PMOD Fusion Tool (PFUS)

USER MANUAL
Version 4.4

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

This page is intentionally left blank.

1 PMOD Fusion Tool (PFUS)

PFUS is PMOD's image registration and fusion tool. Its purpose is to bring images into a common coordinate space where they can be post-processed in different ways.

Depending on the data to be processed, a user is guided in a workflow through the image registration process, so that the procedure is as convenient as possible and the results reproducible. The following types of image registration are available:

- manual, interactive alignment by shifting and rotating the images;
- automatic rigid alignment using different image comparison methodologies;
- SPM5-, SPM8-, SPM12-type elastic deformation to template images;
- SyN methodology of ANTS (Advanced Normalization Tools) for elastic registration
- motion correction of dynamic series;
- registration based on user-defined anatomical or fiducial landmarks.

Once the images are spatially aligned, there are various ways of post-processing supported:

- different variants of fusing the registered images;
- visualization of up to 4 fused images in parallel;
- saving fused images in JPEG/TIFF or as DICOM secondary capture images;
- pixel-wise image algebra of registered images;
- volume-of-interest definition directly in fused images;
- scatter plots of corresponding pixel values in 2 (2D plot) or 3 (3D plot, optional) matched images;
- rotating MIP (Maximum Intensity Projection) images of up to three fused images.

1.1 Operation Principle

The PFUS tool regards one image series as the *Reference.* All other series are adjusted in pixel size and slice thickness to the reference. This is done by interpolating their image information within oblique planes across the image volume, a process called *reslicing*. The images to be adjusted are called the *Input* studies in this text. As a result of reslicing, the *Reference* and the *Inputs* have identical resolution, and fusion images can be easily generated by combining the pixel values in the different series. For instance, the pixel colors (RGB values) can be added *(blending mode)*, or only one of the image values can be shown depending on a threshold value *(overlay mode)*. Furthermore, VOIs can directly be exchanged between all images, and arithmetical operations applied between images.

If the anatomic structures in the *Reference* and the *Input* series are already in spatial agreement, only a mere resolution adjustment of the *Input* series is required, for example to interpolate a 128x128 PET to the 512x512 matrix of the CT (Reference) in a hybrid PET/CT study. In other situations, however, an adjustment of the coordinate system is also required to bring the anatomy in both studies into agreement. For example, in most cases it is required to rotate and shift the images of a brain PET study to match them with the images of an independent MRI study, and interpolate them to the MRI matrix size.

1.2 Spatial Transformations

Spatial Transformations

PMOD supports two types of spatial transformations:

1. **Rigid transformations R** rotate and translate the contents of an image volume, for instance to calculate slices at oblique orientations. Rigid transformations are defined by 6 parameters, the rotation angles and translation distances in the three spatial directions.
2. **Elastic transformations E** allow adjusting the shape of the objects in an image volume to objects with a different shape in another image volume (the template). They have an *affine* part and an *elastic* part. The affine part has 12 parameters to account for an overall rotation, translation, scaling and shearing in the three spatial directions. The elastic part consists of a deformation field which performs the local adjustments.

Combination of Transformations

The PMOD fusion tools support the analytical combination of spatial transformations, avoiding hereby multiple interpolations. For example, when image A is matched to image B by the rigid transformation R1, and B is matched to image C by the rigid transformation R2, A is inherently matched to C by the combination R1*R2 of the transforms.

In PMOD, an arbitrary number of rigid transformations can be combined, but *only one elastic* transformation at the end of the chain. So for example:

- A CT image from a PET-CT study is matched to MR by R1.
- An MR image is elastically matched to the MNI template by E1.
- Then the CT image is matched to the MNI template by the combined transform R1*E1.
- If (and only if) the PET image has the origin at the same anatomical position as the CT, PET can also be matched to the MNI template by R1*E1.

Inverse Transformations

All the automatic methods can not only return the matching transformation, but also the inverse transformation which applies if the role of the *Reference* and the *Input* is reversed. Additionally, PMOD can always calculate the inverse of the current transformation, even if it was created by combining transformations.

Transformation of VOIs

The spatial transformations cannot only be applied to reslice images to reference images, but also to project VOIs from the reference space to the target image space. An application of particular interest is the use of standard VOIs which are defined in the MNI space for the analysis of subject images. This can be achieved with the following steps:

1. The subject images are normalized to a MNI template with **Calculate InverseTransformation** checked.
2. The inverse normalization transform is saved.
3. The subject images are loaded in the PMOD viewing tool.
4. The MNI VOIs are loaded and the inverse normalization transform applied.

As a result the standard VOIs, adjusted for the particular subject anatomy, are available in the VOI tool as outline contours. The user can adjust them if needed, then save them and calculate image statistics on the unchanged subject data.

1.3 Starting PFUS

PFUS is started with the **Fusion** button from the PMOD ToolBox

or by directly dragging image files from the desktop onto the above button.

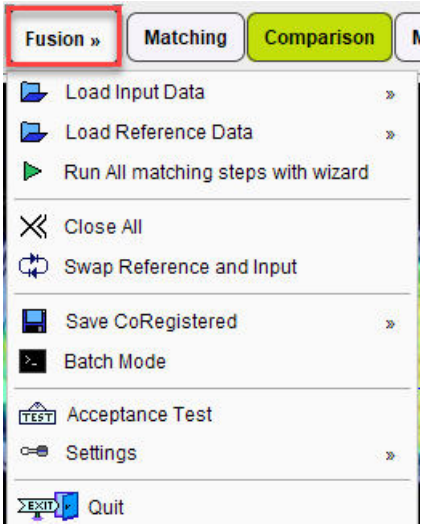
1.4 PFUS Menu Line

The PFUS menu line is located at the top of the docking interface. It consist of the yellow menu button, **Fusion**, followed by the main module pages, functional buttons, an area for progress information and other buttons common for all PMOD modules. The currently active page, e.g. **Comparison** in the example below, is highlighted in green color.

Only the local menu, the configuration, the wizard and protocol functionality are documented in this section, the other elements are standard in PMOD and documented in the *PMOD Base Functionality Users Guide*.

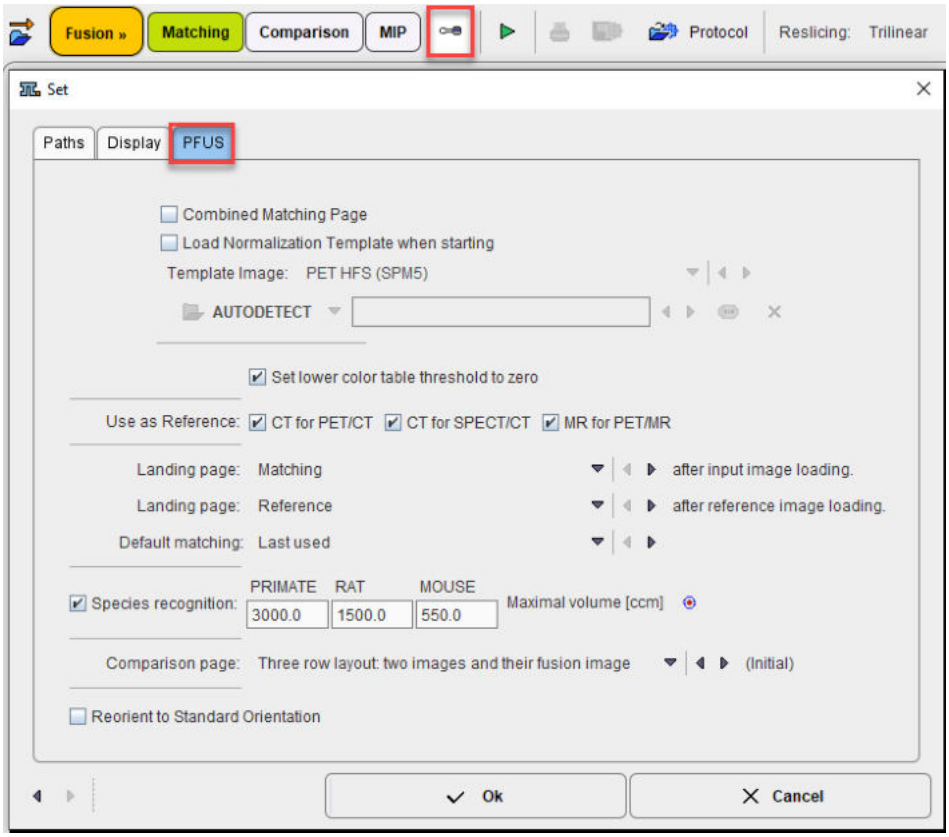
1.4.1 Menu Content

The yellow menu button allows: accessing data loading, the wizard interface, closing all images and swapping the reference and input images, saving of the co-registered, accessing the PFUS batch processing interface, the acceptance test, the local configuration and the PFUS **Quit** button.



1.4.2 Configuration Settings

PFUS has a set of configuration parameters which can be opened with the dedicated button located in the top line menu:



The **Combined Matching Page** allows changing the interface layout: with the box enabled the legacy Fusion interface is shown. See User Interface 12 for details.

Note: Restart PMOD in order to apply the selected layout settings.

The option **Load Normalization Template (Reference) Automatically** is useful if PFUS is mostly applied for the normalization of brain images with the same template.

The **Color table** choices allow to establish default colors for the input and reference images. These defaults can be applied at every loading operation, or only initially.

Use as reference serves for defining the reference image in hybrid situations when more than one image is loaded at once.

Default matching sets the registration method which is applied initially. **Selected** refers to the most recently used method. This setting is particularly relevant for the "Run All" operation mode when the images selected in the database interface are directly submitted for registration.

The **Species recognition** option triggers a species selection based on the loaded image volume. If neither the **PRIMATE**, **RAT** nor the **MOUSE** applies, the **HUMAN** default is applied. A correct species setting is important for proper registration defaults.

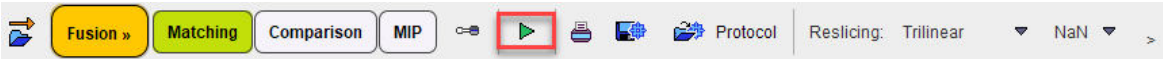
Note: This setting is implemented and works only for the **Default Matching** layout.

If **Reorient to Standard Orientation** is enabled, the images are brought into the radiological HFS orientation after loading. A consistent orientation of the alignment is crucial for working with template images and also provides a better initial alignment of multi-modal images.

1.4.3 Wizard Button

The wizard function makes it possible to launch coregistration workflows in the Fusion tool in a matter of seconds. It allows rapid switching between rigid matching and elastic workflows as well as direct selection of the desired result page.

It is accessible via the dedicate green arrow button available in the top line menu in the Fusion interface, as illustrated in the capture below:



The functionality is described in the Wizard Matching Section 65.

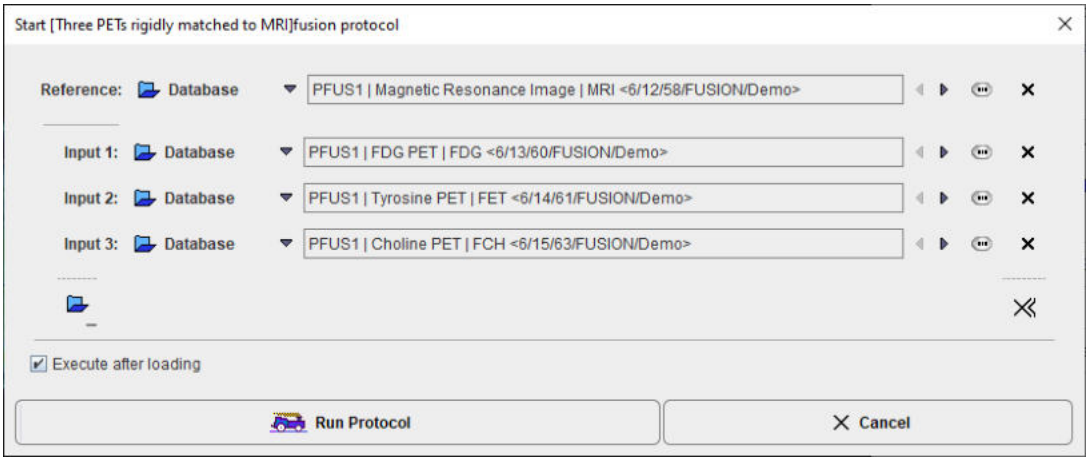
1.4.4 Protocols

PFUS 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 matching processing. The user is advised to save a protocol after a completed data processing workflow, so that at any later time the configuration can be retrieved, and the matching performed.

Protocol Execution

When loading a PFUS protocol a dialog window opens:



If **Execute after loading** is enabled, not only the configuration is retrieved, but the processing is also performed.

1.5 User Interface

PFUS organizes the available tasks on different pages which are explained in the sections below.

The contents of the **Fusion** pages differs based on the Configuration settings 8.

Two options are available:

1. The *Default Matching Layout*: the user is guided step by step through the matching process and can extract optimal results from multi-modal images even in the most challenging situations met in human or preclinical research. Additionally, the user can take advantage of automatic species recognition. The correct species setting is essential for suitable registration defaults.
2. The *Combine Matching Layout*: long term PMOD Fusion users were very familiar with the legacy appearance of the Fusion interface. Therefore with the dedicated box enabled in the local configuration the matching initialization, configuration and results preview steps are available through a single page.

The advantage of the *Default Matching Layout* as compared to the *Combined Matching Layout* are as follows:

- Supports the automatic species recognition based on the image volumes as defined in the local PFUS Configuration 8.
- Implements species-sensitive cropping facility both for the *Input* and the *Reference* images.
- Supports the markers matching workflow.
- Supports the already matched workflow applicable for images from hybrid acquisitions, images like parametric maps derived from a common data set, or images arising in a standard template space.
- Implements the **Dual reference strategy** for the **Motion correction** workflow.

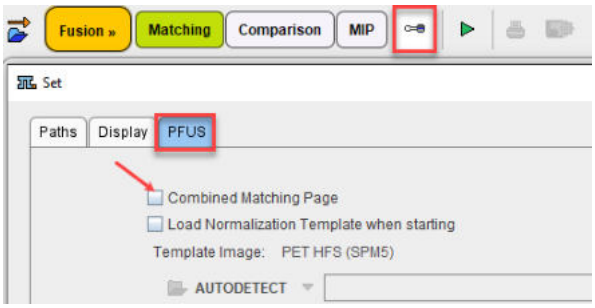
The two layouts share the same **IMAGE ALGEBRA** sub-page, **VOI ANALYSIS** sub-page, **Comparison** page, **MIP** page, **Task** bar and **Status** bar.

Further Information

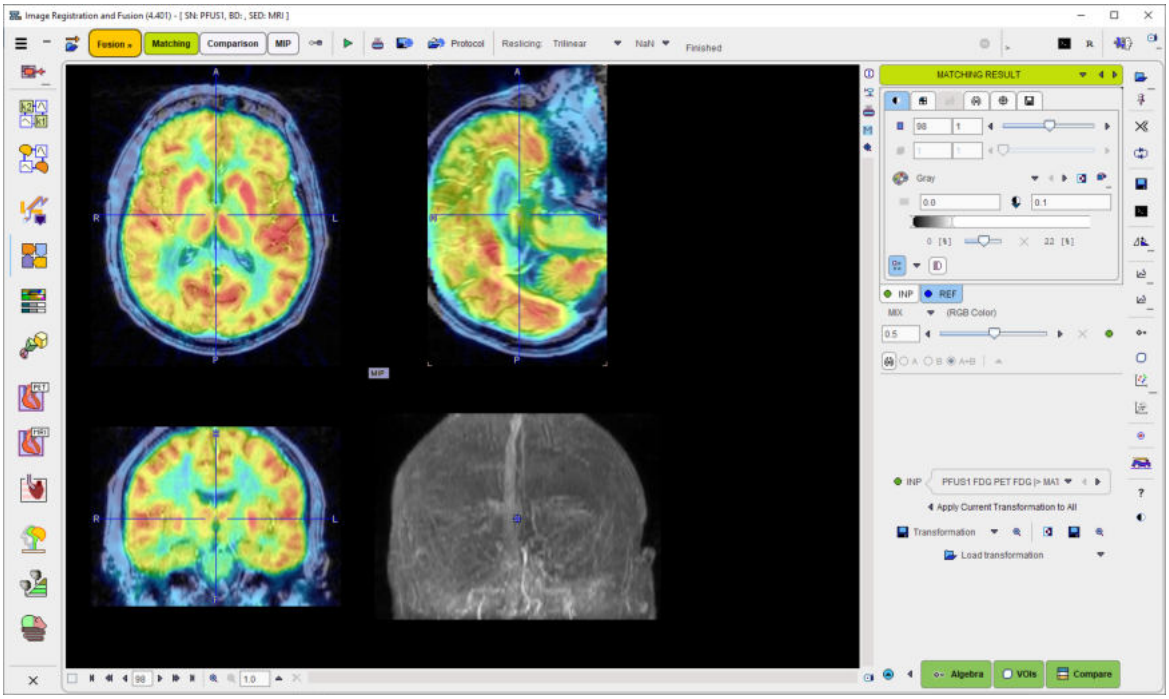
The following description is intended as a reference and not as a tutorial. For practical examples how to work with the PFUS software please refer to the overview video and the training videos which are available in the video and resources area of www.pmod.com .

1.5.1 Default Matching Layout

With the **Combined Matching Page** box disabled:



the PFUS interface is organized on 3 pages as illustrated below:

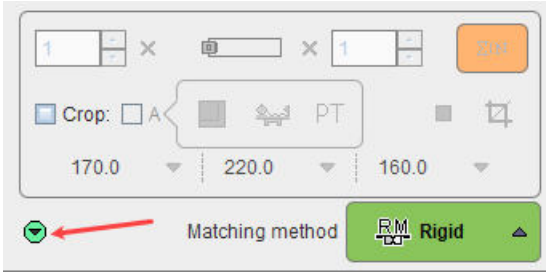


Basically, the **Matching** page serves for image registration, image algebra and VOI definition, **Comparison** for the parallel visualization of up to six matched images, and **MIP** for the generation of rotating fused MIP images.

Hidden Controls

In several places options are hidden to save screen space. This is indicated by a blue up-arrow as in the example below.

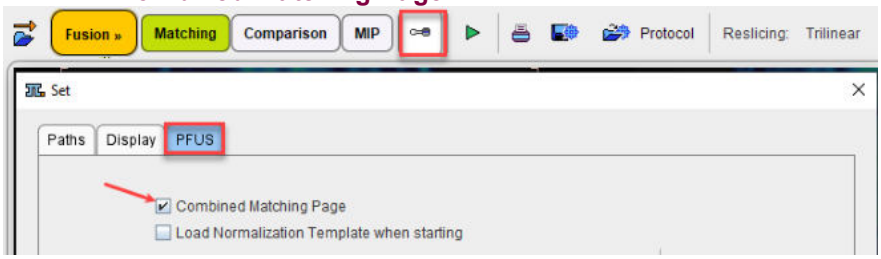
When the button is activated, the area expands, showing all the options.



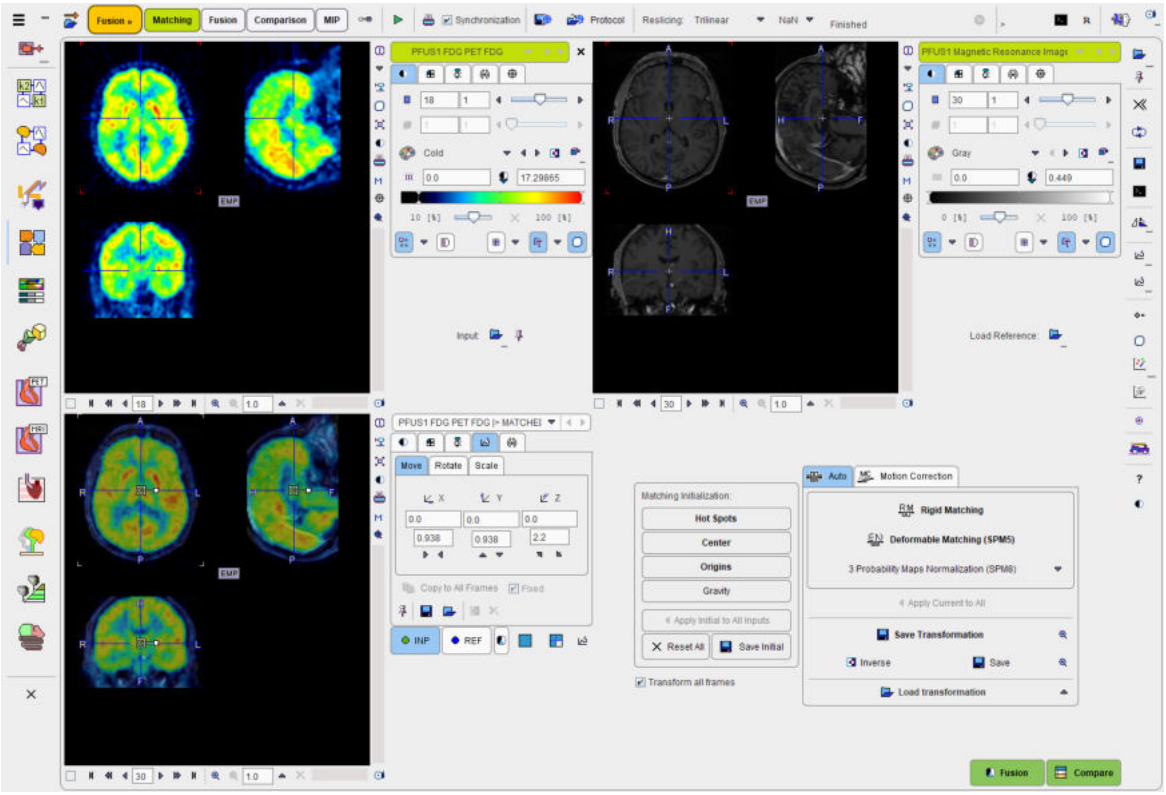
It can be collapsed again with the green down-arrow.

1.5.2 Combined Matching Layout

With the **Combined Matching Page** box enabled:



the PFUS interface is organized on 4 pages as illustrated below:

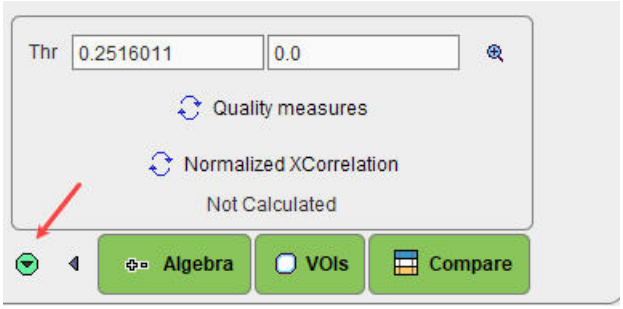


The **Matching** page serves for matching purposes, **Fusion** for fusion display, image algebra and VOI definition, **Comparison** for the parallel visualization of up to six matched images, and **MIP** for the generation of rotating fused MIP images.

Hidden Controls

In the **Fusion** page some options are hidden to save screen space. This is indicated by a blue up-arrow as in the example below.

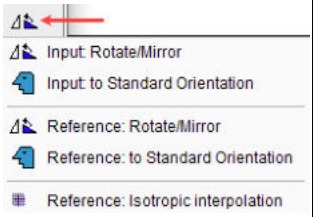
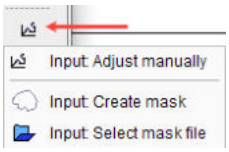
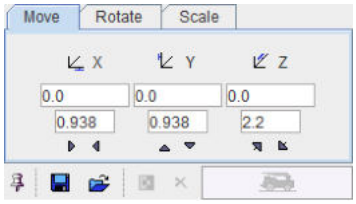
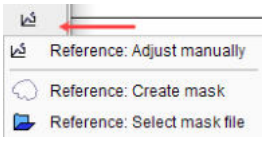
When the button is activated, the area expands, showing all the options.

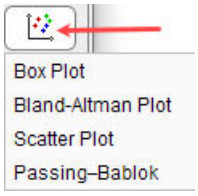


Please refer to previous version documentation (PMOD 4.3) for details about this layout and usability. Please note that the latest Fusion improvements are no longer implemented in this layout.

1.6 Task Bar

The taskbar on the right side of the application window provides shortcut buttons for frequent tasks. Please note the tooltips for hints regarding the button functionality.

	Load images 44.
	Appending toggle button: With  the current **INP** list is cleared when loading a new **INP** series. With  the loaded series are appended to the existing **INP** list.
	Clear all data from PFUS.
	Swap the role of the **REF** and the current **INP**.
	Opens the saving dialog window.
	Opens the Fusion batch dialog window.
	**Rotate/Mirror** opens the panel  of the **INP** or **REF** images respectively for an initial reorientation. **to Standard Orientation** reorients the **Input** or **Reference** images automatically to PMOD's standard orientation depending on species. **Isotropic interpolation** interpolates **Reference** to the smallest pixel size.
	Operations related to the **INP** images. Adjust manually opens the reslicing panel  for applying arbitrary translations and rotations and matching the images manually to the reference. **Create mask** opens the **Segmentation** tool for creating a mask from the **INP** image which can be saved and used in the automatic matching procedures. **Select mask file** allows to pre-select an existing mask for the automatic matching procedures.
	Same as above for the **REF** images. An application of **Adjust manually** is to bring a tilted reference images into better orientation. **Create mask** opens the **Segmentation** tool for creating a mask from the **REF** image which can be saved and used in the automatic matching procedures.

	Select mask file allows to pre-select an existing mask for the automatic matching procedures.
	Opens the **Matching/IMAGE ALGEBRA** sub-page 38. Only useful after successful matching.
	Opens the **Matching/VOIS** sub-page 40. Only useful after successful matching.
	Calculates and shows the Box Plot. Calculates and shows the Bland-Altman Plot. Calculates and shows the 2D scatter plot 107 of VOI pixels in two images. Calculates and shows the Passing-Bablok plot.
	Calculates and shows the 3D scatter plot 117 of VOI pixels in three images.
	Hide/show toggle for the control area to the right. It support image viewing with more space if no manipulations are required.
?	Context-sensitive help, pointing to the html documentation.
	Resets all configurations to the default values.
	"Run all" button to start a matching workflow without interaction 64.

1.7 Image Masking

In certain circumstances, the matching algorithm alone may not be sufficient for a successful result. If this happens, the user can define masks for the reference and /or input image after loading. Masking will exclude pixels outside selected areas from calculations.

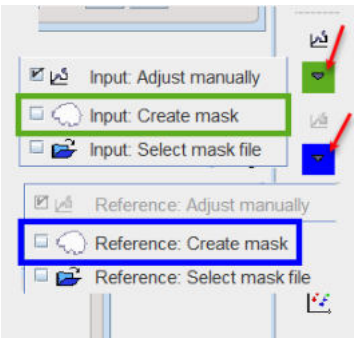
There are three options available for the mask definition:

1. Using the **...Create mask** icon (display definition).
2. Loading an existing mask using the icon (display definition).
3. Defining the mask in the **Basic** parameters tab available for the automatic matching algorithms.

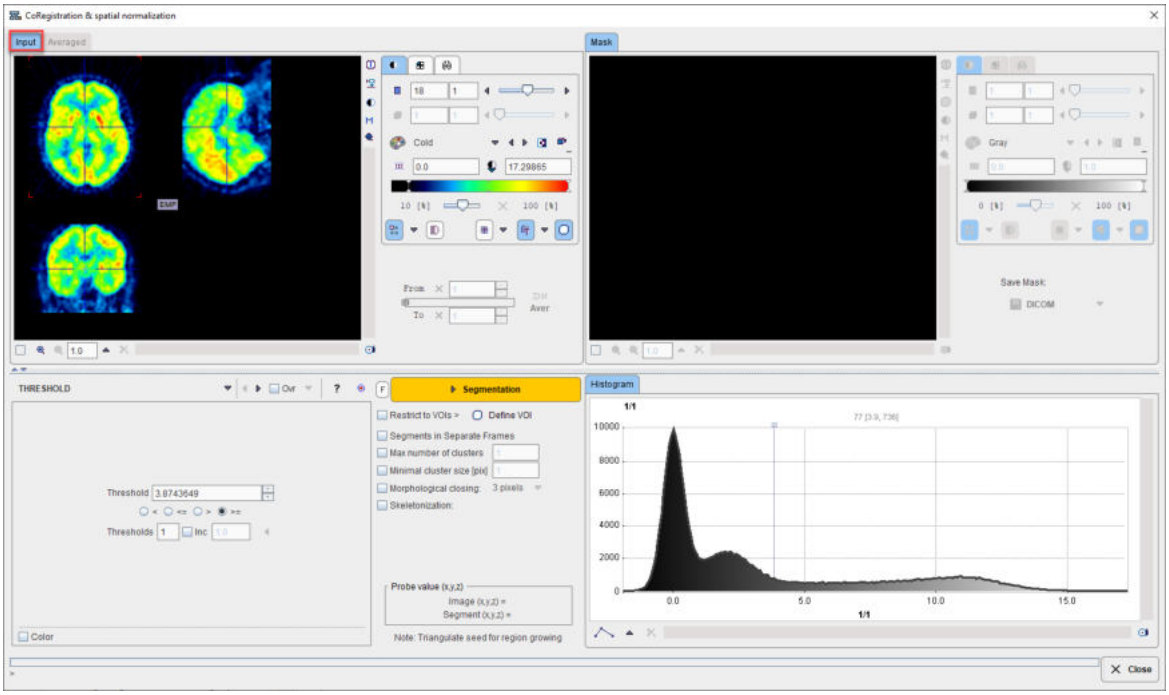
The procedures are described below.

1. Create Mask (display definition).

To create a mask activate the **...Create mask** icon as shown below.



Initially the masking interface appears with the loaded image in the left display area.

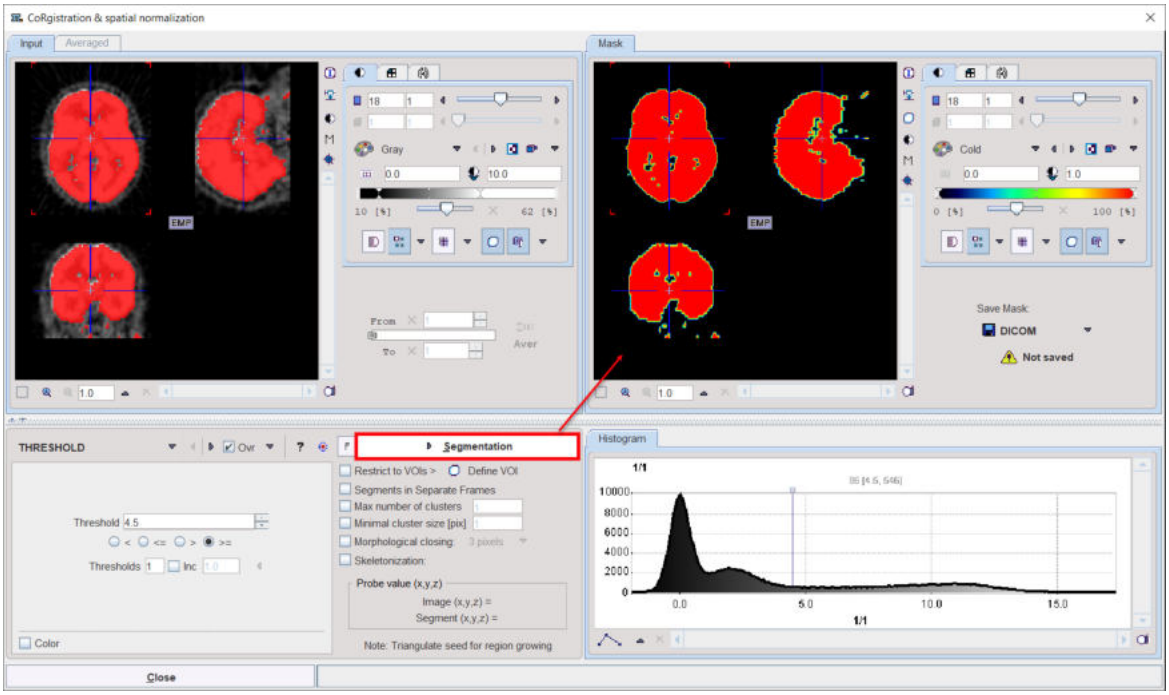


When working with dynamic series, it is recommended to average within an appropriate range in order to obtain an appropriate data set for masking. The range can be specified by the **From** and **To** numbers or using the slider handles. When the **Average** button is activated, the average uptake in the specified frame range is calculated and the result image is shown on the **Averaged** sub-pane.

Segmentation for Creating a Mask

The next step consists of generating segments which represent tissues of interest. Segmentation can be performed on **Input** series but also on the **Averaged** images, depending on which tab is selected. The **Histogram** of the pixel values is updated according to the selected images.

It is recommended to change the color table to **Gray**, and to enable the overlay **Ovr** box. Then, select one of the segmentation methods (described below) to specify an inclusion criterion. The pixels which satisfy the criterion are colored in red in the image overlay. Note that overlay updating might be slow when changing a segmentation parameter, depending on the segmentation method. **Segmentation** performs the actual segmentation and shows the result in the **Mask** tab to the right. While standard segmentations create binary images with 0 (background) and 1 (segment) pixel values, there are clustering approaches which generate multiple segments in a single calculation. These segments are distinguished by increasing integer pixel values. Each **Segmentation** activation overrides the previous contents in **Mask** pane.



In certain circumstances, the segmentation methods alone may not be sufficient to separate an object form other structures. If this happens, the user can defined a VOI which prevents segmentation from leaving the area of main interest. To do so, the **Restrict to VOIs** box has to be enabled and the **Define VOI** button activated. The VOI tools interface appears and allows drawing a VOI. Outline the VOI. Quit the VOI tools with the **OK** button to confirm the VOI selection. Make sure the overlay **Ovr** box is enabled. Finally, activate the **Segmentation** button to perform the actual segmentation within the VOI. The result is shown in the **Mask** tab to the right.

To fill in holes present in the mask segment enable the **Morphological closing** box, select one of the number of pixels available and activate again the **Segmentation** button.

Saving the Mask for Matching Processing

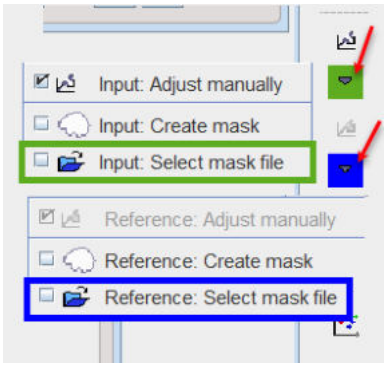
In order to use a generated segment image as a mask in the matching processing it must be saved as a file. Saving can be performed using the **Save Mask** pane in any of the supported image formats. Note that automatically the saved file is configured.

Exit the mask interface activating the **Close** button.

Note that the saved mask is not binary in the case of multiple segments, so that the segments can be recovered. However, during the pixel-wise calculation only the non-zero mask pixels will be processed, while the other pixels are blanked.

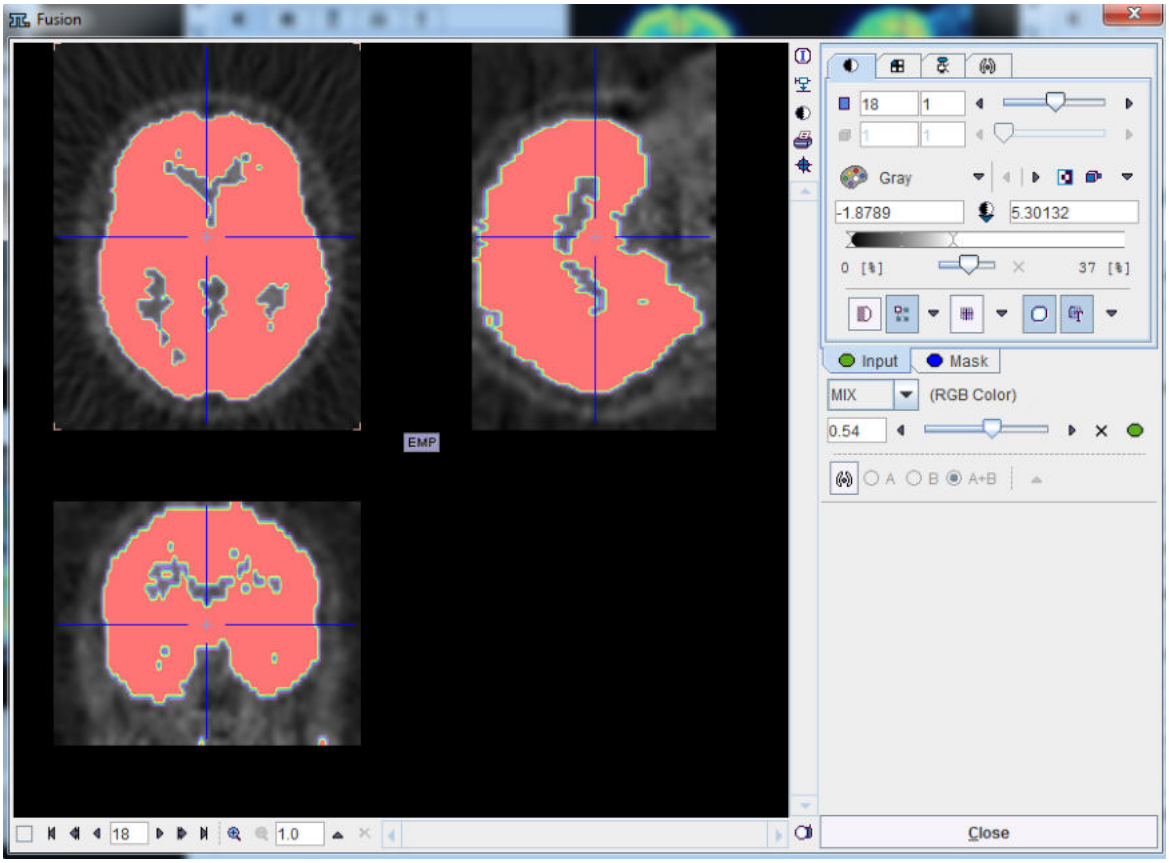
2. Load Mask (display definition)

If a mask file already exists, the interactions described above are not necessary and it can be simply configured with the dedicated icon as illustrated below:



It opens a dialog window as shown below:

The **Attached mask file** radio button need to be enabled. The drop down arrow allows switching between different data formats while the **Change file or directory** button can be used to select the mask. The activation of the button next to mask selection allows loading and viewing the mask in a dialog window.



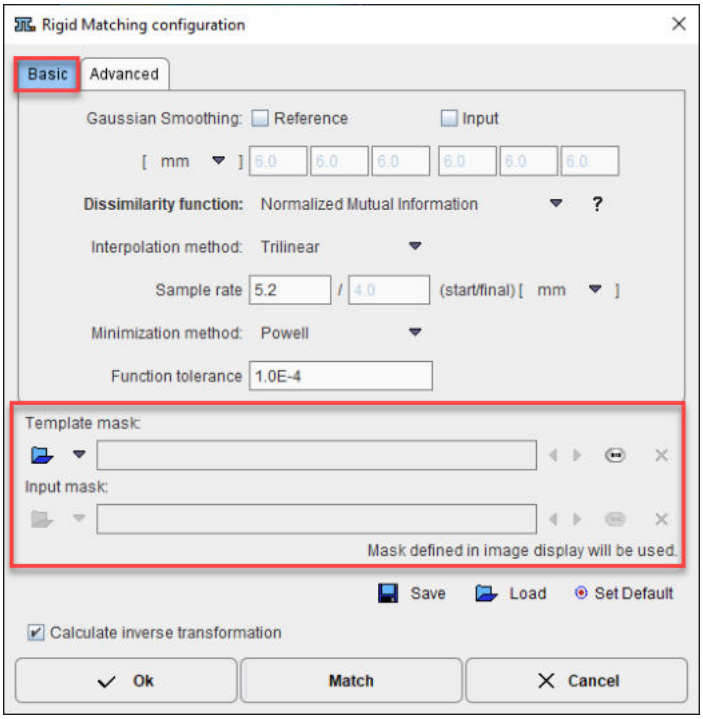
Confirm the selected mask to the protocol activating the **OK** button in the **CoRegistration & spatial normalization** dialog window. Upon confirmation the window is closed.

Remove the Mask from the Matching Processing

To remove the mask from the matching processing activate the the same dedicated icon from the lateral task bar as illustrated above. A dialog window opens. Make sure the **Detach mask file (Set empty to protocol)** radio button is enabled and confirm the selection with OK.

3. Mask definition for Automatic Matching

When the masks are defined in the display, they are immediately set as the corresponding **Basic** parameters to the automatic matching algorithms. Alternatively, the masks can be defined during the setting of the **Basic** parameters for the automatic procedures. In the example below, the *Input* mask was defined in the display while no mask was defined for the *Reference* image. Note the masks section in the **Basic** parameters settings: a message alerts that for the *Input* image the **Mask defined in image display will be used** while for the *Reference* image the mask can be loaded and set to the protocol. Note that in this case the mask need to be created and saved beforehand. The mask file corresponding to the *Input* image will allow restricting the matching algorithm to the meaningful area.



To summarize, there are three potential scenarios:

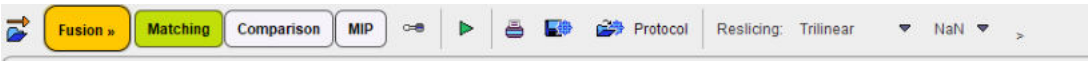
1. if there is a mask defined in the display it will be set and used during the automatic calculation.
2. if there is no mask defined in the display and there is a mask file defined in the **Basic** parameters it will be used for the automatic matching.
3. no mask will be used during the automatic matching algorithms if there is no mask defined in the display and no mask defined as a **Basic** parameter.

1.8 Recommendations

As the *Reference* determines the final image resolution, it is recommended to use the higher resolved image as the *Reference* for avoiding losses in image quality. The user, however, should be aware that the size of the *Input* series may increase dramatically. For instance, if a dynamic PET study is matched to a 256x256 MRI with thin slices, the size of the resliced PET data can easily grow by a factor of 10. Such big data sets can become a problem for the available RAM, and for subsequent processing steps.

2 Processing using Default Matching Layout

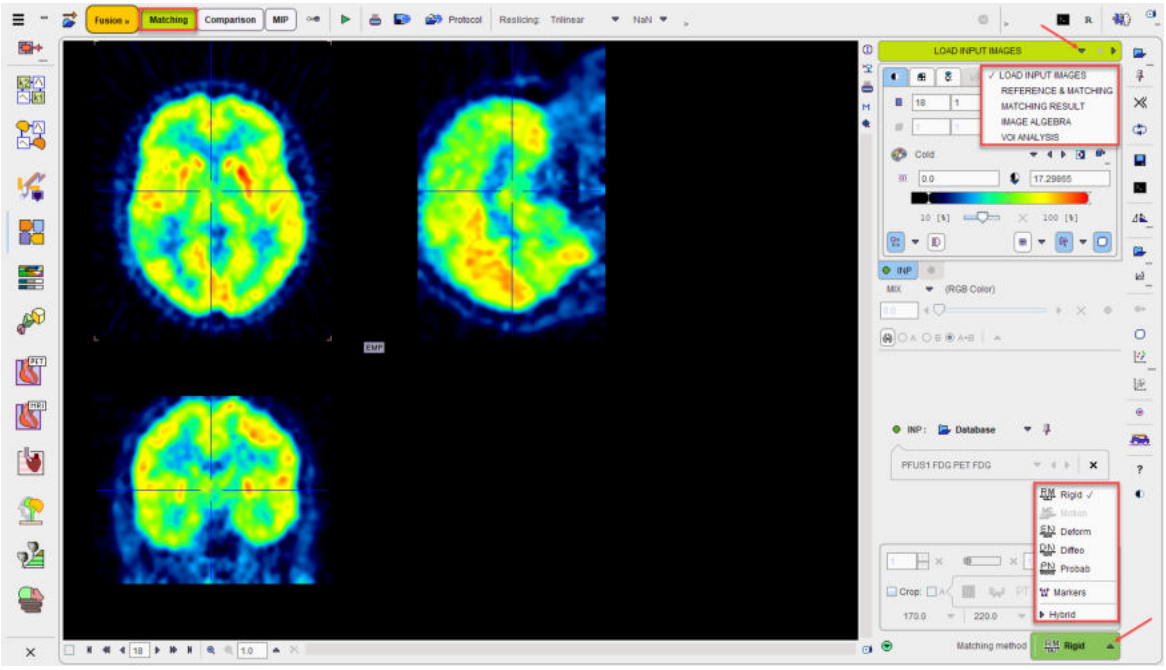
With the default layout PFUS interface is organized on 3 pages as illustrated below:



Basically, the **Matching page** serves for image registration, image algebra and VOI definition, **Comparison** for the parallel visualization of up to six matched images, and **MIP** for the generation of rotating fused MIP images.

2.1 Overview of Matching Sub-Pages

The **Matching** page has five sub-pages, which can be selected with the arrow in the upper right as illustrated below.

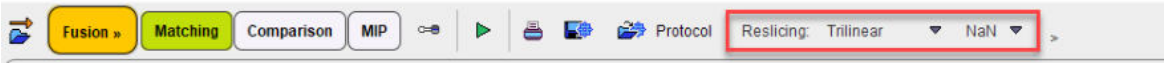


Each page consists of an image area to the left, and a control area to the right. The upper part of the control area relates to the image display and fusion, whereas the lower part is highly page-specific. The red action buttons in the lower right are used for starting a processing step or transferring the matched images to a particular post-processing page.

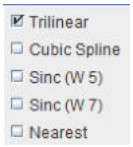
The actual processing works forward through the pages with the red action buttons. After complete processing the pages can be switched without inflicting changes by the selection in the upper right.

Reslicing Options in the Top Line

Transforming the input image to the reference space requires the calculation of pixel values at locations different from the original pixel grid. This value interpolation is governed by settings in the lower status bar as illustrated below.



There are three components:

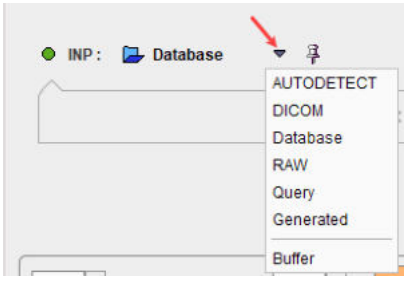
Reslicing	The **Reslicing** method choice lets the user define the interpolation method.  Default is **Trilinear** which is a simple and fast interpolation using all 8 enclosing pixel values. **Cubic Spline** is the best interpolation regarding accuracy and speed. The truncated sinc interpolations **Sinc (Window 5)** and **Sinc (Window 7)** are also accurate, but considerably slower. **Nearest** neighbor interpolation just uses the value of the closest pixel, so it is very fast but in most cases does not provide satisfactory quality. However, it is the method of choice if an object map image containing integer values needs to be resliced.
Undefined Value (NaN)	The appropriate interpolation value for pixels which were outside the original field-of-view is unknown. Per default a **NaN** value is applied, but the behavior can be changed with the selection  to use the **Min**imum of the data set or a fixed value of **0**.

