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Application News

Imaging Mass Microscope, Matrix Vapor Deposition System, Automatic Sprayer for MALDI Imaging, Mass Spectrometry Imaging Data Analysis Software

Mass Imaging Analysis of Organoids on iMScope™ QT

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User Benefits

- Label free image analysis of organoids samples.
- Easy setting up of experiments using Shimadzu's Total Solutions for MALDI Imaging.
- Simple data processing possible with IMAGEREVEAL™ MS.

■ Introduction

Matrix-Assisted Laser Desorption/Ionization (MALDI) Imaging Mass Spectrometry (IMS) is transforming organoid research by providing spatially resolved molecular insights. Organoids—three-dimensional cellular models derived from stem cells that replicate the structure and function of human tissues—are powerful tools for disease modeling, drug screening, and personalized medicine. MALDI Imaging enhances these applications by enabling the spatial analysis of biomolecules such as proteins, lipids, and metabolites, offering a detailed functional understanding of biochemical processes within complex tissue-like structures. This spatial mapping is particularly valuable for studying conditions like cancer, where tissue heterogeneity significantly influences disease progression and drug response.

Fig. 1 Shimadzu's Total Solution for Mass Imaging with A: iMLayer™, B: iMLayer™ AERO, C: iMScope™ QT and D: IMAGEREVEAL™ MS

In this study, we established a standardized workflow using Shimadzu's complete imaging solution to analyze human lung organoids. Sample preparation was carried out using the iMLayer and iMLayer AERO systems to achieve reproducible and homogeneous matrix deposition. Imaging was conducted with the iMScope QT, and data were processed using IMAGEREVEAL MS (Fig. 1). The integration of these tools enabled high-resolution MALDI Imaging with enhanced sensitivity, providing clear lipid distributions within differentiated organoid tissues.

Looking ahead, the clinical potential of MALDI Imaging is immense—especially in the context of personalized medicine. As patient-derived organoids become increasingly prevalent, the ability to map molecular distributions at high spatial resolution will provide important insights into patient-specific molecular profiles.

■ Sample Preparation and Analysis Conditions

Organoids modeling the human lung were derived from human induced pluripotent stem cells (iPSCs) and fixed in 2% paraformaldehyde and 1% glutaraldehyde in DPBS.

Following centrifugation at 100 *g* for 4 minutes, the organoid pellet was embedded in 10% gelatin, cryosectioned, and thaw-mounted onto ITO-coated glass slides.

Matrix application was performed using Shimadzu's two-step deposition method: first, iMLayer was used for sublimation to generate fine DHB crystal layers; this was followed by automated spraying with the iMLayer AERO, which promoted consistent matrix crystal growth. This dual-deposition technique ensured uniform and high-quality matrix coating essential for high-resolution imaging (Fig. 2).

