Goals of this lab

After this lab you will:

1. Be able to examine data using SPM's single- and multi-volume display facilities.
2. Be able to characterize the susceptibility artifacts and signal voids in functional data, as compared to similar structural data.
3. Be able to evaluate the quality of functional image motion correction (realignment).
4. Be able to perform coregistration between the low-resolution and high-resolution structural images. You will understand the implications for an image to be the "Source" vs the "Reference" in terms of the "world space" of each image.
5. Be able to perform spatial normalization, check its success, and apply the transformation.

**Preprocessing Overview**

Processes indicated by gray filled boxes have already been done for you. During this lab you will be verifying that these steps worked as intended as well as performing the coregistration, normalization, and smoothing steps.

**BOX: DICOM files**

During the entire fMRI course you will be working with either Nifti images or header/image pairs. These are not raw MRI data, but reconstructed image files that SPM and many other neuroimaging software packages can work with. Raw MRI data are often exported in the form of DICOM (Digital Imaging and Communications in Medicine) images. DICOM images store only one slice per file, which means that one Nifti image is reconstructed from several DICOM...
files. SPM cannot work directly with DICOM images, so if you start with DICOM files, you have to reconstruct them first. There are several freeware tools that can do this, including SPM and also Chris Rorden's dcm2nii tool which is available for Windows, Linux, and MacOS. Depending on the Manufacturer of your scanner, you may have DICOM files ending in .dcm or ending in .ima. To convert your DICOM images to Nifti using SPM12 click on the 'Batch' button in the main menu. Now select ‘SPM > Util > Import > DICOM Import’. Now you can select all your DICOM files and the output directory where you want your Nifti files to appear.

MATLAB, SPM, Data Setup

The course data is stored under: C:\fMRI_Course\fMRI_Lab\LabAssignments\SPM_Labs_Data. Throughout this lab exercise this marker <<DataDir>> refers to the directory path to the data. Always replace <<DataDir>> with the actual path to the data.

Verify you can see this directory, and that it has data for the 10 subjects listed below. It should also have data for an 11th subject named "subBAD". This subject was included to show you what bad data looks like.

Fortunately, the data for the subjects 01-10 is the opposite of bad. Therefore, the majority of your time will be spent analyze data collected on them. Today, you'll primarily focus your analyses on one of the following 10 subjects. Select your subject and keep note of the subject's number.

Start MATLAB & set MATLAB path
Locate MATLAB under the Start menu and start it (it is also on the desktop).

The first thing you should do after starting MATLAB is to change the working directory to a reasonable place. In the computer lab you do not have write permissions in the default directory. Use the "..." button at the top of the MATLAB window (depending on the version of MATLAB you’re using, this button may be represented by an open folder icon with a downwards pointing green arrow on it), or use the "cd" command to change to the <<DataDir>> directory. If you use the cd command you would have to type:

```
    cd <<DataDir>>
```

Now, add the directory "C:\SPM12" to the path, by either entering "pathtool" in the command window, or by using the "Set path..." button under the "Environment" block in the MATLAB ribbon:

```
    cd C:\SPM12
```

Click the "Add Folder..." button to select the SPM12 folders.

**DO NOT** add with subfolders. Click "Close", and save this path in Documents\MATLAB for your future sessions.

Alternatively you can directly add the folder the path by typing "addpath C:\SPM12"
Check defaults

When SPM is first installed it is important to review and set the defaults, found in spm_defaults.m. Navigate to the C:\spm12 directory and open the SPM12 directory. Find the file "spm_defaults.m" and open it by double clicking or by typing "edit spm_defaults.m" (it should open in the MATLAB m-file editor). Remember that if you always have to restart SPM in order to apply your changes.

---

BOX: Analyze Orientation Defaults

If you have the old style Analyze img/hdr pairs you should educate yourself on the flipping of images based on spm_flip_analyze_images.m. When first installing SPM it is crucial to set the "flip" default correctly. Find the line with flip in spm_flip_analyze_images.m. A value of 0...
indicates that "SPM" Analyze images are expected; such images are often referred to as "Neurological" Analyze. A value of 1 indicates that official Analyze images are expected; these images are sometimes called "Radiological". If your lab uses Analyze format, then this value should be set once, verified, then never changed. (The images you’re about to analyze are in the NIFTI format. Please see the Appendix for more information on NIFTI).

