Houses_RVF</td>
</tr>
<tr>
<td>28 - 35</td>
<td>Fixation</td>
</tr>
<tr>
<td>36 - 43</td>
<td>Faces_CVF</td>
</tr>
<tr>
<td>44 - 51</td>
<td>Fixation</td>
</tr>
<tr>
<td>52 - 59</td>
<td>Houses_LVF</td>
</tr>
<tr>
<td>60 - 67</td>
<td>Fixation</td>
</tr>
<tr>
<td>68 - 75</td>
<td>Faces_RVF</td>
</tr>
<tr>
<td>76 - 83</td>
<td>Fixation</td>
</tr>
<tr>
<td>84 - 91</td>
<td>Houses_CVF</td>
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<tr>
<td>92 - 99</td>
<td>Fixation</td>
</tr>
<tr>
<td>100 - 107</td>
<td>Faces_LVF</td>
</tr>
<tr>
<td>108 - 115</td>
<td>Fixation</td>
</tr>
<tr>
<td>116 - 123</td>
<td>Houses_RVF</td>
</tr>
<tr>
<td>124 - 131</td>
<td>Fixation</td>
</tr>
<tr>
<td>132 - 139</td>
<td>Faces_CVF</td>
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<tr>
<td>140 - 147</td>
<td>Fixation</td>
</tr>
<tr>
<td>148 - 155</td>
<td>Houses_LVF</td>
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<tr>
<td>156 - 163</td>
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</tr>
<tr>
<td>164 - 171</td>
<td>Faces_RVF</td>
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<tr>
<td>172 - 179</td>
<td>Fixation</td>
</tr>
<tr>
<td>180 - 187</td>
<td>Houses_CVF</td>
</tr>
<tr>
<td>188 - 195</td>
<td>Fixation</td>
</tr>
<tr>
<td>196 - 203</td>
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<td>204 - 211</td>
<td>Fixation</td>
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<tr>
<td>212 - 219</td>
<td>Houses_RVF</td>
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<td>220 - 227</td>
<td>Fixation</td>
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<td>228 - 235</td>
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<td>236 - 243</td>
<td>Fixation</td>
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<td>244 - 251</td>
<td>Houses_LVF</td>
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<td>252 - 259</td>
<td>Fixation</td>
</tr>
<tr>
<td>260 - 267</td>
<td>Faces_RVF</td>
</tr>
<tr>
<td>268 - 275</td>
<td>Fixation</td>
</tr>
<tr>
<td>276 - 283</td>
<td>Houses_CVF</td>
</tr>
<tr>
<td>284 - 291</td>
<td>Fixation</td>
</tr>
</tbody>
</table>

**Stimulation Protocol**  
(blocked design, one run, *events.tsv)
Part I: Analysis in Original Space - FMR-STC Documents

Step 1: Launching BrainVoyager and Opening Functional NIfTI Files

In this step we will learn how to launch BrainVoyager and create functional documents from NIfTI files using the NIfTI import. The Neuroimaging Informatics Technology Initiative (NIfTI) file format has been proposed by the Data Format Working Group (DFWG) to facilitate the exchange of neuroimaging data across software packages and is supported for data import and export in BrainVoyager. You can find more information about the use of NIfTI files in BrainVoyager in the User's Guide.

Please note: that you can also create functional documents in BrainVoyager from DICOM files via the “New Document Wizard” or the “New Document” dialog as described in Appendix B: Creation of a Functional Document From DICOM Files.

1. Launch BrainVoyager EDU by clicking the “BrainVoyager EDU” icon. Alternatively, launch the full version by clicking the “BrainVoyager” icon.

2. After opening BrainVoyager EDU, you will see a “Welcome” window giving you the opportunity to load different guides with corresponding example data sets that introduce you to the basic features of the software. Please click on the “Getting Started Guide” button to see the PDF version of the guide while working in BrainVoyager EDU.

Please note: In BrainVoyager you will see a different “Welcome” screen that provides you with additional information via the “Getting Started” link. Both the EDU and the full version of BrainVoyager give you the option to reload all files used in the previous session by selecting the respective option in the “Welcome” screen. After clicking the “Accept” button the “Welcome” window of BrainVoyager is closed.
Step 1: Launching BrainVoyager and Opening Functional NIfTI Files

BrainVoyager consists of the main documents window, a menu bar, one horizontal and one vertical toolbar and a status bar. The usage of the icons in the toolbars will be described in later steps.

![BrainVoyager welcome screen](image)

Within the horizontal icon toolbar you can see 5 pane icons to activate:

a) the “Recent Files Pane”, allowing quick access to recently opened files,
b) the “Info” and “Log Pane”, providing information about various analysis steps (i.e. document creation, coregistration)
c) the “Open Docs Pane”, providing a preview of all open documents
d) the “Zoom View Pane”

3. Some global preferences, e.g. the graphical user interface and the layout, can be set in the “Preferences” menu. This menu can be found by clicking the “Settings” icon in the toolbar (or via “File” -> “Settings”).

4. After you have opened BrainVoyager for the first time, you might see a message in the “Log Pane” stating that “OpenCL is available but not used!”. BrainVoyager supports OpenCL (GP-GPU) acceleration for several compute-intensive operations, if supported by your computer. If you see a message, as stated above, make sure that you go to “File” -> “Settings”, switch to the “Speed” tab and turn on OpenCL acceleration for “Sinc interpolation” and “Sigma filter” as indicated in the next screenshot. For more information please consult the User’s Guide:

5. Now we can create a functional document in BrainVoyager by importing the NIfTI data of a functional run. **Click** on the “Open NIfTI” icon in the horizontal toolbar or go to “File” -> “Open NIfTI…”.

Navigate to “Documents\BVSampleData\GSGData\sub-01\ses-04\func” and **select** the file “sub-01_ses-04_task-blocked_run-1_bold.nii.gz”. Please **click** on “Open” to create an “FMR” document in BrainVoyager. An “FMR” document (FMR = Functional Magnetic Resonance) stores the imaging data belonging to one functional run.

6. After the FMR creation has been completed, you will see the resulting document as well as several new information windows. In a functional recording the brain is scanned repeatedly over time, but BrainVoyager will visualize only the 64 slices of the first volume of the created document. The data of the repeated measurements of the brain, as it is scanned volume by volume during the execution of the “Houses - Faces” experiment, are stored on disc in a single STC (slice time course) file. The resulting files are saved in the “derivatives” folder of the project, i.e. “BVSampleData\GSGData\derivatives\sub-01\ses-04\func”. The name of the FMR document is automatically determined by the name of the NIfTI file, i.e. “sub-01_ses-04_task-blocked_run-1_bold.fmr”.

---

Step 1: Launching BrainVoyager and Opening Functional NIfTI Files
7. The FMR document file is a simple text file, which contains a link to the STC-file and other useful information. After the creation of the FMR document, the “FMR Properties” dialog, shown below, is opened automatically. This dialog allows you to inspect and modify the relevant information of the FMR document. You can call this dialog at any time via the menu item: “File” -> “Document Properties” -> “FMR Properties...”.

8. You might have noticed that BrainVoyager has opened automatically relevant information from the NIfTI header of the selected file. This information has been used to store relevant information in the FMR header for preprocessing of the functional dataset. The first value of the “dim” entry indicates that it is a 4D dataset, with a matrix size of 100 x 100 (2nd and 3rd value), 64 slices (4th value) and 291 volumes (5th value). Values 2 – 5 in the “pixdim” entry indicate the inplane resolution, the slice thickness and the time to repeat (TR) in seconds. Use this information to check the entries made in the FMR Properties. Click on “Options” in the “Voxel resolution definition” field to see the “Matrix size”. 
Step 1: Launching BrainVoyager and Opening Functional NIfTI Files

For statistical analysis of the data, we will use a model of the hemodynamic response function (HRF) in a later step. To correctly apply the HRF model and for temporal preprocessing, correct timing information of the functional recording has to be supplied. When opening a NIfTI file in BrainVoyager it checks whether a JSON sidecar file with the same name exists in the folder, if so, it is automatically read and displayed in a new information window, see below. The JSON file provides additional metadata not stored in the NIfTI header, as for example the slice timing in seconds with respect to the volume onset. This information is especially important for the slice scan time correction. As this NIfTI file was created with the “Create Document Wizard” in BrainVoyager, it contains a “BrainVoyagerInfo” entry that stores information specific to BrainVoyager, i.e. information that is usually stored in the header of the FMR. Explore the information of the JSON file and see whether you can find the “Slice Timing” information.

If the “Verified” options are not checked in the “FMR Properties”, it is important to check the corresponding entries. In our example, you can find the scanning parameters in the “Scanning session information” provided in the beginning of this guide. When the values in the “FMR Properties” are correct, click the “Verified” checkbox. This will automatically save the FMR document to disk with the updated values.

You can now move on to Step 2.
Step 2: Change Layout and Display Options

In this step, we will learn how to change the layout of an FMR document.

1. If you have closed BrainVoyager EDU, start the program now and open the document "sub-01_ses-04_task-blocked_run-1_bold.fmr". Alternatively, all documents used in the previous session will be opened automatically, if you select the “Reload Last Session” option in the “Welcome” window of BrainVoyager EDU.

2. In this example a 8 x 8 layout of the 64 slices is presented, since BrainVoyager attempts to arrange the slices in an optimal way. If you would like to change the way BrainVoyager has arranged the images, you can use the “Decrease Columns/Rows” and “Increase Columns/Rows” icons to change the layout (see below).

   Note: BrainVoyager will initially show documents in a maximized state. You may also display document windows in a smaller size than the workspace window by using “Window -> Sub-Window View Mode”. You may then adjust the display size of the window by moving the mouse to the right lower corner of the window until the cursor changes to a resize shape. Then click+drag the window to the size of your choice. You may notice that the program keeps the aspect ratio of the window constant. This ensures that pixels are displayed as squares. To go back to the maximized document mode, click the sub window’s “Maximize” icon.

3. Click the “Increase Columns” icon to see more columns of slices. Since the layout is also saved in the FMR file, you can permanently keep desired layout settings by saving the file, either by clicking the “Save” icon or by using the “File -> Save” menu item.

4. Click the “Image Border” toggle to enable or disable drawing of separating lines between slices.

5. Click and Drag while holding “Alt” to zoom in a part of one or several of your slices.
Step 2: Change Layout and Display Options

6. You can use the “Page Down” (or Right Arrow) and “Page Up” (or Left Arrow) keys to scroll through the slices. This is convenient if you want to inspect only a single or a few slices. You can “zoom in” a single slice without changing the layout by right-clicking the respective slice while holding down the “CTRL” key. Use the same command to go back to the matrix layout.

7. BrainVoyager also offers another way to change the layout, by means of the “Layout And Display Options” dialog. You can call this dialog by clicking the “Layout...” button in the “FMR Properties” dialog or via the menu “Options -> Layout And Display Options ...”. Use the respective entries to adjust the display to your needs.

8. BrainVoyager allows to change the brightness and contrast of the displayed first volume of the FMR. The example dataset is very bright. By increasing the contrast and decreasing the brightness the visualization of the structural information in the image will be improved. You can invoke the “Contrast and Brightness” dialog via “Options” -> “Contrast and Brightness”.

You can now move on to Step 3.
Step 3: Creation of the Protocol

In this step we will learn how to create protocol files. These files contain information about the experimental design of the functional run. As you will see, these files play an important role in BrainVoyager, simplifying many tasks such as the specification of statistical analyses.

1. Make sure the “sub-01_ses-04_task-blocked_run-1_bold.fmr” file is open.

2. For the current example dataset a stimulation protocol has been created already and was saved in a .tsv file following the BIDS standard (http://bids.neuroimaging.io/). The “sub-01_ses-04_task-blocked_run-1_events.tsv” file is located in the same folder as the original functional NIfTI file and specifies the onset and duration of each trial type in seconds as well as the name of the trial. When importing functional NIfTI files BrainVoyager checks whether a *.events.tsv file with the same name as the NIfTI file exists in the same folder and if so, automatically creates a protocol file and links it to the resulting FMR document. You can see the link to the protocol file in the FMR properties.

3. In order to learn how a protocol is created from scratch in BrainVoyager, remove the link to the current FMR document by clicking “Detach” in the “Referenced protocol (PRT) file” field of the “FMR Properties”.

4. We will now define the Protocol file for the current experimental run. You can find a definition of the protocol at the beginning of this tutorial, see the Scanning Session Information. Click the “Protocol...” menu item in the “Analysis” menu to invoke the “Protocol” dialog.

5. Provide a name for the experiment by entering “Faces Houses in LVF, CVF, RVF” in the “Experiment name” field.

Info: If you want to edit the protocol after it has been saved, you can also open the dialog by clicking the “Edit” button in the “Referenced protocol (PRT) file” field of the “FMR Properties” dialog.
6. We have to define six conditions, “Faces_LVF”, “Houses_RVF”, “Faces_CVF”, “Houses_LVF”, “Faces_RVF” and “Houses_CVF”. To define the first condition, click the “Add” button on the right side of the “Condition list”. You will see “<untitled>” in the “Condition list”. Select “<untitled>” and then click “Edit Name”. Alternatively, you may double click the condition name. Change the name of the first condition to “Faces_LVF” and then press the <RETURN> button.

7. Click the “Intervals >>” button to show the interval definition fields.

8. There are two ways to specify a protocol:
   a. specifying the intervals numerically (the onset and offset of a condition) or
   b. using a graphical procedure based on a segmented visual representation of time.

We use the latter method, which works well with regularly spaced conditions as in our example.

9. Choose the condition “Faces_LVF” from the “Condition list”. Click the “Show Plot” button to call the “Time Course Plot [Protocol]” dialog. This dialog shows segments (vertical lines) in units of 10.

   Note: When the segmentation lines are not visible, click the “Show grid” option.
Step 3: Creation of the Protocol

10. **Click** the spin buttons of the “Size” field to change its value to “8”. This changes the time course segmentation to the correct width in volumes with respect to our condition durations.

   ![Time Course Plot (Protocol)](image1)

   Enter “3” in the “Offset” field. This is necessary because there are three baseline data points before the first main condition starts.

11. **Click** with the left mouse button in all segments belonging to the “Faces_LVF” condition as shown below.

   ![Time Course Plot (Protocol)](image2)

12. **Click** the “Add To PRT” button. This will convert the graphical specification of intervals into a numerical representation, which will be added to the protocol. Note that the intervals defined in the “Time Course Plot” dialog are added to the “current” condition, which is the one highlighted in the “Protocol” dialog.

   ![Time Course Plot (Protocol)](image3)

When a condition has been defined, the “Time Course Plot” window automatically selects the “Show protocol” option in order to present the stimulation protocol defined so far. In our case, we see several grey vertical bars representing the intervals of the “Faces_LVF” condition.

13. Add the next condition by **clicking** the “Add” button on the right of the “Condition list”. **Change** “<untitled>” to “Houses_RVF”. Make sure that the “Houses_RVF” condition is selected in the “Condition list” and **click** in the intervals belonging to this condition in the “Time Course Plot” window as indicated below.

   ![Time Course Plot (Protocol)](image4)
14. **Click** the “Add To PRT” button in the “Time Course Plot” window.

15. Add the condition “Faces_CVF” in the same way as the previous condition. Make sure that the “Faces_CVF” condition is selected in the “Condition list” and **click** in the intervals belonging to this condition in the “Time Course Plot” window.

16. **Click** the “Add To PRT” button in the “Time Course Plot” window.

17. Add the remaining three conditions: “Houses_LVF”, “Faces_RVF” and “Houses_CVF” in the same way.

    **Note:** After having defined all conditions, you may want to deselect the “Show grid” option in the “Time Course Plot” dialog since we do no longer need the grid lines.

18. We now change the display color of our conditions. In the “Condition list”, **click** on the “Faces_LVF” condition and then on the “Edit Color” button. In the appearing “Select Color” dialog, change the “Red” value to 200 and the “Green” and “Blue” values to “43”. **Click** “OK” to accept the new color.
Step 3: Creation of the Protocol

19. **Click** on the “Houses_RVF” condition and then again on “Edit Color”. Change the color for this condition to yellow by entering the values “200”, “200” and “43” in the “Red”, “Green” and “Blue” fields. **Click** “OK” to accept the new color.

20. **Click** on the “Faces_CVF” condition and then again on “Edit Color”. Change the color for this condition to green by entering the values “43”, “200” and “43” in the “Red”, “Green” and “Blue” fields. **Click** “OK” to accept the new color.

