# A spatial multiomics workflow on a new benchtop MALDI-TOF instrument deciphering the lipid and protein landscape of tissues

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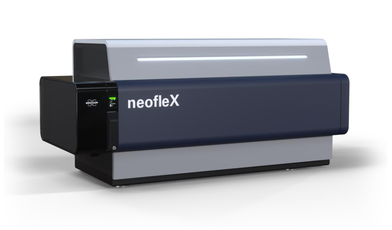
## Introduction

Spatial biology is an emerging field combining spatial cellular information with molecular information of biological tissues, and as such is perfectly complementary to MALDI mass spectrometry imaging (MALDI Imaging). As a non-destructive method, MALDI Imaging can tie various modalities into one dataset by characterizing tissue phenotypes according to lipid or metabolic profiles and integrating spatial targeted protein expression.

Here we present a spatial multiomics workflow combining MALDI Imaging of lipids and MALDI HiPLEX-IHC on a new benchtop axial MALDI-TOF mass spectrometer using a clinically relevant research sample.

## Methods

Formalin-fixed and paraffin-embedded (FFPE) colorectal cancer sections were processed for MALDI Imaging of lipids. Briefly, paraffin was removed by xylene washes and DHB matrix was sprayed using a M3+ sprayer (HTX-Technologies). MALDI Imaging data was acquired on a neofleX Imaging Profiler in positive reflector mode with 20 µm raster width at *m/z* 500-1100. Initial mass calibration was performed on a spot of red phosphorous. During measurement, an online calibration was conducted on endogenous lipid peaks. A consecutive tissue section was measured on the timsTOF fleX to obtain a reference list of annotated lipids for this sample. A MALDI HiPLEX-IHC experiment, using technology from AmberGen, Inc. was conducted on the same section as used before for lipid imaging on the neofleX. The 14 mass tagged antibodies used are listed in (3). After HCCA matrix application, data was acquired on the neofleX using a default acquisition method with 20 µm raster width and SCiLS autopilot for automated setup ensuring controlled instrument conditions for stable measurements. An OME-TIFF file was generated automatically for visualizations in SCiLS Scope. The section was stained with H&E post MALDI, and microscopy images scanned on Hamamatsu nanozoomer. Multimodal data integration, statistical analysis and visualizations were done in SCiLS™ Lab 2024b.



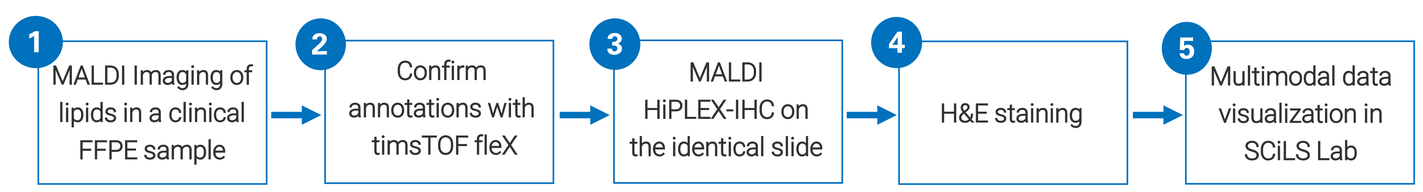




Fig. 1 Spatial multiomics workflow

## Results

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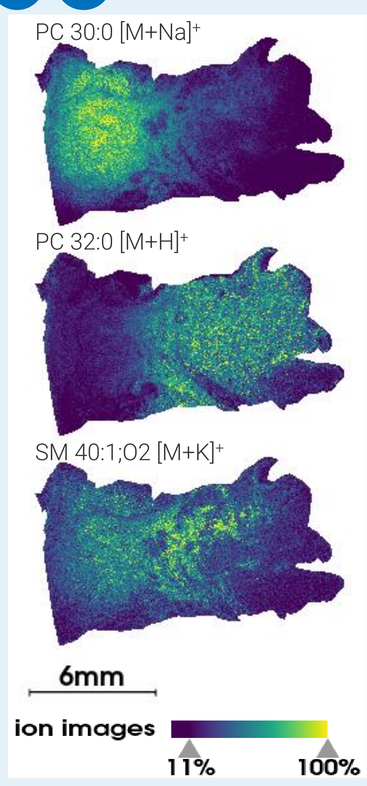


