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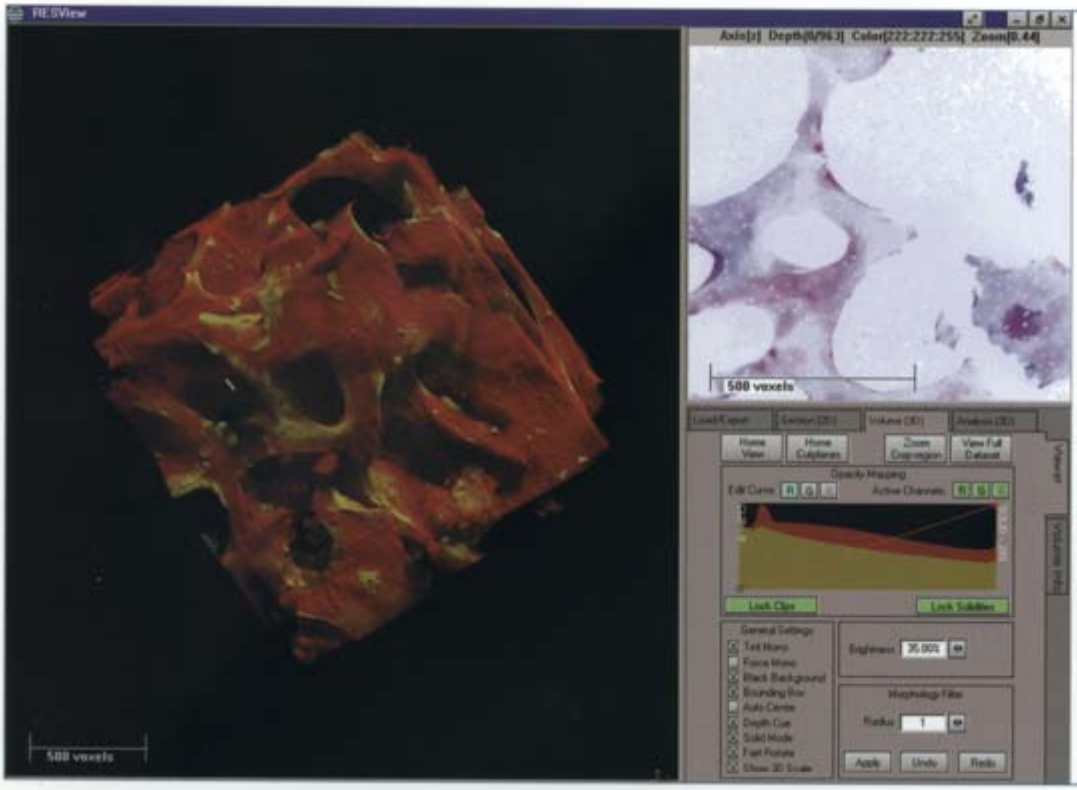
Imaging System Automates Volumetric Microanalysis



RESView visualization and analysis software allows sections of microscope images to be rendered in 3-D.

To accurately replicate volumes of biological tissue and manufactured materials without having to image sections of a sample, Resolution Sciences Corp. (RSC; Corte Madera, CA) has developed a digital volumetric (DVI) system that offers micron-range-resolution visualization and quantitative analysis of samples in volumes of 100 mm3 or more. "DVI accomplishes this by eliminating the need for glass slides," explains Russell Kerschmann, president of Resolution Sciences.

"RSC embeds the sample into a polymer block and images the cut face on the block itself. Acquiring a 2-D image of a volume of the sample situated beneath the cut surface eliminates the need for glass-slide-mounted sections. Because each section is imaged before it is cut from the block, all the 3-D relationship are preserved," he says.

