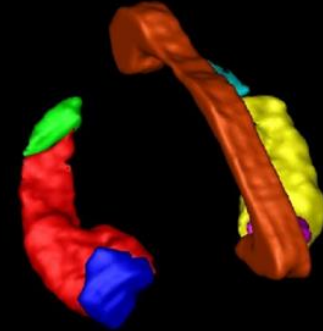
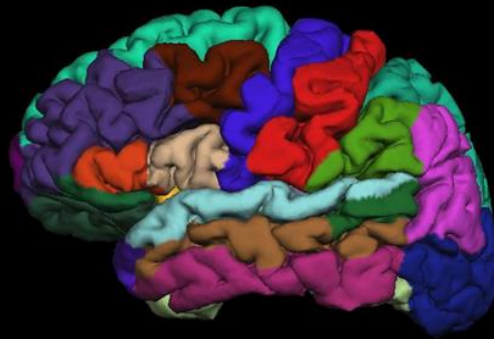
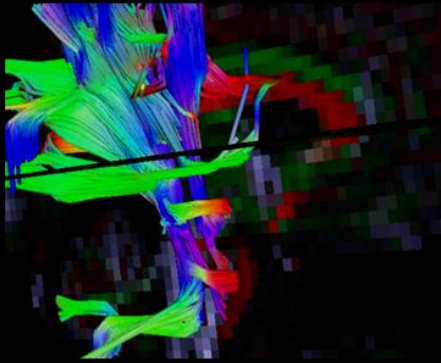


Handbook of Structural Brain MRI Analysis

By Jerome J Maller

With contributions from Rodney Anderson, Melissa Kirkovski, Phillip Law, Kerstin Pannek, and Caley Sullivan



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List of abbreviations

ASL=Arterial spin labelling; CSF=Cerebrospinal fluid; DICOM= Digital imaging and communications in medicine; DTI=Diffusion tensor imaging; DWI=Diffusion weighted imaging; FLAIR=Fluid attenuated inversion recovery; GM=Grey 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

Table of Contents

CHAPTER 1	8
<hr/>	
INTRODUCTION	8
TOPICS COVERED IN THIS HANDBOOK	8
MINIMUM COMPUTER SPECIFICATIONS	8
INSTALLATION OF THESE PACKAGES	10
T1-WEIGHTED AND DIFFUSION TENSOR IMAGING (DTI)	10
OTHER STRUCTURAL SEQUENCES TO CONSIDER	11
WHAT IS T1-WEIGHTED USEFUL FOR?	11
WHAT IS DTI USEFUL FOR?	12
ISSUES TO CONSIDER BEFORE ACQUISITION	13
SOFTWARE COVERED IN THIS HANDBOOK	14
PIPELINES/SCRIPTS WILL SAVE YOU TIME	14
WM BUNDLES: GETTING TO KNOW WHITE MATTER ANATOMY	15
INDEX OF SOFTWARE	15
HOW TO CONVERT INDIVIDUAL DICOMS (SLICES) INTO AN ANALYZE VOLUME (.HDR + .IMG): MRICRO	16
STEPS TO CONVERT DICOMS TO ANALYZE FORMAT (.IMG + .HDR) WITH DCM2NII	18
MRICONVERT	19
SPIN TO CONVERT A DIRECTORY OF DICOMS CONTAINING DIFFERENT SEQUENCES (SERIES)	19
DICOMSORT TO CONVERT A DIRECTORY OF DICOMS AND ANONYMISE DATA	20
PREPARING A T1-WEIGHTED SCAN FOR MINIBIRD	22
USING THE ASCENSION® MINIBIRD (FLOCK OF BIRDS) FOR STEREOTAXIC COREGISTRATION OF MRI-TO_HEAD_AND_BRAIN	24
HOW TO LOCATE THE DLPFC (F3 OR F4) WITHOUT AN MRI BRAIN SCAN	27
EXAMPLE OF TRACING A ROI IN MRICRO. IN THIS CASE, THE CAUDATE	29
MANUAL TRACING OF A ROI IN ANALYZE®	31
STEPS FOR 3D RENDERING IN ITK-SNAP OF ROI (OBJECT MAPS) TRACED IN ANALYZE	33
INTER-RATER RELIABILITY	35
FREESURFER	36
PROTOCOL TO PROCESS SWI IMAGES FROM MAGNITUDE AND PHASE DATA	45
FSL	46
TRACT-BASED SPATIAL STATISTICS (TBSS)	48
DTISTUDIO: HOW TO MEASURE FA (AND OTHER STATISTICS) OF WHITE MATTER TRACTS	61
MEDINRIA	72
MRTRIX	77
SOME USEFUL LINUX COMMANDS	85
REFERENCES	85
COLLECTION OF OUR PUBLISHED ARTICLES THAT HAVE USED THE SOFTWARE DESCRIBED IN THIS HANDBOOK	87
CHAPTER 2	90
<hr/>	
IMAGING GUIDE FOR TRACTOGRAPHY OF MAJOR WHITE MATTER FIBRE BUNDLES	90
TABLE OF CONTENTS	90
INTRODUCTION	90
REGION OF INTEREST (ROI) PLACEMENT	91
SUPERIOR LONGITUDINAL FASCICULUS (SLF)	93
CORTICOSPINAL (THALAMOCORTICAL)	95
OPTIC RADIATION	97

CORPUS CALLOSUM (CC)	98
INFERIOR LONGITUDINAL FASCICULUS (ILF) AND INFERIOR FRONTO-OCCIPITAL FASCICULUS (IFOF)	101
ARCUATE FASCICULUS (AF)	103
UNCINATE FASCICULUS (UF)	107
CINGULATE BUNDLE (CB)	109
SOMATOSENSORY	112
SOME EXAMPLES OF ROI SEEDING FROM THE LITERATURE	116
REFERENCES	121

LIST OF FIGURES

1. ILLUSTRATION OF PRINCIPLES INVOLVED IN DIFFUSION TENSOR IMAGING	12
2. MRICRO INTERFACE	17
3. THE DICOM TO NIFTI GUI INTERFACE	18
4. SORTING DICOM FILES USING SPIN	19
5. DICOMSORT USER INTERFACE	20
6. DICOM PROPERTIES IN DICOMSORT	21
7. GUI FOR MRICRO FOR MRIREG	24
8. MRIREG AND MRICRO ON ONE SCREEN	25
9. THE INTERNATIONAL 10-20 SYSTEM	27
10. BEAM SOFTWARE INTERFACE TO LOCATE THE LEFT DLPFC	28
11. THE IMPORT/EXPORT MODULE IN ANALYZE®	32
12. THE FILE-TO-FILE OPTION IN THE IMPORT/EXPORT MODULE IN ANALYZE®	32
13. FIELDS THAT MUST BE CHANGED TO MATCH VOXEL DIMENSIONS OF THE T1-WEIGHTED SCAN	33
14. THE "UPDATE MESH" BUTTON IN ITK-SNAP	34
15. MAXIMIZING THE 3D-RENDERED ROI IN INTK-SNAP	34
16. AN EXAMPLE OF A LEFT HESCHL GYRUS TRACED IN ANALYZE AND THEN 3D-RENDERED IN ITK-SNAP	34
17. THE QDEC GUI	39
18. THE QDEC GUI WITH A STATS TABLE LOADED	40
19. THE DESIGN SECTION OF QDEC	40
20. THE DISCRETE (FIXED FACTORS) SECTION OF QDEC	41
21. UNCORRECTED RESULTS SUPERIMPOSED OVER THE INFLATED BRAIN IN QDEC	42
22. CORRECTED RESULTS SUPERIMPOSED OVER THE INFLATED BRAIN IN QDEC	43
23. QDEC STATISTICS OUTPUT ARE DISPLAYED IN THE TERMINAL	43
24. OPTIONS WITHIN SPIN FOR PROCESSING SWI IMAGES FROM PHASE AND MAGNITUDE DATA	45
25. THE FSL GUI	46
26. USE THE CORRECT OPTION FOR CONVERTING DICOMS TO FSL (4D NIFTI NII) FORMAT	48
27. THE FDT GUI IN FSL	48
28. THE FSLVIEW INTERFACE DISPLAYING A T2-WEIGHTED IMAGE WITH A MASK SUPERIMPOSED	49
29. TBSS RESULTS PRESENTED FROM AXIAL AND 3D-RENDERED PERSPECTIVES	53
30. THE DTISTUDIO SOFTWARE INTERFACE	62
31. SELECTING "DTI MAPPING"	63
32. SELECT THE TYPE OF DATA	64
33. THE DWI IMAGE PARAMETERS INTERFACE	65
34. DTISTUDIO AFTER THE DICOMS HAVE BEEN IMPORTED	66
35. DTI-MAP OPTIONS	67
36. THE FIBER-TRACKING PARAMETERS BOX	68
37. SELECTING THE COLOR MAP-0 OPTION FROM THE PULL-DOWN MENU	69
38. AN EXAMPLE OF CORPUS CALLOSUM TRACTOGRAPHY AS SEGMENTED FROM SAGITTAL	70
39. CREATING TRACTOGRAPHY STATISTICS	71

40. THE MEDINRIA GUI	73
41. THE MEDINRIA GUI AFTER SELECTING NEW .DTS	73
42. THE DATA IMPORT WIZARD	73
43. LOADING THE SEQUENCE	74
44. LOADING THE DATA	74
45. FLIP THE SCAN DATA IF NECESSARY	74
46. RAW DATA DISPLAYED IN MEDINRIA	75
47. ALTERING THE THRESHOLD	75
48. THE BUNDLE MANAGER IN MEDINRIA	75
49. TRACKING THE FIBERS	76
50. AXIAL PERSPECTIVE MAXIMIZED	76
51. PROBABILISTIC WHOLE-BRAIN TRACTOGRAPHY IN MRTRIX	78
52. THE PULL-DOWN VIEW MENU IN MRTRIX	78
53. WHOLE-BRAIN TRACTOGRAPHY IN MRTRIX WITH ALTERED BRIGHTNESS AND CONTRAST	80
54. DETERMINISTIC OPTIC RADIATION TRACTOGRAPHY WITH EXCLUSION SPHERES	82
55. PROBABILISTIC OPTIC RADIATION TRACTOGRAPHY WITH EXCLUSION SPHERES	83
56. 3-DIMENSIONAL RENDERING OF OPTIC RADIATION TRACK DENSITY SUPERIMPOSED OVER THE SUBJECT'S T1-WEIGHTED MPRAGE SCAN IN MRTRIX3	84
57. COMPARISON BETWEEN POSTMORTEM PREPARATION AND DTI-BASED RECONSTRUCTION RESULTS	92
58. 3D RECONSTRUCTION RESULTS OF SOME ASSOCIATION FIBERS.	92
59. SLF I, II, III FROM DSI OF A MONKEY BRAIN	93
60. THE TRAJECTORY OF THE SLF AND ITS IDENTIFICATION IN THE COLOR MAPS	94
61. ROI PLACEMENT FOR TRACKING OF THE SLF	94
62. CORTEX-SPARING FIBER DISSECTION OF THE SLF	95
63. RELATIONSHIP BETWEEN VARIOUS NOMENCLATURES OF WM TRACTS IN THE INTERNAL CAPSULE	96
64. 3D RECONSTRUCTION RESULTS OF PROJECTION FIBERS	96
65. 3D RECONSTRUCTION RESULTS OF THE PROJECTION FIBERS	97
66. AXIAL TRAJECTORY OF THE OPTIC RADIATION FROM VENTRAL TO DORSAL	97
67. CORONAL TRAJECTORY OF THE OPTIC RADIATION FROM ANTERIOR TO POSTERIOR	98
68. 3D RECONSTRUCTION RESULTS OF COMMISSURAL FIBERS	99
69. TRAJECTORIES OF THE CC AND TAPETUM AND THEIR IDENTIFICATION IN COLOR MAPS	99
70. REPRESENTATIVE COMMISSURAL STREAMLINES EXTRACTED FROM THE B=3000 S MM-2 HARDI DATASET	100
71. PROBABILISTIC CC POPULATION MAPS, AVERAGED ACROSS 8 HEALTHY PARTICIPANTS	101
72. THE TRAJECTORY OF THE ILF AND ITS IDENTIFICATION IN COLOR MAPS	102
73. IFO-ILF	103
74. RELATIONSHIP BETWEEN AF AND SLF	104
75. MAJOR ASSOCIATION, COMMISSURAL, AND PROJECTION FIBER SYSTEMS, AND THE LOCATION OF THE SLF	104
76. METHOD USED FOR LOCATING THE HAF SEEDPOINT IN SAGITTAL, CORONAL AND AXIAL VIEWS IN THE LH	105
77. FIBER TRACTS BETWEEN THE HAF SEEDPOINT AND THE SEPARATE CORTICAL ROI SEEDPOINTS	106
78. DEMARCATION OF THE ROI AROUND THE AF IN THE RIGHT HEMISPHERE	107
79. THE TRAJECTORY OF THE UF AND ITS IDENTIFICATION IN COLOR MAPS	108
80. FIBER DISSECTION OF THE IFOF IN A LEFT HEMISPHERE. AND DTI TRACTOGRAPHY RECONSTRUCTION OF THE IFOF AND UF IN A LEFT HEMISPHERE	109
81. 3D RECONSTRUCTION RESULTS OF ASSOCIATION FIBERS IN THE LIMBIC SYSTEM	110
82. TRAJECTORIES OF THE CINGULUM AND FORNIX / STRIA TERMINALIS AND THEIR IDENTIFICATION IN COLOR	111
83. ROI LOCATIONS USED FOR RECONSTRUCTIONS OF THE TWO SENSORY WHITE MATTER TRACTS	112
84. THREE-DIMENSIONAL VIEW OF RECONSTRUCTED TRACTS USING HIGH SPATIAL RESOLUTION	113
85. STRUCTURAL (T1-WEIGHTED) MRI OF THE SAGITTAL MIDLINE PLANE	113
86. MAPPING OF THE MEDIAL LEMNISCUS, SPINAL LEMNISCUS, AND CENTRAL TEGMENTAL TRACT	114
87. TRANSVERSE DTI COLOR-CODED MAP AND CORRESPONDING T2-WEIGHTED MR IMAGE	115
88. ROIS FOR GENERATING FT IMAGES OF THE MAJOR WHITE MATTER FIBER TRACTS	116

89. DTI TRACTOGRAPHY RECONSTRUCTION OF THE ASSOCIATION BUNDLES OF A LEFT HEMISPHERE	117
90. AVERAGED TRACTOGRAPHY RECONSTRUCTION BY USING A TWO-REGION OF INTEREST APPROACH	117
91. RECONSTRUCTED DIRECT AND INDIRECT PATHWAYS	118
92. COMPARISON BETWEEN PERCENTAGE MAPS BASED ON POST-MORTEM HISTOLOGY	119
93. COMPARISON BETWEEN PERCENTAGE MAPS BASED ON POST-MORTEM HISTOLOGY AND DTI TRACTOGRAPHY OF THE MAJOR ASSOCIATION TRACTS	120

LIST OF TABLES

1. EXAMPLES OF NAMES OF ROIS THAT REPRESENT TALAIRACH CO-ORDINATES	30
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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 the analyses we wanted to carry out. 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 skill set to many students and research groups around the world.

CHAPTER 1

Introduction

Whilst there are many 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 achieve the output 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 Tensor Imaging
- Other structural sequences to consider
- What is T1-weighted useful for?
- What is DTI 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 and most amount of RAM you can access. Same goes for the hard drive capacity: at least 250GB but the bigger the better!

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 (this 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.

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 will 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 at a time, whereas those that are multithreaded can utilise as many CPUs 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. 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 grey/white matter contrast. The word 'contrast' in this context means the clarity of the grey/white matter borders. This is very important when segmenting regions of interest (ROIs) as they rely on clear grey/white matter borders. However, the clarity of these borders is also important in the clinical context as a lack of contrast between grey/white matter can sometimes indicate poor structural integrity as sometimes 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 that is 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 obstructed, leading to the water flow not being parallel anymore 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, 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 WMH 'dots' can be as well (sometimes referred to as 'punctate'). For example, these can be representative of white matter 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 cerebrospinal fluid (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 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 grey 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 grey matter is. Essentially, $volume = thickness \times area$.

- 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 (λ_1), L2 (λ_2), L3 (λ_3), MD, RD. Figure 1 illustrates what the eigenvalues (λ) represent (from Maller et al.,2010).
- Tractography of WM bundles
- Connectivity
- Fibre density

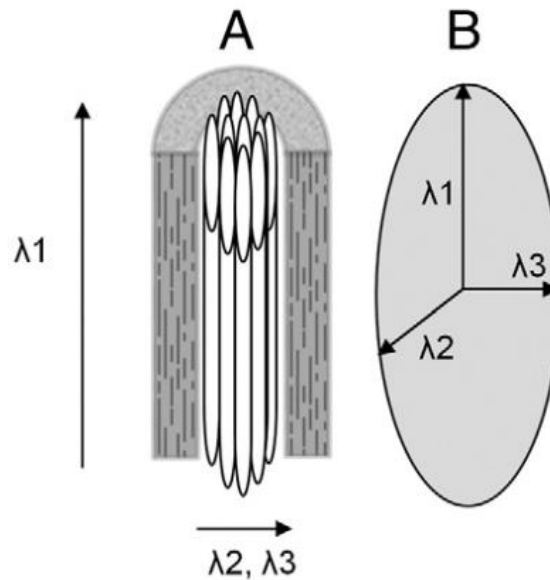


Figure 1. Illustration of principles involved in diffusion tensor imaging. (A) Microtubules of an axon encased in myelin sheath. (B) Eigenvectors represent main axes directions. Water diffusion is restricted along the longitudinal axis (λ_1) and/or perpendicular to the longitudinal axis of the axons (radial diffusion, eigenvalues λ_2, λ_3) due to axonal microstructure.

