Gene expression

CCPLS reveals cell-type-specific spatial dependence of transcriptomes in single cells

Takahiro Tsuchiya, Hiroki Hori and Haruka Ozaki

1Bioinformatics Laboratory, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan, 2Center for Artificial Intelligence Research, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan and 3Doctoral Program in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan

*To whom correspondence should be addressed.

Received on March 10, 2022; revised on August 17, 2022; editorial decision on August 19, 2022; accepted on September 4, 2022.

Abstract

Motivation: Cell–cell communications regulate internal cellular states, e.g. gene expression and cell functions, and play pivotal roles in normal development and disease states. Furthermore, single-cell RNA sequencing methods have revealed cell-to-cell expression variability of highly variable genes (HVGs), which is also crucial. Nevertheless, the regulation of cell-to-cell expression variability of HVGs via cell–cell communications is still largely unexplored. The recent advent of spatial transcriptome methods has linked gene expression profiles to the spatial context of single cells, which has provided opportunities to reveal those regulations. The existing computational methods extract genes with expression levels influenced by neighboring cell types. However, limitations remain in the quantitativeness and interpretability: they neither focus on HVGs nor consider the effects of multiple neighboring cell types.

Results: Here, we propose CCPLS (Cell–Cell communications analysis by Partial Least Square regression modeling), which is a statistical framework for identifying cell–cell communications as the effects of multiple neighboring cell types on cell-to-cell expression variability of HVGs, based on the spatial transcriptome data. For each cell type, CCPLS performs PLS regression modeling and reports coefficients as the quantitative index of the cell–cell communications. Evaluation using simulated data showed our method accurately estimated the effects of multiple neighboring cell types on HVGs. Furthermore, applications to the two real datasets demonstrate that CCPLS can extract biologically interpretable insights from the inferred cell–cell communications.

Availability and implementation: The R package is available at https://github.com/bioinfo-tsukuba/CCPLS. The data are available at https://github.com/bioinfo-tsukuba/CCPLS_paper.

Contact: haruka.ozaki@md.tsukuba.ac.jp

Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Cell–cell communications regulate internal cellular states, e.g. gene expression and cell functions, and play pivotal roles in normal development and disease states (Pelkmans, 2012; Sharpe and Pauken, 2018; Snijder and Pelkmans, 2011). For example, in tumor progression, programmed cell death ligand 1 (PD-L1) is expressed in a tumor cell that binds to the programmed cell death protein 1 (PD-1) of T cells, thus regulating T cell gene expression and weakening the anti-tumor growth response (Shimizu et al., 2020). The PD-1 pathway inhibitors block this cell–cell communication and thus stop the growth of tumor cells (Sharpe and Pauken, 2018). Accordingly, it is essential to elucidate gene expression regulation via cell–cell communications in order to understand and control complex multicellular systems (Pelkmans, 2012; Sharpe and Pauken, 2018; Snijder and Pelkmans, 2011).

Single-cell RNA sequencing (scRNA-seq) methods have been used to study complex multicellular systems. These technologies have revealed cell-to-cell expression variability of highly variable genes (HVGs) even in the same cell type, which is crucial in the normal development and disease states (Ben-Moshe and Itzkovitz, 2019; Satija et al., 2015; Stuart et al., 2019). Nevertheless, the regulation of cell-to-cell expression variability of HVGs via cell–cell communications has not been well characterized (Ben-Moshe and Itzkovitz, 2019; Satija et al., 2015; Stuart et al., 2019). Instead of the expression of HVGs, the expression of ligand and receptor genes has been the focus of the computational tools to infer potential cell–cell communications using scRNA-seq data: these tools compare expression levels of ligand and receptor genes between cell-type pairs (Armingol et al., 2020; Hou et al., 2020; Nagai et al., 2021). However, such approaches have several limitations regarding incompleteness of knowledge of the ligand–receptor pairs, potential
cross-talks among ligands and receptors, and unavailability of spatial contexts of cells (Armingol et al., 2020; Hou et al., 2020; Nagai et al., 2021). Moreover, the ligand–receptor expression approach cannot directly provide insights into the effects of cell–cell communications in most of the HVGs in the same cell type. It is desirable to focus on the regulation of cell-to-cell expression variability of HVGs via cell–cell communications.

As another remarkable aspect, intracellular gene expression regulation is a multiple-input and multiple-output (MIMO) system, and a quantitative understanding is of critical importance (Akimoto et al., 2013; Janes et al., 2001). The regulation via cell–cell communications can also be considered a MIMO system, in which the degree of neighboring cell-type existence regulates the intracellular gene expression values. As an example of the PD-1 pathway, antigen-presenting cells and the tumor cells cooperatively regulate the gene expression of T cells, and the balance of such regulation is crucial to cell-fate decisions (Sharpe and Pauken, 2018). Besides cell–cell communications via known molecular interactions, the cases in which the gene expression in a cell is affected by the spatial arrangement and combinations of the neighboring cells can be interpreted as MIMO systems (Colombo and Cattaneo, 2021; Haanen, 2017; Hui and Bhattacharya, 2007). It is essential to develop computational methods for estimating such a MIMO system of cell–cell communications by using spatial contexts of cells.

The recent advent of spatial transcriptome methods has linked gene expression profiles to the spatial context of single cells, which has provided opportunities to reveal the regulation of cell-to-cell expression variability of HVGs via cell–cell communications (Cho et al., 2021; Eng et al., 2019; Hu et al., 2021b; Marx, 2021). Nevertheless, there is no computational methods based on spatial transcriptome data for estimating regulation on cell-to-cell expression variability of HVGs as a MIMO system of cell–cell communications (Arno et al., 2019; Dries et al., 2021b; Hu et al., 2021a; Rao et al., 2021; Svensson et al., 2018; Tanevski et al., 2022; Velten et al., 2022; Zhu et al., 2021), which could limit quantitiveness and interpretability. For example, Giotto findICG extracts genes with expression levels that are influenced by the neighboring cell types; however, it neither considers the cooperation of neighboring cell types nor quantifies the degree of regulation with each neighboring cell type (Dries et al., 2021b). Other existing methods focus on the spatial gene expression variability itself or gene–gene relationships and do not focus on the effect of neighboring cell types on gene expression variability (Arno et al., 2019; Dries et al., 2021b; Hu et al., 2021a; Rao et al., 2021; Svensson et al., 2018; Tanevski et al., 2022; Velten et al., 2022; Zhu et al., 2021). Moreover, these tools do not explicitly focus on HVGs. Thus, there is a need to develop methods to estimate the MIMO system of cell–cell communications that focus on HVGs.

