MEETING NEWS

Elizabeth Zubritsky reports from the **Human Proteome Organisation's Third Annual World Congress** *—Beijing, China.*

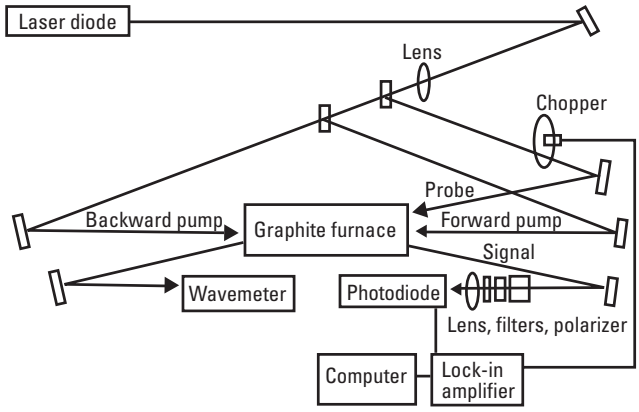
A new wave for wave mixing—proteomics

For the typical proteomics experiment, the list of preferred detection methods probably doesn't include a relatively obscure technique primarily used for the spectroscopic analysis of gas-phase samples. But William Tong of San Diego State University wants to change that. He is applying wave mixing, a label-free method embraced by a handful of analytical chemists, to study biological samples.

Wave mixing began to get noticed in the early 1980s, and the 1983 book *Optical Phase Conjugation,* edited by Robert Fisher, captured much of the important early work. The technique has been used most often with flame atomizers and discharge plasmas for detection of trace isotopes. "Most people didn't think of it at that time for liquid-phase or solid-phase bioapplications," says Tong, but he is convinced that wave mixing is just as good as, if not better than, fluorescence for biological samples.

"Actually, [wave mixing] is easier than fluorescence," Tong says. Those who have seen the traditional setup, known as degenerate four-wave mixing, might question his definition of "easier". Two laser beams are aimed so that they crisscross as they pass through the sample. Where the beams overlap, they interfere with each other and produce gratings—essentially, arrays of tiny perturbations in the refractive index. A third input beam hits these gratings, and part of it scatters to form the output beams. The key to the experiment is the careful alignment of the beams and optics.

However, Tong has a simpler setup for the biological samples. Technically, it's still four-wave mixing, he says, but in this case, a single laser beam is split into two parts that are sent as parallel beams through a lens. The lens focuses and mixes the beams; this creates the grating. Instead of a third input beam, subsequent photons from the original two beams are scattered off the grating. Tong calls it forward-scattering wave mixing because the output beam exits in more or less the same direction as the input beams enter. Because there are fewer beams and the direction of the output beam's propagation can be predicted exactly, alignment of the system is easier.



A typical gas-phase four-wave mixing setup with three input beams (the probe, forward-pump, and backward-pump beams) and one output beam (the signal).

The simpler setup lacks one property that the older configuration is known for: There is no cancellation of Doppler broadening, which is necessary for high resolution in the gas-phase studies. But Tong says the bands are much broader with liquid- or solid-phase samples to begin with, so Doppler cancellation isn't needed.

Because wave mixing uses a collimated laser-like beam, the beam remains compact as it propagates. That makes it easy to collect the signal with little loss, yielding a high S/N. A fluorescence signal, on the other hand, radiates in all directions, so only a fraction of the fluorescence signal reaches the collection lens, he explains.

In wave mixing, it's also possible to precisely define the probe volume—the region where the two beams overlap to form the grating. Tong uses nano- to picoliter volumes, which he can move around within the sample with good spatial resolution. This characteristic makes the approach suitable for reading microarrays or microchips, because the lasers can be positioned within the channels. Tong thinks he will eventually achieve intracellular resolution, "not only two-dimensional but also three-dimensional, because you can probe different depths of the sample."

Tong says that he routinely achieves attomole or better detection limits with forward-scattering wave mixing on liquid samples. He cites a paper published last year (*Anal. Chem.* **2004**, *76*, 1788–1792), in which he reported a dectection limit of 8 zmol for rubidium with the 4-wave, gas-phase configuration. He claims that he has achieved those levels for other samples. "The only requirement is that the sample absorbs light," he says. For biological samples that don't absorb light in their native forms, fluorophores or chromophores can be added.

Tong envisions many applications for wave mixing, including environmental and biomedical studies—for example, DNA curvature and enzyme kinetics. He also sees it as an alternative to high-resolution mass spectrometry for detecting specific isotopes that come from, say, pollutants or explosives. Compared with isotope-capable inductively coupled MS, Tong says, wave mixing is much less expensive and offers significantly better portability. And unlike MS peaks, "the isotope information we get is unambiguous and more information-rich because it's based on wavelength and hyperfine structure." But for now, he's just trying to encourage researchers who aren't spectroscopists, especially bioanalytical scientists, to give this rather unfamiliar method a try.