Linking spatial to functional proteomics on heterogeneous samples: The “pixel-by-pixel” shotgun proteomic approach

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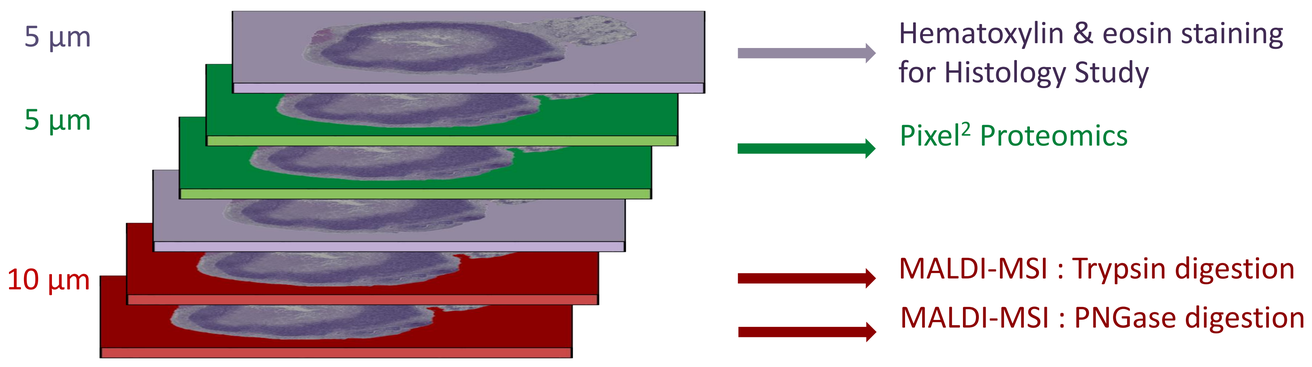
INTRODUCTION

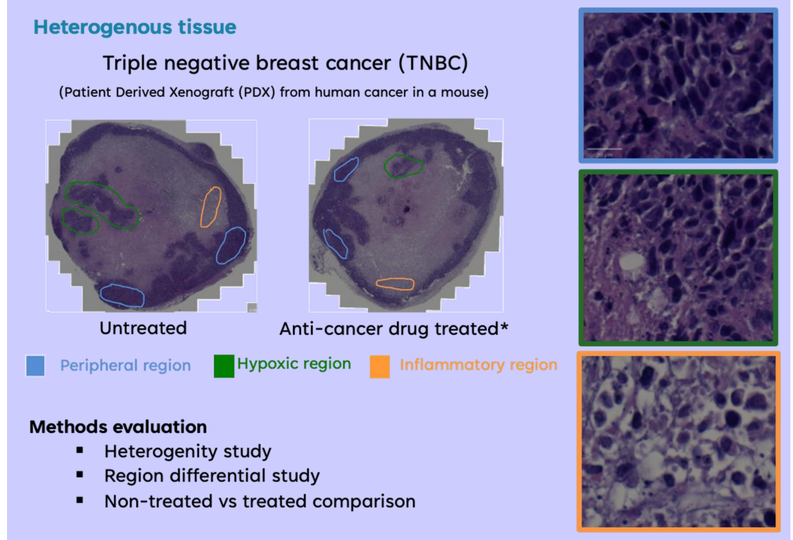
Proteins are crucial for assessing cell health and activity, yet proteomics of biological tissues faces challenges due to sample heterogeneity. MALDI Mass Spectrometry Imaging (MALDI-MSI) offers spatial information with limited functional insights, while laser capture microdissection coupled to shotgun proteomics provides detailed proteome data but generally sacrifices spatial resolution. We propose an advanced technique, called pixel-by-pixel shotgun proteomics (Pixel² Proteomics, P²P), combining systematic laser microdissection with proteomics. This approach was tested on patient-derived xenografts from triple-negative breast cancer tissues, both treated and untreated with anti-cancer drugs.

