Hands-on: Functional Preprocessing using SPM

The instructions below are adapted from the SPM12 manual. If you have a MATLAB license, you may download SPM12 at home for free. The manual and the data sets and do the tutorial yourself. Just go to:
http://www.fil.ion.ucl.ac.uk/spm/software/spm12/

Preprocessing of Student Collected fMRI Data
Motor task

This document describes the preprocessing steps needed to analyze your data. You can also refer to Chapter 29 from the SPM manual. This chapter describes the step-by-step analysis of a sample block fMRI experiment and includes pictures of the output from each step.

Using SPM inside Matlab

We first need to put SPM in the Matlab Path

- Go to **Programs => Matlab** and start the program. Matlab may take a while to load.
- Once the Matlab Window has opened, go to **File => Set Path** and browse for SPM12 folder.
- Click on the button "**add folder with subfolders**" and select the directory where a copy of SPM12 is already located.

Starting SPM:

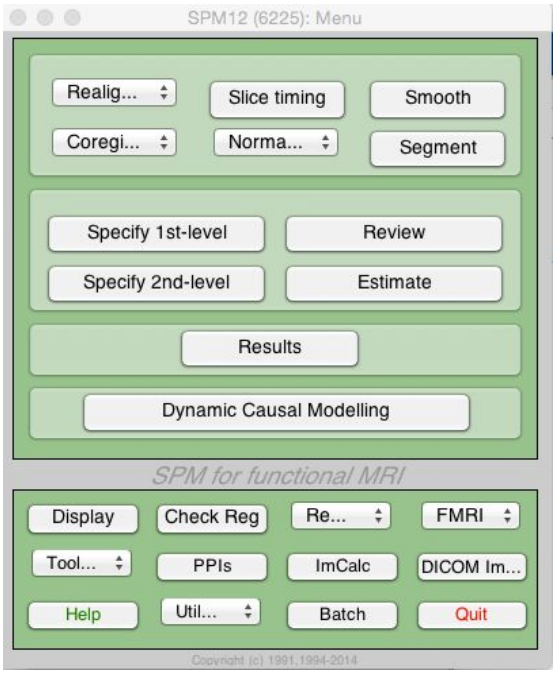
- Now, put the cursor in the **Command Window** (center) and type:

```
spm fmri
```

- The SPM program should start. This may take a while.

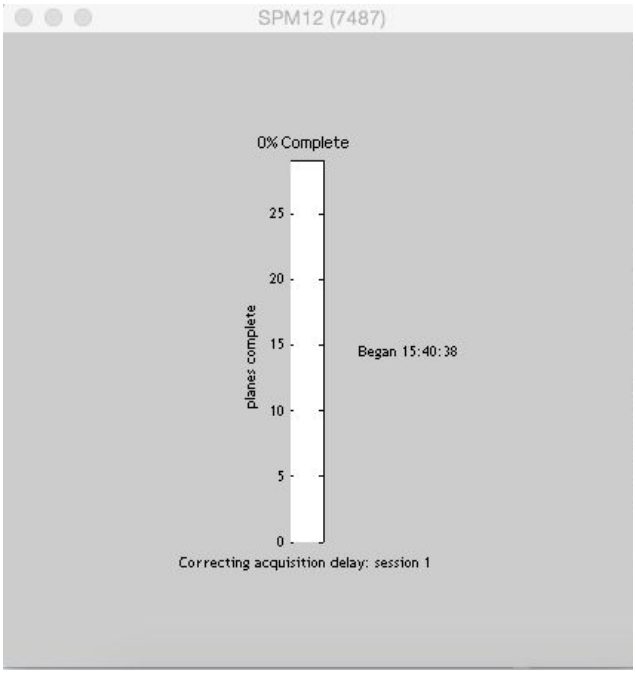
Basics of the SPM Interface:

There are 3 main boxes to the SPM interface shown and described below.



1) Menu box:

This is where you select what operation you want to run. Note the operations are grouped into 4 basic sections: Preprocessing, Statistics Model Specification, Statistics Results, and Utilities.

