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Application News

Imaging Mass Microscope, iMScope™ QT

A Multi-Derivatization Strategy-Based Mass Spectrometry Imaging Technique for Clinical Spatial Metabolomics Studies

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User Benefits

- The first iMScope QT based spatial metabolomics analytical method developed through the application of 3-trinitrophenyl hydrazine concurrent derivatization strategy
- Concurrent targeted derivatization of carbonyl, carboxyl and phosphoryl containing metabolites in a wide coverage for analysis by high-sensitivity MS imaging with simple operating procedures

■ Introduction

Spatial metabolomics is mass spectrometry imaging (MSI) technique based innovative omics study approach which, unlike conventional metabolomics that suffers from the shortcoming of insufficient information dimensions, significantly improves researchers' knowledge of sample information by expanding omics information to spatial level. Through the implementation of in situ testing of tissue samples, MSI is suitable for semi-qualitative and analysis of metabolites of interest without impairing their spatial dimensions information, thereby analyzing sample region or acquiring the spatial distribution profiles of specific metabolites.

MSI analysis generally involves the application of MALDI ion source for ionizing sample molecules. When used for the analysis of compounds with a small mass-to-charge ratio(usually *m/z*:<500), the MALDI ion source may encounter certain limitations. The MALDI matrix materials for their high UV absorption are generally susceptible to ionization that results in a great number of ion peaks in low molecular weight region. This may lead to a suppression effect on the analysis of small molecules, potentially making it challenging to distinguish the target peak from background peaks. Consequently, the sensitivity of analytes in MSI may be significantly affected. Given these challenges, the in vivo analysis of small molecular metabolites such as amino acids, organic acids, and phosphoric acid with high sensitivity may be particularly demanding in MALDI-MSI studies.

We have developed an ultra-sensitive MSI analytical method and applied it in conjunction with the Shimadzu iMScope QT which integrates an optical microscope, MALDI ion source, and Q-TOF mass analyzer, refer to Figure 1, to analyze metabolites containing carbonyl, carboxyl, and phosphoryl groups through multi-derivatization with 3-trinitrophenyl hydrazine (3-NPH). This article serves as an introduction to this method.

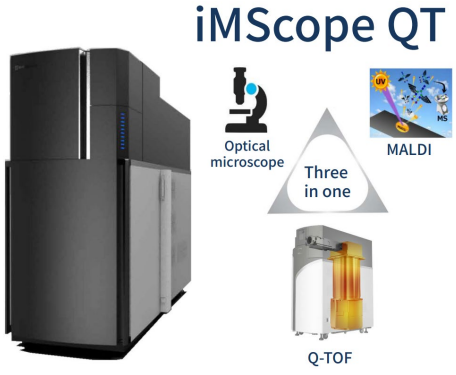

Figure 1 iMScope™ QT

■ Sample Preparation and Analysis

Normal mouse eye tissue was used as samples and 9-AA was used as the matrix in this study. See Figure 2 for sample-handling procedures and Table 1 for analytical conditions.

Step 1: Preparation of tissue slides. Freshly collected mouse eyeballs were embedded in a 10% gelatin aqueous solution in a silicone mold. The mold was then submerged in liquid nitrogen to facilitate the preparation of 10-μm-thick tissue sections using a cryostat microtome. A single section was mounted onto an electrically conductive glass slide and subsequently placed in the iMScope QT for acquisition of optical images at 5× magnification.

Step 2: Derivatization. A mixed reagent containing 25 mM 3-NPH,15 mM 1-(3-Dimethylaminopropyl)-3-ethylcarbodi-imide(EDC), 0.5% pyridine, 70% methanol, and 29.5% water was freshly prepared for derivatization. A volume of 300 μL of this reagent was evenly pipetted and sprayed onto the tissue slice surface. Subsequently, the slice was incubated in a 50% methanol/water solution at 2–8 ˚C for 30 min to allow for the derivatization reaction to proceed.

Step 3: Matrix coating. The tissue slide was placed in the iMLayer™ matrix vapor device for 9-AA matrix deposition (sublimation temperature: 220 ˚C ; deposition thickness: 0.9 μm). Following deposition, the slide was dried under reduced pressure prior to MSI analysis using the iMScope QT.

Figure 2 mouse eyeball slice derivatization flow chart

Table 1 Analytical conditions

Mode of analysis	:	Positive ion mode
Pixel pitch	:	10×10 μm
Laser beam diameter	:	5 μm
Laser energy	:	60
Number of laser shots	:	200 shots
Frequency of laser scanning	:	5000 HZ
Scan range:	:	*m/z* 80-1000

■ Merits of the method

The MSI analysis of small molecular metabolites, such as amino acids, organic acids, and carbohydrates, is often compromised by the ionization suppression effect of the MALDI matrix, which generally diminishes the sensitivity of the method for these metabolites. However, the following derivatization with a combination of 3-NPH, EDC, and pyridine, the signal intensity of metabolites containing carbonyl, carboxyl, and phosphoryl groups is significantly enhanced, thereby markedly improving the imaging results, as demonstrated in Figure 3.

