SpatialSPM: statistical parametric mapping for the comparison of gene expression pattern images in multiple spatial transcriptomic datasets

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Abstract

Spatial transcriptomic (ST) techniques help us understand the gene expression levels in specific parts of tissues and organs, providing insights into their biological functions. Even though ST dataset provides information on the gene expression and its location for each sample, it is challenging to compare spatial gene expression patterns across tissue samples with different shapes and coordinates. Here, we propose a method, SpatialSPM, that reconstructs ST data into multi-dimensional image matrices to ensure comparability across different samples through spatial registration process. We demonstrated the applicability of this method by kidney and mouse olfactory bulb datasets as well as mouse brain ST datasets to investigate and directly compare gene expression in a specific anatomical region of interest, pixel by pixel, across various biological statuses. Beyond traditional analyses, SpatialSPM is capable of generating statistical parametric maps, including T-scores and Pearson correlation coefficients. This feature enables the identification of specific regions exhibiting differentially expressed genes across tissue samples, enhancing the depth and specificity of ST studies. Our approach provides an efficient way to analyze ST datasets and may offer detailed insights into various biological conditions.

Graphical abstract

Introduction

Spatial transcriptomics (ST) has emerged as a powerful technique for obtaining location-specific transcriptomic information in tissues and organs (1–3). This technique provides valuable insights into biological physiology, particularly regarding spatial heterogeneity. Therefore, investigating transcriptomic alterations in ST under pathological conditions, such as injury, inflammatory diseases, or cancer, can enable researchers to uncover the underlying pathogenesis specific to each detailed segment of the organ (4–6). However, the ST dataset itself only provides information about the gene expression level and relative location of each transcript, without any actual anatomical information (1). Furthermore, due to the different shapes and morphologies of each tissue sample for ST dataset, direct comparisons among them become problematic (3). These limitations make it challenging to conduct research solely based on ST dataset.

To overcome the limitations of ST dataset, researchers often incorporate additional information, such as H&E staining images, immunofluorescent staining images based on cell
markers, or single-cell RNA sequencing datasets (1,7). These complementary sources of information enable a comprehensive understanding of the spatial gene expression patterns within organs. Nevertheless, comparing multiple ST datasets for a specific region of interest (ROI) can be challenging, particularly when dealing with organs that have complex anatomical structures, such as the brain (8).

In our study, we introduce a novel methodology, Spatial-SPM, which significantly advances the analysis process of ST datasets by reconstructing gene expression pattern images and facilitating their spatial registration (Figure 1). This approach transforms ST data into two-dimensional (2D) dense matrices, enabling the generation of pixel-wise statistical parametric maps. These maps provide a detailed quantification of gene expression levels across anatomically matched datasets, allowing for precise comparisons at the pixel level. By applying SpatialSPM, researchers can conduct direct comparisons of gene expression within specific anatomical ROI across different biological conditions. This capability offers profound insights into the spatial dynamics of gene expression, shedding light on the molecular mechanisms driving pathogenesis and enhancing our understanding of complex biological processes.

Materials and methods

Acquisition of ST datasets

Two ST datasets generated using the Visium platform technology (10× Genomics, CA, USA) were utilized for this study: the mouse intracranial hemorrhage model (GSE182127) (9) and the amyloid-depositing Alzheimer’s disease mouse model (APPK670/671NL/PSEN1ΔE9; PSAPP) with conditional Inpp5d knockdown in microglia (PSAPP/Inpp5dfl/fl; Cx3cr1CreERT2) (GSE203424) (10). The gene expression matrix and metadata, including location coordinates, were loaded using scanpy version 1.6.1 (11). The expression numbers in the datasets were log-transformed after normalization by dividing the counts within each cell by the total number of transcripts, scaled by 10^6.

In addition to our primary analyses, we extended the application of SpatialSPM to include datasets from various biological contexts and ST platforms to underscore its adaptability and effectiveness. Specifically, we incorporated mouse olfactory bulb (MOB) dataset sourced from a barcode-based ST platform, a precursor to the Visium technology (12). This dataset consists of twelve replicates, characterized by grid-like spots with a fixed distance of 200 micrometers between each pair, presenting a distinct spatial resolution challenge for alignment and registration. Furthermore, we explored the versatility of SpatialSPM by analyzing ST datasets from mouse kidney tissue. These datasets include samples from two different murine models of acute kidney injury (AKI): ischemia/reperfusion injury (IRI) and cecal ligation and puncture (CLP), alongside a sham control (13). Through the application of SpatialSPM to these additional datasets, we aim to showcase its capability for accurate spatial alignment and registration process across different platforms, tissue types, and disease models, further validating its robustness and versatility in the field of ST dataset analysis.