A serial section was measured on a timsTOF fleX and lipids annotated according to exact mass and CCS matching.

| m/z | Name | Notation | Molecular Formula |
|---|---|---|---|
| 518.32 | LPC 16:0 | [M+Na]+ | C24H50NO7P |
| 522.354 | LPC 18:1 | [M+H]+ | C26H52NO7P |
| 524.368 | LPC 18:0 | [M+H]+ | C26H54NO7P |
| 534.293 | LPC 16:0 | [M+K]+ | C24H50NO7P |
| 544.336 | LPC 18:1 | [M+Na]+ | C26H52NO7P |
| 544.338 | LPC 20:4 | [M+H]+ | C28H50NO7P |
| 546.352 | LPC 18:0 | [M+Na]+ | C26H54NO7P |
| 650.43 | HexCer 30:6;O3 | [M+H]+ | C36H59NO9 |
| 672.402 | HexCer 30:6;O3 | [M+Na]+ | C36H59NO9 |
| 688.382 | HexCer 30:6;O3 | [M+K]+ | C36H59NO9 |
| 703.572 | SM 34:1;O2 | [M+H]+ | C39H79N2O6P |
| 706.534 | PC 30:0 | [M+H]+ | C38H76NO8P |
| 711.541 | PE-Cer 36:1;O2 | [M+Na]+ | C38H77N2O6P |
| 714.544 | PC O-32:3 | [M+H]+ | C40H76NO7P |
| 720.551 | PE 34:0 | [M+H]+ | C39H78NO8P |
| 723.542 | SM 34:2;O2 | [M+Na]+ | C39H77N2O6P |
| 725.555 | SM 34:1;O2 | [M+Na]+ | C39H79N2O6P |
| 728.517 | PC 30:0 | [M+Na]+ | C38H76NO8P |
| 731.604 | SM 36:1;O2 | [M+H]+ | C41H83N2O6P |
| 732.551 | PC 32:1 | [M+H]+ | C40H78NO8P |
| 734.567 | PC 32:0 | [M+H]+ | C40H80NO8P |
| 741.529 | SM 34:1;O2 | [M+K]+ | C39H79N2O6P |
| 742.568 | PC O-32:0 | [M+Na]+ | C40H82NO7P |
| 744.491 | PC 30:0 | [M+K]+ | C38H76NO8P |
| 748.584 | PE 36:0 | [M+H]+ | C41H82NO8P |
| 753.584 | SM 36:1;O2 | [M+Na]+ | C41H83N2O6P |
| 754.532 | PC 32:1 | [M+Na]+ | C40H78NO8P |
| 756.55 | PC 32:0 | [M+Na]+ | C40H80NO8P |
| 758.566 | PC 34:2 | [M+H]+ | C42H80NO8P |

