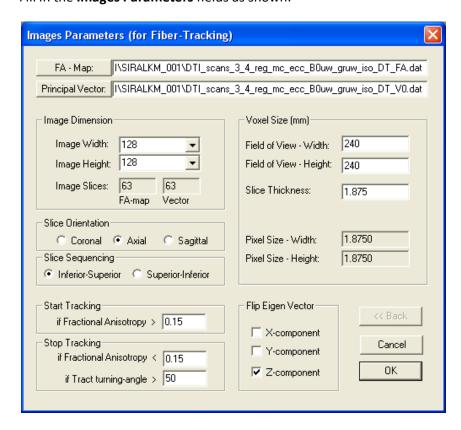
Introduction to DtiStudio

Open DtiStudio
Go to File → Fber-Tracking
In the FA Map field, browse to the *_FA.dat for your data
In the Principal Vector Field, browse to the *_V0.dat file
Fill in the Images Parameters fields as shown:



You can change the **Start Tracking if Fractional Anisotropy** >, **Stop tracking if Fractional Anisotropy** < and **Stop tracking if turning-angle** > thresholds if you want to be more or less conservative about the tracts that are displayed, but those values seem to work well with the DTI protocol commonly used at RIL.

Click OK

The program takes a few minutes to seed tracks.

FA maps will appear on the screen, in 3D, axial, sagittal and coronal views.

The panel on the right side of the screen provides the tools you will need for completing different operations in DtiStudio. Notice that there are 3 tabs at the bottom of the panel – Image, ROI and Fiber. You will mainly be using Image and Fiber.

Use the zoom button at the top of the Image tab to make the maps bigger.

Most of the landmarks used to set ROI's for the fiber tracks assume that the images are being viewed as Color Maps. In order to create a color map from your data, click the **Color Map** button in the **Image Processing** section of the **Image** tab. Under **Select a Vector Image**, highlight your data by clicking on it. Then, under **Select a Weighting-Img**, highlight your data by clicking on it. Click **OK**.

The color maps are based on the directionality of the diffusion of water in the fibers (so, presumably, the color indicates the directional path of the fiber). Right-Left diffusion is indicated by red, Anterior-Posterior diffusion is indicated by green, and Superior-Inferior diffusion is indicated by blue (this is often stated as "RAS maps to RGB" – Right, Anterior, Superior maps to Red, Green, Blue).

Once you've created your color maps, you are ready to start fiber tracking. If you need to switch back to the FA map (without color), go to the drop-down menu in the **Orthogonal Views** section, at the top of the **Image** tab. Color Map – Proc is the color map, so change this to the first option to view the FA map.

Fiber Tracking

To start fiber tracking, go to the **Fiber** tab. In order to start drawing ROI's, you'll need to check the **ROI-Drawing Enable** option. With that box checked, the **ROI - Shape** and **ROI - Operation** tools become active. The **Poly** and **Oval** shapedrawing tools are the most useful for the ROI-defining protocols. For the first ROI of each track, select **or** from the **ROI - Operation** section. Define the ROI. Then un-check **ROI-Drawing Enable** before navigating to the second ROI. Once the region to be defined as a ROI has been located, click **ROI-Drawing Enable** again so that it is checked. Then choose the **ROI - Operation** indicated by the protocol (usually the **and** operation is used for the second ROI). Once the second ROI is defined, DtiStudio will generate the tracts that pass through both ROI's (if the **and** operation was used) or through either ROI (if the **or** operation was used). Sometimes, it is necessary to use the **not** operation to remove fibers that are not actually part of the tract but this operation is used rarely, mainly for "cleaning up" the tract.

The protocol for creating 11 major fiber tracks quoted (below) from

Wakana, et al. 2007. Reproducibility of quantitative tractography methods applied to cerebral white matter.

NeuroImage. 36(3): 630-644

unless otherwise noted.

Saving Tracts

In the Fiber Tracking section at the bottom of the Fiber tab, click the Save icon (the floppy disc).

Choose the **Save selected fibers** option.

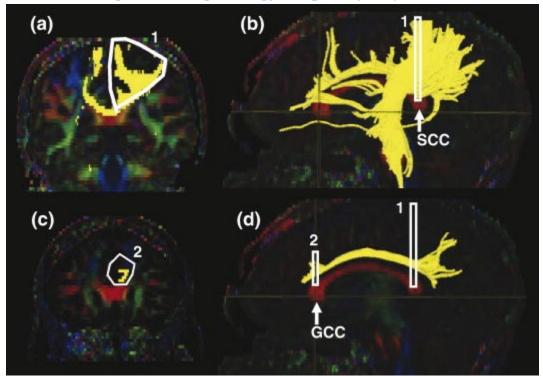
Browse to the appropriate directory and name and save the file.

