Unified integration of spatial transcriptomics across platforms

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Abstract

Spatial transcriptomics (ST) has transformed our understanding of tissue architecture and cellular interactions, but integrating ST data across platforms remains challenging due to differences in gene panels, data sparsity, and technical variability. Here, we introduce LLOKI, a novel framework for integrating imaging-based ST data from diverse platforms without requiring shared gene panels. LLOKI addresses ST integration through two key alignment tasks: feature alignment across technologies and batch alignment across datasets. Feature alignment constructs a graph based on spatial proximity and gene expression to propagate features and impute missing values. Optimal transport adjusts data sparsity to match scRNA-seq references, enabling single-cell foundation models such as scGPT to generate unified features. Batch alignment then refines scGPT-transformed embeddings, mitigating batch effects while preserving biological variability. Evaluations on mouse brain samples from five different technologies demonstrate that LLOKI outperforms existing methods and is effective for cross-technology spatial gene program identification and tissue slice alignment. Applying LLOKI to five ovarian cancer datasets, we identify an integrated gene program indicative of tumor-infiltrating T cells across gene panels. Together, LLOKI provides a robust foundation for cross-platform ST studies, with the potential to scale to large atlas datasets, enabling deeper insights into cellular organization and tissue environments.

Introduction

In single-cell RNA sequencing (scRNA-seq) analysis, dataset integration is crucial for enabling robust comparisons across studies and conditions, with its importance growing alongside large public data repositories such as the Single Cell Portal [1], CellxGene [2], and the Human Cell Atlas [3]. Similarly, integrating spatial transcriptomics (ST) datasets, which contain both spatial coordinates and gene expression, enables comprehensive analysis across samples, technologies, and conditions, revealing cellular spatial organization and dynamics across diverse contexts in health and disease [4]. Although batch integration has been extensively studied in scRNA-seq [5], ST presents unique challenges. A key difficulty is variation in gene panels across samples, even within the same technology. Moreover, ST technologies differ significantly in sensitivity to specific genes [6], and varying sparsity levels of the data further complicate integration. The goal of ST batch integration is to learn a spatially aware embedding function that maps gene expression profiles into a shared feature space, ensuring biologically similar cells remain close, regardless of technology or gene panel.

Existing ST integration methods, such as STAligner [7], SPIRAL [8], DeepST [9], and PRECAST [10], predominantly align tissue slices based on shared genes, limiting their ability to integrate datasets from platforms with differing gene panels. This challenge is further compounded when integrating multiple datasets, as the intersection of gene sets across technologies becomes increasingly small. For example, our results show that datasets from five different technologies share only 19 genes, despite the smallest gene panel containing over 250.

Recently, single-cell foundation models (scFMs) such as UCE [11], Geneformer [12], scGPT [13], and others [14–16] have emerged, leveraging large-scale scRNA-seq data to learn robust, generalizable cell representations. While promising for scRNA-seq batch integration, attempts to train foundation models specifically on ST data remain limited [17, 18] due to challenges such as limited data, non-overlapping gene panels, and pronounced batch effects. Despite this, scFMs remain attractive for ST integration due to their extensive pretraining on full transcriptomes, offering a scalable solution to variability in gene panels across ST technologies. However, a key limitation is that scFMs' cell representations are learned almost exclusively from scRNA-seq data, and the differences in sparsity and gene capture efficiency between scRNA-seq and ST data further complicate their direct application to ST integration.

Here, we introduce LLOKI, a novel framework for scalable ST integration across diverse technologies without requiring shared gene panels. The framework consists of two key components: (1) LLOKI-FP, which leverages optimal transport and feature propagation to transform ST gene expression profiles, aligning their sparsity with scRNA-seq to optimize scGPT embeddings; and (2) LLOKI-CAE, a conditional autoencoder that integrates embeddings across ST technologies using a novel loss function balancing batch integration with the preservation of biological information from LLOKI-FP embeddings. This unique combination ensures both feature and batch alignment, enabling robust ST data integration while preserving biological specificity and local spatial interactions. We reveal the fragility of relying on shared gene panels for integration and demonstrate that LLOKI significantly outperforms state-of-the-art methods across standard batch integration metrics. Additionally, we show that LLOKI's embeddings facilitate important downstream tasks such as physical slice alignment, spatial domain detection, and cross-gene-panel spatially variable gene identification, enabling cross-technology analysis of tissue structure and organization across diverse contexts.

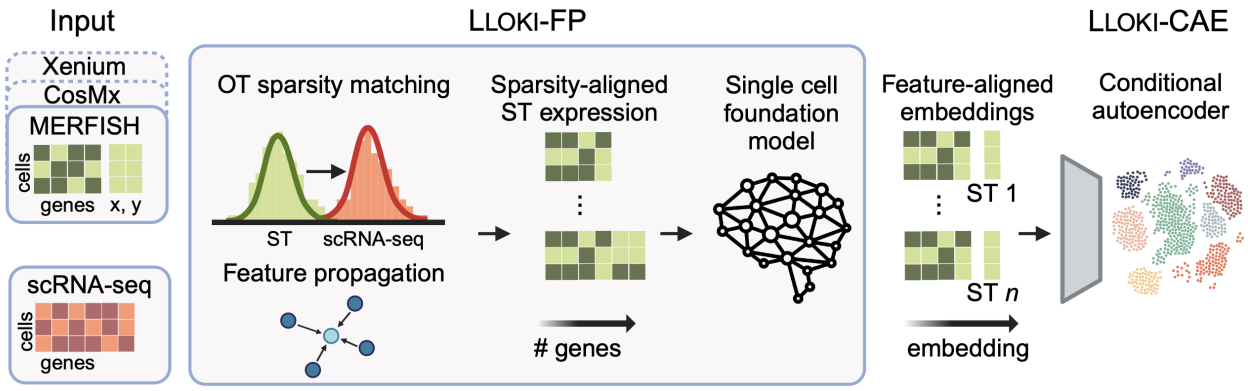


Figure 1: Overview of LLOKI. LLOKI performs a two-stage alignment: first, it unifies the feature space across ST samples, regardless of gene panel, then it removes technology-specific batch effects. LLOKI-FP applies optimal transport-guided feature propagation to impute missing gene expression using cellular spatial organization (i.e., cell graph), mitigating sparsity and gene sensitivity differences. A single-cell foundation model then embeds the data into a shared feature space, LLOKI-CAE further integrates these embeddings across batches using a conditional autoencoder. The final embeddings minimize batch effects and support downstream tasks like cross-technology slice alignment and spatial gene program detection.

