Running TORTOISE (v2.5.2) for DTI data pre-processing by PA Taylor (Aug. 2016)

These are example instructions for using TORTOISE (at present, v2.5.2) for DWI preprocessing. We mostly make use of default options therein. This is **not** an official set of steps-- please see the TORTOISE website for those. These notes take up from the online AFNI-FATCAT help. We describe taking a set of AP and PA phase encoded DWI data sets (TORTOISE calls these blip-down and blip-up) and:

- 1) processing each for subject motion/eddy current/etc. distortion (→DIFF PREP run on each);
- 2) gluing the AP and PA sets together (\rightarrow DR-BUDDI);
- 3) exporting the results to an AFNI/NIFTI format (with DIFF_CALC);
- ... after which results can be used for calculating DTs, DTI parameters and a basic tractography with AFNI-FATCAT (see online AFNI webpage tutorial).

NB: if you don't have both AP and PA data sets, these instructions can still be used to get you through the DIFF_PREP part for the single set, and then go to the DIFF_CALC part.

These instructions are *long* because they are verbose, show lots of screen images of the GUI and terminal, and cover a few different steps. It is probably overly didactic, and users will get comfortable quite quickly with TORTOISE and not need (or want) to refer to it. So, don't fret.

In preparation for processing, we need to have the following data sets for any subject:

- + a reference T2w structural scan-- if this is not available, but a T1w image is, then an 'imitation T2' can be made (see online AFNI webpage tutorial);
- + a set of AP phase encoded DWIs;
- + (optional) a set of PA phase encoded DWIs with same grads as that of AP.

DWI formats/organization for any subject:

To start, we assume that each set of N AP and/or PA DWIs is sitting in its own directory, with only the following 3 files present and in these specific formats (essentially, resembling the output of dcm2nii):

- 1) a 4D volumetric data set of the N DWI images (includes b_0 s); must be a *.nii file, not *.nii.gz;
- 2) a gradient (*.bvec) text file of 3 rows and N values per row;
- 3) a b-value (*.bval) text file of 1 row and N values per row.

Note: official TORTOISE documentation recommends loading DICOM files directly into the software, to reduce chances of misreading header information (orientation, slice order, voxel size, etc.). We have converted to NIFTI to be able to view+kick out bad volumes and visually inspect data afterwards to make sure nothing has gone wrong (if something does, then we know we might have to load DICOMs in directly).

LHS = lefthand side RHS = righthand side

Comments:

1) In order to run, DIFF_PREP requires a "Settings File" (*.dmc), which TORTOISE will look for by default in the following directory:

~/DIFF PREP WORK/

There is an example online here:

https://science.nichd.nih.gov/confluence/display/nihpd/3.2.01+Sample+registration+settings+file This is pretty much what I generally use; probably one thing that could be changed would be saving intermediate outputs, because they take up several gigabytes of space per run (but then it would be harder to troubleshoot any problems).

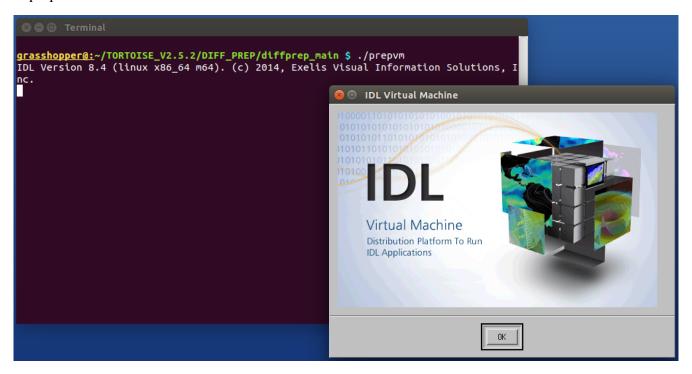
- 2) The final output spatial resolution of the DWI data can also be set in the *.dmc settings file. Based on advice from the TORTOISE gurus, a bit of upsampling is generally advisable. For example, I often go from 2 mm isotropic (acquired) to 1.5 mm isotropic (after processing). Upsampling a lot can lead to huge memory and time demands when processing.
- 3) If loading in DWIs as NIFTIs, there must be a directory with a single *.nii data file, *.bvec gradient file, and *.bval b-value file. TORTOISE looks for these specific extensions-- once you enter the *.nii file into the GUI, it will expect to be able to find the other two text files in the same directory. NIFTI files cannot be zipped.
- 4) TORTOISE will make a processing directory based on the name of the entered *.nii file (in DIFF_PREP) or the entered list file (if DR-BUDDI). For example, <u>if</u> the NIFTI is named "CHEESE_BURGER.nii" and in a directory called "FOOD_DWI/", then the output directory will be called "CHEESE_BURGER_proc/", and this will be parallel to "FOOD_DWI/".

