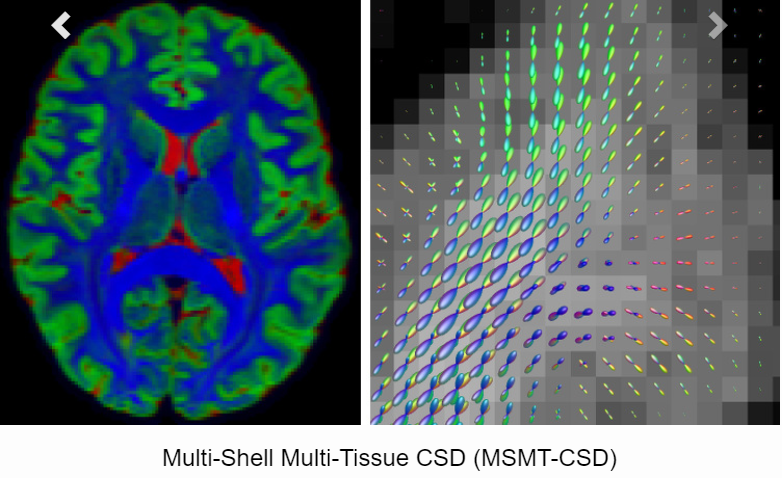
DPRC Diffusion CSD Pipeline

This pipeline takes the data from the preprocessing pipeline. Now, we will do some further analyses, which includes generating *fibre orientation distribution (FOD),* using *constrained spherical deconvolution (CSD)*.

Like the preprocessing steps, most of these postprocessing steps will run through MRtrix3, with some fsl functions. Many of these steps can be found in detail on the mrtrix documentation page.

\*Like what was done with the previous preprocessing script, run this script separately per each group you are interested in analysing in. Each group is separate/unique by the global normalisation step which was applied to them from the preprocessing script.



Resource on CSD and FBA manual: <https://mrtrix.readthedocs.io/en/0.3.16/workflows/fixel_based_analysis.html>

More updated manual (on multi-tisssue CSD: preprocessing, CSD, and FBA): <https://mrtrix.readthedocs.io/en/latest/fixel_based_analysis/mt_fibre_density_cross-section.html>

Steps:

1. Generate response function estimates using multi-tissue CSD
   1. Co-registration t1w and t2 FLAIR to dwi image
   2. Generate 4tt image
   3. Edit pathological image to create 5ttimage
   4. Generate response function (odf.txt file)
2. Compute a group average response function of each tissue
3. Create FOD images (wm, gm, csf)
   1. Upsample dwi image
   2. Upsample brain mask images
   3. FOD estimation (multi-tissue SD)
4. Joint bias field correction and intensity normalistion
5. Generate FOD population template
6. Register all subjects to FOD template
   1. Register to FOD template
   2. Warp mask to template
   3. Compute intersection of masks in template space

On the dementia vm (8 CPU cores with 8 GB RAM), steps 1-6 take approximately ~34 hours with 36 participants. All timings are elapsed from 1 participant, unless otherwise stated.

1. **Generate response function estimation with multi-tissue constrained spherical deconvolution (CSD) (per participant)**

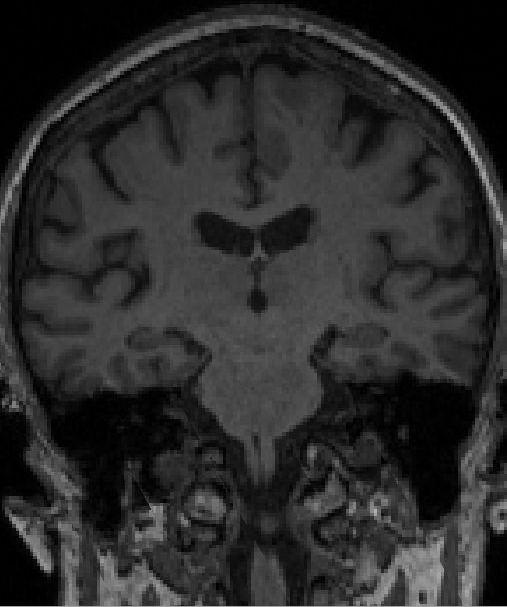
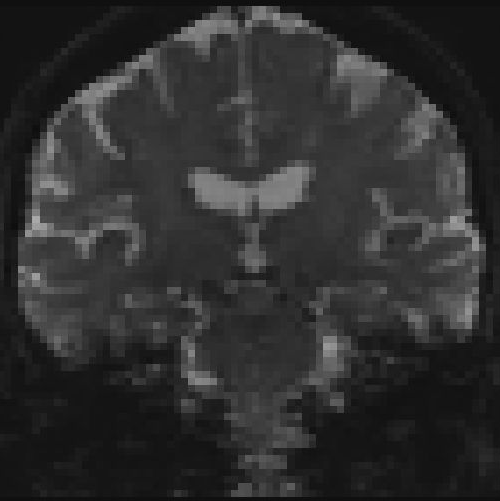
We want to estimate the multi-tissue orientation distribution function (ODF) between the participant groups. Since we have multiple shells, we are separating compartments for each tissue type, which includes a maps of the WM, GM, and CSF signal contributions to dwi data. Three main steps (a, b, c) are carried out in order to do this.

