

# 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 in the main SPM window.

## Select Atlas

The first step is to define the animal species by selecting the atlas. This step is needed to preload some default values.

## Image pre-processing

### Spatial normalization

1. Reset orientation: This function removes any previous transformation stored in the image, and prepares the image for further processing.
2. Coordinates of the "origin": 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. If bregma is not properly defined in the atlas, the center of the image is an alternative reference.
3. Spatial registration. This section of the toolbox use the normalization process implemented in SPM8, and allows the selection of multiple images at once.
  - a. Registration type (for further details see 'spm\_affreg' from SPM)
  - b. Normalize multiple images

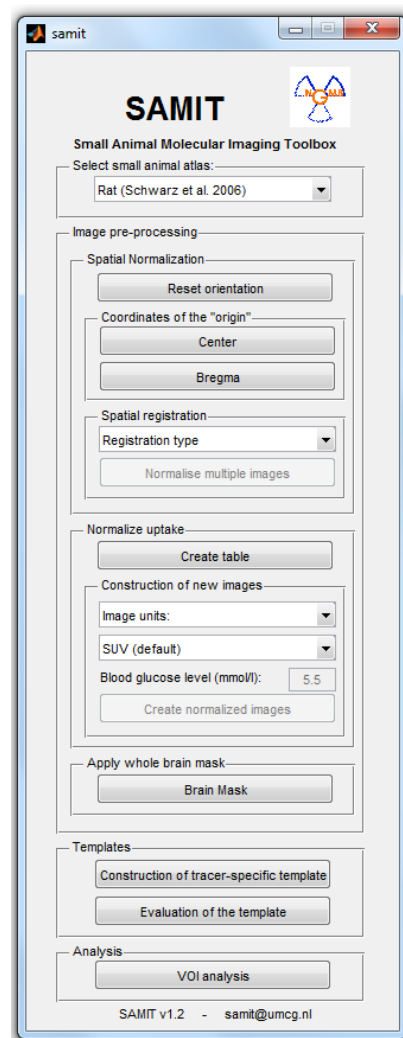
The best results will be obtained when the images and the target template have similar dimensions. Take in consideration that initial alignment between the images must be within about 4 cm and about 15 degrees in order for SPM to find the optimal solution.

### Normalize uptake

This section allows the normalization of the uptake in multiple images at once. This 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 to be normalized and it will generate a template file. The content of this file can be filled with any text or spreadsheet editor, and saved as tabulated file (\*.txt) or Excel file (\*.xls / \*.xlsx).
  - a. First column: Full path and name of the image
  - b. Second column: Activity of the injected tracer (MBq)
  - c. Third column: Animal body weight (gr)
  - d. Fourth column: glucose level in blood (mmol/l)
2. Construction of new images.
  - a. Image units: Select the units of the uptake represented in the image.
  - b. Normalization type:
    - i. SUV (Standardized Uptake Values). Default option. The new image will have the suffix '-SUV'
    - ii. SUVglc (SUV corrected for blood glucose level). The new image will have the suffix '-SUVglc'
    - iii. 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'
    - iv. IDg (Percentage of injected dose per gram). The new image will have the suffix '-IDg'
  - c. Basal Glucose Plasma: This is the reference value for the glucose level
  - d. Create normalized images. The program will ask for the file containing the table with all the information needed for the construction of the new images according to the parameters previously selected.

	A	B	C	D
1	File Name	Dose (MBq)	Weight (gr)	Glucose (mmol/L)
2	C:\Tests\samit\11C-PK11195 Rat 01.nii	50	250	6.5
3	C:\Tests\samit\11C-PK11195 Rat 02.nii	60	280	8



$$\text{SUV} = \frac{\text{Uptake in the Image}}{\text{Injected dose/body weight}}$$

$$\text{IDg} = \frac{\text{Uptake in the Image}}{\text{Injected dose}} \times 100$$

$$\text{SUV}_{\text{glc}} = \text{SUV} \times \frac{\text{Blood glucose level}}{\text{Reference glucose level}}$$

$$\text{SUV}_{\text{w}} = \frac{\text{SUV}}{\text{Whole brain uptake}}$$

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'

## Templates

The construction process to obtain tracer-specific PET and SPECT templates has been automatized as described in Vallez Garcia et al. 2015 ([doi](#)). 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 the 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.