Overall workflow of SpatialSPM for the pixel-wise comparative analysis on the ST dataset

The overall workflow of SpatialSPM is depicted in Figure 1. This method utilized a convolutional operation to estimate gene expression values for locations between the detection spots in ST dataset by superimposing Gaussian values from neighboring spots. The resulting 2D images for each gene were compiled and loaded as matrices. Next, score maps of highly variable genes (HVGs) were constructed, which are used to generate a transformation matrix. This transformation matrix was then applied to all genes in the 2D image matrices, resulting in transformed matrices, which is spatial registration process. Comparative analysis at the pixel level could be performed across the spatially registered images to evaluate gene expression levels in specific ROIs.

Generation of a 2D image matrix of each gene expression based on the ST dataset

Firstly, the multiple ST datasets, comprising each gene expression level data for every detection spot, were converted into 2D image matrices for every gene (Figure 1A). We performed a convolutional operation, assuming that each gene expression value followed a Gaussian distribution based on its distance from the center of the spots. In detail, this calculation process imputed the expression level of the x-th gene at specific locations in the empty space between the spots by superimposing the Gaussian values of the gene expression levels based on the centers of the other neighboring spots (i, j, k, and so on) (Figure 1A). The degree of the Gaussian distribution was specified to measure the count corresponding to a specific range (the value of the probability, 1-alpha) within a specific radius value. The radius value is determined by the factor (v) multiplied by the distance between spots, with alpha set to 0.01 for smoothing the gene expression patterns. The v is a factor that determines the extent of smoothing applied to convert the values of the spots into a 2D dense matrix, so we aimed to assess the subsequent registration performance according to the v. This choice acts as a hyperparameter for Gaussian distribution-based gene expression values that were imputed between the spots. Afterward, the 2D image generated for each gene was compiled and loaded as a matrix to proceed to the next step.

Spatial registration process of 2D image on a template image according to a transformation matrix

Initially, a score map of HVGs was constructed based on the generated 2D images from each ST dataset. To identify HVGs, we employed the ‘sc.pp.highly_variable_genes’ function in the Scanpy package (version 1.9.2) (11). Two thousands HVGs for each dataset were extracted. Subsequently, we determined the intersection of HVGs across all ST datasets to generate score maps for each dataset. This construction was achieved using the ‘scanpy.IL.score_genes’ function in the Scanpy package (version 1.9.2) (11) by calculating every gene score (Figure 1B). By matching the score map of HVGs, which was selected as the template image, with the other HVGs maps, a transformation matrix was generated.

The transformation matrix was constituted by a non-linear transform with a combination of transformation methods. In detail, the implemented method includes four main steps: center-of-mass (COM) matching, translation, rigid, and affine transforms, and non-linear transform. Firstly, the COM matching step and the translation transform step align the images by estimating the translation parameters. The rigid transform step uses rotation and scaling to refine the alignment of the images. The affine transform step applies shearing and...
Figure 1. SpatialSPM: Pixel-wise comparative analysis in spatially registered images generated based on the ST dataset. (A) Generation of a two-dimensional expression image matrix by imputing the gene expression levels in empty spaces between the detection spots. (B) Identification of gene expression patterns for constructing a tissue image with a score map of HVGs for the spatial registration process. (C, D) Pixel-wise investigation for comparing gene expression levels in a specific anatomical region of the spatially registered images. Abbreviation: HVGs, highly variable genes; ST, spatial transcriptomics.

Finally, the non-linear transform step uses the symmetric diffeomorphic registration method to achieve a smooth deformation of the moving image. This step optimizes the spatial registration process by using the cross-correlation metric with Gaussian smoothing. The optimization was performed over multiple resolutions of the images. This transformation matrix was then applied to the matrices of all genes in the generated 2D images, resulting transformed matrices. This spatial registration process was performed using the DIPY package (version 1.4.1) (14) (Figure 1C). Based on these transformed matrices, spatially registered 2D images were portrayed.

