Lecture 19-20: Single-cell genomics

- Introduction, Missing value imputation, Dimensionality reduction
- Trajectory inference, Spatial reconstruction

Single-cell RNA-seq

BREAKTHROUGH OF THE YEAR

Development cell by cell

With a trio of techniques, scientists are tracking embryo development in stunning detail

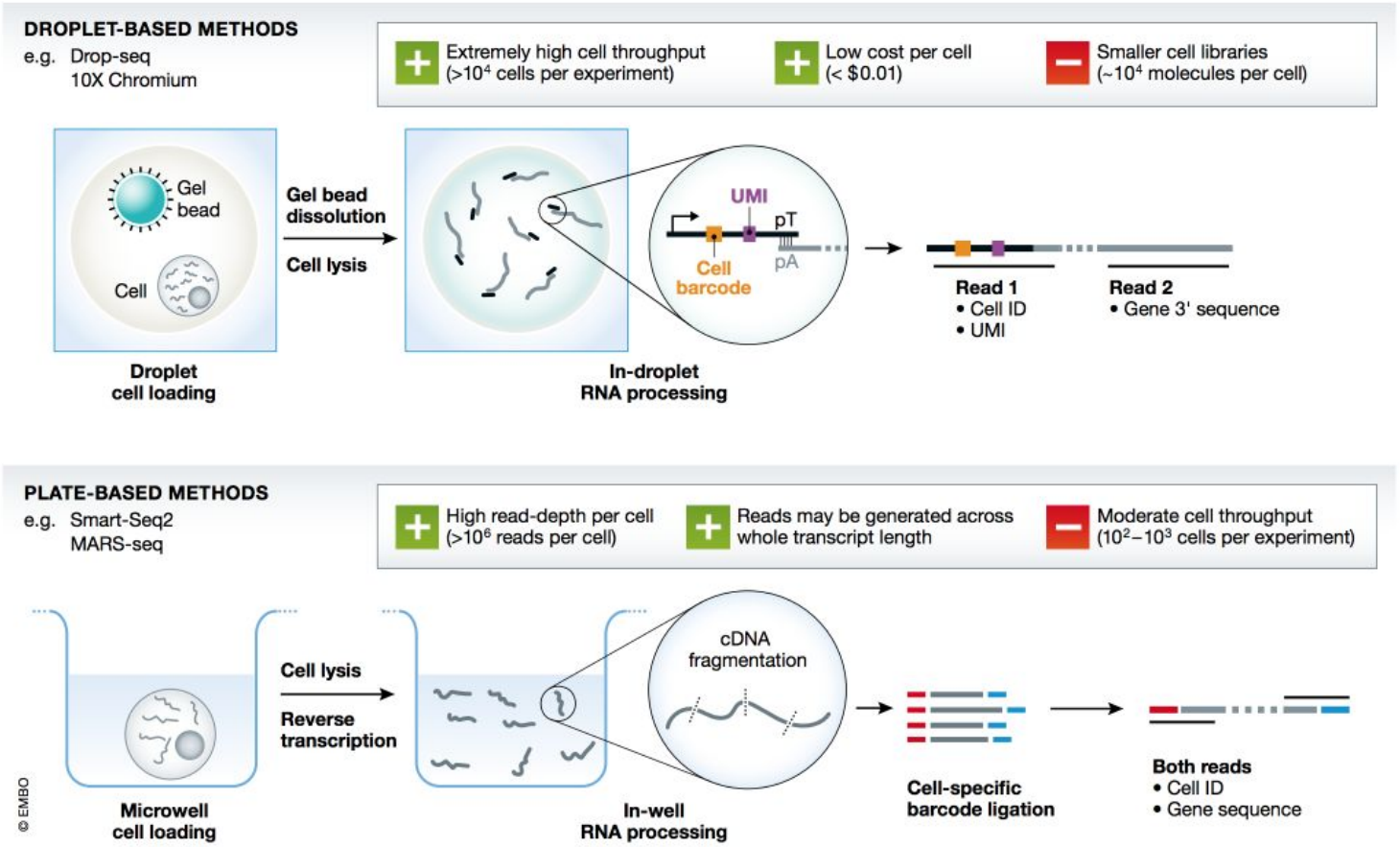
A zebrafish embryo at an early stage of development. Fluorescent markers highlight cells expressing genes that help determine the type of cell they will become. (JEFFREY FARRELL, SCHIER LAB/HARVARD UNIVERSITY)

The single-cell revolution is just starting.

— Elizabeth Pennisi

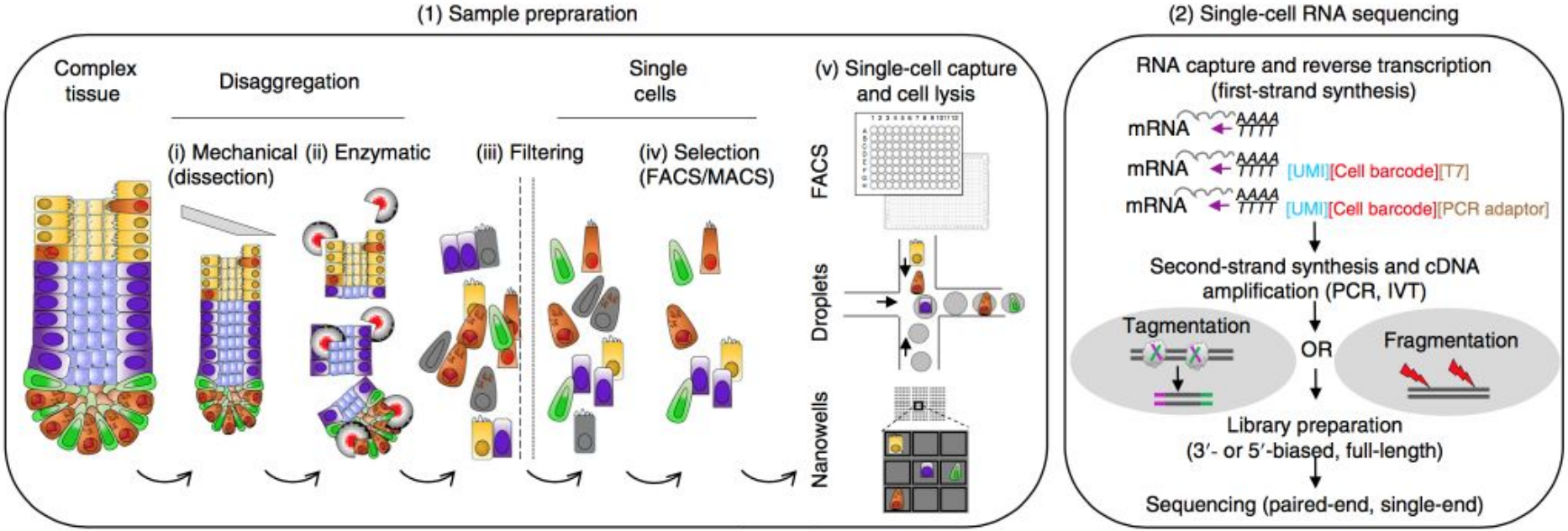
https://vis.sciencemag.org/breakthrough2018/finalists/#cell-development

