PSA as low as 1 ng/ml. Thus the arrays could form the basis of real-time, label-free, multiplexed immunoassays with which to perform high-sensitivity detection of diagnostic profiles for cancer.

The researchers pointed to several advantages with respect to other methods available for detection of molecular bindings. Compared with fluorescence methods, for example, the label-free detection offered by the cantilever platform is "a huge advantage," Yue said. He noted that, if they were to commercialize the technique, the chip would be more expensive than the simple glass slide used in fluorescence detection. "However, keep in mind that the labeling process in fluorescence detection demands more labor and reagents."

Compared with other cantilever platforms based on optical detection, he continued, the 2-D microarray system is easily scaled up, and the readout system is very simple; previous studies offered only one-dimensional arrays and required a more complicated optics hardware setup. "There are two-dimensional piezoresistive cantilever arrays. Even though they don't require optical setup, the chip fabrication is more complicated, so the chips may be more expensive."

The researchers plan to apply the technique to the study of enzymatic activity as well as to small-molecule detection in liquids and gaseous environments. □

Gary Boas

Flow cytometry technique detects translocating proteins

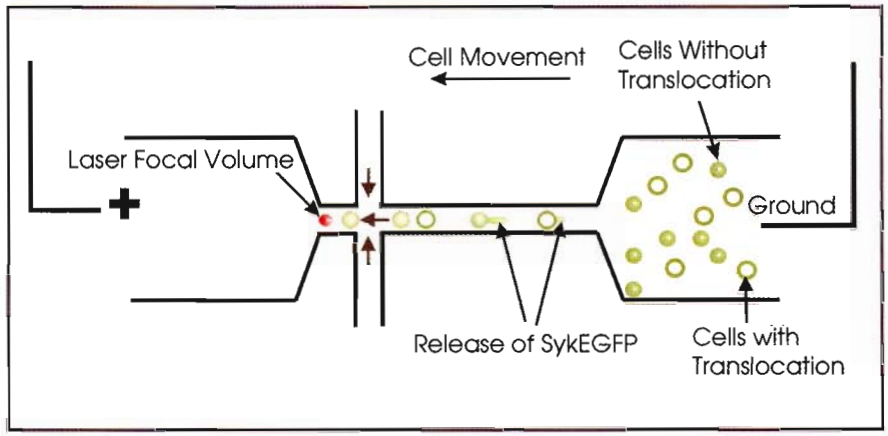
Detecting the translocation of proteins from one subcellular compartment to another is of interest to many researchers because translocation can reflect significant events in signal transduction in living cells. The protein-tyrosine kinase Syk offers an example. Syk is crucial to the survival, proliferation and differentiation of B lymphocytes, and the protein's translocation is part of its role in signal transduction for each process.

Conventional techniques used for such detection are limited, though. Investigators have used subcellular fractionation/Western blotting or have imaged low numbers of cells; however, these techniques either do not offer the high throughput recommended for studying entire cell populations, or they provide only averaged information from the populations.

In a paper published in the Feb. 15 issue of *Analytical Chemistry*, researchers with Purdue University in West Lafayette, Ind., reported a technique they refer to as electroporative flow cytometry, which they used to address these shortcomings. With information acquired from single cells, they were able to distinguish populations in which translocation had occurred from those in which it had not — thus achieving high-throughput detection without resorting to averaged information.

Electroporation is the result of a cell encountering an electrical field with a certain intensity: The electrical field induces the opening of pores in the cellular membrane, leading to the release of intracellular materials into the surrounding solution.

Using a homemade microfluidic device mounted on an Olympus inverted fluorescence microscope, the scientists showed



Researchers have reported a technique they refer to as electroporative flow cytometry. The method enables high-throughput detection of protein translocation because the amount of material remaining in a cell after electroporation — in this case, the material is the protein Syk, tagged with enhanced GFP — correlates with whether or not translocation has occurred. Image courtesy of Chang Lu, Purdue University.

that the amount of enhanced GFP-tagged Syk remaining in the cells after electroporation correlated with whether or not translocation of the protein had occurred. Furthermore, they demonstrated that by detecting translocation based on the release of intracellular kinase in individual cells, they could uncover characteristics of the entire cell population.

The researchers noted that the technique could contribute to kinase-related drug discovery as well as to tumor diagnosis and staging, since the translocations often are involved in oncogenesis and other similar disease processes. □

Gary Boas

Deeper into the plant kingdom

Maldi technique contributes to plant metabolomics

Researchers in a number of areas, from agriculture to environmental processes to pharmacology, want to know more about the structure, function and biosynthetic pathways of metabolites in plants. Because of the number and diversity of metabolites — there are an estimated 200,000 in the plant kingdom — they have had to develop a range of methods to achieve the selectivity and sensitivity needed for analysis in complex mixtures. Methods have included

those based on mass spectrometry, on nuclear magnetic resonance and on vibrational spectroscopy.

Many researchers have come to rely on mass spectrometry in particular, because it offers inherently high sensitivity and specificity. However, many of the techniques currently used involve sample preparation steps that can inhibit the study of live biological samples. Investigators with George Washington University in Washington therefore have reported an atmospheric pressure infrared Maldi imaging mass spectrometry technique for plant metabolomics. As described in the Jan. 15 issue of *Analytical Chemistry*, the technique overcomes the various barriers to probing live biological samples that they might have faced with other similar methods.

