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Protein Analysis Moves into the Fast Lane

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Maldi-Tof technology improves biomarker research.

Protein biomarkers are very early indicators of more than 200 cancer types, but using them for clinical diagnosis and for developing therapies will require considerably more study.

New clinical proteomics technologies are aiding this work by providing faster analysis and simplified visualization of results as researchers look for, identify and validate important proteins. Cancer research centers in the US and Europe already are benefiting from them.

A key technological basis for these studies is matrix-assisted laser desorption/ionization time-of-flight (Maldi-Tof) mass spectroscopy.

Maldi is a soft-ionization technique used in mass spectrometry for desorption/ionization of large molecules such as peptides and proteins. These nonvolatile molecules are embedded in matrix crystals, typically aromatic acids such as benzoic acid derivatives. The matrix isolates individual analyte molecules and absorbs energy from laser pulses. The laser's large energy impact evaporates the matrix, giving the proteins a ride into the gas phase, where the matrix's acidic functionality pronates (ionizes) them.

Time-of-flight analysis basically represents a molecular balance: An electric field accelerates the ions toward a detector, which measures how long it takes them to get there. This time of flight is a simple function of molecular weight-to-charge ratio, thus allowing calculation of mass (Figure 1). The resulting mass spectrum might show single peaks (representing unique molecular species) or peak patterns (representing an extract of the composition of peptides of each sample).

Maldi imaging

Tissue samples can even generate locus-specific spectra: After adding the matrix to a tissue slice on the sample carrier, the researcher can obtain spectra from many spots on the tissue surface.

For example, capturing spectra in a scanning mode across a tissue sample provides the intensity distribution of any given mass spectrometric signal representing a peptide, protein, metabolite or other molecule type. These data produce a color image overlaid on a microscopy image of the sample in a technique called Maldi imaging.

This technique can be used to monitor drug efficacy at the molecular level. After a drug is applied to a sample of, for example, diseased tissue, one can examine the changes at the edges of the sample or track the distribution of the drug candidate. The technique also can reveal how compounds act in different tissue types, without dyes and before the expensive drug development phase.

In the context of clinical proteomics, Maldi imaging enables the study of up- and down-regulation of a potential biomarker in tissue during disease progression or after drug application.

Maldi imaging requires massive software support for data analysis. FlexImaging software from Bruker Daltonics Inc. of Billerica, Mass., can visualize distributions of biomolecules in thin tissue sections as a function of sample morphology or health state. This technology is particularly valuable for biomarker discovery and has a great potential for diagnosis, monitoring of cancer progression and evaluation of surgical removal efficiency by visualizing the spatial distribution of cancer markers within the tissue.

To use the technology, tissue sections are positioned on dedicated glass slides suitable for Maldi imaging, and a matrix

