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# SpatialOMx at its Best: Comparison of different Staining Procedures and Slide Types

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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™. The 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[1]. Defined tissue areas from 0.1 $mm^2$ to 1 $mm^2$ were excised from sections using Laser Capture Microdissection (LCM) and the performance of two different types of slides (IntelliSlides™ or PEN-slides) were compared in Omics experiments. Additionally, different staining procedures were compared to examine their compatibility with the follow-up proteomics experiments.

## Methods

Mouse liver sections (Fresh Frozen, 10µm) were mounted on PEN membrane slides and IntelliSlides™. Slides were coated with DHB matrix (15mg/ml in 90%ACN, 10%H2O, 0.1%TFA) using the HTX TM-sprayer and measured on a timsTOF fleX (Bruker Daltonics). MALDI Imaging experiments were performed on lipids or peptides. Slides were stained with Hematoxylin and Eosin (H&E), H alone, or left unstained. Several areas with defined sizes (0.1 – 1$mm^2$) were selected in SCiLS™ Lab (Bruker Daltonics) and coordinates were transferred to the LMD 7000 device using the SCiLS Region Mapper. Afterwards 4D-Proteomics was performed. For the LC-MS/MS approach, cut areas were extracted and digested. Peptide separation was carried out 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.

## Results

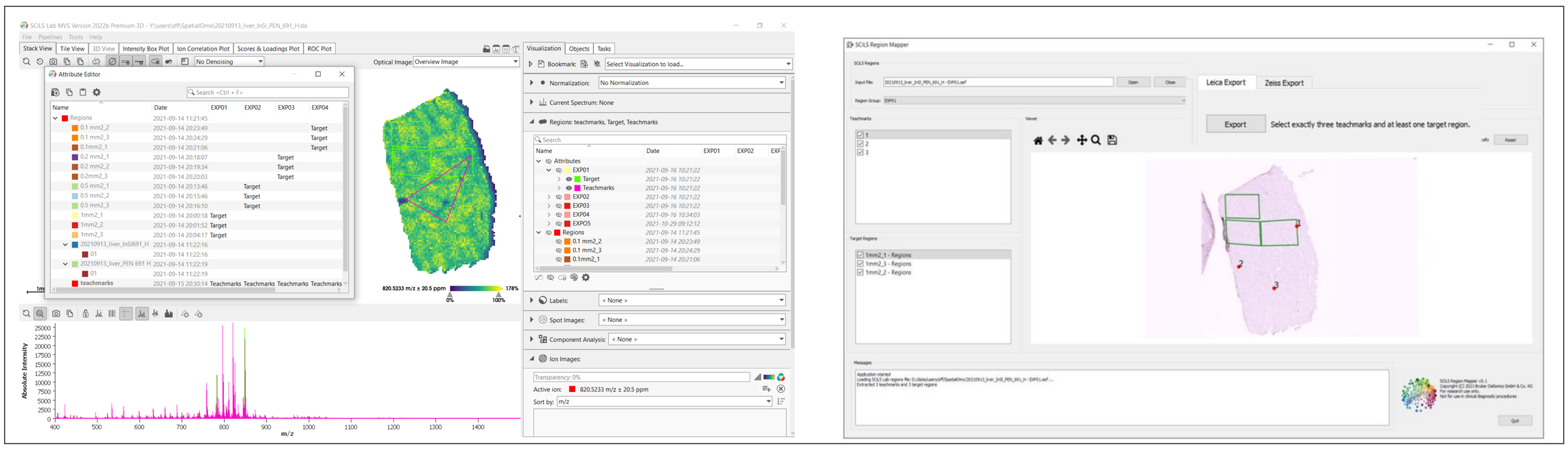


Fig.1: On the left, the SCiLS lab software is shown. The attribute editor offers the possibility to name regions of interest as Target and the points for LCM teaching as Teachmarks. Those elements can then be exported and load into the SCiLS Region Mapper which is shown on the right side. The export button allows a direct export for Leica and Zeiss LCM instrumentation.

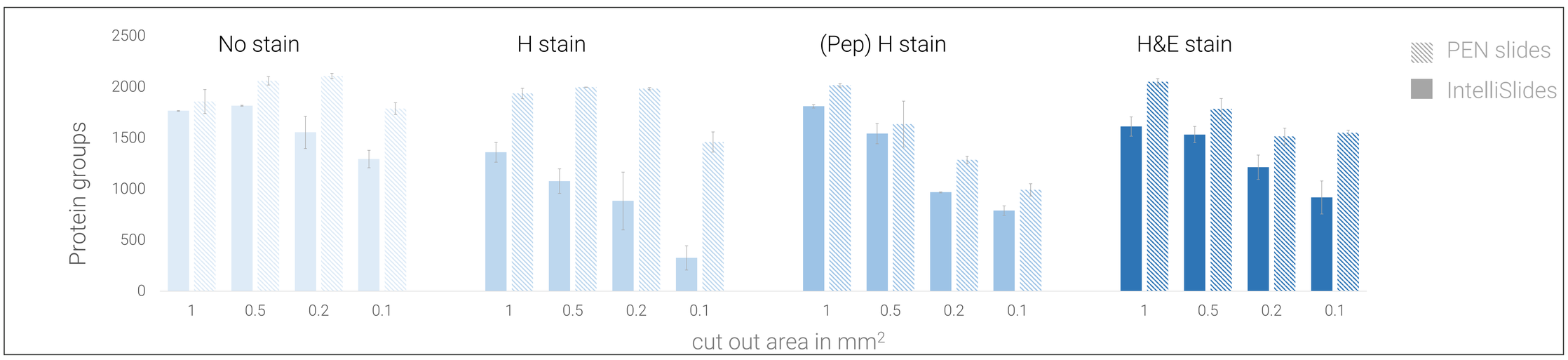


Fig.2: Protein groups identified out of 3 PEN slide experiments or 3 IntelliSlide experiments per condition. Four different cut sizes were compared from 1$mm^2$ (~2000 cells) to 0.1$mm^2$ (~200 cells) per condition whereas the conditions were defined as follows: after a lipid imaging run only matrix was washed off the tissue in 70% EtOH = “no stain” , after the matrix removal the slide was stained with Hematoxylin = “H stain”, after a peptide imaging run and matrix removal the slide was stained with Hematoxylin = “(Pep) H stain”, after a lipid imaging run matrix was washed off the tissue and H&E staining was performed. For the LC-MS/MS approach cut areas were extracted and digested.

- 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.
- Specifically for IntelliSlides; Peptide Imaging prior to protein identification has a positive effect.

- The SpatialOMx workflow allows the combination of MALDI Imaging and in depth 4DProteomics experiments on one tissue section

**Innovation**