**Macaque Segmentation Steps**

Updated by Kep Kee: 11/7/2019

**Required Softwares and Scripts**

1. Neuroimaging softwares (to be installed, and able to run on commandline): ANTs, FSL, AFNI
2. Latest version of NMT repository that contains the template and scripts from <https://github.com/jms290/NMT/tree/master/NMT_v1.2>
3. AtlasBREX (brain extraction script) : <https://github.com/jlohmeier/atlasBREX/blob/master/atlasBREX.sh>
4. Denoising software: <https://sites.google.com/site/pierrickcoupe/softwares/denoising-for-medical-imaging/mri-denoising/mri-denoising-software>
   * Together with Franck Lamberton’s script *meuhmagicfilter.m* (with the commands: [filenameouput, elapsed\_time] = meuhmagicfilter('RAW.nii', '\_denoised', 'aonlm', 0, 'gauss', 1, 1, 3);)

**Paths and directories**

1. Set up paths to ANTs, FSL, AFNI (Ignore if you can already run these on command line):

**export** PATH=$PATH:/hpc/soft/afni/afni/ #add afni to path

**export** PATH=$PATH:/hpc/soft/ANTS/antsbin/bin/ #add ants to path

1. Specify NMT directory:

nmt\_dir=/hpc/meca/users/loh.k/macaque\_preprocessing/NMT\_v1.2/ #directory where the NMT folder is located.

1. Specify Raw directory:

rawdir=/hpc/meca/data/Macaques/Macaque\_hiphop/${site}/sub-${subj}/ #directory where your T1/T2 nii files are located

1. Specify and create Subject directory for output:

subjdir=/hpc/meca/users/loh.k/macaque\_preprocessing/preproc\_cloud/${name}/ #directory where all your preprocessed files will go

subj\_preprocess\_dir=${subjdir}preprocessing/

**mkdir** ${subjdir} #make directory

**cd**${subjdir} #change directory to preprocessing folder

**Copy required scripts and subject data into preprocessing folder (**${subjdir}**):**

**cp** ${rawdir}\*T\*.nii.gz ${subjdir} *#*copy T1 and T2 files

**cp** ${nmt\_dir}NMT.nii.gz ${subjdir} *#*copy NMT template with skull

**cp** ${nmt\_dir}NMT\_SS.nii.gz ${subjdir} *#*copy NMT template without skull

**cp** ${script\_dir}atlasBREX\_fslfrioul.sh ${subjdir} *#*copy atlasBREX script

**PART A: DATA PREPARATION AND CHECKS**

1. Convert T1 and T2 images to NIFTI format (dicom2nii)
2. If there are multiple T1s or T2s, coregister them and average:

**flirt\_average** 2 ${name}\_t1\_1.nii.gz ${name}\_t1\_2.nii.gz  ${name}\_t1.nii.gz -dof 6

**flirt\_average** 2 ${name}\_t2\_1.nii.gz ${name}\_t2\_2.nii.gz  ${name}\_t2.nii.gz -dof 6

1. Coregister T2 to T1 using flirt:

**flirt** -dof 6 -**in** ${name}\_t2.nii.gz -ref ${name}\_t1.nii.gz -out ${name}\_t2.nii.gz

1. Load T1, T2 on fslview/FSLeyes to check for the following

* Orientation - are the images correctly oriented in the 3 views? are the labels correct?
  + if not correctly oriented, use fslreorient, fslswapdim to reorient.
* Check obliquity: **3dinfo** ${name}\_t2.nii.gz
  + if oblique, “de-oblique” using 3drefit: **3drefit -**deoblique ${name}\_t2.nii.gz

**PART B: BRAIN EXTRACTION**

I mainly used [atlasbrex.sh](http://atlasbrex.sh/) for brain-extraction, which depends on registration to a template for brain stripping, and which in turn requires the subject images to be bias-free and denoised. Alternatively, if you don’t have a template, you can directly use fsl-bet for brain extraction.

