Single-Cell Transcriptomics

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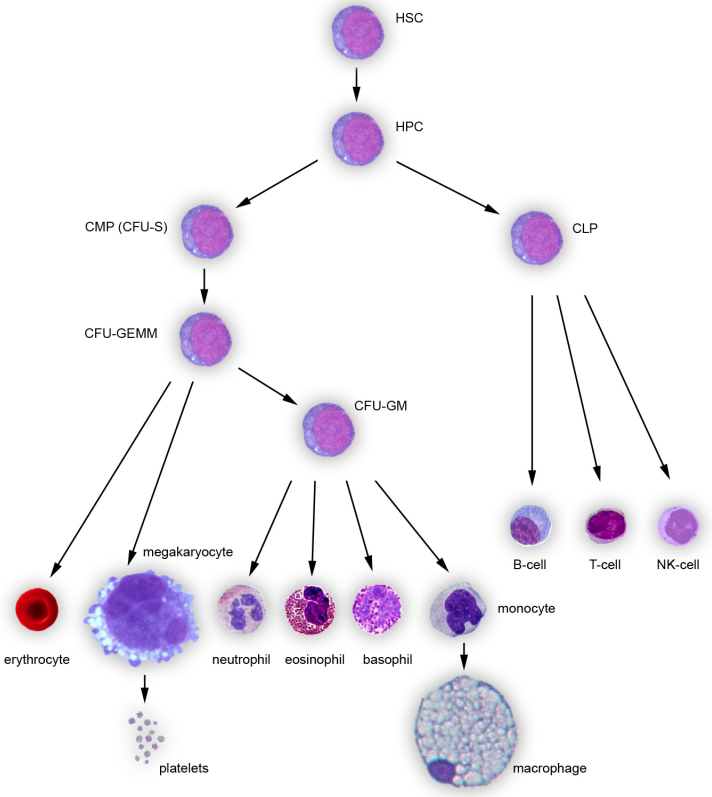
Regan Institute, September 14th 2017

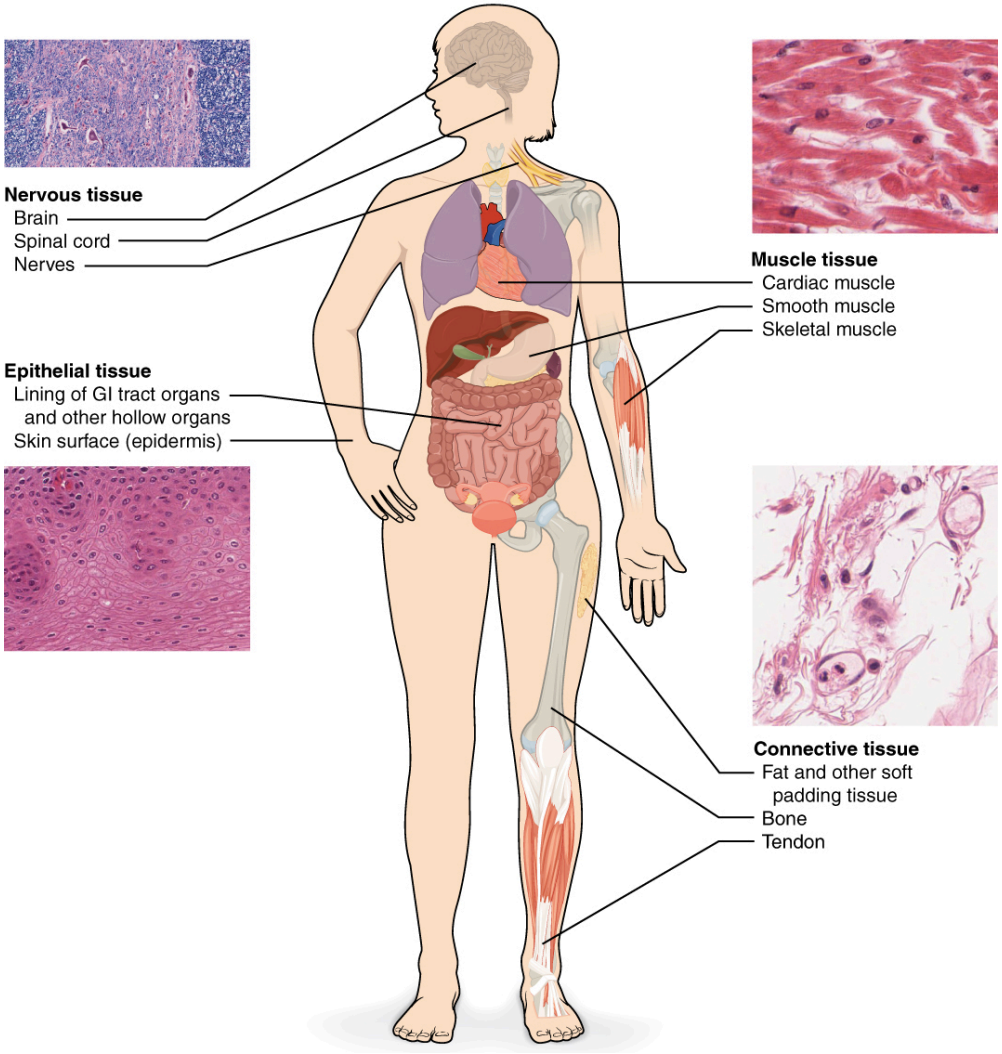
HARVARD MEDICAL SCHOOL | DEPARTMENT OF Biomedical Informatics

HSCI HARVARD STEM CELL INSTITUTE

Cells, Cell Types and Tissues

- Averages of ‘bulk’ samples
  - ~10^7 cells
- Complex tissue organization





Cells, Cell Types and Tissues

- Averages of ‘bulk’ samples
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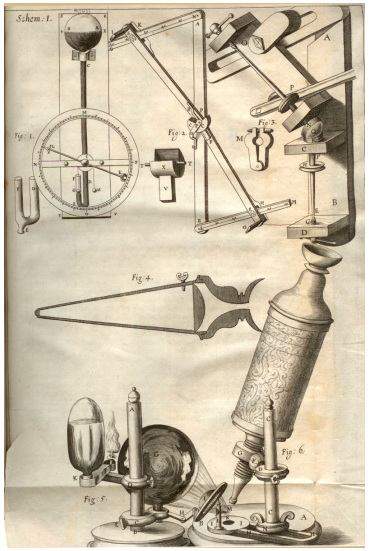
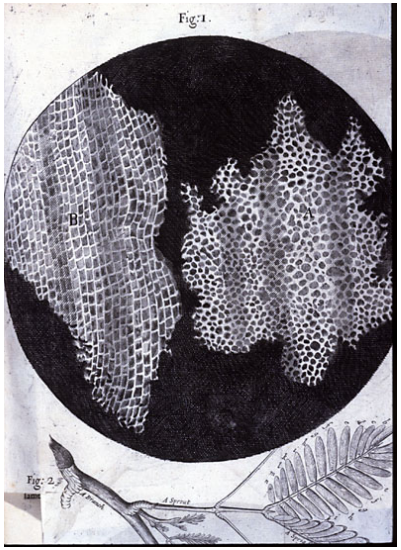
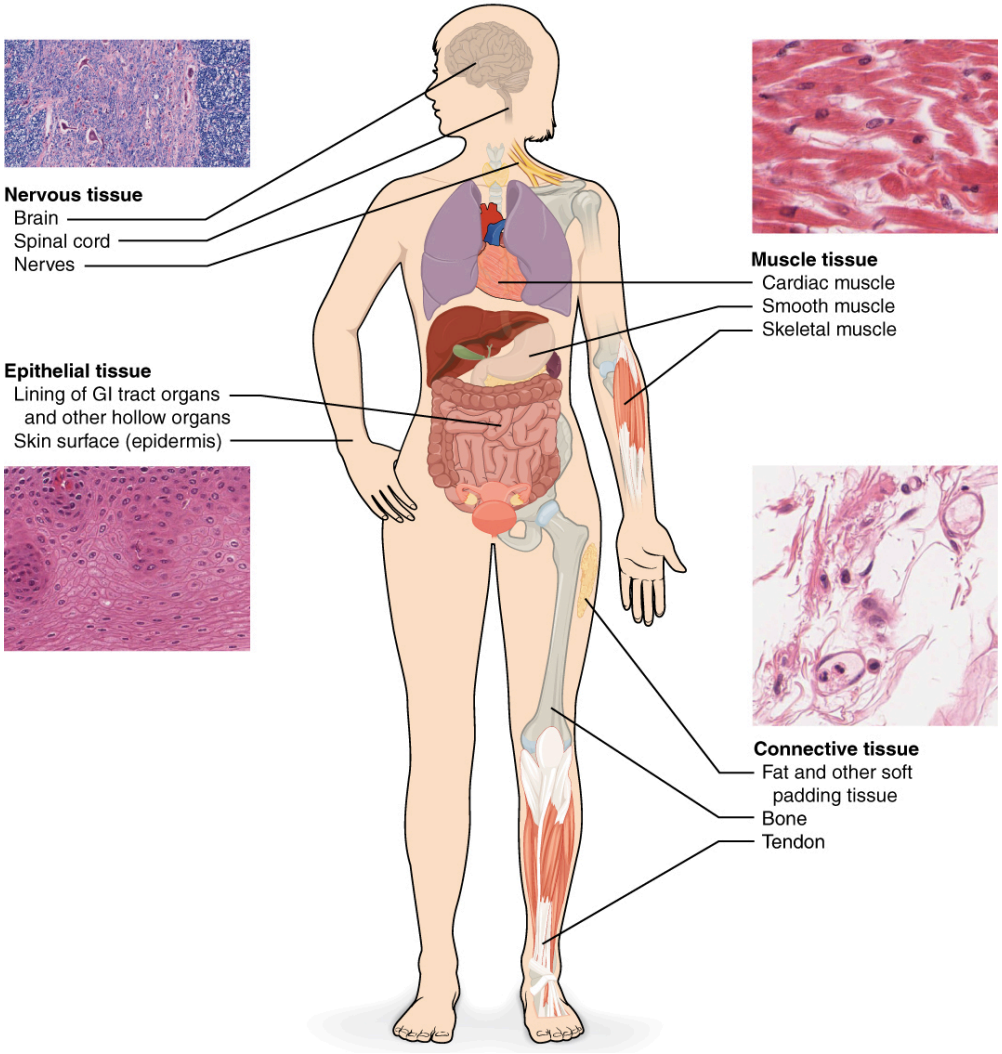
- Complex tissue organization
- Cell Types
  - Early Microscopy
  - Robert Hooke (1635-1703)







Cells, Cell Types and Tissues

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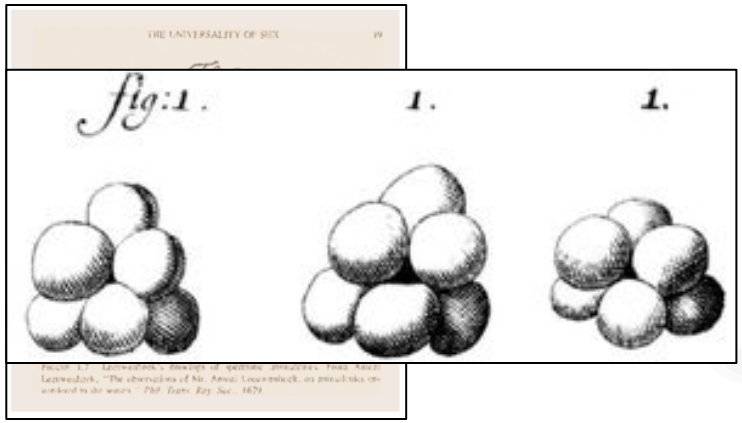

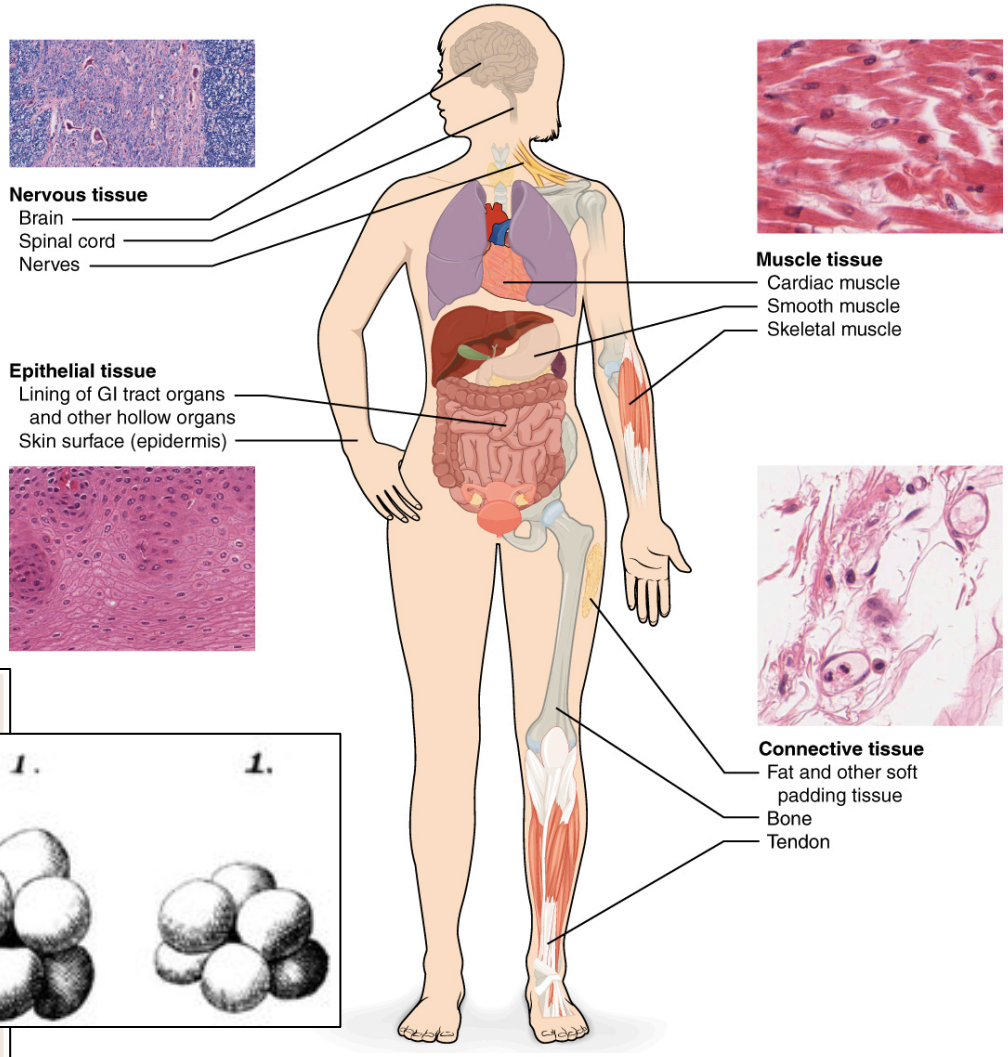

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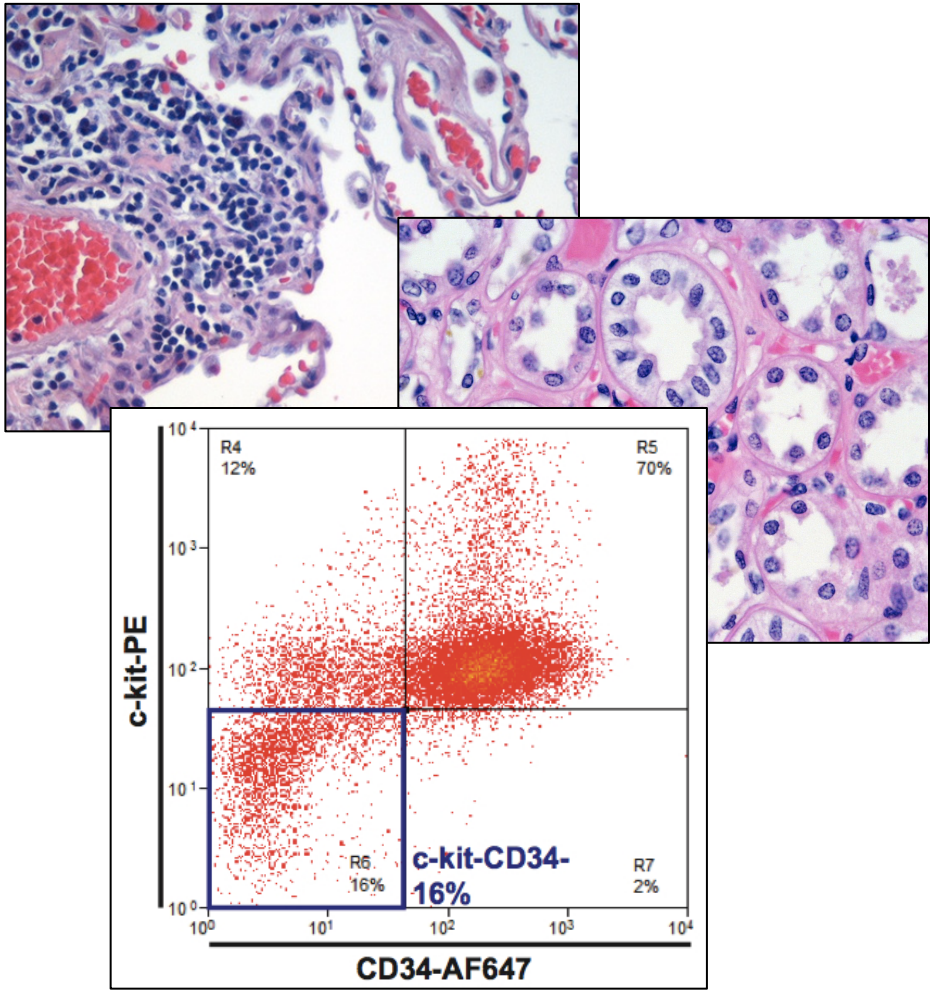
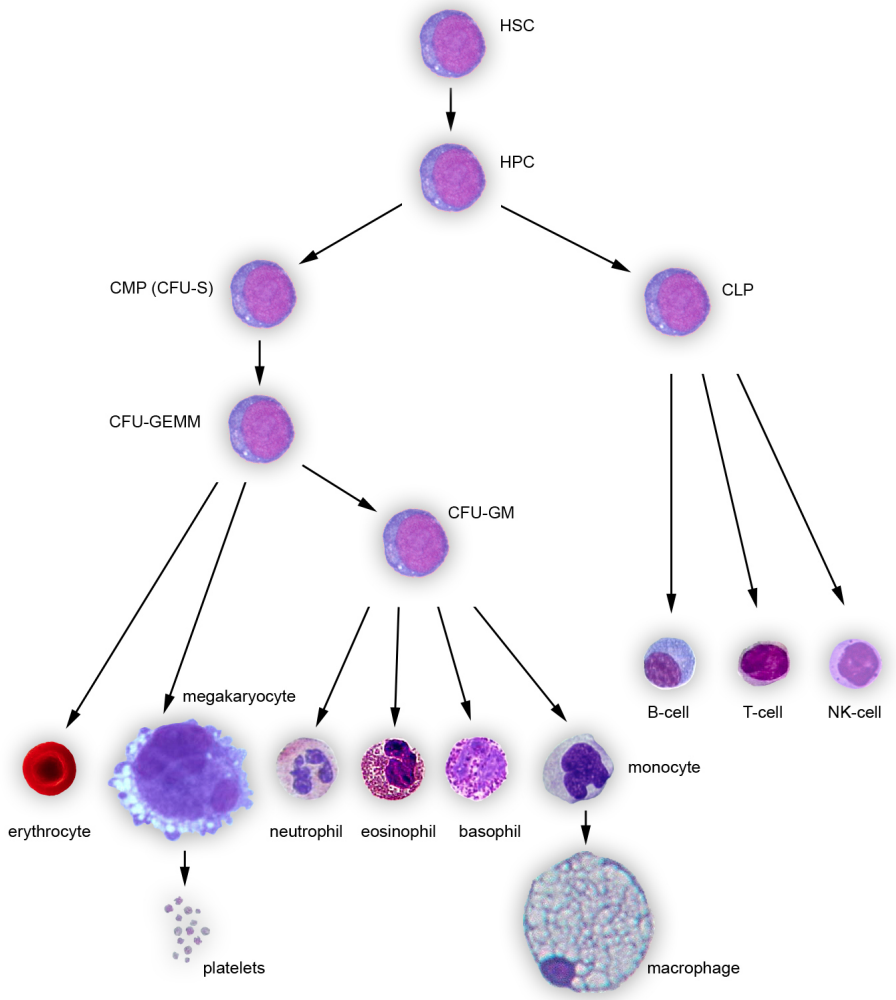
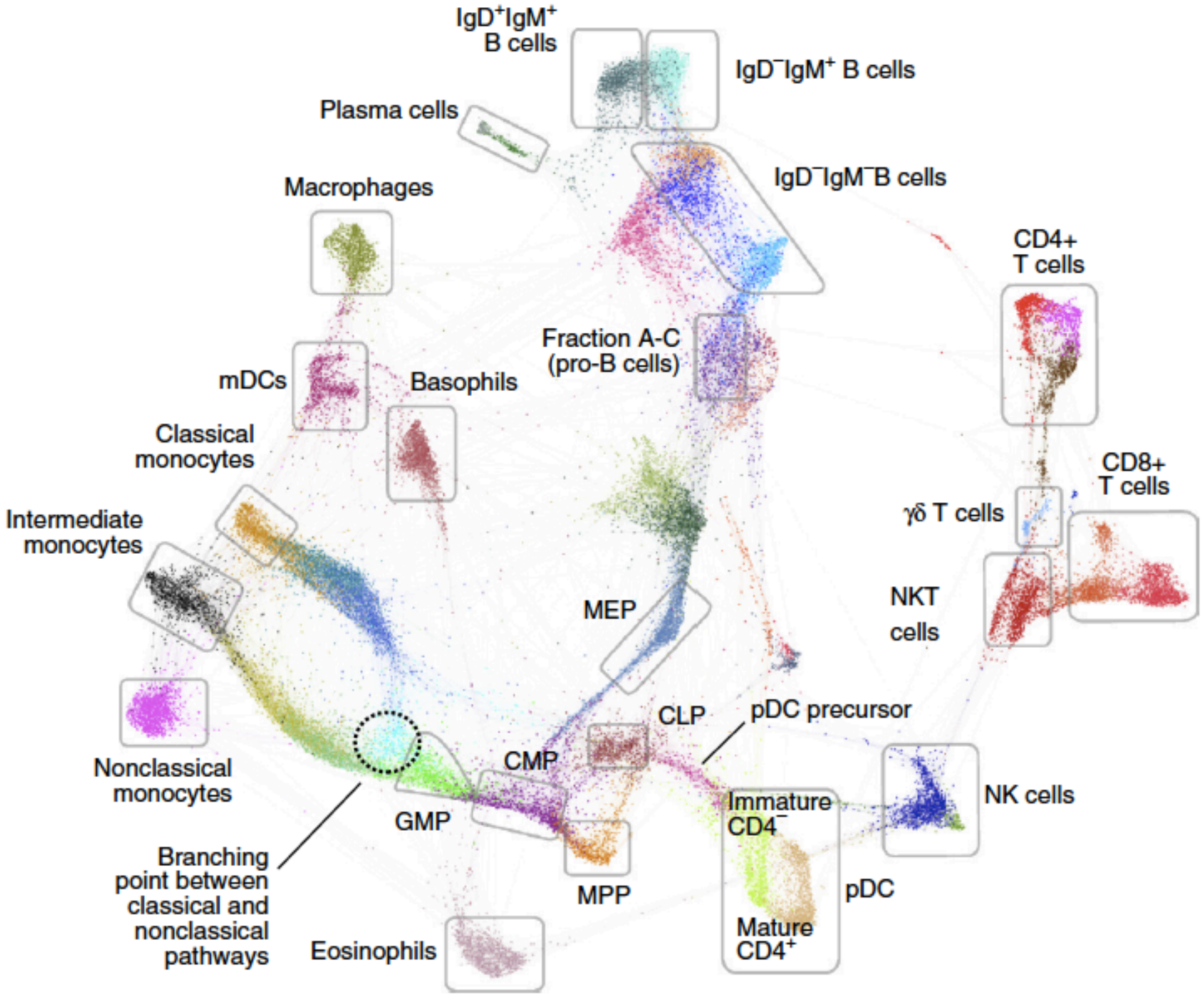
Modern Histology




HSC
HPC
CMP (CFU-S)
CLP
CFU-GEMM
CFU-GM
B-cell
T-cell
NK-cell
megakaryocyte
monocyte
erythrocyte
neutrophil
eosinophil
basophil
platelets
macrophage



Samusik *et al. Nat. Methods*'16

Single-Cell Genomics

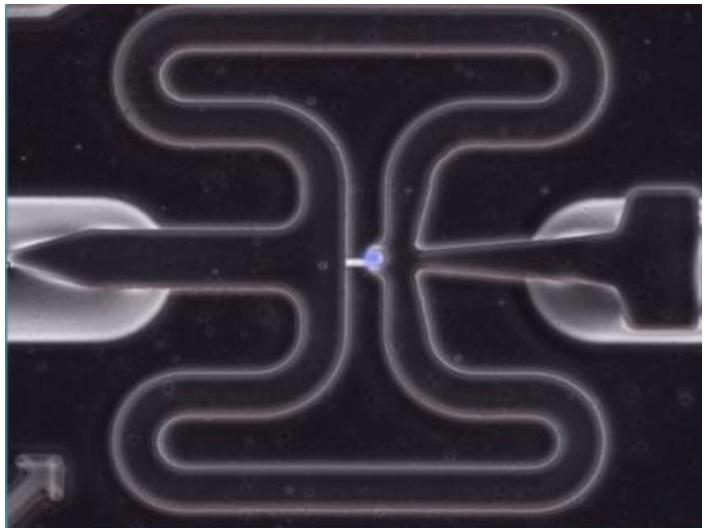
- Aims
  - Classification: cell types, states, subpopulations
  - Mechanisms: molecular differences, dynamics
- Measurements
  - Transcriptional state (single-cell RNA-seq, FISSEQ)
  - Epigenetic state (DNA methylation, accessibility)
  - Genomic sequence (somatic mutations)
- Techniques
  - Scaled-down sequencing assays
  - Microfluidics: valve, droplet-based systems
  - Microscopy, in-situ labeling, sequencing

Single-cell analysis: Why?

