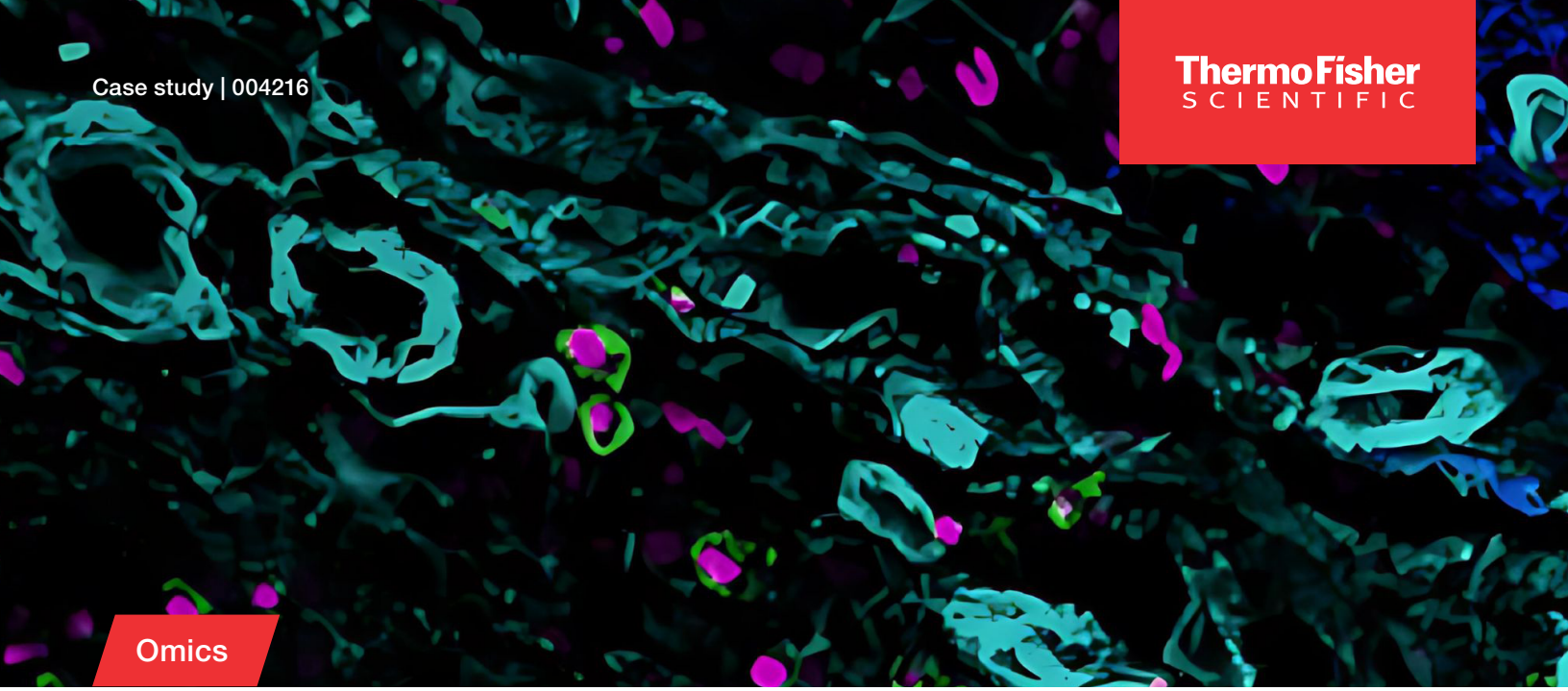

Case study | 004216

Omics

Using mass spectrometry-based proteomics to power spatial biology for deep proteomic profiling

Introduction

Spatial proteomics by LC-MS/MS represents a transformative approach in the field of molecular biology, enabling researchers to explore the intricate landscape of protein expression within the spatial context of tissue architecture. Traditional proteomics methods have provided valuable insights into the protein composition of biological samples, yet they often lack the spatial resolution necessary to fully understand the complex interactions and dynamic states of cells within their native environments.

Recent advancements in mass spectrometry, such as the Thermo Scientific™ OrbitrapT™ Astral™ Mass Spectrometer (MS) coupled with innovative imaging techniques, have paved the way for spatially resolved proteomics. This integrated approach allows for the precise mapping of protein distributions across tissue sections, capturing the heterogeneity of cellular phenotypes and their spatial relationships. By leveraging high-resolution imaging to annotate and segment specific cell populations, followed by ultra-sensitive mass spectrometry to profile their proteomes, researchers can gain a deeper understanding of cellular functions, interactions, and disease mechanisms.

The ability to study proteome heterogeneity at the single-cell level while preserving spatial context is particularly valuable in fields such as oncology, neurobiology, and developmental biology, where cellular microenvironments play critical roles in disease progression and tissue function. Spatial proteomics by mass spectrometry not only enhances our understanding of these complex biological systems; it also opens new avenues for biomarker discovery, therapeutic targeting, and personalized medicine.

