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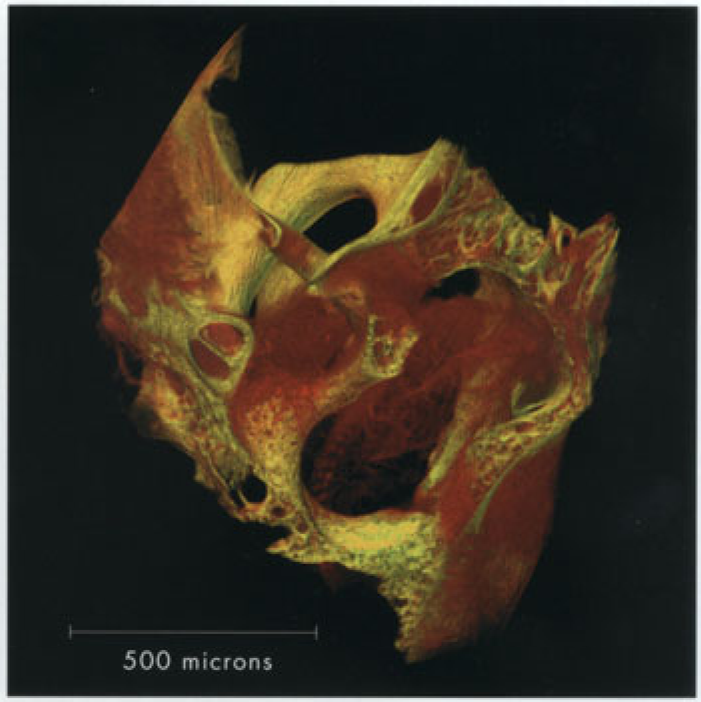
Pretty on the Inside

Resolution Sciences' Digital Volumetric Imaging and RESView software take tissue and materials imaging to a new dimension



Medical grade gauze (left) and bone labyrinth (right) as viewed in RESView.

While new technologies in the fields of proteomics and genomics appear almost daily, histotechnology has remained largely unchanged since its development in the mid-1800s. Tissues are fixed, embedded in wax, sectioned, and stained as needed—a labor-intensive process that generates two-dimensional glass slides that must be viewed one at a time. An anatomical pathologist by training, Russell Kerschmann, founder and president of Corte Madera, Calif.-based Resolution Sciences Corp., is intimately familiar with this classical sample preparation method. As a research fellow at the Massachusetts General Hospital, Kerschmann found the traditional methods of sectioning and slide-making unsatisfactory for his studies of the damage done to blood vessels by lasers used to treat skin diseases. "Blood vessels are intrinsically three-dimensional structures .... I wasn't able to answer such questions as 'Does the earliest damage occur at the branch points?' because the two-dimensional sections didn't show enough of those structures to get a good look," he says.

(over)

Pretty on the Inside *(continued)*

After going on to practice pathology, Kerschmann began to experiment with methods of generating three-dimensional images of the cubic-millimeter-sized samples studied in pathology labs. The resulting technology is called Digital Volumetric Imaging, or DVI. To use DVI, the fixed sample is stained and then embedded in a black polymer. Next, the block is mounted on a microtome, an image is captured directly off the cut surface of the block using a CCD camera, a section is cut to the depth of the first image, and the process is repeated, about 1,000 times on average. The "optical thickness" of each section is controlled by the degree of blackness of the polymer, while interference from the depth of the sample is eliminated by the opacity of the polymer. Because no slides are made and the sectioning is automated, thousands of images can be captured from a sample, allowing micron-range resolution three-dimensional images to be generated for the first time from a 1 to 4.5-mm cube of tissue. "People in the past have tried to make hundreds of glass slides per sample and digitize them under standard digital microscopes and then try to reassemble those images," explains Kerschmann. "No one's ever succeeded in producing an image that recapitulated with any fidelity the architecture of the tissue. We're the first group that's been able to do that with many cubic millimeters per sample." This is because the act of sectioning tends to distort the sample. Because Resolution Sciences images the sample prior to sectioning, this is no longer an issue.

Researchers trim samples to varying sizes depending on the amount of resolution desired (there is an inverse relationship between sample size and resolution), and then send these to Resolution Sciences' laboratories in vials supplied by the company. Resolution Sciences sends back data on a CD or DVD to be processed on a Pentium™ workstation equipped with RESView™ image analysis software. The staining process used by the company generates fluorescent images primarily in shades of red and green. Since the chemistry matches that of hematoxylin and eosin (H&E) staining, the software has been designed to map these colors into the familiar pink and blue of traditional H&E images. The user can sort through the three-dimensional image section by section (in the x, y, and z planes), rotate the image, and perform cell counting through the entire sample in a matter of seconds simply by isolating the nuclei through color segmentation.

The list price of the workstation is $23,500, which includes all hardware and software, a one-year license, and free software upgrades. The maintenance contract can be renewed at the end of the first year. Sample processing costs $1,000 per sample to start, with volume discounting available. Some customers, particularly in academia, get an initial case of "sticker shock" regarding the cost of sample processing, says sales director Colette Saccomanno. "But when they see the amount of information they're starting to be able to pull off of a single sample, it's unbelievable. We're under-priced."

Resolution Sciences started selling systems only last year, and has 10 installed systems across the country, primarily in the pharmaceutical and consumer products industries, according to Saccomanno. Procter and Gamble Pharmaceuticals of Cincinnati, Ohio, for example, uses the technology to study drugs involved in bone formation. Rats are injected with a fluorescing compound, which is incorporated into the bone as it grows; the compound can be viewed with color segmentation to determine the rate of bone formation and to see how the growth regions relate to the overall architecture of the bone. According to researcher Mark Lundy, the technology is too new to determine the full effect that three-dimensional imaging will have on these studies, but early results show that DVI allows investigators to more accurately see how bone cells interact as a group and permits researchers to gather more data per animal, a potentially cost-saving feature.

Applications of the technology are not limited to the life sciences; scientists have successfully used DVI for quality control and measurement in the consumer products industry and in materials science laboratories. Doug Chinn, scientist at Sandia National Laboratories in Livermore, Calif., works with Resolution Sciences to develop methods for imaging micromechanical devices. "In the past we used conventional microscopes—SEM [scanning electron microscopy], optical microscopy—but they're really only two-dimensional imaging techniques. We can really only see surfaces and are limited in the things we can measure using those more conventional techniques," Chinn explains. Using DVI and RESView, however, Chinn can view internal defects and look at the surface plane and sidewall of a part simultaneously to measure the angle between the two. "We're very interested in sidewall angles, and it's very hard to see those with any other technique," he says.

As Kerschmann notes, the company has only started to explore the types of materials that can be investigated with DVI. "We have a technology that encompasses an extremely important range of commercially significant objects ... where the customers want micron-level resolution of data so that they can look at the internal function [of these objects]," says Kerschmann. Markets under development include the agricultural industry, where DVI has been used to look at the internal structure of grain embryos. And each new application leads to new discoveries. As Kerschmann observes, "Almost every day in our lab, I see things that no one's ever seen before in a material, even though people have been looking through microscopes for 150 years."

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