Here, we propose CCPLS (Cell–Cell communications analysis by PLS regression modeling), which is a statistical framework for identifying the MIMO system of cell–cell communications, i.e. regulation of cell-to-cell expression variability of HVGs by multiple neighboring cell types, based on the spatial transcriptome data at a single-cell resolution. CCPLS performs PLS regression modeling for each cell type and reports the estimated coefficients as the quantitative index of the cell–cell communications from each neighboring cell type. Evaluation using simulated data showed our method accurately estimated the effects of multiple neighboring cell types on HVGs. Furthermore, by applying CCPLS to the two real datasets, we demonstrate CCPLS can be used to extract biologically interpretable insights from the inferred cell–cell communications.

2 Materials and methods

2.1 Problem definition

Spatial transcriptome data at a single-cell resolution consist of a gene expression matrix, a coordinate matrix and a cell-type label vector. Let N be the number of cells, G be the number of genes, and M be the number of cell types. The gene expression matrix $Y \in \mathbb{R}^{N \times G}$ contains the expression value $y_{ij}$ for each cell $i (1 \leq i \leq N)$ and gene $g (1 \leq g \leq G)$. The coordinate matrix $P \in \mathbb{R}^{N \times 2}$ contains the 2D spatial coordinate of cells $P(i) \in \mathbb{R}^{2}$ ($1 \leq i \leq N$). The cell-type label vector $L = \{l_i \in \{1, \ldots, M\}\}$ contains the cell-type label $l_i$ of the M unique cell types. We assume the existence of HVGs specific to cell type $m (1 \leq m \leq M)$, i.e. $h^{(m)}$, which is estimated from the expression matrix $Y^{(m)}$ containing cells $i (1 \leq i \leq N^{(m)})$ of cell type $m$ (Hafemeister and Satija, 2019; Stuart et al., 2019).

CCPLS does not estimate cell–cell communications via ligand–receptor interactions but estimates the effect on the gene expression of a cell type due to the arrangement and combination of neighboring cell types as a MIMO system. There are two key assumptions of CCPLS: (i) the cell–cell communications are composed of the linear sum of the effects of neighboring cell types and (ii) the effects of neighboring cell types are the same within each cell type. Accordingly, CCPLS aims to extract HVGs $h^{(m)}$ whose expression values are significantly explained by a linear sum of the effects by neighboring cell types $f$ as follows:

$$y_{ij}^{(m)} = \sum_{f \in L} x_{ij}^{(f)} w_{ij}^{(f)} + \epsilon_{ij},$$

where $h \in h^{(m)}$, $w_{ij}^{(f)}$ denotes coefficient, $x_{ij}^{(f)}$ denotes neighboring cell-type score, and $\epsilon_{ij}$ is the residue term.

Simultaneously, CCPLS aims to estimate the coefficient $w_{ij}^{(f)}$ as the direction and degree of regulation via cell–cell communications by neighboring cell types $f$ for each HVG $h$ in a manner specific to cell type $m$.

2.2 CCPLS

2.2.1 Methods overview

The core of CCPLS is PLS regression modeling performed by setting HVG expression values $Y^{(m)}_{ij}$ as responsive variables and neighboring cell-type scores $x_{ij}^{(f)}$ as explanatory variables for each cell type $m$. After the statistical evaluation, CCPLS performs HVGs $h^{(m)}$ clustering, which is reported with filtered coefficients as the direction and degree of regulation in the cell-cell communication. The input data for CCPLS are the expression matrix $Y$, the coordinate matrix $P$ and the cell-type label vector $L$. CCPLS consists of six main steps as described in Sections 2.2.2–2.2.7, respectively.

Except for the calculation of neighboring cell-type scores, CCPLS is principally based on existing procedures. In other words, by calculating the neighboring cell-type scores, CCPLS realizes a multiple regression approach for estimating the MIMO system of cell–cell communications.

A schematic illustration of the MIMO system of cell–cell communications is shown in Figure 1a, while Figure 1b illustrates the workflow of CCPLS.

2.2.2 Step (i): Extraction of HVG

CCPLS divides the input expression matrix $Y$ into each cell type $Y^{(m)}$, the expression matrix comprised of cells $i$ for each unique cell type $m$. CCPLS filters out the genes with an expression value of 0 in all the cells within cell type $m$ from $Y^{(m)}$, which is subsequently normalized and for each transformed gene $z$-score. Simultaneously, CCPLS extracts cell-type-$m$-specific HVG $h^{(m)}$ and preprocessed HVG expression value $y_{ij}^{(m)}(b = 1, \ldots, H)$ and matrix $Y^{(m)} \in \mathbb{R}^{N^{(m)} \times H}$ (Hafemeister and Satija, 2019; Stuart et al., 2019).

2.2.3 Step (ii): Calculation of neighboring cell-type score

For each cell type $m$, CCPLS calculates the degree of neighboring cell-type existence $x_{ij}^{(m)}$, defined as the neighboring cell-type score, by using the coordinate matrix $P$ and cell-type label vector $L$. CCPLS calculates un-preprocessed neighboring cell-type score $x_{ij}^{(f)}$ based on a function that decays with distance between two cells $i$ and $j$ as follows:

$$x_{ij}^{(f)} = \frac{1}{\sum_{k \neq i} \exp(-d_{ik}^2 / \sigma^2)},$$

where $d_{ik}$ is the distance between cells $i$ and $j$, and $\sigma$ is a decay parameter.
2.2.4 Step (iii): PLS regression modeling

For each cell type \( m \), CCPLS performs PLS regression modeling (Hoskuldsson, 1988). CCPLS employs PLS regression because its advantage is the capability of handling the ‘small N, large P’ problem which is one of the characteristics of the spatial transcriptome data (Abdi, 2010; Nagasawa et al., 2021). The PLS regression model can be understood as two steps regression model (Akimoto et al., 2013; Hoskuldsson, 1988).