Issues to consider before acquisition

- Scanner type (brand, Tesla, gradients, acquisition pulse sequences installed onto the console)
- SPGR (spoiled gradient, used with GE scanners), MPRAGE (Magnetization Prepared Rapid Acquisition GRE (3D Inversion Recovery)), MP2RAGE (MPRAGE with multiple echoes; MPRAGE and MP2RAGE are used with Siemens and Philips scanners)
- DTI, RESOLVE (used for high-resolution q-ball scanning that takes into account crossing fibres)
- Silenz (GE) and PETRA (Siemens) – these are T1-weighted sequences that are less than 10db above ambient noise level
- Fast scanning/GRAPPA, SENSE, SMASH, PILS etc. These usually are parallel imaging methods to reduce scanning times although they can also reduce SNR. MRI is always about give-and-take in terms of 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
- Resolution, repetition time (TR), echo time (TE), slice thickness, signal-to-noise ratio (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 DTI
- DTI number of directions: more directions will yield more accurate tractography (but not necessarily more accurate FA estimation), and more b-zero volumes (T2-weighted scans) 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. AC-PC alignment in the scanner is also important because then you can more accurately compare data across scans
- 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 distribute 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 scan (head) can always be confirmed.

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.

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>). At the end of this handbook we have included some general advice on where to place initial seed points for a variety of major WM bundles.

Organisation of this handbook

Chapter 1 presents T1-weighted and DTI analysis, and Chapter 2 presents tractography.

Index of software and instructions

1. How to convert individual dicoms (slices) into an analyze volume (.hdr + .img): MRlcro
2. Steps to convert dicoms to Analyze format (.img + .hdr) with dcm2nii
3. MRlconvert
4. SPIN to convert a directory of DICOMS containing different sequences (series)
5. DICOMsort (a multi-platform alternative to SPIN) to convert a directory of DICOMS containing different sequences (series) and anonymise data MRlconvert
6. Preparing a T1-weighted scan for MiniBIRD
7. Using the Ascension® MiniBIRD (Flock of Birds) for stereotaxic coregistration of MRI-to-head-and-brain
8. How to locate the DLPFC (F3 or F4) without an MRI brain scan Inter-rater reliability
9. Example of tracing an ROI in MRlcro. In this case, the caudate.
10. Manual tracing of a Region Of Interest (ROI) in Analyze®
11. Steps for 3D rendering in ITK-SNAP of ROI (object maps) traced in Analyze DICOMsort (a multi-platform alternative to SPIN) to convert a directory of DICOMS containing different sequences (series) and anonymise data
12. Inter-rater reliability
13. Freesurfer
14. Protocol to process SWI images from magnitude and phase data
15. FSL
16. Tract-Based Spatial Statistics (TBSS)
17. DTIstudio: How to measure FA (and other statistics) of white matter tracts
18. MedINRIA
19. MRtrix
20. Some useful Linux commands
21. References
22. Collection of our published articles that have used the software described in this handbook
23. Imaging guide for tractography of major white matter fibre bundles

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)

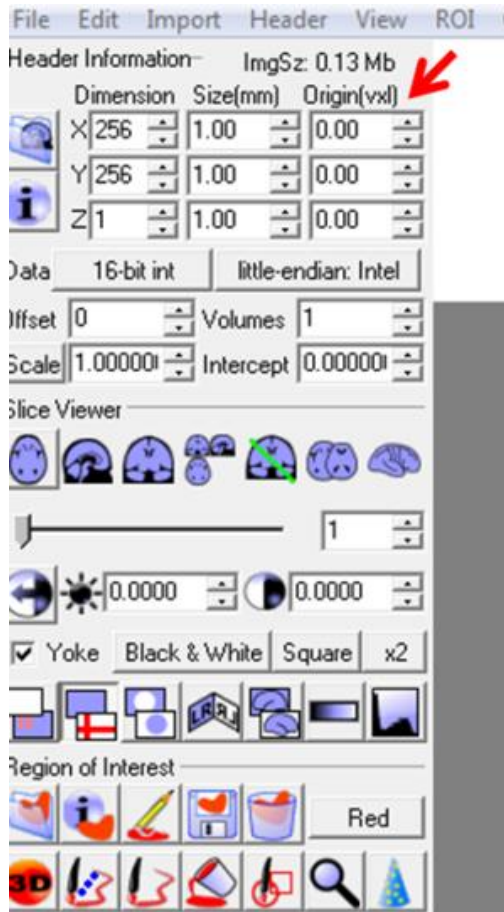


Figure 2. MRICro interface. Red arrow indicates the column where you reset the AC to be 0,0,0

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)
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
7. Click on the “**output format**” and change it to the first option (Figure 3), “**SPM2 (Analyze hdr/img)**”

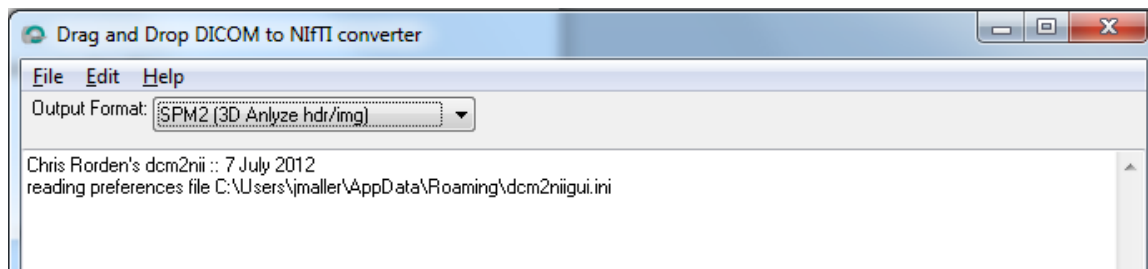


Figure 3. The DICOM to NifTI GUI

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 Metalmage 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).

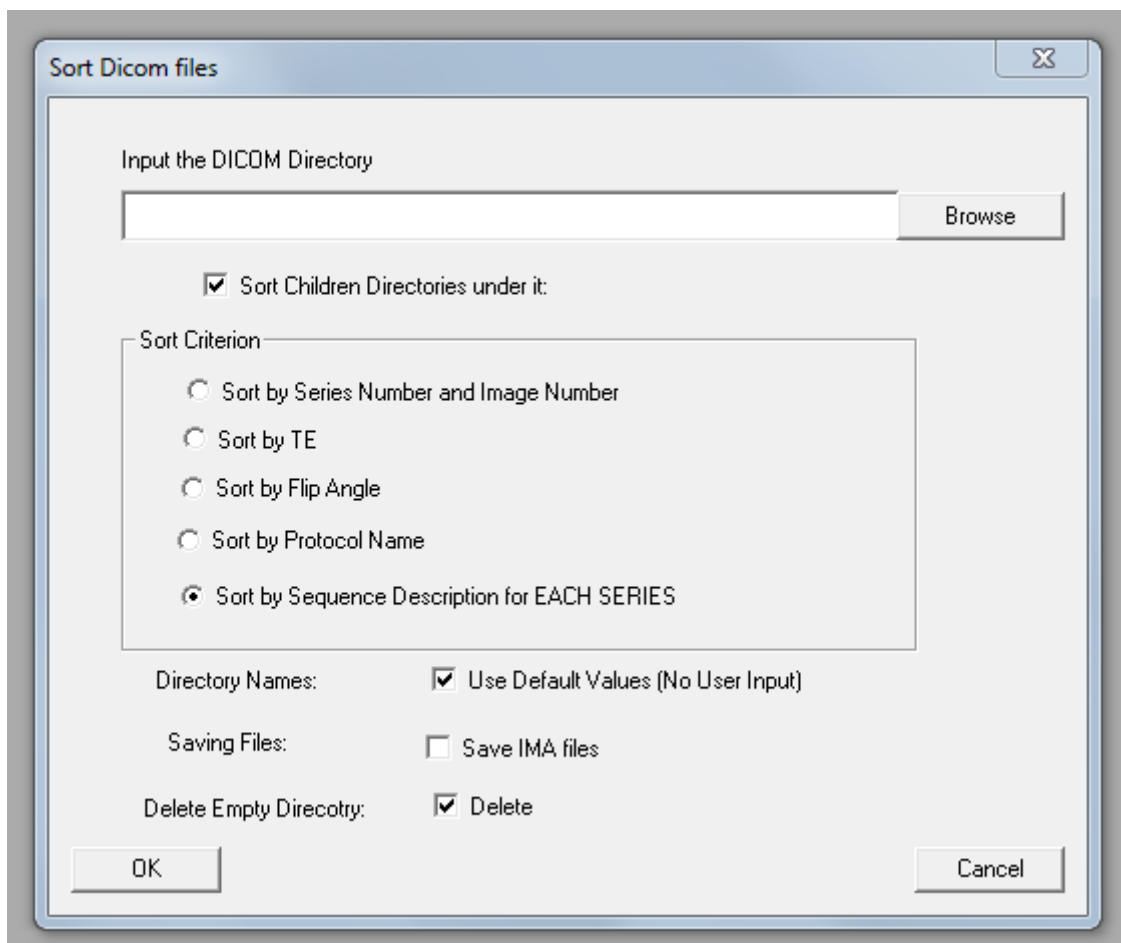


Figure 4. Sorting Dicom files using SPIN.

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.

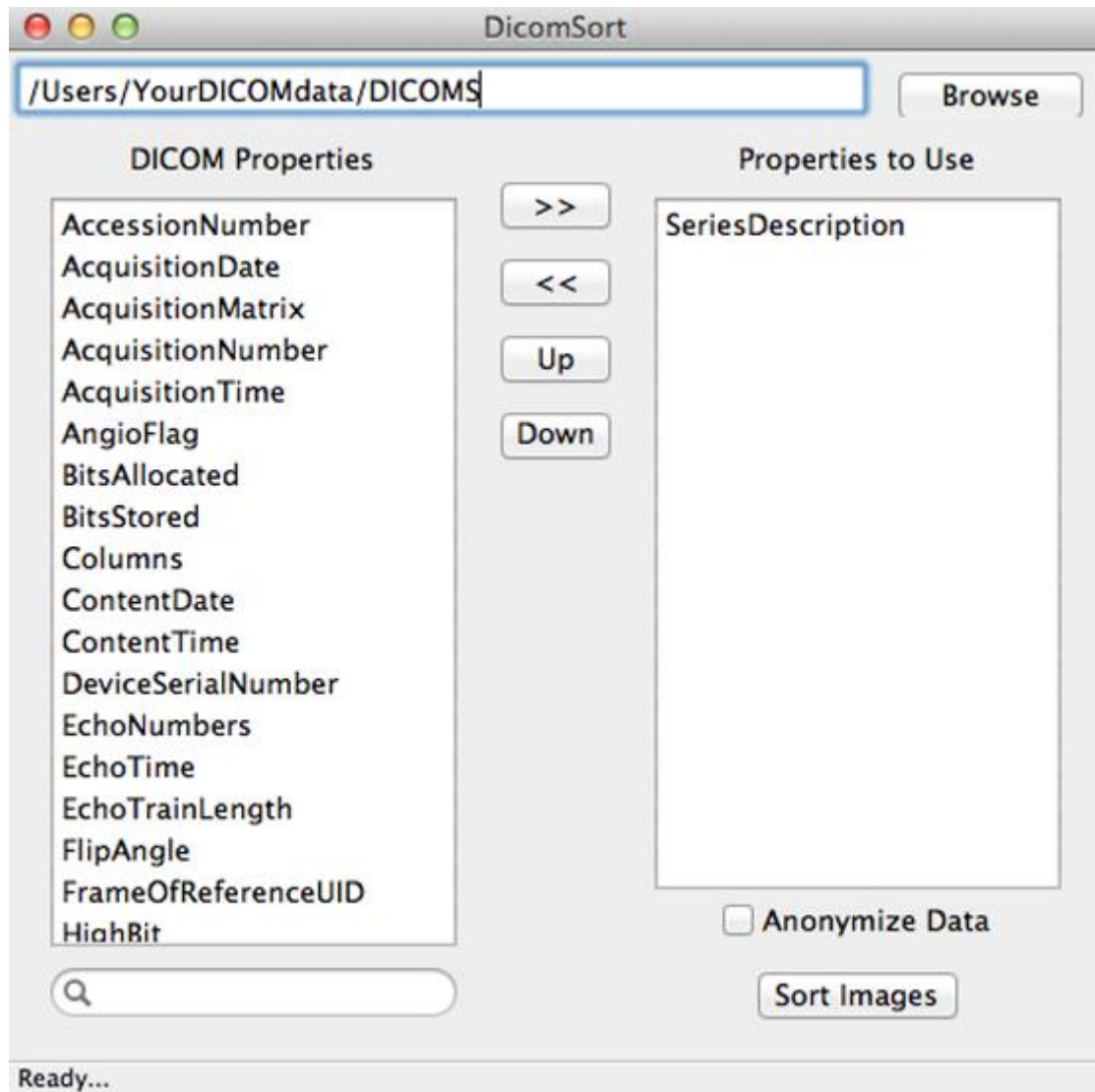


Figure 5. DICOMsort user interface.

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

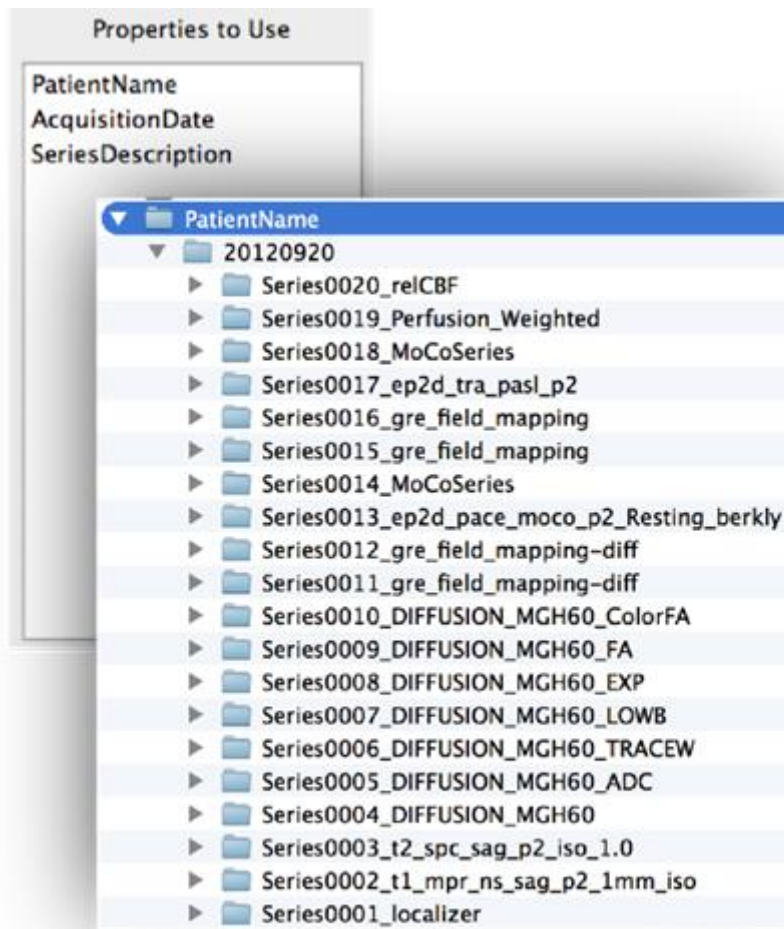


Figure 6. DICOM properties in DICOMsort.

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”**.

6. Preparing a T1-weighted scan for MiniBIRD

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 MRlcro, 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 MRlcro, 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 1mm³**: '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 MRlcro 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. SPM8) and utilise its normalization feature.

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.

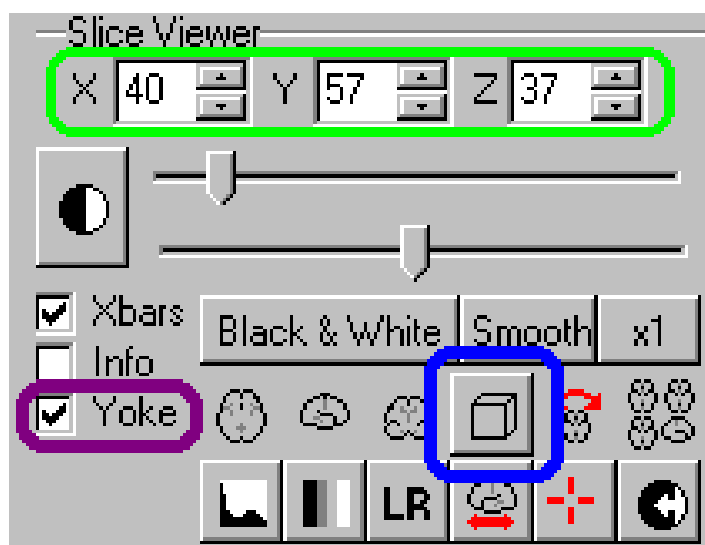


Figure 7. GUI for MRICro for MRIreg.

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).

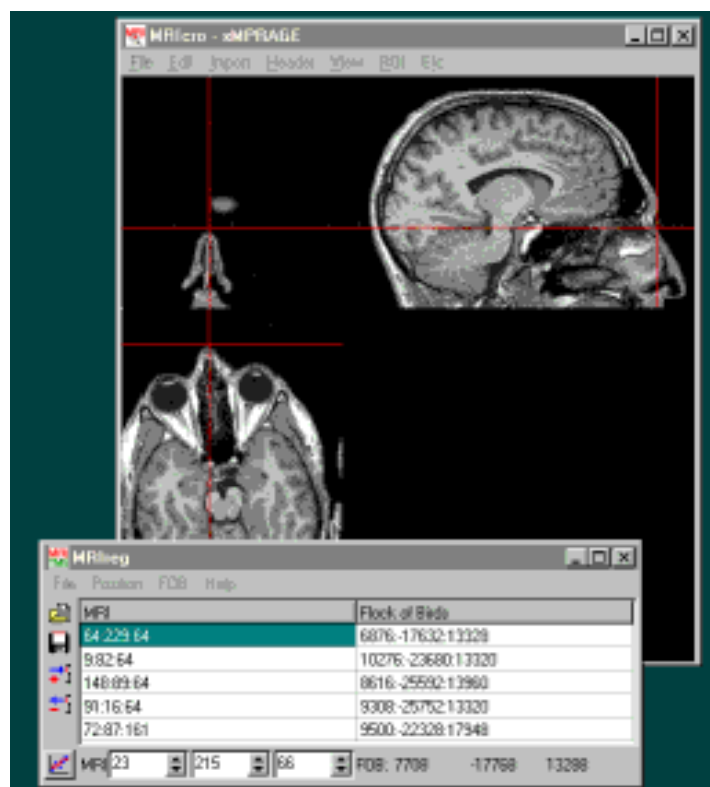


Figure 8. MRIreg and MRIcro on one screen.

