PMOD Neuro Tool (PNEURO)

USER MANUAL
Version 4.3

PMOD is a software
FOR RESEARCH USE ONLY (RUO)
and must not be used for diagnosis or treatment of patients.

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1 PMOD Neuro Tool Introduction (PNEURO)

Precise knowledge of the functional brain areas in the individual subject is crucial for the accurate quantitative analysis of brain PET images and their meaningful interpretation. Often however, functional regions are manually outlined in a casual manner, with subjective results as the outcome. This fundamental flaw can be overcome with PMOD's PNEURO tool which offers both objective region outlining as well as pattern analysis methods via easy step-by-step procedures. These methods are provided as three modules on separate pages of the user interface.

Automatic Brain Regions by Human Maximum Probability Atlas and other Atlases

A well established way of obtaining brain volumes of interest (VOIs) automatically is by leveraging the most likely localization of brain areas as encoded in the maximum probability atlas N30R83 constructed by Hammers et al. [1]. The corresponding PNEURO component allows the user to adjust the atlas to the subject brain anatomy with a spatial normalization procedure, preferably obtained from the T_1-MR image, and the resulting VOI set that can be masked by the individual subject grey matter. Alternatively, the normalization can be directly derived from the PET image, making this solution also applicable for PET-only studies.

Automatic Human Brain Regions by T1-MRI Parcellation

It is desirable that every brain PET study be complemented with a T_1-weighted MRI which precisely represents the subject's brain anatomy. The PNEURO tool includes a sophisticated component which applies knowledge-base technology to accurately segment the cortex and the basal ganglia from the T_1-MRIs. These segments are then converted to VOIs which can be projected to the PET images and used for regional statistics.

Parametric Mapping

If the pixel-wise modeling tool PXMOD has been licensed, PNEURO supports a Parametric Mapping 113 workflow after VOI outlining has been completed. The integration has several advantages: (1) The VOIs generated by PNEURO can be used during the PXMOD model configuration. (2) The resulting parametric maps can immediately be regionally analyzed. (3) The parametric maps can be calculated in an image space or the atlas space.

Normal Brain PET Databases

PET studies with diseased subjects can rarely be done in a fully quantitative manner. Rather, a static image of the PET tracer concentration is acquired after an appropriate equilibration time. In this situation, an image analysis is based on the fact that with consistent study protocols, the normal brain uptake exhibits a characteristic uptake pattern. The database component in the PNEURO tool readily allows for pooling the uptake across a set of normal volunteers, and thus establishing the normal pattern together with its variability. Once such a normal database has been created, the anomalies of the tracer uptake in a subject's brain can be easily localized without any prior assumptions or operator variability and presented as a z-score map.

System Requirements

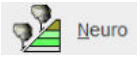
For productively working with the parcellation tool, the following workstation system requirements should be met:

- 64-Bit operating system (Windows, Mac OS X, Linux)
- ≥ 32 GB RAM
- ≥ 8 processing cores (hyper-threading is also viable)

16 GB RAM and 4 cores is at the edge of practicability.

Starting the PNEURO Tool

The PNEURO tool is started with the **Neuro** button from the PMOD ToolBox



or by directly dragging image files onto the above button.

Organization of the PNEURO User Interface

The user interface of PNEURO consists of five pages which can be selected by tabs:

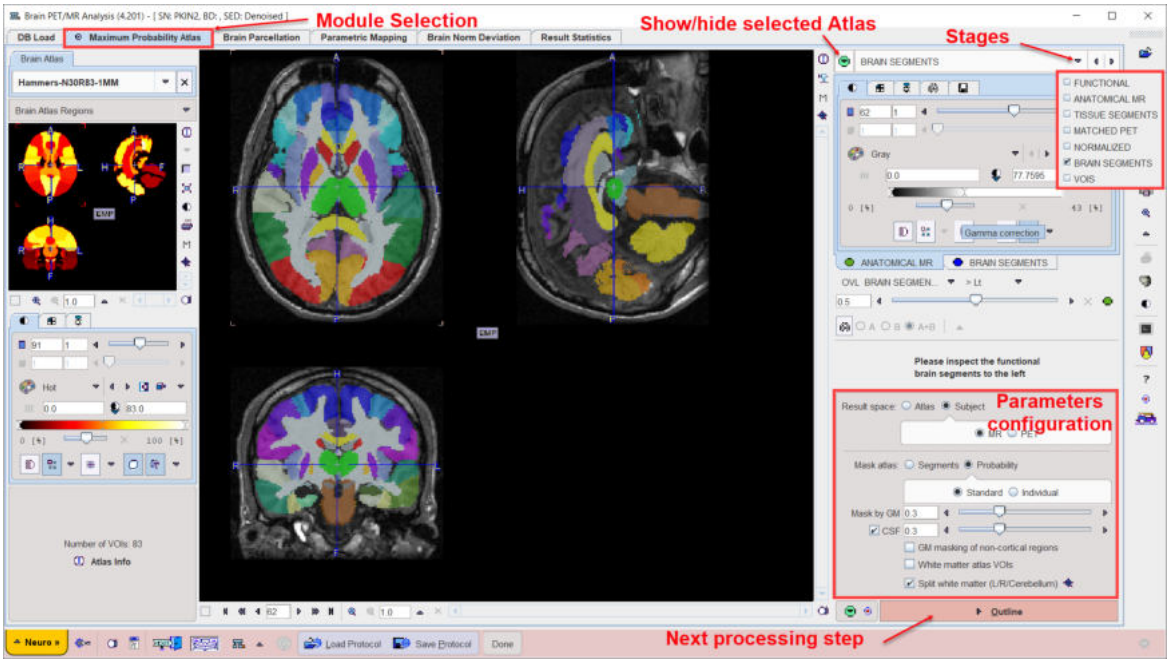
1. **DB Load** page: Loading of images from a PMOD database, making them available for processing in all modules of PNEURO. This page is not shown if the database functionality is disabled. The images may also directly be loaded in the different modules.
2. **Maximum Probability Atlas** page: Creation of brain VOIs by adjusting the N30R83 maximum probability atlas or other atlases to the subject anatomy.
3. **Brain Parcellation** page: Creation of brain VOIs by knowledge-based segmentation of anatomical T_1-MR images.
4. **Mapping** page: When the PXMOD Parametric Mapping Tool has been additionally licensed, parametric mapping may be directly accessed for dynamic PET data processed in the Maximum Probability Atlas or Brain Parcellation workflows.
5. **Compare to Norm** page: Comparison of a static PET image to a Brain Norm. Brain Norms can be created in PNEURO from a set of images acquired with normal controls using the **Edit Norm** tool from the menu.
6. **Statistics** page: The VOIs created by the two methods above can directly be applied to the (dynamic) PET images for calculating the regional uptake, optionally with a partial volume correction. The resulting statistics or curves are shown on the **Statistics** page and from there can be saved or submitted to the kinetic modeling tool.

The user selects the appropriate module for data processing by the corresponding tab in the user interface of PNEURO. Each of the pages is described in a separate section of this guide.

2 Brain VOI Tools: Common Features

2.1 User Interface

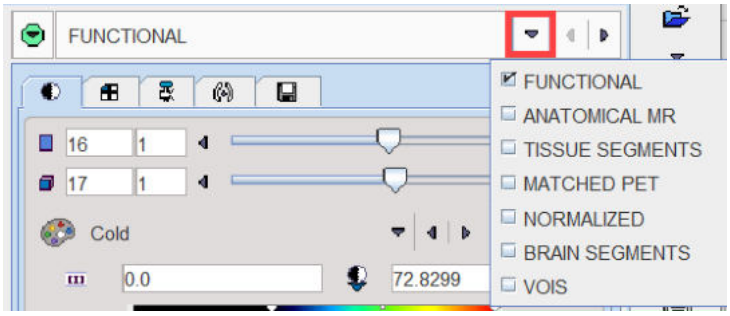
The layout of the pages for the Brain VOIs calculation are illustrated below using the N30R83 **Maximum Probability Atlas.**



The main part is used for displaying the images with some overlay information. The control area is located to the right, and an optional part related to templates is shown to the left. The template area is shown/hidden by the indicated green button in the upper right.

Step-Wise Processing

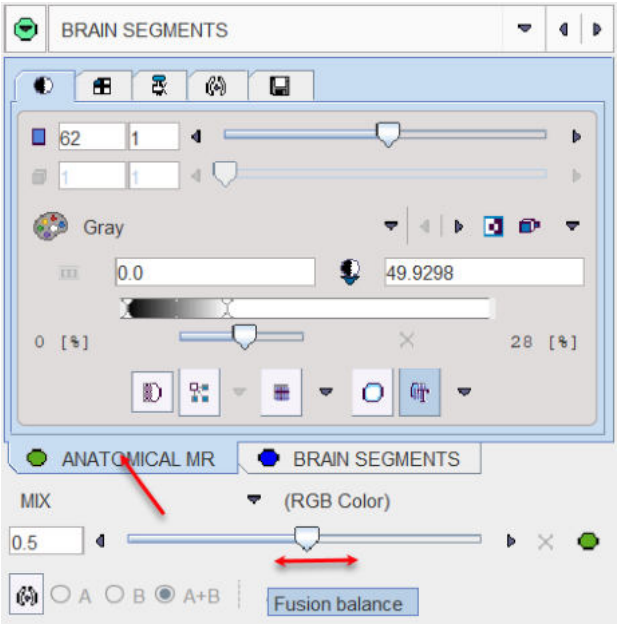
Data processing is consistently organized by a stepwise progression towards the end result. On each processing stage the user has to take some action such as data loading, alignment inspection or parameter configuration, and then start the next processing step with the red action button located in the lower right. As soon as the result is calculated, it will be shown on a new page representing the new processing stage. The cascade of stages is available by the selection area in the upper right.



It conveniently allows inspecting the results of prior stages without initiating any calculations. To repeat a calculation with modified parameters, the action button in the lower right has to be activated again on the actual and all following pages.

Fusion Image Display

The display of the images is controlled in the upper right. In many cases more than one image contributes to the display. In these cases the tab corresponding to an image has to be first activated, before its color table or the color thresholds can be modified. The fusion control section is located below the image control tabs. In the configuration illustrated below the colors of both images are mixed, whereby the weighting can be changed with the slider.



Convenience Buttons

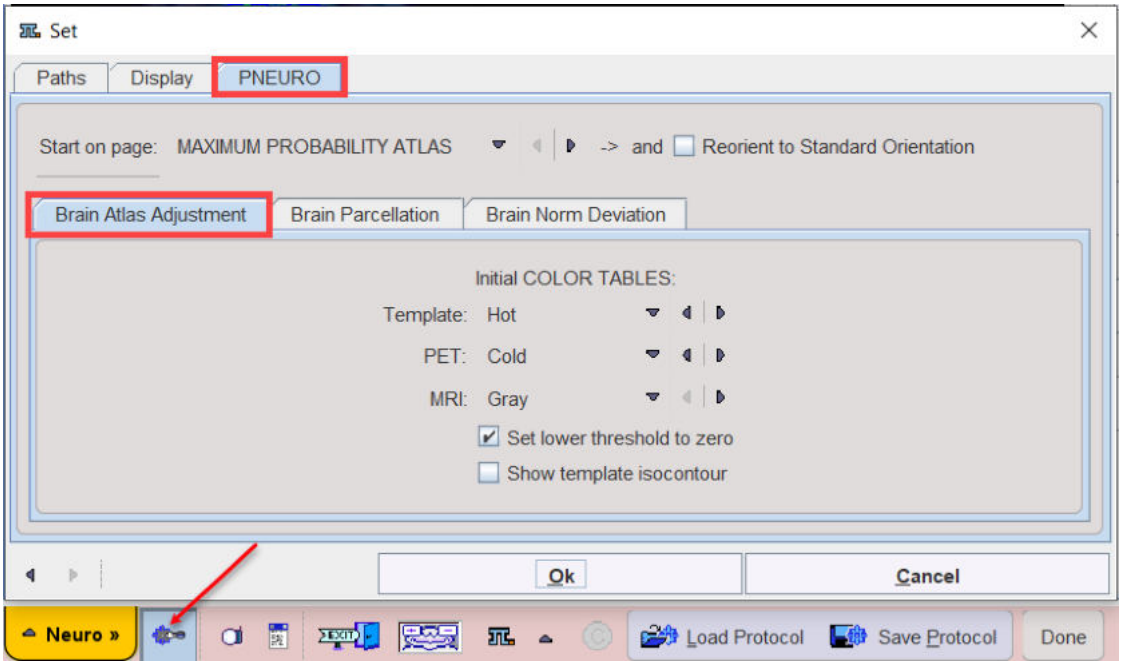
Next to the action button in the lower right is an area with two buttons

offering the following functions:

	Hide the parameters panel to free some space in the user interface. With the panel hidden, the icon changes to . When this button is activated, the panel is shown again.
	Reset the parameters on the page to their default values. If the button in the taskbar to the right is activated, the defaults are reset on all pages.

Configurations

The PNEURO tool can be configured according to user preferences in a dialog window as illustrated below.



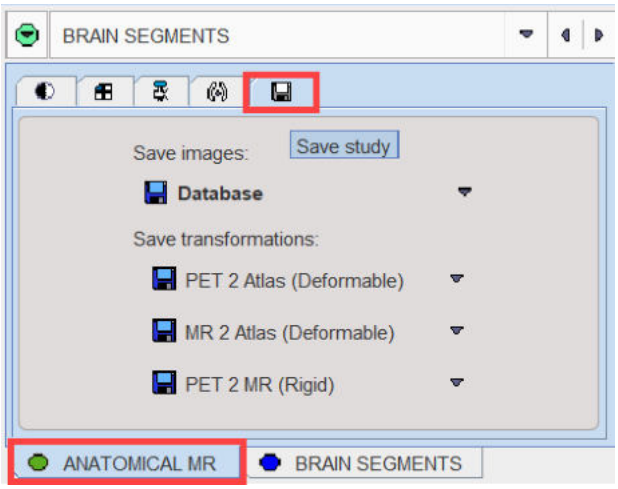
The common configurations are available on the **Paths** and **Display** tabs, and in the upper part of the **PNEURO** tab. Note the **Reorient to Standard Orientation** box. If it is checked, PNEURO tries to orient the brain images such that they appear in the radiological Head First Supine (HFS) order with subject left on the image right. For instance, MR images acquired in sagittal orientation will automatically be reformatted and presented with axial slices. The correct HFS orientation of the data after loading is important for the automatic procedures to work properly.

Further Information

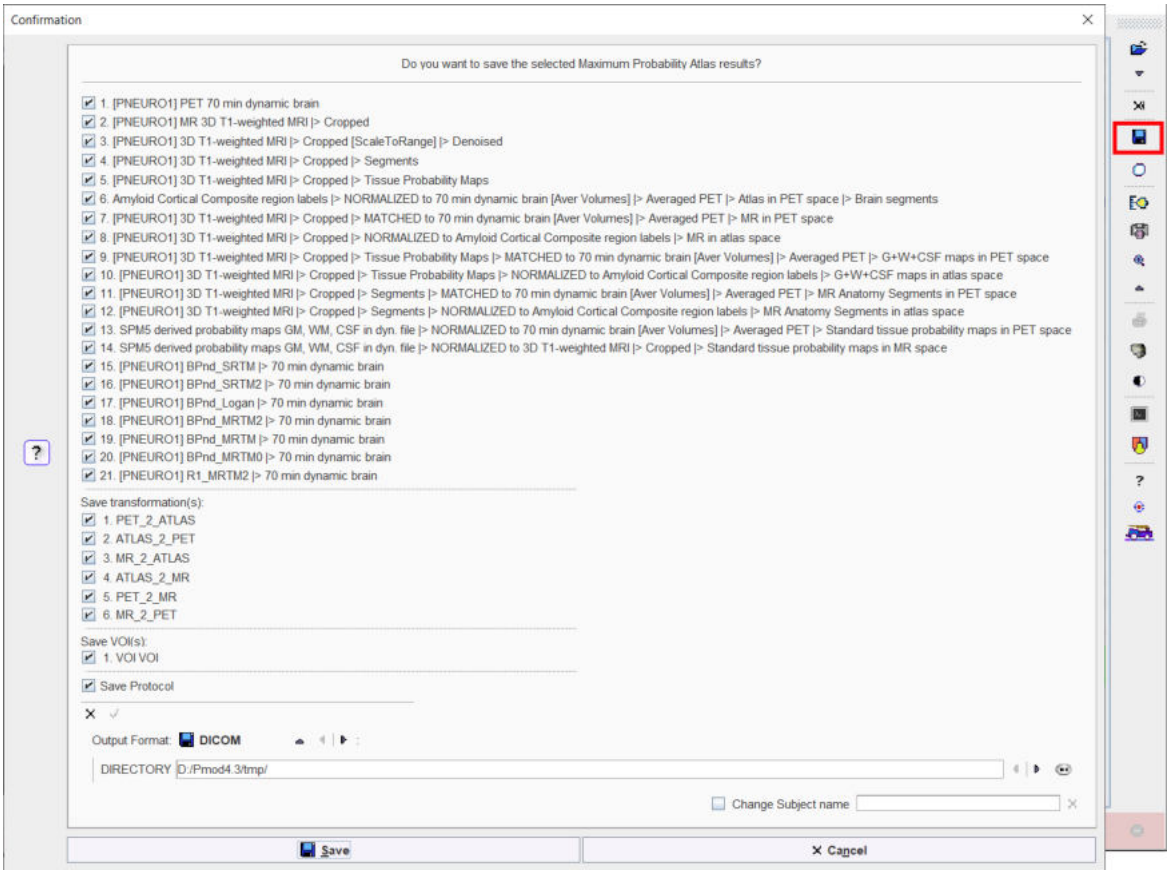
This guide is focused on the brain analysis functionality. Please refer to the *PMOD Base Functionality Guide* for details about general functions such as data loading, image display, and VOI definition.

2.2 Saving Intermediate Results

Intermediate results such as transformed images and the various spatial transformations can be saved from the dedicated saving panels.



The complete set of intermediate information for a Brain VOI processing can be saved at once using the Save all button from the lateral taskbar.



This information may be valuable in order to further exploit the outcome in the viewing and fusion tools.

2.3 Protocols

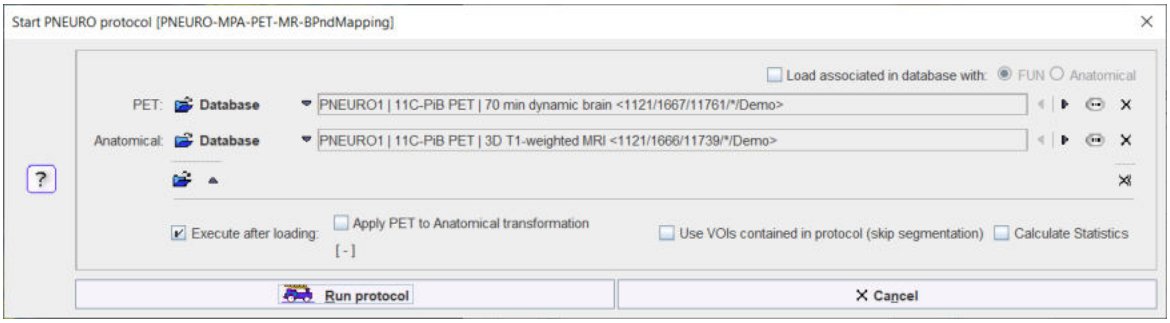
Each module of PNEURO allows saving the final processing configuration as a protocol.

Such a protocol includes definition of the input data as well as the parameters of the different processing stages, including parametric mapping. The user is advised to save a protocol after every completed data processing workflow, so that at any later time the configuration can be retrieved, verified and modified to try variations of the processing parameters.

Note: If parametric mapping should be part of the protocol, page **Parametric Mapping** must be active when saving the protocol.

Protocol Execution

Note that there are differences in the execution of protocols. When loading a **Maximum Probability** protocol a dialog window offers several options.



If **Execute after loading** is enabled, not only the configuration is retrieved, but the processing is also performed, including the MR segmentation. The **Apply PET to Anatomical transformation** allows using a saved transformation instead of performing the actual matching calculations. Either the transformation saved with the protocol can be used (default), or an external transformation file can be loaded. If the **Use VOIs contained in protocol (skip segmentation)** option is enabled, the outlining is not performed, but rather the saved VOIs are retrieved. This has the advantage that any manually adjusted VOIs are recovered. Please note that manual adjustment of the transformation is not possible when VOIs from the protocol are used. With **Calculate Statistics** the VOIs will be applied to the data at the end of the protocol.

When loading a **Parcellation** protocol, the same options are shown. However, note that when the **Use VOIs contained in protocol** option is enabled neither the segmentation nor the parcellation is performed. Consequently, a parcellation protocol will be executed faster than a **Maximum Probability** protocol.

VOIs and Protocol Execution

In case of the step by step interactive procedure the regions for which the atlas description is set to H will NOT be selected on the **Group** tab. The VOIs definitions and settings on the **Group** tab will be store into the protocol.

- If **Execute after loading** is enabled but the **Calculate Statistics** box disabled the processing ends up on the VOIs page with the settings available at the moment of protocol saving. The VOIs outlining is performed as part of the protocol processing.
- If **Execute after loading** and **Calculate Statistics** boxes are enabled the statistics results will be returned for all VOIs, independently of the settings on the **Group** tab at the moment of the protocol saving. The VOIs outlining is performed as part of the protocol processing. This behavior was enforced for the batch processing to return the results for all the corrected regions.
- If **Execute after loading** and the **Use VOIs contained in protocol (skip segmentation)** option is enabled the saved VOIs from the protocol are retrieved. Outlining of the VOIs is not performed anymore. This has the advantage that any manually adjusted VOIs are recovered, while restoring the selection settings on the **Group** tab at the moment of protocol saving.

2.4 Run All

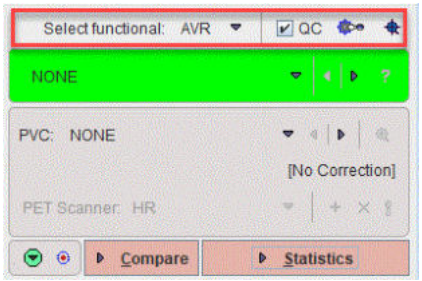
If the desired workflow has proven to be reproducible (i.e. equivalent VOIs can be generated with a stable set of configuration parameters), there is no need to interactively step through the different stages. Rather, the data can be loaded, all processing steps performed unattended, and then the results inspected and saved. To do so please proceed as follows:

1. Make sure the parameters on the all stages are set appropriately. This can also be ensured by loading a specific protocol file.
2. Load the PET image, and define the crop box if necessary.
3. If there is an MR as well, load the image, and define the crop box.

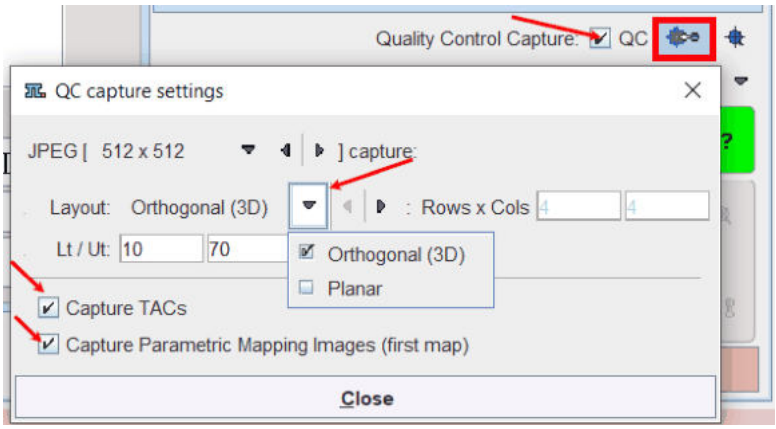
4. Activate the "Run All" button from the lateral taskbar. All steps up to the VOI outlining will be performed.
5. Inspect the relevant intermediate results such as MR segmentation, spatial normalization, rigid matching to ensure the resulting VOIs are meaningful.
6. Save the VOIs and calculate the statistics.
7. Save a protocol file.

2.5 Quality Control Capture

The **Maximum Probability Atlas** and the **Parcellation** workflows in PNEURO allow a quality check preview to be configured on the VOIs page. The functionality is easily accessible above the mapping and PVC pane as illustrated below:



With the **QC** box enabled the configuration button becomes active allowing further setting for the capture:

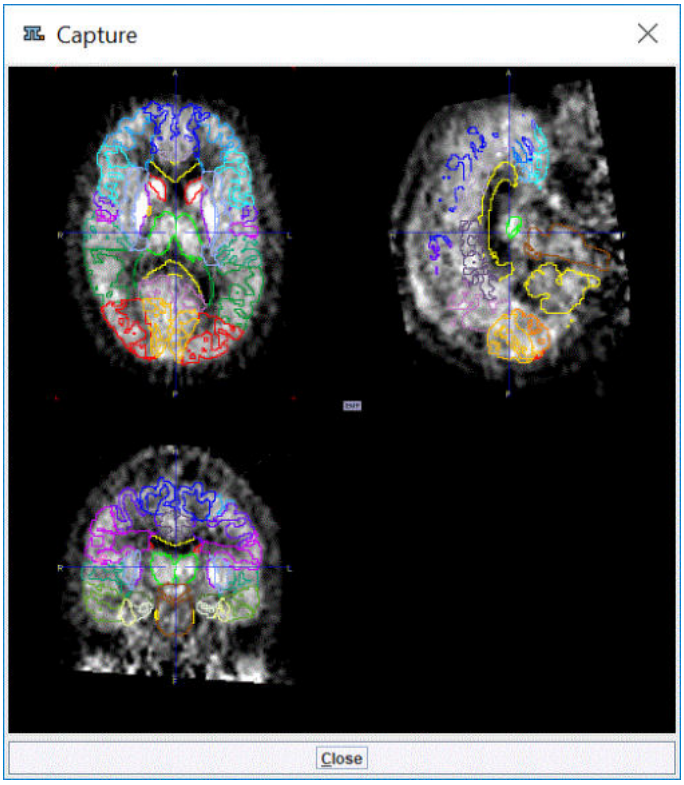


The size of the capture is set under the **JPEG** selection. The recommended **Layout** is the **Orthogonal (3D)** selection. The **Planar** selection requires further definition for the number of rows and columns in the **Rows X Cols** fields.

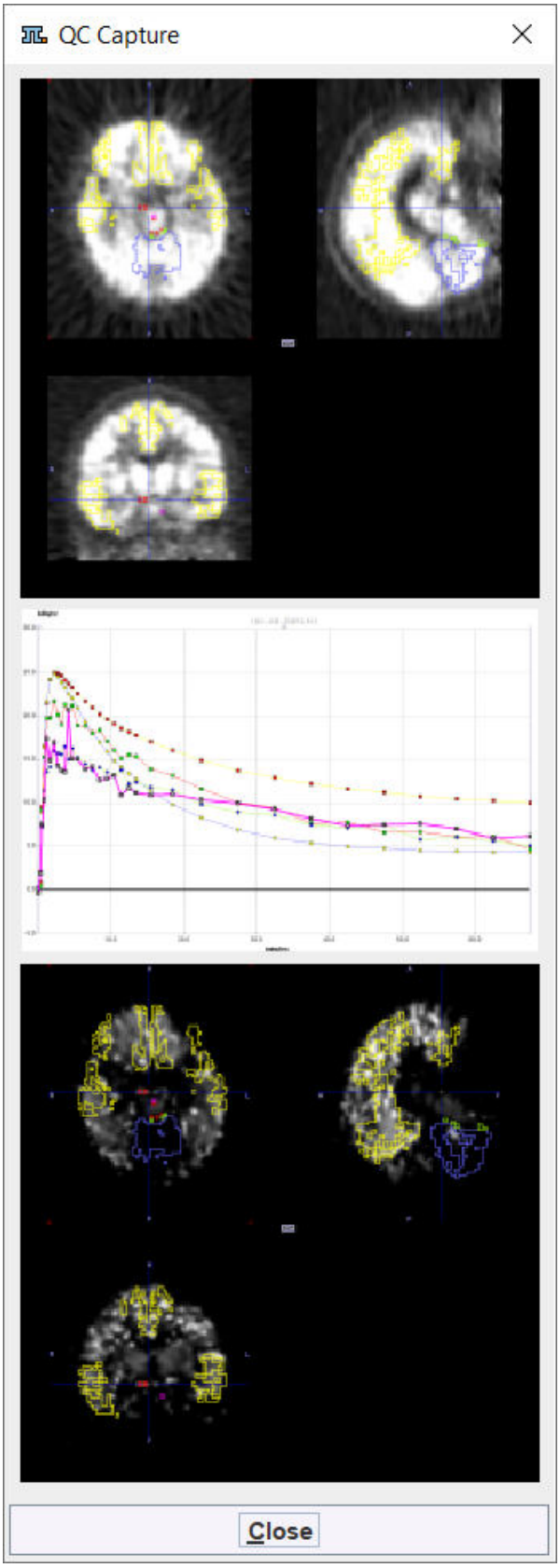
Enable the **Capture TACs** and **Capture Parametric Mapping Images (first map)** boxes to save QC captures for TACs and parametric maps when available.

The Gray color table is used for the JPEG capture. The appearance of the JPEG capture can be optimized by setting appropriate percentage thresholds from the lower and upper (**Lt/Ut**) values of the color table.

The preview button allows a quick inspection of the VOIs overlaid on the **Select functional** image in all planes when the **Orthogonal** layout is used:



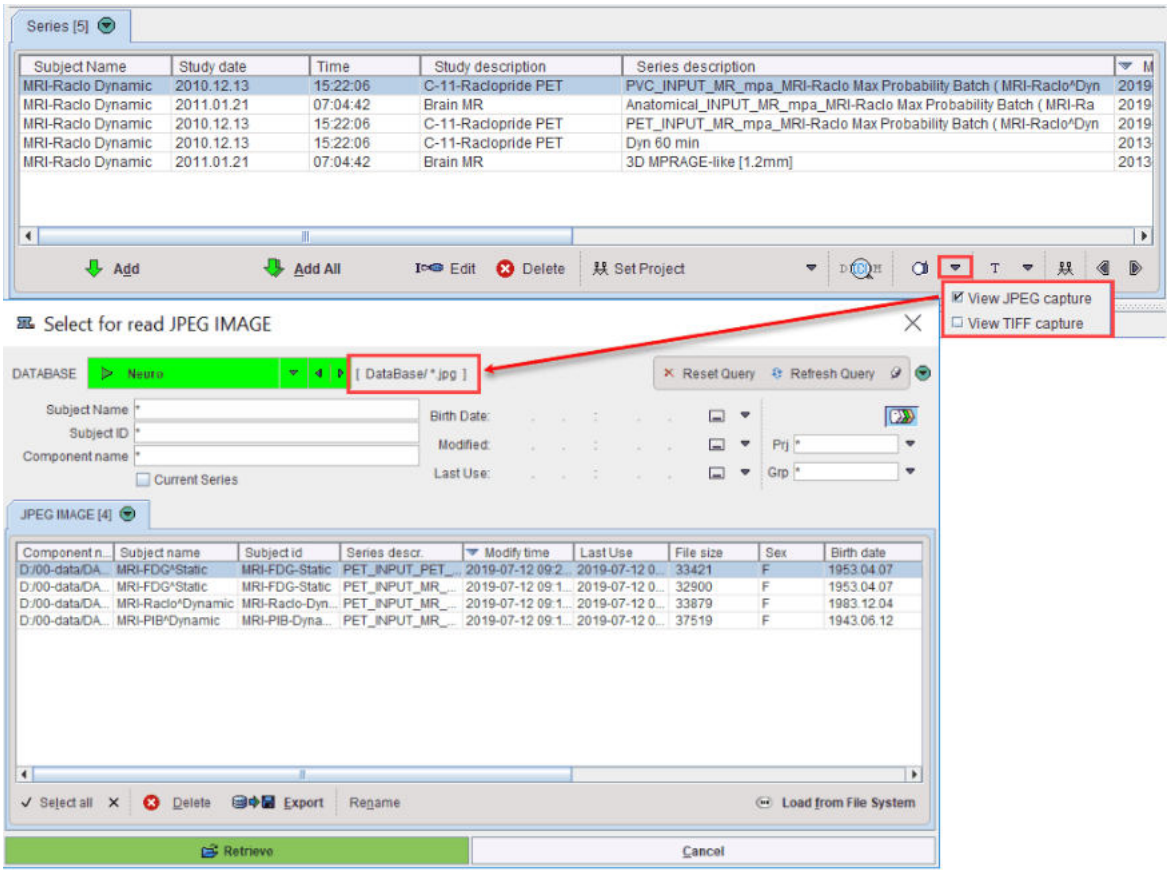
The QC capture content preview of a mapping protocol is illustrated below:



The top panel displays the VOIs overlaid on the **Display functional** image, the mid panel shows the VOIs TACs and the bottom panel displays the VOIs overlaid on the first parametric map in the list.

The **QC** capture settings are stored in the protocol file when the box is enabled. This is relevant for the batch processing: it allows the user to quickly inspect the outcome of the matching procedures, in the selected result space, at the end of the batch processing run.

The JPEG images stored in the database are easily accessible using the **View capture** facility available in the DB Load page on the bottom of the **Series** selection section, as illustrated below:



2.6 Batch Mode

The PNEURO **Batch Mode** menu has two entries:

1. **Maximum Probability Atlas and Brain Parcellation (Brain VOIs)**: this option starts a utility for running a set of pre-configured processing tasks which have been saved as .pbrainProt protocol files.
2. **Compare to Norm**: offers a batch facility which is useful when several studies need to be compared to a brain norm.

2.6.1 Batch Mode Maximum Probability Atlas & Brain Parcellation (Brain VOIs)

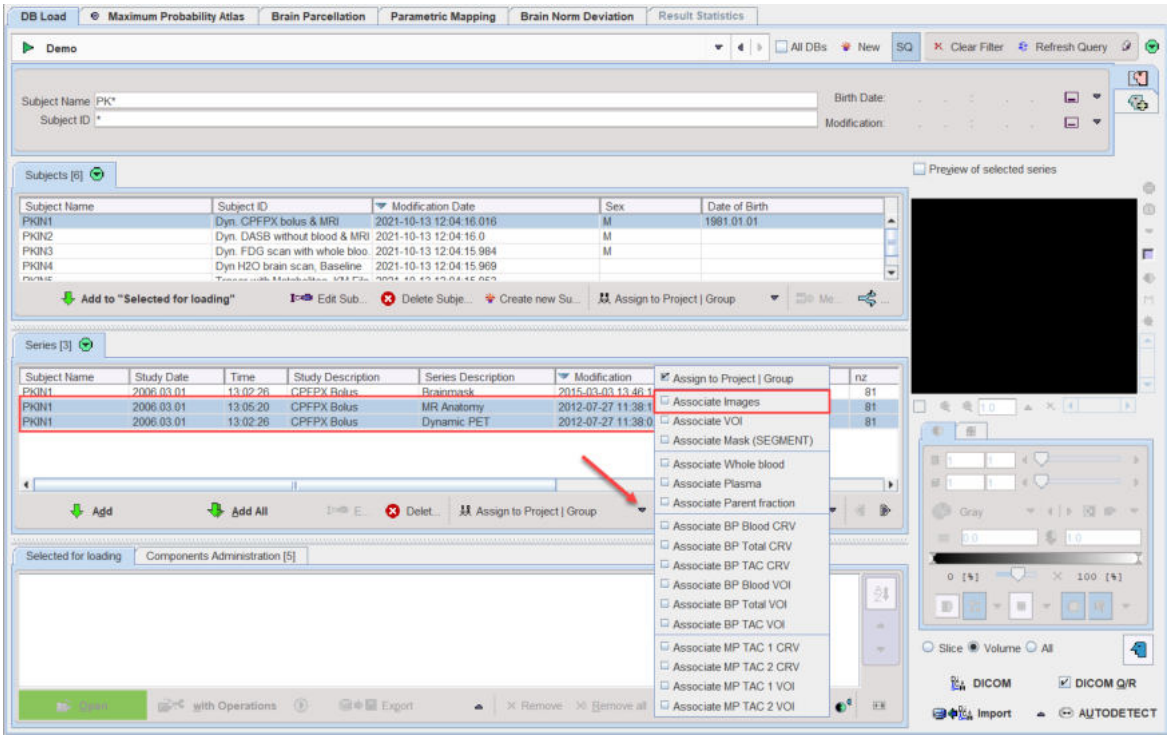
Assumptions and Recommendations

PMOD includes a powerful **database** for the storage and organization of all kinds of relevant data. It is highly recommended that the data for Brain VOIs batch processing is organized in such a database. This will allow the user to take advantage of association between data elements (e.g. identification of PET/MR paired series) which can only be efficiently provided by a database. Furthermore, the output produced during batch processing can directly be inserted at the appropriate database level, which is more difficult when working with directory structures.

Association of MR and PET Series

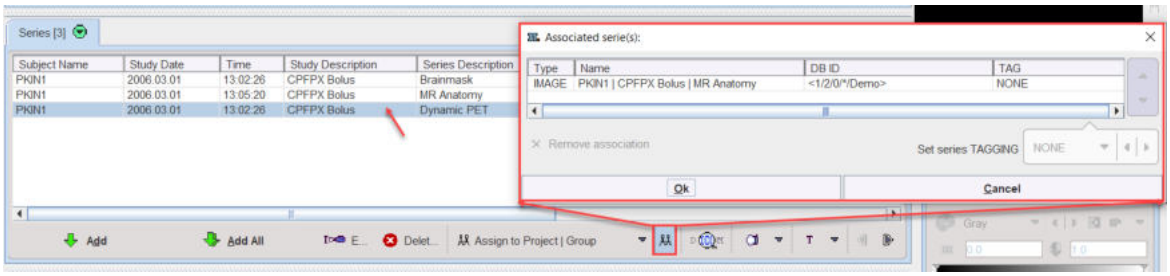
The PMOD database alows the user to define a relationship between images, and other components, called "association". For the analysis of hybrid data such as PET and MRI it is convenient to associate the two image series, because thereafter it is sufficient to select only one of the images in a workflow that will utilise both.

Association of image pairs can be performed on the **DB Load** page of PNEURO as illustrated below.



Select a subject in the **Subjects** list. In its **Series** list select the two image series to be associated. Finally open the **Set Project** option menu and activate the **Associate Images** entry to establish the mutual relation between the image series. This operation has to be repeated for all subjects to be processed. It is a one-time action which however can be taken advantage of in multiple analysis procedures.

The image(s) associated with a given series can be reviewed using the List Associations button:

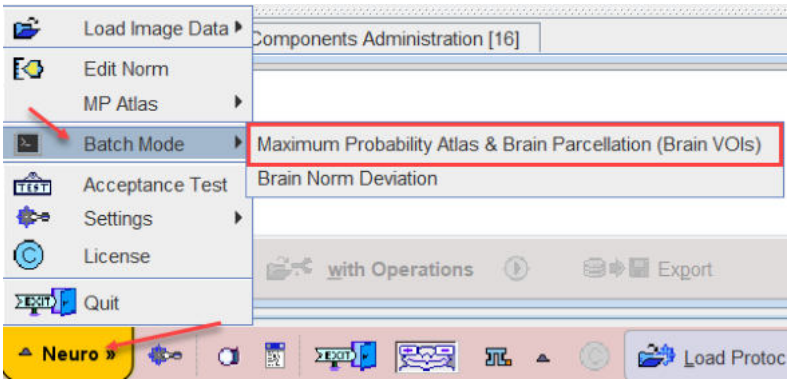


Batch Process Overview

The basic concept of the batch mode is the sequential processing of saved protocol files. Such protocols can be created by loading the data sets (PET and/or MR), defining crop boxes and crop the images, adjusting the processing parameters on the different pages, and saving a protocol file. An alternative is to prepare a master protocol file which can be applied for generating individual protocol files for a whole set of (associated) input data. This procedure is described below.

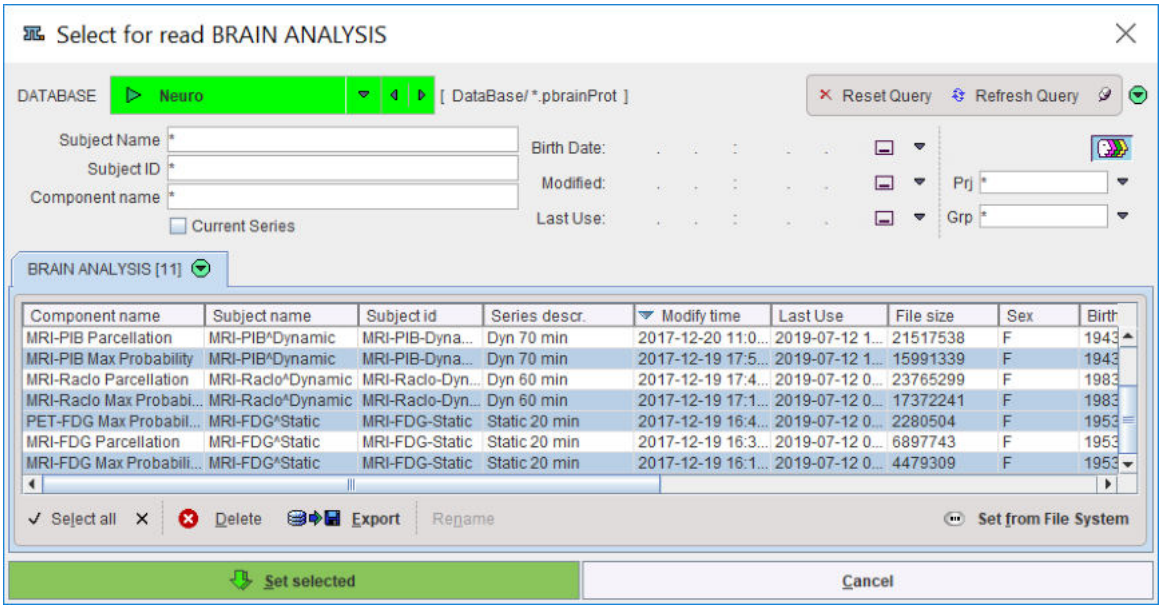
Starting the Batch Brain VOIs Procedure

Use the menu entry **Batch Mode/Maximum Probability Atlas & Brain Parcellation (Brain VOIs)**



or the button from the lateral task bar to start the batch operation.

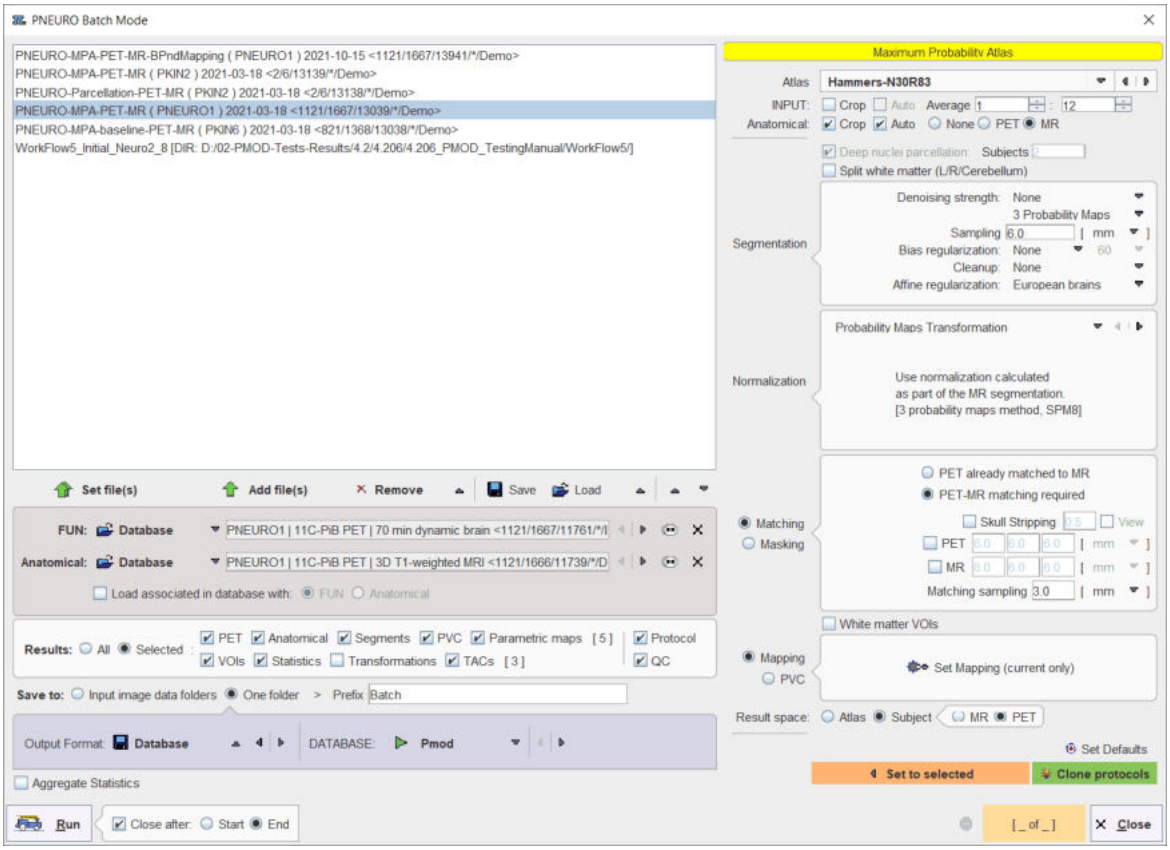
First, a dialog window appears for selecting existing PNEURO protocol(s) (**.pbrainProt**) from a database. Select the appropriate protocols and activate **Set selected**. Use **Set from File System** to load protocols from outside the database.



Note that the method for the VOI generation is defined from the protocol and can't be changed, only its parameters

The Batch Brain VOIs Interface:

The interface of the Batch Brain VOIs is illustrated below:



The window has four areas, which are described below.

Protocols List

The list of the protocols which will be processed is displayed in the upper left. Additional protocols can be added with the **Add file(s)** button, and selected entries removed from the list by **Remove**. When an entry is selected in the protocols list, its data definition is updated in the **FUN** and **Anatomical** text field, and its processing parameters are shown to the right.

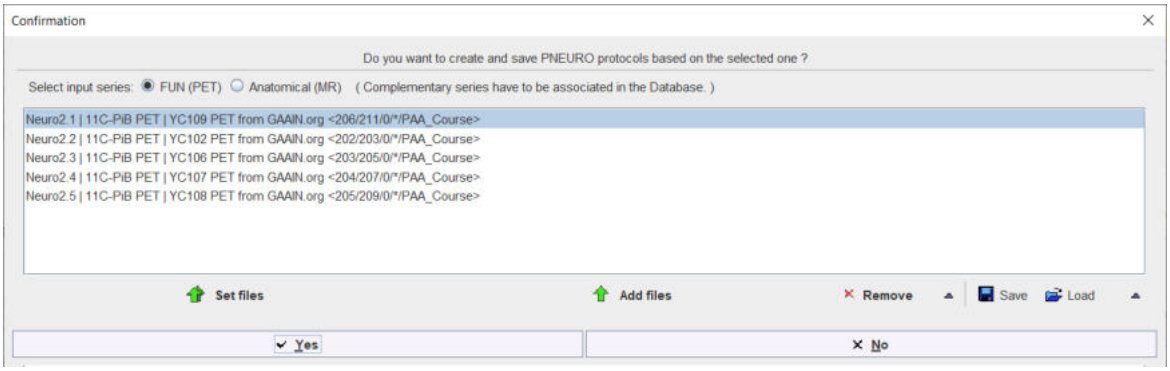
Parameter Settings

The parameters of the protocol (or an entire set of selected protocols) can be edited in the area to the right and then updated with **Set to selected** protocols. This mechanism makes it easy to establish a common processing algorithm.

> **Note**: The **Deep nuclei parcellation** and **Subjects** definitions are active only when a brain parcellation protocol is selected on the protocols list.

Cloning Protocols

Once a protocol with suitable processing parameters has been defined, it can be cloned for application with additional data sets. This is achieved by selecting the template protocol in the list and activating **Clone protocols** the lower right area. A dialog window opens for definition of the input data.



Depending on the type of analysis, the corresponding radio button **FUN (PET)** or **Anatomical (MR)** has to be configured. For workflows with both MR and PET images, the image series have to be associated beforehand as described above.

Please select the appropriate image files with **Set files**, and append additional ones with **Add files**. **Remove** deletes a selected entries from the list, whereas **Remove all** clears the whole list. An input data list can be saved for later use with the **Save** button, and retrieved with **Load**.

Once the data list is complete, activate **Yes** to generate a new protocol list containing a protocol for each of the input data sets. The protocols are saved in the database and associated with the corresponding images.

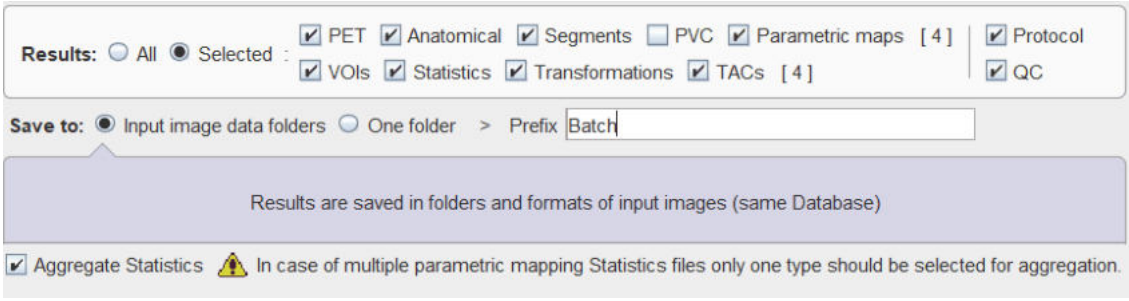
Note: If cropping is used in cloning, it is recommended using the automatic placement of the crop box by enabling the **Crop** and **Auto** boxes in the parameter settings area.

Results and Output Configuration

The granularity of the results saved during the batch processing can be configured in the **Results** section. With **All** enabled, all possible results are stored at the end of processing. With **Selected** enabled, the following components can be enabled for saving:

- **PET (in final space)**: PET image in the space where the VOIs are generated.
- **Anatomical (in final space)**: The anatomical image (MR or FDG) in the space where the VOIs are generated.
- **Segments**: The result of the tissue segmentation into grey matter, white matter, CSF.
- **PVC**: The image results of the partial volume correction configured in the VOIs page.
- **Parametric maps**: The parametric maps results obtained from the mapping procedure. Please note that only the protocols saved at the end of the mapping procedure will generate parametric maps.
- **VOIs**: Contour VOIs in the selected result space.
- **Statistics**: VOIs applied to the target image series (PET, if available, otherwise the anatomical image).
- **Transformations**: The transformations calculated based on the protocol specific workflow .
- **TACs**: The TACs statistics in the selected result space.
- **Protocol**: The protocol after the processing is (re)saved in the specified loaction. This is relevant for the situation when the list of protocols was created using the cloning facility. Such protocol includes definition of the input data as well as the parameters of the different processing stages, including the parametric mapping settings when available.
- **QC**: The quality control capture is generated.

The output destination is set with the **Save to** configuration. A meaningful **Prefix** for the saved data components is recommended. The destination configuration is only relevant when saving to the file system. In that case, the results can be distributed to the **Input image data folders**, or concentrated in **One folder**, which needs to be specified together with the output image format.



Note: The time activity curves generated during parametric mapping protocols may also be saved by enabling the TACs check box.

The **Aggregate Statistics** option is relevant when the batch is setup to run protocols for the same group and condition. If the option is enabled, the results of all data sets are concentrated into a single aggregation table, which can immediately be used for statistical analysis.

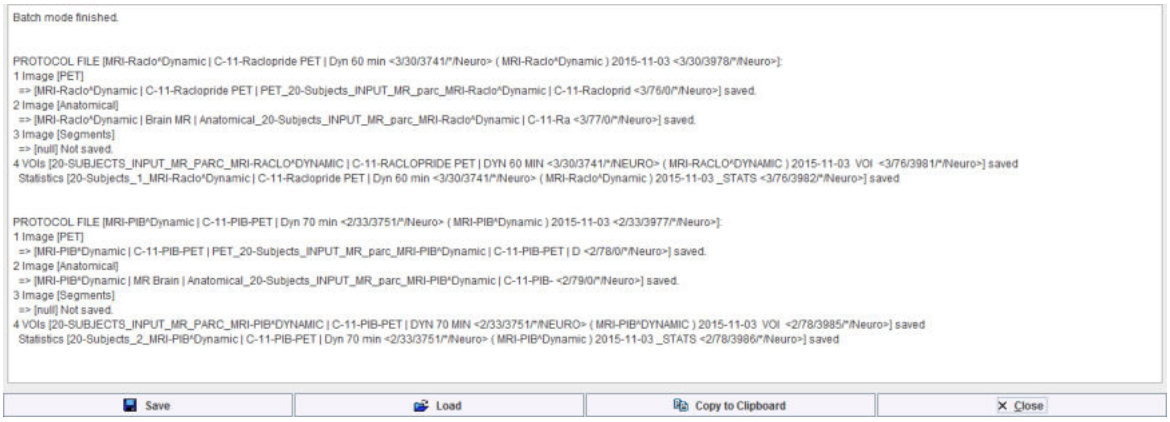
Note: In case of multiple parametric mapping statistics, only one type of statistics should be selected for aggregation at the end of the processing.

Run the Batch

The batch processing is started with the **Run** button.

To run processing without the batch window open (recommended) configure **Close after Start**. While processing is performed, PNEURO will remain blocked and run through the processing steps for each protocol. The **Aggregate Statistics** option is convenient for assembly of the resulting VOI statistics output into an aggregate file for subsequent statistical analysis. In case of multiple parametric mapping statistics files only one type should be selected for the direct aggregation form batch.

After batch processing has completed, a summary is shown in a **Report** window detailing the saved results as well as potential errors. The report can saved using the **Save** button, and added t the clipboard using the **Copy to Clipboard** button.

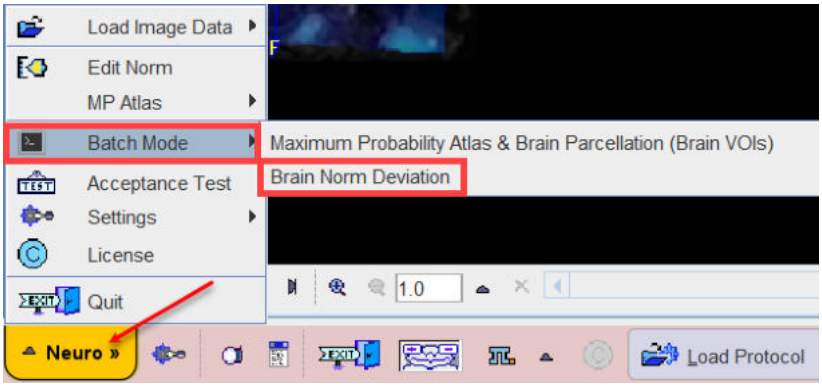


2.6.2 Batch Mode Brain Norm Deviation

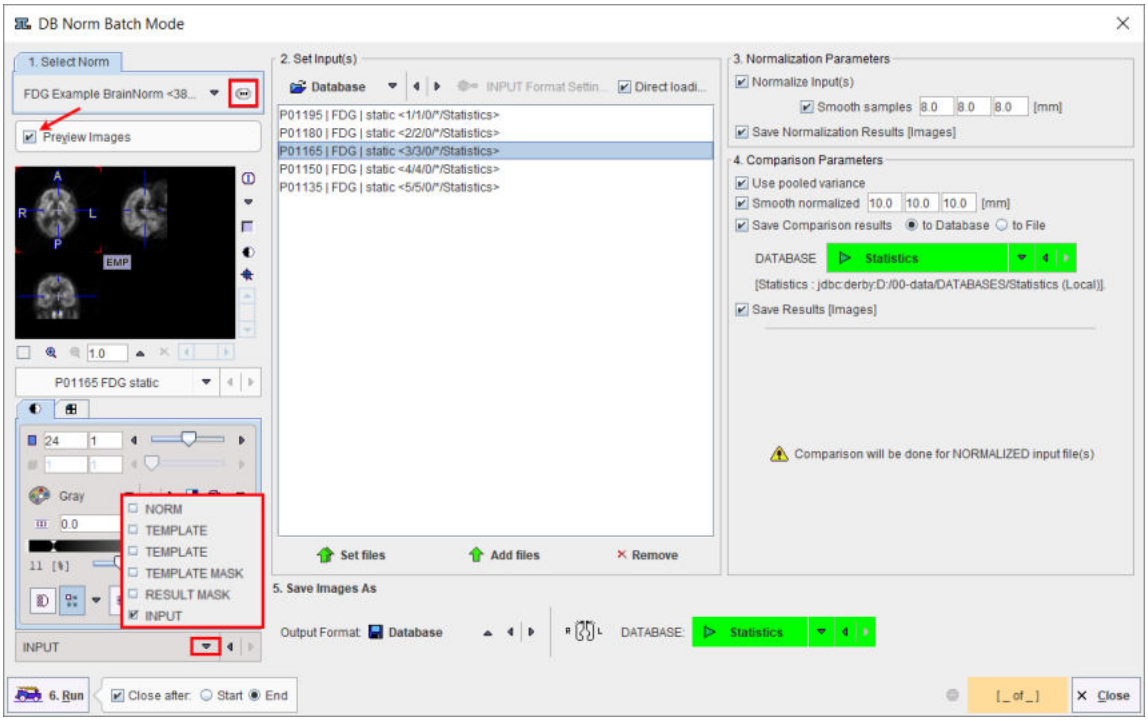
The PNEURO tool offers the Compare to Norm batch facility which is useful when several studies need to be analyzed. The batch mode allows performing the spatial normalization - which is the time-consuming processing step of the whole analysis - for a list of studies and save the normalized images for a later, interactive comparison analysis. It is also possible to perform the comparison analysis at the same time and save its results, but this unsupervised mode is not recommended due to the potential failure of spatial normalization.

Note: The creation of the Brain Norm database is requested for running the batch. This have to be prepared beforehand as explain in the Brain Norm Creation 135 section.

Batch mode is started using the corresponding entry in the Batch Mode menu:



and displays the following dialog window.



There are numbered sections in the dialog which must be configured appropriately:

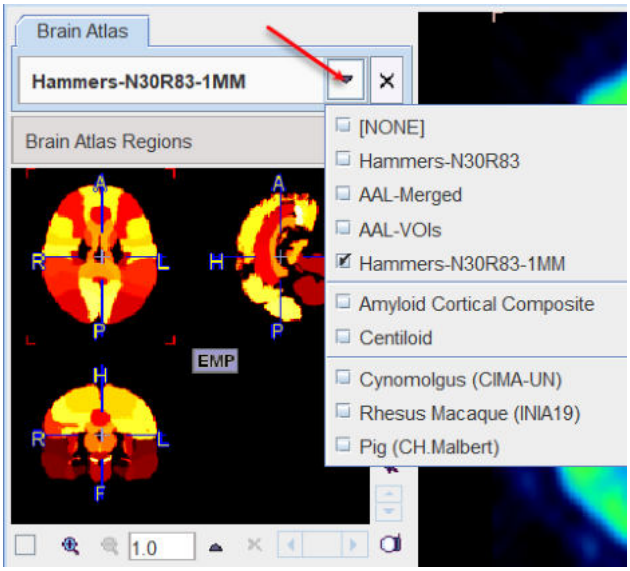
1. Select Norm	The Brain Norm against which the data should be compared can be configured on this section using the button. The images related to the selected Brain Norm are available for inspection on the bottom. The list selection allows switching between the different series such as the normalization **TEMPLATE**, the normalization and result **MASK**, as well as the **NORM** image.
2. Set Input(s)	In this section the studies to be normalized are defined. Please first select the data format (in the example: **Database**) and then add the files to the list to be processed using the **Set files** button. Note the button **Input Format Settings** which allows configuring optional pre-processing steps, such as A-P mirroring, smoothing, time-averaging, etc. **Direct loading** disables these options.

3. Normalization Parameters	The **Normalize Input(s)** check defines whether the input images need to be spatially normalized (this is not necessary if previously normalized images are selected), and the check **Save Normalization Results** whether the resulting images are saved.
4. Comparison Parameters	This section allows the user to perform the comparison analysis in unsupervised mode, which is *not recommended* without saving of the normalization results and user validation of those results. All the comparison results can be saved for post-batch reviewing.
5. Save Images As	If the saving of result images has been configured, the format defined in this section will be applied. First select the data format (the **Database** is recommended), then configure additional parameters for that format.

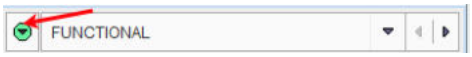
After the configuration is complete, the **6. Run** button can be activated to initiate batch processing.

2.7 Atlas Selection

The atlas to be used for the VOI creation can selected in the **Select Atlas** panel.



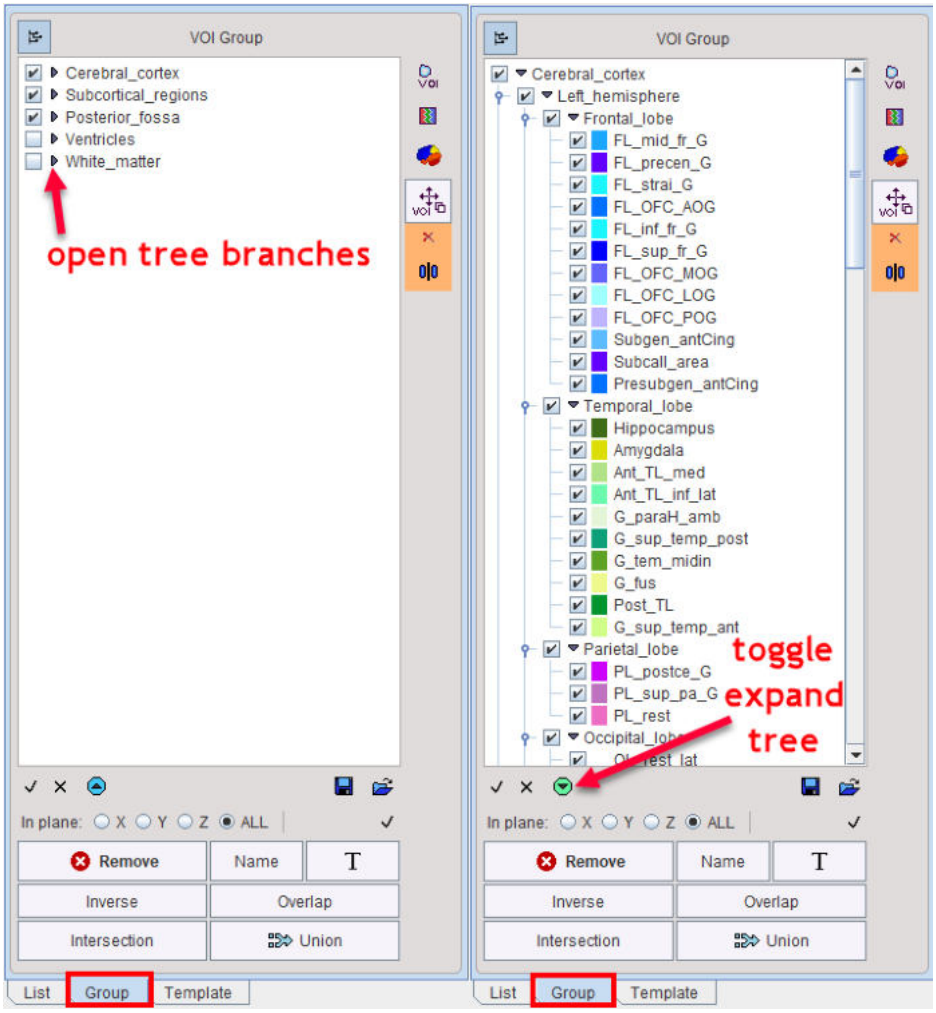
If the panel is not visible, please activate the toggle button

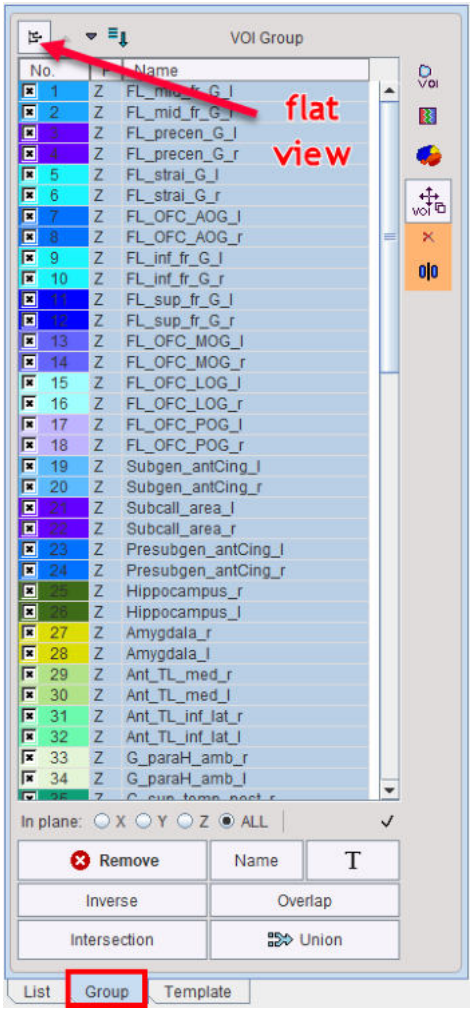


in the upper right. Note that Human, Primates and Pig VOI templates in the sub-directory *resources/templates/voitemplates* are listed.

