Imaging Mass Microscope

iMScope TRIO
Introducing the New Era of Imaging Mass Spectrometry

Imaging mass spectrometry is a revolutionary new technology. The instrument is a combination of an optical microscope which allows the observation of high-resolution morphological images, with a mass spectrometer which identifies and visualizes the distribution of specific molecules. Superimposing the two images obtained based on these very different principles, has created a significant new research tool, the imaging mass microscope. The accurate and high resolution mass images from the iMScope TRIO will drive your research to the next level.

At long last, we have entered the age of imaging mass spectrometry.
Optical microscope
Capture an optical microscope image

Mass Spectrometer
Mass spectra at multiple points
Apply matrix to tissue section and irradiate with laser beam.

Multiplexed imaging
Generate molecular distribution images (multiplexed imaging) based on ms signal intensity for specific ions.
Imaging Mass Spectrometry identifies what you see at the molecular level
iMScope TRIO transforms your data from just "Observation" to "Analysis"

Optical microscopes cannot determine which molecules are localized in the region of interest. On the other hand, the positional information of molecules is lost in mass spectrometric analysis, where sample extraction and homogenization is needed for sample pre-treatment.

What if the qualitative analysis and localization of specific molecules and compositional and morphological observation could be obtained from a single analysis through microscope observation?

Imaging mass spectrometry with the iMScope TRIO realizes this dream.

iMScope TRIO
Superimpose optical and mass microscope images.

Imaging mass spectrometry directly detects both natural and synthetic molecules in tissue sections and measures mass spectra, while retaining their positional information associated with the tissue section.

Then, two-dimensional distributions of specific molecules are visualized by combining the positional information of each mass spectrum and the signal intensity for specific ions in the mass spectrum (MS imaging). The iMScope TRIO imaging mass microscope is an instrument designed specifically for imaging mass spectrometry. It represents a hybrid type microscope that combines both an optical microscope and a mass spectrometer. The iMScope TRIO now makes it possible to identify various substances directly in tissue samples and expands the potential research opportunities in a wide variety of fields.

Ideal for Cutting-Edge Research in a Wide Variety of Fields

**Medical Research**
- Biomarker discovery
- Lipid analysis
- Metabolite analysis
- Pathological studies
- Microstructural analysis

**Pharmaceuticals**
- Pharmacokinetic analysis
- Localization of native and metabolized drugs
- Pharmacological research
- Toxicity mechanism analysis
- DDS research
- Cosmetics development and evaluation

**Food**
- Quality evaluation
- Ingredient research of agricultural products
- Inspection of contaminants

**Industry**
- Surface Analysis
- Homogeneity evaluation
Medical Research

In medical research, the iMScope TRIO is particularly useful for identifying disease-related molecules (biomarker discovery) and rendering two-dimensional distributions of those substances (visualization). Also biological mechanism analysis and pathological research through identifying the location of target molecules (profiling) can be achieved.
Identification of Localized Molecules in Cancerous Mouse Liver Cells

The iMScope TRIO makes it possible to obtain information regarding which molecules are localized within the region of interest in an organ, e.g. cancerous area. Until now, biomarker discovery has been carried out by LCMS or other mass spectrometry techniques for each organ.

GFP-tagged human colon cancer cells (HCT116) were injected into a mouse portal vein so that the cancer spreads to the mouse liver. The bright areas in the fluorescence optical image are the cancerous liver area. Results of analyzing the section showed an increase of UDP-N-acetylhexosamine (UDP-HexNAc), glutathione, and related metabolites in the metastatic cancer. The comparison between the quantitative analysis results of the neighboring tissue and the affected zones enables the highly localized quantitative analysis of many metabolites.

Profiling of Biological Tissue and Organ Cross Sections

The iMScope TRIO, which incorporates database searching with MS and MS\textsuperscript{n} data, can be applied not only to visualize the localization of a certain molecule, but also to identify the molecules relating to the cause of disease.

