High-Parameter Spatial Multi-Omics through Histology-Anchored Integration

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ABSTRACT

Recent advances in spatial omics technologies enable *in situ* molecular profiling while preserving spatial context but face fundamental challenges in achieving high-parameter and multi-omics co-profiling. Spatially resolving complementary panels or distinct omics layers across serial tissue sections circumvents technical trade-offs but introduces the *spatial diagonal integration problem*: reconstructing unified multi-omics states when datasets lack shared molecular features. To address this critical challenge, we present **SpatialEx** and its extension **SpatialEx+**, computational frameworks that leverage histology as a universal anchor to integrate spatial molecular data across tissue sections. The foundational SpatialEx model combines a pre-trained H&E foundation model with hypergraph learning and contrastive learning to predict single-cell omics profiles from histology, encoding multi-neighborhood spatial dependencies and global tissue context. Building upon SpatialEx, SpatialEx+ introduces an *omics cycle module* that encourages cross-omics consistency across adjacent sections via slice-invariant mapping functions, achieving seamless diagonal integration without requiring co-measured multi-omics data for training. Through rigorous validation across three key applications, we demonstrate: (1) **H&E-to-omics prediction** at single-cell resolution, characterizing tumor microenvironments beyond sequencing borders in breast cancer; (2) **panel diagonal integration**, merging non-overlapping spatial gene panels from different slices to resolve immune-stromal boundaries lost by individual panels; and (3) **omics diagonal integration**, revealing Parkinson's disease anatomical domains and context-specific tissue pathologies through integrated transcriptomic-metabolic analysis. The framework scales to datasets exceeding one million cells, maintains robustness with non-overlapping or heterogeneous sections, and supports unlimited omics layers *in principle*. By transforming highly feasible spatial single-omics assays with histology into a holistic spatial multi-omics map, our work democratizes systems-level tissue analysis, bridging fundamental spatial biology and scalable multi-omics research with minimal experimental overhead.

1. INTRODUCTION

The functionality of biological systems is intrinsically linked to the spatial organization of their cells[1]. Recent advances in spatial omics now enable high-resolution, *in situ* profiling of gene expression, epigenomic features, and proteomic distributions[2–4]. These technologies have revolutionized our capacity to map molecular architectures, offering valuable insights into tissue development[5,6], disease progression[7,8], and the cellular interactions[9,10].

Although significant progress has been made, biological complexity requires a multi-layered analysis that goes beyond single-omics measurements[11]. Efforts to profile multiple omics layers from a single tissue slice—using technologies such as DBiT-seq[12], spatial-CITE-seq[13], spatial multimodal analysis (SMA)[14], spatial ATAC-RNA-seq[15], and spatial-Mux-seq[16]—face inherent trade-offs, including limited resolution, low throughput, and technical challenges due to complex processing[17,18]. An alternative approach—profiling distinct single-omics layers (e.g., transcriptomics or metabolomics) on different slices and then aligning them computationally—avoids some pitfalls, but inevitably creates mismatches at cellular level, compromising integration accuracy and downstream analysis[19–21].

Even within a single spatial omics modality, high-parameter profiling remains challenging[3,22,23]. High-resolution spatially resolved transcriptomics (SRT) techniques, such as MERFISH[24] and 10x Xenium[25], achieve single-cell resolution but are limited by pre-defined gene panels, which cover only a fraction of the whole transcriptome. This limitation hinders the detection of subtle cell states and restricts the potential for hypothesis-free discovery[26–28]. Although computational imputation methods using scRNA-seq data have been proposed, they often overlook spatial context and therefore fail to capture local gene expression gradients[29]. Moreover, expanding coverage by profiling different slices with complementary gene panels brings back alignment issues similar to those seen in multi-omics workflows.

To address these limitations, we employed an experimental-computational framework that leverages serial tissue slices analyzed with distinct single-omics techniques. By pairing routine spatial single-omics assays with histology images, our goal is to reconstruct high-parameter multi-omics profiles without the need for simultaneous experimental co-profiling. However, integrating disparate omics layers across non-overlapping slices remains a significant challenge, similar to the diagonal integration issues encountered in single-cell genomics where datasets lack shared omics features[30,31]. In contrast to single-cell data, spatial omics includes additional layers of spatial context and cellular morphology that correlate with molecular profiles[32–34]. Building on these inherent correlations, we introduce the first computational framework for spatial diagonal integration, using histology as a feasible but effective anchor to align complementary omics features from adjacent slides.

We introduce SpatialEx and SpatialEx+. SpatialEx is built on a Hematoxylin and Eosin (H&E) foundation model, hypergraph learning, and a contrastive learning strategy, and it is designed to predict omics profiles directly from H&E images regardless of the spatial resolution of technologies (e.g., single-cell or spot). Building on SpatialEx, SpatialEx+ addresses the spatially resolved diagonal integration problem by connecting multiple SpatialEx models trained on adjacent slices via our newly introduced omics cycle module, which further regularizes the relationships across different omics and gene panels across slices.

Comprehensive benchmarking analyses demonstrate the superior performance of our proposed methods in a variety of tasks. These include predicting single-cell omics profiles from H&E images (i.e., H&E-to-omics prediction), using diagonal integration to expand high-parameter gene panels (i.e., panel diagonal integration), and using diagonal integration to achieve spatial multi-omics (i.e., omics diagonal integration). SpatialEx outperformed existing methods in H&E-to-omics prediction, successfully enabling the characterization of tumor microenvironments outside the sequencing area. In panel diagonal integration, SpatialEx+ successfully aligned spatial domains between two breast cancer slices that underwent SRT with non-overlapping gene panels. Furthermore, the integrated large gene panel achieved through diagonal integration revealed previously indistinguishable distinctions between immune and stromal regions. We evaluated SpatialEx+'s omics diagonal integration capabilities using two distinct cases: spatial transcriptomic-protein profiling and spatial metabolic-transcriptomic profiling. Notably, in the Parkinson's Disease study, the SpatialEx+-generated co-profiling revealed both anatomic and fine-grained pathological domains that were overlooked by spatial single-omics alone. To further validate our approach, we tested SpatialEx+'s scalability using datasets containing over 1 million cells and challenging cases that violate the adjacent slices requirement (including slices with significant differences and no overlapping regions). In principle, SpatialEx+ enables the simultaneous generation of comprehensive high-parameter spatial multi-omics without the limitation on the number of omics layers. Most significantly, SpatialEx+ achieves these capabilities while requiring only spatial single-omics assays paired with histology, thereby eliminating the need for complex paired multi-omics data collection.

2. RESULTS

The Spatial Diagonal Integration Problem

In single-cell genomics, data integration strategies are traditionally categorized into four types: vertical, horizontal, mosaic, and diagonal integration[30]. Among these, diagonal integration presents the most significant challenges due to the absence of overlapping omics features between datasets[35]. Current approaches to diagonal integration rely on weak biological links, such as correlating scATAC-seq data with gene expression through chromatin accessibility patterns around genes, or connecting genes and proteins through protein-coding gene relationships[31,36,37]. While the spatial omics field has made substantial progress in addressing vertical[38,39], horizontal[40,41], and mosaic integration[42] challenges, the problem of spatial diagonal integration—specifically, how to integrate tissue slices profiled with different single-omics measurements to generate comprehensive multi-omics profiles for each cell—remains largely unresolved.

Recent work has expanded the concept of diagonal integration to include the integration of datasets with the same omics type but non-overlapping features[35]. Building on this framework, we define two scenarios in spatial diagonal integration: panel diagonal integration and omics diagonal integration. Panel diagonal integration focuses on integrating data across different slices analyzed with the same omics technology but different feature panels, while omics diagonal integration addresses the integration of different omics types across different slices. These integration approaches respectively address the challenges of achieving high-parameter (i.e., large panel) spatial profiling and spatial multi-omics profiling.

In the approach to spatial diagonal integration, one can leverage histology images as an anchoring mechanism to bridge different tissue slices. Consider a series of tissue sections, each analyzed with a distinct omics technique paired with H&E image—a much more practical approach than simultaneous spatial multi-omics co-profiling. As discussed in a recent Comment by Prof. Mingyao Li's Lab[43], one might initially consider developing individual H&E-to-omics prediction models for each slice, then applying these trained models across slices to predict missing omics data. This consideration led us to develop a technology-agnostic H&E-to-omics prediction model that incorporates both histology and spatial information (SpatialEx's motivation). However, this straightforward approach proves suboptimal, as histological and spatial information alone may not provide sufficient context for accurate omics profile prediction indicated by recent studies[34,44]. This limitation motivated us to develop additional regularization modules that enable collaborative training among omics-specific H&E-to-omics models, thereby enhancing prediction accuracy using omics-omics relationships (SpatialEx+'s motivation).

Overview of SpatialEx and SpatialEx+

Building upon our histology-anchored spatial diagonal integration framework, we first developed SpatialEx, a technology-agnostic H&E-to-omics prediction model. SpatialEx establishes a robust mapping between H&E images and omics expression profiles, enabling precise prediction of omics expression values from histological data (Fig. 1A-D). The model processes input H&E images through cell segmentation[45] and cropping to generate single-cell morphology images, which are then transformed into high-dimensional feature representations using a pre-trained H&E foundation model[46] (Fig. 1B).

While existing approaches typically employ traditional graph structures based on cell-cell adjacency to capture pairwise relationships[47–49], a few studies suggested that such approaches represent the complexity of biological tissues in a sub-optimal manner[50,51]. To address this limitation, we implemented a hypergraph structure that models group-level correlations through hyperedges (Fig. 1C), motivated by established principles in spatial biology, i.e., cellular microenvironments require collective interactions among cell groups to regulate omics states[52]. Our hypergraph design statistically encodes these spatial group dependencies, with ablation studies confirming superior predictive accuracy over pairwise graph models (Supplementary Fig. 15).

To further strengthen the model's capabilities, we incorporated contrastive learning to integrate globally relevant data across the entire tissue (Fig. 1D). This approach involves generating a corrupted hypergraph H' by shuffling cell embeddings while maintaining the topological structure. The contrastive learning mechanism ensures that cells in the original hypergraph are drawn closer to the summary node S while pushing cells in the corrupted hypergraph farther away, thereby effectively capturing both cell expression patterns and their surrounding microenvironments. During testing, while the contrastive learning component is omitted, all other procedures remain operational.

Building on SpatialEx, we developed SpatialEx+ to enable diagonal integration of spatial omics data from adjacent tissue slices (Fig. 1E, F). The framework begins with data preparation (Fig. 1E), where spatial assay and H&E staining are performed on adjacent slices of a tissue sample, generating both spatial omics data and H&E images for various molecular modalities including genes, metabolites, or proteins. SpatialEx+ implements a collaborative training framework that enables mutual guidance between predictive branches of different omics types. When processing H&E images from adjacent tissue slices, the framework simultaneously generates distinct omics data for both slices. Central to SpatialEx+ is the *omics cycle module*, which employs a non-linear transformation to establish explicit omics-omics associations. This module encourages conservation of cross-omics mapping functions between adjacent tissue sections: the relationship f (e.g., gene expression → protein) remains identical between slices, even when omics 1 is measured/predicted in Slice 1 and omics 2 is predicted/measured in Slice 2. By constraining f to align across neighboring sections—where spatial continuity ensures biological similarity—the model avoids conflicting slice-specific predictions, harmonizing multi-omics inferences under unified tissue-wide principles. The effectiveness of these technical components has been validated through ablation studies (Supplementary Fig. 15).

We validated our framework across three experimental scenarios (Fig. 1G): (1) H&E-to-omics prediction, where SpatialEx translates histology to omics at single-cell resolution; (2) panel diagonal integration, where SpatialEx+ generates large-panel SRT data containing both gene panels from adjacent slices; and (3) omics diagonal integration, where SpatialEx+ generates spatial multi-omics data containing both omics from adjacent slices. Detailed procedures can be found in Supplementary Fig. 1 and Methods.

SpatialEx Translates Histology to Omics at Single-Cell Resolution

Current H&E-to-omics prediction methods are mostly limited to spot-level resolution[32,53–55], leaving a critical gap in single-cell resolution prediction. While DeepPT[55] represents the most recent advance and state-of-the-art in this field based on a recent benchmarking study[34], it was originally designed for spot-resolution prediction. We modified DeepPT's implementation to enable single-cell predictions, creating the first baseline for comparison at this resolution. In contrast, SpatialEx was designed as a resolution-agnostic framework, capable of accurate predictions at both spot and single-cell levels without modification. Using formalin-fixed paraffin-embedded (FFPE) sections from a 10x Xenium Human Breast Cancer dataset[25] (see Methods), we evaluated SpatialEx's unprecedented single-cell prediction capability through a train-test framework (Fig. 2A, B). Both training (Slice 1) and test (Slice 2) sections underwent Xenium sequencing for spatially resolved gene expression profiling and H&E staining for histology.

