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Microscopy TODAY

Coming Events

2012

ASU Winter School—HREM
January 4–7, 2012
Tempe, AZ
http://le-csss.asu.edu/winterschool

SPIE—Photonics West
January 21–26, 2012
San Francisco, CA
http://spie.org

High-Throughput Structural Biology
January 22–27, 2012
Keystone Resort, CO
www.keystonesypmposia.org

10th Asia-Pacific Microscopy Conference
February 5–9, 2012
Perth, Australia
www.apmc-10.org

PITTCON
March 11–15, 2012
Orlando, FL
www.pittcon.org

Histochemistry 2012
March 21–23, 2012
Woods Hole, MA
http://immunohistochem.com

MRS Spring Meeting
April 1–4, 2012
San Francisco, CA
www.mrs.org/spring2012

Microscopy & Microanalysis 2012
July 29–August 2, 2012
Phoenix, AZ

European Microscopy Congress
September 16–21, 2012
Manchester, UK
www.emc2012.org.uk
Abstract deadline: March 16, 2012

2013

Microscopy & Microanalysis 2013
August 4–8, 2013
Indianapolis, IN

2014

Microscopy & Microanalysis 2014
August 3–7, 2014
Hartford, CT

2015

Microscopy & Microanalysis 2015
August 2–6, 2015
Portland, OR

More Meetings and Courses
Check the complete calendar near the back of this magazine and in the MSA journal *Microscopy and Microanalysis*.

Carmichael's Concise Review

I Can See Clearly Now!

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An important challenge in microscopy is the development of high-resolution light microscopy methods to label and image cell populations in three dimensions. The ability to achieve this deep into intact specimens is limited by light scattering. Modern technologies, such as two-photon excitation fluorescence microscopy, allow examination of structures at distances of hundreds of micrometers below the surface but are insufficient to image and reconstruct large cell populations that are millimeters in scale and deeper below the surface. Whereas light scattering can be reduced by optical clearing, most of these reagents exhibit limitations such as the quenching of fluorescence. Recently, a clearing agent that spectacularly alleviates these major limitations was developed by Hiroshi Hama, Hiroshi Kurokawa, Hiroyuki Kawano, Ryoko Ando, Tomomi Shimogori, Hisayori Noda, Kiyoko Fukami, Asako Sakaue-Sawano, and Atsushi Miyawaki [1]. They developed a clearing reagent called Sca*l*e that renders mouse brains and embryos transparent while completely preserving fluorescent signals from labeled cells!

The discovery of the Sca*l*e reagent started with the serendipitous observation that polyvinylidene fluoride membranes became transparent when soaked in 4M urea, a reagent that promotes hydration of biological samples. This result inspired Hama et al. to search for an optimal reagent to clear fixed biological samples for light microscopy. They experimented with various concentrations of urea and other ingredients. The most effective solution was composed of 4M urea, 10% glycol, and 0.1% Triton X-100 (a detergent). They quantitatively compared transparency through fixed brain slices using this formulation of Sca*l*e and other available clearing agents. Specimens treated with Sca*l*e permitted substantially more light through in the visible and infrared ranges. Intact fixed mouse brains (and whole mouse embryos) became relatively transparent after incubating in Sca*l*e for 2 weeks or more. Specimens were seen to expand (about 1.25-fold linearly), but careful experiments showed that tissue expansion was isotropic and homogeneous. Interestingly, the term Sca*l*e was chosen as the code word for the project because the word sounds like "something transparent" in Japanese.

Hama et al. next investigated if the capability for fluorescence imaging is retained in Sca*l*e-treated specimens because this is critical for imaging individual neurons in the brain. Experiments *in vitro* and with cultured cells showed that enhanced green fluorescent protein signal was not substantially decreased by treatment with Sca*l*e, whereas a conventional clearing agent dimmed the signal over time. They next treated whole brains of transgenic mice with yellow fluorescent protein. Certain subpopulations of neurons in these brains are known to fluoresce. The whole fixed brain showed homogeneous fluorescence; but, when one half was treated with Sca*l*e and the other half treated with a conventional clearing agent, fluorescence was only retained in the former.