In this interview, we explore principles and methodologies of spatial proteomics, highlighting key innovations and applications that are driving this exciting frontier in biomedical research.

We will discuss how the synergy between high-plex imaging and mass spectrometry enables comprehensive molecular characterization of tissue samples, offering unprecedented insights into the spatial organization and functional states of cells.

We aim to provide a foundational understanding to describe how spatial proteomics by mass spectrometry is revolutionizing approaches to studying biology at the molecular level.

An interview with Dr. Mariya Mardamshina and Dr. Emma Lundberg, Stanford University

Dr. Mariya Mardamshina, MD, PhD, is a post-doctoral researcher in the Lundberg Lab of the Bioengineering Department, Stanford University.

Dr. Emma Lundberg, PhD, is an Associate Professor of Bioengineering and Pathology, Stanford University.

Nicolai Dorka
–PhD Graduate Student at Stanford University

Marvin Thielert
–PhD Graduate Student at Max Planck Institute of Biochemistry

Q: What biological questions or unmet needs inspired your team to develop multiplexed Deep Visual Proteomics (mxDVP)?

A: Our motivation stemmed from the desire to study proteome heterogeneity in single cells with preserved spatial context. While the lab is routinely working with highly multiplexed imaging, the limitation to around 50 antibodies per experiment greatly limits any proteome-scale studies. We saw an unmet need for an integrated approach that could link spatially resolved multiplexed imaging with unbiased, deep proteomics at ultra-low input levels, essentially capturing the molecular phenotype of rare cells *in situ*. Pancreatic islet biology, with its intricate cell state dynamics and rare transitional populations, provided an ideal test bed for such innovation.

Q: For someone new to mxDVP, how would you describe the workflow in your own words?

A: In simple terms, mxDVP starts with high-plex immunofluorescence imaging to map cell types, states, and their spatial context *in situ* in tissue sections. From these images, we segment cells, annotate cell types and states, and computationally select specific cells of interest, including rare and/or spatially defined groups. The multiplexed image and cell contours are aligned to a laser microdissection microscope, and the cells of interest are then physically isolated by laser microdissection, and their proteomes are analyzed using ultra-sensitive mass spectrometry. Finally, we integrate imaging and proteomics data to build a rich spatial proteomics map (Figure 1).

