CELL BIOLOGY

3D laser blasting for biologists

The combination of laser ablation and light sheet–based fluorescence microscopy provides researchers with a powerful way to examine function in living three-dimensional (3D) objects.

If you ask any child what lasers are for they will undoubtedly tell you they are used for blasting something, as is often the case in video games. Although factory robots and surgeons often use lasers in this way—in a carefully controlled manner, of course—biological scientists are more likely to use lasers for microscope-based imaging. But even in biological research, sometimes it can be very useful to blast something away with a precisely controlled burst of laser energy.

Up to now, such laser microsurgery has often been restricted to two-dimensional cultures because of the difficulty in imaging living 3D samples and applying an appropriate 3D-shaped ablation pattern. Ernst Stelzer's laboratory at EMBL has previously developed and used laser microsurgery as well as a light sheet–based method—single plane illumination microscopy (SPIM)—for imaging living 3D samples. "We were thinking it would be very straightforward to combine the two techniques, and you could get a lot of insight in the area of 3D biology," says former thesis student Christoph Engelbrecht.

Their new combined system allows nearly simultaneous 3D microsurgery and optically sectioned fluorescence imaging of living samples. They demonstrated its performance at a wide range of scales from dissecting single microtubules to disrupting single cells in spherical cysts and performing cuts greater than 100 μm long in a zebrafish fin.

"We wanted to be able to perform single-cell ablation anywhere in an object and follow the consequences," says Engelbrecht. To show this, they ablated an area in a Drosophila embryo and watched as GFP-labeled hemocytes migrated to the damaged area over a period of 30 min.

Engelbrecht adds, "We wanted the software to be as user-friendly as possible. We have a live microscope picture on the screen, and we draw the ablation patterns into that picture." EMBL is considering making the software available to interested academic users, and as SPIM becomes more widely used, the potential for being able to integrate laser microsurgery is likely to be a helpful selling point, particularly for the zebrafish community for which this technology seems well suited. Researchers may soon be blasting away in their labs nearly as much as their children are blasting away on their video games at home.

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RESEARCH PAPERS

Engelbrecht, C.J. *et al.* Three-dimensional laser microsurgery in light-sheet based microscopy (SPIM). *Opt. Express* **15**, 6420–6430 (2007).