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.

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).

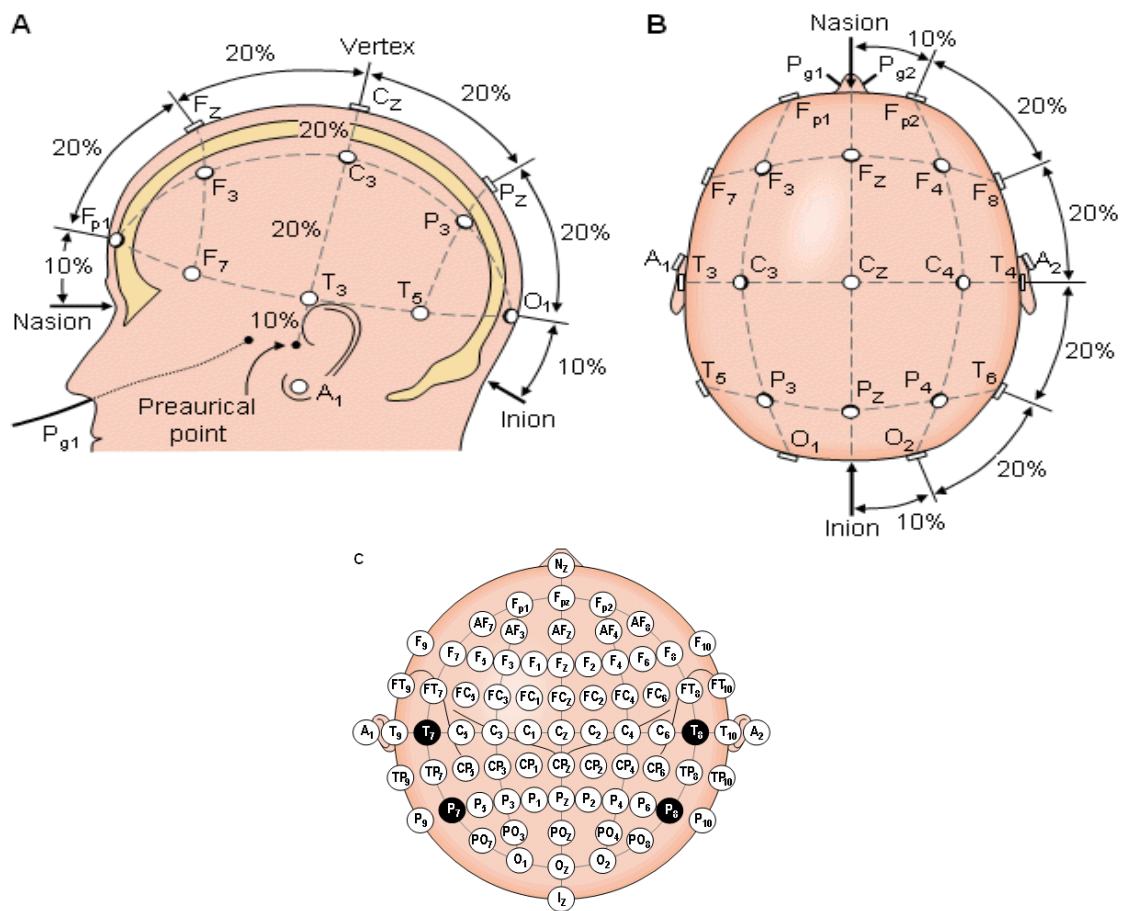


Figure 9. The international 10-20 system seen from (A) left and (B) above the head. A=Ear lobe, C=central, Pg=nasopharyngeal, P=parietal, F=frontal, Fp=frontal polar, O=occipital. (C) Location and nomenclature of the intermediate 10% electrodes, as standardized by the American Electroencephalographic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

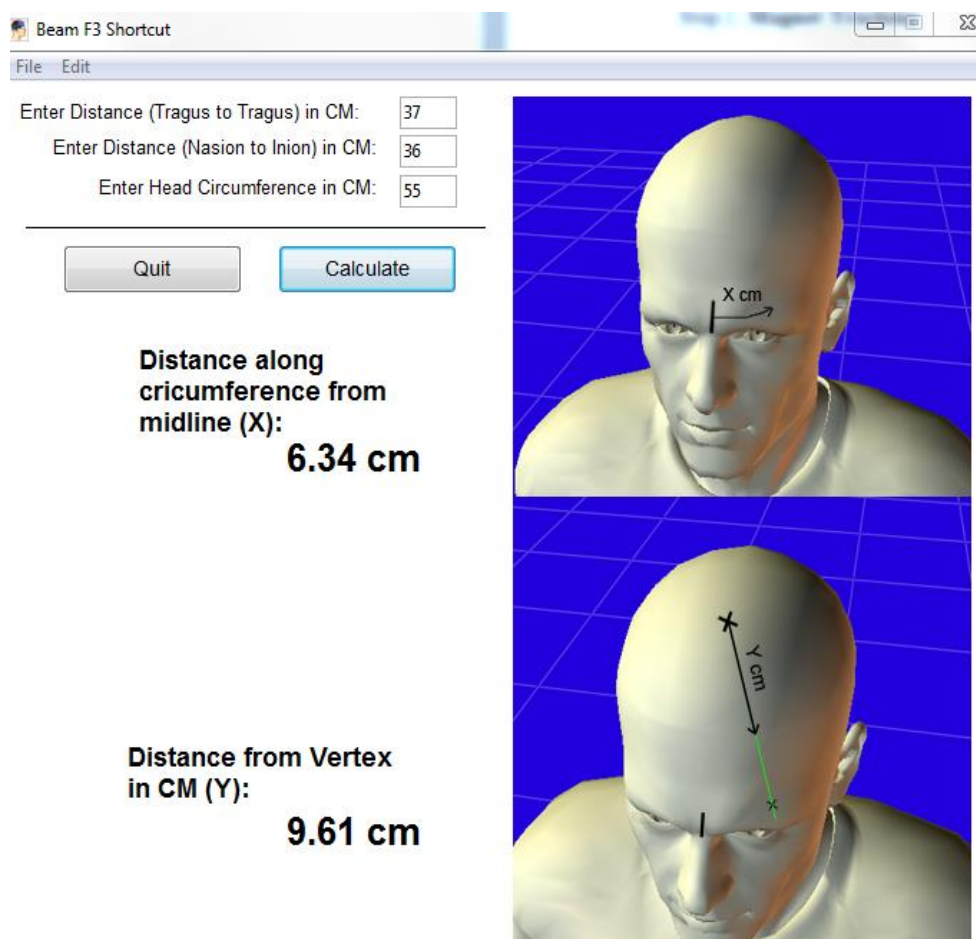


Figure 10. Beam software interface to locate the left DLPFC.

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 grey 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 is often quicker to enter the Intensity Max and Min values manually (once you have established them 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.

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

1. Use Analyze

a. File -> Import/Export

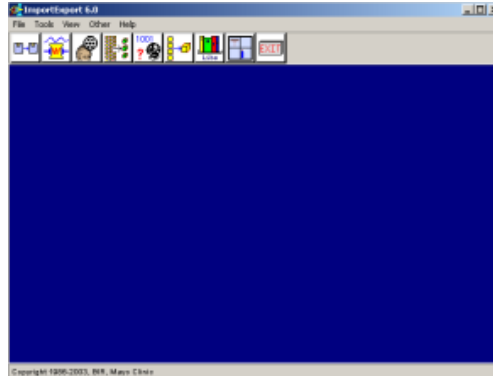


Figure 11. The Import/Export module in Analyze®

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



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

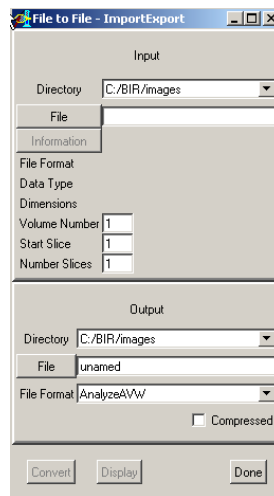


Figure 12. The File-to-File option in the Import/Export module in Analyze®

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

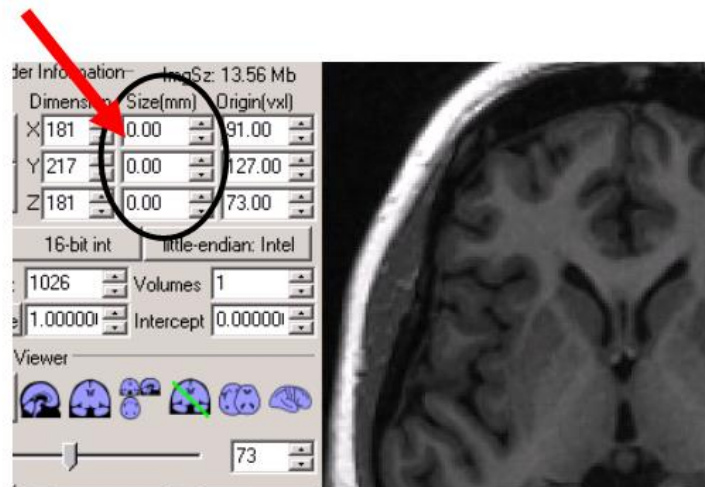


Figure 13. Fields that must be changed to match voxel dimensions of the T1-weighted scan.

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.

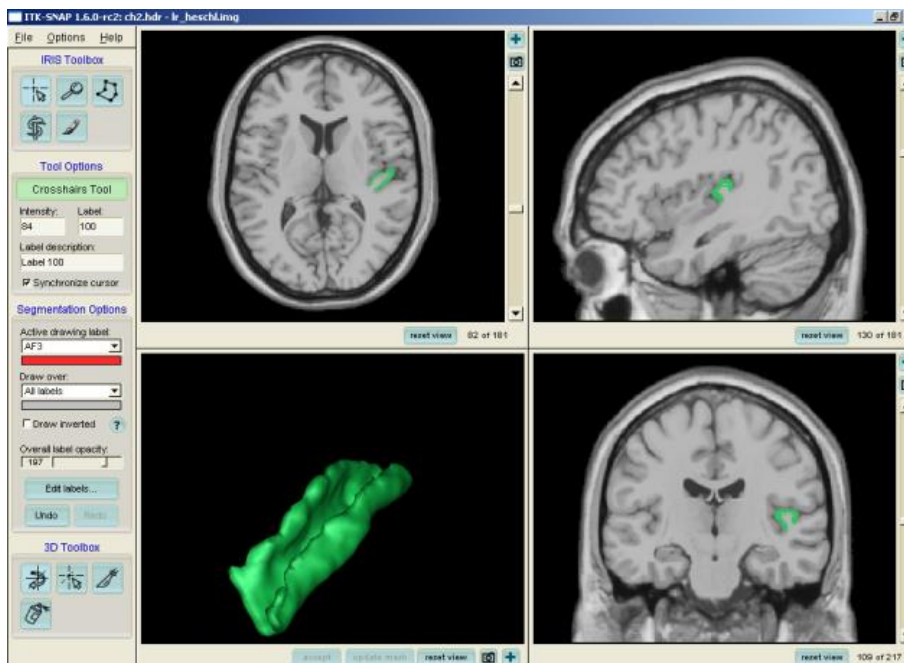


Figure 16. An example of a left Heschl gyrus traced in Analyze and then 3D-rendered in ITK-SNAP.

12. Inter-rater reliability

Inter-rater reliability = $((\text{Rater A} - \text{Rater B}) / ((\text{Rater A} + \text{Rater B}) / 2)) * 100$

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. To process a scan through Freesurfer:

1. Download Freesurfer: 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 **tkviewer 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)

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

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

- In around 8 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

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

ld1 control f 20

ld2 control m 24

ld3 clinical m 21

ld4 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” (in plain text format)
- Now make another file for the “sex” column, as follows:

m

f

And save it as “sex” (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).

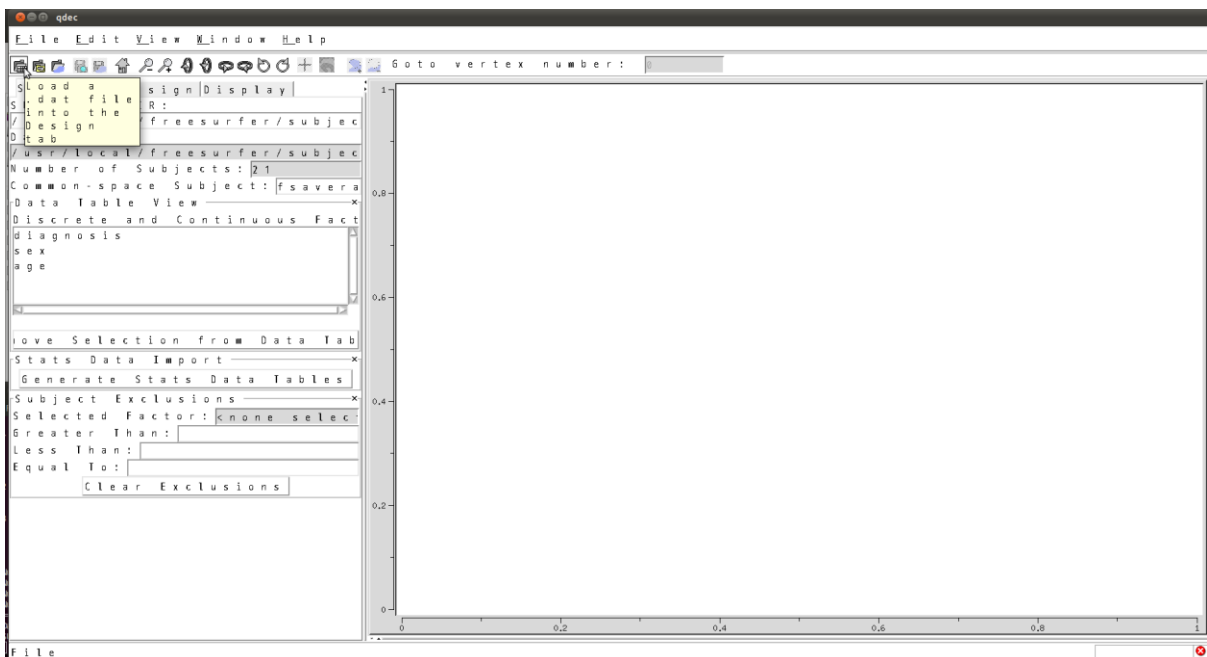


Figure 17. The QDEC GUI.

- 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).

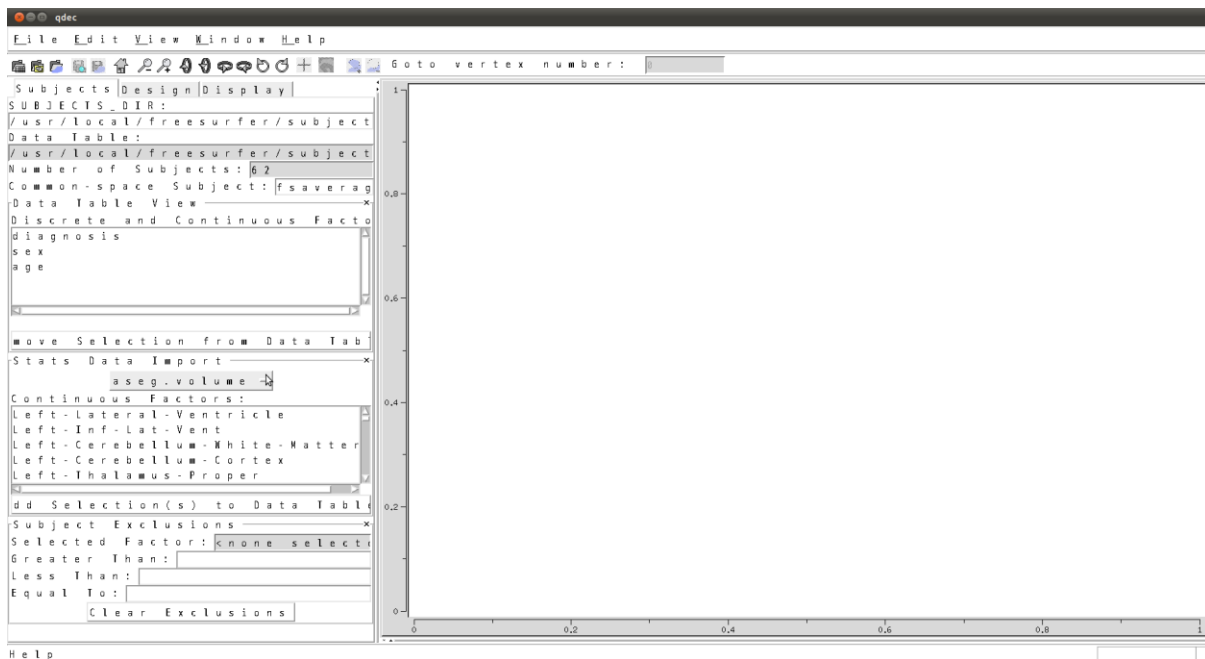


Figure 18. The QDEC GUI with a stats table loaded.