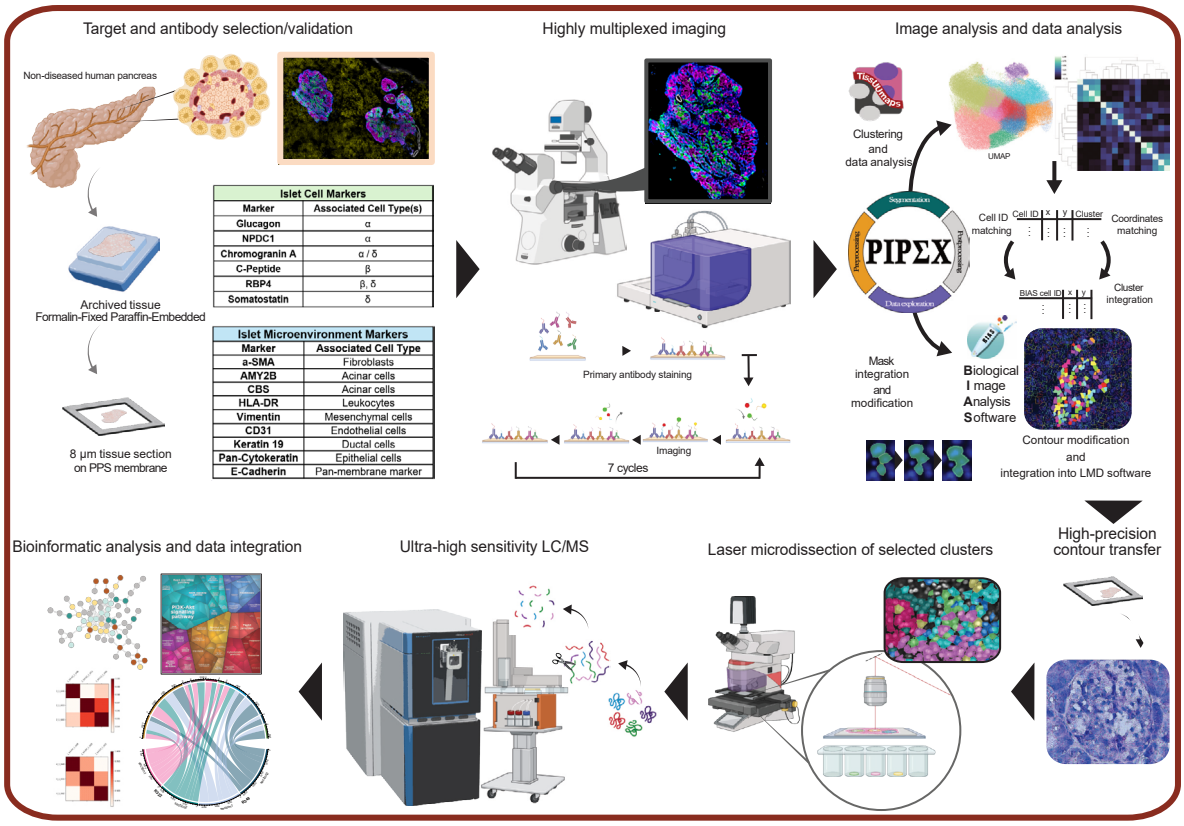

Figure 1. Workflow of the integrated mxDVP pipeline. Schematic representation of the mxDVP pipeline outlining seven key steps: (1) design of a customized antibody panel, (2) acquisition of high-plex spatial proteomic images, (3) image and data analysis using an in-house developed pipeline, (4) application of a polychromatic stain to enable high-precision contour transfer, (5) laser microdissection of selected cell clusters, (6) mass spectrometry-based proteomic profiling of isolated cell populations, and (7) integrated data analysis.

Q: What did your study reveal about pancreatic islet cells? What were the most surprising or significant discoveries from your biological analyses?

A: Our study uncovered striking heterogeneity within human pancreatic islets, including the identification of rare polyhormonal hybrid cells that co-express diverse endocrine markers. These cells exhibited unique proteomic signatures distinct from canonical alpha (glucagon-producing), beta (insulin-producing), or delta (somatostatin-producing) cells. Additionally, we captured gradients of stress and plasticity markers, suggesting dynamic transitional states within the islet niche. One of the most surprising findings was the discovery of rare endocrine cells with a mixed hormonal and progenitor-like phenotype, hinting at potential latent plasticity.

Q: Your antibody findings highlighted intriguing polyhormonal hybrid cells. What implications might these rare cell types have for our understanding of pancreatic function, disease, and plasticity?

A: These polyhormonal hybrid cells could reflect a state of endocrine cell plasticity, possibly playing a role in islet adaptation or early signs of dysfunction in metabolic disease before this disease can fully manifest or develop. Their existence challenges the classical view of endocrine cell identity, opening new avenues for understanding islet resilience, regeneration, or failure in conditions like diabetes.

Q: For your mass spectrometry work, you reported >6,000 proteins from small populations—what innovations made that depth possible, and how does this compare to other spatial proteomics methods?

A: The deep proteome coverage of >6,000 proteins from approximately 100 cell equivalents was only possible because of several tightly integrated innovations across the mxDVP pipeline:

Use of the Orbitrap Astral MS: Its outstanding ion transfer efficiency, high resolution, and speed enabled sensitive and deep proteome coverage from ultra-low inputs, pushing the detection limit beyond what was achievable with previous Orbitrap platforms.

Optimized contour pooling strategy: Through systematic testing, we found that pooling 250 2D cell contours (~100 cells in volume) provided the best balance of depth and rare cell enrichment, ensuring detection of low-abundance transcription factors (e.g., ARX, PAX6, INSM1) critical for capturing endocrine plasticity. The highly multiplexed imaging is critical for efficient selection of cells to pool. With the many markers included, we can ensure to pool cells that express similar cell types and state markers, as well as have a similar spatial positioning (for example, small vs large islets).

Tissue handling innovations: Adoption of PPS membranesover PEN, and the incorporation of Toluidine Blue polychromatic staining post-imaging, preserved tissue integrity, ensured accurate isolation of neighboring cells, and precise contour transfer, which is essential at such small scales.

Integrated image analysis and laser capture: Our PIPΣX1 and BIAS integration enabled precision contour refinement, ensuring the purity of isolated cell populations, which is critical to avoid background proteome noise in low-input mass spectrometry (Figure 2).

