Statistical methods for single-cell RNA sequencing data

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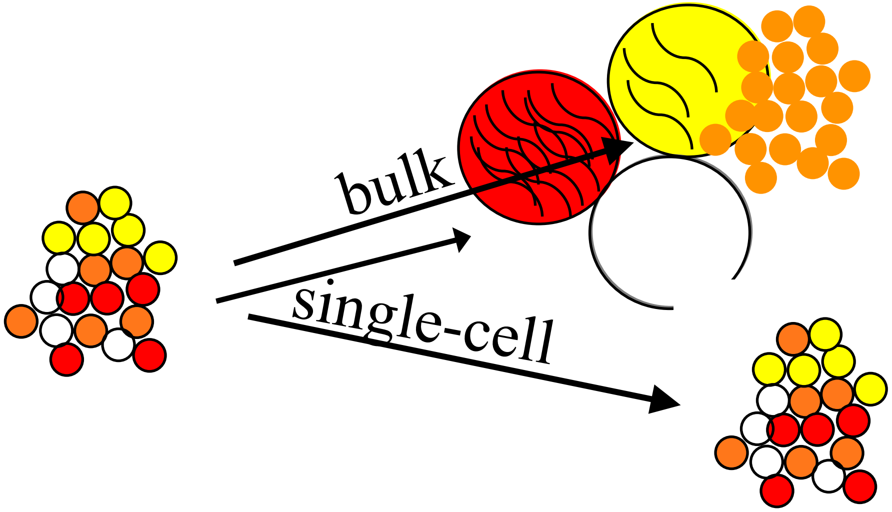
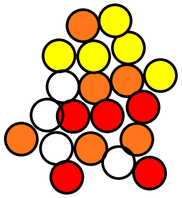
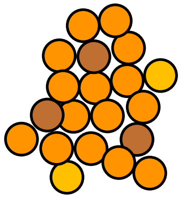
Single-cell vs. bulk RNA-seq



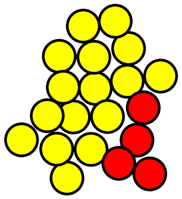
Heterogeneous



Homogeneous

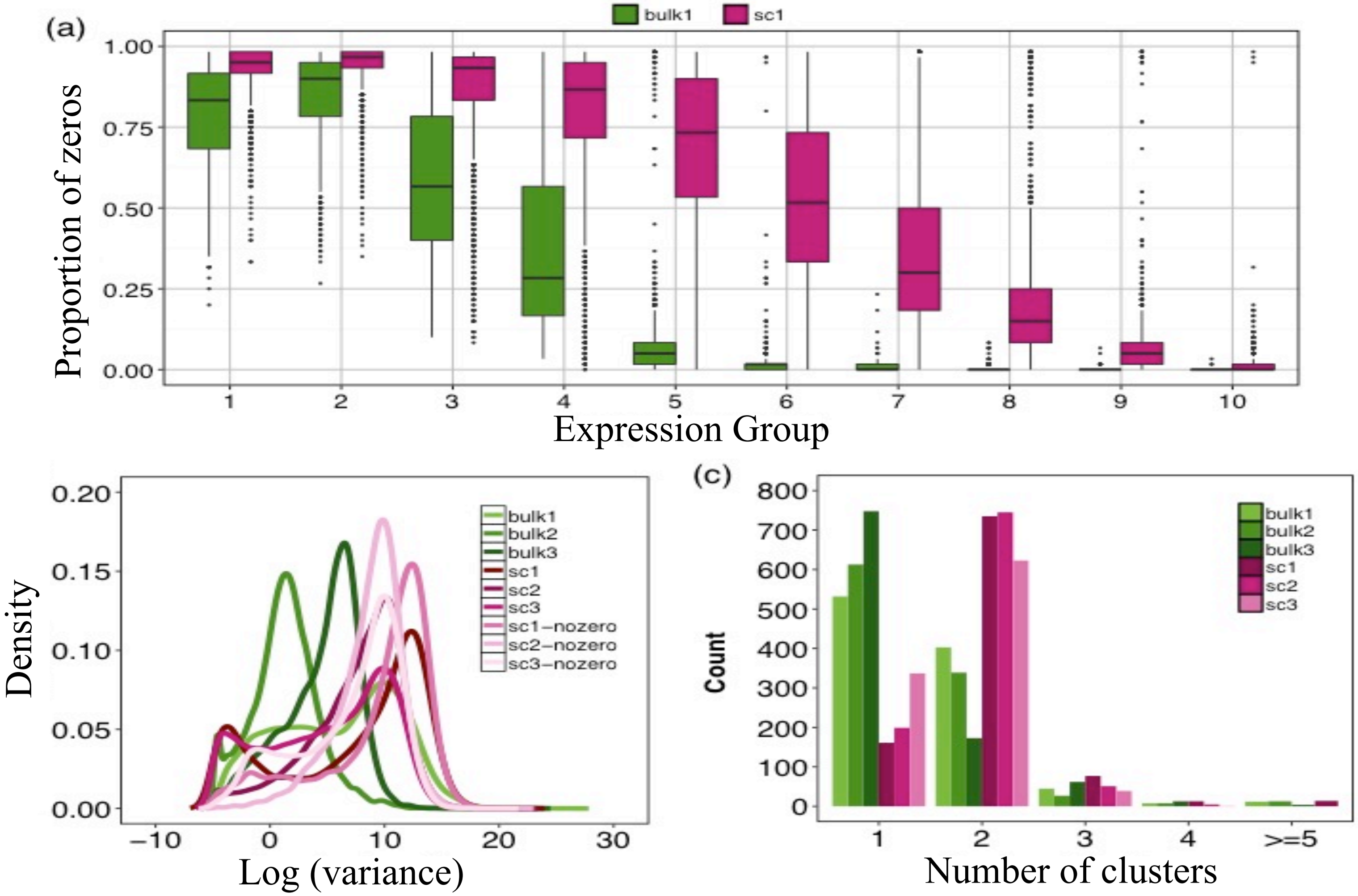


Sub-population



Features of single-cell RNA-seq data

- Abundance of zeros, increased variability, complex distributions



Bacher and Kendziorski, *Genome Biology*, 2016.