**Main steps:**

1. If you have T2, first round quick bias-correction using T2 (HCP method) to facilitate brain extraction (Script from Julien Sein):

    sigma=2 *#I found that this seems to be a better value*

sub=${name} *#subject name*

    T1=${name}\_t1.nii.gz *#input T1*

    T2=${name}\_t2.nii.gz *#input T2*

*#computations*

    fslmaths $T1 -mul $T2 -abs -sqrt "${sub}\_T1mulT2.nii.gz" -odt float

    meanbrainval=`fslstats "${sub}\_T1mulT2.nii.gz" -M`

    fslmaths "${sub}\_T1mulT2.nii.gz" -div $meanbrainval "${sub}\_T1mulT2\_norm.nii.gz"

    fslmaths "${sub}\_T1mulT2\_norm.nii.gz" -bin -s $sigma "smooth\_norm\_s${sigma}.nii.gz"

    fslmaths "${sub}\_T1mulT2\_norm.nii.gz" -s $sigma -div "smooth\_norm\_s${sigma}.nii.gz" "${sub}\_T1mulT2\_norm\_s${sigma}.nii.gz"

    fslmaths "${sub}\_T1mulT2\_norm.nii.gz" -div "${sub}\_T1mulT2\_norm\_s${sigma}.nii.gz" "${sub}\_T1mulT2\_norm\_modulate.nii.gz"

    STD=`fslstats "${sub}\_T1mulT2\_norm\_modulate.nii.gz" -S`

    echo $STD

    MEAN=`fslstats "${sub}\_T1mulT2\_norm\_modulate.nii.gz" -M`

    echo $MEAN

    Lower=`echo "$MEAN - ($STD \* 0.5)" | bc -l`

    echo $Lower

    fslmaths "${sub}\_T1mulT2\_norm\_modulate.nii.gz" -thr $Lower -bin -ero -mul 255 "${sub}\_T1mulT2\_norm\_modulate\_mask.nii.gz"

    fslmaths "${sub}\_T1mulT2\_norm.nii.gz" -mas "${sub}\_T1mulT2\_norm\_modulate\_mask.nii.gz" -dilall "${sub}\_bias\_raw.nii.gz" -odt float

    fslmaths "${sub}\_bias\_raw.nii.gz"  -s $sigma "${sub}\_OutputBiasField.nii.gz"

    fslmaths $T1 -div "${sub}\_OutputBiasField.nii.gz" "${sub}\_t1\_restored\_s${sigma}.nii.gz" -odt float

    fslmaths $T2 -div "${sub}\_OutputBiasField.nii.gz"  "${sub}\_t2\_restored\_s${sigma}.nii.gz" -odt float

*#remove unwanted files*

rm ${sub}\_bias\_raw.nii.gz

    rm ${sub}\_T1mulT2\*.nii.gz

    rm smooth\_norm\_s${sigma}.nii.gz

1. Denoise using ANLM filter (The code below allows us to call the matlab script in bash, alternatively, you can directly run Franck’s denoising script on Matlab)

#denoise biased-corrected t1

    gunzip ${name}\_t1\_restored\_s${sigma}.nii.gz #unzipping because matlab script only works on .nii and not .nii.gz

    matlab -nosplash -nojvm -nodisplay -nodesktop -r     "addpath('/hpc/meca/users/loh.k/macaque\_preprocessing/denoise/');meuhmagicfilter('${name}\_t1\_restored\_s${sigma}.nii','denoised','aonlm',0,'gauss',1,1,3);exit;”

    gzip ${name}\_t1\_restored\_s${sigma}\_\_denoised\_aonlm\_gauss\_1.0\_1\_3.nii #denoised T1

    gzip ${name}\_t1\_restored\_s${sigma}.nii

1. Cropping denoised image using fslroi (open fslview/fsleyes to obtain the exact coordinates for cropping: x, -x, y, -y, z, -z)

    fslroi ${name}\_t1\_restored\_s${sigma}\_\_denoised\_aonlm\_gauss\_1.0\_1\_3.nii.gz ${name}\_t1\_restored\_s${sigma}\_denoised\_cropped.nii.gz 20 192 24 244 288 156

1. Call Atlasbrex script for brain extraction (inputs: -b skull-stripped template, -nb template with skull, -h input image)

    bash atlasBREX.sh -b NMT\_SS.nii.gz -nb NMT.nii.gz -h ${name}\_t1\_restored\_s${sigma}\_denoised\_cropped.nii.gz -f 0.5 -reg 1 -w 5,5,5