Pixel-wise comparative analysis for the gene expression across the spatially registered 2D images

Comparative analysis at the pixel level was performed across the spatially registered images to evaluate gene expression levels in a specific anatomical ROI (Figure 1D). This is
because each specific pixel in the images was matched to the corresponding anatomical location. This study utilized ST datasets from two experimental animal brain models: the mouse intracranial hemorrhage model and the mouse model of Alzheimer’s disease, as working examples. In these two animal models, the levels of transcriptomes that changed in specific anatomical regions of the brain under specific conditions were compared to those of the control group, followed by statistical analysis. In the mouse intracranial hemorrhage model, the statistical association between the concentration of treated Heme-Albumin and the transcriptome levels of genes was evaluated using Pearson correlation coefficients. In the mouse model of Alzheimer’s disease, pixel-wise independent T-tests were used to statistically compare the transcriptomes in the brains of PSAPP control mice with those of PSAPP mice with tamoxifen-sensitive microglial knockdown of 1npp5d.

Evaluation of spatial registration performance
To evaluate the accuracy of spatial registration process, we quantified the alignment of key anatomical locations by examining gene expression scores. Utilizing the spatial gene expression dataset from mouse brain (GSE203424), which had been previously clustered into anatomical structures based on earlier researches (10,15). We first applied batch correction with Scanorama (16). Following this, we employed Leiden clustering via ‘scmapy.leiden’ in the Scanpy package (version 1.9.2) (11), setting the resolution to 0.1. This approach aimed to generate fewer than five clusters corresponding to anatomical structures (Supplementary Figure S1A). For each identified cluster, we selected marker genes (n = 15) and calculated the module score for each set of marker genes across the ST dataset (Supplementary Figure S1B). Subsequently, we converted these cluster marker scores into a 2D dense matrix, following the method outlined in the previous steps. We applied the transformation matrix, derived from the HVG score map, to these cluster marker score matrices. To assess the effectiveness of spatial registration, we computed the cosine similarity across all pairs of different ST datasets for the cluster score images. As a representative cosine similarity score, ‘Cluster0 score’ which highly expressed in cerebral cortex was used (Supplementary Figure S1C).

Comparison with other methods
SpatialSPM was compared with other methods. Firstly, STalign was also applied to the mouse brain image data (17). STalign, initially designed for aligning single-cell resolution ST datasets, could also be adapted for various barcode-based ST data, such as Visium, through a process known as ‘rasterization’. By rasterizing mouse brain data, a transformation mapping matrix was developed to convert from the source ST data to the target. In a manner akin to SpatialSPM, the first ST dataset of the mouse brains (GSE203424) served as the reference or target template, with five additional ST datasets undergoing spatial transformation to align with this reference. Subsequent to this transformation, spatially registered images were produced using the previously mentioned 2D matrix, specifically for ‘cluster0 score’, indicative of the cerebral cortex. The cosine similarity across all sample pairs was calculated for these ‘cluster0 score’ maps, facilitating a comprehensive comparison. Additionally, as a negative control, the cosine similarity for pairs of ‘cluster0 score’ and ‘cluster2 score’ maps, which represented anatomically different structures, was calculated post-registration to assess if the similarity scores remained relatively low, thereby evaluating the effectiveness of the spatial transformation process. As another method, GPSA was tested to compare with SpatialSPM (18). GPSA is employed to transform and map the spots within barcode-based ST data. In this study, we utilized Variational-GPSA for comparative analysis, focusing on spatial alignment using the first ST dataset as a reference. The primary parameters for this process were configured with a learning rate of 0.01 and a total of 1000 epochs for the alignment task.

Results
Investigation of mouse brain ST datasets by SpatialSPM
Our SpatialSPM methodology was applied to ST datasets obtained from mouse animal models with intracranial hemorrhage, induced by Heme-Albumin injection into the striatum (9). Five ST datasets were processed into multiple 2D image matrices of gene expression values, representing brain tissues from the Sham group and Heme-Albumin injection groups with concentrations of 0.03nM, 1.25nM, 5.0nM, and 10.0nM. HVGs score maps were also constructed for each group (Figure 2A). The HVGs score map exhibited clear distinctions based on the specific brain anatomical structure in each group. Subsequently, the images were processed by the spatial registration process, using the image of the sham group as the template. As a result of this process, five spatially registered 2D brain images were obtained, exhibiting identical contours and internal arrangement (Figure 2A).