Results

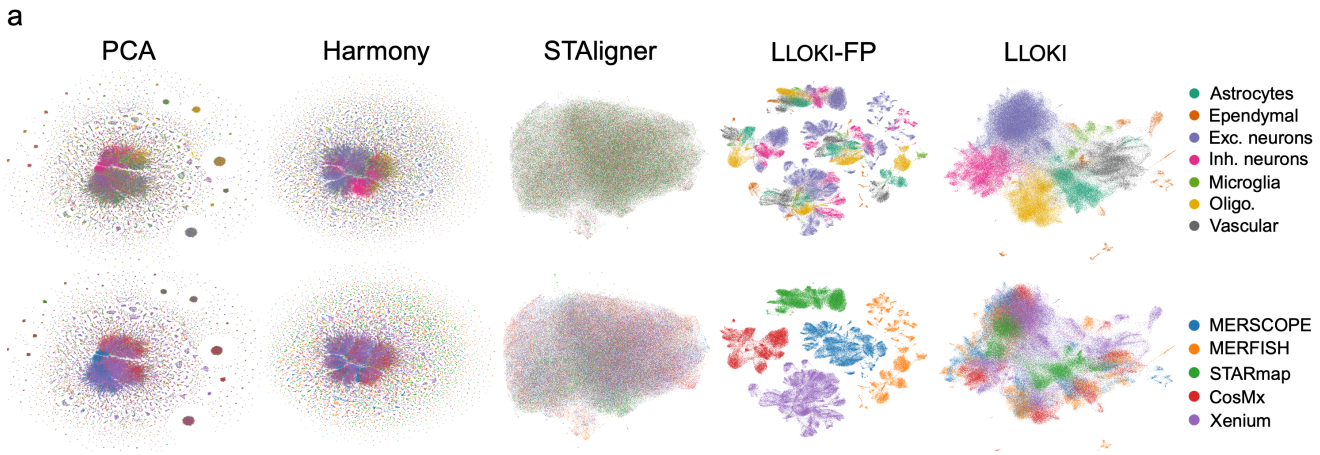
Overview of LLOKI

In LLOKI, we introduce a novel framework for ST integration by decomposing the task into two alignment problems: (1) feature alignment across varying gene panels, and (2) batch alignment across technologies.

For feature alignment with LLOKI-FP, we leverage scGPT [13] to embed data into a shared feature space, independent of gene panel differences. However, scGPT – and most existing scFMs – only process non-zero expression genes, making high sparsity problematic, as ST data falls outside the model's scRNA-seq pretraining distribution. LLOKI-FP adjusts ST data sparsity using spatial information to align with an scRNA-seq reference, ensuring compatibility with pretrained scFMs. To achieve this, we first calculate a target sparsity for each cell using optimal transport, aligning ST data sparsity with the scRNA-seq reference. We then employ a novel feature propagation method that integrates gene similarity and spatial proximity to impute missing features while preserving biological variation. The sparsity-aligned ST gene expression is then processed by scGPT, generating feature-aligned embeddings for each cell.

For batch alignment with LLOKI-CAE, we use a conditional autoencoder to integrate data across ST technologies, using LLOKI-FP embeddings as input features for each cell. Our integration strategy combines three loss functions: (1) reconstruction loss to preserve accurate data representations, (2) triplet loss to enhance clustering and mitigate batch effects, and (3) a new biological conservation loss that maintains the local neighborhood structure from the pre-integration embedding space, ensuring that cell-type relationships established by LLOKI-FP are preserved during batch correction. Together, these loss functions enable robust integration while preserving cell type clusters across diverse ST platforms.

LLOKI introduces several key innovations that distinguish it from existing ST integration methods. Unlike previous approaches that rely on shared gene panels, LLOKI-FP accommodates any gene panel, ensuring all available data contributes to biologically meaningful embeddings. LLOKI also uniquely incorporates spatial information to address differences in technical dropout across technologies without



b

Method	Bio conservation					Batch correction				Aggregate score	
	KMeans NMI	KMeans ARI	cLISI	Silhouette label	Isolated labels	Silhouette batch	iLISI	PCR comparison	Graph connectivity	Bio Conservation	Batch Correction
LLOKI	0.64	0.57	1.00	0.58	0.56	0.88	0.07	0.76	0.91	0.67	0.65
STAligner	0.00	0.00	0.59	0.49	0.49	0.93	0.40	0.86	0.26	0.31	0.61
Harmony	0.00	-0.00	0.58	0.49	0.49	0.92	0.06	0.88	0.07	0.31	0.48
LLOKI-FP	0.13	0.07	1.00	0.51	0.51	0.64	0.00	0.00	0.29	0.44	0.23
PCA	0.14	-0.05	0.93	0.37	0.40	0.67	0.18	0.00	0.53	0.36	0.34

Figure 2: Comparative analysis of batch integration using LLOKI versus baseline methods across five spatial transcriptomics technologies. **a.** UMAP visualizations of integrated data, colored by cell type (top) and by technology (bottom), showing the degree of batch separation and preservation of biological variation. **b.** Quantitative performance evaluation of five methods across nine metrics, measuring both biological variation preservation and integration effectiveness across technologies.

over-smoothing, preserving cell-type specificity rather than collapsing cells into region-specific clusters. Additionally, the three-part loss function in LLOKI-CAE maintains biologically meaningful heterogeneity while enabling robust batch integration. Finally, LLOKI is highly scalable and parallelizable – after initial training, each ST slice is processed individually, and LLOKI embeddings can be computed in under 5 minutes using less than 1 GB of GPU memory.

LLOKI enables cross-technology batch integration

We evaluated LLOKI's performance on cross-technology batch integration using one slice from each of five imaging-based ST datasets of coronal mouse brain sections: MERFISH [19] (1,122 genes), MERSCOPE [20] (550 genes), STARmap [21] (1,022 genes), CosMx [22, 23] (960 genes), and Xenium [24] (248 genes). Despite the smallest panel containing 248 genes, these platforms share only 19 genes, making integration particularly challenging for existing alignment methods. To benchmark LLOKI, we compared it against four baseline methods: PCA, Harmony [25] (for scRNA-seq integration), STAligner [7] (for ST integration), and the raw output of LLOKI-FP (prior to batch alignment via LLOKI-CAE).

We visualized the embedding space from each method using UMAP (**Fig.** 2a), colored either by batch or cell type. For the cell type visualizations, annotations were consolidated into broader labels for consistency across datasets (**Table** S2); for the Xenium dataset, which lacked annotations, cell types were inferred by marker gene analysis.

For quantitative evaluation, we employed nine metrics from scib-metrics [26]. Four metrics assessed

the preservation of biological variation based on cell type separation using annotations provided with each dataset, and five metrics measured batch effect removal, reflecting the degree of integration across technologies into a shared embedding space. For each category, we computed aggregate scores as the mean of their corresponding metrics.

LLOKI achieved the best balance between preserving biological signals and removing batch effects (**Fig.** 2b). It preserved biological variation between cell types, attaining a high biological conservation score (0.65), while also achieving substantial batch correction. In contrast, LLOKI-FP alone, though yielding a moderately high biological conservation score (0.44), performed poorly in batch correction (0.23), highlighting the importance of LLOKI-CAE in aligning LLOKI-FP embeddings across batches. STAligner achieved the second-highest batch correction score (0.61), but had weak biological conservation (0.31), as shown in UMAPs where the embeddings converge into a unified structure with diminished separation between cell type clusters. This suboptimal integration is likely due to the limited overlap of gene panels across technologies – only 19 shared genes for alignment – restricting the ability to capture the full spectrum of biological variation. PCA generated embeddings with a biological conservation score of 0.36 and a batch correction score of 0.34, with UMAPs showing weak cell type specificity. Harmony, designed for scRNA-seq batch correction, produced a lower biological conservation score (0.31) but a higher batch correction score (0.48), indicating that while it mitigates batch effects better than PCA, it does so at the expense of cell type resolution.

