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PRE-PREPROCESSING QA:

Check for motion in FSLview by clicking the filmstrip button. Any clear movement or lines on the sagittal view indicate a reason to exclude and not process.

Make sure image is in the RPI orientation with AFNI command below. If not, follow with the fsl command.

3dinfo <input image.nii.gz>

fslreorient2std <input image.nii.gz> <output image.nii.gz>

PREPROCESSING:

1. *Correct for eddy current distortion using a basic FSL script that uses linear transform to align the diffusion volumes. If b0 is not the first volume, replace "0" with the volume number.*

   eddy_correct <input image.nii.gz> DIFF_${SUBJ}_eddycorrect.nii.gz 0

2. *Create skull-stripping directory ("ss") and copy ONLY the output from Step#1 into this directory. Cd to this directory. Create skull-stripped diffusion image (un-weighted) using fslvbm_1_bet. XYZ coordinates indicate the manually defined center of gravity. CHECK RESULTS IN FSLVIEW by overlaying on original image.*

   fslvbm_1_bet -B -f 0.2 -c <x y z>

   If necessary, decrease –f to 0.18 to include more brain, or increase if SS is too generous. Sometimes it is necessary to do multiple skull strips and combine them [using fslmaths, etc.].

3. *Create binary mask and generate the skull-stripped DWI image with diffusion weighting.*

   fslmaths struc/DIFF_${SUBJ}_struc_brain.nii.gz -bin ../DIFF_${SUBJ}_eddycorrect_ss_mask.nii.gz

   fslmaths DIFF_${SUBJ}_eddycorrect.nii.gz -mas ../DIFF_${SUBJ}_eddycorrect_ss_mask.nii.gz ../DIFF_${SUBJ}_eddycorrect_ss.nii.gz

   cd ../

FIT TENSORS AND QA:

4. *Fit tensors with FSL's DTIFIT.*

   dtifit --data=DIFF_${SUBJ}_eddycorrect_ss.nii.gz --out=dti --mask=DIFF_${SUBJ}_eddycorrect_ss_mask.nii.gz --bvecs=<file.bvec> --bvals=<file.bval>

   Default setting is ordinary least squares (OLS). To switch to weighted least squares (WLS) add argument: "--wls". Changing this parameter without a rationale is not recommended.

5. *Check for errors detecting the primary diffusion direction.*

   fslview dti_FA.nii.gz dti_V1.nii &

   While in the FSLview GUI, change the V1 view options to "Display as: RGB; Modulation: dti_FA.nii".
   Note: The corpus callosum should be red. If not, you have a problem. Stop and troubleshoot.

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RECONSTRUCTION AND TRACKING:

6. *Model white matter pathways using a Mixture of Wisharts (MOW) signal attenuation model.*

   mow_recon -data DIFF_${SUBJ}_eddycorrect_ss.nii.gz -bval <file.bval> -bvec <file.bvec> -mask DIFF_${SUBJ}_eddycorrect_ss_mask.nii.gz -ndir 5 -pthresh 0.5 -delta_lg 42 -delta_sm 9 -radius 13 -odir . -datadir <inclusive path to "track_tools", a child directory within TrackTools program folder>

   Delta and radius values provided by the MRI physicists who wrote TrackTools – do not alter. See script usage for information about 'ndir' and 'pthresh' parameters. Changing these values is also not recommended.

7. *Generate 'wholebrain' tractography file.*

   track_tracker -o TRAC_${SUBJ}_2mm_MOW_wholebrain_ss25_ang50_64s.trk -sd 4 -sp n_cubed -sdm DIFF_${SUBJ}_eddycorrect_ss_mask.nii.gz -ss 0.25 -ang 50 -so

   The output track will be up to ~20GB with these parameters and should NOT be opened in TrackVis with the current computing technology. The final argument '-so' generates an important text file and should not be removed.

   Change the angular deviation cutoff by changing '-ang'. 65 is also acceptable. Change the step length by changing '-ss'. The above command tells track_tracker to search 0.25voxels in the direction of local maxima to make a determination about whether or not to continue a streamline. 0.5 is also acceptable. For these two parameters, troubleshoot on a single subject prior to moving forward to determine the optimal parameters for your dataset.

