MALDI Imaging lipid analysis integrated with histological data for a complete spatial-contextual evaluation

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Histomorphology is key to interpreting MALDI imaging data

In MALDI imaging data analysis, histological evaluation is a key aspect that serves to provide biological context to the imaging results. Therefore, it is critical that software solutions exist to support the integration of histological data with MALDI imaging data.

QuPath is an open-source pathology platform for bioimage analysis [1]. While incredibly powerful for pathological analyses, the software does not work with MALDI imaging data.

Recently, a QuPath plug-in was released for the MALDI imaging data analysis software SCiLS Lab, making it possible to import histological annotations into SCiLS Lab for downstream analysis.

In this study, we demonstrated successful application of this workflow to a rat kidney that revealed specific lipid accumulations in the glomeruli.

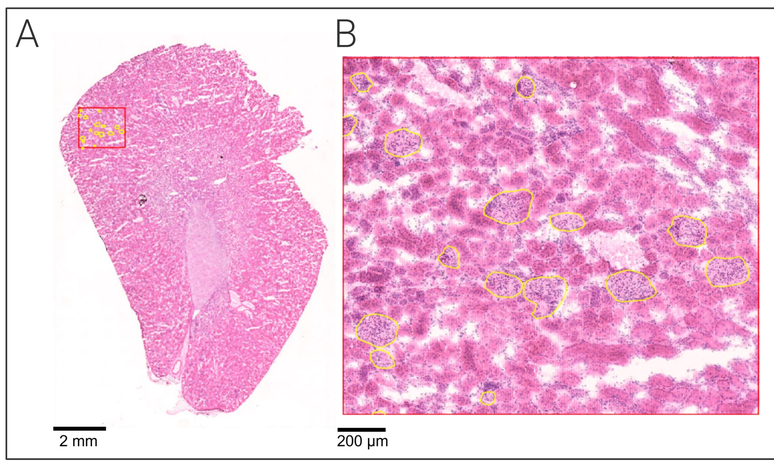

Fig. 1 Hematoxylin and eosin (H&E) image of the frozen rat kidney section used for analysis. Region annotations were performed in QuPath. Glomeruli were localized and borders defined manually with the QuPath polygon tool. Entire section (A) and zoom in of the MALDI imaging measurement area (B). Glomeruli regions are delineated in yellow and the measurement area with a red rectangular.

Methods

A rat kidney section was sublimed with DHB matrix. Lipid imaging data was acquired on a timsTOF fleX instrument (Bruker) at 10 µm pixel size in positive QTOF mode. The data were then imported to SCiLS Lab 2022b (Bruker). After MALDI Imaging, the very same section was stained with H&E and a high-resolution microscopy image was scanned for histological evaluation in QuPath 0.3.2. Glomeruli regions were defined in QuPath based on the histology and exported back to SCiLS. Lipids were automatically annotated using MetaboScape 2022. The SCiLS Lab "co-localization to region" tool was applied to detect annotated lipids occurring with high abundance in glomeruli regions. On-tissue MS/MS was performed on two lipids to confirm automatic annotations.

Results

The aim of this study was an applications test of the QuPath to SCiLS plug-in as a software environment to include histological region annotations in MALDI imaging statistical data analysis.

Glomeruli regions in a rat kidney sample were first defined in QuPath (Fig. 1). After export of these regions back to SCiLS Lab, the corresponding pixel and their spectra can be utilized for statistical data analysis as used here for a co-localization analysis.

To do so, lipid names were assigned to MALDI imaging ion signals using MetaboScape. A list of 75 annotated features comprising phosphatidylcholine (PCs), phosphatidylethanolamines (PEs) and SMs was the outcome of this analysis, as expected in positive mode MALDI imaging data (not shown).

Eight ion species with assigned names occurred with high abundance in the renal glomeruli and were detected by the SCiLS co-localization tool (Tab. 1, Fig. 2).

Putative PC 36:2 and SM 40:1;O2 were found to be localized in the anatomical fine structures of the renal corpuscle (Fig. 3), demonstrating the importance of integrating the histological context for MALDI imaging data interpretation.

Tab. 1. Tentatively assigned lipids found specifically in the glomeruli regions using MetaboScape. The given correlation value is a measure of how well the ion image correlates with the region annotation (correlation value = 1, perfect correlation; correlation value = 0, no correlation; correlation value = -1, perfect anti-correlation).

Number	m/z	Name	Neutral Mass	Notation	Formula	Δm/z [mDa]	Correlation value
1	518.3212	lysoPE 19:0	495.3321	[M+Na]+	C24H50NO7P	-0.55	0.28
2	546.3527	lysoPE 21:0	523.3636	[M+Na]+	C26H54NO7P	-0.10	0.26
3	796.5246	PC 34:2	757.5611	[M+K]+	C42H80NO8P	-0.02	0.29
4	824.5555	PC 36:2	785.5927	[M+K]+	C44H84NO8P	-0.10	0.45
5	731.6060	SM 36:1;O2	730.5985	[M+H]+	C41H83N2O6P	-2.07	0.29
6	753.5875	SM 36:1;O2	730.5985	[M+Na]+	C41H83N2O6P	-0.67	0.30
7	809.6499	SM 40:1;O2	786.6607	[M+Na]+	C45H91N2O6P	-0.67	0.27
8	825.6237	SM 40:1;O2	786.6606	[M+K]+	C45H91N2O6P	-1.75	0.34