Single-cell RNA-seq



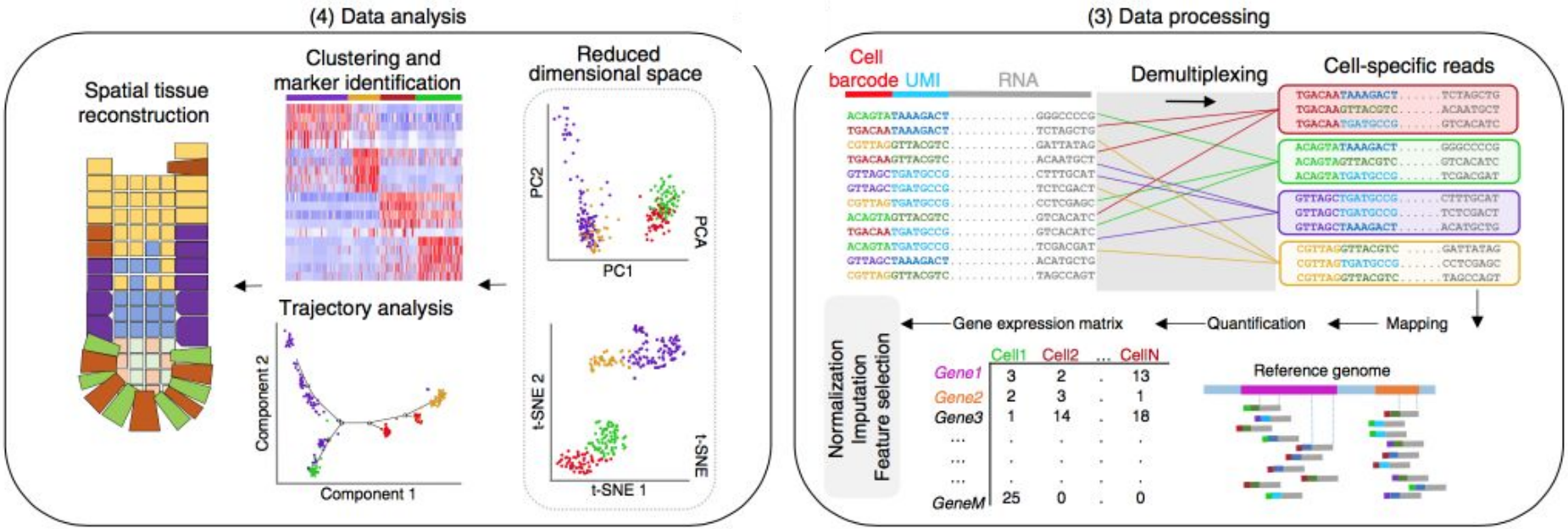
Griffiths (2018) Mol. Sys. Biol.

Single-cell RNA-seq



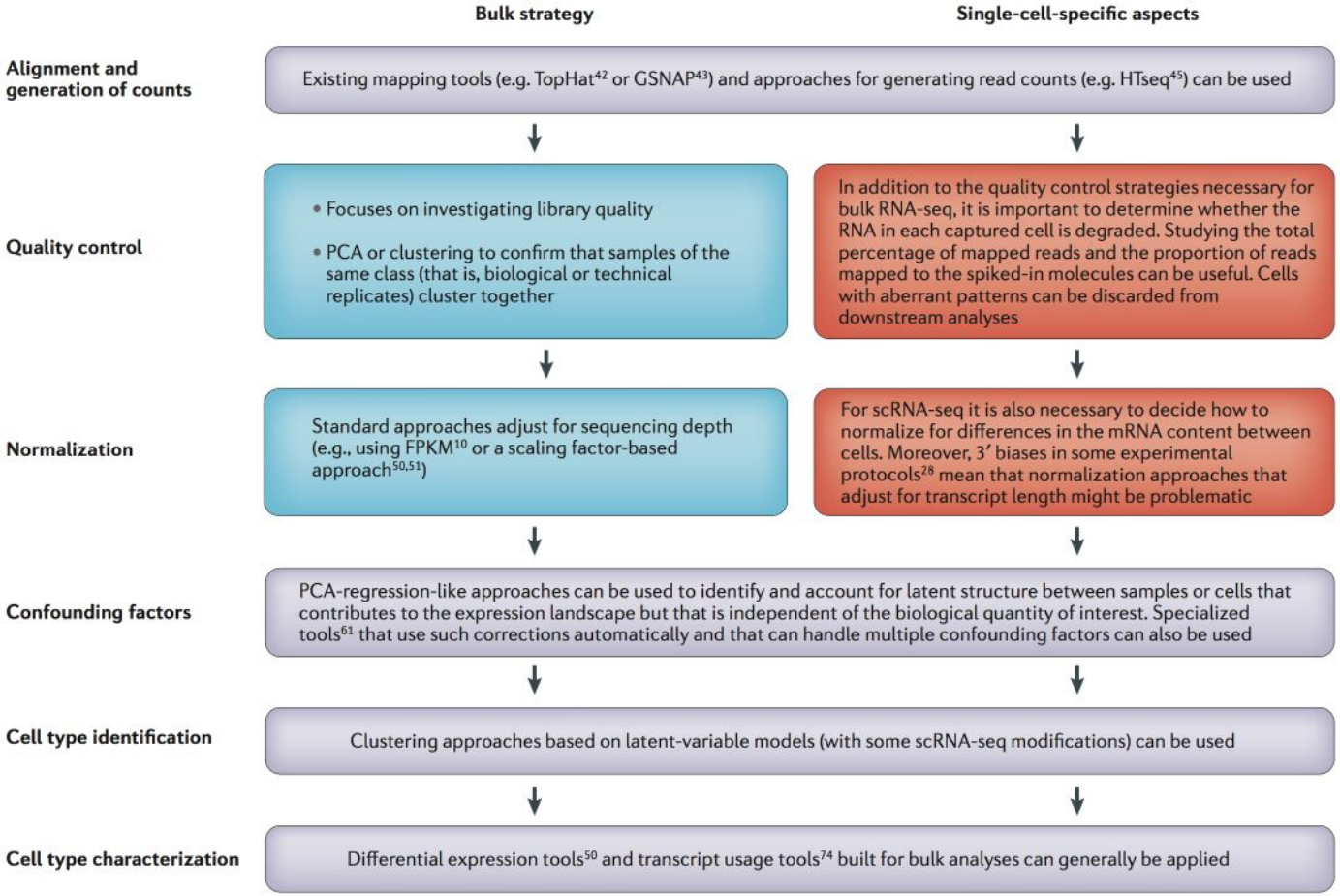
Lafzi (2018) Nat. Prot.

Single-cell RNA-seq



Lafzi (2018) Nat. Prot.

