Геномные Анализы Индивидуальных Клеток

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cbmi
Center for Biomedical Informatics

HSCI
HARVARD STEM CELL INSTITUTE

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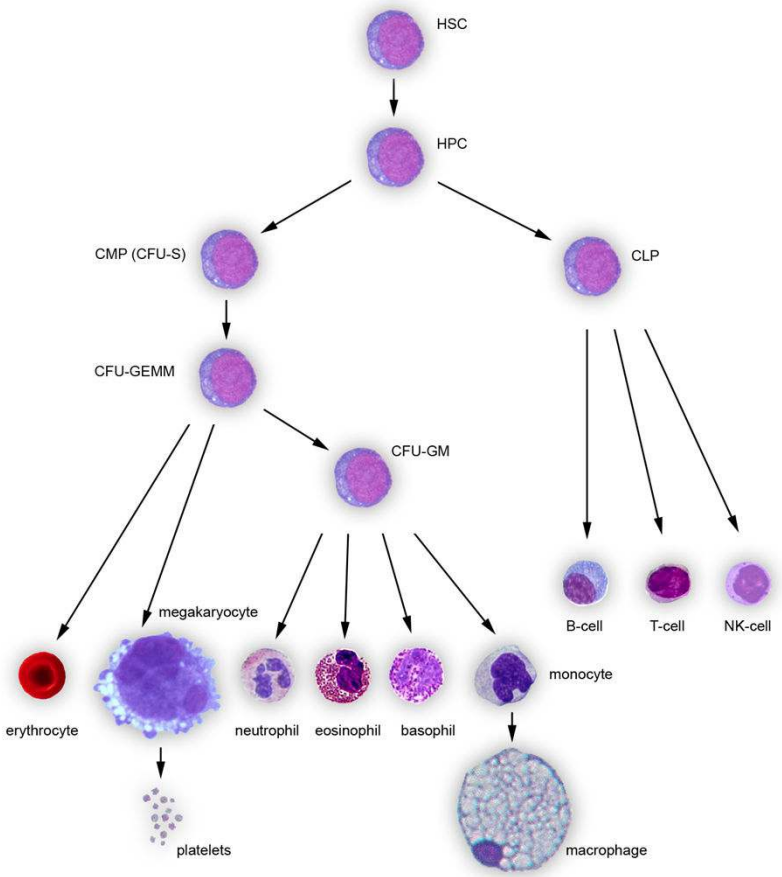
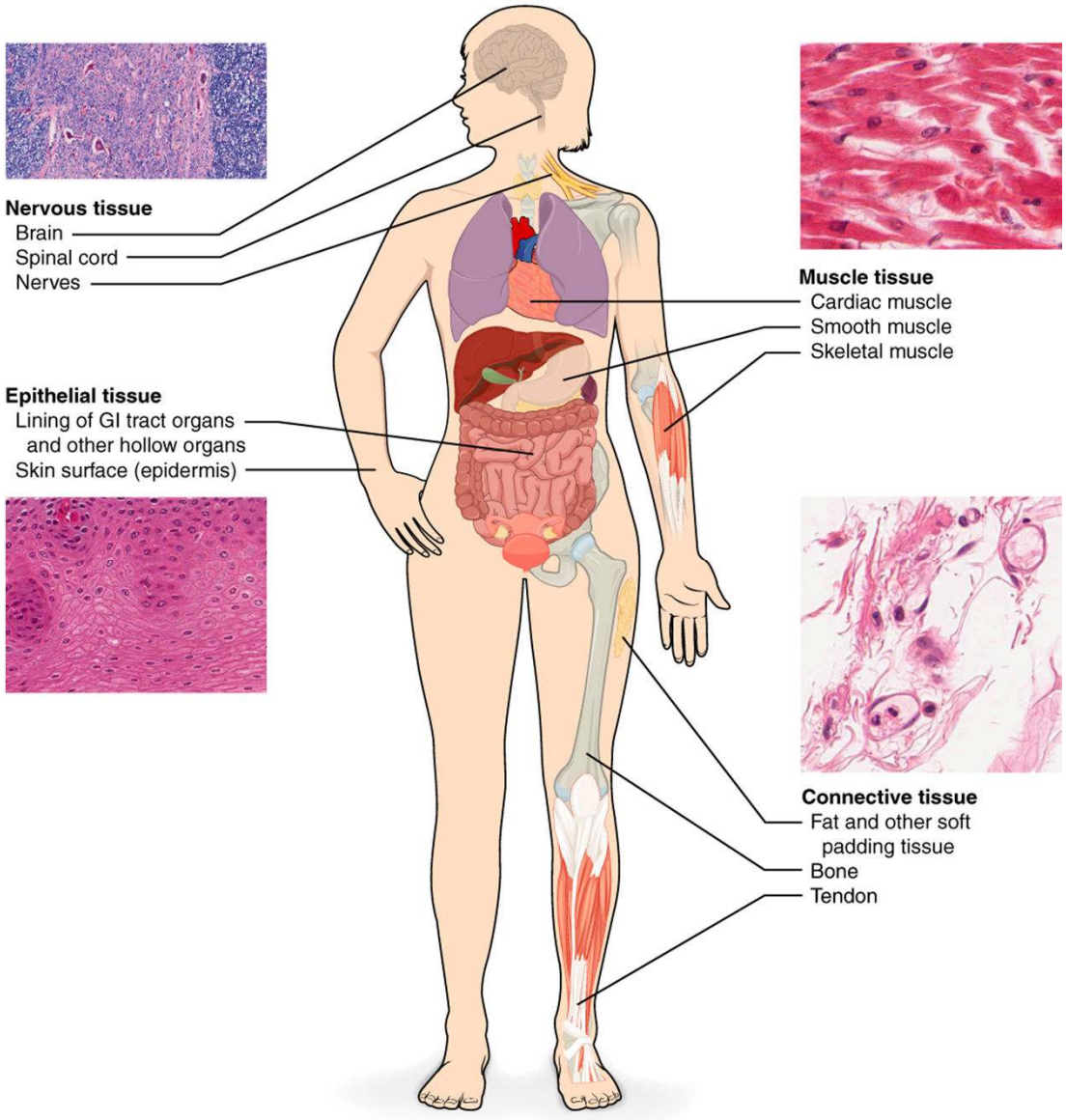
Клетки, Клеточные Типы и Ткани

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





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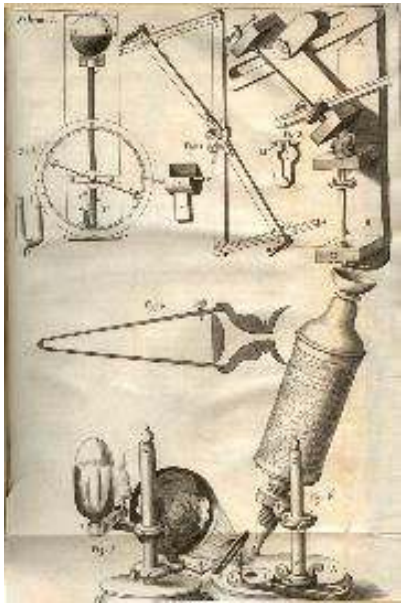
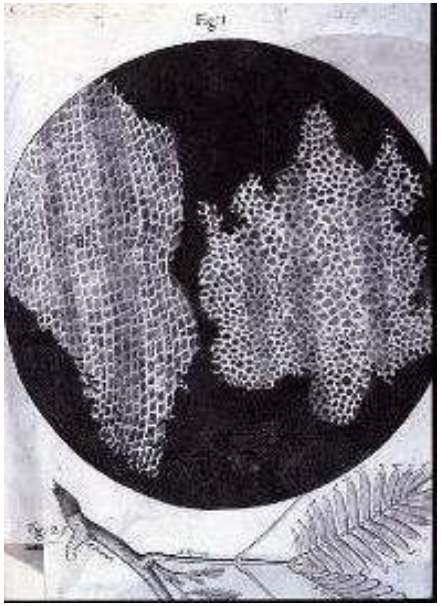
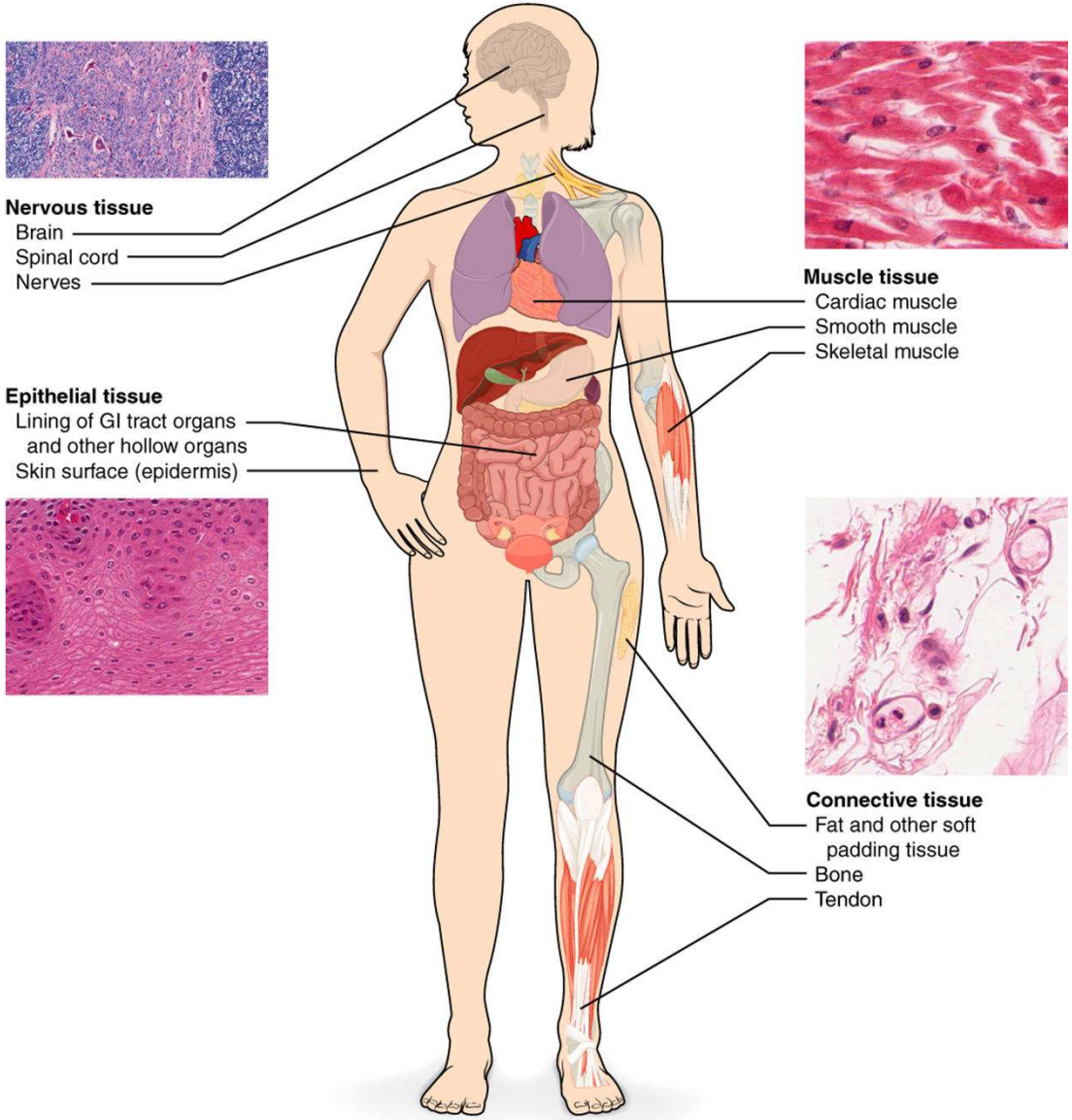
- Cell Types
  - Early Microscopy
  - Robert Hooke (1635-1703)







