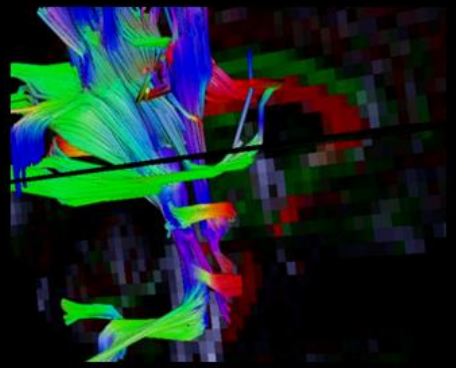
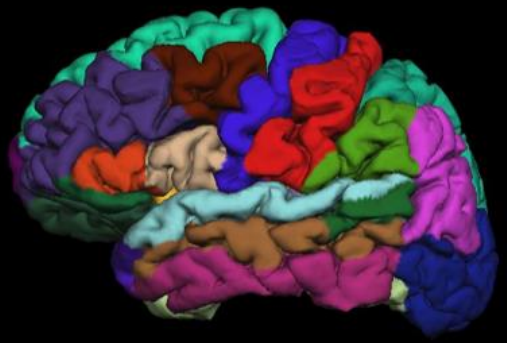
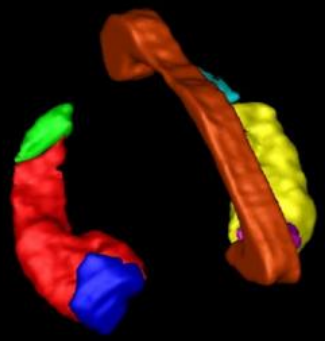
Handbook of Structural Brain MRI Analysis

By Jerome J Maller

With contributions from Rodney Anderson, Melissa Kirkovski, Phillip Law, Kerstin Pannek, Caley Sullivan, & Jonatan Ottino-González







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List of abbreviations

ASL=Arterial spin labelling; BVAL=Gradient value table; BVEC=Gradient vector table; CSD=Constrained Spherical Deconvolution; CSF=Cerebrospinal fluid; DICOM= Digital imaging and communications in medicine; DTI=Diffusion tensor imaging; DWI=Diffusion weighted imaging; FLAIR=Fluid attenuated inversion recovery; FSL=FMRIB Software Library; GM=Gray matter; GUI=Graphical user interface; ICV=Intracranial volume; MPRAGE=Magnetization prepared rapid acquisition gradient recall echo; MP2RAGE=MPRAGE with different inversion times; MRI=Magnetic resonance imaging; NIfTI= Neuroimaging informatics technology initiative; ROI=Region of interest; SPGR=Spoiled gradient; SWI=Susceptibility weighted imaging; TBV=Total brain volume; VM=Virtual machine; WM=White matter

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Preface

Have you ever spent hours going through long software manuals and endless forums trying to work out how to analyze structural MRI scans? We used to do that too, so over time we have documented how to use a wide variety of software packages so we could conduct a large variety of analyses. We have also received training in some of these packages which has enhanced our competence in these programs.

We don't claim to know how to use every module of every program, but we know how to use them to conduct most of the analyses that you are likely to want to run. We have utilized what we've learnt by publishing dozens of peer-reviewed papers but also passing on this skillset to many students and research groups around the world.

Chapter 1 of this Handbook focusses on step-by-step instructions on how to use various packages to process structural MRI data.

Chapter 2 of this Handbook focusses on white matter anatomy and the placement of region-of-interest seeds using diffusion MRI data to perform tractography.

Chapter 3 of this Handbook describes how to setup a virtual machine with software necessary to process structural MRI data.

CHAPTER 1

Introduction

Whilst there are many neuroimaging packages available, we have found that those we describe in this handbook have collectively been sufficient to analyze structural MRI scans. However, we encourage you to seek alternative packages if you do not find these ones adequate to accomplish your tasks.

In this handbook, we provide step-by-step instructions in how to use your data to conduct the analyses you require. The focus of this handbook is T1-weighted and Diffusion data acquisition and analysis.

Topics covered in this handbook

- Minimum computer specifications
- Installation of these packages
- T1-weighted and Diffusion Imaging
- Other structural sequences to consider
- What is T1-weighted useful for?
- What is diffusion imaging useful for?
- Issues to consider before acquisition
- Software
- Pipelines/scripts to save you time

Minimum computer specifications

Windows (pc), Linux/Unix, Macintosh/Apple

The bare minimum specifications are Core2Duo CPU with 4GB RAM, but, of course, it's best to have the fastest CPU with multiple cores and the most amount of RAM you can access. Same goes for the hard drive capacity: at least 250GB but the bigger the better! SSD (Sold State Drive) will also speed things up a lot.

Although you could have a pc running Windows and Linux through a virtual machine (VM), this is not an ideal setup as VMs are notoriously slow. By contrast, a dual-booting pc would be far preferable (that is, Windows and Linux on the same pc with a simple boot menu when you turn on the pc). If you have a Mac/Apple, then the best setup is to run the Mac OS

natively and then install dual-boot software such as Bootcamp or Parallels and then install Windows onto that. Note the Mac/Apple is essentially running on Linux, so you simply open a terminal and you immediately have a type of Linux running natively – very handy. Also, similar, Mac and Linux are not the same.

Of course, if you have access to a supercomputer/cluster via the university then you could run all of your analyses on it, but note that those huge mainframes sometimes have problems and need to shut down to reboot which may interfere with your batched analyses.

Note that not all MRI analysis packages are multi-threaded i.e. they do not use more than one CPU or core at a time, whereas those that are multithreaded can utilise as many CPUs and cores as you have available on the computer. Again, very handy.

Installation of these packages

Most of these packages are simple executable (.exe) files than run natively on Windows. However, some run only on Linux/Mac (e.g. FSL, FreeSurfer) so need to be installed/compiled with the correct paths and calling the correct files; sometimes you will need to install updates via apt-get (depending on your operating system flavour). Some (e.g. FreeSurfer) run in separate shells (e.g. tcsh or csh). If you are a Linux/Mac user, you may find it convenient to run the Windows packages via VMs, or use Windows emulators such as wine.

T1-weighted and Diffusion Tensor Imaging (DTI)

Without going into the physics of it, T1-weighted is essentially the sort of scan you acquire if you want data with good gray/white matter (and cerebrospinal fluid; CSF) contrast. The word 'contrast' in this context means the clarity of the gray/white matter (and CSF) borders. This is very important when segmenting regions of interest (ROIs) as they rely on clear gray/white matter and CSF borders. However, the clarity of these borders is also important in the clinical context as a lack of contrast between gray/white matter can sometimes indicate poor structural integrity as occasionally seen, for example, after traumatic brain injury (TBI). A type of MRI sequence that investigates this more thoroughly is referred to as MTR (magnetisation transfer ratio), but that's beyond the scope of this handbook. Note as well that MP2RAGE (T1-weighted with multiple TIs) is not suitable for most of the software we go through in this Handbook.

Diffusion tensor imaging (DTI) was released in 1994 by Basser and colleagues but not utilised widely until processing power dramatically increased in the early 2000's (see our article reviewing this, Maller et al., 2010). Filler (2009) provides a comprehensive description of its origins. It is based upon DWI (diffusion weighted imaging) but instead of 2-dimensional data, DTI acquires 3-dimensional data (or 4-dimensional, depending on how you look at it). Essentially, DTI produces data based upon Brownian movement i.e. the movement of water between microscopic white matter fibres based on heat. Water will move in a straight line that is directly parallel to the direction of the white matter when the white matter is 'healthy', but the water will move in different directions when the white matter's "integrity" is compromised. As an analogy, think of a bowl of spaghetti… Another useful analogy is a hose with water flowing through it: when the hose is new and has no leaks the water will move parallel to the hose, that is, in an anisotropic direction. Isotropic means the water is moving equally in all directions, hence, anisotropic means in one single direction. When a white matter bundle is damaged, it may be compressed or stretched, leading to leaking in that white matter bundle – this will cause the water flowing within it to leak out the side or its flow to be obstructed, leading to the water flow no longer being parallel to the shape of the hose i.e. the white matter bundle. When the water flows parallel to the hose in one

direction, the 'fractional anisotropy' (FA) has a value of 1; when the hose is leaking and the water flows in all directions equally (e.g. up, down, out the sides), the FA is closer to 0.

The basic physics of NMR is nicely demonstrated here:
http://www.youtube.com/watch?v=1OrPCNVSA4o

Other structural sequences to consider

- T2-weighted, T2-FLAIR, susceptibility-weighted imaging (SWI), arterial spin labelling (ASL)
- T2-weighted is a scan acquired when you want to look at possible pathology, for example, white matter hyperintensities (WMHs), strokes, plaques, tumours, cysts, etc. In normal ageing, WMHs first appear lining the inner border of the lateral ventricles, referred to as periventricular leukomalacia (PVL), which often begins after the age of 40. Large WMHs within cortical or subcortical matter are often representative of pathology, although collections of WMH 'dots' can be as well (sometimes referred to as 'punctate'). For example, these can be representative of WM disease sometimes related to late-onset major depression. The rule-of-thumb is that one WMH per decade is within acceptable limits. T2-FLAIR is like a T2-weighted scan but the colour of the CSF is inverted. That is, instead of CSF appearing white, it is black on a FLAIR scan. Hence, FLAIR stands for FLuid Attenuated Inversion Recovery. SWI/SWAN focusses on the susceptibility of iron and myelination, hence, it is "susceptible" to alterations in iron and myelination. ASL is a sequence used to assess absolute measures of blood flow, rather than relative blood flow such as in dynamic contrast enhanced MRI (DCE-MRI). However, ASL is associated with quite high SAR (specific absorption rate), so sequences cannot be too long.

What is T1-weighted useful for?

- T1-weighted data has a variety of uses, but in the research context, it is used mostly for investigating gray matter (GM), white matter (WM), CSF, and atrophy.
- Volumetrics. GM+WM+CSF=intracranial volume (ICV). Total brain volume (TBV)/ICV = opposite of CSF/ICV. That is, TBV/ICV is a measure of how much of the cranial vault contains brain matter, whereas the opposite (CSF/ICV) is a measure of how much of the cranial vault is not brain matter; hence, CSF/ICV is sometimes referred to as a measure of atrophy. Note that some programs (e.g. FreeSurfer) yield data of TBV and ICV but the TBV does not include blood vessels, choroid plexus, non-brain matter constituents, hence, TBV/ICV in those programs will be smaller than programs that assume everything within the cranial vault is either brain matter or CSF (e.g. FSL's FAST module, or SPM's segment routine).

- Thickness. Cortical thickness is representative of how thick the cortical gray matter is. Essentially, volume = thickness x area.
- Curvature/shape analysis can also be accomplished with T1-weighted data, but its meaning is less straight forward.

What is DTI useful for?

- WM Structural "integrity" and directionality
- FA, L1 (λ1), L2 (λ2), L3 (λ3), MD, RD. Figure 1 illustrates what the eigenvalues (λ) represent (from Maller et al., 2010).
- Tractography of WM bundles
- Connectivity
- Fibre density

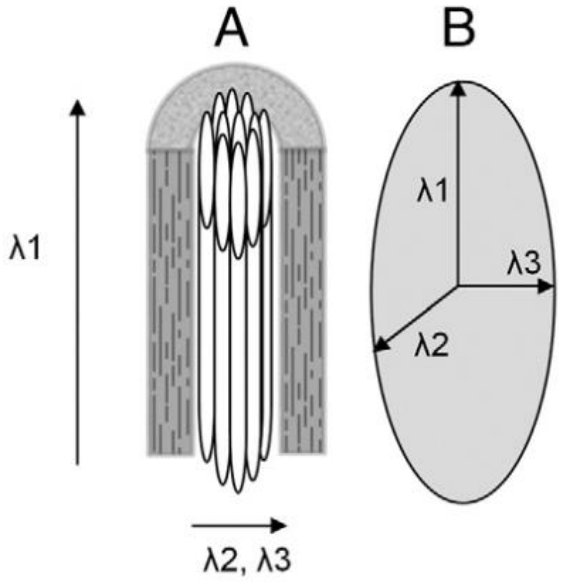

Figure 1. Illustration of principles involved in diffusion tensor imaging. (A) Microtubules of an axon encased in myelin sheath. (B) Eigenvectors represent main axes directions. Water diffusion is restricted along the longitudinal axis (λ1) and/or perpendicular to the longitudinal axis of the axons (radial diffusion, eigenvalues λ2, λ3) due to axonal microstructure.

Note that most diffusion imaging is now not considered to be diffusion 'tensor' imaging (DTI), as the word 'tensor' implies that there is one white matter bundle traversing each WM voxel. By contrast, we know that around 90% of white matter voxels contain at least 2 WM bundles that cross or kiss (hence, the terms 'crossing fibres' and 'kissing fibres'), so we now refer to this technique as dMRI (diffusion MRI). Different variations of dMRI pulse sequences (and analysis techniques) exist, such as HARDI (high angular resolution diffusion imaging), DSI (diffusion spectrum imaging), ball-and-stick, and q-ball imaging.

Issues to consider before acquisition

- Scanner type (brand, Tesla, gradients, acquisition pulse sequences installed onto the console) and reconstruction algorithms available on the workstation
- IR-SPGR, MPRAGE (Magnetization Prepared Rapid Acquisition GRE (3D Inversion Recovery)), MP2RAGE (MPRAGE with multiple TIs; MPRAGE and MP2RAGE are used with GE, Siemens and Philips scanners)
- DTI, RESOLVE (used for high-resolution q-ball scanning that takes into account crossing fibres), FOCUS/ZOOM (used for high-resolution imaging with reduced field of view)
- Silenz (GE) and PETRA (Siemens) – these are T1-weighted sequences that are less than 10db above ambient noise level; some are based on the DUFUS and RUFUS techniques
- Fast scanning/GRAPPA, SENSE, SMASH, PILS etc. These are usually parallel imaging methods to reduce scanning times although they can also reduce SNR. MRI is always about give-and-take in terms of SNR. However, multiband imaging retains most SNR
- Fat suppression and chemical shift
- NEX/number of averages – the more averages, the higher the SNR
- Number of channels (receive coils i.e. antennae) – the more channels the better the resolution but also sometimes lower SNR depending on coil configurations
- Resolution, repetition time (TR), echo time (TE), slice thickness, SNR – you can alter TE, for example, but it must not change between subjects, slice thickness should be less than 1.5mm for T1-weighted and no more than 3mm for dMRI
- dMRI number of directions: more directions will yield more accurate tractography (but not necessarily more accurate FA estimation), and more b-zero (non-diffusion T2-based) volumes will yield better eddy current and movement correction especially when they are interleaved
- Anterior commissure-posterior commissure (AC-PC) alignment – important because if you normalize scans to a template then this will make the analyses more accurate with higher SNR as the scans will not have to be reorientated to match the template
- Length of scanning period and each sequence – some people will not be able to remain very still for long periods of time, plus longer scans may deposit more SAR
- Volume of scanner/patient comfort/movement – wide bore scanners are preferable for patient comfort and the ability to put more equipment into the scanner; patient movement will reduce the quality of the scans
- Knowing for sure which is right and which is left – sometimes software can flip the MRI scan, so it is useful to use fiducials (markers) that show on the scan so that you can always be sure which side is left and which side is right. For example, you can tape a vitamin E pill to the forehead above the right of the subject's eyebrow before they are scanned so that the right-side of the head can always been confirmed.
- CAIPI (Controlled Aliasing in Parallel Imaging) reduces the g-penalty factor
- Compressed Sensing decreases scanning time whilst also increasing SNR

Software covered in this handbook

- MRIcro (visualization, preprocessing, manual segmentation, lesion overlay analysis; http://www.mccauslandcenter.sc.edu/mricro/)
- MRIcron and dcm2nii (format conversion; http://www.mccauslandcenter.sc.edu/mricro/)
- MRIreg (http://www.mccauslandcenter.sc.edu/mricro/)
- BEAM (http://www.clinicalresearcher.org/software.htm)
- MRIconvert (slice conversion http://lcni.uoregon.edu/~jolinda/MRIConvert/)
- ITK-SNAP (T1-weighted visualization http://www.itksnap.org/pmwiki/pmwiki.php)
- Not free: Analyze® (http://www.analyzedirect.com/). Analyze® is the gold standard in manual segmentation software. There is a large variety of freeware that can be used to accomplish a similar task, but Analyze® is established as the most comprehensive and user-friendly package for manual ROI segmentation. It has a number of modules other than the ROI module, but these are beyond the scope of this handbook.
- FreeSurfer (T1-weighted automated segmentation) and QDEC (FreeSurfer GUI for analysis http://freesurfer.net/)
- SPIN (slice conversion and SWI editing http://www.mrimaging.com/category.103.html)
- DICOMsort (multi-platform, Linux, Mac, Windows, for sorting DICOM images by any of the values in the DICOM header. http://www.dicomsort.com)
- FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/): Brain Extraction Tool (BET), Fully Automated Segmentation Tool (FAST), Tract Based Spatial Statistics (TBSS)
- DTIstudio (deterministic tractography https://www.dtistudio.org)
- MedINRIA (deterministic tractography http://www-sop.inria.fr/asclepios/software/MedINRIA/)
- MRtrix (deterministic and probabilistic tractography http://www.brain.org.au/software/)
- SPM (for normalization and GM/WM/CSF segmentation http://www.fil.ion.ucl.ac.uk/spm/)

Pipelines/scripts will save you time

Once you learn how to use a program, particularly those installed in Linux/Unix/Mac, it will save you substantial time (and effort) by writing scripts to conduct multiple-step analyses for multiple scans. You just need to be confident in your scripting! An extreme example of this is referred to as a "pipeline". A good example of utilising both T1-weighted data and DTI data in one 'program' is referred to as the "Connectome pipeline" and another is TRACULA (TRActs Constrained by UnderLying Anatomy; http://surfer.nmr.mgh.harvard.edu/fswiki/Tracula), although they may rely on you first processing the T1-weighted data through FreeSurfer and then inputting the output of that process along with the DTI dataset into the pipeline. However, this can be scripted.

WM bundles: getting to know white matter anatomy

A widely used reference for this is the White Matter Atlas by Mori et al. (2005), but there are many other white matter atlases/articles well worth considering as well, including, Wycoco et al. (2013). We encourage you to search on PubMed using keywords such as "white matter atlas" (http://www.ncbi.nlm.nih.gov/pubmed/?term=white+matter+atlas). In Chapter 2 of this handbook we have included some general advice on where to place initial seed points for a variety of major WM bundles.

Index of software and instructions

1. How to convert individual dicoms (slices) into an analyze volume (.hdr + .img): MRIcro

1. Open MRIcro (Rorden and Brett, 2000)
2. "**Import**" → conversion wizard (bottom option)
3. "**OK**" (conversion options)
4. "**OK**" (flip)
5. A box then appears: select the very first dicom (i.e. the one with the smallest number at the end of its filename: this should be the first [top] file)
6. "**OK**" (call is 'n', the default name)
7. At this stage, all dicoms will now be converted to Analyze format. So now we need to reslice (slice-and-dice) and have the scan in the correct orientation. To do this:
8. Open the Analyze file (Open, then select the file)
9. "**Etc**" → convert anisotropic to isotropic
10. "**OK**"
11. Open the new isotropic file
12. Reslice it to 1mm cubed. To do this, look at the top left of the screen and note the length of the x and y dimensions (e.g. 0.94mm) and enter that box into the reslicing box:
13. "**etc**" → "**rescale**" and enter that number (e.g. 0.94)
14. "**OK**"
15. Open the newly sliced Analyze file
16. Now we need the scan in the correct orientation; that , bottom left should be axial, top left should be coronal and top right should be sagittal (nose pointing to the right of the screen). To do this:
17. Which orientation is currently displayed when you select Axial (horizontal)? E.g. if sagittal appears with nose to the right, then do the following:
18. **File → Save As →** change '**axial**' to '**sagittal right**', then select "**Save Intel**"
19. "**OK**"
20. Note: need to close MRIcro at this stage, and then reopen it
21. If you need to normalize, then you will need to do the following
22. Reorientate the scan parallel to the AC-PC, and
23. Set the AC as 0,0,0 in the "Origin" column (as in Figure 2)

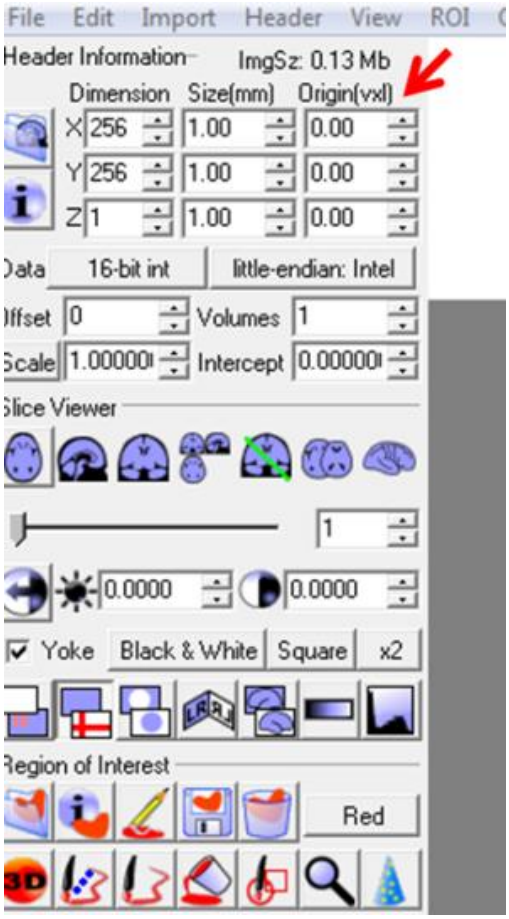

Figure 2. MRIcro interface. Red arrow indicates the column where you reset the AC to be 0,0,0

24. **Save As → Save Intel**

2. Steps to convert dicoms to Analyze format (.img + .hdr) with dcm2nii

1. Install MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/install.html , "download the zipped archive"; Rorden et al., 2007). Note that the latest version of dcm2nii 9called dcm2niix) is within MRIcroGL (http://www.cabiatl.com/mricrogl/)
2. Navigate to the following directory: C:\Program Files (x86)\MRIcroN
3. Right-click on the following file: dcm2niigui.exe
4. Select "**Create shortcut**". There will now be a link on the Desktop to this file
5. Close that directory
6. Double-click dcm2nii on the Desktop (or, if using MRIcroGL, select **"Import"** then **"Convert DICOM to NIfTI"**)
7. Click on the "**output format**" and change it to the first option (Figure 3), "**SPM2 (Analyze hdr/img)**"

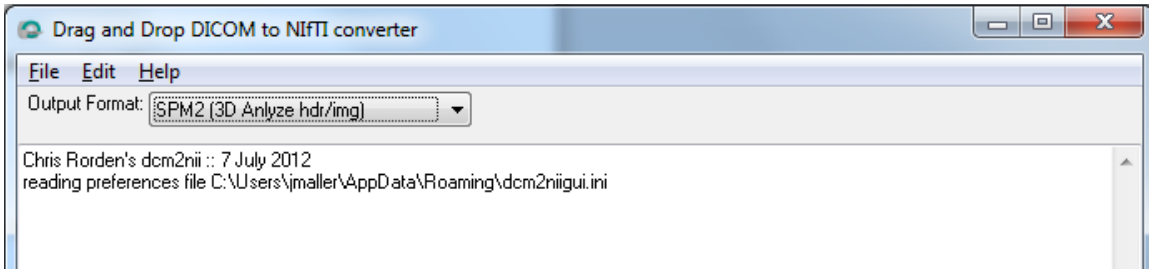

Figure 3. The DICOM to NIfTI GUI

8. Navigate to the directory where the dicoms are
9. Drag the folder of dicoms into the DICOM to NIfTI converter window
10. In a few second the dicoms will be converted to Analyze format and reorientated (if required)

3. MRIconvert

MRIconvert is an easy-to-use medical image file conversion utility that converts DICOM files to NIfTI 1.1, FSL NIfTI, Analyze 7.5 , SPM99/Analyze, BrainVoyager, and MetaImage volume formats. MRIConvert 2.0 is also good for .nii output, Analyze 7.5 output, and automatic generation of diffusion gradient files for Siemens Syngo scanners, Philips, and some GE scanners. It can be installed onto Windows, Mac, or Linux operating systems.

4. SPIN to convert a directory of DICOMS containing different sequences (series)

1. Open SPIN
2. **File → Sort DICOM and convert**
3. A new window opens (Figure 22). Click on the Browse button and navigate to your folder that contains all of the DICOMS
4. Click "**OK**". Within a few seconds all of the DICOMS will have been sorted into their respective directories, and the names of the directories will be based upon the name of the sequence (series).

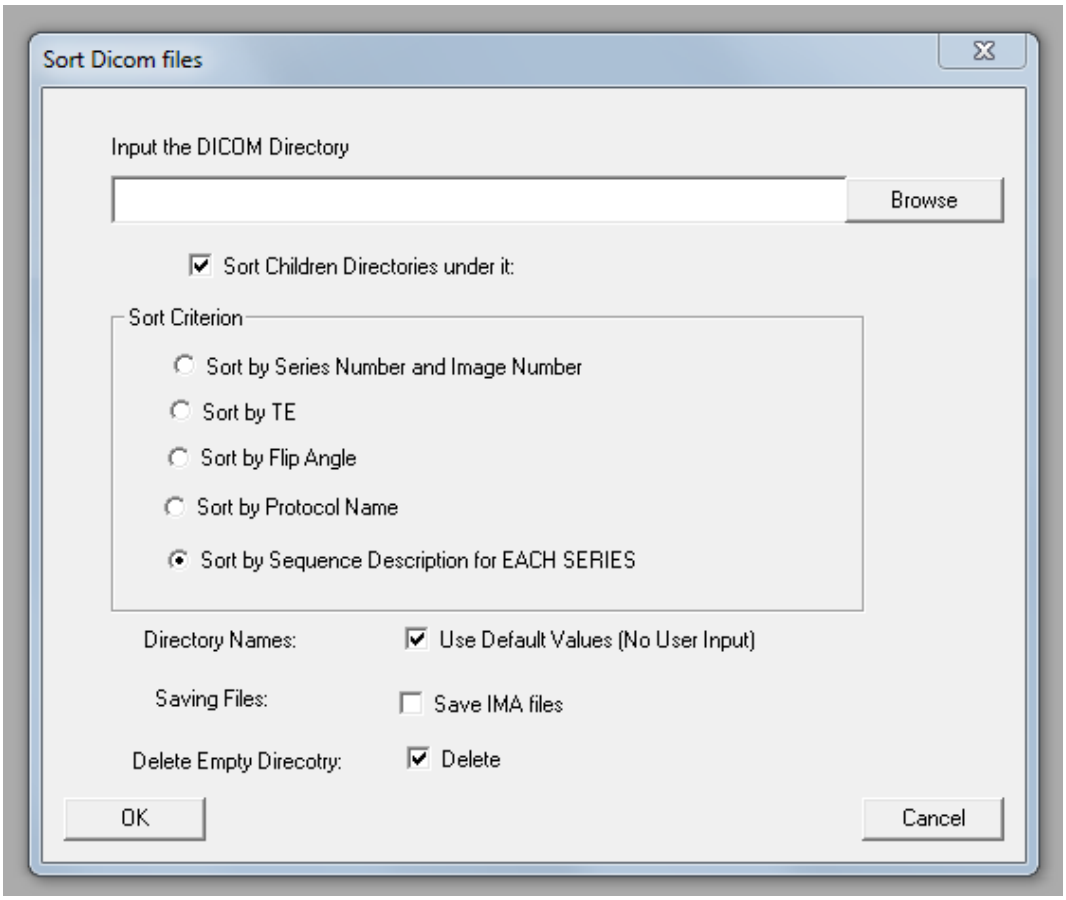

Figure 4. Sorting Dicom files using SPIN.

DTIstudio also has a module to do this.