21. Change the condition “Houses_LVF” to the RGB value 43, 200, 200, the condition “Faces_RVF” to the RGB value 43, 43, 200 and the condition “Houses_CVF” to 200, 43, 200.

22. If you now see a screen like the one below, you have successfully created the stimulation protocol!

![Protocol dialog](image)

**Exercise:** There are further possibilities how you can adapt the appearance of the protocol as well as the time course plot. Check out these features by **clicking** the “Options” button.

23. **Save** the protocol to disk under the name “sub-01_ses-04_task-blocked_run-1_events.prt” by using the button “Save.PRT...”. The protocol is now temporarily linked to the FMR document. To permanently keep that link, save also the “sub-01_ses-04_task-blocked_run-1_bold.fmr” document by **clicking** the “Save” icon in the menu bar. You may now close the “Protocol” dialog by **clicking** the “Close” button.
Note: The consistency of your protocol definitions is constantly checked in the background. The result of this consistency check is shown in the title bar of the “Protocol” dialog. If “OK” appears, your protocol is fine. If, however, “NOT OK” is shown, your protocol contains overlapping condition intervals and you must change the protocol.

You can now move on to Step 4.
Step 4: Statistical Tests and Time Courses

In this exercise, we will learn how to run statistical tests. We will also learn how to inspect time courses of regions-of-interest (ROI's).

1. If you have closed BrainVoyager, start the program now and open the document “sub-01_ses-04_task-blocked_run-1_bold.fmr”.

2. If you did not start this Getting Started Guide from step 1, check if the correct TR is saved in the FMR file. You can check this information in the “FMR Properties” dialog, which should open automatically. If this is not the case, select the “File -> Document Properties -> FMR Properties” menu item. If necessary, enter the value for the “TR”. Then click the “Verified” checkbox to tell the program to use this value.

3. Select “Compute Linear Correlation Maps” in the “Analysis” menu. This will invoke the “Linear Correlation” dialog.

4. We want to compare the condition “Faces_LVF” to the baseline, i.e. the part of the experiment in which subjects were only fixating. This “Fixation” condition was not explicitly defined in the Protocol but is implicitly represented by all non-defined time intervals in the run, i.e. the black intervals in the time course representation. To design a respective “box-car” function for this test click with the right mouse button in one of the red vertical segments and with the left mouse button in all black vertical segments.
5. Click the “HRF” (hemodynamic response function) button, which will modify the box-car function to account for the hemodynamic delay.

![Image](image1.png)

**Note:** The “HRF” button will apply a two-gamma hemodynamic response model. If you would like to change the default parameters of the Two-Gamma HRF, change these values in the “Linear Correlation Options” dialog by clicking the “Options” button. Note that it is normally not recommended to change these values.

![Image](image2.png)

6. Click the “GO” button to run the specified statistical test. The specified reference time course is now correlated with the time course at each pixel of all slices. The results of all individual tests are saved internally in a statistical map which is shown on top of the slices. Those pixels surpassing a specified statistical threshold are shown in red or yellow for a positive correlation and in light and dark blue for a negative correlation. Pixels colored yellow and light blue have obtained a value close to the threshold value while pixels colored in red and dark blue have obtained a statistical value, which is substantially higher than the threshold value.

On the right you see the result of the correlation test for slices 14 and 15. There are only a few pixels in orange color, which surpassed the default threshold of a correlation of 0.32 or greater. This threshold is shown on the left side of the color bar. Note that the statistical values next to the color bar are only visible if the FMR is zoomed.

**Change** the layout in such a way that only slices 14 and 15 are visible in a zoomed state as shown on the right (see Step 2: Change Layout and Display Options).

![Image](image3.png)

Below the color bar the text “r(169)” indicates the statistical test performed (“r” -> correlation) together with the “degrees of freedom” of the test “169”. The latter value is required to compute a probability value “p”, which indicates the “significance” of the test. The “p” value is shown in the right lower corner. BrainVoyager uses the “False Discovery Rate” (FDR) to determine the default threshold. This approach provides thresholds corrected for multiple comparisons. The FDR threshold is shown on top of the color bar as “q(FDR) < 0.05”. For details on the FDR method, check the User’s Guide.
Step 4: Statistical Tests and Time Courses

7. The standard statistical threshold is high, so only few “active” pixels can be seen. To lower the statistical threshold, click the “Decrease Threshold” icon in the toolbar. Observe slices 14 and 15 while clicking the icon repeatedly (i.e. 10 times). You will see more extended brain activation in the right hemisphere. Note that the right hemisphere is shown on the left side!

The observed lateralized activity is expected from knowledge of the visual system. Our statistical test has asked the question “Where does the brain respond to face images presented in the left visual field?” The answer given in form of a statistical map is “Areas mainly in the visual cortex of the right hemisphere”.

8. Now we want to see the time course of a particular brain region of interest (ROI = region-of-interest). This can be done easily by drawing a rectangle around the ROI. Click the left mouse button down at a point where you want to start drawing a rectangle. Keep the mouse button pressed and move the mouse pointer down and to the right until the ROI is located within the rectangle. Release the mouse button. You will see a green rectangle. In addition, a new dialog window pops up showing the “ROI Signal Time Course”. This window shows time on the X axis (in measured scans) and the fMRI signal on the Y axis.

9. The time course window might be shown initially a bit small. You can adjust the display size of the window by moving the mouse to the lower bottom of the window until the cursor changes to a resize shape. Then click+drag the window to the size of your choice.

Remark: The time course window shows the mean time course of all “active” pixels falling within the specified rectangle.

Note: You can also save the selected ROI by using CTRL+R, which invokes the “Region-Of-Interest” dialog. After clicking the “Save...” button you have to specify the target folder and the name of the ROI.

10. As an exercise, inspect additional ROI time courses. If you draw a new rectangle, the previous rectangle is deleted and the “ROI Signal Time Course” window shows the time course for the new selected ROI. Thus, there is only one ROI Signal Time Course window visible. You can, however, see the time courses of more than one ROI at the same time by using the CTRL key. Press the CTRL key and hold it down, then draw the rectangle around a ROI as usual. This will invoke a new “ROI Signal Time Course” window as shown below.

If you have invoked several time course windows, you can keep track which ROI belongs to which window.
by looking at the color of the square at the left lower corner of the “ROI Signal Time Course” window as shown below.

11. To close a “ROI Signal Time Course” window, click the “Close” icon (“x”) on the right side of the title bar.

You can now move on to Step 5.
Step 5: Preprocessing of FMR Documents

In this exercise, we will preprocess the functional MRI data, i.e. the FMR document. This is an important step in improving the power of statistical tests, i.e. by removing drifts in signal time courses and by removing head motion artifacts. To get a "feeling" about the effects achieved by preprocessing, we will run the same statistical test as described in step 4 after the preprocessing is finished.

1. If you have closed BrainVoyager, start the program now and open the document “sub-01_ses-04_task-blocked_run-1_bold.fmr”.

2. Preprocessing requires information about the spatial and temporal resolution of the data. If you have not checked this information in previous steps, you can do this now by inspecting the time and voxel resolution values in the “FMR Properties” dialog. If this dialog has not opened automatically, you can invoke it by clicking the “FMR Properties” item in the “File” menu. As an alternative, you can also change the time and voxel resolution values (if necessary) in the “FMR Data Preprocessing” dialog.

3. To improve the quality of the data, we will preprocess it. Select “FMR Data Preprocessing...” in the “Analysis” menu. This will invoke the “FMR Data Preprocessing” dialog.

4. There are several preprocessing options like “Slice scan time correction”, “3D motion correction” and so on. You can run several preprocessing options at once. The standard settings of BrainVoyager offer good results, and do not include spatial and temporal smoothing.

Note: It is recommended to preprocess the data as much as necessary but as little as possible. For individual data analysis we recommend to first inspect the data without spatial smoothing and if desired add spatial smoothing later in volume time course (VTC) space via “Analysis -> VTC Data Preprocessing...” (for an introduction into VTC space see Part II of this guide).

5. To see further information/options about preprocessing tools, click the “Advanced >>” button which will show an extended “FMR Data Preprocessing” dialog.

The expanded dialog shows more detailed information about the chosen preprocessing options: slice scan time correction and 3D motion correction will be performed on our functional data; in addition, linear (and non-linear) trends (“drifts”) in the data will be removed. Detailed information on the different parameters are described in the section “Preprocessing of Functional Data” in the User’s Guide and are set to reasonable values automatically.

6. Click the “GO” button to run the specified preprocessing options. A “Progress” dialog will appear, which informs you about the state of preprocessing. Additional information about the current preprocessing step will be printed to the log tab.
Parameters used during preprocessing are included in the name of the resulting file. In our case, the explanation of the abbreviations in the file name is as follows:

```
sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c.fmr
```

- **Temporal High Pass (GLM Fourier)** with 2 cycles / points
- **3D Motion Correction** with Trilinear estimation and Sinc interpolation
- **Slice Scan time Correction**, Cubic spline Interpolation, using the Slice Time TaBLE information

You will find more information regarding different preprocessing options in the BrainVoyager User’s Guide and on our support website [http://support.brainvoyager.com](http://support.brainvoyager.com)

7. After preprocessing has been completed, BrainVoyager automatically opens the preprocessed FMR document called “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c.fmr”. 
Step 5: Preprocessing of FMR Documents

BrainVoyager shows a graphical representation of the ongoing 3D motion correction. A specified volume serves as the reference (the first as default) to which all other volumes are aligned in space by rigid body transformations. The detected head motion of a volume with respect to the reference volume results in 3 translation and 3 rotation parameters. These detected values are used to translate and rotate the respective volume accordingly to correct for the detected head motion. During 3D motion correction, the six estimated parameters are displayed incrementally in a time course graph (see below). The 3 translation and 3 rotation parameters are color-coded as follows:

- **Red:** Translation in X direction
- **Green:** Translation in Y direction
- **Blue:** Translation in Z direction
- **Yellow:** Rotation around X axis
- **Magenta:** Rotation around Y axis
- **Cyan:** Rotation around Z axis

Note that the axes are defined in image space (i.e. not in Talairach or MNI space): X refers to the image left-to-right direction, Y refers to the image top-to-bottom direction and Z refers to the direction across slices (first-to-last slice). The display can be altered in the same way as the Event-Related Averaging plot window, which will be described in step 8. You can access these options by clicking with the left mouse button in the plot.

8. It is advised to inspect the data before and after preprocessing. For that purpose, please open the “Time Course Movie” dialog via “Options -> Time Course Movie…”

When clicking the “First <-> Last” button, BrainVoyager will alternate between showing the first and the last volume of the FMR, indicating the amount of motion in this functional run. If you do not see any difference in the spatial location of these volumes, i.e. no “flickering” in the edges of the slices, motion correction was successful. Close the dialog via the button “Close”.

Please do the same for the non-preprocessed original FMR. You will see that the amount of “flickering” in the image when alternating between the first and last volume is quite increased as compared to the preprocessed data set, indicating subject movement in this run.

**Note:** As you can see in the 3D Motion Correction Plot, the subject has moved only minimally in this functional run. When you do have data sets with more motion, the effect of motion correction will be even more pronounced.

**Exercise:** To see the effect of preprocessing on the statistical results, run the same correlation test as in step 4 for both the original and preprocessed data and also inspect the time courses of the same ROI’s as in step 4. Try to reproduce the layout shown on the next page.
Step 5: Preprocessing of FMR Documents

You will see that the statistical map looks slightly different after preprocessing, since preprocessing has removed noise in the data and hence has increased sensitivity of the statistical tests. In addition, the time course shows that there are no more drifts in the data due to the removal of linear and nonlinear trends.

You can now move on to Step 6.
Step 6: Statistical Analysis with the General Linear Model (GLM)

In this exercise, we will use the General Linear Model (GLM) to analyze the data. The GLM goes beyond simple correlation or t-tests by allowing to specify advanced statistical models containing many explanatory variables also called “predictors”. A specified model is “fitted” to the time course of each voxel resulting in a “t”, “F” or “R” value telling us how well the overall model explains the time course. In addition, the fit tells us whether one or more individual explanatory variables contribute significantly to the explanation of the voxel time series. It is also possible to test contrasts, i.e. whether the estimated effect (beta weight) of a predictor A is significantly larger (or smaller) than the estimated effect of a variable B.

1. If you have closed BrainVoyager, **start** the program now and **open** the document “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c.fmr”.

2. **Select** “General Linear Model: Single Study...” in the “Analysis” menu. This will invoke the “Single Study General Linear Model” dialog.

   **Note:** For more details about the GLM consult the topic “The General Linear Model (GLM)” in the section “Statistical Data Analysis” of the BrainVoyager User’s Guide.

3. We want to specify a model with six predictors reflecting our six main conditions “Faces_LVF”, “Houses_RVF”, “Faces_CVF”, “Houses_LVF”, “Faces_RVF” and “Houses_CVF”. The “Predictors” field tells us that the dialog is already prepared for entering the first predictor.

   We can enter the reference function for the first predictor with the mouse: **Click** with the **right mouse button** in one of the red vertical bars to define the first predictor.

   Note that the predictor “Name:” field is automatically set to the name of the corresponding condition, “Faces_LVF”.

4. **Click** the “HRF” button, which will modify the reference function to account for the hemodynamic delay of the fMRI signal.
Step 6: Statistical Analysis with the General Linear Model (GLM)

**Note:** You can also use the “Condition list” on the right side to specify the reference function, by **clicking** with the **right mouse button** on the name of the condition that you would like to specify as a predictor.

5. We now want to enter the second predictor for the “Houses_RVF” condition. **Click** the “Add Pred” button to create a new empty time course for entering the second predictor. In the “Predictors” field, you will now see the entry “2 / 2”, which indicates that we currently see the time course of predictor 2 and that we currently have a model of 2 explanatory variables.

6. Specify the time course of the second predictor by **clicking** with the **right mouse button** in one of the yellow vertical segments. **Click** the “HRF” button to account for the hemodynamic delay of the fMRI signal.

7. **Repeat** these steps for the remaining four conditions in the condition list.

8. We have now defined our simple general linear model consisting of six predictors. You can inspect the specified reference functions for each predictor by clicking the up or down spin of the “Predictor” spin control. You will see the time course of the first (1/6), second (2/6), ..., sixth (6/6) predictor in the window.
Step 6: Statistical Analysis with the General Linear Model (GLM)

9. You can see all predictors simultaneously by **clicking** the “Show all” checkbox. You can additionally visualize the protocol by also **clicking** the “Show protocol” check box.

![Image of the interface showing predictors and protocol visualization]

**Info:** If you made any error entering the predictors, you can use the “Clear Pred” button to set the values of the reference function of the currently selected predictor back to zero. To remove the currently selected predictor from the model, click the “Del Pred” button.

10. As an exercise remove all predictors from the model, by **clicking** the “Del All” button. It is also possible to define all predictors of the model at once by **clicking** the “Define Preds” button, which can be helpful for complicated designs. As default BrainVoyager assumes that you have a baseline (or rest) condition explicitly defined as the first condition in your protocol. This baseline condition should not be included as a predictor in the design matrix of a general linear model. Therefore, when clicking the “Define Preds” button, the first condition (Faces_LVF) in the condition list is ignored and only five predictors will be defined.

You can change this setting in the “Single Study GLM Options”. Please **click** on “Options” in the “Single Study General Linear Model” dialog and **uncheck** the option “Exclude first condition (“Rest”). Afterwards, **click** on “Define Predictors”.

![Image of the Single Study GLM Options dialog]

You will see that all six predictors are defined again.

11. We now save the generated model to disk because we will need it later (see step 13). **Click** the “Save...” button. In the appearing “Save As” dialog, **enter** “FacesHousesDesignMatrix.sdm” and **click** the “Save” button. The saved “SDM” file contains the time courses of the six defined predictors.

![Image of the Save File dialog]

**Info:** When a GLM is computed by clicking the “GO” button, BrainVoyager also automatically saves an .sdm
file, which would be in our case: sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_SD3DSS4.00mm_THPGLMF2c_FMR_autosave.sdm.

12. Now Click the “GO” button to run the GLM. The specified model is fitted to the time course of each pixel in all slices. The results of all individual model fits are saved internally in a glm data structure, from which statistical maps can be derived.