Two metrics, including Pearson Correlation Coefficient (PCC) and Structural Similarity Index Measure (SSIM) are utilized for quantitative performance evaluation, with higher PCC and SSIM values indicating better performance (see Methods). Fig. 2C illustrates the quantitative comparison between SpatialEx and DeepPT in terms of translation evaluation and downstream cell type classification. In the scatter plots for PCC and SSIM (Fig. 2C), each dot represents an individual gene. Notably, compared to DeepPT, the proposed SpatialEx demonstrates significantly improved performance in predicting the gene expression. Additionally, the CMD (see Methods) metric showed substantial improvement (Fig. 2C), with SpatialEx achieving 0.1735 compared to DeepPT's 0.3021 (lower is better).

To assess biological relevance at single-cell resolution, we evaluated cell type annotation accuracy using predicted gene expression profiles. SpatialEx demonstrated superior performance across four major cell types in Receiver Operating Characteristic (ROC) (Fig. 2C), with comprehensive evaluation of additional cell types provided in Supplementary Fig. 2. The spatial distribution of cell types annotated using SpatialEx's predicted gene expression (Fig. 2E bottom) showed strong concordance with annotations based on measured gene expression in Slice 2 (Fig. 2E top). Importantly, SpatialEx enabled cell type annotation in regions where only H&E images were available (beyond the sequenced area), which would be impossible without the SRT predicted by SpatialEx on Slice 2 (Fig. 2E). This predicted result indicates that SpatialEx can easily extend to SRT-unmeasured regions, revealing more comprehensive tissue architecture across larger tissue. More comparations can be found in Supplementary Fig. 4.

In Fig. 2D, we visualized the gene expressions of *EPCAM*, *ESR1*, and *PGR* predicted by SpatialEx and measured by spatial assays (More visualizations of genes can be found in Supplementary Fig. 3). Notably, *ESR1* and *PGR* are genes encoding biomarkers for breast cancer prognosis[25,33]. Based on the comparison, SpatialEx demonstrates a significantly better translation capability (Fig. 2D). The results from DeepPT's exhibit either under-expressions or over-expressions, deviating significantly from the real patterns. Moreover, SpatialEx not only effectively predicts the sequenced regions within the red box (Fig. 2D), with its predicted gene expression closely matching the measured gene expression, but also extends predictions to areas with only H&E available (outside the red box).

Fig. 2F (top) displays the measured spatial distribution of two cell subtypes, Myoepi. *ACTA2*+ and Myoepi. *KRT15*, and the associated histology image in Slice 1. Our proposed SpatialEx effectively captures the gene expression distribution of the marker genes *ACTA2* and *KRT15* for these two cell types on Slice 2 (Fig. 2F

bottom). As shown in Fig. 2E (with the image inside the right box being the histology image from Fig. 2F), in the DCIS_1 region (Fig. 2E top), far from the invasive tumor areas, both *ACTA2*+ and *KRT15*+ myoepithelial cells are prominent. However, near the more invasive DCIS_2 areas (Fig. 2E top), only *ACTA2*+ myoepithelial cells are observed. Then, in invasive tumor regions (Fig. 2E top), both myoepithelial cell subtypes are absent. This finding confirms that the diminishment of myoepithelial cells is a critical hallmark of the transition of cancer from *in situ* to invasive stages[25].

SpatialEx+ Enables Larger Panel Spatial Analysis through Panel Diagonal Integration

High-resolution spatial omics technologies typically face inherent limitations in feature panel size, e.g., spatial transcriptomic or protein profiling[4]. Using spatial transcriptomics as an example, even advanced platforms like 10x Xenium can only profile a restricted number of genes simultaneously. We developed SpatialEx+ to overcome this fundamental constraint through panel diagonal integration (Fig. 3A). The approach combines experimental profiling of different gene panels across tissue sections with computational integration using H&E images as anchors. While we modified DeepPT to enable independent model building for each panel, SpatialEx+ broke this isolation through its novel omics cycle module, which uniquely regularizes each omics-specific prediction.

To evaluate SpatialEx+'s panel expansion capabilities, we applied a complementary panel design using the 10x Xenium Human Breast Cancer dataset[25]. We divided its 313-gene panel into two non-overlapping sets (Panel A: 150 genes, Panel B: 163 genes) and assigned Panel A to Slice 1 and Panel B to Slice 2 for measurement, creating a scenario where adjacent sections have complementary gene coverage. The measured expression of the held-out panel (Panel B in Slice 1 and Panel A in Slice 2) served as ground truth for quantitative evaluation. Quantitative assessment demonstrated SpatialEx+'s superior performance across multiple metrics (Fig. 3B). Gene-level comparisons showed consistently higher PCC and SSIM values compared to DeepPT. CMD metrics confirmed SpatialEx+'s enhanced ability to preserve both cell-to-cell and gene-to-gene relationships present in the original data.

To validate biological relevance, we evaluated spatial domain identification using CellCharter[40] (see Methods). SpatialEx+ achieved (28%-66%) improvement in normalized mutual information (NMI) (computed against CellCharter labels using measured full panel for each clustering resolution) across various resolution scales compared to DeepPT, demonstrating robust performance (Fig. 3B). Cell type annotation accuracy, assessed through 10-fold cross-validation, showed significant improvement when using SpatialEx+'s expanded gene panels compared to single-panel baseline (indicated by gray horizontal line in Fig. 3B). This improvement highlights both the importance of comprehensive gene panels and SpatialEx+'s effectiveness in panel expansion.

In Fig. 3C, we presented the measured expressions of the genes *ESR1* and *ERBB2* in Slice 1, as well as *PGR* and *KRT14* in Slice 2, alongside the predicted expression distributions obtained using DeepPT and SpatialEx+. Among these genes, the estrogen receptor (*ER/ESR1*), progesterone receptor (*PR/PGR*), and human epidermal growth factor receptor 2 (*HER2/ERBB2*) are three crucial ones for breast cancer subtyping[25]. Upon the results in Fig. 3C, it is evident that the gene expressions from DeepPT significantly deviate from the ground truth and are limited to the sequencing-covered regions. The DeepPT predicted *ESR1*+ covers the majority of the slice, conflicting the measured results significantly. Additionally, the *PGR* gene is expressed in only a small subset of cells within the blue box, which can be successfully captured only by SpatialEx+ (Fig. 3C). This finding underscores SpatialEx+'s ability to accurately predict the expression of disease-related genes.

The impact of panel expansion became evident in spatial domain analysis. Initial domain identification using separate panels showed marked inconsistencies between slices (Fig. 3D and Supplementary Fig. 5). After SpatialEx+ integration, spatial domains showed strong alignment between sections (Fig. 3E), enabling unified tissue architecture analysis impossible with single-panel approaches (comparative analysis with DeepPT

shown in Supplementary Fig. 6). This demonstrates SpatialEx+'s ability to overcome coverage limitations while maintaining biological fidelity.

Scalability on Million-Cell Tissue Sections

To validate scalability, we applied SpatialEx+ to consecutive large-scale tissue sections from the 10x Xenium website, each containing ~ 900,000 cells (Fig. 4A). To simulate complementary panel analysis, we divided the 280-gene Xenium panel equally between the two adjacent sections and applied SpatialEx+ for diagonal integration using H&E images as anchors (Fig. 4B).

Quantitative evaluation demonstrated SpatialEx+'s superior performance at scale (Fig. 4C). Gene-level comparisons showed consistently higher PCC and SSIM values compared to DeepPT, while CMD measurements confirmed better preservation of both gene-gene and cell-cell relationships (Fig. 4C). Following the same spatial domain identification setup as in the previous section, SpatialEx+ again significantly outperformed DeepPT across varying numbers of domains (Fig. 4C).

We validated biological relevance through analysis of cancer-associated genes (Fig. 4D). In Fig. 4D, we visualized the gene expressions of *FBLN1* in Slice 2 and *LUM* in Slice 1, predicted by SpatialEx+ and DeepPT. *FBLN1* is associated with cell transformation and tumor invasion[56,57], promoting breast cancer cell survival during doxorubicin treatment[58]. And previous study has shown that lumican[59], encoded by *LUM*, is overexpressed in the breast cancer stroma and may influence tumor invasion and metastasis by modulating matrix structure and collagen arrangement. Based on the visualizations, DeepPT fails to effectively capture the spatial expression patterns of these two genes, as evidenced by numerous instances of abnormal over-expressions or under-expressions (Fig. 4D). In contrast, the gene expression predicted by SpatialEx+ closely resembles the ground truth patterns for both genes. We visualized spatial expression patterns of more genes in Supplementary Fig. 7.

The impact of panel expansion became evident in fine-grained spatial analysis (Fig. 4E). Fig. 4E illustrates the zoomed in tissue region in terms of identified spatial domains in Slice 1 across different analysis settings, including measured panel A only (Fig. 4E middle) and measured panel A and panel B (Fig. 4E bottom). When only measured panel A is used for analysis, the detected domains lead to crucial fine-grained domains being completely overlooked (Fig. 4E middle). By combining the measured genes with the predicted genes, we successfully distinguished the immune area and the stromal area (validated in the following functional analysis), as indicated by the arrows at the bottom of Fig. 4E. The domain distribution closely resembles the results obtained using the two measured full panel in Supplementary Fig. 8. Notably, we consulted senior pathologists to annotate this tissue region (Fig. 4E top), but they were unable to distinguish the immune and stromal regions based on histological features, indicating the additional insights brought by the gene expression information. We next examined these distinctions through functional analysis. Given the domains 0, 4, and 7 are particularly identified after integrating the gene panels, we performed differential gene expression analysis on these domains, and found that the critical markers for these regions, such as *LUM* and *LYZ*, are primarily located on panel B (Fig. 4F and Supplementary Fig. 9). Gene Ontology (GO) analysis of these domains (Fig. 4G) provided molecular characterization of the tissue architecture: Genes highly expressed in Domain 7 were enriched for immune response pathways (GO:0002253, adjusted p-value=4.69e-9), while Domains 0 and 4 showed enrichment for extracellular matrix and cell adhesion processes (GO:0022407, adjusted p-value=5.61e-6). These molecular signatures from the expanded panel were essential for comprehensive characterization of the tissue's functional organization, highlighting the necessity of panel expansion for detailed spatial analysis.

SpatialEx+ Enables Spatial Multi-omics through Omics Diagonal Integration

Given the technique challenges associated with spatial multi-omics sequencing from assay, existing studies generate the spatial multi-omics data by aligning serially profiled single-omics sections to construct a multi-omics dataset. Nevertheless, variations in cellular distribution between sections render this alignment strategy suboptimal. In this section, we employed SpatialEx+ to reconstruct the spatial multi-omics data in an end-to-end function with H&E images as the linkage anchor. The omics diagonal integration analysis was performed on two datasets to demonstrate its potential and applicability.

The first transcriptomic-protein integration dataset is from Human Breast Cancer dataset[25], which consists of two slices, with the first one sequenced by Xenium and Immunofluorescence, and the second one only sequenced by Xenium. We utilized the protein data of Slice 1 and gene expression data of Slice 2 to simulate the serially profiled sections with different omics (Fig. 5A). We presented the spatial expression of the protein HER2 and CD20 in Fig. 5B, including the measured expression on Slice 1, the SpatialEx+ expanded expression to the whole slide on Slice 1, and the whole wide on Slice 2. Additionally, we illustrated the spatial expression of their corresponding marker genes, with *ERBB2* encoding HER2 and *MS4A1* encoding CD20. The visualization demonstrates that SpatialEx+ not only accurately predicted their expression patterns but also expanded the sequencing region, ensuring a comprehensive panorama of the whole breast cancer slice. At the same time, we specifically zoomed in the spatial distribution of CD20 and its corresponding gene *MS4A1*. The remarkable consistency of expressions across different slices validates that SpatialEx+ can precisely capture fine-grained spatial omics patterns. These results demonstrate that our SpatialEx+ effectively performs omics diagonal integration while generating both omics layers for a single tissue slice.