5. DICOMsort to convert a directory of DICOMS containing different sequences (series)

1. Open DICOMsort software (Figure 23)

2. Click the "**Browse**" button and navigate to your DICOM folder

3. The values contained in the DICOM header should populate the "**DICOM properties**" field.

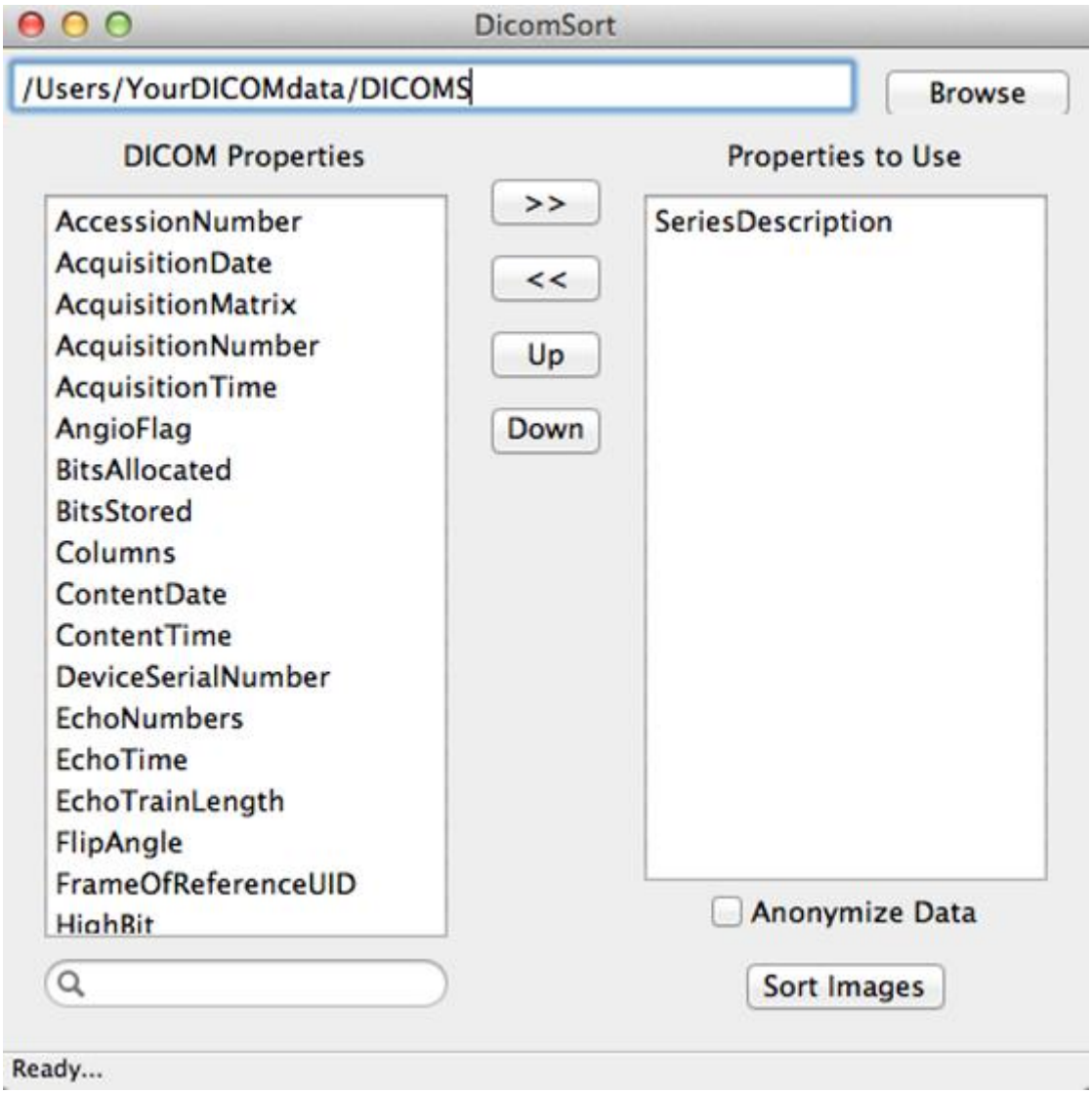

Figure 5. DICOMsort user interface.

4. Choose the values that you wish to sort by moving them from the "**DICOM Properties**" field to the "**Properties to use**" field (Figure 24). The series description is there by default. Note: If you choose multiple values to sort, the one at the top of the "**Properties to Use**" field will be the top-level folder followed by the lower items in subfolders. See figure below for an example of the folder structure that results from the values chosen

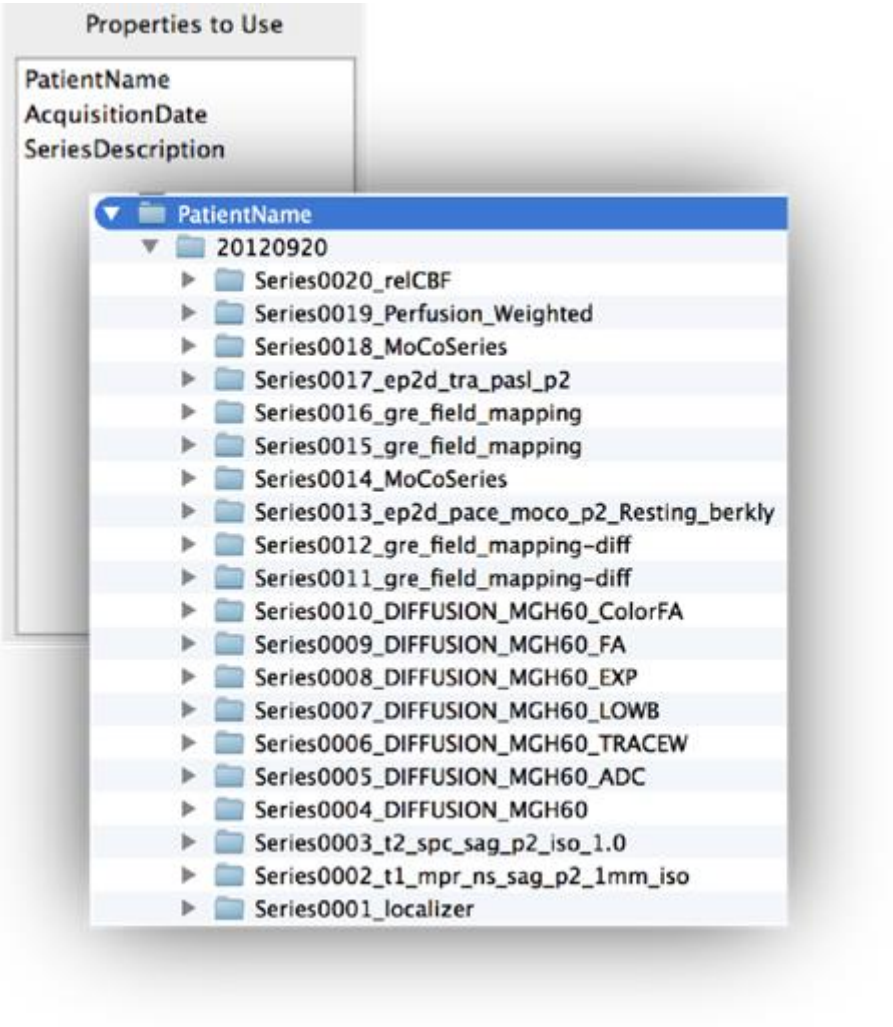

Figure 6. DICOM properties in DICOMsort.

5. Before sorting the images click on "**File**", then "**Preferences**", then "**Filename Format**" and check the "**Keep Original Filename**". (This ensures that other programs, such as FreeSurfer, don't have difficulty reading the files).

6. If you wish to anonymize the data then check the "**Anonymize Data**" button. Then "**File**", then "**Preferences**", then click the "**Anonymizing Fields**" button and choose the values that you wish to anonymize.

7. Click "**Sort Images**".

Another good program to anonymize dicoms is DicomAnonym, which runs directly from the .exe file.

6. Preparing a T1-weighted scan for MiniBIRD stereotaxy

1. **Convert slice-by-slice into a volume** (i.e. scan.img and scan.hdr)
2. 'Import' → 'Conversion wizard'. 'OK'. Select the very first slice (dicom) and then give the scan a new name. The dicoms in the directory will be converted into 2 new files: an Analyze format image (xxx.img) and a matching header file (xxx.hdr)
3. **Locate the new converted scan** ('File' -> 'Open Image'). Select the scan's .img or .hdr and then Open
4. **Reorientate**: 'File' → 'Save As'… Then select 'Format' → 'Sagittal Left', then 'Save [Intel]'; give it a new name, and Save it. Exit MRIcro, wait a few seconds, then open it up again, and load this most recent scan (that has had 'Sagittal Left' applied to it)
5. 'File' → 'Save As'… Then select 'Format' → 'Axial Down', then 'Save [Intel]'; give it a new name, and save it. Exit MRIcro, wait a few seconds, then open it up again, and load this most recent scan (that has had 'Sagittal Left' and 'Axial Down' applied to it)
6. **Convert from non-isotropic (anisotropic) to isotropic**: 'Etc' → 'Convert anisotropic image to isotropic', give it a new name and 'Save'
7. **Reslice voxels to 1mm^3** : 'Etc' → 'Rescale'. Enter the voxel dimension (see the 'Size(mm)' box) and make sure the 'Output Size (mm)' reads 1.00 x 1.00 1.00.
8. **Clip high and low**: 'File' → 'Save As', now increase the values of the 'Clip high' and Clip low' values until the red lines above and below the T1 scan (it should be displayed from sagittal by default) are slightly above the skull and just below the bottom of the cerebellum. Then select 'Save [Intel]', give the scan a new name, and Save. You should now have a saved scan that has 1mm3 isotropic voxels and clipped to only include the brain stem until slightly above the skull
9. **Extract the brain** (include CSF beneath the inner table) – the so-called "BET" process (Brain Extraction Tool): 'Etc' → 'Skull strip image using BET', then 'Go', then give the scan a new name, and Save. A smaller black box will appear as iterations occur on the scan – this should take around 10 to 20 seconds to complete, and then the skull stripped scan will appear in the MRIcro window from 3 orientations. If only brain and no skull is present then BET worked well; if not, you need to reClip the scan (Step 5; so that less skull is present) and then try BET again

Then: Normalize the scan using SPM

1. **Open SPM97**
2. Using a browser (e.g. My Computer), **browse to the location of the scan to be normalized** (i.e. the scan above that still has the skull) and have that screen open on the right-side of the screen
3. In SPM97, 'File' → 'New' → **Normalization**
4. The next screen ('**New spatial normalization'**) presents default values of 1 subject and 'New document'. Just select 'OK'

5. **Click** on the + sign next to Subject 1, and 'Template', 'Image to estimate parameters' and 'Images to normalize appear'. Click on the + sign next to 'Template', and another layer will appear that states something like 'C:\SPM96\Templates\PET.hdr' – you need to change the word PET with T1 – do this by clicking on that line of text until it turns dark blue, and then replace PET with T1
6. Click on the scan.hdr from the My Computer and **drag it over** the 'Image to estimate parameters', and then again click the scan from the My Computer and drag it over the 'Images to normalize
7. '**Calculate**' → 'Settings'
8. Click on the tab that states '**Parameter estimation'**, and change 'Number of nonlinear iterations' to none, and 'Number of basis functions' to none.
9. **Click on the tab** that states 'Reslicing, and change ''Voxel size' from 2 2 2 to 1 1 1, and 'Bounding box' from MNI to 'Template'
10. '**OK**'
11. '**Calculate**' → 'Start'
12. After around 10 to 20 seconds the **scan will be normalized**, but it has to be converted to another format so that you can open it in MRIcro
13. **Close SPM97** (you don't need to save changes), **open My Computer** and **locate the normalized scan**: it will begin with an 'n' by default. Double-click on the scan and it will open in MRIcro. Once it is open, **save it as a new name** ('File' → Save As', 'Save [Intel]'), and it will be saved in the Analyze format that can be opened in MRIcro

Or, if you have Matlab, use a newer version of SPM (e.g. SPM12) and utilise its normalization feature.

The scan preparation process outlined above can also be used in conjunction with the trakSTAR device (updated version of miniBIRD).

7. Using the Ascension® MiniBIRD (Flock of Birds) for stereotaxic coregistration of MRI-to-head-and-brain

You will need to find at least **5** anatomic landmarks.

1. Start MRIcro, and make sure the 'Yoke' and 'XBars' controls are checked [outlined in purple on the picture to the right]. It is generally easiest to find landmarks in the 'projection' view that shows the Axial, Sagittal and Coronal views simultaneously [click on the 3D-cube button outlined in **blue** in Figure 4; Note that different versions of MRIcro will have different button arrangements]
2.

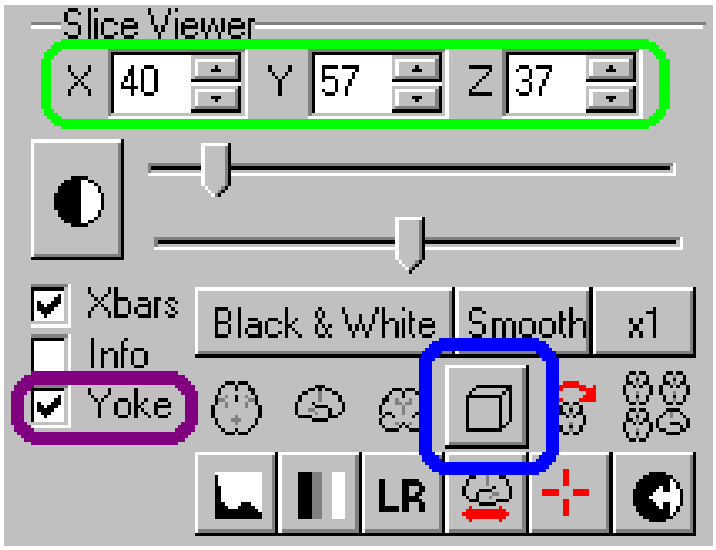

Figure 7. GUI for MRIcro for MRIreg.

3. Load the MRI scan and find your landmarks (regions that you can identify on both the MRI scan as well as the individual's head [e.g. bones, nose, ears, etc]). If there are any anatomical regions of the brain you wish to locate (e.g. the motor hand area), you will also want to work out these coordinates. For all the landmarks, you will want to know where they appear in the X,Y and Z coordinates (outlined in green)
4. Start the MRIreg software
5. There are a few bugs in the software, so make sure to do the following:

i) Select: Tracker (one of the pull-down menus), then “Fastrak”, then change it back to MiniBIRD (i.e. again, select “Tracker” then select “Flock of Birds/Minibird”)

ii) A message will then appear on the screen. At this stage, turn the MiniBIRD switch (on the front of the unit) to 'STBY', then count 5 seconds in your head, and then switch it back to ‘FLY’. If your Bird is connected correctly, the XYZ position of the bird sensor should appear (after a few seconds) at the bottom-right of the MRIreg window (e.g. 'FOB -742 139 892');

this value should change when the Bird sensor is moved. If this does not work, try adjusting the serial-port speed (the 'FOB' menu allows you to choose a speed between 9600 and 57600 baud, as well as letting you select either a miniBIRD or Fastrak system).

5. You are now ready to add in coordinates to MRIreg. At this point, MRIcro should be displaying the MRI scan, and MRIreg should be displaying the position of the bird sensor (at the bottom right of the window) and the MRI scan coordinates (at the bottom left)

Step 1: **Calibration**

1. With MRIcro, click on the projection view to select an anatomical position. (You want to select the location where the sensor will be, e.g. instead of the tip of the nose, select a few millimetres anterior to the tip).
2. Move the magnet sensor to the location displayed in MRIcro (Figure 5).
3. Press the MRIreg '+' button (or press F1). A new spreadsheet row appears in MRIreg, showing the MRI position (left) and the corresponding magnet position (right).
4. Repeat steps 1-3 until you have entered all of your anatomical landmarks. **You must enter at least 5 positions, more positions give better accuracy during magnet tracking.** Pick well-separated points (e.g. select regions on the face, the ears, the back of the head and the top of the head; instead of only points on the face).

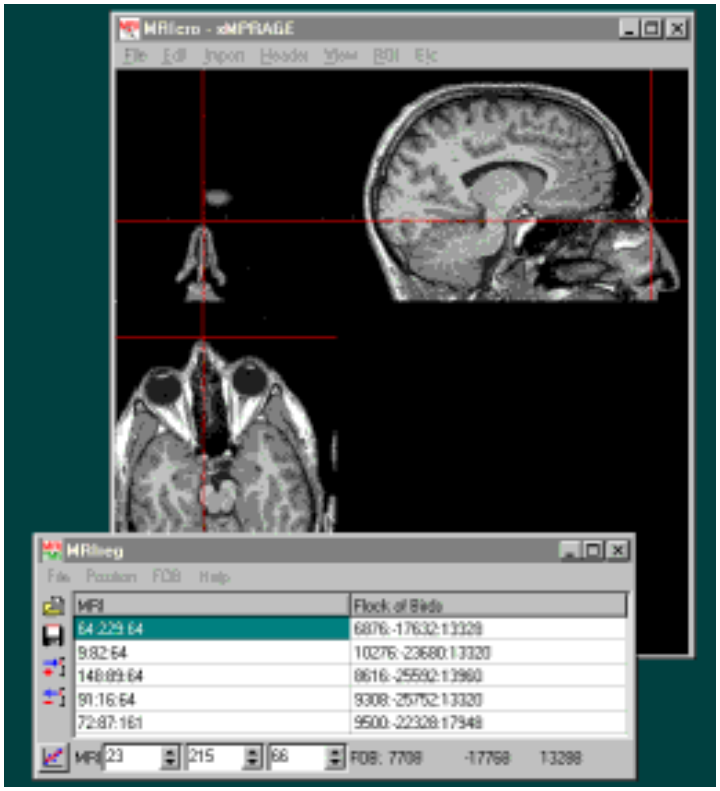

Figure 8. MRIreg and MRIcro on one screen.

Step 2. **Magnet Tracking**

1. Once you have entered all of your anatomical landmarks in step one, we want to check the accuracy of the registration process. To do this: select “Mode” (one of the pull-down menus), and select “Track”. **Now when the MiniBIRD magnetic wand moves, the red cross-bars in MRIcro should move as well**

Example: If you wanted to locate the left DLPFC the Talairach co-ordinates are *-45,45,35* (or *45,45,35* for the right), which is on the border of the anterior and middle thirds of the middle frontal gyrus.

As before, this process can also be used in conjunction with the trakSTAR device (updated version of miniBIRD). Based on our experience, we suggest using the serial port connection (no drivers required) rather than the USB connection when using trakSTAR with MRIreg.

8. How to locate the DLPFC (F3 or F4) without an MRI brain scan

Option A. International 10-20 system

Modified Combinatorial Nomenclature (MCN). This MCN system uses 1, 3, 5, 7, 9 for the left hemisphere which represents 10%, 20%, 30%, 40%, 50% of the inion-to-nasion distance respectively (Figure 6).

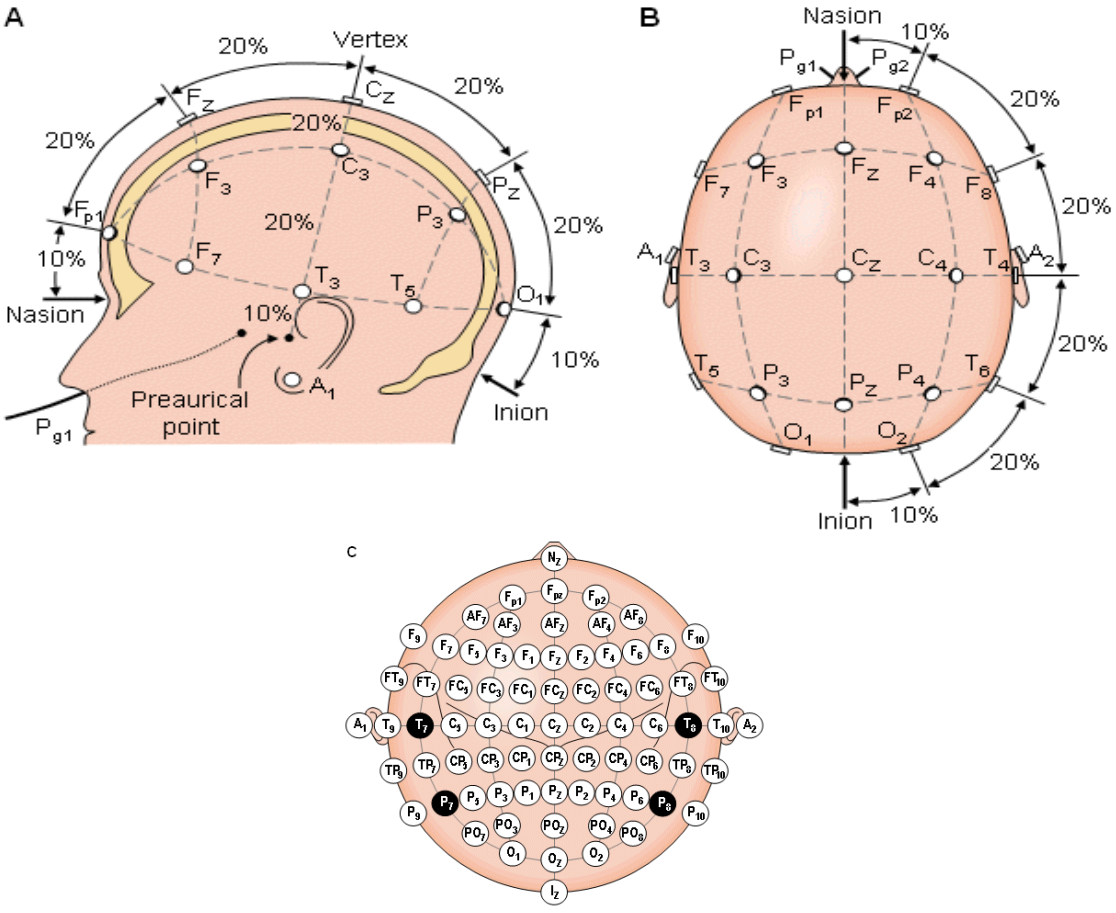

Figure 9. The international 10-20 system seen from (A) left and (B) above the head. A=Ear lobe, C=central, Pg=nasopharyngeal, P=parietal, F=frontal, Fp=frontal polar, O=occipital. (C) Location and nomenclature of the intermediate 10% electrodes, as standardized by the American Electroencephalographic Society. (Redrawn from Lagerlund, 1993).

Option B. "Beam" software (Beam et al., 2009).

1. Measure distance from Tragus to Tragus: make sure it passes through the vertex (AKA CZ in the 10-20 system). Enter this distance into the software
2. Measure distance from nasion to inion: make sure it passes through the vertex. Enter this distance into the software
3. Measure head circumference (above the brow-line); this should measure the largest diameter. Enter this distance into the software
4. Click the "Calculate" button and 2 measurements will appear: "Distance along circumference from midline (X)" and "Distance from Vertex in CM (Y)". Use your tape measure and mark the Xcm distance starting from the midline at the brow-line and mark the spot with a texta, then measure the Ycm distance from the vertex: it should end up at the same spot as X

Figure 10 illustrates an example of locating the left DLPFC.

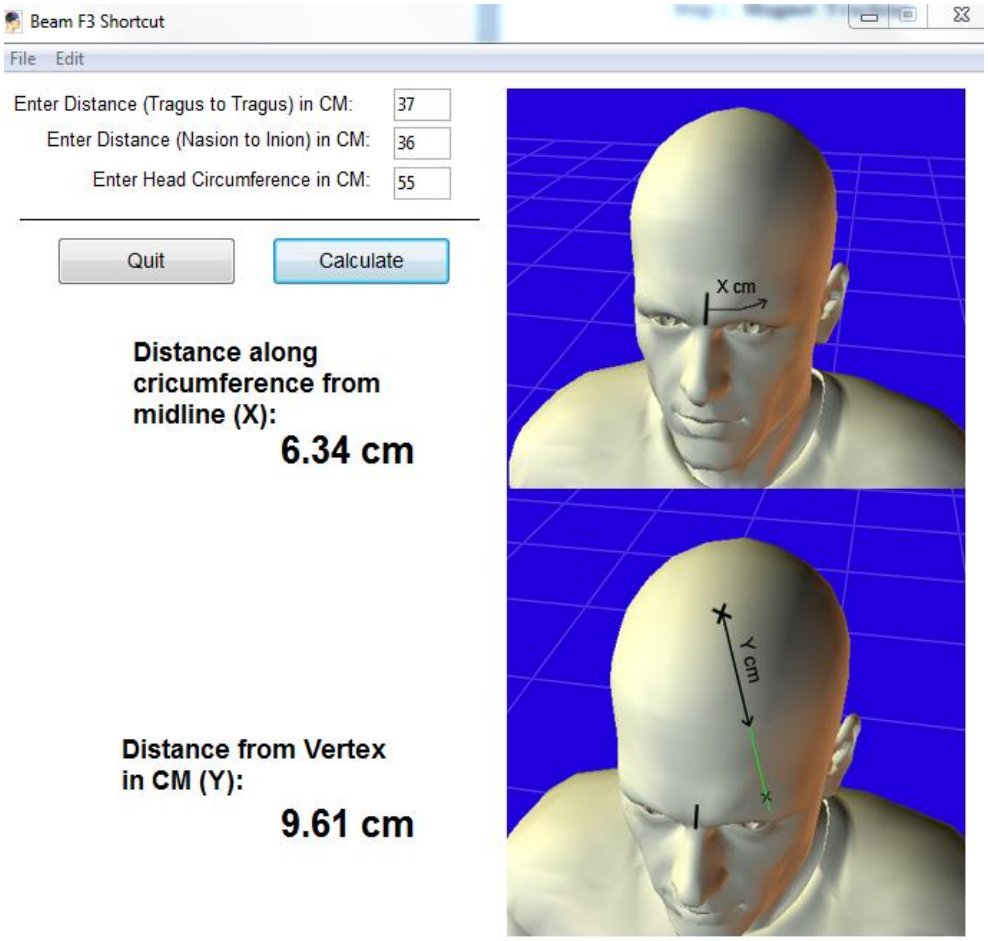

Figure 10. Beam software interface to locate the left DLPFC.

9. Example of tracing an ROI in MRIcro. In this case, the caudate.

The brain MRI scan that you will use in this example (called "ch2") is the 'smoothed' MNI (Montreal Neurological Institute) template (the 27 averages of a 'normal' adult brain). This is included in the MRIcro package. This scan is also included with only the brain (i.e. it has undergone the Brain Extraction Tool (BET) process), hence, no skull appears on it (called "ch2bet"). This scan will be used for task (1) so as to allow for more visually representative 3D renderings.

Task 1: Estimate the volume of the left caudate by manually tracing it using MRIcro

1. **Load T1-weighted volume** (File → Open File → ch2bet.hdr)
2. **Display axial** (transverse) sections (1st icon on the left in the "Slice Viewer" toolbar)
3. **Select the "Pen for drawing object outline"** icon (2nd icon from the left in the lower row in the Region of Interest toolbar)
4. Move to slice 97 and **begin outlining** the superior portion of the caudate. That is, outline this ROI with the left mouse button and then right click inside the outline to flood-fill it. If the entire screen is flood-filled, then the outline has not been closed (i.e. the border has a voxel not traced). If this happens, simply click on the "Undo last drawing" icon (3rd icon from the left on the upper row of the Region of Interest toolbar [it looks like a yellow pencil with a black tip on a 45 degree angle). Note: this undo feature can only be used to undo the previous drawing (that is, you cannot undo 2 or more steps)
5. **Continue to outline** this ROI (i.e. the caudate) on each axial slice (**from superior to inferior**) until the most inferior slice is outlined for the ROI around slice 57; any more inferior [lower] does not contain left-side caudate. You can move from slice-to-slice with the Up and Down arrows on the keyboard, or by changing the number that appears in the Slice Viewer, or, you can simply use the wheel on the mouse between the left and right buttons)
6. Click on the **"View projection"** icon (4th icon from the left in the "Slice Viewer" toolbar). The scan from the midpoint (i.e. the middle slice) of all 3 orientations (axial, sagittal, coronal) should now be displayed simultaneously. The traced ROI should also appear on the coronal (upper left) and axial (lower left) slices. Left clicking on any part of a slice in any of the orientations will automatically change the slice number of the other 2 slice orientations to match the voxel that you have just clicked. For example, clicking in the middle of a large part of the caudate from coronal from slice 126 (you can change the individual slice numbers from any of the 3 orientations whilst they are still displayed simultaneously by altering the slice numbers x,y,z, just below the Slice Viewer toolbar. Using "View projection" **helps to verify** that you have outlined (traced) the structure correctly. That is, it helps to make sure that you have outlined the correct ROI; in this case, it should all be gray matter, hence, if any white matter is traced from any projection then those voxels will need to be erased. **Erasing voxels** (only from a single

orientation, not from View Projection": Go to the slice where part of the ROI needs to be deleted, then move the cursor over the voxel that you wish to remove, then hold down the "Enter" key on the keyboard whilst also holding down the left mouse button. Clicking on the right mouse button whilst holding down the "Enter" key will erase the entire ROI on that slice. If you do this by accident, simply click on the "Undo last drawing" icon

7. Once you are satisfied that your tracing of this ROI is accurate, "**Save the ROI"** (4th icon from the left on the upper row of the Region of Interest toolbar, or, from the ROI pull-down menu) as "ch2bet_right_caudate.roi"
8. **Calculate the volume of the ROI**: the volume appears in the lower left of the "Region of Interest" toolbar when the ROI is initially opened. So, you need to "Open ROIs", select on the ROI that you have just saved (i.e. ch2bet_right_caudate.roi), and click "OK". **Important:** don't move the mouse because as soon as you move the mouse the volume displayed disappears and is replaced by the co-ordinates of the cursor!!
9. **Write down the volume displayed** (which should be around 4.5-5.0cc)

Task 2: Open the "aal" image that is included in the MRIcro directory (C:/ProgramFiles/MRIcro/). Move the mouse to the Talairach co-ordinates below and write down the name of the ROI at these co-ordinates, including whether each set of x,y,z co-ordinates represent the ROI on the right or left of the brain in Talairach space.

Table 1. Example of names of ROIs that represent Talairach co-ordinates.

ROI Number	Talairach co-ordinate			Right or Left	Name of ROI
	x	y	z		
1	-49	6	39	**L**	Precentral gyrus
2	49	-54	32	**R**	Angular gyrus
3	30	-11	-17	**R**	Hippocampus
4	-8	-9	9	**L**	Thalamus
5	-8	18	-2	**L**	Caudate
6	48	18	-20	**R**	Superior temporal gyrus
7	-8	0	60	**L**	Supplementary motor area
8	-4	40	16	**L**	Anterior cingulated gyrus
9	-14	-65	-3	**L**	Lingual gyrus
10	20	3	1	R	Globus pallidum

10. Manual tracing of a Region Of Interest (ROI) in Analyze®

1. Double-click on the Analyze icon
2. Click on the far left icon that looks like a truck ("**Load As**")
3. Click on the "**File**" button, and browse to the directory where the T1-weighted scan is located
4. **Select** the header (.hdr) or image (.img) of the T1-weighted scan
5. Click on the "**Open**" button (bottom right of Load As module), or double click the selected file in Step 4
6. Click on the "**Load**" button (bottom left of Load As module). The brain scan will now load
7. Click on the **ROI icon** (usually left of the EXIT icon on the far left of the tool bar [called a Powerbar])
8. The ROI module should now have opened
9. Move to a slice where the brain is visible (e.g. middle slice). Alter the intensity ("**Intensity**" icon on the far right of the Powerbar [on the top of the screen]) so that the contrast between GM/WM/CSF is optimal. Note: the sliding bar of the Intensity setting is very sensitive, so it often quicker to enter the Intensity Max and Min values manually (once you have established then for one scan, the rest of the scans acquired with that sequence on that scanner should be set to the same (if not identical) intensity levels)
10. **Select the slice** where you have chosen to begin tracing the ROI
11. Select the "**Trace**" icon (on the left) and hold down the left mouse button at the first pixel. Whilst holding down the left mouse button, outline the ROI and then release the left mouse button. A number will appear inside the ROI tracing to represent that ROI.
12. **Name the ROI**: View → Objects. Select the Object (named as its number) and name it as the Name of the ROI. E.g. left_hipp means Left Hippocampus
13. Click on **Done** (lower right)
14. Move to the next slice (+ key on the keyboard or with the "**Arrow**" on the Powerbar) and begin tracing the ROI on that slice. Repeat this until all slices have been traced for the ROI
15. **Save the traced ROI** (File → Save Object Map)
16. To begin tracing a new ROI, select "**New**" from the "**objects to define**" located at the lower right of the screen and repeat Steps 10 to 14 for further ROIs.