Challenges in scRNA-seq

- Normalization
- Technical vs. biological zeros
- Clustering; Identifying sub-populations
- De-noising
    - Adjusting for technical variability
    - Adjusting for biological variability (oscillatory genes)
- Identifying and characterizing differences in gene-specific expression distributions (aka. identifying differential distributions)
- Pseudotime reordering
- Network reconstruction

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Challenges in scRNA-seq

- Normalization → Bacher, Chu *et al.*, *Nature Methods*, 2017
- Technical vs. biological zeros
- Clustering; Identifying sub-populations
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  - Adjusting for technical variability → Leng *et al*. *Bioinformatics*, 2016
  - Adjusting for biological variability (oscillatory genes) → Leng, Chu *et al.*, *Nature Methods*, 2015
- Identifying and characterizing differences in gene-specific expression distributions (aka. identifying differential distributions) → Korthauer *et al.*, *Genome Biology*, 2016
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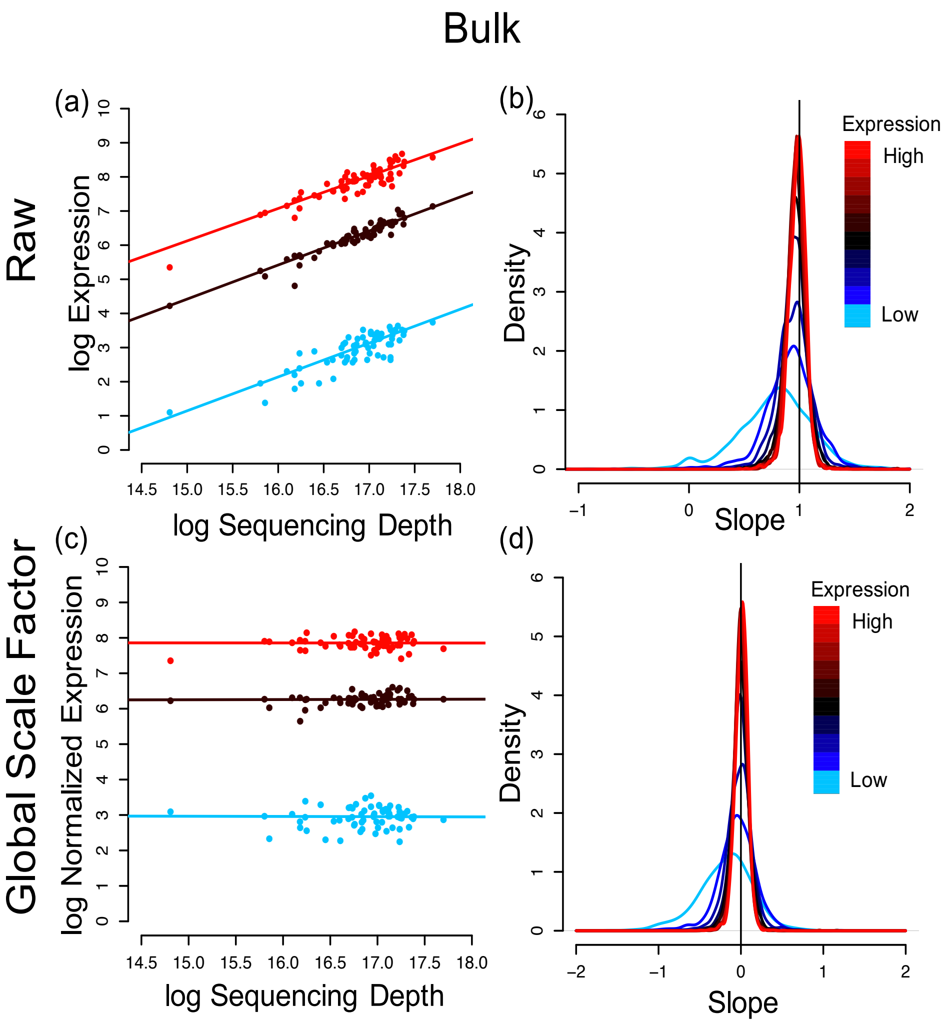
SCnorm: A quantile-regression based approach for robust normalization of single-cell RNA-seq data

