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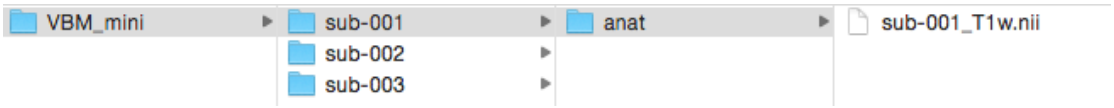
Voxel Based Morphometry Tutorial using the Geodesic Shooting Toolbox

Requirements:

- T1w MRI data for two groups (suggest ~10 subjects in each group)
- Basic demographic details for each subject (Age, Gender + any other covariates)
- MATLAB running SPM12. Make sure SPM12 and all subdirectories is in the MATLAB path (test by typing "which spm" in MATLAB prompt)

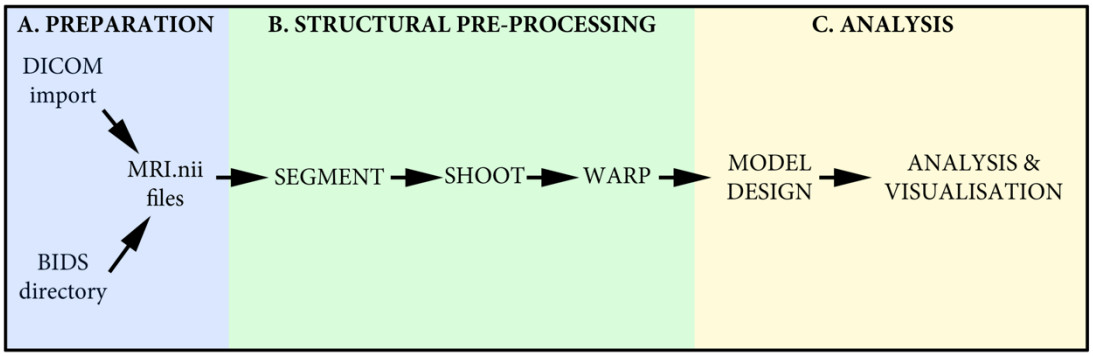
Overview:

This tutorial will go through the full processing pipeline for a two group VBM analysis using the Geodesic Shooting toolbox in SPM. The full analysis, depending on your computer, will take ~6h to run. If this is a live demo, then sub-select three datasets to test, and then set up the full analysis in your own time.



The basic objective is to generate smooth warped modulated tissue classes from T1w MRI data in the native subject space, and used these warped images for a GLM analysis. To achieve this, we need to pre-process the data in several distinct steps that can be broadly divided into:

1. Data preparation
2. Structural pre-processing
3. Analysis



A. DATA PREPARATION

1. Setting up the directory structure

Neuroimaging experiments result in complicated data that can be arranged in many different ways. Historically, individuals have tended to use their own unique naming/directory structures. The "*Brain Imaging Data Structure*" (BIDS) Specification has been designed to standardise this to help others understand the data, aiding replication, data-sharing and collaborations. For more details see: https://bids.neuroimaging.io/bids_spec1.0.0-rc2.pdf

For this demo, we will use the anatomical and naming convention as per BIDS:

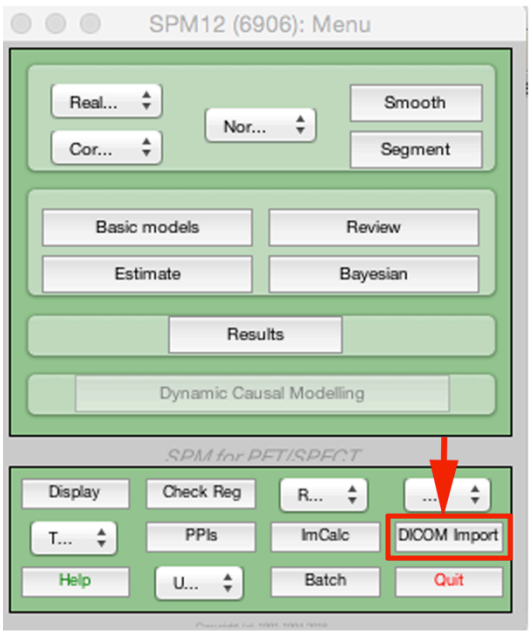
[some path]/vbmdemo/group-01/sub-001/anat/

See the end of this section for a code snippit to combine this with dicom import.

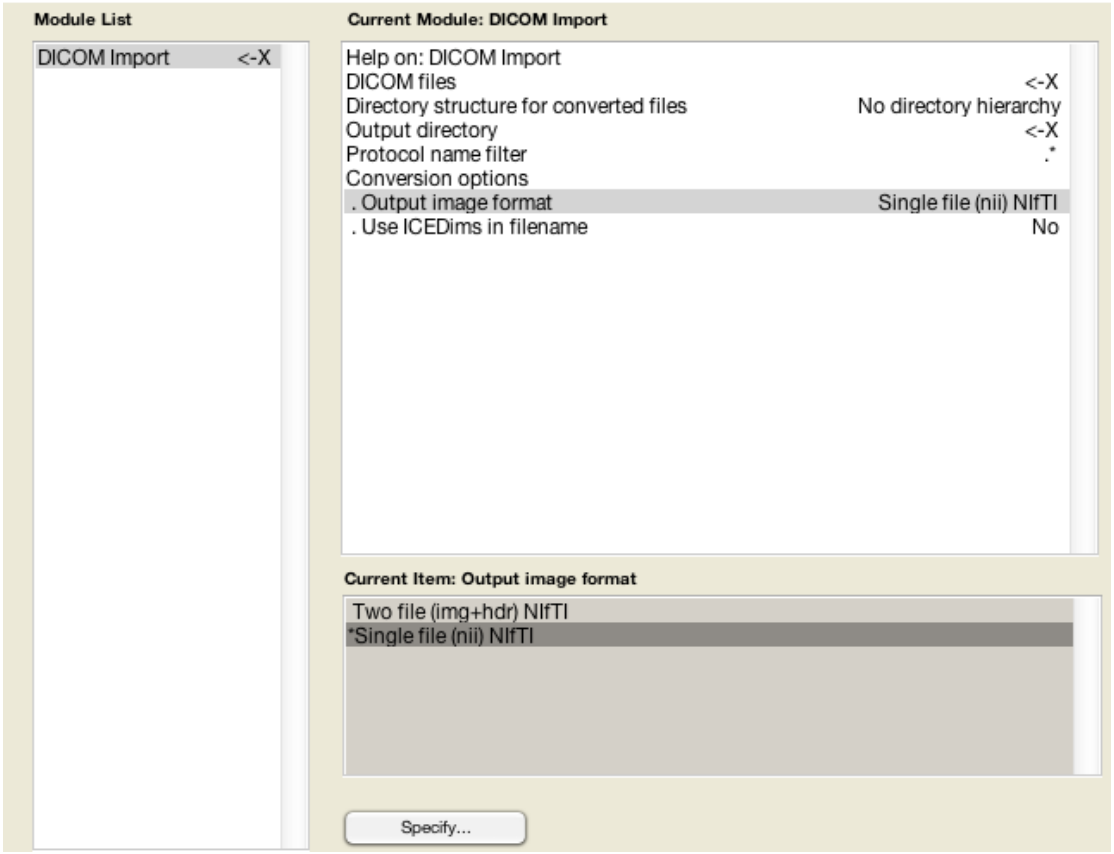
2. DICOM import

Normally, MRI data comes from the scanner in dicom file format (.dcm). To use this we first need to import the data and convert it into nifti file format. It is preferable to use "single file nifti format" rather than two-file (.img & .hdr).

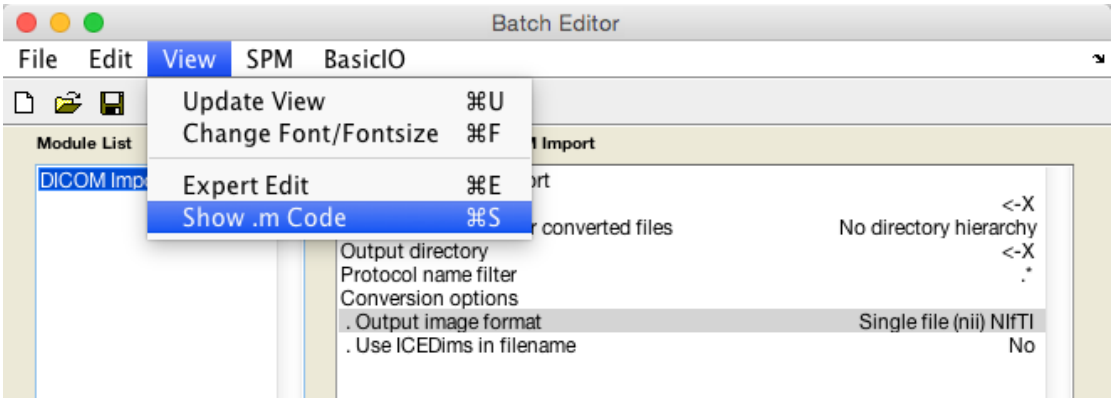
Dicom import is located on the main SPM menu:



This will open up a GUI, where you can select the .dcm data, output directory and output file format.



Obviously this could get a bit tedious with lots of data, so the easiest thing is to write a short section of code to do this. A really useful function in SPM is the View -> Show .m code:

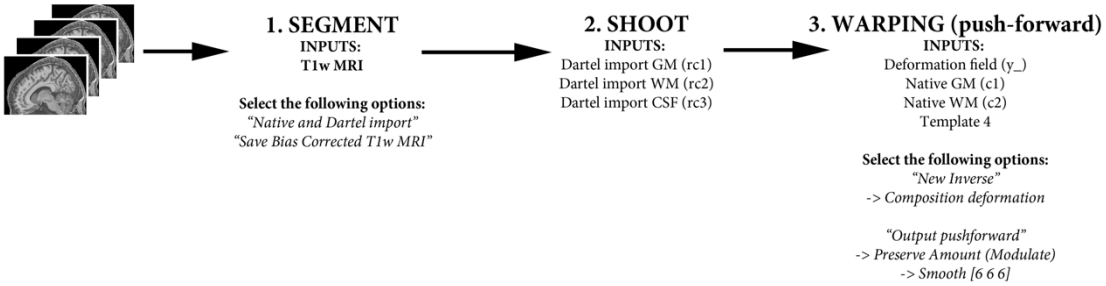


This will open a window with the corresponding code for that function. You can then copy and paste it into your own script. Over is a short example for the data import. I have modified it a bit to restructure the data following BIDs.

```
%Modify naming as required:
study='vbmdemo/';%Study
group{1}='group-01/'; %subgroup1 - For simplicity with analysis
group{2}='group-02/'; %subgroup2

%Select path
OP=spm_select(1,'dir','Select path/location to build data
directory');

%Some BIDS stuff
sub='sub-00n/';%subject numbering (N will be 1 -> max)
bidsanatomy='/anat/'; %bids anatomy format

X{1}=spm_select(inf,'dir','Directories containing GROUP1 .dcm');
X{2}=spm_select(inf,'dir','Directories containing GROUP2 .dcm');

for j=1:2
for i=1:size(X{j},1);
    target=char(strcat(OP,'/',study,group{j},sub(1:end-
2),num2str(i),bidsanatomy));
    mkdir(target);
    clear matlabbatch
    %Select dicom data in directory
    data=spm_select('FPListRec',deblank(X{j}(i,:)),'.*.dcm');

    %This was copied from show .m code, and the variables above
plugged in
    matlabbatch{1}.spm.util.import.dicom.data = cellstr(data);
    matlabbatch{1}.spm.util.import.dicom.root = 'flat';
    matlabbatch{1}.spm.util.import.dicom.outdir = cellstr(target);
    matlabbatch{1}.spm.util.import.dicom.protfilter = '.*';
    matlabbatch{1}.spm.util.import.dicom.convopts.format = 'nii';
    matlabbatch{1}.spm.util.import.dicom.convopts.icedims = 0;

    %Command to make matlabbatch run in spm
    spm_jobman('run',matlabbatch)

    %Now need to rename output to be consistent with BIDS
    NiiFile=spm_select('FPList',target,'.nii');
    newfilename=char(strcat(target,sub(1:end-
2),num2str(i),'_T1w.nii'));
    movefile(NiiFile,newfilename);
end
end

clear X group sub bidsanatomy data matlabbatch target study
```