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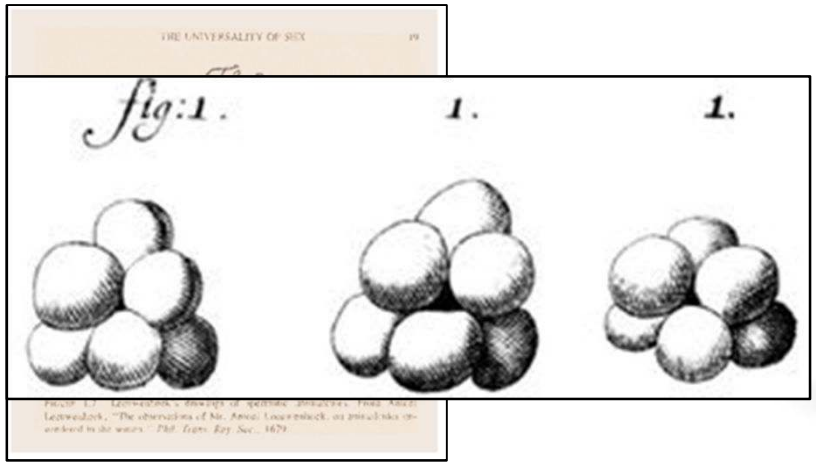

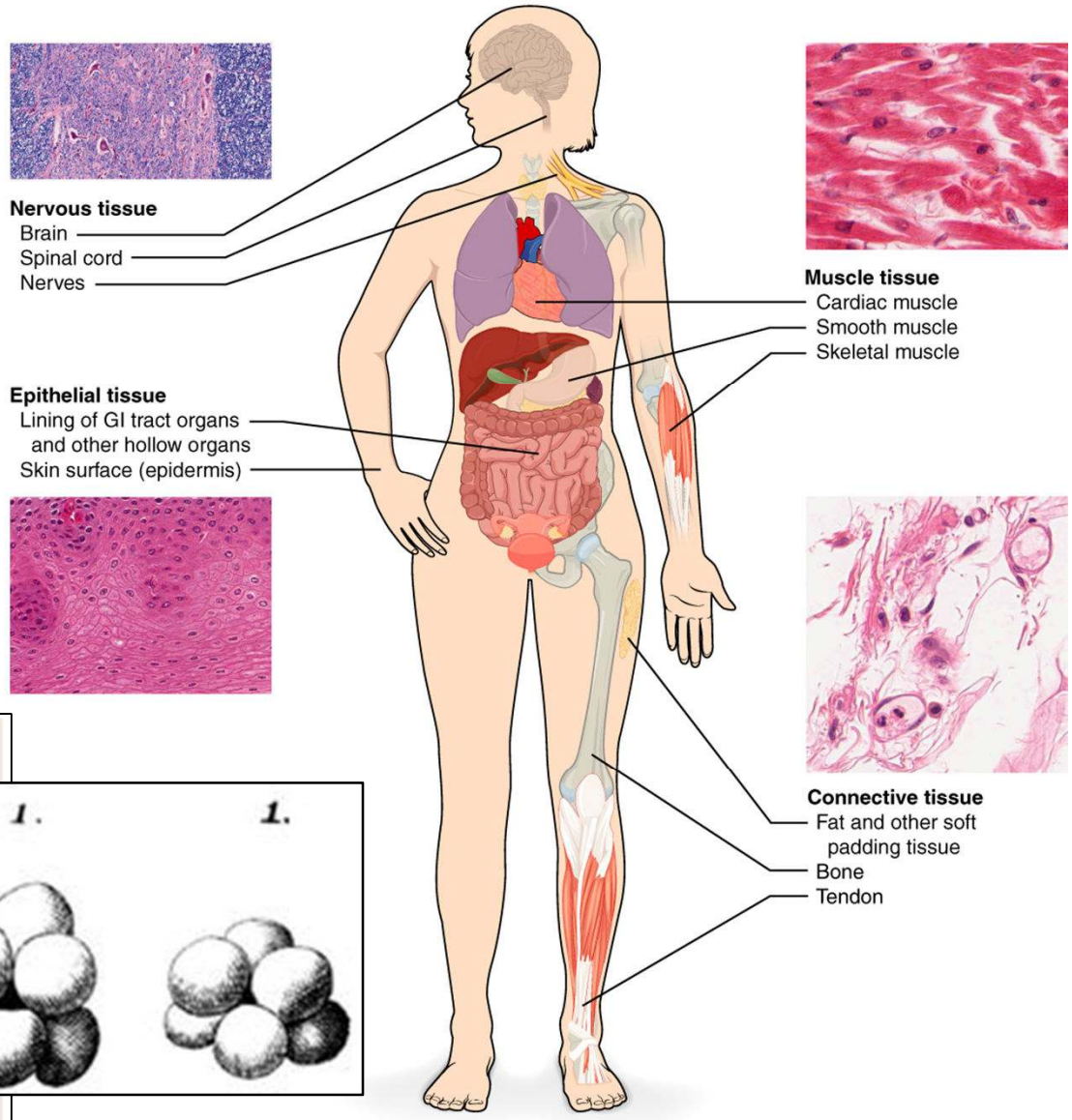

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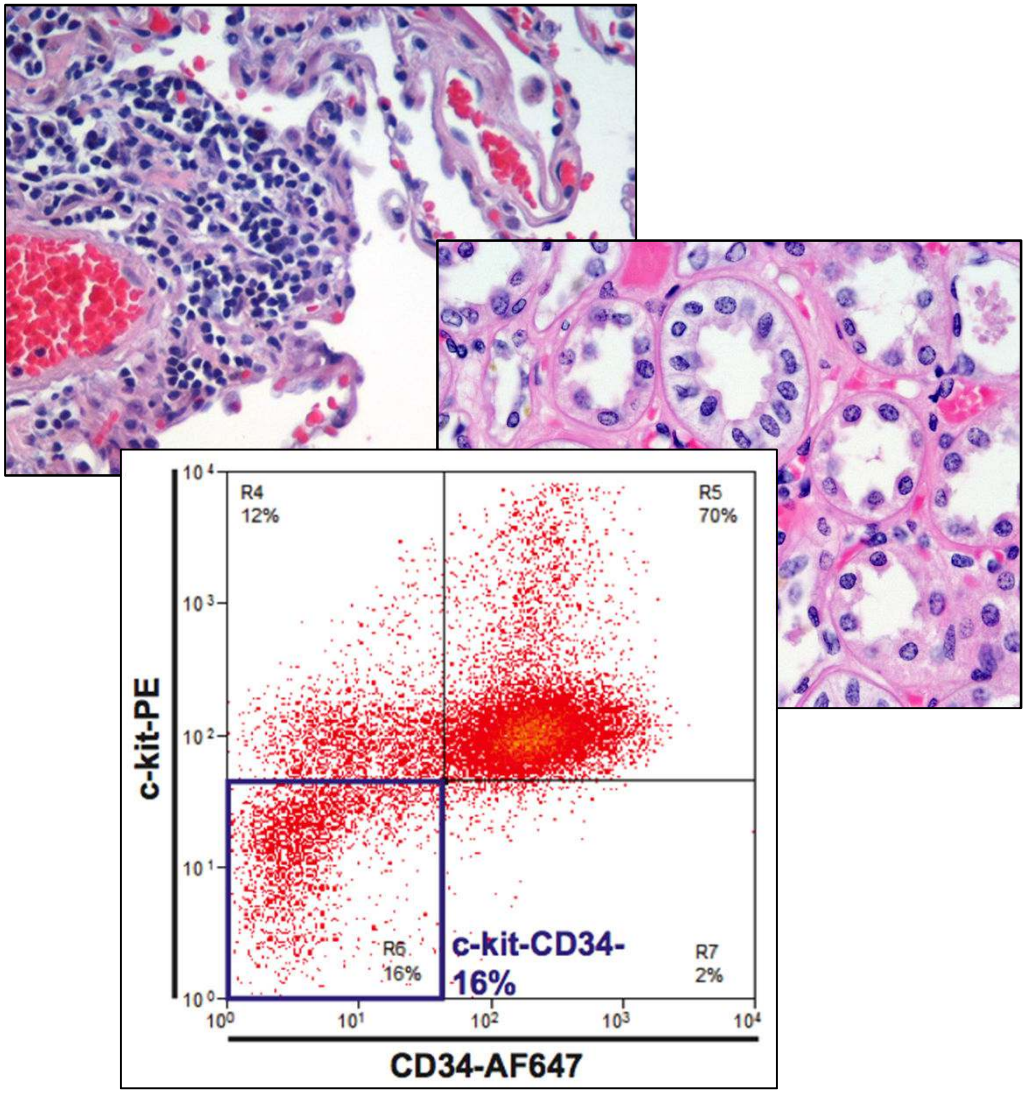
Modern Histology

