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

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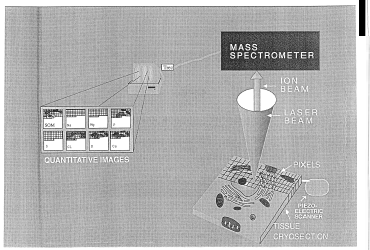
Introduction

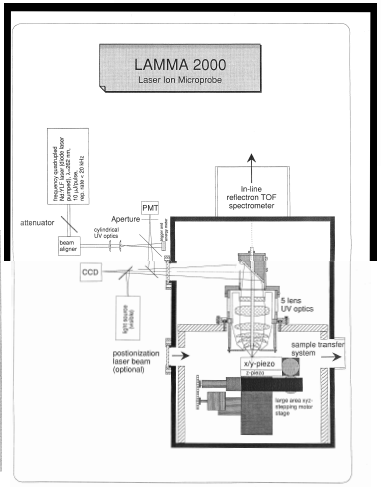
First applications of a new scanning laser ion microprobe to various types of samples are demonstrated.

The instrument allows to perform mass spectrometry of two-dimensional samples with lateral resolution down to 0.6 µm. Typical fields of application are biology, semiconductor engineering and chemistry.

As an example the topological investigation of standard peptide samples for MALDI (matrix assisted laser desorption ionization) analysis is demonstrated.

Method





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.

Output of a frequency quadrupled, diode-laser pumped, Nd:YLF laser is prefocused by a system of two cylindrical suprasil lenses and focused by a high-numerical 5-lens UV objective (numerical aperture 0.6) to a spot size of ≈0.5 µm.

The sample is positioned by an x-y-z stepping motor stage and is scanned by a computer-controlled high-frequency x-y-z piezo stage.

Ions are accelerated and transmitted through the central bore of the objective into the time-of-flight mass spectrometer.

An area of 100x100 µm is scanned by the high-frequency pulsed laser and 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.

Visible-light microscopical sample observation can be performed by a CCD camera. Sample illumination for this mode of operation is done coaxially through the objective lens.

Scanning optical microscopy in the UV can be perfomed by the confocal scanning microscope system using a photomultiplier for light detection.

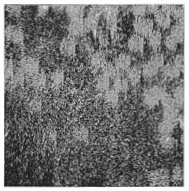
All scanning and imaging procedures are performed under computer control (ULISSES 7.3 data acquisition program).

Acquisition of an ion image with 1 µm resolution takes about 3 to 5 minutes. A confocal optical image with 0.25 µm resolution takes 20 seconds.

For investigating MALDI ion desorption the instrument was operated in a slightly defocused mode (focus diameter ≈ 1µm).

Erythrocytes

K^+ ion image

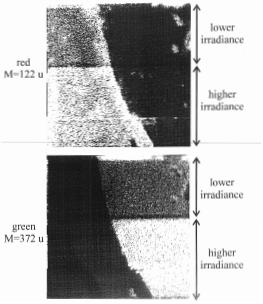


10 µm

Marker

on aluminum substrate

0.5 µm/pixel, 100 x 100 µm

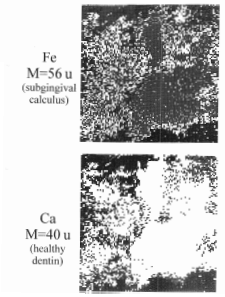


Inorganic Imaging

Human teeth

Iron in subgingival calculus

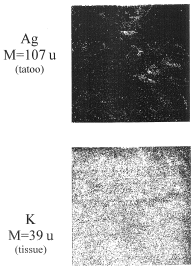
1.0 µm/pixel, 100 x 100 µm



Human gingiva

Silver inclusions from filling tatoos

0.5 µm/pixel, 100 x 100 µm



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The presented ion images demonstrate the instrumental performances with respect to imaging lateral distributions of ion concentrations from various technical and biological samples.

The useful lateral resolution for these kind of samples is in the range of 0.5 µm.

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.

Biomolecular Imaging

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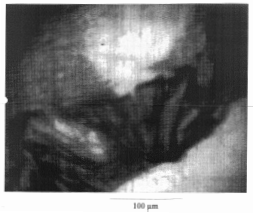
Samples prepared for MALDI (matrix assisted laser desorption ionization) MS analysis of peptides have been investigated by LAMMA 2000 ion imaging. The goal of this study was the development of a method of correlating the preparation protocol used, with the microscopical sample topology and the mass spectrometrical results.

In MALDI MS of biopolymers the preparation protocol plays a major role for the success of analysis, the achievable sensitivity and the topological homogeneity of the sample with respect to analyte ion formation.

For standard preparations of peptides using 2,5-dihydroxybenzoic acid as a matrix it is known that stronger ion signals are obtained from the rim of the dried droplet, where larger matrix crystals form.

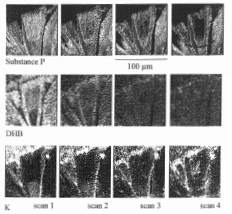
Optical image (CCD camera), visible light



100 µm

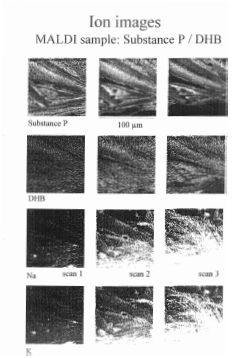
Ion images

MALDI sample: Substance P / DHB



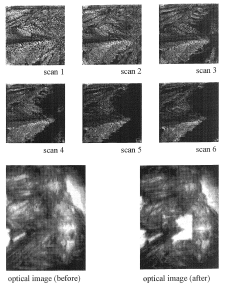
Ion images

MALDI sample: Substance P / DHB



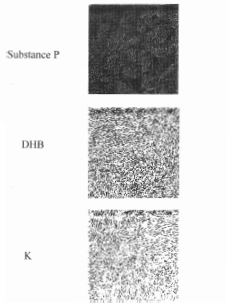
Ion images (Substance P)

MALDI sample: Substance P / DHB



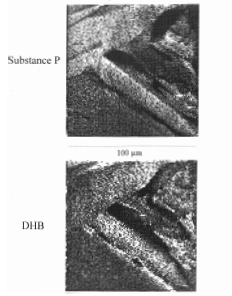
Ion images (inner area)

MALDI sample: Substance P / DHB



Ion images

MALDI sample: Substance P / DHB



Summary

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The presented ion images of 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 impurities and adducts etc.