Integration and Joint Multivariate Analysis of Multimodal Chemical Imaging Data of Hepatocellular Carcinoma in Rat

Katharina Kronenberg[1], Sören-Oliver Deininger[2], Marten Seeba[3], Julia Werner[4], Peter Bohrer[4], Rickmer F Braren[4], Fabian K Lohöfer[4], Heiko Neuweger[2] and Uwe Karst[1], [1]University of Muenster, Institute of Inorganic and Analytical Chemistry, Münster, Germany; [2]Bruker Daltonics GmbH & Co.KG, Bremen, Germany; [3]Bruker Optics GmbH & Co. KG, Ettlingen, Germany; [4]Institute of Diagnostic and Interventional Radiology, School of Medicine, Technical University of Munich, Munich, Germany

Introduction

A plethora of multivariate imaging modalities are available to gain molecular information on tissue sections, all of which visualize different molecular aspects of the sample and therefore are complementary to each other. While it is already common to subject the same sample to different imaging techniques, the joint analysis of different modalities still poses challenges. Specifically, the integration of multiple modalities into a single dataset to find cross-modality correlations which is limited by the availability of accessible software approaches. Here, we present a flexible software workflow that enables the multivariate analyses on multimodal measurements.

Methods

Data were acquired from a rat hepatocellular carcinoma tissue section using the following instruments: CCS-enabled MALDI Imaging: timsTOF fleX at 20 µm spatial resolution (Bruker Daltonics, Bremen, Germany); LA-ICP-MS: iCAP TQ ICP-MS, 25 µm (Thermo Fisher Scientific, Bremen, Germany) coupled to an LSX 213 G2+ laser system (CETAC Technologies, Omaha, NE, USA); µXRF: M4 TORNADO, 30 µm (Bruker Nano, Berlin, Germany), IR imaging: Hyperion II-ILIM, 5 µm (Bruker Optics, Ettlingen, Germany). XRF and IR data were collected from the same section. The data were combined into a single SCiLS™ Lab (Bruker Daltonics, Germany) dataset. Details on the background and sample preparation can be found in [1].

COI Disclosure

Authors SO.D., M.S. and H.N. are employees of Bruker Corporation or one of its subsidiaries ("Bruker"). Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.

Brief Description of the Workflow

1. Create SCiLS Lab dataset from the MALDI imaging measurements.
2. Convert one representative chemical channel of each of the other modalities into an optical image with the native resolution.
3. Co-register the optical images from the previous step into SCiLS Lab as optical images for alignment with the MALDI Imaging data.
4. Retrieve the transformation matrices for the co-registered optical images via the SCILS Lab API.
5. Use an R or Python script to resample all the channels of the other modalities to the SCiLS Lab spot grid and write those intensities into SCiLS Lab as external features.

Note: Since version 2024b, the SCiLS Lab API documentation contains complete example code for the above workflow in both R and Python.

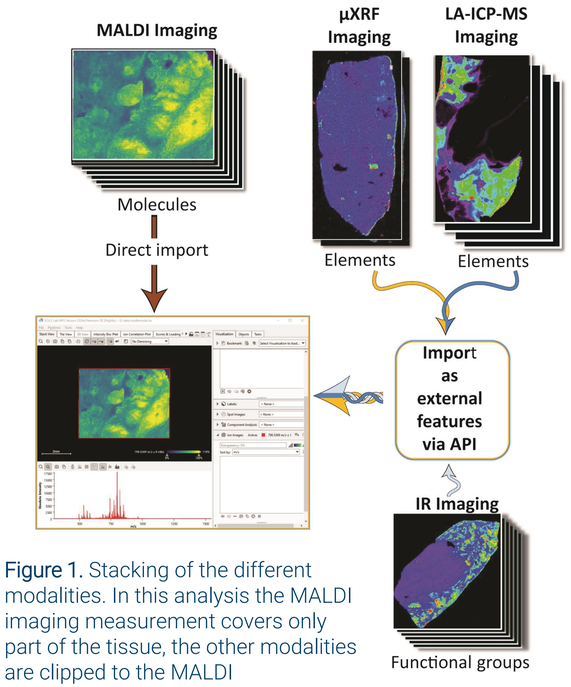

Figure 1. Stacking of the different modalities. In this analysis the MALDI imaging measurement covers only part of the tissue, the other modalities are clipped to the MALDI measurement.

