UCLA Statistics

Categorization of 31 computational methods to detect spatially variable genes (SVGs) from spatial transcriptomics data

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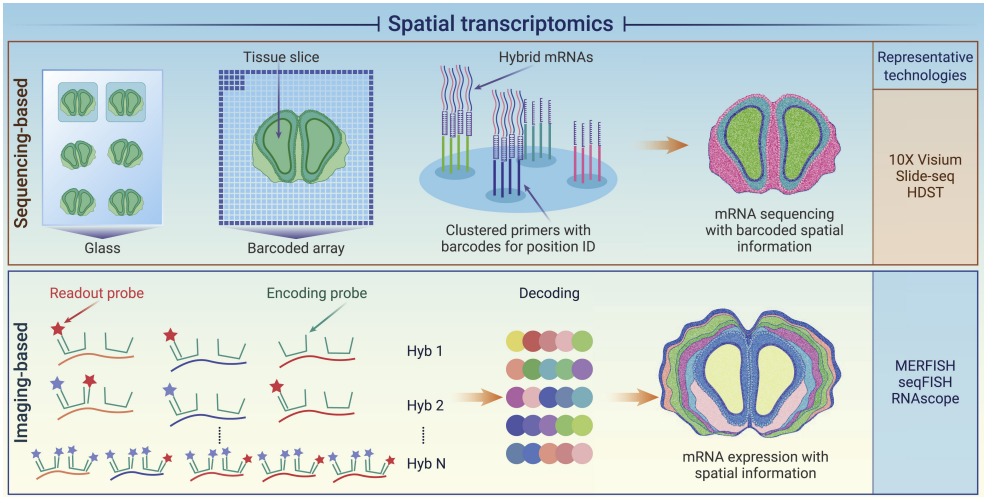
joint work with Guan'ao Yan and Shuo Harper Hua

Spatially Transcriptomics Technologies



metacellular
$\sim 10^4$ genes

subcellular
$\sim 10^2$ genes

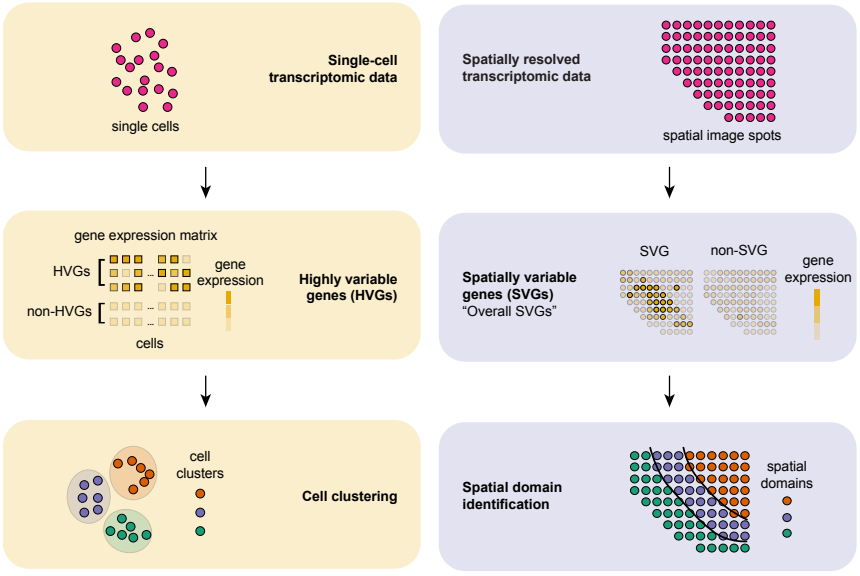
Lu Wen, Guoqiang Li, Tao Huang, et al., Single-cell technologies: From research to application, The Innovation, Volume 3, Issue 6, 2022, 100342.
https://doi.org/10.1016/j.xinn.2022.100342

Highly Variable Genes (HVGs) vs. Spatially Variable Genes (SVGs)

Informative features to screen for before linear dimension reduction and Euclidean distance calculation

- **HVG detection**
  - Used in single-cell transcriptomics data analysis
  - Identifies genes with high expression variability across single cells
  - Helps in clustering cells and identifying subpopulations
- **SVG detection**
  - Used in spatial transcriptomics data analysis
  - Identifies genes with high expression variability across spatial locations
  - Helps in identifying spatial patterns and regions with distinct molecular signatures

Highly Variable Genes (HVGs) vs. Spatially Variable Genes (SVGs)



