NHP Awake fMRI Preprocessing Pipeline

User Guide

LT 2016.11.28
BC 2014.12.17

Software Requirements

UNIX / Mac OS

Learn how to use nano or your command line editor of choice

To edit .bash, .bashrc, .profile, .bash_profile files, the easiest thing is to use the nano editor (e.g., **`nano .bashrc`** in your terminal; to save the files after, you may need root access, so try instead **`sudo nano .bashrc`** and type your user password). Nano is a basic but useful command line editor – Google around for some usage tips.

> **Nano tutorial**
> http://www.howtogeek.com/howto/42980/the-beginners-guide-to-nano-the-linux-command-line-text-editor/

Install MATLAB 2013 or later

- Check with the department IT staffs, or
- If you are a student, get your licence from:
  https://register.it.ox.ac.uk/self/software

Ensure MATLAB command line is working

- while in your home directory, run **`nano .bash_profile`** in your terminal, and add the following lines at the end:

```
PATH=/Applications/MATLAB_R2014b.app/bin:${PATH}
export PATH
```

Make sure to use the correct Matlab path as installed in your Applications directory.
Remember to write-out your changes to .bash_profile (see nano tutorial above)

- then in your home directory, reload the .bash_profile in your terminal:
  `. .bash_profile`

- Test that it's working in the terminal:
  `matlab -nodisplay`

- you should see Matlab open in your terminal (once it's loaded, type **`exit`** to go back to main terminal)

Install FSL

> http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FslInstallation

Install your favourite text editor

- Sublime Text is a good one (https://www.sublimetext.com/) - the trial version is essentially free forever, but "pay for me" messages pop up every so often (tolerable)
- Sublime provides syntax highlighting and code completion for many languages

Install JIP

Download from http://www.nitrc.org/projects/jip/
(note: download **jip-source**, NOT jip-Darwin)

The following steps set up the paths correctly so JIP will be recognized in your terminal command line. To see .bashrc files (files prefixed with . are usually hidden), use **`ls -a`** command in your terminal.

Extract the jip-source directory to the following path:
`/usr/local/share/`

In the jip-source directory, open **`define-jip.bash`** file:
replace **`export JIP_HOME=/homes/me/jip-tools`**
with **`export JIP_HOME=/usr/local/share/jip-source`**
(i.e., where the jip directory is found on your computer)

In the file **`/Users/{your_username}/.bashrc`**, use nano to place the following line:
`source /usr/local/share/jip-source/define-jip.bash`

Install Gitlab and get access to oxneuroanalysis repository

- make a Gitlab account and email it to admin to obtain access
- see Gitlab guide for setup instructions

Access oxneuroanalysis repository (for MRCAT, MATLAB toolboxes, Shell scripts)

From the local **`oxneuroanalysis/nhp_preprocessing`** directory on your computer, **copy over the following files/folders into another directory (do NOT edit or work from the original files in the oxneuroanalysis directory):**

- MrCat-dev folder
- prepare_align_epi folder
- prepare_align_anat folder
- organise_raw_data.sh
- Offline_SENSE.mlappinstall
- Align_EPI.mlappinstall
- Align_Anatomy.mlappinstall

Install MATLAB toolboxes

- double-click on the 3 .mlappinstall files on your computer

Getting data

[NEEDS UPDATING]

New data can be obtained from the external hard-drives in the BSB – though, do not take them out of the MRI control room.

Data backed up on the server can be downloaded from there – make sure that you use a computer with a permitted IP (e.g. via BSB computers, jalapeno, or any desktop inside Plant Sciences).

Backup data server address: **`afp://femur2.imsu.ox.ac.uk`**

You can connect to it on a Mac using the Finder GUI: Finder > Go > Connect to server

You can also connect to it using the terminal:

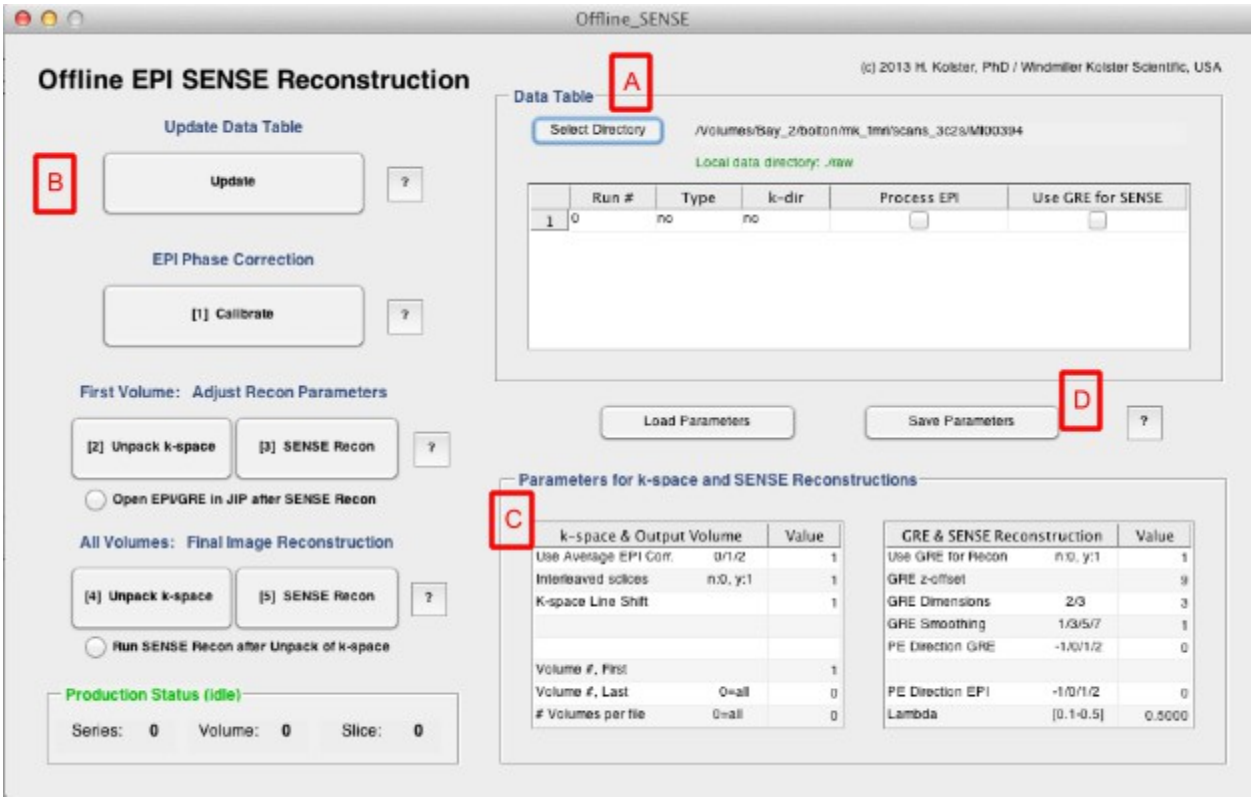
```
scp -r backup_user@mrifmribgw.bms.ox.ac.uk:"/data/raw_backup_3/MI00453
/data/raw_backup_3/MI00455" /Volumes/Bay_3/experiment
```

