Improving MALDI Imaging Throughput by IR Laser Imaging

Introducing a powerful multimodal workflow for downstream MALDI Imaging and saving ~90% analysis time.

Abstract

Imaging whole tissue sections at high spatial resolution (≤10 μm pixel size) entails large number of pixels and requires extensive acquisition times. Here, a multimodal imaging workflow built on the HYPERION II ILIM and timsTOF fleX will be introduced. Both modalities are seamlessly integrated into an end-to-end workflow for the accurate tissue-/cell-specific guidance for MALDI Imaging – thereby increasing sample throughput by significantly reducing acquisition times and data size of mass spectrometry imaging experiments.

Keywords:
Imaging, timsTOF fleX, HYPERION II ILIM, microGRID

Introduction

MALDI mass spectrometry imaging is a proven, powerful tool for biological and pharmaceutical research to interrogate the spatial molecular profile of tissues by detecting hundreds of biomolecules within a single experiment. However, as a large number of pixels correlates to time in MALDI Imaging, a solution to reduce acquisition time is desirable. Thus, researchers are seeking for alternative high-speed imaging techniques, which are conducted prior to the MALDI Imaging experiment to narrow down the regions of interest (ROIs) on large sample areas. Ultimately, this approach would achieve high spatial resolution MALDI Imaging studies to be performed with the highest possible sample throughput.

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In 2018, Rabe *et al.* described a workflow for IR Guided MALDI Imaging to perform exclusive acquisition of regions of interest – thereby achieving >90% reduction of MALDI Imaging acquisition time [1]. However, the employed FT-IR spectrometer was limited regarding speed of acquisition, thus, IR prescreening consumed considerable time.

Empowered by the Quantum cascade laser (QCL) technology, Bruker's recently introduced HYPERION II ILIM microscope provides non-destructive and label-free IR Laser Imaging of whole tissue sections within a few minutes at spatial resolutions equivalent to MALDI Imaging. These spatially-resolved chemical fingerprints include characteristic molecular vibrations for various biomolecular classes (e.g., lipids, proteins, glycans) and can be utilized to guide the chemically specific characterization of individual molecules based on mass-to-charge ratios (*m/z*) by MALDI Imaging. Taking advantage of these recent technical developments, the same working group followed their vision and performed IR Guided MALDI Imaging of various mouse kidney tissue areas and cellular features at unmatched throughput [2].

In this Application Note a new multimodal imaging workflow built on the HYPERION II ILIM and timsTOF fleX is introduced to enable accurate, high-throughput IR Guided MALDI Imaging of tissues and cells at unprecedent speed and molecular detail.

Methods

Sample preparation

Fresh frozen mouse brains were sectioned at 10 µm thickness, thaw-mounted on IntelliSlides® and kept at -80˚C until analysis. Prior to analysis, the slide was brought to room temperature and desiccated in a vacuum chamber for 30 min.

IR Laser Imaging

The IR Laser Imaging experiment was performed using a HYPERION II ILIM FT-IR / QCL microscope (Bruker Optics GmbH & Co. KG, Ettlingen, Germany) in reflection mode. Infrared light generated by a laser source traverses the tissue sample twice while being reflected from the conductive coating of the IntelliSlide onto a detector. This measurement technique is non-destructive and leaves the sample unaltered for further analysis. A 3.5x objective lens was used alongside a room temperature FPA microbolometer for the detector. This detector allows 250 x 250 spectra to be acquired simultaneously at 4.9 µm pixel resolution, covering a sample area of 1.5 mm^2 in one analysis. The laser wavelength was scanned across the fingerprint region from 950-1800 cm^{-1} in order to detect the characteristic molecular vibrations of biochemical components in the tissue. Spectra were recorded at 8 cm^{-1} spectral resolution in each detector pixel. The acquisition time for the whole mouse brain tissue section (12.5 x 8.0 mm) was about 7 minutes and generated 3.8 million IR spectra (~ 9000 pixels/s). Data processing was performed using OPUS (8.7.41).

