**Instructions for running ISAS SPECT in SPM12**

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*Before you start: make sure your SPECT images are in Nifti format and the image spaces are more or less the same. If necessary, set the origins of the SPECT images to the anterior commissure by using the Set Origin function in SPM Display.*

* Open Matlab and start SPM PET.
* Navigate to Utils -> CD and select the folder for results
* Navigate to Batch and open ISAS\_SPM\_part1.mat

*This part does realignment and normalization.*

* *In the Realignment step, click on Sessions and then Specify. Select all interictal and ictal SPECT scans in Nifti format.*
* *In the Old Normalise step, click on Template Image (under Estimate Options) and navigate to the SPECT template. Usually this is stored in your SPM12 folder in: spm12\toolbox\OldNorm\SPECT.nii.*
* Run the script
* Open ISAS\_SPM\_part2.mat in the Batch Editor

*This script does masking, smoothing and statistics.*

* *In the first Image Calculator step, click on Input and then Specify. Select the normalized* ***interictal*** *SPECT (wr\_interictal.nii) and the mask image (spm12\tpm\mask\_ICV.nii).*
* *In the second Image Calculator step, on Input and then Specify and select the normalized* ***ictal*** *SPECT (wr\_ictal.nii) and the mask image (spm12\tpm\mask\_ICV.nii).*
* *In the Factorial Design Specification step, click on Directory, Specify and select the folder to save the results.*
* *Still in the Factorial Design Specification step, navigate to Specify Subjects or all Scans & Factors. The first subject is your subject, these images are already in the editor. For subject 2 to 15, click on Scans, Specify, and select the two processed scans of one subject of the control database. So for the first this would be swrHN001\_D1.img and swrHN001\_D2.img. (Hint: after doing this once, save the batch script with links to your control database location for future use.)*
* Run the script
* Results are saved in PDFs: spm\_date\_001.pdf (hyperperfusion) en spm\_date\_002.pdf (hypoperfusion), and in Nifti files: spmT\_0001\_hyperhypo.nii (hyperperfusion) and spmT\_0002\_hyperhypo.nii (hypoperfusion). Note, these Nifis are in MNI space!
* You can also visualize the results in SPM. In the main menu, navigate to Results and select the SPM.mat that you just generated.
* The contrast manager opens up with a Hyper and a Hypo option. Select Hyper to see the areas of hyperperfusion in the ictal SPECT compared to the interictal SPECT. (Hypo shows hypoperfusion areas in the ictal SPECT.)
* Apply masking: choose None
* P-value adjustment to control: choose FWE (you can be more liberal later on)
* Threshold: 0.05
* Extent threshold: 0
* Now you can navigate through the results. Add an MRI scan by using ‘Overlays’. Since the results are in MNI space, you can use the MNI standard brain as overlay (spm12\canonical\single\_subj\_T1.nii).
* You can change the threshold to uncorrected p<0.001 by navigating to Contrast, Significance level.
* If you want to ‘export’ your results; use them in other software or combine them with other scans; you need to translate back from MNI space to patient space. This is done in ISAS\_SPM\_part3.mat
* *First, co-register the T1 MRI of the patient to the SPECT. As Reference Image, select the mean\_xxx.nii image generated in part 1. As Source Image, select your patients T1 MRI.*
* *In the Segment step, some tissues are segmented to generate the transformation matrix. Navigate to Tissue Probability Map and select spm12\tpm\TPM.nii.*
* *In the Normalise: Write step, navigate to Images to Write and select the spmT\_00XX\_hyperhypo.nii files you want to denormalise.*
* Run the script
* The denormalised file has a preceeding i- in the filename. **Check the registration** by using the SPM Display tool. Select the patient T1 MRI. In the Graphics window, navigate to Add Overlay and select the denormalised file. In the drop down menu click on Red Blobs. The statistical result is now overlaid on the MRI. This should be the same as the saved PDF. The denormalised Nifti file can be used in MRIcron and other software.