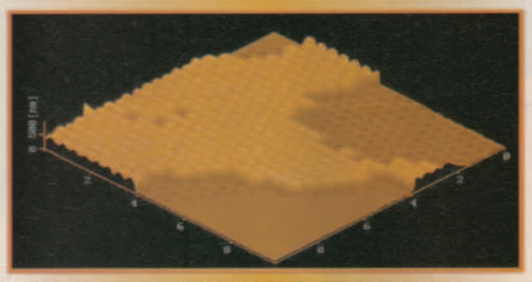
Making a good — and clear — impression

Japanese researchers have shown that, for some imaging applications, such as that of living specimens, the best approach may be to make a good impression — literally. Those are the findings of a team led by Yoshimasa Kawata, associate professor of mechanical engineering at Shizuoka University in Hamamatsu, Japan. The team has developed and demonstrated a lensless near-field optical microscope in which specimens are imaged directly onto photosensitive film, and the resulting film impression is read at the molecular level using an atomic force microscope.

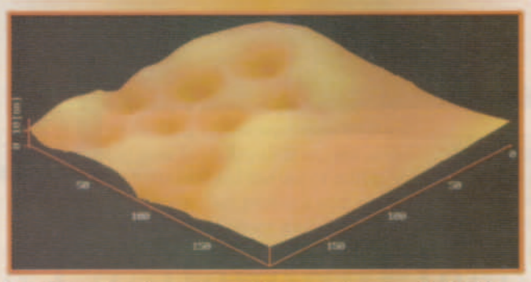
"Our system is a unique near-field optical microscope which can observe moving specimens or very fast phenomena. Other near-field microscopes require very long observation times," said Kawata. Additional advantages of the new approach, he said, include higher resolution, a much simpler optical system, and an expected high signal-to-noise ratio.

Near-field optical microscopes are the subject of research and development because they overcome the resolution limitations imposed by the diffraction limit of light. Therefore, they can capture details and phenomena that are too fine for other types of optical microscopy. At the same time, because they are optical, these microscopes can be used to measure the absorption and refractive index of a sample. These characteristics are invisible to atomic force microscopes or scanning electron microscopes, two powerful tools used to measure the surface of a sample.

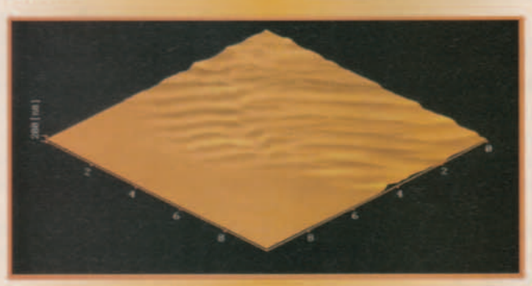
Early near-field optical microscopes circumvented the diffraction limit by using a very small aperture. This pinhole either illuminated the specimen or provided the window through which data were gathered. In any case, a scanning probe tip would have to be moved slowly over the surface of the sample to collect the complete image. Despite recent advances, these microscopes still depend on moving a probe tip over the surface of a specimen — a slow process that requires expertise in setup and operation.



Using a new near-field optical microscopy technique, researchers at Shizuoka University observed 500-nm-diameter spheres. In a 10-μm² scan area, they observed residual particles that were not removed by washing and lattice defects in the arrangement of the particles and holes.



In 50-nm-diameter spheres, the researchers could clearly observe the particles, even though they are 10 times smaller than the wavelength of the illumination. The scan area is 200 nm².



The technique enabled the researchers to observe that each cilium of a paramecium branches into two cilia.

The Japanese team decided to take a different approach. Its method begins with coating a substrate with a photosensitive film, a urethane-urea copolymer. This particular coating was chosen because it doesn't interact with living specimens, and it readily converts changes in light into changes in height.

When light shines on the film, the film undergoes a chemical reaction and a resulting change in thickness. If the light is blocked or altered for some reason (e.g., a sample sitting on top of the copolymer), the film will, in essence, transform the dark and bright spots into hills and valleys. Kawata considers that characteristic important.

"The key point is the use of a photosensitive film as the detection system," he said.

Following construction of a substrate-photosensitive film sandwich, the research team topped it off with a specimen to be imaged. Then they exposed the entire assembly to a pulse of laser light. Because the copolymer has an absorption peak at 476 nm, the researchers used an argon-ion laser at 488 nm with about 100 mW of output for the light source. Exposure times varied from 50 to 200 ms, depending on what was being imaged. To capture a sequential set of images, the team used a shutter and a series of pulses.

After the laser pulse, the researchers washed the specimen from the film and read the resulting topographical changes using an atomic force microscope. Moving over the entire film, the microscope measured the thickness variations created by the differing amounts of light at each point.

