

## Fibre Orientation & Tractography

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### FSL Bedpostx - Estimating fibre orientations using model-based deconvolution

In the previous practical we saw what the diffusion MRI data look like, went through the preprocessing steps that are needed to correct for the distortions before doing any analysis, and fitted the DTI model to the dMRI data. The diffusion tensor model allows a single fibre orientation to be estimated per voxel. For achieving better tractography, we need to consider cases where multiple fibres cross within a voxel. FSL's tool BedpostX includes a range of models for estimating multiple fibre orientations.

This section will familiarise you with the Bedpostx toolbox in FSL, which performs a parametric (model-based) deconvolution of the diffusion signal to fibre orientations. Bayesian inference and Monte-Carlo-Markov-Chain (MCMC) is used on a voxel-by-voxel basis for estimation. Thus, the output comprises samples from the posterior distribution of the model parameters given the data in a voxel, including the fibre orientation distribution that will then be subsequently used for probabilistic tractography.

To start:

```
cd /home/hcpcourse/day3-wednesday/Diffusion_practicals
```

```
cd 100307/T1w/Diffusion.bedpostX
```

Because it takes a considerable amount of time to run, bedpostX has already been run for you. If you were running it yourself on the HCP data, you would use the following command:

```
bedpostx ../Diffusion -n 3 -b 3000 -model 3 -g --rician
```

This models up to n=3 fibres per voxel, uses a "zeppelin" single-fibre response kernel for deconvolution (model=3 - see lecture) and a Rician noise model. We also ask for 3000 burn-in iterations (this is to

achieve better convergence for the MCMC, but the default 1000 iterations would also work reasonably). Gradient nonlinearities can be accounted for using the -g flag (which instructs bedpostx to use the grad\_dev.nii.gz file from the data folder and produce voxel-specific bvals and bvecs). For more information, see the BEDPOSTx section of the user guide at [the FSL Wiki](#). For the HCP data it is really crucial to run bedpostx either on a large computing cluster or using [GPUs , given the high resolution and large data files](#).

- Type “ls” to list the contents of this directory.

For every model parameter a distribution of values is estimated. The mean of this distribution is saved for every parameter (mean\_\*.nii.gz files). For the parameters used for tractography (e.g. orientations), samples from these distributions are also saved (merged\_\*.nii.gz files). For each fibre compartment an orientation and a volume fraction is estimated. The orientation is described in spherical coordinates by two angles, theta and phi.

Thus, for example the files “mean\_f2samples.nii.gz, mean\_th2samples.nii.gz, mean\_ph2samples.nii.gz” describe the mean values of the distributions estimated for the volume fraction and orientation of fibre 2. Files “merged\_f2samples.nii.gz, merged\_th2samples.nii.gz, merged\_ph2samples.nii.gz” describe samples from these distributions. Note: in order for the wb\_command below to run on the course machines and make an orientations file, we had to reduce the number of samples in these files from 50 to 20, hence the file names: reduced\_merged\_f1samples.nii.gz, etc.

## Visualising Orientations in Connectome Workbench

All of these files can be opened as volume NIFTIs in Workbench. However, for better visualisation and more informative option, we convert them to an orientation Workbench file. On your terminal, run wb\_command as follows:

```
wb_command -estimate-fiber-binghams reduced_merged_f1samples.nii.gz \
reduced_merged_th1samples.nii.gz reduced_merged_ph1samples.nii.gz \
reduced_merged_f2samples.nii.gz reduced_merged_th2samples.nii.gz \
reduced_merged_ph2samples.nii.gz reduced_merged_f3samples.nii.gz \
reduced_merged_th3samples.nii.gz reduced_merged_ph3samples.nii.gz \
../Whole_Brain_Trajectory_1.25.nii.gz Whole_Brain_Orientations.fiberTEMP.nii
```

This takes about 30 seconds to run and produces the file Whole\_Brain\_Orientations.fiberTEMP.nii that we can now open in Workbench. Note that files with the “reduced\_” prefix are used, these files have been reduced to the first 20 (out of 50) samples due to the memory constraints of the course machines. For real use on more capable hardware, you would use the “merged\_f1samples.nii.gz” and similar files. The output file summarises all orientation and volume fractions information for all fibre compartments in brain tissue regions. To open it along with a T1w image, enter:

```
wb_view ../T1w_acpc_dc_restore_1.25.nii.gz \
Whole_Brain_Orientations.fiberTEMP.nii &
```

Workbench will open with a volume tab displaying only the T1w Image. To view the fibres:

- *Open* the **Features Toolbox** (from the menu View->Features Toolbox->Attach to Right) and *tick* **Display Fibers**.

What you see in every voxel is the mean fibre orientation from every estimated distribution.

- To make sure that you display fibers from a single slice, *set* the **Slice Above/Below Limit** entries to less than the voxel size (e.g. 0.5 and -0.5).
- *Zoom in* until you can clearly see fibers associated with individual voxels.
- *Change* the view to **coronal**/sagittal by selecting appropriately from the Slice Plane on the toolbar.

There are many options for displaying orientations in the Features Toolbox that you can experiment with. By default, the option **Draw with Magnitude** is selected and displays each orientation with a magnitude proportional to the respective volume fraction.