The first step can be considered as consisting of the development of outer relations (\( X \) and \( Y \) metric individually). These data matrices are decomposed into latent variables plus a residue matrix. The sub-matrices can be represented as the product of the scores and the loadings which can be re-grouped in independent matrices as follows:

\[
X = TP^T + E, \\
Y = UQ^T + F,
\]

where \( T \in \mathbb{R}^{N \times C} \) and \( U \in \mathbb{R}^{N \times C} \) are the score matrices, and \( P^{L \times C} \) and \( Q \in \mathbb{R}^{H \times C} \) are the loading matrices, for the \( X \) and \( Y \) matrices, respectively. \( C \) is the component number for PLS regression. The matrices \( E \in \mathbb{R}^{N \times L} \) and \( F \in \mathbb{R}^{N \times H} \) correspond to the residues associated with the PLS regression modeling.

Fig. 1. Overview of CCPLS. (a) Schematic illustration of the multiple-input and multiple-output (MIMO) system of cell–cell communications that CCPLS aims to identify. (b) Workflow of CCPLS

\[
x^{(m)}_{ij} = \sum b_{ijh} \exp \left( \frac{-dist_{ij}}{dist_0} \right),
\]

where the \( b_{ijh} \) is the binary value indicating whether the cell type \( i \) of cell \( j \) (\( 1 \leq j \leq N, i \neq j \)) belongs to cell type \( f \), and the \( dist_{ij} \) is the Euclidean distance calculated from the coordinates of the two cells \( i \) and \( j \). The value of \( dist_0 \) is the constant set as the minimum value of the Euclidean distance of all the combinations of two cells in the coordinate matrix \( P \). CCPLS performs \( z \)-score transformation on the un-preprocessed neighboring cell-type score matrix \( X^{(m)} \) for each neighboring cell type \( f \) and calculates the preprocessed neighboring cell-type score matrix \( X^{(m)} \). As described here, CCPLS employs a Gaussian kernel similar to the previous studies (Arnol et al., 2019; Dang et al., 2020).
The second step is a linear inner relation linking between $T$ and $U$,

$$U = TD + H,$$

where $D \in \mathbb{R}^{C \times C}$ is the diagonal matrix and $H \in \mathbb{R}^{N \times C}$ denotes the residue matrix. Here, PLS regression modeling yields by,

$$Y = XW + G,$$

where $W \in \mathbb{R}^{L \times H}$ is the matrix of coefficients

$$W = XU(T'XX'T)^{-1}T'Y,$$

and $G \in \mathbb{R}^{N \times H}$ is the residue matrix.

For each cell type $m$, CCPLS estimates this coefficient matrix $W_m^{(i)}$ by the $Y^{(m)}$ and $X^{(m)}$. $W_m^{(i)}$ consists of the each coefficients $w_{ij}^{(h)}$, which are the direction and degree of regulation in the cell–cell communications which CCPLS aims to estimate. The each coefficient consists of each component $c$ as the equation $w_{ij}^{(h)} = \Sigma c w_{ij}^{(h,c)}$.

CCPLS uses component number $C$ that minimizes the mean squared error of 10-folds cross-validation (Bengio and Grandvalet, 2004).

2.2.5 Step (vi): Filtering of coefficients

For each cell type $m$, CCPLS performs two-step filtering, a $t$-test of factor loadings, and a non-parametric test of the coefficients $w_{ij}^{(h)}$ (Yamamoto et al., 2014). In each step, the $P$-values are false discovery rate-adjusted by the Benjamin–Hochberg method, respectively (Benjamini and Hochberg, 1995). The first step filtered out coefficients in each component $w_{ij}^{(h)}$, while remaining summed coefficients $w_{ij}^{(h)} = \Sigma w_{ij}^{(h)}$ with small values. Based on this tendency, CCPLS employed the two steps filtering. See Text S1 in the Supplementary Information for the detailed procedures.

2.2.6 Step (v): Clustering of HVGs

For each cell type $m$, CCPLS performs HVG clustering by the $k$-means method on the filtered coefficients (Yuan and Yang, 2019). The cluster number $k$ is determined by the Silhouette method (Yuan and Yang, 2019). The minimum and the maximum integer values of $k$ are set as 2 and 15, respectively. Before clustering, CCPLS filters out the HVGs whose filtered coefficients are all zero.

2.2.7 Step (viii): Report

CCPLS outputs a heat map and bipartite graph. The heat map reports the filtered coefficients for each combination of HVGs $h$ and neighboring cell types $f$. The bipartite graph visualizes the relationship between the HVG clusters and the neighboring cell types $f$, where the widths of edges are the averaged values of the filtered coefficients of each HVG cluster.

2.3 Datasets

2.3.1 Simulated dataset

For the evaluation of CCPLS performance, we first simulated a dataset according to the following procedures. To realistically simulate the cell positions and ratio of cell types, our simulation experiments were based on the seqFISH+ real dataset (Eng et al., 2019).

We used the coordinate matrix $P$ of the seqFISH+ real dataset. To simplify the cell types, we prepared a cell-type label vector $L$ by substituting cell types A–D as the cell-type label vector of the seqFISH+ real dataset. We set four cell types as inputs and four HVG clusters as outputs, which contained 500 genes, respectively (Fig. 2a). HVG Cluster 1 was a multiple-input case, while clusters 2–3 were single-input cases. HVG Cluster 4 was not affected by neighboring cell types.

We simulated the preprocessed expression value of cell type A according to the equation $\hat{y}_{ij}^{(h)} = \Sigma x_{ij}^{(A)} w_{ij}^{(h)} + x_{0j}^{(h)}$. We prepared coefficient $w_{ij}^{(h)}$ according to Table 1, in which the coefficient is $w_{ij}^{(h)} = 0$. We calculated the preprocessed neighboring cell-type score $x_{ij}^{(A)}$ using the prepared coordinate matrix $P$ and the cell-type label vector $L$. We simulated the term $x_{ij}^{(A)}$ as Gaussian noise, with a mean of 0 and standard deviations of each respective HVG estimated by mean–CV regression modeling of the seqFISH+ real dataset, which was then multiplied by the constant $\lambda$, which were used for the evaluation of CCPLS performance (Fig. 2 and Supplementary Fig. S1). Note that we did not perform experiments in count scale, which was originally un-recommended in the literature (Oshlack and Wakefield, 2009; Van Verk et al., 2013), and instead simulated scaled data.

For further evaluation, instead of the Gaussian noise term $x_{ij}^{(A)}$, we simulated another noise term $\hat{x}_{ij}^{(A)}$ as noise derived from a gamma distribution (Supplementary Fig. S2). We simulated $\hat{x}_{ij}^{(A)}$ for each gene $b$ by using a gamma distribution whose parameters were based on the seqFISH+ real dataset. This noise matrix was log-scaled and z-score normalized as $\hat{x}_{ij}^{(A)}$.

