Preprocessing instructions

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The following instructions rely on your knowledge of quality control and preprocessing in fMRI. For an introduction, read the relevant chapters in the Huettel and Poldrack books and refer to web tutorials such as this one:
https://fmri-training-course.psych.lsa.umich.edu/archived-2017-materials-lecture-slides-lab-materials-lecture-recordings
or the starter's guide available here: http://www.ernohermans.com/wp-content/uploads/2016/09/spm12_startersguide.pdf
as well as the SPM12 manual.
NOTE: There are a lot of fMRI / SPM analysis tutorials online, some of which may be better than the ones above – I did not check them all!

Required Software

- Matlab with the Image processing toolbox
- SPM12 with the following toolboxes:
  - MRTool
  - tsdiffana
  - Fieldmap
  - ArtRepair

Expected folder structure

[directory for all subjects]
    [directory for subject number x]
        [directories for anatomical, MT, fieldmap and functionals in numbered runs]

Working with 4D Nifti files

- The functional data are stored as a single file per run, which includes all 3D volumes in a temporal sequence (4^{th} dimension). To work with or display one of the files, you need to expand the volumes first.
- In the GUI interface, this is done via the line below the filter line: when you open a directory (in the left part of the GUI window), the right side of the window displays all files in that directory. You can use regular expressions* in the line next to "Filter / Reset" to filter the files. This step is the same for all types of files (anatomical, functional, statistics…).
- For functional images, you can then select which frames (=volumes) you want to display using the line below the 'filter' line. In general, you can just enter 1:999 there to display all volumes of that run.
- If you made a mistake in your file selection, you can remove the selected file from the selection list at the bottom by clicking on it. It will not appear again in the list on the top right side unless you click on *reset* first!
- IMPORTANT: If you open a menu that already has a selection at the bottom (e.g. because you are modifying a .mat batch file), you cannot just select a different file in the top-right list (unless you want both files in the selection). If you want to substitute the old selection with the new selection, you have to unselect the file at the bottom first – otherwise the new one will not get selected. There will be no error message to alert you!

*For help on regular expressions (regexp), click on the question mark in the GUI or use google.

SPM coordinates

Each point in the brain can be indexed as coordinates relative to the origin of the image. The origin is the point where coordinates in all three directions are set to 0. When you open an image in SPM using *Display*, you can set the crosshair to the origin by clicking on *Origin*. Each point can be indexed relative to this origin as follows:

x coordinate: lateral-medial (-left, +right)
y coordinate: anterior-posterior (+anterior, -posterior)
z coordinate: superior-inferior (+superior, -inferior)

Steps when starting a new experiment / using a new sequence

- Set up the batch file with your sequence details. To do this, first get a pdf of your fMRI sequences (from the scanner console) and run the dicom import for your pilot subject's data (see step 1 below), from which you will need the .json generated by the import of your EPI and fieldmap sequences and the file names created by the import. Then open the preprocessing_batch template file. Enter the following details from the above files and your PC setup and save the batch under a new filename:
  - directory for your epi template
  - directory for your tissue probability map
  - number of functional runs
  - the name template for your directories (anatomical, MT, fieldmap, functional runs)
  - a file selector for your fieldmap phase and magnitude images
  - directory for all subjects (dir_base)
  - TR of your EPI sequence
  - number of slices of your EPIs
  - The reference slice for slice timing. This is the **temporally** middle slice – with an interleaved sequence, this does not correspond to the middle slice number! If you want additional confirmation, you can look at the .json of your EPI; however, the .json will show slice numbers starting with 0 instead of 1, so you will need to take that into account.
  - The slice order for slice timing. On Siemens scanners, if you have an interleaved sequence with an even number of slices, the order will be 2:2:[topslice] 1:2:[top-1]; if you have an odd number of slices, the order will be 1:2:[topslice] 2:2:[top-1]
  - FWHM for smoothing, usually 2x in-plane resolution
  - The resolution you want after normalization – voxels are resampled during normalization and the resolution applied in this step is usually smaller than the original voxel size.
  - fieldmap echo times for the short and long TE (included in the .json of the fieldmap)
  - Whether your fieldmap is an epi sequence or a non-epi sequence (in the Bion Prisma, it's currently a gradient-echo (GRE) sequence)
  - total readout time of your EPI sequence (included in the .json of the EPI)
  - blip direction (can be hard to determine, in the current Bion sequence it's -1)

    - The rest of the script assumes that your Bion subject name starts with 0 (i.e. your experiment number starts with 0 - the current default is 0xxxyy with x=number and yy= your initials); if it does not, you will need to run search-replace on “^0”,”^a0”, “^ua0” etc.
    - You can also change other parameters in the script, but please discuss such changes with me beforehand.
- As a mandatory next step, set up the preprocessing for your pilot manually using the batch GUI. Save the manually created batch. Check that the parameters in your batch script correspond to the parameters in your manual batch – there are several ways to achieve this: 1) in the batch interface, you can click on view -> show .m code; 2) you can load the manually created .mat batch into the workspace and check the variables and structure there, 3) you can load the individual .mat jobman batches the script creates into the batch interface.
- Run the “steps to follow” for your pilot subject, paying extra special attention to the quality control steps. For this subject, you should check each step of the script carefully (even the steps that do not need to be checked for all subjects later). If you are not sure what to look for after any of the steps, ask me.
- Set up your statistical analysis pipeline for the first subject, so that you can be sure that all the information is in the logfile in usable form (see statistics instructions).
- DO NOT SCAN any more subjects before you are CERTAIN that everything is working correctly for the pilot subject.

