User's Guide

PMOD Neuro Tool (PNEURO)

Version 3.6

PMOD Technologies

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Contents

PMOD Neuro Tool Introduction (PNEURO)

Precise knowledge of the functional brain areas in the individual patient 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 Probabilistic Atlas (N30R83)

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 constructed by Hammers et al. [1]. The corresponding PNEURO component allows the user to adjust the atlas to the individual patient anatomy with a spatial normalization procedure, which is preferably obtained from the T_1-MR image. Alternatively, the normalization can be directly derived from the PET image, making this solution also applicable for PET-only studies.

Automatic 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 patient'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.

Normal Brain PET Databases

PET studies with patients 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 patient's brain can be easily localized without any prior assumptions or operator variability and presented as a z-score map.

System Requirements

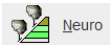
For productively working with the parcellation tool, the following workstation system requirements should be met:

- 64-Bit operating system (Windows, Mac OS X, Linux)
- ≥ 16 GB RAM
- ≥ 8 processing cores (hyper-threading is also viable)

8GB 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 to the patient anatomy.
3) **Brain Parcellation** page: Creation of brain VOIs by knowledge-based segmentation of anatomical T_1-MR images.
4) **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.
5) **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.

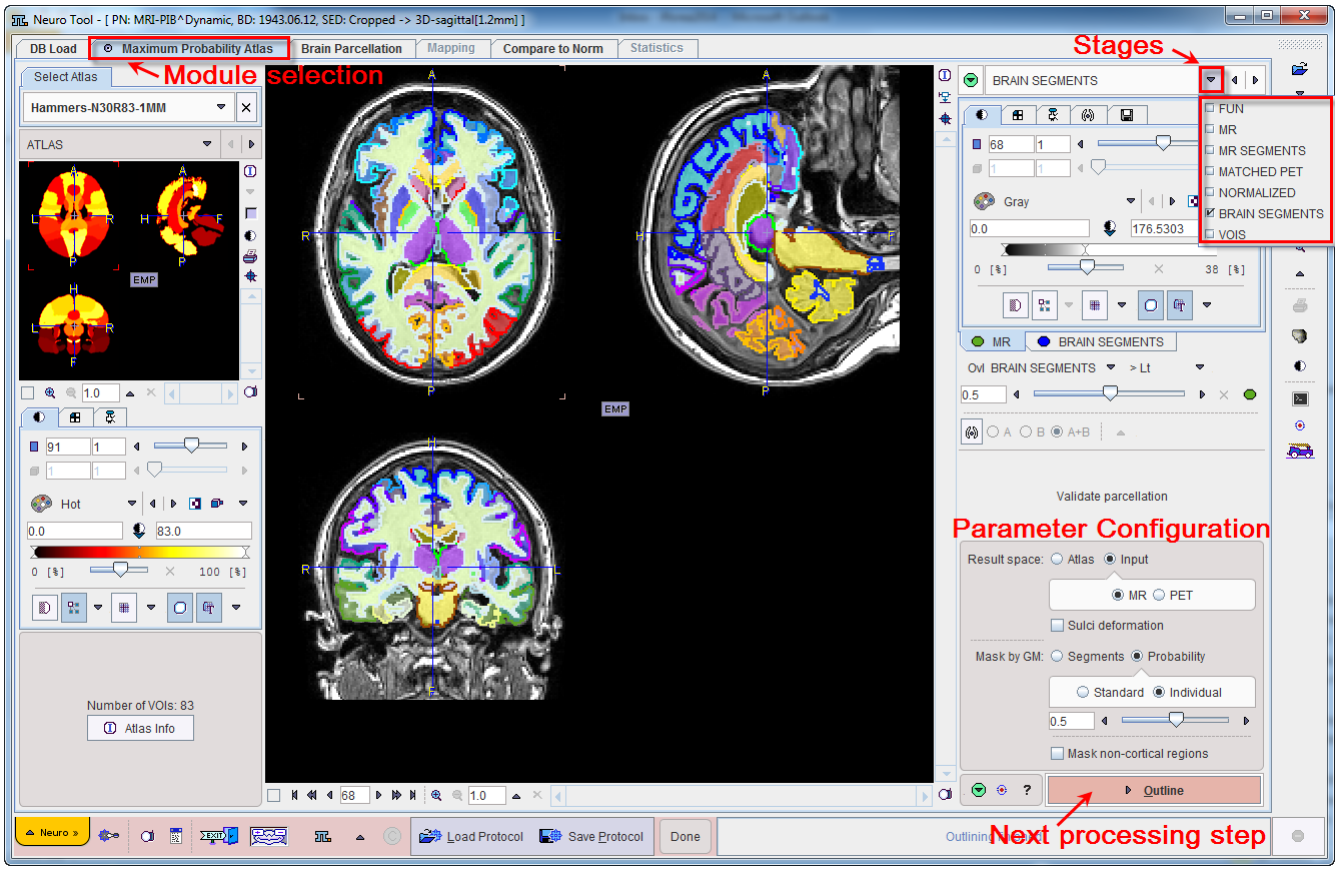
The user selects the appropriate module for data processing by the corresponding tab in the

Brain VOI Tools: Common Features

user interface of PNEURO. Each of the pages is described in a separate section of this guide.

User Interface

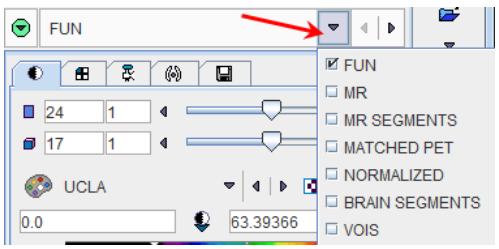
The layout of the pages for the Brain VOIs calculation are illustrated below using the N30R83 **Maximum Probability Atlas.**



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

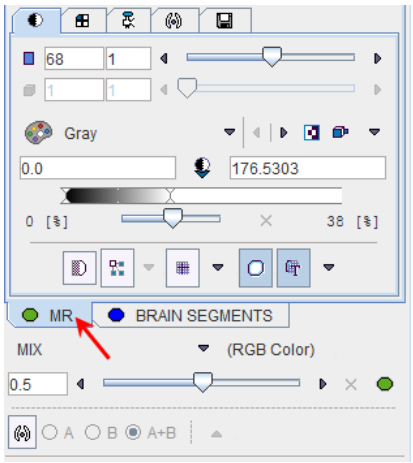
Data processing is consistently organized by a stepwise progression towards the end result. On each processing stage the user has to take some action such as data loading, alignment inspection or parameter configuration, and then start the next processing step with the red action button located in the 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.



Saving of Intermediate Results

Convenience Buttons

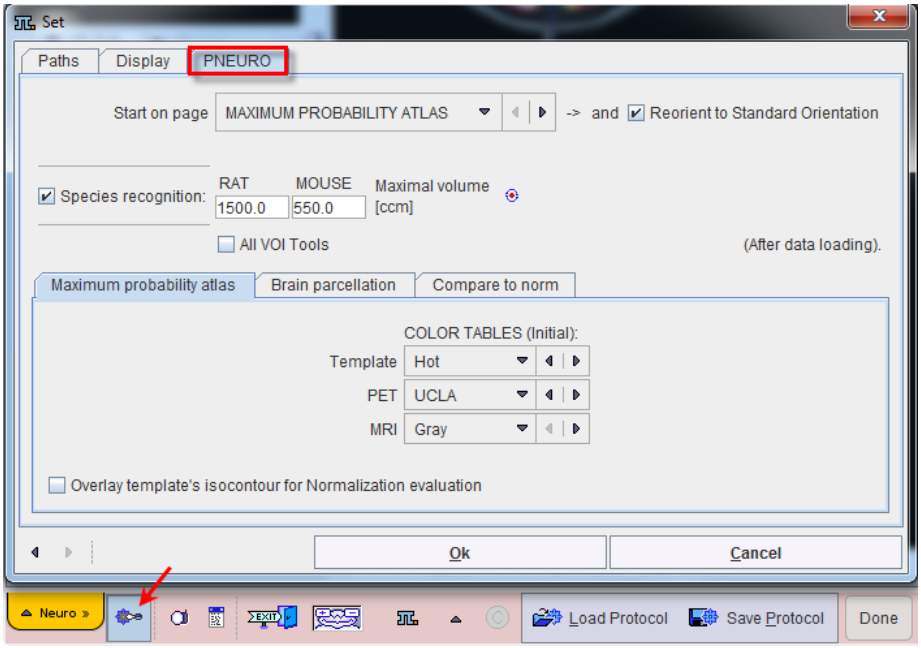
Next to the action button in the lower right is an area with three buttons

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.
- ? Display help information for the current page.

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 patient 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.

If the **Species recognition** is checked, PNEURO uses the defined volumes and tries to guess the species in the loaded images. The default settings for the matching algorithms are species sensitive and are automatically adjusted accordingly.

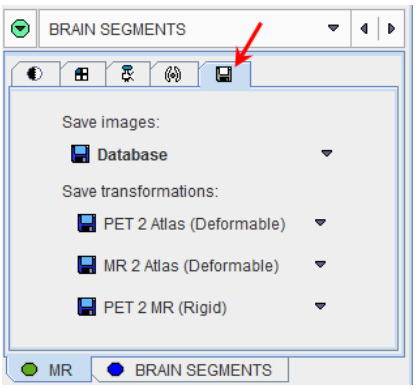
The **All VOI Tools** flag allows defining the functionality level of the **VOIS** page. Default is a reduced set of VOI tools. To enable all possible VOI tools please enable the **All VOI Tools** box and restart.

Further Information

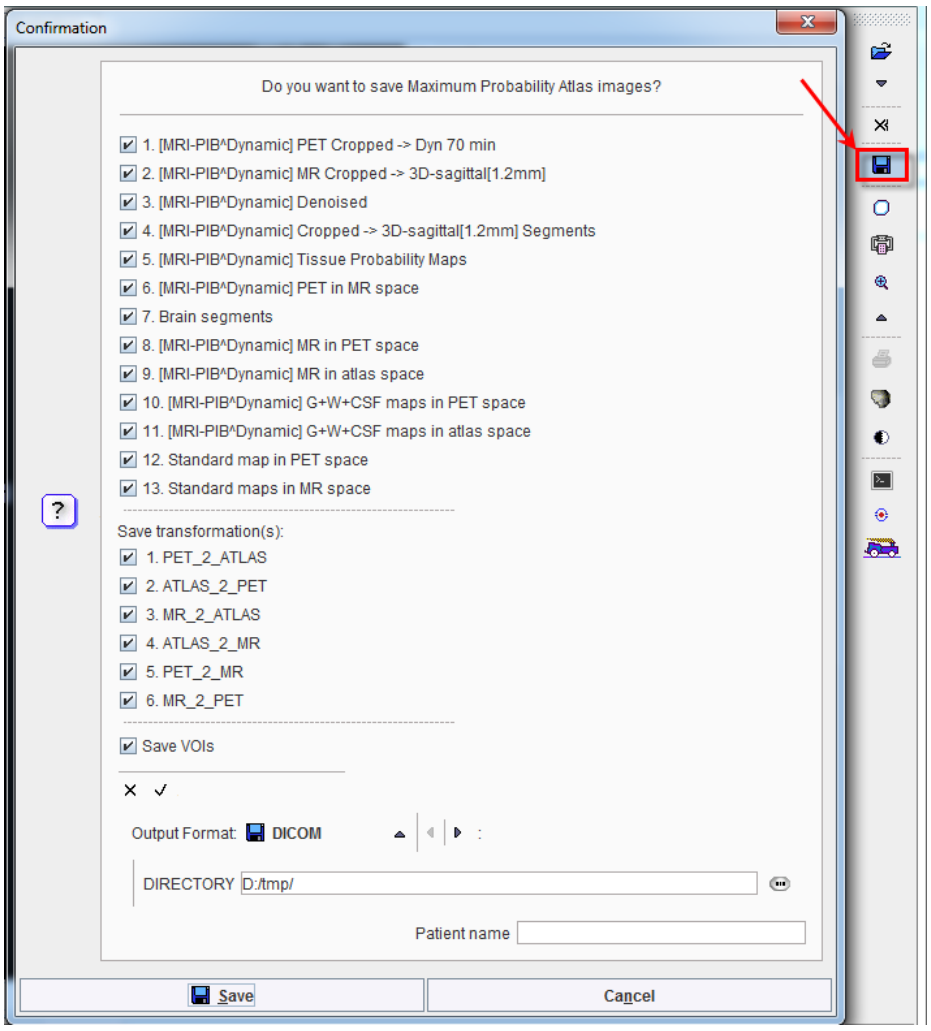
This guide is focused on the brain analysis functionality. Please refer to the *PMOD Base Functionality Guide* for details about general functions such as data loading, image display, and VOI definition.

Saving Intermediate Results

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



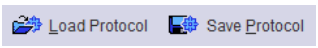
The complete set of intermediate information for a Brain VOI processing can be saved at once using the Save all button from the lateral taskbar.



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

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. It is advised saving a protocol after every completed data processing, so that at any later time the configuration can be retrieved, verified and modified to try variations of the processing parameters.

Run All

If the generation of the VOIs can be done 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 calculated the statistics.

7) Save a protocol file.

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 analyze.

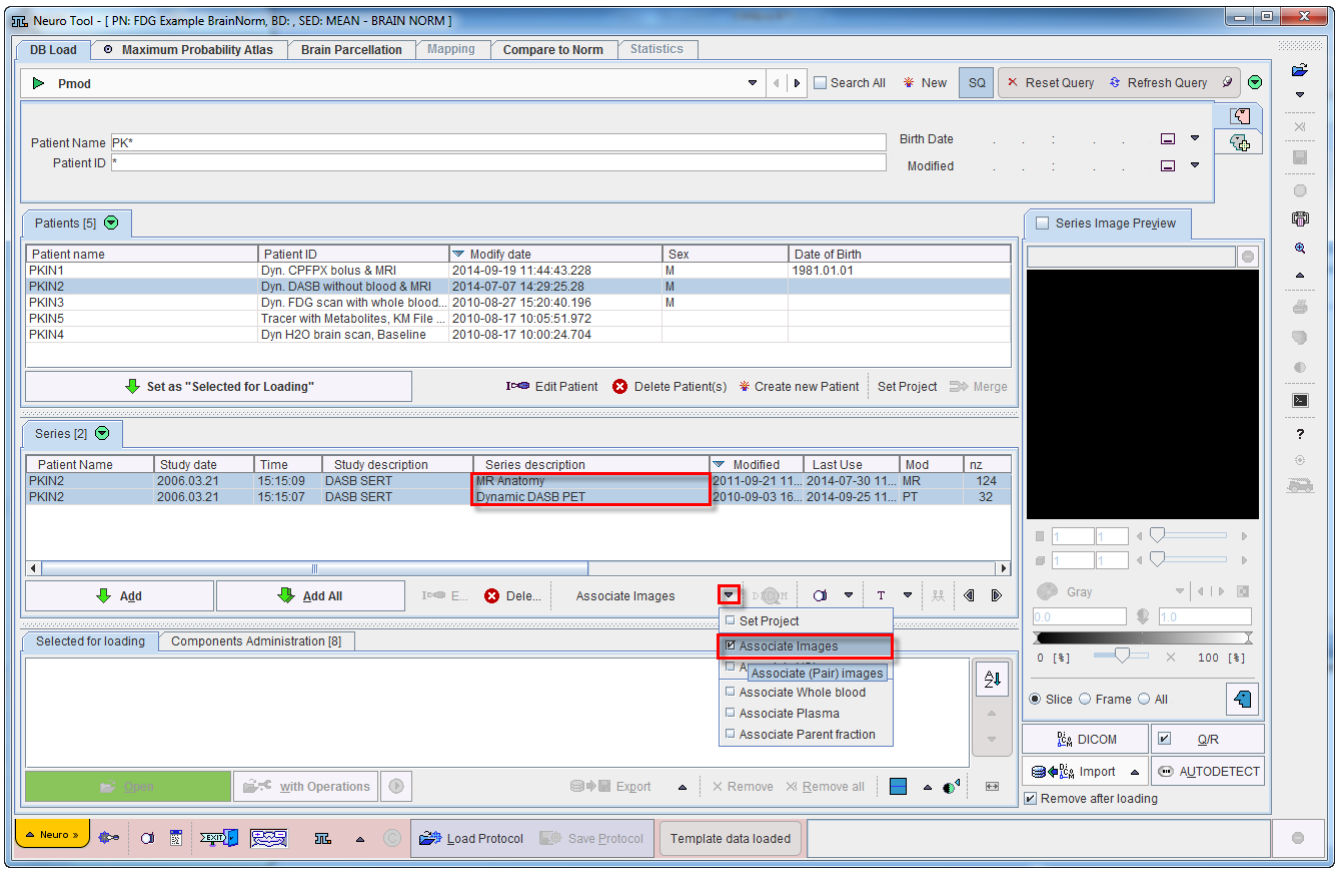
Batch Mode Maximum Probability Atlas & Brain Parcellation (Brain VOIs)

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 taking advantage from specific relationships among data elements which can only be provided by a database. Furthermore, the output produced by the batch can directly be inserted at the appropriate database level, which is more difficult when working with directory structures.

Association of MR and PET Series

Before starting the batch processing, the first step is to prepare the input data. Start the PNEURO tool to perform the image association in the database page as illustrated below.



Select patient **PKIN2** in the **Patients** list. Use SHIFT+Click to select the two image series **MR Anatomy** and **Dynamic DASB PET** which will be used in the pipeline processing. From the **Set Project** list activate the **Associate Images** entry to establish the mutual relation between the image series.

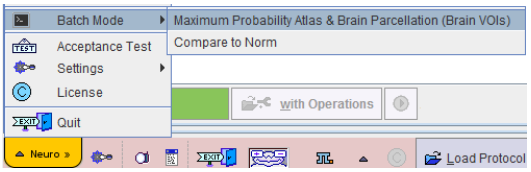
This operation would be repeated for all patients to be processed. It is a one-time action which can be taken advantage of in multiple analysis procedures.

Batch Process Overview

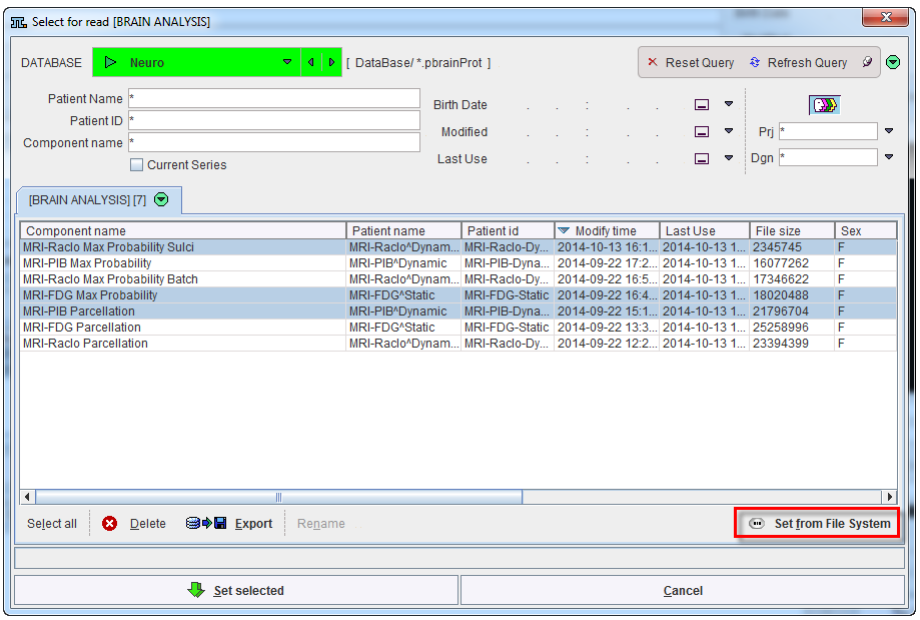
The batch mode essentially consists of a sequential processing of saved protocol files. So the preparation consist of loading a data set (PET and/or MR), defining crop boxes and crop the images, adjusting the parameters on the different pages, and saving a protocol file.

Starting Batch Brain VOIs

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

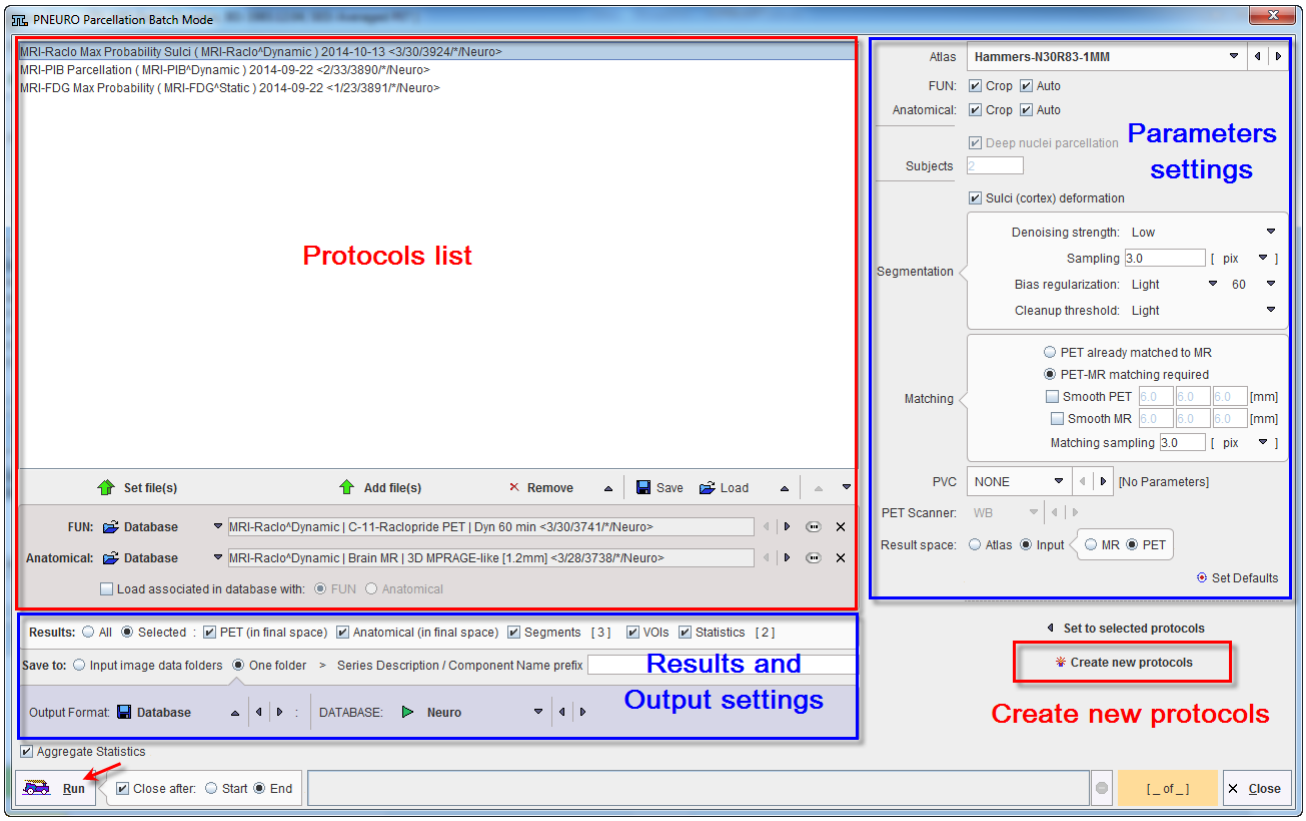


or the button from the lateral task bar to start the batch operation. First, a dialog window appears for selecting PNEURO protocol(s) (**.pbrainProt**) from a database. To load protocols outside the database please use the **Set from File System** button. Select the appropriate protocols and activate **Set selected**.



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 selected protocols is displayed in the upper left. More protocols can be added with the **Add file(s)** button, and selected entries removed from the list by **Remove**.

Parameters Settings

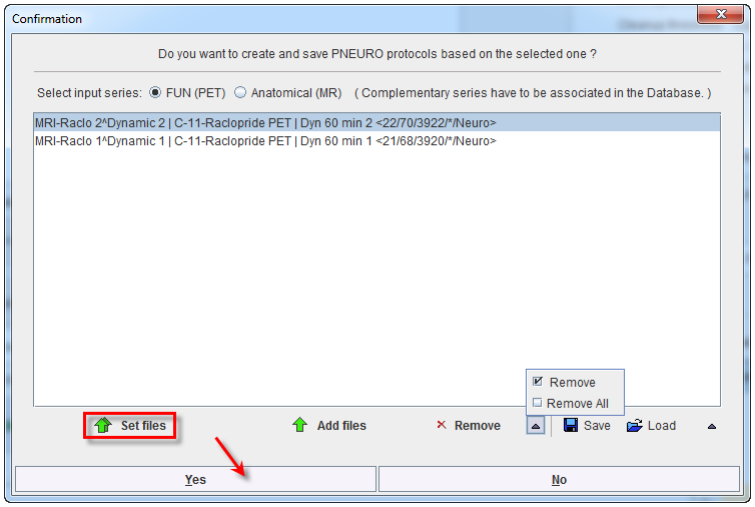
To view or modify the parameters of a protocol click the list entry. The settings of the protocol are shown in the upper right area and can be easily adjusted and **Set to the selected protocols**.

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

Create new protocols

New protocols can be created within the batch interface. To achieve this select a protocol available in the list and activate the **Create new protocols** button in the lower right area. A dialog window opens and allows defining the input data type to be processed: **FUN (PET)** or **Anatomical (MR)**. Please note that for processing studies with PET and MRI, association of the pair studies has to be done beforehand in the Database.

The images to be processed are defined by the **Set files** or **Add files** buttons, which open a dialog window for selecting image files. The data selections build up the data list for processing. While **Remove** deletes a selected entry from the list, **Remove all** clears the whole list. An input data list can be saved for later use with the button right to **Remove**.



To create and save the new protocols activate the **Yes** button as shown in the capture above. The protocols list is replaced by the newly created ones. The protocols are automatically saved and associated in the database with the corresponding images.

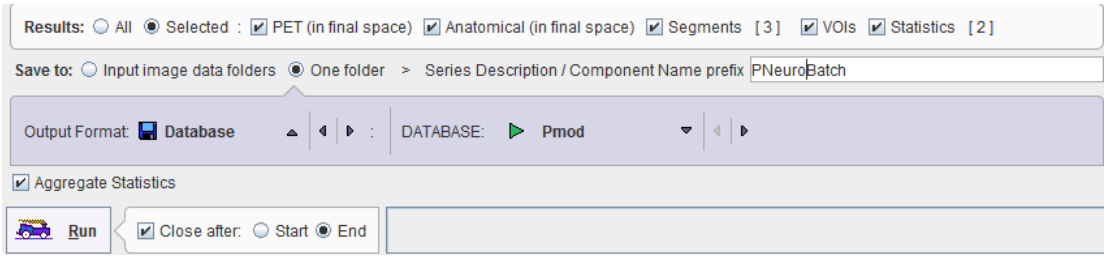
Note: If cropping is required, it is recommended to use auto cropping facility for the protocols created within the Batch interface. To this end, please make sure that both **Crop** and **Auto** boxes are enabled in the Parameters settings area.

Results and Output Configuration

The granularity of the results saved during the batch processing can be configured by the checks in the **Results** section:

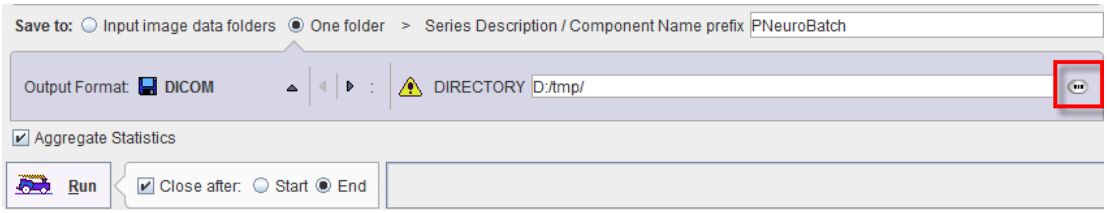
- **All**: PET (in final space), the anatomical image MR or FDG (in final space), VOIs contour and Statistics.
- **PET (in final space)**: PET image in the space where the VOIs are generated.
- **Anatomical (in final space)**: The anatomical image (MR or FDG) in the space where the VOIs are generated.
- **VOIs**: Contour VOIs in the selected result space.
- **Statistics**: VOIs applied to the target image series (PET, if available, otherwise the anatomical image).

The results can either be saved in the same directory as the input images (**Save to: Individual image data folder**) or in a single directory (**Save to: One folder**). A **prefix** string can be defined which will be used for labeling the batch results.



The format of the saved images is defined by the **Output Format** selection. Depending on the format, some parameters need to be configured, such as the output **DATABASE** in the example above.

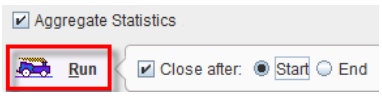
With file-based formats such as **DICOM**



the file system path where all files will be stored needs to be defined with **SET DIRECTORY**.

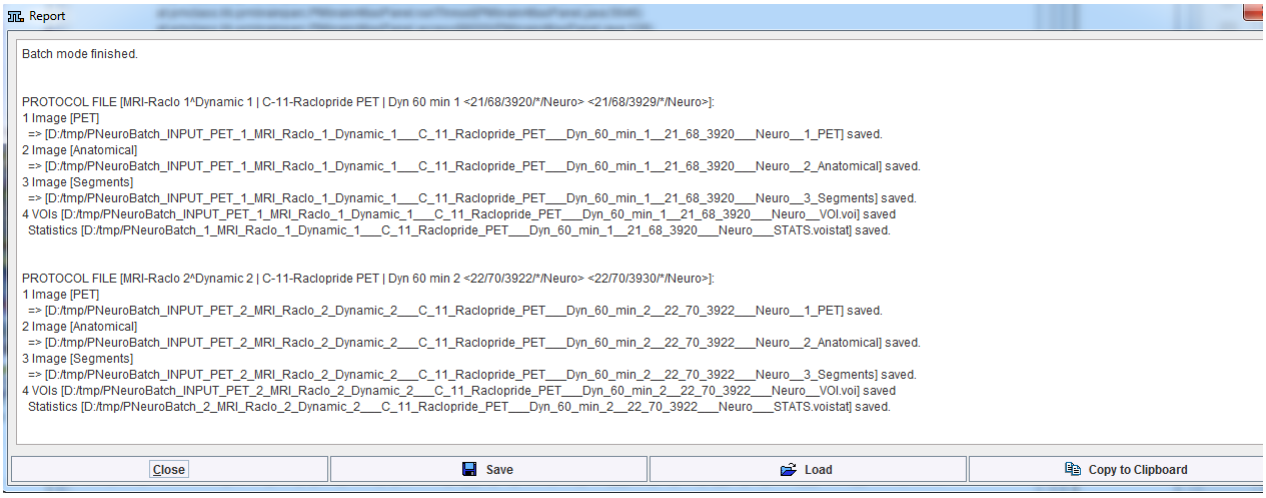
Run the Batch

To start calculation activate the **Run**.



The **Aggregate Statistics** option is only relevant for non-image results such as regional uptakes or regional time activity curve. 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.

Preferably, **Close after Start** is enabled. In this way the dialog window is closed and the processing runs in the background. At the end of processing a dialog window is shown which indicates that processing was ok and provides a report.

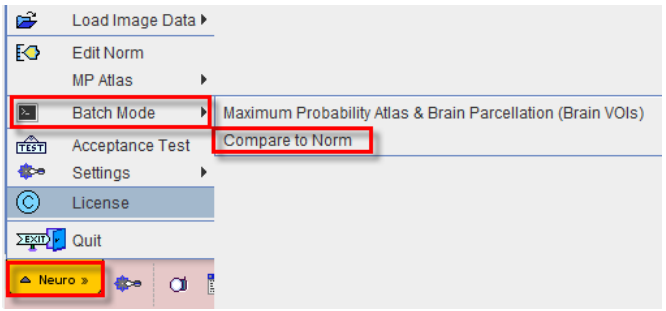


Batch Mode Compare to Norm

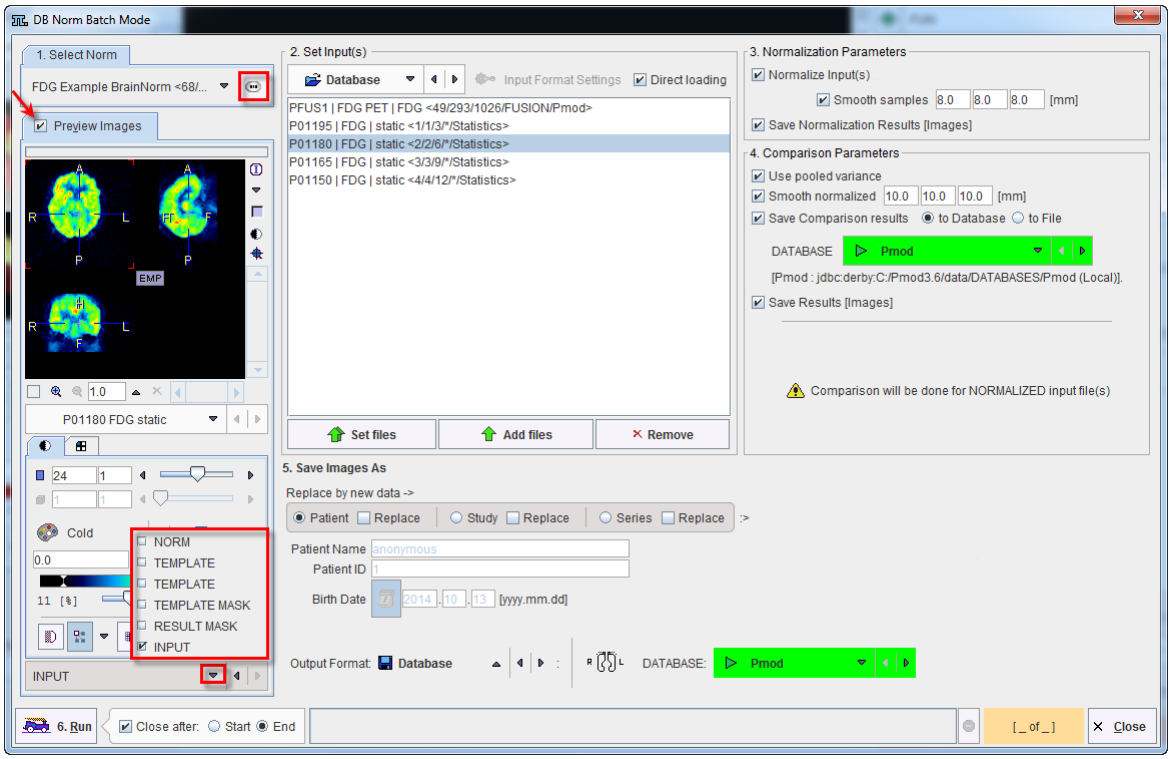
The PNEURO tool offers the Compare to Norm batch facility which is useful when several studies need to be analyzed. The batch mode allows performing the spatial normalization - which is the time-consuming processing step of the whole analysis - for a list of studies and save the normalized images for 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*** (on page 115) 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 to configure 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. The number of basis functions used in the elastic part of matching is currently fixed, but may become relevant in future enhancements.

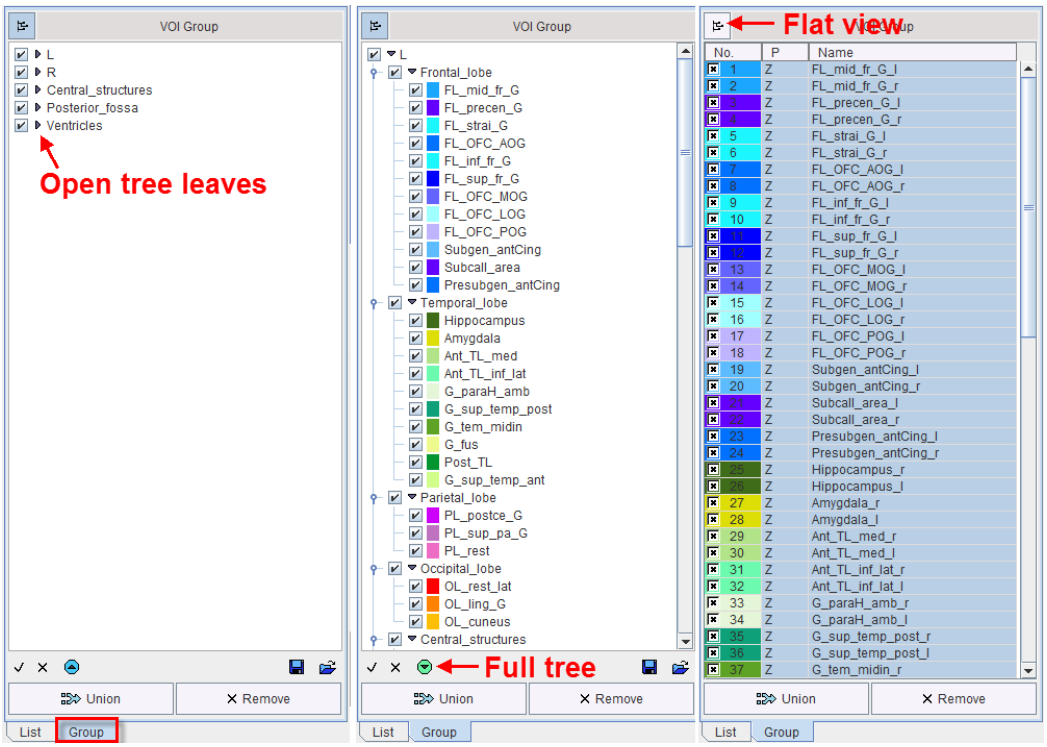
4. Comparison Parameters This section is only relevant if the user wants to perform the comparison analysis in unsupervised mode, which is *not recommended*. All the comparison results can be saved for post-batch reviewing.

5. Save Images As If result images have been configured for saving, the format defined in this section will be applied. First select the data format (recommended is the **Database**), then configure additional parameters of that format. As an additional option, the **Patient**, **Study** or **Series Data** can be replaced.

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

Tree Organization of Brain VOIs

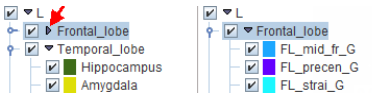
The brain VOIs are structurally organized in a tree on the **Group** tab of the VOI editing page. The selection of a VOI subset is supported by a dedicated user interface illustrated below.



The branches on the top level are the left (**L**) and right (**R**) structures, **Central structures**, **Posterior fossa**, and the **Ventricles** as illustrated in the left panel above. The full tree can be opened easily by the button indicated in the middle panel. The tree view can be flattend to a simple list by the button at the top indicated in the right panel. The statistics will only be calculated for the selected VOIs.

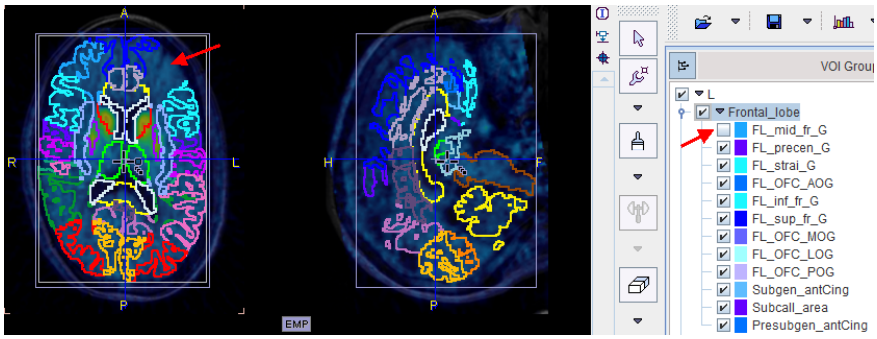
Tree Manipulations

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

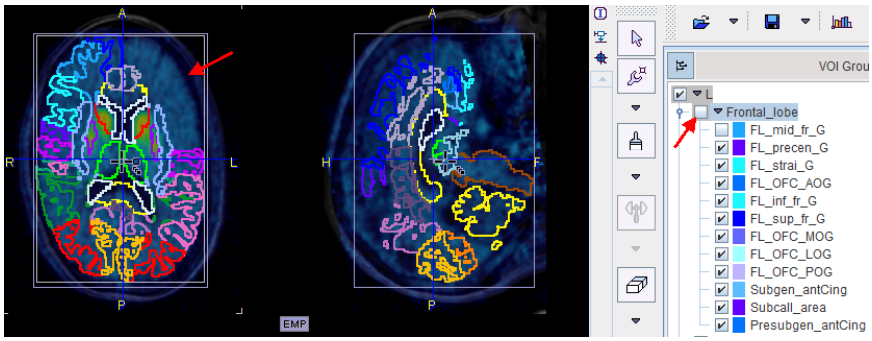


Tree Selections

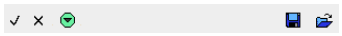
The structures of interest are the ones with checked boxes to the left of their color code. Simply click into a box for changing the selection. In the example below the **FL_mid_fr_G** VOI has been de-selected.