2.8 Tree Organization of Atlas VOIs

The brain VOIs are structurally organized in a tree on the **Group** tab of the VOI editing page. The selection of a VOI subset is supported by a dedicated user interface illustrated below.

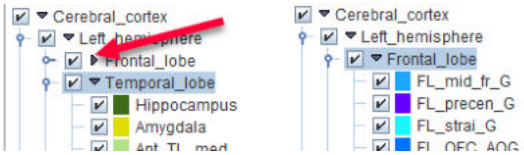

VOI Group
Cerebral_cortex
Subcortical_regions
Posterior_fossa
Ventricles
White_matter
open tree branches
In plane: X Y Z ALL
Remove
Name
T
Inverse
Overlap
Intersection
Union
List
Group
Template
VOI Group
Cerebral_cortex
Left_hemisphere
Frontal_lobe
FL_mid_fr_G
FL_precen_G
FL_strai_G
FL_OFC_AOG
FL_inf_fr_G
FL_sup_fr_G
FL_OFC_MOG
FL_OFC_LOG
FL_OFC_POG
Subgen_antCing
Subcall_area
Presubgen_antCing
Temporal_lobe
Hippocampus
Amygdala
Ant_TL_med
Ant_TL_inf_lat
G_paraH_amb
G_sup_temp_post
G_tem_midin
G_fus
Post_TL
G_sup_temp_ant
Parietal_lobe
PL_postce_G
PL_sup_pa_G
PL_rest
toggle
expand
tree
In plane: X Y Z ALL
Remove
Name
T
Inverse
Overlap
Intersection
Union
List
Group
Template



The branches on the top level are the **Cerebral cortex**, divided into left (**L**) and right (**R**) hemispheres, **Subcortical regions**, **Posterior fossa**, **Ventricles** and the **White matter**, as illustrated in the left panel above. The full tree can be toggled open/closed easily by using the button indicated in the middle panel above. The tree view can be flattened to a simple list using the button at the top, as indicated in the right panel above. The statistics will only be calculated for the selected VOIs.

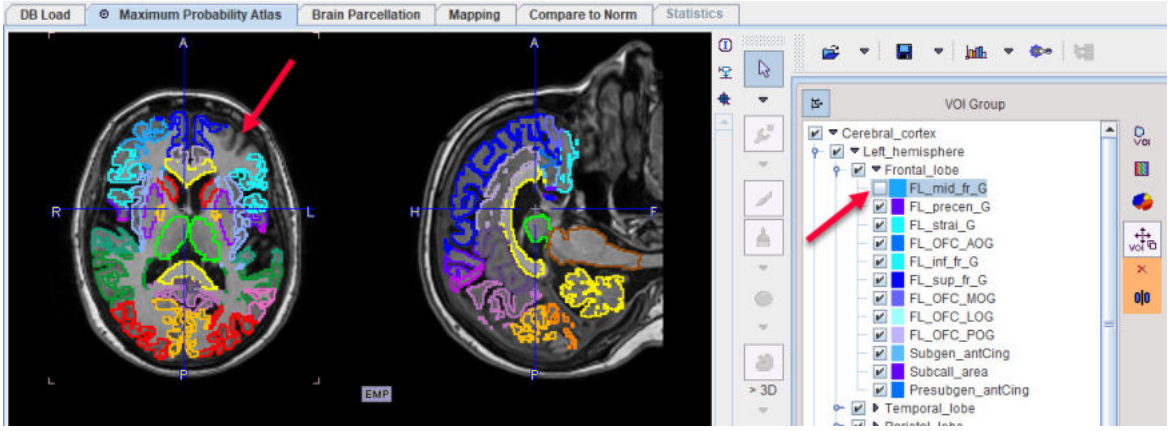
Tree Manipulations

Branches in the tree can be opened/closed with the little arrows left to the branch names.

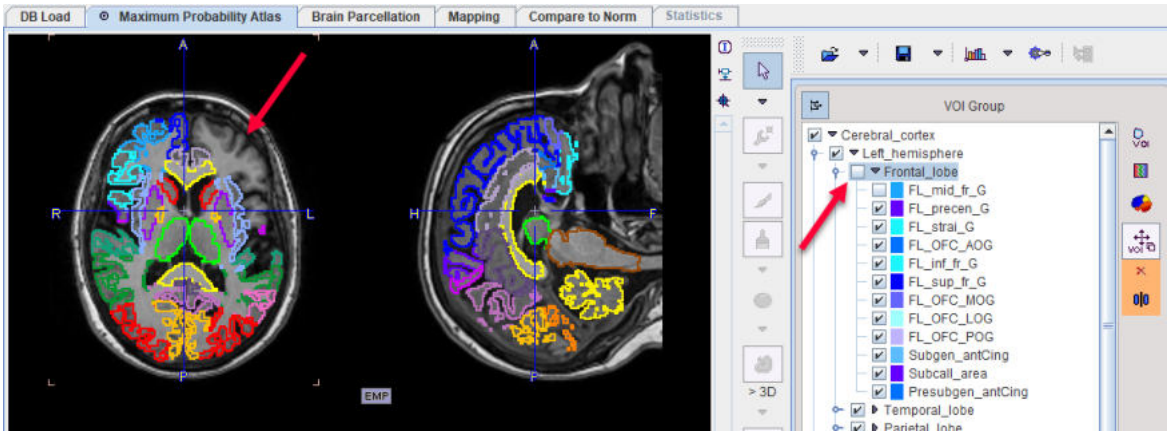


Tree Selections

Checked VOIs in the tree structure are overlaid on the image and used in statistics calculation. Simply uncheck any VOI to exclude it. In the example below the **FL_mid_fr_G** VOI has been de-selected.



If the selection of a branch is removed, all VOIs belonging to it will be de-selected.

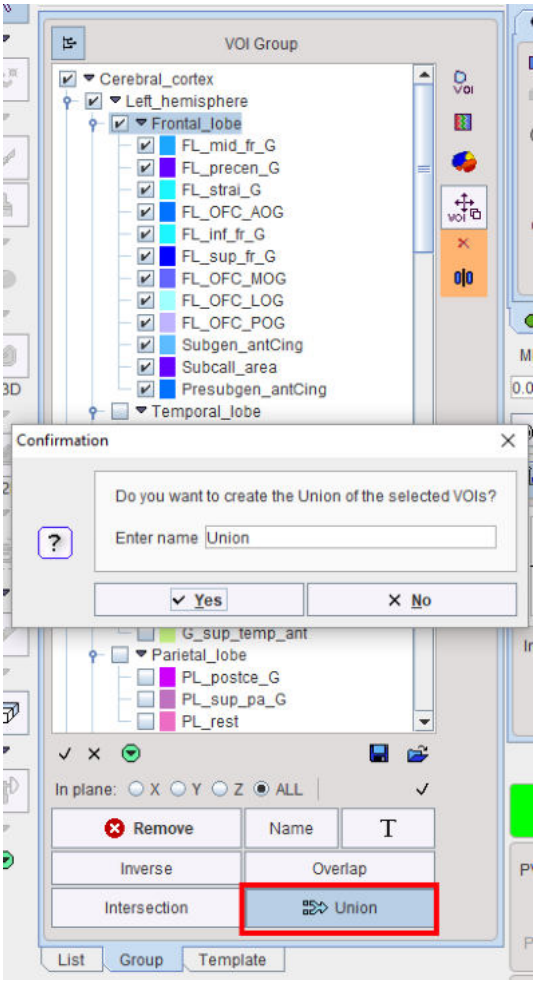


The task of selecting parts in the tree is supported by shortcuts in the area below the VOI list:

✓	Set the selection check of all VOIs
×	Remove the selection check of all VOIs.
	Save the current selection set to a file.
	Load a selection set from a file.

VOI Union

The currently selected VOIs can be combined into a larger structure by the **Union** button. In the example below the selection was first reset by ×, and then the **Frontal_lobe** entry in the **Left_hemisphere** of the **Cerebral cortex** branch was checked.



Note that in PNEURO the original VOIs are removed during the **Union** process. This is necessary for partial volume correction to function correctly. However, the VOIs can easily be recovered by stepping to the prior page and activating the **Outline** button again.

2.9 Recommendations for Brain VOI Calculations

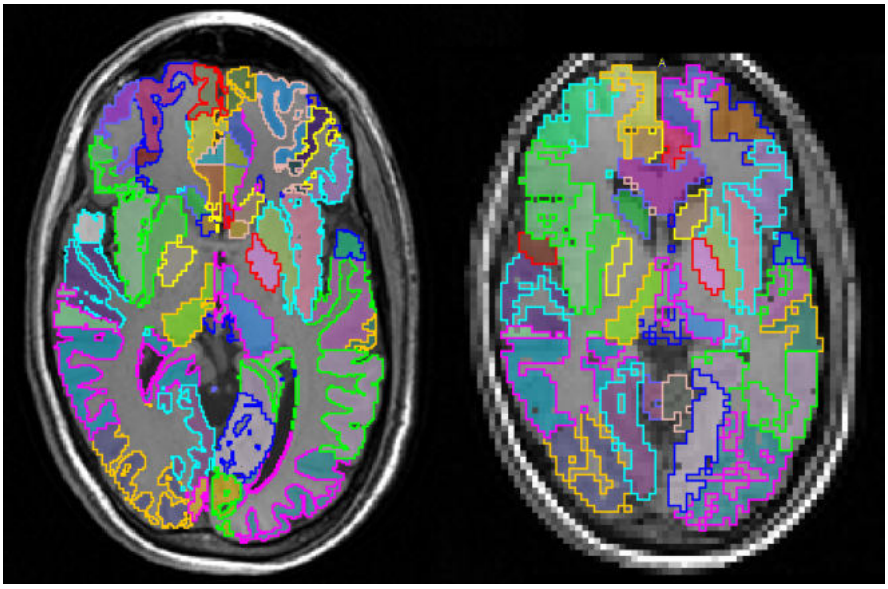
Input Data

Whenever possible, a brain PET study should be complemented by an anatomical T_1-weighted MR study with isotropic high resolution in the order of 1 mm covering the entire brain. This will allow the accurate adjustment of the brain structures to the subject anatomy and conveniently support any interactive fine adjustments.

Evaluation Space for PET Statistics

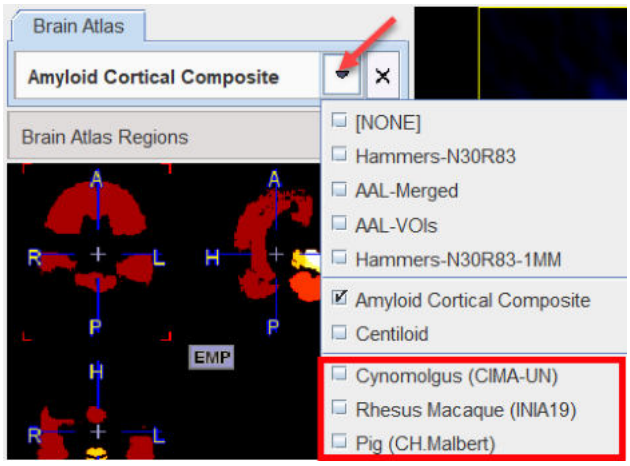
The calculation of the PET VOI statistics can be performed in any of the available image spaces, i.e. depending on the available input data: the MNI template space, the MR space and the original PET space. If the user would like to avoid any interpolation of the original PET values, the original PET space should be used. In this case, the user is strongly encouraged to reconstruct the PET images with a pixel size of about 1 mm. Otherwise, the brain VOIs will be truncated and become coarse.

The resolution effect on the VOIs is illustrated below. The left image shows the brain contours with the resolution of an MR image (0.8mm in-plane), the right with the resolution of a PET image (2.4mm). Note that the correspondence of the slices is only approximate.



Species Selection

Global species selection in PNEURO is governed by the **Atlas** selection . The species is relevant for cropping settings (see below).



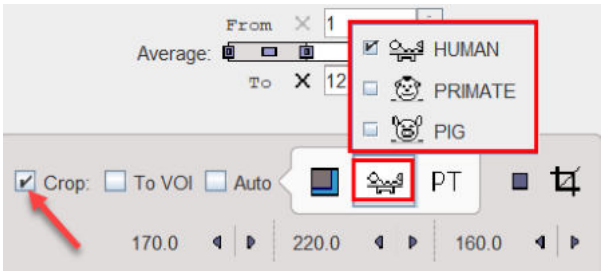
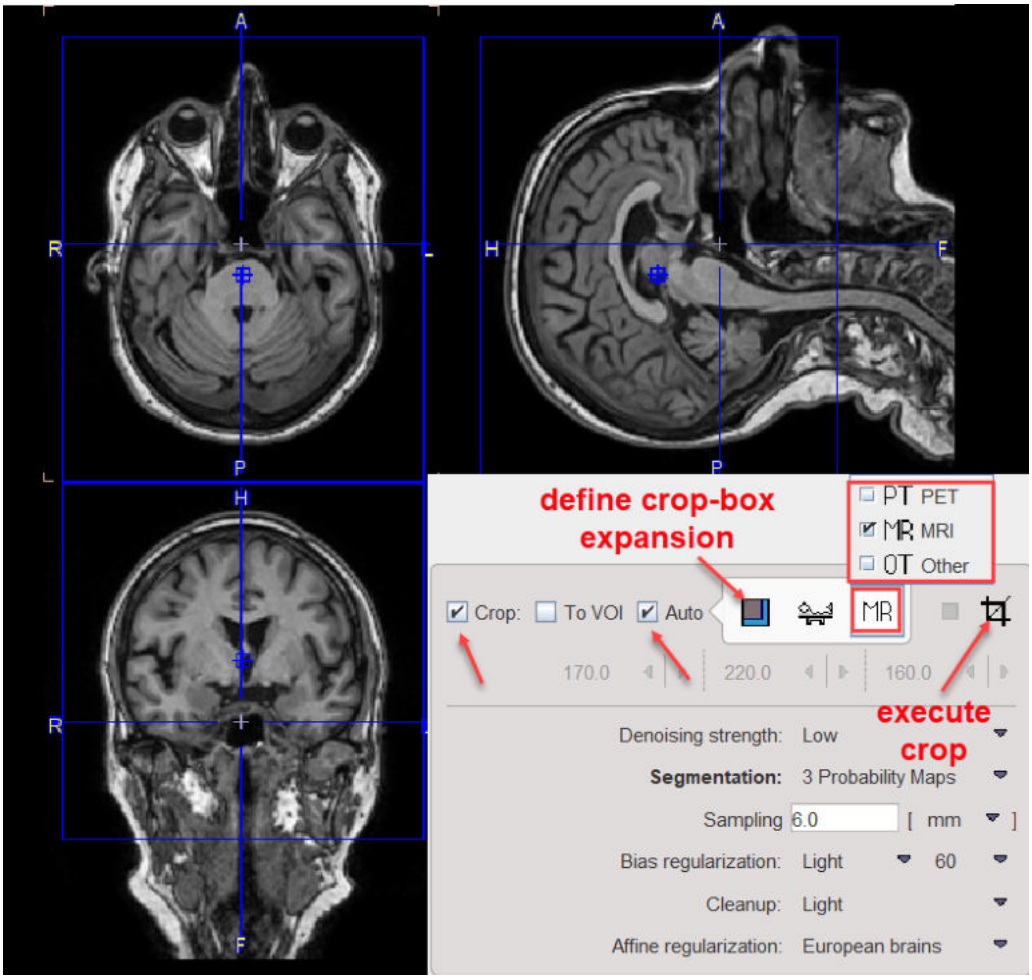
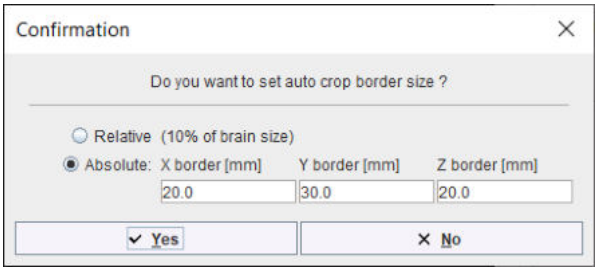


Image Cropping

The algorithms for brain segmentation in the PNEURO modules work best if the images do not contain a large amount of information from outside the brain. Therefore, after loading the MR and PET images, PNEURO offers an automatic cropping facility as illustrated below.

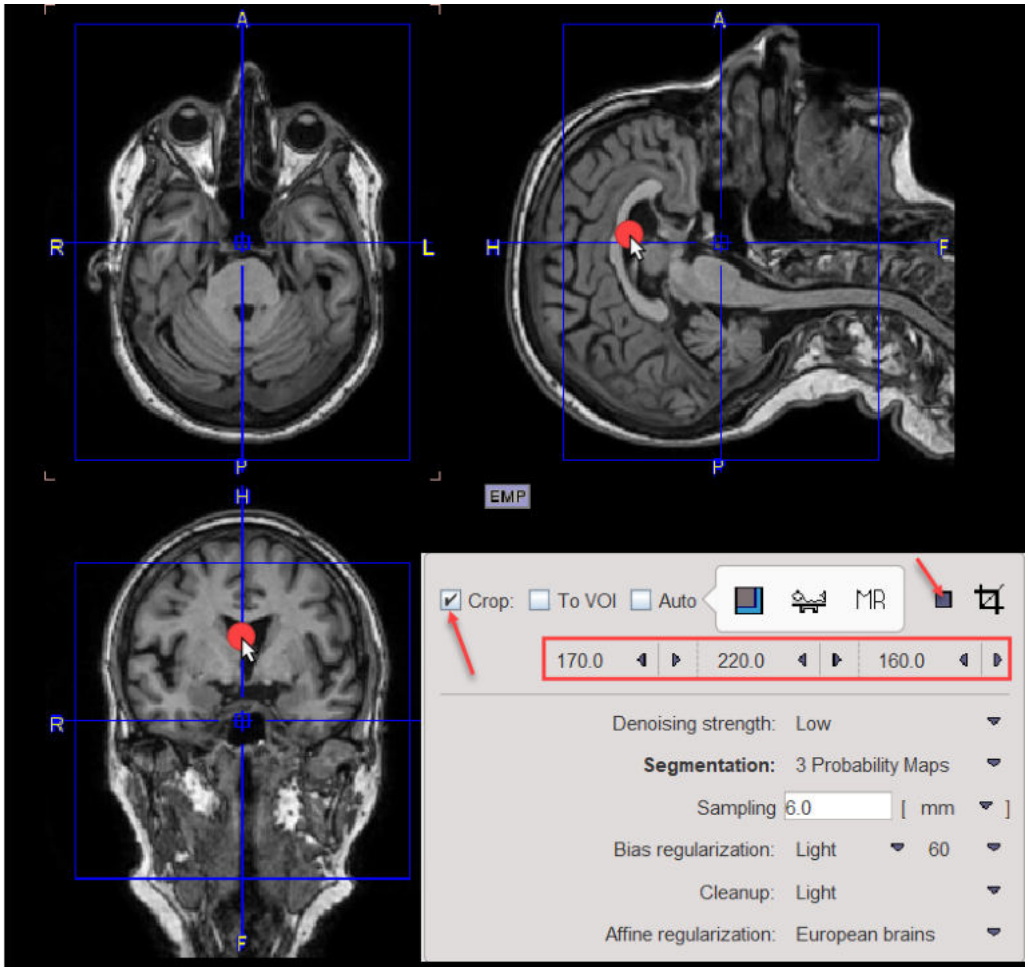


To enable the auto cropping option, please check the **Crop** and the **Auto** boxes. Consequently, blue rectangles are overlaid on the images indicating the extent of the cropping box. Based on the species and modality settings, the program automatically performs a deformable matching to the corresponding template to identify the optimal size and placement of the cropping box. The dimension and location of the cropping box are updated automatically at the end of the calculations. In some cases it can be useful to enlarge the default box used for automatic cropping in either x/y/z dimensions. For example, expansion in y can be used to include the nose in the cropped image, which can be beneficially in PET to MR matching. The icon to the right of the **Auto** checkbox opens the expansion dialog:



Toggling the **Auto** checkbox on/off will update the crop with expansion settings taken into account. Automatic cropping (with expansion) is particularly helpful during batch processing.

In case the automatic procedure fails, the cropping box size and placement can be adjusted manually. Only the **Crop** checkbox need to be checked, as illustrated in the capture below. To move the center of the box simply click into the image. To change the box size in x/y/z directions, use the corresponding selections below the **MR** and **Species** selection, or the indicated button:

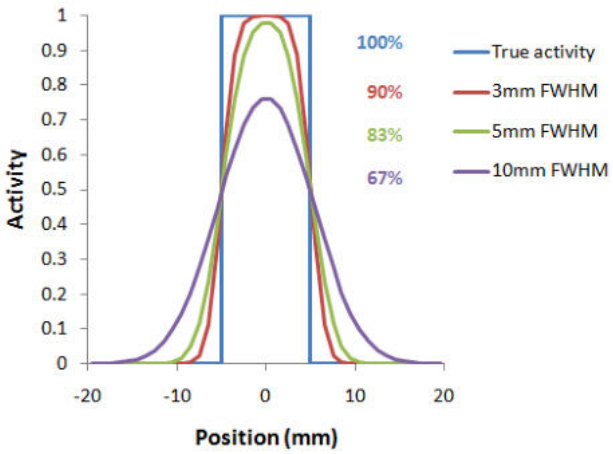


The **To VOI** check box requires the crop box to be defined and saved beforehand (e.g. using the View module) and associated with the image in the database.

Once the cropping box is properly placed, cropping can be started interactively using the **Crop** button . Note that the process is irreversible and the series must be reloaded in the event of mis-positioning. If cropping is not performed interactively a confirmation message will appear when progressing to the next stage. After performing a cropping operation, the function is blocked. In order to apply a different crop box the data has to be loaded again.

2.10 Partial-Volume Correction (PVC)

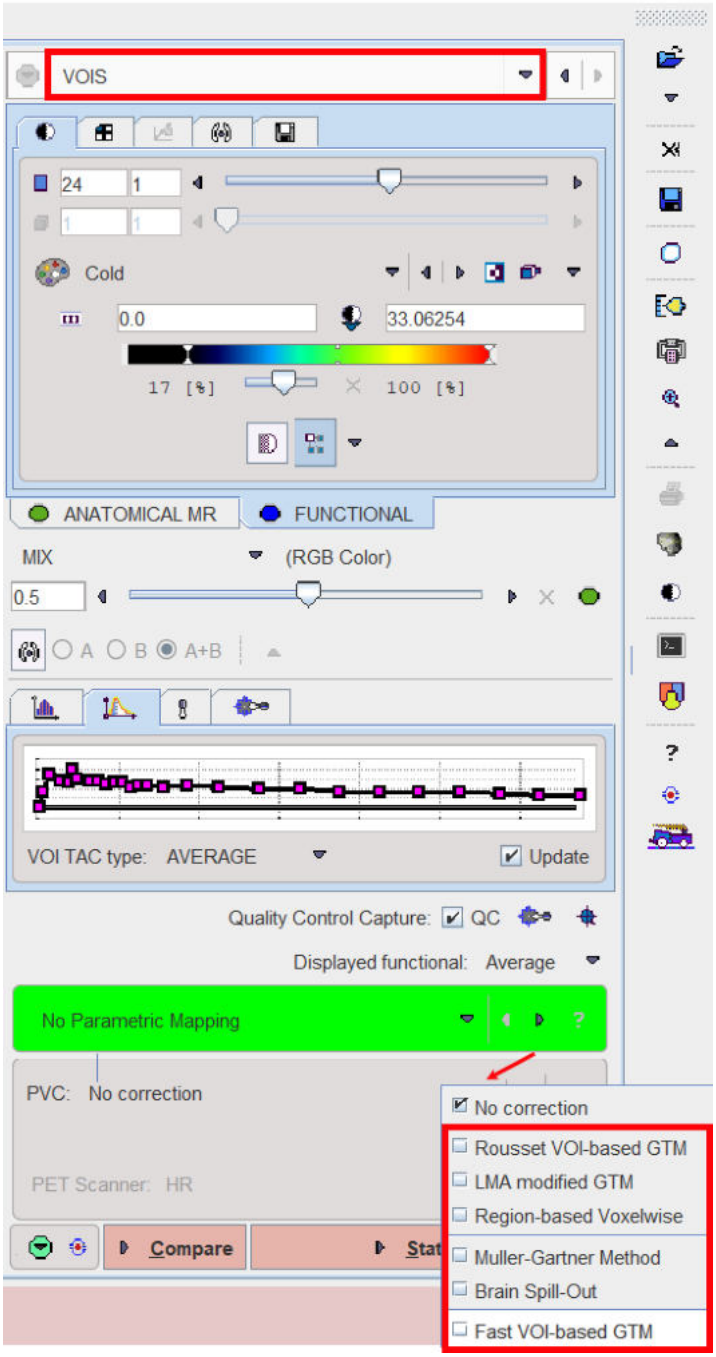
PET images are inherently affected by the partial-volume effect. This means that the measured tracer activity concentration is not accurate due to the relatively low image resolution and the limited tissue sampling. The low spatial resolution of the PET system causes a blurring of the image, so that high activities (from a hot lesion) are spread to the surrounding as illustrated below. This effect is called spill-out. The same effect also causes a spill-in of background activity into the volume of interest.



As a consequence, hot lesions tend to appear less aggressive (reduced maximum) but bigger (spreading) than they are in reality.

Spill-in and spill-out depend on the geometry of the objects, the activity distribution of the tracer, and on the resolution of the scanner which may vary across the imaging field-of-view. Therefore, practical correction approaches have to assume certain conditions and can only be approximate. For a nice overview of the topic please refer to the publication of Soret et al. [1].

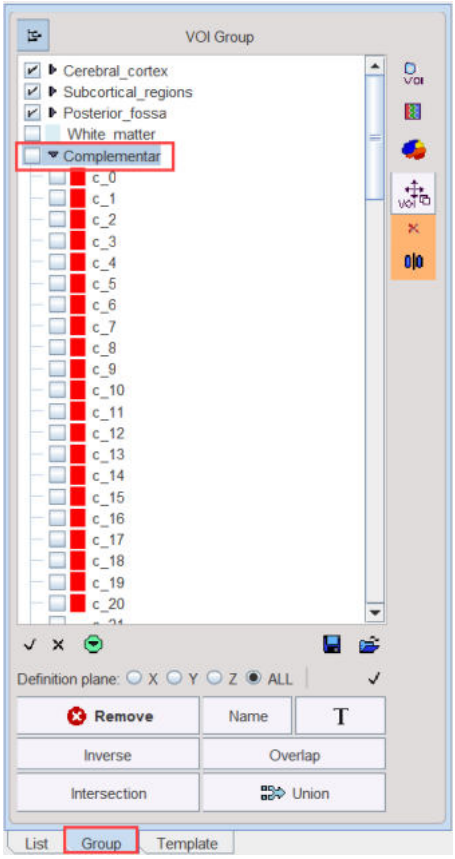
Partial-volume correction in PNEURO is accessed on the VOIs page of either the Maximum Probability Atlas or Brain Parcellation workflows:



The selection and configuration of the methods available is described in more detail in the Workflow sections of this document.

Complementary VOIs

After the PVC correction is performed a list of complementary VOIs appears on the **Group** list under the **Complementar** branch.



The **Complementar** VOIs are representing the regions that were masked out during the intersection of the Atlas structures with the grey/white matter segmentation. These VOIs were created since the very beginning and were generated in order to meet the conditions of the GTM correction method: the area is divided into homogenous regions covering as much as possible. Empty spaces between the corrected VOIs would cause inaccurate correction.

The availability of these VOIs on the list after running the PVC is aimed to increased the transparency such that they can be saved and used for the exact reproduction via the Protocol[10].

When a PVC method is used, both the original and the corrected statistics are calculated. Note that due to the high number of VOIs the PVC calculation may take several minutes and consumes a significant amount of RAM.

Note:The PVC is always calculated for all the created VOIs but statistics results are returned only for the VOIs that are selected on the **Group** tab.

2.10.1 Rousset VOI-based GTM Method

This correction is based on the assumption that the imaging volume can be separated into tissue volumes (VOIs) with homogeneous uptake. If the resolution of the PET scanner is known, the mutual signal contaminations across the VOIs can be calculated and corrected for. This method is known as the GTM (Geometric Transfer Matrix) method and was introduced by Rousset et al.

The relation of the measured average PET values in the VOIs (affected by the partial-volume effect) to the true PET values is given by the matrix equation

$$\vec{C}_{measured} = [GTM] \times \vec{C}_{true}$$

with the following notations:

C_{true}	Vector of the true average activity concentration in the different VOIs of interest. The vector length n equals the number of object VOIs.

$C_{measured}$	Actually measured average activity concentration in the different VOIs. Each VOI is assumed to have a homogeneous concentration.
GTM	Geometric Transfer Matrix which describes the spill-over among all the VOIs. The matrix is square with nxn weighting elements $w_{i,j}$ which express the fraction of true activity spilled over from VOI_i into VOI_j. In practice, $w_{i,j}$ is calculated as follows: A binary map is created with 1 in all pixels of VOI_i and 0 elsewhere. The map is convolved with the imaging Point-Spread Function (PSF), and in the resulting spillover map the weighted average of all VOI_j pixels calculated.

The GTM equation above represents a system of linear equations. Once the weights have been calculated, the system can be solved for the true average concentration values C_{true} in all VOIs by matrix inversion.

Recommendations

According to Rousset et al. [2], the accuracy of the GTM method depends primarily on the proper identification of the tissues which have different functional properties. If this is the case, the GTM algorithm is capable of accurately correcting the regional concentration within small structures such as the human basal ganglia. Furthermore, the propagation of statistical noise during partial-volume correction was found to be easily predictable and suitable for the application in dynamic PET.

2.10.2 LMA modified GTM Method

The LMA (Local Means Analysis) GTM method [4] uses the homogeneous regions localized by the segmentation and calculates the average uptake in the inner of the structures. The percentage of pixels per segment considered for averaging is a parameter of the method. With 100% pixels included, the LMA GTM method equals the standard GTM method.

2.10.3 Region-based Voxelwise Method

The RBV correction introduced by Thomas et al [7] extends the GTM method and performs a voxel-wise correction of the entire image.

In a first step the standard GTM[32] correction is performed, resulting in a synthetic image C_{GTM}, which consist of the VOIs filled with the corrected average values.

In a second step a corrected image is calculated, which is not any more homogeneous within the VOIs, and which shows an image of the entire brain, not just the GM pixels. The calculation uses the formula

$$C_{PVC-RBV} = C_{Measured}\left[\frac{C_{GTM}}{C_{GTM} \otimes PSF}\right]$$

whereby the measured PET image $C_{Measured}$ is multiplied by a correction term calculated from the GTM corrected image and the point-spread function.

Note that the pixelwise correction may be problematic for dynamic data with low signal/noise ratio.

2.10.4 Muller-Gartner Method (Grey Matter Spill-out and White Matter Spill-in)

This method introduced by Muller-Gartner et al. [5] is based on the assumption that white matter (WM) uptake is homogeneous, whereas the uptake in grey matter (GM) is variable in each pixel. All brain pixels are classified as white matter or grey matter and sorted into respective segments. Based on these segments and the assumed PET resolution the spill-out from WM to GM can be

estimated and subtracted. Similarly, the spill-out from GM to the surroundings can be estimated and compensated for. The result is a grey matter image with corrected activity values in all pixels.

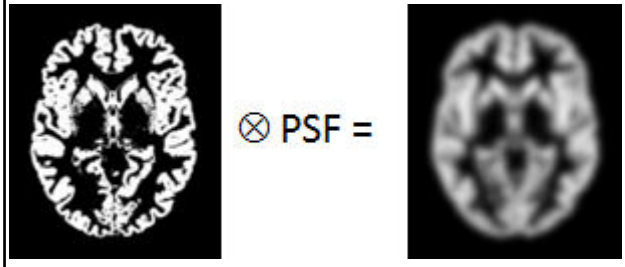
The following correction formula is applied:

$$C_{PVC-GM} = \frac{C_{measured} - C_{WM} \times (WM \otimes PSF)}{GM \otimes PSF}$$

Corrects WM spill-in

Corrects GM spill-out

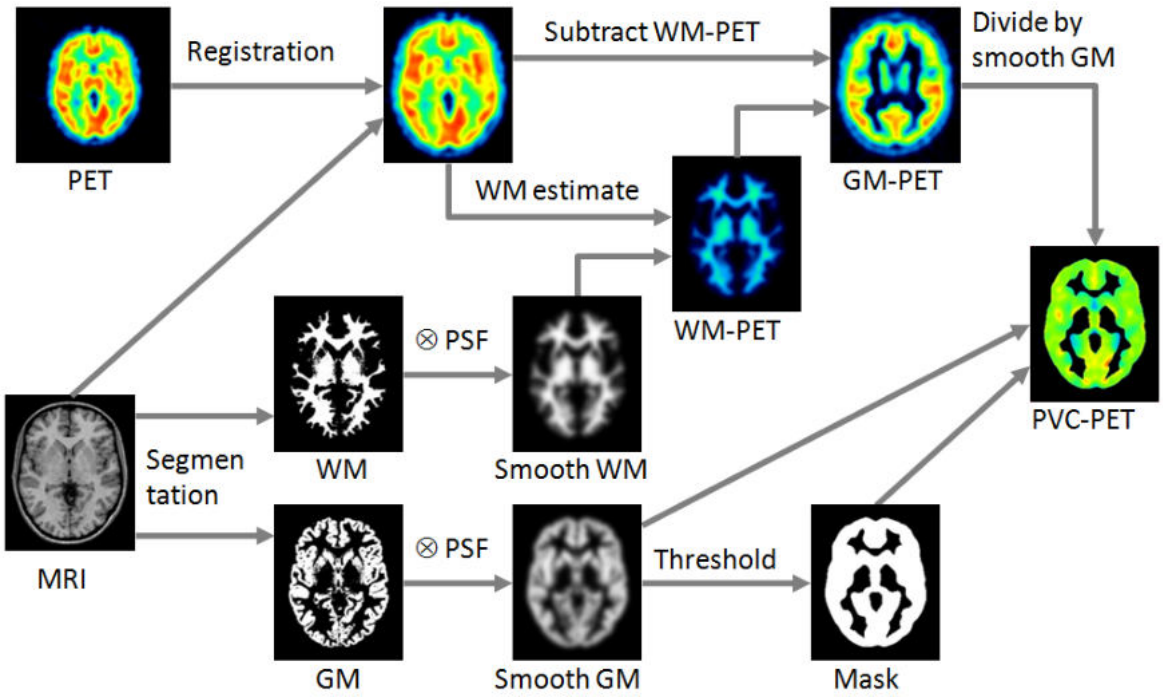
with the following notations:

C_{PVC-GM}	Corrected activity concentration. This is the result of the PVC correction in each GM pixel.
$C_{measured}$	Actually measured activity concentration in a GM pixel which may be distorted due to spill-out and spill-in.
C_{WM}	Activity concentration in WM which is assumed to be homogeneous and just blurred by the PSF.
WM, GM	WM (GM) segment image which represents the WM (GM) probability of each pixel in the image as a value between 0 and 1. These images are assumed to represent the true anatomy with ideal resolution.
PSF	Point-spread function of the imaging system which is assumed to be constant across the image and represented by a three-dimensional Gaussian function.
WM⊗PSF	Mathematical convolution of the WM segment image with the PSF. The result represents the image of the ideal WM segment when detected with a real imaging system characterized by the PSF function. The expected WM image is obtained by scaling WM⊗PSF by the WM concentration C_{WM}. It is subtracted from the measured image to compensate for spill-in from WM to GM.
GM⊗PSF	Mathematical convolution of the GM segment image with the PSF. 

	A division of the WM-corrected image by GM⊗PSF represents a deconvolution operation and corrects for the spill-out effect from GM. The operation of division may introduce over-corrections at the GM boundary. Therefore, the result image is masked at a certain threshold of the convolved GM image

Schematic of the Correction Workflow

The following illustration provides a graphical overview of the different processing steps as well as the resulting intermediate images.



White-Matter Estimate and Masking

Three variants are available for the white matter uptake, which is used to scale the smoothed WM mask: Default is the regression approach of Giovacchini et al. [6]. It collects the PET uptake of all pixels with a WM probability higher than the specified value (e.g. 0.95), and performs a linear regression to estimate the WM value at the probability of 1. A first alternative is calculating the average value of all WM pixels. A second alternative is a fixed value manually entered by the user.

The PVC algorithm is only appropriate for GM pixels. All other pixels in the corrected image should therefore not be evaluated and should be masked. Therefore, a GM threshold should be specified. It is applied to the convolved GM segment (GM⊗PSF) in order to mask background and WM. Note that if the threshold is small, outlier values might appear along the mask boundaries as over-correction artifacts.

2.10.5 Brain Spill-out (Muller-Gartner)

A simplified Muller-Gartner approach can be applied if the segmentation doesn't provide a good separation between GM and WM. In this case the correction is reduced to a spill-out only correction. A brain segment is created by summing the GM and WM probability masks. The brain segment is then convolved with the PSF, and the PET image divided by the result as described by the equation below.

$$C_{PVC-Brain} = \frac{C_{measured}}{Brain \otimes PSF}$$

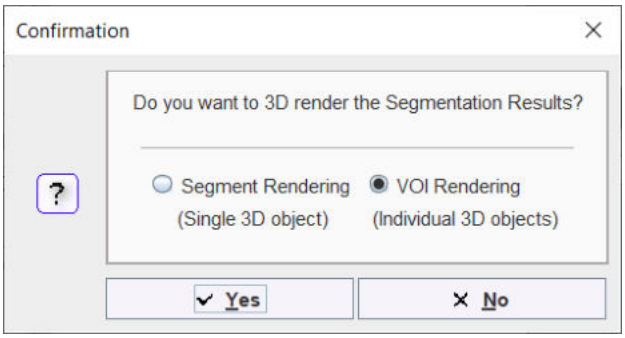
2.10.6 Fast VOI-based GTM method

This is actually the LMA modified GTM method which contains some speed-up improvement for the smoothing. The speed performance might be observe when the selected atlas contain a low number of regions.

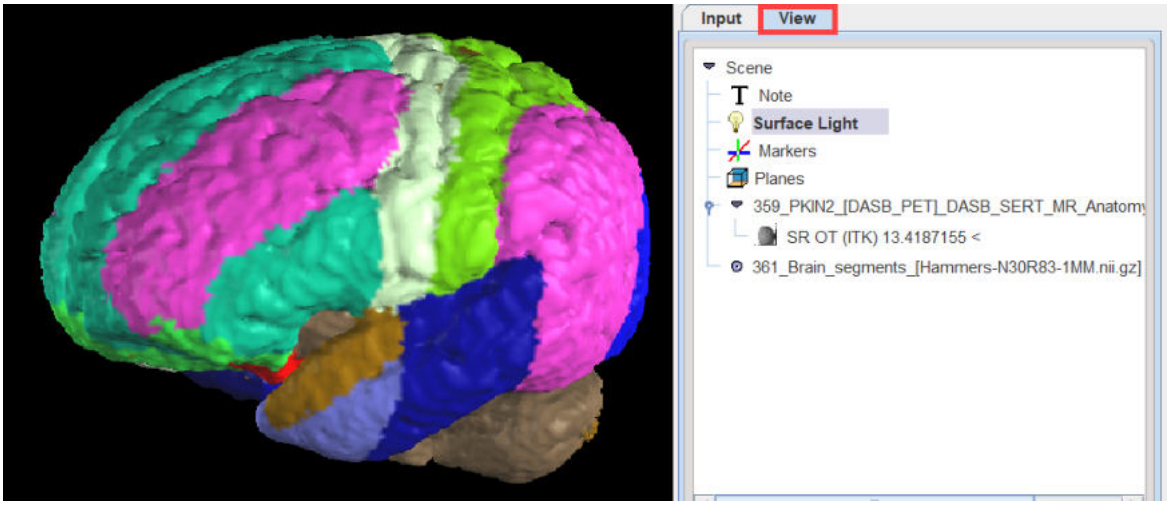
2.11 3D Rendering of Brain VOIs

Once a set of brain VOIs has been calculated it can be visualized in the 3D tool (option) as full surface by activating the button in the lateral taskbar.

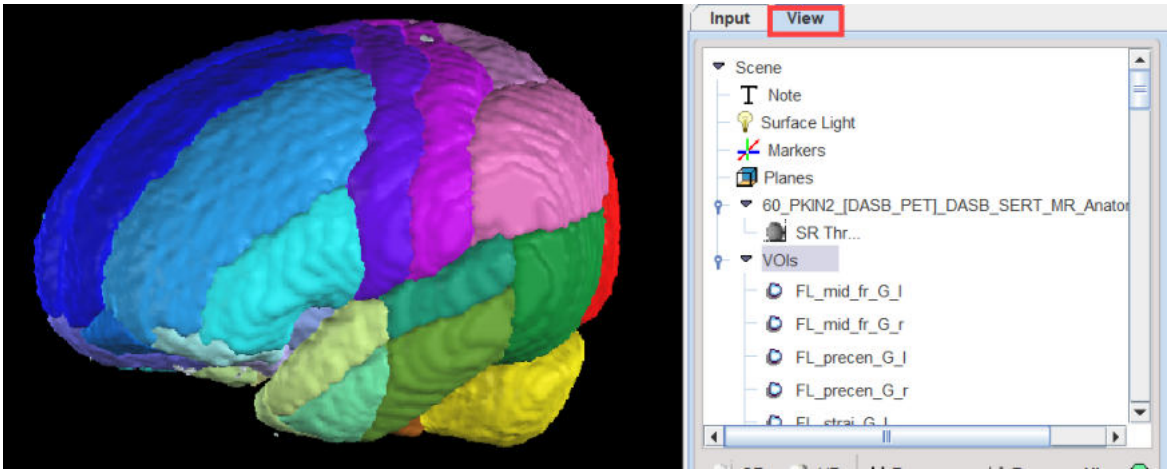
There are two types of renderings to choose from when using the **Start 3D rendering of VOIs** option available on the VOIs Tool tab:



In the **Segments Rendering (Single 3D object)** variant the VOIs are rendered in 3D as a single 3D surface object.



In the **VOI Rendering (Individual 3D object)** variant the VOIs themselves are rendered as individual surfaces. Note that the rendering takes longer in this case.



Please refer to the *PMOD 3D Rendering Tool Users Guide* for information about the operation of this tool.

3 Atlas Methodology

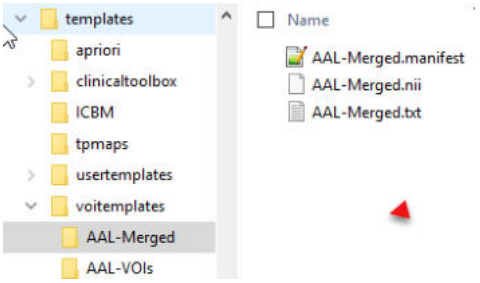
The PNEURO tool includes four human brain atlases for the generation of standard brain VOIs, the N30R83 maximum probability atlas with 1mm and 2mm resolution, the single-subject AAL atlas, an Amyloid Cortical Composite atlas and an atlas for use with the Centiloid method for amyloid PET analysis. Dedicated variants can be derived from the atlases, and user-generated atlases can be added if they conform to PMOD's requirements (maps in template space, correctly inserted into the PMOD installation directory - dedicated functionality to facilitate this process is included in PBAS). Atlases are also provided for Cynomolgus and Rhesus monkeys, and pig.

3.1 Structure of VOI Atlases in PMOD

A VOI atlas in PMOD consists of the following components:

1. *Atlas image:* Image which encodes the atlas VOIs in a stereotactic space as numeric labels.
2. *Label list:* Text file mapping the label values to the VOI names shown in the user interface.
3. *Manifest:* Text file that defines the properties of the atlas.
4. Normalization files for calculation of the transformation between the subject anatomy and atlas anatomy (not needed for MNI atlases).

The atlas information has to be organized in a sub-directory of *resources/templates/voitemplates* exactly as illustrated below for the **AAL-Merged** atlas.



The atlas name (e.g. **AAL-Merged**) has to be used as the sub-directory name, the atlas image name (**AAL-Merged.nii**), the label list name (**AAL-Merged.txt**), and the manifest name (**AAL-Merged.manifest**). If the atlas is not a human atlas in the MNI space, it needs to include an additional *normalization* folder for the templates as described below.

By conforming to this structure it is possible for users to prepare their own VOI atlases. In PNEURO, all atlases can be used for the **Maximum Probability Atlas** approach, whereas the **Brain Parcellation** approach only supports human atlases in the MNI space.

Atlas Image

The atlas image must be prepared as a NifTI file and encode the atlas VOIs as numeric labels. Each pixel has a value of 0 if it is a background pixel, or otherwise an integer number. We recommend using the HFS anatomical orientation (head first, supine = radiological convention) for human data.

Label List File

The label list text file has the minimal form:

```
name1   outlined_name1  label_value1
name2   outlined_name2  label_value2
```

where each VOI is represented by a line.

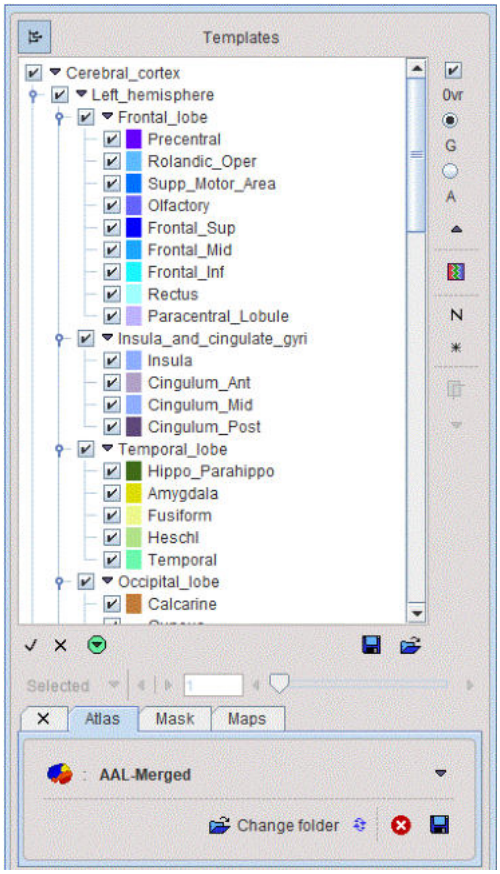
The list can be extended with additional information for the VOI presentation as illustrated below for the **AAL-Merged.txt**. The first column starts with the name followed by a bracket construction

which encodes a tree structure. For instance, **Precentral** belongs to the **Frontal_lobe** which is located in the left **Left** or right **Right_hemisphere** of the **Cerebral_cortex**. The second column indicates the name of a generated contour VOI. The third column contains the label value in the atlas file. Each pixel in **AAL-Merged.nii** with value 1 will belongs to the **Precentral_l** VOI, pixels with value **2** to **Precentral_r**, etc. The third column specifies the RGB color values for showing the VOI, and the last column the text to be shown as a tooltip.

```
Precentral[Frontal_lobe[Left_hemisphere[Cerebral_cortex]]]      Precentral_l    1       (100,0,255)     Precentral gyrus left (PRE)
Precentral[Frontal_lobe[Right_hemisphere[Cerebral_cortex]]]     Precentral_r    2       (100,0,255)     Precentral gyrus right (PRE)
Rolandic_Oper[Frontal_lobe[Left_hemisphere[Cerebral_cortex]]]   Rolandic_Oper_l 3       (93,188,255)    Rolandic operculum left (RO)
Rolandic_Oper[Frontal_lobe[Right_hemisphere[Cerebral_cortex]]]  Rolandic_Oper_r 4       (93,188,255)    Rolandic operculum right (RO)
Supp_Motor_Area[Frontal_lobe[Left_hemisphere[Cerebral_cortex]]] Supp_Motor_Area_l       5       (0,114,255)     Supplementary motor area left (SMA)
Supp_Motor_Area[Frontal_lobe[Right_hemisphere[Cerebral_cortex]]]        Supp_Motor_Area_r       6       (0,114,255)     Supplementary motor area right (SMA)
Olfactory[Frontal_lobe[Left_hemisphere[Cerebral_cortex]]]       Olfactory_l     7       (100,100,255)   Olfactory cortex left (OC)
Olfactory[Frontal_lobe[Right_hemisphere[Cerebral_cortex]]]      Olfactory_r     8       (100,100,255)   Olfactory cortex right (OC)
Frontal_Sup[Frontal_lobe[Left_hemisphere[Cerebral_cortex]]]     Frontal_Sup_l   11      (0,0,255)       Superior frontal gyrus left (F1, F1O, F1M)
Frontal_Sup[Frontal_lobe[Right_hemisphere[Cerebral_cortex]]]    Frontal_Sup_r   12      (0,0,255)       Superior frontal gyrus right (F1, F1O, F1M)
Frontal_Mid[Frontal_lobe[Left_hemisphere[Cerebral_cortex]]]     Frontal_Mid_l   13      (28,167,255)    Middle frontal gyrus left (F2 F2O, FMO)
Frontal_Mid[Frontal_lobe[Right_hemisphere[Cerebral_cortex]]]    Frontal_Mid_r   14      (28,167,255)    Middle frontal gyrus right (F2 F2O, FMO)
Frontal_Inf[Frontal_lobe[Left_hemisphere[Cerebral_cortex]]]     Frontal_Inf_l   15      (28,247,255)    Inferior frontal gyrus left (F3OP, F3T, F3O)
Frontal_Inf[Frontal_lobe[Right_hemisphere[Cerebral_cortex]]]    Frontal_Inf_r   16      (28,247,255)    Inferior frontal gyrus right  (F3OP, F3T, F3O)
Rectus[Frontal_lobe[Left_hemisphere[Cerebral_cortex]]]  Rectus_l 17      (160,255,255)   Gyrus rectus left (GR)
Rectus[Frontal_lobe[Right_hemisphere[Cerebral_cortex]]] Rectus_r 18      (160,255,255)   Gyrus rectus right (GR)
Insula[Insula_and_cingulate_gyri[Left_hemisphere[Cerebral_cortex]]]     Insula_l        19      (143,175,255)   Insula left (IN)
Insula[Insula_and_cingulate_gyri[Right_hemisphere[Cerebral_cortex]]]    Insula_r        20      (143,175,255)   Insula right (IN)
Cingulum_Ant[Insula_and_cingulate_gyri[Left_hemisphere[Cerebral_cortex]]]       Cingulum_Ant_l  21      (178,162,199)   Cingulate gyrus anterior part left (ACIN)
Cingulum_Ant[Insula_and_cingulate_gyri[Right_hemisphere[Cerebral_cortex]]]      Cingulum_Ant_r  22      (178,162,199)   Cingulate gyrus anterior part right (ACIN)
Cingulum_Mid[Insula_and_cingulate_gyri[Left_hemisphere[Cerebral_cortex]]]       Cingulum_Mid_l  23      (143,175,255)   Cingulate gyrus mid part left (MCIN)
Cingulum_Mid[Insula_and_cingulate_gyri[Right_hemisphere[Cerebral_cortex]]]      Cingulum_Mid_r  24      (143,175,255)   Cingulate gyrus mid part right (MCIN)
Cingulum_Post[Insula_and_cingulate_gyri[Left_hemisphere[Cerebral_cortex]]]      Cingulum_Post_l 25      (95,73,122)     Gyrus cinguli posterior part left (PCIN)
```

Note: For all rows, all columns need to be filled. Spaces in the **Name** field are not allowed.

The corresponding atlas VOI structure is illustrated below.



There are additional options to be added to the columns for use in PNEURO:

- **E**: Excluded from masking by the grey matter threshold.
- **O**: Indicates that the VOI is not brain matter.
- **H**: Indicates that the VOI should initially be hidden, i.e. not selected on the **Group** panel.
- **L**, **R**: Indicates that the VOI belongs to the left (L) or right (R) hemisphere. This information is used in sulci deformation.

- **C**: Indicates that the VOI belongs to the cerebellum. This information is also used in sulci deformation.

Example as shown in Excel:

S_nigra[Central_structures[L]]	S_nigra_l	74	(127,127,1	Substantia	E		
S_nigra[Central_structures[R]]	S_nigra_r	75	(127,127,1	Substantia	E		
Insula[Insula_and_cingulate_gyri[L]]	Insula_l	20	(143,175,2	Insula left	L		
Insula[Insula_and_cingulate_gyri[R]]	Insula_r	21	(143,175,2	Insula righ	R		
Cerebellum[Posterior_fossa[R]]	Cerebellum_r	17	(250,239,5	Cerebellun	C		
Cerebellum[Posterior_fossa[L]]	Cerebellum_l	18	(250,239,5	Cerebellun	C		
Brainstem[Posterior_fossa]	Brainstem	19	(151,72,6)	Brainstem	C	E	
FrontalHorn[Ventricles[R]]	FrontalHorn_r	45	(219,238,2	Lateral ver	E	O	H
FrontalHorn[Ventricles[L]]	FrontalHorn_l	46	(219,238,2	Lateral ver	E	O	H
TemporaHorn[Ventricles[R]]	TemporaHorn_r	47	(183,221,2	Lateral ver	E	O	H
TemporaHorn[Ventricles[L]]	TemporaHorn_l	48	(183,221,2	Lateral ver	E	O	H
ThirdVentricl[Ventricles]	ThirdVentricl	49	(146,205,2	Third ventr	E	O	H

Manifest File

The following entries are supported in the manifest text file describing the atlas:

`SPECIES = HUMAN`	The supported species include HUMAN, PRIMATE, PIG, RAT, MOUSE
`SPACE = MNI`	Only the MNI (Montreal Neurological Institute) space for humans is supported. If this line is present, a common set of template files with 2x2x2mm resolution is used.
`APPLICATION = NO_PNEURO`	If this line is present, the atlas is not listed in PNEURO.
`TYPE = PROBABILISTIC, RANGE 0 / 100`	The range specified for the data will be scaled to a probability value in the range [0,1]

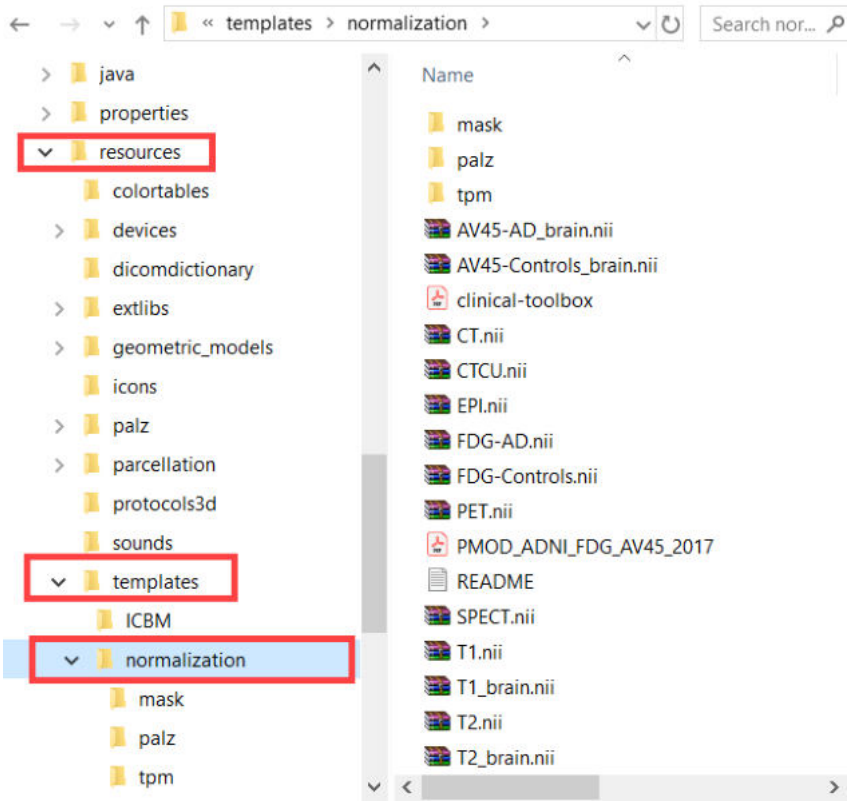
Spatial Normalization Methods

Atlases can only be applied to images if they have the same resolution and show the anatomy with the same geometry. Therefore, images originating from real experiments first need a normalization step for the atlas to be applied. This is done by calculating a normalization transform between the subject image and a "template" image representing the standard anatomy imaged with a certain modality, and using it for warping the VOIs to the subject anatomy.

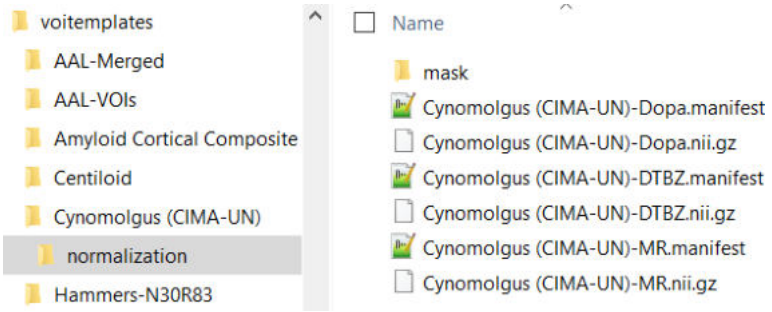
Name of method in PMOD	Methodology and template
Template-based normalization	Implementation of the spatial normalization in SPM5. It requires a template image in the atlas space which has a similar pattern as the image to normalize. The usual reference images are the PET, T1 and T2 SPM5 templates. The appropriate template has to be selected by the user.
3 Probability maps normalization	Implementation of the unified segmentation normalization in SPM8. It requires tissue probability maps of grey matter, white matter and CSF in the atlas space, which is organized in a dynamic file called **tpm.nii**. This normalization is mostly applicable to T_1-weighted MR images.
6 Probability maps normalization	Implementation of the unified segmentation normalization in SPM12. It uses three additional probability maps (bone, tissue and air), which are organized in a dynamic file **tpm_6.nii**. This normalization is mostly applicable to T_1-weighted MR images.

Template Files for Template-based Normalization

For atlases of the human brain anatomy which are defined in the 2x2x2mm MNI space and which have the entry `SPACE = MNI` defined in the manifest file, a common set of template files for the **Template-based normalization** is available in the *resources/templates/normalization* folder.



For atlases in other spaces than MNI corresponding normalization templates have to be included in a *normalization* sub-folder. As an example, for the **Cynomolgus (CIMA_UN)** atlas illustrated below FDOPA, DTBZ and an MRI template are available.

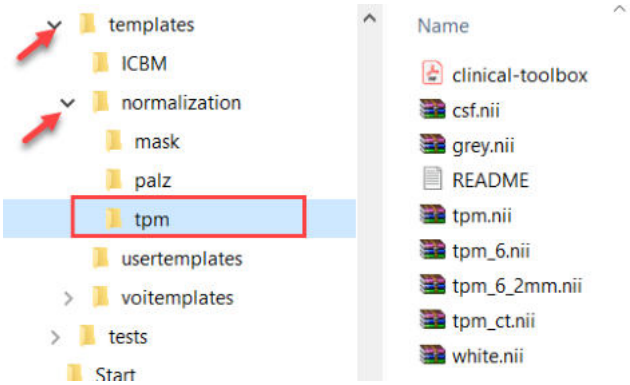


In the example above a manifest file with species definition is available for each normalization template.

Normalization works best if the information is restricted to the relevant image part. Therefore, *normalization* should contain a *mask* sub-folder with a mask file **mask.nii** containing 1 for all relevant pixels and 0 for all others.

Resource Files for Probability Normalization Methods

Again, for a human atlas defined in the MNI space the tissue probability maps are already available in the *templates/normalization/tpm* directory.



To use the methodology for other than MNI atlases, a *normalization/tpm* sub-directory has to be added to the atlas directory with the corresponding tissue probability maps.

3.2 N30R83 Maximum Probability Atlas

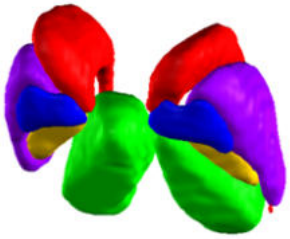
The methodology of the N30R83 maximum probability atlas used in PNEURO is described in detail in the publication by Hammers et al. [3]. The number of subjects in this paper was limited to 20 healthy subjects and 49 brain structures. The population and the number of covered structures was extended in a follow-up study by Gousias et al. [4] to include a total of 30 subjects and 83 brain structures, yielding the current N30R83 atlas. Population: 15 mal, 15 female, median age 31 years (range: 20-54), 25 of the 30 subjects strongly right handed.

Brief summary of the methodology used to construct the N30R83 atlas:

- A T_1-weighted anatomical MR image was acquired for every subject.
- For each MR image, the brain structures were manually outlined in consensus by two neuroanatomically trained operators.
- Each of the 83 brain structures was assigned a unique integer which was used as a label for all pixels belonging to a structure.
- The results of the outlining and the labeling were 30 individual label atlases in the native spaces of the subject MRIs.
- For each MRI, the stereotactic normalization to the MNI T1-MRI template was calculated and applied to the individual atlases for transforming them into the MNI space. Nearest-neighbor interpolation was a used to maintain unique label information.
- These normalizations resulted in 30 atlases representing the individual anatomies in the MNI space.
- For each pixel in the MNI brain mask the label statistics across the 30 individual atlases was calculated. The most frequent label was selected for creating the maximum probability atlas. If several labels had the same maximal frequency, the selection among them was random.
- This statistical analysis resulted in the N30R83 atlas in the MNI space which contains for every pixel a unique label number, corresponding to one of the 83 brain structures.

The illustration below shows a smoothed 3D surface rendering the 83 brain structures of the N30R83 atlas. It is clearly notable that the contours are not restricted to the gray matter pixels.

The deep nuclei N30R83 structures are illustrated below.



Note that there are two variants of the atlas available, with 2mm and 1mm sampling. For data with resolution below 2mm it is recommended using the 1mm variant to take advantage of the smoother anatomical definition.

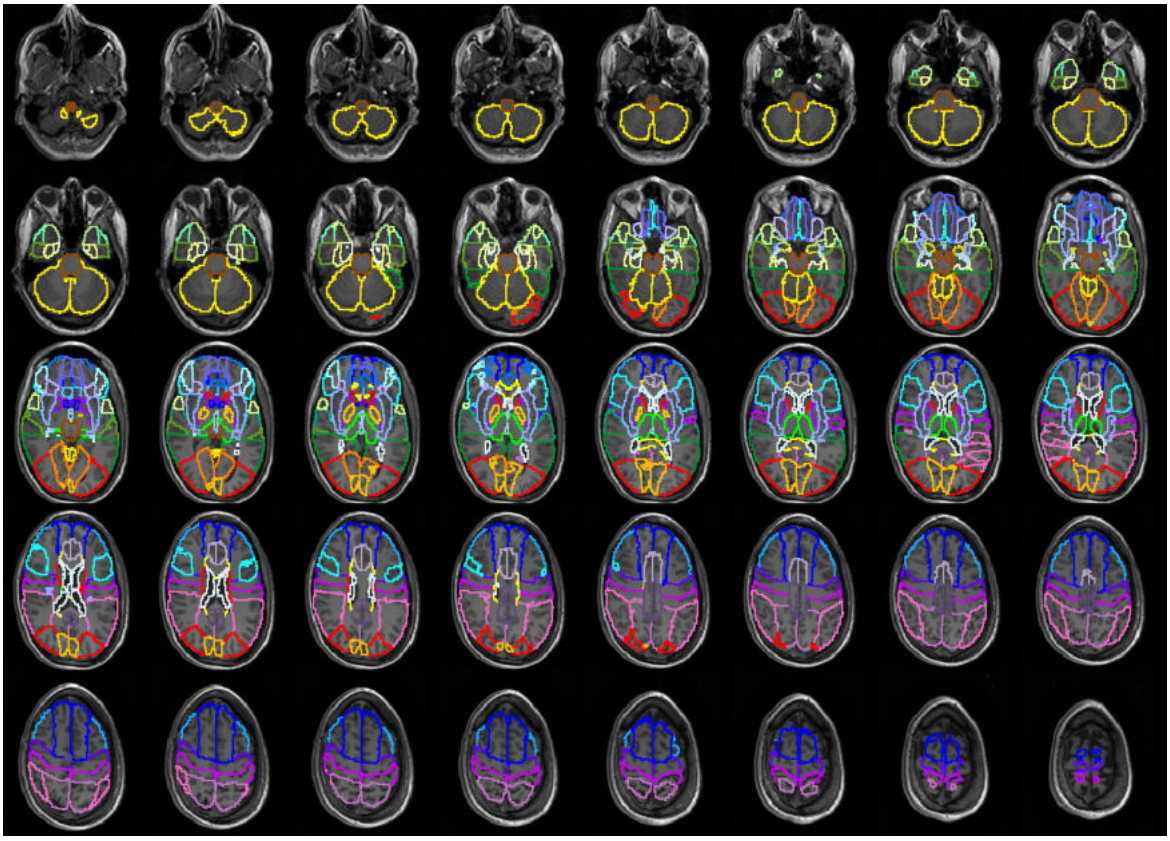
3.2.1 N30R83 Brain Structures

The following 83 brain structures are covered the N30R83 atlas. The even label numbers denote left structures, the uneven numbers right structures.