The group could have used a scanning electron microscope for this. In either case, the high resolution of the atomic force microscope or the scanning electron microscope means that the new near-field optical microscopy technique should have high resolution as well. The lack of a pinhole and lens should eliminate photon losses, improve the signal-to-noise ratio and simplify overall operation. And, because the film is photosensitive, it can detect changes in refractive index, absorption, polarization and other optical characteristics. In theory, this means that the new approach isn't limited to surface features but can detect structures inside cells.

The research group demonstrated the effectiveness of this impression technique by imaging 20-nm spheres, as well as the cilia of a paramecium and the movement of the whiplike tail of a euglena. The researchers plan to image pheochromocytoma cells and to try to observe the growing process of living neurons and propagation of neurotransmitters. They also plan to continue development of the new approach. Other photosensitive films could, for instance, prove to be better for different applications. Some of these development efforts are aimed at making the multistep process more seamless and cutting down on the number of different pieces of gear required. This will make the approach even easier to implement.

"The system is very easy to use, so we think it is very useful to nonspecialists in optics. Our system will in the future be combined with an atomic force microscope as optional equipment," Kawata said. □

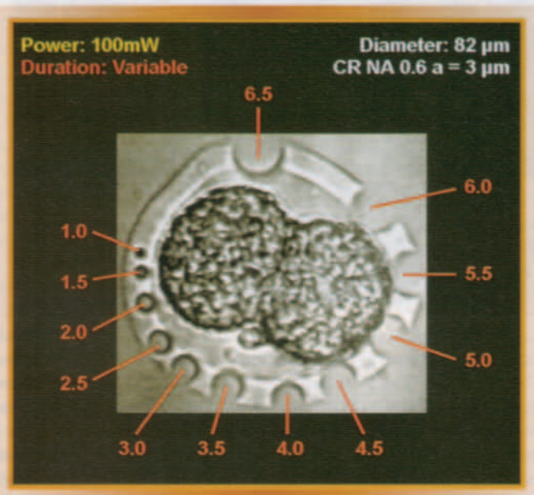
Hank Hogan

Laser-assisted technique aims to improve infertility measures

In vitro fertilization has been used to successfully treat infertility for a number of years. Even though fertilization may be successful, as evidenced by early division in the pre-embryonic stage, implantation into the uterine wall does not always occur. One reason may be that the covering of the pre-embryo, known as the zona pellucida, may be too thick or tough. Since the early 1990s, lasers have been used to cut a notch or groove in the zona to facilitate hatching and subsequent implantation of the pre-embryo.

The zona pellucida is composed of layered glycoproteins. Researchers have found that lasers in the near-IR range, 1480 nm, are most effective for cutting the zona because it strongly absorbs this wavelength. Studies have reported utilizing pulse durations of 3 to 100 ms, power of 22 to 70 mW and spot size of 2 to 8 μm to cut into the zona. Because heat absorption is the mechanism of tissue ablation by laser, the danger is that the cells of the pre-embryo immediately underlying the zona will be damaged in the process.

Diarmaid Douglas-Hamilton of Hamilton Thorne Biosciences Inc. in Beverly, Mass., and Jérôme Conia of Cell Robotics Inc. in Albuquerque, N.M., published a study in the April issue of the *Journal of Biomedical Optics* calculating the amount of heat generated by a laser beam passing through water and the zona pellucida in various configurations. They also calculated the rate of heat diffusion and the tissue temperatures at various distances from the beam, in the hope of determining a protocol of pulse duration, power and spot size that will effectively lyse the zona with the least collateral damage to adjacent pre-embryonic cells.



A series of 100-mW 1480-nm laser beams measuring 6 μm in diameter drilled the zona pellucida of the mouse embryo in this photomicrograph. Pulse duration varied from 1.0 to 6.5 ms.

For the study, Douglas-Hamilton and Conia used a Nikon TE-300 inverted microscope with an IR laser module mounted beneath the stage. The module, the ZLTS made by Hamilton Thorne Research, consisted of a diode laser, control board, adjustable collimating lens and dichroic mirror. It had a maximum power output of 200 mW at 1480 nm, and the pulse duration was adjustable from 0.5 to 25 ms. The beam traveled up through the objective and was tightly focused on the target. The beam diameter at the focal point was approximately 6 μm, and the estimated delivered energy at the focal point through the objective, container and medium was 103 mW.

In the preliminary experiment, the researchers drilled multiple channels into bovine eggs, using tangentially aimed beams with pulse durations of 25 ms and power of 50 mW. Electron micrographs showed sharply drilled channels, about 25 μm long and 12 μm in diameter, virtually cylindrical, and without evidence of beam convergence or divergence. The beams were aimed tangentially so the zone would be notched