    #Note that the following set of parameters (-f 0.5 -reg1 -w 5,5,5) seem to work well for the sbri brains I processed. If they don’t, you can play around with the f value or the type of warping to get the best brain extraction (refer to atlasBREX help for more details)

1. Save extracted brain and brain mask, manually correct (optional), and smooth to produce nice mask

*#Convert stripped brain into brainmask*

    fslmaths ${name}\_t1\_restored\_s${sigma}\_denoised\_cropped\_brain.nii.gz -bin ${name}\_t1\_restored\_s${sigma}\_denoised\_cropped\_brainmask.nii.gz

*#Load mask wih respect to subject t1 for checking*

    fslview ${name}\_t1\_restored\_s${sigma}\_denoised\_cropped.nii.gz ${name}\_t1\_restored\_s${sigma}\_denoised\_cropped\_brainmask.nii.gz

*#EDIT BRAINMASK and save as ${name}\_t1\_restored\_s${sigma}\_denoised\_cropped\_brainmask\_nice.nii.gz*

*#Smooth corrected brain mask*

    fslmaths ${name}\_t1\_restored\_s${sigma}\_denoised\_cropped\_brainmask\_nice.nii.gz -bin -s 1 -thr 0.5 -bin ${name}\_brainmask.nii.gz

**PART C: BRAIN SEGMENTATION**

I mainly used the NMT-template for segmentation which seems to work pretty well. You can substitute the template with any other template. Alternatively, if you don’t have a template, you can directly use fsl-fast for non-template based segmentation.

1. Crop from initial T1 and T2 using same parameters as earlier

    fslroi ${name}\_t1.nii.gz ${name}\_t1\_cropped.nii.gz 20 192 24 244 288 156

    fslroi ${name}\_t2.nii.gz ${name}\_t2\_cropped.nii.gz 20 192 24 244 288 156

1. Run denoise again on cropped T1 and T2

    gunzip ${name}\_t1\_cropped.nii.gz

    matlab -nosplash -nojvm -nodisplay -nodesktop -r     "addpath('/hpc/meca/users/loh.k/macaque\_preprocessing/denoise/');meuhmagicfilter('${name}\_t1\_cropped.nii','denoised','aonlm',0,'gauss',1,1,3);exit;"

    gzip ${name}\_t1\_cropped\_\_denoised\_aonlm\_gauss\_1.0\_1\_3.nii

    gzip ${name}\_t1\_cropped.nii

    gunzip ${name}\_t2\_cropped.nii.gz

    matlab -nosplash -nojvm -nodisplay -nodesktop -r "addpath('/hpc/meca/users/loh.k/macaque\_preprocessing/denoise/');meuhmagicfilter('${name}\_t2\_cropped.nii','denoised','aonlm',0,'gauss',1,1,3);exit;"

    gzip ${name}\_t2\_cropped\_\_denoised\_aonlm\_gauss\_1.0\_1\_3.nii

    gzip ${name}\_t2\_cropped.nii

1. Run T2biascorrection, but this time with the brainmask from Part B.

    sigma=2

    sub=${name}

    T1=${name}\_t1\_cropped\_\_denoised\_aonlm\_gauss\_1.0\_1\_3.nii.gz

    T2=${name}\_t2\_cropped\_\_denoised\_aonlm\_gauss\_1.0\_1\_3.nii.gz

    fsl5.0-fslmaths $T1 -mul $T2 -abs -sqrt "${sub}\_T1mulT2.nii.gz" -odt float

*#mask with nice brain mask that i edited*

fsl5.0-fslmaths "${sub}\_T1mulT2.nii.gz" -mas "${name}\_brainmask.nii.gz" "${sub}\_T1mulT2\_brain.nii.gz"

    meanbrainval=`fsl5.0-fslstats "${sub}\_T1mulT2.nii.gz" -M`

    fsl5.0-fslmaths "${sub}\_T1mulT2\_brain.nii.gz" -div $meanbrainval "${sub}\_T1mulT2\_brain\_norm.nii.gz"

    fsl5.0-fslmaths "${sub}\_T1mulT2\_brain\_norm.nii.gz" -bin -s $sigma "smooth\_norm\_s${sigma}.nii.gz"

    fsl5.0-fslmaths "${sub}\_T1mulT2\_brain\_norm.nii.gz" -s $sigma -div "smooth\_norm\_s${sigma}.nii.gz"     "${sub}\_T1mulT2\_brain\_norm\_s${sigma}.nii.gz"

    fsl5.0-fslmaths "${sub}\_T1mulT2\_brain\_norm.nii.gz" -div "${sub}\_T1mulT2\_brain\_norm\_s${sigma}.nii.gz" "${sub}\_T1mulT2\_brain\_norm\_modulate.nii.gz"

