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| SAMIT |
| Small Animal Molecular Imaging Toolbox |
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| The aim of this toolbox is to facilitate the construction of new tracer specific templates and the subsequent voxel-based analysis of small animal PET and SPECT brain images. |

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# Before to start

## Required Software

Matlab 7.1 or above (<http://www.mathworks.nl/products/matlab/>)

SPM8 or SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>)

SAMIT (<http://mic-umcg.github.io/samit/>)

## 

## Installation of SPM and SAMIT toolbox

1. Install SPM (<http://en.wikibooks.org/wiki/SPM/Download>)
2. Update SPM to its latest version. Type the following command line in Matlab: *spm\_update('upgrade')*
3. Extract the content of the downloaded SAMIT file. Rename the obtained folder as *samit* (e.g. from ‘samit-1.2’ to ‘samit’)
4. Move this *samit* folder inside the ‘toolbox’ directory located in your SPM installation.

## 

## Notes

This manual and the current version of SAMIT were designed to work only with ‘static’ (single frame) images

# SAMIT: Small Animal Imaging Toolbox

The aim of this toolbox is to facilitate the construction of new tracer-specific templates and the subsequent voxel-based and/or volume-of-interest based analysis of small animal PET and SPECT brain images.

After its installation, the *SAMIT* toolbox will be available from the ‘toolbox’ pull-down menu in the main SPM window.

## Select Atlas

The first step is to define the animal atlas. This step is needed to populate some default values and allow the interaction with the rest of the tool.

## Image pre-processing

### **Spatial normalization**

1. Reorient: This option allows to reorient images that were previously registered in PMOD or VINCI software packages, and to relocate the coordinates system of the image.
   1. PMOD2SPM: This option allows to reorient the images created in PMOD to the orientation expected in SPM.
   2. SPM2PMOD: In this case, the images in SPM space are reoriented into PMOD.
   3. VINCI: The NIfTI files created in VINCI present an error in the information stored in the file, which can cause problems with the voxel size and the location of the “origin” of the coordinates.
   4. Bregma: The location of the coordinates system in the image is a crucial step while handling the images. The zero or “origin” of this coordinates is usually located in bregma in the small animals.
   5. Center: If bregma is not properly defined in the atlas, the centre of the image can be used as an alternative reference.
2. Spatial registration. This section of the toolbox use the normalization process implemented in SPM8, and allows the selection of multiple images at once.

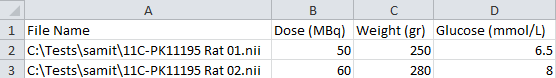
Note that this step is not necessary if the images were aligned to the template previously in another software (e.g. PMOD or VINCI)

* 1. Registration type (for further details see ‘spm\_affreg’ from SPM)
  2. Normalize multiple images

The best results will be obtained when the images and the reference template have similar dimensions and a good initial overlap.

### Normalize uptake

This section allows the normalization of the uptake in multiple images. The procedure has two steps:

1. Create table: The input file can be created manually or with the help of *‘Create table’* button. It will ask for the images that will be normalized, and it will generate a table. The content of this table can be filled with any text or spreadsheet editor, and saved as a tabulated file (\*.txt) or Excel file (\*.xls / \*.xlsx).
   1. First column: Full path and name of the image
   2. Second column: Activity of the injected tracer (MBq)
   3. Third column: Animal body weight (gr)
   4. Fourth column: glucose level in the blood (mmol/l).

*Note:* If the glucose was not measured, or it is not going to be used for the normalization, the whole column can be removed.

1. Construction of new images.
   1. Image units: Select the radioactivity units used when the image was created.

*Note:* By default, PMOD software store the images as kBq/cc

* 1. Normalization type:
     1. SUV (Standardized Uptake Values). The new image will have the suffix ‘*–SUV’*
     2. SUVglc (SUV corrected for blood glucose level). The new image will have the suffix ‘*–SUVglc’*
     3. SUV whole brain. The SUV will be corrected for the mean uptake value of the whole brain. The new image will have the suffix ‘*–SUVw’*
     4. IDg (Percentage of injected dose per gram). The new image will have the suffix ‘*–IDg’*
  2. Basal Glucose Plasma: reference value for the glucose level
  3. Create normalized images. The program will ask for the file created previously. The construction of the new images will be performed according to the parameters previously selected and the information probided in the table.