Single-cell RNA-seq

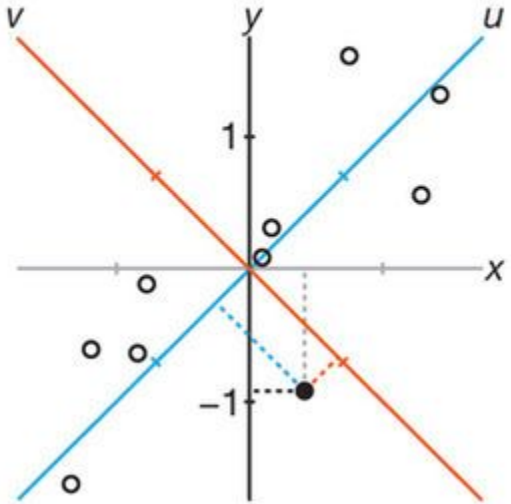


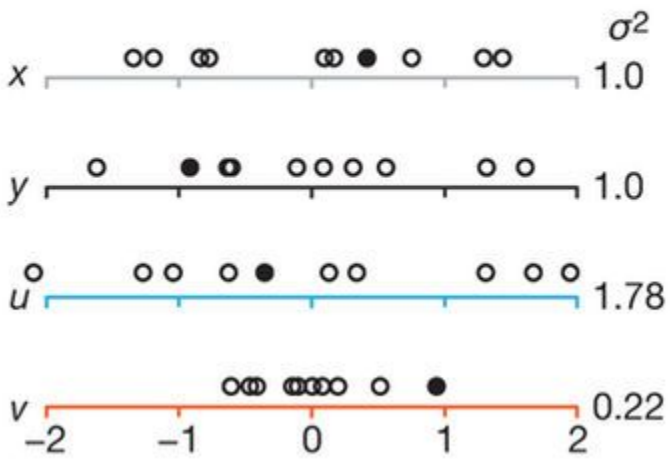
Stegle (2015) Nat. Rev. Genet.

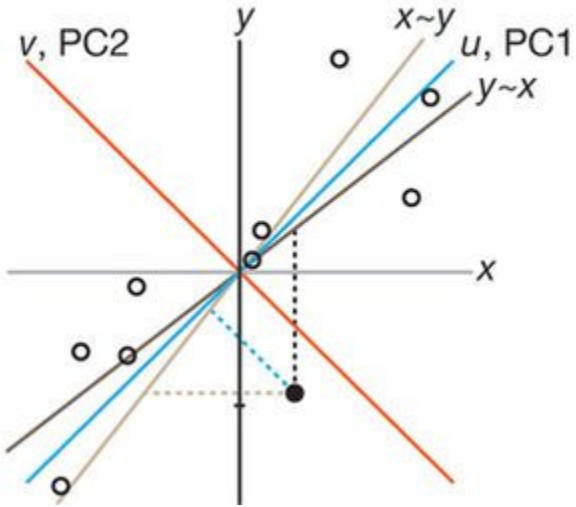
Dimensionality reduction by PCA

PCA geometrically projects data onto a lower-dimensional space

- Each lower dimension is a 'linear' combination of correlated original dimensions.
- The principal components (PCs) represent the directions of maximum variation.