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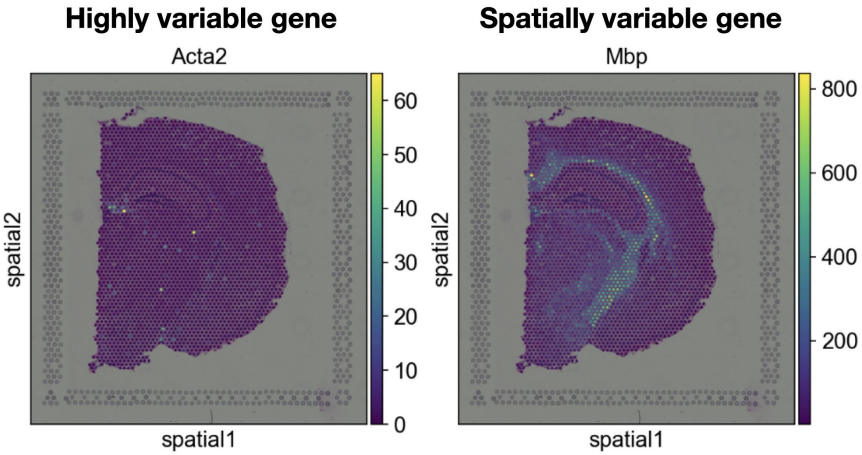
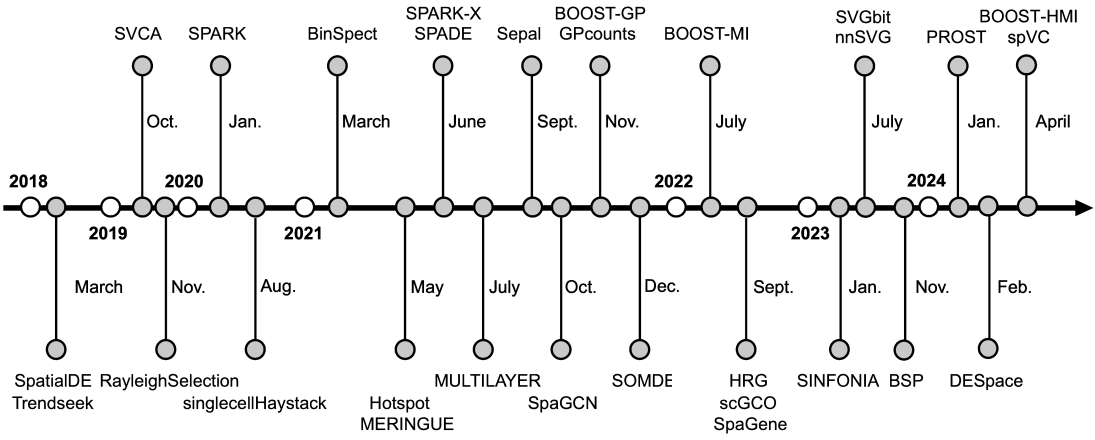


https://www.sc-best-practices.org/_images/svg.jpeg

31 SVG Detection Methods



There is no consensus in SVG definitions

Existing Review and Benchmark Studies

Review

- Adhikari et al., *Computational and Structural Biotechnology Journal*, 2024 (19 methods)

Benchmark studies

- Charitakis et al., *Genome Biology*, 2023 (6 methods)
- Chen et al., *Genome Biology*, 2024 (7 methods)
- Li et al., *bioRxiv*, 2023 (14 methods)

Categorization of SVG definitions is not the focus

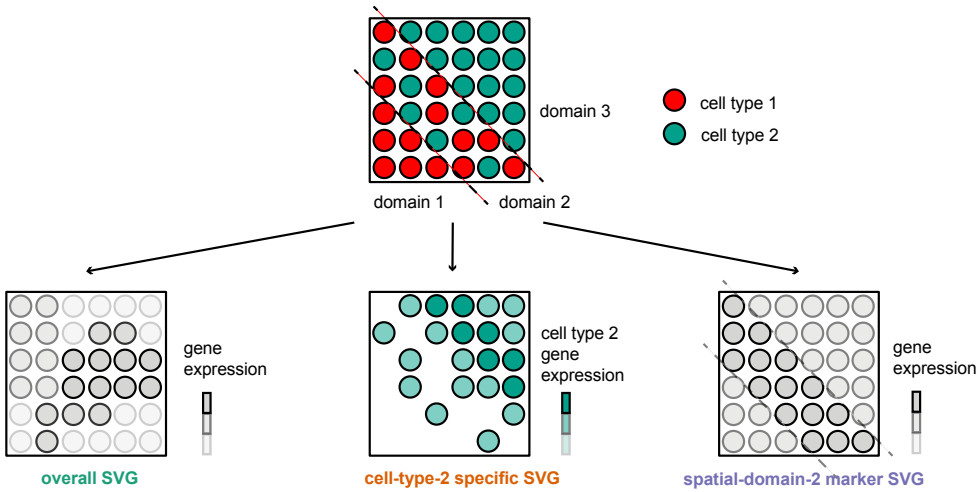
Proposal: Three Categories of SVGs

1. **Overall SVGs**:
   - Informative genes for downstream analysis (e.g., spatial domain identification)
2. **Cell-type-specific SVGs**:
   - Revealing spatial variation within a cell type $\Longrightarrow$ cell subpopulations or states
3. **Spatial-domain-marker SVGs**:
   - Marker genes to annotate and interpret spatial domains already detected

