UNIVERSITÄTS KLINIKUM TÜBINGEN

Hertie-Institut für klinische Hirnforschung

Handout to the hands-on sessions

Practical demonstration I-V

(lesion delineation, spatial normalisation and statistical lesion analysis)

B. de Haan & D. Wiesen

Given in the framework of the Lesion Analysis Workshop

Taking place on 12th & 13th April 2019

Center of Neurology, Division of Neuropsychology
Tübingen, Germany

1. How to delineate the lesion in each individual patient brain with SPM12 and the Clusterize Toolbox:

SPM can be downloaded from http://www.fil.ion.ucl.ac.uk/spm/. Version "SPM12" runs from Matlab versions 7.4 (R2007a) to 9.5 (R2018b). It is not recommended to install SPM in Matlab"s toolbox folder.

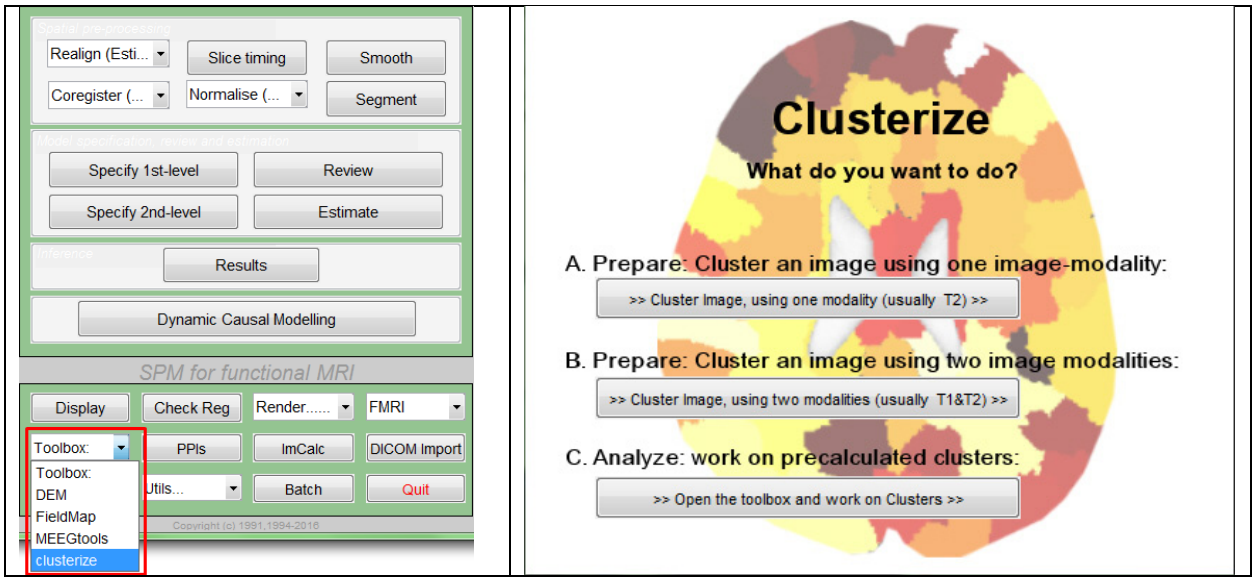
The Clusterize Toolbox can be downloaded from http://www.medizin.uni-tuebingen.de/kinder/en/research/neuroimaging/software/. Version 1.0 only works with MRI data, version 1.0beta works with both MRI and CT data. Simply open the .zip file of choice and extract its contents into a subdirectory "clusterize" to SPM's toolbox directory.

The following 2 papers provide background to the Clusterize Toolbox:

- Clas P, Groeschel S, Wilke M (2012). A semi-automatic algorithm for determining the demyelination load in metachromatic leukodystrophy. *Academic Radiology* 19: 26-34.
- de Haan B, Clas P, Juenger H, Wilke M, Karnath H-O (2015). Fast semi-automated lesion demarcation in stroke. *NeuroImage: Clinical* 9: 69-74.

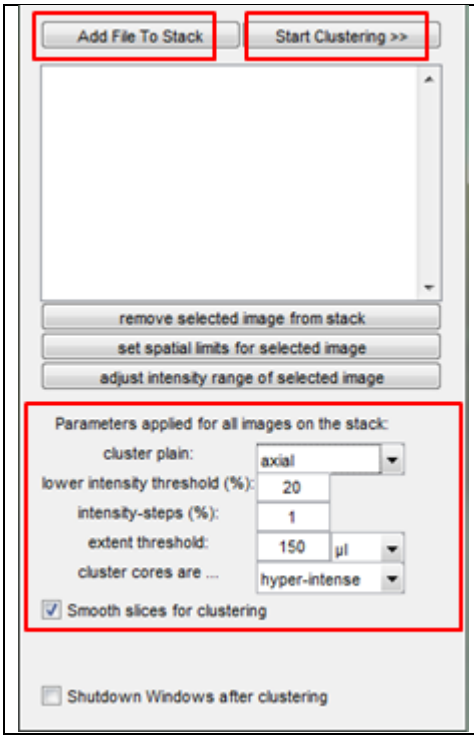
Once the SPM folder has been added to the Matlab path ("File -> Set Path -> Add Folder"), SPM can be started with the command "SPM fmri".

The Clusterize Toolbox can then be found in SPM's Toolbox menu (Click "Toolbox" in SPM's menu window) and select "clusterize").



To delineate the lesion, the image first needs to be prepared (i.e. "clustered"). In this fully automated procedure, each voxel is assigned to a "cluster". Clustering can be done using either a single image modality (option A) or two image modalities (option B). Once the image has been clustered, the clusters are ready to be "analysed" (option C). Here, the clusters-of-interest (i.e. those corresponding to the lesion) are manually and interactively selected and modified.

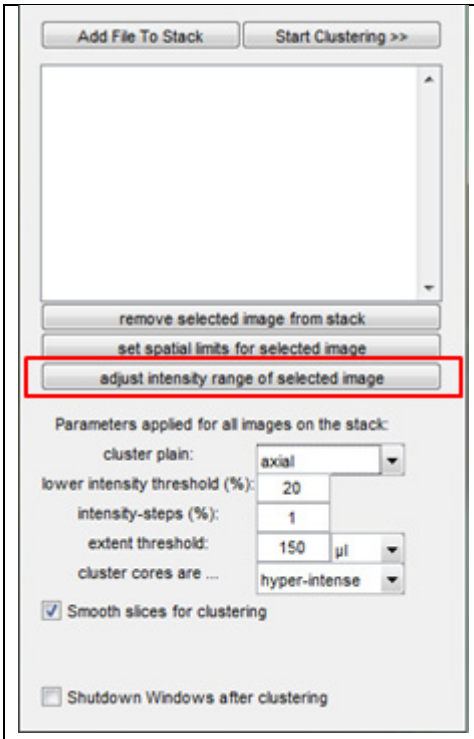
1.1. A. Prepare: Cluster an image using one image-modality

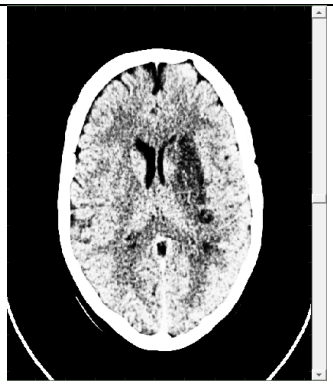


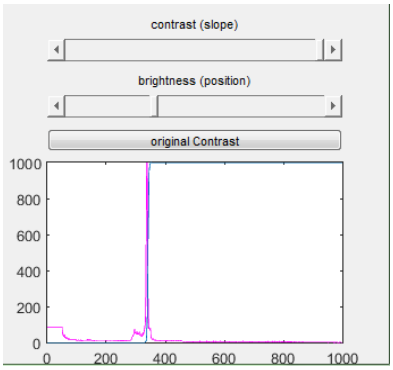
Add File To Stack: Select the image(s) you want to cluster. All files added to the stack, will be processed the same way (as defined under "parameters applied for all images on the stack").

The following processing parameters can be adjusted:
- cluster plane: the option "axial" is usually the easiest one to work with.
- lower intensity threshold: to eliminate non-contributing background voxels. The value of 20% usually works fine.
- intensity steps: the "size" of the "steps" the algorithm takes, can usually be left at 1%.
- extent threshold: the minimum cluster size, serves to avoid oversegmented results.
- cluster cores are: this is usually the only option you might want to modify so that the algorithm knows whether to look for hyperintensities (e.g. acute lesions in T2FLAIR/DWI images, bleedings in CT images) or hypointensities (e.g. acute infarcts in CT images, chronic lesions in T1 images).

When working with CT images, you will additionally have to adjust the intensity range of the selected image. This is because in CT images, the "interesting" intensity range (that corresponds to grey and white matter) is only a small portion of the total intensity range. As such, you need to tell the algorithm where to look.







After adding a CT image to the stack, press "adjust intensity range of selected image".

In the subsequent windows, set the contrast to maximal and adjust the brightness, so that the blue vertical line overlaps with the pink intensity peak. In the window showing your CT image, you should then be able to clearly see the different brain tissues.

When multiple CT images are added to a single stack, this needs to be done for each CT image.

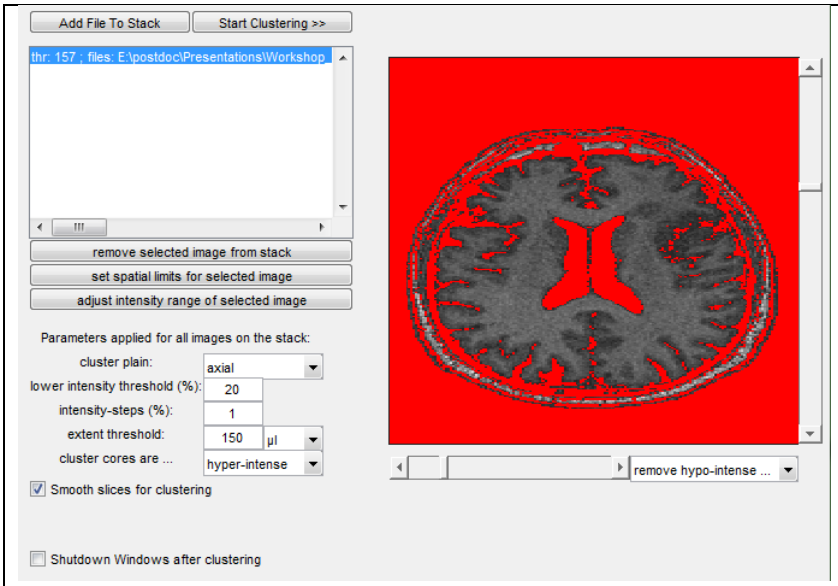
When all parameters are set for all images in the stack, press "Start Clustering" to start clustering. This clustering step is fully automatic and can be left to run overnight.

When clustering is finished (and you did not select the option "Shutdown Windows after clustering"), the Toolbox asks you whether you want to open the Toolbox to start working on the clusters. You can either accept this, or decline this and come back later to manually open the Toolbox to start working on the clusters by selecting option C "Analyze: work on precalculated clusters" (see section 1.3 below).