Example of Measuring Mouse Testis

The images show characteristic distributions of seminolipids (m/z 795.5 and 809.5) and phosphatidylinositols (m/z 885.5) in mouse testis. In this example, 9-aminoacridine was vapor-deposited as a matrix on the sample, and mass spectra were measured in negative ion mode. Negative ion spectra are simpler than those of positive ion, making it easier to identify target molecules. Negative ion analysis is frequently used for low molecular weight metabolite analysis.

Example of Measuring Mouse Kidney

Many phospholipids exist in the mouse kidney, including sphingomyelins (SMs) and phosphatidylcholines (PCs). Using imaging mass spectrometry, it is possible to obtain information about the distribution of the many phospholipids with a single measurement.

Analyzing the entire kidney revealed that the types of molecules that exist in large quantities in the medulla are different than in the cortex. For example, the analysis confirmed that large amounts of PC (34:1) existed in the medulla (top images). Additionally, an even more detailed distribution can be confirmed from a high spatial resolution analysis focused on the region of interest (bottom images).
Visualization of Lipid in a Mouse Cerebellum

A wide variety of molecules relate to diseases states. Imaging mass spectrometry using the iMScope TRIO can detect a wide range of molecules within a defined mass range. Therefore, distribution information for several target molecules with different molecular weights can be determined simultaneously during a single measurement.

Experiment Conditions

Sample: mouse cerebellum  
Matrix: DHB (vapor deposited)  
Measurement pitch: 10 μm  
Laser diameter: 10 μm  
Measurement points: 250 × 250 (62,500 points)  
Measurement time: about 3 hours

Optical Image

MS Images

SM (d18:1/18:0) + K (m/z 769.5)  
PC (16:0/16:0) + K (m/z 772.5)  
PC (18:0/18:1) + K (m/z 826.5)  
GalCer (d18:1/24:1) + K (m/z 848.5)

*Ionization time: 50 ms, mass range: m/z 500–1000, MS mode

By displaying the signal intensity at molecular weight of each target molecule, the 2D location of the target molecules is visualized. All target molecules can be detected simultaneously by a single measurement. Furthermore, samples are measured at a fast 6 pixels per second*, dramatically shortening your experiment time. In this example, the distribution of phosphatidylcholines (PCs) in the mouse cerebellum was successfully visualized within 3 hours (2.5 mm square). Limitations in immunostaining of lipids have previously made visual mapping of these compounds difficult. However this can easily be achieved using the iMScope TRIO, making this technology very powerful, particularly in areas such as brain function analysis and any biological process where lipids are known to play an important role.
In drug discovery, researchers need to do a variety of pharmacokinetics research for many drug candidates in many situations. For instance, a wide range of spatial-resolution observation is needed, from the sub-cellular level high spatial resolution observation to mouse full body large area observation. The iMScope TRIO is useful for pharmacokinetics, pharmacological mechanisms, toxicity testing, and the development of ointments and cosmetics, owing ability to combine morphological observation from the optical microscope and location of target molecules from the mass spectrometer image.
High Spatial Resolution Imaging

High-resolution imaging offered by optical microscopes is required not only for pharmacokinetic analysis, but also for toxicity testing and toxicity mechanism analysis. Analysis of the retina and skin requires imaging with high spatial resolution.

Section with chloroquine administered (retina)

In this experiment, a rat retina administered with chloroquine was measured. High spatial resolution imaging of the retina resulted in visualizing the distribution of chloroquine around the retinal pigment epithelium, which is about 10 μm thick. Therefore, evaluating the safety of phototoxic compounds requires performing detailed analysis near the retina.

High spatial resolution imaging of mouse skin

In this experiment, the fat in rat skin was visualized. High spatial resolution imaging clearly showed localization of ceramide in the horny layer and phosphatidylcholine (PC) in the sebaceous gland.

