Deep molecular coverage at faster throughput: IR Guided MALDI Imaging in cancer research

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Introduction

The throughput of MALDI Imaging samples is negatively correlated with spatial resolution. Utilizing alternative high-speed imaging techniques prior to MALDI Imaging helps narrow down regions of interest (ROIs) on large sample areas, thereby reducing MALDI MS instrumentation acquisition time. In this context, IR Laser Imaging (ILIM) and MALDI Imaging is introduced to overcome the negative time correlation.

Here, IR Guided MALDI Imaging was employed to investigate pancreatic ductal adenocarcinoma tissue (PDAC) at unprecedented speed and molecular detail. Rapid pre-screening by ILIM allowed differentiation between specific morphologies of PDAC, guiding in-depth collision cross section (CCS)-enabled analysis by MALDI Imaging. This approach reduced MALDI MS acquisition time by approximately 90% without sacrificing comprehensive feature finding for ROI characterization.

Methods

Tissue sections (10 µm thickness) from a patient-derived xenograft model for PDAC were mounted on IntelliSlides®. Without any sample preparation, ILIM of the complete tissue section was conducted using the HYPERION II ILIM microscope. This instrument utilizes quantum cascade laser (QCL) technology and a focal plane array (FPA), allowing for the simultaneous acquisition of 250 x 250 pixel grid at a pixel resolution of 5 µm. The laser wavelength was adjusted with a spectral resolution of 8 cm^{-1} across the mid MIR fingerprint region from 950 to 1800 cm^{-1}.

Following ILIM analysis, the same tissue section was spray-coated with NEDC matrix (HTX M3+). Concurrently, k-means clustering was applied to the ILIM data to identify cellular morphological features based on chemical fingerprinting. The spatial coordinates of the selected regions of interest (ROI) were then transferred to a timsTOF fleX equipped with microGRID for 7 µm MALDI Imaging. Data acquisition was performed with TIMS in negative ion mode. SCiLS™ Lab 2024b was utilized for data analysis and multimodal IR and MS feature correlation. Molecular annotation was carried out using MetaboScape® 2023b via the REST API interface, with a scoring of maximum 3 ppm in m/z and 3% in CCS.

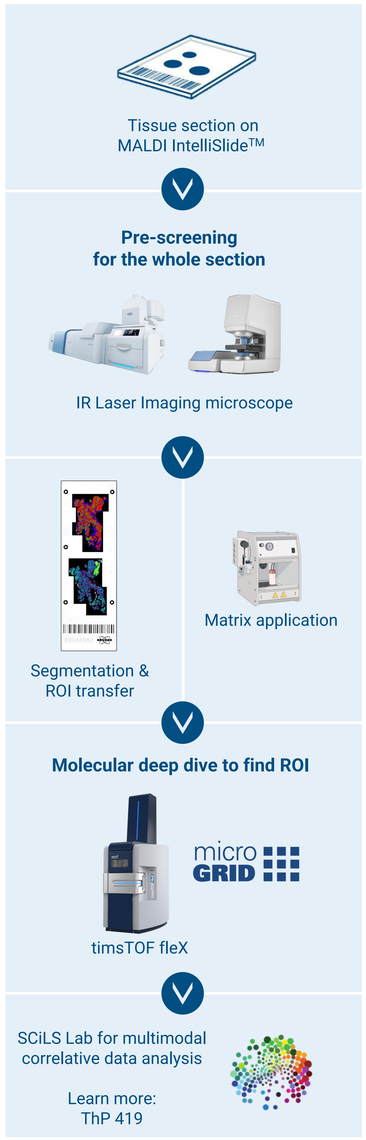

Figure 1. The workflow for multimodal IR Guided MALDI Imaging of pancreatic cancer tissue includes sectioning the tissue samples and pre-screening the entire tissue via ILIM, without further sample preparation. Tissue sections are subsequently spray-coated for MALDI Imaging. For biomarker identification, specific regions of interest (ROI) are defined and measured on the timsTOF fleX instrument. The acquired MALDI Imaging data is then subjected to analysis within SCiLS Lab, where multimodal correlative analysis is performed by integrating IR feature information.

COI Disclosure:

All authors are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumention including mass spectrometers and software used in this study.