1.2. B. Prepare: Cluster an image using two image-modalities

This option can be useful when working with T2FLAIR or DWI images in situations where you also have a T1 image of the same patient. This T1 image can then be used to remove the ventricles, which might improve the separation between ventricles and lesions close to the ventricles.

When pressing "Add File To Stack", the Toolbox will first ask for the file "where you want to find a threshold in". This is the image used to define the ventricles and usually the T1. Subsequently, it will ask for the file "you want to cluster". This is the file that you will use to ultimately define the lesion (i.e. the image that you want to cluster), usually the T2FLAIR or DWI image.



The slider on the right of the T1 image allows you to scroll through the (in this case axial) image slices.

The slider below the T1 image allows you to adjust the intensity cut-off that effectively removes the ventricles.

All options in the left pane are as described under section 1.1. above and allow you to set the parameters for clustering the T2FLAIR or DWI image.

Coregistration of both images is done automatically.

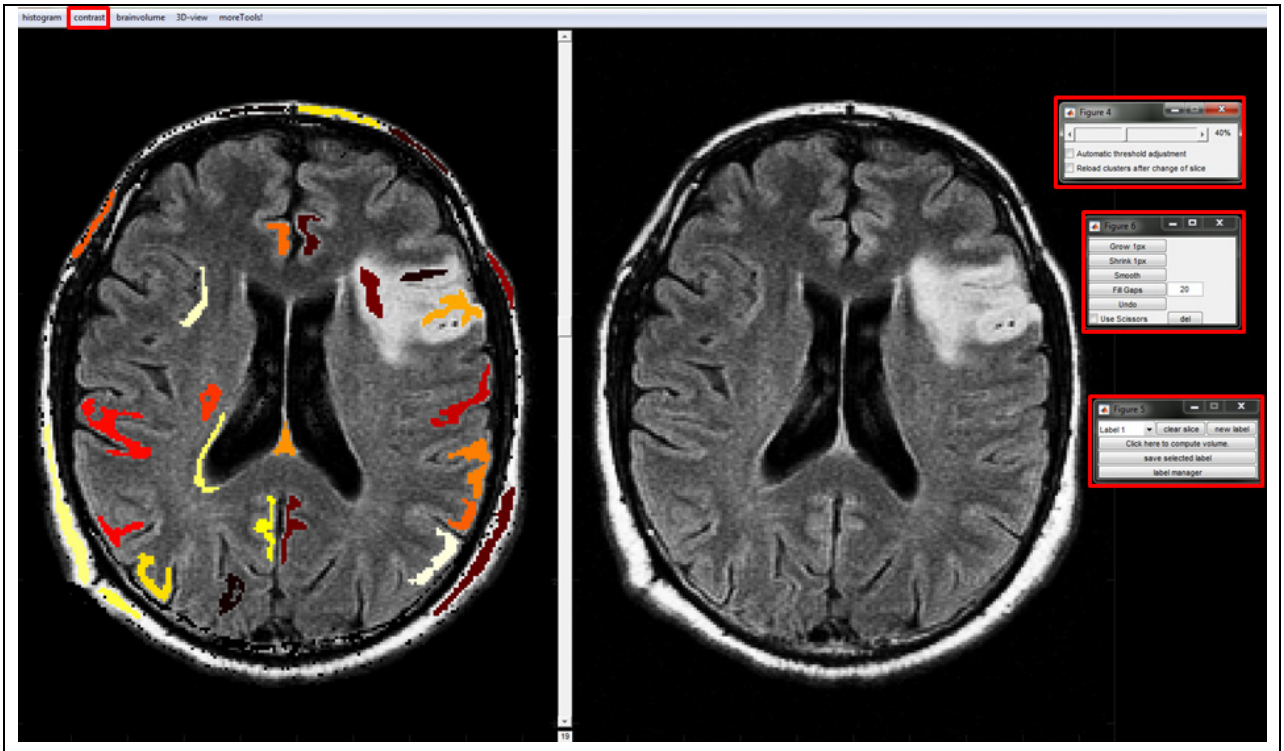
When you're happy with your settings, press "Start Clustering" to start clustering. This clustering step is fully automatic and can be left to run overnight.

When clustering is finished (and you did not select the option "Shutdown Windows after clustering"), the Toolbox asks you whether you want to open the Toolbox to start working on the clusters. You can either accept this, or decline this and come back later to manually open the Toolbox to start working on the clusters by selecting option C "Analyze: work on precalculated clusters" (see section 1.3 below).

1.3. C. Analyze: work on precalculated clusters

This option opens the Toolbox, allowing the manual selection and modification of the cluster(s)-of-interest, i.e. the lesion(s).

Under “Select a file”, select a structural image file (i.e. the CT, T2FLAIR or DWI image) that you previously clustered.



The left pane depicts the structural image with the clusters. In this pane, the cluster(s) corresponding to the lesion(s) can be selected with mouseclick. The right pane depicts the structural image and, if any clusters are selected, the outline of the selected cluster(s).

The slider between the 2 panes allows you to scroll through the (in this case axial) image slices.

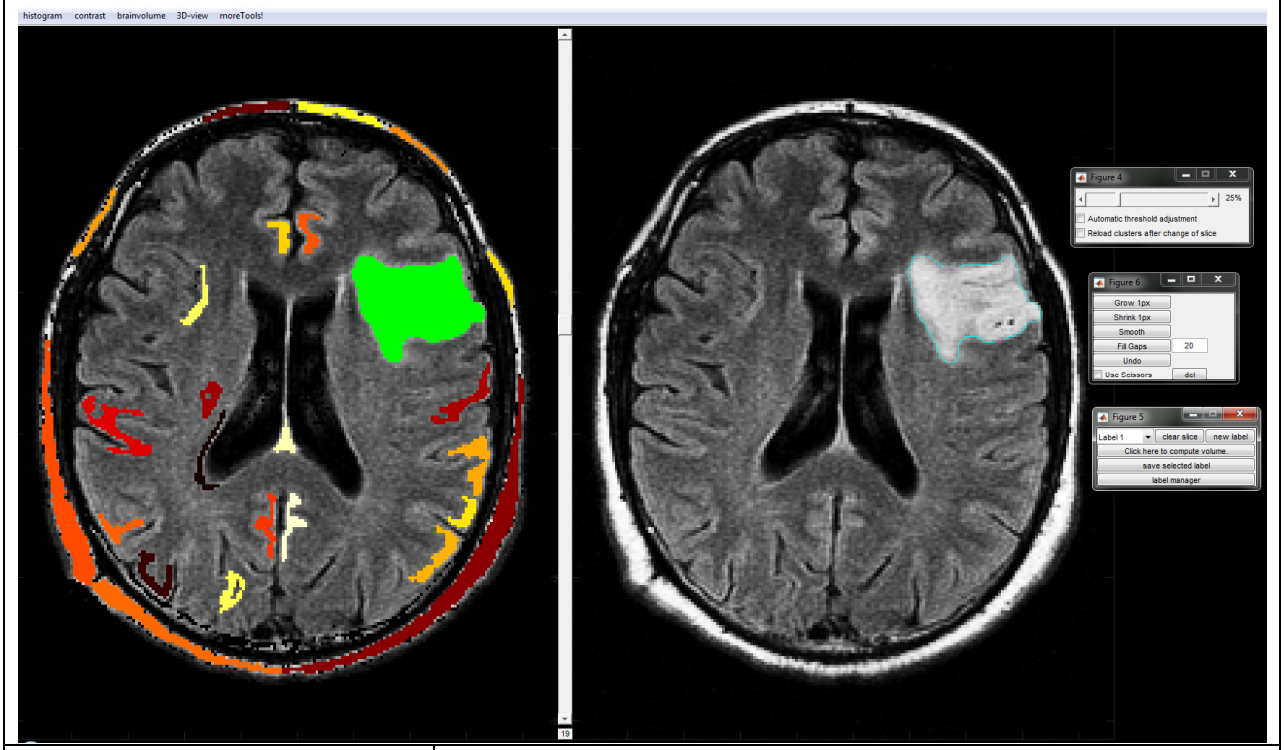
As can be seen here, however, the size of the clusters has to be adapted (which is done by modifying the intensity threshold). Moreover, several additional settings allow further modification of the cluster(s) of interest:

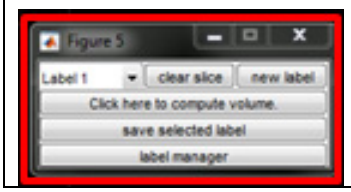
Figure 4: allows you to set the intensity threshold. Changing this threshold will change the size of the clusters. Typically, you want to find a single intensity threshold where the cluster(s) cover the entire lesion(s) in every image slice

Figure 6: allows further small modifications of the cluster(s)-of-interest. The most useful one here is the option to “Fill Gaps”, which allows you to fill any “holes” in your cluster(s)-of-interest.

If necessary, the contrast and brightness of the images can be adjusted in the “contrast” menu.

When you are done modifying the intensity threshold and have selected the cluster(s) corresponding to the lesion(s) in every slice of the image, the view in the Clusterize Toolbox should look something like this:





Subsequently, press “save selected label” to save the label (i.e. the lesion map) to file. Under “label manager” previously created labels can be loaded (and further modified if desired).

2. How to delineate the lesion in each individual patient brain with MRIcroN:

Compared to of manual lesion delineation, the semi-automated Clusterize Toolbox significantly speeds up lesion delineation in the most commonly used image modalities, without loss of either lesion delineation precision or lesion delineation reproducibility. Should you nevertheless prefer manual lesion delineation, this can be done with MRIcroN.

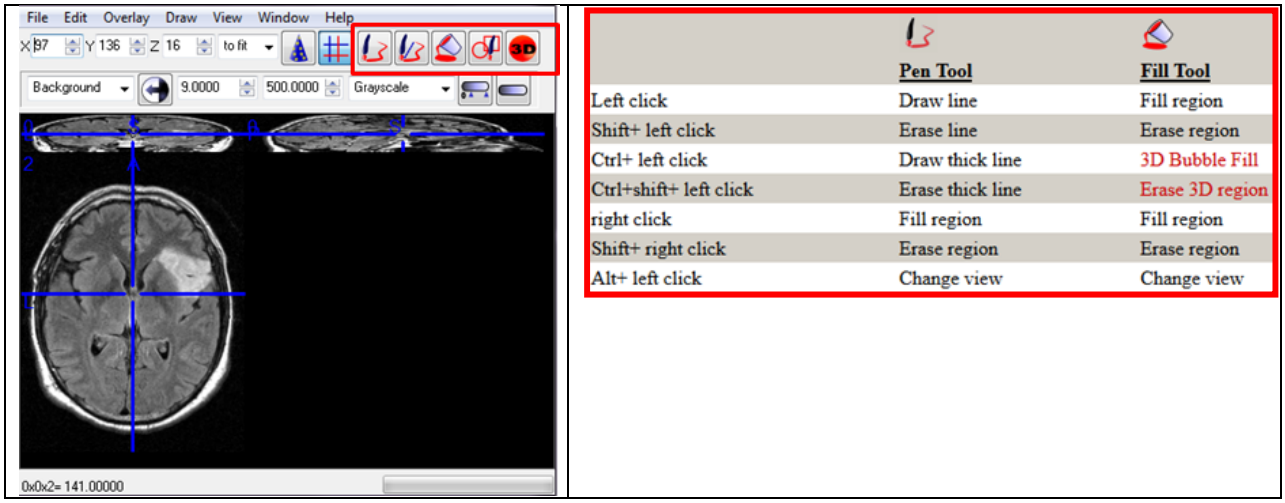
MRIcroN can be downloaded from https://www.nitrc.org/projects/mricron (also includes the program NPM needed for the statistical lesion analysis).