Experiment Conditions

Sample: rat retina with chloroquine administered
Matrix: CHCA (vapor deposited)
Measurement points: 50 μm 81 × 81 (6,561 points)
10 μm 49 × 53 (2,597 points)
Measurement pitch: 50 μm/10 μm
Laser diameter: 50 μm/10 μm
Measurement time: about 18 minutes at 50 μm and about 7 minutes at 10 μm

Experiment Conditions

Sample: rat skin
Matrix: DHB (vapor deposited)
Measurement points: 125 × 215 (26,875 points)
Measurement pitch: 10 μm
Laser diameter: 10 μm
Measurement time: about 1.2 hours
Pharmacokinetic Analysis (whole body and tissue)

In pharmacokinetic analysis, the iMScope TRIO enables the distribution of unchanged and metabolized drugs to be simultaneously mapped in a single measurement, without any labeling. The laser diameter used during mass spectrometry imaging with the iMScope TRIO is continuously variable from 5 to 200 μm, offering low to high spatial resolution. This helps ensure that analyses are performed as efficiently as possible.

Example of measuring section with famotidine administered (full body and kidney)

Famotidine, commonly used as a histamine H2 receptor antagonist, was administered into a mouse vein. Three minutes after administration, the mouse was euthanized and a frozen section was prepared. Famotidine was detected in the kidneys by screening analysis of the full body section at 200 μm pitches. Also, the MS image with 50 μm spatial resolution shows that the famotidine was particularly localized in the kidney pelvis.

Experiment Conditions
Sample: 200 mg/kg full body section of mouse 3 minutes after single administration of famotidine via tail vein
Matrix: DHB (sprayed)
Measurement points: 425 × 107 (45,475 points) (measured in four different regions)
Spatial resolution: 200 μm
Laser diameter: 10 μm

Experiment Conditions
Sample: mouse kidney
Matrix: DHB (sprayed)
Measurement points: 23 × 154 (3,542 points)
Measurement pitch: 50 μm
Laser diameter: 10 μm

Pharmacokinetic analysis of mouse with olaparib administered

Using a mouse administered with olaparib, the pharmacokinetic status of the tumor was visualized. In this way, imaging mass spectrometry is being utilized even in the clinical trial phase of drug discovery, rather than only during basic research.

Samples provided by the Department of Clinical Pharmacology, National Cancer Center Research Institute

Pharmacokinetic analysis of mouse with paclitaxel/micellar paclitaxel administered

This shows that micellation of the known anticancer agent paclitaxel improves its retention within the tumor. Consequently, imaging mass spectrometry is being utilized for DDS research as well.

Samples provided by the Division of Developmental Therapeutics, Research Center for Innovative Oncology, National Cancer Center Hospital East
Example of Measuring Human Hair

Hair grows at a rate of about 1 cm per month (0.3 to 0.4 mm per day), during which pharmaceuticals in the blood are taken into the hair root. From the results of a high-resolution analysis of hair dipped in methoxyphenamine aqueous solution, it was possible to clearly visualize the localization of pharmaceuticals. Applications for such an analysis are anticipated in a wide range of fields, including not only forensic medicine and forensic science, but also medication management, and doping examinations.

Example of Measuring Rat Lung

Lung tissue from a rat administered amiodarone, an antiarrhythmic drug, was measured. The onset of phospholipidosis, as well as foam cell formation/macrophage permeation and other findings, have been observed in rats administered large quantities of amiodarone. In this case, it can be confirmed that amiodarone is accumulating at the lesion sites.
Overlaying Optical and MS Images

Overlaying MS images and optical microscope morphological images reveals the difference between the amounts of specific molecules in each minute organ and can relate molecular distribution to the biological functions of an organelle and morphological changes.

The figures show the resultant images generated by overlaying MS images at different \textit{m/z} (blue and yellow) and optical microscope morphological images. These figures clearly indicate that analysis of the localization of specific molecules combined with morphological images is a useful approach for investigating drug efficiency and toxicity in drug discovery.
In the food industry, breeding improvement is actively done to develop high-value and high productive agricultural foods. Imaging mass spectrometry is useful as a new evaluating tool for monitoring the amount of effective ingredients in foods.