To dissect the contributions of LLOKI-CAE's three-part loss function and to evaluate the importance of the LLOKI-FP feature transformation, we conducted two ablation experiments (**Fig.** S1). In the first experiment, we removed each component of the loss function. Removing the biological conservation loss had the most significant impact, reducing the biological conservation score by 0.28 – underscoring its critical role in maintaining cell type separation in the embedding space. In contrast, removing the triplet loss improved the overall score but compromised batch correction, highlighting the delicate balance required between these objectives. In the second experiment, we compared LLOKI-CAE's performance when using scGPT embeddings versus the transformed LLOKI-FP embeddings as input. While using scGPT embeddings alone improved batch correction by 0.19, it achieved only 0.41 for biological conservation. In contrast, LLOKI-FP's feature alignment strategy significantly enhanced biological conservation (0.58, a 41% improvement) while maintaining strong batch integration, demonstrating its superior ability to balance the critical tradeoff between removing technical variation and preserving meaningful biological signals.

Together, these results suggest that while LLOKI-FP is highly effective at maintaining biological variation and cell type separation, LLOKI-CAE is crucial for integrating slices across technologies and successfully removing batch effects.

LLOKI achieves robust cross-technology spatial alignment

We evaluated LLOKI's ability to align multiple ST slices within a common spatial coordinate framework while ensuring the key tissue features are properly aligned. To assess its robustness, we tested two key aspects: (1) its ability to align slices across different technologies, and (2) its performance when slices have minimal overlap in their gene panels. For slice alignment, LLOKI embeddings were used to generate "landmark pairs" between ST slices by identifying mutual nearest neighbors within the LLOKI embedding space. These landmark pairs were then aligned using the Kabsch algorithm [27], which finds the optimal rotation and translation to minimize root-mean-square deviation (RMSD) between paired

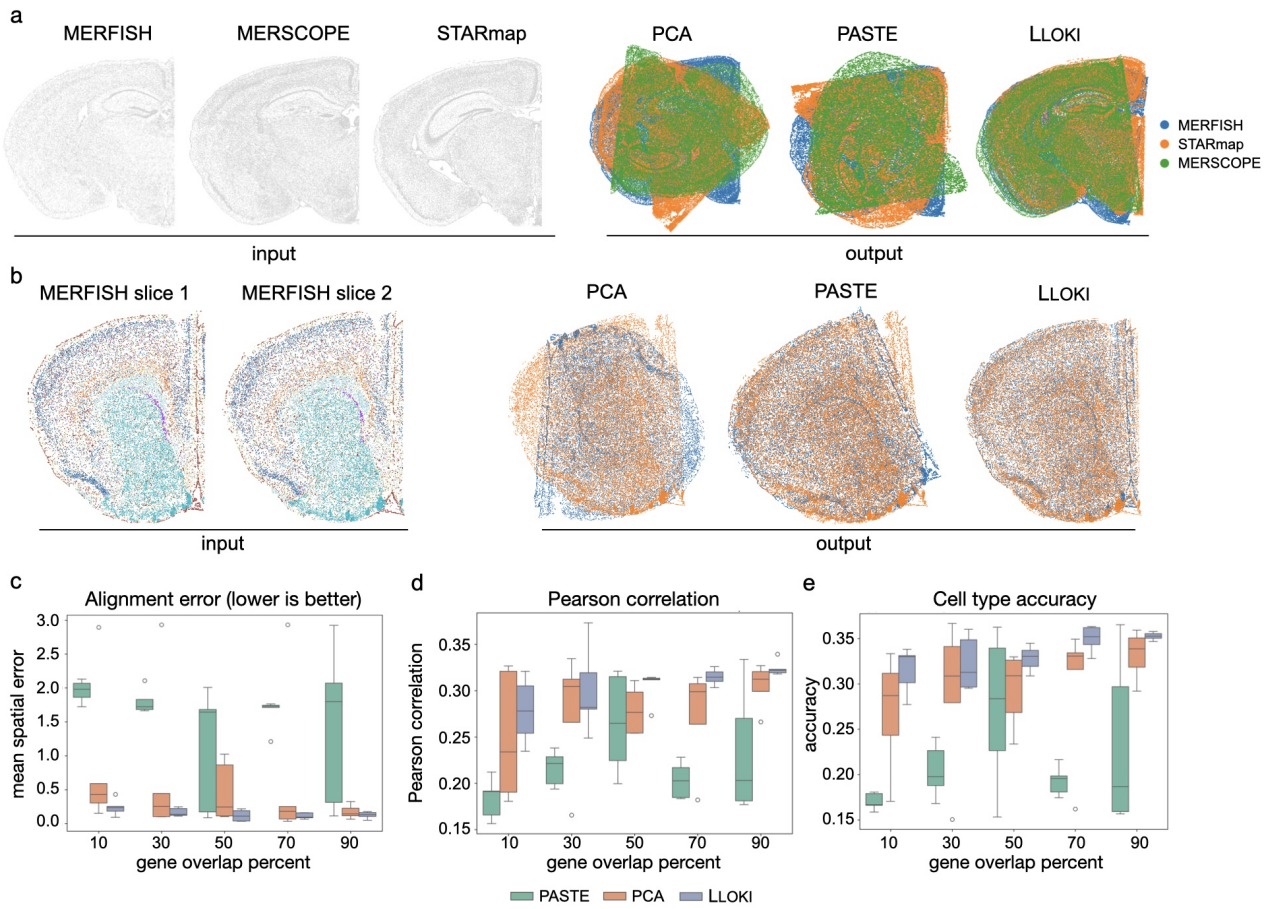


Figure 3: Evaluation of LLOKI's performance in spatial slice alignment. **a.** *In situ* visualizations of three slices from the MERFISH, MERSCOPE, and STARmap datasets, along with alignment results using LLOKI and two baseline methods. **b.** Alignment of two adjacent MERFISH slices with artificially downsampled gene panels to simulate decreasing gene overlap, shown here at 50% overlap. **c-e.** Comparative of LLOKI and two baseline methods as gene overlap decreases, showing results for **(c)** spatial alignment error, **(d)** Pearson correlation, and **(e)** cell type accuracy.

landmarks.

First, we evaluated LLOKI's ability to align slices from different technologies by selecting one slice each from the MERSCOPE, MERFISH, and STARmap datasets (**Fig.** 3a) and performing pairwise alignments using overlapping genes. As baselines, we compared against: (1) the Kabsch algorithm using mutual nearest neighbors from PCA embeddings of shared genes, (2) PASTE [28], a state-of-the-art spatial alignment method that uses fused Gromov–Wasserstein optimal transport to compute alignment based on both transcriptional and spatial similarity, and (3) STAligner [7], which provides a slice alignment module with their integration. Both PCA and PASTE failed to correctly align the slices, whereas LLOKI successfully aligned them such that corresponding regions overlapped. Notably, LLOKI's slice alignment strategy allows only rotation and translation, without scaling or rearranging cells within each slice.