- *Unselect* **Draw with Magnitude**, then *set* **Length Multiplier** to 1, to display all fibre orientations with the same normalised length regardless of their volume fraction.

The volume fractions carry important information, as the Automatic Relevance Determination priors imposed on them indirectly perform model selection (i.e. determine how many fibre orientations are supported by the data in every voxel - see lecture). By changing the **Minimum Magnitude** we can select fibre compartments that are supported by the data at various strengths. A minimum value of 0.05 is good enough for determining compartments with practical relevance.

- *Change* the **Minimum Magnitude** value to 0.

Now all three fibres estimated in every voxel are displayed, but the ones with too small volume fraction (<5%) look particularly noisy, implying that we have fitted more fibres than supported by the data.

- *Change* the **Minimum Magnitude** value back to 0.05 to keep only the relevant fibre orientations. Note that only fibres with  $f > 0.05$  (by default) are considered for tractography.

So far we have displayed the mean of the orientation distributions.

- *Change* the **Symbol** from **Lines** to **Fans** to see a cone of uncertainty drawn for each fibre compartment, reflecting graphically the dispersion of the respective distribution.

The Fan Multiplier entry controls the relative width of these cones for visualisation purposes. A value of 1 corresponds to the width of the cone that reflects one standard deviation of the orientation distribution.

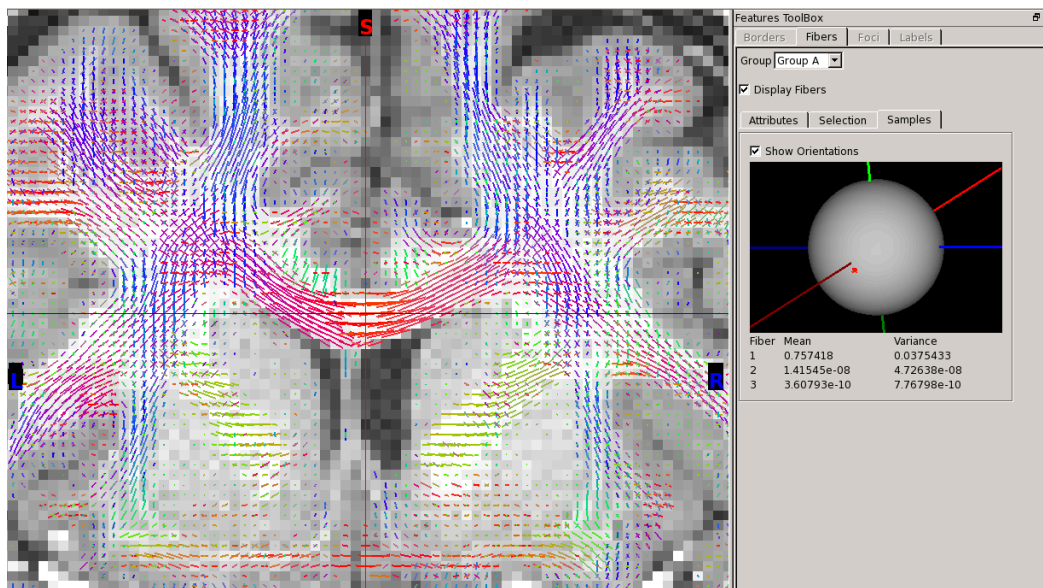
Apart from the mean and uncertainty of the fibre orientation distributions, `wb_view` also enables display all the samples that have been estimated by bedpostX and fully describes the distribution in each voxel. DO NOT do the next two steps, they will crash the course machines.

- *Switch* the Tab from **Attributes** to **Samples**.

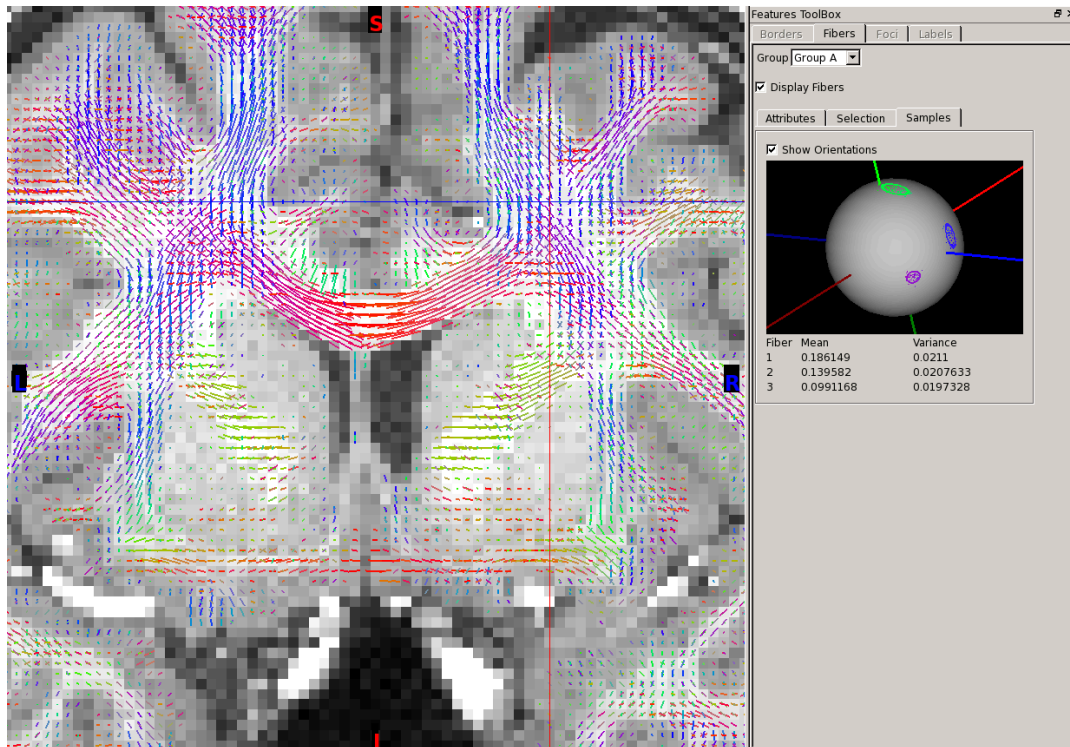
- Select **Show Orientations** to display a spherical plot of all the orientation samples.