2.1.1 LOAD INPUT IMAGES Sub-Page

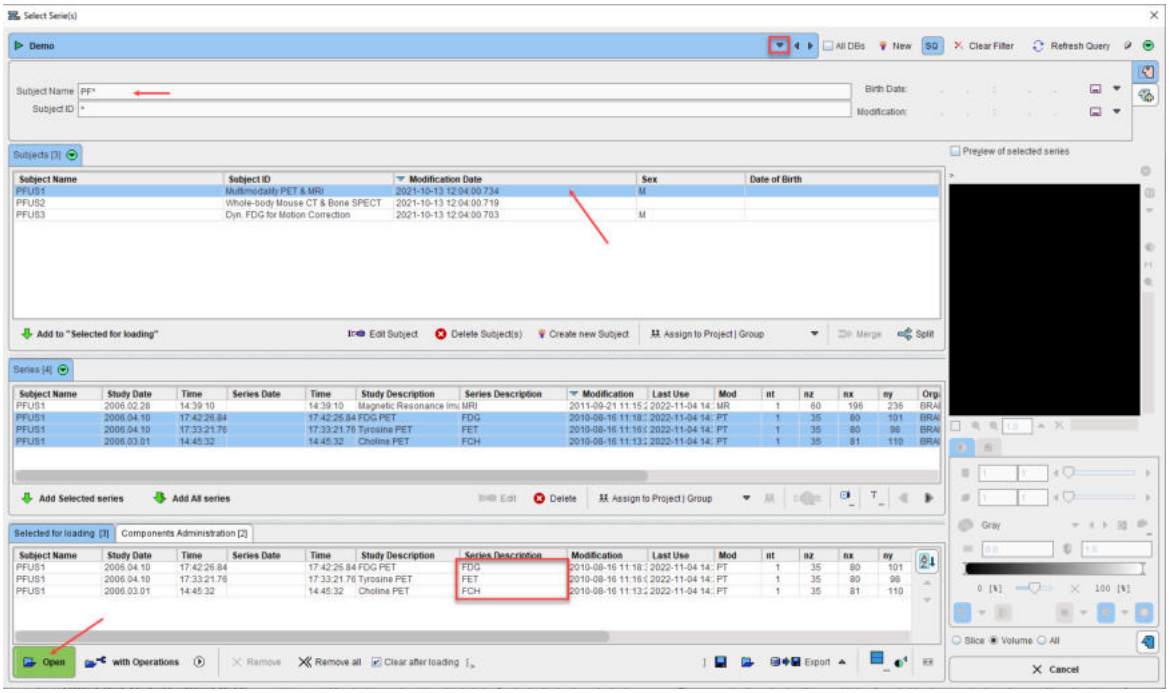
The **INPUT** page is illustrated above. It serves for loading the input images which will be spatially aligned to the reference image.

Image Loading

Image loading is started with the **INP** load button, whereby an appropriate file format can be selected using the down arrow:



Note that several input images can be loaded at once as illustrated in the database loading example below.



After the images have been loaded, the first in the list is shown in the image area. Please use the list selection illustrated below for switching between the input images. Note the **x** button for removing the currently selected series.

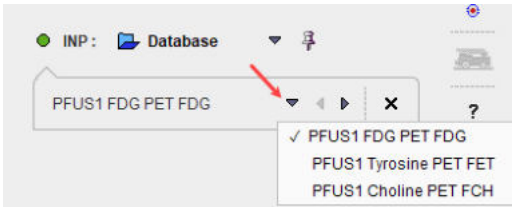
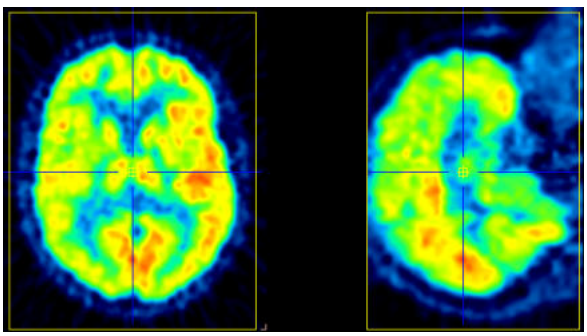


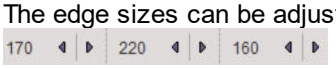
Image Cropping

Image cropping is often useful for discarding irrelevant information and saving RAM. If the cropping controls are not visible, please activate the blue expansion button.

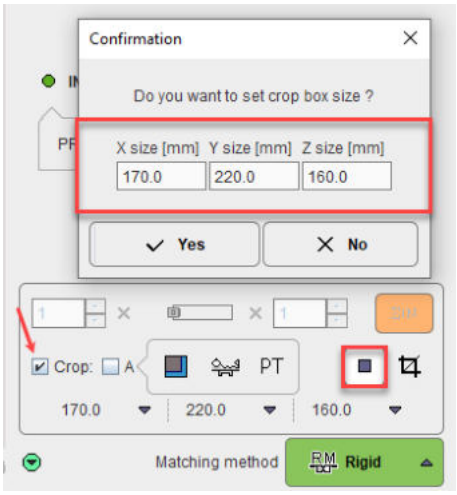
The **Crop** option brings up yellow rectangles in the orthogonal layout. They define the cuboid for the cropping operation.



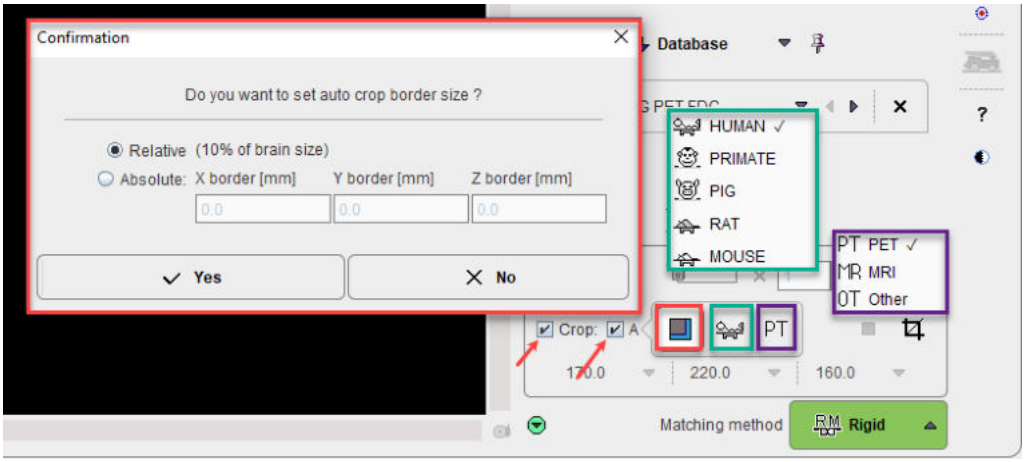
The edge sizes can be adjusted with the arrow buttons,



and the position by clicking at the center of the volume of interest. Alternatively, the edge sizes may be entered as illustrated below.



There is an automatic cropping function available which works for brain images. It is based on the matching of brain templates to the images. To this end the species (red arrow and rectangle) and the modality (blue arrow and rectangle) have to be set properly by the configuration buttons illustrated below.



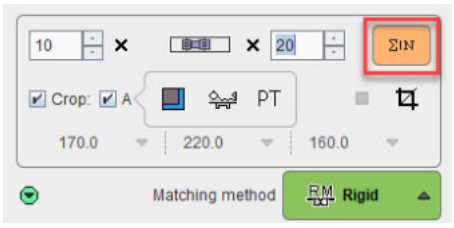
The standard auto-crop bounding box can be expanded by a fixed amount (e.g. inclusion of some non-brain areas, such as the nose, may aid coregistration if the PET include some non-specific binding). This amount can be **Relative,** in percentage of brain size, or defined as **Absolute** size border in [mm] in all direction.

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

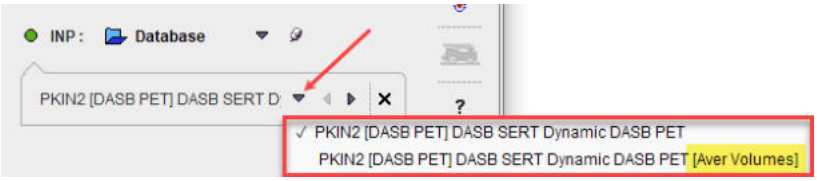
Cropping is started with the  button.

Averaging of Dynamic Images

If dynamic input images are loaded, a frame averaging option is applicable. In the example below a range between frames **10** and **20** is defined.

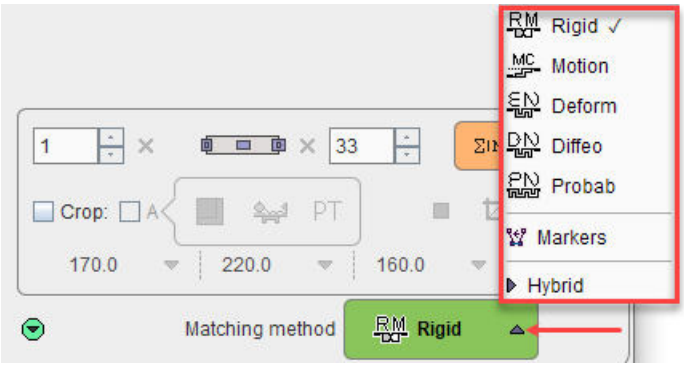


As soon as the indicated averaging button is activated, the time-weighted frame average is calculated and the result added to the list of **INP** images. To label the result the string **[Aver Volumes]** is appended to the series description.



Action Buttons

After the input images have been loaded and the species selection is appropriate, processing can be moved on by selecting the Matching method:

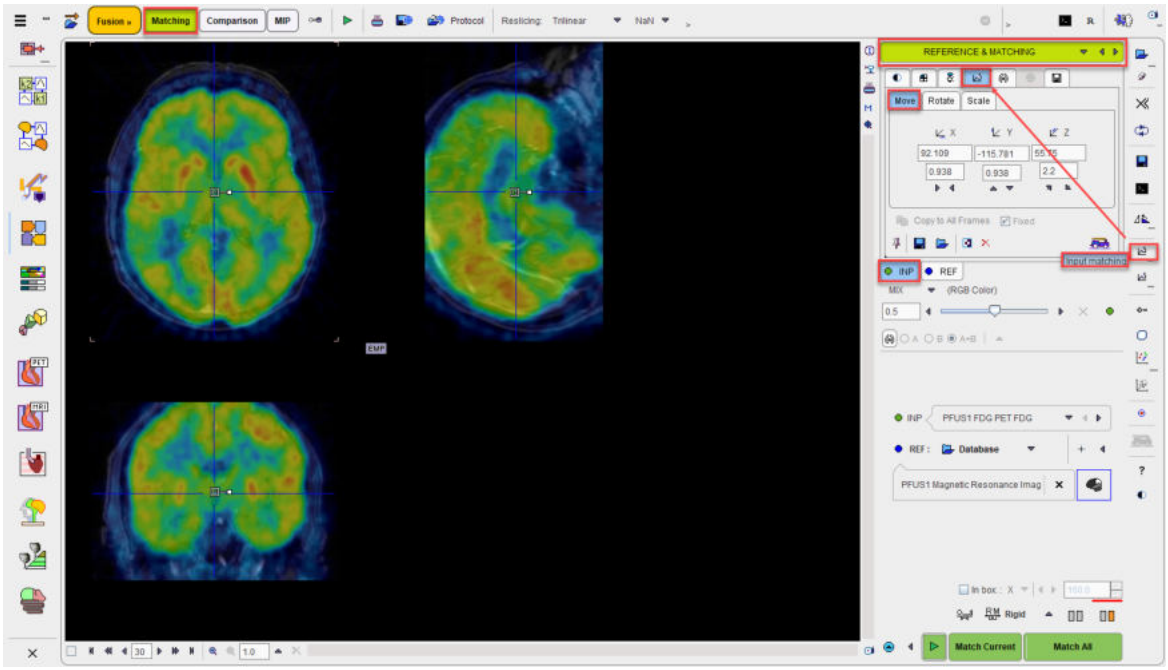


They will open the **REFERENCE & MATCHING** sub-page with an appropriately configured registration method.

RM	Rigid matching
MC	Motion correction. This option is only available for dynamic **INP** images.
EN	Elastic deformation based on a single template reference (SPM5-type).
DN	Not yet available. Will support whole body elastic matching.
PN	Elastic deformation based on a tissue probability maps (SPM8-, SPM12-type), mainly applicable for T_1-weighted MR images.
	Matching based on manually defined landmarks.
	Recommended choice for the hybrid data when the matching is not required.

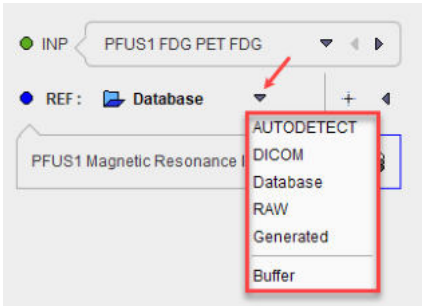
2.1.2 REFERENCE & MATCHING Sub-Page

The **REFERENCE & MATCHING** sub-page is illustrated below. It serves for loading the reference image, matching initialization and for the configuration of the registration method.



Reference Image Loading

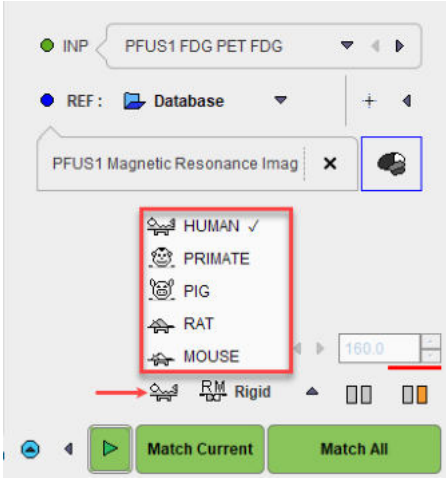
Typically, anatomical images (MR, CT) will serve as the reference, to which the lower-resolved functional input images are registered. Reference image loading is started with the **REF** load button, whereby an appropriate file format can be selected using the down arrow.



Note the convenience button for loading brain templates as the reference in deformable registrations. The loading is species-sensitive and in the **HUMAN** case shows the available brain templates for the selected normalization procedure as described below 85.

Species Selection

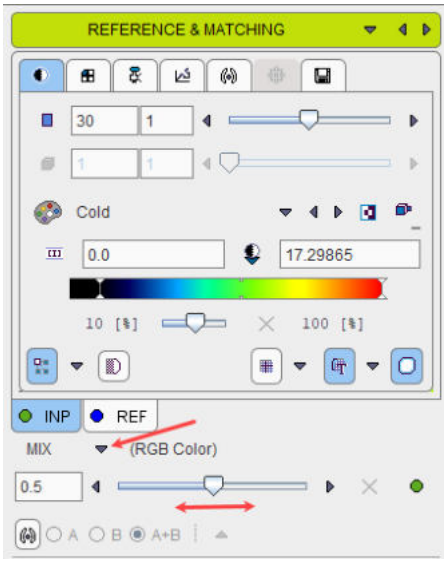
In order to apply tailored presets for the automatic procedures, PFUS tries to guess the **Species** type from the loaded data according to the criteria in the configuration 8. If it is not appropriate, please change the **Species** using the dedicated icon. The available species are **HUMAN**, **PRIMATE**, **PIG**, **RAT** and **MOUSE.**



A correct **Species** setting is important for proper registration defaults.

Fusion Display

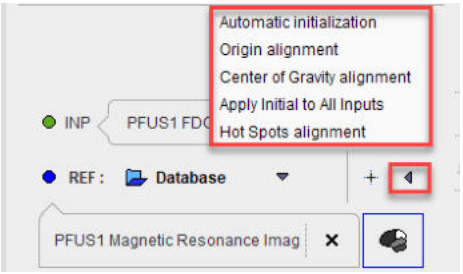
The image display on the **REFERENCE & MATCHING** page shows a fusion of the **REF** image with the currently selected **INP** image. The appearance of the individual images can be adjusted by selecting the corresponding tab and using the image presentation controls, e.g. adjustment of the color thresholds, see below. In the fusion area below the tabs there is a selection arrow for choosing the fusion method (**MIX** as default), and the slider to change the relative emphasis of the two images in the fusion.



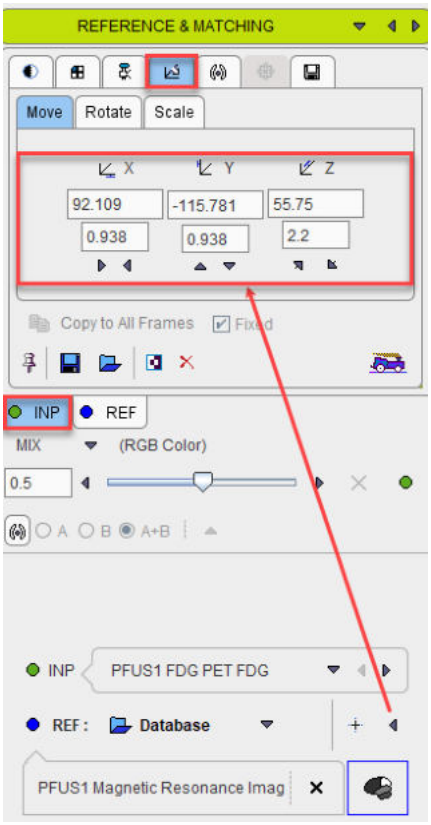
Initialization of Registration

In order to fuse the **INP** and the **REF** images PFUS performs an initial alignment procedure. If the images are from a hybrid acquisition and the **Hybrid** matching method was selected on the **LOAD INPUT IMAGES** sub-page there are good changes, that the resulting alignment is already final. Otherwise, it is only a preliminary starting point for the registration procedure to follow.

It is important for the automatic registrations that the images on the **REFERENCE & MATCHING** page show a sufficient overlap. If this is not the case, other initialization types have to be applied which are available to the right of the **REF** selection:



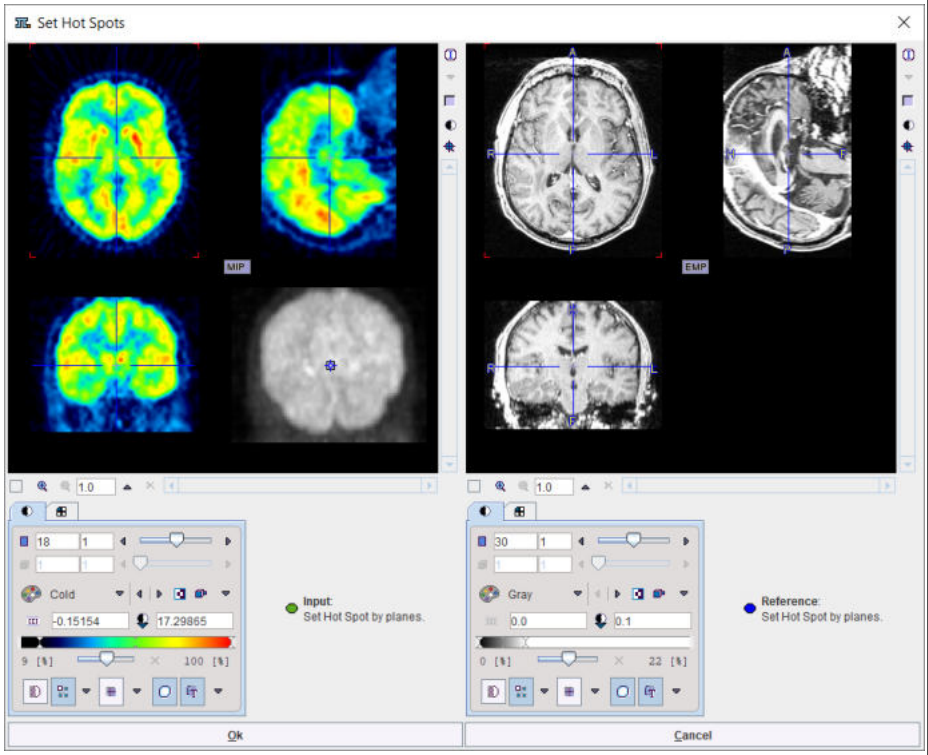
Note that an initialization results in a translation matrix, which can be inspected on the reslicing tab of the INP series:



The initializations behave as follows.

	For current **INP**: Alignment of the **INP** and the **REF** image volume center.
Automatic initialization	Repeats the initial alignment procedure for all loaded **INP** images. This is particularly helpful after unsuccessful matching trials.
Origin alignment	For current **INP**: Alignment of the **INP** and **REF** coordinate origins. This works if the two series have the origin at the same anatomical landmark.
Center of gravity alignment	For current **INP**: Alignment of the **INP** and **REF** gravity centers. This works if the two series have about the same value distribution.
Apply Initial to All Inputs	Use the current transformation for all **INP** series. This works best if the inputs are already aligned.
Hot Spots alignment	Opens a dialog window showing the **Input** and the **Reference** images in orthogonal view on the left and respectively on the right panel. The blue cross

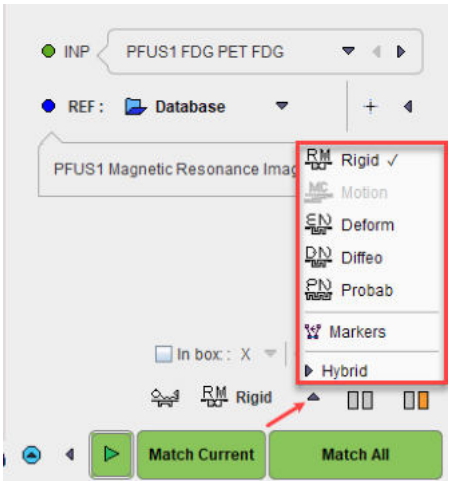
indicating the hot spot should be placed at the same anatomical landmark, as as illustrated below:



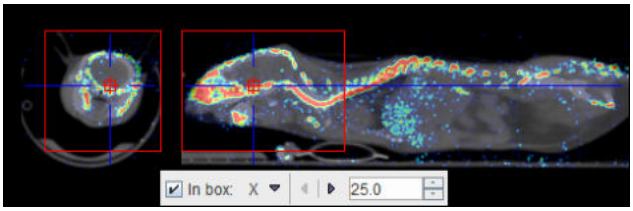
To perform the alignment of the two locations close the window with the **Ok** button. Otherwise select the **Cancel** button.

Registration Configuration

The current automatic registration method is shown next to the species label. It can be switched to another method with the selection arrow. The methods and their parameters are described in a separate section 42.



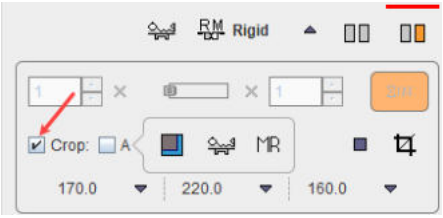
Note the **In box** option. If serves for restricting the operation of the automatic registrations to a sub-volume of the reference image. The location of the sub-volume is indicated by the red rectangles. As the crop box, it can be positioned with clicking at the center of the volume of interest, and the edge sizes can be changed with the direction selection and the edge length to the right of **In box.**



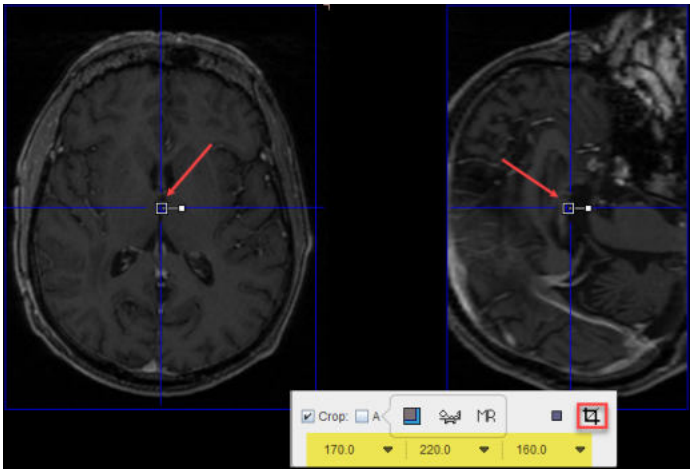
Reference Image Cropping

The reference image can be cropped in the same way as the input images. Please shift the fusion slider to the right to only see the reference image

enable the **Crop** option,



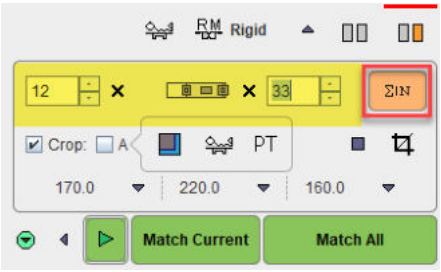
and then adjust the position and size of the blue cropping volume.



The actual cropping has to be started explicitly with the button. The **Auto** cropping works as described for the INPUT page 21.

Averaging of Dynamic Images

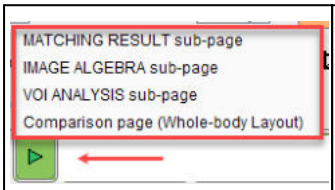
If a dynamic reference image is loaded, a frame averaging option is applicable. In the example below a range between frames **12** and **24** is defined.



As soon as the indicated averaging button is activated, the time-weighted frame average is calculated and the result replaces the reference image.

Action Buttons

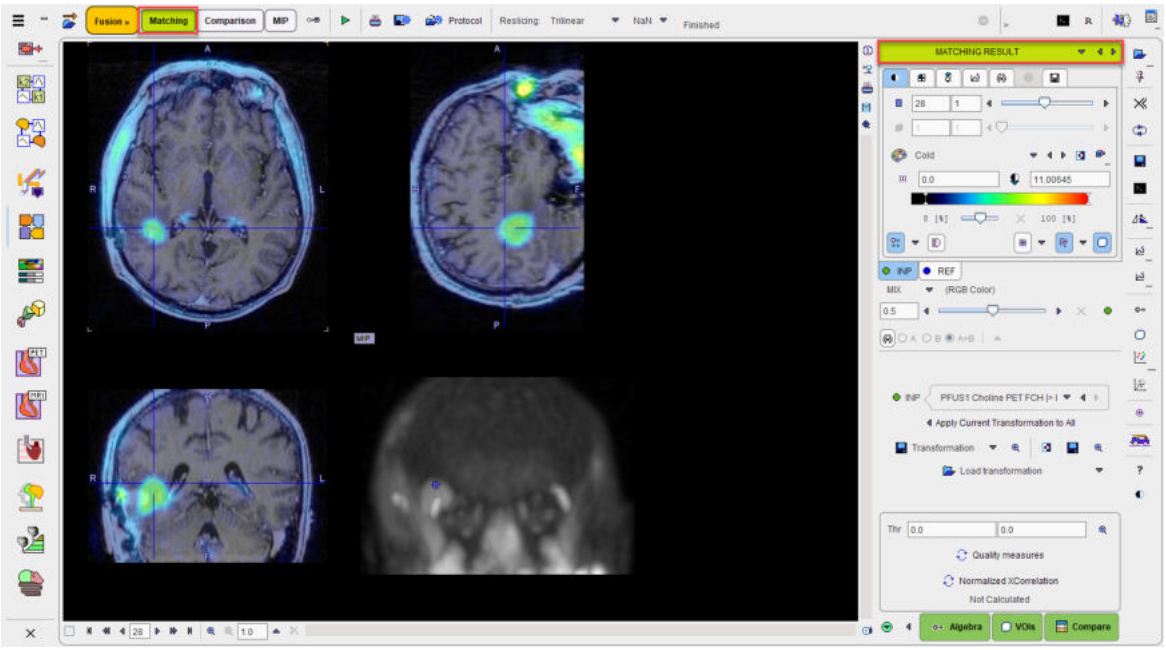
After the reference image has been loaded and prepared by cropping or averaging, the action buttons can be applied.

	No automatic registration is performed and assumes that all input images are already resampled to the reference geometry. For instance, when the **Hybrid** matching method was selected the PET image of a PET/CT hybrid scan will be interpolated to the resolution of the CT image. When activated it allows skipping the matching step and visualizing the result on the selected FuseIt page or sub-page .
Match Current	Starts registration of the selected input image to the reference image using the configured method.
Match All	Starts registration using the configured method and sequentially registers every input image to the reference image.

At the end of the calculations, the result is shown on the **MATCHING RESULTS** sub-page.

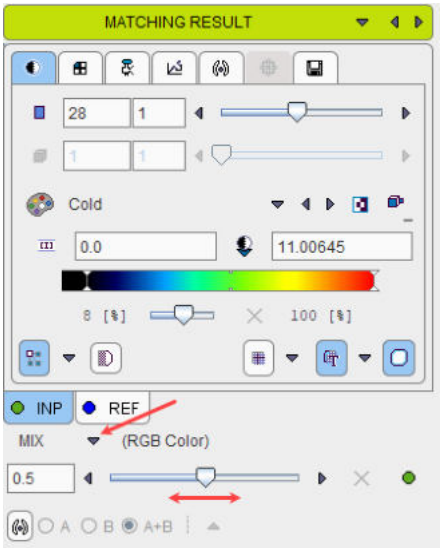
2.1.3 MATCHING RESULT Sub-Page

The **MATCHED** page is illustrated below. It serves for evaluating the matching, manually adjusting the alignment and supports operations with registration transformations.

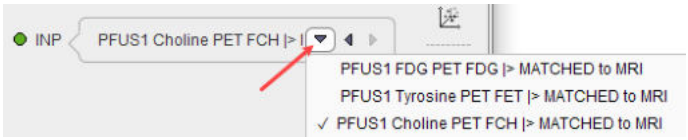


Fusion Display

The image display on the **MATCHING RESULT** page shows a fusion of the **REF** image with the currently selected **INP** image with the usual image fusion controls.

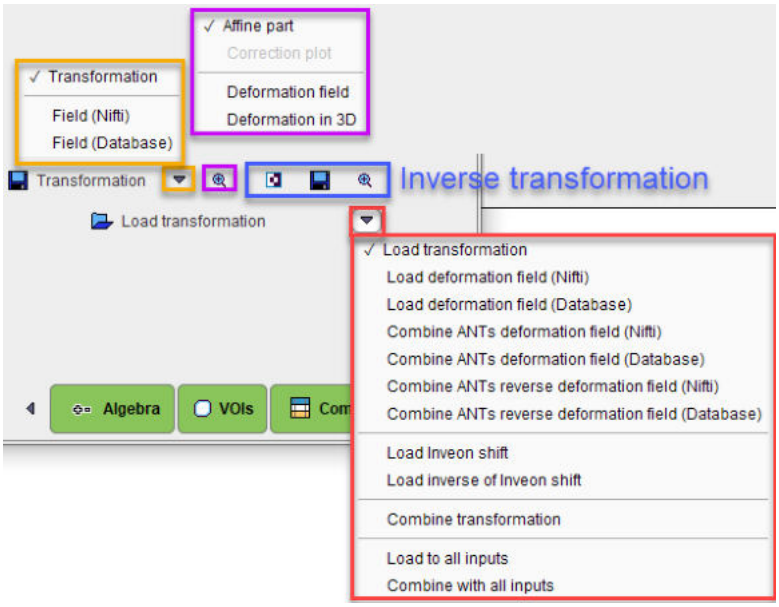


The image used for the fusion display can be selected in the **INP** list.



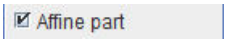
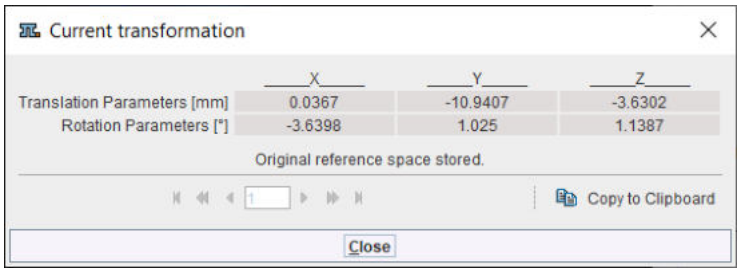
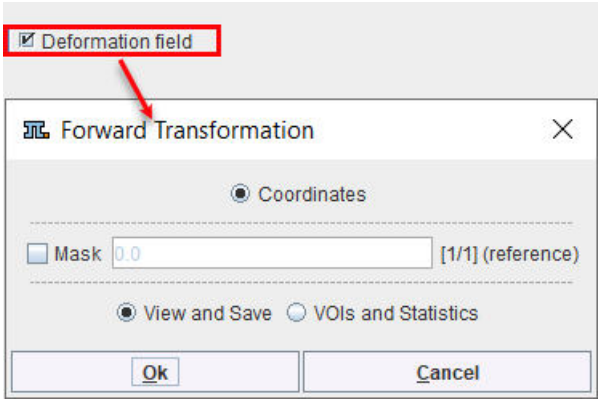
Transformations

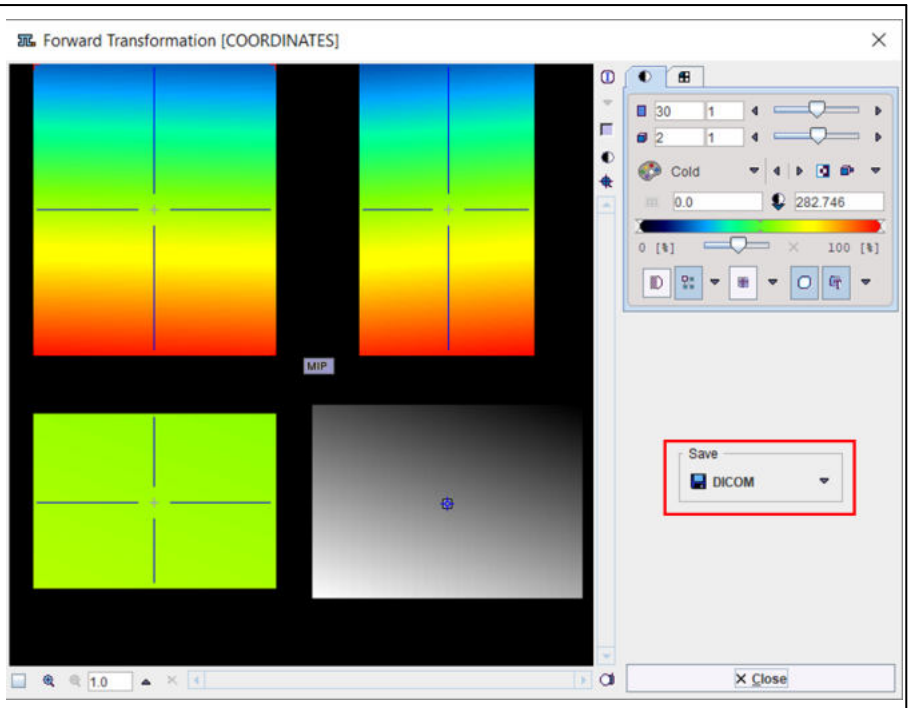
Each of the **INP** images has its own spatial transformation [6] which maps the input image from the original space to the reference space. These transformations as well as their inverse are accessible in the expanded control area at the bottom.



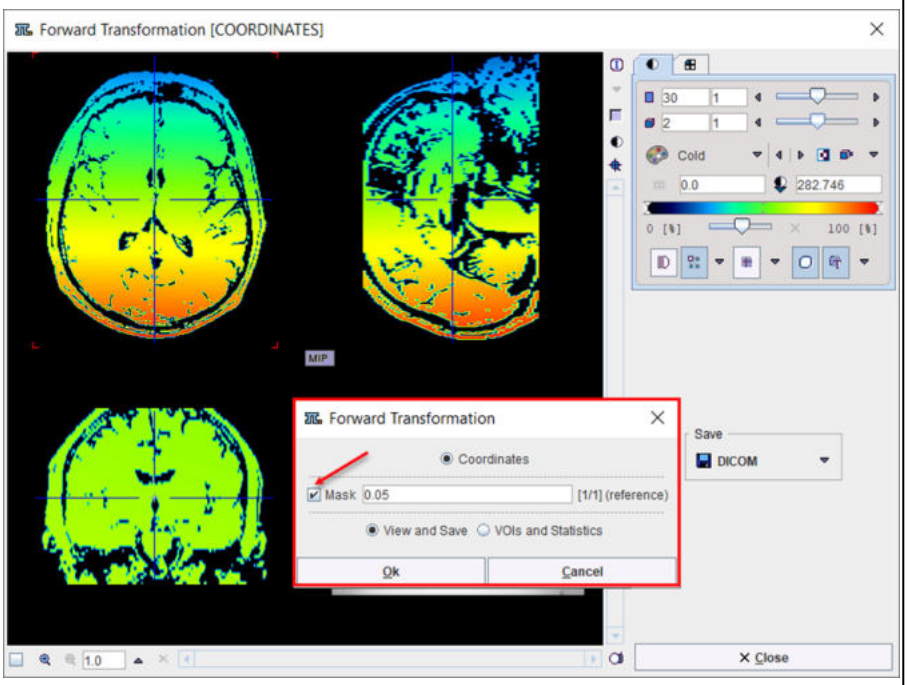
The functionality of these transformation-related elements is as follows:

Transformation	▪ ☑ Transformation

	Save the transformation of the current input image. This includes the automatic registration as well as subsequent manual adjustments. ▪ For the probability maps normalization two additional options become active under the save selection:the two options allow saving the deformation **Field** in **NIftii** or **Database** format.
	▪  Show the affine transformation part of the current input image in a dialog window   ▪ Show the deformation filed of the forward transformation in the three directions, X, Y, Z, as frames. Two options are available for display: the **View and Save** and the **VOIs and Statistics**. The **Mask** option, when enabled, allows visual assessment of the deformation field on the reference image. The **View and Save** allows visual assessment of the deformation field and saving of the deformation field as an image using the **Save** panel. Below is shown the result with the **Mask** option OFF:

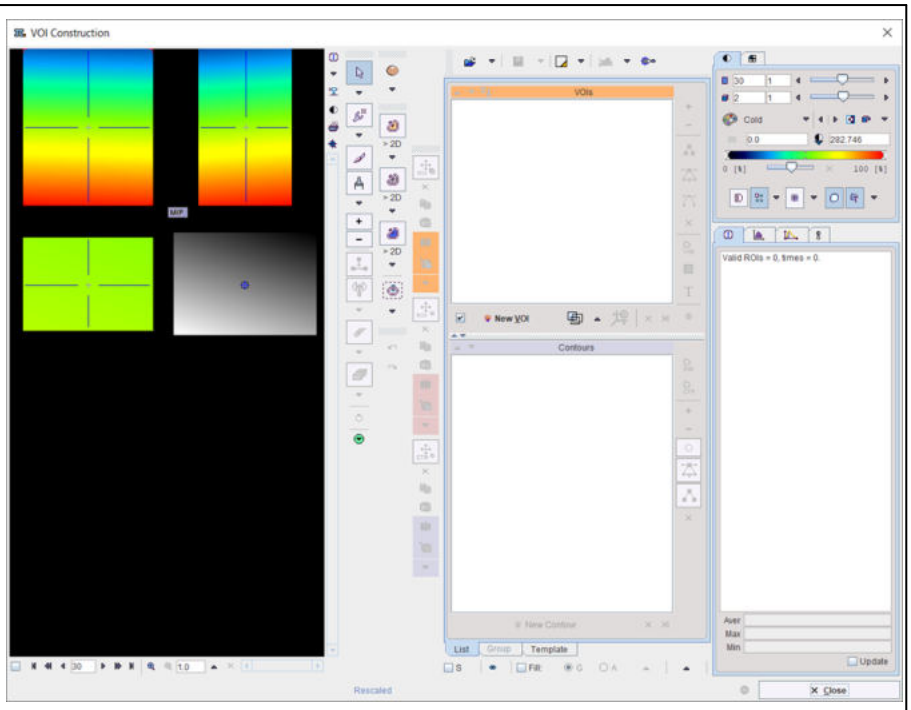


Enable the **Mask** box to view the deformation field on the reference image . Only the pixels with values above the specified value in the text field will be shown for the reference image, e.g 0.05, as illustrated below.

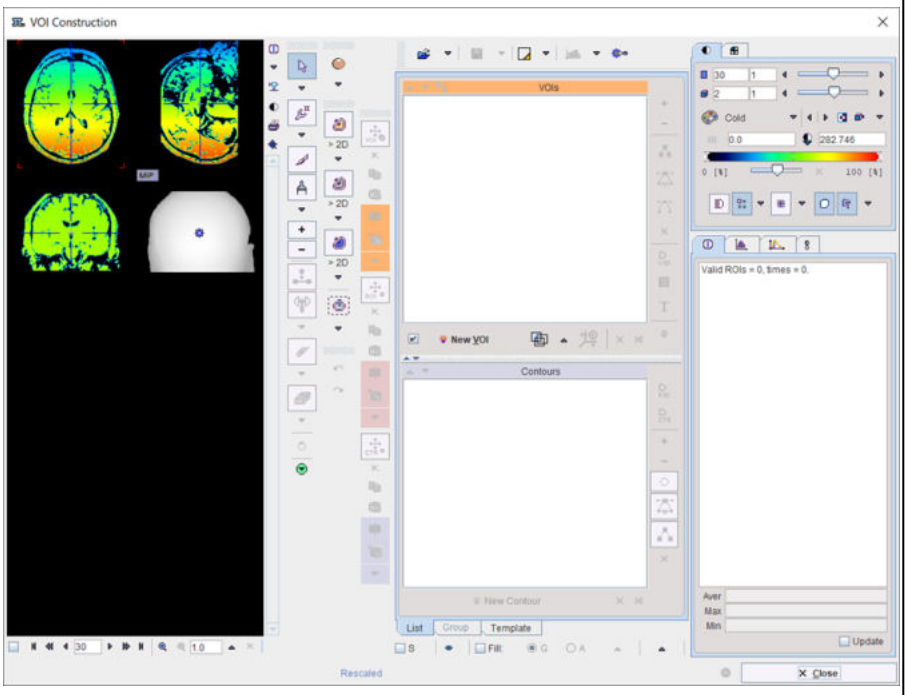


The **VOIs and Statistics** option opens the **VOI Construction** interface allowing to outline VOIs for the calculation of statistics for the deformation field.

Below the interface appearance with **Mask** option OFF**:**



and with **Mask** (0.05 threshold) option **ON**:

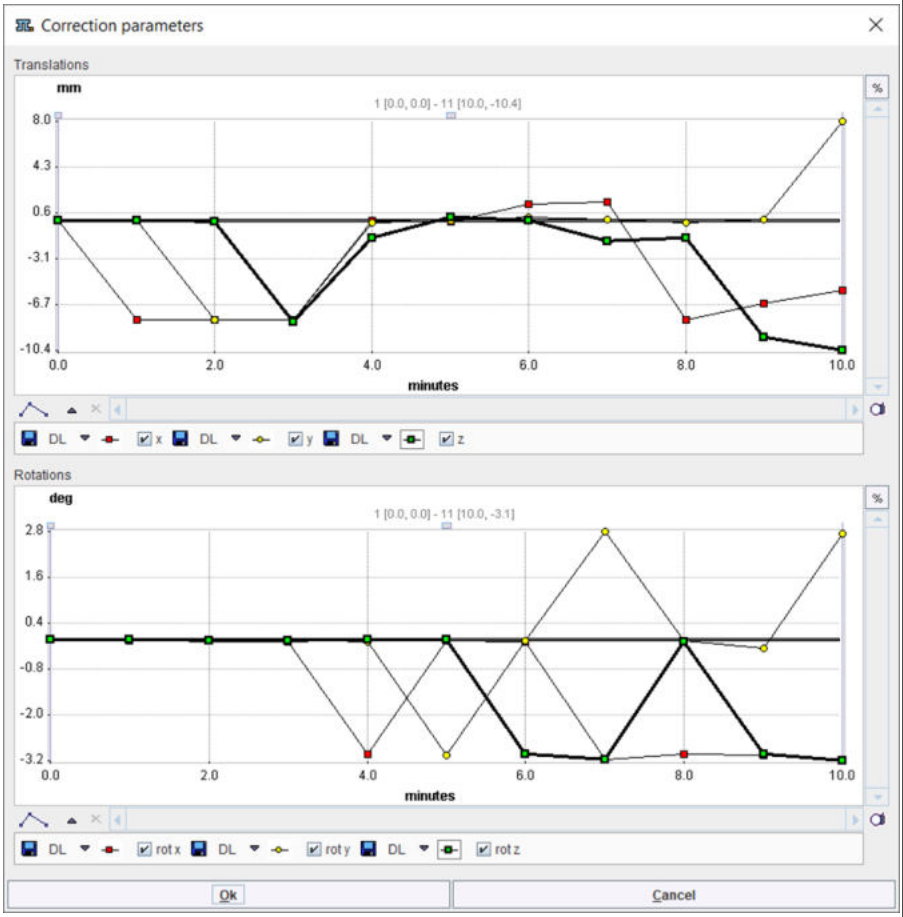


- ☑ Deformation in 3D

Show the deformation field in the 3D window.

