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Targeting single cells with a laser microscalpel

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A new fiber-based probe may enable surgeons to simultaneously view and eliminate individual cells.

Femtosecond laser microsurgery (FLMS), which employs $\sim 10^{-13}$s laser pulses, precisely destroys cells and smaller structures in 3D biological tissue.[1–4] The technique uses high peak intensity pulses to create multiphoton ionization, thereby vaporizing targeted tissue. The process allows for the use of deep-penetrating near-infrared light and requires two to three orders of magnitude less energy than nanosecond pulses.[3] This combination of high precision and tissue penetration makes FLMS appealing for delicate procedures and those requiring vaporization of diseased cells just beneath the surface. Since 2003 femtosecond lasers have been used clinically, specifically for LASIK surgery.[5] Developing the technology for internal surgical procedures could greatly expand its reach.

Unfortunately, delivering the high peak intensity pulses through optical fiber and miniature optics has proven difficult. Furthermore, to fully exploit the precision of this technique, the microsurgery laser must be guided by equally precise and penetrating imaging. These challenges have prevented the integration of FLMS into an endoscopic surgical tool. Towards this goal, we have developed a miniaturized microscope which provides both imaging and femtosecond laser pulses, allowing the user to directly monitor the area of microsurgery.[6]

The miniaturized microscope, shown in Figure 1(a), uses a hollow-core photonic crystal fiber to guide the pulses through air. This approach eliminates the problems of material damage and the nonlinear effects that occur with conventional optical fiber. In addition to the pulses used for microsurgery, we deliver lower-intensity ones for imaging via the same optical pathway. This nonlinear technique, called multiphoton excitation, provides the same resolution and depth penetration as FLMS. By using similar pulses and identical optical pathways, the probe can precisely visualize the exact area being targeted.

Figure 1(a) shows the two-axis microelectromechanical systems (MEMS) scanning mirror, developed by our collaborator

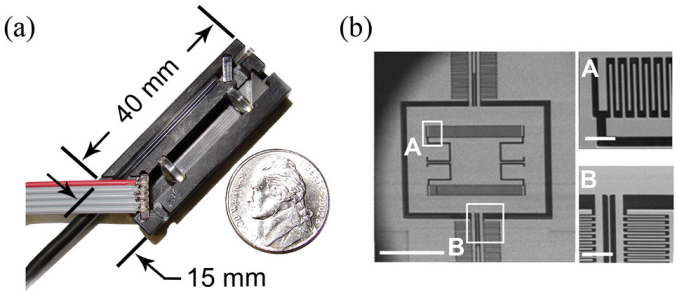

Figure 1. *(a) Photograph of the miniaturized microscope and probe, shown here without the delivery fiber and the sealing lid. (b) Scanning electron micrograph of the MEMS mirror design. The scale bars are 600μm (120μm in inset).*

Olav Solgaard at Stanford Univeristy.[7] It sits inside the probe housing and scans the laser pulses through a miniature lens pair. The pair images the mirror to the back aperture of a gradient-index objective lens. This allows uniform illumination of a wide field of view (FOV) across the mirror's entire scanning range. The lens pair also serves as a beam expander, enabling the imaging and microsurgery laser beams to overfill the back aperture of the objective lens and maximize focusing power.

The system was designed to provide subcellular resolution over a wide FOV and a fast frame rate, both crucial elements for guidance and navigation in vivo. The probe provides two-photon imaging at 10 frames per second, and has a maximum FOV of 310μm and a spatial resolution of 1.6μm. A dedicated collection fiber transmits the fluorescent imaging signal to a photomultiplier tube for image reconstruction.

We have demonstrated the combined imaging and microsurgery capabilities of the probe using breast cancer cells—cultured both in a monolayer and in a 3D collagen-based tissue phantom. In these experiments, we labeled cancer cells with calcein AM, a fluorescent dye that only activates in live cells. We performed microsurgery using one femtosecond pulse of 280nJ ($\sim$14 TW/cm^2 peak intensity), detecting membrane disruption

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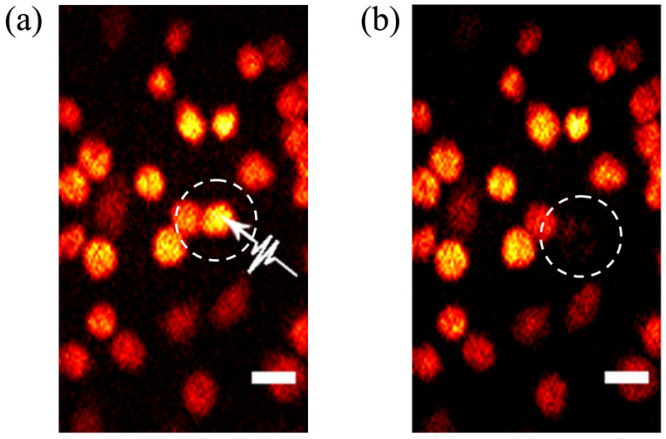

Figure 2. *Combined two-photon microscopy and FLMS can precisely ablate target regions in a single layer of live breast carcinoma cells. (a) Two-photon image of the region prior to irradiation with a high-intensity pulse. (b) The same area immediately after irradiation shows targeted destruction of single cells. Scale bars are 20μm.*

by a sudden loss of fluorescence in a single, targeted cell (see Figure 2). The use of one pulse and a spot size much smaller than the cell diameter eliminated the risk of signal loss due to photobleaching. We also showed the technique's precision in a 3D tissue phantom. In both demonstrations, the cells next to the targeted one remained intact. For clinical applications, the microsurgery laser could be scanned to quickly, and selectively, ablate cells in larger regions of interest.

This probe may allow clinicians to use a microscale, seek-and-treat tool for operating on otherwise inaccessible parts of the body. Our next steps aim to use alternative objective lenses to improve the resolution of the system. We are also miniaturizing the device's overall dimensions to make it small enough for endoscopy.

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