The two figures below show what you would see **IF** you were/are using a computer having plenty of available RAM and were to click on voxels of interest in white matter. The spherical plot can be rotated to observe the sample distribution relative to the three main axes (Superior-Inferior in Blue, Anterior-Posterior in Green, Medial-Lateral in Red).

The first example is from a voxel in the corpus callosum, where fiber bundles tend to be very tightly aligned. Accordingly, this voxel shows only one major orientation and has very low uncertainty (all samples almost overlap, as indicated by the small red patch):



A second example is from a voxel in the centrum semiovale, where there is a three-way fiber crossing (purple, blue, and green). Note that the sample distribution is tightest for the purple fibre bundle. For the other two fibre bundles, the sample distribution is not only broader, it is anisotropic (elliptical rather than circular), which provides evidence for fiber fanning (see below).



Another option is to superimpose cortical surface contours on the fibre orientation plots.

- Select **File->Open File -> Files of Type->Scene Files** and open **Tractography\_Practical.scene** (from /home/hcpcourse/day3-wednesday/Diffusion\_practicals/100307/T1w/Diffusion.bedpostX)
- Show the first scene (100307 pial and white surfaces...).

This scene has loaded surface files (from the ../fsaverage\_LR32k folder) in the subject's T1w space.

The **Overlay Toolbox** at the bottom shows the selection of pial and white surfaces for each hemisphere. In the **Layers** tab, you can toggle different surfaces on or off (including the midthickness surfaces, which are loaded but not displayed in this scene), and in the **Vol/Surface Outlines** tab you can change the colour and/or line thickness of each surface contour.

### Comparison with DTI orientations

We will now load the DTI orientations that we computed in the previous practical and compare with the BedpostX orientations.

- Go to **File->Open File....** Go Up one directory and choose the **Diffusion** folder.
- From the **File types** at the bottom, choose **Connectivity - Fiber Orientations TEMPORARY Files (\*.fiberTEMP.nii)**.
- Choose the **dtiV1.fiberTEMP.nii** file and press **Open**.

The DTI orientations are now loaded and displayed along with the bedpostX orientations.

- Go to the **Features Toolbox** on the right and *toggle on Display Fibers*.
- Choose the **Selection** Tab and *untick* the **dtiV1.fiberTEMP.nii** so that the current window displays only bedpostx orientations.
- *Untick* the **Whole\_Brain\_Orientations.fiberTEMP.nii** file and *choose* the **dtiV1.fiberTEMP.nii** so that the current window displays only DTI orientations.
- *Switch* between the two to appreciate the rich crossing fibre information that is estimated using deconvolution. Notice that the DTI file contains deterministic orientation estimates with their length modulated by the FA, so only displays of Lines are meaningful.

## **FSL ProbtrackX - Tractography**

### **Simple tracking - Connectivity from a single seed point**

- In `wb_view`, *open* the second scene (`Mean_f1samples`, crosshairs on (56,91,58))

This shows a slice through the `mean_f1samples` volume (which has greater contrast between WM and GM than a T1w or T2w volume). The crosshairs (which can be turned on or off in the **Preferences** menu), are centered on a voxel in the internal capsule (the white matter tract shown in the picture; running between the caudate/thalamus and the putamen/pallidum). The specific co-ordinates (useful in a moment to run tractography using FSL) are (56,91,58), as indicated in the **Toolbar (Slice Indices/Coords)**.

- In the terminal, type:

```
Fdt &
```

This brings up FMRIB's diffusion toolbox (Fdt). It should default to "ProbTrackx" with "single voxel" as the selected seed mode.

If you had enough memory on the course computers (DON'T do the following steps), you would:

- *Select* **Diffusion.bedpostx** as the **Bedpostx directory** and *enter* your selected voxel coordinates (above) as the seed. *Set* the Output file to `Tracts/SingleVoxel_paths`.
- *Press Go* (again, DON'T do this). Probabilistic tractography will start running. Alternatively, you can use the following commands in the terminal window.