To test LLOKI's performance under low gene panel overlap, we used two adjacent slices from the MERFISH dataset (**Fig.** 3b), which originally shared approximately 1,100 genes. We created ten simulated datasets by progressively downsampling the shared genes in 10% increments, repeating this process five times per overlap percentage to create replicates. We again compared to the same baselines.

We evaluated alignment accuracy using three metrics: (1) Pearson correlation of gene expression for nearest neighbor pairs across aligned slices, (2) cell type accuracy, measuring how often paired cells

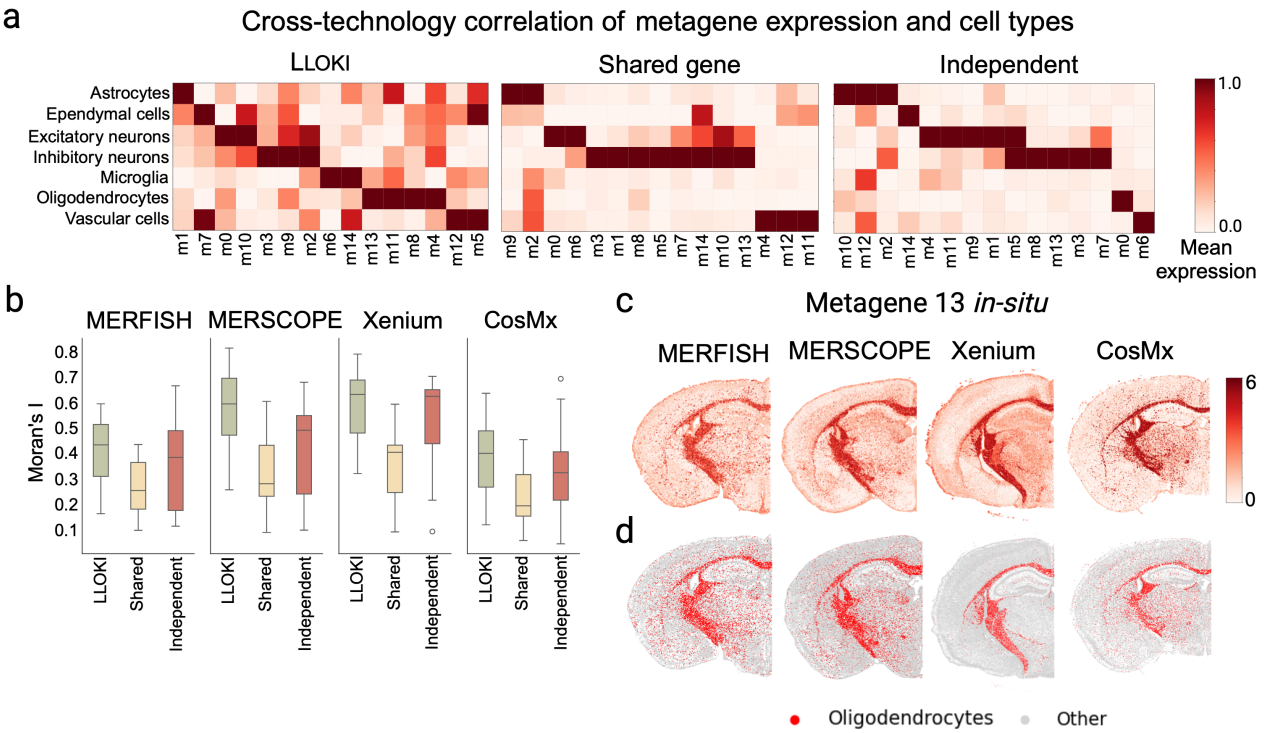


Figure 4: SPICEMIX with LLOKI embeddings enhances cross-technology metagene discovery. **a.** Cell type enrichment of metagenes across MERFISH, MERSCOPE, CosMx, and Xenium datasets, comparing three approaches: LLOKI-based integration, the shared-gene approach (22 common genes), and independent analyses. **b.** Quantitative assessment of metagene quality using Moran's I spatial autocorrelation scores, shown as box plots. **c.** Spatial distribution of metagene 13 across all four technologies, showing consistent patterns using LLOKI embeddings with SPICEMIX. **d.** Spatial distribution of oligodendrocytes across MERFISH, MERSCOPE, and CosMx, demonstrating the correspondence between metagene 13 and this cell type.

shared the same annotated cell type, and (3) spatial distance between aligned pairs in the original dataset, which had been pre-aligned to a common framework. Before alignment, we applied a random rotation and translation to one slice to ensure that alignment methods were actively aligning the data, rather than relying on pre-existing spatial similarity.

LLOKI consistently outperformed both baselines across all three metrics, with significant improvements in nearly every case (**Figs.** 3c, 3d, and 3e). Notably, while baseline performance declined sharply as the percentage of overlapping genes decreased, LLOKI maintained steady performance even with very few overlapping genes. Moreover, LLOKI's alignments never resulted in complete misalignment, such as the 180-degree rotations observed with the baseline methods (**Fig.** 3b).

Overall, these results demonstrate that LLOKI embeddings are highly robust to differences in gene panels, enabling accurate alignment even with minimal overlap between slices. Additionally, LLOKI effectively mitigates batch effects across technologies, facilitating accurate cross-technology slice alignment and enabling more comprehensive integrating of datasets from diverse ST platforms.

LLOKI enhances cross-technology spatial gene program detection

To assess LLOKI's utility for downstream analysis, we integrated data from four ST platforms – MERFISH, MERSCOPE, CosMx, and Xenium – and evaluated whether LLOKI embeddings improve cross-technology analyses using SPICEMIX [29]. Joint analysis across these platforms enables a more comprehensive understanding of tissue biology by revealing conserved spatial patterns that may be missed

when platforms are analyzed in isolation. SPICEMIX models gene expression as combinations of latent factors ("metagenes") that capture both transcriptional programs and spatial organization but requires a common feature space. This dependency limits its effectiveness for cross-dataset analysis when integrating datasets with diverse gene panels. We address this limitation by using LLOKI embeddings as an alternative input to SPICEMIX, providing a unified feature space across platforms.

We evaluated metagene consistency and biological relevance across the four ST technologies using three approaches: (1) LLOKI-based integration, using 128-dimensional LLOKI embeddings as input to SPICEMIX, (2) a shared-gene approach, applying SPICEMIX only to the 22 genes common across all technologies, and (3) independent SPICEMIX runs on each dataset, with optimal mapping of metagenes post hoc.

Mapping cell type annotations onto the learned SPICEMIX metagenes revealed consistent cell type and metagene associations across platforms (**Fig.** 4a). For MERFISH, MERSCOPE, and CosMx, we used the provided cell type annotations, while for Xenium –lacking such annotations – cell types were inferred via marker gene analysis (**Supplemental Information**). Notably, metagene 1 was enriched in astrocytes, metagene 6 in microglia, metagenes 5, 7, and 14 in vascular cells, and metagenes 11 and 13 in oligodendrocytes, with clear neuronal subtype distinctions. In contrast, both the shared-gene and independent approaches captured only partial associations, failing to consistently identify microglial- and oligodendrocyte-specific metagenes.