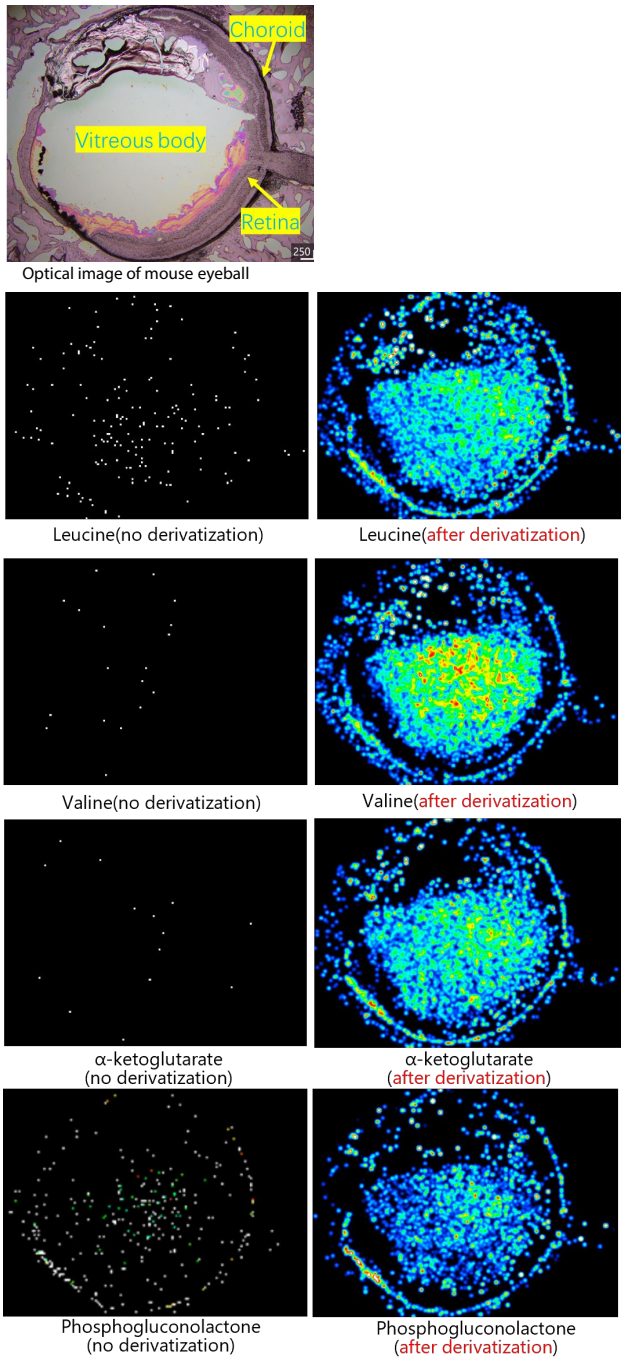

Figure 3. Imaging results of mouse eyeball - Before vs. after 3-NPH derivatization

In metabolomics studies focusing on diabetic eye disorders, researchers typically perform surgical dissection to remove sub-organ tissues from the eyeball. Subsequently, they employ LC-QTOF to determine metabolite levels and identify differences in these excised tissues.

iMScope QT is an integrated high-resolution microscopy and MSI imaging analysis system that enables direct scanning of the eyeball to determine the profile of molecular metabolites without the need to separate the vitreous body, retina, choroid, and other sub-organ tissues. This system can complete the analysis of a tissue section in just 1 hour. This application have revealed that the abundance of relevant small molecular metabolites is relatively high in tissues with active metabolic capabilities and relatively low in regions with lower metabolic activity, such as the optic nerve (e.g., retina). This finding is a typical indication of metabolic heterogeneity at the sub-organ level.

■ Conclusions

The iMScope QT molecular imaging platform integrates an optical microscope with MALDI-MSI. Compared with conventional medical imaging techniques, it can identify the molecular characteristics of early lesions and thus serves as a novel tool for early detection and treatment studies of diseases. High-performance MS-based multi-omics studies are critical for identifying innovative biomarkers for disease screening, early diagnosis, treatment monitoring, and prognosis prediction.

The 3-NPH-based derivatization MSI pretreatment method introduced here is easy to operate and highly sensitive, simplifying spatial metabolomics analysis of glycolysis, the TCA cycle, and other metabolic pathways, and thereby supporting clinical spatial metabolomics studies.

<Acknowledgments>

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<References>

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