Notice that the command-line version of `probtrackx` is more flexible and has options that are not supported in the GUI. If you have enough memory (DON'T run this on the course computers), you could run the same in command line using:

```
echo "56 91 58" >seedvox.txt
```

Then (but DON'T run this on the course computers):

```
probtrackx2 --samples=merged --mask=nodif_brain_mask --seed=seedvox.txt --  
simple --loopcheck --forcedir --opd -V 1 --dir=Tracts --out=SingleVoxel_paths
```

- In `wb_view`, *select* **File: Open File: Volume Files**: Go up one level, *select* **Diffusion.bedpostX/Tracts/SingleVoxel\_paths\_56\_91\_58.nii.gz** to view the resultant connectivity distribution file on top of the `mean_f1samples` map - note the pathway's name ends with the coordinates of the seed voxel (`basename_X_Y_Z.nii.gz`).
- *Select* **SingleVoxel\_paths\_56\_91\_58** volume in the top layer of the **Overlay Toolbox** and *toggle it on*. The connectivity-related voxels will appear black.
- *Show* the third scene, **Single-voxel tractography (56,91,58)**, to view a modified configuration of the same data.
- *Press* the “wrench” icon for **SingleVoxel\_paths\_56\_91\_58**
- In the **Overlay and Map Settings** window, note that the **Palette** is set to red-yellow and the **Threshold** is set to **Type: On** and **Show Data Outside Thresholds**.
- If the connectivity pattern in the white matter appears black instead of yellow-red, *toggle* the **Interpolate Colors** button (under **Palette**) to make the pattern more colorful (This is currently a small glitch in how the scene palette is displayed).
- *Enter* **10** for the **High threshold**, then *press* **Return**.

Note that most of the fibre pathways have disappeared, because they are very sparse.

- *Enter* **20** for the **High threshold**, *press* **Return**, and note that the dominant pathway runs dorsally up to cortex in the medial aspect of the hemisphere.

### Tracking in a standard / non-diffusion space

It is common practice to use an atlas/standard space for defining coordinates and masks, because this improves alignment across subjects and provides standardisation. We have already obtained the transformation between the subject space to FSL's MNINonlinear standard atlas space. We can now use this transform to perform tractography by defining a seed in that standard space. Notice that results will be also produced in the same space as the seed, so in this case standard space.

- *Close* the Workbench window you have opened, as we will open a new instance. In the terminal:

```
cd /home/hcpcourse/day3-wednesday/Diffusion_practicals/100307/MNINonLinear  
  
wb_view Tractography2_Practical.scene &
```

- *Open* the first scene.

This will display the T1w image for subject 100307 transformed to the standard space (`T1w_restore_brain.nii.gz`), with the crosshairs centered on the midbody of the corpus callosum

(129,157,137).

- In the Fdt GUI, remain in "**single voxel**" seed mode, select "**seed space is not diffusion**" –

This should bring up two new dialogues, specifying the Seed space.

- Select "**nonlinear**" and a third dialogue will appear.
- The "**Seed reference image**" is the image you selected the seed point from (T1w\_restore\_brain).
- The seed to diff and diff to seed space registration transforms can be found in the **MNINonLinear/xfms** directory (the registration transforms have already been computed for you). In this case use **standard2acpc\_dc** and **acpc\_dc2standard** respectively.
- Do NOT press "Go" because the course computers lack sufficient memory to successfully run probtrackx!

Probtrackx can also be run by *command* line (but only IF you have enough computer memory). To do this, you would use:

```
echo "129 157 137" >seedvox.txt
```

Then (but DON'T run this on the course computers):

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \  
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask --seed=seedvox.txt \  
--xfm=xfms/standard2acpc_dc --invxfm=xfms/acpc_dc2standard \  
--seedref=T1w_restore_brain --simple --loopcheck --forcedir --opd -V 1 \  
--dir=Tracts --out=SingleVoxel_paths
```

- To view the precomputed results of this process, *open* the second scene in **wb\_view (SingleVoxel paths (129,157,137)...**) to see a restricted set of fiber bundles running through this seed in the corpus callosum.
- Press the "wrench" icon for **SingleVoxel\_paths\_129\_157\_137**, then - If the connectivity pattern in the white matter appears black instead of yellow-red - *toggle* the **Interpolate Colors** button (under **Palette**) to make the pattern more colorful.
- Enter 10 for the High threshold and note that the 'connected' fiber bundles are highly restricted even with this low threshold.

## Using Anatomical priors in tractography

Here we will make use of masks to inform/constrain tractography. There are various options in probtrackX to impose anatomical prior knowledge in the form of:

a) Waypoint Masks (--waypoints option): Curves must go through these masks to be considered valid,



- b) Exclusion Masks ( --avoid option): Curves that go through are invalid,
- c) Termination Masks ( --stop option): Curves terminate when they cross,
- d) Walk-through Termination Masks ( --wtstop option): Curves terminate when they cross the mask for a second time (useful for propagating within subcortical nuclei).

We will see an example of reconstructing the Optic Radiation (pathway connecting the lateral geniculate nucleus (LGN) to the ipsilateral primary visual cortex (V1) that passes via Meyer's loop) with and without constraint masks.

- If you have enough memory, you would run tractography using the LGN mask (file called MNINonLinear/ROIs/SEED\_LGN\_LEFT.nii.gz), which is in standard space, as follows:

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \  
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask --seed=ROIs/SEED_LGN_LEFT \  
--xfm=xfms/standard2acpc_dc --invxfm=xfms/acpc_dc2standard \  
--seedref=T1w_restore.2 --loopcheck --forcedir --opd -V 1 --dir=Tracts \  
--out=LGN_paths
```