Double-click the file “mricron.exe” to open MRIcroN.

Before opening the patient brain image, go to “Help -> Preferences“ and make sure that “Reorient images when loading“ is **unchecked**.

To open a patient brain image, go to “File -> Open“ and select your image.

Use the Draw toolbar to delineate the lesion. If the Draw toolbar is not visible, enable it under “Draw -> Show drawing tools“ or “Help -> Preferences“ (depending on your operating system).



	Pen Tool	Fill Tool
Left click	Draw line	Fill region
Shift+ left click	Erase line	Erase region
Ctrl+ left click	Draw thick line	3D Bubble Fill
Ctrl+shift+ left click	Erase thick line	Erase 3D region
right click	Fill region	Fill region
Shift+ right click	Erase region	Erase region
Alt+ left click	Change view	Change view

The delineated lesion (.voi) can be saved under "Draw -> Save VOI" and a saved .voi can be opened under "Draw -> Open VOI". As delineation of a single lesion can easily take hours, it is wise to save your .voi every now and then.

Once the lesion has been delineated, select "Draw -> Advanced -> Create SPM5 masks" to create the images needed for the normalisation. This will create 2 .nii files: 1) an image of the lesion ("l" prepended to the filename) and 2) an image of the inverse of the smoothed lesion (lesion mask image, "m" prepended to the filename).

3. How to normalise the brain and lesion of each patient with SPM12 and the Clinical Toolbox:

The Clinical Toolbox can be downloaded from http://www.nitrc.org/projects/clinicaltbx/. The "Clinical Toolbox for SPM12" version works, as the name suggests, with SPM12. Simply open the .zip file and extract the subdirectory "Clinical-master" to SPM's toolbox directory. Subsequently, rename the subdirectory "Clinical-master" to "Clinical".

The Clinical Toolbox assumes a typical stroke population (i.e. elderly subjects) and this is reflected in the default spatial normalisation templates used by this toolbox. However, this can easily be modified by supplying your own normalisation templates and modifying the code a bit. Thus, this toolbox is also helpful even if you're not investigating elderly subjects. Please refer to the Appendix for pointers to make the Clinical Toolbox usable in other subject populations.

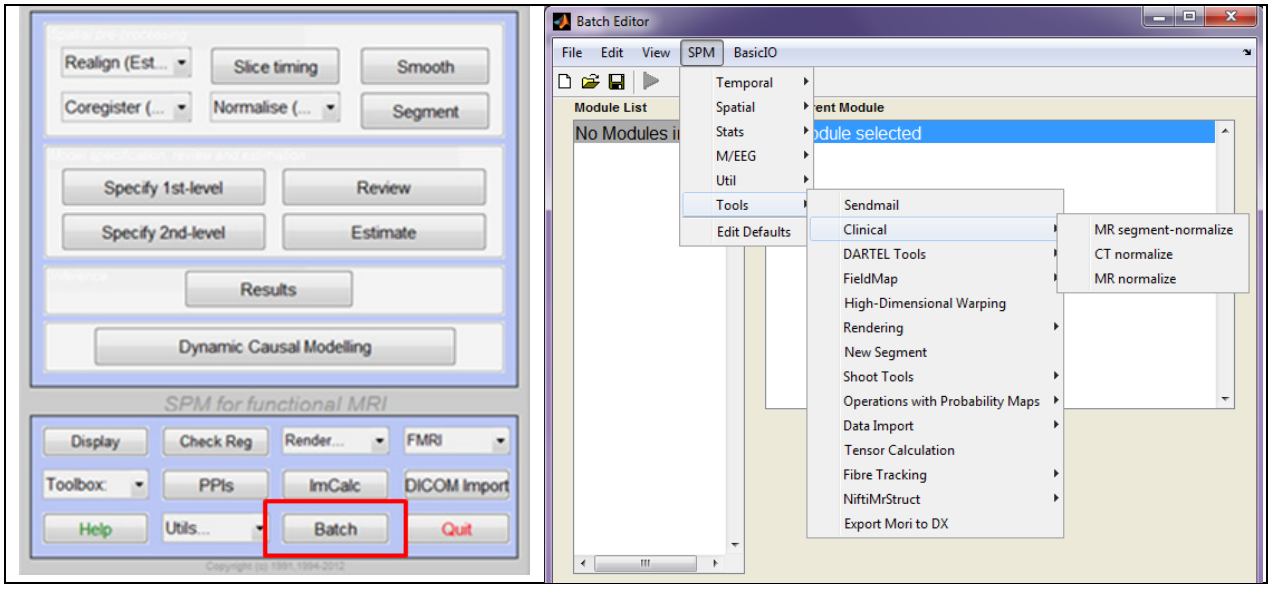
The following 5 papers provide background to the Clinical Toolbox and normalisation of lesioned brains in general:

- Brett M, Leff A P, Rorden C, Ashburner J (2001). Spatial normalization of brain images with focal lesions using cost function masking. *Neuroimage* 14: 486-500.
- Crinion J, Ashburner J, Leff A, Brett M, Price C, Friston K (2007). Spatial normalization of lesioned brains: performance evaluation and impact on fMRI analyses. *Neuroimage* 37: 866-875.
- Nachev P, Coulthard E, Jäger HR, Kennard C, Husain M (2008). Enantiomorphic normalization of focally lesioned brains. *Neuroimage* 39: 1215-1226.
- Andersen SM, Rapcsak SZ, Beeson PM (2010). Cost function masking during normalization of brains with focal lesions: Still a necessity? *Neuroimage* 53: 78-84.

- Rorden C, Bonilha L, Fridriksson J, Bender B, Karnath H-O (2012). Age-specific CT and MRI templates for spatial normalization. *Neuroimage* 61: 957–965.

Once the SPM folder has been added to the Matlab path ("File -> Set Path -> Add Folder"), SPM can be started with the command "SPM fmri".

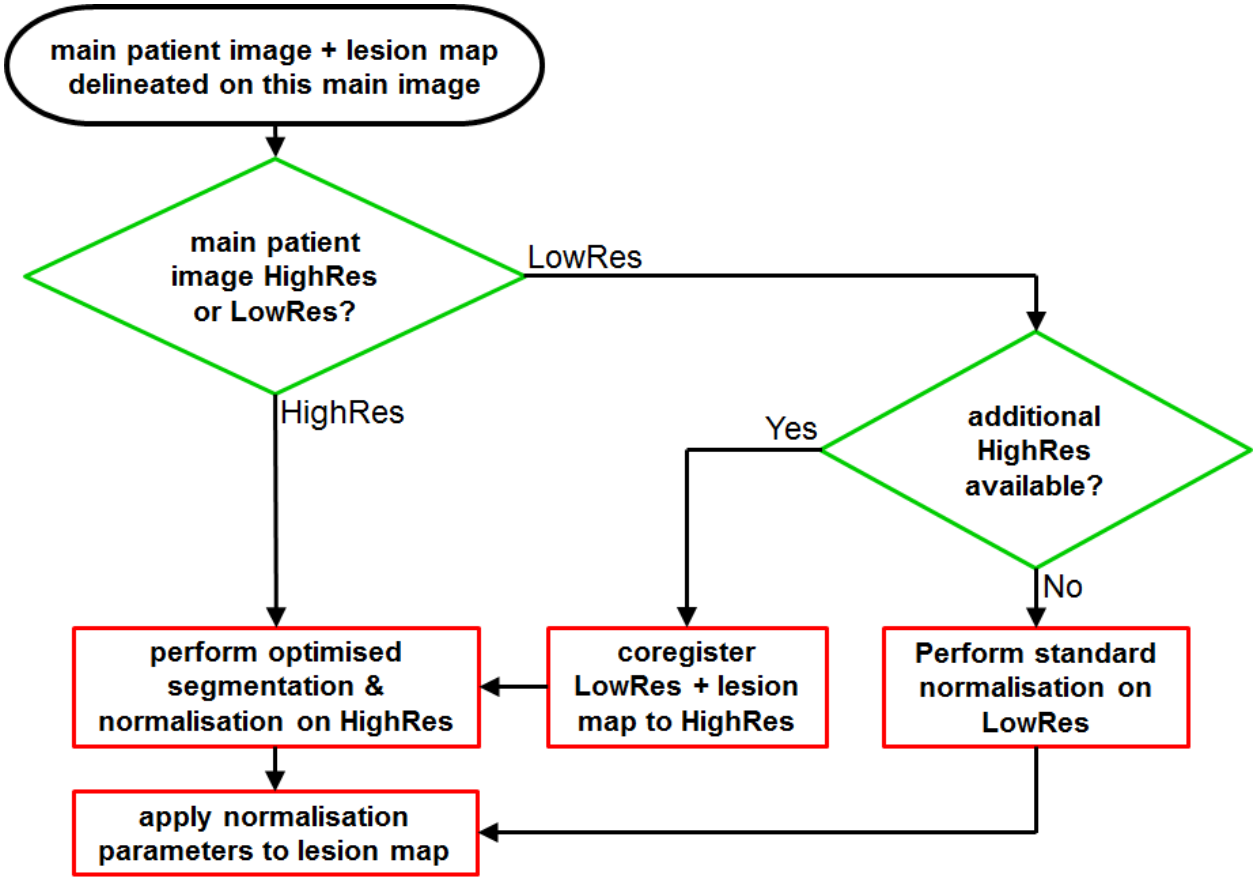
The Clinical Toolbox can then be found in SPM's Batch Editor (Click "Batch" in SPM's menu window) by selecting "SPM -> Tools -> Clinical".



3.1. Normalisation scenarios and definition of terms:

Before explaining the steps needed to normalise our data, it might be helpful to discuss the typical normalisation scenarios used and define a few **terms**.

Flowchart of the typical normalisation scenarios:



The patient image that you used to delineate the lesion will be referred to as **main patient image**.

Scenario 1: If the main patient image has a high (radiometric) resolution, meaning that you can clearly distinguish between grey and white matter tissue in the image, it will be referred to as a **HighRes main patient image**. This might be the case when you study chronic stroke patients and the lesion was delineated directly on the patient's T1 MPRAGE image. The typical normalisation scenario here would be to perform optimised segmentation & normalisation on this HighRes main patient image and apply the normalisation parameters to the lesion map (see section 3.5 below).