After finishing the computation, a statistical “t” map of the “full model” is shown. The full model shows regions in red-to-yellow colors, where one of the predictors, a combination of the predictors or all six predictors together produce a significant effect (see figure on the right). Note the difference to a correlation test (or t-test) where only one individual comparison can be tested (see Step 4: Statistical Tests and Time Courses).

Furthermore, the program will automatically invoke the Voxel Beta Plot showing the beta values for the voxel at the current mouse cursor position (see image above). The beta value represents the contribution of a predictor to the explanation of the time course of a voxel. You can also see the precise statistical value “below” the mouse cursor by looking at the status bar.

13. You will see the map showing a lot of single activated pixels. They are not relevant to our experiment, and we will therefore set a cluster threshold to show only clusters of a certain size. Click on the menu item “Analysis” and choose the option “Overlay Volume Maps...” (or press CTRL+M). This will open the “Statistical Maps” dialog. In this dialog you will see the group “Cluster threshold”, with one spin box called “Size”. Change the default value of “1” to “4” and click the “OK” button.

Now the map should look much cleaner, like the image on the right.
Step 6: Statistical Analysis with the General Linear Model (GLM)

14. We can ask the computed GLM several “questions” with the help of the “Overlay GLM Contrasts” dialog. To invoke this dialog, **click** the “Overlay General Linear Model” item in the “Analysis” menu. You will see a list of the six predictors, which we have defined in the “Single Study General Linear Model” dialog.

**Remark:** Even with automatic naming, it is sometimes helpful to rename predictors. If you would like to do this, **click** on a predictor to make it the currently selected predictor. Then **click** the “Pred name” button, which will invoke the “Change Predictor Name” dialog. Now **enter** the new name for the predictor and then **click** the “OK” button or press the <return> key.

15. On the left side of the predictor list, open squares are shown, which allow to select subsets of predictors. **Click** on the button “Clear Contrast” to clear all predictors. Now **click** on the square of predictor 1 to mark it with a plus sign (+). This corresponds to asking the GLM to show all brain regions that respond significantly to predictor 1 “Faces_LVF” (where a significant amount of the variance of a voxel’s time course is explained by predictor 1). Now **click** the “OK” button.

16. You will see that activity is now mainly in the right hemisphere (shown on the left side of the slices) since the first predictor corresponds to face images in the left visual field.
17. Go back to the “Overlay GLM” dialog. **Click** the “Clear Contrast” button to remove any check marks from the predictor list. **Check** “Faces_RVF” with a plus sign and **click** the “OK” button. You will see activity mainly in the left hemisphere.

   **Tip:** To quickly invoke the “Overlay GLM” dialog, **press CTRL+Y**. Alternatively, **click** the “Increase Threshold” or the “Decrease Threshold” icon while holding down the CTRL key.

18. Invoke the “Overlay GLM” dialog again. **Click** the “Clear contrast” button to remove any check marks from the predictor list. **Check** “Faces_LVF” and “Faces_RVF” with a plus sign and **click** the “OK” button. You will now see activity in regions in which the two predictors together (their mean value) differs significantly from zero.

19. Go back to the “Overlay GLM” dialog. You can check a predictor not only with a plus sign (+) but also with a minus sign (-) by clicking again on a predictor which has already a plus sign. **Check** predictor 1 with a plus sign and predictor 5 with a minus sign. This creates a **contrast** asking for those brain regions where predictor 1 is more active than predictor 5. **Click** the “OK” button to see the result. Compare the outcome with the result of task 15 and try to explain the difference!

20. **Save** the computed GLM under the name: “FacesHouses_FMR.glm” by using the “Save .GLM” button of the “Overlay GLM” dialog. We will be using this file in later steps.

   **You can now move on to Step 7.**
Step 7: Detailed GLM Analysis of Regions-of-Interest (ROI’s)

In this step, we will apply the General Linear Model (GLM) to the time course of a selected region-of-interest (ROI). The “ROI GLM” tool produces statistical tables and graphs with detailed information about how well a chosen statistical general linear model fits to the data. It also provides results of specified contrasts for the data in the ROI. The tool works both for FMR documents and VTC based documents (introduced in Step 12) and is applied here to our preprocessed FMR data.

1. If you have closed BrainVoyager, start the program now and open the document “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c.fmr”.

   To apply the ROI GLM tool, we need the time course of a region-of-interest. To select a time course, we will first overlay a statistical map. Select “Overlay General Linear Model” in the “Analysis” menu. This will invoke the “Overlay GLM Contrasts” dialog.

2. In the invoked dialog, load the General Linear Model “FacesHouses_FMR.glm” saved in Step 6 by clicking the “Open” item in the local “File” menu.

3. Now check both predictors “Faces_LVF” and “Faces_RVF” with a plus sign. Click the “OK” button to overlay the statistical map on the EPI slices. Change the cluster threshold size to 4, as done in step 6.

4. Now select a region-of-interest by dragging a rectangle around an active region. In the figure on the right, a ROI on the left side has been selected encompassing early visual areas of the right hemisphere. The appearing “ROI Signal Time Course” window shows the associated time course of the selected ROI. This time course is the average of the individual time courses of all included voxels.

5. We can now apply the ROI GLM tool to the chosen time course. To get access to further options, right-click at any point in the “ROI Signal Time Course” window. This will show a context menu with further options. Select the “Show/Hide Options” item. As a shortcut to show/hide the advanced options, simply click with the left mouse button in the “ROI Signal Time Course” window.

6. In the expanded “ROI Signal Time Course” dialog, click the “ROI-GLM” button. This will invoke the “ROI GLM Specifications” dialog.
7. In the “Correction / output options” section of the dialog, check “GLM graphs” and “GLM tables”, as well as “% -transform” of the time course. There are additional options producing information about serial correlations. For details about these options, consult the User’s Guide. Before we can run the GLM for the ROI time course, we have to specify a design matrix file. Click the “Browse...” button and select the file “FacesHouses DesignMatrix.sdm”, which we have saved in the previous step. This file contains the time courses for our six defined predictors.

8. Before starting, we can also specify that we want to have analyzed contrasts. Switch to the “Contrasts” tab. To specify a contrast comparing stimulation in the left vs right visual field, check the “Faces_LVF” with a “+” and the “Faces_RVF” with a “-” sign by clicking once or twice, respectively. This contrast asks whether predictor 1 is significantly more (or less) active than predictor 5 within the chosen ROI. Now click the “Fit GLM” button, which will finally calculate the ROI GLM presenting the result in a table and a graph.

9. One of the appearing windows shows the “ROI Time Course – GLM Results” tables.
   The first “ANOVA” table shows how well the chosen General Linear Model fits the ROI time course. The last row shows an overall value, the multiple correlation coefficient “R”, which can have values between 0.0 and 1.0. The value “R=0.836” indicates that our model fits very well to the data.

   The second “Predictor” table shows the so-called beta weights estimated by the GLM together with information about their significance.

   The third “Contrast” table shows that our specified contrast is significant in this ROI.
Step 7: Detailed GLM Analysis of Regions-of-Interest (ROI's)

**Note:** There are more tables displayed in the same window, showing the GLM results after correction for serial correlations. In fMRI data, serial correlations are expected, i.e. high values are followed more likely by high values than low values and vice versa. These correlations lead to biased standard errors of the betas and hence to inflated test statistics. Therefore, it is important to correct for serial correlations and to focus on the test statistics after correction (for more information on serial correlations see the General Linear Model section in the User’s Guide).

The “GLM Results” window is in HTML format and is saved automatically in the current folder as “ROI_GLM_Info.html”. If you want to keep the content of this file permanently, you might want to save it using another name, otherwise it will be overwritten next time when you invoke the ROI GLM tool in the same folder. To save the file you can use the “Floppy Disk” icon.

10. A second and third window show the “GLM – Data, Model and Residuals” graphs without and with (II) correction for serial correlation. Both windows show three curves; the blue curve is the “data”, the time course from our selected ROI. It is the same curve as shown also in the “ROI Signal Time Course” window. The green curve is the “model”, it shows the “fit” of the GLM based on the six beta weights of our predictors. As you can see, the green curve rises and falls nicely with the blue “data” curve. The blue curve is, however, not “explained” completely by the green curve. This is also visible in the red curve, which represents the “residuals”. This curve is obtained by subtracting the model curve from the data curve and adapting these values for visualization purposes.

**Note:** You can show or hide individual curves in the “Plot Options” dialog, which appears after clicking with the left mouse button into the graph window. The Options window is described at the end of the next step. You can save the graph by right-clicking in the window and choosing the “Save snapshot of plot” in the appearing context menu.

11. You can close the graph and table windows by clicking the “x” buttons in the right upper corner.

You can now move on to Step 8.
Step 8: Event-Related Averaging

A helpful tool to visualize and compare effects of different experimental conditions is the event-related averaging plot. In this exercise, we will learn how to create templates for averaging time segments and how to apply them to time courses of any region-of-interest. If you continue directly from step 7, you can go now to point 2.

1. If you have closed BrainVoyager, start the program now.
   Open the document “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c.fmr”.
   Since we will select regions-of-interest based on statistical maps later in this step, repeat (a part of) step 6 now by computing (or loading) a GLM and by overlaying a GLM contrast with both predictors “Faces_LVF” and “Faces_RVF” marked with a plus (+) sign.

2. Select “Event-Related Averaging” in the “Analysis” menu. This will invoke the “Event-Related Averaging Specification” dialog as shown below.

3. In the “Event-Related Averaging Specification” dialog, you may add one or more runs by clicking the “Add Runs” button. This is often, however, not necessary since BrainVoyager places the current functional data file(s) automatically in the “Functional data files” list. For each added functional run (in our case just one), the program looks into the associated stimulation protocol file to get information about the different conditions including their names, color and intervals. The extracted names are listed in the “Available conditions in referenced protocol files” box. In our case these are the condition names “Faces_LVF”, “Houses_RVF”, “Faces_CVF”, “Houses_LVF”, “Faces_RVF”, and “Houses_CVF”.

   In case a GLM has been computed or loaded, the dialog automatically adds all runs, which were used to compute the GLM. This is a very helpful function if you analyze data with many subjects and/or runs.

Note: In principle, you can add additional FMR files allowing to average data from multiple runs. For FMR documents however, this requires that all runs are recorded in the same session, with the same slice parameters and are intra-session motion corrected.
Step 8: Event-Related Averaging

4. Now we select one or more conditions, from which we want to average their associated time course epochs across repetitions. In this example we select all six conditions.

5. When selecting condition names in the “Available conditions in referenced protocol files” list, the “Expected response plot” field presents an idealized expected response profile by plotting each condition in the color defined in the stimulation protocol. This plot is very helpful to adjust the values in the “Number of data points before and after onset of event” field. The “Pre” and “Post” values specify the number of time points before and after the onset of a stimulus condition used for averaging. The “Post” value should be large enough to see the entire time course but not larger than necessary to avoid the inclusion of subsequent events in the averaging.

6. If the “Expected response plot” shows that the window is not large enough to include the whole condition-related time course, increase the “Post” value in the “Number of data points before and after onset of event” field. Any change in the “Pre” and “Post” field will be immediately reflected in the plot of the expected time courses. You can play around with different numbers to see how the plot changes. In our example there is no need to change the default value of 16 time points, as this time window is long enough to “capture” the entire expected fMRI signal.

Note: The height of the response for each condition is randomly chosen just to separate the curves from each other. To get a new random selection of the simulated response strength for each condition, simply click in the plot window.

7. Now click the “Create AVG” button. In the invoked “Save As” dialog, enter “FacesHouses_1.avg” and click the “Save” button.
8. Now we can apply event-related averaging as specified and saved in the “FacesHouses_1.avg” file to any ROI time course. **Use the left mouse button** to select a rectangle encompassing a statistically significant region in the left hemisphere as shown below.

![Image](image1)

9. Now, **click** somewhere in the appearing “ROI Signal Time Course” window, which will expand the dialog showing several options. One of these options is the application of event-related averaging files. **Click** the “Browse…” button in the “Event-related averaging” field (see figure on the right). In the appearing “Open File” dialog, select our saved file “FacesHouses_1.avg” and **click** “Open”.

![Image](image2)

10. You will now see a new window on the screen called the “Event-Related Averaging Plot” or “AVG Plot” window. The “AVG Plot” window shows the result of averaging all time course epochs evoked by the same condition as indicated for the “Faces_RVF” condition below. The colors of the curves correspond to the condition colors assigned in the stimulation protocol.

![Image](image3)
Step 8: Event-Related Averaging

11. To change the size of the AVG plot window, simply move the mouse cursor to the border of the window until it changes its shape to a sizing cursor. Then use the left mouse button to change the window size. The X axis is labelled “Time (scans)” in the plot reflecting the selection of “Volumes” resolution for time resolution in the “Event-Related Averaging Specification” dialog.

12. The displayed axes labels as well as many other options can be modified in the “Plot Options” dialog. To invoke this dialog, simply click with the left mouse button in the “Event-Related Averaging Plot” window.

Exercise: Explore the options of this dialog. Check the “Horizontal arrangement” item to display the averaged curves side-by-side. Hide the error bars for the six curves by clicking each curve name in turn in the “Curve properties” list. After clicking on a curve name, you can change its display properties including color and curve size. Add a title by moving to the “Options” tab, checking “Enable” in the “Title” field and entering “Early visual areas – LH” in the “Label” edit box of the “Title” field. Change the separation of ticks on the X-Axis to 4. Try to achieve a display like the one shown below.
13. Since the program keeps the changes of the plot settings, you can select another ROI without having to change the plot layout again. Select a ROI in the right hemisphere as shown on the right. You will immediately see an update of both the “ROI Signal Time Course” window and the “Event-Related Averaging Plot” window as shown below. Note that the red and the light blue curve now show a strong response while the dark blue and yellow curve are essentially flat. This is the opposite from what we have seen in the previous “Event-Related Averaging Plot” window.

Exercise: Correct the title from showing “LH” to showing “RH” instead. This correction has been made in the plot below.

Note: If you want to display several “Event-Related Averaging Plot” windows at the same time, you must first display several “ROI Signal Time Course” windows using the CTRL button. For each “ROI Signal Time Course” window, invoke an “AVG Plot” window as described above. The settings from one AVG window do not transfer automatically to a newly created one, but you can change the settings in all open “AVG Plot” windows in the same way using the “Plot Options” dialog of each window.

14. We will create a second .AVG file with a time resolution of seconds instead of volumes (scans). A time resolution of volumes is used by default showing the data in its recorded resolution. The recorded time resolution (volumes, scans) is characterized by the TR value, which is 2 seconds in our case. Sometimes it is convenient to present the data in a time resolution of seconds. Select “Event-Related Averaging” in the “Analysis” menu to invoke the “Event-Related Averaging Specification” dialog again. Our functional data file will be automatically added to the “Functional data files” list as before. Select the six main conditions in the “Available conditions in referenced protocol files” list.
Step 8: Event-Related Averaging

15. In the “Resolution of data points” field, check now the “Seconds” option. With this option, the expected responses as well as later the averaged data will be shown in resolution of seconds. Since the real temporal resolution of the data is 2 seconds (the TR), the program will interpolate the ROI time course data to the higher resolution of seconds prior to averaging.

16. Since we now have higher temporal resolution, the default settings in the “Number of data points before and after onset of event” field will not be adequate anymore. These values specify how many data points are used for averaging before and after the beginning of the stimulus event. Change the “Pre” value to “4” and the “Post” value to “32” seconds.

17. We also adjust the “Average From” value in the “Percent signal change” field to “-4”. Together with the “Average To:” value of “0”, these parameters define that the mean signal intensity of the 4 seconds before the beginning of every event are used as baseline for computing % signal change values.

18. Another option is the possibility to compute either standard deviations or standard errors for each data point. The resulting values can be shown as “error bars” in an event-related averaging plot. Standard errors are the default option and it is recommended to keep that setting. Now click the “Create AVG” button to create the .AVG file and save it as “FacesHouses_2.avg”.

Apply the saved AVG file to a region-of-interest as described before. You will see that the X axis is labelled “Time (sec)”, reflecting our specification of a “Seconds” resolution in the “Event-Related Averaging Specification” dialog.

You can now move on to Step 9.
Part II: Analysis in Standard 3D Space - VMR-VTC Documents

Step 9: Creating 3D Anatomical Documents from NIfTI Files

In this step, we will learn how to create 3D anatomical documents from NIfTI files. The 3D data set will be used in the next step for coregistration with the functional data. As an alternative to the way described below, you can create new documents also from DICOM files via the “Document Creation Wizard” or via “Create Document” dialog as described in Appendix B: Creation of a 3D Anatomical Document.