A) RUNNING TORTOISE: DIFF PREP

NB: This DIFF_PREP step would be run separately on each the AP and PA set of DWIs (the filtered sets, if filtering was performed). In this example, we just go through the DIFF_PREP steps for the 'AP' set-- the same set of steps applies to the PA case. Both runs of DIFF_PREP could be run simultaneously on the AP and PA sets (subject to computational power/memory; there is no interaction among them, and you will need both for the next step).

A1) Starting DIFF_PREP: stage one, to be run on a set of DWIs, using T2w for reference.

Go to diffprep_main/ directory of TORTOISE on the computer, and start the virtual machine by typing "./prepvm" on the command line:



and start the IDL (ugh) virtual machine by hitting "OK".

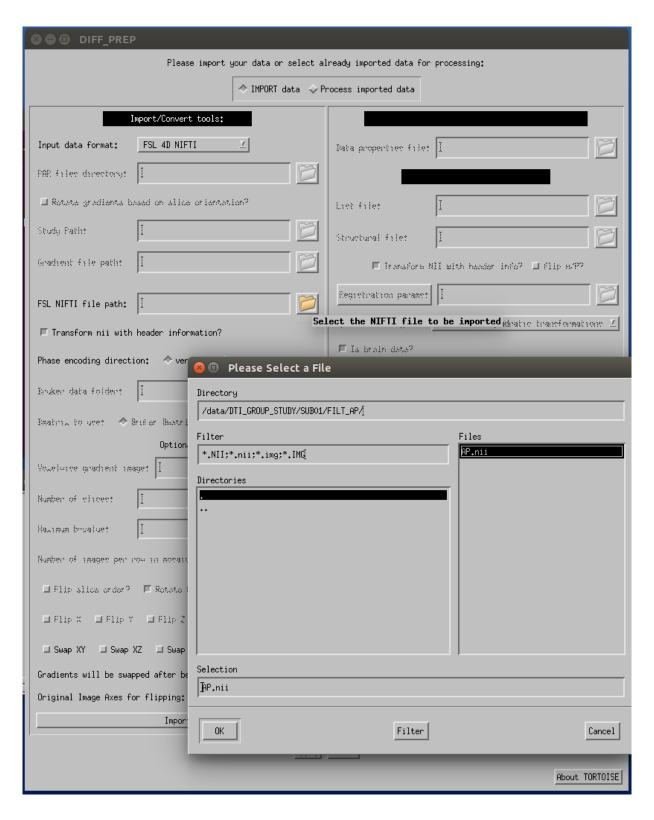
A2) The DIFF_PREP GUI: where basic information gets loaded in.

This will start a two step process: importing (DWI) data in the left column, and then the structural data and running options file in the right column.

	⊗ ⊜ □ DIFF_PREP		
	Please import your data or select already imported data for processing:		
	↑ IMPORT data ❖ Pr	rocess imported data	
⊗ □ Terminal	Import/Convert tools:		
grasshopper@:~/	Input data format:	Lata properties files [
IDL Version 8.4 nc.	PAR files directory: []		
Warning: Cannot	■ Rotate gradients based on slice orientation?	List file:	
so8859-1" to ty	Study Path:	Structural fale:	
	Gradient file path:	□ Iransform NII with hooder info? □ flip m/P?	
	FM. NIFTI file path:	Esquetration paraset	
	Transform mil with hooder information?	Optimization Type: Human brain/quadratic transformations I	
	Frace encoding direction:	■ Is brain data?	
	Eruker data folder:	Optional properties:	
	Ematry, to ure:		
	Optional	liverdefined output name	
⊗ 🖨 🗈 Registratio	Vowelwice gradient image: I	RO nonce meant	
I Registrati	Number of classes:	IO noise obdevt	
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	Number of images per row in mosaic images:	Structural more etdev:	
	■ Flip slice order? ■ Rotate Gradients?		
	Selip x Selip y Selip 2	■ Display images and Log?	
	□ Suap X7 □ Suap X2 □ Suap Y2		
	Gradients will be swapped after being flipped.		
4	Original Image Alec for flipping: None		
	import		
	App Ly	Cancel Cancel	
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A3) DIFF PREP GUI LHS: Import data.

From 'Input data format' dropdown list, select 'FSL 4D NIFTI'. This unfreezes the 'FSL NIFTI file path:' box, and click on the folder icon there. Then navigate in the file structure to where your input DWI NIFTI file of interest is (here, AP.nii). The *bval and *bvec files must be in the same directory, and the NIFTI file cannot be zipped (*.nii.gz). After selecting the correct file, click "OK":



