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

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Histomorphology is key to interpret 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 renal corpuscule.

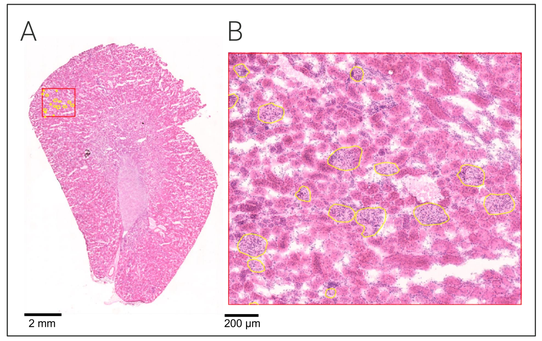

Fig. 1 Hematoxylin and eosin (H&E) image of the frozen rat kidney section. Region annotations were performed in QuPath. Renal corpuscules were localized and borders defined manually with the QuPath polygon tool. Entire section (A) and zoom in of the MALDI imaging measurement area (B). Renal corpuscule regions are delineated in yellow and the measurement area in 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 imported to SCiLS Lab 2022b (Bruker). 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. Renal corpuscule 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 renal corpuscule regions. On-tissue MS/MS was performed on two lipids to confirm automatic annotations.

Tab. 1. Tentatively assigned lipids using MetaboScape which were found specifically in the renal corpuscule regions. The given correlation value is a measure 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	NeutralMass	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

Results

- Histology can be linked with MALDI imaging data through the QuPath integration with SCiLS Lab (Fig. 1, Fig. 3).
- MetaboScape served as a tool for automatic assignment of names to lipids in MALDI imaging data and resulted in a list of 75 lipid features .
- Eight ion species with assigned names occurred with high abundance in renal glomeruli and were detected by the SCiLS co-localization tool (Tab. 1, Fig. 2).
- We confirmed the annotations of sphyingomyelin (SM) 36:1;O2 and SM 40:1;O2 by on-tissue MALDI TIMS MS/MS analysis in glomeruli (not shown).

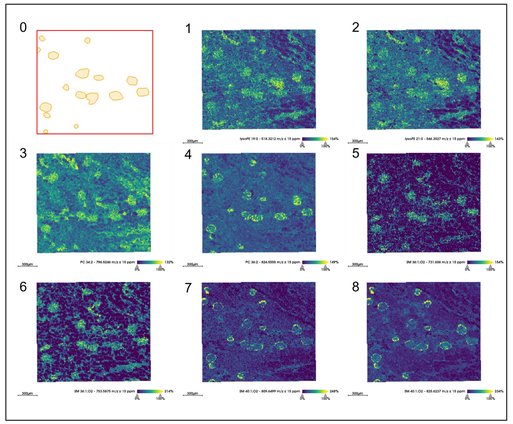

Fig. 2 Lipid ion species colocalizing with annotated renal corpuscule regions as defined by QuPath (panel 0). The numbering corresponds to the order listed in Table 1.

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

Renal corpuscule 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.

For that, 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.

The distribution maps of eight ion species including adducts, or six different lipids correlated well with the renal corpuscule regions (Tab. 1, Fig. 2).

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

Summary

The QuPath plug-in to SCiLS Lab is the first fully integrated tool for combining histology with MALDI imaging statistical analysis.

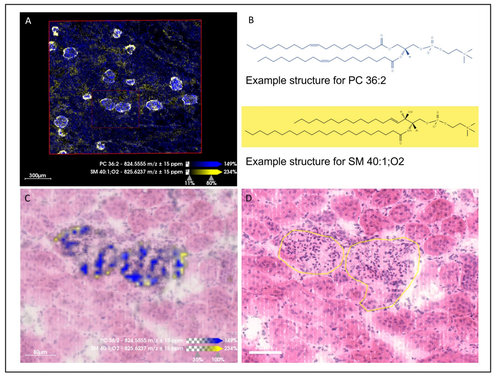

Fig. 3 . Lipids in the renal corpuscles. Two-channel image of a putative PC 36:2 (in blue) and SM 40:1;O2 (in yellow) in the cortex of the measured rat kidney sample (A). Enlarged view of two renal corpuscules as indicated in the inlet in A (C). Low intensity pixels were made transparent. H&E stain and 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).

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

Conclusion

- 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 makes it easy to find compound distributions associated with relevant histopathological regions.
- It is important to correlate data with context especially for fine anatomical structures and high spatial resolution MALDI imaging.

Technology