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Spatial Proteomics Imaging to Decode Disease Progression: A Perspective on Tumor Microenvironments

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KEYWORDS

ABSTRACT

Protein spatial imaging, rooted in spatial proteomics, enables the localization of proteins within their native environment, thus preserving structural and functional context. Its integration into cancer research has provided insights into tumor progression, especially in breast cancer, where ductal carcinoma in situ (DCIS) may transition into invasive breast cancer (IBC). Multiplexed ion beam imaging by time-of-flight (MIBI-TOF) has emerged as a powerful method to visualize protein distribution, tumor–stroma interactions, and immune cell dynamics. Evidence indicates that the tumor microenvironment (TME)—including stromal architecture, extracellular matrix (ECM), and immune cell infiltration—plays a central role in malignant progression. Here, we highlight recent advances in protein spatial imaging, discuss optimization strategies, and examine its implications for breast cancer progression and other diseases.

INTRODUCTION

Proteins are the primary effectors of nearly every cellular process. Their location—both within specific subcellular compartments such as nucleus, cytosol, plasma membrane, endosomes, organelles, and extracellular matrix (ECM) — and their interactions with surrounding proteins and structures determine their functional state. Changes in localization can therefore reflect early steps in dysfunction, even before gross changes in protein abundance are detectable (Rehman and Botelho., 2024).

Spatial proteomics focuses on understanding where proteins reside within cells and tissues, a key determinant of their function. Protein localization is not random but tightly regulated, and aberrations can contribute to pathological processes. While bulk proteomics provides quantitative abundance data, it often neglects spatial heterogeneity (Pinto et al., 2015).

Recent advances in spatial imaging now allow direct in situ visualization of proteins at subcellular resolution (Lundberg & Borner, 2019). Techniques such as multiplexed ion beam imaging (MIBI-TOF), imaging mass cytometry, and expansion-based imaging enable simultaneous detection of dozens of proteins while preserving tissue architecture. This is critical because proteins frequently exert activity at specific subcellular sites, and mislocalization is closely linked to functional dysregulation (Palla et al., 2022). In cancer, aberrant localization and expression patterns are associated with tumor progression and metastasis, offering potential biomarkers for early detection, prognosis, and therapeutic targeting (Brožová et al., 2023).

Existing spatial proteomics methods include organelle fractionation coupled with MS, protein proximity labeling, and spatial imaging using fluorescent or antibody-based labeling (Mou et al., 2022). Among these, spatial imaging uniquely preserves the spatial context of proteins, enabling direct visualization of cell-to-cell variability and tissue architecture. The integration of spatial proteomics with computational analysis and multi-omics approaches now allows quantitative assessment of protein networks, cellular interactions, and microenvironmental influences, thereby providing a comprehensive view of cellular function and disease progression.

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BREAST CANCER PROGRESSION AND THE TUMOR MICROENVIRONMENT

Breast cancer (BC) is highly heterogeneous, spanning carcinoma in situ (ductal or lobular) and invasive disease, each with distinct histological and molecular traits (Granat et al., 2019). The transition from DCIS to IBC is not only dependent on genetic changes within epithelial cells but also heavily shaped by the surrounding TME, which includes stromal fibroblasts, vasculature, immune infiltrates, and myoepithelial cells (Risom et al., 2022). Importantly, BC cells adapt their metabolism during progression, with altered pathways and metabolite utilization (Brožová et al., 2023). These metabolic shifts can reprogram local environments and influence immune or stromal behavior, reinforcing malignant traits.

Risom et al. (2022) examined 79 tissue samples (9 normal, 58 DCIS, 12 IBC) using MIBI-TOF with 37-plex antibody staining. They found that spatial positioning strongly correlated with cell morphology and function, and that myoepithelial and stromal alterations were more predictive of invasive transition than epithelial tumor cell changes. This highlights the importance of TME as both a biomarker and a therapeutic target.

Recent studies reinforce this perspective. ECM composition and collagen remodeling discriminate DCIS from IBC with high sensitivity (Hulahan & Angel, 2024). Moreover, DCIS lesions proximal to invasive regions exhibit ECM peptide signatures more similar to IBC than distal lesions, suggesting a "field effect" (Siragusa et al., 2024). Immune infiltration also differs: B-cell abundance around DCIS ducts correlates with metabolic gene expression and appears protective in HER2-positive lesions (Bergholtz et al., 2025).

METHODOLOGICAL ADVANCES AND OPTIMIZATION

MIBI-TOF integrates multiplexed ion beam imaging with mass spectrometry to generate high-resolution protein expression maps (Keren et al., 2019). Recent advances have focused on optimizing data accuracy and interpretability. For instance, MAUI facilitates denoising and artifact removal (Baranski et al., 2021), while deep-learning tools such as Mesmer improve single-cell segmentation (Greenwald et al., 2022). Clustering methods like FlowSOM enable classification of diverse cell types but require cautious validation to prevent over-clustering (Quintelier et al., 2021). Additional algorithms such as IMC-Denoise further reduce pixel-level noise and enhance signal fidelity (Lu et al., 2023). Beyond protein mapping, emerging frameworks like scSpaMet integrate proteomic and metabolomic data, revealing cell-type–specific metabolic interactions and competitive dynamics within tissues (Zhang et al., 2023). Together, these methodological advances greatly expand the resolution and interpretive power of spatial imaging, enabling the detection of subtle microenvironmental features that shape disease progression.