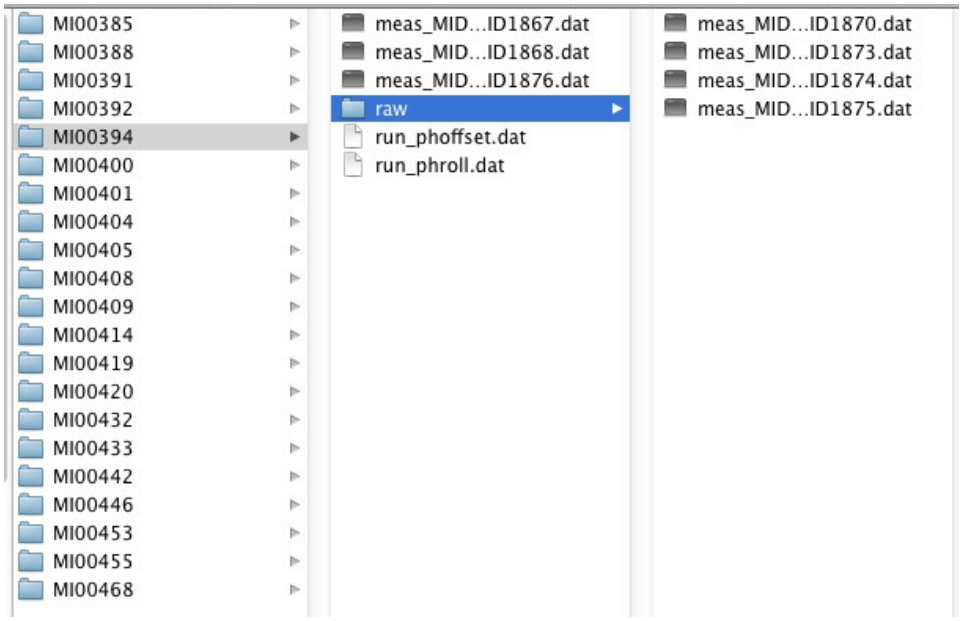
Setting up the data folder structure

Hauke's toolboxes (Offline_SENSE, Align_EPI, Align_Anatomy) depend on a specific folder structure for your data:

- Put all scanning sessions (e.g., MI01035, MI01036... etc. folders) into one “Experiment folder” named whatever you like
- In each session folder, create a folder called “raw”
- Move the EPI (e.g. meas_MID896_ep2d_fmri_FOV128_1_5mm_TE30_FID1870.dat) and GRE (e.g. meas_MID901_gre_3D_fmri_base128_fov186_4chcoil_FID1875.dat) raw data into the “raw” folder

- **Note: if you have many sessions, there is a sample script (`organise_raw_data.sh`) which you can edit and use to organise your folder automatically for each session (be careful – Shell commands cannot be undone!)**





Experiment folder ⇒ Session folder ⇒ “raw” folder

OVERVIEW OF PREPROCESSING STEPS

1. Data unpacking and source reconstruction (Offline_SENSE)
2. Setting up necessary files for slice alignment (Preparing for Align_EPI)
3. Aligning slices (Align_EPI)
4. Setting up necessary files for registration (Preparing for Align_Anatomy)
5. Registering EPI, GRE, structural images to common space (Align_Anatomy)

1. Offline_SENSE

This toolbox reconstructs raw EPI data from k-space and performs ghost correction with the use of GRE data.

You will need:

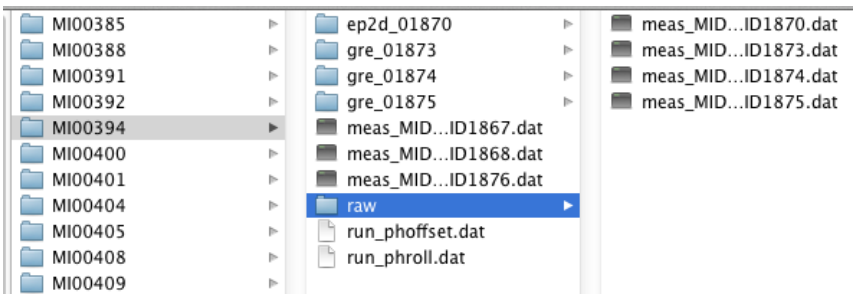
1. Offline_SENSE toolbox installed as an app on your MATLAB

To start preprocessing

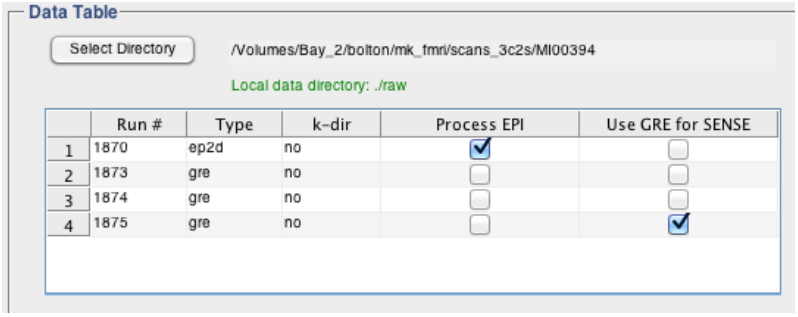
1. Turn on the Offline_SENSE script in MATLAB

2. Click “Select Directory” (A)
   Select the session folder (e.g. MI00394), but not the “raw” folder under the session folder

3. Click “Update” (B).
   For each EPI and GRE data placed inside the raw folder a folder is created in the session.



4. Under (A), choose the EPI and GRE files



If you have more than one GRE, choose the one that you think has the best quality (least motion). The GRE file is important for steps B3 and B5.

If you have more than one EPI in the session for the preprocessing, do not select all of them but do it one at a time. The script does not count the volume number for multiple EPI runs properly.

5. Click “Calibrate” (B1)

6. Click “Unpack k-space” (B4), it may run overnight. It is normal if it crashes at the end. Normally I suggest not to follow the order of running B2, B3 and then B4 if you do not know how many volumes the EPI contains. The script normally crashes after the last volume, because we manually stop the EPI sequence during scanning and the script expects to have all the volumes indicated in the scanning sequence.

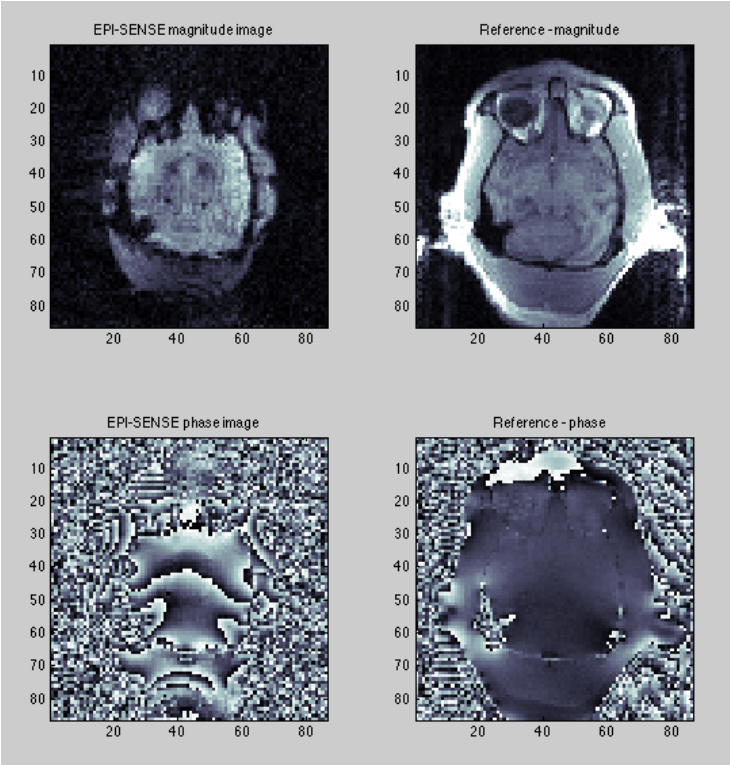
7. Set parameters in (C), change “Volume #, Last” to the total number of volumes.

k-space & Output Volume		Value
Use Average EPI Corr.	0/1/2	1
Interleaved sclices	n:0, y:1	1
K-space Line Shift		1
Volume #, First		1
Volume #, Last	0=all	0
# Volumes per file	0=all	0

GRE & SENSE Reconstruction		Value
Use GRE for Recon	n:0, y:1	1
GRE z-offset		9
GRE Dimensions	2/3	3
GRE Smoothing	1/3/5/7	1
PE Direction GRE	-1/0/1/2	0
PE Direction EPI	-1/0/1/2	0
Lambda	[0.1-0.5]	0.5000

- “Volume #, Last” has to be changed because we usually stop the scanner manually, otherwise the script will crash.
- Turn “Use GRE for Recon” to 0 if there is no GRE or only very bad GRE.
- “GRE z-offset” is usually 9 and sometimes 18, important for aligning with EPI.
- “Lambda” is usually 0.5 with GRE and 0.25 without GRE. Smaller numbers remove more ghost but introduce more noise.

