MULTI-IMMERSION AXIALLY SWEPT LIGHT-SHEET MICROSCOPY FOR LARGE-SCALE TISSUE IMAGING WITH ISOTROPIC SUB-MICRON RESOLUTION.

Tonmoy Chakraborty, Bo-Jui Chang, Kevin M. Dean, and Reto Fiolka.
Department of Cell Biology, UT Southwestern Medical Center
6000 Harry Hines Blvd., NL5.116A. Dallas, TX, USA.
E-mail: kevin.dean@utsouthwestern.edu

KEY WORDS: 3D Imaging, Axially Swept Light-Sheet Microscopy, Optically Cleared Tissue.

Expansive interdisciplinary efforts currently aim to generate comprehensive atlases of human cells in a diverse array of tissue types. To date, this has largely relied on massively parallel sequencing and machine learning-based analyses to identify unique sub-populations of cells. Nevertheless, it remains challenging to infer cellular function from high-dimensionality clusters in gene expression. Furthermore, given that protein activity is frequently regulated via post-translational modifications, gene expression does not necessarily provide insight into protein activity. In contrast, advanced imaging-based approaches provide information on both protein localization and activity, as well as the cellular structure and the biological context in which the cell operates in.

Here, we present a scalable imaging platform that provides sub-cellular anatomical detail in any spatial dimension across millimeter cubes of tissue, and nicely complements sequencing-based Human Cell Atlas efforts. The system, which we refer to as multi-immersion Axially Swept Light-Sheet Microscopy, miASLM [1], is to the best of our knowledge compatible with any optical clearing technique, which is necessary since each tissue requires unique clearing conditions. By developing a high-speed voice coil-based remote focusing system, and overcoming residual aberrations due to refractive index mismatches, we achieve ~800 nm isotropic image resolution throughout a volume of 870 x 870 x 1,000 microns with unparalleled optical sectioning [1]. By tiling and stitching the data [2], miASLM permits routine visualization of sub-cellular features throughout millimeters of tissue, including dendritic spines (Figure 1). Moving forward, we will apply robust computer vision-based analyses, including machine learning and deep learning approaches, to identify how sub-cellular states alter global tissue-level architectures in diverse populations of mice in both health and disease.

1. Kevin M. Dean, P. Roudot, Erik S. Welf, G. Danuser, and R. Fiolka, Biophysical Journal **108**, 2807-2815 (2015).
2. D. Hörl, F. Rojas Rusak, F. Preusser, P. Tillberg, N. Randel, R. K. Chhetri, A. Cardona, P. J. Keller, H. Harz, H. Leonhardt, M. Treier, and S. Preibisch, (2018).

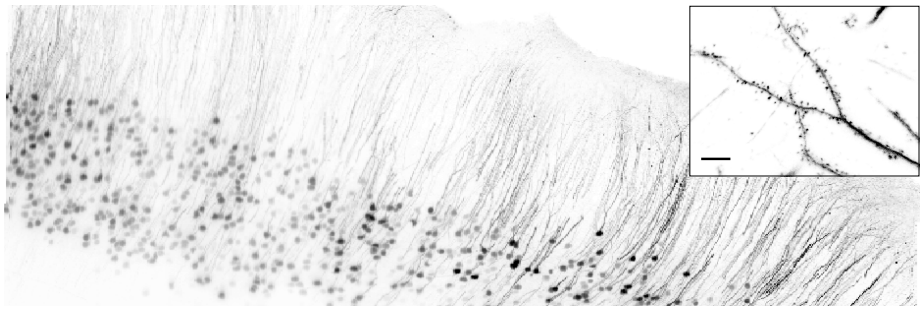

Figure 1: PEGASOS-cleared cortical neurons, as imaged with miASLM. Axial maximum intensity projection over ~135 microns, lateral field of view ~1.6 x 1 mm (scale bar 200 microns). Inset, magnified view at full resolution reveals synapses (scale bar 20 microns).