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 at the tree bottom:



 Remove the selection check of all VOIs.

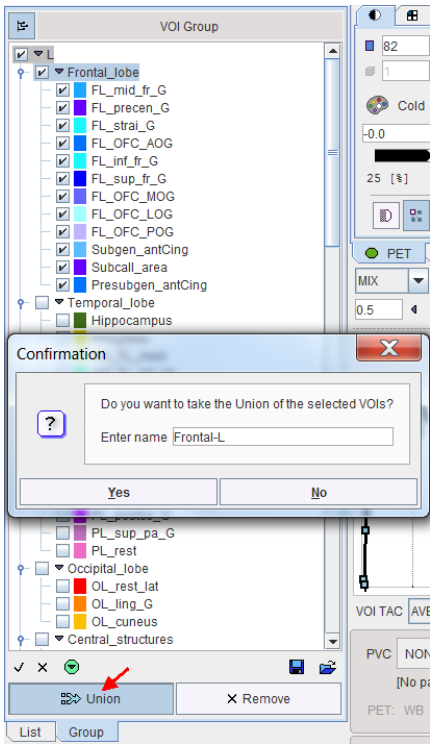
 Set the selection check of all VOIs

 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 all left frontal lobe VOIs selected by checking the **Frontal_lobe** entry.



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

Recommendations for Brain VOI Calculations

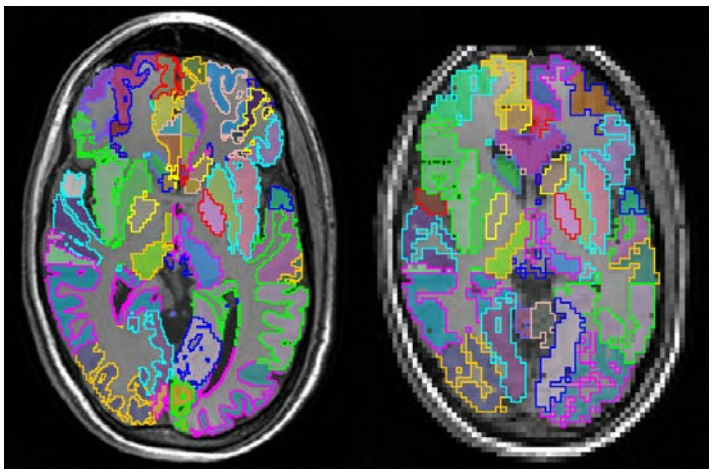
Input Data

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 patient anatomy and conveniently support any interactive fine adjustments.

Evaluation Space for PET Statistics

The calculation of the PET VOI statistics can be performed in different spaces, 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, he should evaluate the VOIs in the PET space. In this case he 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

In order to apply tailored presets for the automatic procedures, PNEURO tries to guess the **Species** type from the loaded data. If it is not appropriate, please change the **Species** using the selection button.

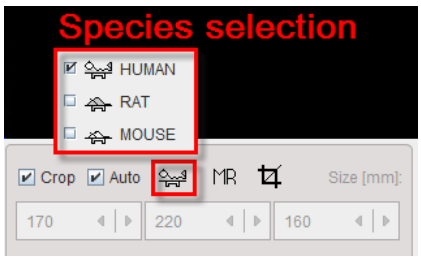
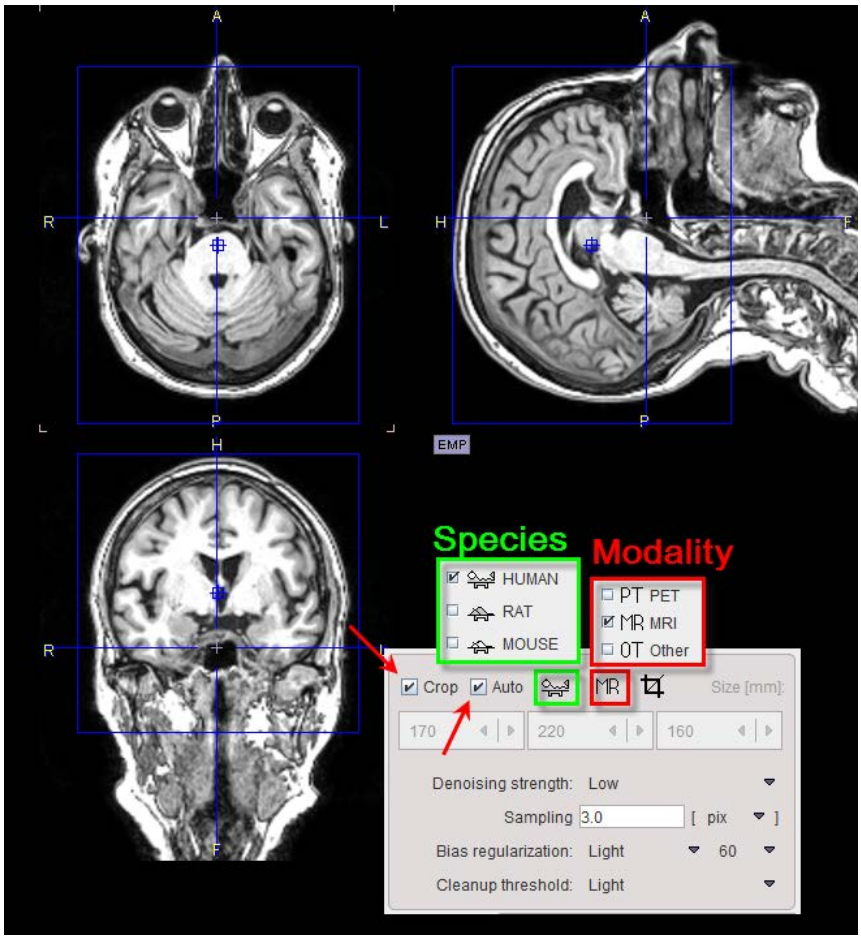


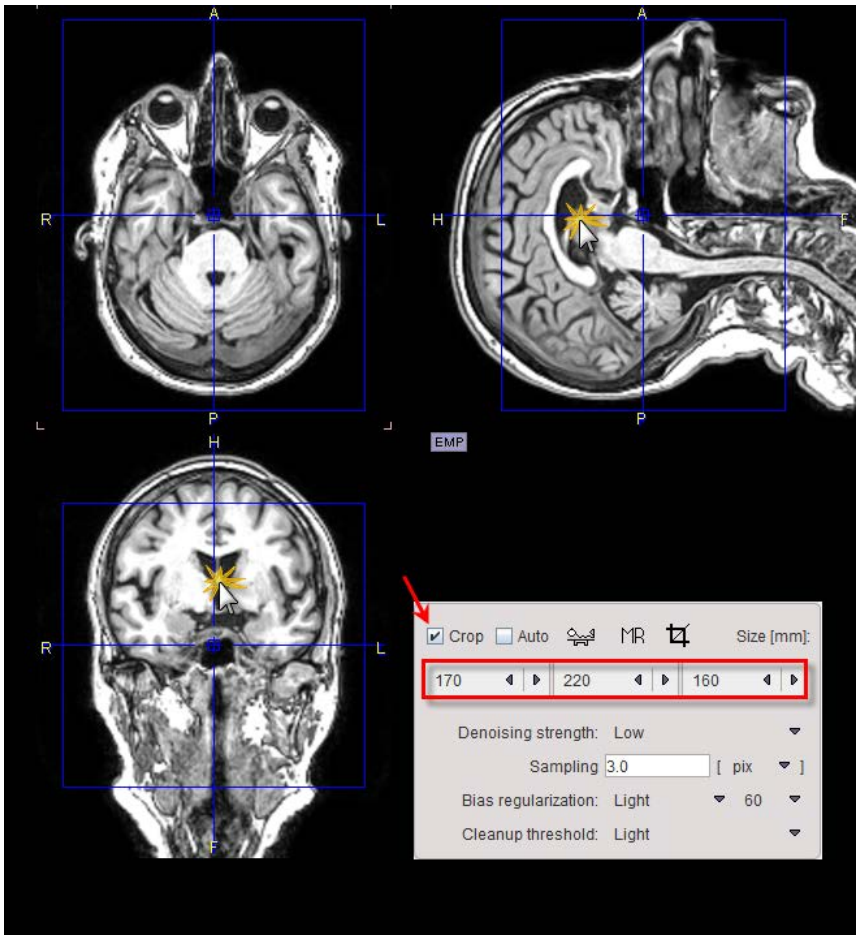
Image Cropping

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

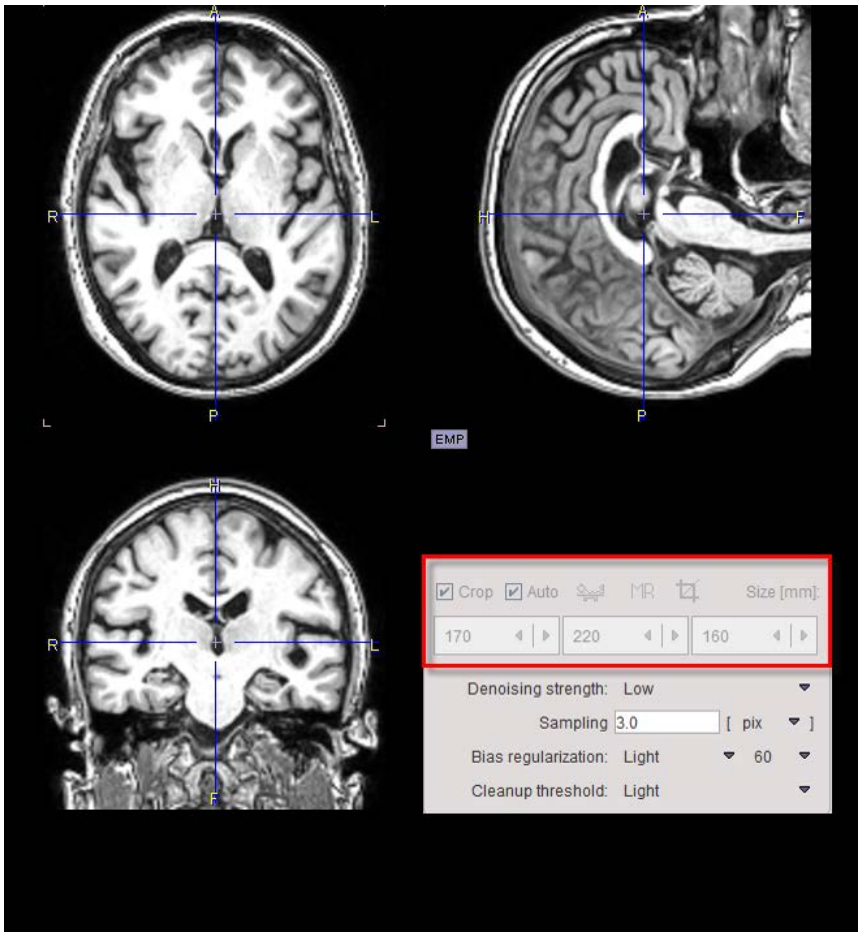


To enable the auto cropping option, please check the **Crop** and the **Auto** boxes. Consequently, blue rectangles are overlaid on the images indicating the extent of the cropping box. Based on the species and modality settings, the program is performing automatically a deformable matching to the corresponding 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 case the automatic procedure fails, the cropping box size and placement can be adjusted manually. To this end only the **Crop** box need to be checked as illustrated in the capture below. To move the center of the box, please click into the image. To change the box size in the three directions, use the corresponding selections below the **MR** and **Species** selection.

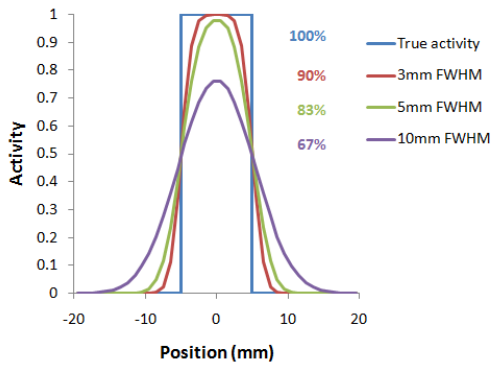


Once the cropping box is properly placed, activate the **Crop** button to perform the (irreversible) image cropping. If the cropping is not done, a confirmation message will appear when progressing to the next stage. After performing a cropping operation, the function is blocked. In order to apply a different crop box the data has to be loaded again.



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.

Partial-volume effects are complex: 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.

VOI Based Partial-Volume Correction (GTM Method)

The Geometric Transfer Matrix (GTM) method according to Rousset et al. [1] restricts partial volume correction to the signal of the true objects which are constituted by VOIs. The relation of measured PET values (affected by the partial-volume effect) to the true PET values is given by the matrix equation below

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

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.

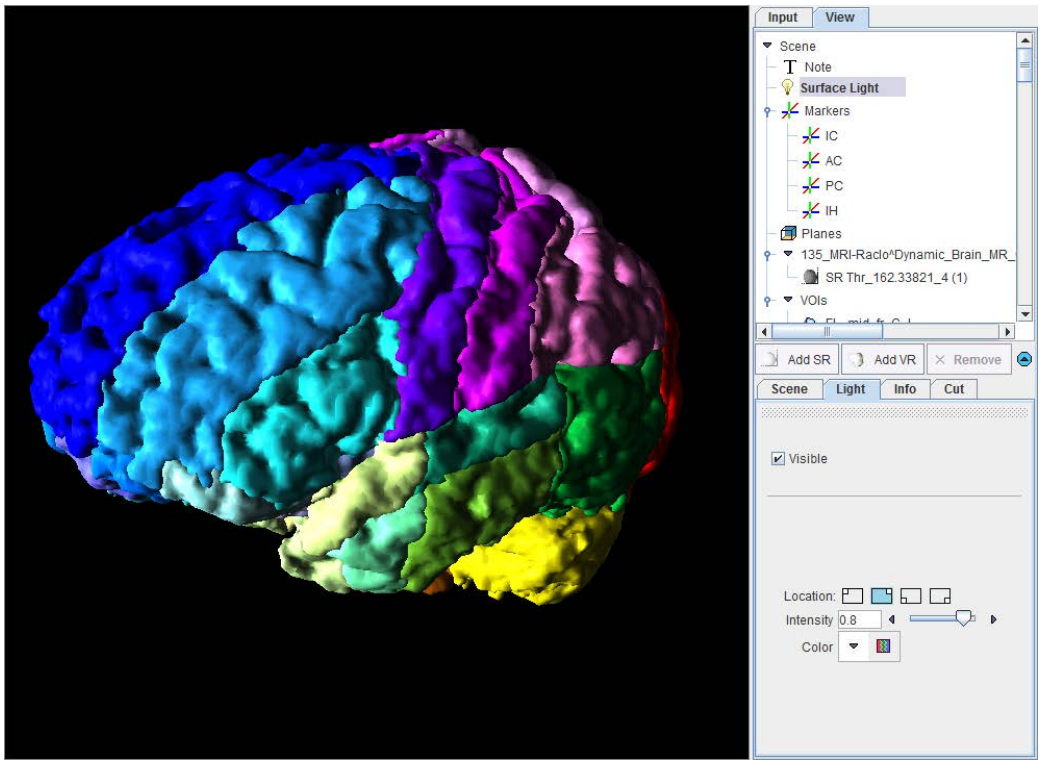
The GTM equation above represents a system of linear equations. Once the weights have been calculated, the system can be solved for the true values C_{true} by matrix inversion. It has been shown [4] that this algorithm is robust to noise propagation during the correction process.

LMA Variant of GTM Method

The LMA (Local Means Analysis) GTM method [2] 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.

3D Rendering of Brain VOIs

Once a set of brain VOIs has been calculated it can be visualized in the 3D tool (option) by activating the button in the lateral taskbar. Please refer to the *PMOD 3D Rendering Tool Users Guide* for information about the operation of this tool.



Brain VOIs Based on Probabilistic Atlas

The functionality of this module is based on the N30R83 maximum probability atlas of brain structures [3,4], which was officially licensed from the Imperial College in London, UK. 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 calculating regional statistics or Time-Activity Curves (TAC), which may optionally be corrected for the partial-volume effect.

Atlas Methodology

The PNEURO tool includes two human brain atlases for the generation of standard brain VOIs, the N30R83 maximum probability atlas, and the single-subject AAL atlas. Optional user-defined atlases are also supported.

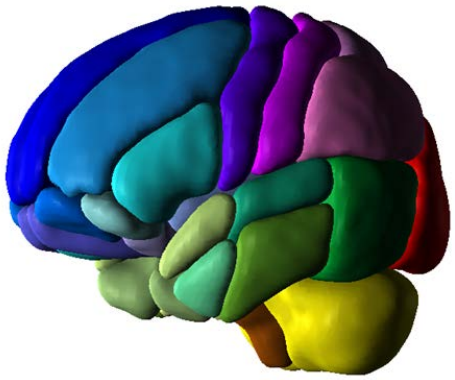
N30R83 Maximum Probability Atlas

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.

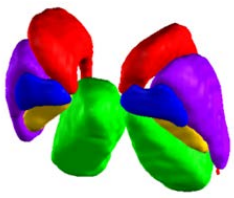
Brief summary of the methodology used to construct the N30R83 atlas:

- 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.



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	Hippocampus
3; 4	Amygdala
5; 6	Anterior temporal lobe, medial part
7; 8	anterior temporal lobe, lateral part
9; 10	Parahippocampal and ambient gyri
11; 12	Superior temporal gyrus, posterior part
13; 14	Middle and inferior temporal gyrus
15; 16	Fusiform gyrus
30; 31	Posterior temporal lobe
82; 83	Superior temporal gyrus, anterior part

Posterior Fossa

17; 18	Cerebellum
19	Brainstem

Insula and Cingulate Gyri

20; 21	Insula
24; 25	Cingulate gyrus (gyrus cinguli), anterior part
26; 27	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

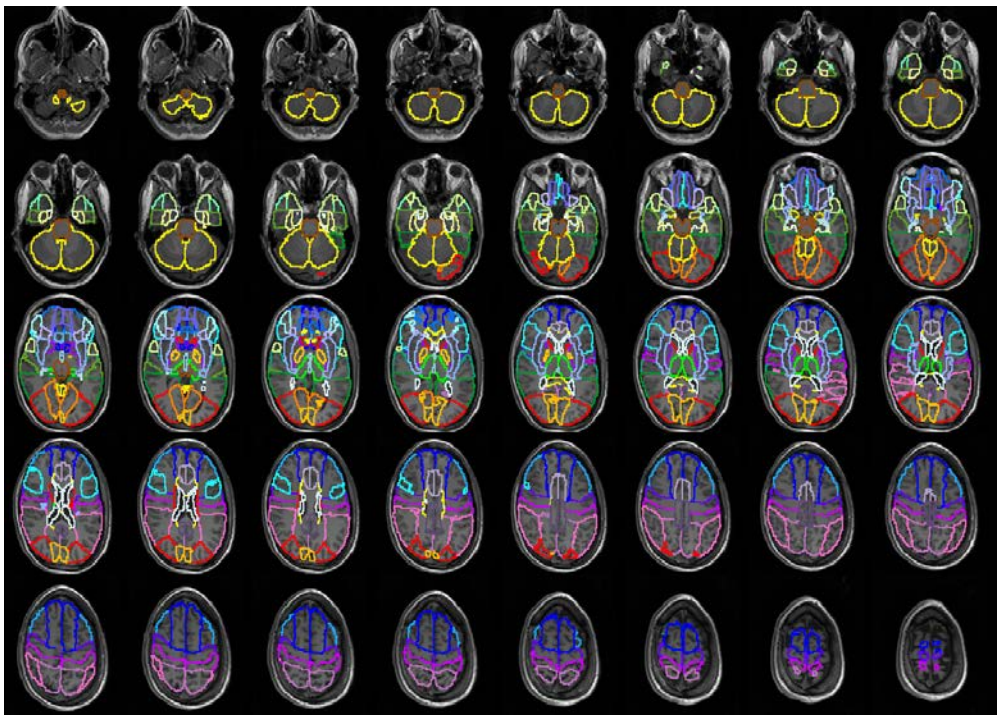
Parietal Lobe

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 patient 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.

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.

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

37; 38	Hippocampus (HIP)
39; 40	Parahippocampus (PHIP)
41; 42	Amygdala (AMYG)
55; 56	Fusiform gyrus (FUSI)
79; 80	Heschl gyrus (HES)
81;82	Superior temporal gyrus (T1)
83; 84	Temporal pole: superior temporal gyrus (T1P)
85; 86	Middle temporal gyrus (T2)
87; 88	Temporal pole: middle temporal gyrus (T2P)
89: 90	Inferior temporal gyrus (T3)

Posterior Fossa

91; 92	Cerebellum crus 1
93; 94	Cerebellum crus 2
95; 96	Cerebellum 3
97; 98	Cerebellum 4 5
99; 100	Cerebellum 6
101; 102	Cerebellum 7
103; 104	Cerebellum 8
105; 106	Cerebellum 9
107; 108	Cerebellum 10
109	Vermis 12
110	Vermis 3
111	Vermis 4 5
112	Vermis 6
113	Vermis 7
114	Vermis 8
115	Vermis 9
116	Vermis 10
117	Cerebellar white matter
118	Medulla
119	Midbrain
120	Pons

Insula and Cingulate Gyri

29; 30	Insula (IN)
31; 32	Cingulate gyrus, anterior part (ACIN)
33; 34	Cingulate gyrus, mid part (MCIN)
35; 36	Cingulate gyurs, posterior part (PCIN)

Frontal Lobe

1; 2	Precentral gyrus (PRE)
3; 4	Superior frontal gyrus, dorsolateral (F1)
5; 6	Superior frontal gyrus, orbital (F1O)
7; 8	Middle frontal gyrus (F2)
9; 10	Middle frontal gyrus, orbital (F2O)
11; 12	Inferior frontal gyrus, opercular (F3OP)
13; 14	Inferior frontal gyrus, triangular (F3T)
15; 16	Inferior frontal gyrus, orbital (F3O)
17; 18	Rolandic operculum (RO)
19; 20	Supplementary motor area (SMA)
21; 22	Olfactory cortex (OC)
23; 24	Superior frontal gyrus, medial (F1M)
25; 26	Superior frontal gyrus, medial orbital (F1MO)
27; 28	Gyrus rectus (GR)
69; 70	Paracentral lobule (PCL)

Occipital Lobe	
43; 44	Calcarine fissure and surrounding cortex (V1)
45; 46	Cuneus (Q)
47; 48	Lingual gyrus (LING)
49; 50	Superior occipital lobe (O1)
51; 52	Middle occipital lobe (O2)
53; 54	Inferior occipital lobe (O3)

Parietal Lobe	
57; 58	Postcentral gyrus (POST)
59; 60	Superior parietal gyrus (P1)
61; 62	Inferior parietal gyrus (P2)
63; 64	Supramarginal gyrus (SMG)
65; 66	Angular gyrus (AG)
67; 68	Precuneus (PQ)

Central Structures	
53; 54	Caudate nucleus (CAU)
55; 56	Putamen (PUT)
57; 58	Pallidum (PAL)
59; 60	Thalamus (THA)

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	Temporal, superior, mid, inferior, poles (T1, T1A, T2, T2A, T3)
29; 30	Amygdala (AMYG)
27; 28	Hippocampus and parahippocampus (HIP, PHIP
39; 40	Fusiform gyrus (FUSI)
61; 62	Heschl gyrus (HES)

Posterior Fossa	
67	Vermis
68; 69	Cerebellum crus
70; 71	Cerebellum
72	Cerebellar white matter
73	Medulla
74	Midbrain
75	Pons

Insula and Cingulate Gyri	
19; 20	Insula (IN)
21; 22	Cingulate gyrus, anterior part (ACIN)
23; 24	Cingulate gyrus, mid part (MCIN)
25; 26	Cingulate gyurs, posterior part (PCIN)

Frontal Lobe	
1; 2	Precentral gyrus (PRE)
3; 4	Rolandic operculum (RO)
5; 6	Supplementary motor area (SMA)
7; 8	Olfactory cortex (OC)
11; 12	Superior frontal gyrus (F1, F1O, F1M)
13; 14	Middle frontal gyrus (F2, F2O, FMO)
15; 16	Inferior frontal gyrus (F3OP, F3T, F3O)
17; 8	Gyrus rectus (GR)
51; 52	Paracentral lobule (PCL)

Occipital Lobe	
31; 32	Calcarine fissure and surrounding cortex (V1)
33; 34	Cuneus (Q)
35; 36	Lingual gyrus (LING)
37; 38	Lateral remainder of occipital lobe (O1, O2, O3)

Parietal Lobe	
41; 42	Postcentral gyrus (POST)
45; 46	Supramarginal gyrus (SMG)
47; 48	Angular gyrus (AG)
49; 50	Precuneus (PQ)
63; 64	Parietal, superior and inferior (P1, P2)

Central Structures	
53; 54	Caudate nucleus (CAU)
55; 56	Putamen (PUT)
57; 58	Pallidum (PAL)
59; 60	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.

User-Defined Atlases

It is possible for users to prepare their own VOI atlases for use in PNEURO. Note, however, that they are only applicable for **Maximum Probability Atlas** approach, not for the **Brain Parcellation** approach.

The following sets of data are need to be prepared for a user-defined atlas:

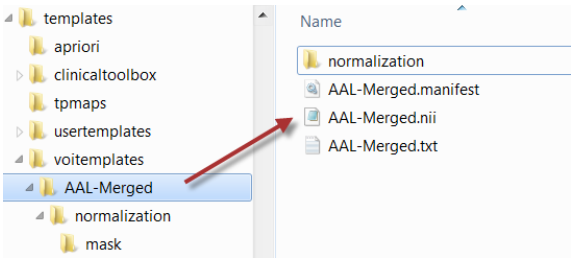
1) *Atlas image:* Image which encodes the atlas VOIs as numeric labels.

2) *Label list:* Text file mapping the label values to VOI names.

3) *Manifest: T*ext file for the defining of the species type

4) *Normalization* files for calculating the transformation between the subject anatomy and atlas anatomy.

This information has to be organized in a subdirectory of *resources/templates/voitemplates* in a particular way as illustrated below.



The atlas name (e.g. **AAL-Merged**) has to be used as the name of the sub-directory, the atlas image (**AAL-Merged.nii**), the label list (**AAL-Merged.txt**), and the manifest (**AAL-Merged.manifest**).

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

The label list text file has the minimal form:

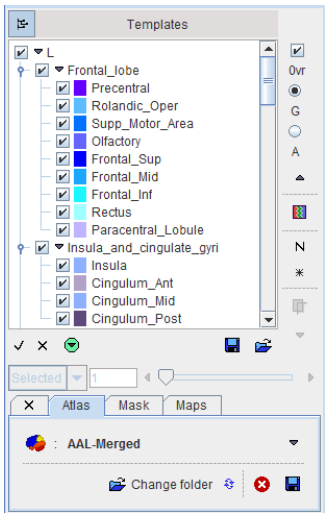
```
name1   outlined_name1  label_value1
name2   outlined_name2  label_value2
```

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 **L** or right **R** hemisphere. 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 belong the **Precentral_l** VOI, pixels with value **2** to **Precentral_l**, 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.

Name	Outlined	Label	Color	Tooltip
Precentral[Frontal_lobe[L]]	Precentral_l	1	(100,0,255)	Precentral gyrus left (PRE)
Precentral[Frontal_lobe[R]]	Precentral_r	2	(100,0,255)	Precentral gyrus right (PRE)
Rolandic_Oper[Frontal_lobe[L]]	Rolandic_Oper_l	3	(93,188,255)	Rolandic operculum left (RO)
Rolandic_Oper[Frontal_lobe[R]]	Rolandic_Oper_r	4	(93,188,255)	Rolandic operculum right (RO)
Supp_Motor_Area[Frontal_lobe[L]]	Supp_Motor_Area	5	(0,114,255)	Supplementary motor area left (SMA)
Supp_Motor_Area[Frontal_lobe[R]]	Supp_Motor_Area	6	(0,114,255)	Supplementary motor area right (SMA)
Olfactory[Frontal_lobe[L]]	Olfactory_l	7	(100,100,255)	Olfactory cortex left (OC)
Olfactory[Frontal_lobe[R]]	Olfactory_r	8	(100,100,255)	Olfactory cortex right (OC)
Frontal_Sup[Frontal_lobe[L]]	Frontal_Sup_l	11	(0,0,255)	Superior frontal gyrus left (F1, F1O, F1M)
Frontal_Sup[Frontal_lobe[R]]	Frontal_Sup_r	12	(0,0,255)	Superior frontal gyrus right (F1, F1O, F1M)

The corresponding atlas VOI structure is illustrated below.



There are additional options to be added in the columns:

- **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 used in sulci deformation.

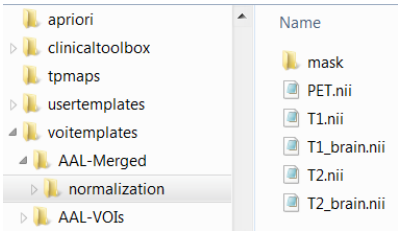
Example as shown in Excel:

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

Normalization Files

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.

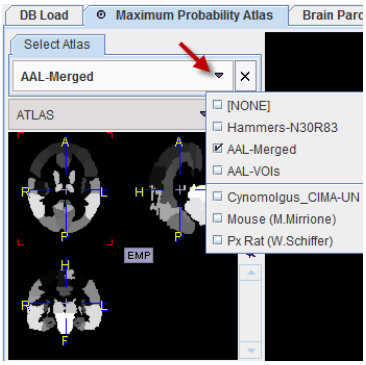
Appropriate template images need to be copied to a **normalization** sub-folder. In the example below **normalization** contains T1 and T2 MR templates with and without skull, as well as a PET template. All of these templates show the anatomy in the MNI space in which the AAL VOIs were defined.



The 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 **brainmask.nii** containing 1 for all relevant pixels and 0 for all others.

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 all VOI templates in the sub-directory *resources/templates/voitemplates* are listed.

Maximum Probability Atlas Implementation in PNEURO

In PNEURO the use of the N30R83 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.

5) Rigid matching of the PET image to the MR image and interactive visual assessment of the alignment by the user.

6) Spatial normalization of the MR image to the MNI T_1 template and interactive visual assessment of the alignment by the user.

7) Transformation of the label atlas to the MR space and display of the result as an overlay on the MR image.

8) Intersection of the cortical structures with the gray matter probability map at a user-defined probability level.

9) 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.

10) 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.

11) Dynamic PET case: The resulting TACs can directly be transferred to the kinetic modeling tool.

Note that instead of defining the VOIs in the MR space as described above, they may be defined in the MNI 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 patient 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 which has been transformed to the patient space. The user can define the probability level used for masking, and whether the central 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.

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 MNI 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 MNI 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 patient and the template. Optionally, a user-defined normalization template may be used instead of the standard MNI PET template.

4) The label atlas is transformed to the patient 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 which has been transformed to the patient 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.

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 MNI 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 MNI 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.

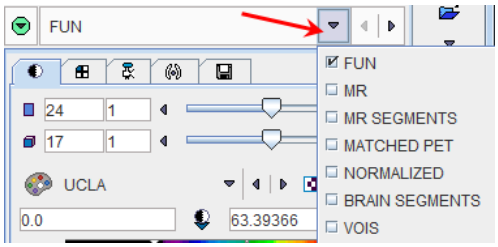
7) Application of the VOIs for calculating statistics. In the absence of PET the statistics are reduced to the calculation of the VOI volume.

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*** (on page 47) if any questions arise.

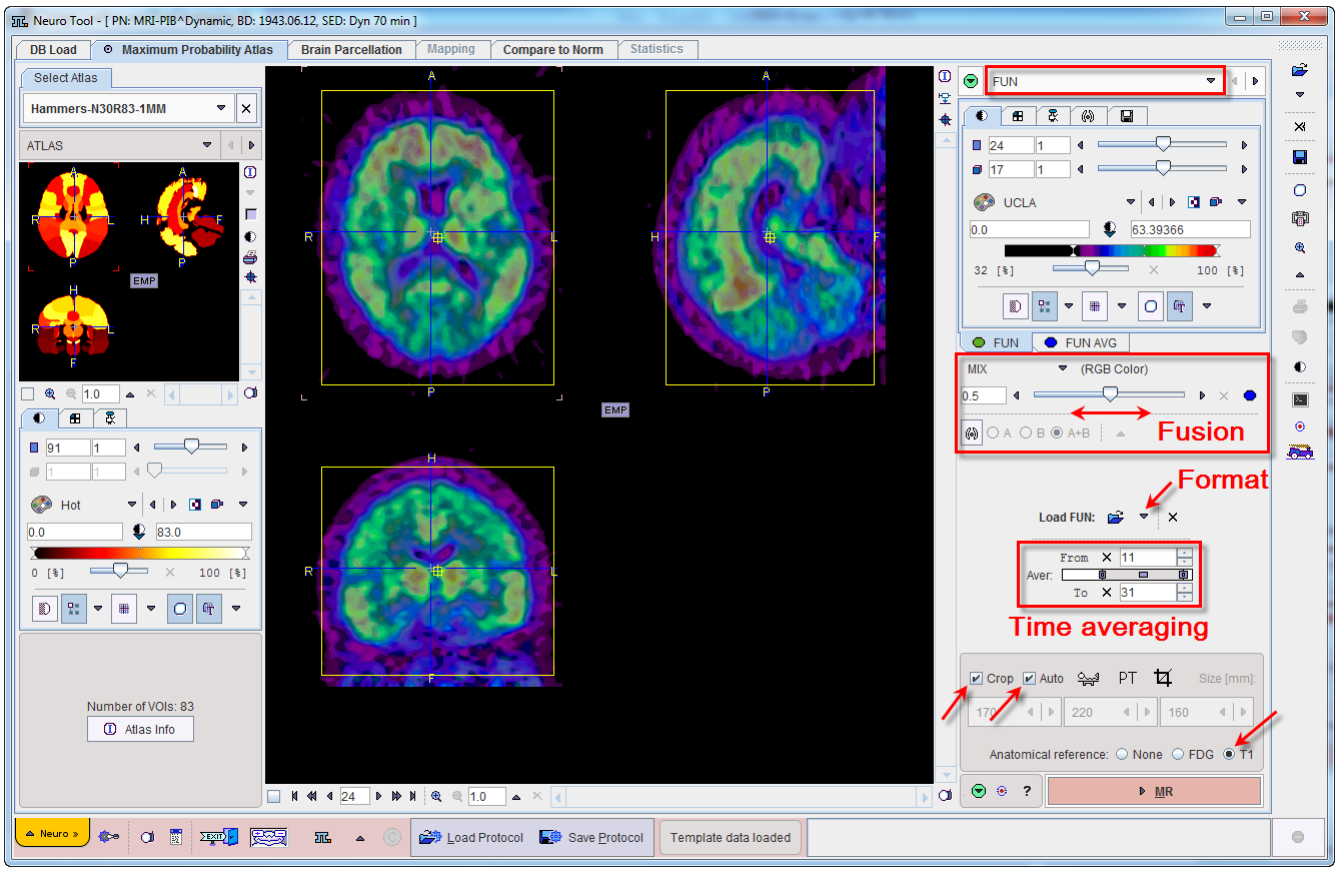
Workflow for Studies with PET and MRI

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



PET Image Loading and Time Averaging

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



The **Load FUN** 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 it 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 **FUN AVG** tab. The averaging range can be defined by the **From** and **To**

number fields, or dragging the range indicators in the **Aver** 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

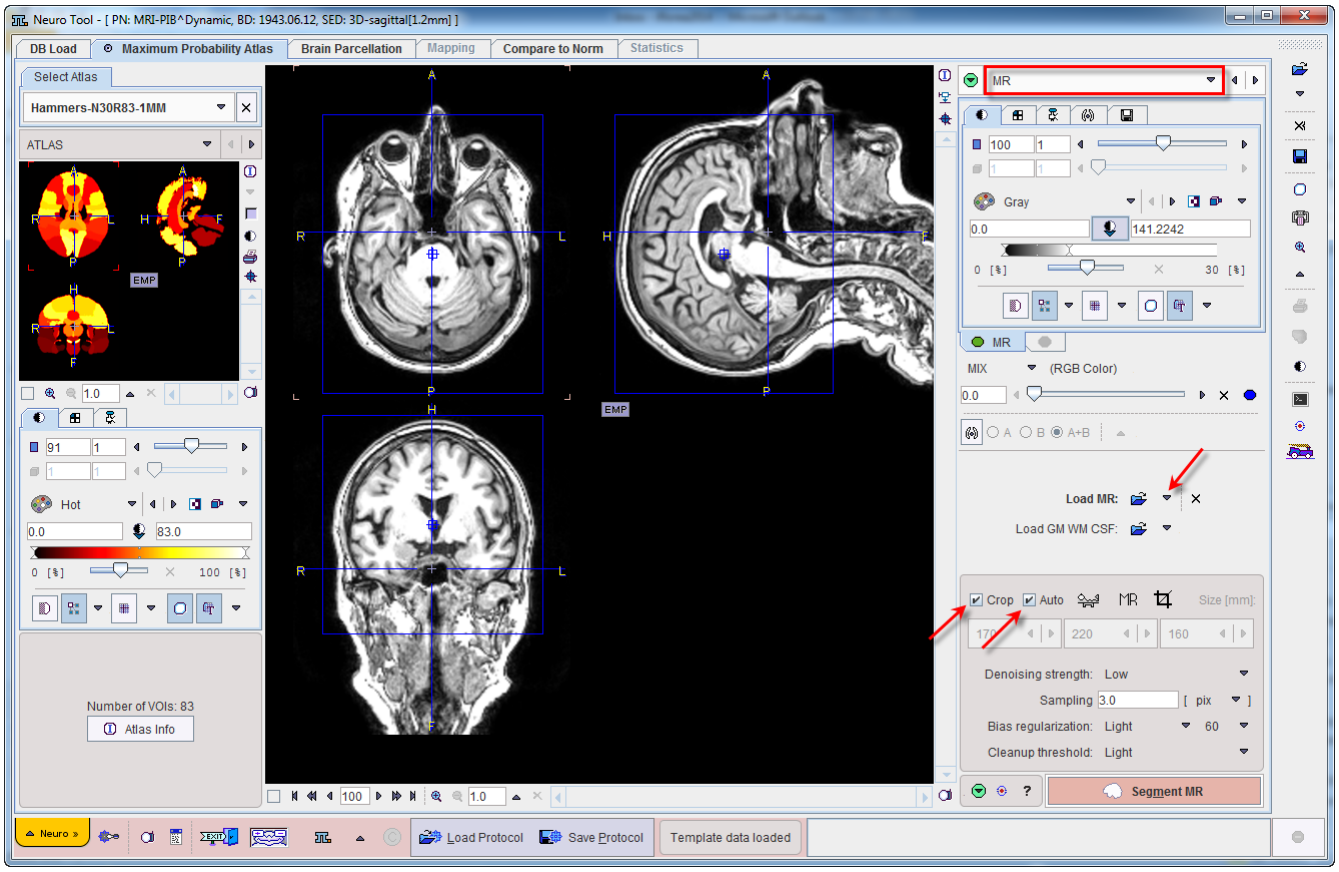
If the PET field-of-view is larger than the brain, the data set should be reduced in order to save RAM and optimize processing. This can be achieved automatically enabling the **Crop** box and the **Auto** box. A yelow crop volume appears on the image while the optimal size and placement is calculated in the background based on species and modality selection.

In case the automatic procedure fails, the cropping box size and location can be adjusted manually. Make sure only the **Crop** box is enabled. Place the yellow crop volume indication by clicking at the brain center so that the brain is fully enclosed. The edge size in **[mm]** can be adjusted for each direction by selecting the size in the corresponding list. The **Crop** 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.

To continue loading the MR images please make sure the **T1** radio button is selected and activate the **MR** action button in the lower right.

MR Image Loading and Segmentation

The **MR** page allows loading the T_1-weighted brain MR image of the same patient using the **Load MR** button.



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 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 four 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.
Sampling	Density of pixels considered in the calculation.

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.