Bacher, Chu *et al.*, *Nature Methods*, 2017

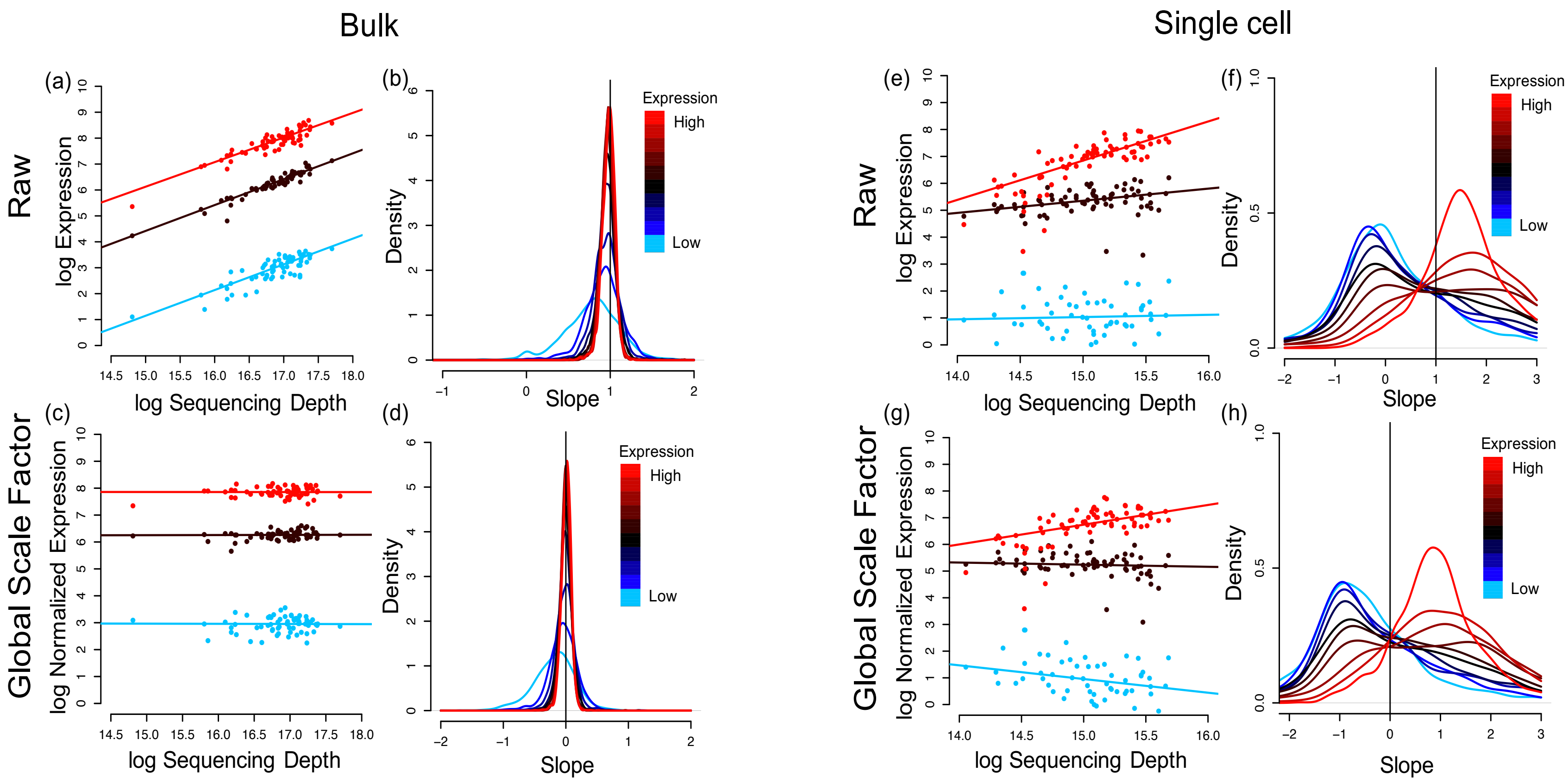
Background

- Goal: correct for technical artifacts and/or gene-specific features
  - Sequencing depth
  - Length, GC content
  - Amplification and other technical biases
- Without UMIs/spike-ins, most single-cell methods calculate global scale factors as in bulk RNA-seq
  - One scale factor is calculated per sample and applied to all genes in that sample.

Bulk: Global scale-factor normalization for sequencing depth



Expression vs. depth varies with expression in scRNA-seq



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We see the count-depth relationship varying with expression in many datasets

Overview of SCnorm

- Identify gene groups based on the count-depth relationship.

Within each group,

- Quantile polynomial regression is used to quantify the group-specific relationship between expression and sequencing depth. The quantile is chosen iteratively.
- Predicted values are used to calculate group-specific scale factors for each cell.

SCnorm

- Filter: genes having greater than 10% expression values nonzero and median nonzero expression greater than 2.
- Let $Y_g = (y_{g1}, \ldots, y_{gJ})$ denote log non-zero expression for gene g in cell j ; X_j denote log sequencing depth.
- The gene-specific count-depth relationship is estimated by:

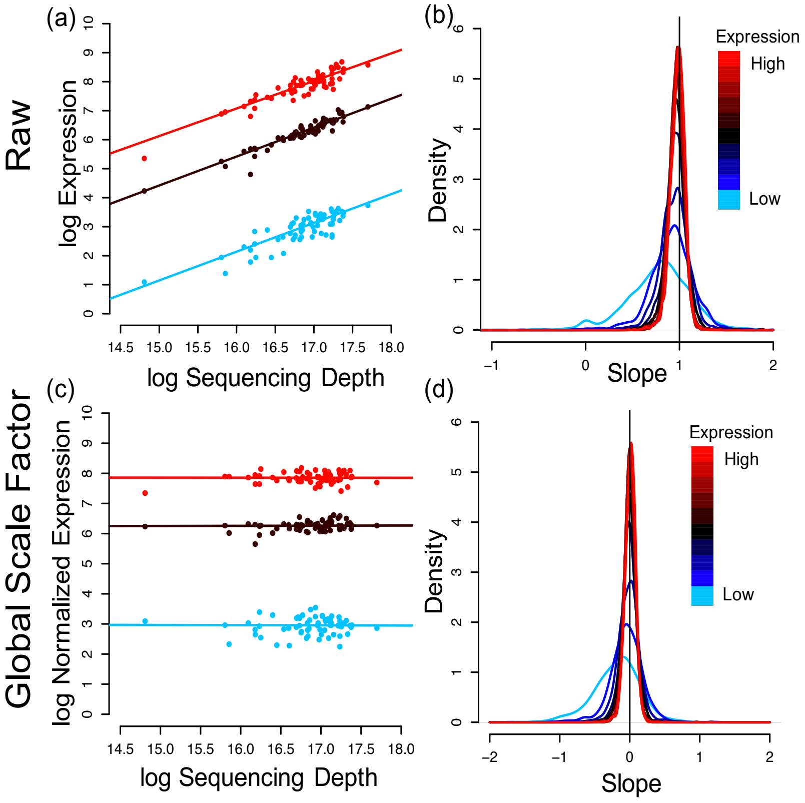
$$Q^{0.5}\left(Y_{g,j} | X_j\right) = \beta_{g,0} + \beta_{g,1} X_j$$

- Genes are split into K groups. The group specific count-depth relationship is estimated by:

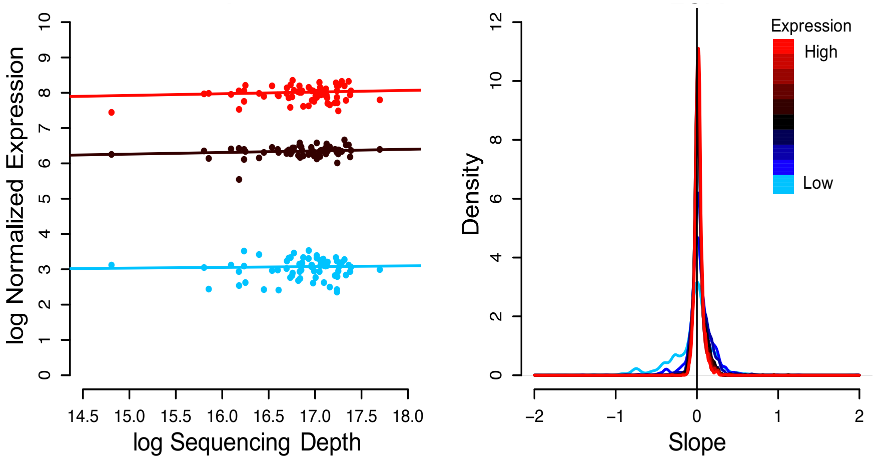
$$Q^{\tau_k, d_k}\left(Y_j | X_j\right) = \beta_0^{\tau_k} + \beta_1^{\tau_k} X_j + \cdots + \beta_d^{\tau_k} X_j^{d_k}$$

- Estimates of τ_k and d_k minimize $\left|\hat{\eta}_1^{\tau_k} - {}^{mode}_{g}\hat{\beta}_{g,1}\right|$; where $\hat{\eta}_1^{\tau_k}$ represents the count-depth relationship among predicted values.
- K is chosen so that the absolute value of the maximum normalized slope mode is < 0.1 within each of ten groups.