Steps to follow for all subjects

After setting up the batch file with your sequence details, go through the following steps for each subject. Do so soon after acquiring the data, so that you can detect artifacts and other problems early.

1) Dicom import
    - Use dcm2niix.exe for your dicom import
    - Open a command prompt (in Windows, click on the start icon and type *cmd*)
    - Change the directory to the directory where dcm2niix is located using *cd* (if it is on a different drive, change the drive first by typing the drive letter, e.g. *d:* and hitting enter).
    - If the exe is in the (supra-) directory where your dicoms are, you don’t need to specify an input directory, it’ll take the directory where it is located plus all subdirectories. In this case, you can use the following code to run it:
      dcm2niix -f %n_%p_%s_%e -z n . (include the dot!)
    - If the exe isn’t in the directory where your files are, you can use the following code to run it:
      dcm2niix -f %n_%p_%s_%e -z n "your_dicom_directory"
    - The script generates 4D nifti files plus files ending in .json. When you run a new study with a new EPI sequence, you will need the json of the EPI sequence to set up the fieldmap parameters in the preprocessing batch script. After that, you can create matlab code to automatically delete your jsons.

2) Visual quality control

   - Use the *CheckReg* button to display up to four files at a time. Select 3-4 pseudorandom files from each epi run (beginning, middle, end), the structural and the MT. (Not all at the same time!)
   - Inspect the images for abnormalities. If you're not sure about something, ask me.
   - Open a functional image using *CheckReg*, then right-click and select *Browse…* -> select all functional images -> click on the bottom-right play button (>) to see a movie. Artifacts and other signs of bad quality should pop out. You can save the movie if you want by clicking on Browse… again.
   - Alternatively, you can use art_movie to display the movie (see ArtRepair instructions at the end). Examples of good and bad quality images are provided in the ArtRepair documentation, e.g. in the file Alphascript_v1.0.pdf.

3) Manually reorient each subject's scans to match the template orientation.

   - First, get to know the orientation of the MNI template brains. Open the single_subject_T1 (found in spm12\canonical) using *Display*. Click on *Origin* and take note of the approximate crosshair position, especially in the y direction. Look at the orientation of the brain – the frontal pole and occipital pole are approximately on the same horizontal line. The brain is approx. symmetrical both in the horizontal and the coronal views (there is no rotation).
   - Next, open one of your subject's scans (functional or structural, doesn't matter). Click on *Origin* and compare the crosshair position to the position in the canonical image. If it is not approx. in the same position, move the brain by changing the relevant values in the bottom left table. The most likely shift is in y direction – try getting the origin closer to the canonical origin by changing the "forward" value (unit: mm; e.g. -10) and clicking enter. You will see the crosshair move. Do this until it is in the correct position.
   - Then, use the rotation values as necessary to align your brain with the canonical orientation. It is almost always necessary to change the pitch (rotation along the z axis in the sagittal plane) to a more positive value (unit: radian; e.g. 0.3). If the subject was tilted in the scanner, you may also have to correct the roll (rotation along the z axis in the coronal plane) or yaw (rotation along the y axis in the horizontal plane).
   - Finally, apply these values to **all scans** (all functionals, structural, MTs, etc.) by clicking *Reorient* and selecting them using the GUI (all in one step). If you leave one scan out, it will not be aligned with the other scans anymore!

4) Run the preprocessing script, which includes the following steps:

   - Extraction of the brain (skull stripping) from the structural scan. This step helps with the anatomical images from the Bion Prisma, which may otherwise appear dark in the standard SPM display. For other scanners/sequences, this step is not necessary but can potentially still help with normalization.
   - tsdiffana: checks for volume-to-volume variance, mean voxel intensity and slice variance

- slicetiming, fieldmap generation, realignment, coregistration, normalization and smoothing: standard preprocessing steps, refer to the literature or ask me if you have any questions
- 4D to 3D conversion of the smoothed images, for use in ArtRepair

You can run the script as a function: enter the function name in the Matlab command line, followed by the index number of the first and last subjects you want to include in preprocessing (e.g. *preprocessing_batch(1,10)*). If you want to skip some subjects, include the following code just inside the loop *for s0 = [first:last]* in the script*:*
if s0 = index_to_be_excluded then continue; end