## 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).

## 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:

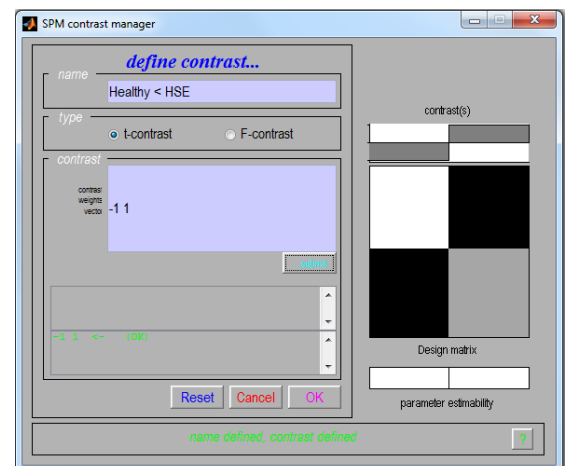
1. Select the appropriate small animal atlas
2. Perform spatial normalization of the images to the reference template
3. Normalize the uptake signal
4. Apply the brain mask to all the images
5. Smooth the images (Implicit masking : Yes)
6. Define the statistical model
7. Estimate the model
8. Display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

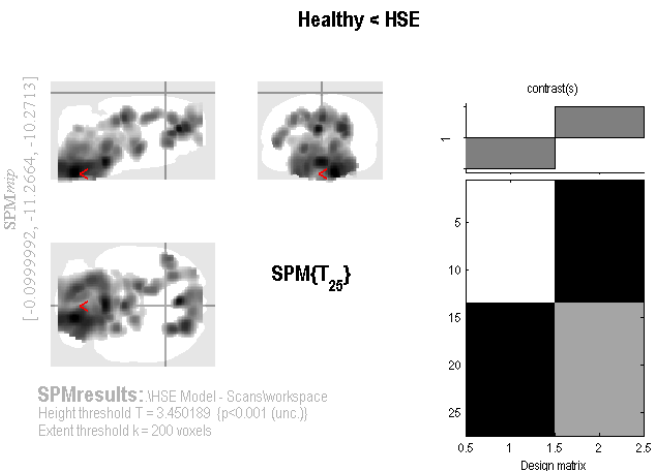
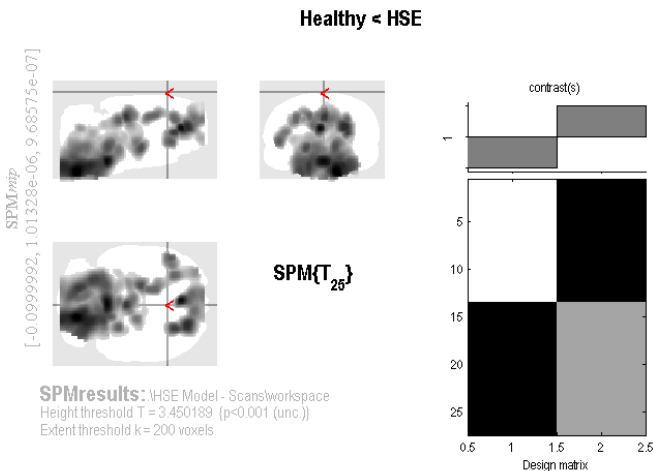
### Example