- Variability between cells
  - Stereotypical subsets of cells
    - Cell types
    - Subclones
    - Composition of the sample
  - Cell state can reflect multiple concurrent processes
    - Cell cycle vs. cell type
    - Multiple axes of variation (cell types, cell states)
  - Gradual differences between cells
    - Differentiation trajectories
- Co-variation of individual characteristics
  - Transcriptional correlations
  - CNV co-occurrence

Progress in Single-Cell RNA Sequencing Techniques

- Cell separation and handling
  - Commercial C1 platform from Fluidigm



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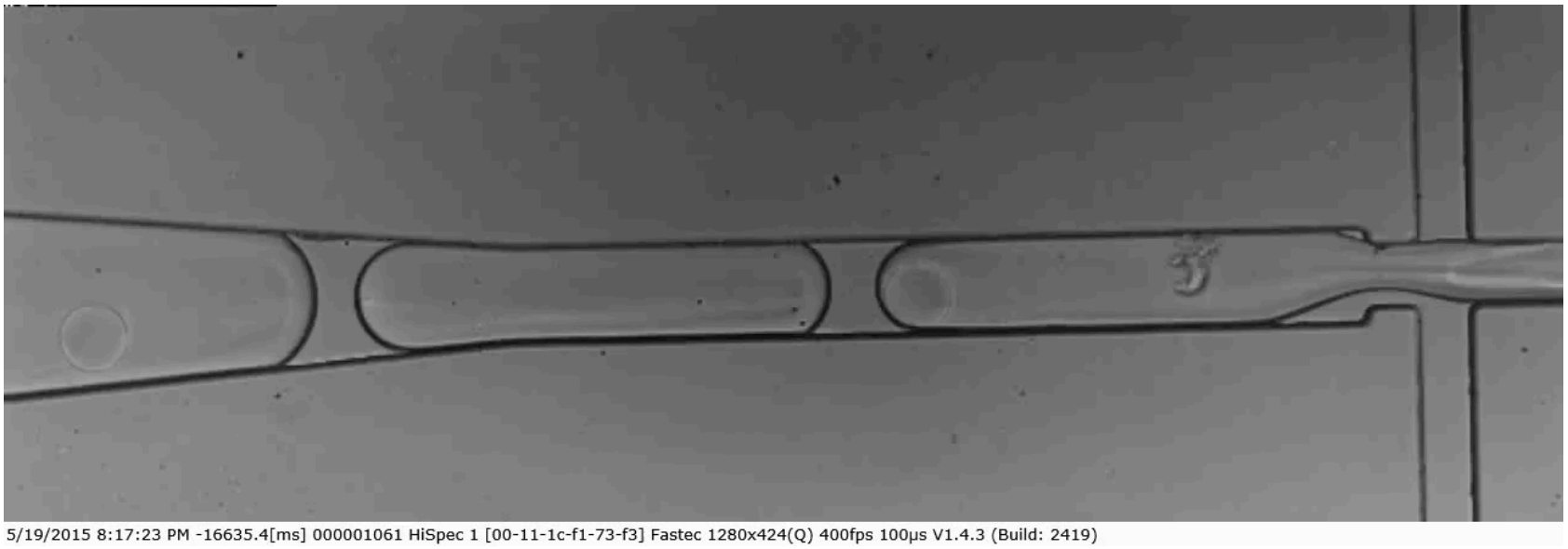
  - Using Cell Sorters to place cells
    - 384-well plates
  - Droplet microfluidics (~10K cells/run)



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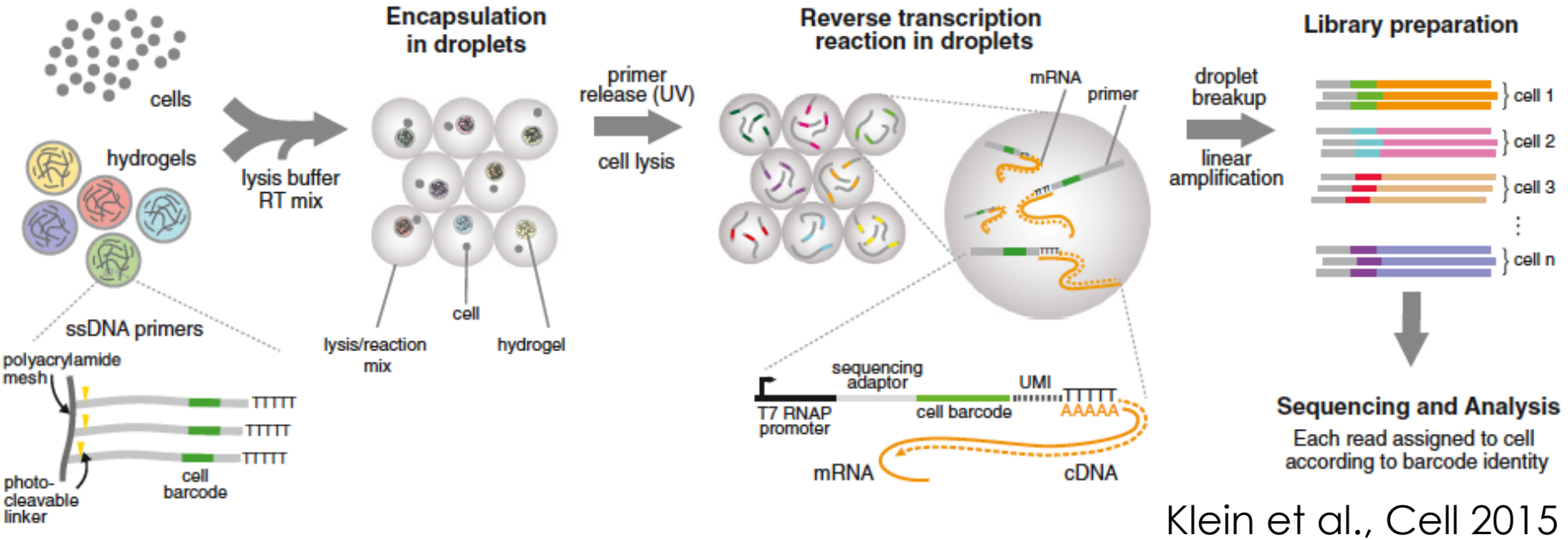
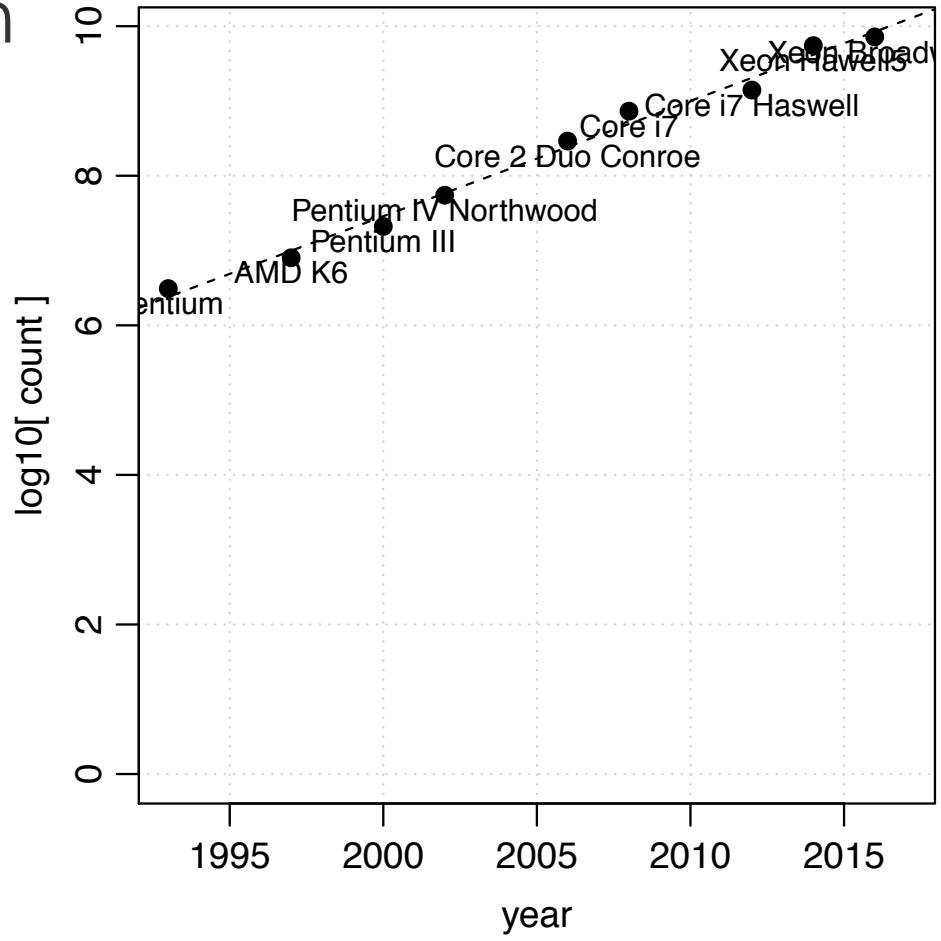


Klein et al., Cell 2015

Progress in Single-Cell RNA Sequencing Techniques

- Rapid data growth
  - Cell numbers



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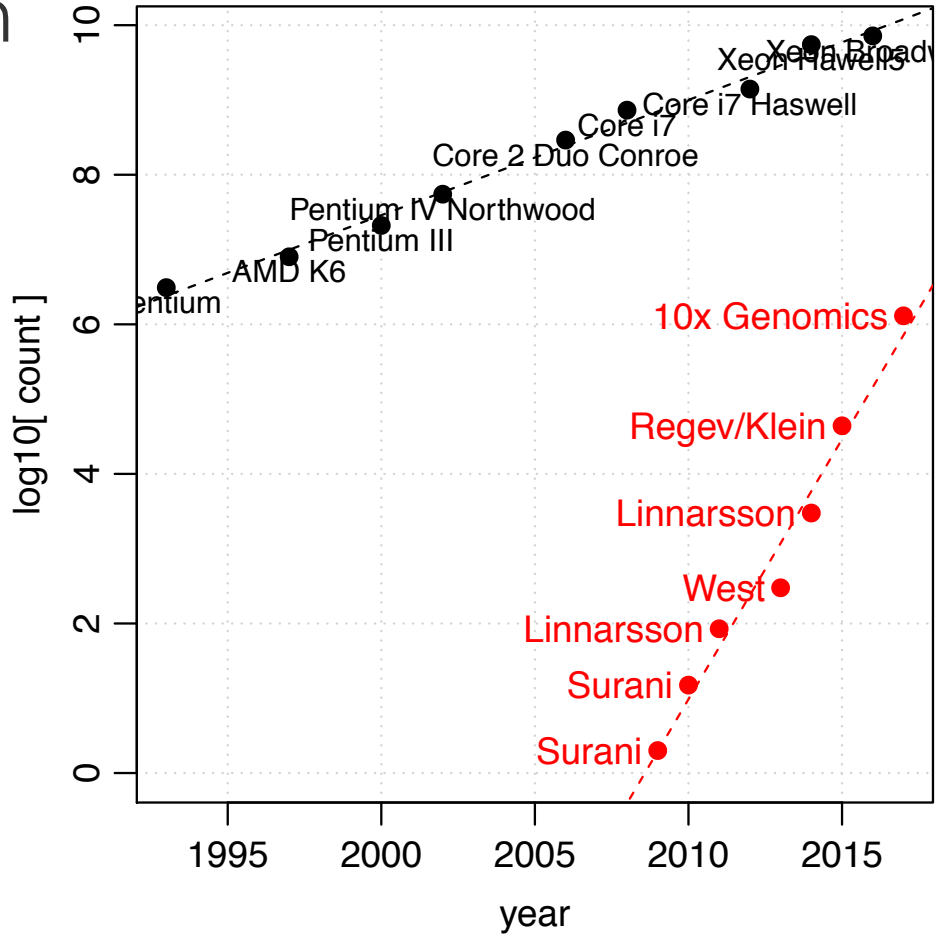
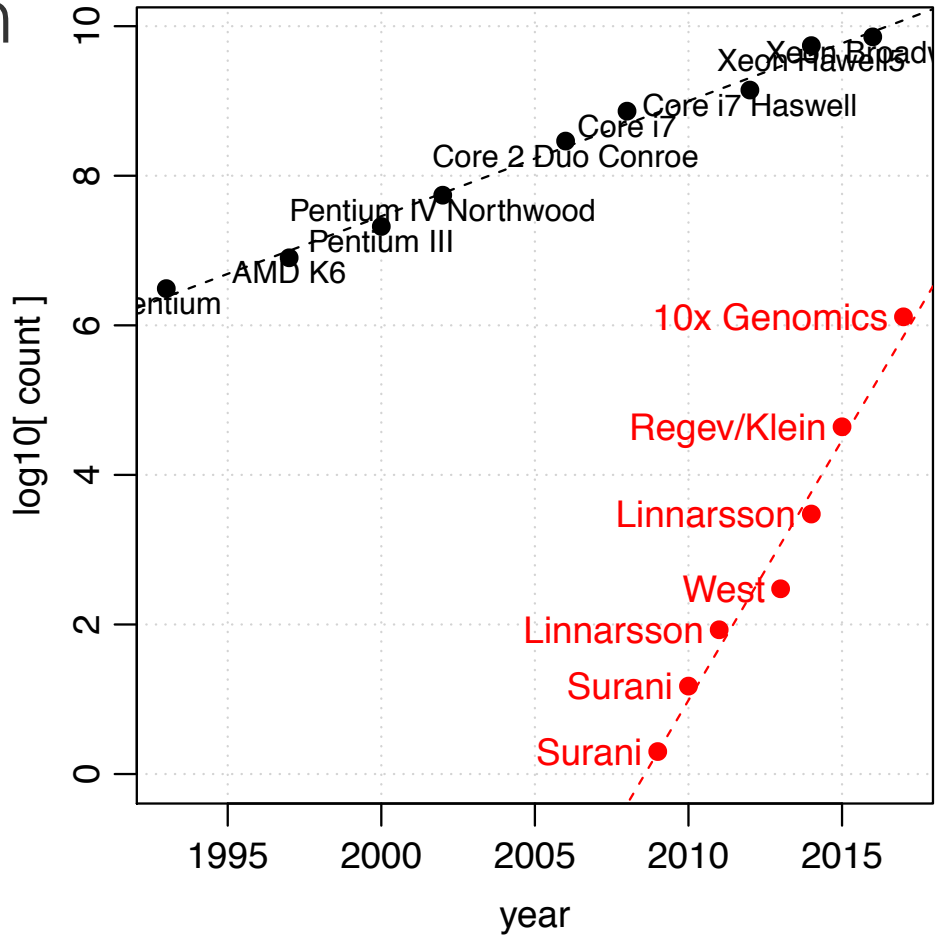


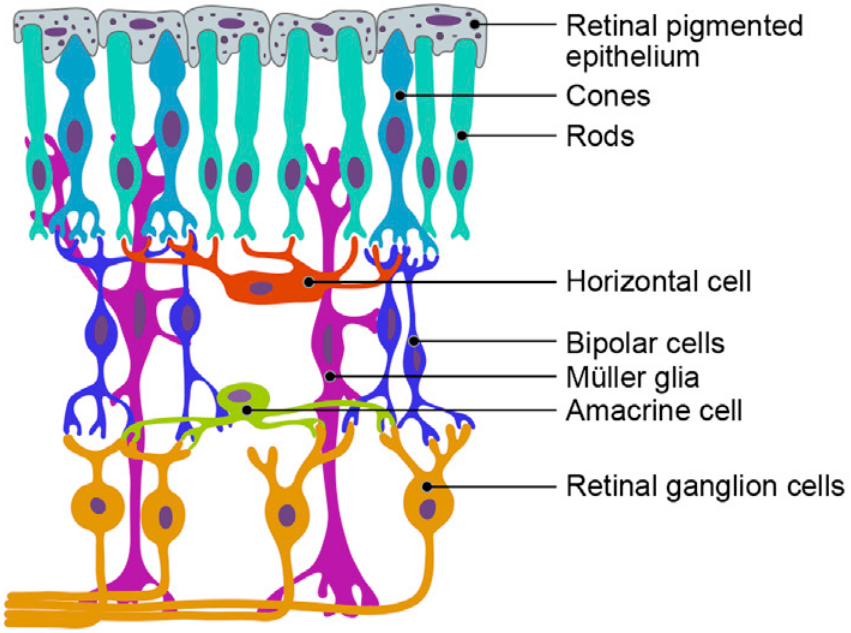
Progress in Single-Cell RNA Sequencing Techniques

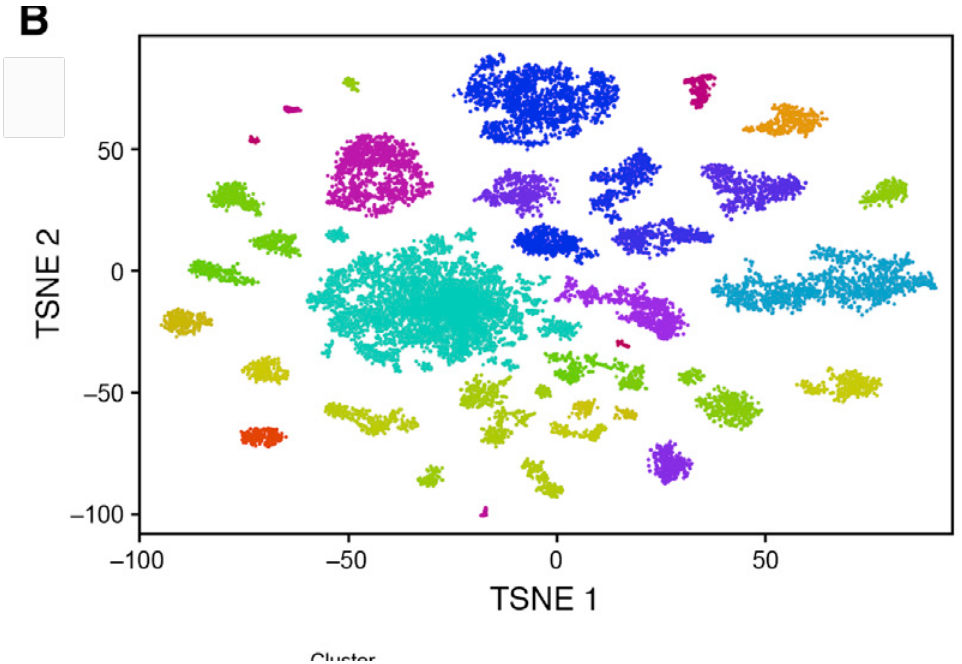
- Rapid data growth
  - Cell numbers
  - High data volumes
    - 10^5 cells by 10^4 genes
    - ~30GB sparse data
  - More samples
    - Multi-patient cohorts
    - Consortium-scale reference datasets
- Computational task
- Statistical treatment



Single-Cell RNA-seq: tissue composition

- Mouse Retina: Macosco et al. Cell 2015

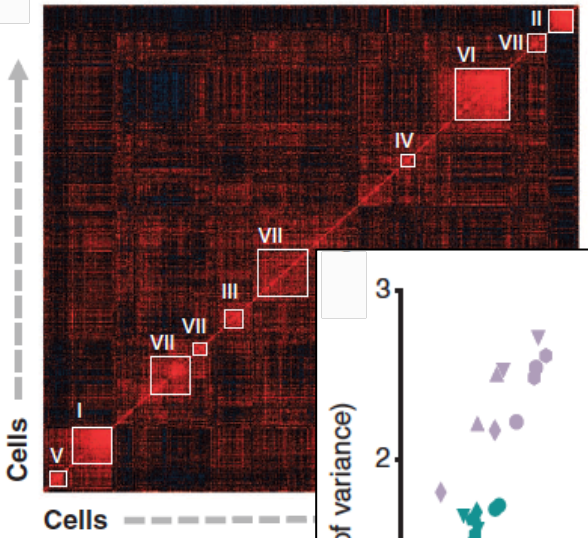




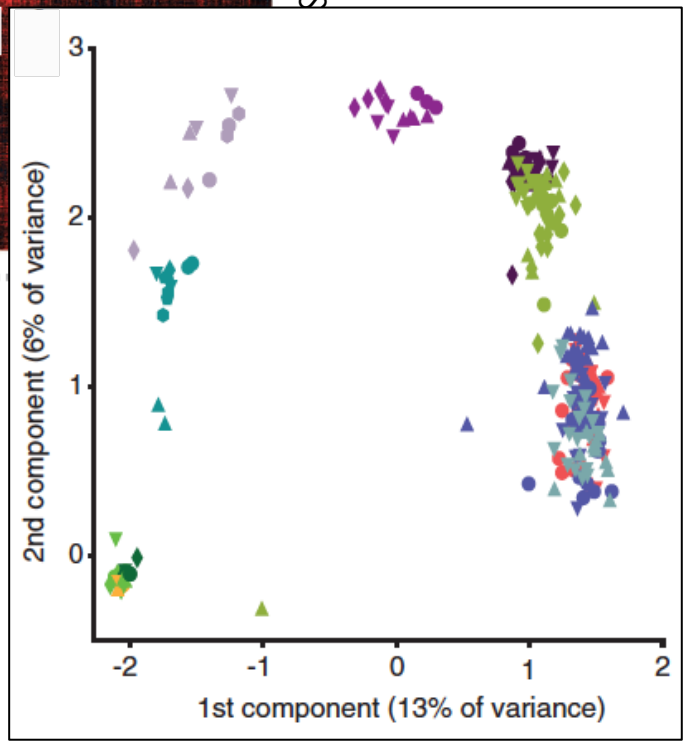
- 50 thousand cells collected using droplet method
- Recovers most known subtypes of cells

Identifying Transcriptionally Distinct Subpopulations

- Clustering
  - K-means, affinity
  - Challenging topology
  - Single, hard, partition



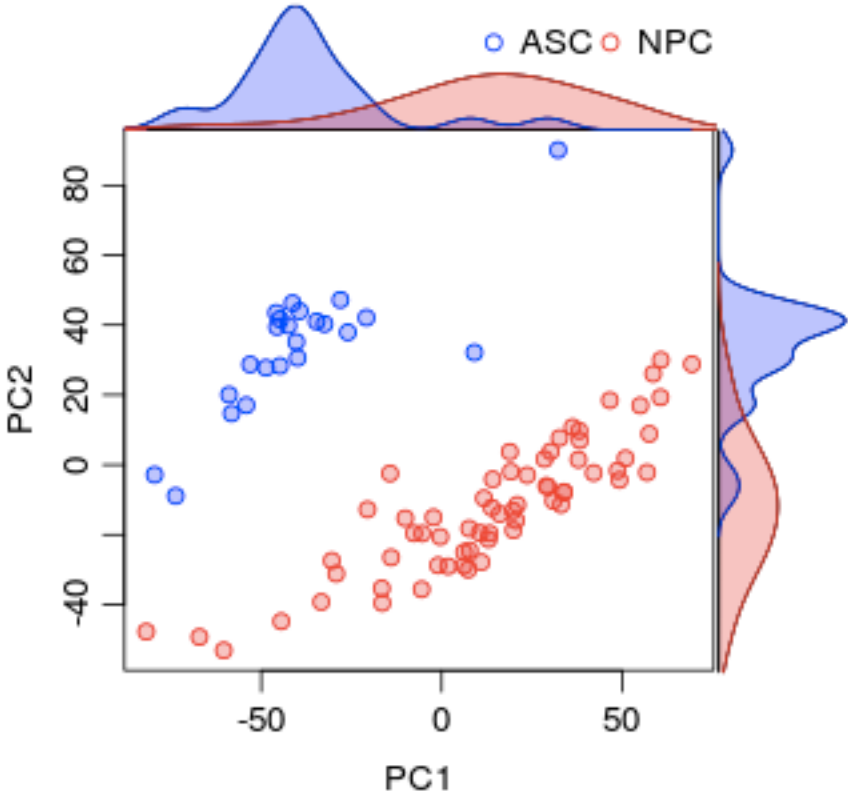
Jaitin *et al.* S

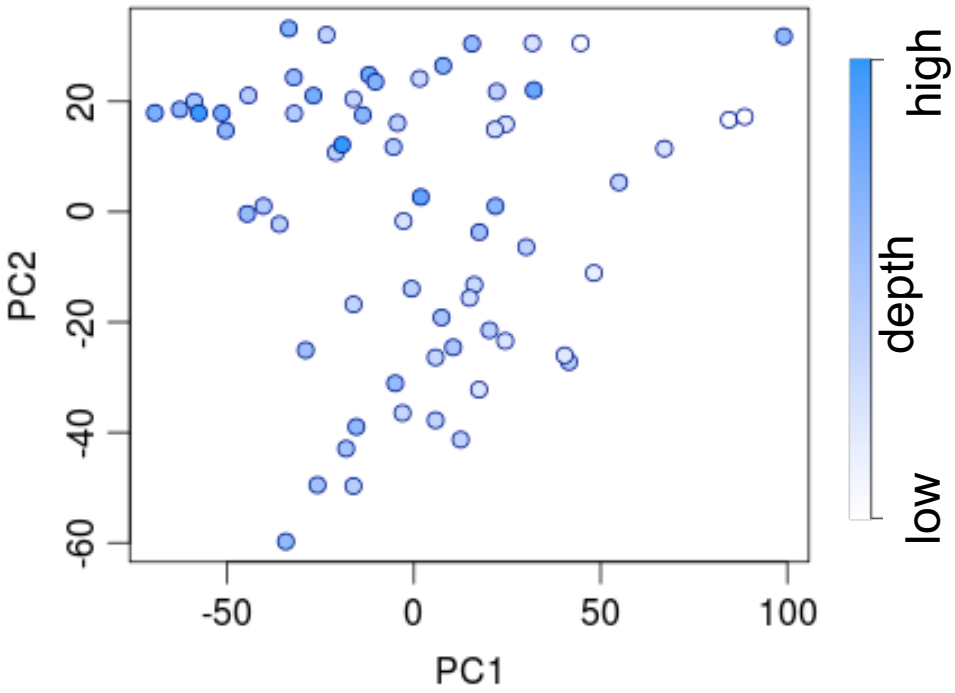


Deng *et al.* Science 2014

- Dimensionality Reduction
  - PCA, ICA
  - Can detect gradual differences
  - Sensitivity, significance