In Section 3.1, we present the application of CCPLS to this simulated dataset, including an evaluation of the results of cell type A. We first set the $a_{\max}$ and $a$ to 1 in Figure 2a and then changed in Supplementary Figure S1a. Next, we changed the noise term $x_{ij}^{(A)}$ with $\hat{x}_{ij}^{(A)}$ (Supplementary Fig. S2a). See Text S2 in the Supplementary Information for the detailed procedures.

2.3.2 SeqFISH+ real dataset

For the demonstration of CCPLS with a real dataset, we selected the seqFISH+ mouse somatosensory cortex dataset provided by Giotto (https://rubd.github.io/Giotto_site/articles/mouse_seqFISH_cortex_200914) (Dries et al., 2021b). This seqFISH+ dataset profiled 10 000 genes in 523 cells with 12 distinct cell types at a single-cell resolution by using a super-resolved imaging technique (Eng et al., 2019).

In Section 3.2, we describe the results of applying CCPLS to this seqFISH+ real dataset. We further assigned the contributor cell types, which were the common neighboring cell types in each HVG cluster. We performed gene ontology (GO) enrichment analysis for each HVG cluster to investigate the associated biological insights. See Text S2 in the Supplementary Information for the detailed procedures.

2.3.3 Seq-Scope real dataset

For the demonstration of CCPLS with a real dataset, we selected the Seq-Scope colon dataset, which can be acquired from the repository of Deep Blue Data (https://doi.org/10.7302/cjfe-wa35) (Cho et al., 2021). This Seq-Scope dataset profiled 10 806 genes in 4489 cells (subcellular spots) with nine distinct cell types at subcellular resolution by using Illumina flow-cell-based spatial barcoding techniques (Cho et al., 2021).

In Section 3.3, we describe the results of applying CCPLS to this Seq-Scope real dataset, including the assignment of contributor cell types and GO enrichment analysis according to the same procedure referred to in Section 2.3.2.

3 Results

3.1 CCPLS yields an accurate estimation of cell–cell communications

To evaluate CCPLS performance, we prepared simulated data with parameters based on the seqFISH+ real dataset and then applied CCPLS to this simulated data as described in Section 2.3.1 (Fig. 2a). We obtained a heat map and bipartite graph, which indicate the coefficient as the direction and degree of regulation in the cell–cell communication for each combination of HVGs and neighboring cell types by CCPLS (Fig. 2b and c). Cell types C and B exhibited up-regulation of HVG Clusters 1 and 2, respectively (Fig. 2b and c), while cell types A and C cooperatively up-regulated HVG Cluster 3 (Fig. 2b and c). Three cell types, A, B and C, exhibited up-regulation of HVG Cluster 4, whose degree of cell–cell communications was relatively low (Fig. 2c).

CCPLS: cell–cell communication analysis by PLS regression modeling
Recall the effect of neighboring cell types (Arnol et al., 2019; Dries et al., 2021b; Hu et al., 2021a; Rao et al., 2021; Svensson et al., 2018; Tanevski et al., 2022; Velten et al., 2022; Zhu et al., 2021) (Supplementary Table S1).

Next, we examined the performance limits of CCPLS by changing $w_{\text{max}}$ and $\alpha$, the parameters for adjusting the degree of the cell–cell communications and Gaussian noise, respectively (Supplementary Fig. S1a). According to the same procedures shown in Figure 2, we calculated the ten indexes (Supplementary Fig. S1b). In each $w_{\text{max}}$, these 10 indexes decreased when $\alpha$ increased. These 10 indexes were greater than 0.97 for conditions 1–5 and 7, which indicated the estimations were successful. In Conditions 6 and 8, these ten indexes were relatively lower and moderate. The estimation failed under only Condition 9, for which the eight indexes were less than 0.02 (Supplementary Fig. S1b).

We further investigated the difference between the failed and successful conditions by calculating the index of variance proportion (VP) as the ratio of the variance derived from the regression model term not but the Gaussian noise term. In each $w_{\text{max}}$, VP decreased when $\alpha$ increased, which indicated that the higher $\alpha$ conditions were more severe, such that the variance associated with cell–cell communications was more difficult to distinguish from noise (Supplementary Fig. S1c). The VP of the failed conditions was less than 0.04, while the VP of the successful conditions were greater than 0.61. The estimation did not work well under excessively severe VP conditions, while CCPLS estimated the prepared coefficients and the predefined HVG clusters with high performance in the relatively higher VP cases. Note that CCPLS did not estimate any HVGs and cell–cell communications from randomly generated data (data not shown).

We also generated another simulated dataset by replacing Gaussian noise $\epsilon_{i,j}$ with noise derived from gamma distribution $\epsilon_{i,j}'$. Table 1. Coefficients $w_{i,j}$ in the simulation experiments

| Neighboring cell type | HVG cluster | HVG cluster | HVG cluster | HVG cluster |
|-----------------------|-------------|-------------|-------------|-------------|
| Cell type             | 1           | 2           | 3           | 4           |
| A                     | $w_{\text{max}}$ | 0           | 0           | 0           |
| B                     | 0           | $w_{\text{max}}$ | 0           | 0           |
| C                     | $w_{\text{max}}$ | 0           | $w_{\text{max}}$ | 0           |
| D                     | 0           | 0           | 0           | 0           |

In Figure 2, we calculated the ten indexes (Supplementary Fig. S1b). The color of each edge indicates the average coefficients for each combination of HVG clusters and neighboring cell types. (d) Performance indexes and comparison with Giotto findICG. The calculated indexes are as follows: adjusted rand index, Pearson correlation coefficient, precision and recall of each HVG cluster. Red and green indicate CCPLS and Giotto findICG results, respectively (A color version of this figure appears in the online version of this article).
(see Section 2.3.1) and evaluated the performance of CCPLS on the Gamma-noise simulated dataset. The result showed similar performance and tendency with the cases by using Gaussian noise (Supplementary Fig. S2).

These simulation experiments showed that CCPLS yielded an accurate estimation of the MIMO system of cell-cell communications.

### 3.2 Application to seqFISH+ real dataset reveals biologically consistent insights associated with oligodendrocyte differentiation

To demonstrate CCPLS with a real dataset, we applied CCPLS to the seqFISH+ real dataset (Eng et al., 2019; Dries et al., 2021b) (Fig. 3a). Procedures were as described in Section 2.3.2. Before we applied CCPLS, we examined the overlap of the extracted HVGs among all 12 of the cell types in the seqFISH+ real dataset (Fig. 3b). The top 10 GO terms are indicated. (Supplementary Fig. S2).