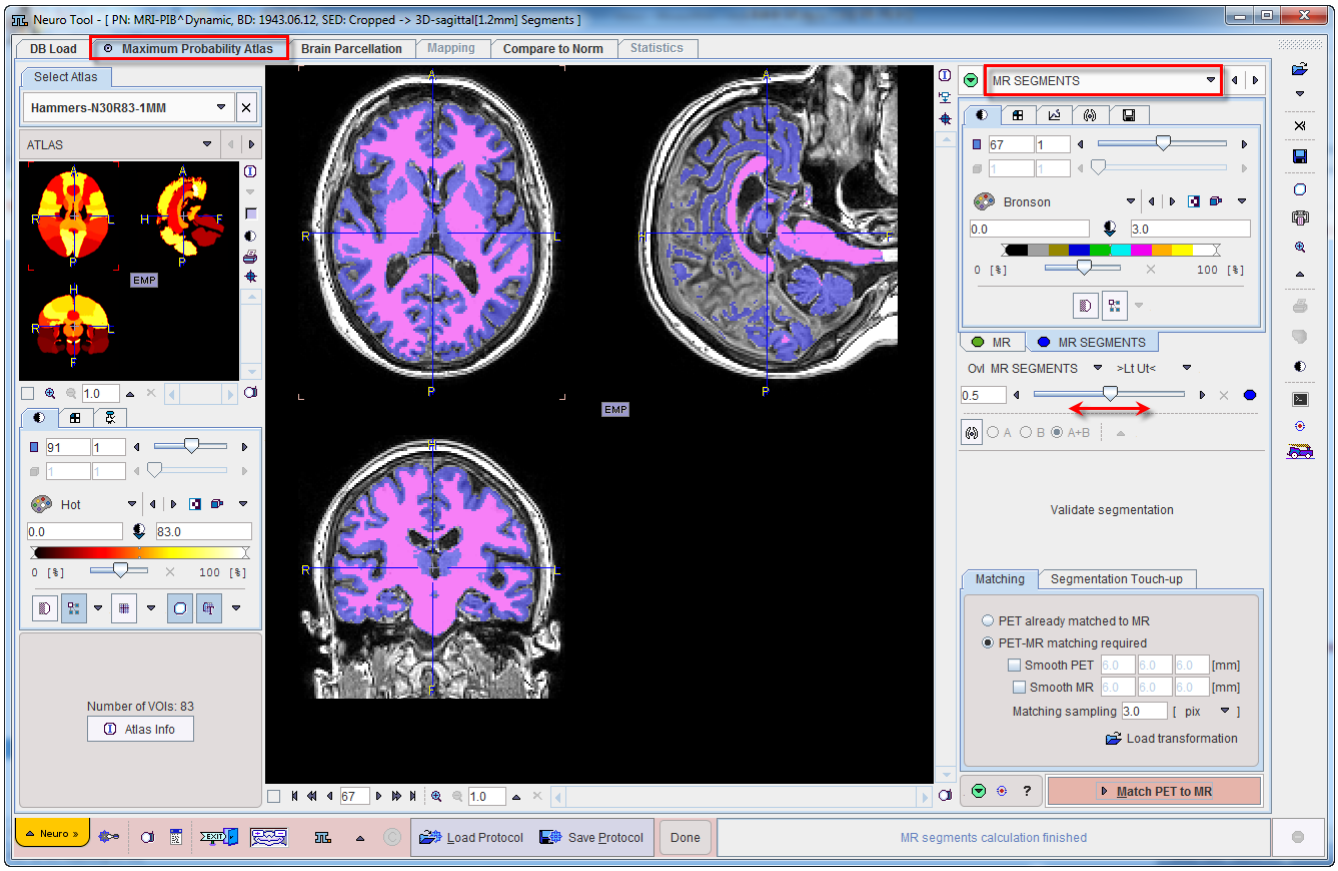
Cleanup threshold Procedure for rectifying the segmentation along the boundaries.

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 MR** action button. Note that the denoising and segmentation process may take several minutes.

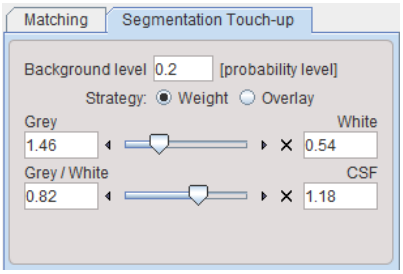
PET to MR Matching

The result of the segmentation is shown as a fusion of the tissue segment map with the MR image on the **MR SEGMENTS** page. Note that the **MR 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 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.

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 MR** again.

PET to MR Matching

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

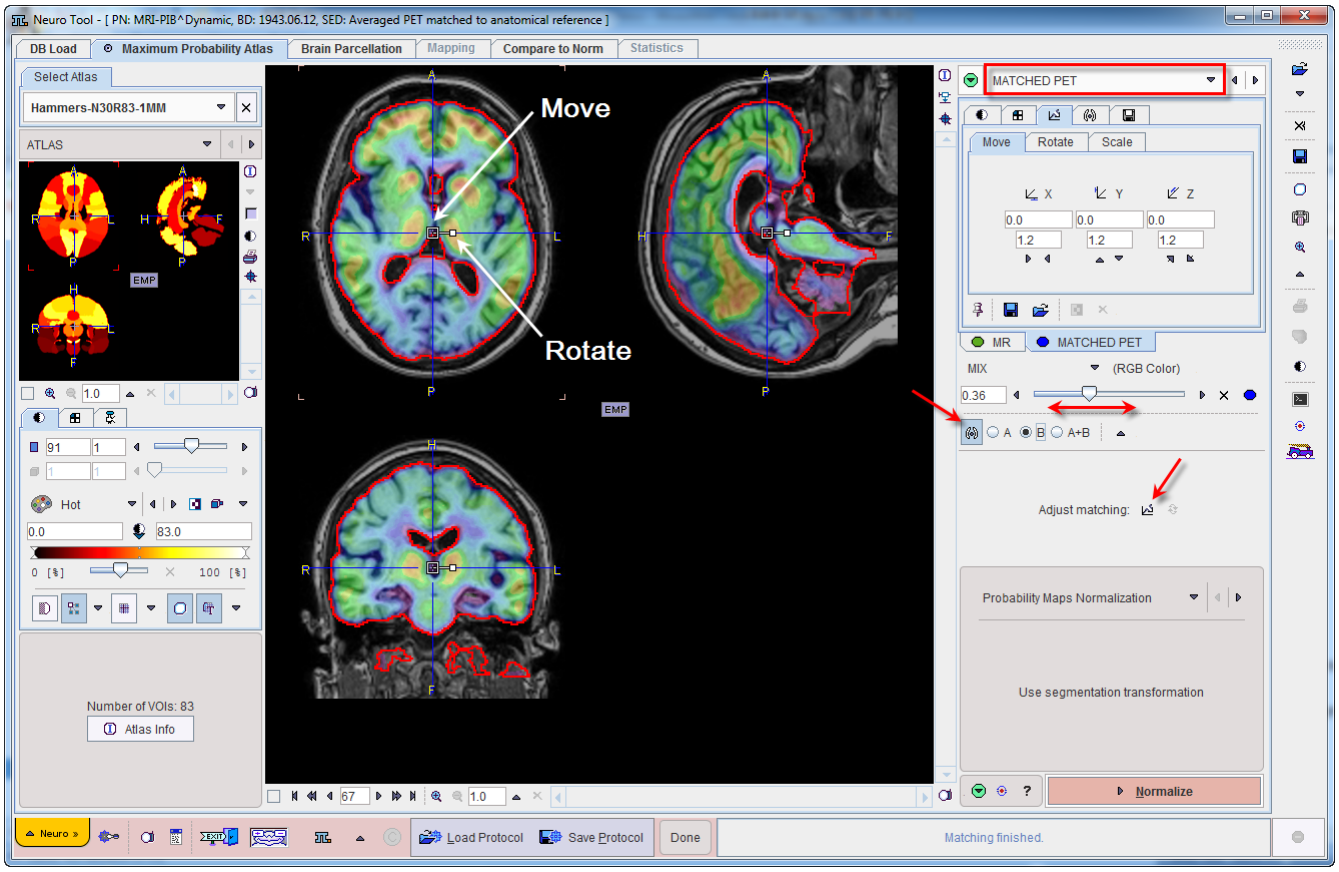
Otherwise, PNEURO will apply a rigid matching procedure based on the Normalized Mutual Information criterion with **Matching sampling** as the main parameter. Optionally, if the result is not satisfactory, the PET and/or the MR image may be smoothed.

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

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:

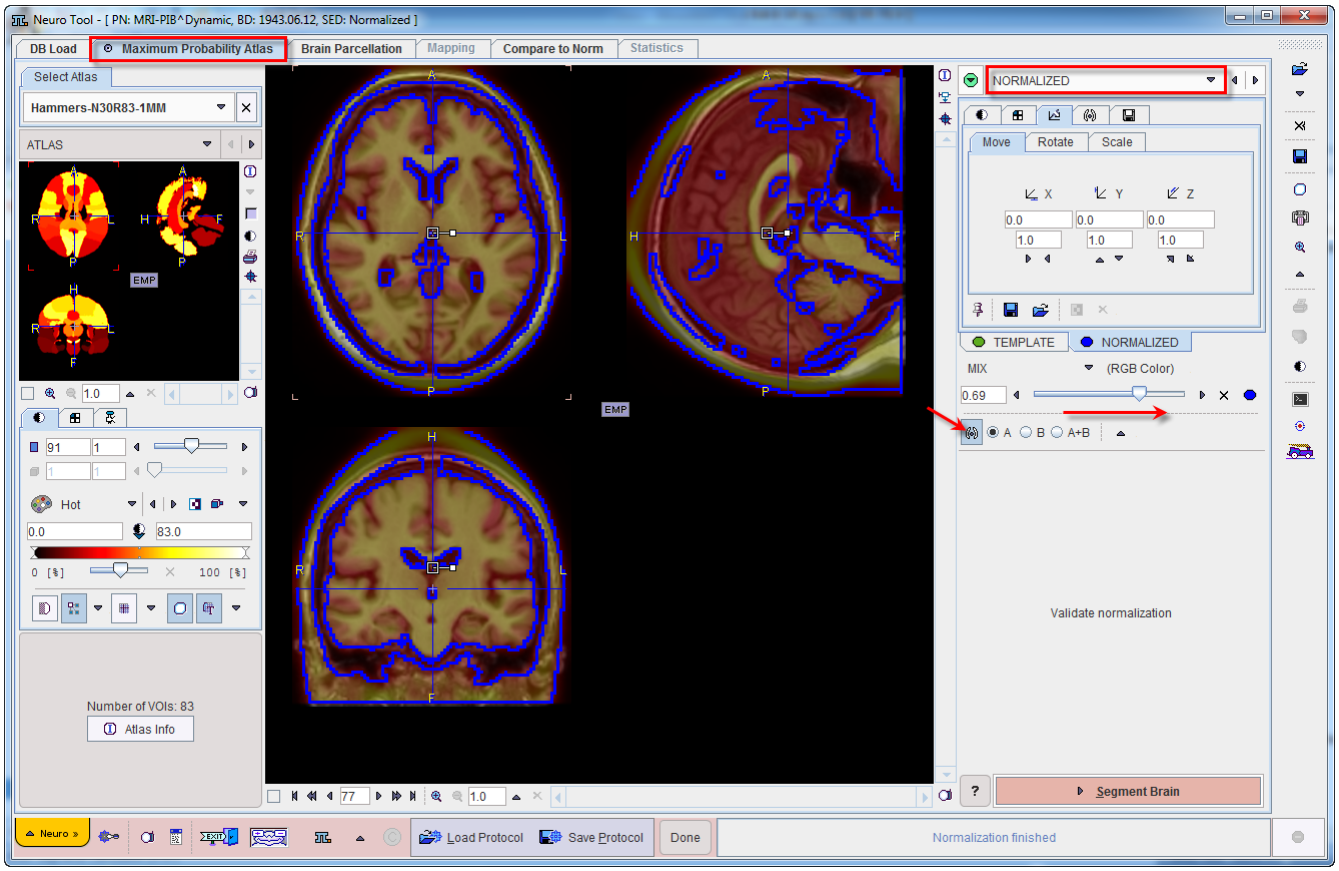
1) Return to the previous page, change the sampling and smoothing parameters and try the automatic matching again, or

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.

Please activate the **Normalize** action button to proceed. Because the normalization has already been calculated in the process of the GM/WM/CSF segmentation, it is simply applied to the data at this stage.

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 MNI 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 MNI space.

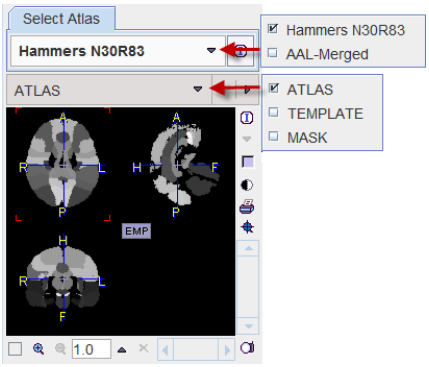
As all the transformations can be inverted, the MNI space can also be mapped to PET and the MR image space. Consequently, the brain structures which are defined in the MNI space can be mapped to the PET and MR patient space and shown in the overlay.

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 in the MNI space. Currently the Automatic Anatomic Labeling (AAL) atlas is available as an

alternative definition. 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. 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.

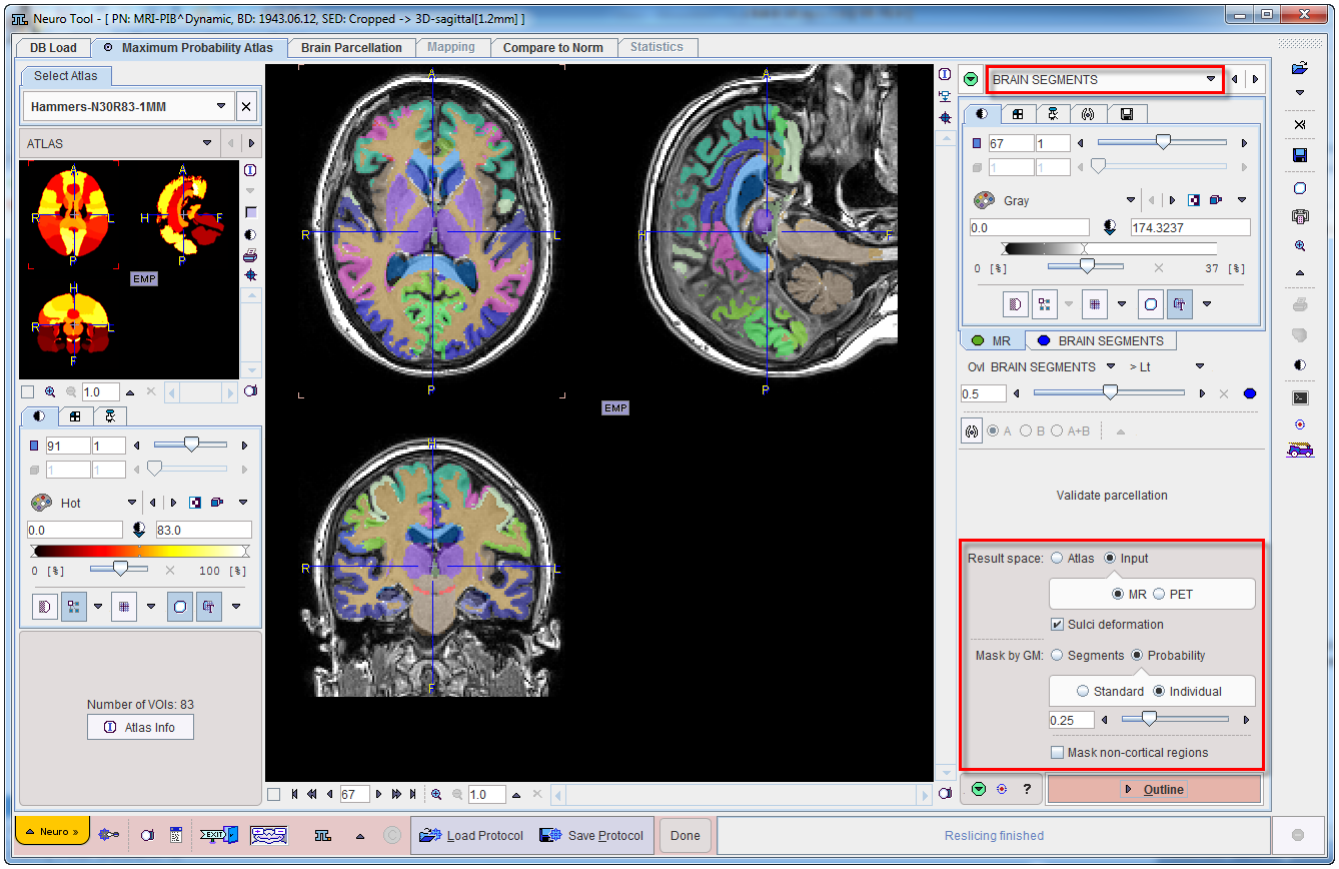


To hide the atlas selection window please activate the show/hide button again.

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

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.

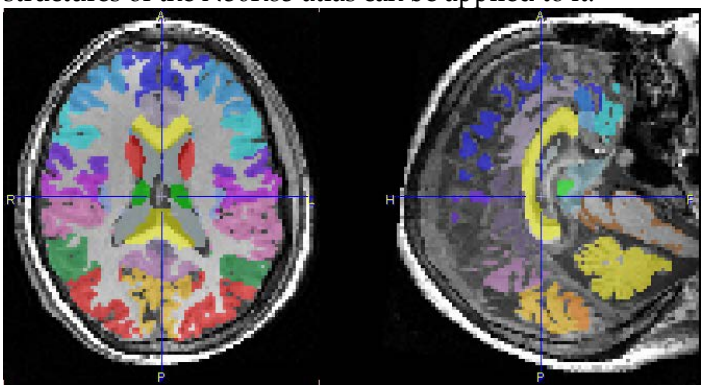


Result Space

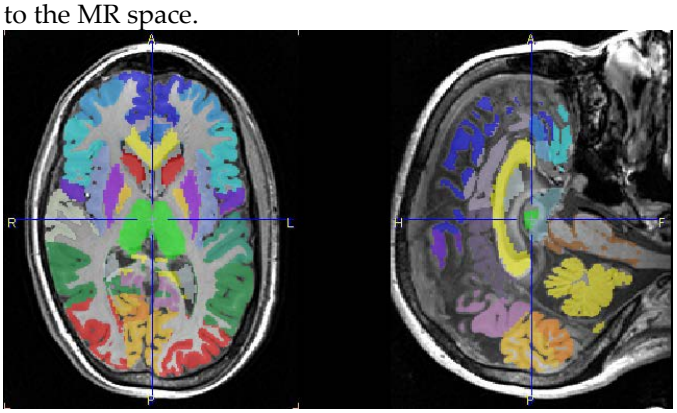
There are three different options to evaluate the PET image, which can be configured using the **Result space** radio button. The information visualized on the page is updated as soon as the configuration is changed. The image display shows the MR image transformed to the selected result space together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

The **Result space** options are:

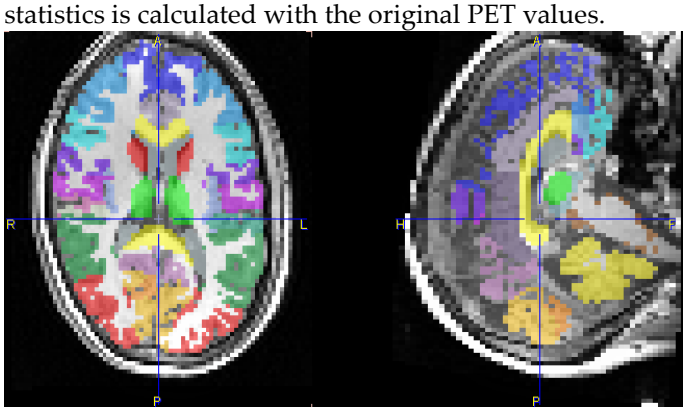
1) **Atlas:** With this setting the PET image is transformed to the MNI space and the original structures of the N30R83 atlas can be applied to it.



2) **Input, MR:** With this setting the PET image and the N30R83 structures are transformed to the MR space.



Input, PET: With this setting the N30R83 structures are mapped to the PET space and statistics is calculated with the original PET values.

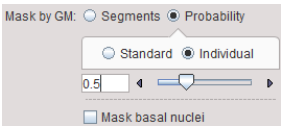


Sulci Deformation

The **Sulci deformation** option instructs the program to perform an anatomical optimization of the VOIs obtained from the maximum probability map approach. Based on the MR image, the VOI boundaries are adjusted such that the border should essentially follow the sulci bottoms.

Intersection of N30R83 Structures with Gray Matter

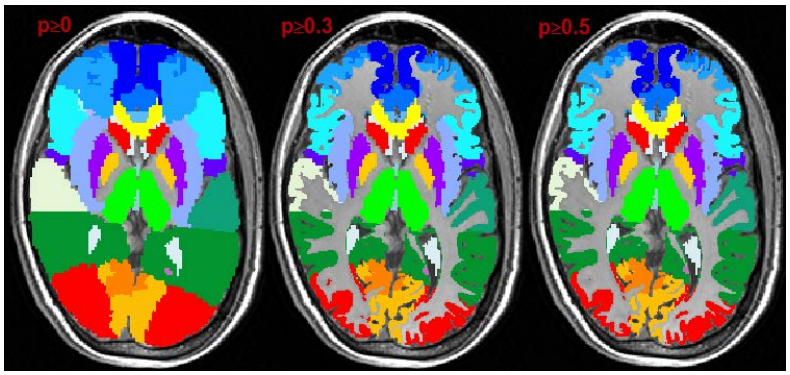
The original N30R83 structures cover the whole brain. Users can take advantage of the gray matter probability to restrict the structures to pixels with a high probability of belonging to gray matter. This masking is controlled by the elements in the **Mask by GM** area.



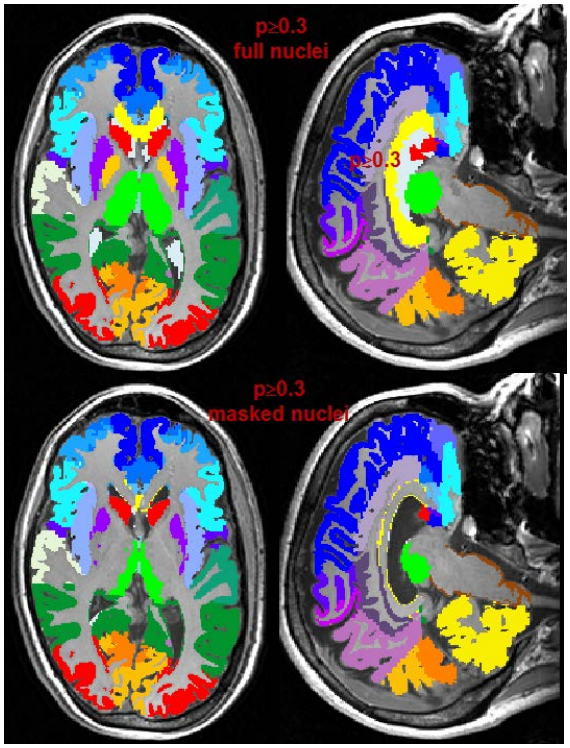
There is a choice between two types of probability information:

- **Segments** means that the binary information in the ***segment label image*** (on page 50) is applied. Here, no thresholding is necessary to obtain a GM mask.
- **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 threshold slider allows defining the lower threshold of the probability map for creating the mask. The higher the threshold value, the thinner the cortical structures become. The illustration below shows individual masking at three increasing probability thresholds. The standard mask will result in broader structures at the same probability threshold.



Note that the central structures are not affected by masking in the example above, because the box **Mask basal nuclei** is not enabled. If the option is enabled, the central structures are also shrinking, as illustrated below. However, because of the low probability levels in that area, the reduction may become too severe. Therefore, masking of the basal nuclei will usually remain disabled.



Intersected VOIs and Partial Volume Correction (PVC)

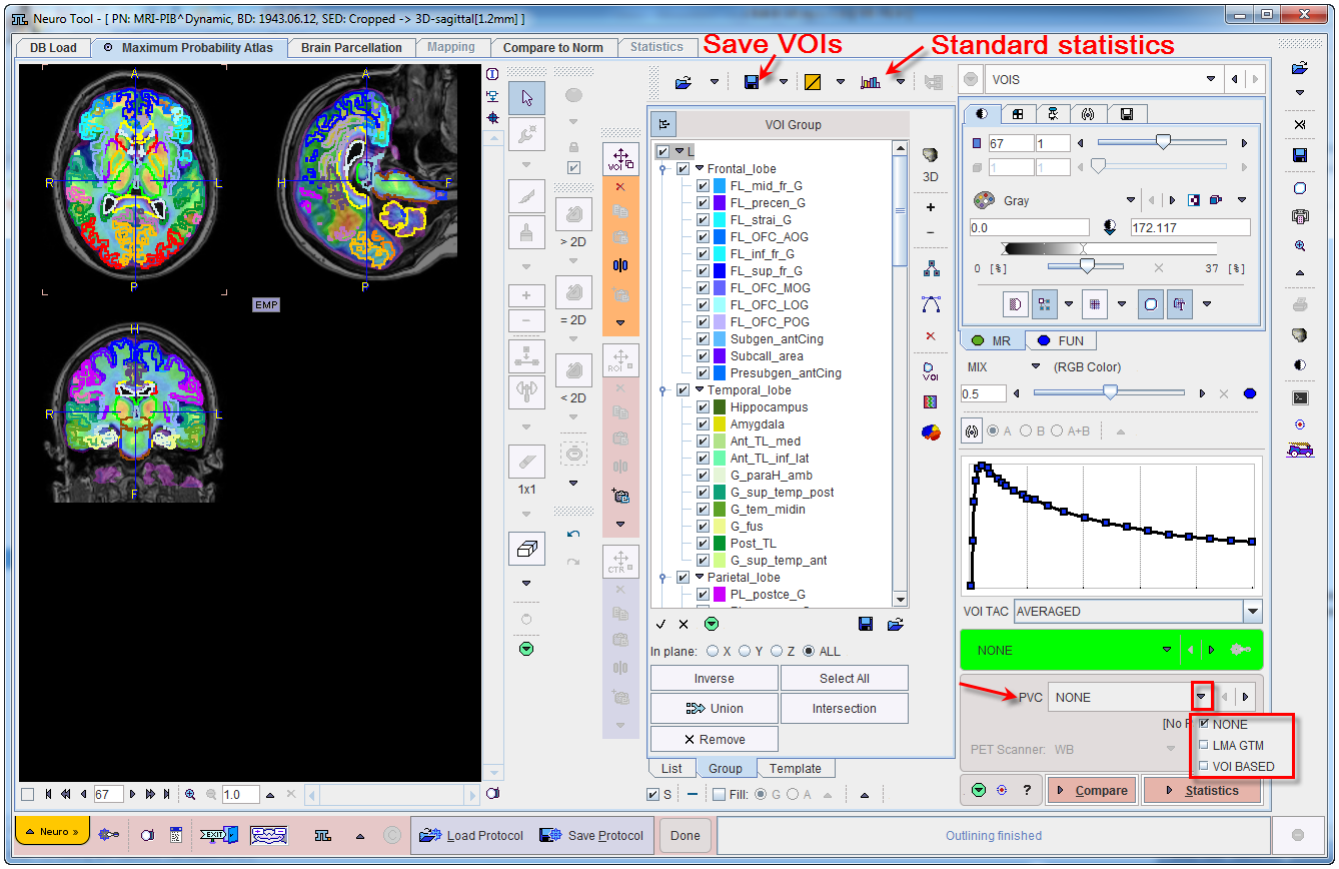
VOI-based PVC correction methods only work properly when all the activity contained in the brain is included in VOIs. Therefore, in the case of VOI masking, PNEURO will automatically create the complementary VOIs, i.e. the VOIs masked with the gray matter pixels below the threshold. These complementary VOIs will be used for the PVC calculation, but will not be visible in the user interface and in the result statistics.

Structure Outlining

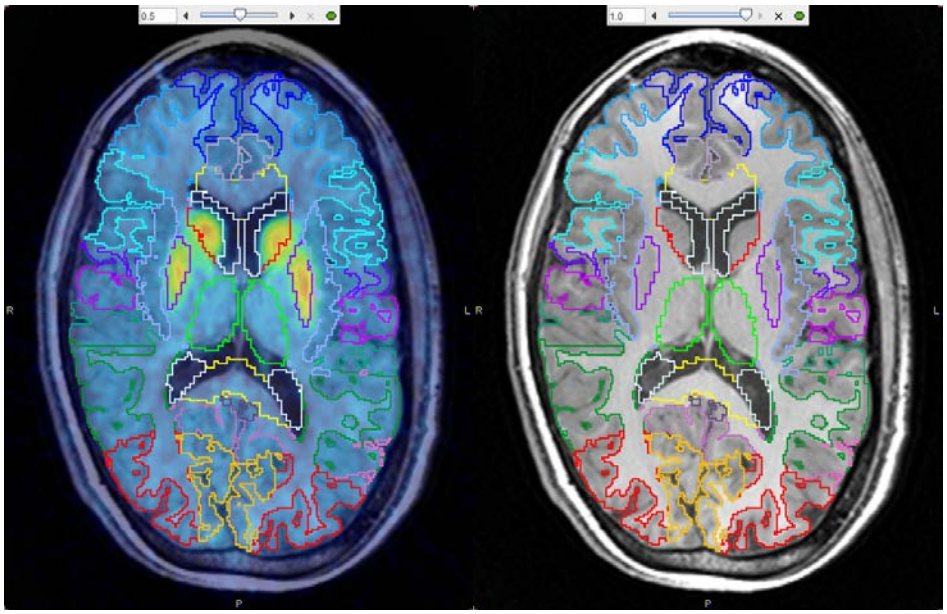
Once the result space and gray matter masking have been specified, the brain structures are fully defined and can be outlined to create contour VOIs. This process is started with the **Outline** action button.

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 and MR only.



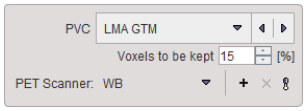
VOI Editing and Selection

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*** (on page 6) only a reduced set of VOI tools may be available.

A subset of the VOIs can easily be selected on the **Group** tab as described ***above*** (on page 21).

Partial-Volume Correction (PVC) Option

The PNEURO tool supports the GTM-based ***partial-volume correction*** (on page 28) (PVC) of the PET signal.



The **PVC** selection has three choices:

- **NONE:** No partial-volume correction is applied (default).
- **LMA GTM:** A variant of the Rousset correction method is applied, whereby only a percentage of the pixels in the inner of the VOI is used for calculating the VOI average. This percentage can be set by the **Voxels to be kept** parameter.
- **VOI BASED:** The original Rousset correction method is applied.

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.

Statistics Calculation

Once the VOIs are acceptable, it is recommended to first save them and then proceed with statistics calculation by the **Statistics** action button. The result (of the selected VOIs only) is shown on the separate ***Statistics*** (on page 106) 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*** (on page 103).

Normal Database Comparison

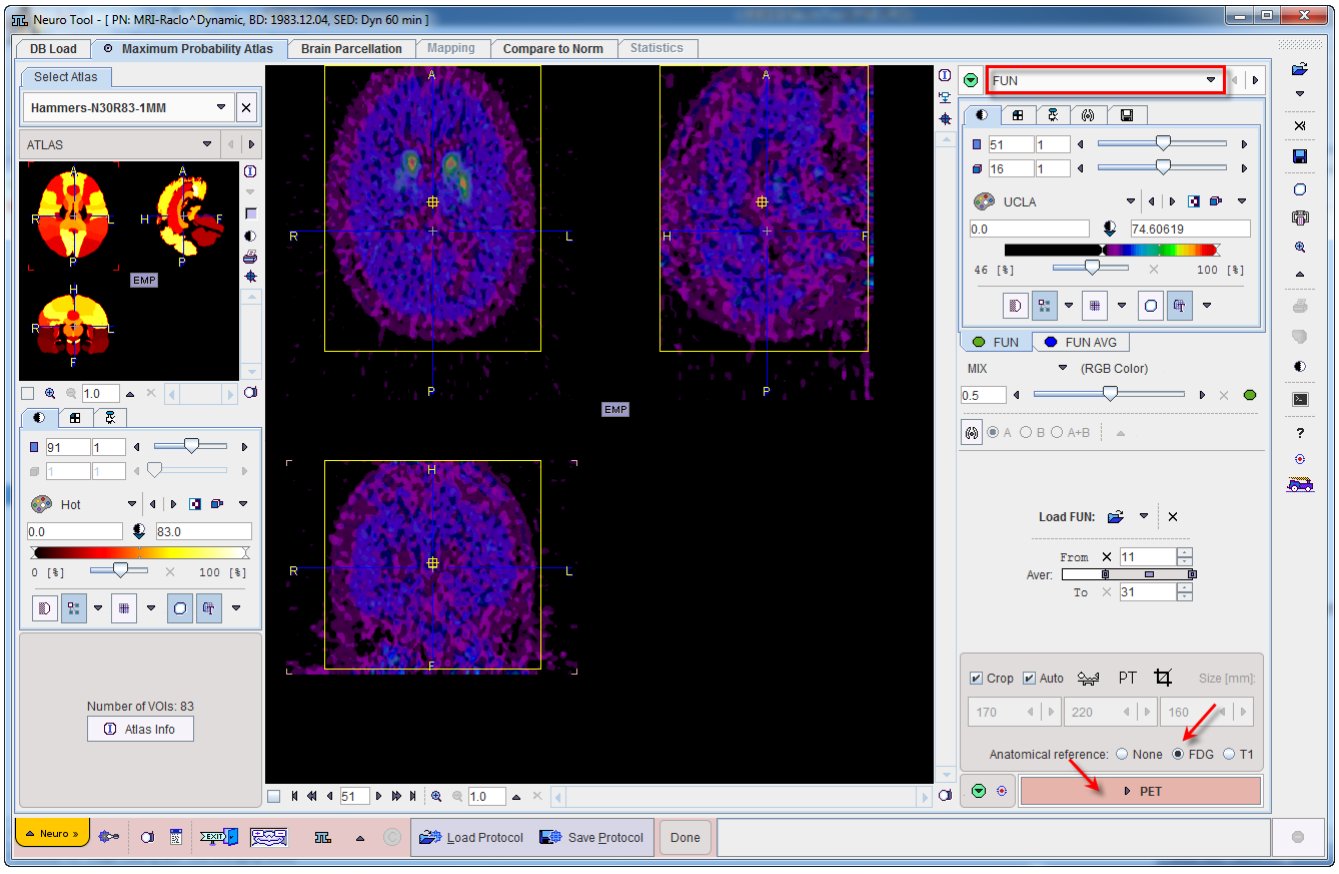
As PNEURO is able to provide the PET images normalized to the atlas space, there is a direct link with the ***Brain Norm Functionality*** (on page 103). The **Compare** button copies the PET images as they appear on the VOIs page to the **Compare To Norm** page where they can be compared against a normal uptake pattern of a normal database. Conveniently, if the two spaces match, there is no need to perform a normalization.

Workflow for Functional and Anatomical PET

In some situations a patient who has undergone a functional PET might also have had a more "anatomical PET" (e.g. FDG). In this case, the "anatomical PET" should be used for the 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 the "anatomical PET" series will be called "FDG PET".

Functional PET Image Loading and Time Averaging

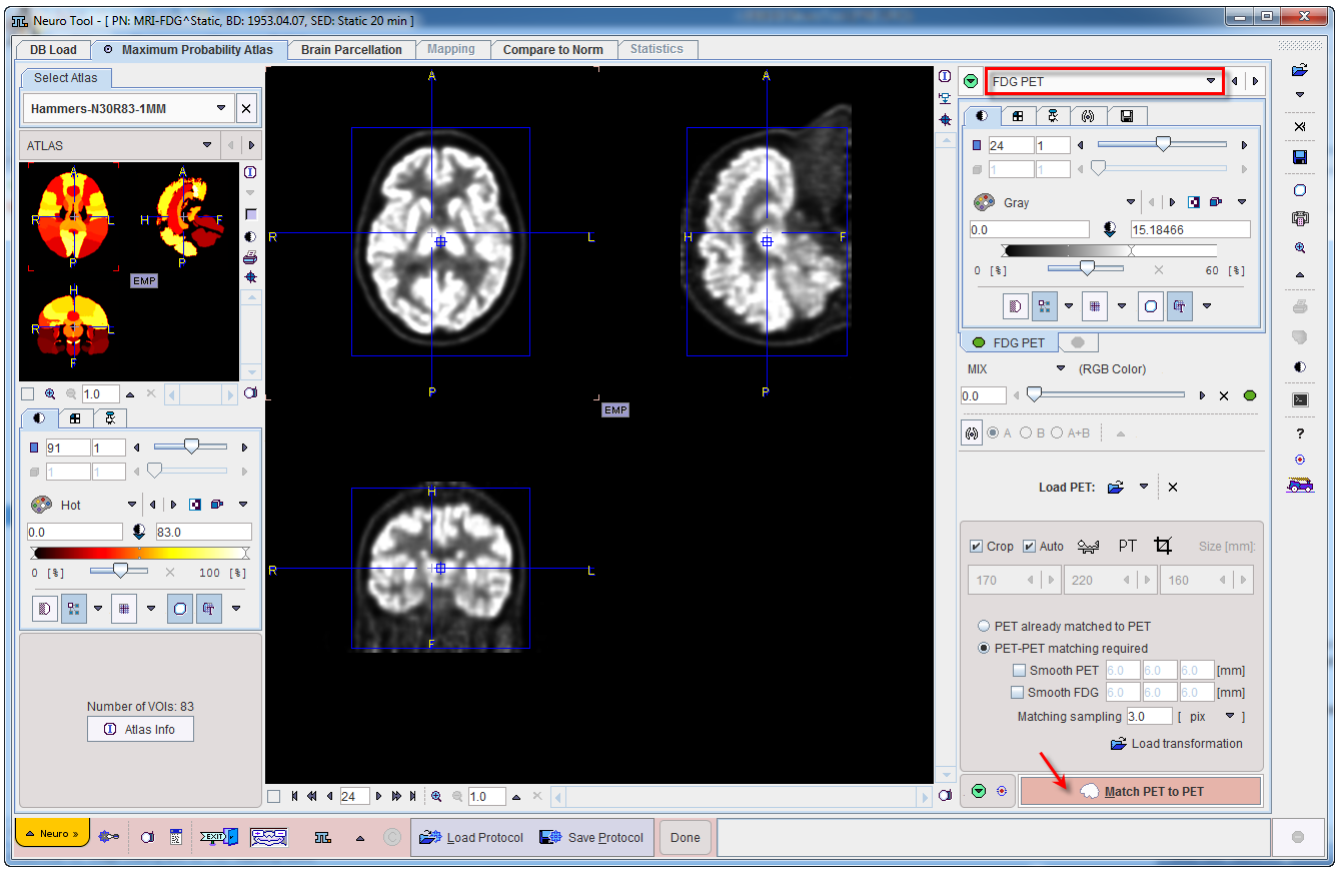
Please use the **Load FUN** button for loading the functional PET series which may be static or dynamic.



In the case of a dynamic PET series define the averaging range for creating a new series **FUN AVG**, which will be used for matching with the FDG PET. Apply a crop window, select the **FDG** radio box to indicate the PET-PET workflow to the program, and proceed with the **PET** action button.

Anatomical PET Image Loading and PET-PET Matching

The **FDG PET** page allows loading the anatomically weighted brain PET image of the same patient using the **Load PET** button.



Please apply a crop box if appropriate.

