RESEARCH PROFILES

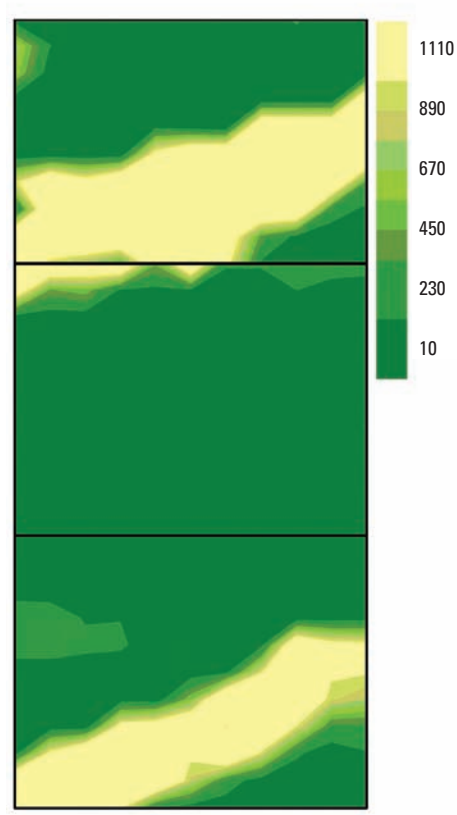
In vivo molecular imaging by LAESI MS

To perform MS analyses on living organisms, researchers have developed atmospheric-pressure ionization techniques such as desorption electrospray ionization (DESI) and electrospray-assisted laser desorption/ionization (ELDI) that don't require the addition of a matrix. Several groups quickly extended this concept to a whole range of organisms and to additional applications, such as imaging. In their recent *AC* paper (**2008**, *80*, 4575–4582), Akos Vertes and his colleagues at George Washington University performed in vivo molecular imaging and depth profiling on the leaves of plants with their own matrixless technique, laser ablation ESI (LAESI).

LAESI combines atmospheric pressure (AP) IR/MALDI with electrospray postionization. First, a 2.94 µm laser ablates the water-rich sample in a resonant manner. Next, the ejected neutral species are intercepted by an electrospray plume and form multiply charged ions, which are sent into the TOF mass analyzer. The technique is quantitative over 4 orders of magnitude, does not require an internal standard, and has femtomole detection limits for small molecules.

LAESI has some distinct advantages over AP IR/MALDI. "When you use LAESI, because of the electrospray postionization, large ions become available again," explains Vertes. "We demonstrated this [in a previous paper (*AC* **2007**, *79*, 8098–8106)]." LAESI can detect molecules as large as 66 kDa, whereas AP IR/MALDI MS cannot detect ions from species >3 kDa.

In their most recent work, the researchers imaged the spatial distribution of metabolites in a zebra plant (*Aphelandra squarrosa*) leaf with LAESI. This plant is chimeric, which means that the yellow areas on its variegated leaves contain different DNA than the green areas do. The team hypothesized that the differently colored areas of the leaf might have different metabolic pathways.



Molecular-imaging map showing the differential distribution of methoxykaempferol glucoronide (*m/z* 493) in the zebra plant leaf with peak areas depicted by color.

After analyzing spots 400 µm apart over a 4 × 12 mm grid, the group detected >40 primary and secondary metabolites. Then, they mapped the peak areas for the ions detected at *m/z* 493, 663, and 813 by selected ion monitoring. The smallest ion was present only in the yellow areas, the middle one was uniformly distributed throughout the green and yellow areas, and the largest one was more abundant in the green areas. One mystery the group is currently trying to solve is why metabolites and lipids were detected in the plant sample, but nucleotides, peptides, and proteins were not.

The researchers also performed depth profiling on a leaf from the peace lily plant, *Spathiphyllum lynise*, by firing multiple laser pulses in the same spot, progressively removing layers of plant tissue. To test the tissue removal rate, the researchers marked the underside of the leaf with ink; the red dye in the ink could be detected after three or four laser pulses, yielding an average tissue removal rate of ~50–60 µm/pulse. The group achieved an average depth resolution of ~50 µm and a lateral resolution of ~350 µm; the lateral resolution in LAESI is fundamentally limited by the focal spot size of the laser.

AP ionization techniques cannot be combined with separation methods such as HPLC and electrophoresis because the ions are formed directly from the sample. Consequently, there is no simple way to preseparate neutral species prior to MS analysis. Vertes and colleagues say that LAESI and other AP techniques could possibly be combined with ion mobility spectrometry, which would preseparate ions but not neutral molecules.

Some potential applications for LAESI include single-cell and surface analysis. "With this technique, we can actually analyze one cell at a time, especially with further improvement in focusing and sensitivity," says Vertes. Already, the group has analyzed single plant cells with LAESI and is currently studying plant–herbivore interactions with collaborators at the Max Planck Institute for Chemical Ecology (Germany). In addition, the team has successfully analyzed samples with low water content by first misting them with water.

Because LAESI can directly analyze surfaces, it might be incorporated into a portable instrument. LAESI could one day be used to distinguish cancerous from healthy cells during surgery, Vertes says. "I really want to diversify now in the applications and answer real biological and medical questions that couldn't be answered with other techniques, because that's the ultimate test." ■

—Christine Piggee