- To view the precomputed file, in wb\_view, first *open* the third scene (“**LGN seed**”) to see the LGN seed used for this analysis.
- *Click* on the wrench icon for this layer (**SEED\_LGN\_LEFT.nii.gz (../ROIs)**) and toggle **Interpolate colors** (if needed for coloring)
- In the **Overlay Toolbox**, *toggle* the top layer on (“**LGN\_paths**”) to see that without thresholding or other constraints, it contains a vast number of paths arising from the LGN.
- *Click* on the wrench icon for this layer (**LGN\_PATHS.nii.gz (../Tracts)**) and *toggle* **Interpolate colors** (if needed for coloring)
- *Adjust* the **high threshold** to **200** by typing “**200**” into the box at the end of the slider by “High” in the palette settings window and press Enter, and note that the optic radiation is one of several major pathway that persist. Then *close* the **Overlay and Map Settings** window.
- *Toggle off* the top layer (“**LGN\_paths**”) and *toggle on* the second layer (“**TARGET\_V1\_LEFT.nii.gz (../ROIs)**”) to see left area V1, which can be used as mask to isolate tracts that reach V1. This can be done using V1 as both a waypoint mask and as a termination mask to avoid tracts that reach V1 and then continue to other parts of the brain.
- *Click* on the wrench icon for this layer (**TARGET\_V1\_LEFT.nii.gz (../Tracts)**) and toggle **Interpolate colors** (if needed for coloring).

Finally if you had enough computer memory, you could use an exclusion mask that rejects paths that go to the frontal areas:

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \  
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask --seed=ROIs/SEED_LGN_LEFT \  
--xfm=xfms/standard2acpc_dc --invxfm=xfms/acpc_dc2standard \  
--seedref=T1w_restore.2 --waypoints=ROIs/TARGET_V1_LEFT.nii.gz \  
--stop=ROIs/TARGET_V1_LEFT.nii.gz --avoid=ROIs/EXCLUSION --loopcheck \  
--forcedir --opd -V 1 --dir=Tracts --out=LGN_constrained_paths
```

- To view the precomputed results, *open* the fourth scene ("**Connections – LGN seeded, with masking**") and compare with the previous unconstrained version. The optic radiation forms the core of the distribution in the constrained scenario.
- *Click* on the wrench icon for this layer, adjust the **high threshold** to **10**, and note that the optic radiation is now more crisply delineated.

### Using Surfaces in Tractography

So far we have used volumetric seeds/constraint masks. Probtrackx also supports using surface masks as seeds/constraints, which is important because long-distance pathways generally run exclusively through white matter and only branch locally in their cortical gray matter origins and terminations. For more details see the relevant FSL [documentation](#). Here, we will see an example, where in order to avoid false positive connections that connect adjacent gyri, we impose the convoluted WM/GM boundary surface as a termination mask. Tracking will be performed again in standard space as before. We will first run an example without any surface constraints from a seed close to the cortex.

IF you had sufficient computer memory, in the terminal window you would run:

```
echo "151 196 183" >seedvox.txt
```

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \  
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask -x seedvox.txt \  
--xfm=xfms/standard2acpc_dc --invxfm=xfms/acpc_dc2standard \  
--seedref=T1w_restore_brain --loopcheck --simple --forcedir --opd -V 1 \  
--dir=Tracts --out=NoSurf_constraints
```

- To view the precomputed results, *open* the 5<sup>th</sup> scene ("**seed 151, 196, 183, no surface constraints**"), which uses output **Tracts/NoSurf\_constraints**.
- Examine the coronal slice at position Y = 203, where it is evident that a spurious tract is connecting two adjacent gyri via an anatomically implausible path. This arises from the apposition of gyral gray matter and the limits in MRI resolution.

We will use the left and right WM/GM boundary surfaces as termination masks to prohibit this type of implausible "jumps" (similarly you can use the pial surfaces or midthickness surfaces). We will first convert the GIFTI surface provided with the HCP release to a more friendly ASCII format for probtrackx. We will use the tool surf2surf to perform the conversion and also remove from the HCP GIFTI surface the medial wall.