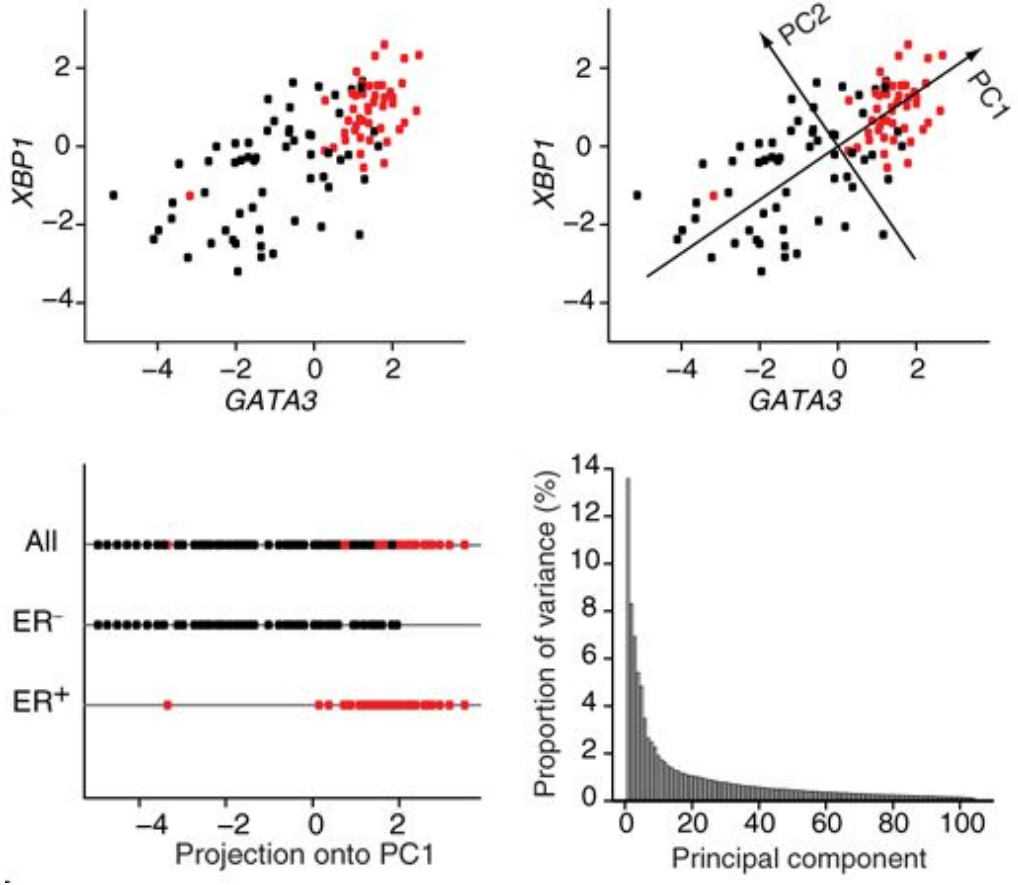






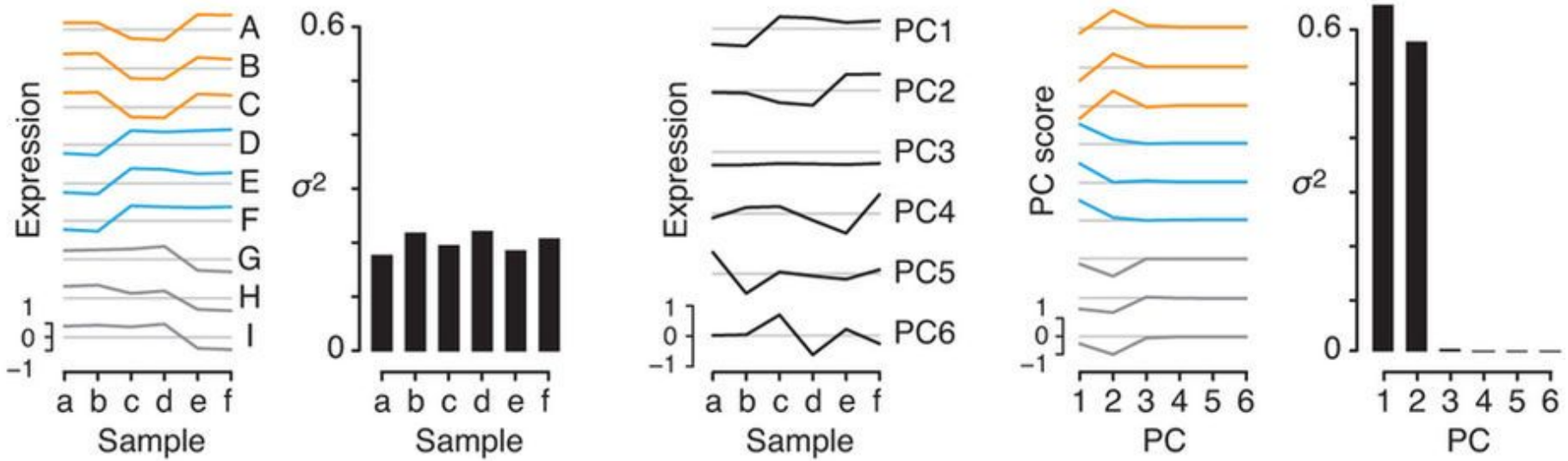
Lever, Krzywinski, & Altman (2017) Nat. Meth.

Dimensionality reduction by PCA



Ringnér (2008) Nat. Biotech

Dimensionality reduction by PCA



Lever, Krzywinski, & Altman (2017) Nat. Meth.

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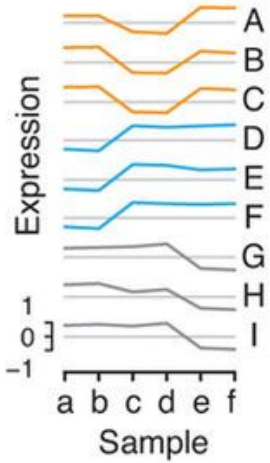

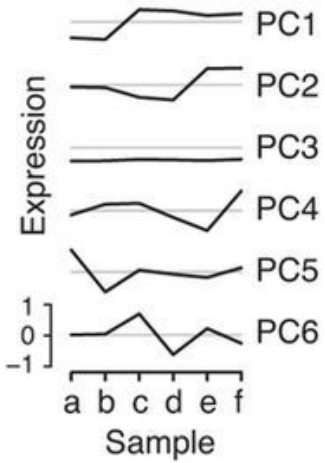

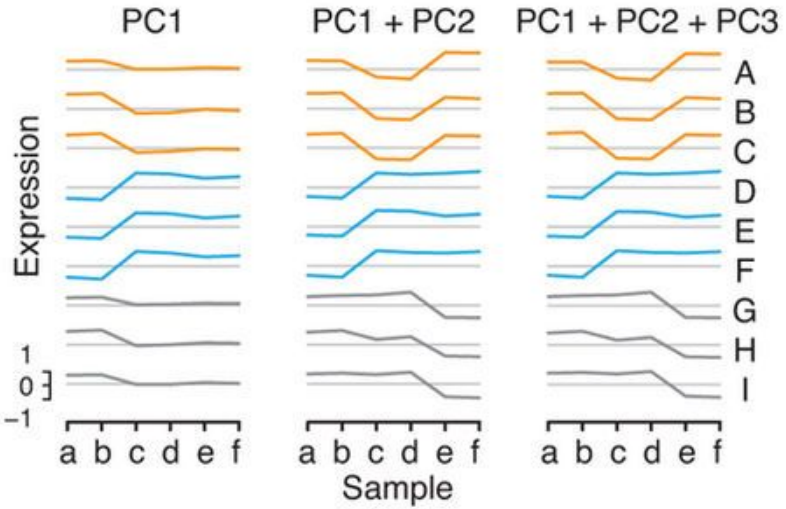

Lever, Krzywinski, & Altman (2017) Nat. Meth.

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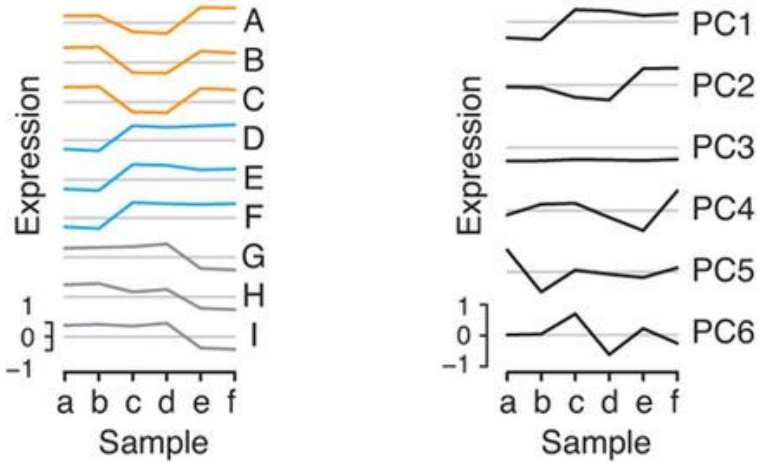

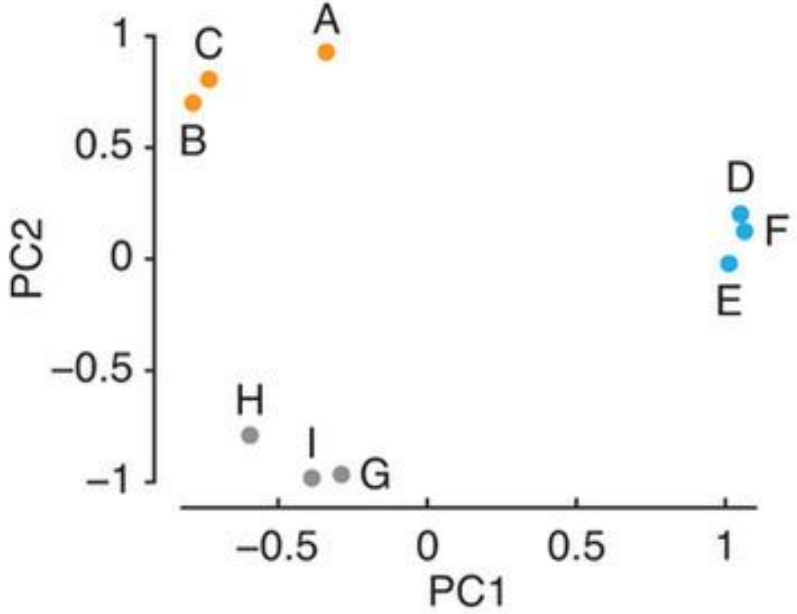

Lever, Krzywinski, & Altman (2017) Nat. Meth.