Easy and Challenging Mixtures





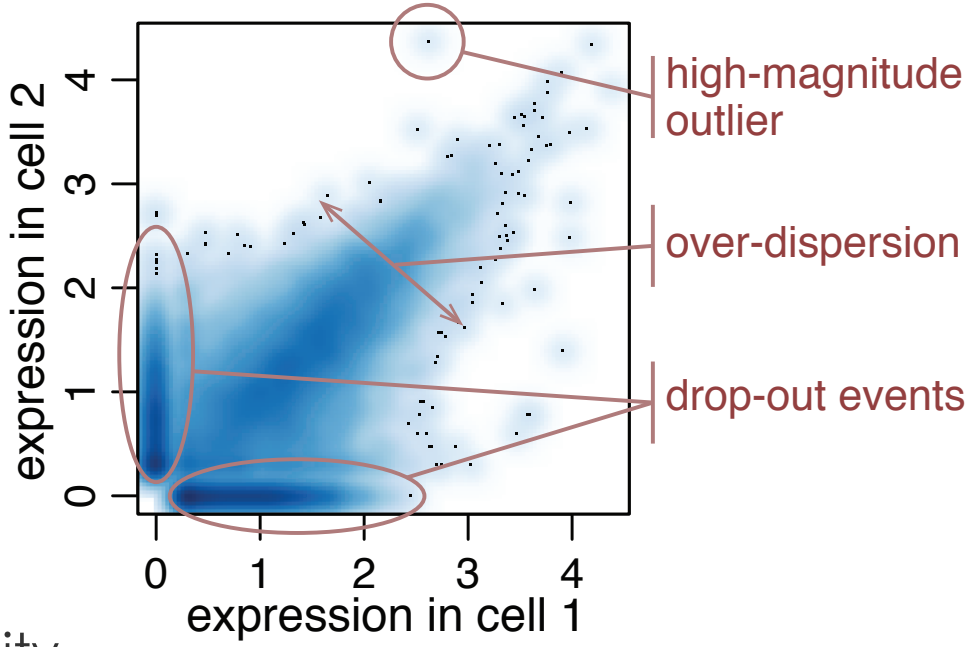
- Distant cell types easily separate
- Analysis of closely related cell types is challenging

Transcriptional Heterogeneity: Improving Statistical Sensitivity

- Get a better handle on technical noise
  - Account for possible drop-out events
  - Estimate true biological variability of a gene

Variability in single-cell RNA-seq data

- Differences between individual cells (of the same type)
- Overdispersion
- Measurement failures
- Cells vary in "quality"
- Problems for PCA, etc.
  - Non-Gaussian
  - Noisy cells form outliers
  - Can mask true heterogeneity

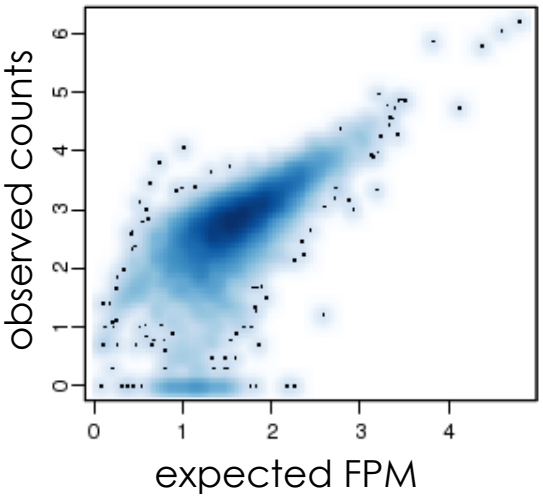


- Biological and technical variability
  - Cell-specific noise models
  - Accurate variance normalization

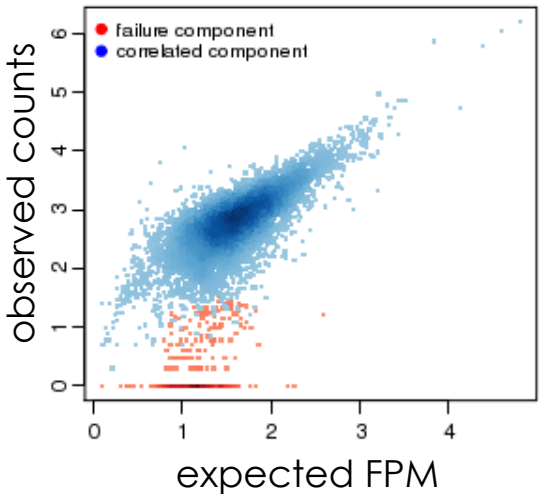
Modeling Noise of an Individual Cell

- Mixture: may amplify or drop-out, depending on expression level
- $count_i \sim NegativeBinomial(M_i)$ | $count_i \sim Poisson()$
  - M_i – expected expression magnitude for gene *i*
    (based on consensus of non-drop-out measurements within a group)
- Mixing between the two options depends on the magnitude itself
  - probability of drop-out is modeled using logistic regression

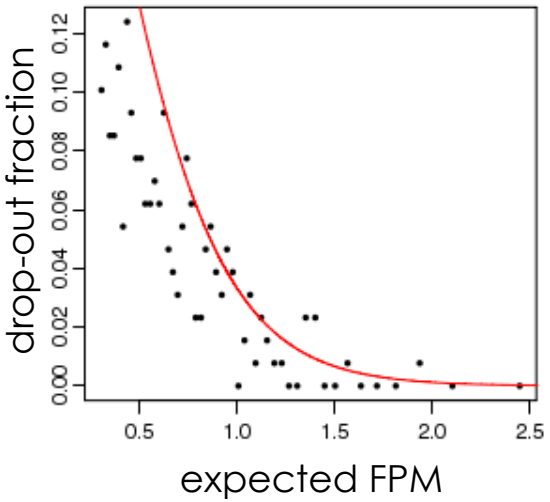
observed vs. expected



mixture fit



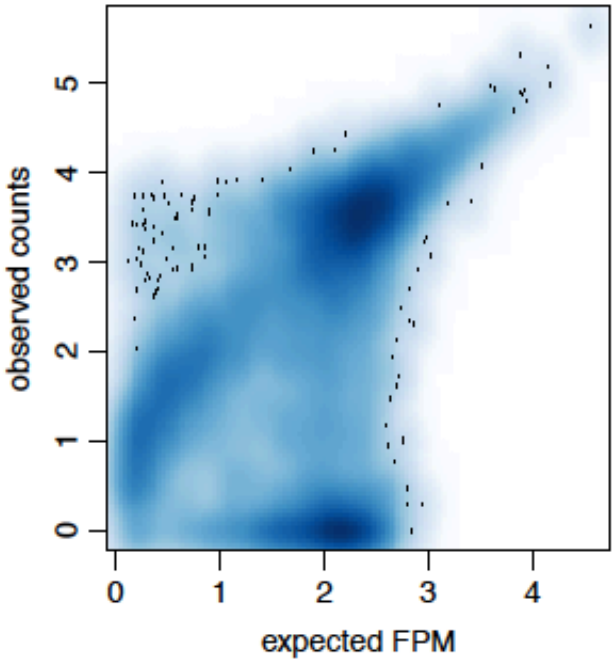
drop-out dependency

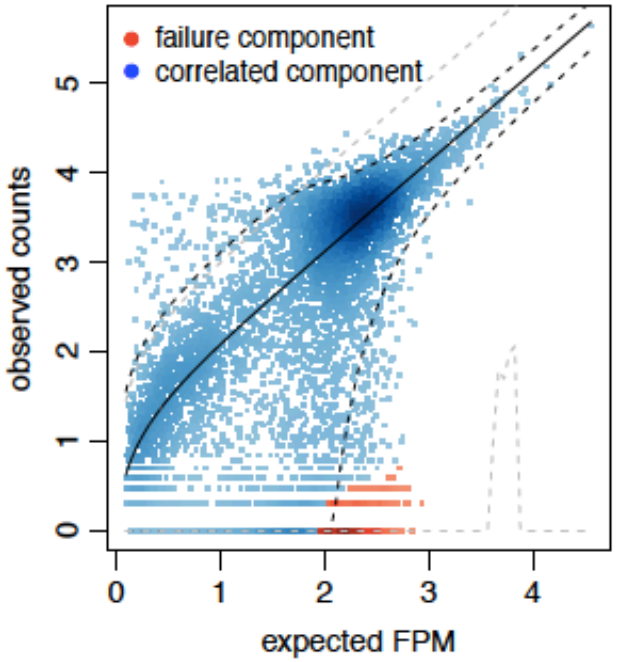


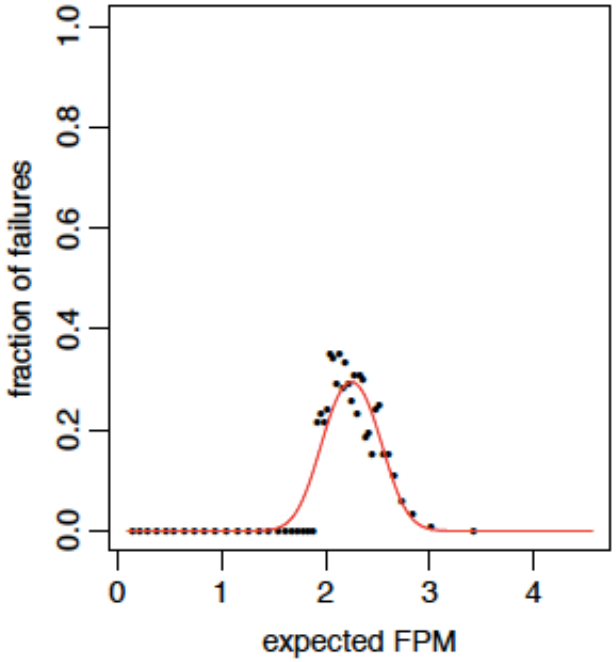
Kharchenko *et al.* Nature Methods 2014

Modeling Noise of an Individual Cell

- Fluidigm C1
  - Generally high overdispersion
  - Compressed dynamic range
  - Exceptionally high amplification rate at low magnitudes

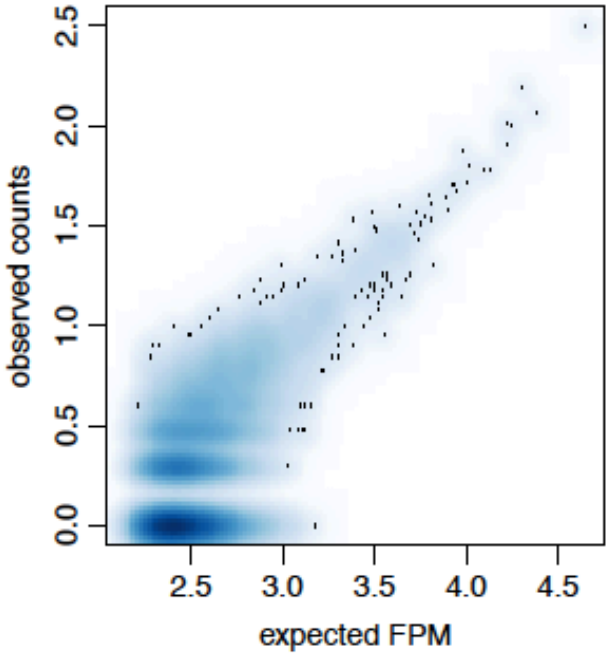


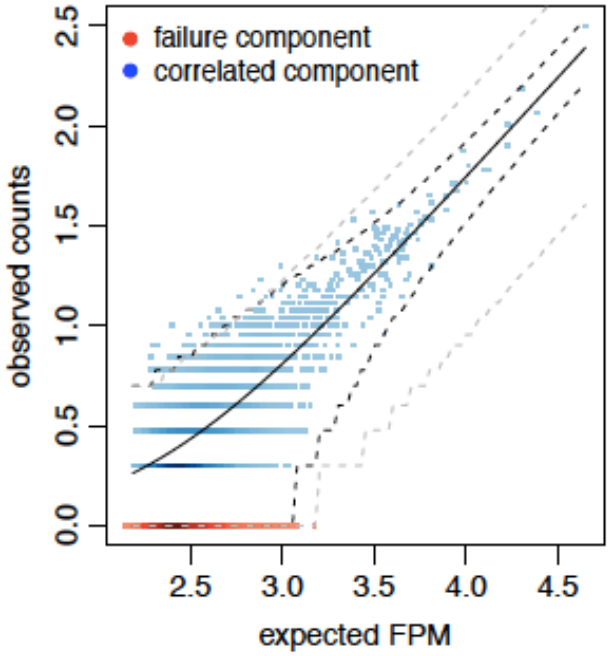


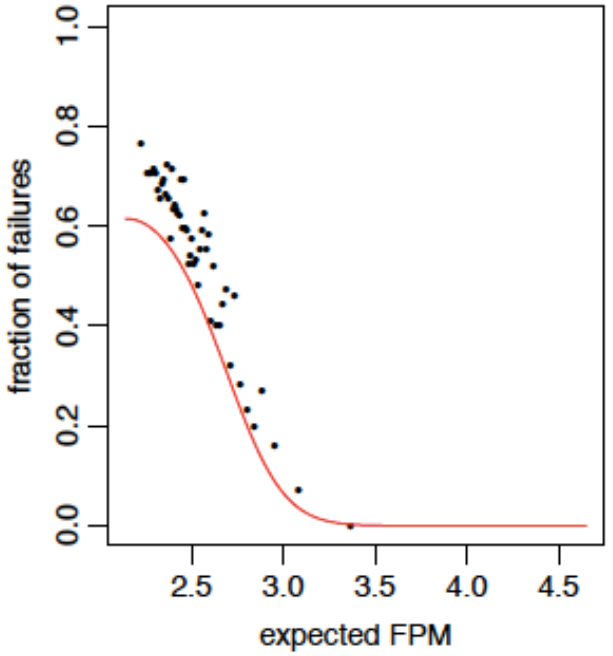


Modeling Noise of an Individual Cell

- Indrop
  - Relatively sparse: 20-50k UMIs/cell, 3-5K genes/cell
  - Much lower overdispersion
  - Elevated drop-out rates due to inefficient capture and RT
  - … but with many more cells (3K/batch, multiplexed)

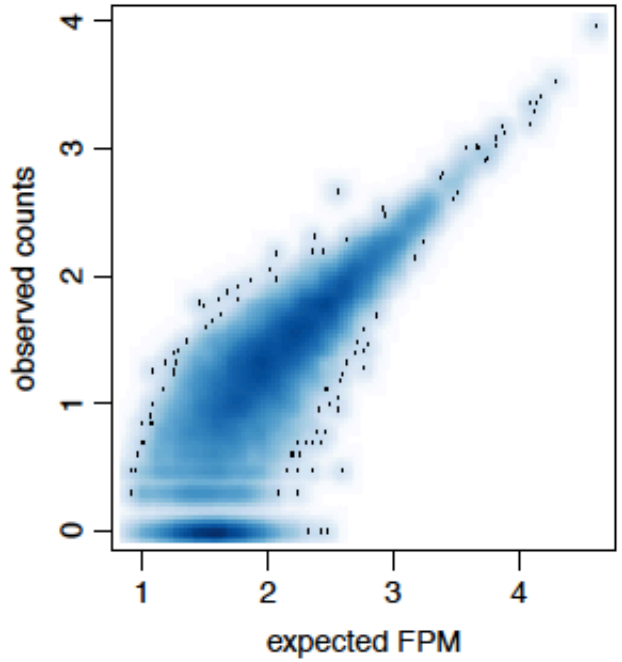


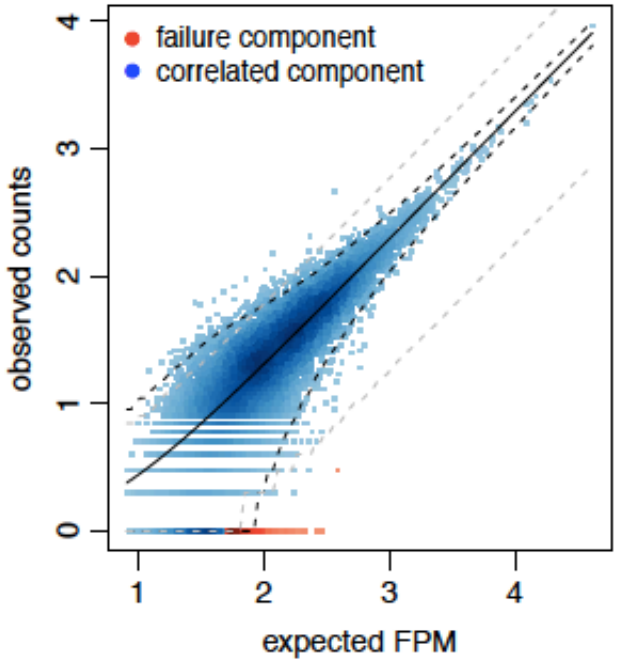


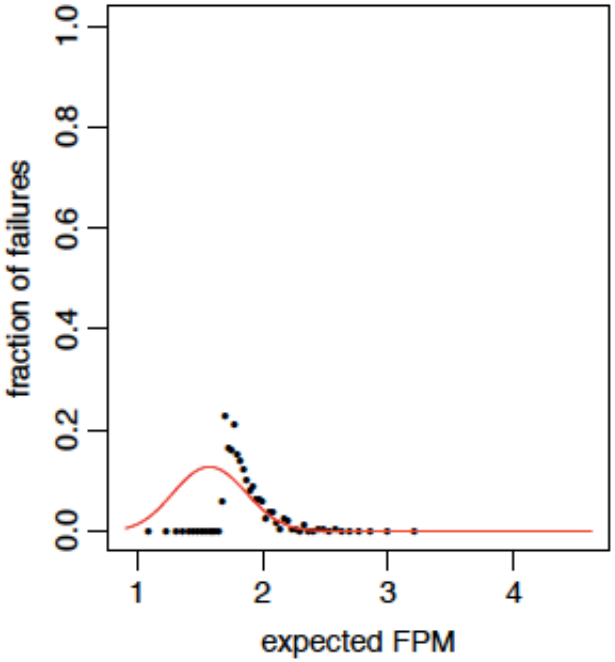


Modeling Noise of an Individual Cell

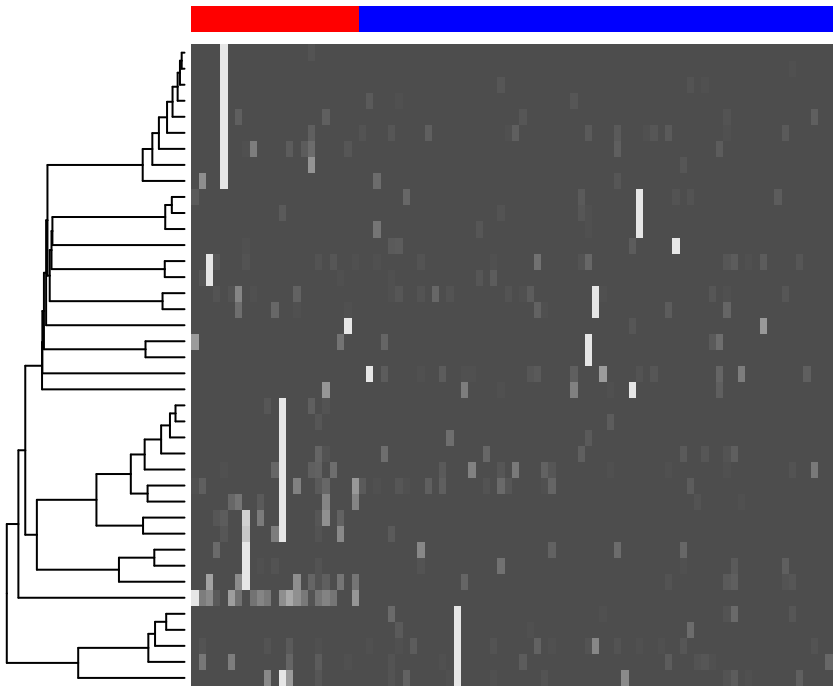
- Optimized Smart-seq2
  - High coverage: 200k reads/cell
  - Wide dynamic range
  - Very low drop out rates
  - Very low overdispersion (comparable with UMI-based techniques)



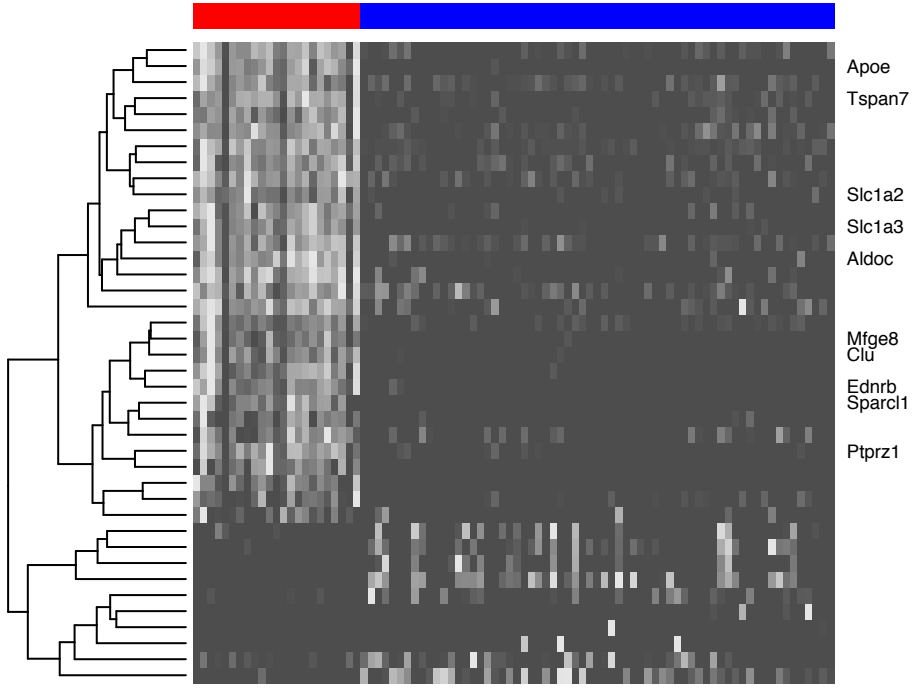




Robust inference of most variable genes



Brennecke et al. Nat. Methods 2013



Cell-specific models (PAGODA)

Robust inference of most variable genes

Astrocytes NPCs Astrocytes NPCs

PLOS | COMPUTATIONAL BIOLOGY

RESEARCH ARTICLE

BASiCS: Bayesian Analysis of Single-Cell Sequencing Data

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1 MRC Biostatistics Unit, Cambridge Institute of Public Health, Cambridge, United Kingdom, 2 EMBL European Bioinformatics Institute, Cambridge, United Kingdom

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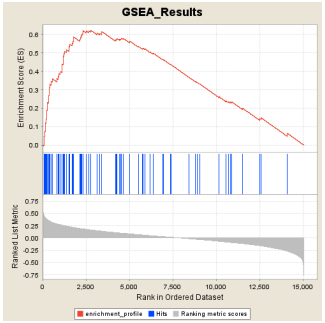
Brennecke et al. Nat. Methods 2013

Cell-specific models (PAGODA)