To validate the neighboring cell-type effects detected by CCPLS, we plotted the positions of the cells in the 2D space and showed the expression level of Mag, which is a gene annotated with the GO term ‘glial cell differentiation’ (Supplementary Fig. S7). This visualization showed that the OPCs near astrocytes, Olig and OPCs showed high expression of Mag genes. The other genes in HVG Clusters 1 and 2 also showed similar spatial expression patterns in OPCs (Supplementary Fig. S7), possibly because the HVGs might be over-clustered with the minimum number of clusters set to 2 in Step (v). Moreover, we visualized the expression of the Clusters 1 and 2 genes of each OPC against the nearest distance to astrocytes, olig and OPCs (Supplementary Fig. S8). We found that the cells expressing these genes generally have shorter nearest distance to astrocytes,

![Fig. 3. Application to the seqFISH+ real dataset.](image)

(a) Schematic illustration of the seqFISH+ dataset. (b) Overlap of highly variable genes (HVGs) between cell types. Rows and columns both correspond to cell types. The color of the heat map indicates the ratio of the overlapping HVGs. Neuron: inhibitory neuron, endothelial: endothelial cells, eNeuron: excitatory neuron, mural: mural cells, Olig: oligodendrocyte. (c) Heat map of oligodendrocyte precursor cells (OPCs) generated by CCPLS. Rows correspond to neighboring cell types. Columns correspond to HVGs. The color of the heat map indicates the coefficient. (d) Bipartite graph of OPCs generated by CCPLS. The width of the edge indicates the averaged coefficients for each combination of HVG clusters and neighboring cell types. (e) Gene ontology (GO) enrichment of HVG Clusters 1 and 2 for OPCs. The top 10 GO terms are indicated. (f) Schematic illustration of the obtained biological insights on OPCs

In addition, we performed GO enrichment analysis to investigate associated biological insights. The GO enrichment analysis was performed on the mixed gene list of HVG Clusters 1 and 2, as HVG Clusters 1 and 2 have the same contributor cell types. The GO enrichment of HVG Clusters 1 and 2 in OPCs indicated the 25 enriched GO terms (Supplementary Fig. S6). The top 10 GO terms are indicated in Figure 3c. GO terms related to differentiation were enriched, such as ‘glial cell differentiation,’ indicating that differentiation of OPG was induced by communicating with astrocytes, Olig, and OPCs itself (Fig. 3f). This visualization showed that the OPCs near astrocytes, Olig and OPCs showed high expression of Mag genes. The other genes in HVG Clusters 1 and 2 also showed similar spatial expression patterns in OPCs (Supplementary Fig. S7), possibly because the HVGs might be over-clustered with the minimum number of clusters set to 2 in Step (v). Moreover, we visualized the expression of the Clusters 1 and 2 genes of each OPC against the nearest distance to astrocytes, olig and OPCs (Supplementary Fig. S8). We found that the cells expressing these genes generally have shorter nearest distance to astrocytes,
Fig. 4. Application to the Seq-Scope real dataset. (a) Schematic illustration of the Seq-Scope dataset. (b) Overlap of highly variable genes (HVGs) between cell types. Rows and columns both correspond to cell types. The color of the heat map indicates the ratio of the overlapping HVGs. (c) Heat map of the immature B cell generated by CCPLS. Rows correspond to neighboring cell types. Columns correspond to HVGs. The color of the heat map indicates the coefficient. (d) Bipartite graph of the immature B cell generated by CCPLS. The width of each edge indicates the average coefficients for each combination of HVG clusters and neighboring cell types. (e) Gene ontology (GO) enrichment of HVG Cluster 4 of the immature B cell. (f) Schematic illustration of the obtained biological insights on the immature B cell.

indicated the capability of CCPLS to extract biologically interpretable insights.

3.3 Application to Seq-Scope real dataset suggests epithelial cell development in immature B cell

For the further demonstration of CCPLS using a real dataset, we applied CCPLS to the Seq-Scope real dataset (Cho et al., 2021) (Fig. 4a). The procedures followed are as described in Section 2.3.3. Before we applied CCPLS, we examined the overlapping of the extracted HVGs between all nine cell types in the Seq-Scope real dataset (Fig. 4b). The mean value of the ratio of the overlap HVG was 0.31. The majority of the HVGs differed between each comparison, which indicated that the HVGs were uniquely characterized for each cell type.

To estimate cell–cell communications associated with these HVGs for each cell type, we applied CCPLS. We obtained the HVGs and the cell–cell communications for eight cell types out of all nine cell types (Fig. 4c and d and Supplementary Figs. S11 and S12). Focusing on the immature B cell, nine HVG clusters were estimated (Fig. 4c and d). We assigned the contributor cell types (Supplementary Fig. S13), which indicated that the immature B cell and the fibroblast cell types cooperatively down-regulated HVG Cluster 1 in the immature B cell (Fig. 4d). In HVG Clusters 2–9, olig and/or OPCs, further supporting the effect of neighboring cell types on gene expression found by our method.

These obtained insights were principally consistent with previous in vivo mouse experiments showing that astrocytes induce the differentiation of OPCs into oligodendrocytes (Nutma et al., 2020) (Fig. 3f). In contrast, when we applied Giotto findICG (Dries et al., 2021) on the seqFISH+ dataset, few overlaps were observed between the detected genes by Giotto findICG and CCPLS (Supplementary Fig. S9). Moreover, for the cell type pairs where the receiver cell type is OPCs and the neighboring cell type is astrocyte, Olig or OPCs itself, we found no GO enrichment in the genes detected by Giotto findICG (data not shown). Furthermore, these genes showed fewer overlap with the genes annotated with the GO term ‘glial cell differentiation’ than CCPLS (Supplementary Fig. S10). Considering that CCPLS can handle combinations of neighboring cell types, these results suggest that CCPLS detects cell–cell communications that cannot be detected by Giotto findICG.