Saving files in this way will save the data in a format that can be imported into spreadsheets (check with Don or Josh).

Re-opening Saved Tracts

Open DtiStudio
Go to File →Fber-Tracking
Load FA Map and Principle Vector files for the appropriate data.
Go to File → Load Fiber-Data ...
In Files of Type:, choose All Files
Choose the fiber tract files
Click Open.

Tract #1: Cingulum, cingulate gyrus part (CCG):



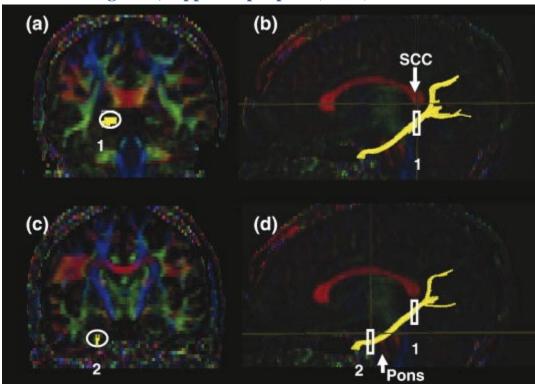
The cingulum is defined as two separate segments; the upper part along the cingulate gyrus (CGC: cingulum cingulate gyrus

part) and lower segment (Tract #2) along the ventral side of the hippocampus (CGH: cingulum hippocampal part).

For CGC (Fig. 2), a coronal plane is selected at the middle of splenium of the corpus callosum (CC) using the mid-sagittal plane

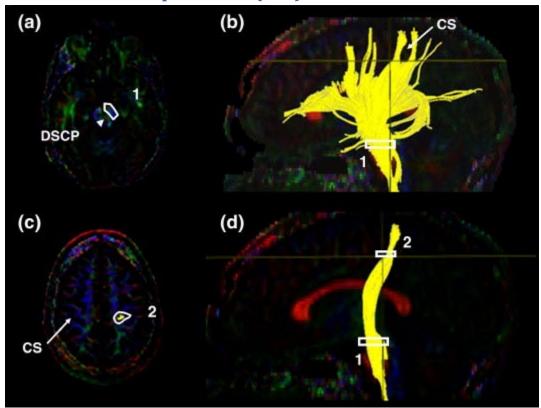
(Fig. 2b) and a **ROI** as shown in Fig. 2a is drawn. For the **second ROI**, a coronal plane in the middle of genu of CC is selected using the mid-sagittal plane (Fig. 2d) and a second ROI is drawn to include the cingulum (Fig. 2c). The size of the second ROI does not affect the result as long as only the labeled cingulum is included.

Tract #2: Cingulum, hippocampal part (CGH)



The inferior segment of the cingulum runs along the ventral aspect of the hippocampus. For the **first ROI**, a coronal plane in the middle of the splenium of corpus callosum is selected using the mid-sagittal plane (Fig. 3b) and the cingulum below the corpus callosum is delineated. For the **second ROI**, a coronal slice anterior to the pons is selected using the mid-sagittal plane (Fig. 3d). The second ROI includes the cingulum which is already labeled by the tracking (Fig. 2c).

Tract #3: Cortico-spinal tract (CST)