Results

Infrared Laser Imaging

- ILIM pre-screening of the whole tissue section was finished in 13 minutes at 9,000 spectra/s.
- Unsupervised segmentation clustered tissue areas with similar IR molecular fingerprints (lipids, metabolites, proteins, glycans). (Figure 2B)
- ROI was narrowed down to discrete specific cell types, including stroma tissue (S), healthy pancreatic acinar cell regions (A), advanced tumor tissue regions (T) and regions related to pancreatic intraepithelial neoplasias (P). (Figure 2C)

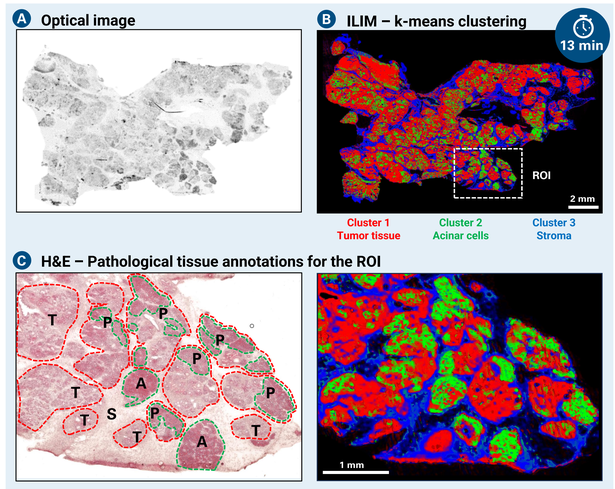

Figure 2. (A) Optical image of the PDAC tissue section. (B) RGB tissue segmentation map based on ILIM k-means clustering. (C) Magnified ROI showing morphological subtypes of PDAC. ILIM clustering results were confirmed by pathological annotation of the adjacent H&E-stained section (T: advanced tumor tissue regions, P: precursor lesions with acinar cells outlined in green, A: discrete healthy acinar cell regions).

MALDI Imaging

- IR Guided MALDI Imaging based on ROI was performed in 7 hours, achieving an acquisition time reduction of 88% compared to untargeted whole slide imaging.
- CCS-enabled analysis delivered confident molecular annotations to support biomarker identification.
- Multimodal statistical data analysis, was enabled by importing the ILIM segmentation map into SCiLS Lab. IR cluster regions were linked with mass spectrometric data (i.e., Pearson correlation) to identify molecular signatures. (Figure 3)
- Oleic acid, monounsaturated phospholipids, and glutathione showed correlation ($r \geq 0.26$) with IR cluster 1 (tumor tissue). In contrast, arachidonic acid and polyunsaturated phospholipids displayed correlation ($r \geq 0.28$) with IR cluster 2 (acinar cells).

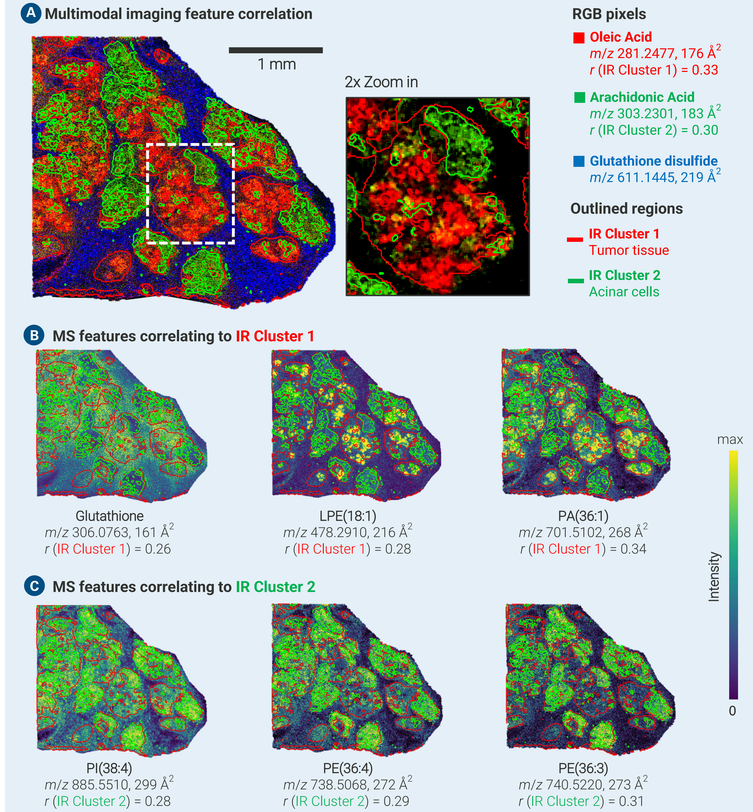

Figure 3. Multimodal feature correlation for PDAC. Region outlines for IR Cluster 1 (red) and IR Cluster 2 (green) were overlayed onto all MS images. (A) RGB MS image for the whole ROI and 2x zoom in for pancreatic intraepithelial neoplasia. (B) Additional metabolites and phospholipids correlating ($r \geq 0.26$) with IR cluster 1 (tumor tissue). (C) Phospholipids correlating ($r \geq 0.28$) with IR cluster 2 (acinar cells).

Conclusion

- Rapid elucidation of tumor-associated tissue areas across the entire section within a few minutes by ILIM.
- Semi-automated and accurate tissue specific guidance for MALDI Imaging.
- Faster sample throughput in combination with maximized analytical depth regarding spatial resolution and CCS-enabled molecular characterization.
- Complementary multimodal information from a single tissue section.

Method Development