The heat map, bipartite graph, contributor cell type assignment, and GO enrichment analysis results for all cell types are shown in the Supplementary Information (Supplementary Figs. S3–S6, respectively). The correspondence tables between gene and cluster number are shown in our github repository (https://github.com/bioinfo-tsukuba/CCPLS_paper/tree/main/Work/Gene_and_cluster_number/seqFISHplus). This application to the seqFISH+ real dataset indicated the capability of CCPLS to extract biologically interpretable insights.
there was one contributing cell type associated with up-regulation. The contributor cell types in Clusters 2–9 were immature B cell, DCSC, IgA B cell, macrophage, smooth muscle, stem, Paneth-like and IgG B cell, respectively.

In addition, we performed GO enrichment analysis to investigate potential biological insights. The GO enrichment of Cluster 4 for the immature B cell indicated, among the eight enriched GO terms, ‘epithelial cell development’ was the most enriched term (Fig. 4e).

We further plotted the positions of the cells in the 2D space and visualized the expression level of Gpx1 gene, a gene annotated with the GO ‘epithelial cell development’, in B cell-Immature (Supplementary Fig. S14), showing that the B cell-Immature cells on the boundary between B cell-Immature and B cell-IgA showed high expression of Gpx1 genes. The gradual change in expression suggests the involvement of paracrine signals rather than direct physical contact with adjoining cells (Armingol et al., 2020; Longo et al., 2021).

These results suggested that epithelial cell development of the immature B cell occurred through communicating with IgA B cells (Fig. 4f), which have not been reported previously to the best of our knowledge (Goto, 2019) and would be deserving of further investigation. In contrast, when we applied Giotto findICG (Dries et al., 2021b) on the Seq-Scope dataset, less than 30% of genes were overlapped on average (Supplementary Fig. S15). For the cell type pairs where the receiver cell type is immature B cell and the neighboring cell type is IgA B cell, we found no GO enrichment in genes detected by Giotto findICG (data not shown). Furthermore, we examined the number of overlap between genes of GO ‘epithelial cell development’, genes detected by CCPLS, and genes detected by Giotto findICG in those cell type pairs (Supplementary Fig. S16), revealing the genes annotated with the GO term ‘epithelial cell development’ were detected by only CCPLS (15 of 15 genes).

The heat map, bipartite graph, contributor cell type assignment and GO enrichment analysis results for all the cell types are shown in the Supplementary Information, respectively (Supplementary Figs S11–S13 and S17). The correspondence tables between gene and cluster number are shown in our github repository (https://github.com/bioinfo-tsukuba/CCPLS_paper/tree/main/Work/Gene_and_cluster_number/Seq-Scope). The application to the Seq-Scope real dataset demonstrates the capability of CCPLS to extract biologically interpretable insights.

4 Discussion

In this study, we propose CCPLS, which is a statistical framework for identifying the MIMO system of cell–cell communications, i.e. gene expression regulation by multiple neighboring cell types, based on the spatial transcriptome data at a single-cell resolution. CCPLS estimates regulation on cell-to-cell expression variation of HVGs via cell–cell communications not limited to ligand–receptor pairs. The core of CCPLS is PLS regression modeling performed by setting the HVGs expression values as the responsive variables and the neighboring cell-type scores as the explanatory variables for each cell type. After the statistical evaluation, CCPLS performs HVG clustering, which is reported with coefficients as the direction and degree of regulation in the cell–cell communication. Evaluation using simulated data showed accurate estimation by CCPLS. The application of CCPLS to the two real spatial transcriptome datasets demonstrated the capability to extract biologically interpretable insights.

CCPLS differs from the existing method Giotto findICG in that CCPLS quantitatively evaluates cell–cell communications as a MIMO system by including cooperation of neighboring cell types, which may have revealed the biological insights derived from the applications to the two real datasets. In the simulation experiments, CCPLS outperformed Giotto findICG, especially with regard to HVG Cluster 1, which is a multiple-input case.

The application of CCPLS to the scqFISH+ real dataset showed that the differentiation in the OPCs occurred through communicating with astrocytes, Olig and OPCs itself, which was principally consistent with previous in vivo mouse experiments and not identified by Giotto findICG (Dries et al., 2021b; Nutma et al., 2020). The application to the Seq-Scope real dataset suggested that epithelial cell development is induced in the immature B cell by communicating with the IgG B cells, which has not been reported previously to the best of our knowledge (Goto, 2019). Accordingly, further experimental follow-up studies are merited. Other existing methods, which do not focus on the effect of neighboring cell types, may have missed these findings (Arnol et al., 2019; Dries et al., 2021b; Hu et al., 2021a; Svensson et al., 2018; Tanevski et al., 2022; Velten et al., 2022; Zhu et al., 2021). As another characteristic, CCPLS focuses on HVGs not limited to ligand–receptor pairs, which may have also enabled it to reveal the biological insights noted above.

The exploration of cell–cell communications in combination with spatial transcriptome data is still largely incomplete. CCPLS would be effective for exploring drug targets as in the case of the PD1 pathway, which led to a breakthrough drug discovery (Sharpe and Pauken, 2018). For example, the treatment of cold tumors, which are composed of tumor cells under a severe microenvironment with few neighboring T cells, is still considered to be a particular challenge (Haanen, 2017). The severe tumor progression possibly derived from the cold tumor-specific combinations of HVGs and neighboring cell types, which thus may be candidates for drug targeting. Moreover, cell–cell communications involve various molecular mechanisms (Armingol et al., 2020; Longo et al., 2021). For example, recent studies reported the effect of adjoining cell types on gene expression in developing mouse embryos based on RNA sequencing of physically interacting cells and single cells (Kim et al., 2021) and in the mouse embryo and hippocampus based on the comparison of heterotypic and homotypic spots in spot-based spatial transcriptome data (Kim et al., 2022). CCPLS would be suitable in cases where cell arrangement causes gene expression changes.

The limitations and perspectives in this study are as follows. The first is the model assumption of CCPLS. In this study, we assumed that HVGs can be explained by a linear combination of neighboring cell-type scores. Several studies exemplify changes in gene expression are associated with or induced by the placement of surrounding cell types in multicellular systems (Colombo and Cattaneo, 2021; Haanen, 2017; Hui and Bhatia, 2007). Such a method of detecting the effect of surrounding cell types on gene expression could be biologically useful. For CCPLS, we decided to assume a linear combination of neighboring cell-type scores is a simple model, which is easy to interpret and computationally tractable. However, it is possible that the refinement of the mathematical model of the effect of cell type on gene expression (e.g. nonlinear relationships among cell types) is an important issue to be addressed in the future.