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

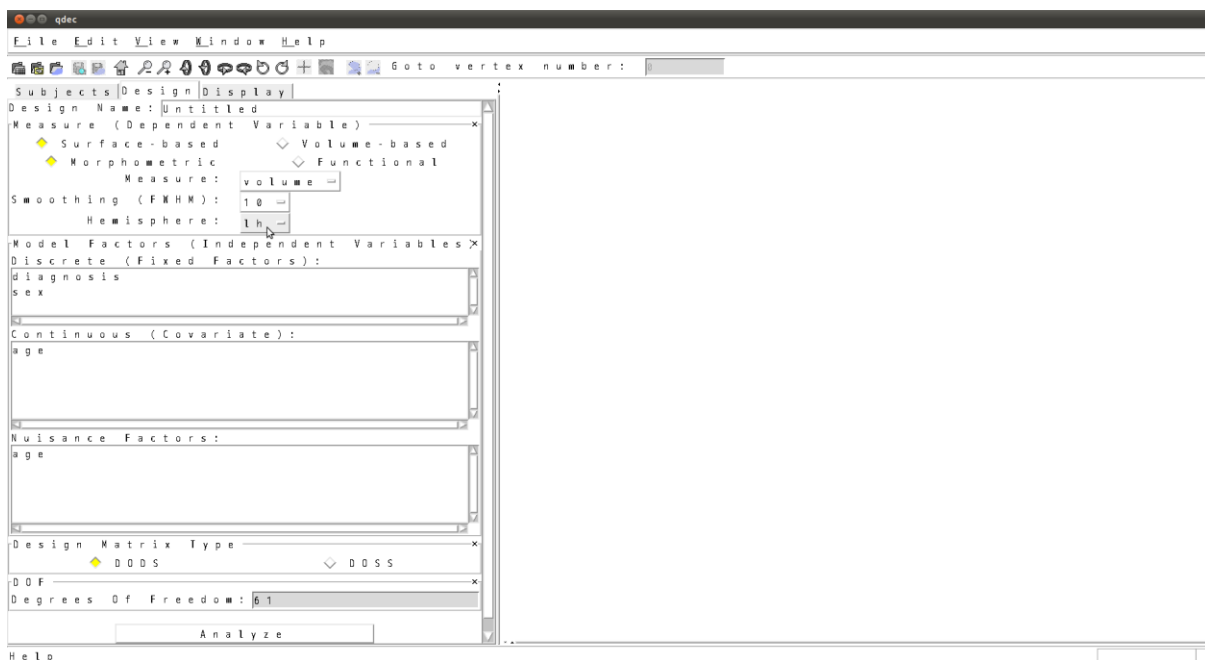


Figure 19. The Design section of QDEC.

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

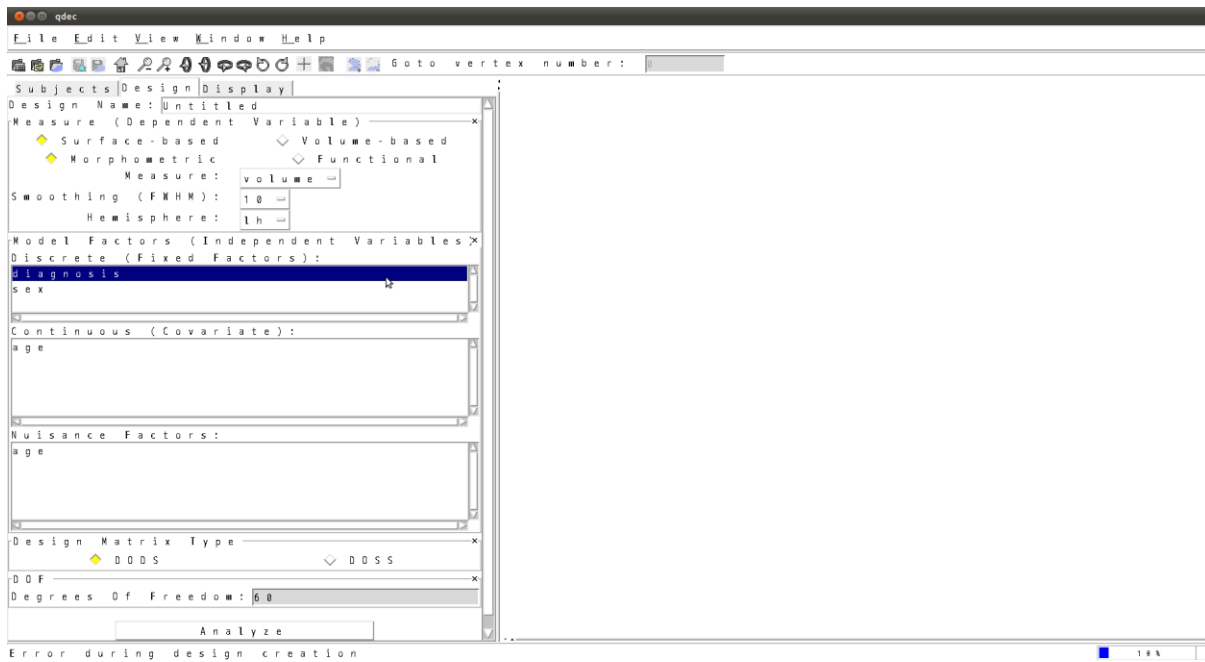


Figure 20. The Discrete (Fixed Factors) section of QDEC.

- 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

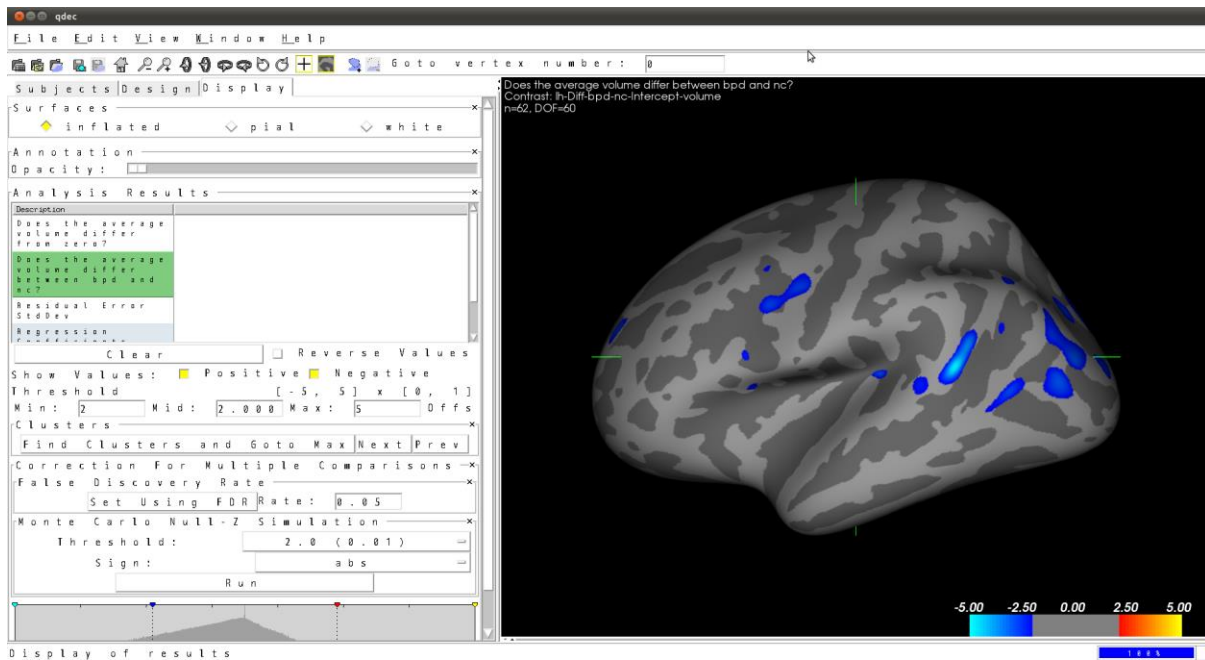


Figure 21. Uncorrected results superimposed over the inflated brain in QDEC.

- 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 $p=0.05$.

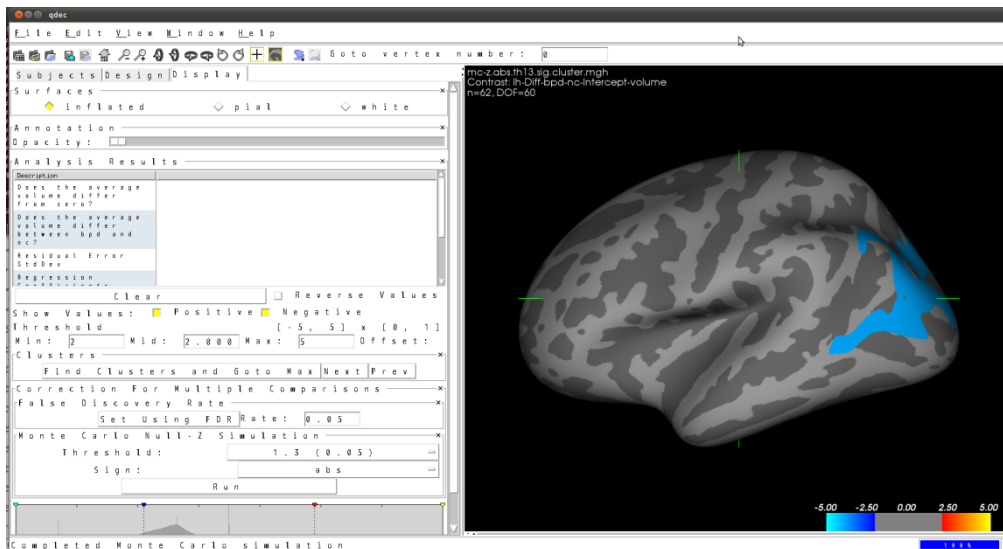


Figure 22. Corrected results superimposed over the inflated brain in QDEC.

```

user@user: ~
File Edit View Search Terminal Help
# Adjust Threshold: 1
# CVP Value Threshold: 0.05
# Area Threshold: 0 mm^2
# CSD thresh: 1.300000
# CSD nreps: 10000
# CSD signtype: null-z
# CSD contrast: NA
# CSD confint: 90.000000
# Overall max: 2.1485 at vertex 2429
# Overall min: -4.94487 at vertex 44549
# Clusters: 1
# Total Cortical Surface Area: 65416.6 (mm^2)
# FixMN: 1
#
# ClusterNo Max VtxMax Size(mm^2) Tal X Tal Y Tal Z CVP CVPLow C
VPH Ntxs Annot
1 -4.124 10868 3785.71 -14.3 -81.5 37.3 0.00010 0.00000 0.
00020 6266 superiorparietal
    
```

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

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 will 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**)

14. Protocol to process SWI 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

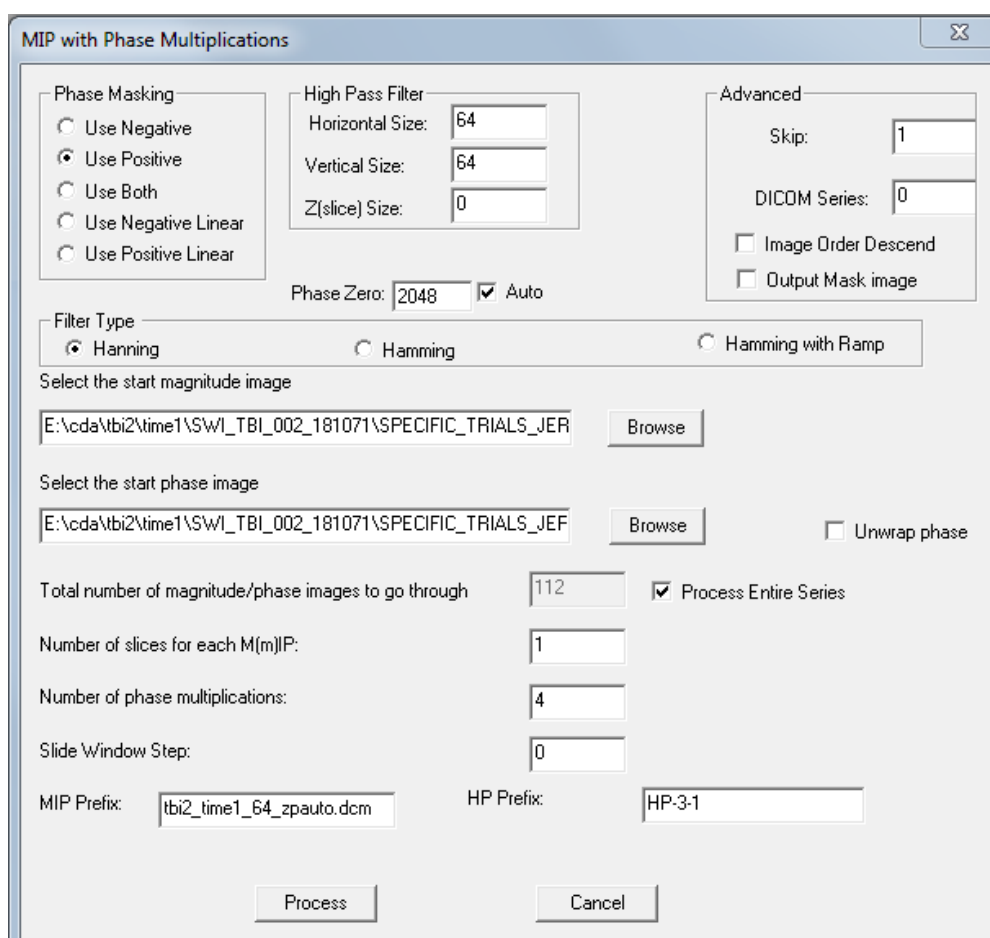


Figure 24. Options within SPIN for processing SWI images from phase and magnitude data

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.

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
```

```
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).

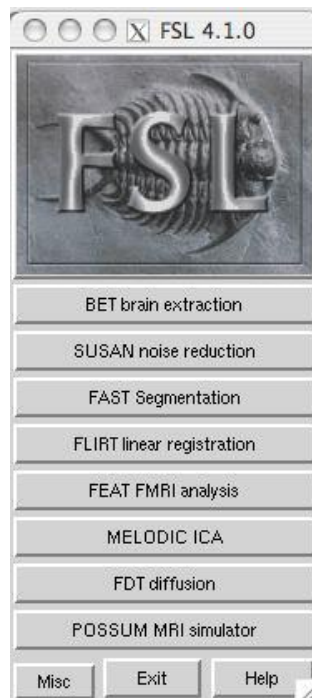


Figure 25. The FSL GUI.

BET (Brain extraction tool): This strips the skull and eyes from the scan to leave just brain. Very useful, very accurate, and very quick.

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 grey matter, white matter, and CSF volumes from T1-weighted scans, including FSL (FAST), SPM, Freesurfer, and many more...

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)

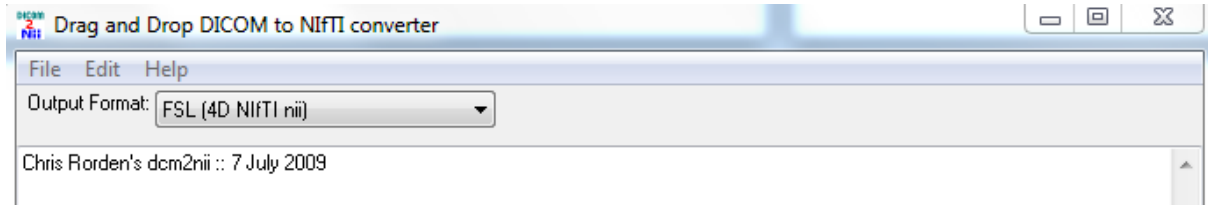


Figure 26. Use the correct option for converting dicoms to FSL (4D Nifti nii) format.

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"**

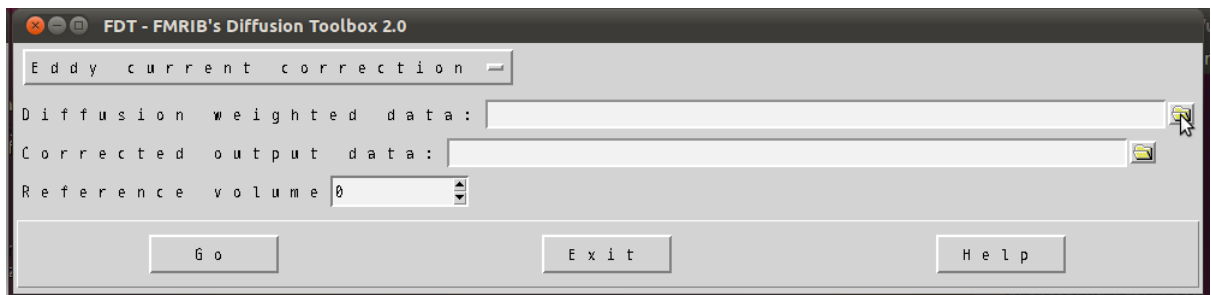


Figure 27. The FDT GUI in FSL.

6. Open the directory where your .nii file is and select it. The default output file will be called **"data.nii.gz"**
7. 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.
8. Exit FSL
9. 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):
10. **fslroi data.nii.gz nodif 0 1**

11. **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)
12. Then, type in **“fslview”**
13. **File → Open** (select the output of the eddy current correction process called “data”)
14. 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).
15. **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 28. The FSLview interface displaying a T2-weighted image with a mask superimposed.

16. When you have finished editing the mask, **File → Save As**, and save the mask as “mask1”.
17. 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

18. **Select the correct files** (data, mask1, output name, gradient directions (.bvec) and b-values (.bval).
19. Click “Go”

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 -l all_FA_skeletonised -o tbss -m mean_FA_skeleton_mask -d design.mat -t design.con -n 5000 -T2 -V**
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_corrpt_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_corr_p_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 $1-0.95=0.05$).