Table 1
Comparison of the experimental conditions of IR Laser Imaging and MALDI Imaging

	QCL	MALDI
Area	100 mm^2	8 mm^2
Pixel Count	3800 k	316 k
Resolution	5 µm	5 µm
Acquisition time	7 min	6.6 hours

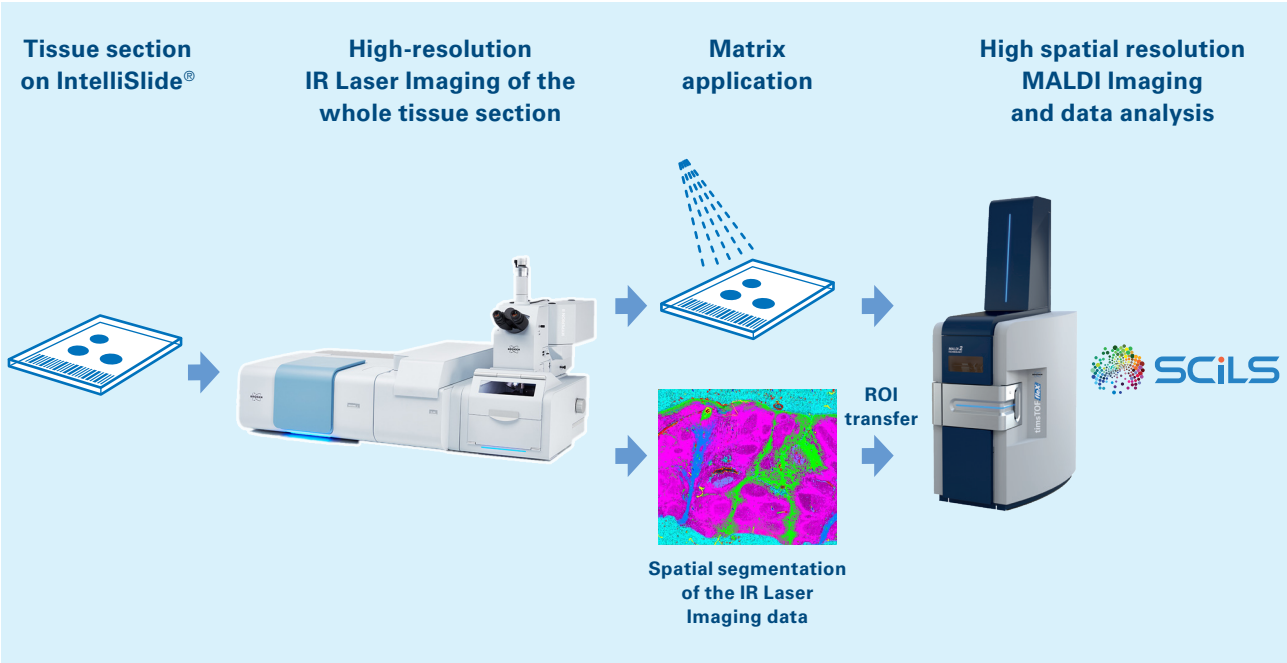

Figure 1
Workflow for multimodal IR Guided MALDI Imaging of tissues and cells.

MALDI Imaging

After rapid pre-screening via IR Laser Imaging, the entire slide was immediately spray-coated with 2,5-DHAP matrix solution using an HTX M3+ Sprayer. MALDI Imaging was conducted on a timsTOF fleX MALDI-2 system equipped with microGRID and automatically set up with the SCiLS™ autopilot workflow in flexImaging 7.2. Using the ILIM teaching image generated by the Tissue Imaging Workflow software package (1.0) teaching marks of the IntelliSlide® were co-registered in flexImaging to teach spatial coordinates of the sample and to align with the sample stage positions. The cerebellum region was chosen for analysis at 5 μm spatial resolution in positive ion mode from *m/z* 300–1000 with lock mass enabled. Upon completion, the dataset was imported into SCiLS™ Lab 2023b for data visualization, with annotations conducted based on exact mass.

Results and Discussion

The multimodal tissue imaging workflow consists of three major steps and is depicted in Figure 1. First, a cryo-sectioning protocol was performed to obtain tissue sections for the organ of interest on IntelliSlides®. Next, the speed and ease of IR Laser Imaging was used to perform rapid pre-screening of the complete tissue section within a few minutes. In parallel to matrix application, spatial segmentation results of the IR Laser Imaging data based on k-means clustering or integration were obtained to determine potential regions of interest (ROI). In the final step, the spatial coordinates of the selected ROI were accurately transferred to the timsTOF fleX instrument to guide the in-depth spatial multiomic characterization by MALDI Imaging for the same tissue section.