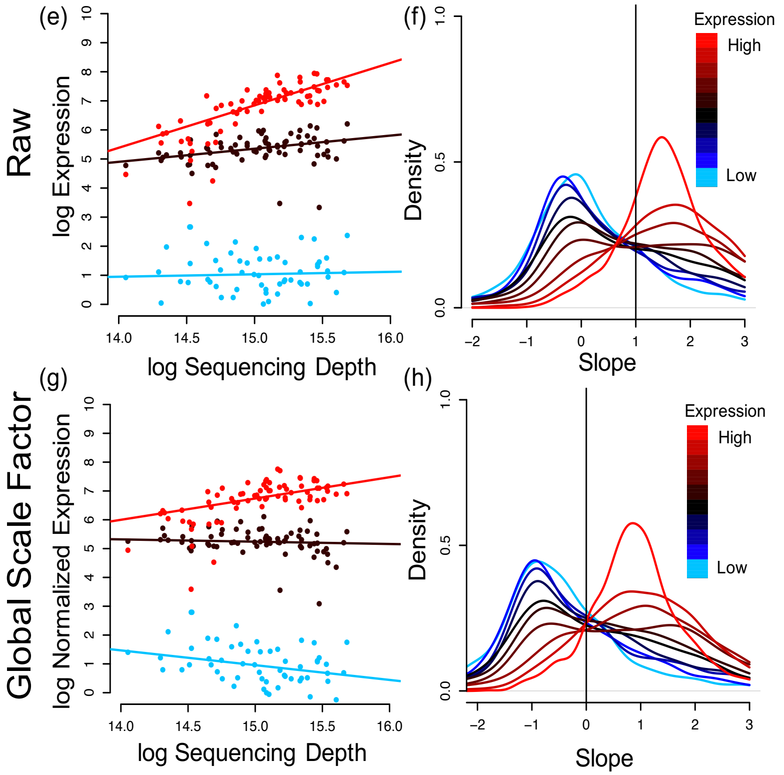
Bulk RNA-seq



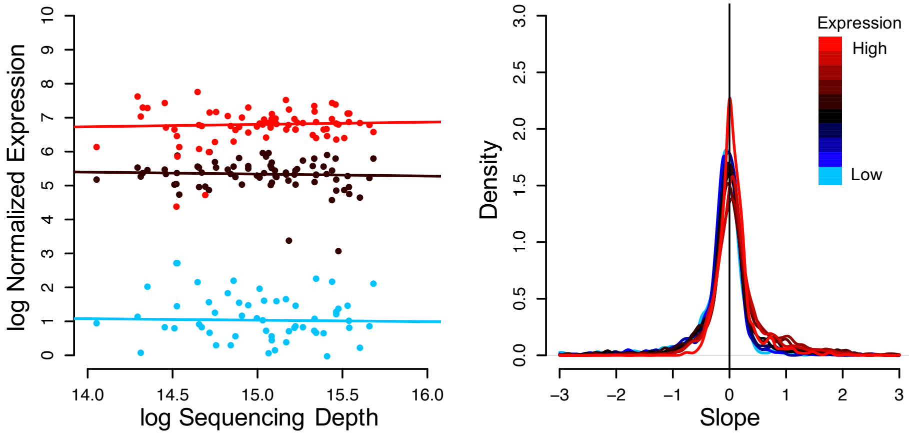
SCnorm



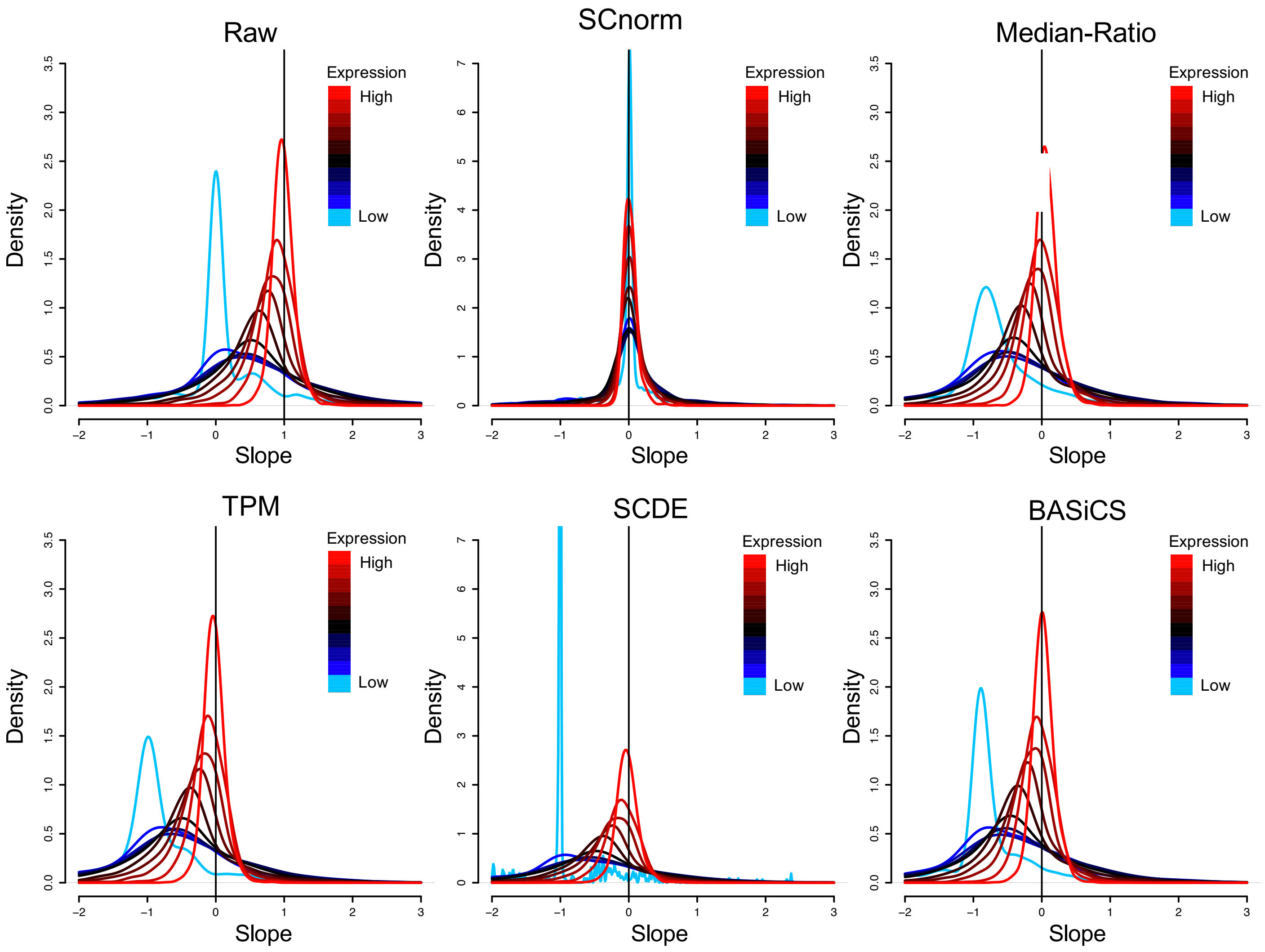
Single-cell RNA-seq



SCnorm



H1 - 1 (~ 1 million reads per cell)