- In the terminal window run:

```
surf2surf -i fsaverage_LR32k/100307.L.white.32k_fs_LR.surf.gii -o \
L.white.asc --outputtype=ASCII \
--values=fsaverage_LR32k/100307.L.atlasroi.32k_fs_LR.shape.gii surf2surf \
-i fsaverage_LR32k/100307.R.white.32k_fs_LR.surf.gii -o R.white.asc \
--outputtype=ASCII \
--values=fsaverage_LR32k/100307.R.atlasroi.32k_fs_LR.shape.gii
```

Then, run tractography using these surfaces L.mid.asc and R.mid.asc as stopping masks:

```
echo "L.white.asc" >stopsurfaces.txt
echo "R.white.asc" >>stopsurfaces.txt
```

IF you had sufficient computer memory, you could also run:

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask -x seedvox.txt \
--xfm=xfms/standard2acpc_dc --invxfm=xfms/acpc_dc2standard \
--seedref=T1w_restore_brain --loopcheck --simple --forcedir --opd -V 1 \
--dir=Tracts --stop=stopsurfaces.txt --out=Surf_constraints
```

- To view the precomputed tracking results, toggle on the top layer ("Surf\_constraints\_156\_193\_183...") and toggle off the second layer ("NoSurf\_constraints..."). Looking again at coronal slice 203 as before, the spurious connection should now be absent; the overall tracking results are now better delineated and more anatomically plausible.

## Connectivity-based segmentation

Now that we have seen how to run some basic tractography, we will look into using tractography results to classify/segment an area by finding differences in the connectivity profiles of each voxel in that area. A connectivity-based seed classification analysis for the Thalamus has been run for you and is saved in a sub-directory called THAL2CTX\_right within your MNINonLinear directory. This analysis was carried out in standard space, by seeding tractography from all voxels in the right thalamus and using cortical regions as target masks.

We have volume masks that define seven different cortical regions(THAL2CTX\_right/MASK\_\*.nii.gz. The objective of this type of analysis is to ask the following question: "For each location in the seed mask (thalamus), what is the probability of connection to each of my target masks". The output is therefore a single image for each target mask in which only voxels within the seed mask contain data.

We could use directly the volume cortical masks, but to make the problem more interesting we will first convert the volume masks to cortical surface masks. We will then perform tractography-based parcellation of the thalamus using surfaces as targets. First we need to project the volume masks to a surface.

For each of the seven masks, do the following (as an example for the primary motor cortex M1 region):

```
wb_command -volume-to-surface-mapping THAL2CTX_right/MASK_average_M1_right.nii.gz \
fsaverage_LR32k/100307.R.white.32k_fs_LR.surf.gii \
THAL2CTX_right/M1_atlasROI.roi.gii - \ enclosing
then
```

```
surf2surf -i fsaverage_LR32k/100307.R.white.32k_fs_LR.surf.gii -o \
THAL2CTX_right/M1.white.asc --outputtype=ASCII \
--values=THAL2CTX_right/M1_atlasROI.roi.gii
```

The seed mask will be ROIs/THALAMUS\_RIGHT. The file THAL2CTX\_right/targets.txt contains a list of the target surface masks used in this analysis.

Then IF you had sufficient computer memory, you would run tractography as:

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask \
--xfm=xfms/standard2acpc_dc --invxfm=xfms/acpc_dc2standard \
--seedref=T1w_restore.2 --seed=ROIs/THALAMUS_RIGHT.nii.gz --loopcheck \
--onewaycondition --forcedir --opd --dir=THAL2CTX_right \
--targetmasks=THAL2CTX_right/targets.txt --os2t
```

As mentioned before, the output is a single image for each target mask in which only voxels within the seed mask contain data. At each voxel within the seed mask the voxel value is the number of samples which reached this target mask from this seed voxel. These outputs can be found in THAL2CTX\_right/seeds\_to\_\* files.

- In `wb_view`, *open* the sixth scene (“**Thalamic connectivity with pmc mask (../THAL2CTX)**”) to see thalamic connectivity with the premotor cortex (pmc) target mask (shown in yellow – after *toggling Interpolate Colors*, if necessary).
- *Toggle on* the top two layers (“**seeds\_to\_ppc....**” and “**MASK\_averge\_ppc...**” to see the different pattern of thalamic connectivity with posterior parietal cortex.

Other newly generated masks and thalamic seeds can be loaded from the THAL2CTX\_right/ directory.

Now we will use these files to classify the thalamus based on the connection probabilities to different target. Run `find_the_biggest` on the outputs of seeds to targets to generate a hard segmentation of the thalamus:

```
find_the_biggest THAL2CTX_right/seeds_to_* \
THAL2CTX_right/biggest_segmentation
```

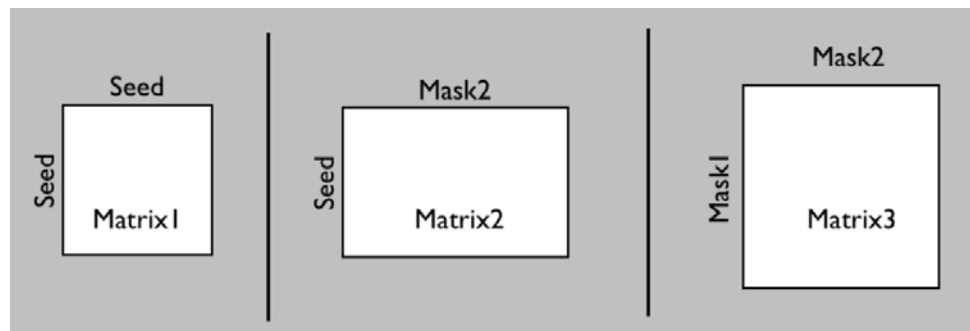
- *Open* the seventh scene (“**Thalamic connectivity – find the biggest**”) to view this segmentation on the 100307 brain. The numbers in the image each correspond to a single target surface. The different numbers in the output image (`biggest_segmentation`) each relate to a different target mask, i.e. they tell you to which of the target regions each voxel in the thalamus is more likely connected. Note that the palette used for this display is JET256.