- ☑ Correction plot

Displays plot graphs of the parameters resulting from the motion correction of a dynamic image

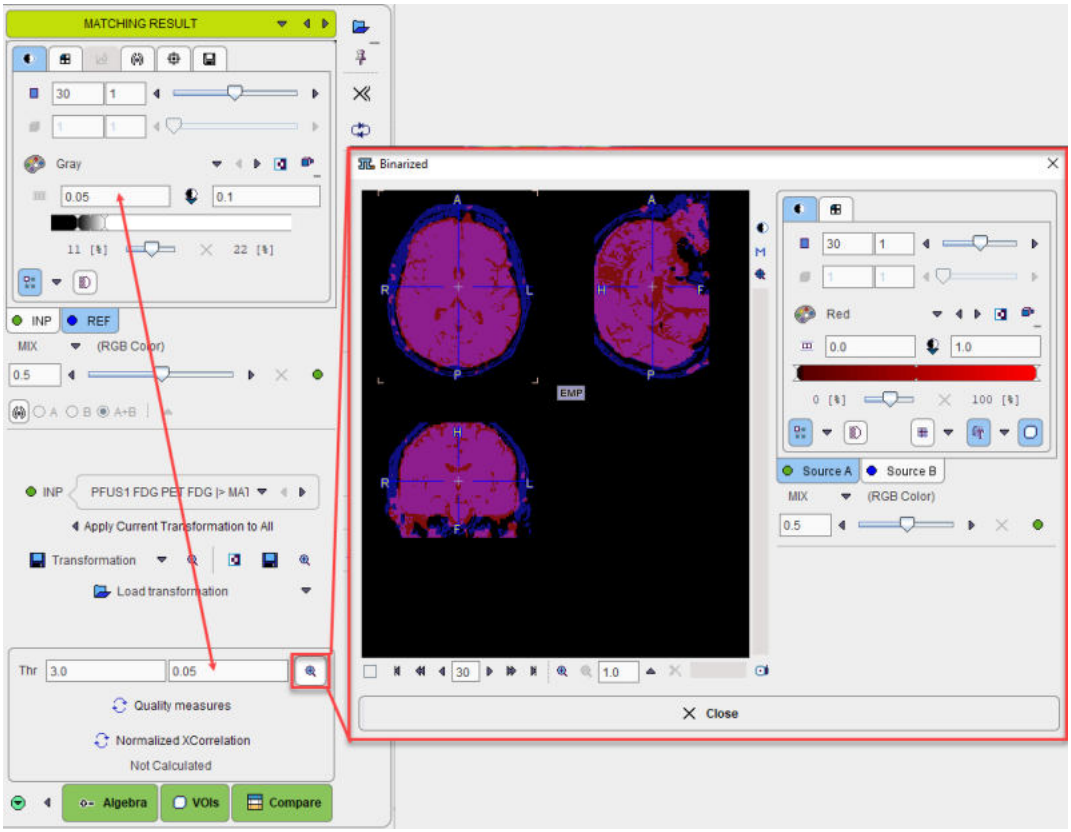
	
	Calculate the inverse of the current transformation.
	Save the inverse of the current transformation.
	Show the affine part of the inverse transformation in a dialog window.
Load Transformation	Load a transformation, replacing the transformation of the current input image.
Load Deformation Field (Niftii)	Load a probability maps deformation field saved in Nifti format, replacing the transformation of the current input image.
Load Deformation Field (Database)	Load a probability maps deformation field saved in Database format, replacing the transformation of the current input image.
Combined ANTS deformation field (Niftii)	Load an ANTS deformation filed saved in Niftii format and combine it with the transformation of the current input image. Note that the combined transformation becomes the current one and can be inspected with the
Combined ANTS deformation field (Database)	Load an ANTS deformation filed saved in Database format and combine it with the transformation of the current input image. Note that the combined transformation becomes the current one and can be inspected with the

Combined ANTS reverse deformation filed (Niftii)	Load an ANTS reverse deformation filed saved in Niftii format and combine it with the transformation of the current input image.
Combined ANTS reverse deformation field (Databse)	Load an ANTS reverse deformation filed saved in Database format and combine it with the transformation of the current input image.
Load Inveon shift	Read the *image_ref_shift* field from Inveon microPET files and apply it as transformation. It is required for some versions of Inveon microPET for the alignment of the PET and CT images
Load inverse of Inveon shift	Read the *image_ref_shift* field from Inveon microPET files and apply the inverse as transformation. It is required for some versions of Inveon microPET for the alignment of the PET and CT images.
Combine Transformation	Load a transformation and combine it with the transformation of the current input image. Note that the combined transformation becomes the current one and can be inspected with the .
Load to All Inputs	Load a transformation, replacing the transformation of all input images. This makes sense if all input images are in the same space, for instance for a set of parametric maps generated from a single series.
Combine with All Inputs	Load a transformation and combine it with the current transformation of each of the input images.

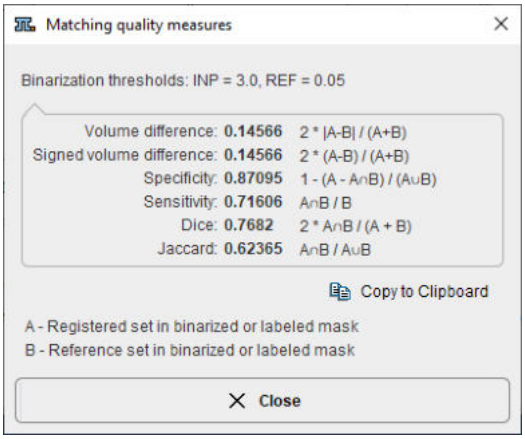
The button **Apply current Transformation to All** allows propagating the current transformation to all input series. This operation is applicable if all input images are in the same space. A typical application case is that the registration calculation has been performed with a frame average of a dynamic series, and the result transformation is now applied to the dynamic series itself. Another application case is the matching of a set of parametric maps generated from a single series.

Overlap Indexes

PFUS supports the calculation of overlap indexes as follows: In the **Thr** area threshold values can be entered for the registered **INP** and the **REF** image. Alternatively, the lower threshold of the color table can be adjusted, whereby the **Thr** values are modified accordingly. The two binary volumes can then be visualized as a fusion image with the button.

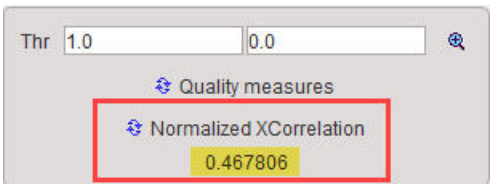


The overlap criteria are then calculated based on the two masks with the **Quality measures** button.



Normalized XCorrelation

After the matching had been performed, FuseIt supports the calculation of the normalized cross correlation with the **Normalized XCorellation** button. It shows the correlation coefficient result below:

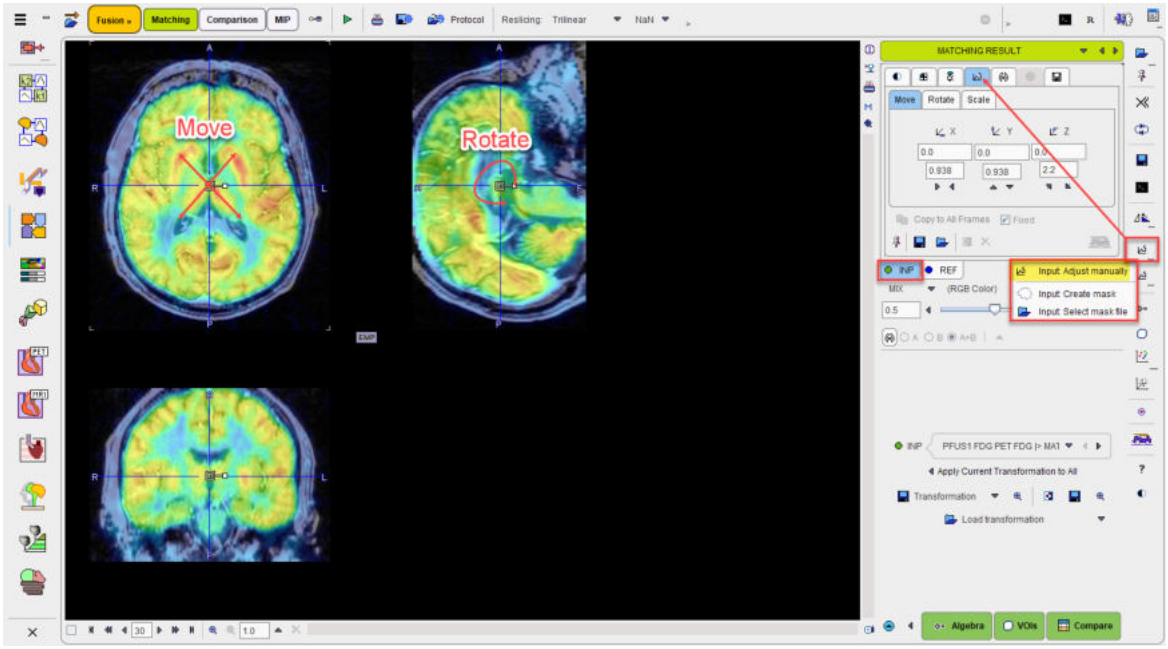


The correlation coefficient is estimated based on the overalpping criteria set in the **Thr** fields. The closer this value is to 1 the better the matching outcome.

One application for this index could be the selection of the best reference image to be used in the matching procedure. The results of the matching outcome (correlation coefficient) of e.g. INP image to the REF1 and subsequently to the REF2 image should be recorded e.g. in an excel or text file.

Manual Adjustments

After automatic registration, the input images can be manually shifted and rotated to improve the alignment, if necessary. The same applies, if automatic registration has been skipped altogether in order to perform a fully manual alignment. Manual adjustment is started with the  button in the lateral taskbar as illustrated below. It opens the reslicing tab of the **INP** images, and shows handles in the image overlay for dragging/rotating the images interactively, as described below 47.



Action Buttons

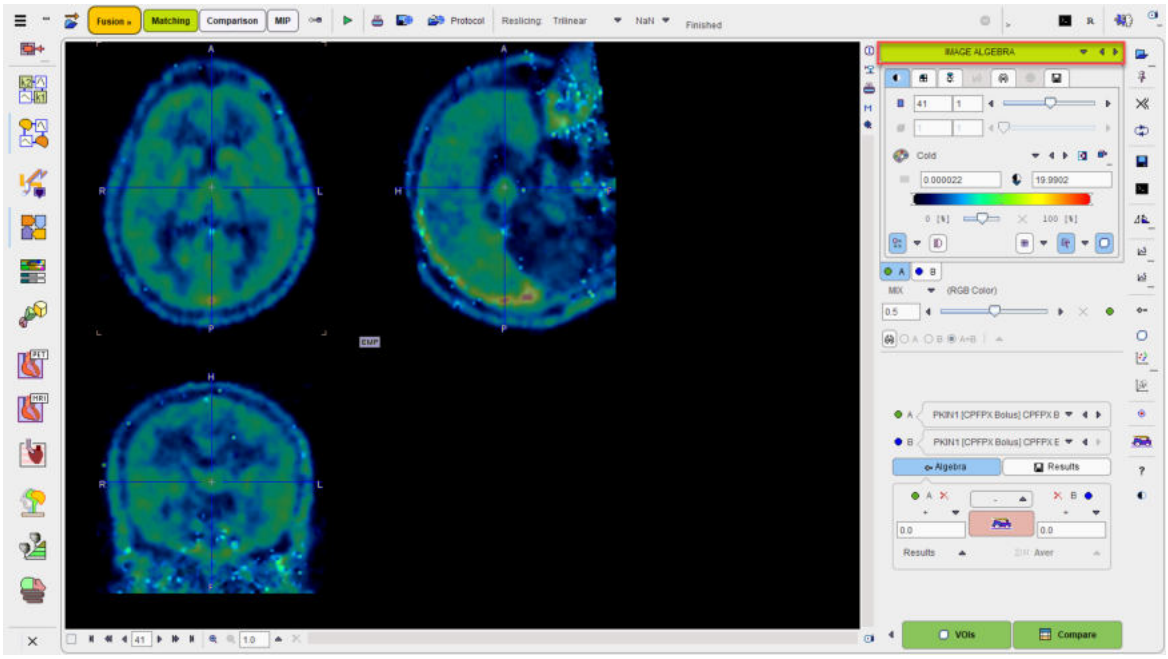
Assuming that all input images have been registered to the reference, the user can proceed to the various post-processing pages with the two action buttons

Algebra	Switches to the **IMAGE ALGEBRA** sub-page for performing pixelwise image arithmetics.
VOIs	Switches to the **VOIS** sub-page for outlining VOIs directly in fused images.
Compare	Switches to the **Comparison** page

Alternatively the main pages **Comparison** and or **MIP** can be selected.

2.1.4 IMAGE ALGEBRA Sub-Page

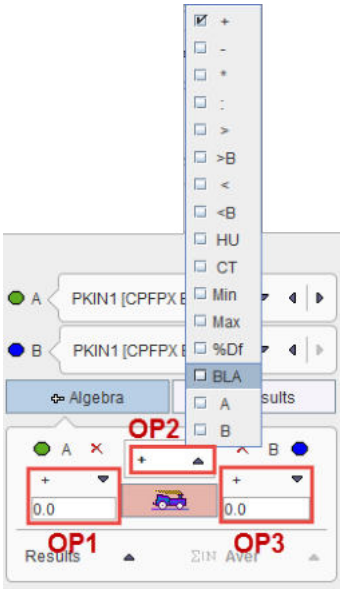
The **IMAGE ALGEBRA** page is illustrated below. It serves for applying pixel-wise operations between the registered images. Examples are the calculation of the difference image between two functional maps, or the multiplication of a mask image with a target image.



Operation Principle

The principle is that an algebraic operation is defined between two images, resulting in a new image which can also be used for further operations. The input images are defined via the **A** and **B** list selections

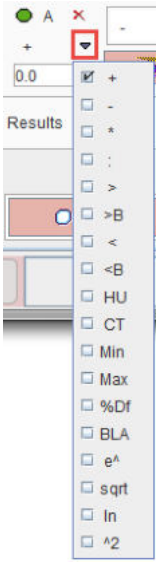
The operation between **A** and **B** is configured with the Algebra area and has the general form `(A OP1 number) OP2 (B OP3 number).`



The calculation is then started with the  button and adds the result image to the selection lists.

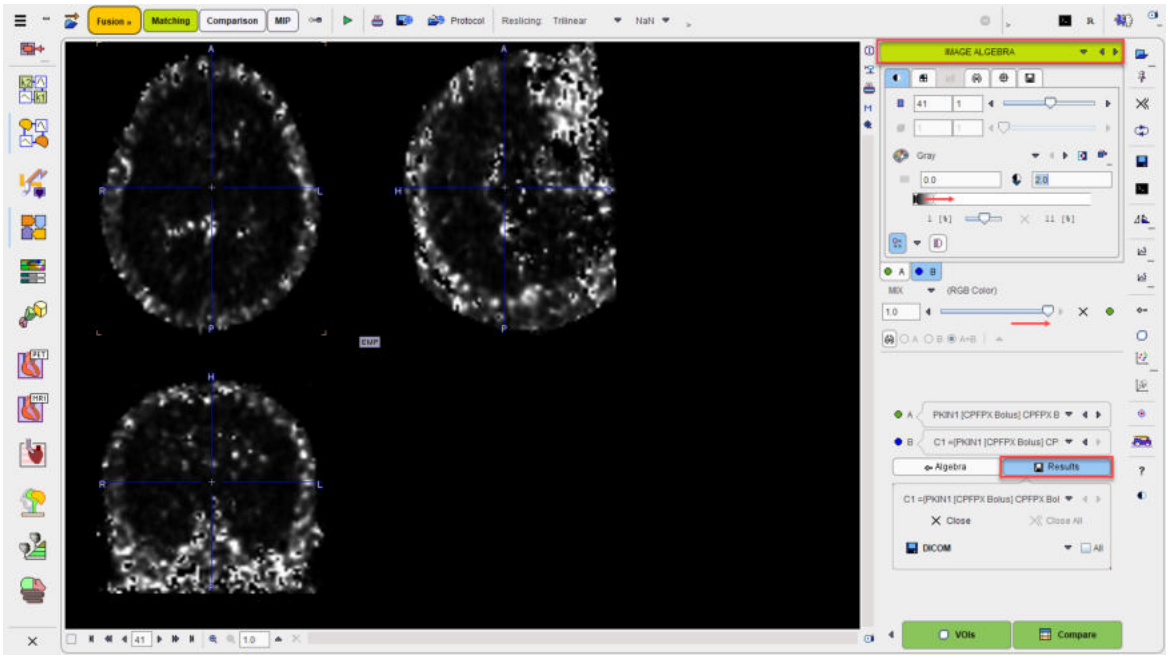
Available Operations

The following operation can be applied to the individual images (as `OP1` or `OP3`):



Results

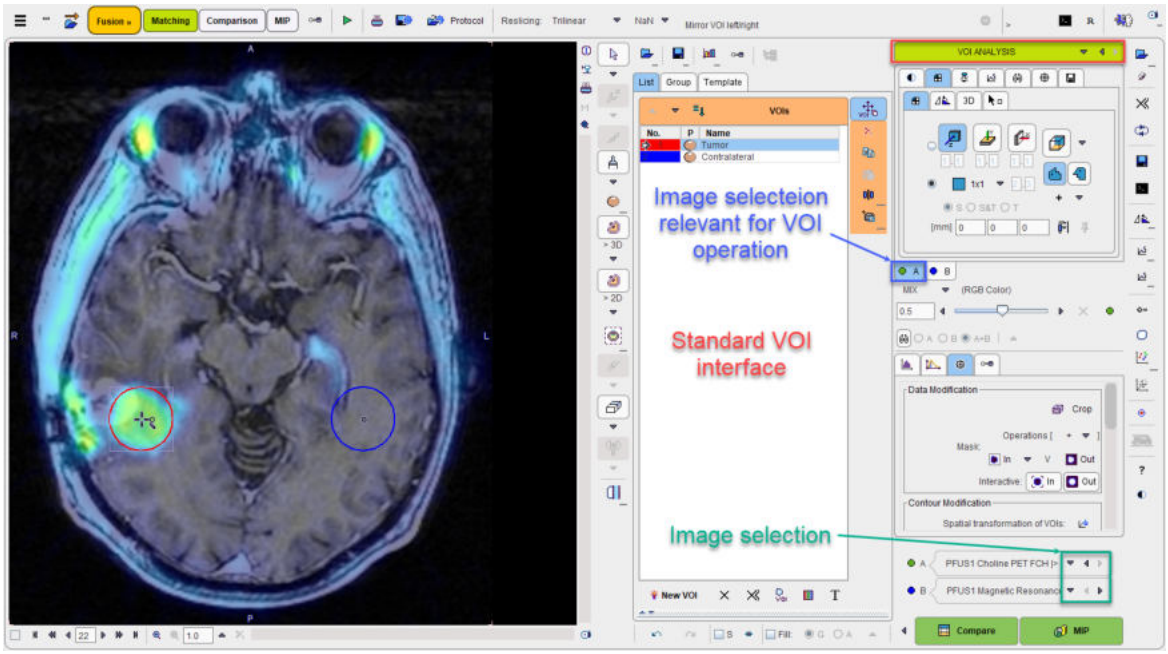
The operation results are automatically selected as the image **B** and shown in the fusion display. The color table may need some adjustments, and to only see the result image the fusion slider should be set to the right. The example below shows the difference between the Vt maps calculated with two different methods.



The **Results** button gives access to the created result images in a dedicated area. There are buttons for selecting among the results, closing or saving a result.

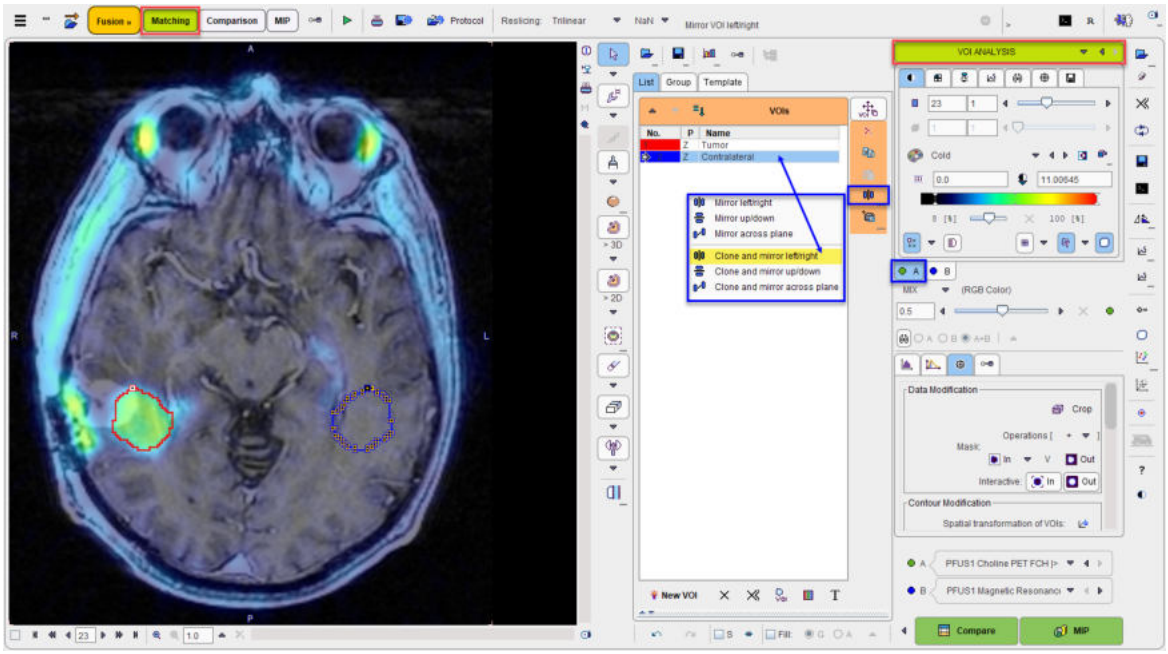
2.1.5 VOI ANALYSIS Sub-Page

The **VOIs** page is illustrated below. It serves for outlining volumes-of-interest directly in the fused images.

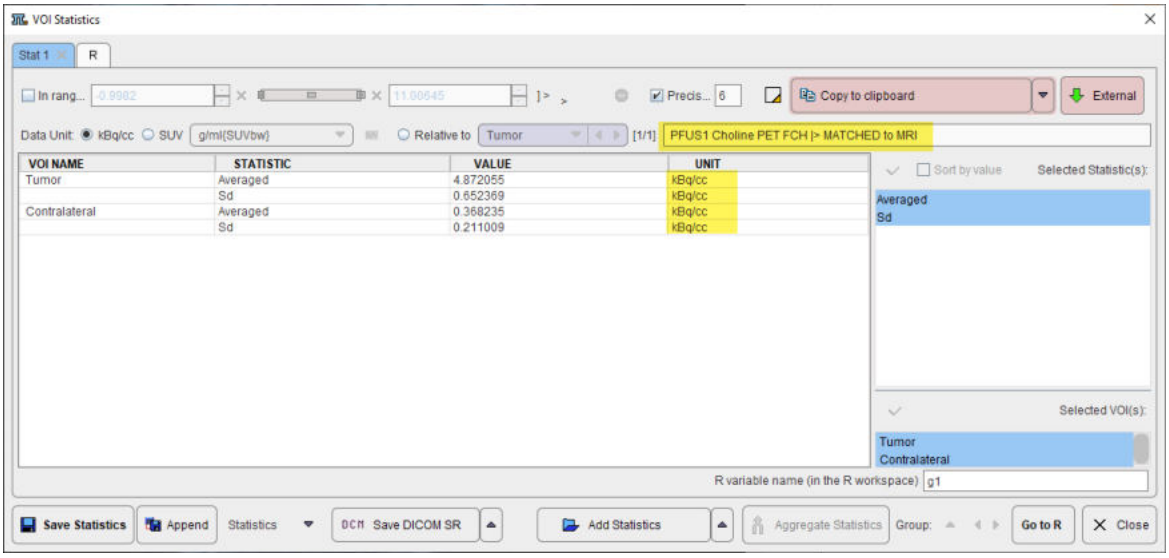


VOI Definition and Evaluation

The standard VOI options are available for the VOI creation. Please refer to the *PMOD Base Functionality Guide* for explanations of the VOI functionality. The only distinctive thing to consider is, that the series selected on the tab to the right (**A** or **B**) is relevant for VOI definition and evaluation. In the example below, the choline PET series **A** is selected, so that the hot iso-contouring was successful in detecting the tumor boundary. The Contralateral VOI was obtained using the **Clone and mirror left/righ** the Tumor VOI.



When the statistics is calculated with the button, the choline uptake in the tumor uptake is obtained:



Otherwise, had the tab **B** been selected, iso-contouring would have operated on the MRI and failed in the tumor outlining task.

Image Selection

If more than one input series has been processed or image algebra results were generated, there are several candidate images for the VOI statistics. The two selections in the lower right allow freely defining which series is configured on the **A** and **B** tabs. After a suitable configuration of the image presentation and the selection of the appropriate source the VOI controls can be minimized using the button indicated above.

Action Buttons

Assuming that all input images have been registered to the reference, the user can proceed to the various post-processing pages with the two action buttons.

Compare	Switches to the **Comparison** main page 69 for visualizing multiple fused images.
MIP	Switches to the **MIP** main page 79 for creating rotating fusion MIP renderings.

Alternatively the main pages **Comparison** and or **MIP** can directly be selected with the tabs.

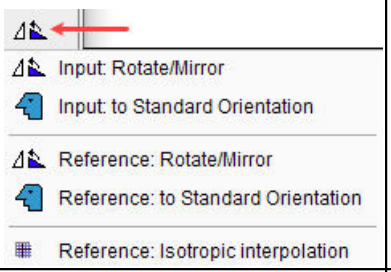
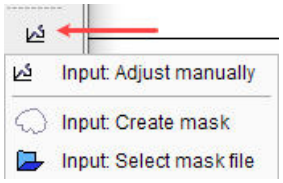
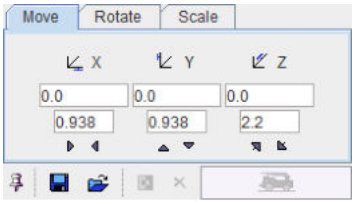
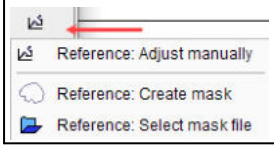
2.2 Matching Workflows

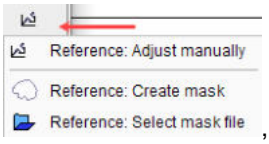
The following sections describe popular matching scenarios. In most cases it is assumed that the input and reference images have been loaded as described above 44.

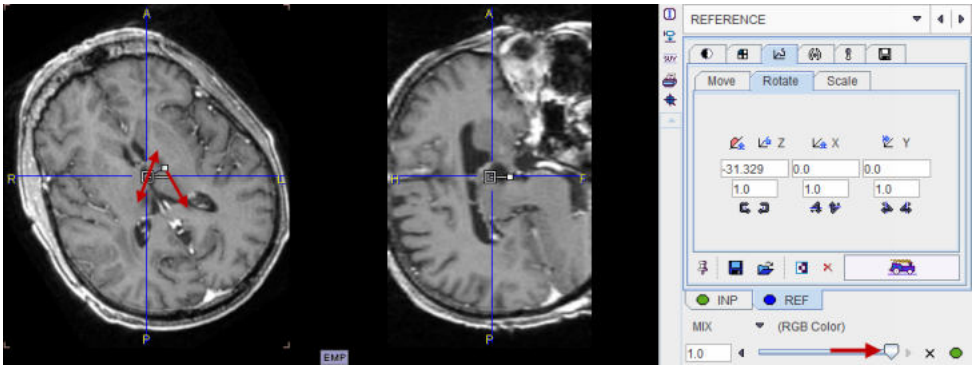
2.2.1 Recommendations

Initial Reorientation

Before the actual registration is addressed, the images should be brought into a consistent orientation. If this is not the case after loading, the images may be reoriented. There are shortcut buttons in the lateral taskbar to achieve this conveniently.

	Initial rearrangement of the **INP** images by mirroring and rotations with the panel  Reorient the image to Standard orientation HFS
	Similar for the **REF** images..
	Initial reorientation of the **INP** images by arbitrary translations and rotations with the reslicing panel. 
	Similar for the **REF** images by arbitrary translations and rotations with the reslicing panel.

In the example below, the reference needs to be straightened. Activate  , shift the fusion balance fully to **REF**, and then rotate the axial image.



Transformation Initialization

The next step is to ensure that the initialization is appropriate. This means that the images are either already aligned on the **REFERENCE** sub-page, or that they are brought into a reasonable overlap as described **above** 24.

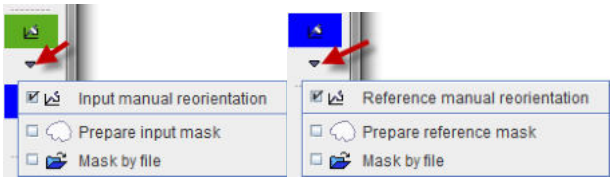
Layout Adjustments

Initially the images will appear in orthogonal layout (`Ctrl+D`) which allows working easily in all 3 dimensions. For fine adjustments it may be preferable to switch to the axial (`Ctrl+Z`), coronal (`Ctrl+Y`) or sagittal (`Ctrl+X`) single-plane layout.

Restriction of Matching Volume

In some cases the automatic matching procedure needs to be restricted to a sub-volume of the data. This can be achieved in different ways.

As described above 24, the **In box** option allows defining a box in the reference image, top which the registration algorithm will be confined. An alternative is to define a free-form masking volume on the input or reference image using the selections from the lateral taskbar illustrated below.



Prepare input mask opens the segmentation tool described in the *PMOD Base Functionality Guide* for generating a mask file. **Mask by file** allows selecting an existing mask file

which can be inspected with the button. Note that each input file has its own mask definition

Registering Dynamic Images to a Reference

In the case of a dynamic **INP** series it is recommended to proceed as follows:

1. Check whether there is motion in the data. If there is, a motion correction 50 should first be applied.
2. Calculate an average image from some dynamic frames. Typically, early PET frames will result in a perfusion-related image which provides a good pattern for registration to an MR image.
3. Match the average image to the reference.
4. Apply the resulting transformation to the dynamic series, as described above 30.

2.2.2 Image Loading

There are several alternatives for loading images in PFUS.

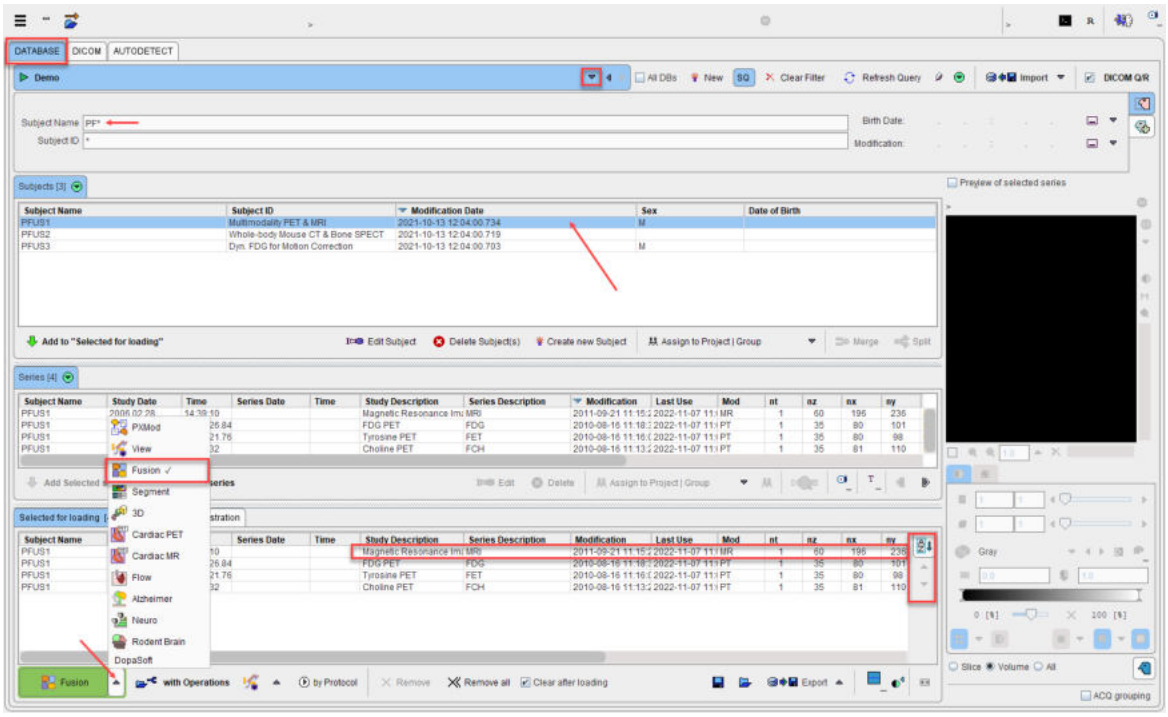
Step-wise Loading

If the user directly starts working on the **Matching** page image loading is straightforward: All images loaded on the **LOAD INPUT IMAGES** sub-page are treated as the input images for registration. The image which is loaded on the **REFERENCE & MATCHING** sub-page serves as the registration reference. Only one reference image is supported, a successive loading will overwrite the current reference.

Loading from the Unified Data Loader

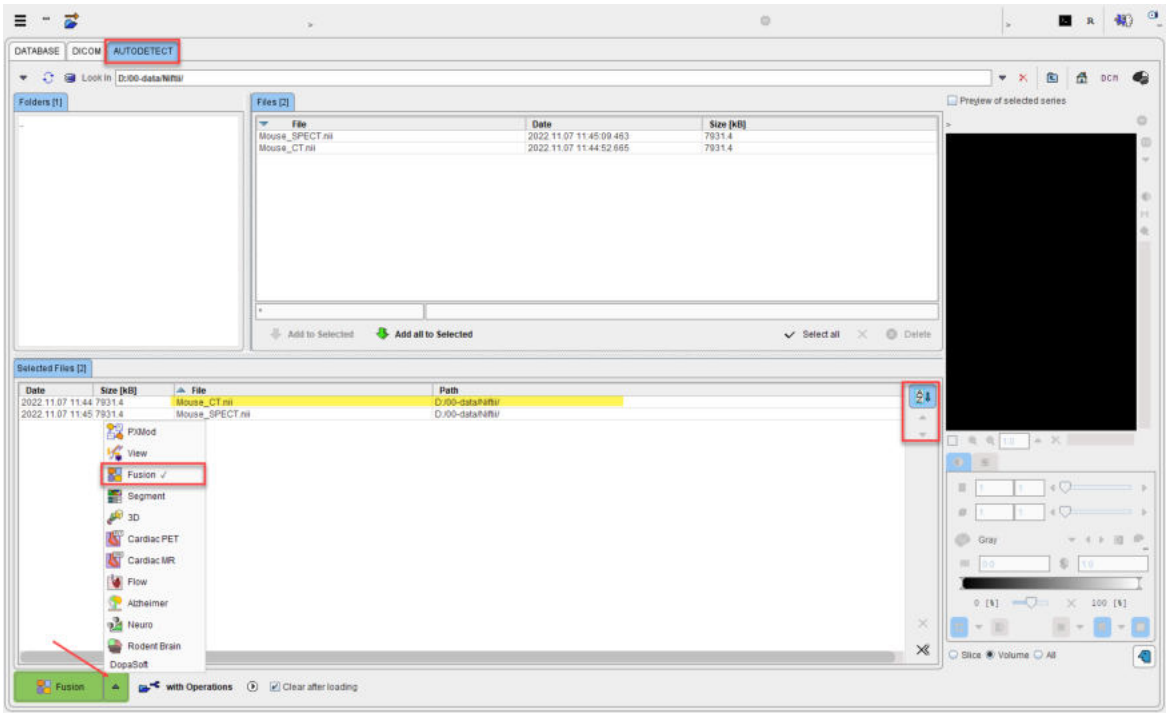
Loading from DATABASE Tab

The loading of multiple images is supported when using the **Unified Data Loader, DATABASE** page. The basic rule is, that the first entry in the **Selected for loading** list is treated as the reference, all others as input images. Note the arrow to the right of the list for changing the list order, and the button for enabling alphabetical sorting by the column headers. Finally, select the **Fusion** tool to load the data.



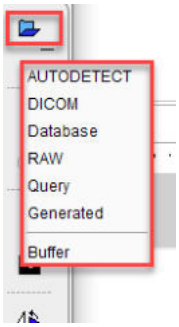
Load From Autodetect Tab

Illustrated below is **AUTODETECT** format loading. The first entry in **Selected Files** will be loaded as the reference, the following entries as input images.



Loading from Lateral Taskbar

Loading from the taskbar works similarly, but supports different image data formats.



Reference Defaults

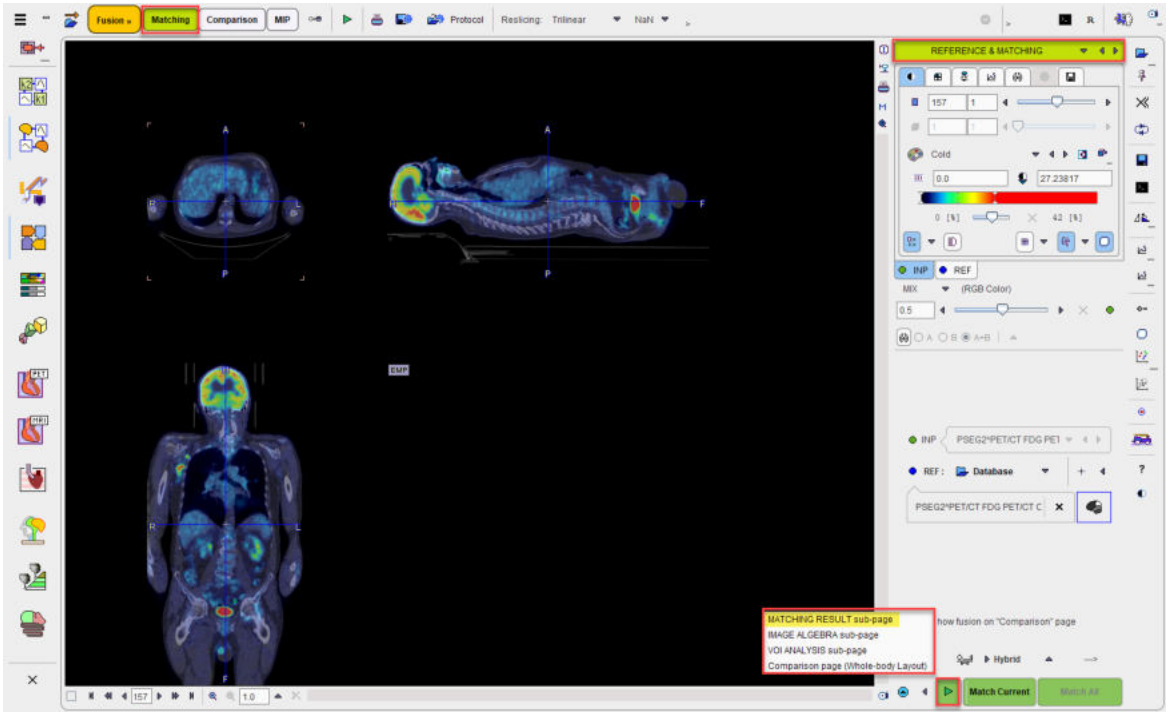
The configuration facility allows establishing convenient defaults for multi-modality situations.

If the modality is encoded in the data format (DICOM, Database), this configuration will take precedence over the order in the loading list and the anatomical image will always appear as the reference. Hence it is not necessary any more to bring the anatomical reference to the first position in the selection list.

2.2.3 Already-matched Workflow

The simplest case is the situation that the input and the reference images are already registered. Examples are images from hybrid acquisitions, images like parametric maps derived from a common data set, or images arising in a standard template space.

In this case, the images should already be aligned on the **REFERENCE & MATCHING** sub-page after loading. Please simply proceed to one of the post-processing options using the "Already matched" button indicated below.

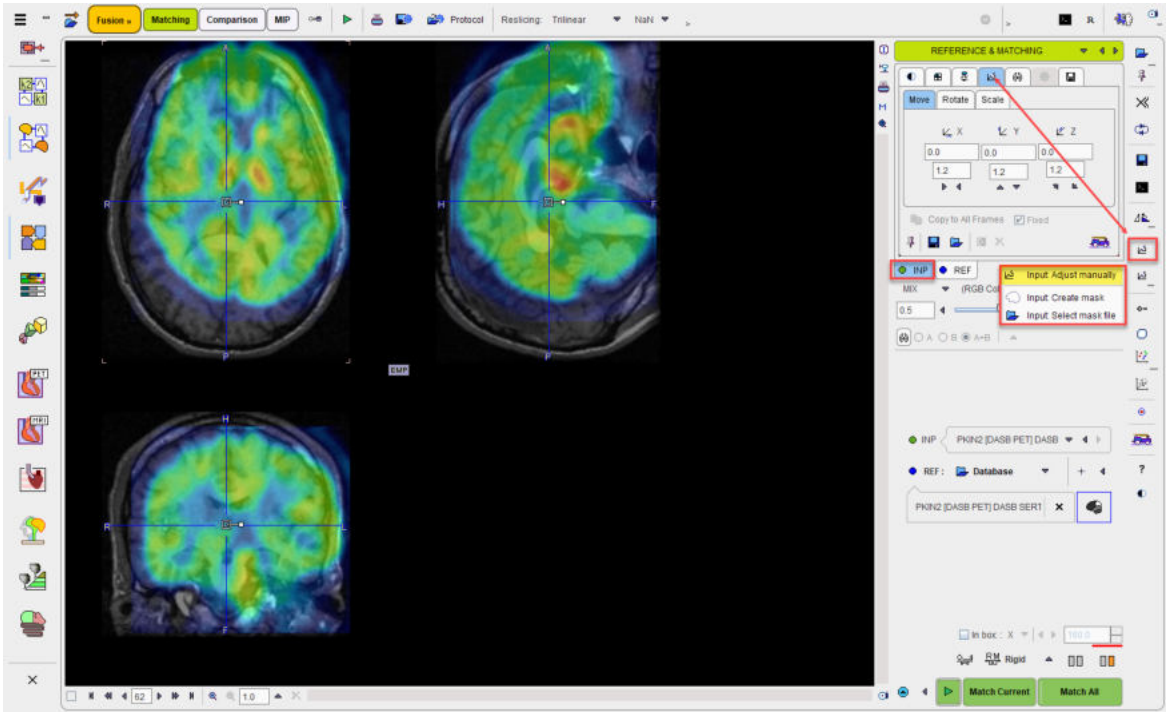


It allows continuing in the selected environment without applying any matching procedure and. It assumes the images are aligned and that the input image was reslice to the resolution of the reference image.

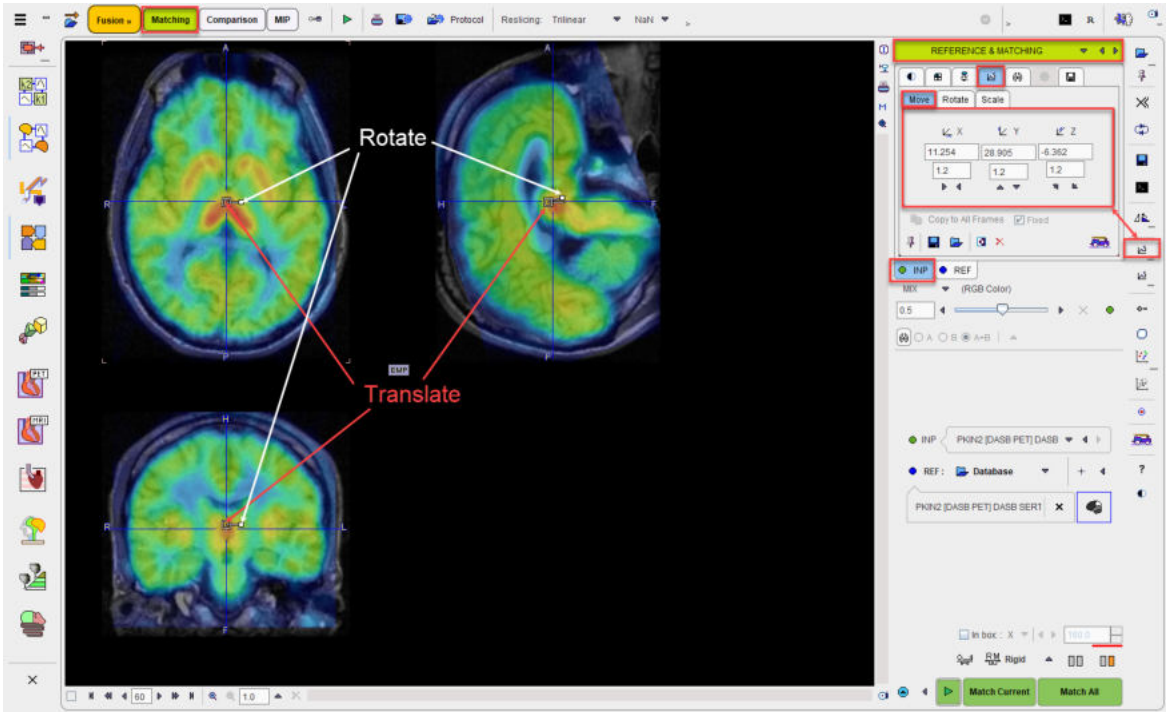
2.2.4 Manual Interactive Matching Workflow

If the loaded images don't appear to be aligned on the **REFERENCE & MATCHING** page start by try using one of the initialization of registration option described previously 24. This should give a preliminary reasonable starting point for the procedure to follow.

In the example below the centers of the **INP** and **REF** images are aligned using the initialization indicated in the capture belo:



To see the parameters of the results of the initialization select the **INP** reslicing shortcut in the lateral taskbar:

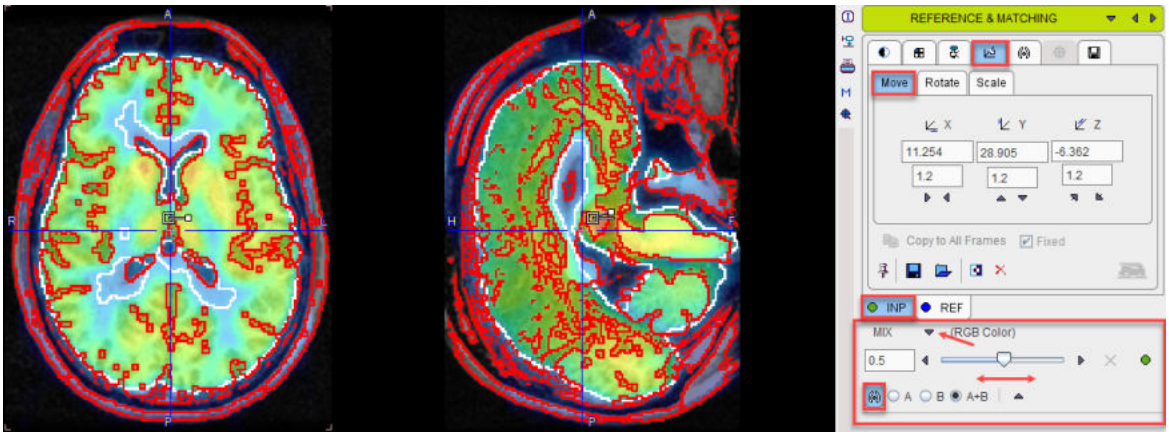


To further refine the alignment, shift and rotate the **INP** image until it aligns with the **REF**. Shifting can be done by entering offsets in the **Move** panel, or dragging the open rectangle directly in the images. Rotation angles can be numerically entered in the **Rotate** panel, or the image interactively rotated by dragging the small filled rectangle in the image overlay. Adjust the **INP** image position and orientation until the anatomy in both images is aligned.