Here is a link to the Analyze document that we contributed to which provides step-by-step instructions for manually segmenting the hippocampus from T1-weighted images:
http://analyzedirect.com/documents/guides/hippocampal_volume_assessment.pdf

A visual guide of the conversion of .obj files to Analyze format:

1. Use Analyze

a. File -> Import/Export

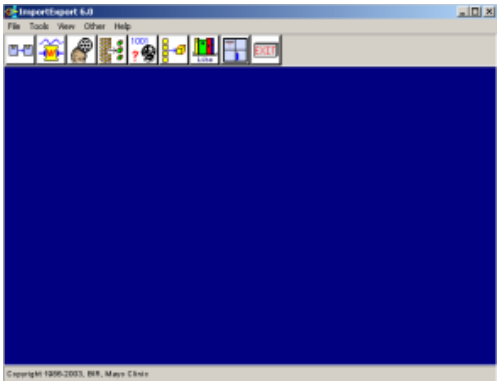

Figure 11. The Import/Export module in Analyze®

b. Click on the 'File-to-File button 

c. Select the object map to be modified (by using the 'File' button to browse)

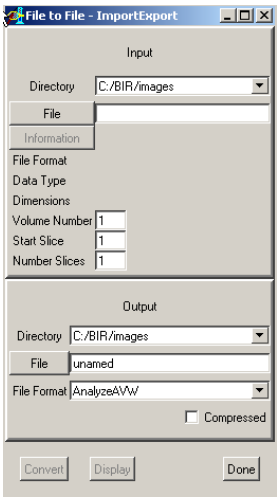

Figure 12. The File-to-File option in the Import/Export module in Analyze®

d. Rename the output file

e. Assign the output file the 'AnalyzeImage 7.5' file format. **IMPORTANT**: A bug in this module is as follows: if you Assign the output file format BEFORE naming the new output file then this entire file-to-file process will NOT work! SO, BE SURE TO CARRY OUT STEP D. BEFORE STEP E AND F.

f. Click on the 'Convert' button

g. Click on the 'Done' button

11. Steps for 3D rendering in ITK-SNAP of ROI (object maps) traced in Analyze

1. Open Analyze
2. **File → Import/Export**
3. **File-to-File icon** (far left icon)
4. Browse for the tracing (object map) to be converted, and select it
5. Change output name to something relevant
6. Change new format to "**Analyze 7.5**"
7. **Convert**
8. **Done**
9. Open ITK-SNAP (Yushkevich et al., 2006)
10. Open T1-weighted image (.hdr) as "**Load Data/Greyscale Image**"
11. Open the newly converted Analyze 7.5 format object map (.img) as "**Load Data/Segmentation Data**"
12. Then fiddle with label colours, transparency, and intensity (Options , Display curves, then hold-and-move the 2 dots along the graph's diagonal line)
13. NOTE: you **must** change the voxel dimensions of the Analyze 7.5 format tracing; USE MRIcro → note that voxels are 0 x 0 x 0 mm in MRIcro → change these number to 1.00 x 1.00 x 1.00mm (NOTE: figure below) so that they match the T1-weighted scan voxel dimensions

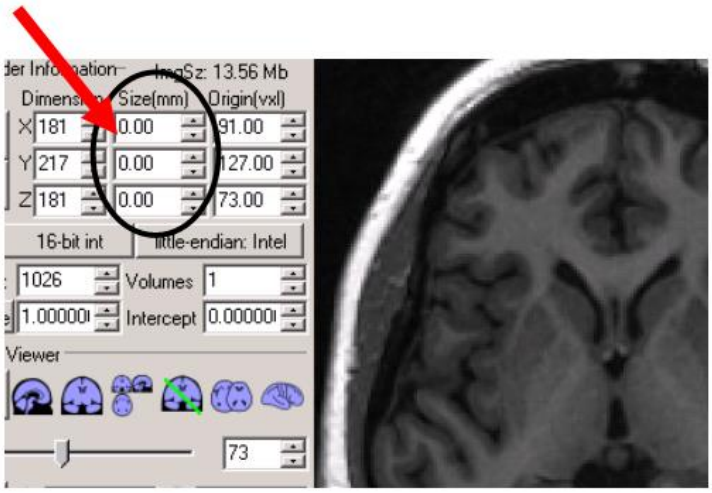

Figure 13. Fields that must be changed to match voxel dimensions of the T1-weighted scan.

14. 12) “Update mesh” (bottom of screen)

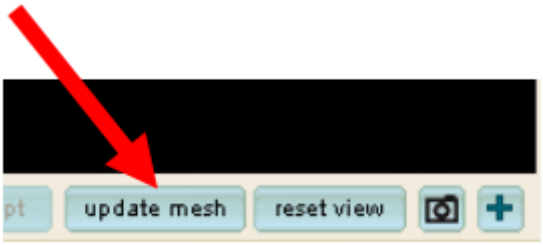

Figure 14. The “update mesh” button in ITK-SNAP

15. Then fiddle with label colours, transparency, and intensity (Options , Display curves, then hold-and-move the 2 dots along the graph’s diagonal line)
16. To view the 3D rendering in its own screen, press “**+**” at the bottom of the screen, as follows:

Figure 15. Maximizing the 3D-rendered ROI in INTK-SNAP.

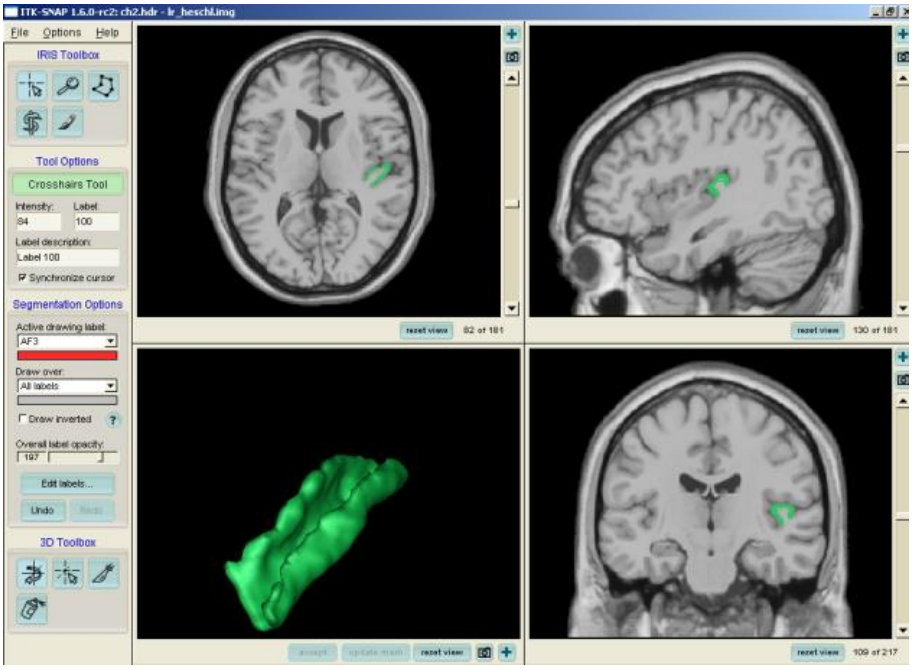

Figure 16. An example of a left Heschl gyrus traced in Analyze and then 3D-rendered in ITK-SNAP.

12. Inter-rater reliability

Inter-rater reliability = ((Rater A – Rater B) / ((Rater A + Rater B) / 2)) * 100

In other words, the percentage difference between the Raters.

The other way to calculate inter-rater reliability is in SPSS with "**intraclass correlation**". The reference is Shrout & Fleiss (1979). Or, simply calculate "**kappa**".

13. FreeSurfer

FreeSurfer (Fischl et al., 1999) is a great program for conducting group T1-weighted (structural) analyses. It calculates volume, thickness, area and curvature of dozens of ROIs. Apart from the FreeSurfer forum and wiki, an excellent FreeSurfer resource is located here: http://vitallongevity.utdallas.edu/cnl/wp-content/uploads/2015/10/FREESURFER_GUIDE_CNL_2015Oct.pdf . To process a scan through FreeSurfer on Linux:

1. Download FreeSurfer (latest version): note whether your computer is 32-bit or 64-bit

2. Copy it to the desktop of your Linux operating system

3. Open a terminal

4. Type the following: **sudo mv freesurfer-<version> /usr/local/**

5. Extract the freeSurfer file by typing the following (from /usr/local/):

sudo tar xzvf freesurfer-<version>-full.tar.gz

6. Copy your .license into the /usr/local/freesurfer directory by typing:

sudo mv .license /usr/local/freesurfer

7. Download and install tcsh by either downloading and installing online by:

a) typing the following: **sudo apt-get install tcsh**

or

b) Download it from another computer and then copy it across to the desktop of your Linux operating systems and type in the following from a terminal (from ~/Desktop):

sudo dpkg –i tcsh

8. Then modify the tcsh file so that the following 2 lines appear:

setenv FREESURFER_HOME <freesurfer_installation_directory>/freesurfer

(which in our case would be: setenv FREESURFER_HOME /usr/local/freesurfer)

source $FREESURFER_HOME/SetUpFreeSurfer.csh

If done correctly then those 2 lines should load when you type in 'tcsh' from a terminal

9. Give permission to write in the /usr/local/freesurfer/subjects/ directory by typing:

sudo chmod 777 /usr/local/freesurfer/subjects

10. Test the installation by typing in: tkmedit bert orig.mgz (to see an example) and then tksurfer bert rh pial (to see an example). Have a play and then close the program.

11. Copy a subject's dicoms into a directory under the directory /usr/local/freesurfer/subjects/

12. Let's say the subjects is called 'control1' and the first dicom is called IM1.dcm

13. From a terminal in the /usr/local/freesurfer/subjects/ directory type in 1 single command to execute all 31 steps of Freesurfer:

recon-all -s control1new -i control1/IM1.dcm -all

Note: for Step 8: cd (to make sure you are in your home directory). Note as well that if you have scans with higher resolution than 1.00 isotropic, add the following flag to the end of the recon-all command: **-highres**

It's also a good idea to include T2 data to optimise the pial segmentation. To perform recon-all to incorporate the T2 data:

recon-all -s <SUBJECT_ID> -i <SUBJECT_ID>/T1/IM1.dcm -T2 <SUBJECT_ID>/T2/IM1.dcm -all -T2pial

- In around 7 to 24 hours (depending upon the scan contrast and computer resources) the scan will be fully processed. It takes a long time because 31 processes are occurring for each hemisphere of data
- FreeSurfer is multi-threaded, so it can use all of the CPU cores you have available (To run recon-all with multiple cores, type this is in at the end of the command:

-openmp number_of_cores . For example, if your CPU has 8 cores, then: **-openmp 8**

14. After each T1-weighted scan has been processed via recon-all, you can view the results by typing in the following (as a single command line):

tkmedit nameofscan norm.mgz -segmentation aseg.mgz $FREESURFER_HOME/FreeSurferColorLUT.txt

15. To view the 3D rendering, type in the following:

tksurfer nameofscan rh pial

(To look at the left hemisphere type **tksurfer nameofscan lh pial**)

Note: to visualize only specific gyri using tksurfer; follow these instructions (contributed by Phillip Law):

1. Load existing label: File -> Label -> Load Label -> Browse -> <select label file> (e.g., *lh.aparc.annot* for left hemisphere or *rh.aparc.annot* for right hemisphere) -> Press 'OK' -> Press 'OK'.
2. Select and mark ROI(s): Left click on ROI and press *Mark Label* icon ( ; marked ROI will turn white).
    - To select another ROI, repeat step 2.
3. Create marked label from selected ROI(s): Click *New Label from Marked* icon ().
4. Save custom marked label: File -> Label -> Save Selected Label -> <enter filename> -> Press 'OK'.
5. Restart FreeSurfer
6. Load custom marked label: File -> Label -> Load Label -> Browse -> <select label saved in step 4> -> Press 'OK' -> Press 'OK'.
7. Change color of marked label: Click *Change label color* icon ().

16. For group analysis, you then need to smooth the data via the "qcache" command. That is, for each scan, type in the following:

recon-all -s control1new -qcache

- The -qcache takes around 10 to 30 minutes for each scan, depending upon how many scans you are simultaneously processing, and the available computer resources
- After each scan has finished the qcache procedure, you need to create a "plain text" document that is the matrix. For example,

Fsid diagnosis sex age

Id1 control f 20

Id2 control m 24

Id3 clinical m 21

Id4 clinical f 24

- Then save it as something like "qdec_table" (in plain text format)

- Next, you need to make a “diagnosis” plain text file, something like:

control

clinical

- Then save it as “diagnosis.levels” (in plain text format)
- Now make another file for the “sex” column, as follows:

m

f

And save it as “sex.levels” (in plain text format).

- Now run the FreeSurfer GLM program called “qdec”: Type in “**qdec**”
- A GUI (graphical user interface) will now open (Figure 17).

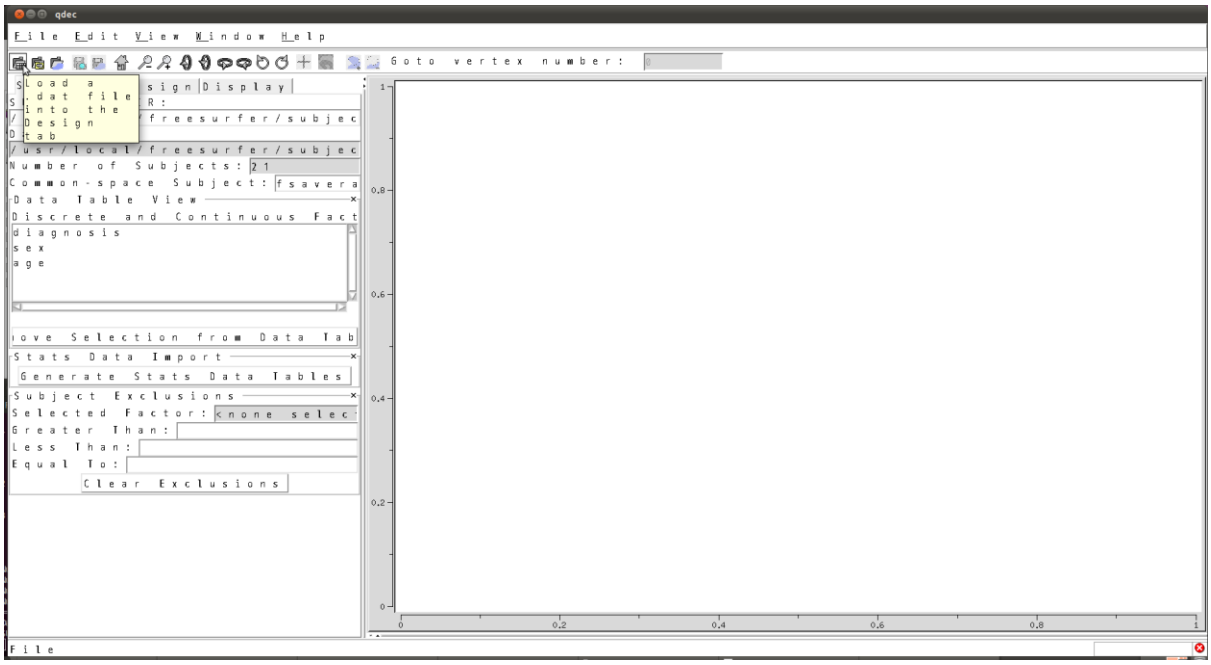

Figure 17. The QDEC GUI.

- Open the matrix table (the .dat file you made that contains the matrix data, the one called “**qdec_table.dat**”)
- Then, click on the “**Generate Stats Data Tables**”. This will import all of the data pulled from the scans’ “surf” directories included in the qdec_table file

- Select the pull-down menu from that tab (which now states "**none selected**") and select "**aseg.volume**" (Figure 18).

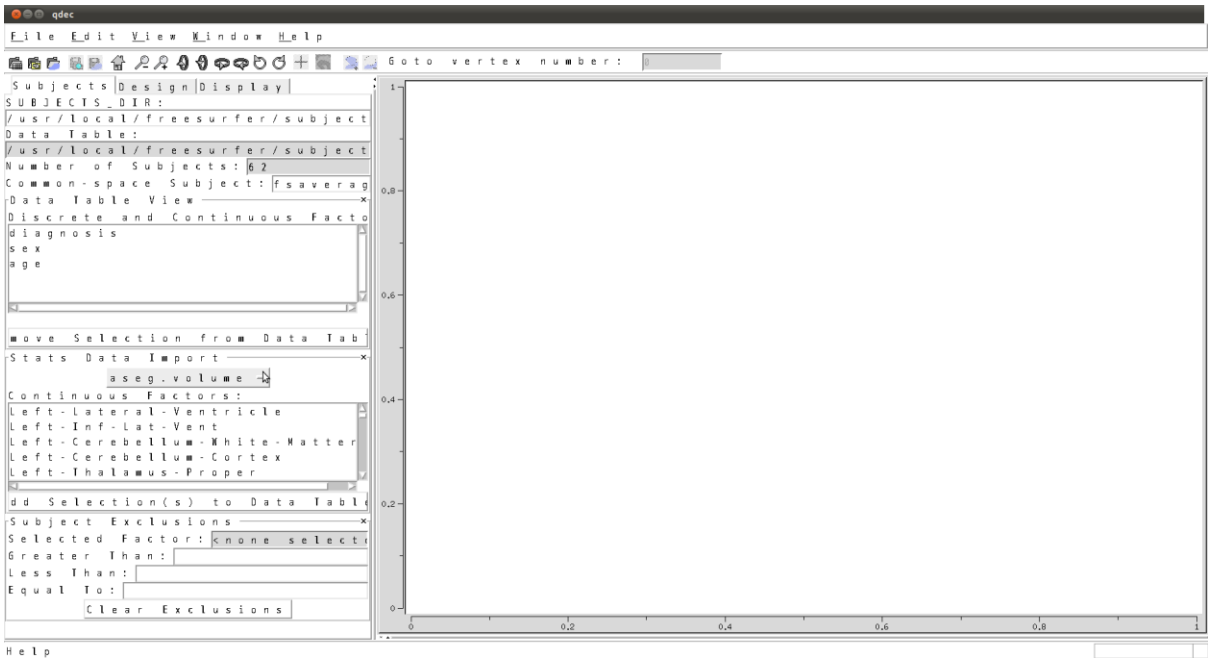

Figure 18. The QDEC GUI with a stats table loaded.

- Select the "**Design**" tab, and change "**thickness**" to be "**volume**" (Figure 19).

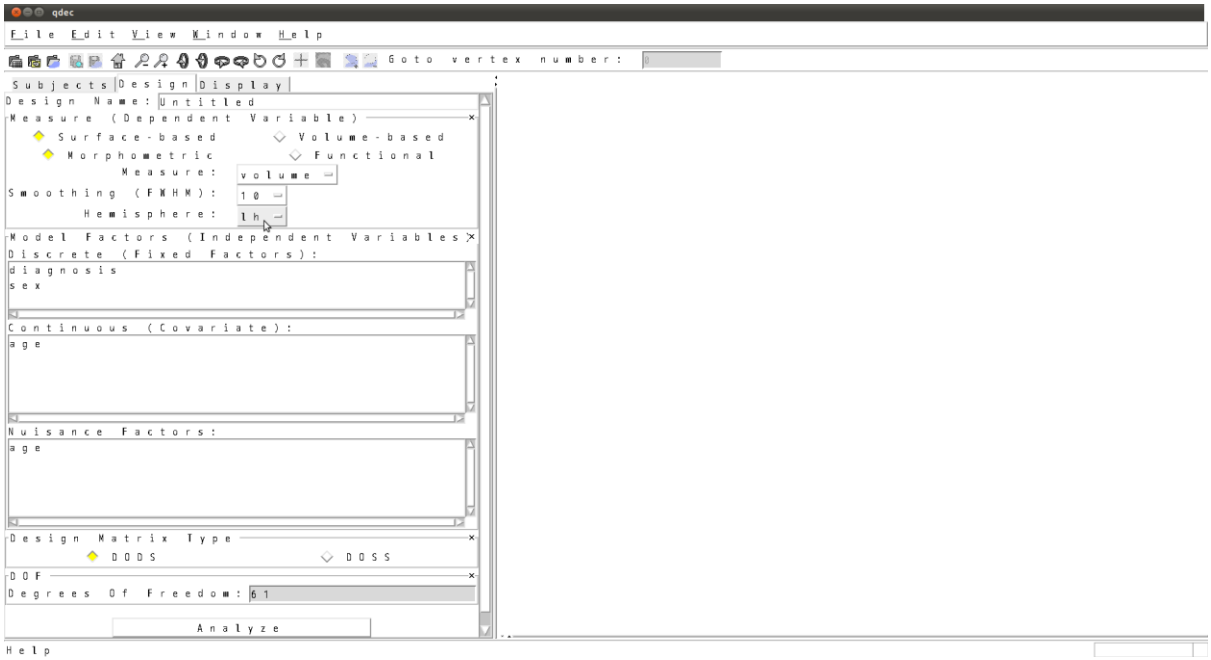

Figure 19. The Design section of QDEC.

- Click on the "**diagnosis**" option in the "**Discrete (Fixed Factors)**" window (Figure 20).

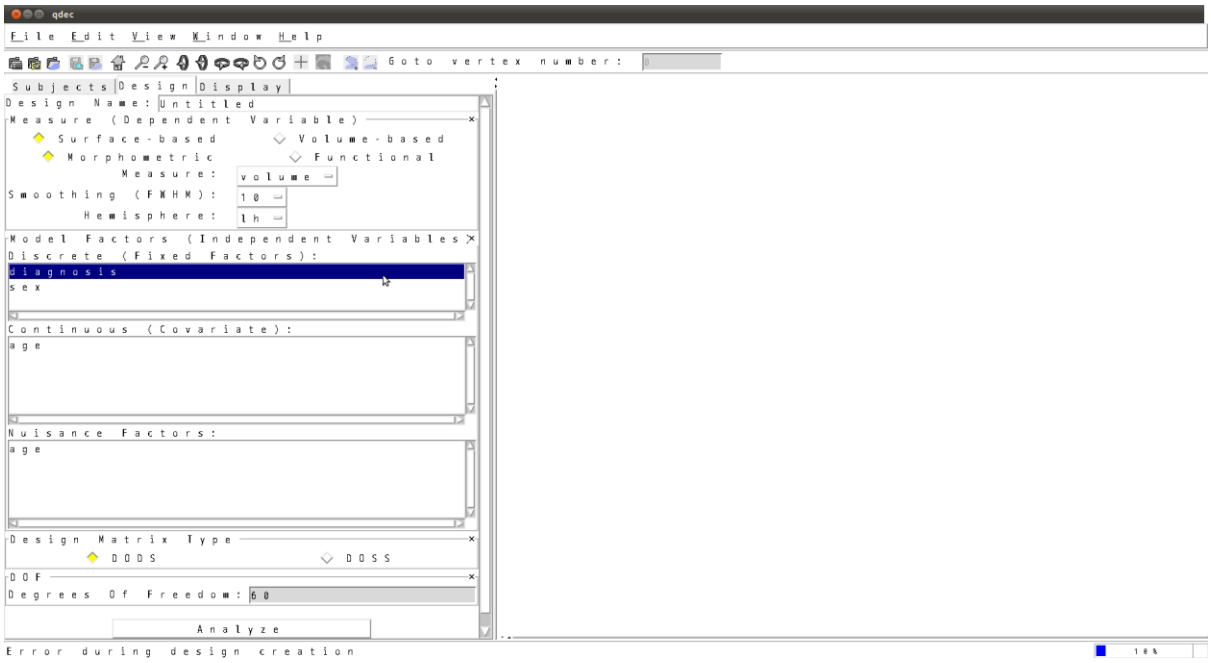

Figure 20. The Discrete (Fixed Factors) section of QDEC.

- Click on the "**Analyze**" button at the bottom. You will see the progress bar (in blue) moving in the bottom right of the screen from 0% to 100% (can take a few moments to process)
- A new window will now appear called "**Display**" that shows the "**inflated**" brain in 3D
- Select the second option in the left window (e.g. "Does the average volume differ between control and clinical?")
- The *uncorrected* results (vertices) will now appear over the inflated brain (Figure 21). To visualise the pial surface, click on "**pial**" (near the top of the Display tab) or "**white**" to see white matter

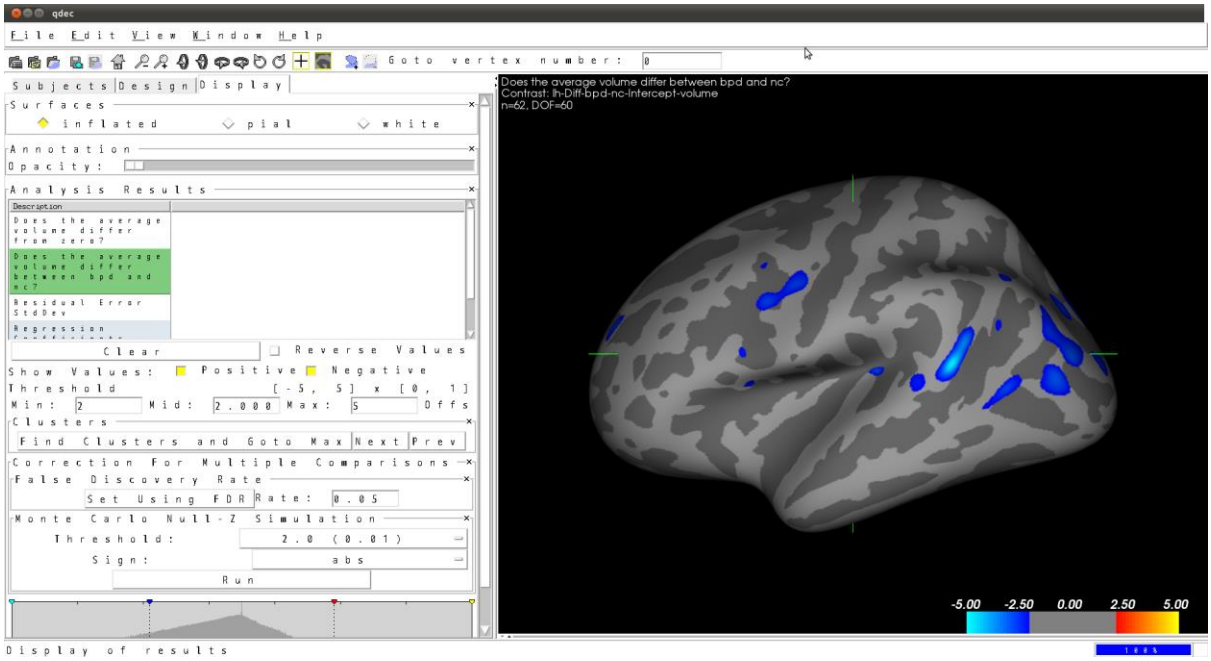

Figure 21. Uncorrected results superimposed over the inflated brain in QDEC.

- To analyze results for multiple-comparisons, used either FDR (false discovery rate), or the less stringent Monte Carlo Null-Z simulation:
- Change the default Threshold of 2.0 (0.01) to 1.3 (0.05) from the drop-down menu next to the word "**Threshold**"
- Click on the "**Run**" button
- Within a few seconds, the multiple-comparisons results will be superimposed in 3D over the inflated brain (Figure 22). In the example below, there are significant differences over the left superior parietal lobe, as seen in the terminal (Figure 23) which also states the significance values for that cluster as well as its Talairach co-ordinates and size (in mm^2). Note that the significance values in FreeSurfer are stated as -log(10). As a guide, a value of 2.0 is equivalent to p=0.01, and 1.3 is equivalent to p=0.05.

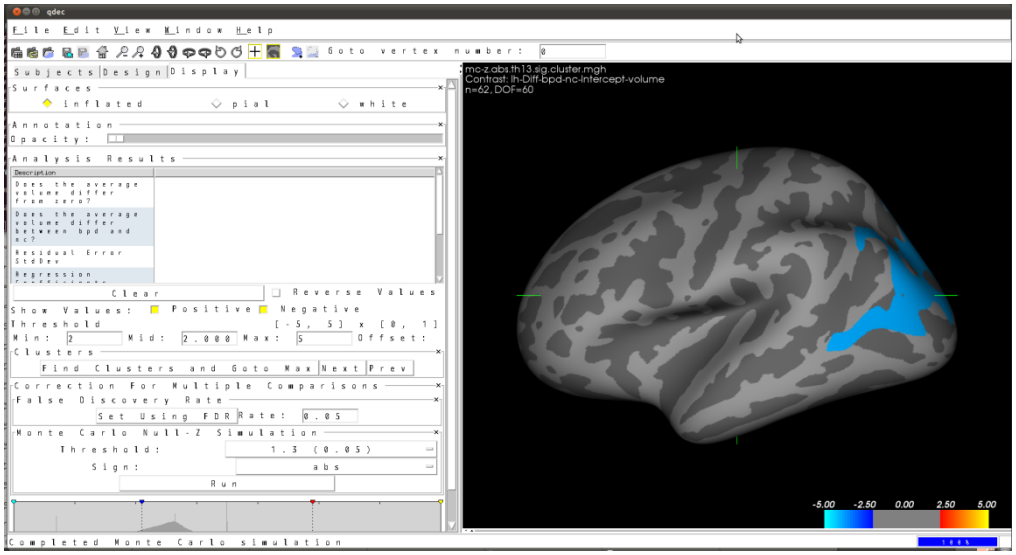

Figure 22. Corrected results superimposed over the inflated brain in QDEC.