Temporal Lobe	
1; 2 3; 4 5; 6 7; 8 9; 10 11; 12 13; 14 15; 16 30; 31 82; 83	Hippocampus Amygdala Anterior temporal lobe, medial part anterior temporal lobe, lateral part Parahippocampal and ambient gyri Superior temporal gyrus, posterior part Middle and inferior temporal gyrus Fusiform gyrus Posterior temporal lobe Superior temporal gyrus, anterior part
Posterior Fossa	
17; 18 19	Cerebellum Brainstem
Insula and Cingulate Gyri	
20; 21 24; 25 26; 27	Insula Cingulate gyrus (gyrus cinguli), anterior part Cingulate gyurs (gyrus cinguli), posterior part
Frontal Lobe	

28; 29	Middle frontal gyrus
50; 51	Precentral gyrus
52; 53	Straight gyrus
54; 55	Anterior orbital gyrus
56; 57	Inferior frontal gyrus
58; 59	superior frontal gyrus
68; 69	Medial orbital gyrus
70; 71	Lateral orbital gyrus
72; 73	Posterior orbital gyrus
76; 77	Subgenual anterior cingulate gyrus
78; 79	Subcallosal area
80; 81	Pre-subgenual anterior cingulate gyrus
Occipital Lobe	
64; 65	Lingual gyrus
66; 67	Cuneus
22; 23	Lateral remainder of occipital lobe
Parietal Lobe	
60; 61	Postcentral gyrus
62; 63	Superior parietal gyrus
32; 33	Inferiolateral remainder of parietal lobe
Central Structures	
34; 35	Caudate nucleus
36; 37	Nucleus accumbens
38; 39	Putamen
40; 41	Thalamus
42; 43	Pallidum
44	Corpus callosum
74; 75	Substantia nigra
Ventricles	
45; 46	Lateral ventricle (excluding temporal horn)
47; 48	Lateral ventricle, temporal horn
49	Third ventricle

The example below illustrates the structures adjusted to a brain MRI. Only every third image slice is shown.



Intersection with Gray Matter Probability Map

The definition of the cortical brain structures in N30R83 is not limited to the gray matter. In order to trim the VOIs to brain pixels with a high likelihood of belonging to gray matter, the VOIs can be intersected with a gray matter probability mask thresholded at a certain probability level. It has been noted that this masking tends to excessively reduce the area of the central structures. Therefore, PNEURO offers the option to exclude the central structures from the intersection process. As the ventricular regions have no relation to the gray matter, they are always disregarded by the intersection.

3.3 AAL Single-Subject Atlas

The **AAL-VOIs** atlas is the automatic anatomical labeling result [5] of the spatially normalized, single subject, high resolution T_1 MRI data set provided by the Montreal Neurological Institute (MNI)[6]. It includes 120 structure definitions. By merging some small structures an **AAL-Merged** atlas was created. Both **AAL-VOIs** and **AAL-Merged** can be used alternatively to the N30R83 atlas. There is a slight asymmetry in the AAL VOIs which corresponds to the natural asymmetry of normal brains and which is also part of the MNI template.

In general, it should be noted that from a theoretical point of view a maximum probability atlas is preferable, because it accounts for the normal variation of the human brain anatomy.

3.3.1 AAL-VOIs Atlas

The following 120 brain structures are included in the AAL-VOIs atlas. The first column indicates the label number, the second the name and abbreviation). For paired structures the first and second numbers refer to the left and right part, respectively.

Temporal Lobe

4101; 4102	Hippocampus (HIP)
4111; 4112	Parahippocampus (PHIP)
4201; 4202	Amygdala (AMYG)
5401; 5402	Fusiform gyrus (FUSI)
8101; 8102	Heschl gyrus (HES)
8111;8112	Superior temporal gyrus (T1)
8121; 8122	Temporal pole: superior temporal gyrus (T1P)
8201; 8202	Middle temporal gyrus (T2)
8211; 8212	Temporal pole: middle temporal gyrus (T2P)
8301: 8302	Inferior temporal gyrus (T3)
Posterior Fossa	
9001; 9002	Cerebellum crus 1
9011; 9012	Cerebellum crus 2
9021; 9022	Cerebellum 3
9031; 9032	Cerebellum 4 5
9041; 9042	Cerebellum 6
9051; 9052	Cerebellum 7
9061; 9062	Cerebellum 8
9071; 9072	Cerebellum 9
9081; 9082	Cerebellum 10
9100	Vermis 12
9110	Vermis 3
9120	Vermis 4 5
9130	Vermis 6
9140	Vermis 7
9150	Vermis 8
9160	Vermis 9
9170	Vermis 10
	Cerebellar white matter (added by PNEURO)
	Medulla (added by PNEURO)
	Midbrain (added by PNEURO)
	Pons (added by PNEURO)
Insula and Cingulate Gyri	
3001; 3002	Insula (IN)
4001; 4002	Cingulate gyrus, anterior part (ACIN)
4011; 4012	Cingulate gyrus, mid part (MCIN)
4021; 4022	Cingulate gyurs, posterior part (PCIN)
Frontal Lobe	
2001; 2002	Precentral gyrus (PRE)
2101; 2102	Superior frontal gyrus, dorsolateral (F1)
2111; 2112	Superior frontal gyrus, orbital (F1O)
2201; 2202	Middle frontal gyrus (F2)
2211; 2212	Middle frontal gyrus, orbital (F2O)
2301; 2302	Inferior frontal gyrus, opercular (F3OP)
2311; 2312	Inferior frontal gyrus, triangular (F3T)
2321; 2322	Inferior frontal gyrus, orbital (F3O)
2331; 2332	Rolandic operculum (RO)
2401; 2402	Supplementary motor area (SMA)
2501; 2502	Olfactory cortex (OC)
2601; 2602	Superior frontal gyrus, medial (F1M)
2611; 2612	Superior frontal gyrus, medial orbital (F1MO)
2701; 2702	Gyrus rectus (GR)
6401; 6402	Paracentral lobule (PCL)
Occipital Lobe	

4001; 4002 5011; 5012 5021; 5022 5101; 5102 5201; 5202 5301; 5302	Calcarine fissure and surrounding cortex (V1) Cuneus (Q) Lingual gyrus (LING) Superior occipital lobe (O1) Middle occipital lobe (O2) Inferior occipital lobe (O3)
Parietal Lobe	
6001; 6002 6101; 6102 6202; 6203 6211; 6212 6221; 6222 6301; 6302	Postcentral gyrus (POST) Superior parietal gyrus (P1) Inferior parietal gyrus (P2) Supramarginal gyrus (SMG) Angular gyrus (AG) Precuneus (PQ)
Central Structures	
7001; 7002 7011; 7012 7021; 7022 7101; 7102	Caudate nucleus (CAU) Putamen (PUT) Pallidum (PAL) Thalamus (THA)

3.3.2 AAL-Merged Atlas

The following 71 brain structures are covered the AAL-Merged atlas. For paired structures the first and second numbers refer to the left and right part, respectively.

Temporal Lobe	
65; 66 29; 30 27; 28 39; 40 61; 62	Temporal, superior, mid, inferior, poles (T1, T1A, T2, T2A, T3) Amygdala (AMYG) Hippocampus and parahippocampus (HIP, PHIP Fusiform gyrus (FUSI) Heschl gyrus (HES)
Posterior Fossa	
67 68; 69 70; 71	Vermis Cerebellum crus Cerebellum Cerebellar white matter (added by PNEURO) Medulla (added by PNEURO) Midbrain (added by PNEURO) Pons (added by PNEURO)
Insula and Cingulate Gyri	
19; 20 21; 22 23; 24 25; 26	Insula (IN) Cingulate gyrus, anterior part (ACIN) Cingulate gyrus, mid part (MCIN) Cingulate gyurs, posterior part (PCIN)
Frontal Lobe	
1; 2 3; 4 5; 6 7; 8 11; 12	Precentral gyrus (PRE) Rolandic operculum (RO) Supplementary motor area (SMA) Olfactory cortex (OC) Superior frontal gyrus (F1, F1O, F1M)

13; 14 15; 16 17; 8 51; 52	Middle frontal gyrus (F2, F2O, FMO) Inferior frontal gyrus (F3OP, F3T, F3O) Gyrus rectus (GR) Paracentral lobule (PCL)
Occipital Lobe	
31; 32 33; 34 35; 36 37; 38	Calcarine fissure and surrounding cortex (V1) Cuneus (Q) Lingual gyrus (LING) Lateral remainder of occipital lobe (O1, O2, O3)
Parietal Lobe	
41; 42 45; 46 47; 48 49; 50 63; 64	Postcentral gyrus (POST) Supramarginal gyrus (SMG) Angular gyrus (AG) Precuneus (PQ) Parietal, superior and inferior (P1, P2)
Central Structures	
53; 54 55; 56 57; 58 59; 60	Caudate nucleus (CAU) Putamen (PUT) Pallidum (PAL) Thalamus (THAL)

In comparison to the the original AAL atlas the following subregions were pooled:

- Vermis: Vermis_1_2, Vermis_3, Vermis_4_5, Vermis_6, Vermis_7, Vermis_8, Vermis_9, Vermis_10.
- Cerebellum crus: Cerebellum_Crus1, Cerebellum_Crus2.
- Cerebellum: Cerebellum_3, Cerebellum_4_5, Cerebellum_6, Cerebellum_7b, Cerebellum_8, Cerebellum_9, Cerebellum_10.
- Frontal Mid: Frontal_Mid, Frontal_Mid_Orb, Frontal_Med_Orb.
- Frontal Sup: Frontal_Sup, Frontal_Sup_Orb, Frontal_Sup_Medial.
- Frontal Inf: Frontal_Inf_Oper, Frontal_Inf_Tri, Frontal_Inf_Orb.
- Hippocampus and parahippocampus.
- Occipital: Occipital_Sup, Occipital_Mid, Occipital_Inf.
- Parietal: Parietal_Sup, Parietal_Inf.
- Temporal: Temporal_Sup, Temporal_Pole_Sup, Temporal_Mid, Temporal_Pole_Mid, Temporal_Inf.

3.4 Amyloid Cortical Composite Atlas

In the last years the analysis of the amyloid deposition in the brain using composite cortical VOI became common. There have been slight variations regarding which brain regions are included in amyloid cortical composites. The most common amyloid cortical composite includes frontal, temporal, and parietal cortices, including cingulate regions as well as precuneus [1-8]. These amyloid cortical composite regions have been published and validated using various amyloid PET radioligands such as and not limited to [11C]PIB, [18F]Florbetapir (Amyvid, Eli Lilly), [18F] flutemetamol (Vizamyl, GE Healthcare) and [18F]florbetaben (NeuraCeq, Piramal Pharma).

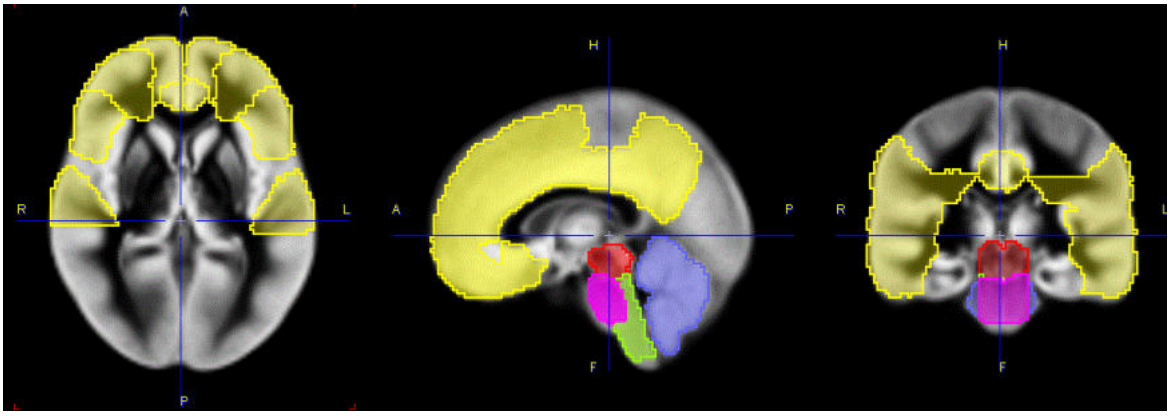
Various reference regions have been used to calculate standardized uptake value ratio (SUVr) such as whole cerebellum, cerebellar grey matter, pons, brainstem and white matter. In addition, an amyloid cortical composite combined with a composite reference region (e.g., whole cerebellum, brainstem/pons and white matter) has been shown to improve the longitudinal measures of amyloid [3, 9].

Thus, we provide our users the most common pre-defined amyloid cortical composite template including various reference regions used for calculating SUVr.

VOI Atlas

The VOI atlas **Amyloid Cortical Composite** can be selected in the list of included VOI atlases. The corresponding files can be found in the *resources/templates/voitemplates/Amyloid Cortical Composite* directory.

The capture below illustrates the VOIs structures overlaid on the gray matter probability map in MNI space.



There are 5 brain structures included in the Amyloid Cortical Composite atlas. In comparison to the the original Hammers N30R83 atlas the *Cortical Composite* and the *Cerebellum* listed structures were created pooling the specified regions. The even label numbers denote left structures, the uneven numbers right structures in the Hammers N30R83 atlas. The *Midbrain*, *Medulla Oblongata* and the *Pons* were manually segmented from the original Hammers N30R83 Brainstem structure. The segmentation was performed in such manner to avoid contour overlapping in the new structures.

1. Cortical Composite [1-9]	
28; 29 52; 53 58; 59 68; 69 70; 71 72; 73 76; 77 78; 79	Middle frontal gyrus Straight gyrus Superior frontal gyrus Medial orbital gyrus Lateral orbital gyrus Posterior orbital gyrus Subgenual anterior cingulate gyrus Subcallosal area
11; 12 13; 14 82; 83	Superior temporal gyrus posterior part Middle and inferior temporal gyrus Superior temporal gyrus, anterior part
62; 63 32; 33	Superior parietal gyrus Inferiolateral remainder of parietal lobe
24; 25 26; 27	Cingulate gyrus (gyrus cinguli), anterior part Cingulate gyurs (gyrus cinguli), posterior part
2. Cerebellum	
17; 18	Cerebellum
3. Midbrain *	
19	Structure of the Brainstem

4. Medulla Oblongata *	
19	Structure of the Brainstem
5. Pons *	
19	Structure of the Brainstem

References

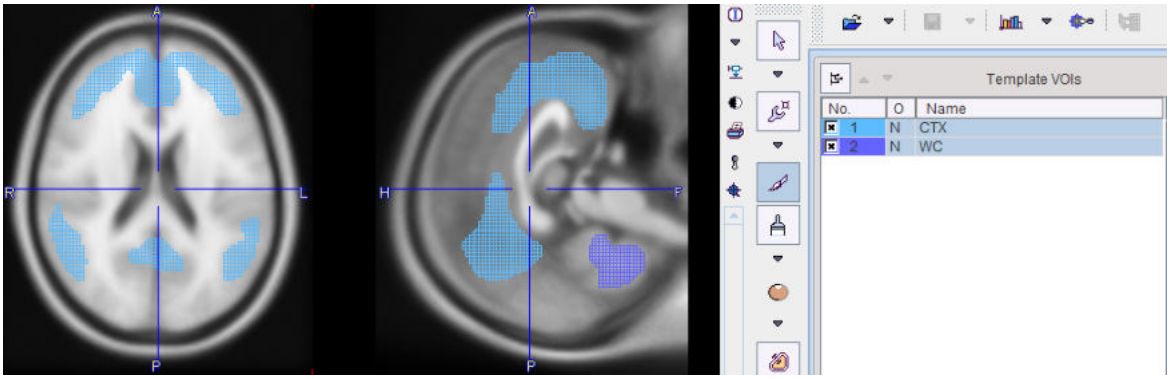
1. Hutton, C., et al., Quantification of [18F]florbetapir PET: comparison of two analysis methods. Eur J Nucl Med Mol Imaging, 2015. 42(5): p. 725-32.
2. Johnson, K.A., et al., Florbetapir [18F]AV-45) PET to assess amyloid burden in Alzheimer's disease dementia, mild cognitive impairment, and normal aging. Alzheimers Dement, 2013. 9(5 Suppl): p. S72-83.
3. Landau, S.M., et al., Amyloid-beta imaging with Pittsburgh compound B and florbetapir: comparing radiotracers and quantification methods. J Nucl Med, 2013. 54(1): p. 70-7.
4. Liu, E., et al., Amyloid-beta [11C]PiB-PET imaging results from 2 randomized bapineuzumab phase 3 AD trials. Neurology, 2015. 85(8): p. 692-700.
5. Nayate, A.P., et al., Use of Standardized Uptake Value Ratios Decreases Interreader Variability of [18F]Florbetapir PET Brain Scan Interpretation. AJNR Am J Neuroradiol, 2015. 36(7): p. 1237-44.
6. Ostrowitzki, S., et al., Mechanism of amyloid removal in subjects with Alzheimer disease treated with gantenerumab. Arch Neurol, 2012. 69(2): p. 198-207.
7. Sevigny, J., et al., Amyloid PET Screening for Enrichment of Early-Stage Alzheimer Disease Clinical Trials: Experience in a Phase 1b Clinical Trial. Alzheimer Dis Assoc Disord, 2016. 30(1): p. 1-7.
8. Wolk, D.A., et al., Amyloid imaging in Alzheimer's disease: comparison of florbetapir and Pittsburgh compound-B positron emission tomography. J Neurol Neurosurg Psychiatry, 2012. 83(9): p. 923-6.
9. Landau, S.M.a.J., W., Florbetapir processing methods. ADNI PET core, 2015.

3.5 Centiloid Atlas

“Centiloid” was introduced as a “standard” method for the quantification of the amyloid load by Klunk et al. [1]. It uses a lumped SUVr value obtained with PiB PET as the standard, and describes a scaling method to transform the outcome using other amyloid PET tracers into a comparable measure.

The lumped SUVr is defined by a cortical target region **CTX** and a reference region in the MNI space. **CTX** is a data-driven cortex VOI which includes the typical brain regions with high amyloid load in Alzheimer's Disease including the frontal, temporal and parietal cortices, precuneus, the anterior striatum and insular cortex. While different reference regions were tested, use of whole cerebellum **WC** is the final recommendation.

The **Centiloid** atlas in PMOD only includes the **CTX** and **WC** VOIs, as illustrated below on top of the ICBM152T1 template. Note that the locations are correct, although they don't follow the anatomical boundaries. This behavior is due to the PET data-driven process for their derivation, and has been confirmed by the authors.



Reference

1. Klunk WE, Koeppe RA, Price JC, Benzinger TL, Devous MD Sr, Jagust WJ, Johnson KA, Mathis CA, Minhas D, Pontecorvo MJ, Rowe CC, Skovronsky DM, Mintun MA. The Centiloid Project: standardizing quantitative amyloid plaque estimation by PET. Alzheimers Dement. 2015; 11(1):1-15.e1-4. DOI:

3.6 Cynomolgus Monkey Atlas

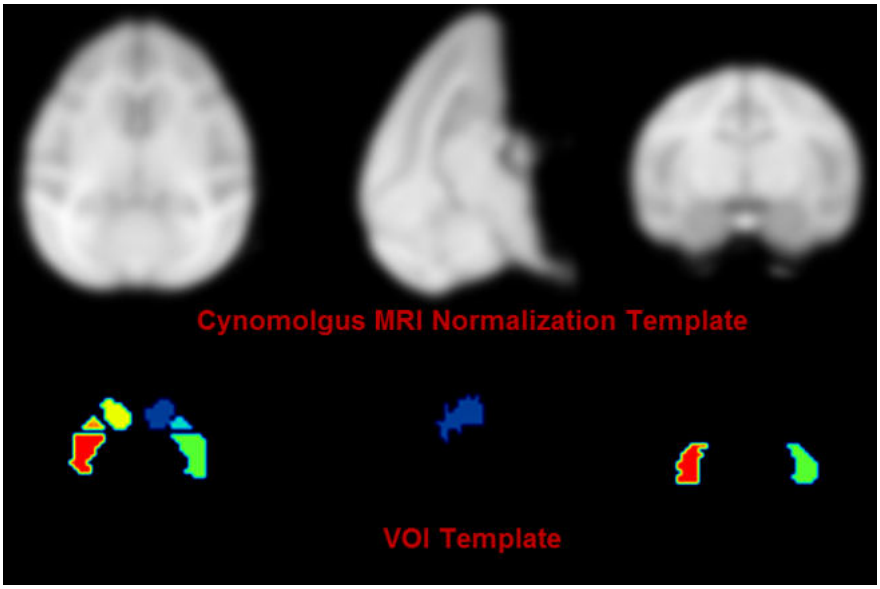
For the analysis of cynomolgus monkey (Macaca Fascialis) brain data the **Cynomolgus_CIMA-UN** VOI atlas [1] is available. We would like to thank Elena Prieto and Maria Collantes from the Centro de Investigacion Medica Aplicada (CIMA), Universidad de Navarra, for providing the data and helping with the integrations.

Spatial Normalization

A T_1-weighted MRI normalization template created from 15 healthy animals is available as **Cynomolgus_CIMA-UN-MRI**. Two PET templates in the same space are also provided: **Cynomolgus_CIMA-UN-Dopa** and **Cynomolgus_CIMA-UN-DTBZ**. These images are located in the *resources/templates/voitemplates/Cynomolgus (CIMA-UN)/ normalization* folder.

VOI Atlas

The VOIs were hand drawn in the striatum (VOI size 400 mm³) and occipital lobe (VOI size 310 mm³) on axial MRI slices based on anatomical borders.

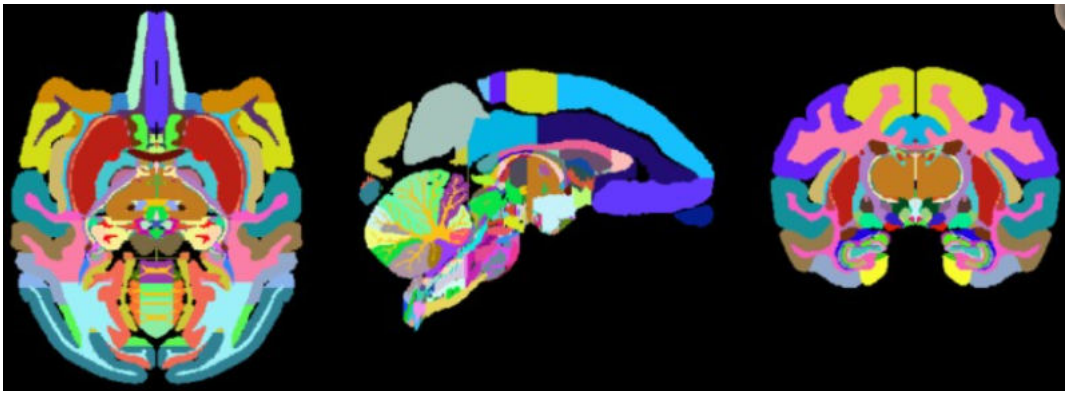


Reference

[1] Collantes M, Prieto E, Penuelas I, Blesa J, Juri C, Marti-Climent JM, Quincoces G, Arbizu J, Riverol M, Zubieta JL, Rodriguez-Oroz MC, Luquin MR, Richter JA, Obeso JA. New MRI, 18F-DOPA and 11C-(+)-alpha-dihydrotetrabenazine templates for Macaca fascicularis neuroimaging: advantages to improve PET quantification. Neuroimage. 2009;47(2):533-9. DOI

3.7 Rhesus Macaque (INIA19)

The INIA19 atlas has been developed for the rhesus macaque by Rohlfing et al. [1] and made available to the scientific community (http://nitrc.org/projects/inia19/). It includes 1014 VOI definitions, an MR template for template-based normalization and three tissue probability maps for normalization using the unified segmentation approach.



The atlas was created from high-resolution, T1-weighted MR images of 19 rhesus macaque (Macaca mulatta) animals. Combined with the comprehensive cortical and sub-cortical label map, the INIA19 is equally suitable for studies requiring both spatial normalization and atlas label propagation.

Reference

1. Rohlfing T, Kroenke CD, Sullivan EV, Dubach MF, Bowden DM, Grant KA, Pfefferbaum A: The INIA19 Template and NeuroMaps Atlas for Primate Brain Image Parcellation and Spatial Normalization. Frontiers in neuroinformatics 2012, 6:27. DOI

3.8 Domestic Pig / Minipig Brain Atlas (CH.Malbert)

Overview

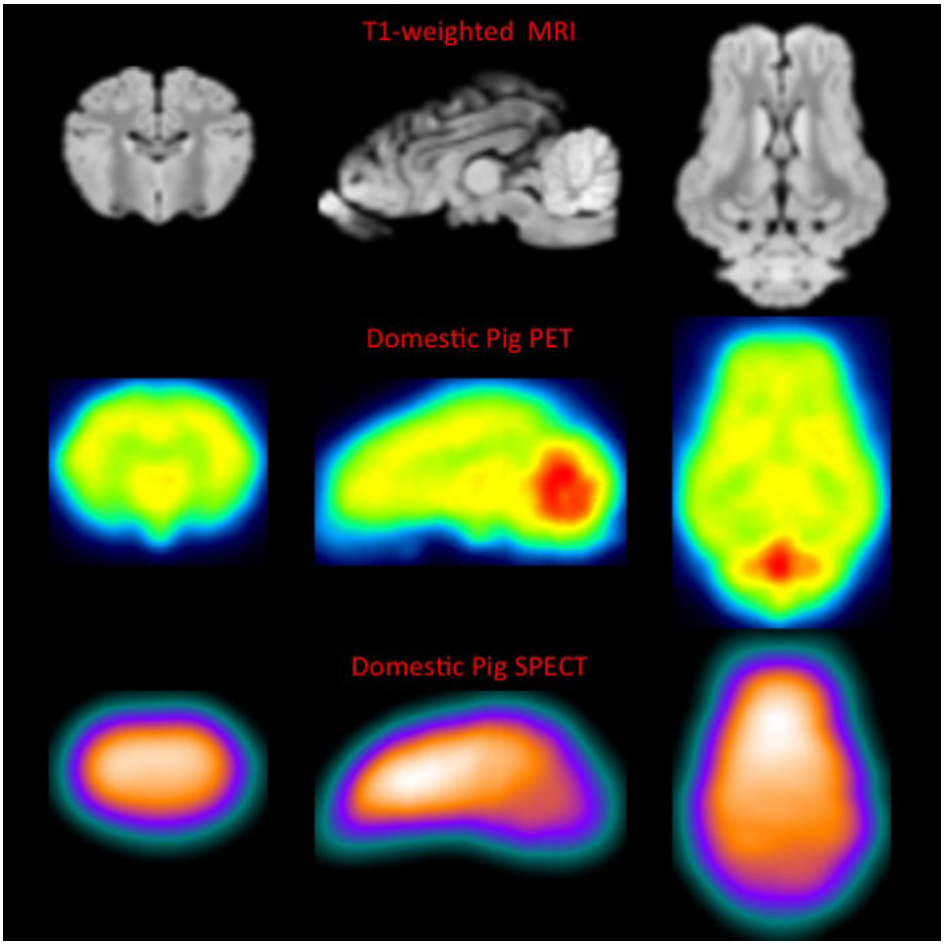
The **Pig (CH.Malbert)** atlas is based on brain scans of 5 6-month-old female domestic pigs (Sus scrofa domesticus). An ex vivo T2-weighted MRI was used by Saikali et al. [1] to construct the atlas, and a T1 MRI is provided for image coregistration. Subsequently, the same group constructed a 99mTc-HMPAO SPECT template [2] and FDG PET template [3] in the same image space. Additionally, an FDG PET template for Yucatan miniature pigs was developed in the same image space as the domestic pig atlas [4]. The atlas was initially constructed at high resolution (100 x 150 x 100 um) in one hemisphere, and then mirrored to create the full brain atlas. The VOI atlas contains 173 cortical and subcortical regions (reduced from 178 in the original high resolution atlas). The atlas is distributed with PMOD by courtesy of Dr. Charles-Henri Malbert, director of research at the Unité Ani-Scan, Département de Nutrition Humaine, INRA, France.

Spatial Normalization

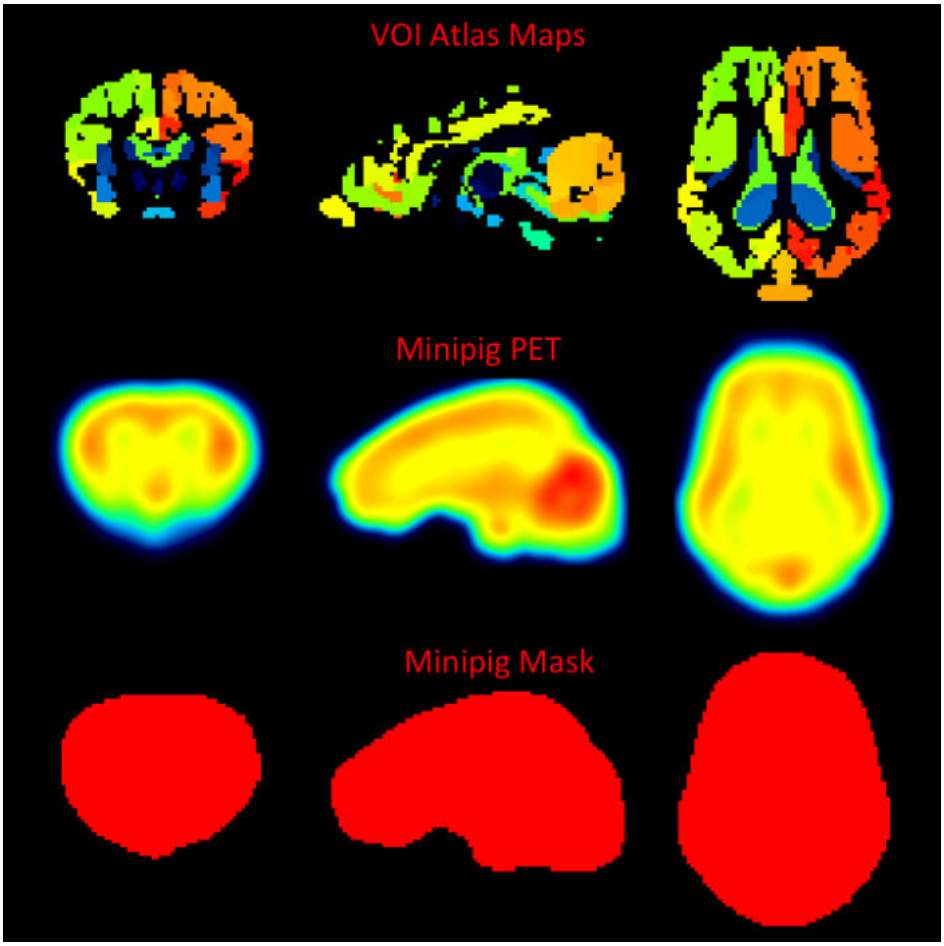
Four normalization templates are available in the Fuse It tool. They can be selected on the Reference page using the Template button when Pig is selected as Species:

- **Pig (CH.Malbert)-FDG**: This is an FDG PET template derived from static scans in 4 month old domestic pigs, shown below.
- **Pig 2y (CH.Malbert)-FDG**: This is an FDG PET template derived from dynamic scans in 2 year old minipigs, shown below. Correspondence to the domestic pig template and MRI is high.
- **Pig (CH.Malbert)-SPECT:** This is a 99mTc-HMPAO SPECT template derived from scans in 4 month old domestic pigs.
- **Pig (CH.Malbert)-T1**: This is a T1-weighted MRI PET template in the same image space as the PET templates. The VOI atlas was derived from this image.

The image files corresponding to these templates can be found in the *resources/templates/voitemplates/Pig (CH.Malbert)/normalization* folder. Additionally, mask files corresponding to each template, and tissue probability maps are available.

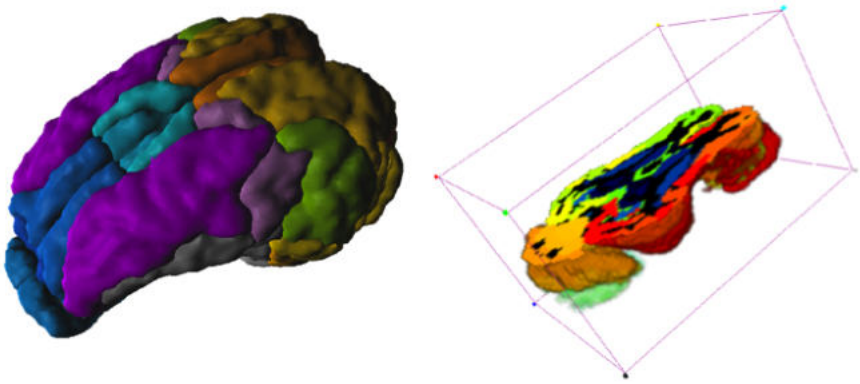


Selection and generation of appropriate masks is critical to successful spatial normalization with these templates. The mask corresponding to the template selected should be identified in the **Normalization Settings** dialog. The PBAS functionality (e.g. via the **View** tool) can be used to generate a mask for the input image. Accurate exclusion of activity outside the brain, and the olfactory bulbs, is necessary. This user-defined mask should also be identified in the **Normalization Settings** dialog.



VOI Atlas

The VOI atlas Pig (CH.Malbert) can be selected in the list of included VOI atlases. The corresponding map files in Nifti format can be found in the *resources/templates/voitemplates/Pig (CH.Malbert)* directory. The brain VOIs are structurally organized in a tree on the **Group** tab of the VOI editing page. The selection of a VOI subset is supported by a dedicated **user interface**[22].



References

1. Saikali S, Meurice P, Sauleau P, Eliat PA, Bellaud P, Randuineau G, Vérin M, Malbert CH. A three-dimensional digital segmented and deformable brain atlas of the domestic pig. J Neurosci Methods. 2010; 192(1):102-9. **DOI** link: http://dx.doi.org/10.1016/j.jneumeth.2010.07.041.

2. Boubaker J, Val-Laillet D, Guérin S, Malbert CH. Brain processing of duodenal and portal glucose sensing. J Neuroendocrinol. 2012 Aug;24(8):1096-105. DOI.
3. Gaultier A, Meunier-Salaün MC, Malbert CH, Val-Laillet D. Flavour exposures after conditioned aversion or preference trigger different brain processes in anaesthetised pigs. Eur J Neurosci. 2011 Nov;34(9):1500-11. DOI.
4. Ochoa M, Malbert CH, Meurice P, Val-Laillet D. Effects of Chronic Consumption of Sugar-Enriched Diets on Brain Metabolism and Insulin Sensitivity in Adult Yucatan Minipigs. PLoS One. 2016 Sep 1;11(9):e0161228. DOI.

4 Brain VOIs Based on Maximum Probability Atlas

The functionality of this module was initially developed for use with the N30R83 maximum probability atlas of brain structures [3,4], which was officially licensed from Imperial College London, UK. Meanwhile, however, additional atlases can also be used in the same manner.

Given brain PET and/or MR images, it allows the user to calculate objective brain structure outlines in a guided step-by-step fashion. These definitions can then be applied to PET images for the calculation of regional statistics or Time-Activity Curves (TAC), either of which may optionally be corrected for partial-volume effect.

4.1 Maximum Probability Atlas Implementation in PNEURO

In PNEURO the use of the N30R83 atlas (and the other atlases with PET and MR normalization templates) is supported in four situations, namely studies with PET and T_1-MR, studies with a functional and an "anatomical" PET, PET-only studies, and MR-only studies. The corresponding workflows are outlined in the following.

Analysis of Study with PET and T1-weighted MRI

This is the most accurate workflow. The other workflows are essentially adapted subsets.

1. Loading of the PET image series which may be static or dynamic.
2. Dynamic PET case: Averaging of the PET series in a specified acquisition range. The averaged PET image is used in the following for all steps except for the statistics calculation.
3. Loading of the T_1-weighted MR image series.
4. Calculation of the individual gray matter probability map by segmentation of the MR image. This only possible if the atlas definition includes tissue probability maps.
5. Splitting of the brain into the left and right hemispheres as well as cerebellum. This step is relevant for separating the white matter parts correspondingly.
6. Rigid matching of the PET image to the MR image and interactive visual assessment of the alignment by the user.
7. Spatial normalization of the MR image to the MNI T_1 template and interactive visual assessment of the alignment by the user.
8. Transformation of the label atlas to the MR space and display of the result as an overlay on the MR image.
9. Intersection of the cortical structures with the gray matter probability map at a user-defined probability level.
10. Calculation of the outline contours of the masked structures. They are presented in a VOI editor together with the MR images, so that the user can adjust them interactively and save the final VOI set.
11. Application of the VOIs to the matched PET series for calculating statistics. This results in TACs in the case of a dynamic PET series, and simple statistics otherwise. Optionally, a partial-volume correction can be applied during the statistics calculation.
12. Dynamic PET case: The resulting TACs can directly be transferred to the kinetic modeling tool. Alternatively, pixel-wise models can be applied for parametric mapping.

Note that instead of defining the VOIs in the MR space as described above, they may be defined in the atlas or the PET space, and the statistics calculated after transforming the PET series into the selected space.

Analysis of Study with Functional and Anatomical PET

If no T_1-weighted MRI is available, but an additional PET with more anatomical information (e.g. FDG), the role of the MRI for the normalization can be taken over by the "anatomical" PET (called "FDG PET" in the following).

1. Loading of the PET image series which may be static or dynamic.
2. Dynamic PET case: Averaging of the PET series in a specified acquisition range. The averaged PET image is used in the following for all steps except for the statistics calculation.
3. Loading of the FDG PET image series.
4. Rigid matching of the two PET images, and interactive visual assessment of the alignment by the user.
5. Spatial normalization of the FDG PET image to the MNI PET template and interactive visual assessment of the alignment by the user. Optionally, a user-defined normalization template may be used instead of the standard MNI PET template.
6. The label atlas is transformed to the subject space and shown as an overlay on the PET image.
7. The cortical structures in the transformed label atlas can be intersected with a standard gray matter probability map (if available for the atlas) which has been transformed to the subject space. The user can define the probability level used for masking, and whether the non-cortical structures are masked or not.
8. The structures resulting from transforming and masking are outlined and shown in a VOI editor, so that the user can adjust them interactively and save the final VOI set.
9. The VOIs are applied to the PET series for calculating statistics. This results in TACs for a dynamic PET series, and simple statistics otherwise. Optionally, a partial-volume correction can be applied during the statistics calculation.
10. Dynamic PET case: The resulting TACs can directly be transferred to the kinetic modeling tool, or alternatively pixel-wise models applied for parametric mapping.

As an alternative to the workflow described above which performs the calculations in the individual's anatomy on the original PET images, the PET images can be transformed to the atlas space and all calculations performed in analogy.

Analysis of PET-only Study

If an anatomical image series is lacking the processing sequence reduces to the following steps:

1. Loading of the PET image series which may be static or dynamic.
2. Dynamic PET case: Averaging of the PET series in a specified acquisition range. The averaged PET image is used in the following for all steps except for the final statistics calculation.
3. Spatial normalization of the PET image to the atlas PET template. The user has to visually check that the transformed PET image is in reasonable spatial alignment with the template. If this is the case, the normalization transform establishes a bidirectional mapping between the space of the subject and the template. Optionally, a user-defined normalization template may be used instead of the standard atlas PET template.
4. The label atlas is transformed to the subject space and shown as an overlay on the PET image.
5. The cortical structures in the transformed label atlas can be intersected with a standard gray matter probability map (if available for the atlas) which has been transformed to the subject space. The user can define the probability level used for masking, and whether the central structures are masked or not.
6. The structures resulting from transforming and masking are outlined and shown in a VOI editor, so that the user can adjust them interactively and save the final VOI set.
7. The VOIs are applied to the PET series for calculating statistics. This results in TACs for a dynamic PET series, and simple statistics otherwise. Optionally, a partial-volume correction can be applied during the statistics calculation.

8. Dynamic PET case: The resulting TACs can directly be transferred to the kinetic modeling tool, or alternatively pixel-wise models applied for parametric mapping.

As an alternative to the workflow described above which performs the calculations in the individual's anatomy on the original PET images, the PET images can be transformed to the atlas space and all calculations performed in analogy.

Analysis of MR-only Study

In this case the workflow reduces to the following steps:

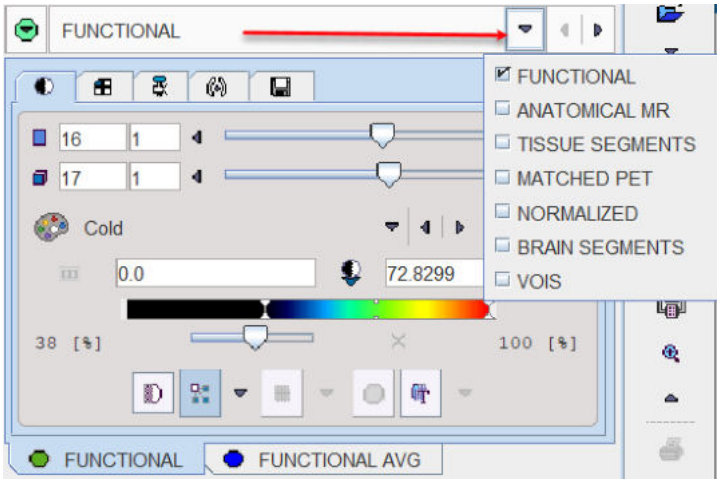
1. Loading of the T_1-weighted MR image series.
2. Calculation of gray matter probability maps by segmentation of the MR image.
3. Spatial normalization of the MR image to the atlas T_1 template and interactive visual assessment of the alignment by the user.
4. Transformation of the label atlas to the MR space and display of the result as an overlay on the MR image.
5. Intersection of the cortical structures with the gray matter probability map at a user-defined probability level.
6. Calculation of the outline contours of the masked structures. They are presented in a VOI editor together with the MR images, so that the user can adjust them interactively and save the final VOI set.
7. Application of the VOIs for calculating statistics. In the absence of PET the statistics are reduced to the calculation of the VOI volume.

Documentation of the Workflows

The implementation of the workflows described above in PNEURO is very similar. Therefore, only the first workflow with PET and a T_1-weighted MRI will be described in full detail, while the others are restricted to the essential parts. Please refer to the first workflow description[58] if any questions arise.

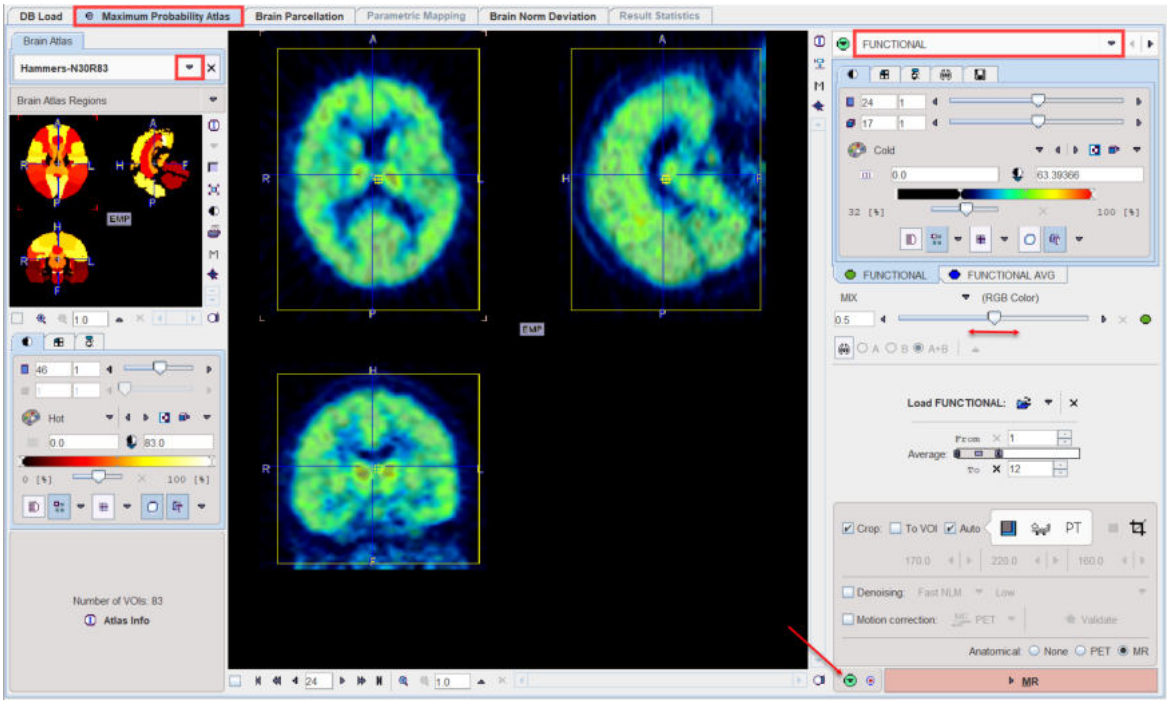
4.2 Workflow for Studies with PET and MRI

The workflow will run through the following pages of the **Maximum Probability Atlas** module:



4.2.1 PET Image Loading and Time Averaging

Stepwise processing is started by selecting the **Maximum Probability Atlas** tab.



The **Load FUNCTIONAL** button for loading the PET images is located in the right control area. As usual it is an option button which needs to be set to the appropriate data format with the indicated arrow. For loading images which are not saved in a PMOD database i is recommended to use the **Autodetect** format. Note that the PET series may be static or dynamic.

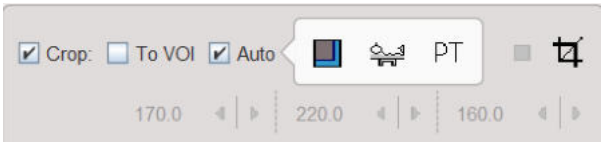
In the case of a dynamic PET series, a new series is generated by averaging a range of frames and assigned to the **FUNCTIONAL AVG** tab. The averaging range can be defined by the **From** and **To** number fields, or dragging the range indicators in the **Average** bar. After any modification of the range, the average is recalculated and the display updated.

The original and the averaged images are shown in a fusion rendering which can be controlled in the area below the controls of the individual images.

The aim of the averaging is to generate an image with as detailed anatomy as possible for the rigid matching with the MR image.

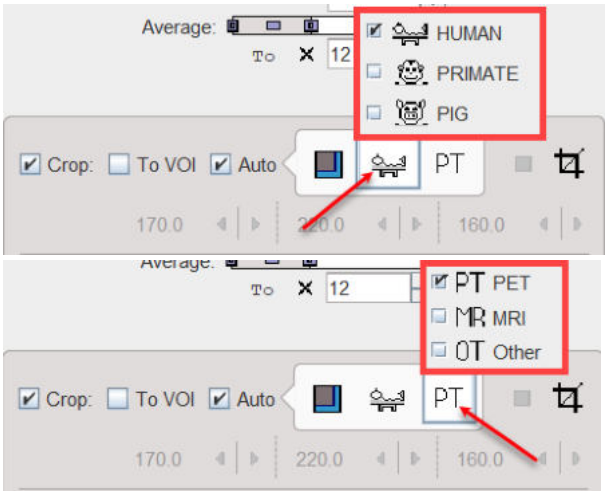
PET Image Cropping

If the PET field-of-view is larger than the brain, the data set should be reduced in order to save RAM and optimize processing. This can be achieved by enabling the **Crop** box and defining a crop box which appears as yellow rectangles in the image overlay.



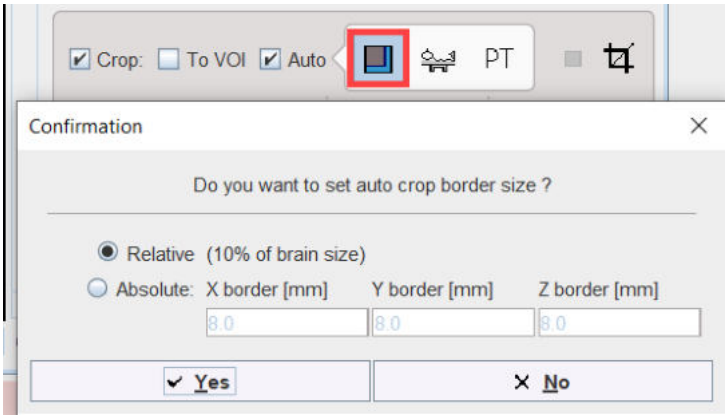
Note: The cropping elements are only shown when the option area is expanded by the colored arrow indicated above.

There is an automatic cropping function available. It is based on the matching of brain templates to the images. To this end the species and the modality have to be set properly by the configuration buttons illustrated below.

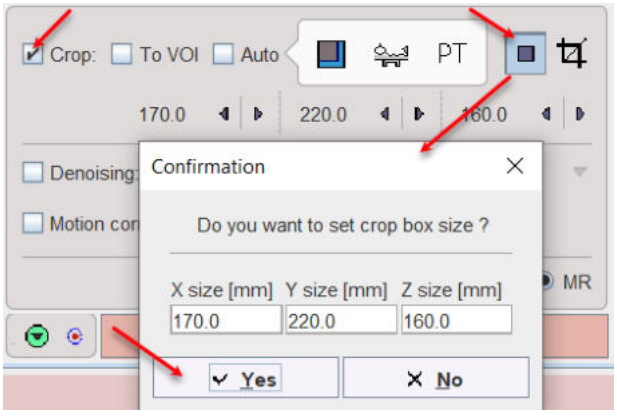


As soon as the **Auto** option is checked, the process is started. It results in the placement of the yellow cropping box, which can be inspected by the user.

Non-specific tracer uptake outside the brain itself can still be of use in rigid matching to the MRI, particularly the nose area. The box-shaped icon to the right of the **Auto** checkbox opens a dialog for definition of the auto crop border size. Two options are supported: the **Relative** selection sets the border size at a fix value of 10% of the brain size while the **Absolute** method allows precise mm extension in x/y/z:



Alternatively, the crop box can be set manually as follows. Make sure the **Auto** box is disabled. Place the yellow crop box by clicking at the brain center. The brain should be fully enclosed. If this is not the case, the edge size in **[mm]** can be adjusted for each direction by selecting the size in the corresponding list. An alternative is entering the edge sizes using the button indicated below.

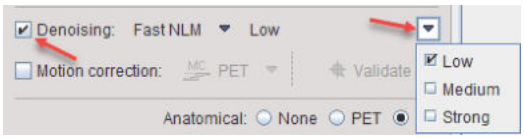


The **To VOI** check box requires the crop box to be defined and saved beforehand (e.g. using the View module) and associated with the image in the database.

The actual cropping has to be started explicitly with the button. Otherwise, a confirmation will be shown when proceeding to the next analysis step. Note that cropping cannot be repeated.

Image Denoising

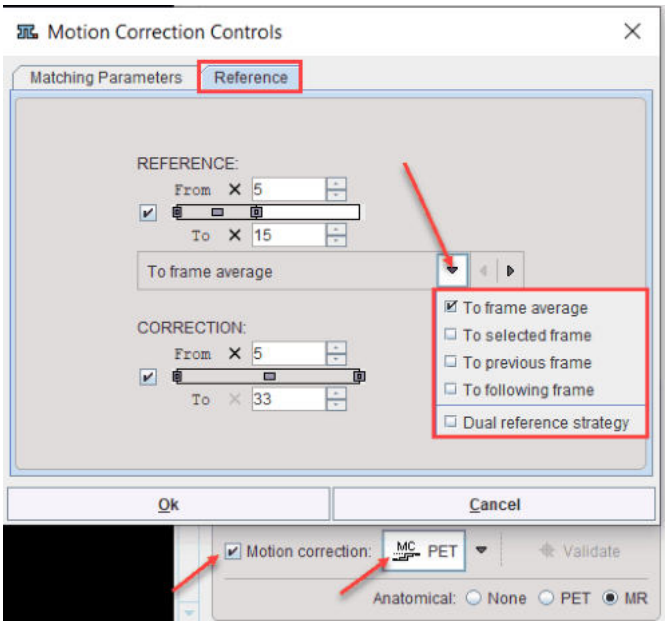
If the functional image is very noisy the data set should be denoised in order to improve the results of further steps such as segmentation, matching or quantification. This can be achieved by enabling the **Denoising** box and defining the level of denoising, **Low**, **Medium** or **Strong**, for the non-local means **Fast NLM** algorithm:



Note that **Denoising** cannot be repeated.

Motion Correction

The dynamic PET acquisition may be affected by motion. The data has to be inspected in order to identify where the motion occurs. This can be easily achieved by stepping through the frames using the slider or by playing a cine across time on the **FUNCTIONAL** tab. In order to apply the correction the **Motion correction** box has to be activated and the reference image created based on the available options:



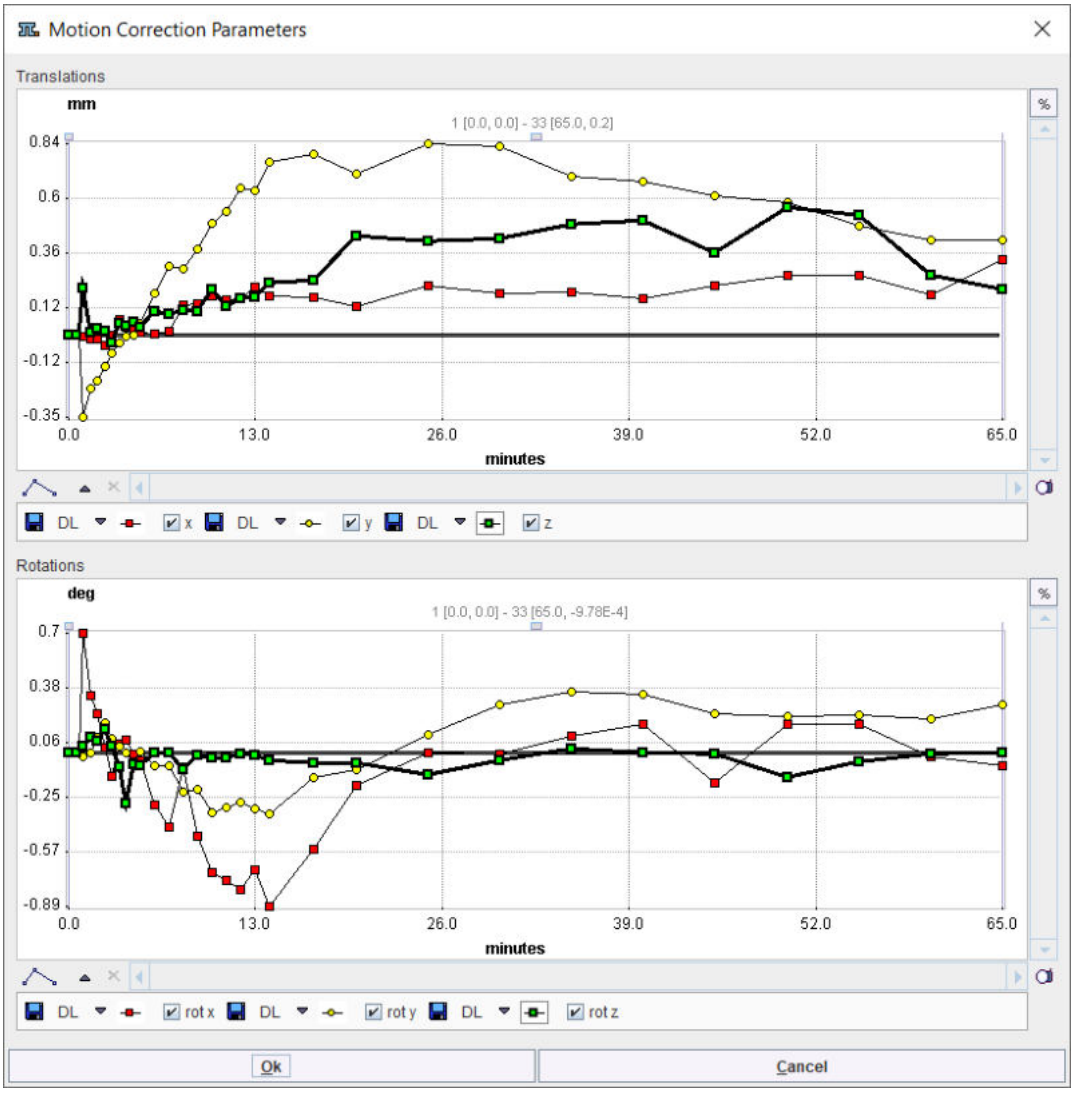
The example above illustrates how the reference image is created using the **To frame averaged** selection. With this approach, a reference image is created by averaging the frames between **From** and **To** in the **REFERENCE** section. In this example a range from **5** to **15** is applied, omitting the noisy short frames at the beginning.

The **CORRECTION** section specifies the frames which will be matched to the frame average which serves as the reference. Assuming that there was negligible motion in the initial frames, a range from **5** to **33** is specified.

Please refer to the *PMOD Image Fusion Guide* for additional information about the other options.

Note: The **Matching Parameters** are tailored to the selected **Species** but can be further adjusted if needed. The motion correction cannot be repeated.

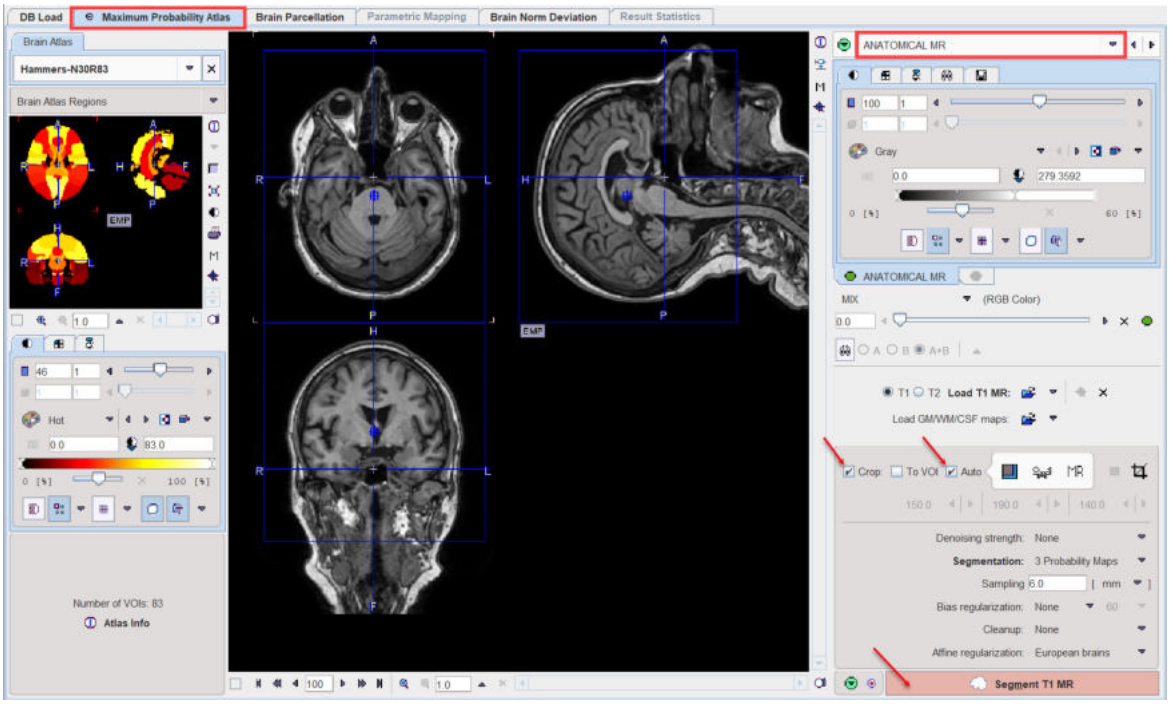
The outcome of the motion correction can be inspected on **FUNCTIONAL** page by activating the **Validate** motion correction button. A dialog window opens displaying the **Translation** and **Rotation** parameters for each time frame in each of the **x**, **y** and **z** direction as illustrated below:



To continue loading the MR images please make sure the **MR** radio button is selected and activate the **MR** action button in the lower right.

4.2.2 MR Image Loading and Segmentation

The **ANATOMICAL MR** page allows loading the T_1-weighted brain MR image of the same subject using the **Load T1 MR** button. The MR image has to appear in the HFS orientation as illustrated below. If there are two matched MR images with different contrasts (e.g. T_1 and T_2) both can be loaded and used for a multi-spectral segmentation using the **6 Probability Maps** (SPM12) method.



MR Image Cropping

The MR image will be the basis for determining the stereotactic normalization. Experience has shown that problems may occur if the MR field-of-view is much larger than the template as occurs for instance with sagittal MR acquisitions. Therefore, please use the **Crop** facility as described before for reducing the MR data set to the relevant portion with skull and brain, but without the neck.

MR Image Segmentation

The MR image will be segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The algorithm uses the following parameters:

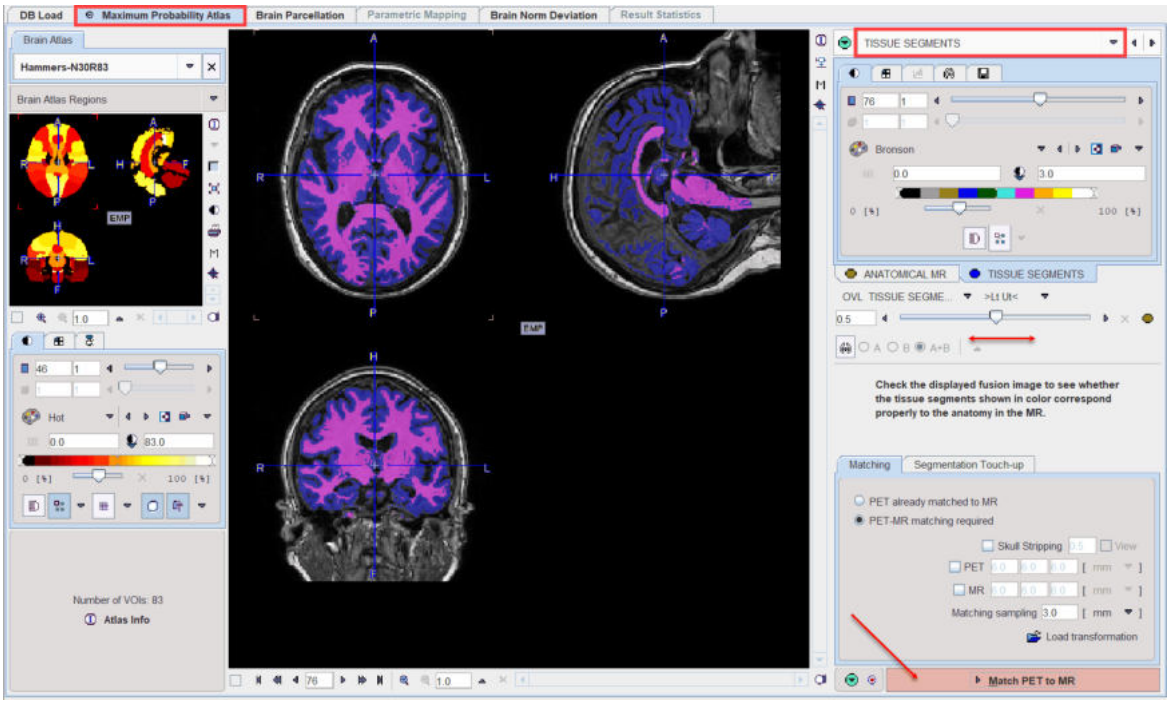
Denoising strength	Denoising of the MR image may improve the segmentation of gray matter, white matter and CSF. If a **Denoising strength** other than **None** is selected, a non-local means denoising algorithm is applied which preserves structure boundaries unless the strength is too high.
Segmentation	Two SPM-type segmentation variants are supported, the **3 Probability Maps** (SPM8) and the **6 Probability Maps** (SPM12) variant. Note that the normalization transform which is obtained as part of the segmentation can be applied for the spatial normalization of the subject brain anatomy in later stages.
Sampling	Density of pixels considered in the calculation. It can be specified in **pix**el or **mm** units.
Bias regularization	Serves for compensating modulations of the image intensity across the field-of-view. Depending on the degree of the modulation, a corresponding setting can be selected from the list. The parameter to the right indicates the **FWHM [mm]** to be applied. The larger the FWHM, the smoother the variation is assumed.
Cleanup	Procedure for rectifying the segmentation along the boundaries.
Affine regularization	Two different initializations of the affine registration are supported, **European brains** and **East Asian brains**, as well as **No regularization**. The setting should correspond to the nature of the subject under study.