| m/z | Name | Notation | Molecular Formula |
|---|---|---|---|
| 759.638 | SM 38:1;O2 | [M+H]+ | C43H87N2O6P |
| 760.582 | PC 34:1 | [M+H]+ | C42H82NO8P |
| 768.586 | PC O-34:1 | [M+Na]+ | C42H84NO7P |
| 770.506 | PC 32:1 | [M+K]+ | C40H78NO8P |
| 772.524 | PC 32:0 | [M+K]+ | C40H80NO8P |
| 774.598 | PE 38:1 | [M+H]+ | C43H84NO8P |
| 780.548 | PC 34:2 | [M+Na]+ | C42H80NO8P |
| 782.564 | PC 34:1 | [M+Na]+ | C42H82NO8P |
| 785.648 | SM 40:2;O2 | [M+H]+ | C45H89N2O6P |
| 786.598 | PC 36:2 | [M+H]+ | C44H84NO8P |
| 787.666 | SM 40:1;O2 | [M+H]+ | C45H91N2O6P |
| 788.612 | PC 36:1 | [M+H]+ | C44H86NO8P |
| 796.58 | PC 34:2 | [M+K]+ | C43H84NO8P |
| 798.538 | PC 34:1 | [M+K]+ | C42H82NO8P |
| 807.631 | SM 40:2;O2 | [M+Na]+ | C45H89N2O6P |
| 808.578 | PC 36:2 | [M+Na]+ | C44H84NO8P |
| 809.648 | SM 40:1;O2 | [M+Na]+ | C45H91N2O6P |
| 810.596 | PC 36:1 | [M+Na]+ | C44H86NO8P |
| 813.683 | SM 42:2;O2 | [M+H]+ | C47H93N2O6P |
| 815.696 | SM 42:1;O2 | [M+H]+ | C47H95N2O6P |
| 824.553 | PC 36:2 | [M+K]+ | C44H84NO8P |
| 825.62 | SM 40:1;O2 | [M+K]+ | C45H91N2O6P |
| 826.565 | PC 36:1 | [M+K]+ | C44H86NO8P |
| 832.579 | PC 38:4 | [M+Na]+ | C46H84NO8P |
| 833.646 | SM 42:3;O2 | [M+Na]+ | C47H91N2O6P |
| 835.664 | SM 42:2;O2 | [M+Na]+ | C47H93N2O6P |
| 837.677 | SM 42:1;O2 | [M+Na]+ | C47H95N2O6P |
| 851.636 | SM 42:2;O2 | [M+K]+ | C47H93N2O6P |
| 853.653 | SM 42:1;O2 | [M+K]+ | C47H95N2O6P |

↑ Example ion images from neofleX.

↓ Mean spectrum with annotated features indicated by a triangle.

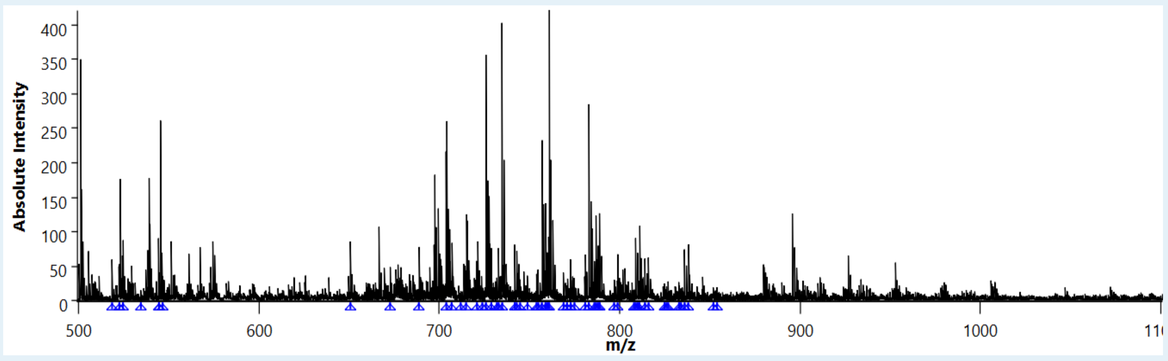


Fig. 2 MALDI Imaging of lipids of a colorectal cancer sample and confirmation of annotations.

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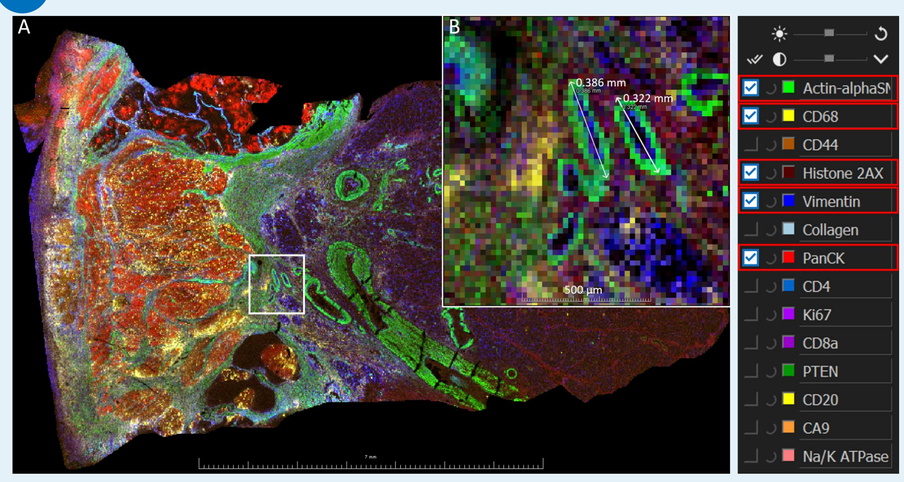