8. Click “SENSE Recon” (B3).



Make sure the two magnitude images (top left and right) are aligned in z coordinate, adjust “GRE z-offset” if they are not.

9. The first EPI volume is now reconstructed. Check the ghost level of ep2d_XXXXX/fe.nii and its alignment with gre_XXXXX/fg.nii in z axis using FSLVIEW.
   fslview ep2d_XXXXX/fe.nii gre_XXXXX/fg.nii
   Do not use JIP to check, it is harder to visualize the ghosting using JIP.
   Ghosting is seen best in the axial slices (and sometimes the sagittal)s

10. Optimize by changing the Lambda value (or try another GRE if any) and re-do Step 8.
    - It is very helpful to have a good quality GRE.
    - **If there is more than one GRE, try them one by one, using lambda 0.5, open each fe.nii in FSLVIEW after each reconstruction as the next reconstruction will write over it (or just copy over each reconstruction to new folder so they are not overwritten) → flip between the different reconstructions with each GRE**
    - Pick the best result, a good quality GRE helps to reduce a lot of noise
    - Try using a lower lambda (e.g. try 0.1, then 0.25), if the GRE quality is good it should not increase much noise but should reduce more ghost.

11. Enter “Volume #, Last” in (C)

You can check this number by looking at the number of k-space data files under the folder ep2d_XXXXX/k (excluding the last one called epi_9999.k)

12. Click “Save Parameters” (D) for your record.

13. Click “SENSE Recon” (B5), it normally takes a few hours.
    Beware of RAM capacity if running many sessions in parallel.

14. The 4D EPI data is now reconstructed and ghost corrected. Check the quality. Keep only the folder of the GRE that was used, remove all unused GRE folders (not the raw data in the “raw folder”, but still those raw data won’t be used anymore).
    fslview ep2d_XXXXX/f.nii

2. Preparing for Align_EPI

This step prepares everything needed for the Align_EPI toolbox. It is designed to be most convenient if you are preprocessing multiple sessions for a given animal, but this is not at all necessary.

You will need:

1. **`prepare_align_epi.sh`**
2. **`MRCAT toolbox`**
3. **`create_overlay`** (directory with Matlab functions used in prepare_align_epi.sh – should be in the same directory as prepare_align_epi.sh)
4. **`inspect_target_volume.sh`**
5. **`manual_target_volume.sh`**

To begin:

open **`prepare_align_epi.sh`** in your text editor:

```
# ////////////////////////////////////////////////////////////////////////////////////////////////////////////////////
# ____________________________________________________________________________________________________________________
# SUBJECT / SESSION SETUP

# path to main study/data directory
study_dir=/Users/rushworth/seqodr_data/mri_data

# subject (only 1 per run)
subj="puck"

# session folder IDs (separated by spaces)
sessions_to_do="MI01001 MI01002 MI01003"

# IMPORTANT: flip orientations and correct labels? should only be done * ONCE * for a given session
flip_orient=1

# MRCAT directory
mrcat_dir=/Users/rushworth/dropbox/Work/seqodr/preprocessing/MrCat-dev
```

1. input your data paths into the **`study_dir`**, **`subj`**, and **`sessions_to_do`** variables (the script can run multiple sessions in a loop but only for a single animal – easy to add a loop over animals if you wish)

2. set **`flip_orient=1`** if you want to correct the orientations and labels of all the images created in the previous step → if you have fresh data from Offline_SENSE, the data will be incorrectly oriented, so you will need to run this step **(note: only run this ONCE on a given session, or else it will orient things back to the incorrect state)**

3. set **`mrcat_dir`** to the correct path

4. Save and run the script in your terminal with: **`sh prepare_align_epi.sh`**

This script calculates the mean variance of each volume in the EPI and finds the one with the least variance as the "target" volume for alignment. Ideally, this target volume has no misalignment of slices. The target volume is saved as an image in your session folder (**`session_folder/f_t.nii`**) and its volume number is in the filename of an empty textfile (e.g., **`session_folder/TARGETVOLUME_fsl_605_jip_606)`**. The volume number is different for FSL and JIP, since FSL uses 0-based indexing, and JIP uses 1-based indexing.

This script also corrects orientations for all images in session (except BACKUPS), as described above.

It also makes runs **`bet_macaque`** (from MRCAT) on the target volume to obtain a very rough brain mask (**`f_t_brain_mask.nii`**) which is then dilated to ensure full coverage of brain (and usually some muscle/skull – this is good, see note from Bolton below). Note: if you have run the script multiple times, it will make a backup of existing masks in the same folder.

It finally makes an overlay file from **`f_t_brain_mask.nii`** (**`session_folder/overlay.dat`**), which Align_EPI uses in the next step.

open **`inspect_target_volume.sh`** in your text editor:

*this is simply a convenience wrapper script which, for each session, opens the EPI (**`f.nii`**), the target volume (**`f_t.nii`**) and the target volume brain mask (**`f_t_brain_mask.nii`**) in fslview for you to check.*

1. as before, input your data paths into the **`study_dir`**, **`subj`**, and **`sessions_to_do`** variables

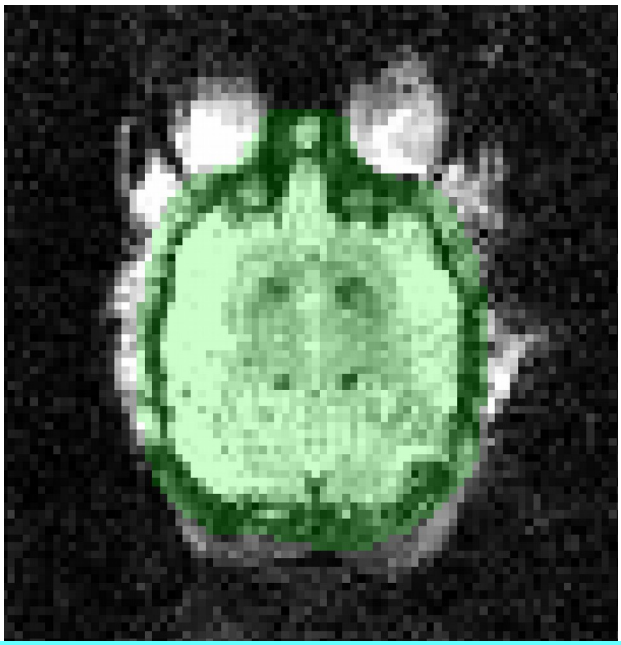
2. Save and run the script in your terminal with: **`sh inspect_target_volume.sh`**

3. Check if the target volume looks okay → it should have as little motion artefact as possible. If in the rare case it's not okay: make a note, and look through the EPI volumes to find an alternative target volume (one with minimal misalignment of slices. **Note down this "manually" obtained target volume number for the next step.)**

4. Check if the brain mask looks okay – it should cover the entire brain and also include a few bits of muscle around the head (this actually helps with alignment). The mask should be okay most of the time. Note: if the auto-detected target volume is bad, ignore this mask as a new one will be made anyway with your manually-selected volume.