In five spatially registered 2D brain images, glial fibrillary acidic protein (GFAP) (9) expression level was displayed as a demonstrative example of a specific gene (Figure 2B). GFAP is a signature intermediate filament of astrocytes and its expression level is indicative of the severity and extent of intracranial pathologic status after traumatic brain injury (19). The expression level of GFap in striatum and cortex of brain was tightly correlated with the Heme-Albumin concentration. Additionally, the pixel-wise correlation analysis was performed and represented as a parametric map showing Pearson’s correlation coefficients for each gene (Figure 2B). Notably, the expression level of GFap in the center of striatum (Heme-Albumin injection site) showed statistically less correlated with the concentration, suggesting that GFap expression levels in the injection site were not affected by Heme-Albumin concentration. Also, parametric maps can be used to display and statistically analyze all other gene features in the ST datasets. Supplementary Figure S2 shows the representative parametric maps of the top 30 genes that were highly correlated (Pearson’s correlation coefficient > 0.3) with Heme-Albumin concentration.

SpatialSPM can also be applied for comparative analysis between two groups. Total six ST datasets from the amyloid-depositing Alzheimer’s disease mouse model (APP/K670N/671NL.PSEN1Δexon9; PSAPP) with conditional 1npp5d knockdown in microglia (PSAPP/1npp5dfl/fl/Cx33xFR2ER) were utilized (10). Among them, three datasets were acquired from corn oil (CO) treated mice (PSAPP-CO) and the other datasets were from tamoxifen (TAM) treated mice (PSAPP-TAM) with down-regulation of 1npp5d in microglia.
Figure 2. Investigation of the intracranial hemorrhage mouse model brain ST dataset by SpatialSPM. (A) Five ST datasets were obtained from five groups: Sham, 0.03, 1.25, 5.0 and 10.0 nM of Heme-Albumin injection groups. Each dataset was processed into a 2D image matrix representing gene expression values. The HVGs score maps was constructed based on these matrices. The spatial registration process resulted in five spatially registered 2D brain images. (B) Gfap expression levels in spatially registered brain images of intracranial hemorrhage mouse model. Heatmaps illustrated based on Gfap expression levels across five groups (Sham, 0.30, 1.25, 5.0 and 10.0 nM of Heme-Albumin injection groups). Heatmaps illustrated based on the Pearson's correlation coefficient between Gfap expression and Heme-Albumin concentration. (asterisk: Heme-Albumin injection site) Abbreviation: Gfap, glial fibrillary acidic protein.
The ST datasets were processed into 2D image matrix of each gene expression value, constructing the HVGs score map images, respectively (Figure 3A). Despite all the maps being images of coronal brain tissue sections, direct comparison and analysis of gene expression status in specific anatomical locations was challenging due to variations in image morphologies. The spatial registration process of the images on a template image (in this case, the first image of the PSAPP-CO group) was performed, resulting six spatially registered 2D brain images with same configuration (Figure 3A). The scores of HVGs were clearly distinguishable according to the brain anatomic structure.

After the spatial registration process, Cst7 gene, a well-known biomarker of neuro-inflammation related to the amyloid beta plaque in Alzheimer’s disease, was selected as an example for investigation and the gene expression levels were shown in the images (Figure 3B). The expression level of Cst7 was higher in the brain of the PSAPP-TAM group compared to the PSAPP-CO group. The pixel-wise T-test was performed between two groups (PSAPP-CO and PSAPP-TAM with three biological replicates in each group) for mapping the statistics (presented as T-score) for Cst7 gene expression alteration in each anatomic location according to the conditional Inpp5d knockdown in brain microglia (Figure 3B). It was found that Cst7 expression was elevated in striatal and cortex area of PSAPP-TAM brain with statistical significance.

In Supplementary Figure S3, the representative parametric T-score maps were shown for the most significantly upregulated 30 genes in mice in PSAPP-TAM group, compared to those in PSAPP-CO group.