We then validated SpatialEx+ on the second metabolomic-transcriptomic data. The dataset consists of mouse brain slices generated using the SMA protocol[14] (Fig. 5C). We processed the dataset to accommodate the omics diagonal integration, i.e., H&E image and metabolomics data on Slice 1, H&E image and transcriptomics data on Slice 2. Spatial metabolomics data on Slice 1 are obtained using the Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) technique, while spatial gene expression data on Slice 2 are generated through Visium sequencing. Notably, each tissue slice comprises an intact hemisphere of healthy tissue alongside a lesioned hemisphere, modeled to represent Parkinson's Disease (PD) through unilateral 6-hydroxydopamine (6-OHDA) administration (Fig. 5D, first row). First, we performed spatial domain identification on the metabolic data from Slice 1 and the transcriptomics data from Slice 2, respectively. The results reveal that the metabolic data is insufficient to resolve the brain anatomy structures, while using SRT data fails to distinguish the lesioned and intact choroid plexus (CP) (Fig. 5D, second row). After using SpatialEx+ on this data, each slice is equipped with both SRT and spatial metabolomics. Feeding the generated spatial multi-omics data into the state-of-the-art multi-omics analysis algorithm SpatialGLUE[38], both the anatomic and pathological domains, including CP (lesion) and CP (intact), could be identified successfully (Fig. 5D, third row), demonstrating the necessity of the generated different omics data and the utility of SpatialEx+ in a wide range analysis. A plausible reason for this phenomenon is that SpatialEx+ not only links histology and omics, but also introduces the omics cycle module to establish omics-omics associations. Quantitative performance metrics are detailed in Supplementary Figs. 10 and 11.

Fig. 5E (left) quantitatively shows the expression of a selected differentially expressed (DE) metabolic *m/z* 674.28592, as well as two genes, *Tac1* and *Rgs7bp*, in CP (intact) and CP (lesion). Fig. 5E (right) and

Supplementary Fig. 12 qualitatively illustrate the spatial distribution of these DE metabolites or genes. Here, *m/z* 674.28592 has been identified as highly associated with PD by the original paper[14], and its accurate identification is crucial for building a robust multi-omics transition model. The *Tac1* gene encodes tachykinin peptide[60], which is believed to function as a neurotransmitter, and the *Rgs7bp* gene may be associated with risk of aspirin-exacerbated respiratory disease[61]. SpatialEx+ complements the gene and metabolite expression in the cortical areas outside the original sequencing area, enabling multi-omics data of the mouse brain across the whole section. The *m/z* 674.28592 are primarily presented in the intact caudate putamen, while the spatial expression patterns of *Tac1* and *Rgs7bp* exhibit a unilateral enrichment trend. The metabolic expressions generated by our proposed SpatialEx+ closely resemble the ground truth distribution. Specifically, the metabolic expression levels across different functional regions exhibit a high degree of similarity to the ground truth (Fig. 5E right). The predicted distributions of the two genes (*Tac1* and *Rgs7bp*) also closely align with the measured ground truth gene distributions. Moreover, we performed GO enrichment analyses on the genes in the intact region of the mouse brain sample, and the results indicated that this group of genes is associated with dopamine (Fig. 5F). The aforementioned two sets of multi-omics experiments demonstrate that by using SpatialEx+ to complete multi-omics data on the serial tissue slice, it provides a more comprehensive understanding of disease mechanisms from different biological layers.

SpatialEx+ is Robust Even When There is Weak or No Overlap between Slices

While previous sections demonstrated SpatialEx+'s capabilities on adjacent serial sections, here we evaluate its performance under more challenging conditions that violate the adjacent section requirement. Specifically, we test scenarios with significant spatial differences and sections lacking overlapping regions, conditions commonly encountered in practical applications.

To systematically evaluate robustness, we simulated different sequencing area configurations between adjacent slices using a sliding window approach, while using the actual measured data from the entire sequencing area for evaluation (Fig. 6A). Starting from the center of the maximum overlapping region between two slices (Supplementary Fig. 13A), we systematically shifted the simulated sequencing windows along opposite X-axis directions while maintaining consistent cell numbers through dynamic selection (Supplementary Fig. 13B). This generated a spectrum of test cases from fully overlapping sequencing areas (no sliding) to completely non-overlapping areas (stride 9) (Fig. 6B). Importantly, while we used these sliding windows to simulate different sequencing area configurations during training, all performance evaluations were conducted using the true measured data across the entire sequencing area.

SpatialEx+ demonstrates strong robustness, with no significant performance decline observed across varied overlap rates (evidenced by the flat trend of both within window and within sequencing area curves in Fig. 6C). This highlights the potential capability of SpatialEx+ in scenario that the sequencing area can be expanded through serial slices. The quantitative results across the entire sequencing area show only a small difference compared to the results within the window (evidenced by the small difference between purple and green bars for each stride in Fig. 6C), providing quantitative evidence of the generalizability of SpatialEx+ in extending the sequencing area to larger area. The immune cell marker *CD3E*[62] and the tumor cell marker *CTTN*[63] are visualized in Fig. 6D and Supplementary Fig. 14. The results demonstrate that SpatialEx+ is capable of accurately reconstructing the true spatial distribution of important markers, regardless of whether the serial slices are fully overlapping or completely non-overlapping. Notably, each slice used for training contained only 20,000 to 30,000 cells within the windows, compared to over 100,000 cells across the entire sequencing area, which demonstrated that SpatialEx+ was able to generalize accurately on H&E images that were not available during training. Subsequently, under the experimental setup with no overlap (Stride 9), the gene expression predicted by SpatialEx+ on the two slices was input into Cellcharter for joint spatial domain identification. We first input the genes predicted by SpatialEx+ on the two slices into Cellcharter to generate spatially aware embeddings of each cell, and then visualize the results using SOView[64], which is designed for visualization and analysis of high-plex spatial omics data. The spatial domain results are consistent across the both slices, and the tissue structure is clearly delineated (Fig. 6E).

3. Discussion

In this study, we addressed important challenges in spatial multi-omics analysis through SpatialEx and SpatialEx+, frameworks that enable high-parameter spatial profiling and multi-omics integration using histology as an anchor. Our approach overcomes key limitations of existing methods by enabling comprehensive molecular profiling without requiring complex simultaneous multi-omics co-measurements.

Our comprehensive evaluations across multiple experimental scenarios—from small-scale validations to million-cell tissue sections, from single-modality expansions to multi-omics integrations, and from ideal adjacent sections to challenging non-overlapping configurations—demonstrate SpatialEx+'s capabilities as a versatile and robust solution for spatial molecular data analysis.

However, several important challenges remain for advancing spatial diagonal integration. First, current implementations assume consistent spatial resolution across different measurements. Future developments should address cross-slice integration where spatial resolutions vary between modalities, potentially through adaptive resolution mapping, multi-scale integration approaches, or super-resolution approaches similar to iSTAR[33]. Second, H&E image batch effects and style variations between sections can impact prediction accuracy. Incorporating style transfer networks or domain adaptation techniques could help normalize histological features across batches[65,66]. Third, while our framework successfully handles paired sections, extending to simultaneous integration of multiple adjacent slices (>2) would enable more comprehensive tissue analysis, requiring sophisticated multi-slice alignment strategies and expansion of the omics cycle module.

Technical improvements could further enhance performance, as evidenced by the hyperparameter sensitivity in Supplementary Fig. 16. While we utilized the UNI-pathology foundation model, incorporating more advanced histology image foundation models could improve feature representation. Additionally, leveraging newer developments in deep hypergraph learning could better capture cellular interactions and microenvironmental information.

The growing importance of spatial multi-omics in understanding cellular behaviors and biological processes makes our framework particularly valuable for standard laboratories unable to conduct spatial multi-omics experiments directly. By lowering the barrier to spatial multi-omics analysis while maintaining high accuracy and robustness, SpatialEx+ can accelerate applications in disease modeling, drug discovery, and tissue characterization. As spatial omics technologies continue to evolve, frameworks like SpatialEx+ that enable flexible, scalable integration will become increasingly crucial for comprehensive tissue analysis.

4. Methods

The proposed method comprises three components—H&E foundation model, hypergraph encoder, and omics cycle module—with the first two forming SpatialEx and all three together constituting SpatialEx+. Briefly, the H&E foundation model is responsible for capturing cellular morphology features from H&E images and embedding the biologically meaningful knowledge in the feature space. The hypergraph encoder enhances cellular representations by incorporating spatial contextual information, and further refines these representations by integrating global tissue context through contrastive learning. The omics cycle module facilitates the mapping of omics-omics correlations, enabling flexible omics transition between different omics modalities. The subsequent subsections provide a detailed exposition of each module.

H&E Foundation Model

Given a histology image $\mathrm{X} \in \mathbb{R}^{h \times w \times 3}$, our objective is to encode informative morphology features of each individual cell into the feature space. To achieve this, we cropped square patches centered on each cell with a pre-defined radius *r*; the set of patches can be denoted as $\mathrm{P} \in \{p_1, p_2, \dots, p_N\}$ with each patch $p_i \in \mathbb{R}^{r \times r \times 3}$ and N representing the total patch number. Subsequently, these patches were processed through a pre-trained encoder to obtain the cellular *d*-dimensional cellular representations. In this computational process, the general-purpose self-supervised pathology foundation model UNI[46] is chosen given its exceptional feature extraction capability across a wide range pathology tasks. After the histology feature extraction, the cells are transformed from their original image space into the high-dimensional feature space, enabling efficient computation even with a mass of cells.

Hypergraph Encoder

To fully exploit the spatial contextual information in terms of complex and higher-order cellular interactions, we incorporated a hypergraph structure into the computational framework. Specifically, a hypergraph can be defined as $\mathcal{G} = \{\mathcal{V}, \mathcal{E}\}$, where $\mathcal{V} = \{v_1, v_2, \cdots, v_N\}$ represents the set of nodes characterized by the extracted histology features and $\mathcal{E} = \{e_1, e_2, \cdots, e_M\}$ denotes the set of hyperedges derived from the spatial contextual relationships. Each hyperedge e_j is a non-empty subset of nodes, *i.e.*, $\emptyset \neq e_j \subseteq \mathcal{V}$. The hypergraph structure is mathematically formulated using an incidence matrix $\mathrm{A} \in \mathbb{R}^{N \times M}$ and a diagonal weight matrix $\mathrm{W} \in \mathbb{R}^{M \times M}$. The elements in A and W indicate the node-hyperedge relationship as well as the corresponding hyperedge weights. In this study, we first identified *k* spatially neighboring cells for each cell and grouped them together to form a hyperedge. This construction process ensures each hyperedge contains multiple cells and each cell participates in multiple hyperedges. As a result, this hypergraph-based representation effectively models high-order interactions, facilitating more expressive and context-aware feature learning in the subsequent computation.

Following the hypergraph construction, we leveraged the hypergraph neural networks (HGNNs)[67,68] for computation. Methodologically, we applied a two-stage message-passing mechanism[69,70], which involves node-to-hyperedge (cell-to-microenvironment) and hyperedge-to-node (microenvironment-to-cell) aggregation. Specifically, in the first stage, each hyperedge representation is iteratively updated by aggregating the features of its constituent nodes. In the second stage, each node feature is refined by aggregating the updated hyperedge representations. The whole computation process can be formally expressed as follows:

$$\mathrm{H}_{\mathcal{E}}^{(\ell)} = \sigma(\mathrm{D}_{\mathcal{E}}^{-1}\mathrm{A}^{\top}\mathrm{H}_{\mathcal{V}}^{(\ell-1)}\Theta_{\mathcal{E}}^{(\ell)}), \ (1)$$

$$\mathrm{H}_{\mathcal{V}}^{(\ell)} = \sigma(\mathrm{D}_{\mathcal{V}}^{-1}\mathrm{AWH}_{\mathcal{E}}^{(\ell-1)}\Theta_{\mathcal{V}}^{(\ell)}), \ (2)$$

where $\mathrm{H}_{\mathcal{E}}^{(\ell)} \in \mathbb{R}^{M\times d'}$ and $\mathrm{H}_{\mathcal{V}}^{(\ell)} \in \mathbb{R}^{N\times d'}$ indicate the hyperedge and node representations, respectively, $\sigma(\cdot)$ is the Rectified Linear Unit (ReLU) function[71], and $\Theta_{\mathcal{E}}^{(\ell)} \in \mathbb{R}^{d\times d'}$ and $\Theta_{\mathcal{V}}^{(\ell)} \in \mathbb{R}^{d'\times d'}$ are the layer-specific trainable weights. $\mathrm{D}_{\mathcal{E}} \in \mathbb{R}^{M\times M}$ and $\mathrm{D}_{\mathcal{V}} \in \mathbb{R}^{N\times N}$ represent the hyperedge diagonal degree matrix and the node diagonal degree matrix, respectively, where $\mathrm{D}_{\mathcal{E},j} = \sum_{v_i\in e_j}\mathrm{A}_{i,j}$ and $\mathrm{D}_{\mathcal{V},j} = \sum_{e_i\in\mathcal{E}} w_j\mathrm{A}_{i,j}$.