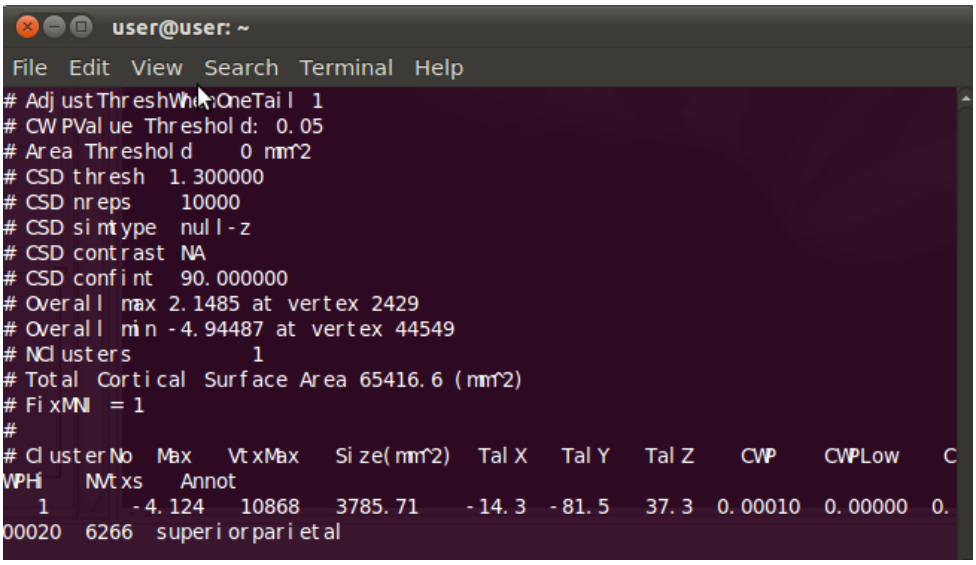

Figure 23. QDEC statistics output are displayed in the terminal.

- To process thickness, area, or curvature analysis, navigate back to the "**Subjects**" tab, drop down the pull-down menu, and select the type of analysis you want to investigate. Then, navigate to the "**Design**" tab and change "**volume**" to what you want to look at, then "**Analyze**". Note that the left hemisphere was processed first, so you need to select the right hemisphere if you want to look for significant differences in the right hemisphere
- Note that you can also use covariates or nuisance factors (in the "Design" tab) by clicking on them

NOTE: To change the working directory, for example, to a directory called "mydirectory", type in the following BEFORE typing in tcsh:

export SUBJECTS_DIR=/usr/local/freesurfer/subjects/mydirectory

NOTE: when running the recon-all command you must have the fsaverage subdirectory in your working directory, however in recent versions (e.g. 6.0) this is not required.

How to create volume and thickness statistics of BA regions and V1 and V2 (primary and secondary visual areas)

First extract the data from the FreeSurfer output (this may take a few hours per subject):

recon-all -s control1new -qcache

Then extract the thickness data into a text file (replace "thickness" with "volume" for volumetrics):

aparcstats2table --hemi lh --subjects subject1 subject2 subject3 --parc BA --meas thickness --tablefile ba_v1_v2_lh_thickness.txt

aparcstats2table --hemi rh --subjects subject1 subject2 subject3 --parc BA --meas thickness --tablefile ba_v1_v2_rh_thickness.txt

How to export the data from FreeSurfer into SPSS

1. After you have conducted a QDEC analysis, a new directory will have been created in the "**qdec**" directory called "**stats_tables**". Navigate to this directory
2. Copy all of the files in the directory (they will all have a .dat extension) onto a Windows computer
3. Open MS Excel
4. Select on a .dat file (although it may appear with a .stats extension) and then open it (you may have to select "**delimited**", then "**tab**")
5. **Save the file in Excel 97-2003 format**
6. Close Excel
7. Open SPSS
8. Change the option in the navigation window to show "**All files**"
9. Select the Excel file which you have just saved
10. The Excel file will now open in SPSS
11. Save As
12. Complete this process for all of the .dat files
13. Once all .dat files have been converted to SPSS format, merge them (using the merge feature in SPSS: **Data → Merge Files → Add variables**)

How to extract a ROI from FreeSurfer output and use it as a seed for tractography in MRtrix

mri_label2vol --seg aparc.a2009s+aseg.mgz --temp fa_coreg.nii --o aparc.a2009s+aseg-in-fa.mgz --regheader aparc.a2009s+aseg.mgz

Note: Don't confine to 'interp nearest', and the fa_coreg is the FA map converted to .nii.

A simple way to convert an ROI from FreeSurfer (e.g. caudal anterior cingulate) into nii format is as follows:

mrconvert rh.caudalanteriorcingulate.nii.gz rh.caudalanteriorcingulate.mif

How to import a volume-based mask into surface space to extract the thickness values in FreeSurfer (Contributed by Jonatan Ottino-González)

(1) to check if the mask is in the correct space

tkmedit -f $FSL_DIR/data/standard/MNI152_T1_2mm.nii.gz -overlay /path/to/mask/mask.nii.gz -fthresh 0.5

(2) cd to $SUBJECTS_DIR/fsaverage/surf

(3) register the MNI152 template to the 'fsaverage' space

fslregister --s fsaverage --mov $FSL_DIR/data/standard/MNI152_T1_2mm.nii.gz --reg MNI152_to_fsaverage.dat

(4) Check if everything's ok

tkmedit fsaverage T1.mgz -overlay /path/to/mask/mask.nii.gz -overlay-reg MNI152_to_fsaverage.dat -fthresh 0.5 -surface lh.white -aux-surface rh.white

(5) Transform the volume-ROI into a surface-ROI

mri_vol2surf --mov /path/to/mask/mask.nii.gz --reg MNI152_to_fsaverage.dat --projdist-max 0 1 0.1 --interp nearest --hemi lh --out lh.surface_mask.mgh

(6) Quality check

tksurfer fsaverage lh inflated -overlay lh.surface_mask.mgh -fthresh 0.5

(7) Convert surface (*.mgh) to a .label file

mri_vol2label --i lh.surface_mask.mgh --id 1 --surf fsaverage lh inflated --l lh.surface_mask.label

Note that the last step can cause problems because the mri_vol2label command (now called cor2label) needs an ID number in a COR-file, so the --surf flag saves you on this one.

FS hippocampal subfields

To perform hippocampal subfield segmentation, add the following flag to the end of your recon-all command:

-hippo-subfields

sudo gedit .tcshrc

From version 5.3, FreeSurfer can segment hippocampal subfields. However, always try to use the most recent version of FreeSurfer as each update contains more accurate segmentation and additional features. At the moment, FreeSurfer 6 is the most recent. The updated feature of this version is the enhanced accuracy of the hippocampal subfield segmentations (instead of -hippo-subfields, on Version 6 you add **-hippocampal-subfields-T1**). You can have multiple versions of FreeSurfer installed on the same computer, just make sure to change the path to point to the version you want to use. Also, when using version 6, you can add the -qcache flag to the original recon-all command; that is, you no longer have to run recon-all -all and then run recon-all -qcache afterwards (you can now do it all in one command). Note that the hippocampal calculations can require around 10GB of RAM per scan.

You can run the hippocampal subfield segmentation routine on previously processed recon-all data, but they *must* have been processed using version 5.3 and above. The results are contained in each subject's /mri/ directory in files named "lh.hippoSfVolumes-T1.v10.txt" and "rh.hippoSfVolumes-T1.v10.txt". To gather the hippocampal subfield volumes from all subjects, type in: **quantifyHippocampalSubfields.sh <T1>-<analysisID> <output_file> <OPTIONAL_subject_directory>** (note that the third [last] argument is not necessary if the environment variable SUBJECTS_DIR has been defined).

IMPORTANT: To run the hippocampal subfield segmentation for version 6 ("dev"), you need to download and install Matlab Runtime:

LINUX:

cd /usr/local/freesurfer

curl "http://surfer.nmr.mgh.harvard.edu/fswiki/MatlabRuntime?action=AttachFile&do=get&target=runtime2012bLinux.tar.gz" -o "runtime2012b.tar.gz"

tar xvf runtime2012b.tar.gz

MAC:

cd /usr/local/freesurfer

curl "http://surfer.nmr.mgh.harvard.edu/fswiki/MatlabRuntime?action=AttachFile&do=get&target=runtime2012bMAC.tar.gz" -o "runtime2012b.tar.gz"

tar xvf runtime2012b.tar.gz

Now all you need is to append the flag -hippocampal-subfields-T1 to your recon-all command. For example, to analyze your subject "bert", you would type:

recon-all -all -s bert -hippocampal-subfields-T1

Or, if Bert has already undergone the FreeSurfer pipeline (recon-all -all), you can just run:

recon-all -s bert -hippocampal-subfields-T1

Contrast-to-noise ratio (CNR)

To calculate the contrast-to-noise ratio (CNR) of a T1-weighted scan, type in the following:

mri_cnr "$SUBJECTS_DIR"/surf "$SUBJECTS_DIR"/mri/norm.mgz

Signal-to-noise-ratio (SNR)

To calculate SNR:

wm-anat-snr --s "$SUBJECTS_DIR"/subjectID

Euler

To extract the Euler statistics for your scans (i.e. 2 - 2*number of surface defects) before being fixed (before any smoothing operations are applied and estimates the topological correctness of one's surface model), type in the following:

grep - A 2 "Computing euler" "$SUBJECTS_DIR "/scripts/recon-all.log

If that doesn't work, then the words "Computing euler" are not in the all.log files. So, leave out the word "Computing" and just type in:

grep - A 2 "euler" "$SUBJECTS_DIR "/scripts/recon-all.log

To have no defects (holes, handles) is quite unusual; I have found that on average each scan has around 15 to 20 holes/handles (so the Euler values would be around -28 to -38). Chalavi et al. (2012) provides some normative data based on a set of scans.

Viewing FreeSurfer hippocampal subfield output: Freeview

The alternative FreeSurfer viewer is called Freeview and is very easy to use.

For example, to open a subject's T1-weighted scan and then superimpose the hippocampal subfields segmentations, type in:

freeview

- Select **File → Load Volume** (or use the Load button on the left toolbar)
- Select the **T1.mgz** file in the subject's "mri" directory
- Open another volume (**File → Load Volume**, or use the Load button [green +])
- Select the left (or right) hippocampal segmentations i.e. **lh.hippSfLabels-T1.v10.mgz**
- Change the **Color Map** (on the left toolbar) to "**Lookup Table**". The hippocampal subfields will now appear as different colours.
- Click the box "**Show existing labels only**"

NOTE: *If you encounter an error that states "error while loading shared libraries: libjpeg.so.62: cannot open shared object file: No such file or directory", then run the following from a terminal (https://surfer.nmr.mgh.harvard.edu/fswiki/ReleaseNotes):*

sudo apt-get install libjpeg62-dev

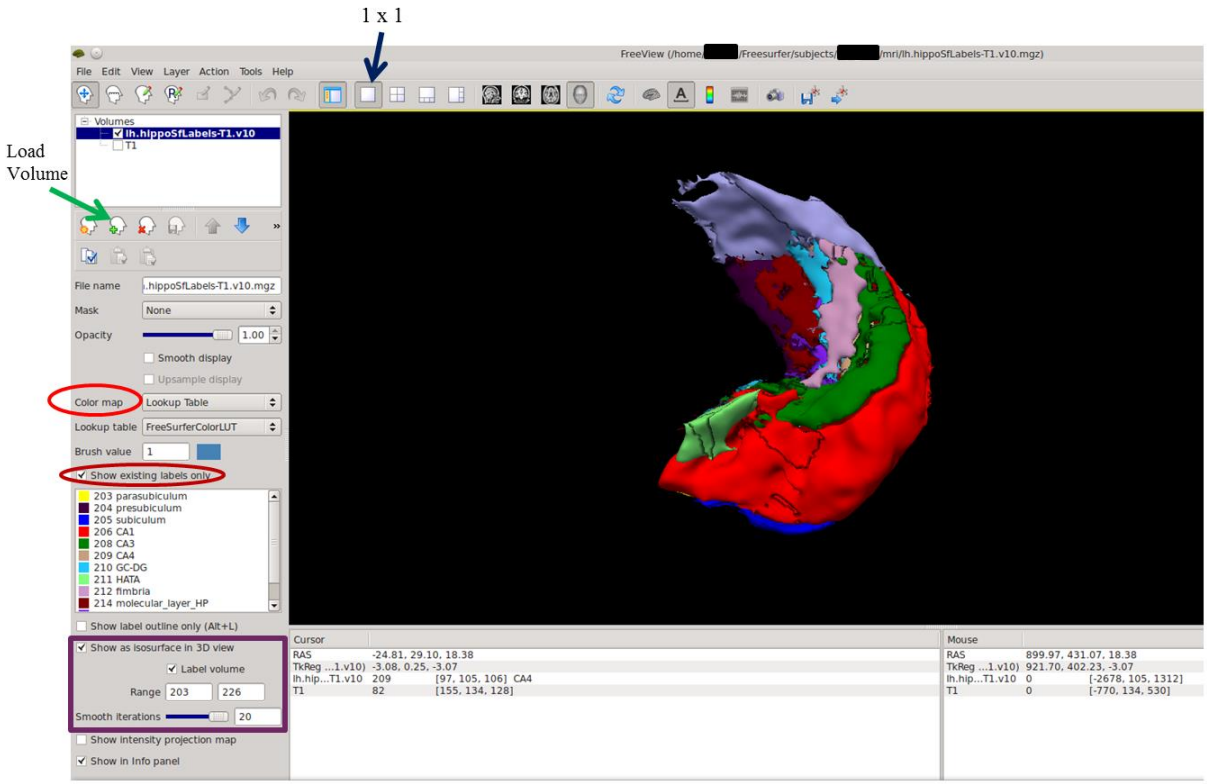

Figure 24. A 3-dimensional rendering of hippocampal segmentation in freeview.

- To render the subfields without the T1 scan in 3D, unclick the T1 scan (upper left), and then click "**Show as isosurface in 3D view**"
- In the "**Range**" boxes, type in **203** and **226** (these are the subfield label numbers). Press Enter.
- Select the **1x1 box** (top toolbar)
- **Right-click** anywhere on the 3D image and select **Show Slice Frames (3D View)**, the frames will now be removed
- Slide the mouse whilst holding down the right mouse button to zoom, the left button to rotate, and the middle button to move the object.
- To generate a smoother rendering, slide the "**Smooth iterations**" bar to the right (maximum value is 20).

Note: After loading the qdec.dat into Qdec and clicking on "Generate Stats Data Tables", several .dat files containing, left and right volumes, thickness etc will be produced in the /qdec/stats folder. To merge all of those.dat files into one single file:

paste -d lh*.dat > all_left.dat

paste -d rh*.dat > all_right.dat

paste -d aseg*.dat all_left.dat all_right.dat > all_subjects_output.dat

14. Protocol to process SWI/SWAN images from magnitude and phase data

1. Use SPIN
2. Process →MIP/SWI processing (Figure 24)
3. Option 2 (MIP with Phase multiplication)
4. Phase masking: use "positive"
5. Select Phase (2nd dicom folder)
6. Select Magnitude (1st dicom folder)
7. Number of slices for each MIP: 1
8. Number of multiplications: play with it, but default is 4 (watch for false positives if use a higher value)
9. MIP prefix (=SWI image): change to a meaningful name
10. High pass filter: 160
11. Vertical slice: 160
12. Note: reduce HP filter and Vertical slice (e.g. 64) to show "more" vasculature but will increase artefact

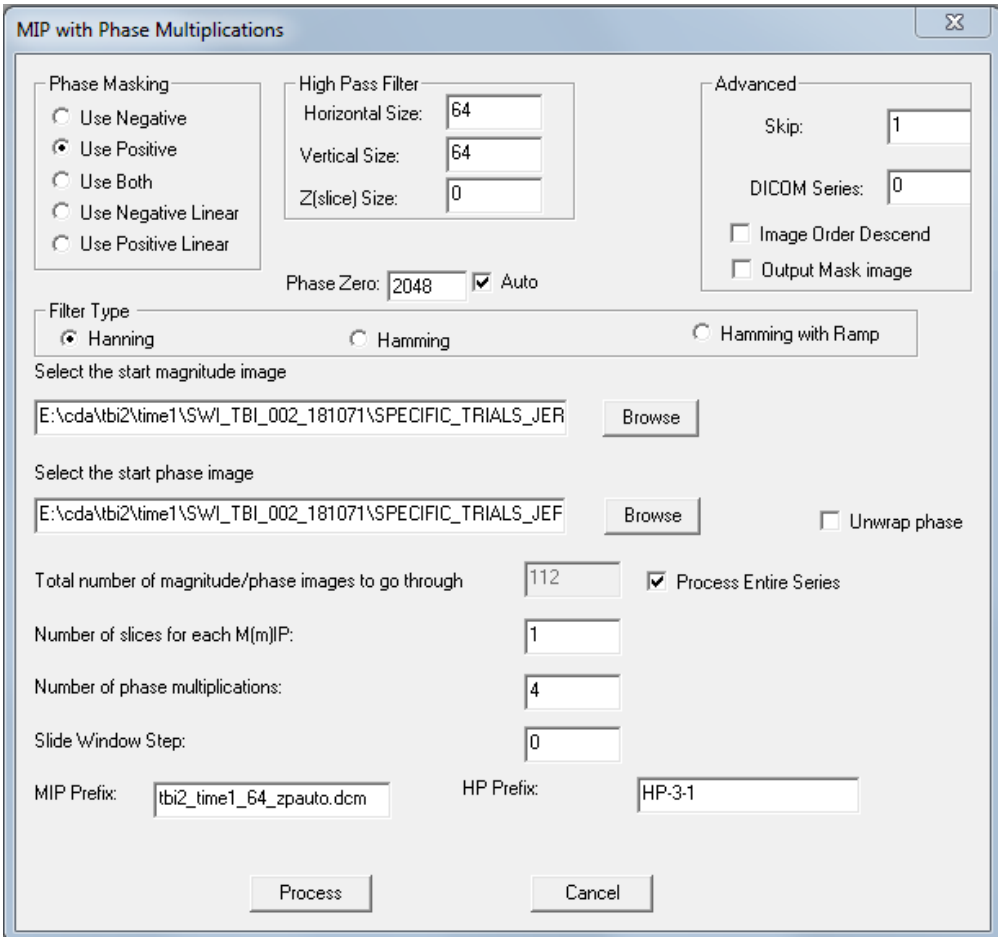

Figure 25. Options within SPIN for processing SWI images from phase and magnitude data

15. FSL (Linux/MAC)

FSL (Smith et al., 2004) includes a number of modules for T1-weighted and DTI analyses, although it can also be used for fMRI processing. Always make sure to download the most current version. If you encounter an error during installation that states something like fsl-complete is available but not downloaded, just try another server.

To install FSL in bash, type in the following (it is best to add this to the setup file):

FSLDIR=/usr/local/fsl

. ${FSLDIR}/etc/fslconf/fsl.sh *# note the space between the . and $*

PATH=${FSLDIR}/bin:${PATH}

export FSLDIR PATH

Alternatively, you can download an automated installation script (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FslInstallation)

After typing in "fsl" in the console a GUI will appear (Figure 25).

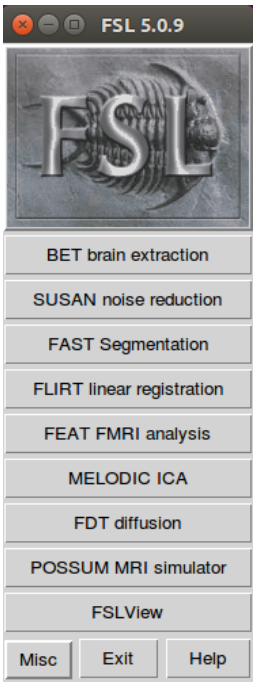

Figure 26. The FSL GUI.

BET (Brain extraction tool): This strips the skull and eyes from the scan to leave just brain. Very useful, very accurate, and very quick. Hint: If you T1-weighted scan has a lot of neck in it, consider clipping the scan to remove the neck by typing in the following:

robustfov "name_of_t1_scan"

FAST (Fully Automated Segmentation Tool): input your BET'ed T1-weighted scan, and after a few second the output will be GM, WM, and CSF, saved as separate images as well as volumetrics for each of these components.

Some tips on settings and interpretation of FSL FAST output viewing in MRIcro

Change the colour to HOT METAL

Change the following intensity and contrast value as follows:

Black = 0

White = 4

Colours for each component:

TIS1 (WM) = orange

TIS2 (GM) = red

CSF = blue

NOTE: There are MANY packages available to compute gray matter, white matter, and CSF volumes from T1-weighted scans, including FSL (FAST), SPM, FreeSurfer, and many more…

Useful in dMRI processing:

To flip gradients automatically in a bvec, for example the x-gradients, try this:

cat bvec.bvec | awk '{ print -$1, $2, $3; }' > new_bvec.txt

16. Tract-Based Spatial Statistics (TBSS) which is part of FSL

For every subject's DTI scan, you must complete the following pre-processing steps:

1. **Convert each scan to FSL 4D NIfTI nii format** (via dcm2nii; this produces 3 files: a big data file (.nii) + a small .bvec file + a small .bval file; Figure 26)

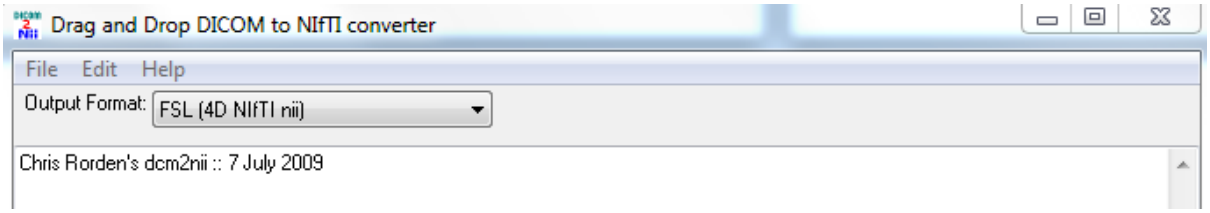

Figure 27. Use the correct option for converting dicoms to FSL (4D NIfTi nii) format.

2. **Eddy current correct** the niifti scan by doing the following:
3. Type in "**fsl**"
4. Select "**FDT diffusion**" (Figure 27).
5. Click on the drop-down box and select "**Eddy current correction**"

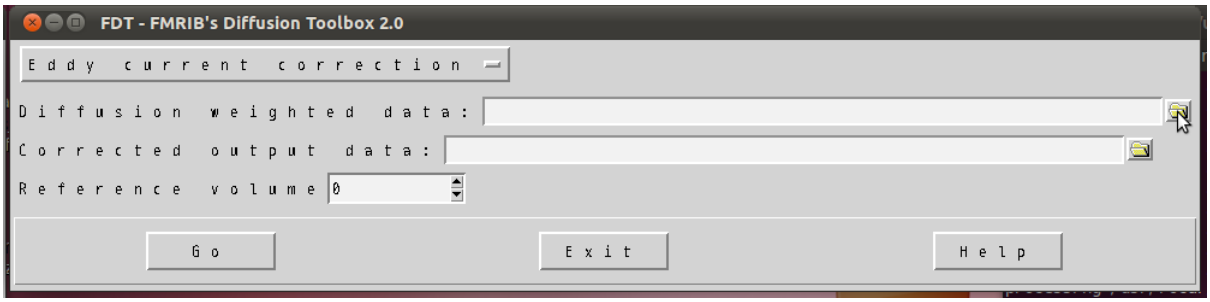

Figure 28. The FDT GUI in FSL.

6. Open the directory where your .nii file is and select it. The default output file will be called "**data.nii.gz**"

Click "**Go**". The eddy current correction takes at least 5 minutes to process, depending upon the number of directions and number of slices to be processed. After it has completed, click on the "**OK**" (in the "Done" box), then "**Exit**" on the FDT box. ***It is now known that the eddy in FSL versions before 5.0.9 may make the output worse. So, a new version of eddy was introduced in 5.0.9 which also produces a rotated bvec file. We strongly urge you to use version 5.0.9 (or newer). UPDATE: If you have downloaded the Neurodebian eddy patch (***http://neuro.debian.net/pkgs/fsl-5.0-eddy-nonfree.html ***), then eddy can only utilize one of your CPU cores. To make eddy much faster, you can download eddy_openmp (see Part 3***

of this Handbook) and then eddy will use as many cores as you have available. Just place eddy_openmp in the bin directory where your eddy is (to locate the directory, type in 'which eddy'), and then you can use it. Then, instead of typing in 'eddy', just type in 'eddy_openmp'. Make sure to give yourself permission to use this new file (sudo chmod 775 eddy_openmp).

7. Exit FSL
8. In the terminal, navigate to the directory you are working with, and type in the following which will extract the first volume of your data (i.e. the b-zero [T2-weighted scan]) and labels it as nodif):
9. **fslroi data.nii.gz nodif 0 1**
10. **bet nodif.nii.gz nodif_brain -m -f 0.3** (this command BETs the no diffusion (b=zero) scan and then masks it as a value of 0.3 which works well for most head sizes)
11. Then, type in "**fslview**"
12. **File → Open** (select the output of the eddy current correction process called "data")
13. Open the mask in fslview (**File → Add**, then select the file **nodif_brain_mask**) and then edit the mask (don't forget to **click the 'lock' box** [icon at the bottom of the screen that looks like a padlock]). **Tools → Single**, then axial view of the scan and mask will appear on the right-hand side of the screen (Figure 28).
14. **Click on the button that looks like a pencil**, and change the numbers in the 2 boxes from 1 to 5 (these represent the number of voxels (in 3 directions). Use the + and – buttons (or the scroll button on the mouse, or PageUp and PageDown) to navigate up and down the axial slices)

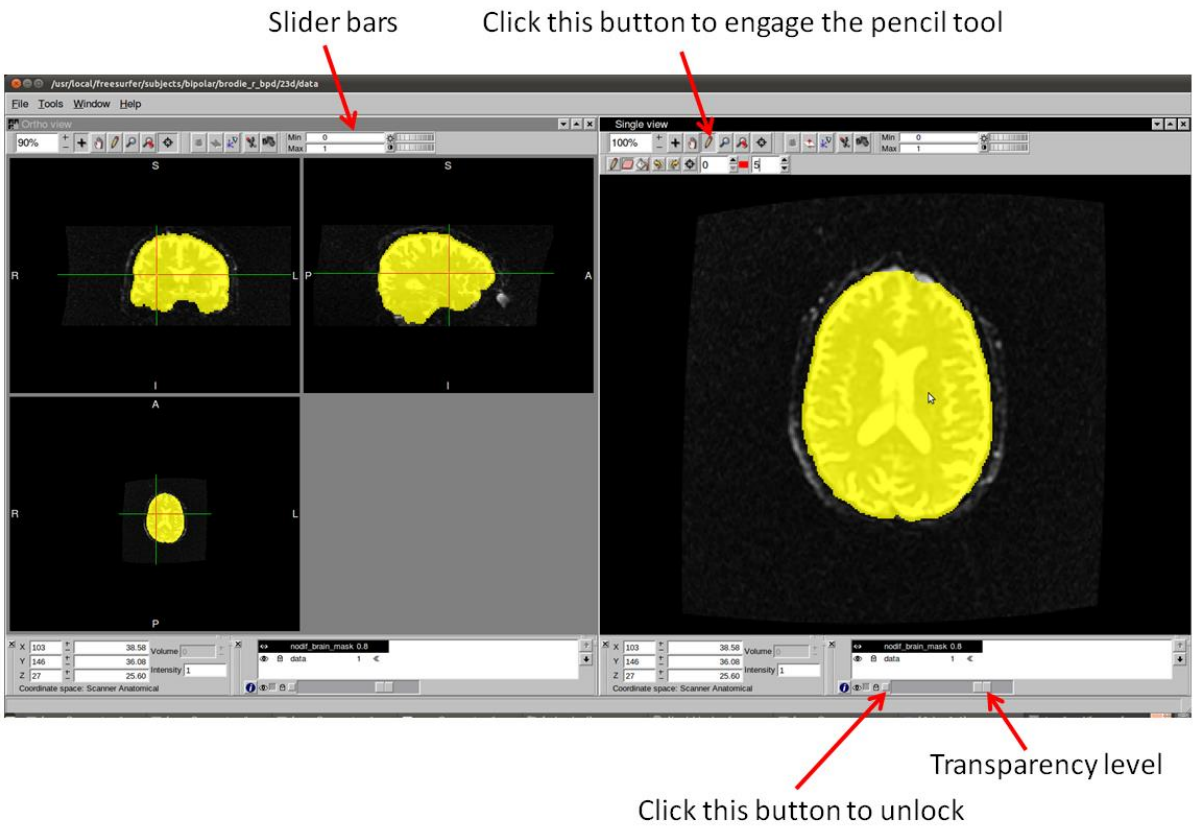

Figure 29. The FSLview interface displaying a T2-weighted image with a mask superimposed. *Note: if you receive an error such as "unable to load AtlasWidget" or an error when you try and load an atlas, download the fsl-atlases package from neuro.debian.net to fix this - it seems to be simply a lack of error handling for the case where atlases are unavailable. Note: FSL version 5.0.10 has a new viewer interface called FSLeyes (pronounced "fossilize").*

15. When you have finished editing the mask, **File → Save As**, and save the mask as "mask1".
16. Fit the diffusion tensor model using the **DTIFIT** toolbox (within FSL; i.e. select FDT Diffusion button), then DTIFIT Reconstruct diffusion tensors, and then select the box "**specify input files manually**". To create the .bvec and .bval files, open them in Excel (separately), transpose them, copy them into Notepad, then save them as separate plain text files
17. **Select the correct files** (data, mask1, output name, gradient directions (.bvec) and b-values (.bval).
18. Click "**Go**"

It's important to know all of these pre-processing steps, so now I can tell you that they can all be carried out with one simple recently-released command called "dti_preprocess". It also dewarps Bzero inhomogeneity distortion. Furthermore, if you add the -B flag at the end of the command, then a rotated bvec will also be produced. To install this script, follow the instructions on this url: http://www.bic.mni.mcgill.ca/~thayashi/dti.html . Make sure to check the mask before viewing the output; if the mask is not accurate then you will need to

fix it, modify the script to call your edited mask, and then rerun the script. Some good editors are vi, nano, and kate. *However*, it is based on the old eddy command, not the one introduced in 5.0.9.

