

Tiny Optics for In Vivo Imaging

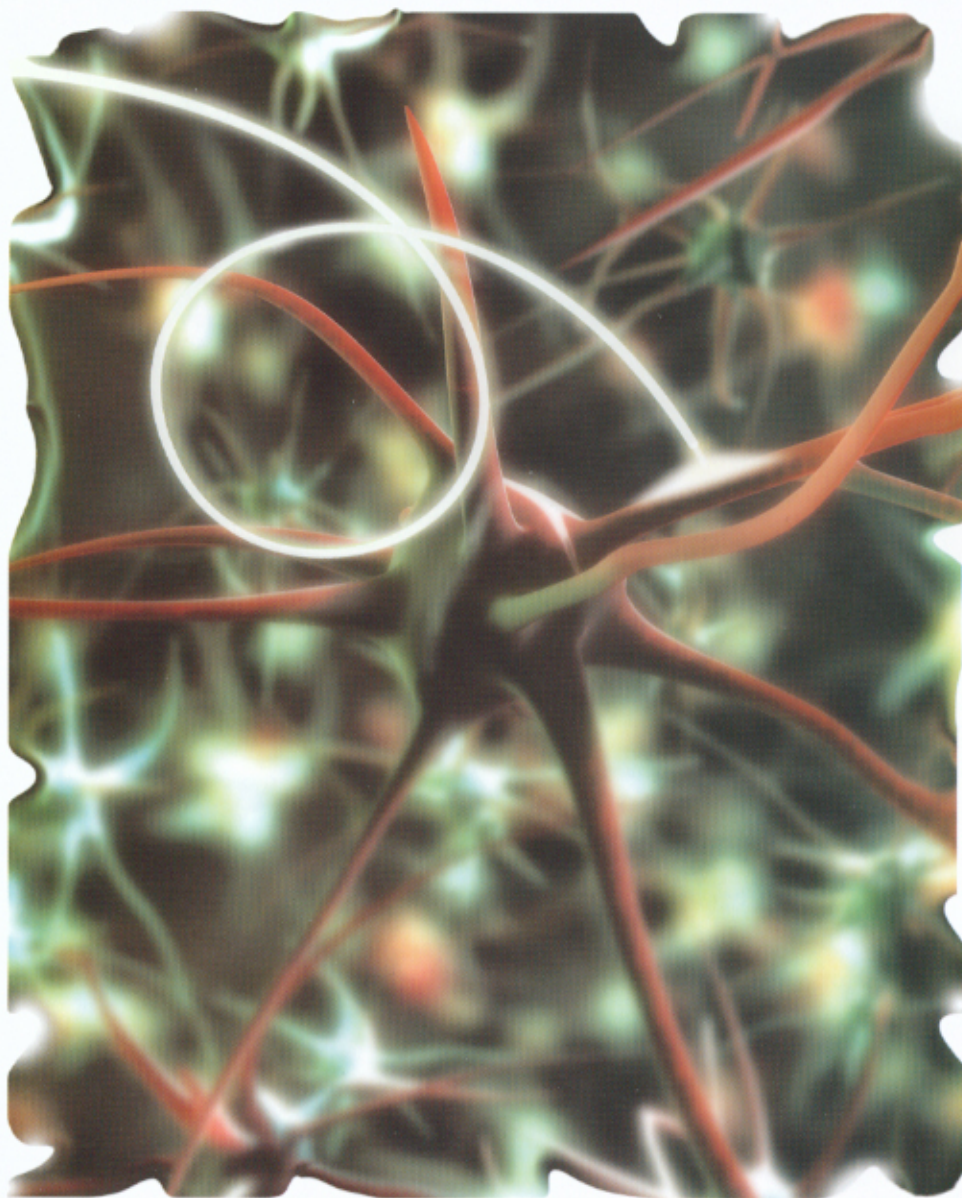
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Optofluidic microscope enables lensless imaging of microorganisms

In biological research or clinical applications, optical imaging typically is performed using large microscope systems with expensive optics. Now researchers at California Institute of Technology in Pasadena have developed a device that integrates optics and microfluidics on the same platform and offers resolution comparable to that of conventional microscopes.