After obtaining the encoded cellular representations, we map them to the gene expression space using a Multilayer Perceptron (MLP) decoder. Specifically, the decoder follows a two-layer linear architecture with 512 hidden units, incorporating batch normalization (BN) and the ReLU activation function, i.e., linear → ReLU → BN → linear.

Contrastive learning is known for enhancing representation learning across various classification and regression tasks[72,73]. Here, we employed contrastive learning to refine the omics-related features by encoding the global tissue context. Formally, a corrupted hypergraph is first created as $\mathcal{G}' = \{\mathcal{V}', \mathcal{E}\}$, where $\mathcal{V}'$ represents a set of nodes with shuffled features and $\mathcal{E}$ is the original unchanged hyperedges. This corrupted hypergraph is then fed into the HGNN encoder, producing the corrupted representations $\mathrm{H}'_{\mathcal{V}}$. The message-passing mechanism used for this computation follows the formulation described in Equations (1) and (2). Referring the local-global contrastive framework of Deep Graph Infomax (DGI)[74], we generated the global context representation S using an average readout operation, and maximized the mutual information between the local representation of each cell and its global context. Unlike conventional contrastive learning methods that explicitly compute loss using positive and negative sample pairs, this strategy instead associates all node embeddings—both from the original and corrupted graphs—with a single global summary vector, reducing the computational complexity from O(N^2) to O(N). Moreover, in contrast to the standard DGI model, we opted not train a separate discriminator to assess the global information contained in the local representations and instead used a non-parametric cosine similarity objective function. In all, the global summary vector and the contrastive loss can be formulated as follows:

$$\mathrm{S} = \chi(\mathrm{H}_{\mathcal{V}}) = \frac{1}{N}\sum_{i=1}^{N}\mathrm{H}_i, \ (3)$$

$$\mathcal{L}_{cl} = \begin{cases} 1-\cos(\mathrm{H}_{\mathcal{V}},\mathrm{S}), & \text{if } \mathcal{V}\in\mathcal{G} \\ \max(0,\cos(\mathrm{H}_{\mathcal{V}'},\mathrm{S})), & \text{if } \mathcal{V}'\in\mathcal{G}' \end{cases}. \ (4)$$

Omics Cycle Module

The histology feature extractor, hypergraph representation learning, and contrastive learning jointly enable SpatialEx to translate histology to spatial omics at single-cell resolution. Building upon this foundation, SpatialEx+, an extension of SpatialEx with omics cycle modules, integrates the prediction of two distinct omics into a collaborative training framework, ensuring diagonal integration of multi-omics analysis. Specifically, each omics cycle module follows an identical structure to the MLP decoder in SpatialEx, consisting of a sequence of computations: linear → ReLU → BN → linear. It takes one type of omics data as input and translates it to another. The input omics data can either be experimentally measured or predicted

from SpatialEx, enabling flexible and scalable integration. As shown in Fig. 1F, SpatialEx+ comprises omics cycle module 1 (OC_1) and omics cycle module 2 (OC_2). The details for module training can be found in Supplementary Fig. 1 and Methods (Model optimization).

Model Optimization

SpatialEx: The reconstruction loss ($\mathcal{L}_{mse}$) and contrastive loss ($\mathcal{L}_{cl}$) are jointly utilized to optimize SpatialEx. In terms of the first one, it targets at aligning the predicted omics expression profiles and the measured ones by minimizing the mean square errors as follows:

$$\mathcal{L}_{mse} = \frac{1}{IJ}\sum_i^I \sum_j^J ||\,\mathrm{Y}_{ij} - SpatialEx(\mathrm{X})_{ij}||^2, \quad (5)$$

where i is the index for the I dataset-specific cells or spots and j is the index for the J genes. Y is the measured omics data. The overall loss function for SpatialEx is shown as follows:

$$\mathcal{L}_{SpatialEx} = \mathcal{L}_{mse} + \mathcal{L}_{cl}. \quad (6)$$

SpatialEx+: For optimizing SpatialEx+, we in total introduced four sets of loss functions, comprising two sets for the two SpatialEx networks and two sets for the two omics cycle modules. Here, we indicate the two SpatialEx networks as $\mathrm{SpatialEx}_1$ and $\mathrm{SpatialEx}_2$ and two omics cycle modules as OC_1 and OC_2. Taking the OC_1 as an example, its optimization process involves

$$\mathcal{L}_{\mathrm{OC}_1} = \mathcal{L}_{mse}(\mathrm{SpatialEx}_1(\mathrm{X}_1), \mathrm{OC}_1(\mathrm{Y}_1)) + \mathcal{L}_{mse}(\mathrm{Y}_2, \mathrm{OC}_1(\mathrm{SpatialEx}_2(\mathrm{X}_2)) \quad (7)$$

In all, the total loss for SpatialEx+ can be formulated as follows:

$$\mathcal{L}_{SpatialEx+} = \mathcal{L}_{SpatialEx_1} + \mathcal{L}_{SpatialEx_2} + \mathcal{L}_{\mathrm{OC}_1} + \mathcal{L}_{\mathrm{OC}_2} \quad (8)$$

Data Preprocess

In processing H&E images, one critical step is locating each individual cell for subsequent feature extraction. For the data labeled with cell boundaries, e.g., Xenium, we directly utilized the provided information to locate cells in images. In terms of the tissues using other techniques (not providing cell boundaries) and exceeding the sequencing area, we applied Cellpose[45] to identify the cellular position.

Xenium *in situ* human breast cancer data. We used two Xenium breast cancer datasets for experiments, including spatial transcriptomics-proteomics human breast cancer dataset and spatial transcriptomics human breast using the entire sample area. Both datasets comprise two consecutive Xenium slices with the panel size of 313 genes and 280 genes, respectively. Additionally, in the first dataset, Slice 1 includes a paired immunofluorescence (IF) image with markers for HER2 and CD20 proteins. For both datasets, we employed identical preprocessing operations for gene expressions, following the Squidpy tutorial [Analyze Xenium data — squidpy documentation]. Specifically, we first filtered out cells with fewer than 10 detected gene expression counts, and then used *scanpy.pp.normalize_total* to normalize the total gene expression across all cells. Afterward, we applied *scanpy.pp.log1p* for log transformation. For the IF data, we followed NicheTrans for image registration and protein quantification. This process enables the precise alignment of IF images with spatial transcriptomics data. Following this integration, each cell was characterized by a comprehensive molecular profile, incorporating both gene expression and protein expression values. Considering the small proteomics panel size, we only applied *scanpy.pp.scale* for data preprocessing. We then followed the

[Understanding Xenium Outputs - Official 10x Genomics Support] tutorial to align the H&E image pixels to the sequenced physical locations using Xenium Explorer.

Spatial transcriptomics-metabolomics mouse brain data. The spatial multimodal analysis (SMA) workflow enables spatial multi-omics profiling in a single tissue section; however, the measured omics data reside on distinct coordinate systems and exhibit differing resolutions. We followed the multi-omics alignment procedures in NicheTrans[75] to generate the fully aligned spatial multi-omics data. The input transcriptomic panel is defined as the intersection of the top 1,000 spatially variable genes across the three slices, while the metabolite panel consists of the intersection of the top 50 spatially variable metabolites shared among them. We first perform log transformation for gene expression data, while leaving the metabolomic expression data unchanged. Afterwards, we scale the two types of omics data uniformly to the range of 0-1 using max-min normalization.

Implementation Details

For hypergraph construction, we set k as 7, identifying the 7 nearest cells along with the center cell to construct the hyperedges. Additionally, all edge weights in the hypergraph are set to 1, i.e., W is an identity matrix. We employed a two-layer (i.e., ℓ=2) HGNN to obtain the cell representations. We trained the model using the Adam[76] optimizer with a learning rate of 0.001, and set the number of training epochs to 500. All experiments were conducted on one 24 GB Nvidia GeForce RTX 3090, using PyTorch 2.3.1 and Python 3.8 environment.

SOView visualization. We conducted joint spatial domain identification on two slices using the aligned gene expression using Cellcharter. Specifically, the process begins by applying scVI[77] for dimensionality reduction to obtain a low-dimensional representation. Next, it aggregates representations from varying numbers of neighboring cells based on their spatial locations, and concatenates them to create spatially-aware embeddings for each cell. These embeddings are then input into a Gaussian mixture model for clustering. To visualize the spatially-aware embeddings, we employed SOView[64]. The first three components of the UMAP embeddings are rescaled to the range 0–255, forming an RGB color matrix. A color plot is then generated based on this RGB color matrix and the spatial location matrix. In this way, the color similarities between spots reflect the similarities in gene expression, allowing tissue heterogeneity to be visualized in a single plot.

Cell type transferring. We jointly performed Principal Component Analysis (PCA) on the measured gene expressions of the slice 1 and the predicted gene expression of the slice 2, extracting 50 Principal Components (PCs). Afterwards, the data of slice 1 was utilized to optimize the MLP classifier, with the PCs serving as inputs and cell type annotations as targets. Here, the MLP classifier is constructed by three linear layers, where the first two layers are followed by a $LeakyReLU$ activation function and the last layer followed by a $Softmax$ function. Considering the inherent long-tail distribution of different cell types, focal loss[78] was utilized to optimize the MLP classifier. Additionally, cell types with limited samples were augmented by adding Gaussian noise, maintaining balanced class distribution in each batch. The model parameters were updated using the Adam optimizer with a learning rate of 0.001 and no weight decay.

To thoroughly evaluate transferability, we performed 10-fold cross-validation on Slice 1. In each fold, nine parts were used for training and the remaining part for validation. The best-performing model on the validation set was then tested on Slice 2.

GO enrichment analysis. We first used the *scanpy.tl.rank_genes_groups()* function from Scanpy to rank gene importance within each spatial domain. Genes with a logfoldchange > 1.5 were selected as marker genes for each spatial domain. These marker genes were then converted to gene IDs and subjected to GO functional enrichment using the *enrichGO* function from the R package clusterProfiler. Multiple testing correction was performed using the Benjamini-Hochberg FDR method (pAdjustMethod = 'fdr'), with p-value and q-value thresholds of 0.05 and 0.2, respectively.

Evaluation

In this study, three evaluation metrics, including Pearson correlation coefficients (PCC), correlation matrix distance (CMD), and the structural similarity index measure (SSIM), were utilized for quantitatively assessing the model's performance.

PCC serves to quantify the linear correlation between two variables, and its definition is shown as follows:

$$\mathrm{PCC}(x,y) = \frac{\sum_1^n (x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum_1^n (x_i-\bar{x})^2}\sqrt{\sum_1^n (y_i-\bar{y})^2}}, \quad (9)$$

where x_i represents the expression of gene i. $\bar{x}$ and $\bar{y}$ denotes the mean values of x and y.

CMD is a general measurement of the difference between two correlation matrices R1 and R2. A lower CMD value indicates better results. CMD can be formulated as follows:

$$d(R1,R2) = 1 - \frac{trace(R1\times R2)}{||R_1||_F||R_2||_F}, \quad (10)$$

where *trace*(.) calculates the trace of the matrix, and $||\cdot||_F$ represents the Frobenius norm of the matrix.

SSIM is used to evaluate the similarity between the ground truth and the predicted spatial structure of gene expression, with higher values indicating greater similarity. In this study, we used SSIM to evaluate the structural similarity of the hypergraph formed by spatial information of cells. Specifically, it can be formulated as follows:

$$\mathrm{SSIM}(x,y) = \frac{(2\mu_x\mu_y+c_1)\left(2v_{xy}^{\frac{1}{2}}+c_2\right)(v_{xy}+c_3)}{(\mu_x^2+\mu_y^2+c_1)(v_x+v_y+c_2)(v_{xy}^{\frac{1}{2}}+c_3)}, \quad (11)$$

where x and y denote measured and predicted gene expression, respectively. The μ_x and μ_y represent the spatially Gaussian-filtered gene expression of x and y. Correspondingly, v_x and v_y indicate the spatially Gaussian-filtered variance of x and y, while v_{xy} is the spatially Gaussian-filtered covariance between x and y. The c_1 and c_2 are predefined constant, $c_1 = (0.01(max(x) - min(x)))^2$, $c_2 = (0.03(max(x) - min(x)))^2$ and $c_3 = (\frac{0.03}{\sqrt{2}}(max(x) - min(x)))^2$.

Data availability

The datasets used in this study are publicly accessible. The Xenium Human Breast Cancer tissue dataset is available at https://www.10xgenomics.com/products/xenium-in-situ/human-breast-dataset-explorer. The 10x Xenium Human Breast Using the Entire Sample Area dataset is publicly available at

https://www.10xgenomics.com/datasets/ffpe-human-breast-using-the-entire-sample-area-1-standard. The Visium Spatial Multimodal Analysis dataset is available at https://data.mendeley.com/datasets/w7nw4km7xd/1.