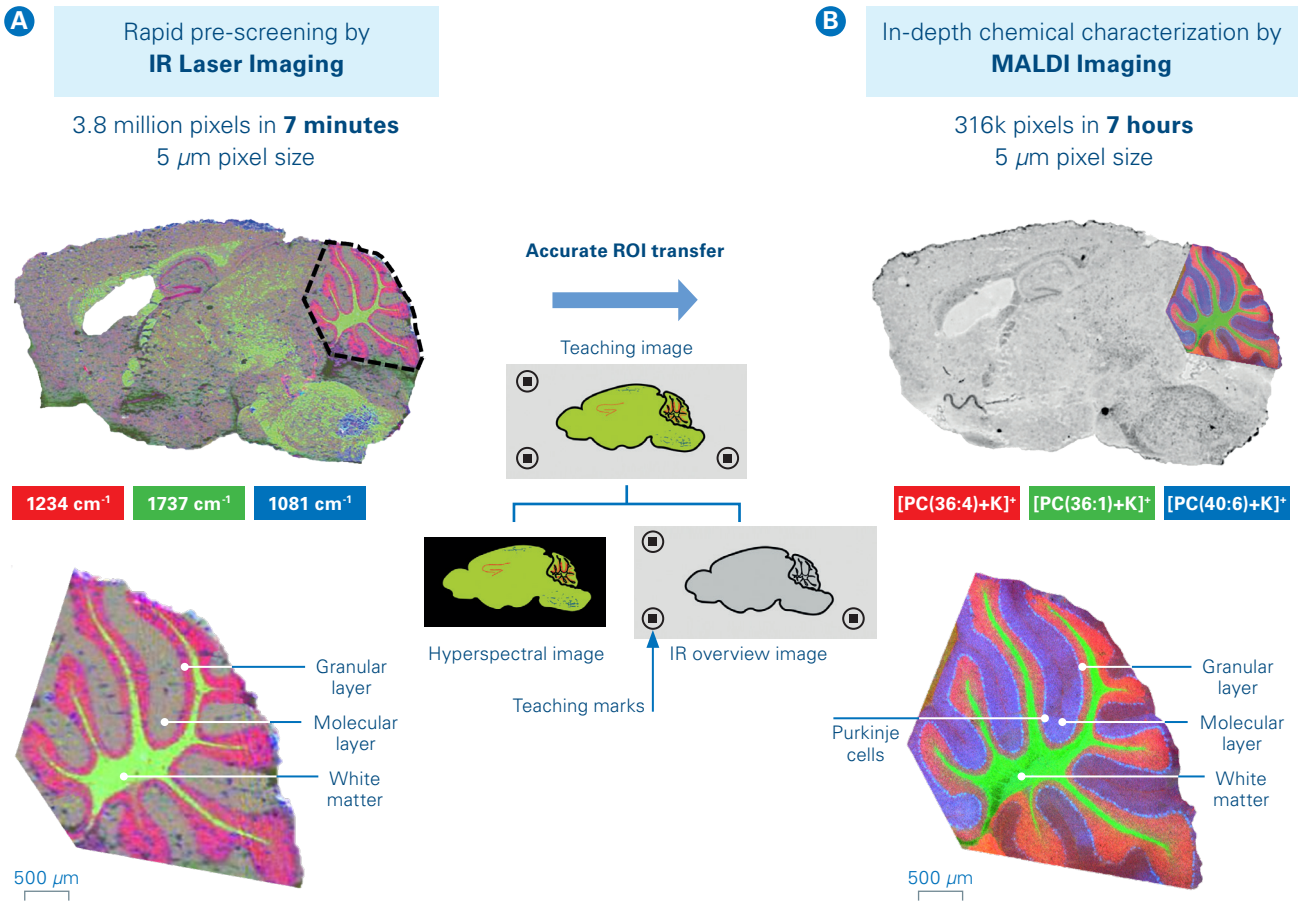

Figure 2

Multimodal IR Guided MALDI Imaging of mouse brain.