Results

Table 1. Correlation table with selected features from each modality. For MALDI- TIMS, m/z and ccs indicate m/z in Da and collision cross section in Å², for LA-ICP-MS and µXRF, the measured element is indicated, for IR measurements the wavenumber is shown.

	m/z:741.527_ccs:287.7	m/z:760.58_ccs:288.913	µXRF Fe	LA-ICP-MS Fe	IR WN 1238	LA-ICP-MS Gd	LA-ICP-MS Pt	m/z:848.548_ccs:298.577	m/z:796.521_ccs:289.161	m/z:824.551_ccs:295.57	m/z:758.564_ccs:285.919	IR WN 1030
m/z:741.527 ccs:287.7	1.00	0.14	-0.18	-0.15	-0.47	-0.56	-0.65	-0.67	-0.54	-0.61	-0.61	-0.52
m/z:760.58 ccs:288.913	0.14	1.00	-0.08	-0.08	-0.11	-0.11	-0.18	-0.48	-0.37	-0.16	0.06	-0.10
µXRF Fe	-0.18	-0.08	1.00	0.33	0.20	0.23	0.25	0.22	0.10	0.12	0.15	0.10
LA-ICP-MS Fe	-0.15	-0.08	0.33	1.00	0.14	0.23	0.26	0.20	0.09	0.11	0.14	0.08
IR WN 1238	-0.47	-0.11	0.20	0.14	1.00	0.58	0.55	0.50	0.37	0.35	0.47	0.49
LA-ICP-MS Gd	-0.56	-0.11	0.23	0.23	0.58	1.00	0.85	0.54	0.31	0.31	0.56	0.28
LA-ICP-MS Pt	-0.65	-0.18	0.25	0.26	0.55	0.85	1.00	0.66	0.47	0.46	0.63	0.50
m/z:848.548 ccs:298.577	-0.67	-0.48	0.22	0.20	0.50	0.54	0.66	1.00	0.67	0.70	0.57	0.47
m/z:796.521 ccs:289.161	-0.54	-0.37	0.10	0.09	0.37	0.31	0.47	0.67	1.00	0.72	0.65	0.50
m/z:824.551 ccs:295.57	-0.61	-0.16	0.12	0.11	0.35	0.31	0.46	0.70	0.72	1.00	0.51	0.44
m/z:758.564 ccs:285.919	-0.61	0.06	0.15	0.14	0.47	0.56	0.63	0.57	0.65	0.51	1.00	0.52
IR WN 1030	-0.52	-0.10	0.10	0.08	0.49	0.28	0.50	0.47	0.50	0.44	0.52	1.00

Since not all datasets were acquired from the same tissue section, distortion between the modalities was observed. Still, the registration was viable to perform cross-modality correlation analysis. The rat was treated with Cisplatin and underwent a contrast-enhanced MRI scan prior to tissue collection. Since both platinum and the gadolinium from the contrast agent was seen in the ICP-MS dataset, one can look for which molecular features from the MALDI measurement are most or least correlated with those elements. Similarly, it can be analyzed which molecular features correlate most or least with the infrared signal at 1030 cm^{-1} (characteristic for glycogen) or 1238 cm^{-1} (nucleic acids). Lastly, the iron signals from µXRF and ICP-MS correlated most with each other. These findings are summarized in Table 1.

With the combined dataset, it was also possible to perform cross-modality multivariate analyses, such as principal component analysis (data not shown) or spatial segmentation. A spatial segmentation analysis based on a selection of features from all modalities is shown in Figure 2, which shows agreement with the histology.

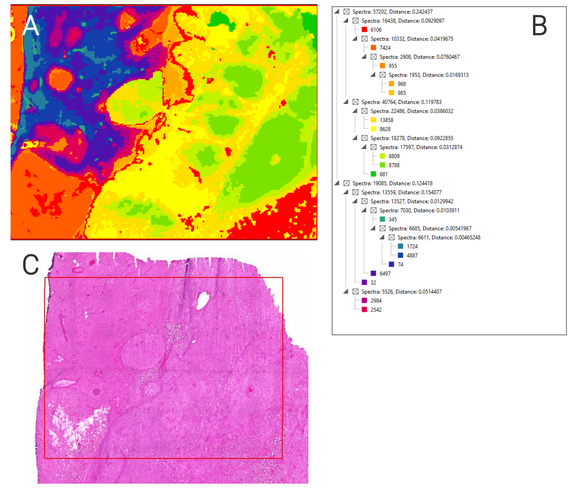

Figure 2. Spatial segmentation based on combined features from all modalities (MALDI: 17, IR: 7 µXRF: 2, LA-ICPMS: 6 features). A) Segmentation map, B) Segmentation tree, C) Microscopy image of H&E stained section with the relevant tissue area outlined in red.

Summary

Integration of multimodal data into single datasets provides a complete way to mine information across modalities.

Reference

[1] Kronenberg K, Werner J, et al. ChemRxiv. 2023; doi:10.26434/chemrxiv-2023-85hbd

Conclusion

- Possible to combine features from multiple modalities into a single dataset, which enabled the quantification of correlations across modalities
- Multivariate analysis such as spatial segmentation using information from different modalities mimics expected tissue morphology

MALDI Imaging