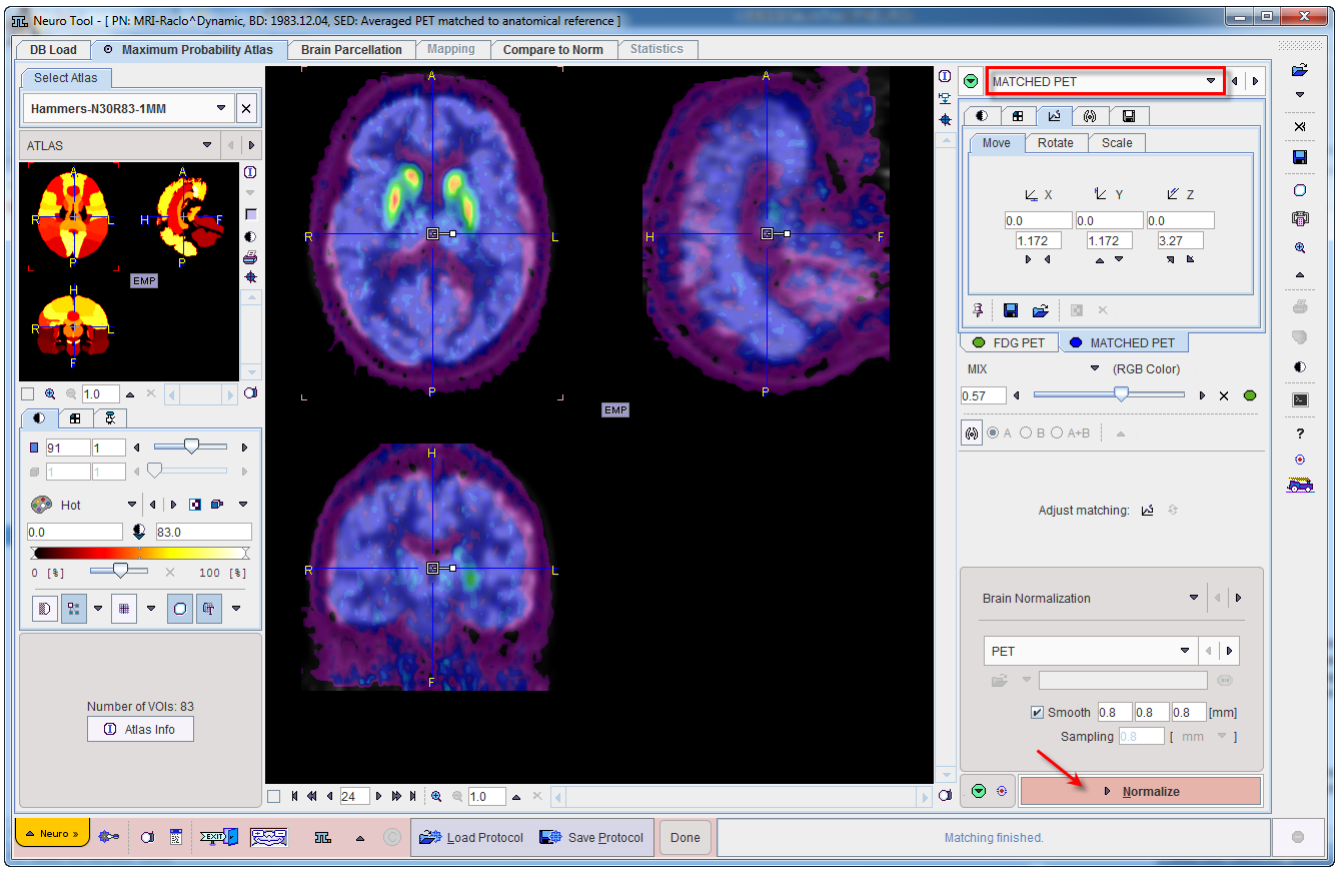
The next step consists of rigidly matching the averaged PET image to the anatomical PET image. If the data is already matched, the calculation can be skipped by activating the **PET already matched to PET** box. If the matching has been performed before and the transformation saved, it can be loaded 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 images may be smoothed.

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

PET-based Normalization

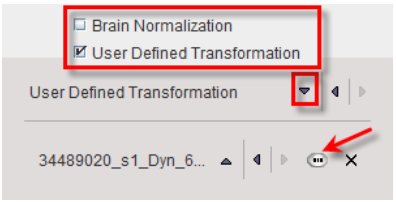
The result of the PET-PET matching is shown on the **MATCHED PET** page and should be validated using the available fusion functionality.



Stereotactic Normalization

The configuration of the **Brain Normalization** 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** template. Often, such a pattern can be obtained by averaging the early phase of PET tracer uptake.

If the matching has been performed before and the transformation saved, it can be used selecting the **User Defined** entry instead of the **Brain Normalization** option. Finally load the transformation with the load button as shown in the capture below.



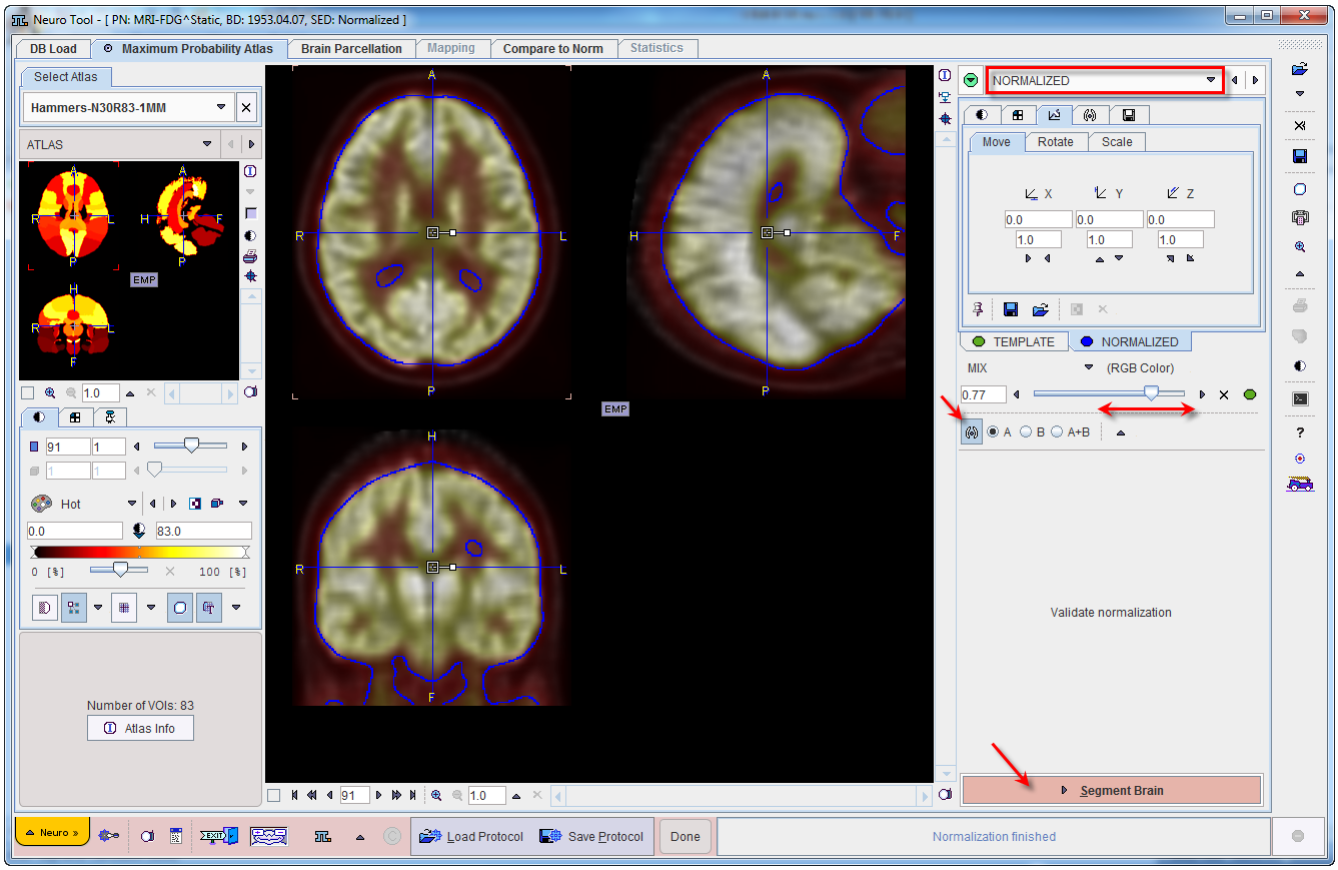
User-defined Templates

If the normalization results are not satisfactory, the user is advised creating his own PET template representing the uptake pattern of the specific tracer in the MNI space. Such a template can be constructed from the images of normal volunteers with the ***Brain Norm Creation*** (on page 114) facility of the PNEURO tool. Once such a template is available, select the **User Defined** entry in the **Brain Normalization** list and configure the template with the load button as illustrated below.

The other normalization parameters are the **Smooth** option which will smooth the input image with a Gaussian filter of the specified half-widths. 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 with the **Normalize** action button.

Brain Segments Calculation

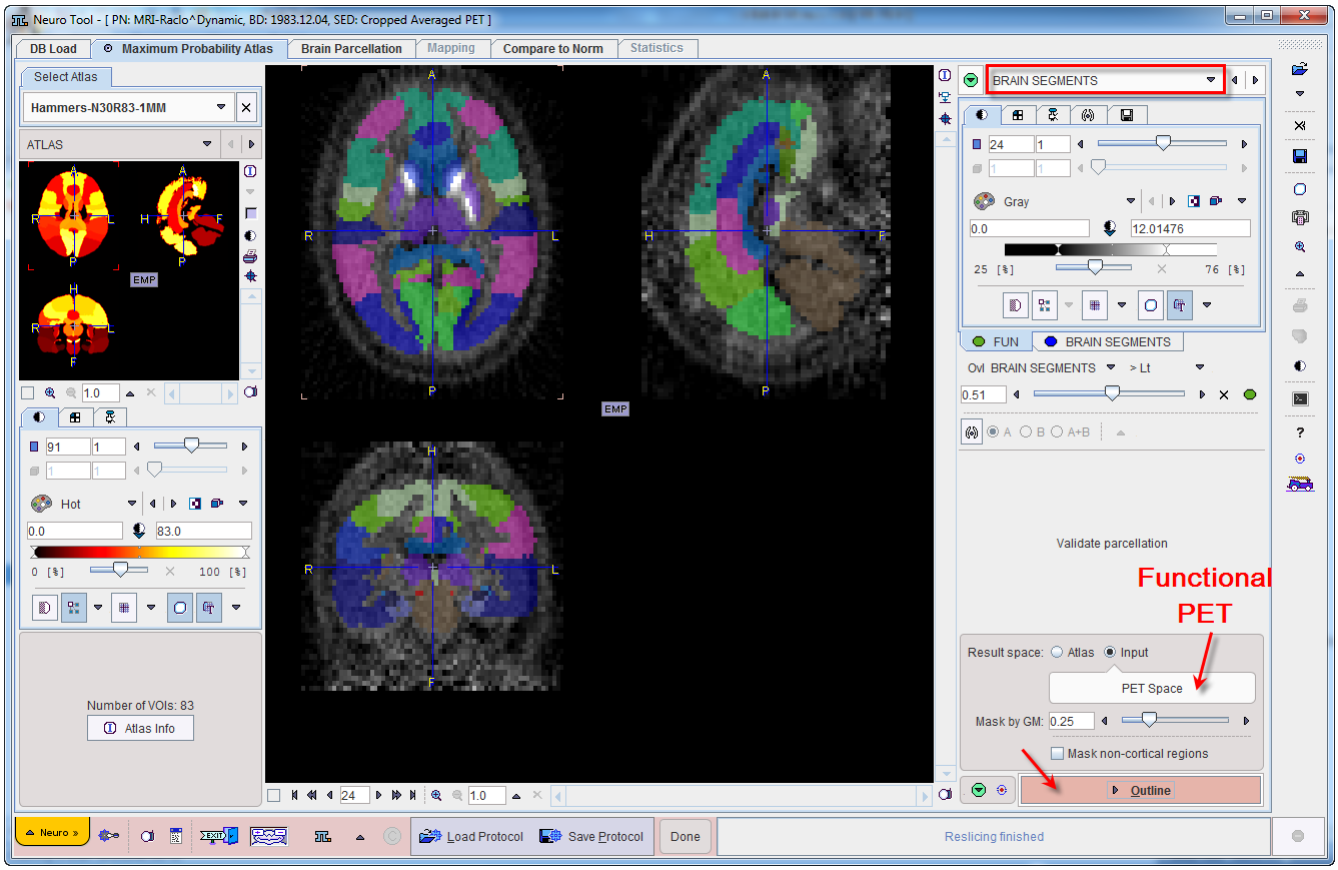
The stereotactic normalization result is shown on the **NORMALIZED** page. Please verify that the normalization procedure was successful.



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

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 averaged functional PET.

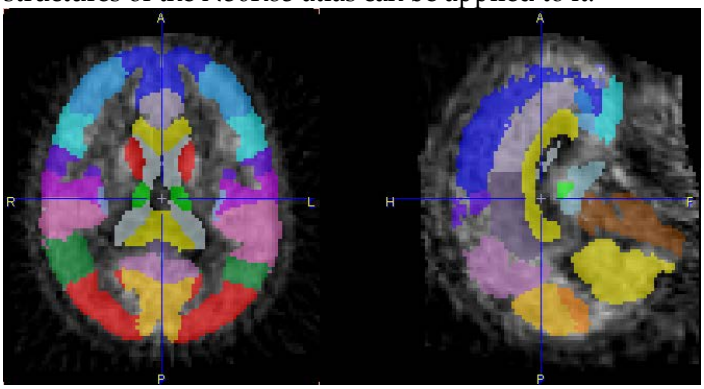


Result Space

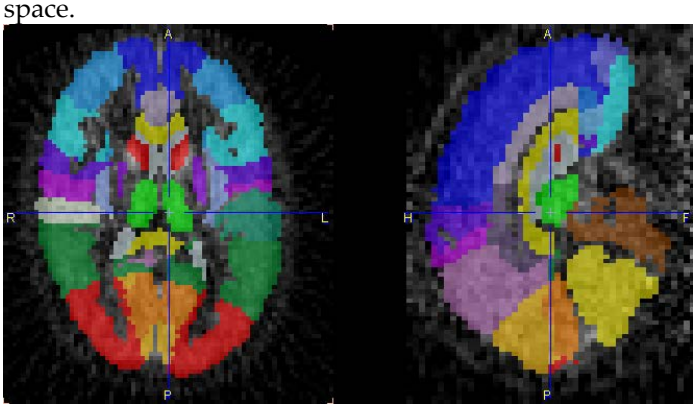
There are two options for evaluating the PET image which can be configured with the **Result space** box. The information shown on the page is updated as soon as the configuration is changed. It shows the PET image in the selected result space together with the brain structures.

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.



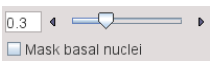
2) **Input, PET Space:** With this setting the N30R83 structures are transformed to the PET space.



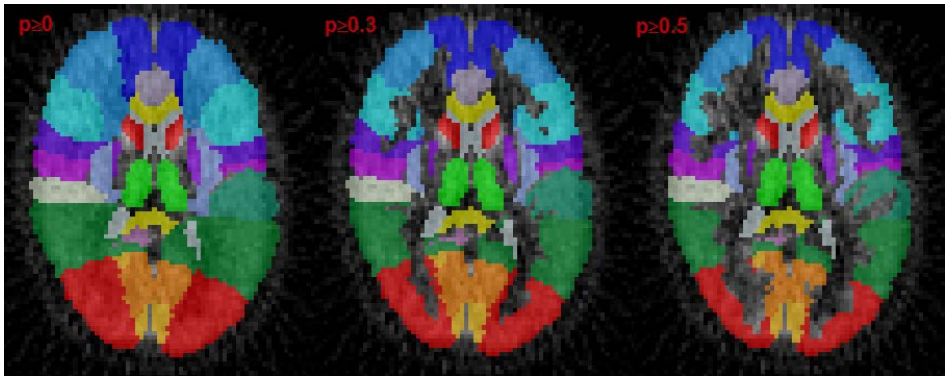
Note that due to the relatively large size of the PET pixels in this example the structure outlines look rugged. To avoid this effect the PET image should be reconstructed with small pixel sizes.

Intersection of N30R83 Structures with Gray Matter

The original N30R83 structures cover the whole brain. Users can take advantage of a population average gray matter probability map and restrict the structures to pixels with a high probability belonging to gray matter. This operation is controlled by the elements in the masking area.



The slider allows defining the lower threshold which is applied to the probability map for creating the mask. The higher the probability threshold, the thinner the cortical structures become. The illustration below shows masking in the PET space at three increasing probability thresholds.



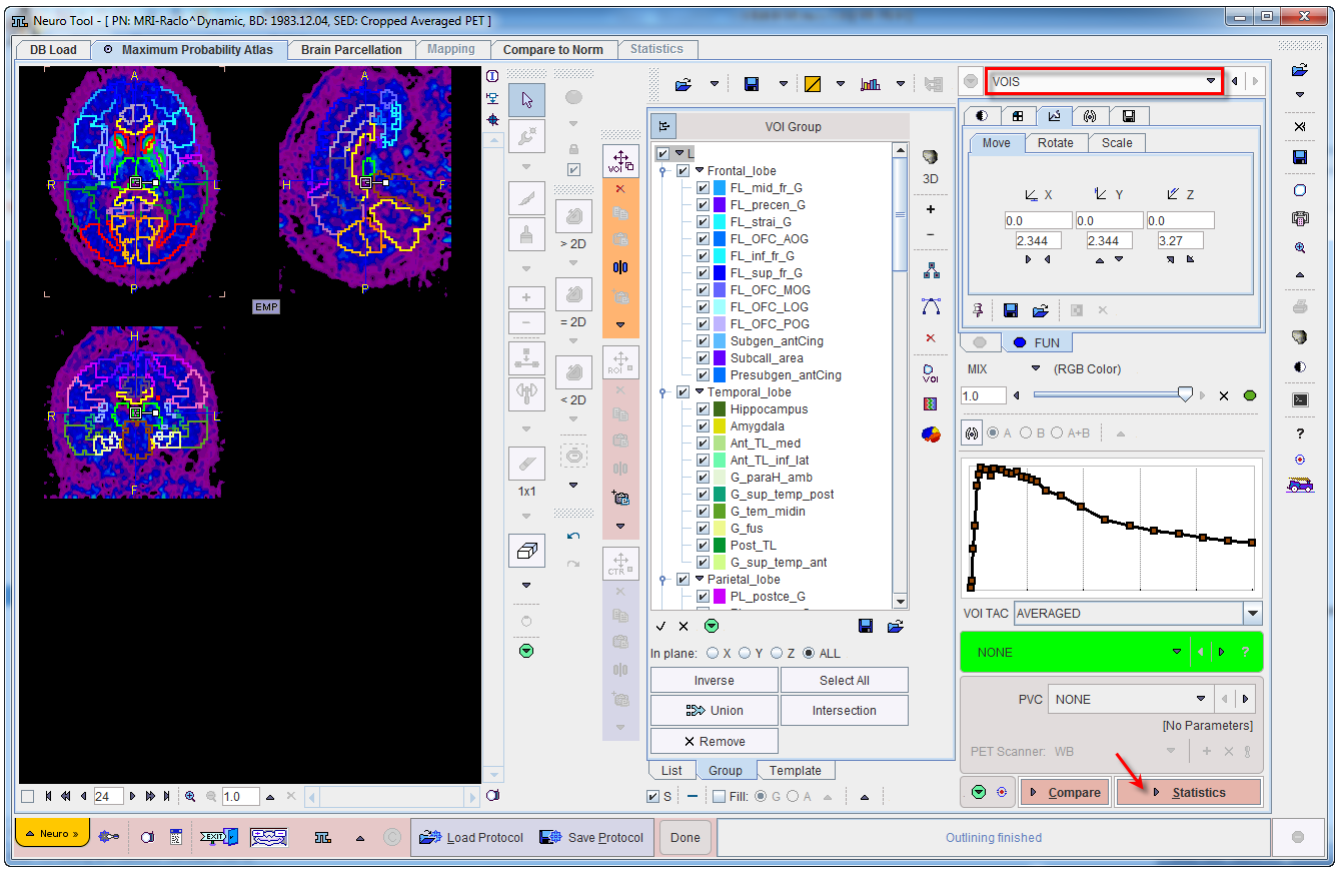
Note that the central structures were not affected by masking in the example above, because the box **Mask basal nuclei** was not enabled. If this option is enabled, the central structures are also shrinking. However, because of the low probability levels in that area, the reduction may become too severe. Therefore, masking of the basal nuclei will usually be disabled.

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.

Brain VOI Editing and Statistics Calculation

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



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 ***Statistics*** (on page 106) page.

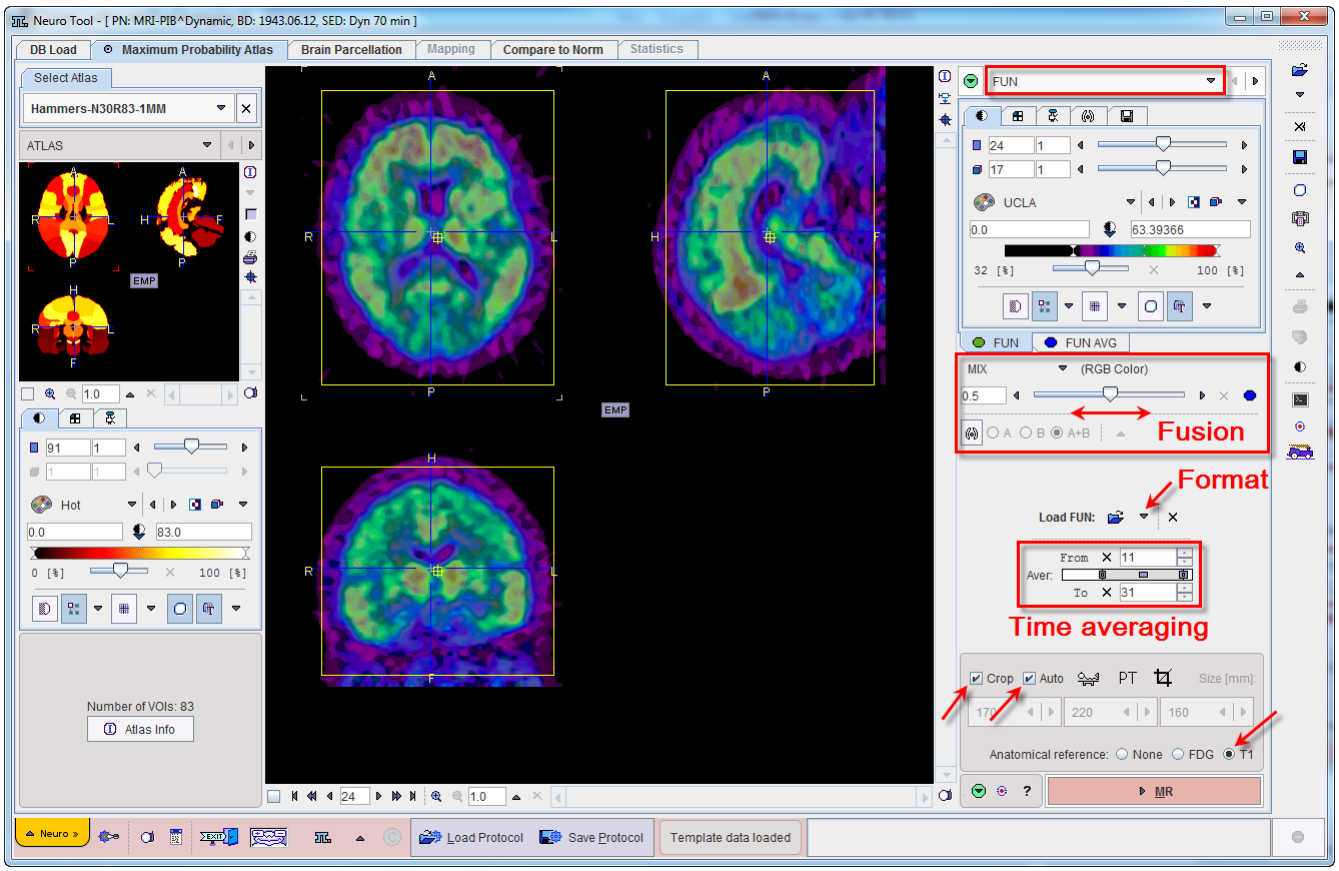
Workflow for PET-only Studies

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 available in the MRI.

PET Image Loading and Time Averaging

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



The **Load FUN** 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 it is recommended to use the **Autodetect** format. Note that the PET series may be static or dynamic.

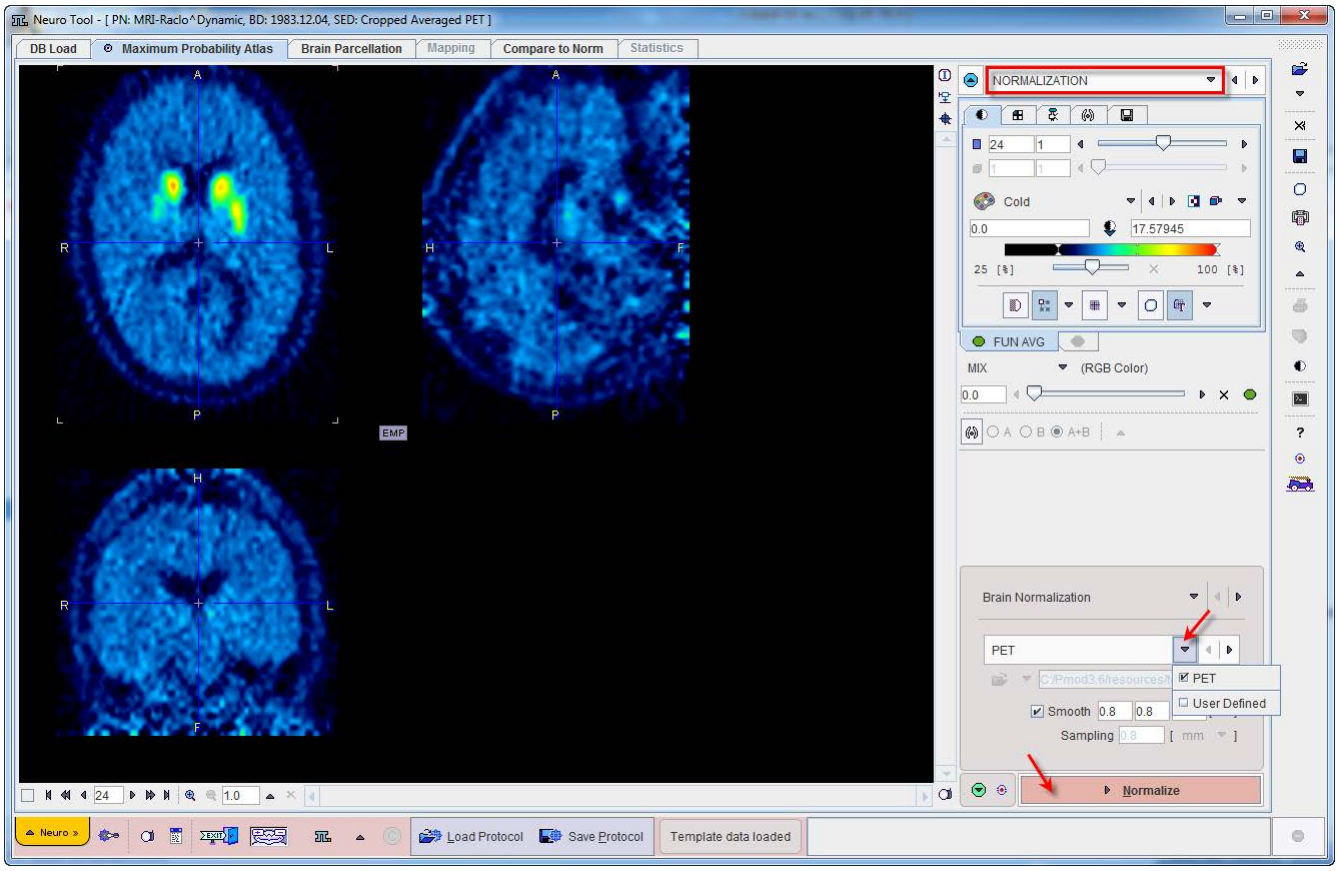
In the case of a dynamic PET series, a new series is generated by averaging a range of frames and assigned to the **FUN AVG** tab. The averaging range can be defined by the **From** and **To** number fields, or dragging the range indicators in the **Aver** bar. After any modification of the range, the 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 which resembles as good as possible to the PET template used for the spatial normalization. To continue please select the **None** radio button activate the action button in the lower right which has changed to **Norm**.

PET-based Normalization

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

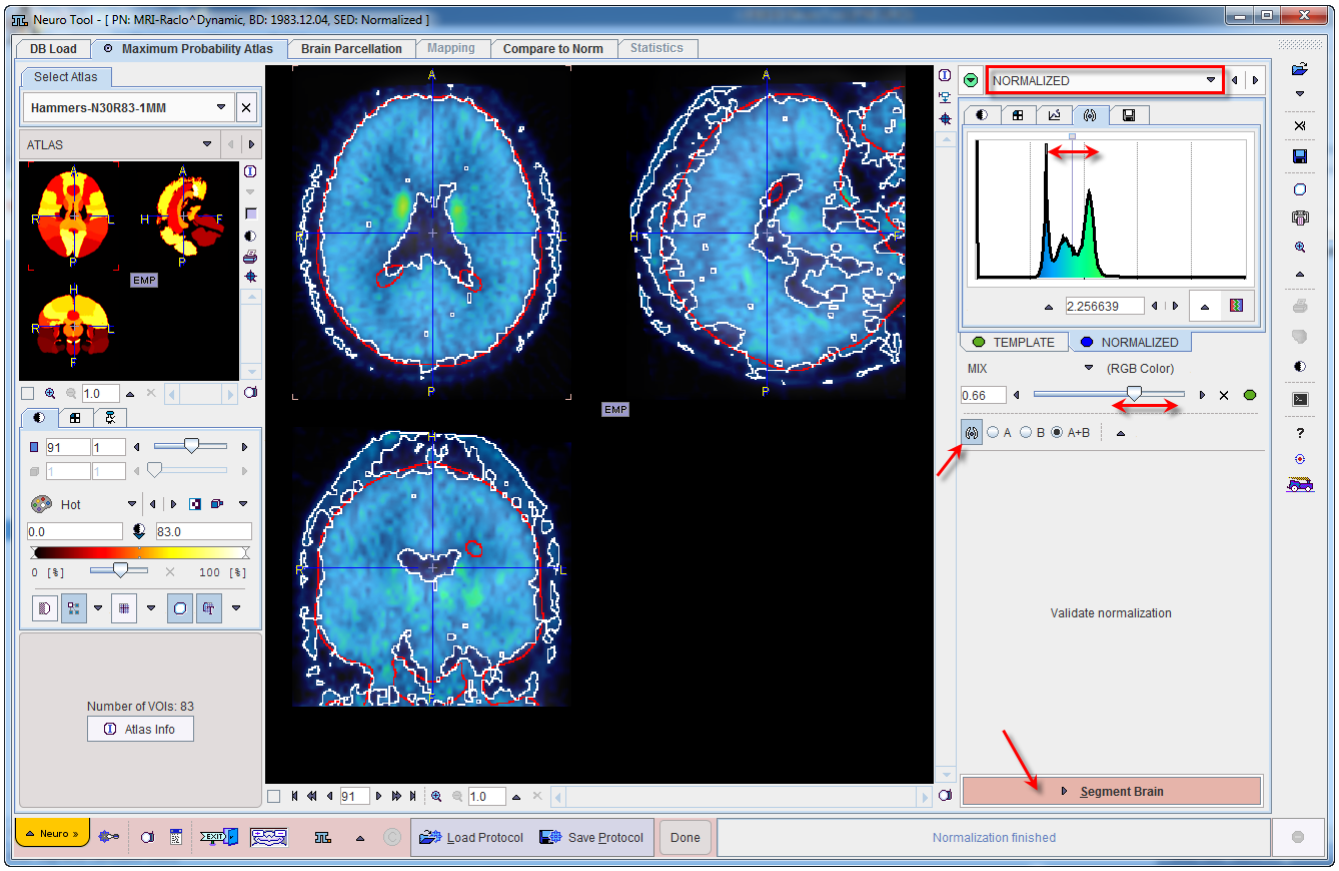


The configuration of the **Brain Normalization** step is crucial. **PET** is the default selection and refers to the perfusion-weighted PET template provided with SPM5. Please refer to the section ***above*** (on page 63) for more information about the **PET** template and **User Defined** templates.

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

Brain Segments Calculation

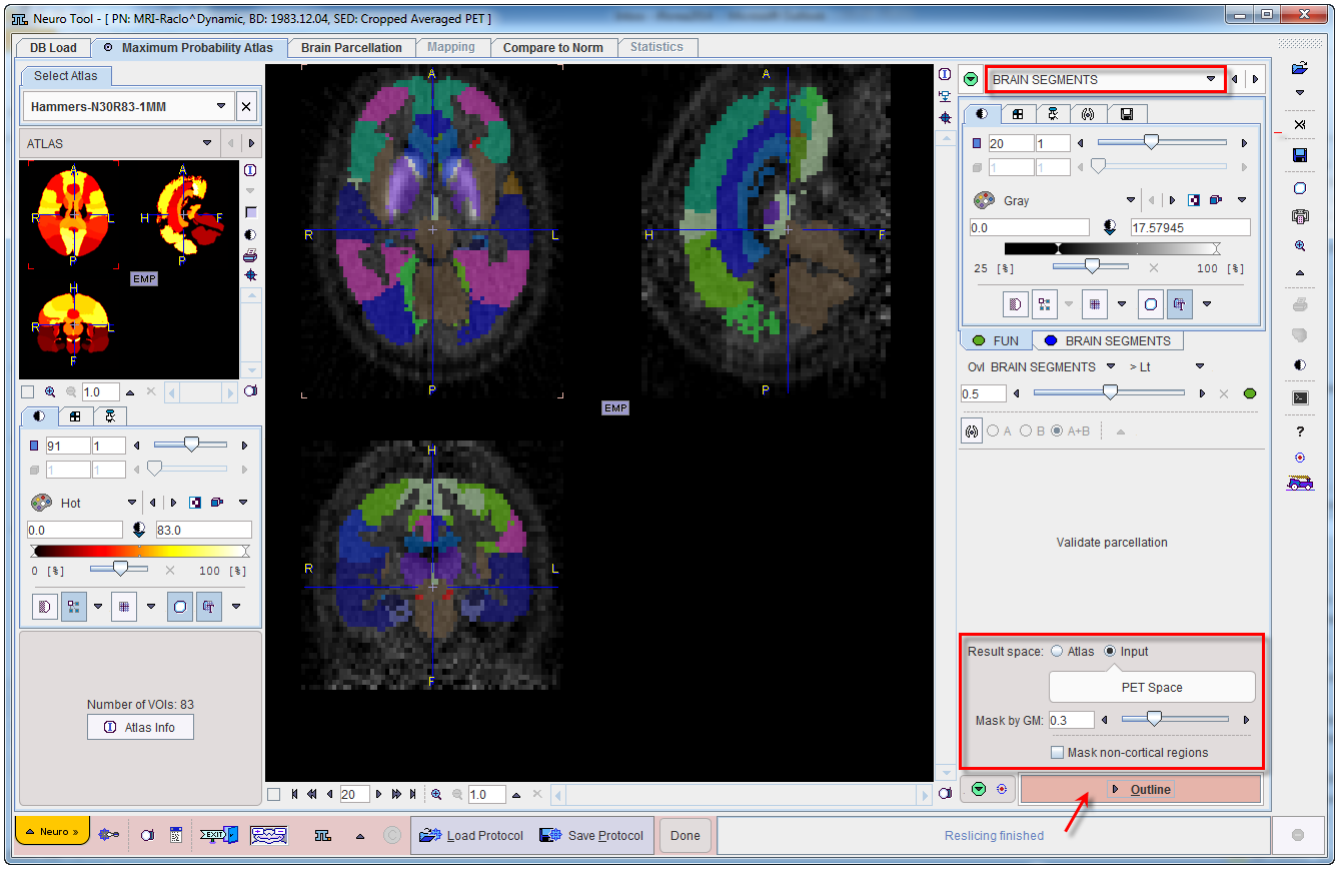
The stereotactic normalization result is shown on the **NORMALIZED** page. Please verify that the normalization procedure was successful by evaluating the alignment in different parts of the brain. Particularly helpful are the iso-contour lines. The thresholds on the **TEMPLATE** and the **NORMALIZED**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



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

Outlining of Brain Structures

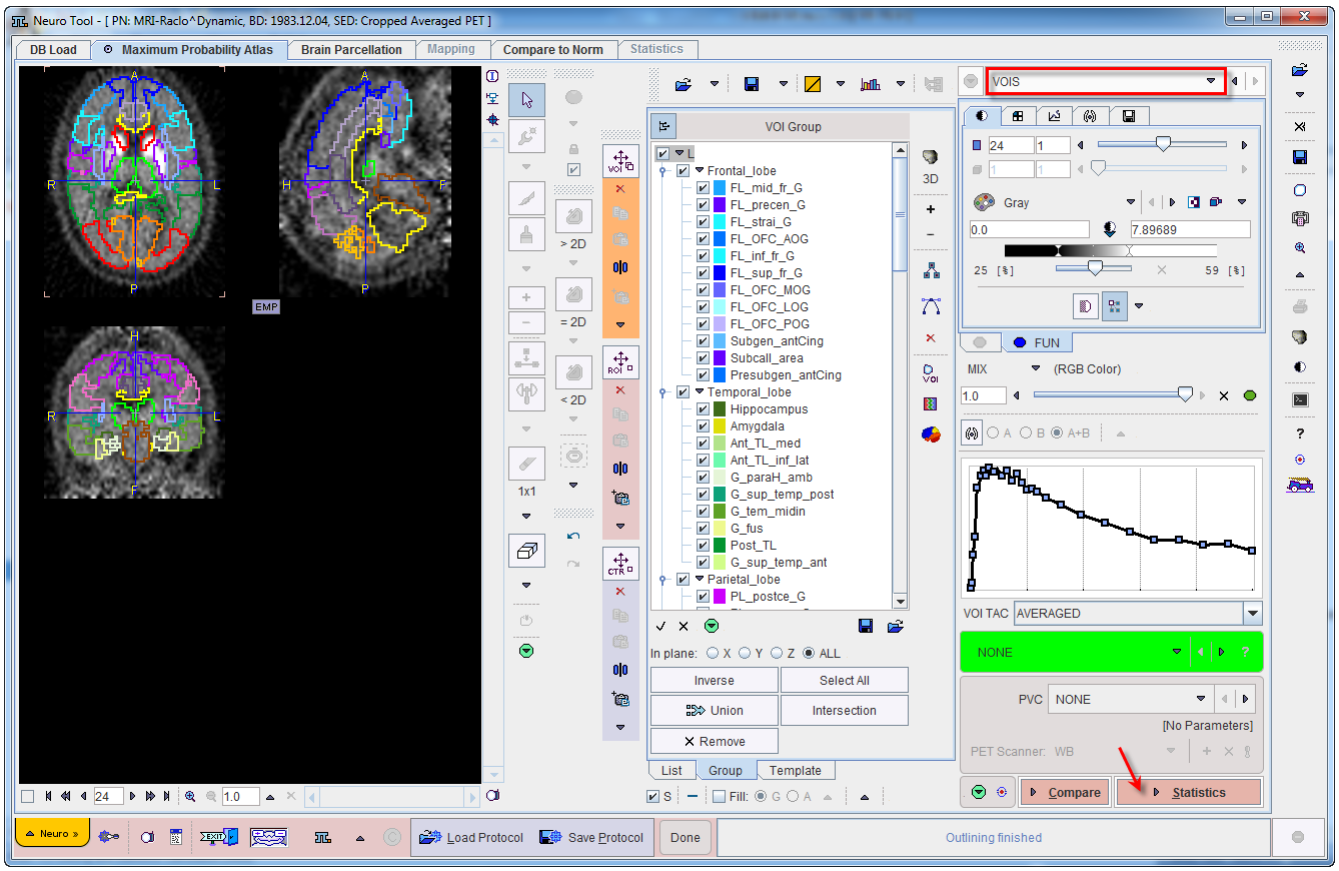
The result of the brain structure transformation is shown on the **BRAIN SEGMENTS** page.



Please define the result space and gray matter masking, and start the VOI outlining process with the **Outline** action button.

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**.



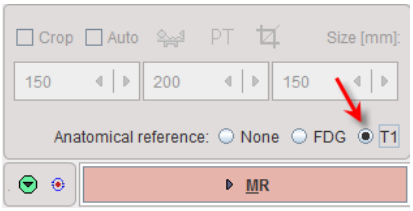
After editing the VOIs and configuring the optional partial volume correction **PVC**, please proceed with the **Statistics** action button. The result is shown on the ***Statistics*** (on page 106) page.