Transcriptional Heterogeneity: Improving Statistical Sensitivity

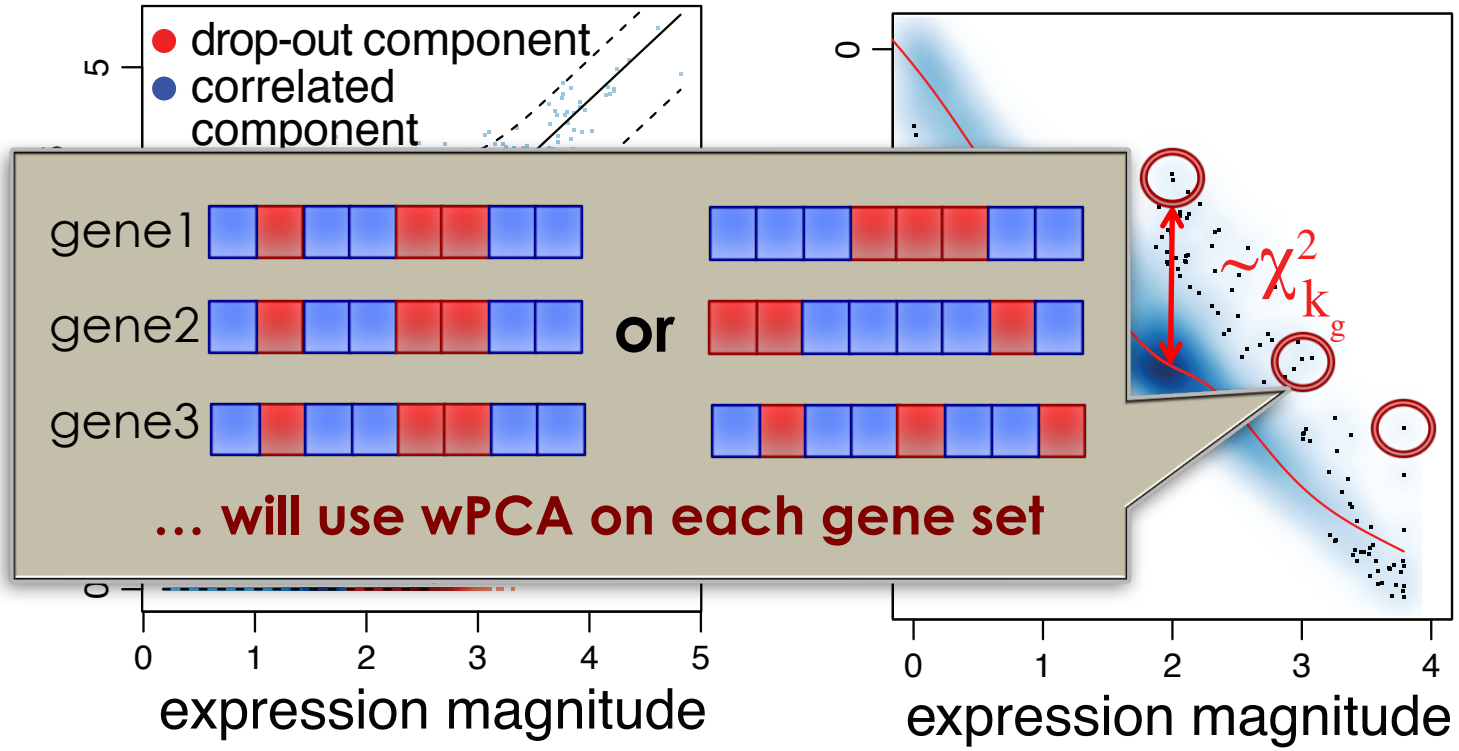
- Get a better handle on technical noise
  - Account for possible drop-out events
  - Estimate true biological variability of a gene

- Look for broader patterns of variability
  - Gene sets: annotated pathways, computationally derived sets
    - GO statistics, GSEA, widely used
  - Coordinated patterns of variability of genes linked to function/phenotype – a strong signal
  - Increases statistical power



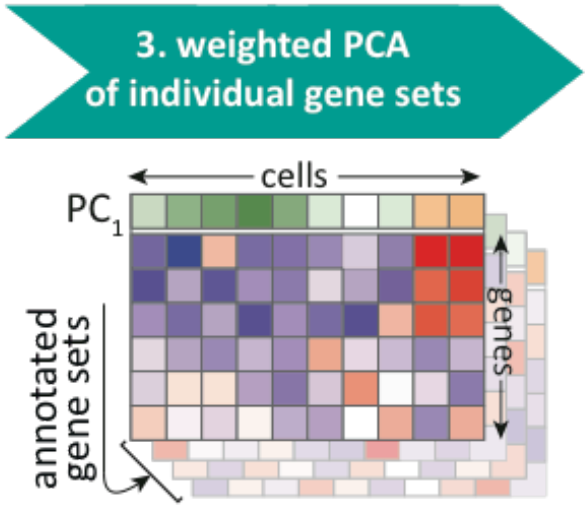
Variance Normalization

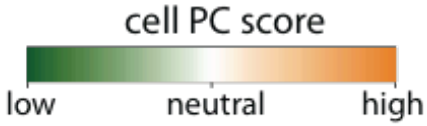
- Account for cell- and gene-specific uncertainty
  - Cell-specific error models. Expression-dependent size
  - NB/Poisson sample variance ➔ χ^2 statistic
- Account for expression magnitude dependency
  - Adjusting with local regression fit

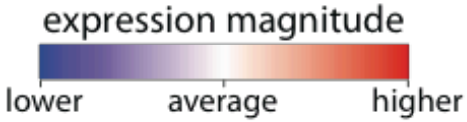


Kharchenko *et al.* Nature Methods 2014

Heterogeneity Overview: Pathway Clustering







Fan *et al.* Nat. Methods 2016

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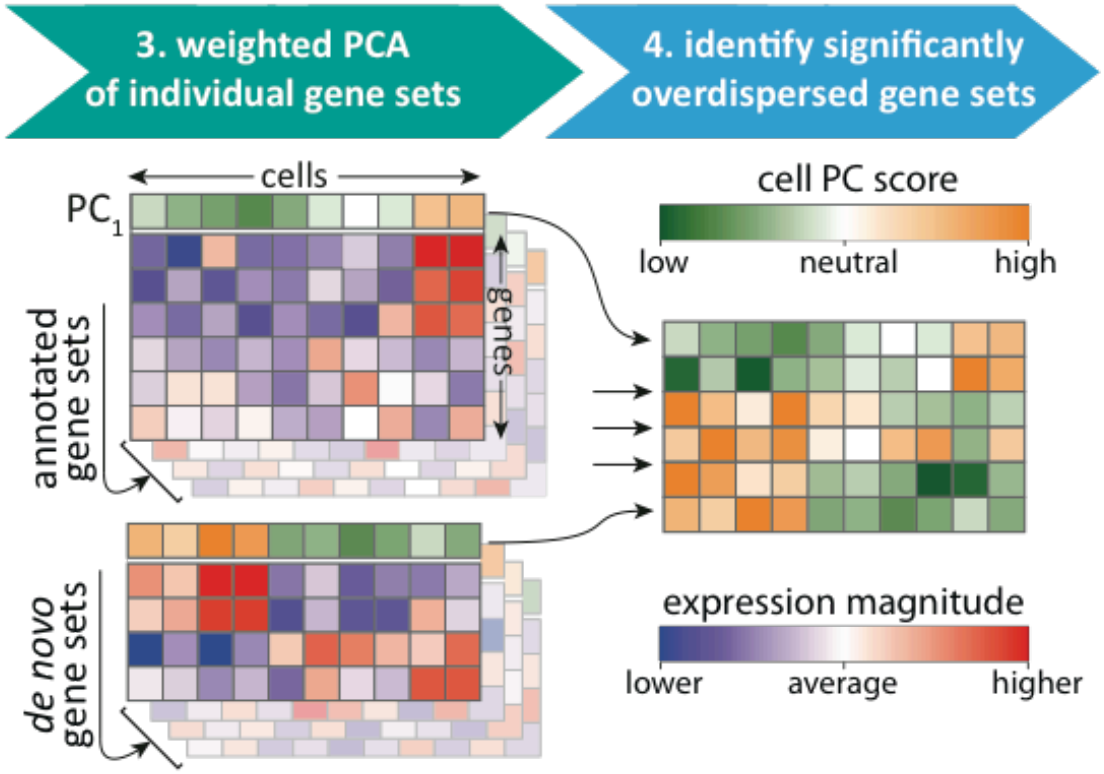

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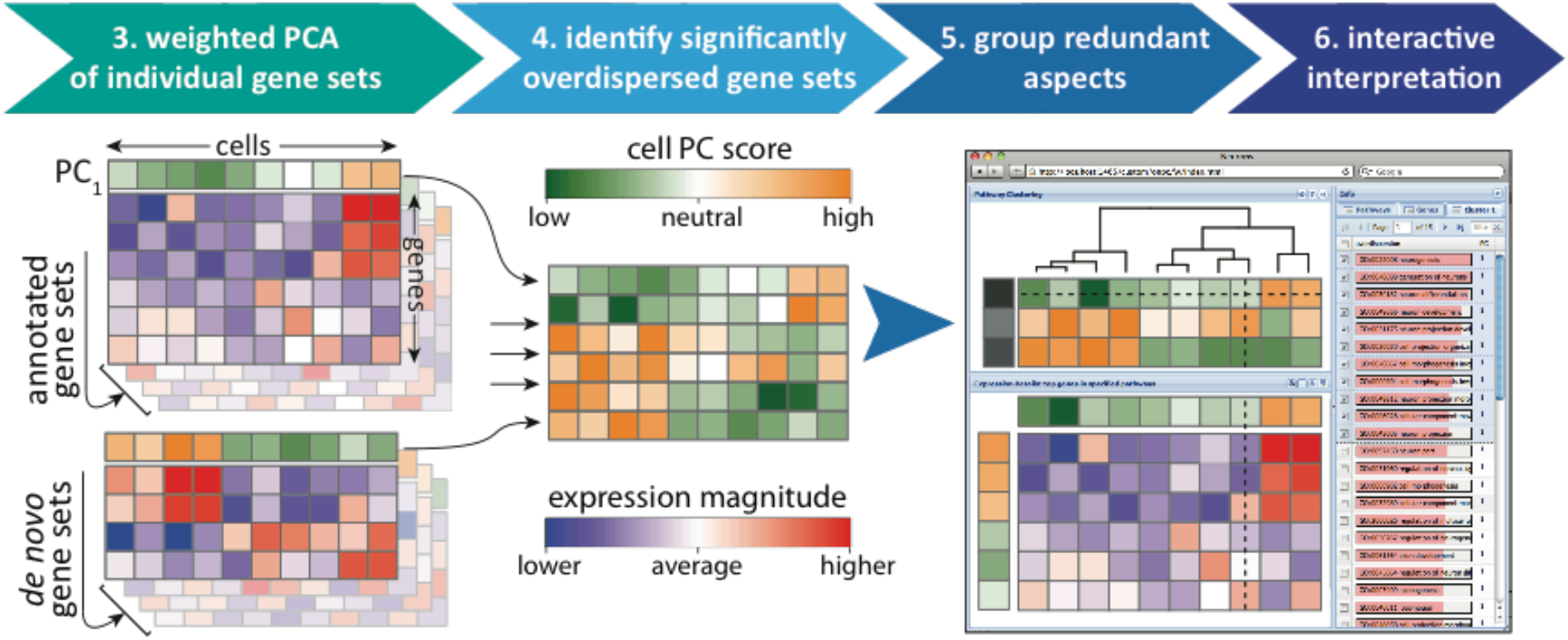
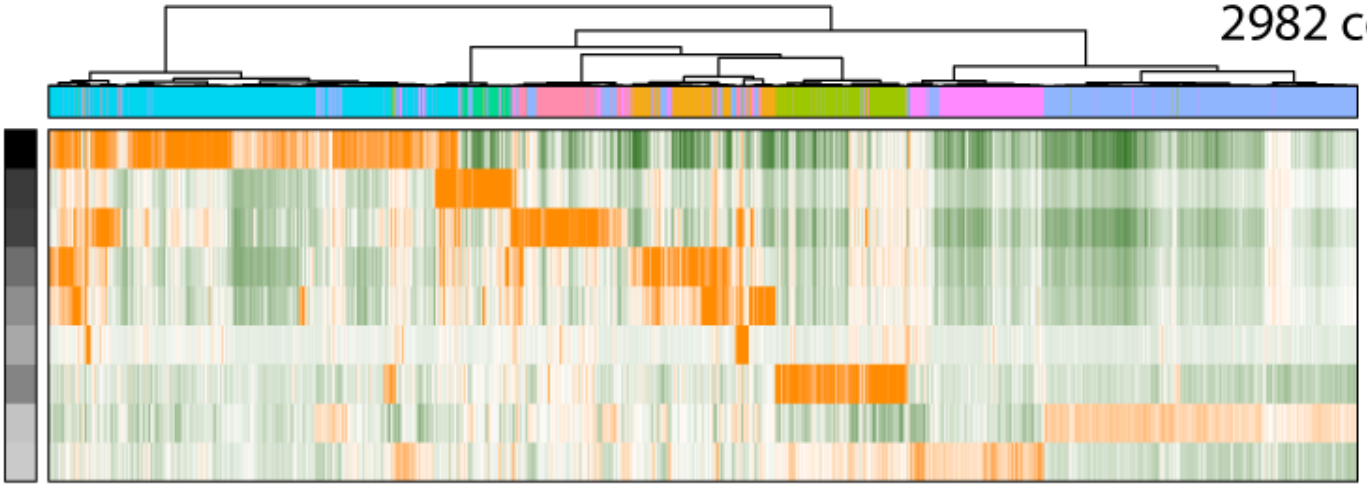


Fan *et al.* Nat. Methods 2016

Heterogeneity in Mouse Cortex and Hippocampus

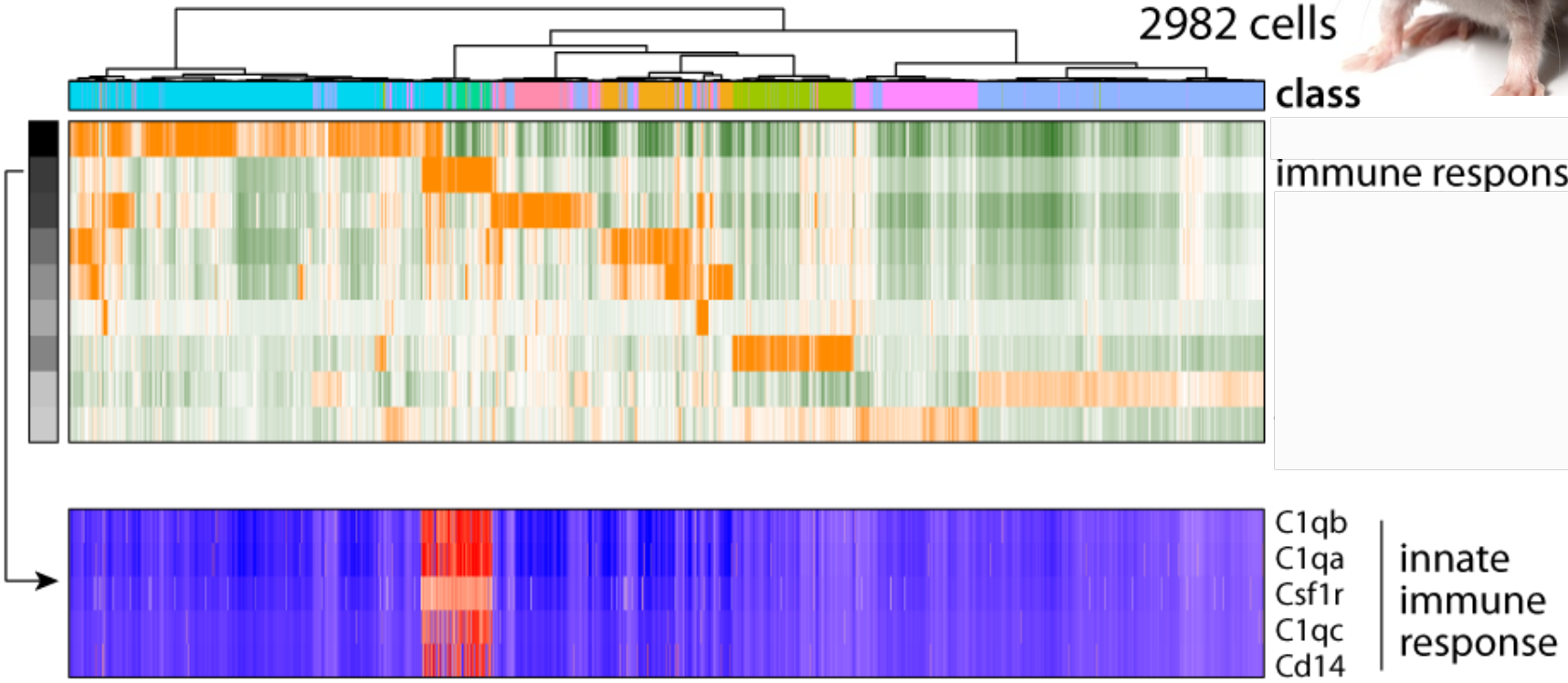


data from Zeisel *et al.*, Science 2015

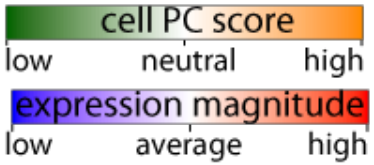
cell PC score
low neutral high

expression magnitude
low average high

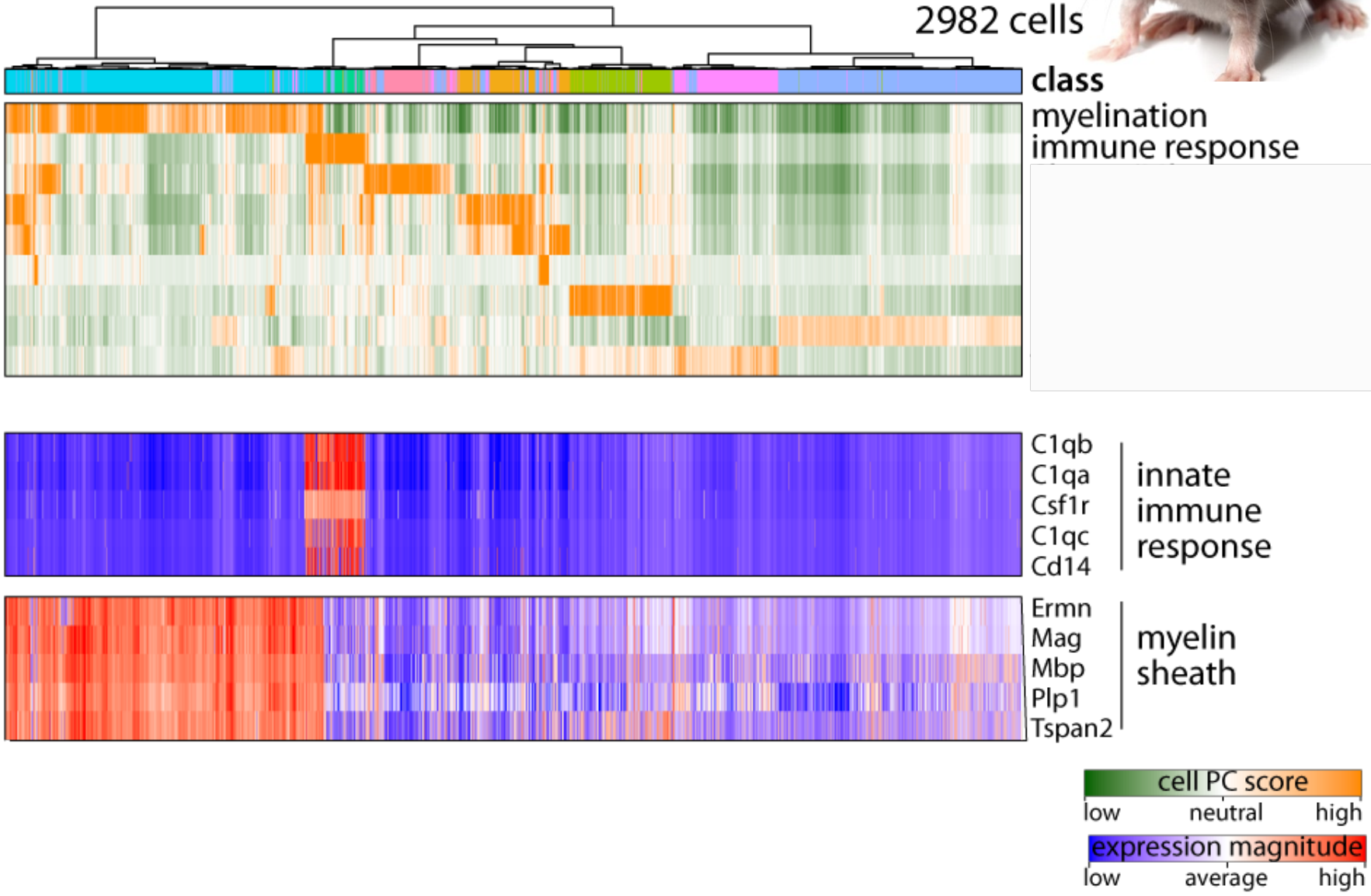
Heterogeneity in Mouse Cortex and Hippocampus



data from Zeisel *et al.*, Science 2015



Heterogeneity in Mouse Cortex and Hippocampus



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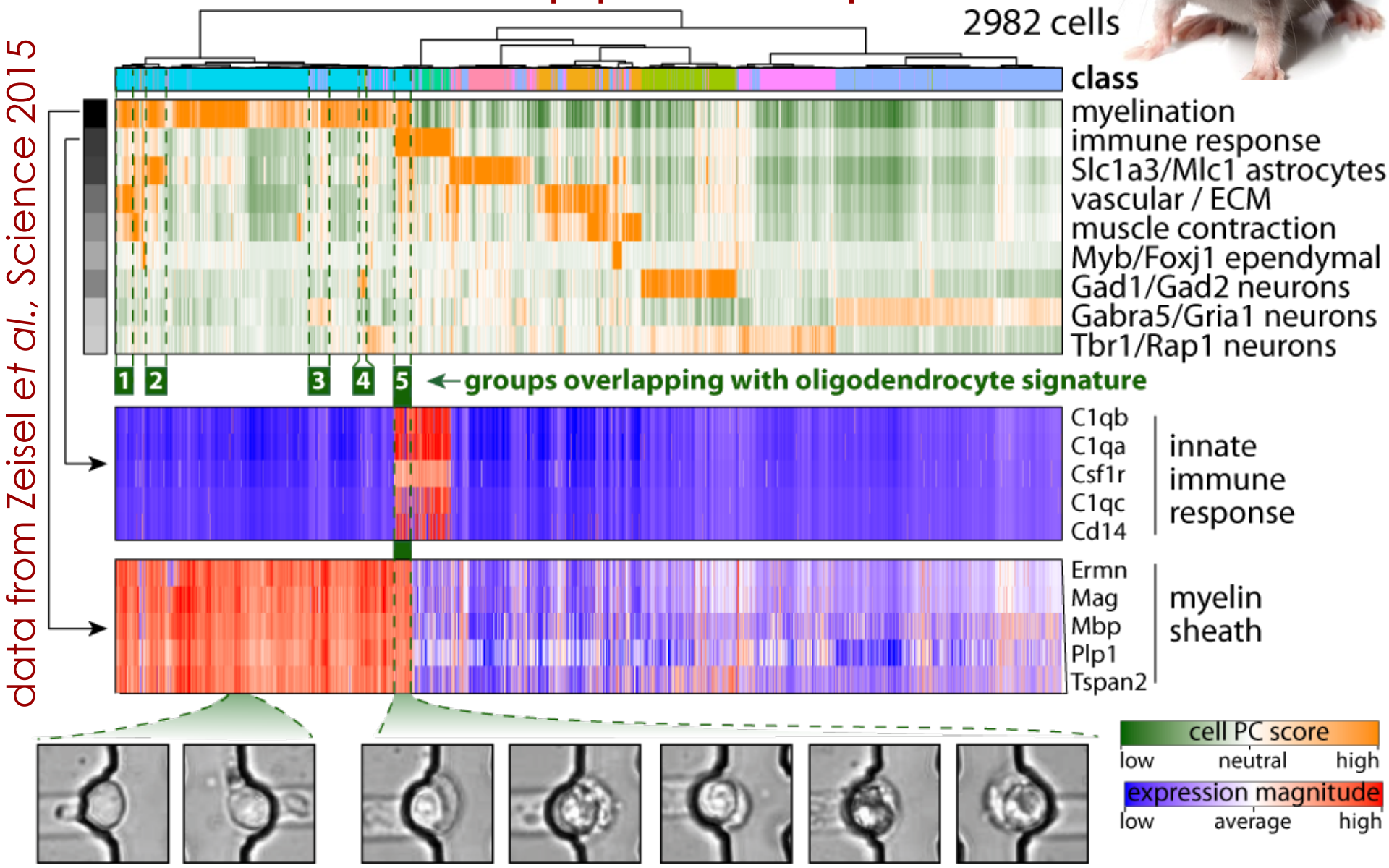
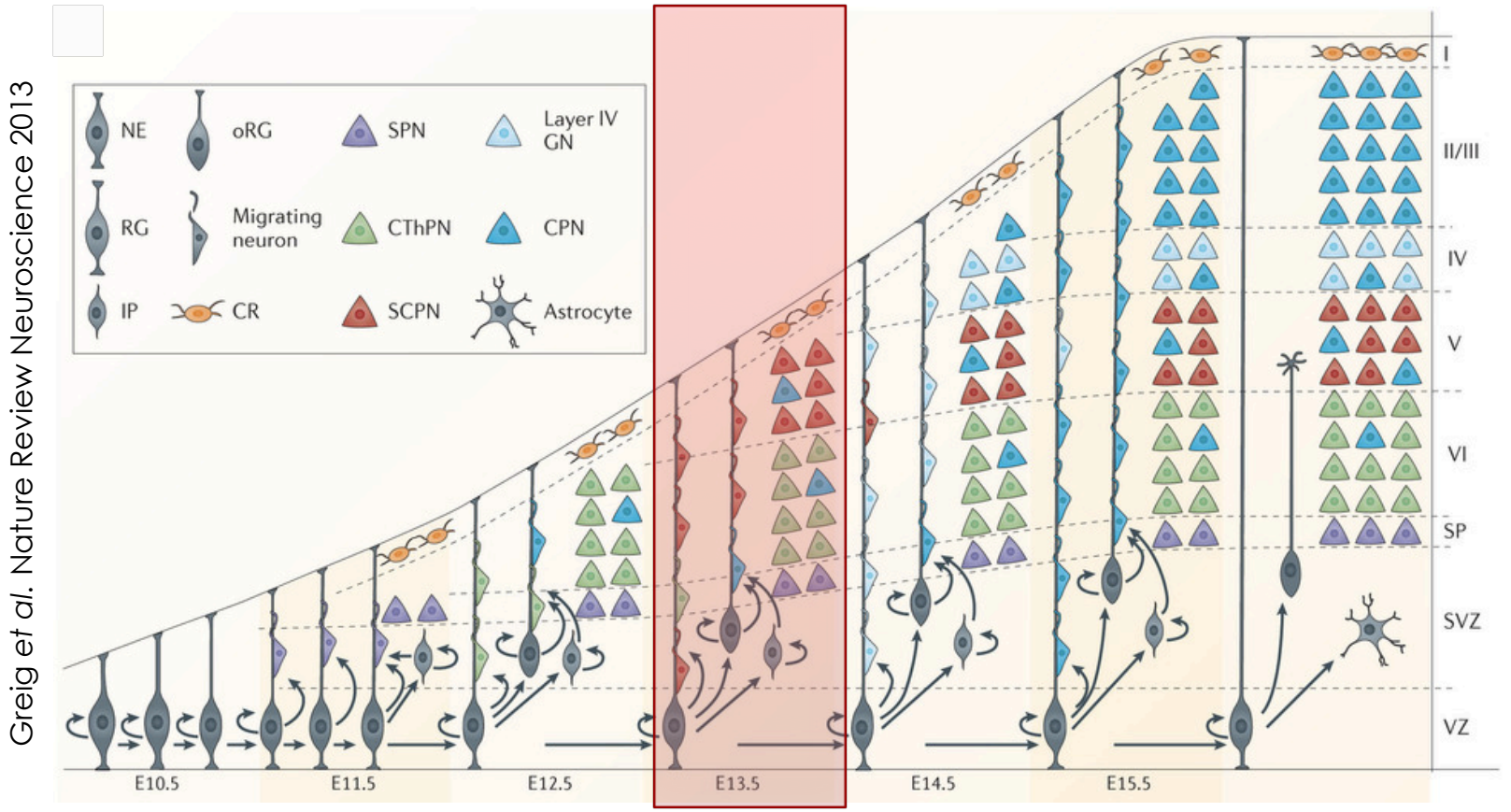
Heterogeneity in Mouse Cortex and Hippocampus