We further evaluated metagene quality using spatial autocorrelation, quantified by Moran's I (**Fig.** 4b). In brain tissue, where functionally similar cells tend to cluster spatially, metagenes that recapitulate known cell-type patterns indicate robust spatial organization. Our results show that LLOKI consistently produces metagenes with strong spatial structure across all technologies, whereas the shared-gene approach exhibits the lowest autocorrelation and independent analyses show high variability.

To assess biological relevance, we examined metagenes with consistent spatial and cell-type associations across platforms. For example, metagene 13 showed highly consistent spatial expression patterns across all four technologies (**Fig.** 4c). Notably, regions of high metagene 13 expression were strongly enriched for oligodendrocytes (**Fig.** 4d), indicating that this metagene captures a conserved, spatial localized, cell type-specific gene program. This cross-platform consistency underscores that supplementing SPICEMIX with LLOKI's integrated embeddings facilitates the identification of conserved biological signals.

Together, these results show that LLOKI's integrated embeddings enable SPICEMIX to delineate biologically meaningful spatial gene programs, overcoming the limitations imposed by platform-specific gene panels and enhancing cross-technology analyses. For further validation, *in situ* metagene plots for LLOKI+SPICEMIX, shared-gene+SPICEMIX, and independent SPICEMIX runs are provided in **Figs.** S2, S3, and S4, respectively.

LLOKI identifies shared gene program for tumor infiltration in ovarian cancer

We next applied LLOKI to integrate five ST datasets of human ovarian cancer [30], which differ in both gene panel composition and technology (**Fig.** 5a). These datasets include three from CosMx, one from Xenium, and one from MERFISH. The CosMx datasets include: (1) 960 genes measured across 100 small tissue samples (0.9 mm $\times$ 0.6 mm), (2) 1,000 genes measured across 4 large whole-tissue samples (up to 2 cm), and (3) 6,175 genes measured across 62 small tissue samples (0.5 mm $\times$ 0.5 mm). The Xenium dataset contains 240 genes from 32 small tissue samples (1.5 mm $\times$ 1.5 mm), while the

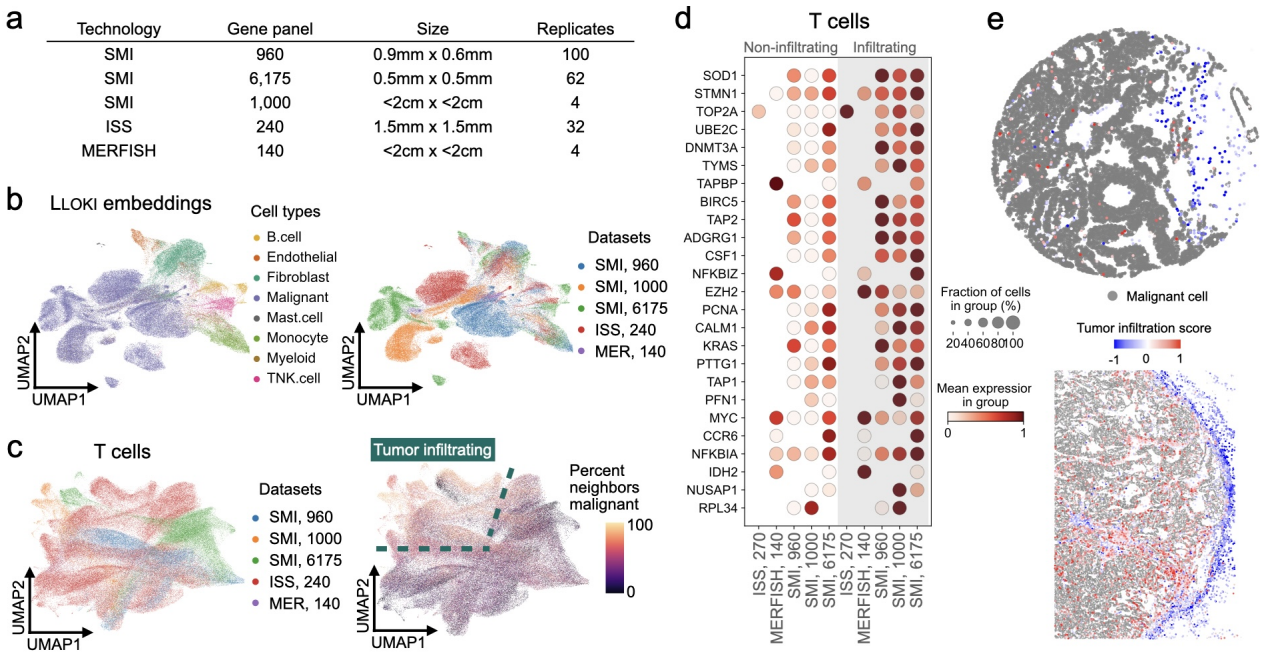


Figure 5: LLOKI identifies cross-technology gene program indicative of tumor infiltration in T cells in ovarian cancer. **a.** Summary of the five ovarian cancer ST datasets used in this analysis. **b.** UMAP visualization of LLOKI embeddings shows successful integration of non-malignant cells across datasets, while transcriptionally distinct tumor cells remain separate. **c.** UMAP visualization of LLOKI embeddings of T cells, colored by dataset (left) or by the proportion of 100-nearest spatial neighbors that are malignant. Dashed outlines indicate regions identified as tumor-infiltrating. **d.** Expression levels of upregulated genes from the cross-technology tumor infiltration gene program in infiltrating vs. non-infiltrating T cells. **e.** Representative *in situ* visualization showing T cells colored by the tumor infiltration score and malignant cells in gray.

MERFISH dataset includes 140 genes from 4 large whole-tissue samples (up to 2 cm). Integration via LLOKI achived robust mixing of shared cell types across these technologies (**Fig.** 5b), yielding a biological conservation score of 0.47 and a batch correction score of 0.23 when evaluated on non-malignant cells. Malignant cells remained largely distinct between datasets, reflecting tumor-specific transcriptional differences from diverse origins.

Using LLOKI embeddings, we further investigated T cell heterogeneity with a focus on tumor infiltration. We combined T cells from all datasets and clustered them based solely on their LLOKI embeddings. This unsupervised approach partitioned T cells into two groups that corresponded well with the percentage of malignant cells among their 100 nearest spatial neighbors – a proxy for local tumor infiltration (**Fig.** 5c).

To characterize the genes responsible for this tumor infiltration program, we performed integrated differential expression analysis in two stages: (1) comparing T cells against all other cell types to obtain T cell-specific genes for each dataset's gene panel, and (2) identifying differentially expressed genes between the two T cell groups detected via LLOKI embeddings. Genes consistently up- or downregulated across datasets were retained, yielding a shared gene panel that reliably distinguishes tumor-infiltrating T cells (**Fig.** 5d). In particular, this gene panel includes markers associated with cell proliferation (e.g., STMN1, TOP2A, UBE2C, BIRC5, NUSAP1) and antigen processing (e.g., TAP1, TAP2, TAPBP), suggesting that these processes are correlated with tumor infiltration.