There is an option in that choosing only 1 single subject per each group for the estimate response should be OK. This is because the shape of the estimated white matter response function varies little across subjects. But we can also take the **group average response function (which is what my script does)**, after taking the response function for every participant. So, perform all of these steps on every participant per each group, then take the average response per group.

1. **Co-registration of t1w and t2 FLAIR with dwi image**

Linear registration with 6 degrees of freedom (dof) are used. Do this registration with both the t1w and t2 FLAIR images to the dwi image (first b0 vol in dwi sequence - captured as the ‘bestb0’ from the previous script).

Command: -flirt (fsl)

 **+** 

T1w dwi (b0)

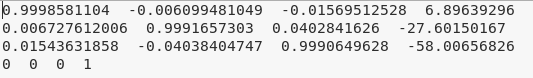


Co-registered (t1\_flirt)

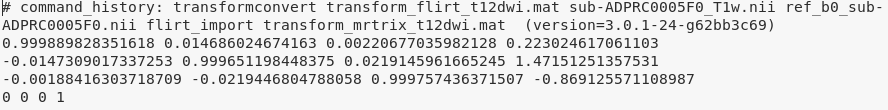
Once you have applied co-registration using FLIRT, you need to create linear transform matrices to apply to the images. This is because by default, FLIRT applies a direct *resampling* of the input image to match the target image, which is unnecessary. It is better to have flirt provide the calculated transformation matrix, then *apply* that linear transformation to the T1 image (either using flirt again, or combination of transformconvert and mrtransform), such that the image header transformation is altered but the image grid / intensities are not.

Command: transformconvert

mrtransform



*transform\_flirt\_t12dwi.mat*



*transform\_mrtrix\_t12dwi.mat*

See posts on mrtrix community forum for more info: <https://community.mrtrix.org/t/5ttgen-error/1138>; <https://community.mrtrix.org/t/5ttgen-waiitng-for-creation-of-new-file/1044/2>

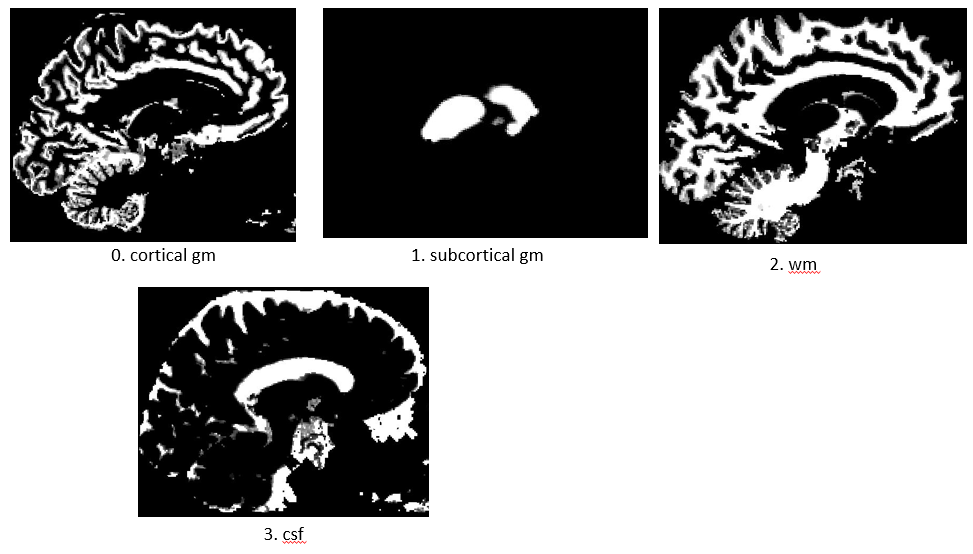
In addition, the provided T2 FLAIR image must be resampled to match the T1 image: <https://community.mrtrix.org/t/5ttgen-provided-t2-image-does-not-match-input-t1-image/1204>

Elapsed time: 59 sec

1. **Generate a ‘Five-Tissue-Type’ (5tt) image**

The 5 tissues include: cortical gm, subcortical gm, wm, csf, pathological tissue(\*). Inputs to the 5ttgen include the t1w, t2 FLAIR image, the brain mask (generated from the preprocessing script), and the preprocessed/segmented t2 FLAIR (see below) as the pathological tissue. The 5ttgen script using the fsl algorithm interfaces with FSL to generate the necessary image data from the raw T1 and T2 FLAIR image, using BET, FAST and FIRST.