<table>
<thead>
<tr>
<th>Convention</th>
<th>Glib descriptions seen everywhere</th>
<th>Slice orientation</th>
<th>Viewer software displays [Note 1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiological</td>
<td>&quot;Images are viewed as though looking upward from the feet of the subject.&quot;</td>
<td>Axial</td>
<td>Looking towards pt</td>
</tr>
<tr>
<td></td>
<td>&quot;Right is Left&quot;</td>
<td>Superior</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterior</td>
<td>Up</td>
</tr>
<tr>
<td>Coronal</td>
<td>&quot;Images are viewed as though looking from the top of the head downward.&quot;</td>
<td>Posterior</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>&quot;Right is right&quot;</td>
<td>Superior</td>
<td>Up</td>
</tr>
<tr>
<td>Sagittal</td>
<td>(Think brain-surgeon-view.)</td>
<td>Left?</td>
<td>Anterior</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Superior</td>
<td>Up</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Convention</th>
<th>Glib descriptions seen everywhere</th>
<th>Slice orientation</th>
<th>Viewer software displays [Note 1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological</td>
<td>&quot;Images are viewed as though looking upward from the feet of the subject.&quot;</td>
<td>Axial</td>
<td>Looking towards pt</td>
</tr>
<tr>
<td></td>
<td>&quot;Right is Left&quot;</td>
<td>Inferior</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterior</td>
<td>Up</td>
</tr>
<tr>
<td>Coronal</td>
<td>&quot;Images are viewed as though looking from the top of the head downward.&quot;</td>
<td>Anterior</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Superior</td>
<td>Up</td>
</tr>
<tr>
<td>Sagittal</td>
<td>(Think brain-surgeon-view.)</td>
<td>Left?</td>
<td>Anterior</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Superior</td>
<td>Up</td>
</tr>
</tbody>
</table>
Maximum Memory default:
The amount of data acquired during a functional brain scanning session is large. Computers need to analyze these data in portions and in order to do that, the matlab package SPM processes data in "chunks". The maximum chunk size is determined by the `defaults.stats.maxmem` setting in the `spm_defaults.m` file. In the factory, this is set to "2^29" (i.e., 512 MB), which allows SPM to run on even the most feeble computer.

However the computers you are using in this lab can process larger chunks of information therefore we increased this limit to 2^30 (i.e., 1 GB). When you are running SPM in your own computer you can safely set this to as much as half of your computer's total RAM. So for instance, if you intended to use 2 Gigabytes of memory you would type in:

```
defaults.stats.maxmem = 2^31;
```

in `spm_defaults.m` and then save the file.

Now we have set the defaults such that we can begin using SPM. (You may close the editor window displaying `spm_defaults.m`). Remember that if you always have to restart SPM in order to apply your changes.
Start SPM

In order to start SPM, back in the Matlab command window, type `spm` to start SPM12 and select "fMRI". (You can also just type `spm fmri` in the command window.) You should get windows like the ones below...
Selecting files in SPM12 & examining functional data

You should always examine your functional images to check orientation and to detect any possible catastrophic problems. SPM package has tools that make it easy to do this. SPM has a user interface, which allows the user to choose the images that they intend to explore. This is accomplished by the file selection box, which is brought whenever you try to display images. For those of you not familiar with SPM, this box has certain features that might be counterintuitive. Let’s walk through the steps:

1. Use the "Display" button to bring up the dialog that will allow you to choose the functional image you are intending to explore. The SPM12 select dialog, which is invoked by clicking the display button, shows folders on the left and files on the right. Once you select a file it will show up on the bottom of the dialog. You may navigate through folders with a *single* click.

2. Now you need to navigate to \<<DataDir>> where you will find subject directories named after the date of the acquisition and subject's initials (e.g. "sub05"). Functional data are in each subject's directory in "func/whyhow/run_01"; "rrun_01.nii" is the data after motion correction as was covered in lecture. Note that these are 4D NIFTI images, with all of the acquired 3D images in one file.