Code availability

Our code is available on GitHub at https://github.com/KEAML-JLU/SpatialEx. The implementations of Spatial and SpatialEx+ as well as tutorials can be found at https://spatialex-tutorials.readthedocs.io/en/latest.

Author contributions

Z.Y., X.F., and R.G. conceived and supervised the study, secured funding, and provided analytical guidance. Y.L. implemented the algorithm with assistance from C.W. and Z.W. C.W. performed the main analyses with support from Y.L. and Z.W. C.W. generated the figures with input from Z.W. and L.C. Z.L. conducted the tissue annotation. J.S., B.Q., Q.Z., and R.G. provided valuable suggestions on model design. Z.Y., X.F., R.G., Y.L., and Z.W. wrote the manuscript with the input from all authors. All authors reviewed and approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

Inclusion & Ethics

Not relevant.

Figure Legend

Fig. 1: **Overall architecture of SpatialEx and SpatialEx+**. **A.** SpatialEx processes an H&E image with cell segmentation and cropping to generate images corresponding to each cell. These single-cell images are fed into an H&E foundation model, transforming them into low-dimensional embeddings in the feature space. Subsequently, a cellular hypergraph is constructed based on the spatial contextual information, and then a hypergraph encoder is employed to learn enhanced cellular features. These features are further refined through contrastive learning and are passed through a decoder to predict the spatial omics expression profiles. **B.** Cells in each H&E image are segmented according to the provided spatial coordinates or using a cell segmentation algorithm. Afterwards, the single-cell images are generated by cropping square patches with each cell centered. **C.** A hypergraph is represented by the cellular features and hyperedges, the second of which is constructed by each cell and its *k* nearest spatial neighbors. **D.** A corrupted hypergraph $\{H_1', H_2', \ldots, H_N'\}$ is generated by randomly shuffling the cell embeddings $\{H_1, H_2, \ldots, H_N\}$ of the original hypergraph while maintaining the hypergraph's topological structure. The global summary node S is derived from the original hypergraph using a graph pooling operation. The contrastive learning strategy seeks to minimize the distance between the summary node and the original hypergraph while maximizing the distance between the summary node and the corrupted hypergraph. **E.** Illustration of data preparation for SpatialEx+, i.e., two slices both having H&E images but measured with different omics data types. **F.** Building upon SpatialEx, SpatialEx+ introduces omics cycle modules, enabling diagonal integration across panels and omics of two slices and complementing the missing omics profiles in each tissue slice. **G.** Three key applications of this study include: (1) H&E-to-omics prediction, (2) Panel diagonal integration, (3) Omics diagonal integration.

Fig. 2: **SpatialEx translates histology to omics at single-cell resolution. A.** The experimental process for SpatialEx. Taking serial human breast cancer tissues, we operated H&E imaging for both slices and only *in situ* sequencing for Slice 1. **B.** The computational schematic for SpatialEx. SpatialEx predicts omics data at single-cell resolution from H&E image encoded features. **C.** Quantitative comparison of SpatialEx and DeepPT in terms of PCC, SSIM, and CMD. The accuracy of the downstream cell type transfer task using predicted gene expression in the bar plot. The ROCs of SpatialEx and DeepPT on cell type transfer task for four major cell types in the bottom-right plot. **D.** Gene expression visualization of *EPCAM*, *ESR1*, and *PGR*. Left: Ground truth. Center: DeepPT predicted spatial expression. Right: SpatialEx predicted spatial expression. The red box indicates the sequencing tissue area, whereas its outside only has the H&E image information. **E.** Visualization of cell type transfer results. Top: Cell type annotations on Slice 2. Bottom: Cell type transferring results from Slice 1 to Slice 2 using spatial predicted gene expressions. The zoomed-in ROI (left black box) illustrates the transferred cell types outside the sequencing area. **F.** The zoomed-in ROI (right black box) in Fig. 2E. Top: The H&E image and spatial distribution of Myoepi. *ACTA2*+ and *KRT15*+ cells in Slice 1. Bottom: The transferred cell type as well as corresponding marker genes (both measured and SpatialEx predicted) spatial distributions (*ACTA2*+ and *KRT 15*) in Slice 2.

Fig 3. SpatialEx+ enables larger panel spatial analysis through panel diagonal integration. A. The schematic diagram for spatial panel diagonal integration with SpatialEx+. In the experimental process: Two consecutive tissue sections were both sequenced using Xenium analyzer, measuring Panel A on Slice 1 and Panel B on Slice 2. In the computational process: SpatialEx+ bridges the two slices through H&E images, enabling more comprehensive and consistent gene expression across consecutive sections. **B.** Quantitative comparison between SpatialEx+ and DeepPT**.** The first row: Scatter plots of PCC and SSIM, with each dot

being one gene, and x- and y-axis indicating the performance of DeepPT and SpatialEx+, respectively. The second row: Left: The scatter plot of the CMD between SpatialEx+ and DeepPT on the two sections, with the x-and y-axis representing the gene dimension and cell dimension, respectively. Center: The line plot of NMI for spatial domain delineation with predicted panels across varying domain numbers. Right: The boxplot of ACC from 10-fold cross-validation for cell type transfer task using SpatialEx+ enabled large-panel gene expression. **C.** Visualization of gene expression: *ESR1* and *ERBB2* on Slice 2, and *PGR* and *KRT14* on Slice 1. Top: Measured. Center: DeepPT predicted spatial expression. Bottom: SpatialEx+ predicted spatial expression. The red box indicates the sequencing tissue area, whereas its outside only has the H&E image information. **D.** Visualization of the identified domains using distinct panels on the two serial slices. Left: Spatial domains detected on Slice 1 based on panel A. Right: Spatial domains detected on Slice 2 based on panel B. **E.** Visualization of the identified domains using SpatialEx+ enabled large-panel (Panel A + Panel B) spatial transcriptomics data.

Fig. 4. Scalability on Million-Cell Tissue Sections. A. The consecutive large-scale tissue sections from the Xenium Human Breast Cancer Using the Entire Sample Area dataset. **B.** The computational process for analyzing two large tissue sections. **C.** Quantitative comparison of DeepPT and SpatialEx+ performance on large tissue slices. From left to right: the scatter plots of PCC and SSIM for each gene, with the x-axis representing the performance of DeepPT and the y-axis representing the performance of SpatialEx+; the scatter plot of the CMD between SpatialEx+ and DeepPT on the two sections, with the x-axis representing the gene dimension and the y-axis representing the cell dimension; the line plot of NMI for spatial domain delineation using predicted panels across varying domain numbers. **D.** Spatial visualization of measured, DeepPT predicted, and SpatialEx+ predicted genes (*FBLN1* and *LUM*) on Slice 1 and Slice 2. **E.** Spatial domain identification results for the ROI in Slice 1. Top: The annotated H&E images. Center: The spatial domains generated using only measured panel A. Bottom: The spatial domains identified using SpatialEx+ enabled large-panel (panel A + panel B). **F.** Left: Dot plots showing differentially expressed genes in domain 0, 4, and 7. Right: The measured and SpatialEx+ predicted spatial expressions of *LYZ* and *LUM*. **G.** GO terms generated by the markers of domain 7, and domain 0&4.

Fig 5. SpatialEx+ enables spatial multi-omics through omics diagonal integration. A. The schematic diagram illustrates the diagonal integration of spatial transcriptomics-proteomics data using SpatialEx+, encompassing both the experimental and computational processes. **B.** Left: Visualizations of CD20 and HER2 protein expression measured on Slice 1, alongside predicted by SpatialEx+ on both Slice 1 and Slice 2. Right: Visualizations of the coding genes *MS4A1* and *ERBB2* measured on Slice 1 and predicted by SpatialEx+ on both Slice 1 and Slice 2. **C.** The schematic diagram of spatial transcriptomics-metabolomics data diagonal integration using SpatialEx+. **D.** Top: The spatial domain annotations of both Slice 1 and Slice 2. Center: The identified spatial domains on Slice 1 using metabolomics data and on Slice 2 using transcriptomic data. Bottom: Multi-omics spatial domain identification results using SpatialGLUE after SpatialEx+ supplements the transcriptomics information for Slice 1 and the metabolomic information for Slice 2. **E.** Quantitative (left) and qualitative (right) visualizations of the metabolites m/z 674.28592, and genes *Tac1* and *Rgs7bp*. **G.** GO enrichment analyses on the genes highly expressed within the CP (intact) region.

Fig 6. SpatialEx+ is robust even when there is weak or no overlap between slices. A. A schematic diagram of the whole slide image, sequencing area, and the sliding window of input data. **B.** Illustration of sliding windows transitioning from highly overlapping to low overlapping configurations. **C.** The performance of

SpatialEx+ under different overlapping scenarios. The original H&E images were evenly divided into 10 strides, with the x-axis representing the sliding step size and the y-axis denoting performance metrics. A bar chart showing the PCC values of SpatialEx+ at each step size, with values calculated within the window (green) and across the entire sequencing area (purple). The two curves are obtained through fitting. **D.** Spatial visualizations of selected genes (*CD3E* and *CTTN*) at Stride 1 (complete overlap) and at Stride 9 (Non-overlap) from SpatialEx+. **E.** SOView visualization of SpatialEx+ predicted expression at Stride 9 (non-overlap). In the no-overlap experimental setting, the gene expressions predicted by SpatialEx+ on two slices were input into Cellcharter to generate spatially informed embedding and then visualized the results using SOView.

Supplementary Figure 1. Detailed training procedure of SpatialEx+. In this framework, Slice 1 is provided as input to SpatialEx 1, which generates the predicted omics 2 $\widehat{Y}_2$, along with the calculation of loss. Similarly, Slice 2 is fed into SpatialEx 2, producing the predicted omics 1 $\widehat{Y}_1$ and calculating the loss. Omics cycle 1 is optimized using the loss $\mathcal{L}_{OC_1}$. Subsequently, the measured omics 1 Y_1 and predicted omics 2 $\widehat{Y}_2$ are input into omics cycle 1 to calculate the loss. Likewise, the measured omics 2 Y_2 and the predicted omics 1 $\widehat{Y}_1$ are input into omics cycle 2 to obtain the loss. Omics cycle 2 is optimized using the loss $\mathcal{L}_{OC_2}$. The solid and dashed lines in the figure represent corresponding input and generated loss values for the omics cycle module.

Supplementary Figure 2. ROC curves and AUC values for cell type classification using gene expression predicted by SpatialEx and DeepPT. For clarity, the curves are organized into three panels according to the number of cells of each cell type. Different colors are used to represent distinct cell types, with solid and dashed lines corresponding to SpatialEx and DeepPT, respectively. The AUC values for each cell type are provided in legends.

Supplementary Figure 3. Visualization of gene expression on Slice 1. Left: Measured. Center: DeepPT predicted. Right: SpatialEx predicted. The region enclosed by the red box represents the sequencing area, whereas the surrounding outer region contains only H&E images.

Supplementary Figure 4. Visualization of cell types classified using DeepPT predicted gene expression. (A) Cell type annotations on Slice 2 and spatial distribution of cell types classified using DeepPT-predicted gene expression. (B) Top row: Spatial distribution of two myoepithelial cell subtypes (Myoepi. *ACTA2*+ and Myoepi. *KRT15*+) predicted by DeepPT within the zoomed region. Second row: Measured spatial distribution of marker genes (*ACTA2* and *KRT15*) corresponding to the two subtypes in the zoomed region. Bottom row: DeepPT-predicted spatial distribution of marker genes in the zoomed region.

Supplementary Figure 5. Spatial Domain identification across different conditions on Xenium *in situ* breast cancer dataset. Spatial domains derived from experimentally measured full-panel gene expression were used as the ground truth. The identified domains using SpatialEx+ and DeepPT predicted Panel B are illustrated in the second and third columns, respectively. NMIs are utilized as quantitative metrics. Notably, DeepPT fails to distinguish between invasive ductal carcinoma (IDC) and ductal carcinoma *in situ* (DCIS) on the first tissue slice.

Supplementary Figure 6. Integrated spatial domain identification across the two slices using DeepPT expanded large-panel expression. Given the inferior prediction capability of DeepPT, the clustering results using the predicted large-panel expression fail to distinguish DCSI and IDC. This limitation also results in inconsistent spatial domain assignments for cells at identical anatomical locations, for example in zoomed-in areas.

Supplementary Figure 7. Visualization of gene expression on the big dataset. The left half of the page shows the spatial expression of panel B on Slice 1, while the right half displays the spatial expression of panel A on Slice 2. For each gene, from left to right columns indicate the measured expression, the expression predicted by SpatialEx+, and the expression predicted by DeepPT, respectively.