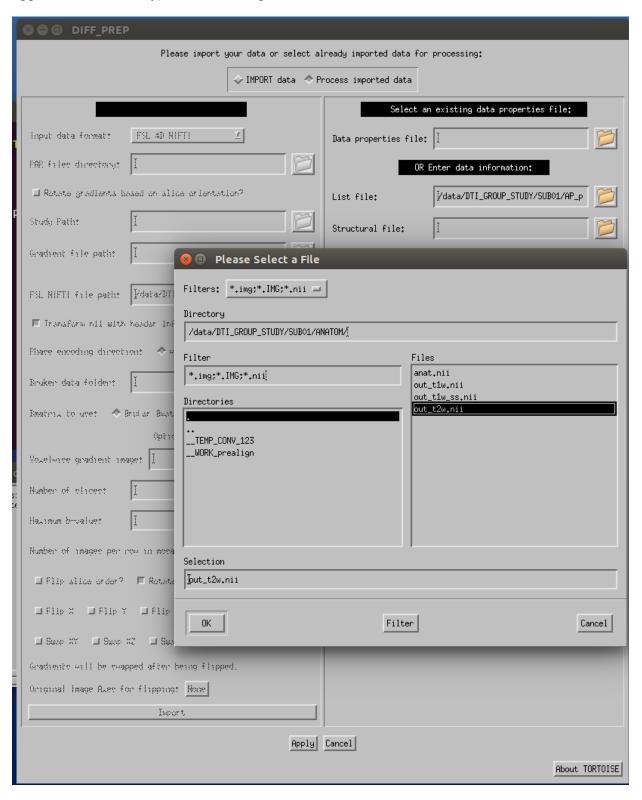
A4) DIFF_PREP GUI: Import data.

After the NIFTI file path has been given, (probably) nothing else has to be selected on the LHS, and you can select the 'Import' button at the bottom of the left column.

⊗ ● ® DIFF_PREP			
Please import your data or select already imported data for processing:			
↑ IMPORT data Process imported data			
Import/Convert tools:			
Input data format: FSL 4D NIFTI	Nata properties file:		
PAR files directorys			
☐ Rotate gradients based on slice orientation?	List file:		
Study Path:	Structural file:		
Swadnent file path:	□ Transform NII with header info? □ flip m/P?		
FSL NIFTI file path: //data/DTI_GROUP_STUDY/SUB01/FILT	Eggistration paramet		
☐ Transform nii with header information?	Optimization Type: Human brain/quadratic transformations I		
Phase encoding direction: ♦ vertical ♦ horizontal	□ is brain data?		
Enuken data folden:	Optional properties:		
Ematro, to use: ♦ Bruler Beatrix ♦ Original gradients	Ucerdefined output name		
Optional			
Vowelwice gradient image:	IO noise meant		
Number of circect [IO nonce cideu:		
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Number of images per now in mosaic images:	Structural nonce obdev:		
☐ Flip slice order? ☐ Rotate Oradients?			
□Flip × □Flip Y □Flip 2	□ Display images and Log?		
□ Swap XY □ Swap XZ □ Swap YZ			
Gradients will be swapped after being flipped.			
Original Image Axes for flipping:			
Import			
App by	Cancel		
	About TORTOISE		

A5) DIFF PREP GUI: Process imported data.

After importing the data successfully on the LHS of the GUI, the RHS ("Process imported data") unfreezes. The 'List file' should have been automatically populated. At this point, we will just need to load in the 'Structural file' and the 'Registration params'. Start by clicking on the folder to the right of "Structural file," and navigating the file structure to where the T2w reference anatomical is (again, unzipped NIFTI file, only). After selecting the correct file, click "OK":



A6) DIFF_PREP GUI: registration parameters.

Click on the folder icon by the 'Registration params:' option. A selection GUI should open up the ~/DIFF_PREP_WORK directory. Select a desired file (here, "FOR_DIFF_PREP_T2REG_1.5.dmc") and click "OK". You are then all set, and you can click "Apply" at the bottom of the GUI (sometimes I first unselect "Display images and Log" of the bottom of the GUI just to not have windows opening).

⊗ ⊜ ® DIFF_PREP			
Please import your data or select already imported data for processing:			
	Select an existing data properties file:		
input data format: FSL 40 NIFTI	Data properties file:		
PAR files directory:	OR Enter data information:		
□ Rotate gradients based on slice orientation?	List file: [/data/DTI_GROUP_STUDY/SUB01/AP_p]		
Study Path:	Structural file: [/data/DTI_GROUP_STUDY/SUB01/ANAT		
Gradient frie path:	☐ Transform NII with header info? ☐ flip A/P?		
	Registration params: FOR_DIFF_PREP_T2REG_1,5.dmc		
FSL NIFTI file path: Ydata/ITI_GEOUF_STURY/SUROL/FILT	Optimization Type: Human brain/quadratic transformations 1		
Transform mil with hooder information?	■ Is brain data?		
Fixee encoding direction:	Optional properties:		
Enuken data folder:	Recrientation file:		
Ematers to use: 🔷 Bruler Beatrix 💠 Original gradients	Userdefined output name		
Optronal	BO noise mean:		
Vowelware gradient amage:			
Number of clices:	B0 noise stdev:		
Havinum b-value:	Structural noise mean:		
Number of images per row in mosaic images:	Structural noise stdev:		
☐ Flim slice order? ☐ Rotate Gradients?			
□ Flip X □ Flip Y □ Flip 2	□ Display images and Log?		
□ Swep XY □ Swep X2 □ Swep Y2			
Gradients will be swapped after being flipped.			
Original Image Akes for flippings None			
Import.			
Apply	Cancel		
	About TORTOISE		
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A7) DIFF PREP running+finishing.