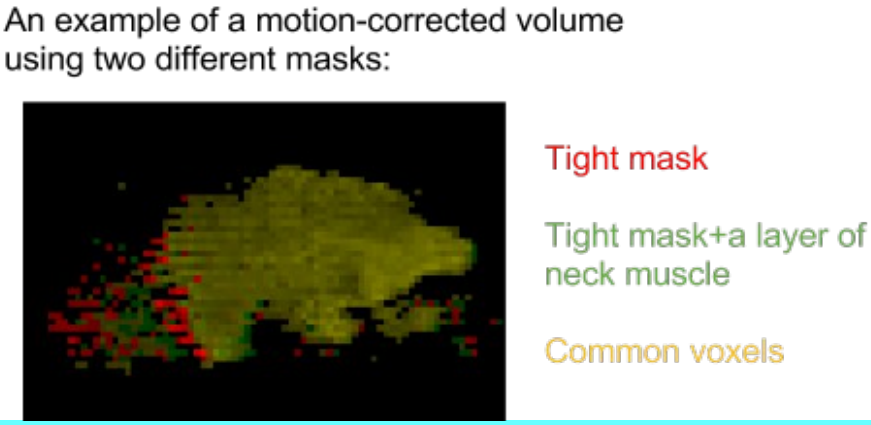
   **** If the mask is NOT okay **:** re-run **`prepare_align_epi`** for that subject (remembering to turn `flip_orient` off if already done before) and in the

bet_macaque command try setting size option to "bet" (add `-s "bet"` to **bet_macaque** command). This uses bet to estimate brain size instead of the default intensity estimate.



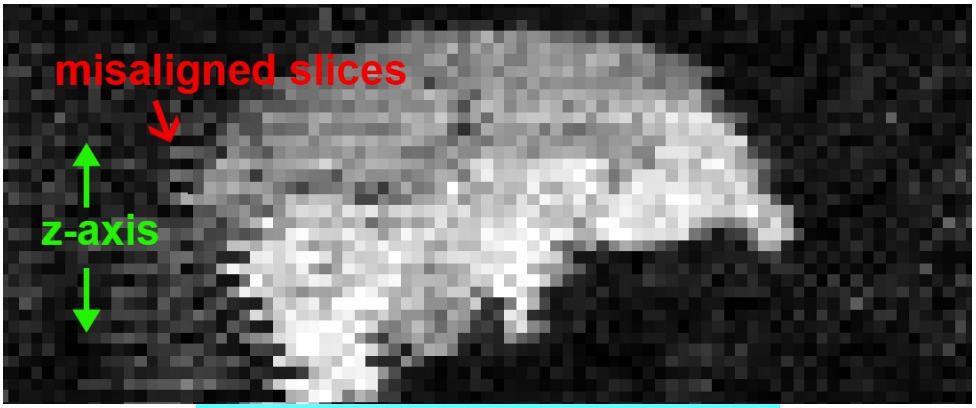
Good EPI brain mask (rough, with muscles included on purpose, see below)

Note from Bolton (2014-12-15): the correction at the occipital lobe can be much improved by involving a layer of muscle of a 2-3 voxels wide, it adds information for the registration. But don't include the whole neck muscle as the shape can change when the animal moves.



Example volume showing alignment improvement when including neck (from Bolton)

5. Look through the full EPI (f.nii) and find a **particularly BAD axial slice (z coordinate)** which is most misaligned with the rest of the brain on average (by flipping through volumes) → **make a note of this z coordinate in JIP convention (+1) for the next step**, as this will be used as a **"preview slice"** which you will check for corrected alignment before running the correction on the full dataset, and which will be used as to tune parameters by Align_EPI toolbox (note: this slice tends to be similar across sessions).



Example of misaligned axial slices (in z-axis)

[NOTE: maybe possible to automate the selection of the preview slice?]

IF YOU MANUALLY FOUND A BETTER TARGET VOLUME (rare!), open **`manual_target_volume.sh`** in your text editor:

1. Set the data paths again as before *but only for the sessions in which you want to use a manually found target volume*

2. Set the manually found target volumes in **`target_volumes`** variable (one target volume for each session in **`sessions_to_do`**)

3. Save and run the script in your terminal with: **`sh manual_target_volume.sh`**

   As before, this script creates the target volume image, the brain mask, and the overlay based on your manually chosen volume.

   The previous target volume image is saved as a backup, the overlay is overwritten, and the target volume number is saved in a new filename (e.g., **`MANUAL_TARGETVOLUME_fsl_605_jip_606`**)

3. Align_EPI

This toolbox re-aligns EPI slices which are misaligned due to motion by using a motion-free volume (the target volume from before) as a reference.

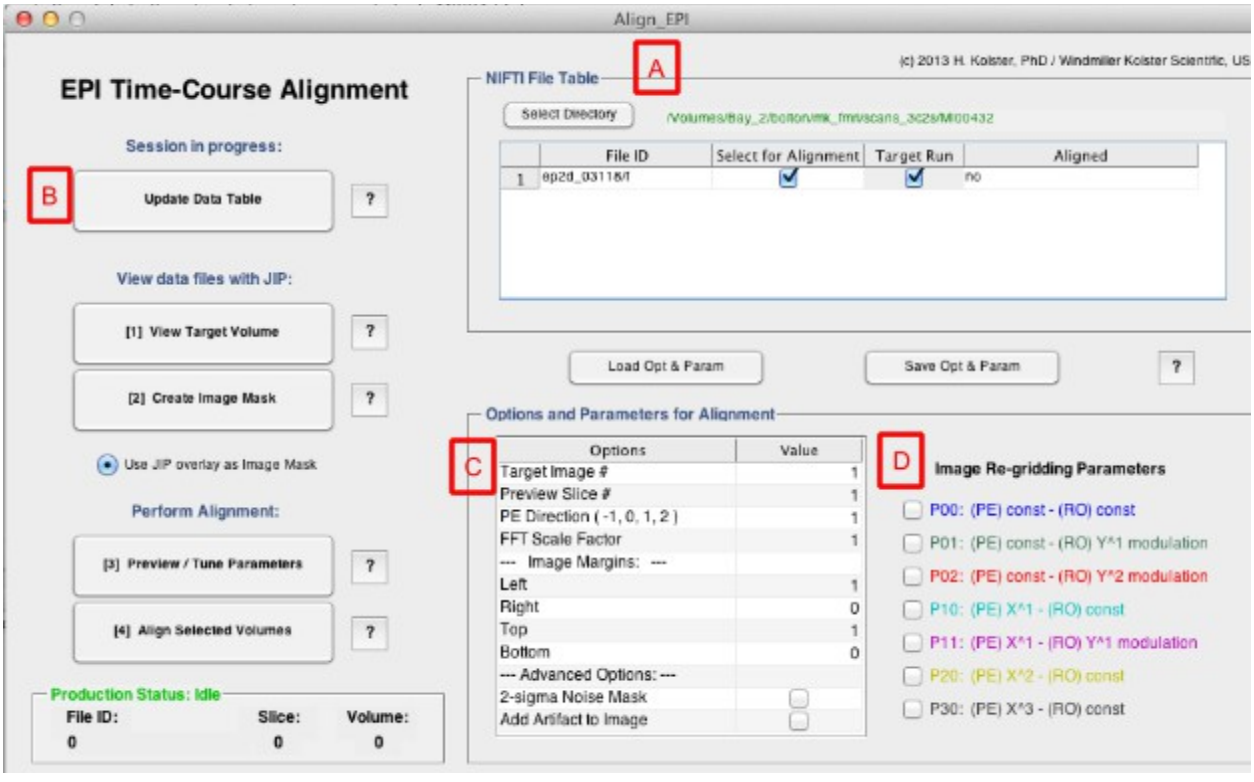
You will need:

1. the target volume (**`f_t.nii`**) and its volume number (in JIP index)
2. preview slice number
3. Align_EPI toolbox installed as an app in your MATLAB

To begin:

1. Turn on the “Align_EPI” MATLAB script

2. Click “Select Directory” (A) and select the session folder

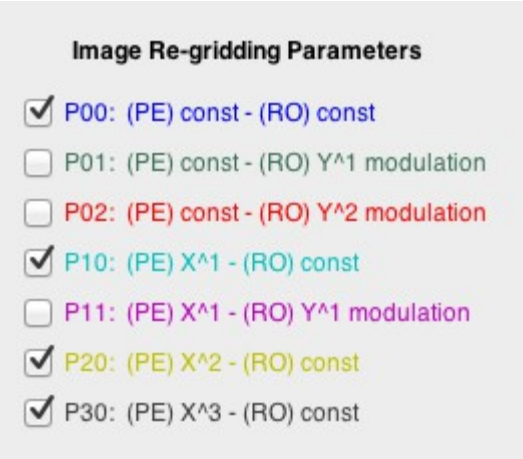


3. In (A), check “Select for Alignment” and “Target Run” as above, then click “Update Data Table” (B).

4. Click “Use JIP overlay as Image Mask” (below B2).

5. In (C) input the target volume number (JIP convention) as “Target Image #”, and the preview slice number (JIP convention) as “Preview Slice #”

6. In (D), select the parameters for the alignment.
   Normally start with P00, P10, P20, P30.

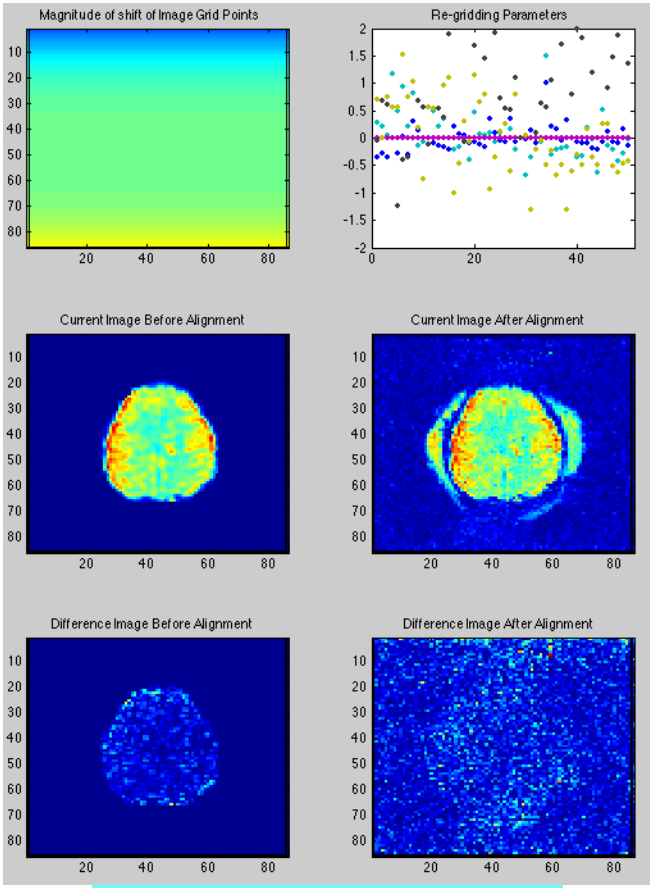


Parameters to set for alignment

7. Make sure that **PE Direction is set to 0**.

8. Click “Preview/Tune Parameters” (B3).

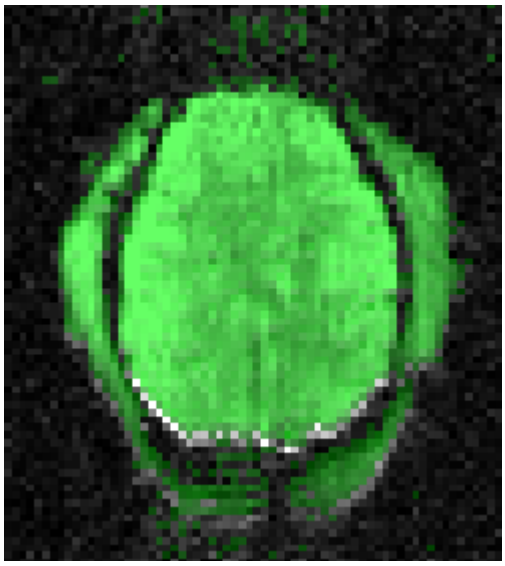
   Check that the mask is applied to your scan – it should only show the brain in the mid-left figure. If you could see the whole slice, you might have not clicked “Use JIP overlay as Image Mask”



Preview/tuning window that pops up

9. Compare the preview slice (prelim.nii) with the original 4D data (f.nii).

   fslview f.nii prelim.nii



Example of the unaligned original with aligned preview slice

You have to adjust the intensity to visualize the preview slice (e.g. min 400, max 2000), and set its colour to something else (e.g. green)

Turn on movie mode and see if the aligned preview slice (green) remains stable across volumes.

If alignment is bad (preview slice still jumps around across volumes):

- try changing parameters (e.g. removing P30 sometimes helps)
- try making the mask bigger in the back, to cover more neck area
- re-run preview/tuning and check again

10. Click “Align Selected Volumes” (B4) to align the whole 4D data.

11. Check the quality of the aligned data (fa.nii).

```
fslview f.nii fa.nii
```

4. Preparing for Align_Anatomy

This step prepares everything needed for the Align_Anatomy toolbox. It is designed to be most convenient if you are preprocessing multiple sessions for a given animal, but this is not at all necessary.

You will need:

1. **`prepare_align_anat.sh`**
2. **`MRCAT toolbox`**
3. **`preproc_fieldmap.sh`**
4. **`resample_struct_to_GRE`** (folder with JIP and Matlab scripts used in prepare_align_anat.sh – should be in the same directory as prepare_align_anat.sh)
5. **`JIP toolbox`**
6. **`edit_epi_mask.sh`**

To begin:

*** Remove all **unused** GRE folders in the session folder and keep the used GRE folder if you have not yet done this. ***

open **`prepare_align_anat.sh`** in your text editor:

```
# STEPS TO RUN:

    create_epi_mask=1 # run EPI mask steps at all?
    borrow_epi_mask=1 # 1 = yes (for all sessions), 0 = no (make a new mask for all sessions)

    create_gre_mask=1 # run GRE mask steps at all?
    borrow_gre_mask=1 # 1 = yes (for all sessions), 0 = no (make a new mask for all sessions)
    create_gre_overlay=1 # 1 = yes, 0 = no

    create_structural_mask=1 # 1 = yes, 0 = no
    create_structural_overlay=1 # 1 = yes, 0 = no

# SETTINGS:

    # path to main study/data directory
    study_dir="/Users/rushworth/seqodr_data/mri_data"

    # subject
    subj="puck"

    # session folder IDs (separated by spaces)
    sessions_to_do="MI01002 MI01003 MI01004"

    # paths to structural (excluding .nii extension!)
    anat_full="$study_dir/$subj/structural/structural_restore"
    anat_crop="$study_dir/$subj/structural/structural_restore_brain"

    # MRCAT directory
    mrcat_dir="/Users/rushworth/dropbox/Work/seqodr/preprocessing/MrCat-dev"

    # JIP directory
    jip_dir="/usr/local/share/jip-source"

    # degrees of freedom for FLIRT (default is 6) if borrowing GRE mask
    borrow_gre_dof=6

    # degrees of freedom for FLIRT (default is 6) if borrowing EPI mask
    borrow_epi_dof=6

    # session name from which to borrow GRE/GRE mask
    borrow_gre_session="MI01001"

    # session name from which to borrow EPI mask
    borrow_epi_session="MI01001"

    # if session(s) listed in sessions_to_do is missing a GRE, mark it here (the entire GRE will be borrowed in addition to the mask)
    missing_gre_session="MI01003"
```

1. Select what steps you want to run (note: **`create_epi_mask/create_gre_mask`** means running any of the EPI or GRE mask steps at all (i.e., borrowing and creating new masks), while b**`orrow_epi_mask/borrow_gre_mask`** specifically sets borrowing the masks rather than making new ones for a session)

2. Input your data paths into the **`study_dir`**, **`subj`**, and **`sessions_to_do`** variables (the script can run multiple sessions in a loop but only for a single animal – easy to add a loop over animals if you wish)

3. set paths to your structural images, **`anat_full`** (full structural) and **`anat_crop`** (just the brain), both bias-corrected

4. set **`mrcat_dir`** and **`jip_dir`** paths

5. if you're borrowing an EPI and/or a GRE mask from another session (instead of making brand new masks for each session), choose which session you want to borrow from in

borrow_gre_session/borrow_epi_session (this borrowed session will be applied to all sessions in **sessions_to_do**)

6. If any sessions are missing GREs, list them in **missing_gre_session** (in addition to `sessions_to_do`) – once these sessions are processed, the entire GRE (in addition to the mask) will be borrowed from the session defined in **borrow_gre_session**

7. set **borrow_gre_dof/borrow_epi_dof** (degrees of freedom) for FLIRT if borrowing GRE/GRE mask and/or EPI mask

8. Save and run the script in your terminal with: **sh prepare_align_anat.sh**

This script creates a folder called **align_anatomy** in the session folder, and copies into there the structural image, renamed as **brain.nii**).