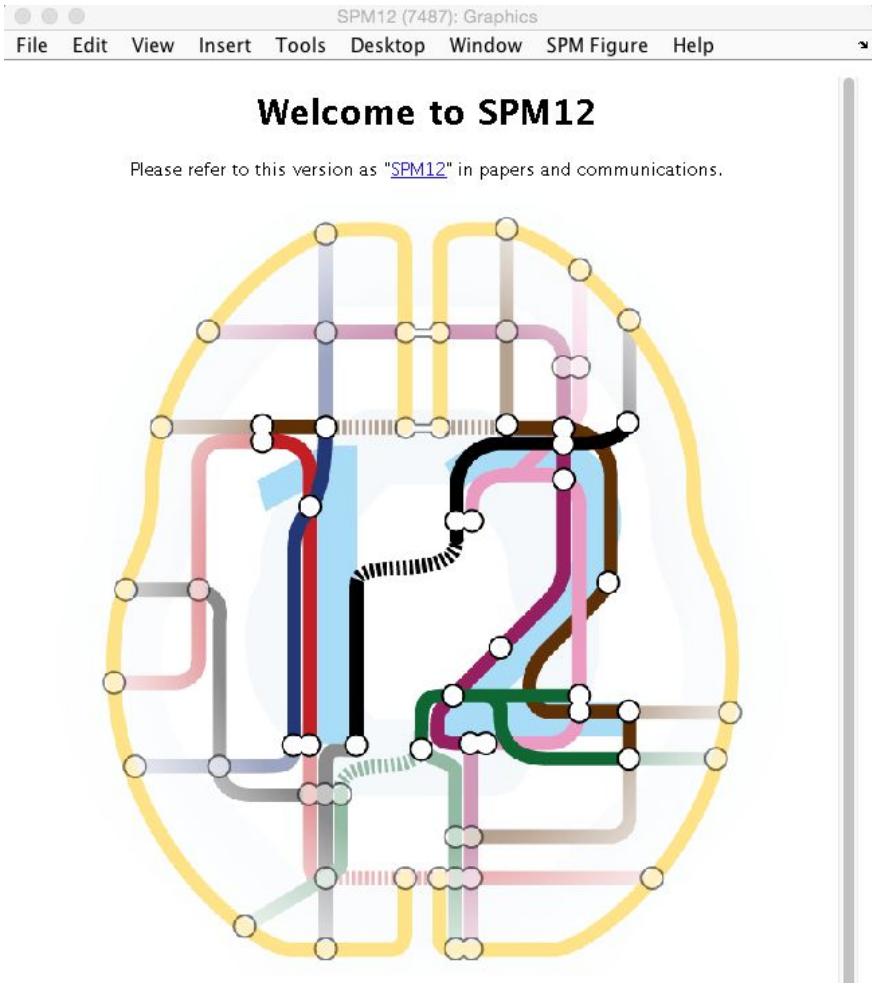


2) Progress Box:

This is where a progress indicator will appear when an operation is running showing how far along the operation is towards completion. In addition, a number of options for the Statistics -> Results are entered.

3) Graphics box:

This box is where you enter the values for any option for the selected operation. It is also where results are displayed when an operation finishes.



IMPORTANT:

Information needed for data analysis: the parameters are specific for the experiment carried out in the course. If you do this elsewhere or in another study, the parameters have to be changed accordingly.

ABOUT THE MRI DATA:

- Data is saved on your computer **(P001 = Katherine)** .
- Within each subject's folder there are two folders: **ANAT** and **MOT**, containing structural and motor task data, respectively.
- The fMRI data in the **MOT** folder are named **P001.MOT.nii**.
- The SPGR, the high-resolution structural image, is stored in the **ANAT** folder. We will use the data to co-register your functional data, it is named **P001.ANAT.nii**.