Workflow for MR-only Studies

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

MR Loading Configuration

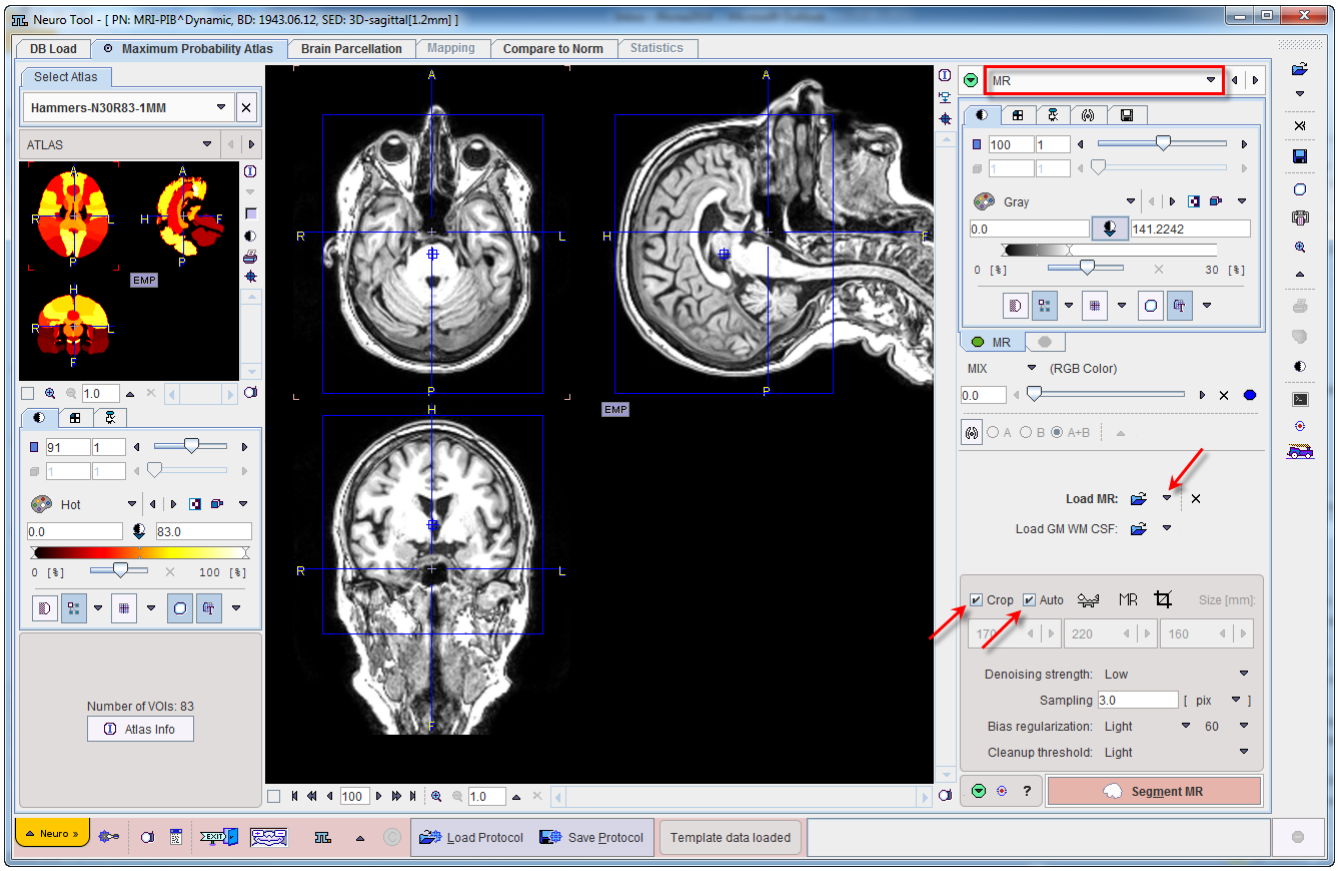
In order to configure PNEURO for the MR-only workflow please select the **T1** radio button on the **FUN** page.



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

MR Image Loading and Segmentation

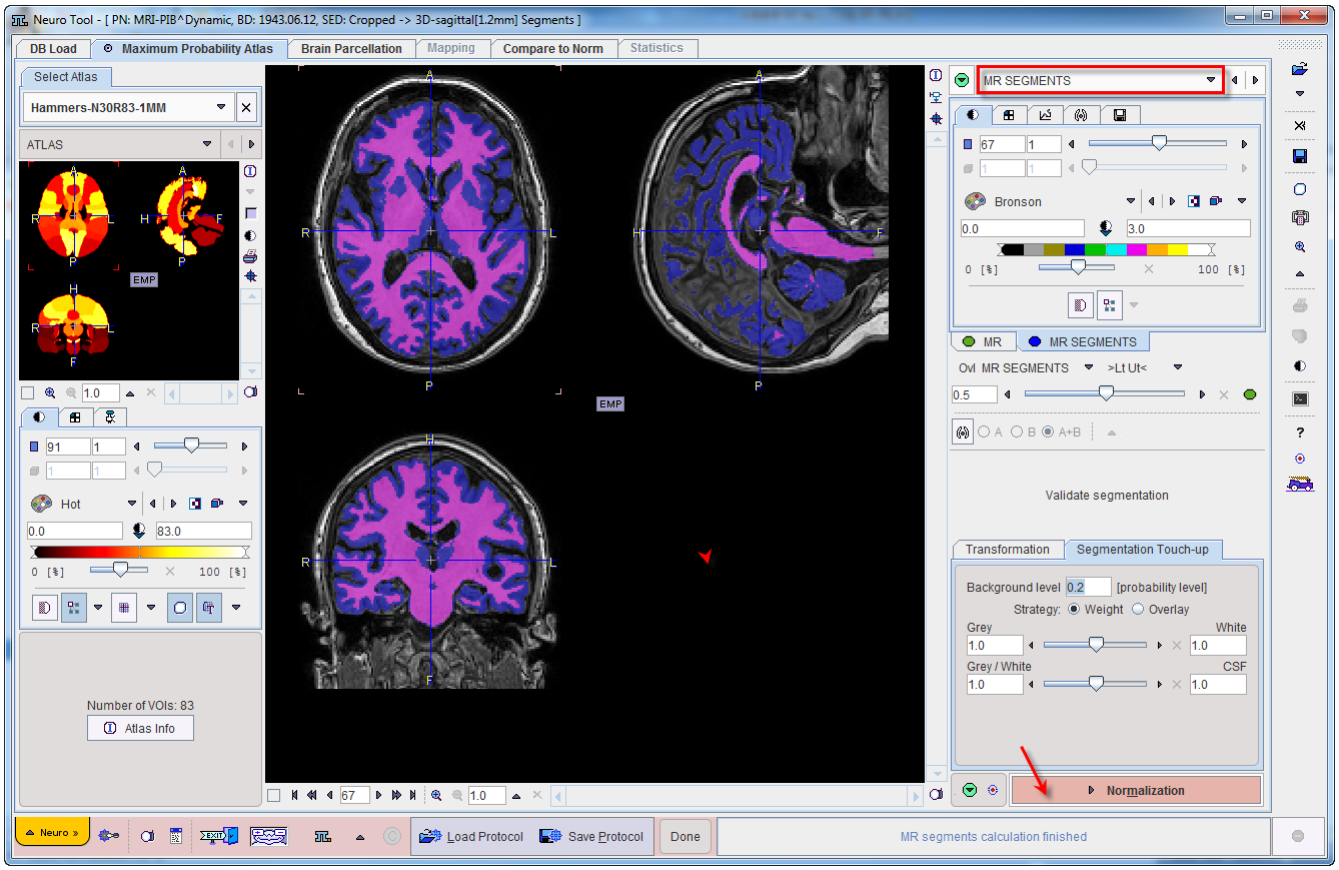
The **MR** page allows loading the T_1-weighted brain MR series using the **Load MR** button.



Please define an appropriate crop window, configure appropriate denoising and segmentation parameters, and proceed with the **Segment MR** action button.

MR-based Normalization

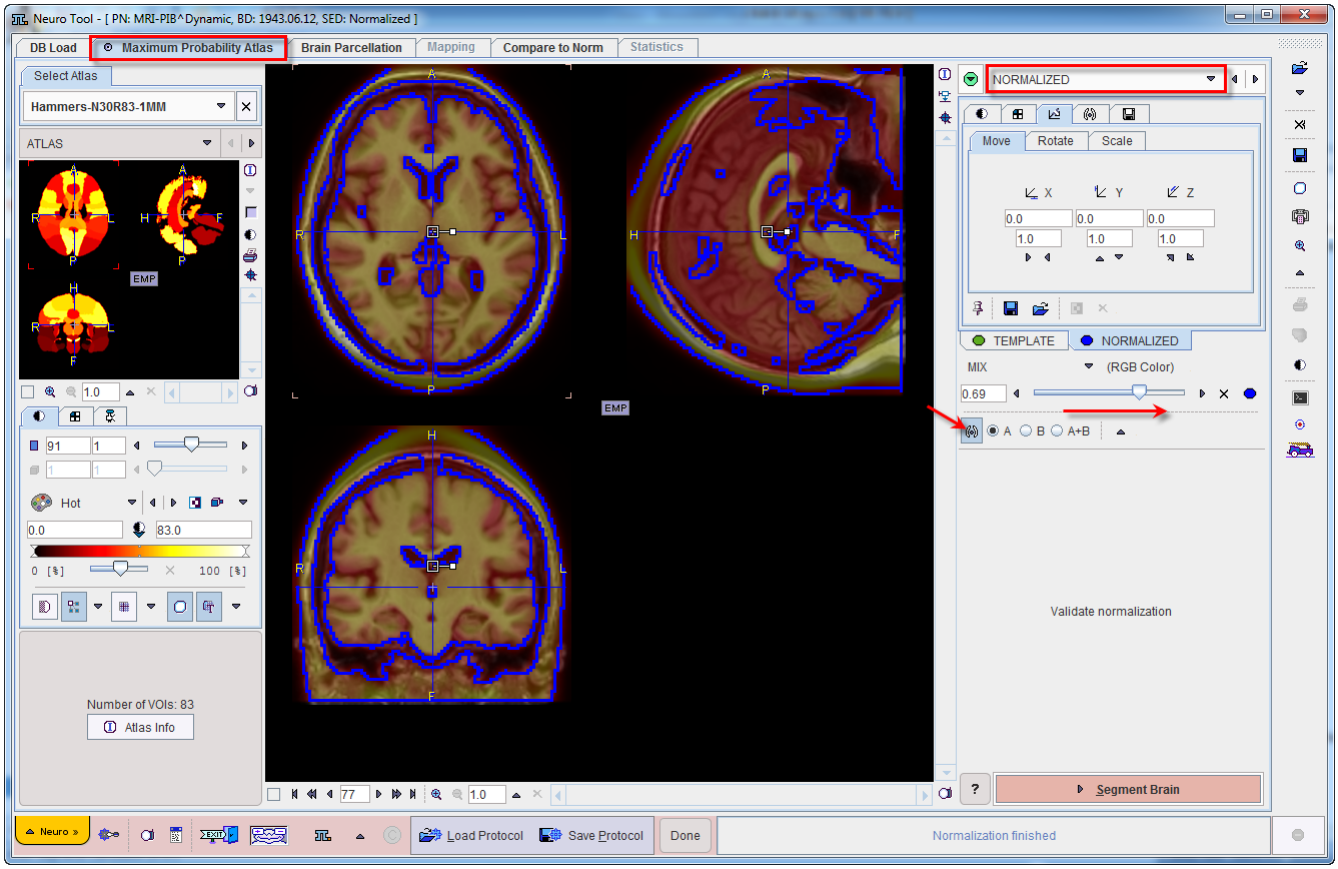
The result of the segmentation is shown as a fusion of the tissue segments with the MR image on the **MR SEGMENTS** page.



Please activate the **Normalization** action button to start the stereotactic normalization using the T_1 MNI template as the reference.

Brain Segments Calculation

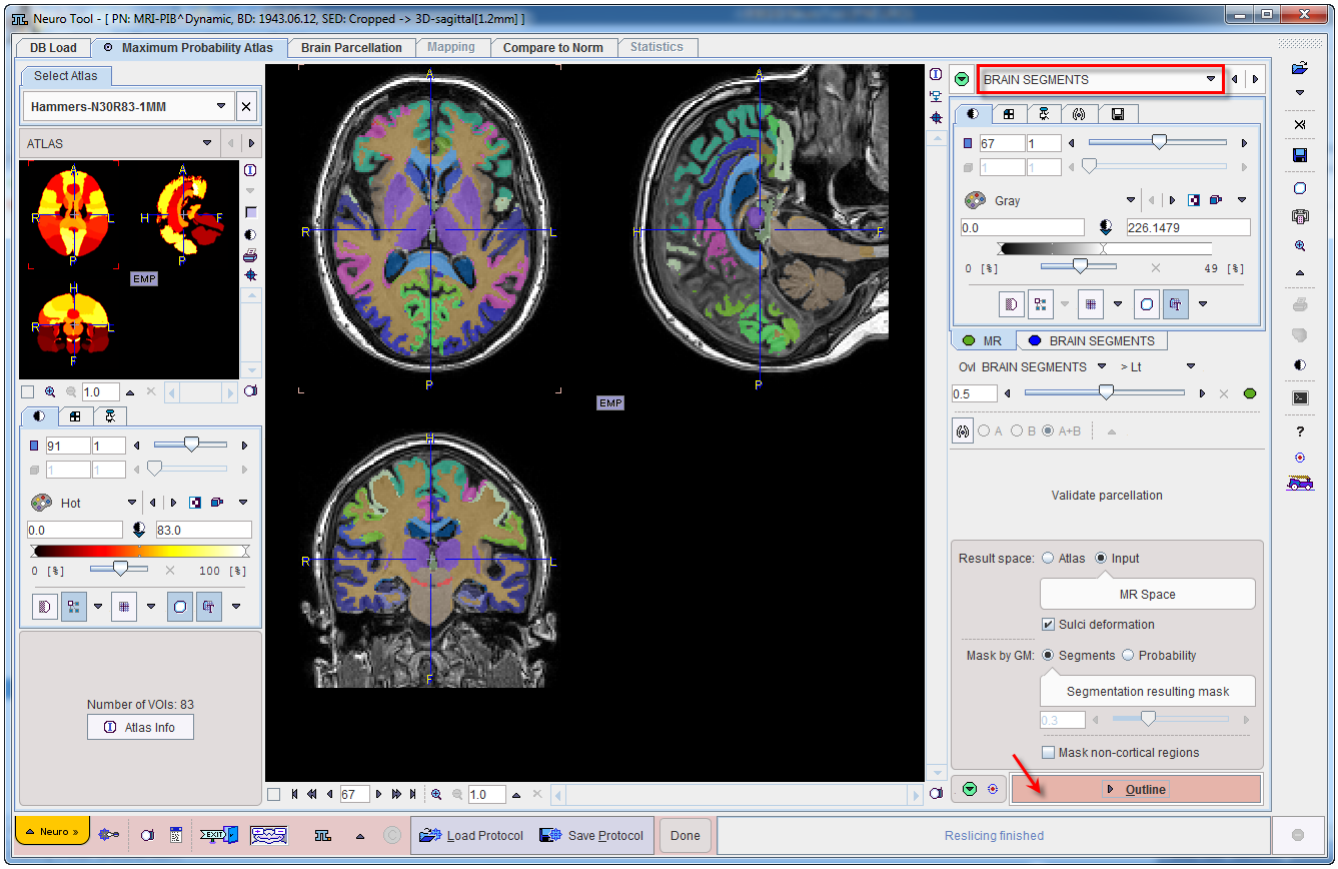
The stereotactic normalization result is shown on the **NORMALIZED** page.



Please verify that the normalization procedure was successful, then start the mapping of the brain structures to the MR space by the **Segment Brain** action button.

Outlining of Brain Structures

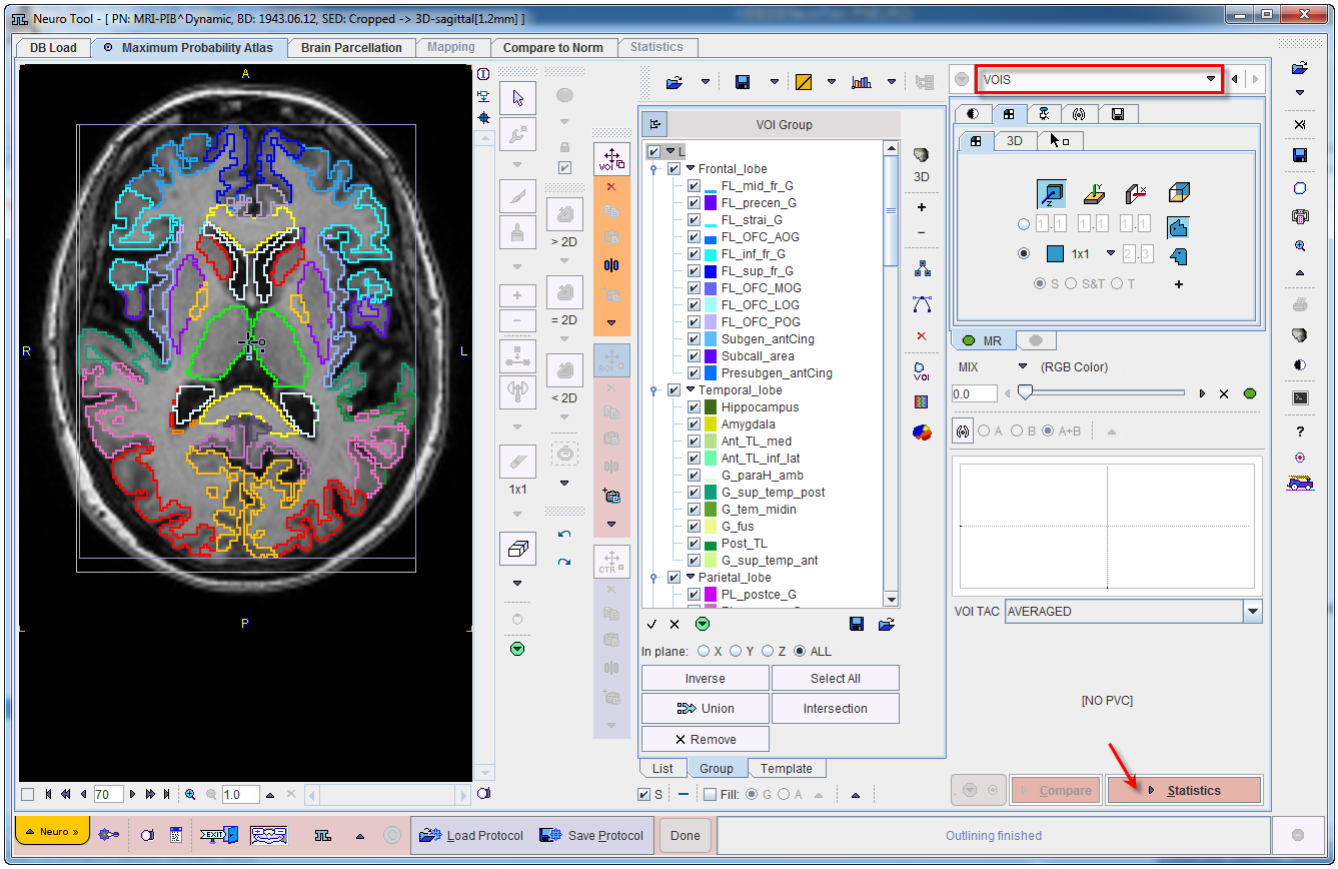
The result of the brain structure transformation is shown on the **BRAIN SEGMENTS** page.



Please configure the result space (usually **Input/MR Space**) and gray matter masking, and start the VOI generation with the **Outline** action button.

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 **Statistics** *page* (on page 106).

Brain VOIs Based on T1-MRI Segmentation

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 patient 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 applied sophisticated methodology allows detecting the structure boundaries with high accuracy, so that the result may even be used for morphometric analyses.

Methodology

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 29). 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 interactively specified by the operator: 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 to calculate 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 patients: a combined ROI-based and voxel-based morphometric study. Neuroimage 2006, 32(4):1562-1575. ***DOI http://dx.doi.org/10.1016/j.neuroimage.2006.05.057***

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.

Brain Parcellation Implementation in PNEURO

Brain parcellation in PNEURO supports two situations:

- PET and MR: The patient 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 patient 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 which 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 in the following for all steps except for the statistics calculation.

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 hemispheres. This requires the interactive definition of 4 anatomical landmarks.

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 patient'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 the interactive definition of 4 anatomical landmarks.

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 patient'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

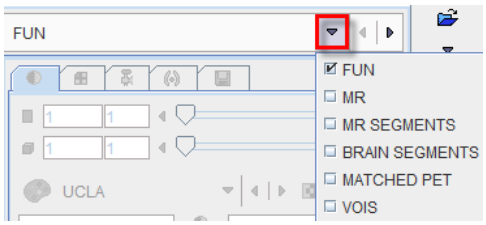
6) Optional: application of the VOIs to the MR series, mainly for calculating the VOI volumes.

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.

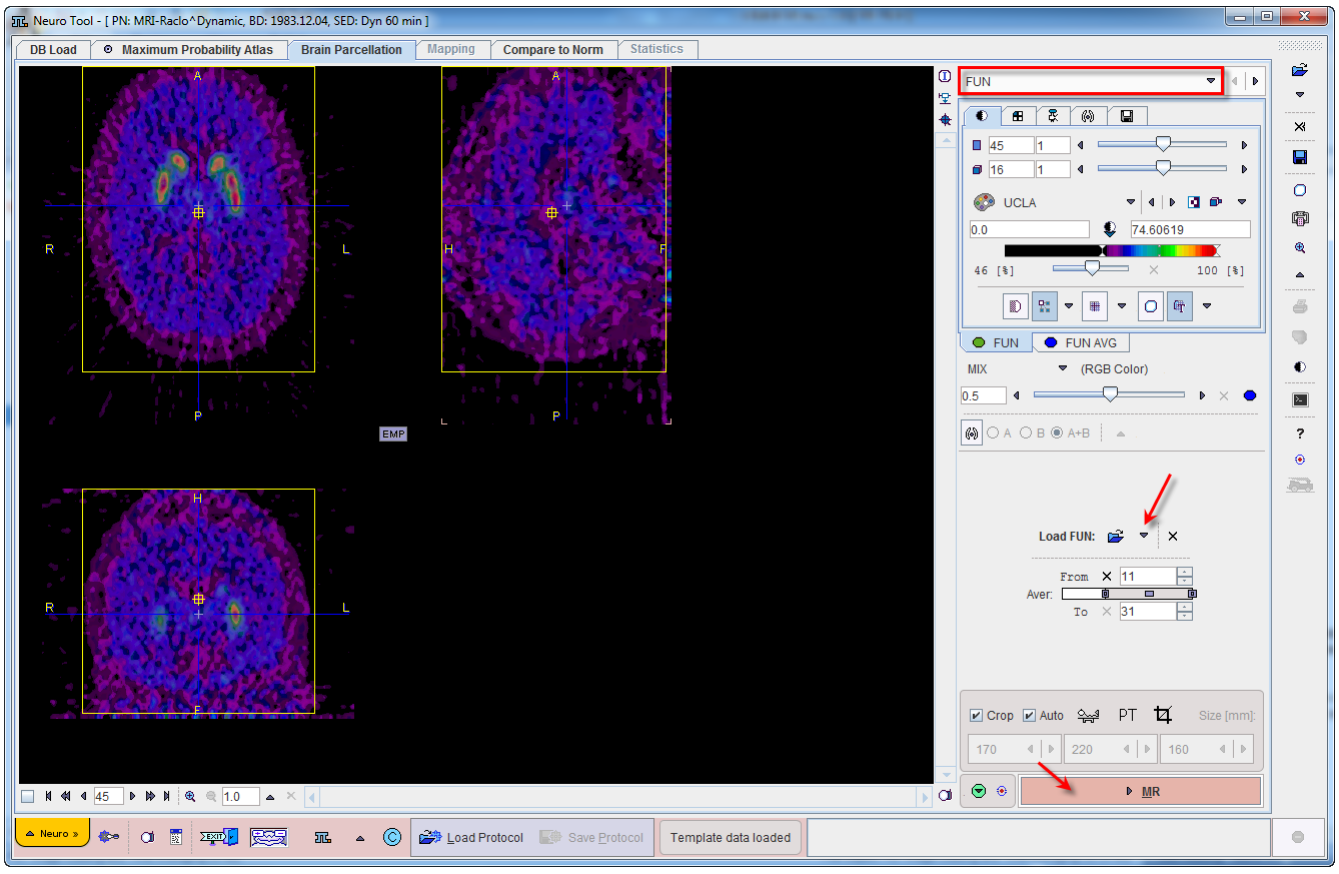
Workflow for Studies with PET and MR

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



PET Image Loading and Time Averaging

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



PET Image Loading

The **Load FUN** 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 it 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 **FUN AVG** tab. The averaging range can be defined by the **From** and **To**

number fields, or dragging the range indicators in the **Aver** 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.

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 automatically enabling the **Crop** box and the **Auto** box. A yelow crop volume appears on the image while the optimal size and placement is calculated in the background based on species and modality selection.

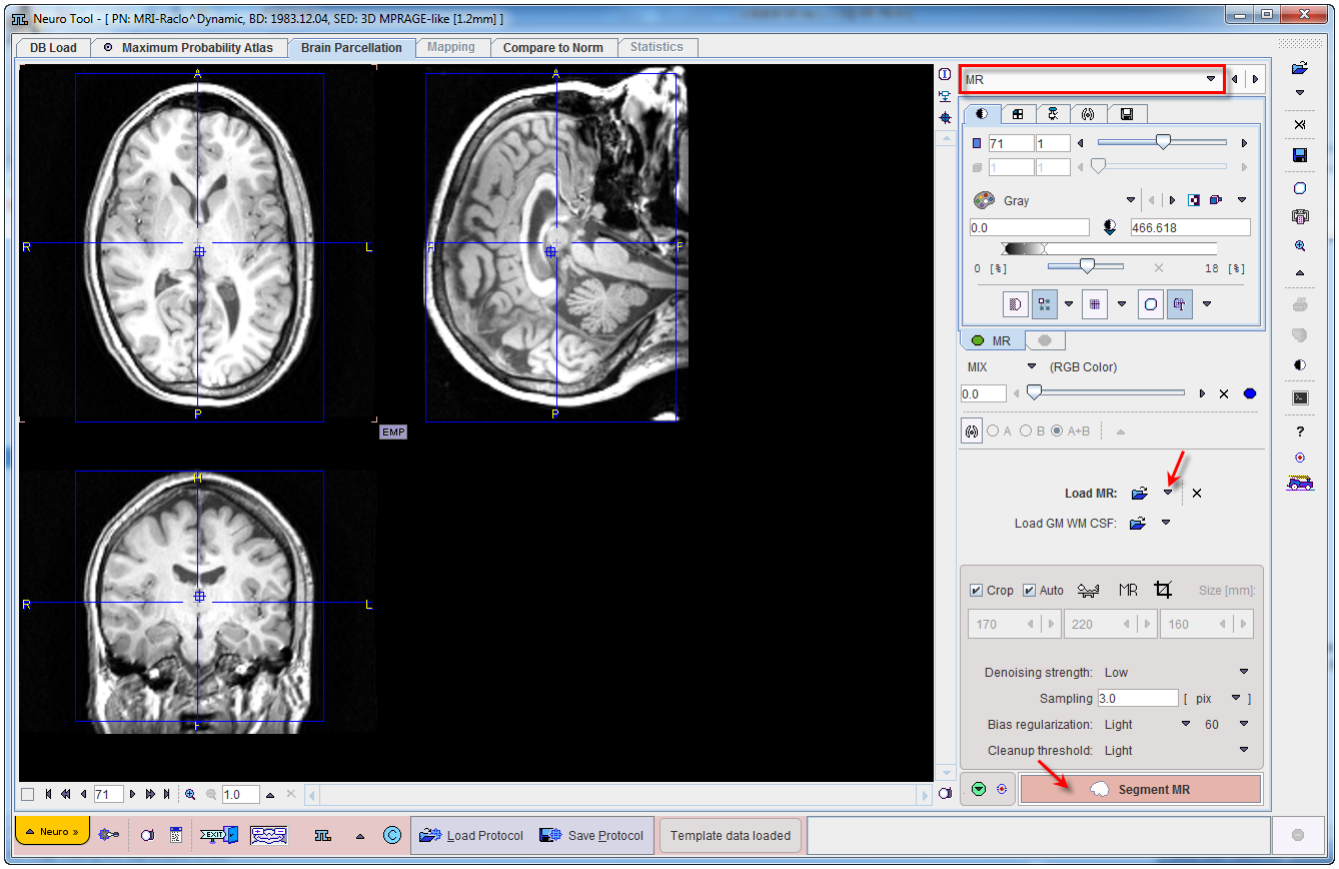
In case the automatic procedure fails, the cropping box size and location can be adjusted manually. Make sure only the **Crop** box is enabled. Place the yellow crop volume indication by clicking at the brain center so that the brain is fully enclosed. The edge size in **[mm]** can be adjusted for each direction by selecting the size in the corresponding list. The **Crop** 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.

To continue with loading the MR images please select the **MR** action button in the lower right.

MR Image Loading and Segmentation

The **MR** page allows loading the T_1-weighted brain MR image of the same patient using the **Load 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 will be segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The algorithm uses four 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.

Sampling Density of pixels considered in the calculation.

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.

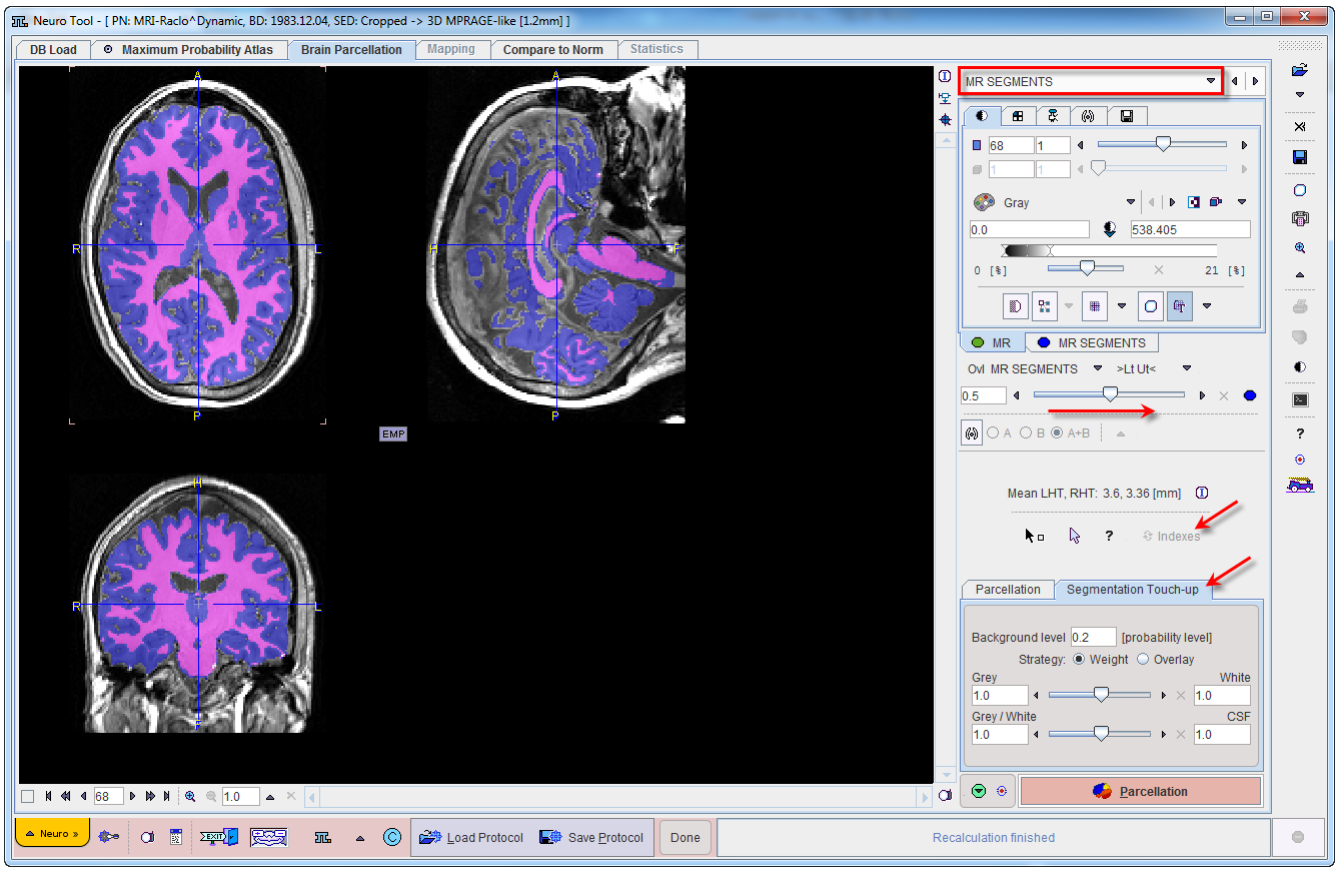
Cleanup threshold Procedure for rectifying the segmentation along the boundaries.

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 MR** action button. Note that the denoising and segmentation process may take several minutes.

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 **MR SEGMENTS** page. Note that the **MR 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, but requires that the **Indexes** are recalculated before the parcellation can be started.

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 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.

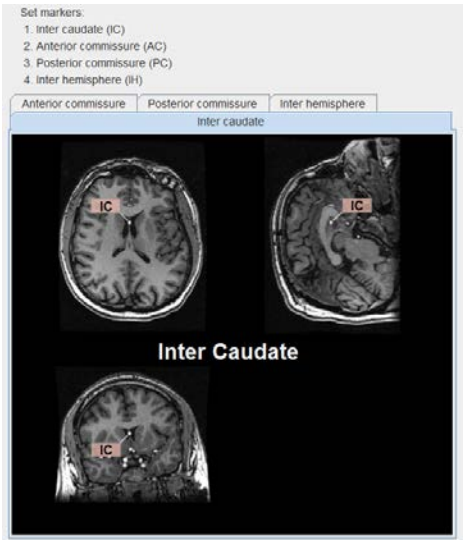
Please use the fusion slider to evaluate the segmentation quality. In case the result is not satisfactory, return to the previous page, modify the segmentation parameters, then activate **Segment MR** again.

Landmark Definition

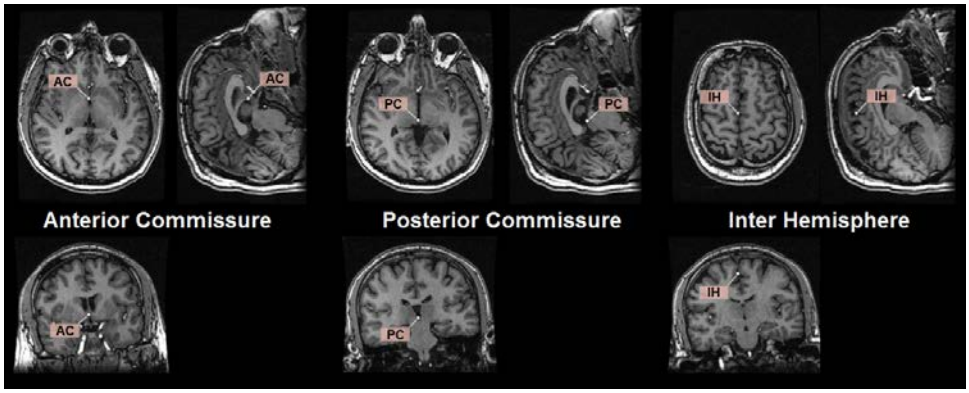
The parcellation procedure requires four anatomical landmarks

- 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.

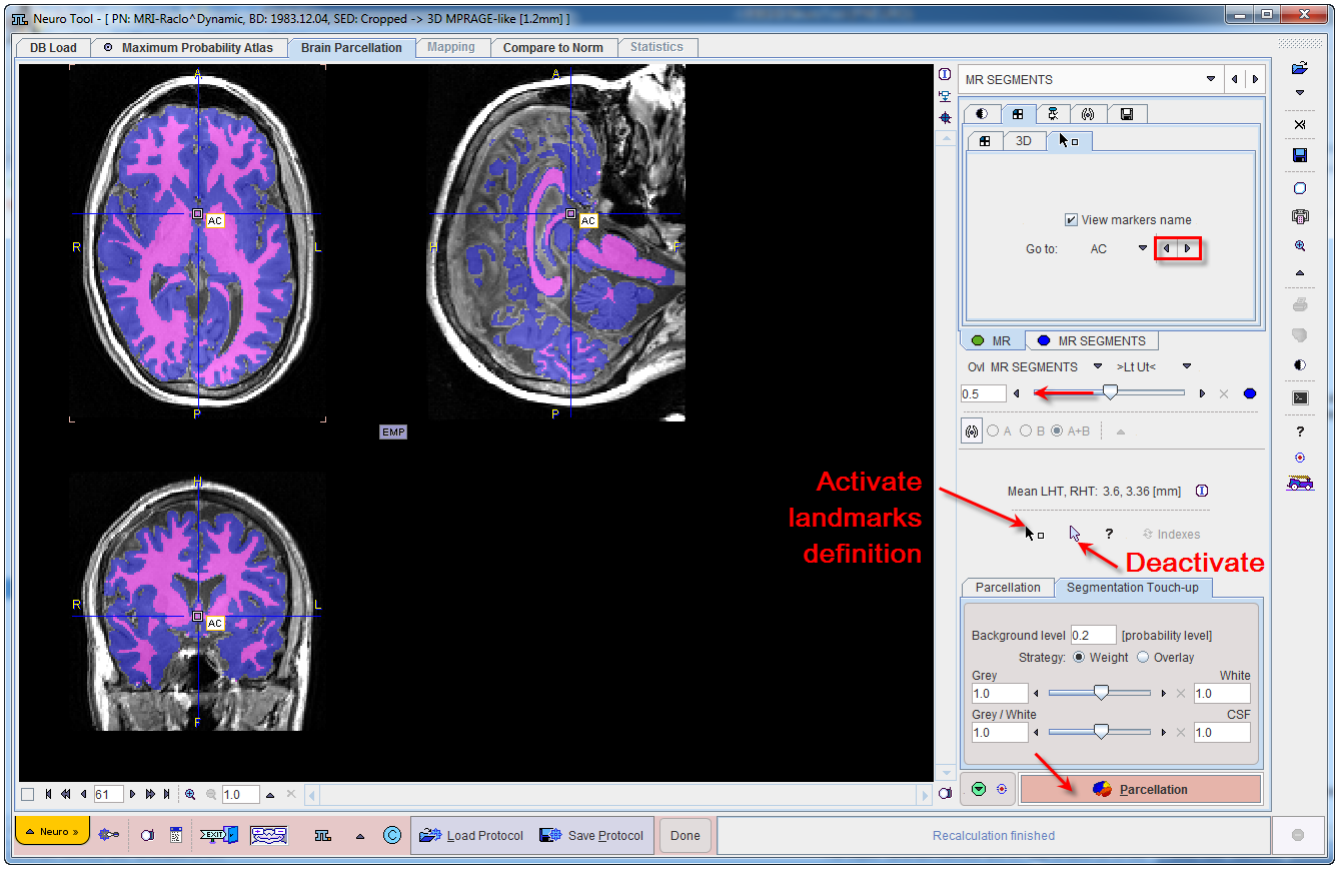
A visual help for this task can be opened with the **?** button to the left of the **Indexes** button. It appears with the IC landmark on front as illustrated below.



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. The **Go to** list allows to easily select 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.



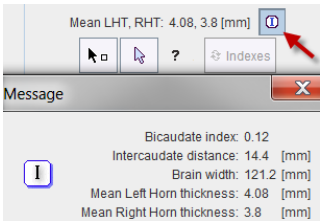
To avoid marker placement when clicking into the images for triangulation, hold down the SHIFT+CTRL keys. Note the indication of the landmark which needs to be placed (IC, AC, PC, IH) as part of the cursor symbol.

Brain Indexes

Based on the segmentation 3 indexes are calculated which will be further employed for the selection of suitable hemispheres from the knowledge base:

- Bi-caudate index: ratio of caudate distance and brain width.
- Left horn thickness (LHT): mean thickness of left horn in coronal section.
- Right horn thickness (RHT): mean thickness of left horn in coronal section

A summary of the indexes is shown in the user interface, and complete information can be opened with the button indicated below.