It is recommended to use the default settings and only experiment with other parameter values if the segmentation fails. The default settings can be recovered by the button.

The actual segmentation is started with the **Segment T1 MR** action button. Note that the denoising and segmentation process may take several minutes.

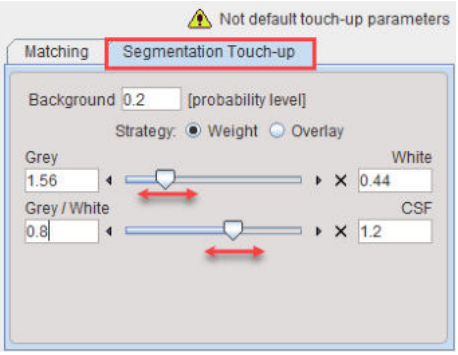
4.2.3 PET to MR Matching

The result of the segmentation is shown as a fusion of the tissue segment map with the MR image on the **TISSUE SEGMENTS** page. Note that the **TISSUE SEGMENTS** image tab contains a label image with gray matter, white matter and CSF represented by the label values 1, 2 and 3, respectively.



Segmentation Touch-up

The label map is calculated from the probability maps of GM, WM and CSF. The relative extent of the tissue categories can be modified on the **Segmentation Touch-up** panel using two different methods. Any modification is immediately reflected in the display.



If the probability value in all of the GM, WM, and CSF maps is below the **Background level,** a pixel is classified as background and assigned the background label value of 0.

With the **Weight** strategy the boundary between GM and WM can be shifted. The following procedure is applied in all non-background pixels: The GM and WM probabilities are multiplied by the factor in the **Grey/White** field, and the CSF probability by the value in the **CSF** field. If the scaled probability of CSF is higher than the scaled GM and WM probabilities, the pixel is assigned

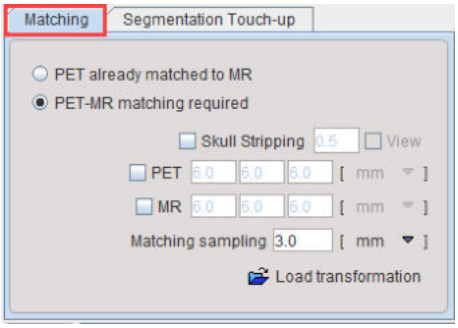
the CSF label value of 3. Otherwise, a similar comparison is done between GM and WM: The GM probability is multiplied by the factor in the **Grey** field, and the WM probability by the value in the **White** field. If the scaled GM probability is higher than the scaled WM probability, the pixel is assigned the GM label value of 1, otherwise the WM label value of 2.

The **Overlay** strategy simply uses two thresholds for the GM and the WM probability map, with white matter having higher priority.

Please use the fusion slider to evaluate the segmentation quality. In case the result is not satisfactory, return to the previous page, modify the segmentation parameters, then activate **Segment T1 MR** again.

PET to MR Matching

The next step consists of rigidly matching the averaged PET image to the MR image, with the options



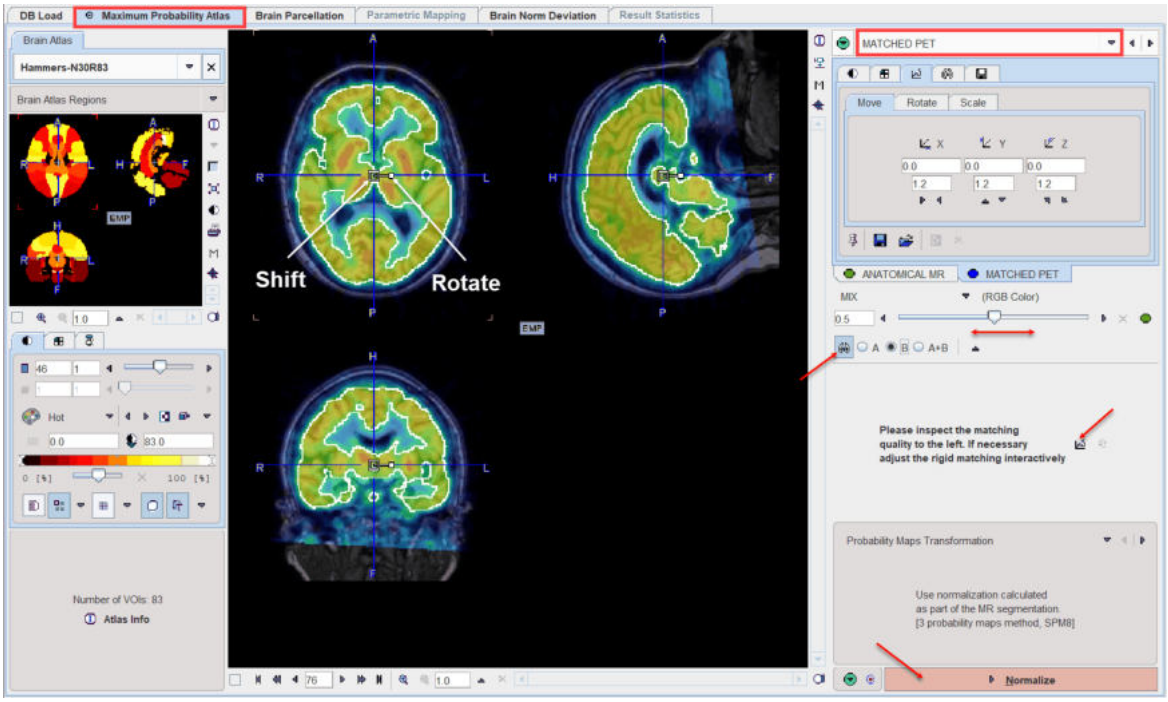
If the data is already matched, the calculation can be skipped by activating the **PET already matched to MR** radio button. If the matching has been performed before and the transformation saved, it can be loaded and applied with the **Load transformation** button.

Otherwise, PNEURO will apply a rigid matching procedure based on the Normalized Mutual Information criterion with **Matching sampling** as the main parameter. Optionally, if the result is not satisfactory, the PET and/or the MR image may be smoothed.

Please activate the **Match PET to MR** action button to start matching.

4.2.4 MR-based Normalization

The result of matching is shown on the **MATCHED PET** page. Please verify that matching was successful by evaluating the alignment in different parts of the brain. Particularly helpful to do so is to interactively drag the fusion balance left/right, and to enable contour outlines.

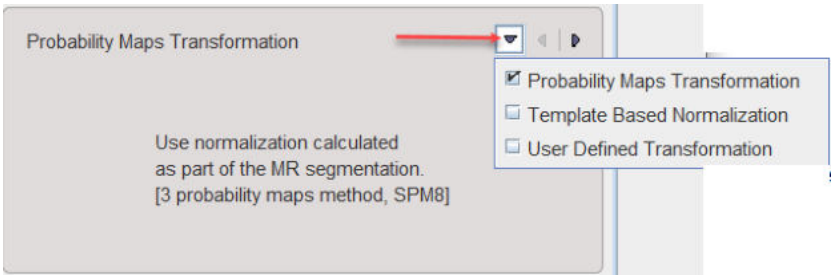


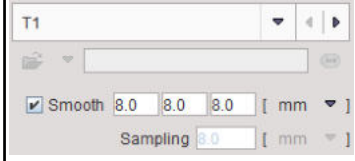
If the match is not satisfactory, there are two options to rectify the situation:

1. Return to the previous page, change the sampling and smoothing parameters and try the automatic matching again, or
2. Activate the **Adjust matching** button and shift/rotate the PET image interactively by dragging the handles in the image or entering offsets/angles on the **Move**/**Rotate** tabs. Finally the transformation needs to by applied with the button.

Normalization

The next step is to spatially normalize the subject images. There are three options as illustrated below.

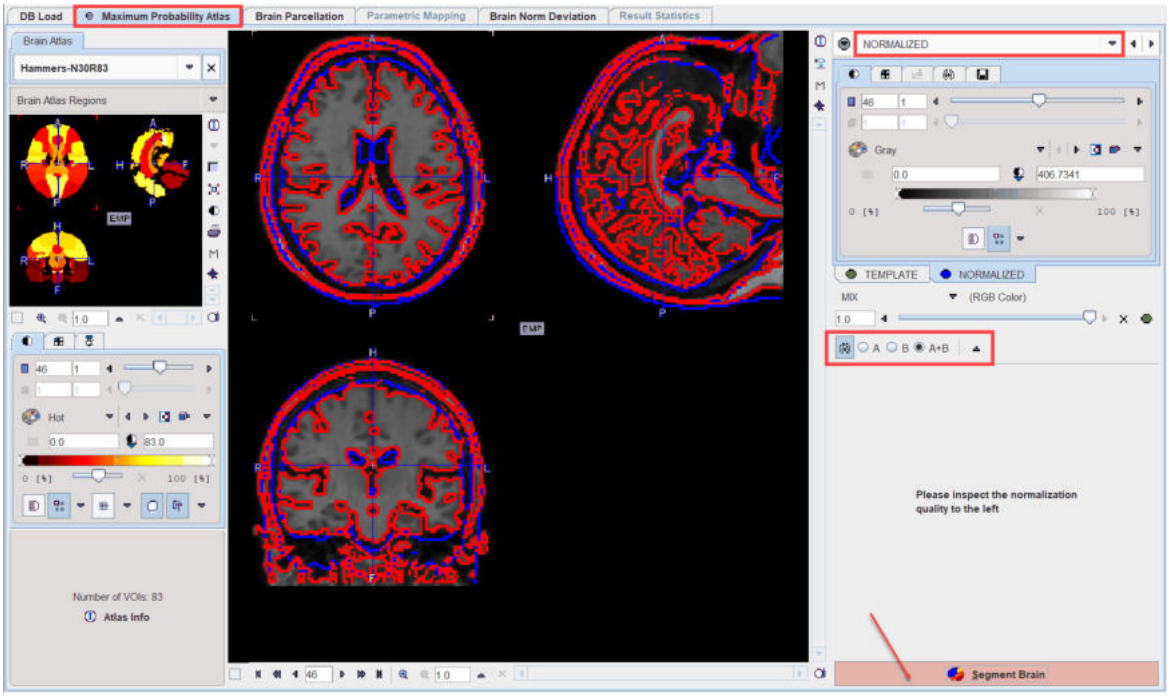


Probability Maps Transformation	Uses the normalization resulting from the GM/WM/CSF MR segmentation procedure.
Template Based Normalization	Performs an SPM5-type normalization between the subject MR and the T1 atlas template image with the usual options 
User Defined Transformation	For loading and applying a normalization transformation which has previously been calculated for the MR image and saved.

Please activate the **Normalize** action button to proceed.

4.2.5 Brain Segments Calculation

The stereotactic normalization result is shown on the **NORMALIZED** page. Please verify that the normalization procedure was successful by evaluating the alignment in different parts of the brain. Particularly helpful are the iso-contour lines. The thresholds on the **TEMPLATE** and the **NORMALIZED** should be adjusted so that the lines follow the same structures. The final brain structure outlines will only be adequate if the normalization succeeded.



After successful normalization the mapping between the different image spaces is established:

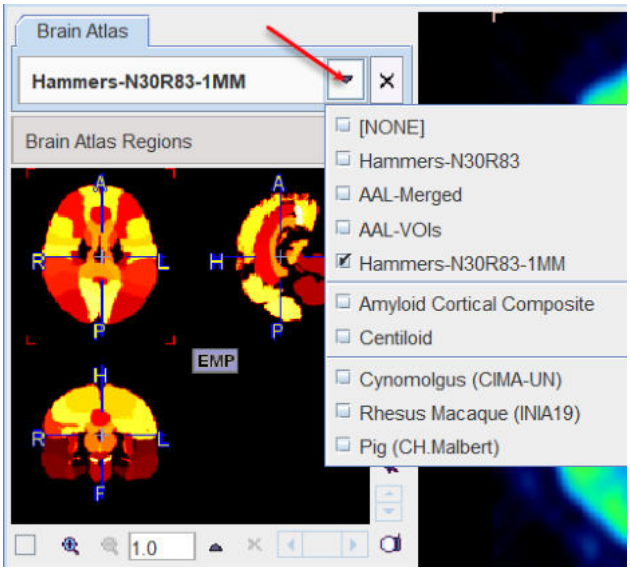
- the normalization maps the MR to the atlas space;
- the rigid transform maps the PET to MRI space;
- the rigid transform combined with the normalization results in a transform which maps the PET to the atlas space.

As all the transformations can be inverted, the atlas space can also be mapped to PET and the MR image space. Consequently, the brain structures which are defined in the atlas space can be mapped to the PET and MR subject space and shown in the overlay.

Atlas Selection

The **Maximum Probability Atlas** module is aimed at leveraging the brain structures defined in the N30R83 atlas. However, the same workflow can also be applied to other atlases, even to atlases defined for other species. For the selection of the atlas please activate the blue show/hide button left to **NORMALIZED**.

As a consequence, a window appears to the left of the page which allows selecting the atlas from a list. To hide the atlas selection window please activate the show/hide button again.

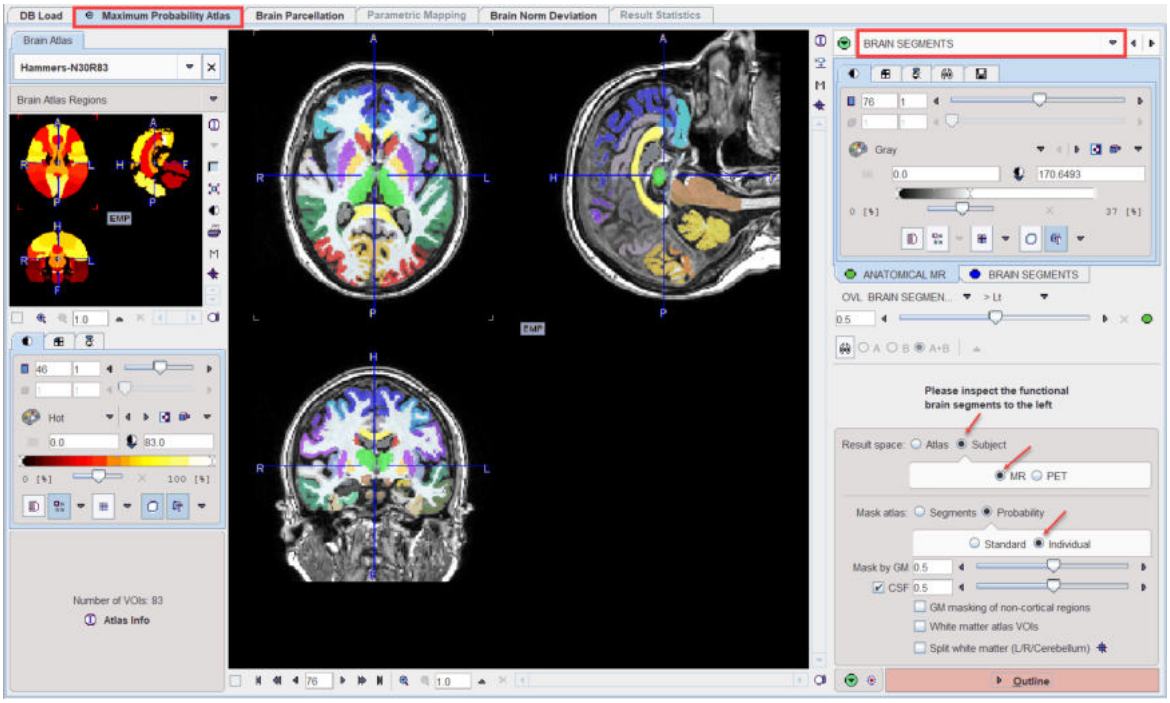


For each of the selections, the **ATLAS** (defining the structures), the **TEMPLATE** (pattern for the spatial normalization) and the **MASK** of the atlas can be inspected.

Please activate the **Segment Brain** action button to start the mapping of the brain structures of the selected atlas.

4.2.6 Outlining of Brain Structures

The result of the brain structure transformation is shown on the **BRAIN SEGMENTS** page. The image on the **BRAIN SEGMENTS** tab is the transformed atlas with integer labels as the pixel values. It is fused with the MR image.

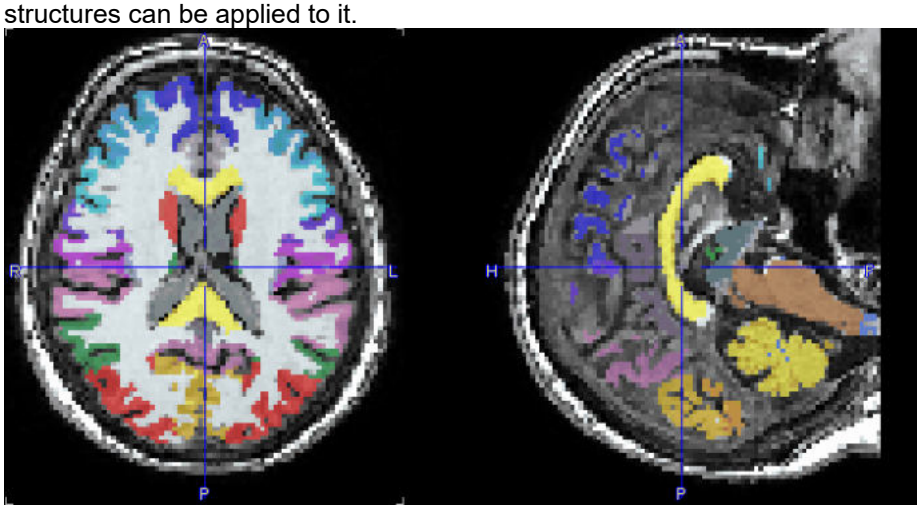


Result Space

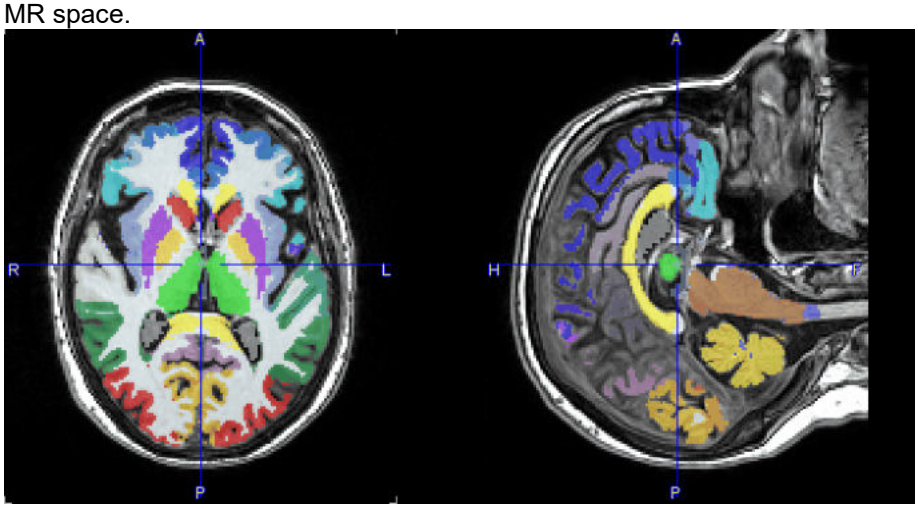
There are three different options to evaluate the PET image, which can be configured using the **Result space** radio button. The information visualized on the page is updated as soon as the configuration is changed. The image display shows the MR image transformed to the selected result space together with the brain structures.

The **Result space** options are:

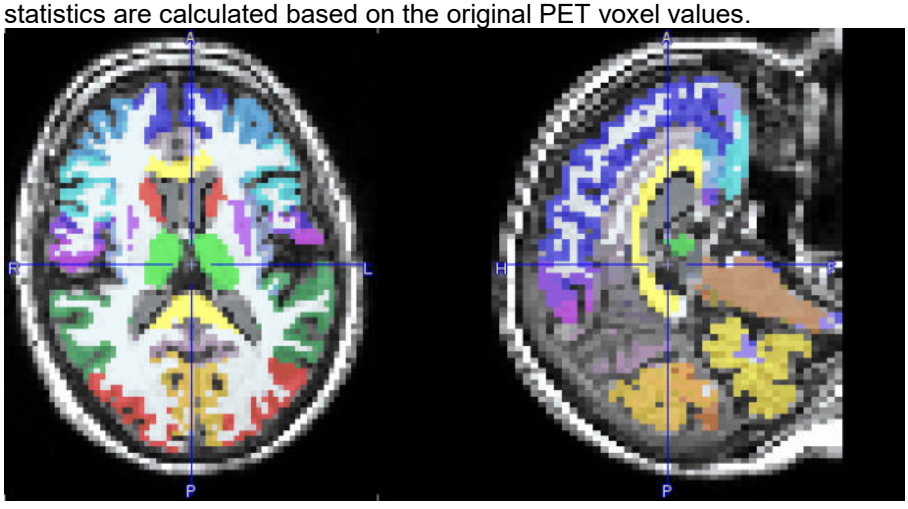
1. **Atlas:** With this setting the PET image is transformed to the atlas space and the original atlas structures can be applied to it.



2. **Subject, MR:** With this setting the PET image and the atlas structures are transformed to the MR space.



3. **Subject, PET**: With this setting the atlas structures are mapped to the PET space and statistics are calculated based on the original PET voxel values.



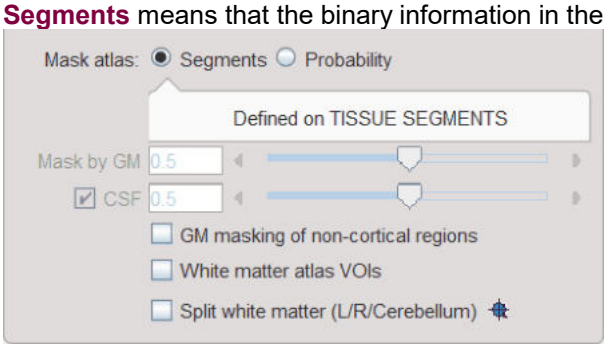
Intersection of the Atlas Structures with Gray Matter

The original atlas structures are typically not restricted to gray matter. Users can take advantage of the gray matter probability calculated during MR segmentation to restrict the structures to pixels

with a high probability of belonging to gray matter. This masking is controlled by the elements in the **Mask atlas** area.

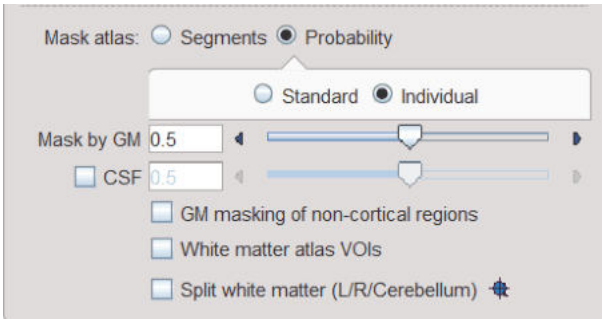
There is a choice between two types of probability information:

- **Segments** means that the binary information in the segment label image[64] is applied.



Here, no thresholding is necessary to obtain a GM mask because the binarization was performed at the time of the segment calculation.

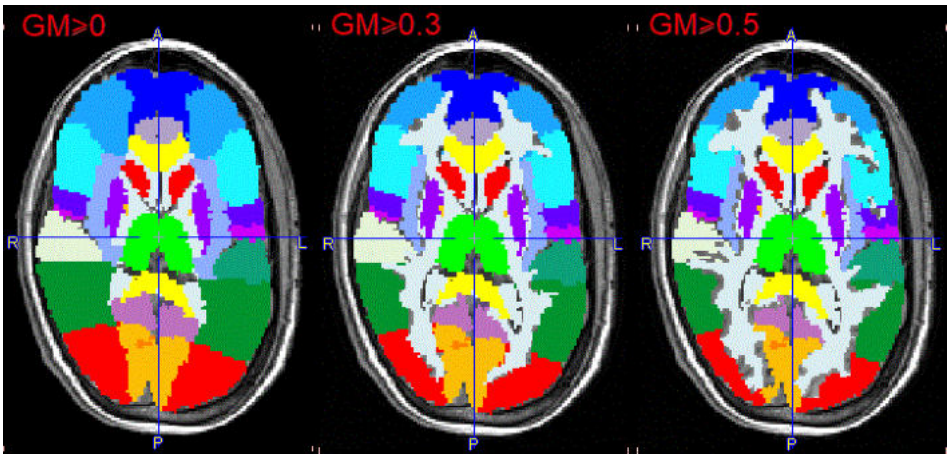
- **Probability** means that a simple threshold is applied to a GM probability map to create a GM mask.



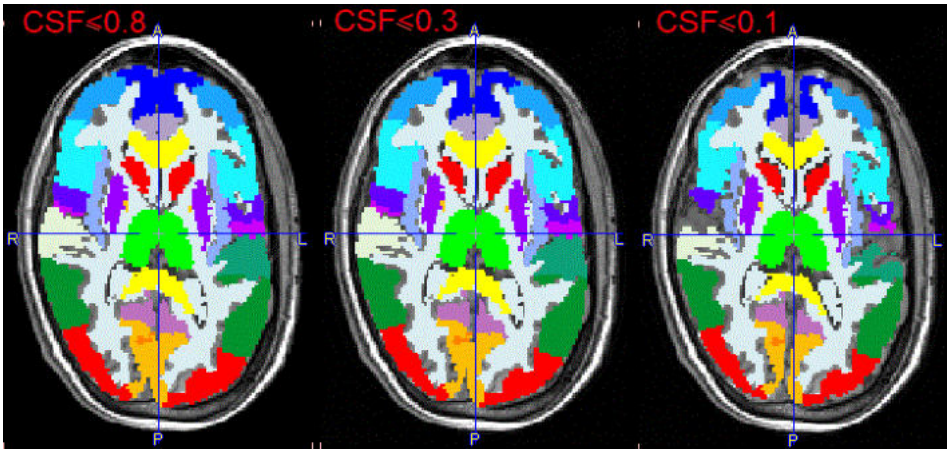
In this case, a **Standard** probability map can be used which represents a population average, or the **Individual** GM probability map resulting from the actual MR segmentation. This latter should be the preferred choice unless the segmentation fails.

The GM threshold slider of **Probability** masking allows a lower probability threshold to be defined. This threshold is applied to the GM probability map in order to create a mask. The higher the threshold value, the thinner the cortical structures become. With the CSF box enabled, the CSF threshold slider allows the GM mask to be further trimmed based on voxels with a high probability of being CSF: the higher the threshold the thicker the cortical structures become.

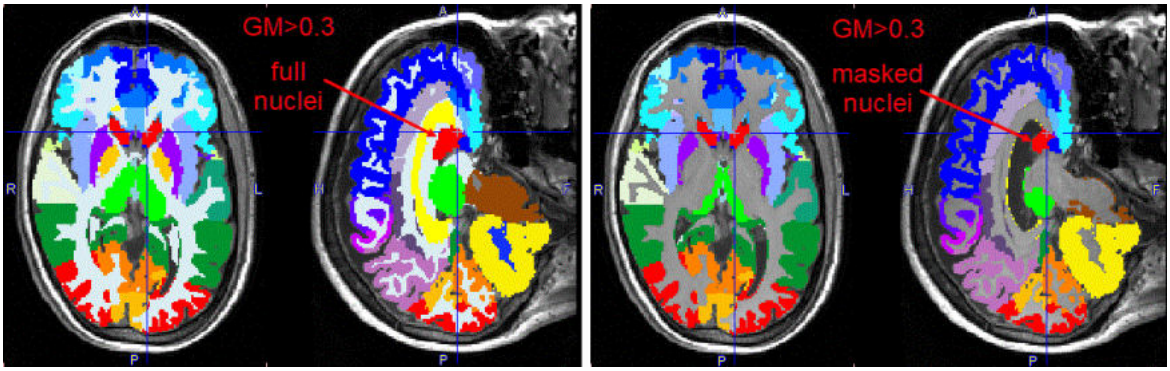
The illustration below shows individual masking at three increasing GM probability thresholds while the CSF box is disabled. The **Standard** mask will result in broader structures at the same probability threshold.



The illustration below shows individual masking at three decreasing CSF probability thresholds and a constant GM probability threshold of 0.5. The **Standard** mask results in narrower structures as CSF probability threshold decreases.



Note that the central structures are not affected by masking in the example above, because the box **GM masking non-cortical regions** is not enabled. If the option is enabled, the central structures will also be limited based on the grey matter tissue probability mask, as illustrated below. However, because of the low probability levels in that area, the reduction may become too severe. Therefore, masking subcortical regions is disabled by default.

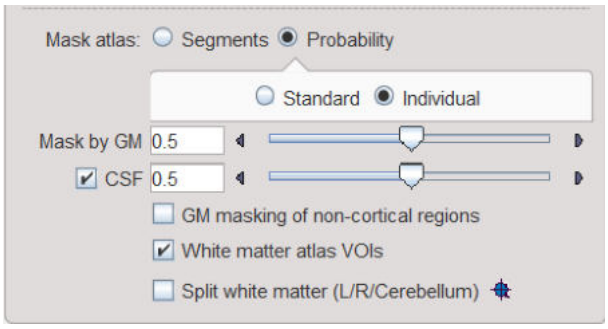


Intersected VOIs and Partial Volume Correction (PVC)

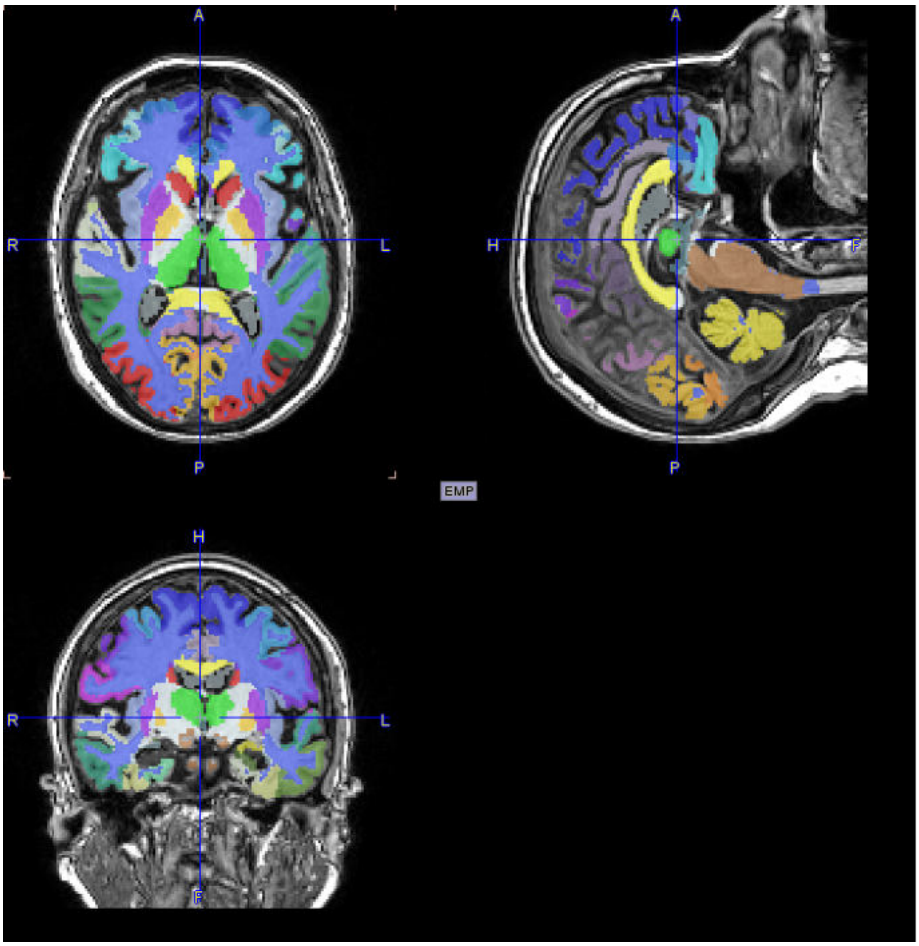
VOI-based PVC correction methods only work properly when all the activity contained in the brain is included in VOIs. Therefore PNEURO automatically creates the full set of complementary VOIs, i.e. GM, WM and CSF fractions of all atlas VOIs, in the memory buffer, regardless of WM masking settings. This full set of VOIs will be used for the PVC calculation, but will not be visible in the user interface and in the result statistics.

White Matter Atlas VOI

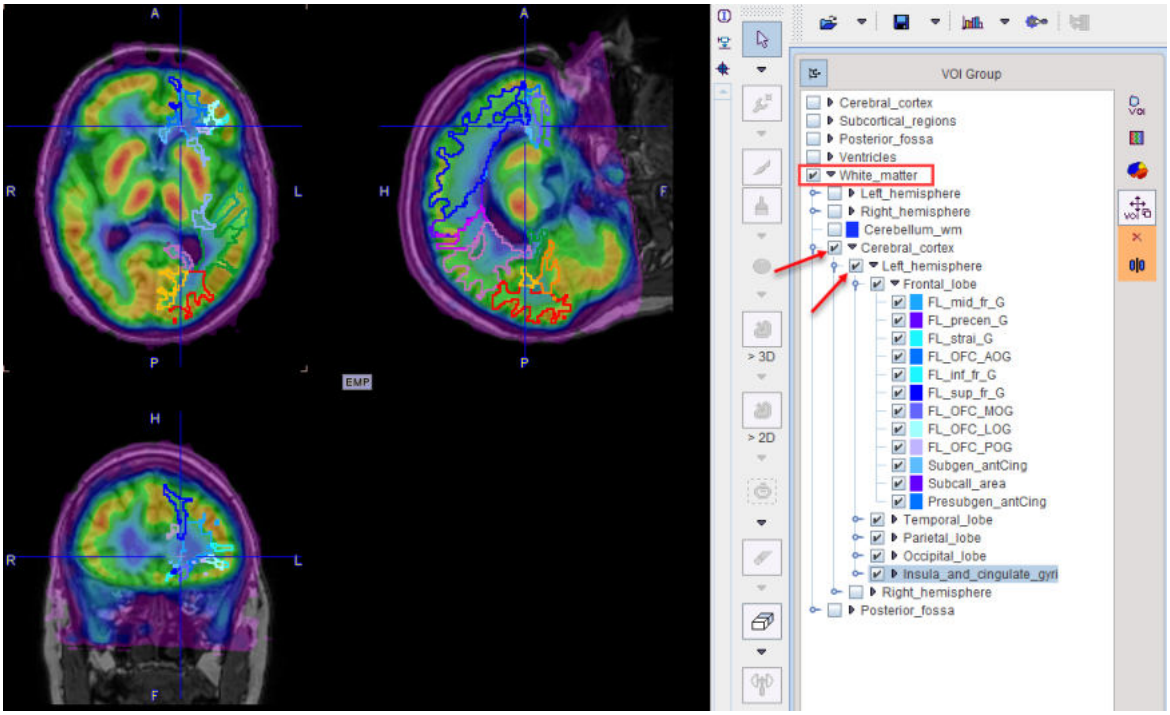
The **White matter atlas VOI** box, when enabled, allows dividing the white matter region according to the VOIs in the selected atlas.



Result on **Brain Segments** page:

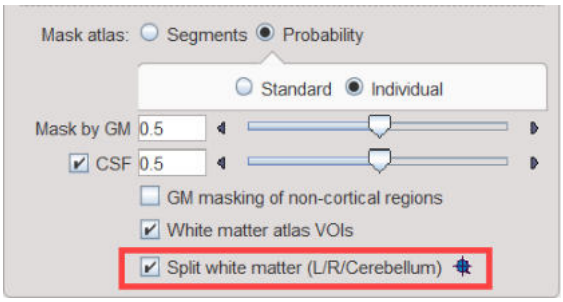


and **White_matter** VOIs with tree structure:



Note: The white matter voxels which do not have any definition in the atlas will be classified as general white matter.

Split Brain

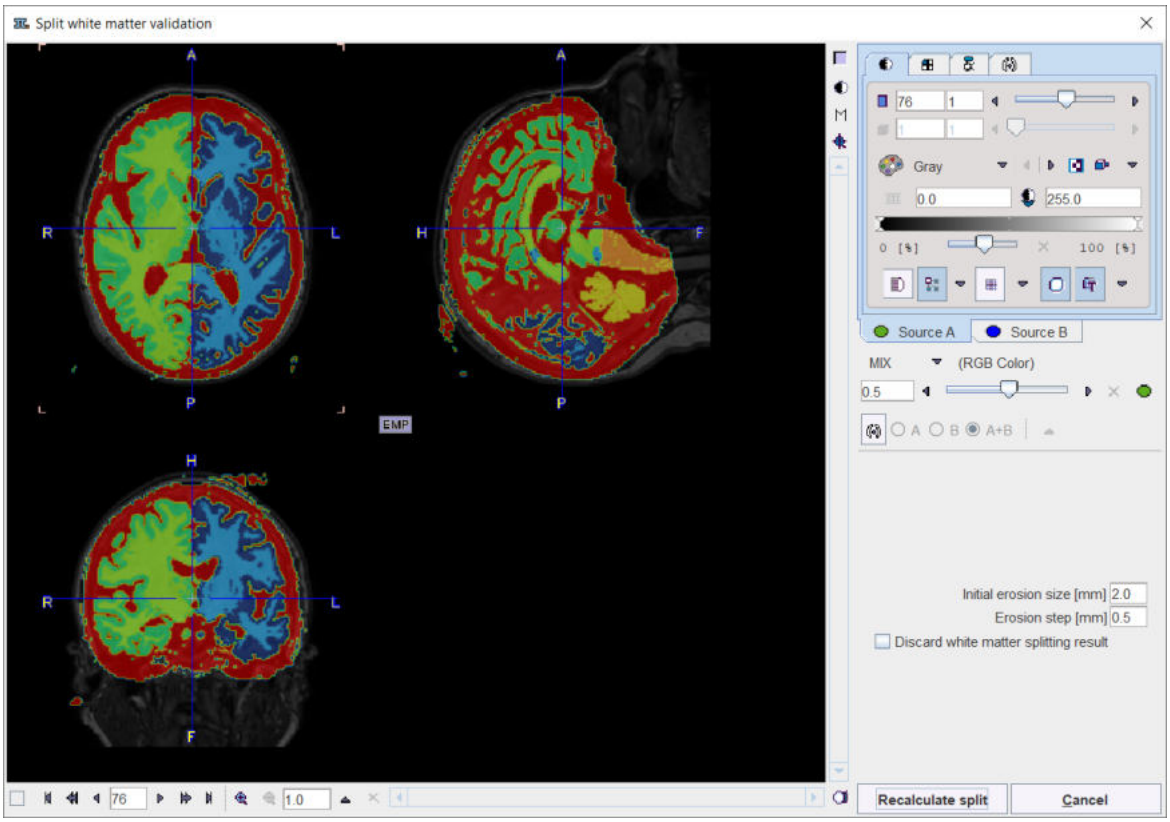


The **Split white matter (L/R/ Cerebellum)** option is only relevant for the separation of the white matter into left hemispheric, right hemispheric, and cerebellar parts. If the procedure is not enabled or fails, then a global white matter region is created.

Note: The brain split division uses a template in the MNI space. This template consists of three labels corresponding to the left hemisphere, the right hemisphere and the cerebellar part. In the brain split process the brain mask is eroded with an initial erosion size and the separation into individual segments is verified. The result must consist in at least 3 separate segments. If it is not the case the erosion size is incremented with the erosion step. The algorithm verifies each separate region to which label in the template belongs. The front propagation is used to divide the whole brain mask only when all the three region seeds are found to be geodesic.

Inspect the Left/Right Split Outcome

The **Inspect left/right split** button opens a dialog window for inspecting the result of the brain splitting procedure.



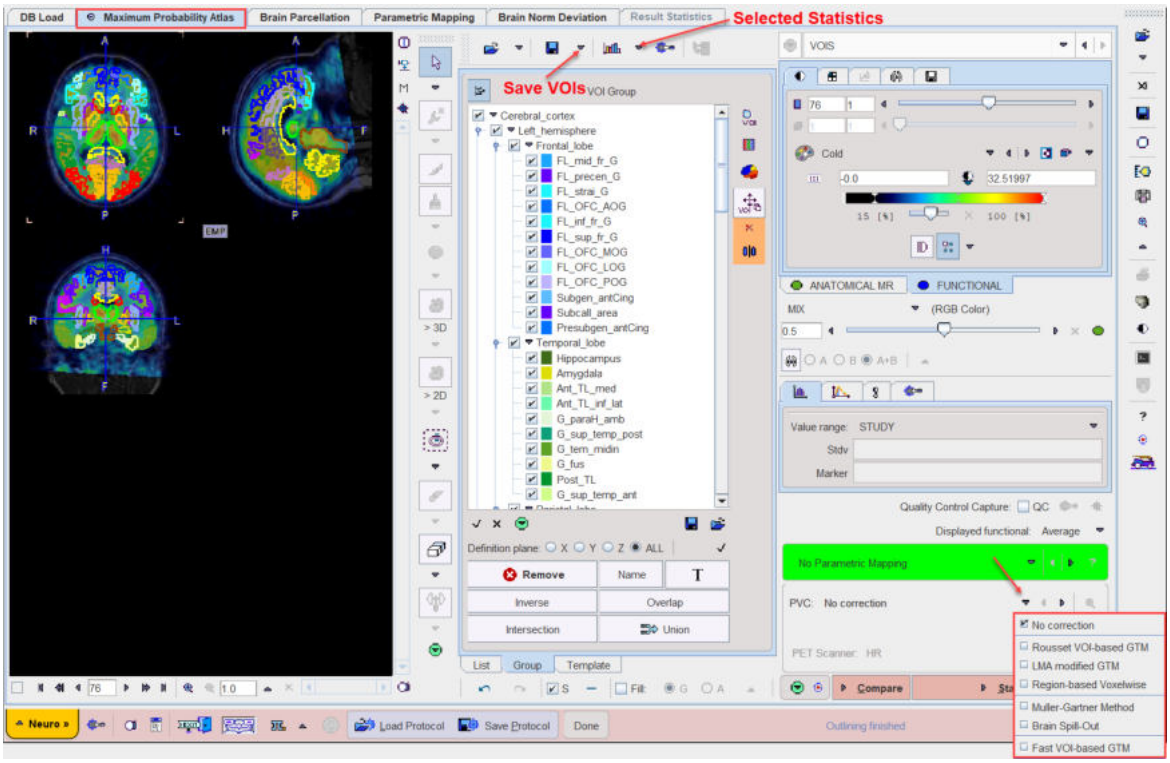
There are 7 resulting segments, with are shown in the **Source B** tab. They correspond to left white matter, left gray matter , right white matter, right gray matter, cerebellar gray matter (including pons), cerebellar white matter and CSF. If the procedure doesn't come up with a reasonable splitting into right and left parts, there are two options to proceed. The **Initial erosion size [mm]** and **Erosion step[mm]** parameters can be modified and the procedure restarted using **Recalculate split**, or splitting can be switched off by the **Discard white matter splitting result** flag. In the latter case please confirm with the **Discard split** button to close the window.

Structure Outlining

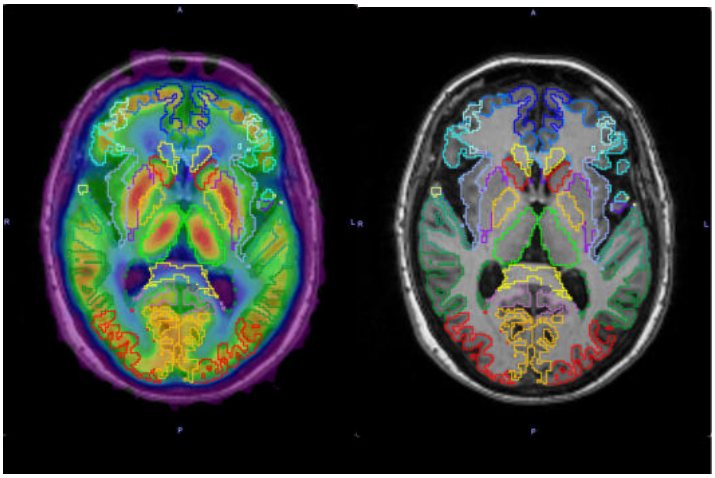
Once the result space and gray matter masking have been specified, the brain structures are fully defined and can be outlined to create contour VOIs. This process is started with the **Outline** action button.

4.2.7 Brain VOI Editing and Statistics Calculation

The result of structure outlining is shown on the **VOIS** page.



The image display shows a fusion of the MR image with the averaged PET image in the selected **Result space**. Please use the fusion slider to change the weight between the image contributions, and use the individual image control tabs for the changing the individual image displays. The example below shows the contour VOIs with 50% mixing (on the left) and MR only (to the right).



VOI Editing and Selection

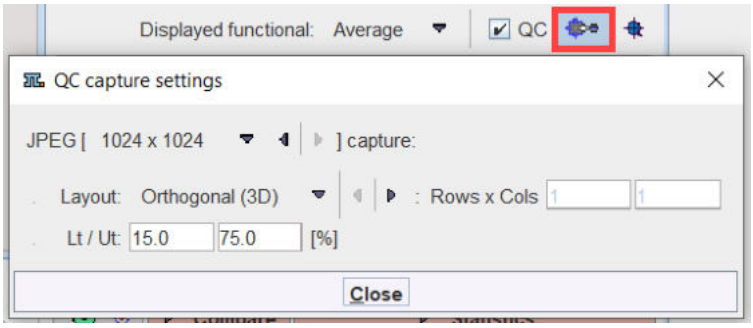
At this time the contour VOIs can be interactively adjusted using the VOI features of PMOD, which are described in the *PMOD Base Functionality Guide*. Note that the **List** tab should be selected for the adjustment, and that depending on the configuration[7] only a reduced set of VOI tools may be available.

Please note that for the step by step interactive procedure the regions for which the atlas description is set to H will NOT be selected on the **Group** tab.

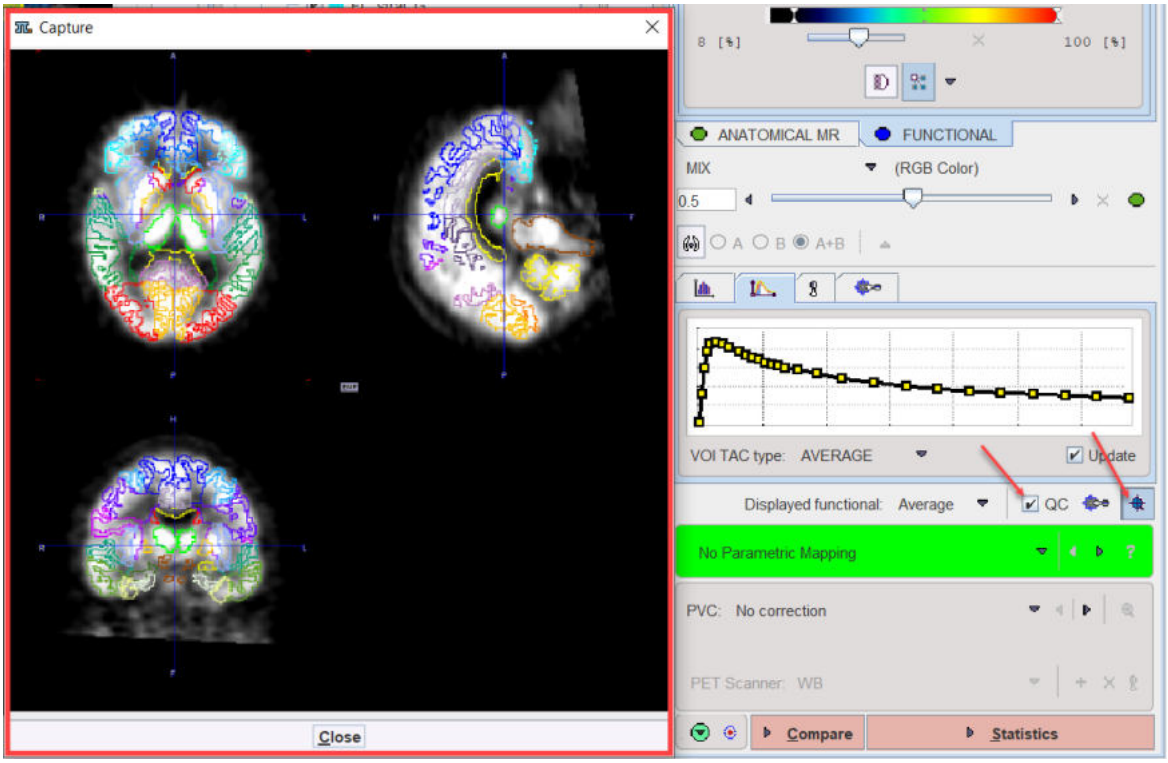
A subset of the VOIs can easily be selected on the **Group** tab as described above[22].

Quality Control

If the **QC** box is enabled, a screen capture of the VOIs overlayed on the selected functional image will be saved, based on the capture settings as illustrated below:

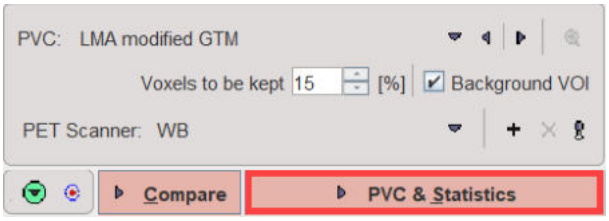


This setting is saved in the protocol and is relevant for batch processing: it allows the user to quickly inspect the outcome of the matching procedures, in the selected result space, at the end of the batch processing.



Partial-Volume Correction (PVC)

The PNEURO tool supports GTM-based partial-volume correction[29] (PVC) of the PET signal. Note that the corrections are sensitive to the signal-to-noise ratio and might produce large artifacts for noisy dynamic data.

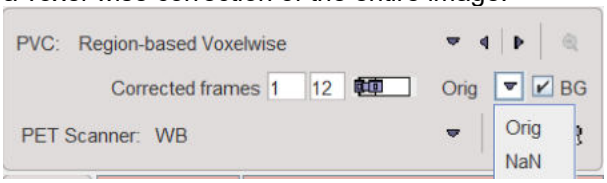


When PVC correction is enabled the right most action button label becomes **PVC & Statistics**.

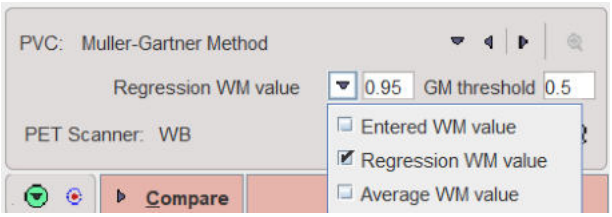
The **PVC** selection has five choices:

- **No correction:** No partial-volume correction is applied (default).
- **Rousset VOI based GTM:** The original Rousset GTM correction[32] method is applied.
- **LMA modified GTM:** A variant[33] of the Rousset correction method is applied, whereby only a percentage of the pixels in the inner of the VOI is used to calculate the VOI average. This percentage can be set using the **Voxels to be kept** parameter.

- **Region-based voxelwise:** Extension of the GTM[33] method by Thomas et al which performs a voxel-wise correction of the entire image.

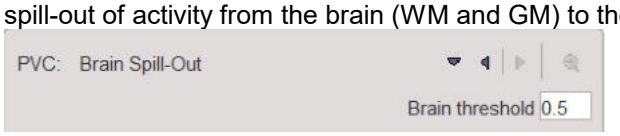


- **Muller-Gartner Method:** The original Muller-Gartner[33] correction which calculates the corrected activity in the GM pixels by compensating spill-out from the gray matter and spill-in from the white matter.



The WM value used for the correction can be specified in 3 ways: as a fix **Entered WM value** to be entered, as a value determined by linear **Regression WM value** at WM probability of 1 using the values of all WM pixels, and as the **Average WM value** in the WM segment above the specified probability threshold.

- **Brain Spill-Out:** A variant of the Muller-Gartner[35] correction which compensates only for the spill-out of activity from the brain (WM and GM) to the CSF.



The **Brain threshold** is used to mask the pixels with brain probability above the specified value.

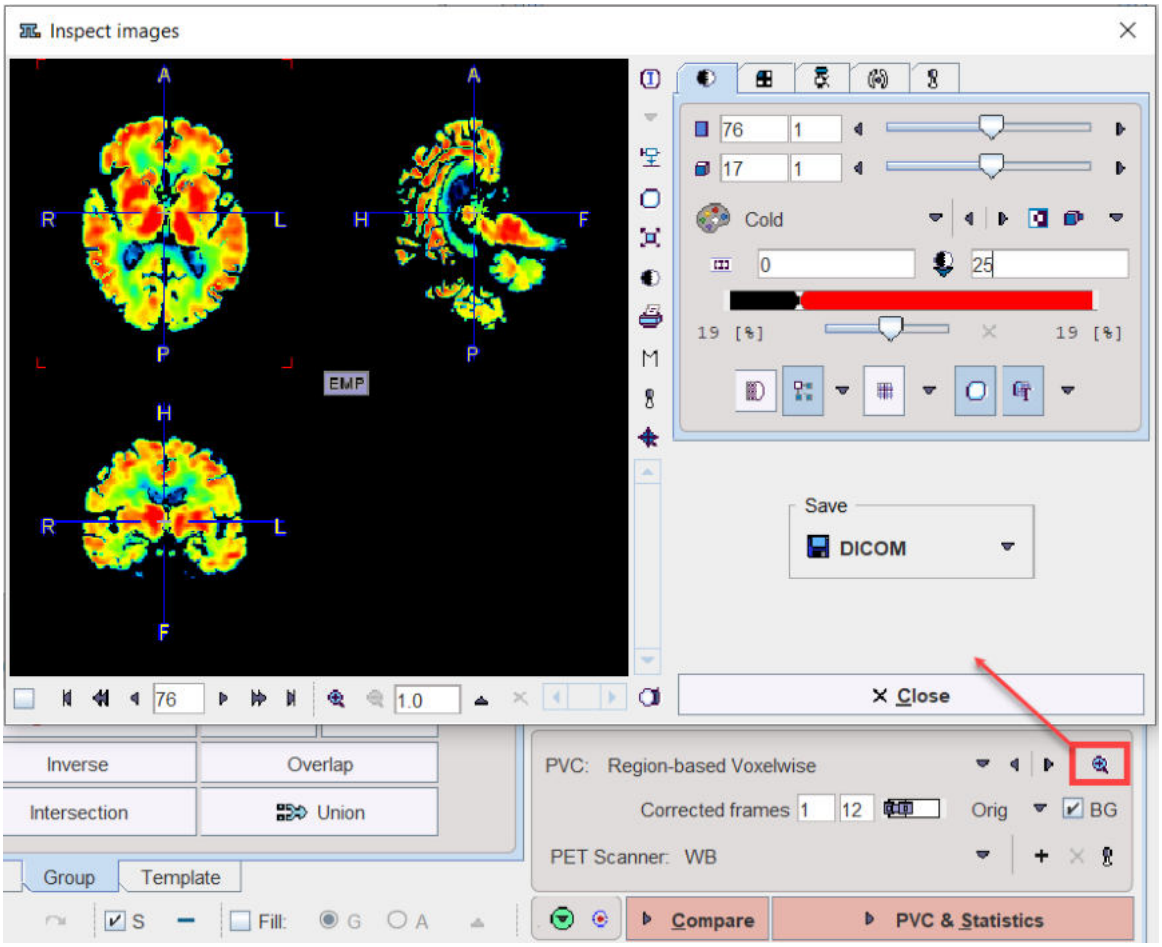
- **Fast VOI-based GTM**: represents actually the **LMA modified GTM** method which contains some speed-up improvement for the smoothing. The speed performance might be observe when the selected atlas contain a low number of regions.

For the purpose of VOI-based PVC methods, the VOIs complementary to the gray/white matter masked VOIs are taken into account. These VOIs are listed on the **Group** tab under the **Complementar** branch once the PVC calculation has been completed.The availability of these VOIs on the list after running the PVC is aimed to increased the transparency such that they can be saved and used for the exact reproduction via the Protocol[10].

If a PVC method is used, both the original and the corrected statistics are calculated. Note that due to the high number of VOIs the PVC calculation may take several minutes and consumes a significant amount of RAM.

PVC-corrected Images

Note that the PVC-corrected images are available for inspection after the **PVC&Statistics** calculation. When returning to the **VOIS** page, the inspection button to the right of the PVC method indicated below has become active. It opens the corrected images in a dialog window where they can be interactively inspected and saved.



Statistics Calculation

Once the VOIs have been outlined and carefully checked by the user, it is recommended to first save them, then proceed with calculation of statistics using the **Statistics** action button.

When PVC correction is enabled the right most action button label becomes **PVC & Statistics**. The PVC is always calculated on all created VOIs but the statistics values are returned only for the VOIs selected on the Group tab.

The result (for the selected VOIs only) is shown on the separate Result Statistics[119] page of the PNEURO tool, from where it can be further evaluated.

Parametric Mapping

If the PET images are dynamic and the PXMOD option is included in the license, parametric mapping using pixel-wise models can be directly applied within PNEURO, as described in a separate section[113].

When a parametric mapping model is selected the right most action button label becomes **Mapping**.

Normal Database Comparison

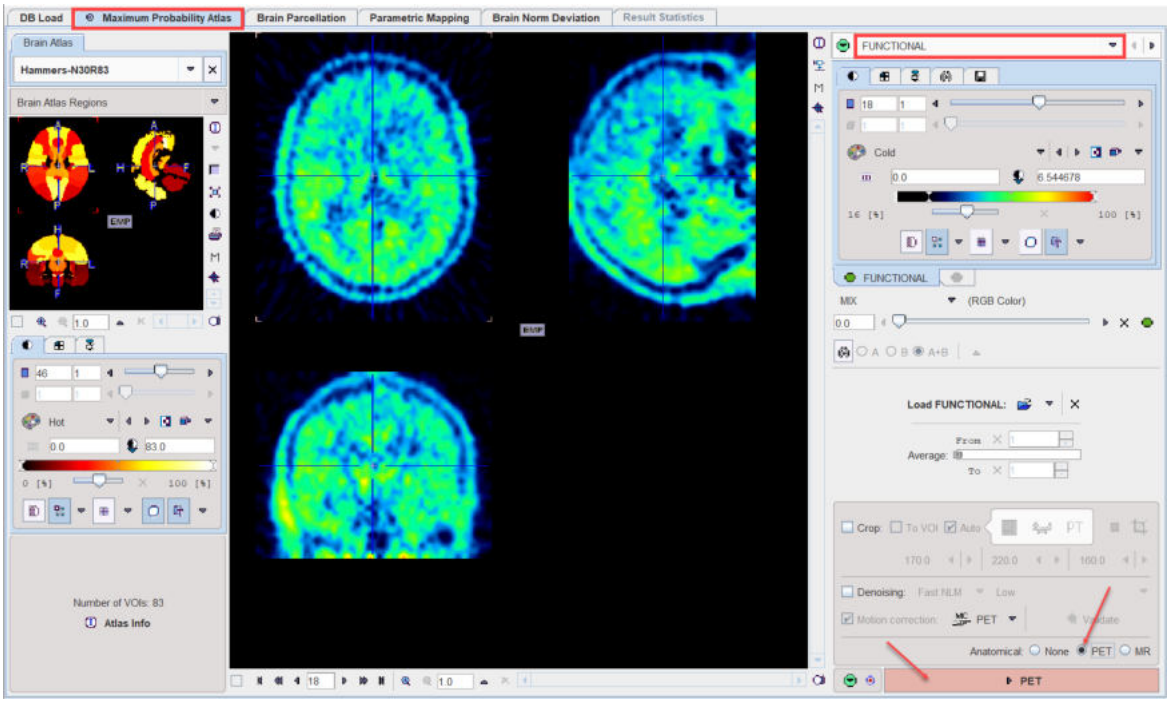
As PNEURO is able to provide the PET images normalized to the atlas space, there is a direct link with the Brain Norm Functionality[113]. The **Compare** workflow button copies the PET images as they appear on the VOIs page to the **Brain Norm Deviation** page where they can be compared against the normal uptake pattern for a given tracer, as defined in a normal database (to be defined by the user, see Brain Norm Functionality)[125]. Conveniently, if the two spaces match, there is no need to perform a normalization.

4.3 Workflow for Functional and Anatomical PET

In some situations a subject who has undergone a functional PET, but not an MRI, might also have had a more "anatomical PET" (e.g. FDG). In this case, the "anatomical PET" can be used for stereotactic normalization, and the resulting transformation applied to the functional PET. Essentially, the "anatomical PET" will take the role of the MRI in the PET-MRI workflow described above. In the following workflow the "anatomical PET" series will be called "FDG PET".

4.3.1 Functional PET Image Loading and Time Averaging

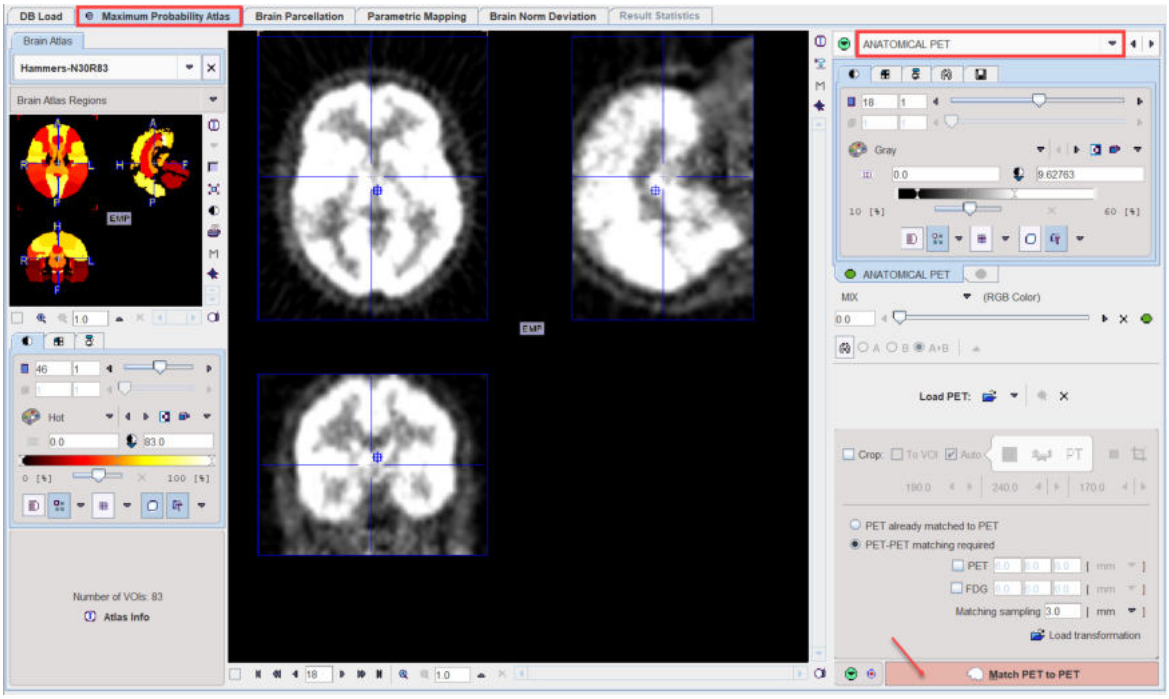
Please use the **Load FUNCTIONAL** button to load the functional PET series. Note that this series may be static or dynamic.



In the case of a dynamic PET series, define the averaging range for creation of a new series, **FUNCTIONAL AVG**, which will be used for matching with the FDG PET. Enable the **Denoising** box if the data are very noisy in order to improve the further steps (e.g. matching and quantification). Inspect the dynamic series for subject motion, and if desired correct for it by enabling the **Motion correction** setting. Apply cropping if desired, select the **PET** radio button to indicate the PET-PET workflow to the program, and proceed using the **PET** action button.

4.3.2 Anatomical PET Image Loading and PET-PET Matching

The **ANATOMICAL PET** page allows the brain PET image with better anatomical correspondence (e.g. FDG PET) to be loaded using the **Load PET** button.



Please apply a crop box if appropriate.