1. If you have closed BrainVoyager, start the program now and click on the “Open NIfTI icon” icon. Alternatively, go to “File -> Open NIfTI...” and navigate to “Documents\BVSampleData\GSGData\sub-01\ses-04\anat”. In this folder please select the file “sub-01_ses-04_acq-nondistorted_T1w.nii.gz” and confirm your choice by clicking on “Open”.

2. The assembled images appear in the workspace in a so called VMR (Volume Magnetic Resonance) document and a file called “sub-01_ses-04_acq-nondistorted_T1w.vmr” will be automatically written to the derivatives folder of the “GSGData” project (Documents\BVSampleData\GSGData\derivatives\sub-01\ses-04\anat).

Note: BrainVoyager shows useful information from the header of the NIfTI file in the Info Pane. The first value of the “dim” entry indicates that it is a 3D dataset, with a matrix size of 256 x 256 (2nd and 3rd value) and 192 slices (4th value). Values 2 – 4 in the “pixdim” entry indicate the voxel size of 1 x 1 x 1 mm. If the voxels have different dimensions, BrainVoyager automatically proposes to interpolate the data set to iso-
Step 9: Creating 3D Anatomical Documents from NIfTI Files

voxel dimensions. In case the 3D data set is not scanned in sagittal slices, it will also automatically propose to change the orientation of the data set.

The accompanied .json file that is located in the same folder as the NIfTI file is automatically read and displayed in BrainVoyager when importing the NIfTI data. This file stores additional information about the data that cannot be found in the NIfTI header but might be useful for data interpretation.

3. In order to adjust the brightness and contrast of the created document open the “Contrast and Brightness” dialog via “Options -> Contrast and Brightness …”. In this dialog you can change the brightness and contrast
Step 9: Creating 3D Anatomical Documents from NIfTI Files

of the data set. As a rule of thumb, the grey matter intensity should be around 100, while the white matter intensity should be between 120 - 160. Since the “Apply immediately” check-box is checked, you will see the VMR document changing immediately. By default BrainVoyager cleans the VMR, meaning that all voxels in the background will be set to an intensity value of 0, which corresponds to black. This improves different procedures, such as coregistration of data sets and brain peeling. **Click** the “OK” button to close this dialog.

**Note:** If you would like to change the intensities at a later stage, you can use the menu option: “Volumes” - > “Inhomogeneity Correction, V16 Tools...” to open the “16 Bit 3D Tools” dialog. In this dialog you would have to load the 16 bit version of the 3D dataset (sub-01_ses-04_acq-nondistorted_T1w.v16) via the button “Load.V16...”.

4. You can explore this 3D data set by **clicking** with the left mouse button at any point within the “SAG”, “COR” and “TRA” view. This will define the “current” voxel highlighted by the white cross. The three views are automatically updated to show a sagittal, axial and coronal slice running through the specified 3D point. You can also change the position of the white cross by holding down the left mouse button while moving the mouse. To cycle through an enlarged view of the “SAG”, “COR” and “TRA” window, press **CTRL + T** multiple times. Notice that the intensity value and current position of the mouse cursor is shown in the Info Pane, even without clicking in the data set.

**Note:** You can see that the data set is a bit inhomogeneous. Since intensity inhomogeneities in the image might have negative effects on the outcome of coregistration and segmentation procedures, we will improve the quality of the anatomical data set by applying an automatic intensity inhomogeneity correction. This becomes particularly important with data sets from ultra-high field scanners.

5. First, open the “16 Bit 3D Tools” dialog by going to the “Volumes” menu and Select “Inhomogeneity Correction, V16 Tools...”.

6. The automatic intensity inhomogeneity correction includes 4 steps:
   1) Background cleaning (all intensities of voxels in the background are set to 0)
   2) Brain extraction (the brain is segregated from the head tissue)
   3) White matter detection
   4) Bias field estimation within white matter voxels (voxels labeled as white matter are used to estimate the variability of white matter intensities across 3D image space)

The white matter detection and bias field estimation is usually applied several times to improve the result; by default three times as indicated by the “No. of cycles:” entry in the “Intensity inhomogeneity correction (IIHC)” field.
2. Click the “GO” button to start the inhomogeneity correction.
Step 9: Creating 3D Anatomical Documents from NIfTI Files

For each step of the intensity inhomogeneity correction process, BrainVoyager saves one new file in the same folder as the original VMR sub-01_ses-04_acq-nondistorted_T1w.vmr. You can track the different processing steps that BrainVoyager applies to the VMR, as depicted in the images below.

**Step 1 and 2:**
Background cleaning and Brain extraction

Resulting file:
sub-01_ses-04_acq-nondistorted_T1w_BrainMask.vmr

**Step 3 and 4:**
White matter detection and Bias field estimation (1st cycle)

Resulting file:
sub-01_ses-04_acq-nondistorted_T1w_IlH-BiasField-1.vmr

Separation of white matter and grey matter before (blue) and after (yellow) the first bias field estimation and removal step
Step 5 and 6:
White matter detection and
Bias field estimation (2nd cycle)

Separation of white matter and grey matter before (blue) and after (yellow) the second bias field estimation and removal step

The result of all three cycles of the intensity inhomogeneity correction procedure is a more homogeneous and peeled VMR data set
sub-01_ses-04_acq-nondistorted_T1w_IIHC.vmr (and the corresponding V16 file: sub-01_ses-04_acq-nondistorted_T1w_IIHC.v16)

You can now move on to Step 10.
Step 10: Coregistration of Functional and Anatomical Data

In this step, we will learn how to align functional slice-based data of an FMR document with a 3D data set. This allows us to relate brain activity more easily to anatomical locations and it prepares the transformation of the functional data into normalized (Talairach or MNI) space. In this step, we consider the case that the two data sets are recorded in the same session, which allows us to coregister them by using positioning information stored in the image file headers. Such a mathematical coregistration produces optimal results assuming no head motion takes place between the two measurements. After the position-based (mathematical) coregistration, small head motions can be corrected by manual or automatic fine adjustments.

1. If you have closed BrainVoyager, start the program now.
   Open the file “sub-01_ses-04_acq-nondistorted_T1w_IIHC.vmr” by clicking its name in the Files Pane.

   Alternatively, you can use the “Open” icon or select the “File -> Open” menu item. In the “Open” dialog, navigate to the folder “BVSampleData\GSGData\derivatives\sub-01\ses-04\anat”. Select the file “sub-01_ses-04_acq-nondistorted_T1w_IIHC.vmr” and click “Open”.

2. We want to align our functional data recorded in the same session to this 3D data set. The necessary routines can be accessed from the “3D Volume Tools” dialog which appears automatically after opening a 3D data set. You can always show or hide this dialog by using the “3D Volume Tools” icon in the vertical toolbar of BrainVoyager. The dialog might be in “Mini Dialog” state when it appears. Use the button “Full Dialog>” to see the full dialog. The “3D Coords” tab is visible initially and shows, among other things, the coordinates of the current voxel (indicated by the position of the white cross) in the “System coords” field. Click the “Coregistration” tab to switch to the options we need for our task.

3. Click the “Select FMR...” button in the “FMR-VMR coregistration” field.
   In the appearing “Open” dialog navigate to the folder “BVSampleData\GSGData\derivatives\sub-01\ses-04\func”, select the file “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c.fmr” and click “Open”.

4. To coregister the selected functional data with the loaded intra-session 3D data set, the program can use information from the headers of the raw data. This information is extracted automatically during document creation and is stored directly in the FMR and VMR files.
Step 10: Coregistration of Functional and Anatomical Data

You will see that the document window has changed from a 2 x 2 display to a 2 x 3 display. The upper row shows the 3D data set and the lower row (coregistration row) shows the functional data (purple-blue) overlaid on the 3D data set. You will notice that the functional data is still in a different orientation than the anatomical data set. This will be corrected as soon as BrainVoyager has read the positioning information from the header.

5. Click the “Align” button in the “FMR-VMR coregistration” field which will invoke the “FMR-VMR Coregistration” dialog. Note that BrainVoyager proposes file names for the transformation files (*.trf) holding the information of the Initial Alignment and Fine-Tuning Alignment. These files will be saved when the alignment is finished.

6. Click the “Source Options” tab. You will see that the “Invert intensity” option is checked in the “Options for FMR/AMR source” field which will lead to the functional data being visualized as anatomical data. Furthermore, the checkbox “Create edge display for FMR/AMR data” is marked. This setting will help to evaluate the quality of the alignment after the coregistration is done.

Note: By default, BrainVoyager will also correct for inhomogeneities in the first volume of the FMR after the initial position information-based alignment has been performed. This will yield improved fine-tuning coregistration results especially for data sets with substantial inhomogeneities as the current example data. These inhomogeneities in the functional data also affect the visualization of statistical maps, therefore BrainVoyager will use the inhomogeneity corrected FMR automatically for visualization when opening the preprocessed FMR. Please consult the User’s Guide for more details on the functional inhomogeneity correction.

7. To start the mathematical initial alignment and the gradient-based affine alignment (for fine-tuning) press the “GO” button. BrainVoyager will align the data using positioning information found in the header of the raw data (Initial Alignment), and by comparing the data sets on basis of their edges (Fine-Tuning Alignment). In gradient-based (affine) image registration, the rotation, translation and scaling parameters for each voxel are found via optimization. Each voxel will be transformed in the same way according to the parameters in the transformation matrix of the transformation file (*.trf). The previously specified transformation files (*.trf) will be saved to disk.
**Note:** After the initial alignment step and during the functional inhomogeneity correction you will see a display as shown on the right. In this view the functional data has been transformed into volume space, based on the positioning information saved in the FMR and VMR. The green box in the coregistration row shows the area in the 3D data set, i.e. volume space, where no functional data is available. This information is used for the functional inhomogeneity correction.

**Please note** that the fine-tuning alignment profits from edge information in the functional data set; using spatial smoothing during preprocessing however results in less anatomical or edge information in the functional images. This might result in a sub-optimal fine alignment of the functional and anatomical data as well as a constrained visualization of the functional - anatomical overlay in the coregistration dialog. Therefore, if you use spatial smoothing during FMR preprocessing, you should follow a slightly different pathway for aligning your functional and anatomical data:

During FMR document creation BrainVoyager uses the first scan of the FMR to create a pseudo-anatomical image (or AMR) which is linked for display purposes to the FMR document. The AMR is not affected by the spatial smoothing step in the preprocessing routine and can therefore be used to improve the alignment and visualization of functional-anatomical overlay in case of smoothed data. **Switch** from the default option “Use FMR data (EPI slices)” to “Use linked AMR (coplanar T1 or T2 weighted slices)”. This switch is not needed if you use a non-smoothed FMR for the coregistration.

It is important to evaluate the coregistration by checking whether the coregistered functional data indeed matches the corresponding anatomical data. This can be done by browsing to various regions in the data set and by switching between different display views. The display option “Blend: Edges” is marked by default, which shows the “edge” display of the coregistered functional data in green overlaid on the anatomical data in the coregistration row. If the anatomical data is not clearly visible in the coregistration row, you can reduce the “Blend mode transparency” by clicking the “Options” button.
9. **Click** the “Blend: Mosaic” option for yet another view. Now you see a checkerboard pattern in the coregistration row with half of the checks showing the anatomical data and half of the checks showing the coregistered functional data.

*Note:* Since BrainVoyager 20.2 boundary-based registration is included for fine-tuning alignment. More information about this can be found in the User’s Guide ([https://download.brainvoyager.com/bv/doc/UsersGuide/Coregistration/Boundary-BasedRegistration.html](https://download.brainvoyager.com/bv/doc/UsersGuide/Coregistration/Boundary-BasedRegistration.html))

*You can now move on to Step 11.*
Step 11: MNI Normalization of Anatomical Data

In this step, we will learn how to normalize a recorded 3D data set into MNI space, which is a commonly used as "standard" space for reporting locations of activated brain regions and for averaging data across subjects.

1. If you have closed BrainVoyager, start the program now. Open the file “sub-01_ses-04_acq-nondistorted_T1w_IIIHC.vmr”.

2. Select the “Normalize to MNI Template Space” option from the “Volumes” menu.

3. Leave the options for the “Template-Based Normalization” as they are, unless your data set requires specific settings and click “GO”. In contrast to the Talairach normalization, no manual intervention is necessary.

4. The VMR data has been normalized to the MNI-ICBM 152 template and is stored with a different name (sub-01_ses-04_acq-nondistorted_T1w_IIIHC_MNI.vmr). BrainVoyager will also automatically create the corresponding transformation files (*.trf), necessary for the transformation of the functional data into MNI space.
5. Using the “Show secondary VMR” option in the “Spatial Transf” tab of the “3D Volume Tools” allows to check the match of the transformed anatomy and the underlying template file (showing the contour of the transformed anatomy in orange).

You can now move on to Step 12.
Step 12: MNI Normalization of Functional Data – Creation of VTC Documents

In this step, we will learn how to transform our functional data into MNI space. The result of this process is a “VTC” file (VTC = volume time course), containing the data from a corresponding FMR document. For this purpose, we will use several pieces of information, which we have produced during previous steps, namely the intra-session anatomical and functional coregistration transformation matrix and, finally, the MNI a12 transformation matrix created during the anatomical normalization into MNI space.

1. If you have closed BrainVoyager, **start** the program now and **open** the file “sub-01_ses-04_acq-nondistorted_T1w_IHLC_MNI.vmr”. This is the anatomical data set we will use to display the functional data.

2. **Use** the menu item “Analysis” -> “Create Normalized VTC from FMR Data...” to open the “Create VTC” dialog. The invoked “Create VTC” dialog contains several empty slots, which have to be filled to define the transformation “pipeline”.

3. **Click** the “Browse” button on the right of the “Functional slice-based data file (FMR)” text box. In the appearing “Open” dialog, **navigate** to the “GSGData\derivatives\sub-01\ses-04\func” folder, **select** the file “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c.fmr” and **click” “Open”. This is the source file containing functional data. Since we would like to create a VTC in MNI space make sure that the radio button “To MNI” is selected.

4. **Click** the “Browse” button on the right of the “FMR -> VMR coregistration file 1, e.g. header-based (_IA.TRF)” text box. In the appearing “Open” dialog, **select** the file “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c-TO-sub-01_ses-04_acq-nondistorted_T1w_IHLC_IA.trf” and **click” “Open”.

5. **Click** the “Browse” button on the right of the “FMR -> VMR coregistration file 2, e.g. fine-tuning (_FA.TRF)” text box. In the appearing “Open” dialog, **select** the file “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c-TO-sub-01_ses-04_acq-nondistorted_T1w_IHLC_FA.trf” and **click” “Open”.

6. **Click** the “Browse” button on the right of the “12 parameter MNI transformation file (_MNI_a12.TRF)” text box. In the appearing “Open” dialog, **navigate** to the folder “GSGData\derivatives\sub-01\ses-04\anat” and **select** the file “sub-01_ses-04_acq-nondistorted_T1w_IHLC_TO_MNI_a12.trf” and **click” “Open”.

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Step 12: MNI Normalization of Functional Data – Creation of VTC Documents

**Note:** The VTC creation process does **not require** that the functional data is transformed into a normalized space like MNI or Talairach. You can, for example, create a VTC file, which is only coregistered with the intra-sessio 3D data set. For this you select the radio button “To Native” and fill only the ".IA.TRF and *.FA.TRF file entries in the “Create VTC” dialog. Although it is possible to create a VTC in any 3D space, MNI as well as Talairach space allow to analyze data across subjects. For the analysis of several experiments from the same subject, however, it is fully valid to align all data sets to a 3D space other than Talairach, preferably ACPC space. The advantage of not going to Talairach or MNI space is that with rigid body transformations (only translations and rotations), the geometry of the cerebrum is not changed. In this case, select “To ACPC“ and fill all entries except the “Cerebrum border..” text box.

7. After having specified these files, the “Resulting VTC file:” field shows the name of the VTC file that will be written to disk, it consists of:
   - the name of the originating FMR (“sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c”),
   - the target bounding box (“_256”, for 256 x 256 x256),
   - the interpolation used to transform the functional data to VTC space (“_trilin”, for trilinear),
   - the resulting voxel size (“_3x1.0”, for three times the resolution of the current VMR, which is 1.0 mm iso-voxel. Therefore, the VTC data will have a resolution of 3.0 mm iso-voxel.)
   - and the resulting space (“_MNI.vtc”, for MNI standard space).