Parcellation

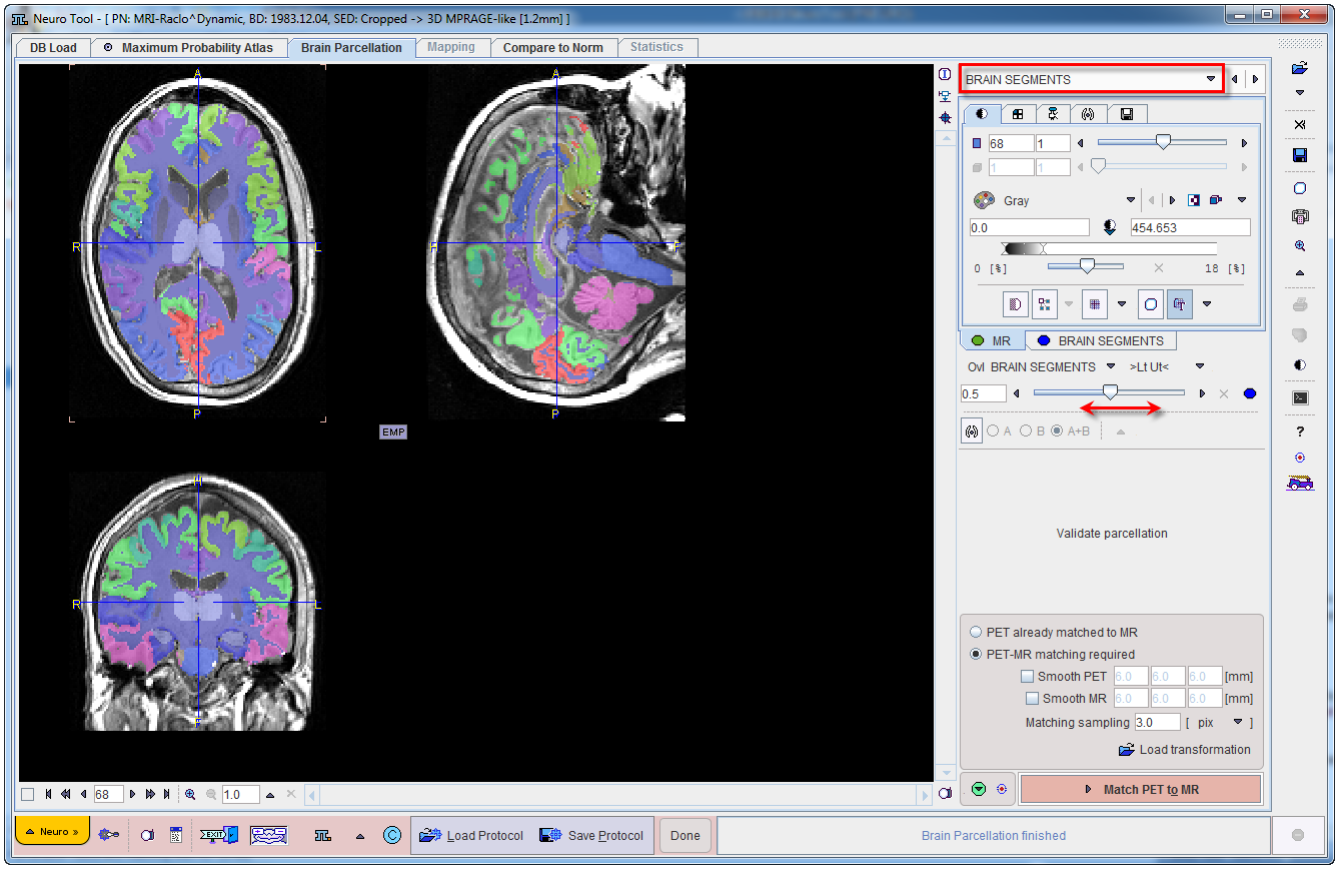
Deep nuclei parcellation calculates the brain structure shapes based on the knowledge base and the segmented MR image. Each hemisphere is processed individually. The user may select the **Number of subjects** (<=26) which are taken into account during parcellation. A higher number tends to provide more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

Sulci 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.

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.

Parcellation Result and Matching with PET

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 found brain structures. Please check the alignment of the structure information with the patient image with the fusion slider. In the case of a mismatch try changing the GM segment definition on the **MR SEGMENTS** page and/or the number of included subjects and repeat parcellation. Alternatively, the structure definitions can be adjusted manually after the outlining step.

PET to MR Matching

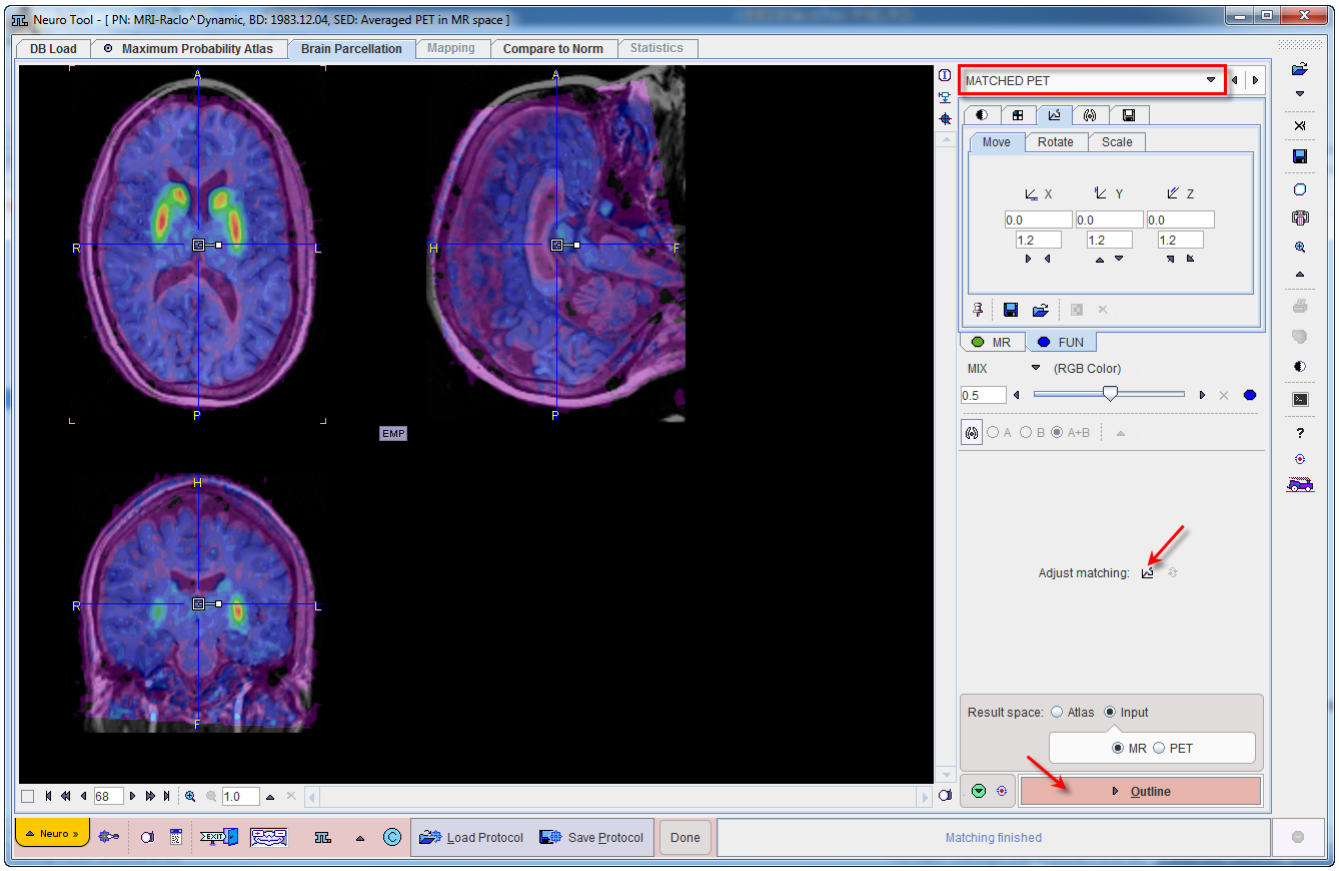
The next step consists of rigidly matching the averaged PET image to the MR image. If the data is already matched, the calculation can be skipped by activating the **PET already matched to MR** box. If the matching has been performed before and the transformation saved, it can be loaded and applied with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

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.

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 in the Atlas space and statistics are calculated with interpolated PET values

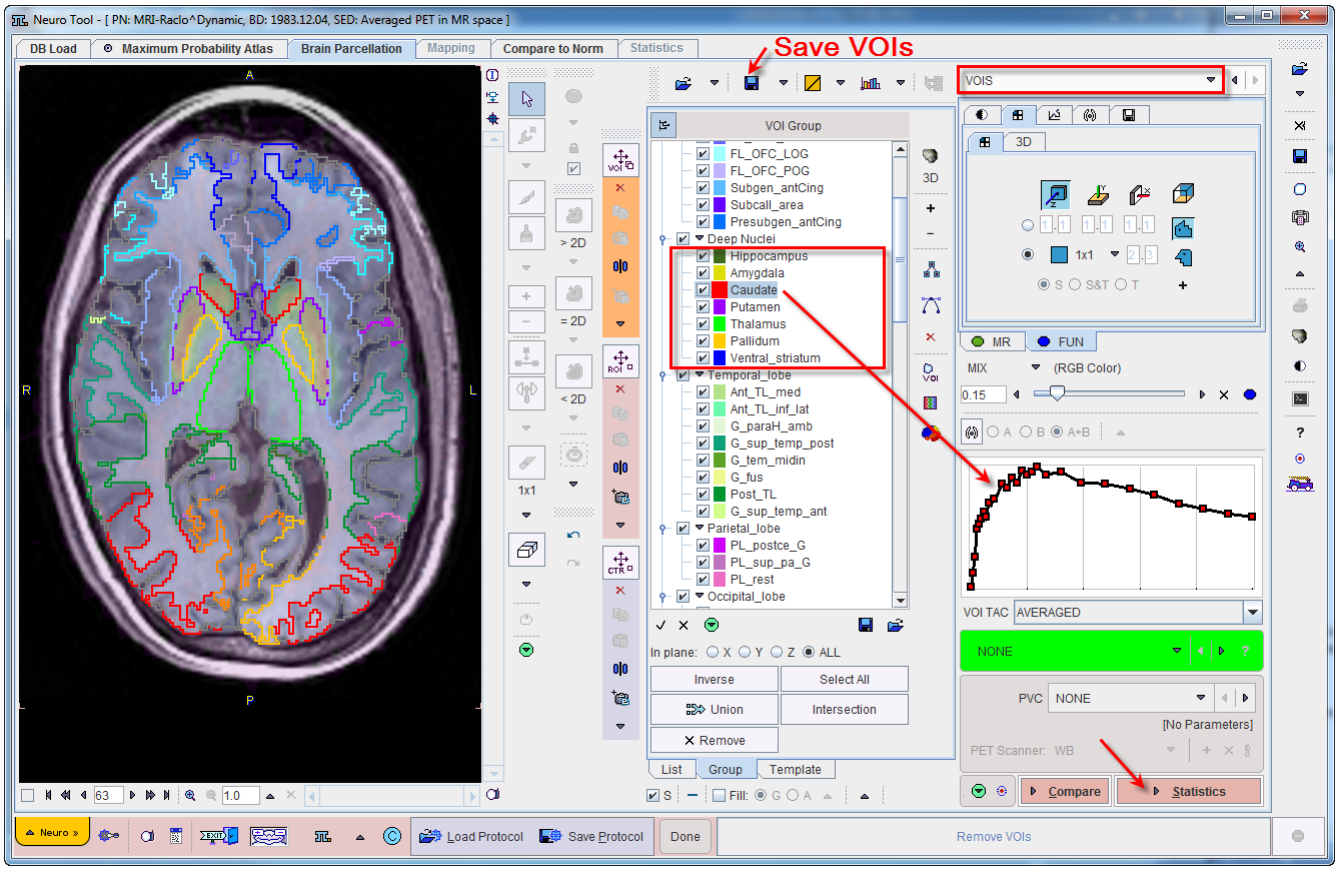
MR: The PET image is transformed to the MR space and statistics are calculated with interpolated PET values.

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.

Brain VOI Editing and Statistics Calculation

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.



VOI Editing and Selection

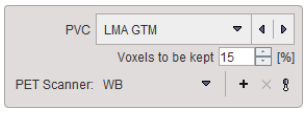
At this time the contour VOIs can be interactively adjusted using the VOI features of PMOD, which are described in the *PMOD Base Functionality Guide* and in the ***Introduction*** (on page 21). Note that the **List** tab should be selected for the adjustment, and that depending on the ***configuration*** (on page 6) only a reduced set of VOI tools is available.

Statistics Calculation

Once the VOIs are acceptable, it is recommended to first save them and then proceed with statistics calculation by the **Statistics** action button. The result (of the selected VOIs only) is shown on the separate ***Statistics*** (on page 106) page of the PNEURO tool, from where it can be further evaluated.

Partial-Volume Correction (PVC) Option

The PNEURO tool supports the GTM-based ***patial-volume correction*** (on page 28) (PVC) of the PET signal.



The **PVC** selection has three choices:

- **NONE:** No partial-volume correction is applied (default).
- **LMA GTM:** A variant of the Rousset correction method is applied, whereby only a percentage of the pixels in the inner of the VOI is used for calculating the VOI average. This percentage can be set by the **Voxels to be kept** parameter.
- **VOI BASED:** The original Rousset correction method is applied.

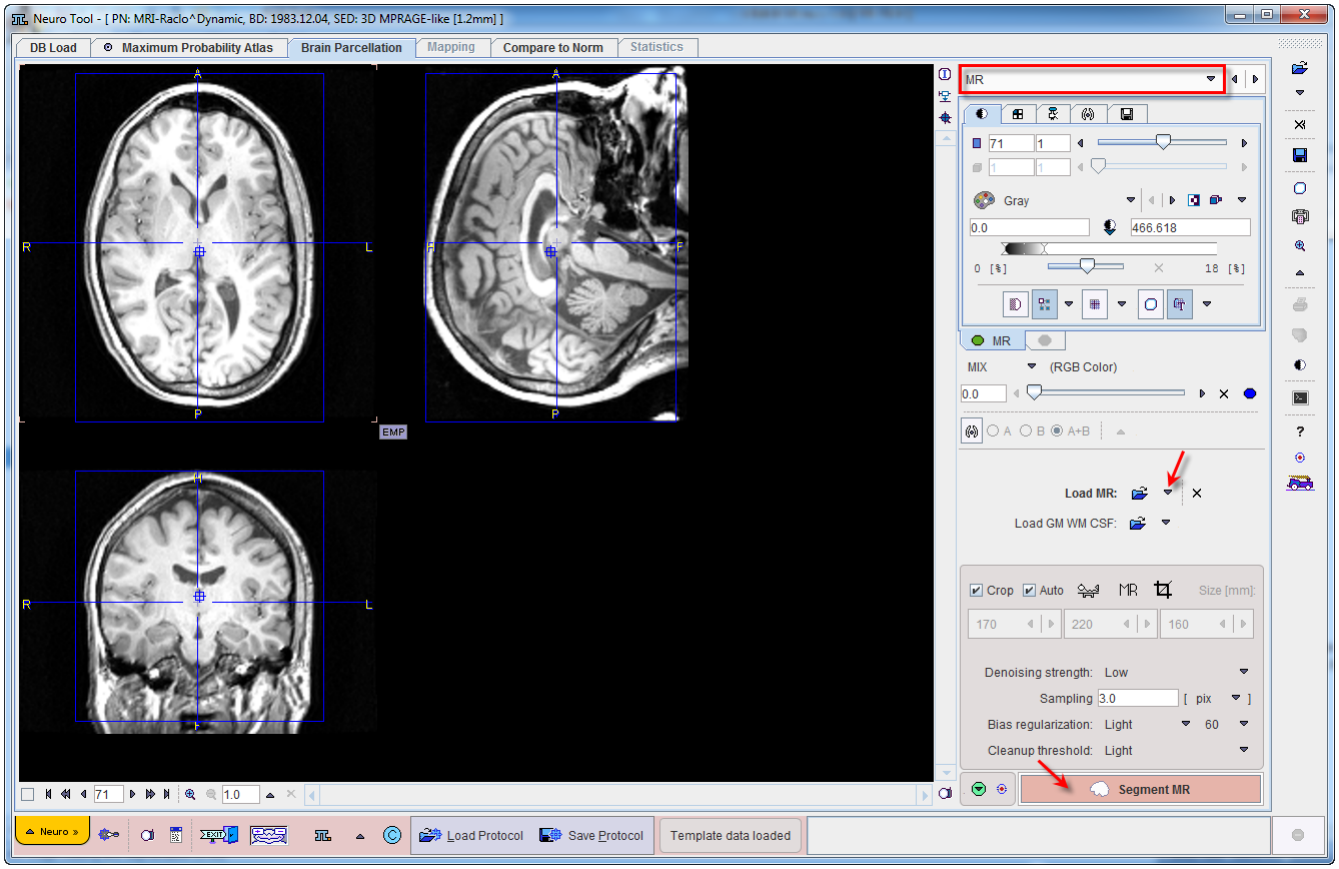
If a PVC method is used, both the original and the corrected statistics are calculated. Note that due to the high number of VOIs the PVC calculation may take several minutes and consumes a significant amount of RAM.

Workflow for MR-only Studies

MR Image Loading and Denoising

To start parcellation of a brain PET series, select the **Brain Parcellation** tab of PNEURO and activate the **MR** button in the lower right for arriving at the **MR** page.

The **MR** page allows loading the T_1-weighted brain MR image of the same patient using the **Load 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 will be segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The algorithm uses four parameters:

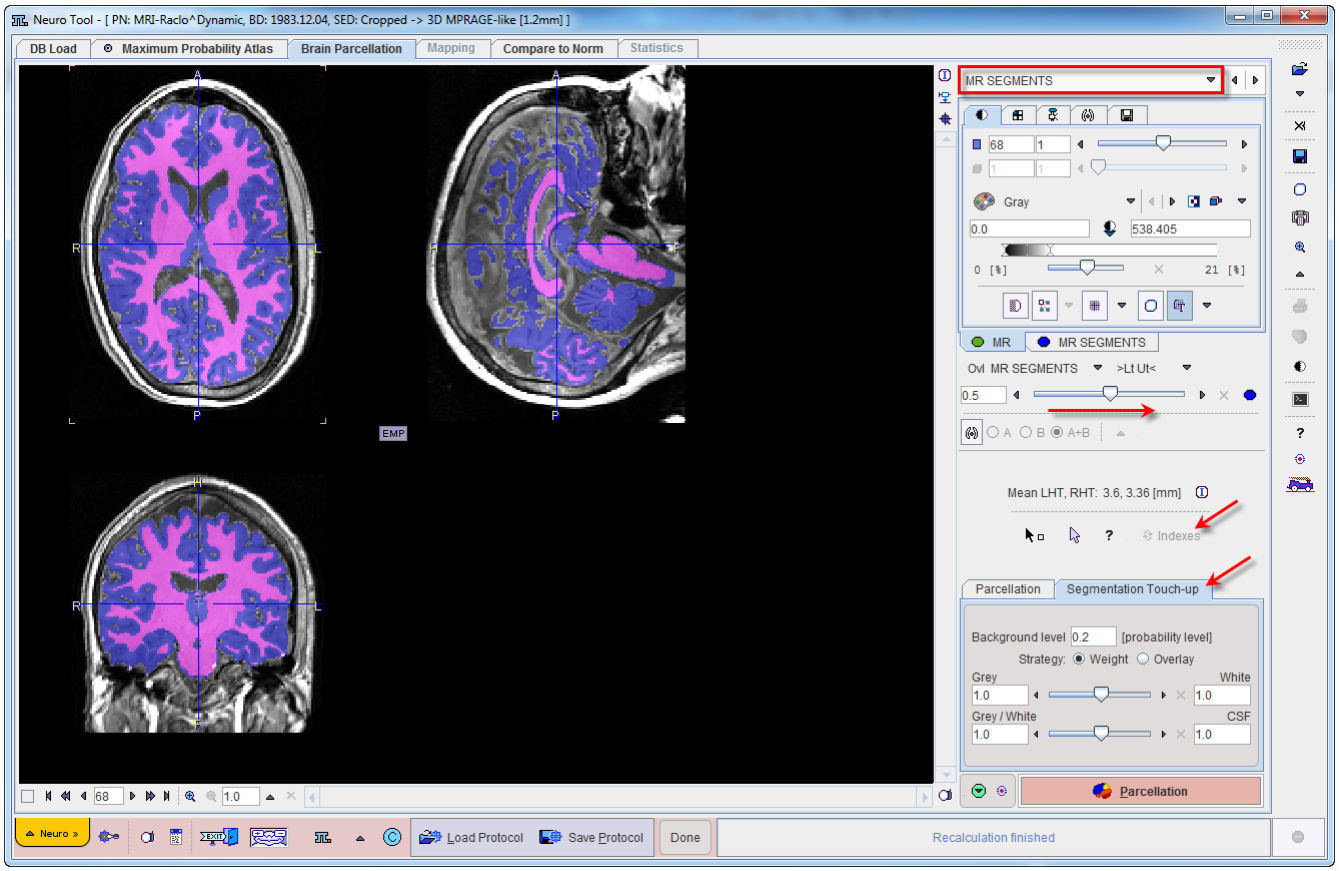
Denoising strength	Denoising of the MR image may improve the segmentation of gray matter, white matter and CSF. If a **Denoising strength** other than **None** is selected, a non-local means denoising algorithm is applied which preserves structure boundaries unless the strength is too high.
Sampling	Density of pixels considered in the calculation.
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 threshold	Procedure for rectifying the segmentation along the boundaries.

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 MR** action button. Note that the denoising and segmentation process may take several minutes.

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 **MR SEGMENTS** page. Note that the **MR 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, but requires that the **Indexes** are recalculated before the parcellation can be started.

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 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.

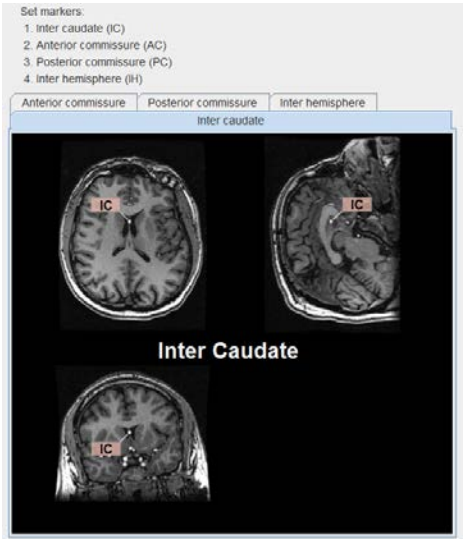
Please use the fusion slider to evaluate the segmentation quality. In case the result is not satisfactory, return to the previous page, modify the segmentation parameters, then activate **Segment MR** again.

Landmark Definition

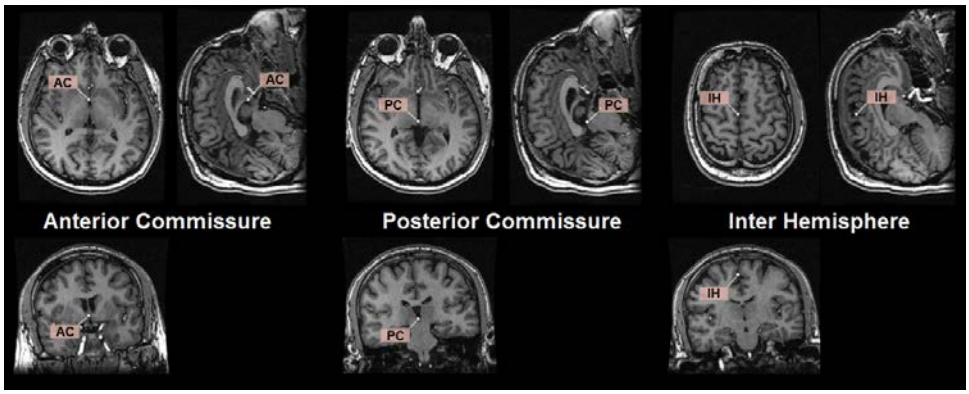
The parcellation procedure requires four anatomical landmarks

- 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.

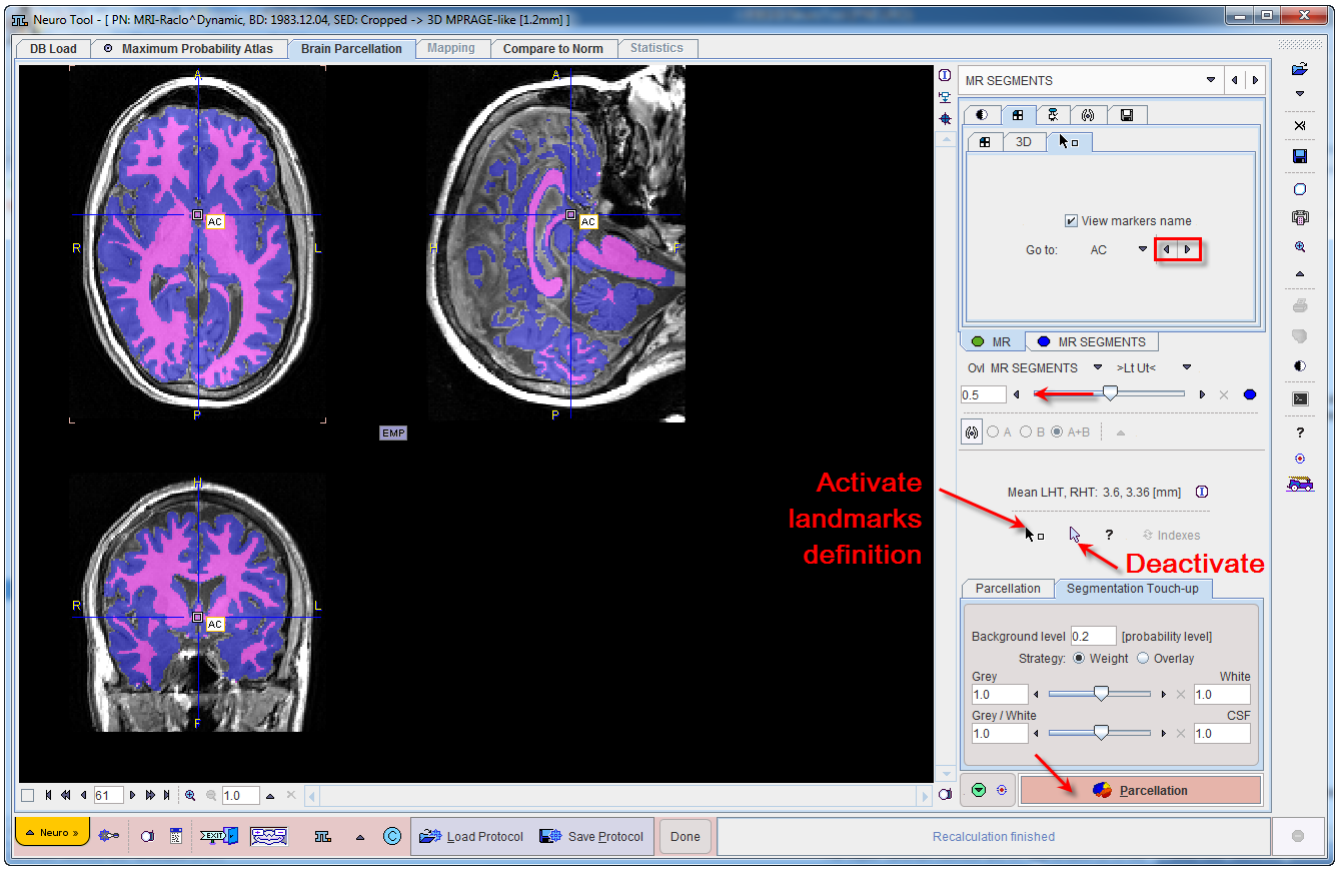


The location of the other three landmarks is illustrated below.



Estimates of the landmark locations are obtained as part of the segmentation. They can be inspected by activating the  button. The **Go to** list allows to easily select 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.



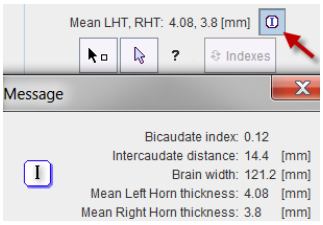
To avoid marker placement when clicking into the images for triangulation, hold down the SHIFT+CTRL keys. Note the indication of the landmark which needs to be placed (IC, AC, PC, IH) as part of the cursor symbol.

Brain Indexes

Based on the segmentation 3 indexes are calculated which will be further employed for the selection of suitable hemispheres from the knowledge base:

- Bi-caudate index: ratio of caudate distance and brain width.
- Left horn thickness (LHT): mean thickness of left horn in coronal section.
- Right horn thickness (RHT): mean thickness of left horn in coronal section

A summary of the indexes is shown in the user interface, and complete information can be opened with the button indicated below.



Parcellation

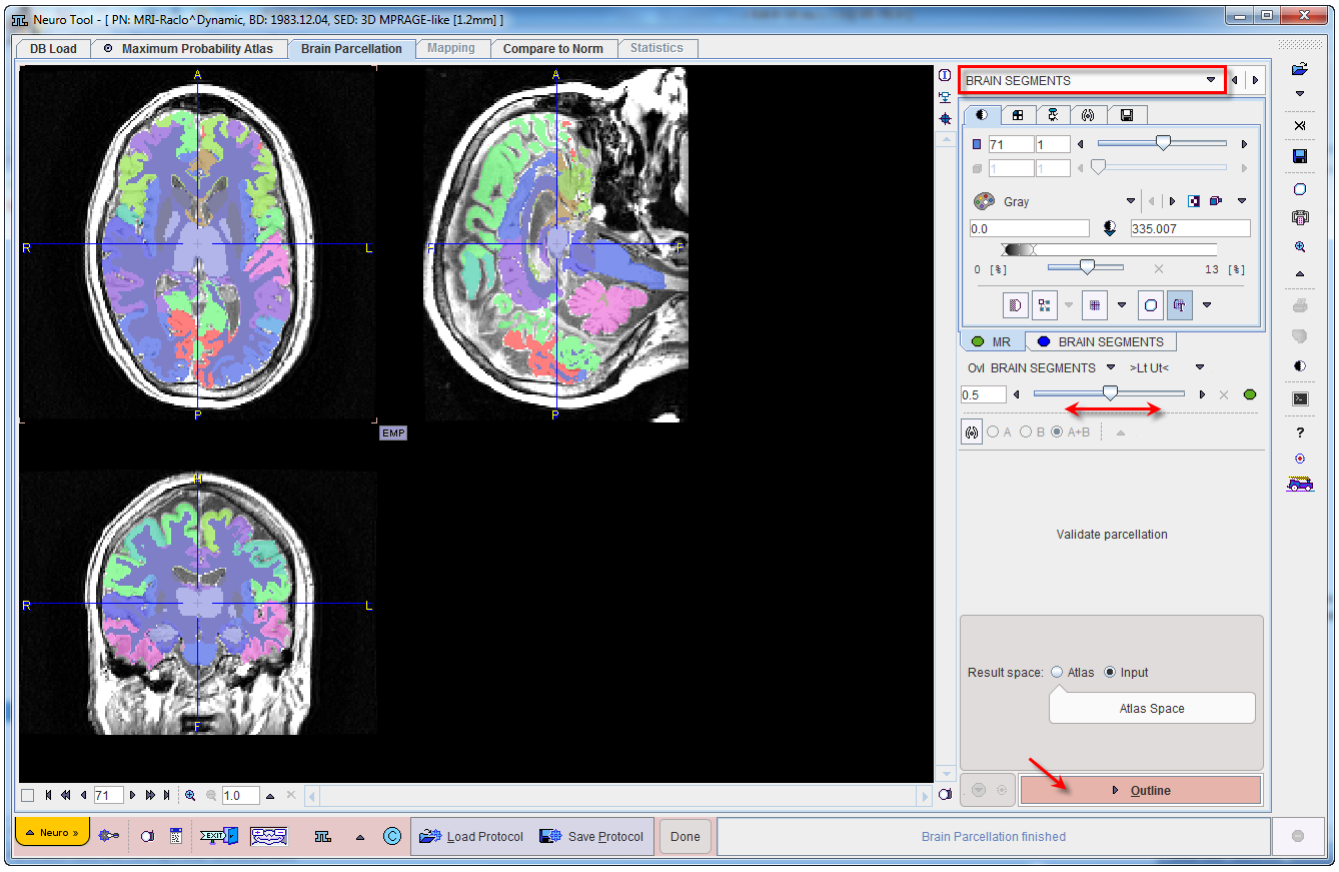
Deep nuclei parcellation calculates the brain structure shapes based on the knowledge base and the segmented MR image. Each hemisphere is processed individually. The user may select the **Number of subjects** (<=26) which are taken into account during parcellation. A higher number tends to provide more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

Sulci 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.

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.

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 found brain structures. Please check the alignment of the structure information with the patient image with the fusion slider. In the case of a mismatch try changing the GM segment definition on the **MR SEGMENTS** page and/or the number of included subjects 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 **Result space** which defines, where the MR statistics are calculated:

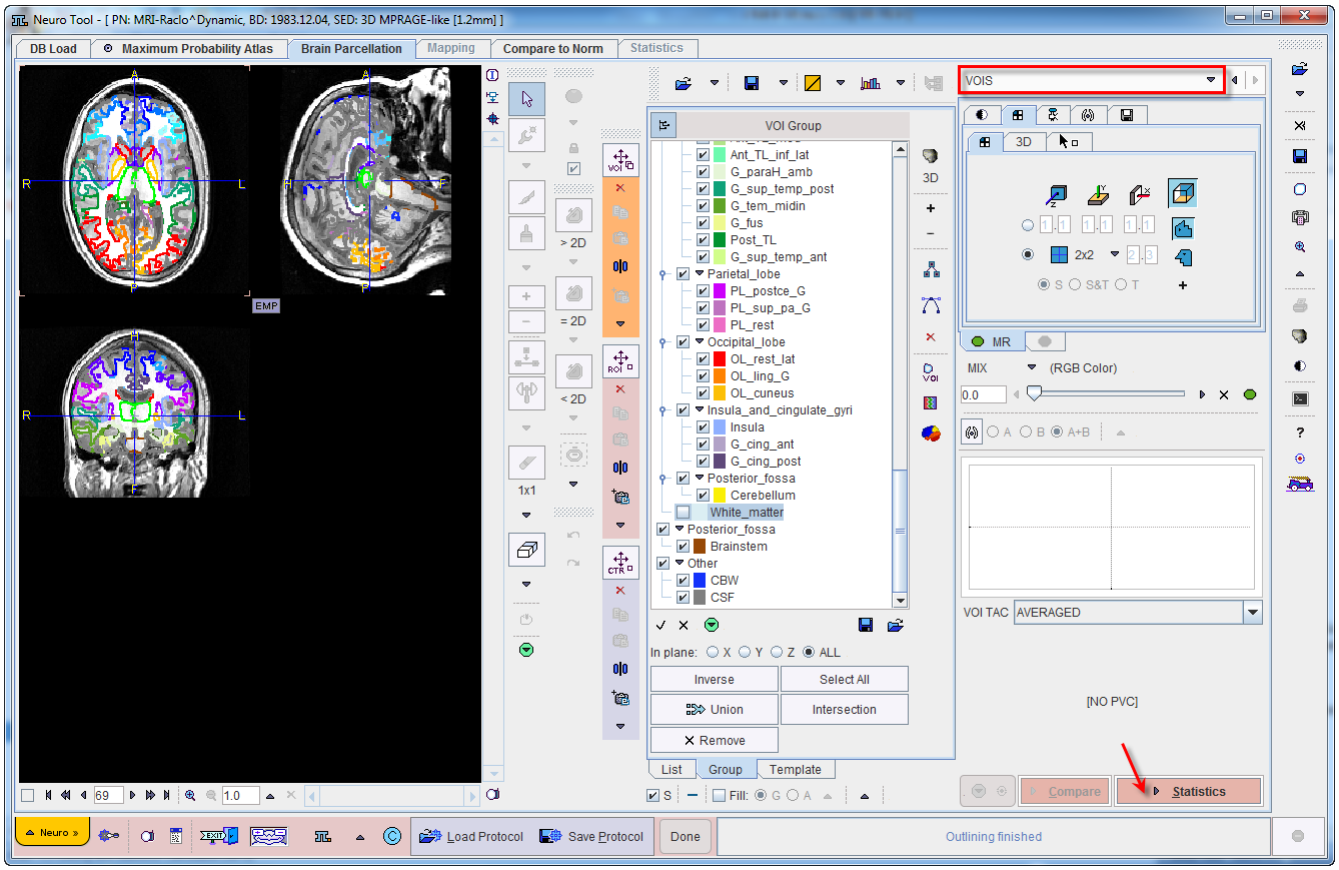
Atlas: The MR image is transformed in the Atlas space and statistics are calculated with interpolated MR values

Input: The statistics are calculated with original MR values.

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

Brain VOI Editing and Statistics Calculation

The **VOIS** page shows a fusion of the MR image and the brain segments, as well as the outlined brain structures.



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* and in the ***Introduction*** (on page 21). Note that the **List** tab should be selected for the adjustment, and that depending on the ***configuration*** (on page 6) only a reduced set of VOI tools is available.

Statistics Calculation

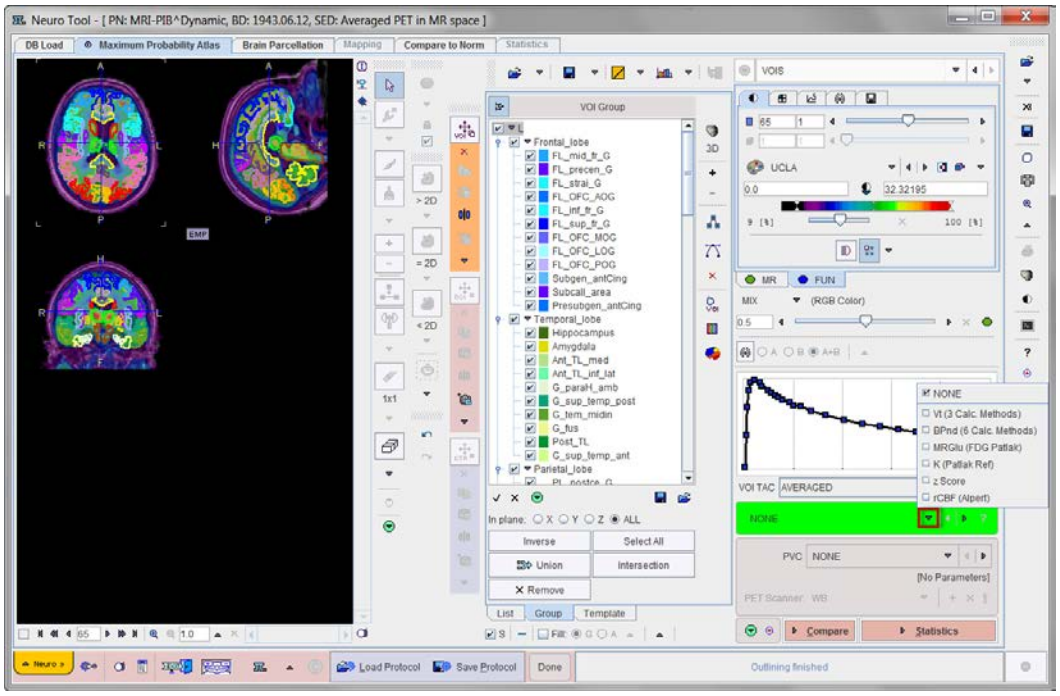
Once the VOIs are acceptable, it is recommended to first save them and then proceed with statistics calculation by the **Statistics** action button. The result (the volume of the selected

VOIs only) is shown on the separate ***Statistics*** (on page 106) page of the PNEURO tool, from where it can be further evaluated.