3. The select interface initially assumes that all files are 3D Analyze files, i.e., that they only contain a single volume where a volume refers to the signal acquired from the subjects' brain at
one time point in the experiment. This is reflected in the ",1" after the filename. Since we are intending to display image number 100 we need to be able to see all the volumes. In order to do this we need to **change the volume specification to "1:300"** in order to see all the volumes in the directory (as displayed in the screenshots below). Now you can scroll down until you find `run_01.nii,100`. Click on the filename so that it appears in the bottommost window, as in the screenshot below. Then click **Done**. When you change the volume specification SPM will automatically expand all files in the directory. To filter only the files that we are interested in, we can make use of regular expressions (see box **Regular Expressions**).

![Select image dialog](image.png)

**BOX: select images dialog**

If you accidentally select a file (removing from the list on the right, moving it to the list on the bottom) you can de-select it by clicking on it. Unfortunately, that file does not re-appear in the list on the right. To refresh the list of files on the right, hit the "Filt" button.

**BOX: Analyze Orientation Defaults**

Regular expressions (regex) are special text strings that define a search pattern. These patterns can help you select for example only those files that contain a certain string, or that start or end with a certain string. A short overview on regular expressions can be found [HERE](#). In short: when you open a SPM dialog you will see `.*` already filled out in the search box. This means ‘show all files’. If you replace `.*` with a single character in the search box it will match all files that contain that character. Regex distinguishes between upper and lower case
characters. If you want to select only files that start with a certain pattern you can add a ‘^’ before your query. For example: ‘^w’ will filter all files that start with w. If you want to select all files that start with ‘w’ and also contain ‘subject_001’, you can enter ‘^w.*subject_001’. The ‘.*’ in the middle will allow other characters between the leading ‘w’ and the following ‘subject_001’. Regex have many other sophisticated features that are worth looking into.
Using the SPM Display Dialog
Now that the display dialog is open you may click around the orthogonal views of the brain (coronal, sagittal, transverse) in order to explore your images.

Try to answer the following questions:

1. What is the voxel size?
2. What are the image dimensions?
3. What are typical gray matter intensity values?
4. On the lower right hand side of the display dialog you may see the kind of interpolation being used in visualizing the images. Trilinear interpolation is default. First select Nearest Neighbor interpolation and explore the image. Next select Sinc interpolation and again explore the image.
   a. Which interpolation do you prefer?
   b. Why? It doesn't really change anything substantial.

Compare the functional data to the anatomical data
Now that we have explored the functional image of our choice we should make sure that the functional images are in the same space as the low-resolution T2 overlay anatomical images that we
have acquired for each subject. These data are found within each subject’s directory:

i.e. <<DataDir>>\sub05\anatomy

Anatomical low-resolution T2 overlay images (not to be confused with the high-resolution T1 anatomical images) in general have smaller in-plane voxels (i.e. greater in-plane resolution) but they have the same number of slices with the same spatial location. In order to ensure that functional and anatomical images are lined up (coregistered) we need to check their registration. This is accomplished by clicking the **Check Reg** button in the SPM dialog

**Click the Check Reg button:**

1. Note that the Check Reg button brings up a dialog box that is very similar to the one you have seen in the Display example in the previous section. One of the differences is that the Check Reg dialog will allow you to choose multiple files for you to be able to check their coregistration.

Since we are checking the registration of the anatomical (anat_lores.nii) and functional (e.g., rrun_01.nii,100) we need to make sure that they are both present in the "Images To Display" portion of the dialog box. To do this, navigate to the anatomical subdirectory mentioned above and select **anat_lores.nii** and then navigate back to the directory in which functional images reside and select rrun_01.nii,100. Then click **Done**.
**BOX: Windowing**

You can modify the contrast of the images by playing with the range of min-max values to be displayed as described below and shown in the figure above. In one of the 3 views, right-click; a context menu will appear, showing you the location of the
cursor and the intensity of the voxel under the cursor (shown as "Y = 415.141", for example). From the context menu, select:

**Image:Window:local:manual**

and enter a new minimum and maximum value. Try 0 and 110% of typical values. For example, if most of the brain is around intensity 1000, a good maximum is probably 1050 and you would enter "0 1050".

2. Note that now you see both the anatomical and functional images in the same window. If you click around one, note that the crosshair in the other will also move. This is how one checks whether two images are correctly coregistered. Specifically, there are certain landmarks in the brain, which are used to ensure that all parts of the brain are correctly coregistered.

