ToF-SIMS and MALDI ToF MS imaging of whole mouse sections to monitor the distribution of small molecules and proteins in different organs

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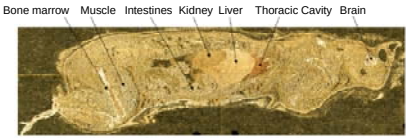
Abstract

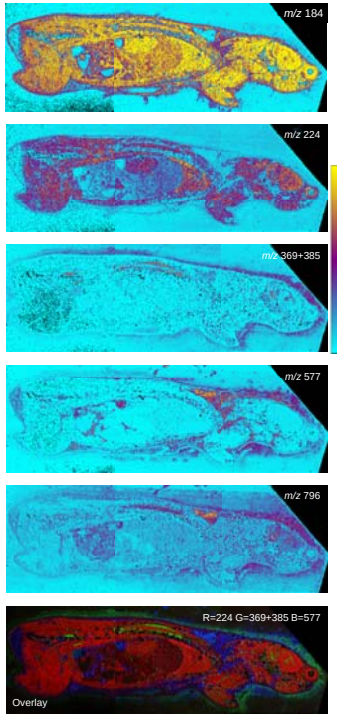
Time-of-flight secondary ion mass spectrometry has become in recent years a powerful tool for imaging biological samples[1]. Until recently, only small parts of animals like brain, could be analyzed. However, analysis of the animal in its entirety is of great interest, especially for monitoring the metabolism of drugs and their effects on metabolite and protein expression. This kind of work has already been reported by Caprioli *et al.* by MALDI-TOF mass spectrometry imaging[2]. We present here for the first time the parallel investigation of serial whole mouse sections with cluster-TOF-SIMS and MALDI TOF MS.

[1] A. Brunelle, D. Touboul and O. Laprévote, J. Mass. Spectrom. 40, 985 (2005)

[2] S. Khatib-Shahidi, J. Herman, E. Wickremsinhe, T. A. Gillespie, R. M. Caprioli, Proc. of the 53rd ASMS, San Antonio, TX

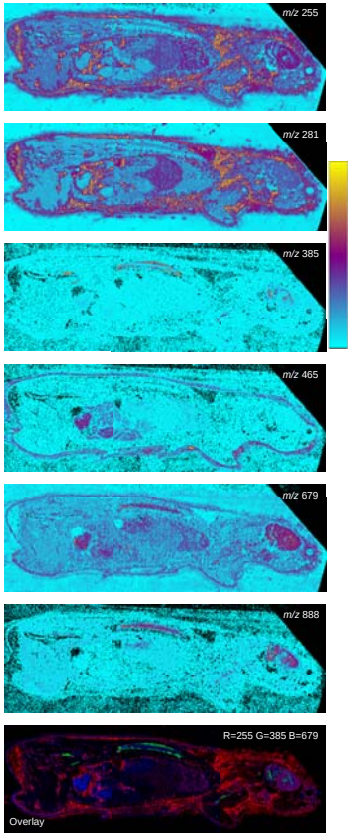


Positive Ion Images ToF-SIMS



ToF-SIMS

Negative Ion Images ToF-SIMS



Ions in the ToF-SIMS Images

m/z	Negative ions
255	C16 fatty acid carboxylate
281	C18 fatty acid carboxylate
385	Cholesterol
465	Cholesterol sulfate
679	Phosphatidic acid
888	Sulfatide

m/z	Positive ions
184	Phosphocholine
224	Phosphoinositol fragment
369+385	Cholesterol fragment + Cholesterol
577	Diglyceride
796	Sum of Phospholipides

Material and Methods

A control mouse was sacrificed and frozen in an ice block. Full body sections were cut on a microtome at a thickness of 20 µm and transferred on silicon wafers.