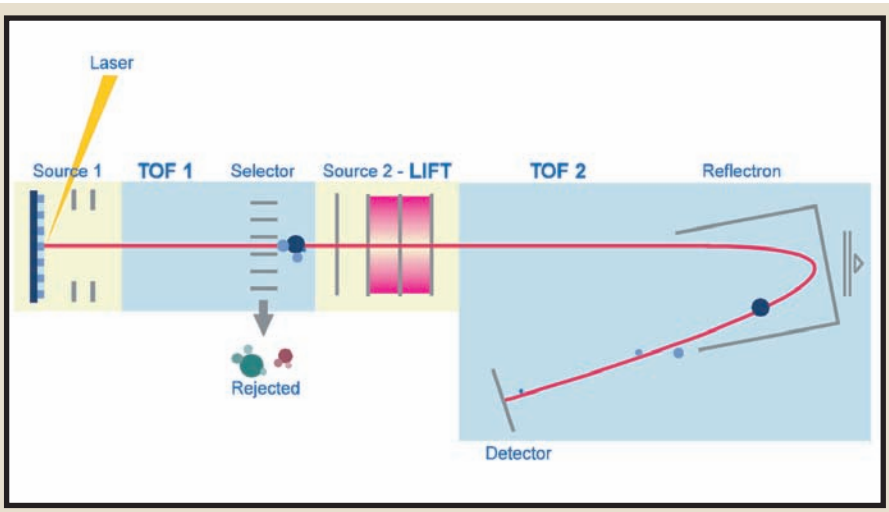

Figure 1. After ion formation in the first source, ions are separated in the mass analyzer (TOF 1, TOF 2) and detected with a linear detector or after passage through a reflector. Using a second time-of-flight measurement (LIFT), full-fragment ion spectra can be acquired in a single scan.

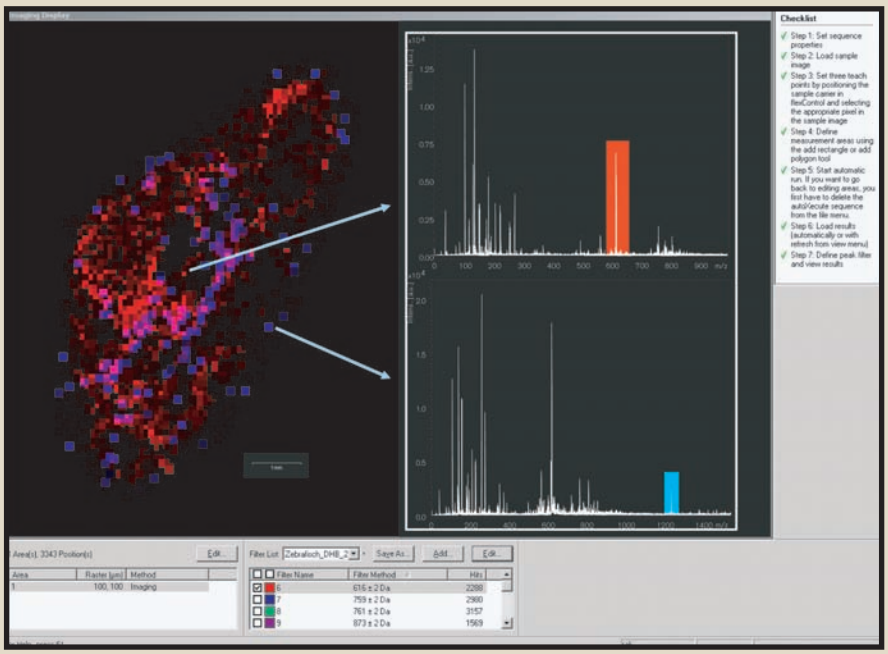

Figure 2. Maldi imaging was used on a sagittal section of zebra fish. The 616- (red) and 1231-dalton (blue) masses were selected and represented in the following mass spectra. Color intensities correlate to signal intensities in the mass spectra.