The resolution of the functional data set that we use in the current tutorial is 2.0 mm iso-voxel. You can find this information in the FMR properties as described in step 1. Therefore, it makes sense to change the
default resolution of three times the 1 mm VMR resolution to twice the VMR resolution. Please **click** on “Options” to open the “Create VTC Options” dialog and **change** the “Target resolution” to “2x2x2”. You can **close** the dialog via “OK”.

![Create VTC Options dialog](image)

**Note:** you will see that the resulting “VTC file” entry has changed to: “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c_256_trilin_2x1.0_MNI.vtc”

8. **Click** the “GO” button to start the transformation process. A progress dialog will appear to inform you about the ongoing computations: For each of the 291 time points/volumes, the 64 functional slices are coregistered with the intra-session 3D data set and then transformed into MNI space (see schema below).
Overview alignment and normalization

- sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c-TO-sub-01_ses-04_acq-nondistorted_T1w_IIHC_IA.trf
- sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c-TO-sub-01_ses-04_acq-nondistorted_T1w_IIHC_FA.trf
- sub-01_ses-04_acq-nondistorted_T1w_IIHC_TO_MNI_a12.trf
9. We can connect or link the functional data in MNI space to the anatomical data set in MNI space. This linking possibility provides high flexibility. We can link the functional data from any experiment of a subject to a 3D data set and analyze across the functional data from different experiments. If all the data from the same subject has been transformed into MNI space, detected brain activity can be located reliably and precisely. In the “Analysis” menu, click the “Link Volume Time Course (VTC) file...” item, which invokes the “Link Volume Time Course (VTC)” dialog.

10. Click the “Browse...” button on the right of the “3D volume time course (VTC) file name” text box. In the appearing “Open” dialog, navigate to the “GSGData\derivatives\sub-01\ses-04\func” folder, select the file “sub-01_ses-04_task-blocked_run_1_bold SCCTBL_3DMCTS_THPGLMF2c_256_trilin_2x1.0_MNI.vtc” and click “Open”.

11. As default, the “Store reference to VTC file” item is checked after selecting a VTC file, which means that the data in that file is accessed from the file when needed. You can also check the other option “Load VTC into working memory”, which will load the complete data in the referenced file into memory. You should use this option only if the VTC file is not too big with respect to the installed working memory. Click the “OK” button to accept the link to the VTC file.

12. There is no visible effect of establishing the link so far. Click with the right mouse button on an arbitrary position of the cerebrum. Click on “Show ROI time course”. A “ROI Signal Time Course” window will pop-up showing the time course of our “Faces-Houses-Left-Right-Center” experiment at the location selected with the mouse. This shows that the functional data is now indeed linked to the 3D data set. As we will see in the next step, we can now perform statistical tests and event-related averaging in the same way as we have done with the FMR document.

**Note:** A more convenient way to get the ROI time course is by clicking with the left mouse button, while holding down the CTRL key. (CTRL+Left Mouse Button)
13. Another possibility to visualize the functional VTC data provided, which is very important for checking whether the transformation was successful and whether anatomical and functional data are in good spatial alignment. Switch to the “Spatial Transf” tab of the “3D Volume Tools” dialog and click the “Show VTC Vol” button in the “Show a volume of attached VTC data” field. The anatomical images in the “SAG”, “COR” and “TRA” view will disappear and images from one volume of the VTC data will be shown instead. If everything went well during VTC creation, these images should be in good spatial registration, as indicated on the right. You can explore the depicted functional volume the same way as an anatomical volume by clicking and moving the white cross with the left mouse button.

14. In order to see the anatomical images again, click the “Show primary VMR” option in the “Two VMR display options” field, or use the F8 key. With this function key, you can toggle between the anatomical and functional view. You can also blend the VTC volume with the anatomical dataset, by using the blend options. After having used the “Show VTC Vol” feature once, you can also save the functional volume as a VMR document to disk. In the “File” menu, click the “Save secondary VMR...” item. In the appearing “Save As” dialog, enter “sub-01_ses-04_task-blocked_run-1_VTC_MNI” and click the “Save” button.

**Note:** The performed task is similar to the creation of a pseudo-anatomical AMR document from functional slices of an FMR document. This allows you, for example, to visualize functional clusters over the VTC volume.

15. You can “browse” to any location and check the correspondence between anatomical and functional data by clicking the “F8” key or by clicking the “Show primary VMR” or “Show Secondary VMR” option. With the “F9” key you can see both datasets blended in one view. The title bar of the “Volume” window shows the names of both loaded data sets, the name of the primary data set on the left side and the name of the secondary data set on the right side.

**Note:** A VTC file contains a 4D data set: 3D space x 1D time. A “functional VMR” data set shows only one of many 3D volumes of the 4D data set. The “Show VTC Vol” function transforms by default the first volume of a linked VTC file into the VMR format.

You can now move on to Step 13.
Step 13: Statistical Analysis of 3D Functional Data

In this step, we will learn how to run statistical tests with VTC functional data. The good news is that this works in the same way as we have learned for slice-based (FMR) documents. We refer therefore to steps 4, 6 and 7 and show here only a short summary of a GLM analysis. You will, however, learn about small differences in how to access time courses in 3D functional documents as opposed to slice-based FMR documents.

1. If you have closed BrainVoyager, start the program now. Open the document “sub-01_ses-04_acq-nondistorted_T1w_IlHC_MNI.vmr”.

2. Link the VTC file “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c_256_trilin_2x1.0_MNI.vtc” to the opened 3D data set (see step 12). If you have performed the previous step, you can click on the name of the VTC file in the Sidebar.

3. Select “General Linear Model: Single Study...” in the “Analysis” menu. This will invoke the “Single Study General Linear Model” dialog.

4. We load the simple six predictor model, which was saved in step 6. If you have not saved this file, go back to step 6 and create the model as described. Click the “Load...” button of the “Single Study General Linear Model” dialog and in the appearing “Open” dialog, navigate to the “GSGData\derivatives\sub-01\ses-04\func” folder, select the file “FacesHousesDesignMatrix.sdm” and click the “Open” button.

5. Click “GO” to run the GLM. After a few seconds, the statistical map of the full model is superimposed on the 3D data set.
Step 13: Statistical Analysis of 3D Functional Data

6. You can now “browse” to any point in 3D space by clicking with the left mouse button in the “SAG”, “COR” and “TRA” view. You may also move the mouse while holding down the left mouse button, which will update the three views to show orthogonal planes through the “current” voxel. You will also see a “Voxel Beta Plot” window, which opens automatically. If you move the mouse button over the brain (with or without pressing the left mouse button), the estimated beta values for the voxel under the mouse will be plotted in the “Voxel Beta Plot”.

7. To inspect a time course of a certain region of interest, click with the right mouse button at any “hot” voxel as shown above. Then select “Show ROI Time Course” in the appearing context menu. As a faster method, simply CTRL-click a “hot” voxel. A “ROI Signal Time Course” window will pop up showing the time course of a small volume around the 3D coordinates selected by the mouse. The border of this volume is marked with a white line. The cluster is computed via region growing in 3D “statistical” space. You can control the maximum extent by changing the “Range” value in the “Options” field of the “3D Coords” tab of the “3D Volume Tools” dialog. If you use a range value of “1”, the time course of a single voxel will be selected when CTRL-clicking with the left mouse button in the “VMR” window.

8. Since we need the computed GLM in a later step, we save it now to disk. Select the “Overlay General Linear Model...” item in the “Analysis” menu.
Step 13: Statistical Analysis of 3D Functional Data

9. In the appearing “Overlay GLM Contrasts” dialog, **click** on “Save GLM...”.

10. In the appearing “Save As” dialog, **navigate** to the folder “GSGData\derivatives\sub-01\ses-04\func”, **enter** “FacesHouses_VTC.glm” and **click** on “Save”.

11. Now clear the contrast by clicking “Clear Contrast”. Afterwards check “Faces_LVF”, “Faces_RVF” and “Faces_CVF” with a plus sign (+) and “Houses_LVF”, “Houses_RVF” and “Houses_CVF” with a minus (-) and confirm your selection by clicking “OK”. BrainVoyager will compute a statistical 3D map showing brain regions that respond to face stimuli and will superimpose this map on the anatomical data, as shown below.

*Note*: The 3D statistical map is internally stored in a VMP (**volume map**) data structure. Note that you can create many different VMPs from a single GLM file. VMPs can be created also with other statistical tests like linear correlation or t-tests.

12. A VMP can also be saved to disk by using the “Overlay Volume Maps” item in the “Analysis” menu. In the appearing “Volume Maps” dialog, **click** the “Save As...” button.
13. Usually, you want to know some details about the activated clusters for publication purposes, e.g. the MNI coordinates and the t-value of the peak voxel of the respective clusters. This can be easily achieved by creating volumes of interest (VOIs) from all map clusters. We would like to focus on clusters of relevant size; therefore, we enable the “Cluster threshold” and set it to 12 functional (2x2x2 mm$^3$) voxels in the “Statistics” tab of the “Volume Maps” dialog. Please note that this number is only a suggestion and depends on the details of your analysis. Now we can create VOIs from these clusters by clicking on the item “Convert Map Clusters to VOI(s)...” in the “Options” menu.

14. In the appearing dialog you have to choose one of the two options: creating separate VOIs for all active clusters (“Create VOI for each cluster”) or creating one single VOI containing all significantly active voxels in the map (“Create one VOI from active voxels”). Since we want to get an overview of all distinguishable clusters and their statistical values, we select the former option. This dialog gives you the opportunity to restrict the minimal cluster size also for VOI creation. To do so, we enter 12 functional voxels (2x2x2 mm$^3$) in the cluster threshold field, the same number that we have specified in the “Volume Maps” dialog. Click “GO” to start the VOI creation.

15. After BrainVoyager has finished the computation, the “Volume-Of-Interest Analysis” dialog is invoked with a list of VOIs representing the activation clusters displayed in the statistical map. We might need the definition of these VOIs later again. Therefore, we save the VOI file by clicking on “Save”. Then we navigate to the folder “GSGData\derivatives\sub-01\ses-04\func” and enter “Faces-Houses” in the invoked “Save VOI File” dialog.

16. Now we have our map converted into VOIs, but we still don’t have any detailed information on the clusters. To obtain the required information we click on the “Options” button and switch to the “VOI Functions” tab in the “VOI Analysis Options” dialog. There we can access, among other things, the peak voxel of each cluster with its associated MNI coordinates, its t-value and its p-value. For this purpose, you simply have to click on the button “Table ...” in the “VOI Map Peak Voxels” field.

Remark: The information is displayed in a table that can be saved to disk. Furthermore, by marking one
cluster and **clicking** the button “Show Voxel” BrainVoyager automatically directs the white cross to the peak voxel of the respective cluster in the map.

You can now move on to Step 14.
Step 14: Preprocessing of VTC Documents

In this step we will learn how to preprocess VTC documents. By adding the preprocessing step of spatial smoothing only at the VTC stage and not at the FMR level, you will have the option to explore the effect of different spatial smoothing kernels on your data without having to recreate new VTC documents.

1. If you have closed BrainVoyager, start the program, open the file “sub-01_ses-04_acq-nondistorted_T1w_ILHC_MNI.vmr” and link the VTC “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c_256_trilin_2x1.0_MNI.vtc” by clicking its name in the “Recent Files” pane.

2. Invoke the “VTC Data Preprocessing” dialog by selecting the entry with same name in the “Analysis” menu.

3. Here we keep the default option of spatial smoothing but reduce the kernel size to twice the voxel resolution, i.e. 4 mm. The result of the spatial smoothing will be automatically saved to disc in form of a new VTC file called: “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c_256_trilin_2x1.0_MNI_SD3DVSS4.00mm”, as indicated in the “Output file options”. Start the preprocessing by clicking on “GO”.

Note: The progress of the preprocessing step is indicated by a progress bar. After the spatial smoothing is finished, the resulting VTC document will be automatically linked to the current VMR.

4. To explore the effect of spatial smoothing on your data analysis, please repeat points 3-5 of step 13.

5. You will see that the resulting map looks smoother, more extended, but also less specific than the overall model map in step 13 which was based on unsmoothed data.

You can now move on to Step 15.
Step 15: Event-Related Averaging of 3D Functional Data

In this step, we will learn how to create templates for averaging time segments and how to apply them to time courses of any region-of-interest. Like with statistical tests, this works the same as in slice-based FMR documents. The only difference is that VTC files - instead of FMR files - will be used for the averaging procedure.

1. If you have closed BrainVoyager, start the program now and open the document “sub-01_ses-04_acq-nondistorted_T1w_ILHC_MNI.vmr”. Link the VTC file “sub-01_ses-04_task-blocked_run-1_bold_SCCTBL_3DMCTS_THPGLMF2c_256_trilin_2x1.0_MNI.vtc” to the opened 3D data set (see step 12).

2. Select “Event-Related Averaging” in the “Analysis” menu. This will invoke the “Event-Related Averaging Specification” dialog.

   ![Event-Related Averaging Specification dialog]

   Our VTC, which we have linked above to the VMR document, will be automatically placed in the “Functional data files” list box and the condition names from the attached protocol will be listed in the “Available conditions in referenced protocol files” box.

   **Note:** You can add additional VTC files allowing to average across multiple runs from the same subject or across runs from different subjects. This requires only that all VTC files are in the same space, typically Talairach or MNI space.

3. We now select conditions, from which we want to have their associated time course epochs averaged across repetitions. We select all six main conditions. The “Expected response plot” panel shows the predicted time course, which we can use to see whether the complete expected time course is visible or whether we need to increase the “Post” value. In the current example this value does not need to be changed.

   **Note:** You will see that the “Volumes” option in the “Resolution of data points” field is checked. This will show the averaged data in the recorded time resolution, as we will see shortly. The recorded time resolution (volumes, scans) is characterized by the TR value, which in our case is 2 seconds.
4. Now click the “Create AVG” button. In the invoked “Save As” dialog, navigate to the folder “GSGData/derivatives/sub-01/ses-04/func” and enter “FacesHouses_VTC.avg” and click the “Save” button.

5. We can now apply event-related averaging as specified and saved in the .AVG file to any ROI time course. If you cannot see any activation at this point, run a GLM as described in step 13. Use the right mouse button to select a small volume encompassing a statistically significant region and its associated time course as shown on the right.

6. In the appearing “ROI Signal Time Course” dialog, click in the plot which will expand the window showing several options. You can also expand the dialog by clicking with the right mouse button in the main window. In the appearing context menu choose the “Show/Hide Options” item.

One of the appearing options is the application of event-related averaging files. Click the “Browse...” button on the right side within the “Event-related averaging” field. In the appearing “Open File” dialog, select our saved file “FacesHouses_VTC.avg” and click “Open”.

7. You will now see the “Event-Related Averaging Plot” window. As described in step 8, you can click into the window to access several options to change the appearance of the “Event-Related Averaging Plot” window.

You can now move on to Step 16.
Step 16: Surface Reconstruction of the Head

Part III: Analysis in Surface Space - SRF-MTC Documents

Step 16: Surface Reconstruction of the Head

In this step, we will learn how to invoke the 3D Viewer. The 3D Viewer of BrainVoyager (before BrainVoyager 21: surface module) can be used to create advanced 3D renderings and to apply various surface-based methods like cortical flattening. Here we will learn how to create a 3D model of a subject’s head from a 3D MRI data set. The resulting visualizations are instructive in itself but they are also important for advanced applications such as the spatial coregistration of a subject’s MRI 3D coordinate system with that obtained from other imaging modalities, like EEG or MEG.

1. If you have closed BrainVoyager, **start** the program now.

2. **Click** in the Files Pane on the file: “sub-01_ses-04_acq-nondistorted_T1w.vmr”.

3. In the “3D Volume Tools” dialog, click on “3D Viewer” button when the dialog is still in mini-state. If the “3D Volume Tools” is in full dialog-state **select** the “3D Coords” tab and **click** the “3D Viewer” button. A new window called “3D Viewer” will be invoked allowing you to load and operate surface meshes.

4. **Click** the “Create Sphere Mesh” icon in the “Mesh Tool Box” on the right side of the BrainVoyager window. The 3D Viewer will show a sphere, which is the mesh created as default.
5. **Click** the “Wrap-Volume Mesh Morphing” icon to start a “shrink-wrap” reconstruction procedure. The invoked process reduces the radius of the sphere until the vertices of the mesh “detect” tissue, which corresponds in our case to the skin of the subject’s head. The procedure iterates until the complete head is reconstructed as shown below.