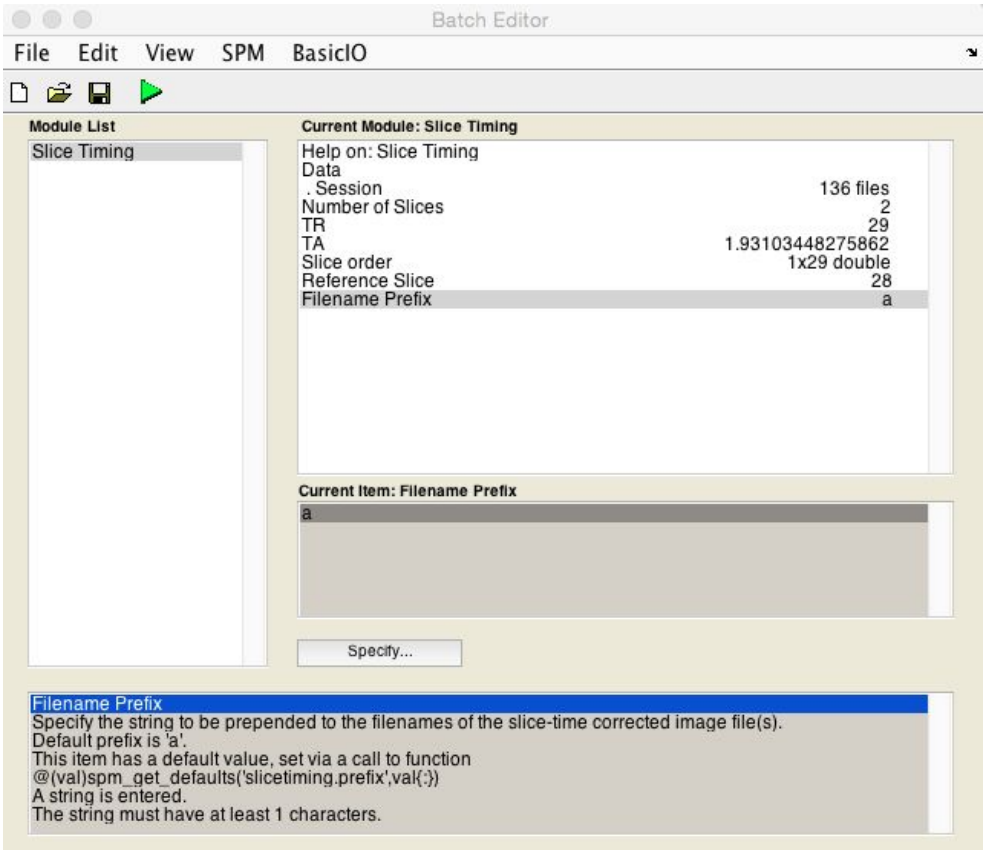
Before we start, we need to **delete the first 3 timepoints (images)** from our functional data. The first timepoints are affected by T1 equilibrium effects and need to be removed from the analysis [**Note:** we already did this before the class].

STEP 1 - SLICE TIMING CORRECTION:

There is a difference in the timing of each slice is acquired. So, we are going to correct that.

1. Click on **Slice Timing** in the Menu Box.
2. Click on **Data**, then **New Session**. First, we need to choose the **P001.MOT.nii** for the Data, then highlight **Session** and click on **Specify Files**. In the file selection box, you can enter **P001*** in the entry next to the Filter to get just a list of the P001.MOT.nii files. On Frames, we need to fill the box with **1:128**. This will show all the 128 volumes acquired. This will become increasingly necessary as you go through the analysis as several versions of the functional images are going to be generated.
3. Given a **TR = 2s**.
4. Given a **Number of Slices = 29**.
5. The TA (acquisition time) is calculated by **TR – (TR/slices)**. So our TR is 2 and our number of slices is 29. Enter: **2 – (2/29)**.

6. We used an INTERLEAVED acquisition. For the **Slice Order** option you can enter the following: **[1:2:29 2:2:28]** to automatically generate the slice order. This is a short-hand way of telling SPM to generate a sequence of numbers.
7. If you have a pair number of slices, make the number of slices acquired and subtract 1 (e.g. 29 - 1) use that as the reference slice. It is the middle slice in the acquisition. [**Note:** if you do this procedure in a different experiment, these parameters will change according to the number of slices and slice acquisition you use].
8. Save the job as **slice_timing.mat** in your computer's folder and then press **Run**.
9. This will produce a new set of files (timepoints) with an '**a**' prepended to the filename. So, you will have files named **aP001.MOT.nii**.