Supplementary Figure 8. Spatial domain identification across different conditions on the big human breast cancer dataset. The first and second rows illustrate the results on Slice 1 and Slice 2, respectively. Column 1: Joint spatial domain identification on both slices using measured full-panel gene expression data. Column 2: Independent spatial domain identification of each slice with measured partial panels (Panel A on Slice 1; Panel B on Slice 2). Column 3: Joint spatial domain identification on the two slices using SpatialEX+ predicted full panel (Panel A + Panel B).

Supplementary Figure 9. Visualization of representative DEGs regarding spatial domain 0&4, and domain 7 in Slice 1 on the big breast cancer dataset. The predictions from SpatialEx+ demonstrate more close spatial patterns to the measured gene expression, whereas DeepPT failed to recapitulate the transcriptional distinctions between these domains.

Supplementary Figure 10. Quantitative evaluation of SpatialEx+ on the human breast cancer spatial transcriptome-proteome multi-omics dataset. From left to right: Pearson correlation coefficient (PCC), structural similarity index metric (SSIM), and correlation matrices difference (CMD). In PCC and SSIM plots, each teal point represents an individual gene, and the black points indicate mean values across all genes.

Supplementary Figure 11. Quantitative evaluation of SpatialEx+ on the mouse brain spatial transcriptome-metabolome multi-omics dataset. (A) From left to right: PCC, SSIM, and CMD. In PCC and SSIM plots, each teal point represents an individual metabolite, and the black point indicates the mean value across all genes. (B) From left to right: PCC, SSIM, and CMD. For clarity, gene points were not illustrated, and the black points represent the mean values across all genes.

Supplementary Figure 12. Visualization SpatialEx+ predicted metabolites and genes against ground truth. (A) Slice 1: SpatialEx+ expanded the measured metabolite expression to the whole slide image (WSI) wide. Slice 2: SpatialEx+ predicted metabolite expression. (B) Slice 1: SpatialEx+ predicted gene expression. Slice 2: SpatialEx+ expanded the measured gene expression to the WSI wide.

Supplementary Figure 13. Training Data Selection in Sliding Window Experiments. (A) Illustration of the overlapping regions of the two slices regarding original sequencing areas. (B) Visualization of the cellular distributions along with sliding window striding on. The total number of cells is attached below each window.

Supplementary Figure 14. Visualization of gene expression predicted by SpatialEx+ at each stride. From stride 0 to 9, the overlapping area between the two sections progressively diminishes until complete non-overlap. The red dashed box enclosed the region involved in model training at each stride. The measured expression of each gene is illustrated on the bottom row.

Supplementary Figure 15. Ablation study. The bar plot is about the ablation study of SpatialEx+ on the Xenium *in situ* breast cancer dataset regarding mean PCC. The results demonstrate that each introduced model plays a critical role in the computation and they jointly corporate to achieve the best performance.

Supplementary Figure 16. Parameter sensitivity analysis on Xenium *in situ* breast cancer dataset. (A)

Keeping other parameters at their default settings (num_neighbors = 7, patch_radius = 64)，the bar plot illustrates the mean PCC of SpatialEx+'s results using different image encoders. (B) Keeping other parameters at their default settings (num_neighbors=7, image_encoder='UNI')，the bar plot illustrates the mean PCC of SpatialEx+'s results using different patch radiuses. (C) Keeping other parameters at their default settings (patch_radius=64, image_encoder='UNI')，the bar plot illustrates the mean PCC of SpatialEx+'s results using different spatial context—different number of neighbors.

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Figure 1

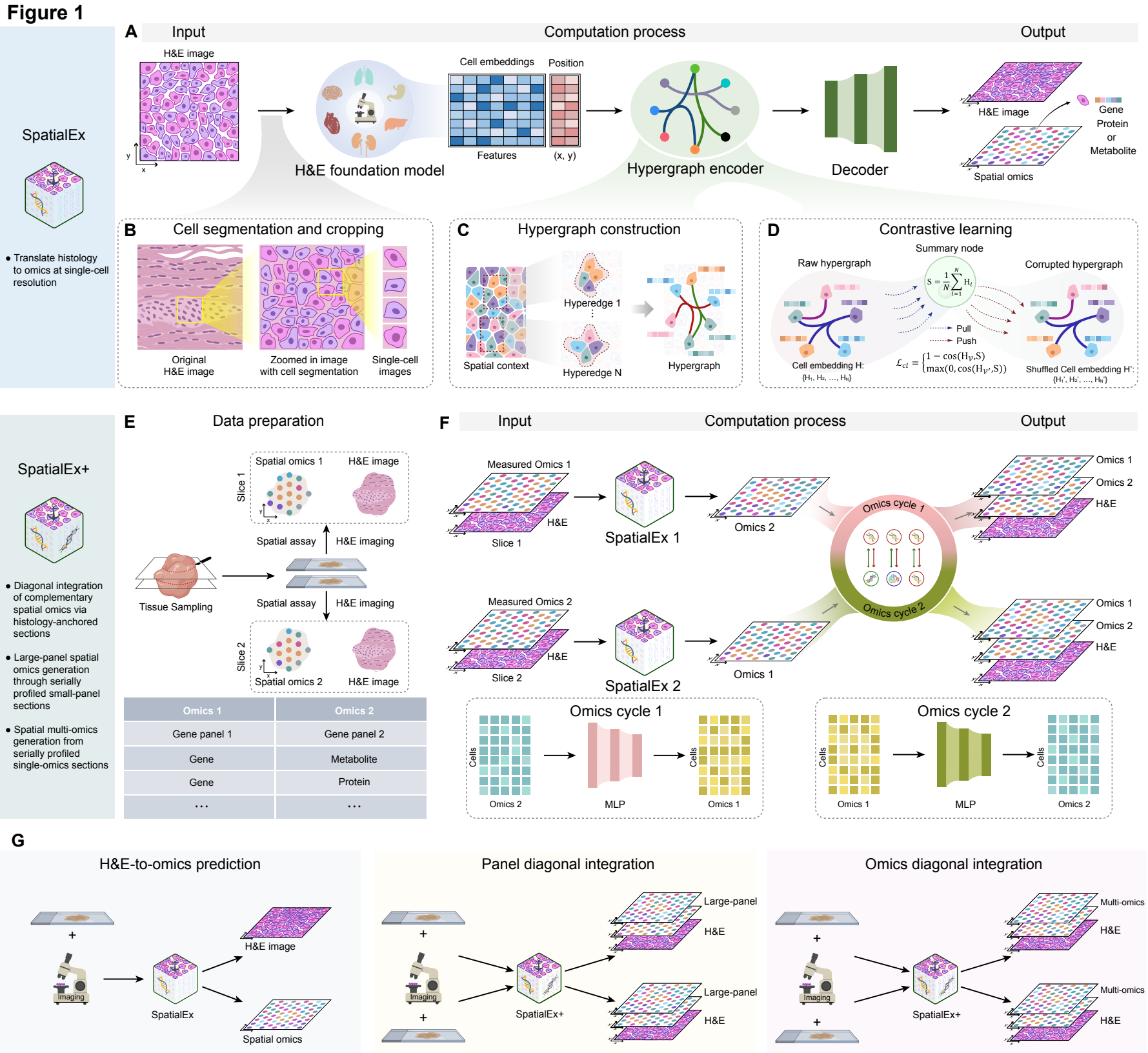

A
Input
Computation process
Output
SpatialEx
• Translate histology to omics at single-cell resolution
H&E image
H&E foundation model
Cell embeddings
Position
Features
(x, y)
Hypergraph encoder
Decoder
H&E image
Gene
Protein
or
Metabolite
Spatial omics
B
Cell segmentation and cropping
Original H&E image
Zoomed in image with cell segmentation
Single-cell images
C
Hypergraph construction
Spatial context
Hyperedge 1
Hyperedge N
Hypergraph
D
Contrastive learning
Raw hypergraph
Summary node
Corrupted hypergraph
Pull
Push
Cell embedding H:
Shuffled Cell embedding H':
E
Data preparation
SpatialEx+
• Diagonal integration of complementary spatial omics via histology-anchored sections
• Large-panel spatial omics generation through serially profiled small-panel sections
• Spatial multi-omics generation from serially profiled single-omics sections
Slice 1
Spatial omics 1
H&E image
Spatial assay
H&E imaging
Tissue Sampling
Slice 2
Spatial omics 2
Omics 1
Omics 2
Gene panel 1
Gene panel 2
Gene
Metabolite
Gene
Protein
F
Input
Computation process
Output
Measured Omics 1
H&E
Slice 1
SpatialEx 1
Omics 2
Omics cycle 1
Omics 1
Omics 2
H&E
Measured Omics 2
Slice 2
SpatialEx 2
Omics 1
Omics cycle 2
Omics 1
Omics 2
H&E
Omics cycle 1
Cells
Omics 2
MLP
Omics 1
Omics cycle 2
Omics 1
MLP
Omics 2
G
H&E-to-omics prediction
Imaging
SpatialEx
H&E image
Spatial omics
Panel diagonal integration
SpatialEx+
Large-panel
H&E
Omics diagonal integration
Multi-omics
H&E