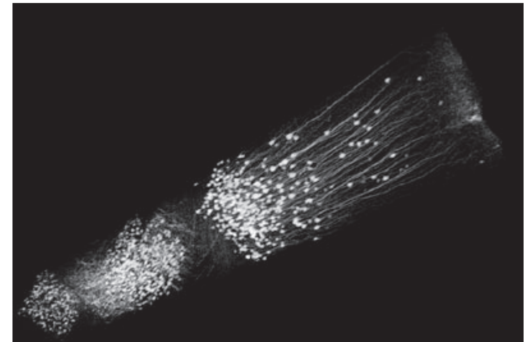

Figure 1: Three-dimensional reconstruction of yellow fluorescence protein-expressing neurons in Sca*l*e-treated mouse brain. The imaging depth is approximately 1.6 mm.

Using one- and two-photon excitation fluorescence microscopy, Hama et al. demonstrated

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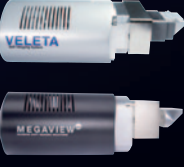

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that three-dimensional neuronal structures could be reconstructed using sophisticated imaging software. They found that in brains cleared with Sca*l*e, the imaging depth was limited by the working distance of the objective lens. They even asked the lens manufacturer Olympus to develop a customized 25× objective lens with a working distance of 4 mm and a numerical aperture (1.0) that allows for high resolution.

Using various formulations of Sca*l*e, Hama et al. could vary the time required for clearing whole brains from months to days. Also, various light microscopy modes were used to demonstrate additional aspects of brain cytoarchitecture such as specific axonal projections and imaging discrete cell populations, including quantitative measurement of their geometric properties. They thoroughly demonstrated proof of principle by reconstructing networks involving cortical, callosal, hippocampal, and neurogenic populations (see Figure 1). Sca*l*e promises to revolutionize future light microscopy studies of the brain and possibly other organs!

References

[1] H Hama, H Kurokawa, H Kawano, R Ando, T Shimogori, H Noda, K Fukami, A Sakaue-Sawano, and A Migawaki, *Nature Neurosci* 14 (2011) 1481–88.
[2] The author gratefully acknowledges Dr. Atsushi Miyawaki for reviewing this article and explaining what Sca*l*e meant.

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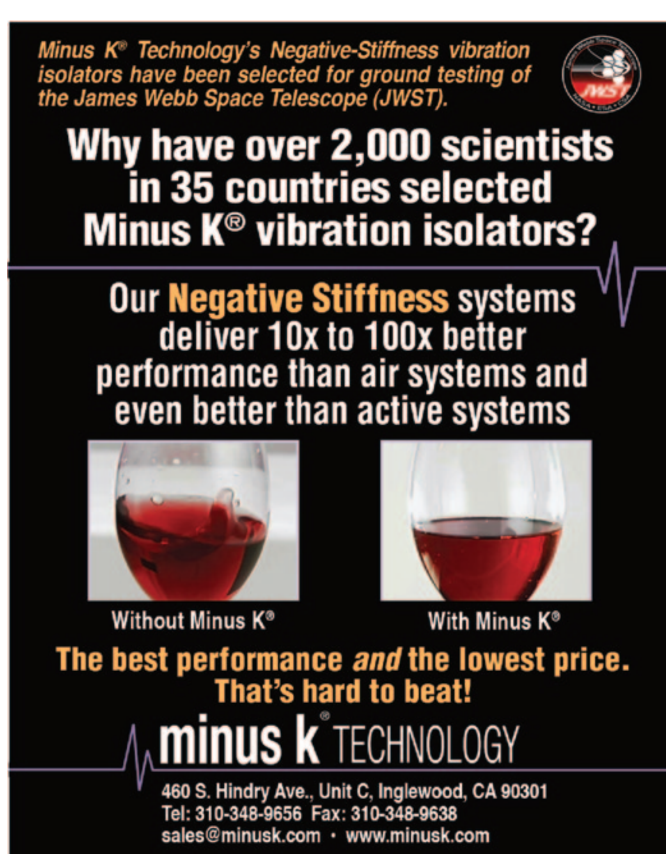

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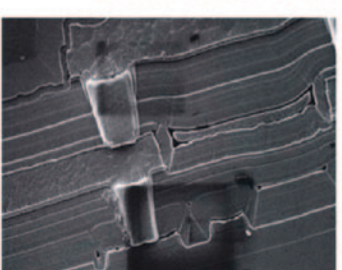
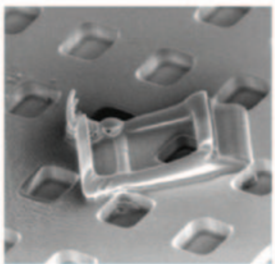