Command: 5ttgen -fsl



1. Cortical grey matter
2. Sub-cortical grey matter
3. White matter
4. CSF
5. Pathological tissue\*

The highlighted white areas represent the specific tissue type.

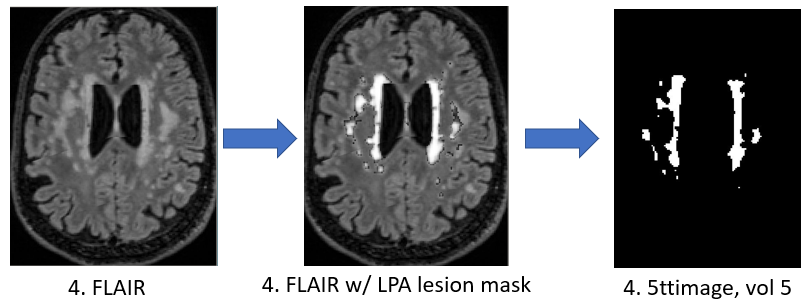
Elapsed time: 12 min 52 sec

1. **Generate a ‘Five-Tissue-Type’ (5tt) image**

\*To add in the pathological tissue (in this case, with the white matter hyperintensities (WMHs) using the t2 FLAIR which is preprocessed and segmented with the lesions as a mask), you will need to edit and add in the image to the 5tt image. Using the toolbox Lesion Segmentation Tool (LST) under SPM, the lesion prediction algorithm (LPA) has done the preprocessing and segmentation of the WMHs. Apply same linear registration steps to the pathological image, as the 4ttimage above

Command: 5ttedit

mrtransform



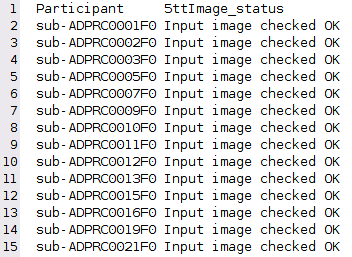
More information about dealing with WMHs from the MRtrix forum: <https://community.mrtrix.org/t/5ttgen-recommended-approach-to-dealing-with-white-matter-hyperintensities-wmhs-for-act/3753/3>

Maybe better to use 5ttgen -hsvs algorithm instead – combination of fsl and FreeSurfer tools. Hsvs = hybrid surface and volume segmentation.

<https://community.mrtrix.org/t/5ttgen-hsvs-template-option/3593/7>

Elapsed time: 2 sec

You can quickly and easily evaluate each 5tt image by running the 5ttcheck command; this will thoroughly check that the images conform to the expected ACT five-tissue-type (5TT) format. My script does this with the **FiveTTImageCheck.m** function, and the output is a text file (5ttImageCheck.txt), which displays the status of the 5tt image for all participants.



1. **Generate response function estimation**

This will be a text file for each of the 3 tissue types. Use the brain mask again to provide and initial mask for response voxel selection.

Command: dwi2response msmt\_5tt

Elapsed time: 36 sec

1. **Compute a group average response function of each tissue**

Take the group average response function per each of the 3 tissues (wm, gm, csf).

%Sample input: responseFunctionCSF

responseFunctionGM 🡪 folders holding all participant response files

responseFunctionWM

Command: responsemean

%Sample output: group\_average\_responseCSF.txt

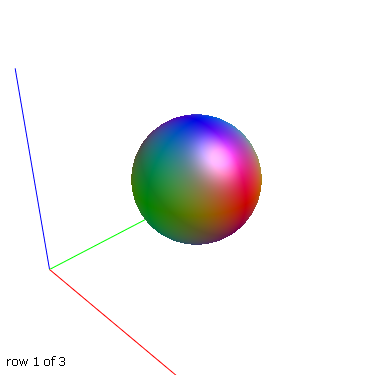
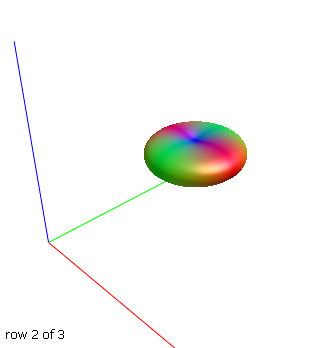
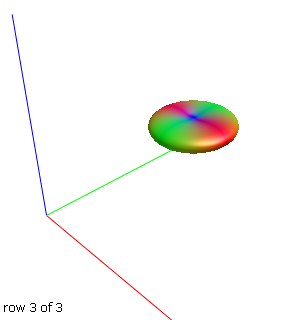
group\_average\_responseGM.txt

group\_average\_responseWM.txt

For a sanity check: you can inspect the basis functions for each tissue (wm, gm, csf) generated at each shell.