Methodology : serial tissue sections analysis

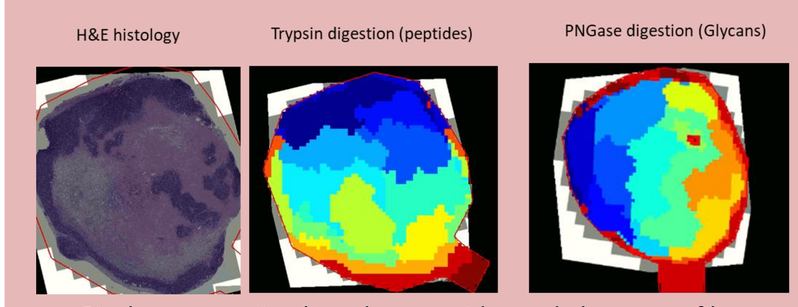
Xenografts of triple-negative breast cancer tissues initially preserved in formalin-fixed paraffin-embedded (FFPE) form were sectioned into 5 µm and 10 µm slices. A classical hematoxylin and eosin staining was performed on 5 µm slices for **Histology Study**. The 10 µm slices underwent matrix-assisted laser desorption/ionization mass spectrometry imaging (**MALDI-MSI**) following on-tissue digestion with trypsin or PNGase to release peptides and glycans. Tissue sections of 5 µm thickness were placed on polyethylene naphthalate (PEN) slides and subsequently microdissected using a Leica LMD7000 Microdissection Laser (**Pixel² Proteomics**). The microdissection area was set to 2,500 µm², representing the minimal size necessary to maintain sufficient proteomic analysis performance. These samples are then prepared, applying an adapted “one pot strategy”(1), for shotgun proteomics and analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS). A Waters Acquity M-Class LC system coupled to a Bruker timsTOF SCP was employed. The MS/MS acquisition is conducted using the dia-PASEF technology. Protein identification and quantification are computed using DIA-NN with an in-house spectral library.



Histology Study

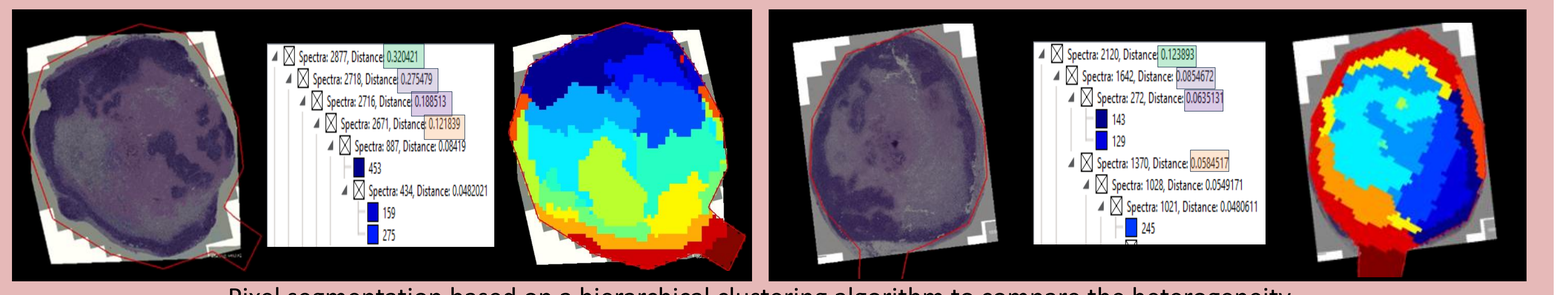


MALDI-MS Imaging



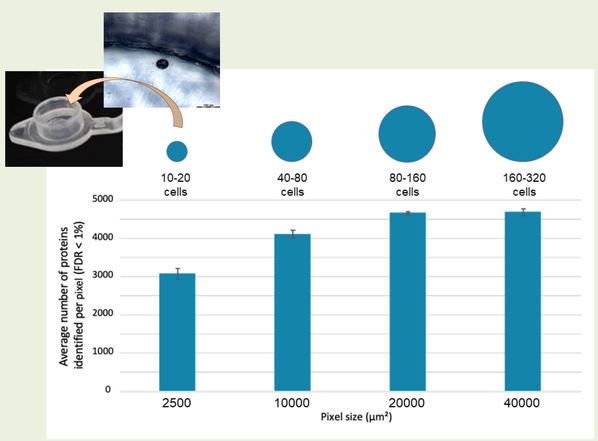
Pixel segmentation based on peptides and glycans profiles.