The large GUI closes, and the gray 'Registration Status Report Window' remains open, with things churning by in the terminal. It may take a few+ hours or more per data set, depending on the number DWIs, the spatial resolution, and the amount of distortion.

When DIFF_PREP has finished running successfully, the remaining gray GUI closes, and you WILL see an error about 'arithmetic error: Floating underflow' in the terminal. That's just part of the joy of IDL. The following is a standard example of the terminal output at the end:

```
source_low, source_high, target_low, target_high
0.100000 174.897
                                                            0.100000
                                                                            1943.51
Trans.p:
                                                                                0.00000
      0.00000
                     0.00000
                                    0.00000
                                                  0.00000
                                                                 0.00000
                                    0.00000
      0.00000
                     1.00000
                                                   0.00000
                                                                 0.00000
                                                                                0.00000
      0.00000
                     0.00000
                                    0.00000
                                                   0.00000
                                                                  0.00000
                                                                                0.00000
      0.00000
                     0.00000
                                    0.00000
endian_raw_in=BIG
original_columns=128
original_rows=128
slice=78
nim=23
phase_encode_direction='vertical'
x_field_of_view=256.000
y_field_of_view=256.000
rawimageformat='float
bmatrixfile='AP.bmtxt
slice_gap=0
slice_thickness=2.00000
image_plane='axial
raw_image_path_filename='AP.path'
Loaded bmatrix
% Program caused arithmetic error: Floating underflow
grasshopper@:~/TORTOISE_V2.5.2/DIFF_PREP/diffprep_main $
```

```
🕽 🖨 🕕 Terminal
grasshopper@:/data/DTI_GROUP_STUDY $ ls SUB01/
01_dicom_dir_anat 01_dicom_dir_PA AP_proc FILT_PA UNFILT_AP
01_dicom_dir_AP ANATOM FILT_AP PA_proc UNFILT_PA
grasshopper@:/data/DTI_GROUP_STUDY $ ls SUB01/AP_proc/
                   AP.path
AP.bmtxt
                                                                                 AP_up_rpd.bmtxt
                            AP_up_rpd.bmtxt
AP_up_b0_orig_crop.nii
AP_up_rpd.list
AP_up.bmtxt
AP_up.list
AP_up.list
AP_up.list_deformation_field_output
AP_up.list_step4
AP_up.rpd.transformations
AP_DMC.bmtxt
AP_DMC_corims
AP_DMC.list
AP_DMC.path
AP_DMCstructural.nii
AP_DMCtemplate.nii
AP_DMC.transformations AP_up.path
AP.list
                               AP_up_RAWFLOAT
                                                                                 timing.txt
grasshopper@:/data/DTI_GROUP_STUDY $
```

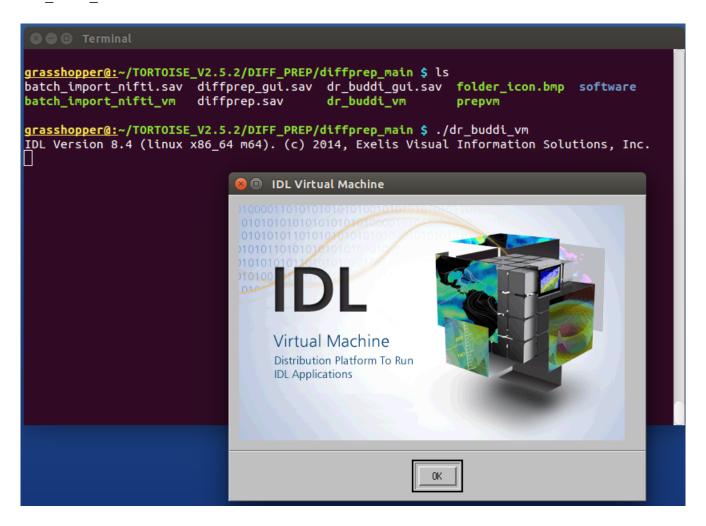
... And the DIFF_PREP stage is complete. If you don't have both AP and PA data, skip the the Part B "DR_BUDDI" and go to exporting data into usable formats in Part C "DIFF_CALC".

B) RUNNING TORTOISE: DR BUDDI

After DIFF_PREP has been run separately on the subject's AP and PA DWI sets, we can now perform the actual EPI distortion correction using TORTOISE's DR-BUDDI tool on the pair of sets (including the anatomical for reference/registration).