THEN, you are ready for group analyses, as follows:

1. Navigate to the "mytbss" directory and type in the following (analyses **MUST** be run from a directory called mytbss, and you **MUST** have the template in that directory called FA_2_FMRIB58_1mm.cnf, unless you choose to use your own template)
2. **tbss_1_preproc *.nii.gz**
3. After a few minutes, a message will appear in the terminal suggesting step 2 (which will take around 5 to 15 minutes per scan, depending upon the scan dimensions and your computer's resources), but first, check the output of tbss_1 by navigating to the mytbss/FA/slicesdir directory and double-clicking on the file called "**index.html**": this will display 9 slices from each subject's scan – check that none are upside down, back to front, or too bright or dull. To flip a scan along x, y, or z, try different combinations of the following command: fslswapdim data.nii.gz z y x dataoutput.nii.gz. If upside down, try: fslswapdim data.nii.gz –x y –z dataoutput.nii.gz
4. After you are sure each scan is in the correct orientation, proceed to the next step:
5. **tbss_2_reg -T**
6. **tbss_3_postreg -S**
7. Now you can check the output by typing the following:
8. **cd stats**
9. **fslview all_FA -b 0,0.8 mean_FA_skeleton -b 0.2,0.8 -l Green**
10. For Step 4 (thesholding, which will take a minute or two), type in the following:
11. **tbss_4_prestats 0.2**
12. Now run the group stats. For this, first check the order of your scans by typing:
13. **cd FA**
14. **imglob *_FA.*** This will list all of the subjects' data in the order to be processed
15. Then run the analysis:
16. **cd ../stats**
17. **design_ttest2 design 7 11** (this makes the matrix; note the numbers in this example are for comparing group 1 with N=7 versus group 2 with N=11)
18. Then type the following (will take a few hours to process)
19. **randomise -i all_FA_skeletonised -o tbss -m mean_FA_skeleton_mask -d design.mat -t design.con -n 5000 --T2 -v** ***IMPORTANT! Note the double small dash before T2 i.e. --T2, not dash T2 i.e. -T2***
20. To view the results, type in the following:
21. **fslview $FSLDIR/data/standard/MNI152_T1_1mm mean_FA_skeleton -l Green -b 0.2,0.8 tbss_corrp_tstat1 -l Red-Yellow -b 3,6 tbss_tstat2 -l Blue-LightBlue -b 3,6**
22. Note: tbss_p_tstat is the UNCORRECTED result, tbss_corrp_tstat is the CORRECTED result.

Note: to view significant differences at p=0.05, change the first slider bar (Min) to 0.95 (enter it manually rather than using the slider bar), and the value below it (Max) to 1 (as 1-0.95=0.05). **Update:** To produce uncorrected results from randomize on FSL 5.0.9 (and above) you now need to add the -uncorrp flag.

To process non-FA data:

1. Create a directory for each non-FA dataset (e.g. L1, L2, L3, MD, radial diffusivity)
2. Copy over the relevant data into those directories
3. Rename each file in the non-FA folders to be **EXACTLY** the same names as listed in the original FA dataset (which will now be in a directory called "origdata"). For example, if your original (FA) data ended in the filename _FA.nii.gz then you must rename your non-FA data file to also end in _FA.nii.gz . You can do this by carrying out the following commands *within* each directory:

   for i in ./*L1*;do mv -- "$i" "${i//L1/FA}";done
   for i in ./*L2*;do mv -- "$i" "${i//L2/FA}";done
   for i in ./*L3*;do mv -- "$i" "${i//L3/FA}";done
   for i in ./*MD*;do mv -- "$i" "${i//MD/FA}";done

4. Type in "**tbss_non_FA L1**" (from the mytbss directory) to begin processing the L1 data
5. **cd stats**
6. **randomise -l all_L1_skeletonised -o tbss_L1 -m mean_FA_skeleton_mask -d design.mat -t design.con -n 5000 --T2 -V**

To name the white matter bundle where there are significant voxels, open the built-in atlases (**Tools → Toolbars → Atlas tools**) and then select the voxel of interest: the atlases will state the name of the white matter fibre that click on.

NOTE: *to create radial diffusivity data (the average of L2 and L3), type in the following script (created by Dr Kerstin Pannek) from your working directory*:

```
l2dir="L2" #edit this line
l3dir="L3" #edit this line
cd $l2dir
list=`ls *.nii.gz`
cd ../
mkdir radial_diffusivity
for x in $list
do
        echo "calculating radial diffusivity for $x"
        fslmaths $l2dir/$x -add $l3dir/$x -div 2 radial_diffusivity/$x
```

done

Presenting the results

If you don't like typing commands, then follow these steps to show the results (assuming there is greater FA in the first group):

1. Open **fslview**
2. **File → Open**
3. Select the file called "**all_FA.nii.gz**"
4. Select the file called "**all_FA_skeletonized.nii.gz**"
5. Select the file called "**tbss_tfce_corrp_tstat1.nii.gz**"
6. Change the **Min value to 0.95** and the **Max value to 1**
7. The red-yellow voxels are the voxels of FA that are significantly greater in the first group (if there is greater FA in the second group, then these voxels will appear after loading tbss_tfce_corrp_tstat2.nii.gz)
8. To show the results in 3d, select "**Tools**" then "**3D Viewer**" and adjust the transparencies accordingly
9. If you want to show the differences more easily, then you need to use the "**fill**" command that makes the white matter voxels thicker so they are more easily visualised:

tbss_fill tbss_tfce_corrp_tstat1 0.95 mean_FA tbss_fillFAp95

That command will apply the "tbss_fill" command to the file called "tbss_tfce_corrp_tstat1", threshold it at a p-value of 0.95, and save the output as "tbss_fillFAp95"

The result (tbss_fillFAp95) can then be added in fslview.

If you want to show these results using a Windows program, then use MRIcro:

1. **File → Open**
2. **Select** the T1-weighted or MNI DTI template
3. **Overlay → Load image overlay**
4. Select "**tbss_fillFAp95**"
5. The tbss_fillFAp95 will now be superimposed onto the template
6. To show the differences slice-by-slice, click on the "**Etc**" pull-down menu then select "**Options**"
7. Enter the slice numbers you want to display, and whether you want to show left hemisphere over right (or the other way around), the extent of slice overlap, and whether you want to display transverse (axial) or coronal slices, then click "**OK**". Figure 29 is an example of the screen after you have completed this (the top row is slice by slice with significant FA voxel at a p-value of 0.05, and the bottom row is fslview using the 3d viewer; merged in Powerpoint):

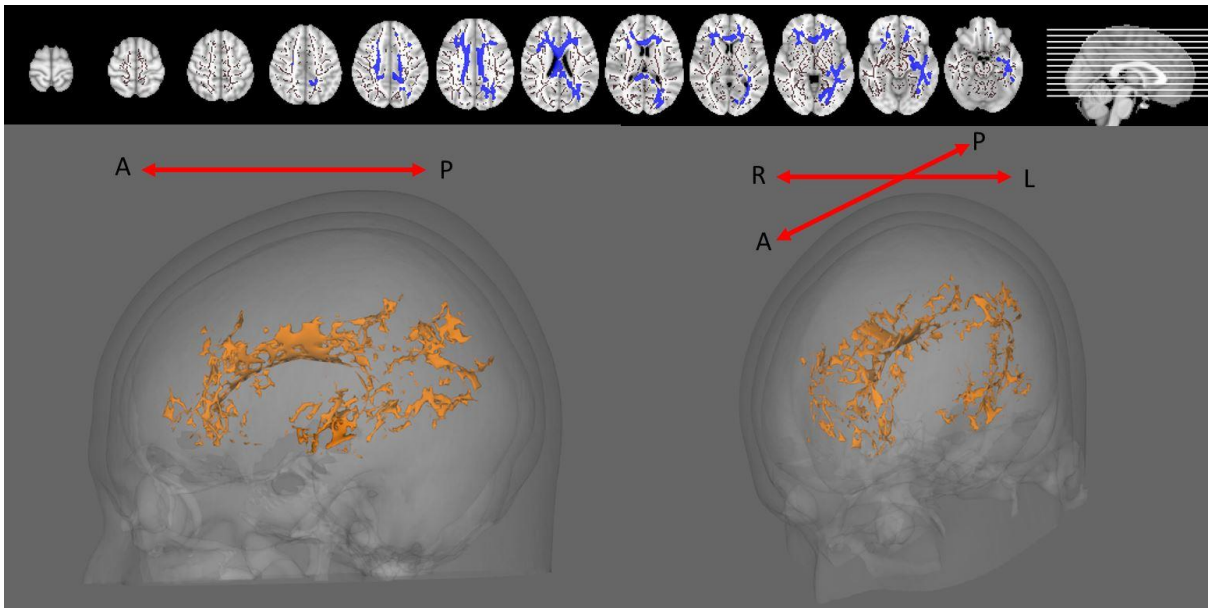

Figure 30. TBSS results presented from axial and 3D-rendered perspectives.

Note about eddy current and movement correction:

If you have used the older version of the FSL GUI for eddy and movement correction (and not 'eddy' or 'dti-preprocess'), then you will need, for each subject, to create individualised gradient tables (.bvec) based on the ecclog (called "data.ecclog", which is an output file after correction is complete in FSL), as suggested by Leemans and Jones (2009) and Rohde et al. (2004). To do this, type in the following fdt_rotate_bvecs command:

fdt_rotate_bvecs nameoforiginalgradienttable.bvec nameofnewgradienttable.bvec data.ecclog

Sometimes you will need to transpose (easiest to do this in Excel) the original gradient table because fdt_rotate_bvecs expects the original bvec file to be 3 columns (not 3 rows). Note that dti_process rotates the bvec for you if you add a -B flag at the end of the command.

To extract mean value for ROIs according to the JHU White Matter Atlas

You need to extract the ROIs from the Atlas and convert them into masks; they are numbered as "intensities" when viewed with FSLview. For example, corpus callosum (middle) is 4.

First, copy the file "JHU-WhiteMatter-labels-1mm.nii.gz" from the fsl/atlases directory into your tbss/stats directory. Then extract the ROIs (to make them masks) with the following commands (script created by Dr Melissa Kirkovski):

```
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 1 -uthr 1 cer_peduncle
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 2 -uthr 2 pontine_crossing_tract
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 3 -uthr 3 ant_CC
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 4 -uthr 4 CC
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 5 -uthr 5 post_CC
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 7 -uthr 7 R_corticospinal_tract
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 8 -uthr 8 L_corticospinal_tract
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 11 -uthr 11 R_Inf_cer_peduncle
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 12 -uthr 12 L_Inf_cer_peduncle
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 13 -uthr 13 R_Sup_cer_peduncle
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 14 -uthr 14 L_Sup_cer_peduncle
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 15 -uthr 15 R_cer_peduncle
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 16 -uthr 16 L_cer_peduncle
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 17 -uthr 17 r_alic
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 18 -uthr 18 l_alic
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 19 -uthr 19 r_plic
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 20 -uthr 20 l_plic
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 23 -uthr 23 R_Ant_cor_rad
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 24 -uthr 24 L_Ant_cor _rad
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 25 -uthr 25 R_Sup_cor_rad
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 26 -uthr 26 L_Sup_cor_rad
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 27 -uthr 27 R_Post_cor_rad
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 28 -uthr 28 L_Post_cor_rad
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 31 -uthr 31 R_ilf
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 32 -uthr 32 L_ilf
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 33 -uthr 33 R_unc
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 34 -uthr 34 L_unc
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 35 -uthr 35 R_cing
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 36 -uthr 36 L_cing
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 37 -uthr 37 R_cing_hipp
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 38 -uthr 38 R_cing_hipp
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 39 -uthr 39 R_hipp
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 40 -uthr 40 L_hipp
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 41 -uthr 41 R_slf
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 42 -uthr 42 L_slf
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 45 -uthr 45 R_unc_needsTBC
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 46 -uthr 46 L_unc_needsTBC
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 47 -uthr 47 R_tapetum
fslmaths JHU-WhiteMatter-labels-1mm.nii.gz -thr 48 -uthr 48 L_tapetum
```

Then, apply the "fslmeants" command to extract the values within each ROI (mask) from your all_FA.nii.gz file:

FA

```
fslmeants -i all_FA.nii.gz -o FA_mean_cer_peduncle.txt -m cer_peduncle.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_pontine_crossing_tract.txt -m pontine_crossing_tract.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_ant_CC.txt -m ant_CC.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_CC.txt -m CC.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_post_CC.txt -m post_CC.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_corticospinal_tract.txt -m R_corticospinal_tract.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_corticospinal_tract.txt -m L_corticospinal_tract.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_Inf_cerebellar_peduncle.txt -m R_Inf_cer_peduncle.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_Inf_cer_peduncle.txt -m L_Inf_cer_peduncle.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_Sup_cer_peduncle.txt -m R_Sup_cer_peduncle.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_Sup_cer_peduncle.txt -m L_Sup_cer_peduncle.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_cer_peduncle.txt -m R_cer_peduncle.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_cer_peduncle.txt -m L_cer_peduncle.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_r_alic.txt -m r_alic.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_l_alic.txt -m l_alic.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_r_plic.txt -m r_plic.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_l_plic.txt -m l_plic.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_Ant_cor_rad.txt -m R_Ant_cor_rad.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_Ant_cor_rad.txt -m L_Ant_cor_rad.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_Sup_cor_rad.txt -m R_Sup_cor_rad.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_Sup_cor_rad.txt -m L_Sup_cor_rad.nii.gz
```

```
fslmeants -i all_FA.nii.gz -o FA_mean_R_Post_cor_rad.txt -m R_Post_cor_rad.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_Post_cor_rad.txt -m L_Post_cor_rad.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_ilf.txt -m R_ilf.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_ilf.txt -m L_ilf.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_unc.txt -m R_unc.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_unc.txt -m L_unc.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_cing.txt -m R_cing.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_cing.txt -m L_cing.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_cing_hipp.txt -m R_cing_hipp.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_cing_hipp.txt -m L_cing_hipp.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_hipp.txt -m R_hipp.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_hipp.txt -m L_hipp.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_slf.txt -m R_slf.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_slf.txt -m L_slf.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_unc_needsTBC.txt -m R_unc_needsTBC.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_unc_needsTBC.txt -m L_unc_needsTBC.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_R_tapetum.txt -m R_tapetum.nii.gz
fslmeants -i all_FA.nii.gz -o FA_mean_L_tapetum.txt -m L_tapetum.nii.gz
```

L1

```
fslmeants -i all_L1.nii.gz -o L1_mean_cer_peduncle.txt -m cer_peduncle.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_pontine_crossing_tract.txt -m pontine_crossing_tract.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_ant_CC.txt -m ant_CC.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_CC.txt -m CC.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_post_CC.txt -m post_CC.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_corticospinal_tract.txt -m R_corticospinal_tract.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_corticospinal_tract.txt -m L_corticospinal_tract.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_Inf_cerebellar_peduncle.txt -m R_Inf_cer_peduncle.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_Inf_cer_peduncle.txt -m L_Inf_cer_peduncle.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_Sup_cer_peduncle.txt -m R_Sup_cer_peduncle.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_Sup_cer_peduncle.txt -m L_Sup_cer_peduncle.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_cer_peduncle.txt -m R_cer_peduncle.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_cer_peduncle.txt -m L_cer_peduncle.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_r_alic.txt -m r_alic.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_l_alic.txt -m l_alic.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_r_plic.txt -m r_plic.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_l_plic.txt -m l_plic.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_Ant_cor_rad.txt -m R_Ant_cor_rad.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_Ant_cor_rad.txt -m L_Ant_cor_rad.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_Sup_cor_rad.txt -m R_Sup_cor_rad.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_Sup_cor_rad.txt -m L_Sup_cor_rad.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_Post_cor_rad.txt -m R_Post_cor_rad.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_Post_cor_rad.txt -m L_Post_cor_rad.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_ilf.txt -m R_ilf.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_ilf.txt -m L_ilf.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_unc.txt -m R_unc.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_unc.txt -m L_unc.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_cing.txt -m R_cing.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_cing.txt -m L_cing.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_cing_hipp.txt -m R_cing_hipp.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_cing_hipp.txt -m L_cing_hipp.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_hipp.txt -m R_hipp.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_hipp.txt -m L_hipp.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_slf.txt -m R_slf.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_slf.txt -m L_slf.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_unc_needsTBC.txt -m R_unc_needsTBC.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_unc_needsTBC.txt -m L_unc_needsTBC.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_R_tapetum.txt -m R_tapetum.nii.gz
fslmeants -i all_L1.nii.gz -o L1_mean_L_tapetum.txt -m L_tapetum.nii.gz
```

L2

```
fslmeants -i all_L2.nii.gz -o L2_mean_cer_peduncle.txt -m cer_peduncle.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_pontine_crossing_tract.txt -m pontine_crossing_tract.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_ant_CC.txt -m ant_CC.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_CC.txt -m CC.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_post_CC.txt -m post_CC.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_corticospinal_tract.txt -m R_corticospinal_tract.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_corticospinal_tract.txt -m L_corticospinal_tract.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_Inf_cerebellar_peduncle.txt -m R_Inf_cer_peduncle.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_Inf_cer_peduncle.txt -m L_Inf_cer_peduncle.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_Sup_cer_peduncle.txt -m R_Sup_cer_peduncle.nii.gz
```

```
fslmeants -i all_L2.nii.gz -o L2_mean_L_Sup_cer_peduncle.txt -m L_Sup_cer_peduncle.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_cer_peduncle.txt -m R_cer_peduncle.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_cer_peduncle.txt -m L_cer_peduncle.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_r_alic.txt -m r_alic.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_l_alic.txt -m l_alic.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_r_plic.txt -m r_plic.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_l_plic.txt -m l_plic.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_Ant_cor_rad.txt -m R_Ant_cor_rad.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_Ant_cor_rad.txt -m L_Ant_cor_rad.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_Sup_cor_rad.txt -m R_Sup_cor_rad.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_Sup_cor_rad.txt -m L_Sup_cor_rad.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_Post_cor_rad.txt -m R_Post_cor_rad.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_Post_cor_rad.txt -m L_Post_cor_rad.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_ilf.txt -m R_ilf.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_ilf.txt -m L_ilf.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_unc.txt -m R_unc.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_unc.txt -m L_unc.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_cing.txt -m R_cing.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_cing.txt -m L_cing.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_cing_hipp.txt -m R_cing_hipp.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_cing_hipp.txt -m L_cing_hipp.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_hipp.txt -m R_hipp.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_hipp.txt -m L_hipp.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_slf.txt -m R_slf.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_slf.txt -m L_slf.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_unc_needsTBC.txt -m R_unc_needsTBC.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_unc_needsTBC.txt -m L_unc_needsTBC.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_R_tapetum.txt -m R_tapetum.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_tapetum.txt -m L_tapetum.nii.gz
```

L3

```
fslmeants -i all_L3.nii.gz -o L3_mean_cer_peduncle.txt -m cer_peduncle.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_pontine_crossing_tract.txt -m pontine_crossing_tract.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_ant_CC.txt -m ant_CC.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_CC.txt -m CC.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_post_CC.txt -m post_CC.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_corticospinal_tract.txt -m R_corticospinal_tract.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_corticospinal_tract.txt -m L_corticospinal_tract.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_Inf_cerebellar_peduncle.txt -m R_Inf_cer_peduncle.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_Inf_cer_peduncle.txt -m L_Inf_cer_peduncle.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_Sup_cer_peduncle.txt -m R_Sup_cer_peduncle.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_Sup_cer_peduncle.txt -m L_Sup_cer_peduncle.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_cer_peduncle.txt -m R_cer_peduncle.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_cer_peduncle.txt -m L_cer_peduncle.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_r_alic.txt -m r_alic.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_l_alic.txt -m l_alic.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_r_plic.txt -m r_plic.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_l_plic.txt -m l_plic.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_Ant_cor_rad.txt -m R_Ant_cor_rad.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_Ant_cor_rad.txt -m L_Ant_cor_rad.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_Sup_cor_rad.txt -m R_Sup_cor_rad.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_Sup_cor_rad.txt -m L_Sup_cor_rad.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_Post_cor_rad.txt -m R_Post_cor_rad.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_Post_cor_rad.txt -m L_Post_cor_rad.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_ilf.txt -m R_ilf.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_ilf.txt -m L_ilf.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_unc.txt -m R_unc.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_unc.txt -m L_unc.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_cing.txt -m R_cing.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_cing.txt -m L_cing.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_cing_hipp.txt -m R_cing_hipp.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_cing_hipp.txt -m L_cing_hipp.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_hipp.txt -m R_hipp.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_hipp.txt -m L_hipp.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_slf.txt -m R_slf.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_slf.txt -m L_slf.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_unc_needsTBC.txt -m R_unc_needsTBC.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_unc_needsTBC.txt -m L_unc_needsTBC.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_R_tapetum.txt -m R_tapetum.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_tapetum.txt -m L_tapetum.nii.gz
```

MD

```
fslmeants -i all_MD.nii.gz -o MD_mean_cer_peduncle.txt -m cer_peduncle.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_pontine_crossing_tract.txt -m pontine_crossing_tract.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_ant_CC.txt -m ant_CC.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_CC.txt -m CC.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_post_CC.txt -m post_CC.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_corticospinal_tract.txt -m R_corticospinal_tract.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_corticospinal_tract.txt -m L_corticospinal_tract.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_Inf_cerebellar_peduncle.txt -m R_Inf_cer_peduncle.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_Inf_cer_peduncle.txt -m L_Inf_cer_peduncle.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_Sup_cer_peduncle.txt -m R_Sup_cer_peduncle.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_Sup_cer_peduncle.txt -m L_Sup_cer_peduncle.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_cer_peduncle.txt -m R_cer_peduncle.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_cer_peduncle.txt -m L_cer_peduncle.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_r_alic.txt -m r_alic.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_l_alic.txt -m l_alic.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_r_plic.txt -m r_plic.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_l_plic.txt -m l_plic.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_Ant_cor_rad.txt -m R_Ant_cor_rad.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_Ant_cor_rad.txt -m L_Ant_cor_rad.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_Sup_cor_rad.txt -m R_Sup_cor_rad.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_Sup_cor_rad.txt -m L_Sup_cor_rad.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_Post_cor_rad.txt -m R_Post_cor_rad.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_Post_cor_rad.txt -m L_Post_cor_rad.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_ilf.txt -m R_ilf.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_ilf.txt -m L_ilf.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_unc.txt -m R_unc.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_unc.txt -m L_unc.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_cing.txt -m R_cing.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_cing.txt -m L_cing.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_cing_hipp.txt -m R_cing_hipp.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_cing_hipp.txt -m L_cing_hipp.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_hipp.txt -m R_hipp.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_hipp.txt -m L_hipp.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_slf.txt -m R_slf.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_slf.txt -m L_slf.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_unc_needsTBC.txt -m R_unc_needsTBC.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_unc_needsTBC.txt -m L_unc_needsTBC.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_R_tapetum.txt -m R_tapetum.nii.gz
fslmeants -i all_MD.nii.gz -o MD_mean_L_tapetum.txt -m L_tapetum.nii.gz
```

Radial_diffusivity

```
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_cer_peduncle.txt -m cer_peduncle.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_pontine_crossing_tract.txt -m pontine_crossing_tract.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_ant_CC.txt -m ant_CC.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_CC.txt -m CC.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_post_CC.txt -m post_CC.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_corticospinal_tract.txt -m R_corticospinal_tract.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_corticospinal_tract.txt -m L_corticospinal_tract.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_Inf_cerebellar_peduncle.txt -m R_Inf_cer_peduncle.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_Inf_cer_peduncle.txt -m L_Inf_cer_peduncle.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_Sup_cer_peduncle.txt -m R_Sup_cer_peduncle.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_Sup_cer_peduncle.txt -m L_Sup_cer_peduncle.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_cer_peduncle.txt -m R_cer_peduncle.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_cer_peduncle.txt -m L_cer_peduncle.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_r_alic.txt -m r_alic.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_l_alic.txt -m l_alic.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_r_plic.txt -m r_plic.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_l_plic.txt -m l_plic.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_Ant_cor_rad.txt -m R_Ant_cor_rad.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_Ant_cor_rad.txt -m L_Ant_cor_rad.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_Sup_cor_rad.txt -m R_Sup_cor_rad.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_Sup_cor_rad.txt -m L_Sup_cor_rad.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_Post_cor_rad.txt -m R_Post_cor_rad.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_Post_cor_rad.txt -m L_Post_cor_rad.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_ilf.txt -m R_ilf.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_ilf.txt -m L_ilf.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_unc.txt -m R_unc.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_unc.txt -m L_unc.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_cing.txt -m R_cing.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_cing.txt -m L_cing.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_cing_hipp.txt -m R_cing_hipp.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_cing_hipp.txt -m L_cing_hipp.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_hipp.txt -m R_hipp.nii.gz
```

```
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_hipp.txt -m L_hipp.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_slf.txt -m R_slf.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_slf.txt -m L_slf.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_unc_needsTBC.txt -m R_unc_needsTBC.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_unc_needsTBC.txt -m L_unc_needsTBC.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_R_tapetum.txt -m R_tapetum.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_tapetum.txt -m L_tapetum.nii.gz
```

To Extract TBSS skeleton

```
fslmeants -i all_FA.nii.gz -o FA_mean_skel.txt -m all_FA_skeletonised.nii.gz

fslmeants -i all_L1.nii.gz -o L1_mean_skel.txt -m all_L1_skeletonised.nii.gz

fslmeants -i all_L2.nii.gz -o L2_mean_skel.txt -m all_L2_skeletonised.nii.gz

fslmeants -i all_L3.nii.gz -o L3_mean_skel.txt -m all_L3_skeletonised.nii.gz

fslmeants -i all_MD.nii.gz -o MD_mean_skel.txt -m all_MD_skeletonised.nii.gz

fslmeants -i all_radial_diffusivity.nii.gz -o RD_mean_skel.txt -m all_radial_diffusivity_skeletonised.nii.gz
```

You will now have many .txt output files. To merge them into a single file with individual variable labels, use the following concatenate Matlab script (created by Caley Sullivan):

```
%A simple script to import and concatenate .txt files

workingfolder = 'E:\workingdirectory'; %locate your files
outfolder =  'E:\workingdirectory';        %location your desired output
outfile = 'Output.txt';                % save as 'something.txt'
m= 42;                             % m= no. data points in .txt files
decimal = '%.9f'  ;                % data precision to 9 decimel points

outpath = fullfile(outfolder, filesep, char(outfile));
cd(workingfolder);

dirListing= dir(fullfile('*.txt'));  % Imports ALL .txt files in folder
                          % Can Edit wilcards if needed
                          %e.g. dir(fullfile('*_ant_CC.txt'));
names={dirListing(:).name};
count= numel(names);
outPath= strcat(outfolder);
ALLdata = NaN(m,count);

for i=names;
n= find(ismember(names,i));
filename= char(i);
DELIM = ' ';
NHEADER= 0;
data = importdata(filename,DELIM,NHEADER);savefile = 'Output.mat';
ALLdata(:,n)=data';
end

fid = fopen(outpath,'wt');
header= sprintf('%s\t',names{:});
header(end)='';
dlmwrite(outpath,header,'delimiter','');
dlmwrite(outpath, ALLdata,'delimiter','\t','precision',decimal,'-append')
fclose(fid);
```

```
cd(outfolder);

clear all;
```

Paired t-test in TBSS

Say you are comparing 12 subjects' data before and after treatment, it might be good to try a paired t-test instead of the normal t-test. Set this up as follows:

1. In all_FA_skeletonised you should have as first 12 subjects the first scan and as second 12 subjects the second scan

2. Use the Glm interface to set up the design and contrast: type **Glm**

3. Change from "timeseries design" to "higher order / non timeseries design"

4. Number of inputs 24

5. Click again on "higher order / non timeseries design" (otherwise it doesn't accept the 24; it's a bug…)

6. Click on "Wizard"

7. Choose "two groups, paired"; "process"

8. Save as design_paired (or something similar)

9. This gives you (among other things) the files design_paired.con and design_paired.mat, which you can use in randomise.

To test for Left vs Right symmetry, type in the following:

tbss_sym FA

Then run the single randomize command as follows:

randomise -i all_FA_skeletonised_left_minus_right.nii.gz -o tbss -m mean_FA_symmetrised_skeleton_mask.nii.gz -1 -n 5000 --T2 –V

To test for L1 symmetry

tbss_sym L1

then:

randomise -i all_L1_skeletonised_left_minus_right.nii.gz -o tbss -m mean_FA_symmetrised_skeleton_mask.nii.gz -1 -n 5000 --T2 -V

Correlating or covarying in TBSS

Using the Glm module in fsl, you can create matrices to correlate DWI data with other variables (e.g. age) or covary for a variable (e.g. severity of depression). This can be accomplished via the FEAT module in FSL, or by typing in **Glm** into a terminal. The following wiki is very useful is creating the appropriate matrix: http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM#Two-Group_Difference_Adjusted_for_Covariate

TBSS cluster stats

Using the 'cluster' command.