- *Exit wb\_view*

### Connectome Matrix options in probtrackx: Matrix1, Matrix2, Matrix3

Probtrackx supports the generation of various Connectome Matrices (see lecture). As shown in the figure below, there are choices for outputting different types of summary matrices (--omatrix1, --omatrix2, --omatrix3 options). The seed and the target masks can be volumes, surfaces or a combination of the two, as is the case for HCP dense connectomes of GrayOrdinates x GrayOrdinates, where GrayOrdinates={Surface Vertex coordinates, Voxel coordinates}.

We will focus on Matrix1 and Matrix3 here, but you can learn more about Matrix2 and how it can be used to perform connectivity-based parcellation from the relevant FSL [documentation](#).



If we are after a GrayOrdinates x GrayOrdinates square connectome matrix, then Matrix1 can be obtained by giving the set of GrayOrdinates as Seeds. This effectively means seeding from everywhere in the WM/GM boundary and the subcortical volumes and record propagation of curves to the same set of GrayOrdinates. For Matrix3, the Seed can be different from the target GrayOrdinates. Obviously these matrices can take very long to generate. We will see a simple example here of generating a Matrix1 parcellated connectome using a small number of surface and volume ROIs. In the following section we will navigate through some precalculated dense connectomes.

Let's assume we want to obtain the connectome matrix between N regions. As an illustration, we will choose two surface ROIs, the left M1 and the left occipital pole and one volume ROI, the left thalamus. All ROIs are in standard space. The thalamic ROI is in NIFTI format as ROIs/THALAMUS\_LEFT.nii.gz and have been extracted from an atlas. Let's imagine we want to extract the surfaces that correspond to precentral and occipital regions.

In the terminal:

```
cd /home/hcpcourse/day3-wednesday/Diffusion_practicals
cd 100307/MNINonLinear/fsaverage_LR32k
wb_view 100307.32k_fs_LR.wb.spec &
```

Once loaded, the displayed surfaces will have overlaid some label files that parcellate them into different ROIs.

- *Click* on the Precentral gyrus (Blue Area) on one of the surfaces, the **Information Box** appears,

informing you of the name of the chosen ROI for the different label files that are loaded. We will go with the Freesurfer-based parcellation 100307.aparc.32k\_fs\_LR, for which the name of the desired ROI is "precentral". Similarly, if we click at the occipital region (blue at the posterior part of the brain) we get the name "lateraloccipital".

Extract the "precentral" and "lateraloccipital" surfaces from the label files, by running the following two commands:

```
wb_command -gifti-label-to-roi 100307.L.aparc.32k_fs_LR.label.gii \  
../ROIs/LPrecen.func.gii -name L_precentral
```

```
wb_command -gifti-label-to-roi 100307.L.aparc.32k_fs_LR.label.gii \  
../ROIs/LOccip.func.gii -name L_lateraloccipital
```

We will also convert the obtained GIFTI surface files to ASCII as before:

```
surf2surf -i fsaverage_LR32k/100307.L.white.32k_fs_LR.surf.gii -o \  
ROIs/LPrecen.asc --values=ROIs/LPrecen.func.gii --outputtype=ASCII \  
surf2surf -i fsaverage_LR32k/100307.L.white.32k_fs_LR.surf.gii -o \  
ROIs/LOccip.asc -- \ values=ROIs/LOccip.func.gii --outputtype=ASCII
```

We can now run tractography using the surface ROIs and the thalamic volume ROI and obtain a Matrix1 parcellated Connectome.

IF you had sufficient computer memory, your probtrackx call would be:

```
echo "ROIs/LPrecen.asc" > seeds.txt
```

```
echo "ROIs/LOccip.asc" >>seeds.txt
```

```
echo "ROIs/THALAMUS_LEFT.nii.gz" >>seeds.txt
```