6. You will notice that the created polygon mesh shows some “holes” in the surface of the head, to correct these flaws, we need to adjust the “Shrink mesh force to find VMR intensity”, i.e. the minimum intensity in the image that is considered to belong to the outer surface of the image, which is in our case the skull of the participant. Please **open** the “Mesh Morphing” dialog via “Meshes -> Mesh Morphing”. **Select** the “Find VMR mode”, **change** the value in the “VMR intensity” field to 10 and start the mesh morphing process by **clicking** on “GO”.

---

![Image of mesh reconstruction process](image-url)
7. We now save the created polygon mesh. **Click** the “Meshes->Save Mesh As ...” menu item. In the appearing “Save As” dialog **enter** “sub-01_ses-04_acq-nondistorted_T1w_Head.srf” and **click** the Save” button.

*You can now move on to Step 17.*
Step 17: Navigation in the 3D Viewer

In this step, we will learn how to navigate within the surface window. If you continue directly from the previous step, you can go to point 3 now.

1. If you have closed BrainVoyager, start the program now.

2. In the “Recent Files” Pane, click on the files: “sub-01_ses-04_acq-nondistorted_T1w.vmr” and “sub-01_ses-04_acq-nondistorted_T1w_Head.srf”. Alternatively, you could load the files using the toolbar and menu. Click the “Open” icon or select the “File -> Open” menu item. In the “Open” dialog select the file “sub-01_ses-04_acq-nondistorted_T1w.vmr” and click “Open”. Select the “Meshes” -> “Load Mesh” menu item. In the “Open” dialog, select the file “sub-01_ses-04_acq-nondistorted_T1w_Head.srf”.

3. The program now displays a surface window as shown on the right. The window shows the surface reconstruction created in the previous step. With this mesh you can learn to navigate the camera position of the 3D scene also called the viewpoint.

   Click the right mouse button at an arbitrary point in the 3D Viewer, hold it down and move the mouse. You will see that the mesh moves left-right and up-down as the mouse is moved left-right and up-down.

   You can also move the viewpoint in the third dimension by holding down the left and right mouse button at the same time and by moving the mouse up and down. This movement of the viewpoint resembles “zooming” because the viewpoint is moved towards / away from the mesh. Alternatively use Shift + Ctrl + left click-dragging for zooming.

   You can also rotate the viewpoint around the center of the scene by holding down the left mouse button and by moving the mouse left / right and up / down. This will rotate around two out of three possible axes.
4. A helpful tool to navigate in the 3D Viewer is the “Viewpoint Navigation” Panel. Press the corresponding button in the 3D Viewer toolbox to invoke it. This dialog offers 6 sliders that allow to change the three translation and rotation parameters just described. In addition, it offers six standard viewpoints, which you can apply by clicking the respective brain icons (“Left”, “Right”, “Top” etc.) on the left side of the dialog.

![Viewpoint Navigation Panel](image)

**Note:** You can keep the dialog open and continue working in the 3D Viewer.

*You can now move on to Step 18.*
Step 18: Slicing a Polygon Mesh

In this step, we will learn how to slice a loaded or created polygon mesh. Sliced views of the head or brain provide useful information about the spatial relation of 3D renderings and the 2D images from which they have been built. If you continue directly from the previous step, you can go to point 3 now.

1. If you have closed BrainVoyager, start the program now.

2. In the Files Pane, open the files: “sub-01_ses-04_acq-nondistorted_T1w.vmr” and “sub-01_ses-04_acq-nondistorted_T1w_Head.srf” by clicking on them.

3. Click the “Mesh Slicing Panel” icon in the Surface tool box. The “Mesh and Volume Slicing” dialog will open. You can use the checkmarks to activate the mesh slicing and the corresponding sliders to change the slice line in axial, coronal and sagittal mode.

4. Activate the axial slicing. Click the checkmark next to the “TRA” slide. Now change the cut slice plane. The “TRA Slicing Mode” mode is automatically enabled when the “TRA Cut” mode is selected. Use the slider next to the TRA and see that the cut slice plane moves upward or downward, respectively. Hide the transparent rectangle around the slice by clicking the respective button in the “Mesh And Volume Slicing” dialog.
5. Besides transversal slicing, you can select sagittal and coronal slicing. Click the checkmarks next to the “COR” and “SAG”. A screen capture of the result is presented below.

6. A cut shows one of two possible halves of the sliced object. You can also show the other half of the sliced object. Click the “Flip” checkmark next to the slider. You will see a scene like the one on the right, revealing the half of the object on the other side of the slice. You can do this with any of the three cut planes after having selected the respective slicing mode icon.

**Note:** In BrainVoyager 21.4 the option of oblique slicing has been introduced, providing even more degrees of freedom for visualizing 3D renderings and the 2D images from which they have been built. This option can be enabled using the upper right button in the “Mesh And Volume Slicing” dialog.

*You can now move on to Step 19.*
Step 19: Automatic Cortex Segmentation

In this step, we will learn how to automatically segment and reconstruct the cortex of both hemispheres of the brain.

1. If you have closed BrainVoyager, **start** the program now. If BrainVoyager is running, close any open document.

2. **Click** the “Open” icon or **select** the “File -> Open” menu item. In the “Open” dialog, **navigate** to the folder “GSGData/derivatives/sub-01/ses-04/anat” folder. **Select** the “sub-01_ses-04_acq-nondistorted _T1w_IIHC_MNI.vmr” and **click** “Open”.

3. **Switch** to the “Segmentation” tab of the “3D Volume Tools” dialog and then **click** the “Autom. Segm.” button.

4. **You will now see** the “Automatic Cortex Segmentation and Reconstruction” dialog with a set of options, most of them are checked by default. **Check** the options “Create surfaces of outer grey matter (pial) boundary”, “Remove bridges LH” and “Remove bridges RH”. Keep all other default settings and **click** the “GO” button.

Several steps are now executed to properly segment the cortex along the white-grey matter boundary. The processes involve edge-preserving smoothing (sigma filter), filling of ventricles, application of masks to label subcortical structures, creation and analysis of intensity histograms to detect white and grey matter peaks, a region growing process and several small morphological operations. Finally, the hemispheres are disconnected and reconstructed. The segmentation can be further improved by removing topological errors (‘bridges’ and ’handles’) via a bridge removal algorithm. After a successful segmentation of both hemispheres, they are saved to disk. Then the 3D Viewer is used to reconstruct and smooth the boundary of the cortex.

**Note**: More details about these segmentation steps can be found in the User’s Guide and in Kriegeskorte & Goebel (2001).
5. During segmentation, a window displaying a set of histograms is shown. The different curves represent histograms for different axial slices. The left peak in the histograms corresponds to grey matter and the right peak to white matter. Another dialog appears allowing to adjust the white matter (WM) / grey matter (GM) cut point. If necessary, adjust the cut value in such a way, that the white line is between the two peaks and click the “OK” button. If the automatic segmentation has been completed, you can simply close the “Smoothed intensity histogram” window (as well as a second window with additional histogram information) by clicking the “Close” button (“x”) at the right upper corner.

Info: The following files will be saved to disk during the segmentation process (“<MNI>” = “sub-01_ses-04_acq-nondistorted_T1w_ILHC_MNI”):

<table>
<thead>
<tr>
<th>File Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;MNI&gt;<em>WM_LH</em>(BL2).vmr</td>
<td>The volume segmented at the white/grey matter boundary (WM) for the left hemisphere (LH), with topological errors removed (BL2) in case bridge removal was selected.</td>
</tr>
<tr>
<td>&lt;MNI&gt;<em>WM_RH</em>(BL2).vmr</td>
<td>The volume segmented at the white/grey matter boundary (WM) for the right hemisphere (RH), with topological errors removed (BL2) in case bridge removal was selected.</td>
</tr>
<tr>
<td>&lt;MNI&gt;_WM_LH_RECO.srf</td>
<td>The reconstructed surface mesh (RECO) of the left hemisphere (LH) without smoothing.</td>
</tr>
<tr>
<td>&lt;MNI&gt;_WM_RH_RECO.srf</td>
<td>The reconstructed surface mesh (RECO) of the right hemisphere (RH) without smoothing.</td>
</tr>
<tr>
<td>&lt;MNI&gt;_WM_LH_RECOSM.srf</td>
<td>The reconstructed mesh after smoothing (RECOSM), left hemisphere (LH); used as reference mesh.</td>
</tr>
<tr>
<td>&lt;MNI&gt;_WM_RH_RECOSM.srf</td>
<td>The reconstructed mesh after smoothing (RECOSM), right hemisphere (RH); used as reference mesh.</td>
</tr>
<tr>
<td>&lt;MNI&gt;_WM_LH.srf</td>
<td>An optimized mesh of the white/grey matter boundary (WM) for the left hemisphere (LH).</td>
</tr>
<tr>
<td>&lt;MNI&gt;_WM_RH.srf</td>
<td>An optimized mesh of the white/grey matter boundary (WM) for the right hemisphere (RH).</td>
</tr>
</tbody>
</table>
6. Since we have checked the “Create..pial” option, the program also creates and saves two VMR files named: “sub-01.ses-04.acq-nondistorted_T1w_IIHC_MNI_GM_LH.vmr” and “sub-01.ses-04.acq-nondistorted_T1w_IIHC_MNI_GM_RH.vmr” and two surfaces named: “sub-01.ses-04.acq-nondistorted_T1w_IIHC_MNI_GM_LH.srf” and “sub-01.ses-04.acq-nondistorted_T1w_IIHC_MNI_GM_RH.srf”. When the process is finished, the 3D Viewer shows the reconstructed pial boundary (“GM”) of the right hemisphere.

**Note:** The surfaces of the outer grey matter boundary are only for visualization purposes. For inflation and flattening we will use the reconstructed and smoothed white matter surfaces.

7. We will now load the mesh: “sub-01.ses-04.acq-nondistorted_T1w_IIHC_MNI_WM_RH_RECOSM.srf”, which was created during the automatic segmentation by selecting “Load Mesh..” in the “Meshes” menu. This mesh resembles a slightly dilated version of the grey/white matter boundary. Alternatively, you can use the “Load Mesh” icon in the toolbar to open an existing surface file (.srf).

8. It is important to check the quality of the achieved segmentation. Once the 3D Viewer is selected, click the “Meshes -> Spatial Transformations...” menu item. In the appearing “Mesh Transformations” dialog, click the “Mesh -> VMR” button.
9. The program now automatically switches to the “Volume” window. The voxels that reflect the mesh in the 3D Viewer are shown in yellow. You can now navigate within the “SAG”, “COR” and “TRA” views to visually check whether the mesh contour matches the white/grey matter boundary.

Note: With most data sets, the achieved quality of the automatic segmentation procedure can be further improved. For assistance consult the Image Segmentation guides located on the BrainVoyager support website (https://support.brainvoyager.com/) for possible improvements including manual segmentation procedures.

You can now move on to Step 20.
Step 20: Cortex Inflation

In this step, we will learn how to inflate a mesh, representing the cortical sheet, and how to show curvature information of the folded cortex on the inflated cortex representation.

1. If you have closed BrainVoyager, **start** the program now. If BrainVoyager is running, close any open document.

2. **Click** the “Open” icon or **select** the “File -> Open” menu item. In the “Open” dialog, **select** the file “sub-01_ses-04_acq-nondistorted_T1w_IIHC_MNI.vmr” and **click** “Open”.

3. **Click** on the “Meshes -> Load mesh…” menu item. In the appearing “Open” dialog, **select** “sub-01_ses-04_acq-nondistorted_T1w_IIHC_MNI_WL_RECOSM.srf” and **click** “Open”.

   **Note:** We use the “RECOsM” version of the reconstructed cortex and not the “WM” version. For the inflation process it does not matter which one is chosen, but for curvature coloring the “RECOsM” version is more appropriate than the “WM” version.

   **Exercise:** In this step, inflation is performed only for one hemisphere. As an exercise, you can run the same procedure for the other hemisphere.

4. **Click** the “Mesh Morphing” menu item in the “Meshes” menu.

5. In the appearing “Mesh Morphing” dialog, **check** the “Inflation mode” option in the “Morphing modes and forces” field. This will set appropriate default values for morphing forces and it will automatically specify the loaded mesh as the reference mesh. The reference mesh is used to keep the surface area constant while unfolding the cortex and the respective option “Keep surface area constant” is automatically enabled. The reference mesh can be also used for showing curvature information on the inflated cortex.

6. The inflation process is performed by repeatedly executing small morphing steps. The “Number of iterations” entry is set to “800” iterations. The “Update every: .. Iterations” entry specifies that the screen (3D Viewer) will be updated every n morphing steps. **Change** the settings from 800 to 500 iterations and from 30 to 50 iterations and **click** the “GO” button to start the inflation process.
7. The screen will be updated repeatedly, showing the progression of the inflation process. On the right, you see the state of the inflation process after 171 iterations. The next figure shows the state of the mesh after all requested 500 iterations have been performed.

8. **Optional**: Since there are still some folds visible (for example the central sulcus), we will run additional inflation steps. **Invoke** the “Mesh Morphing” dialog again and **enter** the value “300” in the “Number of iterations” entry. Keep all other settings and **click** “Go”. Now 300 more inflation steps are performed, further removing folds.

**Note**: You can stop the inflation process by pressing the ESCAPE key. Depending on the speed of your computer, you might hold the ESCAPE key down for a few seconds to stop the process. You can easily restart the inflation process by clicking the “Morph Mesh” icon.

9. We will now visualize curvature information from the folded cortex on the inflated representation, which allows to localize sulci and gyri. **Invoke** the “Background and Curvature Colors” dialog by selecting the respective item in the “Meshes” menu. There are two options to control the resulting curvature display. If the “Use two colors for convex and concave” option is clicked, one color will be used for concave (corresponding roughly to sulci) and another color for convex (corresponding roughly to gyri) regions. If the “Use graded
Step 20: Cortex Inflation

curvature colors (SMP-based)” option is checked (default), the degree of convexity or concavity is visualized by a whole range of colors. In this example we switch to the “Use two colors for convex and concave” option. **Click** the “Curvature” button in the “Calculate curvature” field. Then **click** on the “Smooth” button to slightly smooth the visualized contours. You can close the dialog by **clicking** on “OK”.

10. We now save the inflated cortex mesh. **Click** the “Save Mesh As ...” item in the “Meshes” menu. In the appearing “Save As” dialog, enter “sub-01_ses-04_acq-nondistorted_T1w_IHIC_MNI_WM_LH_RECOSM_INFL.srf” and **click** the “Save” button

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You can now move on to Step 21.
Step 21: Changing Mesh Colors

In this step, we will learn how to change the two basic colors of a mesh. Optimized mesh colors can improve the visualization of statistical maps (see next step).

1. If you have closed BrainVoyager, start the program now. If BrainVoyager is running, close any open documents.

2. Click in the Sidebar on the file: “sub-01_ses-04_acq-nondistorted_T1w_IIHC_MNI.vmr” and “sub-01_ses-04_acq-nondistorted_T1w_IIHC_MNI_WM_LH_RECOSM_INFL.srf”.

3. You will now see the inflated hemisphere as saved in the last step. We will now change the color of the mesh to grey because functional data can be better visualized on a “neutral” background color. Click on the “Background and Curvature Colors” menu item in the “Meshes” menu.

4. In the appearing dialog, click the “color display and selection” button to choose the “Default and convex color”.

5. In the appearing “Color” dialog, enter the value “120” in the “Red:”, “Green:” and “Blue:” entries. Click the “OK” button to accept the new mesh color.
Step 21: Changing Mesh Colors

6. A mesh has two default colors; the second is used during curvature coloring for visualizing concavely curved surface patches. To change the concave curvature color also to grey, **click** the corresponding “color display and selection button”.

7. In the appearing “Color” dialog, **enter** the value “60” in the “Red:”, “Green:” and “Blue:” entries. **Click** the “OK” button to accept the new concave curvature mesh color.

8. Back in the “Background and Curvature Colors” dialog change the setting again to “Use two colors for convex and concave”, **click** the “Curvature” button, smooth the contours with the “Smooth” button and close the dialog with “OK”. You will now see that our inflated map has changed its color from blue to grey.

9. Since we want to keep the grey coloring of the mesh, we now save the mesh to disk. **Click** the “Save Mesh ...” item in the “Meshes” menu.

