ALL-OPTICAL HISTOLOGY FOR THE LARGE-SCALE MAPPING OF CELLULAR AND VASCULAR DISTRIBUTIONS IN RODENT BRAIN

Philbert S. Tsai[1], Beth Friedman[2], Augustin I. Ifarraguerri[5], Chris B. Schaffer[1], Varda Lev-Ram[3], Roger Y. Tsien[3, 4], and David Kleinfeld[1]
[1]Dept. of Physics and CTBP, [2]Dept. of Neurosciences, [3]Dept. of Pharmacology, and [4]Howard Hughes Medical Institute, University of California at San Diego, La Jolla, CA.
[5]Science Applications International Corporation, Arlington, VA.

KEY WORDS: Femtosecond laser light, Ablation, Two-photon microscopy, Anatomy, Serial tissue processing

We quantify the large-scale, three-dimensional distributions of brain cells and neighboring vasculature in neocortex from mice. These distributions provide insight into the extent of cortex that can be serviced by individual capillaries. They bear directly on our understanding of neurovascular coupling in normal and diseased states of the brain, with particular relevance to vasculature-related diseases such as stroke, vascular dementia, and Alzheimer's disease.

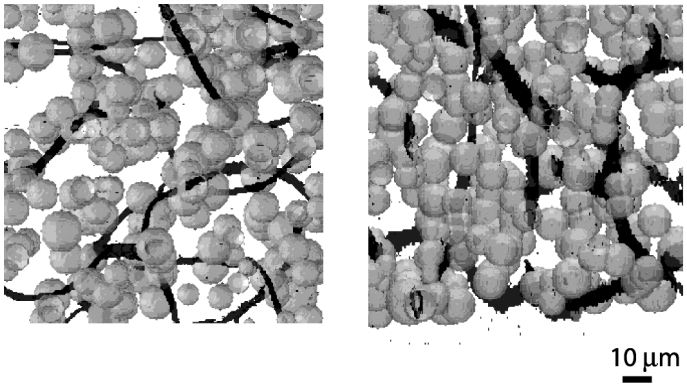

Figure 1. Two orthogonally rendered views of fluorescein-filled blood vessels and Hoechst 33342-stained cell nuclei in mouse neocortex.

Current techniques in histology involve the manual slicing of frozen or embedded tissue to generate thin tissue sections for individual imaging. Construction of three-dimensional maps from these two-dimensional images is highly labor intensive and is complicated by warpage of the thin tissue sections. To circumvent these issues, we demonstrate the use of ultrashort laser pulses for an all-optical histology technique [1] that provides an automated means to perform *in-situ* histology of fluorescently labeled brain tissue. The all-optical histological process involves the use of two-photon laser scanning microscopy, at a fluence of < 10 mJ/cm^2, to image the top layers of a fixed tissue preparation *in-situ*. The imaged tissue is then ablated with amplified ultrashort laser pulses, at a fluence of 1 to 10 J/cm^2, to expose previously underlying layers for imaging. The imaging and ablation processes then proceed serially until the entire tissue volume is processed. Image registration is intrinsic to the technique.

We use the all-optical histology technique to visualize and reconstruct fluorescently-labeled brain vasculature and cell nuclei in fixed tissue from mouse cortex. Either all cell nuclei (Figure 1) or only neuronal nuclei are labeled. Our present mapping effort focuses on the multiple vibrissa sensory areas in neocortex, a tissue volume of approximately 30 mm^3.

[1] P. S. Tsai, B. Friedman, A. I. Ifarraguerri, B. D. Thompson, V. Lev-Ram, C. B. Schaffer, Q. Xiong, R. Y. Tsien, J. A. Squier, and D. Kleinfeld. All-Optical Histology Using Ultrashort Laser Pulses, *Neuron* **39**, 27-41 (2003).