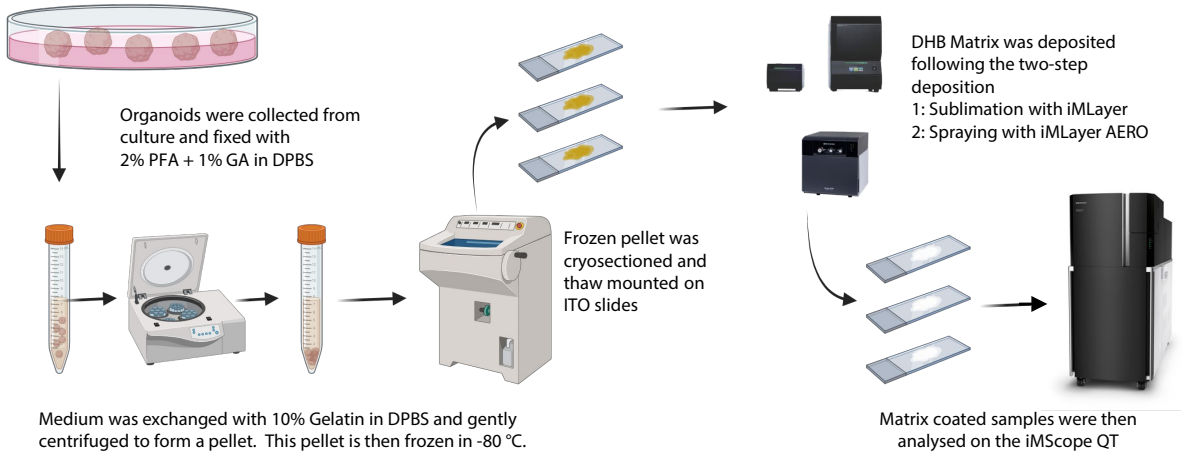


Fig. 2 Sample Preparation

MALDI Imaging was performed on the iMScope QT in positive ion mode. A 40 mg/mL DHB solution in acetonitrile was also spotted onto the slides and used for external mass calibration based on DHB and its' adducts. Full data acquisition parameters are detailed in Table 1.

Table 1 Analysis Conditions for MS Imaging

Matrix Coating (2-Step Vapour Deposition)

Instrument Name	: iMLayer
Matrix Used	: DHB
Coating Method	: Time Deposition for 5 min
Instrument Name	: iMLayer AERO
Matrix Used	: DHB
Matrix Solution	: 30 mg/mL in 0.1% TFA in 30:70, H_2O:MeOH
Number of Layers	: 1
Stage Speed	: 10 mm/s
Nozzle distance	: 5

Mass Spectrometry

Instrument Name	: iMScope QT
DL temperature	: 250 °C
Heat block temperature	: 450 °C
Detector voltage*	: 2.48 + 0.02 kV
Spatial Resolution (Pitch)	: 20 µm
Polarity	: Positive
Mass Range	: *m/z* 500-1000
Laser Irradiation Number	: 50
Laser Repetition Frequency	: 2 [kHz]
Laser Diameter Setting	: 2
Laser Intensity	: 65.0

*: Reference value
The optimum detector voltage depends on the degree of detector degradation and the ease of ionization of the sample. Some detector voltage settings may accelerate detector deterioration. Please refer to the instruction manual for details.

■ Image Processing and Analysis with IMAGEREVEAL MS

Post-acquisition, regions of interest (ROIs) were identified based on the optical and mass spectral images. Lipid mapping was conducted using Principal Component Analysis (PCA), Partial Least Squares (PLS), and p-value analysis within the "Collective Analysis" function in IMAGEREVEAL MS. ROIs were classified into distinct spatial clusters—ROI001–002 (Red), ROI003–004 (Blue), and ROI005–007 (Green), corresponding to different organoid microenvironments (Fig. 3). Red and blue regions are expected to be gelatin matrix regions whilst green are organoids embedded in the gelatin matrix.

This automated analysis pipeline enabled rapid screening of lipid profiles, revealing biochemical signatures consistent with organoid morphology observed under optical microscopy (data not shown). The proximal airway organoids, approximately 500 µm in size, displayed molecular features characteristic of human lung tissues, including discrete epithelial-like layers containing airway basal cells, club cells and epithelial cells. While optical microscopy provides only structural outlines, mass spectrometry imaging offered a direct molecular readout, allowing the visualization of lipid classes essential to lung physiology. Table 2 summarizes the compound list generated by IMAGEREVEAL MS, highlighting the *m/z* values of key lipid species such as fatty acylcarnitines, diacylglycerols (DAG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) (Fig. 4).

The spatial organization of these lipid groups could be resolved within the organoids, offering insight into metabolic activity and membrane composition. Fatty acylcarnitines marked zones of active fatty acid metabolism, while diacylglycerols highlighted regions of phospholipid turnover and signaling. Together, these distributions reinforce the resemblance of the organoids to native human lung tissue, both structurally and biochemically.