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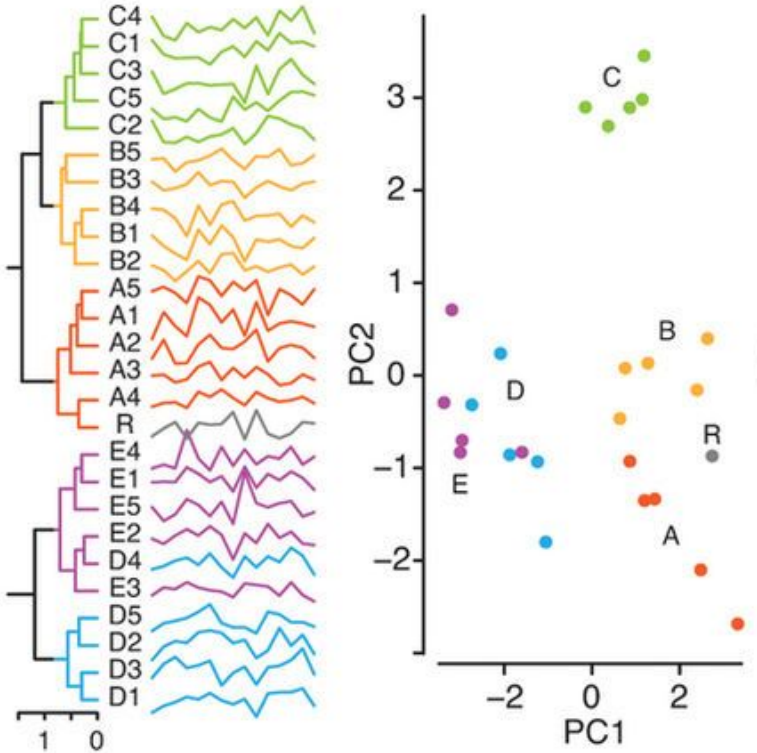
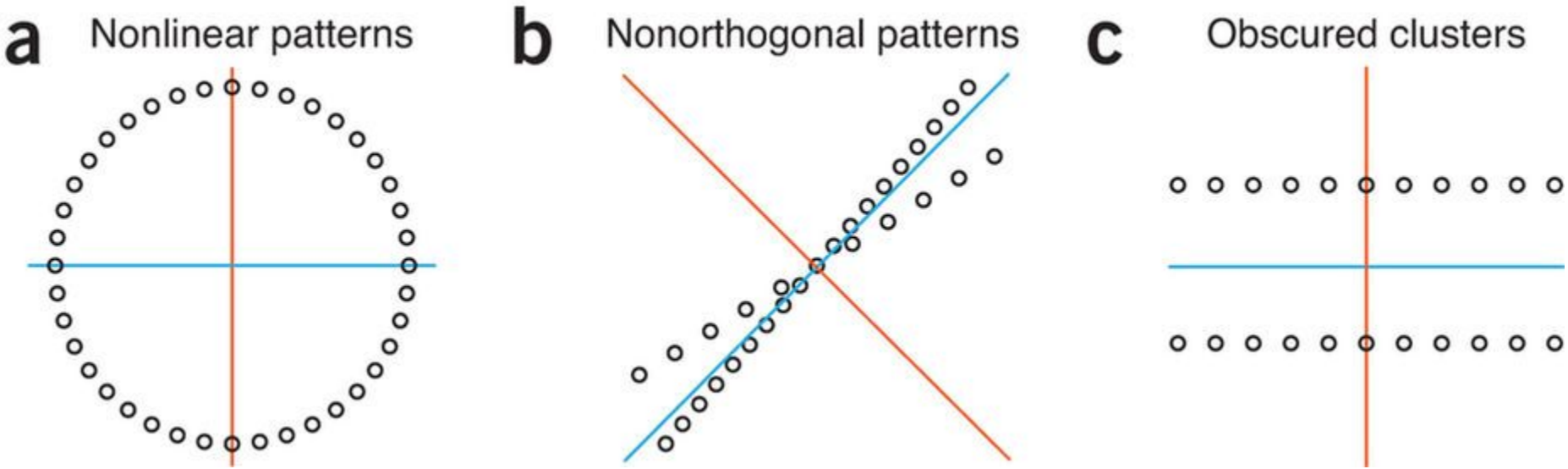


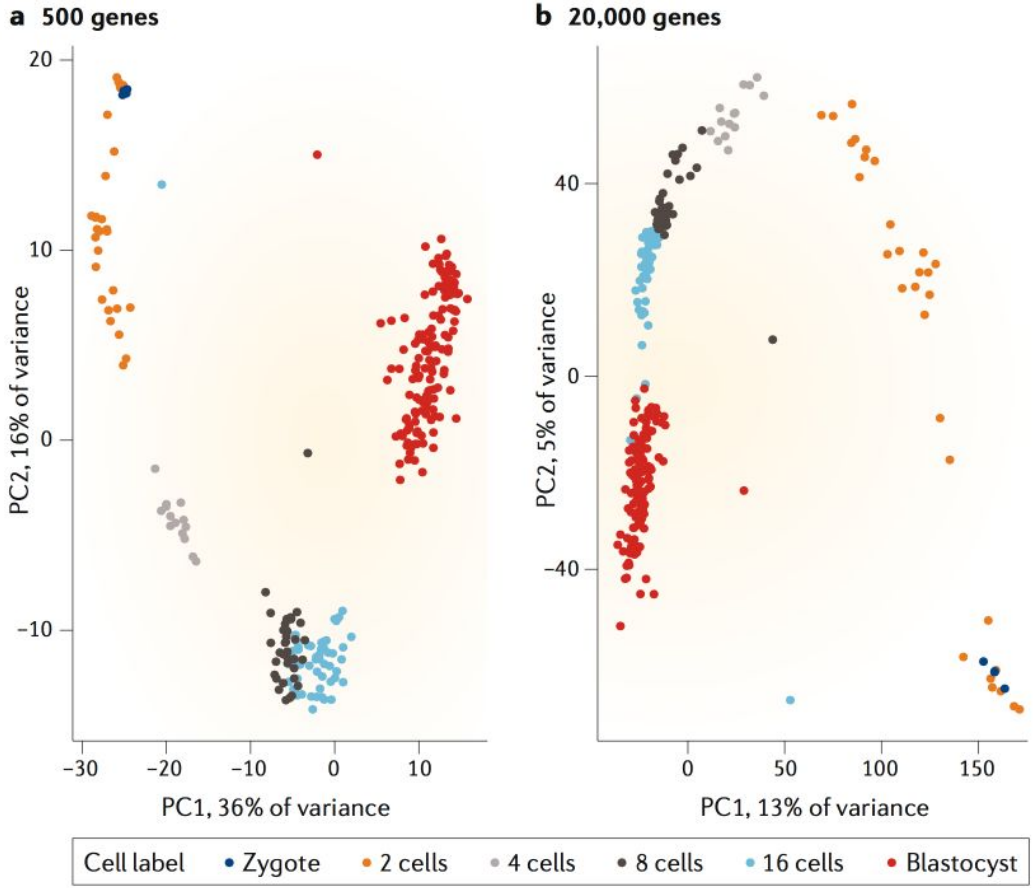
Lever, Krzywinski, & Altman (2017) Nat. Meth.