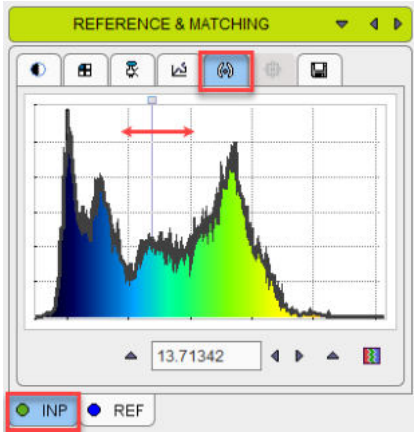
Evaluating the Alignment

The evaluation of the alignment is a subjective and iterative process. It is recommended verifying the result in all plane orientations and using different fusion techniques such as iso-contours and overlay windows which are described in more detail separately.

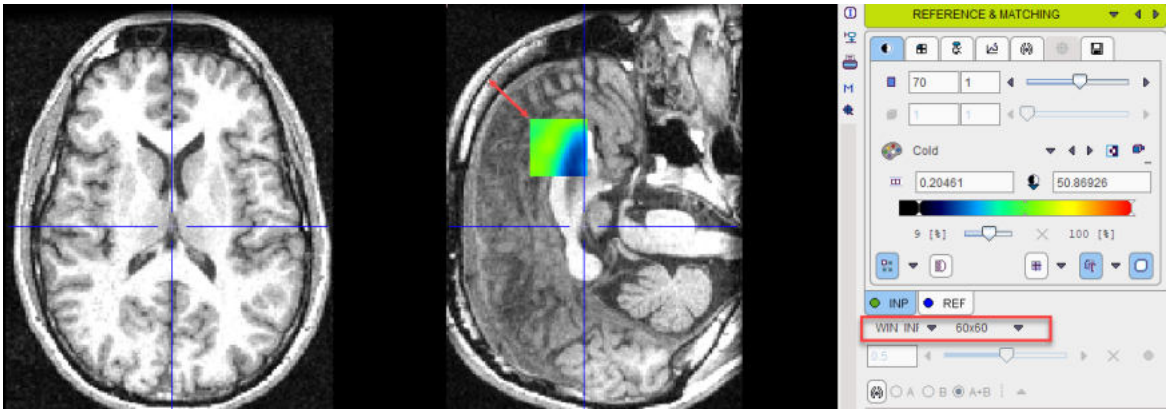
Often the iso-contours are helpful because they highlight boundaries which might be common in both images.



If the contouring level is not appropriated, adjustments can be made on the corresponding panels of the **INP** and **REF**.

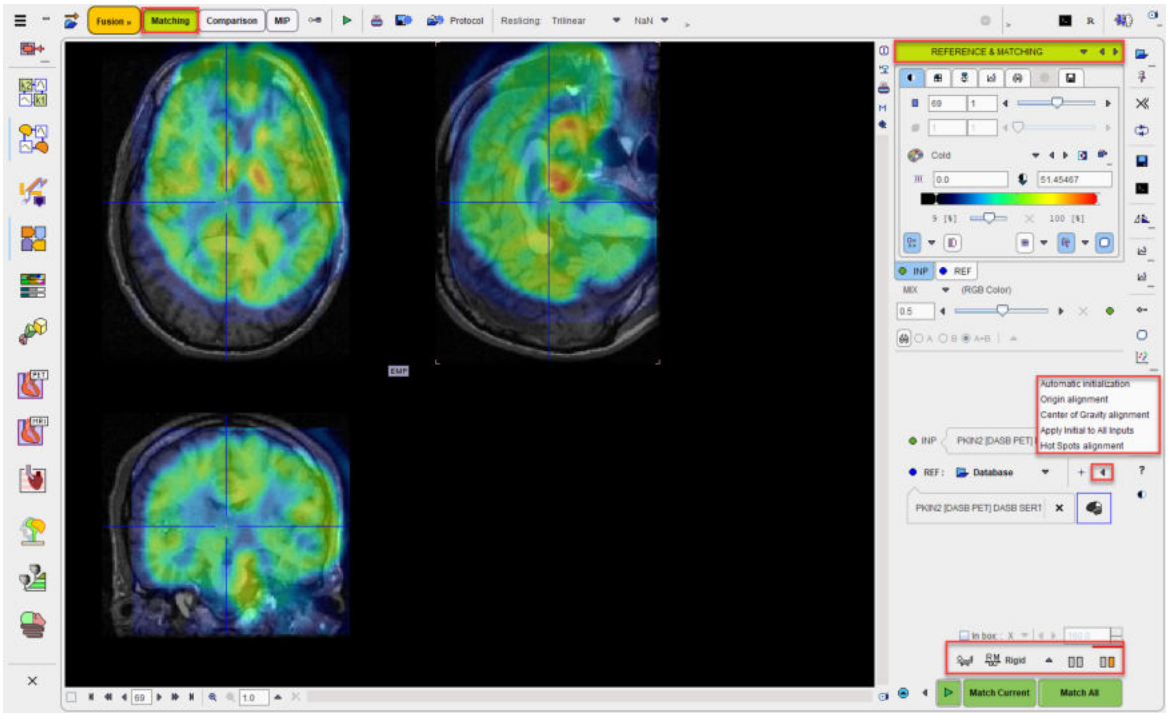


An alternative method for checking the boundaries is with the **Win INP** fusion method. The display only shows the **REF** image, but when the left mouse is clicked in the images the **INP** content at this location is shown in a window of configurable size.



2.2.5 Rigid Matching Workflow

The rigid matching approach is applicable for images of the same subject if there is no significant deformation in the anatomy of the target tissue. Note that an appropriate initialization is required so that the image volumes overlap sufficiently. Make sure that the proper species is selected (e.g. **HUMAN**), and the registration method is set to **Rigid** as illustrated below.

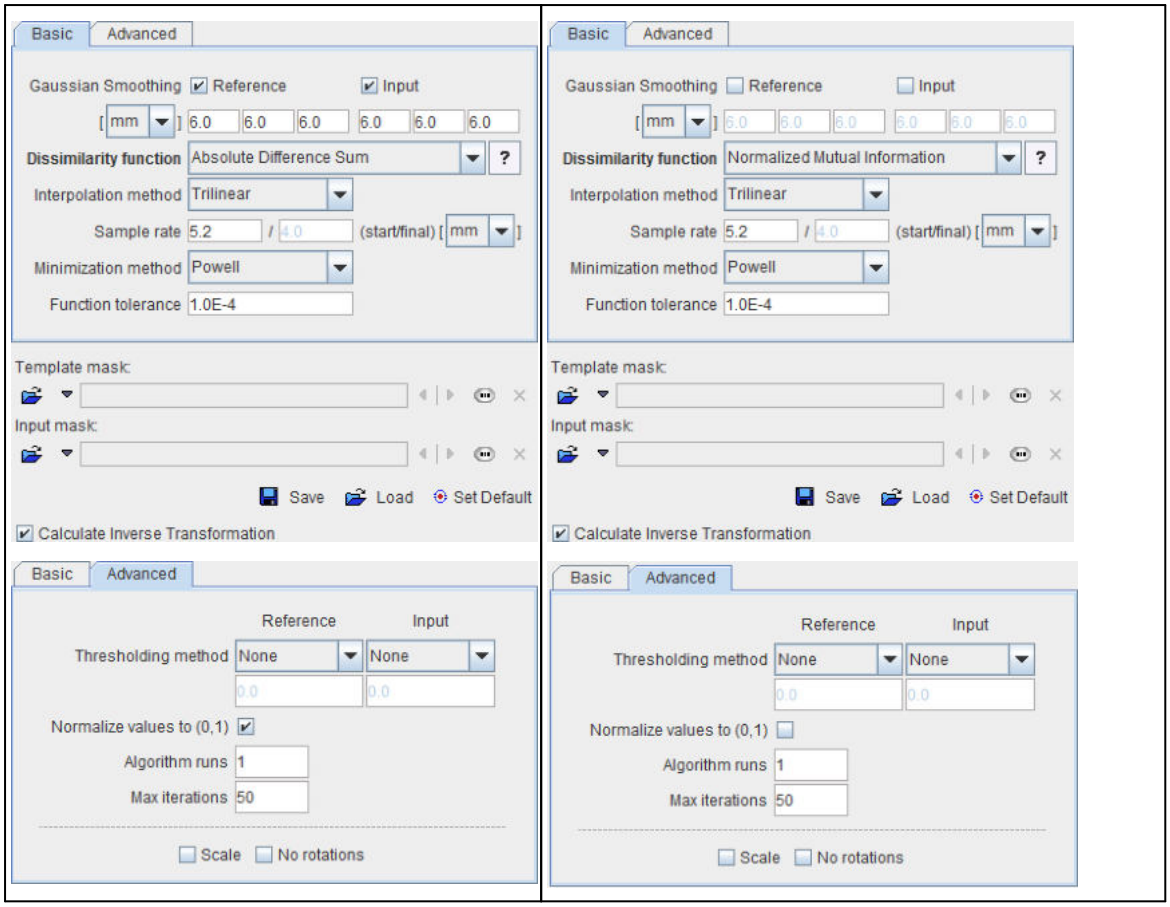


Rigid Matching Parameters

The **Rigid** matching algorithm uses several parameters, which are hidden from the user interface. There are two presets, ▯▯ for matching images with similar values (same-modality situation), and ▯▯ otherwise (cross-modality situation). The red bar above the buttons indicates which preset is active.

To enable a preset and edit the parameters please select one of the two buttons. A dialog window opens and shows the current configuration. The **HUMAN** default settings are shown below and can always be restored with the **Set Default** button.

▯▯	▯▯



Note the differences in the **Dissimilarity function**, the **Gaussian Smoothing**, and the **Normalize values**. The parameter details are described in a separate section 84.

Important: The parameter settings are serialized. The next time **Rigid** matching is selected for the same species, the last parameter configuration will be applied. This is particularly relevant for the Matching without Interaction 64 functionality.

Starting the Registration

Please use the **Match Current** button to start the registration of the currently selected **INP** series to the **REF** series. In the case of multiple **INP** series the Match All is also active. It serves for matching each **INP** series to the **REF** applying the same registration parameters.

In the case of a dynamic series one would rather perform the registration with a frame average, and use **Apply Current Transformation to All** on the **MATCHING RESULT** sub-page to bring the dynamic series also into alignment.

2.2.6 Motion Correction Workflow

Motion correction can only be applied to a dynamic input series. The aim is to correct for subject motion which is visible in the images and bring the anatomy into agreement across all the dynamic frames. The implementation uses the rigid matching approach, so it is only suitable when the motion doesn't result in deformation of the target tissue. Note that most appropriate way for PET and SPECT data is to correct motion during the image reconstruction, because otherwise the attenuation correction will not be fully accurate.

Please first load the dynamic images on the **LOAD INPUT IMAGES** sub-page 21. Proceed selecting the **Motion** correction **Matching method**.

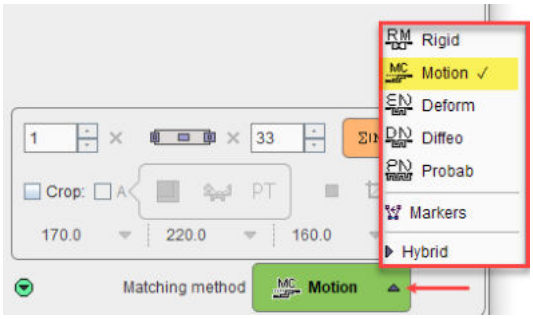
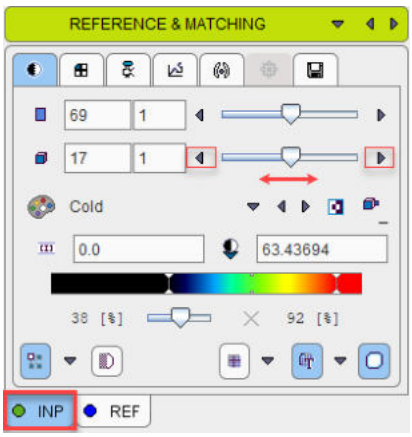
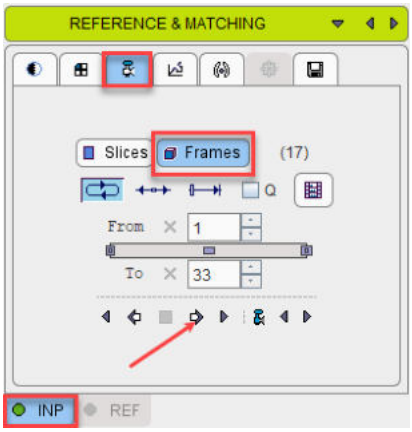


Image Inspection

On the **REFERENCE & MATCHING** sub-page 24 inspect the motion in the data in order to see where the motion starts. This can be achieved by stepping through the frames using the slider



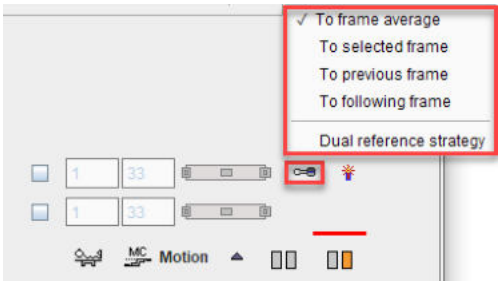
or by playing a cine across time



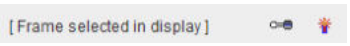
Reference Image for Motion Correction

There are various approaches for using rigid matching in the context of motion correction. One approach is to use **REF Load** for loading a suitable image to which the frames of the dynamic series are rigidly matched.

Alternatively, a reference can be created out of the series itself in different ways with the button as illustrated below.



The choices work as follows:

To frame averaged	 An average image is calculated from a range of frames and serves as the reference for the correction of the frames. The average is calculated from the sub-range defined by the upper selection range, which should only have negligible subject motion. Use the button for the actual reference creation.
To selected frame	 The frame shown in the display will serve as the reference. Please note that if the pattern in the image changes significantly over time it will be difficult to motion correct successfully using a single frame. The use of markers is a way to potentially alleviate this problem. Use the button for the actual reference creation.
To previous frame	 In this mode, motion correction matches each frame to its previous with the advantage that gradual pattern changes are less of a problem. On the other hand, successive matching errors might accumulate with this strategy. The final transformation per frame is obtained by combining the transformation matrices of all preceding frames. In this way multiple interpolations in the final image reslicing are avoided.
To following frame	 This is the same principle as the **To previous frame** mode, but the method works from the last frame in the selection through the earliest one.
Dual reference strategy	 This option uses the selected frame to correct the upper range of frames. Next, an average image is created from the matched frames and set as reference. This new reference is used to correct the lower range of frames.

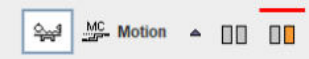
Correction Range

Optionally, configure a sub-range, wherein motion correction will be performed.

A reason to exclude a range of frames may be the lack of signal in the initial frames, and/or frames with a short acquisition duration during which subject motion is less likely. Excluded frames will be copied to the corrected series without changes, and the correction matrix of these frames will contain zero for all rotations/translations.

Matching Parameters

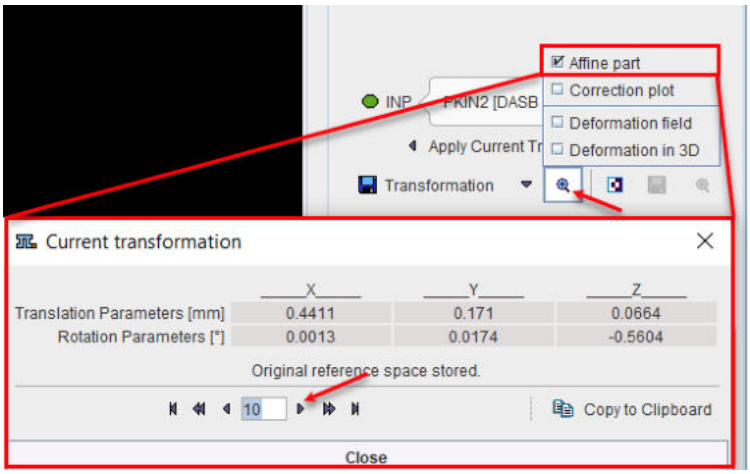
Motion correction uses the rigid matching technology and has the same two parameter presets and (default). The red bar above the buttons indicates which preset is active. As described for rigid matching 49, the parameters can be tailored if needed.



Make sure the **Species** setting is correct.

Starting the Motion Correction

Please use the **Match current** button to start the process, and inspect the results which are shown on the **MATCHING & RESULT** sub-page. The resulting transformation is a sequence of rigid transformations as illustrated below.



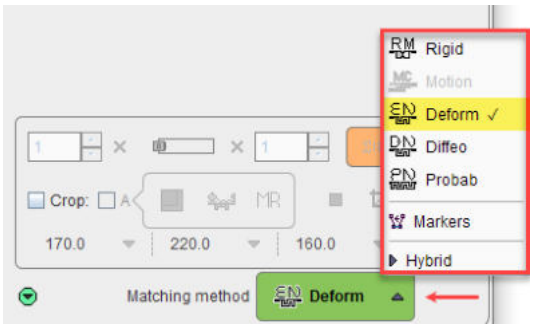
Correction plot

This option displays plot graphs of the parameters resulting from the motion correction of a dynamic image as illustrated below:

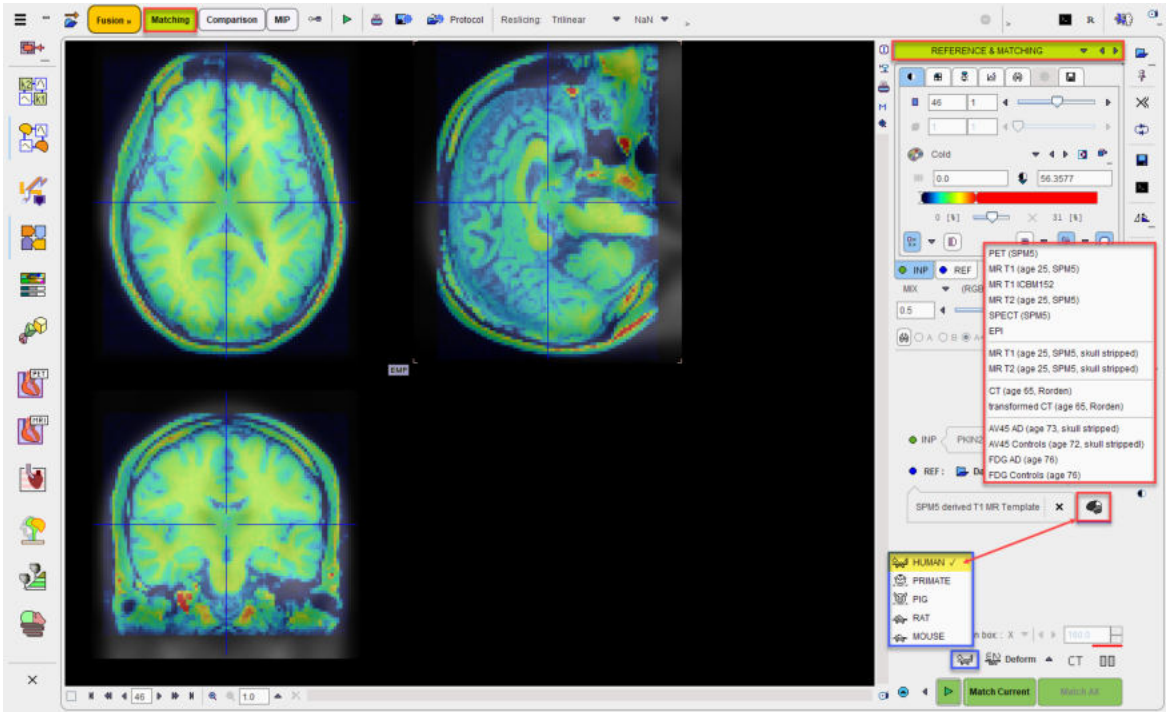
2.2.7 Elastic Deformation Workflow

The deformable registration approach is the template-based normalization[87] of SPM8 mainly suited for the stereotactic normalization of brain images using appropriate template images which can be loaded with the button. However, application to different scenarios is also possible.

Please first load the input images on the **LOAD INPUT IMAGES** sub-page[21]. To proceed select the **Deform**able registration as **Matching method**.



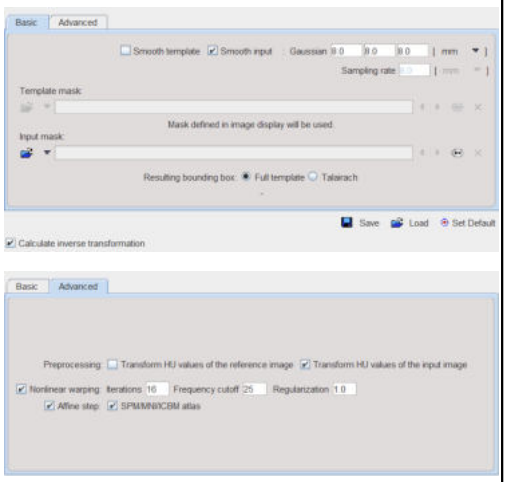
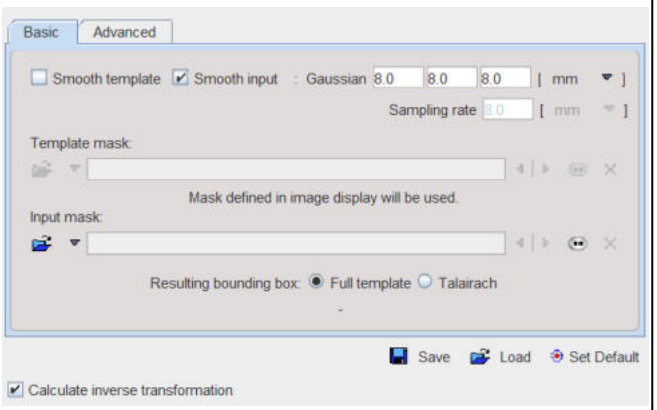
On the **REFERENCE & MATCHING** sub-page[24] make sure the **Species** setting is correct and load the reference image either with **REF** load button, or using the shortcut to load an in-built template[85]. Note that the selection of built-in templates changes according to the species selection.

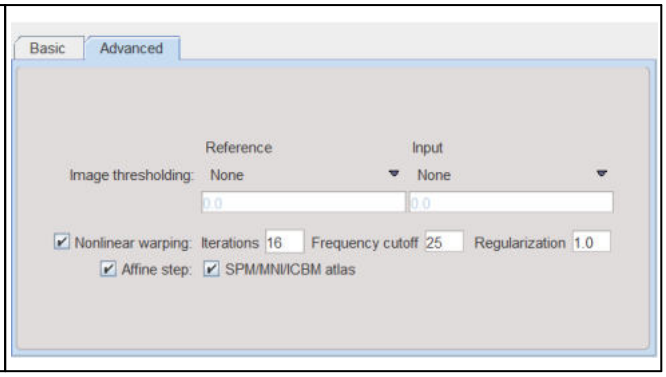


Deformable Matching Parameters

The **Deform**able matching algorithm uses several parameters, which are hidden from the user interface. For the **HUMAN** species there are two presets, CT for the normalization of CT brain images, and ▯▯ otherwise. The red bar above the buttons indicates which preset is active. For other species, the CT preset is absent.

To enable a preset and edit the parameters please select one of the buttons. A dialog window opens and shows the current configuration. The **HUMAN** default settings are shown below and can always be restored with the **Set Default** button.

CT	▯▯
	



Note the **Transform HU values** options which transform the values in the CT image such that the contrast between bone and soft tissue is reduced and they are more similar to the usual anatomical images. The parameter details and the deformation method are described in a separate section 87.

Important: The parameter settings are serialized. The next time **Deform**able matching is selected for the same species, the last parameter configuration will be applied. This is particularly relevant for the Matching without Interaction 64 functionality.

Starting the Registration

Please use the **Match Current** button to start the registration of the currently selected **INP** series to the **REF** series. In the case of multiple **INP** series the Match All is also active. It allows matching each **INP** series to the **REF** applying the same registration parameters.

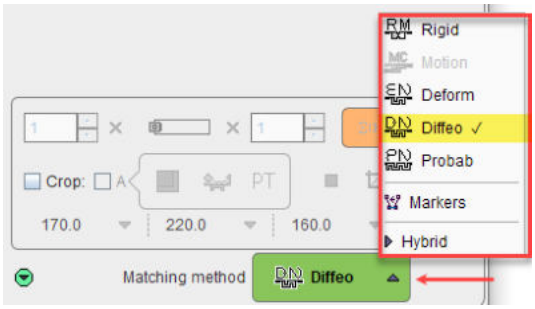
2.2.8 Diffeomorphic Elastic Matching (ANTS) Workflow

The **Diffeo** elastic matching is a native Java implementation of the SyN method within the ANTS tools and therefore portable. Please note, that this method is much slower than the SPM based elastic mapping. It will depend on the application, whether the improved accuracy is worth the long waiting times, and if yes batch processing might be a helpful option to consider.

The method may be applied to both 3D and 2D (single sliced) images.

Input Images

Please first load the input images on the **LOAD INPUT IMAGES** sub-page 21. To proceed select **Diffeo** as **Matching method**



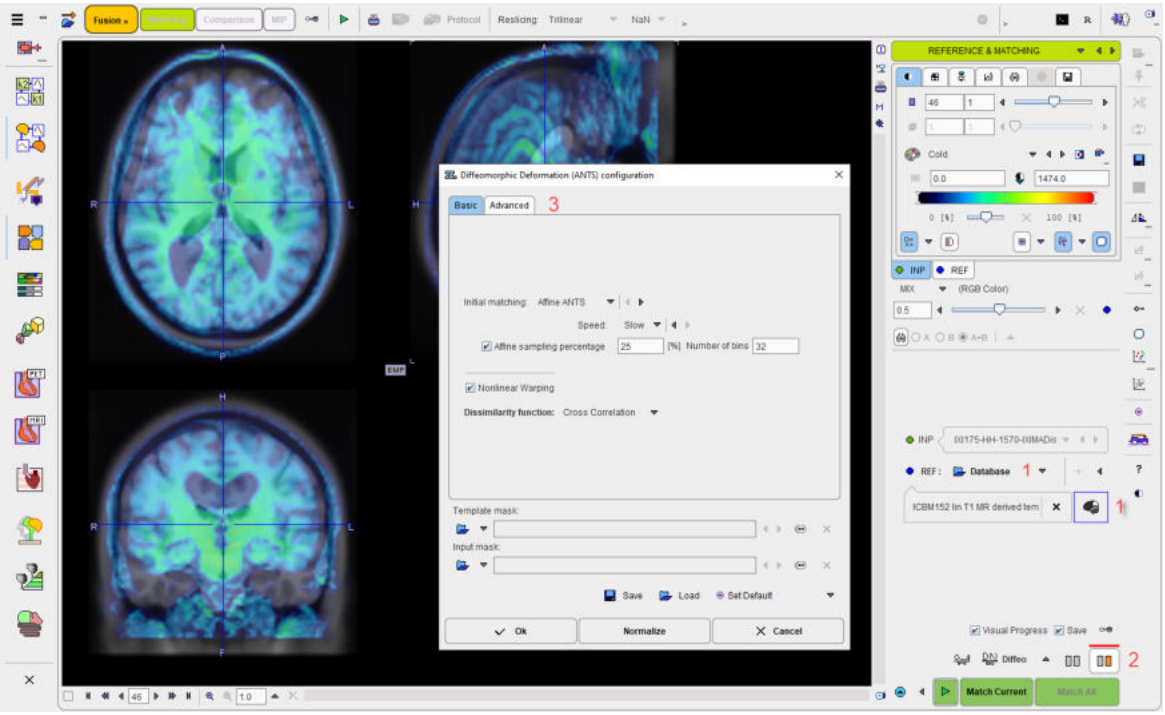
and proceed to the **REFERENCE & MATCHING** sub-page by activating the **Diffeo** button.

Reference Image

There are two ways for loading the reference image, using the **REF** load button for selecting an image from a database or the file system, or using the button for selecting among the appropriate system templates which is dependent on the species setting. If the species was not correctly detected, it can be easily switched

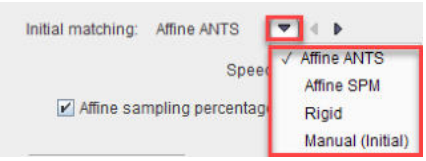
Diffeo Matching Parameters

With the correct species selected, open the parameter configuration dialog window.



In brief, as the method details are described in a dedicated section 95.

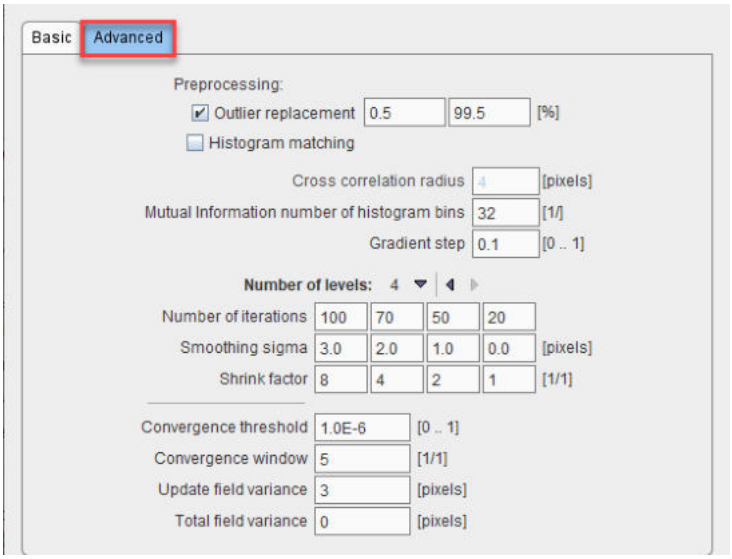
On the **Basic** panel select of the procedure for the **Initial Matching**, before the elastic deformations start. The choices are



with **Affine Ants** as the default. The **Affine SPM** will apply the standard SPM initialization matching parameters. **Rigid** will apply the standard rigid matching procedure, and **Manual (Initial)** will use the current location of the input image and not do any further alignment

Nonlinear Warping should be enabled, otherwise the elastic part will be skipped. This can be helpful to assess the effect of the initial step.

The main setting on the **Advanced** parameter panel



is the **Number of levels** for the hierarchical matching. It has a direct impact on accuracy and calculation time. Default is the full procedure with 4 levels, but they can be reduced down to 1 level. The compromise between accuracy and calculation time will require initial experiments with typical data for a task.

Image Masking

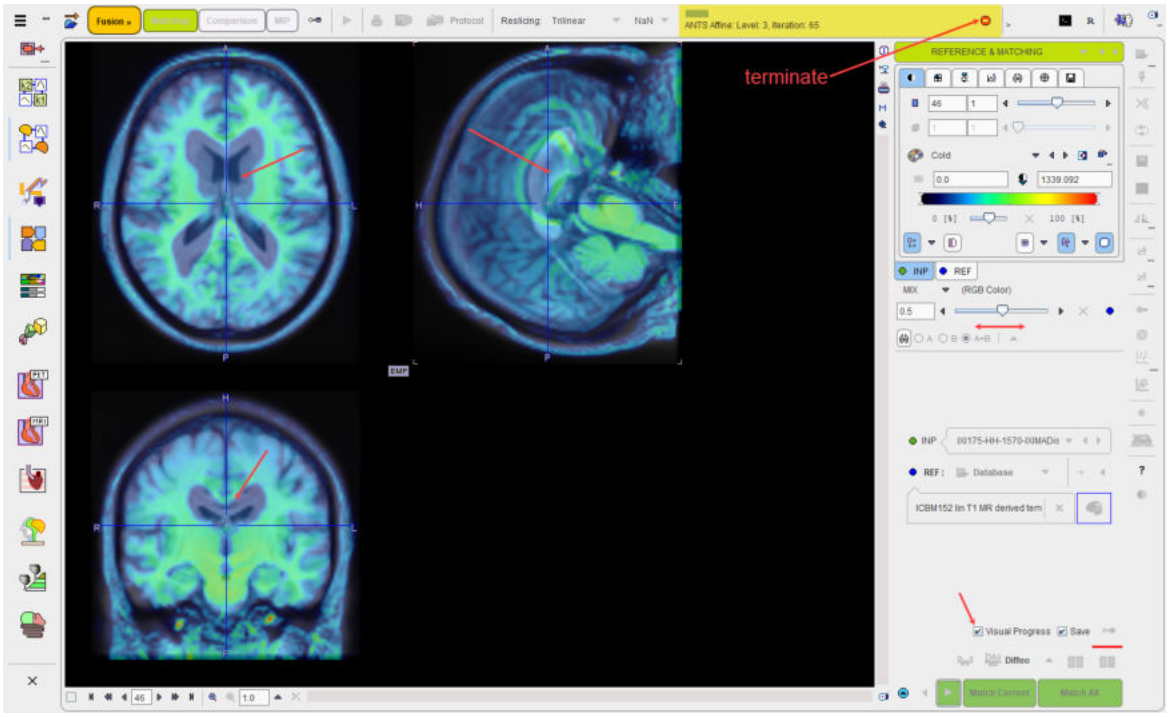
The dialog windows offers the configuration of an **Input mask** and **Template mask** for restricting the volume considered for the matching.

Caveat: While masking improves the calculation speed, ANTS users have repeatedly reported that artefacts are hereby introduced.

Visual Progress and Manual Termination

As the processing can take long, a facility is offered for visually monitoring the matching progress. It is switched on by checking the **Visual Progress** box.

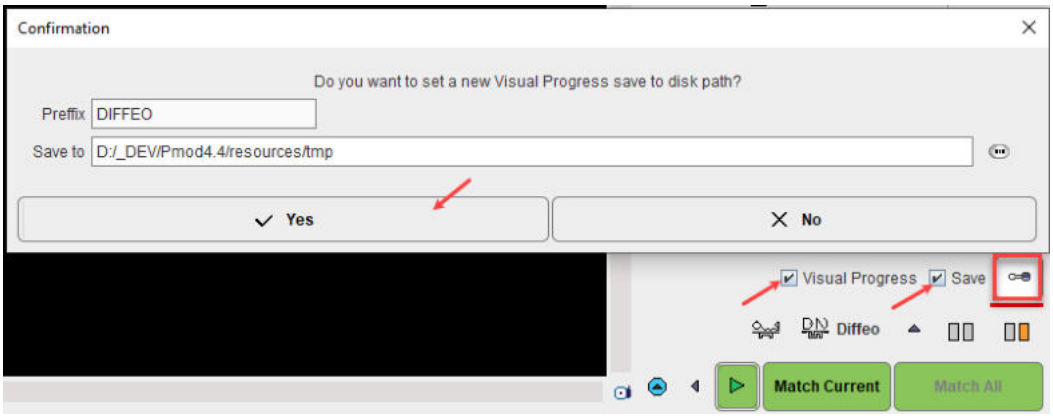
The effect is that whenever an iteration ends, the fusion display is updated with the current result. The fusion image can be adjusted and explored as usually, while the registration continues.



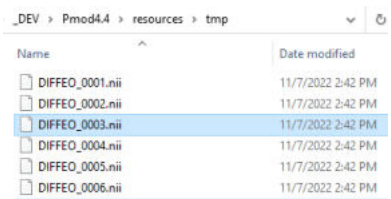
If the user decides that the registration is good enough, registration can be stopped with the button in the lower right, and the last state returned as the result.

Deformations during Registration

During the **Diffeo** process, the input image undergoes a sequence of deformation which should progress towards optimal alignment. In order to review these stages the intermediate images can be saved to disk, and then for instance played as a movie. This functionality is enabled with the **Save** check box. The configuration button next to it allows changing the destination folder from the default illustrated below.



Result is a series of Nifti images which can be loaded, merged in a dynamic series and inspected.



Starting the Registration

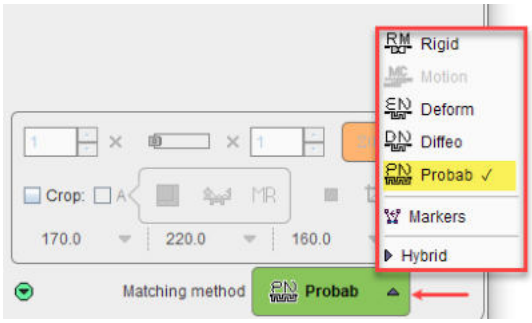
Please use the **Match Current** button to start the registration of the currently selected **INP** series to the **REF** series. In the case of multiple **INP** series the Match All is also active. It allows matching each **INP** series to the **REF** applying the same registration parameters.

2.2.9 Probability Maps Normalization Workflow

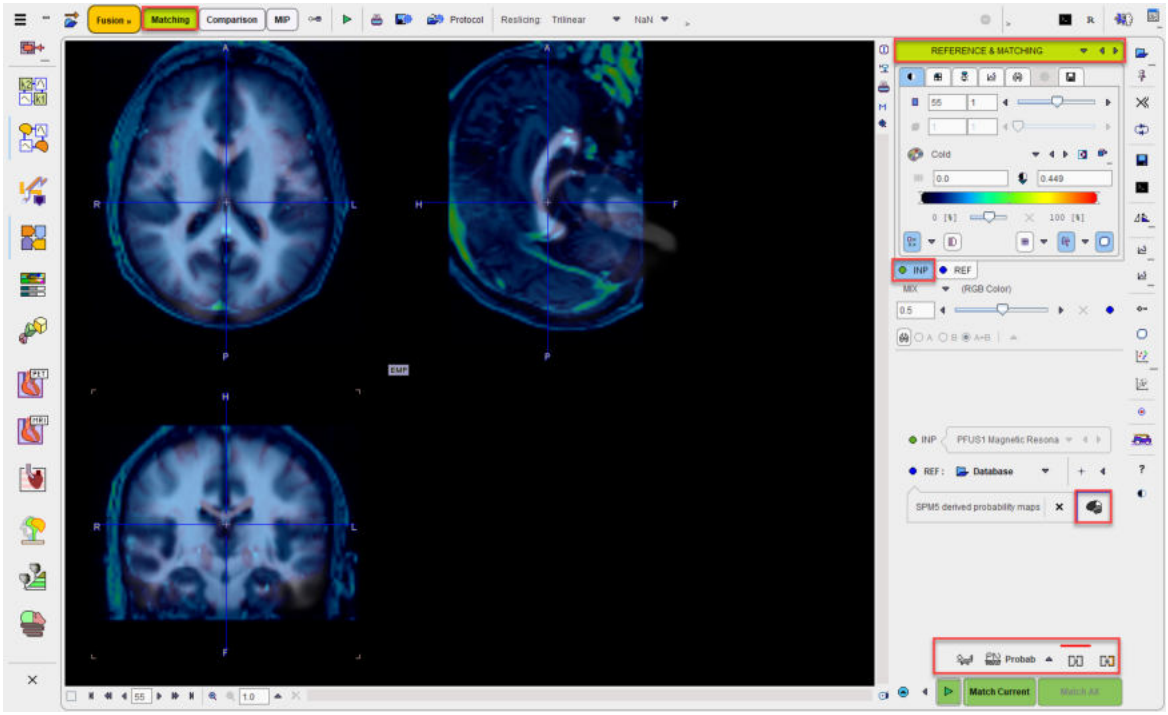
The probability maps normalization approach is an implementation of the Unified Segmentation procedure developed by Ashburner et al [11]. The two variants using 3 tissue probability maps (SPM8) 91 and using 6 probability maps (SPM12) 93 are supported. Note that the method is only applicable for the stereotactic normalization of T_1-MRI brain images to appropriate template images which can be loaded with the button.

Loading of the MR Image

Please first load the T_1-MRI brain image on the **LOAD INPUT IMAGES** sub-page 21 and make sure the **Species** setting is set to **HUMAN**. To proceed select the **Probab**ility maps normalization as **Matching method**.



which switches to the **REFERENCE & MATCHING** sub-page.



Normalization Method Configuration

On the **REFERENCE & MATCHING** sub-page 24 select the normalization method by activating one of the buttons

	3 probability maps normalization
	6 probability maps normalization

and edit the parameters in the dialog window if necessary. Note that the active method is indicated by the red bar above the button. Close the dialog window with **Ok**, or directly start processing with **Normalize** if the reference is already loaded.

Important: The parameter settings are serialized. The next time **Probab**ility matching is selected for the same species, the last parameter configuration will be applied. This is particularly relevant for the Matching without Interaction 64 functionality.

Normalization Template Loading

Use the shortcut to load an in-built template 85 as the reference image. Their details are described in the reference section.

	MR GM/WM/CSF Probability (age 25, SPM8) MR GM/WM/CSF Probability (age 73, Rorden)
	MR 1.5 mm GM/WM/CSF/T/B/A Probability (SPM12) MR 2.0 mm GM/WM/CSF/T/B/A Probability (SPM12)

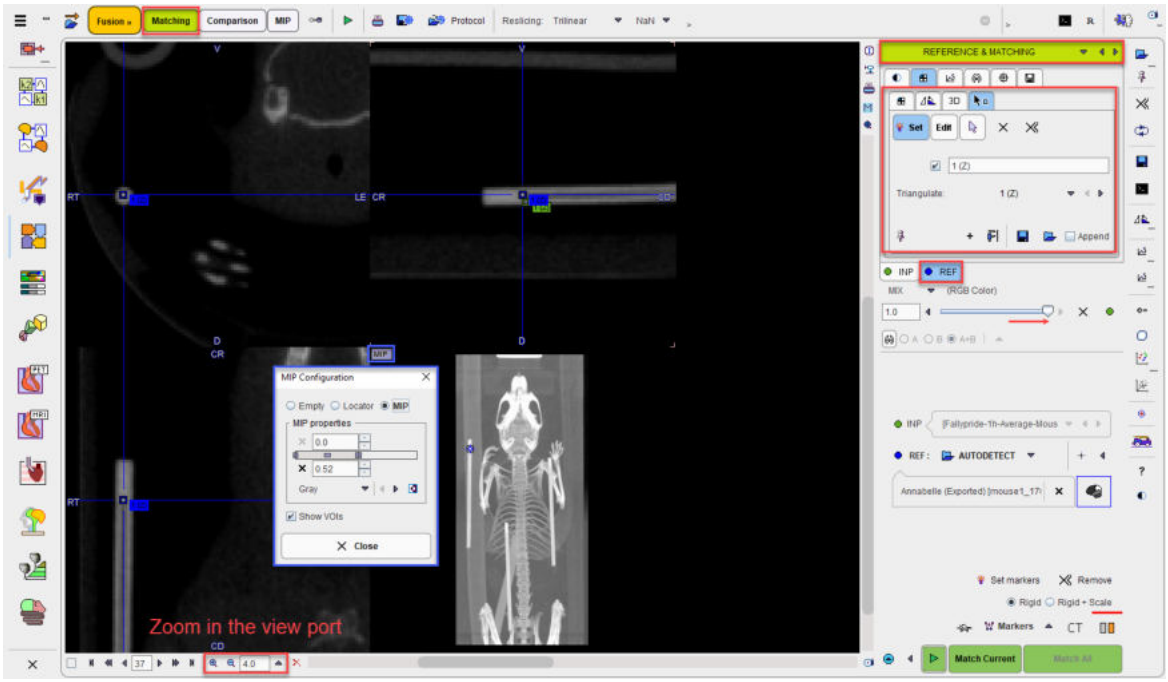
Starting the Registration

Please use the **Match Current** button to start the registration of the currently selected **INP** series to the **REF** series. In the case of multiple **INP** series the Match All is also active. It allows matching each **INP** series to the **REF** applying the same registration parameters.

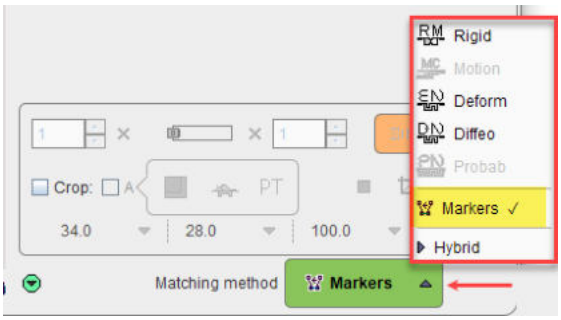
2.2.10 Marker Matching Workflow

If the automatic matching rigid matching is not working properly for a combination of images, the use of fiducial markers should be considered. In the example below three capillaries filled with activity were attached to the bed of the mouse, and then imaging performed on separate CT and PET systems. The capillaries are clearly visible in the CT, whereas the activity in the inner of the capillaries is picked up by PET. The tubes were plugged by a small plasticine plugs, which can be seen by zooming in on the CT image. Consequently, the end of the capillary activity in PET should correspond to end of the plug in CT.

For marker matching, the user explores the two image sets and marks corresponding locations, i.e. markers. A transformation is then calculated which brings the two spatial arrangements of markers into optimal agreement.

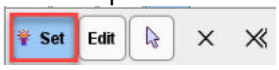


Please first load the input images on the **LOAD INPUT IMAGES** sub-page 21 and make sure the **Species** setting is correct. To proceed select the **Markers** matching as **Matching method**.



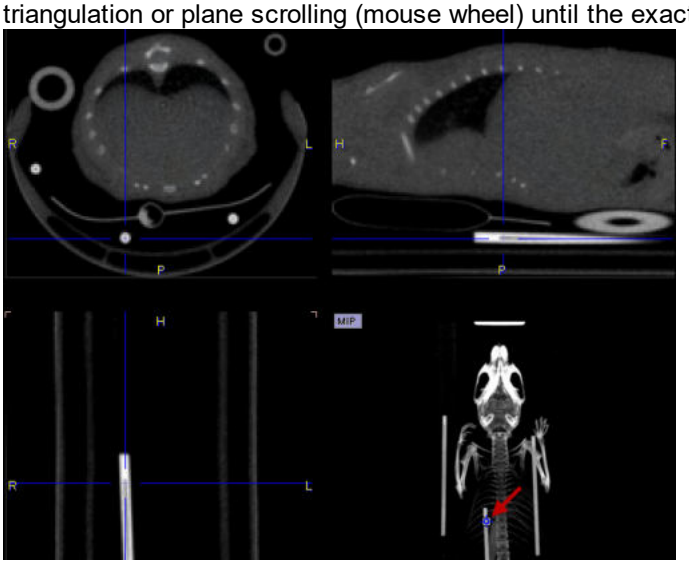
On the **REFERENCE &Matching** sub-page 24 load the reference image with the **REF: Load** button. Next start landmark definition with the **Set markers** button.