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \  
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask --seed=seeds.txt \  
--xfm=xfms/standard2acpc_dc --invxfm=xfms/acpc_dc2standard \  
--seedref=T1w_restore.2.nii.gz --loopcheck --forcedir --network --omatrix1 \  
-P 1000 --avoid=ROIs/CSFmask.nii.gz -V 1 --dir=Network
```

The output will now be in the **Network** directory. The file fdt\_network\_matrix is a 3x3 matrix containing the number of streamlines that start from ROI i and end up in ROI j: cat **Network/fdt\_network\_matrix** will display this matrix.

The first row corresponds to the connections of the Precentral ROI to the other two, the second to the connections of the Occipital ROI and the third row to the connections of the thalamic ROI. Notice that these are unnormalised values, to normalise them each row can be divided by the respective rowsum (or entries in the Network/waytotal file). After such a division the matrix becomes: 0 0.0188 0.9812 0.112 0 0.888 0.797 0.203 0 which suggests two strong projections between the thalamus and the surface ROIs. Notice the asymmetry in the Matrix, as tractography is not necessarily symmetric. I.e. it is

easier to track from the Cortex to the thalamus, than from the thalamus to the cortex. Why do you think that is the case? Finally, the ROIs are different in size and also surface ROIs will in general have less streamlines passing through them than volume ROIs (it is more difficult to hit a plane than a cube). These considerations need to be taken into account when interpreting this type of matrices.

## Dense Connectomes

Dense Connectomes take a long time to compute and are very greedy on computational resources (using the standard 92,000 Grayordinates of the HCP, gives us a final size of ~33 GB for the 92k x 92k connectome matrix). We have precomputed some dense connectomes using a computing cluster.

The Grayordinates are defined in the file Connectomes/GrayOrdinates.txt and are all in standard space. The Left WM/GM boundary surface defines about ~30k vertices, followed by another ~30k vertices for the Right WM/GM boundary surface. The last ~32k grayordinates are voxel coordinates described in the NIFTI files Connectomes/CIFTI\_STRUCTURE\_\*.nii.gz. To obtain a connectome using the Matrix1 option, we would seed tractography from each of these 92k Grayordinates and record the streamlines arriving to the rest. To obtain a connectome using the Matrix3 option, we would seed tractography from every voxel in the brain and for every obtained curve record which of the two grayordinates it intersects.

In theory (don't run), the probtrackx call for each of the two options would be (when called from the MNINonLinear directory:

For Matrix1:

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \  
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask \  
--xfm=xfms/standard2acpc_dc \  
--invxfm=xfms/acpc_dc2standard --seedref=T1w_restore.2.nii.gz \  
--loopcheck --forcedir -c 0.2 --sampvox=2 --randfib=1 \  
--stop=Connectome/stop --wtstop=Connectome/wtstop --forcefirststep \  
--waypoints=ROIs/Whole_Brain_Trajectory_ROI_2 \  
-x Connectomes/GrayOrdinates.txt --omatrix1 --dir=Connectomes
```

For Matrix3:

```
probtrackx2 --samples=../T1w/Diffusion.bedpostX/merged \  
--mask=../T1w/Diffusion.bedpostX/nodif_brain_mask \  
--xfm=xfms/standard2acpc_dc \  
--invxfm=xfms/acpc_dc2standard --seedref=T1w_restore.2.nii.gz \  
--loopcheck --forcedir -c 0.2 --sampvox=2 --randfib=1 \  
--stop=Connectome/stop --wtstop=Connectome/wtstop \  
--waypoints=ROIs/Whole_Brain_Trajectory_ROI_2 \  
-x ROIs/Whole_Brain_Trajectory_ROI_2 --omatrix3 \  
--target3=Connectomes/GrayOrdinates.txt --dir=Connectomes
```

(Notice that only the last arguments change). In practice however, as this above commands would need impractically large amounts of memory and computing resources, we need to split and parallelise the

computation by using the `--rseed` option in `probtrackx` and using a small number of samples `--nsamples` per instance.

That is, instead of running the above command, which will attempt to propagate 5000 curves per seed, we can run, e.g., 100 instances of the above commands, using `--nsamples=50` and `--rseed=i`, `i=1..100`. This will make sure that every instance runs with fewer curves per seed and a different random seed generator to produce a different stochastic result; thus, all these instances can be parallelised.

The results at the end, obtained from all instances, can be merged using the FSL command `fdt_matrix_merge`. Notice that the final result of the above processes will be a matrix in standard sparse `.dot` format (which can be imported e.g. in MATLAB using the function `spconvert()`). To convert this result to a CIFTI dense connectome file, a final call of `wb_command` is needed with the option `wb_command -probtrackx-dot-convert matrix1.dot Conn1.dconn.nii ....`

We have prepared some Workbench scene files to visualise the dense connectomes.

- In your current directory (**100307/MNINonLinear/fsaverage\_LR32k**), *select File->Open File...* and *select* from the **MNINonLinear/Connectomes** directory the **Conn.scene** file.

After loading the scene file, you will see a montage view (tabs in 2 rows and 3 columns). Each row shows a different dense connectome and the columns show Left and Right hemispheres and subcortical volumes. The first row corresponds to a Matrix3 connectome, the second row corresponds to a Matrix3 connectome with path distance correction (i.e. using the `--pd` option in `probtrackx` that instead of counting the number of streamlines, it weights the counts by the streamline length; thus longer connections are upweighted).

- *Click* at different locations in one of the surfaces to update the connectome values for all panels.
- *Click* around to get a feel of the differences between the different connectome types. For instance, *click* on the sensorimotor areas of the thalamus and see respective connections to the cortex. Or *click* on the frontal regions to see contralateral and subcortical connections.

A parcellated version of these dense connectomes can be obtained using `wb_command -cifti-parcellate` and your favourite parcellation label file. Some parcellated connectomes have been obtained for you using standard Freesurfer parcellations in the **Connectomes/Parcellated** directory. Open them in Workbench and explore them (as well as exploring the "Chart View" option).

### **End of Tractography Practical**

### **Authors:**

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