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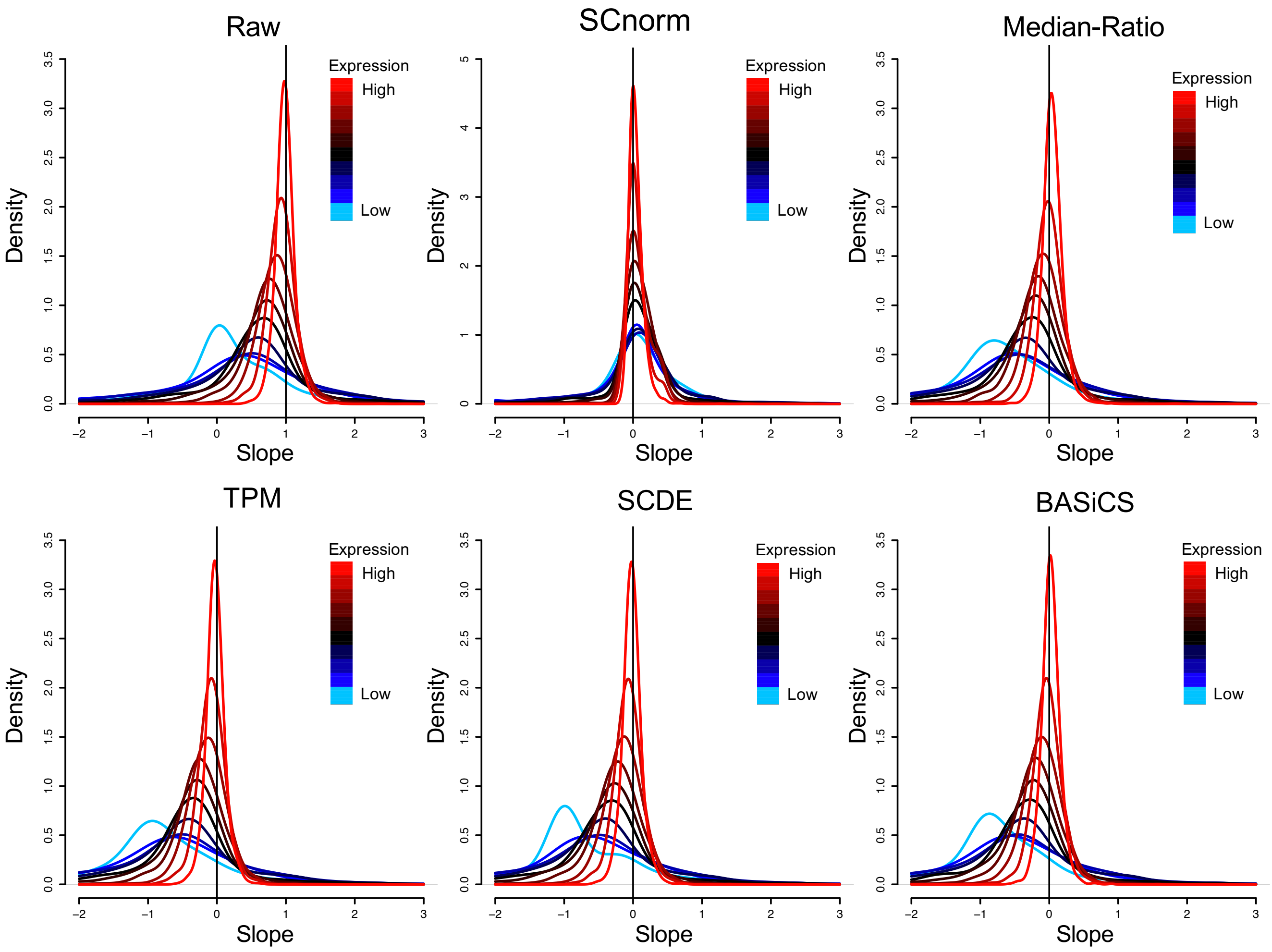
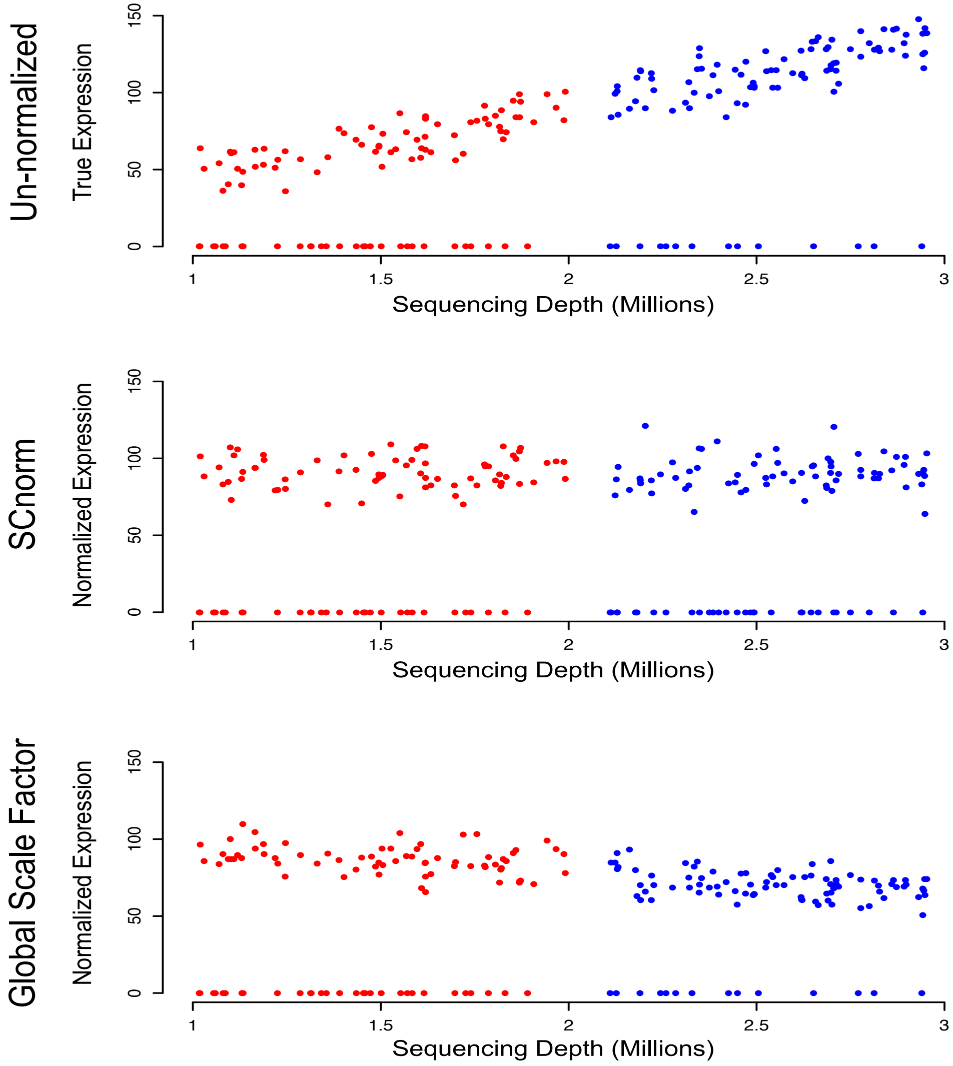
H1 - 4 (~4 million reads per cell)