Marker Definition for the Reference

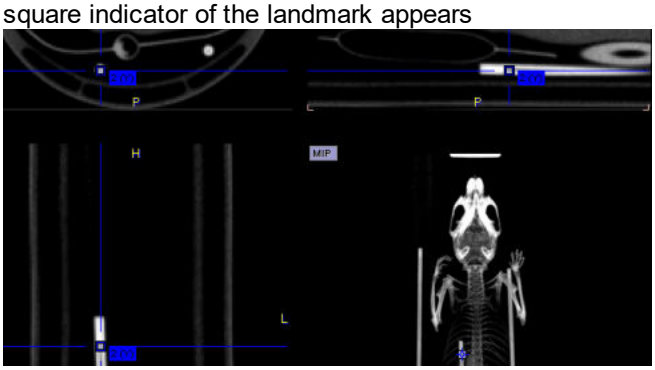
1. Shift the fusion slider fully to the right, so that only the **REF** image is shown.
2. Select the **REF** panel.
3. Note the panel for markers definition which is already open. The buttons 

define the behavior when clicking into the image. With **Set** active, each click into the image generates a marker. With **Edit** active, markers can be dragged to different locations. The third button is the neutral mode for triangulating the images until the marker position has been found.
4. Enable the MIP image in the 4th quadrant with the **MIP** button indicated above and adjust the color thresholds such that the markers are well visible.

5. Click at the landmark position in the MIP image and then adjust the plane locations by triangulation or plane scrolling (mouse wheel) until the exact position is seen in the images.

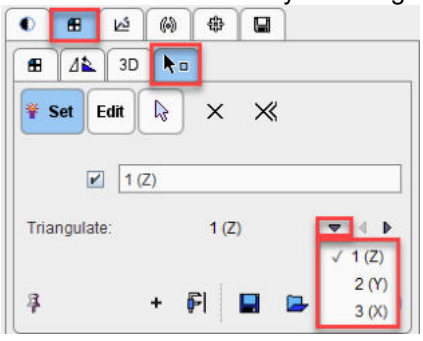


6. Enable the **Set** mode and click at the landmark position in one of the plane images. A numbered square indicator of the landmark appears



7. Switch back to the neutral mode for triangulating the next landmark position, and then define the second landmark in **Set** mode.

8. It is recommended to repeat landmark definition for more points in order to improve the accuracy. The landmarks can easily be triangulated later by selecting a marker in the **Go to** list:

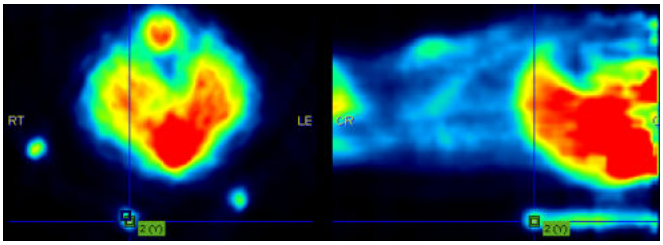


Marker Definition for the Input

The next task is the definition of the corresponding landmarks for the input image.

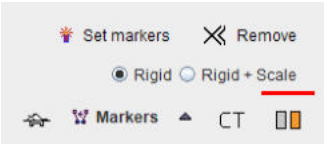
1. Shift the fusion slider fully to the right, so that only the **INP** image is shown.
2. Select the **INP** panel.

3. Define the landmarks in the same order as described above.



Matching Parameters

As the image content is not used for the registration, only parameter is whether the transformation is strictly rigid, or whether a scaling is allowed (**Rigid + Scale** option).



Starting Markers Matching

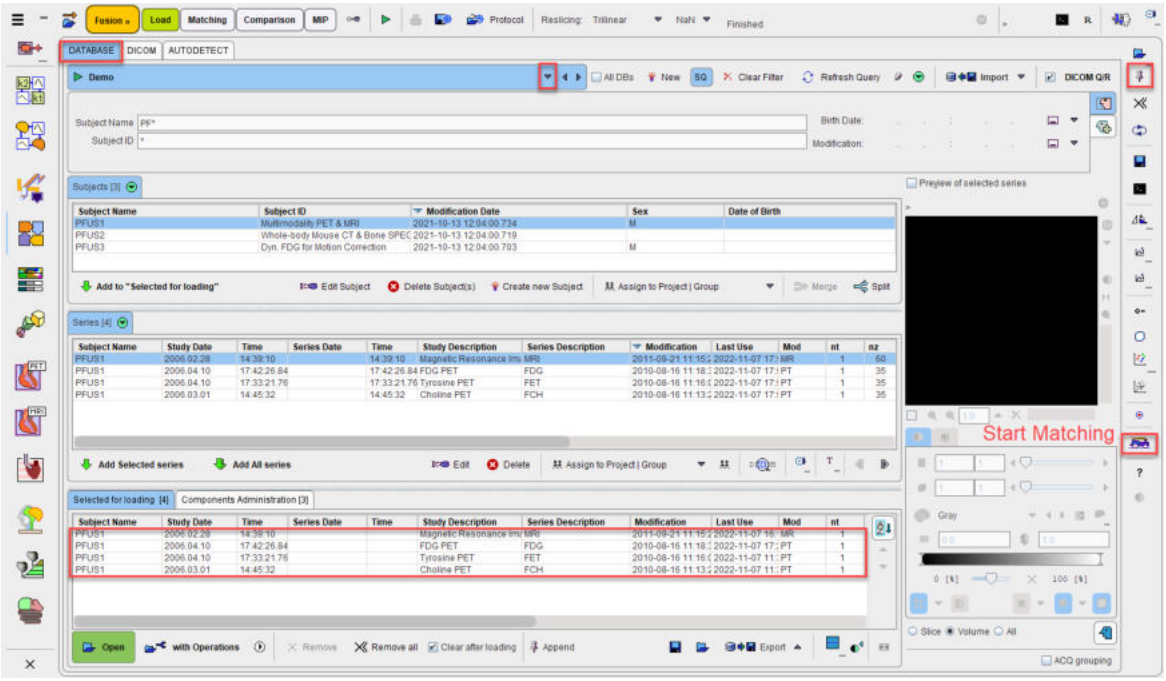
Please use the **Match Current** button to start the registration of the two sets of landmarks.

2.2.11 Maching Without Interaction

In some situation it is not necessary to step-wise run through the matching. For instance, if a similar matching task is repeated and it doesn't require any interactive adjustments, the data can simply be selected and the processing started.

Data Definition

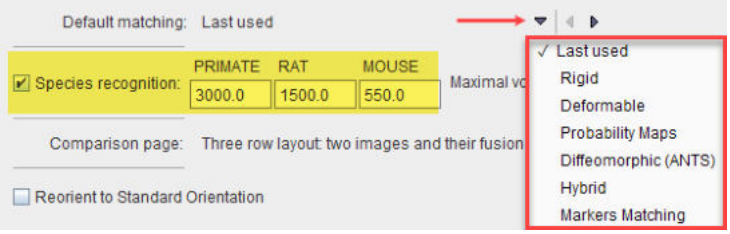
The automatic approach requires that the images reside in the database and that **Load** page is configured in the main PMOD configuration. The images have to be brought to the **Selected for loading** area, and then the matching can be started with the  button in the lateral taskbar as illustrated below.



Please note that the push-pin button for the loading need to be set to "overwrite mode" as indicate din the capture above by the red rectangles.

Registration Method

The applied registration depends on **Default matching** method specified in the configuration 8.

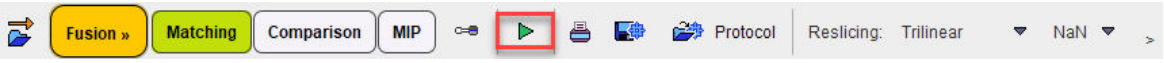


With **Last used** the most recently applied registration is used with all its parameter settings, whereas for the other choices the default species-dependent parameters will be applied.

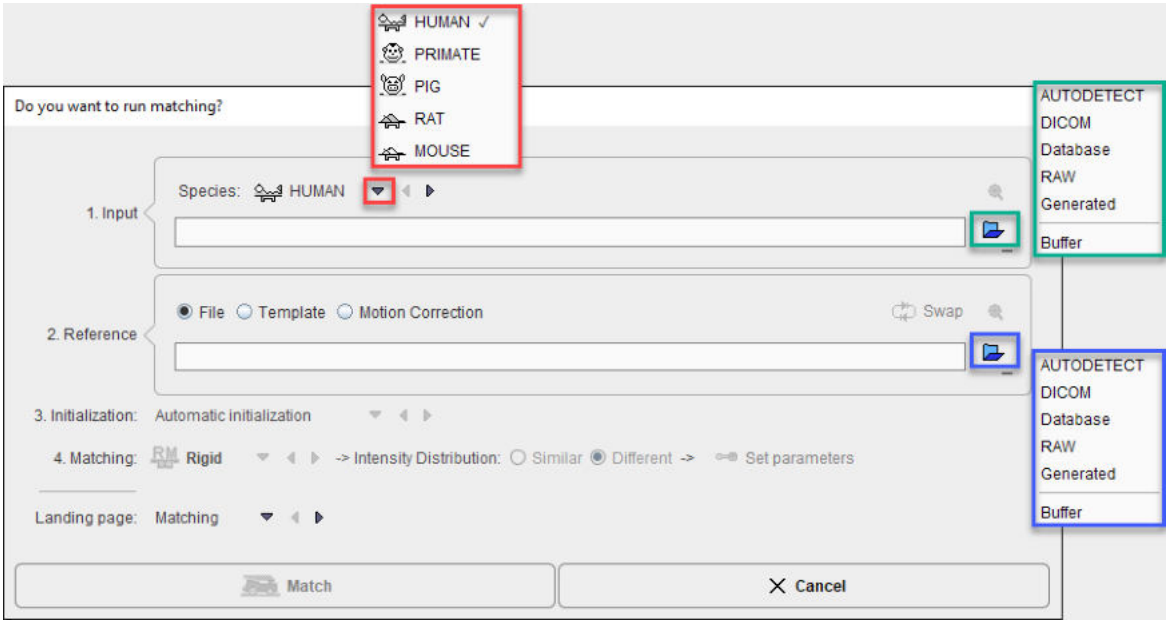
2.2.12 Wizard Matching

The wizard function makes it possible to launch coregistration workflows in the Fusion tool in a matter of seconds. It allows rapid switching between rigid matching and elastic workflows as well as direct selection of the desired result page.

It is accessible via the dedicate green arrow button available in the top line menu in the Fusion interface, as illustrated in the capture below:



The activation of the wizard button opens a dialog window for configuration:



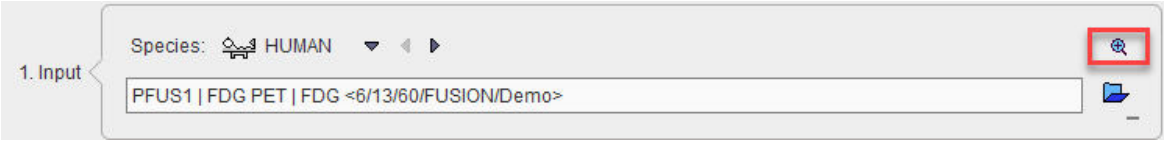
The window is organized in four main section described below.

Input Section

It is recommended to start the configuration with the selection of the **Species**. The correct species setting is important for proper registration defaults parameters.

Input image definition is started with the dedicated load button. It allows selecting the appropriate file format. Once the image is selected the **View input image** button becomes active and when

initiated the image is previewed in the **LOAD INPUT IMAGE** sub-page while the Wizard is still accessible:



Note that only one single **Input** image definition is supported in the wizard interface and cropping is not supported.

Reference Section

There are three types of reference images that are supported in the wizard interface:

1. **File**: this option allows loading an images file after selecting the appropriate file format.
2. **Template**: this option allows loading a template from a predefined list of built-in template 85. Note that the selection of built-in templates changes according to the **Species** selection. In case of **Human Species**, the available predefined template list also depends of the **Matching** method.
3. **Motion Correction**: allows creating a reference region and bringing the anatomy into agreement across all the dynamic frames. The procedure works as described in the Motion Correction Workflow 50

Once the **Reference** image is defined the **Go to Reference Step** button becomes active and when initiated the image is previewed in the **REFERENCE & MATCHING** sub-page while the wizard is still opened:

The **Swap** button allows swapping the **Reference** and **Input** image

Note that cropping is not supported through the wizard interface.

Initialization

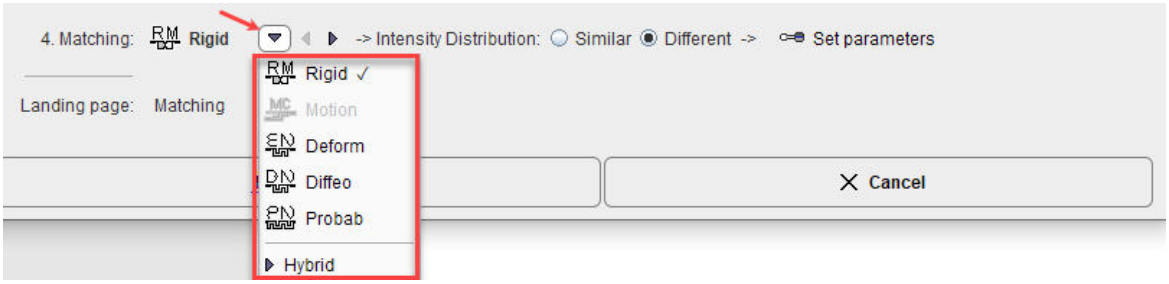
It is important for the automatic registrations that the images have a sufficient overlap. The initialization supported in the wizard interface is available for selection with the drop down arrow as illustrated below:

Automatic initialization	Alignment of the **Input** and the **Reference** images centers.
Origin alignment	Alignment of the **Input** and the **Reference** coordinate origins. This works if the two series have the origin at the same anatomical landmark.
Center of gravity alignment	Alignment of the **Input** and the **Reference** gravity centers. This works if the two series have about the same value distribution.

Center of images	Alignment of the **Input** and the **Reference** image volume center

Matching

The matching selection reduces complexity and improves the speed for standard matching situations.



The **Intensity distribution** and the **Set parameters** settings correspond to the selected **Matching** method. The details settings for each **Matching** method is described in the **Matching Workflow** section above.

Landing Page

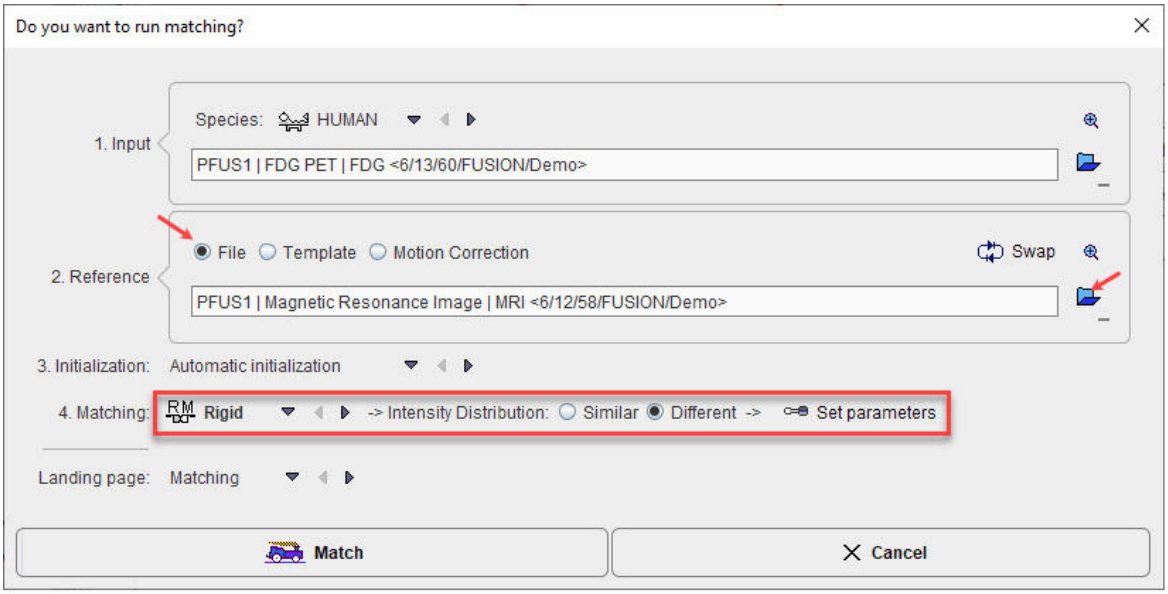
The **Landing page** selection allows choosing on which page the matching result will be shown:

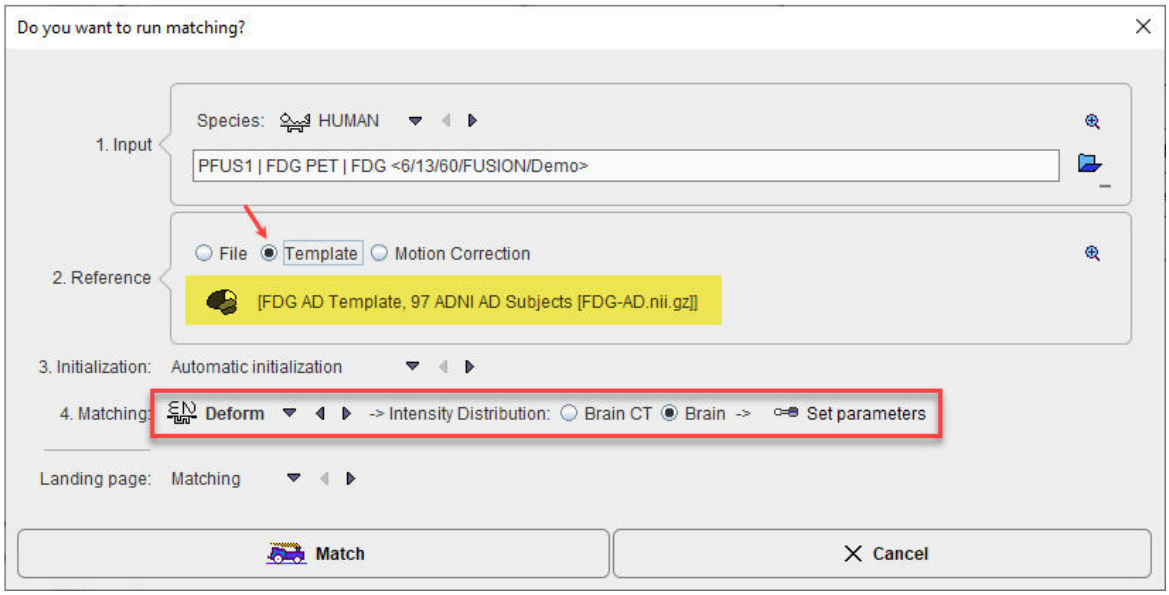
Start the Matching

Finally, activate the **Match** button to start the matching procedure. The wizard window is closed, the matching procedure is started and the results are returned on the selected **Landing page**.

Wizard Easy Switch

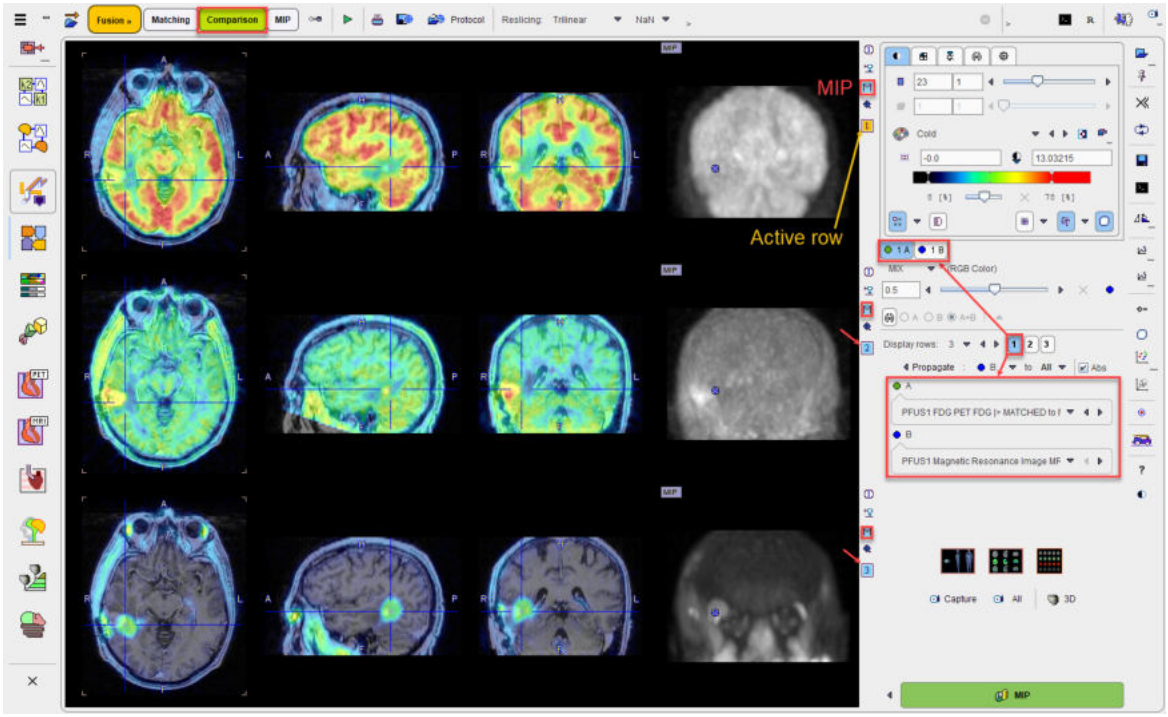
In the example below it is illustrated how a **Rigid Matching** configuration for a **Human** brain can be easily switched by simply enabling the **Template** radio button for the **Reference** and selecting one of the images from the predefined list available in combination with the **Deformable Matching** configuration:


Do you want to run matching?
1. Input
Species: HUMAN
PFUS1 | FDG PET | FDG <6/13/60/FUSION/Demo>
2. Reference
File
Template
Motion Correction
Swap
PFUS1 | Magnetic Resonance Image | MRI <6/12/58/FUSION/Demo>
3. Initialization:
Automatic initialization
4. Matching:
Rigid
-> Intensity Distribution:
Similar
Different
->
Set parameters
Landing page:
Matching
Match
Cancel


Do you want to run matching?
1. Input
Species: HUMAN
PFUS1 | FDG PET | FDG <6/13/60/FUSION/Demo>
2. Reference
File
Template
Motion Correction
[FDG AD Template, 97 ADNI AD Subjects [FDG-AD.nii.gz]]
3. Initialization:
Automatic initialization
4. Matching:
Deform
-> Intensity Distribution:
Brain CT
Brain
->
Set parameters
Landing page:
Matching
Match
Cancel

3 Comparison Page

The **Comparison** main page allows viewing fused images in varying layouts.



Note the All button in the lower right for creating a capture of the arrangement in the display area.

Note the **M** buttons aside each row for activating with the single click the MIP for all configured rows.

3.1 Layouts with Multiple Rows

The image display can be configured for up to 4 rows, each showing a fused image.

Layout Configuration

The **Number of rows** selection serves for defining the number of rows into which the display area is split.

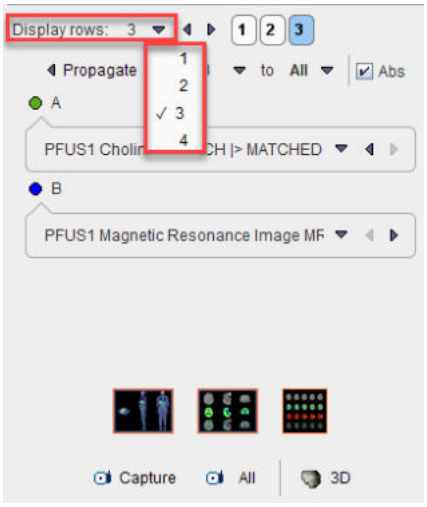
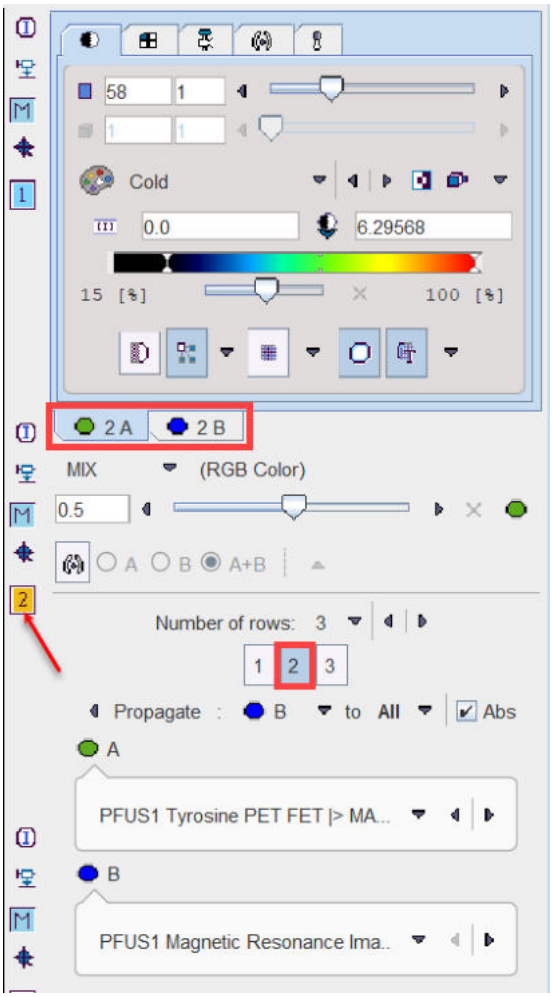


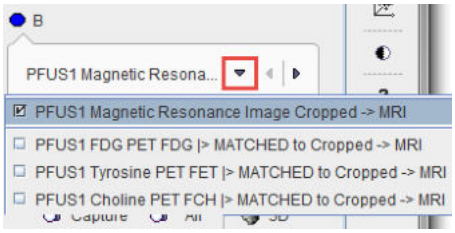
Image Selection

The fused images shown in a particular row are configured with the **A** and **B** selections. Initially, the reference series is set as **B** series for all rows, although with separate display controls. This means that changing the display characteristics in one row has no effect on the reference display characteristics in the other rows.

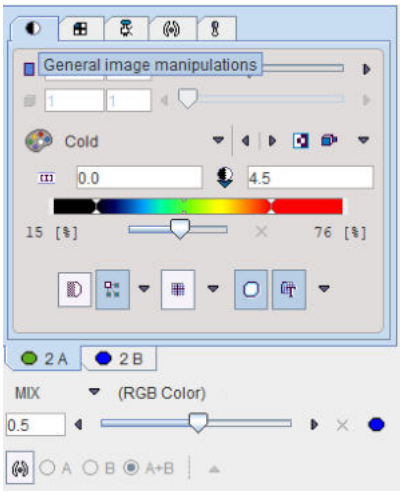
To change the fused images in a row the row has to be activated first. This can be done by clicking the number to the right of the image, or by selecting the number in the button row below **Number of rows** as illustrated below:



The number next to the selected row is highlighted in yellow (e.g. 2), and the image tabs are labeled accordingly with the number (e.g. **2A** and **2B**). Next, any series can be selected for the **A** and **B** tabs using the corresponding selections.



Thereafter, the display characteristics of the two series as well as their fusion can be tailored in the upper right area.

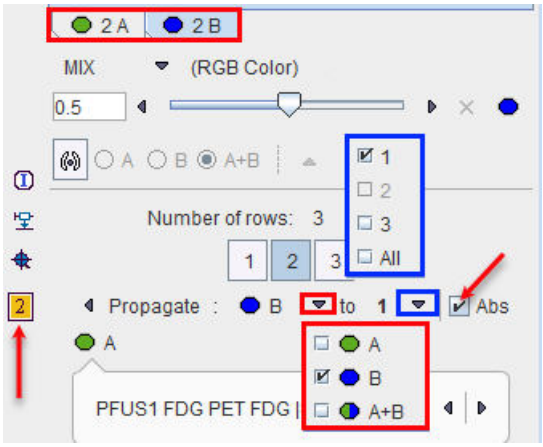


Color Propagation

With multiple rows configuration is rather tedious to change the color scale settings separately in each row for the **A** or for the **B** images or for both. FuseIt implements a **Propagate** facility described below.

Each row shows a fusion image generated from an **A** and a **B** series, whereby **B** plays the role of the reference per default. Therefore, the MR is initially set as **B** series for all rows.

To propagate for example the **B** series color adjustments in absolute values from one row (e.g. row **2**) select **B** and enable the **Abs** box. With the black down arrow select the row to which the same color settings should be applied (e.g. row **3**). With the **All** option the propagation is applied to all the other configured rows.

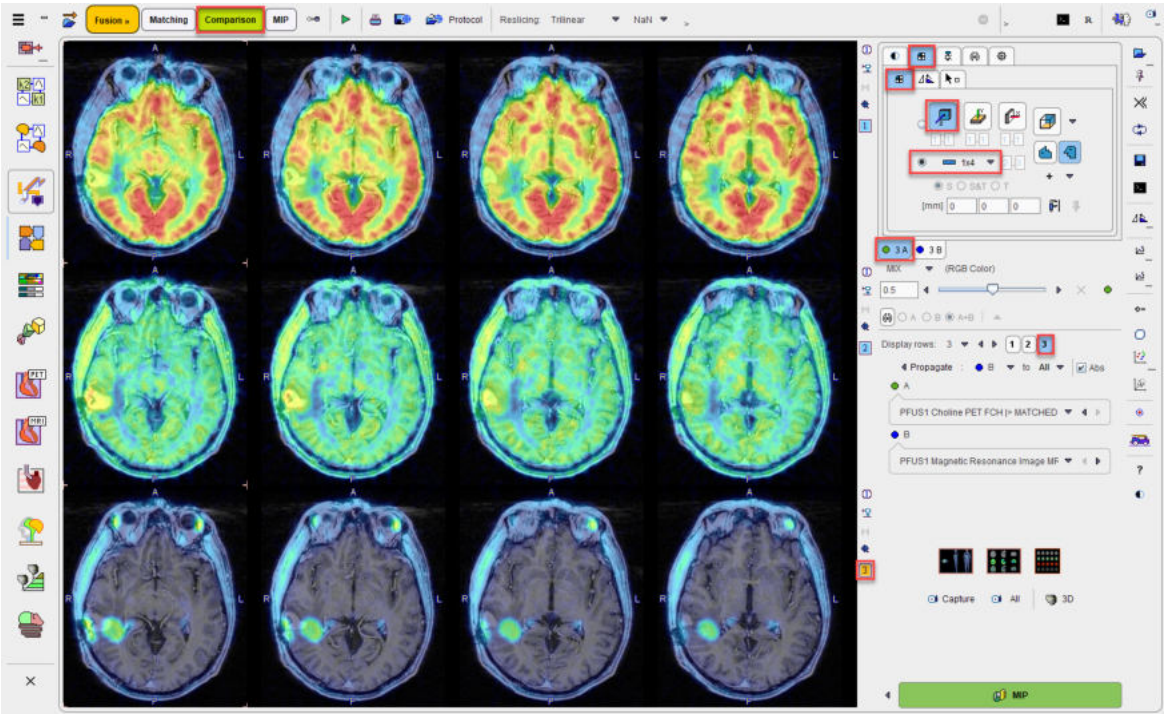


To individually adjust a row it has to be activated first either by clicking the number to the right of the image, or by selecting the row number below the **Number of rows** as illustrated above. The number next to the selected row is highlighted in yellow (2), and the image control tabs are labeled accordingly (e.g. **2A** and **2B**). Any series can be selected for the **A** and **B** tabs using the corresponding image selections.

To propagate the color adjustments in percentage threshold of both **A** and the **B** series disable the **Abs** box, select the A+B option followed by the row selection e.g **1** or **All**.

Layout Changes

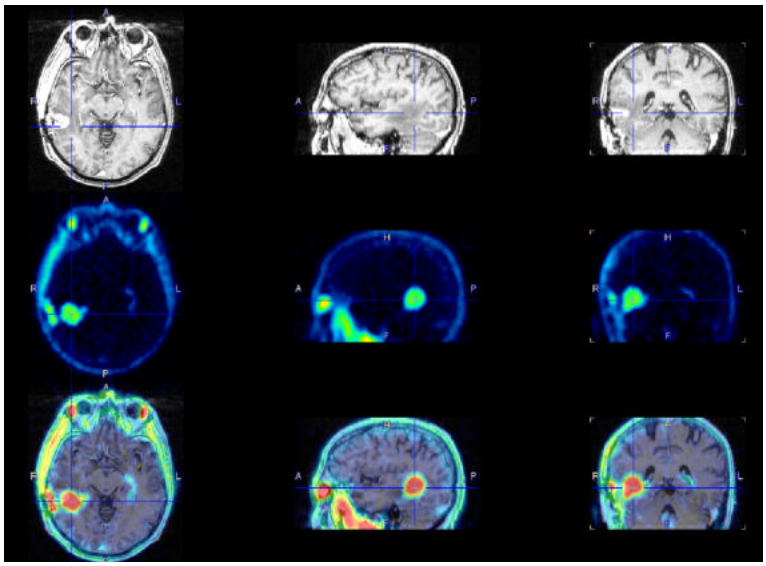
While the orthogonal planes are the default layout which is appropriate for approximately cubic data volumes, the layout can be changed to only show a number of axial, coronal or sagittal slices as illustrated below. The layout change to any image will immediately be applied to the images of all rows, since they are always synchronized.



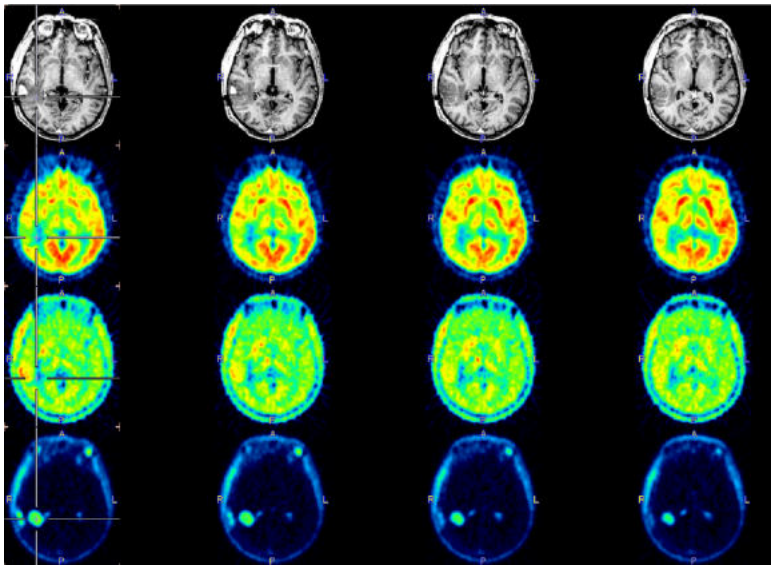
The **M** button for enabling/disabling the MIP in the image is available only for the orthogonal planes view

Layout Presets

There are presets for some popular layouts in the lower right.

	Switches to a single-row whole-body layout as described below 73.
	Switches to a three-row layout which mimics the behavior of the legacy fusion tool: Row 1 shows the reference, row 2 the matched input, and row three their fusion.  This appearance is obtained by selecting the same matched input and reference as images **A** and **B**, respectively in all rows. Further, the fusion slider is set to the right, the left and the middle in the rows one, two and three, respectively.

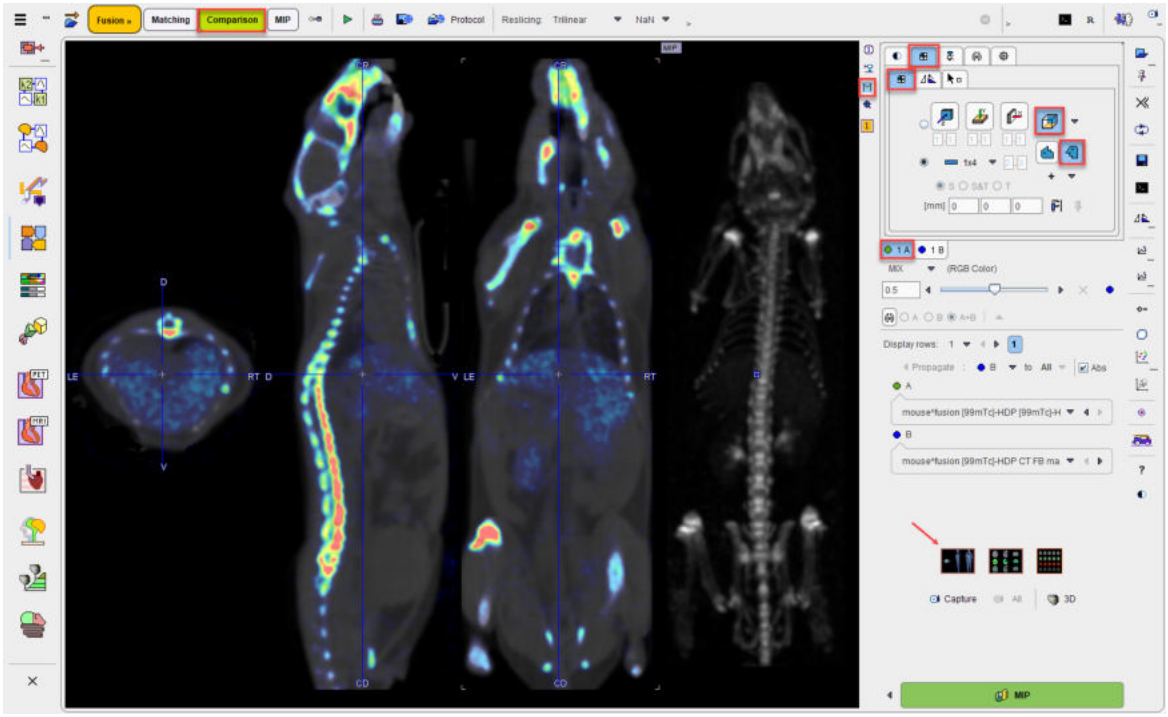
This preset arranges all available images in a separate row and shows them as axial slices. The number of rows therefore depends on the number of loaded and matched images, including the reference.



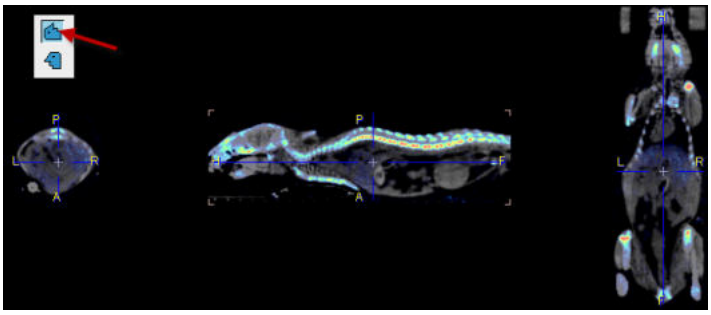
The appearance is obtained by selecting the series sequentially as image **A** in all rows, and setting all fusion sliders to the left.

3.2 Whole-body Layout

The whole-body preset aims at a better use of the display are for non-cubic image volumes which arise in whole-body imaging. It works best in the single-row layout, but can also be used with multiple rows.



Note the non-standard orientation selection in the layout tab which is enabled by the preset. The effect is that the sagittal slice is arranged in parallel to the coronal slice. Otherwise the arrangement would look as illustrated below.



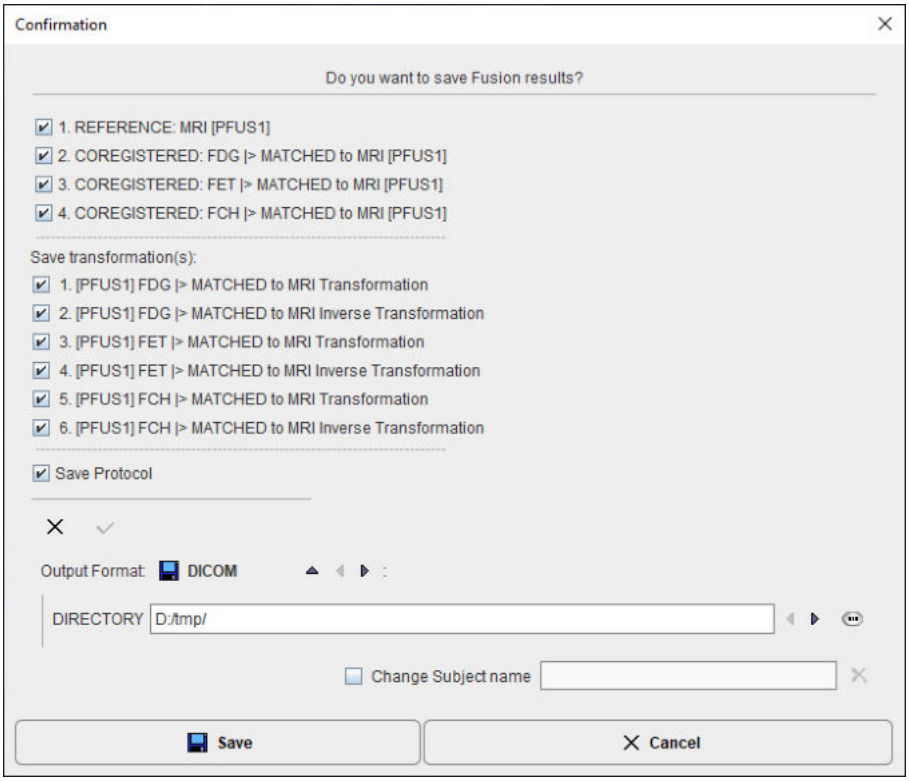
Action Button

The only action button on the **Comparison** page

transfers the images to the **MIP** main page for creating rotating fusion Maximum Intensity Projection images.

4 Results Saving

Saving of the matching results is started with the button from the lateral taskbar. A dialog window is shown with the image list in the upper part, transformation list, VOIs and protocol in the middle part and an **Output Format** selection below. By default, all entries in the lists are checked.



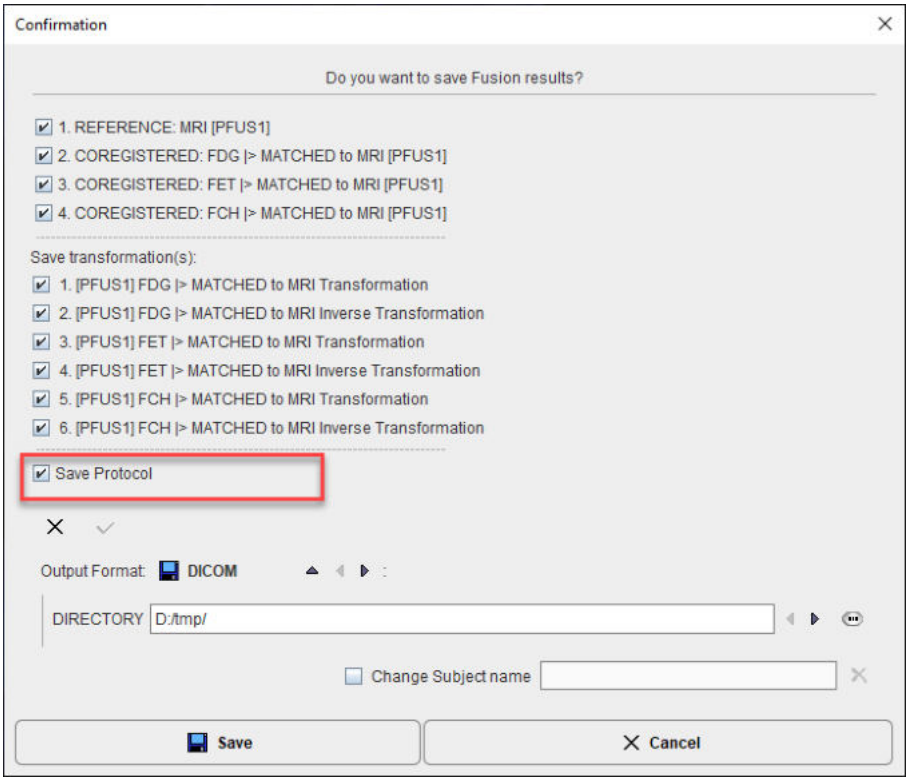
With the **Save** button, the selected entries in the lists are finally saved.

4.1 Protocol Saving

The best way for reproducing a registration result is to save the entire configuration with the save **Protocol** button in the Menu line.

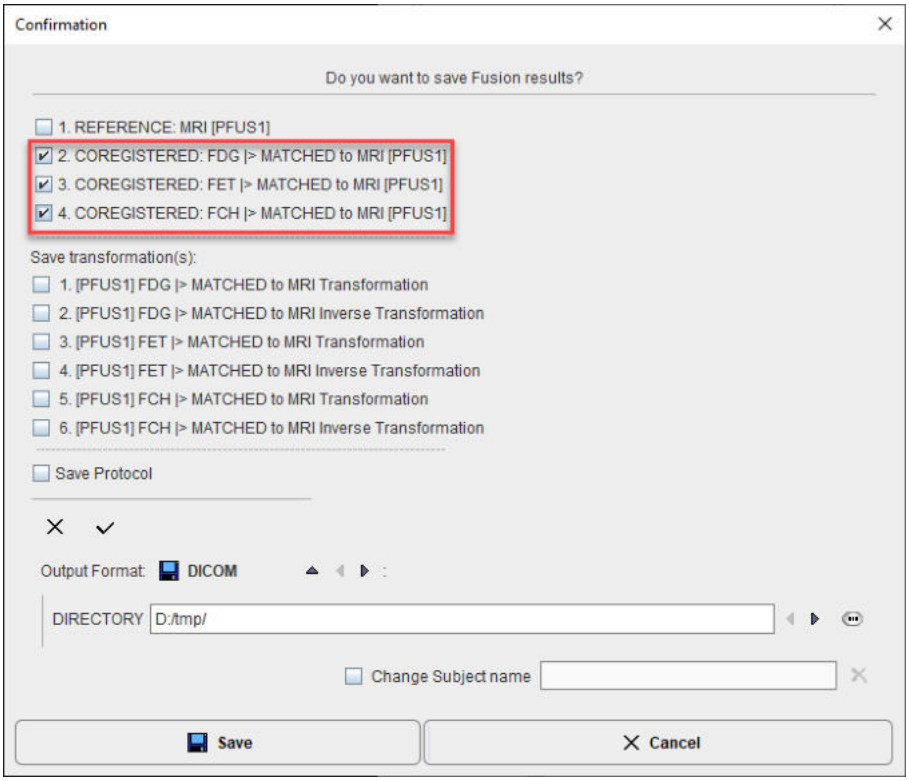
By simply replaying the registration using load **Protocol** icon, the registration is recovered. Note that derived information such as VOIs or image algebra results are not included in the protocol and will be missing.