Dimensionality reduction by PCA



Lever, Krzywinski, & Altman (2017) Nat. Meth.

Dimensionality reduction by PCA



Lever, Krzywinski, & Altman (2017) Nat. Meth.

Imputing missing values

scRNA-seq data has:

- **a high frequency of zero values**, often referred to as dropouts, and
- **high levels of noise** due to the low amounts of input RNA obtained from individual cells.

Zero values in scRNA-seq may arise due to:

- low experimental sensitivity, e.g. sequencing sampling noise, technical dropouts during library preparation, or
- biologically the gene is not expressed in the particular cell.

Imputing missing values

Zero values in scRNA-seq may arise due to:

- low experimental sensitivity, e.g. sequencing sampling noise, technical dropouts during library preparation, or
- because biologically the gene is not expressed in the particular cell.

Imputation is a common approach when dealing with sparse genomics data: predict missing values from the rest of the measured values.

One challenge when imputing expression values is to **distinguish true zeros from missing values**.

scRNA-seq data imputation methods use information internal to the dataset to be imputed.

- Some degree of circularity → false positive results when identifying marker genes, gene-gene correlations, or testing differential expression.

Imputing missing values

Many imputation methods:

- **SAVER, DrImpute & scImpute**: use models of the expected gene expression distribution to distinguish true biological zeros from zeros originating from technical noise.
    - Assume homogenous cell populations → identify clusters of similar cells to which an appropriate mixture model is fitted.
    - Values falling above a given probability threshold to originate from technical effects are subsequently imputed.
- **MAGIC & knn-smooth**: perform data smoothing.
    - Infer values of missing data + reduces noise present in observed values (using information from neighbouring data points).
    - Use each cell's k nearest neighbours either through the application of diffusion models or weighted sums respectively.

Imputing missing values

Many imputation methods:

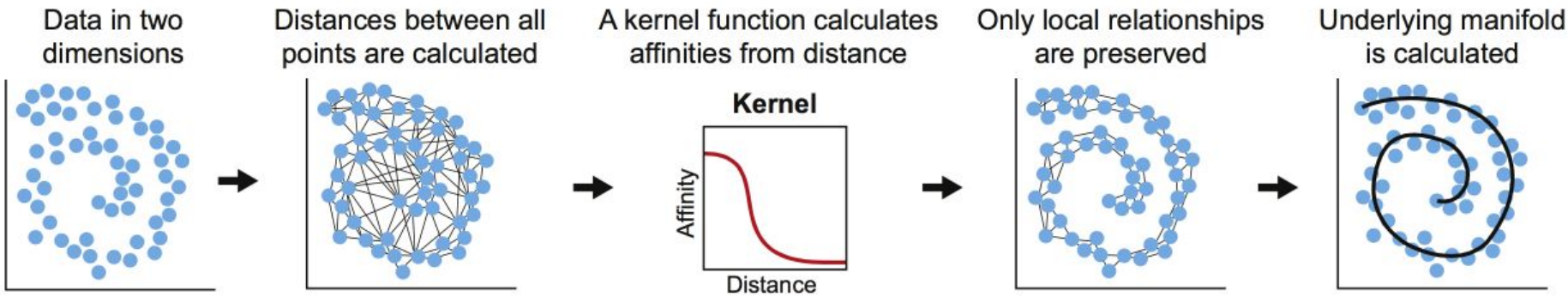
	Designed for single cell	Local or global	Beyesian method	Need other information	Imputation strategy
LLSimpute	N	local	N	No. of nearest genes	1
Low-rank	N	global	N	error tolerance δ	2
BISCUIT	Y	global	Y	dispersion parameter	1 and 2
scUnif	Y	global	Y	cell labels	2
MAGIC	Y	global	N	diffusion time	2
scImpute	Y	local	N	dropout rate cutoff	2
DrImpute	Y	local	N	cluster numbers	2
SAVER	Y	global	Y	size factor	1

Strategy 1 represents imputing dropout based on co-expressed or similar genes, while strategy 2 denotes imputing dropout by borrowing information from similar cells.

Zhang & Zhang (2017) bioRxiv

Imputing missing values

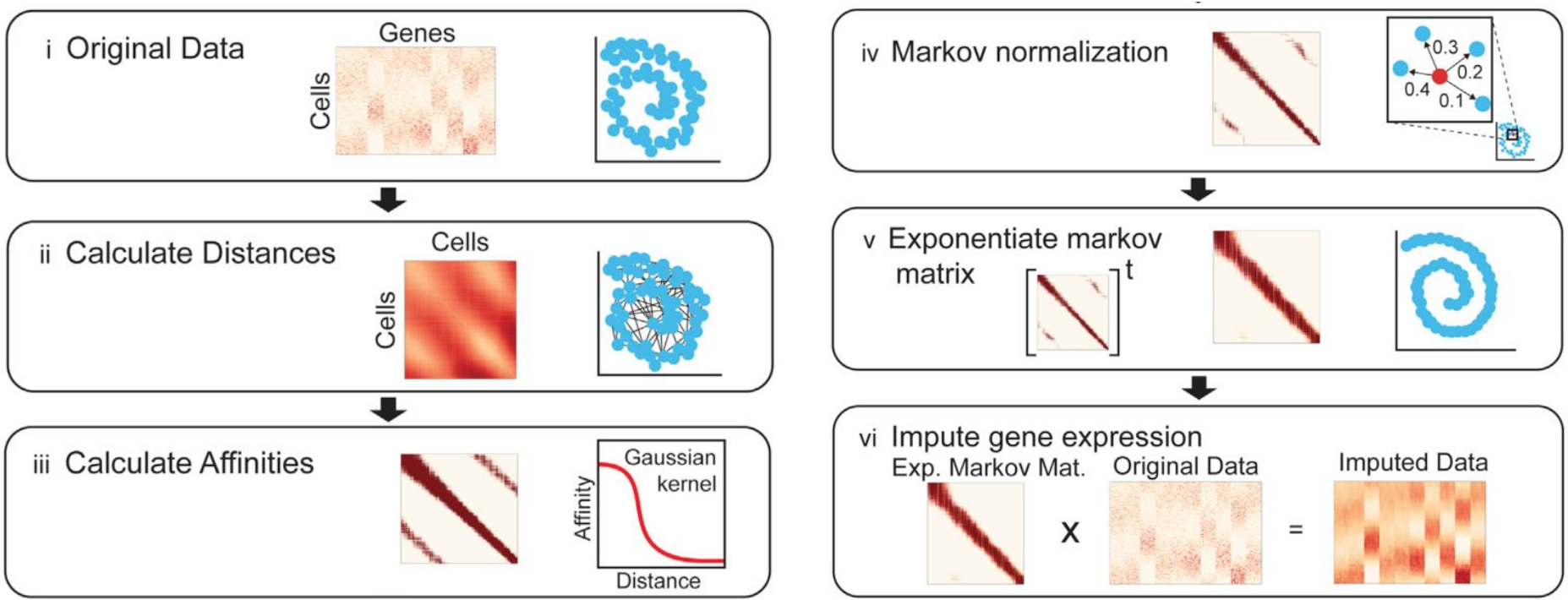
Manifold learning



Moon (2018) Curr. Opin. Sys. Biol.