Optimization of SpatialSPM and comparison with other methods

SpatialSPM is based on the process including generation of 2D dense matrix from barcode-based ST data followed by rigid, affine transformation and symmetric diffeomorphic registration. In this process, 2D dense matrix transformation is a critical step for image-based pixelwise comparison as well as registration performance. To identify the optimal parameter for creating a 2D dense matrix, we varied the radius factor (ψ) and assessed the impact on registration performance. The radius parameter plays a crucial role in this context, as it is used to calculate the count of occurrences within a pre-determined range. This calculation is grounded in the Gaussian distribution, specifically targeting a probability value of 1 − α, which dictates the extent of spread or influence each data point has within a defined radius. The essence of this approach is to ensure that the degree of the Gaussian distribution directly influences how counts are aggregated over a specified spatial area. Furthermore, the radius factor ψ is employed as a scaling factor that adjusts the distance between spots, effectively tuning the spatial resolution of the matrix based on the distribution of data points. The efficacy of the registration process was evaluated based on the degree of similarity between brain structures, specifically through the ‘cluster0 score’, which represents the cerebral cortex, as shown in Supplementary Figure S1. The HVG score maps, corresponding to variations in radius factors (ψ), were illustrated in Figure 4A. Following the registration, ‘cluster0 score’ maps for different values of radius factor ψ were depicted in Figure 4B. It was observed that a higher ψ resulted in smoother images, whereas a smaller ψ retained grid-like appearances. The performance of registration, quantified using cosine similarity, improved with increasing ψ. However, beyond a radius factor of ψ = 3, the qualitative improvements in similarity reached a plateau (Figure 4C). Given that larger values of ψ produced smoother images, a radius factor of ψ = 3 was determined to be optimal for this Visium dataset (Figure 4C). For additional brain structures delineated by different cluster scores, analogous outcomes were observed, as detailed in Supplementary Figure S4. The similarity scores improved with increasing values of ψ, but plateaued around ψ = 3, indicating a consistent trend across various brain regions assessed by distinct cluster scores.

To evaluate the robustness of the spatial registration method employed by SpatialSPM, we conducted a series of experiments using various references. Specifically, we sequentially used each of the six ST datasets as a reference and then performed spatial registration on the remaining five ST datasets, comparing their alignment against the ‘cluster0 score’ image, which serves as a benchmark for assessing cerebral cortex representation. This process was repeated for each ST dataset, effectively cycling through all six as potential references. The outcomes of these experiments revealed that the registration method’s performance remained consistently stable, irrespective of which ST dataset was chosen as the reference (Supplementary Figure S5).

The effectiveness of spatial registration techniques for ST datasets was evaluated. Firstly, STalign employed diffeomorphic metric mapping to adjust for local non-linear distortions in various ST datasets, following the rasterization of ST data cells (17). While SpatialSPM utilizes Gaussian distribution factors to create 2D dense matrices from barcode-based ST datasets, STalign relies on a cell density map for its computations. GPA, another method introduced, leverages a probabilistic model to map ST datasets onto a common coordinate system using a foundational two-layer Gaussian processing model that encompasses spatial warping and phenotype matching (18). The performance of SpatialSPM was benchmarked against STalign and GPA using cosine similarity of the ‘cluster0 score’, which represents maps of the cerebral cortex across six datasets. The results, illustrated in Figure 5A, show the cosine similarities for all pairs in Figure 5B. SpatialSPM achieved a cosine similarity of 0.989 ± 0.007, while STalign and GPA recorded similarities of 0.822 ± 0.067 and 0.728 ± 0.084, respectively. To evaluate the specificity of the alignment, the cosine similarity between ‘cluster0 score’ maps and ‘cluster2 score’ maps was examined, representing different anatomical structures. Here, SpatialSPM demonstrated distinct patterns in the cosine similarity plot, exhibiting high similarity values between the same cluster scores of different samples while maintaining low similarity values between different cluster scores. Specifically, the similarity scores between ‘cluster0’ and ‘cluster2’ pairs were 0.693 ± 0.027 for SpatialSPM, 0.580 ± 0.111 for STalign, and 0.632 ± 0.055 for GPA (Supplementary Figure S6). This underscores the precision of SpatialSPM in distinguishing between similar and different anatomical structures.