Explore the pair of images, making sure to compare the following anatomical regions:

i. Frontal pole
ii. Occipital (posterior) pole
iii. Left & Right sides (e.g. superior temporal gyrus)
iv. Corpus callosum:
   a. Most anterior
   b. Most superior
   c. Most posterior extent

Do the functional and structural line up well? If not, how so?

Explore the regions of signal loss as we described in the morning lecture. This usually happens in the temporal poles and the orbitofrontal cortex.

<table>
<thead>
<tr>
<th>Temporal Poles</th>
<th>Orbitofrontal Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Temporal Poles" /></td>
<td><img src="image" alt="Orbitofrontal Cortex" /></td>
</tr>
</tbody>
</table>

On the anatomical image, go to the medial orbitofrontal cortex and look at the sagittal image; click on the coronal or axial until you have nice view on the sagittal image---that is, not right on the mid-sagittal plane (to avoid the cerebral fissure), but just off mid-sagittal.
Now click around on the sagittal view, keeping an eye on the axial view, and carefully compare the T2 and the functional.

Similarly, find the signal void in the temporal lobe (due to the ear canal). Observe cortex visible in the T2 anatomical image not visible in functional (T2*) image.

When we get thresholded activation maps, we typically overlay them on the structural images since they have more anatomical detail. But why should we *also* check localization of activation on the functionals?

**BOX: Despiking**

Brief global signal change due to artifacts (such as noise spikes during acquisition leading to "white pixel" effects) can be detrimental to functional MRI studies. Further, the presence of these artifacts can reduce the efficacy of other preprocessing steps; e.g. motion correction. Thus, detection and correction for these artifacts is important in functional MRI.

Typical despiking routines will look for significant deviation from the average signal intensity (such as flagging points that fall 3 standard deviations from the mean), then replace those points with the average of the nearest non-corrupted timepoints. This can be done in k-space (typically called "despiking") or in image space (sometimes called "scrubbing"). One advantage of doing it in k-space is that it is prior to reconstruction and other processing steps, and can remove a smaller amount of degrees of freedom (one k-space timepoint versus one image timepoint). In UM spiral imaging data, we apply despiking in the k-space data before image reconstruction.

**BOX: Slice time correction**

The 3D volumes (TR's) in fMRI that form 4D datasets are commonly collected in 2D slices. This results in a temporal offset between slices, meaning that one Because timing is important for functional MRI we want to adjust the slice timing trough temporal interpolation, so that all slices show the activation that the would have shown if they would have been collected at the same time. SPM has a build in tool that can be found on the main menu (‘Slice timing’). The first thing you need to do is click ‘Data’, specify ‘new session’ and select all 3D images in your data set. In SPM’s slice timing tool you further have to enter the number of slices in each 3D image, the TR (acquisition duration of each 3D volume), the TA (TR-(TR/number of slices), the slice order (see THIS explanation by Chris Rorden), and a reference slice. For a reference slice, often the middle slice that was collected is selected to ensure that the maximum interpolation is minimized. After everything has been filled out you can click on the play button to obtain slice time corrected 4D images. The data that you will be working with today is already corrected for slice timing.
Preprocessing I: Checking motion correction

Two key preprocessing steps have already been applied to the functional data: functional image registration ("realign"ment, or motion correction), and coregistration of anat_lores.nii with the realigned functional images. In the previous section we have inspected the quality of the coregistration of the anatomical and functional images. In this section, we'll be evaluating the results of the image realignment step. As noted earlier, subjects 01-10 in this dataset were all stellar participants in that they all kept their head very still while their functional images were being acquired. The subject named "subBAD" did not. Therefore, let's temporarily focus on the functional data in <<DataDir>>\subBAD\func\whyhow\run_01.

A particularly powerful way to check motion is to view a movie of the image timeseries before and after motion correction. You may have noticed that subBAD has an m-file named "Show4DMovie.m" in their functional image folder. To start the function, navigate to the above folder and enter the following at the command line:

```
>> Show4DMovie
```

Once you hit enter, you will then be prompted to select three files that will be used to make the movie:

1. run_01.nii - the 4D file before motion correction
2. rrun_01.nii - the 4D file after motion correction
3. rp_run_01.txt - the realignment parameters file
Once the program loads the data, the movie will start in a figure window like the one shown below:

- The top two images show the timeseries before and after motion correction from the same sagittally oriented slice.
- The bottom two images plot the timeseries of the estimated head translations (in mm) and estimated head rotations (in degrees).
- The position of the moving vertical lines corresponds to the number of the image being show
Once the movie finishes, you should see a prompt at the MATLAB command window asking you if you’d like to re-play. Once you’re satisfied, answer the questions on the following page.

