Combination of fluorescence and IR images with protein and lipid MALDI Imaging allows insight into disease phenotypes

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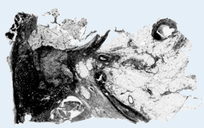
Introduction

The MALDI HiPLEX-IHC antibody-based workflow in combination with spatial multiomics data obtained on our high-resolution timsTOF fleX platform, has opened new and exciting possibilities to answer disease-related spatial biology questions. The possibility to integrate and align MALDI Imaging data with fluorescence images based on dual-labelled antibodies, enables the alignment of individual cells within a tissue. As a further source of information, the HYPERION II ILIM provides infrared laser imaging information and can stratify cancer and inflammatory areas at high spatial resolution within minutes. Here, we combined MALDI Imaging of lipids and targeted proteins, fluorescence imaging, and IR microscopy on a single FFPE section while highlighting the new visualization options in SCiLS Lab 2024b for image overlay to enable a fully integrated analysis (Fig. 1).

Multimodal Workflow



1. Lipid MALDI Imaging
2. HiPLEX-IHC staining (dual labeled fluorescence probes)
3. Fluorescence microscopy
4. IR Laser Imaging
5. MALDI HiPLEX-IHC
6. H&E stain

Figure 1. After lipid imaging (1), HiPLEX staining, including two fluorescence tags, was performed (2) and the two fluorescence channels were scanned (3). Afterwards the IR scan was done (4) and the HiPLEX tags were UV cleaved and measured by MALDI HiPLEX-IHC. Lastly, the tissue was stained by H&E.

COI Disclosure

C.H., B.W., T.B, B.H, A.B. T.Bo., S.D., S.S. and K.S. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.

Methods

An FFPE tissue section of human colorectal cancer was first prepared for lipid imaging. The tissue was incubated at 80°C for 15 min and washed with xylene two times for 5 min each. The slide was measured on a Bruker timsTOF fleX MALDI-2 instrument with microGRID at 20 µm pixel size using DHAP matrix (sprayed with a HTX M3+ sprayer) and measured with MALDI-2. MALDI HiPLEX-IHC staining, using 12 photocleavable mass-tagged antibodies from AmberGen Inc., was performed as previously described [1]. Two antibodies were also labelled by the fluorophores DyLight 650 (Na/K ATPase-α1) and DyLight 550 (Histone H2A.X) and scanned on an Olympus VS200 scanner. Next, the slide was scanned by the HYPERION II ILIM IR microscope. Lastly, after photocleavage of the antibody mass tags, the analysis was performed as described previously, albeit using sublimated 2,5-DHB as the MALDI matrix. All data analysis, annotation, and co-registration was performed in SCiLS™ Lab 2024b. Combined data from lipids and proteins were obtained using the SCiLS Ion Image Mapper tool.

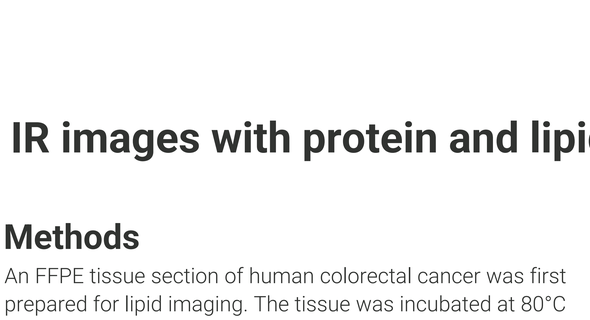


Figure 2. Segmentation analysis based on lipid, HiPLEX and IR measurements. Additionally, a corresponding H&E image is shown.

Results

A spatial multimodal and multiomics workflow was successfully performed on a human colorectal carcinoma sample. Even when utilizing FFPE tissue, lipid classes, including phosphatidylcholines, sphingomyelins, phosphatidylethanolamines, and other species were accessible for analysis. The multimodal segmentation analysis based on the three different datasets (lipids, HiPLEX-IHC, IR) showed comparable results (Figure 2). The IR segmentation is based on the overall abundance of compound classes that exhibit similar IR signatures, such as proteins, lipids, or glycans, that can help indicate cellular specificity.