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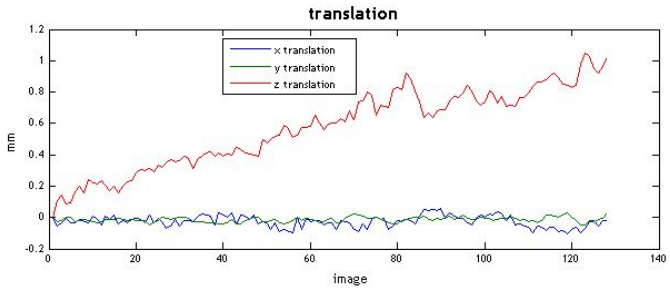
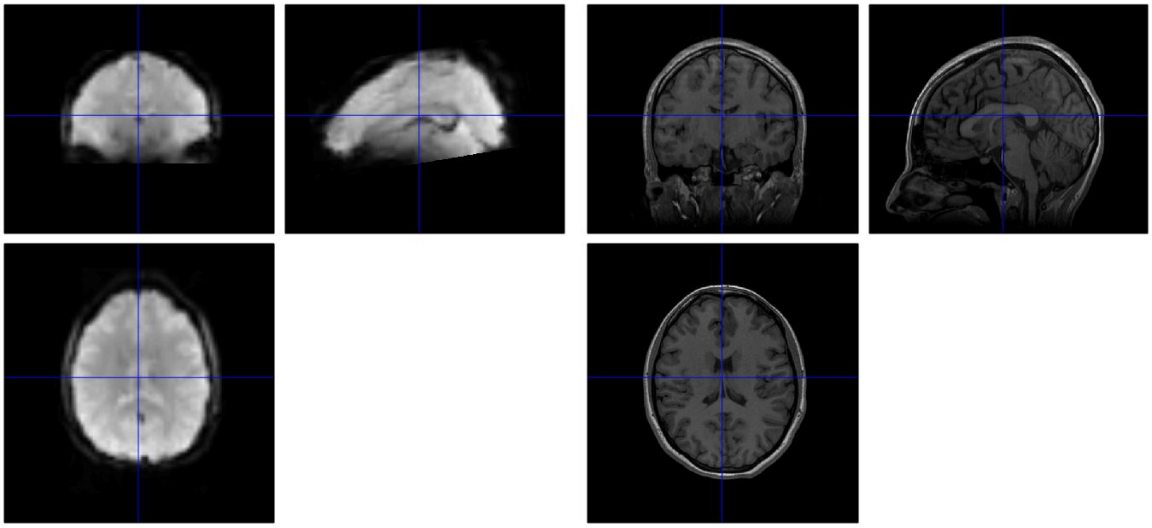
STEP 2 - REALIGNMENT:

1. Choose **Realign (Est & Res)** from the Menu box.
2. Add a New Session.
3. In the Data part of the session enter the **aP001.MOT.nii files**. Remember to only select the images that were the output of the slice-timing correction step.
4. For the rest of the parameters, let's just leave the default parameters for now.
5. Save the job as **realign.mat** in your folder and then press Run.
6. The results for this operation will be the motion plots relative to the first scan. You will want to save this by selecting **File -> Save As** from the Graphics Box menu bar; be sure to change the format to PDF. Also, make a note of how much motion there was. The y-axis scale gives you a sense of the amount of motion.
7. This will produce a new set of files (timepoints) with an '**r**' prepended to the filename. So, you will have files named **raP001.MOT.nii**. In addition, this operation will generate a mean image of all the realigned timepoints with a file called **meanaP001.MOT.nii**. Another file called **rp_aP001.MOT.txt** will be generated in both the folders. This text file has listed by row the movement parameters for each timepoint. The columns correspond to the X-, Y-, and Z-translations followed by the rotations Pitch, Roll, and Yaw.
8. Finally, this step will generate a graph with the motion estimation in the graphics box. Look at these data to see if there was too much motion.



STEP 3 - COREGISTRATION:

1. The mean fMRI image will be in the MOT folder.
2. Click on **Coregister (Estimate)**.
3. Enter as the Reference image the mean file (**meanaP001.MOT.nii**) from the Realignment step.
4. Enter as the Source image the SPRG (**P001.ANAT.nii**) inside the **ANAT** folder.
5. Save the job as **coreg.mat** in your folder and then press **Run**.
6. This operation will coregister the mean fMRI image to the SPRG (structural image).
7. The results for this operation will be shown in the Graphics Box. The left columns are the coronal, sagittal, and axial slices of the co-registered mean fMRI image. The right columns are the SPRG. Also, you can click around in either of these images and the images will update to show the same location in the other image. This is a good way to judge how well the co-registration worked.
8. You will want to save this figure by selecting **File -> Save As** from the Graphics Box menu bar.



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STEP 4 - NORMALIZATION:

1. In this step we will calculate the warping parameters of the SPRG (T1) image to the Talairach space (MNI).
2. Select **Normalize (Estimate)**
3. Choose the **P001.ANAT.nii** from the Structural folder for the data file.
4. Select the Template Image: **T1.nii (??)**
5. Save the job as **normalize.mat** in your folder and then press Run
6. This operation will generate a deformation field with the prefix "**y_**". For us this will be "**y_P001.ANAT.nii**".