Try to answer the following questions:

1. On which dimension of translation did subBAD move most?
2. On which axis of rotation did subBAD move most?
3. Can you see any residual head motion in the image time series after motion correction?
4. Should subBAD be excluded from the study? Why or why not?

---

**Preprocessing II: Coregistration**

SPM's "Coregister" facility is used for registering different types of images from the same subject (it is a "intermodality, intrasubject" registration). To use it you must specify a "Reference" image, which does not move, and a "Source" image, which is transformed to match the reference.

**Historical Note:** Beginning with SPM2, the rigid body transformation was recorded in an auxiliary .mat file named like the source image. With either SPM2, SPM5, SPM8 or SPM12 it is not necessary to write out the transformed image (the Source transformed into the space of the Reference), though it is sometimes convenient to do so.

Before coregistering the low- and high-resolution structural images we need to (I) make sure the two images are in the same orientation and not too far from one another, and (II) correct for intensity inhomogeneities in the high-resolution T1 image.

1. First see how close the anat_lores and anat_hires images are to start with. What button do you use to view more than one image?

   Multiple images are displayed in the space of the first image. If they are not previously registered this simply means that their origins are lined up.

   Neither image has had their origin set. The default origin is the center of the volume. To see the specific voxel that is defined as the origin, use 'Display' and look in the lower right panel.

2. If the images were very out of register we could try to get the images closer by manually setting the origin to the Anterior Commissure (AC).
A. Here is a diagram of how to find the AC on the midsagittal plane:

B. Now use the Display button to view the anat_lores image and find the location, as best as possible, of the AC. You should be able to see it on the axial slice.

*Hint: Once you are close, it helps to "zoom in". In the display facility, click on the "Full Volume" pop-up menu; select "80x80x80 mm"

- What is the location of anat_lores's AC in voxels?

C. Use the Display button view the T1 image and locate the AC, as best as possible.

- What is the location of T1's AC in voxels?
- What is the location of T1's AC in mm?
D. At this point we *could* set each image's origin but we *won't* (just for reference).

**BOX: How to change the origin**  
*(again, don't do it now!)*

View the image whose origin you want to set, using the 'Display' button. By default, the crosshairs start at the currently-defined origin. (If you've previously been using 'Display' facility, and you're not at the 0,0,0 mm point, click the "Origin" button.

To change the origin, locate the AC and put your cursor on it. Click the "Set Origin" button. Next, click the "Reorient..." button. Select the set of images (any number!) in the same space whose origin you want to change.

We're not changing the origin for two reasons. First, the images *should* already be quite close, but there's a more important reason. By setting the origin on the Reference image (anat_lores), we change its "world space"; specifically, the origin of its world space moves from the center of the volume to the AC. Hence it will no longer have the same world space as the functional data *unless* we identically change the origin on all the functional data and any results (beta*, con*, spm_T* etc) we already created.

To summarize, if the images are way off, we can set the origin manually to help the Coregistration. But if we change the origin of the image representing the functional space, we have to similarly change the origin on all functional images.

3. Two processing steps have already been performed on the structural images. They have had been scalp-edited and corrected for inhomogeneity.

   1. Scalp-editing (also known as skull-stripping) is the deletion of non-brain regions of the
image volume, i.e., the scalp and skull. Since this extraneous information can throw off subsequent preprocessing the images have been scalped-edited with the Brain Extraction Tool (BET) from the FMRIB Software Library (FSL).

2. Correction for inhomogeneity (also known as bias correction) accounts for the fact that anatomical images obtained with high-field magnets (> 2T), tend to be brighter in the center of the field of view than at the edge. This inhomogeneity can throw-off subsequent preprocessing, especially segmentation. Therefore, the images have been corrected for inhomogeneity. The uncorrected images have "_nobiascorrect" appended to them. See sidebar for further details.

4. Use Check Reg to compare anat_lores.nii to anat_lores_nobiascorrect.nii.
   1. Can you see the greater intensity in the center of the uncorrected image?
   2. Does the corrected image look better?