Example of Measuring Tomato Stem

Matrix application methods include the vapor deposition method, in which matrix is applied by sublimation to the tissue surface, and the two-step application method, in which the matrix solution ($\alpha$-CHCA concentration: 10 mg/mL; solvent: 30 % acetonitrile, 10 % isopropanol, 0.1 % formic acid mixed solution) is applied by spraying after vapor deposition. The figures above show a comparison of the matrix application methods on stem sections from tomatoes exposed for 1 day to imidacloprid at 100 ppm. They show that the peak intensity is higher with the vapor deposition method and that the imidacloprid has leached outside the tissue with the two-step application method, so that the distribution is not accurately reflected. These results suggest that the use of the vapor deposition method is better suited for applying matrix to plant tissue.
Example of Measuring Tomato Seedlings

The figure above shows the visualization of the metabolite distribution in tomato seedlings. Imaging mass spectrometry is expected to be useful not only for developing improved varieties, but also for analyzing metabolites with functional characteristics for use for processed foods or crude drugs.

Samples provided by Innovation Center for Medical Redox Navigation, Kyushu University

Example of Measuring Rice

The figure above shows that a specific fat is distributed throughout the entire endosperm of rice. By measuring the distribution of various substances, the technology is anticipated for use in a diverse range of applications, such as for the development of safe and high quality food products or for developing new varieties more resistant to changes in growing environments.
Industry

In industrial fields, various surface inspection technologies are widely used to ensure stable production of high quality products. Imaging mass spectrometry is capable of analyzing various contaminants that cannot be detected by conventional surface inspection methods, and it is possible to gain new knowledge for higher quality production.

Example of measuring IC chip

An example using IC chip is shown on the left. A wide variety of optical images and mass spectrometry imaging technologies are used to support cutting-edge research.

<table>
<thead>
<tr>
<th>m/z</th>
<th>174.98</th>
<th>216.95</th>
<th>217.17</th>
</tr>
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<tbody>
<tr>
<td>m/z</td>
<td>276.98</td>
<td>294.91</td>
<td>313.94</td>
</tr>
</tbody>
</table>

**Experiment Conditions**
- Sample: IC chip
- Matrix: none (LDI)
- Measurement points: 80 × 60 (4,800 points)
- Measurement pitch: 5 μm
- Laser diameter: 5 μm
- Measurement time: about 15 minutes

Example of measuring printed circuit boards

In the example shown on the left, imaging mass spectrometry technology is used to analyze the causes of soldering defects or contaminants on printed circuit boards.

| m/z   | 456.710 |

**Experiment Conditions**
- Sample: printed circuit board
- Matrix: none (LDI)
- Measurement points: 40 × 80 (3,200 points)
- Measurement pitch: 10 μm
- Laser diameter: 10 μm
- Measurement time: about 9 minutes
Integration of Intelligent Advanced Technologies

The instrument features simple design with indicator lights that allow the user to confirm the operating status from a distance combined with smooth and highly accurate analysis. It also comes with dedicated software for rapid analysis and processing of massive amounts of data.

The imaging mass microscope, iMScope TRIO, features extensive functionality and straightforward operation that has been designed with users in mind.

Overlaid optical and MS images

Best-in-class 5 μm spatial resolution

Structural analysis by highly accurate MS\textsuperscript{n} analysis

6 pixel per second, high-speed analysis\textsuperscript{*}

*Ionization time: 50 ms, mass range: 500–1000, MS mode
A Revolutionary Analysis System That Puts State-of-the-Art Technologies Together

The imaging mass microscope, iMScope TRIO, is a high-performance, de novo and progressive analysis system, featuring:
• proprietary technology to combine optical and mass spectrometric images
• 5 μm laser diameter—the world’s highest level MS image resolution
• high-precision tandem mass spectrometer to perform structural analysis

Optical Microscope
Magnification is variable from 1.25 to 40 times for observing from small to wide field to high detail. Bright field and fluorescence modes are both available.