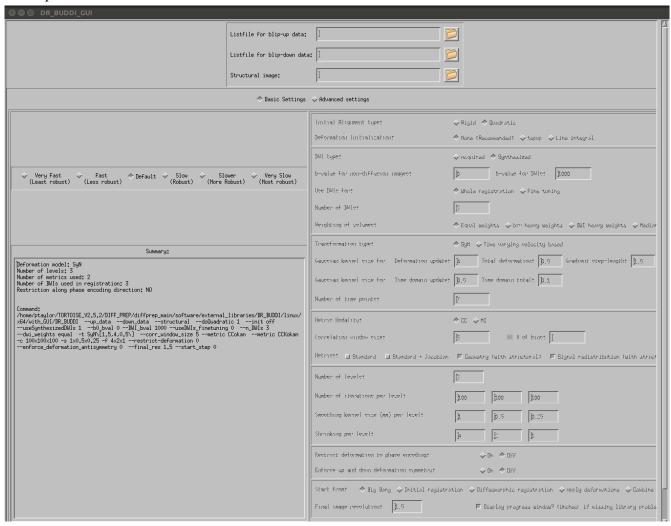
B1) open the DR-BUDDI GUI.

From the same DIFF_PREP/diffprep_main/ directory, fire up the IDL virtual machine using './dr buddi vm'.



B2) DR-BUDDI GUI.

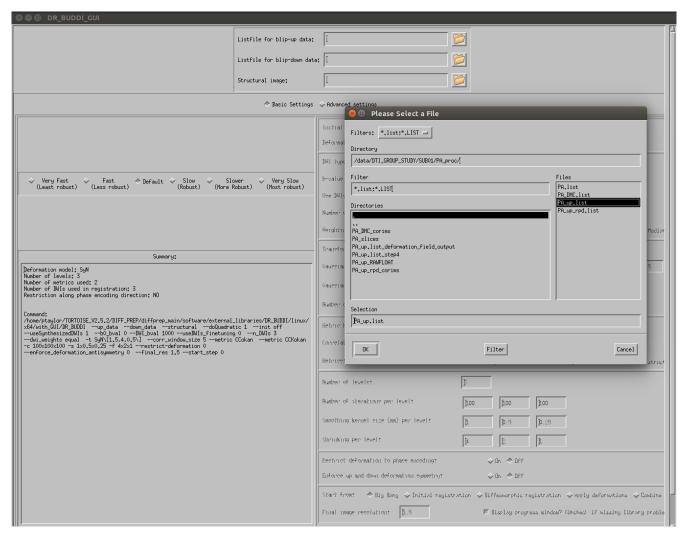
This opens the DR-BUDDI GUI:



We will use pretty much all the defaults and just enter the locations of the "Listfiles" of the blip-up/blip-down (what we've mainly been calling AP/PA) data from the DIFF_PREP runs, as well as the T2w NIFTI volume that we had used previously.

B3) DR-BUDDI: blip-up data.

Click on the folder icon for loading in 'Listfile for blip-down data:'. For the subject being processed, go in to the DIFF_PREP-processed PA directory (SUB01/PA_proc/), and select the 'PA_up.list' there. Then click OK.



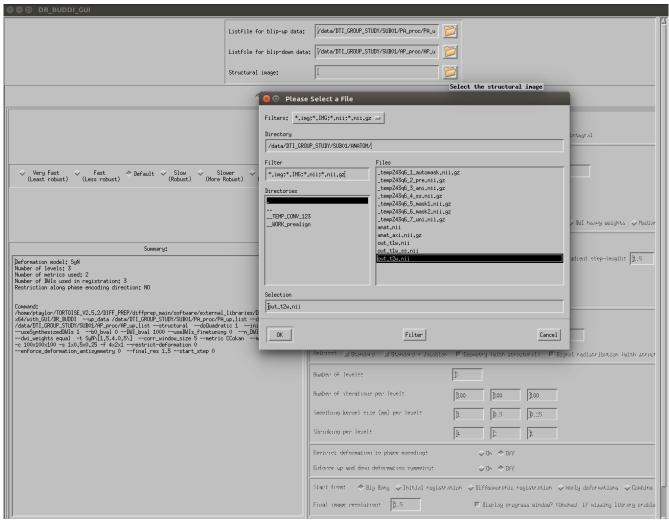
B4) DR-BUDDI: blip-down data.

Essentially, repeat the previous step for the other blipped data--

Click on the folder icon for loading in 'Listfile for blip-down data:'. For the subject being processed, go into the DIFF_PREP-processed AP directory ([somewhere]/AP_proc/), and select the 'AP_up.list' there. Then click OK.

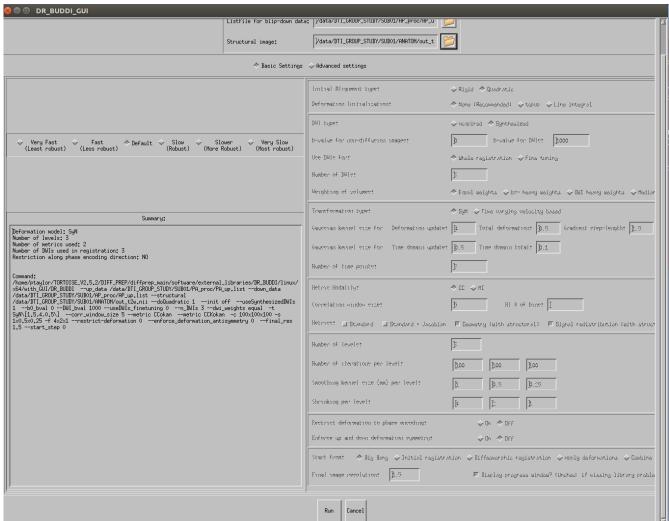
B5) DR-BUDDI: structural data.