   Do not change '-sp', as this indicates that seeds should be uniformly distributed throughout the voxel. The seed density '-sd' can be increased, but keep in mind that this and increases the size of the file dramatically and can result in generating spurious streamlines. However, for graph metrics a seed density increase stabilizes edge weight (Colon-Perez et al., 2015). Test changes on a single subject prior to moving forward to determine the optimal parameters for your dataset.

REGIONS OF INTEREST:

The following steps refer to only two regions of interest for network tractography. The two regions, ordered arbitrarily, will be referred to as ${roi1} and ${roi2}. The names of these regions, without filename extensions, are referred to as ${roi1name} and ${roi2name}. See note about registration at the end of this document.

TRACTOGRAPHY INITIAL STEPS:

8. *Filter wholebrain file to only include streamlines that pass through a given region of interest.*

   track_intersect -r TRAC_${SUBJ}_2mm_MOW_wholebrain_ss25_ang50_64s.trk ${roi1} TRAC_${SUBJ}_ss25_ang50_64s_${roi1name}.trk

9. *Filter file from Step #8 to only include streamlines that also pass through the second region of interest.*

   track_intersect -r TRAC_${SUBJ}_ss25_ang50_64s_${roi1name}.trk ${roi1} TRAC_${SUBJ}_ss25_ang50_64s_${roi1name}_${roi2name}.trk

   Steps #8-9 CAN be eliminated, in theory. However, this changes the final output and is less consistent with publications that should be cited when using this method. Additionally, processing networks directly from the wholebrain file is significantly more time-consuming. Thus, eliminating these steps is NOT recommended.

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NETWORK TRACTOGRAPHY:

10. *Combine regions of interest into a single file. Intensity values must be unique integers.*

```
fslmaths ${roi1} -add ${roi2} ${roi1name}_${roi2name}.nii.gz
```

11. *Eliminate streamlines that aren't direct network connections.*

```
track_network -r -m pass -save-matching TRAC_${SUBJ}_ss25_ang50_64s_${roi1name}_${roi2name}.trk
${roi1name}_${roi2name}.nii.gz TRAC_${SUBJ}_ss25_ang50_64s_${roi1name}_${roi2name}_mpass_network
```

ALTERNATIVE TO STEPS #8-11: Process network tractography with PanTrack

PanTrack is an in-house program that produces intra-structure white matter tracing and increases the robustness of results. Processing time is significantly longer (24hrs+). Process all networks for a single subject in the same folder.

```
mkdir <directory name>
cd <directory name>
```

12. *Run the PanTrack preparation script (optional – if skipped, add arguments to pantrack command in #13).*

```
pantrack_prepare -s <subject_ID_no_whitespace_or_plus_signs> \
-t ../TRAC_${SUBJ}_2mm_MOW_wholebrain_ss25_ang50_64s.trk
```

Note: this script only needs to be run once. When processing additional networks, neither -s nor -t is needed.

13. *Run PanTrack.*

```
pantrack -R ${roi1} -N ${roi1name} -r ${roi2} -n ${roi2name}
```

Primary output file will be Tracks/TRAC_${SUBJ}_${roi1name}_${roi2name}_pan.trk. 1-2 additional, upsampled files will be created in the same directory. Using the smallest resolution is recommended, as it increases the specificity of the track volume metric and any nifti files based on your network tractography.

Note: PanTrack is still in beta testing. If an error occurs, please email Simone with the output, the error message if one exists, and the text files within the folder "tmp/".

MAXIMIZE RESOLUTION FOR DATA ANALYSIS: ***(optional)***

14. *Run the PanTrack upsampling script to increase track resolution to 1mm^3 and 0.5mm^3. This allows track volume and all other quantitative metrics to be calculated with increased specificity while maintaining the sensitivity achieved from processing in acquisition space. Upsampling does not interpolate data.*

```
pan_upsample -n <skull-stripped nifti brain OR brain mask.nii.gz; 2mm/1mm voxel size> \      (REQUIRED ARG)
             -t <user specified track file.trk> \                                          (REQUIRED ARG)
             -o <output.trk; default = original filename amended> \
             -b  (upsample and save the nifti file specified for -n argument)
```

Output tracks and optional, resampled niftis will be generated in the directories containing the original files.