The second is the potential modifications of CCPLS. Although CCPLS employed PLS regression, the other sparse regression can be candidates such as lasso or ridge regression, whose implementations and evaluations are an intended focus of our future work (Tibshirani, 1996). CCPLS employed a Gaussian kernel for calculating the neighboring cell-type score similar to the previous studies (Arnol et al., 2019; Dang et al., 2020). However, the other kernels such as Matern kernel or exponential kernel can be possible, whose implementations and evaluations are also an intended focus of our future work (Pustokhina et al., 2021). CCPLS selected dist0 as the minimum value of the Euclidean distance of all the combinations of two cells, while the other selection procedures may work. The selection of parameter dist0 is also one of our future works. Besides, there is a room for improvement in the choice of other hyperparameters (e.g. the numbers of clusters in the HVG clustering step).

The third is the incorporation of cell–cell communications other than the neighboring cell effects. CCPLS explicitly handles combinations of neighboring cell types as advantages compared to the existing methods (Arnol et al., 2019; Dries et al., 2021b; Hu et al., 2021a; Rao et al., 2021; Svensson et al., 2018; Tanevski et al., 2022; Velten et al., 2022; Zhu et al., 2021) (Table S1), while CCPLS neither extracts non-linear relationships nor estimates the cell–cell communications derived from factors beyond the neighboring cell-type score, such as ligand–receptor pairs and cell–cell communications (Dries et al., 2021b; Nutma et al., 2020; Goodman, 2018; Longo et al., 2021; Palla et al., 2022; Rao et al., 2021; Sapir and Tzill, 2017). Handling molecular pathways
involved in the MIMO system is an intended focus of our future work.

The fourth is the extension of CCPLS to other types of spatially resolved omics data. CCPLS does not receive as input spatial transcriptome data with a multiple-cell resolution as is characteristic of 10× Genomics Visium and Slide-seq datasets (Rodrigues et al., 2019; Stickels et al., 2021; Asp et al., 2020). Expansions of CCPLS to address these limitations are an intended focus of our future work. In addition, the responsive expression values can be replaced by the other omics data such as spatial metabolome or proteome data, which is also an intended focus of our future work (Alexandrov, 2020; Bhatia et al., 2021; Drying-Andersen et al., 2020; Geier et al., 2020; Liu et al., 2020; Seydel, 2021).

Currently, collaborative consortia are accumulating large-scale spatial transcriptome data (BRAIN Initiative Cell Census Network (BICCN), 2021; Regev et al., 2017; Zhang et al., 2021), and CCPLS can be readily applied to these large-scale datasets. CCPLS is provided as a package in the R statistical computing environment, and its calculation time was less than 10 min for each demonstration presented in this study. In summary, CCPLS provides novel insights into regulation of cell-to-cell expression variability of HVGs via cell–cell communications.

Acknowledgements

We thank Drs Wataru Iwasaki, Hirotaka Matsumoto, Tsukasa Fukanaga and Kentaro Kawata for critical reading of this manuscript. The computational analysis of this work was performed in part with the support of the supercomputer system of the National Institute of Genetics (NIG), Research Organization of Information and Systems (ROIS). We thank kango-roo (www.kango-roo.com) for providing illustrations.

Funding

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI [20K19915, 17H06300, 21H03124, 19H03656, and 19K20394].

Conflict of Interest: none declared.

Data availability

The R package is available at https://github.com/bioinfo-tsukuba/CCPLS. The data are available at https://github.com/bioinfo-tsukuba/CCPLS_paper.

References

Abdi,H. (2010) Partial least squares regression and projection on latent structure regression (PLS regression). WIREs Comp. Stat., 2, 97–106.

Akimoto,Y. et al. (2013) The extraction of simple relationships in growth factor-specific multiple-input and multiple-output systems in cell-fate decisions by backward elimination PLS regression. PLoS One, 8, e72780.

Alexandrov,T. (2020) Spatial metabolomics and imaging mass spectrometry in the age of artificial intelligence. Annu. Rev. Biomed. Data Sci., 3, 61–87.

Armingol,E. et al. (2020) Deciphering cell–cell interactions and communication from gene expression. Nat. Rev. Genet., 22, 71–88.

Arnold,D. et al. (2019) Modeling cell–cell interactions from spatial molecular data with spatial variance component analysis. Cell Rep., 29, 202–211.e6.

Asp,M. et al. (2020) Spatially resolved transcriptomes: next generation tools for tissue exploration. BioSays, 42, e1900221.

Ben-Moshe,S. and Irzovits,S. (2019) Spatial heterogeneity in the mammalian liver. Nat. Rev. Gastroenterol. Hepatol., 16, 395–410.

Bengyo,Y. and Grandvalet,Y. (2004) No unbiased estimator of the variance of k-fold cross-validation, J. Mach. Learn. Res., 5, 1089–1105.

Benjamini,Y. and Hochberg,Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc., 57, 289–300.

Bhatia,H.S. et al. (2021). Proteomics of spatially identified tissues in whole organs. https://doi.org/10.1101/2021.11.02.466753.

BRAIN Initiative Cell Census Network (BICCN) (2021) A multimodal cell census and atlas of the mammalian primary motor cortex. Nature, 598, 86–102.

Cho,C.-S. et al. (2021) Microscopic examination of spatial transcriptome using Seq-Scope. Cell, 184, 3559–3572.e22.

Colombo,E. and Cattaneo,M.G. (2021) Multicellular 3D models to study Tumour-Stroma interactions. Int. J. Mol. Sci., 22, 1633.

Dang,Y. et al. (2020) Cellular dialogues: cell–cell communication through diffusible molecules yields dynamic spatial patterns. Cell Syst., 10, 82–98.e7.

Dries,R. et al. (2021a) Advances in spatial transcriptomic data analysis. Genome Res., 31, 1706–1718.

Dries,R. et al. (2021b) Giotto: a toolbox for integrative analysis and visualization of spatial expression data. Genome Biol., 22, 78.

Drying-Andersen,B. et al. (2020) Spatially and cell-type resolved quantitative proteomic atlas of healthy human skin. Nat. Commun., 11, 5587.

Eng,C.-H.L. et al. (2019) Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH. Nature, 568, 235–239.

Fechner,S. and Goodman,M.B. (2018) Synaptic communication upon gentle touch. Neuron, 100, 1272–1274.

Geier,B. et al. (2020) Spatial metabolomics of in situ host-microbe interactions at the micrometre scale. Nat. Microbiol., 5, 498–510.