Scenario 2: If the main patient image has a low (radiometric) resolution, meaning that you cannot clearly distinguish between grey and white matter tissue in the image, it will be referred to as a **LowRes main patient image**. This is usually the case when you study acute stroke patients and the lesion was delineated directly on the patient's CT or T2FLAIR image (as these lesions are typically not visible in T1). The typical normalisation scenario here would be to perform standard normalisation on this LowRes main patient image and apply the normalisation parameters to the lesion map (see section 3.3 for CT or section 3.4 for MR below).

Scenario 3: The most complicated normalisation scenario occurs when you have a **LowRes main patient image** and a **HighRes additional patient image**. This can occur when you study acute stroke patients where the lesion is visible only in the T2FLAIR, but were also able to additionally

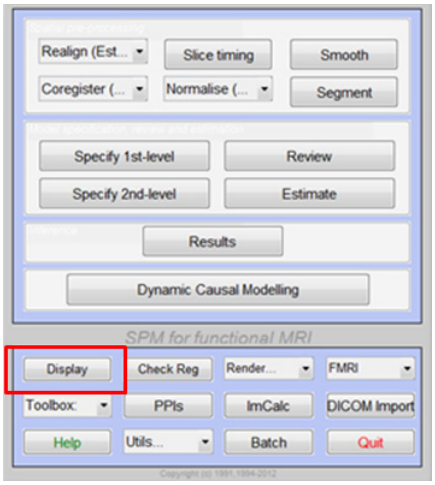
obtain a T1 MPRAGE from the same patient. Alternatively, this might be the case when you study acute stroke patients and collected DWI data. In clinical settings, DWI data is typically collected with different b-values. Higher b-values (e.g. b1000) allow good visualisation of the lesion and are thus normally used for lesion delineation, but do not allow good differentiation between grey and white matter. The b0 image, however, often allows good differentiation between grey and white matter. The optimal normalisation scenario here would be to first coregister the LowRes main patient image (e.g. the T2FLAIR or b1000 DWI image) and lesion map to the HighRes additional patient image (e.g. the T1 MPRAGE or b0 DWI image), then perform optimised segmentation & normalisation on the HighRes additional patient image and finally apply the normalisation parameters derived from the HighRes additional patient image to the coregistered LowRes main patient image and lesion map (see section 3.5 below).

3.2. Reorienting:

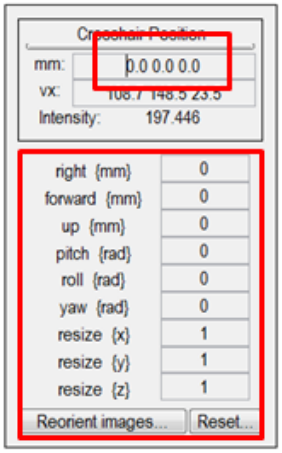
Normalisation requires a rough match between the image origin and orientation of the patient image and the template image used for normalisation. The Clinical Toolbox contains an routine to do this, that is automatically called when you normalise your images. In most cases, this will work fine and you can ignore this section. In rare cases, however, starting estimates may be off to such an extent that normalisation fails (i.e. normalisation gets stuck in a local minimum). In these cases, you may need to manually reorient the patient image, so that the image origin and orientation of this patient image roughly match the image origin and orientation of the template image used for normalisation.

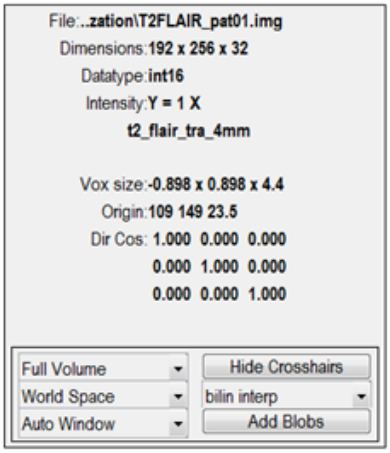
For scenario 1 and 2 above, we reorient the (LowRes or HighRes) main patient image. For scenario 3 above, we reorient the HighRes additional patient image.

Open your patient image by clicking "Display" in SPM's menu window:



In SPM's graphics window, make sure the crosshair position is "0 0 0" and, if necessary, move and rotate the image, by modifying the values in the lower red square (resizing is usually not necessary.



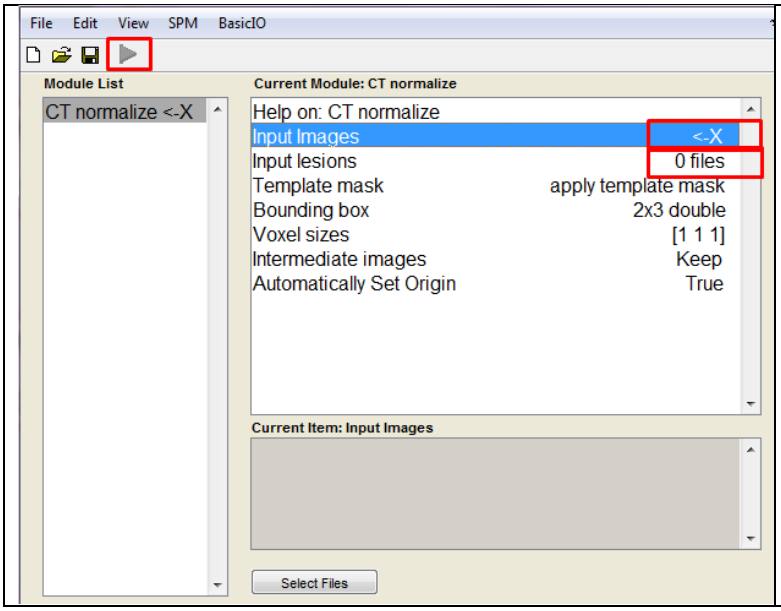


Once you are happy, press "Reorient images" and select the image(s) you want to reorient. When reorienting the (LowRes or HighRes) main patient image in scenarios 1 and 2, don't forget to select the associated lesion map as well so that the main patient image and lesion map remain coregistered.

3.3. Normalisation – CT normalize

Typical example: Your main patient image that you used to delineate the lesion is a LowRes CT image and you have no other usable images from the same patient.

Select the option “CT normalize” from the Clinical Toolbox.



Input Images: select your LowRes main patient image (CT).

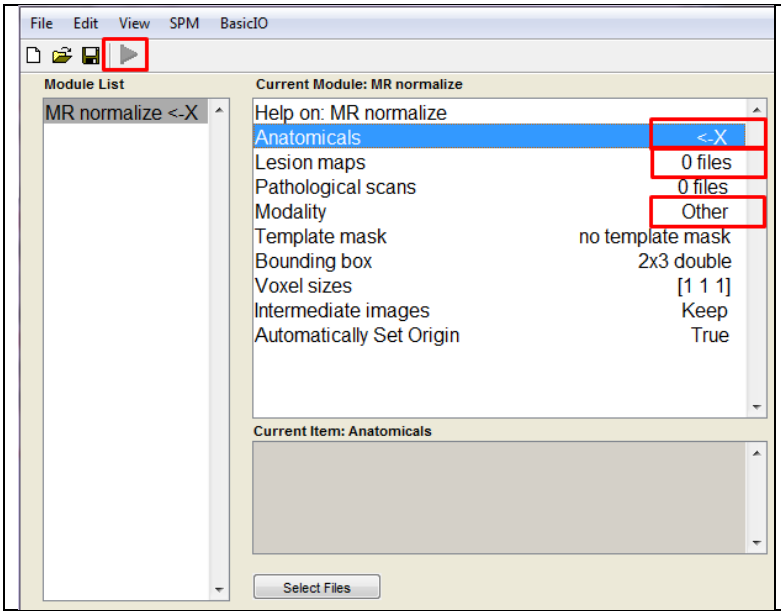
Input lesions: select your lesion map.

Press the green arrow to start the analysis.

3.4. Normalisation – MR normalize

Typical example: Your main patient image that you used to delineate the lesion is a LowRes MR image and you have no other usable images from the same patient.

Select the option “MR normalize” from the Clinical Toolbox.



Anatomicals: select your LowRes main patient image.

Lesion maps: select your lesion map.

Modality: select your image modality. “T1” and “T2” are the default young adult SPM templates, “FLAIR” uses templates developed by the Glahn Group and “Other” uses a weighted average of the above mentioned T1 and T2 templates.

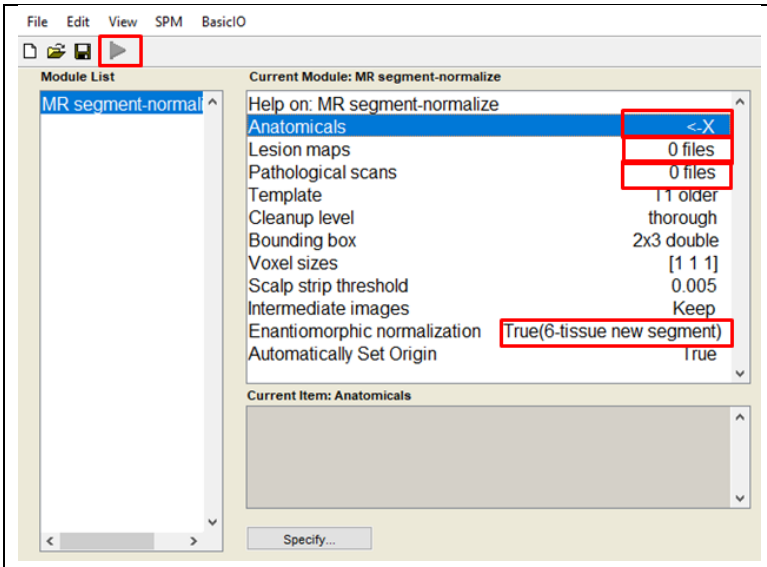
Press the green arrow to start the analysis.

Note, however, that this routine also supports the less common normalisation scenario where you have a LowRes main patient image and a LowRes additional patient image and want to derive your normalisation parameters from the LowRes additional patient image to be applied to the LowRes main patient image and associated lesion map. This might be the case when you have very poor quality T2FLAIR scans and somewhat less poor quality T1 scans that does not allow segmentation, but still allows better standard normalisation than the very poor quality T2FLAIR image. In this case you would select your LowRes additional patient image under "Anatomical", your LowRes main patient image under "Pathological scans" and your lesion map under "Lesion maps". The Clinical Toolbox will then first coregister your LowRes main patient image and the lesion map to your LowRes additional patient image before deriving standard normalisation parameters from the LowRes additional patient image and applying them to the coregistered LowRes main patient image and associated lesion map.

3.5. Normalisation – MR segment-normalize

Typical examples:
1) Your main patient image that you used to delineate the lesion is a HighRes MR **or**
2) Your main patient image that you used to delineate the lesion is a LowRes MR image and you have a HighRes additional patient image. In this case, the Clinical Toolbox will automatically first coregister your LowRes main patient image (and associated lesion map) to your HighRes additional patient image, before deriving the normalisation parameters from the HighRes additional patient image.

Select the option "MR segment-normalize" from the Clinical Toolbox.



Anatomicals: if example 1) select your HighRes main patient image, if example 2) select your HighRes additional patient image.

Lesion maps: select your lesion map.