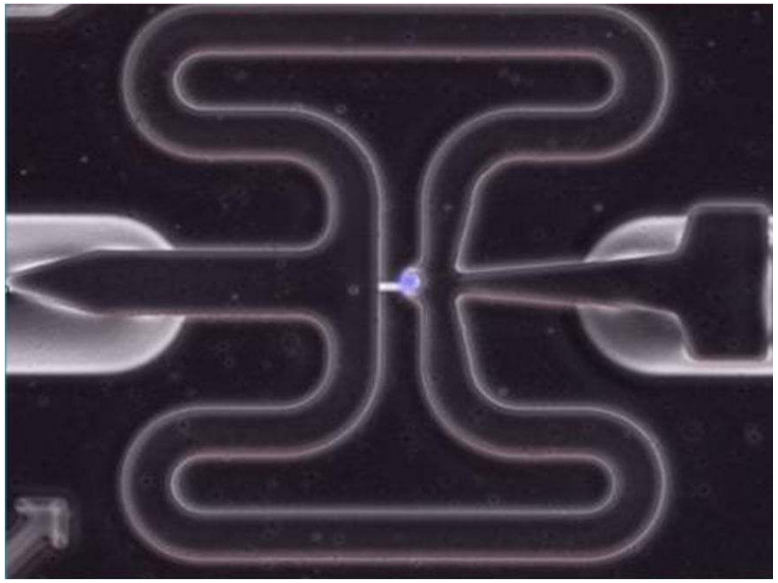


Single-Cell Genomics

- Цели
  - Классификация: клеточные типы, подтипы, состояния
  - Механизмы: молекулярные отличия, динамика
- Измерения
  - Транскрипционное состояние (single-cell RNA-seq, FISSEQ)
  - Епигенетическое состояние (DNA methylation, accessibility)
  - Геномная последовательность (somatic mutations)
- Технологии
  - Миниатюризированные протоколы
  - Микрофлюидика: клапаны, пузырьки
  - Микроскопия: in-situ labeling, sequencing

Транскриптомика:
Single-Cell RNA Sequencing

- Разделение и манипулирование клетками
  - Commercial C1 platform from Fluidigm



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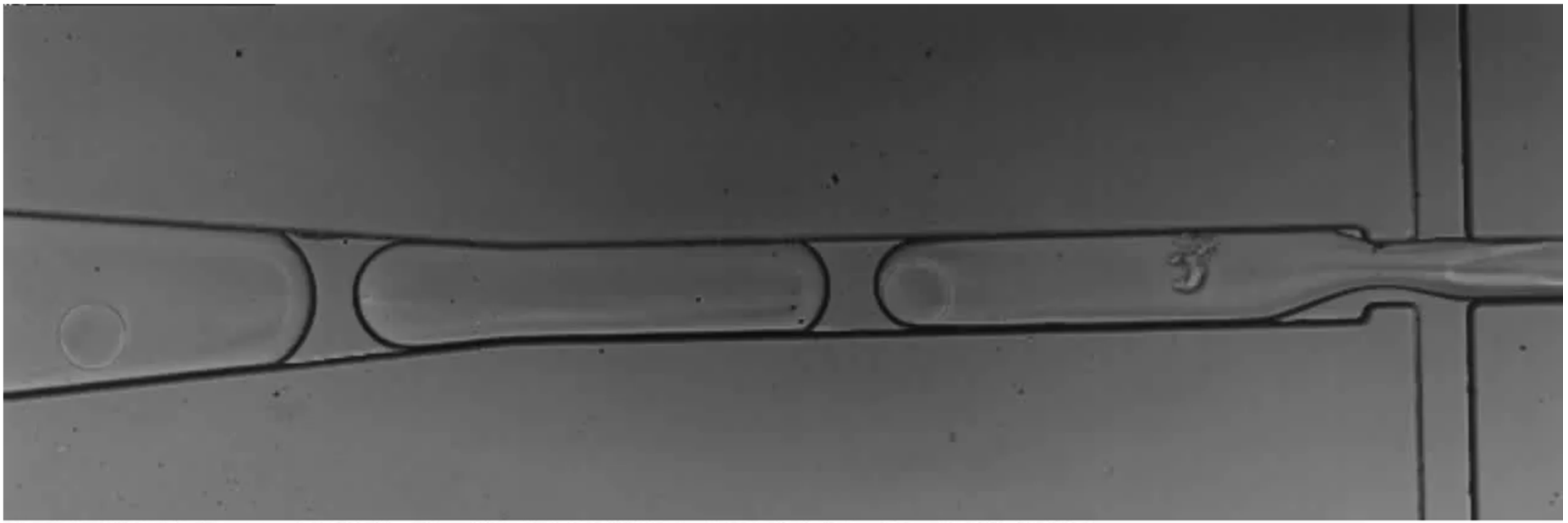

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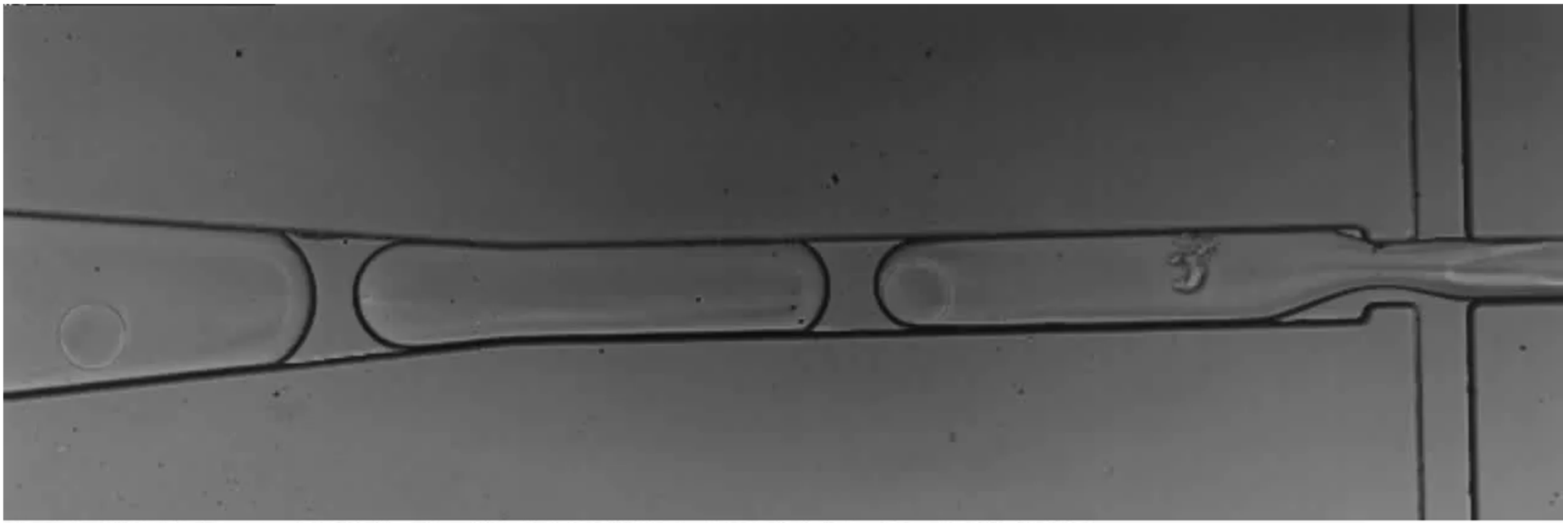

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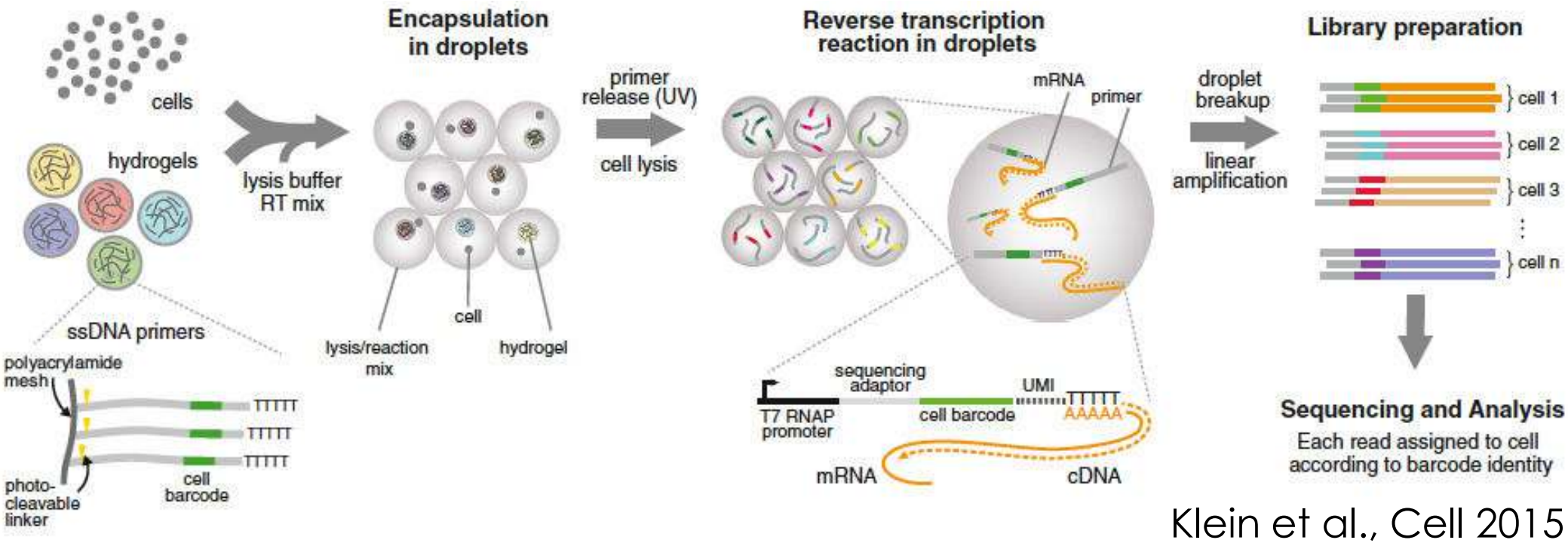
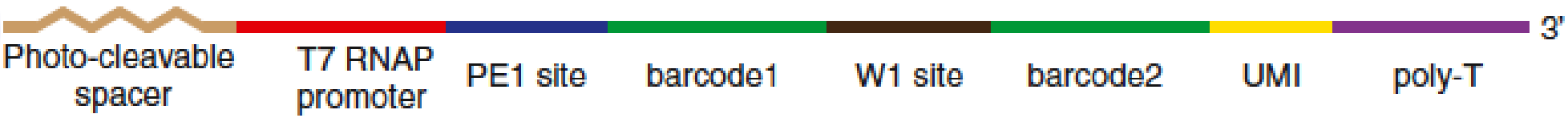