Hint: To view options for the cluster command type in to a terminal: cluster -h

Example:

Using the examples below will output a text file with a unique number for each of the significant clusters at the threshold that you set. It will also give location information and the size of the clusters.

Commands for FA, radial diffusivity (RD), mean diffusivity (MD) and the first eigenvalue or axial diffusivity (AD).

-i is the input file from the 'stats' folder

-t is the threshold. In the example 0.95 (i.e., p=0.05).

cluster -i tbss_tfce_corrp_tstat2.nii.gz -t 0.95 --mm > cluster_t2_95_C_FA.txt

cluster -i tbss_L1_tfce_corrp_tstat2.nii.gz -t 0.95 --mm > cluster_t2_95_C_AD.txt

cluster -i tbss_RD_tfce_corrp_tstat2.nii.gz -t 0.95 --mm > cluster_t2_95_C_RD.txt

cluster -i tbss_MD_tfce_corrp_tstat2.nii.gz -t 0.95 --mm > cluster_t2_95_C_MD.txt

cluster -i tbss_tfce_corrp_tstat1.nii.gz -t 0.95 --mm > cluster_t1_95_C_FA.txt

cluster -i tbss_L1_tfce_corrp_tstat1.nii.gz -t 0.95 --mm > cluster_t1_95_C_AD.txt

cluster -i tbss_RD_tfce_corrp_tstat1.nii.gz -t 0.95 --mm > cluster_t1_95_C_RD.txt

cluster -i tbss_MD_tfce_corrp_tstat1.nii.gz -t 0.95 --mm > cluster_t1_95_C_MD.txt

The output file format will look like this:

Cluster Index	Voxels	MAX	MAX X (mm)	MAX Y (mm)	MAX Z (mm)	COG X (mm)
7	16	0.992	-23	-23	-23	-24.5
6	5	0.991	-19	20	55	-18.2
5	5	0.995	40	14	-6	39.6
4	4	0.993	34	18	-1	34
3	2	0.991	-17	21	53	-17
2	1	0.999	6	11	37	6
1	1	0.991	36	18	-2	36

17. DTIstudio: How to measure FA (and other statistics) of white matter tracts

1. Open DTIstudio (Jiang et al., 2006; Figure 30)
2. **File → DTI Mapping** (Figure 31)
3. Select the "**Siemens, GE or Philips DICOM**" option. "**Continue**" (Figure 32)
4. A page entitled, "**DWI-Image Parameters**" will appear: **select** your customized Gradient Table file, and values will then appear in each Section of that page. Note that DTIstudio expects the gradient table file to be in a specific format, such that 0: x,y,z is the first b-zero, etc. That is, the first number represents the volume number, and the three numbers after the semi-colon represent the x,y,z directions of the gradients for that volume (Figure 33)
5. **MAKE SURE THAT THE BOX IS UNTICKED NEXT TO "BYTE SWAP", AND THAT "ALL SLICES" IS SELECTED, "INFERIOR-SUPERIOR**". Make sure the b-zeros look like T2's and you can clearly see the CC
6. Check that each parameter in each Section is correct; edit if required. "**OK**"
7. A new screen will appear with 4 windows (all 3 x 2D orientations + 1 x 3D; Figure 34)
8. Select the **DtiMap tab** at the bottom left of the page
9. Correct for movement by using the **AIR (automatic image registration)** tool: select all diffusion (i.e. not the b-zero) data then move it to the box on the right. "**OK**"
10. Click on the "**Tensor, Colour Maps etc**" tab in the Calculations area (in the right hand column; Figure 35)
11. DTI-Maps options windows will appear. Don't change any of the Defaults. "**OK**". A new window may appear, "**Reject Outliers?**" For the moment, "**Yes**", then "**OK**"
12. Select the **Fibre Tracking button** on the bottom of the right-hand column
13. You may have to **flip** the gradient tables in x, y, or z, depending upon your acquisition parameters "**OK**" (Figure 36)
14. A **new tab** will appear in the very bottom right, "**Fiber**"
15. In the upper region of that right-hand column, Click on "**ROI-Drawing Enable**"
16. **Choose an ROI shape**
17. Apply the shape to one of the 3 quadrants on the screen (i.e. axial, sag, or coronal)
18. Statistics: Click on the wide rectangular "**Statistics**" button (Figure 39)
19. Note in the database (taken from the "**Fiber Statistics; Select Fibers**" output box):
20. Number of fibres
21. Max length (mm)
22. Min length (mm)
23. Average length (mm)
24. # of voxels that fibers go
25. Anisotropy – FA: Max, Min, Mean, Std
26. Anisotropy – RA: Max, Min, Mean, Std

NOTE: DTIstudio uses the FACT tracking algorithm, which means the tractography is deterministic (not probabilistic)

Figure 31. The DTIstudio software interface.

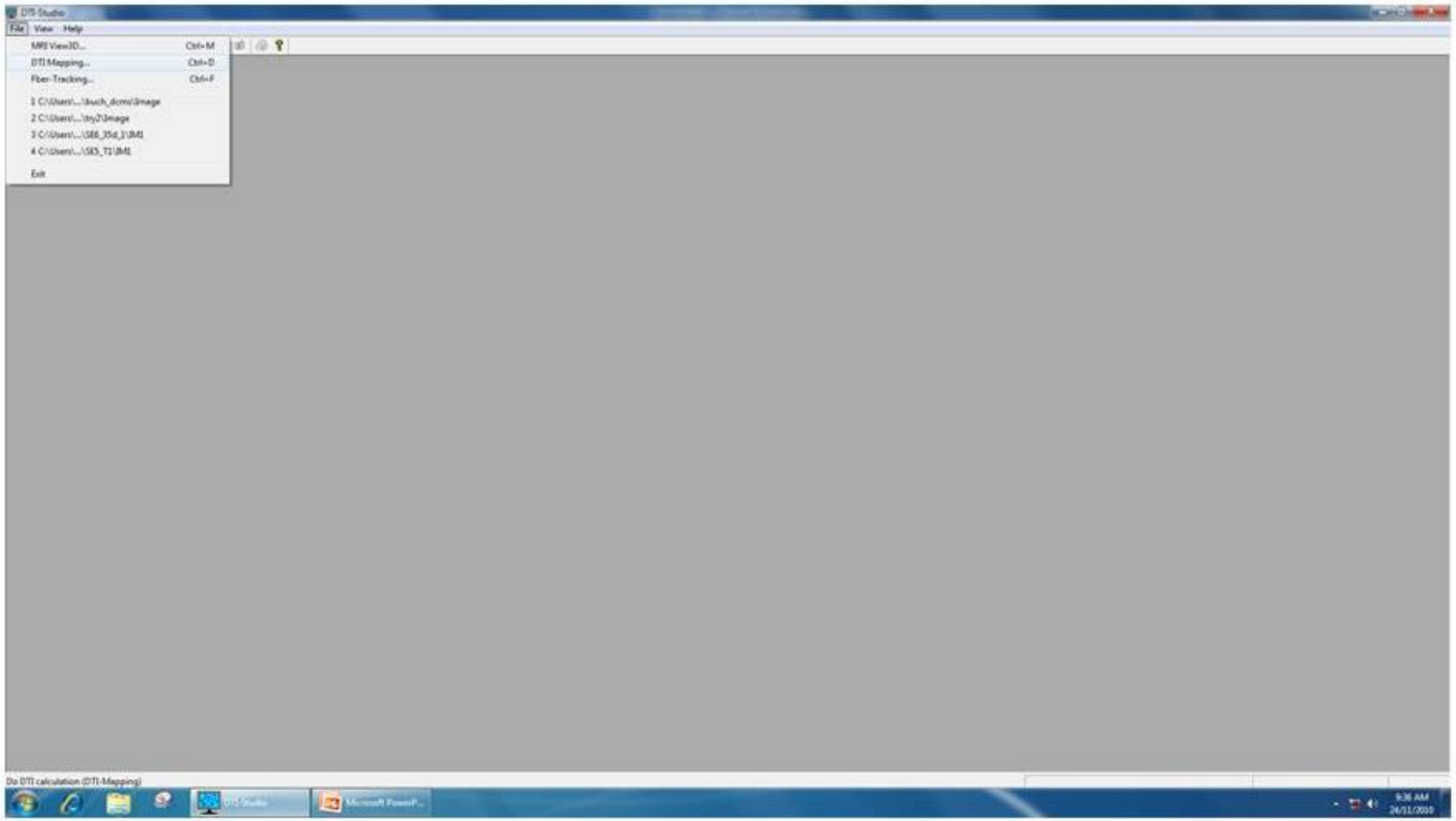

Figure 32. Selecting “DTI Mapping”

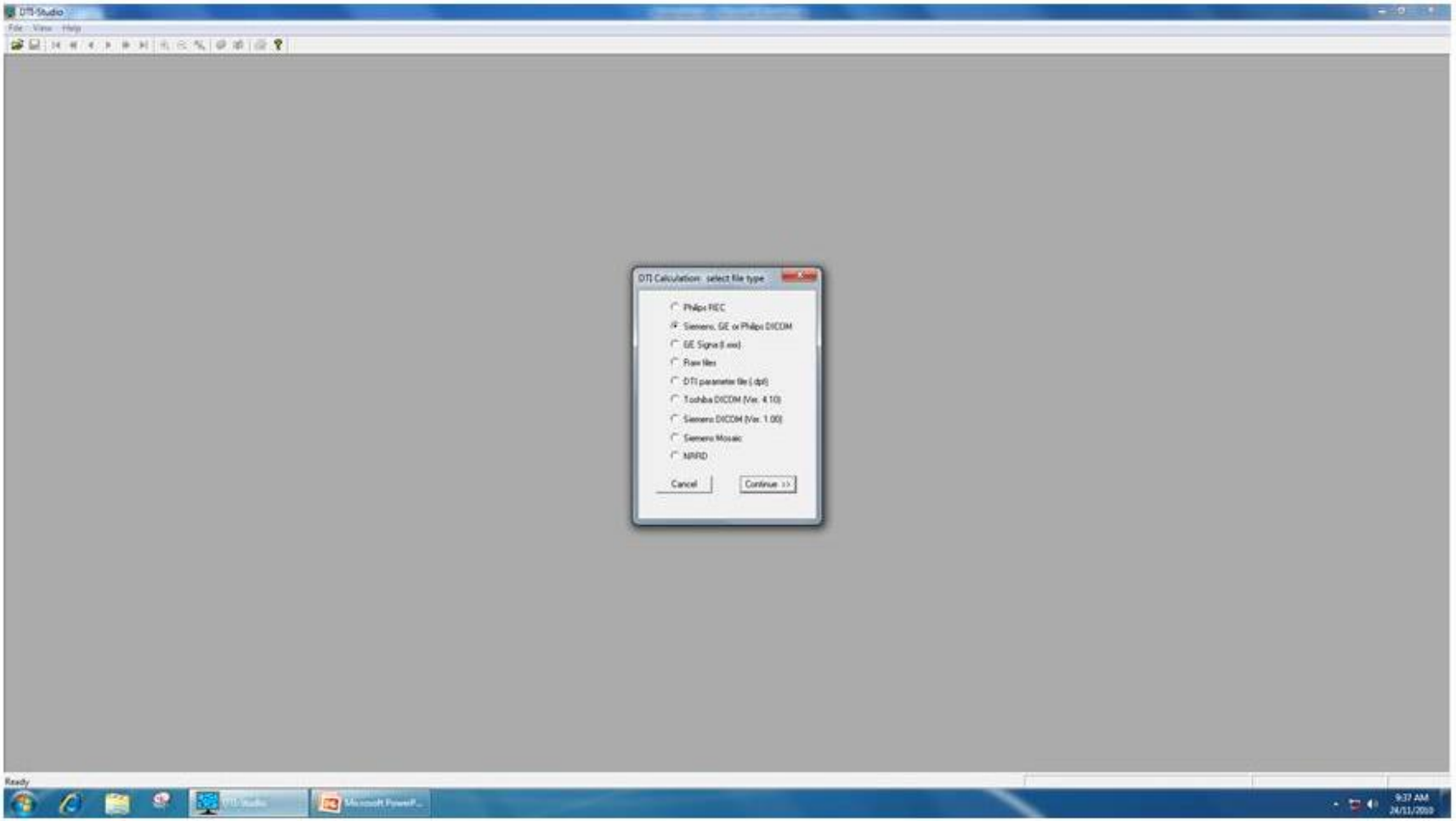

Figure 33. Select the type of data.

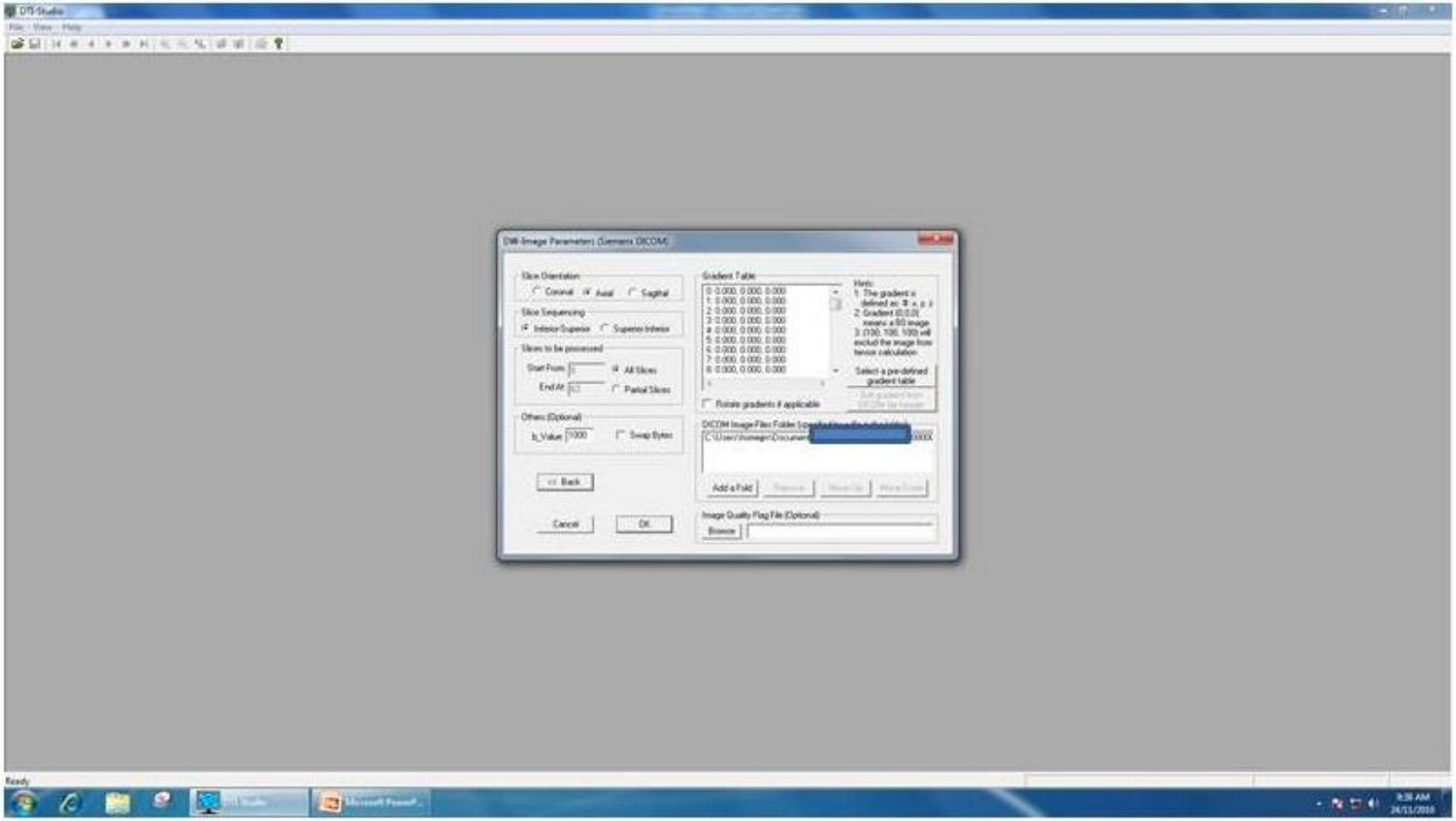

Figure 34. The DWI Image Parameters interface.

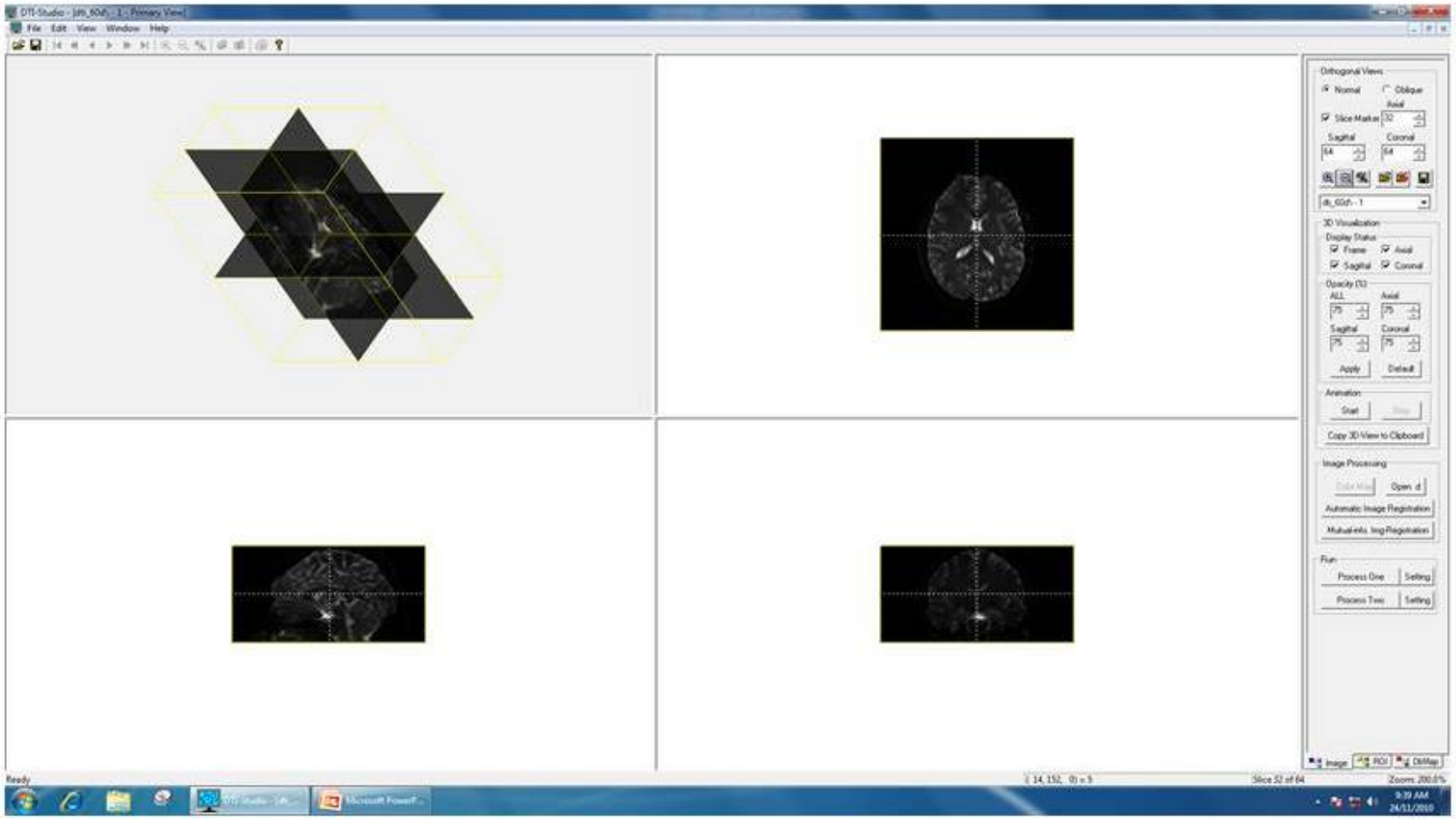

Figure 35. DTIstudio after the DICOMS have been imported.

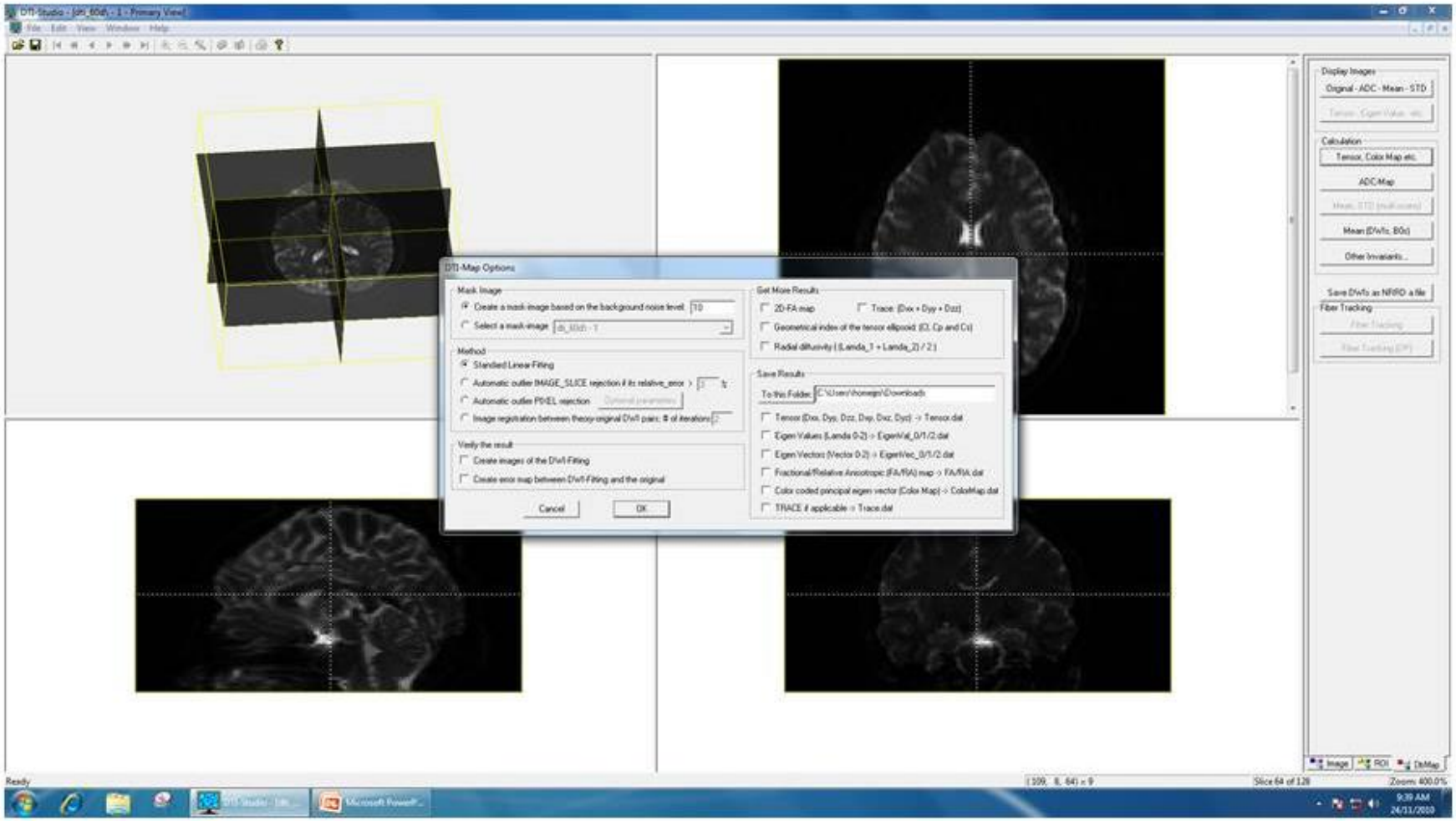

Figure 36. DTI-Map options.

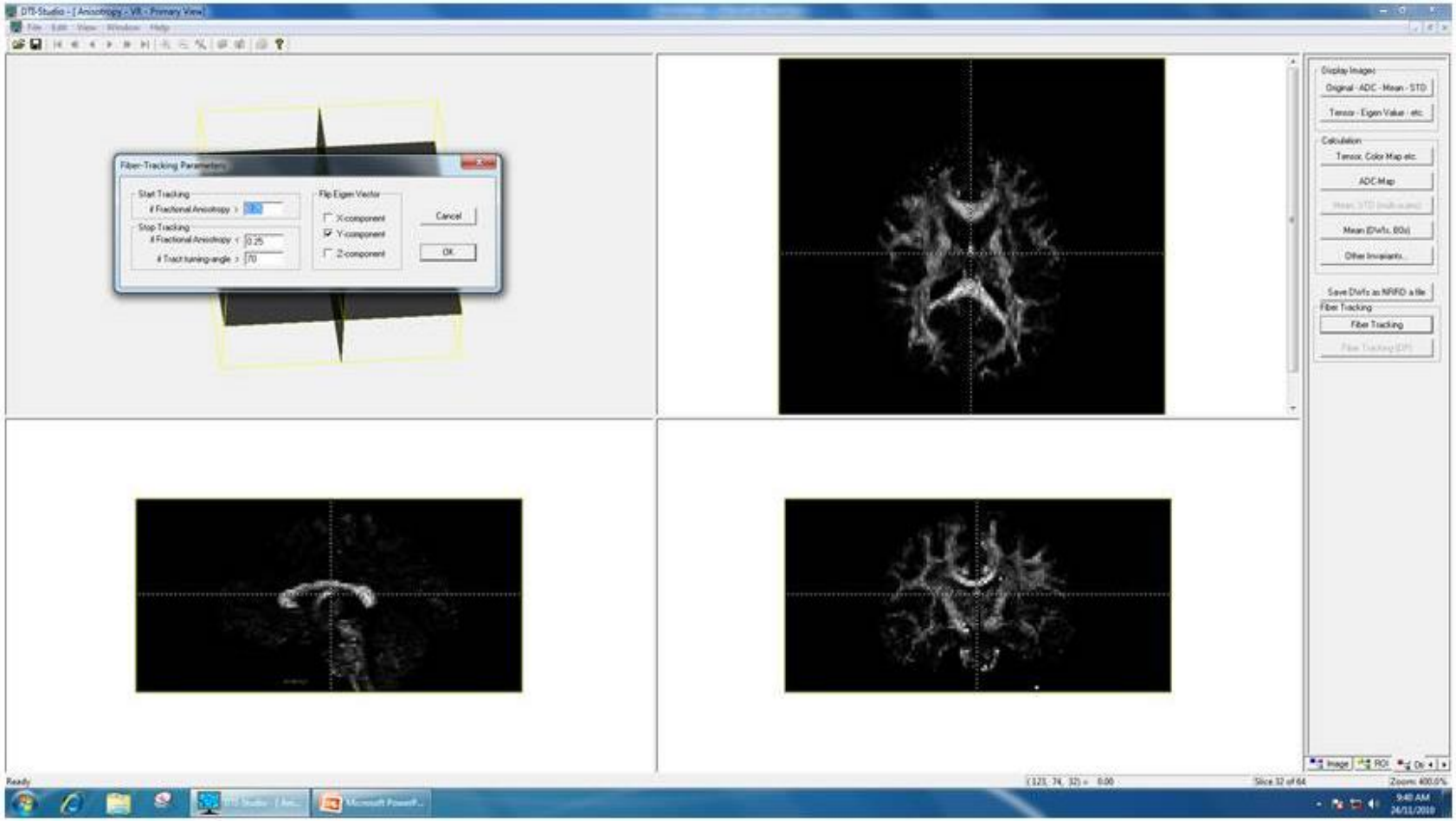

Figure 37. The Fiber-Tracking Parameters box.

Click on the pull-down menu on the right, and select “Color Map – 0” (Figure 37). This will show the RGB colours representing fibre directions. Use the right mouse button to zoom in/out on the 3d (top left) image. Press the “+” button (right-side menu) twice to maximize the 2d images.

