Development and Evaluation of New Stigmatic Mass Microscope with High Mass and Spatial Resolving Power using Multi-Turn Time-of-Flight Mass Spectrometer

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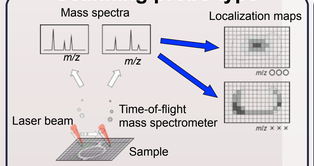
Overview

A stigmatic mass microscope based on matrix-assisted laser desorption/ionization (MALDI) and a multi-turn time-of-flight (TOF) mass spectrometer, MULTUM-IMG 2, was developed.
The ion images were maintained after round in orbit of MULTUM-IMG 2. The mass resolving power $m/\Delta m$ ~ 10000 and the spatial resolution of 1 μm were achieved.

Introduction

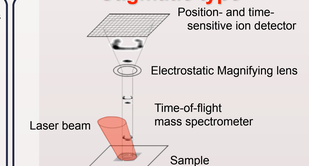
Measurement methods of spatial distribution of molecules such as proteins and drugs at cellular-scale are required in many fields including pathology, pharmacology, etc. Recently, scanning type imaging mass spectrometry (IMS) with matrix-assisted laser desorption/ionization (MALDI) is intensively used for biomolecular analysis. However, the spatial resolution of scanning MALDI-IMS is limited by the laser focus diameter to about 10–100 μm and inadequate for cellular-scale observation. Therefore, we are developing a stigmatic MALDI imaging mass spectrometer, in which spatial resolution of sub-micron can be achieved irrespectively to the laser focus diameter. We obtained a basic property of stigmatic IMS from our prototype experiment and now we are developing a new apparatus to improve measurement performance.

Scanning probe type



- Spatial resolution is limited by the laser spot size to 10 ~ 100 μm.
- Measurement time becomes extremely long.

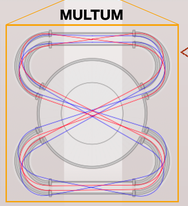
Stigmatic type



- Spatial resolution is not restricted by the laser spot size.
- Measurement time can be reduced.

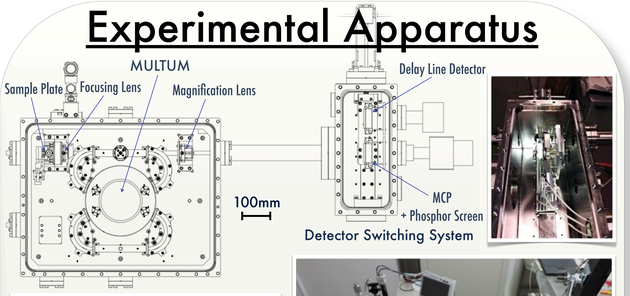
Ion trajectory of focusing lens system

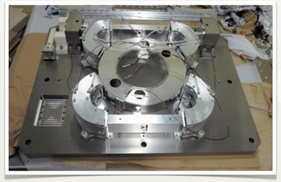
Sample molecules ionized by laser irradiation are extracted and accelerated by extraction electrode and focused on detector plane by einzel lens. Space between sample plate and detector functions as a flight path for TOF measurement.

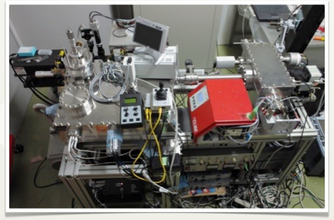


The ion optical system of MULTUM satisfy the "spatial perfect focusing condition". Ions injected to the orbit of MULTUM return in same position and angle at the ejection. Thus the spatial distributions of ions is optically conserved after circulation.

Experimental Apparatus



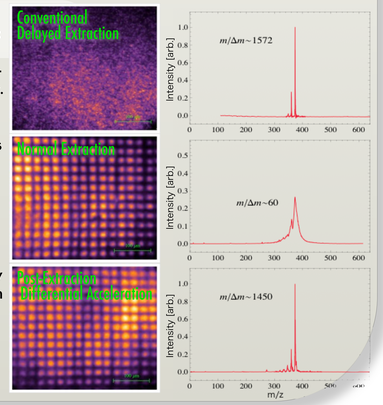




The experimental apparatus consists of a matrix-assisted laser desorption/ionization (MALDI) ion source, a multi-turn time-of-flight mass spectrometer and a time and position sensitive ion detector. The all components of the ion optical system are designed and assembled to achieve high accuracy alignment. Vacuum chamber is made of titanium for high rigidity, low thermal dilation and low outgassing property. Electrodes of MULTUM and electro static lens system are rigidly fixed on a titanium base plate. Ion distributions at the sample plate are magnified and projected with an ion optical lens system onto the detector. MULTUM-IMG which has four toroidal sector electric fields constitute a figure-eight ion trajectory is inserted into the ion flight path.