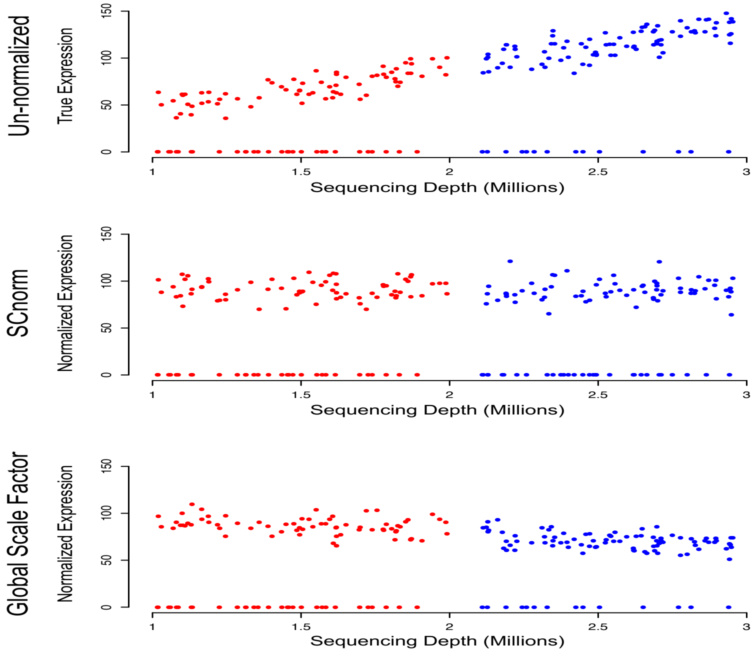


Implications for DE analysis



FC= H1-1/H1-4

- H1-1: ~100 H1 cells profiles at ~1 million reads per cell
- H1-4: Same H1 cells profiled at ~4 million reads per cell
- Prior to normalization, H1-1/H1-4 should be about ¼
- Post normalization, H1-1/H1-4 should be about 1
- If over-normalization is going on, H1-1/H1-4 will be greater than 1.