B. STRUCTURAL PRE-PROCESSING

Here is an overview of the structural pre-processing steps we are aiming to achieve:

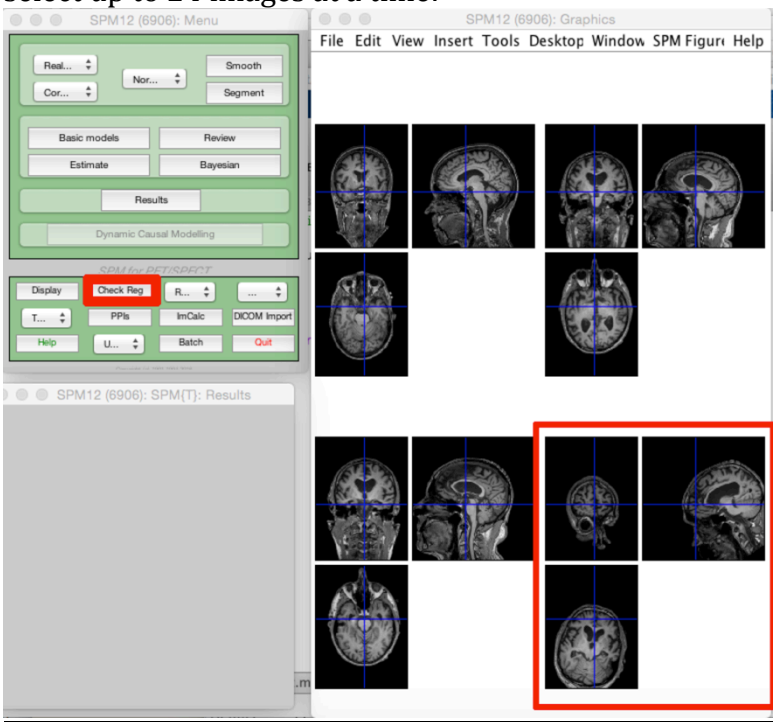


****IMPORTANT BEFORE STARTING****

While the spm pipelines do an excellent job of combining and realigning data, they do rely on the source data (i.e. all of the T1w MRIs in native subject space) being in reasonably close alignment to one another.

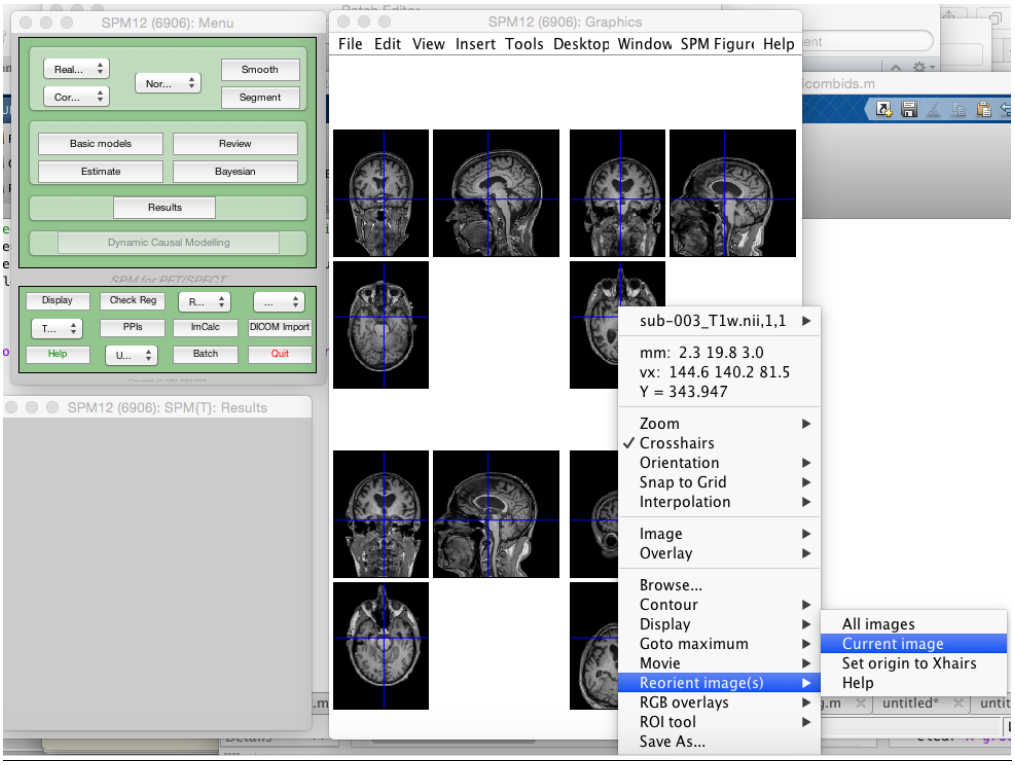
Before starting any analysis, you should ALWAYs visually check your data. This is by far and away the most common source of errors/mistakes, and it is much easier to correct at the start.

To do this, use the "Check Registration" function in the spm main menu. You can select up to 24 images at a time:

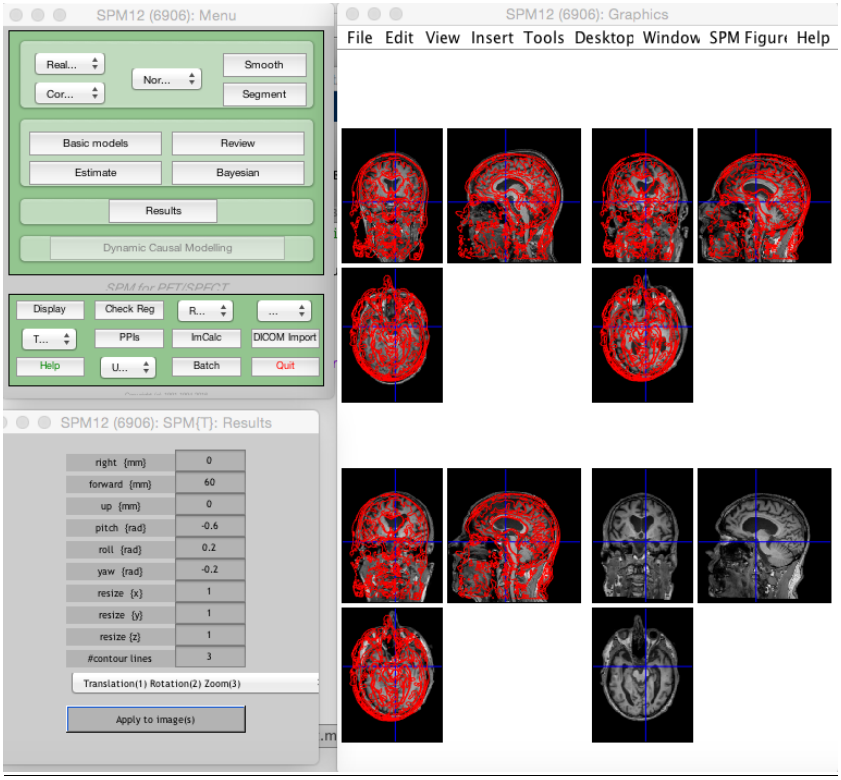


In the example above, we can see that the bottom right MRI is very poorly aligned with the rest. This is unlikely to be successfully processed and may cause problems later. It is easier to manually realign images like these at the start.

To do this, right click on the MRI with the issue, and select “Re-orientate image” and “Current image” from the menu:



This will open a window with 9 affine transforms you can manually specify until you have reasonably good alignment. Then just “Apply to images”. It is a good idea to store the transformation matrix for reference.



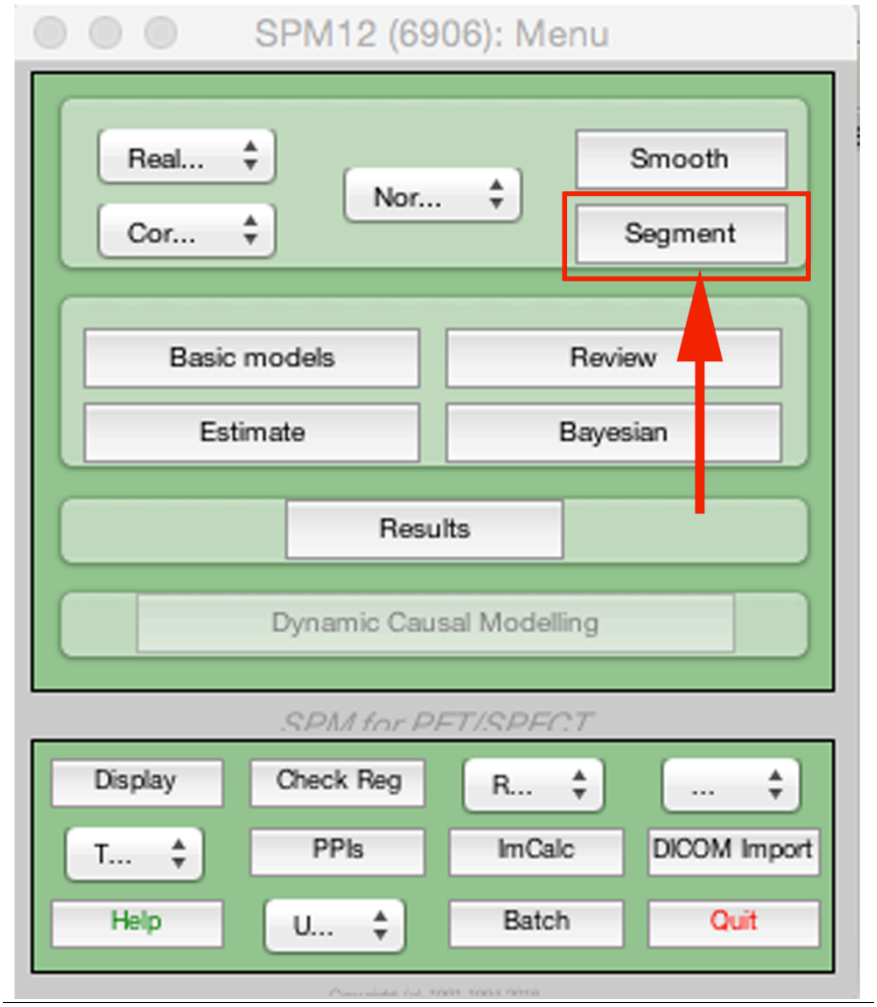
1. SEGMENTATION

Assuming your data has been successfully imported, you have checked it for any problems and corrected any issues, the next step is to segment the brain into different tissue types. SPM12 will produce up to six tissues:

- c1 – Grey Matter
- c2 – White Matter
- c3 - CSF
- c4 - Bone
- c5 – Soft tissue
- c6 – Everything else

For VBM, we are interested in analyzing the grey and white matter. In these processing steps, we will also include the CSF for generating the group template, brain mask, and calculating total intracranial volume.