To assess the biological relevance of this gene panel, we devised a tumor infiltration score, weighting upregulated genes positively and downregulated genes negatively. When spatially projected onto indi-

vidual T cells, this score revealed a clear pattern: T cells within tumor regions exhibited consistently higher scores than those outside the tumor (**Fig.** 5e). We further quantified the correlation between this tumor infiltration score and the percentage of malignant cells among the 100 nearest spatial neighbors. Across 170,000 T cells from all datasets, we observed a significant correlation (Pearson $R = 0.326$, $P < 1 \times 10^{-200}$; Spearman $\rho = 0.366$, $P < 1 \times 10^{-200}$), confirming the biological relevance of our scoring approach.

These findings further demonstrate that LLOKI not only enables effective integration of heterogeneous spatial transcriptomics data but also facilitates the discovery of biologically meaningful gene programs, such as the one defining tumor-infiltrating T cells in ovarian cancer.

Discussion

In this work, we developed LLOKI, a novel framework for integrating ST data across diverse platforms without the need for shared gene panels. LLOKI combines feature alignment and batch integration through two complementary components: LLOKI-FP, which aligns gene panels into a shared feature space by addressing differences in data sparsity, and LLOKI-CAE, which mitigates batch effects across ST technologies while preserving biological specificity. By leveraging both components, LLOKI enables robust cross-technology integration while retaining meaningful biological variation.

Our results demonstrate that LLOKI outperforms baseline methods across key integration metrics, maintaining biological variation and effectively correcting batch effects – even when gene panel overlap is minimal. LLOKI's integrated embeddings support challenging downstream tasks such as spatial gene program identification and slice alignment, underscoring its versatility in addressing the inherent complexities of ST data integration and paving the way for more comprehensive analysis of tissue architecture across diverse technologies.

Several avenues exist for extending LLOKI's capabilities. Refinements to LLOKI-FP – such as improved mitigation of technology-specific biases via optimal transport – could enhance the alignment between ST and scRNA-seq data. Similarly, the conditional autoencoder design of LLOKI-CAE offers promising generalization capabilities: by updating only the conditional weights while retaining shared parameters, LLOKI can be readily adapted to new ST platforms without full retraining. As the field of scFMs evolves, our approach – currently leveraging scPGT to create a unified feature space – can be extended to incorporate emerging models, further enhancing integration performance.

Beyond methodological advancements, LLOKI has several practical applications. One notable use case is leveraging samples from one ST technology as controls for disease samples collected using another, reducing data requirements and conserving resources. Moreover, LLOKI could be applied to the integration of atlas-scale datasets, enabling large-scale, cross-technology analyses to identify rare cellular niches without necessitating prohibitively large sample sizes. Overall, LLOKI provides a powerful tool for harmonizing ST datasets across different platforms and gene panels, facilitating a deeper understanding of spatial cellular organization in diverse biological contexts.

By addressing key challenges in ST data integration, LLOKI lays the groundwork for scalable, cross-technology spatial transcriptomics analysis, empowering researchers to uncover biologically meaningful patterns across diverse tissue samples and experimental conditions.

Methods

LLOKI is a framework for integrating spatial transcriptomics (ST) data across diverse platforms by addressing two key alignment challenges (**Fig.** 1): (1) feature alignment across varying gene panels, achieved through LLOKI-FP, which imputes gene expression and embeds cells using a single-cell foundation model (scFM); and (2) batch alignment across different ST technologies, handled by LLOKI-CAE via a conditional autoencoder with a novel three-part loss function. This approach ensures unified analysis of heterogeneous ST datasets while preserving essential biological signals.

Feature alignment and denoising with LLOKI-FP

scFMs offer a promising solution for aligning features across ST datasets without requiring shared genes [11–13]. However, ST data often appears out-of-distribution for scFMs pretrained on scRNA-seq due to differences in sparsity and gene detection sensitivity. Additionally, scFMs process only genes with non-zero expression, making higher sparsity a major challenge, as it reduces the effective feature dimensionality and introduces variability in embedding quality across ST platforms.

To address this, LLOKI-FP employs a sparsity-matching and denoising approach, combining Wasserstein optimal transport [31] with graph-based feature propagation [32]. Rather than performing conventional gene imputation, LLOKI-FP transforms the sparsity profile of ST data to better match the scRNA-seq distribution, optimizing compatibility with scFMs and improving embedding quality. Unlike prior feature propagation methods for single-cell data [33], LLOKI-FP integrates spatial information and applies a sparsity prior via optimal transport, shifting the focus from mere imputation to distribution alignment.

At a high level, LLOKI-FP first determines a target sparsity for each ST cell using optimal transport. It then constructs a cell-cell graph incorporating both gene similarity and spatial proximity, which is used to iteratively impute missing gene expression until each cell's target sparsity is reached.

Graph Construction

LLOKI-FP begins by constructing an undirected graph $\mathbf{G} = (\mathbf{V}, \mathbf{E})$, where nodes $v_i \in \mathbf{V}$ represent cells, and edges $e_{ij} \in \mathbf{E}$ encode similarity between cells. Since feature propagation assumes high graph homophily – where connected cells share similar gene expression – it is critical that the graph reflects true biological relationships.

To achieve this, LLOKI-FP integrates gene expression and spatial proximity into graph construction. Each cell's *k*-nearest neighbors (*k*-NNs) are identified in gene expression space to establish graph connectivity, while edge weights ($w(e_{ij})$ for edge e_{ij}) are further refined using a Radial Basis Function kernel based on spatial distance:

$$w(e_{ij}) \;=\; \exp\left(-\frac{d_{ij}^2}{2\sigma^2}\right) \tag{1}$$

where d_{ij} is the spatial distance between cells i and j and σ is a bandwidth parameter computed as the mean distance from a random sample of 100 cell pairs. This weighting reinforces graph homophily by leveraging the biological insight that physically adjacent cells tend to share microenvironments and gene expression patterns. The graph is then symmetrically normalized to ensure balanced feature propagation across cells. This graph-guided feature propagation aligns ST sparsity distributions with scRNA-seq while ensuring imputation is informed by both transcriptional and spatial information.

Feature propagation for data imputation with optimal transport

To impute sparse gene expression values, LLOKI-FP employs feature propagation [32], enhanced with optimal transport [31] to align ST sparsity with scRNA-seq references. Given that ST data is typically more sparse than scRNA-seq data, our approach selectively imputes missing values while preserving a degree of inherent sparsity – since completely eliminating sparsity would reduce embedding diversity and obscure biologically meaningful variation.

This process starts with the constructed similarity graph $\mathbf{G}$ with spatially informed, normalized adjacency matrix $\mathbf{A}$ and the original gene expression matrix $\mathbf{X}$. Gene expression is iteratively updated as follows:

1. *Feature propagation with controlled sparsity*. Imputed gene expression values are updated iteratively as:

$$\mathbf{X}^{(t+1)} = (1 - \mathbf{M}) \circ \mathbf{A}\mathbf{X}^{(t)} + \mathbf{M} \circ \mathbf{X}^{(0)}, \tag{2}$$

   where $\mathbf{X}^{(0)}$ is the original gene expression matrix, $\mathbf{M}$ is a binary mask indicating observed (nonzero) entries in $\mathbf{X}^{(0)}$, and $\circ$ denotes the element-wise Hadamard product. This update minimizes the graph Dirichlet energy as shown in [32], ensuring that imputation is both spatially coherent and biologically relevant.