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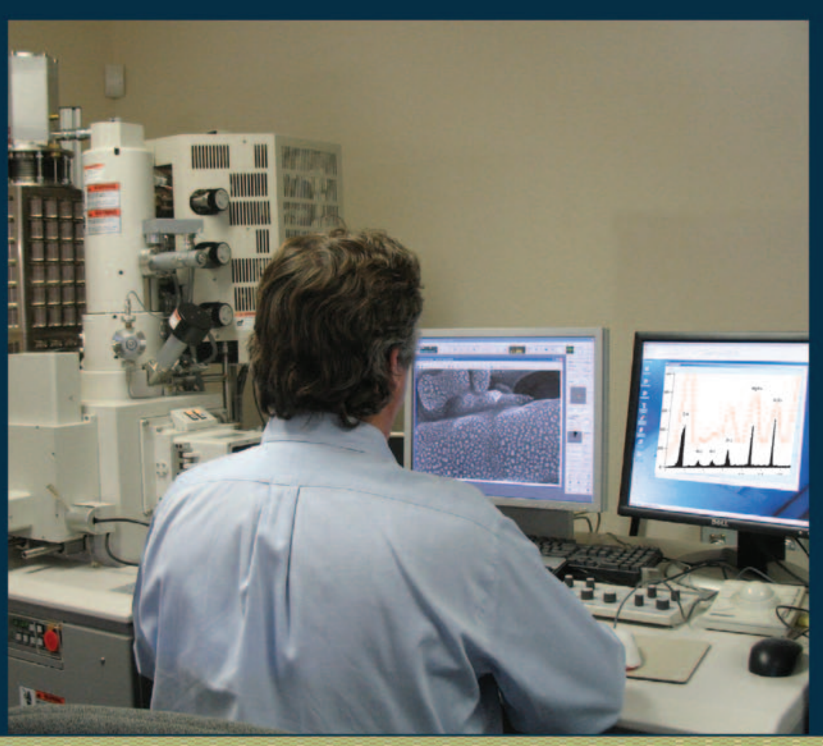

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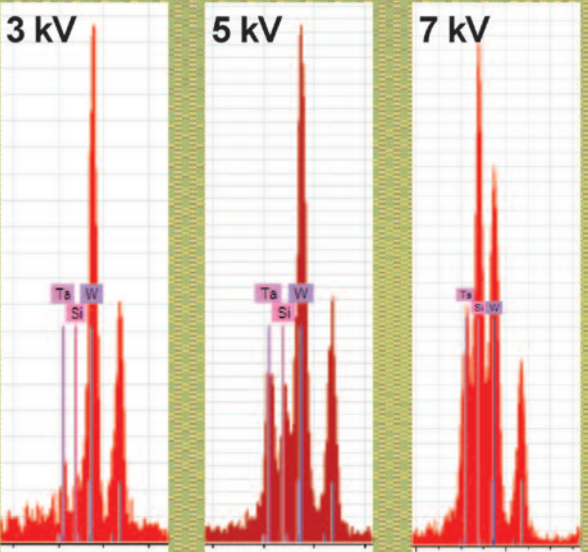

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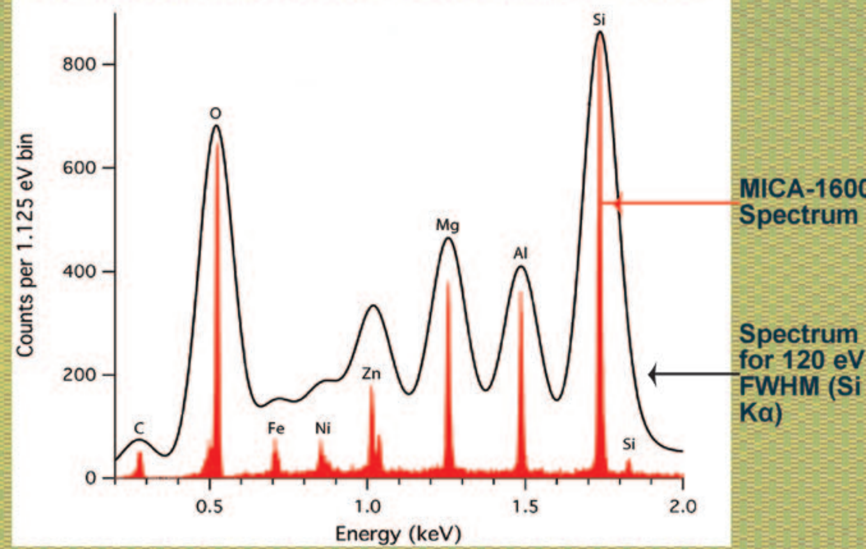

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