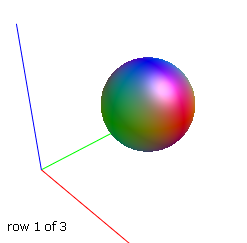
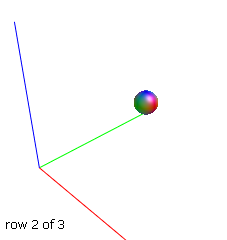
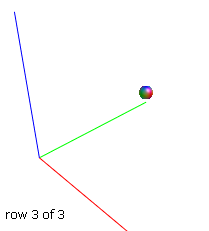
Command: shview

WM (more anisotropic):

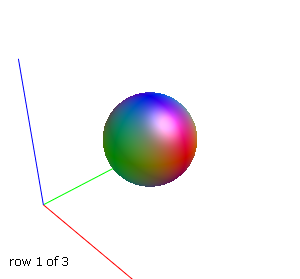
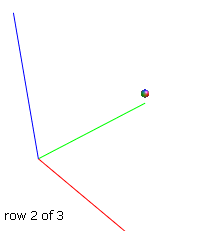
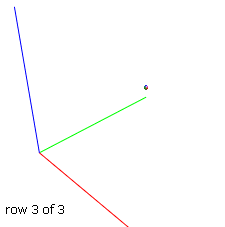
B0 B1000 B2000

GM (more isoptropic):

B0 B1000 B2000

CSF (even more isotropic):

B0 B1000 B2000

Elapsed time: 1.5 sec

1. **Create FOD images (wm, gm, csf)**

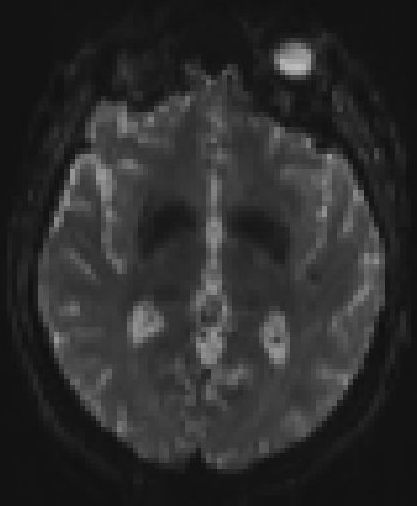
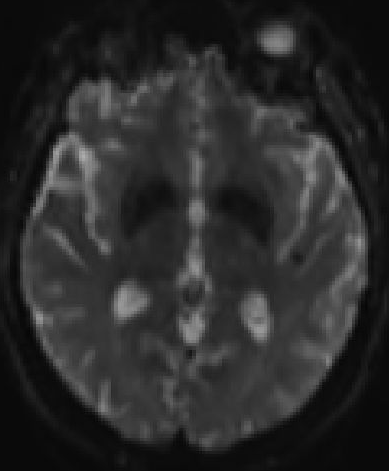
Using the response estimate functions, we will create the FOD images of each tissue.

1. **Upsample DW images**

Upsampling DWI data *before* computing FODs increases anatomical contrast and improves downstream template building, registration, tractography and statistics.

With this step, you have the option to modify the grid of an image without interpolation (cropping or padding) or by regridding (this is what my script does) to an image grid with modified orientation, location and or resolution. The image content remains in place in real world coordinates.

Command: mrgrid -regrid

Pre-upsample dwi data Gaussian smoothing (-regrid)