Klein et al., Cell 2015

Клеточные и Молекулярные Штрих-Коды

- Read structure



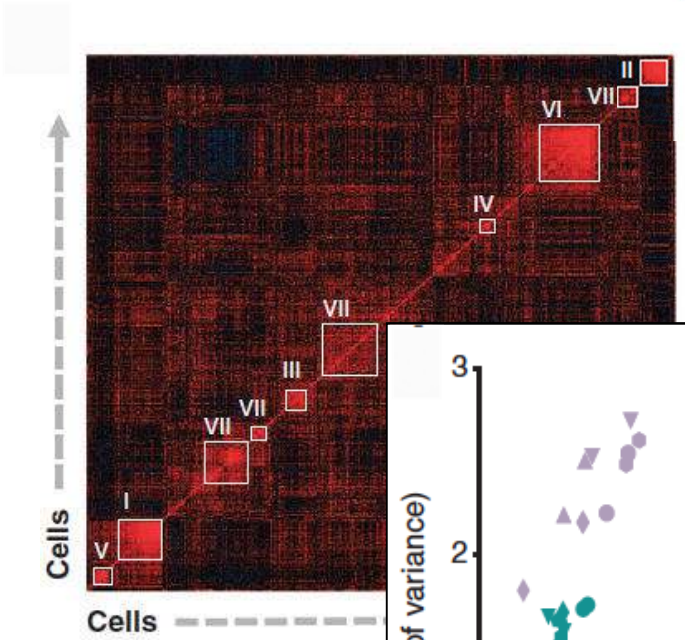
- Barcodes have to be extracted prior to alignment
- Molecular barcode tabulation, collisions
  - Expected number of colliding cells: N barcodes; k cells

$$k_d = k\left(1-(1-1/N)^{k-1}\right) \quad f_d = k_d/(k-k_d) \sim 1\%$$

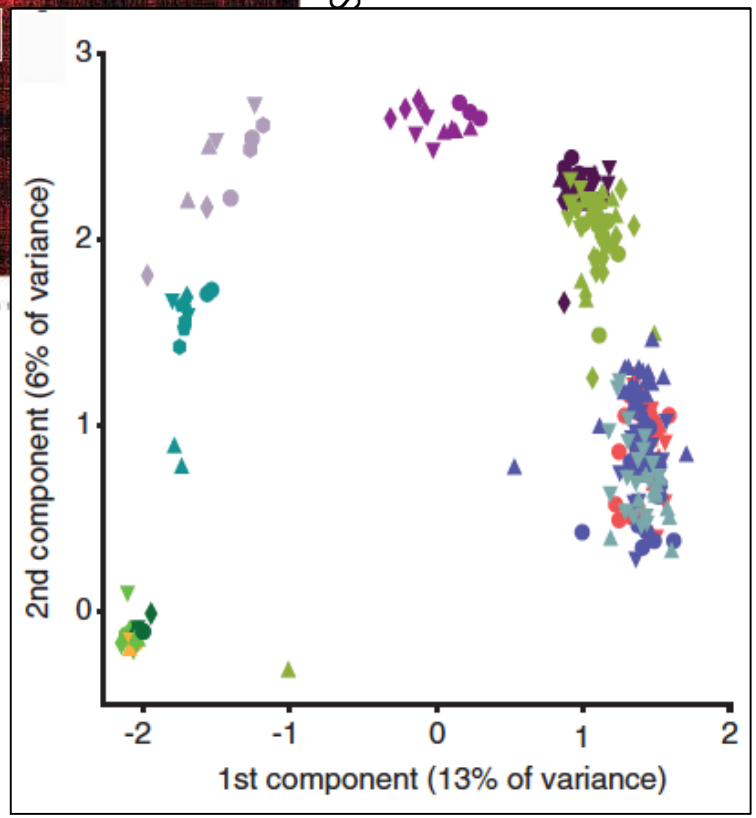
- UMI reduction
  - Count the number of unique molecular barcodes per transcript

Поиск транскрипционно-различных подгрупп

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



Jaitin *et al.* S

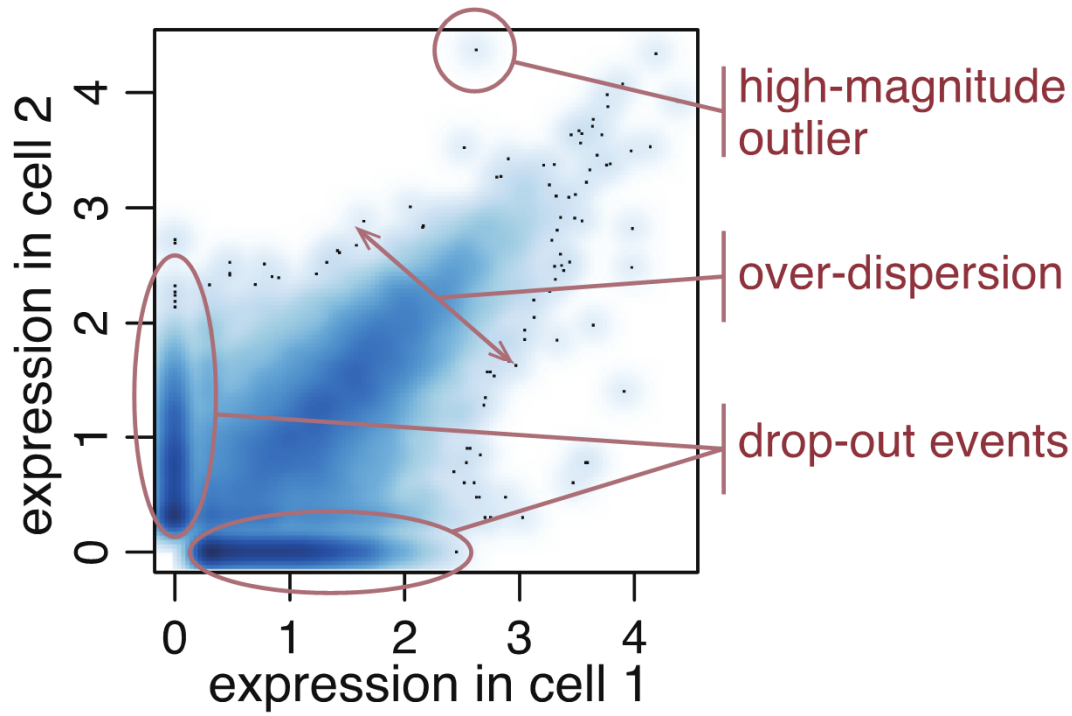


Deng *et al.* Science 2014

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

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
  - Non-Gaussian
  - Noisy cells form outliers
  - Drop-out events can mask true covariance structure

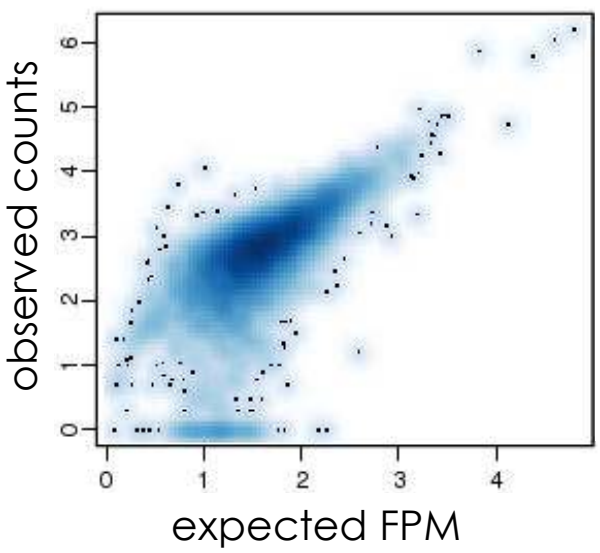


- Biological and technical
  - Control for the technical variability
  - Focus on the biological variability