Application to various datasets

To reveal that SpatialSPM could be used in various types of datasets, we additionally focused on MOB datasets, which precede the development of Visium technology (12). A total of twelve ST datasets from the MOB were selected for detailed analysis within the framework of SpatialSPM. The
Figure 3. Investigation of the Alzheimer’s disease mouse model brain ST dataset by SpatialSPM. (A) Six ST datasets were obtained from two groups: PSAPP-CO and PSAPP-TAM. Each dataset was processed into 2D image matrix representing each gene expression value. The HVGs score maps were constructed based on these matrices. The spatial registration process resulted in six spatially registered 2D brain images. (B) Cst7 expression levels in spatially registered brain images of Alzheimer’s disease mouse model are illustrated across two groups (PSAPP-CO and PSAPP-TAM). Heatmaps illustrated based on the T-score estimated by pixel-wise T-test between two groups. Abbreviation: CO, corn oil; PSAPP, APP*M670I/V717I; /PSEN1ΔE39, TAM, tamoxifen.
initial step involved the extraction of HVGs, from which we then constructed 2D dense matrices reflecting the distribution of HVG scores (Figure 6A). SpatialSPM registration process was proceeded, aiming to achieve spatial alignment across the ST datasets to compare gene expression patterns across different samples within the spatial context of the MOB. To demonstrate the effectiveness of this spatial alignment, we focused on the expression patterns of representative genes, Pcp4 and Calb2. These genes are well-known markers for distinct anatomical layers within the MOB: Pcp4 for the granule cell layer and Calb2 for the glomerular layer (Figure 6A). This showcased the potential of SpatialSPM for detailed spatial analyses in other tissue environments as well as different platforms for barcode-based ST data.

In addition to MOB datasets, mouse kidney ST data were additionally tested (13). We analyzed mouse kidney datasets featuring two murine models of AKI: IRI and CLP, alongside a sham control. We generated score maps for HVGs and transformed into 2D dense matrices. Following spatial registration, we focused on the distribution of key marker genes for a comparative analysis across the models. Podxl, identified as a marker for the proximal tubule, and Nphs1, a marker for the glomerulus, along with Pvalb, indicative of the collecting duct, were mapped onto the spatially registered datasets. These mappings revealed consistent spatial patterns for these markers across the three datasets, highlighting the robustness of our spatial registration method in preserving biological structures. We observed a notable increase in the expression of Atf3, a marker associated with kidney injury, specifically within the IRI model (Figure 6B).

Discussion
As ST technology becomes more ubiquitous, there is increasing demand to investigate the spatial gene expression patterns in complex organs such as the kidney, brain, intestines, and liver (1,2). Researchers acquire ST datasets from tissues exhibiting specific diseases or pathological conditions, detect alterations in gene expression within regions of anatomical interest and investigate their clinical implications. However, the lack of a methodology to align ST data anatomically for the various shape and coordinates of histologic tissues limits the
Figure 5. Benchmarking spatial registration methods compared with SpatialSPM. (A) Six ST data of mouse brain illustrates the example of registered images of ‘cluster0 score’. It represented comparison of SpatialSPM, STalign, and GPSA in aligning ST datasets. (B) Using the ‘cluster0 score’ as a measure for cerebral cortex representation, SpatialSPM demonstrates superior alignment with a cosine similarity of 0.989 ± 0.007 outperforming STalign and GPSA which exhibit similarities of 0.822 ± 0.067 and 0.728 ± 0.084, respectively. Abbreviation: ST, spatial transcriptomics.

direct comparison of gene expression patterns across various types of samples (20).

One of the main contributions of SpatialSPM is the development of a method that overcomes this challenge. Pixel-wise or voxel-wise statistical analysis, such as the well-established method called statistical parametric mapping (21), is commonly used for analyzing images with complex structures, such as the brain (22). SpatialSPM applies this method to transform ST data into image matrices, portraying every gene expression level of each ST dataset on a common template image, referred to as spatially registered 2D images. This process could facilitate direct comparison of gene expression patterns across similar regions in different histologic samples by pixel-wise statistical analyses, such as correlation analyses or T-tests, for specific target genes.

