SERIAL BLOCK FACE SECTIONING & LIGHT SHEET FLUORESCENCE EXPANSION MICROSCOPY

Complete Mapping of Extended Neuronal Circuits at Super Resolution

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The combination of tissue expansion and Light Sheet Fluorescence Microscopy (LFSM) allows extended volumetric super resolution imaging of large mouse brain samples at high speed (1, 2). Recently, we demonstrated the capabilities of this method by performing three color imaging of mouse CA1 and dentate gyrus molecular-, granule cell- and polymorphic layers. This approach features (i) high imaging rates, (ii) high contrast, (iii) low photobleaching, (iv) lateral sample extensions in the centimeter range and (v) effective optical super resolution. A careful sample preparation allows preserving the fluorescence of autofluorescent proteins, avoiding the use of antibodies, which do not penetrate thick samples well and produce considerable background noise (3). However, when imaging expanded brain slices, their thickness easily exceeds the working distance of high resolution objective lenses (4). Sectioning the sample before imaging results in artifacts and distortions that prohibit reconstructing intact neuronal circuits.

Here we approach this problem by combining serial block face sectioning and LFSM of the expanded samples in the same instrument using a custom-developed microtome featuring a 40 μm thin steel wire. Using the microtome we cut and eliminated physically the specimen slab already imaged and could therefore examine samples with a practically unlimited axial extension. Distortions introduced by the cutting process reached approximately 200 micrometers deep into the sample. We present super resolved images of expanded samples with a thickness greater than 4 mm expressing autofluorescent proteins.

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(3) Kohl, J. *et al*. Ultrafast tissue staining with chemical tags. *PNAS* **111(36)** E3805-14 (2014).
(4) Perkel, J. The microscope makers putting ever-larger biological samples under the spotlight. *Nature* **575**, 715-717 (2019)