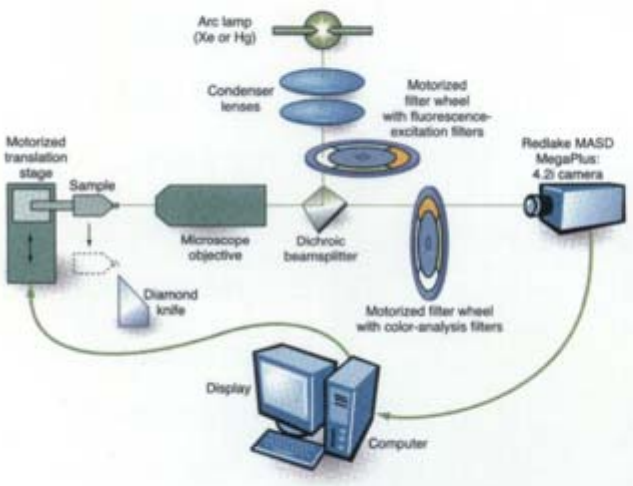
Sectioning and block-face imaging are repeated 1000 times or more per sample, converting the piece of tissue or material into a digital 3-D replica. Each cut slice can be saved for later biomolecular analysis or discarded. "The stack of digital images becomes the volume," says Benn Herrera, RSC senior software engineer.

(over)

Imaging System Automates Volumetric Microanalysis *(continued)*

The plastic-embedded sample is physically sectioned on a diamond blade from MicroStar (Huntsville, TX) in a robotically controlled microtome from Olympus (Melville, NY). Rather than imaging the sections cut from the block, the DVI imaging platform digitally captures an image of the cut face on the block. It acquires a 2-D image of a volume of the sample situated beneath the cut surface of the block.

To digitally capture the image of a block face, the RSC system uses a 2029 x 2044-pixel MegaPlus: 4.2I CCD camera from Redlake MASD (San Diego, CA) with 200-mm-focal-length lenses from Nikon (Melville, NY). It is coupled to a computer-controlled surface-imaging microscope.



In the design of the Digital Volumetric Imaging system, a motorized translation stage is used to prepare images that are then digitized using a high-resolution CCD camera.

Under control of a PC, a DCX-AT2000 motion-control board from Precision MicroControl (Carlsbad, CA) moves the stage of the microtome to cut sections of the sample. Images of the cut face are then captured to PC memory using a PCI-DVK digital camera interface from Engineering Design Team (Beaverton, OR). To capture multichannel data sets, the platform also includes a dichroic beamsplitter and several optical bandwidth filters, all under computer control.

At capture time, a 2:1 software reduction is applied to reduce the 2k x 2k images from the camera to produce 1k x 1k-output images. DVI images are then stored on disk for postprocessing in a proprietary file format. Finally, data sets are recorded on DVD and shipped to customers for visualization and analysis software, data can be displayed as 3-D images and used for counting cells or other biological structures and materials.

"The rendered in RESView 2.2 is based on a shear-warp method created by Philippe Lacroute of the Computer Graphics Laboratory at Stanford University (Stanford, CA)," explains Herrera. "Our implementation can handle 8-bit gray-scale volumes of up to 384 x 384 x 768 pixels or 24-bit color volumes of up to 384 x 384 x 384 pixels, rendered at several frames per second." The current RESView system can produce images as large as 24 Gbytes.

"Since data sets are generally around 1000 voxels per side, interaction is performed with either a reduced volume or a user-selected subvolume of the data set," adds Herrera.

RESView supports full color, lighting controls, variable alpha blending, cropping, thresholding, physical segmentation, chromatic segmentation, and translucency rendering. The 3-D model can be manipulated in the same manner as a real object. Regions of interest can be selected by rotating and cutting the model to produce 2-D sections. Resultant 2-D images look similar to those obtained through a light microscope. The user can also pan, magnify, and scroll through any plane of view within the whole imaged sample.

The software package also allows DVI data to be imported, displayed, analyzed, and exported. To facilitate particular research applications, a number of specialized RESView application modules have been created, including one tailored for the analysis of linear elements such as fibers in manufactured materials. There are plans to create a package for the analysis of bone growth and another for the quantification of large populations of cells.

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