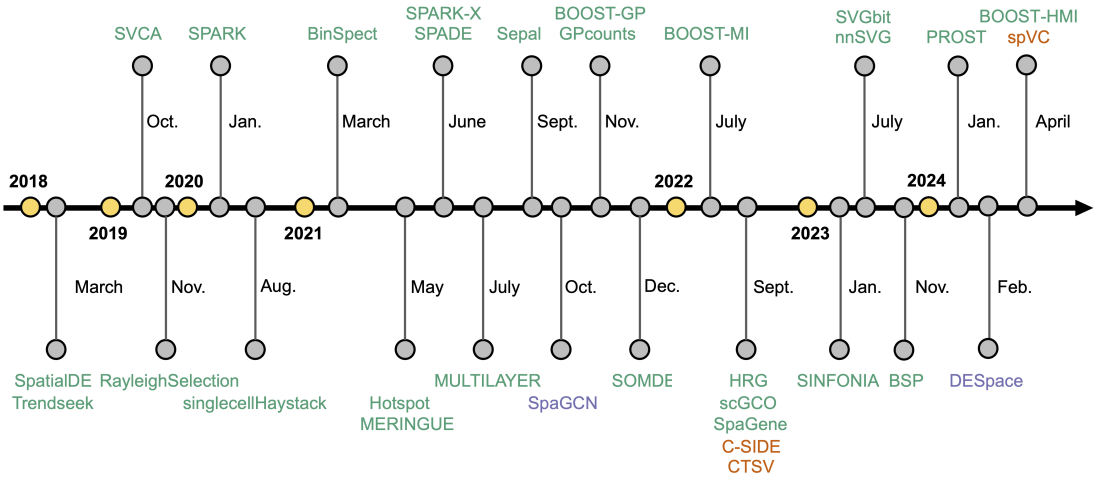
Relationships among the three categories depends on

- Detection methods' null and alternative hypotheses

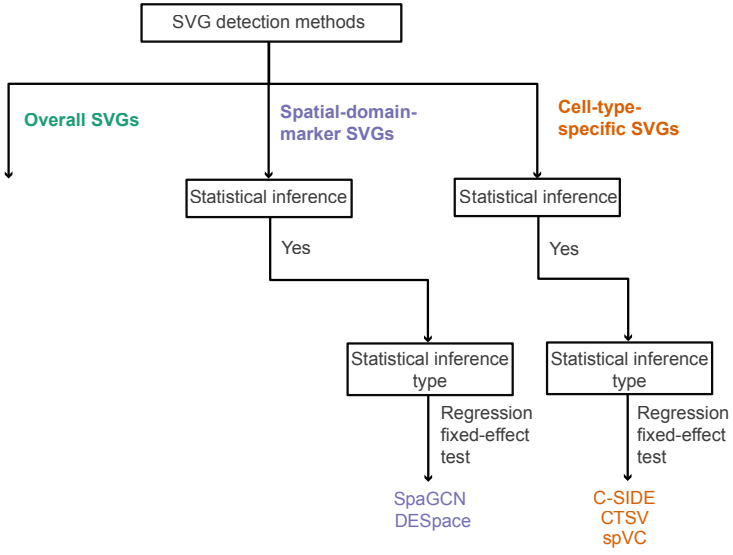
SVG Categories: Overall, Cell-type-specific, and Spatial-domain-marker SVGs



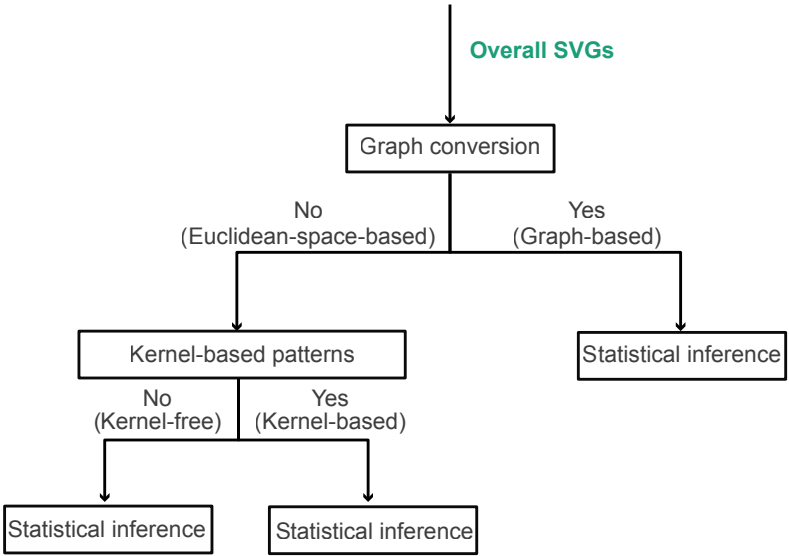
Categorization of 31 SVG Detection Methods