Figure 2

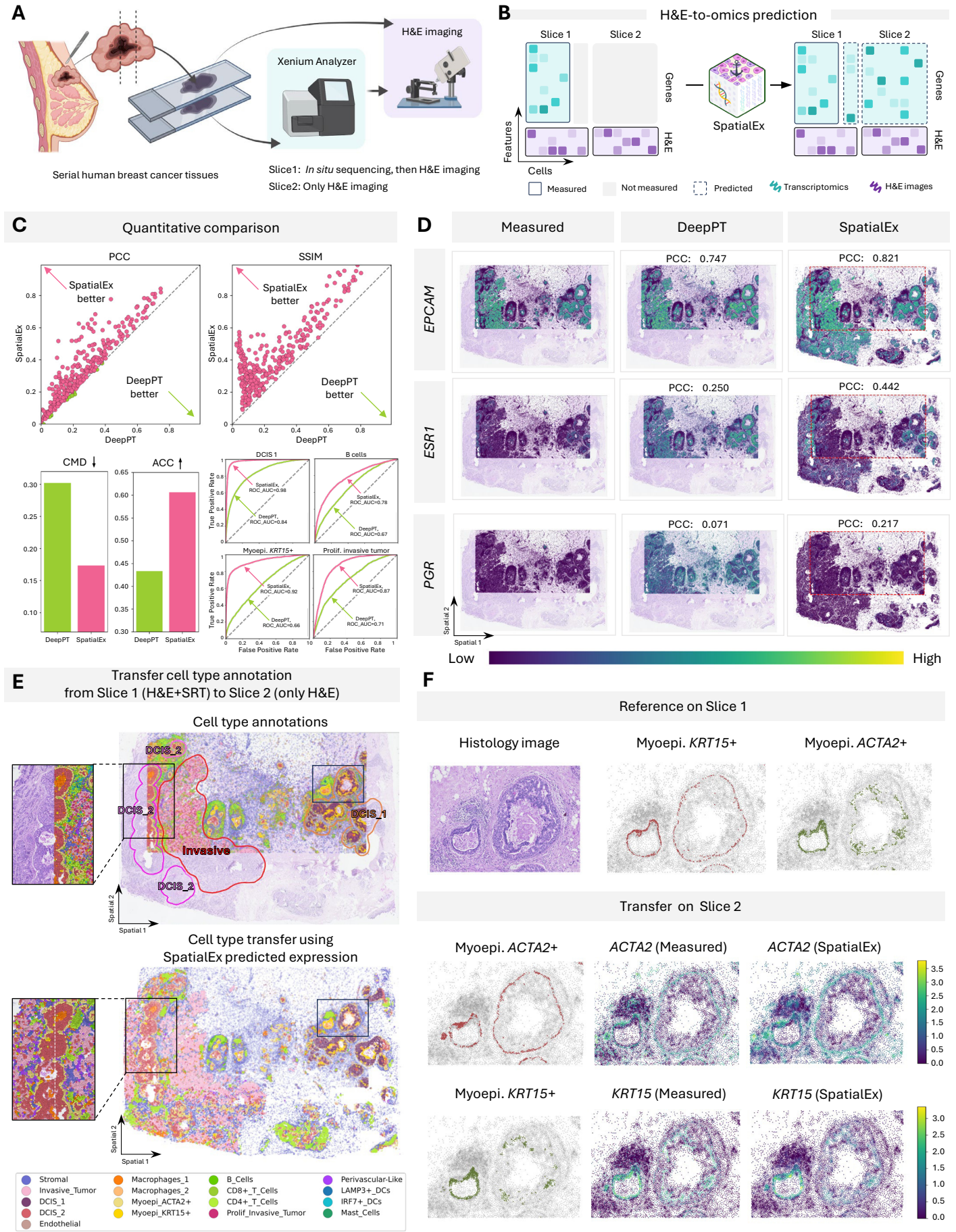

A
Xenium Analyzer
H&E imaging
Serial human breast cancer tissues
Slice1: In situ sequencing, then H&E imaging
Slice2: Only H&E imaging
B
H&E-to-omics prediction
Slice 1
Slice 2
Genes
H&E
Features
Cells
SpatialEx
Measured
Not measured
Predicted
Transcriptomics
H&E images
C
Quantitative comparison
PCC
SSIM
SpatialEx better
DeepPT better
SpatialEx
DeepPT
CMD ↓
ACC ↑
DCIS 1
B cells
Myoepi. KRT15+
Prolif. invasive tumor
True Positive Rate
False Positive Rate
SpatialEx, ROC_AUC=0.98
DeepPT, ROC_AUC=0.84
SpatialEx, ROC_AUC=0.78
DeepPT, ROC_AUC=0.67
SpatialEx, ROC_AUC=0.92
DeepPT, ROC_AUC=0.66
SpatialEx, ROC_AUC=0.87
DeepPT, ROC_AUC=0.71
D
Measured
DeepPT
SpatialEx
EPCAM
ESR1
PGR
PCC: 0.747
PCC: 0.821
PCC: 0.250
PCC: 0.442
PCC: 0.071
PCC: 0.217
Spatial 1
Spatial 2
Low
High
E
Transfer cell type annotation
from Slice 1 (H&E+SRT) to Slice 2 (only H&E)
Cell type annotations
DCIS_2
DCIS_1
Invasive
Cell type transfer using
SpatialEx predicted expression
Stromal
Invasive_Tumor
DCIS_1
DCIS_2
Endothelial
Macrophages_1
Macrophages_2
Myoepi_ACTA2+
Myoepi_KRT15+
B_Cells
CD8+_T_Cells
CD4+_T_Cells
Prolif_Invasive_Tumor
Perivascular-Like
LAMP3+_DCs
IRF7+_DCs
Mast_Cells
F
Reference on Slice 1
Histology image
Myoepi. KRT15+
Myoepi. ACTA2+
Transfer on Slice 2
Myoepi. ACTA2+
ACTA2 (Measured)
ACTA2 (SpatialEx)
Myoepi. KRT15+
KRT15 (Measured)
KRT15 (SpatialEx)

Figure 3

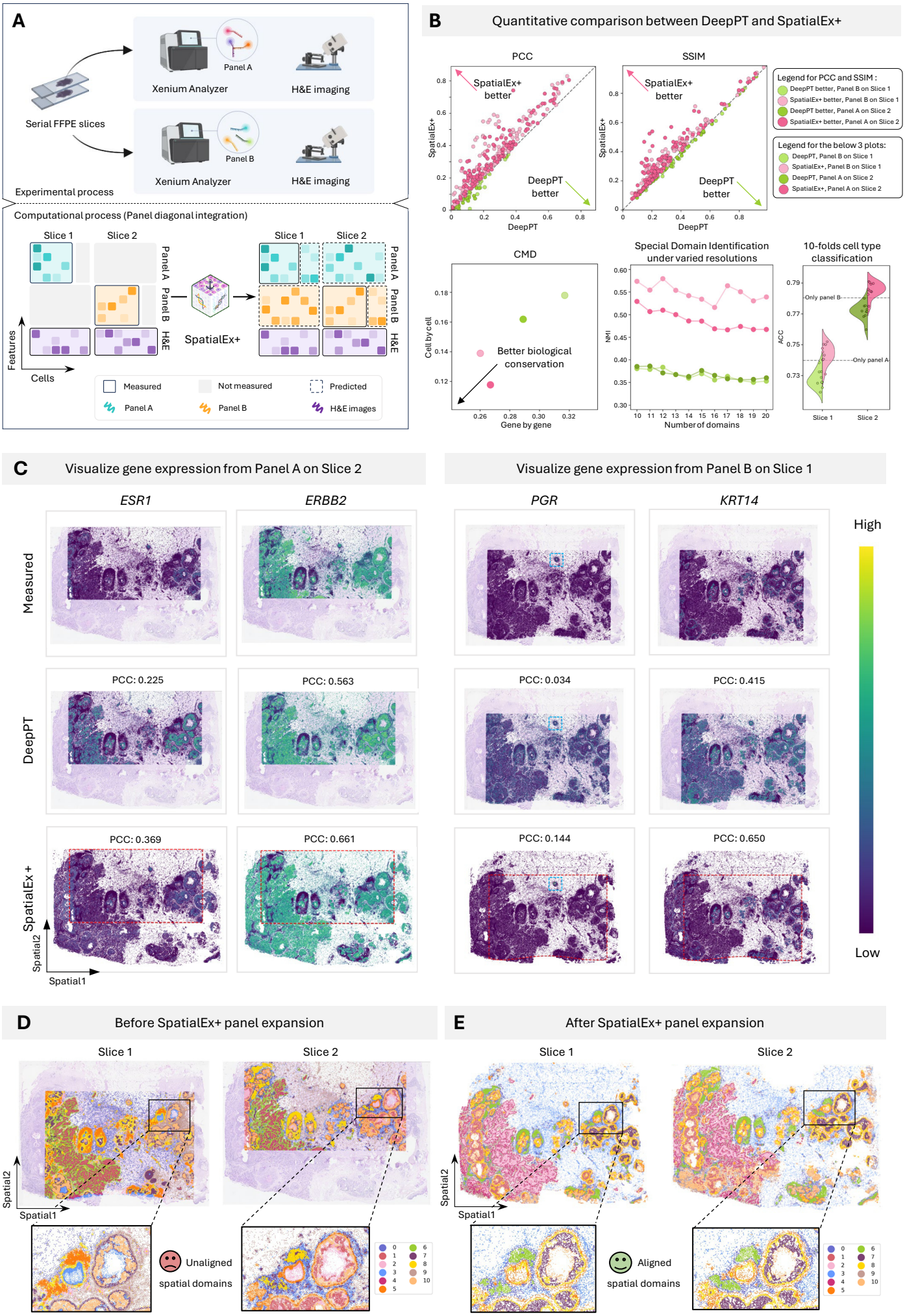

A
Serial FFPE slices
Panel A
Xenium Analyzer
H&E imaging
Panel B
Xenium Analyzer
H&E imaging
Experimental process
Computational process (Panel diagonal integration)
Slice 1
Slice 2
Panel A
Panel B
H&E
Features
Cells
SpatialEx+
Measured
Not measured
Predicted
Panel A
Panel B
H&E images
B
Quantitative comparison between DeepPT and SpatialEx+
PCC
SSIM
SpatialEx+ better
DeepPT better
SpatialEx+
DeepPT
Legend for PCC and SSIM :
DeepPT better, Panel B on Slice 1
SpatialEx+ better, Panel B on Slice 1
DeepPT better, Panel A on Slice 2
SpatialEx+ better, Panel A on Slice 2
Legend for the below 3 plots:
DeepPT, Panel B on Slice 1
SpatialEx+, Panel B on Slice 1
DeepPT, Panel A on Slice 2
SpatialEx+, Panel A on Slice 2
CMD
Cell by cell
Gene by gene
Better biological conservation
Special Domain Identification under varied resolutions
NMI
Number of domains
10-folds cell type classification
ACC
Only panel B
Only panel A
Slice 1
Slice 2
C
Visualize gene expression from Panel A on Slice 2
Visualize gene expression from Panel B on Slice 1
ESR1
ERBB2
PGR
KRT14
Measured
DeepPT
SpatialEx +
PCC: 0.225
PCC: 0.563
PCC: 0.034
PCC: 0.415
PCC: 0.369
PCC: 0.661
PCC: 0.144
PCC: 0.650
High
Low
Spatial1
Spatial2
D
Before SpatialEx+ panel expansion
Slice 1
Slice 2
Unaligned
spatial domains
E
After SpatialEx+ panel expansion
Aligned
spatial domains