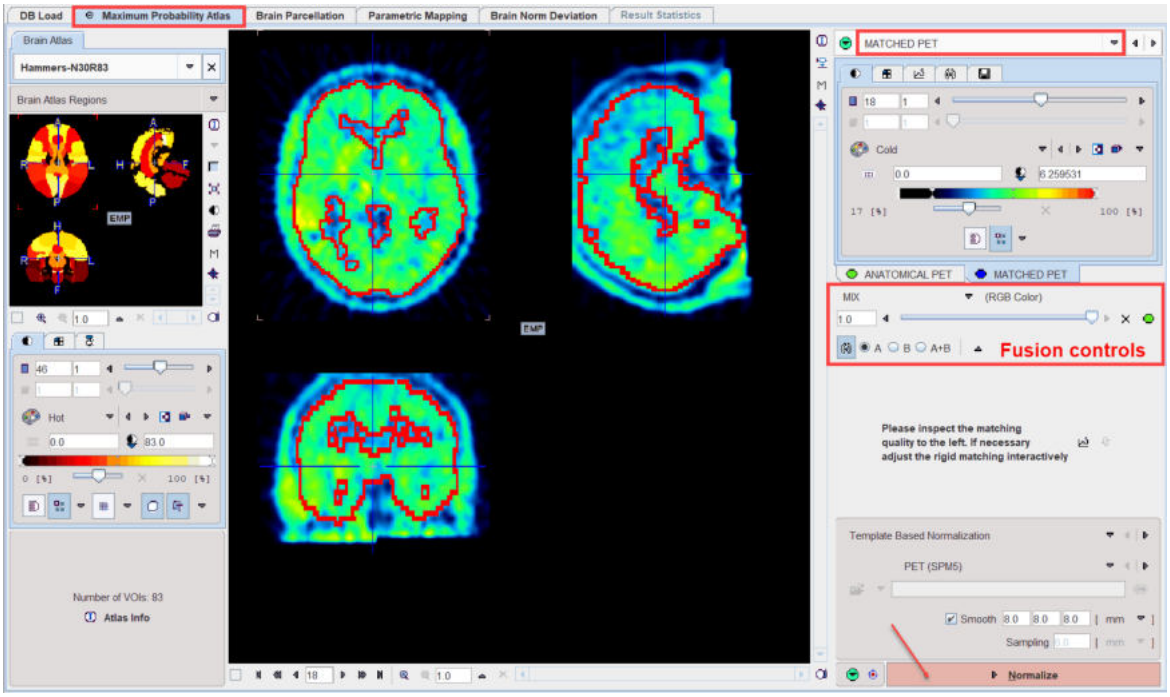
The next step consists of rigidly matching the averaged PET image to the ANATOMICAL PET image. If the data is already matched, the calculation can be skipped by activating the **PET already matched to PET** radio button. If the matching has been performed before and the transformation saved, it can be loaded and applied using the **Load transformation** button.

Otherwise, PNEURO will apply a rigid matching procedure based on the Normalized Mutual Information criterion with **Matching sampling** as the main parameter. Optionally, if the result is not satisfactory, the PET images may be smoothed.

Please activate the **Match PET to PET** action button to start matching.

4.3.3 PET-based Normalization

The result of the PET-PET matching is shown on the **MATCHED PET** page and should be validated using the available fusion functionality.

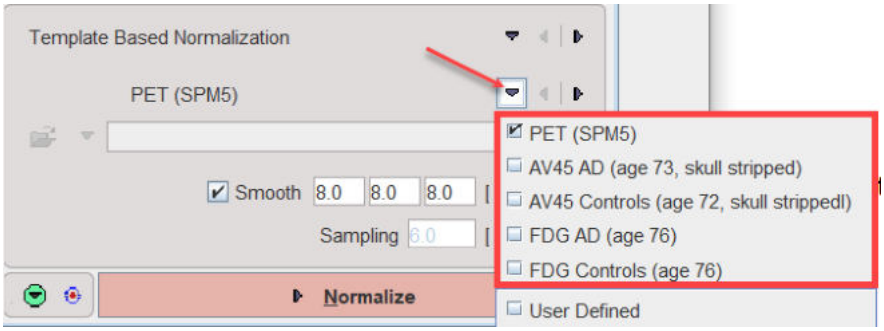


Stereotactic Normalization

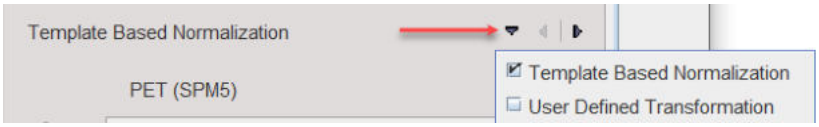
The configuration of the **Template BrainNormalization** is crucial. **PET** is the default selection and refers to the PET template provided with SPM5 (Statistical Parametric Mapping). It was constructed by Friston et al. at the Wellcome Department of Cognitive Neurology (University College London, UK) using Oxygen-15 water PET images of 12 normal subjects scanned in resting condition with eyes closed. In order to get a reasonable stereotactic normalization, the averaged PET image should have sufficient resemblance with the perfusion-weighted pattern of the **PET (SPM5)** template. Often, such a pattern can be obtained by averaging the early phase of PET tracer uptake, and FDG uptake usually resembles perfusion (in the absence of substantial pathology such as tumours).

The amyloid tracers, e.g. AV45, have different pattern distribution as compared to the SPM5 Oxygen-15 water PET template. When such images data are analyzed the PET based normalization will not work. Therefore, we created group specific (Controls and AD) AV45 PET templates using ADNI data. The description of the template methodology is available for direct download. The same methodology was used to create group specific FDG PET templates using FDG images available via ADNI repository database.

The additional templates are available for selection in the predefined PET template list:



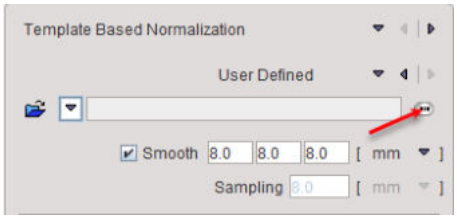
If the normalization has been performed before and the transformation saved, it can be used selecting the **User Defined Transformation** entry instead of the **Template Based Normalization** option.



Finally load the transformation with the button.

User Defined Templates

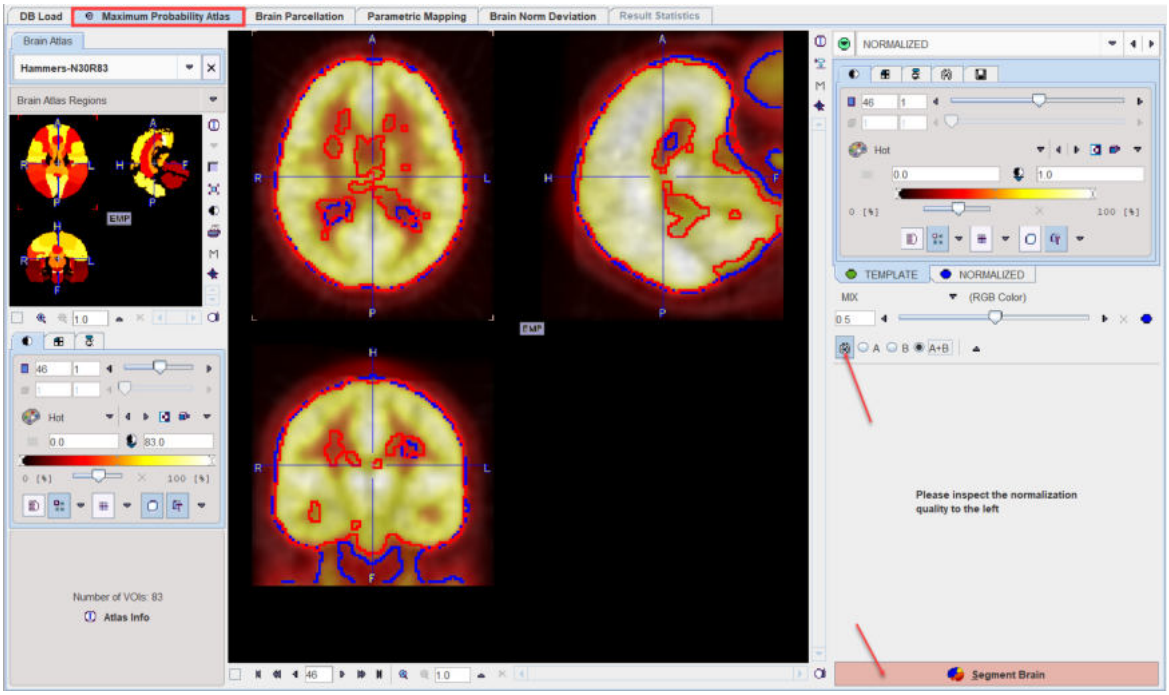
If the normalization results are not satisfactory, the user is advised to create their own PET template representing the uptake pattern of the specific tracer in the atlas space. Such a template can be constructed from the images of normal volunteers using the Brain Norm Creation[125] facility of the PNEURO tool. Once such a template is available, select the **User Defined** entry in the **Template Brain Normalization** list and configure the template with the load button as illustrated below.



The other relevant normalization parameter is the **Smooth** option which will smooth the input image with a Gaussian filter of the specified FWHM. Smoothing should usually be applied, because the template images are normally averaged across a population and therefore smoother than the image from an individual subject. An alternative to specifying the smoothing is to define directly the **Sampling** increment. The normalization is started using the **Normalize** action button.

4.3.4 Brain Segments Calculation

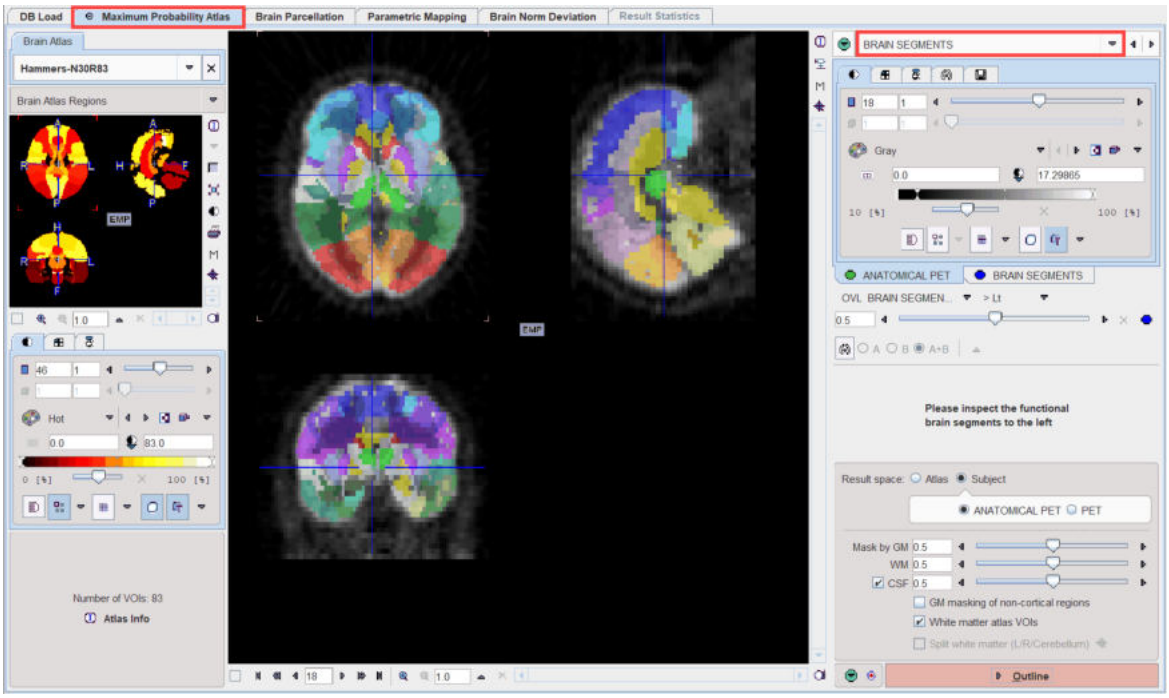
The stereotactic normalization result is shown on the **NORMALIZED** page. Please verify that the normalization procedure was successful.



To start the mapping of the brain structures of the selected atlas please activate the **Segment Brain** action button.

4.3.5 Outlining of Brain Structures

The result of the brain structure transformation is shown on the **BRAIN SEGMENTS** page. It shows the transformed atlas together with the anatomical PET.

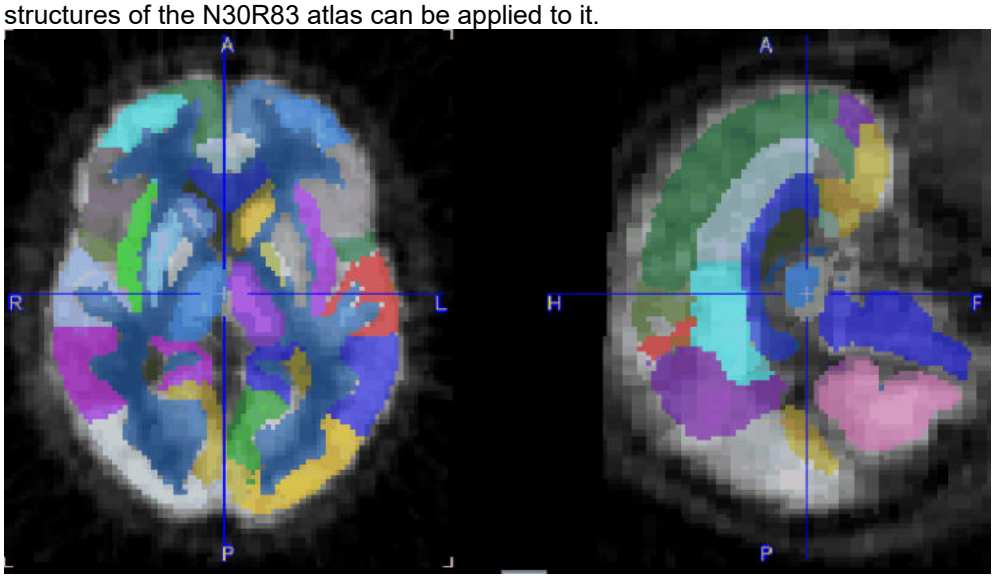


Result Space

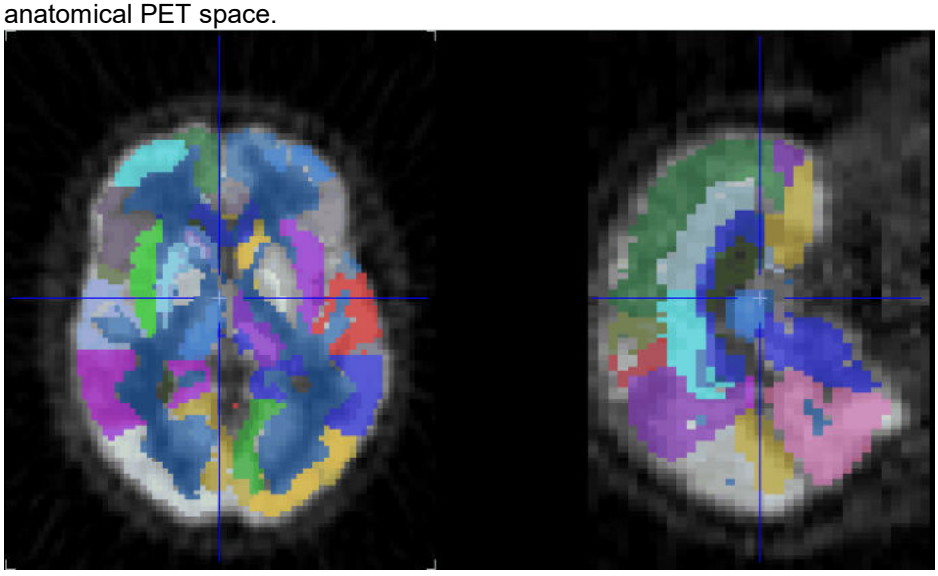
There are three options for evaluating the PET image. These can be configured using the **Result space** radio buttons. The information shown on the page is updated as soon as the configuration is changed. It shows the PET image in the selected result space together with the brain structures.

The **Result space** options are:

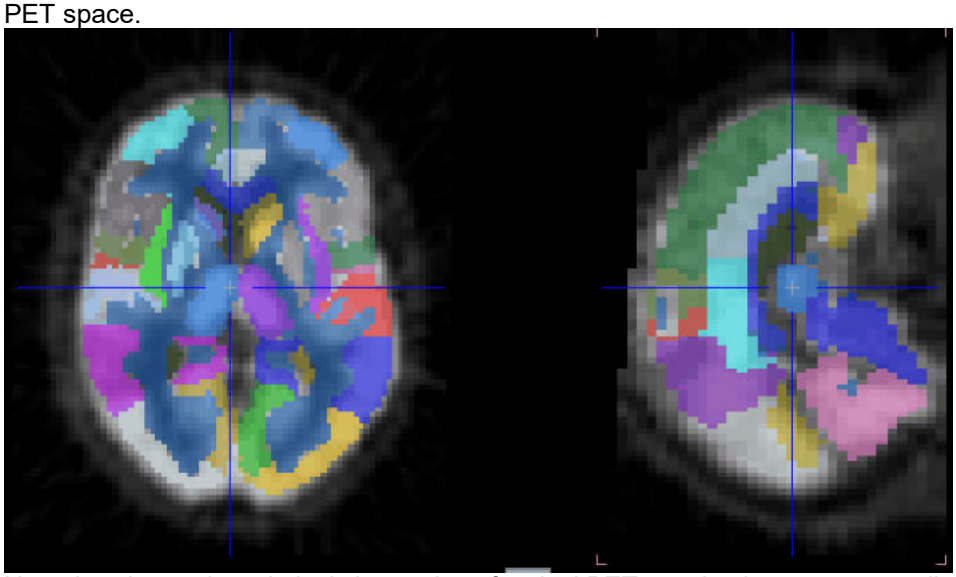
1. **Atlas:** With this setting the PET image is transformed to the MNI space and the original structures of the N30R83 atlas can be applied to it.



2. **Subject**, **Anatomical PET**: With this setting the atlas structures are transformed to the anatomical PET space.



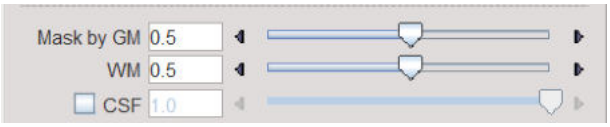
3. **Subject**, **PET**: With this setting the atlas structures are transformed to the (original) functional PET space.



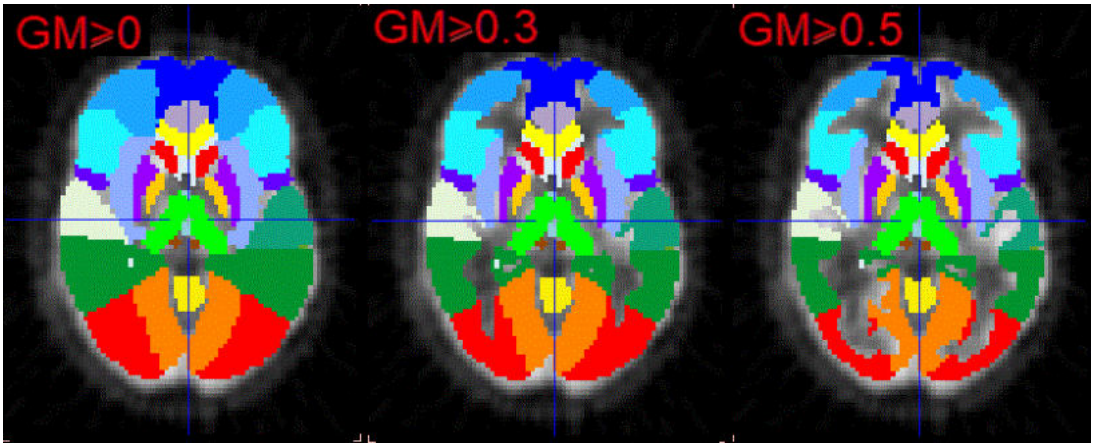
Note that due to the relatively large size of typical PET voxels, the structure outlines are likely to appear pixelated. To avoid this effect the PET image should be reconstructed with small pixel sizes.

Intersection of Atlas Structures with Gray Matter

For atlases with population tissue probability maps users can take advantage of restricting the structures to pixels with a high probability of belonging to gray matter.



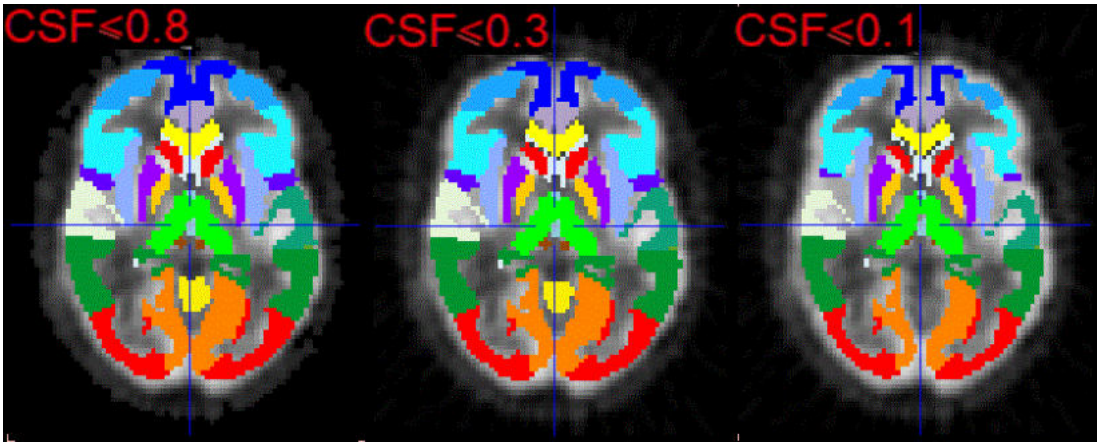
The **Mask by GM** slider allows the lower threshold to be applied to the GM probability map for creating the mask to be defined. The higher the probability threshold, the thinner the cortical structures become. The illustration below shows masking in the PET space at three increasing GM probability thresholds (CSF box disabled and WM slider set to 1).



Note that the central structures were not affected by masking in the example above, because the box **GM masking of non-cortical regions** was not enabled. If this option is enabled, the central structures also shrink. However, because of the low probability levels in that area, the reduction may become too severe. Therefore, masking of non-cortical regions is disabled by default.

The white matter tissue probability map also can be thresholded at a certain theshold in order to generate a white matter VOI (or VOIs, if **White matter atlas VOIs** was activated).

With the **CSF** box enabled, the CSF threshold slider allows further trimming of the GM mask: the higher the threshold the broader the cortical structures become.The illustration below shows masking in the PET space at three decreasing CSF probability thresholds while the GM probability threshold is set to 0.5 and WM probability is set to the value of 1.



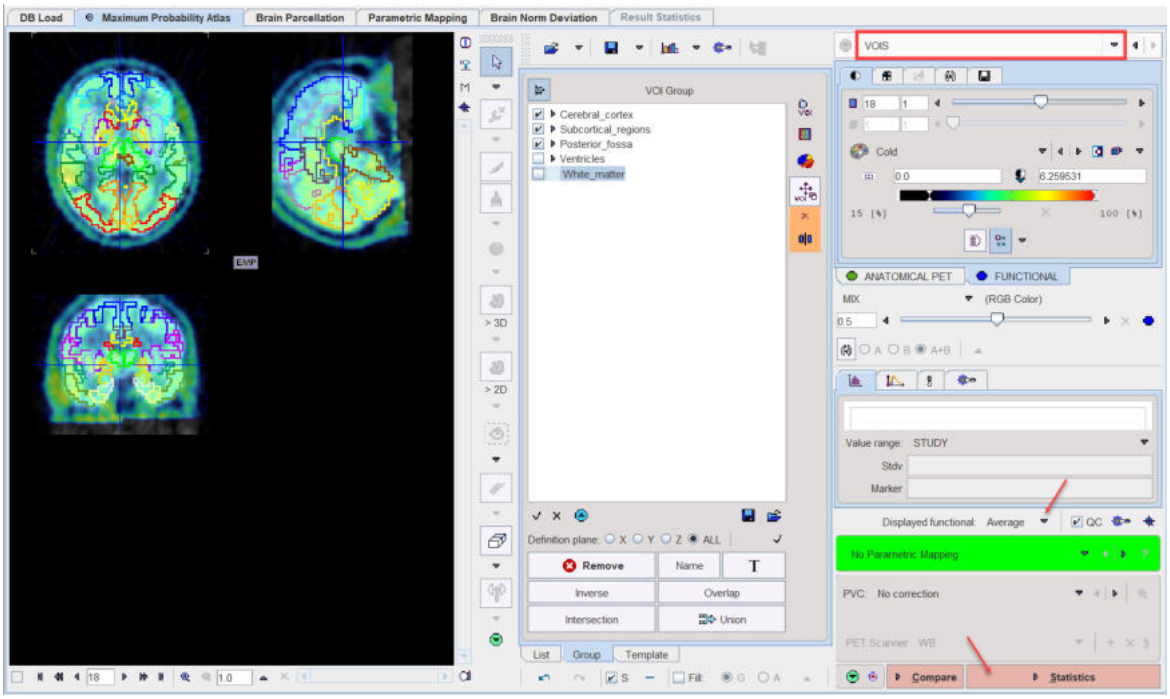
For the atlases with population tissue probability maps the white matter tissue probability map can be used to allow the intersection of the population white matter region with the selected atlas. This can be easily achieved enabling the **White matter atlas VOI** box.

Structure Outlining

Once the result space and gray matter masking have been specified, the brain structures are fully defined and can be outlined to create contour volumes-of-interest. This process is started with the **Outline** action button.

4.3.6 Brain VOI Editing and Statistics Calculation

The result of structure outlining is shown together with the averaged functional PET on the **VOIS** page.



If the functional PET was dynamic, statistics can be calculated on either the original dynamic data (transformed to the selected image space) or the averaged image used for matching to the ANATOMICAL PET (e.g. FDG). The user can switch between the dynamic and averaged images using the Select functional menu, highlighted in the screenshot above. After editing the VOIs and configuring the optional partial volume correction **PVC**, please proceed with the **Statistics** action button. The result is shown on the Result Statistics 119 page.

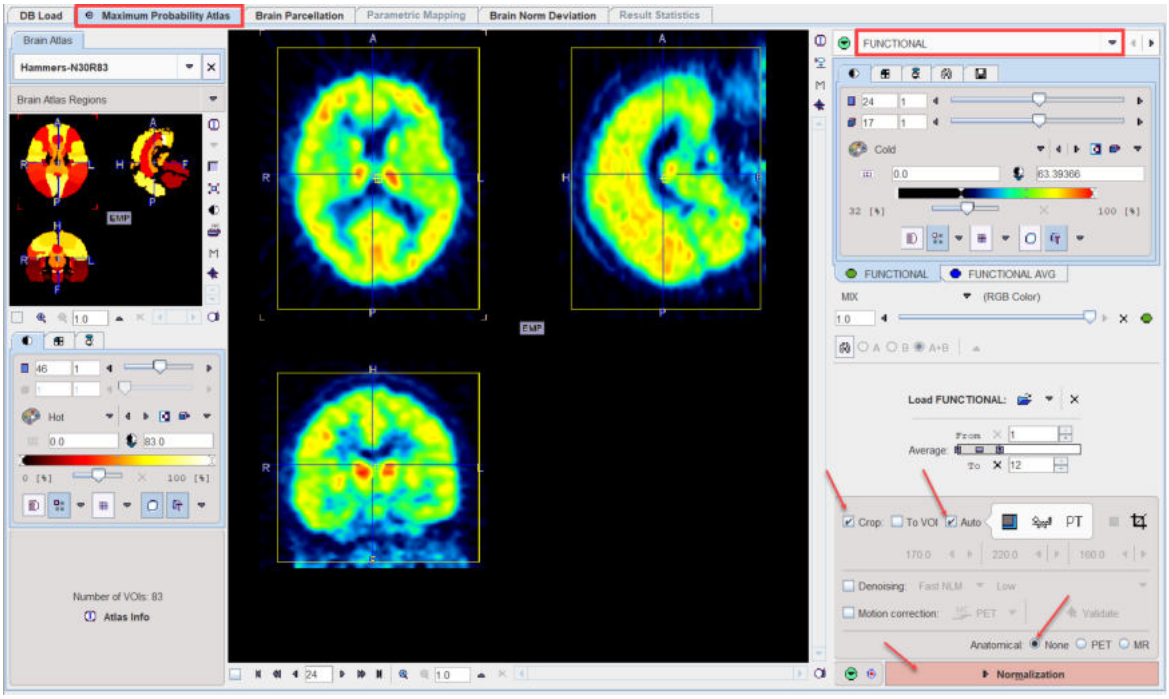
4.4 Workflow for PET-only Studies

The workflow described in this section applies in the case of brain PET studies for which no anatomical image series is available.

Note: If an MRI is available it is recommended to use the MR-based workflow which takes advantage of the better anatomical information in the MRI.

4.4.1 PET Image Loading and Time Averaging

Please use the **Load FUNCTIONAL** button to load the functional PET series. The series may be static or dynamic.



In the case of a dynamic PET series the user should define the averaging range that will be used to create a new series, **FUNCTIONAL AVG**, which will be used for spatial normalization. Often, the early frames are perfusion-related and thus an average of these frames is better suited for normalization with the water PET template than later frames with a more specific uptake pattern.

The original and the averaged images are shown in a fusion rendering which can be controlled in the area below the controls of the individual images.

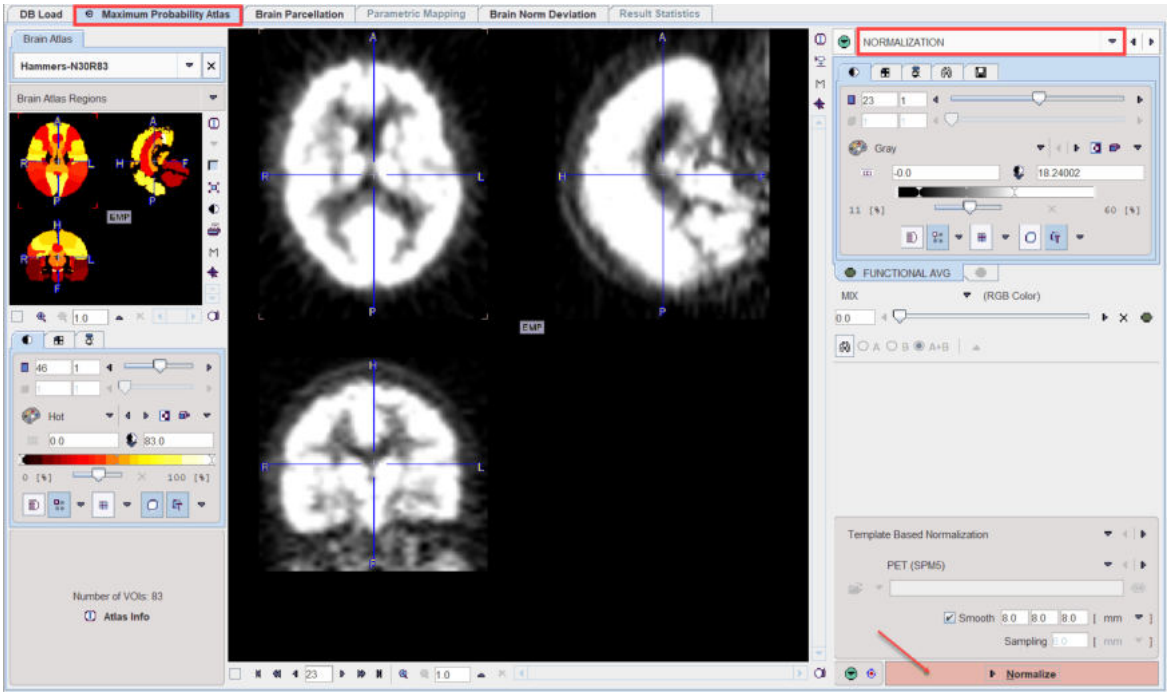
If the PET field-of-view is larger than the brain the data set should be reduced in order to save RAM and optimize processing. This can be achieved by enabling the **Crop** and the **Auto** boxes. It results in the placement of the yellow cropping box, which can be inspected by the user. Alternatively, the crop box can be set manually when the **Auto** box is disabled. Place the yellow crop box by clicking at the brain center. The brain should be fully enclosed. If this is not the case, the edge size in **[mm]** can be adjusted for each direction by selecting the size in the corresponding list.

Enable the **Denoising** box if the data are very noisy, in order to improve later steps such as matching and quantification. Inspect the dynamic series to identify motion, and optionally correct for it using the **Motion correction** setting.

To continue please select the **None** radio button (no anatomical reference available) and proceed using the **Normalization** action button.

4.4.2 PET-based Normalization

The normalization is configured on the **NORMALIZATION** page which shows the averaged PET image.

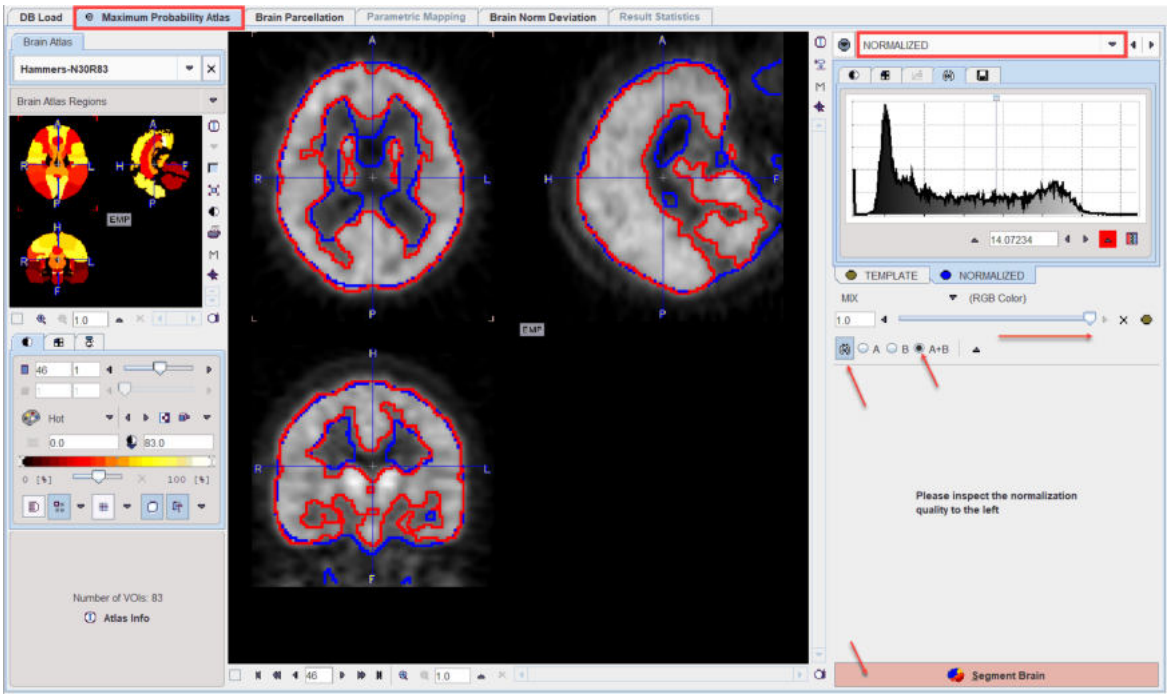


The configuration of the **Template Based Normalization** is crucial. **PET (SPM5)** is the default selection and refers to the perfusion-weighted PET template provided with SPM5. Please refer to the section above 80 for more information about the **PET** template and **User Defined** templates.

The spatial normalization is started with the **Normalize** action button.

4.4.3 Brain Segments Calculation

The stereotactic normalization result is shown on the **NORMALIZED** page.

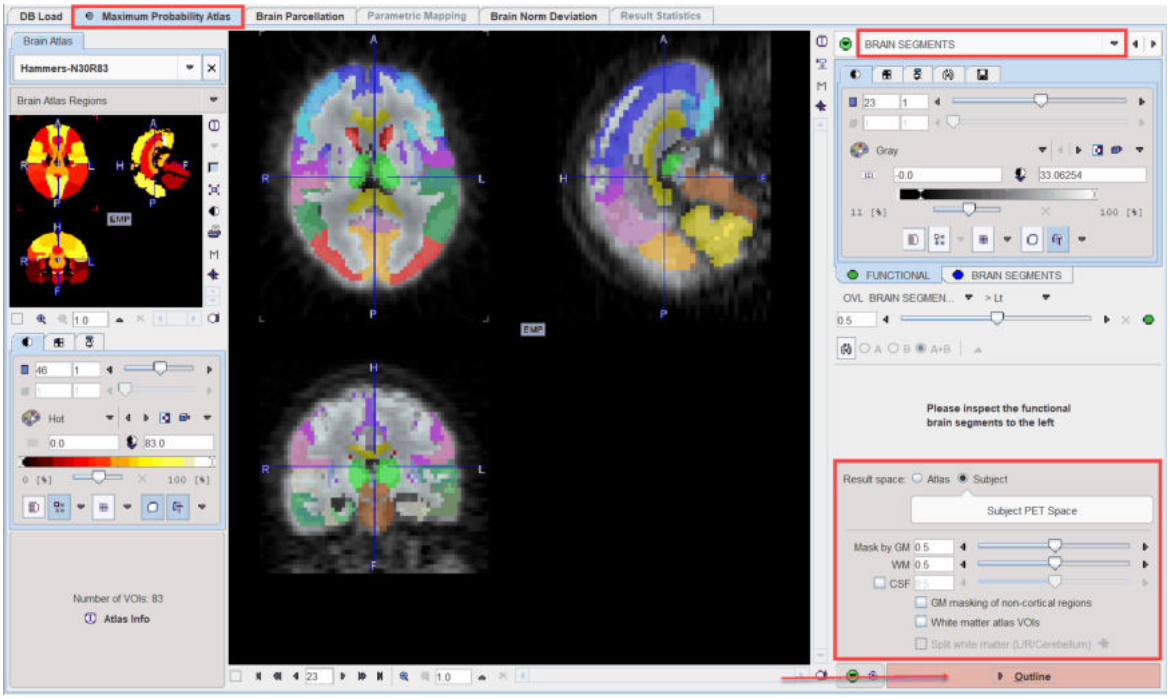


Please verify that the normalization procedure was successful by evaluating the alignment in different parts of the brain. Activation of the iso-contour lines can be particularly helpful. The thresholds on the **TEMPLATE** and the **NORMALIZED** panel should be adjusted so that the lines follow similar structures (e.g. approximating the outer contour of the cerebral cortex). The final brain structure outlines will only be adequate if the normalization was successful.

To start the mapping of the atlas brain structures to the subject space please activate the **Segment Brain** action button.

4.4.4 Outlining of Brain Structures

The result of the brain structure transformation is shown on the **BRAIN SEGMENTS** page.



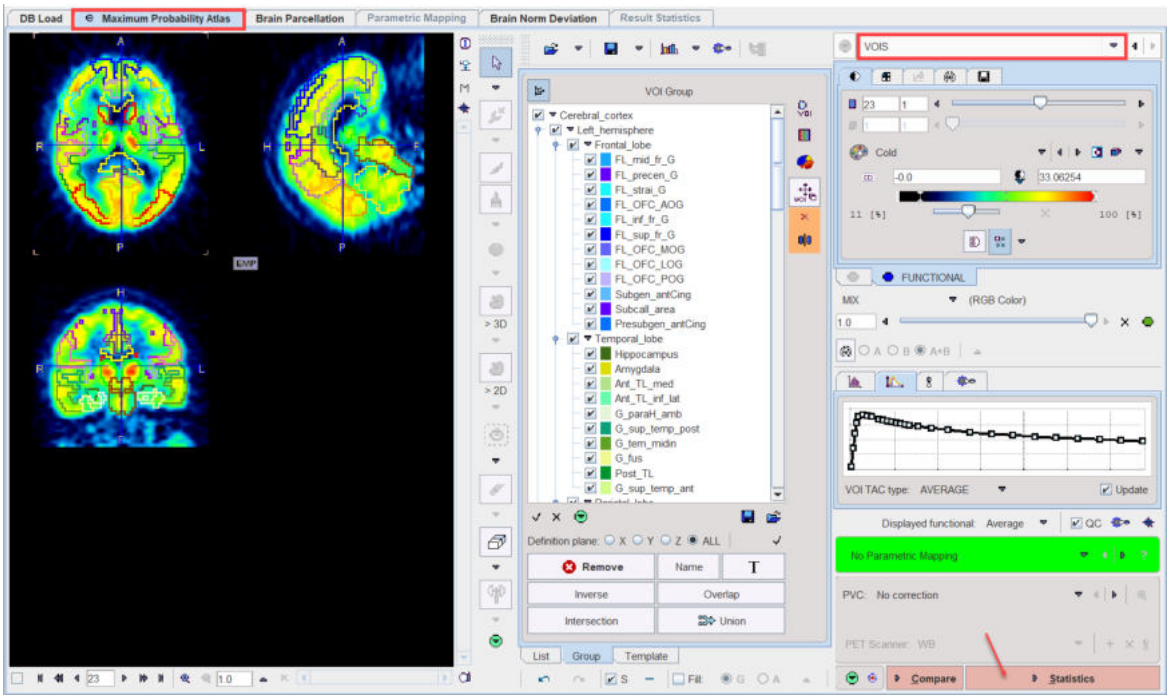
For the atlases with population tissue probability maps the white matter tissue probability map can be used to allow the intersection of the population white matter region with the selected atlas. This can be easily achieved by enabling the **White matter atlas VOIs** box.

Please define the result space, the gray and white matter masking, optionally CSF masking, and start the VOI outlining process using the **Outline** action button.

The GM, WM and CSF masking process is identical to the one described in the Workflow for Functional and Anatomical PET 83.

4.4.5 Brain VOI Editing and Statistics Calculation

The result of structure outlining is shown on the **VOIS** page as an overlay on the averaged PET image in the selected **Result space**.



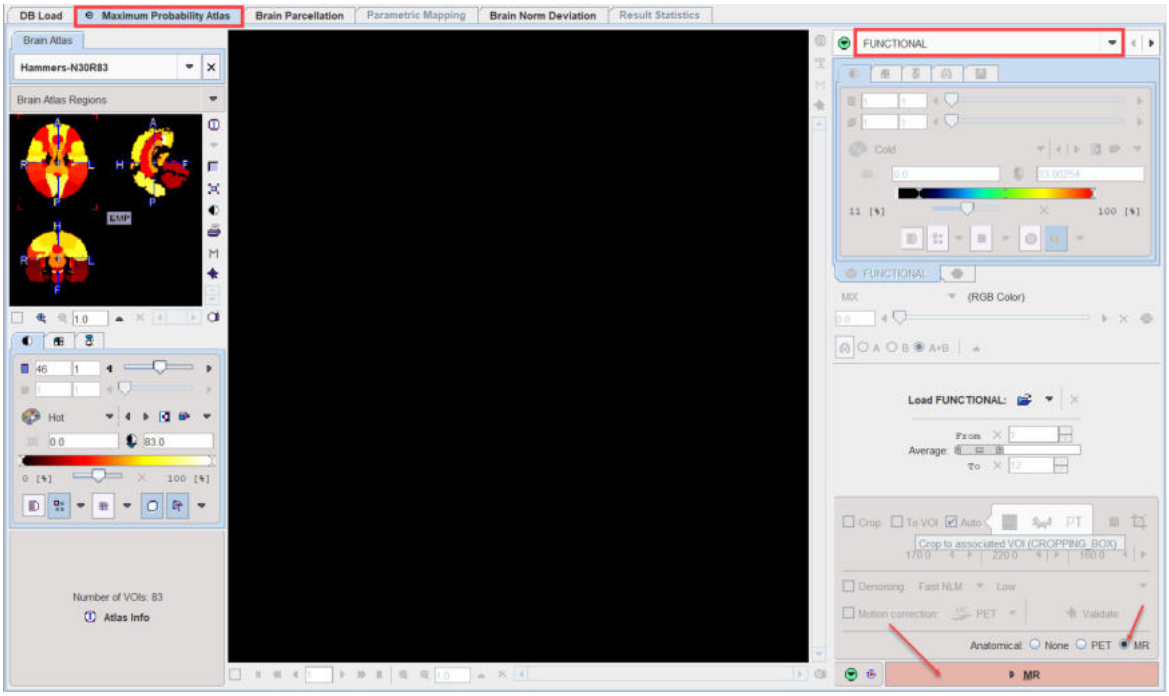
After editing the VOIs and configuring the optional partial volume correction **PVC**, please proceed with the **Statistics** action button. The result is shown on the Result Statistics 119 page.

4.5 Workflow for MR-only Studies

The workflow described in this section applies in the case of T_1 brain MR studies for which the user wants to calculate structural brain VOIs.

4.5.1 MR Loading Configuration

In order to configure PNEURO for the MR-only workflow please select the **T1** radio button on the **FUNCTIONAL** page.

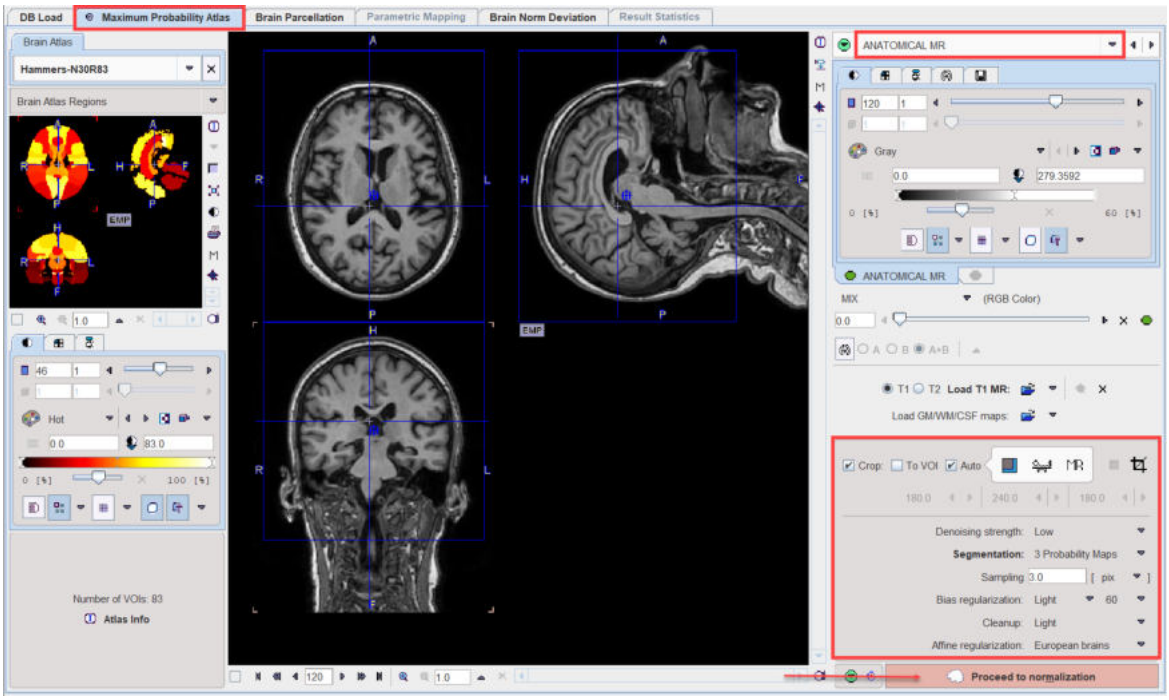


.

Then, proceed with the **MR** action button.

4.5.2 MR Image Loading and Segmentation

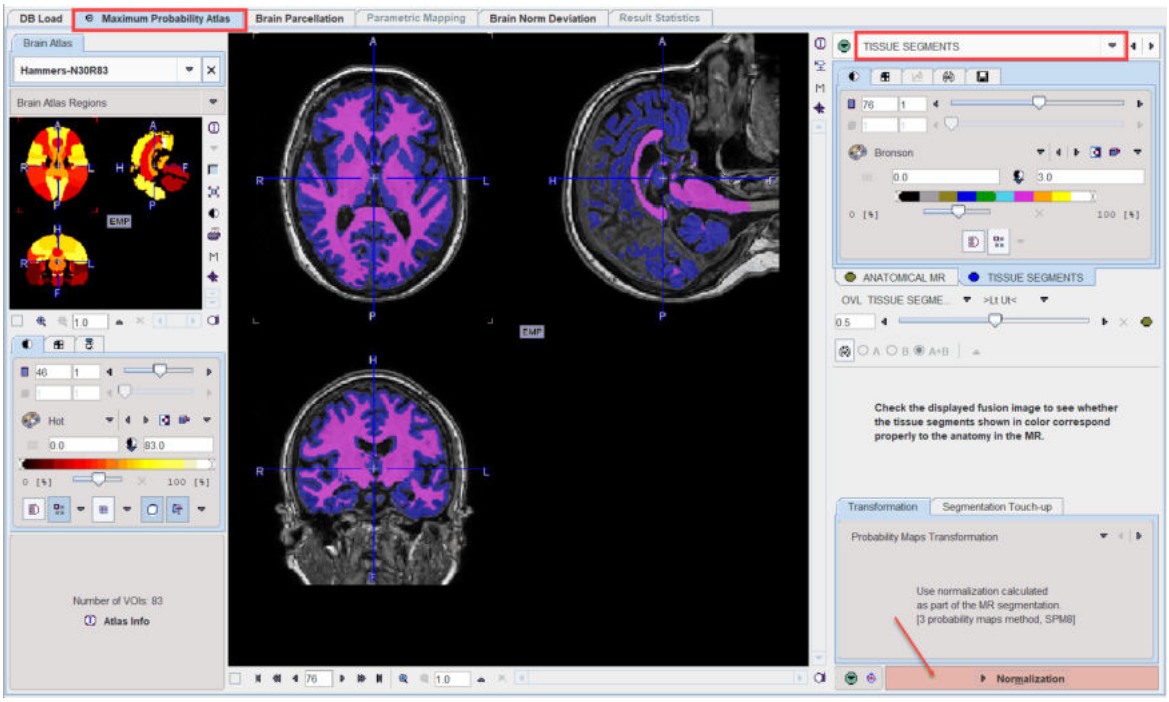
The **MR** page allows the T_1-weighted brain MR series to be loaded using the **Load MR** button.



Please define an appropriate cropping window, configure the denoising and segmentation parameters described above 62, and proceed using the **Proceed to normalization** action button.

4.5.3 MR-based Normalization

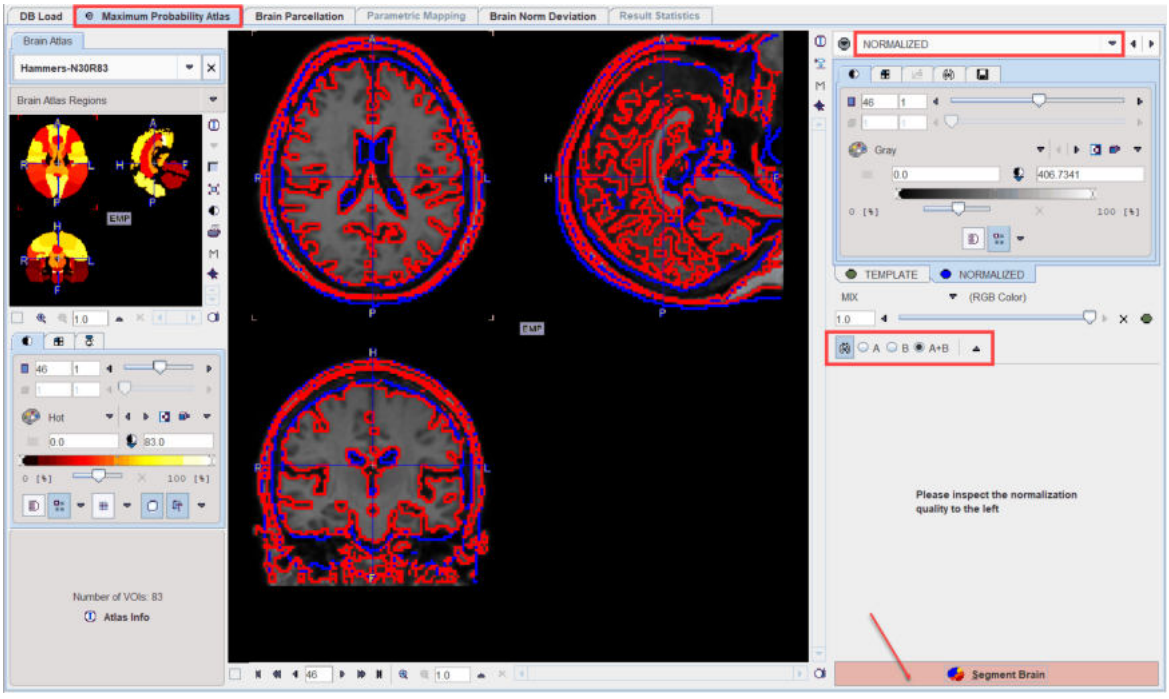
The result of the segmentation is shown as a fusion of the tissue segments with the MR image on the **TISSUE SEGMENTS** page.



Please configure the normalization in the lower right as explained above[65] and proceed with the **Normalization** action button.

4.5.4 Brain Segments Calculation

The stereotactic normalization result is shown on the **NORMALIZED** page.

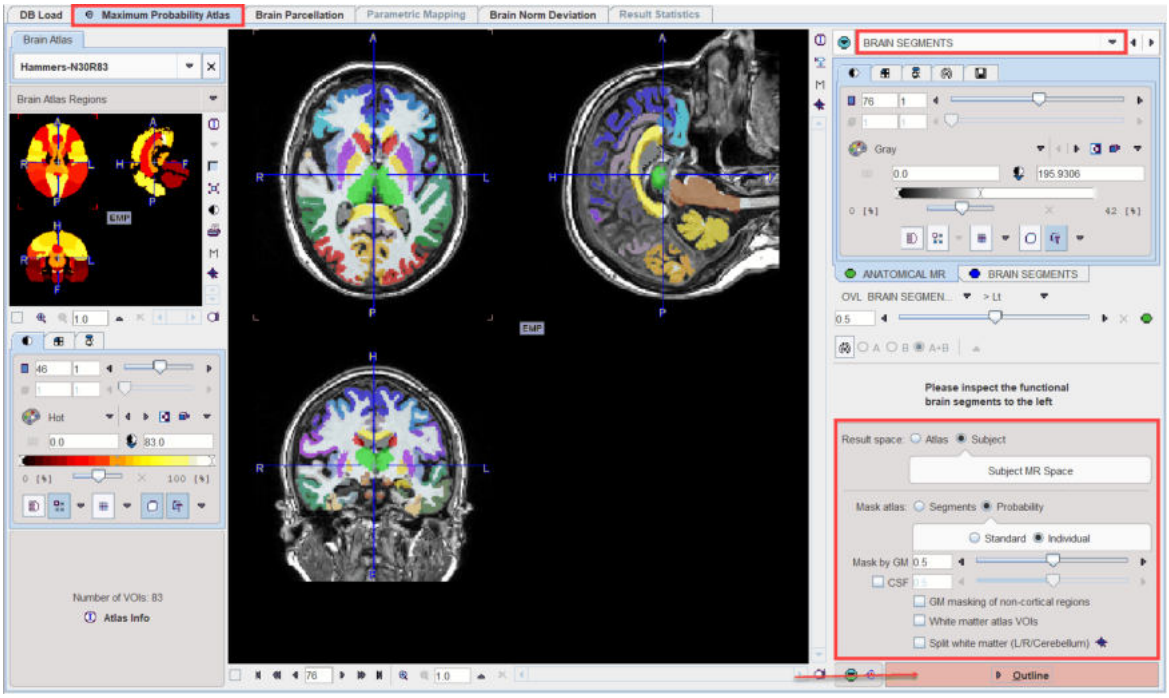


The normalized MR image is shown together with the MR template image for verification purposes.

If the normalization was successful, start mapping of the brain structures to the MR space using the **Segment Brain** action button.

4.5.5 Outlining of Brain Structures

The result of the brain structure transformation is shown on the **BRAIN SEGMENTS** page.

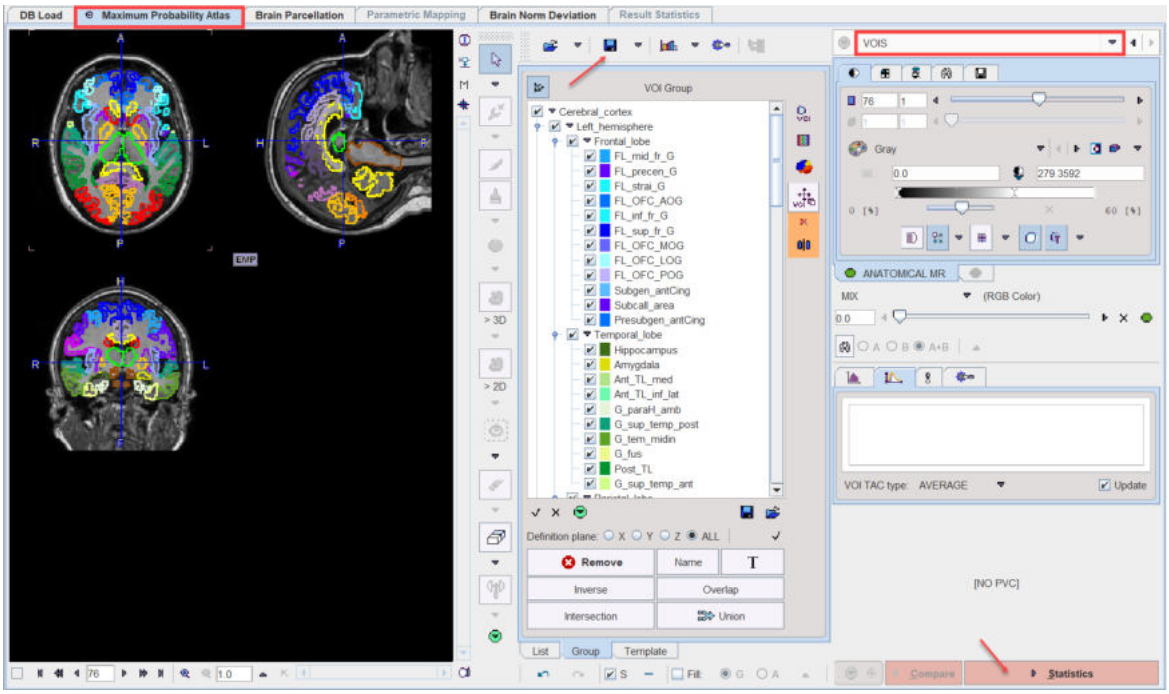


Please configure the result space and and the masking options as described above[68], and start the VOI generation with the **Outline** action button.

Optionally, if enabled, the **White matter atlas VOIs** box allows the white matter segment to be intersected with the selected atlas. Please note that the white matter voxels which do not have any definition in the atlas will be classified as general white matter.

4.5.6 Brain VOI Editing and Statistics Calculation

The result of structure outlining is shown on the **VOIS** page.



If necessary, the VOIs can be adjusted, and should then be saved. Then activate **Statistics** to proceed. The volume of the VOIs will be calculated and shown on the **Result Statistics** page[119].

5 Brain VOIs Based on T1-MRI Database

The functionality of this module is based on methodology developed by Renaud Maroy and licensed from the Commissariat à l'Energie Atomique (CEA), Orsay, France. Given an anatomical T_1-MR brain series, it allows the user to calculate objective brain structure outlines in a guided step-by-step fashion. These definitions can then be applied to PET images of the same subject for calculating regional statistics or Time-Activity Curves (TAC), which may optionally be corrected for the partial-volume effect.

A particular strength of this module is its ability to precisely segment the deep nuclei (caudate, putamen, ventral striatum, globus pallidus, thalamus). This is a difficult task because of the complex structure shapes as well as the low contrast and high noise of the MR in this image area. The sophisticated methodology applied allows detection of the structure boundaries with high accuracy, so that the result may even be used for morphometric analyses.

5.1 Methodology

5.1.1 Brain Parcellation

The brain parcellation methodology is based on a database of 26 normal T_1-MR brain scans (all non-smokers; female: 3, left-handed:1, age: 34±12, min 19, max 39). The images were manually segmented by neuroanatomically trained operators as described in Dououd et al. [1]. The parcellation of an individual T_1-MR brain scan consists of the following processing steps:

1. Reduction of the noise in the MR image by a non-local means algorithm.
2. Segmentation of the gray matter (GM), white matter (WM) and the cerebrospinal fluid (CSF).
3. Splitting of the left and right hemispheres. This step requires three anatomical points: the anterior commissure (AC), the posterior commissure (PC) and an inter-hemispheric point (IHP). Both hemispheres are processed separately in the following.
4. Definition of a fourth anatomical point located between the caudates, allowing calculation of the average thickness of the frontal horn of the left and right ventricles. The ventricles are known to increase in volume with age and as a consequence of a number of neurological conditions and diseases.
5. Selection of the N most comparable brain hemispheres in the knowledge base using the frontal horn thickness and an inter-caudate point (IC) specified by the user. The optimal number of hemispheres to include is in the order of 8.
6. Each of the selected knowledge base hemispheres is elastically matched to the subject hemisphere using a hierarchical approach. It starts with a global affine transformation and then adjusts each structure separately with a free form deformation algorithm. The result is a set of N structure definitions in the geometry of the subject hemisphere.
7. A maximum probability atlas is derived from the N structure definitions as well as the GM and WM segments, resulting in 16 structures: Structures with separate left/right parts are gray matter, caudate, putamen, thalamus, globus pallidus; structures without laterality are cerebellum and liquor.
8. The gray matter structure is further parcellated into cortical regions by means of an atlas.

Reference

1. Douaud G, Gaura V, Ribeiro MJ, Lethimonnier F, Maroy R, Verny C, Krystkowiak P, Damier P, Bachoud-Levi AC, Hantraye P et al: Distribution of grey matter atrophy in Huntington's disease subjects: a combined ROI-based and voxel-based morphometric study. Neuroimage 2006, 32(4):1562-1575. DOI

5.1.2 Brain Structures

Currently, segments and outline definitions of the following structures are obtained:

- Structures with separate left/right parts: gray matter, caudate, putamen, thalamus, globus pallidus
- Structures without laterality: cerebellum, liquor
- Cortical structures: intersection of the grey matter segment at p>0.3 with the selected atlas[38].

5.2 Brain Parcellation Implementation in PNEURO

Brain parcellation in PNEURO supports two situations:

- PET and MR: The subject had a brain PET study as well as a corresponding T_1-weighted anatomical MR study. In this case the MR-derived brain VOIs are applied for calculating statistics of brain PET uptake.
- MR only: The subject had an anatomical T_1-weighted MR study covering the entire brain. In this case the MR-derived brain VOIs are the main outcome of the analysis and might be applied for morphometric or other purposes.

The corresponding PNEURO workflows are outlined in the following.

Analysis of Study with PET and T1-weighted MRI

1. Loading of the PET image series. The series may be static or dynamic.
2. Dynamic case only: Averaging of the PET series in a specified range of the acquisition. The averaged PET image is used for all following steps except for the calculation of statistics.
3. Loading of the T_1-weighted MR image series.
4. Denoising of the MR images.
5. Segmentation of gray matter (GM), white matter (WM) and the cerebrospinal fluid (CSF) and splitting of the brain into the left and right hemispheres as well as cerebellum. This requires 4 anatomical landmarks which are automatically placed, but can be corrected if needed.
6. Interactive adjustment of the boundaries between the segments and definition of the number of hemispheres in the knowledge base to compare with. Parcellation of the brain structures based on the hemispheres which are most similar to the subject's hemispheres.
7. Rigid matching of the PET image to the MR image.
8. Presentation of the brain contours in a VOI editor together with the MR images, so that the user can adjust them interactively and save the final VOI set.
9. Application of the VOIs to the matched PET series for calculating statistics. This results in TACs for a dynamic PET series, and simple statistics otherwise. Optionally, a geometric partial-volume correction can be applied during the statistics calculation.
10. Dynamic case only: The resulting TACs can directly be transferred to the kinetic modeling tool or saved.

There is an alternative with respect to the recommended workflow above. Instead of defining the VOIs in the MR space, they may be transformed to the PET space, and the statistics calculated using the PET series in the original space.