Click on the folder icon for loading in 'Structural image', and then click OK.



B6) DR-BUDDI: running.

I typically leave the remaining options/settings at their default values. You can select your degree of Fast/Default/Slow speed for the relative robustness you want. Then click 'Run' at the bottom (you might have to scroll down in the menu to see it, depending on your screen size/resolution):



B7) DR-BUDDI: running.

The main GUI closes, and there is a stream of text in the terminal. DR-BUDDI will likely take many hours to run, again, depending on the number of DWIs, voxel resolution, quality of data, etc.

```
paul@paul:~/TORTOISE V2.1.0/DIFF PREP/diffprep main $ ./bup bdown vm
IDL Version 8.2.1 (linux x86 64 m64). (c) 2012, Exelis Visual Information Solutions, Inc.
/home/paul/TORTOISE_V2.1.0/DIFF_PREP/diffprep_main/software/external_libraries/bup_bdown/linux/x64/bup_bdown/
TORTOISE /home/paul/TORT EXAMPLE UCT/SUBJ 01/INTERMED/PA_proc/PA_up.list /home/paul/TORT_EXAMPLE_UCT/SUBJ_0
1/INTERMED/AP proc/AP up.list /home/paul/TORT_EXAMPLE_UCT/SUBJ_01/ANATOM/T2F_RPI_SS.nii 0 0 1.50000 N 1.5000
0 7 0 1 1 1 0 0 1 6 --doQuadratic true --dwi-weights 0 --force-dwis-through-structural true --move-gradually
-from-structural-to-duo false -r Gauss[5.00000,1.50000]
No extra fine tuning registration!
No equality constraint on moving-to-middle and fixed-to-middle deformations
No restriction along phase encoding!
Transforming All DWIs...
Transforming Volume:0
Transforming Volume:1
Transforming Volume:2
Transforming Volume:3
Transforming Volume:4
Transforming Volume:5
Transforming Volume:6
Transforming Volume:7
Transforming Volume:8
```

B6c) DR-BUDDI: output. *******************

DR-BUDDI finishes discretely (i.e., no error message like at the end of DIFF_PREP):

```
⊗ □ □ paul@paul: ~/TORTOISE V2.1.0/DIFF PREP/diffprep main
Combining vol: 7
Combining vol: 8
Combining vol: 9
Combining vol: 10
Combining vol: 11
Combining vol: 12
Combining vol: 13
Combining vol: 14
Combining vol: 15
Combining vol: 16
Combining vol: 17
Combining vol: 18
Combining vol: 19
Combining vol: 20
Combining vol: 21
Combining vol: 22
Combining vol: 23
Combining vol: 24
Combining vol: 25
Combining vol: 26
Combining vol: 27
Combining vol: 28
Combining vol: 29
Combining vol: 30
[-87.5, 124.512, -124.512]
paul@paul:~/TORTOISE_V2.1.0/DIFF_PREP/diffprep_main $
```

The final results are stored in a TORTOISE-made directory, parallel to the DIFF_PREP-produced ones. The name will be derived from the blip-up directory, plus 'up bupdown proc' as a postfix:

```
paul@paul:~/TORT_EXAMPLE_UCT/SUBJ_01/INTERMED $ ls
AP_proc FILT_AP FILT_PA PA_DMC_bupdown_proc PA_proc UNFILT_AP UNFILT_PA
paul@paul:~/TORT_EXAMPLE_UCT/SUBJ_01/INTERMED $
```

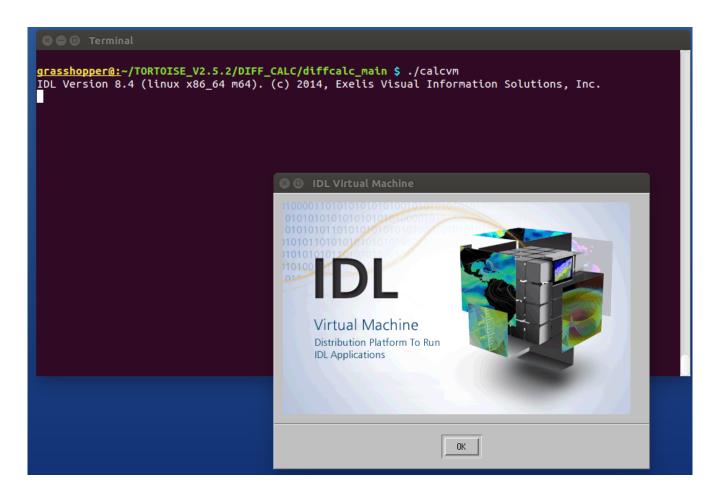
The next step will be to convert the results to usable NIFTIs...