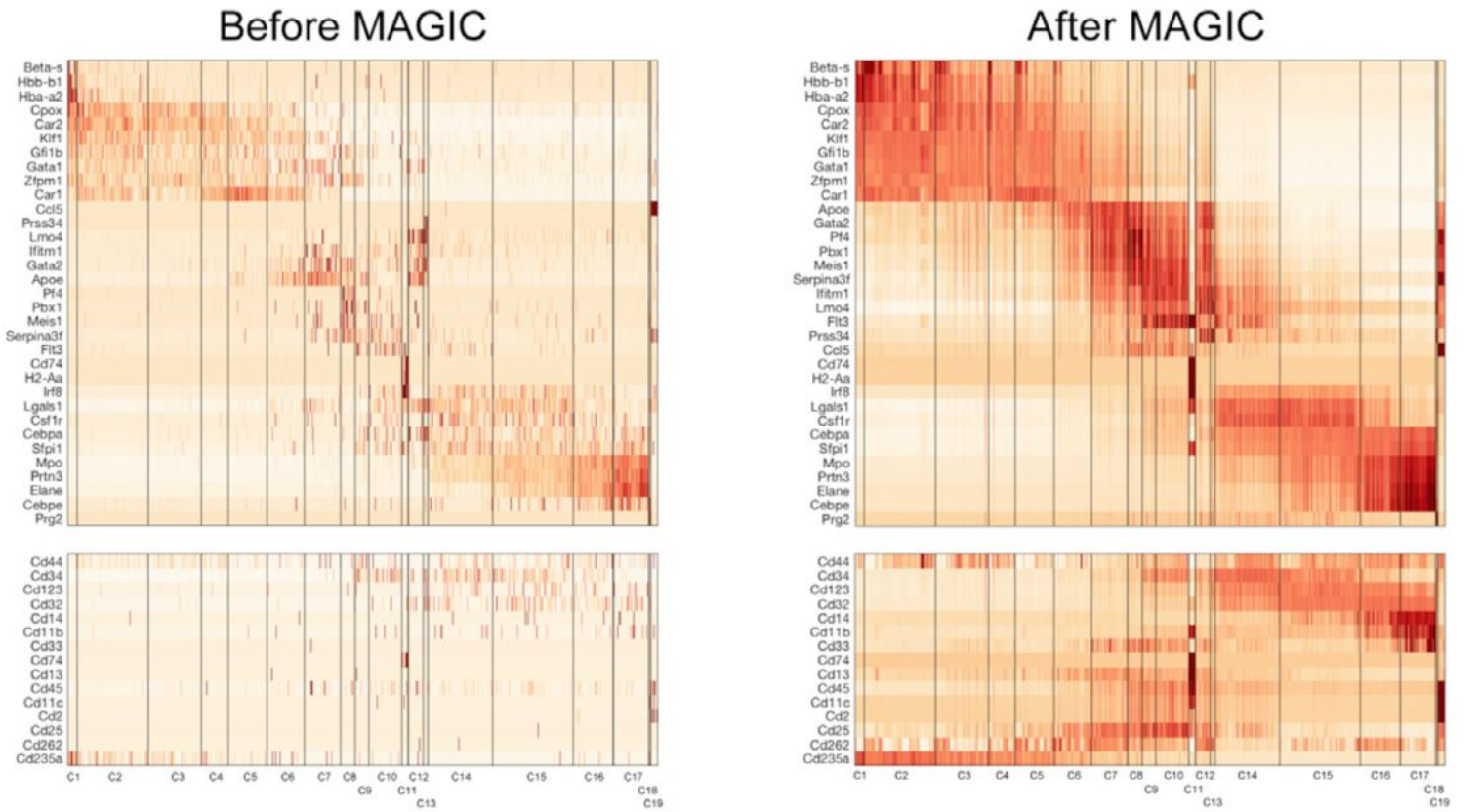
Imputing missing values

MAGIC



Vandijk (2018) Cell

Imputing missing values



Vandijk (2018) Cell

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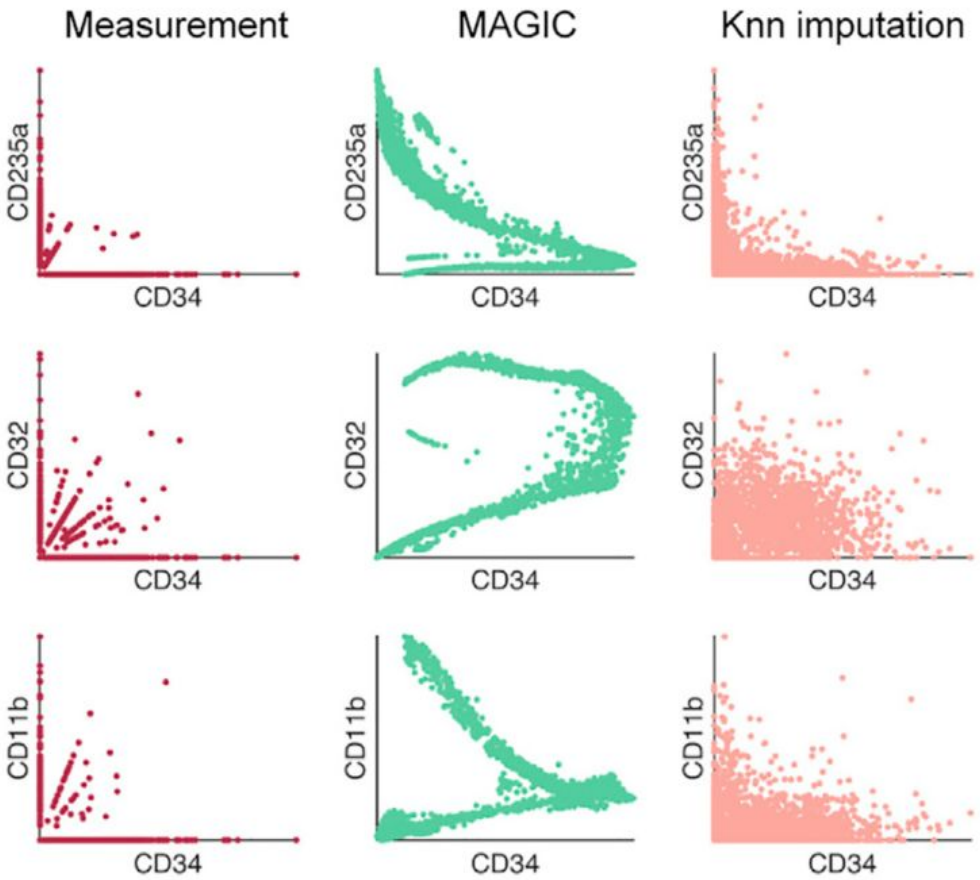