Pathological scans: if example 1) leave empty, if example 2) select your LowRes main patient image.

Template: select if you want to use spatial tissue priors derived from younger or older subject population.

Additionally, if "Enantiomorphic normalization" is set to "True", the Toolbox will use enantiomorphic correction instead of cost function masking to correct for the lesion during normalisation. The "True(6-tissue new segment)" option, however, often fails, so you may want to select "True(3-tissue old segment)" instead.

Press the green arrow to start the analysis.

3.6. Normalisation – always check your results

Always check the quality of the normalisation by comparing the normalised patient brain to the template brain. The easiest way to do this is to open multiple instances of MRIcroN (not possible on a Mac OS) and separately load the normalised patient brain (optionally with the normalised lesion image loaded under "Overlay -> Add") and the template brain in the different instances of MRIcroN. By selecting "View -> Yoke" (ticked when selected) in each instance of MRIcroN the information can be yoked (i.e. if you scroll through the brain in one instance of MRIcroN, the brain in the other instance of MRIcroN will also be scrolled). After successful normalisation, the size, shape, orientation and image origin of the normalised image should match those of the template. If normalisation fails, it typically fails spectacularly. Thus, small differences are no cause for concern.

4. How to perform a univariate voxel-based statistical analysis with NPM:

Before starting a voxel-based statistical analysis, copy all the .voi files of the normalised patient lesions into a single directory.

The following 2 papers provide background to (statistical) lesion analysis:

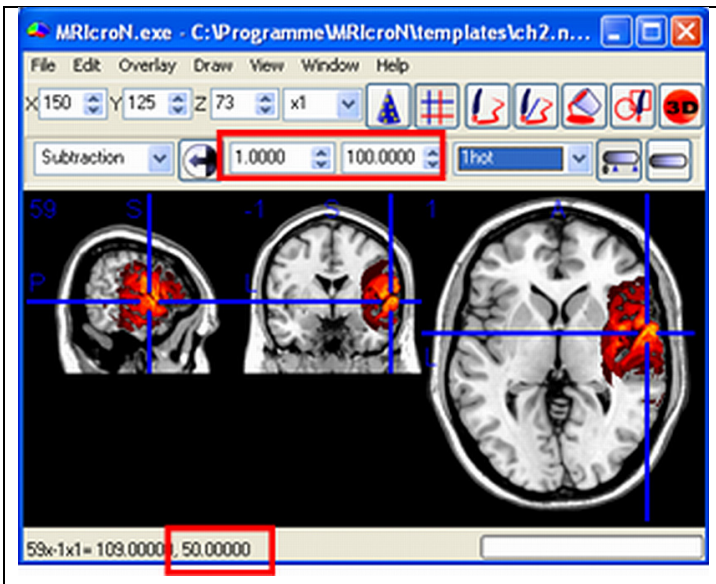
- Rorden C, Karnath H-O (2004). Using human brain lesions to infer function: a relic from a past era in the fMRI age? *Nature Reviews Neuroscience* 5: 813-819.
- Rorden C, Karnath H-O, Bonilha L (2007). Improving lesion-symptom mapping. *Journal of Cognitive Neuroscience* 19: 1081-1088.

4.1. Subtraction analysis:

Sometimes we simply do not have enough patients to perform a voxel-based statistical analysis. If our patient group can be divided into 2 subgroups on the basis of their behaviour, we can perform a subtraction analysis. This does not allow any statistical inferences however.

First create a lesion overlap image for each patient subgroup (usually one patient subgroup with a deficit and a control patient subgroup without that deficit) by selecting in MRIcroN "Draw -> Statistics -> Create overlap images".

When we have an overlap for each of the patient subgroups, we can perform a subtraction analysis: In MRIcroN select "Draw -> Statistics -> Subtraction Plots". Under "Select POSITIVE overlap image" select the overlap image of the patient subgroup with a deficit and under "Select NEGATIVE overlap image" select the overlap image of the control patient subgroup without the deficit. Saving the subtraction plot sometimes needs to be repeated several times (just keep reselecting the originally chosen filename and ignore the "overwrite" warning).



In MRIcroN the subtraction plot can be loaded as an overlay (over a template image) by selecting "Overlay -> Open".

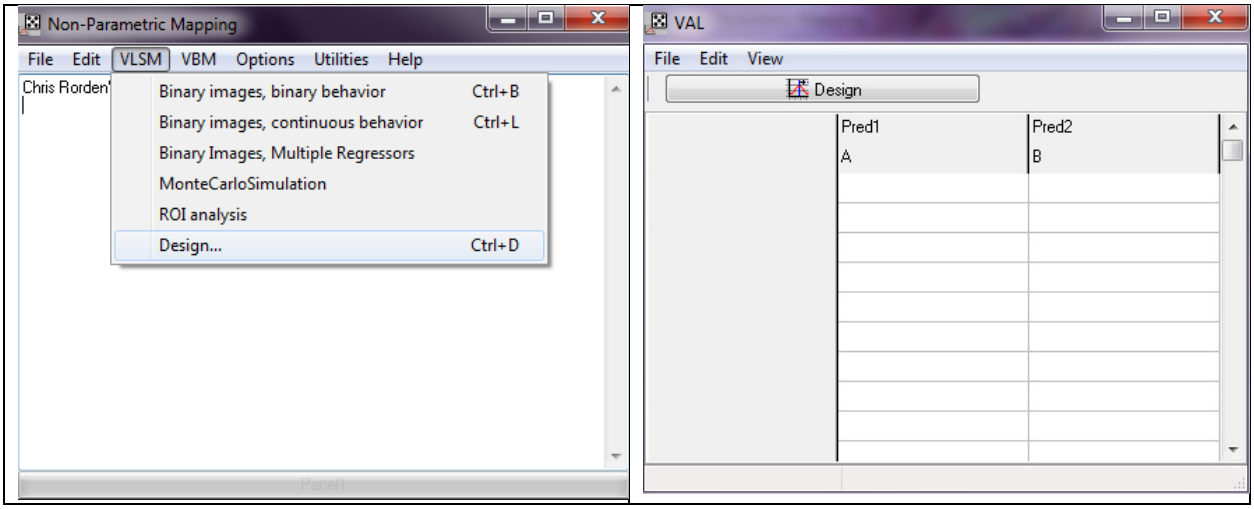
Values 1 to 100 show voxels more often damaged in group 1 (with deficit) than in group 2 (without deficit), whereas values -1 to -100 show the reverse.

Values reflect relative frequency of damage, e.g. value of 50 reflects that that voxel is damaged 50% more frequently in group 1 than in group 2. Assuming n = 10 in both groups, this could mean that the voxel is damaged in 9 of 10 patients (i.e. 90%) in group 1 and 4 of 10 patients (i.e. 40%) in group 2 (or 7 patients in group 1 vs. 2 patients in group 2, etc.).

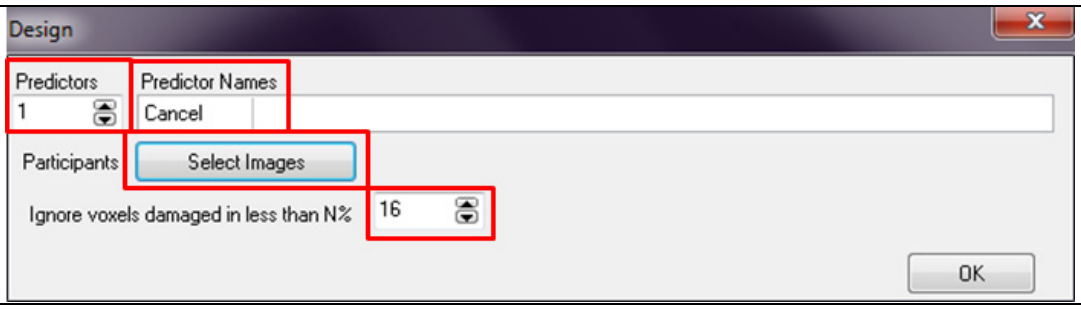
4.2. Voxel-based statistical analysis with a single behavioural predictor:

The program NPM can be found in the MRIcroN directory. Open NPM by double-clicking the file "npm.exe". NPM works best on a Windows OS.

In the main NPM window, select "VLSM -> Design". This will open a VAL window where we can later enter the patients' behavioural scores.



First, however, press “Design” in this VAL window to select the patients’ lesion maps and to specify the analysis design:



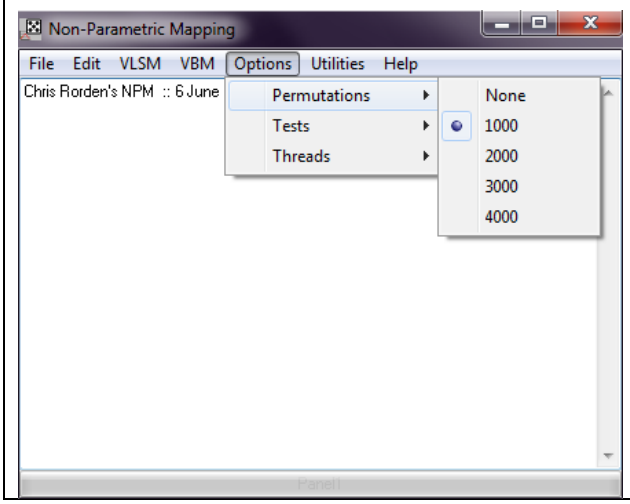
- Predictors: Specify how many behavioural variables you have (typically 1).
- Predictor Names: Specify a sensible and not overlong name for each behavioural variable.
- Select Images: Click to select the .voi files of all patients that you want to include in the analysis.
- Ignore voxels damaged...: Specify a threshold to exclude voxels never or rarely damaged. This is specified as % of total number of patients in the analysis. Thus, if you have 10 patients in your analysis and specify 20% here, the analysis will ignore voxels damaged in less than 2 patients.

Some versions of NPM also offer the option to “Select Template”, which is prefilled with “template.img”. Just leave this as is.

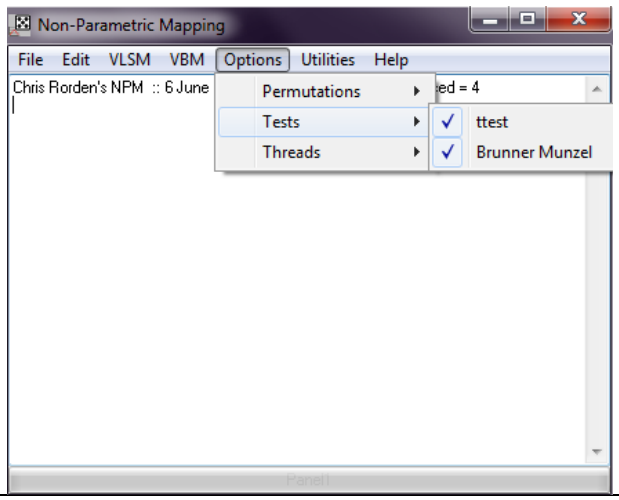
Once the analysis design is specified and the lesion maps are selected, press “OK” to return to the VAL window. In this VAL window, each row depicts the .voi file and associated behavioural score(s) of a single patient. Behavioural score can be continuous or binomial. **Higher scores are assumed to reflect better performance.** Once you’ve entered the behavioural scores of the patients, press “File -> Save“.

