channel snaked around in a helical pattern many times from the two inlets to the single outlet. The third was a vortex mixer with two 100-µm inlets, a 2-mm-diameter central blending chamber and a single outlet.

The scientists imaged fluid mixing in these structures using both light microscopy and OCT. For the light microscopy studies, they injected two fluids into the mixers and colored one with a red food dye and the other with a blue one. They acquired images with a Sony digital color video camera and used software to analyze the data and determine the degree of mixing.

For the OCT research, the investigators used two different concentrations of skim milk diluted in water. The optical source was a mode-locked Ti:sapphire laser at 800 nm. They broadened the laser's spectral output to about 100 nm using a fiber and split this into two beams. One was used as a reference, and the other was sent into the sample. They combined the beams in a detector, with the interference between the two providing information about the depth and magnitude of the backscattered signal. The researchers analyzed this data to obtain the degree of mixing. By extending the technique, they could use Doppler methods to get velocity profiles as well.

Comparing the results of light microscopy with OCT, they found that with the Y channel, both methods showed complete diffusion-based mixing. Velocity measurements put the fastest flow at the center of the channel and the slowest flow near the walls. That was consistent with standard models of laminar flow in a narrow channel.

The two techniques used with the serpentine and vortex mixers produced differing results. Light microscopy indicated complete mixing with both. OCT measurements, on the other hand, revealed bands of two distinct fluids in the output. These bands, when superimposed, could produce an image of complete mixing. The results, according to the researchers, show that complex shapes improve mixing efficiency but not as much as had been thought previously.

The OCT results are persuasive, and the technique doesn't suffer from the same problems as light microscopy. However, Boppart noted that the validity of the technique still needs a final check. "The next step would be to compare our results with computational models," he said. "We believe this experimentally obtained data will help validate current models as well as help answer some questions regarding fluid mixing and performance."

Other plans by the researchers call for scaling up the OCT system for high-speed imaging and Doppler measurements in the coming months. These improvements will be useful, according to Boppart, in looking for transients and pulses in the fluid flow. □

Hank Hogan

Taking cells for a spin

Microvortices and optical trapping could be used to study the effect of rotation on cells

At the University of Washington in Seattle, scientists have set a speed record. They found that single living cells twirled as much as 200 times a second when optically trapped in microvortices they created in microfluidic structures. According to researcher Daniel Chiu, that rotation rate is a record for single mammalian cells.

This miniature merry-go-round isn't for fun and games, however. "This ability, we believe, may allow us to study the effect of rotation on the behavior of single cells, a regime that has not been studied much in the past," Chiu said. Studies have shown that cells that adhere, such as endothelial cells, change behavior and function as a result of hydrodynamic forces. This technique may be useful for studying the hydrodynamic forces on other cells, such as those flowing through arteries.

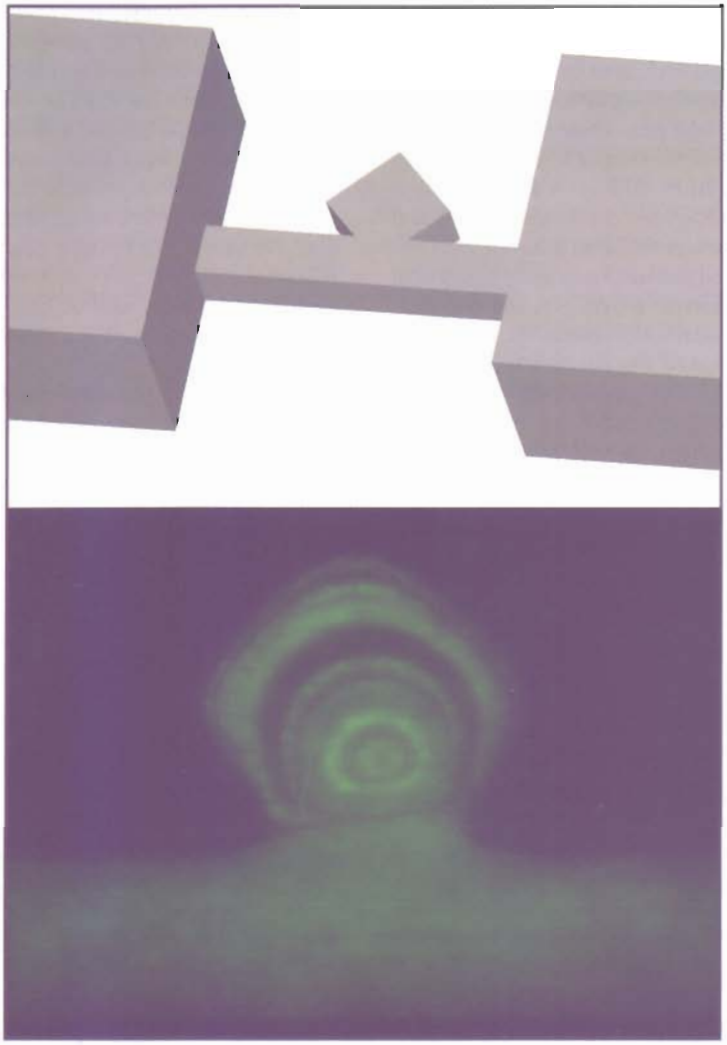
Creating the microvortices required structures with stable centers of fluid rotation that, ideally, are unaffected by changing fluid flow rates. After analyzing various structures, the researchers concluded that the best arrangement

consisted of a chamber in the shape of a diamond with three smaller diamonds placed at its corners that was attached to a linear channel. They used photolithographic techniques to construct such a microfluidic shape.