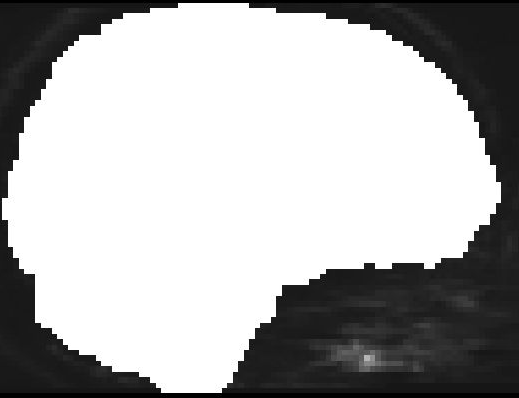
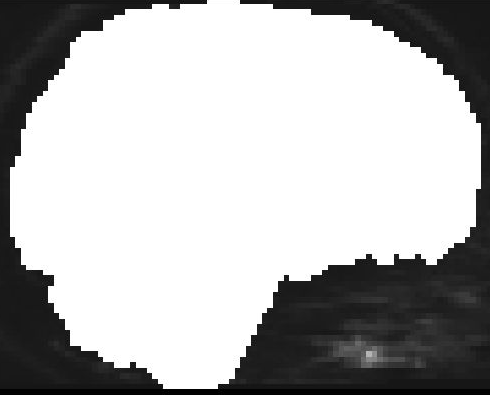
The -regrid option performs changes of the voxel grid that require interpolation of the image such as changing the resolution or location and orientation of the voxel grid. If the image is down-sampled, the appropriate smoothing is automatically applied using Gaussian smoothing unless nearest neighbour interpolation is selected or oversample is changed explicitly. The resolution can only be changed for spatial dimensions. It is recommended to upsample to an isotropic voxel size of 1.25 mm for human brains (if your original resolution is already higher, you can skip this step).

Elapsed time: 1 min 10 sec

1. **Upsample brain mask images**

Compute a whole brain mask from the upsampled DW images. We will use BET again for this. Since topup has already been applied in the previous preprocessing script, we will just use the first b0 (ref\_b0) as an input to create the new upsampled brain mask.

Command: bet threshold FA set at 0.2

brain mask (no manual corrections) upsampled mask (less non-brain parts covered)

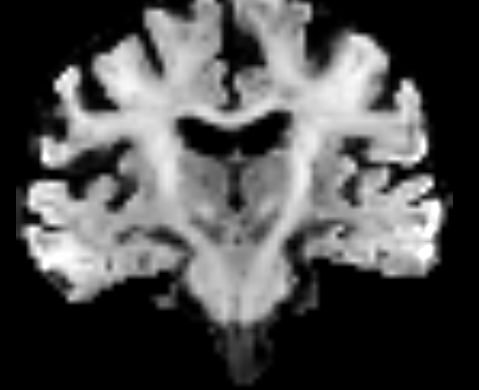
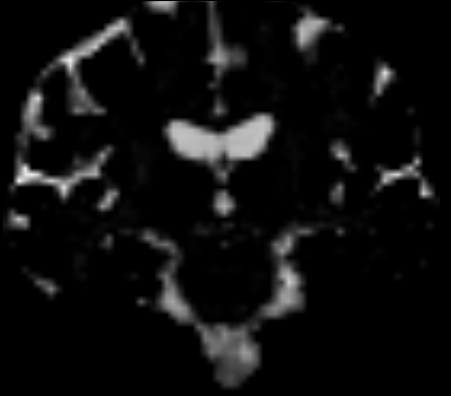
\*note that upsampled images take a lot of space – 2.6 GB for main dwi data

Elapsed time: 3 sec

1. **FOD estimation (multi-tissue SD)**

Use the group average response function estimates calculated from the previous step for this.