data from Zeisel *et al.*, Science 2015

Cortical Neuron Progenitors

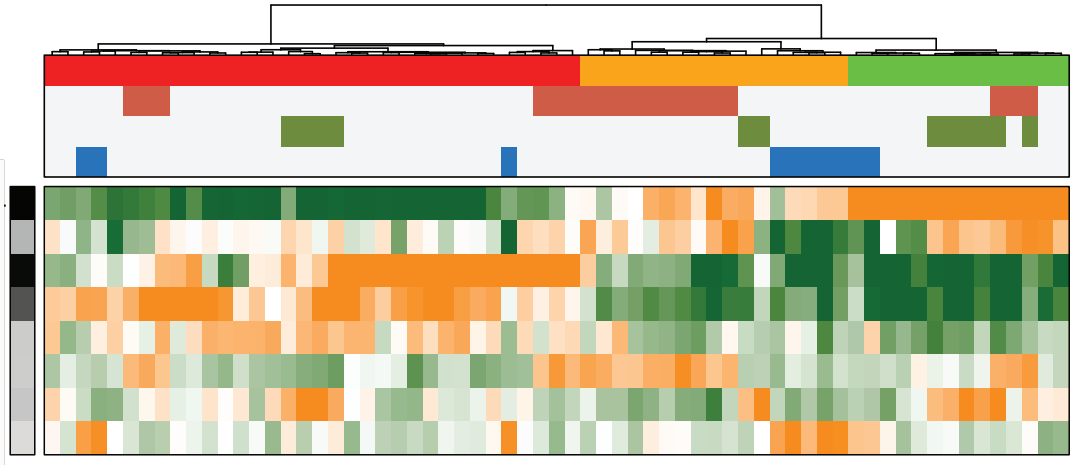
- Formation of Cortical Layers



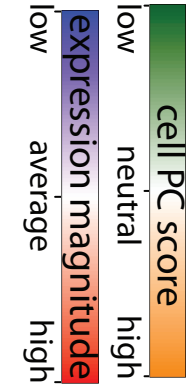
Greig *et al.* Nature Review Neuroscience 2013

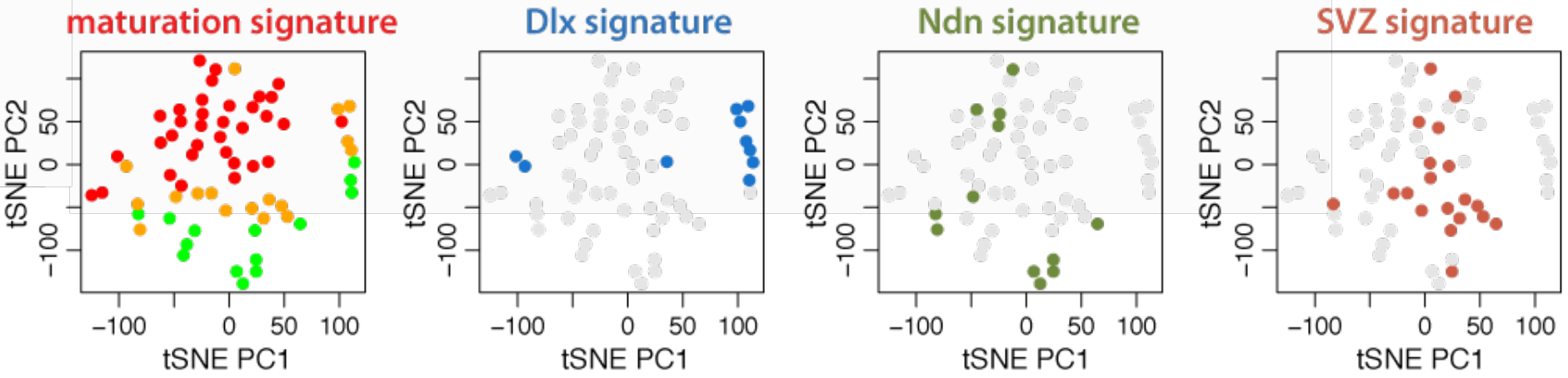
- Neuronal progenitor cells (NPCs)
  - Purified from E13.5. ~84 NPCs, 25 ASCs

More challenging cells: NPC



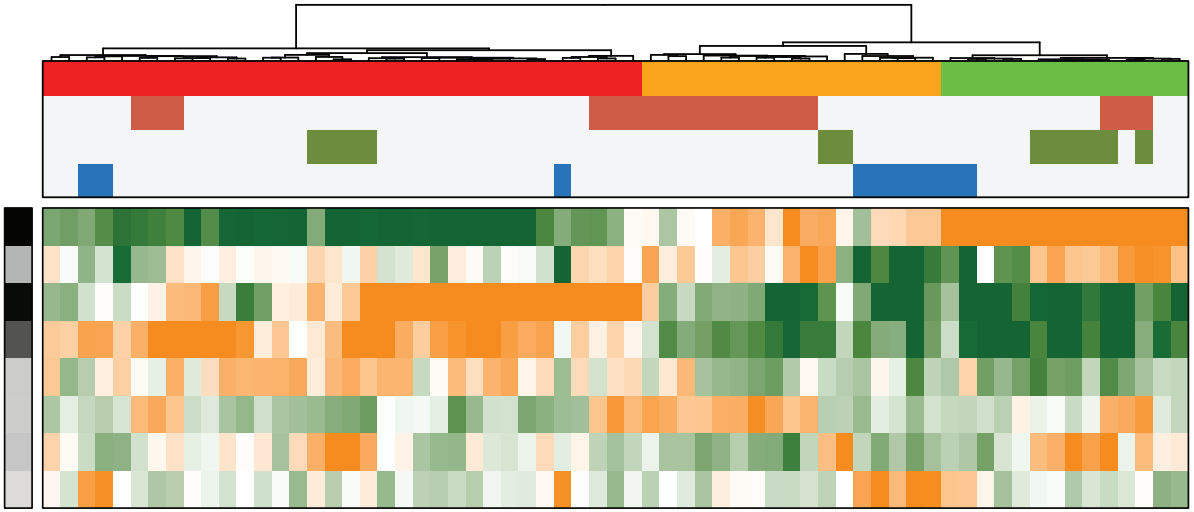
early / intermediate / mature
SVZ signature / Eomes
Ndn-expressing
Dlx signature
nervous system development
hindbrain development / Nfib
mitosis (M phase)
DNA replication (S phase)
Prdx4 / Mest - driven
neurogenesis regulation / Eomes
Ndn - expressing
proximal-distal / Dlx





tSNE re-analysis

Cortical Neuron Progenitors



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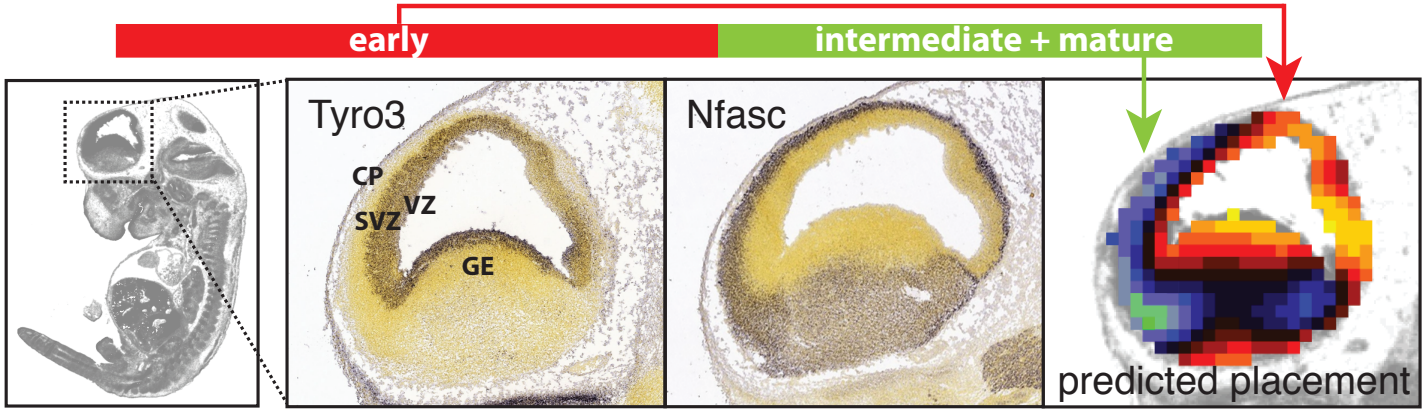
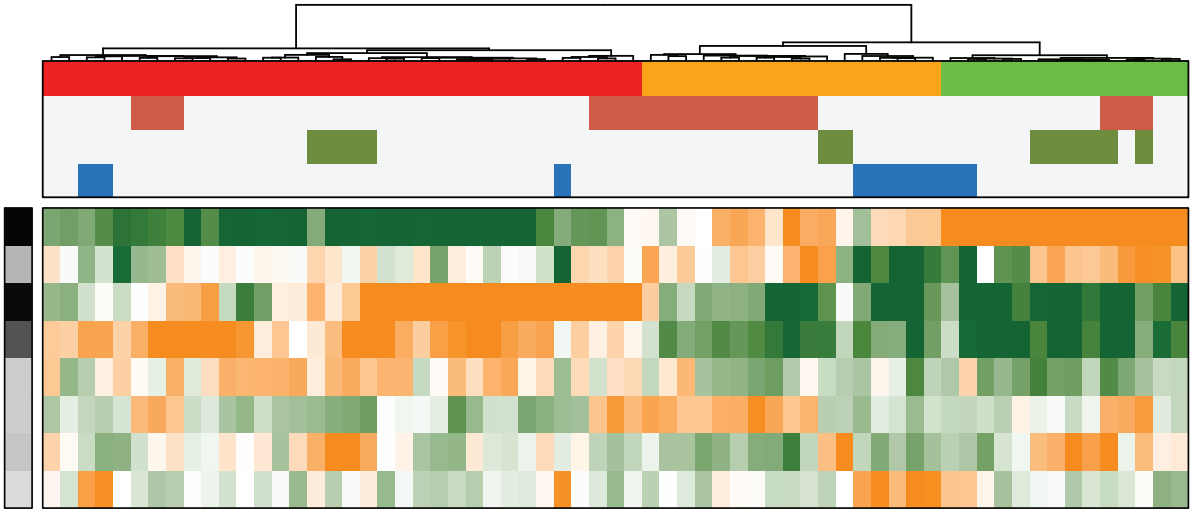


early/mature NPC density
early
mature

cell PC score
low
neutral
high

expression magnitude
low
average
high

Cortical Neuron Progenitors



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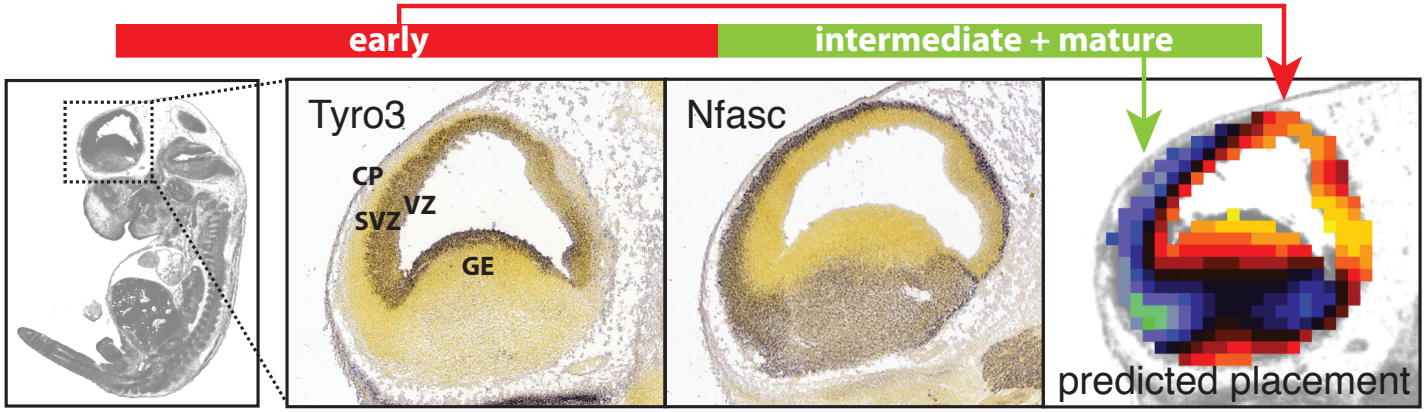

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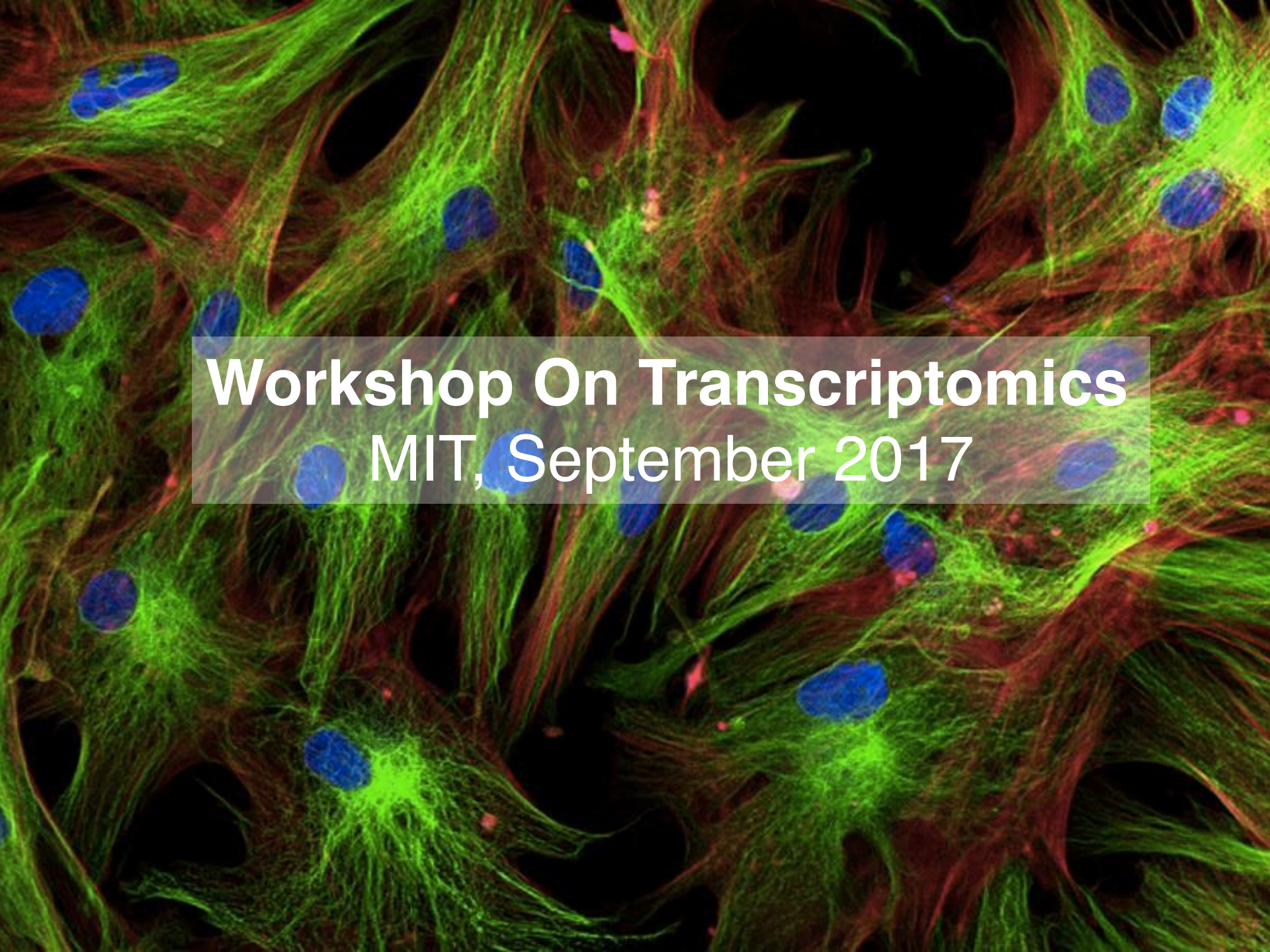
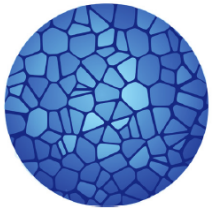
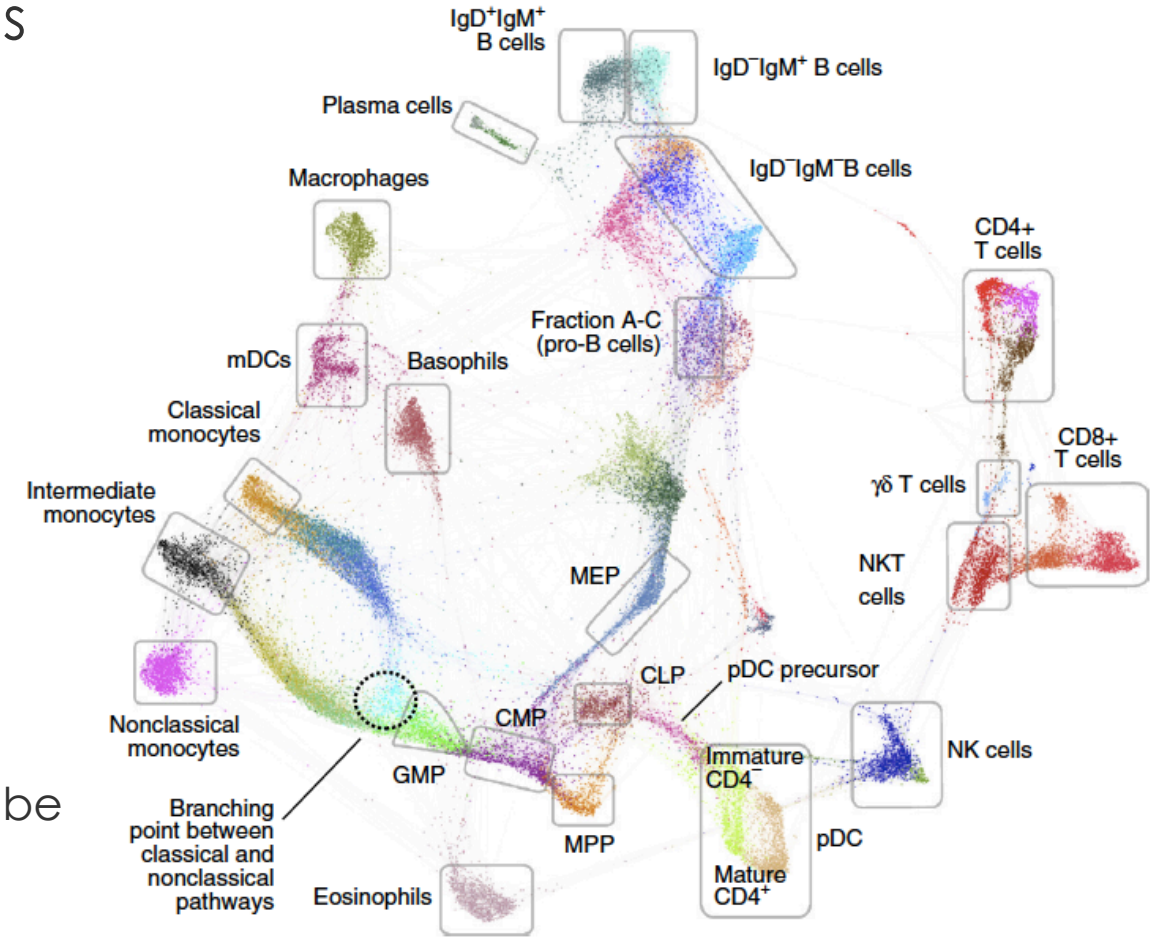

Workshop On Transcriptomics
MIT, September 2017

Cell Clusters: Units of Interpretation

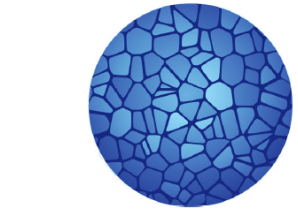


- Aim: Group similar cells
- Clusters are used for
  - Illustrating heterogeneity
    - Visualization, Navigation
  - Assigning interpretation
  - Estimating pooled signals
    - Differential expression
- Many clustering methods
  - Cluster assignments vary
  - Key clusters/groups tend to be stable

