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Brain CABLE analysis

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is for using the cytoarchitecture-based link estimation (CABLE) method to infer fiber tracts in the brain by analyzing nuclear/somal anisotropy and spatial arrangement of adjacent cells. This method was developed based on the high-resolution fluorescence imaging data of mouse, marmoset, macaque, and human brains obtained with the serial sectioning-and-clearing, 3D microscopy, semi-automated reconstruction and tracing (SMART) strategy. CABLE effectively resolves intersecting and bending fibers, enhancing structural connectivity mapping of primate brains to the cellular resolution. The method is compatible with other micrometer-resolution imaging datasets, provided the cytoarchitecture is labeled via nuclear or somal staining.

CABLE analysis for 3D images

1 Obtain 3D-reconstructed brain images

1.1 We recommend the SMART brain mapping pipeline for obtaining the 3D whole-brain images, with a spatial resolution of 1 µm × 1µm × 2.5µm.

1.2 (optional) Get the ground-truth virus-labeling axons.

2 3D imaging with other methods is also applicable, given the spatial resolution is beyond 5 µm.

3 Convert the image to a single HDF5 file.

4 Run the CABLE analysis tool available at: **BrainCABLE/CABLE**

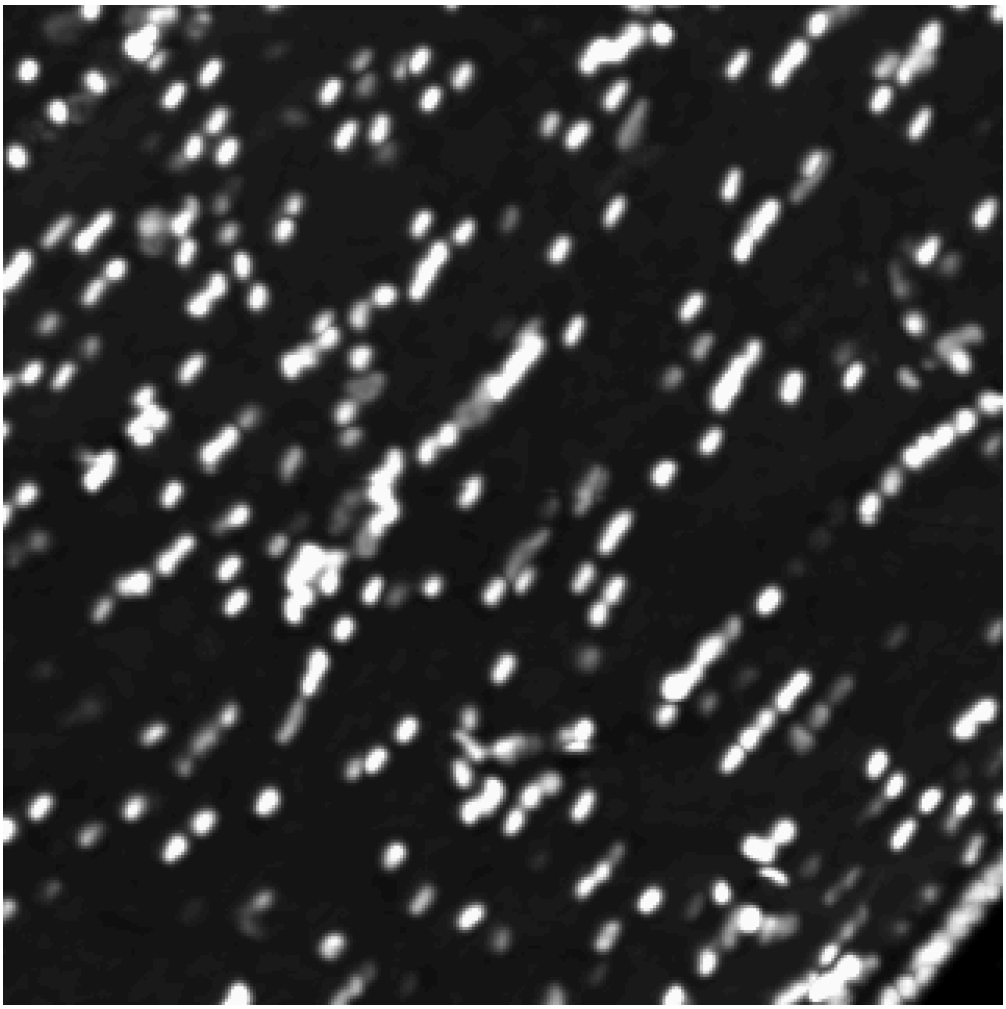
CABLE analysis for 2D images

5m

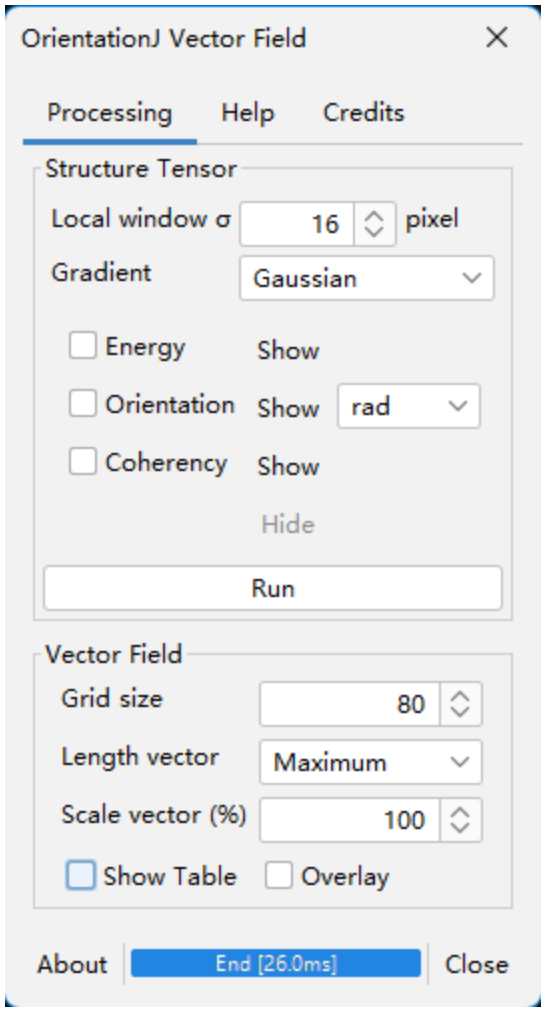
5 Install **OrientationJ** in **FIji**.

5m

6 Open a 2D brain image in Fiji. Navigate to **Plugins–OrientationJ–Orientation vector field**.



DAPI staining of the corpus callosum.

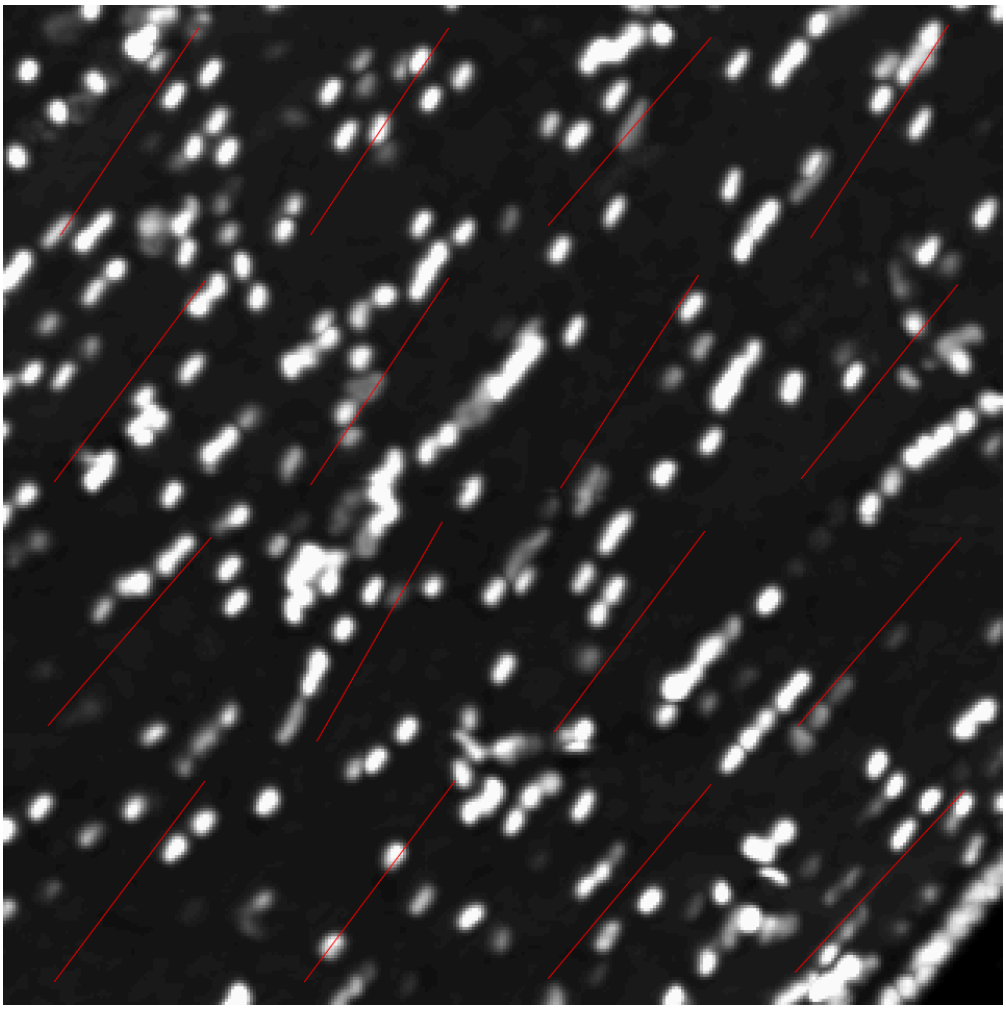


Orientation vector field analysis window.