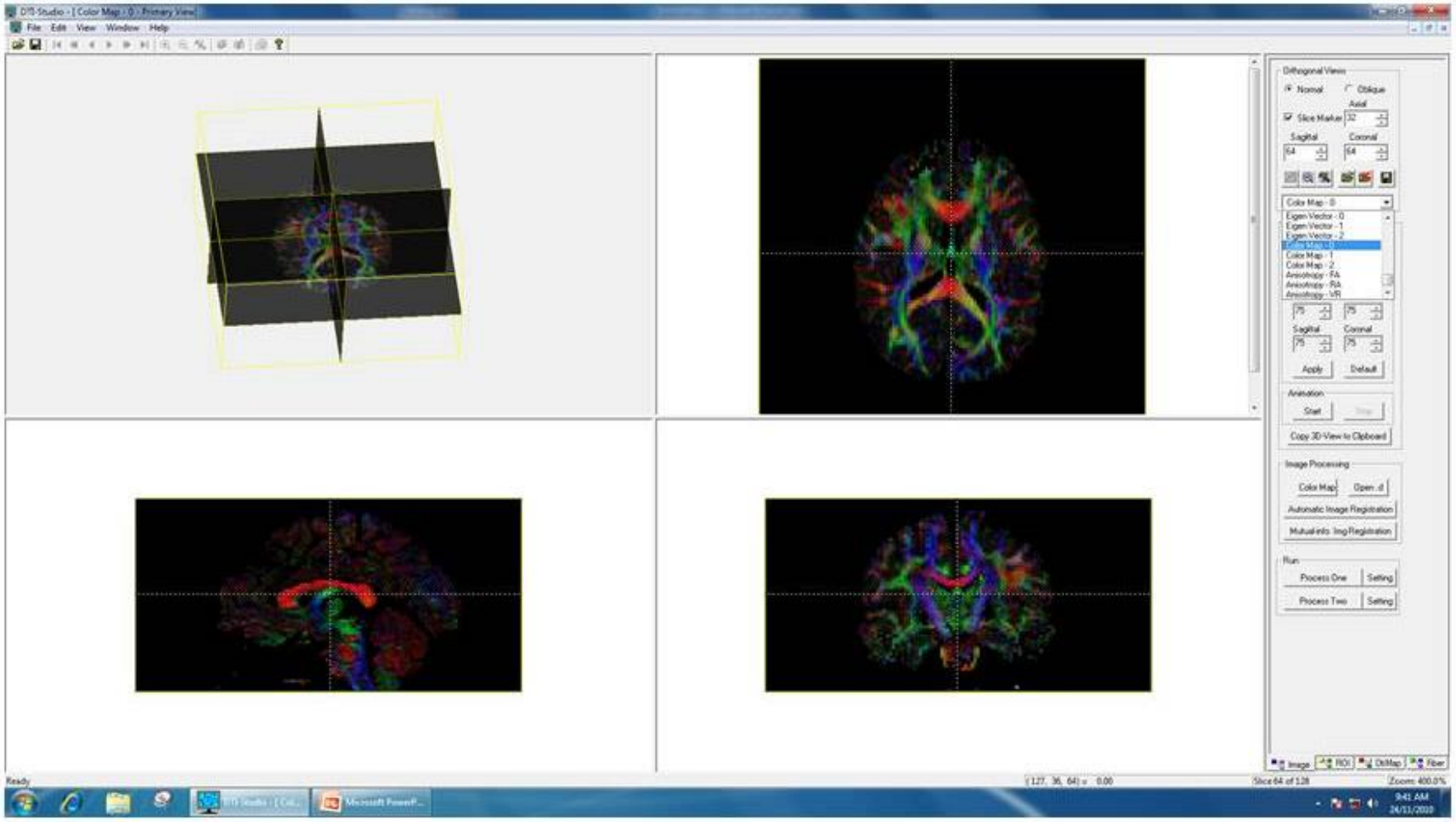

Figure 38. Selecting the Color Map-0 option from the pull-down menu.

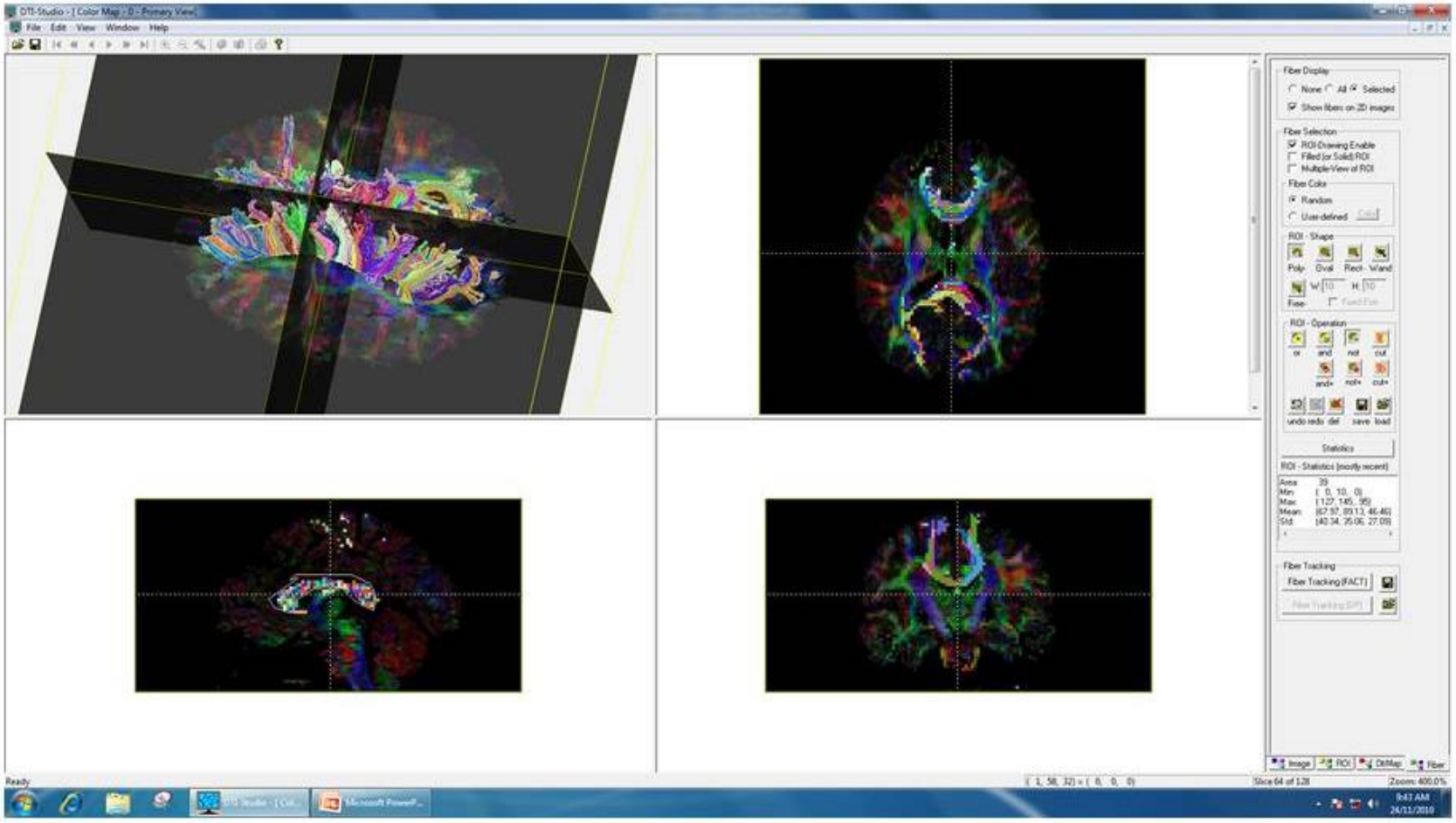

Figure 39. An example of corpus callosum tractography as segmented from sagittal (bottom left).

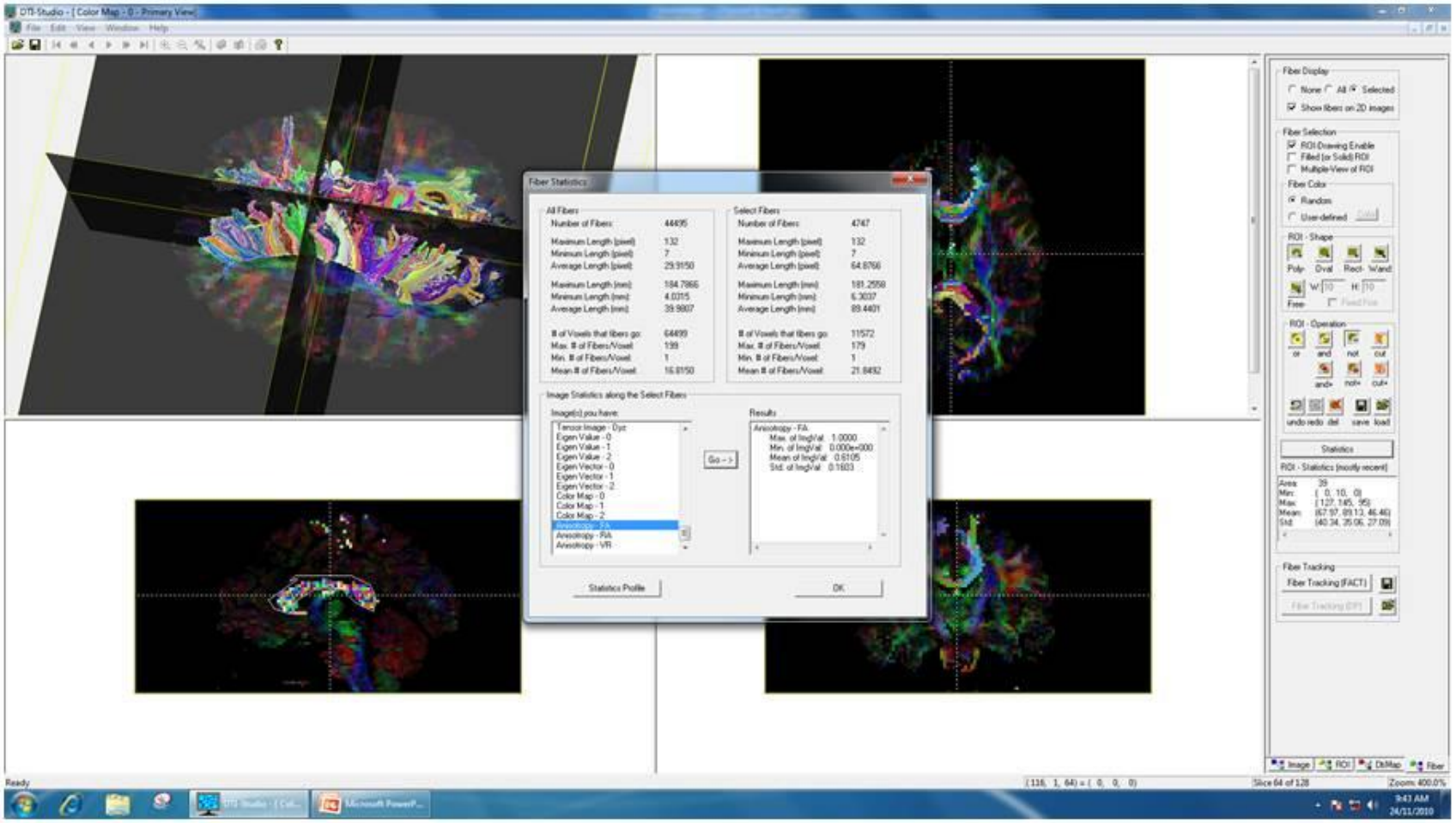

Figure 40. Creating tractography statistics.

If you have more complex diffusion-weighted data, such as multishell (e.g. DSI), a good starting point for analysis is DSIstudio (http://dsi-studio.labsolver.org/). The online documentation is excellent (http://dsi-studio.labsolver.org/Manual) and shows how processing can be completed in 3 simple steps (reading data, reconstruction, and fiber tracking) with a great GUI (no typing in code! Although there is also a DSIstudio version which allows you to use code). Our preferred software is MRtrix3.

18. MedINRIA

1. Click on the MEDINRIA (Ayache et al., 2007) icon (Figure 40)
2. Select "**Modules**" then "**DTI Track**"
3. Select the far left icon on the top row of icons ("**New .dts**"; Figure 41)
4. A new window appears ("**Diffusion weighted MRI dataset importer wizard**"; Figure 42)
5. Click "**Next**"
6. Click "**Open**" and navigate to the directory where your diffusion data is located and select the .nii file
7. Click "**Load sequence**" (Figure 43) and select the .bvec file from the same directory (make sure you have already processed the scans via dcm2nii)
8. Click "**Next**" (Figure 44)
9. Flip the scans as appropriate (Figure 45)
10. Click "**Next**", then "**Finish**". Data will then be displayed (Figure 46)
11. Click on the "**Tensor Processing**" tab, and then click the colored "**FA**" button
12. A few second later the colour maps will appear. If there are any black holes in the images, reduce the threshold and then press the FA button again (Figure 47)
13. Click on the "**Bundle Manager**" tab (Figure 48)
14. Click the 14th button from the left ("**Track fibers**")
15. It will take a few minutes to track all of the fibers in the data (very computer resource intensive)
16. Click "**j**" on the keyboard – this will engage the tracing tool
17. Using the left mouse button, trace around a ROI. In Figure 48 I have roughly traced around the corpus callosum in the bottom left window
18. Click on the big green "**tick**" (validate fiber bundle)
19. Give the ROI a name and then select a colour after the color grid appears
20. Click on the ROI name (in the case below, it is called CC) and then click on the big blue arrow ("**Project all fiber bundles on to 2D views**")
21. Deselect the bottom left box ("**Uniform/Orientation Coded Color**"), and after a few moments, all of the colour fibers will appear on the scans from each orientation (Figure 49)

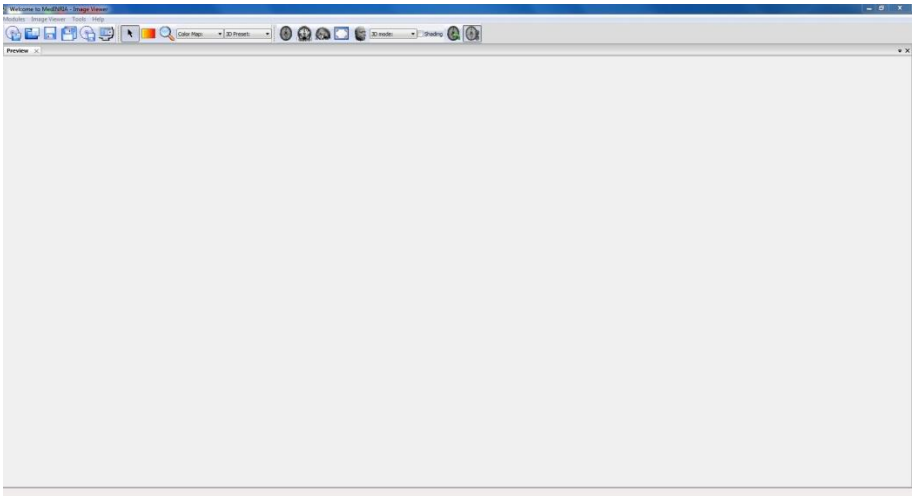

Figure 41. The MedINRIA GUI.

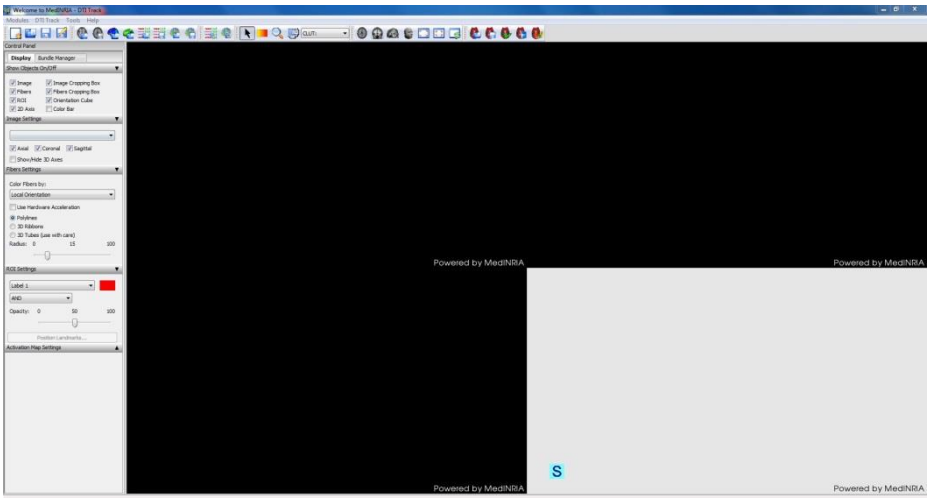

Figure 42. The MedINRIA GUI after selecting New .dts

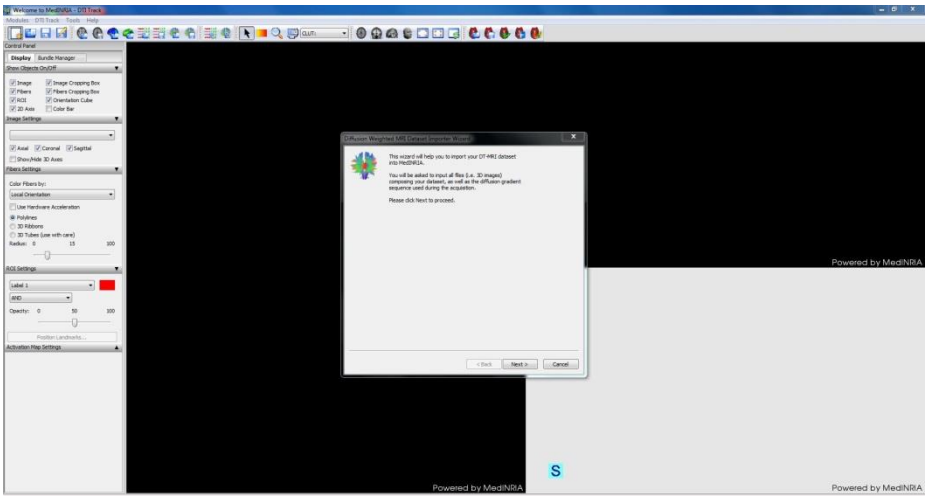

Figure 43. The data import wizard.

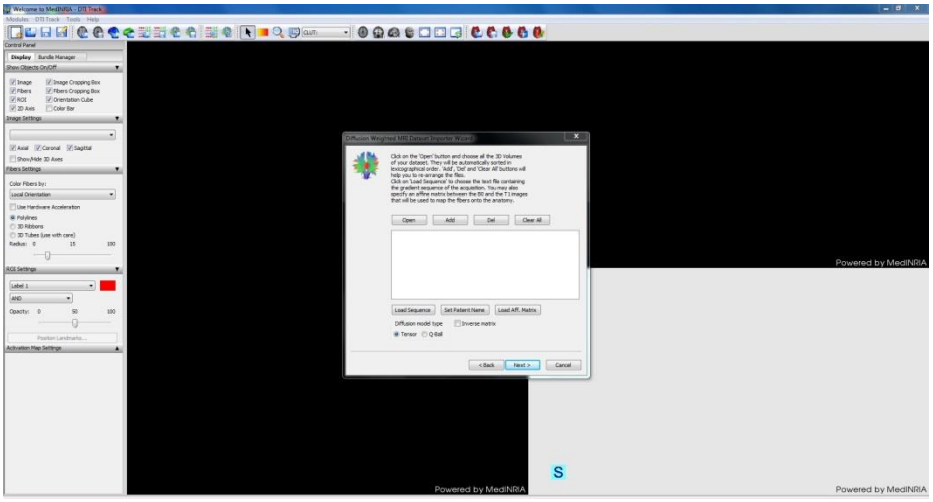

Figure 44. Loading the sequence.

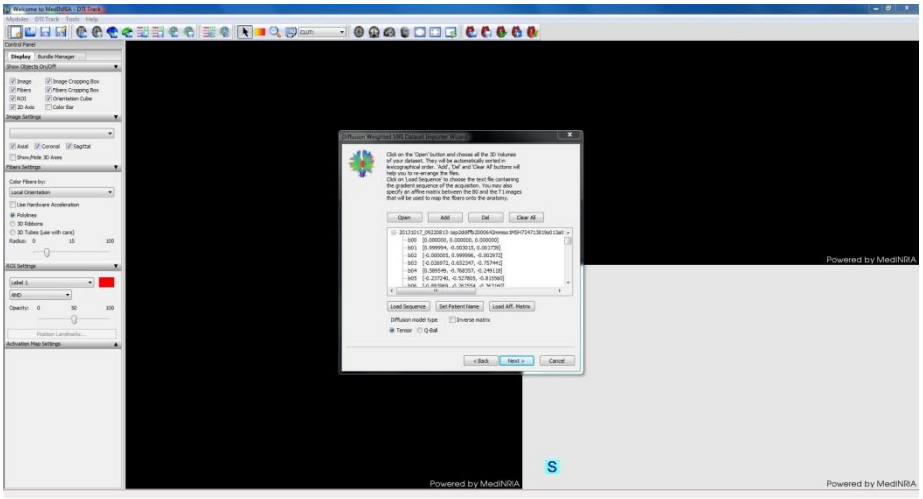

Figure 45. Loading the data.

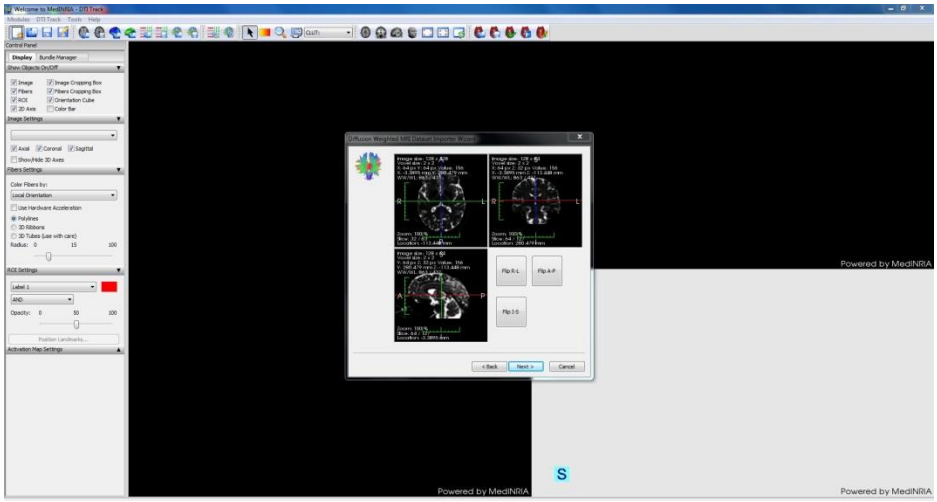

Figure 46. Flip the scan data if necessary.

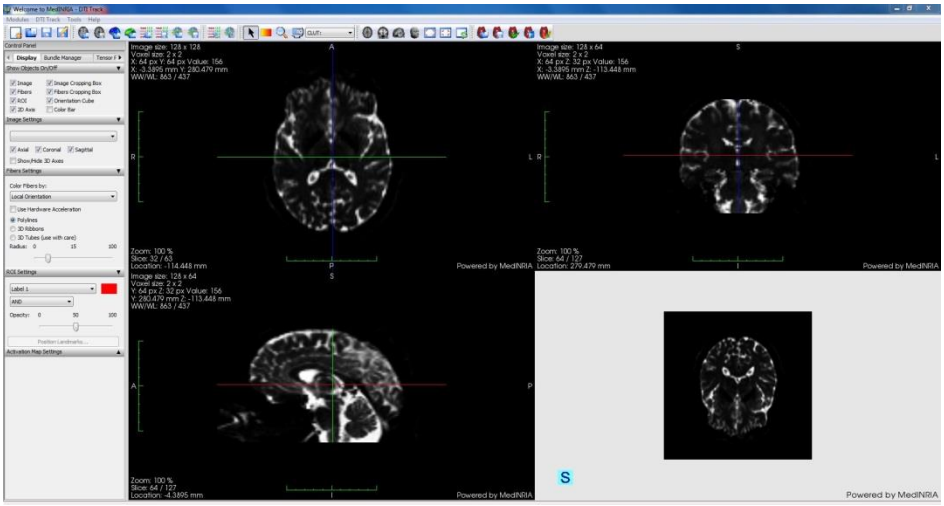

Figure 47. Raw data displayed in MedINRIA.

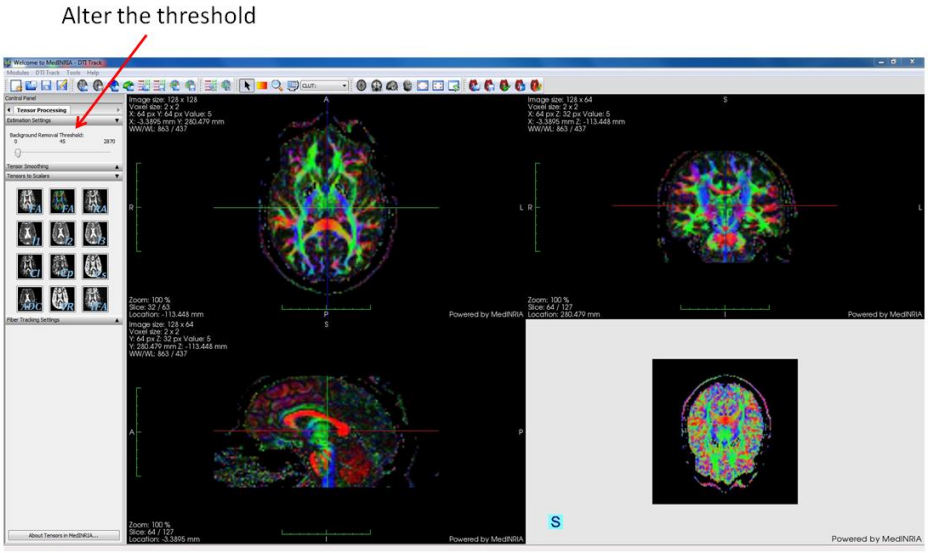

Figure 48. Altering the threshold.

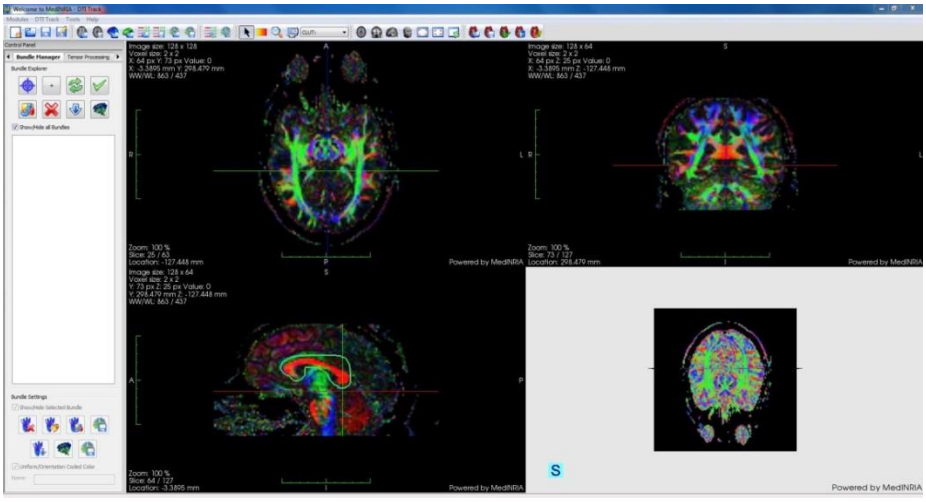

Figure 49. The bundle manager in MedINRIA.

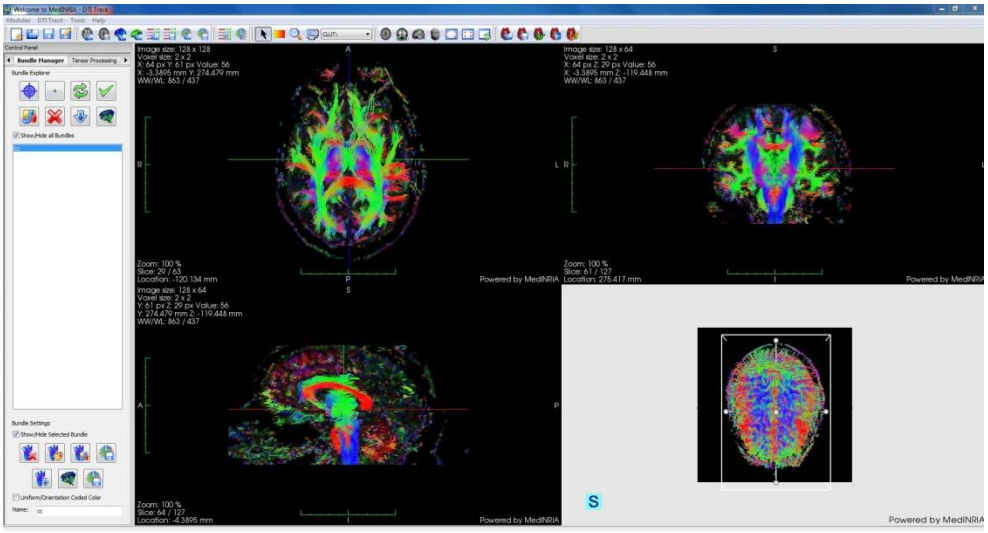

Figure 50. Tracking the fibers.

Click on one of the 2d buttons (top row) to show just one orientation maximized (Figure 50).

Figure 51. Axial perspective maximized.