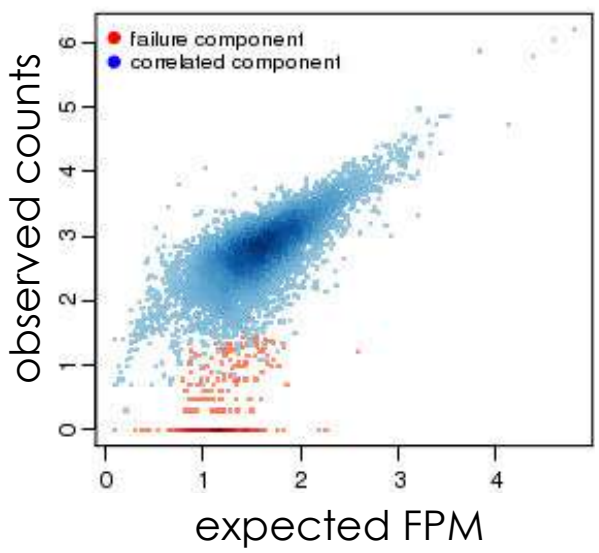
Модель Ошибок в Отдельной Клетке

- 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

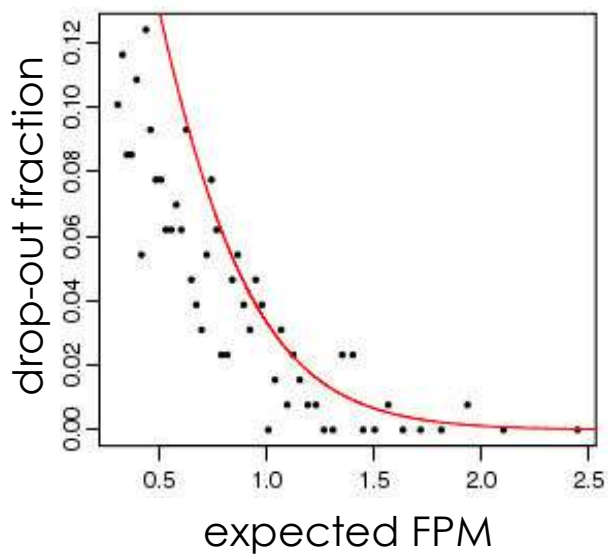
observed vs. expected



mixture fit



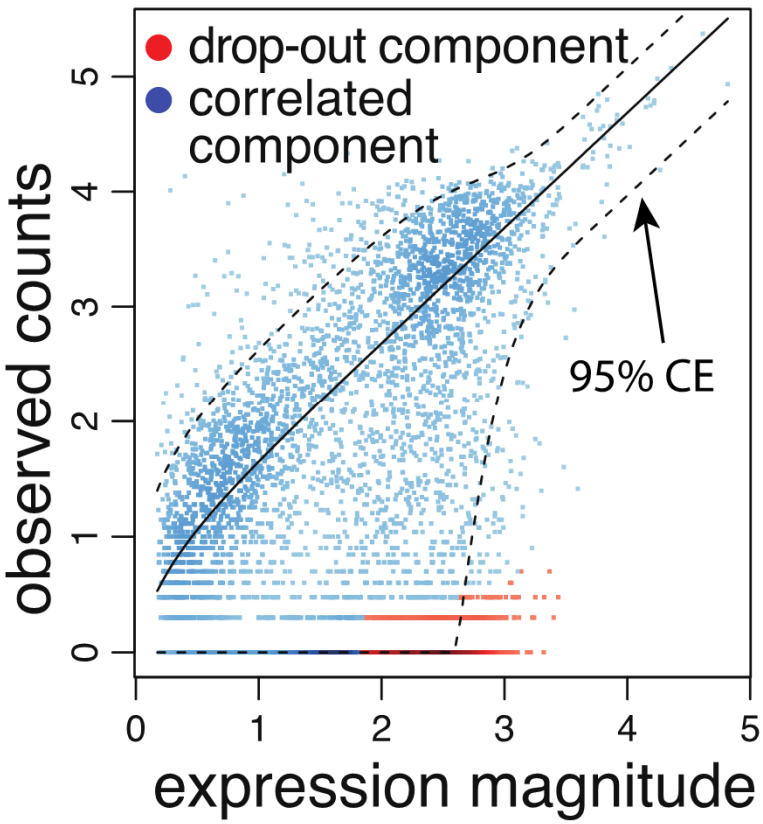
drop-out dependency

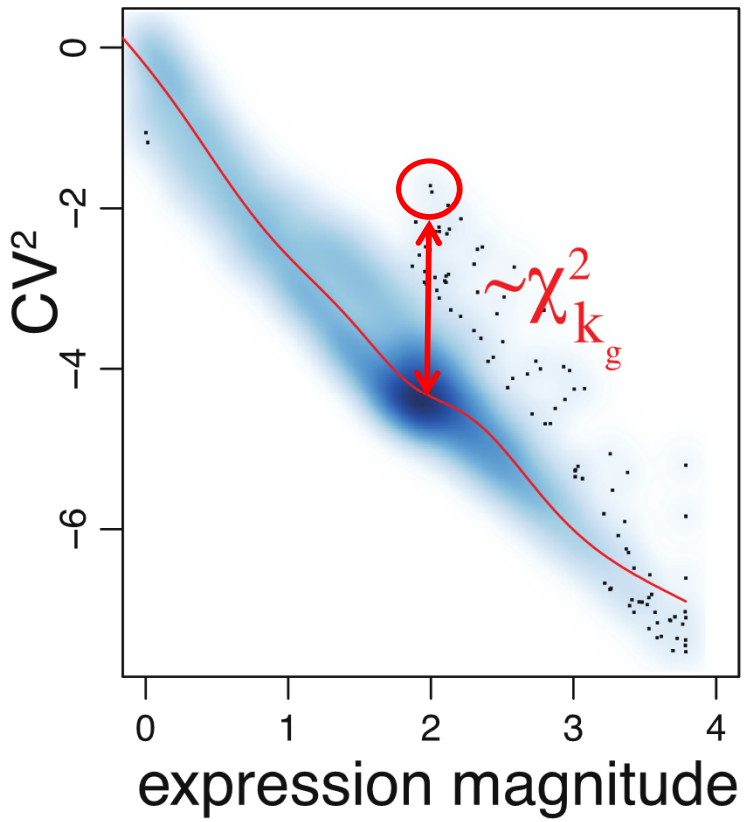


Kharchenko *et al.* Nature Methods 2014

Нормализация Дисперсии

- Account for cell- and gene-specific uncertainty
  - Cell-specific error models
  - Translate into χ^2 statistic
- Account for expression magnitude dependency
  - Adjusting with local regression fit



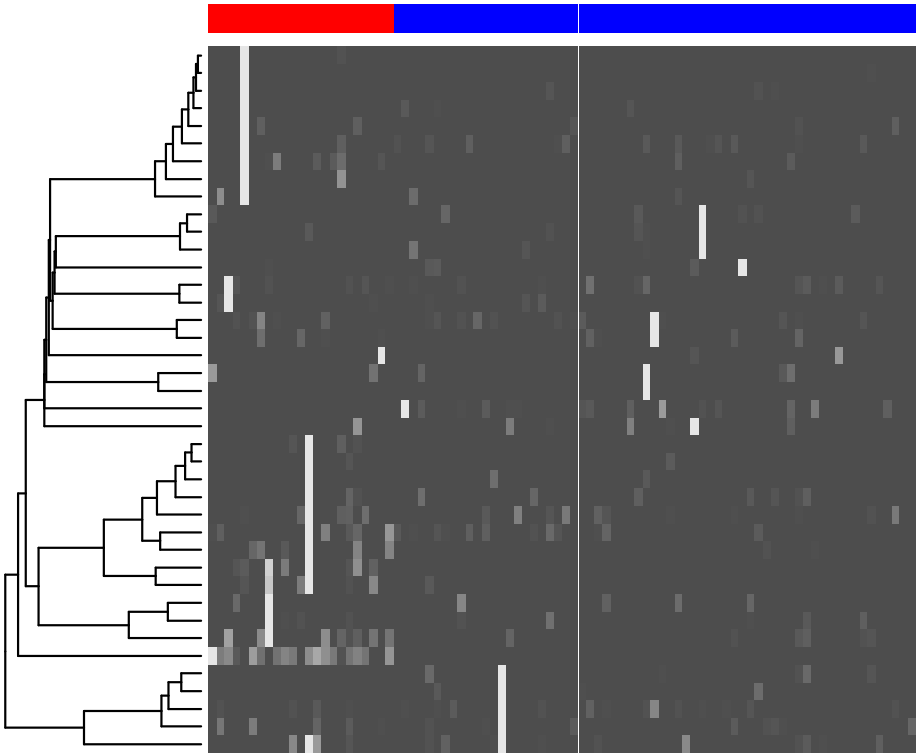


Kharchenko *et al.* Nature Methods 2014

Определение Высоко-Дисперсных Генов

Brennecke et al. Nat. Methods 2013

Astrocytes **NPCs**



Cell-specific models (PAGODA)

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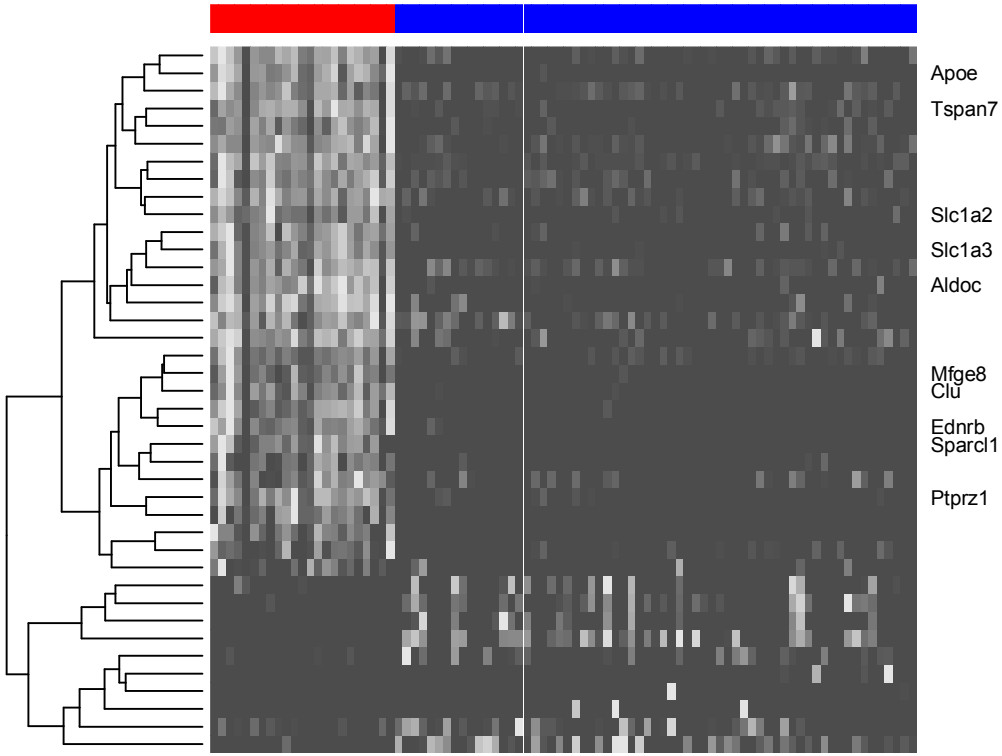


Stochastic neighbor embedding (SNE)

- 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

$$q_{j|i} = \frac{\exp\left(-\|y_i - y_j\|^2\right)}{\sum_{k \neq i} \exp\left(-\|y_i - y_k\|^2\right)}$$

- Note: If the map points y_i and y_j correctly model the similarity between the high-dimensional datapoints x_i and x_j, the conditional probabilities $p_{j|i}$ and $q_{j|i}$ will be equal
  - Probability of picking each other as neighbors is same regardless of dimension

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

Stochastic neighbor embedding (SNE)

- SNE finds the set of y_i and y_j s that minimize the sum of Kullback-Leibler divergences between conditional probabilities $p_{j|i}$ and $q_{j|i}$ over all datapoints by gradient descent

$$D_{\mathrm{KL}}(P\|Q) = \sum_i P(i) \ln \frac{P(i)}{Q(i)}.$$

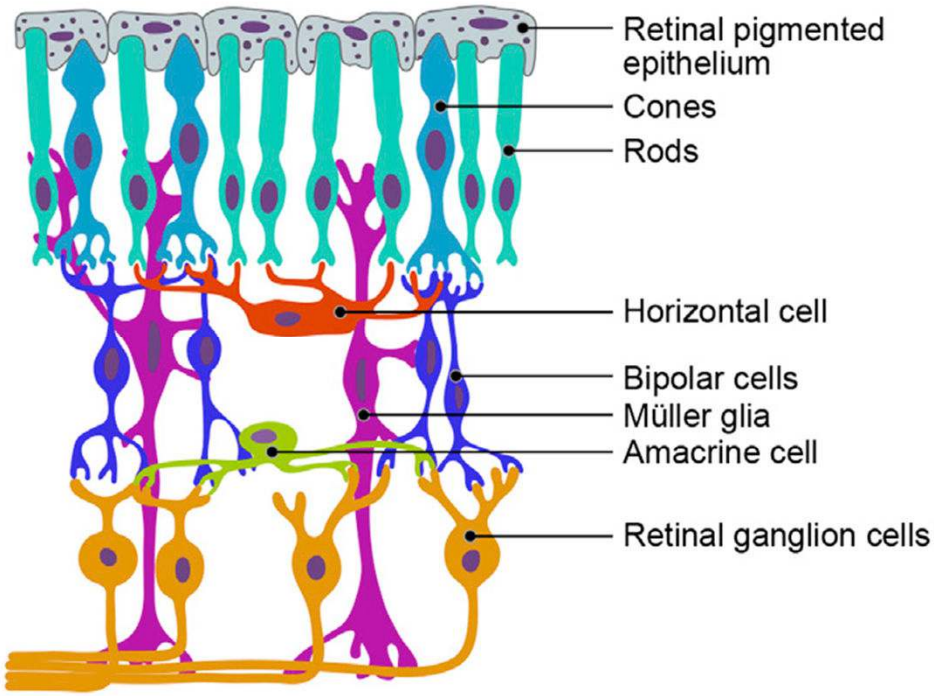
$$C = \sum_i KL(P_i||Q_i) = \sum_i \sum_j p_{j|i} \log \frac{p_{j|i}}{q_{j|i}}$$

