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Application News

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Imaging Mass Microscope

High Spatial Resolution Imaging by iMScope *TRIO* - Imaging of Chloroquine Distribution in Rat Retina -

Pharmacokinetic analysis of candidate compounds is a critical step in drug development, for elucidating the site and mechanism of activity and for evaluating local cytotoxicity. Whole body autoradiography or fluorescence imaging are the conventional methods for tracing the fate of molecules in *vivo*. Despite the high cost of tagged compound production, these methods give ambiguous results for two reasons: first, it is impossible to distinguish between signals derived from intact or metabolites of tagged compounds; and secondly, tagged compounds could behave differently from untagged target compounds.
Imaging mass spectrometry (MS) is a recently emerging technology as an alternative to conventional methods because it can quantify and give spatial distribution of both intact and metabolized forms of target compounds simultaneously. This label-free strategy is expected to drive drug development processes towards breakthrough discoveries. Here we present how imaging mass microscope iMScope *TRIO* was used for analysis of rat retina administered with chloroquine.

High-Resolution Imaging of Chloroquine in Rat Retina

In this experiment, chloroquine, a well known drug against malaria infection, was administered to a rat to elucidate its distribution in retina.
Fig. 1 shows the structure of chloroquine. Detection of chloroquine standard was optimized prior to analysis by selecting a suitable MALDI matrix compound and instrument parameters as listed in Table 1. High resolution imaging of chloroquine distribution using a 10 µm section of pigment epithelium of retina was then performed by iMScope *TRIO*. The MS/MS product ion spectrum of chloroquine (*m/z* 320.190) and the plot of product ion intensity (*m/z* 247.095) were displayed as Fig. 2 and 3, respectively.
Conventional imaging analyses by MS mode sometimes fail to give a resolved image due to interference of noise signals, and this was the case for chloroquine (data not shown). In contrast, data acquisition by MS/MS mode can resolve target-derived signal from noise to improve the sensitivity of the imaging analysis. The so-called "MS/MS imaging" strategy was applied to chloroquine to acquire a high-sensitivity, 10 µm spatial resolution image.

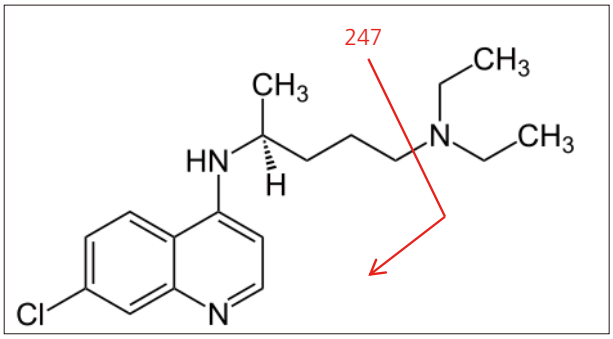


Fig. 1 Structure of Chloroquine
Red arrow indicates the site of MS/MS fragmentation

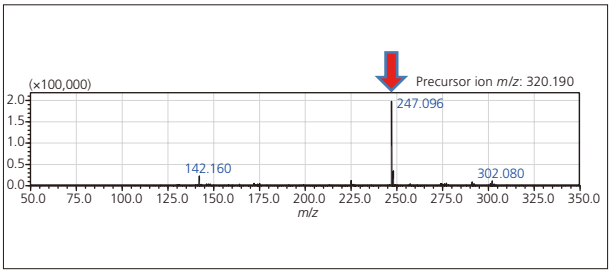
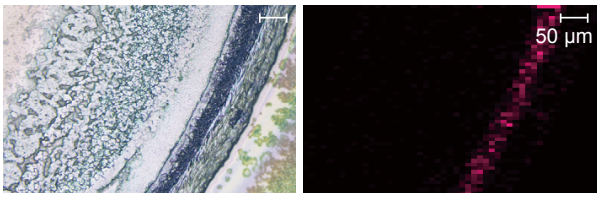


Fig. 2 MS/MS Product Ion Spectrum of Chloroquine Acquired Directly from Tissue Section



Optical image | MS/MS image of *m/z* 320.190 > 247.095

Fig. 3 MS/MS Imaging of Rat Retina, Showing the Spatial Distribution of Chloroquine (*m/z* 320.190 > 247.095)

Table 1 Analytical Conditions

Sample Type and Preparation	
Sample	: Chloroquine treated (20 mg/kg, po) rat retinal section (10 µm, OCT embedded)
MALDI Matrix	: CHCA applied by sublimation
Analytical Conditions	
Polarity	: positive mode
Precursor Ion	: *m/z* 320.190
Laser Diameter	: 10 µm
Acquisition Pitch	: 10 µm

High-Speed Imaging of Chloroquine in Rat Eyeball

In pharmacokinetics analysis, information about molecular distribution is needed at both high and medium resolutions, for elucidating site-specific localization at cellular level and at organ level, respectively. Here, MS/MS imaging of chloroquine was performed at medium (50 µm) resolution over a large area of rat eyeball section. The analytical conditions were as listed in Table 2. Even when wide laser diameter was used, where high level of noise and ion suppression becomes problematic, iMScope *TRIO* still detected chloroquine specifically and at high signal to noise ratio needed for clear molecular imaging.
The speed of image acquisition in imaging MS is governed by the number of points (pixels) inside the area of interest. iMScope *TRIO* can change both laser diameter and acquisition pitch independently to each other to easily control acquisition time and image size without compromising quality of the data.