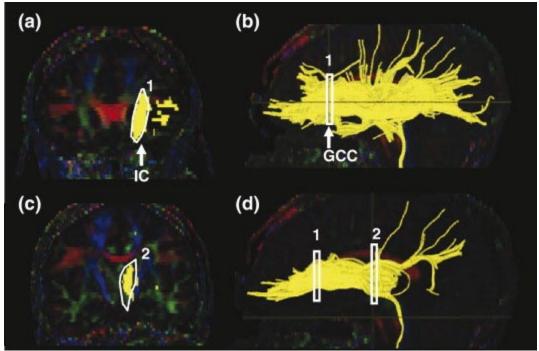


In this protocol, we define the CST between the primary motor cortex and the midbrain. The **first ROI** defines the entire cerebral peduncle in an axial plane at the level of the decussation of the superior cerebellar peduncle (Fig. 4a), indicated by the arrow. [Note: *The decussation of the superior cerebellar peduncle (DSCP, aka "brachium conjunctivum") appears as a red dot in the center of the axial slice at the level of the eyes or slightly below. The ROI is the blue region anterior and lateral to the red dot. (Note added by M. Perry)*] By inspecting the reconstruction result from the first ROI, a bundle of trajectories that reach the primary motor cortex and the location of the central sulcus can be identified. The most ventral axial slice that can clearly identify the cleavage of the central sulcus in the tracking result (Fig. 4b) **is selected (ROI#2)** and the bundle in the primary motor cortex is defined (Figs. 4c and d). [Note: *Look for the break between the fibers of the motor cortex and sensory cortex. The more anterior fibers are the motor cortex.* (Note added by M. Perry)] As long as only the trajectories to the primary motor cortex are defined, the size of the second ROI can be arbitrary. The trajectories outside the two ROIs may cross the midline via the pontine crossing fibers and re-enter the contralateral hemisphere, which interferes with subsequent quantification procedures. These tracts should be removed by using the **not** operation across the entire mid-sagittal slice. [Note: use the midsagittal slice to define a **not** ROI. Any fibers that pass through the midsagittal slice should be removed. (Note added by M. Perry)]

Please note that if the **cut** operation is used, which retains only coordinates between the two ROIs, this extra editing procedure using the **not** operation is unnecessary.

The tracking method described in this protocol usually reconstructs only the CST projecting to the medial cortical regions. The CST projection to the lateral areas of motor cortex cannot be accurately reconstructed because there is a significant mixing of fibers with different orientations within the pixels as the CST passes through the massive bundle of association fibers.

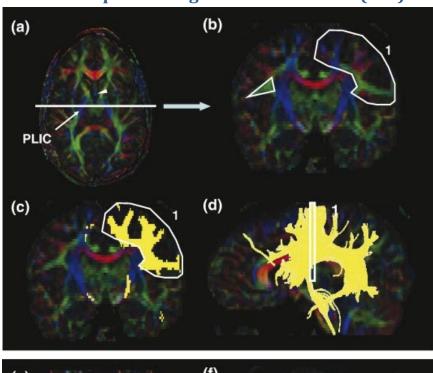
Tract #4: Anterior thalamic radiation (ATR)

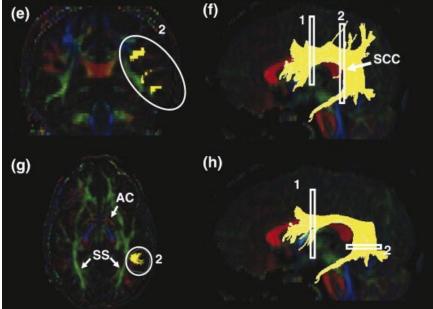


A coronal slice is selected at the middle of the genu of corpus callosum (Figs. 5a, b). The **first ROI** defines the anterior limb of internal capsule (Fig. 5a). For the **second ROI**, a coronal plane at the anterior edge of pons (Fig. 5d) is selected and the entire thalamus is delineated (Fig. 5c). [*Note: When defining the 2nd ROI*, uncheck the **Show fibers in 2D images** option in the **Fiber Display** section of the **Fiber** tab. This makes it easier to exclude the posterior limb of the internal capsule (the blue area). (Note added by M. Perry)]

The trajectories outside the two ROIs may cross the corpus callosum and enter the contralateral hemisphere. These trajectories are removed by a **not** operation across the entire mid-sagittal slice.

Tract #5: Superior longitudinal fasciculus (SLF)



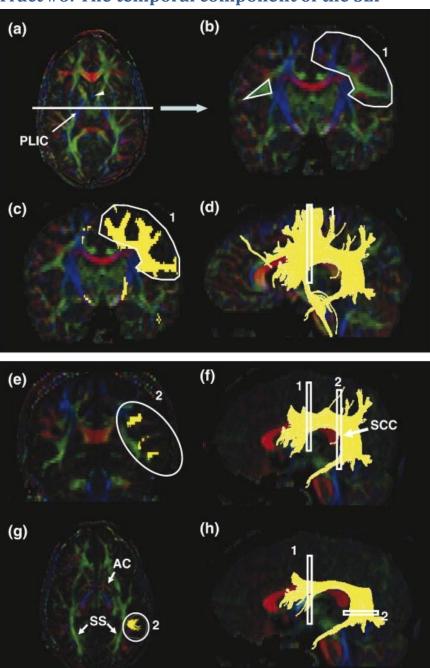


We tested two different protocols to define the SLF. One is used to reconstruct the SLF as comprehensively as possible and the other is selective for isolating the trajectories to the temporal lobe (named SLFt in this paper).