First select Segment from the SPM menu:



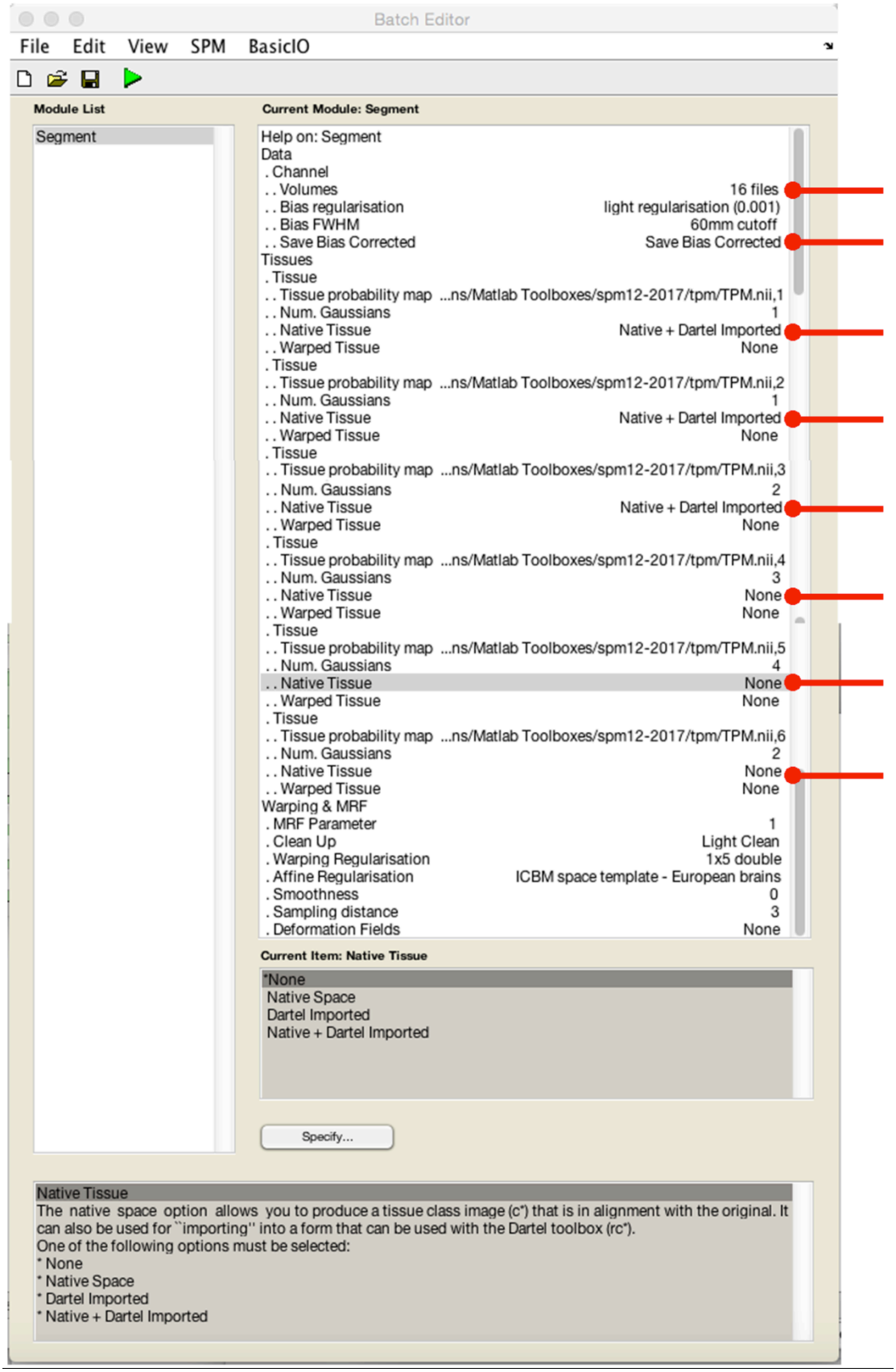
This will open up the Segment batch menu. There are a lot of options available that we will not explore here.

First double click on volumes. This will open a file select box, where we will select all of the T1w images we want to analyse:

Rather than selecting each file manually, you can speed this up using recursive search (shown above). At the end of the filter add .nii (to specify you want to look for all of the nifti files within the directories above) and then hit Rec (arrow). We will use this again later with other file specifiers.

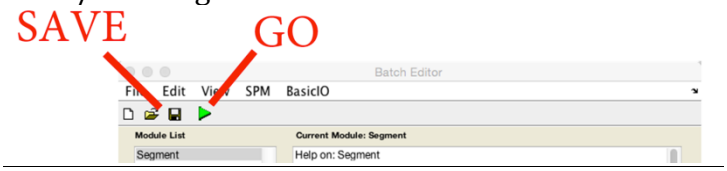
You will now need to modify the following options (summarized on the figure over):

- Save Bias Corrected: Change to Save bias corrected (for making group average brain to project results on later).
- Native Tissue: Native and Dartel imported for tissue classes 1-3
- Native Tissue: None for tissue classes 4-6



Above: Summary of Segment settings. Note this is a merged image and you will need to scroll down to see all the options.

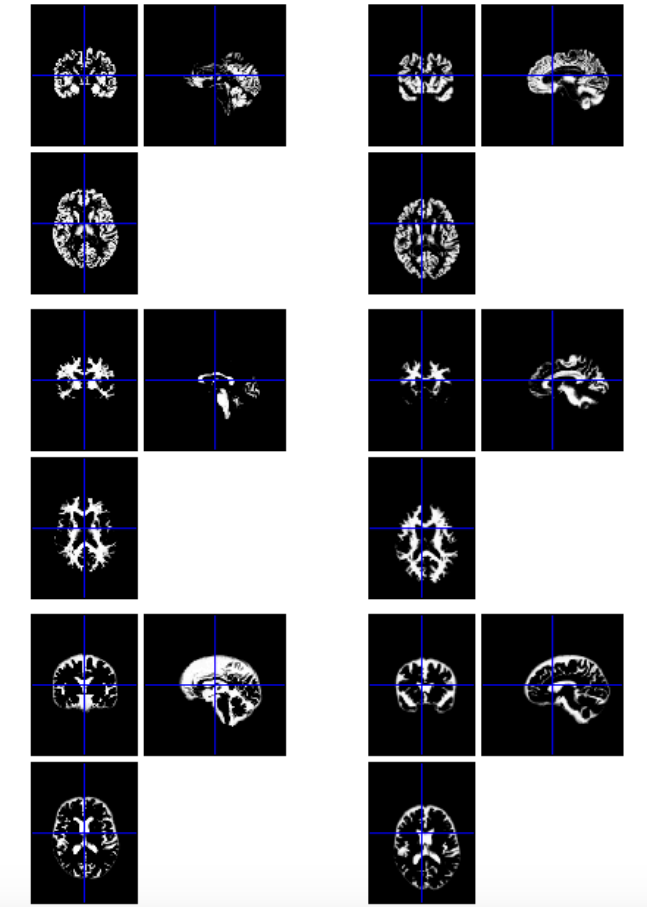
Before processing the data, it is a good idea to save the batch incase you need to re-do/check again later.



This may take a little while to complete, depending on the size of your dataset and computer. The output in each file directory will be:

- c1[filename].nii – Native space grey matter segmentation
- c2[filename].nii – Native space white matter segmentation
- c3[filename].nii – Native space CSF segmentation
- rc1[filename].nii – Dartel import space grey matter segmentation
- rc2[filename].nii – Dartel import space white matter segmentation
- rc3[filename].nii – Dartel import space CSF segmentation
- m[filename].nii – Native space bias corrected T1w
- [filename]_seg8.mat – Various normalization transform data

Below is a figure showing native c1 (left) and dartel import (right) segmentations. Basically, dartel import is orientated (rigidly aligned) with MNI space, meaning every subject's data is in reasonably close alignment with one another which will help produce better results using the warping algorithms.

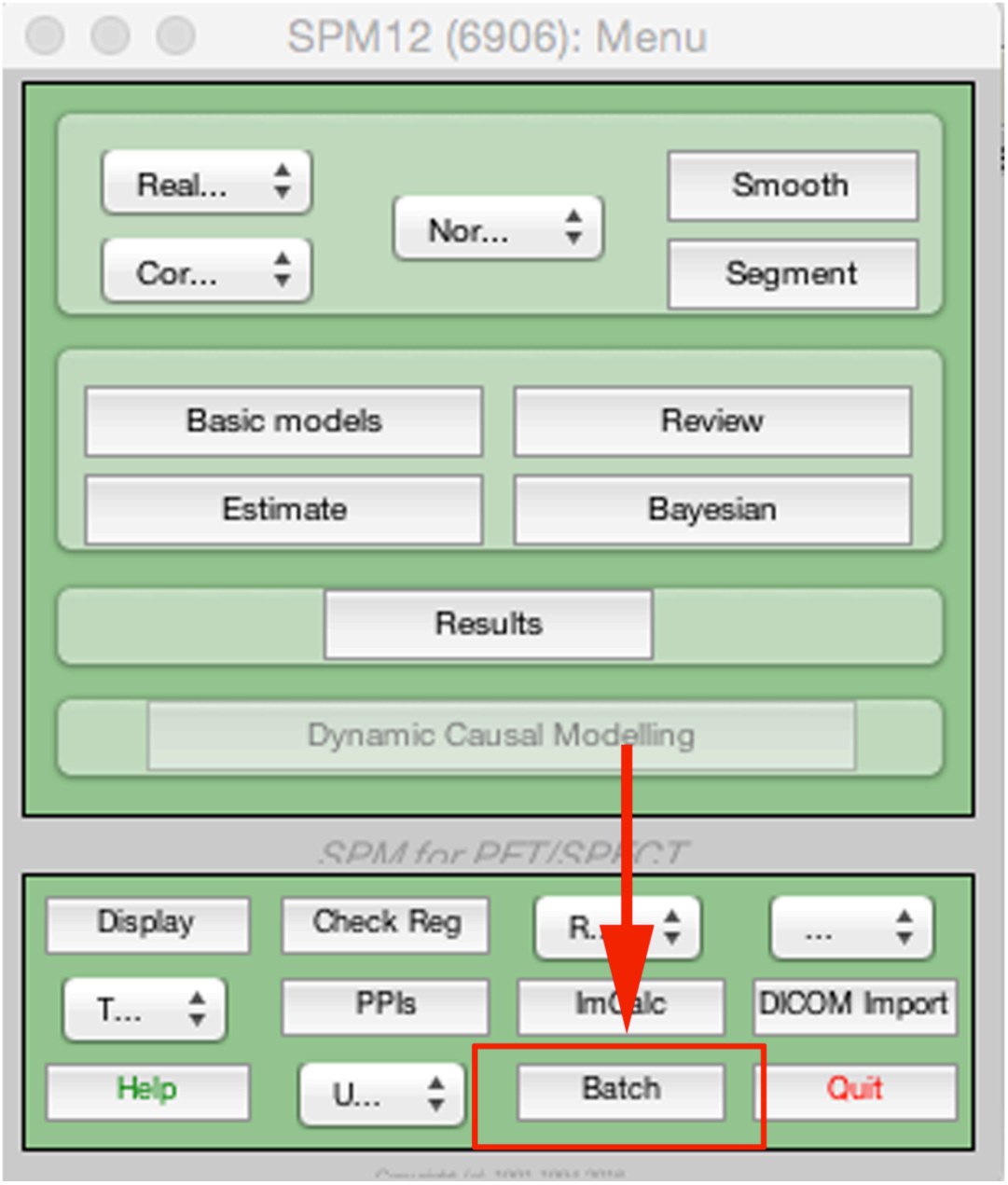


<u> It is a good idea to visually check your segmentation results for errors/problems at this stage before moving onto Shoot.**</u>**

2. SHOOT

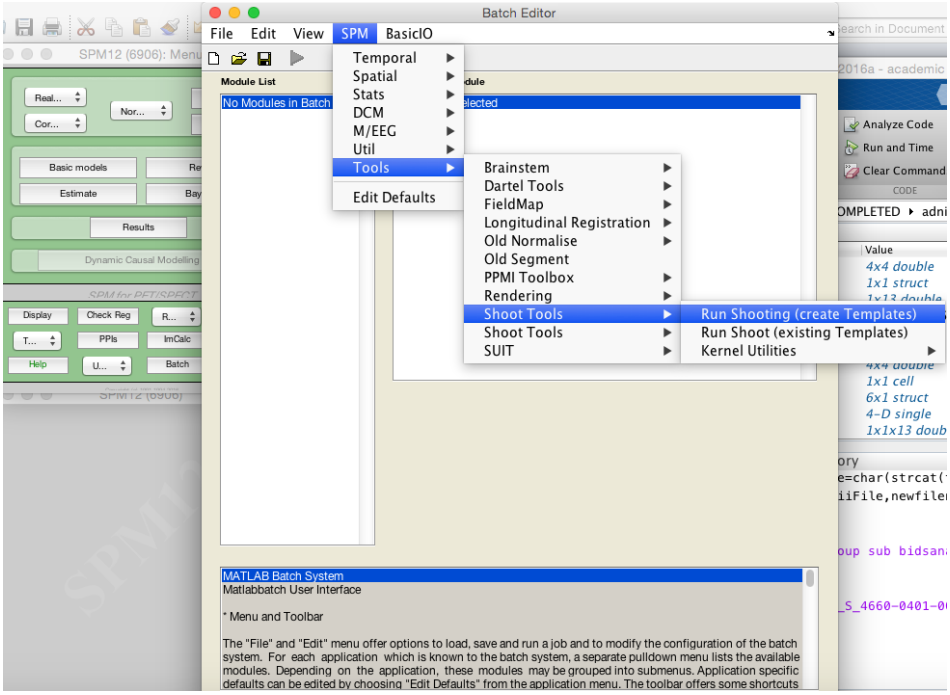
Geodesic shooting is a diffeomorphic warping algorithm designed to non-linearly align the brain data. It has recently been developed building on the earlier "Dartel" pipeline. As such, it does not yet have as many additional functions compared to Dartel (e.g. Normalise to MNI space, warp many etc.,), and therefore requires some additional manual steps to use (covered here). However, the overall impression is that it is better than Dartel and will be increasingly used in the future, therefore it is the pipeline used here.