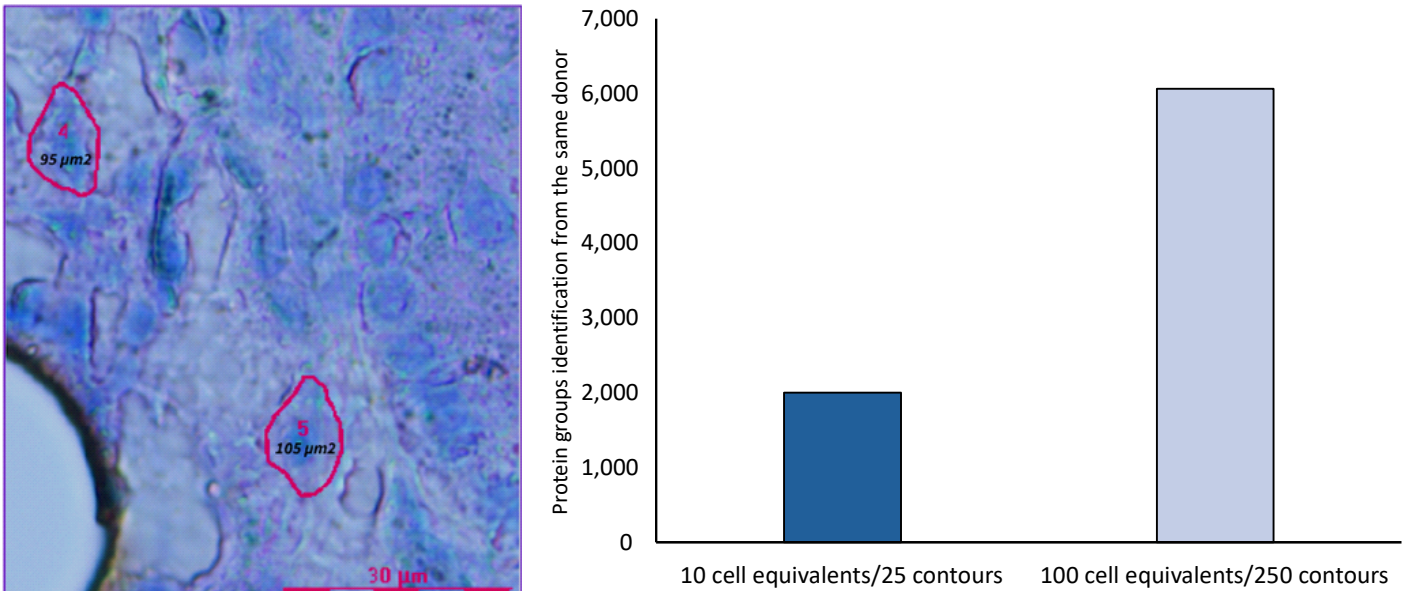

Figure 2. Integrated image analysis and laser capture. Our PIPΣX1 and BIAS integration enabled precision contour refinement, ensuring the purity of isolated cell populations, which is critical to avoid background proteome noise in low-input mass spectrometry.

Q: How did your deep proteomic data specifically contribute to your new discoveries about cell types and states that other methods couldn't reveal?

A: Our deep proteomic data provided an unbiased, discovery-driven molecular readout that extended well beyond what we could capture with multiplexed imaging alone. While imaging is a powerful tool that allows spatial context and can now reach over 100-plex panels, it is fundamentally constrained by the pre-selection of known markers and high-quality antibodies, which inherently limit the discovery of unexpected cell states or plasticity markers that were not initially targeted.

By integrating unbiased mass spectrometry-based proteomics, we could achieve deep proteome profiling of specific, spatially resolved cell populations, revealing metabolic, stress response, and plasticity-associated proteins that are not represented in standard antibody panels. For example, in polyhormonal hybrid cells, we detected mitochondrial, metabolic, and stress adaptation proteins that would have been completely missed by targeted imaging panels alone.

And vice versa, without the spatial profiling provided by high-plex imaging, we would not have been able to uncover spatial relationships such as the association between islet size, polyhormonal states, and the vascularization network, insights that rely entirely on spatially resolved phenotyping. This integration of refined, knowledge-based cell state selection via imaging with deep, unbiased proteomics allowed us to uncover nuanced cell identities, transitional states, and rare phenotypes, enabling biological discoveries that challenge classical models of islet cell identity and function (Figure 3).

Q: How did you ensure that cells analyzed by immunofluorescence imaging were precisely the same cells captured for deep visual proteomics?

A: We ensured precise alignment by using the same tissue section for both imaging and laser microdissection. Our custom pipeline, PIPΣX, integrates interactive image analysis (via TissUUmaps) with spatial annotations that guide downstream cell isolation. PIPΣX allows interactive exploration to visually inspect and validate annotated cell populations with precision prior to isolation.