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 $I2dir/$x -add $I3dir/$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_corr_p_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_corr_p_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_corr_p_tstat1 0.95 mean_FA tbss_fillFAp95
```

That command will apply the "tbss_fill" command to the file called "tbss_tfce_corr_p_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):

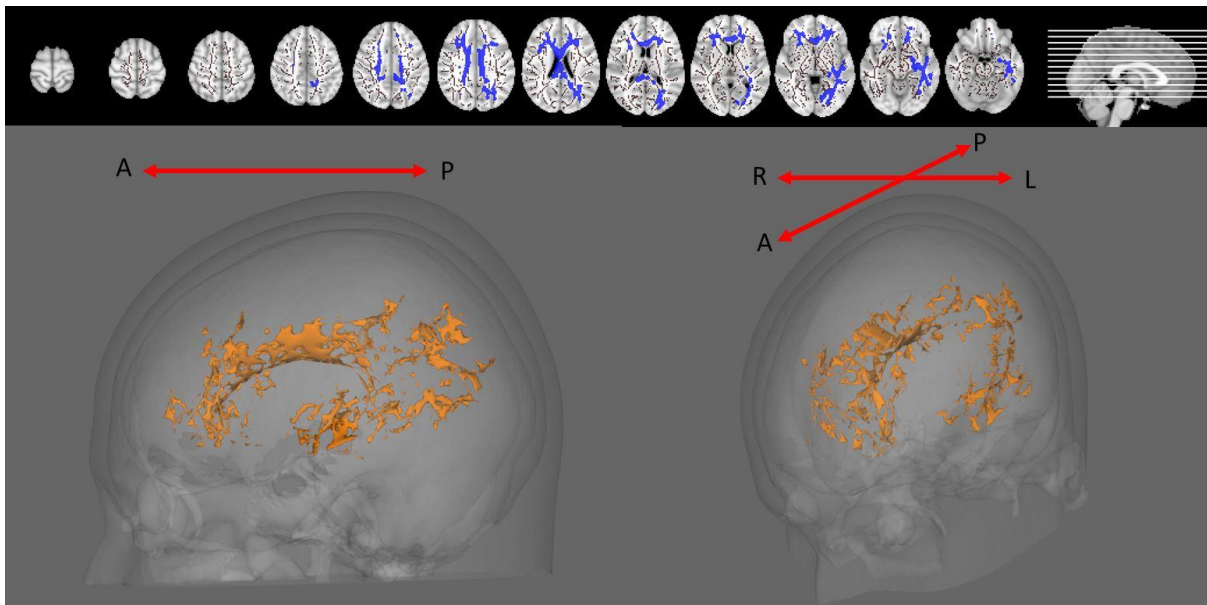


Figure 29. TBSS results presented from axial and 3D-rendered perspectives.

Note about eddy current and movement correction:

For each subject, it is advisable 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)

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/atlas directory into your tbss/stats directory. Then extract the ROIs (to make them masks) with the following commands (script created by 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_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_ilm.txt -m R_ilm.nii.gz
fslmeants -i all_L2.nii.gz -o L2_mean_L_ilm.txt -m L_ilm.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_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_ilm.txt -m R_ilm.nii.gz
fslmeants -i all_L3.nii.gz -o L3_mean_L_ilm.txt -m L_ilm.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_ilmf.txt -m R_ilmf.nii.gz