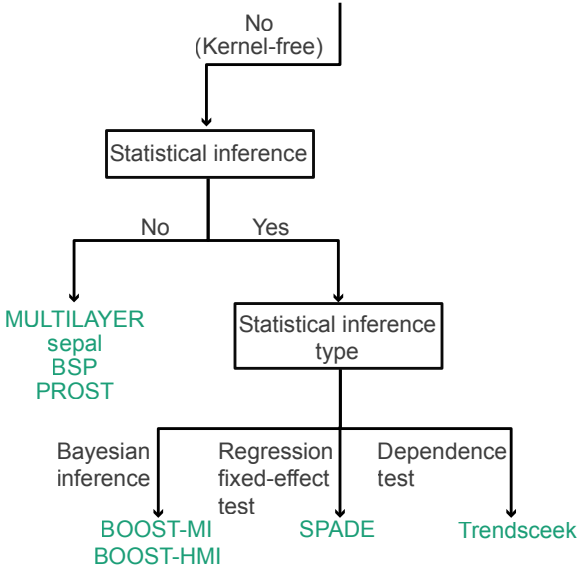
Hierarchy of 31 SVG Detection Methods (Part 1: Three Categories)



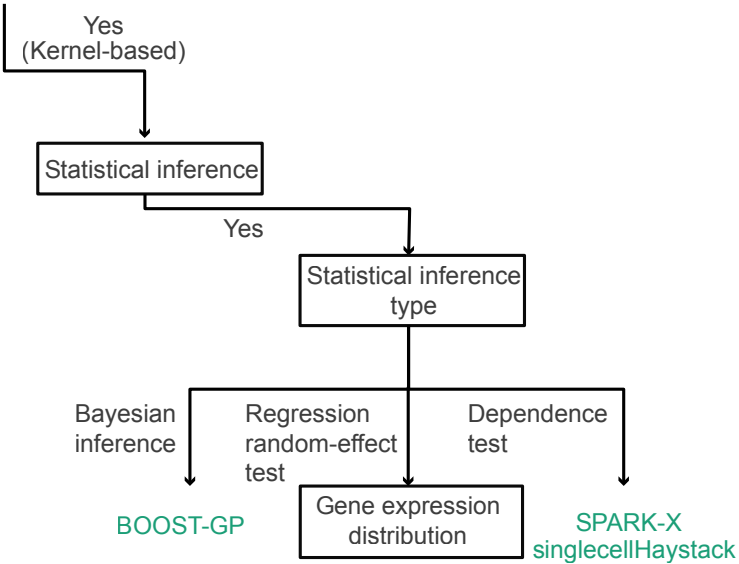
Hierarchy of 31 SVG Detection Methods (Part 2: Overall SVGs)



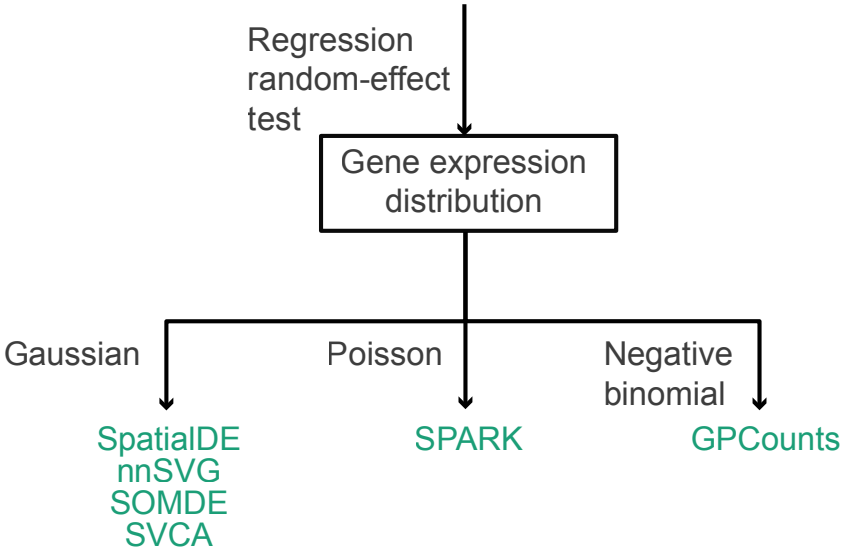
Hierarchy of 31 SVG Detection Methods (Part 3: Kernel-free Methods)



