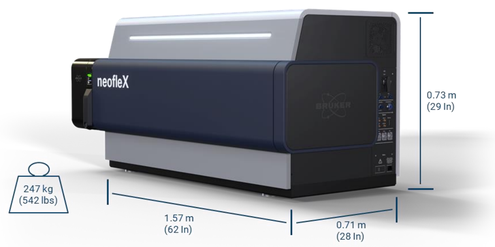
A spatial multiomics workflow on a new benchtop MALDI-TOF instrument for deciphering the protein and lipid landscape in tissues



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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.

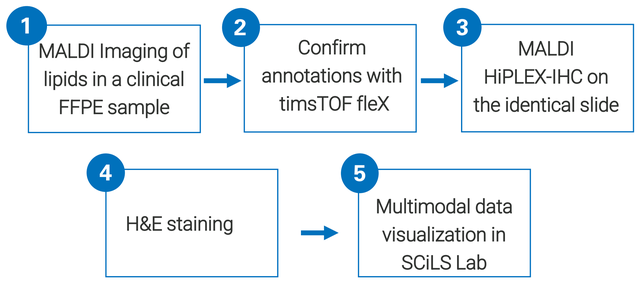
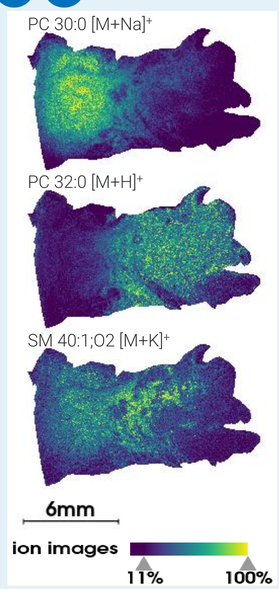


Figure 1 Spatial multiomics workflow

1 2



Results:

m/z	Name	Notation	Molecular Formula	m/z	Name	Notation	Molecular Formula
518.32	LPC 16:0	[M+Na]+	C24H50NO7P	759.638	SM 38:1;02	[M+H]+	C43H87N2O6P
522.354	LPC 18:1	[M+H]+	C26H52NO7P	760.582	PC 34:1	[M+H]+	C42H82NO8P
524.368	LPC 18:0	[M+H]+	C26H54NO7P	768.586	PC O-34:1	[M+Na]+	C42H84NO7P
534.293	LPC 16:0	[M+K]+	C24H50NO7P	770.506	PC 32:1	[M+K]+	C40H78NO8P
544.336	LPC 18:1	[M+Na]+	C26H52NO7P	772.524	PC 32:0	[M+K]+	C40H80NO8P
544.338	LPC 20:4	[M+H]+	C28H50NO7P	774.598	PE 38:1	[M+H]+	C43H84NO8P
546.352	LPC 18:0	[M+Na]+	C26H54NO7P	780.548	PC 34:2	[M+Na]+	C42H80NO8P
650.43	HexCer 30:6;03	[M+H]+	C36H59NO9	782.564	PC 34:1	[M+Na]+	C42H82NO8P
672.402	HexCer 30:6;03	[M+Na]+	C36H59NO9	785.648	SM 40:2;02	[M+H]+	C45H89N2O6P
688.382	HexCer 30:6;03	[M+K]+	C36H59NO9	786.598	PC 36:2	[M+H]+	C44H84NO8P
703.572	SM 34:1;02	[M+H]+	C39H79N2O6P	787.666	SM 40:1;02	[M+H]+	C45H91N2O6P
706.534	PC 30:0	[M+H]+	C38H76NO8P	788.612	PC 36:1	[M+H]+	C44H86NO8P
711.541	PE-Cer 36:1;02	[M+Na]+	C38H77N2O6P	796.58	PC 34:2	[M+K]+	C43H84NO8P
714.544	PC O-32:3	[M+H]+	C40H76NO7P	798.538	PC 34:1	[M+K]+	C42H82NO8P
720.551	PE 34:0	[M+H]+	C39H78NO8P	807.631	SM 40:2;02	[M+Na]+	C45H89N2O6P
723.542	SM 34:2;02	[M+Na]+	C39H77N2O6P	808.578	PC 36:2	[M+Na]+	C44H84NO8P
725.555	SM 34:1;02	[M+Na]+	C39H79N2O6P	809.648	SM 40:1;02	[M+Na]+	C45H91N2O6P
728.517	**PC 30:0**	**[M+Na]+**	**C38H76NO8P**	810.596	PC 36:1	[M+Na]+	C44H86NO8P
731.604	SM 36:1;02	[M+H]+	C41H83N2O6P	813.683	SM 42:2;02	[M+H]+	C47H93N2O6P
732.551	PC 32:1	[M+H]+	C40H78NO8P	815.696	SM 42:1;02	[M+H]+	C47H95N2O6P
734.567	**PC 32:0**	**[M+H]+**	**C40H80NO8P**	824.553	PC 36:2	[M+K]+	C44H84NO8P
741.529	SM 34:1;02	[M+K]+	C39H79N2O6P	**825.62**	**SM 40:1;02**	**[M+K]+**	**C45H91N2O6P**
742.568	PC O-32:0	[M+Na]+	C40H82NO7P	826.565	PC 36:1	[M+K]+	C44H86NO8P
744.491	PC 30:0	[M+K]+	C38H76NO8P	832.579	PC 38:4	[M+Na]+	C46H84NO8P
748.584	PE 36:0	[M+H]+	C41H82NO8P	833.646	SM 42:3;02	[M+Na]+	C47H91N2O6P
753.584	SM 36:1;02	[M+Na]+	C41H83N2O6P	835.664	SM 42:2;02	[M+Na]+	C47H93N2O6P
754.532	PC 32:1	[M+Na]+	C40H78NO8P	837.677	SM 42:1;02	[M+Na]+	C47H95N2O6P
756.55	PC 32:0	[M+Na]+	C40H80NO8P	851.636	SM 42:2;02	[M+K]+	C47H93N2O6P
758.566	PC 34:2	[M+H]+	C42H80NO8P	853.653	SM 42:1;02	[M+K]+	C47H95N2O6P