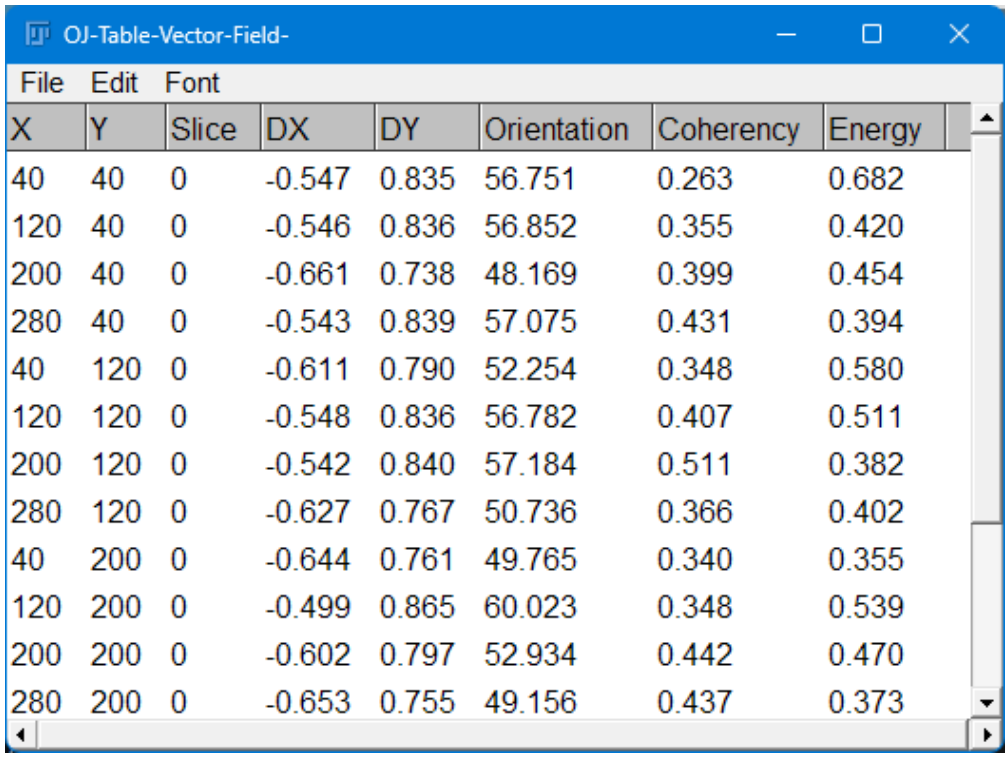
7 (optional) **Apply a Gaussian blur** with a kernel size of 1 or 2µm for denoising.

8 Configure the **structure tensor analysis window** and **vector field window size** based on the scale under analysis.

9 **Enable the "overlay" and "show table" options**. This will display the local orientation on the image and present quantitation results in a pop-up table.



The red lines represent the local directions.



OJ-Table-Vector-Field-

File Edit Font

X	Y	Slice	DX	DY	Orientation	Coherency	Energy
40	40	0	-0.547	0.835	56.751	0.263	0.682
120	40	0	-0.546	0.836	56.852	0.355	0.420
200	40	0	-0.661	0.738	48.169	0.399	0.454
280	40	0	-0.543	0.839	57.075	0.431	0.394
40	120	0	-0.611	0.790	52.254	0.348	0.580
120	120	0	-0.548	0.836	56.782	0.407	0.511
200	120	0	-0.542	0.840	57.184	0.511	0.382
280	120	0	-0.627	0.767	50.736	0.366	0.402
40	200	0	-0.644	0.761	49.765	0.340	0.355
120	200	0	-0.499	0.865	60.023	0.348	0.539
200	200	0	-0.602	0.797	52.934	0.442	0.470
280	200	0	-0.653	0.755	49.156	0.437	0.373

Corresponding to the relevant attributes of each line segment.

Protocol references

1. Xu, F. *et al.* High-throughput mapping of a whole rhesus monkey brain at micrometer resolution. *Nat. Biotechnol.* **39**, 1521–1528 (2021).
2. Schurr, R. & Mezer, A. A. The glial framework reveals white-matter fiber architecture in human and primate brains. *Science* **374**, 762–767 (2021).