STEP 5 - QUALITY CONTROL - Spatial Normalization Structural Data:

1. It is important to make sure your data has been put into Talairach space correctly before proceeding.
2. Click on **Normalize** and select **Normalize (Write)**.
3. Double click on **Data**.
4. Select the deformation field file **y_P001.nii** in the **ANAT** folder.
5. Now click on **Images to Write** and select the intensity SPRG file **P001.ANAT.nii** file.
6. On **Writing Options**, select voxel sizes and change the default **[2 2 2] to [1 1 1]**, which matches the original resolution of the images [1 1 1].
7. **Run** the normalization.
8. After the normalization is performed, the file **wP001.ANAT.nii** is created.
9. Now we need to check how well the Normalization performed.
10. Click on **Check Reg** in the Menu Box from the bottom set of buttons.
11. A file selection box will pop-up. In this box you will want to select 2 images.
12. First, from the "**spm12/tmp**" folder choose the **TMP.nii** file.
13. Second, choose the normalized image from the Structural folder the SPRG, **wP001.ANAT.nii**
14. In the Results Window/Graphics Box you will see the T1 template on top and the SPRG on the bottom. When you click around in one of the images at the same location from the other will be shown. Click on a few different locations and verify the two are aligned.

15. If the images are not aligned then contact Augusto/Nathalia to get help figuring out why this happened and how to fix it.

STEP 6 - QUALITY CONTROL - Spatial Normalization Functional Data:

1. Now let's perform the same normalization, but now with the functional data. Use the mean functional image (**meanaP001.MOT.nii**) from the Realignment step.
2. Choose **y_P001.ANAT.nii** as Deformation Field
3. Choose **meanaP001.MOT.nii** as Images to Write.
4. The only difference is to now change the voxel sizes to **[3 3 3].**
5. Check the results in the same way as the previous Normalization (Steps 10 to 15).

STEP 7 - SPATIAL NORMALIZATION OF THE FUNCTIONAL DATA:

1. Choose **Normalize (Write)**.
2. Highlight **Data** and add a **New Subject**.
3. Open the Subject.
4. Choose the deformation field file **y_P001.nii** in the **ANAT** folder as the **Deformation Field File**.
5. Choose for the Images to Write all of the realigned and slice time corrected images (**raP001.MOT.nii**) files and the mean fMRI image.
6. Open **Writing Options**, and change **Voxel sizes** from **[2 2 2] to [3 3 3]**. Note do not include the square brackets when you type in the numbers.
7. Save the job as **SN_Write_fMRI.mat** in your folder and then press **Run**.

STEP 8 - SMOOTHING:

1. Choose **Smooth**.
2. On **Images to smooth**, choose the Talairach' functional MRI (**wraP***) files from the **MOT**.
3. I would recommend changing the smoothing to **6 6 6** to indicate that the FWHM should be 6 mm in each dimension, which is 2 times your voxel size.
4. Save the job as **smooth.mat** in your folder and then press **Run**
5. The output of this step will be smoothed files called **swraP001.MOT.nii**.

At the end of this preprocessing you should have **swraP001.MOT.nii** files in the **MOT** folder for your subject. These will be used for the next class.

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QC' ing your data

After you have finished preprocessing the data from your subjects, it is important to carefully examine the results and QC (quality control) your data to ensure each step worked as intended. Failure to do so could easily lead to erroneous statistical results both false negatives (failure to correctly identify areas of activation) and false positives (incorrectly identifying an area as active). At a minimum, I recommend examining the degree to which motion correction worked, the accuracy of the co-registration, and how well the data were transformed into the MNI template space.

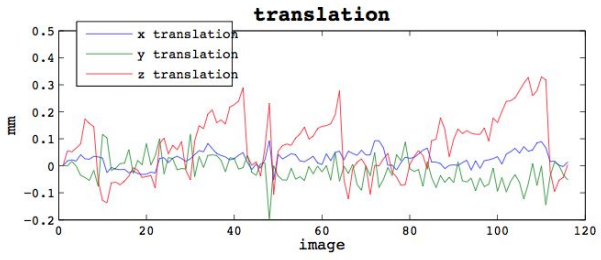
a. Motion Correction QC

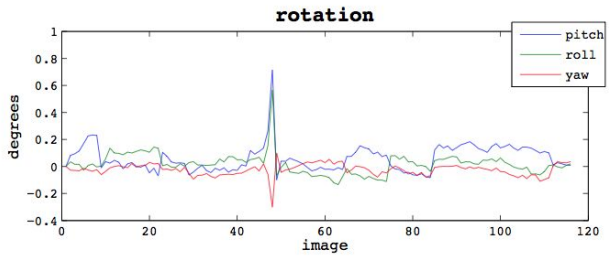
There are three things that I recommend examining with regards to motion correction: 1) motion correction plots; 2) movies of both the uncorrected and motion corrected timeseries in a cross sectional plane (e.g., sagittal or coronal); and 3) plots of various metrics that are sensitive to motion.

