Editor's Page

Microscopy: Imaging Broader, Diving Deeper

by Barbara Foster

In previous articles and editorials, the life sciences have been defined as the "next big growth area" in microscopy, spectroscopy, and related imaging techniques. Numerous trends are emerging. Some are functional, such as leaps in glassware and coatings driven by both performance and ecological concerns, or new forms of illumination such as typified by the rise of light-emitting diodes (LEDs), or on-line core facility management software from companies such as **ideaElan** (Herndon, VA). Others are more profound, such as live animal/intravital imaging or the correlative techniques resulting from the recent shift of atomic force microscopy (AFM) into the biological sciences; the Shuttle & Find, an integrated solution for correlative microscopy from **Carl Zeiss** (Thornwood, NJ); and **FEI's** (Hillsboro, OR) acquisition of TILL (the latter two bridging the electron microscopy-to-optical gap).

Two of the new landscape-shifting trends are taking microscopy broader and deeper: whole slide scanning (WSI) and a new constellation of technologies, enabling super-deep imaging.

Diving deeper

The **Olympus America** (Center Valley, PA) booth at the Society for Neuroscience (November 12–16, 2011, Washington, DC) featured an eye-catching panel: an 8-mm-deep 3-D section of mouse brain. No, the "mm" is not a typo. Imaging through tissues to depths like these has long been a holy grail for anyone working with tissue sections or small animal models. It opens new worlds for context and interrelated structures and functions.

A convergence of new technologies makes imaging at these great depths possible. The first centers on advances in "clearing agents," new preparatory methods that convert previously opaque tissue to clear supportive material. Leading the pack is ScaleViewA-2, developed by a research team at RIKEN Brain Science Institute (Saitama, Japan), where Dr. Atsushi Miyawaki works on imaging mouse brain as part of the worldwide Connectome Project. Connectome is a global effort to understand the structure, interconnectivity, and function of animal (and ultimately, human) brains. With all pun intended, ScaleView's impact is clear, gifting previously opaque samples with the same transparency biologists have only seen with animal models such as the zebrafish.

While developed for brain tissue, this agent has broad application to other tissue types. Next on Dr. Miyawaki's agenda: a reagent for living tissue. One caveat: As with all preparation techniques, we advise that you try them with your own samples to assure that they are not causing swelling or other aberrations.

The second contributor to this trend is new glassware. **Olympus** has pioneered in this field, announcing two ScaleView objectives in late 2011: a 25×/1.0 4-mm working distance (WD) and a 25×/0.8 8-mm WD. Both are engineered to match the refractive index of tissue cleared with ScaleViewA-2.

Multiphoton imaging

Multiphoton imaging (MP) completes this picture and is a story in and of itself. Fluorescence-based, this technique excites fluorophores in the sample using red and infrared illumination. These longer wavelengths provide two key advantages. They are absorbed only at specific planes, eliminating out-of-plane excitation, and they undergo less scattering than shorter excitation wavelengths. As a result, they image deeper in the sample than either widefield fluorescence or confocal microscopy.

However, MP has been limited by both its expense (a typical system can run $500–800K) and by Cornell University (Ithaca, NY) patents, which have limited manufacture. Cornell's patents in both Europe and the U.S. have expired over the past three years, opening the field to a technology that many are predicting will rapidly supplant its time-honored predecessor, confocal microscopy.

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Together, ScaleView, the new objectives, and MP imaging are taking microscopy to new depths.

Expanding the micro landscape: Whole slide scanning for digitizing slides, telepathology, and clinical diagnostics

A 1993 visit with Dr. Alvar Gustafson (Tuft's Medical School, Boston, MA) made clear three reasons why whole slide scanning is important. It efficiently converts information from traditional glass slides to digital slides, having profound impact on slide storage and management, comparison, and access. As Dr. Gustafson pointed out, computer disk space was much more space-effective than storing and maintaining tens of thousands of glass slides for

student and staff review. Also, digital slides made it possible not only to compare one area to another across the large expanse of a single slide, but to compare one slide to another, visibly demonstrating subtle variations important both in cell studies and for tumor boards. Finally, digital slides opened the imaging library to anyone with permission, anywhere—from students in their dorm rooms at midnight to research colleagues and pathologists in far-flung corners of the world. The bottom line: WSI is important not just for digitizing slides, but also for telepathology and even clinical diagnostics.

The Neuroscience meeting revealed just how involved our industry is in this arena. Large, dedicated WSI systems were on display from **Leica** (Buffalo Grove, IL), **Olympus**, and **Zeiss**, as well as digital pathology leader **Aperio** (Vista, CA). Other players also have systems, ranging from TissueScope, the unique scanning confocal system from **Huron** (Waterloo, Canada), and the emerging baby uScope from **Microscopes International** (Plano, TX). And, of course, most image analysis packages provide stage control for scanning and algorithms for stitching smaller areas together.

But the story goes further. While WSI has been an important foundation tool in research and education for over 15 years, it is now moving aggressively into clinical diagnosis. Pathologists see this trend as freeing them from the microscope. But is this really a good move? Good diagnosis depends so strongly on both good sample preparation and the eye-brain-hand coordination of the pathologist at the microscope to accurately and consistently classify, score, and then count each cell. Is it time to take this leap?

This editor believes that there is strong evidence supporting the move. Currently, large medical service labs already use digital imaging and analysis for blood counts, with algorithms tightly written so that anything out of the ordinary gets kicked out of the automated queue for live review by a pathologist. Radiology made the move to digital years ago.

In the microscopy arena, cameras have improved dramatically over the intervening 20 years, providing the high pixel densities necessary to image at the lower magnifications typically used by WSI. Algorithms have reached an exceedingly high level of sophistication. Tissue preparation is readily controlled, and companies such as **ThinPrep** (part of **Hologic**, Bedford, MA) are addressing cell-based preps. As for the pathologists themselves, if they were able to identify key markers through the traditional bewildering debris of mucus, stacked cells, and other artifacts, the assumption could easily be made that they should be able to see much more in the clean, clear field presented by a digitally scanned slide.

What does the FDA have to say about all of this? Preliminary rulings made in 2009 required only Class 2 premarket notification for approval for diagnosis based on a digital image. However, the current thinking, reported at Pathology Visions (October 30–November 2, 2011, San Diego, CA) in the Regulatory Panel section, "Navigating Digital Pathology's Path to Patients," was for more stringent Class 3 validation. Class 3 requires clinical trials for each test. It is important to note that this report was a reflection of current FDA thinking, not an FDA ruling.

What is the impact on microscopy and related life-science imaging techniques? First, our market tends to provide components rather than the all-encompassing integrated hardware/software solutions that seem to be FDA's focus. Secondly, the FDA is most concerned about primary diagnosis, not the consultation, research, or education that comprise at least 60% of the estimated $3B market digital pathology market. Also, as has long been the custom, individual clinicians can internally validate a process and, once validated, even commercialize it. Finally, the U.S. lags the global market in its decision not to use WSI as a basis for primary diagnosis, leaving open the rest of the world. The bottom line: WSI is now available and free to provide a whole new, broader landscape for researchers, students, and clinicians alike.

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