MALDI mass spectrometry imaging guided LC-MS on the same instrument

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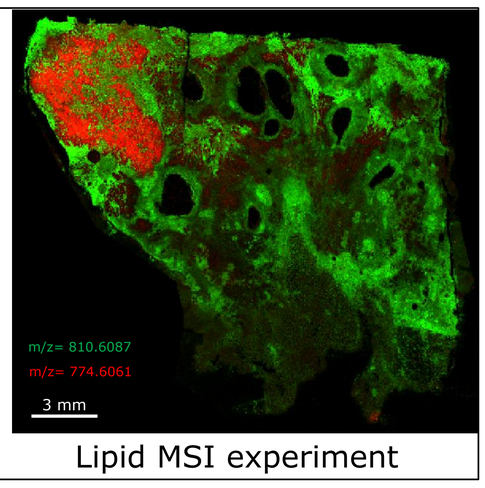
Introduction

Mass spectrometry imaging (MSI) is an analytical technique for the unlabeled and multiplex analysis of molecular spatial distributions in biological tissues. Unfortunately, due to lack of purification steps, the analytical depth of MSI for a comprehensive *in situ* molecular characterization is still limited [1]. Liquid chromatography mass spectrometry (LC-MS), in contrast, gives the possibility to obtain proteomic or metabolomic data for thousands of molecules even from smallest amounts of samples [2]. In the presented project, the goal is to combine MSI and LC-MS by designing a pipeline where MSI can guide a laser microdissection system (LMD) to accurately isolate regions of interest (ROIs) and perform microproteomics on the same instrument.

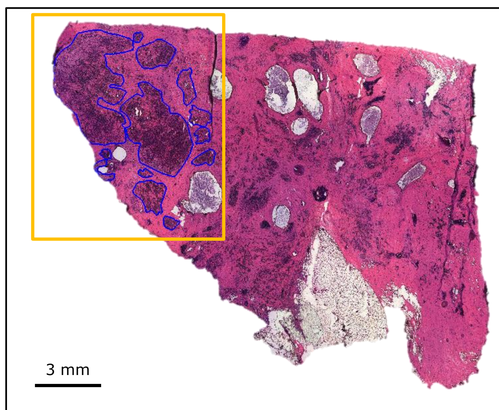
I. Lipid MSI of breast cancer tissue section

Tims TOF Flex

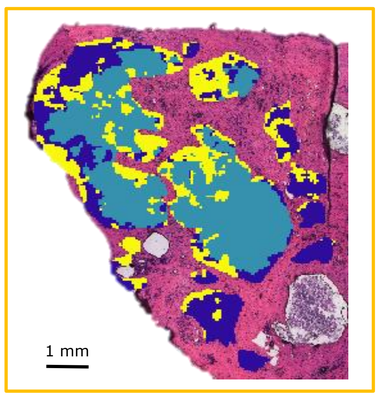


Lipid MSI experiment

II. Segmentation of tumor areas



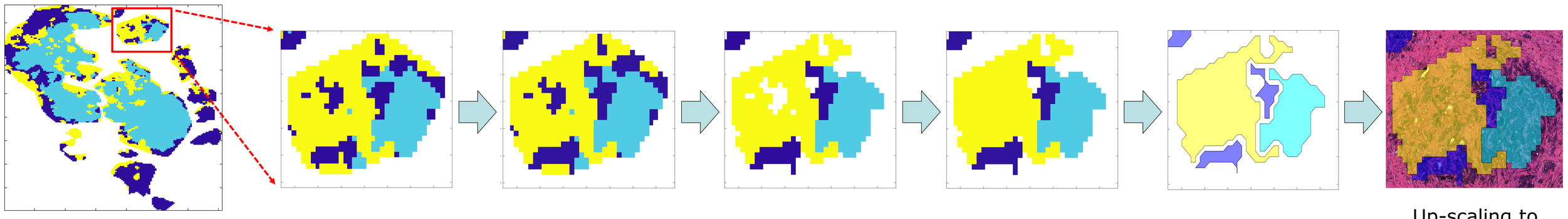
Pathologist annotations



k-means segmentation

MSI experiment of lipids was performed on a fresh frozen breast tumor section mounted onto a PEN (PolyEthylene Naphthalate) membrane slide compatible with LMD. The data were acquired in positive ionization mode at 50 µm pixel size on a tims TOF flex (Bruker Daltonik GmbH, Bremen, Germany). The MSI data were then imported into SCiLS Lab MVS 2019c (Bruker Daltonik) for data analysis. K-means segmentation (k=3) was performed on the annotated tumor areas by a pathologist.