High-Speed and High Precision Sample Stage
The stage below the section sample moves at high speeds and stops at the desired point instantaneously and precisely.
High-Speed Laser Unit with Variable Beam Diameter
The laser beam diameter can be set from 5 μm to 200 μm. After screening the wide area with the 200 μm beam diameter, detailed analysis can be carried out with a 5 μm beam using the serial section function. Extensive and efficient data acquisition is supported by the 1 kHz laser frequency.

Versatile and Reliable Quadrupole-Ion-Trap
Even though the mass spectra are directly acquired from a tissue section, high mass accuracy is achieved independent of the sample surface state, because the ions are cooled and focused in the ion-trap prior to TOF-MS. Multi-stage (MS\textsuperscript{n}) target molecule fragmentation, which is easy to set up, provides definitive molecular structural information.

A Proprietary Ion Transfer System Ensures High Sensitivity Analysis
The laser generated plume of ionized molecules is transferred efficiently and quantitatively from the sample stage into the mass spectrometer.
Work flow

With the iMScope TRIO, the entire workflow from setting the sample, acquiring an optical image, setting and acquiring MS data to data analysis and statistical treatment is completely seamless and follows the intuitive flow of process of the experiment.

1. Place the sample on the stage.
Place a thinly sliced sample (e.g. tissue section) directly on the optical microscope sample stage.

2. Capture an optical microscope image.
Observe the sample via the optical microscope and capture an image. The objective lens magnification can be varied from 1.25 times to 40 times, which enables high-resolution images to be captured.

3. Apply matrix.
Apply the matrix, which assists ionization and desorption, to the tissue section by vapor deposition. The matrix transfers the laser light energy into heat via UV light absorption, with the resultant thermal energy releasing the target molecules from within the tissue section and at the same time ionizing them.

4. Acquire mass spectra at multiple points across the sample.
A mass spectrum of the ionized molecules is acquired at each laser irradiation point. By irradiating the whole sample area in a grid pattern, a mass spectral "image" of the whole sample is built up.

5. Visualize the molecular distribution (multiplexed imaging).
Visualize a two-dimensional distribution of specific molecules, based on the signal intensity of selected ions of interest in the mass spectra.

MS and MS/MS Imaging
Even if a molecule cannot be identified in the MS spectra, due to multiple molecules coexisting at the same mass (peak), these can be distinguished and visualized (rendered to images) by MS/MS analysis.

Rat Brain

Optical Image
MS Image

m/z 772.530

Optical Image
MS/MS Images

m/z 713.462
m/z 589.469

6. Overlay the optical microscope and MS images.
By overlaying the optical microscope image, which provides the morphological information, and MS image, which reveals the localized molecules in the region of interest, a single tissue section reveals the molecular makeup and distribution of specific tissue features.

7. Use the software for statistical analysis.
Data reduction tools built in to the software help the user to efficiently extract and analyze the necessary data from the high-volume, high-resolution mass spectrometer images.
Imaging MS Solution™ Software

Imaging MS Solution is a new software program designed specifically for the iMScope TRIO, which integrates optical and mass image acquisition. All imaging mass spectrometry parameters can be set on the optical images. Visualization processing is added to mass spectrum acquisition, and high-speed imaging processing up to 6 pixels per second is available. Furthermore, this analysis and display software incorporates statistical tools to analyze the huge amounts of MS-imaging data combined with detailed optical microscope images.

Data Acquisition Software Designed for Easy Operation

The acquisition area, mass range, and other parameter settings can be specified based on an image captured by the built-in microscope in the iMScope TRIO. Settings for multiple acquisition areas can be configured with a variety of conditions, enabling more efficient analysis.
Data Analysis Software Capable of Statistical Analyses and Compound Searches

The analysis software is specially designed for the imaging mass spectrometer data. In addition to overlaying mass spectrometer images on the microscope optical images, it is equipped with functions enabling statistical analysis using three methods: PCA, HCA, and ROI analyses.

Statistical Analysis

The detection of characteristic peaks is easily implemented via statistical analysis procedures.

