Mass cytometry (CyTOF) is a breakthrough technology, enabling the simultaneous detection of over 50 cellular markers at single-cell resolution without signal overlap. This is achieved using antibodies conjugated to stable metal isotopes, allowing for highly multiplexed analysis of complex biological systems. Imaging Mass Cytometry (IMC) extends this platform to tissue sections by combining CyTOF with high-resolution laser ablation, providing spatially resolved visualization of cellular architecture and tissue interactions.

Taking advantage of the high dimensionality and spatial resolution of these technologies, mass cytometry and IMC was applied to study breast cancer — one of the most preva- lent and deadly malignancies worldwide. Despite progress in diagnostics and treatment, patient outcomes remain highly variable, especially in regions with limited access to early care. A critical knowledge gap concerns the mechanisms behind poor responses to stan- dard chemotherapy or immunotherapy and the role of the tumor microenvironment in therapy resistance.

The main research hypothesis of this thesis is that proteolytic profiling using mass cytometry can improve patient stratification. Detecting specific patterns of protease ac- tivity and co-expression may guide the development of more personalized and effective treatment strategies. A secondary hypothesis suggests that deeper insights into protease- driven tumor progression could support more conservative surgical approaches, reducing unnecessary lymph node resections and related complications.

This study explored the protease landscape in breast cancer. Proteases regulate key biological processes, including signaling, immune modulation, and extracellular matrix remodeling. Their dysregulation is linked to tumor invasion, metastasis, and treatment failure. However, protein abundance alone does not necessarily reflect enzymatic activity. Therefore, in addition to expression profiling, activity-based probes (ABPs) were used to directly detect active proteases in frozen tissue sections. These small molecules covalently bind to active sites, enabling detection of the activome—the functionally active subset of the proteome. The proteolytic analysis was complemented with an immunological profile and the assessment of clinically standardized markers.

Analyzed samples included tumor tissue, blood, and plasma from patients with varying molecular subtypes and clinical stages. Protease profiles were integrated with stan- dard markers such as HER2, ER, and Ki-67. IMC enabled spatial mapping of protease distribution within tumors, revealing associations with immune infiltration, tissue orga- nization, and the stromal compartment.

Conducted at the Wroclaw University of Science and Technology under the supervision of Prof. Marcin Poręba, it provides new methodological and biological insights into oncology, mass cytometry, and functional proteomics.