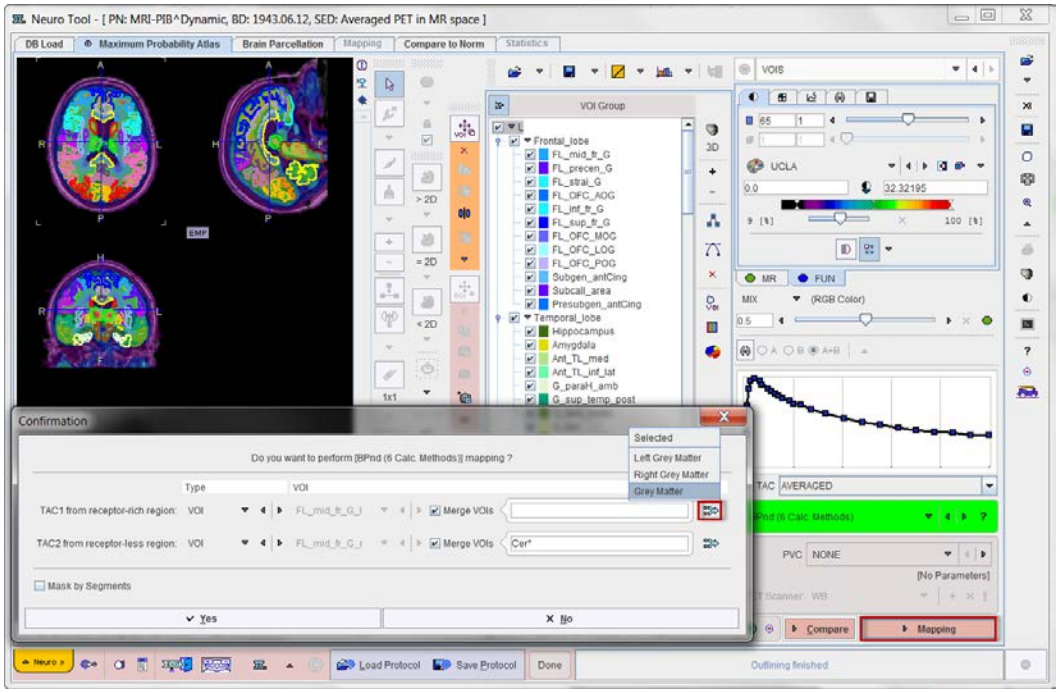
Parametric Mapping

If the pixel-wise modeling tool PXMOD has been licensed, PNEURO includes a parametric **Mapping** page. This integration has the advantage, that the VOIs generated by PNEURO can be used during the PXMOD model configuration, and that the resulting parametric maps can directly be regionally analyzed.

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.



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.



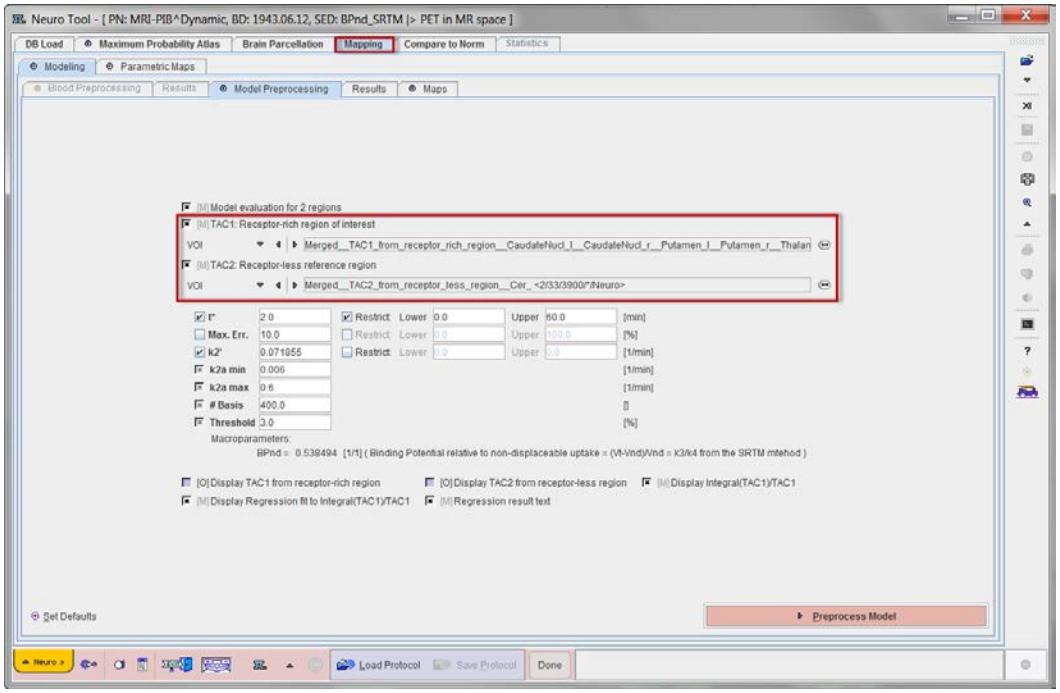
It requires two tissue time-activity curves for a preprocessing step, one representing a **receptor-rich region**, and one a **receptor-less** reference region. There are three options to specify them: **FILE**, **VOI** and **TAC(DB)**. **VOI** is the obvious choice, as the PNEURO-generated VOIs can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

There are two ways of defining 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**) VOI lists can be generated. **Selected** shows a dialog window for defining any VOI combination.

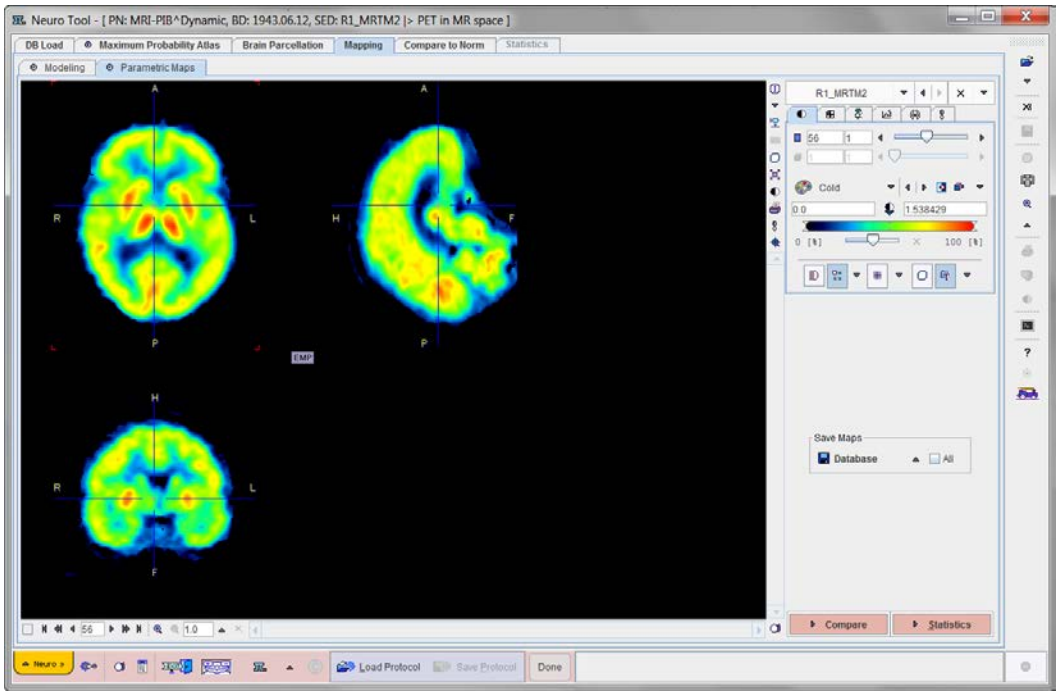
The **Mask by Segments** option serves for restricting the mapping procedure to the brain pixels. This might speed calculation significantly up.

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.

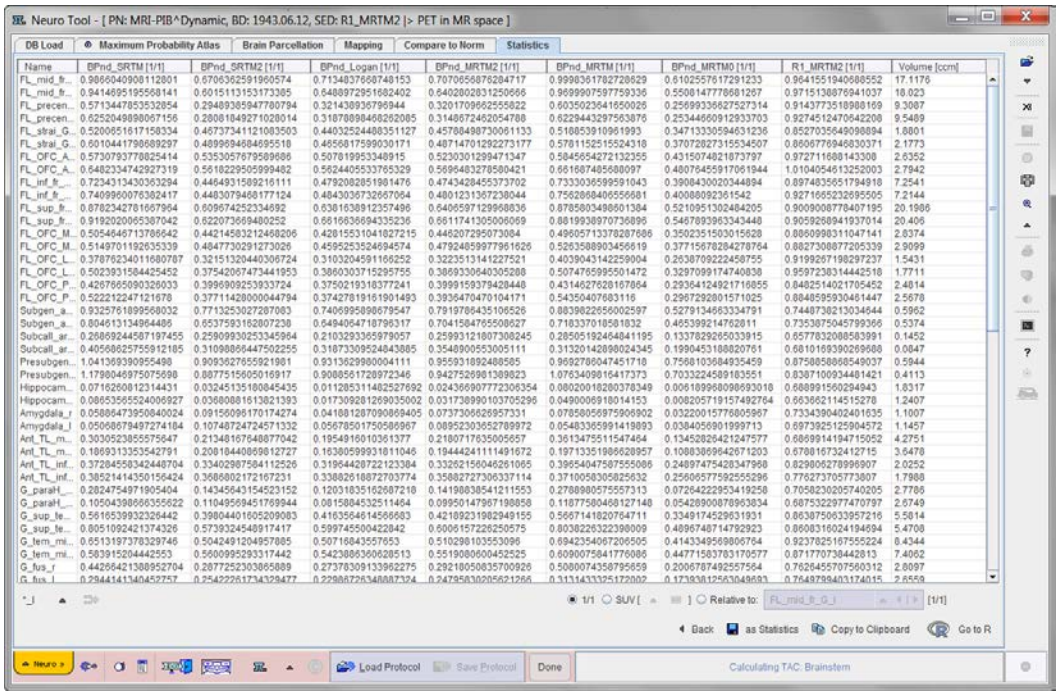
After confirming with **Yes** the program enters the **Mapping** page which hosts the PXMOD functionality. In the example below mapping start at the **Model Preprocessing** page, because there is no blood data involved. Note that the VOI definitions are already configured according to the TAC1/TAC2 definition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



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



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 parametric maps can be sent to the **Compare to Norm** 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

Statistics Results

PNEURO has to be compliant with the space of the normal database.

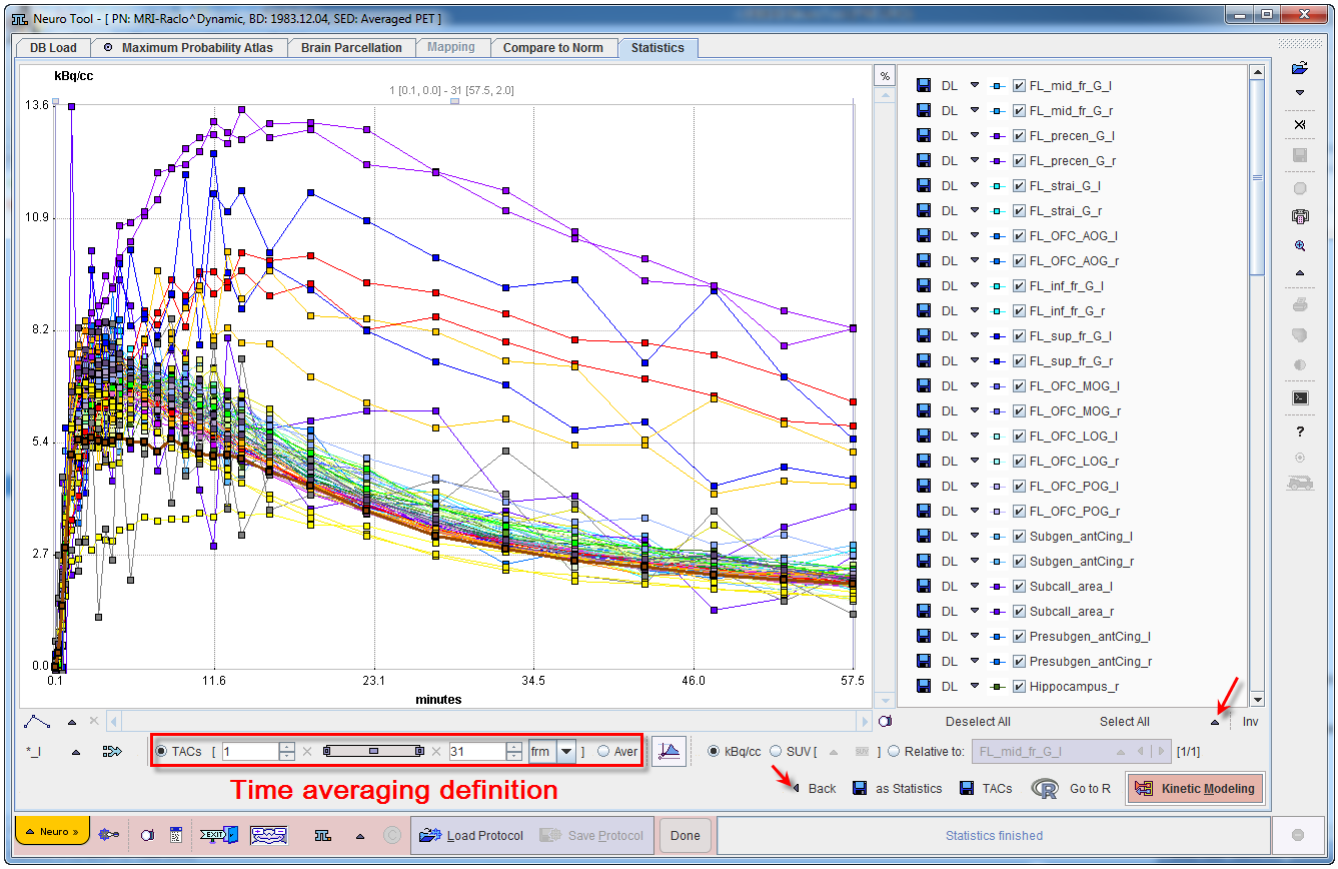
The statistics output from the PNEURO VOI modules is shown on the dedicated **Statistics** page. It shows

- 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% .

Statistics of Dynamic PET Data

The example below illustrates the result of deriving brain VOIs by a T_1-weighted MRI parcellation, and applying them to a dynamic raclopride PET in order to calculate regional TACs.

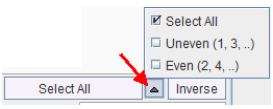


The **Back** arrow in the lower right is a convenience button for switching back to the PNEURO module which generated the statistics.

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* (on page 59) before calculating the actual statistics.

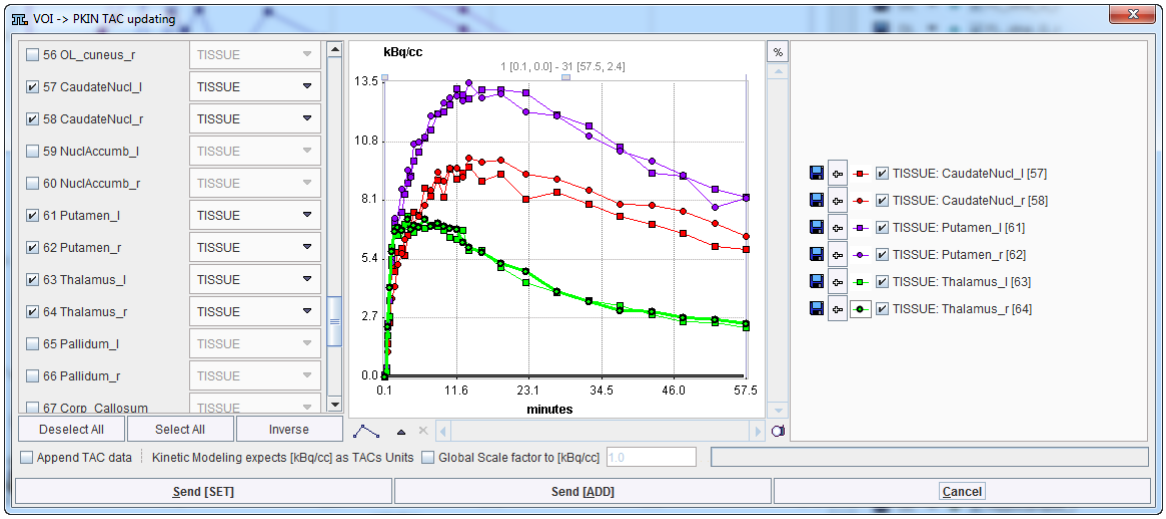
An alternative is to select the relevant curves in the list to the right. There are some convenience buttons supporting this selection: **Deselect All** switches all curves off. **Select All** switches all curves on. **Inverse** inverts the current selection. Note the arrow button which allows to quickly 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 directly transferring dynamic tissue TACs 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 [SET]** 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 [ADD]** first creates a new workspace in PKIN before actually transferring the selection.

Saving the Statistics

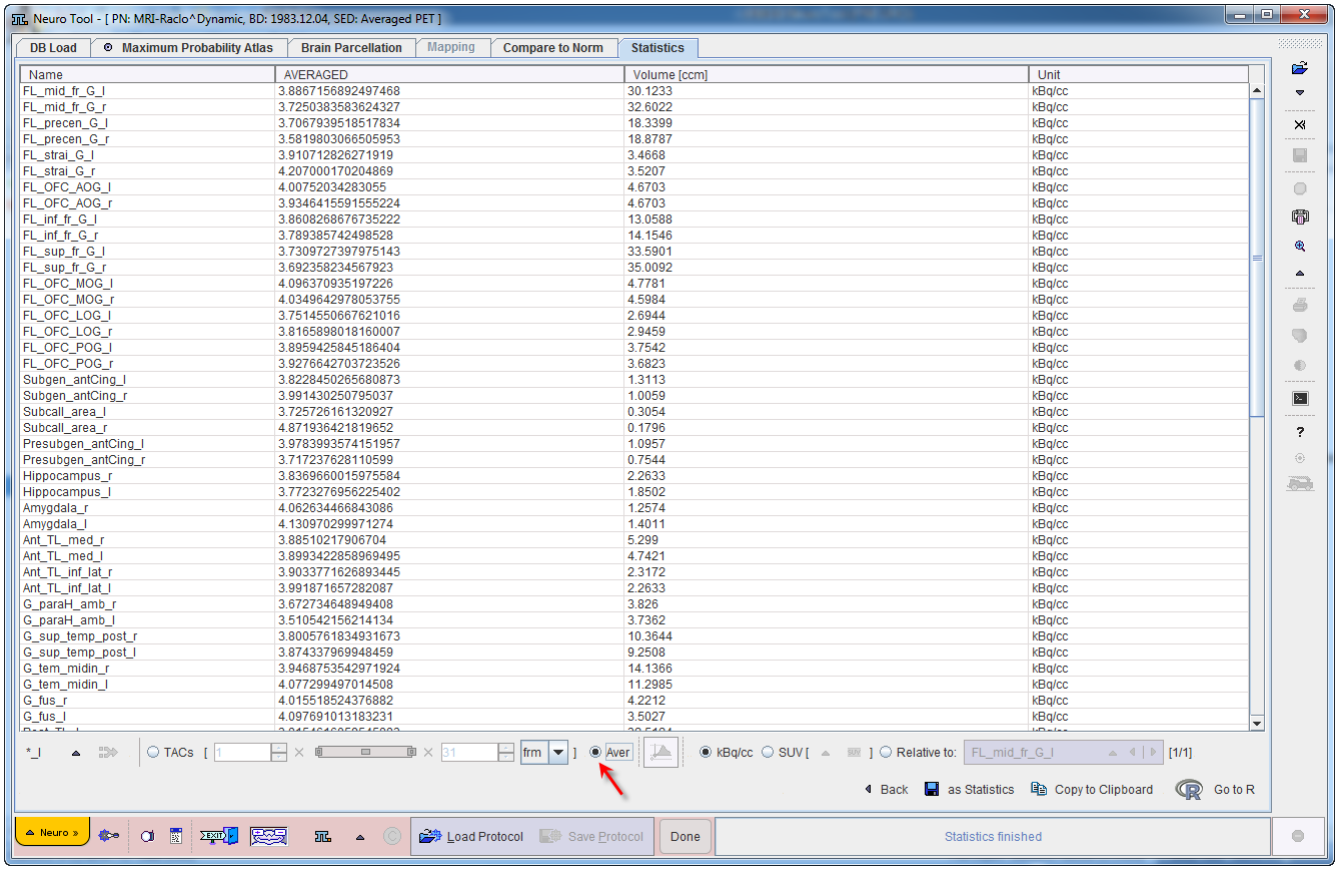
The **Save TACs** button allows saving all curves in a simple tab-delimited text file as illustrated below. Such a file can easily be imported into other programs for processing and visualization.

	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.

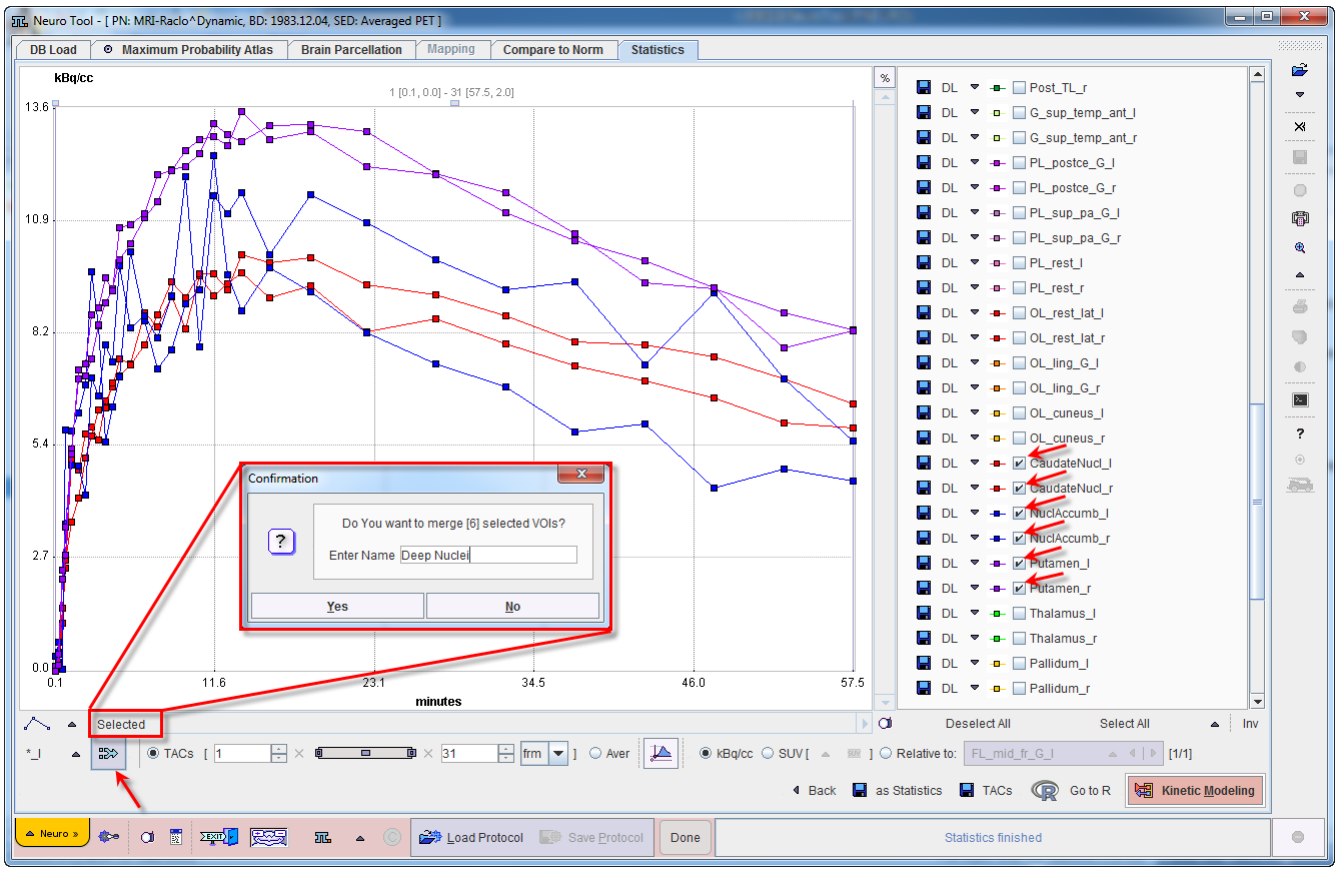
Average in Frame Range

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.

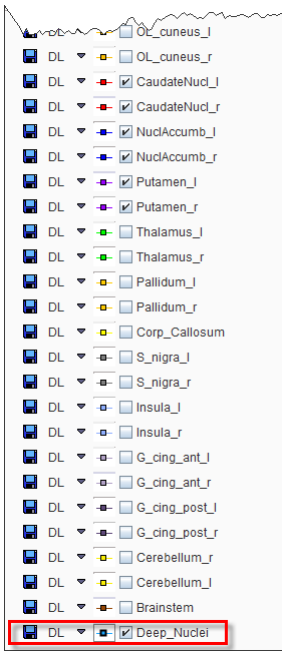


Average TACs

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



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

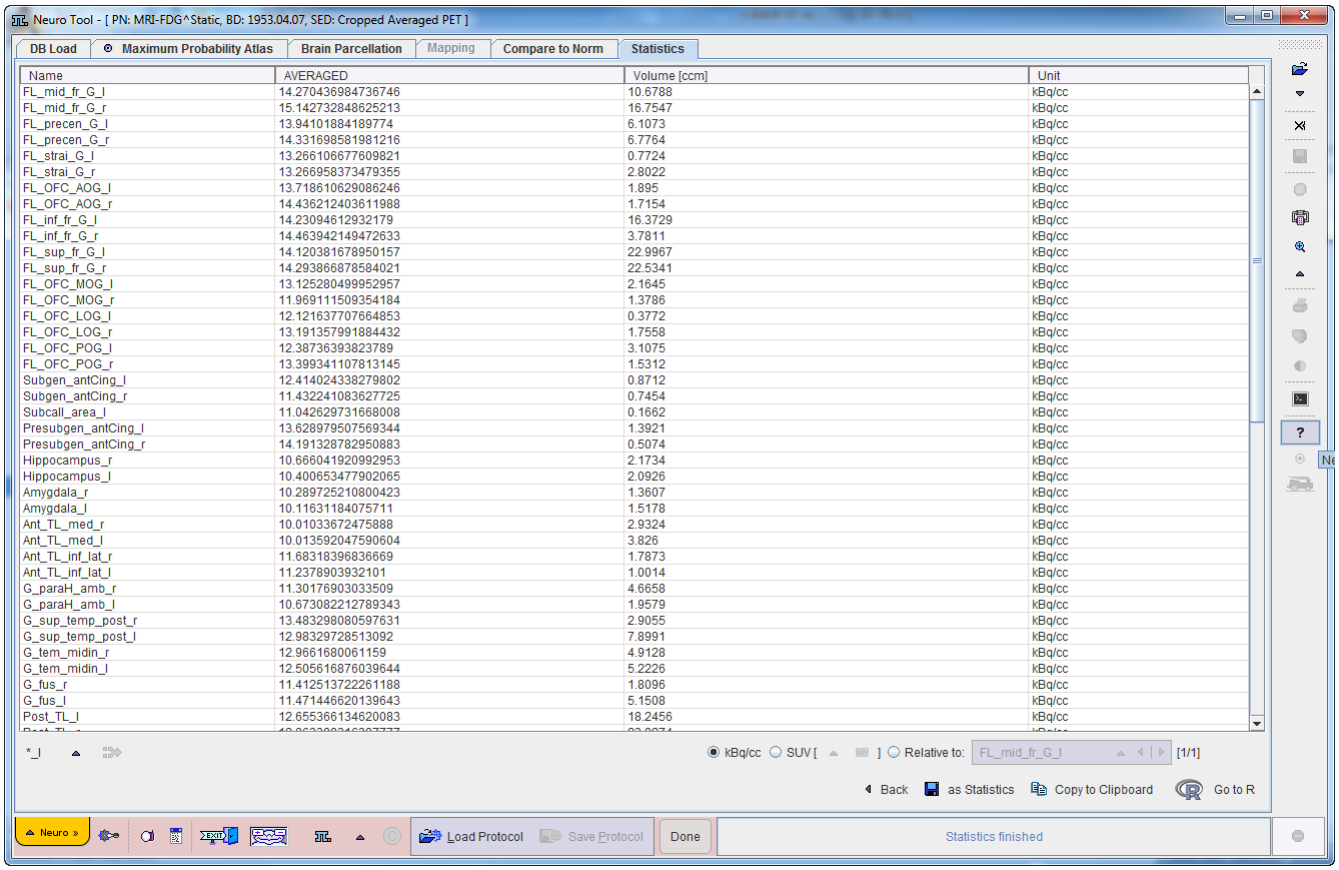


Transfer of Statistics to R

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

Statistics of Static Data

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



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

Transfer of Statistics to R

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

Brain Norm Functionality

The **Compare to Norm** module of PNEURO supports the creation of normal brain PET databases from a consistent set of normal volunteer images and its application for analyzing patient images.

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 patient 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 patient can be compared with the normal pattern. To this end, the patient 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 patient 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 patient images and 3D rendering (separate option).

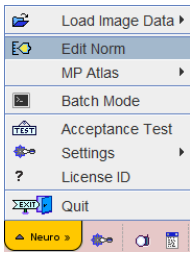
Note: The same type of analysis is also possible with SPECT images.

Brain Norm Creation

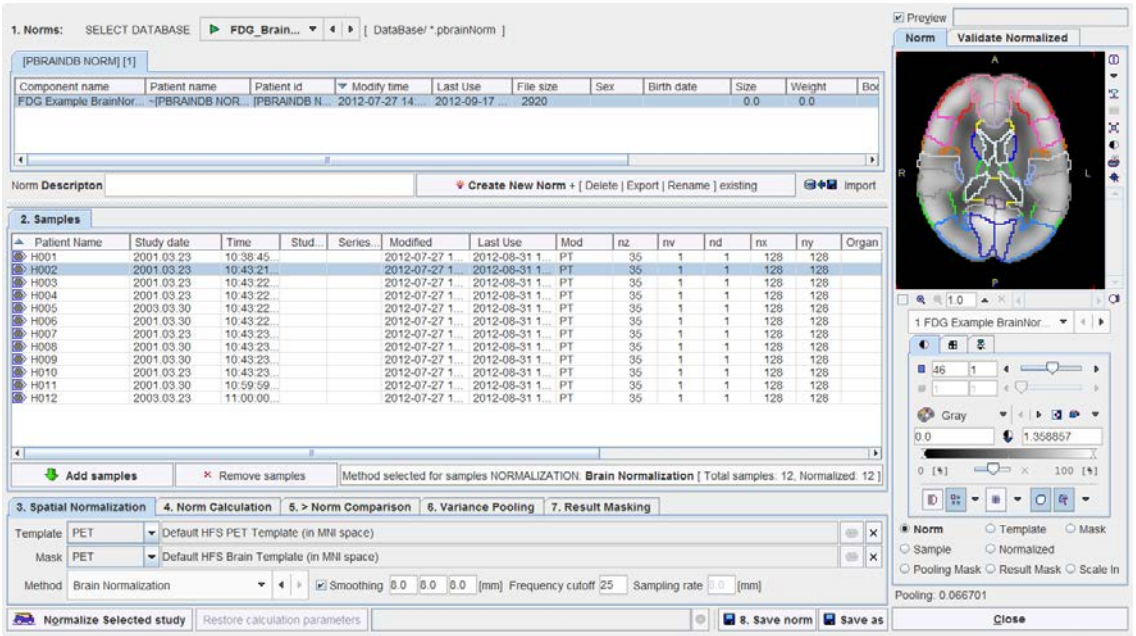
The creation of a Brain Norm requires the availability of a number of control images. These images must beforehand be saved in a PMOD database, where they are in DICOM format and include information such as the age or sex of the controls, and the anatomical image orientation. New controls can always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

Brain Norm Editor

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



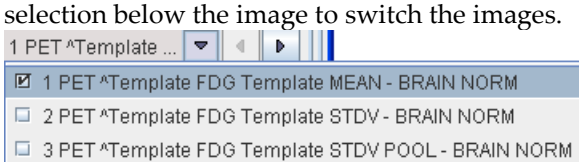
A dialog window appears which supports the creation of a new Brain Norm in a step-by-step manner. These steps are explained in the following sections.



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

Template Show the template for stereotactic normalization.

Mask Show the normalization mask.

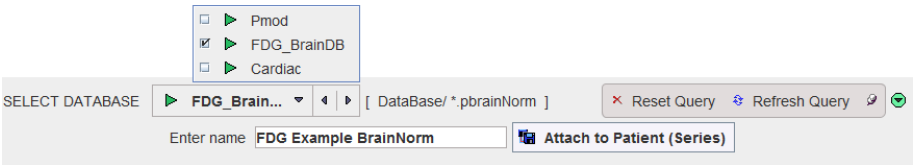
Sample	Show the original images of the currently selected control sample.
Normalized	Show the images of the currently selected control sample after spatial normalization.
Scale In	Show the mask used for finding the reference value for scaling.
Pooling Mask	Show the mask used for the averaging of the standard deviations.
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. 
Result Mask	Mask outside which the database comparison is cleared.

Brain Norm Creation

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

1. Create empty Brain Norm

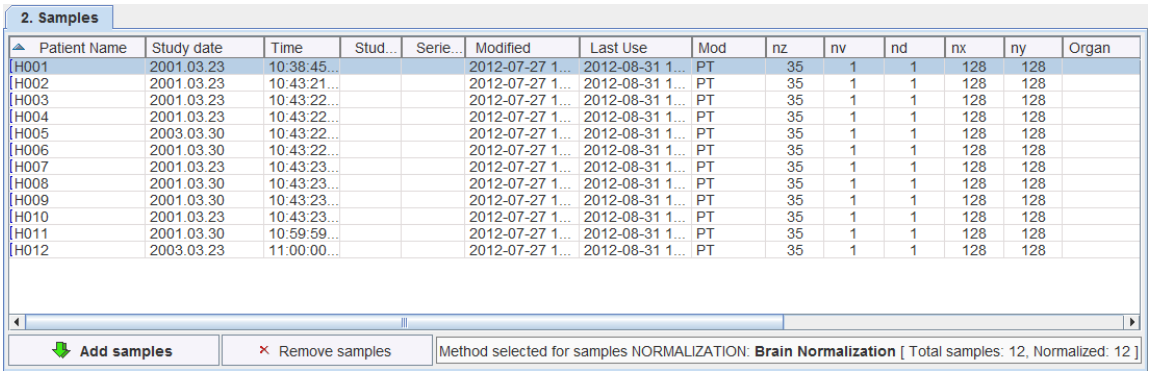
Activate the **Create New Norm** button. In the appearing dialog select the PMOD database in which the Brain Norm definition will be saved,



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

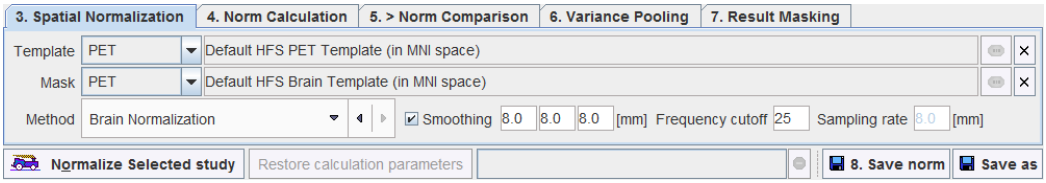
2. Add Control Samples

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



3. Specify Normalization Template and Normalize Samples

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



The **Template** selection serves for defining the template image which will be the reference for normalizing to the stereotactic anatomy. It offers the choice of the **PET** and **SPECT** MNI templates, as well as a **User defined** entry for using custom templates. With **User defined** 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 the PNEURO tool and import it into the database.

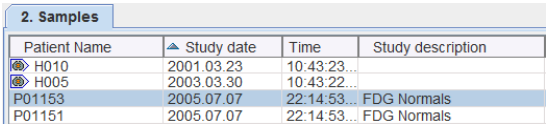
A mask is required to cut off signal from outside the brain during the normalization. It is specified using the **Mask** selection. For the standard **PET** and **SPECT** templates there are default masks available, for **User defined** templates the user has to select an appropriate mask series in the database.

The **Method** selection allows choosing the method applied for the stereotactic normalization. It is recommended using **Brain Normalization**, but an older version is still available for compatibility purposes.

To the right of the method, its parameters are available for editing. 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). Based on the smoothing, 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.

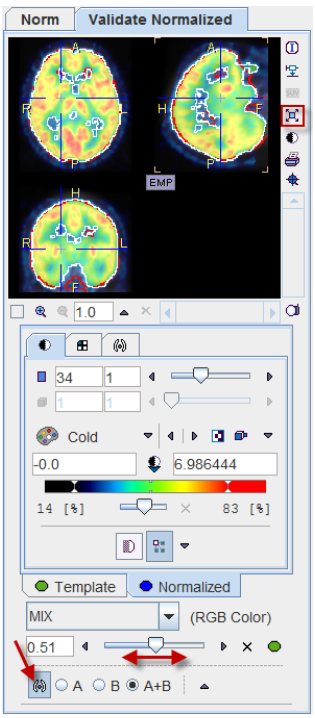
After the normalization method has been fully specified, the samples in the list can be selected and normalization started with the **Normalize Selected** button.



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 patient anatomy to the stereotactic template cannot be avoided, samples showing gross deviations should be excluded from the analysis.

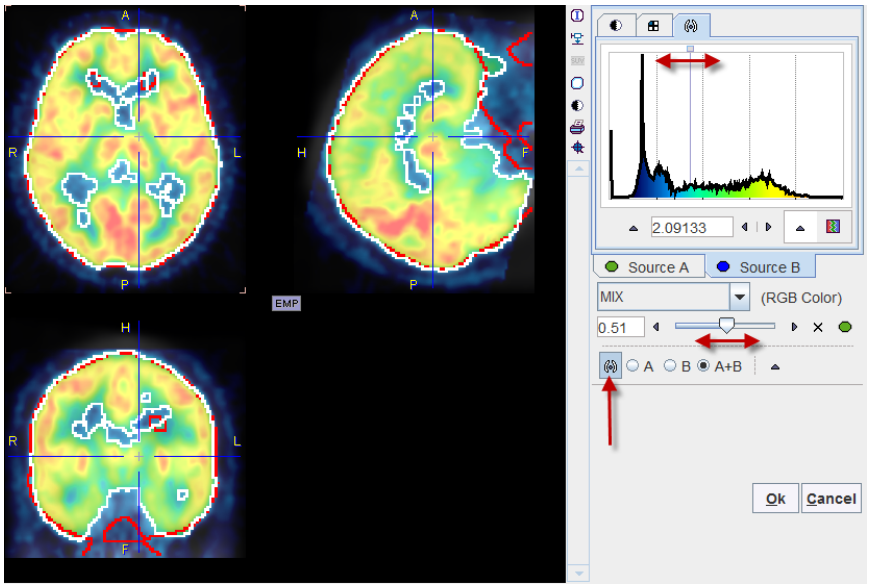
A fusion display supporting the quality control of spatial normalization is integrated in the **Edit Norm** tool. When the **Preview** box is checked and the **Validate Normalized** tab is selected, the image display shows a fusion of the template with the normalized sample image.



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.

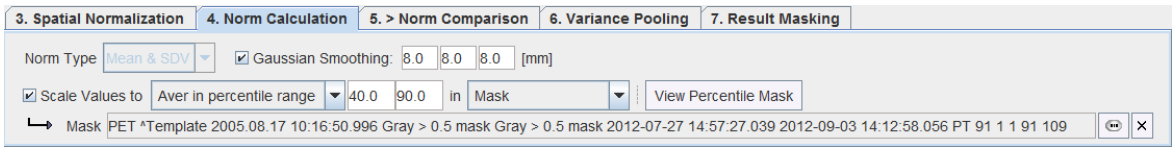
The easiest way to quickly check all normalized samples in the database is to switch to the orthogonal view of the **Validate Normalized** tab, select the first entry in the **2. Samples** list, and with the ARROW DOWN keyboard key browse through the list entries. Each time a new sample is selected the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

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



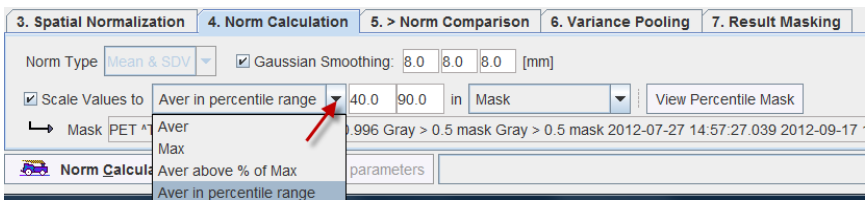
4. Define and Calculate the Database

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.

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.

In the example shown above the average is calculated of all pixels of a gray matter probability mask with p>0.5.

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**.