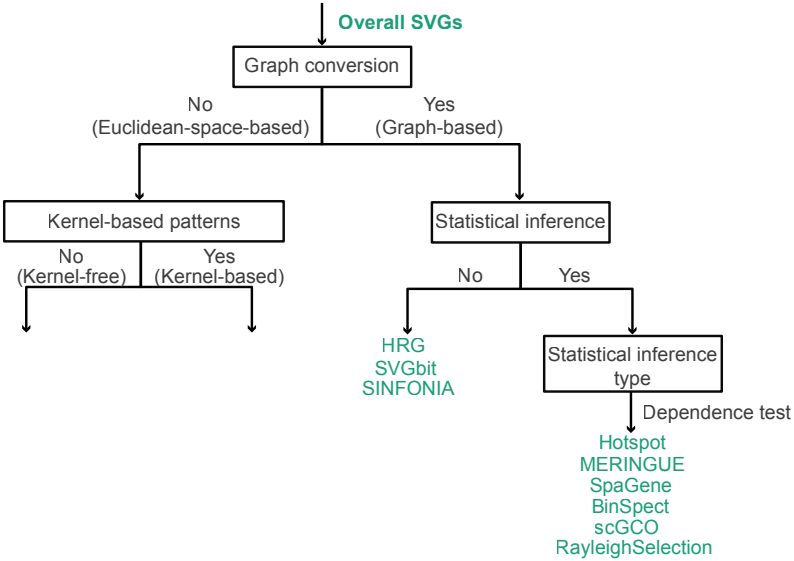
Hierarchy of 31 SVG Detection Methods (Part 4: Kernel-based Methods)



Hierarchy of 31 SVG Detection Methods (Part 5: Kernel-based Methods)



Hierarchy of 31 SVG Detection Methods (Part 6: Graph-based Methods)



Notations for SVG Detection (Per Gene)

For a given gene with expression levels measured at n spatial spots

Observed variables at spot $i = 1, \ldots, n$

- Gene expression level
  - $y_i \in \mathbb{R}$
  - $Y_i \in \mathbb{R}$: random variable notation
- 2D spatial location
  - $\mathbf{s}_i = (s_{i1}, s_{i2})^\top \in \mathbb{R}^2$
  - $\mathbf{s} = [\mathbf{s}_1, \ldots, \mathbf{s}_n]^\top \in \mathbb{R}^{n \times 2}$: spatial location matrix

Inferred variables at spot $i = 1, \ldots, n$

- Spatial-domain indicator vector
  - $\mathbf{d}_i = (d_{i1}, \ldots, d_{iL})^\top \in \{0, 1\}^L$, with $\sum_{l=1}^{L} d_{il} = 1$
- Cell-type proportion vector
  - $\mathbf{c}_i = (c_{i1}, \ldots, c_{iK})^\top \in [0, 1]^K$, with $\sum_{k=1}^{K} c_{ik} = 1$

Hypothesis Tests Used for SVG Detection

Among the 31 SVG detection methods, 21 use frequentist inference to detect SVGs:

- Define a test statistic
- Derive the test statistic's null distribution
- Convert the test statistic value to a p-value

Types of null hypotheses:

- **Dependence tests:** a gene's expression level is independent of spatial location
- **Regression-based tests:** spatial location has no "effect" on a gene's expression level
  - **Fixed-effect tests**
  - **Random-effect tests (variance component tests)**

Dependence Tests

Null hypothesis:

$$H_0 : Y \perp \mathbf{S}$$

Assume that $(y_1, \mathbf{s}_1), \ldots, (y_n, \mathbf{s}_n)$ are independently sampled from the distribution of $(Y, \mathbf{S})$

If H_0 is rejected, the gene is detected as an overall SVG

Nine methods adopt the dependence test formulation:

- **Conventional test statistics** (with theoretical null distribution):
  SPARK-X, Hotspot, MERINGUE, BinSpect, scGCO
- **Unconventional test statistics** (with permutation-based null distribution):
  Trendsceek, singlecellHaystack, RayleighSelection, SpaGene

SPARK-X (Zhu et al., Genome Biology, 2021)

SPARK-X: a non-parametric test that compares two $n \times n$ spot similarity matrices:

- Matrix 1 based on the gene's expression levels at the n spots
- Matrix 2 based on the kernel-transformed spatial locations of the n spots

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To detect diverse spatial patterns, SPARK-X transforms the spatial locations $\mathbf{s}_i = (s_{i1}, s_{i2})$, $i = 1, \ldots, n$, using two kernel-based functions:

- Gaussian transformation $s'_{il} = \exp\left(\frac{-s_{il}^2}{2\sigma_l^2}\right)$, $l = 1, 2$, to detect clustered or focal patterns
- Cosine transformation $s'_{il} = \cos\left(\frac{2\pi s_{il}}{\phi_l}\right)$, $l = 1, 2$, to detect periodic patterns

where σ_1, σ_2, ϕ_1, and ϕ_2 are tuning parameters

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Test statistic: Pearson correlation of the two matrices

Theoretical null: mixture chi-square distribution

singlecellHaystack (Vandenbon and Diez, Nature Communications, 2020)

singlecellHaystack: a unconventional test involves two pre-processing steps:

- Binarize the gene's expression levels at spots into **two states**: detected and undetected
- Divide the 2D Euclidean space into **grid points** as coarse spatial coordinates

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singlecellHaystack uses a 2D independent Gaussian kernel, assuming independence of the two dimensions of the Euclidean space, to define **three distributions** of grid points:

- A reference distribution based on all grid points
- A conditional distribution based on grid points in the detected state
- Another conditional distribution based on grid points in the undetected state

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Test statistic: sum of Kullback-Leibler divergences of the two conditional distributions from the reference distribution

Permutation null

Regression-based Tests

Two types: **fixed-effect tests** and **random-effect tests**

Linear mixed-effect model (LMM) for a given gene:

$$Y_i = \beta_0 + \mathbf{x}_i^\top \boldsymbol{\beta} + \mathbf{z}_i^\top \boldsymbol{\gamma} + \epsilon_i$$

- Y_i: a gene's expression level at spot i (response variable)
- β_0: (fixed) intercept
- $\mathbf{x}_i \in \mathbb{R}^p$: fixed-effect covariates of spot i
- $\boldsymbol{\beta} \in \mathbb{R}^p$: fixed effects

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- $\mathbf{x}_i \in \mathbb{R}^p$: fixed-effect covariates of spot i
- $\boldsymbol{\beta} \in \mathbb{R}^p$: fixed effects
- $\mathbf{z}_i \in \mathbb{R}^q$: random-effect covariates of spot i
- $\boldsymbol{\gamma} \in \mathbb{R}^q$: random effects with zero means $\mathbb{E}[\boldsymbol{\gamma}] = 0$ and covariance matrix

$$\mathrm{Cov}(\boldsymbol{\gamma}) \in \mathbb{R}^{q \times q}$$

- ϵ_i: independent random error at spot i with $\mathbb{E}[\epsilon_i] = 0$
- $\boldsymbol{\gamma} \perp \boldsymbol{\epsilon} = (\epsilon_1, \ldots, \epsilon_n)^\top$

Fixed-effect Tests

$$Y_i = \beta_0 + \mathbf{x}_i^\top \boldsymbol{\beta} + \mathbf{z}_i^\top \boldsymbol{\gamma} + \epsilon_i$$

Fixed-effect tests examine whether $\mathbf{x}_i$ contribute to $\mathbb{E}[Y_i]$

If $\mathbf{x}_i$ makes no contribution, then $\mathbb{E}[Y_i|\mathbf{x}_i] = \mathbb{E}[Y_i]$

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Null hypothesis

$$H_0 : \boldsymbol{\beta} = \mathbf{0}$$

implies $\mathbb{E}[Y_i|\mathbf{x}_i] = \mathbb{E}[Y_i]$, $i = 1, \ldots, n$

Random-effect Tests

$$Y_i = \beta_0 + \mathbf{x}_i^\top \boldsymbol{\beta} + \mathbf{z}_i^\top \boldsymbol{\gamma} + \epsilon_i$$

Random-effect tests examine whether $\mathbf{z}_i$ contribute to $\mathrm{Var}(Y_i)$:

$$\mathrm{Var}(Y_i) = \mathrm{Var}(\mathbb{E}[Y_i|\mathbf{z}_i]) + \mathbb{E}[\mathrm{Var}(Y_i|\mathbf{z}_i)] = \mathbf{z}_i^\top \mathrm{Cov}(\boldsymbol{\gamma})\mathbf{z}_i + \mathrm{Var}(\epsilon_i)$$

If $\mathbf{z}_i$ makes no contribution, then $\mathrm{Var}(\mathbb{E}[Y_i|\mathbf{z}_i]) = 0$

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Generalization of LMM

Assume $\epsilon_i \overset{\text{iid}}{\sim} N(0, \sigma^2)$ and $\boldsymbol{\gamma} \perp \boldsymbol{\epsilon} = (\epsilon_1, \ldots, \epsilon_n)^\top$

$$Y_i = \beta_0 + \mathbf{x}_i^\top \boldsymbol{\beta} + \mathbf{z}_i^\top \boldsymbol{\gamma} + \epsilon_i \quad \Longleftrightarrow \quad \begin{cases} Y_i \mid \mu_i \overset{\text{ind}}{\sim} N(\mu_i, \sigma^2) \\ \mu_i = \beta_0 + \mathbf{x}_i^\top \boldsymbol{\beta} + \mathbf{z}_i^\top \boldsymbol{\gamma} \end{cases}$$

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Generalized LMM (GLMM): The distribution of Y_i can be non-Gaussian

$$\text{e.g.,} \quad \begin{cases} Y_i \mid \mu_i \overset{\text{ind}}{\sim} \text{Poisson}(\mu_i) \\ \log(\mu_i) = \beta_0 + \mathbf{x}_i^\top \boldsymbol{\beta} + \mathbf{z}_i^\top \boldsymbol{\gamma} \end{cases}$$

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Generalized non-parametric mixed-effect model:

The effects of $\mathbf{x}_i$ is modeled as non-parametric:

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Q: Is spatial location $\mathbf{s}_i$ modeled as $\mathbf{x}_i$ or $\mathbf{z}_i$?