To use, first open the SPM batch window:

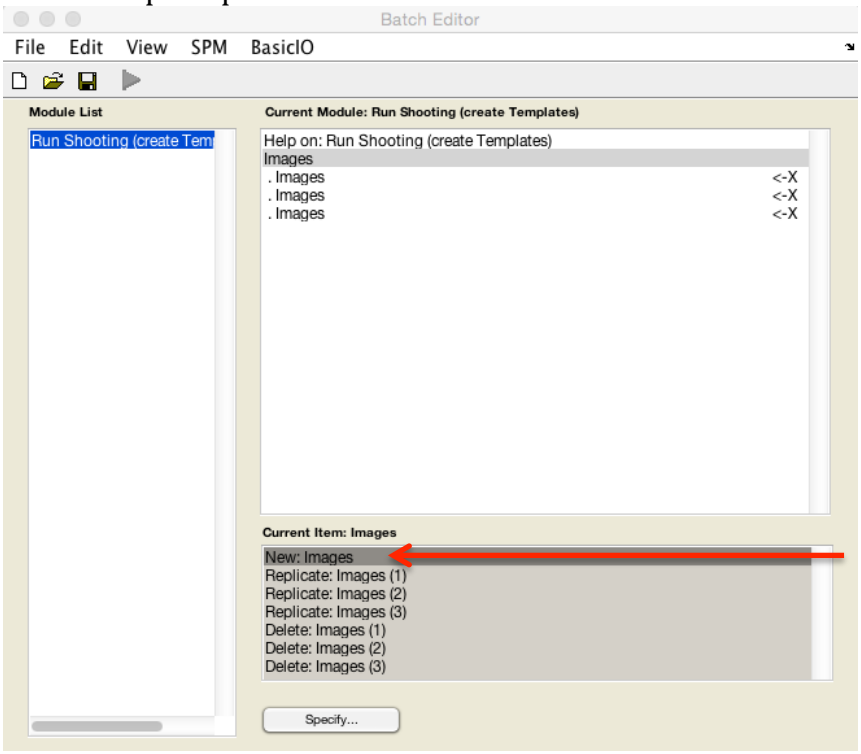


From there, you want to select:

- SPM – Tools – Shoot Tools – Run Shooting (Create Templates)



This will open up the Shoot batch window:



Click on "New: Images" (arrow) for each tissue class you are going to use for generating the population average template. Here I am going to use GM, WM and CSF so I create three "Images" channels.

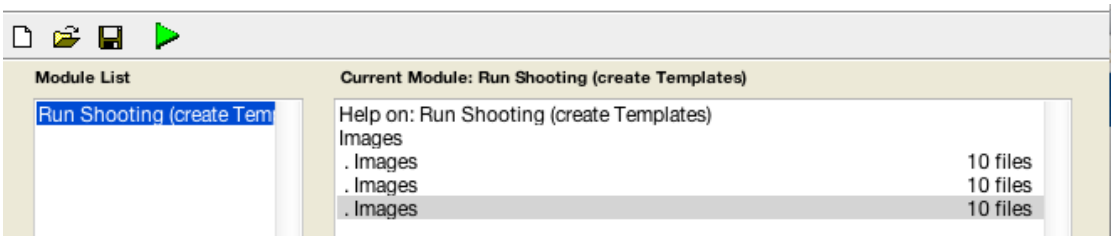
Now double click on each image channel to add the data. Here you will need to select your "Dartel Import" tissue classes. Again I'm going to use recursive search by typing:

^rc1.*

And pressing Rec (arrow). This will find all the GM. Then click done.

Repeat this process for the remaining two channels, using ^rc2.* for WM and ^rc3.* for CSF.

When you inputted the data correctly, the arrow should go green. Again it is sensible to save your batch at this stage.

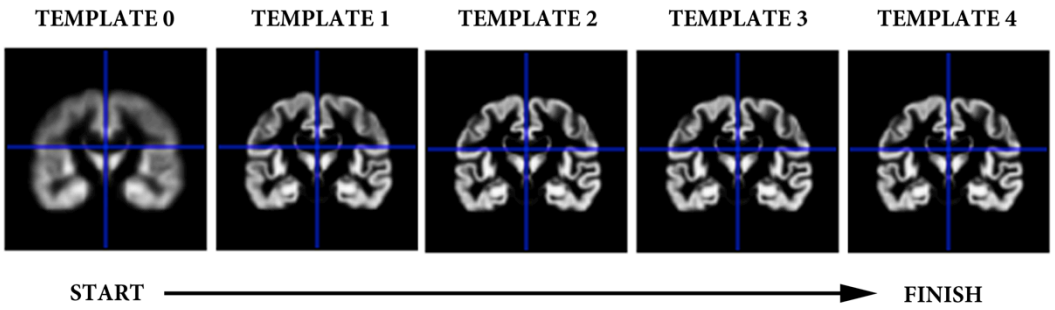


<u>This is the slowest part of the entire pipeline, and may take hours-days depending on data size/computer.</u>

The output in each file directory will be:

- y_rc1[filename].nii – Deformation field
- j_rc1[filename].nii – Jacobian determinants
- v_rc1[filename].nii – Velocity field

Additionally, in the first directory you will find five Template images (0-4). These are 4D files (containing GM, WM, CSF, Other) showing various stages in estimating the POPULATION AVERAGE:



Importantly, this process estimates a POPULATION AVERAGE TEMPLATE. This is a type of group average space that is unique to your study population. This is NOT MNI space (MNI is a type of population average space), however it will be very close.

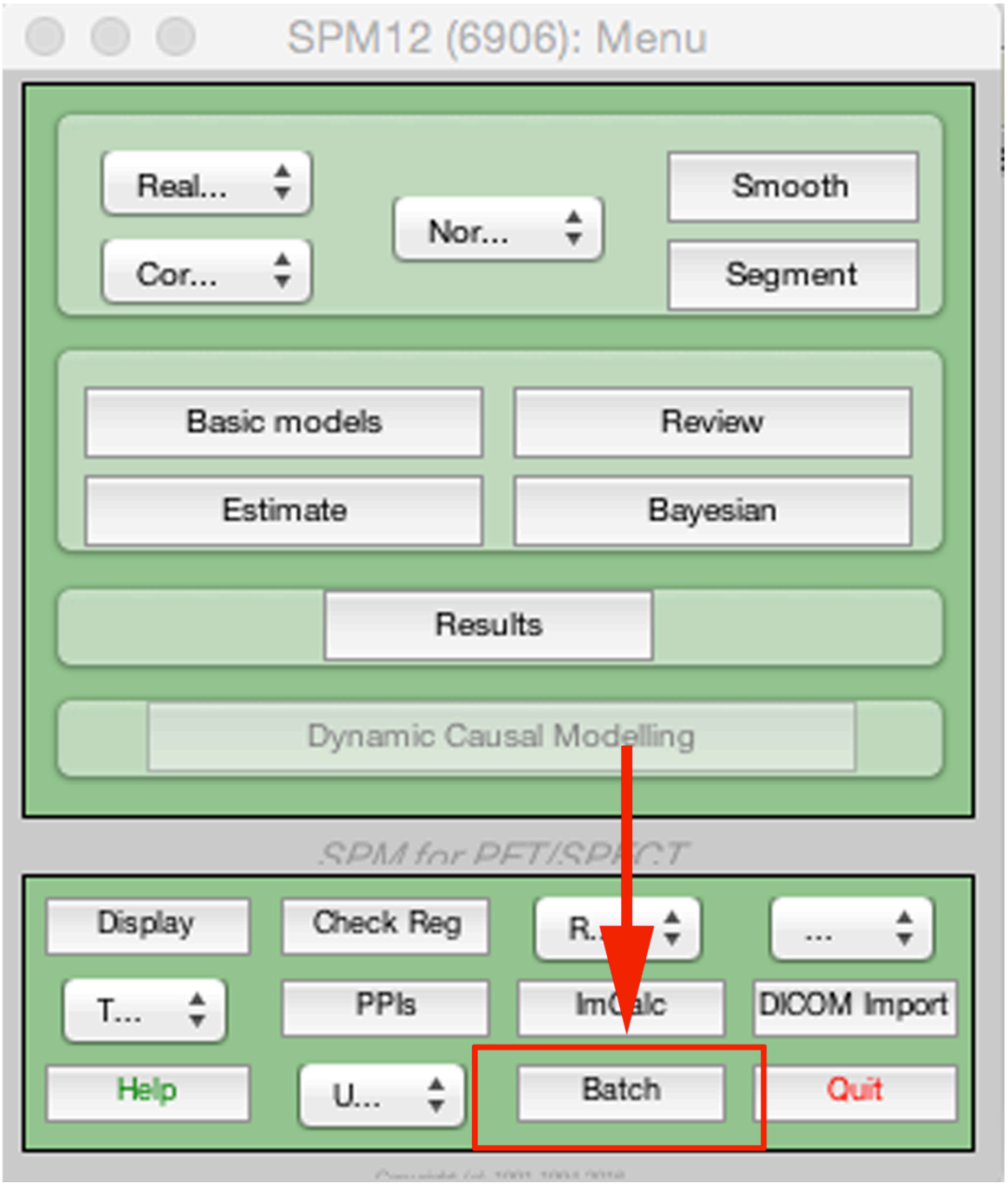
You can do and publish your entire analysis in your specific population average space.

If you really want your results in MNI space (e.g. for data-sharing results maps/coordinates), you can do this using the Co-registration function (MNI = reference, population average = source, other = Any results/data you want to move to MNI).