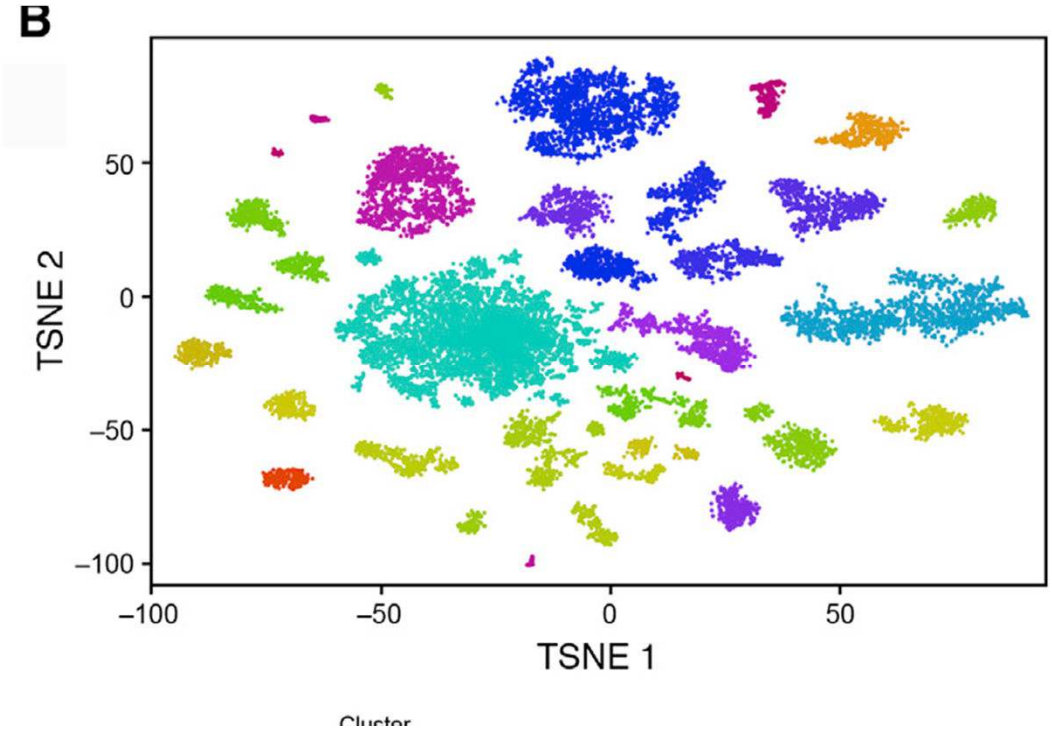
$$\frac{\delta C}{\delta y_i} = 2 \sum_j (p_{j|i} - q_{j|i} + p_{i|j} - q_{i|j})(y_i - y_j)$$

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

Single-Cell RNA-seq: tissue composition

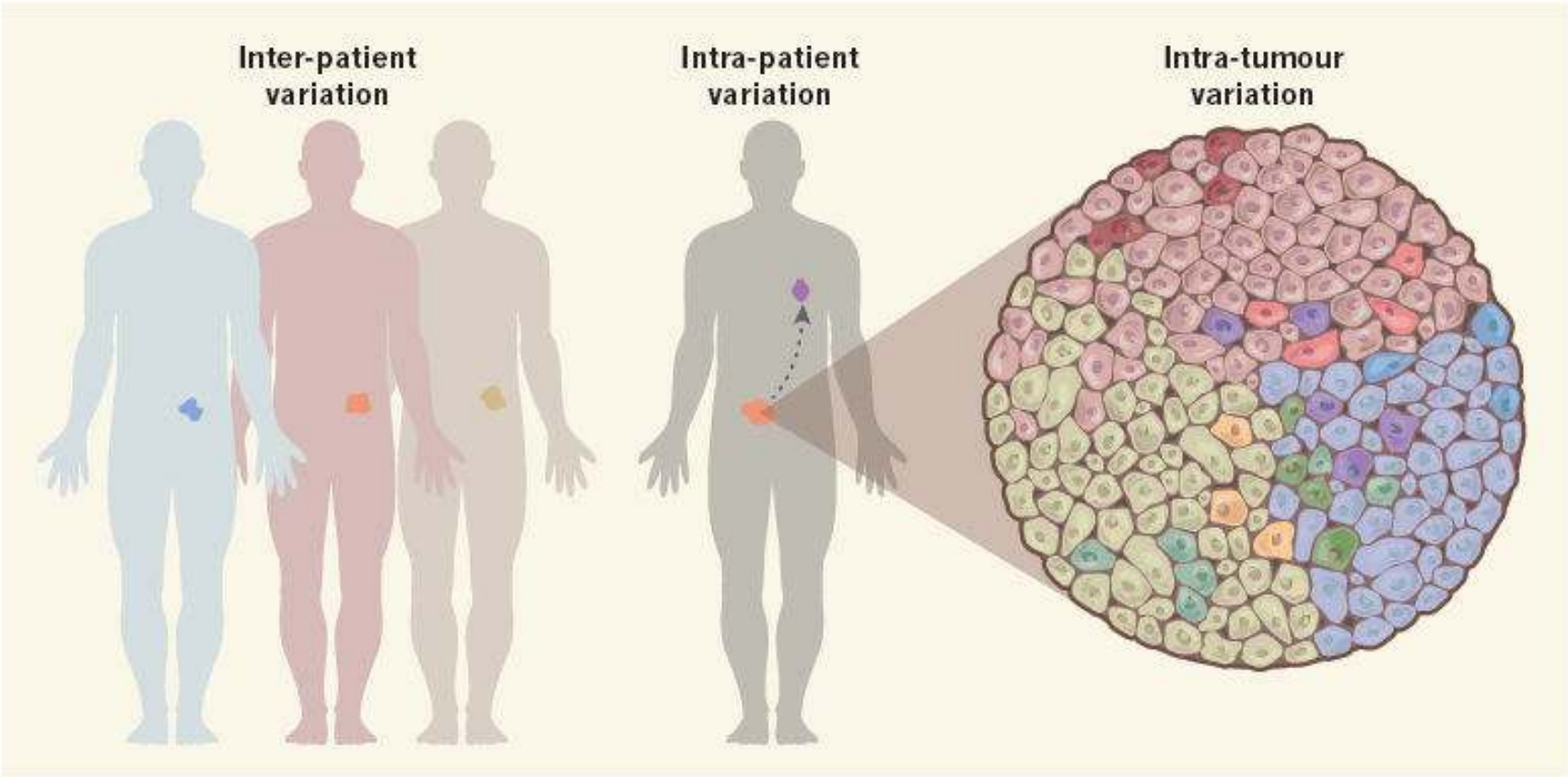
- Mouse Retina: Macosco *et al.* Cell 2015





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

Гетерогенность Раковых Тканей



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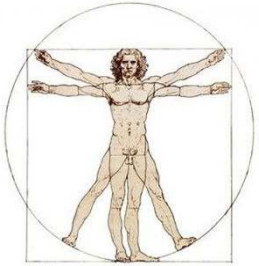
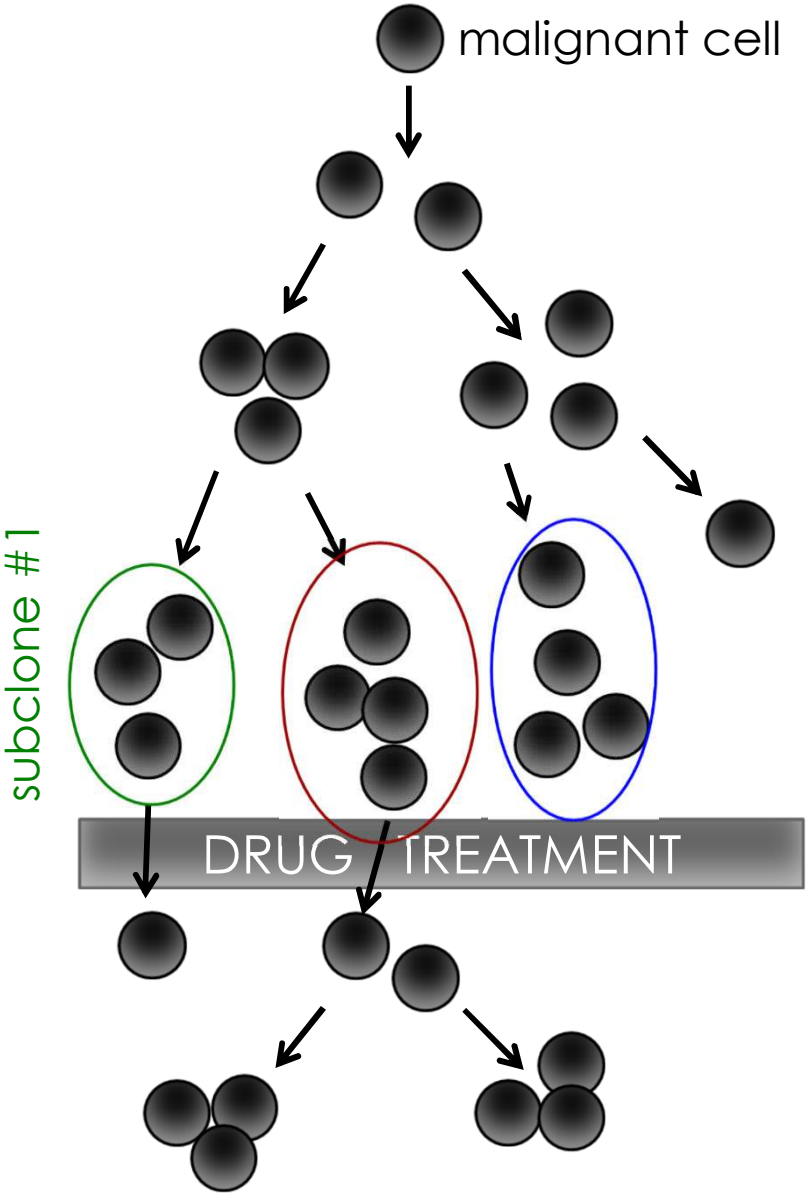


- Clinical relevance
  - Progression
  - Drug Resistance
- Genetic Heterogeneity
  - Clonal evolution
  - Genome sequencing