A key aspect of SpatialSPM’s methodology is its transformation of spot-based ST data into a 2D dense matrix. This transformation facilitates the application of a wide array of image processing tools, including spatial registration, to ST data. The transition from spot-based methods to a 2D dense matrix presents notable challenges, particularly in accurately representing the spatial distribution of RNA molecules. To address these challenges, our method leverages Gaussian smoothing, a technique chosen for its effectiveness in mirroring the natural diffusion characteristics of RNA. This smoothing process plays a crucial role in attenuating noise, thereby ensuring a representation of gene expression that is both coherent and biologically meaningful across the spatial matrix. The decision to apply Gaussian smoothing is grounded in the need to approximate the gradual dispersion of RNA signal from the central point of detection outward, reflecting the inherent diffusion process. By employing this approach, we aim to capture a more accurate spatial representation of gene expression, enhancing the biological relevance of the resultant data.
Figure 6. Application of SpatialSPM to diverse tissue types. (A) The use of SpatialSPM in analyzing MOB ST datasets is presented. It showcases 2D dense matrices derived from HVG scores to align and compare gene expression across twelve ST datasets. It is noteworthy that these datasets were sourced from an earlier generation of ST technology, characterized by a greater distance between spots compared to that of Visium. The expression patterns of Pcp4 and Calb2, markers for the granule cell layer and glomerular layer respectively, are used to demonstrate the efficacy of spatial alignment within MOB. (B) The application to mouse kidney ST data was presented. It features two AKI models: IRI and CLP, plus a sham control. Key marker genes such as Podxl, Nphs1, and Pvalb are mapped to illustrate the consistency of spatial patterns post-registration, with an observed upregulation of the injury marker Atf3 in the IRI model. Abbreviation: AKI, acute kidney injury; CLP, cecal ligation and puncture; HVGs, highly variable genes; IRI, ischemia/reperfusion injury; MOB, mouse olfactory bulb; ST, spatial transcriptomics.

2D dense matrix. Looking ahead, our research will further explore the underlying distributional assumptions integral to the SpatialSPM framework (23). This future work is essential for advancing our understanding of the behavior of the model in various biological scenarios, ensuring that SpatialSPM remains a robust and versatile tool for the spatial analysis of transcriptomics data.

The spatial investigation of molecular change plays a significant role in the analysis of disease pathophysiology and the mode of action of drugs (8). In this regard, SpatialSPM is a powerful technique that offers new insights into the underlying molecular mechanisms of various diseases, particularly in the brain. This is particularly relevant given the intricate relationship between anatomical structures and brain function. Furthermore, by utilizing cellular deconvolution for ST dataset (24), SpatialSPM empowers researchers to investigate the density of specific cell groups or pathway signatures. The cellular map can be inferred from the ST dataset and subsequently compared the groups using this proposed method.
In comparing SpatialSPM with STalign and GPSA, it is evident that SpatialSPM demonstrates superior performance in terms of the cosine similarity metric, particularly in mapping the cerebral cortex across various datasets. This strength likely stems from its specific approach to interpreting ST data, utilizing Gaussian distribution factors to generate 2D dense matrices from barcode-based ST datasets. STalign, while not achieving the same level of performance as SpatialSPM in this comparison, offers complementary advantages, particularly in its ability to adjust for local non-linear distortions through diffeomorphic metric mapping. This method is adept at creating cellular-level maps and is particularly well-suited for image-based ST datasets such as MERFISH, where cellular density and arrangement play a critical role. It is not specifically optimized for alignment between barcode-based ST data, which may limit its effectiveness in certain scenarios. GPSA introduces a different strategy by focusing on altering the coordinates of spots themselves within a probabilistic model framework. This two-layer Gaussian process model, encompassing spatial warping and phenotype matching, is pivotal for aligning ST observations. GPSA's focus on changing spot coordinates may not provide the same level of registration accuracy as methods utilizing an image matrix approach for fitting the registration algorithm. The discrepancy in performance can be attributed to the inherent differences in how each method conceptualizes and processes spatial information within the ST data. Each method's strengths and limitations underscore the importance of selecting an appropriate spatial registration technique based on the specific characteristics and requirements of the ST data being analyzed.

SpatialSPM has been primarily applied to ST datasets involving anatomically consistent tissues such as the brain, MOB and kidneys, showcasing its capability for precise anatomical registration across these varied contexts. This methodological approach facilitates the detailed comparison of ST datasets by aligning them based on anatomical features, thereby enabling a deeper understanding of tissue-specific gene expression patterns. However, the extension of SpatialSPM to disease tissues with structure changes such as cancer introduces significant challenges that stem from the dynamic and complex nature of biology. Heterogeneous changes in tumors can significantly impact the spatial organization of the tissue, making the anatomical registration of cancerous and normal tissues more complicated than in more homogeneous and structurally stable tissues. To address these challenges and improve the utility of SpatialSPM in cancer research, future developments should focus on adapting the method to better handle the altered tissue architecture and cellular composition typical of tumor environments. These adaptations may include the integration of other computational models that can discern and accommodate the spatial heterogeneity of disease tissues, thereby enabling more accurate comparison and analysis of tumor and adjacent normal tissues within the ST domain.