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](#) and extract its content into a new folder. The data set consists 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. al 2006)*
6. Define the location of the 'origin'. In this data set the current location of the origin is in the center of the image. This information can be checked using the 'Display' option in SPM. In SAMIT interface, press in 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.
  - a. Select the 'almost rigid body' option in the 'Spatial Registration' section
  - b. Click in 'Normalise multiple images'
  - c. Select all the images from the healthy and HSE folders
  - d. 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'
  - a. Click on 'Create table'
  - b. Select all the files created recently with the prefix 'w'
  - c. Select a name for the new file that will be created
  - d. 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 as Excel file
  - e. Select the image units. In this study, the original images were acquired in *Bq/cc*
  - f. Select the normalization procedure. For this example we will use the *SUV (default)* option
  - g. Click in '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
  - a. Click in the 'Brain Mask' button
  - b. Select the images to which apply the mask (i.e. those with the suffix -SUV)
  - c. The new images will be located in the same folder than the previous ones with the prefix 'm'
10. Apply smoothing to the image
  - a. Click in 'Smooth' button from the SPM interface
  - b. Select the images to smooth (i.e. those with the prefix 'm')
  - c. Select the desired FWHM. For this example we can leave the default value (recommended values are between 0.8 and 0.12 mm)

- d. Select 'Implicit masking' to 'Yes'. This will avoid the smoothing to go outside the masked region
  - e. Run the batch: Click on the icon of a green arrow or select *File > Run Batch*
  - f. 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
- a. 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)
    - i. Click on '*Basic Models*' button in the main SPM window
    - ii. *Directory*: choose the directory where data will be written (e.g. '*workspace*')
    - iii. Select the '*Design*' as 'Two-sample t-test'
    - iv. Select the Healthy group as 'Group 1 scans'
    - v. Select the HSE group as 'Group 2 scans'
    - vi. Select 'Explicit Mask', using the whole brain mask located in SAMIT folder (i.e. Schwarz\_intracranialMask.nii)
  - b. Estimate the model
    - i. In the menu of the Batch Editor select *SPM > Stats > Model estimation*
    - ii. Select the new module 'Model estimation' from the left section of the Batch Editor
    - iii. Click on '*Dependency*' button, located in the lower right section
    - iv. Select the Factorial design option
    - v. Save the batch for future modifications
  - c. Run the batch: Click on the icon of a green arrow or select *File > Run Batch*
  - d. Show the results
    - i. Click on '*Results*' button in the main SPM window
    - ii. Select the "*SPM.mat*" file located in the directory selected previously during the specification of the model
    - iii. 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'
    - iv. Apply masking: *none*
    - v. Title for comparison: leave the current title and press enter
    - vi. Choose the p value adjustment (FEW or uncorrected). In this example select *none*
    - vii. Choose the p value. In this example type *0.001*
    - viii. Extent threshold (voxels). In this example type *200*
  - e. 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
  - f. To visualize the results over the MRI go to *Display > Overlays... > Section* and choose the rat MRI template located in the SAMIT folder





Statistics: *p-values adjusted for search volume*

set-level		cluster-level				peak-level							mm mm mm		
P	C	P <sub>FWE-clust</sub>	q <sub>FDR-clust</sub>	k <sub>E</sub>	P <sub>unc-clust</sub>	P <sub>FWE-peak</sub>	q <sub>FDR-peak</sub>	T	(Z)	P <sub>unc-peak</sub>					
0.066	2	0.000	0.000	33600	0.000	0.017	0.314	5.45	4.39	0.000	1.7	-12.2	-10.1		
						0.052	0.314	4.90	4.06	0.000	0.1	-5.6	-9.1		
		0.000	0.000	8416	0.000	0.023	0.314	5.30	4.30	0.000	-0.5	1.6	-4.1		