C) RUNNING TORTOISE: DIFF CALC

After finishing with either DIFF_PREP or DR-BUDDI, the processed data can now be exported. In this case, we will just use DIFF_CALC to export a DWI NIFTI file and the gradient information; we won't do further, fancier processing with RESTORE and tensor fits, etc. But that is possible, if you wish.

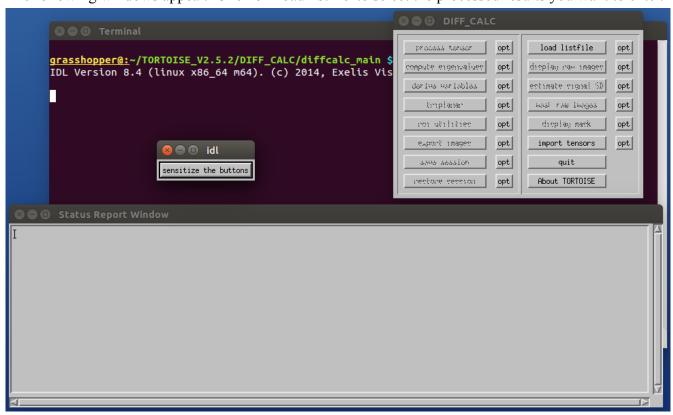
C1) Starting DIFF_CALC GUI.

Go into the DIFF_CALC/diffcalc_main/ directory within TORTOISE, and enter './calcvm' on the command line:



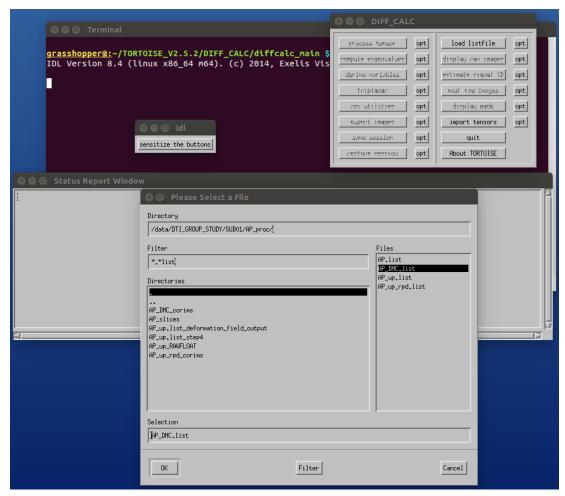
C2) DIFF_CALC GUI: loading in listfile of data to convert.

The following windows appear. Click on 'load listfile' to select the processed results you want to enter.



C3) Entering listfile of processed data.

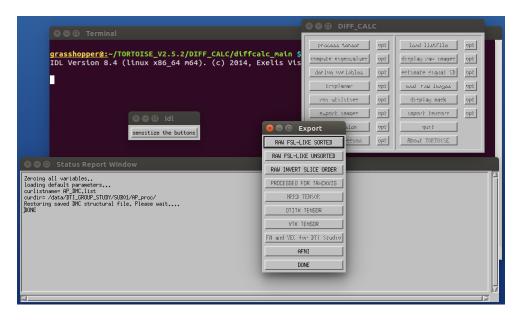
In the window that opens, navigate to the *_proc/ directory that you want to export from. Whether you are exporting data from DIFF_PREP or DR-BUDDI, you will select the *_DMC.list listfile from that directory. This is an example of exporting data from DIFF_PREP (from AP_proc/):



... then hit "OK"

C4) Exporting images.

In the left column, the 'export images' button (LHS, third from the bottom) should become unfrozen. Click on it, and the following menu appears:



Click on "AFNI" for the basic export of the processed DWI volumes (called "DWI.nii") and the gradient information (TORTOISE will output a single b-matrix file, instead of separate byec and byal files). The b-matrix file will have updated the gradients during processing if rotation of a volume was performed.

You may then hit the "DONE" button in the menu and enter a new list to export, or just close the thing.

These two files are exported into a single directory, a subdirectory of the current *_proc/ directory, ending in "SAVE_AFNI":

```
grasshopper@:/data/DTI_GROUP_STUDY/SUB01/AP_proc $ ls
                                                                 AP_up_rpd_corims
                         AP.path
                                                                 AP_up_rpd.list
AP_DMC.bmtxt
                         AP slices
AP_DMC_corims
                         AP_up_b0_orig_crop.nii
                                                                 AP_up_rpd.path
                         AP_up.bmtxt
AP_DMC.list
                                                                 AP_up_rpdstructural.nii
AP_DMC.path
                         AP_up.list
                                                                 AP_up_rpdtemplate.nii
                         AP_up.list_deformation_field_output AP_up_rpd.transformations
AP_DMC_SAVE_AFNI
AP_DMCstructural.nii
                         AP_up.list_step4
                                                                 AP.xml
AP_DMCtemplate.nii
AP_DMC.transformations
                         AP_up.path
AP_up_RAWFLOAT
                                                                 timing.txt
AP.list
                         AP_up_rpd.bmtxt
grasshopper@:/data/DTI_GROUP_STUDY/SUB01/AP_proc $ ls AP_DMC_SAVE_AFNI
BMTXT_AFNI.txt DWI.nii
grasshopper@:/data/DTI_GROUP_STUDY/SUB01/AP_proc $
```

NB: TORTOISE will have averaged all b=0 images together and put them as the 0th volume automatically during processing (and this will also be consistently reflected in the BMTXT.txt entries).

