

HIGH-LIGHTS

URLs
yellow fluorescent protein
<http://us.expasy.org/cgi-bin/nice-prot.pl?P21578>

IMAGING TECHNIQUE

Mind-reader

The ability to image the molecular structure of the brain has provided us with insights into its normal and pathological functions. However, the technologies that are available to image neuroanatomy are generally slow. Automated, high-throughput methods similar to those applied to the field of proteomics would therefore be invaluable, and such a method might now be available. In *Neuron*, Kleinfeld and colleagues present a technique — all-optical histology — for the automated, three-dimensional (3D) histological analysis of brain tissue.

Recent work indicated that ultrashort amplified laser pulses could be used to section or remove layers of tissue. The potential to use these lasers as a histological tool fits well with the fact that similar nonamplified laser pulses are used in the imaging technique two-photon laser-scanning microscopy (TPLSM). TPLSM can be used to image fluorescent molecules to depths of hundreds of microns from the brain surface.

Repeated rounds of ablation and imaging could therefore be used to obtain diffraction-limited volumetric data that can be used to reconstruct fluorescently labelled tissue in 3D. Advantages of this method include the fact that it can be carried out on unfrozen samples and that the physical location of the sample can be maintained, which means that this process could be automated. However, are there disadvantages?

Kleinfeld and co-workers showed

that 1–10 μJ laser pulses could be used to make cuts that could ablate tissue with micron precision, and that the roughness of the optically ablated surface is comparable to that of frozen or unfrozen surfaces that have been cut using a knife. The roughness of the block face is also well within the imaging depth of TPLSM, which makes the ablation and imaging methods compatible. Furthermore, they tested this method on fragile, unfrozen embryonic mouse brain and found that both the gross and fine structure appeared normal after large-scale laser tissue removal.

Next, the authors showed that, after ablation, normal tissue properties are retained in the adjacent, unablated regions. For example, they applied fluorescent dyes to show that the physical integrity of the cell surface and organelles had been preserved. Furthermore, they showed the preservation of antigenic response, and the retention of fluorescence in tissue from transgenic animals expressing fluorescent proteins.

In the final part of this work, Kleinfeld and colleagues used all-optical histology to generate 3D reconstructions of labelled tissue. In

the first example, they imaged, with micron resolution, the fixed neocortex of transgenic mice in which certain neurons were selectively expressing **yellow fluorescent protein**. In the second example, they imaged, again with micron resolution, the microvasculature in a block of fixed neocortex from transgenic mice expressing cyan fluorescent protein. This work has therefore shown that the use of laser pulses to ablate and then image fixed and fresh tissue could provide us with a powerful method for the automated, high-throughput, 3D histological analysis of brain tissue.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Tsai, P. S. *et al.* All-optical histology using ultrashort laser pulses. *Neuron* **39**, 27–41 (2003)

WEB SITE

David Kleinfeld's laboratory: <http://www-physics.ucsd.edu/neurophysics/>