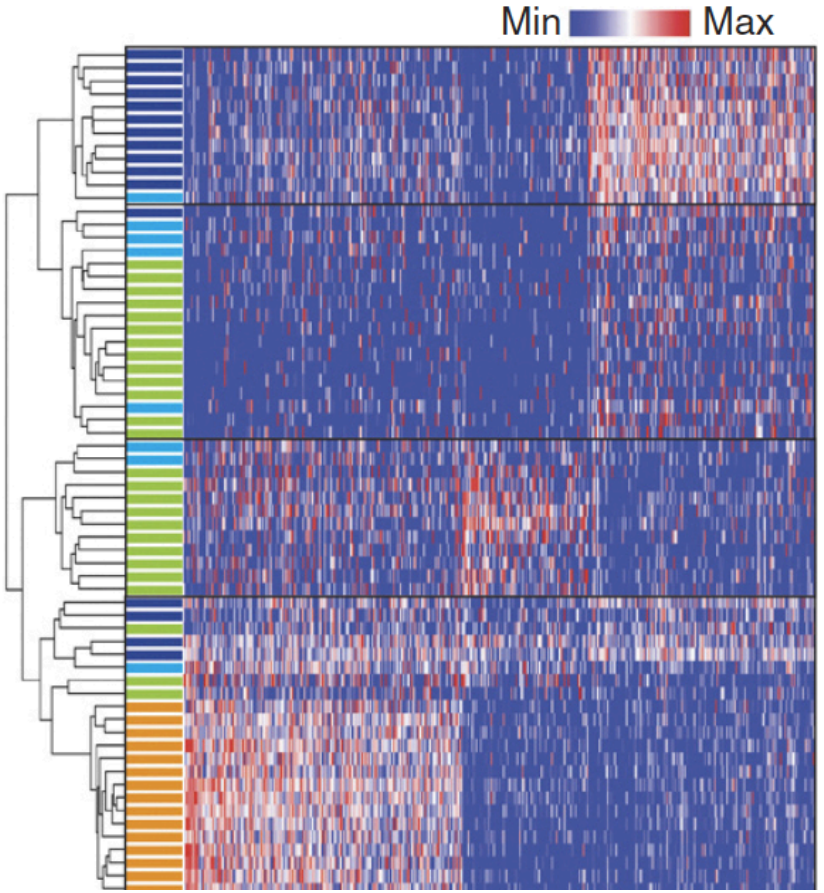


Samusik *et al. Nat. Methods*'16

Clustering Approaches

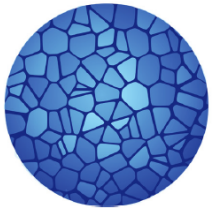


- Direct clustering methods
  - Hierarchical clustering

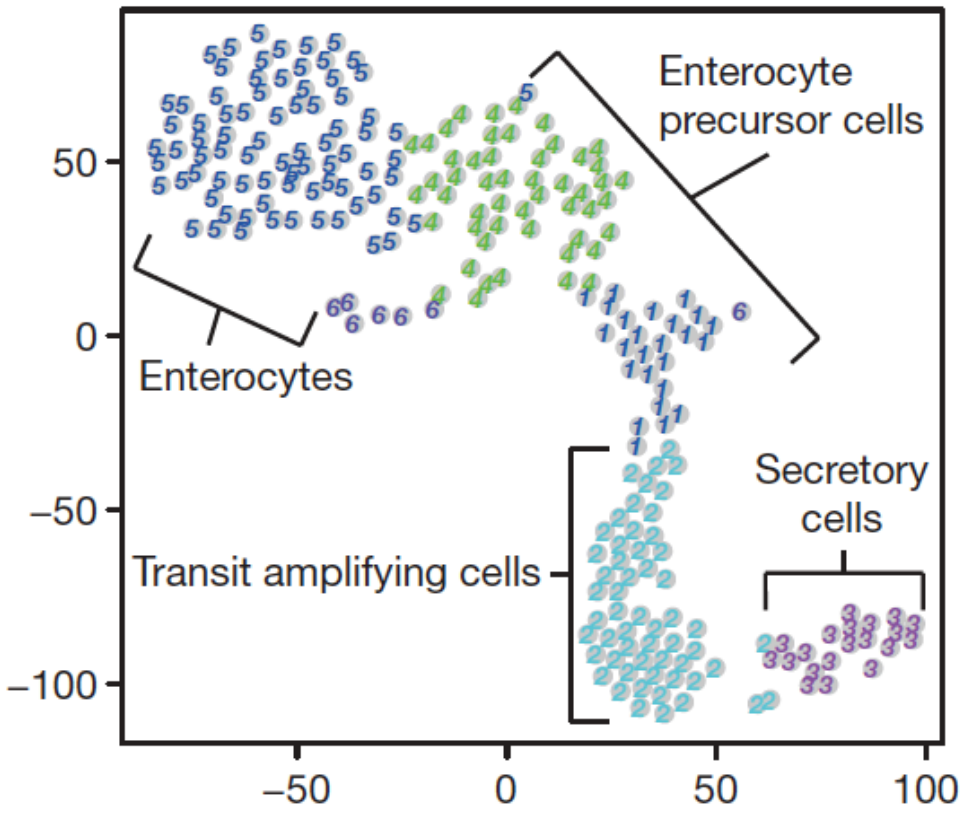


Pollen *et al., Nat. Biotech*'14

Clustering Approaches



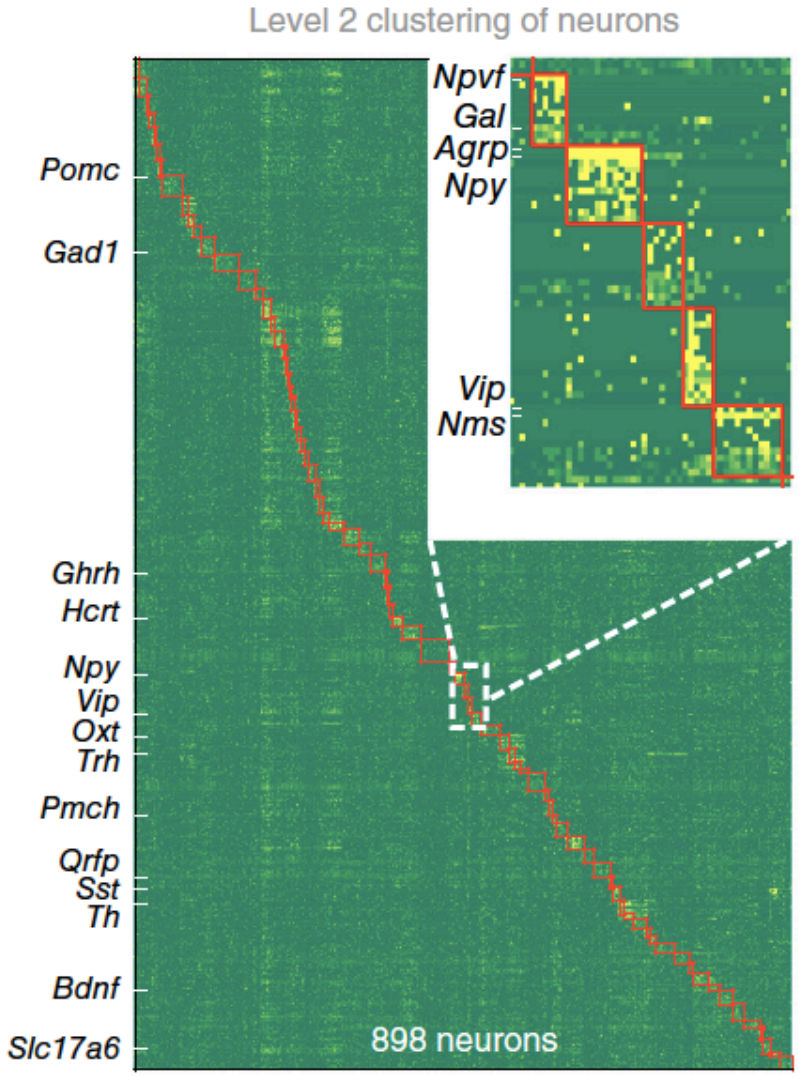
- Direct clustering methods
  - Hierarchical clustering
  - K-means, PAM



Grun *et al., Nature*'15

Clustering Approaches

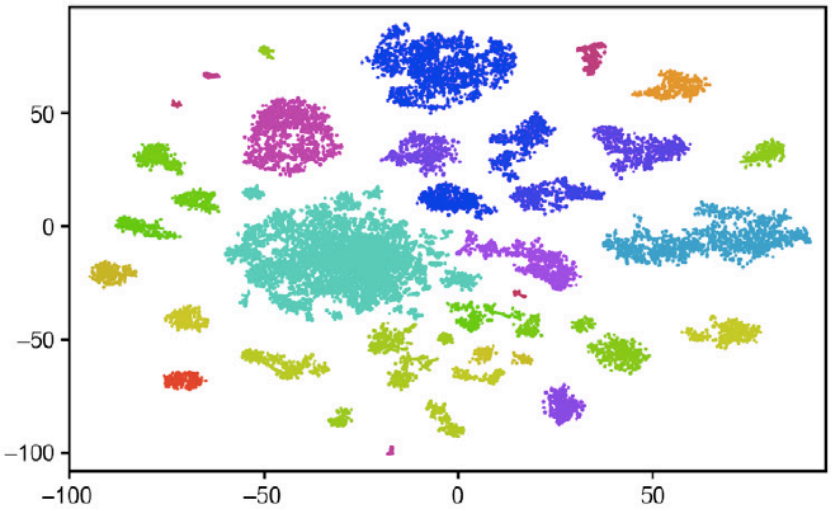
- Direct clustering methods
  - Hierarchical clustering
  - K-means, PAM
  - BackSpin (Zeisel *et al.*, Science'15)



Romanov *et al.*, *Nat Neuro*'17

Clustering Approaches

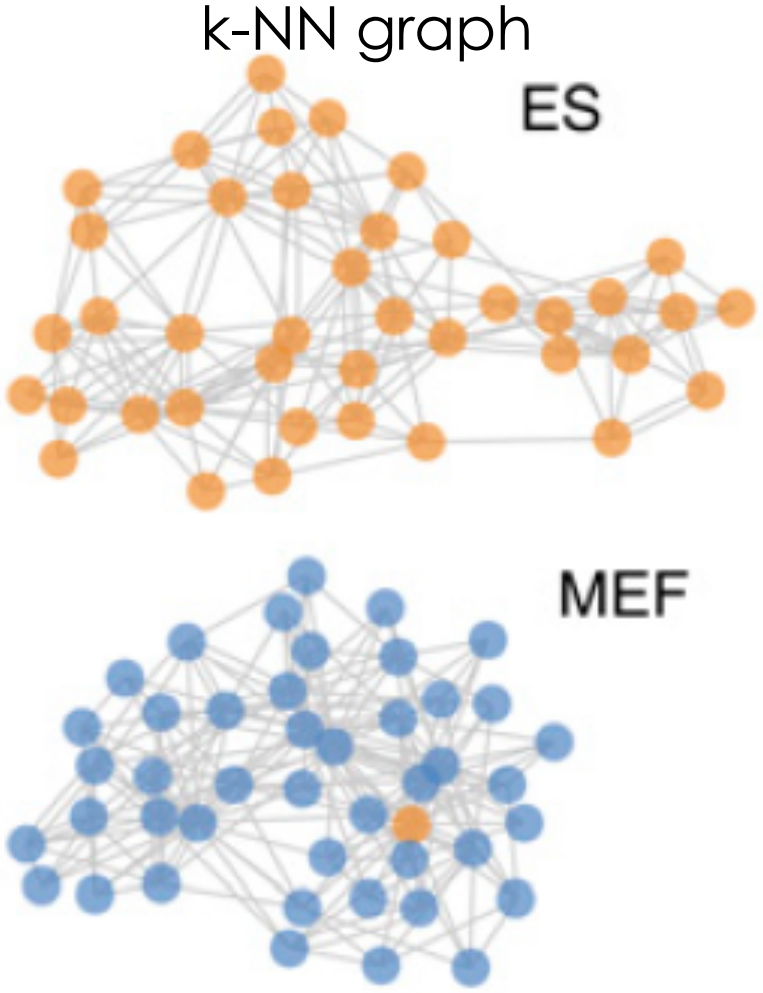
- Direct clustering methods
  - Hierarchical clustering
  - K-means, PAM
  - BackSpin (Zeisel *et al.*, Science'15)
- Density clustering
  - Find cell density clumps
  - Embed cells into low dimensions



Macosco *et al.*, *Cell*'15

Clustering Approaches

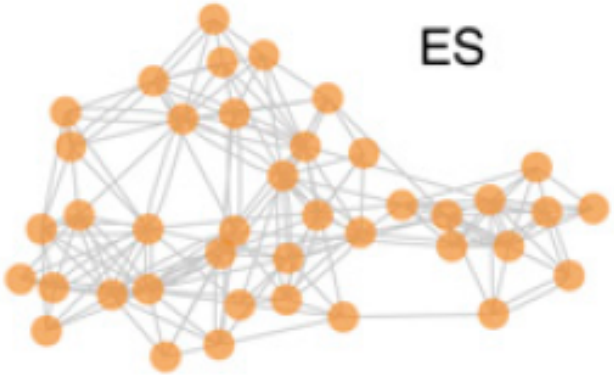
- Direct clustering methods
  - Hierarchical clustering
  - K-means, PAM
  - BackSpin (Zeisel *et al.*, Science'15)
- Density clustering
  - Find cell density clumps
  - Embed cells into low dimensions
- Graph-based clustering
  - k-nearest neighbor graph
  - Community detection methods
    - Modularity, Edge betweenness, Laplacian eigenvectors, InfoMap
    - Phenograph, SLM (Seurat)



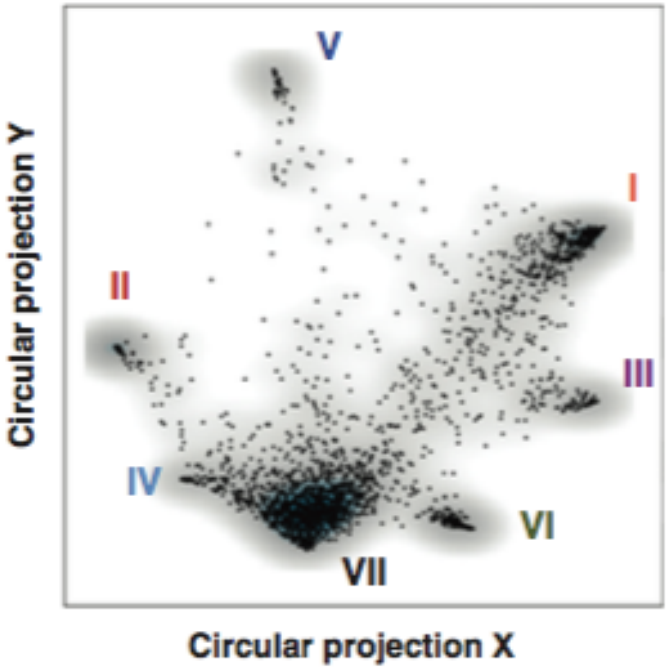
Islam *et al.*, Gen. Res'11

Clustering Approaches

- Traditional distance measures
  - Euclidian, L1, Correlation, Canberra, Jensen-Shannon
  - Transformed expression values
    - log scale
    - Reduced dimensions
    - Graph-based (scTDA)
  - Multi-scale distance (SIMLR)
  - Down-weighting of drop-outs
  - Restricted gene sets
- Model-based distances
  - Poisson
- Biological significance unclear



Islam *et al.*, Gen. Res'11

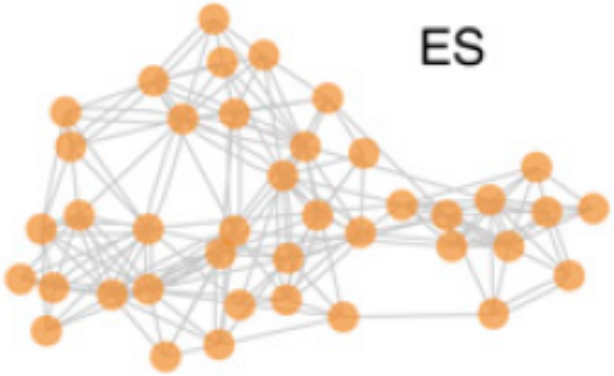


Jaitin *et al.*, Science'14

Clustering Approaches

Distance interpretation:

1. Deviation from equality
   - *e.g.* Jensen-Shannon. Larger cells will appear more distant then cells with few molecules
   - *e.g.* Poisson. Change in a highly-expressed gene can drive the overall likelihood
   - Relative distances of other distinct clusters
2. Amount of transcriptional change
   - *e.g.* L1. All genes are equal?
   - Low-dimensional dangers
   - Likelihood of transcriptional change
     - *e.g.* Weighting by observed variation
     - Stability, Assessing distant transitions
3. "Biological" distance
   - Extent of phenotypic difference



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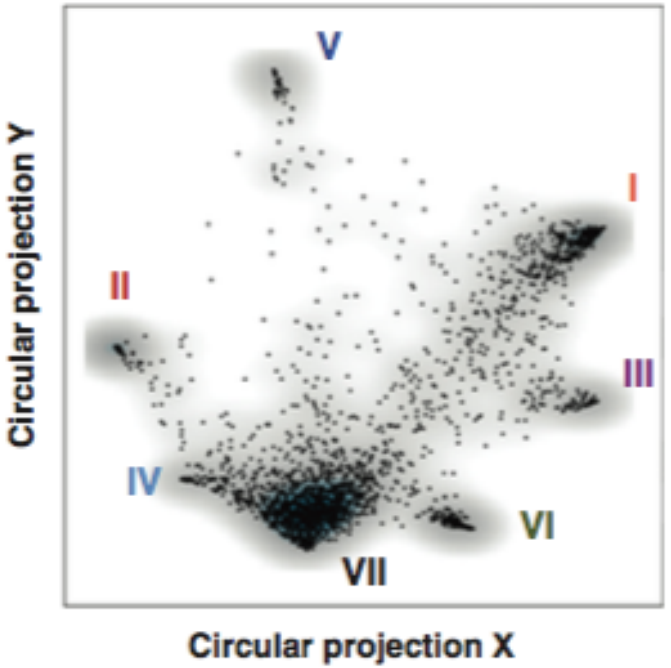
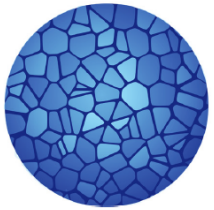
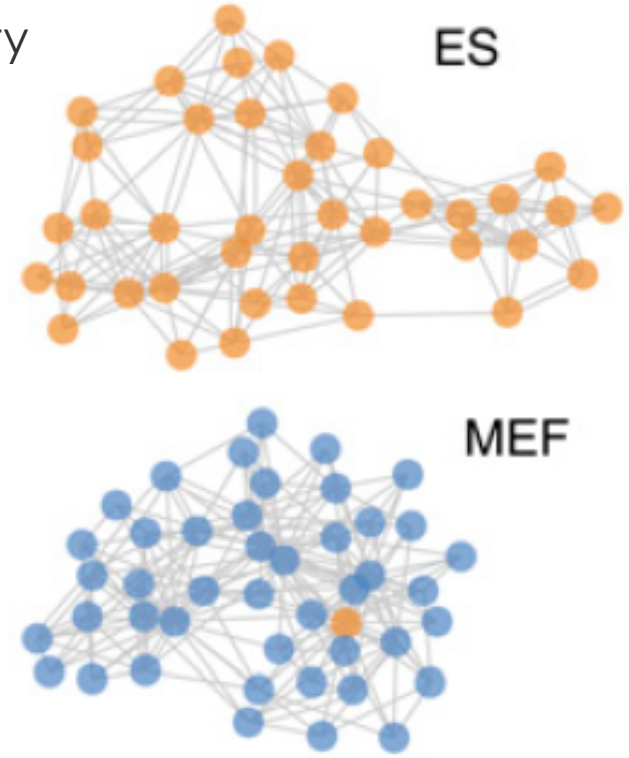


Jaitin *et al.*, Science'14

Challenge: Is that a real cluster?

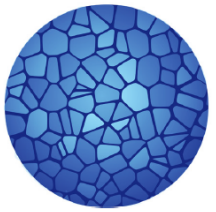


- Should a given subpopulation be split?
  - Cluster granularity varies between methods
  - Hierarchical clustering: improved stability
- Assessing statistical significance
  - Likelihood of observing the cluster
    - Under measurement noise
    - Cell subsampling
    - Across replicates
  - Robustness of expression signature
    - Gene subsampling
- Is there a distinct phenotype?

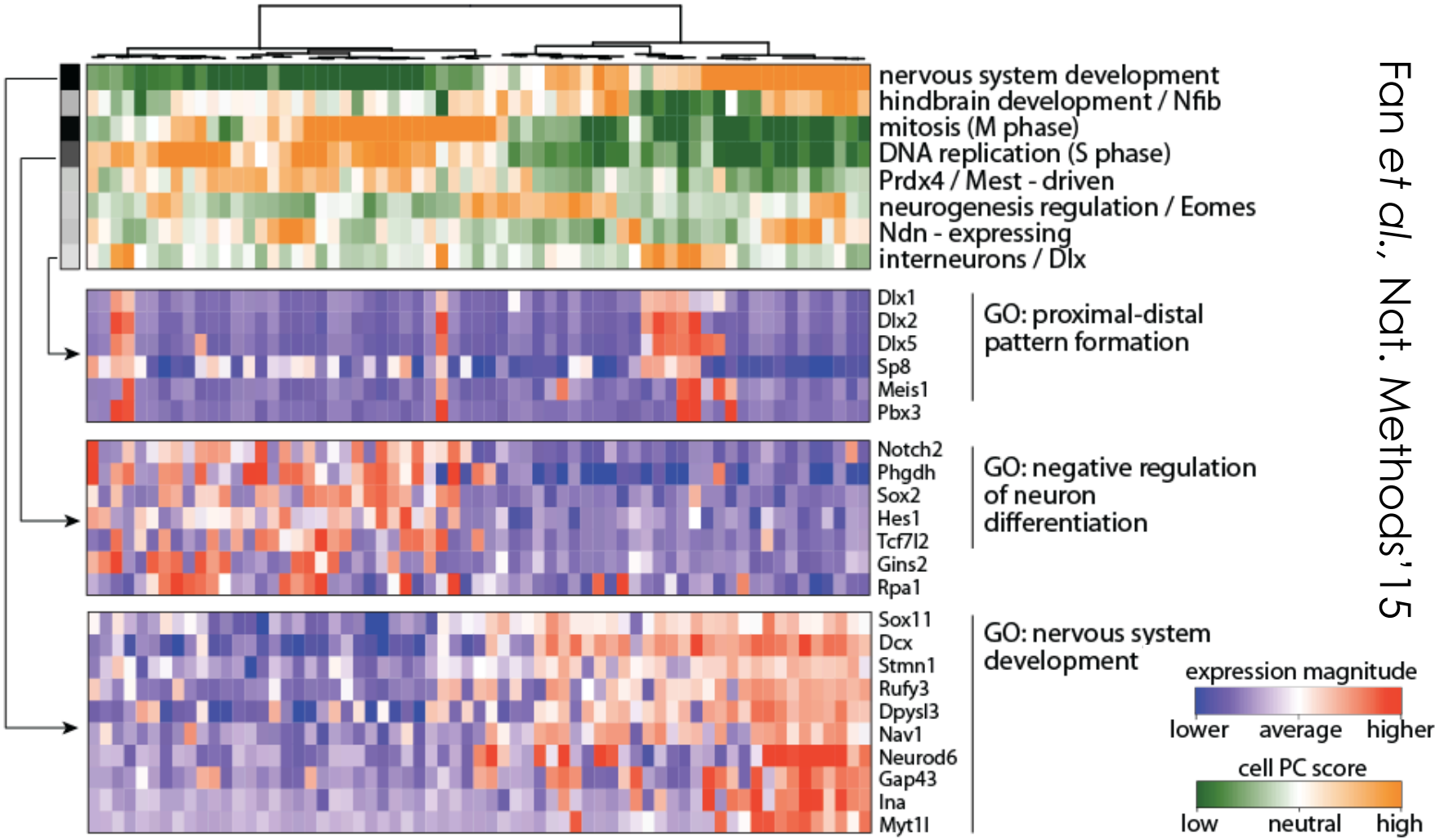


Islam *et al.*, Gen. Res'11

Challenge: Is that the whole story?



- Cross-cutting classifications



Fan *et al.*, Nat. Methods'15

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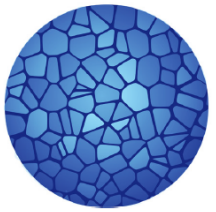
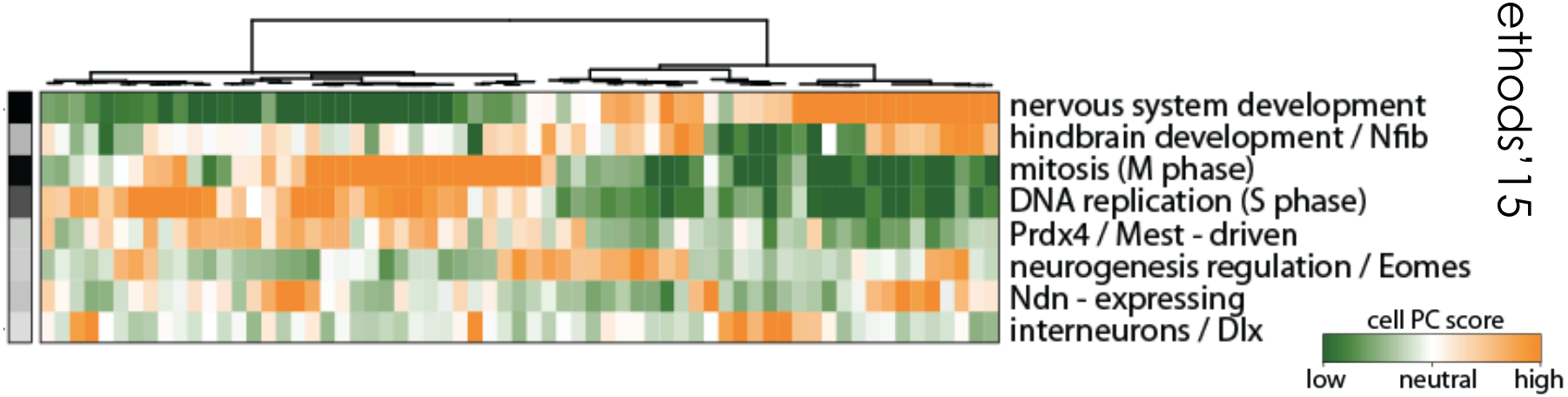


- Cross-cutting classifications
  - Cells have many physiologically-important properties
  - Clustering segments by some subset of properties
    - Limit -> by a combination of all properties
- How to identify and visualize cluster correspondence?