In our study, we explored the application of SpatialSPM for spatial registration when dealing with partially matched datasets. The potential for limited usage of this method arises from its dependency on the context of image-generated datasets, suggesting that its applicability may vary based on the specific characteristics of the data being analyzed. To illustrate this, we utilized spatially partially matched samples from different Visium datasets, including sagittal mouse brain data with fields of view (FOV) of 11 mm and 6.5 mm, respectively (as available at https://www.10xgenomics.com/datasets). For practical application, the larger 11 mm FOV dataset served as the template, which we manually cropped and rotated as an initial processing to align with the 6.5 mm FOV dataset (Supplementary Figure S7A). Following the estimation of the transformation matrix, we were able to highlight anatomical markers (Pcp4 and Pvalb), demonstrating the ability to align key features across different FOVs (Supplementary Figure S7B). However, the application of SpatialSPM requires manual cropping for partially matched datasets. Although designed for detailed pixel-wise comparisons with minimal manual intervention, the diversity and specificity of different datasets present challenges. Future improvements will aim to better accommodate this variability, enhancing SpatialSPM’s usability and applicability across various ST data.

However, some limitations to the use of SpatialSPM should be noted. Firstly, barcode-based ST technology provides sparse transcriptomic data with spots located apart from each other, rather than a complete pixel image or matrix. To address this issue, we generated 2D image matrices with continuous location information by assuming a Gaussian distribution of transcript data, followed by the spatial registration process. In this line, it is important to consider the technology platform used for generating ST dataset. It is necessary to expand the application and provide more detailed explanations of this method on various technology platforms, including image-based ST (such as Xenium, CosMX and MERFISH) (1). The limitation is the exclusion of image-based ST data, primarily due to its characteristic non-uniform spatial distribution of cell types, which poses challenges in generating feature maps as 2D dense matrices. While the ‘rasterization’ method employed by STalign offers a potential solution for this issue, it was not incorporated within the scope of our current application of SpatialSPM. More specifically, the Gaussian distribution approach used by SpatialSPM could lead to potential biases, such as inflated values in areas of high cell density, when transforming a specific gene expression map into a 2D dense matrix in scenarios where spatial cell distribution is non-uniform. This issue could impact the accuracy of comparison of different samples after registration. Additionally, employing a Gaussian distribution to model single-cell resolution data might not capture the detailed cell-type composition accurately, underscoring the necessity for additional exploration and explanation of this limitation. However, the advent of VisiumHD (25), which offers high-resolution barcode-based ST data, presents a promising opportunity for the future application of SpatialSPM. The enhanced resolution and uniformity provided by next-generation barcode-based ST could facilitate the effective application of SpatialSPM to a broader array ST dataset, overcoming the current limitations and expanding the technique's utility in analysis. Furthermore, it is important to consider a technical issue that arises during the tissue acquisition process for the ST dataset. For example, when analyzing brain tissue, the orientation or anatomical location of sectioning can result in completely different ST datasets. Such variations can introduce errors in the analysis process, potentially leading to false positive or false negative findings. Nevertheless, by employing well-structured histology and ST dataset, we believe that SpatialSPM can overcome these challenges and yield reliable results.
In conclusion, SpatialSPM marks a pivotal leap forward in ST analysis, transcending traditional approaches that focus solely on identifying differentially expressed genes. By enabling pixelwise comparison across tissue samples, Spatial-SPM offers a more nuanced and detailed examination of gene expression patterns, particularly within structurally complex tissues such as the brain and kidney. This ability of Spatial-SPM to facilitate direct comparisons of gene expression within identical anatomical regions—despite variations in tissue shape and spatial anatomy—enriches our understanding of disease-specific gene expression changes. Such in-depth analysis not only enhances our comprehension of the underlying mechanisms of pathogenesis but also opens new avenues for the development of targeted therapeutic interventions.

Data availability
The data underlying this article are available in Gene Expression Omnibus (GEO) under the accession number GSE182127, GSE20342, and GSE171406. The mouse olfactory bulb data can be downloaded from https://www.spatialresearch.org/resources-published-datasets/. The code for the SpatialSPM is available in Figs hare at https://doi.org/10.6084/m9.figshare.25538311. It is also available at the GitHub repository (https://github.com/portrai-io/spatialspm).

Supplementary data
Supplementary Data are available at NAR Online.

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Conflict of interest statement
D.L. and H.C. are the co-founders and shareholders of Portrai, Inc.

References