If `create_epi_mask` is set:

It creates a folder called **brain_mask** inside the EPI folder (ep2d_xxx), within the session folder.

If **borrow_epi_mask** is set, it uses FLIRT (with the chosen DOF) to register the borrowed mean EPI image to current mean EPI image, and applies that transformation to the borrowed EPI mask to create a new EPI mask (**brain_mask/fm_brain_mask.nii**) which will need to be edited at a later stage.

If **borrow_epi_mask** is NOT set, it runs **bet_macaque** (from MRCAT) on the mean EPI image to create a rough brain mask (**brain_mask/fm_brain_mask.nii**) which will need to be edited at a later stage.

Note: if you have run the script multiple times, it will make a backup of existing masks in the same folder. It also saves a copy of the mean EPI (**brain_mask/fm_whole.nii**), since the original (**fm.nii**) will be masked later on.

*NOTE: Bolton's old scripts named the files **whole_fm.nii** and **mask_fm.nii** and did not create a separate **brain_mask** folder.*

If `create_gre_mask` is set:

If **borrow_gre_mask** is set, it uses FLIRT (with the chosen DOF) to register the borrowed mean EPI image to current mean EPI image, and applies that transformation to the borrowed GRE mask to create a new GRE mask (**gre_xxxx/fg_brain_mask.nii**).

If a session is listed in **missing_gre_session**, the script also applies the transformation to the borrowed GRE itself to create a new GRE for the current session in a new folder (**gre_99999/fg.nii**).

If **borrow_gre_mask** is NOT set, it runs **preproc_fieldmap** script which automatically creates a good/tight GRE mask for the current session (**gre_xxxx/fg_brain_mask.nii**). This takes ~20 min.

If **create_structural_mask** is set:

It resamples the high-res structural image to GRE space (**align_anatomy/struct_gre_space.nii**), and creates a structural brain mask in this space (**align_anatomy/ref_brain_mask.nii**).

If **create_gre_overlay** is set:

It **creates a GRE overlay** based on the GRE brain mask (**align_anatomy/overlay_gre.ovl**).

If **create_structural_overlay** is set:

It creates a structural overlay based on the structural brain mask. (**align_anatomy/overlay_ref.ovl**).

Check structural mask – open `check_structural_mask.sh` in your text editor:

1. as before, input your data paths into the **study_dir**, **subj**, and **sessions_to_do** variables

2. the script will open the first session for checking (structural image in GRE space: **struct_gre_space.nii**, and structural brain mask: **ref_brain_mask.nii**)

3. Check that the mask is good.

4. Close fslview when finished to load the next session.

Edit GRE mask – open `edit_gre_mask.sh` in your text editor:

1. as before, input your data paths into the **study_dir**, **subj**, and **sessions_to_do** variables

2. the script will open the first session for editing (GRE image: **fg.nii**, and GRE brain mask: **fg_brain_mask.nii**)

3. edit the mask

   The GRE mask is important for the non-linear registration. It is important to include all brain voxels and to exclude:

   - the optic nerves near the anterior brain,
   - the non-brain high intensity voxels near vlPFC/OFC and ventral temporal lobe. (esp. ventral temporal lobe)
   - the olfactory bulb
   - the neck muscles near the posterior brain.

4. Save the mask manually in fslview

   NOTE: after a save, fslview for some reason flips the mask in the L-R orientation as you see it in the program. The saved mask on file is NOT flipped, but will become flipped if you then save again (because you will overwrite the non-flipped mask with the displayed flipped mask) – thus, **do not save a given mask more than once within a single instance of fslview.**

   If you want to save a mask and come back to it later (which involves saving twice...), see step 5 below.

5. Once the mask is saved, close fslview. This will prompt the terminal to display some options:

   `...Create GRE mask overlay?? >> Y - yes and continue / N - no and continue / Q - exit script`

   If you are finished editing the mask and you want to create the overlay, choose Y. The overlay will be created and the next session will be loaded into fslview.

   If you are NOT finished editing the mask but you want to move on to the next session for whatever reason, choose N. The overlay will not be created and the next session will be loaded into fslview.

   If you are NOT finished editing the mask but you want to quit this script and re-run it later to finish editing the mask you're working on, choose Q. (Remember to update `sessions_to_do` in the script as necessary). Once the script is re-run and the mask is loaded again in a new instance of fslview, it shouldn't be flipped, and you can now save once again after editing. The basic principle is not to save a given mask more than once within a single instance of fslview.

Edit EPI mask – open `edit_epi_mask.sh` in your text editor:

1. as before, input your data paths into the **`study_dir`**, **`subj`**, and **`sessions_to_do`** variables

2. the script will open the first session for editing (mean EPI image: **`fm_whole`**, and EPI brain mask: **`fm_brain_mask`**)

3. edit the mask

   The EPI mask is important for the non-linear registration. It does not need to be excellent, but it is important to include all brain voxels and to exclude:

   - the optic nerves near the anterior brain,
   - the non-brain high intensity voxels near vlPFC/OFC and ventral temporal lobe. (esp. ventral temporal lobe)
   - the olfactory bulb
   - the neck muscles near the posterior brain.

4. Save the mask manually in fslview

   NOTE: after a save, fslview for some reason flips the mask in the L-R orientation as you see it in the program. The saved mask on file is NOT flipped, but will become flipped if you then save again (because you will overwrite the non-flipped mask with the displayed flipped mask) – thus, **do not save a given mask more than once within a single instance of fslview.**

   If you want to save a mask and come back to it later (which involves saving twice...), see step 5 below.

5. Once the mask is saved, close fslview. This will prompt the terminal to display some options:

   `...Mask mean EPI using brain mask? >> Y - yes and continue / N - no and continue / Q - exit script`

   If you are finished editing the mask and you want to apply the mask to the mean EPI image, choose Y. The mask will be applied and the next session will be loaded into fslview.

   If you are NOT finished editing the mask but you want to move on to the next session for whatever reason, choose N. The mask will not be applied and the next session will be loaded into fslview.

   If you are NOT finished editing the mask but you want to quit this script and re-run it later to finish editing the mask you're working on, choose Q. (Remember to update

`sessions_to_do` in the script as necessary). Once the script is re-run and the mask is loaded again in a new instance of fslview, it shouldn't be flipped, and you can now save once again after editing. The basic principle is not to save a given mask more than once within a single instance of fslview.