In the main NPM window, we can optionally specify further analysis details under “Options“:

The number of permutations for permutation thresholding (to correct for multiple comparisons):



Ticked tests are the tests performed (only applies when behavioural data is continuous:



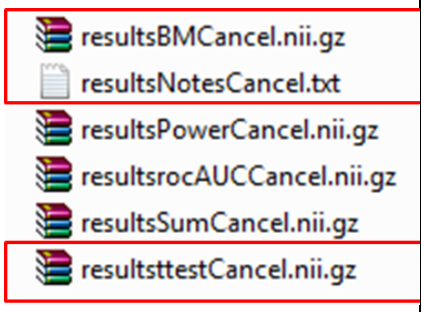
To start the voxel-based statistical analysis, go to “VLSM“ in the main NPM window and select “Binary images, binary behavior/groups“ when behavioural data is binomial and “Binary images, continuous behavior/groups“ when behavioural data is continuous. Specify your .val file.

When it asks for “Base Statistical Map“, enter a filename. The results from the analysis will then be saved as <filename><StatisticalTestUsed><Predictorname>

When it asks for “Select explicit mask [optional]“, you can optionally select an explicit mask to restrict the analysis to the voxels included in this mask. This allows a region of interest analysis. If you want to use an explicit mask, make sure the image dimensions of the mask match that of the lesion data. If you’re not interested in doing so, simply press “cancel“.

4.2.1 Output interpretation and visualisation when behavioural data is continuous:

If under “Options“ in the NPM main window both “ttest“ and “Brunner Munzel“ are ticked, the VLSM will produce output for both the t-test and the Brunner-Munzel test.

	The file ending with “BM<predictorname>“ is the file containing the standardised test statistic (Z-value) for each voxel for the Brunner Munzel analysis. The file ending with “ttest<predictorname>“ is the file containing the standardised test statistic (Z-value) for each voxel for the t-test analysis. The file ending with “Notes<predictorname>“ is the text file containing a summary of the analysis and the cut-off Z-values of different p-thresholds for the different methods of correcting for multiple comparisons.

Critical part of the text file:

```
57390 test Std Bonferroni FWE Z 0.050=4.781, 0.025=4.919, 0.01=5.095
539 test Number of Unique Lesion Patterns Bonferroni FWE Z 0.050=3.738, 0.025=3.909, 0.01=4.12
ttest Range -2.277...5.435
ttest +FDR Z 0.050=1.99511700, 0.01=2.74344100
ttest -FDR Z 0.050=9.20000000, 0.01=9.20000000
ttest+: permutationFWE , 0.050=3.37321100, 0.025=3.58051900, 0.01=3.93811000
ttest-: permutationFWE , 0.050=-2.68733900, 0.025=-2.90089900, 0.01=-3.09330500
BM Range -2.197...3.891
BM +FDR Z 0.050=2.08466200, 0.01=3.06181400
BM -FDR Z 0.050=9.20000000, 0.01=9.20000000
BM+: permutationFWE , 0.050=3.10543400, 0.025=3.32005400, 0.01=3.61530000
BM-: permutationFWE , 0.050=-2.90266700, 0.025=-3.26361600, 0.01=-3.61530000
```

- ttest Range: Range of Z-values in the dataset for the t-test analysis (-2.277 to 5.543).
- BM Range: Range of Z-values in the dataset for the Brunner-Munzel test analysis (-2.197 to 3.891).

- 57390 test Std Bonferroni FWE: Cut-off Z-value when using Bonferroni correction for multiple comparisons for different p-thresholds (at p=0.05 Zcrit=4.781) for both t-test and Brunner-Munzel test.

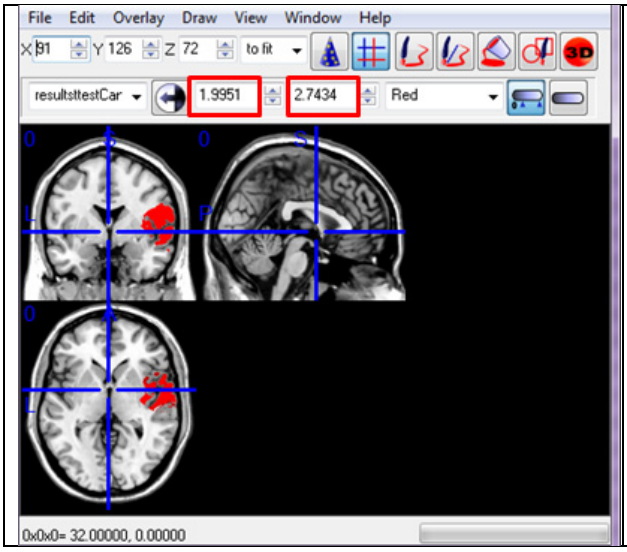
- ttest +FDR: Cut-off Z-value when using False Discovery Rate correction for multiple comparisons for different q-thresholds (at q=0.05 Zcrit=1.99511700) for the t-test analysis.
- ttest +permutationFWE: Cut-off Z-value when using Permutation Thresholding to correct for multiple comparisons for different p-thresholds (at p=0.05 Zcrit=3.37321100) for the t-test analysis.

- BM +FDR: Cut-off Z-value when using False Discovery Rate correction for multiple comparisons for different q-thresholds (at q=0.05 Zcrit=2.08466200) for the Brunner-Munzel test analysis.
- BM +permutationFWE: Cut-off Z-value when using Permutation Thresholding to correct for multiple comparisons for different p-thresholds (at p=0.05 Zcrit=3.10543400) for the t-test analysis.

Significant voxels are those voxels where the Z-value surpasses the cut-off Z-value (i.e. you can already see here whether you have significant results: if the highest Z-value in the dataset (5.435 for t-test and 3.891 for BM-test) exceeds one of the cut-off Z-values, you have at the very least 1 significant voxel.

The significant results denote the voxels where the behavioural score of patients with damage to that voxel is significantly lower than the behavioural score of patients without damage to that voxel.

The critical Z-value of 9.20000000 that can occur when using FDR deserves special mention. This is not so much an actual critical Z-value, this value is NPM's way of saying "Infinite". In other words, **when you obtain a critical Z-value of 9.20000000 when using FDR in any type of analysis (e.g. binomial, continuous), your cut-off Z-value is infinitely high and thus by definition there are no significant voxels**.

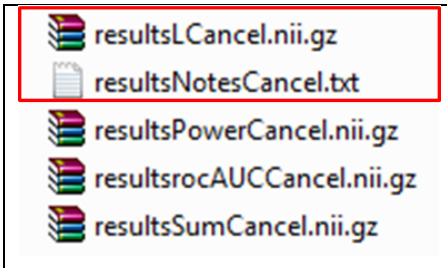


To visualise the significant voxel(s) in MRIcroN, load the map containing the Z-value for each voxel
Depending on whether you want to visualise the results of the t-test or the Brunner-Munzel test, load either the file ending with "ttest<predictorname>" or the file ending with "BM<predictorname>".

As display range enter the cut-off Z-value on the left and the maximum Z-value observed in the data (indicated under "ttest Range" or "BM range") on the right.

Your overlay now contains the significant voxels.

4.2.2 Output interpretation and visualisation when behavioural data is binomial:



The file ending with "L<predictorname>" is the file containing the standardised test statistic (Z-value) for each voxel (in this example the predictor was called "Cancel").

The file ending with "Notes<predictorname>" is the text file containing a summary of the analysis and the cut-off Z-values of different p-thresholds for the different methods of correcting for multiple comparisons.

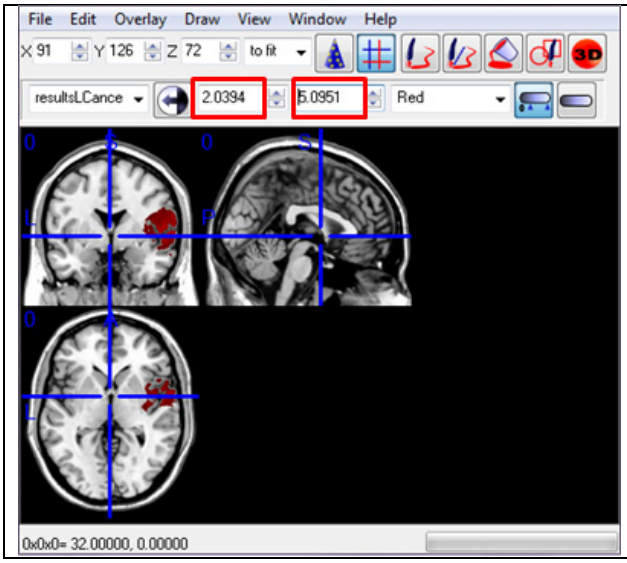
Critical part of the text file:

```
57390 test Std Bonferroni FWE Z 0.050=4.781, 0.025=4.919, 0.01=5.095
539 test Number of Unique Lesion Patterns Bonferroni FWE Z 0.050=3.738, 0.025=3.909, 0.01=4.1
L Range -2.435...3.873
L +FDR Z 0.050=2.03944800, 0.01=9.20000000
L -FDR Z 0.050=9.20000000, 0.01=9.20000000
L+: permutationFWE , 0.050=2.53742700, 0.025=2.79329600, 0.01=3.14734600
L-: permutationFWE , 0.050=-2.43463500, 0.025=-2.79329600, 0.01=-2.91058900
```

- L Range: Range of Z-values in the dataset (-2.435 to 3.873). We performed the statistical test for each voxel, this is the range of the (standardised) test statistic values obtained.
- 57390 test Std Bonferroni FWE: Cut-off Z-value when using Bonferroni correction for multiple comparisons for different p-thresholds (at p=0.05 Zcrit=4.781).
- L +FDR: Cut-off Z-value when using False Discovery Rate correction for multiple comparisons for different q-thresholds (at q=0.05 Zcrit=2.03944800).
- L +permutationFWE: Cut-off Z-value when using Permutation Thresholding to correct for multiple comparisons for different p-thresholds (at p=0.05 Zcrit=2.53742700).

Significant voxels are those voxels where the Z-value surpasses the cut-off Z-value.

The significant results denote the voxels where there is a significant association between the frequency of damage to the voxel and the frequency of behavioural impairment in the patients.



To visualise the significant voxel(s) in MRIcroN, load the map containing the Z-value for each voxel (filename ending with "L<predictorname>") as overlay (over a template image) in MRIcroN.

As display range enter the cut-off Z-value on the left and the maximum Z-value observed in the data (indicated under "L Range") on the right.

Your overlay now contains the significant voxels.

4.3. Voxel-based statistical analysis with multiple predictors:

For more complex designs with multiple predictors (e.g. using lesion size as a covariate), you will have to turn to NiiStat (https://www.nitrc.org/projects/niistat/). The NiiStat website contains a detailed description of the toolbox and installation instructions, as well as a well-documented manual and tutorial.