In addition to selection from the spectrum, \( m/z \) images can easily be added from peaks extracted via statistical analysis.

Principal Component Analysis (PCA)

PCA organizes all detected peaks (\( m/z \) values) based on loading factor (weight coefficients assigned to individual peaks). When the peaks are arranged in an array, the MS images can form several patterns representing principal components. Therefore, PCA can be especially useful for finding ions that show specific localizations. This feature helps provide more multifaceted data analysis.

Hierarchical Cluster Analysis (HCA)

By analyzing images of a mixture of substances with different properties, hierarchical cluster analysis (HCA) creates clusters (groups) of similar substances, in order to classify target substances and analyze them based on distribution information. Even when the substances of interest are determined in advance, other substances with a similar distribution can be found. For example, by selecting substance groups with the same distribution as the administered drug, it is possible to apply the results to study pharmacological mechanisms or to evaluate toxicity.
Region of Interest (ROI) Analysis

ROI analysis helps investigate and compare substances in two specified regions of interest (regions A and B, in the example above) to determine which substances are increased or decreased in each region. For example, if the amount of a given molecule increases in a cancerous region, then that is treated as a candidate cancer biomarker.

Compound Search Function

Compound search based on MS analysis
Compounds can be screened prior to MS/MS analysis. Screening can determine whether a peak originates from the matrix or whether the compound corresponding to a peak is registered in a library.

Library search based on MS/MS analysis
Molecules can be identified using MS/MS spectral library. Accuracy of identification can be improved by subtracting spectra outside of the ROI from those of the ROI. MS/MS libraries can also be added or deleted.
Matrix Vapor Deposition System
iMLayer™ (option)

High Reproducibility and Minute Matrix Crystals
This system ensures reliable vapor deposition of matrices to assist the consistent ionization of samples. Vapor deposition parameters can be changed for each type of matrix. It also includes an auto control function of matrix layer thickness.

User-friendly Design
Front opening chamber for easy sample loading
Intuitive recognition of operation status with indicator LEDs.
New Method of Applying a Matrix

After forming fine crystals with the iMLayer, the matrix is overlaid by spraying. This results in higher resolution images and higher signal intensity.

Inside the iMLayer

- Apply fine matrix crystals by vapor deposition.
- Overlay matrix by spraying.

Increasing imaging resolution and signal intensity (mouse cerebellum analysis example)

Deposition + spray

- Deposition
- Spray

m/z 772.53 (yellow) / 838.62 (blue)

Scale bar: 200 μm

m/z 772.53 Overlay
m/z 838.62

Deposition & spray

m/z 838.62
m/z 848.648
m/z 866.635

Scale bar: 200 μm
Biomarker search of effect drug intake from comparative analysis using LC-MS, succeeded to confirm the distribution of candidate molecules by MS imaging.

Table 1  Lipid Component Variation Due to Administration of AAPH

<table>
<thead>
<tr>
<th></th>
<th>m/z</th>
<th>m/z</th>
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<tbody>
<tr>
<td>A</td>
<td>786.568 (+)</td>
<td>E   832.5429 (+)</td>
</tr>
<tr>
<td>B</td>
<td>810.5616 (+)</td>
<td>F   822.5151 (+)</td>
</tr>
<tr>
<td>C</td>
<td>806.5357 (+)</td>
<td>G   780.4237 (+)</td>
</tr>
<tr>
<td>D</td>
<td>785.5436 (+)</td>
<td>H   801.4930 (+)</td>
</tr>
</tbody>
</table>

Experiment Conditions
Sample: Mouse liver
Matrix: DHB
Point: 250 × 250
Pitch: 5 μm
Laser diameter: 5 μm
Time: 2.7 hours

Imaging data combined with LC-MS results is expected to provide more informative analysis results.
LC-MS option for iMScope TRIO can be provided on request. (Setup by Shimadzu field engineers.) (ESI only)

Sample data: Innovation Center for Medical Redox Navigation, Kyushu University