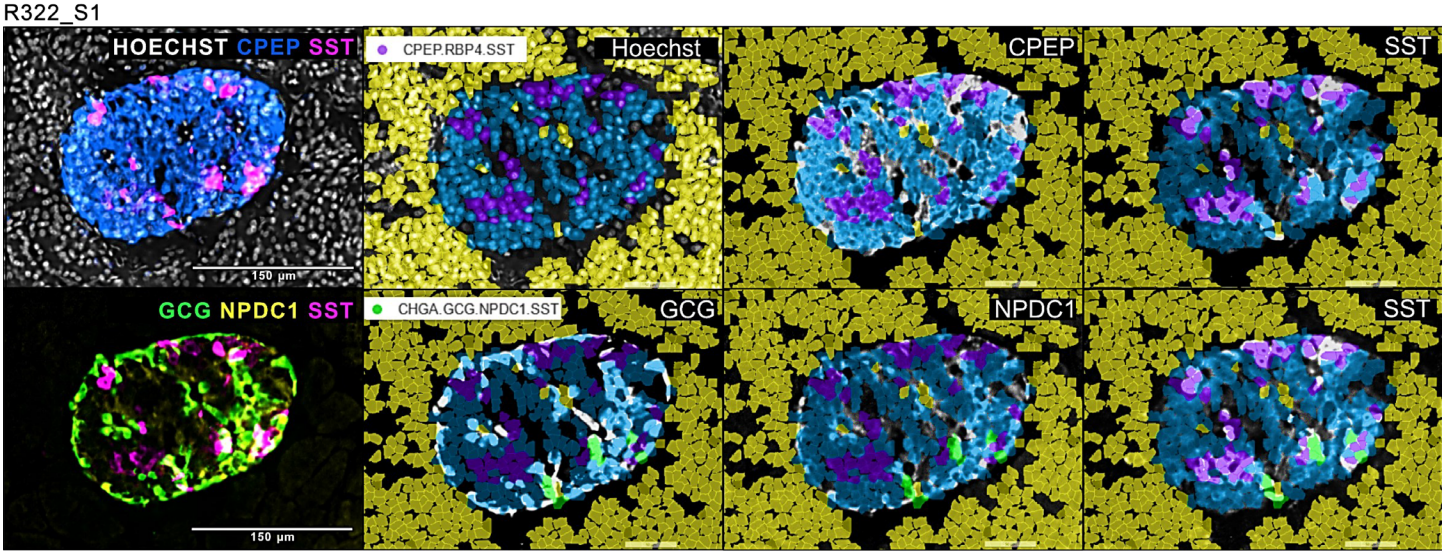

Figure 3. Examples of cell population co-expression.

Top panel: Example of a cell population co-expressing CPEP, RBP4, and SST. Cell segmentation masks are overlaid on a multiplex composite image of islet marker expression. Co-expressing cells are shown in purple, other endocrine cells in blue, and non-endocrine cell types in yellow.

Bottom panel: Example of a GCG-, NPDC1-, and SST-co-expressing polyhormonal cell population within the same islet. Segmentation masks are overlaid on a multiplex composite image, with co-expressing cells highlighted in green, other endocrine cells in blue, and other cell types in yellow.