On the first section, images were acquired on a ToF-SIMS IV spectrometer (Ion Tof GmbH) fitted with a bismuth cluster ion source.[3] The kinetic energy of these ions is 25 keV, with an angle of incidence of 45°. The secondary ions are accelerated to a kinetic energy of 2 keV and are post-accelerated to 10 keV before hitting the detector. The primary ion dose density was 6.10^8 ions.cm^{-2}. The whole mouse image is recorded in three parts, the head, upper body and lower body sections. Each image is 28x28 mm^2, 256x256 pixels (65 536 pixels), pixel size 109 µm. The resulting images which are shown are 28x84 mm^2, *i.e.* 768x256 pixels (196 608 pixels). No averaging, compression or normalisation was applied on the images. The images were acquired using IonImage software (ION-TOF GmbH). The acquisition time for each image was ~4h (total 12h for each polarity).

On the second section, images were acquired by MALDI TOF MS on a Bruker Autoflex (Bruker Daltonics, Billerica, MA) equipped with Smartbeam™ technology at 20 kV of accelerating potential in the linear mode after matrix (sinapinic acid) spray deposition. The whole mouse was analyzed in two parts, a head and tail section. For the whole mouse image, spectra were acquired at a lateral resolution of 300 µm, for a total of 18311 pixels. The images were obtained using Biomap software (Novartis, Basel, Switzerland). The acquisition time for each image was ~7h (total 14h).

[3] D. Touboul, F. Kollmer, E. Niehuis, A. Brunelle, O. Laprévote, J. Am. Soc. Mass. Spectrom. 16, 1608 (2005).

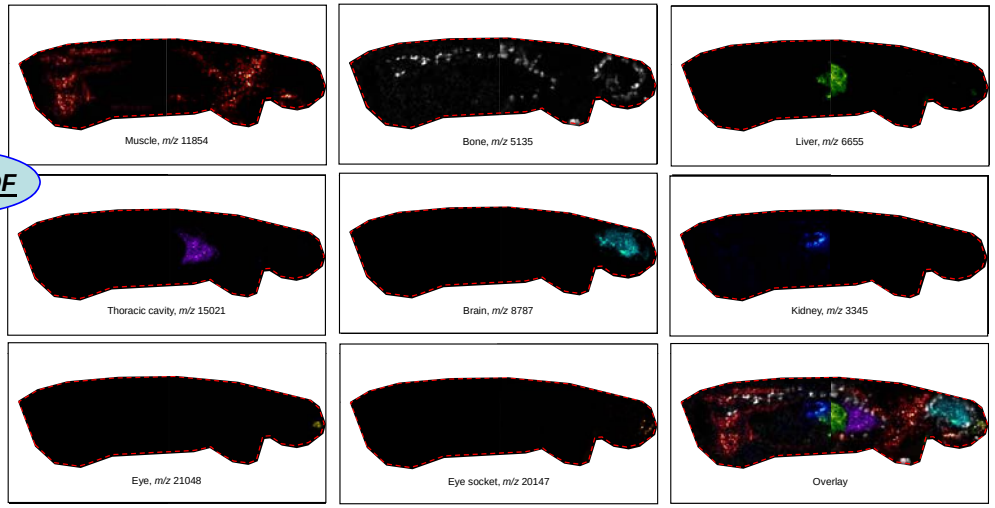
Use of Silicon Wafers

The main advantages of using silicon wafers (4 inches) are the following:

- The surface is absolutely flat,
- The surface is free of contaminants for SIMS (no alkali ions in positive ion mode, no metal oxide ions in negative ion mode),
- The surface is conductive: the electron flood gun which is commonly used when analysing tissue sections was in the present case not necessary.

Positive Ion Images MALDI -ToF

MALDI-TOF



Conclusions

With ToF SIMS, images of signals from lipids at *m/z* 281, 283 and 886 were found to be highly expressed in some organs such as brain, lung and liver. Numerous other signals gave highly regionalized and organ specific images. Similar results were obtained by MALDI MS for proteins of different molecular weights. For example, proteins at *m/z* 5135 and 11854 were observed to be specific for bone and muscle, respectively. Ultimately, this set of data demonstrates that images of drug treated mice, sacrificed at different times after administration will allow us to observe the kinetic of metabolism of a drug in the different organs and correlate this information with variations in lower molecular weight molecules such as phospholipids, and in protein expression.

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