5. How to perform a multivariate voxel-based statistical analysis using the SVR-LSM approach:

There are several tools available for performing a multivariate support vector regression based lesion symptom mapping (SVR-LSM) Analysis. Please note, that this is currently the most used technique for multivariate lesion behaviour mapping.

The following 2 papers provide background to multivariate (statistical) lesion analysis with SVR-LSM:

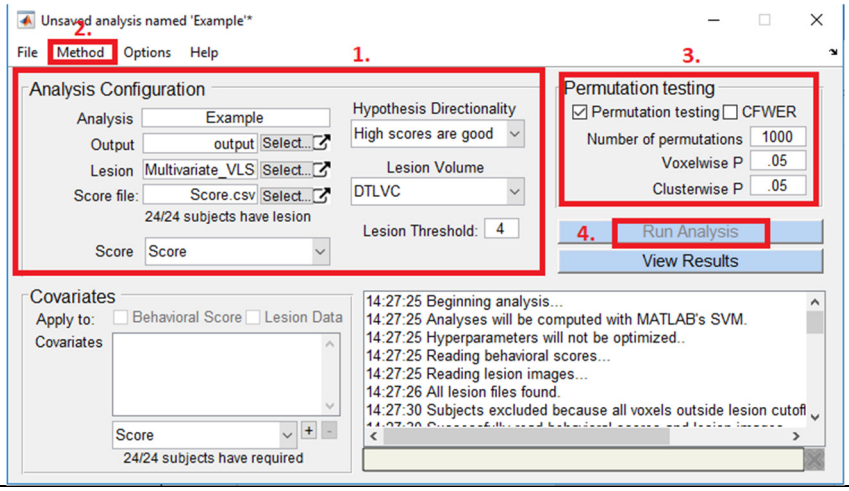
- Zhang, Y., Kimberg, D.Y., Coslett, H.B., Schwartz, M.F., Wang, Z., 2014. Multivariate lesion-symptom mapping using support vector regression. *Hum. Brain Mapp.* 35, 5861–5876.
- DeMarco, A.T., Turkeltaub, P.E., 2018. A multivariate lesion symptom mapping toolbox and examination of lesion-volume biases and correction methods in lesion-symptom mapping. *Hum. Brain Mapp.* 21, 2461–2467.

As the analysis technique is relatively new, most of the tools are still in development. This means that none of the tools is currently able to perform everything in this domain. In most cases, they are restricted to linear or non-linear processing or to specific statistical thresholding procedures (e.g. FDR, FWE voxelwise, FWE clusterwise, …). Here, we only go through one of the tools, SVR-LSM GUI, which has a graphical user interface. Alternatively, you can also use the SVR-LSM tool by Zhang and colleagues (2014), or the NiiStat toolbox (see Appendix).

5.1. SVR-LSM GUI (DeMarco & Turkeltaub, 2018):

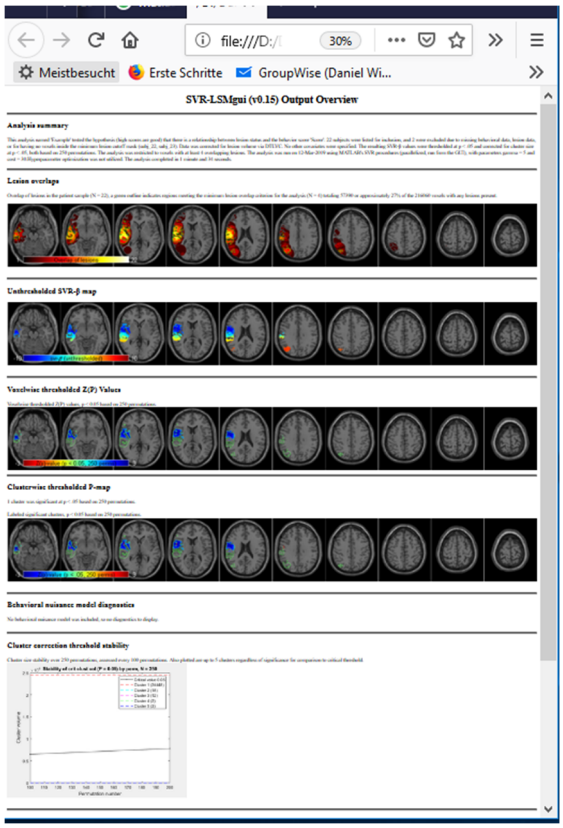
This toolbox can be recommended, if you are not familiar with Matlab scripting and can be downloaded from https://github.com/atdemarco/svrlsmgui. You need at least Matlab 9.2 (2017a) and should optimally install SPM12 as well as the Matlab Statistics and Machine Learning toolbox. The analysis performed with the toolbox is non-linear (e.g. epsilon-SVR with Radial Basis Function Kernel). This corresponds to the most frequently used analysis procedure currently employed in the field. One downside is that the toolbox is not able to compute a FDR correction out of the box, but relies either on cluster-wise FWE, or a new technique called CFWER (for details, see Mirman et al., 2015). Nevertheless, the results contain an unthresholded p map, which can be manually FDR corrected using online available Matlab scripts.

Once the SVR-LSM toolbox folder has been added to the Matlab path ("File -> Set Path -> Add Folder"), the SVR-LSM graphical user interface (GUI) can be started with the command "svrlsmgui".



1. Specify your input (Score file & Lesion data path) and output path, lesion volume correction (dTLVC recommended), minimum number of lesion affection ("Lesion Threshold").
2. Under "Methods" you can define different settings, as for example the SVR implementation and the parameters C, ε and γ. For a basic SVR-LSM analysis, you can leave the defaults. [Note that γ is handled differently for the Matlab SVR or libSVM SVR implementation. If you want to specify a γ of 4 for the Matlab SVR implementation, you need to transform γ into sigma = $1/\sqrt{(\gamma)}$ and enter that value].
3. Specify the number of permutations (at least 4000) and type of multiple comparisons thresholding. Note, that the voxelwise thresholded p parameter creates a map uncorrected for multiple comparisons at the specified p threshold. Therefore, we largely recommend to either use the cluster level FWE approach, the new CFWER approach or applying an FDR correction manually on your resulting untresholded voxelwise p map.
4. If you want to perform an FDR correction after running the analysis, you should keep the permutation number high (e.g. ~10.000), as **low permutation numbers** might result in an **inaccurate FDR** application. If you have behavioural covariates included in the behavioural score file, you can easily add them at the covariates section.
5. After setting up the analysis parameters, push the "Run Analysis" button.

Once the analysis is finished, a quick overview about the results can be found in the "overview.html" file, which can be opened in nearly every internet browser. Additionally, you are able to find unthresholded and thresholded p maps with original p values as well as an inverse p map in the output folder. In most cases, the inverse p maps are those you want to use for visualization.



The text in the "Analysis Summary" section will give you a report about the settings with which you ran the analysis. The "Lesion overlaps" section show a lesion overlap plot overlaying the lesions in your dataset. The "unthresholded SVR-β map" section shows the beta map weights before statistical inference via permutation testing. Please do not interpret these beta weights directly, as they differ from classical regression weights in univariate testing. Hence there is no linear relationship between p-values and SVR-β weights. The next 1-2 sections are defined by the correction for multiple comparisons you used. The "Voxelwise thresholded Z(P) Values" show the results as z-statistic, unthresholded for multiple comparisons at the defined p cut-off level. The "Clusterwise thresholded P-map" show if there are any clusters surviving the cluster-wise FWE multiple comparisons correction. The "Behavioral nuisance model diagnostics" section show the results and effect of your nuisance variable corrections as for example lesion volume control by regression. Finally there is a report about "cluster size stability" (if cluster-wise FWE is used) and a "Hyperparameter quality report" showing how good the multivariate model performs with the used hyperparameter set (C, ε and γ). If the performance is very low (low prediction accuracy and large mean absolute difference), you should consider running an optimization procedure as explained in the Appendix.

For visualization of the results in MRIcroN, you need to load the inverse p map ".nii" file as overlay over the background "ch2" template. For cluster-wise FWE correction, you can load the "Signif clusts cluster pvals (inv).nii" file as overlay and set the lower bound intensity to 1 - your critical p value (for $p < 0.05$ you enter 0.95) and the upper bound to 1. Then you will see all significant clusters in your results. Each cluster will have a unique intensity value.

If you aim to use FDR correction and have determined manually the critical threshold, then open up the "Untresholded P map (inv).nii" file as overlay and enter the critical FDR corrected p value again as 1 – p, while setting the upper bound to 1. Alternatively, you can also check the unthresholded results, "Unthresholded P map (inv).nii" and enter an uncorrected inverse p threshold as lower bound (e.g. 1 - 0.01 = 0.99).

For generating an atlas report, you can use the built-in functionality in MRIcroN (→ Load the atlas as background → Overlay your thresholded statistics image → Draw → Descriptives).

6. How to perform a multivariate voxel-based statistical analysis using the SCCAN approach:

Sparse canonical correlations based lesion symptom mapping is a completely different approach. It has only very recently been introduced and validated and is currently only available in one single toolbox (LESYMAP) which relies on the statistical software package R.

The following paper provides background to multivariate (statistical) lesion analysis with SCCAN:

- Pustina, D., Avants, B., Faseyitan, O.K., Medaglia, J.D., Coslett, H.B., 2018. Improved accuracy of lesion to symptom mapping with multivariate sparse canonical correlations. *Neuropsychologia* 115, 154–166.

6.1 LESYMAP (Pustina et al., 2018):

LESYMAP is available under the following link (https://dorianps.github.io/LESYMAP/). The package is completely command line based. Therefore, you should have some basic programming knowledge before using it. The most complicated part of this tool is however the installation process, as it relies on a relatively large number of R packages. Most errors encountered during installation will probably be based on missing dependencies of different R sub packages. In case of any installation errors, the first step would be to check the error output and to install the missing dependencies individually following the error output recommendations. The author also provides a video on about the installation process, which can be found here: https://www.youtube.com/watch?v=HSK2txFvbMU&feature=youtu.be. After successful installation, the use of the toolbox is straight forward, as it comes with a well-documented manual and an example dataset. Note that this toolbox is also able to perform univariate lesion-behaviour mapping and will be complemented by SVR-LSM in a future release. For better folder visualization and navigation of the command line interface and path structure, you can use Rstudio (https://www.rstudio.com/).

First create a folder "lesydata" with two subfolders "behavior" and "lesions". In the "behavior" folder, you store your behavioural data as a text file. Your binary lesion data should be stored in the "lesions" folder (either as .nii or .nii.gz files). Then, enter the following commands to read in the data:

```
lesydata = file.path(["exchange with path to your lesydata folder"])

filenames = Sys.glob(file.path(lesydata, 'lesions', 'Subject*.nii.gz'))

behavior = Sys.glob(file.path(lesydata, 'behavior', 'behavior.txt'))

template =
antsImageRead(Sys.glob(file.path(find.package('LESYMAP'),'extdata',
'template', 'ch2.nii.gz')))
```

To run the SCCAN analysis, save the results and to get a simple visualization of the results you can use the following commands:

```
lsm = lesymap(filenames, behavior, method = 'sccan',
optimizeSparseness=TRUE)

save.lesymap(lsm, saveDir='[specify the path where you want to save the
results]')

plot(template, lsm$stat.img, window.overlay = range(lsm$stat.img))
```

For further information on the different parameter definitions and functionalities, please read the manual of the toolbox.