The instrument, called an optofluidic microscope, does not require bulky optical elements for imaging. It consists of an illumination source, a CCD sensor and a slanted linear array of apertures etched onto an opaque metal film that is bonded to a microfluidic chip.

The scientists fabricated the aperture array and microfluidic chip in two steps: First, using reactive ion etching, they created a series of 600-nm-diameter holes spaced 5 μm apart on a layer of aluminum. They then constructed a microfluidic structure using photolithography techniques and transferred it onto a

polydimethylsiloxane elastomer.

To image with the device, they flowed a sample through the 30 \times 15- μm microfluidic channel until it passed over the aperture array, which was uniformly illuminated from above by a white LED source. Light transmitted through the apertures was detected by a CCD sensor from Princeton Instruments. Each hole scanned a line across the object as it was conveyed through the channel. The composite of the line scans from all of the apertures generated a transmission image of the object.

To evaluate the performance of their prototype, they flowed *Caenorhabditis elegans* through the channel and used an inverted microscope from Olympus to relay the transmitted light through the aperture array to the CCD; however, the inverted microscope played no direct role in imaging.

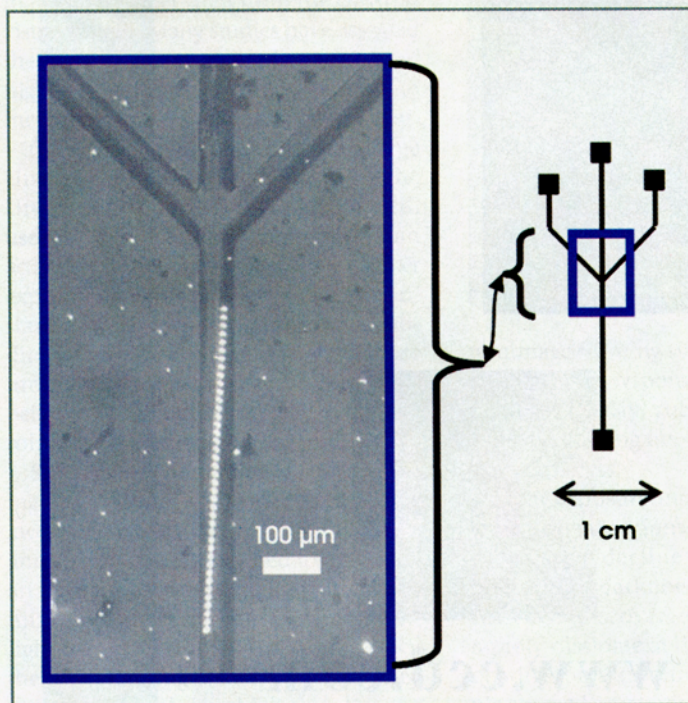
They demonstrated that the device has an imaging throughput rate of about 40 worms per minute and a resolution limit of ~490 nm. To achieve this resolution,

the sample must be as close as possible to the aperture array.

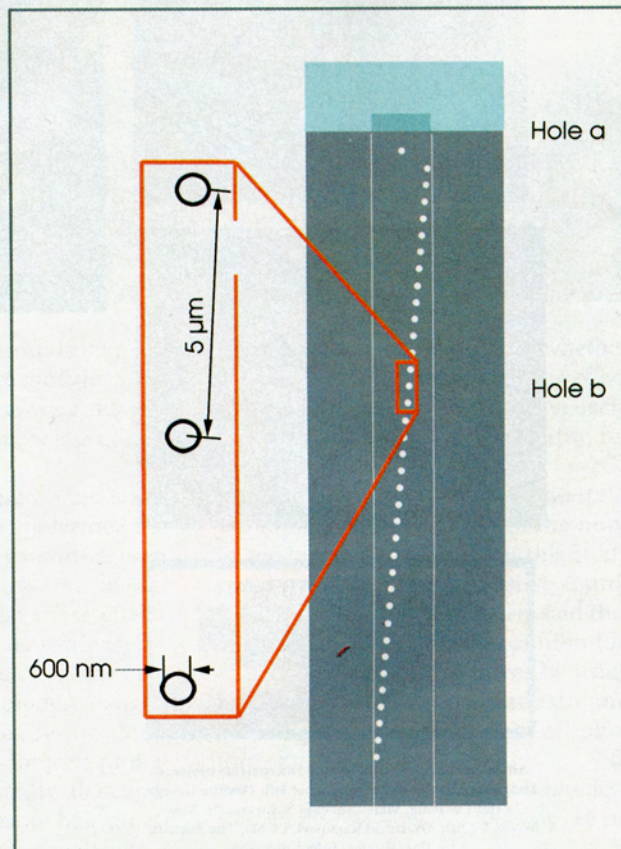
Changhuei Yang, an assistant professor at the institute and associate director of the Defense Advanced Research Projects Agency's center for optofluidic integration (which provided support for the project), said that resolution limits as low as 100 nm are possible with smaller aperture diameters. In comparison, conventional microscopes provide resolutions from about 0.2 to 1 μm .