Analysis of MR-only Study

If the PET information is lacking, the processing sequence reduces to the following workflow steps:

1. Loading of the T_1-weighted MR image series.
2. Denoising of the MR images.

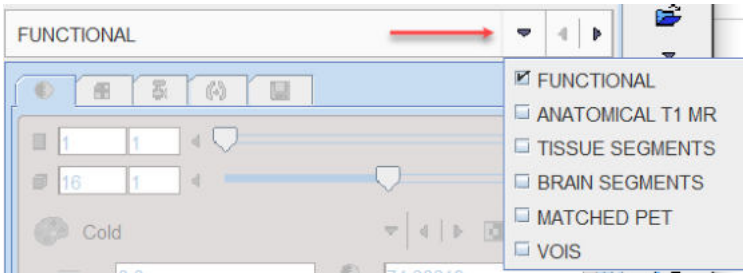
3. Segmentation of gray matter (GM), white matter (WM) and the cerebrospinal fluid (CSF) and splitting of the hemispheres. This requires 4 anatomical landmarks which are automatically placed, but can be corrected if needed.
4. Interactive adjustment of the boundaries between the segments and definition of the number of hemispheres in the knowledge base to compare with. Parcellation of the brain structures based on the hemispheres which are most similar to the subject's hemispheres.
5. Presentation of the brain contours in a VOI editor together with the MR images, so that the user can adjust them interactively and save the final VOI set
6. Optional: application of the VOIs to the MR series, mainly for calculation of the VOI volumes.

Documentation of the Workflows

The implementation of the two workflows described above in PNEURO is very similar. Therefore, only the first workflow with PET and a T_1-weighted MRI will be described in full detail, while the MR-only workflow is restricted to the essential parts.

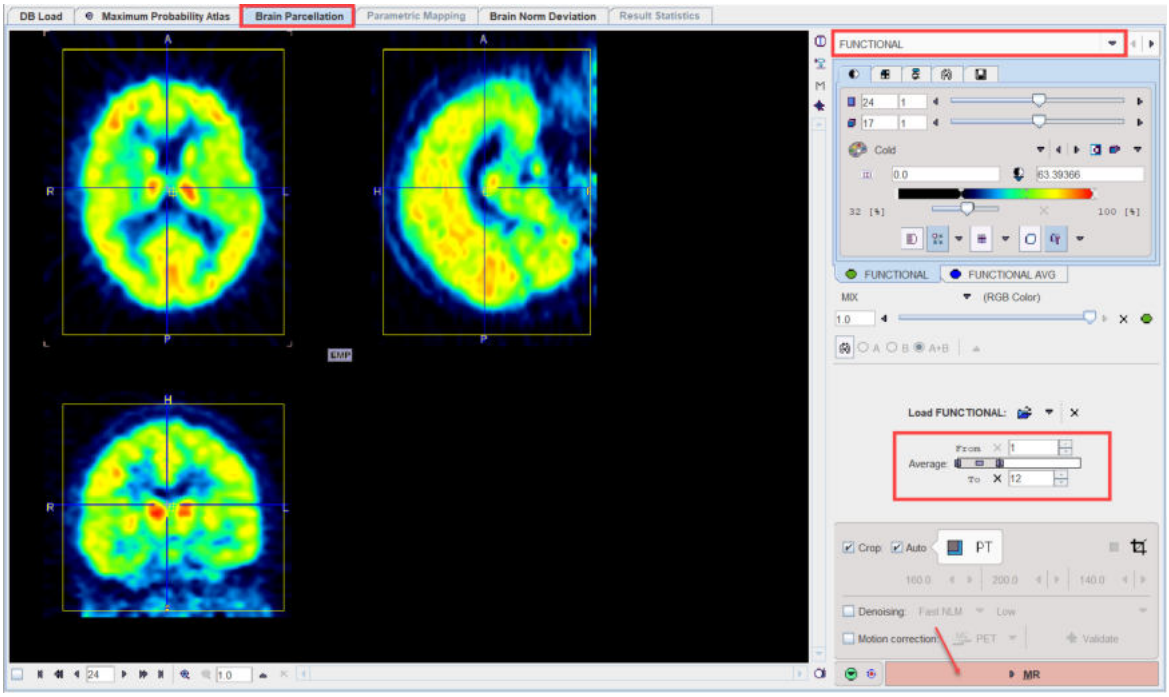
5.3 Workflow for Studies with PET and MR

The workflow will run through the following 6 pages of the **Brain Parcellation** module:



5.3.1 PET Image Loading and Time Averaging

The easiest way to start stepwise processing is to select the **Brain Parcellation** tab.

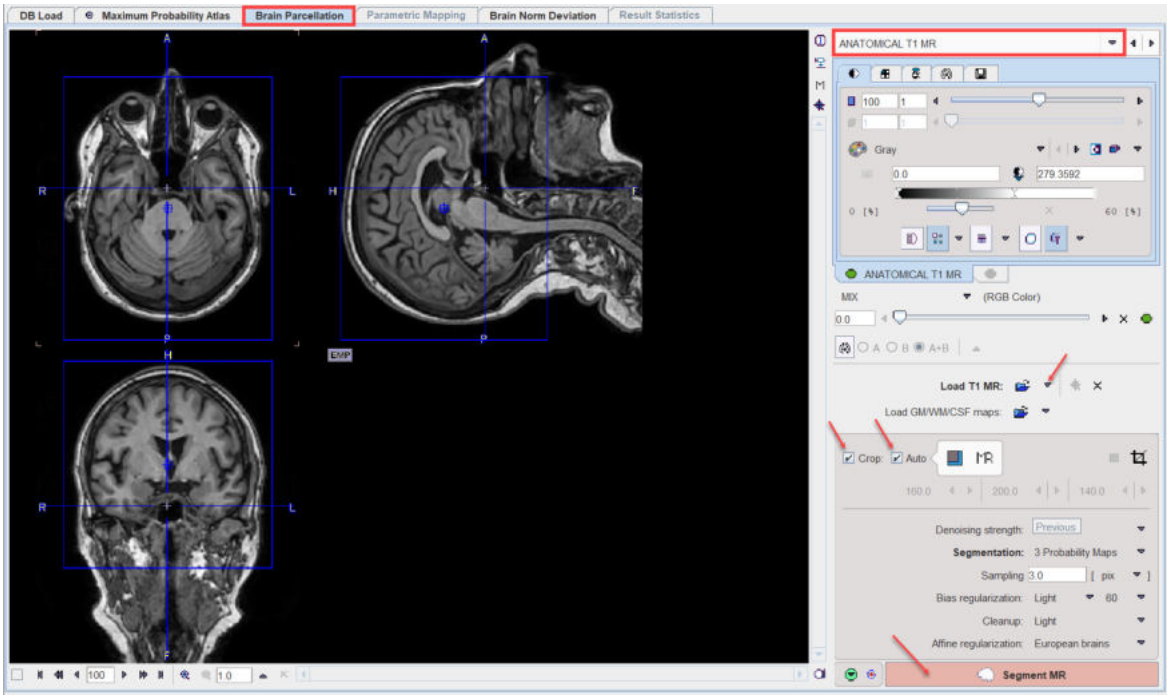


PET image loading, the averaging of a dynamic series, the cropping, the denoising and the motion corection are working exactly as described[79] for the Maximum Probability Atlas workflow.

Continue to loading of the MR images with the **MR** action button in the lower right.

5.3.2 MR Image Loading and Segmentation

The **ANATOMICAL T1 MR** page allows loading the T_1-weighted brain MR image of the same subject using the **Load T1 MR** button. The image should be T_1-weighted, cover the entire brain and have a pixel size of about 1mm. After loading, the image should appear in radiological HFS orientation.



MR Image Cropping

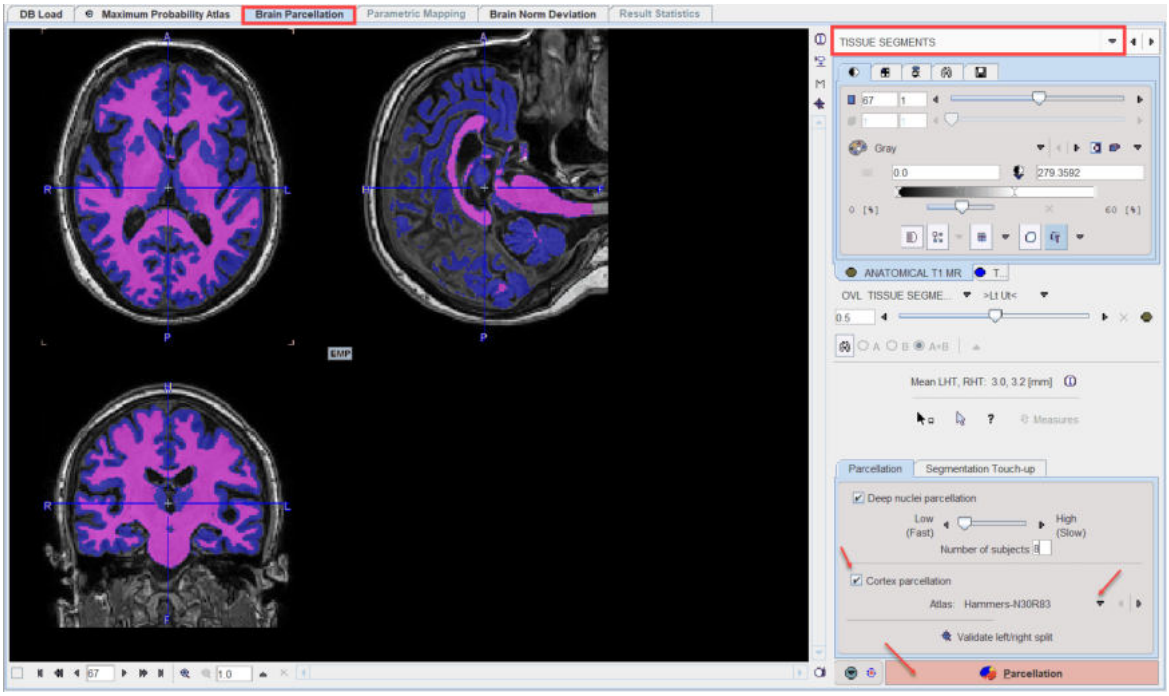
The MR image is the basis for the parcellation. Experience has shown that problems may occur if the MR field-of-view is much larger than the brain as occurs for instance with sagittal MR acquisitions. Therefore, please use the **Crop** facility for reducing the MR data set to the relevant portion with skull and brain, but without the neck in the same way as the PET image is cropped.

MR Image Segmentation

The MR image needs to be segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The options of the algorithm are described above[62]. The actual segmentation is started with the **Segment MR** action button. Note that the denoising and segmentation process may take several minutes.

5.3.3 Landmark Definition and Parcellation

The result of the segmentation is shown as a fusion of the tissue segment map with the MR image on the **TISSUE SEGMENTS** page. Note that the **TISSUE SEGMENTS** image tab contains a label image with gray matter, white matter and CSF represented by the label values 1, 2 and 3, respectively. The segmentation can be trimmed using the controls in the **Segmentation Touch-up** panel as described above[64].

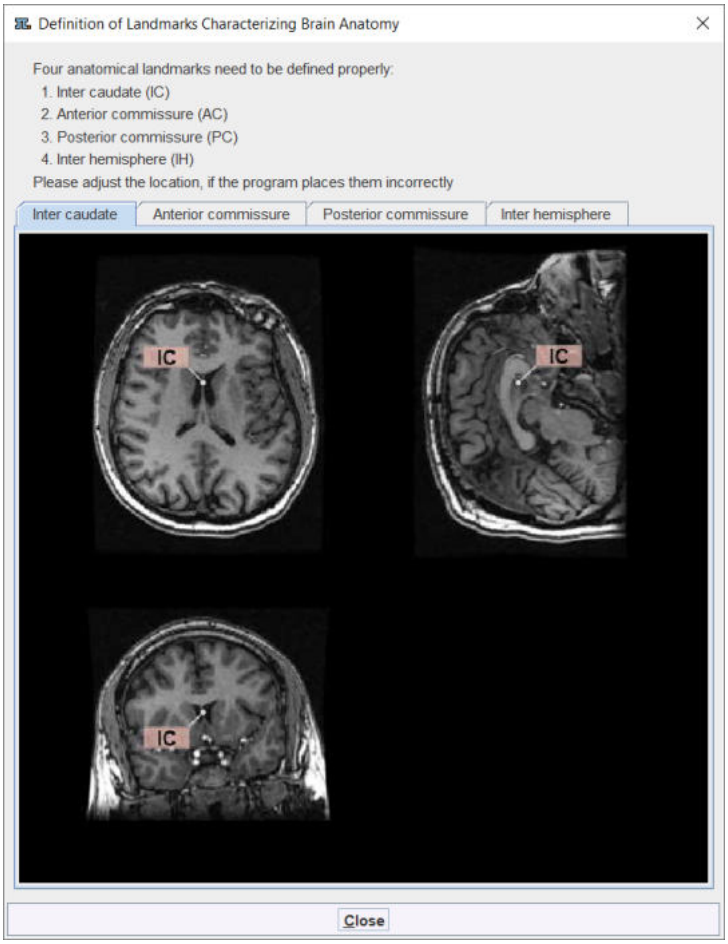


Landmark Definition

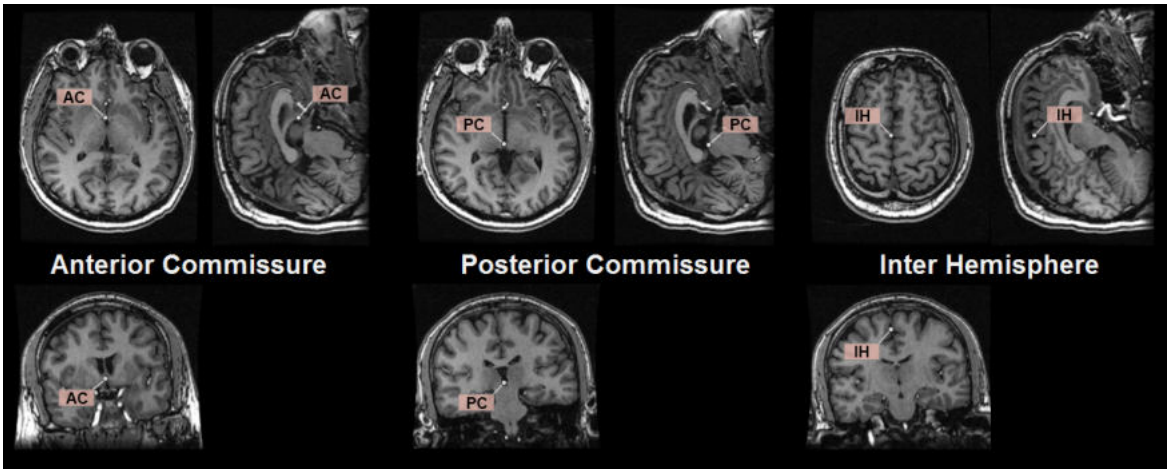
The parcellation procedure requires four anatomical landmarks:

- an inter-caudate point (IC) for separating the ventricles,
- the anterior commissure (AC),
- the posterior commissure (PC)
- an inter-hemispheric point (IHP) for defining the plane which divides the hemispheres.

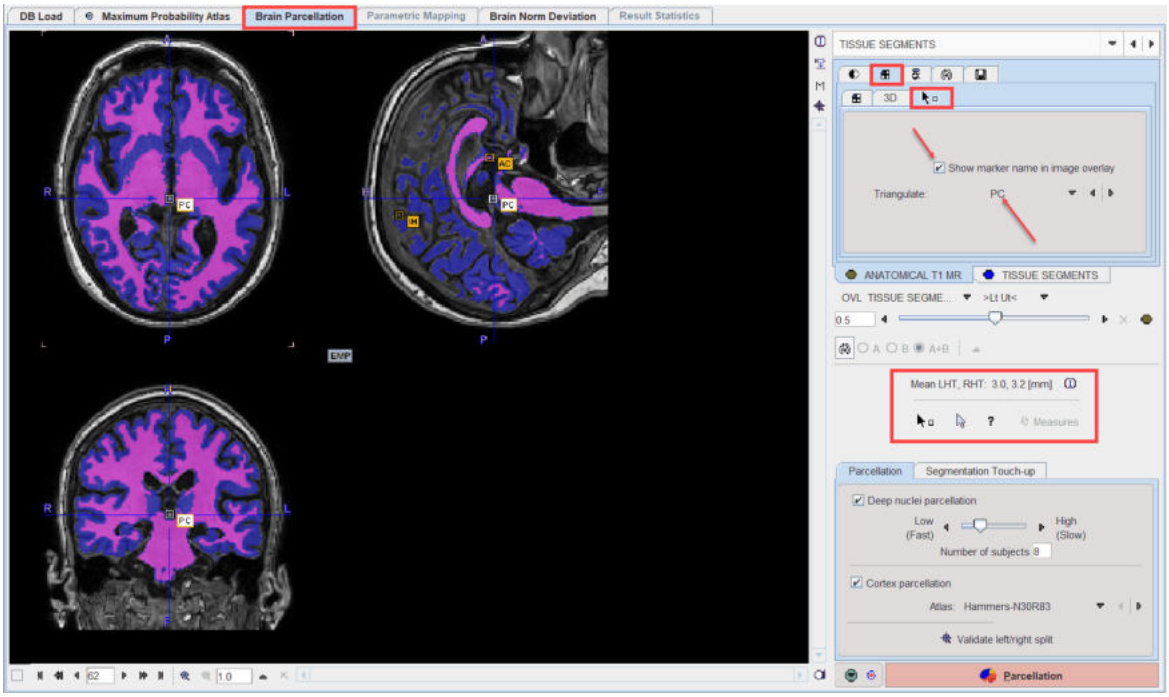
A visual help for this task can be opened with the **?** button to the left of the **Indexes** button. It appears with the IC landmark on front as illustrated below.



The location of the other three landmarks is illustrated below.



Estimates of the landmark locations are obtained as part of the segmentation. They can be inspected by activating the button, which also opens the markers panel. There the**Traingulate** list allows easily selecting any of the 4 landmarks, which is shown as a marker in the triangulated image. If the location is not accurate within a few millimeters, please adjust the position by dragging the marker. You may need to shift the fusion slider to the left in order to see the markers on top of only the MR.



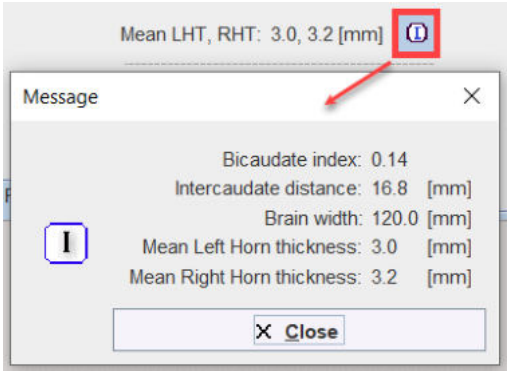
To avoid marker placement when clicking into the images for triangulation, hold down the SHIFT+CTRL keys. Note the indication of the landmark which needs to be placed (IC, AC, PC, IH) as part of the cursor symbol.

Brain Indexes

Based on the segmentation five indexes are calculated which will be further employed for the selection of the most similar hemispheres from the knowledge base:

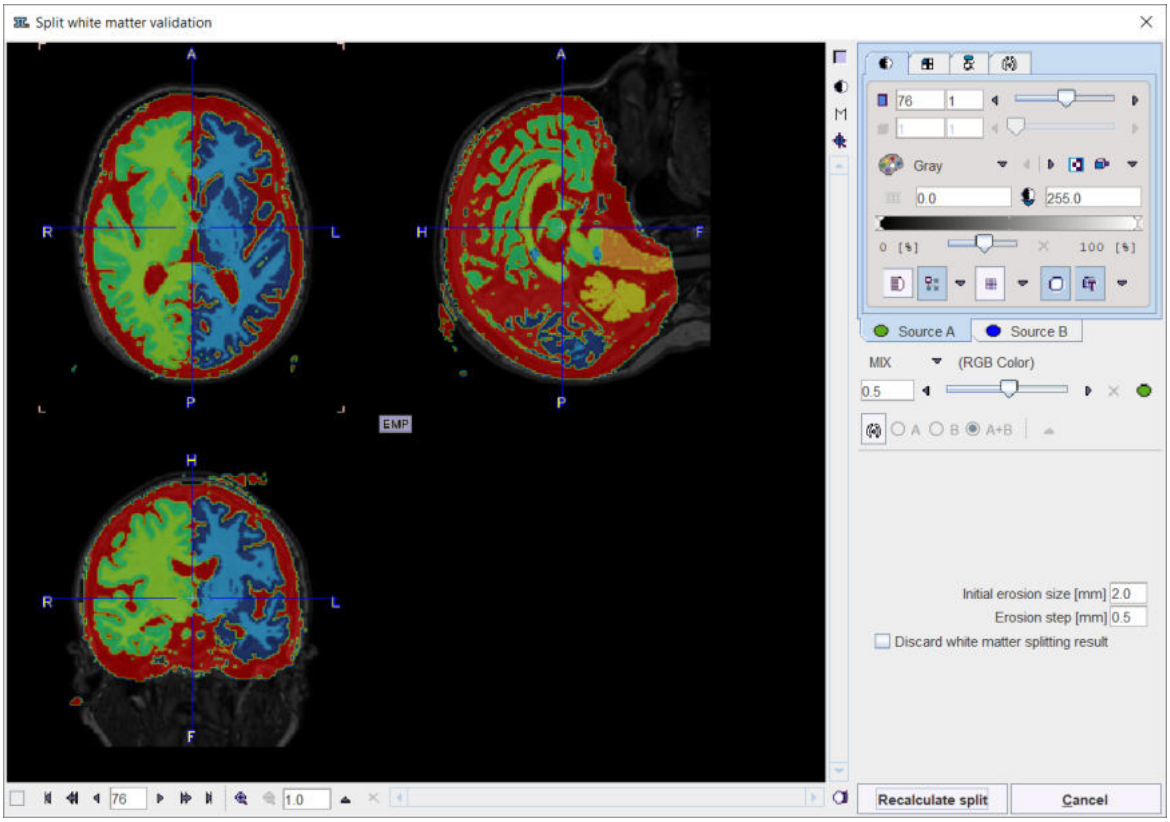
- Bi-caudate index: ratio of caudate distance and brain width.
- Intercaudate distance: minimal distance between the caudate heads in the axial plane.
- Brain width: size of the brain in the left-right direction.
- Left horn thickness (LHT): mean thickness of left horn in coronal section.
- Right horn thickness (RHT): mean thickness of right horn in coronal section

The horn thicknesses are shown in the user interface, and complete information can be opened with the button indicated below.



Split Validation

The **Validate left/rightsplit** button opens a dialog window for inspecting the result of the brain splitting procedure.



There are 7 resulting segments, which are shown in the **Source B** tab. They correspond to the left white matter, left gray matter, right white matter, right gray matter, cerebellar gray matter(including pons), cerebellar white matter and CSF. If the procedure doesn't come up with at reasonable splitting into right and left parts, there are two options to proceed. The **Initial erosion size [mm]** and **Erosion step [mm]** parameters can be modified and the procedure restarted using **Recalculate split**, or splitting can be switched off by the **Discard white matter splitting result** flag. In the latter case please confirm with the **Discard split** button to close the window.

Note: A successful brain splitting is mandatory for the brain parcellation approach. If it fails and adjustment of parameter settings cannot fix the problem, parcellation will not proceed any further.

Parcellation

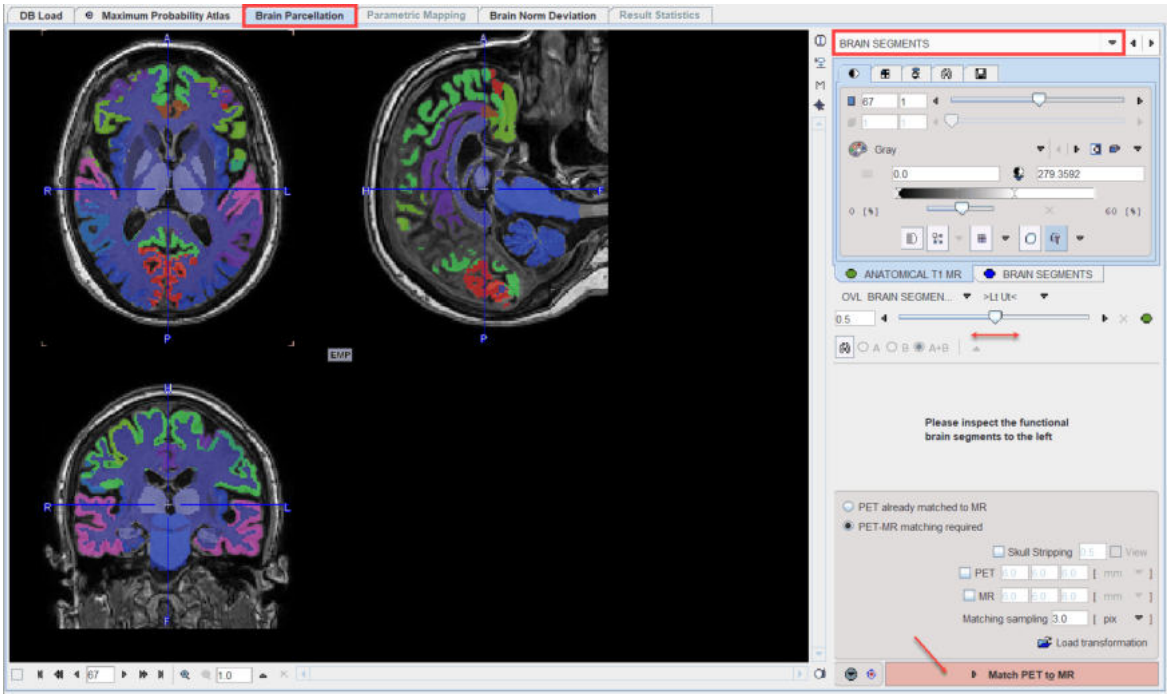
Deep nuclei parcellation calculates the brain structure shapes based on the knowledge base and the segmented MR image. Each hemisphere is processed individually. The user may select the **Number of subjects** (<=26) which are taken into account during parcellation. A higher number tends to provide more accurate results, but 8 has been found to be the optimal compromise between speed and accuracy.

Cortex parcellation uses the grey matter segment and a transformed atlas to derive cortical VOIs. The VOI **Atlas** to be used can be selected in the list. Note that the atlas will be intersected with the GM segment for creating the cortical VOIs.

Calculation is started with the **Parcellation** button. Its duration highly depends on the system performance (RAM, processors) and the selected number of subjects, and can range from minutes to hours.

5.3.4 Parcellation Result and Matching with PET

The **BRAIN SEGMENTS** page shows the parcellation result as an overlay onto the **MR** image.



The **BRAIN SEGMENTS** image represents a label atlas of the brain structures identified by the segmentation algorithm. Please check the alignment of the segments with the subject image using the fusion slider. In the case of a mismatch, try changing the GM segment definition on the (previous) **TISSUE SEGMENTS** page and/or the number of subjects included and repeat parcellation. Alternatively, the structure definitions can be adjusted manually after the outlining step.

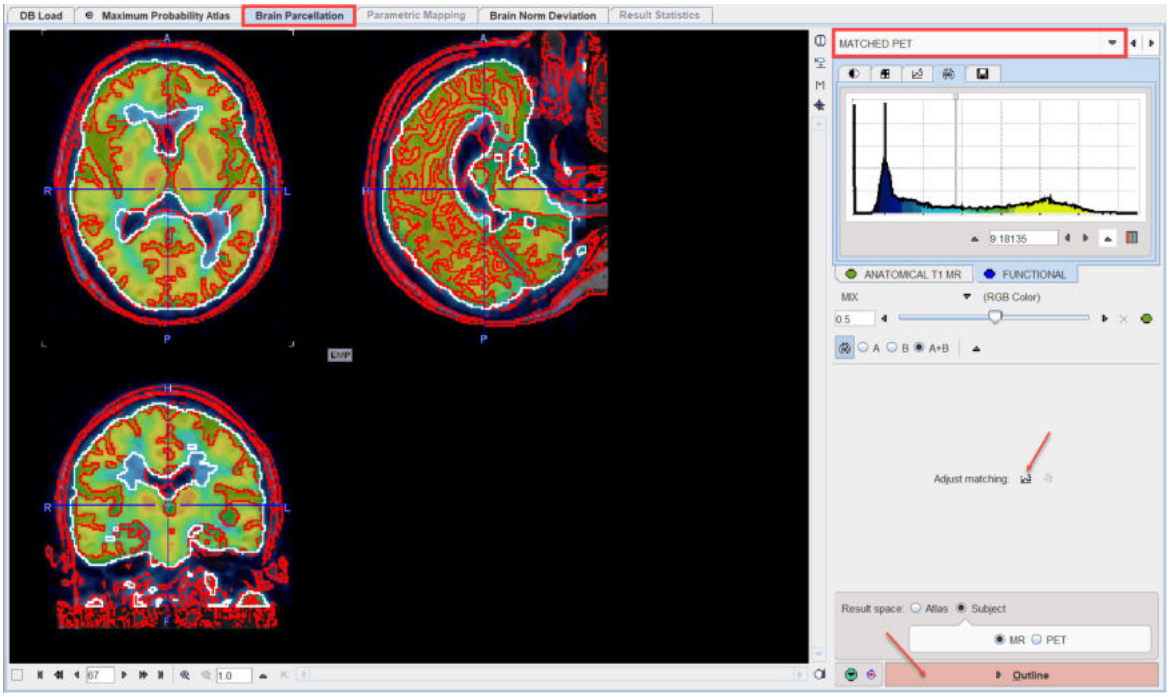
PET to MR Matching

The next step consists of rigidly matching the averaged PET image to the MR image. The matching options are in the lower right and described above 64.

Please activate the **Match PET to MR** action button to start matching.

5.3.5 Outlining of Brain Structures

The result of matching is shown on the **MATCHED PET** page. Please verify that matching was successful by evaluating the alignment in different parts of the brain. Particularly helpful to do so is to interactively drag the fusion balance left/right, and to enable contour outlines.

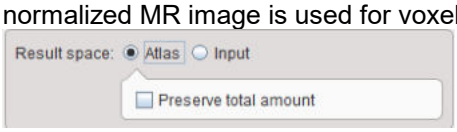


If the match is not satisfactory, there are two options to rectify the situation:

1. Return to the previous page, change the sampling and smoothing parameters and try the automatic matching again, or
2. Activate the **Adjust matching** button and shift/rotate the PET image interactively by dragging the handles in the image or entering offsets/angles on the **Move**/**Rotate** tabs.

Result Space

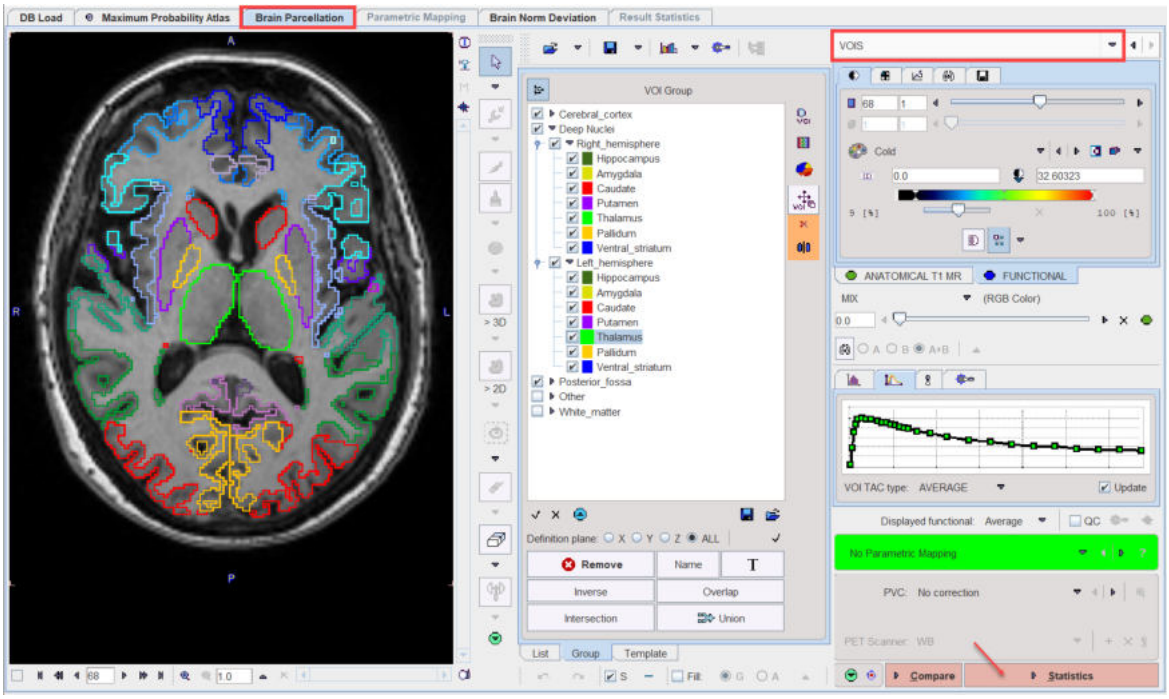
The next step consists of outlining the brain segments in the **Result space** which defines, where the PET statistics are calculated:

Atlas	The PET image is transformed to the Atlas space and the statistics are calculated with interpolated PET values. The **Preserve total amount** corrects for volume changes during the spatial normalization step. Image intensities are scaled by the amount of contraction that has occurred during spatial normalization, so that the total amount of gray matter remains the same as in the original image. This option should be used when the normalized MR image is used for voxel-based morphometry (VBM). 
MR	The PET image is transformed to the MR space and statistics are calculated with interpolated PET values.
PET	The brain structures are mapped to the PET space and statistics are calculated with the original PET values.

Outlining of the brain structures in the **Result space** is started with the **Outline** button.

5.3.6 Brain VOI Editing and Statistics Calculation

The **VOIS** page shows a fusion of the MR image and the average PET in the chosen result space, as well as the outlined brain structures.



On this page, the same procedures and options are available as described previously[74]:

- manual adjustments of the VOIs,
- calculation of the PET statistics with and without partial-volume correction,
- parametric mapping of the dynamic PET,
- comparison of the uptake pattern with a brain norm.

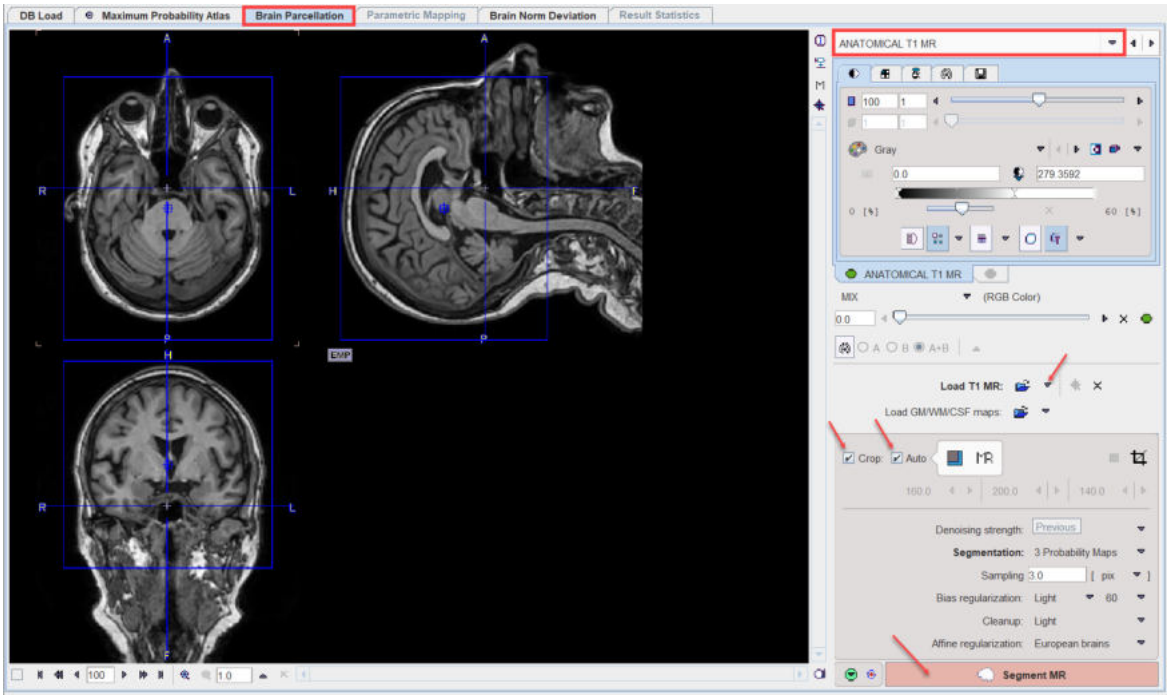
Statistics Calculation

Once the VOIs have been outlined and carefully checked by the user, it is recommended to first save them, then proceed with calculation of statistics using the **Statistics** action button.

5.4 Workflow for MR-only Studies

5.4.1 MR Image Loading and Denoising

To start parcellation of a brain MR series, select the **Brain Parcellation** tab of PNEURO and activate the **MR** button in the lower right of the **FUNCTIONAL** page to move to the **ANATOMICAL T1 MR** page. The image should be T_1-weighted, cover the entire brain and have a pixel size of about 1mm. After loading, the image should appear in radiological HFS orientation.



MR Image Cropping

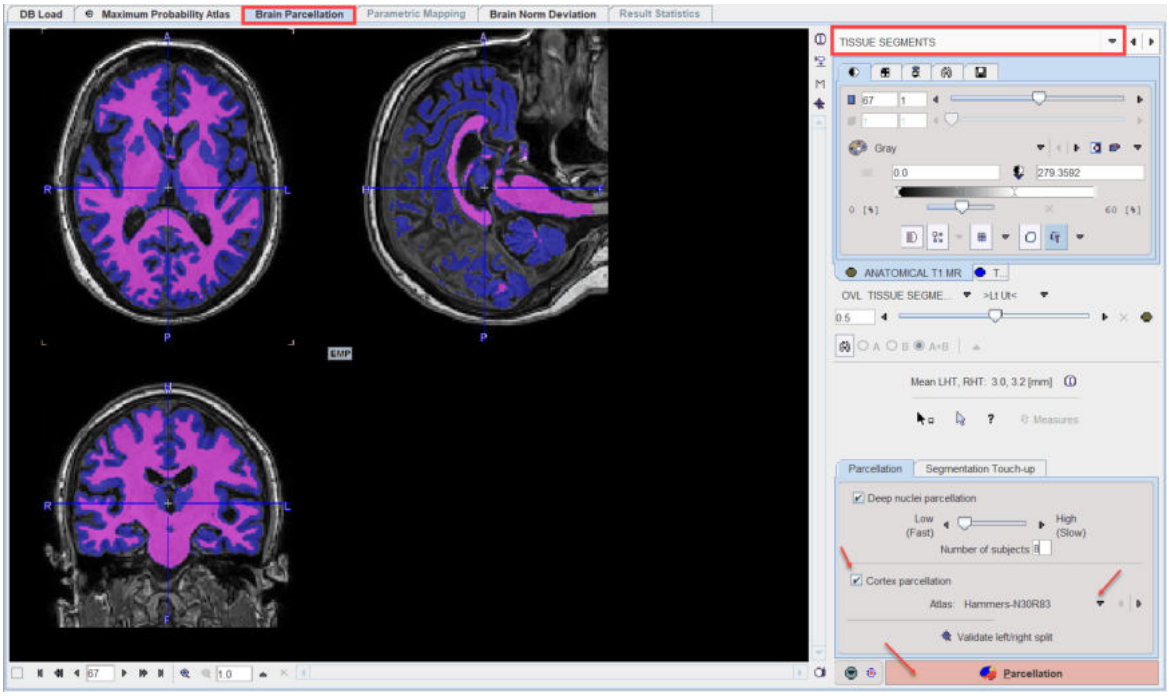
The MR image is the basis for the parcellation. Experience has shown that problems may occur if the MR field-of-view is much larger than the brain as occurs for instance with sagittal MR acquisitions. Therefore, please use the **Crop** facility to limit the MR data set to the relevant portion with skull and brain.

MR Image Segmentation

The MR image needs to be segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The settings that influence the algorithm are described above 62. The actual segmentation is started with the **Segment MR** action button. Note that the denoising and segmentation process may take several minutes.

5.4.2 Landmark Definition and MR Segmentation

The result of the segmentation is shown as a fusion of the tissue segment map with the MR image on the **TISSUE SEGMENTS** page. Note that the **TISSUE SEGMENTS** image tab contains a label image with gray matter, white matter and CSF represented by the label values 1, 2 and 3, respectively. The segmentation can be trimmed using the controls in the **Segmentation Touch-up** panel as described above 64.

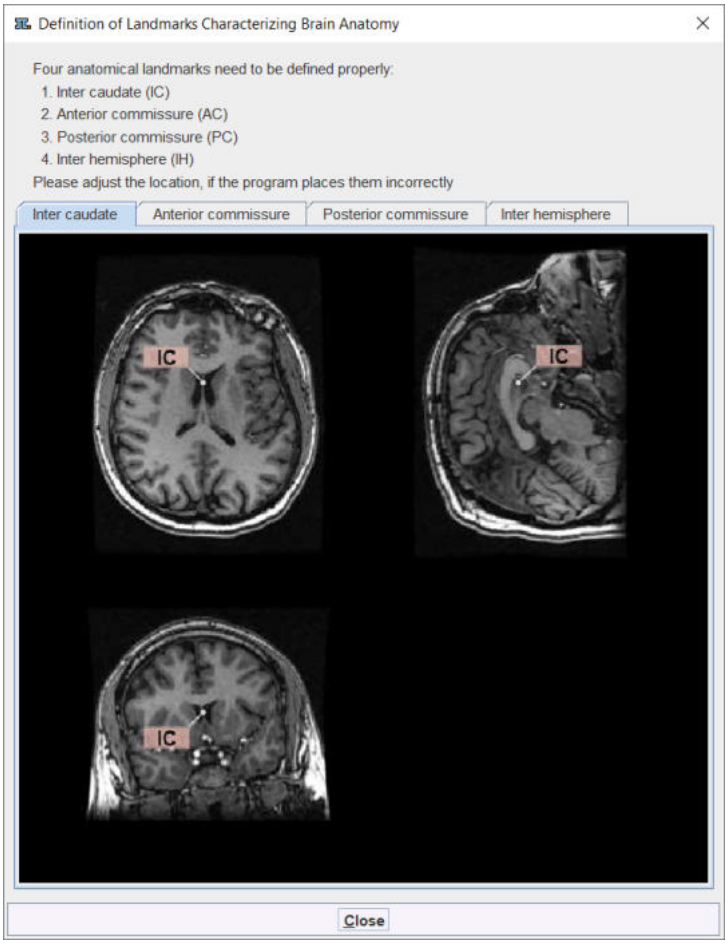


Landmark Definition

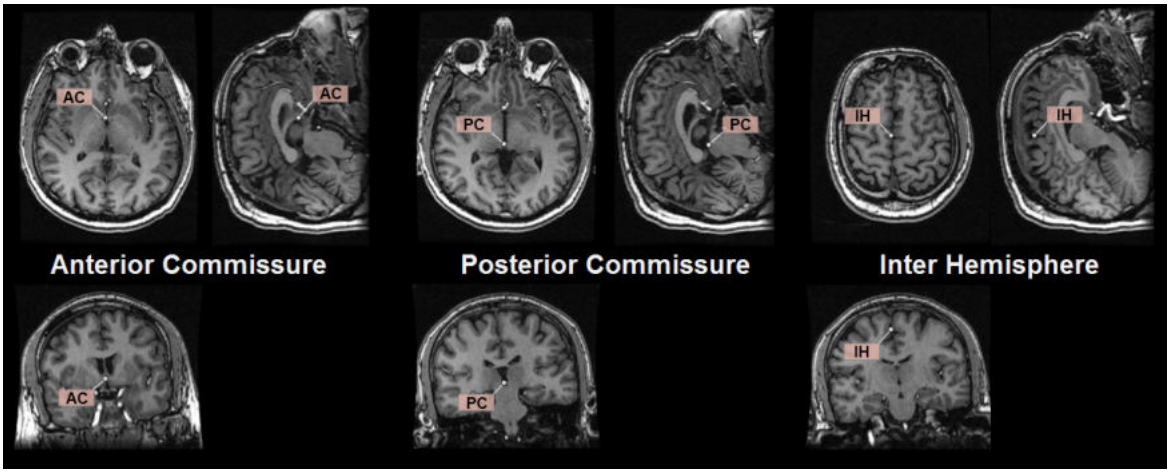
The parcellation procedure requires four anatomical landmarks:

- an inter-caudate point (IC) for separating the ventricles,
- the anterior commissure (AC),
- the posterior commissure (PC)
- an inter-hemispheric point (IHP) for defining the plane which divides the hemispheres.

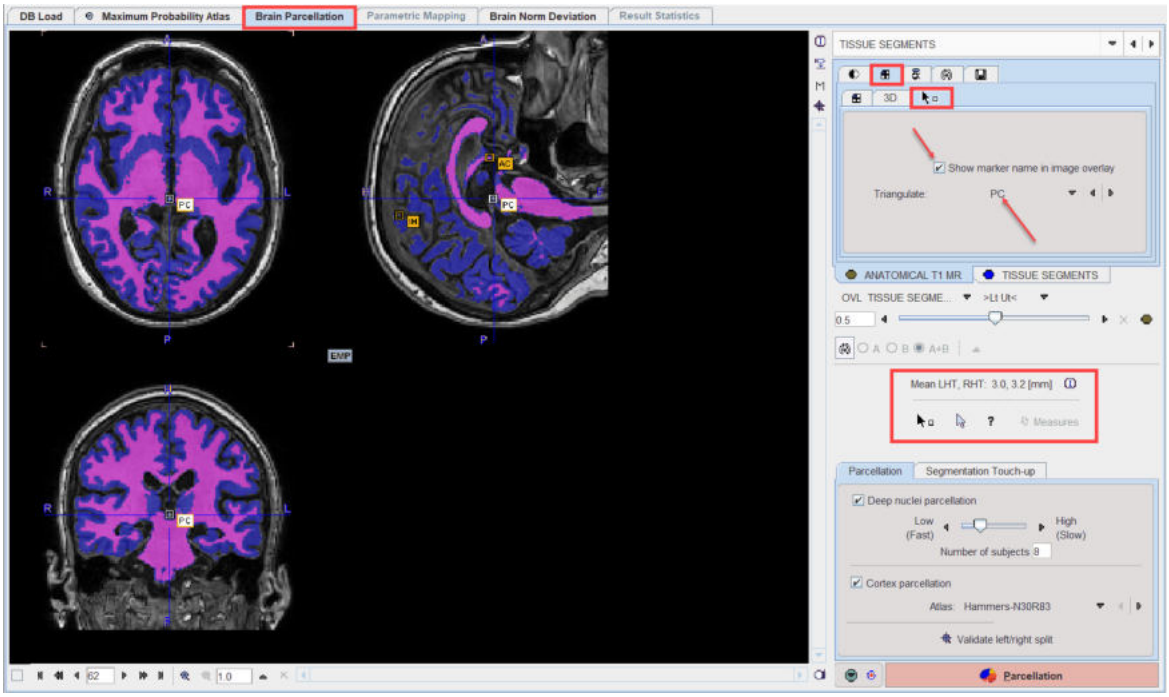
A visual help for this task can be opened with the **?** button to the left of the **Indexes** button. It appears with the IC landmark on front as illustrated below.



The location of the other three landmarks is illustrated below.



Estimates of the landmark locations are obtained as part of the segmentation. They can be inspected by activating the button, which also opens the markers panel. There the**Traingulate** list allows easily selecting any of the 4 landmarks, which is shown as a marker in the triangulated image. If the location is not accurate within a few millimeters, please adjust the position by dragging the marker. You may need to shift the fusion slider to the left in order to see the markers on top of only the MR.



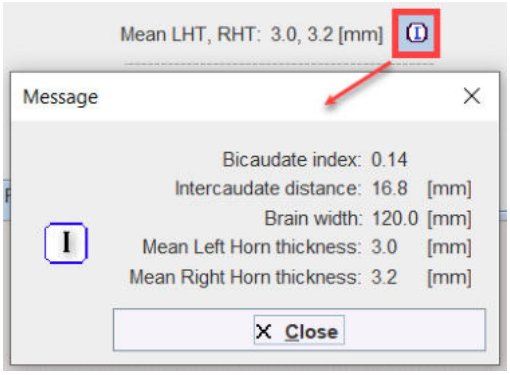
To avoid marker placement when clicking into the images for triangulation, hold down the SHIFT+CTRL keys. Note the indication of the landmark which needs to be placed (IC, AC, PC, IH) as part of the cursor symbol.

Brain Indexes

Based on the segmentation five indexes are calculated which will be further employed for the selection of the most similar hemispheres from the knowledge base:

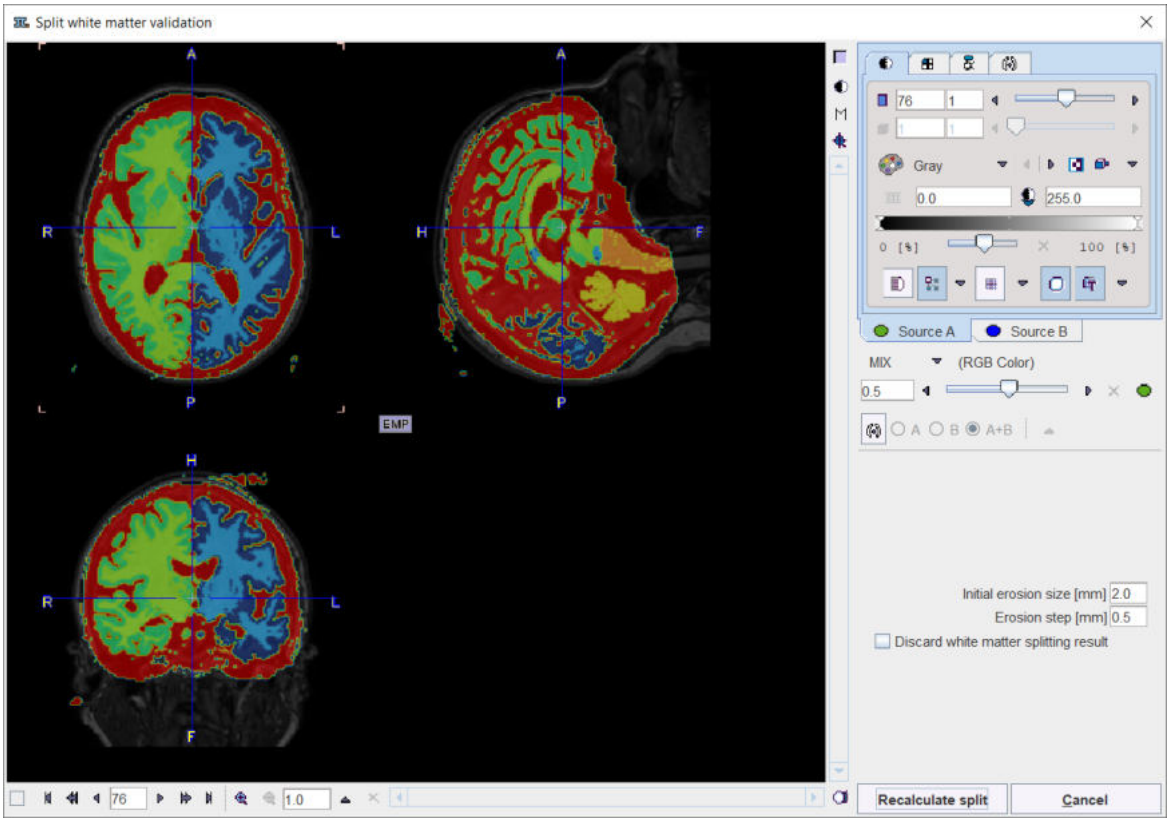
- Bi-caudate index: ratio of caudate distance and brain width.
- Intercaudate distance: minimal distance between the caudate heads in the axial plane.
- Brain width: size of the brain in the left-right direction.
- Left horn thickness (LHT): mean thickness of left horn in coronal section.
- Right horn thickness (RHT): mean thickness of right horn in coronal section

The horn thicknesses are shown in the user interface, and complete information can be opened with the button indicated below.



Split Validation

The **Validate left/rightsplit** button opens a dialog window for inspecting the result of the brain splitting procedure.



There are 7 resulting segments, which are shown in the **Source B** tab. They correspond to the left white matter, left gray matter, right white matter, right gray matter, cerebellar gray matter(including pons), cerebellar white matter and CSF. If the procedure doesn't come up with at reasonable splitting into right and left parts, there are two options to proceed. The **Initial erosion size [mm]** and **Erosion step [mm]** parameters can be modified and the procedure restarted using **Recalculate split**, or splitting can be switched off by the **Discard white matter splitting result** flag. In the latter case please confirm with the **Discard split** button to close the window.

Note: A successful brain splitting is mandatory for the brain parcellation approach. If it fails and adjustment of parameter settings cannot fix the problem, parcellation will not proceed any further.

Parcellation

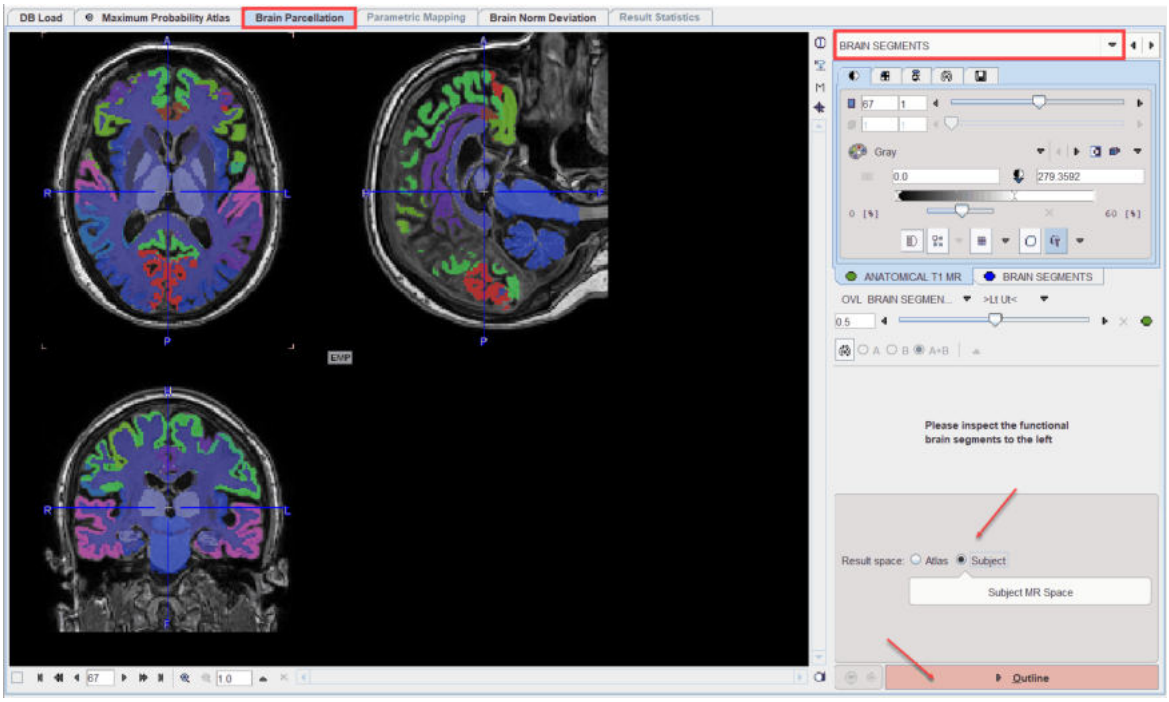
Deep nuclei parcellation calculates the brain structure shapes based on the knowledge base and the segmented MR image. Each hemisphere is processed individually. The user may select the **Number of subjects** (<=26) which are taken into account during parcellation. A higher number tends to provide more accurate results, but 8 has been found to be the optimal compromise between speed and accuracy.

Cortex parcellation uses the grey matter segment and a transformed atlas to derive cortical VOIs. The VOI **Atlas** to be used can be selected in the list. Note that the atlas will be intersected with the GM segment for creating the cortical VOIs.

Calculation is started with the **Parcellation** button. Its duration highly depends on the system performance (RAM, processors) and the selected number of subjects, and can range from minutes to hours.

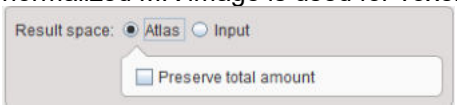
5.4.3 Parcellation Result and Outlining of Brain Structures

The **BRAIN SEGMENTS** page shows the parcellation result as an overlay to the **MR** image.



The **BRAIN SEGMENTS** image represents a label atlas of the brain structures identified by the segmentation algorithm. Please check the alignment of the structure information with the subject image with the fusion slider. In the case of a mismatch, try changing the GM segment definition on the (previous) **TISSUE SEGMENTS** page and/or the number of subjects included, and repeat parcellation. Alternatively, the structure definitions can be adjusted manually after the outlining step.

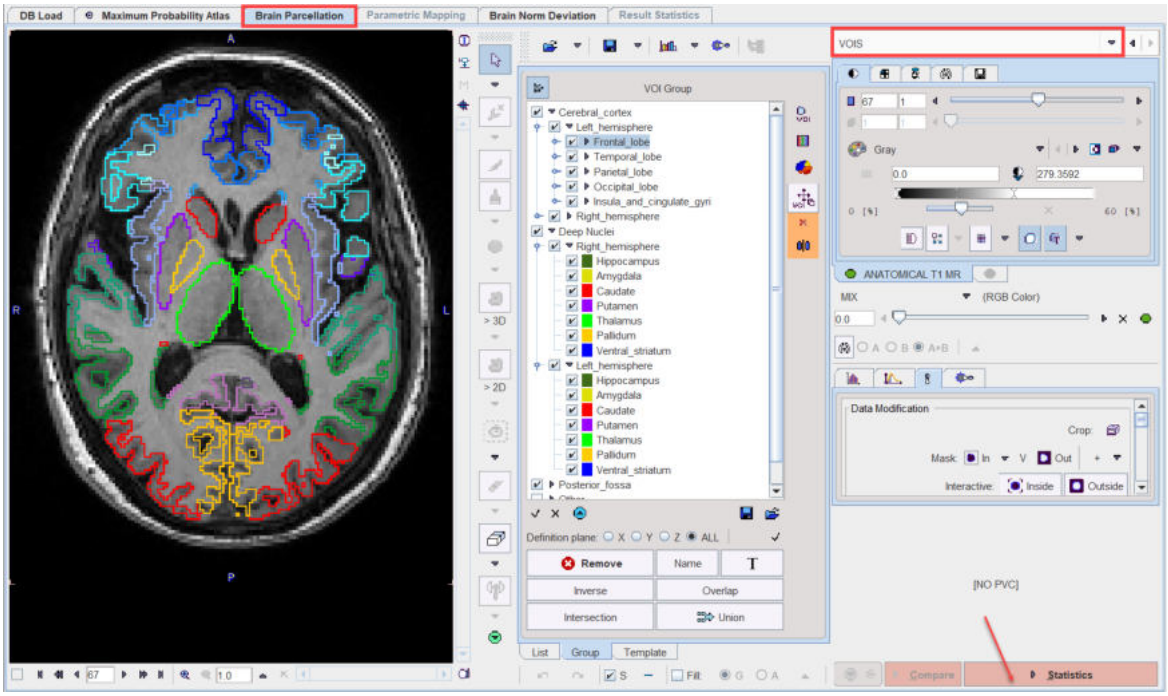
The next step consists of outlining the brain segments in the desired **Result space** :

Atlas	The MR image is transformed into the Atlas space and statistics are calculated with interpolated MR values. The **Preserve total amount** option corrects for volume changes during the spatial normalization step. Image intensities are scaled by the amount of contraction that has occurred during spatial normalization, so that the total amount of gray matter remains the same as in the original image. This option should be used when the normalized MR image is used for voxel-based morphometry (VBM). 
Subject	The statistics are calculated with original MR values.

Outlining of the brain structures is started with the **Outline** button.

5.4.4 Brain VOI Editing and Statistics Calculation

The **VOIS** page shows the MR image as well as the outlined brain structures.



VOI Editing and Selection

At this time the contour VOIs can be adjusted interactively using the VOI features, as described in the *PMOD Base Functionality Guide* and in the Introduction[22]. Note that the **List** tab should be selected while making adjustments, and that depending on the configuration[7] only a reduced set of VOI tools is available.

Statistics Calculation

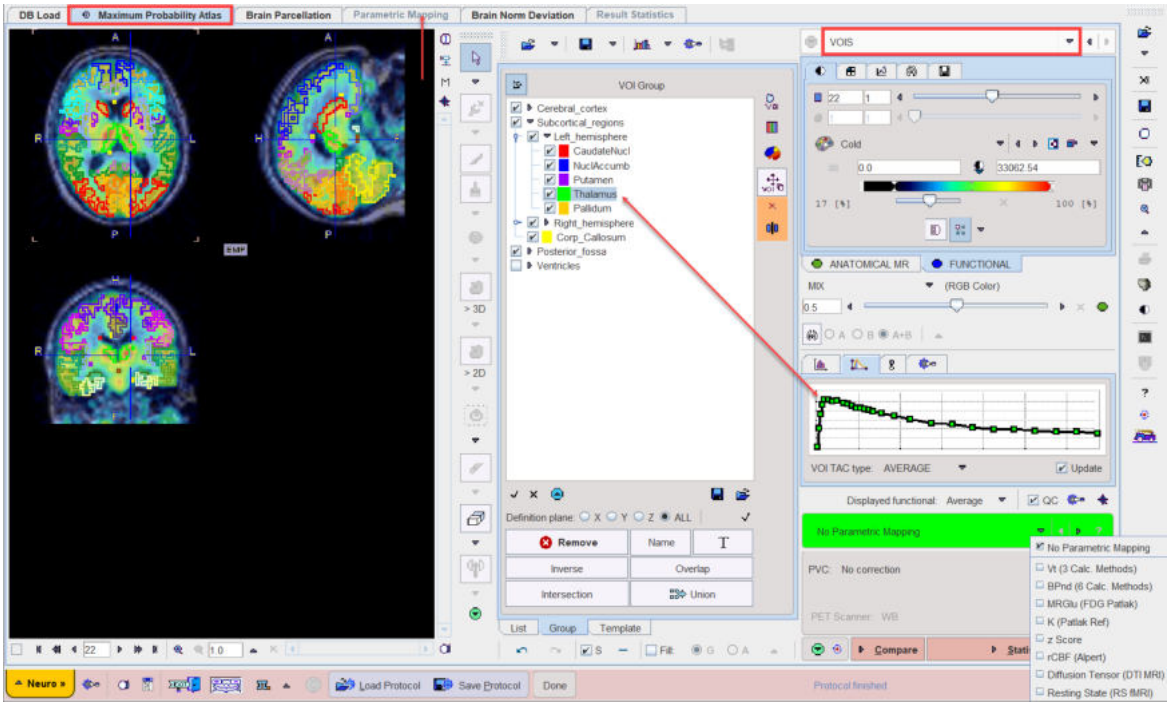
Once the VOIs have been outlined and carefully checked by the user, it is recommended to first save them, then proceed with calculation of statistics using the **Statistics** action button. The result (for the selected VOIs only) is shown on the separate Statistics[119] page of the PNEURO tool, from where it can be further evaluated.

6 Parametric Mapping

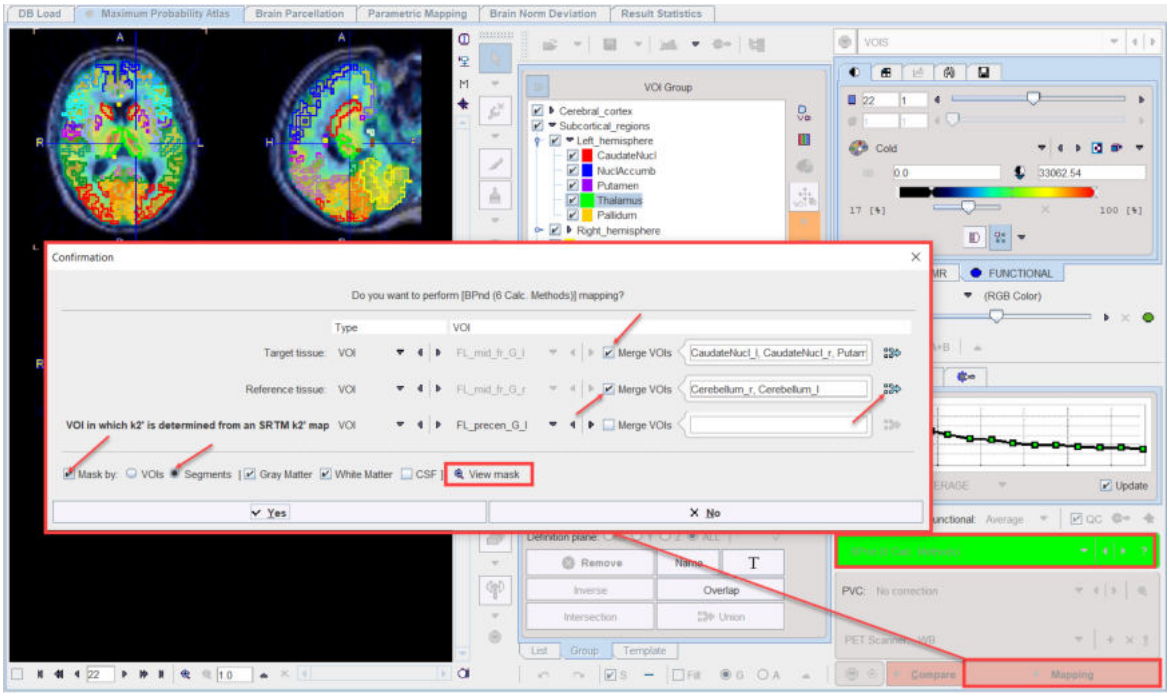
If the pixel-wise modeling tool PXMOD has been licensed, PNEURO includes a parametric **Parametric Mapping** page. An advantage of this integration is that the VOIs generated by PNEURO can be used during the PXMOD model configuration, and that the resulting parametric maps can immediately be regionally analyzed using those same VOIs. A further advantage is the choice of image space in which parametric maps should be calculated. For example, **Atlas** space may be useful when image algebra between subjects will be performed later, or **Input** (original PET) space may be preferred in order to work with the original pixel TACs.