i. Motion Correction Plots

Image realignment

```
1   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
2   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
3   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
4   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
5   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
6   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
7   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
8   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
9   /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
10  /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
11  /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
12  /Volumes/mirror/cfmi/processed3/NSCI-521/Manipulable/Man-Test3/Man
.............. etc
```





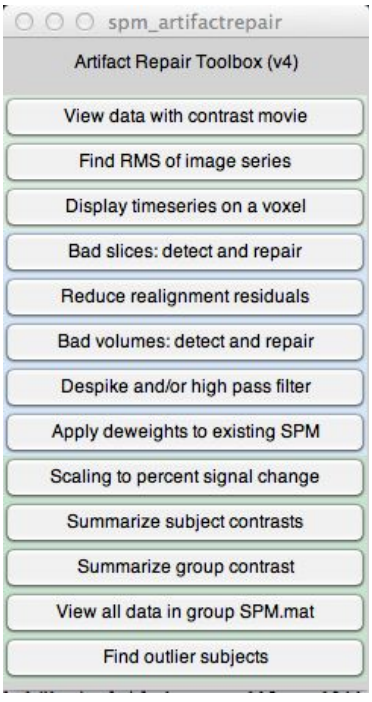
The motion correction plots generated by the Realignment step in the SPM preprocessing give you an overall sense of the amount of motion for each subject at least with respect to what the Realignment algorithm determined computationally. The output from your Batch Job will be stored in a file called spm_DATE.ps, where DATE is the date that you ran the batch script. An example realignment report is shown below. The top graph shows the translations in mm and the bottom graph shows the rotations in degrees. Both are relative to the first volume. From this plot you can quickly see the overall magnitude of the movement and

specific time points that may need to be either repaired or unweighted in the statistical analysis. Ideally, you would like to limit the motion to no more than 0.75 mm and 0.5°. However, this is not written in stone and sometimes you need to be more lenient. In addition, you need to keep in mind that just because the motion shown in these plots is greater than these ideal thresholds that does not necessarily mean that realignment was not successful. Indeed, it is entirely possible that the realignment worked and that motion was removed. These plots should be interpreted as a guide to which time points bear further investigation and overall how much motion there was across the whole run. The other motion correction QC steps will give you more information about whether realignment succeeded.

ii. **Motion Movies**

One way to assess how well realignment worked in terms of removing motion is to look at movie of the realigned time series. In SPM this can be done by using the ArtRepair toolbox.

