Gaining High Resolution with Nanoaperture Grid

Researchers at California Institute of Technology in Pasadena have demonstrated a subwavelength-resolution microscopy technique that uses a two-dimensional grid of tiny holes to increase resolution in a manner similar to that of a near-field scanning optical microscope (NSOM). The novel method could be the first step toward a high-throughput, low-cost system that is less expensive and simpler to operate than current NSOMs. The prototype of the device, dubbed an optofluidic microscope because it maintains and transports samples inside microfluidic chambers, operates with liquids, an important benefit for biological studies.

The device has several innovations over previous NSOM designs. One is that, rather than moving the samples on a typical microscope stage, it uses an optical tweezer. "Although it might prove to be hard to miniaturize the size of an optical tweezer, its superior ability in terms of accurately controlling the sample transportation should be critical for high-resolution optofluidic microscope imaging," said Xin Heng, a graduate student at Caltech at the time of the microscope's development.

Another key innovation is the use of the 2-D grid instead of the linear arrays that had been employed previously. The grid, whose apertures are placed between the illumination source and the sample rather than being used as light-collection units, allows for parallel near-field scanning microscopy.

A white LED from Lamina Lighting Inc. of Westampton, N.J., provides illumination through a grid of 100-nm holes spaced 2.5 µm apart (see figure). The point sources transmit through the holes, with the signal changing wherever the light strikes the sample. The light is collected with an Olympus 0.8-NA, 40× objective lens and imaged onto a Princeton Instruments CCD camera.

To move the sample across the grid, the researchers used an optical tweezer comprising optics and an 850-nm Ti:sapphire laser from Spectra-Physics of Mountain View, Calif. This process interrupts the light passing through the apertures. A cold mirror and a shortwave filter from Newport Corp. of Irvine, Calif., ensured that backscattered light did not oversaturate the CCD's pixels.

The microscope's ultimate resolution was determined by the size of the nanoapertures. Tests showed that the achievable limit under optimal conditions was about 110 nm. The scientists successfully imaged polystyrene beads and pollen spores measuring between 10 and 17 µm.

The microscope has some constraints. For example, vibrations in the fluid in the chamber cause jitter and blur the image. The researchers found that using a 1-mm-diameter fluidic chamber resulted in a sample jitter of less than 25 nm as measured by the standard deviation. Moreover, the sample must not change shape and must remain flat when moved across the array. Using a grid reduced the scan distance over which the object had to remain stable. A linear array, for example, would have required a scan distance of 300 µm, whereas the 2-D grid needed only 83 µm for a 9-µm-diameter cell.

Possible improvements include faster imaging speed and a smaller optical tweezer module. "The goals are to make the system cheaper, smaller and faster with better image quality," Heng noted. □

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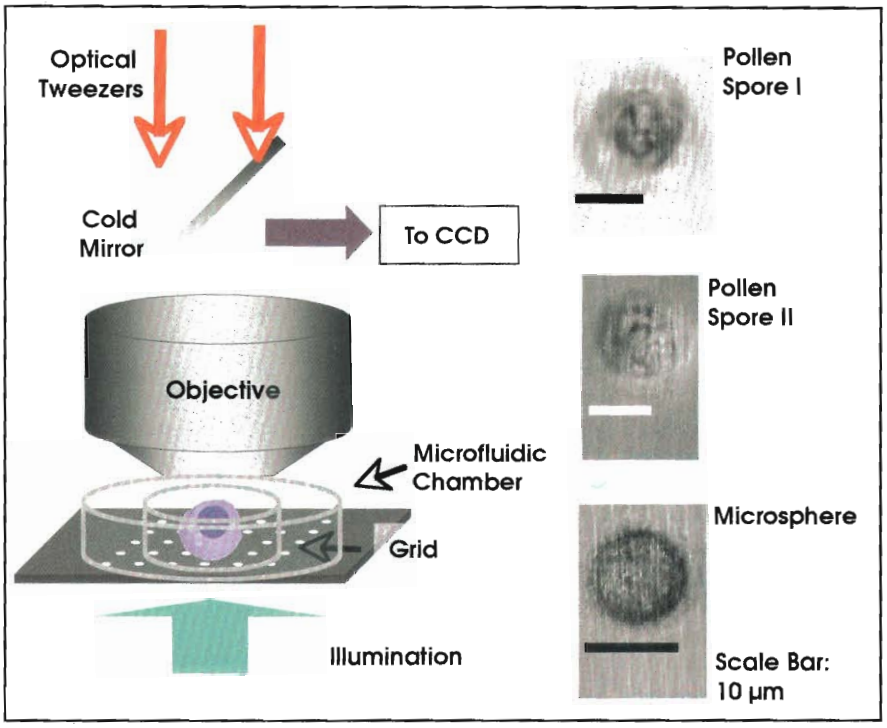

In a novel high-resolution microscope (left), a sample is placed in a microfluidic chamber and illuminated from below by light passing through a nanoaperture grid. Optical tweezers entering from above allow investigators to move the sample across the grid. The interrupted light is captured by a CCD, thus imaging the sample. Images of a pollen spore and a microsphere, each about 10 µm in diameter, are shown (right). Courtesy of Xin Heng and the Biophotonics Group at Caltech.