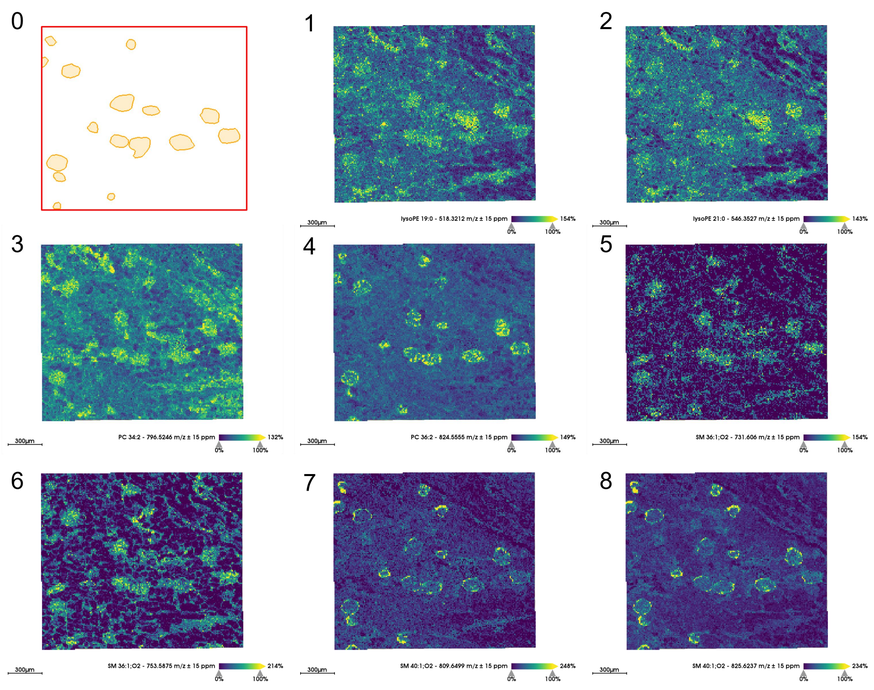

Fig. 2 Lipid ion species colocalizing with annotated glomeruli regions as defined by QuPath (panel 0). The numbering corresponds to the order listed in Table 1.

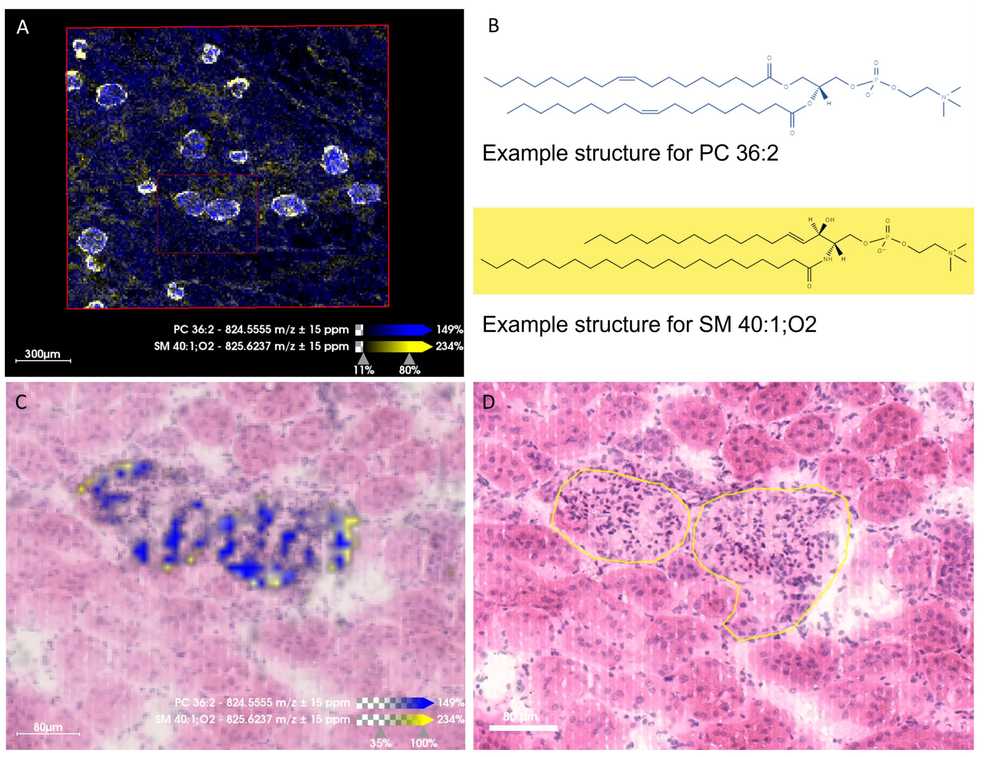

Fig. 3 . Lipids in the renal corpuscles. Two-channel image of a putative PC 36:2 (in blue) and SM 40:1;O2 (in yellow) in the cortex of the measured rat kidney sample (A). Enlarged view of two renal corpuscles as indicated in the inlet in A (C). Low intensity pixels were made transparent. H&E stain and region annotations in QuPath for the same area (D). SM 40:1;O2 (yellow) was present in the parietal layer of the Bowman's capsule while PC 36:2 was mainly found in the interior of the renal corpuscle. Example structures for both compounds, PC 18:1/18:1 and SM 16:1/22:0 are shown in (B).

Conclusion

- It is important to correlate data with context, especially for fine anatomical structures and high spatial resolution MALDI imaging.
- The QuPath to SCiLS plug-in provides an integrated software solution to connect MALDI imaging data with histology.
- The SCILS Lab tools for statistical data analysis make it easy to find compound distributions associated with relevant histopathological regions.

Reference:
1. Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. Scientific Reports (2017). https://doi.org/10.1038/s41598-017-17204-5