Protocol is available for saving using the save button in the lateral taskbar, as shown below:



4.2 Image Saving

Saving of the images with the button from the lateral taskbar is straightforward. The image list is available in the upper part of the dialog window. Select the images to be saved and set the **Output Format**. Depending on the format chosen, information such as the target directory, a prefix or the transfer syntax have to be specified.

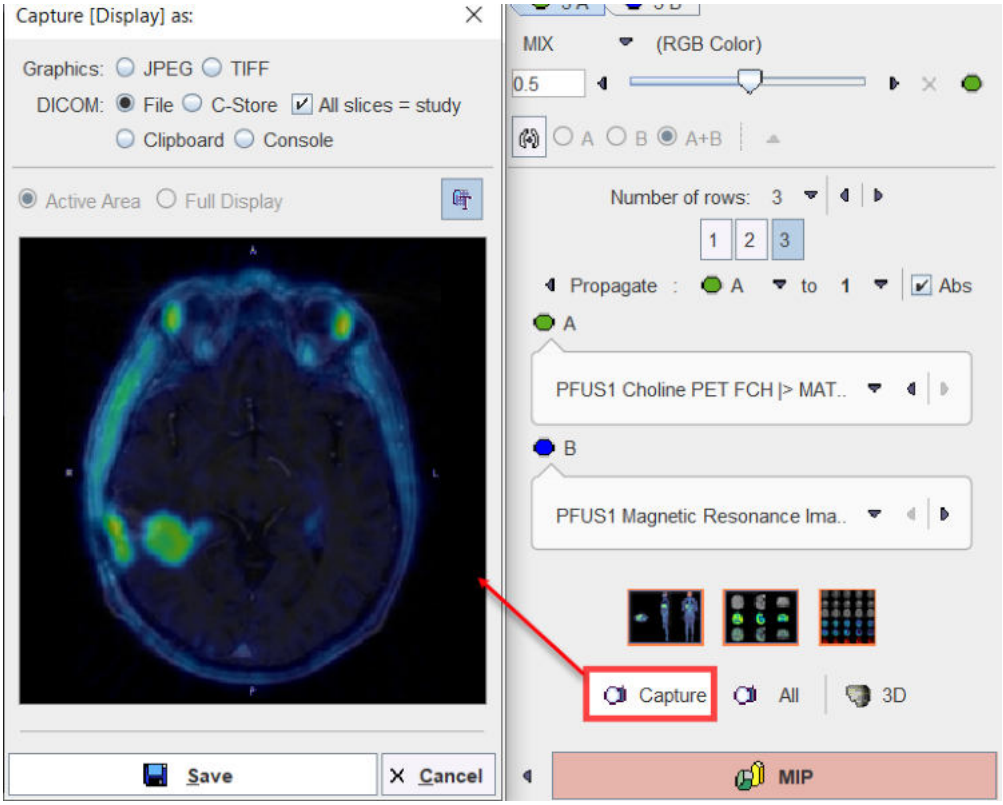


With the **Save** button, the selected images are finally saved.

4.3 Fused Images Saving

In addition to saving the individual images, explicitly fused images can also be saved using the **Capture** button on the **Comparison** page. A dialog window appears for defining the format of the RGB images.

The **DICOM** output is of particular interest because with the **All slices** option it can create a full volume of fused images which can be saved for archival in a PACS system and inspected with any reviewing workstation. With the **File** the DICOM SC images are saved to disk, whereas the **C-Store** option supports direct network transfer to a DICOM server.

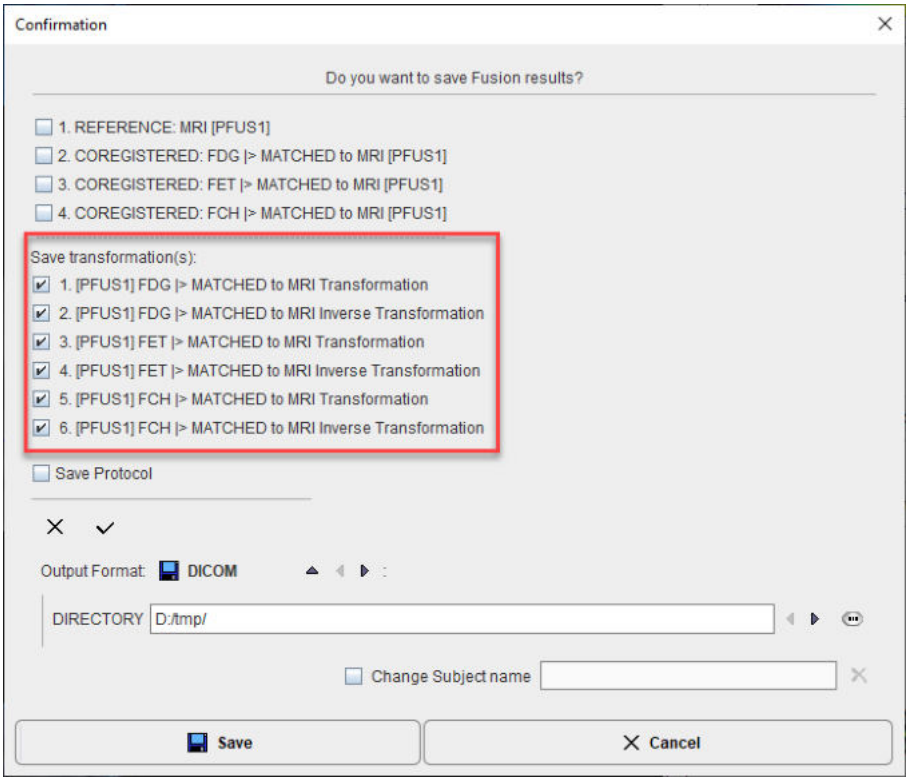


4.4 Transformation Saving

The spatial transformations between the reference and the input images are saved from the **MATCHED**sub-page 30.

The spatial transformation between the reference and the input images when **Combined Matched** layout is set are saved from the **Initialize/Match** page.

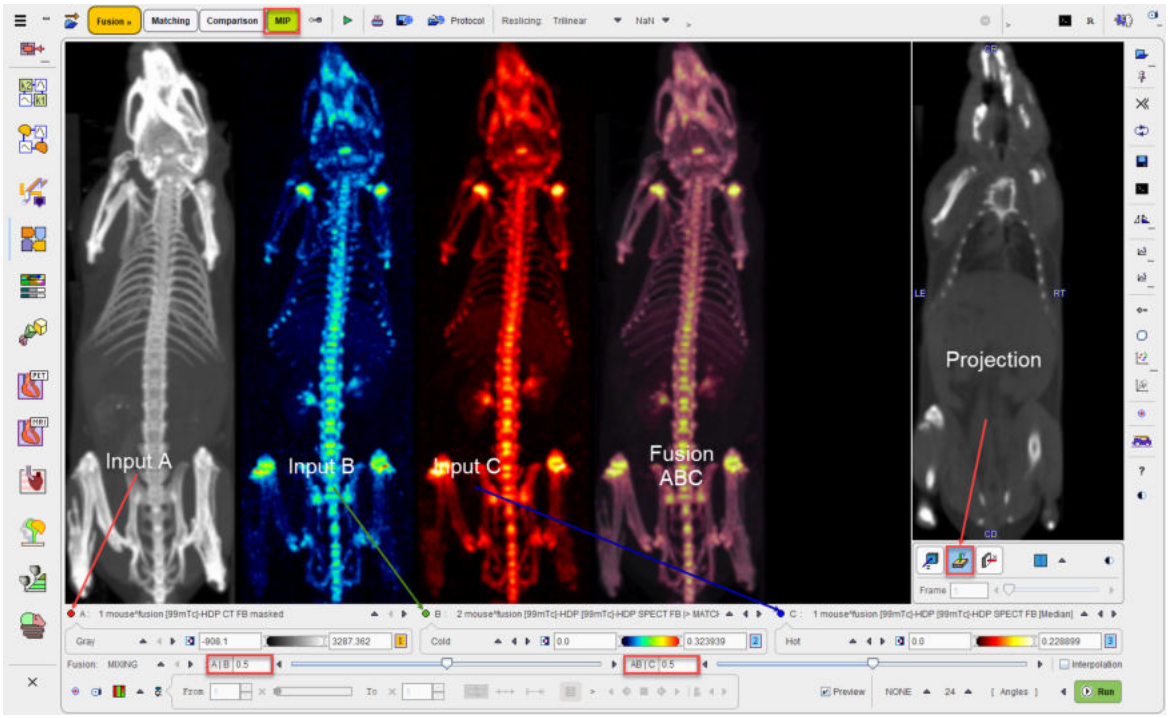
Note, however, that reproducing the final state may require the saving of more than a single transformation, because the reference image might have been reoriented as well. An easy alternative is using the save button from the lateral taskbar. Select the transformations to be saved among the entries available in the **Save Transformations(s)** list.



With the **Save** button, the selected transformations are finally saved.

5 MIP Page

The **MIP** main page for generating **M**aximum **I**ntensity **P**rojection cines has the layout illustrated below.

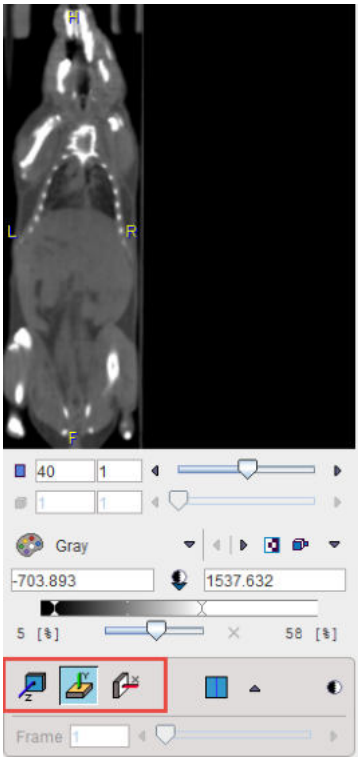


- The upper left image area shows a preview of the input images (**A**, **B**, **C**) and their fusion. Each input image has a color bar associated to adjust the image coloring. The upper right area serves for defining the projection direction, coronal in the example above.
- The fusion image is obtained by first fusing **A** and **B**, and then fusing the result with **C**. The mixing is defined by the two corresponding **Fusion** sliders **A|B** and **AB|C** below the colorbars.
- The control of the MIP characteristics and movie generation is located at the bottom.

Please proceed as described below.

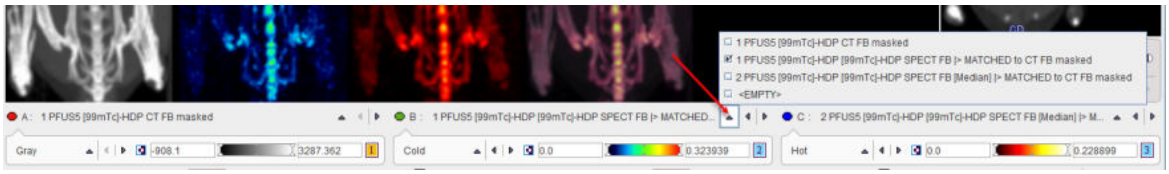
5.1 Projection Direction

The MIP projection direction is set using the plane orientation buttons in the image area to the right. If necessary, the displayed image can be switched as illustrated above.



5.2 Input Image Selection

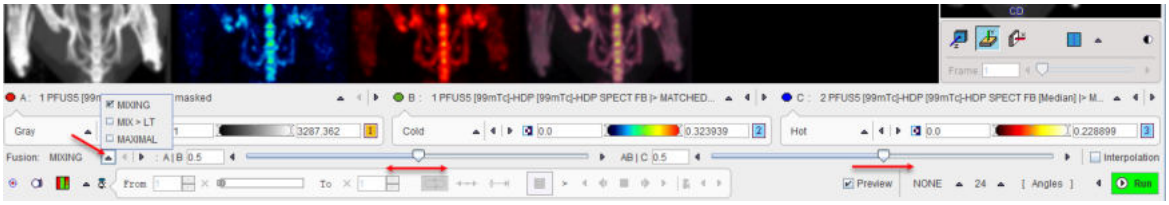
Per default, the reference image is arranged as series **A**, and the matched input images in sequentially as series **B** and **C**. This arrangement can be changed using the series selection as illustrated below.



Note the EMPTY entries which are only available for **B** and **C** which allow excluding those images from MIP generation.

5.3 Fusion Configuration

Each series has its own colorbar for adjusting the image presentation. The color choices should be such that the different image components can be distinguished in the fusion. As a default configuration 8, the reference is shown with **Gray** colors.



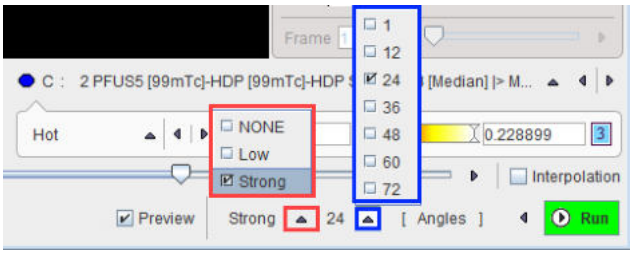
There are three fusion options available:

MIXING	Simple weighted averaging of the RGB values, whereby the relative contributions are defined by the fusion slider.

MIX>LT	Weighted RGB averaging considering only pixels which are above the respective lower thresholds, hereby removing the background. The relative contributions are defined by the fusion slider.
MAXIMAL	With this setting no color averaging of the two inputs is performed. Rather, the bigger of the two contributions is selected.

5.4 MIP Configuration

The **MIP** calculation performs a ray tracing from different angles and selects the maximal value on a ray for display in the MIP image. There are two MIP calculation parameters in the lower right.



1. Distance waiting with the settings **NONE** (default), **Low**, **Strong**. This option emphasizes objects closer to the observer by multplying the value with a factor which decreases with distance.
2. Number of projection angles. The selection ranges between **1** and **72** angles. The more angles are chosen, the smoother the rotation cine will appear, however at the cost of longer preparatory calculations.

5.5 Cine Control

Image Display Selection

Per default, all the input MIPs as well as the fusion MIP are rotated. However, there is a choice in the lower left which allows showing subsets of these images.

ABC+F	Show the three input MIPs as well as the fusion MIP (default).
ABC	Show only the three input MIPs.
Fusion	Show only the fusion MIP.

Projection Calculation

Calculation of the configured projections is started with the Run button. After the calculation is completed, the rotation cine is immediately started.

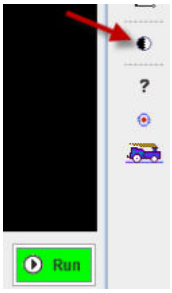
Cine Controls

The direction of the cine, the speed and the behavior after a rotation can be configured with the usual cine control elements.

If any of the image presentation options is activated, the cine stops. However, in most cases a projection recalculation is not required, so the rotation can be simply restarted.

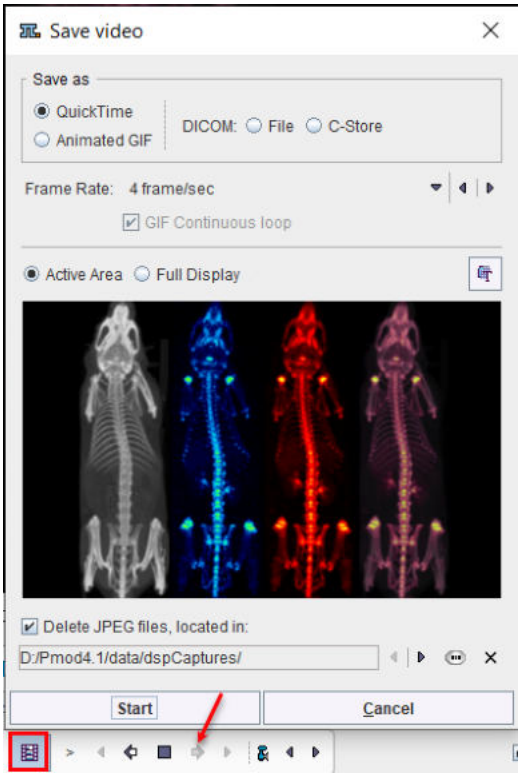
Maximizing the Display Area

In order to maximize the image area for watching the cine, the controls can be minimized with the show/hide taskbar button indicated below. They are recovered by activating the same button again.



Movie Generation

In order to create a movie file, the  button has to be activated and then the cine started. A dialog window is shown for configuring the movie format **QuickTime**, **Animate GIF** or **DICOM**.

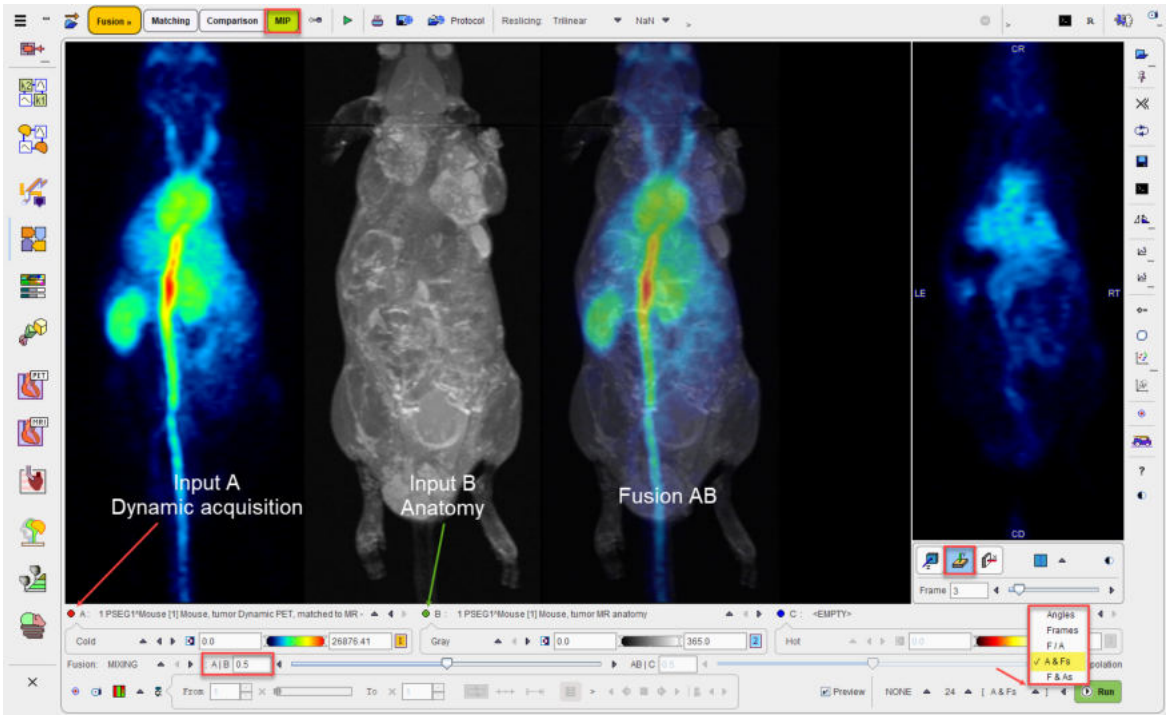


The movie will be assembled from JPEG files which are saved to a folder which is to be configured in the lower part. The JPEG images may be persistent, depending on the **Delete JPEG files** option.

After activating the **Start** button, the JPEG images corresponding to the different angles are written to disk and a dialog window opened defining a location and a name for the movie file.

5.6 MIPs using Dynamic Series

In the special case that an image series is dynamic, there are additional control options. To configure the MIP fusion of the dynamic uptake with the anatomy, the dynamic series have to be set as image series A as illustrated in the capture below:



Angles	Standard rotating MIP cine of the current frame.
Frames	MIP across all dynamic frame in the selected projection direction.
F/A	Mode in which the angle and the frame are simultaneously incremented. As a consequence, the number of angles equals the number of frames. The effect is, that the image changes during the rotation.
A & Fs	In this mode, the projection angle is fixed, while all frames are MIP rendered. This rendering is sequentially performed for all angles.
F & As	In this mode, a full rotation MIP is generated for a fixed frame. This rendering is sequentially performed for all frames.

6 Registration Methods Reference

6.1 Rigid Registration Parameters

The settings available in the rigid matching panels allow fine-tuning the basic procedure in a multitude of ways. While there are successful settings (as the predefined ones), experimenting with these configurations may result in improved or faster matches in specific situations.

Basic Parameters

Smoothing window	A Gaussian filter with configurable width in mm or pixels can be separately enabled for the *Reference* and the *Reslice* study. While this introduces an additional performance burden during start-up, iterations are less likely to get trapped in a local optimum with smoothed images.
Dissimilarity function	This is the main definition of the matching algorithm. Note that a short explanation of the selected dissimilarity function can be shown with the **?** button besides the selection. The selections are ▪ **Absolute Difference Sum,** and ▪ **Squared Difference Sum**: These are measures based on image subtraction and therefore require images of the same modality. ▪ **Woods**: Partitioned Intensity Uniformity for the registration of MRI-PET images [7], [8]. ▪ **Mutual Information**, *Intra- and Cross-Modality:* Mutual information (MI) is a term from information theory [1]-[6]. Mutual information can be expressed as the sum of individual entropy terms of the random variables less their joint entropy. MI normalizes the joint entropy with respect to the partial entropies of the contributing signals. The dissimilarity function value is calculated from joint histogram of resampled reference and input data. ▪ **Mutual Information (PV)**, *Intra- and Cross-Modality:* In this MI variant, a partial volume interpolation algorithm is used as a part of the joint histogram construction. The histogram calculations are performed directly on the reference and input data. As a consequence the interpolation method selection in the matching parameters configuration has no relevance for this dissimilarity function. ▪ **Normalized Mutual Information**, *Intra- and Cross-Modality:* The normalized MI variant also uses partial volume interpolation and additionally a normalization scheme proposed by Studholme [5]. This variant has become very popular in the recent years and performs well in many multi-modality situations.
Interpolation method	Type of interpolation used during reslicing. Has an impact on speed, and may also influence convergence.
Sample rate	Density of resampling the original images during the matching process. Coarse sampling increases speed dramatically, but too coarse images may not allow any more for accurate matching. 6 or 8 mm is often satisfactory for MRI/PET matching. A strategy with multiple searches can be implemented in combination with the **Algorithm runs** option: the first matching runs are performed at a coarse resolution, but the last one with a fine sampling rate for an accurate final match.

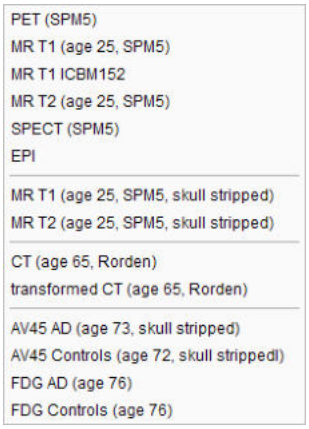
Minimization Method	**Powell** usually finds the optimal match faster than **Downhill Simplex**.
Function tolerance	Termination criterion for the iterations.
Template Mask	Allows defining a mask for the reference image if none was created and/or set to the matching protocol after the reference image was loaded. To discard the mask activate the **Clear file or directory** button
Input Mask	Allows defining a mask for the input image if none was created and/or set to the matching protocol after the input image was loaded. To discard the mask activate the **Clear file or directory** button
Save Parameters	Save the parameter settings for later use.
Calculate Inverse Transformation	If the box is checked, the inverse transformation is also calculated once the matching completed.

Advanced Parameters

Thresholding method	The image volume considered during matching can be restricted to a sub-volume by thresholding, eg. by excluding the image background. Note: selecting a background separation option reduces the time for dissimilarity function evaluation, but it may also worsen convergence, especially with a poor initial overlap of the segmented objects. Absolute values can be defined when **User defined** option is selected as thresholding method.
Normalize values to (0,1)	When this box is checked, the image values are normalized to the numeric range [0,1]. Note that the operation is a scaling, not a binarization of the image. This transformation may be required when applying one of difference criteria, if the dynamic range of the matched images is different for instance because of different administered tracer doses.
Algorithm runs	A value > 1 configures multiple successive matching runs, whereby a run is started with the result parameters of the preceding run.
Max iterations	A maximal number of optimization steps can be configured to avoid "endless" looping.
Scale	If box is checked allows scaling the image during rigid matching
No rotation	If box is checked no rotation is performed during the automatic rigid matching.

6.2 Normalization Templates

The normalization templates serve as the reference images for the elastic deformation algorithm. They usually represent the standard anatomy imaged with a certain modality. Currently the following brain templates for different modalities are available via the shortcut :



PET (SPM5)	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. The template is in MNI (Montreal Neurological Institute) coordinates.
MR T1 (age 25, SPM5)	T1 template provided with SPM5. The image was derived from the ICBM152 image which represents the average of 152 healthy T1 brain images by reducing it to 2mm isotropic resolution and smoothing with an 8mm FWHM Gaussian filter. The original ICBM152 data originates from Alan Evans, MNI, Canada (ICBM, NIH P-20 project, Principal Investigator John Mazziotta).
MR T1 ICBM152	Represents the average of 152 healthy T1-weighted MRI scans, linearly transformed to Talairach space. The template has been reduced to 2mm isotropic resolution and smoothed with an 8mm FWHM Gaussian filter.
MR T2 (age25, SPM5)	The same as the **T1** above, but with the T2 MR images.
SPECT (SPM5)	SPECT template provided with SPM5. It was created by Leighton Barnden et al from the Department of Nuclear Medicine at the Queen Elizabeth Hospital in Adelaide 22 normal female subjects. Each was scanned after injection of Tc-99m HMPAO on a triple head camera with ultra-high resolution fanbeam collimators.
EPI	SPM5 EPI derived template.
MR T1 (age 25, SPM5, skull stripped)	As the **T1** above, but with the skull part of the image removed.
MR T2 (age 25, SPM5, skull stripped)	As the **T2** above, but with the skull part of the image removed.
CT (age 65, Rorden)	CT template for an older population created from 30 healthy individuals with ages similar to what is commonly seen in stroke (mean 65 years). Developed for the SPM8 Clinical Toolbox by Rorden et al [10].
transformed CT (age 65, Rorden)	CT template as above but with the converted units (CU) which improves the contrast for soft tissue and CSF so that the normalization procedure works better.

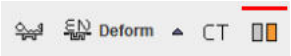
	Conversion procedure : HU values -1000..-100 are mapped to 0..900, values from -99..100 are linearly scaled to the range 911...3100, and values i>100 become [i+3000] [10]. CT images to be normalized with this template must also be converted by enabling the **Transform HU values** option.

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

6.3 Template-based Normalization (SPM5)

The template-based normalization is an implementation of the SPM5 methodology. It adjusts the input image to a template image by applying an affine transformation first, followed by iterative elastic adjustments.

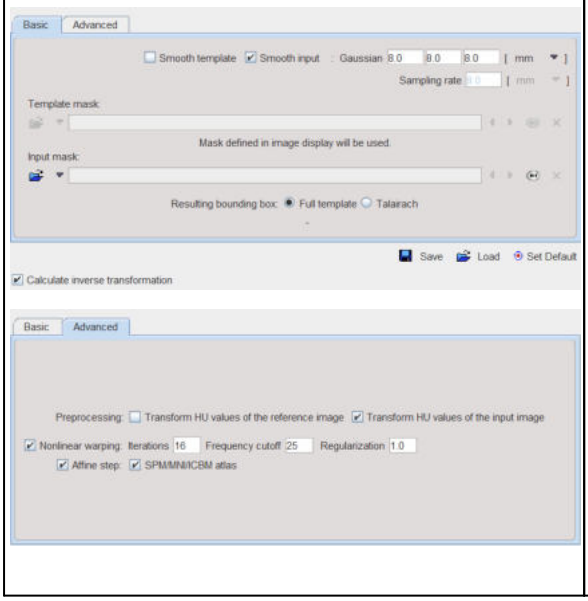
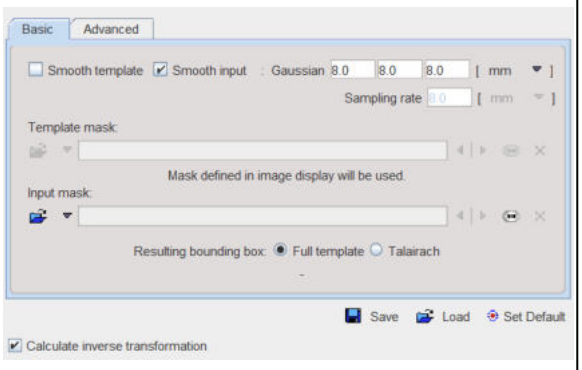
In the user interface the template-based normalization is configured as the **Deform** method.

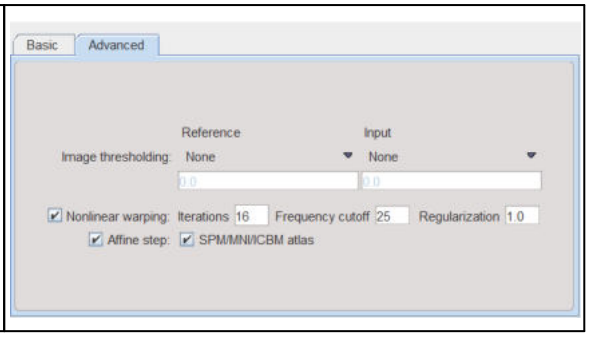


6.3.1 Parameters

The template-based algorithm uses several parameters, which are hidden from the user interface. For the **HUMAN** species there are two presets, CT for the normalization of CT brain images, and ▯▯ otherwise. The red bar above the buttons indicates which preset is active. For other species, the CT preset is absent.

To enable a preset and edit the parameters please select one of the buttons. A dialog window opens and shows the current configuration. The **HUMAN** default settings are shown below and can always be restored with the **Set Default** button.

CT	▯▯
	

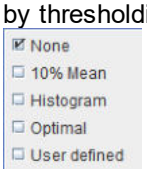
Note the **Transform HU values** options which transform the values in the CT image such that the contrast between bone and soft tissue is reduced and they are more similar to the usual anatomical images.

Basic Parameters

Smooth atlas, Smooth input	If either box is checked, an initial **Gaussian** smoothing of the respective data is performed. Both smoothing operations use the same configurable FWHM parameters. Usually, the template has already been smoothed beforehand so its smoothing is normally not required for the normalization.
Sampling rate	The sampling rate of the method is derived from the **Smooth Input** filter size. If no smoothing is applied, the sampling rate needs to be specified by the user.
Template Mask	This selection allows defining a mask to be applied to the template during the normalization procedure. If one of the standard templates 85 is used, its mask is implicitly defined and the selection is therefore inactive.
Input Mask	A mask file can be selected which masks the part of the input image which should be disregarded in the normalization. To discard a selected mask activate the **Clear file** button ×.
Resulting bounding box	The radio box for defining the extent (bounding box) of the resulting normalized images. ▪ **Full atlas**: The result image has the size of the template. ▪ **Talairach**: The result image is trimmed to the bounding box of the Talairach brain atlas as in the SPM99 program. It is only applicable for MNI brain templates.

Advanced Parameters

The **Advanced** parameters are usually only changed if a normalization fails or if the user aims at a specific effect.

Thresholding method	The image volume considered during matching can be restricted to a sub-volume by thresholding, e.g. to exclude the image background.  Absolute values can be defined when **User defined** option is selected as thresholding method.
Nonlinear Warping	If this box is not checked, only the affine (translation, rotation, scaling, shearing) part of the normalization is performed.

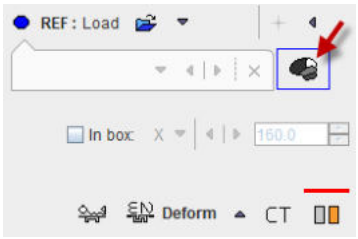
Iterations	Number of nonlinear iterations. The higher the iterations number, the more deformations may occur.
Frequency cutoff	The specified **Frequency cutoff** (default = 25) is used together with the **Bounding box** size to calculate the number of basis functions. Higher cutoff values result in fewer basis functions.
Affine calculations	Estimate and apply an affine transformation before the nonlinear warping iterations start.
SPM/MNI/ICBM atlas	Use settings which are appropriate for the templates of these standard atlases.

Non-Human Species

With a non-human species selected, the parameter windows show the same sets of parameters, but initialized with settings corresponding to the expected pixel size.

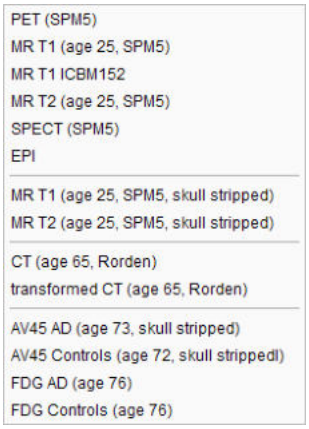
6.3.2 Normalization Templates

The standard normalization template can be loaded with the button indicated below. A list will be shown, which corresponds to the selected species.



HUMAN Normalization Templates

The following brain templates are provided for the **HUMAN** species.



PET (SPM5)	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. The template is in MNI (Montreal Neurological Institute) coordinates.
MR T1 (age 25, SPM5)	T1 template provided with SPM5. The image was derived from the ICBM152 image which represents the average of 152 healthy T1 brain images by reducing it to 2mm isotropic resolution and smoothing with an 8mm FWHM

	Gaussian filter. The original ICBM152 data originates from Alan Evans, MNI, Canada (ICBM, NIH P-20 project, Principal Investigator John Mazziotta).
MR T1 ICBM152	Represents the average of 152 healthy T1-weighted MRI scans, linearly transformed to Talairach space. The template has been reduced to 2mm isotropic resolution and smoothed with an 8mm FWHM Gaussian filter.
MR T2 (age25, SPM5)	The same as the **T1** above, but with the T2 MR images.
SPECT (SPM5)	SPECT template provided with SPM5. It was created by Leighton Barnden et al from the Department of Nuclear Medicine at the Queen Elizabeth Hospital in Adelaide 22 normal female subjects. Each was scanned after injection of Tc-99m HMPAO on a triple head camera with ultra-high resolution fanbeam collimators.
EPI	SPM5 EPI derived template.
MR T1 (age 25, SPM5, skull stripped)	As the **T1** above, but with the skull part of the image removed.
MR T2 (age 25, SPM5, skull stripped)	As the **T2** above, but with the skull part of the image removed.
CT (age 65, Rorden)	CT template for an older population created from 30 healthy individuals with ages similar to what is commonly seen in stroke (mean 65 years). Developed for the SPM8 Clinical Toolbox by Rorden et al [10].
transformed CT (age 65, Rorden)	CT template as above but with the converted units (CU) which improves the contrast for soft tissue and CSF so that the normalization procedure works better. Conversion procedure : HU values -1000..-100 are mapped to 0..900, values from -99..100 are linearly scaled to the range 911...3100, and values i>100 become [i+3000] [10]. CT images to be normalized with this template must also be converted by enabling the **Transform HU values** option.

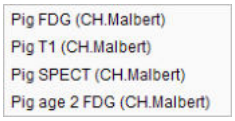
The amyloid tracers, e.g. AV45, have different pattern distribution as compared to the SPM5 Oxygen-15 water **PET** template. When such images data are analyzed the PET based normalization will not work. Therefore, we created group specific (**AV45 Controls** and **AV45 AD**) AV45 PET templates using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

PRIMATE Normalization Templates

The following brain templates are provided for the **PRIMATE** species.

PIG Normalization template

The following templates are provided for the PIG species:



RAT Normalization Templates

The following brain templates are listed for the **RAT** species.

MOUSE Normalization Templates

The following brain templates are listed for the **MOUSE** species.

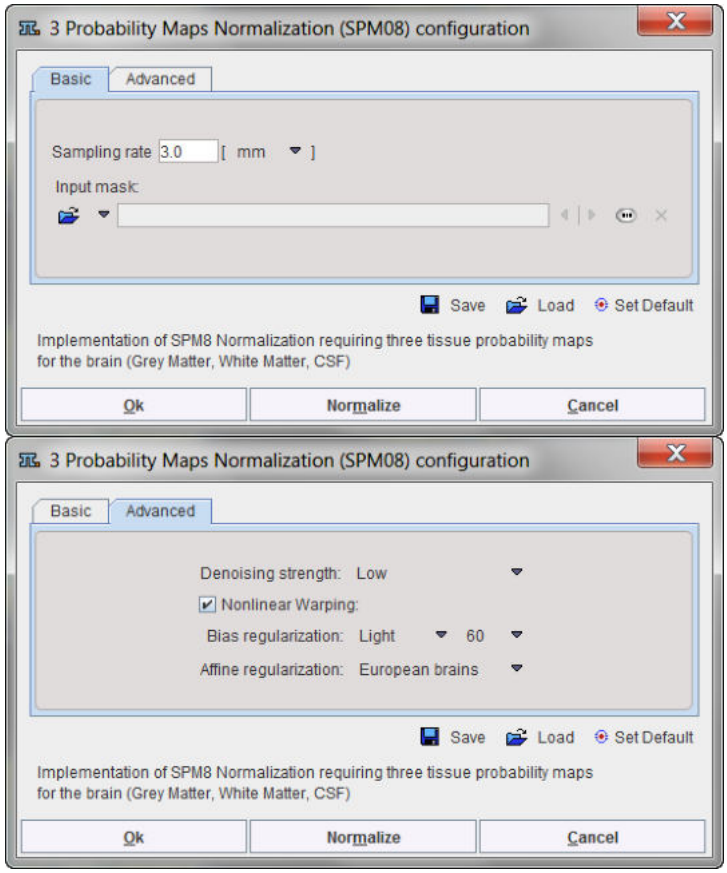
6.4 3 Probability Maps Normalization (SPM8)

The **3 Probability Maps Normalization** is based on PMOD's Java implementation of the Unified Segmentation methodology developed in SPM8 by Ashburner et al [11].

In the user interface the **3 Probability Maps Normalization** is configured as the **Probab** method and activating the preset so that is marked by the red bar.

6.4.1 Parameters

The following parameters allow fine-tuning the algorithm.



Basic Parameters

Sampling rate	Pixel sampling rate for the calculation.
Input mask	A mask file can be selected which masks the part of the input image which should be disregarded in the normalization.

Advanced Parameters

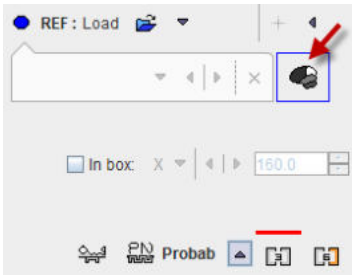
The **Advanced** parameters are usually only changed if a normalization fails or if the user aims at a specific effect.

Denoising	Image denoising prior to the normalization using the fast Non Local Means Analysis method with settings **None**, **Low**, **Medium**, **Strong**.
Nonlinear Warping	Enable elastic deformation in addition to the affine transformation.
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: **None**, **Very Light**, **Light**, **Medium**, **Heavy**, **Very Heavy**. The parameter to the right indicates the **FWHM [mm]** to be applied. The larger the FWHM, the smoother the variation that is assumed.
Affine regularization	Two different initializations of the affine registration are supported, **European brains** and **East Asian brains**, as well as **No regularization**. The setting

	should correspond to the nature of the subject under study.

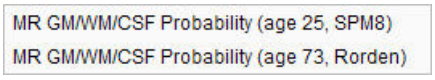
6.4.2 Normalization Templates

The templates for the **3 probability maps** normalization are expected to include tissue probability maps for grey matter, white matter and CSF, arranged in a dynamic series. If such a template has been prepared by the user, it can be loaded with the **REF Load** button. Alternatively, a standard normalization template can be loaded with the button indicated below. A list will be shown, which corresponds to the selected species.



HUMAN Normalization Templates

The following brain probability map templates are provided for the **HUMAN** species.



MR GM/WM/CSF Probability (age 25, SPM8)	Brain template consisting of probability maps of SPM8 for Grey Matter, White Matter and CSF arranged as three frames in a dynamic series.
MR GM/WM/CSF Probability (age 73, Rorden) (Clinical Toolbox)	Brain template of an older population created from 50 healthy individuals (mean 73 years). Developed for the SPM8 Clinical Toolbox by Rorden et al [10]. Note that although the anatomy is in the MNI space, the grey matter is thinner and the ventricles larger, so that the standard AAL and Hammers VOIs appear too large. Intersection with the grey probability map (as in PNEURO) should be used for trimming those VOIs.

RAT Normalization Templates

When the RAT species is selected, there is only one template listed

It corresponds to the **Wistar Rat (Tohoku)** atlas which was developed by Valdes-Hernandez et al [1] using 7T T_2-MRIs from 30 Wistar rats. Please refer to the atlas description in the *PMOD Base Functionality User Guide.*

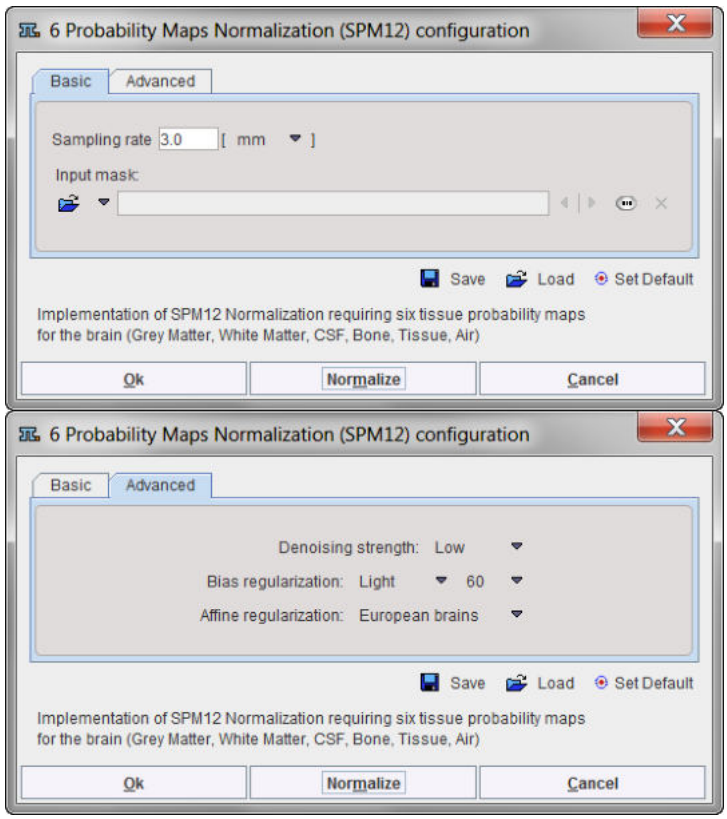
6.5 6 Probability Maps Normalization (SPM12)

The **6 Probability Maps Normalization** is based on PMOD's Java implementation of the Unified Segmentation methodology in SPM12 developed by Ashburner et al [11]. In addition to the probability maps of grey matter, white matter and CSF it uses probability maps of bone, soft tissue and air/background.

In the user interface the **6 Probability Maps Normalization** is configured as the **Probab** method and activating the preset so that is marked by the red bar.

6.5.1 Parameters

The following parameters allow fine-tuning the algorithm.



Basic Parameters

Sampling rate	Pixel sampling rate for the calculation.
Input mask	A mask file can be selected which masks the part of the input image which should be disregarded in the normalization.

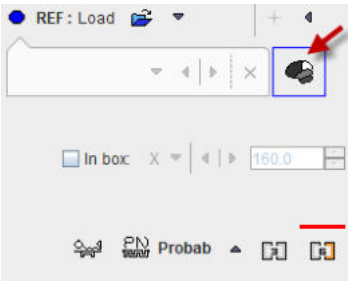
Advanced Parameters

The **Advanced** parameters are usually only changed if a normalization fails or if the user aims at a specific effect.

Denoising	Image denoising prior to the normalization using the fast Non Local Means Analysis method with settings **None**, **Low**, **Medium**, **Strong**.
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: **None**, **Very Light**, **Light**, **Medium**, **Heavy**, **Very Heavy**. The parameter to the right indicates the **FWHM [mm]** to be applied. The larger the FWHM, the smoother the variation that is assumed.
Affine regularization	Two different initializations of the affine registration are supported, **European brains** and **East Asian brains**, as well as **No regularization**. The setting should correspond to the nature of the subject under study.

6.5.2 Normalization Templates

The templates for the 6 **probability maps** normalization are expected to include tissue probability maps for grey matter, white matter, CSF, bone, soft tissue and air/background arranged in a dynamic series. If such a template has been prepared by the user, it can be loaded with the **REF Load** button. Alternatively, a standard normalization template can be loaded with the button indicated below. A list will be shown, which corresponds to the selected species.



HUMAN Normalization Templates

The following probability map templates are provided for the **HUMAN** species.

MR 1.5mm GM/WM/CSF/T/B/A Probability (SPM12)	Brain template consisting of probability maps of SPM12 at 1.5mm resolution.
MR 2.0mm GM/WM/CSF/T/B/A Probability (SPM12)	Brain template consisting of probability maps of SPM12 at 2.0 mm resolution.

6.6 Diffeomorphic Elastic Matching (ANTS)

The ANTS (Advance Normalization Tools) have been extensively used for many image processing tasks, as described in the overview article by Tustison et al. The SyN algorithm has been proven to be a top performer in the registration of brain, lung and cardiac images.

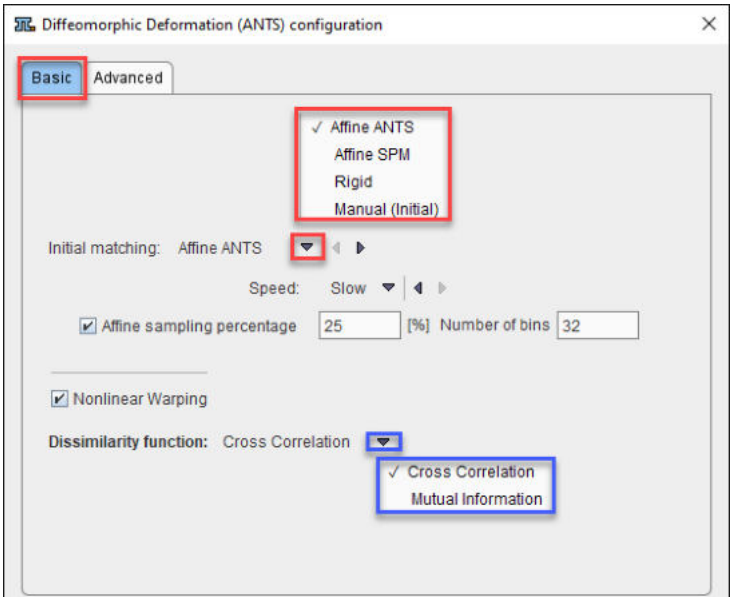
PMOD has ported the SyN algorithm to Java in order to make it portable and embed it fully into the software framework. PMOD users can now take advantage of an integrated solution, rather than having to bother with command scripts with a big number of options.