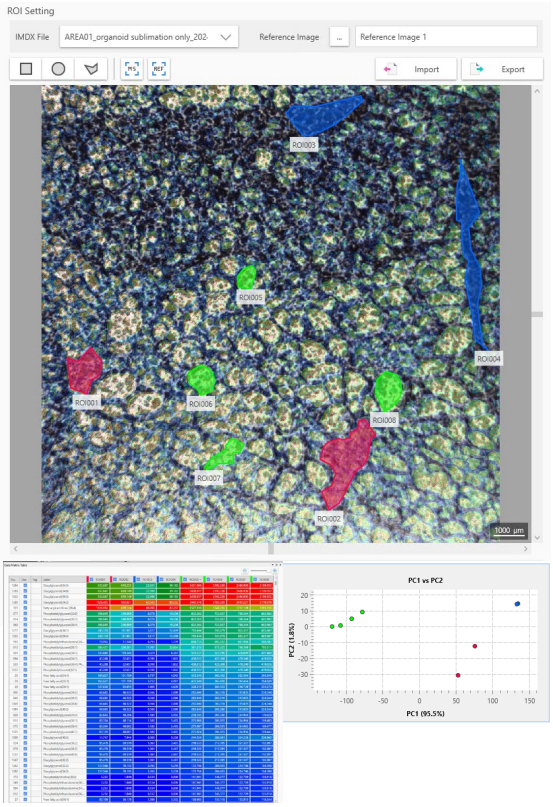


Fig. 3 MS Imaging analysis using the "Collective Analysis" function in IMAGEREVEAL MS.

Table 2 Compound list generated by IMAGEREVEAL MS.

Compound	*m/z*
Fatty acylcarnitine(26:4)	552.285
Diacylglycerol(34:2)	653.452
Diacylglycerol(34:1)	655.467
Diacylglycerol(36:4)	655.470
Diacylglycerol(34:3)	657.444
Diacylglycerol(34:0)	657.486
Diacylglycerol(36:3)	657.486
Phosphatidylethanolamine(34:1)	718.538
Phosphatidylethanolamine(30:0)	740.403
Phosphatidylcholine(30:2)	740.463
Phosphatidylethanolamine(36:4)	740.522
Phosphatidylglycerol(32:0)	799.428
Phosphatidylglycerol(36:1)	799.546
Phosphatidylglycerol(38:4)	799.548
Phosphatidylglycerol(34:1)	809.471
Phosphatidylglycerol(36:4)	809.474

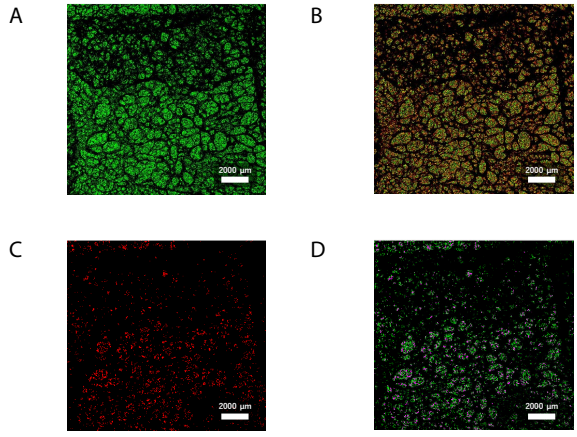


Fig. 4 MS Images from ROI005-007 filtered for the following classes of lipids associated with human lung tissues[1] ;
A: Fatty acylcarnitines, B: Diacylglycerol, C: Phosphatidylcholine, and D: Phosphatidylethanolamines.

At the species level,

- Fatty acylcarnitines (Fig. 4A and 5A), known intermediates in mitochondrial β-oxidation, were predominantly localized in regions resembling alveolar zones, indicating high metabolic activity.
- Diacylglycerols (Fig. 4B), involved in phospholipid turnover and intracellular signaling, can be enriched in ECM-associated regions where active remodeling and cell-matrix signaling occur (Fig. 5B).
- Phosphatidylcholine (Fig. 4C) in proximal airway organoids reflects club cell secretory activity, supporting airway surface fluid stability and barrier function.
- Phosphatidylethanolamine (Fig. 4D), abundant in alveolar epithelial membranes, were also spatially resolved across the organoid structures.