(A) Results of pre-screening IR Laser Imaging of mouse brain showing spatial distribution of three different molecular groups identified by integration of characteristic streching bands. (B) MALDI Imaging result of selected ROI determined by IR Image. Three different species are identified by exact mass visualizing the main histological regions of the cerebellum.

For this technical proof-of-concept experiment, the workflow was applied to mouse brain as a model organ with complex tissue architectures. Figure 2 displays an overview of the multimodal imaging results. Using a HYPERION II ILIM, the IR pre-screening of the whole mouse brain section was carried out in 7 minutes (~ 3.8 million pixels, 5 µm pixel size). The overlay image was generated by integrating three different spectral features that correspond to characteristic functional groups. The C=O stretching band at 1737 cm^{-1} is characteristic for lipids and is shown in green [3]. The vibrational bands for phosphodiesters at 1234 cm^{-1} and 1081 cm^{-1} are shown in red and blue, respectively [4]. The final displayed pixel color is a combination of the three colors depending on their contribution. In particular, for the cerebellum, IR Imaging revealed tissue-dependant chemical heterogeneity. Whereas lipid-rich myelin was predominantly detected in white matter, phosphodiesters originated from nucleic acids were exclusively detected in the granular cell layer (overlay of red and blue: pink). In contrast, the molecular layer showed homogenous molecular abundance for both functional groups (overlay of red, green and blue: grey).

Based on this chemical overview of the complete mouse brain tissue section, a region of interest in the cerebellum was selected for the spatially-directed chemical characterization via high spatial resolution MALDI Imaging using a timsTOF fleX (Figure 2B). The spatial coordinates of the ROI were transferred via the ILIM teaching image. This teaching image was automatically generated after the ILIM acquisition and displayed an overlay of the IR overview image (including teaching marks) with the selected ROI (cerebellum region) based on the hyperspectral image. In total, the acquisition time for MALDI Imaging was ~7 hours (316k pixels, 5 μm pixel size). The generated overlay ion image shows the spatial distribution of $[PC(36:4)+K]^+$ (*m/z* 820.5255, red) in the granular cell layer, $[PC(36:1)+K]^+$ (*m/z* 826.5707, green) in the white matter and $[PC(40:6)+K]^+$ (*m/z* 872.5547, blue) in the Purkinje cells and molecular layer (Annotations by exact mass).

Collectively, our MALDI Imaging results provided in-depth spatial lipidomic analysis at the cellular level and displayed a high level of alignment with the IR laser chemical image. Both modalities successfully revealed major molecular differences in the main histological regions of the cerebellum. Hence, IR Laser Imaging serves as a powerful and non-destructive method to reveal and guide downstream MALDI Imaging experiments for the same tissue section – thereby enabling accurate guidance and excellent spatial match of the multimodal imaging results.

Conclusion

The workflow described herein combines state-of-the-art imaging technologies into an intuitive workflow for multimodal tissue imaging. IR Laser Imaging provides for the unbiased chemical imaging of whole tissue sections within a few minutes. Given its excellent compatibility with established MALDI Imaging sample preparation protocols, IR Laser Imaging represents an ideal method for pre-screening to guide high spatial resolution MALDI Imaging experiments towards specific tissue and cellular regions of interest. As a result, the total acquisition time is drastically reduced utilizing both modalities. In conclusion, the presented multimodal IR Guided MALDI Imaging workflow provides both, in-depth spatially-resolved molecular information at high spatial resolution, and increased sample-throughput to enable large-scale imaging studies – thereby paving the way for next-generation bioanalytical imaging.

timsTOF fleX

Dual ion source design brings unparalleled versatility to enable ESI/MALDI with zero compromises. More molecular information behind every pixel with SpatialOMx®. TIMS to solve the highest molecular complexity. Smartbeam 3D and microGRID for unmatched spatial resolution.

HYPERION II ILIM FT-IR / QCL Microscope

Our pioneering QCL technology delivers exceptional IR Laser Imaging performance that is up to 180 times faster than traditional FT-IR Imaging. Innovative hardware design for coherence reduction to acquire artifact-free IR imaging data with high fidelity unlike any other IR instrument – all while still being augmented by FT-IR.

References

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