6. Once you are finished editing all of the masks, double-check that they have all been applied to the mean EPI image (fm.nii)

5. Align Anatomy

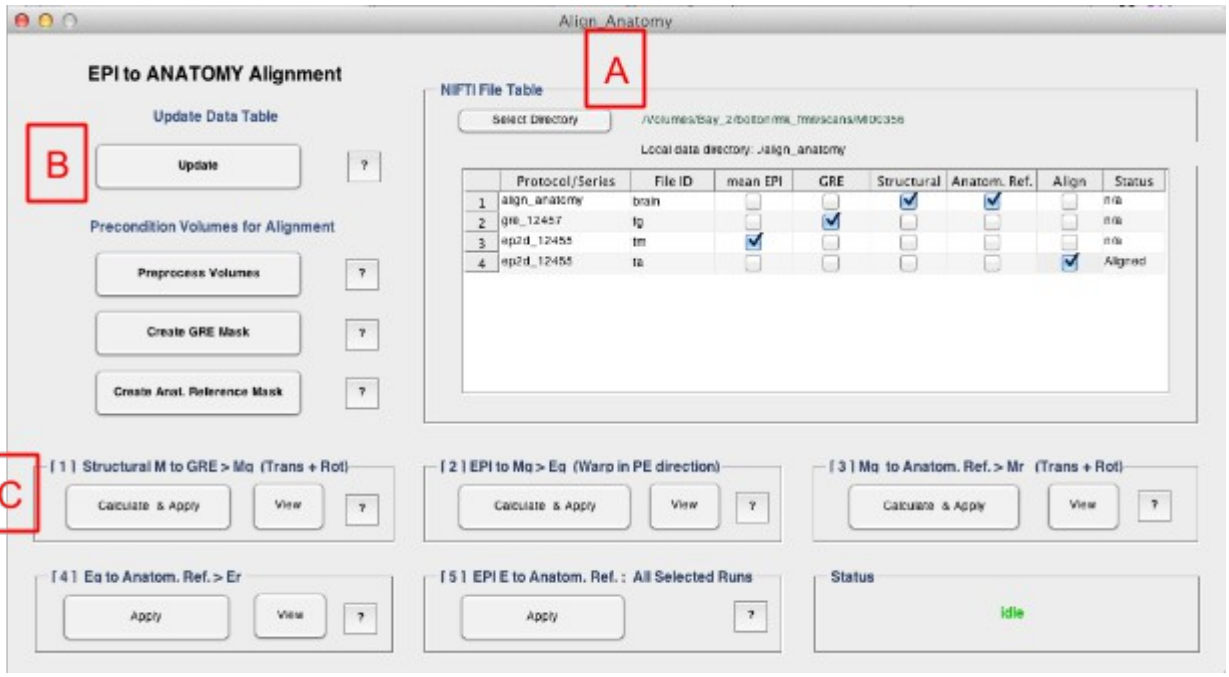
This toolbox (with the help of JIP) registers the EPI and structural images to the GRE image for each session to bring them into the same space.

You will need:

1. The mean EPI image (fm.nii) masked by the detailed/tight EPI brain mask (done in previous step)
2. the structural image (brain.nii) in the align_anatomy folder (done in previous step)
3. the GRE (fg.nii) and the slice-aligned EPI image (fa.nii) (done in previous steps)
4. the GRE overlay (overlay_gre.ovl) and structural brain mask overlay (overlay_ref.ovl) in the align_anatomy folder (done in previous step)
4. Align_Anatomy toolbox installed as an app in your MATLAB
5. JIP installed

To begin:

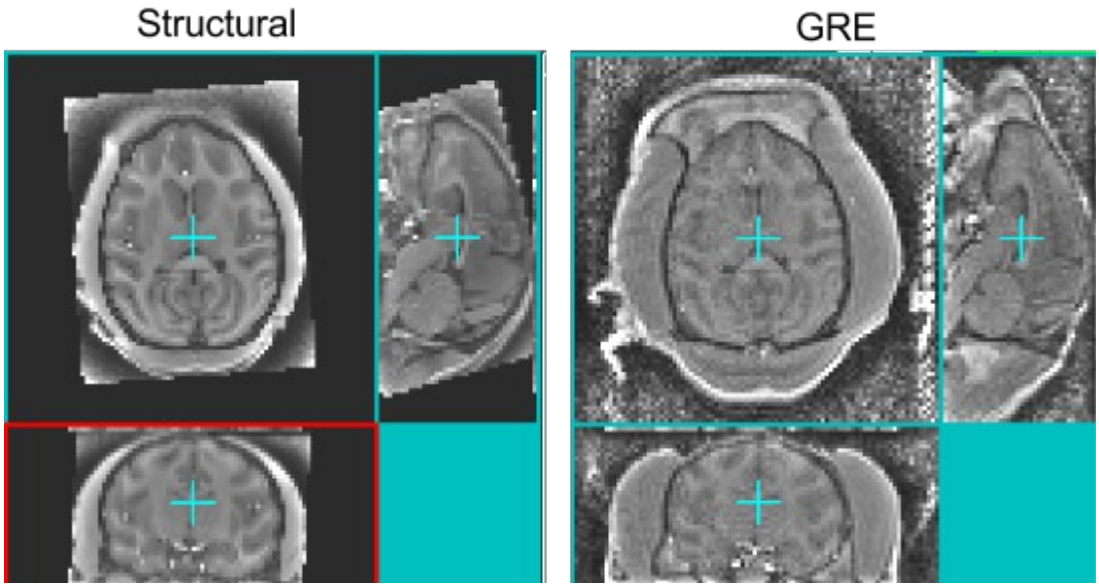
1. Turn on the “Align_Anatomy” toolbox in MATLAB
2. Press “Select Directory” and choose the session folder
3. Press “Update”
4. in the file table (A), set the “brain” file as the Structural and Anatomical Ref; the “fg” file as the GRE; the “fm” file as the mean EPI; the “fa” file as the Align
5. Press “Preprocess Volumes”



JIP usage tips (the Align_Anatomy toolbox uses JIP to do registration):

- JIP is mainly controlled through the 4 arrow keys and the cursor position: depending on where the cursor is in the JIP window, different options will be highlighted and available to select (so always be aware of where the cursor is within the JIP window before setting options with the arrow keys!)
- To look through images slice by slice, place the cursor in one of the image displays and use the arrow keys to look through the slices
- **To change luminance/contrast** of the image displays, place the cursor in the empty area next to the image displays, and use arrow keys (luminance = UP/DOWN, contrast = LEFT/RIGHT)
- **Press the spacebar to flip back and forth between image types** to check the registration quality between them (e.g., checking that ventricles, major sulci are aligned)
- **Press shift+z to enlarge JIP window** for better viewing
- Clicking on the settings bar to the left will flip through the different settings options (SETUP, AFFINE, NON-LINEAR)
- To edit settings: make sure cursor is in the settings bar, use UP/DOWN to scroll through settings, LEFT/RIGHT to set/unset a given option
- In the bottom-right corner, click on “select all”/”select none” to quickly set/unset all registration parameters