Figure 4

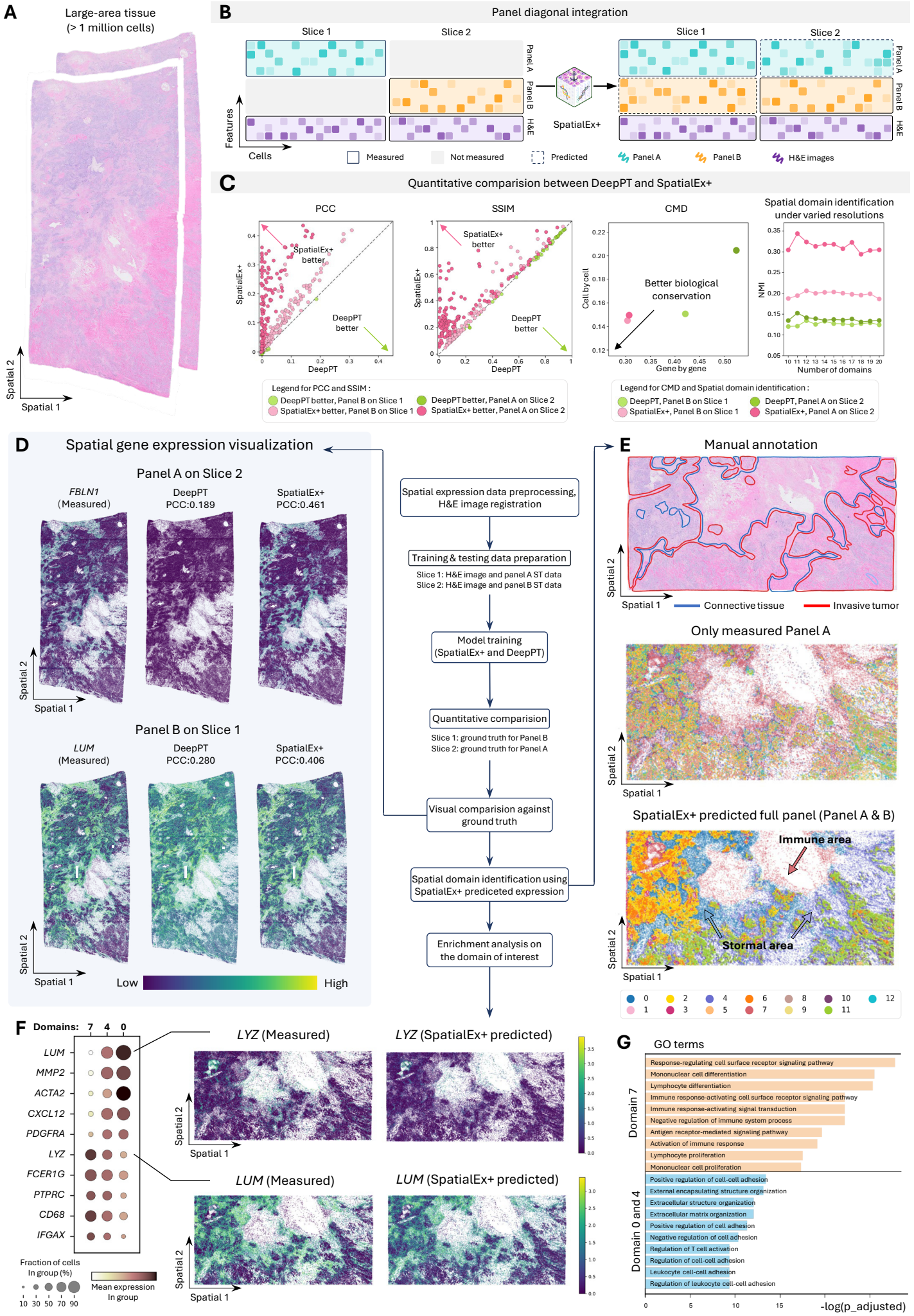

A
Large-area tissue
(> 1 million cells)
Spatial 2
Spatial 1
B
Panel diagonal integration
Slice 1
Slice 2
Panel A
Panel B
H&E
Features
Cells
SpatialEx+
Measured
Not measured
Predicted
Panel A
Panel B
H&E images
C
Quantitative comparision between DeepPT and SpatialEx+
PCC
SSIM
CMD
Spatial domain identification under varied resolutions
SpatialEx+ better
DeepPT better
SpatialEx+
DeepPT
Cell by cell
Gene by gene
Better biological conservation
NMI
Number of domains
Legend for PCC and SSIM :
DeepPT better, Panel B on Slice 1
SpatialEx+ better, Panel B on Slice 1
DeepPT better, Panel A on Slice 2
SpatialEx+ better, Panel A on Slice 2
Legend for CMD and Spatial domain identification :
DeepPT, Panel B on Slice 1
SpatialEx+, Panel B on Slice 1
DeepPT, Panel A on Slice 2
SpatialEx+, Panel A on Slice 2
D
Spatial gene expression visualization
Panel A on Slice 2
FBLN1 (Measured)
DeepPT PCC:0.189
SpatialEx+ PCC:0.461
Panel B on Slice 1
LUM (Measured)
DeepPT PCC:0.280
SpatialEx+ PCC:0.406
Spatial 2
Spatial 1
Low
High
Spatial expression data preprocessing, H&E image registration
Training & testing data preparation
Slice 1: H&E image and panel A ST data
Slice 2: H&E image and panel B ST data
Model training (SpatialEx+ and DeepPT)
Quantitative comparision
Slice 1: ground truth for Panel B
Slice 2: ground truth for Panel A
Visual comparision against ground truth
Spatial domain identification using SpatialEx+ prediceted expression
Enrichment analysis on the domain of interest
E
Manual annotation
Connective tissue
Invasive tumor
Only measured Panel A
SpatialEx+ predicted full panel (Panel A & B)
Immune area
Stormal area
F
Domains: 7 4 0
LUM
MMP2
ACTA2
CXCL12
PDGFRA
LYZ
FCER1G
PTPRC
CD68
IFGAX
Fraction of cells In group (%)
10 30 50 70 90
Mean expression In group
LYZ (Measured)
LYZ (SpatialEx+ predicted)
LUM (Measured)
LUM (SpatialEx+ predicted)
G
GO terms
Domain 7
Response-regulating cell surface receptor signaling pathway
Mononuclear cell differentiation
Lymphocyte differentiation
Immune response-activating cell surface receptor signaling pathway
Immune response-activating signal transduction
Negative regulation of immune system process
Antigen receptor-mediated signaling pathway
Activation of immune response
Lymphocyte proliferation
Mononuclear cell proliferation
Domain 0 and 4
Positive regulation of cell-cell adhesion
External encapsulating structure organization
Extracellular structure organization
Extracellular matrix organization
Positive regulation of cell adhesion
Negative regulation of cell adhesion
Regulation of T cell activation
Regulation of cell-cell adhesion
Leukocyte cell-cell adhesion
Regulation of leukocyte cell-cell adhesion
-log(p_adjusted)

Figure 5

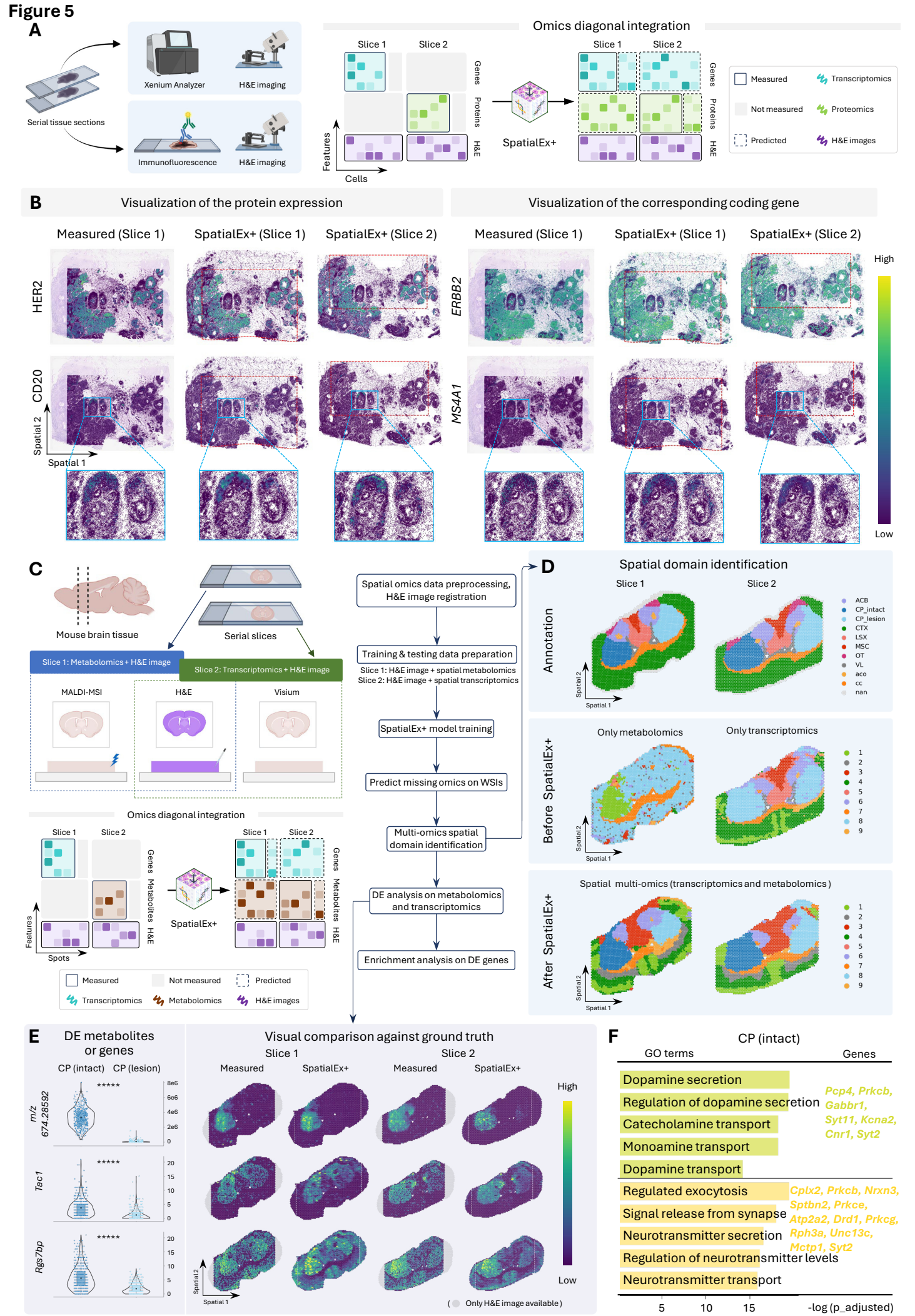
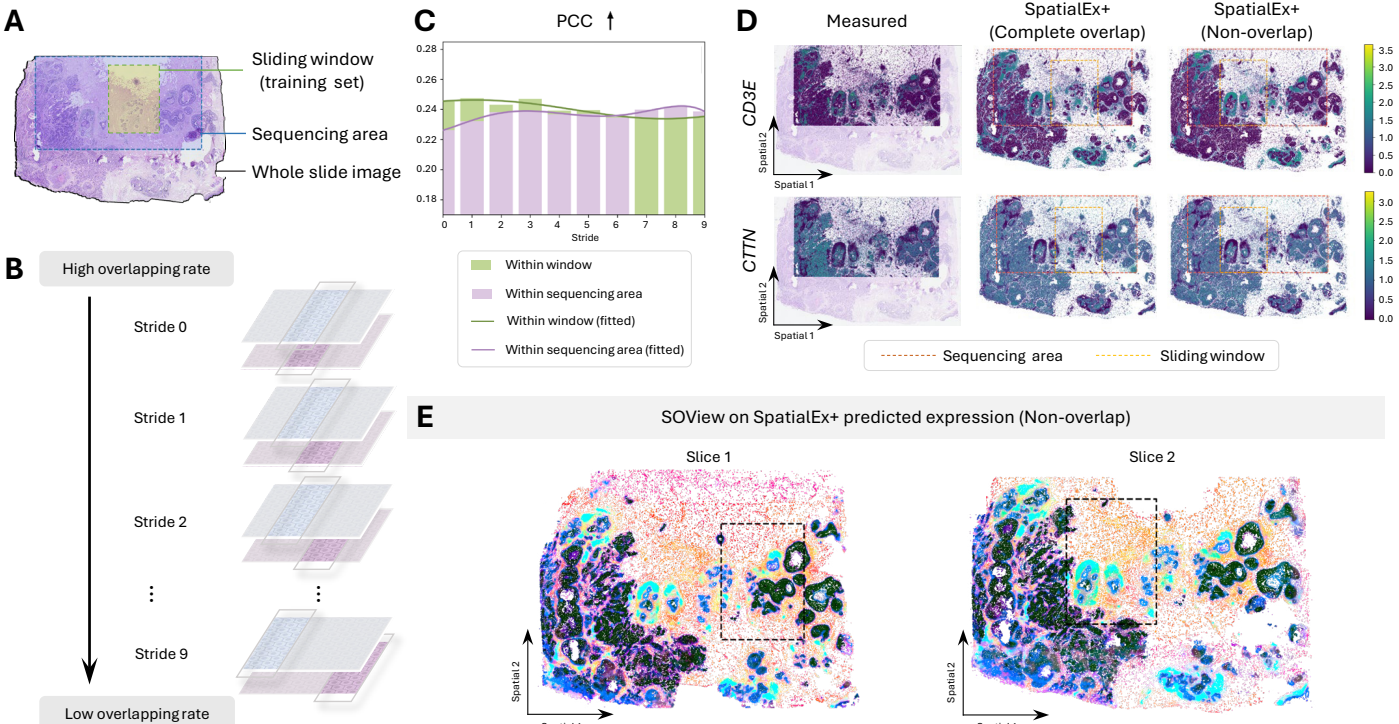

A
Serial tissue sections
Xenium Analyzer
H&E imaging
Immunofluorescence
H&E imaging
Omics diagonal integration
Slice 1
Slice 2
Genes
Proteins
H&E
Features
Cells
SpatialEx+
Measured
Not measured
Predicted
Transcriptomics
Proteomics
H&E images
B
Visualization of the protein expression
Visualization of the corresponding coding gene
Measured (Slice 1)
SpatialEx+ (Slice 1)
SpatialEx+ (Slice 2)
HER2
CD20
ERBB2
MS4A1
Spatial 2
Spatial 1
High
Low
C
Mouse brain tissue
Serial slices
Slice 1: Metabolomics + H&E image
Slice 2: Transcriptomics + H&E image
MALDI-MSI
H&E
Visium
Omics diagonal integration
Slice 1
Slice 2
Genes
Metabolites
H&E
Features
Spots
SpatialEx+
Measured
Not measured
Predicted
Transcriptomics
Metabolomics
H&E images
Spatial omics data preprocessing, H&E image registration
Training & testing data preparation
Slice 1: H&E image + spatial metabolomics
Slice 2: H&E image + spatial transcriptomics
SpatialEx+ model training
Predict missing omics on WSIs
Multi-omics spatial domain identification
DE analysis on metabolomics and transcriptomics
Enrichment analysis on DE genes
D
Spatial domain identification
Slice 1
Slice 2
Annotation
Before SpatialEx+
After SpatialEx+
Only metabolomics
Only transcriptomics
Spatial multi-omics (transcriptomics and metabolomics)
ACB
CP_intact
CP_lesion
CTX
LSX
MSC
OT
VL
aco
cc
nan
E
DE metabolites or genes
CP (intact)
CP (lesion)
m/z 674.28592
Tac1
Rgs7bp
*****
Visual comparison against ground truth
Slice 1
Slice 2
Measured
SpatialEx+
High
Low
(Only H&E image available)
F
CP (intact)
GO terms
Genes
Dopamine secretion
Regulation of dopamine secretion
Catecholamine transport
Monoamine transport
Dopamine transport
Pcp4, Prkcb, Gabbr1, Syt11, Kcna2, Cnr1, Syt2
Regulated exocytosis
Signal release from synapse
Neurotransmitter secretion
Regulation of neurotransmitter levels
Neurotransmitter transport
Cplx2, Prkcb, Nrxn3, Sptbn2, Prkce, Atp2a2, Drd1, Prkcg, Rph3a, Unc13c, Mctp1, Syt2
5
10
15
-log (p_adjusted)

Figure 6


A
Sliding window
(training set)
Sequencing area
Whole slide image
B
High overlapping rate
Stride 0
Stride 1
Stride 2
Stride 9
Low overlapping rate
C
PCC
0.28
0.26
0.24
0.22
0.20
0.18
0 1 2 3 4 5 6 7 8 9
Stride
Within window
Within sequencing area
Within window (fitted)
Within sequencing area (fitted)
D
Measured
SpatialEx+
(Complete overlap)
SpatialEx+
(Non-overlap)
CD3E
CTTN
Spatial 2
Spatial 1
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Sequencing area
Sliding window
E
SOView on SpatialEx+ predicted expression (Non-overlap)
Slice 1
Slice 2
Spatial 2
Spatial 1