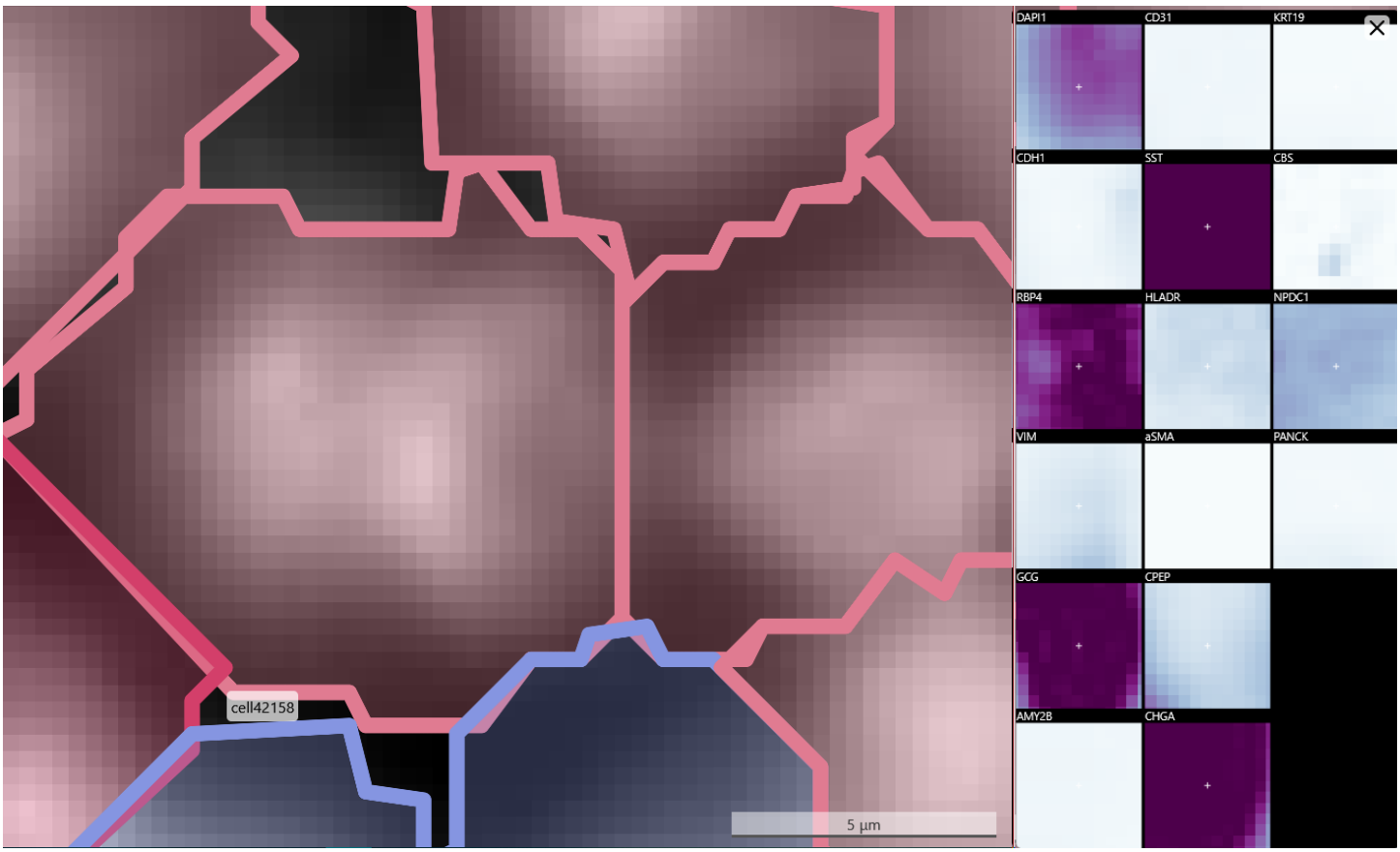

Figure 4. Example of visualization of the segmentation mask of pancreatic tissue using the TissUUmaps Spot Inspector plugin. Islet cells selected from PIPΣX output are interactively overlaid onto the original image. Right: Close-up views of selected cell highlights marker expression patterns consistent with rare hybrid cell annotations co-expressing CHGA, GCG, NPDC1, and SST.

This human-in-the-loop step ensures that the cells selected for mass spectrometry are exactly those phenotyped in the multiplexed images, even in highly complex tissue architectures. This guarantees that the cells profiled by mass spectrometry are exactly those annotated in the multiplexed images (Figure 4).

Q: Your approach integrates highly multiplexed antibody imaging with mass spectrometry on the same tissue section. How do these two technologies complement each other in mxDVP, and did the presence of antibodies raise any concerns about interference with the biological signal?

A: Our mxDVP approach leverages the synergy between multiplexed antibody imaging and mass spectrometry, capitalizing on the strengths of each modality. High-plex imaging provides detailed spatial and phenotypic maps of cell populations using well-characterized markers, enabling us to navigate complex tissues and annotate rare or transitional phenotypes. In parallel, mass spectrometry offers an unbiased, comprehensive proteome readout that extends beyond the limitations of targeted panels, capturing metabolic, stress response, and signaling pathways not represented in antibody libraries.

A key consideration was the potential for interference from residual antibody reagents or imaging buffers in downstream mass spectrometry analysis. To address this, we optimized post-imaging washing steps and rigorously evaluated the impact on proteome profiles. Control experiments confirmed minimal residual contamination, with no observable distortion in protein identification or quantification. These findings validate that antibody-based imaging and mass spectrometry can be seamlessly combined in mxDVP without compromising data quality or depth.

Q: Reproducibility is critical in mass spectrometry. What metrics or quality control measures did you use to demonstrate the robustness and reproducibility of your mxDVP approach?

A: Robustness and reproducibility were foundational to the validation of our mxDVP workflow. We implemented multilayered quality control measures across cell capture, proteomic depth, and quantitative consistency. For laser microdissection, precision and reproducibility of cell population isolation were confirmed through replicate captures of annotated regions, ensuring consistent targeting accuracy.

On the proteomics side, we assessed technical replicates across independent tissue sections and biological conditions, achieving coefficients of variation (CV) below 10% for quantitative proteome data. Protein identification overlap consistently exceeded 85%, and Pearson correlation coefficients between replicates were typically above 0.95, reflecting high reproducibility both in identification and quantitation.

Together, these metrics demonstrate that mxDVP provides a reliable, reproducible platform capable of delivering both deep and consistent spatial proteomics data, suitable for application in complex tissues and diverse experimental conditions (Figures 5 and 6).