Command: dwi2fod msmt\_csd

wmfod.mif gmfod.mif csffod.mif

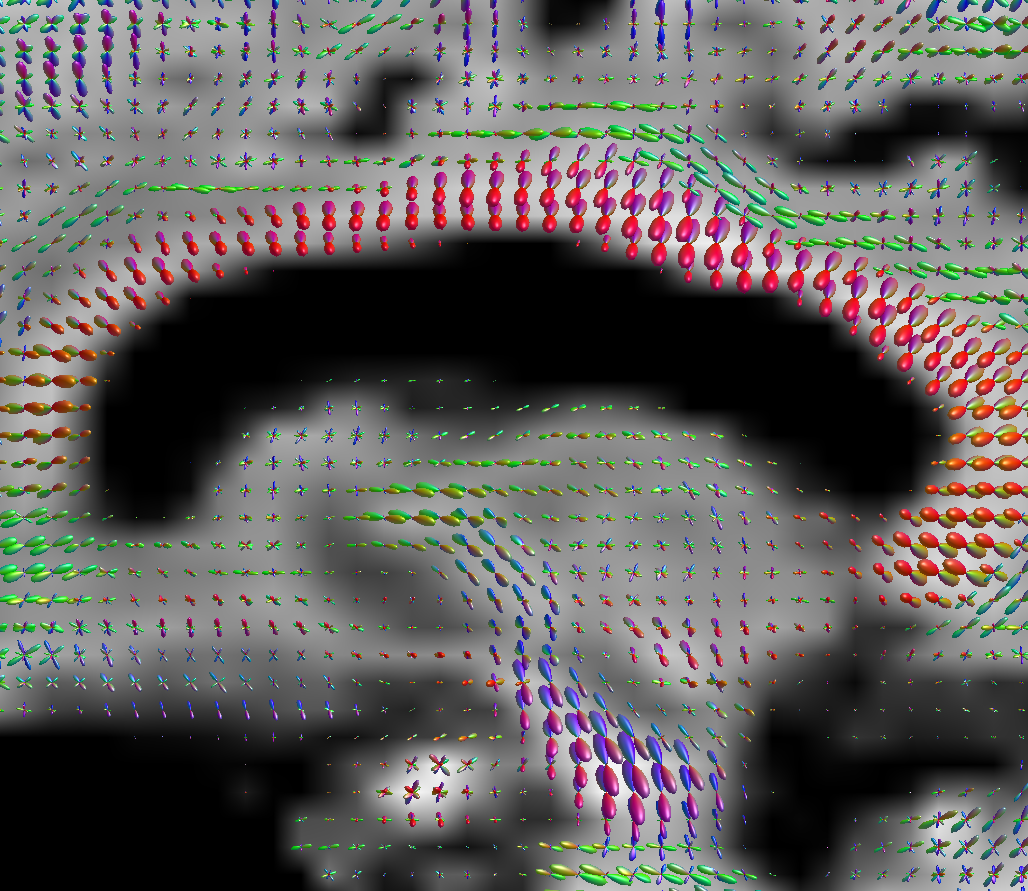
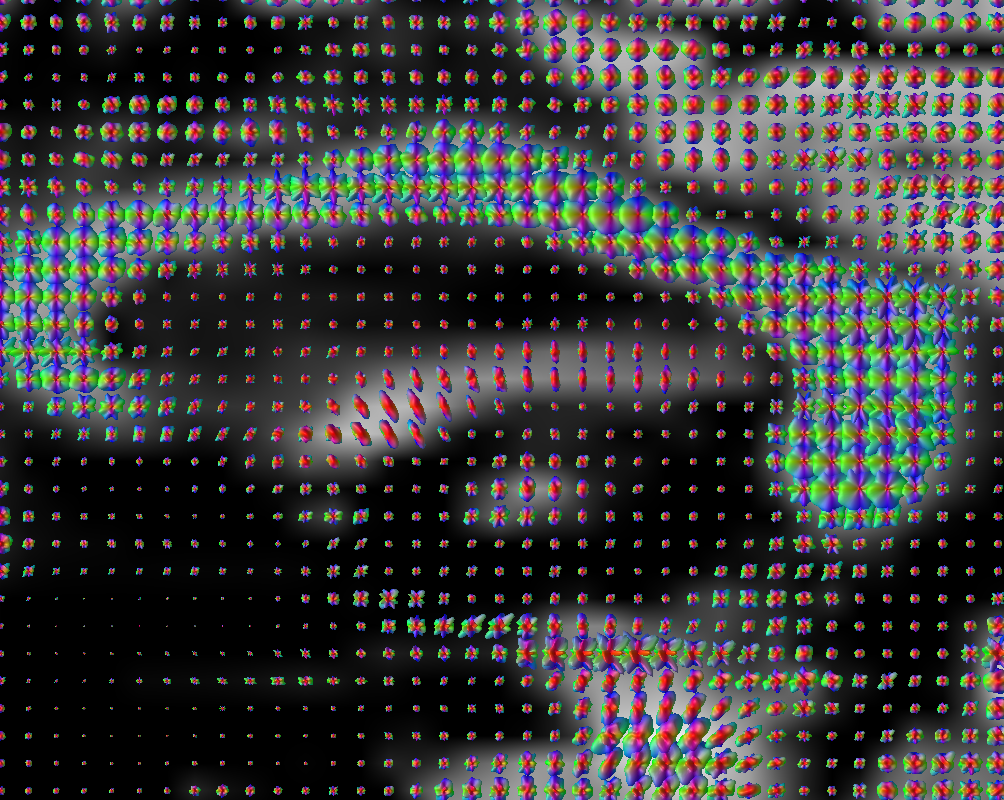


Image of the FOD loaded onto the wmfod template of a participant.

Elapsed time: 11 min

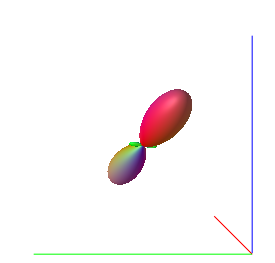
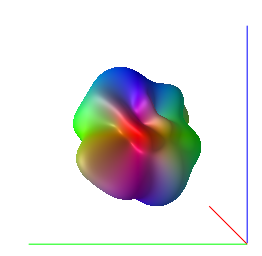
You can also compare this with to the diffusion data before spherical deconvolution was applied to it.