New Ion Extraction Method

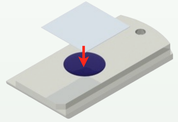
We have developed a new ion extraction method for a stigmatic matrix-assisted laser desorption/ionization (MALDI) imaging time-of-flight (TOF) mass spectrometer. The conventional delayed extraction method is unsuitable for a stigmatic imaging TOF mass spectrometer because the ions disperse until extraction and the ion distribution is not preserved for imaging. In the proposed method, temporal and spatial focusing can be simultaneously achieved, enabling a high-fidelity image to be obtained with a high mass resolution. This method is expected to be applicable to cellular-level observations by imaging mass spectrometry.



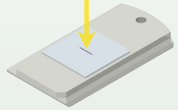
Results (Evaluation of Spatial Resolution)

Making of Evaluation Sample

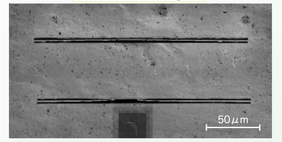
① A dried droplet of crystal violet dye is covered by a thin aluminum foil (1.5 μm thick).

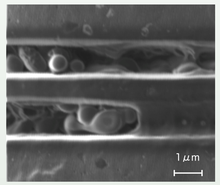


② Fine slit is formed by irradiation of focused ion beam.

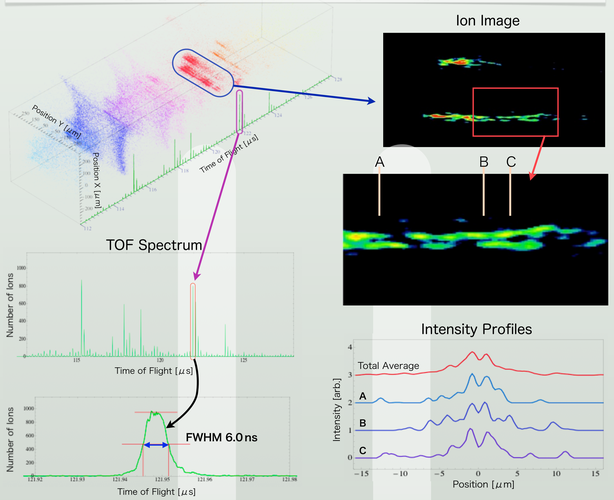


SEM image





Mass Imaging Data acquired by Delay Line Detector

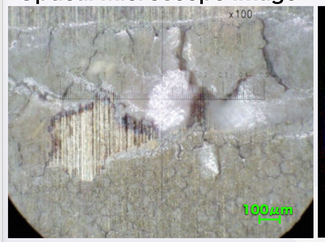


- mass resolving power $m/\Delta m$ > 10000
- High spatial resolution ~ 1 μm

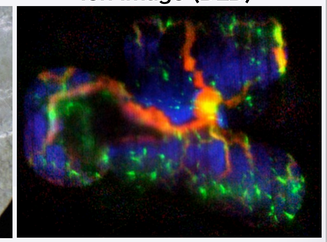
Observation of Biological Sample

A liver of a mouse was sliced to a thickness of 10 μm. The section of the liver was sprayed with matrix (DHB), and coated by gold with a thickness of 8 nm. Ion image was obtained by delay line detector with linear mode (flight length of 1 m). 12 images were combined into one image.

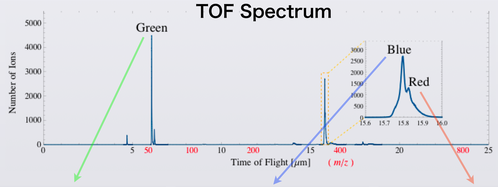
Optical Microscope Image

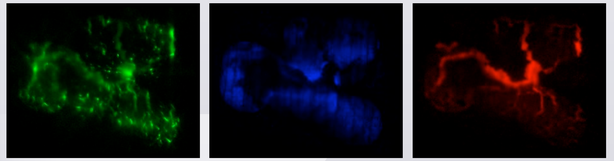


Ion Image (DLD)



TOF Spectrum





Conclusion

- We have developed a new stigmatic multi-turn TOF imaging mass spectrometer, MULTUM-IMG 2.
- We have obtained ion images with the mass resolving power of 10000 and the spatial resolution of 1 μm.
- As the next step, we plan to apply MULTUM-IMG 2 to biological applications.

Acknowledgement
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