    STD=`fsl5.0-fslstats "${sub}\_T1mulT2\_brain\_norm\_modulate.nii.gz" -S`

    echo $STD

    MEAN=`fsl5.0-fslstats "${sub}\_T1mulT2\_brain\_norm\_modulate.nii.gz" -M`

    echo $MEAN

    Lower=`echo "$MEAN - ($STD \* 0.5)" | bc -l`

    echo $Lower

    fsl5.0-fslmaths "${sub}\_T1mulT2\_brain\_norm\_modulate.nii.gz" -thr $Lower -bin -ero -mul 255 "${sub}\_T1mulT2\_brain\_norm\_modulate\_mask.nii.gz"

    fsl5.0-fslmaths "${sub}\_T1mulT2\_brain\_norm.nii.gz" -mas "${sub}\_T1mulT2\_brain\_norm\_modulate\_mask.nii.gz" -dilall "${sub}\_bias\_raw.nii.gz" -odt float

    fsl5.0-fslmaths "${sub}\_bias\_raw.nii.gz"  -s $sigma "${sub}\_OutputBiasField\_new.nii.gz” #save bias field for viewing

    fsl5.0-fslmaths $T1 -div "${sub}\_OutputBiasField\_new.nii.gz" -mas "${name}\_brainmask.nii.gz" "${sub}\_t1\_brain\_restored\_s${sigma}.nii.gz" -odt float #bias-corrected T1 brain

    fsl5.0-fslmaths $T1 -div "${sub}\_OutputBiasField\_new.nii.gz" "${sub}\_t1\_restored\_s${sigma}\_new.nii.gz" -odt float #bias-corrected T1

    fsl5.0-fslmaths $T2 -div "${sub}\_OutputBiasField\_new.nii.gz" "${sub}\_t2\_restored\_s${sigma}\_new.nii.gz" -odt float #bias-corrected T2

    fsl5.0-fslmaths $T2 -div "${sub}\_OutputBiasField\_new.nii.gz" -mas "${name}\_brainmask.nii.gz"  "${sub}\_t2\_brain\_restored\_s${sigma}.nii.gz" -odt float #bias-corrected T2 brain

*#remove non-used files*

    rm ${sub}\_bias\_raw.nii.gz

    rm ${sub}\_T1mulT2\*.nii.gz

    rm smooth\_norm\_s${sigma}.nii.gz

1. Run additional N4 with parameters from Jerome Sallet (Oxford) on the skull-stripped T1 brain image. (Seems to improve bias correction based on the images I have worked on so far).

    N4BiasFieldCorrection -d 3 -b [200] -c [50x50x40x30,0.00000001] -i ${sub}\_t1\_brain\_restored\_s${sigma}.nii.gz -o ${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -r 0 -s 2 --verbose 1

1. Call NMT\_subject\_align script (from NMT), that computes all transformation between subject T1 and NMT skullstripped brain template and priors. (You can change the templates to any other template.)

    dset=${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz #denoised debiased subject brain

    base=${nmt\_dir}NMT\_SS.nii.gz #skullstripped template

    tcsh -x ${nmt\_dir}NMT\_subject\_align.csh $dset $base #command to call NMT\_subject\_align script

1. Transform NMT segmentation priors into subject space.

croppedfileprefix=${sub}\_t1\_brain\_restored\_s${sigma}\_N4

mkdir NMT\_${name}\_segmentation

cd ./NMT\_${name}\_segmentation

#Apply computed warps+linear transformations to transform NMT priors and template to subject space

#apply warp

3dNwarpApply -prefix ${name}\_NMT\_prior.nii.gz ${name}\_NMT\_brainmask\_prob\_prior.nii.gz ${name}\_NMT\_brainmask\_prior.nii.gz tmp\_01.nii.gz tmp\_02.nii.gz tmp\_03.nii.gz \