2. WARPING

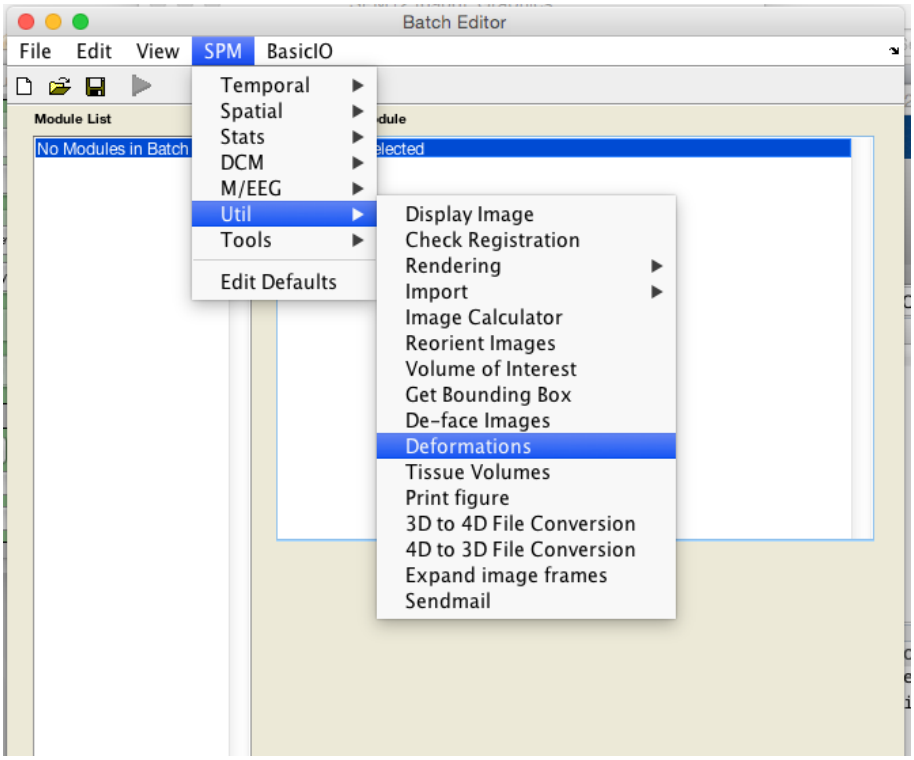
To analyse our brain data, we need to warp it to the group average space. To do this, we will use the deformation fields (y_[filename].nii) we have just generated using the Shoot Toobox.

To do this, we need to open the spm batch menu again



From here we need to select:

- SPM -> Util -> Deformations



This will provide a menu with a LOT of options for normalizing data. The approach we will use is called "Push-forward". This has the advantage that we can also simultaneously modulate and smooth the data, cutting down the number of steps we have to do.

To use push-forward, we need to select:

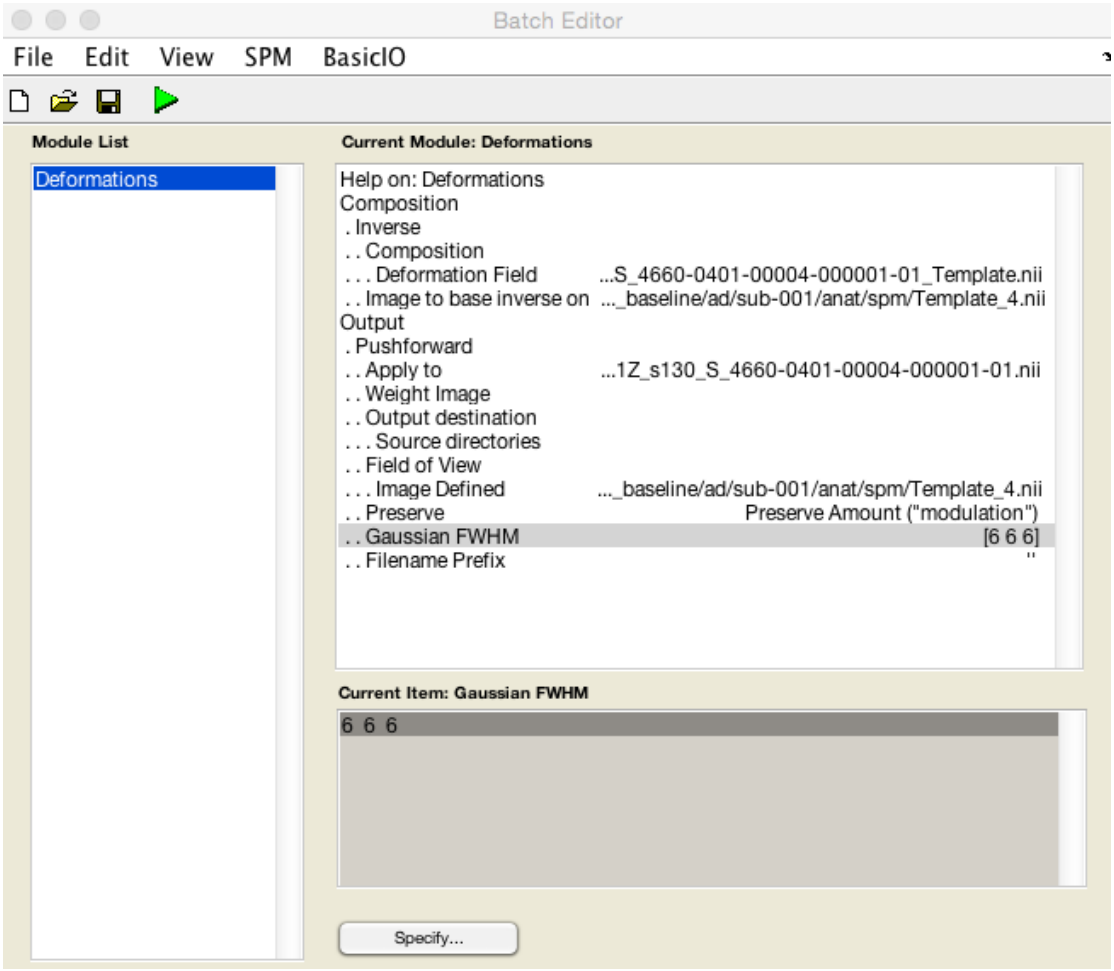
Composition: New Inverse (*this will add a new submenu*)

Inverse
-Composition: New Deformation
---> Deformation Field: Input the y_[filename].nii
---> Image to base inverse on: Input Template_4.nii

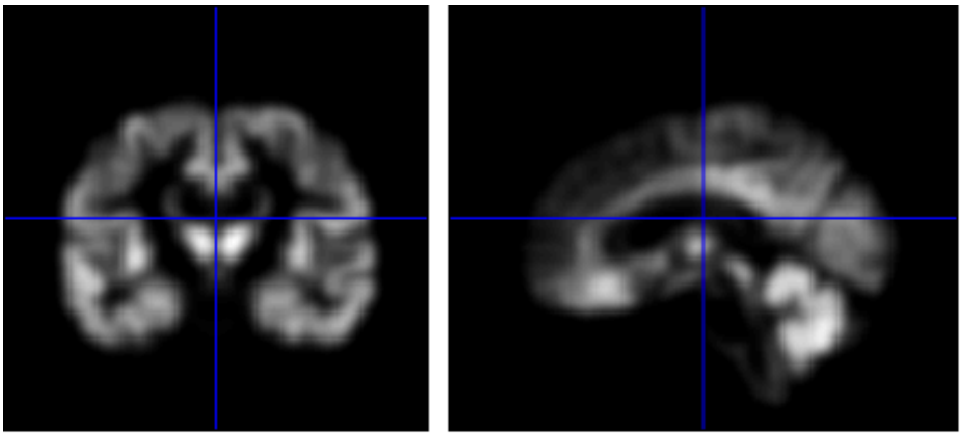
Output: New Pushforward (*this will add a new submenu*)

Pushforward
-Apply to: Put in image(s) to be warped (i.e. c1 and c2, NOT dartel import)
--> Output destination: Source directory
--> Field of view: Image Defined (*this will add a new submenu*)
----->Image Defined: Input Template_4.nii
--> Preserve: Preserve Amount ("Modulation")
--> Gaussian FWHM: [6 6 6] (*smoothing kernel*)

This is summarized in the figure over



Save the batch. When you hit go, it should output smw[inputfilename].nii files in the folder containing the MRI data. This is the **S**moothed **M**odulated **W**arped images that you will use for your VBM analysis:



Repeating this for every subject would be a bit tedious. Unfortunately there is not yet an "apply to many" option for this (though may be soon). Attached is a script to do that for you for now. Copy and paste into a blank script, save as spm_warpshoot, make sure it is in your path and simply type "spm_warpshoot" to run. This can also be used to create a group average brain (with adaptations, detailed later).

```
function spm_warpshoot
%Geodesic shooting does not currently have a GUI for warping many
subjects
%This is a simple wrapper script to help.
%-----------------------------------------------------------------
------
%C.Lambert, July 2018
%-----------------------------------------------------------------
------

%Just check number of tissue classes to normalise
answer = inputdlg('Number of tissue classes (default 2)','INPUT');
if isempty(str2num(answer{1})),classes=2;else
classes=str2num(answer{1});end

%Modulate or not
answer = inputdlg('Modulate (0 = No, 1 = Yes, default YES)','INPUT');
if isempty(str2num(answer{1})),mod=1;else mod=str2num(answer{1});end
if mod~=1,mod=0;end %Make sure no silly inputs

answer = inputdlg('Smoothing FWHM (default 0 0 0)','INPUT');
if isempty(str2num(answer{1})),fwhm=[0 0 0];else
fwhm=str2num(answer{1});end

%SELECT WARP FIELDS:
W=spm_select(inf,'any','INPUT WARP FIELDS');Sw=size(W,1);
T=spm_select(1,'any','TARGET TEMPLATE');

%Now select INPUTS SEGMENTATIONS:
for i=1:classes
    S{i}=spm_select(inf,'any',char(strcat('Input tissue
class',32,num2str(i))));
end

for i=1:Sw,
    INPUT=deblank(S{1}(i,:));[OP,~,~]=fileparts(INPUT);clear
matlabbatch

    if classes>1
        for j=2:classes,INPUT=char(INPUT,deblank(S{j}(i,:)));end
    end

    matlabbatch{1}.spm.util.defs.comp{1}.inv.comp{1}.def =
cellstr(deblank(W(i,:)));
    matlabbatch{1}.spm.util.defs.comp{1}.inv.space = cellstr(T);
    matlabbatch{1}.spm.util.defs.out{1}.push.fnames = cellstr(INPUT);
    matlabbatch{1}.spm.util.defs.out{1}.push.weight = {''};
    matlabbatch{1}.spm.util.defs.out{1}.push.savedir.saveusr =
cellstr(OP);
    matlabbatch{1}.spm.util.defs.out{1}.push.fov.file = cellstr(T);
    matlabbatch{1}.spm.util.defs.out{1}.push.preserve = mod;
    matlabbatch{1}.spm.util.defs.out{1}.push.fwhm = fwhm;
    matlabbatch{1}.spm.util.defs.out{1}.push.prefix = '';
    spm_jobman('run',matlabbatch);
end
end
```

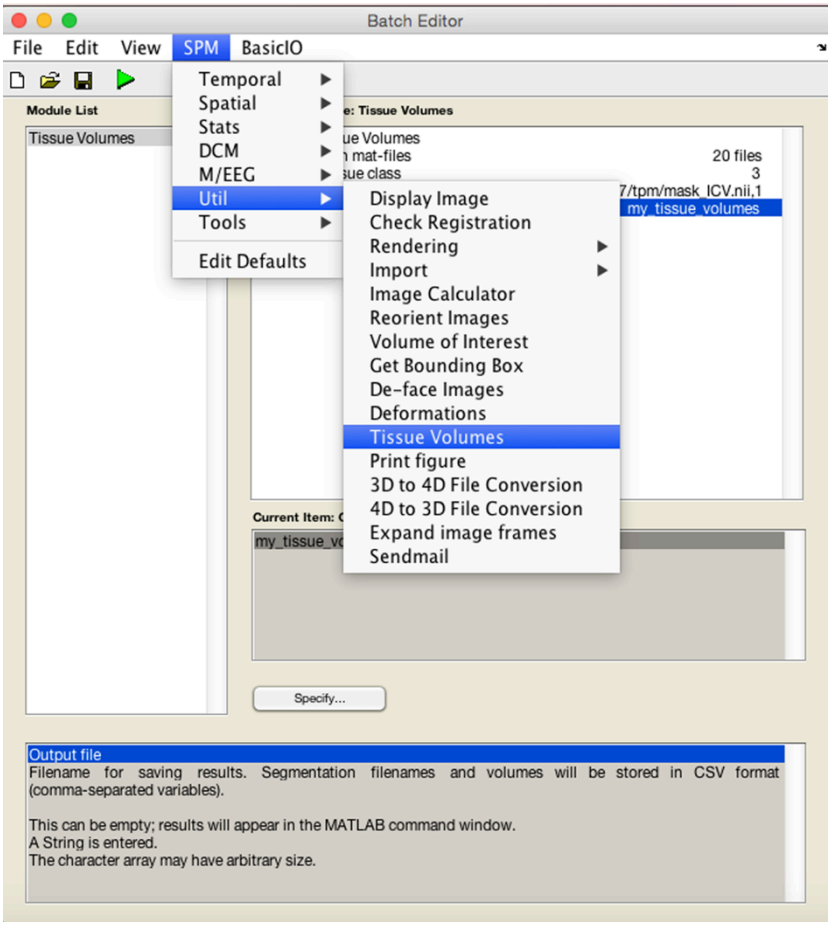
C. ANALYSIS

If you have got to this stage, then hopefully in every subjects folder you have a swm[tissue-class][filename].nii