Fixed-effect Tests for SVG Detection

Six methods use regression fixed-effect tests, covering all three SVG categories:

- **Overall SVGs:** SPADE
  - $\mathbf{x}_i$ includes $\mathbf{s}_i$
- **Cell-type-specific SVGs:** C-SIDE, CTSV, and spCV
  - $\mathbf{x}_i$ includes $\mathbf{s}_i$ and $\mathbf{c}_i$ (cell-type proportion vector)
- **Spatial-domain-marker SVGs:** SpaGCN and DESpace
  - $\mathbf{x}_i$ includes $\mathbf{s}_i$ and $\mathbf{d}_i$ (spatial-domain indicator vector)

SPADE (Bae et al., Nucleic Acids Research, 2021)

SPADE: linear-model fixed-effect test that detects **overall SVGs**:

$$\mu_i = \beta_0 + \mathbf{x}_i(\mathbf{s})^\top \boldsymbol{\beta}$$

- $\mathbf{x}_i(\mathbf{s})$: principal components of 512 features from a pre-trained convolutional neural network applied to the n spots' spatial locations $\mathbf{s}$ in an H&E image

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Null hypothesis:

$$H_0 : \boldsymbol{\beta} = \mathbf{0}$$

If H_0 is rejected, the gene is detected as an overall SVG

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Test: R package `limma`
(Smyth, G. K., 2005 $\Rightarrow$ Ritchie et al., *Nucleic Acids Research*, 2015)

spVC (Yu and Li, Genome Biology, 2024)

spVC: fixed-effect test that detects **cell-type-specific SVGs**

Assume

$$Y_i \mid \mu_i \overset{\text{ind}}{\sim} \text{Poisson}(\mu_i)$$

Two-step procedure:

1. A reduced model without interactive effects between $\mathbf{c}_i$ and $\mathbf{s}_i$:

$$\log(\mu_i) = \beta_0 + \sum_{k=1}^{K} c_{ik}\beta_k + f_0(\mathbf{s}_i)$$

It tests two null hypotheses:

- $H_0 : \boldsymbol{\beta} = (\beta_1, \ldots, \beta_K)^\top = \mathbf{0}$ using the likelihood ratio test
- $H_0 : f_0(\cdot) = 0$ using the Wald test

If both null hypotheses are rejected, it proceeds to the second step

2. A full model with interactive effects between $\mathbf{c}_i$ and $\mathbf{s}_i$:

$$\log(\mu_i) = \beta_0 + \sum_{k=1}^{K} c_{ik}\beta_k + f_0(\mathbf{s}_i) + \sum_{k=1}^{K} c_{ik} f_k(\mathbf{s}_i)$$

It tests if any of the interactive effects $f_1(\cdot), \ldots, f_K(\cdot)$ are zero using the likelihood ratio test

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$$\log(\mu_i) = \beta_0 + \sum_{k=1}^{K} c_{ik}\beta_k + f_0(\mathbf{s}_i) + \sum_{k=1}^{K} c_{ik} f_k(\mathbf{s}_i)$$

It tests if any of the interactive effects $f_1(\cdot), \ldots, f_K(\cdot)$ are zero using the likelihood ratio test

If

$$H_0 : f_k(\cdot) = 0$$

is rejected, the gene is detected as a SVG specific to cell type k

DESpace (Cai et al., Bioinformatics, 2024)

DESpace: fixed-effect test that detects **spatial-domain-marker SVGs**

Assume

$$Y_i \mid \mu_i \overset{\text{ind}}{\sim} \text{NegativeBinomial}(\mu_i, \phi)$$

$$\log(\mu_i) = \beta_0 + \sum_{l=1}^{L} d_{il}\beta_l$$

where β_l indicates the effect of spatial domain l

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DESpace: fixed-effect test that detects **spatial-domain-marker SVGs**

Assume

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$$\log(\mu_i) = \beta_0 + \sum_{l=1}^{L} d_{il}\beta_l$$

where β_l indicates the effect of spatial domain l

If

$$H_0 : \beta_l = 0$$

is rejected, the gene is detected as a marker SVG of spatial domain l

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Six methods use regression random-effect tests to detect **overall SVGs**:

SpatialDE, nnSVG, SOMDE, SVCA, SPARK, and GPcounts

$$Y_i = \beta_0 + \mathbf{x}_i^\top \boldsymbol{\beta} + \mathbf{z}_i^\top \boldsymbol{\gamma}(\mathbf{s}) + \epsilon_i$$