**BOX: Inhomogeneity Correction**
Images were corrected for inhomogeneity using two SPM8 functions. The first, `spm_bias_estimate.m`, estimates the image inhomogeneity. This estimate is saved in a "bias field" matrix that is used to correct for inhomogeneity using the second function, `spm_bias_apply.m`. In the following example, these functions are used to correct anat_lores.nii. It assumes that you are working in your subject's anatomical directory:

```matlab
>> biasfield = spm_bias_estimate('anat_lores_nobiascorrect.nii');
>> spm_bias_apply('anat_lores.nii', biasfield);
```

If successful, this should yield a bias corrected version of the image with "m" prepended to the name (in this case, "manat_lores.nii").

5. You will now "Coregister" the high-resolution anatomical image (anat_hires) to the low-resolution anatomical image (anat_lores). The anat_lores image has the same space as the functionals, and hence this coregistration will set anat_hires' world space to correspond to the functionals. Make sure you know answers to the following:

   A. What is your "Reference" image?
   B. What is your "Source" image?
   C. Click on the Coregister button. In the Batch Editor select
      New Coreg: Estimate
      i. Double click Coreg: Estimate, then
      ii. Set the Reference and Source images you identified above.
      iii. Now "Run" the job to perform the coregistration.
The transformation parameters are written into the NIFTI file of the source image anat_hires (if a file initially has an Analyze header, it is silently converted to a NIFTI header). Please see the Appendix for more information on NIFTI. This action sets the world space of anat_hires to match that of anat_lores. Given that the world space of anat_lores already matched that of the functional images, this means that the anat_hires is now in the world space of the functional images.

D. **Note**: changes in world spaces.
   - "Display" anat_lores.nii and use the "World Space"/"Voxel Space" pop-up button to change between the two spaces.
   - Does the image move as you flip between the two spaces? If so, do you understand why?
   - "Display" anat_hires.nii and do the same again.
   - Does the image move as you flip between the two spaces?
   - Why do the images move between spaces?

E. Check the success of the Coregistration.
Use check reg to check the registration. Check the points mentioned before:

i. Frontal pole
ii. Occipital (posterior) pole
iii. Left & Right sides (e.g superior temporal gyrus)
iv. Corpus Callosum
   a. Most anterior
   b. Most superior
   c. Most posterior extent
v. Trace the sulci

Has the coregistration succeeded?

---

**Preprocessing III: Spatial Normalization**

Spatial Normalization is used for intersubject (between subject) registration. It is essential for performing intersubject analyses for determining Talairach/MNI coordinates of activation foci.

While the "Normalise" button can accept any kind of image (T1, T2, etc), to register your subject into the standard atlas space, it is important to use the highest resolution structural data.

1. For us, that is which image?

   (Check your answer before continuing on!)

Spatial normalization (or, with a British spelling, normalisation) takes three types of images

i. **Image to Align**
   These are the high resolution anatomical images from which the spatial transformation is determined. Typically you only specify *one* such image per subject.

ii. **Images to Write** (normalised)
   These are other images *with* *the* *same* *world* *space* as image (i) above. Typical examples would be statistic or contrast images, or whole set of ra* functional images.

iii. **Tissue probability map**
   The normalization parameters are obtained through segmentation (see box: SPM12 normalization). The tissue probability map (TMP.nii) is a 4D image that contains a total of 6 3D images of the following modalities: gray matter, white matter, cerebrospinal fluid, fat tissue, bone tissue, and air. The atlas space of this TPM defines the space that you warp your images to. In short: the TPM defines the world space of your normalized images. In SPM12, the world space of the standard TPM is MNI152.

   It produces a "y_.nii" deformation field image (see box: SPM12 normalization), which records the nonlinear transformation *from* the *world* *space* of image (i) *to* the
2. Now that we have coregistered, what does the world space of anat_hires correspond to?

3. Thus if we spatially normalize anat_hires the resulting anat_hires_sn.mat file will not just be good for anat_hires, but also for ...?

Click 'Normalise' to start the spatial normalization process. Select "Normalise: Estimate & Write" in the drop-down menu, find the "Data" item in the hierarchy and click "New Subject" (for now, just do one subject). Under "Subject", for "Image to Align", select anat_hires.nii, the homogeneity-corrected, scalp-edited, hi-res anatomical image that is now in the space of the functionals. For "Images to write", select again anat_hires.nii.