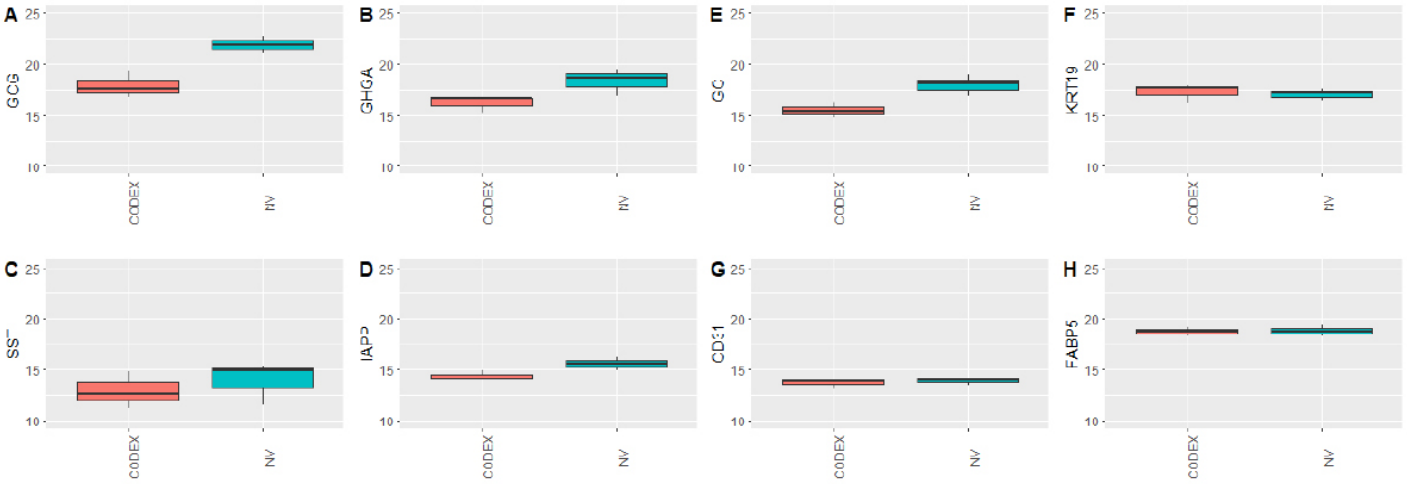

Figure 5. Boxplots illustrate selected marker expression levels quantified from mass spectrometry data in Naïve and CODEX-stained sections. Expression levels were comparable, with no significant differences detected between conditions.

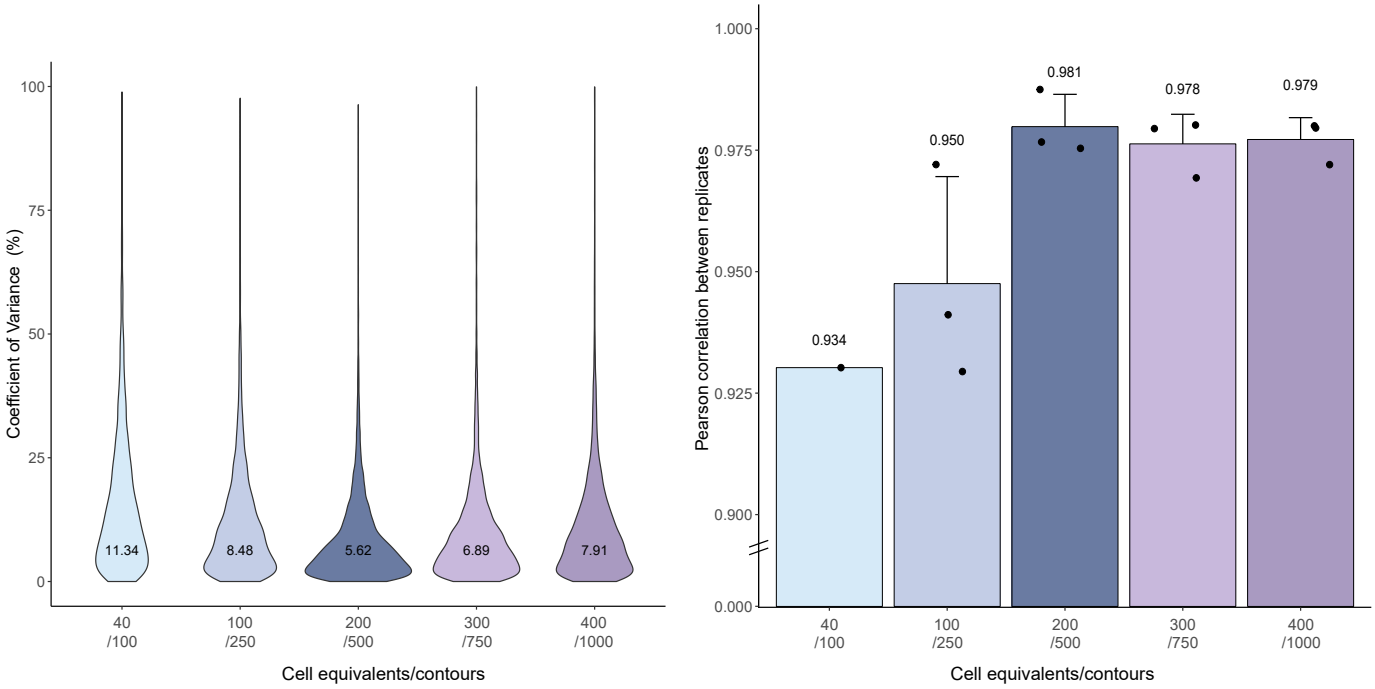

Figure 6. CVs and Pearson correlation between replicates.

Left: CVs between replicates of the contour dilution series across the workflow. n = 2 workflow replicates.