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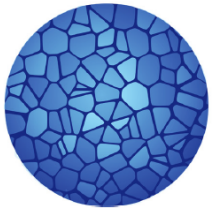
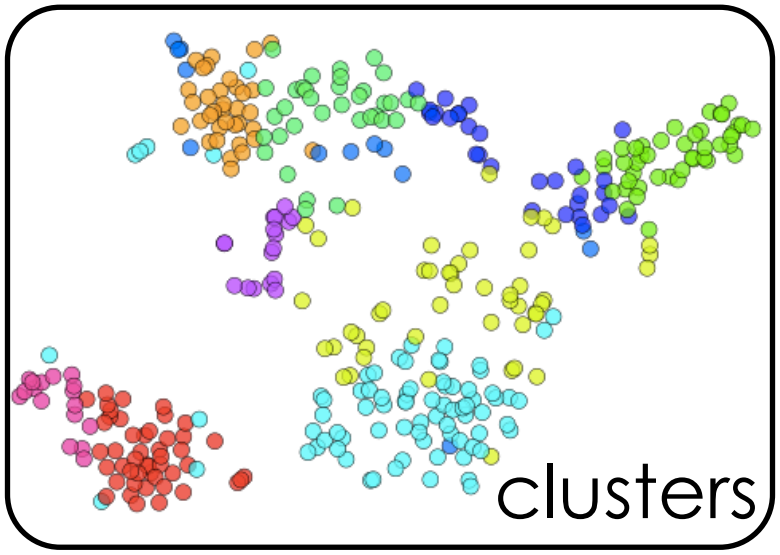
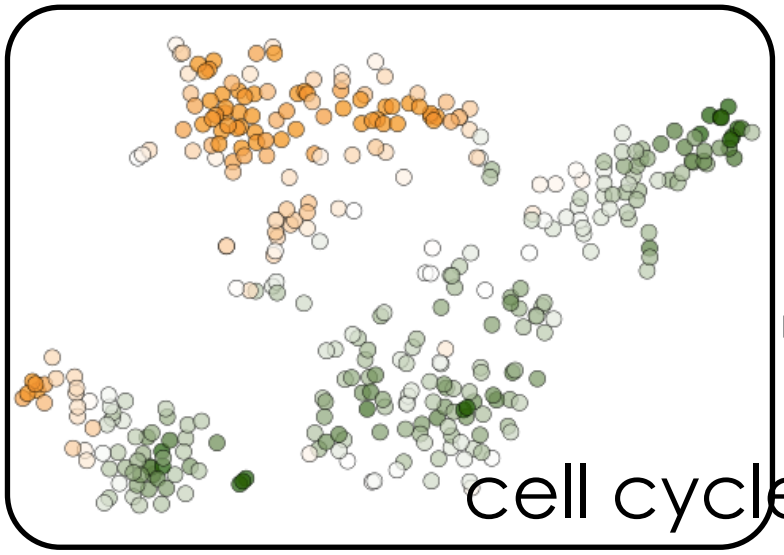
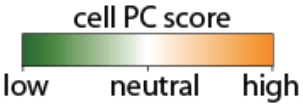


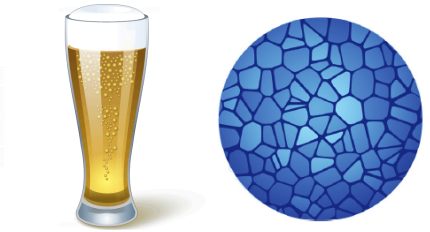
- Cross-cutting classifications
  - Cells have many physiologically-important properties
  - Clustering segments by some subset of properties
    - Limit -> by a combination of all properties
- How to identify and visualize cluster correspondence?
- Preferred configuration depends on a biological question







Cell Type vs. Cell State

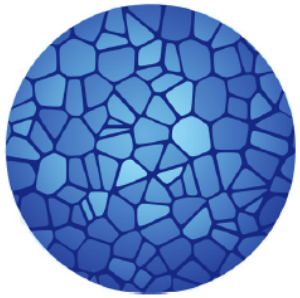


"Cellular states span configuration space within which cells of a given type can move in a reversible manner under physiological conditions"

- Discrete / enumerable states - local maxima within the state configuration space
- Common processes can give rise to similar transcriptional states within different cell types (*e.g.* cell cycle phases)
- Types are separated by irreversible transitions*
  - Rare reversible events
- Intended as a guideline, not a strict definition

Baker *et al.* Cell Stem Cell '12

Fast Processing of Sparse Measurements



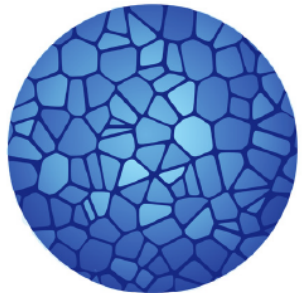
- Current single-cell datasets
  - 10^4-10^6 cells
  - With molecular barcoding
  - As few as a hundred molecules per cell

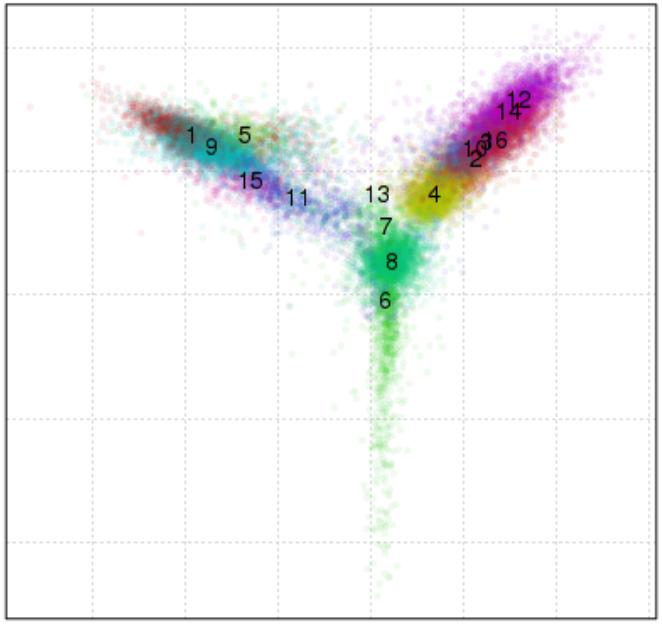
Fast Processing of Sparse Measurements

- Current single-cell datasets
  - 10^4-10^6 cells
  - With molecular barcoding
  - As few as a hundred molecules per cell
- Pagoda2
  - Basic workflow for large sparse measurements
    - Residual variance normalization, corrections
    - Cell size estimation
    - PCA
    - Differential expression
    - Clustering
    - Visualization
    - Gene clustering
    - Overdispersion analysis
    - Differential expression

http://github.com/hms-dbmi/pagoda2/

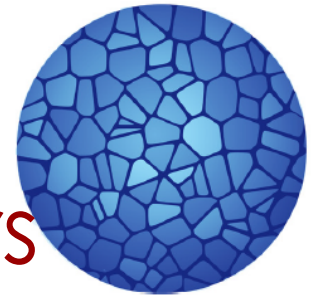
Fast Processing of Sparse Measurements: PCA





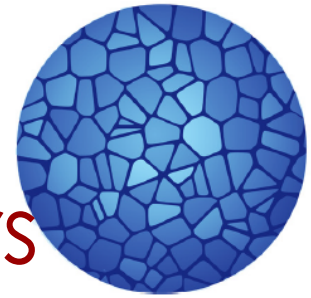
- Run PCA on a sparse matrix with:
  - ~10^3-10^4 variables
  - ~10^5-10^6 observations
- Lanczos algorithm
  - $x_{n+1} = Ax_n \; ; \quad x_n / \|x_n\|$
  - Krylov subspaces
    - $\mathcal{K}_r(A, b) = \operatorname{span}\{b, Ab, A^2b, \ldots, A^{r-1}b\}.$
  - Orthogonalize Krylov space of *A* to obtain Arnolodi vectors, which are approximations of the largest eigenvectors
  - Restart with multiple random vectors *b*
  - Implemented by irlba package
  - 10 largest eigenvectors on 10^6 cells by 10^3 features:

Fast Processing of Sparse Measurements: nearest neighbors



- Looking for nearest k (20-100) neighbors for ~10^5-10^6 cells
- A relatively common problem
  - Typically <1000 variables
  - Approximate search is OK
  - Tree-based approaches (kd, PCA)
  - Locale-sensitive Hashing

Fast Processing of Sparse Measurements: nearest neighbors



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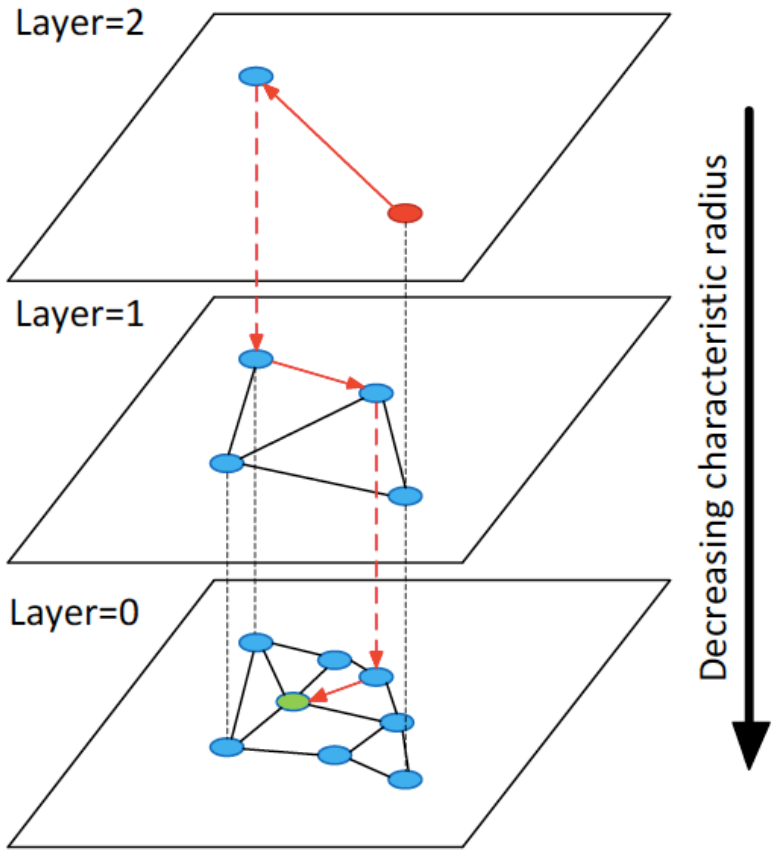
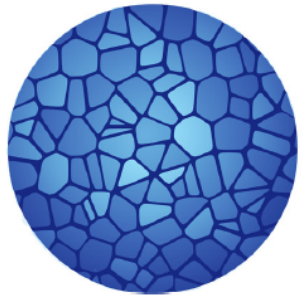
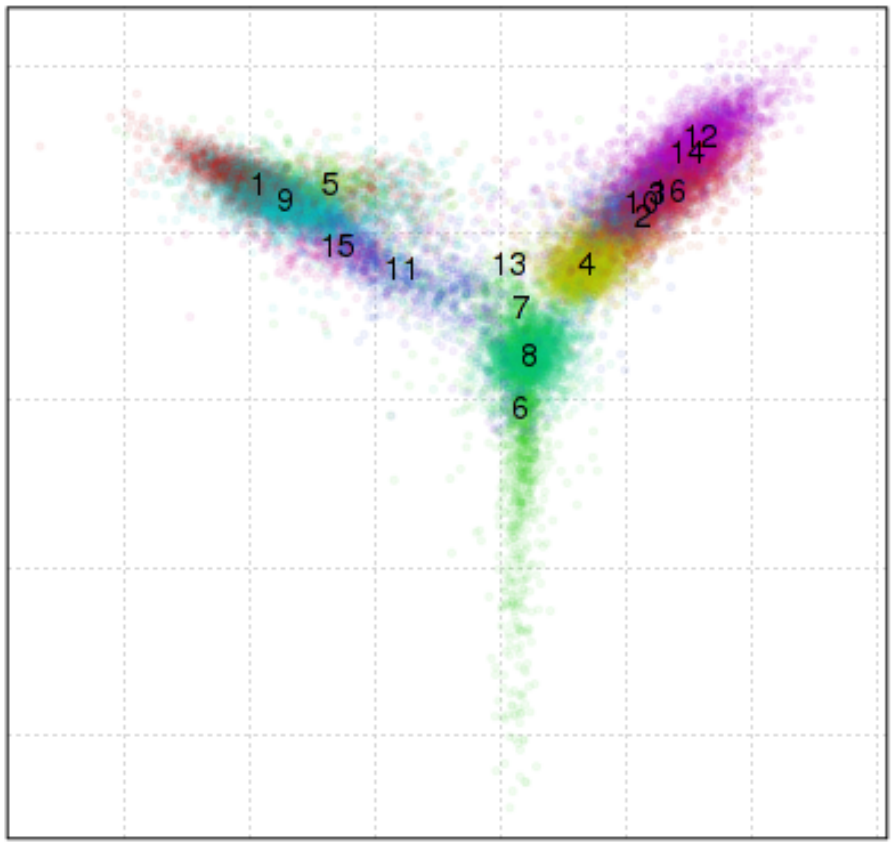
- A relatively common problem
  - Typically <1000 variables
  - Approximate search is OK
  - Tree-based approaches (kd, PCA)
  - Locale-sensitive Hashing
- Navigable Small World Graphs
  - Malkov, Yashunin'16
  - Two-phase: graph insertion, search
  - Cell-cell correlation networks are great small-world graphs
  - Based on nmslib implementation



Fast Processing of Sparse Measurements: cluster, layout



- Interactive exploration is key to getting biological insights
- Effective visualization is important



Stochastic neighbor embedding (SNE)

- Convert the high-dimensional Euclidean distances between data points into conditional probabilities that represent similarities

$$p_{j|i} = \frac{\exp\left(-\|x_i - x_j\|^2 / 2\sigma_i^2\right)}{\sum_{k \neq i} \exp\left(-\|x_i - x_k\|^2 / 2\sigma_i^2\right)}$$

Gaussian:

$$\frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

- probability that x_i would pick x_j as its neighbor conditional on neighboring being picked in proportion to their probability density under a Gaussian centered at x_i with variance σ_i

Maaten L, Hinton G. Visualizing Data using t-SNE. 9(Nov):2579--2605, 2008.

Stochastic neighbor embedding (SNE)

- Define a similar conditional probability / similarity metric for the low-dimensional counterparts y_i and y_j

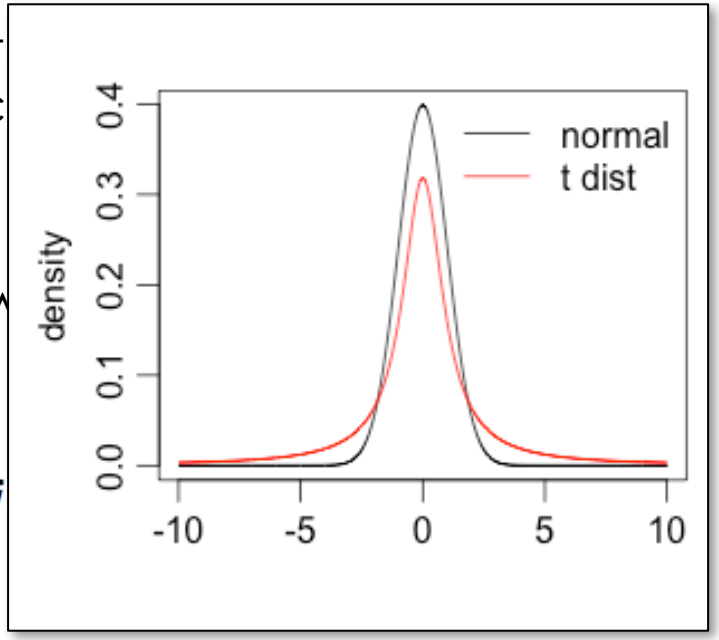
$$q_{ij} = \frac{\left(1 + \|y_i - y_j\|^2\right)^{-1}}{\sum_{k \neq l} \left(1 + \|y_k - y_l\|^2\right)^{-1}}$$

t-SNE

- If the map points y_i and y_j correctly model t
high-dimensional datapoints x_i and x_j, the c
and $q_{j|i}$ will be equal

- Optimize Kullback-Leibler divergences betw
probabilities $p_{j|i}$ and $q_{j|i}$ over all datapoints

$$C = \sum_i KL(P_i||Q_i) = \sum_i \sum_j p_j$$



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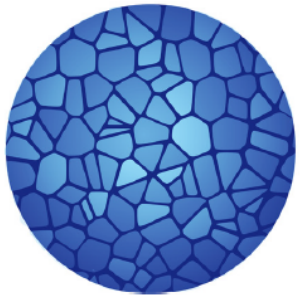
Diffusion Maps



- Problems for PCA
  - Count values are noisy
  - Are not normally distributed
- Transformations of the count values
  - log
  - Imputation (MAGIC, scImpute)
  - Cell-cell similarity
- Diffusion probabilities

Diffusion maps for high-dimensional single-cell analysis of differentiation data

Laleh Haghverdi[1,2], Florian Buettner[1,*,†] and Fabian J. Theis[1,2,*]

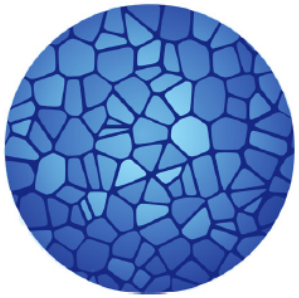
"wavefunction"

$$Y_x(x') = \left(\frac{2}{\pi\sigma^2}\right)^{1/4} \exp\left(-\frac{||x'-x||^2}{\sigma^2}\right)$$

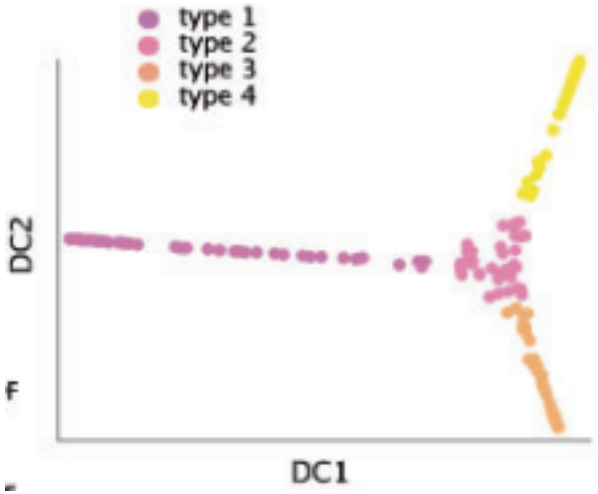
"interference" -> diffusion p

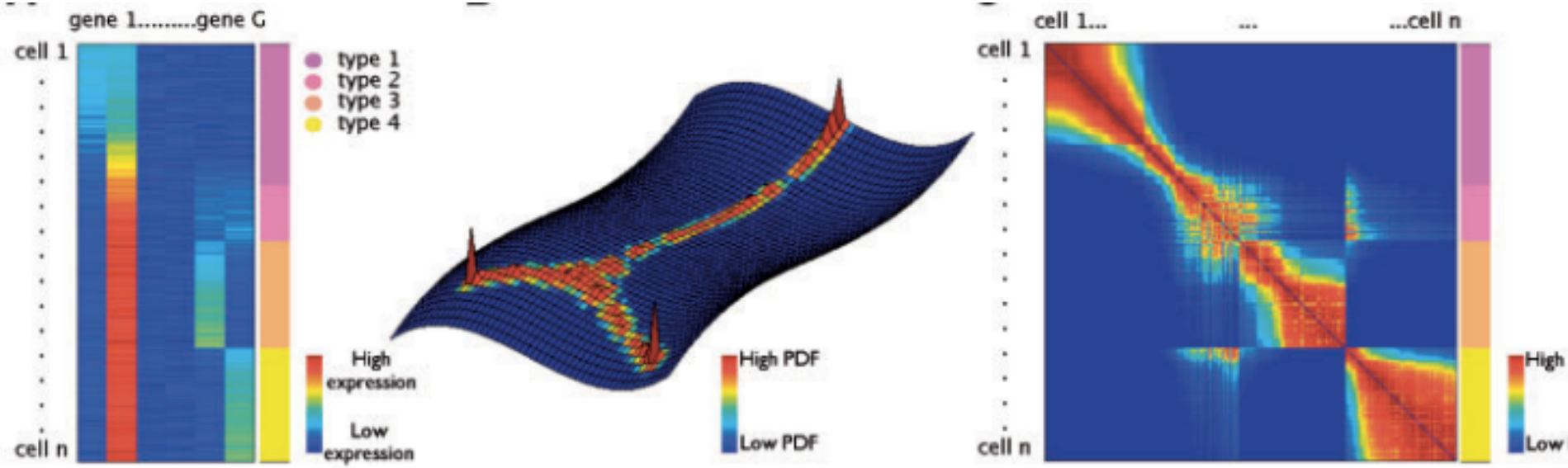
$$P_{xy} = \frac{1}{Z(x)} \exp\left(-\frac{||x-y||^2}{2\sigma^2}\right)$$

Diffusion Maps



- Diffusion p
- PCA of the diffusion matrix
- Project on the first two PCs





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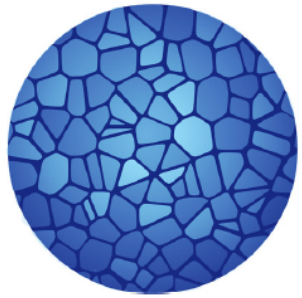

- Interactive exploration is key to getting biological insights
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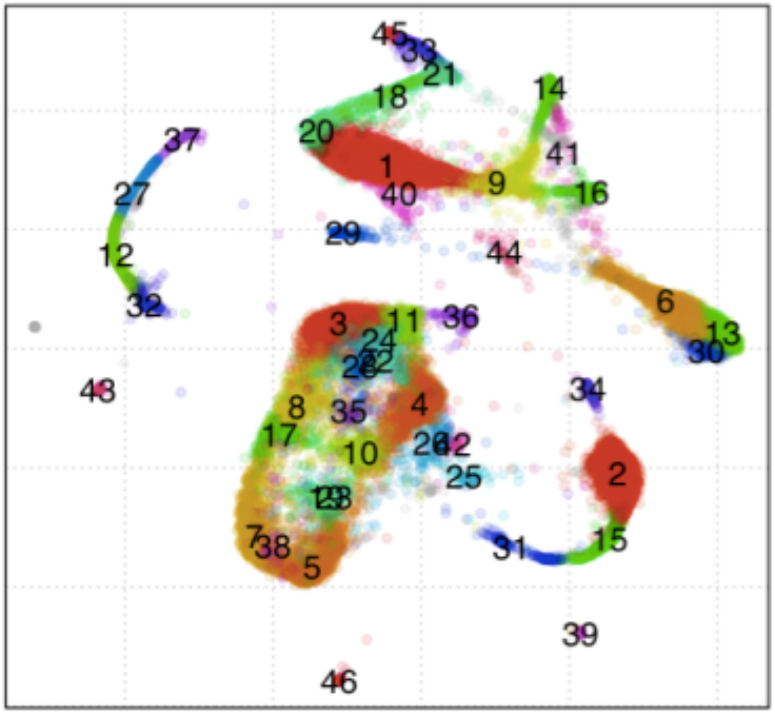
- Popular embedding methods
  - PCA, MDS
  - tSNE
  - Graph layout



Computer Science > Learning

Visualizing Large-scale and High-dimensional Data

Jian Tang, Jingzhou Liu, Ming Zhang, Qiaozhu Mei

(Submitted on 1 Feb 2016 (v1), last revised 5 Apr 2016 (this version, v2))

Fast Processing of Sparse Measurements: cluster, layout

- Interactive exploration is key to getting biological insights
- Effective visualization is important
- Popular embedding methods
  - PCA, MDS
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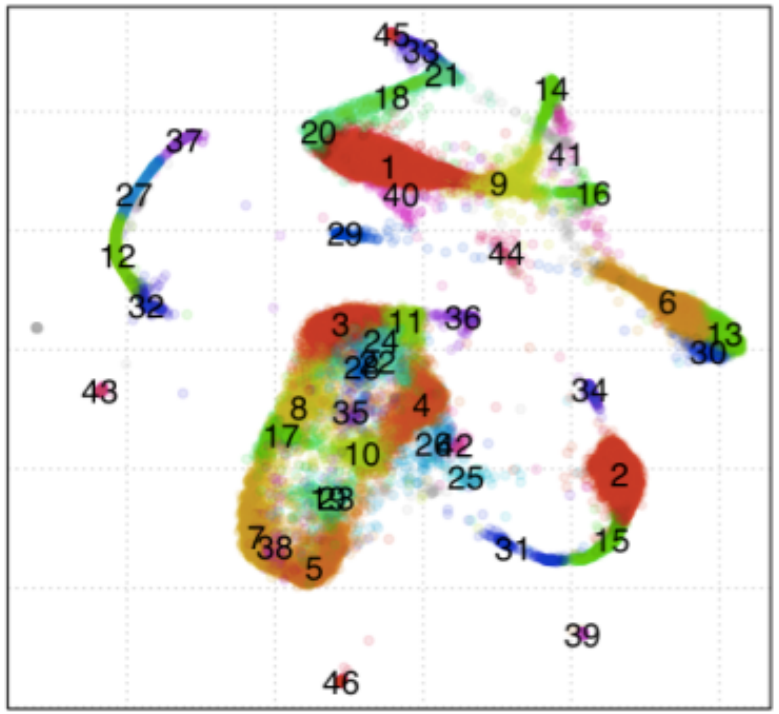
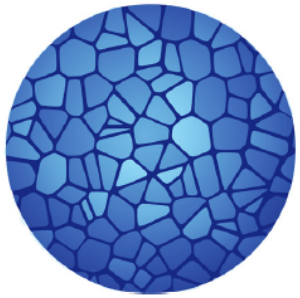
  - Graph layout
- Clustering
  - Density clustering
  - Graph community clustering
    - Common problem
    - Multilevel community detection
      - Near-linear performance on large sparse graphs