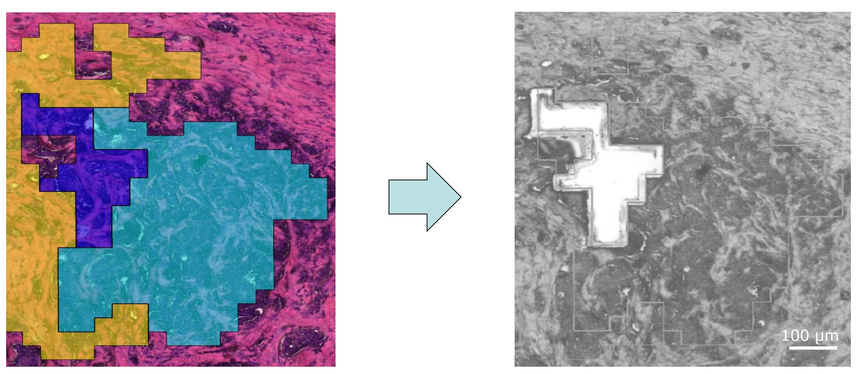
III. Image processing of segmentation



Segmentation image | Raw data | Smoothing | Removal of small objects | Filling holes | Boundary detection | Up-scaling to optical image

In order to increase the viability of microdissection, the segmentation data of the tumor areas was first exported from SCiLS Lab (.csv) and then imported into Matlab R2018a for image processing. First, a smoothing was performed by *opening* the image with a 2x2 square as structuring element (*imopen*). Then, small objects composed of less than 30 pixels based on a 4-connected neighborhood were removed (*bwareaopen*) and holes were filled in the 8-connected neighborhood (*imfill*). Finally, the boundaries (as polygonal areas) of the MSI segments were determined and up-scaled to the resolution of the optical image for a later coregistration to the LMD system.

IV. Coregistration of MSI data to LMD



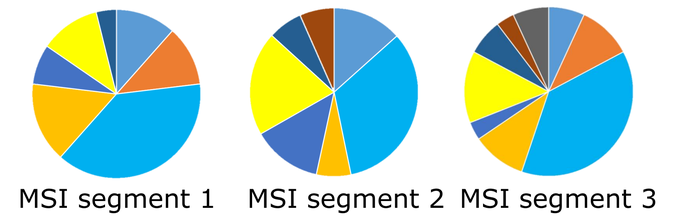
Coregistration to consecutive section and LMD

The coordinates of the MSI segments were then transferred to the LMD system (LMD 7000, Leica Microsystems GmbH, Wetzlar, Germany) by recalculating the boundary coordinates using one common reference point that is visible in the LMD as well as in the digital optical image [3].

V. Microproteomics analysis of microdissected MSI segments

Microproteomics analysis using tims TOF fleX

	Number of protein IDs
Segment 1	5040
Segment 2	2224
Segment 3	3445



Biological processes characterization

Regions of interest (~2000 cells) from the three MSI clusters were then microdissected for the subsequent protein characterization using the bottom-up proteomics capability of the tims TOF fleX. The molecular properties of the different tumor subpopulations were then characterized using the gene ontology tool PANTHER V.13.1.

Conclusions

Here we present a pipeline where MSI can spatially guide the LMD for subsequent microproteomics molecular characterization of regions of interest.
The tims TOF fleX is an ESI instrument associated with a MALDI source. Combining with this workflow, this will bring together the MSI spatial dimension to the molecular information using X-Omics analyses on the very same instrument for a more comprehensive molecular characterization of in situ biological processes.

References

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3. Dewez F et al. Precise co-registration of mass spectrometry imaging, histology, and laser microdissection-based omics. Anal Bioanal Chem.

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