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### Apply whole brain mask

A new image will be created in the same location as the original one, removing the signal from the outside of the brain. The new images will have the prefix *‘m’*

## 

## VOI Analysis

The purpose of this function is to facilitate the extraction of the mean values from the images, for further volume of interest (VOI) analysis. The program will ask you for the image containing the VOIs, followed by the image(s) from which to calculate the values. The results will be saved in a tabulated text file (\*.txt) and in a Matlab file (\*.mat).

## Templates

The construction process to obtain tracer-specific PET and SPECT templates have been automatized as described in Vallez Garcia et al. 2015 ([doi](http://dx.doi.org/10.1371/journal.pone.0122363)). The program assumes that the images are already aligned between them in space and in its uptake. The first selected image will be defined as the reference, and all the other images will be aligned to this one in the first step of the template construction. It is recommended to check the images that will be used for the construction of the template with Check Image Registration (*Check Reg*) in SPM, to select the most appropriate reference image and to confirm that the images are correctly aligned between them.

The steps performed by the program are:

1. Spatial normalization of the selected images to the first one
2. Construction of a mean symmetrical image
3. Co-registration of the symmetrical image to the reference MRI template
4. Co-registration of the images used for the construction of the template with the parameters obtained in the previous step. This new images are saved with a prefix *‘r’* and can be used for the evaluation of the template
5. The final version of the template and its registration with the MRI is displayed

Several files will be created when the construction of the template is completed:

* *Name*\_coreg.mat The co-registration matrix obtained in step 3
* *Name*\_MRI\_Size.nii. The new template with the same dimensions and voxel size as the reference MRI
* *Name*\_Original\_Size.nii. The same template as before but preserving the original dimensions of the image. The co-registration matrix is stored in the file. This image can be used for the construction of other versions of the template with different dimension size.

The evaluation of the registration accuracy of the images to the template can be performed by selecting ‘Evaluation of the template’. The previously constructed template must be selected (\*\_MRI\_Size.nii), followed by the new version of the images used for the construction of the template.

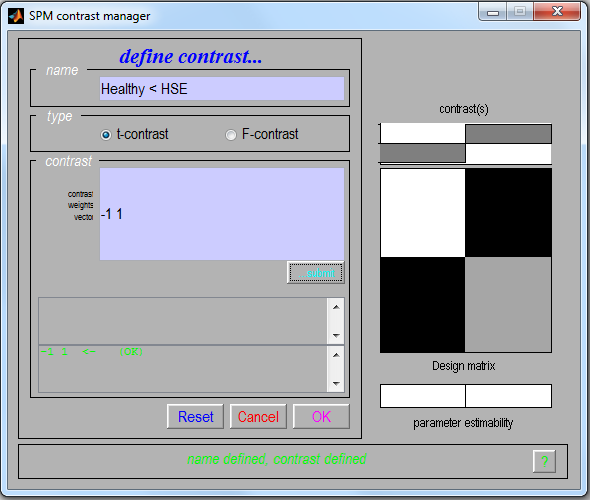
# Voxel-based Analysis in SPM

The voxel-based analysis is performed in a similar way as it is done in humans. The following steps are recommended:

1. Select the appropriate small animal atlas
2. Perform spatial normalization of the images to the reference template
3. Normalize the uptake signal
4. Apply the brain mask to all the images
5. Smooth the images (Implicit masking: Yes)
6. Define the statistical model
7. Estimate the model
8. Display the results

## Example

In this section, we will go through all the steps needed to perform the data analysis using a sample data set.