E Final conversions: DT, DT parameters and a whole brain tracking example.

Time for last (pre-) processing step: getting DTI parameters.

E1) Running script to calculate DTs.

One more script is run (this one is currently still 'tcsh'...) from the *SAVE_AFNI/ directory: $\frac{\text{SAVE_AFNI/directory:}}{\text{SCRIPTS/do_TORT_AFNI_proc.tcsh}}$.../.../ANATOM/T2F_RPI_SS.nii

It will: make sure that diffusion data is in the same space as the anatomical volume (that's why it takes the original anatomical as its single argument); mask the data using the b_0 image; use 3dDWItoDT to estimate diffusion tensors (DTs) and DTI parameters; and perform a simple, whole brain tractography to make sure that everything is alright (and that the gradients don't need to be flipped!...)

```
paul@paul:~/TORT_EXAMPLE_UCT/SUBJ_01/INTERMED/PA_up_bupdown_proc/PA_up_bupdown_DMC_SAVE_AFNI $ tcsh ~/TORT_SC
RIPTS/do_TORT_AFNI_proc.tcsh ../../ANATOM/T2F_RPI_SS.nii
```

Successful execution of the script will produce something like what follows:

```
++ Number of ROIs in netw[0] = 1
++ No refset labeltable for naming things.
++ SEARCHING for files with prefix 'DTI/DT*'
++ Obtained 9 prefix-matching files to sort
++ SCALAR FINDINGS:
         'FA' 'L1' 'L2' 'L3' 'MD'
++ Done with scalar search, found: 6 parameters (well, including internal RD calc)
        --> so will have 17 output data matrices.
++ Calculating RD
++ VECTOR FINDINGS:
        'V1' 'V2'
                        'V3'
++ With '-logic OR', the '-cut_at_rois' option will be automically turned off ('-uncut_at_rois').
++ Tracking progress count: start ...
++ Done tracking, tidying up outputs..
++ From tracking, net[0] has 89606 tracks.
++ Writing output (RPI, same as your input): DTI/o.WB ...
paul@paul:~/TORT EXAMPLE UCT/SUBJ 01/INTERMED/PA up bupdown proc/PA up bupdown DMC SAVE AFNI $
```

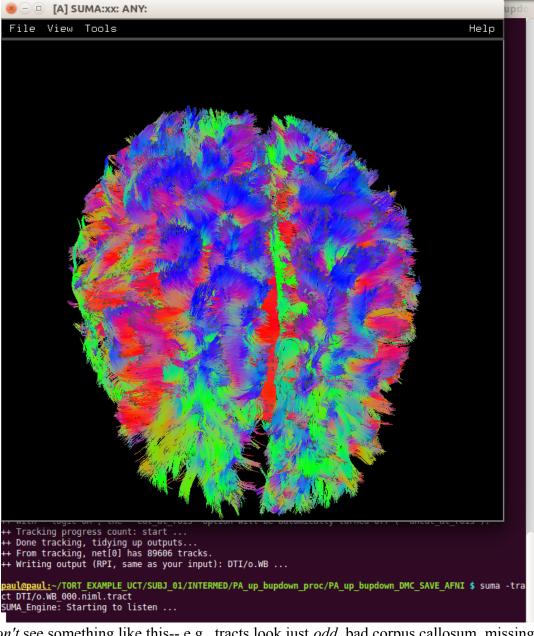
E2) Viewing results.

You can use AFNI viewer to view DTI fit things, for example to make sure the masking actually was good, that the FA>0.2 map looks like a reasonable proxy for WM (if you are analyzing adults), etc. Consider running, for example:

\$ afni DTI/

You can also use SUMA to view the automatic, whole brain tracking that was done. A very basic call might be:

\$ suma -tract DTI/o.WB_000.niml.tract and, if all went well during processing, you will hopefully see a nice, full set o' tracts:



If you *don't* see something like this-- e.g., tracts look just *odd*, bad corpus callosum, missing chunks, no cingulate bundles, weird spikiness-- then see the online help about flipping gradients, which can be done by editing the 'ldDW_Grad_o_Mat' command in the

'~/TORT_SCRIPTS/do_TORT_AFNI_proc.tcsh' script. (Also, first check that the whole brain mask was ok.)

Happy DTI analysis!