2. *Sparsity matching with optimal transport*. To harmonize sparsity profiles between ST and scRNA-seq data, we compute per-cell sparsity as $s_i = 1 - \frac{\text{nonzero}(x_i)}{N}$, where $\text{nonzero}(x_i)$ is the number of nonzero gene expression values in cell i and N is the total number of genes. We then compute the empirical cumulative distribution function (CDF) for the sparsity values. For a sorted set $\{s_{(1)}, s_{(2)}, \ldots, s_{(n)}\}$, the CDF is defined as:

$$F(s_{(i)}) = \frac{i}{n}. \tag{3}$$

   Denoting the CDFs for the ST data and the scRNA-seq reference as $F_{\text{ST}}(s)$ and $F_{\text{scRNA}}(s)$ respectively, we align the distributions by mapping each ST sparsity value s to a target value s^* via:

$$s^* = F_{\text{scRNA}}^{-1}\Big(F_{\text{ST}}(s)\Big). \tag{4}$$

   This quantile alignment minimizes the Wasserstein distance between the distributions, yielding target sparsity levels that mirror the scRNA-seq profile. Subsequently, the same feature propagation update is applied iteratively until each cell's imputed expression conforms to its target sparsity. To prevent over-imputation – where the imputed data becomes denser than intended – we monitor the number of nonzero entries per cell and reintroduce zeros as needed to recapitulate the natural dropout observed in ST technologies.

This iterative process – alternating between controlled feature propagation and sparsity adjustment via 1D optimal transport mapping – ensures that LLOKI-FP embeddings preserve critical biological signals while aligning with scRNA-seq sparsity, thereby enhancing compatibility with scFMs.

Denoising with feature diffusion

Following optimal transport-enhanced feature propagation, we refine gene expression data using feature diffusion. We construct a new cell similarity graph based on the imputed gene expression matrix $\mathbf{X}_{\text{imputed}}$,

with $\mathbf{A}_{\text{imputed}}$ as the corresponding adjacency matrix. During this phase, the original gene expression matrix is diffused across the new graph without modifying non-zero values, using:

$$\mathbf{X}_{\text{denoised}} = \alpha\, \mathbf{A}_{\text{imputed}}\, \mathbf{X}^{(0)} + (1-\alpha)\, \mathbf{X}^{(0)} \tag{5}$$

This diffusion step propagates gene expression features across the spatially weighted k-NN graph, updating missing or noisy values while preserving the original gene expression measurements. The hyperparameter $\alpha \in [0, 1]$ controls the extent to which information from neighboring cells (via $\mathbf{A}_{\text{imputed}}\, \mathbf{X}^{(0)}$) influences the denoised output. A value of α close to 1 emphasizes the diffused signal from neighboring cells, while a value closer to 0 favors retaining the original gene expression values. This formulation leverages the assumption that cells that are both spatially and transcriptionally similar share comparable true gene expression patterns.

Feature alignment using a single-cell foundation model

Once ST sparsity is aligned to match the scRNA-seq reference and the data is denoised, we proceed with feature alignment via a scFM pretrained on scRNA-seq data. This step ensures that all datasets, regardless of their original gene panels, share a common feature space, allowing the full gene panel to inform cell embeddings.

scGPT [13] demonstrated strong performance across cell type clustering metrics (**S**upplementary Results). It is not only computationally efficient but also particularly adept at handling smaller gene panels due to its autoregressive pretraining strategy. The final output of LLOKI-FP is a 512-dimensional embedding space, consistent across all input datasets, enabling robust feature alignment across diverse ST technologies.

We compare LLOKI-FP to scGPT alone (without feature propagation) across five ST technologies (**Supplemental Information**). LLOKI-FP consistently outperforms scGPT, with the most significant improvement observed on the STARmap dataset (ARI=0.423 vs. ARI=0.197). We also conducted an ablation study evaluating the impact of the optimal transport sparsity alignment step in LLOKI-FP (**Supplemental Information**). This analysis revealed that incorporating optimal transport alignment significantly enhanced performance for the MERSCOPE and STARmap Plus datasets, confirming the importance of this step in our workflow.

Batch integration with LLOKI-CAE

The cell embeddings from LLOKI-FP effectively capture biological variation within each ST dataset, but they still exhibit large batch effects across different technologies. To address this, we develop a conditional autoencoder (CAE) for batch integration, conditioned on both ST technology and specific gene panel. This approach allows the model to differentiate datasets collected from different technologies or with distinct gene panels. Integration is achieved through a novel three-part loss function that aligns embeddings across technologies while preserving the structure of cell-type clusters within each dataset.

Conditional autoencoder architecture

Our CAE architecture follows a standard design with some key new developments. Both the encoder and decoder comprise three layers, reducing the dimensionality of the 512-dimensional input embeddings from LLOKI-FP down to 128. Many downstream tasks require positive embeddings, so we apply a ReLU activation function in the final layer of the encoder to ensure all embeddings are positive.

The conditional component of the autoencoder is implemented as a learnable embedding that maps a

batch token to a 10-dimensional embedding, appended to the input of both the encoder and decoder.

Three-part loss function

Conventional methods for integrating ST datasets – such as STAligner [7] – typically balance cell-type heterogeneity and batch integrating using a combination of reconstruction loss and triplet loss. While triplet loss effectively promotes batch integration, we found that reconstruction loss alone does not sufficiently preserve critical biological information. We therefore introduce a third loss function, the biological conservation loss, designed to maintain the local structure of the original LLOKI-FP embeddings, which inherently capture robust biological signal across cell types. By jointly optimizing reconstruction loss, triplet loss, and our new biological conservation loss, our approach aligns embeddings across batches while preserving essential biological information.

1. **Reconstruction loss.** The reconstruction loss seeks to recreate the original LLOKI-FP embeddings for each cell X. Given the decoder output Y, we minimize the mean squared error (MSE) between X and Y, ensuring that the CAE preserves original information through the encoding-decoding process:

$$\boldsymbol{\mathcal{L}}_{rec} = \frac{1}{n}\sum_{i=1}^{n}(X_i - Y_i)^2 \tag{6}$$

2. **Triplet loss**. Triplet loss encourages embeddings of biologically similar cells from different technologies to be closer, while pushing apart embeddings of dissimilar cells within the same technology. Anchor-positive pairs consist of a cell from one technology (anchor) and its mutual nearest neighbor from another. Negative pairs are randomly selected cells from the same slice as the anchor, which are likely to be dissimilar. When reliable cell type annotations are available, we optionally refine this selection by: (1) restricting positive pairs to cells sharing the same cell type across batches, and (2) selecting negative pairs using hard negative sampling, where the negative pair is chosen as the closest cell from the same batch that belongs to a different cell type than the anchor. The triplet loss is defined as:

$$\boldsymbol{\mathcal{L}}_{trip} = \frac{1}{n}\sum_{i=1}^{n}\max\left(0, \|Z_i^{anchor} - Z_i^{positive}\|^2 - \|Z_i^{anchor} - Z_i^{negative}\|^2 + \alpha\right) \tag{7}$$

   where Z_i represents the CAE embeddings, and α is the margin separating positive and negative pairs.