A list of predefined brain templates based on species selection is available in a similar manner like for the Template based normalization matching algorithm.

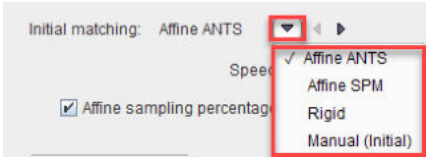
6.6.1 Parameters

Most of the parameters correspond to the parameters of the original ANTS software, for which a manual page is available. Please refer to the ANTS user community for hints regarding tailoring of the parameters for specific tasks.

Basic Parameters



Initial Matching offers four methods to roughly align the data, before the elastic deformations start. The choices are



with **Affine ANTS** as defaults. It estimates and apply an affine transformation before the nonlinear warping iterations start. The amount of voxels used for calculation is specified by the sampling percentage parameter and represents a regular sampling strategy in combination with the histogram **Number of bins**. The initialization **Speed** has two options: **Slow** (4 Levels, [1000, 500, 250, 100] iterations) and **Fast** (3 Levels, [1000, 500, 250] iterations). The **Slow Speed** is more time consuming but the result are expected to be most accurate.

Affine SPM from Template-based Normalization (SPM5) 87 represents the second choice.

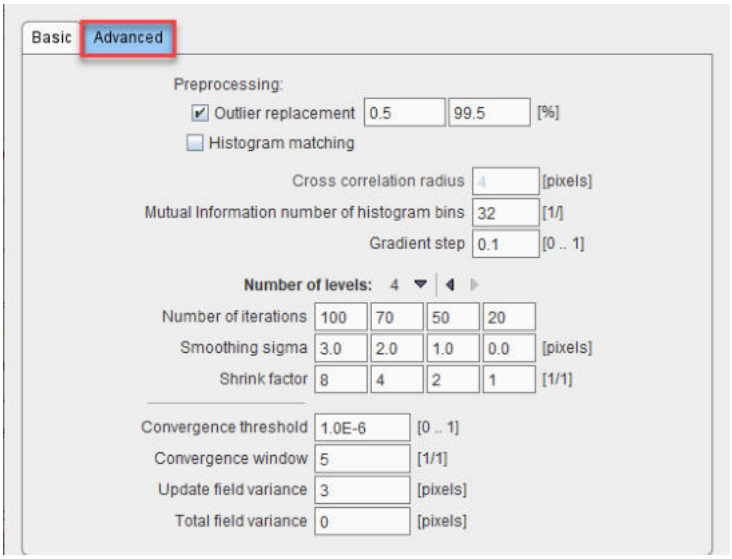
Rigid will apply the standard Rigid Registration 84 procedure, whereas **Manual (Initial)** will use the current location of the input image and not do any further alignment. The parameters of these methods (if applicable) appear after selecting them.

Nonlinear Warping should be enabled, otherwise the elastic part will be skipped. This can be helpful to assess the effect of the initial step.

The **Dissimilarity function** represents the definition of the matching algorithm. The available selections are:

- **Cross correlation:** to be considered when matching images with similar pixel distribution (same image modality). It is a time consuming calculation.
- **Mutual information**: to be considered when matching images with different pixel distribution (cross modality). It is a faster algorithm

Advanced Parameters



With **Outlier replacement** enabled, extreme pixel values are replaces by the winsorization method. **Histogram matching** normalizes the pixel values, and is recommended when matching images from the same modality.

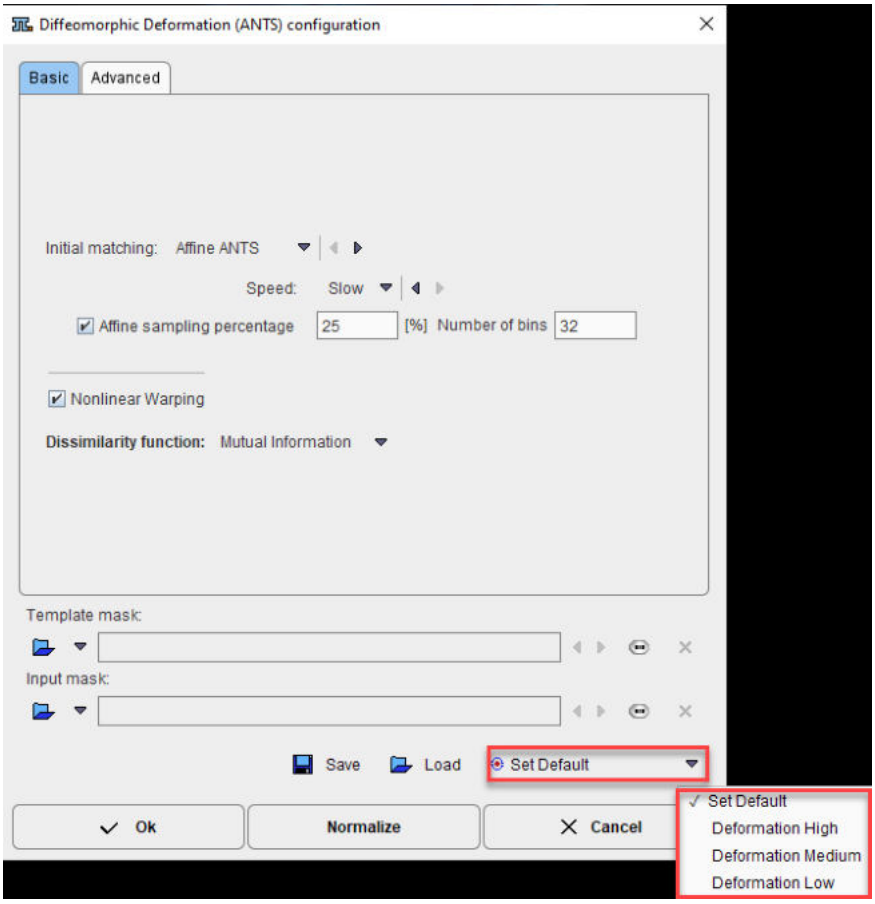
The **Histogram matching**, when enabled, allows normalizing the pixels values by the histogram matching. It is a parameter to be used for matching images acquired using the same modality.

The **Cross correlation radius** determines the number of pixels in the neighborhood, which are used for calculating the cross correlation cost function.

The **Gradient step** characterizes the gradient descent optimization.

Number of levels determines the levels of hierarchical matching, working from coarse towards fine resolution. On each level, three parameters are configured: **Shrink factor** defines the sub-sampling in each direction. A factor of 8 reduces the number of pixels by a factor 8^3. **Smoothing sigma** is the Gaussian smoothing kernel size, and **Number of iterations** the number of optimizations at each level.

The **Convergence** parameters determine whether the iterations can be stopped before the configured number of iterations are exhausted. The **Field variance** parameters may be useful when adjusting to published methods.



The **Template mask** allows defining a mask for the reference image if none was created and/or set to the matching protocol after the reference image was loaded. To discard the mask activate the **Clear file or directory** button ×

The **Input mask** allows defining a mask for the input image if none was created and/or set to the matching protocol after the input image was loaded. To discard the mask activate the **Clear file or directory** button ×

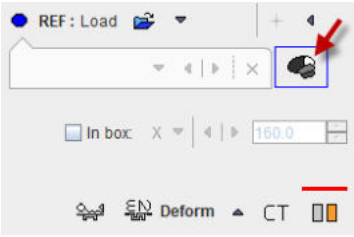
The **Save** icon allows saving the parameters set in the **Basic** and **Advanced** panel for later use.

The **Load** icon allows retrieving parameters previously saved and set them to the current matching procedure.

The **Set Default** options allows resetting the **Basic** and **Advance** parameters to their default values. The default values will depend on the selected entry in the default list. With **Deformation High** there will be long calculation times but with high quality deformation results while **Deformation Low** will return the results faster but the deformation quality will be lower. When hoovering with the mouse cursor above the selection, a tool tip will display the matching method (cross correlation=CC or mutual information=MI) and the associated default parameters. Please note that this parameters can be further modified on the **Basic** and/or **Advanced** panel and the new parameters saved using the **Save** icon.

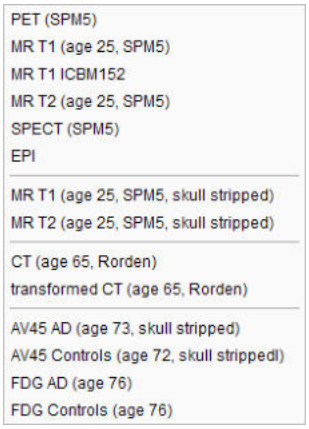
6.6.2 Normalization Templates

The standard normalization template can be loaded with the button indicated below. A list will be shown, which corresponds to the selected species.



HUMAN Normalization Templates

The following brain templates are provided for the **HUMAN** species.



PET (SPM5)	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. The template is in MNI (Montreal Neurological Institute) coordinates.
MR T1 (age 25, SPM5)	T1 template provided with SPM5. The image was derived from the ICBM152 image which represents the average of 152 healthy T1 brain images by reducing it to 2mm isotropic resolution and smoothing with an 8mm FWHM Gaussian filter. The original ICBM152 data originates from Alan Evans, MNI, Canada (ICBM, NIH P-20 project, Principal Investigator John Mazziotta).
MR T1 ICBM152	Represents the average of 152 healthy T1-weighted MRI scans, linearly transformed to Talairach space. The template has been reduced to 2mm isotropic resolution and smoothed with an 8mm FWHM Gaussian filter.
MR T2 (age25, SPM5)	The same as the **T1** above, but with the T2 MR images.
SPECT (SPM5)	SPECT template provided with SPM5. It was created by Leighton Barnden et al from the Department of Nuclear Medicine at the Queen Elizabeth Hospital in Adelaide 22 normal female subjects. Each was scanned after injection of Tc-99m HMPAO on a triple head camera with ultra-high resolution fanbeam collimators.
EPI	SPM5 EPI derived template.
MR T1 (age 25, SPM5, skull stripped)	As the **T1** above, but with the skull part of the image removed.

MR T2 (age 25, SPM5, skull stripped)	As the **T2** above, but with the skull part of the image removed.
CT (age 65, Rorden)	CT template for an older population created from 30 healthy individuals with ages similar to what is commonly seen in stroke (mean 65 years). Developed for the SPM8 Clinical Toolbox by Rorden et al [10].
transformed CT (age 65, Rorden)	CT template as above but with the converted units (CU) which improves the contrast for soft tissue and CSF so that the normalization procedure works better. Conversion procedure : HU values -1000..-100 are mapped to 0..900, values from -99..100 are linearly scaled to the range 911...3100, and values i>100 become [i+3000] [10]. CT images to be normalized with this template must also be converted by enabling the **Transform HU values** option.

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

PRIMATE Normalization Templates

The following brain templates are provided for the **PRIMATE** species.

- Cynomolgus T1 (CIMA-UN)
- Cynomolgus DTBZ (CIMA-UN)
- Cynomolgus FDOPA (CIMA-UN)
- Rhesus Macaque T1 (INIA19)
- Rhesus Macaque T1 (INIA19, skull stripped)

PIG Normalization template

The following templates are provided for the PIG species:

- Pig FDG (CH.Malbert)
- Pig T1 (CH.Malbert)
- Pig SPECT (CH.Malbert)
- Pig age 2 FDG (CH.Malbert)

RAT Normalization Templates

The following brain templates are listed for the **RAT** species.

- Rat FDG (W.Schiffer)
- Rat FDG (W.Schiffer, skull stripped)
- Rat T2 (W.Schiffer)
- Rat CT (W.Schiffer)
- Rat T2 (Tohoku)
- Rat T2 (A.Schwarz)
- Rat Flumazenil (Groningen)
- Rat MeDAS (Groningen)
- Rat PBR28 (Groningen)
- Rat PK11195 (Groningen)
- Rat Raclopride (Groningen)
- Rat FDG (Groningen)
- Rat SPECT (Groningen)
- Rat T2 (Groningen)
- Rat T2 (Waxholm)

MOUSE Normalization Templates

The following brain templates are listed for the **MOUSE** species.

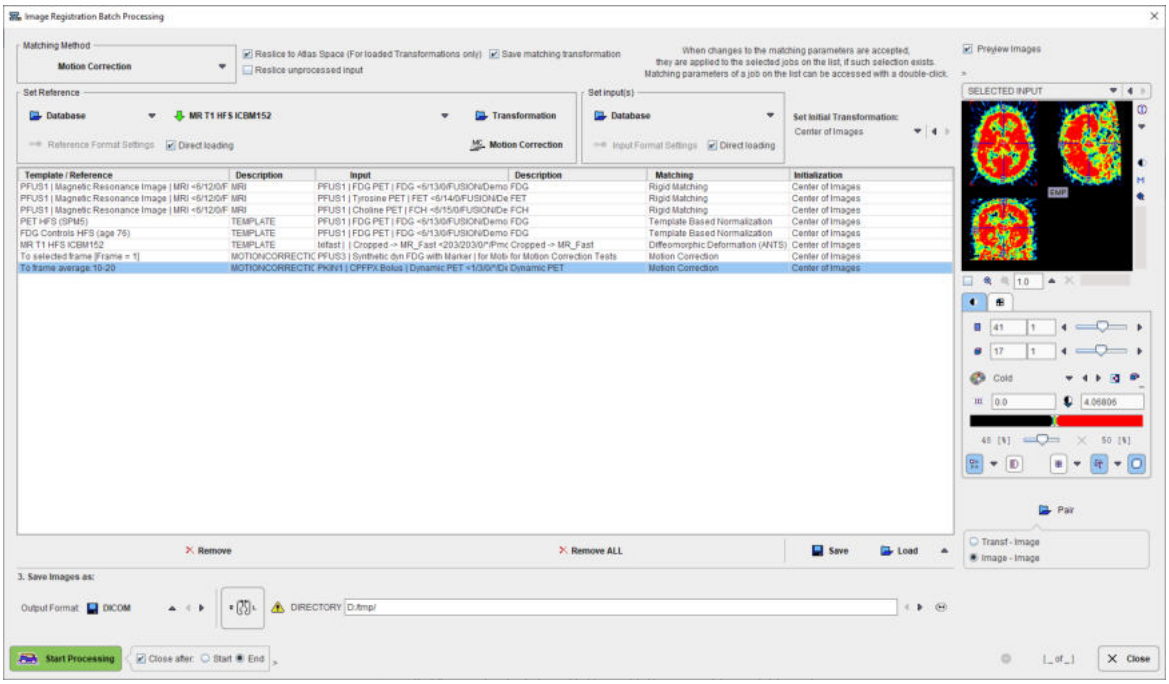
- Mouse FDG (Ma-Benveniste-Mirrione)
- Mouse T2 (Ma-Benveniste-Mirrione)
- Mouse CT (Ma-Benveniste-Mirrione)
- Mouse T2 (Waxholm)

7 Matching and Reslicing in Batch Mode

The **Fusion** tool offers a batch matching facility which is useful for different scenarios:

1. Definition of multiple jobs for Single Reference/Single Input or Single Reference/Multiple Inputs.
2. Definition of the single job for multiple Reference/Input pairs.
3. Application of previously calculated transformations to a series of data sets using **Transformation-Image** pairs.

Batch matching is started using the button from the taskbar or **Fusion/Batch Mode** and displays the dialog window illustrated below.



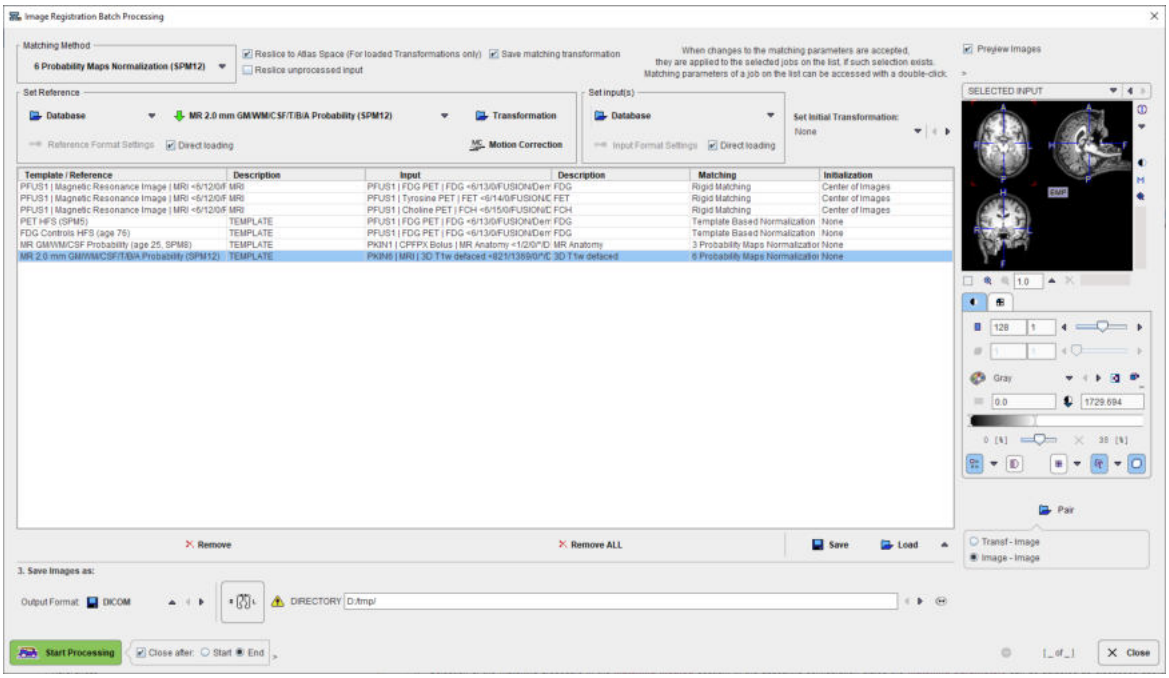
The following configuration tasks must first be completed for running matching in a batch procedure.

1. Selection of the matching procedure in the **Matching Method** section. If the **Save matching transformation** box is checked, the resulting transformation parameters will be saved in addition to the resliced images.
2. Optionally, the matching initialization is possible selecting one of the available entries from the **Set Initial Transformation** list
3. Definition of the job(s) according to the working scenario. The **Save** and **Load** buttons allow saving/retrieving jobs definition.
4. Specification of the output in the **Save Images as** section. First select the data format, and then configure the parameters of that format. Finally, specify the output path (or the target database). As an option, the **subject**, **Study**, **Series** data information can be replaced by an arbitrary string to create anonymous data sets in the corresponding sub-tabs.
5. Finally, the **Start Processing** button can be activated to initiate batch processing. The matching jobs are processed one after the other and the results saved according to the specification.

7.1 Multiple Jobs Definition for Single Reference/Single(Multiple) Input

Different type of matching procedures can be defined for such scenario. In the example below 3 PET studies of one subject are to be rigidly matched to one *Reference:* the subject MRI. The **Center of Gravity alignment** was selected for the 3 PET studies as matching initialization in the **Set Initial Transformation** list. Spatial normalization to an atlas template is also illustrated for the PET FDG study of the same subject. For example, all images of a clinical trial could be normalized by batch matching to a common template.

The 3 Probability Maps Normalization (SPM8) is configured for a subject MRI and a 6 Probability Maps Normalization (SPM12) for another subject MR



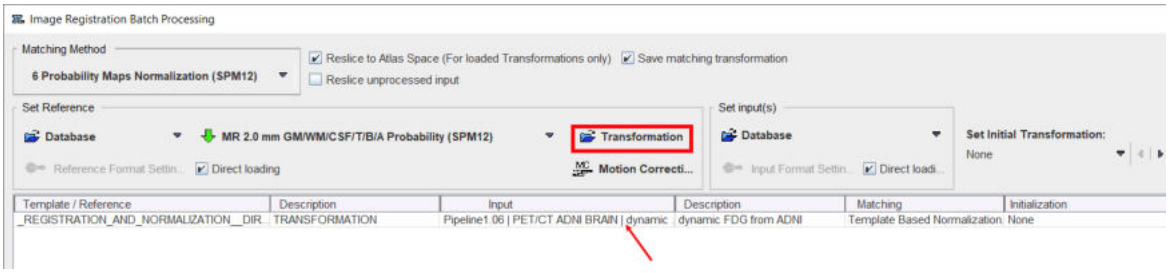
The following steps need to be completed for the jobs definition:

1. Selection of the matching procedure in the **Matching Method** section. In the appearing configuration dialog the **Matching parameters** can be adjusted as discussed above for the different methods. If the **Save matching transformation** box is checked, the resulting transformation parameters will be saved in addition to the resliced images.

2. Definition of the job(s) for Single Reference/Multiple Input. As a first step select the data formats correctly, in the example above **Database**. Then proceed by selecting a reference data set using the **Database** button, and subsequently the input data set(s) to be matched to this reference. The result is a job entry in the list as illustrated above. Continue these configuration steps until all matching pairs are listed.
   Note that only a single data format is supported for a batch procedure. For normalization procedures a template can easily be configured using the dedicated selection button in the **Set Reference** section, here labeled **PET HFS**. If there are pre-processing transformations which should be applied during loading, these can be configured using the **Input Format Settings** buttons. Note that, in this case, the **Direct loading** box have to be unchecked.

3. The matching method can be selectively adjusted for each job in the list by clicking at it and then changing the **Matching Method** selection.

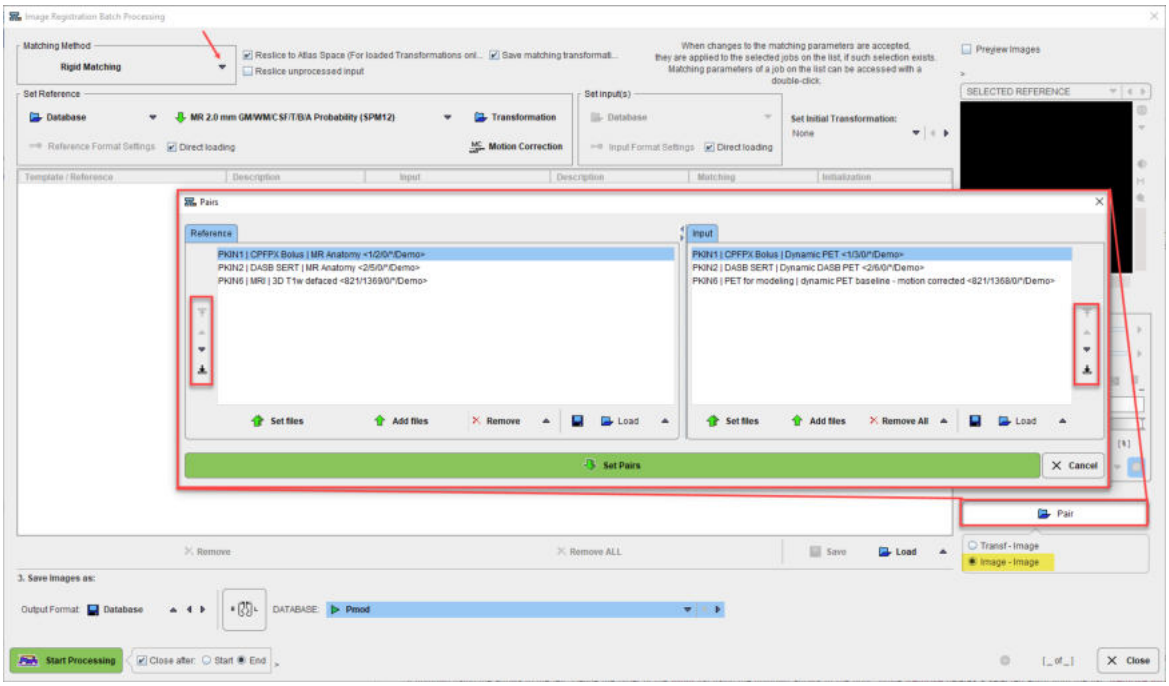
In addition to matching the batch mode can also be used to apply previously calculated transforms to a series of data sets as illustrated below. In this case **Transformation** is used to select a transformation instead of a reference.



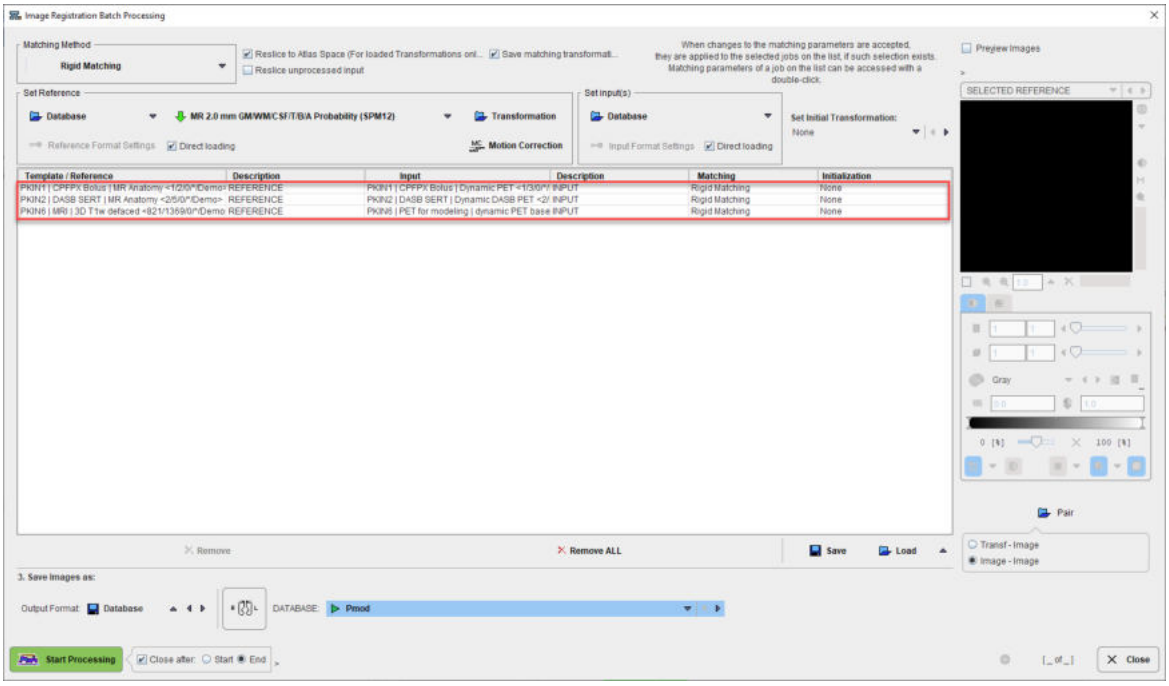
7.2 Single Jobs Definition for Multiple Reference/Input Pairs

One type of matching method can be easily defined for multiple Reference/Input pairs in the **Batch Mode** interface.

As a first step select the **Matching Method** and adjust the **Matching parameters** as discussed above for the different methods. Then set the data formats correctly, in the example below **Database**. If there are pre-processing steps which should be applied during loading for the **Reference** or **Input** data, these can be configured using the **Reference Format Settings** and **Input Format Settings** buttons respectively. Note that, in this case, the **Direct loading** box have to be unchecked. Proceed by enabling the **Image-Image** radio button. Activate the **Pair** icon to open the Reference/Input dialog definition:



The window has two main areas: the **Reference** data specifications are on the left side while the corresponding **Input** specification on the right side. 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 **Reference** and **Input** data list for processing. To modify the order how the data appears in the **Reference** list please select the entry and move it up/down using the arrows to the left. Define the order of the **Input** list using the up/down arrows to the right. While **Remove** deletes a selected entry from the list, **Remove all** clears the whole list. A data list can be saved for later use with the **Save** button right to **Remove**. Finally confirm the lists with the **Set Pairs**. The data are transfer automatically in the **Image Registration Batch Processing** window as illustrated below:

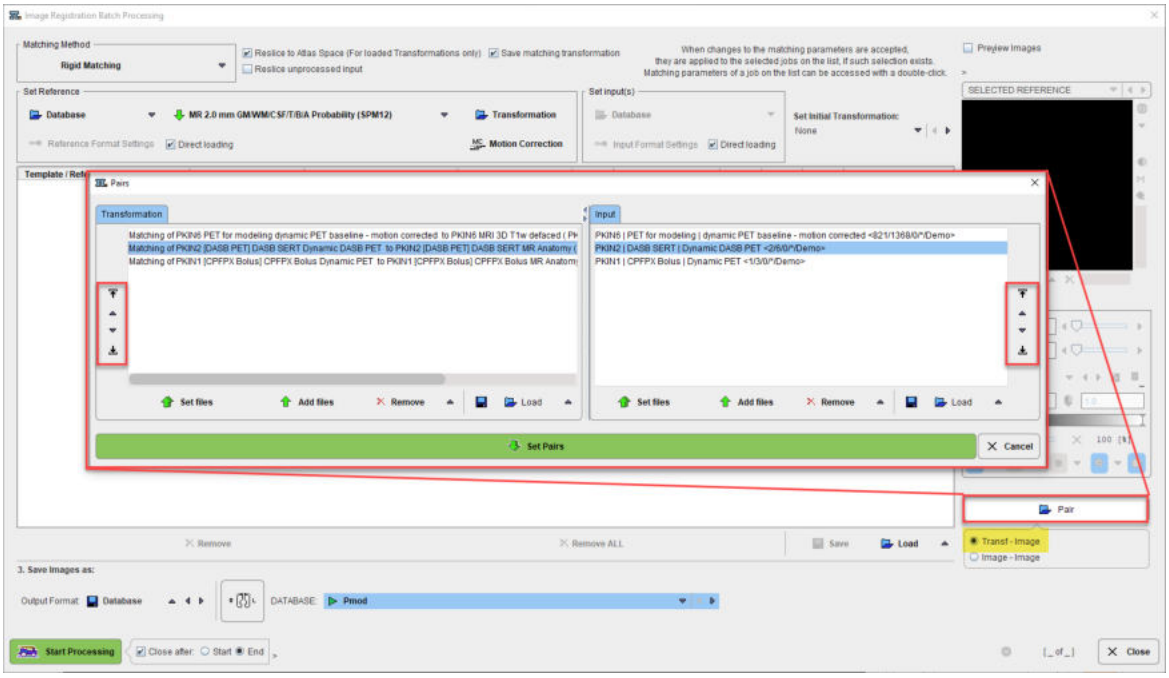


Activate the **Start Processing** button to run the batch.

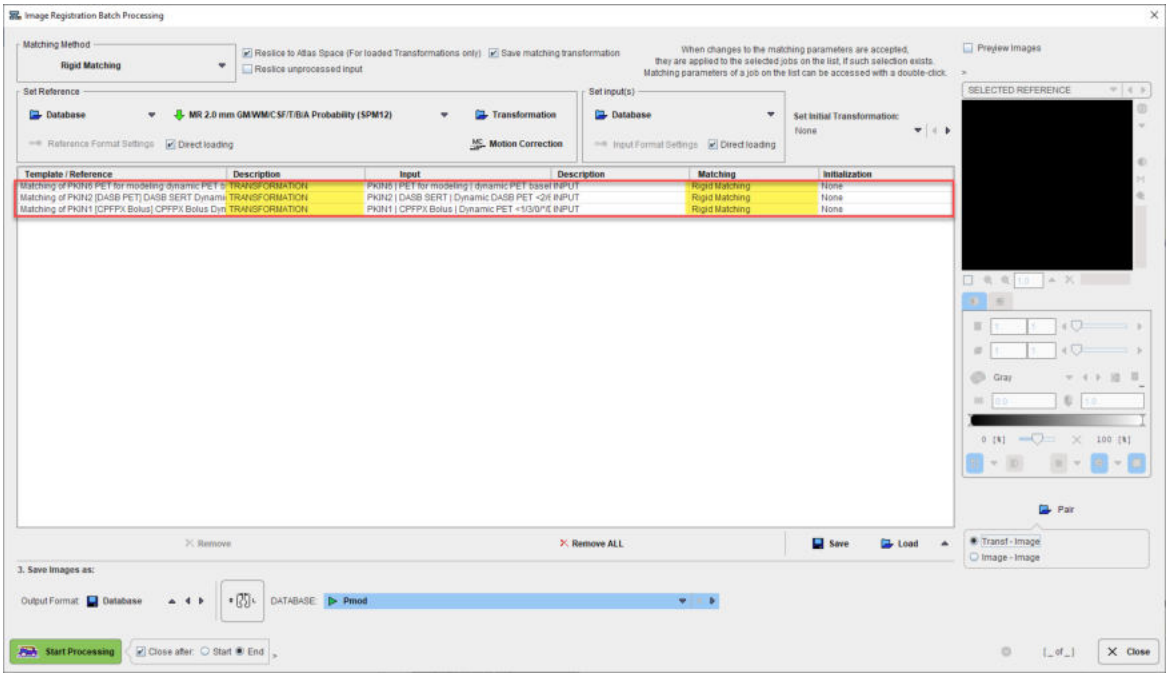
7.3 Transformation-Image Pairs

Transformations previously calculated can be applied to a series of data sets using **Transformation-Image** pairs facility in the Batch mode interface.

Start setting the input data formats correctly, in the example below **Database**. If there are pre-processing steps which should be applied during loading for the **Input** data, these can be configured using the **Input Format Settings** buttons. Note that, in this case, the **Direct loading** box have to be unchecked. Proceed by enabling the **Transf-Image** radio button. Activate the **Pair** icon to open the Transformation/Input dialog definition:



The window has two main areas: the **Transformation** matrices specifications are on the left side while the corresponding **Input** specification on the right side. The transformations to be applied are defined by the **Set files** or **Add files** buttons, which open a dialog window for selecting transformation matrix files. Similarly, the image data to which the transformations will be applied can be defined in the Input section. The data selections build up the **Transformation** and **Input** data list for processing. To modify the order how the data appears in the **Transformation** list please select the entry and move it up/down using the arrows to the left. Define the order of the **Input** list using the up/down arrows to the right. While **Remove** deletes a selected entry from the list, **Remove all** clears the whole list. A data list can be saved for later use with the **Save** button right to **Remove**. Finally confirm the lists with the **Set Pairs**. The data are transfer automatically in the **Image Registration Batch Processing** window as illustrated below:



Activate the **Start Processing** button to run the batch.

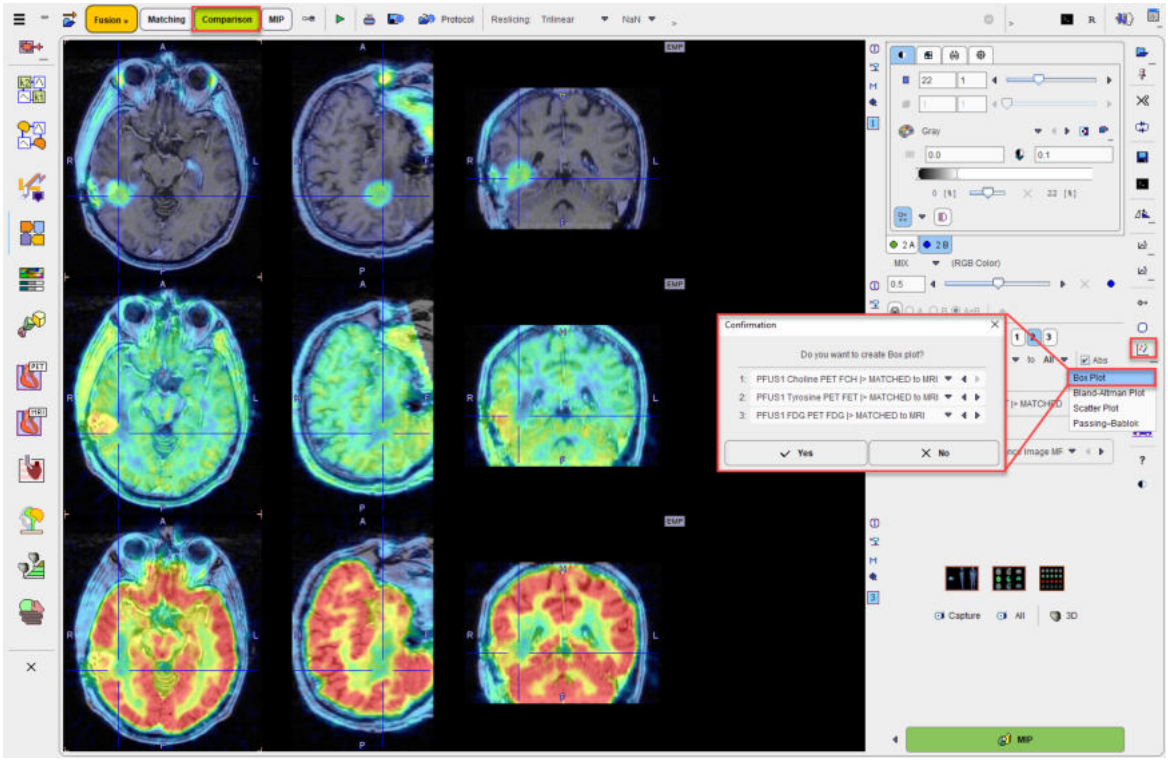
8 Plots of VOI Pixel Values in 2D and 3D

For matched images, there is a 1:1 correspondence of the pixel values. The PFUS tool supports the analysis of corresponding pixel values across images, limited within VOIs.

8.1 2D Plots

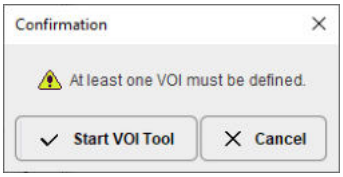
8.1.1 Box-Plot

The best way to prepare the data of interest is arranging them on the **Comparison** main page in a three-row layout, and then activating the **Box Plot** selection with the black down arrow located under the  icon in the lateral taskbar:

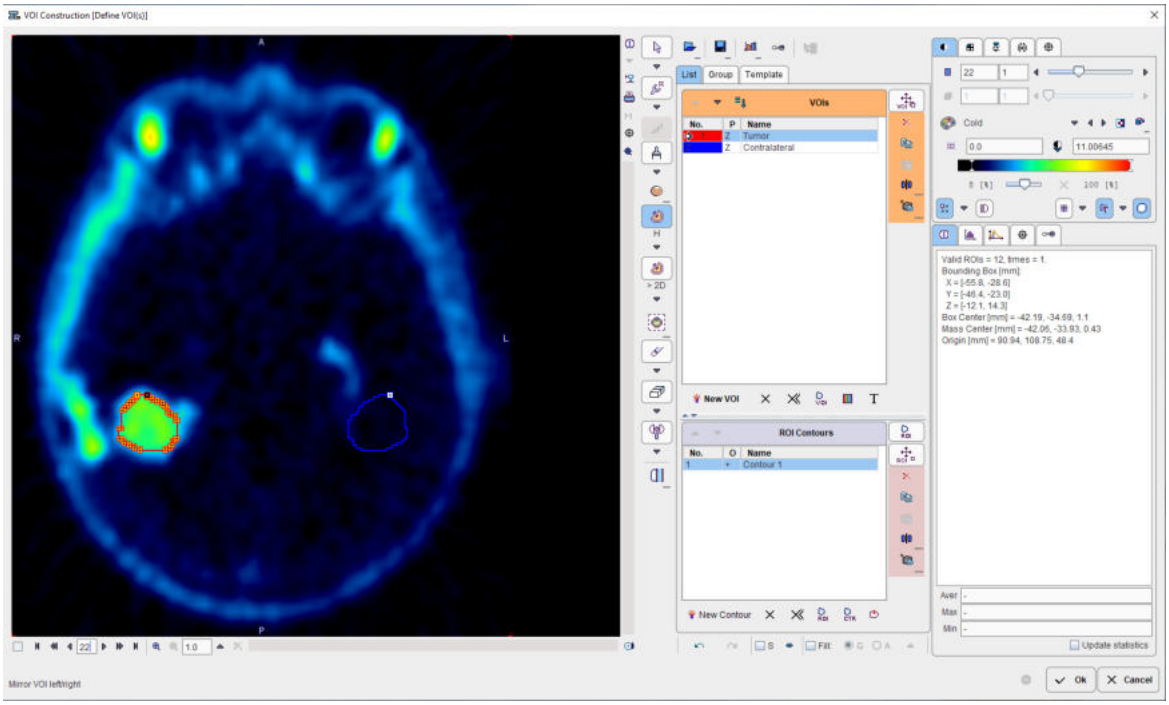


A dialog window is shown for configuring the 3 image series for which the result will be shown. If the series selection is not yet proper, it can be corrected before proceeding.

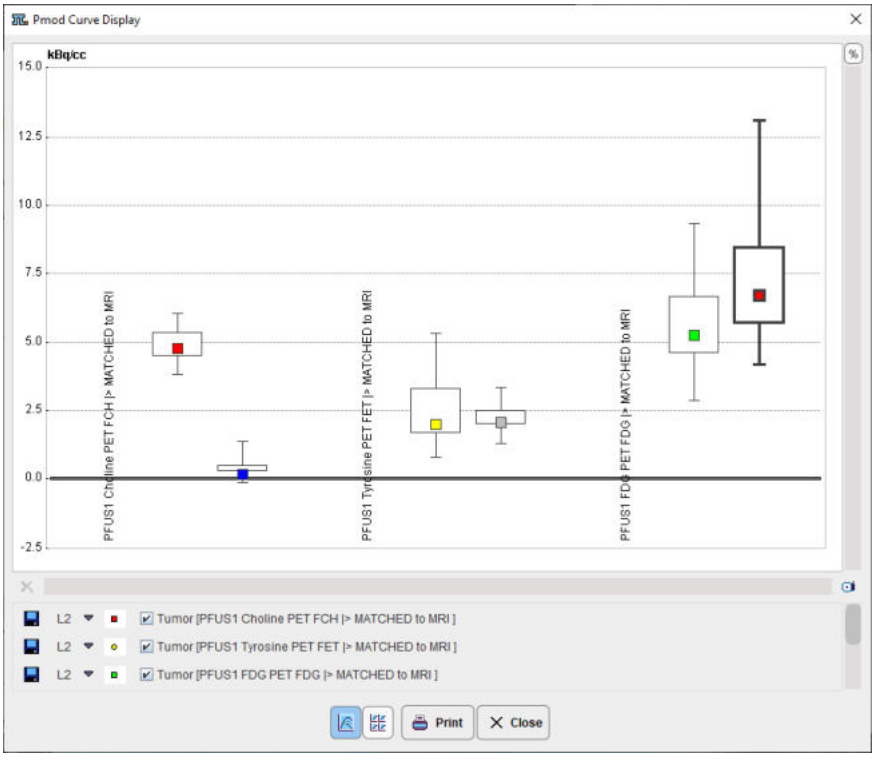
The box plot plot requires VOIs to exist in the first series. If this is not the case, a dialog window appears



and **Start VOI Tool** used to enter VOI definition. The usual PMOD functionality can be applied for defining a list of VOIs.



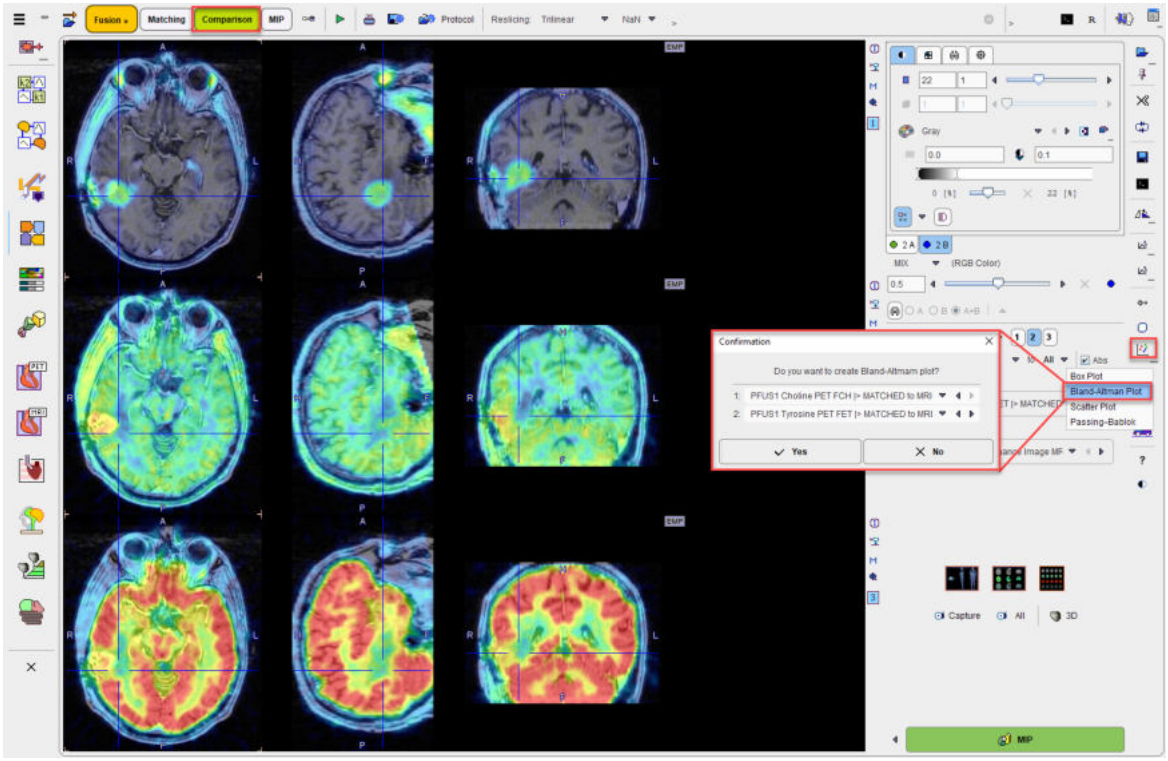
After confirming with **Ok**, the **Box Plot** calculates a box plot representation of the VOI pixel values for all three image sets.



The boxplot is a simple box and whiskers and allows to compare easily across series. Boxplot summarizes a sample data using 25th, 50th and 75th percentiles. These percentiles are also known as the lower quartile, median and upper quartile.The value is plotted on the vertical axis and grouped by the series on the horizontal axis. The actual data values can be exported by right-clicking into the plot and selecting **Value table of visible curves** or **Save multiple curves**. To switch ON/OFF the boxplot in the plot area use the boxes available in the plot control area.