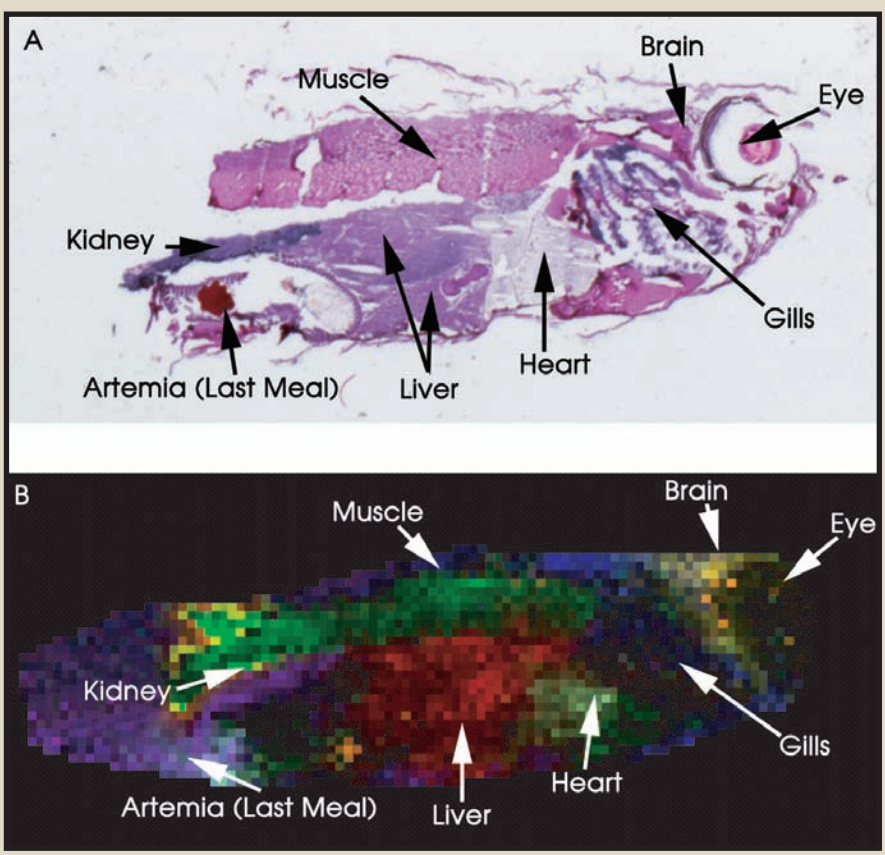

Figure 3. A zebra fish sagittal section was analyzed. A histological reference section is used to assign the organs (A), while Maldi imaging illustrates an adjacent section (B)

is applied to the surface, either by spraying or by spotting. The slides are mounted to an industry-standard microtiter plate slide adapter and inserted into mass spectrometers.

Reduces analysis time

The company's Maldi imaging mass spectrometers use trademarked smartbeam laser technology, which combines the high (200 Hz) acquisition speed of all-solid-state lasers such as Nd:YAG with the high Maldi-imaging spectral quality and sensitivity provided by the much slower (20 to 50 Hz) nitrogen lasers.

In many imaging studies, the laser scans across a significant tissue area to generate high-resolution Maldi images. Typically, each mass spectrum is averaged from 50 to 500 laser shots. A data array in Maldi imaging comprises 1000 to 30,000 spots, depending on image resolution.

At approximately 2.5 s per time cycle at a laser frequency of 20 Hz,[1] increasing the frequency to 200 Hz greatly reduces analysis time. Switching from nitrogen to smartbeam either reduces the analysis time by 90 percent or triples the spatial resolution at a given analysis time.

In addition, the researcher can adapt the images' spatial resolution to the diagnostic requirements (resolution vs. analysis speed), as the laser spot size can also be adjusted between 10 and 200 µm under software control.

The laser system allows a variety of matrices to be used in the Maldi process, providing opportunities to adapt Maldi-Tof to the respective sample. For example, researchers involved with intact protein analysis in clinical proteomics can benefit from using matrices such as sinapinic acid, resulting in significantly better spectra quality and signal intensity because samples can be considerably more durable.

Flexible visualization

The Maldi-specific software provides visualization of biomolecules with a color-coding system that highlights locations of biomarker mass signals. This is important because correlating the mass signal intensity distribution and the morphology/health state of the sample is the major factor that suggests conclusions with regard to diagnosis. It is a first step on the way to identifying biomarkers that may be of prognostic use on the tissue level (e.g., biopsies from colonoscopy) before morphological changes become

obvious to a histologist. Such molecules also may become relevant for serum-based diagnostics.

The software can map the abundance distributions of various analyte mass values onto the tissue sample area. For example, we examined a sagittal section of zebra fish (*Danio rerio*) at a raster width of 100 µm in a polygonal area across the whole tissue slice. To generate an image, we included 3343 total spots containing masses (m/z values) of 616 (red) and 1231 daltons (blue). Color intensity correlates to signal intensity (Figure 2). The zebra fish system, with its combination of forward genetics and vertebrate biology, is used as a model system because its cancer biology is much like that of humans.[2]

The imaging system software generates filter lists for interesting molecular masses to be considered in the Maldi imaging experiment. The colors are assigned automatically by the software, and a name may be assigned to each mass expected in the experiment. The filter list shows all masses, and the number of hits for this specific mass range from the whole measured array of the section.

