**FreeSurfer Segmentation/Recon-all (all in server, run from MATLAB)**

1. **Segmentation:**

**Organize the data:**

In Linux Server (possible also via MobaXterm):

Segment MP2RAGE data

* Copy and Cd to ‘<your\_path>/Segmentations\_FS’
* Open new folder with the project name, e.g., ‘qMRI\_mapping’
* Open new folder with the Volunteer “name”, e.g., ‘V0351’
* Copy the anatomy T1w folder with the DICOMs in it. Make sure that folder name includes the string ‘rage’, e.g., ‘4\_t1\_mp2**rage**\_sag\_p3\_iso\_UNI\_Images’. For MP2RAGE, use the “UNI” T1w folder.

In MATLAB:

* cd to ‘…/Segmentations\_FS’
* Open/edit the script: ‘Parallel\_Segmentation.m’
* Change the path to your project folder: ‘Data\_dir='…/Segmentations\_FS/<Project name>';’
* Run the script (Perform reconstruction for multiple volunteers, using parfor loop)
* Resulted segmentation map filename is “aparc+aseg.mgz” and will appear under

… /Segmentations\_FS/<Project name>/<Volunteer name>/00\_FSseg/mri/

Segment mrQ output’s T1w data

* Segmentation of mrQ results can be applied directly on the output maps space.
* During the mrQ process, a synthetic T1w image is produced in the qT1 map space, and can be used for reconstruction in FreeSurfer. The T1w image can be found under: ‘<your\_path>/mrQ-master/DATA/<Project name>/<Volunteer name>/Analysis/mrQ/OutPutFiles\_1/T1w’
* Use the “T1w\_Nii\_scaling.m” function under ‘<your\_path>/Segmentation\_FS/mrQ\_related’, which expand the T1w images dynamic range (by multiplying the original values times 1000) and creates the “tmp\_T1w”.
* To run the reconstruction with FreeSurfer use “Parallel\_recon\_mrQ.m” script and run it. Make sure the directories are OK (particularly the data dir) in both .m functions.

**Possible errors in segmentation pipeline:**

* P: Reconstruction fails
* S: The common cases:

(1) convert the input data to int16

(2) expand the images dynamic range, e.g. multiply the images by 1e4

(3) Poor image SNR/cannot differentiate WM – use FreeSurfer solutions: https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/TopologicalDefect\_freeview.

1. **Registration:**

* Registration is performed using the different “Register\_FS\_BA\_#” .m files (e.g., Register\_FS\_BA\_ihMT.m for ihMT). The registration can be performed on DICOMs, NIFTI and mgz formats.
* Per map type, data format and registration direction (i.e., to which map’s space) – the Register\_FS\_BA\_#” script and how to run information are delineated under Appendix A: registration of different qMRI maps.
* Registration options:

**Inv\_reg\_flg** (perform regular or inverse registration):

=0 : register new map (e.g. T2) -> to T1w map (holes and errors may appear)

=1 : register T1w map -> new map (preferred for current study)

**bb\_register** (Choose if the image contrast is like T1w or T2w – based on the WM to GM contrast), e.g. (qT1 ->t2, T2s->t2, diff->t2). >> \_\_t1 / \_\_t2

* Registrations results will appear under:

‘…/Segmentations\_FS/<Project name>/<Volunteer name>/temp’

* In MATLAB: some resulting segmentation maps might require either flipud (upside-down) or fliplr (left-right) – decide by observing the registered-segmentation map and the relevant map/image.

1. **View segmentation + registration results:**

**(Option A) - MATLAB**

**Load .mgz file to MATLAB:**

>> FS\_mtl\_Fld = ‘<your\_path>/FreeSurfer\_related/freesurfer\_MATLAB\_scripts’;

>> addpath(FS\_mtl\_Fld)

>> load\_mgh(<file name>.mgz);

**(Option B) – FreeSurfer GUI**

**in Moba/command shell:**

>> export FREESURFER\_HOME=/usr/local/freesurfer

>> source $FREESURFER\_HOME/SetUpFreeSurfer.sh

>> freeview &

In the FS GUI:

* Ctrl+O (open)
* Load .mgz files. e.g:

Graphical user interface, text, application

Description automatically generated

* Use lookuptable colors to view segmentation on top of other maps:

Graphical user interface, text, application

Description automatically generated

1. **References:**
2. Fischl, B. (2012). FreeSurfer. In *NeuroImage* (Vol. 62, Issue 2, pp. 774–781). https://doi.org/10.1016/j.neuroimage.2012.01.021
3. *GitHub - freesurfer/freesurfer: Neuroimaging analysis and visualization suite*. (n.d.). Retrieved January 29, 2022, from https://github.com/freesurfer/freesurfer
4. Greve, D. N., & Fischl, B. (2009). Accurate and robust brain image alignment using boundary-based registration. *NeuroImage*, *48*(1), 63–72. https://doi.org/10.1016/J.NEUROIMAGE.2009.06.060
5. Lin, L. (2007). *Cleaning Data the Chauvenet Way*. http://analytics.ncsu.edu

**Appendix A: registration of different qMRI maps**

Assuming FreeSurfer segmentation maps from MPRAGE or other T1w data are already reconstructed (i.e., “aparc+aseg.mgz” exists).