Example ion images from neofleX.

Mean spectrum with annotated features indicated by a triangle.

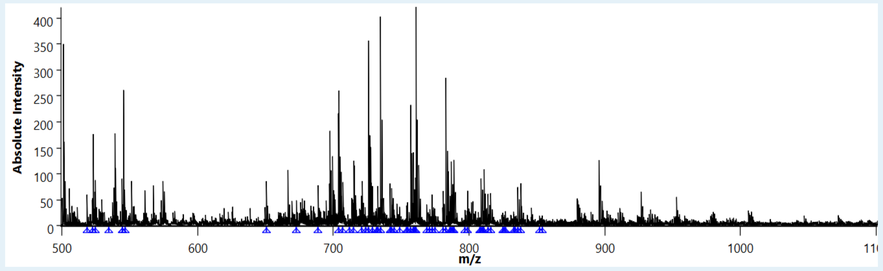


Figure. 2 MALDI Imaging of lipids of a colorectal cancer sample and confirmation of annotations.

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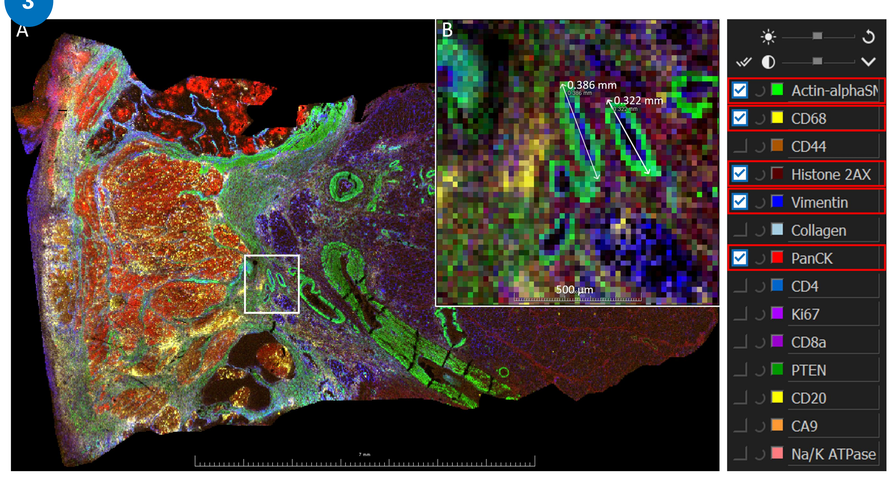
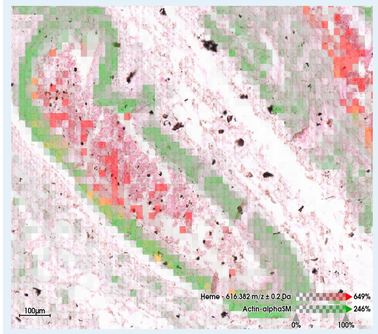


Figure 3 MALDI HiPLEX-IHC of the same tissue in Figure 2

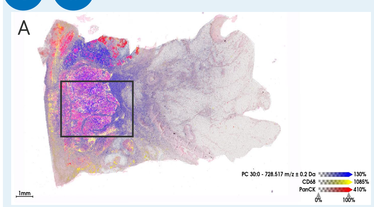
A) A 14-plex HiPLEX experiment with the antibodies indicated on the right of the figure was conducted on the same colorectal cancer tissue section used for MALDI Imaging of lipids. An **OME-TIFF** file was automatically generated and visualized in the new software, **SCiLS Scope**. The ion images of 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.

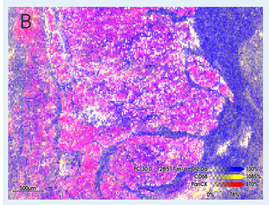


With the **high-resolution optical image** support, a pyramidal, multi-resolution file of the optical image is stored externally. This file can be accessed and visualized within SCiLS Lab.

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.

4 5





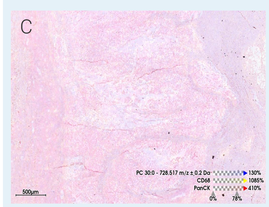


Figure 4 MALDI Imaging of lipids of a colorectal cancer sample and confirmation of annotations.

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 rectangle 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.

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

Technology

COI Disclosure: C.L., J.O., C.H., D.N., U.S.H., T.B., S.B., C.N., A.S., K.S., M.E., A.K., are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation and products, including mass spectrometers and software used in this study.

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