http://cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html



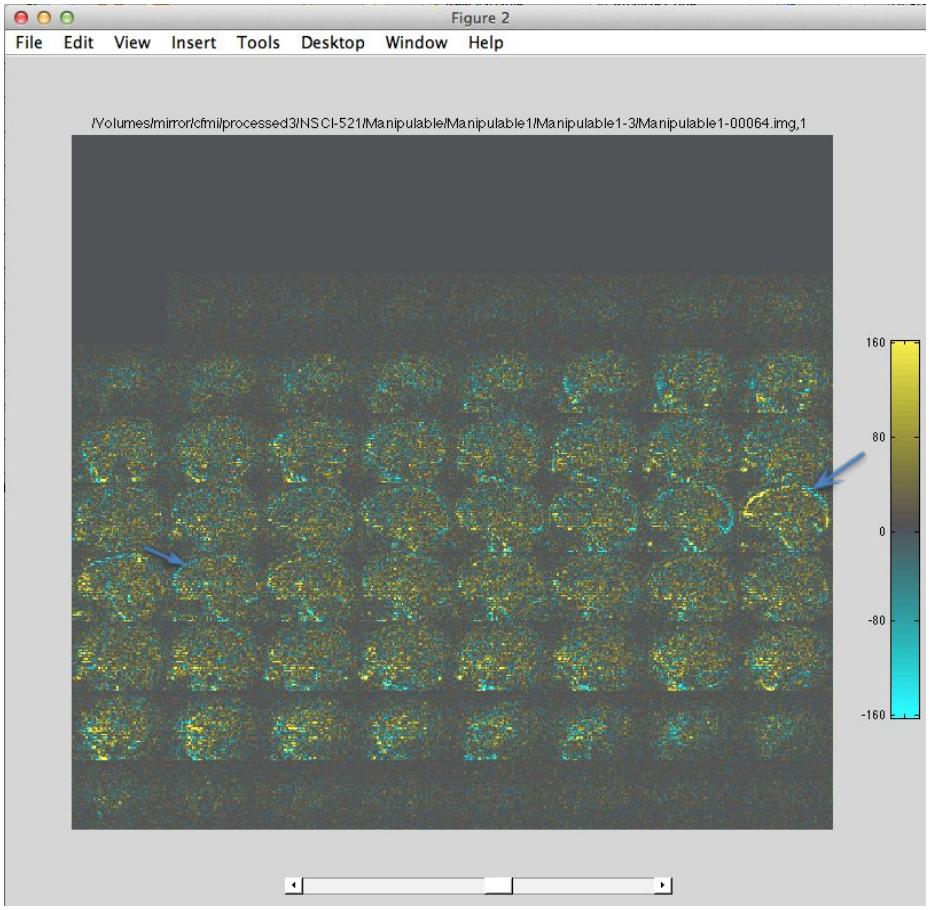
1. In the main SPM Menu box choose Toolbox -> ArtRepair to pull up the ArtRepair interface (see above).
2. Click on the "View data with contrast movie".
3. In the File Selection box choose the ra*.nii images.
4. At this point you can choose what slice orientation in which to view the images. Since motion that occurs during the acquisition of volume is best seen in an orientation that cuts through the slices, I recommend choosing either Sagittal or Coronal.
5. Enter the range of time points to view. Enter 1:128 to view all of the time points.
6. The next option allows you to choose to view all of the slices or focus on a subset of 20 slices. For now choose All; later you can experiment with this option if you want.
7. The "Select Data Magnification" allows you to either view the images, "Raw Image Data", or to examine different images, "Contrast" and "High Contrast". The latter options will create a difference image based on subtracting a given Reference image from each of the time points in the time series. The differences

images are then displayed in a color scale with yellows showing voxels that have an intensity greater than the reference and blues for voxels with an intensity less than the reference. The manual recommends choosing High Contrast for motion corrected data. I find this to be less informative in runs with very little motion and thus for data from the class I would choose Contrast.

8. Select Reference Image – If there is not a lot of residual T1 contrast in the data then the default of using the 2nd volume is fine. On our scanner, this will generally work.
9. Select viewing mode – The slider option is probably best as it allows you to interactively scroll through the data and identify which time points are problematic.
10. It will take a couple of minutes for the data to be prepared – so just wait. When it is done, you will see 2 windows pop-up.

The window titled 'Figure 4' shows you the first volume as a reference showing the anatomy in each slice. The window titled 'Figure 2' shows the Contrast or difference images with a slider underneath. The brighter the color in either direction the more motion there is in that time point. You will want to note which time points have a lot bright colors. Do not worry about being precise at this point as the next step will provide greater quantitative assessment of the time points that are problematic.

In figure below, there are some possible slices that indicate this time point might be problematic.



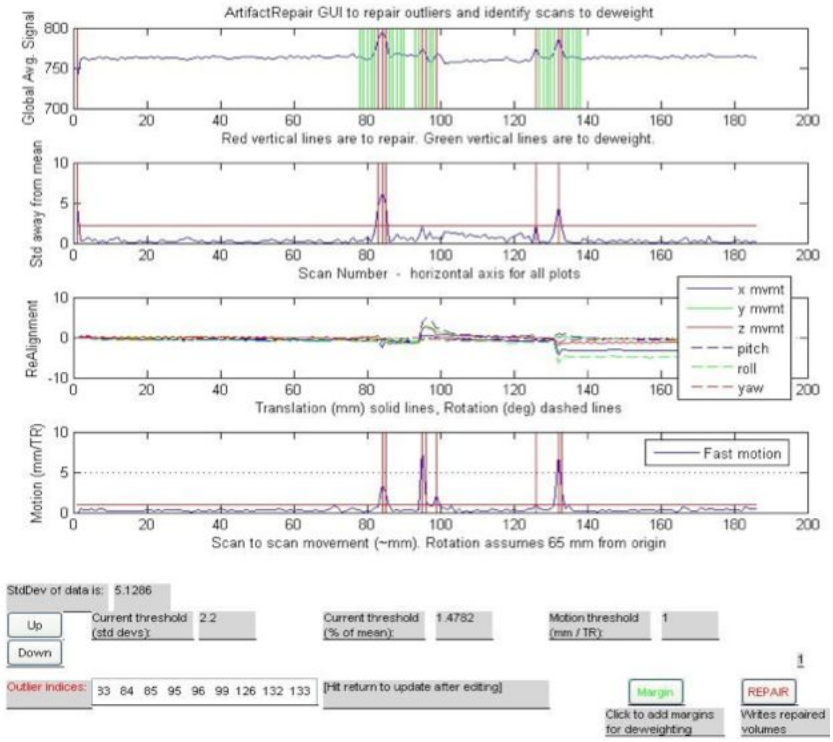
iii. **Quantitative Detection of Bad Time Points**