   *You can now move on to Step 22.*
Step 22: Statistical Maps on Cortex Meshes

In this step, we will learn how to visualize statistical maps on inflated cortex meshes. We will also learn how to define surface patches as regions-of-interest, and to invoke time course plots for these regions.

1. If you have closed BrainVoyager, start the program now. If BrainVoyager is running, close any open document.

2. Open the file “sub-01_ses-04_acq-nondistorted_T1w_IWHC_MNI.vmr” and load the mesh “sub-01_ses-04_acq-nondistorted_T1w_IWHC_MNI_WM_LH_RECOSM_INFL.srf”. You will now see the inflated left hemisphere as saved in the last step.

3. One way to create a new statistical map for a cortex mesh (SMP) is to convert a computed or loaded statistical map in volume space (VMP). Make sure that the VMR window is active (e.g. by clicking on the window title). Click the “Overlay General Linear Model” item in the “Analysis” menu. In the appearing “Overlay GLM Contrasts” dialog, click the “Load .GLM” button. In the appearing “Open” dialog, navigate to the “GSGData/derivatives/sub-01_ses-04/func” folder. Select the file “FacesHouses_VTC.glm” and click “Open”. Now check all six predictors with a plus sign or click on “Fill Contrast”. Next, click the “OK” button.

4. Check if a statistical map has indeed been superimposed on the anatomical data. If you have the surface window maximized, click the “3D Volume Tools” icon, since this will switch to the “3D Volume” window.

5. If the statistical map was superimposed on the data, click on the title of the 3D Viewer to switch back.

Note: If you did not save the “Faces-Houses” GLM file in a previous step, recompute the GLM as described in step 13.
6. Before we can create a surface-based statistical map, we have to make sure that the inflated map is linked to the correct folded cortex representation, as described in previous steps. By default, the “RE-COSM” mesh is linked, and in the “Mesh Morphing” dialog you can check whether the correct link is established. **Click** the “Mesh Morphing” item in the “Meshes” menu and make sure that the “Use information from file:” field contains the file “sub-01_ses-04_acq-nondistorted_T1w_IIHC_MNI_WM_LH_RECOSM.srf”.

7. **Click** the “Quit” button to close the “Mesh Morphing” dialog.

**Note:** If no link to a folded mesh (RE-COSM or WM) would have been established, the 3D coordinates of the inflated plane would be used to “sample” the functional data in the volume space. This would lead to useless results. In establishing a link to the folded cortex, the inflated map can “sample” the correct voxel space using the 3D coordinates of its vertices from the linked folded mesh.

8. Now **click** the “Surface Maps” item in the “Meshes” menu. This will invoke the “Surface Maps” dialog.

9. We can create a surface map by **clicking** the “Create SMP” button. This procedure will create a statistical surface map (SMP) by sampling the voxel-based statistical map (VMP) at the vertex positions of the folded cortex mesh. The way this will be done can be specified in the invoked “Depth Integration” dialog. We will keep the default settings and close this dialog by **clicking** “OK”. Then **click** the “Close” button to close the “Surface Maps” dialog. You will now see several activation clusters super-imposed on the inflated map, most of them are in the temporal and occipital lobe.

10. After linking a VTC to the VMR document, we can also inspect time courses for surface-defined regions-of-interest (patches of interest, *.pol). An easy way to get a time course plot is by clicking on a “hot” (yellow or red) surface patch while holding down the CTRL key. You will see a “ROI Signal Time Course” window showing the time course of the selected surface patch. The boundary of the selected region is marked with a white contour line.
11. Close the “ROI Signal Time Course” window now by **clicking** the “Close” icon in the right upper corner.

12. **Now click** the “Surface Maps” item in the “Meshes” menu to re-enter the “Surface Maps” dialog. The “SMP” list box shows the map we have created earlier. In this list, many maps can be available simultaneously, allowing to superimpose multiple maps, each with a different color range. To change the properties of a surface map, it must be selected by **clicking** with the left mouse button in the row of the respective surface map. If a surface map has been selected, several properties of it can be changed. Change the lower threshold of the surface map to a value of “3.0”. We will also slightly smooth the statistical map on the surface. For that you have to switch to the tab “Advanced” and **click** the “Smooth SMP” button. **Click** the “Close” button to close the dialog. You will see the changes performed as shown in the figure below.
13. It is helpful to see a voxel view and a surface view of the data at the same time. To achieve this, click the “Tile” item in the “Window” menu. This will arrange the “Volume” and the “Surface” windows side by side (with the active window docked to the left side of the screen). If you now also select a time course, you will at the same time have information about the location of your activation in MNI space as well as on a (inflated) cortex representation. In addition, you can access any ROI time course by clicking in either the “Volume” window or the 3D Viewer.

14. We now save the created (and slightly smoothed) surface map to disk. Click the “Surface Maps” item in the “Meshes” menu to re-enter the “Surface Maps” dialog.

15. Click the “Save As...” button in the “Surface Maps” dialog. In the appearing “Save As” dialog, navigate to the folder “GSGData\derivatives\sub-01\ses-04\func”, enter “FacesHouses_LH.smp” and click the “Save” button.

16. We can now load the saved surface map to any version of the same mesh, for example the folded one. Because an “SMP” is a statistical data file, you can use a surface map without having to have a voxel-based statistical map (VMP) present. Close the 3D Viewer by clicking its “Close” icon in the right upper corner. Relaunch the 3D Viewer by clicking the “3D Viewer” button in the “3D Volume Tools” dialog.

17. We now load the folded left hemisphere. Click on the “Meshes -> Load Mesh” menu item. In the appearing “Open” dialog, select “sub-01_ses-04_acq-nondistorted_T1w_IIHC_MNI_WM_LH_RECOSM.srf” and click “Open”.

**Exercise:** Change the color of the folded mesh to grey, as described in step 21.
18. Click on the “Surface Maps” item in the “Meshes” menu. In the “Surface Maps” dialog, click the “Load .SMP” button. In the appearing “Open” dialog, navigate to the folder “GSGData\derivatives\sub-01\ses-04\func”, select the file “FacesHouses_LH.smp” and click the “Open” button.

19. You will see a single entry in the SMP list box - the map we have saved earlier. If the square on the left side of the map entry is empty, click the square, which will fill the square with a “+” sign. Finally, click the “Close” button.

20. You will now see the surface map on the folded cortex.
Alternative Pathways and Appendix

Appendix A: Renaming DICOM Files

The raw data of the “Faces-Houses-Left-Right-Center” experiment are stored in DICOM image format. Since DICOM files are named by the scanner in a complex way, it is often helpful to let BrainVoyager rename the files using information stored in the header of the images. This will avoid any potential problems during data import and the files will become easier to read. While the “Create Document Wizard” is doing this step automatically (if necessary), you can call this function anytime, e.g. before using the “Create Document” dialog. The usage of the “Rename DICOM Files...” dialog is shown below for the anatomical dataset.

1. Select “File”->“Rename DICOM Files...” to invoke the “Rename DICOM Files” dialog.

2. In this dialog, click the “Browse...” button, and navigate to the “BVSampleData\GSGData\sub-01\ses-04” folder. Now select the “anat” folder and click the “OK” button.

3. With the correct folder selected, click the “GO” button. The program will start renaming the files, which will be completed in a short time. The progress of the renaming is plotted in the “Log” tab of the sidebar.

Info: The names are changed to the format depicted in the screenshot. It is easily readable, showing a unique identifier provided by the experimenter, followed by three numbers indicating the <series>, <volume> and <image> of the data. Note that renaming does not change the content of the files.
Appendix B: Creation of a Functional Document from DICOM Files

In this step, we learn how to create BrainVoyager documents from raw data. Documents can be created by using the “Create Document Wizard” dialog in the “File -> New Doc Wizard” menu. Alternatively, you can use “File -> New Document”, which opens the “Create Document” dialog with more advanced options for document creation. Because BrainVoyager supports the native file format of many scanner types, the data has to be converted to BrainVoyager’s own file formats, so that all subsequent steps can be performed in the same way with all data. For the used sample data, original DICOM images are coming from a 3T Magnetom Prisma Fit scanner.

1. Launch BrainVoyager by clicking the “BrainVoyager” icon.

2. We will now begin importing the data into BrainVoyager by creating a document. Select “File” -> “New Document Wizard...” or click the “New Document Wizard” icon in the toolbar.

3. The Document Creation Wizard will guide you through the creation process step by step. To start, click the Next button.
Appendix B: Creation of a Functional Document from DICOM Files

4. First choose the type of document you want to create. Toggle through all four possible selections, FMR, AMR, VMR and DMR and read their descriptions. For a functional document we thus have to choose an FMR document. It will contain the repeated measurements of the brain as it is scanned volume by volume during the execution of the “Faces-Houses” experiment.

Click “Next” to continue.

5. Choose the type of raw data format as “DICOM”.

6. Change the name of the document to “sub-01_ses-04_task-blocked_run-1_bold”. To choose the source directory click “Browse” to navigate to the “GSGData\sub-01\ses-04\func” folder in the “BVSampleData” directory (e.g. C:\Users\<USER>\Documents\BVSampleData), select any file within the directory and click “Open”.

The wizard will detect if the names of your raw files have to be renamed into a more readable format. This is not necessary for our sample dataset. For more details about renaming DICOM files, please see Appendix A.
7. The Document Creation Wizard detects one set of DICOM files in this directory and determines from the header, what type of document it is and how many files it contains. **Choose** “JudEck_20181128_BI_Exercises_sess4-0002-0001-00001.dcm” (the only FMR present) and click “OK”. Note that 291 files (= volumes) have been detected.

8. BrainVoyager selects automatically the first source file and reads all required information from the header, as for example the mosaic image resolution (800x800) containing the 8x8 individual slices (100x100 each) making up one complete volume of the brain measured at one timepoint. **Click** “Next” to continue.

9. In the next menu you can change the number of slices to 64, if not recognized correctly.
10. In the last menu you can check the number of brain volumes detected (291). Since the first scans in a functional run contain data with very high intensity values (due to T1 saturation), it is recommended to skip a few volumes (e.g. 2-4). However, for newer sequences this is already done during scanning. Therefore, it is not necessary to skip files for the example data set. Despite skipping volumes for analysis, BrainVoyager will use the first (skipped) volume for display since it contains more anatomical information than later volume scans. In addition, the user can already decide to link an existing protocol file at this point (if available). We will discuss the creation of the protocol file in step 3 of this guide.

11. The Create Document Wizard ends with an overview containing the most important settings. Click “Finish” to create the document.
12. If you now see a screen like the one below, you have successfully built the FMR document. The data of the repeated measurements are now stored on disc in a single STC (slice time course) file.
Appendix C: Creation of a 3D Anatomical Document from DICOM Files

In this step, we will learn how to create 3D anatomical documents. The 3D data set will be used in the next step for coregistration with the functional data. As an alternative to the way described below, you can create new documents via the “Create Document” dialog (File -> New Document...).

1. If you have closed BrainVoyager, start the program now and click on the “Document Creation Wizard” icon to open the “Create Document Wizard”. This time we create an anatomical document (VMR) of the “intra-session” 3D data set. “Intra-session” means that the 3D data set was recorded in the same scanning session as the functional data.

2. Click “Next” to begin.

3. In the “Document Type” section, check VMR document as shown on the right. Click “Next” to continue.

4. In the “Raw data format” section, select “DICOM” as file type.
5. **Enter** the name of the document “sub-01_ses-04_acq-nondistorted_T1w”. To choose the source directory, **click** on “Browse” to navigate to the “BVSampleData\GSGData\sub-01\ses-04\anat” folder.

6. The Document Wizard automatically detects the set of DICOM files in this directory and determines what type of document it is and how many files it contains from the header of the files. Choose “JudEck_20181128_BI_Exercises_sess4-0008-0001-00001.dcm” (the only VMR file present) and click “OK”. Note that 192 files (=slices) have been detected.

7. The first source file is transferred to the appropriate line. BrainVoyager automatically detects the image resolution (256*256), typical for a T1 scan of the brain.

8. In the next menu you can change the number of slices in case they were not recognized correctly. Check if the correct number of slices is entered (192).
Appendix C: Creation of a 3D Anatomical Document from DICOM Files

9. The Create Document Wizard ends with an overview containing the most important settings. **Click** “Finish” to build the VMR document. After the document creation is finished the assembled images will appear in the workspace and the file “sub-01_ses-04_acq-nondistorted_T1w.vmr” will be automatically saved to disk.

**Note:** BrainVoyager shows useful information about the data in the Info Pane, as found in the header of the first image. In our case the voxel size is 1 x 1 x 1 mm. If the voxels have different dimensions, BrainVoyager automatically proposes to interpolate the data set to iso-voxel dimensions. In case the 3D data set is not scanned in sagittal slices, BrainVoyager will also automatically propose to change the orientation of the data set.
Appendix D: Automatic Talairach Transformation

In this step, we will learn how to transform a recorded 3D data set into Talairach space, an alternative option to the MNI normalization.

1. If you have closed BrainVoyager, start the program now. Open the file “sub-01_ses-04_acq-nondistorted_T1w_HIC.vmr”.

2. Talairach transformation is performed in two major steps. In the first step, the cerebrum is translated and rotated into the AC-PC plane (AC = anterior commissure, PC = posterior commissure). In the second step, the borders of the cerebrum are identified; in addition to the AC and PC points, the size of the brain is fitted into standard space. These steps are performed in the “Talairach” tab of the “3D Volume Tools” dialog. Switch to the “Talairach” tab of the “3D Volume Tools” dialog.

Note: Talairach transformation can be performed either automatically or manually. By default, the automatic Talairach transformation option is checked in BrainVoyager. However, for some non-standard data sets (e.g., lesion data, data sets from children or elderly) it might be advised to perform the Talairach transformation manually. This manual transformation procedure is described in Appendix E.

Note: The 3D data set used here is recorded as sagittal slices and thus fits into BrainVoyager’s scheme. Data sets recorded axially or coronally must be “standardized” first, i.e. transformed to a sagittal orientation.

3. Select the “Visualize intermediate results” checkbox and click on “Auto-ACPC-TAL”. This will initialize the automatic Talairach transformation. The “Visualize intermediate results” option gives you the opportunity to review all steps performed during the transformation process in the so called “Image Reporter”; a new window that is opened by BrainVoyager. Figures on the following page, labeled with Image Reporter, were copied from this additional review window.
Appendix D: Automatic Talairach Transformation

Note: BrainVoyager automatically performs the following steps:
It identifies the mid-sagittal plane (MSP) and uses this information to detect, segment and rotate the corpus callosum (CC), which is, in turn, removed to identify the fornix. The location information of the fornix is used to detect the anterior commissure (AC), which is the landmark that defines the origin of the Talairach coordinate system and will become the new center of the transformed data set. The figures below illustrate these steps.

Image Reporter:

Mid-Sagittal Plane (MSP)  Sigma smoothed MSP  Binarized MSP – iteration 2, threshold 170  Binarized MSP – iteration 3, threshold 150

Segmented Fornix  Remove CC to detect Fornix  Rotated CC  Segmented CC

The x- and y-axes of the Talairach coordinate system are defined by the horizontal plane, connecting the AC and the posterior commissure (PC). The latter is identified by detecting the brain stem.

Find Brainstem (BS)  Segmented BS  Estimated AC, PC, and posterior BS (red points)

4. After the AC and PC are specified, the 3D data set is translated and rotated into the AC-PC plane. This is indicated by a progress window, as shown on the right.
5. After a few seconds, the transformation procedure is completed, and the resulting new 3D volume has been computed and saved to disk as sub-01_ses-04_acq-nondistorted_T1w_HIHC _aACPC.vmr. In addition, it is shown automatically in a new window. The “aACPC” in the new file name indicates that the transformation was applied automatically and that the brain is located in the AC-PC plane. This can be seen clearly in the “TRA” view, which shows both the AC and PC point (see figure on the right).

In the second automatic step of Talairach transformation, the 3D data set has to be transformed from ACPC space into Talairach space by specifying eight landmarks within the AC-PC transformed data set: AC, PC, AP (the most anterior point of the cerebrum), PP (the most posterior point), SP (the superior point), IP (the inferior point), RP (the most right point) and LP (the most left point).

In order to identify those points, BrainVoyager cleans the background of the data set and removes non-brain tissue. Afterwards the image is binarized so that the background intensity is 0 (black) and the brain tissue has an intensity value close to 225 (white).