This step is redundant if PanTrack is used. PanTrack does this operation automatically for tracks.

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SOURCES TO CITE OR ACKNOWLEDGE:

Prior research using this method:

Bohsali, A., Triplett, W., Sudhyadhom, A., Gullett, J. M., McGregor, K., FitzGerald, D. B., … Crosson, B. (2015). Broca's area - Thalamic connectivity. *Brain and Language*, *141C*, 80–88. doi:10.1016/j.bandl.2014.12.001

Ford, A., Colon-Perez, L., Triplett, W. T., Gullett, J. M., Mareci, T. H., & Fitzgerald, D. B. (2013). Imaging white matter in human brainstem. *Frontiers in Human Neuroscience*, *7*(July), 400. doi:10.3389/fnhum.2013.00400

Steps #6-7:

Jian, B., & Vemuri, B. C. (2007). A unified computational framework for deconvolution to reconstruct multiple fibers from diffusion weighted MRI. *IEEE Transactions on Medical Imaging*, *26*(11), 1464–71. doi:10.1109/TMI.2007.907552

Jian, B., & Vemuri, B. C. (2007). Multi-Fiber Reconstruction from Diffusion MRI Using Mixture of Wisharts and Sparse Deconvolution, *Inf Process Med Imaging*. *20*, 384–395.

Jian, B., Vemuri, B. C., Özarslan, E., Carney, P. R., & Mareci, T. H. (2007). A novel tensor distribution model for the diffusion-weighted MR signal. *NeuroImage*, *37*(1), 164–76. doi:10.1016/j.neuroimage.2007.03.074

Step #11:

Colon-Perez, L. M., Spindler, C., Goicochea, S., Triplett, W., Parekh, M., Montie, E., … Mareci, T. H. (2015). Dimensionless, Scale Invariant, Edge Weight Metric for the Study of Complex Structural Networks. *Plos One*, *10*(7), 1-29. doi:10.1371/journal.pone.0131493

TrackTools:

Developed in Dr. Thomas Mareci's research lab at the University of Florida (http://marecilab.mbi.ufl.edu). Written by William Triplett.

PanTrack:

Developed in Dr. Bruce Crosson's research lab at Georgia State University/Emory University. Written by Simone Roberts. Still cite all other sources if used. If not used, AFNI and TrackVis do not need to be cited. However, cite TrackVis if using this program for streamline viewing purposes.

FSL:

Jenkinson M. & Smith S.M. (2001). A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*, *5*(2):143-156.

Jenkinson M., Bannister P.R., Brady J.M., & Smith S.M. (2002). Improved optimisation for the robust and accurate linear registration and motion correction of brain images. *NeuroImage, 17*(2):825-841.

Smith S.M., Jenkinson M., Woolrich W.M., Beckmann C.F., Behrens T.E.J., Johansen-Berg H., Bannister P.R., De Luca M., Drobnjak I., Flitney D.E., Niazy R., Saunders J., Vickers J., Zhang Y., De Stefano N., Brady J.M., and Matthews P.M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*(S1):208-19.

AFNI: (http://afni.nimh.nih.gov)
TrackVis: (http://trackvis.org)

NOTE.
It is recommended to keep intermediate files produced during processing, Files remaining in the "ss" skull-stripping directory are an exception.

NOTE.
Precise registration of the skull-stripped T1 image into diffusion-space is critical for registering ROIs drawn/segmented in T1-space and for visual inspection of tractography. Upsampling the diffusion image to 1mm^3 prior to registration, to maintain the integrity of the original T1 and ROIs, and then spline registration of ROIs into 2mm^3, is suggested. Use AFNI's '3dresample' with '-dxyz'.

REQUIRED SOFTWARE:

FSL	[download from http://fsl.fmrib.ox.ac.uk]
AFNI	[download from http://afni.nimh.nih.gov]
PanTrack	[contact Simone]
TrackTools	[download from http://marecilab.mbi.ufl.edu/software/TrackTools]
TrackVis Diffusion Toolkit	[download from http://trackvis.org]