-source ${nmt\_dir}/NMT\_SS.nii.gz ${nmt\_dir}/masks/probabilisitic\_segmentation\_masks/NMT\_brainmask\_prob.nii.gz ${nmt\_dir}/masks/anatomical\_masks/NMT\_brainmask.nii.gz ${nmt\_dir}/masks/probabilisitic\_segmentation\_masks/NMT\_segmentation\_CSF.nii.gz \

${nmt\_dir}/masks/probabilisitic\_segmentation\_masks/NMT\_segmentation\_GM.nii.gz ${nmt\_dir}/masks/probabilisitic\_segmentation\_masks/NMT\_segmentation\_WM.nii.gz \

-master ../${croppedfileprefix}\_shft\_aff.nii.gz -nwarp ../${croppedfileprefix}\_shft\_WARPINV.nii.gz -ainterp NN -overwrite

#apply linear transform

3dAllineate -base ../${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -source ${name}\_NMT\_brainmask\_prob\_prior.nii.gz -1Dmatrix\_apply ../${croppedfileprefix}\_composite\_linear\_to\_NMT\_inv.1D  -final NN -prefix ${name}\_NMT\_brainmask\_prob\_prior.nii.gz -overwrite

3dAllineate -base ../${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -source ${name}\_NMT\_brainmask\_prior.nii.gz -1Dmatrix\_apply ../${croppedfileprefix}\_composite\_linear\_to\_NMT\_inv.1D  -final NN -prefix ${name}\_NMT\_brainmask\_prior.nii.gz -overwrite

3dAllineate -base ../${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -source ${name}\_NMT\_prior.nii.gz -1Dmatrix\_apply ../${croppedfileprefix}\_composite\_linear\_to\_NMT\_inv.1D  -final NN -prefix ${name}\_NMT\_prior.nii.gz -overwrite

3dAllineate -base ../${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -source tmp\_01.nii.gz -1Dmatrix\_apply ../${croppedfileprefix}\_composite\_linear\_to\_NMT\_inv.1D  -final NN -prefix tmp\_01.nii.gz -overwrite

3dAllineate -base ../${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -source tmp\_02.nii.gz -1Dmatrix\_apply ../${croppedfileprefix}\_composite\_linear\_to\_NMT\_inv.1D  -final NN -prefix tmp\_02.nii.gz -overwrite

3dAllineate -base ../${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -source tmp\_03.nii.gz -1Dmatrix\_apply ../${croppedfileprefix}\_composite\_linear\_to\_NMT\_inv.1D  -final NN -prefix tmp\_03.nii.gz -overwrite

1. Run AntsAtroposN4 in subject space with NMT priors.

cp ../${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz ${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz *#copy subject image to segmentation folder*

*#make brainmask from brain image because antsAtroposN4 needs a brainmask*

fsl5.0-fslmaths ${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -bin ${sub}\_t1\_brain\_restored\_s${sigma}\_N4\_mask

#run antsAtroposN4.sh

bash [antsAtroposN4.sh](http://antsatroposn4.sh/) -d 3 -a ${sub}\_t1\_brain\_restored\_s${sigma}\_N4.nii.gz -x ${sub}\_t1\_brain\_restored\_s${sigma}\_N4\_mask.nii.gz -c 3 -p tmp\_%02d.nii.gz -o ${name}\_segmentation\_

cp ${name}\_segmentation\_SegmentationPosteriors01.nii.gz ${name}\_segmentation\_CSF.nii.gz *# change filenames*

cp ${name}\_segmentation\_SegmentationPosteriors02.nii.gz ${name}\_segmentation\_GM.nii.gz *# change filenames*

cp ${name}\_segmentation\_SegmentationPosteriors03.nii.gz ${name}\_segmentation\_WM.nii.gz *# change filenames*

cp ${name}\_segmentation\_Segmentation.nii.gz ${name}\_segmentation.nii.gz *# change filenames*

*#remove extra files*

rm -r ${name}\_segmentation\_SegmentationPosteriors\*.nii.gz *# remove default ANTs outputs*

rm -r ${name}\_segmentation\_ *# remove default ANTs outputs*

rm -r tmp\_\*.nii.gz *# remove temporary NMT segmentation priors*

rm -r ${name}\_segmentation\_Segmentation.nii.gz

rm -r ${name}\_segmentation\_Segmentation0N4.nii.gz