When a dynamic PET image is available, parametric mapping is started after the PNEURO VOI outlining step as illustrated below. The model to be applied is selected from a subset of the PXMOD models in the green area (which initially shows **NONE**). Please refer to the *PXMOD Users Guide* for a description of the procedure and the models.

Note: If a PVC method is enabled which generates corrected dynamic images, parametric mapping will be applied to the PVC corrected images.



As a consequence of model selection, the **Statistics** action button is replaced by a **Mapping** button. When continuing with **Mapping**, a model-dependent configuration utility is show. The example below illustrates the case of mapping the binding potential with the **BPnd (6 Calc methods)** model.

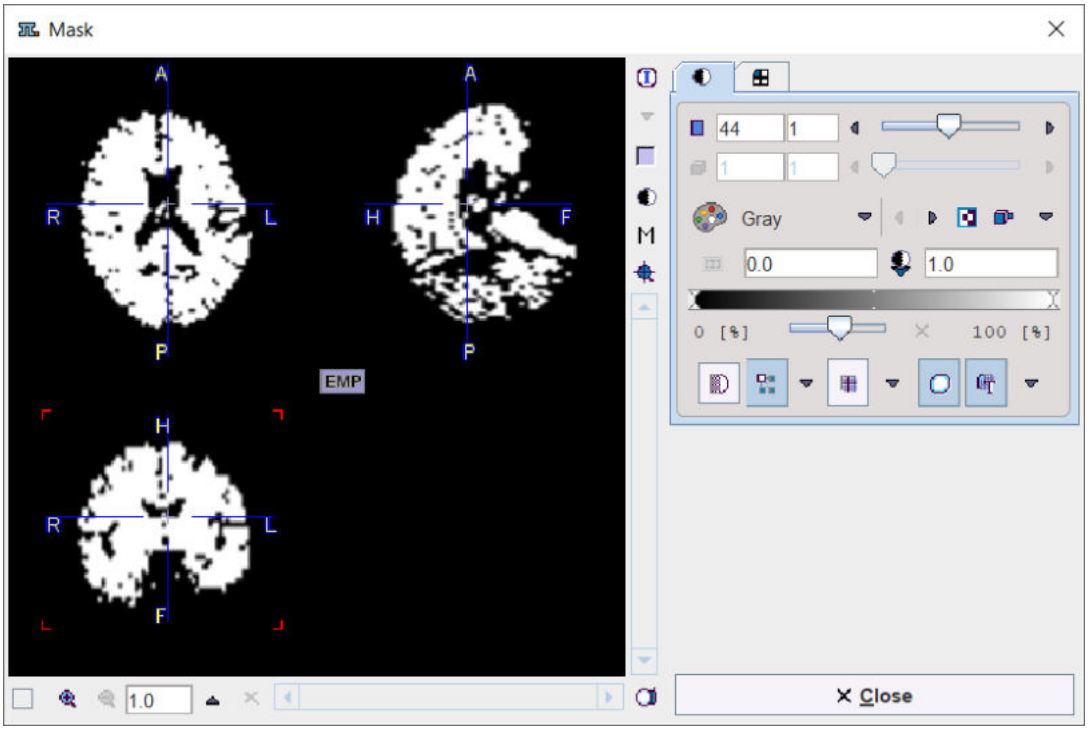


It requires two tissue time-activity curves for a preprocessing step, one representing a **receptor-rich region**, and one a **receptor-less** reference region. There are three options to specify them: **FILE**, **VOI** and **TAC(DB)**. **VOI** is the obvious choice, as the PNEURO-generated VOIs can be employed. Either a VOI can be selected from the list, or the **Merge VOIs** option can be enabled in order to create a VOI from a subset of the existing VOIs.

There are two ways to define a VOI subset: (1) by the specification of a list of VOIs or a pattern such as **Cer*** (select all VOIs with a heading "Cer" in the name) in the text field, or (2) by selecting the merge button indicated above. With the latter, predefined (**Left Grey Matter**, **Right Grey Matter**, **Grey Matter, Cerebellum**) VOI lists can be generated. **Selected** shows a dialog window within which any VOI combination can be defined.

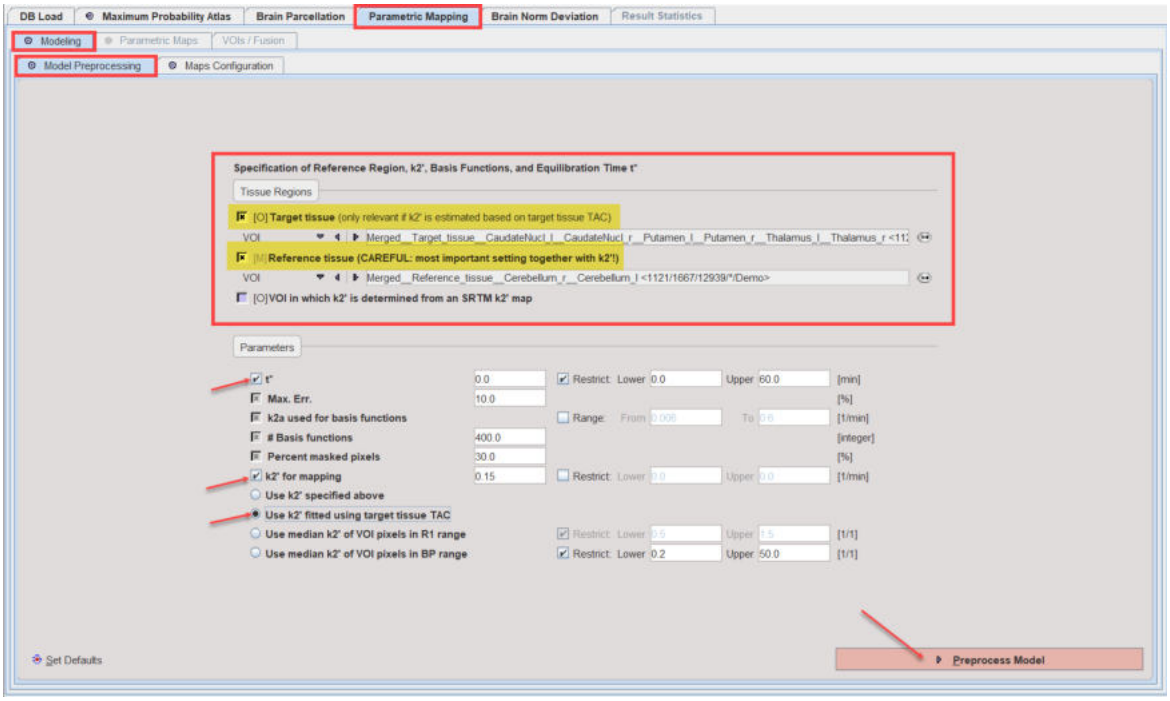
The **Mask by** options allow the user to restrict the mapping procedure to within the VOIs or the brain tissue segments. This may substantially reduce the time needed for map calculation, and allows non-specific tracer uptake outside the brain to be ignored.

Use of the **Mask by: Segments** option allows the user to leverage the tissue segments calculated earlier in the workflow to create a well-defined brain mask. The resulting mask can be inspected using the **View mask** button. For example, the combination of **Gray Matter, White Matter** above produces:

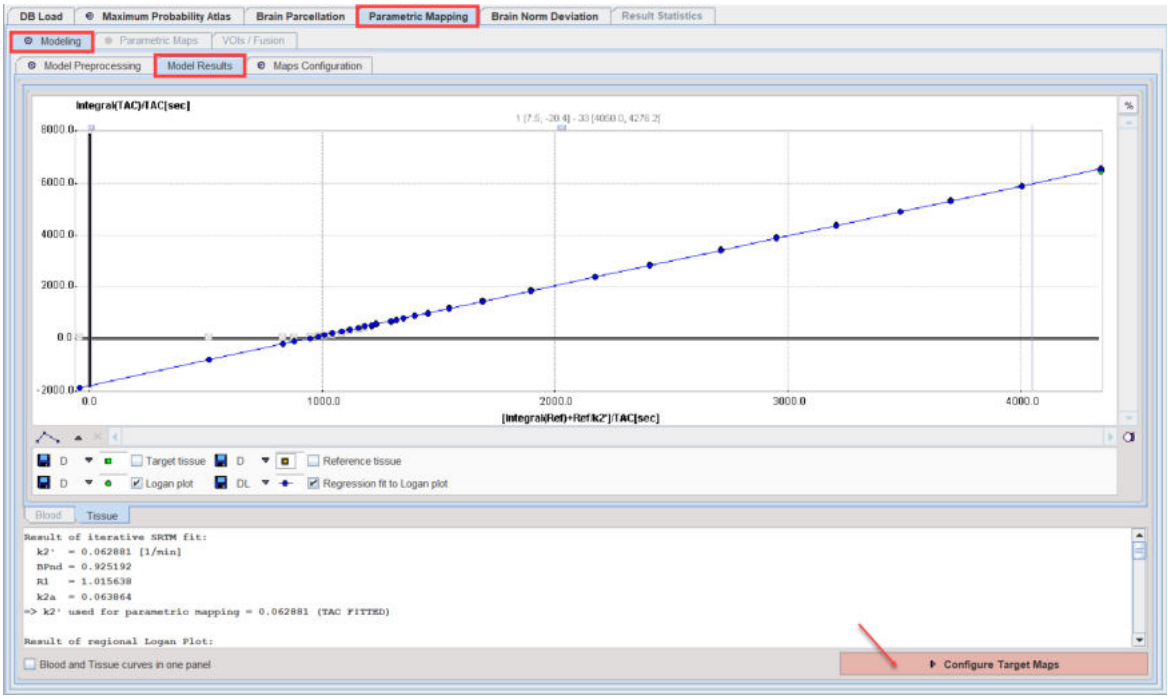


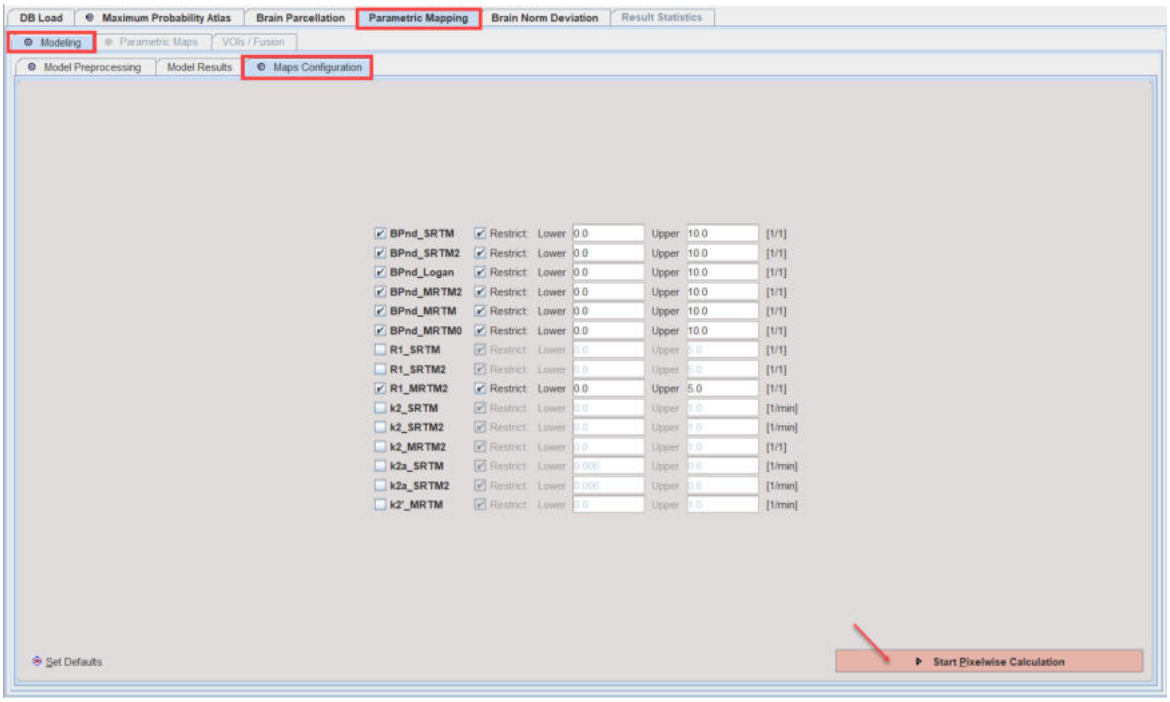
Note: Mapping is performed in the selected result space and can take much longer in a highly-resolved MR space than in the PET space.

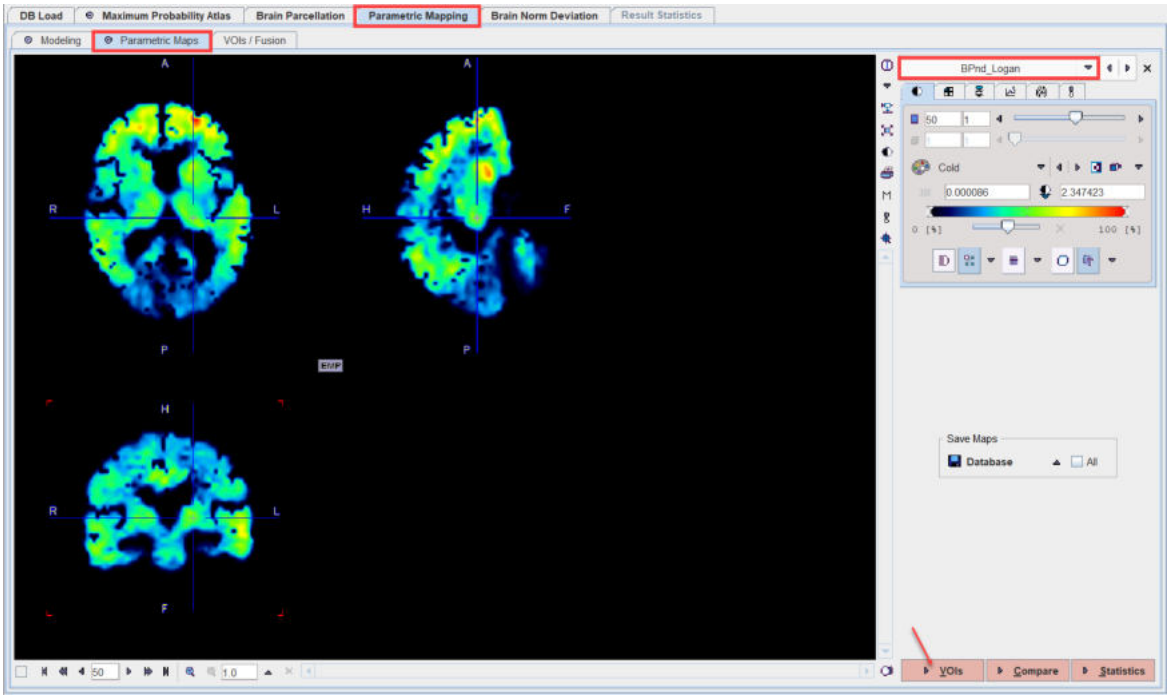
After confirming with **Yes** the program enters the **Parametric Mapping** page which hosts the PXMOD functionality. In the example below mapping started at the **Model Preprocessing** page, because there is no blood data involved. Note that the VOI definitions are already configured according to the TAC1/TAC2 definition provided by the user in the previous dialog window.



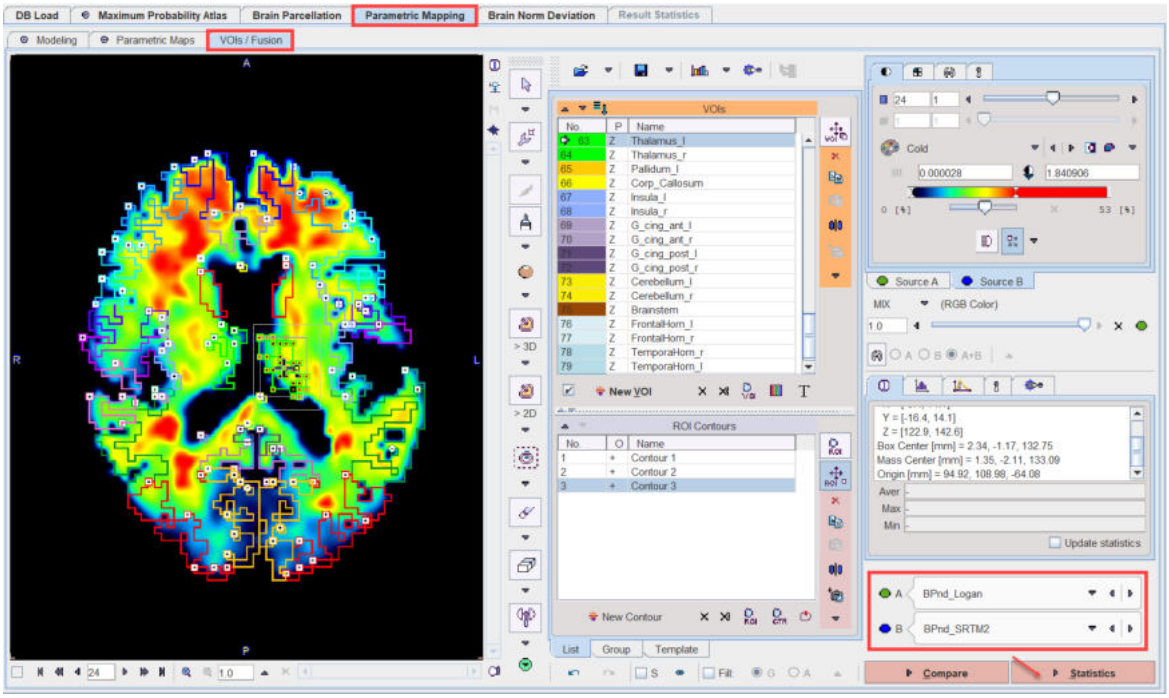
The remaining processing steps are described in the PXMOD documentation. Basically, the action buttons in the lower right have to be activated consecutively. After parametric mapping has completed, the results are shown on the **Parametric Maps** page.


DB Load
Maximum Probability Atlas
Brain Parcellation
Parametric Mapping
Brain Norm Deviation
Result Statistics
Modeling
Parametric Maps
VOIs / Fusion
Model Preprocessing
Model Results
Maps Configuration
Integral(TAC)/TAC[sec]
8000.0
6000.0
4000.0
2000.0
0.0
-2000.0
0.0
1000.0
2000.0
3000.0
4000.0
[Integral(Ref)+Ref.k2']/TAC[sec]
Target tissue
Reference tissue
Logan plot
Regression fit to Logan plot
Blood
Tissue
Result of iterative SRTM fit:
k2' = 0.062881 [1/min]
BPnd = 0.925192
R1 = 1.015638
k2a = 0.063864
=> k2' used for parametric mapping = 0.062881 (TAC FITTED)
Result of regional Logan Plot:
Blood and Tissue curves in one panel
Configure Target Maps

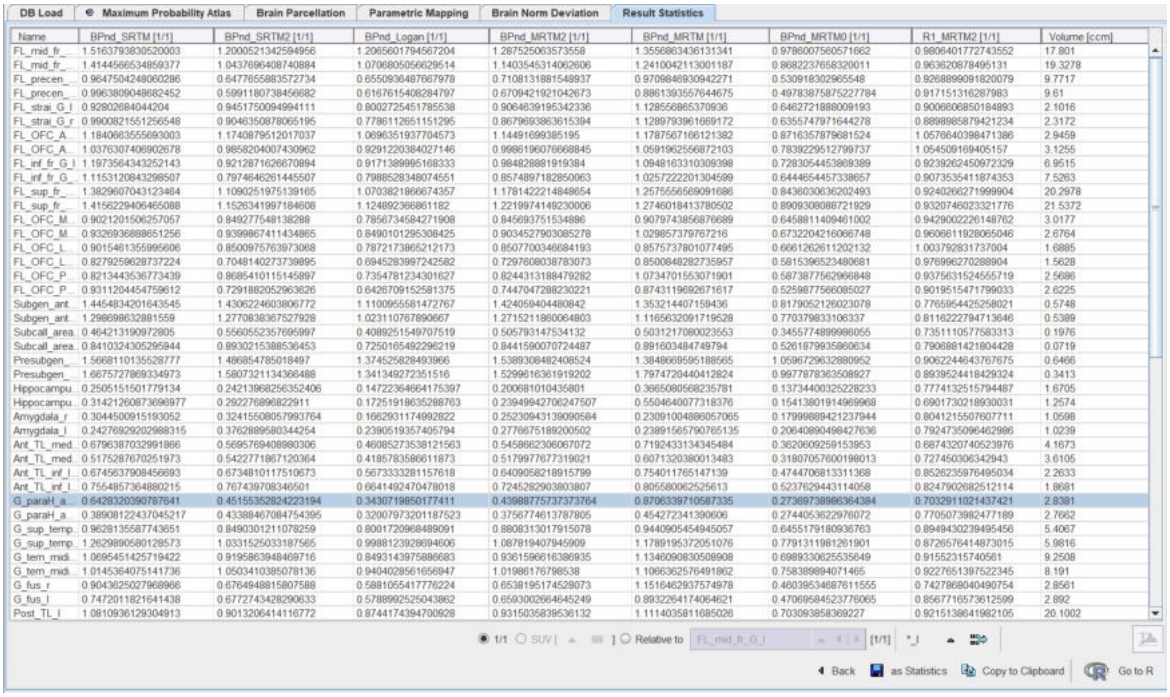

DB Load
Maximum Probability Atlas
Brain Parcellation
Parametric Mapping
Brain Norm Deviation
Result Statistics
Modeling
Parametric Maps
VOIs / Fusion
Model Preprocessing
Model Results
Maps Configuration
BPnd_SRTM
BPnd_SRTM2
BPnd_Logan
BPnd_MRTM2
BPnd_MRTM
BPnd_MRTM0
R1_SRTM
R1_SRTM2
R1_MRTM2
k2_SRTM
k2_SRTM2
k2_MRTM2
k2a_SRTM
k2a_SRTM2
k2'_MRTM
Restrict
Lower
Upper
[1/1]
[1/min]
Set Defaults
Start Pixelwise Calculation



The **VOIs** action button opens the VOI page with the resulting parameteric maps and the VOIs loaded.



The **Statistics** action button calculates the regional average of all VOIs in all parametric maps and shows the resulting table in the main **Statistics** page. Note also the last column which lists the VOI volumes for convenience.



As an alternative, the currently selected parametric map can be sent to the **Brain Norm Deviation** functionality with the **Compare** action button. This is valuable, if a normal database has been established against which the result can be compared. Note that in this case the selected result space of PNEURO has to be compliant with the space of the normal database.

Note: If parametric mapping should be part of the protocol, the **Parametric Mapping** page must be active when saving the protocol.

7 Statistics Results

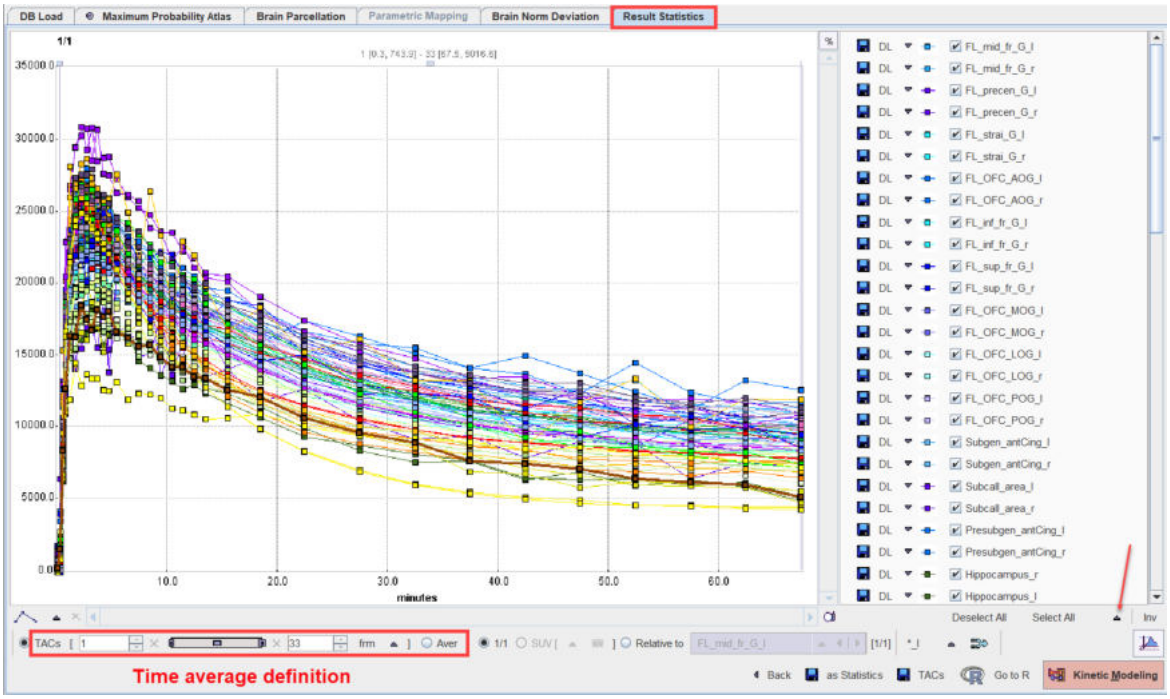
The statistics output from the PNEURO VOI modules is shown on the dedicated **Result Statistics** page. It shows

- time-activity curves in the case of a dynamic PET series,
- average regional uptake in the case of static PET series,
- volume in the case of an MRI series.

Note:
• In PNEURO, when new VOIs are created, the VOI voxel classification mode is forced to binary 100% (in preparation for partial-volume correction, in which overlaps are not permitted).
• The Statistics results are returned only for the VOIs selected on the Group tab .
• When a PVC method is used, both the original and the corrected statistics are calculated. Note that due to the high number of VOIs the PVC calculation may take several minutes and consumes a significant amount of RAM.

7.1 Statistics of Dynamic PET Data

The example below illustrates the result of the Maximum Probability Atlas workflow for a dynamic 11C-PiB PET followed by the calculation of regional TACs.

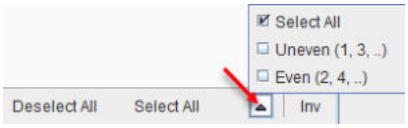


The **Back** arrow in the lower part is a convenience button for switching back to the PNEURO workflow which generated the statistics.

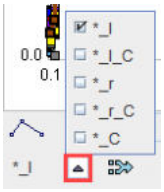
Selection of Curve Subsets

The PNEURO tools create a large number of VOIs. Sometimes, only a subset may be relevant for further processing. In this case the VOIs of interest are preferably selected on the VOIs page[74] before calculating the actual statistics.

An alternative is to select the relevant curves in the list to the right. There are some convenience buttons supporting this selection: **Deselect All** switches all curves off. **Select All** switches all curves on. **Inverse** inverts the current selection. Note the arrow button which allows the user to quickly select dedicated subsets.

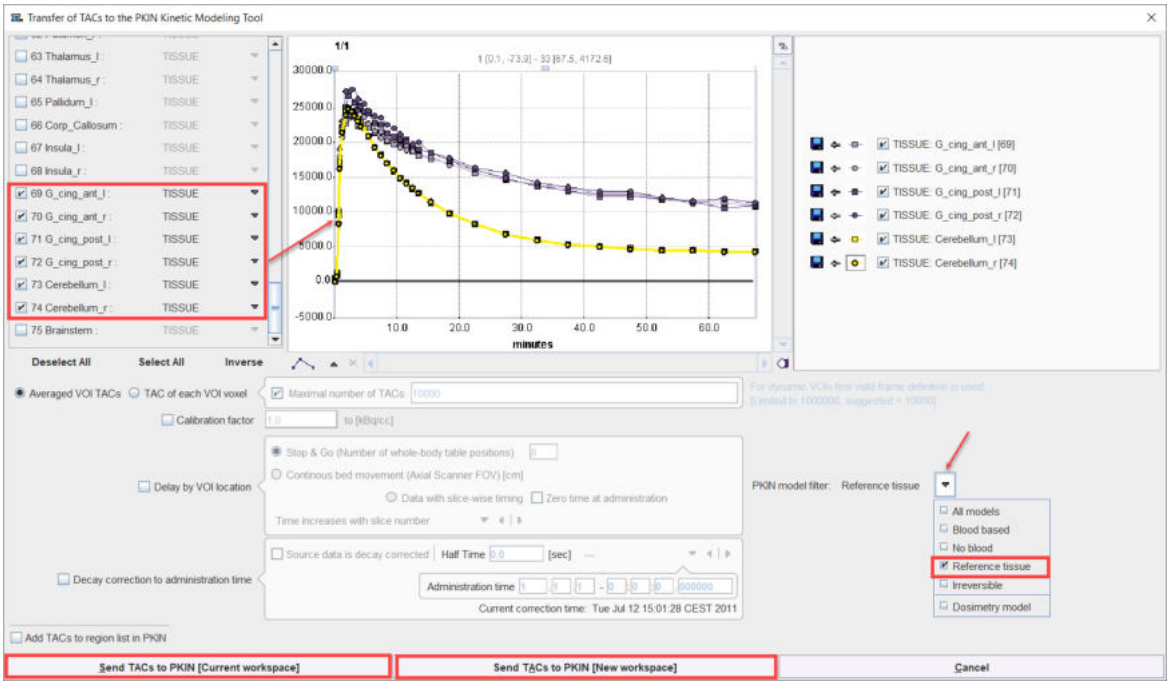


For instance, if the optional partial-volume correction is enabled for the statistics calculation there will be pairs of curves for all VOIs: one curve representing the original data, and one curve ending with **_C** representing the partial volume corrected data.



Transfer of TACs to the PKIN Tool

The **Kinetic Modeling** button allows dynamic tissue TACs to be directly transferred to the PKIN tool for modeling. It opens the following dialog window.



The TACs to be transferred can be selected by checking the box in the left column. The right side lists the currently selected TACs. **Send TACs to PKIN [Curent workspace]** transfers the selection to the active workspace in PKIN. If the **Append TAC data** is enabled, the TACs will be added as additional tissue regions, otherwise the existing tissue data is overwritten. **Send TACs to PKIN [New workspace]** first creates a new workspace in PKIN before actually transferring the selection.

Saving the Statistics

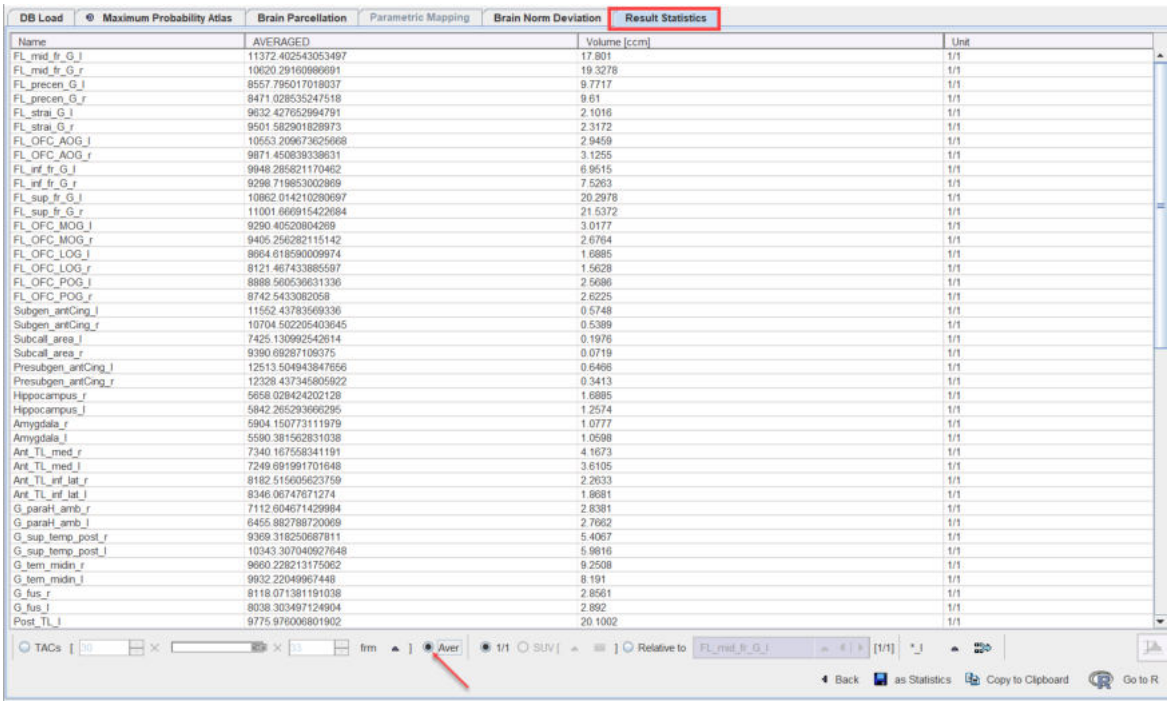
The save **TACs** button allows saving all curves in a simple tab-delimited text file as illustrated below. Such a file can easily be imported into other programs for processing and visualization.

	A	B	C	D	E	F	G	H	I
1	start[seconds]	end[kBq/cc]	FL_mid_fr_G_	FL_mid_fr_G_	FL_mid_fr_G_	FL_mid_fr_G_	FL_precen_G_	FL_precen_G_	FL_precen_G_
2	0	15	-0.01805802	-0.0237901	-0.00809923	-0.01044849	-2.80E-04	-0.00175514	0.01678049
3	15	30	0.18238082	0.17940207	0.17277237	0.17323294	0.17513265	0.17628254	0.18365888
4	30	45	1.48355082	1.54653299	1.37899899	1.40065527	1.38176575	1.41704893	1.64404241
5	45	60	2.85032315	3.00546241	2.92950542	3.06743169	2.72632691	2.86039639	2.72433356
6	60	90	4.88213226	5.19129419	4.70627261	4.90000296	4.62729848	4.82096481	4.50529739
7	90	120	5.97886228	6.37790203	5.67886381	5.97654629	5.50321562	5.77289343	5.43741881
8	120	150	6.29164095	6.73310232	5.96296405	6.24871445	5.66183036	5.90993452	5.91597253
9	150	180	6.24211139	6.63681841	5.94574397	6.22122765	5.95691097	6.28271723	5.63898401
10	179	209	6.17611005	6.52946091	5.94075375	6.2096324	6.05175658	6.39006615	5.70305054
11	209	239	6.2144399	6.57365131	6.0084352	6.28019571	5.8288573	6.09601021	5.56641523
12	240	270	6.21646508	6.59142828	6.00638489	6.27205563	5.83429973	6.10338783	5.71702874
13	270	300	6.22066609	6.58430815	6.05484013	6.33700275	5.8790232	6.16334867	5.85685307
14	300	360	6.17567308	6.53216267	6.02100392	6.30852175	5.78979378	6.04351091	5.69640827
15	360	420	6.17867916	6.54855061	5.83683003	6.08304644	5.79610749	6.0796051	5.74529036
16	420	480	5.96746211	6.29536724	5.78396531	6.01878643	5.60821518	5.85697508	5.74256271
17	480	540	5.91729331	6.25040293	5.73132909	5.97638178	5.66247183	5.96205616	5.41579735
18	540	600	5.85853982	6.18818474	5.67395434	5.93448591	5.51844713	5.78429985	5.38698142
19	600	660	5.67935879	5.98871708	5.46173548	5.685009	5.4860704	5.77765369	5.4313084
20	660	720	5.63046951	5.9691205	5.32891463	5.56270647	5.36284302	5.65853786	5.28426302
21	720	780	5.41255657	5.71388817	5.09929017	5.29820299	5.21115898	5.48118591	5.1606334
22	780	840	5.15910065	5.42277193	5.00602194	5.21456432	5.11606861	5.41962767	4.96614755
23	840	1020	4.95138175	5.22675276	4.6982705	4.87556744	4.83949982	5.10412455	4.77162598

Save **as Statistics** allows storing the information in a format suitable for statistical analysis.

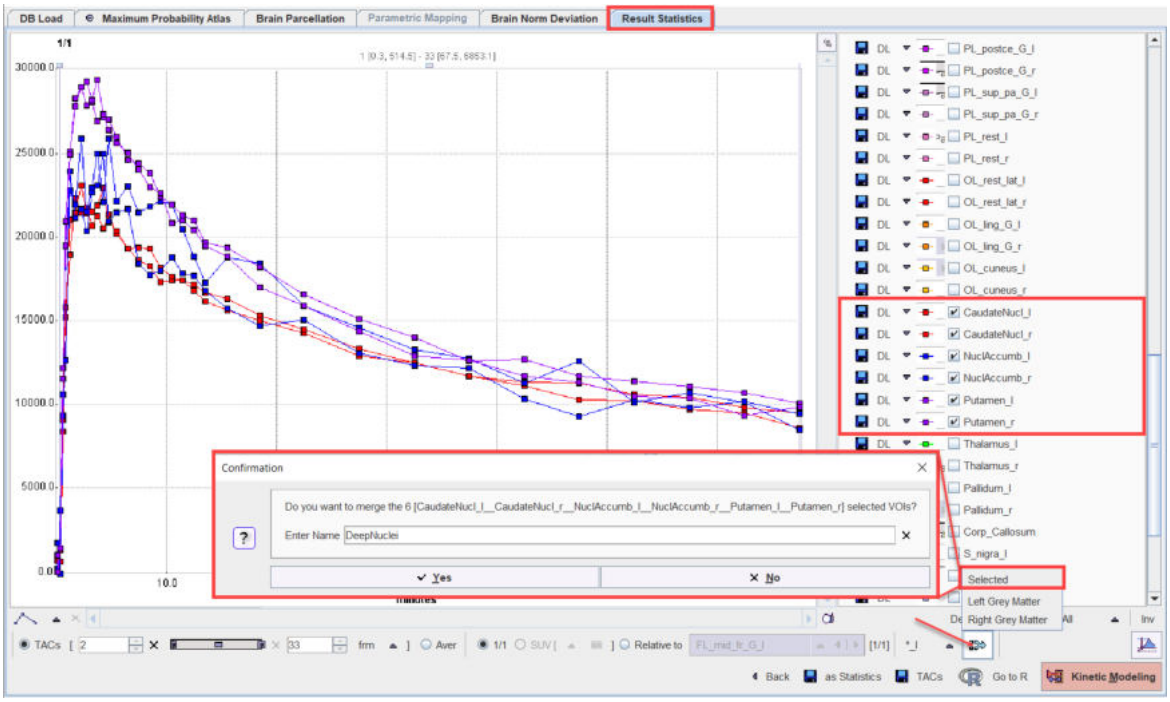
Average in Frame Range

For dynamic data there is an easy way to calculate the average regional uptake in the regions in a certain frame range: with the **TACs** radio button selected define the range to be averaged. As soon as the **Aver** radio button is switched on the uptake statistics is calculated and listed, replacing the curves display.

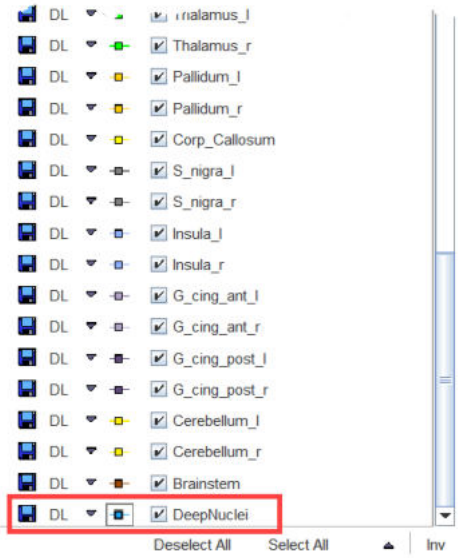


Average TACs

The **Merge (volume weighted) selected VOIs** button allows averaging of selected TACs. When the **Selected** option is activated a confirmation window appears. It allows the name for the new TAC to be defined.



As soon as the **YES** button is pressed the new TAC is calculated and appended at the bottom of the currently available TACs list.

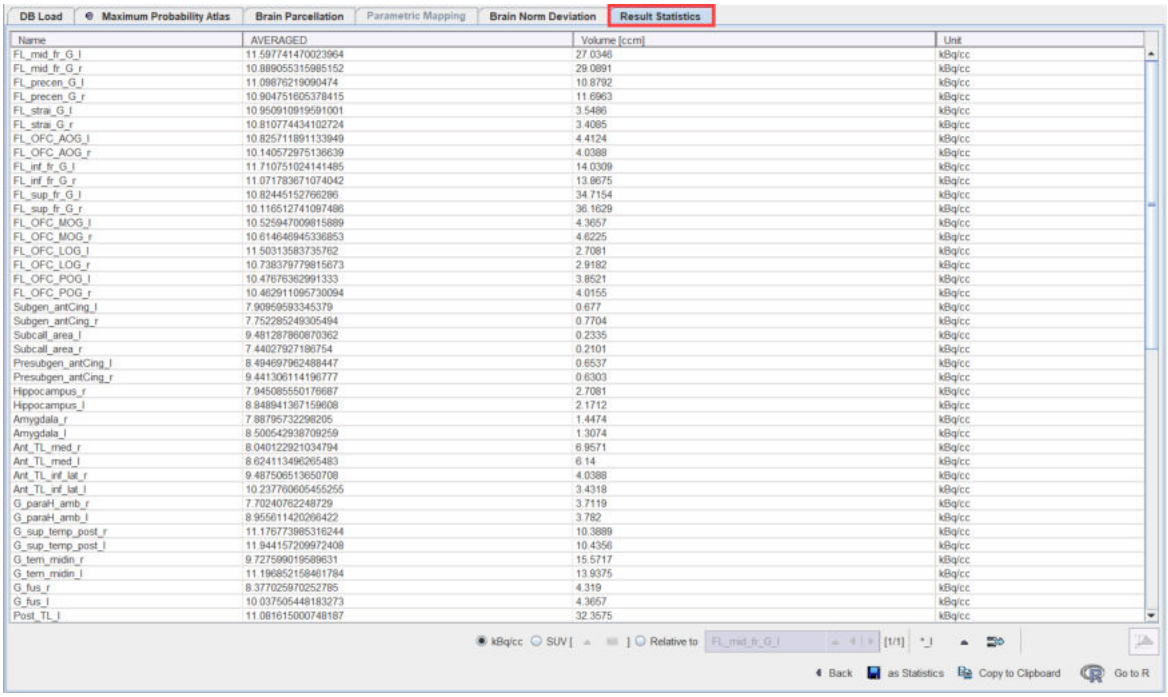


Transfer of Statistics to R

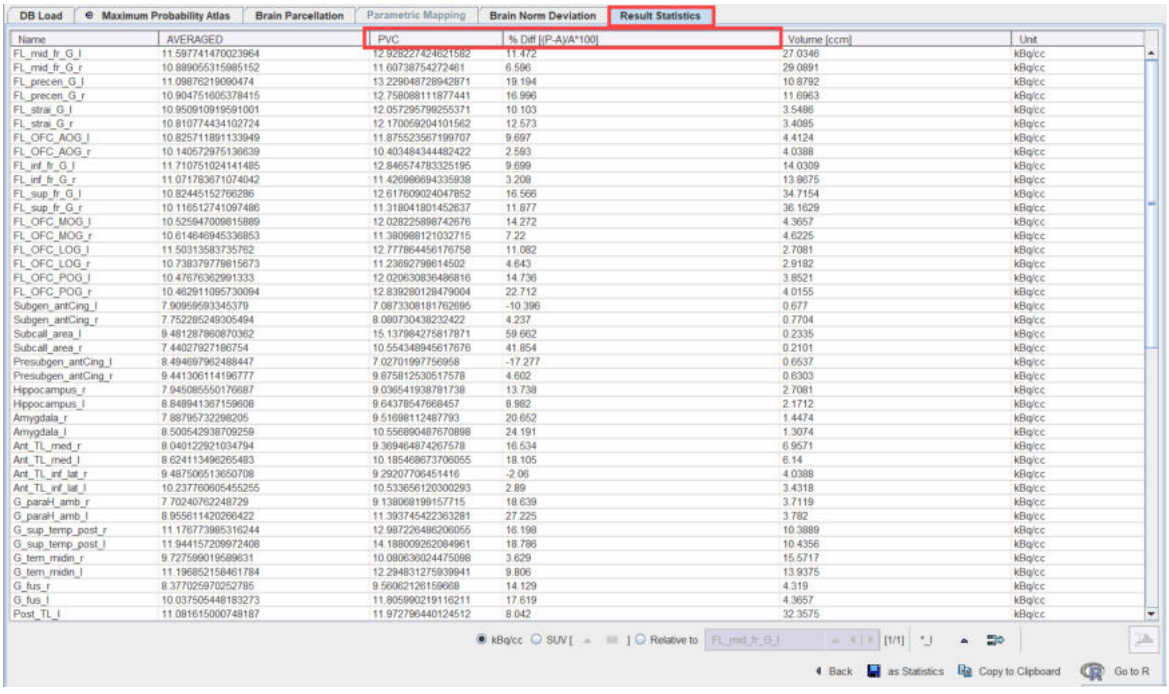
The **Go to R** button transfers the average and volume statistics of the selected TACs to the R server, generating one R variable in the R workspace.

7.2 Statistics of Static Data

If the VOIs are applied to static data, the **Results Statistics** page only shows a table of the main outcome parameter. In the case of a static PET the list shows the tracer average uptake in kBq/cc (**AVERAGED**) and the VOI volume in ccm.



When a PVC method is applied, two additional columns are available in the list after the tracer average uptake: the tracer average **PVC** corrected value and the percentage difference between the non corrected and the corrected value:



Please use the regular statistics button on the **VOIs** page to get the full statistics output.

SUV Statistics

If the statistics are calculated for activity concentration images, the uptake results can be converted to different types of SUV images, assuming that the related activity information and subject weight/height is available in the image header. If the information is not available in the image header, but is available via lab records etc., it can be entered after activating the SUV button.

SUVR Statistics

The so-called SUVR statistic is the uptake divided by a reference region uptake. It can easily be obtained by switching to the **Relative to** radio button and selecting the reference region from the VOI list. Note that the ratio is also calculated for all the other statistical measures.

Transfer of Statistics to R

The **Go to R** button transfers the average and volume statistics to the R server, generating one R variable in the R workspace.

8 Brain Norm Deviation Functionality

The **Brain Norm Deviation** module of PNEURO supports the creation of normal brain PET databases from a consistent set of normal volunteer images and its application for analyzing the images of test subjects.

The process of constructing the normal pattern - named *Normal Brain Database* or short *Brain Norm* - in principle consists of the following steps:

- The acquisition of images from a set of normal volunteers (controls). Preferably the same acquisition and image reconstruction protocols should be used as in the test subject studies.
- The stereotactic normalization of the control images, so that the anatomy of the normalized images is comparable across the controls.
- The scaling of the pixel values in the normalized images relative to an internal reference. The resulting normalized values allow pooling of the data.
- The analysis of the values across the control collective in each pixel of the stereotactic anatomy. Hereby the normal values and their deviation across the set of normal controls is established in each pixel.

With a database-assisted analysis, the brain PET uptake pattern of a subject can be compared to the normal pattern. To this end, the subject images are normalized and scaled in the same way as the control images, and the resulting pixel values compared with the established normal values. This process results in a map showing the differences between the subject images and the normal pattern, expressed as a z-score value. The z-score map can be investigated in a multitude of ways including fusion with the subject images and 3D rendering (separate option).

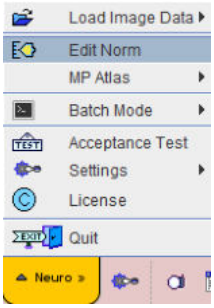
Note: The same type of analysis is also possible with SPECT images, and with MR images using the option for voxel-based morphometry (VBM)

8.1 Brain Norm Creation

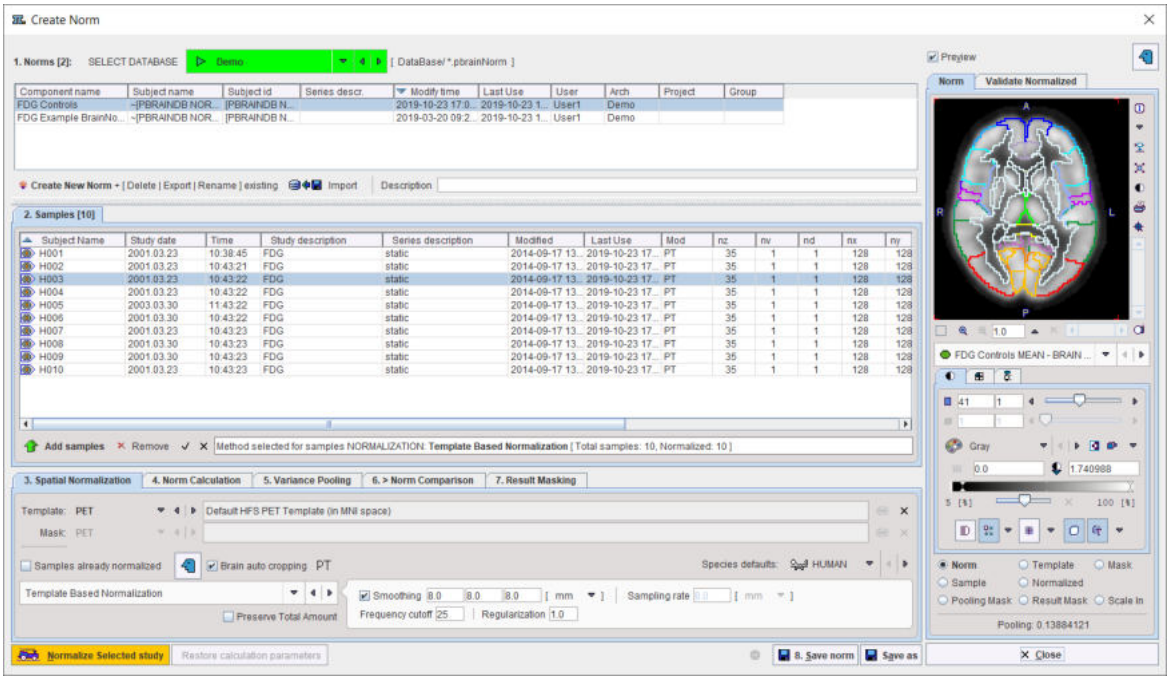
The creation of a Brain Norm requires the availability of a number of control images. These images must be saved beforehand in a PMOD database, where they are in DICOM format and include information such as the age or sex of the controls, and the anatomical image orientation. New controls subjects can always be added to an existing Brain Norm for improving the statistical power of the analysis.

8.1.1 Brain Norm Editor

The tool for Brain Norm creation and maintenance can be started using the **Edit Norm** entry in the **Neuro** menu.



A dialog window appears which supports the creation of a new Brain Norm in a step-by-step manner. These steps are explained in the following sections.



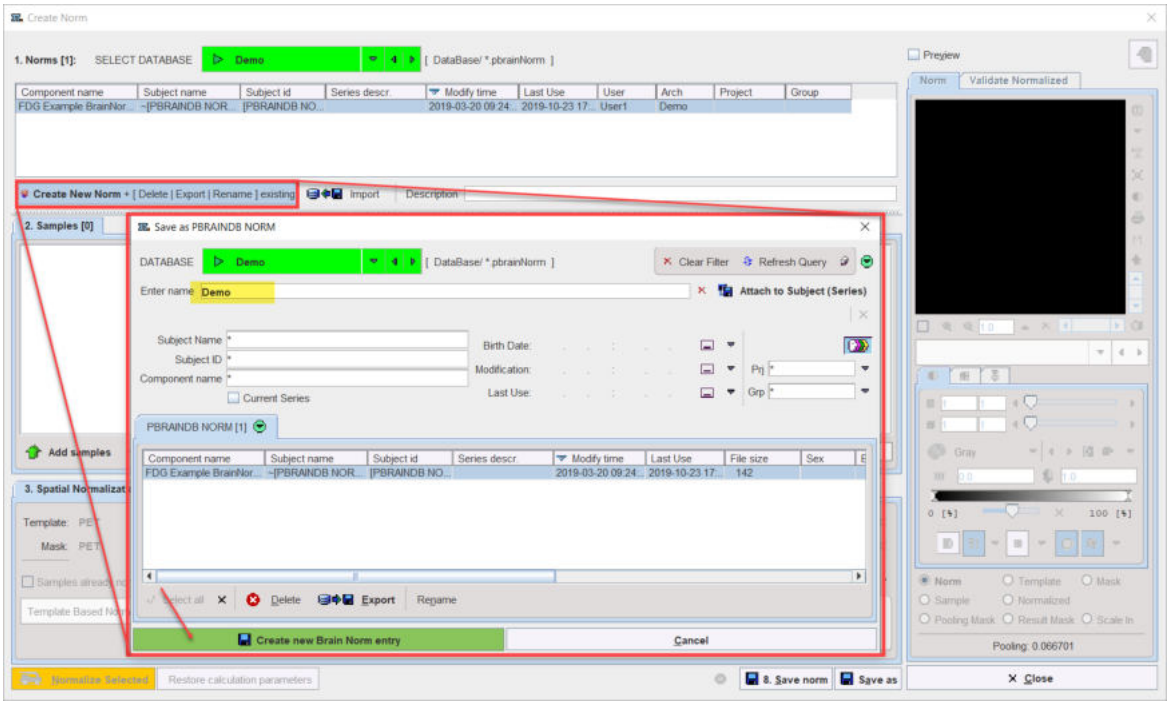
The image viewer to the right of the dialog window allows displaying the different data sets involved in the Brain Norm calculation:

Norm	Show the result of the database calculation. The **MEAN**, the **STDV** and the **Pooled STDV** image are available here. Note the selection below the image to switch the images. 1 PET ^Template ... 1 PET ^Template FDG Template MEAN - BRAIN NORM 2 PET ^Template FDG Template STDV - BRAIN NORM 3 PET ^Template FDG Template STDV POOL - BRAIN NORM
Template	Show the template for stereotactic normalization.
Mask	Show the normalization mask.
Sample	Show the original images of the currently selected control sample.
Normalized	Show the images of the currently selected control sample after spatial normalization.
Pooling Mask	Show the mask used for the averaging of the standard deviations.
Result Mask	Mask outside which the database comparison is cleared.
Scale In	Show the mask used for finding the reference value for scaling.

The principle in the Brain Norm editor is to work from top to bottom following the numbered user interface elements.

8.1.2 1. Empty Brain Norm Creation

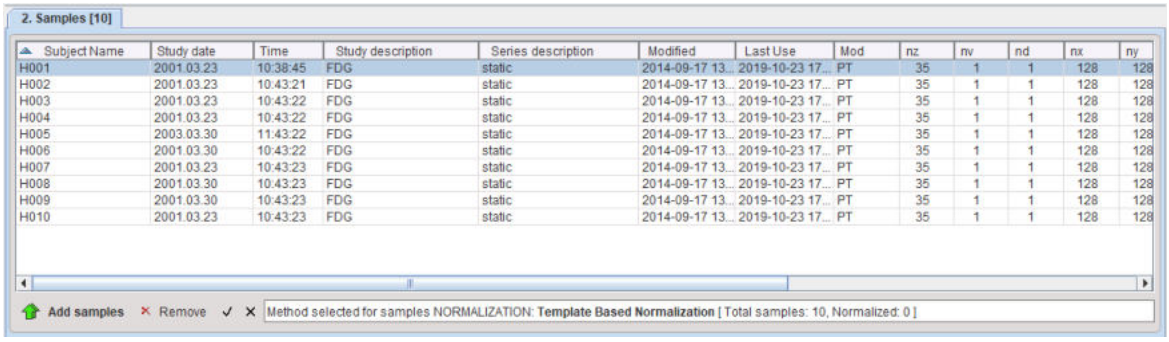
Activate the **Create New Norm** button. In the dialog that appears, select the PMOD database in which the Brain Norm definition will be saved (Demo database in the example below),



enter a name into the **Enter name** field, and activate the **Create new Brain Norm entry** button to create an empty Brain Norm definition. Note the norm **Description** text field which allows explanatory comments to be added to the definition.

8.1.3 2. Control Samples Addition

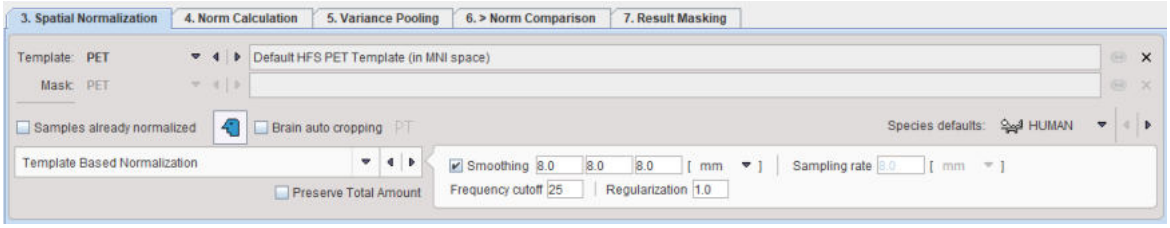
The button **Add samples** brings up a database selection dialog window. In this window bring one or more image sets of normal control subjects into the **Selected for loading** area and confirm with the **Set series** button. The samples are then listed in the **2. Samples** section.



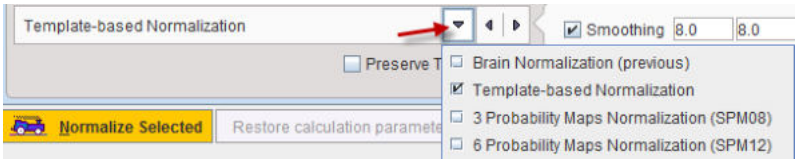
Subject Name	Study date	Time	Study description	Series description	Modified	Last Use	Mod	nz	nv	nd	nx	ny
H001	2001.03.23	10:38:45	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H002	2001.03.23	10:43:21	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H003	2001.03.23	10:43:22	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H004	2001.03.23	10:43:22	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H005	2003.03.30	11:43:22	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H006	2001.03.30	10:43:22	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H007	2001.03.23	10:43:23	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H008	2001.03.30	10:43:23	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H009	2001.03.30	10:43:23	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128
H010	2001.03.23	10:43:23	FDG	static	2014-09-17 13...	2019-10-23 17...	PT	35	1	1	128	128

8.1.4 3. Normalization Definition and Sample Normalization

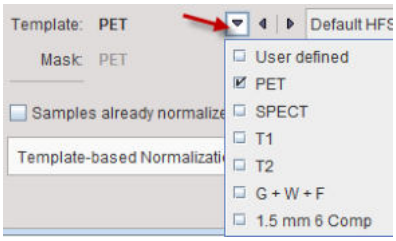
The stereotactic normalization to be used for the database is defined on the **3. Spatial Normalization** tab.



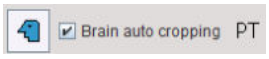
The main choice is selection of the spatial normalization procedure.

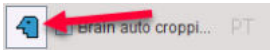


and a corresponding template image.



Normalization Methods

The normalization procedures are summarized below. The parameter presets are species dependent and can be set using the **Species defaults** selection. If the image field-of-view is significantly larger than the brain, **Brain auto cropping** 

should be enabled, and the proper modality (**PT**, **MR**) set. If the data are not available in the HFS orientation, the button left of **Brain auto cropping** 

should be enabled to automatically perform the reorientation.

Normalization methods:

Brain Normalization (previous)	SPM99-type normalization with parameters Because the normalization template is typically a relatively smooth image, some smoothing of the target image is needed to make it more similar to the template. To this end a **Gaussian smoothing** filter may be defined by its full width at half maximum in all directions (x,y,z). **No. of Basis Functions** from the cosine transformation in each direction. Suitable template images are the **PET** and **SPECT** templates in the MNI space, or a proprietary **User defined** template image.
Template-based Normalization	SPM5-type normalization with the same template images as above. Based on the **Gaussian smoothing** as above, the **Sampling rate** is determined. Alternatively, **Gaussian smoothing** can be disabled and a dedicated **Sampling rate** specified. The specified **Frequency cutoff** (default = 25) is used for calculating the number of basis functions. Higher cutoff values

	result in fewer basis functions. Regularization controls the smoothness of the deformation field. The higher regularization, the smoother the deformation.
3 Probability Maps Normalization	Normalization applicable to 3D T1-weighted MR images (parameters[62]) Denoising strength: Low | Sampling rate 3.0 [mm] Bias regularization: Light 60 | Affine regularization: European brains It uses the normalization resulting from the SPM8-type unified MR segmentation and requires the **G + W + F** template, which is automatically selected.
6 Probability Maps Normalization	Normalization applicable to 3D T1-weighted MR images with (parameters[62]) Denoising strength: Low | Sampling rate 3.0 [mm] Bias regularization: Light 60 | Affine regularization: European brains It uses the normalization resulting from the SPM12-type unified MR segmentation and requires the **1.5 mm 6 Comp** template, which is automatically selected.

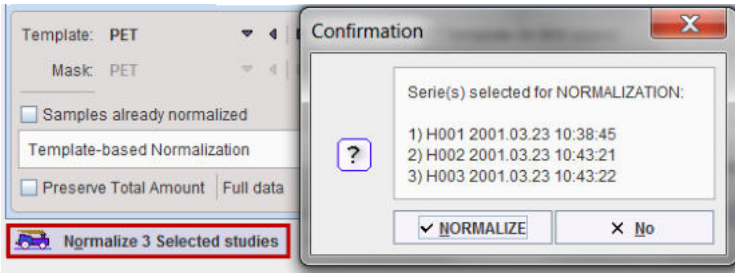
With **User defined** templates please use the button to select the template image, which must be available in the database. For new tracers, the user may first have to generate a suitable template outside PNEURO and import it into the database.

A mask is required to cut off signal from outside the brain during the normalization. It is specified using the **Mask** selection. While the standard templates already have associated mask files for use during normalization, **User defined** templates require specification of a dedicated **Mask** series in the database.

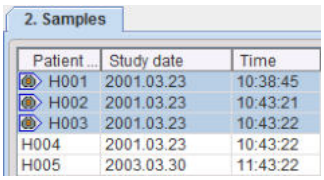
The **Preserve Total Amount** option enables volume modulation during image reslicing as used for VBM applications (Voxel Based Morphometry). Rather than preserving the concentration in the image, the total amount is preserved.

Normalization of the Control Images

After the normalization method has been fully specified, the samples in the list can be selected and their normalization started with the **Normalize Selected** button.

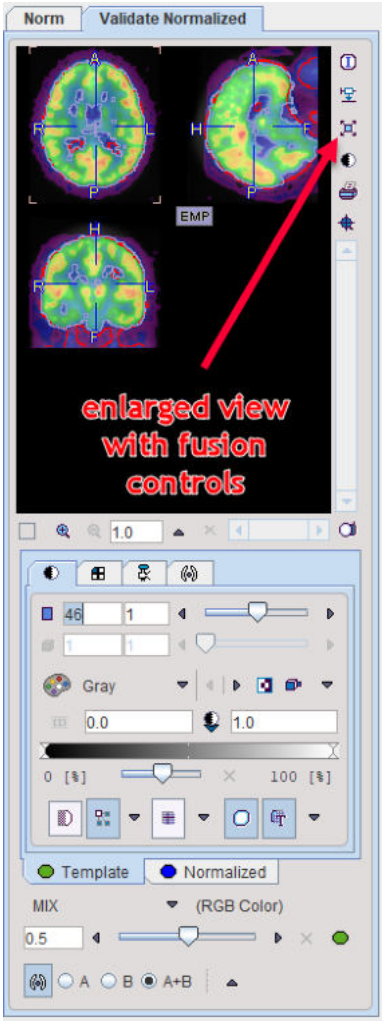


The normalization is performed in the background, and finally confirmed by a message. The normalized images are saved in the database as new series of the controls, labeled with **NORMALIZED** in the series description. Although smoothing is applied during the process of normalization, the **NORMALIZED** images are not smoothed. Sample images which have been normalized are marked in the **Samples** list with the symbol.