Optofluidic devices offer several advantages over conventional microscopes for bioscience and clinical applications: They are smaller, are less expensive, can be easily reconfigured to handle a variety of samples and potentially can be used to perform high-resolution, high-throughput optical analysis.

Their simplicity and compactness facilitate fabrication of multiple units on a single microfluidic chip. Using such devices in parallel, biologists could increase imaging throughput of microorganisms



A transmission microscope image of an optofluidic microscope — a lensless imaging device that integrates optics and microfluidics — shows the pattern of apertures that are used to capture line scans of a sample as it flows through a microfluidic channel.

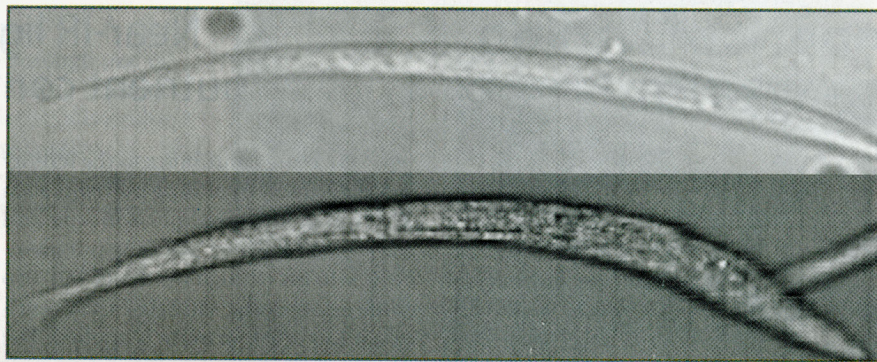


for phenotype characterization. The optofluidic microscopes also could be used to directly image individual cells in suspension, enabling more accurate type-sorting in clinical blood sample analyses.

The metal film can be deposited directly onto a CCD or CMOS sensor to create compact, portable imaging devices. Direct imaging of cells achieved with a portable version may aid definitive diagnoses of parasitic infections such as malaria, which is currently diagnosed by examining blood samples for infected cells using a 100× immersion objective on a conventional microscope.

The main limitation of the optofluidic microscope is that the sample effectively must be static during the imaging process. According to Yang, there are two ways to overcome this limitation.

“For nonmotile objects, such as blood cells, we simply need to control the flow well enough so that the cells don’t tumble or roll. Electro-osmotic-driven flow should help because it generates a flat flow front.” In addition, if the imaging time is sufficiently short, motile objects



A prototype device with 600-nm-diameter apertures captured images of *C. elegans* with comparable resolution (bottom) to images acquired using a conventional microscope with a 40× objective (top).

will not have time to change shape or orientation significantly.

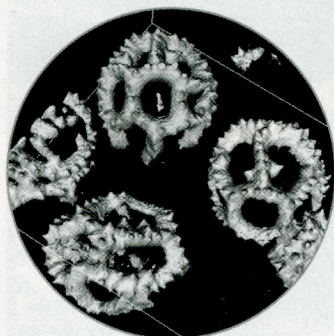
In the experiment, flow was actuated by gravitational pull — tilting the chip caused the *C. elegans* to slide down the microfluidic channel. The researchers are investigating other mechanisms to induce flow, including pressure, electro-osmosis and dielectrophoresis. They

also are working on incorporating all components on a single platform, reducing the aperture diameter to improve resolution and developing versions for fluorescence and differential interference contrast imaging. □

Gwynne D. Koch

Lab on a Chip, online edition, Aug. 4, 2006, doi: 10.1039/b604676b.

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