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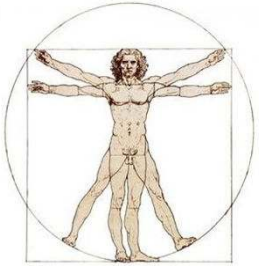

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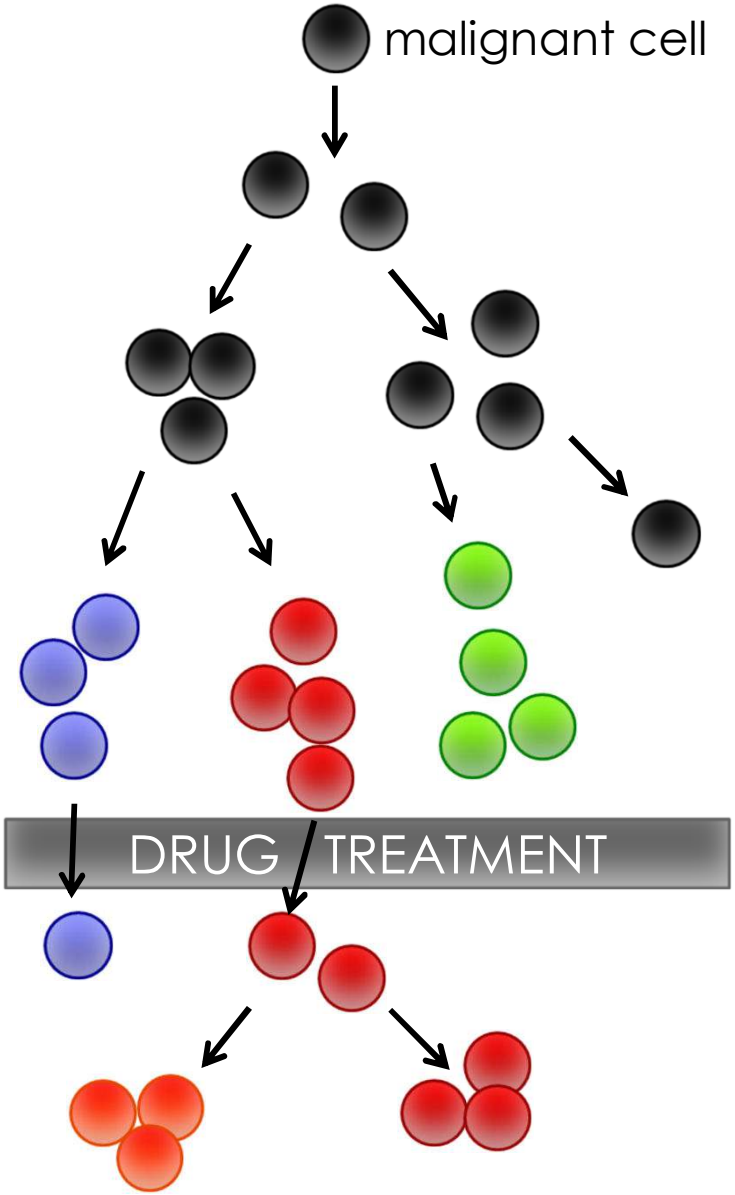
- Transcriptional Heterogeneity
  - Transient vs. stable states



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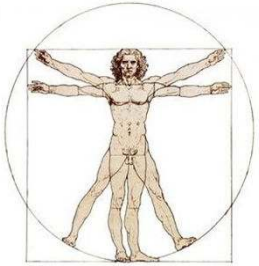

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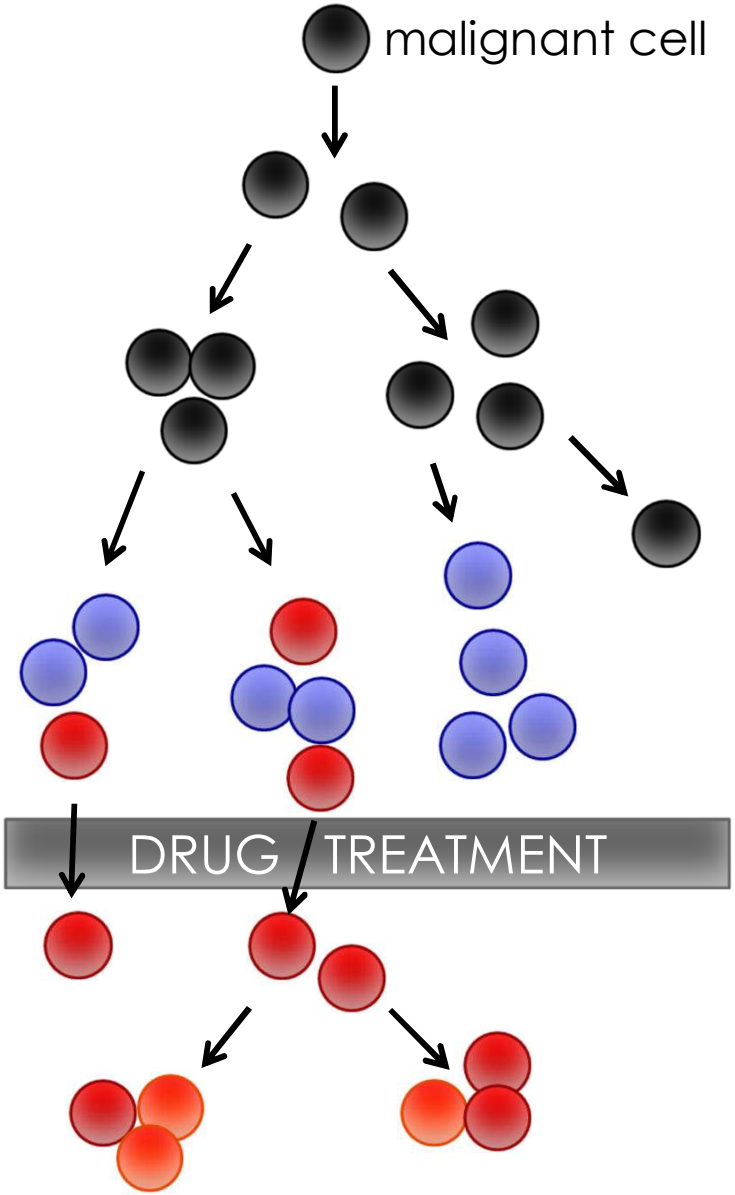
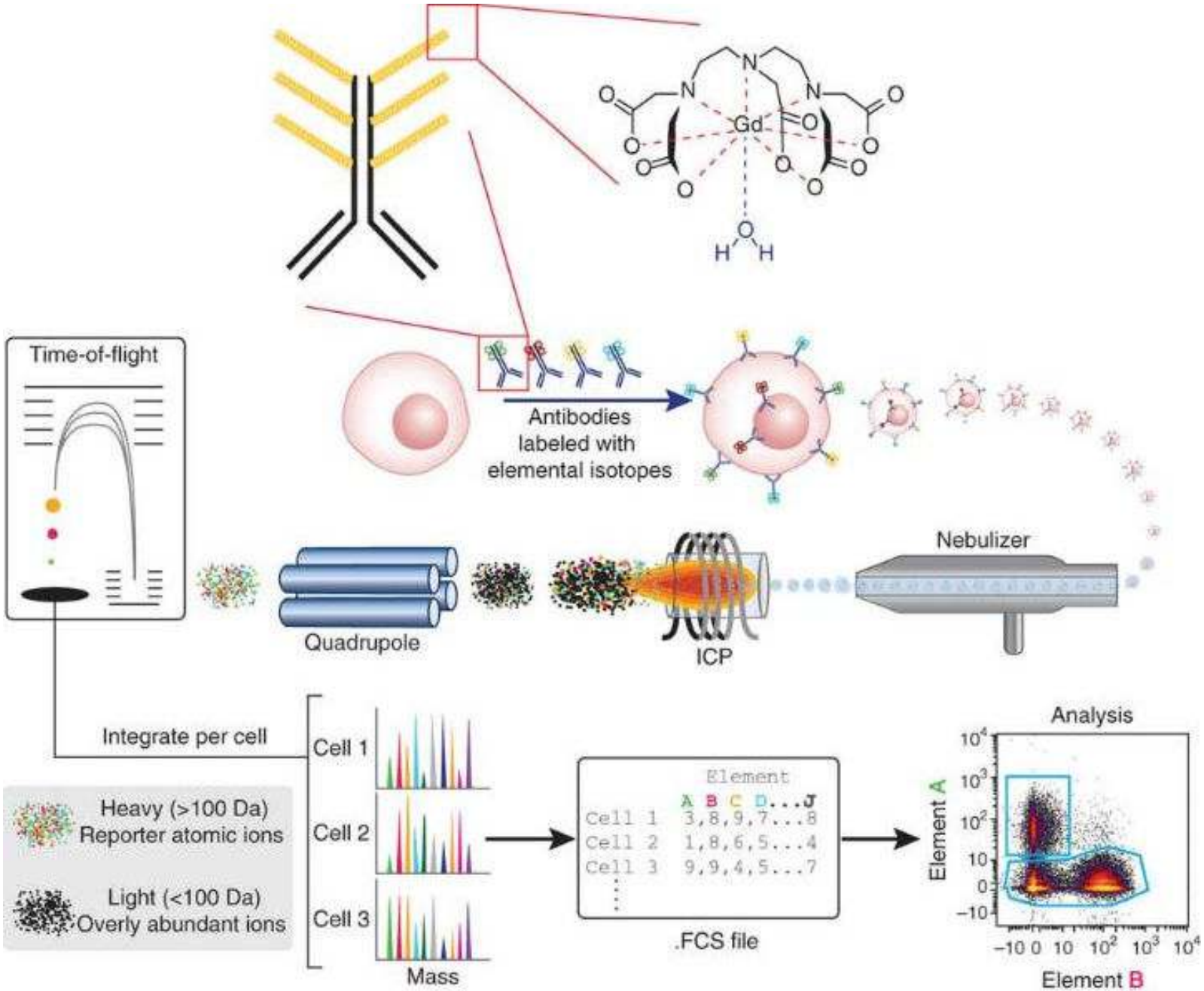
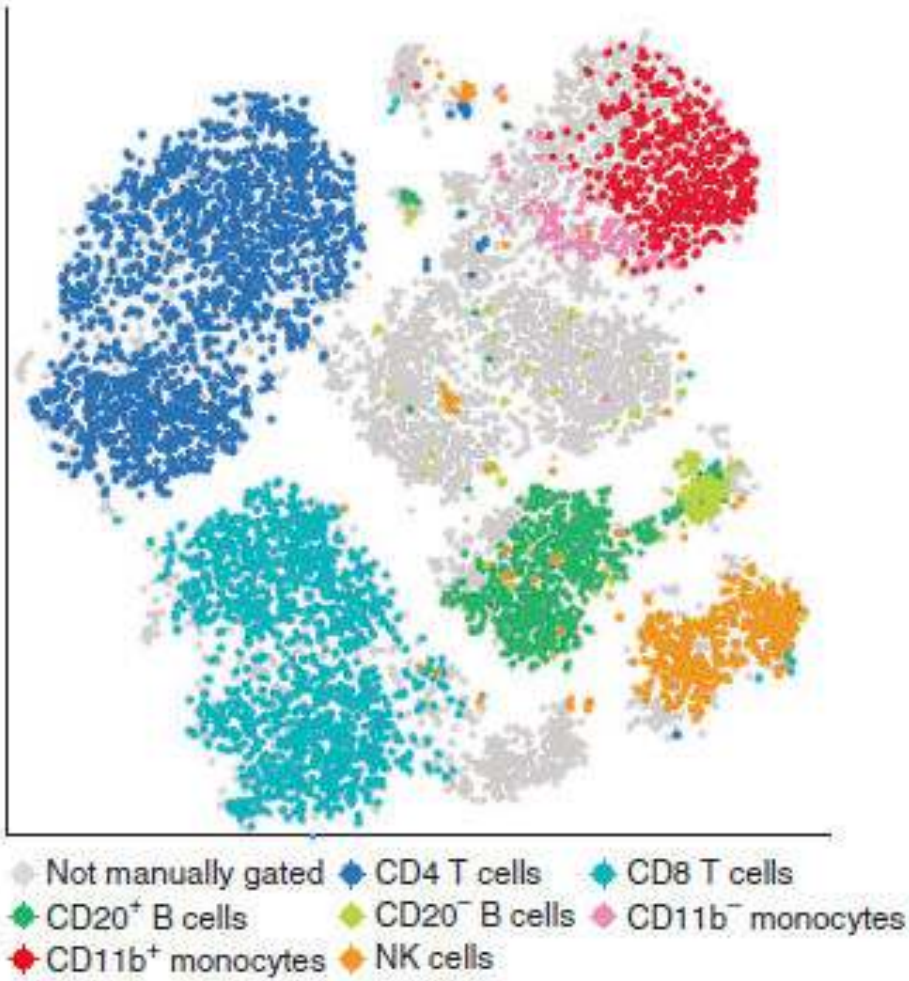