19A. MRtrix

MRtrix (Tournier et al., 2012) is originally a Windows program that is easily installed (just download it and run the .exe file). One of the great features of MRtrix is that you don't have to make your own bvec matrix. *NOTE: there are MANY freeware packages to conduct DTI analyses, comprehensively reviewed in Soares et al. (2013).*

1. Open a terminal by clicking the Windows start button
2. type in **cmd**
3. A black terminal window will now open
4. Navigate to your dataset (T1-weighted and diffusion data) by using the cd (change directory command) and dir command (to list what is in the directory)
5. Type in the following:

mrconvert . T1.mif (the program will then ask you to select the directory number of the T1-weighted dataset)

mrconvert . dwi.mif (the program will then ask you to select the directory number of the diffusion-weighted dataset; alternatively, replace the "." with the name of the directory to convert)

mrconvert T1.mif T1.nii

mrconvert dwi.mif dwi.nii

mrinfo -grad grad.txt DWI.mif

average DWI.nii -axis 3 - | threshold - - | median3D - - | median3D - mask_DWI.mif

dwi2tensor DWI.nii -grad grad.txt dt_DWI.mif

tensor2FA dt_DWI.mif - | mrmult - mask_DWI.mif fa_DWI.mif

tensor2vector dt_DWI.mif - | mrmult - fa_DWI.mif ev_DWI.mif

erode mask_DWI.mif -npass 3 - | mrmult fa_DWI.mif - - | threshold - -abs 0.7 sf_DWI.mif

streamtrack DT_STREAM DWI.nii -grad grad.txt -seed mask_DWI.mif -mask mask_DWI.mif DWI_whole_brain.tck -num 10000

Alternatively, try this:

mrconvert {DWI path} diff.mif

mrconvert {MP2RAGE INV1 path} mp2rage_inv.mif

average diff.mif -axis 3 - | threshold - - | median3D - - | median3D - mask_DWI.mif

dwi2tensor diff.mif dt_DWI.mif

tensor2FA dt_DWI.mif - | mrmult - mask_DWI.mif fa_DWI.mif

tensor2vector dt_DWI.mif - | mrmult - fa_DWI.mif ev_DWI.mif

erode mask_DWI.mif -npass 3 - | mrmult fa_DWI.mif - - | threshold - -abs 0.7 sf_DWI.mif

streamtrack DT_STREAM diff.mif -seed mask_DWI.mif -mask mask_DWI.mif DWI_whole_brain.tck -num 10000

Note: *if you use the eddy current and movement corrected scans (e.g. output from FSL eddy correct) then use the -grad command (e.g. -grad eccgrad.txt) in the "dwi2tensor" command line. Make sure to first convert your new gradient table (described on page 53 of this Handbook) into MRtrix format by adding the b-value to end of each line of gradients and then saving the new gradient table under a new name.txt. However, if you are using MRtrix3 (page 90), then this is no longer necessary (as there is a flag available in MRtrix3 (-fslgrad) that allows you to use the rotated bvec in FSL format).*

For probabilistic, replace the last command with this:

estimate_response DWI.nii -grad grad.txt sf_DWI.mif -lmax 8 response_DWI.txt

To perform deconvolution (takes time):

csdeconv DWI.nii -grad grad.txt response_DWI.txt -lmax 8 -mask mask_DWI.mif CSD_DWI.mif

Then:

streamtrack SD_PROB CSD_DWI.mif -seed mask_DWI.mif -mask mask_DWI.mif cst_CSD_DWI.tck -num 10000

In a single script:

estimate_response DWI.nii -grad grad.txt sf_DWI.mif -lmax 8 response_DWI.txt

csdeconv DWI.nii -grad grad.txt response_DWI.txt -lmax 8 -mask mask_DWI.mif CSD_DWI.mif

streamtrack SD_PROB CSD_DWI.mif -seed mask_DWI.mif -mask mask_DWI.mif cst_CSD_DWI.tck -num 10000

If the tracks don't look right, it may be due to an incorrect response file. To display the response function:

disp_profile -response response_DWI.txt

To open the viewer, type in:

1. **mrview**
2. Maximize the screen
3. **File → open**
4. Open the T1-weighted file ending in .mif
5. Hold the CONTROL keyboard key and then use the mouse scroller to change the zoom
6. **View → sidebar**
7. Hold down the right mouse button and move it up and down for brightness, and left and right for contrast
8. Right click in the "**tracks**" window, and select the tractography (called "**whole_brain.tck**")
9. Change the value from 5.0 to 20.0 (Figure 51)

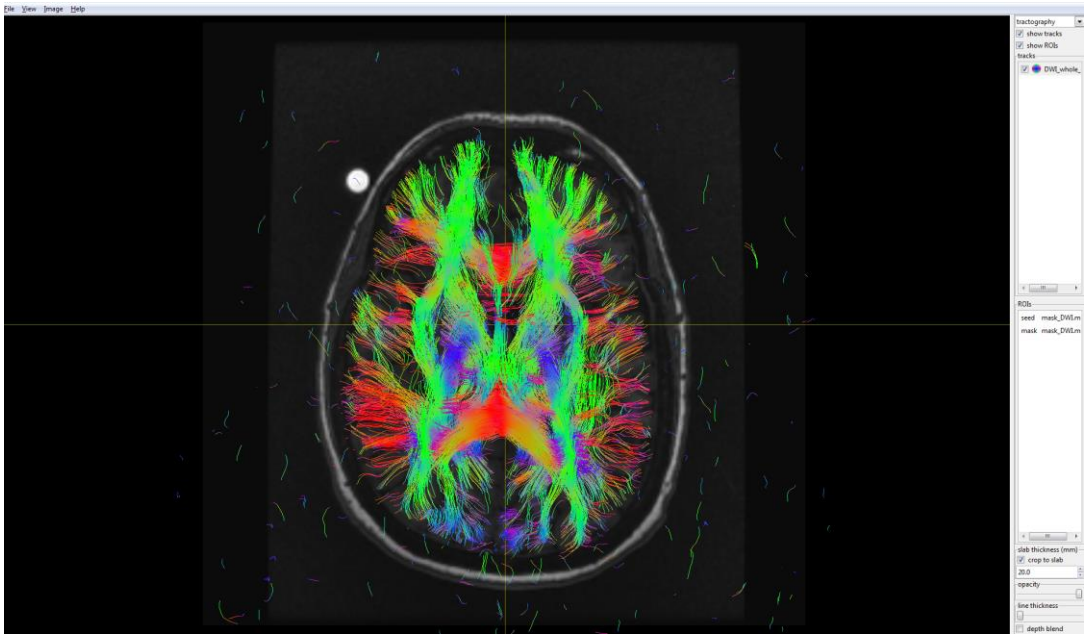

Figure 52. Probabilistic whole-brain tractography in MRtrix.

- Use the mouse scroller to navigate through the slices
- Press S for sagittal, A for axial, or C for coronal
- Click on "**depth blend**" to see the tracks in depth (i.e. 3d)
- To see the scan in 3d, untick the "Lock to images axes (under the "**View**" pull-down menu; Figure 52) and untick the "**crop to slab**" option (bottom right)

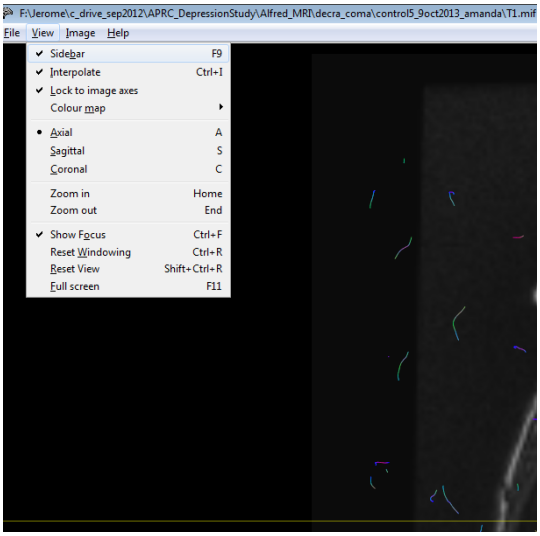

Figure 53. The pull-down View menu in MRtrix.

Click the CONTROL key and the middle mouse key to reorientate the scan and play around with the "**opacity**" and "**line thickness**"

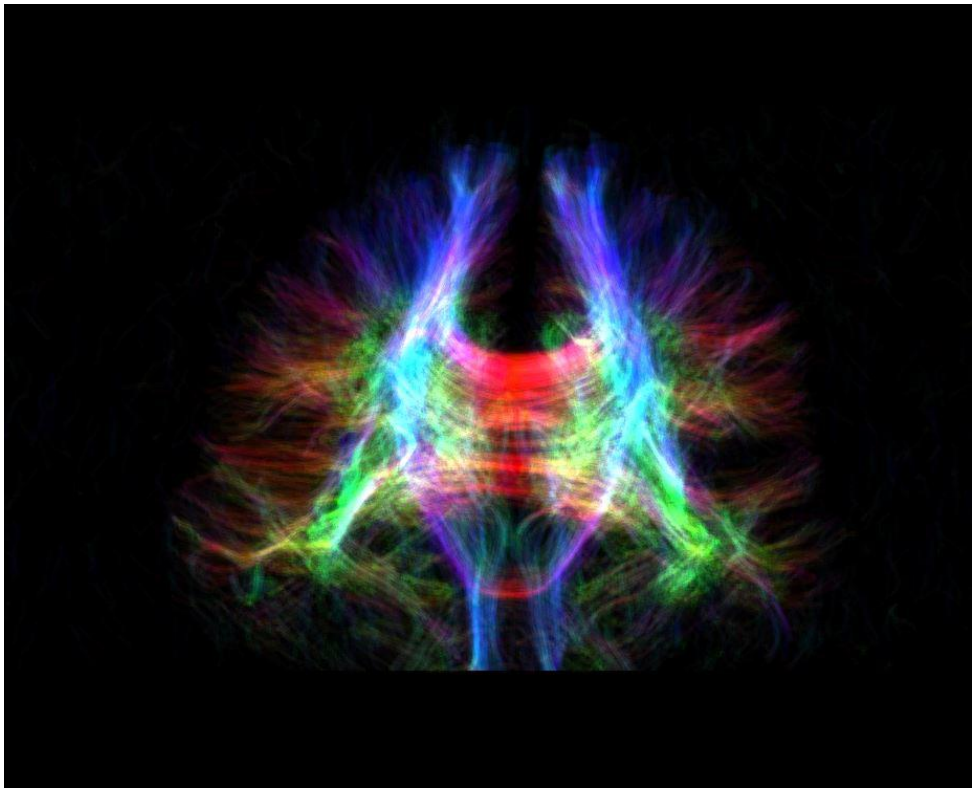

Figure 54. Whole-brain tractography in MRtrix with altered brightness and contrast.

DRAWING ROIs:

1. Load up T1 using mrview

2. Go to View, Sidebar

3. Select ROI Analysis in drop down

4. Right click in blank box on right, click New

5. Specify filename

6. To draw, Shift + left click

7. To remove, Ctrl + Shift + left click

SPHERICAL ROIS:

e.g.

streamtrack SD_PROB CSD_DWI.mif -seed 24.52,-25.48,9.39,5 -include 9.76,-86.94,-2.16,10 -mask mask_DWI.mif LGN_01Lp.tck -num 10000

Spherical ROIs are xco-ordinate, yco-ordinate, zco-ordinate, size

Get co-ordinates from T1 in mrview, bottom left ('position')

Commands:

-seed

-include (only track if it goes from seed->include region)

-exclude (don't track if it goes from seed->exclude region)

tck file is a tract file, you can load this in via the sidebar

To show whole brain tractography:

1. Unclick “crop to slab” (bottom right)
2. Uncheck “lock to axes” (through the View pull-down menu)
3. Control-middle-mouse to rotate

Example of using ROIs. In this example, using deterministic tracking of the left optic radiations from a band of Gennari ROI, trigone ROI, and LGN ROI, and saving the tractography as left_optic:

streamtrack DT_STREAM DWI.nii -grad grad.txt -seed left_bog.mif -include left_lgn.mif –include left_trigone.mif -mask mask_DWI.mif left_optic2.tck -num 10000

For probabilistic tractography, try this:

streamtrack SD_PROB DWI.nii -seed left_bog.mif -include left_lgn.mif -mask mask_DWI.mif cst_csd.tck

Figures 55 and 56 show the result after adding in a few -exclude co-ordinates (red spheres):

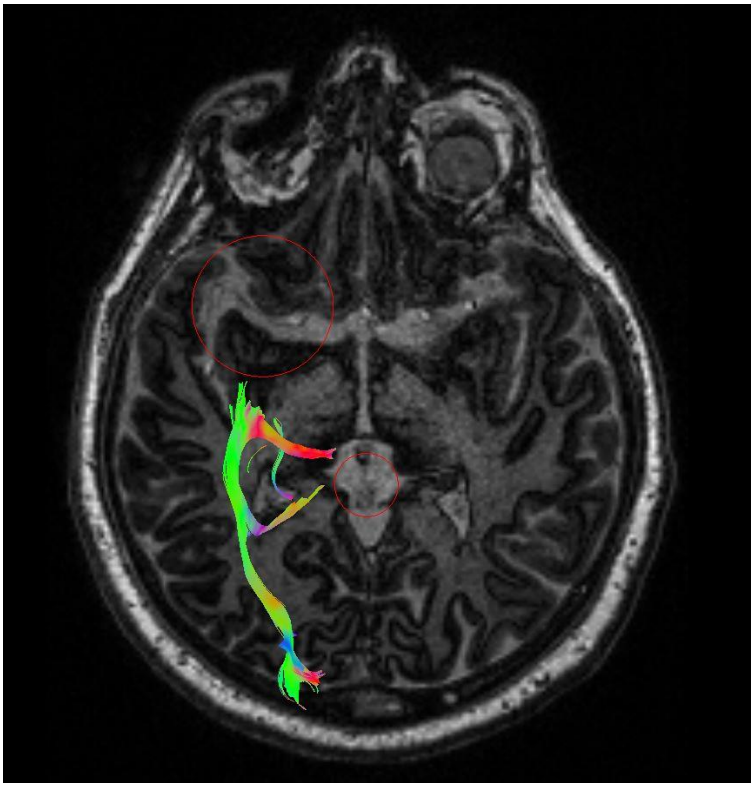

Figure 55. Deterministic optic radiation tractography with exclusion spheres.

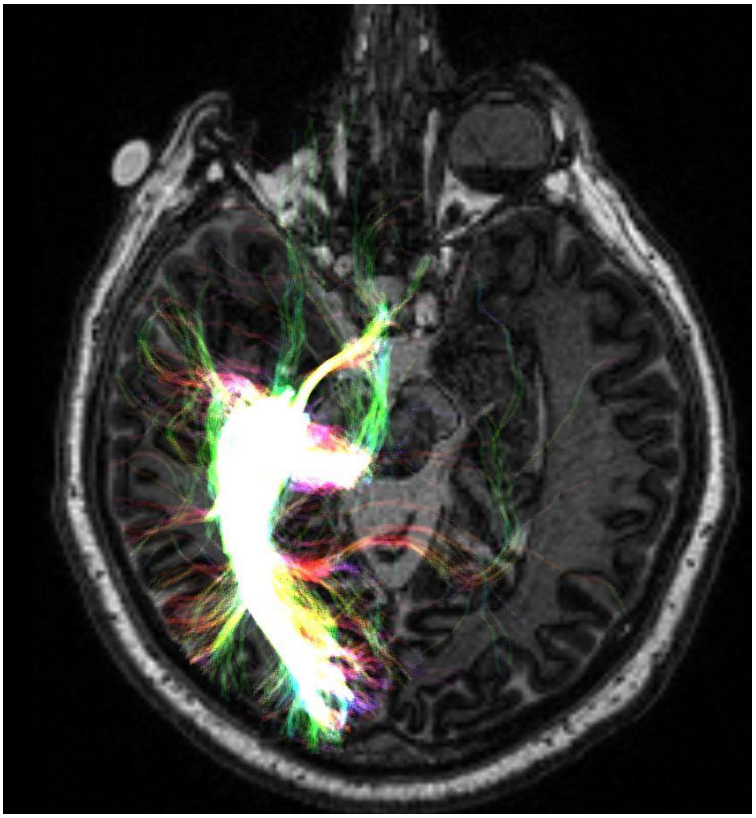

Figure 56. Probabilistic optic radiation tractography with exclusion spheres.

If your DWI data does not align accurately with the T1-weighted data, we recommend using FLIRT (part of the FSL package) to align the scans. That is, align the T1-weighted scan to the DWI data (not the other way around). To achieve an even more accurate alignment, first extract a b-zero volume from the DWI dataset using the **fslroi** command:

fslroi dwi.nii.gz nodif 0 1

The output from that command will be a separate file called nodif.nii.gz

Then use FLIRT as follows:

flirt -in t1.nii -ref nodif.nii -out t1_reg.nii -omat t1_reg.mat -bins 256 -cost normmi -searchrx -5 5 -searchry -5 5 -searchrz -5 5 -dof 6 -interp trilinear

The output from that command will be a separate file called t1_reg.nii

Then open mrview, open t1_reg.nii and the dwi.nii should align accurately to it.

To extract FA values from tractography

tracks2prob -template fa_DWI.mif nameoftrack.tck FA_mask_track.mif

mrstats fa_DWI.mif -mask FA_mask_track.mif

To extract FA values from tractography within a ROI mask

mrstats fa_DWI.mif -mask name_of_roi.mif

Note that there is a 'track density' feature in MRtrix; however, this is meant for the purpose of validating neuroanatomy. That is, metrics derived from this feature are not clinically interpretable (although track *weighted* can be quantitatively useful). Note as well that it is suggested that you extract at least 1million tracts before running the track density command.

To run track density at 500 micron resolution:

tracks2prob DWI_whole_brain.tck -vox 0.5 tdi.mif

To run TDI on specific tracts that you have already created:

tracks2prob name_of_track_1million.tck -vox 0.5 tdi_1million.mif

19B. MRtrix3

Note that the examples shown above are from using MRtrix version0.2.x. However, there is a newer (and far more comprehensive) version of MRtrix called MRtrix3 (https://github.com/MRtrix3/mrtrix3/wiki; https://media.readthedocs.org/pdf/mrtrix/latest/mrtrix.pdf). Some of the commands are different (https://github.com/MRtrix3/mrtrix3/wiki/MRtrix3-commands ; http://mrtrix.readthedocs.io/en/latest/reference/mrtrix2_equivalent_commands.html), but it offers better algorithms, and additional features such as orthogonal views (i.e. display coronal, axial, and sagittal simultaneously), 3-dimensional rendering (Figure 56), flood-fill ROI module, SIFT (Spherical-deconvolution Informed Filtering of Tractograms; Smith et al., 2013), connectome modules, as well as dixel and fixel analysis. Note that to get the best results from SIFT, the authors advise that a b-value of at least 3000 is used, and that reverse-phase encoded bzero volumes (also known as blip-up-blip-down) are acquired as well. However, there is talk that SIFT may not be required for such blipped data. Note that in MRtrix3, spherical harmonic basis is now orthonormal. This suggests that the old CSD files generated in MRtrix0.2.x are not valid for analysis by MRtrix3 without conversion. And the FOD file generated in MRtrix3 is also not reciprocally valid for MRtrix0.2.

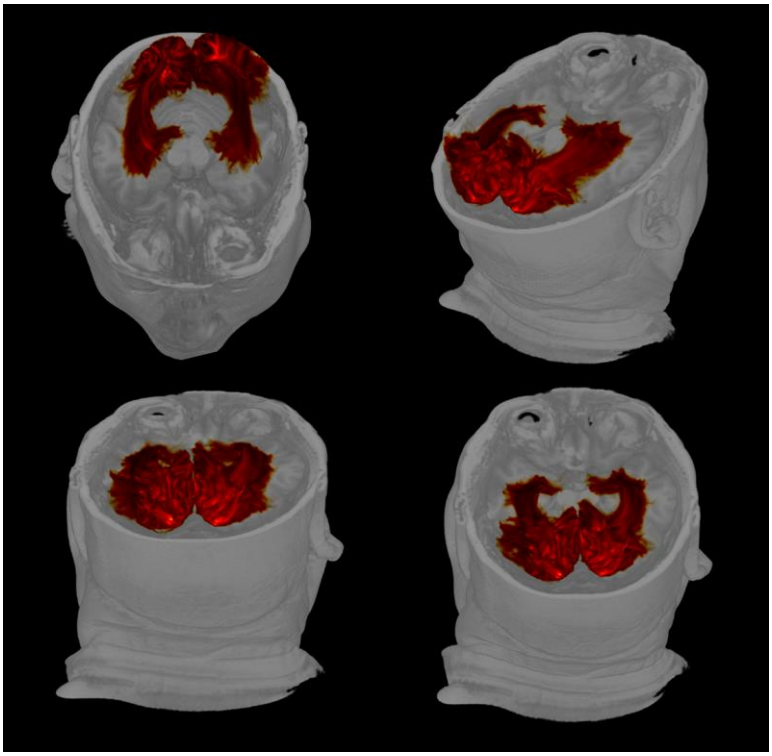

Figure 57. 3-dimensional rendering of optic radiation track density superimposed over the subject's T1-weighted MPRAGE scan in MRtrix3.

As always, before undertaking the steps below, cd to your DICOM folder.

Example 1.

Whole brain tracking using constrained spherical deconvolution (CSD) and the probabilistic tracking algorithm iFOD2 (the default). After step 3 (dwi2mask), check the accuracy of the mask using mrview and edit if necessary.

mrconvert . T1.mif

mrconvert . DWI.mif

dwi2mask DWI.mif DWI_mask.mif

dwi2response dwi.mif response.txt -mask DWI_mask.mif

dwi2fod dwi.mif response.txt FOD.mif -mask DWI_mask.mif

tckgen fod.mif wholebrain_CSD.tck -seed_image DWI_mask.mif -mask DWI_mask.mif -number 100000

Example 2.

This example lists the commands for whole-brain tractography using the rotated bvecs from FSL. Tractography is using CSD and the probabilistic tracking algorithm iFOD2. After step 3, check the accuracy of the mask using mrview and edit if necessary.

mrconvert . T1.mif

mrconvert -fslgrad filename_rot.bvec values_file.bval filename_ecc.nii.gz DWI.mif

dwi2mask DWI.mif DWI_mask.mif

dwi2response dwi.mif response.txt -mask DWI_mask.mif

dwi2fod dwi.mif response.txt FOD.mif -mask DWI_mask.mif

tckgen fod.mif wholebrain_CSD.tck -seed_image DWI_mask.mif -mask DWI_mask.mif -number 10000

To display the response profile:

shview -response response.txt

Note: to extract and check the bvec and bval inside the dwi.mif:

mrinfo dwinii2mif.mif -export_grad_fsl bvec bval

Creating a track density image

Example 1.

Using a wholebrain track file (as created in previous examples).

tckmap -dec -vox 0.25 wholebrain_CSD.tck wholebrain_TDI.mif

-vox voxel size provide either an isotropic voxel size (in mm^3), or comma-separated list of 3 voxel dimensions. This can be smaller than the acquired voxel size.

-dec option performs track mapping in directionally-encoded colour (DEC) space

To generate maps of tensor-derived parameters

Examples:

dwi2tensor -mask DWI_mask.mif DWI.mif DTI.mif

For FA:

tensor2metric -fa fa.mif DTI.mif

For ADC:

tensor2metric -adc adc.mif DTI.mif

First eigenvalue:

tensor2metric -num 1 -value firstEV.mif DTI.mif

Second eigenvalue:

tensor2metric -num 2 -value secondEV.mif DTI.mif

Third eigenvalue:

tensor2metric -num 3 -value thirdEV.mif DTI.mif

To calculate radial diffusivity in MRtrix3:

tensor2metric -num 2,3 -mask binarymask.mif diffusiontensorimage.mif -value - | mrmath - mean -axis 3 radialdiffusivity.mif

To create colour FA maps:

dwi2tensor dwi.mif tensor.mif

tensor2metric tensor.mif -vec dec.mif

You can also do it in one go using Unix pipes:

dwi2tensor dwi.mif - | tensor2metric - -vec dec.mif

If you want to clean up your results, you can also supply a mask to either command using the -mask option. And if you want other types of tensor metrics, you can use e.g. the -fa, -adc, -ad, rd, etc. options to tensor2metric.

Note that if you're using the updated_syntax (or final MRtrix3 release) branch, you can already use the -rd option of tensor2metric to get the radial diffusivity directly. The axial diffusivity is simply the first (i.e., largest) eigenvalue. Soon also available via the -ad option of tensor2metric as well.

The most recent version of MRtrix3 *before* the final release offered an add-on development kit referred to as "updated_syntax". To install it, it is best to remove your current MRtrix3 installation and start fresh:

Install MRtrix3

Install the eigen3 dependencies

Install the updated_syntax:

cd /path/to/your/mrtrix3

git fetch

git checkout updated_syntax

./build

The majority of the development version (updated_syntax) of MRtrix3 was related to analysis of multishell diffusion data via the new commands "msdwi2fod" and "msdwi2response" which work very well. However, in the 'final' release (0.3.15), these commands were deprecated, so multishell data can be processed with the conventional "dwi2fod" and "dwi2response" commands.

Note: When converting dicoms to .nii or .mif format for use in MRtrix, you do not have to use dcm2nii (or the newer version, dcm2niix). Instead, you can use the mrconvert command. If you are having trouble with MRtrix3 not recognizing the diffusion encoding in further processing steps, then use the following command:

mrconvert DICOM -stride 1:4 -export_grad_fsl bvecs bvals DWI.nii

Remember to use your ***corrected*** dwi.nii and rotated bvecs when analysing data.

Final release of MRtrix3 (version 0.3.15 at June 2016) for single-shell data:

mrconvert . T1.mif

mrconvert . DWI.mif

dwi2mask DWI.mif DWI_mask.mif

mrinfo dwi.mif -export_grad_fsl bvecs bvals

dwi2response tax -lmax 8 -mask DWI_mask.mif DWI.mif response.txt

dwi2fod csd -mask DWI_mask.mif dwi.mif response.txt odf.mif

tckgen odf.mif wholebrain_CSD.tck -seed_image DWI_mask.mif -mask DWI_mask.mif -number 10000

For non-CSD deterministic:

mrconvert . T1.mif

mrconvert . DWI.mif

dwi2mask DWI.mif DWI_mask.mif

mrinfo dwi.mif -export_grad_fsl bvecs bvals

dwi2response tax -lmax 8 -mask DWI_mask.mif DWI.mif response.txt

dwi2fod csd -mask DWI_mask.mif dwi.mif response.txt odf.mif

tckgen -algorithm tensor_det dwi.mif wholebrain_CSD.tck -seed_image DWI_mask.mif -mask DWI_mask.mif -number 10000 (that is, replace the odf.mif with dwi.mif)

For CSD-deterministic, replace the last line with:

tckgen -algorithm sd_stream odf.mif wholebrain_CSD.tck -seed_image DWI_mask.mif -mask DWI_mask.mif -number 10000

Note: Conversion options to dcm2nii:

If you do not have dcm2nii, you can convert dicoms to .nii or .mif using MRtrix:

T1-weighted: **mrconvert t1_directory/ t1.mif** (or **mrconvert t1_directory/ t1.nii**)

Diffusion: **mrconvert dti_directory / -stride -1,2,3,4 dwi.mif** (and then convert the dwi.mif to .nii: **mrconvert dwi.mif -stride -1,2,3,4 -export_grad_fsl bvecs bvals dwi.nii**)

Note: It is advisable to first preprocess your data after conversion from dcm to .mif format:

dwidenoise dwi.mif dwi_denoised.mif

preproc -rpe_none AP dwi_denoised.mif dwi_denoised_preproc.mif #Note that this step includes carrying out eddy_openmp

dwibiascorrect dwi_denoised_preproc.mif dwi_denoised_preproc_bias_corrected.mif -fsl

The output of all that preprocessing will be dwi_denoised_preproc_bias_corrected.mif

Here is a nice pipeline: http://community.mrtrix.org/t/beginner-connectome-pipeline-mrtrix3-fsl-and-freesurfer/981/1

MAJOR UPDATE!

Instead of using dwi2response manual -shell XX -lmax YY etc etc, you can use the **dhollander** option to carry out unsupervised estimation of WM, GM and CSF response functions WITHOUT a T1 image or segmentation. ***Make sure to first do a git pull to get access to this (and other) updated scripts.***

Try this:

mrconvert your_dwi_dataset.nii dwinii2mif.mif -fslgrad bvecs bvals

dwi2response dhollander dwinii2mif.mif wm.txt gm.txt csf.txt

dwi2mask dwinii2mif.mif mask.mif

dwi2fod msmt_csd dwi.mif wm.txt fod_wm.mif gm.txt gm.mif csf.txt csf.mif -mask mask.mif

Note that this last line is for MULTISHELL MULTITISSUE (msmt). For single shell, replace the last line with this (*although msmt works well with single shell, and sometimes better*):

dwi2fod csd dwi.mif wm.txt fod_wm.mif -mask mask.mif

Note: you may have to delete the first line of the wm.txt file before using it for tckgen:

tail -n +2 wm.txt > "wm_new.tmp" && mv wm_new.tmp wm.txt

To obtain directionally encoded colour FA maps with MRtrix3:

fod2dec odf.mif dec_fa.mif

MAJOR UPDATE! July 2017

In late July 2017 MRtrix3 underwent another major revision. Of note, when specifying the number of streamlines to track, the flag **-number** has now been replaced with **-select**.

Note: *Calamante (2017) suggests using the terms "streamline" and "track" interchangeably, to represent a mathematical representation (i.e. a three-dimensional curve generated using a tractography algorithm). In contrast, the terms "tract" and "white matter pathway" are also used interchangeably to represent the actual biological structure in the brain.*

MAJOR UPDATE! May 2018

Major update to candidate 3 in May 2018 which fixes bugs, adds new flags and replaces some commands: http://community.mrtrix.org/t/mrtrix-3-0-release-candidate-3/1624

To merge 2 datasets:

Convert each image to a format that can hold the DW scheme in the header (i.e. .mif), importing the relevant bvecs/bvals in the process, then use **mrcat** to merge them. So this might look like:

mrconvert dwi1.nii -fslgrad bvecs1 bvals1 dwi1.mif

mrconvert dwi2.nii -fslgrad bvecs2 bvals2 dwi2.mif

mrcat dwi1.mif dwi2.mif -axis 3 dwi_all.mif

20. Some useful Linux commands

- cd (change directory)
- cd .. (go one directory up)
- ls (list the directory)
- ll (instead of ls) or ls -1: this will list the directory as well as the attributes of every in the directory (i.e. not just the filenames)
- exit
- to force program to close, press CONTROL-C in the terminal
- mkdir (make a new directory). To remove a directory, type in rmdir
- which 'command' (the directory the command is located in as per the bash file)
- what is 'command' (this displays a single line description about a command)
- pwd (shows the present working directory)
- df -k: (this will display the file system disk space usage in bytes)
- sudo (this will run a command as super user)
- arrow up (this displays the previous commands used in a terminal)

There are many script languages available to write code in order to automate image processing (e.g. bash, Matlab, R, Python, C++). Here are some nice pieces of advice for bash scripting:

https://fsl.fmrib.ox.ac.uk/fslcourse/lectures/scripting/all.htm

http://tldp.org/LDP/Bash-Beginners-Guide/html/chap_08.html

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22. Collection of our published articles that have used the software described in this handbook

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