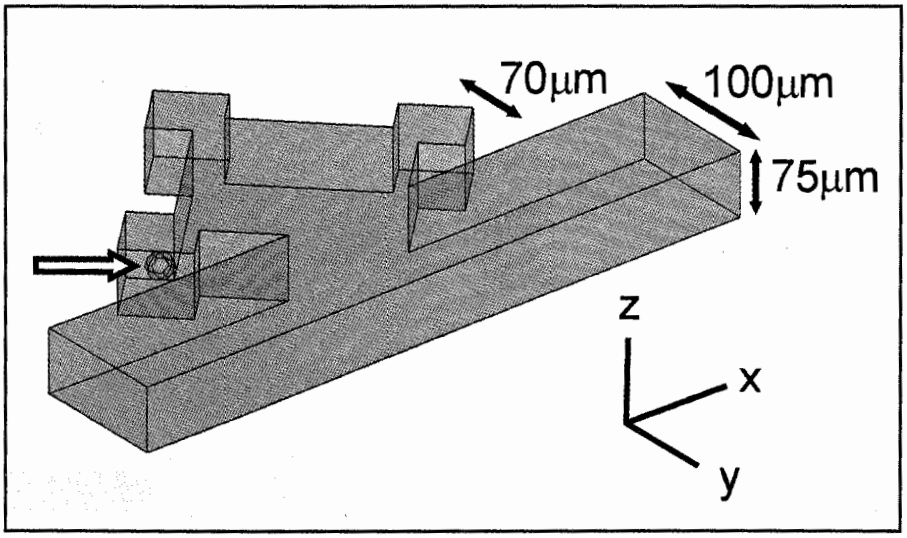
The overall structure had a height of 75 µm, the rectangular duct was 100 µm wide, and the three smaller diamonds were 70 µm wide. Fluid flowing through the channel formed three microvortices, one at the center of each of the small diamonds. This arrangement produced a slower rotation and fluid flow than other arrangements that were tried, but the researchers felt that the increase in the vortices' stability was worth the decrease in velocity.

To investigate the rotation of microparticles and single cells, they injected either microparticles or cells into a fluid flowing through the microstructure. They then optically trapped and positioned the moving particles within a microvortex using the 1064-nm line of a Spectra-Physics Nd:YAG laser. An argon-ion laser from the same company provided a 488-nm line for single-molecule confocal detection.

Measuring particle rotations required an optically visible mark. The investigators marked the microparticles using a



University of Washington researchers constructed diamond-shaped chamber designs to generate microvortices (top panel). The resulting microvortex (bottom panel as visualized by tracing of the flow lines by fluorescent beads) can be used to spin cells and nanoparticles.



The researchers found that a chamber shaped like a diamond with three smaller diamonds produced the most stable vortices. Reprinted with permission of the American Chemical Society.

337-nm pulsed nitrogen laser from Laser Science, part of Spectra-Physics. The focused beam from this laser created 1- to 3-μm spots on a series of 10- to 25-μm-diameter polystyrene beads trapped within a microvortex. As a marked bead rotated, the researchers captured the backscattered photons using an avalanche photodiode detector. Because the ablated spot moved in and out of the observation volume, the result was a series of peaks and valleys in the photon count.

They found that the microparticles rotated at a rate between 0.15 and 100 Hz. The experiment showed that the rate was a function of the location of the particle in the vortex, which was expected. However, they also discovered that the trap tended to slow the rotation. Further optimization of the trap and better designs, Chiu and the other researchers predict, will enable the technique to hold particles at the center of the vortices during high fluid flow.

In the case of living cells, ablation wasn't an option. Therefore, the researchers selectively stained portions of mouse B-lymphocyte cells with a fluorescent membrane dye from Molecular Probes Inc. of Eugene, Ore. Because the dye penetrates an exposed surface and then works its way into the cell, they could stain only parts of cells resting on a culture flask by removing the dye after less than a minute. This procedure selectively stained more than 90 percent of the cells.

After adjusting laser power to minimize photobleaching, they monitored the rotation of single living cells. They found that, because the cells are larger than the beads, they rotated more slowly.

The researchers plan to improve the optical trap and work on making microfluidic structures that create more efficient microvortices. The microvortex technique could be used to study the effect of rotation on single cells without subjecting them to the distorting forces of centrifuges or other equipment. Such information could be important in understanding how blood cells react to the rotational and shear stress they experience in the bloodstream. The scientists have already noted visible changes in cell content during rotation.

The technique also could be used to study microfluidic flow. Placing microparticles in different areas within a microfluidic structure and mapping the resulting rotation can provide a validation of computational models of flow.

Chiu is quick to note, however, that there could be applications not yet foreseen. "Rather than starting with a hypothesis and working towards it, we start with a very interesting and neat phenomenon — microvortices — and capability — rotation — and see what will come out of them." □

Hank Hogan