When creating a Brain Norm it is important to ensure that only correctly normalized samples without truncated areas are included. Although a certain inaccuracy of the mapping from the subject anatomy to the stereotactic template cannot be avoided, samples showing gross deviations should be excluded from the analysis.

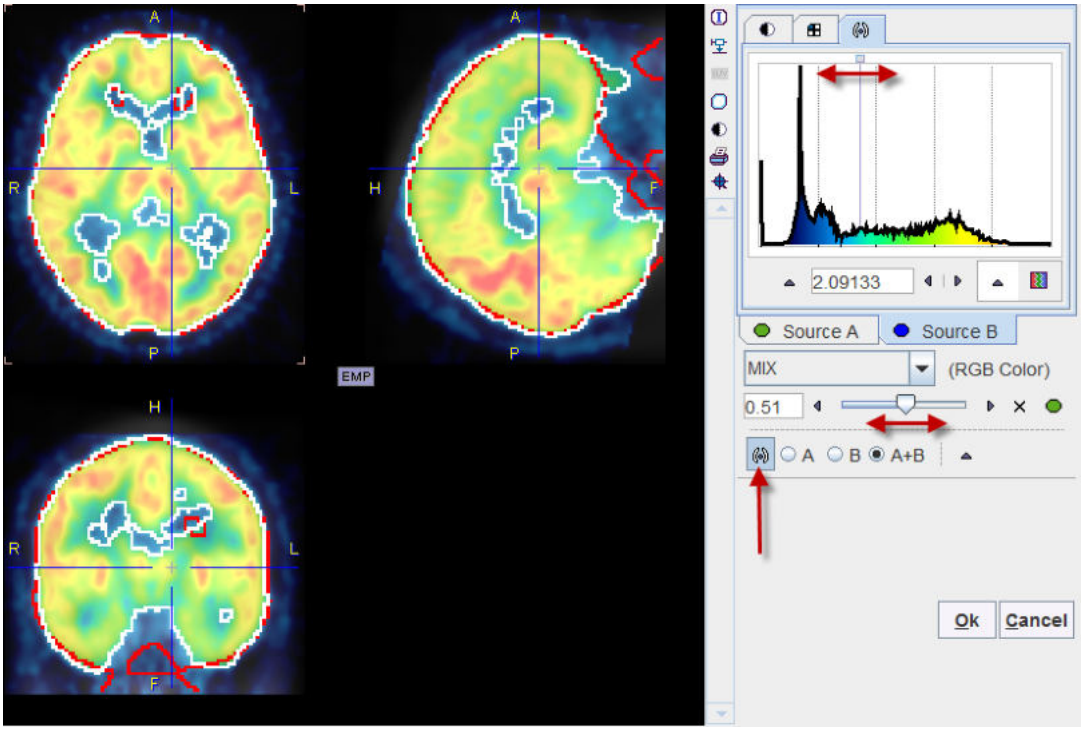
A fusion display supporting the quality control of spatial normalization is integrated in the **Edit Norm** tool. When the **Preview** box is checked and the **Validate Normalized** tab is selected, the image display shows a fusion of the template with the normalized sample image.



The tabs allow switching between the **Template** and **Normalized** images, for example to adjust the color tables or to define the iso-contour level. The relative contribution of the two components to the fusion image is governed by the fusion balance slider.

The easiest way to quickly check all normalized samples in the database is to switch to the orthogonal view of the **Validate Normalized** tab, select the first entry in the **2. Samples** list, and with the ARROW DOWN keyboard key browse through the list entries. Each time a new sample is selected the view gets updated.

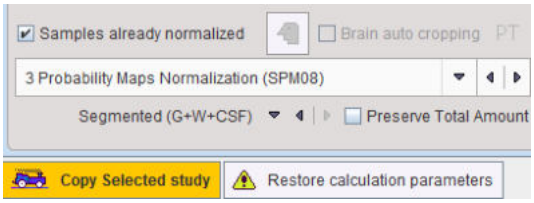
For a detailed analysis the fusion display can be blown up using the large view button indicated above.



Already Normalized Control Images

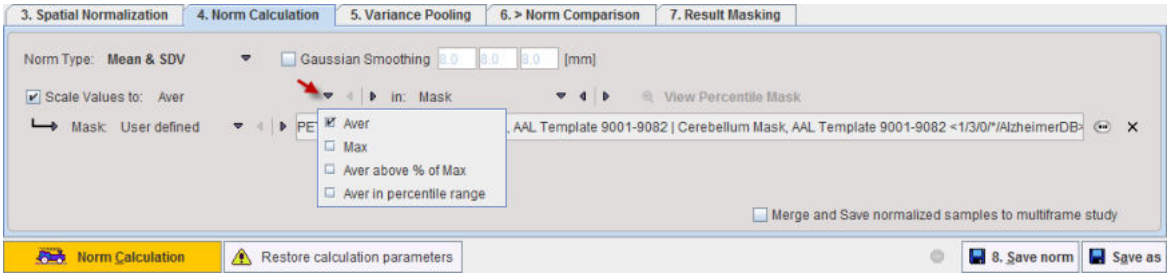
A special case is the use of control images which have already been normalized externally and saved in the database, for instance during the Maximum Probability workflow of PNEURO. Note that such images have to be calculated in the space of the atlas which is used in the **Brain Norm Deviation** workflow.

The flag **Samples already normalized** allows indicating this situation. As a consequence, normalization of the control data is not necessary, and therefore the button **Normalize Selected study** changes to **Copy Selected study** as illustrated below. Note that the normalization definition is still required to normalize test subject images for comparison with the Brain Norm.



8.1.5 4. Calculation of Normal Pattern

The database properties are defined on the **4. Norm Calculation** tab.



A **Gaussian smoothing** filter is available for smoothing the normalized control images before the database calculations. However, as the pooling of controls also has a smoothing effect, the filter should be set moderately.

In order to perform a pooled analysis it is required that the control images have comparable pixel values. In most cases the image values must therefore be scaled. This is achieved by dividing the pixel values by the average value in a stable reference tissue. Several user interface elements in the **Scale Values to** line allow flexible scaling strategies. The first selection lets define how the reference value is calculated within a spatial domain defined by the second selection. The calculation choices are:

Aver	Average value in the reference pixels.
Max	Maximal value in the reference pixels.
Aver above % of Max	Average of those pixels which are above the entered percentage of the maximum.
Aver in percentile range	A pixel value histogram is calculated, and the average value in the specified percentile range calculated. The setting in the example above only considers grey matter pixels which are in the 40% to 90% percentile range. This may represent an approach for excluding diseased hypometabolic (low values) and activated (highest values) pixels. The **View Percentile Mask** provides a visualization of the pixels which are used for calculating the reference value. As soon as it is activated, the currently selected sample is analyzed and the pixels shown which qualify for the criterion.

The spatial domain choices are:

VOI	A volume-of-interest which can be selected in the database.
Mask	A binary mask file which can be selected in the database.
Normalized Sample	No restriction, all data pixels are used.

Finally, the database needs to be calculated with the **Norm Calculation** button. The following processing steps are performed:

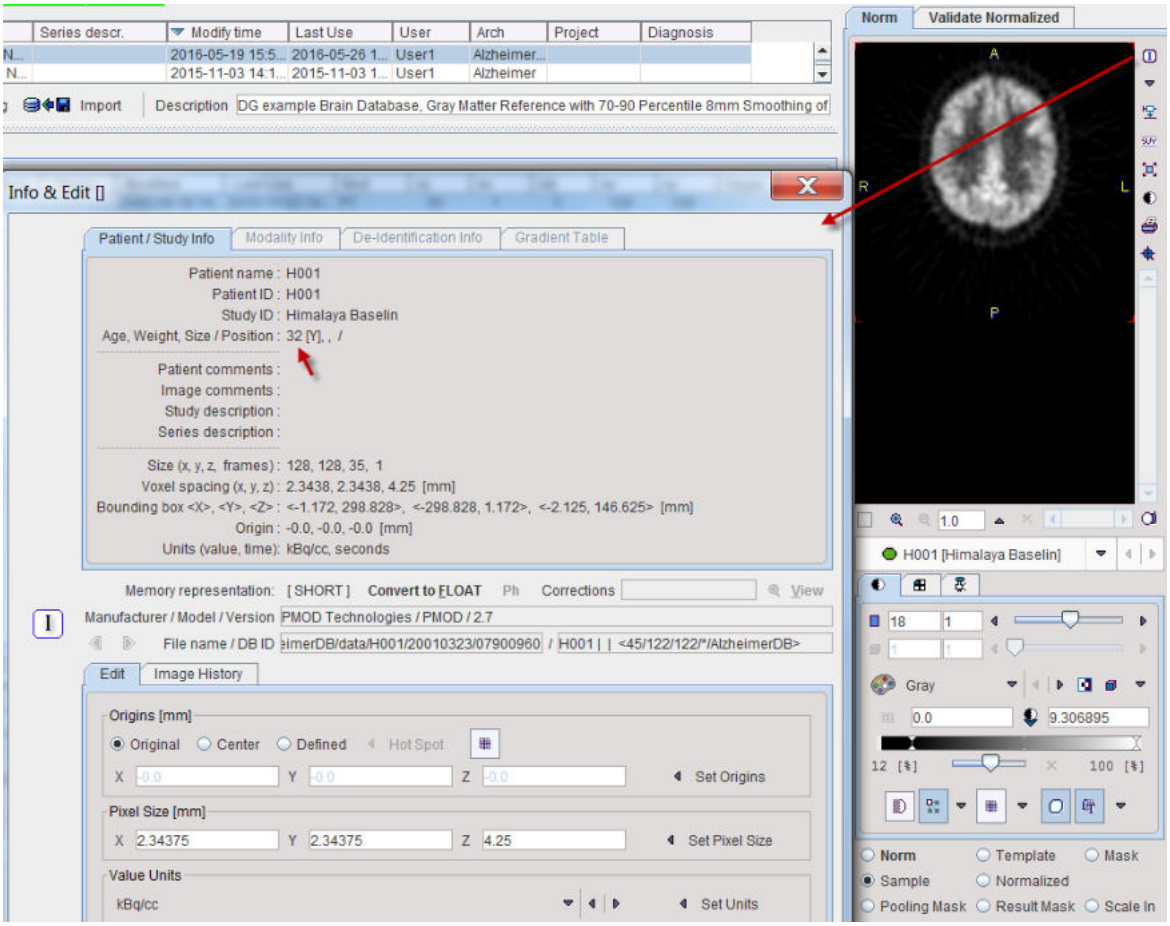
1. For each normalized control sample the reference value is calculated, and all pixels values are divided by it. The resulting normalized values typically range from 0 to somewhat above 1.
2. The scaled images are smoothed with the Gaussian filter.
3. For each pixel the distribution of the scaled values is calculated in all control samples.

As a result, the average value (representing the expected normal value) and the standard deviation (a measure of uncertainty) are known per pixel. These results are saved in the database as new image series of the normalization template study and marked in the description as **MEAN** and **STDV**.

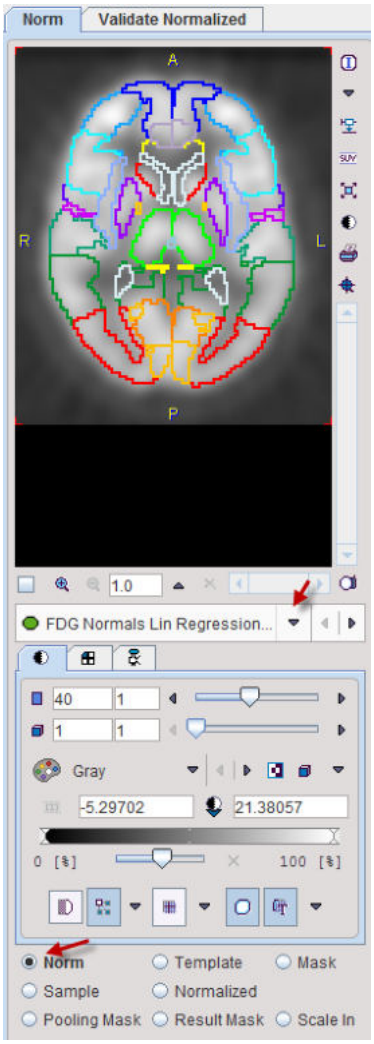
If the **Merge and Save normalized samples to multiframe study** is enabled, all normalized control images are arranged in a dynamic series and saved in the database when the **Norm calculation** completes. This allows for a simple export of the data for other purposes.

Age Regression

If the image pattern is dependent on the age of the controls, the **Norm Type** can switched to **Linear Regression**. Note that it is required that the age of the subject is available in the data. This can be inspected as illustrated below. If it is not encoded as age in the data, the age is calculated as the difference between birthdate and scan date.

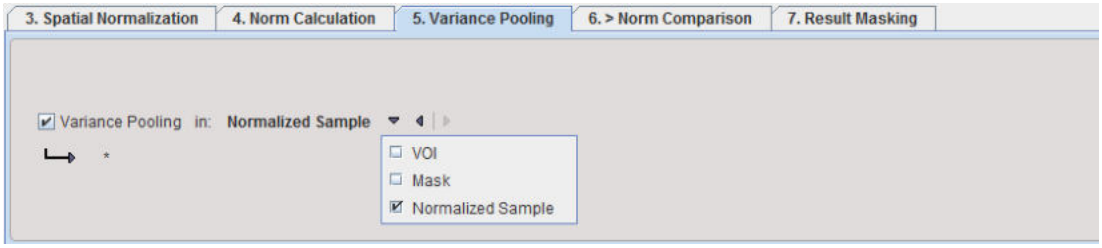


The result of a norm calculation with age regression is a linear regression at each pixel. It can be inspected by selecting **Norm** in the preview window and is presented as a dynamic series with the y-intercept as the first frame an the slope as the second frame. When applying an age-regression norm, the normal pattern at the subject age will be calculated and compared with the subject image.



8.1.6 5. Variance Definition for z-Score Calculation

When a normalized subject image is compared against the Brain Norm, a z-score value is calculated in each image pixel. This operation requires the standard deviation of the normalized values across the controls data sets. If the number of control samples is low, the statistical power may not be sufficient for calculating reliable standard deviations in each individual pixel. In this case **Variance Pooling** on the **5. Variance Pooling** tab should be enabled.



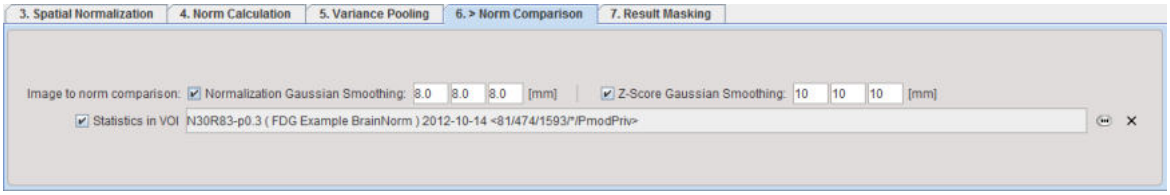
With this setting the user needs to define a spatial domain, within which the pixel standard deviations are averaged to derive a pooled standard deviation which will be used for all pixels. A **VOI**, a **Mask**, or the whole **Normalized sample** can be employed for variance pooling.

Note: After changing the **Variance Pooling** method the database has to be recalculated with the **Norm Calculation** button.

8.1.7 6. Comparison Settings

The subject images which are to be compared against the Brain Norm are also first normalized. The smoothing filter during this normalization may differ from the filter applied to the control image normalization and can be specified with the **Normalization Gaussian Smoothing** values.

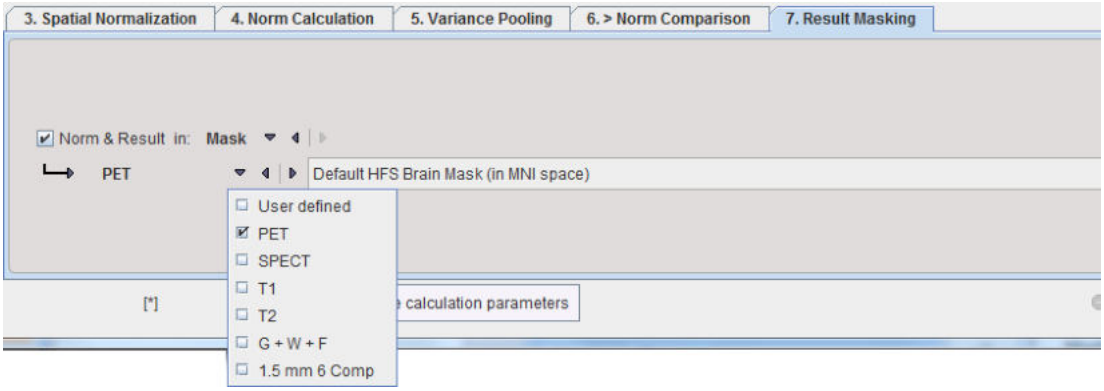
Due to the pooling of many samples and some optional filtering, the database is inherently smoothed. Therefore, some smoothing of the normalized subject images is most likely required to reduce disturbing edge artifacts. This smoothing can be specified with **Z-Score Gaussian Smoothing** values.



The **Statistics in VOI** facility allows to specify a VOI set for calculating regional statistics in the resulting z-score map. These VOIs have to be prepared in the normal space, and can be selected using the button. Hint: An easy way to generate a comprehensive VOI set is to save an outline result[74] from one of the VOI outlining modules in PNEURO, after selecting **Atlas** as the result space.

8.1.8 7. Result Mask Specification

A **Result Mask** may be defined outside which the calculation results are cleared. This definition is available on the **7. Result Masking** tab, and a **VOI** or a **Mask** can be employed. The masks for the standard MNI templates are directly listed, proprietary masks can be selected via **User defined**.



8.1.9 8. Brain Norm Saving

The last step after the Brain Norm has been calculated and the comparison parameters have been set is saving with the **Save Norm** button. **Save as** serves for saving variants of the Brain Norm with different comparison strategies under different names.

Note: New samples can incrementally be added. In this case, and if one of the definitions has been modified, it is required to recalculate and save the Brain Norm.

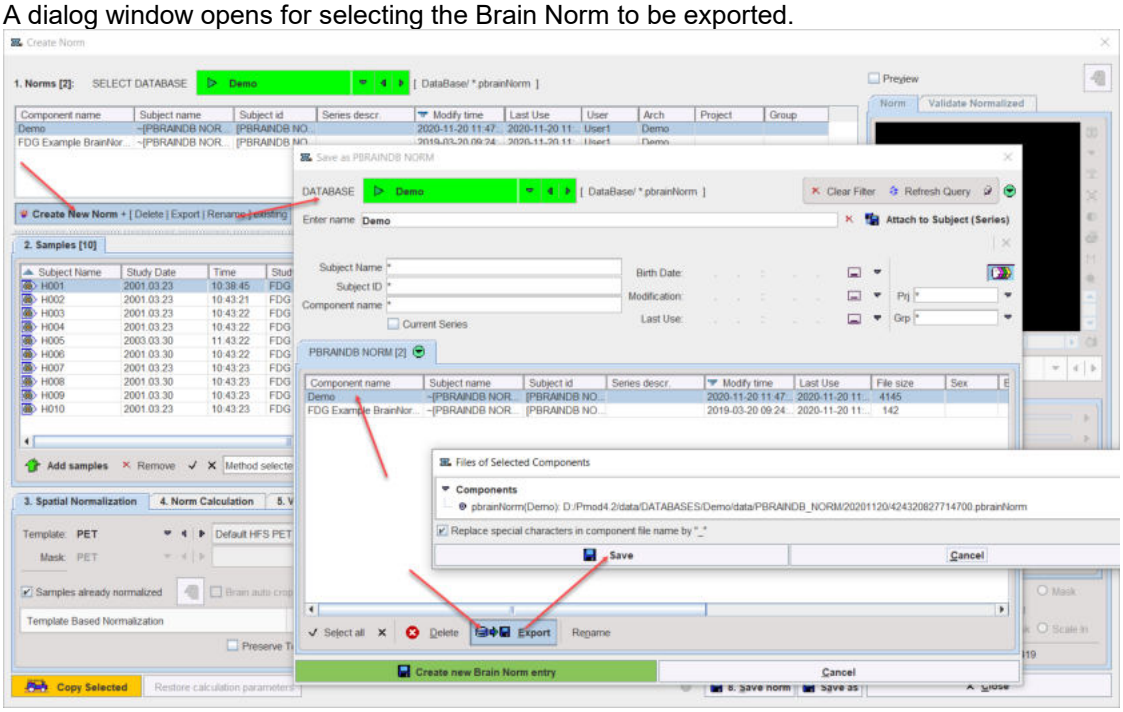
8.2 Sharing Databases

Establishing a Brain Norm requires a substantial effort. Therefore, a brain database represents a valuable asset which should be sharable with others. The **Edit Norm** tool supports the easy

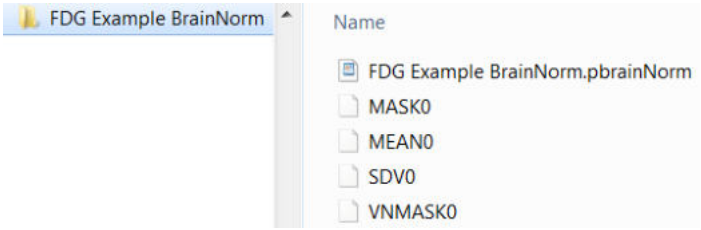
export/import of databases. Note that only the compiled information is exported, not the original control data sets, so it can only be modified by the owner of the control image data.

Exporting a Brain Norm

1. Open **Edit Norm**.
2. Activate the button **1. Create New Norm + [Delete|Export|Rename] existing**.
3. A dialog window opens for selecting the Brain Norm to be exported.



4. In the **DATABASE** list select the database containing the Brain Norm to be exported, and then select the appropriate entry in the list of **PBRAINDB NORM**.
5. Activate the **Export** button. A new dialog window appears indicating the exported information. When the **Save** button is activated, another window opens for defining the export directory. After confirming the directory path with **Select** a new subdirectory is created wherein the definition files are saved as illustrated below.



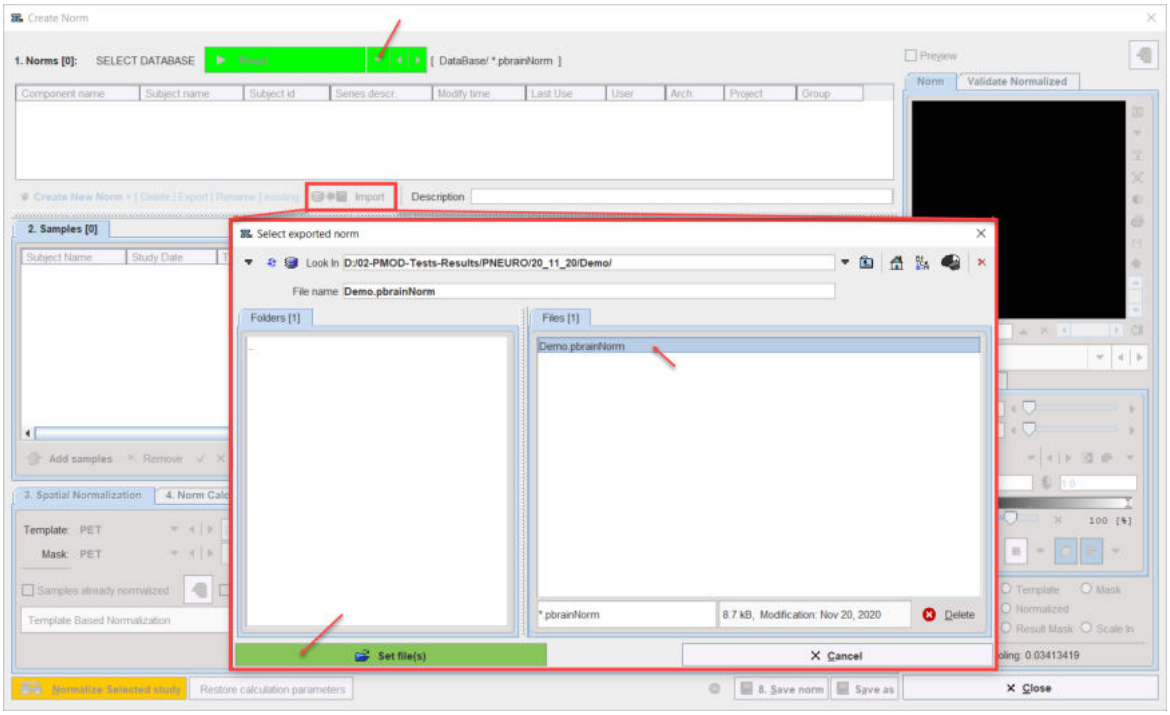
Close the dialog window with **Cancel**.

Importing a Brain Norm

A database import requires the whole contents of the directory created during the Brain Norm export.

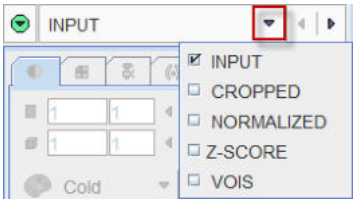
1. Open **Edit Norm**.
2. In the **SELECT DATABASE** list select the database into which to import the Brain Norm definition.
3. Activate the **Import** button. In the appearing file selection dialog navigate to the directory containing the exported files, and select the definition file (e.g. **FDG Controls.pbrainNorm**).
4. When **Set file(s)** is activated the relevant files are loaded and stored together with the processing definition in the database.

Immediately after importing the Brain Norm it can be used for analysis.



8.3 Compare to Norm Patient Data Analysis

The workflow for comparing the brain PET images of a test subject with the established normal uptake pattern will run through the following 5 pages of the **Compare to Norm** module:

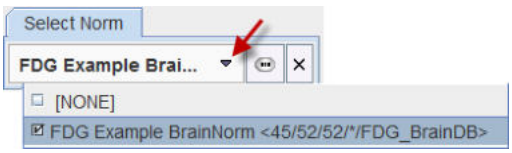


To start a subject brain PET analysis select the **Compare to Norm** tab.

Brain Norm Selection

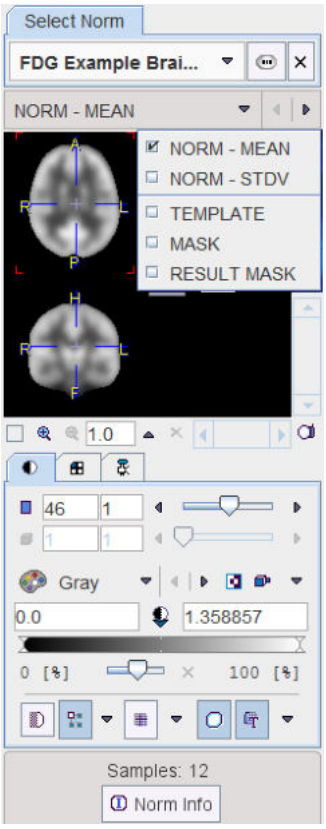
The Brain Norm against which the data should be compared can be configured on the **Select Norm** panel to the left of the **Compare to Norm** page. If the panel is hidden, it can be shown with the button in the upper right,

which will become .



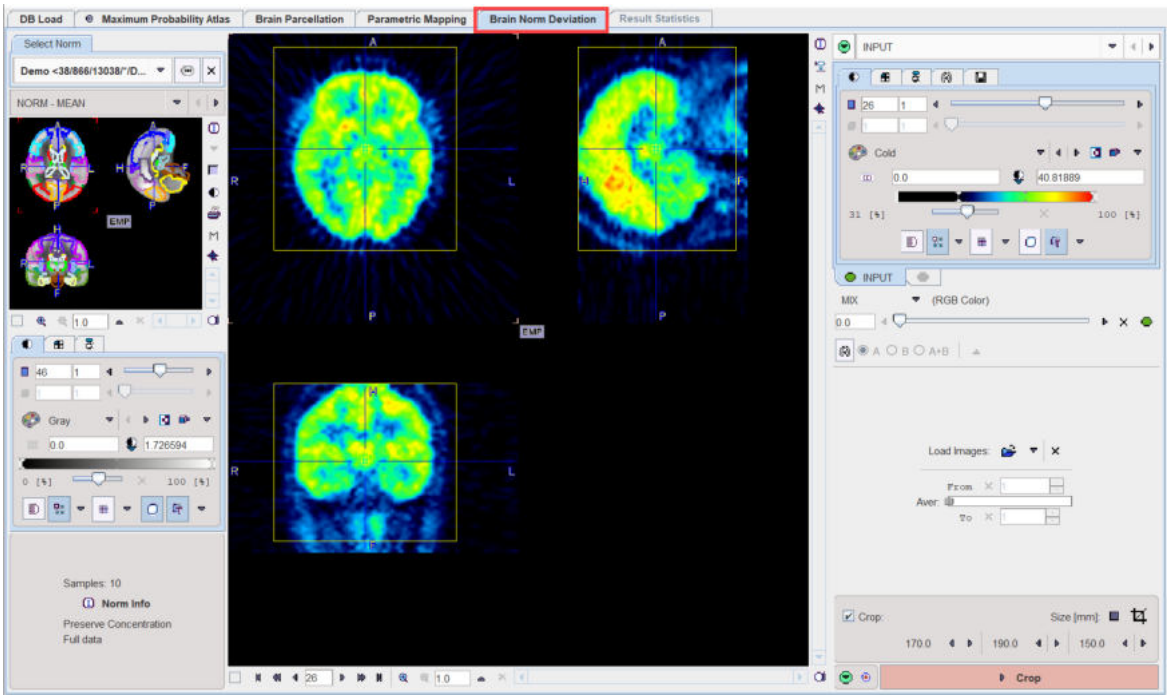
Use the arrow indicated above to show the list of Brain Norms available in the current database and select the appropriate one. If the Brain Norm is stored in a different database, the current database can be changed using the button.

The images related to the selected Brain Norm are available for inspection. The list selection allows switching between the different series such as the normalization **TEMPLATE**, the normalization and result **MASK**, as well as the **NORM** images (**MEAN**, **STDV**). The **Norm Info** button pops up a dialog window summarizing the database parameters.



Note: The last used database will be loaded whenever starting PNEURO. In order to avoid this lengthy operation while the **Compare to Norm** functionality is not used, the selection can be set to **[NONE]**.

8.3.1 PET Image Loading



The **Load Images** button for loading the brain PET images is located in the right control area. It is an option button which needs to be set to the appropriate data format. For loading images which are not saved in a PMOD database it is recommended to use the **Autodetect** format.

After loading, the images should appear with the same orientation as the template images. If this is not the case, please use the reformatting facilities in the PMOD viewing tool to correct the orientation.

Frame Averaging

In the case of loading a dynamic PET series, a new series is automatically generated by averaging a range of frames and assigning it to the **INPUT AVG** tab. The averaging range can be defined by the **From** and **To** number fields, or by dragging the range indicators in the **Aver** bar. It should correspond to the range used for creating the Brain Norm. After any modification of the range, the average is recalculated and the display updated. The original and the averaged images are shown in a fusion rendering which can be controlled in the area below the controls of the individual images. To see only the averaged images, move the fusion slider entirely to the right.

PET Image Cropping

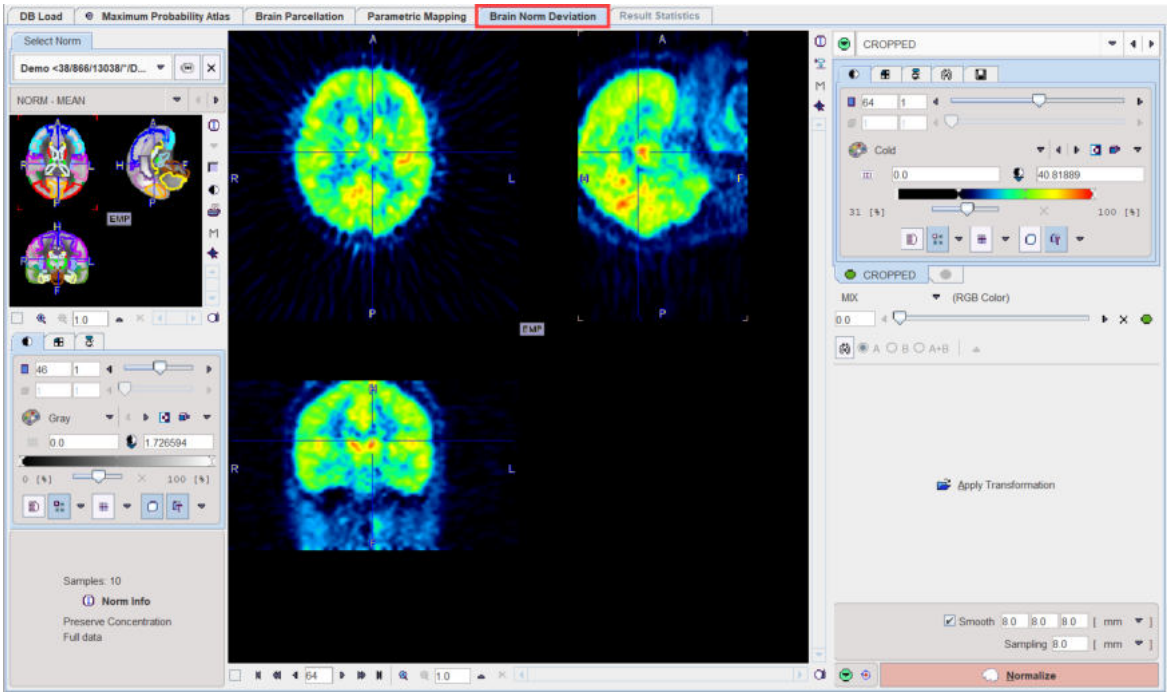
If the PET field-of-view is substantially larger than the brain, the data set should be reduced in order to improve the processing reliability. In this case, enable the **Crop** check and place the yellow crop box so that the brain is fully enclosed. The box center location can be changed by clicking into the image. The edge size in **[mm]** can be adjusted for each direction by selecting a size in the corresponding list. The **Start data cropping of Input Image** button initiates cropping, whereby the original data are replaced. If cropping is not initiated manually, a request will be shown when proceeding to the next step.

Note: The cropping operation is only allowed once. When returning to this page it will be deactivated.

To continue activate the **Crop** action button in the lower right.

8.3.2 Template-based Normalization

The cropped and time-averaged image is shown on the **CROPPED** page.

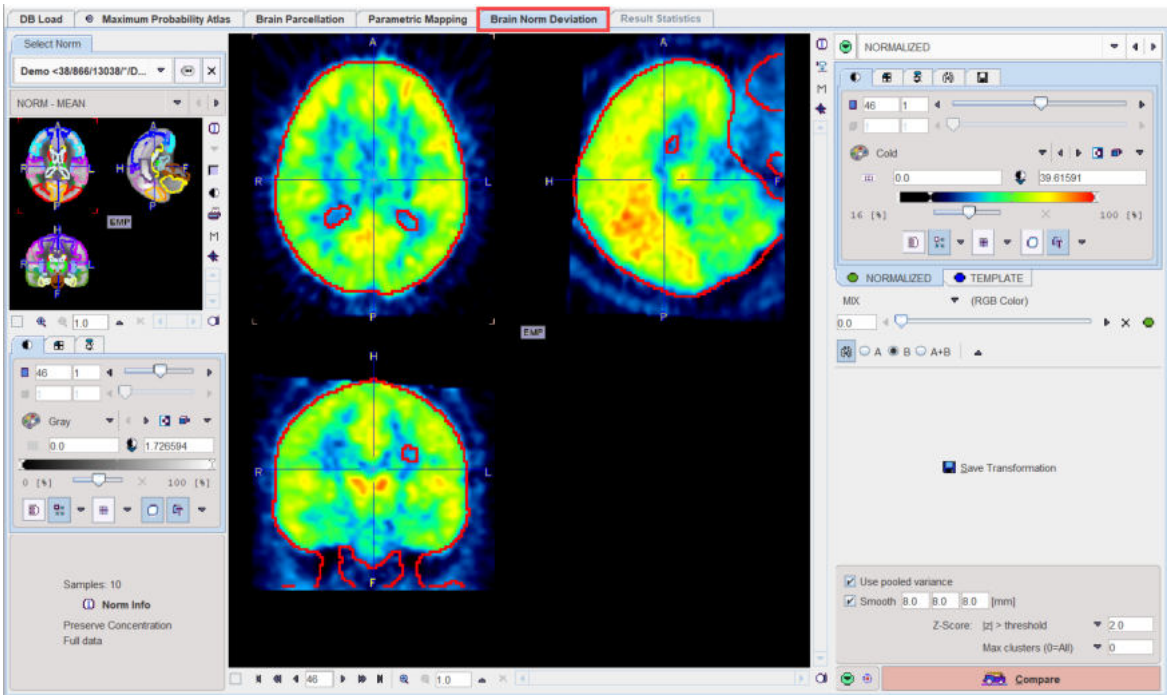


Assuming that the appropriate Brain Norm has been selected, the only task on this page consists of configuring the spatial normalization in the lower right. These parameters are already set based on the Brain Norm definition parameters. The proposed values can be overwritten if needed, for instance if a normalization procedure fails.

The **Normalize** button starts the stereotactic normalization. In case the normalization has been calculated and saved before, it can be retrieved with the **Apply Transformation** button and will be automatically applied to the subject images.

8.3.3 Quality Control of Normalization

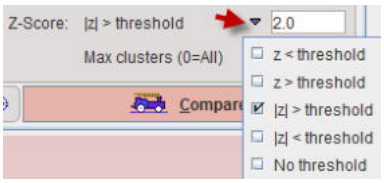
The result of the normalization is shown on the **NORMALIZED** page as a fusion of the normalized subject image with the template image.



The purpose of this page is to verify that the anatomical agreement in both images is sufficient. To this end it is recommended moving the fusion slider left/right, enable the iso-contours, and clicking at different brain locations.

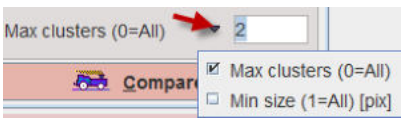
The area in the lower right serves for configuring and starting the z-score calculation. The initial configuration is derived from the Brain Norm, but it can be modified according to the current image quality. If the size of the control group is small, the **Use pooled variance** box should be checked to use a single averaged standard deviation value for all pixels. Otherwise, the individual pixel-wise standard deviation determined will used. A Gaussian **Smooth**ing filter should be applied to the normalized images before comparing them with the Brain Norm, because the reference was obtained by smoothing and pooling. It will be a matter of some optimizations to determine the filter parameters which bring both data sets to about the same resolution.

A z-score map will be calculated for each pixel of the result mask. This z-score map can be restricted to values in a certain range by the **Z-Score** selection.



With **z< threshold**, only pixels with z-scores below the entered threshold value will be retained, whereas the value in the other pixels is set to zero. The typical setting is **|z|>threshold** to retain pixels which deviate from the normal value by 2 standard deviations. With **No threshold** the z-score value in all pixels is retained.

The filtered results may be restricted with respect to connected cluster size. There are two ways to specify this filter.

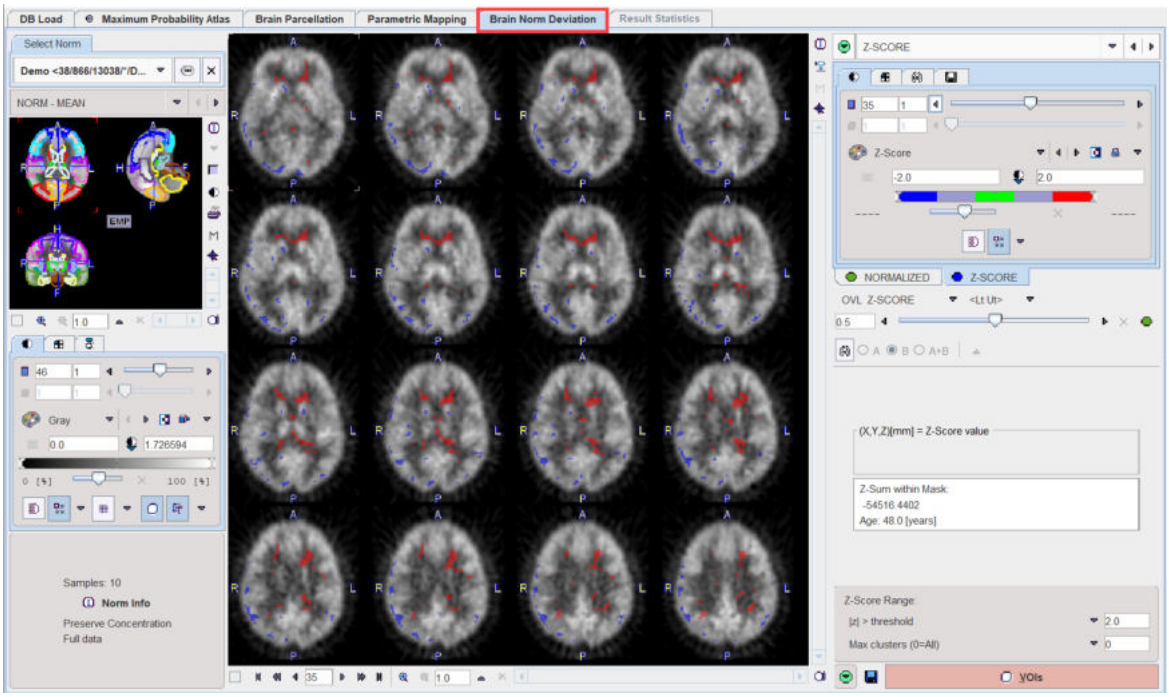


With **Max clusters** the result can be restricted to the number of largest clusters specified. No filtering is applied for the value of 0. With **Min size** only clusters with the minimal number of pixels specified will be shown.

Calculation is started with the **Compare** button.

8.3.4 Z-Score Analysis

The result of the z-score calculation is shown on the **Z-SCORE** page as a fusion of the **NORMALIZED** subject image with the **Z-SCORE** image map.



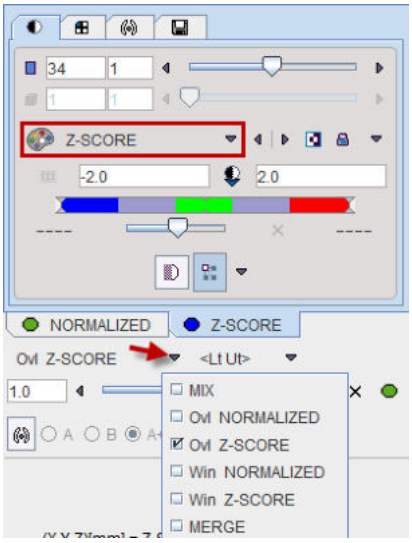
The z-score defines the deviation of a sample with respect to the mean of a distribution. It is defined by the formula

$z = (x-m)/\sigma$

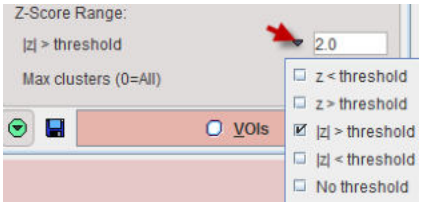
where x is the sample value, m the sample mean, and σ the standard deviation of the distribution. Therefore z describes the deviation from the mean in number of standard deviations and is positive, when the sample is above the mean, and negative when below.

Z-Score Thresholds

Per default the **Z-SCORE** image is shown with a dedicated color table as illustrated below. It will show all pixels with values below the lower threshold in blue, and pixels above the upper threshold in red.

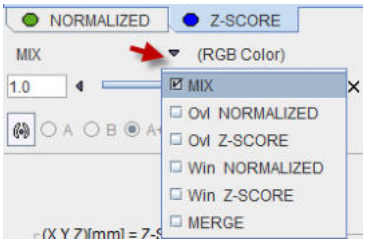


To allow a clear view of the z-score clusters on the normalized subject images the default fusion mode is set to **OVL B** with filter **<Lt Ut>**. This means that only **Z-SCORE** pixels with values below/above the lower/upper thresholds are shown. These thresholds are updated by any change in the **Z-Score Range** settings.



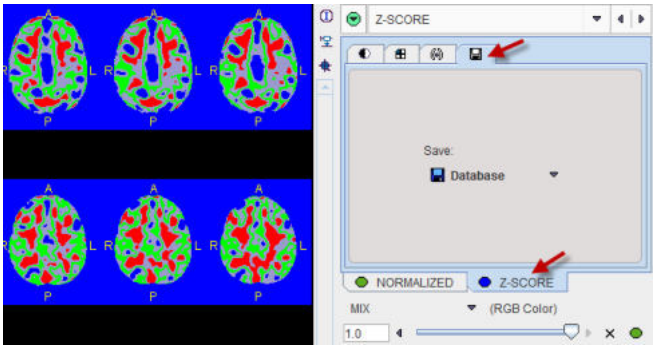
The threshold can be entered numerically, whereas the filtering criterion can be selected from a list. In this way z-score pixels below a threshold, above a threshold, or with an absolute value beyond the threshold can be shown.

The setting **No threshold** removes any data filtering. In this case the fusion mode should be switched to **MIX** in order to actually see the full z-score map. This setting should also be selected for saving the unfiltered z-score map.

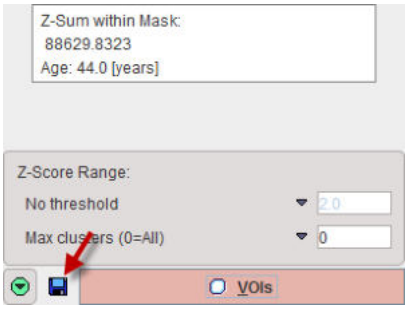


Saving Results

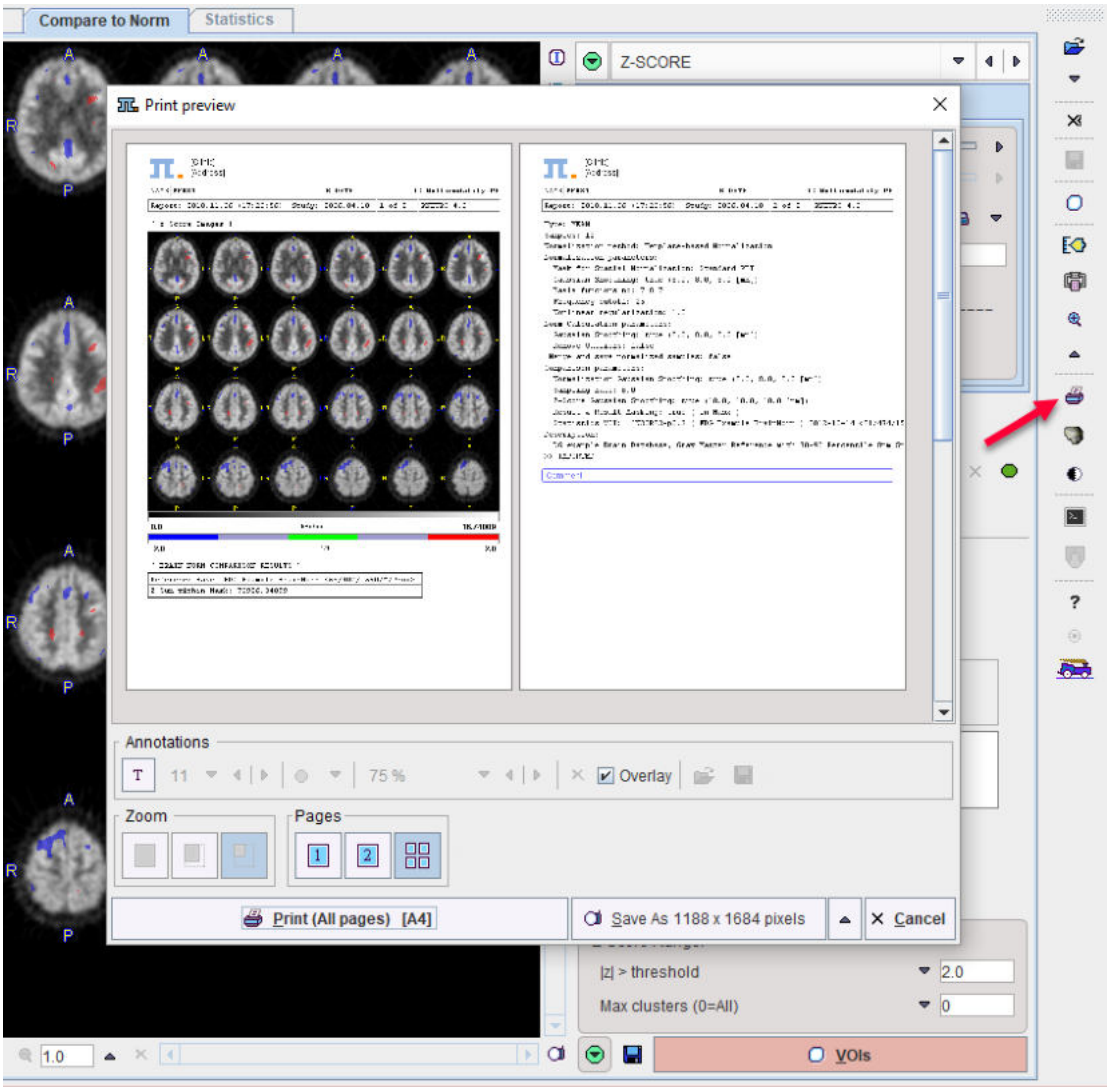
The **Z-SCORE** panel has a dedicated tab for saving the currently shown z-score map for use in another context. Configure the appropriate image format, and then select the **Save** button for saving.



The program always calculates the sum of all z-score values within in the result mask. This information is displayed together with the age of the subject. It can be saved to the database or a text file using the saving button indicated below.



When the button is activated, a dialog window pops up showing two minimized report pages, one with the current image display as illustrated below, the other containing a summary of the Brain Norm parameters.



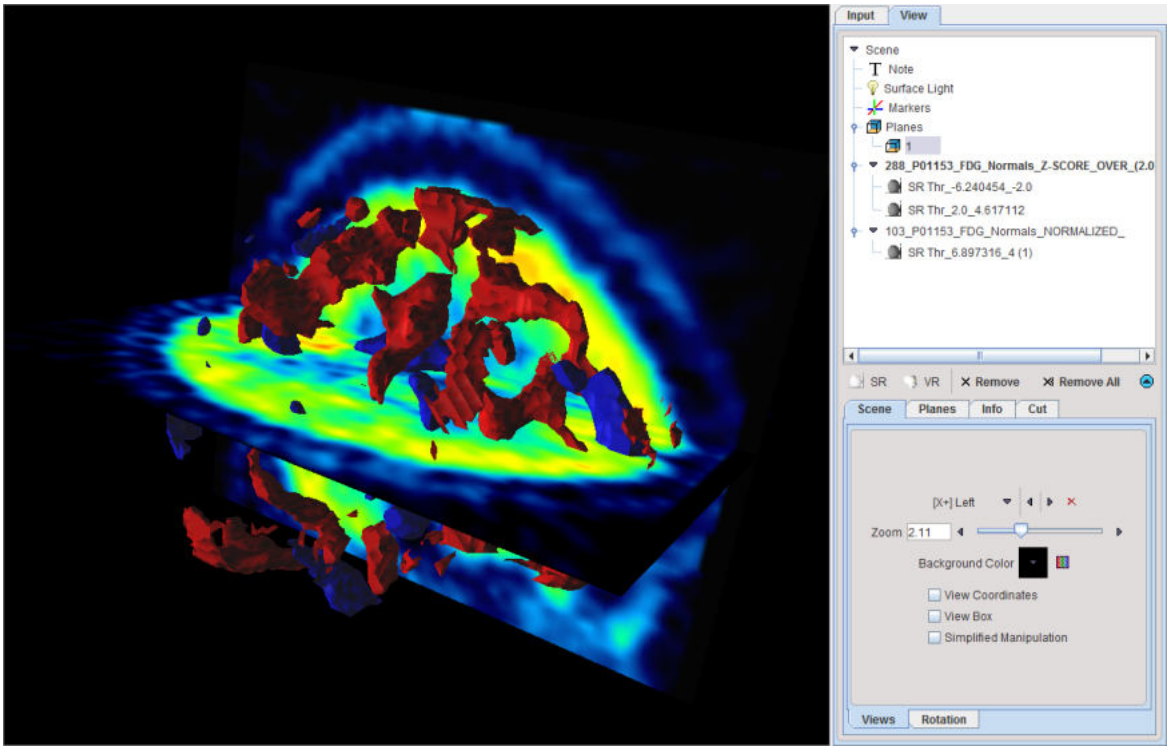
Select the page of interest in the **Pages** section. It can be annotated, printed, and saved as a JPEG/TIFF file or as a DICOM secondary capture object.

VOI Statistics of z-score Values

The z-score maps can be used for VOI statistics calculation. To do so, please activate the **VOIs** action button in the lower right.

3D Rendering (Option)

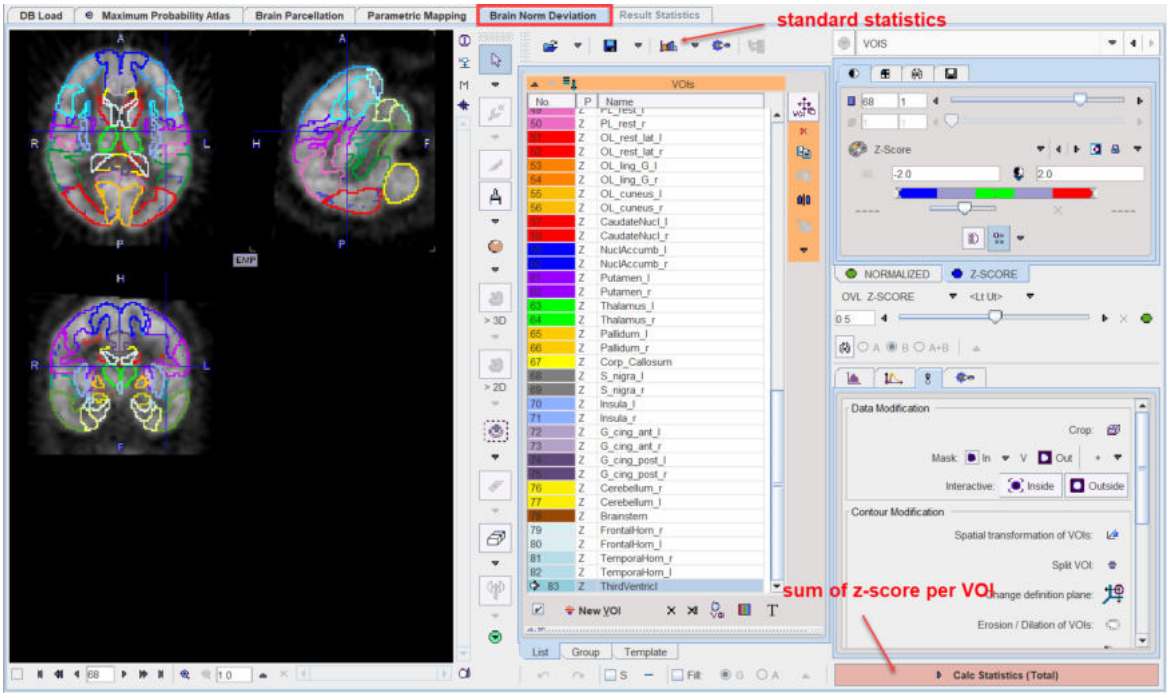
If the 3D option has been purchased and installed the button is available in the lateral taskbar and can be used to rapidly transfer the **NORMALIZED** subject images and the **Z-SCORE** map to the 3D tool. Rendering is immediately initiated and a result shown such as the example below.



Here the truncated z-score maps were calculated with |z|>2. Therefore, the red objects enclose all areas with a z-score above 2, while the blue objects enclose the areas with z-score below -2. The scene can interactively be rotated and zoomed. Additionally, more information can be added, for example the brain shape, and/or planes of the normalized images.

8.3.5 VOI Analysis

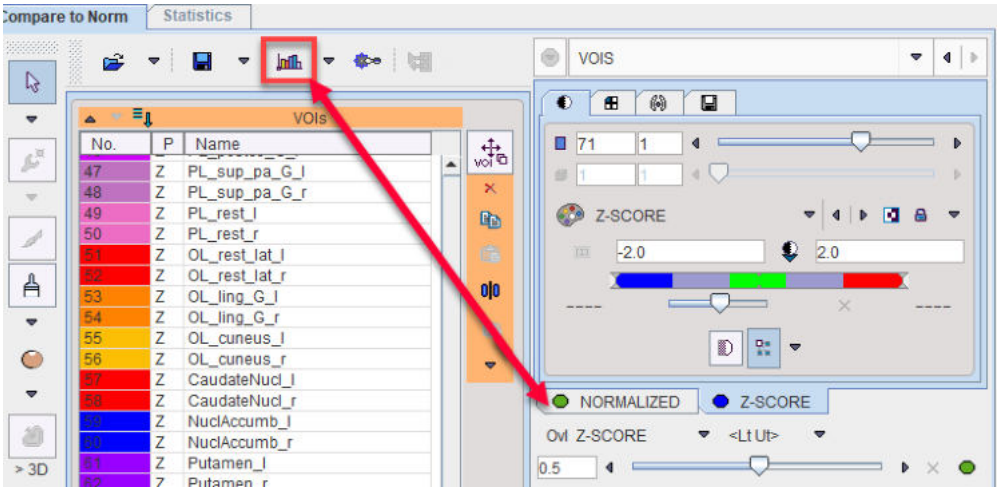
The **VOIS** page shows the filtered **Z-SCORE** map fused with the **NORMALIZED** subject images together with the VOI editor.



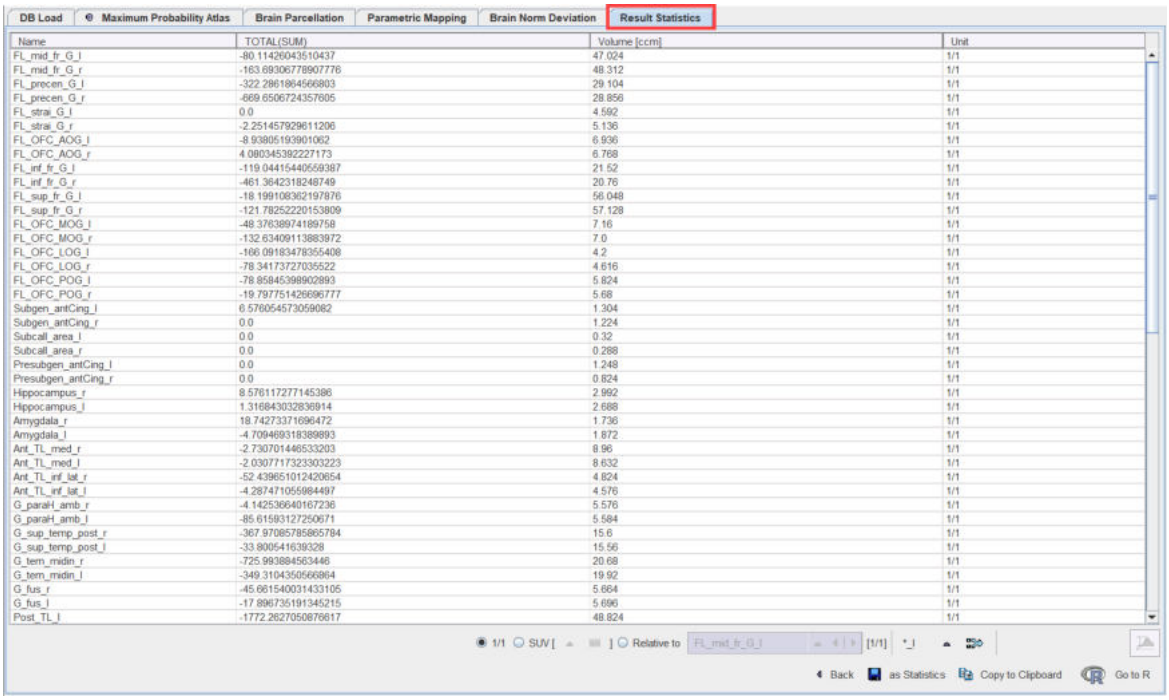
If a VOI set has been defined in the Brain Norm, it is directly available for adjustments and statistics evaluation. The alignment of the VOIs with the brain structures can be verified using the

NORMALIZED images. If needed, individual VOIs can be scaled, reshaped, or cleared and completely be redefined from scratch.

There are two ways of calculating statistics. The standard statistics button applies the VOIs to the images in the selected tab and shows the results in a dialog window. In the example illustrated below the mean, stdv, max, etc. of the **NORMALIZED** subject images will be calculated in kBq/cc.



With the **Calc Statistics (Total)** action button the VOIs are applied to the filtered z-score map and only the sum of z-scores per VOI is calculated. The result is shown on the dedicated **Statistics** page of the PNEURO tool.



8.3.6 Application Notes

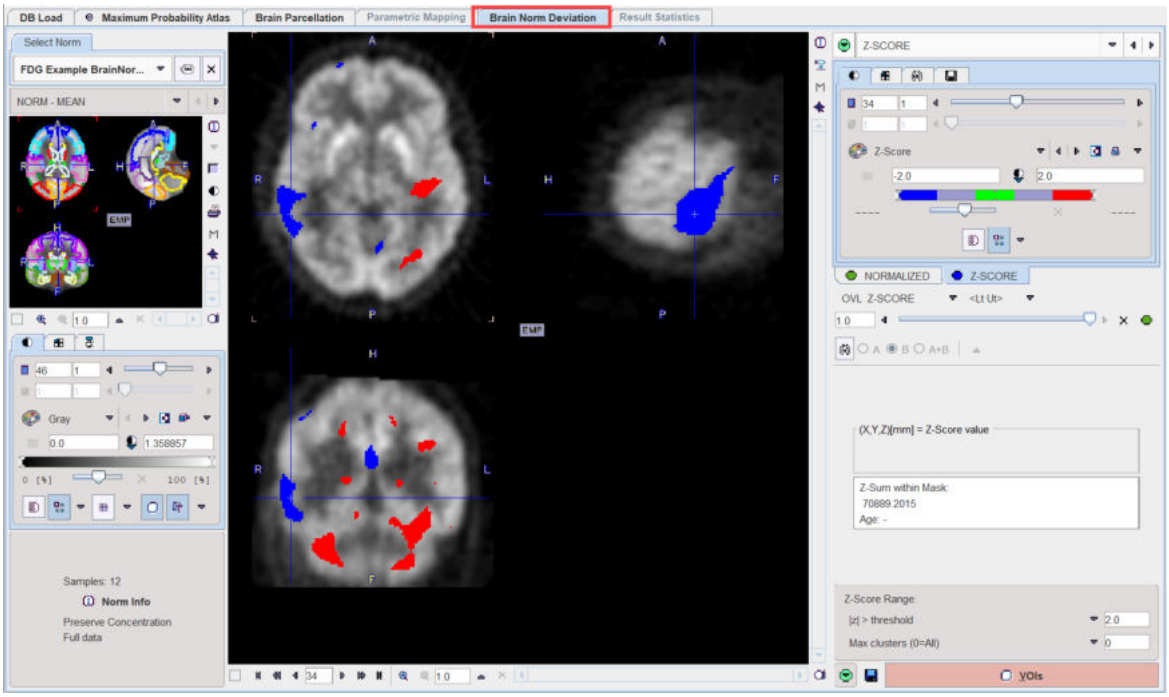
The results of database comparisons will crucially depend on the parameters used for database creation.

- When calculating the reference value for scaling the pixel values, diseased tissue should always be avoided. Therefore it might be helpful to have databases available with differing reference calculation, for example one using a gray matter mask, and another using a cerebellum mask.
- Resolution mismatches between the database template and the processed subject image and inaccuracies in spatial normalization will result in edge artifacts in the z-score maps. This effect

can be reduced by sufficient image smoothing. Again, it might be helpful to have databases available with different smoothing, to be applied depending on the size of the effect one is looking for.

8.3.7 Example Database

Included in the distributed Pmod database is an example **FDG Example BrainNorm** database. FDG images for testing the Brain DB functionality are available under the PALZ1 and PFUS1 example subjects. Shown below is the PFUS1 FDG example. The extended blue area clearly marks the location of the tumor, while red areas indicate activations. Peripheral differences can be attributed to inaccuracies in stereotactic normalization.



DISCLAIMER: The example database is only intended for testing the functionality of the Compare to Norm tool, and is in no way a validated FDG Brain Norm.

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