1. **QSM and T2\***

* The resulting susceptibility maps are expected to be aligned with the original data (magnitude images).
* Register Segmentation map to either (both works fine):
  + Single-echo series, choose the best contrast (e.g., 3rd TE~14ms) –
  + Copy the Echoe’s magnitude images folder (DICOMs-all slices for single TE) under the segmentations folder:

‘…/Segmentations\_FS/<Project name>/<Volunteer name>/QSM/<3rd echo magintude>’

* + run the script:

‘…/Segmentations\_FS/Register\_FS\_BA\_QSM.m’

* + T2\* maps, assuming those maps were already reconstructed –
  + Copy the T2\* maps DICOMs under the segmentations folder: ‘…/Segmentations\_FS/<Project name>/<Volunteer name>/T2\_star’
  + run the script:

‘…/Segmentations\_FS/Register\_FS\_BA\_T2s.m’

1. **ihMT**

* Copy the resulting ihMTR.nii file to a new folder “ihMT” under the segmentations folder: ‘…/Segmentations\_FS/<Project name>/<Volunteer name>/ihMT’
* Register Segmentation map to the resulting ihMTR map, using the script: ‘Register\_FS\_BA\_ihMT.m’ under ‘…/Segmentations\_FS’
  + Validate directories
  + Inputs are: Cur\_Sbj\_dir –

full path for subject folder

vol\_ID – volunteer name, e.g. ‘V0351’

1. **T2**

**EMC and prepare for registration**

* Run the EMC pipeline (Windows can be used for the T2 fitting process)
* Copy the EMC\_results.mat to the volunteer folder, for example:

Graphical user interface, text

Description automatically generated

To convert the .mat results into DICOMs (for FreeSurfer):

* Open/Edit ‘mat\_2\_DCM\_T2maps\_BA.m’ script under: ‘…/T2\_fit\_BrainAtlas’
* Change directories for your project:
  + Line 3 – Output folder (for FS), e.g.:

>> T2\_base\_path='/home/noambe/Public/Segmentations\_FS/<Project name>';

* + Line 4 – Project folder, e.g.,:

>> main\_org\_DCM\_path= '…/T2\_fit\_BrainAtlas/< Project name >';

* + Line 10 – Volunteer “name” – e.g., find all folder names starting with ‘V’
* Run the script
* Resulting DICOMs will appear under:

‘…/Segmentations\_FS/<Project name>/<Volunteer name>/qT2’

**Registration –**

* Assuming that T2 maps were converted to DICOMs and are under:

‘…/Segmentations\_FS/<Project name>/<Volunteer name>/qT2’

* Register Segmentation map to the T2 maps:
* Open/Edit the script:

…/Segmentations\_FS/Register\_FS\_BA\_qT2.m

* Inputs:
* Cur\_Sbj\_dir = full path to volunteers folder, e.g. –

‘…/Segmentations\_FS/<Project name>/<Volunteer name>’

* vol\_ID = Volunteer name, e.g. ‘V099’
* Resulting registered-segmentation map will appear under: ‘…/Segmentations\_FS/<Project name>/<Volunteer name>/temp’

**Apply MP-PCA Denoising for T2 images (not mandatory):**

Open/edit ‘apply\_PCA\_on\_complex\_images.m’ function, under: “…/T2\_fit\_BrainAtlas/PCA/Denoising\_T2\_Atlas”

Before 1st run (when starting a new project):

* In the pipeline – correct the following parameters for your project:
  + Line 6 – Kernel size, e.g., ws\_list = [5];
  + Line 7 – Number of slices, e.g., num\_of\_slices = 32;
  + Line 8 – Echo Train Length, e.g., NEchoes = 12;
  + Line 11 - your data directory- All\_data\_path='…/T2\_fit\_BrainAtlas/Brain\_Atlas';
  + Line 17 – Volunteer “name” – e.g., find all folder names starting with ‘V’
* Run the Denoising script:
* Open/Edit apply\_PCA\_on\_complex\_images project .m script
* Validate the data directory.
* Run the script, duration depends on the matrix size.
* The result .mat file will be saved under:

‘… T2\_fit\_BrainAtlas/<ProjectName>/<VolunteerName>/ ‘

* + e.g. result name file: DAT\_V001\_result\_3d\_mat\_denoised\_ws\_5
* Convert .mat result to DICOMs (for EMC fitting):
  + Open/Edit the ‘mat\_2\_DCM\_T2\_BA.m’ script (WARNING! Do not use the ‘mat\_2\_DCM\_T2maps\_BA.m’)
  + Change the directories for your project:
  + Line 3 - Set the project folder: e.g., T2\_base\_path=

'… /T2\_fit\_BrainAtlas/Brain\_Atlas';

* + Line 10 – Volunteer “name” – e.g., find all folder names starting with ‘V’
  + Run the script.
  + Resulting denoised DICOMs will appear under: ‘…/T2\_fit\_BrainAtlas/<Project\_name>/<Volunteer name>/den\_DCM’