Goto,Y. (2019) Epithelial cells as a transmitter of signals from commensal bacteria and host immune cells. Front. Immunol., 10, 2057.

Haanen,J.B.A.G. (2017) Converting cold into hot tumors by combining immunotherapies. Cell, 170, 1055–1056.

Hafemeister,C. and Satija,R. (2019) Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. Genome Biol., 20, 296.

Hsingulsdot,A. (1988) PLS regression methods. J. Chemometrics, 2, 211–228.

Hou,R. et al. (2020) Predicting cell–cell communication networks using NATMI. Nat. Commun., 11, 5011.

Hu,j. et al. (2021a) SpaGCN: integrating gene expression, spatial location and histology to identify spatially distributed and spatially variable genes by graph convolutional network. Nat. Methods, 18, 1342–1351.

Hu,j. et al. (2021b) Statistical and machine learning methods for spatially resolved transcriptomics with histology. Comput. Struct. Biotechnol. J., 19, 3829–3841.

Hu,E. and Bhatia,S.N. (2007) Micromechanical control of cell–cell interactions. Proc. Natl. Acad. Sci. USA, 104, 5722–5726.

Janes,K.A. et al. (2005) A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis. Science, 310, 1646–1653.

Kim,H. et al. (2022). Detecting cell contact-dependent gene expression from spatial transcriptomics data. bioRxiv.

Kim,J. et al. (2021). Neighbor-specific gene expression revealed from physically interacting cells during mouse embryonic development. bioRxiv.

Liu,Y. et al. (2020) High-spatial-resolution multi-omics sequencing via deterministic barcoding in tissue. Cell, 183, 1665–1681.e18.

Longo,S.K. et al. (2021) Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. Nat. Rev. Genet., 22, 627–644.

Marx,V. (2021) Method of the year: spatially resolved transcriptomics. Nat. Methods, 18, 9–14.

Nagai,S. et al. (2021) CrossTalkRe: analysis and visualisation of ligand receptor networks. Bioinformatics, 37, 4263–4265.

Nagasawa,a. et al. (2021) Single-cell and spatial analyses of cancer cells: towards elucidating the molecular mechanisms of clonal evolution and drug resistance acquisition. Inflamm. Regen., 41, 22.

Nutma,E. et al. (2020) Astrocyte and oligodendrogliocyte cross-talk in the central nervous system. Cells, 9, 600.

Oshlack,A. and Wakefield,M.J. (2009) Transcript length bias in RNA-seq data confounds systems biology. Biol. Direct., 4, 1–10.

Palla,G. et al. (2022) Spatial components of molecular tissue biology. Nat. Biotechnol., 40, 308–318.

Pelkmans,L. (2012) Cell biology using cell-to-cell variability—a new era in molecular biology. Science, 336, 425–426.

Pustokhina,I. et al. (2021) Developing a robust model based on the gaussian process regression approach to predict biodiesel properties. J. Chemometrics, 35, 235–246.

Qiu,2. et al. (2021) Giotto: a toolbox for integrative analysis and visualization of spatial expression data. Genome Res., 31, 1706–1718.

Qiu,2. et al. (2020) Spatial metabolomics of in situ host-microbe interactions at the micrometre scale. Nat. Microbiol., 5, 498–510.

Qiu,2. et al. (2021) Spatial metabolomics of in situ host-microbe interactions at the micrometre scale. Nat. Microbiol., 5, 498–510.

Rao,A. et al. (2021). Exploring tissue architecture using spatial transcriptomics. Nature, 596, 211–220.

Regen,A. et al. (2017) Human Cell Atlas Meeting Participants. (2017) The human cell atlas. Elife, 6, e27041.

Rodrigues,S.G. et al. (2019) Slide-seq: a scalable technology for measuring genome-wide expression at high spatial resolution. Science, 363, 1463–1467.

Sapir,L. and Tzlil,S. (2017) Talking over the extracellular matrix: how do cells communicate mechanistically? Semin. Cell Dev. Biol., 71, 99–105.

Sarria,R. et al. (2015) Spatial reconstruction of single-cell gene expression data. Nat. Biotechnol., 33, 495–502.
Seydel, C. (2021) Single-cell metabolomics hits its stride. *Nat. Methods.*, 18, 1452–1456.
Sharpe, A.H. and Pauken, K.E. (2018) The diverse functions of the PD1 inhibitory pathway. *Nat. Rev. Immunol.*, 18, 153–167.
Shimizu, K. et al. (2020) PD-1 imposes qualitative control of cellular transcriptomes in response to T cell activation. *Mol. Cell*, 77, 937–950.e6.
Snijder, B. and Pelkmans, L. (2011) Origins of regulated cell-to-cell variability. *Nat. Rev. Mol. Cell Biol.*, 12, 119–125.
Stickels, R.R. et al. (2021) Highly sensitive spatial transcriptomics at near-cellular resolution with slide-seqV2. *Nat. Biotechnol.*, 39, 313–319.
Stuart, T. et al. (2019) Comprehensive integration of single-cell data. *Cell*, 177, 1888–1902.e21.
Vesanto, J. et al. (2018) SpatialDE: identification of spatially variable genes. *Nat. Methods.*, 15, 343–346.
Tanevski, J. et al. (2022) Explainable multiview framework for dissecting spatial relationships from highly multiplexed data. *Genome Biol.*, 23, 97.
Tibshirani, R. (1996) Regression shrinkage and selection via the lasso. *J. R. Stat. Soc.*, 58, 267–288.
Van Verk, M.C. et al. (2013) RNA-seq: revelation of the messengers. *Trends Plant Sci.*, 18, 175–179.
Velti, B. et al. (2022) Identifying temporal and spatial patterns of variation from multimodal data using MEFISTO. *Nat. Methods*, 19, 179–186.
Yamamoto, H. et al. (2014) Statistical hypothesis testing of factor loading in principal component analysis and its application to metabolite set enrichment analysis. *BMC Bioinformatics*, 15, 51.
Yuan, C. and Yang, H. (2019) Research on K-Value selection method of K-Means clustering algorithm. *J.*, 2, 226–235.
Zhang, M. et al. (2021) Spatially resolved cell atlas of the mouse primary motor cortex by MERFISH. *Nature*, 598, 137–143.
Zhu, J. et al. (2021) SPARK-X: non-parametric modeling enables scalable and robust detection of spatial expression patterns for large spatial transcriptomic studies. *Genome Biol.*, 22, 184.