absorption for sectioning by imaging 5-µm silica beads surface-stained with the dye. They also performed axial sectioning by imaging lamin proteins stained with the dye in the nucleus of human glioma. Both cases showed good lateral resolution with optical sections of less than 1 µm and, in the case of the cells, did so with a sample greater than 6 µm thick. The work is detailed in the Aug. 20 issue of *Angewandte Chemie*.

The researchers expect multicolor applications, a possibility because the emission spectrum of the dye can be shifted by changing the rhodamine in the compound. In polar solvents, the markers can be localized several times by introducing a lag time between frames, which will allow the thermal relaxation to the non-fluorescent state to take place between images. That could be useful during live-cell experiments, the researchers noted. ☐

Hank Hogan

Going in circles to detect single molecules

Optical sensing of single molecules performed with whispering-gallery microcavities

Quality counts. One result of that reality is a label-free single-molecule detection scheme demonstrated by researchers at California Institute of Technology in Pasadena, who used an optical sensor based on an ultrahigh-quality (Q)-factor whispering-gallery microcavity, with a Q factor of greater than 100 million. Because of the high Q factor, they could detect the effect of single molecules as a shift in the microcavity's resonant frequency, something that would not be possible with a lower Q factor. The scheme might find use in applications such as studying cell signaling.

In a whispering-gallery microcavity, light orbits inside a structure that is typically circular in shape to maximize the number of round-trips a photon makes. Because the broadband spectrum is very restricted, a single resonant mode within the microcavity can be isolated.

As the name implies, the structure is microscopic in scale. This setup means that a molecule will be optically sampled many times, leading to a more pronounced and more easily detected effect. The Q factor is a measure of how good the resonator is and how much light is lost during each pass, with a higher Q factor meaning less light lost.

Andrea M. Armani, who was a graduate student at the time of the research and who is now doing postdoctoral work at California Institute of Technology, explained that this work extends earlier investigations conducted at Rockefeller University and at Polytechnic University, both in New York, on microcavity detection of molecules (see "Silica microspheres enable sensitive DNA detection"; *Biophotonics International, October 2003, p.34*).

Those studies were performed using resonance at 1300 nm, a part of the spectrum where the absorption of water is very high. As a result, the Q factor of the microcavity plunged from more than 500 million in air to about 1 million in water, which diminished the ability to detect molecules in solution.

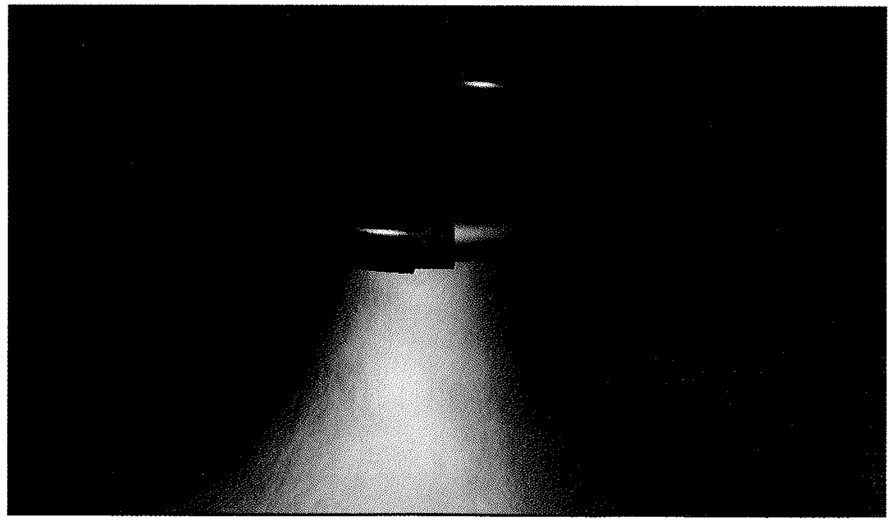
The Caltech scientists conducted experiments in the visible range, using a 680-nm light source.

To do this, they needed several enabling technologies. One was thinner optical fibers so that the source light could be injected into the microcavity. They manufactured these by heating fiber while stretching it until they achieved an average fiber waist diameter of 500 nm. The researchers also needed a tunable, continuous-wave, narrow-linewidth visible laser. In a development that Armani characterized as key to conducting the research, such lasers became available from New Focus Inc. of San Jose, Calif., shortly before studies got under way.