Right: Pearson correlation between replicates of the contour dilution series. n = 3 workflow replicates.

Q: You imaged almost one million cells (864,000) for this study and performed mass spectrometry on carefully selected subpopulations. Roughly how many cells were analyzed by mass spectrometry overall, and what does that tell us about the scalability of mxDVP for larger tissue banks or clinical studies? Is there a limit?

A: mxDVP offers scalability across multiple dimensions, from whole-slide imaging of over 864,000 cells to targeted deep proteomics of selected subpopulations comprising approximately 3,600 cells across diverse biological states and experimental conditions. This capacity to flexibly navigate between tissue-wide surveys and focus deep dives into rare or transitional phenotypes is a unique strength of the approach.

Currently, throughput is primarily limited by mass spectrometry acquisition times and sample processing steps like laser capture microdissection, particularly in low-input formats where sensitivity demands careful handling. However, the modular design of mxDVP, combining established imaging platforms, automated contouring and cell capture pipelines, and scalable proteomic workflows, positions it well for adaptation to larger-scale studies.

Moreover, advances in high-throughput mass spectrometry instrumentation, ultra-high sensitivity acquisition methods, and the automation of laser microdissection workflows are rapidly accelerating. These developments will further enhance the scalability of mxDVP, facilitating its application in larger clinical cohorts, tissue banks, and biomarker discovery pipelines.

Q: What were some of the biggest workflow and mass spectrometry challenges that you faced developing and applying the mxDVP workflow—and how did your team overcome them?

A: One key challenge was maintaining proteome depth and data quality with such small inputs, while also ensuring precise spatial registration. We overcame these challenges through iterative pipeline optimization, combining sensitive sample prep, cutting-edge mass spectrometry, and robust image-to-proteome alignment tools like PIPΣX.

Q: For researchers interested in adopting mxDVP for their own spatial proteomics questions, what practical guidance or lessons learned can you share from your experience?

A: Start by defining clear biological questions, particularly where understanding of rare, transitional, or spatially restricted cell states can provide new insights into tissue organization, disease mechanisms, or therapeutic response. Whether your focus is on deep molecular profiling of specific cell populations or mapping their interactions within complex tissue microenvironments, mxDVP's modular design allows you to tailor the workflow to your scientific priorities.

For projects emphasizing cell state plasticity, stress responses, or metabolic adaptation, the workflow can be tuned for maximum proteomic depth. Conversely, if your questions center on spatial relationships, such as cell-cell communication, niche occupancy, or vascular proximity, mxDVP enables integration of spatial context at single-cell or neighborhood resolution.

To help ensure success, invest in rigorous sample preparation, high-quality image analysis workflows, and automation of key steps like contour export and cell isolation, which are essential for reproducibility, particularly when working with low-input materials. Finally, always validate the compatibility of your antibody panels with downstream mass spectrometry workflows; small optimizations in washing or staining protocols can have significant impacts on the sensitivity levels required for deep proteomic analysis.

Overall, mxDVP empowers researchers to align technological precision with their most pressing biological questions, enabling discoveries that integrate spatial context, cell phenotyping, and deep molecular characterization in a single, harmonized workflow.

Q: Looking ahead, how do you envision mxDVP evolving? Where do you see the greatest potential for spatial proteomics to impact biomedical research or clinical practice in the future?

A: mxDVP represents a transformative step toward truly integrative spatial proteomics, where the power of unbiased molecular depth meets the precision of spatially resolved phenotyping at single-cell resolution. By bridging these modalities in a modular, flexible workflow, mxDVP empowers researchers to move beyond static cell classifications and explore dynamic cell states, rare phenotypes, and tissue ecosystems in unprecedented detail. As technology continues to scale, we envision mxDVP becoming a foundational tool for translational research, enabling the discovery of new biomarkers, mechanisms of disease progression, and therapeutic vulnerabilities within the spatial complexity of human tissues.

Summary

- Spatial biology supported by mxDVP enables the study of proteome heterogeneity down to single cells while preserving spatial context to capture the phenotype of rare cells *in situ*
- The Orbitrap Astral MS achieved deep proteome coverage of >6000 proteins from ~100 cell equivalents across ~3,600 cell subpopulations
- High reproducibility with coefficients of variation below 10% and Pearson correlation coefficients typically above 0.95 were achieved with the mxDVP and LC-MS/MS workflow
- Mass spectrometry-based proteomics offered comprehensive, unbiased proteome profiling to allow the discovery of unexpected cell states, new biomarkers, mechanisms of disease progression, and potentially the ability to target therapeutic vulnerabilities

References

1. Mardamshina, M. et al. Streamlining Multiplexed Tissue Image Analysis with PIPΣX: An Integrated Automated Pipeline for Image Processing and EXploration for Diverse Tissue Types. *bioRxiv* **2025**.05.04.652145; doi: https://doi.org/10.1101/2025.05.04.652145

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