To further resolve lipid distributions at the cellular level, MS imaging conditions were optimized to enhance ionization efficiency and spatial resolution. An additional layer of DHB matrix was applied using the iMLayer AERO to improve co-crystallization and signal uniformity, and acquisition parameters (Table 3) were refined. Increasing the laser irradiation number and repetition frequency improved ion yield, while reducing the laser diameter enabled sharper delineation of cellular boundaries. These adjustments facilitated a shift from organoid-scale mapping to cell-level visualization within the three-dimensional structures.

Table 3 Analysis Conditions for Higher Resolution MS Imaging

Mass Spectrometry		
	Instrument Name	: iMScope QT
	DL temperature	: 250 °C
	Heat block temperature	: 450 °C
	Detector voltage*	: 2.68 + 0.02 kV
	Spatial Resolution (Pitch)	: 10 µm
	Polarity	: Positive
	Mass Range	: *m/z* 500-900
	Laser Irradiation Number	: 500
	Laser Repetition Frequency	: 2 [kHz]
	Laser Diameter Setting	: 1
	Laser Intensity	: 71

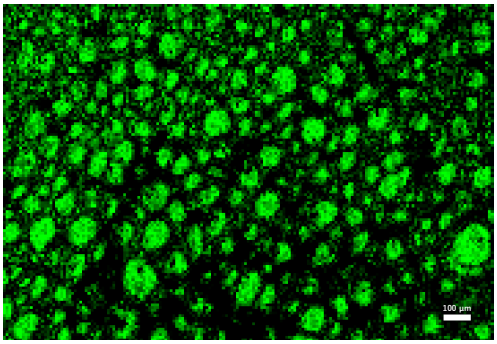
*: Reference value
The optimum detector voltage depends on the degree of detector degradation and the ease of ionization of the sample. Some detector voltage settings may accelerate detector deterioration. Please refer to the instruction manual for details.

Under these optimized conditions, fatty acylcarnitine (26:4) and diacylglycerol (34:2) were clearly resolved within the organoid sections (Fig. 5). Distinct spatial morphologies were observed: fatty acylcarnitine (26:4) (Fig. 5A) appeared in rounded, globular distributions resembling individual alveolar-like cells, consistent with its role in mitochondrial and peroxisomal lipid metabolism. In contrast, diacylglycerol (34:2) (Fig. 5B) exhibited an interconnected, web-like pattern surrounding the cellular structures, reflecting its dual function as a lipid intermediate in membrane remodeling and a second messenger in signaling pathways. These complementary patterns suggest that while fatty acylcarnitines emphasize metabolic activity within specific cell types, diacylglycerols trace broader membrane-associated processes and intercellular interactions within the lung-mimicking organoid environment.

■ Conclusion

This study demonstrates the effective application of Shimadzu's

A



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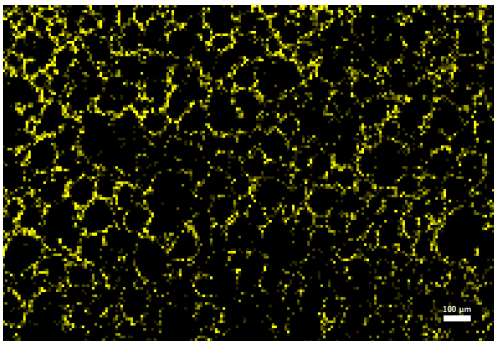

Fig. 5 MS Images of cells within organoids; A: Fatty acylcarnitines (26:4), and B: Diacylglycerol (34:2).

end-to-end MALDI Imaging solution —including iMLayer, iMLayer AERO, iMScope QT, and IMAGEREVEAL MS— in profiling the molecular composition of human iPSC-derived lung organoids. This streamlined workflow enables high-resolution, label-free imaging of complex 3D tissues, eliminating the need for conventional staining methods that may disrupt native molecular distributions.

By enabling the spatial mapping of lipids associated with tissue differentiation and metabolic activity, this approach provides a powerful tool for assessing the functional maturity of organoid models. It lays the groundwork for future applications, where high-content imaging of patient-derived tissues can support studies on patient-specific biological characteristics.

■ Acknowledgements

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