**Image Reporter:** Binarized slices

In a last step, BrainVoyager uses this binarized version of the original data set to detect the smallest and biggest coordinate for each axis of the coordinate system with an intensity value above 0, which corresponds to the following 6 landmarks:

- RP - smallest coordinate on x axis
- AP - smallest coordinate on y axis
- SP - smallest coordinate on z axis
- LP - biggest coordinate on x axis
- PP - biggest coordinate on y axis
- IP - biggest coordinate on z axis
All Talairach landmarks have now been specified. This information is used by BrainVoyager to change and fit the size of the brain to the size of the standard Talairach brain. This fitting procedure is done separately for 12 subvolumes, which are defined by the 8 landmarks. After a few seconds, the resulting new 3D volume has been computed and saved to disk. In addition, it is also automatically shown in a new window (see figure on the right). The name of the new file is “sub-01_ses-04_acq-nondistorted_T1w_ILHC_aTAL.vmr” and indicates that the data set is in Talairach space and that the automatic transformation procedure was used.

6. The center of the new Talairach data set is still the AC point and the brain is still located in the AC-PC plane. The cerebrum’s size is, however, adjusted to fit into Talairach space. This can be easily tested by showing the Talairach grid over the new data set. The grid is shown by default when the transformation process is finished. In the “Talairach tab” of the “3D Volume Tools” dialog, you can choose whether you want to see the full or the partial grid, or no grid at all. Switch to the “Talairach tab” of the “3D Volume Tools” dialog. Check the “Display full grid” item in the “Specification and visualization of Talairach landmarks” field.
7. Another option to verify that the cerebrum’s size is correctly adjusted to Talairach space is to review the location of the landmarks. To do this, select a landmark in the drop-down list of the “Specification and visualization of Talairach landmarks” field, for example “LP”. The white cross will immediately jump to the respective coordinates.

8. After Talairach transformation, the “3D Coords” tab also shows Talairach coordinates in addition to the system coordinates. You can test this by switching to the “3D Coords” tab and by clicking on any point in the data set.

In total 7 new files are saved to disk during Talairach transformation:

- sub-01_ses-04_acq-nondistorted_T1w_IIHC_ToMSP.trf -> mid-sagittal transformation information
- sub-01_ses-04_acq-nondistorted_T1w_IIHC_aACPC.trf -> ACPC transformation information sub-sub-
- sub-01_ses-04_acq-nondistorted_T1w_IIHC_aACPC.vmr -> data set in ACPC space
- sub-01_ses-04_acq-nondistorted_T1w_IIHC_aACPC.v16 -> 16bit data set in ACPC space
- sub-01_ses-04_acq-nondistorted_T1w_IIHC_aTAL.vmr -> list with the 8 Talairach landmarks sub-
- sub-01_ses-04_acq-nondistorted_T1w_IIHC_aTAL.v16 -> data set in Talairach space
- sub-01_ses-04_acq-nondistorted_T1w_IIHC_aTAL.trf -> 16bit data set in Talairach space

Remark: For details about the sub-steps performed during automatic Talairach transformation, please consult the User’s Guide.
Appendix E: Manual Talairach Transformation

In this step, we will learn how to transform a recorded 3D data set into Talairach space, which is a commonly used “standard” space for reporting locations of activated brain regions and for averaging data across subjects.

1. If you have closed BrainVoyager, start the program now. Open the file “sub-01_ses-04_acq-nondistorted _T1w_IHIC.vmr”.

2. Talairach transformation is performed in two major steps. In the first step, the cerebrum is translated and rotated into the AC-PC plane (AC = anterior commissure, PC = posterior commissure). In the second step, the borders of the cerebrum are identified; in addition to the AC and PC points, the size of the brain is fitted into standard space. These steps are performed in the “Talairach” tab of the “3D Volume Tools” dialog. Switch to the “Talairach” tab of the “3D Volume Tools” dialog and switch from “Automatic” to “Manual” AC-PC transformation.

   Note: The 3D data set used here is recorded as sagittal slices and thus fits into BrainVoyager’s scheme. Data sets recorded axially or coronally must be “standardized” first.

3. Click the “Find AC Point…” button in the tab of the “3D Volume Tools” dialog. The “3D Volume Tools” dialog will be replaced by the “Find AC Point” dialog. You can directly move the cross by clicking the spin controls of the “Find AC Point” dialog. The most convenient, but still precise navigation method is using the cursor keys, try the left, right, up and down cursor key, as well as SHIFT-UP and SHIFT-DOWN.

4. Now click the “OK” button in the “Find AC Point” dialog. The dialog will be replaced by the “3D Volume Tools” dialog and appropriate translation parameters (in the x, y, and z direction) will be specified for the AC-PC transformation. The display now also changes into a 2 x 3 arrangement. The upper row shows the original data set, while the lower row shows the data set after the application of the specified transformation.
**Note:** You can inspect the defined translation parameters in the “Coregistration” tab of the “3D Volume Tools” dialog. The values in the “Translation” field reflect the shifts \((x = 0.0, y = 14.0, z = 16.0)\) necessary to move the original center of the 3D data set to the AC point, which will become the new center of the transformed data set. If desired, you can fine-adjust the translation values further.

5. After having specified the AC point, the “Find AC Point” button is disabled and the “Find AC-PC Plane...” button is enabled in the “Talairach” tab of the “3D Volume Tools” dialog. The next task is to rotate the data set in the coregistration window (lower row) in such a way, that we also see the posterior commissure in the axial slice. To find the posterior commissure, click the “Find AC-PC Plane...” button. The “Find AC-PC Plane” dialog will appear on the right side of the “VMR” window.

6. Use the “x” spin control in the “Rotation” field to rotate the dataset in the lower row until the view shows the posterior commissure. The rotation is executed around the position of the green cross. Since the cross is located at the anterior commissure, it will remain visible in the coregistration window, despite the rotation.

7. The green cross can be used to adjust two additional angles, if necessary. Change the “y” and “z” angle to rotate the dataset in the coregistration window, so that the green cross runs through the middle of the brain. Now click the “OK” button in the “Find AC-PC Plane” dialog.

**Tip:** It might be helpful to temporarily hide the green and white cross. Deselect the “Show cross” item in the “3D Coords” tab. More conveniently, you can also press the “A” key to display or hide the green / white cross.

**Info:** The “Find AC Point” and “Find AC-PC Plane” dialogs provide a convenient way to specify the AC-PC transformation values. You can also do the same steps directly in the “Coregistration” tab. After you have placed the green cross on the AC, click the “Set Translation” button to fixate this point. If you click in the dataset afterwards, you can get back to the AC by clicking the “Center” button. Then adjust the rotation parameters by using the spin controls in the “Rotation” field on the “Coregistration” tab.
Appendix E: Manual Talairach Transformation

8. After having specified the AC-PC plane, the “Find AC-PC Plane” button is disabled and the “Transform to ACPC” button in the “AC-PC transformation” field of the “Talairach” tab is enabled. To save the specified transformation (translation and rotation values) and to apply them to the 3D data set, click the “Transform to ACPC...” button.

9. After clicking the “Transform to ACPC...” button a “Spatial Transformation of VMR” dialog opens automatically. BrainVoyager will suggest the filename “sub-01_ses-04_acq-nondistorted_T1w_IIHC_ACPC”, which we will keep. It is used to name both the spatial transformation (TRF) file as well as the resulting VMR file. The TRF file specifies a desired spatial (rigid body) transformation, while the resulting VMR file is the result of the application of the spatial transformation. In order to save the TRF file and to create the resulting AC-PC aligned VMR file, click the “GO” button.

The specified spatial transformation is now applied to the data set. After a few seconds, the resulting new 3D volume has been computed and saved to disk. In addition, it is also automatically shown in a new window. The center of the new data set is now the AC point, and the brain is in the AC-PC plane. This can be seen clearly in the “TRA” view, which shows both the AC and PC point (see figure on the right).
10. In the second step of Talairach transformation, eight landmarks have to be specified within the AC-PC transformed data set: AC, PC, AP (the most anterior point of the cerebrum), PP (the most posterior point), SP (the superior point), IP (the inferior point), RP (the most right point) and LP (the most left point). Switch to the “Talairach” tab of the “3D Volume Tools” dialog.

11. Click on the “Talairach landmarks” list box in the “Specification and visualization of Talairach landmarks” field. In the opened list box, you will see some landmarks and a spin control. You can use the spin control to navigate to a desired landmark. The first landmark is “AC”, which does not need to be specified again. Select the “PC” landmark. This point is easy to find in the data set, because it must be in the same axial plane as “AC”. Note that BrainVoyager automatically jumps to a location in the vicinity of the PC. Press repeatedly SHIFT-DOWN to move the white cross from the AC point to the PC point (see figure on the right).

12. Click the “Set point” button to define the “PC” point as the current location of the white cross.

13. Select the “AP” landmark. Click the “Set point” button to define the “AP” point as the current location of the white cross.

Tip: Although instructive, it is not necessary to set the AP point precisely in all dimensions. The only critical dimension is the y-axis or anterior-posterior axis. The z-coordinate (superior-inferior dimension) and the x-coordinate (left-right dimension) are irrelevant. This holds true also for all remaining points - only one dimension is critical.

Note: This is also the reason why Talairach transformation is performed in two steps: The AC-PC rotation step has already matched the three orthogonal axes between the original data set and the final Talairach space, allowing to specify the landmarks, as described. It would be much harder to find the borders of the cerebrum correctly in an obliquely rotated brain.
14. **Select** the “PP” landmark. **Click** the “Set point” button to define the “PP” point as the current location of the white cross.

**Note:** You can easily verify and adjust the location specified for any landmark. To do this, **select** a landmark in the landmarks list, for example “AP”. The white cross will immediately jump to the respective coordinates. If you adjust the location of the landmark, do not forget to **click** the “Set point” button to accept the new coordinates.

15. **Select** the “SP” landmark. **Click** the “Set point” button to define the “SP” point as the current location of the white cross.
16. Select the “IP” landmark. Click the “Set point” button to define the “IP” point as the current location of the white cross.

17. Select the “RP” landmark. Please note that in BrainVoyager, MRI images are displayed in radiological convention. This means that LEFT is RIGHT and RIGHT is LEFT! Therefore, you need to locate “RP” on the left side. In radiological convention, BrainVoyager shows an “R” symbol on the left side of the “COR” and “TRA” view to remind you of the reversal of left and right. After having located “RP” on the left side, click the “Set point” button.
Appendix E: Manual Talairach Transformation

18. Select the “LP” landmark on the right side of the cerebrum (see remarks above). After having located “LP”, click the “Set point” button.

![Image](image.png)

19. Now all Talairach landmarks have been specified. Since we will need this information later to transform our functional data into Talairach space, we save the list of defined landmarks to disk. Click the “Save .TAL...” button. In the appearing “Save As” dialog, enter “sub-01_ses-04_acq-nondistorted_T1w_ILHC_ACPC.tal” and click the “Save” button.

![Image](image.png)

20. Up to now, we have only specified the landmarks, but the cerebrum is still in AC-PC space. To “warp” it finally into Talairach space, click the “ACPC -> TAL...” button. In the appearing “Talairach Transformation” dialog, make sure that the name “sub-01_ses-04_acq-nondistorted_T1w_ILHC_TAL.vmr” appears in the “File name” box within the “Resulting .VMR file” field, and click “GO”.

![Image](image.png)

Note: The specified landmarks are now used to change the size of the brain in such a way that it fits into the size of the standard Talairach brain. This fitting procedure is done differently for 12 subvolumes, which are defined by the 8 landmarks. After a few seconds, the resulting new 3D volume has been computed and saved to disk. In addition, it is also automatically shown in a new window.
21. The center of the new Talairach data set is still the AC point and the brain is still located in the AC-PC plane. The cerebrum’s size is, however, adjusted to fit into Talairach space. This can be easily tested by showing the Talairach proportional grid over the new data set. Check the “Display partial grid” item in the “Specification and visualization of Talairach landmarks” field. The appearing grid is a reduced version of the full proportional grid, which you can also show by checking the “Display full grid” item.

22. The “3D Coords” tab also shows Talairach coordinates in addition to the system coordinates. You can test this by switching to the “3D Coords” tab and by clicking on any point in the data set.

Remark: Please note that you might have defined slightly different landmarks for the Talairach transformation compared to the automatic definition of the landmarks. Therefore, you might notice some small, but negligible differences in the Talairach coordinates of significant voxels, when you move on in the Getting Started Guide from this point.
Appendix F: Data Analysis Manager

A new addition to BrainVoyager is the so-called Data Analysis Manager. The aim of this tool is to create and preprocess multiple data sets at once. More information about how to use this tool can be found in the User’s Guide.
Appendix G: Defacing the VMR

To satisfy data privacy and protection laws, e.g. the EU General Data Protection Regulation (GDPR), it may become necessary to remove facial features from the 3D anatomical dataset (VMR and surface mesh) – this will make sure nobody will be able to identify the subject from any of the data representations within BrainVoyager.

From BrainVoyager version 21 on, we offer a “defacing” option for the VMR. Alternatively, users may already apply a defacing operation to their raw data if it is stored in DICOM format.

1. The “deface” option can be found in the “File” menu.

2. Open the file “sub-01_ses-04_acq-nondistorted_T1w.vmr”, created in step 9.
Appendix G: Defacing the VMR

3. Click the “Deface VMR” entry in the “File” menu and start the procedure. After several automatically applied steps, we receive a new VMR called “sub-01_ses-04_acq-nondistorted _T1w_defaced.vmr”) with all facial features removed.

4. We test the result of the defacing in surface space by repeating the head mesh creation demonstrated in step 16.

5. In the next screenshot, we compare the result of the head mesh reconstruction before (left side) and after (right side) the defacing.
Appendix H: Summary of Keyboard Shortcuts

(different shortcuts for Mac OS X are indicated in blue)

**Keyboard and Mouse Functions in FMR documents**

**Invoking time course plots**

- **Left-drag**
  Shows time course plot for the selected region-of-interest in *current* time course plot window (only voxels above threshold).

- **CTRL + Left-drag**
  Shows time course plot for the selected region-of-interest in a *new* time course plot window (only voxels above threshold).

- **SHIFT + Left-drag**
  Adds time course of selected region-of-interest to *current* time course plot window (only voxels above threshold).

- **CTRL + R**
  Saves or loads a region-of-interest.

**Keyboard and Mouse Functions in VMR-VTC documents**

**Invoking time course plots**

- **CTRL + Left-click**
  Shows time course plot for the selected region-of-interest in *current* time course plot window.

**Display options**

- **‘A’ key**
  Shows / hides the cross marking the current voxel.

  - **CTRL + ‘A’ key**
    Hides a statistical map, if present.

- **CTRL + Cursor Down**
  Cycles through an enlarged SAG, COR, TRA and the standard 4-window view.

  - **CMD + Cursor Down**
    (multiple times)

**Translations and Rotations**

- **Cursor LEFT**
  Moves cross along the X axis to lower system coordinate values (changes sagittal slice plane).

- **Cursor RIGHT**
  Moves cross along the X axis to higher system coordinate values (changes sagittal slice plane).

- **Cursor UP**
  Moves cross along the Z axis to lower system coordinate values (changes axial slice plane).

- **Cursor DOWN**
  Moves cross along the Z axis to higher system coordinate values (changes axial slice plane).

- **SHIFT + Cursor UP**
  Moves cross along the Y axis to lower system coordinate values (changes coronal slice plane).

- **SHIFT + Cursor DOWN**
  Moves cross along the Y axis to higher system coordinate values (changes coronal slice plane).
Appendix H: Summary of Keyboard Shortcuts

Two VMR data sets

‘F8’ key  
Toggle between primary (‘<’)and secondary (‘>’) VMR. In 3D-3D fusion mode, secondary VMR shows fused image (‘<>’ or ‘<=>’).

‘F9’ key  
Cycle through fusion mode 1 (‘<>’), fusion mode 2 (‘<=>’) and standard non-fused view of VMRs.

Keyboard and Mouse Functions in the 3D Viewer

Mouse-driven translations and rotations

SHIFT + Left-Move  
Translate camera horizontally and vertically moving the mouse left / right and up / down.

CTRL + SHIFT + Left-Move  
Zooming. Move the mouse down (up) to move the camera towards (away) from mesh.

CMD + SHIFT + Left-Move  
Rotate around object’s Z axis (screen’s Y axis) by moving the mouse left / right (up / down).
## General Keyboard Functions

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