fslmeants -i all_radial_diffusivity.nii.gz -o radial_diffusivity_mean_L_ilmf.txt -m L_ilmf.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 decimal
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 in creating the appropriate matrix:

http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM#Two-Group_Difference_Adjusted_for_Covariate

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

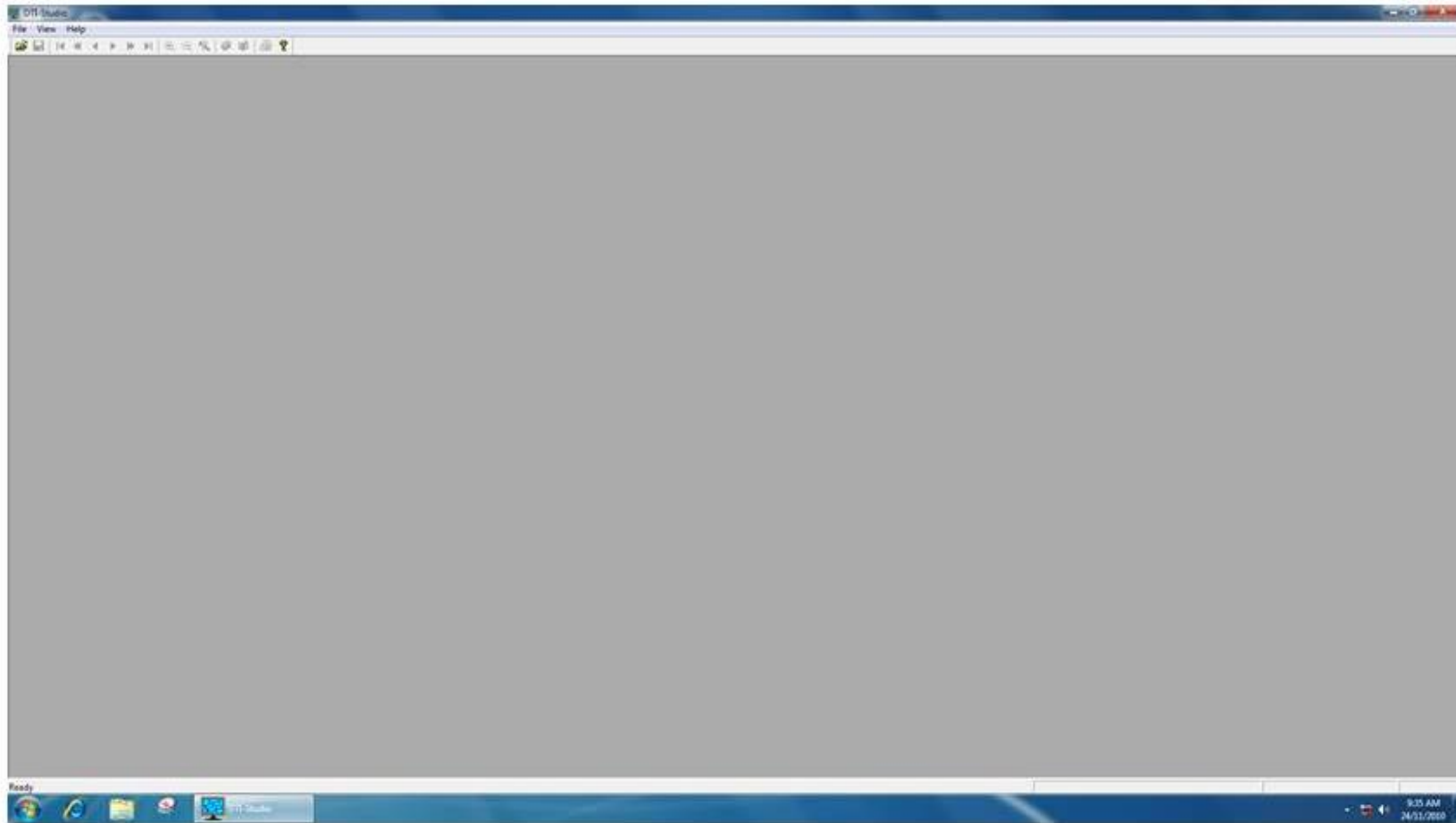


Figure 30. The DTIstudio software interface.

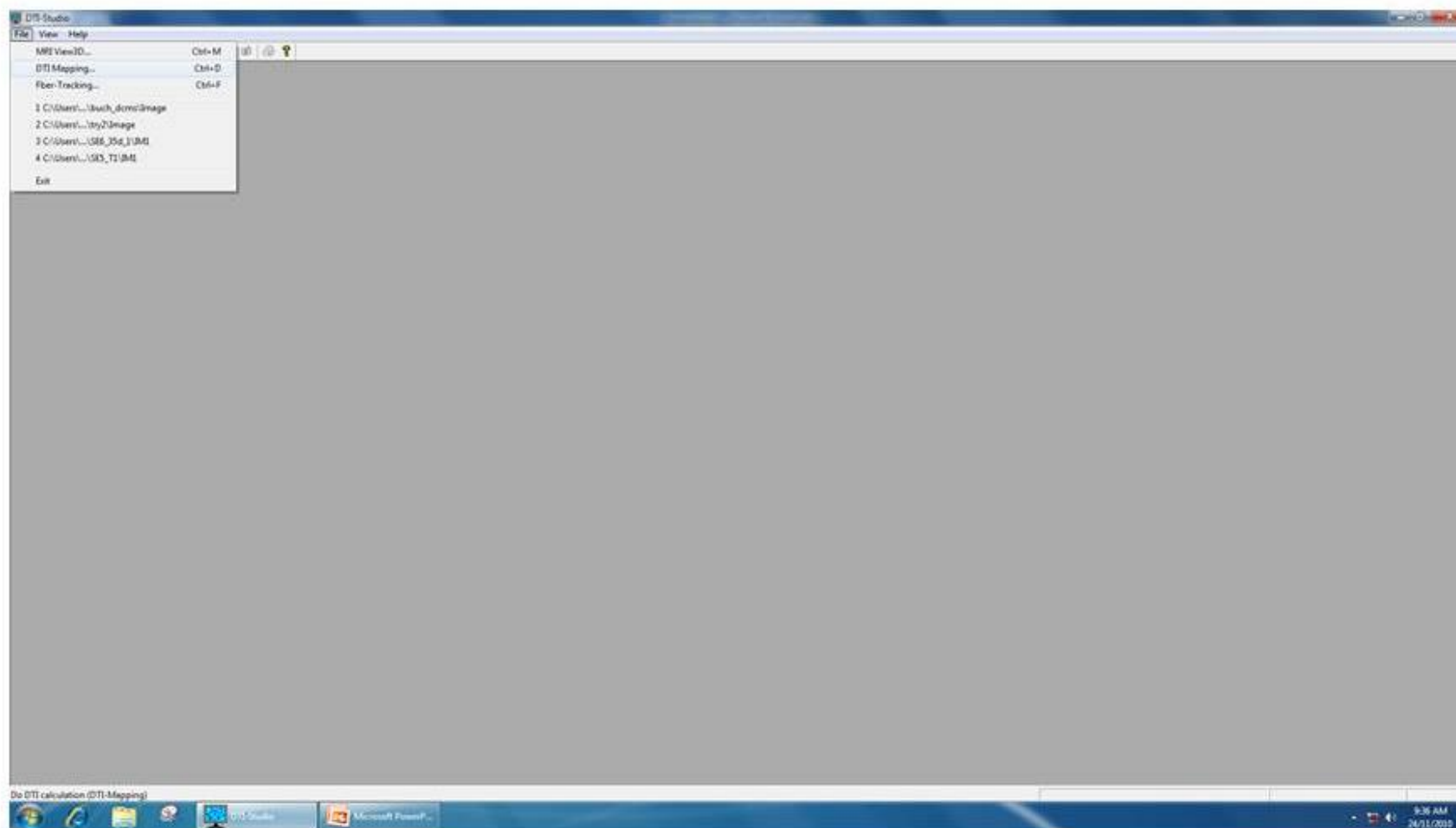


Figure 31. Selecting “DTI Mapping”

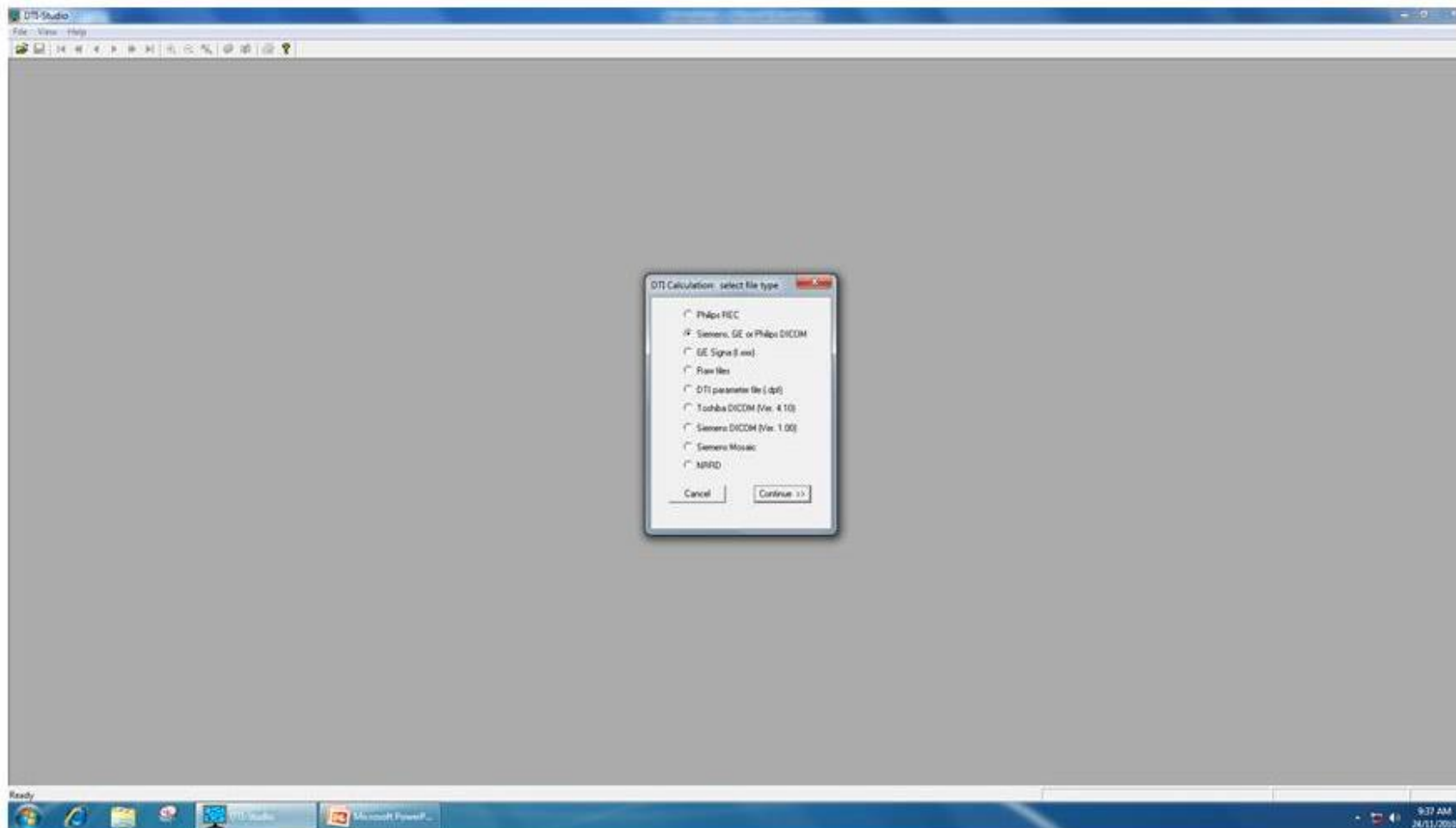


Figure 32. Select the type of data.

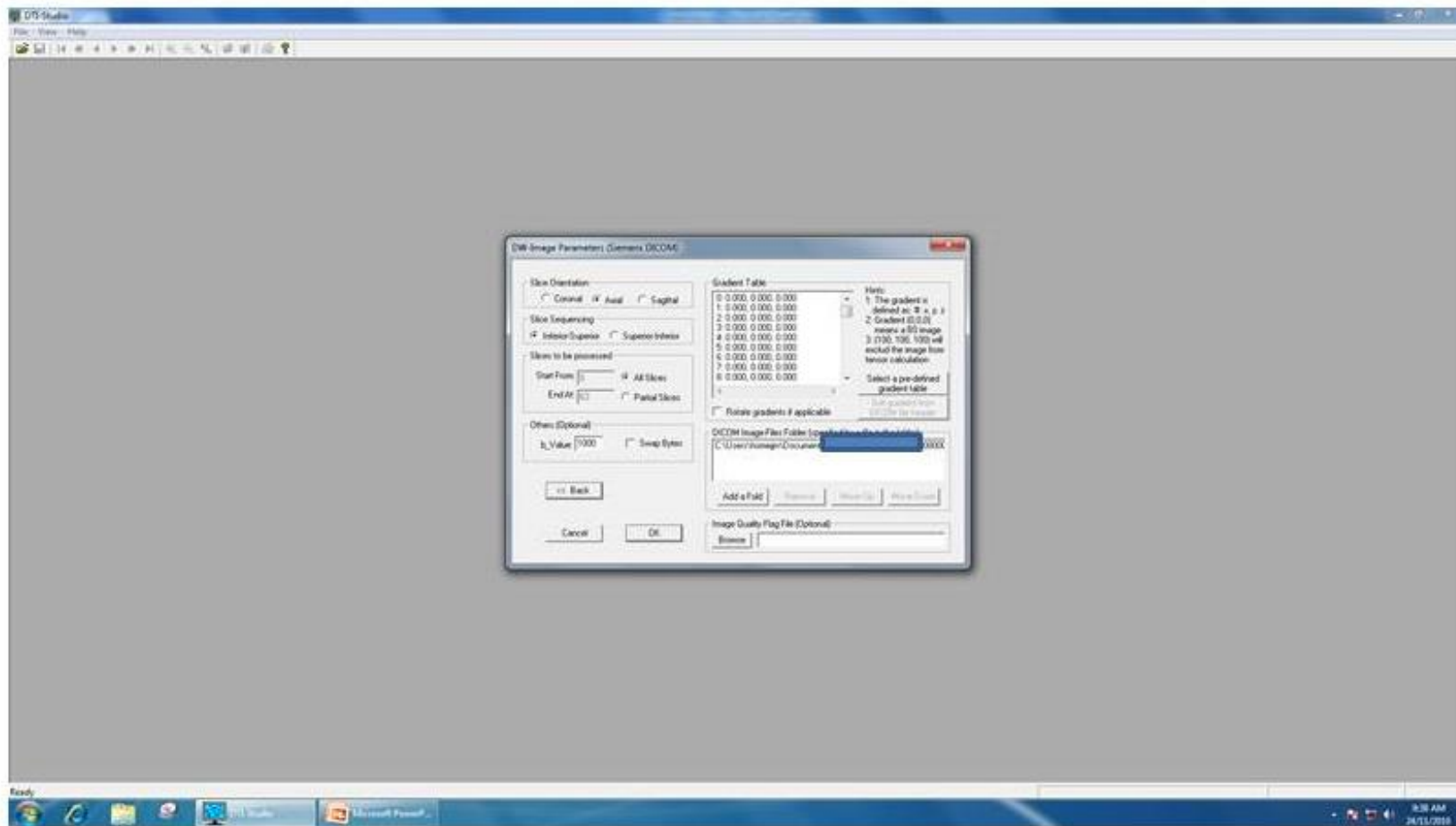


Figure 33. The DWI Image Parameters interface.

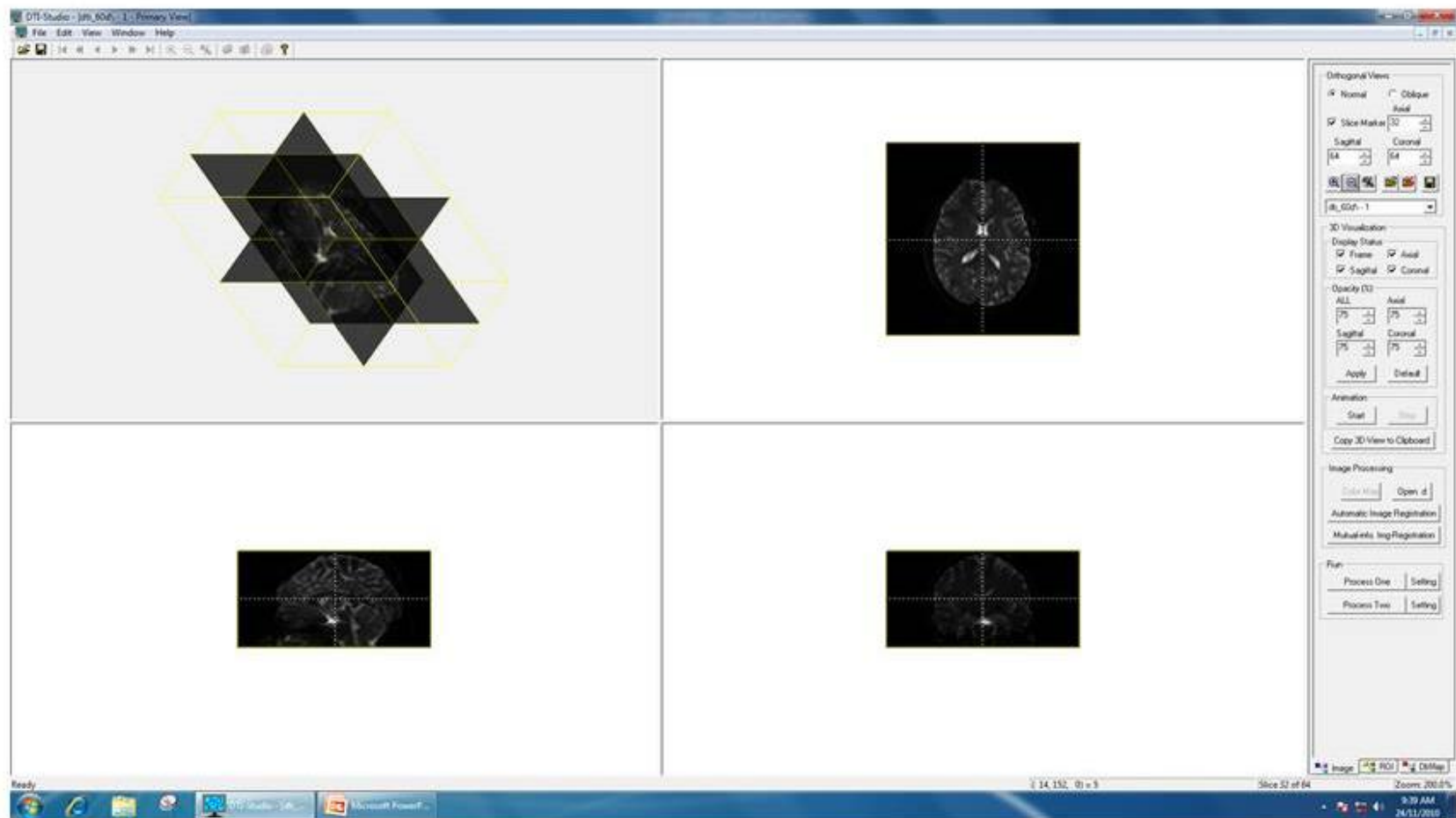


Figure 34. DTIstudio after the DICOMS have been imported.

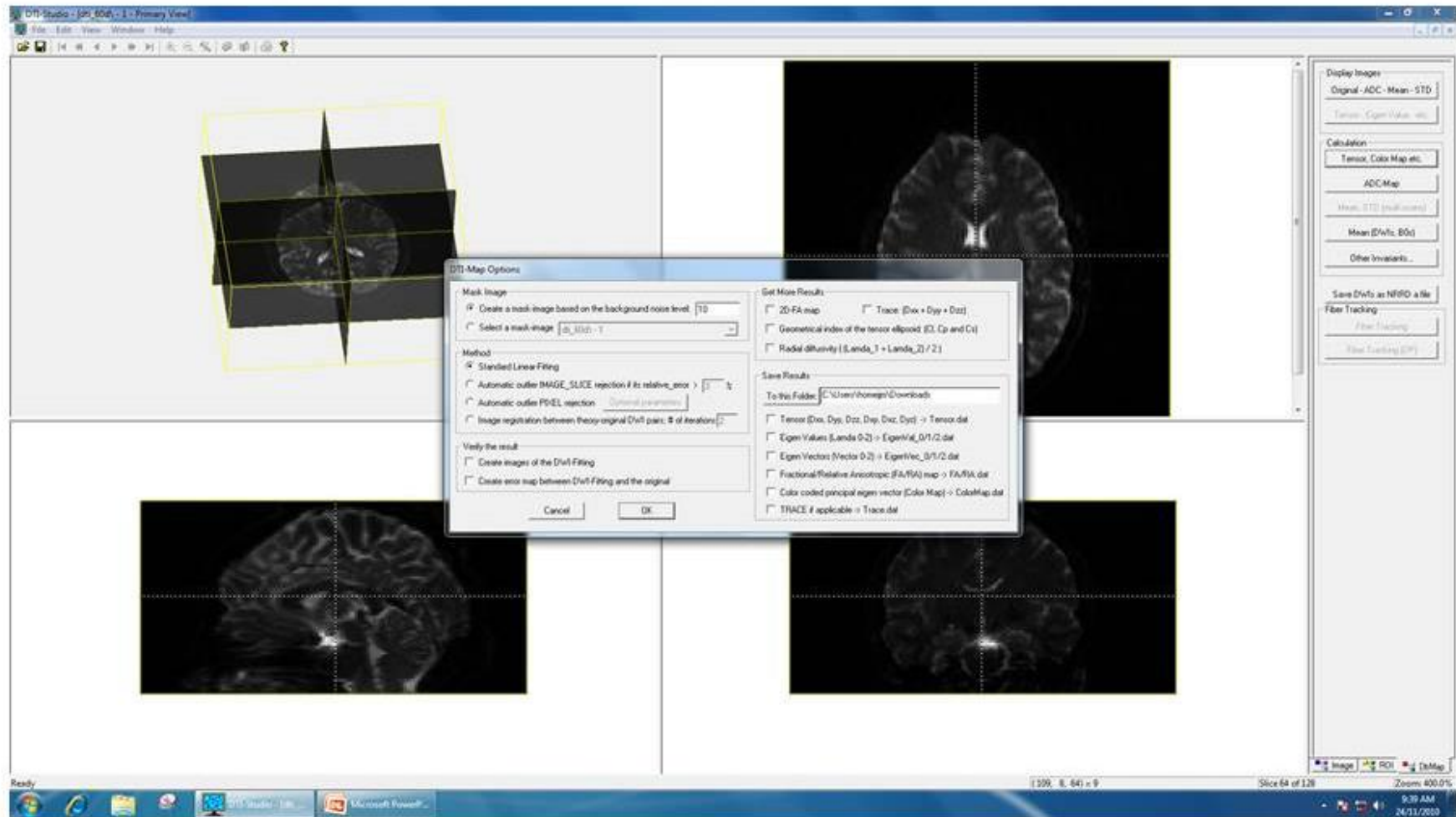


Figure 35. DTI-Map options.

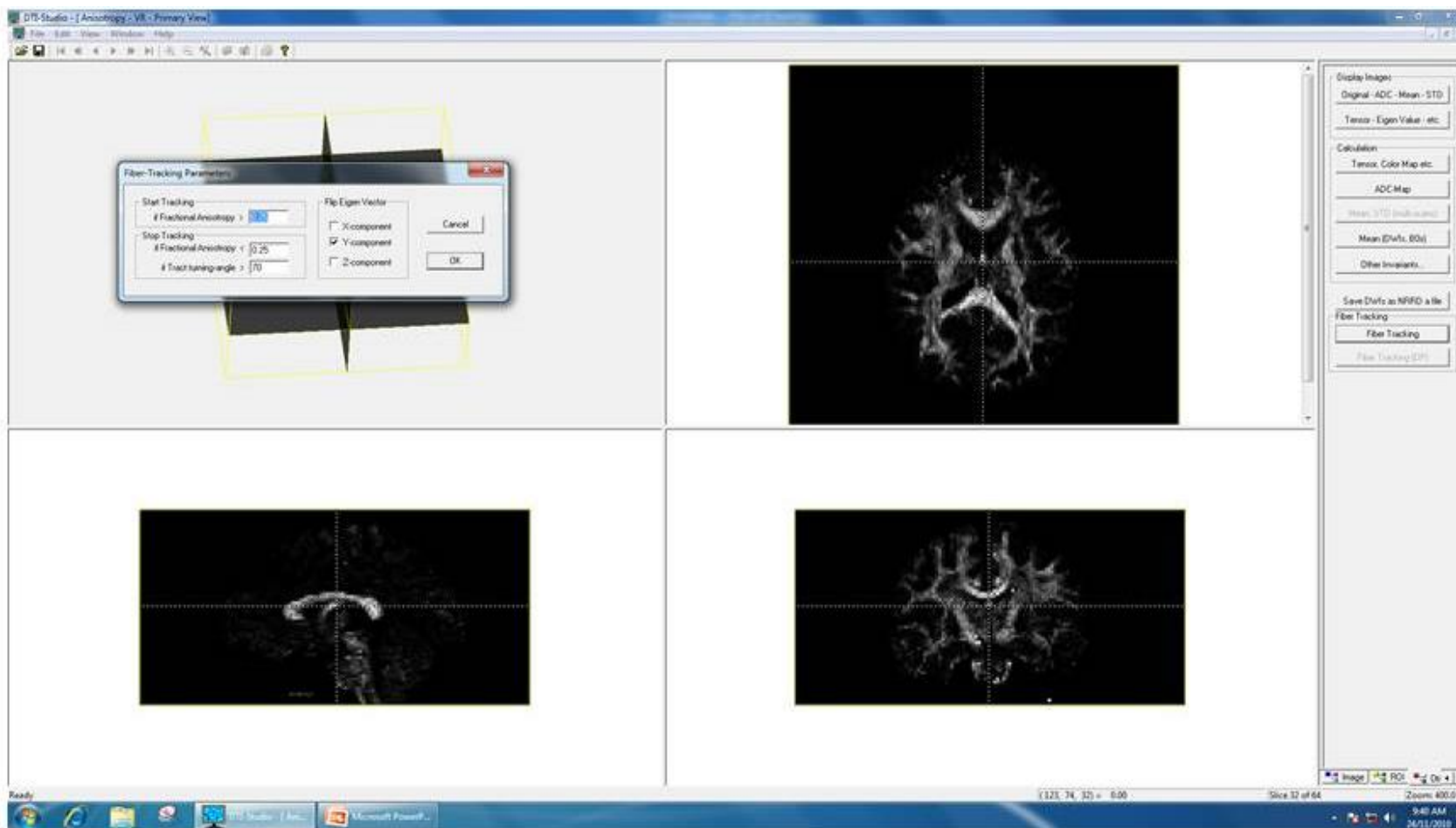


Figure 36. The Fiber-Tracking Parameters box.

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.

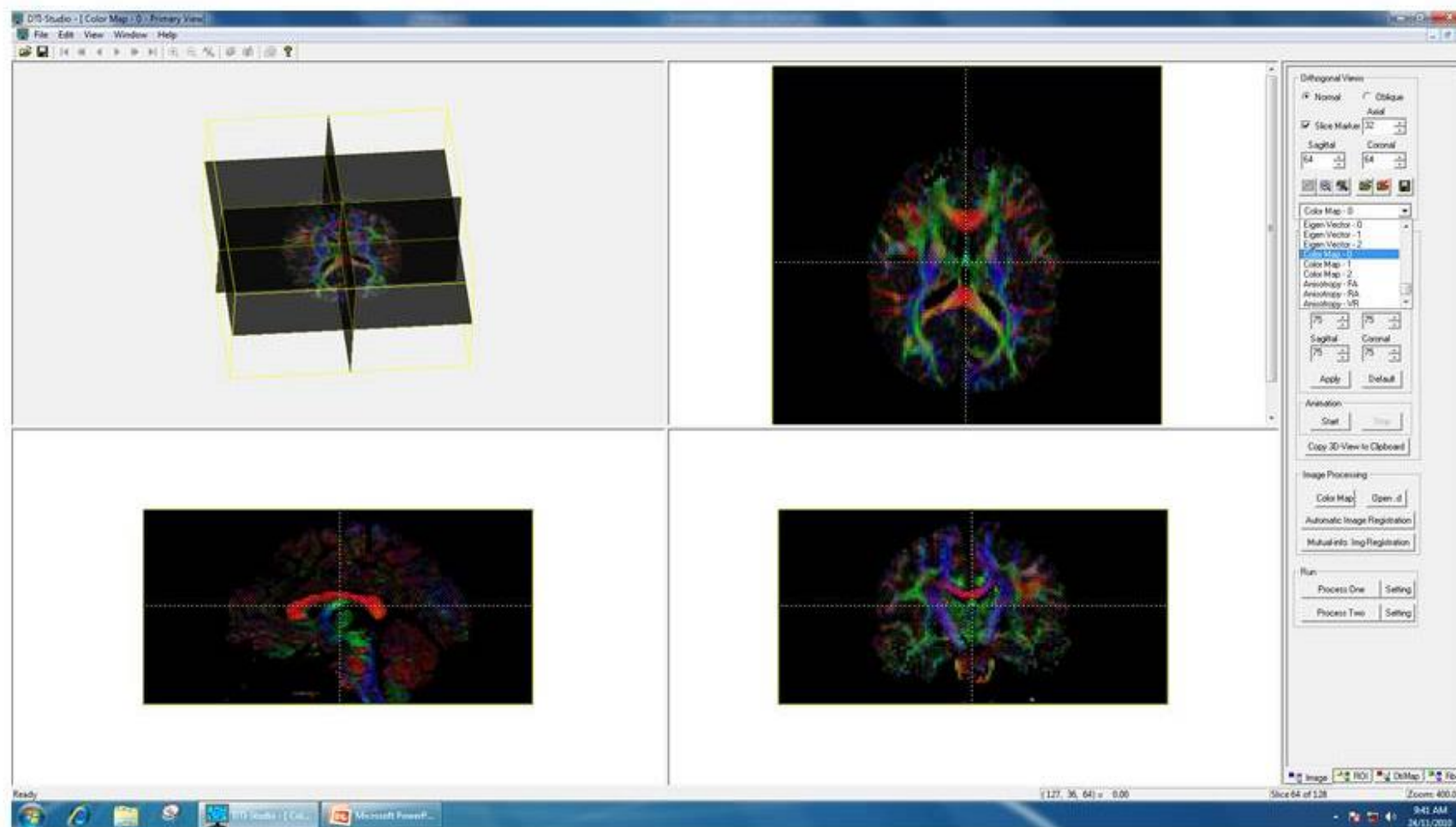


Figure 37. Selecting the Color Map-0 option from the pull-down menu.

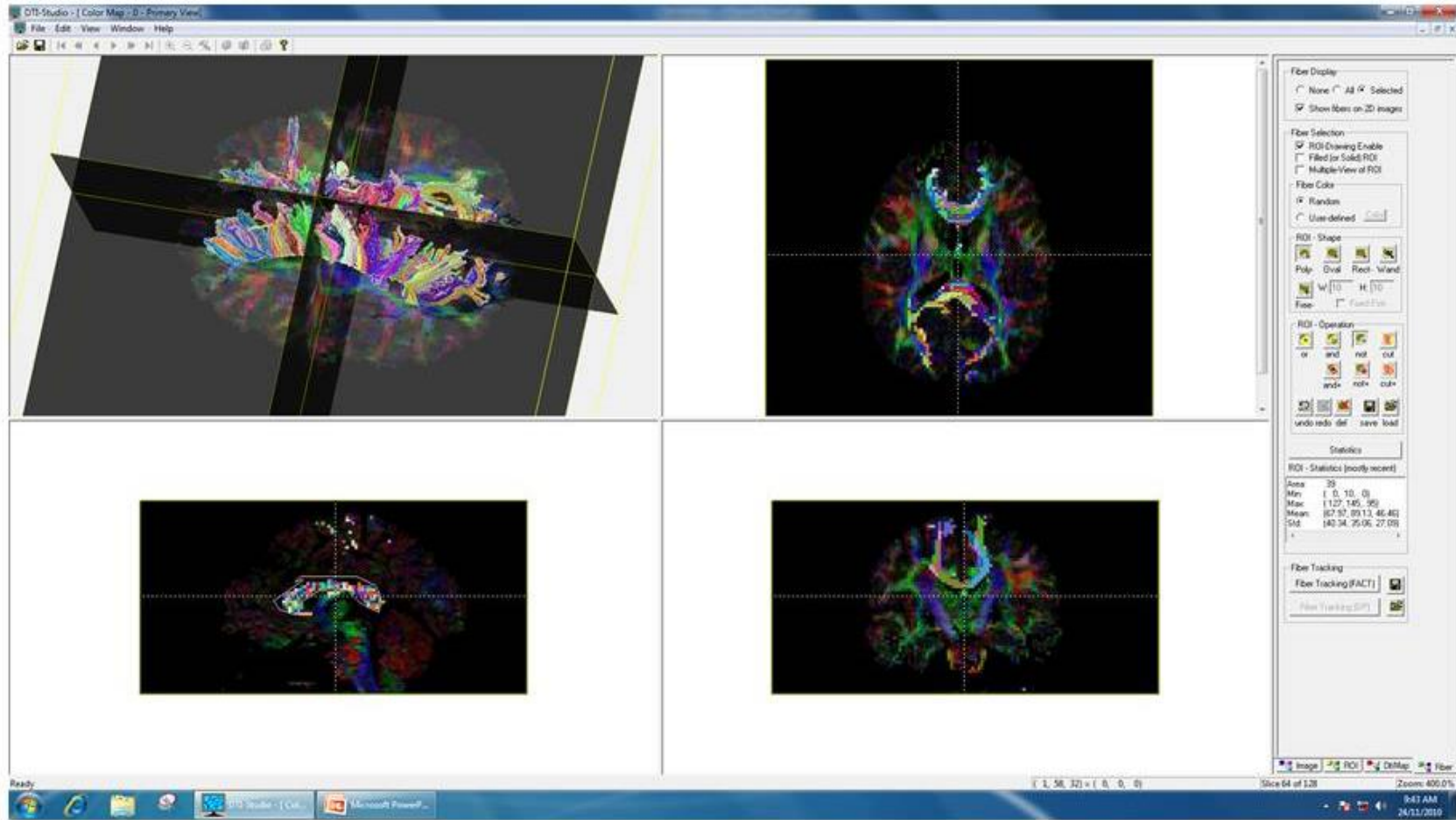


Figure 38. An example of corpus callosum tractography as segmented from sagittal (bottom left).