5. Define Comparison Filters

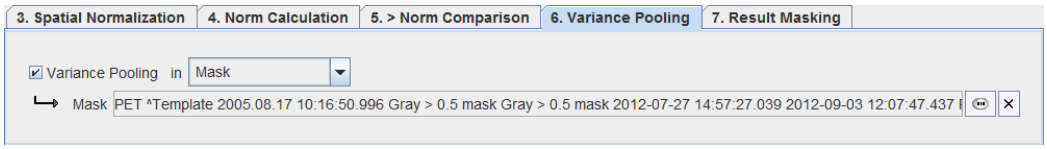
The patient images which are to be compared against the Brain Norm are also first normalized. The smoothing filter during this normalization may differ from the filter applied to the control image normalization and can be specified with the **Normalization Gaussian Smoothing** values.

Due to the pooling of many samples and some optional filtering, the database is inherently smoothed. Therefore, some smoothing of the normalized patient 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*** (on page 59) from one of the VOI outlining modules in PNEURO.

6. Define the Variance used for z-Score Calculation

When a normalized patient 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 **6. Variance Pooling** tab should be enabled.

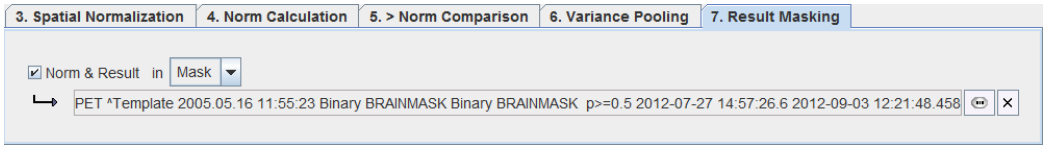


With this setting the user needs to define a spatial domain, within which the pixel standard deviations are averaged to derive a pooled standard deviation which will be used for all pixels. A **VOI**, a **Mask**, or the whole **Normalized sample** can be employed for variance pooling.

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

7. Define Result Mask

Finally, a **Result Mask** may be defined outside which the calculation results are cleared because they are meaningless. This definition is available on the **7. Result Masking** tab, and a **VOI** or a **Mask** can be employed.



8. Save Brain Norm

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.

Sharing Databases

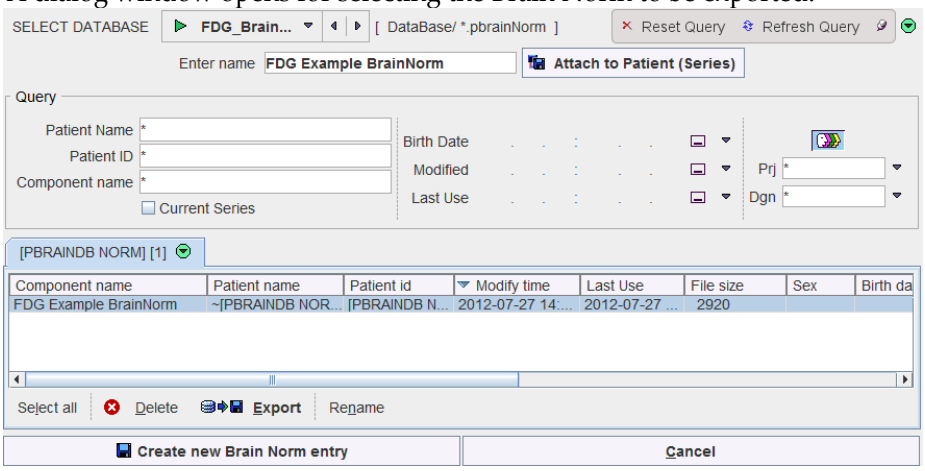
Establishing a Brain Norm requires a substantial effort. Therefore, a brain database represents a valuable asset which should be sharable with others. The **Edit Norm** tool supports the easy export/import of databases. Note that only the compiled information is exported, not the original control data sets, so it can only be modified by the owner of the control image data.

Exporting a Brain Norm

1) Open **Edit Norm**.

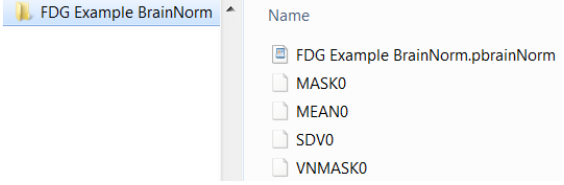
2) Activate the button **1. Create new norm + [Delete|Export|Rename] existing**.

3) A dialog window opens for selecting the Brain Norm to be exported.



4) In the **SELECT DATABASE** list select the database containing the Brain Norm to be exported, and then select the appropriate entry in the list of Brain Norms.

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.



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.

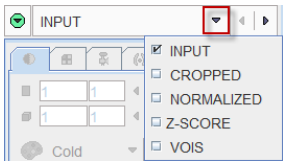
3) Activate the **Import** button. In the appearing file selection dialog navigate to the directory containing the exported files, and select the definition file (e.g. **FDG Example BrainNorm.pbrainNorm**).

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 analyses.

Compare to Norm Patient Data Analysis

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

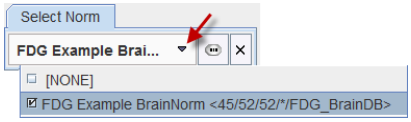


To start a patient brain PET analysis select the **Compare to Norm** tab.

Brain Norm Selection

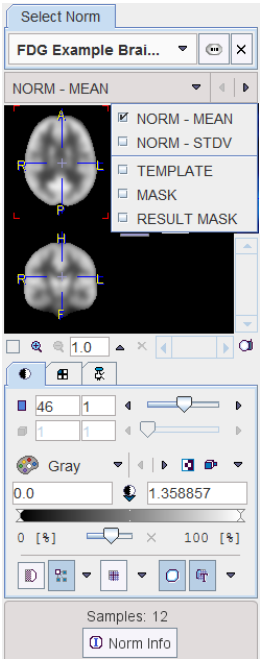
The Brain Norm against which the data should be compared can be configured on the **Select Norm** panel to the left of the **Compare to Norm** page. If the panel is hidden, it can be shown with the button in the upper right,

which will become .



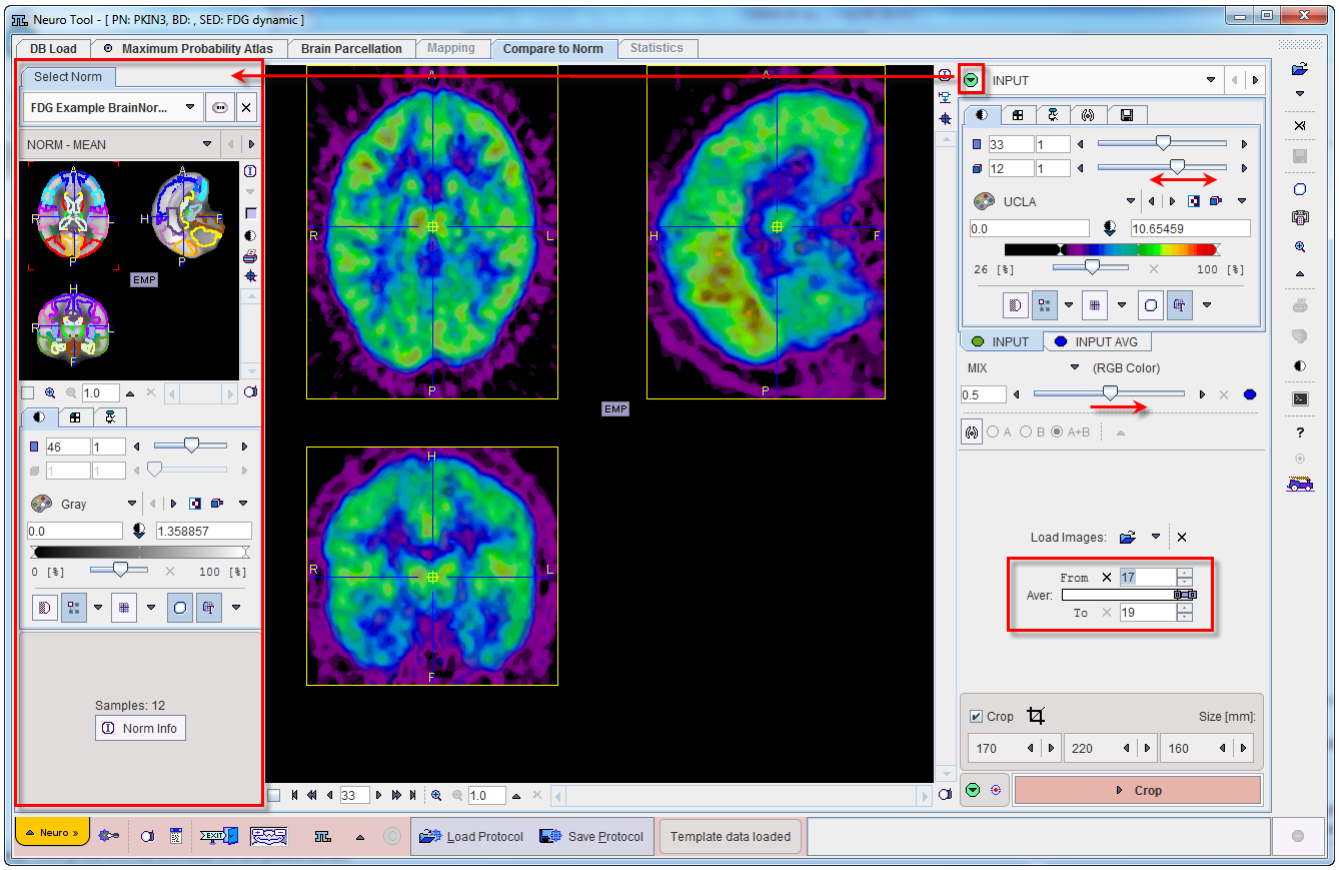
Use the arrow indicated above to show the list of Brain Norms available in the current database and select the appropriate one. If the Brain Norm is stored in a different database, the current database can be changed using the button.

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]**.

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.

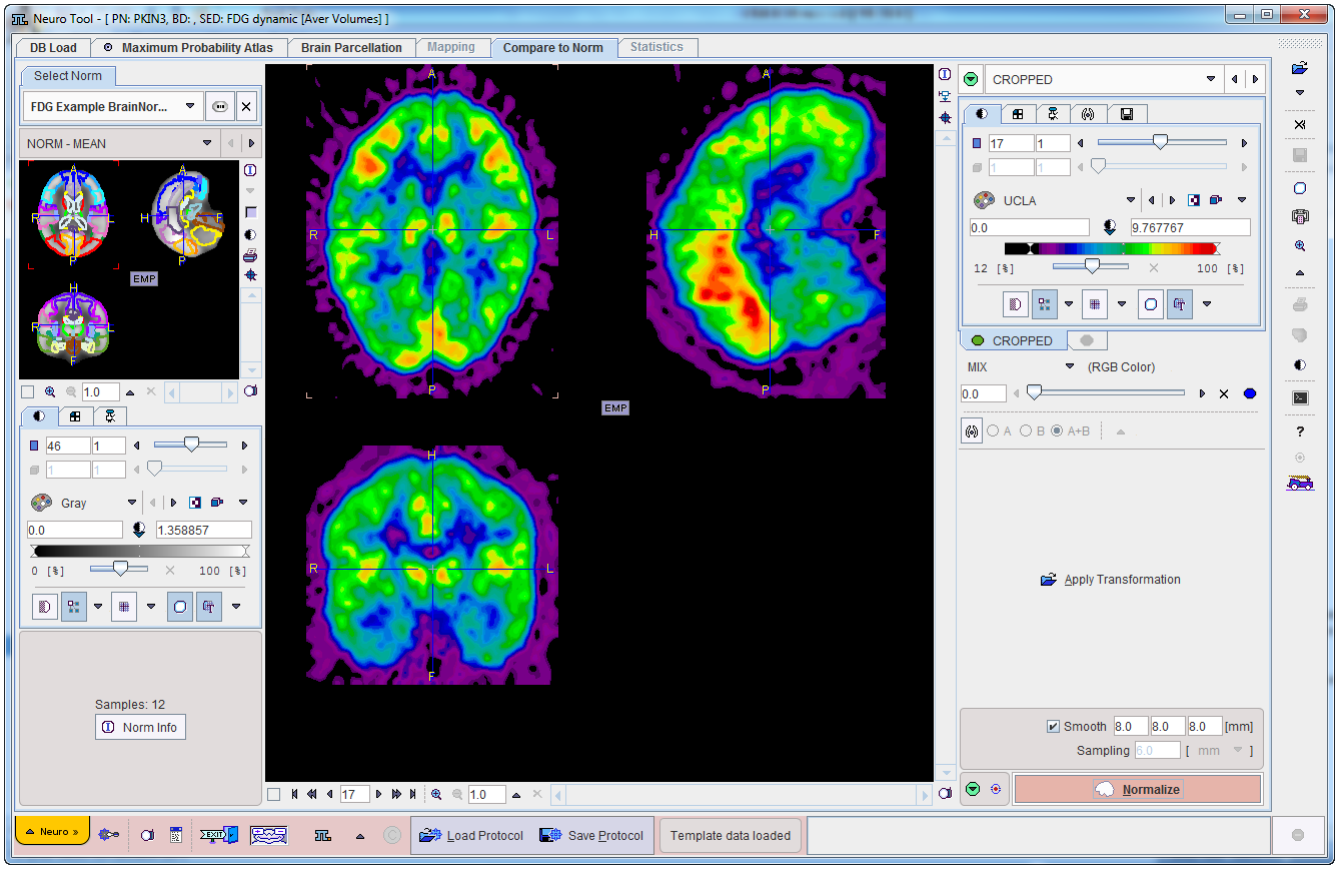
PET Image Cropping

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 **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.

Template-based Normalization

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

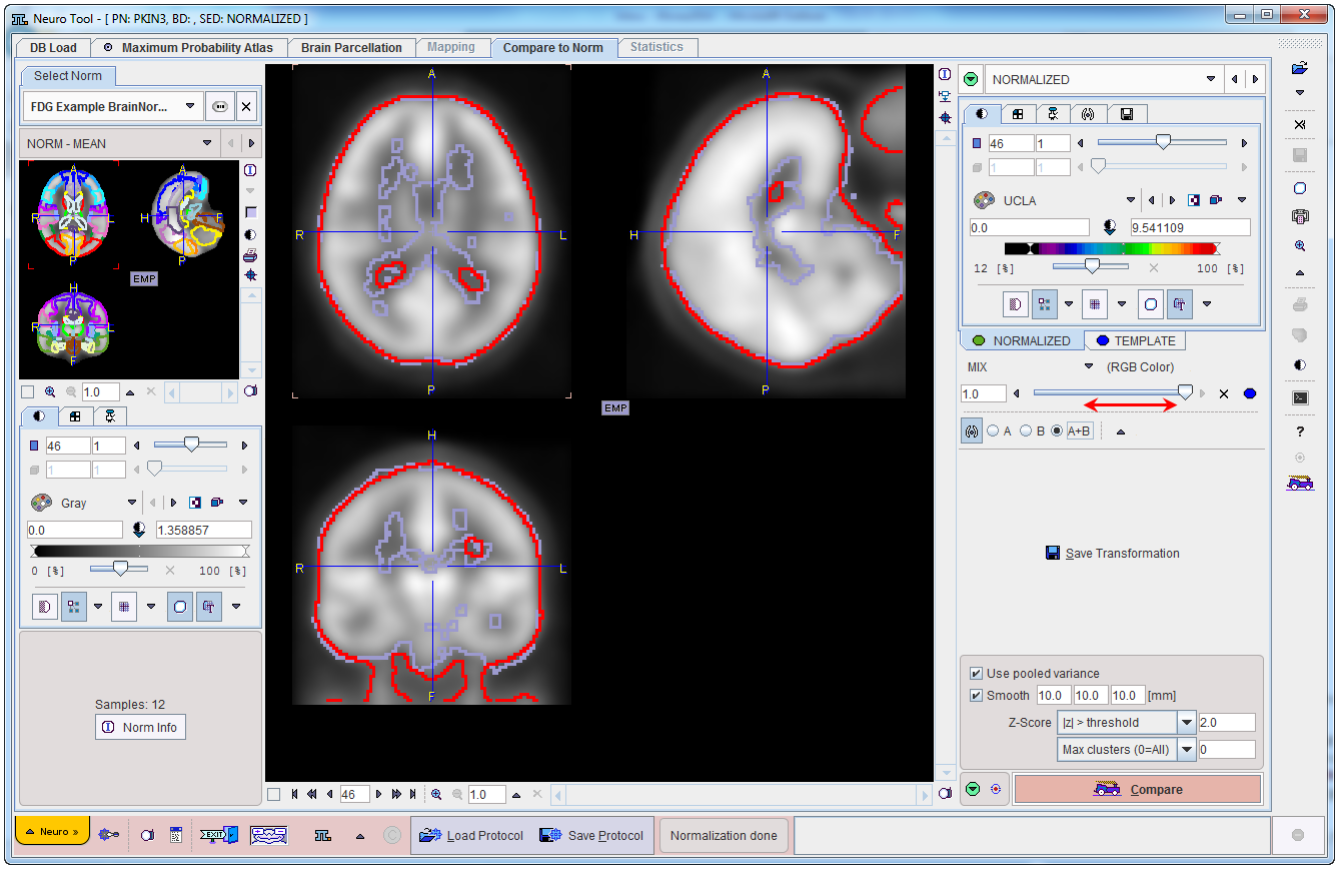


Assuming that the appropriate Brain Norm has been selected, the only task on this page consists of configuring the spatial normalization in the lower right. These parameters are already set based on the Brain Norm definition parameters. The proposed values can be overwritten if needed, for instance if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

Quality Control of Normalization

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



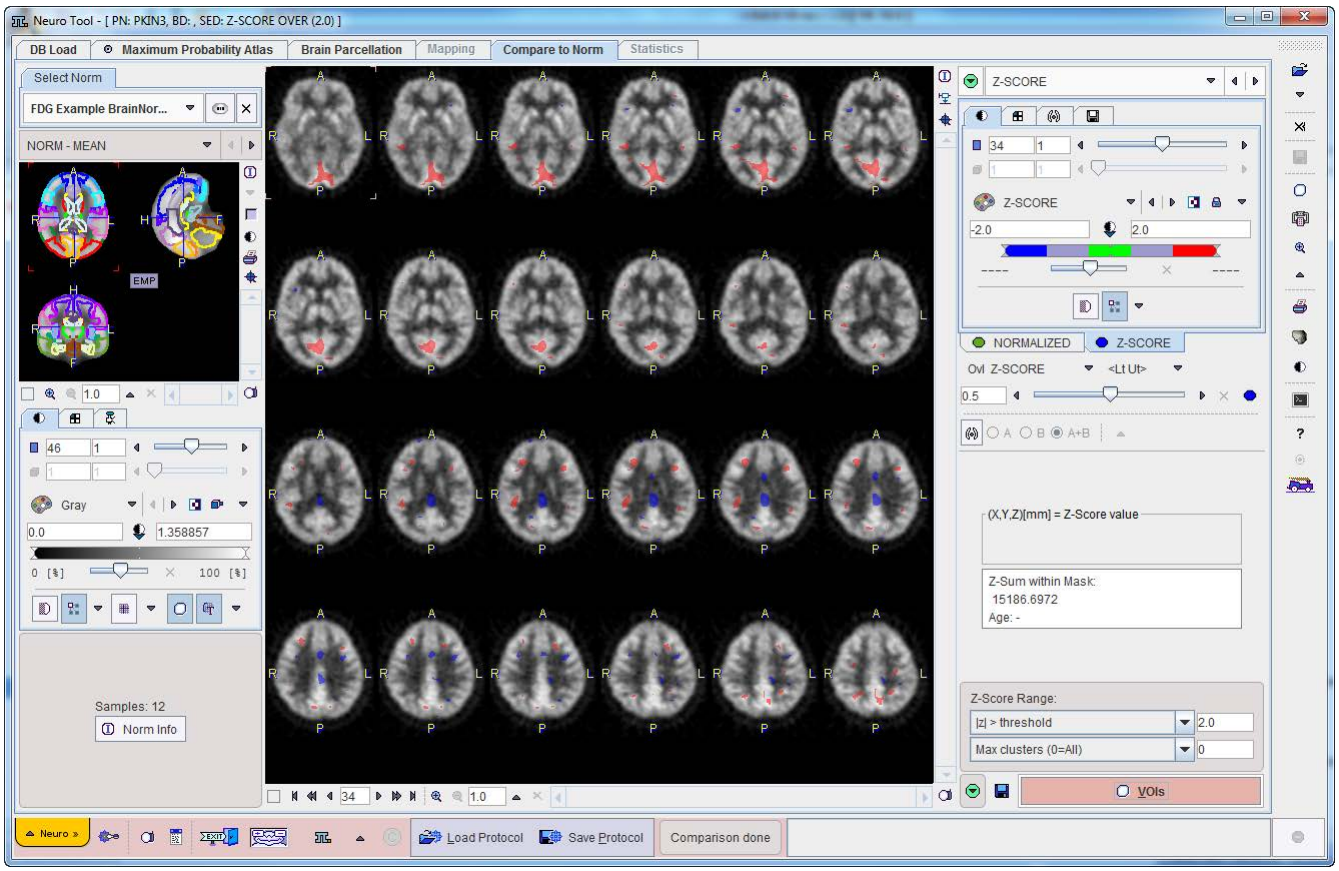
The purpose of this page is to verify that the anatomical agreement in both images is sufficient. To this end it is recommended moving the fusion slider left/right, enable the iso-contours, and clicking at different brain locations.

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 obtained by calculating the z-score value for each pixel of the result mask. Calculation is started with the **Compare** button, with the filter pre-configured in the **Z-Score** section.

Z-Score Analysis

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



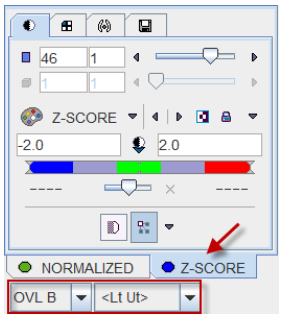
The z-score defines the deviation of a sample with respect to the mean of a distribution. It is defined by the formula

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

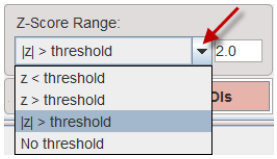
where x is the sample value, m the sample mean, and σ the standard deviation of the distribution. Therefore z describes the deviation from the mean in number of standard deviations and is positive, when the sample is above the mean, and negative when below.

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.

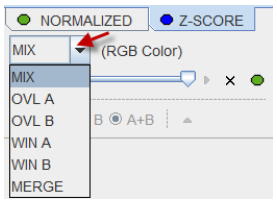


To allow a clear view of the z-score clusters on the normalized patient images the default fusion mode is set to **OVL B** with filter **<Lt Ut>**. This means that only **Z-SCORE** pixels with values below/above the lower/upper thresholds are shown. These thresholds are updated by any change in the **Z-Score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



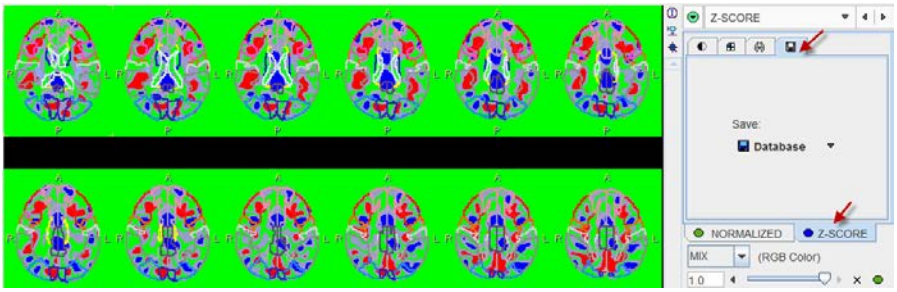
The threshold can be entered numerically, whereas the filtering criterion can be selected from a list. In this way z-score pixels below a threshold, above a threshold, or with an absolute value beyond the threshold can be shown.

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.

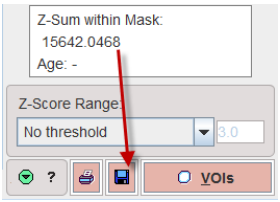


Saving Results

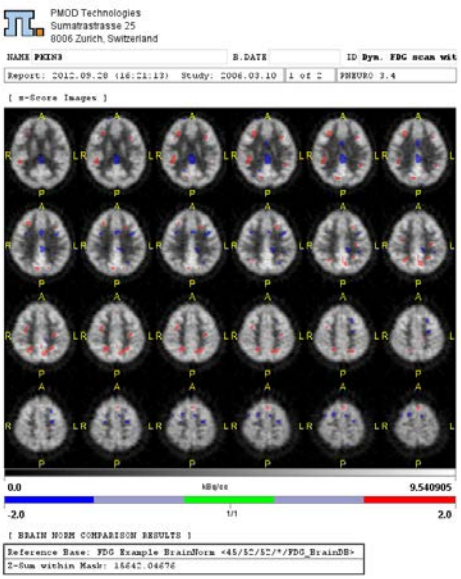
The **Z-SCORE** panel has a dedicated tab for saving the currently shown z-score map for use in another context. Configure the appropriate image format, and then select the **Save** button for saving.



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 patient. 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.



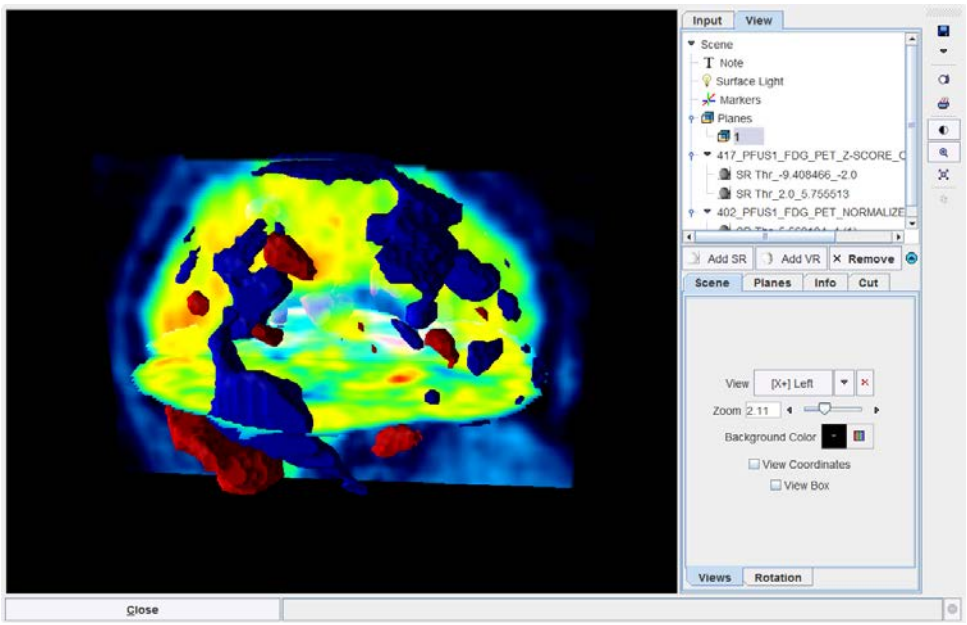
Select the page of interest in the **Pages** section. It can be annotated, printed, and saved as a JPEG/TIFF file or as a DICOM secondary capture object.

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)

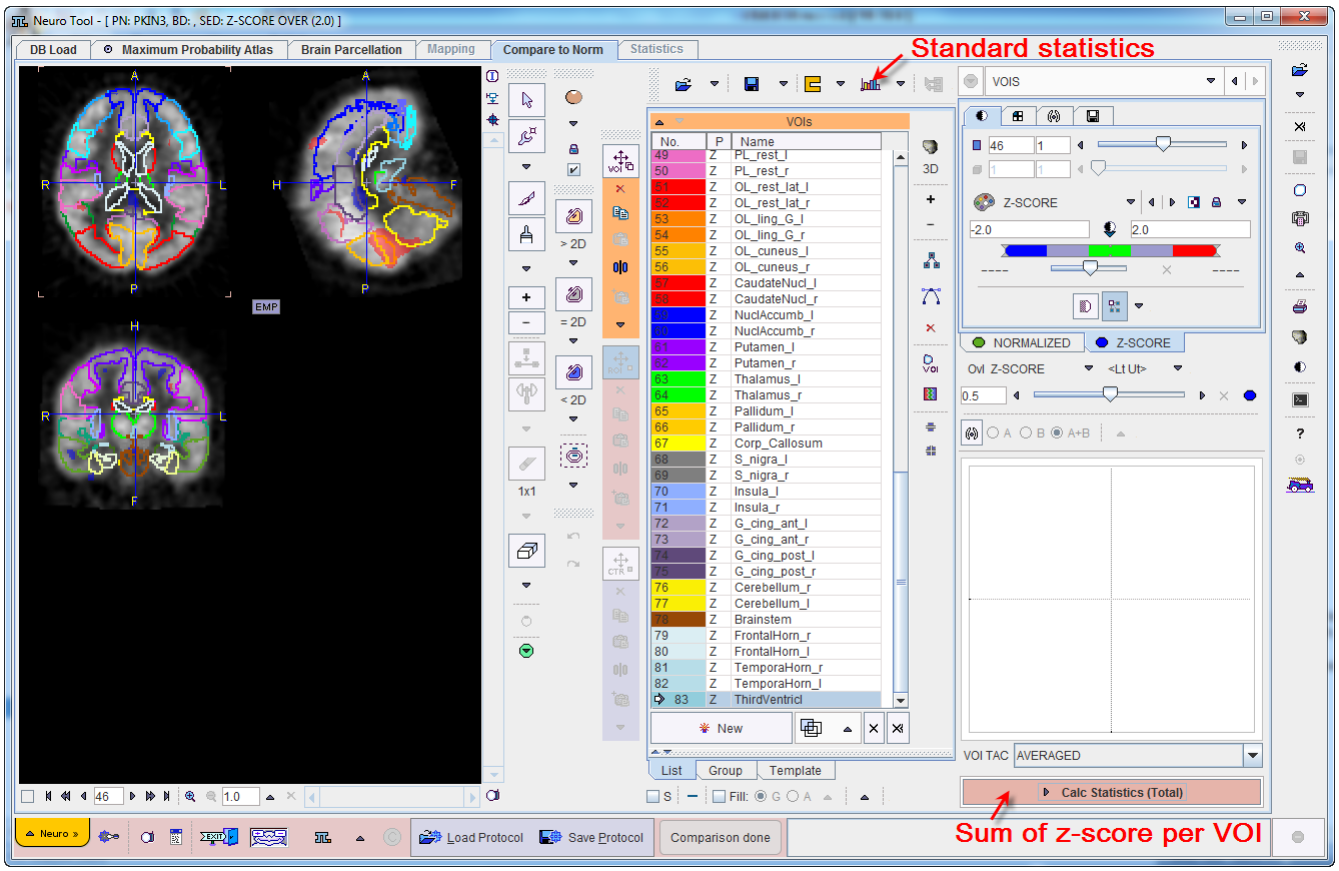
If the 3D option has been purchased and installed the button is available in the lateral taskbar and can be used to rapidly transfer the **NORMALIZED** patient images and the **Z-SCORE** map to the 3D tool. Rendering is immediately initiated and a result shown such as the example below.



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.

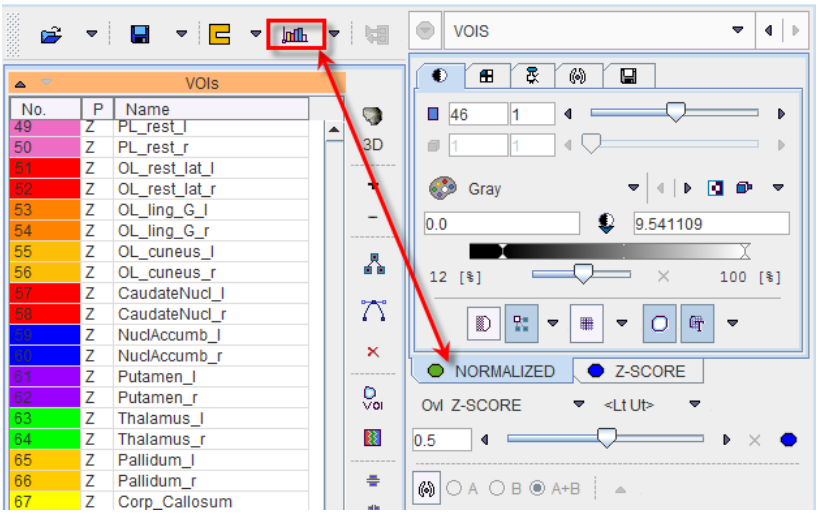
VOI Analysis

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

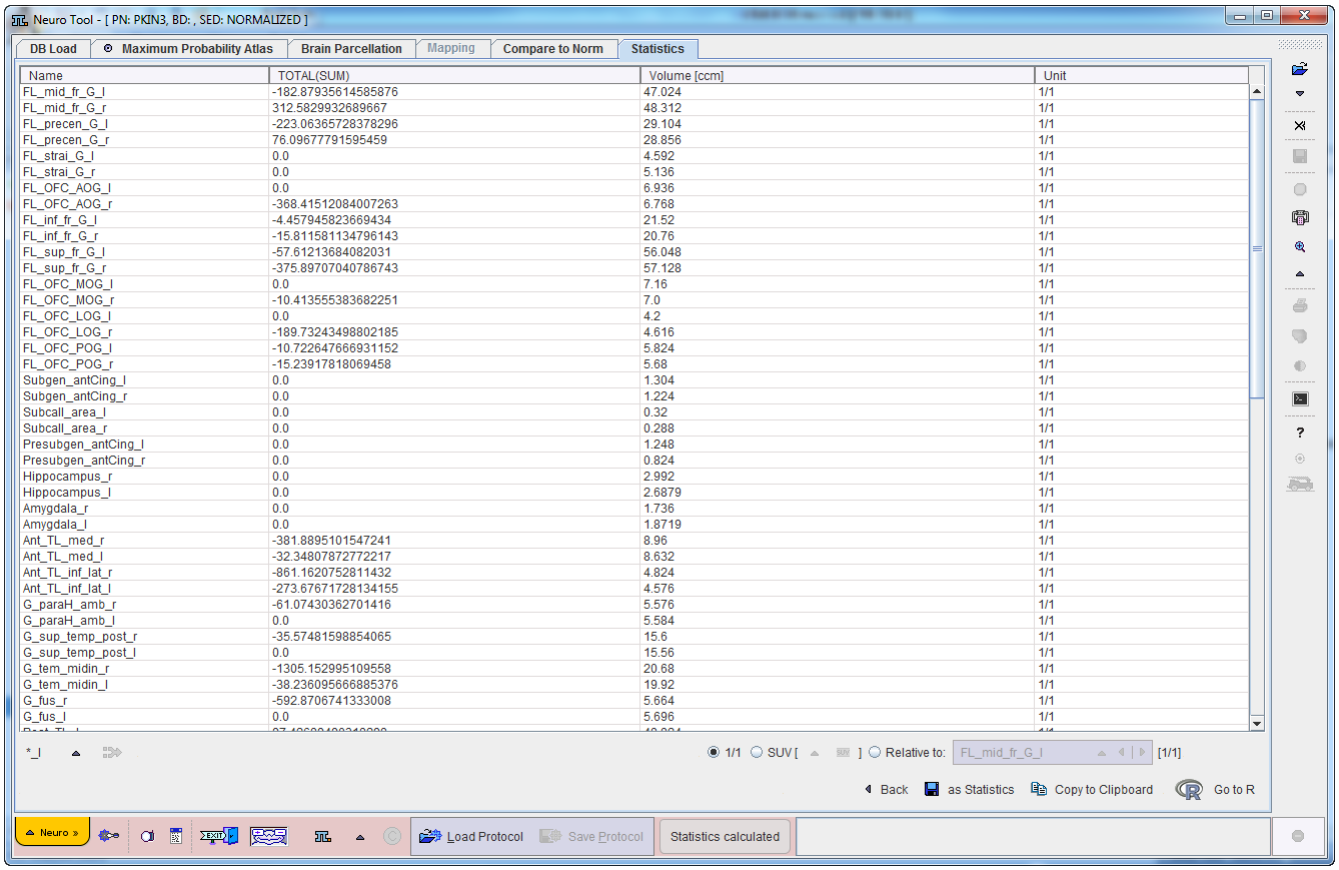


If a VOI set has been defined in the Brain Norm, it is directly available for adjustments and statistics evaluation. The alignment of the VOIs with the brain structures can be verified using the **NORMALIZED** images. If needed, individual VOIs can be scaled, reshaped, or cleared and completely be redefined 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** patient 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.



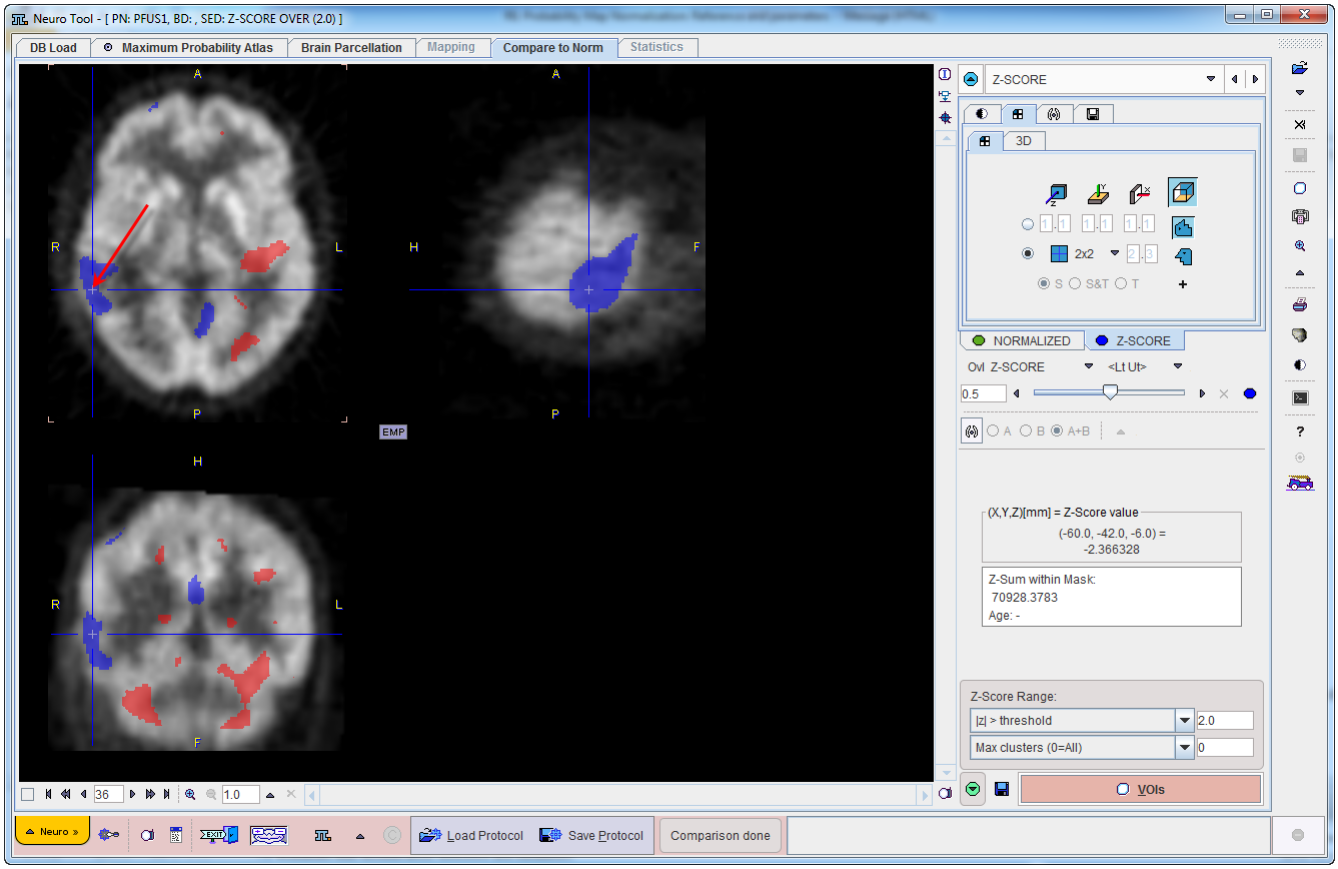
Application Notes

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 patient 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.
- As the **Compare to Norm** module does not provide functionality to account for the age distribution of the controls, it may be reasonable to set up separate age-matched Brain Norm databases among which the most appropriate one is selected when analyzing patient images.

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 patients. 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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[5] Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in spm using a macroscopic anatomical parcellation of the MNI MRI single subject brain. Neuroimage 2002; 15: 273-289. ***DOI http://dx.doi.org/10.1006/nimg.2001.0978***

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