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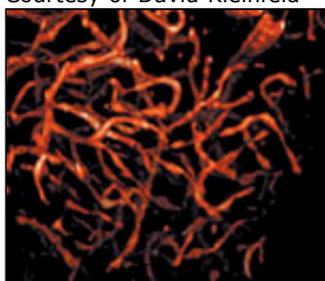
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Slice of Life

Researchers develop new techniques to unveil the architecture of tissues

Courtesy of David Kleinfeld



Many techniques for creating three-dimensional images of tissues have accompanying problems. Image reconstruction from serial tissue sections suffers because sections can warp as they dry, distorting the composite picture. Prolonged photoexcitation can bleach fluorescent dyes used to provide subcellular detail, eventually rendering the sections useless. And, even two-photon microscopy can reach only 100-600 microns deep into a sample. But a new crop of imaging technologies may someday completely automate this process, simplifying the analysis of 3-D tissue samples.

David Kleinfeld, a physicist and neuroscientist at the University of California, San Diego, developed an optical ablation technique in which a femtosecond laser is used to turn the tissue into plasma. In essence, he vaporizes his way deeper and deeper in an iterative imaging and cutting process. "We just had to cut on a scale that wasn't deeper than we could see into the tissue," says Kleinfeld, who claims that with laser cutting, the surface gets rough at approximately 1.1 microns. "You go as deep as you can [with two-photon microscopy], then cut in a way that doesn't cause any physical distortion or affect the optical properties of the tissue." Despite the advanced optics required, Kleinfeld says, "If you can do two-photon microscopy, you can do the cutting; it takes no more skill."

A SLICE OFF THE OLD BLOCK Scott Fraser of the California Institute of Technology uses a different approach to the same problem. His technique, called surface imaging microscopy (SIM), dates back to 1990.^{2,3} This block-imaging technique is similar to cryosectioning,⁴ but in SIM, the specimen is embedded in black plastic. Fraser explains that the

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plastic is used to tune the depth of the light going into and out of the sample. "You control the light by how opaque the plastic is, by adding more of the black pigment," he says.

Fraser's technique is an academic version of work that was commercialized by Russell Kerschmann, who dubbed the technique digital volumetric imaging (DVI). The company he created to market the idea, Resolution Sciences,⁵ fell victim to the biotechnology crash, and Kerschmann is now selling the remaining DVI equipment to academic and commercial users through Corte Madera, Calif.-based Microscience Group.

DVI offers true high-resolution, 3-D images of solid samples. Fraser explains that, in the past, people who examined 3-D data sets could always determine which dimensions were high-resolution and which was low; one axis was always more blurry than the other two. "With Kerschmann's approach, I've had to look back at our notes to see which way it was sectioned," he says.

COST-BENEFIT ANALYSIS With both techniques, higher resolution comes at a cost. Kleinfeld's technique, for instance, allows for use of multiple fluorescent proteins, but SIM is a lot harsher, Fraser allows. "It's probably not as good [for the proteins] as David's technique because he doesn't have to embed [a specimen] into the plastic or expose it to the solvents we use," Fraser says.

Both techniques completely destroy the starting sample. "You can build up this very-high-resolution, three-dimensional rendering of what the specimen once was," says Fraser, "but of course, you've destroyed it in generating that image." Kerschmann, a pathologist, notes that at least with SIM/DVI, the sections still exist in slides. On the other hand, since specimens to be optically ablated can be placed in water, they can be alive, at least initially.

Both methods pose the risk of unintended artifacts. "[Our technique] requires that the tissue be infiltrated with plastic. We might end up with one set of artifacts from that," says Fraser. "[Kleinfeld] might end up with his own set of distinct artifacts, from ablating the surface and having to hold the specimen the way he does."

Ultimately, says Kleinfeld, these techniques "will take anatomy, which is an art form, and turn it into an automated technique. Anatomy has to become automated or we're never going to understand algorithms in the brain." Fraser concurs: "Each one of these techniques gives us a different, somewhat limited view. By assembling together all of these techniques we're going to end up with some novel insights."

--Karen Heyman

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