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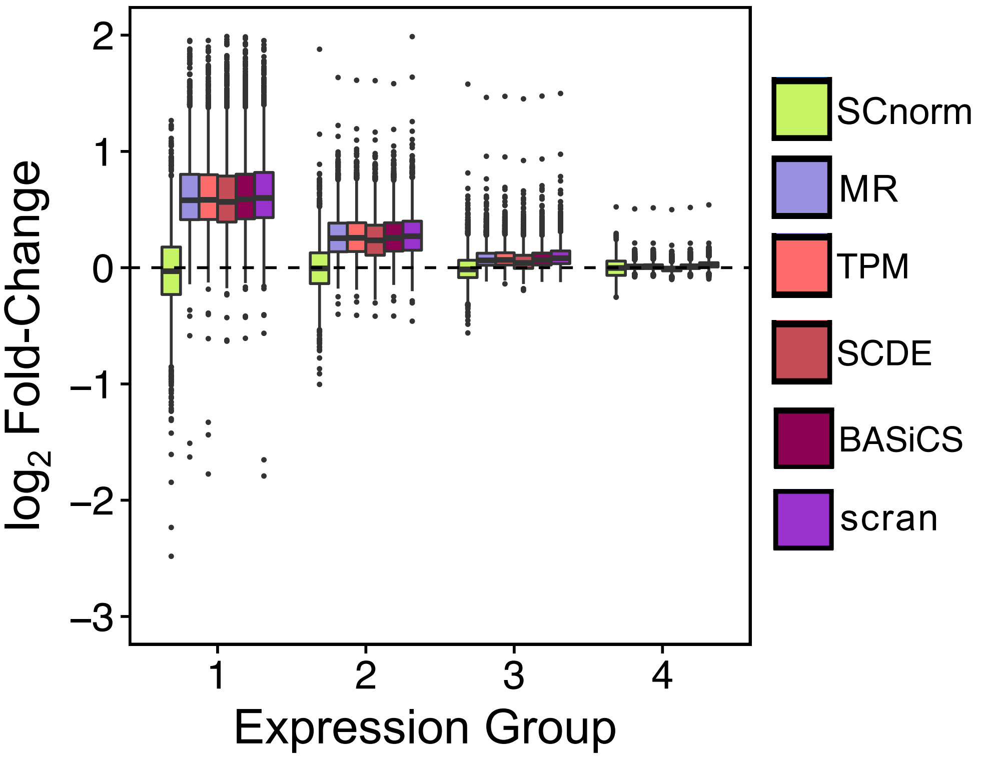
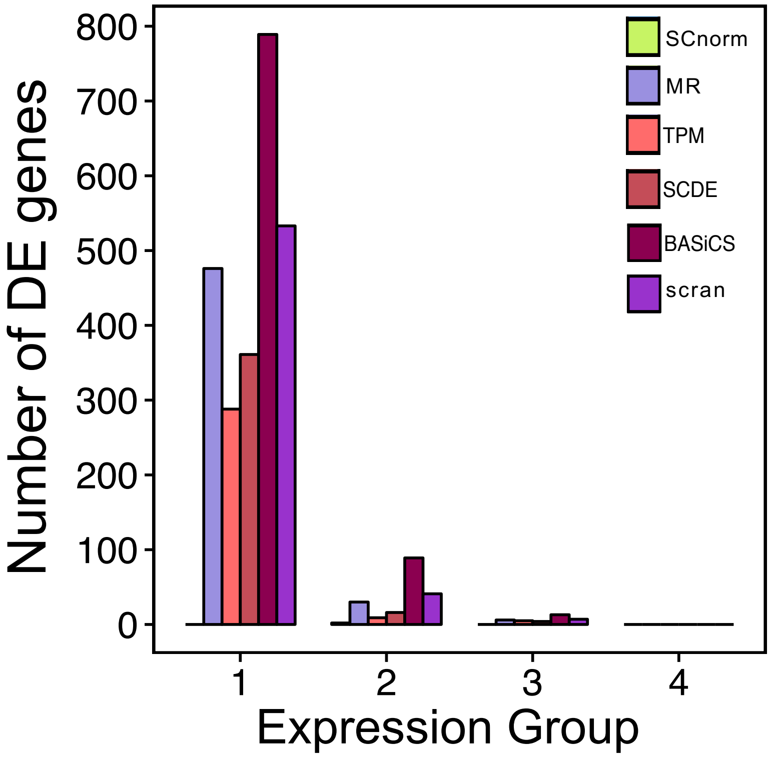
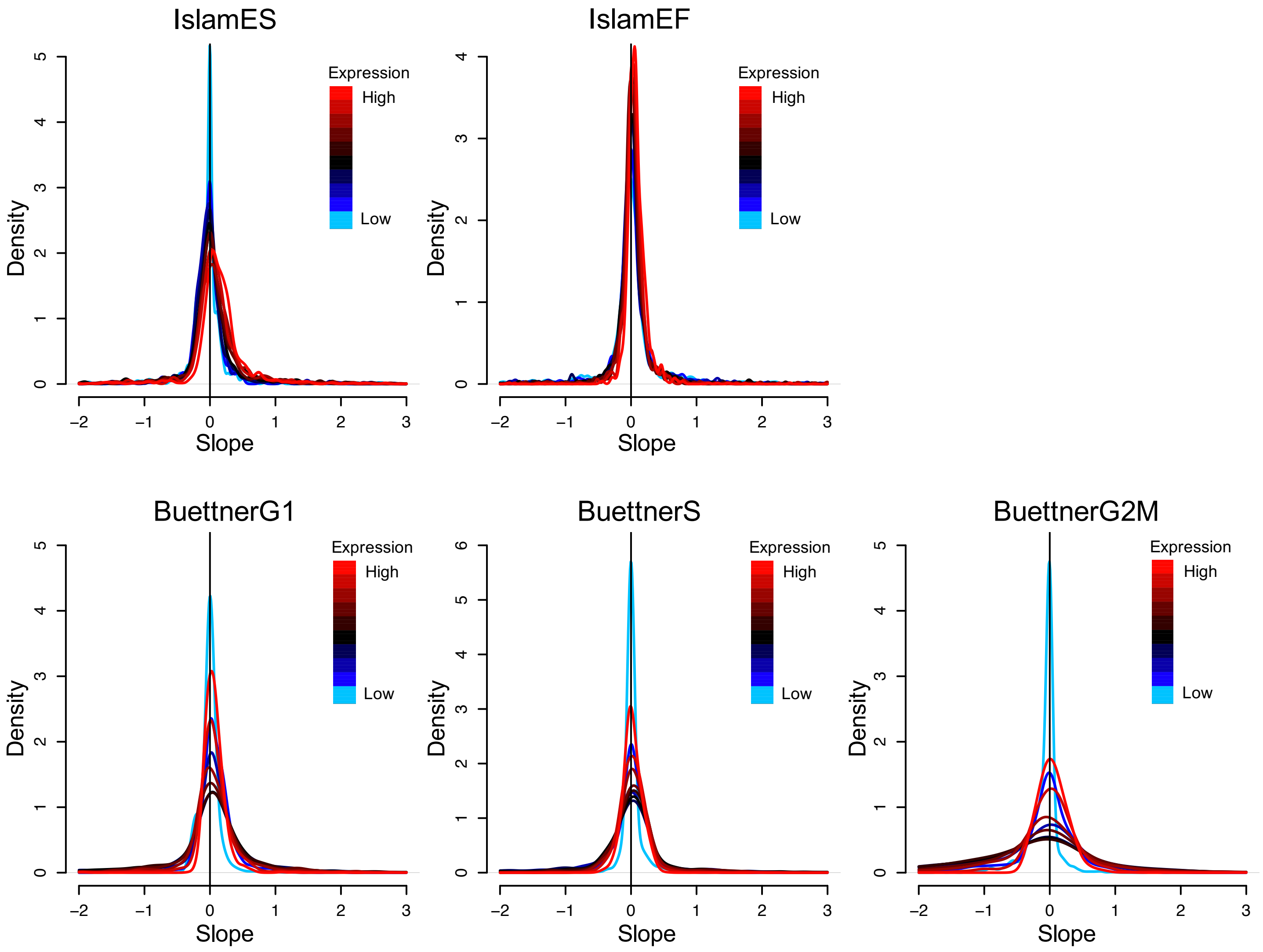