Up to this point we have qualitatively assessed using different tools the degree of residual motion in our data. Now, we want to use some quantitative measure of which time points in our time series that we might want to eliminate from our statistical analyses. For this we will use the "Bad volumes: detect and repair" option in ArtRepair.

1. In the main SPM Menu box choose Toolbox -> ArtRepair to pull up the ArtRepair interface (see above).
2. Click on the "Bad volumes: detect and repair".
3. The first question, "Which global mean to use?," gives you options as to what to use as a reasonable subject-specific mask. I would use the "Auto (Generates ArtifactMask and can Calculate Movement)" option.
4. Click Yes for "Have realignment files?".
5. In the file select dialog box choose the rafPXXX.MOT_YYY.nii volumes.

6. The next file section is asking for the output text file generated by SPM Realignment. Choose the rp_afPXXX.MOT_004.txt file.
7. For the "Always repair the first volume?" option choose No.

The output of "Bad volumes: detect and repair" (aka art_global) is four graphs as shown below.



The top graph shows the global mean intensity within the head for each volume. Since this is plotting the average intensity a good run should have a fairly flat line with only a few small bumps (less than 1.5% of the mean value). The y-axis is in image intensity, which is unit-less.

The second is standard deviation of the global mean intensity relative to the mean across the whole run. The red horizontal line is by default set at at 1.5% variation from the mean. In a good run all of the peaks are below this line.

The third graph shows both translations and rotations from SPM realignment all on one graph. If you are using data that has not been realigned then this graph shows a crude estimate of the 3 translational estimates of centroid motion.

The fourth graph shows the scan-to-scan movement calculated as the net movement relative to the first volume. The vertical scale is mm/TR, which calculated using the following formula:

$$d^2 = \Delta x^2 + \Delta y^2 + \Delta z^2 + C^2(\Delta pitch^2 + \Delta roll^2 + \Delta yaw^2)$$ *

* from http://cibsr.stanford.edu/documents/ClinicalSubjectMotionHBM2011.pdf

where Δ is the difference between successive volumes and $C=65\pi/180$ (converts degrees to radians) for a voxel 65 mm from the origin. The default threshold for the red horizontal line is 0.5 mm/TR. Again, the ideal would be for all scans to be below this red line.

In addition to the four graphs, this dialog box has options for adjusting the threshold used to identify volumes that should either be repaired or unweighted in the statistical analysis. I personally do not recommend using the "Repair" option as I do not believe it is possible to faithfully recreate the data in the volumes that will be repaired by using an average of the surrounding volumes. When we do the first-level single subject analysis we might use the "Outlier indices" shown in the bottom right corner. These correspond to the time points that exceed the red-line threshold in either the 2nd or 4th graph.

b. **Co-registration QC**

We have already performed this during the preprocessing steps. Make sure you do not skip these steps.

c. **Spatial Normalization QC**

Finally, it is important to visually inspect how well the spatial normalization worked to warp the mean functional volume into the MNI template space. The easiest way to do this is to use the "Check Reg" option.

1. In the main SPM Menu box choose "Check Reg".
2. In the File Select box you want to choose 2 volumes in the following order: first choose the /Volumes/mirror/apps/spm/8/templates/EPI.nii volume and then wmeanaManipulalbe1-00001.img volume.
3. Click Done in the File Select box after choosing both volumes.

As with the Co-Registration QC, you are trying to visually assess how well you volume (shown on the bottom) matches the MNI EPI template. Click on several different locations to see how well the two match up.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