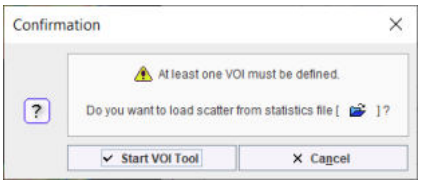
8.1.2 Bland Altman Plot

The best way to prepare the data of interest is arranging them on the **Comparison** main page in a three-row layout, and then activating the **Bland-Altman Plot** selection with the black down arrow located under the  icon in the lateral taskbar:

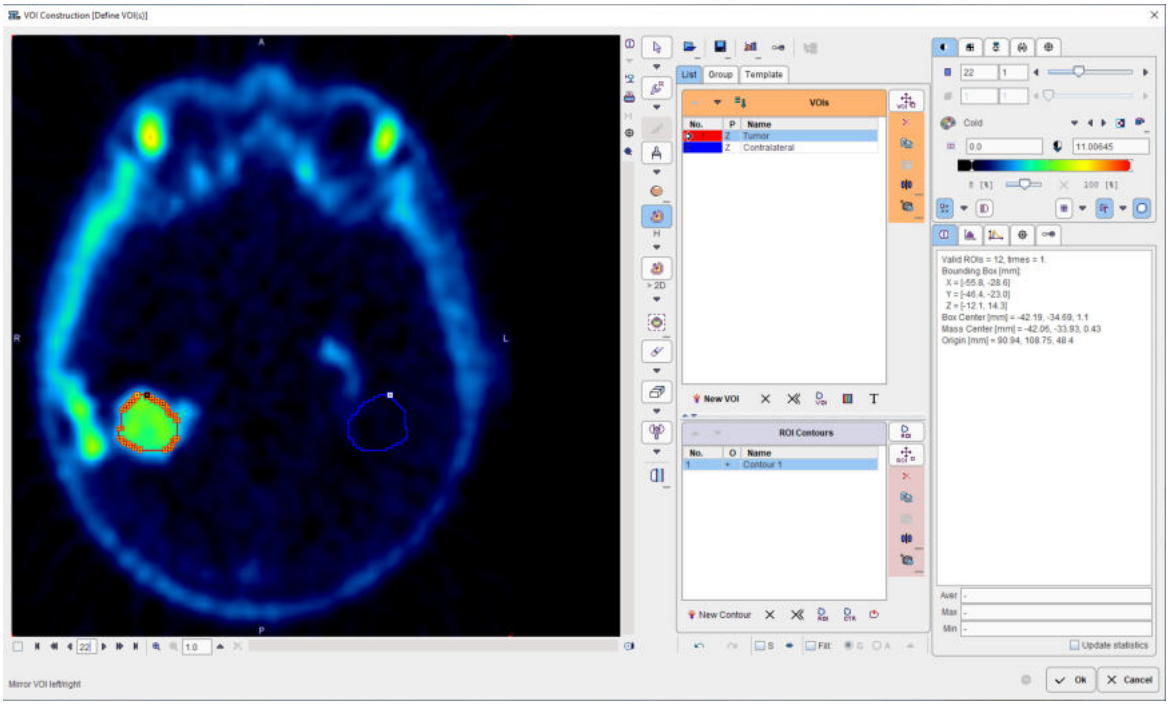


A dialog window is shown for configuring the 2 image series for which the result will be shown. If the series selection is not yet proper, it can be corrected before proceeding.

The plot requires VOIs to exist in the first series. If this is not the case, a dialog window appears:

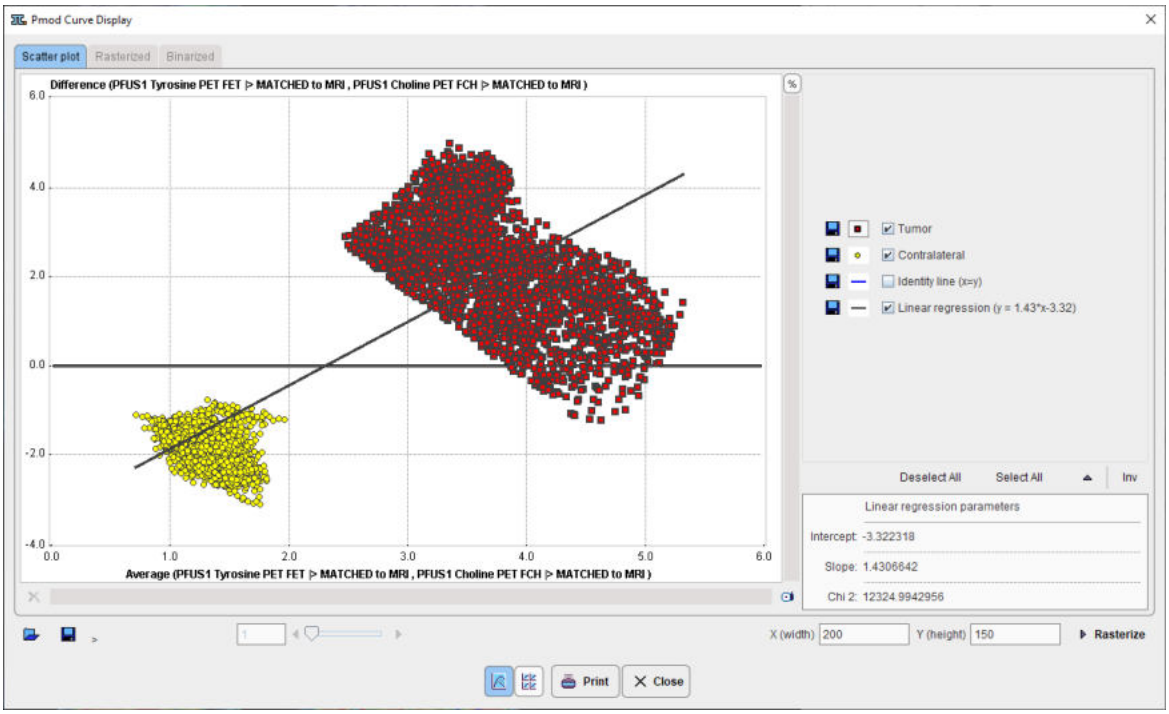


and **Start VOI Tool** used to enter VOI definition. The usual PMOD functionality can be applied for defining a list of VOIs.



After confirming with **Ok**, the plot is generated.

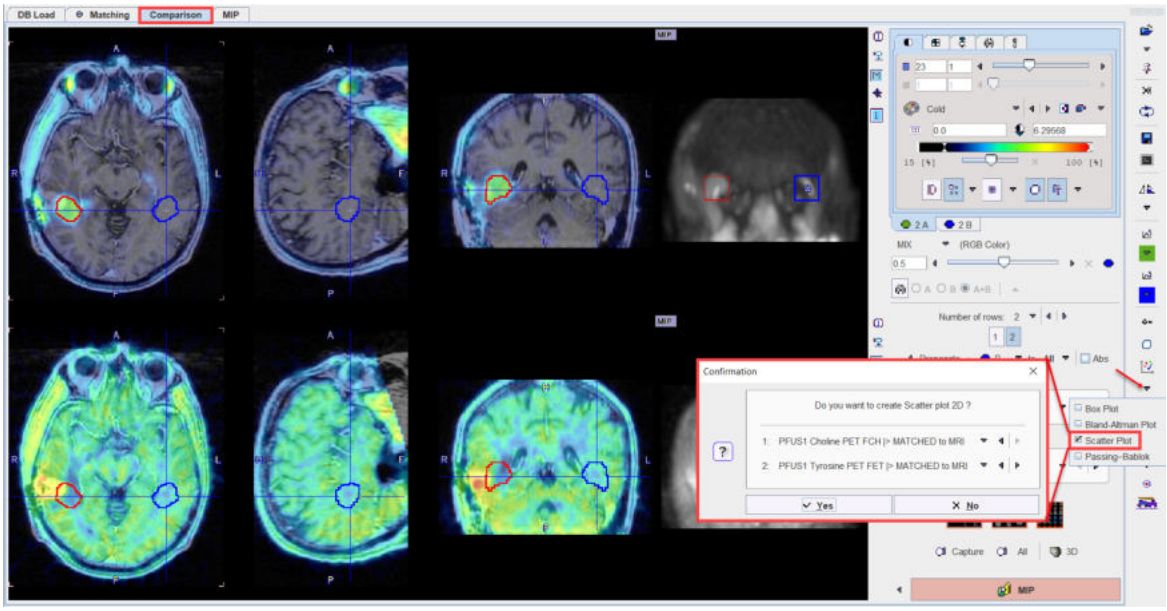
The **Bland-Altman** plot can be used for a quick method comparison. For each VOI pixel it calculates the average and the difference across the images in the upper two rows, and plots the result (average on horizontal axis, difference on vertical axis).



8.1.3 2D Scatter Plot Generation

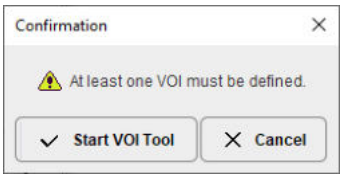
The scatter plot functionality allows investigating the values of pixels in two or three image series.

The best way to prepare the data of interest is arranging them on the **Comparison** main page in a two-row layout, and then activating the button in the lateral taskbar.

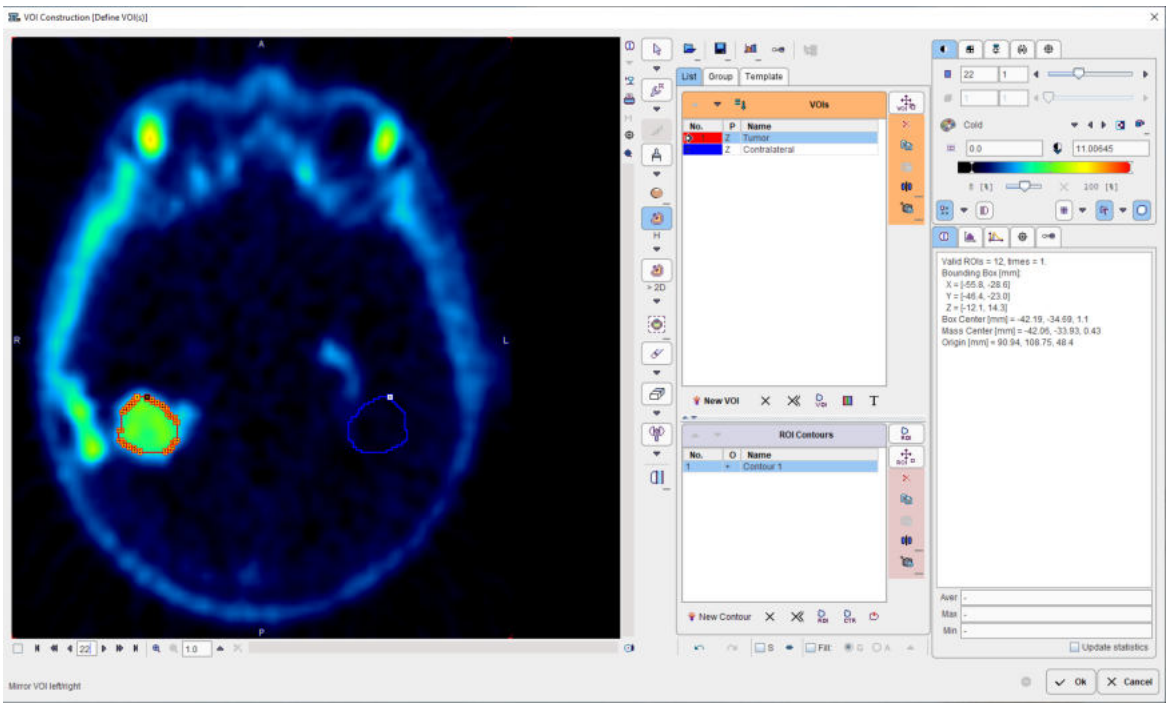


A dialog window is shown for configuring the 2 image series to compare. If the series selection is not yet proper, it can be corrected before proceeding.

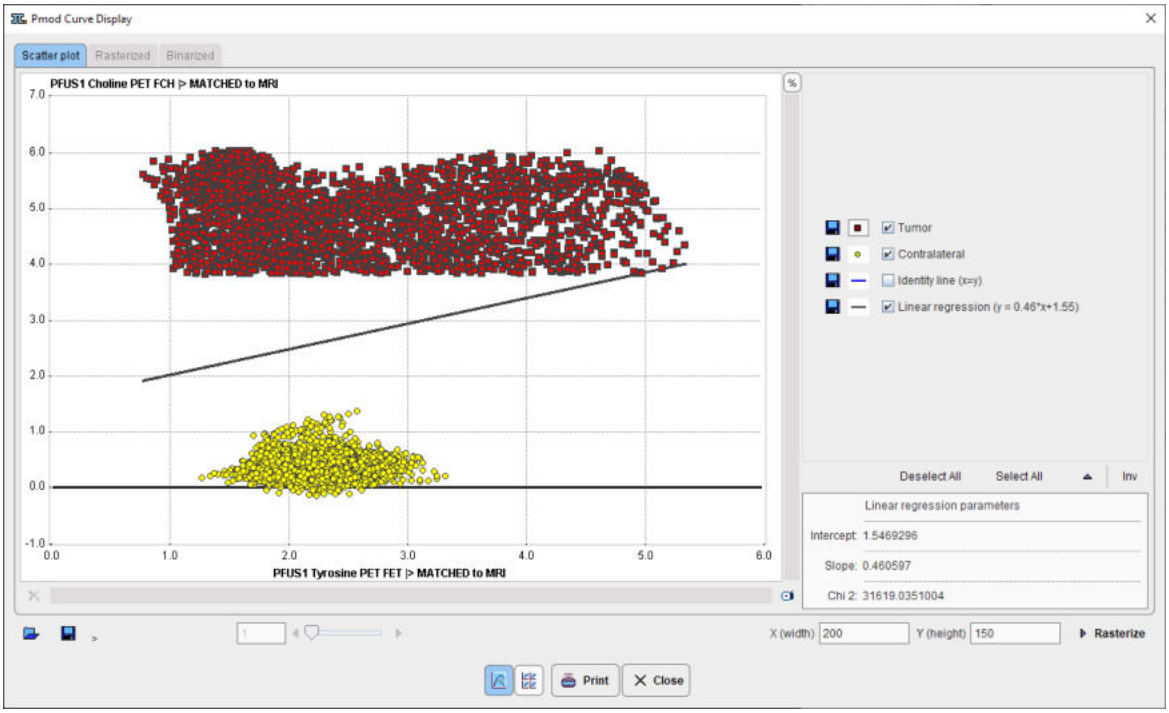
The scatter plot requires VOIs to exist in the first series. If this is not the case, a dialog window appears



and **Start VOI Tool** used to enter VOI definition. The usual PMOD functionality can be applied for defining a list of VOIs.



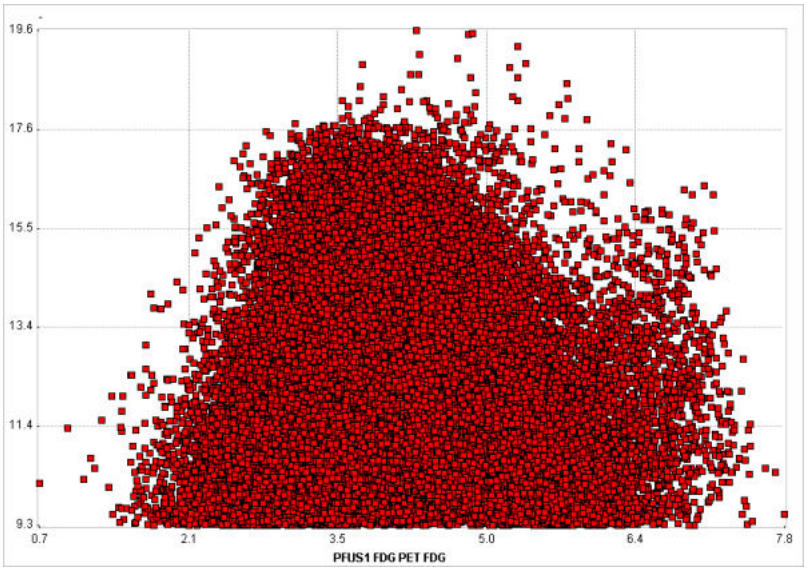
After confirming with **Ok**, the value of each VOI pixel is calculated in both series, and a scatter plot generated.



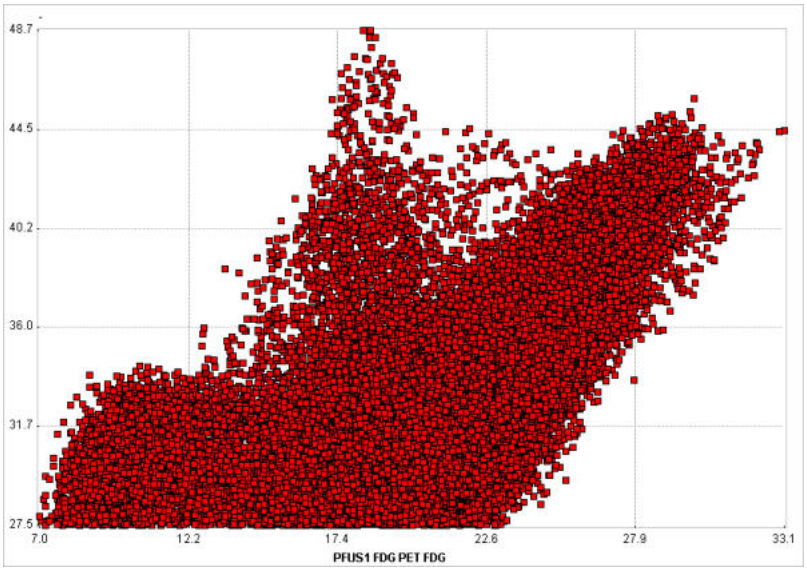
In this scatter plot, each point represents a VOI pixel. The color serves for labeling the different VOIs. The pixel value in the first series is plotted on the vertical axis, and the value in the second series in the horizontal axis. The actual data values can be exported by right-clicking into the plot and selecting **Value table of visible curves** or **Save multiple curves.**

8.1.3.1 Shape Analysis of Scatter Plot

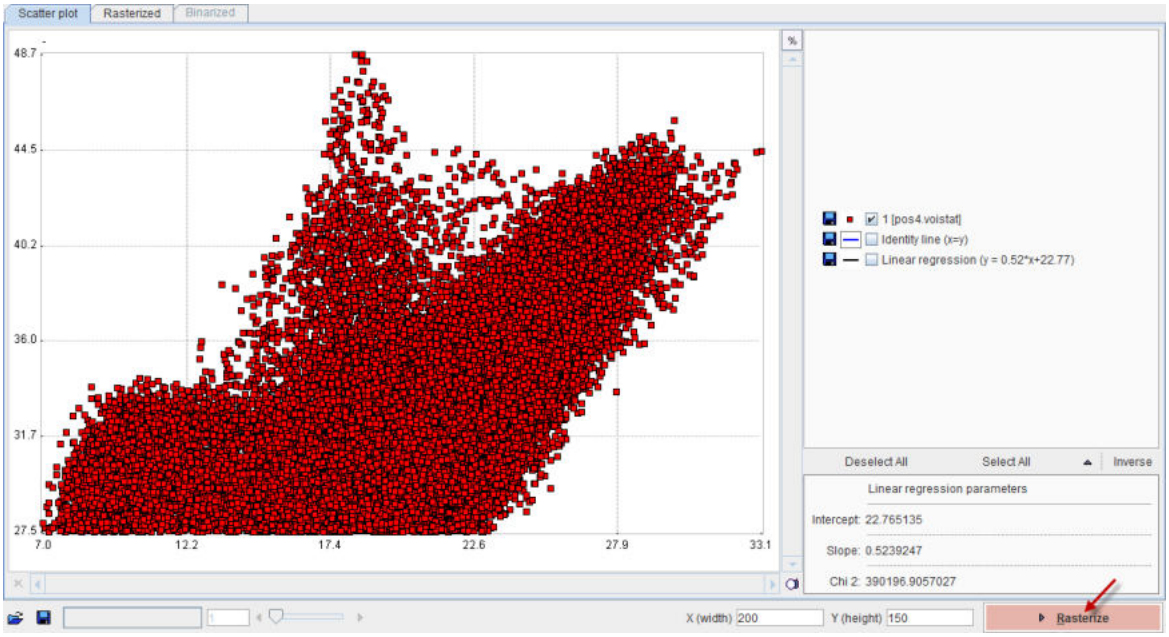
The shape of a scatter plot from a single VOI may contain diagnostic information. As an application example consider the case where the scatter plot of healthy controls is simple and symmetric



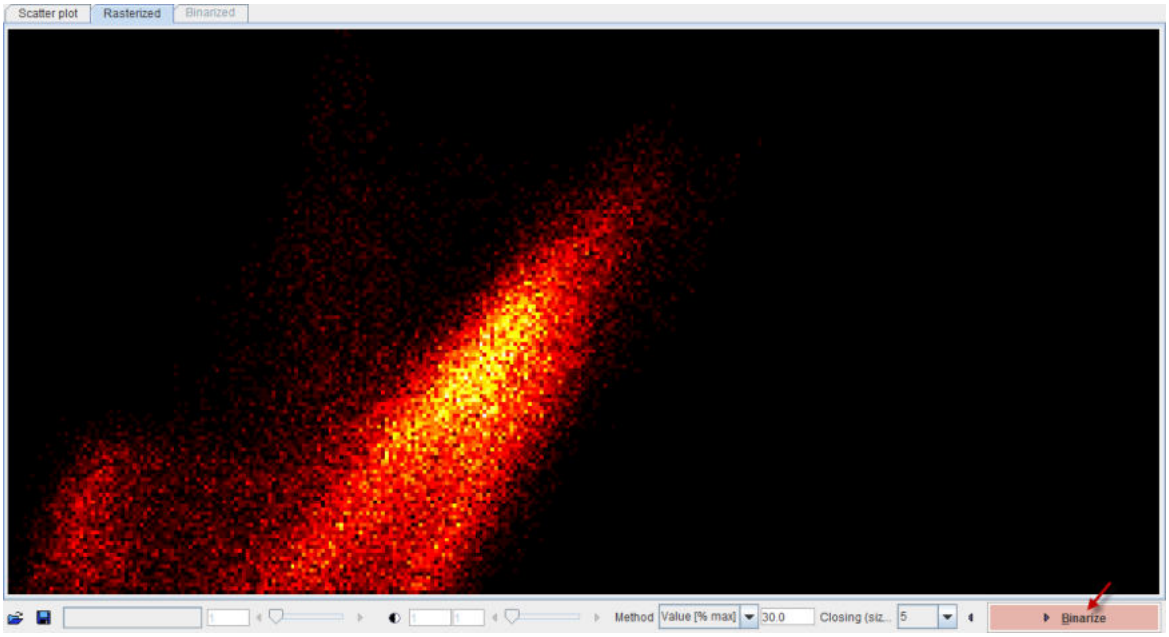
whereas subject scatter plots show distinct irregularities such as



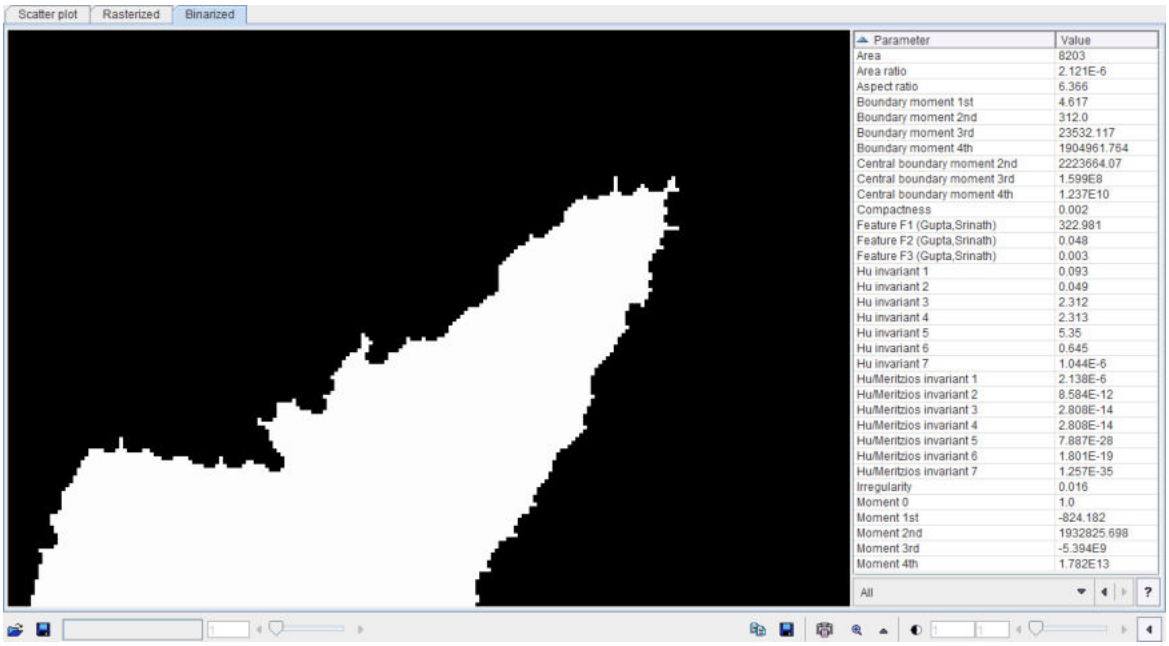
In order to perform the shape analysis, the scatter plot is converted into a binary image in two steps.



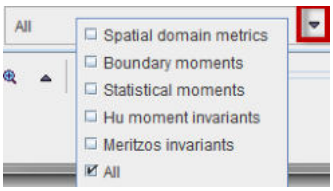
The **Rasterize** step creates an image from the scatter plot with dimensions **X(width)** and **Y(height)**. The value of each pixel in the generated image corresponds to the number of scatter points included in the pixel area.



A threshold value is applied to this image in order to convert it into a binary image. The threshold can be set by choosing one of the **Method** selections: **Optimal**, **Mean**, **Value [%max]**. In order to fill small inner holes, a **Closing** morphologic operation can optionally be set with structure sizes of **5** and **7** pixels. After applying **Binarize**, the result is shown on the **Binarized** panel. Note that the result will depend on the image dimensions as well as the threshold setting.



The binary structure who's shape is analyzed is shown to the left, and the resulting parameters in the list to the right. There are different metric types which can be selected from the list



Please refer to Haidekker [1] for details about the metrics.

As usually in PMOD, these metrics can be saved to the clipboard, to a file, and aggregated for later statistics using the

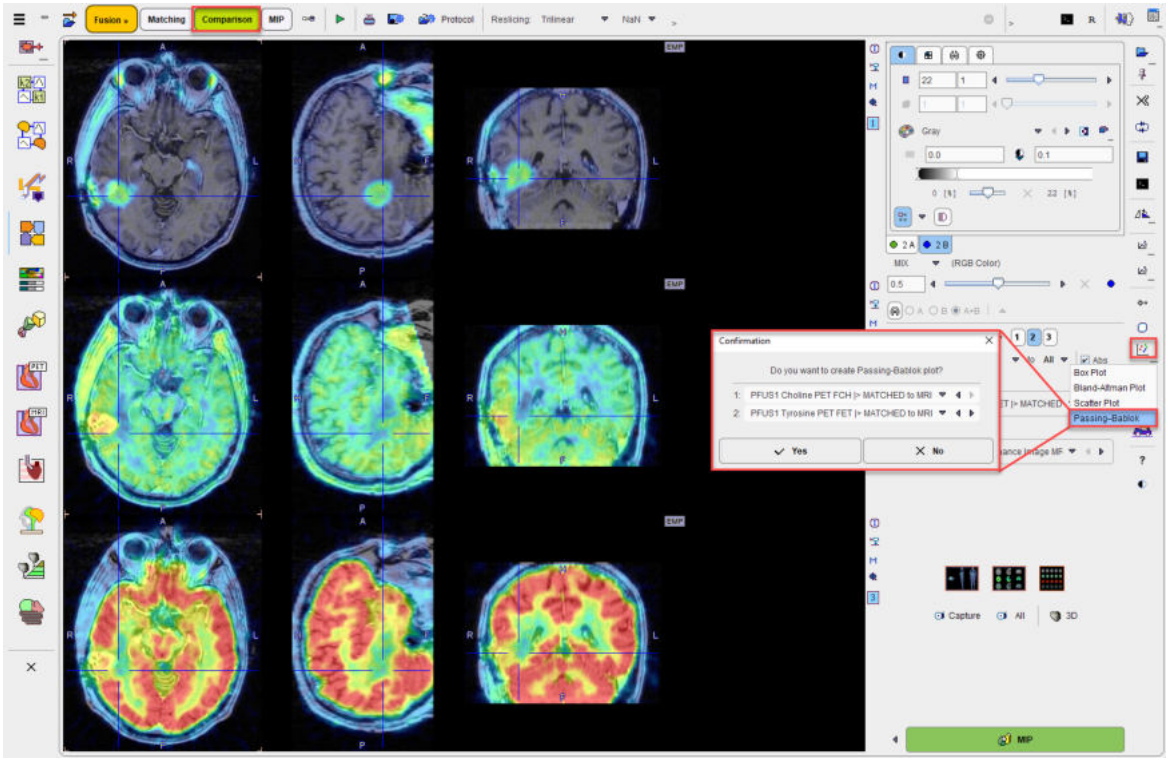
buttons. By comparing the classification results between populations of controls and subjects, it may be possible to develop a criterion of disease.

Reference

1. Haidekker, M. A. (2010) Shape Analysis, in Advanced Biomedical Image Analysis, John Wiley & Sons, Inc., Hoboken, NJ, USA.

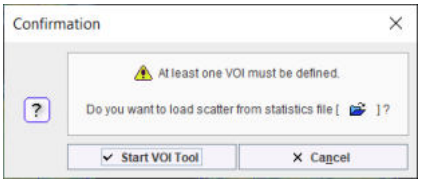
8.1.4 Passing-Bablok plot

The best way to prepare the data of interest is arranging them on the **Comparison** main page in a three-row layout, and then activating the **Passing-Bablok** selection with the black down arrow located under the  icon in the lateral taskbar:

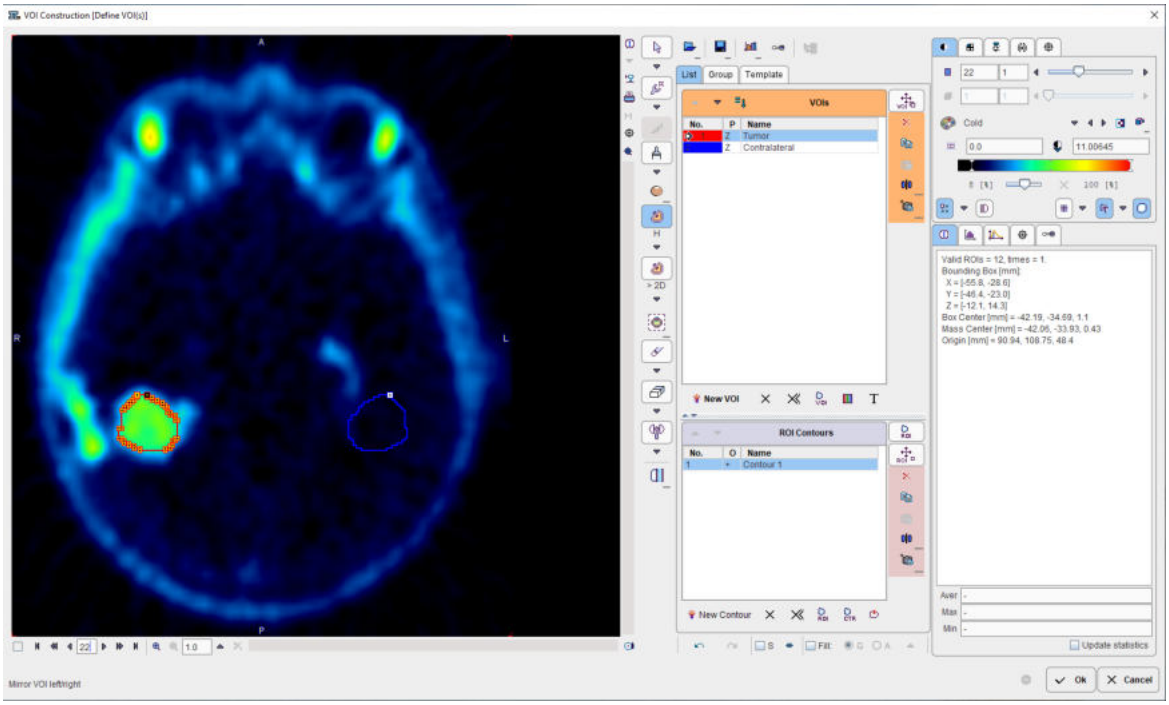


A dialog window is shown for configuring the 2 image series for which the result will be shown. If the series selection is not yet proper, it can be corrected before proceeding.

The plot requires VOIs to exist in the first series. If this is not the case, a dialog window appears:



and **Start VOI Tool** used to enter VOI definition. The usual PMOD functionality can be applied for defining a list of VOIs.

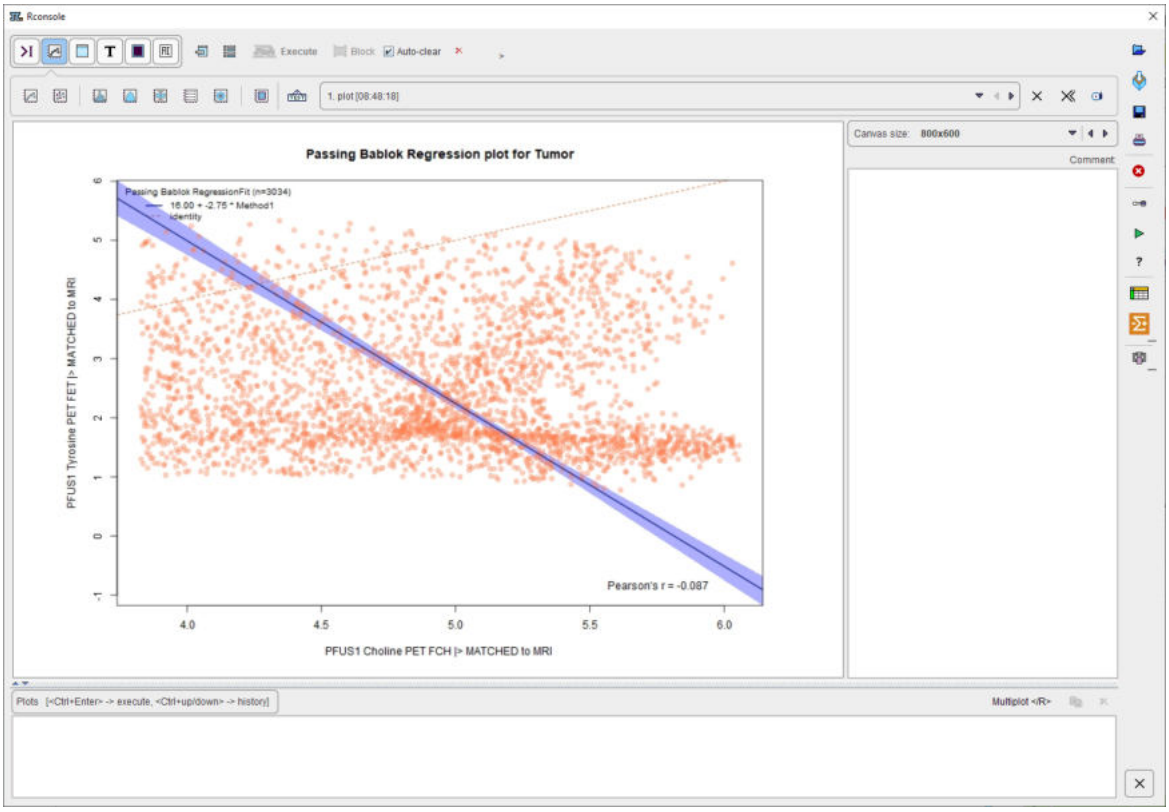


After confirming with **Ok**, the plot is calculated and generated in the PMOD-R interface.

The **Passing–Bablok** regression is a statistical method for non-parametric regression analysis suitable for method comparison studies. The Passing-Bablok procedure fits the parameters a and b of the linear equation y = a + b x using non-parametric methods. Please note that this procedure is valid only when a linear relationship exist between x and y variables.

The calculation for single VOI is time consuming. It is recommended to configure locally R if such regression analysis is of interest.

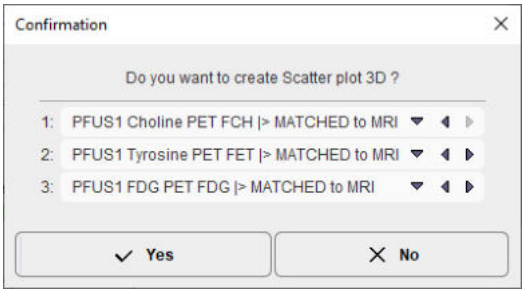
The graph shows the observations with the regression line (solid blue line), the confidence interval for the regression line (the violet interval) and the identity line (x=y, dotted red line):



Particularly, in the scatter plot above, each point represents a VOI pixel. The pixel value in the first series (x, Choline in the example above) is plotted on the horizontal axis, and the value in the second series in the vertical axis (y, Tyrosine in the example above).

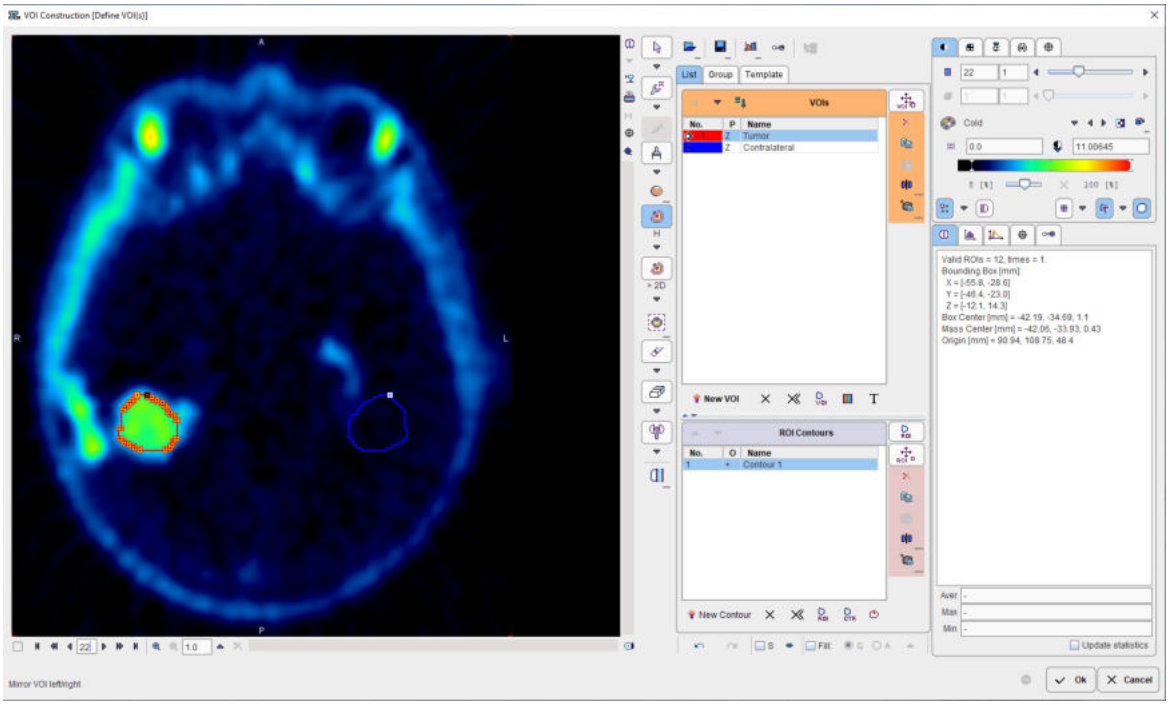
8.2 3D Scatter Plots (P3D Option Required)

The 3D scatter plot works along the lines of the 2D scatter plot 110 and requires 3 registered images. After activating from the lateral taskbar a dialog window appears for configuring the 3 image series to compare.

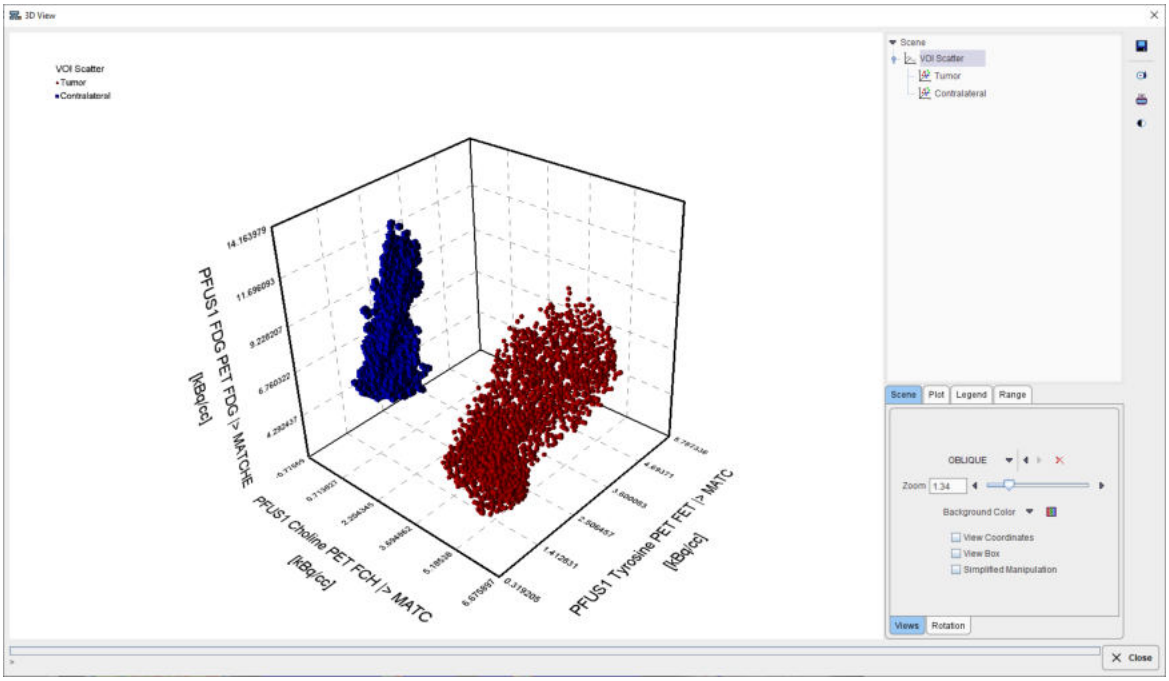


If the series selection is not yet proper, it can be corrected before proceeding. Note that the VOI of the first series will be used. If none exist, the following dialog window shows up.

Start VOI Tool starts VOI definition, and the usual functionality can be applied for defining a list of VOIs.



After confirming with **Ok**, the value of each VOI pixel is calculated in all three series, and a 3D plot generated.



In this scatter plot, each point represents a VOI pixel. The color serves for labeling the different VOIs. Please refer to the *P3D Users Guide* for information about the 3D rendering options.

The actual data values can be exported by the save button in the lateral taskbar.

9 References

1. P. A. Viola, Alignment by maximization of mutual information. Ph.D. thesis, Massachusetts Institute of Technology, 1995.
2. W.M. Wells, P. Viola, R. Kikinis, Multi-modal Volume Registration by Maximization of Mutual Information, Medical Robotics and Computer Assisted Surgery (1995), Wiley-Liss, New York, 155-162.
3. Maes F, Collignon A, Vandermeulen D, Marchal G, Suetens P. Multimodality image registration by maximization of mutual Information. In: Proceedings of the Workshop on Mathematical Methods in Biomedical Image Analysis, San Francisco, Calif., USA, 21–22 June 1996, pp 14–22.
4. P. Viola, W. Wells, Alignment by Maximization of Mutual Information, International Journal of Computer Vision (1997), 24:137-154
5. C. Studholme, D.L.G. Hill, D.J. Hawkes, An overlap invariant entropy measure of 3D medical image alignment, Pattern Recognition (1999), Vol.32:71-86.
6. J. Michael Fitzpatrick, Derek L.G. Hill, and Calvin R. Maurer, Jr., Image Registration, Chapter 8 p488-496, Handbook of Medical Imaging - Volume 2, Medical Image Processing and Analysis, Editors: M. Sonka, J.M. Fitzpatrick, SPIE PRESS, Bellingham, Washington 2000.
7. R.P. Woods, S.R. Cherry, J.C. Mazziotta, Rapid Automated Algorithm for Aligning and Reslicing PET Images, Journal of Computer Assisted Tomography (1992), 16(4):620-633.
8. R.P. Woods, J.C. Mazziotta, S.R. Cherry, MRI-PET Registration with Automated Algorithm, Journal of Computer Assisted Tomography (1993), Vol. 17(4):536-546.
9. K. J. Friston, S.Williams, R. Howard, R. S. Frackowiak, and R. Turner, Movement related effects in fMRI time- series, Magn. Reson. Med., vol. 35, pp. 346–355, 1996.
10. Rorden C, Bonilha L, Fridriksson J, Bender B, Karnath HO: Age-specific CT and MRI templates for spatial normalization. Neuroimage 2012, 61(4):957-965. DOI
11. Ashburner J, Friston KJ: Unified segmentation. Neuroimage 2005, 26(3):839-851. DOI
12. Ashburner J et al, SPM 8 Manual.
13. Valdes-Hernandez PA, Sumiyoshi A, Nonaka H, Haga R, Aubert-Vasquez E, Ogawa T, Iturria-Medina Y, Riera JJ, Kawashima R: An in vivo MRI Template Set for Morphometry, Tissue Segmentation, and fMRI Localization in Rats. Frontiers in neuroinformatics 2011, 5:26. DOI

10 PMOD Disclaimer

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

11 PMOD Copyright Notice

PMOD Technologies LLC
Industriestrasse 26
8117 Fällanden
Switzerland
support@pmod.com
http://www.pmod.com

This page is intentionally left blank.

- 2 -

- 3 -

- 6 -

- A -

- C -

- D -

- E -

- F -

- I -

- L -

- M -

- N -

- O -

- P -

- R -

- S -

- T -

- U -

- V -

- W -