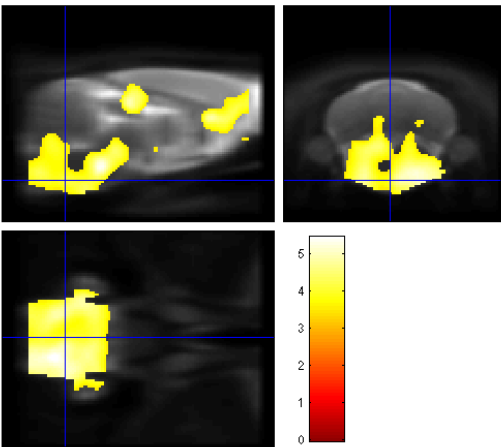


table shows 3 local maxima more than 6.0mm apart

Height threshold: T = 3.45, p = 0.001 (0.583)  
Extent threshold: k = 200 voxels, p = 0.477 (0.341)  
Expected voxels per cluster, <k> = 417.119  
Expected number of clusters, <c> = 0.42  
FWEp: 4.914, FDRp: Inf, FWEc: 8416, FDRc: 8416

Degrees of freedom = [1.0, 25.0]  
FWHM = 3.3 3.4 3.4 mm mm mm; 16.4 17.2 16.8 (voxels)  
Volume: 1875 = 234415 voxels = 46.0 resels  
Voxel size: 0.2 0.2 0.2 mm mm mm; (resel = 4751.62 voxels)



## Flexible Factorial Design: simplified method to input 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 in different time points)
1. Create a table with the following data (e.g. in Excel)
    - a. First column: location of the image file
    - b. Second column: Index number of the image
    - c. Third column: Index number of the animal
    - d. Fourth column: Index number of the group
    - e. Fifth column: Index number of the condition

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

2. 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).
3. Double click in *Scans*. In the new window do click on the button located at the left side "**Ed**". Copy & Paste here the first column of the table, with the address of the image files. Accept & Done.
4. Double click in *Factor matrix*. Copy & Paste here the last four columns of the table.

## References

### Atlases

- Ma et al., 2005. A three-dimensional digital atlas database of the adult C57BL/6J mouse brain by magnetic resonance microscopy. *Neuroscience*, 135(4) doi: [10.1016/j.neuroscience.2005.07.014](https://doi.org/10.1016/j.neuroscience.2005.07.014)
- Ma, Y. et al., 2008. In Vivo 3D Digital Atlas Database of the Adult C57BL/6J Mouse Brain by Magnetic Resonance Microscopy. *Frontiers in neuroanatomy*, 2(April) doi: [10.3389/neuro.05.001.2008](https://doi.org/10.3389/neuro.05.001.2008)
- Schwarz et al., 2006 A stereotaxic MRI template set for the rat brain with tissue class distribution maps and co-registered anatomical atlas: application to pharmacological MRI. *NeuroImage* 32(2) doi: [10.1016/j.neuroimage.2006.04.214](https://doi.org/10.1016/j.neuroimage.2006.04.214)

### SAMIT and tracer-specific templates

- Vázquez García et al., 2015. A Standardized Method for the Construction of Tracer Specific PET and SPECT Rat Brain Templates: Validation and Implementation of a Toolbox. *PLoS One*, 10(3) doi: [10.1371/journal.pone.0122363](https://doi.org/10.1371/journal.pone.0122363)
- Casteels et al., 2013. Construction and evaluation of quantitative small-animal PET probabilistic atlases for [18F]FDG and [18F]FECT functional mapping of the mouse brain. *PLoS One*, 8(6) doi: [10.1371/journal.pone.0065286](https://doi.org/10.1371/journal.pone.0065286)
- Casteels et al., 2006. Construction and evaluation of multitracer small-animal PET probabilistic atlases for voxel-based functional mapping of the rat brain. *J Nucl Med*, 47(11) ([link](#))

### List of Publications that used SAMIT

- Vázquez García et al., 2015. Evaluation of [11C]CB184 for imaging and quantification of TSPO overexpression in a rat model of herpes encephalitis. *Eur J Nucl Med Mol Imaging*. 2015 doi: [10.1007/s00259-015-3021-x](https://doi.org/10.1007/s00259-015-3021-x)