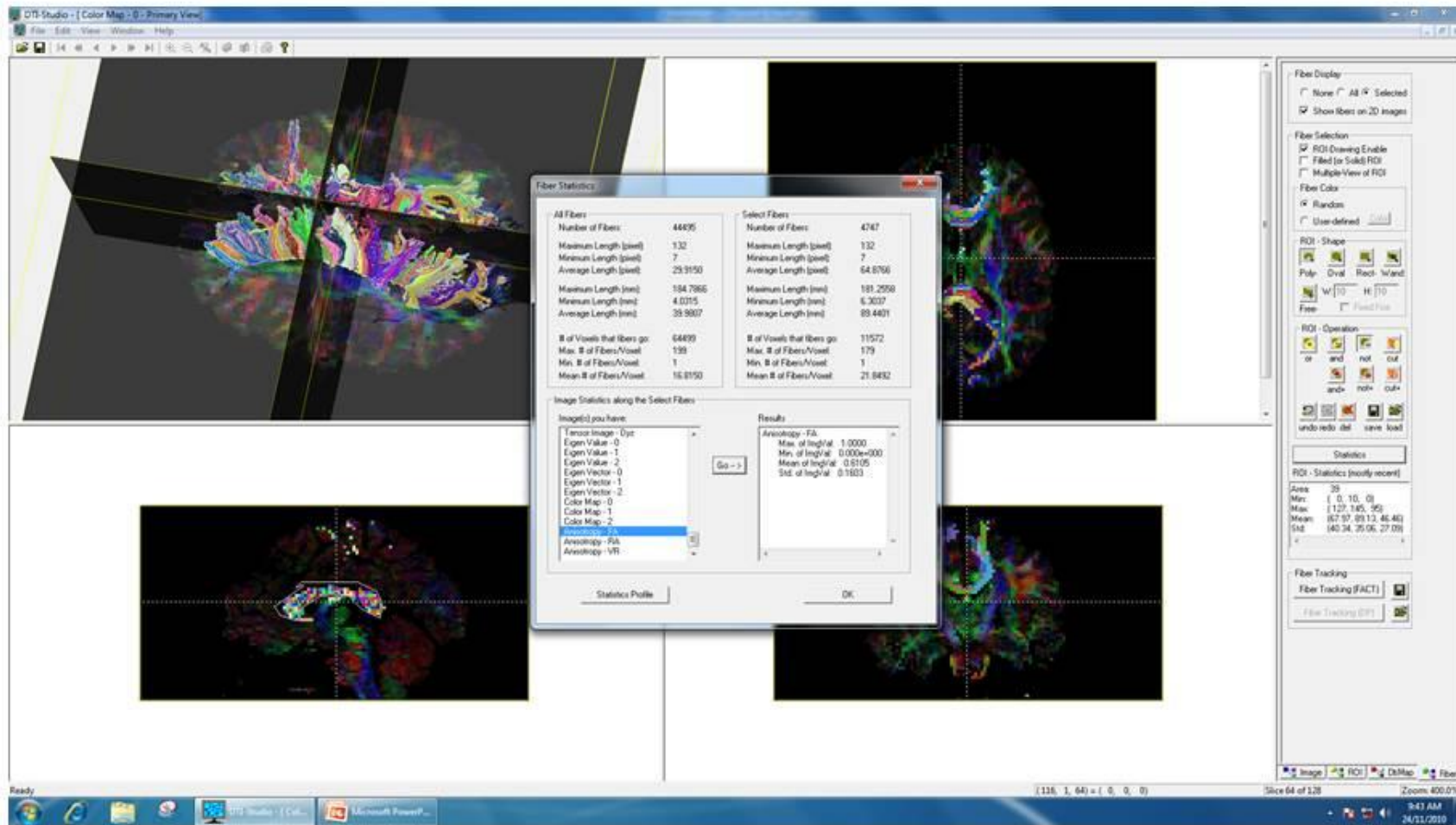


Figure 39. Creating tractography statistics.

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)

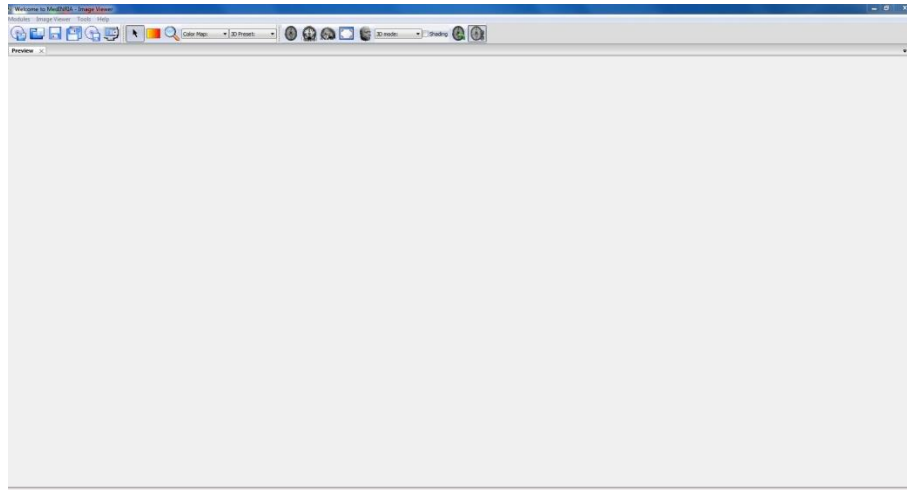


Figure 40. The MedINRIA GUI.

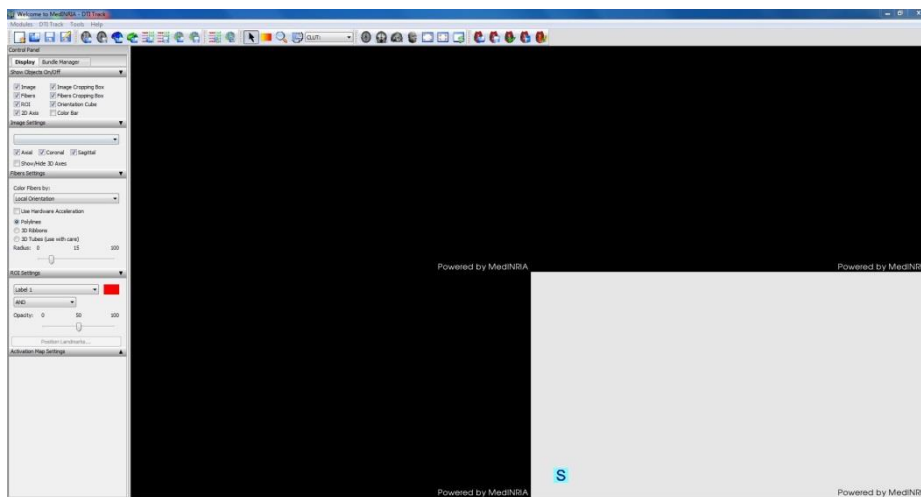


Figure 41. The MedINRIA GUI after selecting New .dts

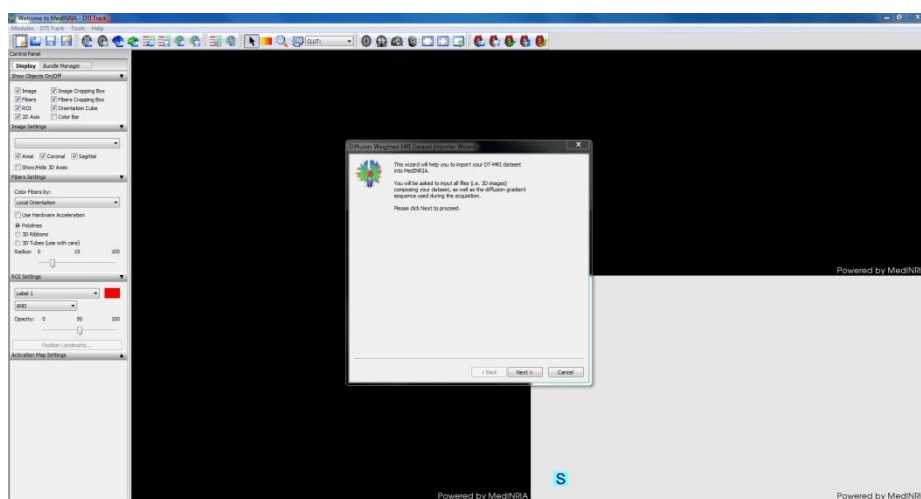


Figure 42. The data import wizard.

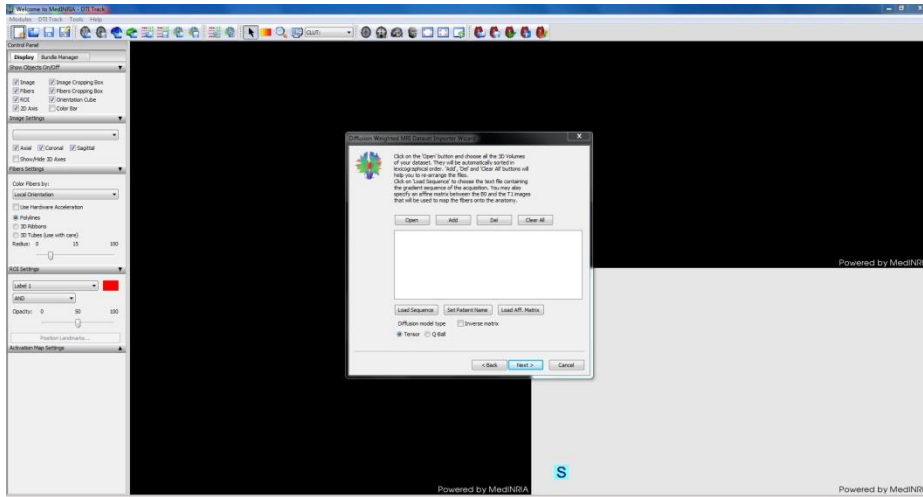


Figure 43. Loading the sequence.

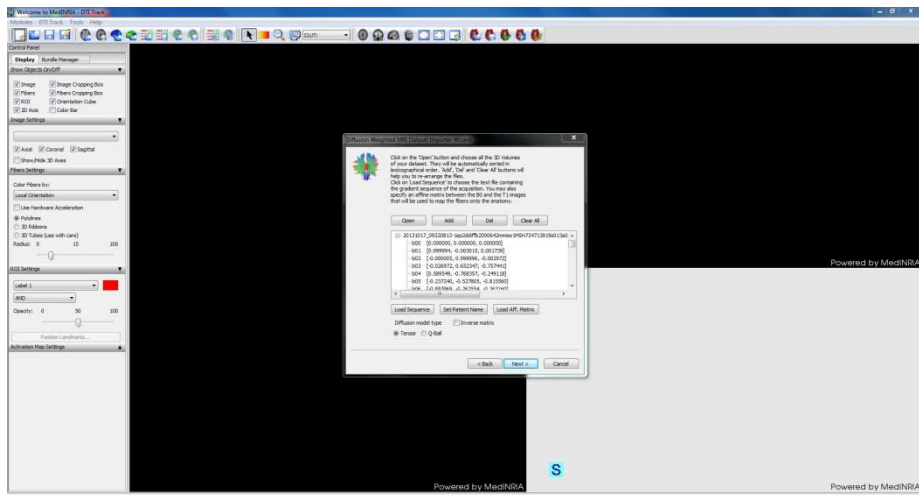


Figure 44. Loading the data.

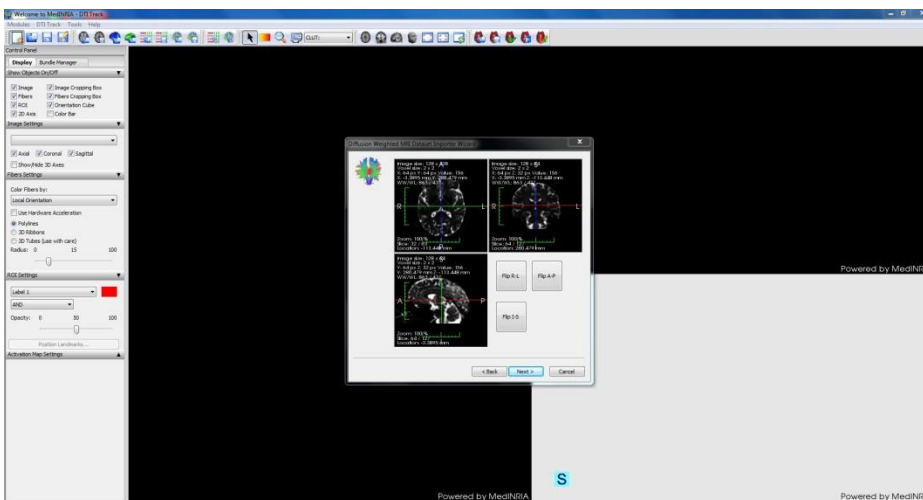


Figure 45. Flip the scan data if necessary.

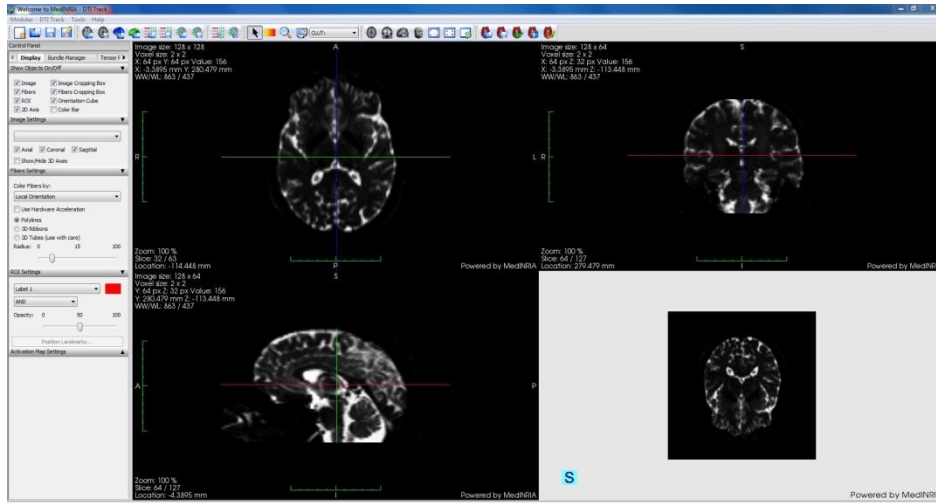


Figure 46. Raw data displayed in MedINRIA.

Alter the threshold

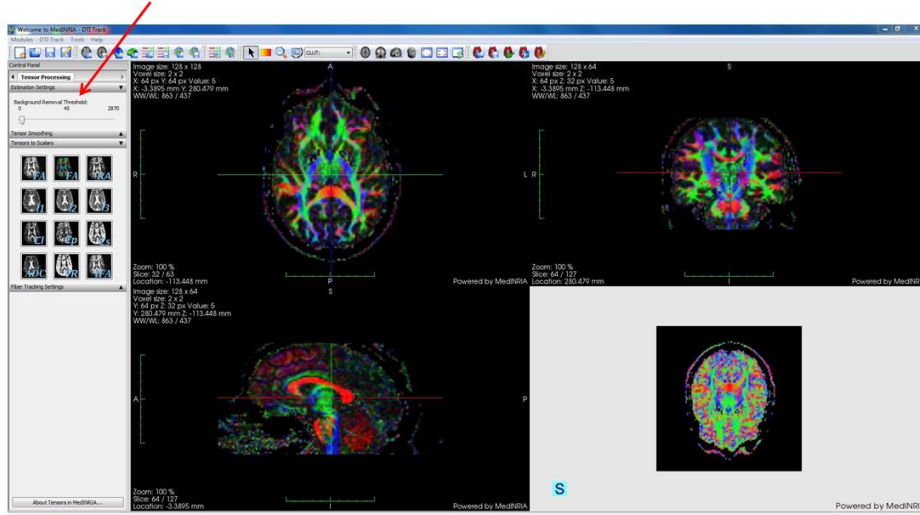


Figure 47. Altering the threshold.

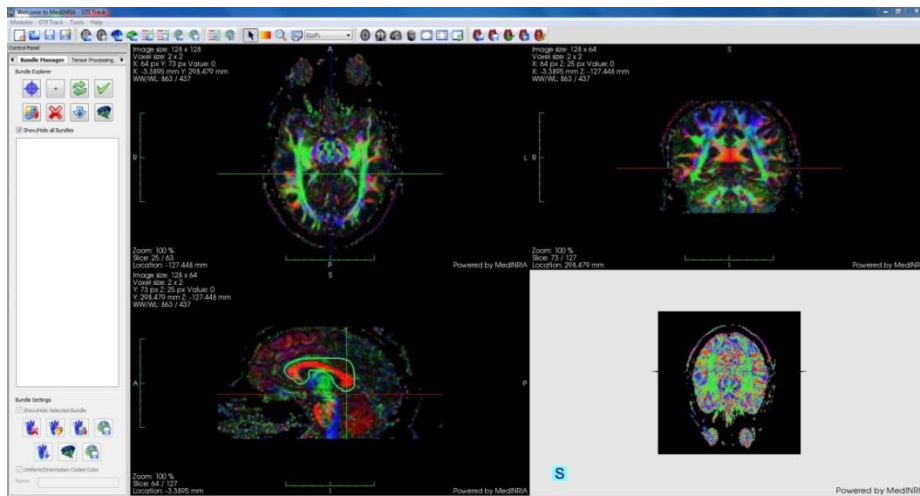


Figure 48. The bundle manager in MedINRIA.

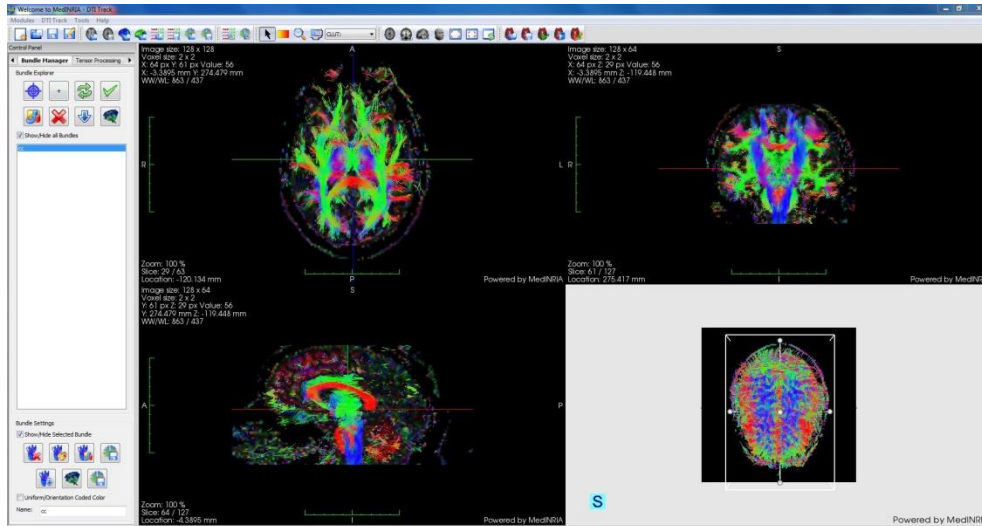


Figure 49. Tracking the fibers.

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

Show just axial enlarged

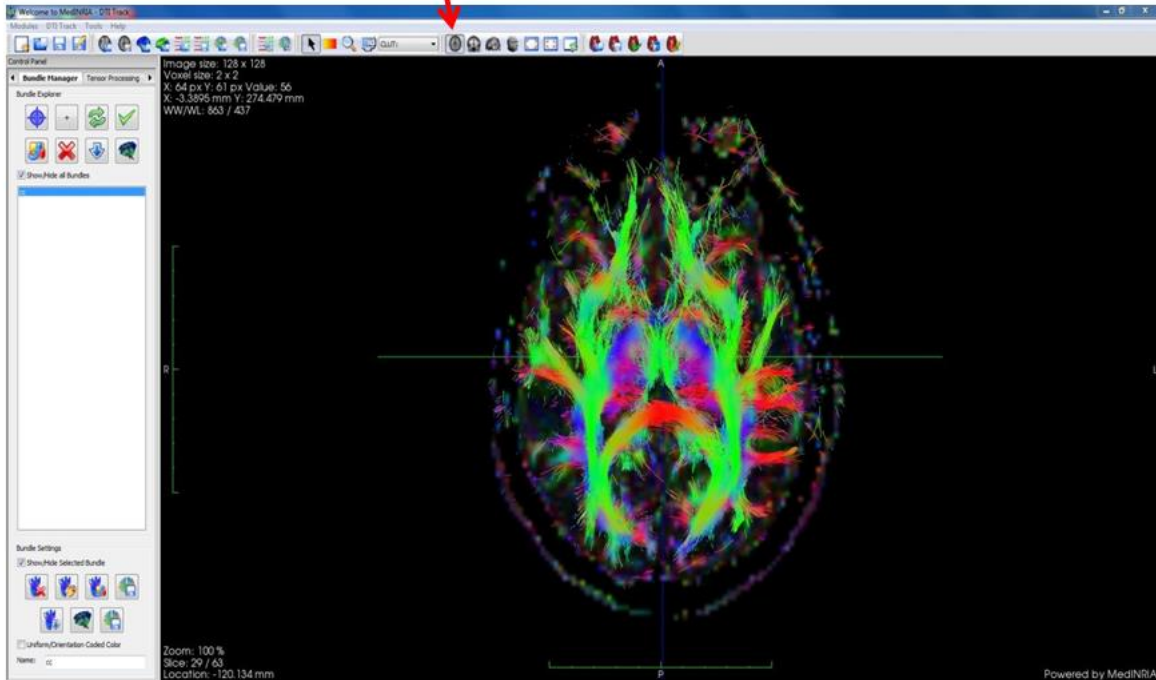


Figure 50. Axial perspective maximized.

19. MRtrix

MRtrix (Tournier et al., 2012) is 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)

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.

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
```