1. [Download the data set](https://unishare.nl/public.php?service=files&t=000dae353d05c0ebbf9251881cd3bfdd) an extract its content into a new folder. The dataset consist of PET brain images from male Wistar rats, and include one healthy group (n=13) and one intervention group with Herpes Encephalitis (HSE)
2. In Matlab, go to the folder where the data set has been extracted. It is always recommended to define a working folder, since several files may be generated in SPM and located by default in the current directory
3. Start SPM by typing in Matlab: *spm pet*
4. From the Toolbox menu in the SPM interface select ‘samit’. The SAMIT interface must remain open during the whole procedure otherwise the default human parameters will be used
5. Select the small animal atlas to be used. In this example select *Rat (Schwarz et. all 2006)*
6. Define the location of the ‘origin’. In this data set, the current location of the origin is in the centre of the image. This information can be checked using the ‘*Display’* option in SPM. In SAMIT interface, press the ‘*Bregma*’ button and select all the images located in the folders ‘HSE’ and ‘Healthy’. This process will relocate the origin in a position of the image close to where bregma is expected.
7. An automatic registration of the images was performed during its pre-processing. The alignment between images can be observed by selecting few images with *‘Check Reg’* in SPM interface. Nevertheless, we are going to perform again the automatic registration process on the images, for a better alignment with the template.
   1. Select the ‘almost rigid body’ option in the *‘Spatial Registration’* section
   2. Click in *‘Normalise multiple images’*
   3. Select all the images from the healthy and HSE folders
   4. Select the tracer-specific template located inside the SAMIT folder. The new images will be located in the same folder than the original ones but with the prefix ‘w’
8. Normalize uptake: Information about the injected dose and the weight of the animals is provided in the file ‘SUV table.txt’
   1. Click on *‘Create table’*
   2. Select all the files created recently with the prefix ‘w’
   3. Select a name for the new file that will be created
   4. Open the file with a text or spreadsheet editor. Fill the file information according to the one provided in ‘SUV table.txt’. No data was collected for the glucose level, so there is no need for the last column and it can be removed. Save the file as tabulated text file or Excel file
   5. Select the image units. In this study, the original images were acquired in *Bq/cc*
   6. Select the normalization procedure. For this example, we will use the *SUV (default)* option
   7. Click on *‘Create normalized images’*. The new images will be located in the same folder than the previous ones but including the suffix ‘-SUV’
9. Apply a mask to the whole brain. This step will avoid the influence of uptake outside the brain during the smoothing procedure, and it will avoid errors if global normalization is used during the statistical model
   1. Click on the *‘Brain Mask’* button
   2. Select the images to which apply the mask (i.e. those with the suffix –SUV)
   3. The new images will be located in the same folder than the previous ones with the prefix ‘m’
10. Apply smoothing to the image
    1. Click in *‘Smooth’* button from the SPM interface
    2. Select the images to smooth (i.e. those with the prefix ‘m’)
    3. Select the desired FWHM. For this example, we can leave the default value (recommend values are between 0.8 and 0.12 mm)
    4. Select ‘Implicit masking’ to ‘Yes’. This will avoid the smoothing to go outside the masked region
    5. Run the batch: Click on the icon of a green arrow or select *File > Run Batch*
    6. The new images will be located in the same folder than the previous ones with the prefix ‘s’. These are the final images to be used in the voxel-based analysis
11. Statistical model. In this section, we are going to define the statistical model, estimate the values and display the results
    1. Basic model. First, we have to define the model. In the present example, it is a simple two-sample t-test design (for further details, please refer to the SPM manual)
       1. Click on *‘Basic Models’* button in the main SPM window
       2. *Directory*: choose the directory were data will be written (e.g. ‘*workspace*’)
       3. Select the *‘Design’* as ‘Two-sample t-test’
       4. Select the Healthy group as ‘Group 1 scans’
       5. Select the HSE group as ‘Group 2 scans’
       6. Select ‘Explicit Mask’, using the whole brain mask located in SAMIT folder (i.e. Schwarz\_intracranialMask.nii)
    2. Estimate the model
       1. In the menu of the Batch Editor select *SPM > Stats > Model estimation*
       2. Select the new module ‘Model estimation’ from the left section of the Batch Editor
       3. Click on *‘Dependency’* button, located in the lower right section
       4. Select the Factorial design option
       5. Save the batch for future modifications
    3. Run the batch: Click on the icon of a green arrow or select *File > Run Batch*
    4. Show the results
       1. Click on *‘Results’* button in the main SPM window
       2. Select the *“SPM.mat”* file located in the directory selected previously during the specification of the model
       3. Click on *‘Define a new contrast’*
          1. In the name type “Healthy < HSE”
          2. Type: t-contrast
          3. Contrast: -1 1
          4. Click on *‘OK’*
          5. Select the contrast and press *‘Done’*
       4. Apply masking: *none*
       5. Title for comparison: leave the current title and press enter
       6. Choose the p-value adjustment (FWE or uncorrected). In this example select *none*
       7. Choose the p-value. In this example type *0.001*
       8. Extent threshold (voxels). In this example type *200*
    5. Results are presented in a maximum intensity projection (MIP) display on a glass rat brain. Since the MRI rat template was spatially aligned with the Paxinos atlas the resulting coordinates are equivalent to those in the atlas
    6. To visualize the results on the MRI go to *Display > Overlays… > Section* and choose the rat MRI template located in the SAMIT folder