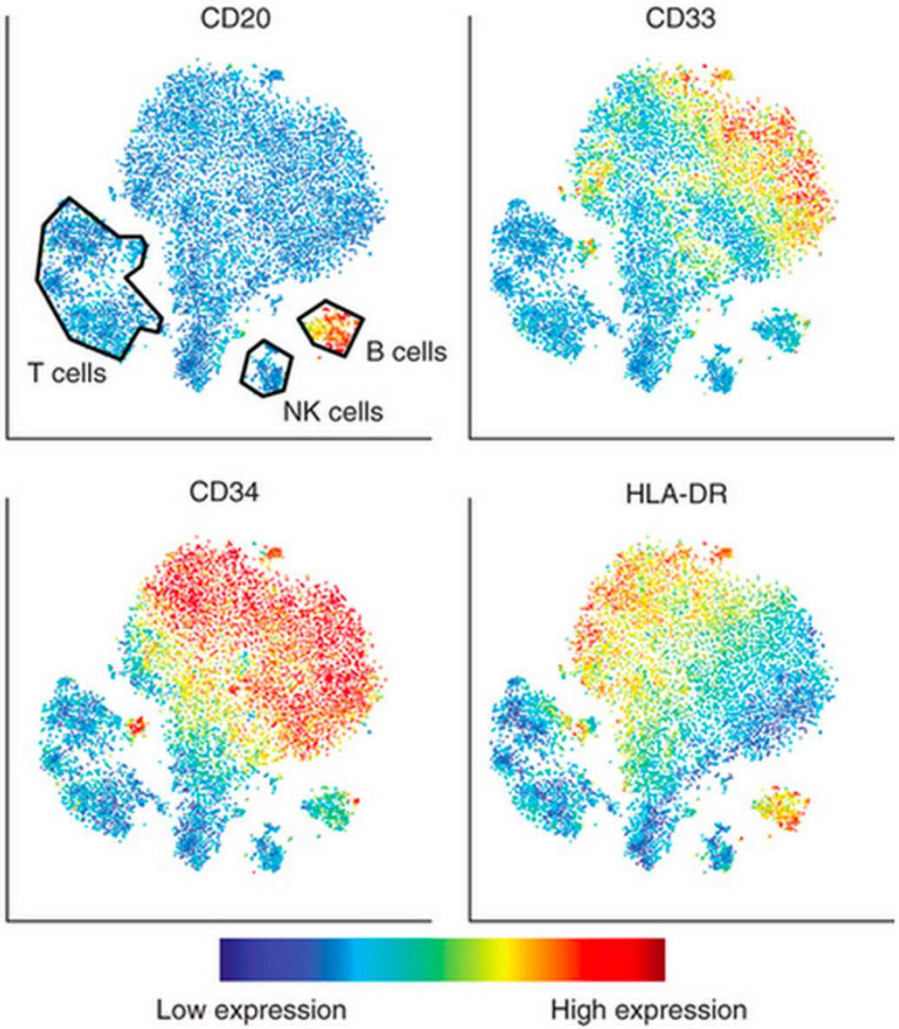
Mass Cytometry: CyTOF



viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia

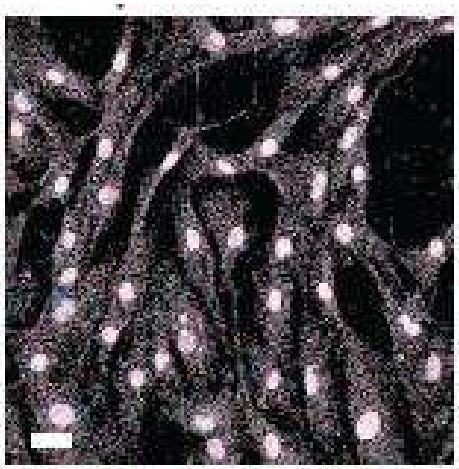
El-ad David Amir[1], Kara L Davis[2,3], Michelle D Tadmor[1,3], Erin F Simonds[2,3], Jacob H Levine[1,3], Sean C Bendall[2,3], Daniel K Shenfeld[1,3], Smita Krishnaswamy[1], Garry P Nolan[2,4] & Dana Pe'er[1,4]

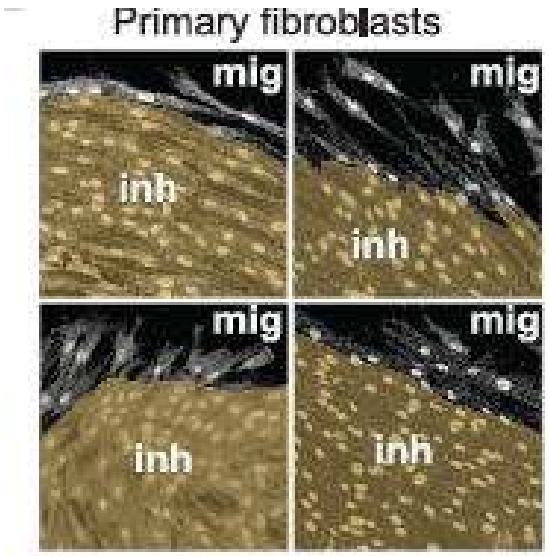


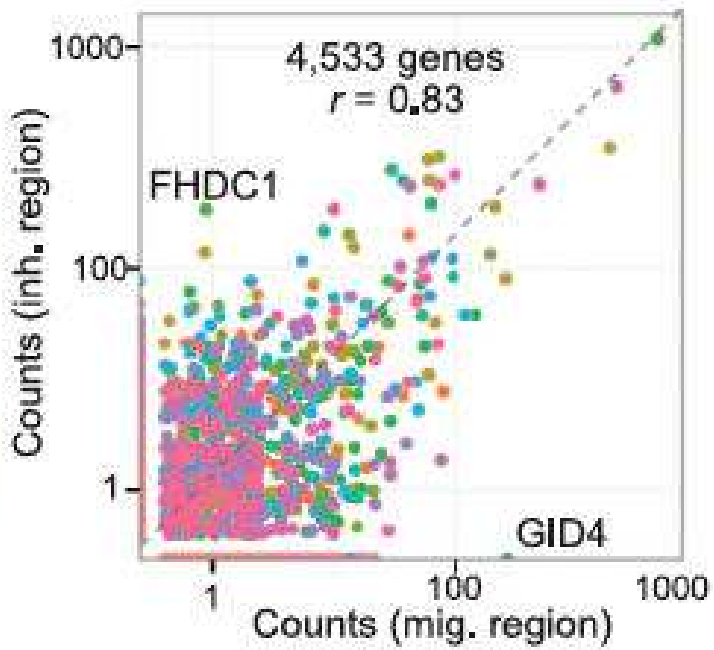


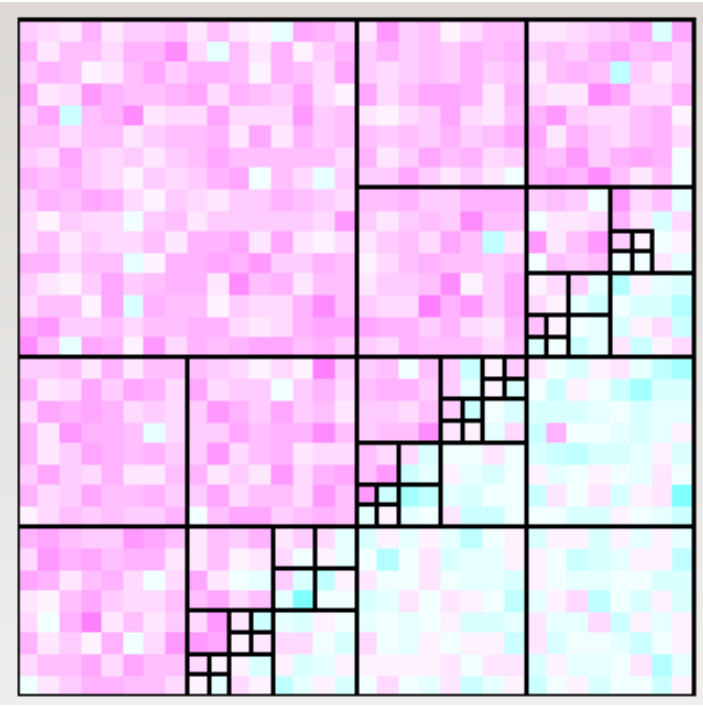
In situ RNA sequencing: FISSEQ

- Lee et al. Science 2014









Multi-scale Poisson models