Untreated — Anti-cancer drug treated



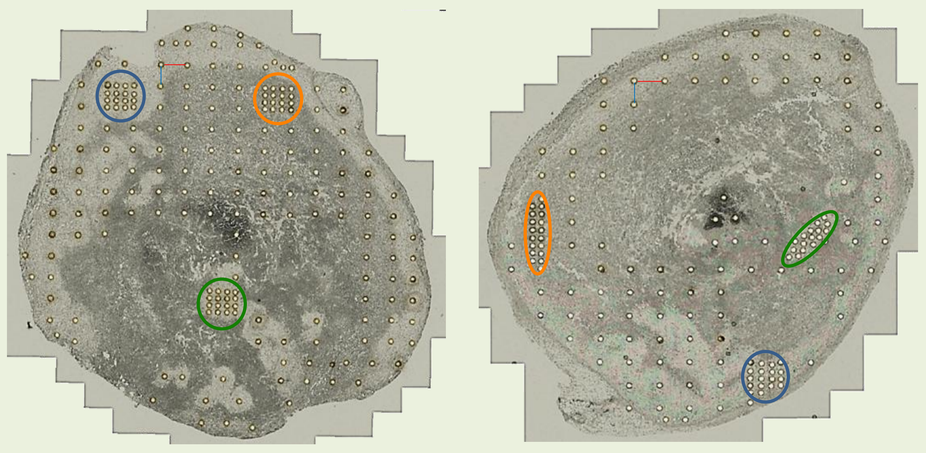
Pixel segmentation based on a hierarchical clustering algorithm to compare the heterogeneity of untreated tissue and treated tissue.

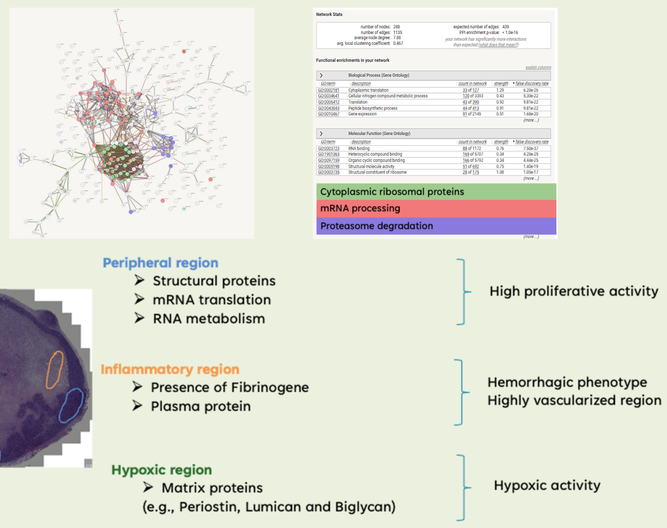
Pixel² Proteomics



2500µm² tissue area showed that is sufficient to achieve a fairly deep proteome with ~3000 identified proteins.

Untreated — Anti-cancer drug treated





Microdissected tissues areas were analyzed by shotgun proteomics. Lists of identified proteins were generated for each pixel after database searches (800 to 3000 proteins per pixel). To evaluate the pixel-to-pixel variation, a supervised differential analysis between areas of biological interest was performed to highlight a list of differential proteins.

CONCLUSION

This analysis highlighted proteins specific to peripheral, inflammatory, and hypoxic regions and the impact of the anti-tumor treatment. This method provides detailed spatial and functional insights into tissue heterogeneity, demonstrating its potential to advance tissue-based proteomic studies. In perspective, we plan to combine single-cell proteomic (SCP) analyses from the same tissue and deconvolute pixel-by-pixel microdissection data using SCP data, enabling the identification and spatial distribution of different cellular phenotypes within heterogeneous tissues.

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(1). C. Kune et al. , Significant impact of consumable material and buffer composition for low-cell number proteomic sample preparation, ChemRvix, 2024, DOI: 10.26434/chemrxiv-2024-dw5zg-v2