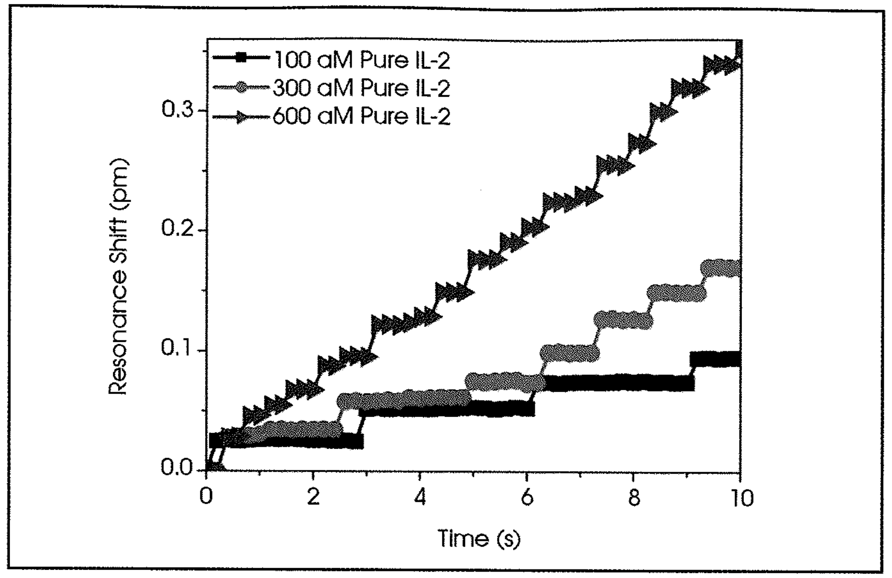
The researchers fabricated an ultrahigh-Q-factor microtoroid resonator. They patterned oxide pads on a silicon wafer, etched the silicon — using the oxide as a mask — then used a CO_2 laser to heat and reflow the microdisks. The result was ringlike silica structures sitting atop a mesa. Light of the proper wavelength would propagate around the ring with a Q factor of 100 million or more.

The researchers fabricated devices with an average major diameter of 80 µm and an average minor diameter of 4 µm. The major diameter is the width of the entire structure, whereas the minor diameter is the distance across the ring at the structure's edge.

They placed a low-loss thinned-waist fiber waveguide near a microtoroid, through which they could couple light into the resonator. After immersing everything in water, they functionalized the silica surface of the ring, using interleukin-2 for an antigen and its corresponding polyclonal antibody, attaching the antibody to the toroid surface. They did this by adhering a monolayer of a protein to the microtoroid, which redshifted the resonant frequency. They used the affinity of the antibody for the protein to affix the antibody to the resonator, which again red-shifted the resonant frequency.



This artist's rendering shows a cutaway view of a microtoroid measuring about 80 × 4 µm. Light is injected via an optical fiber (red line angling up from left to right and passing behind microtoroid). Because photons make many passes around the ring, they are impacted as single molecules bind to the outside of the microtoroid. That binding changes the structure's resonant frequency, which enables single-molecule detection. Image courtesy of Andrea M. Armani, California Institute of Technology.



In these plots of resonance shift versus time, individual molecule-binding events can be seen as a series of steps. As the concentration increases, the slope becomes greater because of an increasing number of binding events (IL = interleukin). Reprinted with permission of Science.

After rinsing the functionalized microtoroid and immersing it in a fresh solution, they flowed the antigen past the resonator and looked for frequency shifts by monitoring the power transmission spectra of the devices. They scanned the wavelength of the laser over a 0.03-nm range and recorded the resonance position using an oscilloscope.

As detailed in the Aug. 10 issue of *Science*, the concentrations used in these experiments varied from 1×10^{-19} M to as much as 1×10^{-6} M. The minimum detectable concentration was 100 aM, and the system had a response curve that covered 12 orders of magnitude. At very low concentrations and with suitable flow rates, the resonance change took place in easily seen steps. This, the researchers concluded, indicated single-molecule events.

They also performed experiments using biotin and streptavidin — two biomolecules with a high affinity for each other. Again, they saw single molecule shifts.

Armani noted that the group was somewhat surprised by these results. "Previously proposed resonant cavity detection theories assumed that the molecule was 'lossless,' or did not contribute a significant amount of optical loss to the resonant cavity system."

She added that this was known to be true at longer wavelengths where the Q factors are lower. However, at shorter wavelengths where the Q factor is higher, even small losses are amplified.

In considering their results and setup, the researchers concluded that the interaction of molecules on the surface of the toroid led to a temperature change. That change, slight though it might be, in turn led to a change in resonant frequency and to the ability to detect single molecules without labels.

Research in this area continues. As for possible uses of the technique, Armani noted that it could have a variety of applications.

"There are opportunities in studying cell signaling, by placing the sensor between two cells. Additionally, it would be interesting to investigate its application in a completely different arena — gas detection in space." ☐

Hank Hogan