For the **1st ROI**, the lowest axial level in which the fornix can be identified is selected as a single intense structure (Fig. 6a, the fornix is indicated by an arrowhead). Then a coronal slice is selected at the middle of the posterior limb of internal capsule (Fig. 6a; white line). The core of the SLF can be identified as an intense green tract with a triangular shape (Fig. 6b, white triangle). The first ROI includes this core and all branches coming out from the triangular area (Figs. 6b and c).

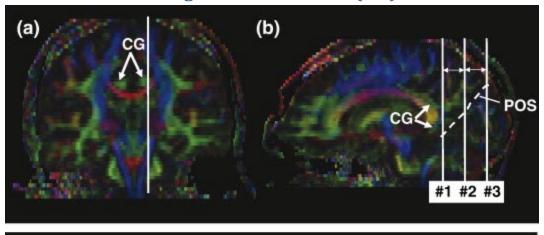
For the **second ROI**, a coronal slice is selected at the middle of the splenium of corpus callosum using the midsagittal level (Figs. 6e and f). The second ROI includes all labeled fibers (Fig. 6e).

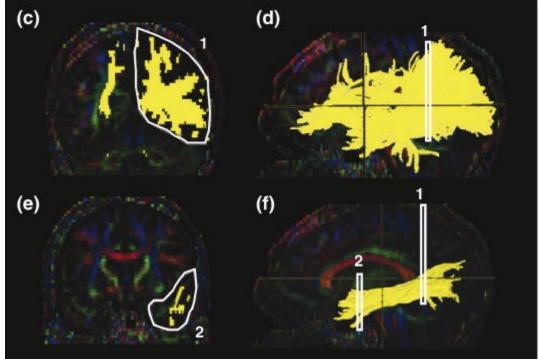
Tract #6: The temporal component of the SLF



For the temporal component of SLF (SLFt), the **first ROI** is same as the body of SLF. For the **second ROI**, an axial slice is selected at the level of the anterior commissure (AC in part g of the figure) and the projections located laterally to the sagittal stratum (SS in part g of the figure) are delineated by the second ROI.

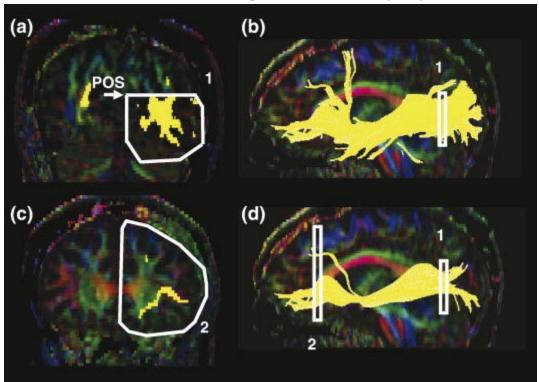
Tract #7: Inferior longitudinal fasciculus (ILF)





Using a parasagittal slice, the posterior edge of the cingulum is identified (indicated by an arrow in part b of the figure). A coronal slice is selected at that edge (coronal level #1 in part b of the figure). The **first ROI** includes the entire hemisphere (part c of the figure). For **second ROI**, the most posterior coronal slice in which the temporal lobe is not connected to the frontal lobe is selected (in part e of the figure). The second ROI includes the entire temporal lobe.

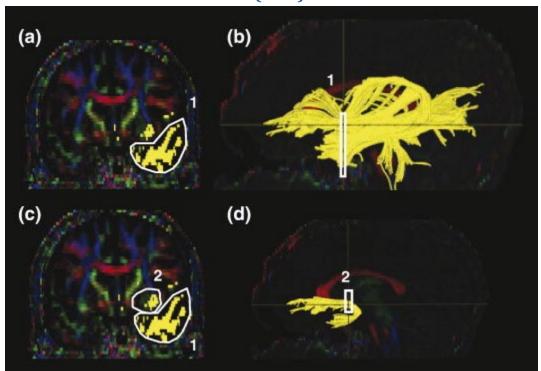
Tract #8: Inferior fronto-occipital fasciculus (IFO)