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$$Y_i = \beta_0 + \mathbf{x}_i^\top \boldsymbol{\beta} + \mathbf{z}_i^\top \boldsymbol{\gamma}(\mathbf{s}) + \epsilon_i$$

With n spots, $\mathbf{z}_i = (z_{i1}, \ldots, z_{in})^\top \in \{0,1\}^n$ is a binary indicator vector for spot i s.t.

$$z_{ii} = 1\,; \quad z_{ij} = 0 \text{ if } j \neq i$$

Random-effect Tests for SVG Detection

Six methods use regression random-effect tests to detect **overall SVGs**:

SpatialDE, nnSVG, SOMDE, SVCA, SPARK, and GPcounts

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If

$$H_0 : \mathrm{Cov}(\boldsymbol{\gamma}(\mathbf{s})) = \mathbf{0}$$

is rejected, the gene is detected as an overall SVG

SpatialDE (Svensson et al., Nature Methods, 2018)

SpatialDE: a linear random-effect model:

$$Y_i = \beta_0 + \mathbf{z}_i^\top \boldsymbol{\gamma}(\mathbf{s}) + \epsilon_i$$

- The random errors $\epsilon_1, \ldots, \epsilon_n \overset{\text{iid}}{\sim} N(0, \delta)$
- The random effects $\boldsymbol{\gamma}(\mathbf{s}) \sim \text{MVN}(\mathbf{0}, \sigma_s^2 \cdot \mathbf{K}(\mathbf{s}))$
  The kernel matrix $\mathbf{K}(\mathbf{s}) = [K(\mathbf{s}_i, \mathbf{s}_j)]_{n \times n}$ is specified by a kernel function $K(\cdot, \cdot)$

This model is essentially a Gaussian process

If

$$H_0 : \sigma_s^2 = 0$$

is rejected, the gene is detected as an overall SVG

Discussion: Power vs. Specificity Trade-off

26 methods for detecting overall SVGs:

9 kernel-based methods vs. 17 other methods (kernel-free or graph-based)

Kernel-based methods have

- Higher specificity for targeted patterns
- Lower overall power for other patterns

Discussion: Challenges in Detecting Non-Global Expression Patterns

1. **Small regions of interests (ROIs)**
   - Spatial-domain-marker SVGs by first identifying ROIs as spatial domains (e.g., SpaGCN)
2. **Spatial-Domain-Specific SVGs**
   - Genes with spatial patterns in small ROIs but not marker genes
   - No existing methods
3. **Cell-Type-Specific SVGs**
   - Easily missed if cell types have small proportions
   - Existing methods' model goodness-of-fit
4. **Sharp Expression Changes**
   - Genes with sharp changes at tissue layer boundaries (e.g., Belayer)
   - Adding H&E image can help refine tissue boundaries

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Future direction: Incorporate knowledge on "interesting genes" to improve specificity

Discussion: Scalability

1. Calculate a summary statistic for each gene.
2. Convert the summary statistic to a p-value (frequentist methods only)

Summary Statistic Calulation (n: number of spatial spots)

- Gaussian process: $O(n^3)$ in SpatialDE and SPARK
- Nearest-neighbor Gaussian process approximation: $O(n)$ in nnSVG

p-value Conversion

- Fast if closed-form null distribution is available (conventional statistics)
- Computationally intensive if by permutation (unconventional statistics)

Improving Scalability

- Use approximation algorithms to speed up summary statistic calculation
- Reduce number of permutations in the p-value conversion step

Future Direction 1: Accommodating Technological Differences

Two Key Differences:

- **Spatial Resolution**
  - Imaging-based Technologies: Single-cell or subcellular resolution
  - Sequencing-based Technologies: Multicellular level, coarser resolution
- **Positional Randomness**
  - Structured grids (e.g., Spatial Transcriptomics, 10x Visium)
  - Unstructured spots (e.g., Slide-seq, MERFISH, SeqFISH)

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Current Limitations:

- Most SVG detection methods lack consideration of these technological differences
- Lack of consensus on pre-processing and modeling SRT data

Future Direction 2: Enhancing Statistical Rigor and Method Benchmarking

Challenges:

- Double-dipping: Same data analyzed more than once, leading to confirmation bias
- Example: Spatial-domain-marker SVG detection

Strategies:

- Use *in silico* negative control data to remove spurious discoveries (e.g., ClusterDE)
- Develop fast visualization tools for interpreting top-detected SVGs

Method Benchmarking:

- Benchmarking requires well-annotated datasets with SVG ground truths
- Synthetic datasets and realistic simulators (e.g., SRTsim, scDesign3)
- No method is optimal in every aspect; benchmarking should be specific to data characteristics and align with biological questions

Preprint

Yan, G., Hua, S. H., & Li, J. J. (2024). Categorization of 31 computational methods to detect spatially variable genes from spatially resolved transcriptomics data. *arXiv*. https://arxiv.org/abs/2405.18779