MALDI Ion Imaging and Biological Ion Imaging with a new Scanning UV-Laser Microprobe

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LAMMA 2000 is a new scanning laser ion microprobe, developed in our laboratory, for inorganic and organic mass spectrometrical analysis of e.g. biological or technical samples.

An area of 100x100 µm is scanned by a high-frequency pulsed UV laser with a lateral resolution of ≈0.5 µm (≈1.0 µm for MALDI samples). Time-of-flight mass spectra of each pixel are evaluated with respect to several ion signals and are transformed into two-dimensional ion distribution plots by the data acquisition program (ULISSES 7.3). For detailed technical description see other paper on this conference.

Ion images obtained so far demonstrate the instrumental performances with respect to imaging lateral distributions of ion concentrations from various technical and biological samples. For non-flat samples, signal intensities are not a direct measure of substance concentrations, but are convoluted with a variation of the total ion current. This is due to the fact that the focus depth is in the µm range, which additionally allows to develop three-dimensional mass spectrometry techniques.

Samples prepared for MALDI (matrix assisted laser desorption ionization) MS analysis of peptides have been investigated by LAMMA 2000 ion imaging. The goal of this study was the development of a method of correlating preparational protocols with microscopical sample topology and mass spectrometrical results. In MALDI MS of biopolymers the preparation protocol plays a major role for analytical success, achievable sensitivity and topological homogeneity of the sample with respect to analyte ion formation.

Ion images obtained from MALDI samples demonstrate that
- MALDI-MS is possible even under strongly focused conditions (focus diameter ≈ 1 µm), suggesting the development of sensitivity-enhanced micro-preparation procedures,
- analyte ion intensities basically image the physical structure of matrix crystals,
- analyte ion intensities and alkali ion intensities are in general mutually exclusive,
- alkali ions are mainly located between larger crystals and (homogeneously dispersed) in the inner part of the sample,
- alkali ions are not incorporated into matrix crystals,
- analyte ions are incorporated into matrix crystals,
- analyte ion images usually look less smooth from the first laser shot per pixel, compared to the following shots,
- the method allows to investigate dynamical sample erosion, preparational effects, influences of e.g. impurities and adducts.

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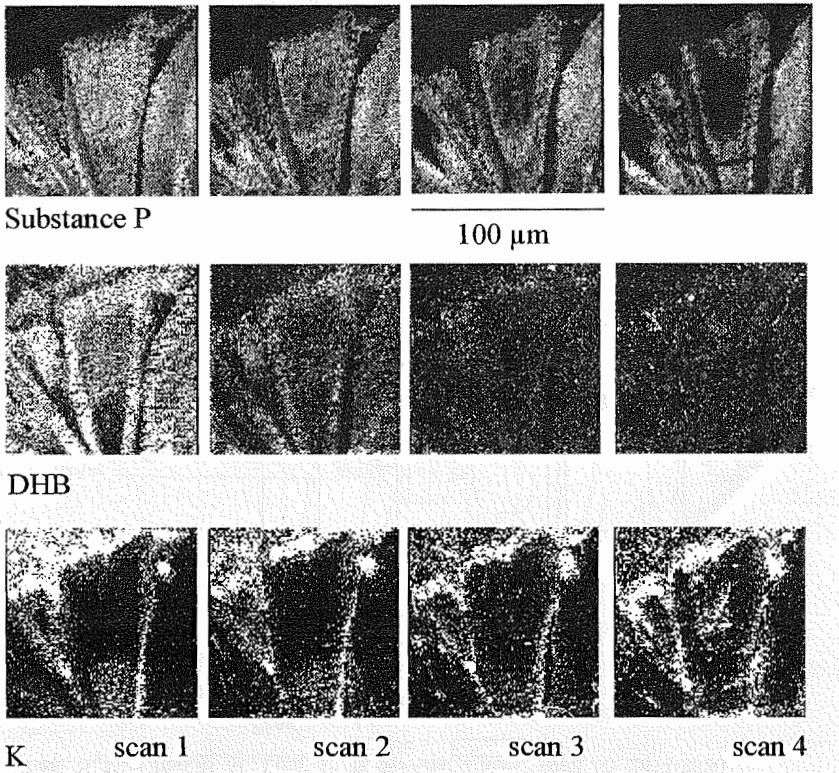

Ion images of MALDI samples of substance p (MW=1348 u) in 2,5-dihydroxy-benzoic acid as matrix. Ion signals of analyte, matrix and potassium are imaged with one laser shot per pixel. Four consecutive scans are shown.
The area scanned is part of the crystalized rim of a dried droplet, with the inner area of the droplet on top.
White = high ion intensity; Black = low ion intensity

Development of a new Scanning UV-Laser Microprobe for Ion Imaging and Confocal Microscopy

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The technical design of a new reflectron time-of-flight laser microprobe mass spectrometer (LAMMA 2000), developed in our laboratory, is described. Instrumental features are fast ion imaging and optical sample imaging with lateral resolution of ≈0.5 µm. Sample illumination, sample observation, laser irradiation, confocal sample imaging and ion extraction are all performed coaxially through a high-numerical UV-transmitting 5-lens objective equipped with a central bore. A diode-pumped Nd:YLF laser has been frequency-quadrupled to 262 nm and is used for both, laser desorption ionization and sample irradiation for confocal scanning microscopy. Advantages of this type of laser are its small physical dimensions and the opportunity to run at high repetition rates of up to 10 kHz, which is a prerequisite for fast imaging.

Sample positioning is performed by a stepper motor driven x-y-z stage. Scanning of an area of 100 x 100 µm is done by a high-speed x-y-z piezo stage. For UV confocal scanning microscopy the same quadrupled Nd:YLF laser is used, running at low pulse energy and high repetition rate. As a result of this optical design scanned areas in the optical confocal imaging mode and in the ion imaging mode are exactly identical, allowing high resolution optical control of mass spectral data acquisition.

Data aquisition for optical and ion imaging as well as instrument control is software driven by a Windows-based software package. Acquired mass spectra of each sample pixel are either stored individually or are processed directly to form a set of two-dimensional ion images. A transient recorder board for PC allows an aquisition rate of more than 50 spectra per second. In the optical imaging mode x/y position and photomultiplier signal are acquired by a high-speed 12-bit A/D converter to build up a confocal image of 400 x 400 pixels within 20 seconds. In addition to that an on-line video camera image is displayed on the PC screen using a PC overlay board combined with frame grabbing functionality to save on-line pictures to hard disk.

First applications of the new instrument are presented in another paper on this conference.

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