Bringing Physiological Content to OMICS Studies by Optimizing the Pixel to Chromatogram Connection through SpatialOMx

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Introduction

SpatialOMx® on the timsTOF fleX provides high ID-rates from small sample amounts to combine regiospecificity from MALDI Imaging with PASEF empowered X-Omics. Here we present the SpatialOMx workflow in combination with 4D-Proteomics™. Aim of this study was to find the optimal experimental setup to perform SpatialOMx using MALDI Imaging in combination with Proteomics from the same tissue section. Small tissue pieces were excised from sections using laser microdissection (LMD) and two different types of slides (IntelliSlides™ or PEN-slides) were compared with regards to their performance in the Omics experiment. Additionally, different staining procedures were compared to examine their compatibility with the follow-up proteomics experiments.

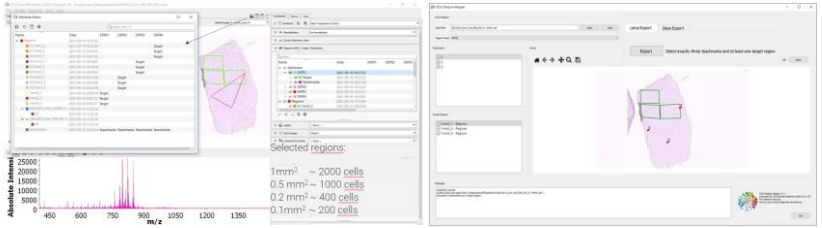
Methods

SpatialOMx workflow

- Liver sections were mounted on IntelliSlides and PEN slides
- MALDI imaging with DHB matrix on lipid or peptide (Pep) level on a timsTOFfleX
- Staining protocols; H&E and Hemalaun
- Define regions of interest (ROI) with SCiLS Lab™
- Export ROI to Laser Microdissection device (Leica, LMD 7000)
- Tissue areas were cut out in draw and cut (PEN) or draw and scan (IntelliSlides) mode
- 4D-Proteomics combined with PaSER GPU-based searches
- **In depth proteomics profiles with regiospecific content**

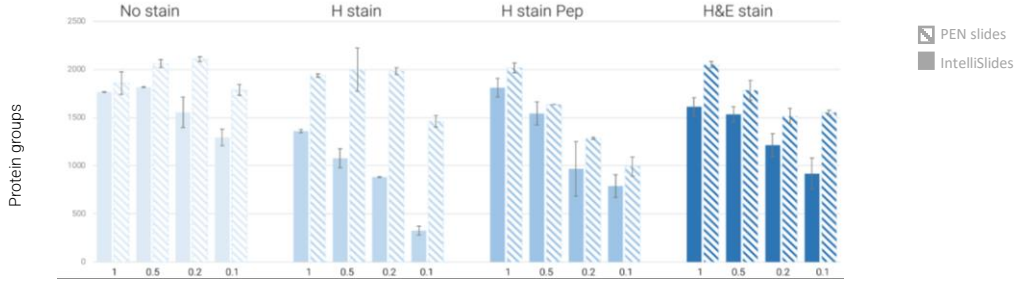
Results

Different staining protocols were performed after the MALDI Imaging experiment which was carried out on lipid or peptide level ("Peptide H staining"). For the LMC cut areas and in house extraction and digest protocol was used. Peptide separation was carried with a 25 cm Aurora (75 µm ID, ionOpticks, Australia) column using a 35 minute run time. Data was acquired using a DDA PASEF method and raw data were processed using Fragpipe + Msfragger tool.



Region coordinates were assigned in the Attribute editor from SCiLS lab 2021b. Additionally, a triangle was created to get three teachmarks for the correct coordinate transfer to the LMD instrument. Area sizes and numbers of cells are shown (left image).
For the transfer to the Leica LMD instrument the SCiLS Region Mapper was used (right image).

Protein groups identified out of 3 PEN slide experiments or 3 IntelliSlide experiments per condition.
A lipid imaging or peptide imaging (H stain Pep) was performed followed by staining and PASEF LC-MS/MS analysis



Conclusions

- TimsTOF Flex is designed for MALDI guided SpatialOMx.
- Entire SpatialOMx workflow could be successfully performed independent on slide type.
- PEN slides gave slightly more protein groups, albeit less consistent results.
- Non stained samples resulted in highest amount of protein groups for PEN and IntelliSlides
- Low H&E staining interference with the protein identification.
- Especially for IntelliSlide; Peptide Imaging prior to protein identification has a positive effect.

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