Table 2 Analytical Conditions

The Sample and Its Preparation	
Sample	: Chloroquine treated (6 mg/kg, po) rat retinal section (OCT embedded)
MALDI Matrix	: CHCA applied by sublimation
Analytical Conditions	
Polarity	: positive mode
Precursor Ion	: *m/z* 320.190
Laser Diameter	: 50 µm
Acquisition Pitch	: 50 µm

Comparison of Matrix Deposition Method

Two methods of MALDI matrix deposition were compared for suitability to chloroquine imaging. The imaging result obtained by sublimation method is shown in Fig. 5 (a schematic of sublimation process is shown in Fig. 6). Sublimation method is performed automatically by iMLayer instrument, and spray method is a manually operated technique. The result obtained by spray method is shown in Fig. 7.
In comparison, the signal derived from chloroquine gave a sharp image by sublimation method (Fig. 5) whereas that given by spray method seemed "dispersed" (Fig. 7).
Nonetheless, the optimum preparation method depends on the nature of the tissue slide as well as the matrix compound. As demonstrated, sample preparation substantially affects the quality of final image; not only the slicing conditions but also MALDI matrix deposition matter.

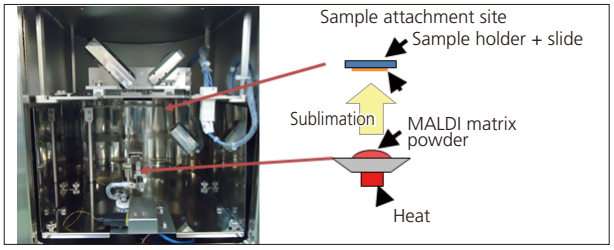


Fig. 6 A Schematic of iMLayer and Sublimation Process

Combination of MS and MS/MS Imaging on a Single Tissue Section

In imaging MS, data can only be acquired once from the same position. However, using iMScope *TRIO* that can adjust laser diameter and acquisition pitch, it is possible to leave unaffected spaces in between the points of data acquisition to enable more rounds of imaging analysis. Fig. 8 schematically shows the arrangement of acquisition points for four rounds of imaging analysis performed on a single area of interest, using 5 µm laser diameter and 10 µm acquisition pitch.

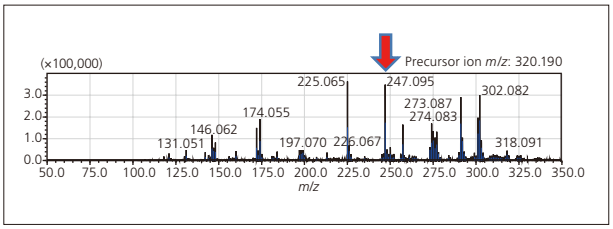
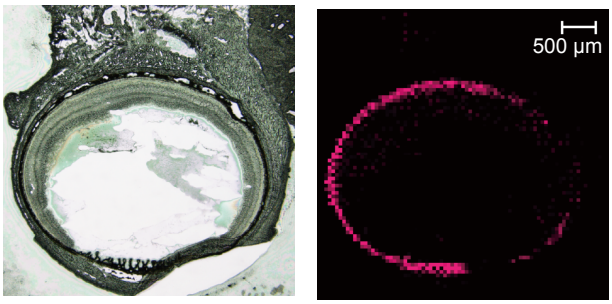
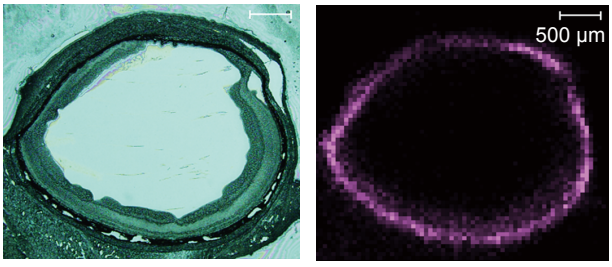


Fig. 4 MS/MS Product Ion Spectrum of Chloroquine Acquired Directly from Tissue Section, Using Laser Diameter of 50 µm



Optical image | MS/MS image of 320.190 > 247.095

Fig. 5 Image of Chloroquine Distribution Obtained by Sublimation Method



Optical image | MS/MS image of 320.190 > 247.095

Fig. 7 Image of Chloroquine Distribution Obtained by Spray Method

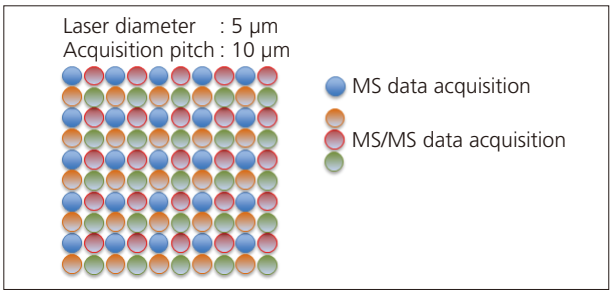


Fig. 8 An Example of Data Acquisition Point Arrangement for Performing a MS Survey Scan and Up to Three MS/MS Imaging on a Single Area of Interest

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