A) A 14-plex HiPLEX experiment with the antibodies indicated on the right of the figure was conducted on the same colorectal cancer tissue section used for MALDI Imaging of lipids. An **OME-TIFF** file was automatically generated and visualized in the new software, **SCiLS Scope**. The ion images of the mass tags from the selected antibodies were visualized by false-color coding in a multichannel image. High intensity of tumor marker PanCK (pan cytokeratin) was observed in a specific region (red) where also immune cells infiltrated (CD68, macrophage marker, yellow). B) SCiLS Scope enables measurement of distances as shown here for two blood vessels.

Fig. 3 MALDI HiPLEX-IHC of the same sample

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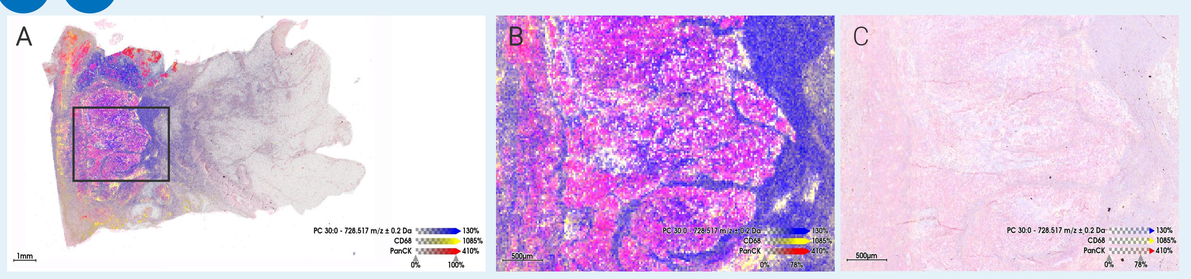
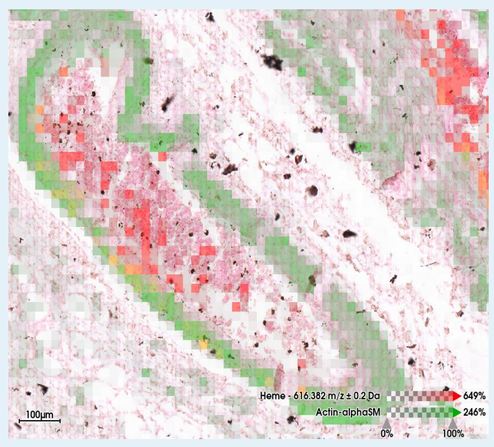



A) Overlay of three modalities in SCiLS Lab 2024b integrating lipid distribution, expressed protein landscapes and the optical microscopy image of the same tissue section after H&E staining in one dataset. The lipid PC_30:0 colocalizes with the distribution of tumor marker PanCK. CD68 is a marker for macrophages and indicates immune cell infiltration. B) Zoom into the tumor area as indicated by a rectangular in A). C) Same image as B) applying 90% transparency so that the underlying histology can be seen. Linear transparency was applied to all images making low intensity pixel transparent.




With the **high-resolution optical image** support, a pyramidal, multi-resolution file of the optical image is stored externally. This file can be accessed and visualized within SCiLS Lab.

Here, a 40-fold magnification of the H&E microscopy image of the colorectal cancer tissue section is combined with the MALDI HiPLEX-IHC image of Actin-alpha SM and with the MALDI image of heme zooming on a blood vessel. As expected, Actin-alpha SM was detected on the epithelial cell layer while heme was detected with high intensity within the blood vessel.

Fig. 4 MALDI Imaging of lipids of a colorectal cancer sample and confirmation of annotations.

## Conclusion

- Our new spatial multiomics workflow on the neofleX Imaging Profiler provides insight into the lipid and targeted protein expression landscapes of tissues
- SCiLS Lab 2024b enables multimodal data integration and high-resolution optical image support
- Automatically generated OME-TIFF files can readily be visualized with SCiLS Scope

**Imaging: Spatially-Resolved Omics**