Command: amp2sh



Preprocessed dwi with no spherical deconvolution applied.

You can view a stark difference within *a same, single ODF* as well.

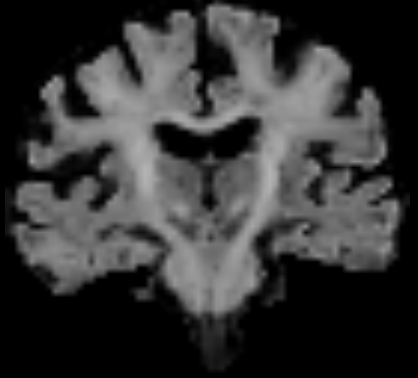
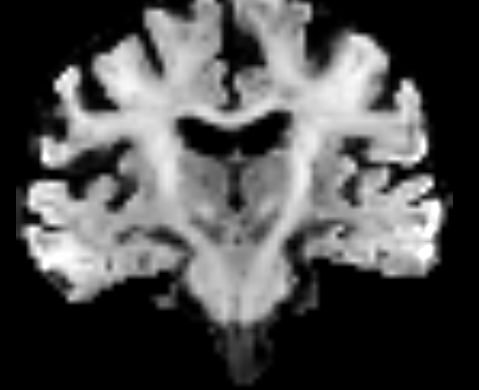
 

Spherical deconvolution no spherical deconvolution

1. **Joint bias field correction and intensity normalisation**

Perform joint bias field correction and global intensity normalisation of the multi-tissue compartment parameters. This command takes as input any number of tissue components (e.g. from multi-tissue CSD) and outputs corresponding normalised tissue components corrected for the effects of (residual) intensity inhomogeneities. Intensity normalisation is performed by optimising the voxel-wise sum of all tissue compartments towards a constant value, under constraints of spatial smoothness (polynomial basis of a given order). Different to the Raffelt et al. 2017 abstract, this algorithm performs this task in the log-domain instead, with added gradual outlier rejection, different handling of the balancing factors between tissue compartments and a different iteration structure. This step is crucial for the FBA pipeline. Use the upsampled masks in this command. This was also done with a similar cohort (HC, MCI, and AD) in Mito et al. (2018) *Fibre-specific white matter reductions in Alzheimer’s disease and mild cognitive impairment*.

Command: mtnormalise

viewed in *mrview*

wmfod.mif normalised (wmfod\_norm.mif)

  viewed in *fsleyes*

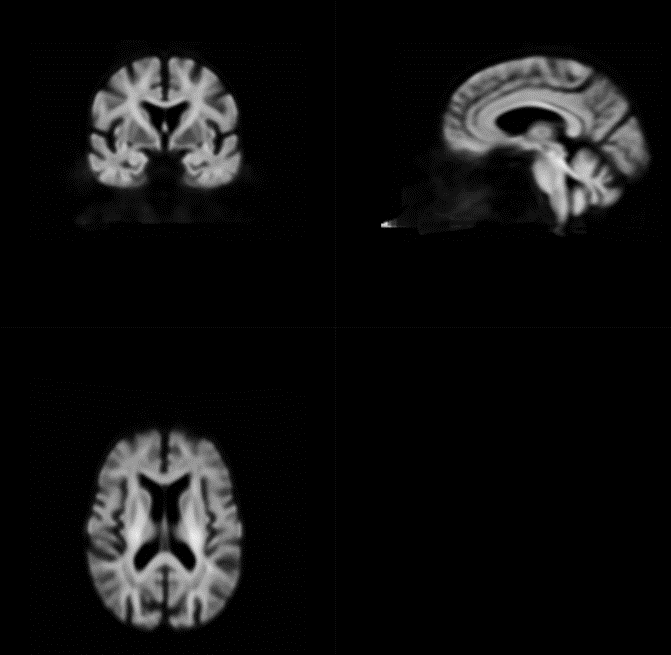
wmfod.mif normalised (wmfod\_norm.mif)

Elapsed time: 14 sec

1. **Generate FOD population template**

Generate a study-specific unbiased FOD template. Generating a population template is one of the most time consuming steps in a fixel-based analysis. If you have a very large number of subjects in your study, you can opt to create the template from a limited subset of 30-40 individuals. Typically, subjects are chosen so the generated template is representative of your population (e.g. similar number of patients and controls, though avoid patients with excessive abnormalities compared to the rest of the population). To build a template, you will use all FOD images, which are segregated into separate folder (e.g. wmFODimages) and put a set of corresponding mask images (with the same prefix as the FOD images) in another folder (using masks speeds up registration significantly).