Maldi images can be represented with or without the underlying microscopic image. In an experiment with zebra fish, for example, specific masses from 2500 to 25,000 daltons can be represented in a color-coded manner (Figure 3). Single organs can be clearly determined, comparing the microscopy image with the Maldi image (raster width 200 µm and 2126 measuring positions). Altering the filter list presents a different set of masses (biomarkers), which enables screening for either single or combinations of biomarkers.

Scientists in the pathology and gastroenterology departments of Otto von Guericke Universität Magdeburg in Germany, as another example, recently performed Maldi imaging on gastric cancer. They compared the tumor area with a tumor-free area on the same section (Figure 4). This technique provides a potential for fast and specific analysis, such as for direct classification of tumors based on biomarker analysis. By resolving mass signals, it provides more detailed information than conventional histological staining and, from the simultaneous evaluation of a broad mass range in Maldi-imaging analysis, allows a much broader view of the tissue sample than immunostaining.

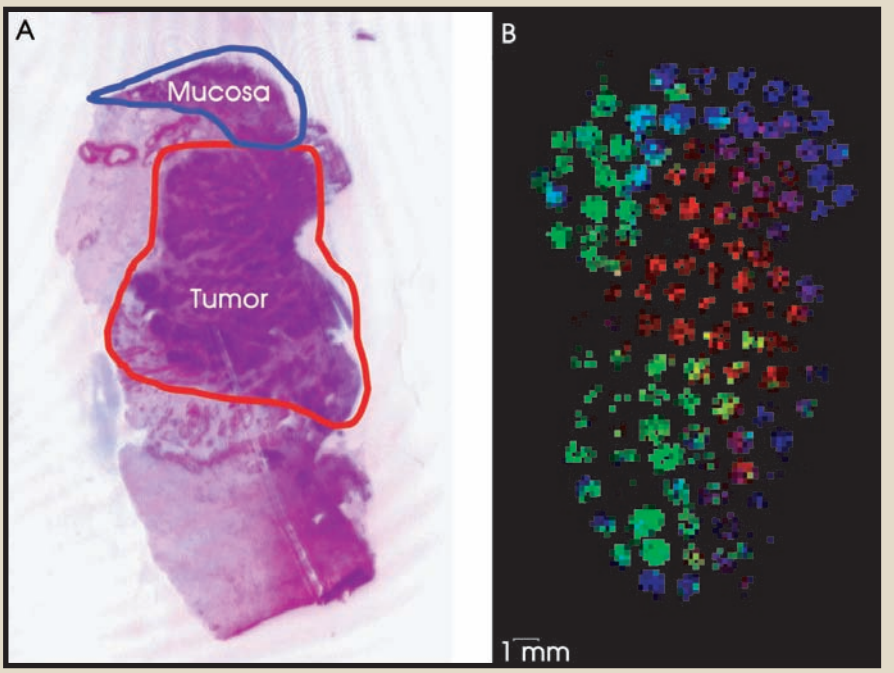

Figure 4. A reference section of a tumor is stained to assign the tumor margin (A). Maldi imaging illustrates an adjacent section of the same tumor. The matrix was applied as small droplets, leading to the dotlike appearance of the image. Sample courtesy of Cristoph Roecken and Matthias Ebert, Otto von Guericke Universität.

The image analysis provides molecular weights of biomolecules that can be used as a handle for their isolation and identification downstream in a marker characterization project. This initial identification and characterization must follow any assignment of a molecular mass as a putative biomarker, which will require more technologies such as laser capture microdissection of the tissue expressing the marker, combined with classical protein fractionation and mass spectrometric identification technology. Such projects are ongoing with zebra fish. □

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