Making use of reference data



- Reference cell collections
  - Organism, tissue-specific data
- *De novo vs.* reference-based analysis
- Reference-augmented
  - Will capture both dataset-specific and reference-based signatures
  - Facilitates interpretation
  - Higher statistical power, stability

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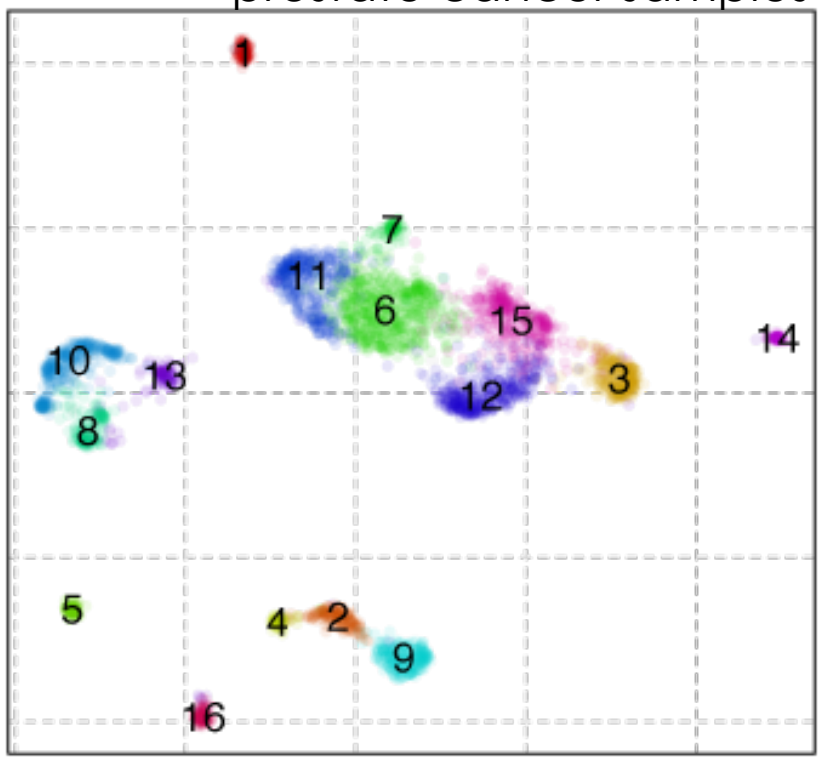
prostate cancer samples



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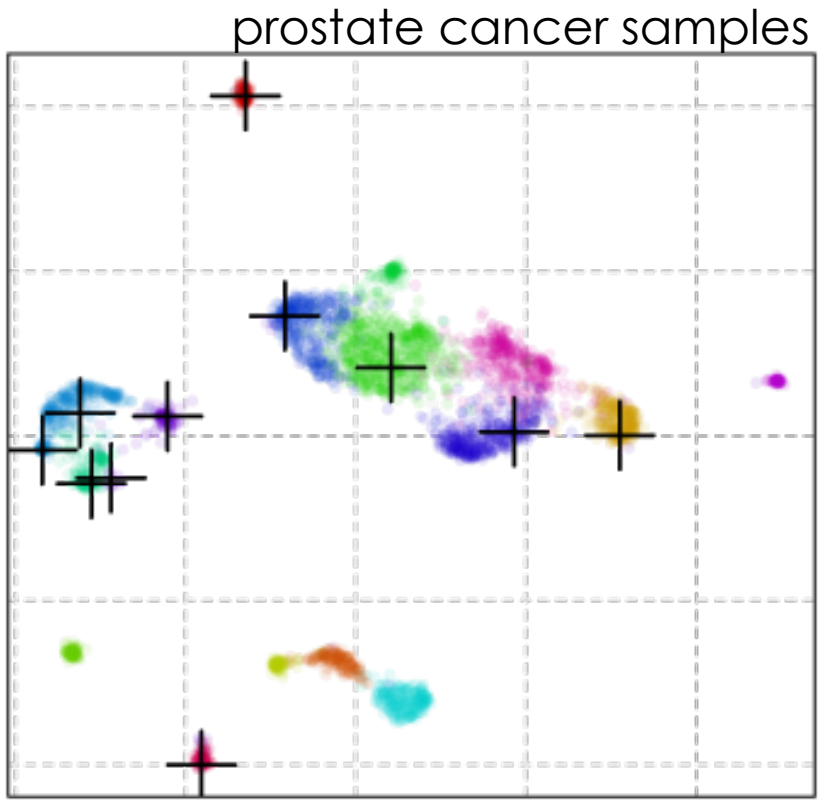

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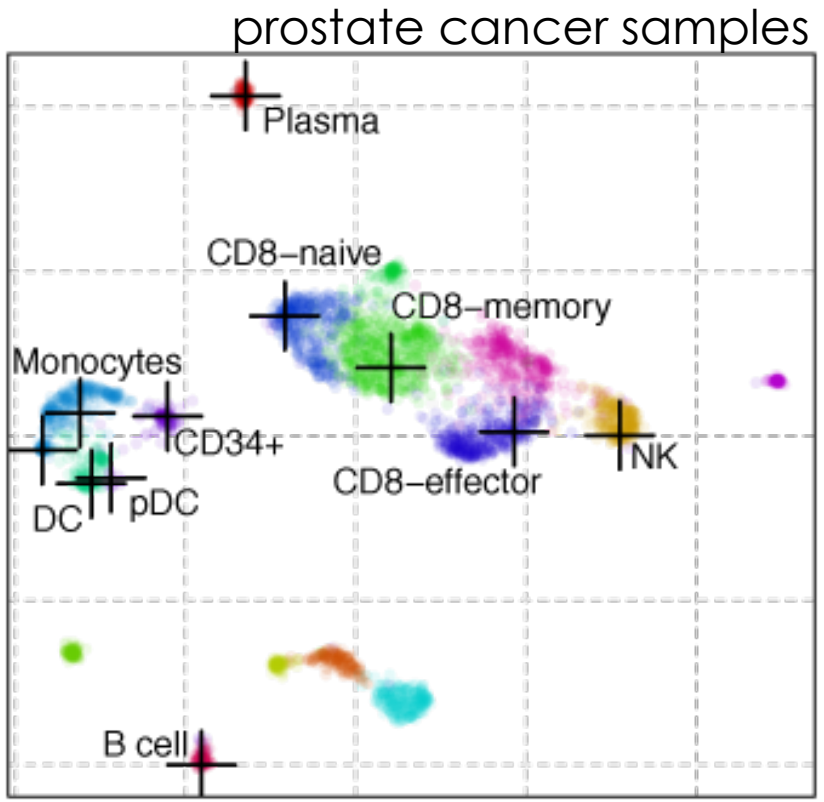

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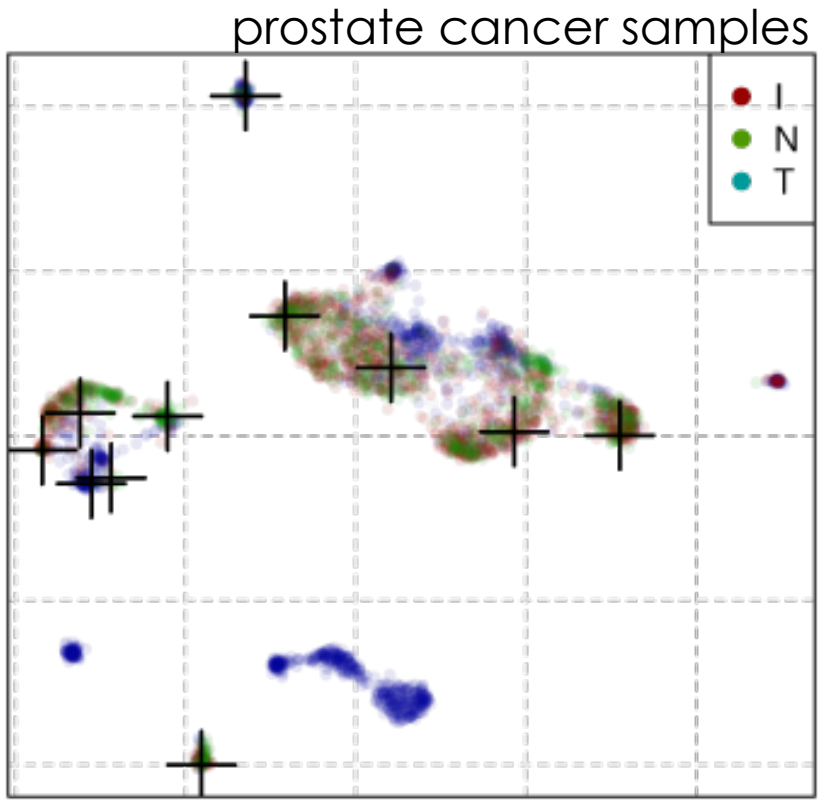
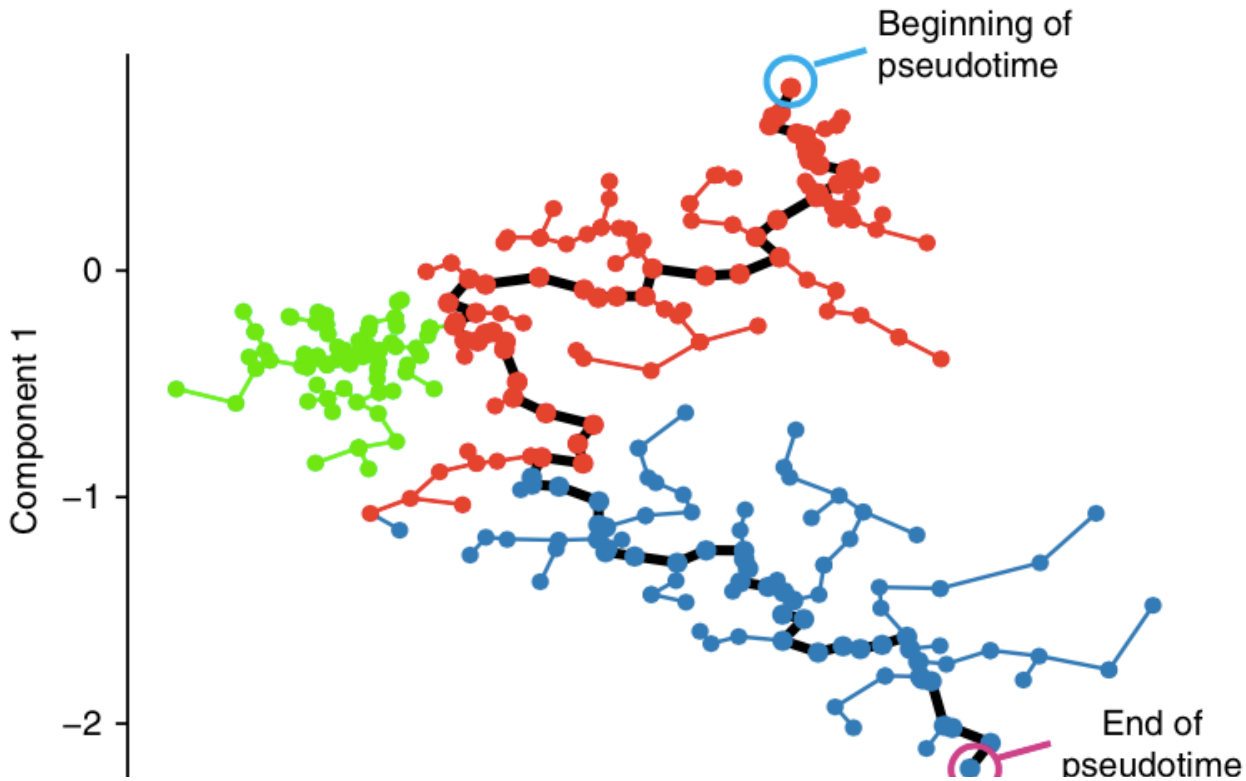
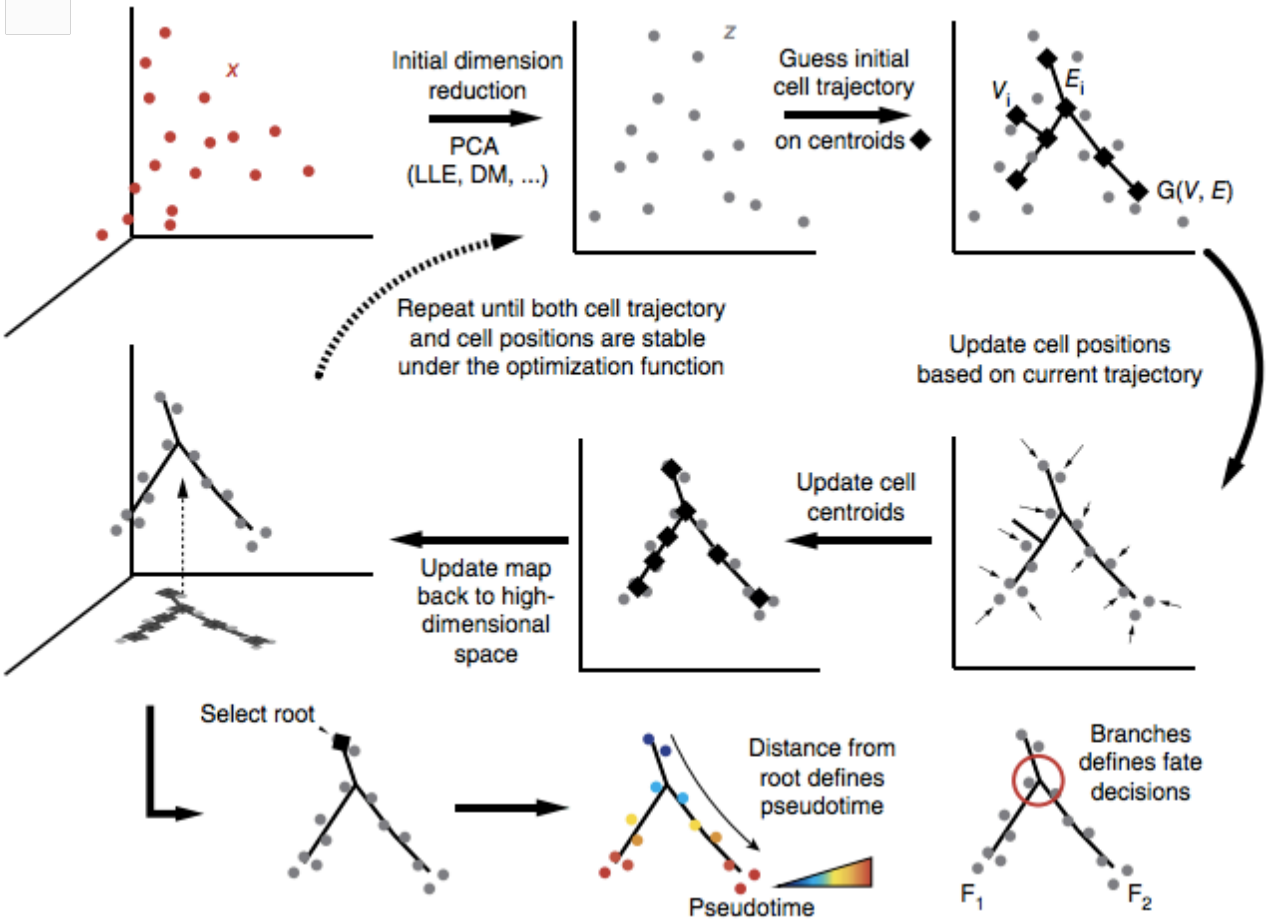


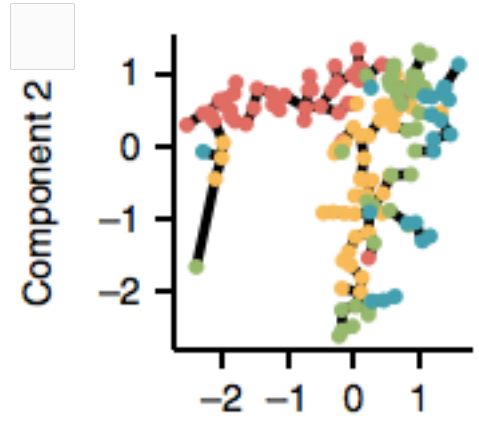
Trajectory Tracing: Monocle

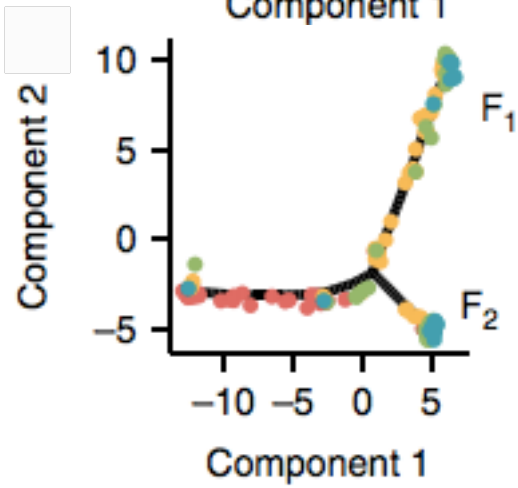


Trapnell et al., Nat. Biotech 2015

Trajectory Tracing: Monocle2



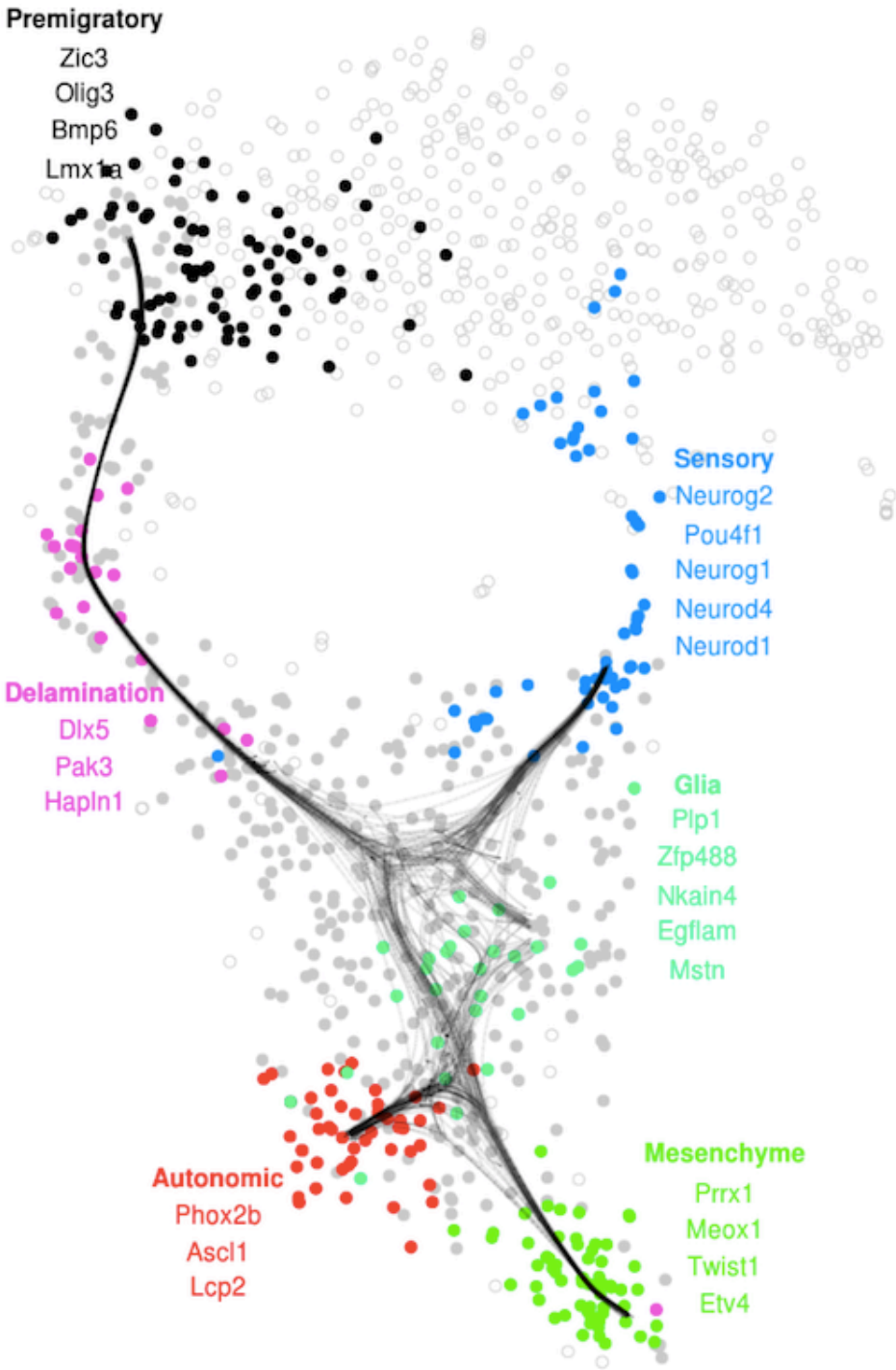




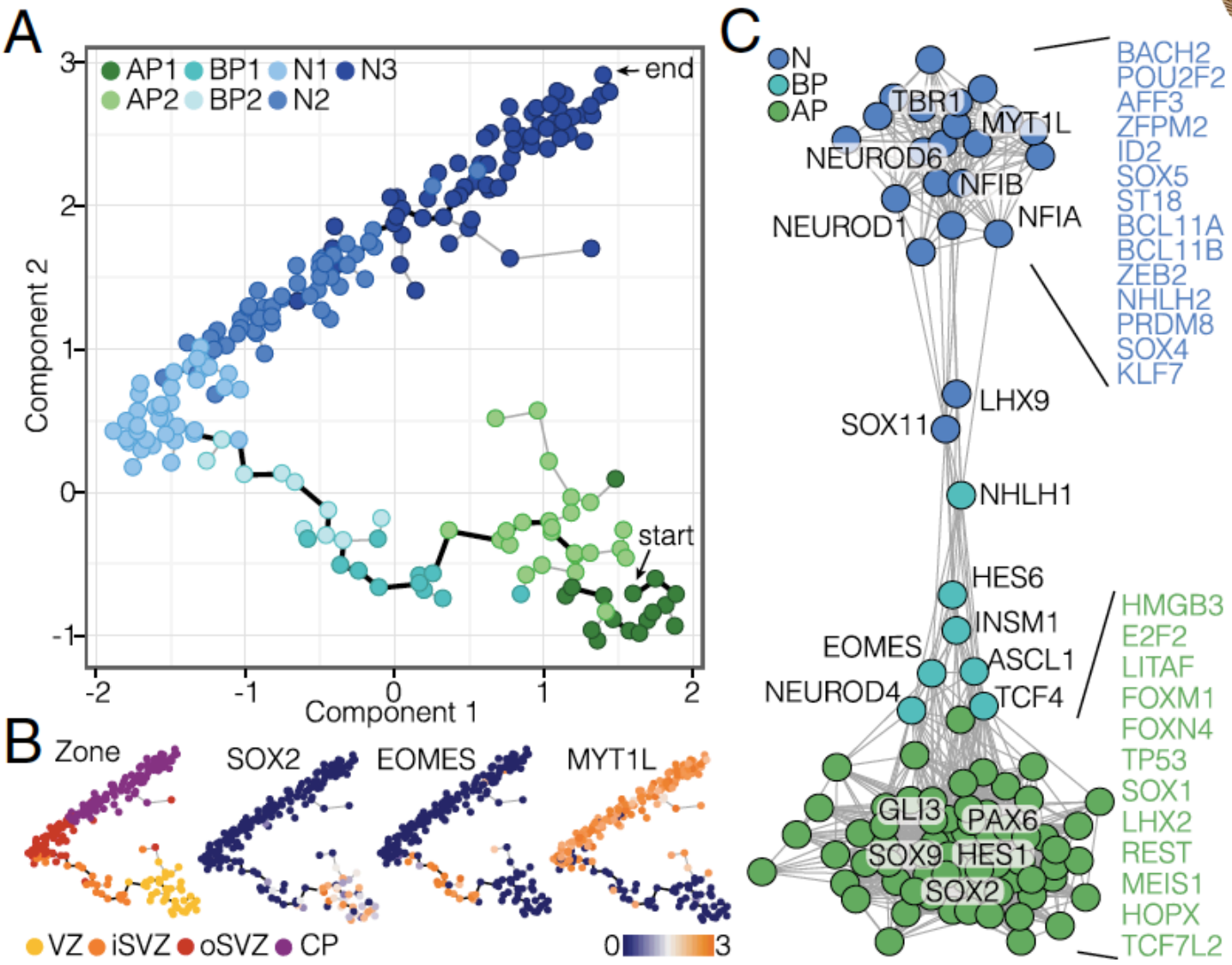
Qui *et al.*, Nat. Methods 2017

Trajectory Tracing

- Trying to reconstruct likely trajectory that the cells have taken
- Requires assumptions
  - Ergodicity
  - Continuity
  - Directionality
- Path uncertainty
  - How many decisions does a cell make?
  - Are they reversible?



Gene-gene correlations



Camp et al. PNAS 2015