**BOX: SPM12 Normalization**

Normalization consists of several steps which can generally be divided into an initial linear (affine) part that uses 12 degrees of freedom (translations, rotations, shearing, and stretching) and a consecutive non-linear component.

In SPM12 spatial normalization is no longer based on minimizing the mean squared difference between a template and a warped version of the image. Instead, it is now done via segmentation, as this provides more flexibility. I.e., a nonlinear deformation field is estimated that best overlays the atlas on the individual subjects' image. The older way of spatially normalizing images that was used in SPM8 and older versions is still available via the "Old Normalise" Tool. However, the aim is to try to simplify SPM and eventually remove the older and less effective routines.

**BOX: Deformation fields**

During the non-linear transformation, for each voxel it is calculated how it should move and shrink or expand to fit the template image. This information is stored in the deformation field \( y_* \).nii instead of sn.mat files which was the case in previous SPM versions. The current format allows much more precise alignment. It contains three image volumes encoding the x, y and z coordinates (in mm) of where each voxel maps to.

The images below show an example of 1 3D volume of the flowfield that encodes for the x (coronal; left image) and y (sagittal; right image) deformations. The right image shows the deformation field overlaid on the high-resolution anatomical image.
Click "Run" to run the job, after a short while it will finish. This will create a `y_anat_hires_.nii` and reslice the requested file, creating a `wanat_hires.nii`.

Check the success of the registration, comparing the normalized image (`wanat_hires.nii`) to the unsmoothed version of the template image that is `avg152T1.nii` in the `SPM12\canonical` directory.

4. Using the landmarks suggested above, has the spatial normalization succeeded?

5. Above, we noted that the "Estimation" part of "Estimate & Write" saves the nonlinear transformation necessary for normalizing any image that is in the same space as (or co-registered with) the `anat_hires.nii`. The file it saves should be located in the same directory as `anat_hires.nii`, and should be named `y_anat_hires.nii`. Now, we can use this file to normalize the functional images. To do so, first click 'Normalise' as before. Given that we've already estimated the nonlinear transformation (and have the `y_*.nii` file to go along with it), we should select "Normalise: Write" in the menu. Find the "Data" item in the hierarchy and click "New Subject" as before. Under "Subject", for "Deformation Field", select the `y_anat_hires.nii` we just estimated. For "Images to write", you'll want to select all 300 functional images. Navigate to that subject's "func/run_01" directory and change the volume specification to 1:300 as before. Then, first select `rrun_01.nii`, 1, and then scroll down and shift-select `rrun_01.nii`,300 to grab all 300 images. The select dialog should report "Selected 300 files". (If you select the wrong files right-click in the bottom selected file box, and select "Unselect All".)
6. Save and run your job. Given that this job will write a new 4D file, it will take a couple minutes to finish. When it does finish, you should have a file named "wrrun_01.nii" in the same directory as your original 4D functional file. Use the "Check Reg" function to visually examine the quality of the registration between the normalized anatomical (wanat_hires.nii) and one of the normalized functionals (e.g., wrrun_01.nii,300).

Preprocessing IV: Smoothing
The only preprocessing step left to do for an intrasubject analysis is spatial smoothing (we'll use 8mm). This is done in order to increase signal to noise ratio (SNR) and the ability of statistical techniques to detect true and task related changes in the signal. We'll use the Batch Editor module Spatial:Smooth to implement this.
1. Click the "Smooth" button in the top-left window. The graphics window now shows a 4-panel interface. The upper left shows the hierarchy of the current job; the bottom panel displays help messages (these are often quite helpful!); the right column of panels are used to input new values and review current values in the job specification. The top-left panel should show: Smooth <-X The "<-X" marker indicates that there are required options that have not been specified. As long as there are any "<-X " markers present the job cannot be run (notice that the Run button is grayed-out)).

2. If the window with the title "Current Module" is empty, double click "Smooth <-X ". Six sub-options appear. The first item is SPM's built in help on the topic of smoothing. The second item, "Images to Smooth", since it bears the "<-X " marker must be set. The other four already have default values set.