5) Go through the following quality control steps **as soon as possible after data acquisition**:
   - Visually check that the .vdm-files look fine.
   - Check the subject's movement using the .ps file in a subdirectory of your subject called 'realign_figure'. If a subject shows volume-to-volume movement above approx.1 mm of translation or 0.5° of rotation, it should probably be excluded. Discuss the options with me.
   - Open the mean functional image using *CheckReg*, then right-click and select *Browse…* -> select all normalized functional images -> click on the bottom-right play button (>) to see a movie. Artifacts and other signs of bad quality should pop out. You can save the movie if you want by clicking on Browse… again.
   - Visually check the normalization. When you open two or more images with CheckReg, the crosshairs are linked: when you click on a particular position in one image, the crosshair in the other image(s) will move to a corresponding position. After normalization, the positions should match closely.
     Check the normalization using the following steps:
     - *CheckReg* on the structural (wua0*.nii) and the canonical T1.
     - *CheckReg* on the mean functional (wmeanua*.nii) and the canonical T1.
     - *CheckReg* on the MT (wua0.*nii) and the canonical T1.
     - For each of these steps, check the quality of the normalization by:
       - Clicking on the outline of the brain to see whether the positions match. Check a number of anterior, posterior and lateral positions in different axial scans (i.e. along the inferior-superior axis).
       - Clicking on clearly visible structures in the brain, mostly around the corpus callosum and boundaries of the ventricles.
       - Clicking on the outline of the midbrain (dorsal, ventral, lateral).
     - For each of these steps, additionally right-click in the figure, select *Contour -> Display onto -> all but current*. This will overlay the contour of the image which you selected by right-clicking onto all the other images in the figure. Check that the outlines correspond to the underlying tissue. [After collecting all subjects, you can also use this function to check the overall normalization.]
- Check the tsdiffana output together with the next step (ArtRepair). The output is in a postscript file in a subdirectory of your subject called 'tsdiffana'. (There are many postscript viewers available – an easy one is 'Postscript Viewer' by Rampant Logic.) Each run is displayed on a single page. Both give the volume-to-volume

variance and the variance in global signal, but tsdiffana also gives the slice-by-slice variance. The latter usually drops off from the first to the last volume. If it looks different, it could be a sign of artifacts.

6) Run ArtRepair using the Toolbox button in the SPM GUI.

   1) Run art_movie by clicking on the first green option in the ArtRepair menu (*View data with contrast movie*).

      - Select all functional images using the GUI. Select your preferred slice orientation. You can then select only some volumes (check all volumes per default, only subselect if you want to specifically check volumes highlighted in other quality control steps).
      - Select Data Magnification: Contrast or High contrast
      - Choose a Reference Image: Static-default
      - Select your preferred viewing mode (with Slider mode, you can move at your own pace).
      - Parse & Display bad slices: No
      - You will then see a static b/w image and a movie or slider. If there are big orange or blue clusters in the latter, this is an indication that artifacts will impact your analysis -> a sign that you need to re-check and re-do your preprocessing. Other artifacts may also be visible. Examples of good and bad quality images are provided in the ArtRepair documentation, e.g. in the file Alphascript_v1.0.pdf or ArtRepairOverview.pdf.

   2) Run art_global by clicking on the middle blue option (*Bad volumes*: …)

      - Which global mean to use: Auto
      - Have realignment files: Yes -> select your swuaf.*00.* files for the first run (the 00 is necessary to not include the 4D file), then select the realignment parameters file (rp*.txt) for the first run. In theory, there should be an option in ArtRepair to indicate more than one session; however, it does not seem to be working, so you will have to run art_global separately for each session.
      - Always repair 1st scan: No
      - You will then get the output display. I would suggest to use the default cutoff for repair (0.5 mm/TR). If it flags a lot of scans, discuss the options with me.
      - Follow these guidelines from the ArtRepair documentation (with modified percentages for our use):
        Look for scans with excessive repairs, excessive motion, sudden shifts in motion, and global change in signal. If a scan has more than one of these issues, it will probably be unusable.
        - Global Average Signal: count # of red vertical lines, or count # of entries in art_repaired.txt file (also located in preproc_data. Log # and % in excel spreadsheet. Make a note if repaired scans exceed 5% of total nframes. Also note if Global Average Signal appears to exhibit a significant linear drift (Fig O).
        - Standard Deviation Plot: Check maximum std dev. Any values > 4 are probably unusable. Log in excel spreadsheet.

  - Realignment Plot: Check motion. Consistent motion > 3mm probably unusable. Log in excel spreadsheet.
  - Motion (mm/TR) Plot: Check for scans flagged for fast motion. If these exceed 5% of total nframes, probably unusable. Log in excel spreadsheet.
- Click on *REPAIR*, then *Interp*. ArtRepair writes all scans with a prefix v.
- Use the *Display* button for a sample of the corrected files for an additional check.

Preprocessing is now **finished**, use the files called **vswuaf*** for statistical analysis!

Additional options

After scanning and analysing all subjects, you can use art_groupsummary, art_groupcheck and art_groupoutlier (the last three green options in the ArtRepair menu) for additional quality control. Refer to the ArtRepair documentation for these steps.