Light-Sheet Microscopy of Cleared Tissues with Isotropic, Subcellular Resolution.

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Cleared tissue imaging is an emerging technique where the entire tissue/organs are made transparent, allowing light to probe deeper into the tissue without the need to slice them. Light-sheet illumination, compared to confocal, offers a much faster and light-efficient option when it comes to 3D imaging of large samples. Historically, because each clearing protocol utilizes solvents with distinct optical properties, the microscope had to be designed from scratch to accommodate the refractive index of the clearing media. In addition to this, in order to image bigger volumes faster, the quest to cover large field-of-view often limits the z-resolution of light-sheet microscopes.

Here, we have developed a light-sheet based cleared tissue microscope that can image over the entire refractive index range (1.33-1.56) with an isotropic resolution of 260nm[1] and a field of view of 327 microns. The working principle behind the microscope can be seen in Fig. 1b, where the light-sheet is axially swept along its propagation direction with aberration free remote focusing synchronously with the camera rolling shutter readout[2]. Because the full illumination NA is used throughout the scan, the resolution achieved is the highest reported for any diffraction limited light-sheet microscopy technique.

Using this microscope, we imaged large organs such as mouse brain with spine level details (Fig. 1c-d), whole mouse kidney with enough resolution to resolve the individual endothelial cells of each glomeruli (Fig. 1e-f). Owing to the high optical sectioning of this light-sheet we could image previously unseen interactions like the one between stem-cells, vasculature and nerves in bone marrow (Fig. 1g-k). Today, this microscope forms the basis for many exciting collaborations, evaluating subjects that include renal lymphatic development, Alzheimer's disease, glomerular composition, stem cell niche maintenance, and more.

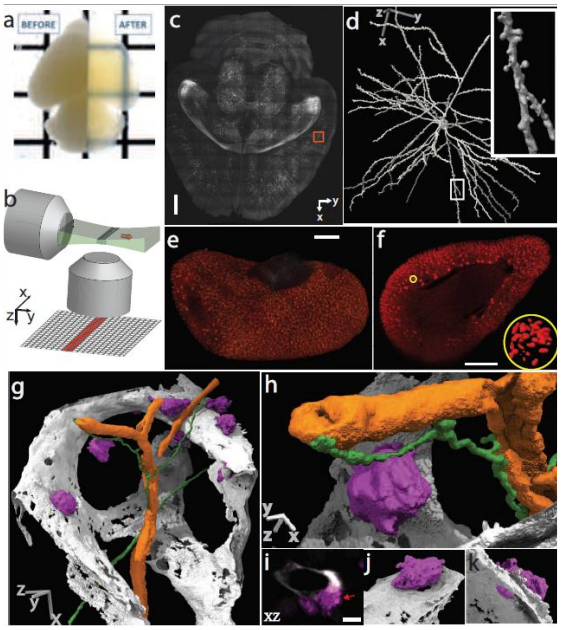

Figure 1: *(a) Cleared mouse brain. (b) Working principle of the microscope. (c-d) Thy1GFP mouse brain and segmented neuron showing spine level details. (e-f) Whole mouse kidney with glomeruli structure from a section. (g) 3D rendering of a mouse bone marrow perisinusoidal-nich. c-kit+ cells magenta) can be seen contacting the blood-vessels (orange and white) and nerve (green). (f) c-kit+ cells positioned along fenestration*

[1] Chakraborty, Tonmoy, et al. "Light-sheet microscopy of cleared tissues with isotropic, subcellular resolution." Nature methods 16.11 (2019): 1109-1113.

[2] Dean, Kevin M., et al. "Deconvolution-free subcellular imaging with axially swept light sheet microscopy." Biophysical journal 108.12 (2015): 2807-2815.