You should pick around ~40 participants total to compose the population template. For my interested study, for example, I would run 10 participants from each of the 4 groups (HC, SCD, MCI, AD) to create the template to represent them all. Note that when selecting from the clinical group (e.g. AD), you need to be wary *not* to select AD participants who have a lot of degeneration. You should select participants who are ‘representative’ of each group.



wmfod\_template.mif (with 36 participants)

Elapsed time: 33 hours and 16 min with 36 participants.

\*\*If 2 people on the dementia vm (Nikki and I) are simultanesouly running intensive mrtrix commands, then the processing time would increase to 55 hours with 36 participants.

1. **Register all subjects to FOD template**
2. **Register all subjects to FOD template**

Command: mrregister

Elapsed time: 20 min

1. **Warp mask to template**

Different subjects have different brain coverage. To ensure subsequent analysis is performed in voxels that contain data from all subjects, we warp all subject masks into template space and compute the template mask as the intersection of all subject masks in template space.

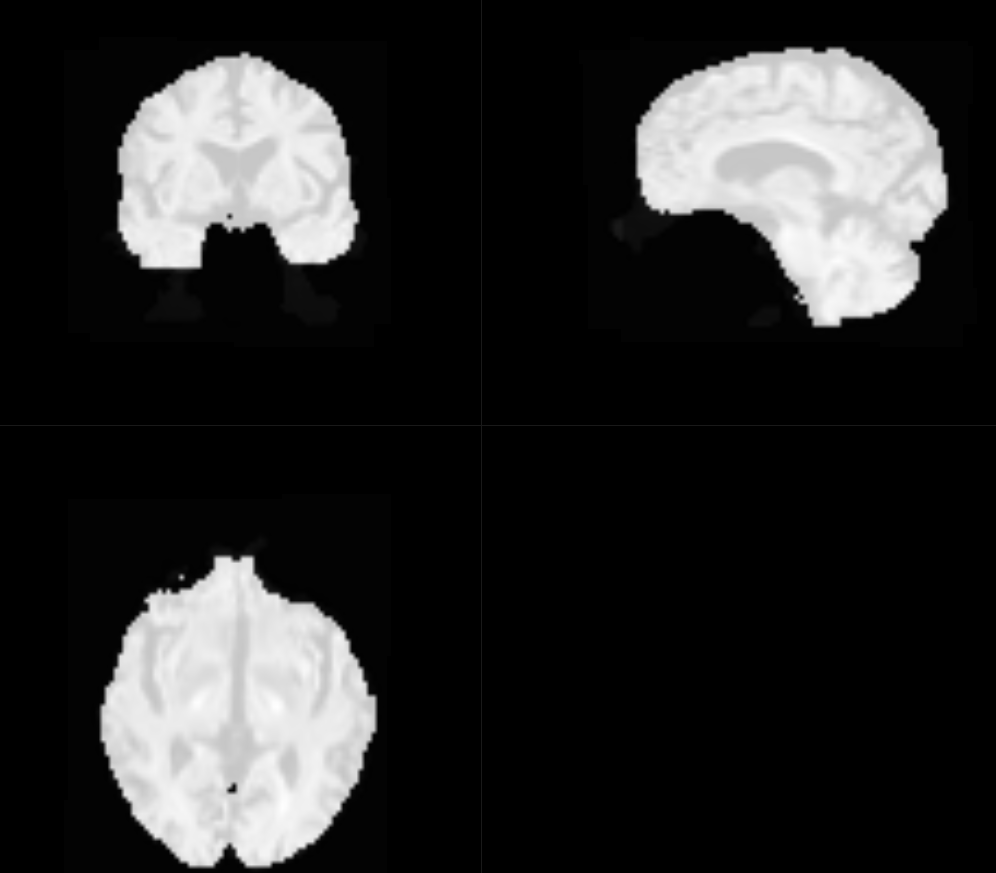
Command: mrtransform

Elapsed time: 3 sec

1. **Compute intersection of masks in template space**

Compute the template mask as the intersection of all warped masks.

Command: mrmath



template\_mask\_intersection.mif

Elapsed time: 11 sec for 36 participants

References

Mito, R., Raffelt, D., Dhollander, T., Vaughan, D. N., Tournier, J. D., Salvado, O., … Connelly, A. (2018). Fibre-specific white matter reductions in Alzheimer’s disease and mild cognitive impairment. *Brain*, *141*(3), 888–902. https://doi.org/10.1093/brain/awx355