Wakana, et al., Fig. 8

For the **first ROI**, a coronal slice (the coronal level #2 in Fig **7b**) is identified at the middle point between the posterior edge of the cingulum (slice level #1 in Fig. **7b**) and the posterior edge of the parieto-occipital sulcus (slice level #3 in Fig **7b**). Although a color map is shown in Fig. 7b, another anatomical image such as the least-diffusion-weighted image better defines the parieto-occipital sulcus. The first ROI delineates the occipital lobe. The boundary between the occipital and parietal lobes is defined by linearly extrapolating the parieto-occipital sulcus (POS) to the lateral region (in part a of the figure). For the **second ROI**, a coronal slice is selected at the anterior edge of the genu of corpus callosum (Fig. 8c) and the entire hemisphere is delineated. These two ROIs are sometimes shared by the cingulum and some fibers relayed at the thalamus. These fibers clearly do not belong to the IFO and should be manually removed using **not**.

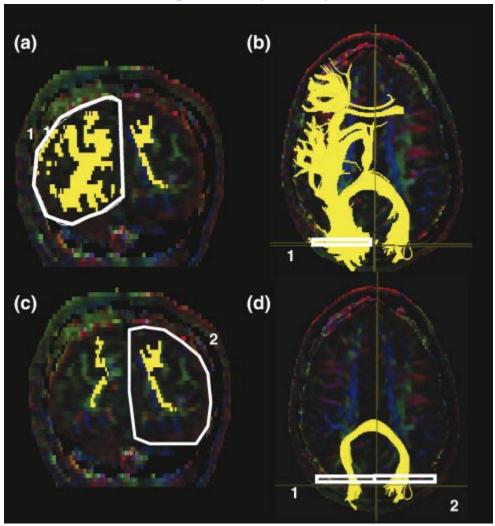
Tract #9: Uncinate fasciculus (UNC)



Wakana, et al., Fig. 9

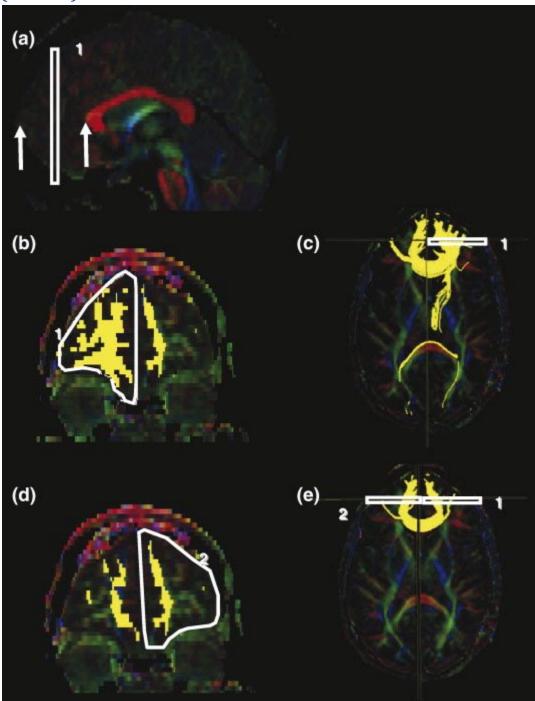
The most posterior coronal slice in which the temporal lobe is separated from the frontal lobe is selected (Figs. 9a and c). The **first ROI** includes the entire temporal lobe and the **second ROI** includes the entire projections toward the frontal lobe.

Tract #10: The forceps major (Fmajor)



It is difficult to devise a protocol that can reconstruct the entire corpus callosum reproducibly. In this paper, reconstruction of callosal connections to the occipital lobe via the splenium of corpus callosum (forceps major) and to the frontal lobe via the genu of corpus callosum (forceps minor) are devised. For the 1st ROI for the forceps major (Fmajor), a para-sagittal plane at the level of cingulum is selected (Fig. 7a) and the parietooccipital sulcus is identified. A coronal plane is selected at the most posterior edge of this sulcus (coronal slice #3 in Fig. 7b) so that the coronal plane includes only the occipital lobe. The least-diffusionweighted image may be used for better identification of the sulcus. The first and second ROIs delineate right and left occipital lobe as shown in Figs. 10a and c.

Tract #11: The frontal projection of the corpus callosum (the forceps minor) (Fminor)



For the **first ROI** (Fig. 11b), a coronal plane at the middle point between the anterior tip of frontal lobe and the anterior edge of the genu of the CC is selected using the mid-sagittal plane (Fig. 11a, between the two arrows). The **first and second ROIs** delineate the entire frontal lobe of each side as shown in Figs. 11b and d).