Infrared Maldi

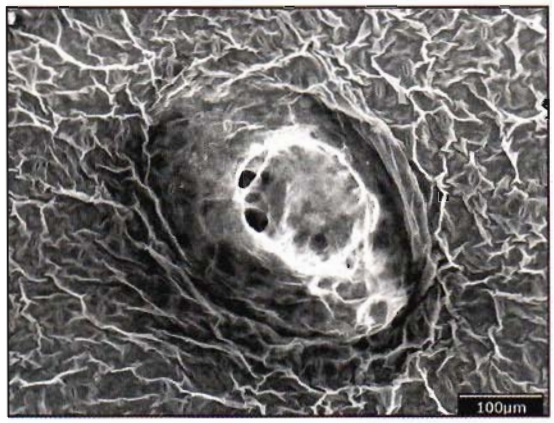
The researchers achieved this through the combination of infrared and atmospheric pressure Maldi. They noted that infrared Maldi has not caught on generally because it is not as analytically robust as conventional UV Maldi and because of the relatively high cost of mid-IR sources. However, it has advantages for particular applications. First, the technique has more potential matrixes because many molecules offer strong absorption in the mid-infrared. Also, investigators can couple it directly with some liquid-phase separation techniques.

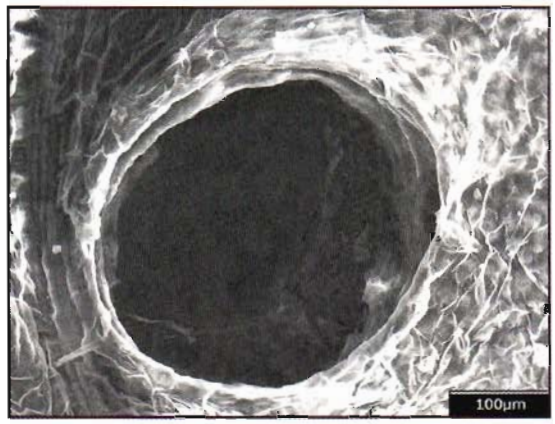
Similarly, although keeping the sample at atmospheric pressure can result in slightly lower sensitivity with respect to transferring it to the vacuum system of the mass spectrometer, it can be favorable in some cases. Because of the reduced sample handling involved with the technique and the applicability of a broader range of matrixes, the researchers wrote, atmospheric pressure Maldi could contribute to in vivo studies, among others.

Akos Vertes, the principal investigator of the study, explained that research groups have experimented with infrared excitation and atmospheric pressure sources since the early 1990s and since early in this decade, respectively. "A few years ago, people started putting two and two together and combining mid-IR and atmospheric pressure techniques." However, the researchers probed dried samples in these studies; they did not look at biological tissues directly. One of the two new components of the *Analytical Chemistry* paper, Vertes said, was the application of atmospheric pressure infrared Maldi mass spectrometry for investigations of biological tissues.

The other was refinement of the technique for imaging. Early last year, the researchers described a study in which they achieved atmospheric pressure infrared Maldi imaging using native water in plant tissue as a matrix for positive ion production with infrared laser irradiation: Thus, they showed they could map the spatial distributions of surface peptides and various small molecules in the tissue without resorting to any external matrixes. In the current study, they reported experiments with a wide range of plant tissue types in both positive and negative ion modes as well as imaging of fluid transport resulting from plant transpiration.

They used a homebuilt atmospheric





Researchers have reported an atmospheric pressure infrared Maldi imaging mass spectrometry technique for plant metabolomics that offers a variety of advantages over others available for the same purpose. It allows quick identification and imaging of metabolites in live samples, for example. Also, it enables ablation of subsurface layers, facilitating depth profiling and possibly 3-D imaging of samples. Shown here are scanning electron microscopy images of infrared laser ablation of a cilantro leaf, after one laser shot (top) and five laser shots (bottom).

pressure Maldi ion source with a 30-mm inlet capillary mounted on a Q-TOF mass spectrometer made by Waters Co. of Milford, Mass. Excitation was provided by a 10-Hz-repetition-rate Nd:YAG laser, the output of which was converted to 4-ns mid-infrared (2940-nm) pulses by an optical parametric oscillator. Beam steering was achieved using gold mirrors and beam focusing with a 50-mm-focal-length planoconvex lens. To achieve maximal ion signal intensity while avoiding an electrical breakdown, the researchers maintained a distance of approximately 2 mm between the mass spectrometer inlet orifice and the sample. They collected positive and negative ion spectra for all samples using the atmospheric pressure interface.

Using this setup, they identified more than 50 small metabolites and various lipids, including, for instance, 70 percent of the intermediates in the citric acid cycle. Thus, they demonstrated the potential of the technique for quick identification of a wide range of metabolites. The technique recommends itself especially for analysis of living organisms because it enables analysis at atmospheric pressure and does not involve extensive sample preparation.

The researchers noted, however, that there still is room for improvement. Demonstration of reliable quantitation of metabolites in tissue samples would advance the technique considerably. Investigators have reported absolute quantitation of a range of metabolites using gas chromatography mass spectrometry. Comparative studies with atmospheric pressure infrared mass spectrometry could help to establish these numbers for the latter technique.

In addition, the technique would benefit from improvements in instrumentation. Better focusing would result in improved spatial resolution, for example. At the same time, higher repetition rates would facilitate more reasonable collection times. The investigators would like to move from the 10 to 20 Hz of the current setup to the kilohertz scale. No such lasers are available, so they are working with a company to develop a 100-Hz laser.

Finally, they hope to explore further the technique's potential for depth profiling. "If you expose the same spot on the sample to multiple laser shots, you can actually dig into the sample and ablate material from subsurface layers, resulting in depth profiling," Vertes explained. To exploit this possibility, they first will need to establish the removal rates of tissues through laser ablation. The payoffs could be considerable, however. "If you combine depth profiling with lateral imaging, you get 3-D imaging," Vertes said, opening up a range of additional applications. □

Gary Boas