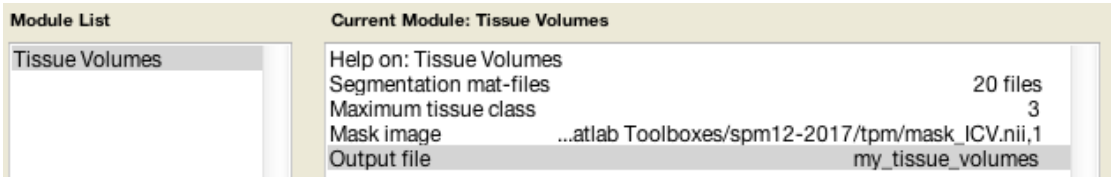
Before we construct a model, we will need one additional covariate in addition to those we already have for our study population – Total intracranial volume

In SPM, there is a function to do this. Open the batch menu:

- SPM -> Util -> Tissue Volumes



You will need to input the seg8.mat from the segmentation steps earlier. Recursively search using .*seg8.mat

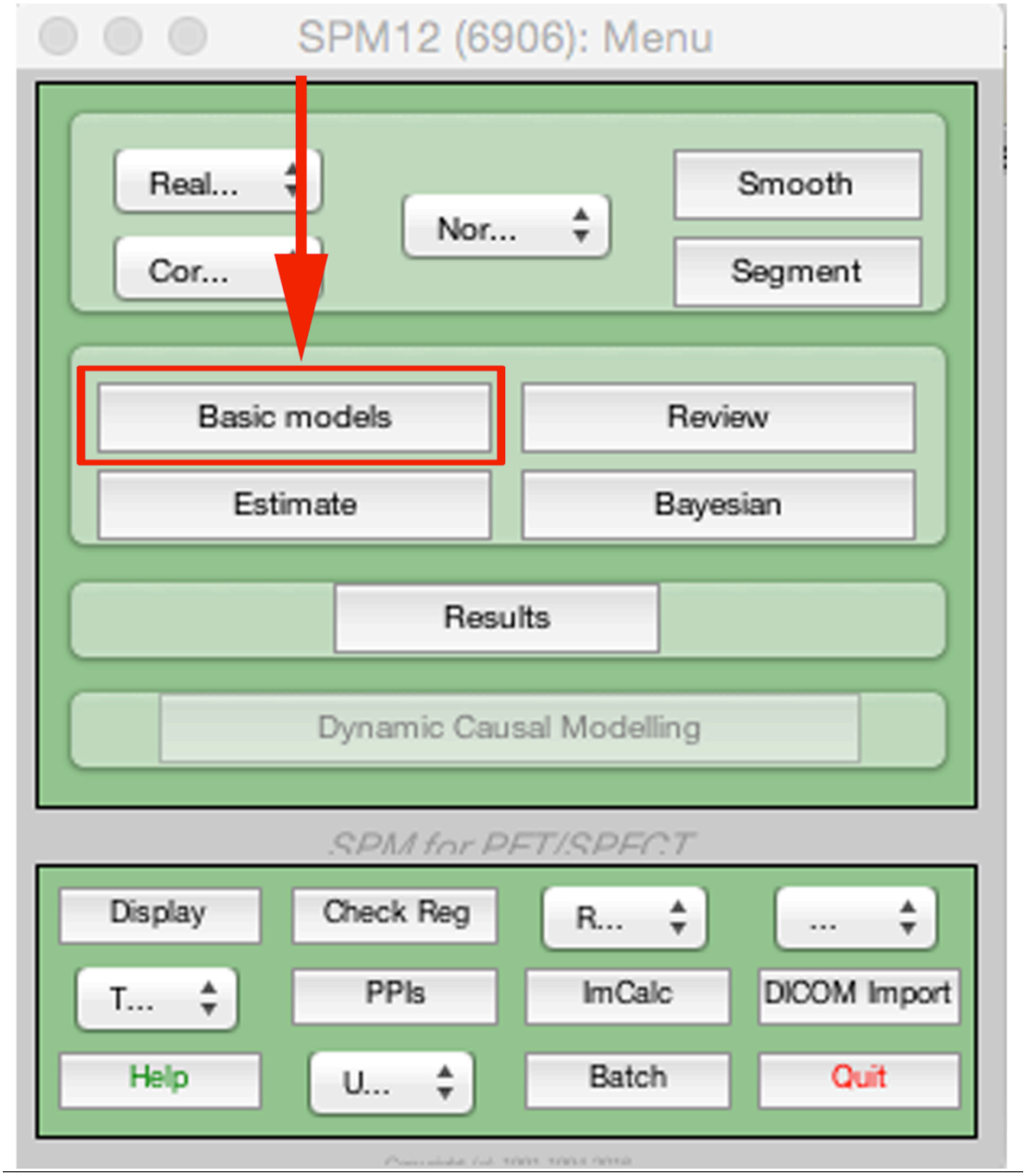


It will either output in MATLAB prompt, or if you specify a file it will generate a .csv you can use in excel. You will need to sum the three tissue classes (GM, WM, CSF) to obtain a volume in litres for each subject.

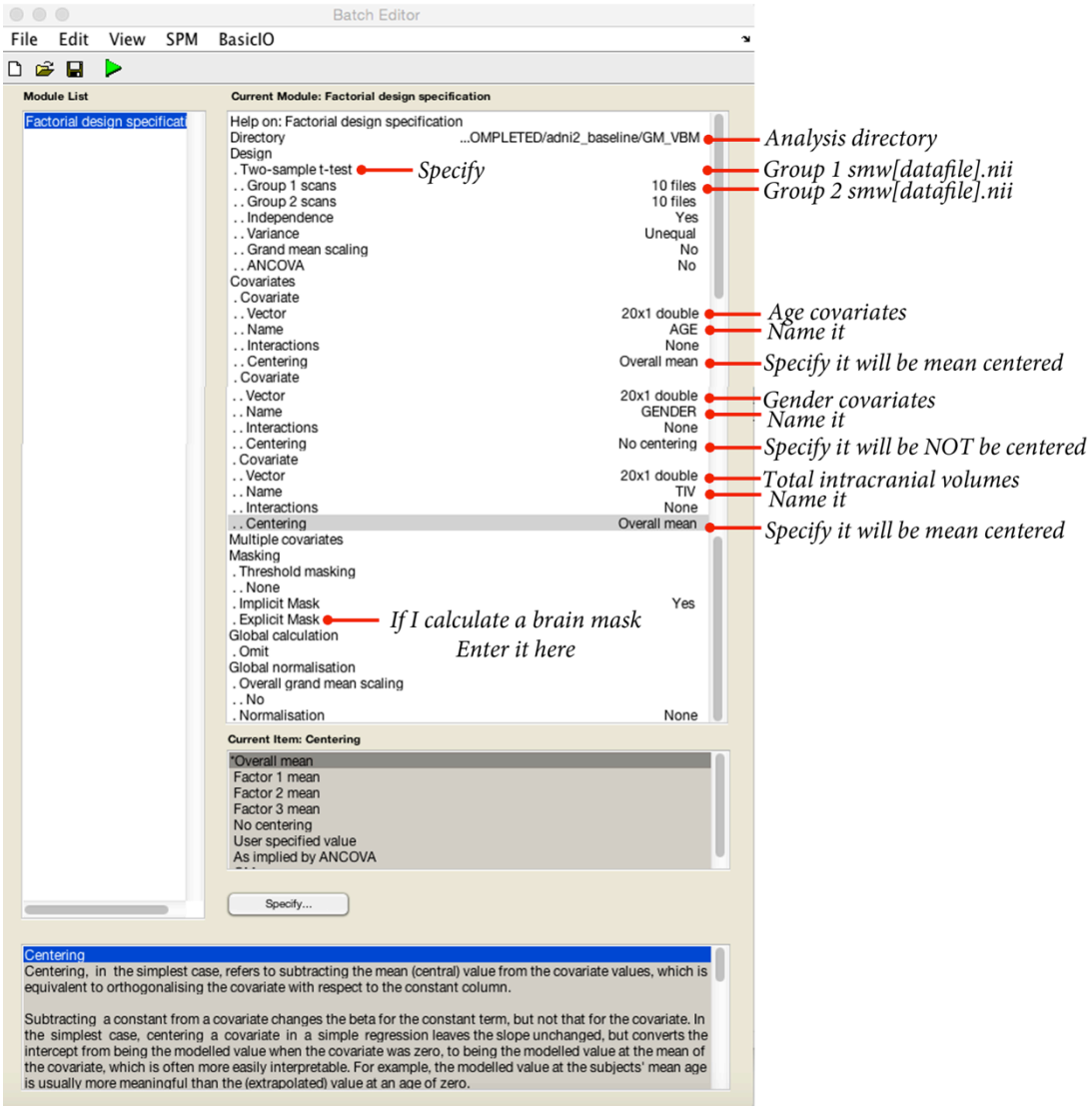
1. MODEL

First make a new directory for your specific analysis. You will need one directory for each analysis you do.

Then from the SPM menu, select "Basic Models"



The precise set-up will depend on your study. Over I have provided an example for a two-sample T-test, controlling for age, gender and total intracranial volume.

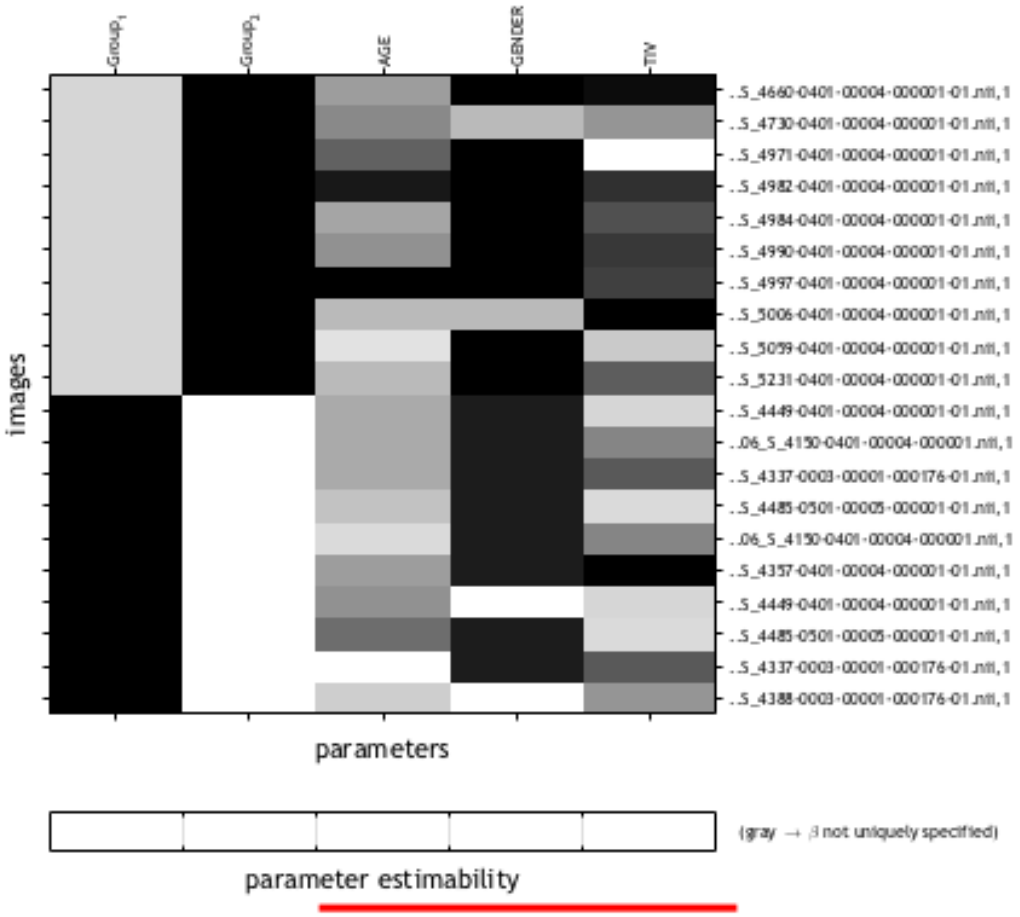


Again save your batch before proceeding. If you have done everything right, you will see the design matrix corresponding to your model (see next page). Now select "Estimate" from the SPM menu, select the SPM.mat in the folder you are using, and hit go.