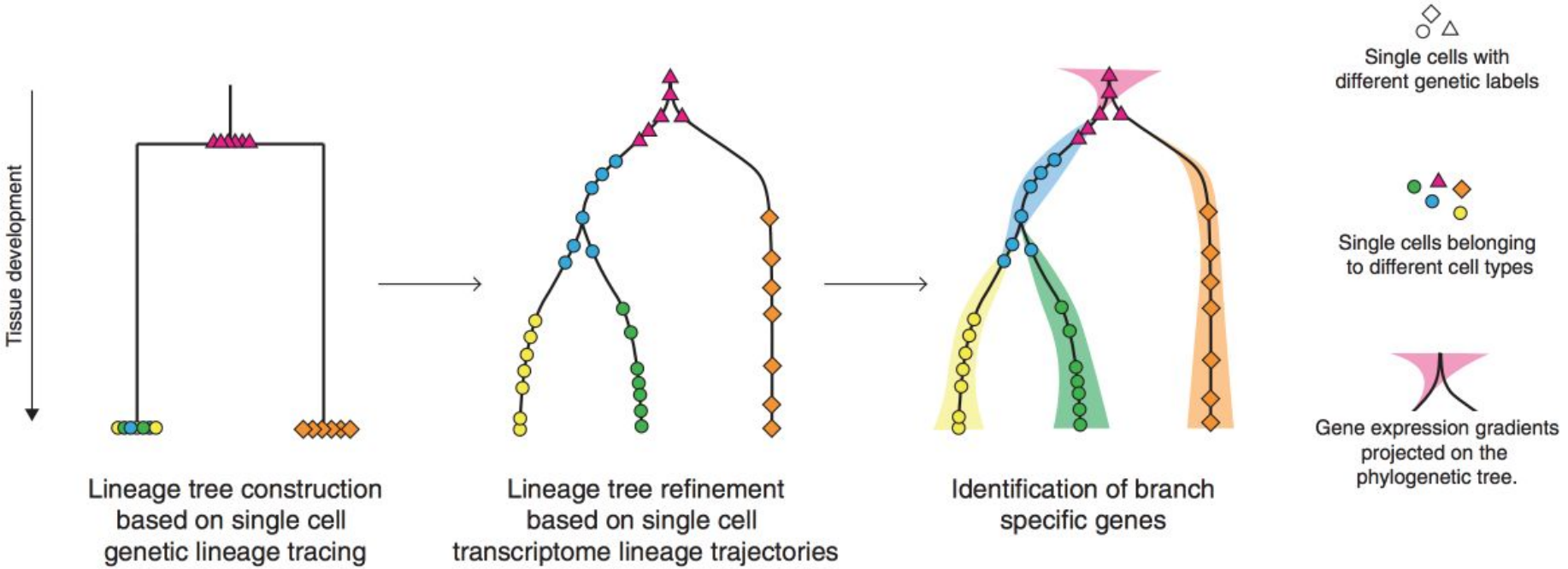


Vandijk (2018) Cell

Lineage tracing



Vandijk (2018) Cell

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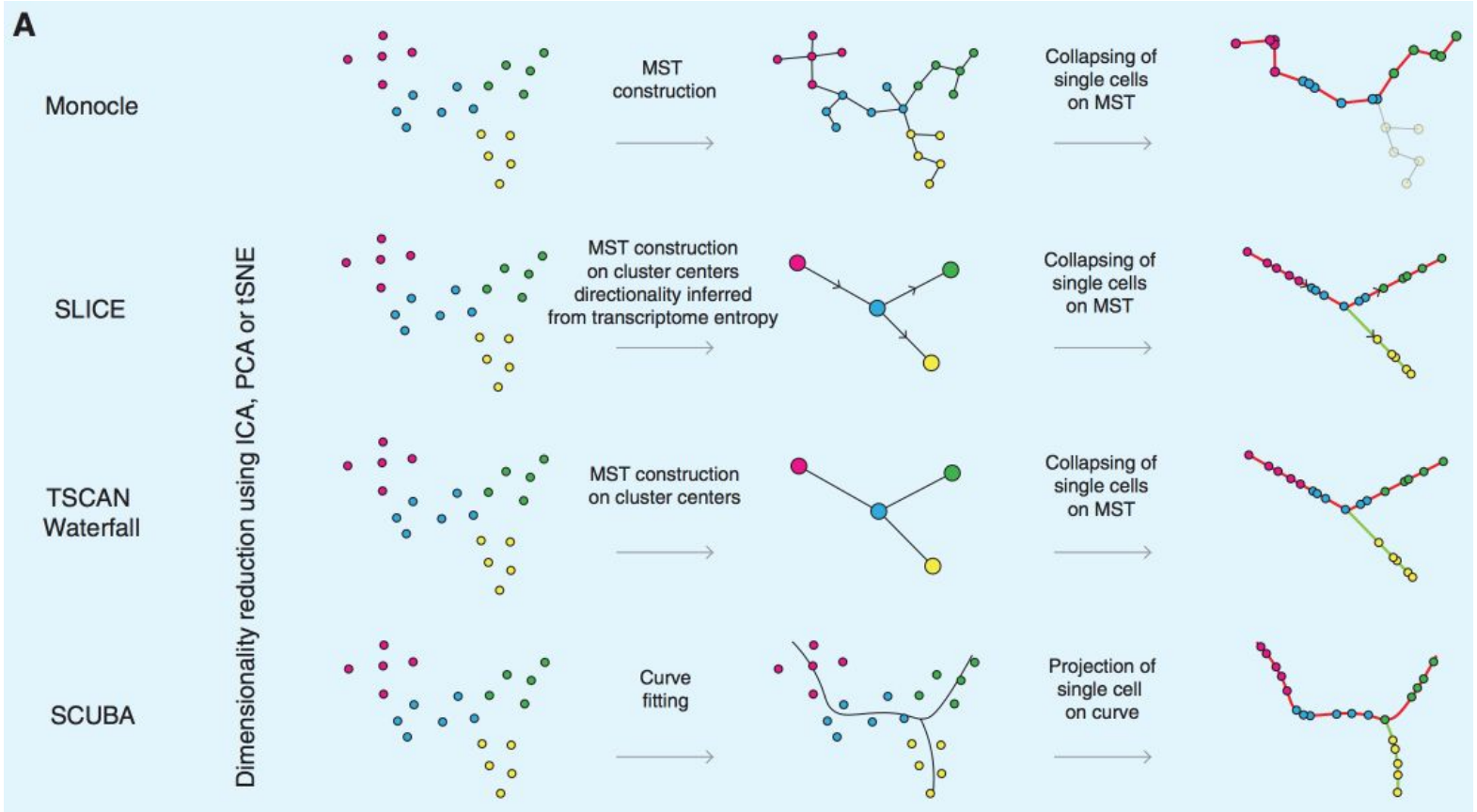

Kester (2018) Cell Stem Cell