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)

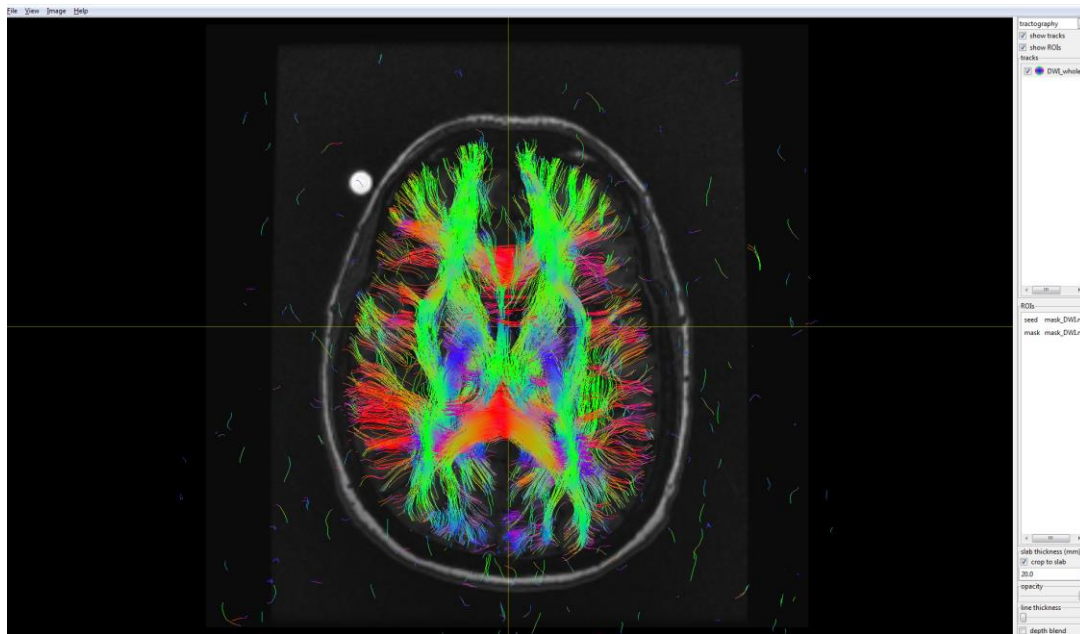


Figure 51. Probabilistic whole-brain tractography in MRtrix.

- 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)

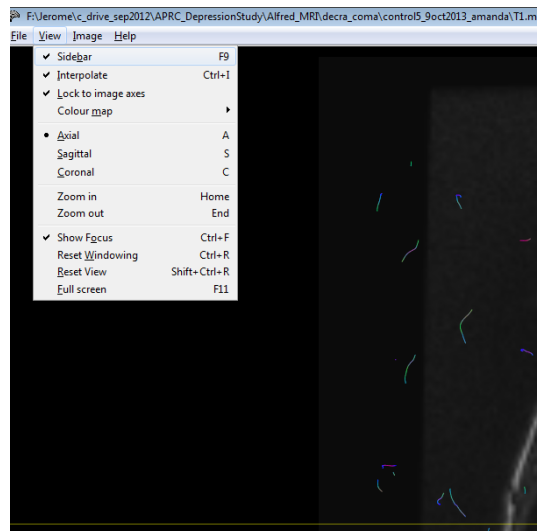


Figure 52. The pull-down View menu in MRtrix.

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

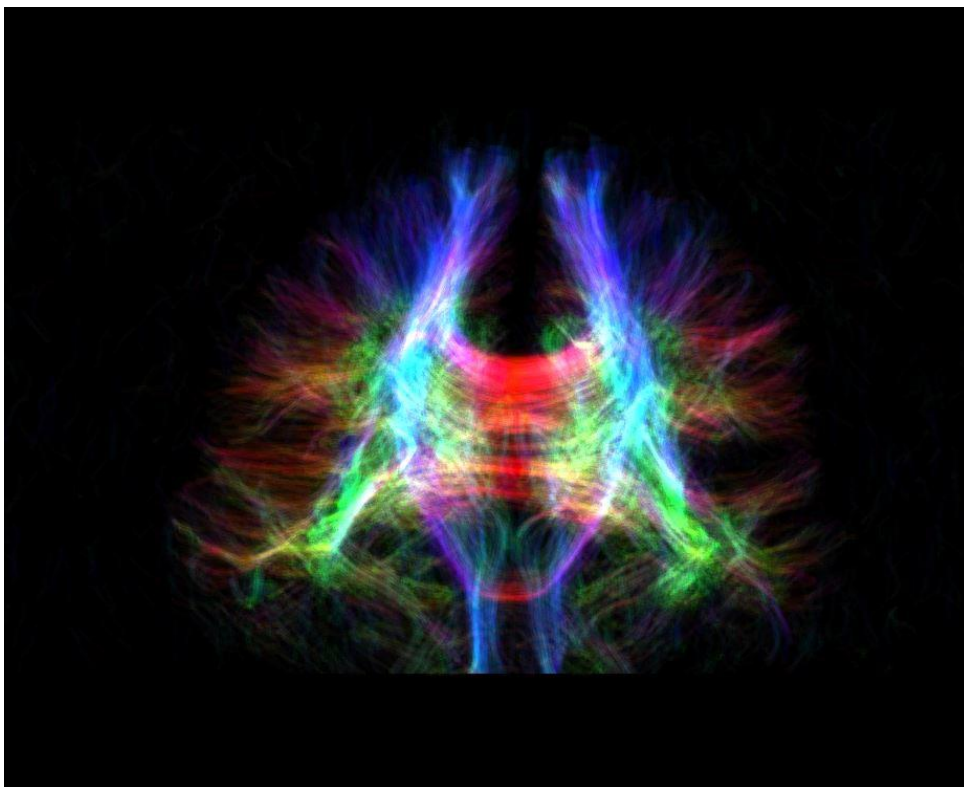


Figure 53. Whole-brain tractography in MRtrix with altered brightness and contrast.

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 mrvuew, 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 54 and 55 show the result after adding in a few –exclude co-ordinates (red spheres):

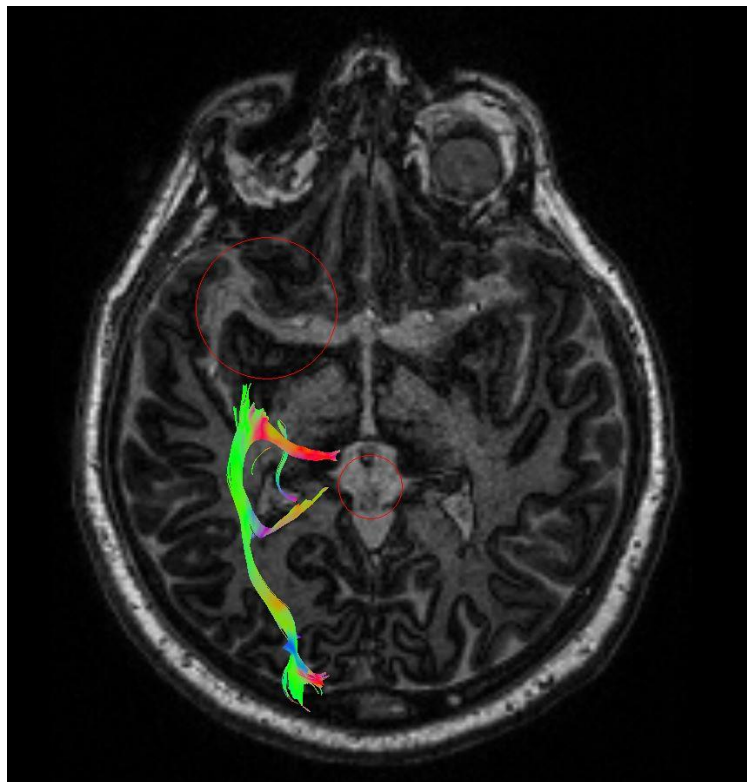


Figure 54. Deterministic optic radiation tractography with exclusion spheres.

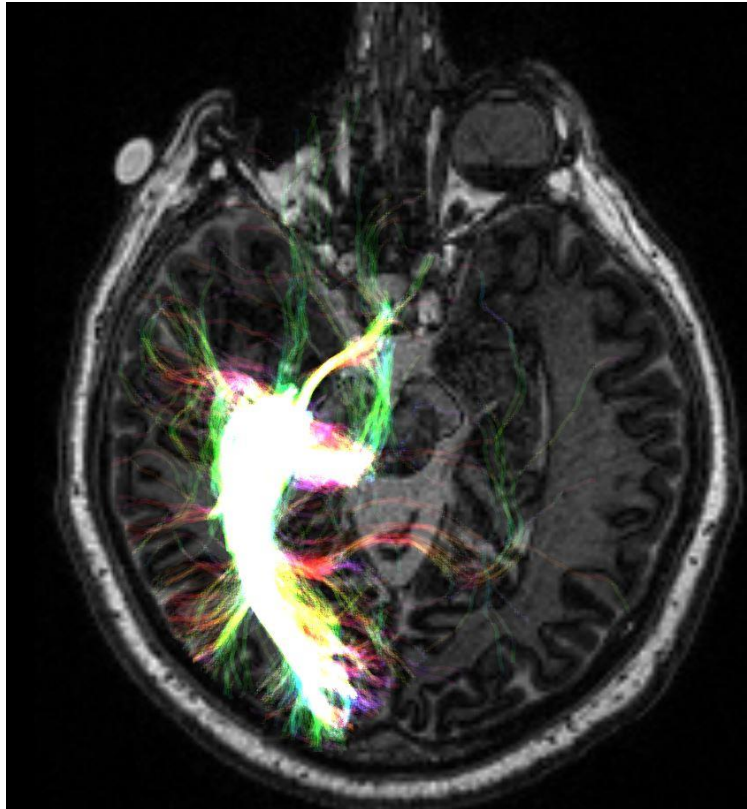


Figure 55. Probabilistic optic radiation tractography with exclusion spheres.

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 `fsroi` command:

```
fsroi 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. Note as well that it is suggested that you extract at least 1million tracts before running the track density command.

To run track density:

```
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
```

Note: The examples shown above are from using MRtrix version 2.11. However, there is an updated version of MRtrix called MRtrix3. Some of the commands are different, but it offers better algorithms, and addition features such as orthogonal views (i.e. display coronal, axial, and sagittal simultaneously), 3-dimensional rendering (Figure 56), and the SIFT module (Spherical-deconvolution Informed Filtering of Tractograms; Smith et al., 2013). However, to get the best results from SIFT, the authors advise that 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.

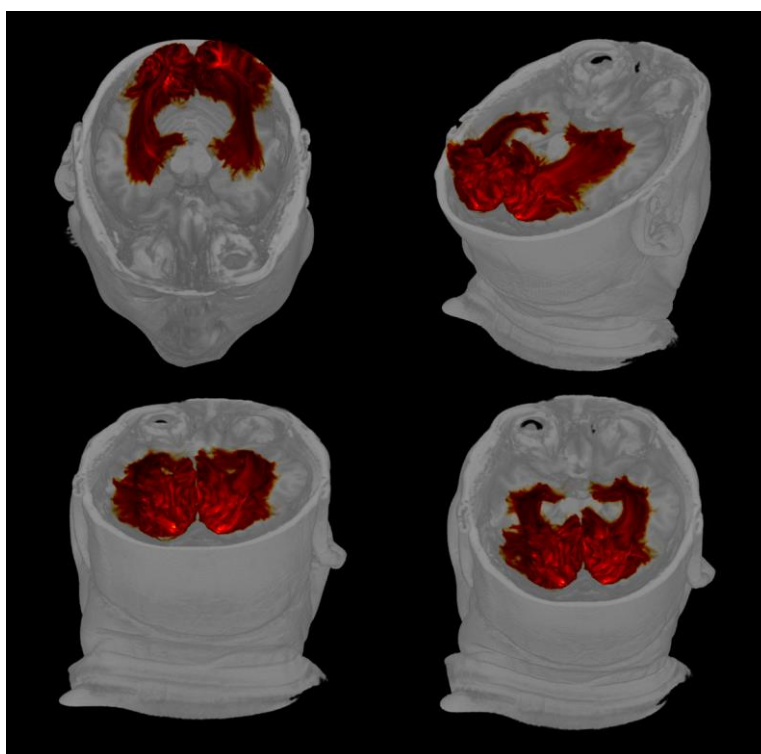


Figure 56. 3-dimensional rendering of optic radiation track density superimposed over the subject's T1-weighted MPRAGE scan in MRtrix3.

20. Some useful Linux commands

- cd (change directory)
- cd .. (go one directory up)
- ls (list the directory)
- exit
- to force program to close, press CONTROL-C in the terminal
- mkdir (make a new directory)

21. REFERENCES

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

MRicro and MRIreg

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FSL and/or DTIstudio

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