3. Select "Images to Smooth" by single-clicking it. Then in the lower right, single-click "Select Files". Alternatively, you can double click "Images to Smooth". Once you have the dialog open, select all 300 normalised images within your freshly written "wrrun_01.nii" 4D file. The selection procedure is the same as in the "Normalise: Write" job described above, but make sure you're selecting the 4D file with "w" prepended to it. Once you're sure you've selected all 300 normalised images, click 'Done', and confirm that the correct files were selected (as shown in the 2nd box in the right-hand column of the Batch Editor).
4. Set smoothing. The interface allows us to specify things such as the width of the filter. Since we accomplish filter/smoothing by simply convolving our signal with a three dimensional Gaussian function we can specify the extent of smoothing by using the full width at half maximum (FWHM) of the Gaussian Kernel. This can be done by clicking "FWHM". Note that a default smoothing of "8 8 8" has already be set, so there is no need to change anything.

5. Now it is time to start the job. You can do this by clicking the green filled triangle button or by selecting "Run Batch" under the file drop-down menu. (Note that at this point we could have clicked File> Save Batch to save the job in .mat, .m or .xml formats. For such a simple job, there is no point in saving the job.) You will notice that the window on the lower left hand side will display a progress bar.

6. Smoothing creates images with a "s" prefix. Use "Check Reg" to compare the first and last image (1 and 300) from both the unsmoothed (wrrun*) and smoothed (swrrun*) 4D image files. (This will ensure that you smoothed all the images, and give you a visual feel for the impact of 8mm smoothing).
APPENDIX

Notes on preprocessing

There are several ways to check the quality of preprocessing stages in fMRI. You can check most of them using spm_check_registration ("Check Reg" in the GUI). If you or someone in your lab can do some scripting, it may be a good idea to automatically generate images that show the following for each subject and save them in an electronic "log book." Here’s a suggested list:

1. Do the functional images appear coregistered with the anatomical image (overlay) you will normalize to the template?
2. Are the anatomical images you will normalize roughly in alignment with the template?
3. Do the normalized anatomical images seem closely matched to the template?
4. Do the normalized functional images seem to be in reasonable alignment with the template?

BOX: NIFTI (Neuroimaging Informatics Technology Initiative)
The purpose of the Neuroimaging Informatics Technology Initiative is to support service,
training and research to develop and enhance the utility of informatics tools used in neuroimaging, with a focus on functional magnetic resonance imaging (fMRI).

NIFTI is jointly sponsored by National Institutes of Mental Health (NIMH) and the National Institutes of Neurological Disorders and Stroke (NINDS) which are part of the National Institutes of Health (NIH) and Department of Health and Human Services (DHHS). The Data Format Working Group (DFWG) of NIFTI is in charge of coming up with a technical solution to the problem of multiple data formats used in fMRI research. The NIFTI header that we refer to in this tutorial is the supplemental information that is placed at the beginning of the fMRI data. This header has the parameter settings for aspects of the data such as voxel size, affine transformation and number of slices. When two images are coregistered, the rigid body transformation that maps one image to the other is stored as quaternions, which are short forms of rotation/scaling/translation/shearing matrices, in the NIFTI header. A schematic description of how this information is used is included below.
user interface. Experience with this will increase your speed and power in processing your data.

The following code uses the saved normalization job from above.

1. The following commands let you bring up and run the Batch Editor from the command line in MATLAB.

   % brings up Batch Editor in interactive mode
   spm_jobman('initcfg')
   spm_jobman('interactive')
   % You can then create, edit, load, save, and run batch jobs; try loading the normalization batch file

2. Alternatively, you can also load and run batch jobs from the command line.

   batchname=spm_select(1,'mat','Select batch file with normalization')
   load(batchname)
   spm_jobman('run', matlabbatch)

3. You can also load batch jobs, and use the results in any other spm modules. This code loads the normalization batch file, and brings up the check registration window:

   % Select and load batch file
   batchname=spm_select(1,'mat','Select batch file with normalization');}
   load(batchname})

   % Get file names
   src = matlabbatch{1}.spm.spatial.normalise.estwrite.subj(1).source{1}
   template = matlabbatch{1}.spm.spatial.normalise.estwrite.eoptions.template{1}

   % Append w to src name to select normalized file
   [dd, ff, ee] = fileparts(src)
   wsrc = fullfile(dd, [‘w’ ff ee])

   % Compare images
   spm_check_registration(char(template, wsrc));