Applications Beyond Breast Cancer

MIBI-TOF is increasingly applied outside oncology, underscoring its versatility in mapping immune–tissue interactions across diverse diseases. In autoimmune gastritis triggered by nivolumab therapy, spatial imaging revealed enhanced epithelial proliferation and IFN-γ secretion, highlighting how immune checkpoint blockade can inadvertently remodel epithelial–immune crosstalk and potentially initiate gastric carcinogenesis (Ferrian et al., 2021). Similarly, McCaffrey et al. (2022) used MIBI-TOF to dissect the spatial architecture of human tuberculosis (TB) granulomas, profiling over 30 proteins simultaneously. Their analysis linked microanatomical immune niches to systemic immune responses, revealing that granuloma heterogeneity may underlie divergent clinical outcomes in TB.

Beyond these conditions, spatial protein imaging is increasingly being explored in neurodegenerative diseases. Recent studies applying MIBI-TOF to Alzheimer's disease (AD) brain tissue have revealed microglial activation states and region-specific protein expression changes associated with disease progression (Vijayaragavan et al., 2022; Mrdjen et al., 2023). Moreover, advanced spatial proteomics frameworks integrating deep-learning–based super-resolution prediction (NPF) enhance detection of subcellular protein patterns in human neuropathology (Vijayaragavan et al., 2022).

In cardiovascular research, spatial proteomics is being applied to map immune infiltration and extracellular matrix remodeling in atherosclerotic plaques, linking protein localization to tissue remodeling and functional outcomes (Hu et al., 2025; Zhao et al., 2025). These studies demonstrate that aberrant protein localization underlies pathological changes in the heart and vasculature, providing insights not accessible through bulk proteomics.

Overall, these applications highlight the broader clinical potential of spatial proteomics to uncover mechanisms of immune regulation, tissue remodeling, and chronic inflammation. Integration with transcriptomics, metabolomics, and multi-modal imaging could accelerate translation into diagnostic and therapeutic strategies across multiple disease domains.

Advantages, Limitations, and Future Directions

Spatial protein imaging offers unparalleled resolution of protein localization, enabling simultaneous study of tumor cells, stroma, vasculature, and immune infiltrates. It preserves tissue architecture while delivering quantitative, multidimensional data. Recent advances further extend these strengths. Techniques such as PSERP allow proteome-wide spatial mapping across entire tissue sections with improved reproducibility and protein coverage (Xu et al., 2025). Similarly, FAXP, combining tissue expansion with laser-capture microdissection, achieves near single-cell resolution in FFPE specimens, enhancing structural fidelity and detection sensitivity (Dong et al., 2024). These innovations support integration with transcriptomics and metabolomics, enabling multimodal mapping of how protein, gene, and metabolite distributions converge in the tumor microenvironment (Chen et al., 2025).

Nevertheless, several limitations remain. The methods are labor-intensive and technically demanding, often requiring specialized instrumentation, costly reagents, and computational expertise, restricting widespread scalability (Risom et al., 2022). Batch effects, tissue deformation during expansion, and peptide loss during sample preparation remain technical concerns (Čuklina et al., 2021). Antibody-based approaches also suffer from issues of specificity and epitope masking, particularly in archived FFPE tissues (Xu et al., 2025). Furthermore, while throughput has improved, cover-

age across large clinical cohorts and heterogeneous breast cancer subtypes is still limited. These constraints highlight the need for cost-effective, clinically adaptable platforms.

Looking ahead, translational adoption will require validation of candidate biomarkers—such as ECM-derived peptides or immune spatial signatures—using scalable methods like immunohistochemistry. Future integration of spatial proteomics with metabolite mapping could clarify how metabolic rewiring reshapes the tumor microenvironment. Finally, prospective studies with long follow-up of DCIS patients are essential to distinguish indolent from progressive lesions, reducing overtreatment and improving precision oncology.

CONCLUSION

Spatial protein imaging, particularly through MIBI-TOF, has emerged as a powerful tool for dissecting the complexity of breast cancer and other pathological conditions. It demonstrates that the tumor microenvironment—including extracellular matrix remodeling, stromal interactions, and immune infiltration—plays a central role in driving the transition from DCIS to invasive disease, while breast cancer cells concurrently rewire their metabolism to reinforce malignant crosstalk. By integrating advanced computational analyses and multi-omics approaches, spatial proteomics can uncover early drivers of disease, identify prognostic biomarkers, and highlight potential therapeutic targets.

Beyond breast cancer, these approaches are increasingly applied in autoimmune, infectious, neurodegenerative, and cardiovascular diseases, providing unprecedented insights into tissue-specific protein networks, immune–tissue interactions, and subcellular heterogeneity. Although challenges related to technical complexity, cost, and scalability remain, continued methodological innovation—including high-resolution mapping, antibody validation, and integration with transcriptomics and metabolomics—will enhance both mechanistic understanding and translational potential. Overall, spatial protein imaging represents a transformative approach for precision medicine, offering opportunities to improve early detection, stratify patients, and guide targeted therapeutic interventions across multiple disease contexts.

Declaration of interests The authors declare no competing interests.

Declaration of generative AI During the preparation of this manuscript, the authors used ChatGPT (OpenAI) Grammarly solely to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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