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## Flexible Factorial Design: simplified method to organize the data

Here it is described how to simplify the input of data in the flexible factorial design. For example, assuming we have:

* Factor 1: *subject* (4 animals)
* Factor 2: *group* (2 groups: ‘Control’ and ‘Intervention’)
* Factor 3: *condition* (3 scans performed at different time points)

1. Create a table with the following data (e.g. in Excel)
   1. First column: location of the image file
   2. Second column: Index number of the image
   3. Third column: Index number of the animal
   4. Fourth column: Index number of the group
   5. Fifth column: Index number of the condition

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| --- | --- | --- | --- | --- |
| **File** | **Index** | **Animal** | **Group** | **Condition** |
| D:\Matlab\Test\Rat01 Control Scan1.nii | 1 | 1 | 1 | 1 |
| D:\Matlab\Test\Rat01 Control Scan2.nii | 2 | 1 | 1 | 2 |
| D:\Matlab\Test\Rat01 Control Scan3.nii | 3 | 1 | 1 | 3 |
| D:\Matlab\Test\Rat02 Control Scan1.nii | 4 | 2 | 1 | 1 |
| D:\Matlab\Test\Rat02 Control Scan2.nii | 5 | 2 | 1 | 2 |
| D:\Matlab\Test\Rat02 Control Scan3.nii | 6 | 2 | 1 | 3 |
| D:\Matlab\Test\Rat03 Intervention Scan1.nii | 7 | 3 | 2 | 1 |
| D:\Matlab\Test\Rat03 Intervention Scan2.nii | 8 | 3 | 2 | 2 |
| D:\Matlab\Test\Rat03 Intervention Scan3.nii | 9 | 3 | 2 | 3 |
| D:\Matlab\Test\Rat04 Intervention Scan1.nii | 10 | 4 | 2 | 1 |
| D:\Matlab\Test\Rat04 Intervention Scan2.nii | 11 | 4 | 2 | 2 |
| D:\Matlab\Test\Rat04 Intervention Scan3.nii | 12 | 4 | 2 | 3 |

1. Then, in the Batch Editor of SPM, under the *Factorial design specification* select *“Design > Flexible Factorial > Specify Subjects or all Scans & Factors >* ***Specify all****”* (by default “Subjects” is used).
2. Double click in *Scans*. In the new window do click on the button located on the left side “**Ed**”. Copy & Paste here the first column of the table, with the address of the image files. Accept & Done.
3. Double click in *Factor matrix*. Copy & Paste here the last four columns of the table.

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## Atlases

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## SAMIT and tracer-specific templates

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* David Vállez García, Andreas Otte, Rudi A.J.O. Dierckx, and Janine Doorduin. 2016. “Three Month Follow-Up of Rat Mild Traumatic Brain Injury: A Combined [ 18 F]FDG and [ 11 C]PK11195 Positron Emission Study.” *Journal of Neurotrauma* 33 (20):1855–65. <https://doi.org/10.1089/neu.2015.4230>.
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