7. Suggested reading

Clusterize Toolbox for semi-automated lesion delineation

- Clas P, Groeschel S, Wilke M (2012). A semi-automatic algorithm for determining the demyelination load in metachromatic leukodystrophy. *Academic Radiology* 19: 26-34.
- de Haan B, Clas P, Juenger H, Wilke M, Karnath H-O (2015). Fast semi-automated lesion demarcation in stroke. *NeuroImage: Clinical* 9: 69-74.

Clinical Toolbox for automatic normalisation of brains and lesions:

- Rorden C, Bonilha L, Fridriksson J, Bender B, Karnath H-O (2012). Age-specific CT and MRI templates for spatial normalization. *Neuroimage* 61: 957–965.

Spatial normalisation of lesioned brains:

- Brett M, Leff A P, Rorden C, Ashburner J (2001). Spatial normalization of brain images with focal lesions using cost function masking. *Neuroimage* 14: 486-500.
- Crinion J, Ashburner J, Leff A, Brett M, Price C, Friston K (2007). Spatial normalization of lesioned brains: performance evaluation and impact on fMRI analyses. *Neuroimage* 37: 866-875.
- Nachev P, Coulthard E, Jäger HR, Kennard C, Husain M (2008). Enantiomorphic normalization of focally lesioned brains. *Neuroimage* 39: 1215-1226.
- Andersen SM, Rapcsak SZ, Beeson PM (2010). Cost function masking during normalization of brains with focal lesions: Still a necessity? *Neuroimage* 53: 78-84.

(Statistical) Lesion analysis:

- Rorden C, Karnath H-O (2004). Using human brain lesions to infer function: a relic from a past era in the fMRI age? *Nature Reviews Neuroscience* 5: 813-819.
- Rorden C, Karnath H-O, Bonilha L (2007). Improving lesion-symptom mapping. *Journal of Cognitive Neuroscience* 19: 1081-1088.
- Zhang, Y., Kimberg, D.Y., Coslett, H.B., Schwartz, M.F., Wang, Z., 2014. Multivariate lesion-symptom mapping using support vector regression. *Human Brain Mapping* 35, 5861–5876.
- Pustina, D., Avants, B., Faseyitan, O.K., Medaglia, J.D., Coslett, H.B., 2018. Improved accuracy of lesion to symptom mapping with multivariate sparse canonical correlations. *Neuropsychologia* 115, 154–166.
- DeMarco, A.T., Turkeltaub, P.E., 2018. A multivariate lesion symptom mapping toolbox and examination of lesion-volume biases and correction methods in lesion-symptom mapping. *Human Brain Mapping.* 21, 2461–2467.

8. Full download links

- MRIcroN & NPM: https://www.nitrc.org/projects/mricron (if the latest version [2MAY2016] does not work on your OS [e.g. if you still have a 32-bit Windows OS: in the newest version NPM is only 64-bit compatible], try older versions, by selecting "See All Files").
- Clusterize Toolbox for SPM: http://www.medizin.uni-tuebingen.de/kinder/en/research/neuroimaging/software/
- SPM12: http://www.fil.ion.ucl.ac.uk/spm/software/spm12/
- Clinical Toolbox for SPM: http://www.nitrc.org/projects/clinicaltbx/
- NiiStat: https://www.nitrc.org/projects/niistat/
- SVR-LSM GUI: https://github.com/atdemarco/svrlsmgui.
- SVR-LSM (Zhang et al., 2014): https://github.com/yongsheng-zhang/SVR-LSM or https://github.com/dmirman/SVR-LSM
- LESYMAP: https://dorianps.github.io/LESYMAP/

9. Appendix

9.1. Clinical Toolbox modifications:

The Clinical Toolbox assumes a typical stroke population (i.e. elderly subjects) and this is reflected in the default spatial normalisation templates used by this toolbox:

- The "CT normalize" routine uses a CT template derived from older subjects (population described in Rorden et al., 2012)
- The "MR normalize" routine offers the choice between using a T1 template derived from younger subjects (the standard SPM T1 template), a T2 template derived from younger subjects (the standard SPM T2 template), or a T2FLAIR template from the Glahn Group (mean age 35.44 years [std. dev.: 11.11, min.: 18, max.: 69], with 205 females and 161 males, population described on https://brainder.org/download/flair/).
- The "MR normalize-segment" routine offers the choice between spatial tissue priors derived from older subjects (population described in Rorden et al., 2012) or spatial tissue priors derived from younger subjects (the standard SPM priors).

Thus, if you want to use the Clinical Toolbox in other populations (e.g. children), you will have make a few changes:

First, of course, you will need to find or make spatial normalisation template(s) appropriate for your population. If you have these templates, place them in the "Clinical" directory. Secondly, you will have to slightly modify the code of the Clinical Toolbox so that it will use your template instead of one of its default templates (Note: more elegant solutions are possible, but would require extensive modifications to the code, I have simply listed the simplest solution here):

- If you want to change the template used in the "CT normalize" routine, you will need to edit the file "**clinical_ctnorm.m**" in the "Clinical" directory. The critical line that defines the template is line 22, which reads
  "**cttemplate = fullfile(fileparts(which(mfilename)), 'scct.nii');**".
  This line defines that the template file "scct.nii" is used by default when you select the "CT normalize" option of the Clinical Toolbox. Thus, if you want to use a different CT/CAT template, enter the filename of your own CT/CAT template instead of "scct.nii". Now when

you run the "CT normalize" routine of the Clinical Toolbox, it will use your preferred template instead.

- If you want to change the template used in the "MR normalize" routine, you will need to edit the file "**clinical_mrnorm.m**" in the "Clinical" directory. The critical line is line 152, which reads
  "**FLAIRtemplate = fullfile(fileparts(which(mfilename)),'GG-366-FLAIR-2.0mm-8mmFWHM.nii');**".
  This line defines that, when selecting "FLAIR" as modality in the "MR normalize" option of the Clinical Toolbox, the template file "GG-366-FLAIR-2.0mm-8mmFWHM.nii" is used by default. Thus, if you want to use a different MR template, enter the filename of your own MR template instead of "GG-366-FLAIR-2.0mm-8mmFWHM.nii". Now when you run the "MR normalize" option of the Clinical Toolbox and select "FLAIR" under "Modality", it will use your preferred template instead.
- If you want to change the spatial tissue priors used in the "MR normalize-segment" routine, you will need to edit the file **"clinical_mrnormseg.m"** in the "Clinical" directory. The critical lines are lines 154-156, that read
  "gtemplate = fullfile(fileparts(which(mfilename)),"scgrey.nii");
  wtemplate= fullfile(fileparts(which(mfilename)),"scwhite.nii");
  ctemplate = fullfile(fileparts(which(mfilename)),"sccsf.nii");"
  These lines define that the spatial priors "scgrey.nii" (grey matter prior), "scwhite.nii" (white matter) and "sccsf.nii" (csf) are used by default when selecting "T1 older" as template in the "MR segment-normalize" option of the Clinical Toolbox. Thus, if you want to use different spatial tissue priors, enter the filenames of your own spatial priors instead (gtemplate = grey matter prior; wtemplate = white matter prior; ctemplate = csf prior). Now when you run the "MR normalize-segment" option of the Clinical Toolbox and select "T1 older" under "Template", it will use your preferred spatial priors.

With these changes, the Clinical Toolbox can be used in all populations where spatial normalisation is possible (i.e. for which spatial normalisation templates are available).

9.2. SVR-LSM (Zhang et al., 2014) and optimization procedure:

Zhang and colleagues (2014) published a publicly available repository of scripts to perform a SVR-LSM analysis. These scripts can be downloaded here: https://github.com/yongsheng-zhang/SVR-LSM or https://github.com/dmirman/SVR-LSM. The latter contains additionally a 5-fold cross-validation routine for finding the optimal set of analysis parameters and was added by Daniel Mirman. To our knowledge, this is the only tool, which allows a parameter optimization. This can be an important step in improving your results. However, these scripts require to handle a command line interface (e.g. Matlab) and therefore, if you don´t have any programming knowledge we largely recommend to use either NiiStat (for SVR-LSM with linear kernel) or SVR-LSM GUI (for SVR-LSM with RBF, non-linear kernel). It might be that you need to install further Matlab packages to be able to use the tool.

The first lines of the main script "SVR_LSM_toolbox.m" (12-36) specify the path structure of your lesion and behavioural files as well as the specific settings for the analysis. This main script controls and targets all the other functions of the tool. The epsilon-SVR with RBF (default) allows to specify three parameters, C, ε and γ. In the tool, these parameters are defined by default as 20, 0.01 and 2 respectively. For the best performance you should utilize the "optimize_parameters.m" function from the Daniel Mirman variant of the SVR-LSM toolbox.

To do the optimization, first run the main script "SVR_LSM_toolbox.m" manually until line 88 while omitting line 61. After completion, the necessary data is loaded in the Matlab workspace and can be send to the "optimize_parameters.m" function by entering the following commands into the Matlab command line:

```
svr_cost = 1:50;
svr_gamma = 0.5:10;
[cost_best, gamma_best, acc] = optimize_parameters(variables, svr_cost, svr_gamma);
```

This will run a 5-fold cross validation grid search with a parameter range of 1-50 for C and 0.5-10 for γ in steps of 1. If another parameter range is needed, svr_cost and svr_gamma should be modified before running the "optimize_parameters.m" function. cost_best and gamma_best will return the best C and γ for the dataset. The variable "acc" is returned, which is the prediction accuracy of the multivariate model. After that, the final analysis can be run by changing the general parameter variables for C and γ, running the following commands:

```
parameters.cost = cost_best;
parameters.gamma = gamma_best;
```

Now the remaining lines of the "SVR_LSM_toolbox.m" script can be run from line 90 on. Alternatively, you use the SVR-LSM GUI tool with the now detected optimal parameters.

========== FDR q < 0.0500, equivalent to p < 0.0140 index | atlas_name | voxel number 1, Precentral_L, 13787 out of 28174 voxels 3, Frontal_Sup_L, 26407 out of 28915 voxels	The tool creates an unthresholded probability map which can be opened up in visualization software packages as MRIcron. If some voxels are surviving the FDR thresholding, the critical p is saved to the generated report. For instructions on how to visualize in MRIcroN, we refer to 5.1.

9.3. SVR-LSM using Niistat:

The NiiStat toolbox can be downloaded from https://www.nitrc.org/projects/niistat/. A detailed description of the toolbox and installation instructions is given at: https://www.nitrc.org/plugins/mwiki/index.php/niistat:MainPage. This toolbox currently runs an epsilon-SVR with a linear kernel as default and does not provide an optimization routine to find the optimal SVR-LSM hyperparameter C. Moreover, it relies on a default setting of 1 for C and 0.01 for ε. A large benefit of this toolbox is, that it is able to run also univariate analyses.