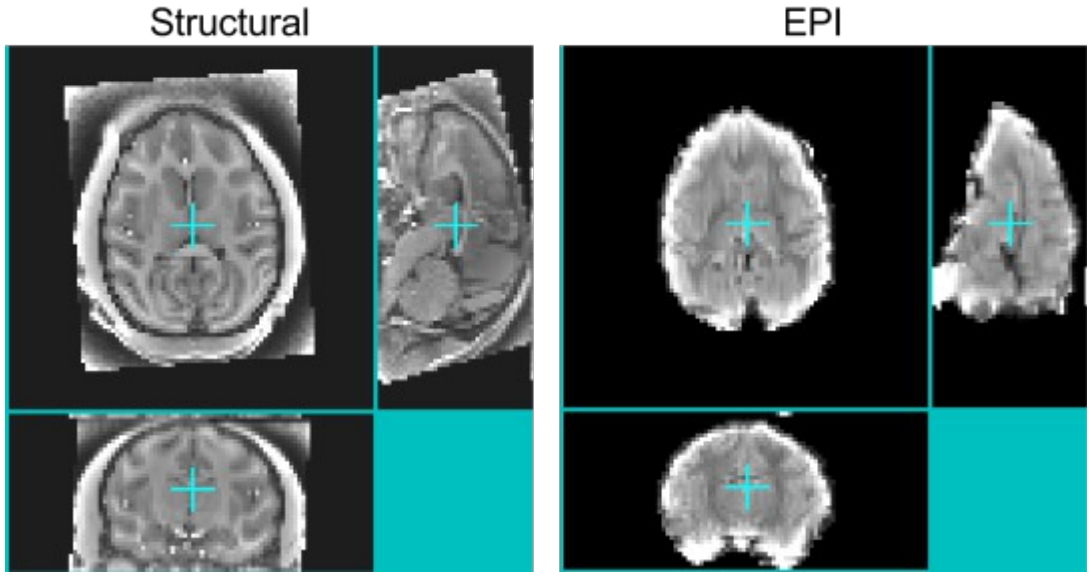
6. **Align structural to GRE (C1)**
   - Click “Calculate & Apply”, this will open JIP
   - On SETUP:
     Apply overlay (the background should turn black)
     down-sampling x,y,z=1
   - On AFFINE:
     Hauke’s suggestion: first 6 parameters (x to phi_xz)
     Bolton’s suggestion: first 6 parameters then all 12
   - Manually change the starting values of the parameters to roughly align the two images
   - Click “auto-align”
   - **It is important to get good quality on this step.** You’ll need to re-do all the alignments if you, at the end, realize that the poor alignment is mainly contributed by this step. So slightly change the values and re-run “auto-align”.
   - Press “Q” then “Y” to quit JIP.
   - In MATLAB popup window, Click “Yes” to save transformation data.



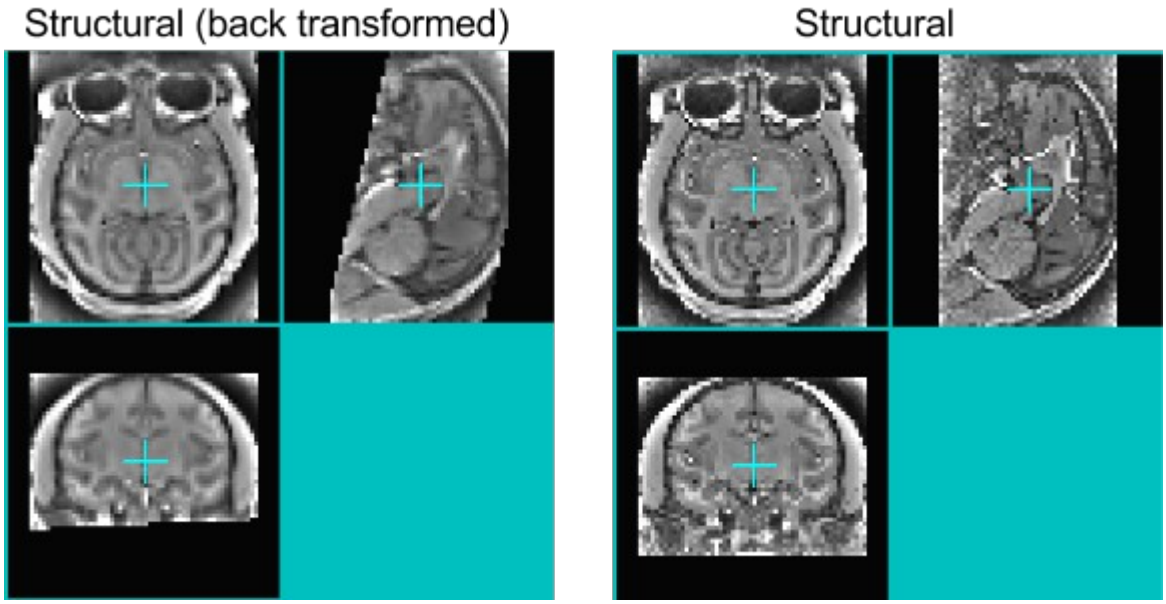
7. **Align mean EPI to structural (in GRE space) using non-linear transformation (C2)**
   - Click “Calculate & Apply”, this will open JIP
   - On SETUP:
     apply overlay
     down-sampling x,y,z=1
   - On AFFINE:
     Hauke’s suggestion: select none

Bolton's suggestion: align y and then also size-y (remember to run "auto-align")

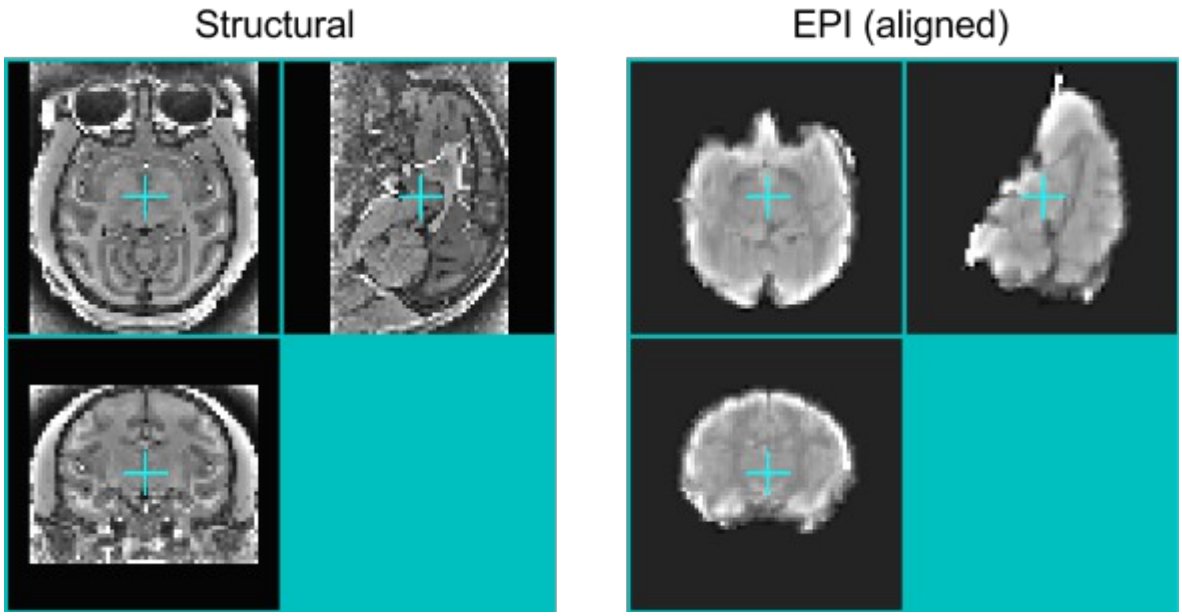
- On NON-LINEAR:
  activate Y Field, down-sample 1, Rigidity 3, Jacobian 5
- Click "auto-align" twice. **It could take 15 min.**
- Re-run "auto-align" immediately if poor quality.
- Press "Q" then "Y" to quit JIP.
- In MATLAB popup window, click "Yes" to save transformation data.



8. **Back transform the structural in GRE space to structural space (C3)**
   - Click "Calculate & Apply", this will open JIP
   - On SETUP:
     apply overlay (the background should turn black)
     down-sampling x,y,z=1
   - On AFFINE:
     Hauke's suggestion: first 6 parameters (x to phi_xz)
     Bolton's suggestion: first 6 parameters then all 12
   - Manually change the starting values of the parameters to roughly align the two images
   - Click "auto-align"
   - If poor quality, slightly change the values and re-run "auto-align".
   - Press "Q" then "Y" to quit JIP.
   - In MATLAB popup window, click "Yes" to save transformation data.



9. **(C4) click “Apply” and click “View” to check registration quality.**
    - Check that ventricles, major sulci, and any other obvious markers are aligned between image types (change luminance/contrast and enlarge JIP window to see more detail if needed)



10. **(C5) click “Apply” to register all volumes (will take some time)**