SPM12 (6906): Graphics

File Edit View Insert Tools Desktop Window SPM Figure Help

Statistical analysis: Design



Design description...

Design : Two-sample t-test
Global calculation : omit
Grand mean scaling : <no grand Mean scaling>
Global normalisation : <no global normalisation>
Parameters : 2 condition, +3 covariate, +0 block, +0 nuisance
5 total, having 5 degrees of freedom
leaving 15 degrees of freedom from 20 images

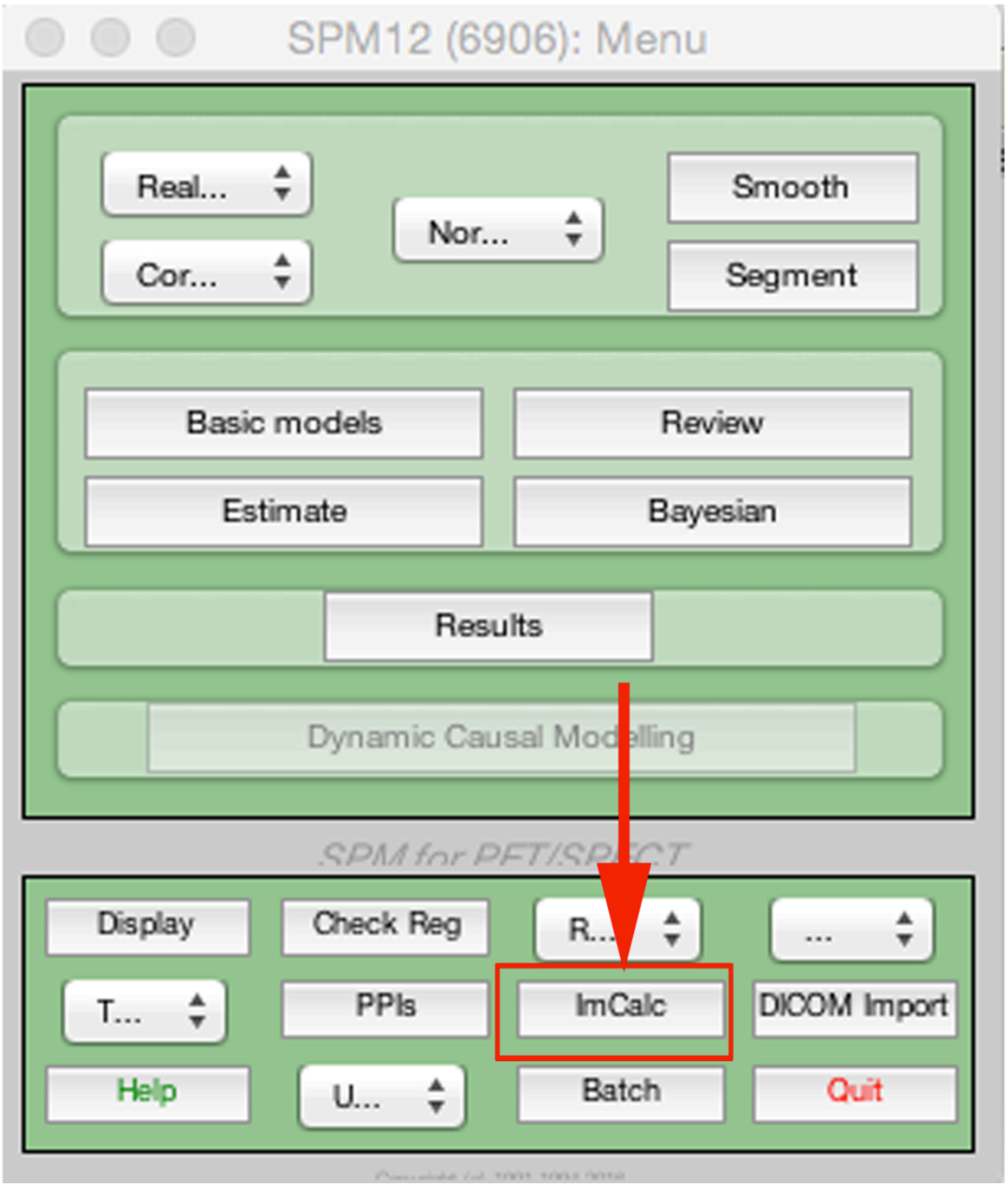
2. ANALYSIS AND VISUALISATION

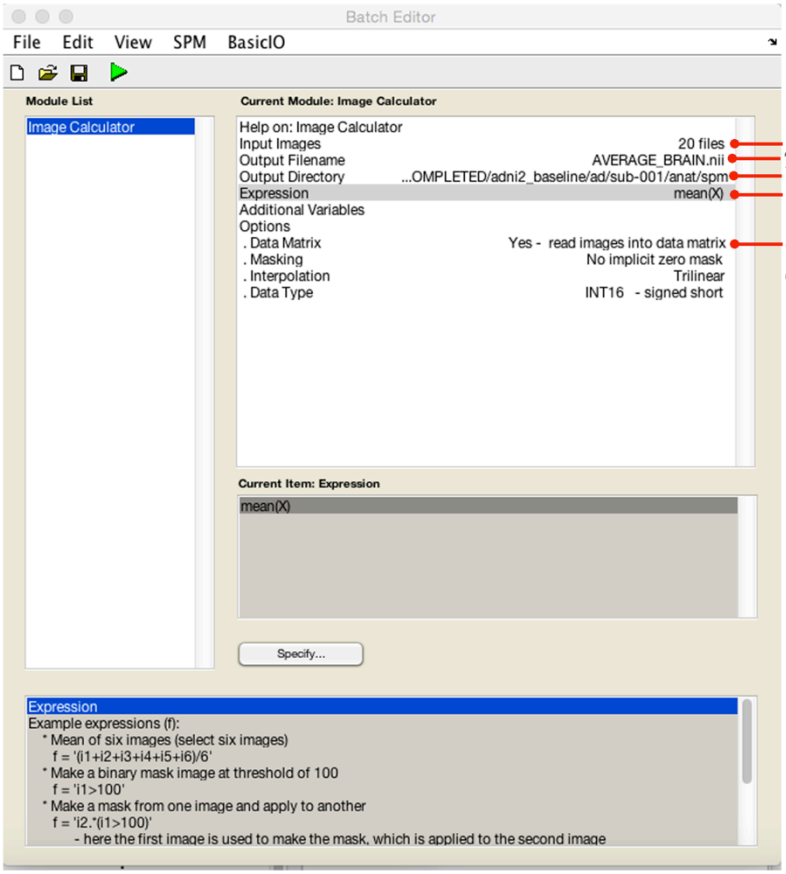
Before we finish, it is a good idea to generate a population average brain for projecting our data onto (because it is not precisely mni). To do this, we simply warp the bias corrected T1s to our group average space. You can do this using spm_warpshoot script on page 18. Make sure to input the following options:

- Tissue class = 1
- Modulation = 0 (i.e. no modulation)
- FWHM = 0 0 0

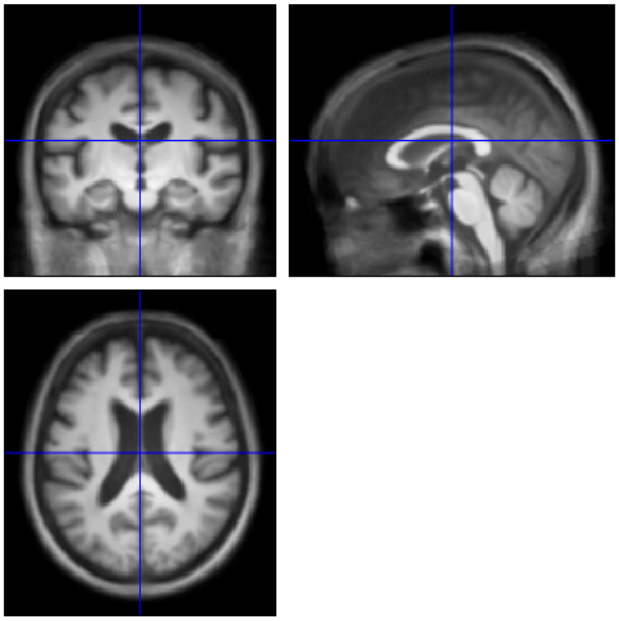
All being well, this will generate a w[filename].nii in each folder.

Now to generate an average image, select Imcalc from the SPM menu:

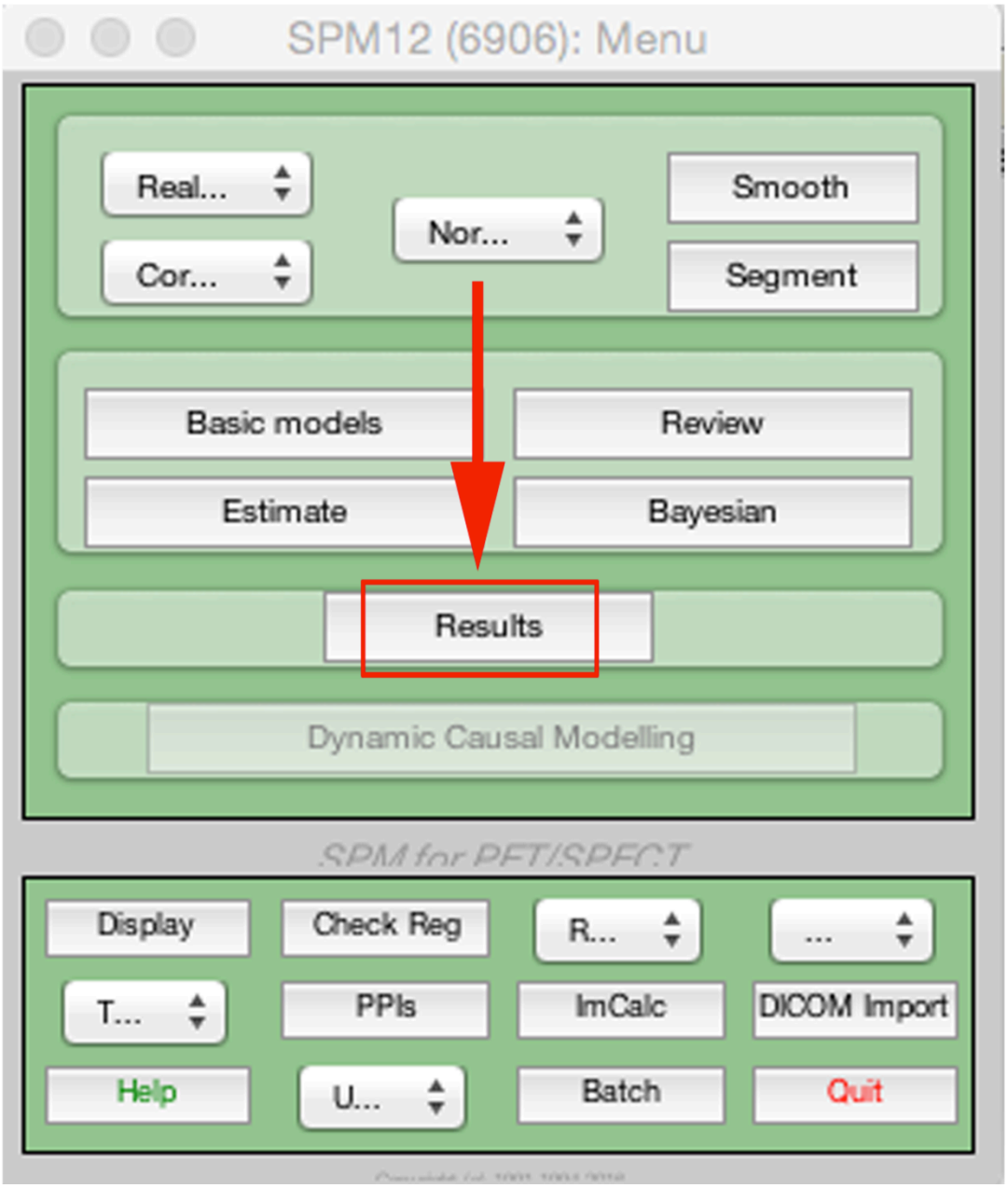




This will read in all the w[filenames].nii and calculate a population average:

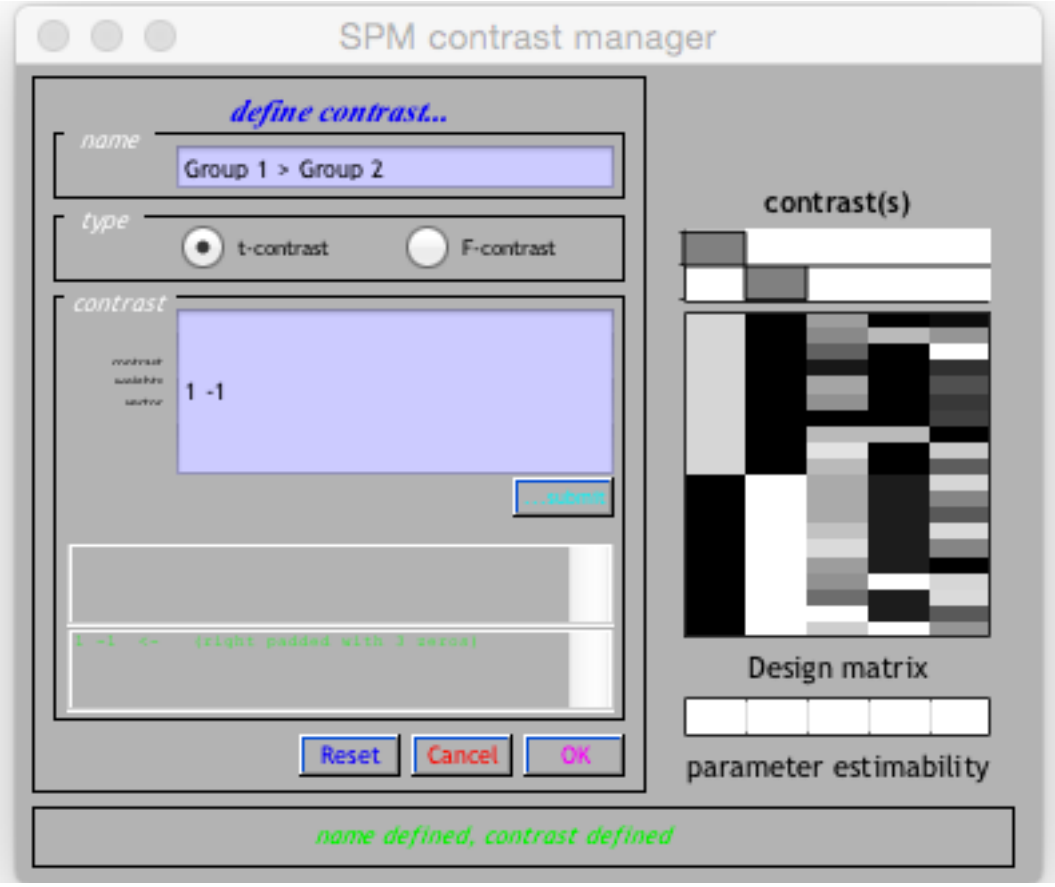


Now onto looking at our results:



This will open a file select, you need to go to your analysis folder and pick the SPM.mat

Following this, the contrast manager will open. Click “Define new contrast”

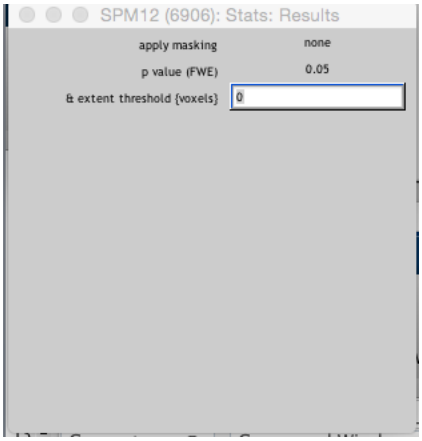


Enter a name.

To test for regions of the brain significantly bigger in group 1 vs. group 2, enter 1 -1 into the contrast, then click ok. To do the opposite analysis (group 2 regions > group 1) enter -1 1.

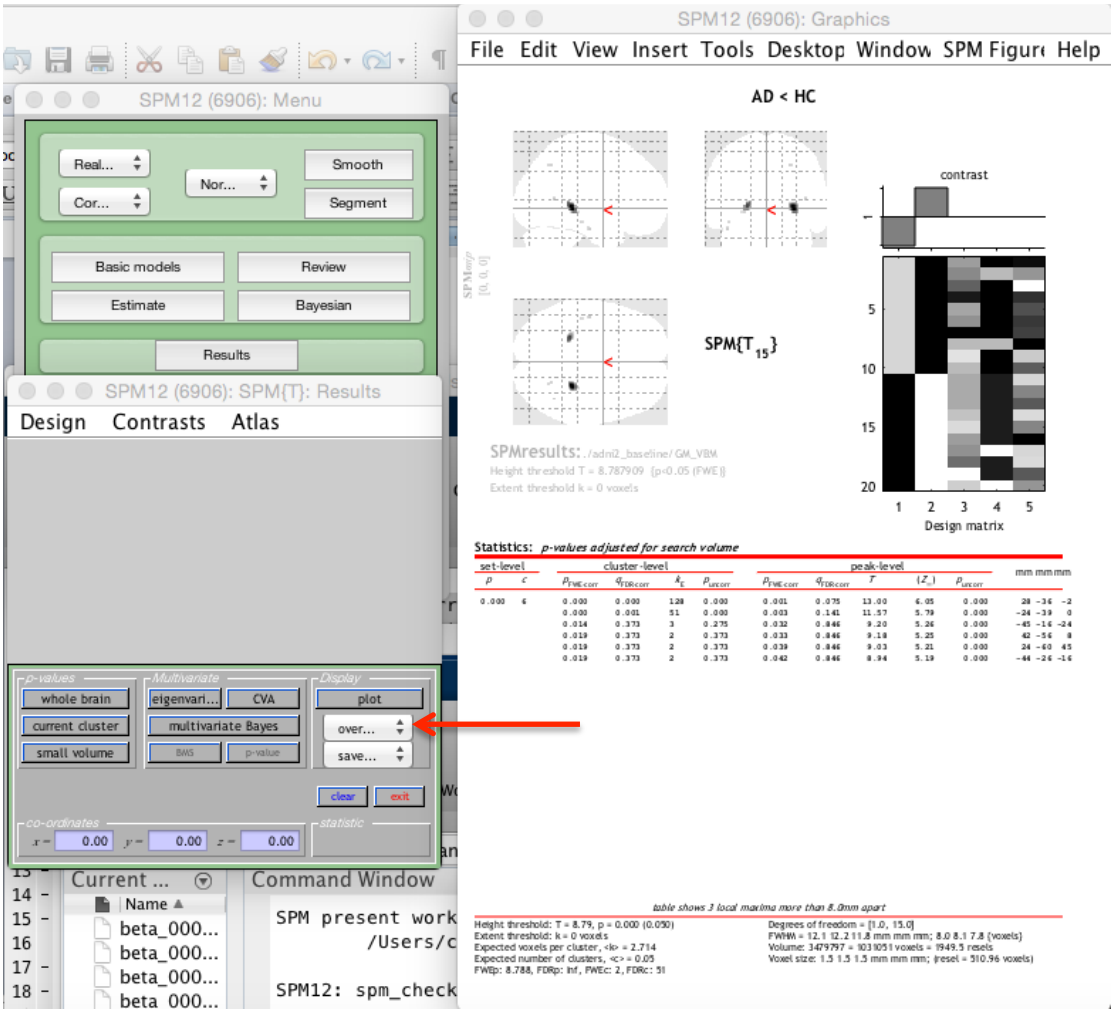
Then:

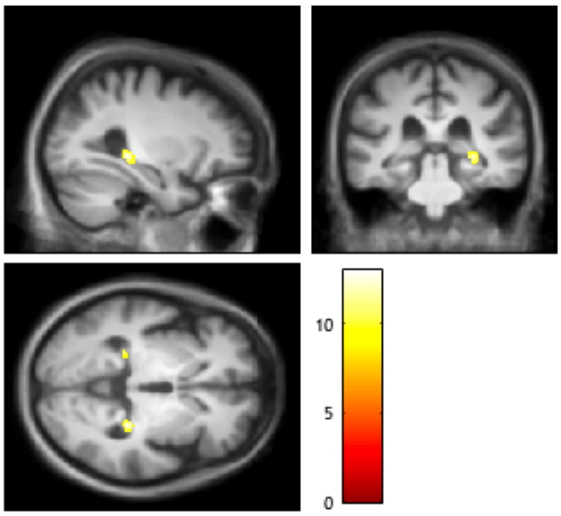
- Apply masking: None (or enter a mask if you have one)
- Correction: FWE
- P value: 0.05
- Extent threshold: 0



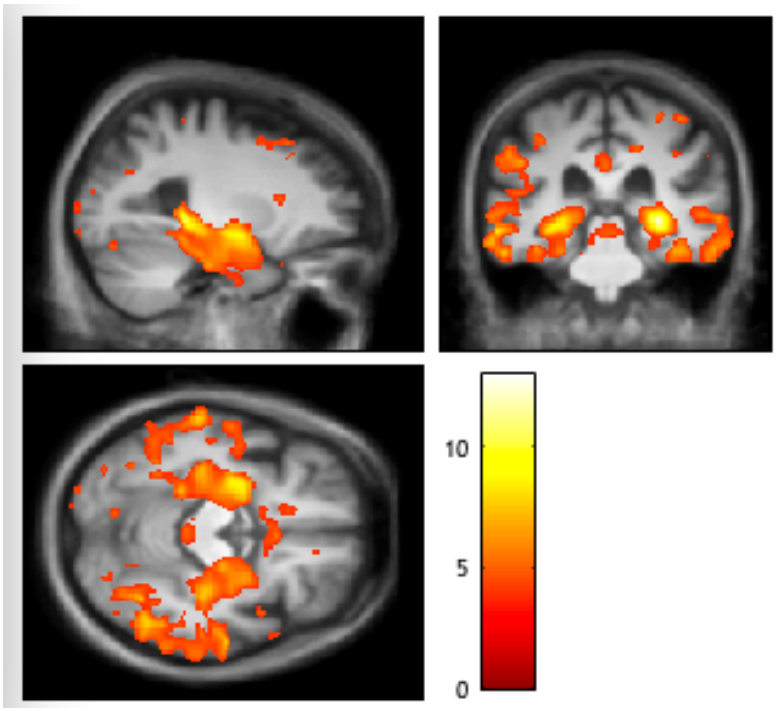
The next window displays any results that survive FWE correction at P < 0.05, displayed on a glass brain.

If you want to see these results on an actual MRI, click (overlay) and select "sections" and then input the average brain you just calculated.





If you want to change the contrast, or inspect a different threshold (P < 0.001 uncorrected), use the Contrasts menu:



Control > Alzheimer's

You can save your thresholded SPM result in the save menu (the one under the overlay menu (arrow)). You can then use your data visualization program of choice to generate results.