3. **Biological conservation loss**. Our new biological conservation loss maintains the local structure of the LLOKI-FP embeddings. For each cell, we identify its *k*-NNs in the original LLOKI-FP embedding space and aim to preserve these relative distances in the CAE embedding space. The biological conservation loss is defined as:

$$\boldsymbol{\mathcal{L}}_{bc} = \frac{1}{n}\sum_{i=1}^{n}\sum_{j\in\boldsymbol{\mathcal{N}}_i}\left(\|Z_i - Z_j\| - \|X_i - X_j\|\right)^2 \tag{8}$$

   where $\boldsymbol{\mathcal{N}}_i$ is the set of nearest neighbors of cell i in the original embedding space.

To balance these three components, we introduce weighting parameters λ_{rec}, λ_{trip}, and λ_{bc}, which are tuned to determine the optimal balance among the three competing objectives. The total loss is defined as:

$$\boldsymbol{\mathcal{L}}_{total} = \lambda_{rec}\boldsymbol{\mathcal{L}}_{rec} + \lambda_{trip}\boldsymbol{\mathcal{L}}_{trip} + \lambda_{bc}\boldsymbol{\mathcal{L}}_{bc} \tag{9}$$

These weights are optimized to ensure that each aspect of the loss appropriately influences training, preserving cell-type clustering while effectively reducing batch effects across technologies.

Further details about hyperparameter choices for LLOKI-CAE are in **Supplemental Information**.

Evaluation metrics for batch integration

To quantitatively evaluate LLOKI and compare it to baselines for batch integration we used ten metrics from scib-metrics [26]. Five of these metrics assessed the biological variation preserved by the embeddings, based on cell type separation using cell type annotations provided with each dataset: isolated labels silhouette score, *k*-means clustering NMI and ARI (KMeans NMI and KMeans ARI), silhouette width (Silhouette label), and cell-type local inverse Simpson index (cLISI). The remaining five metrics evaluated batch effect removal, measuring how well technologies were integrated into a shared embedding space: silhouette batch score, integration local inverse Simpson index (iLISI), *k*-nearest-neighbor batch-effect test (KBET), batch connectivity in a cell-type specific subgraph (Graph connectivity), and principal component regression comparison (PCR comparison). Scores were normalized across tools, and aggregate scores were computed by averaging biological conservation and batch correction metrics.

Scalability of LLOKI

LLOKI demonstrates robust scalability for processing large ST datasets. The computational framework consists of two main components with complementary performance characteristics. LLOKI-FP processes each ST slice independently, resulting in computational requirements that scale linearly with the number of slices. For a typical slice containing around 50,000 cells, running LLOKI-FP takes approximately 5 minutes and uses 20 GB of memory. Importantly, this component can be fully parallelized across compute nodes to process multiple slices simultaneously, offering substantial acceleration for large-scale studies. LLOKI-CAE employs an efficient batching approach during training and maintains a lightweight memory footprint of less than 2 GB regardless of dataset size. For the five-slice integration task described in our results, model training was completed in under one hour on a single GPU. Post-training embedding generation is computationally efficient, requiring only a single forward pass operation through the trained encoder. This process takes less than one second to generate embeddings for cells from all five slices (250,000 cells), enabling rapid integration of new datasets without retraining. Notably, only one slice of each technology is needed to train the model; processing all slices from large datasets after training takes only 5 minutes each and is fully parallelizable.

Slice alignment

For slice alignment with LLOKI, we implemented the Kabsch algorithm [27] for image alignment and used our LLOKI embeddings to determine landmark pairs between two slices. The algorithm then seeks to minimize the root-mean-squared distance (RMSD) for all landmark pairs. For baselines, we ran PASTE and a version of the Kabsch algorithm that uses PCA embeddings of shared gene expression instead of LLOKI embeddings. We ran PASTE with default parameters and five different values for alpha (0.01, 0.05, 0.001, 0.005, 0.0001), using the best-performing parameter set for our comparison.

We note that we run PASTE instead of PASTE2 [34], because the advantage of PASTE2 is in partial alignment, and all of our analysis was on full-slice alignments.

SPICEMIX related analysis

For the SPICEMIX analysis on the four mouse brain coronal slices from MERFISH, MERSCOPE, Xenium, and CosMx, we ran SPICEMIX with the following parameter settings: We set the number of metagenes $K = 15$ and the spatial affinity regularization to 10^{-4}. We performed pretraining without optimizing spatial affinity parameters for 10 iterations, followed by 200 iterations with full optimization.

To assess the correspondence of metagenes identified by SPICEMIX with cell type annotations, we computed an enrichment matrix where each row represents a cell type and each column a metagene, using only high-confidence cell type annotations. For each dataset, we excluded "Other/Unannotated" cells, retaining seven cell labels. For each cell type, we aggregated the corresponding cell embedding values by calculating the mean expression over non-zero entries. Metagenes were then reordered based on their maximum enrichment across cell types to highlight cell type-specific patterns and enable cross-technology comparisons.

We used Moran's I to evaluate spatial autocorrelation of metagene expression for each dataset. Spatial neighborhood graphs were constructed using Squidpy's `spatial_neighbors` [35]. For each metagene, Moran's I was computed to quantify how strongly its expression was spatially clustered.

Ovarian cancer analysis

We integrated the ovarian cancer datasets using LLOKI with the following hyperparameters: $\lambda_{bc} = 500.0$, $\lambda_{trip} = 0.2$, $\lambda_{recon} = 1.0$, a learning rate of 0.0005, a chunk size of 16,000, and a batch dimension of 10. Hyperparameter tuning was performed to optimize for both a high biological conservation score and effective batch correction.

To identify tumor infiltrating T cells, we first performed Leiden clustering on T cells from all datasets combined, using LLOKI embeddings. We then selected the clusters corresponding to regions in the UMAP with a high percentage of malignant neighbors, thereby defining a cross-technology group of tumor infiltrating T cells.

To derive a gene list associated with tumor infiltration, we used a two-stage process. In the first stage, for each dataset, we performed differential expression analysis comparing T cell populations to identify genes that were enriched in T cells. We applied filters to retain only genes expressed in at least 5% of T cells and exhibiting a minimum fold change of 0.3. This yielded 816 T cell-enriched genes across all datasets. In the second stage, we split T cells by infiltration status and repeated differential expression analysis on the subset of genes identified in the first stage. These results were aggregated using a weighted rank-based aggregation method: for each dataset, each gene received a normalized ranking and the final ranking was averaged across datasets.

Finally, using the shared gene list, we scored T cells for their expression of the tumor infiltration program. We applied the `score_genes` function from `scanpy`, using positive weights for upregulated genes and negative weights for downregulated genes. This provided a robust metric for quantifying T cell infiltration across the integrated ovarian cancer datasets.

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Code Availability

The source code for LLOKI can be accessed at: https://github.com/elliehaber07/LLOKI.

Author Contributions

Conceptualization, E.H., S.K., J.M.; Methodology, E.H., S.K., J.M.; Software, E.H., A.D., S.K.; Investigation, E.H., S.K., J.M.; Writing, E.H., S.K., J.M.; Funding Acquisition, J.M.

Competing Interests

The authors declare no competing interests.

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