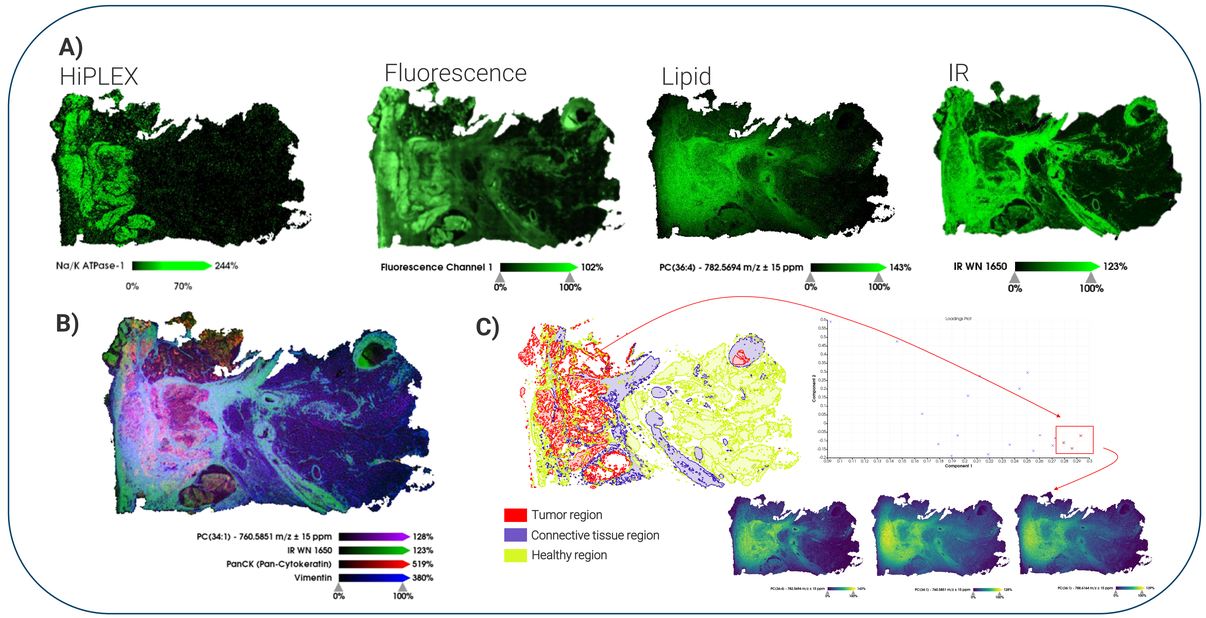


Figure 3. A) NaATPase-1 was analyzed using HiPLEX and fluorescence visualization. Additionally, a co-localization analysis was performed on the lipid dataset and the most equal one was added. The IR wavelength 1650 reflects the overall protein content measured by IR. B) Overlay of all the different channels in one image. C) Combination of HiPLEX-IHC regions (gained by segmentation, red tumor) with lipid PCA results to find tumor relevant lipid markers by selecting component specific (red region) *m/z* values from the loadings plot.

Contrary to the IR segmentation, the HiPLEX-IHC segmentation is exclusively based on specific protein markers for different tissue types. Lastly, the lipid segmentation was based on annotated lipid features. Due to lipid delocalization from the deparaffination process, the segmentation resulted in a less precise morphological representation. PCA analysis of the lipid information allowed the identification from the loadings plot the most variable lipid markers for the tumor region, as defined by the HiPLEX-IHC segmentation. The three most specific lipid markers were annotated as PC(36:4), PC(34:1), PC(36:1) (Fig. 3C). The fluorescence images of the two dual labelled antibodies were co-registered with the MALDI Imaging data and allowed for cross-correlation with the HiPLEX-IHC and lipid images (Fig. 3A). A full multimodal interpretation of the study was enabled within SCiLS Lab.

Reference

[1] Yagnik *et al. J. Am. Soc. Mass Spectrom.* 2021, *32*, 977-988.

Conclusions

- Measurements of lipid MALDI imaging, fluorescence microscopy, IR imaging and HiPLEX-IHC provide complementary information from the same tissue
- Multimodal segmentation was visualized for different analyses and showed clear segmentation of different tumor areas, blood vessels and connective tissue
- Integrative multiomic data analysis within SCiLS Lab allowed identification of variable lipid species within the tumor micronenvironment

Method Development