Normalization via SCnorm



Challenges in scRNA-seq

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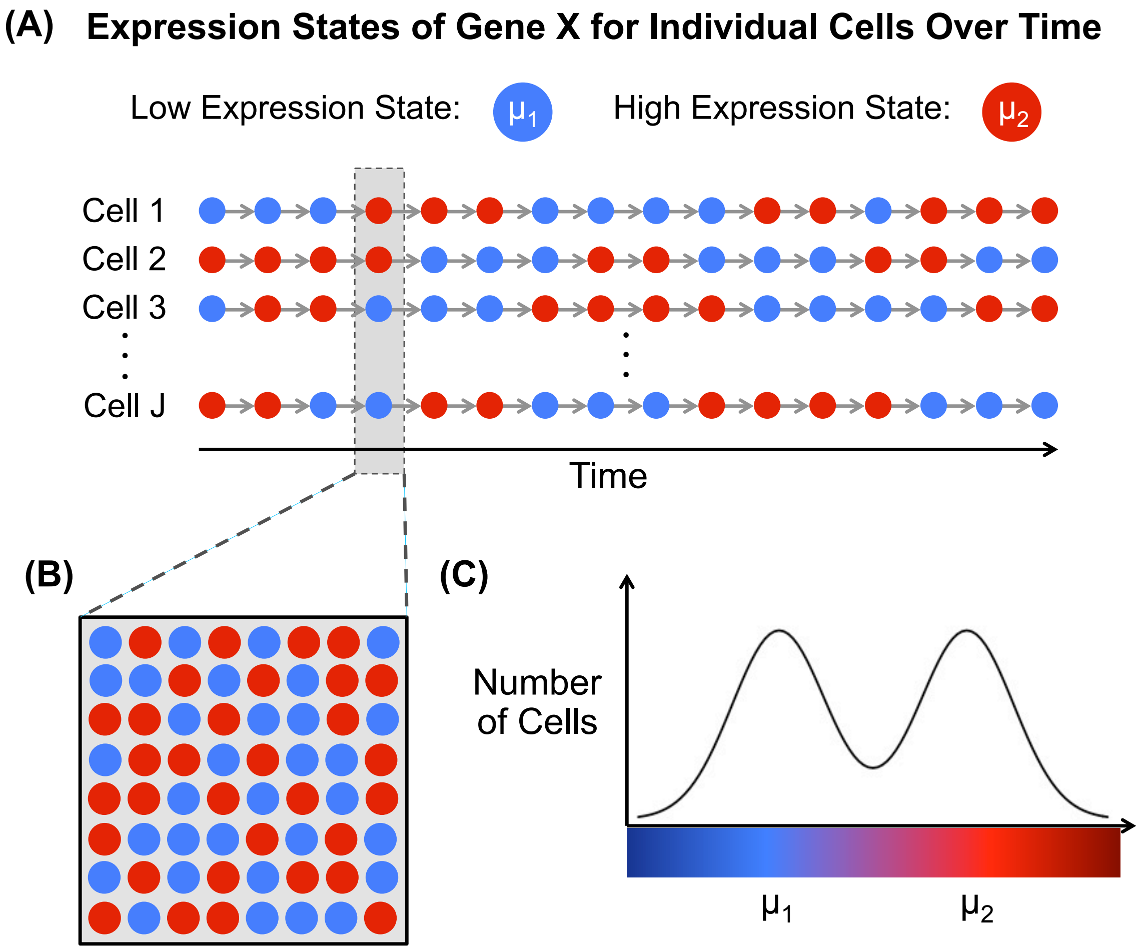
scDD: A Dirichlet mixture model based approach for identifying differential distributions in scRNA-seq experiments

Korthauer *et al*., *Genome Biology,* to appear, 2016

Gene-specific multi-modality

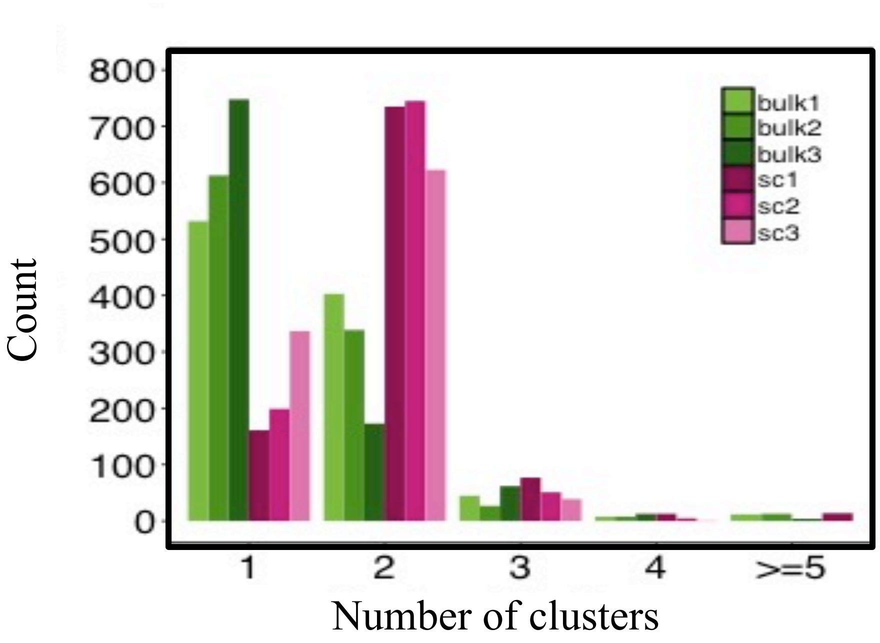


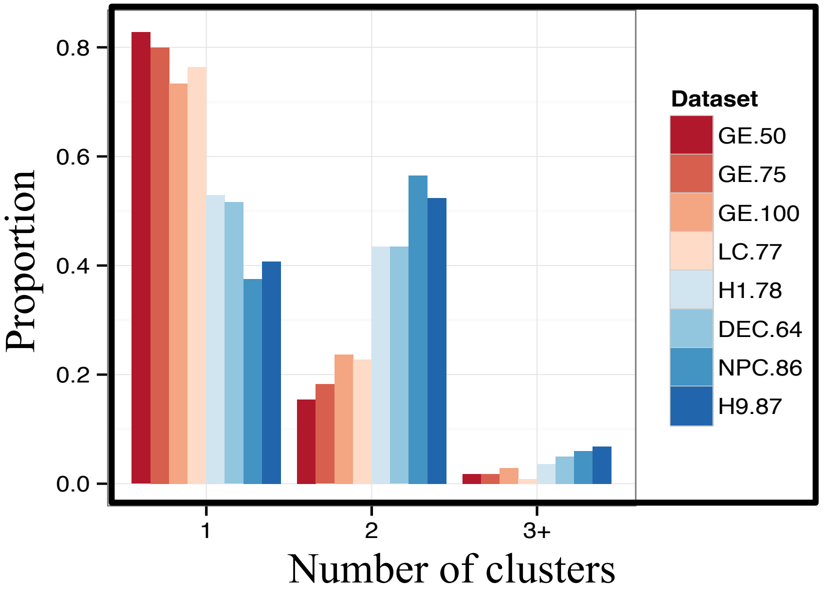
(A) Expression States of Gene X for Individual Cells Over Time

Snapshot of Population of Single Cells

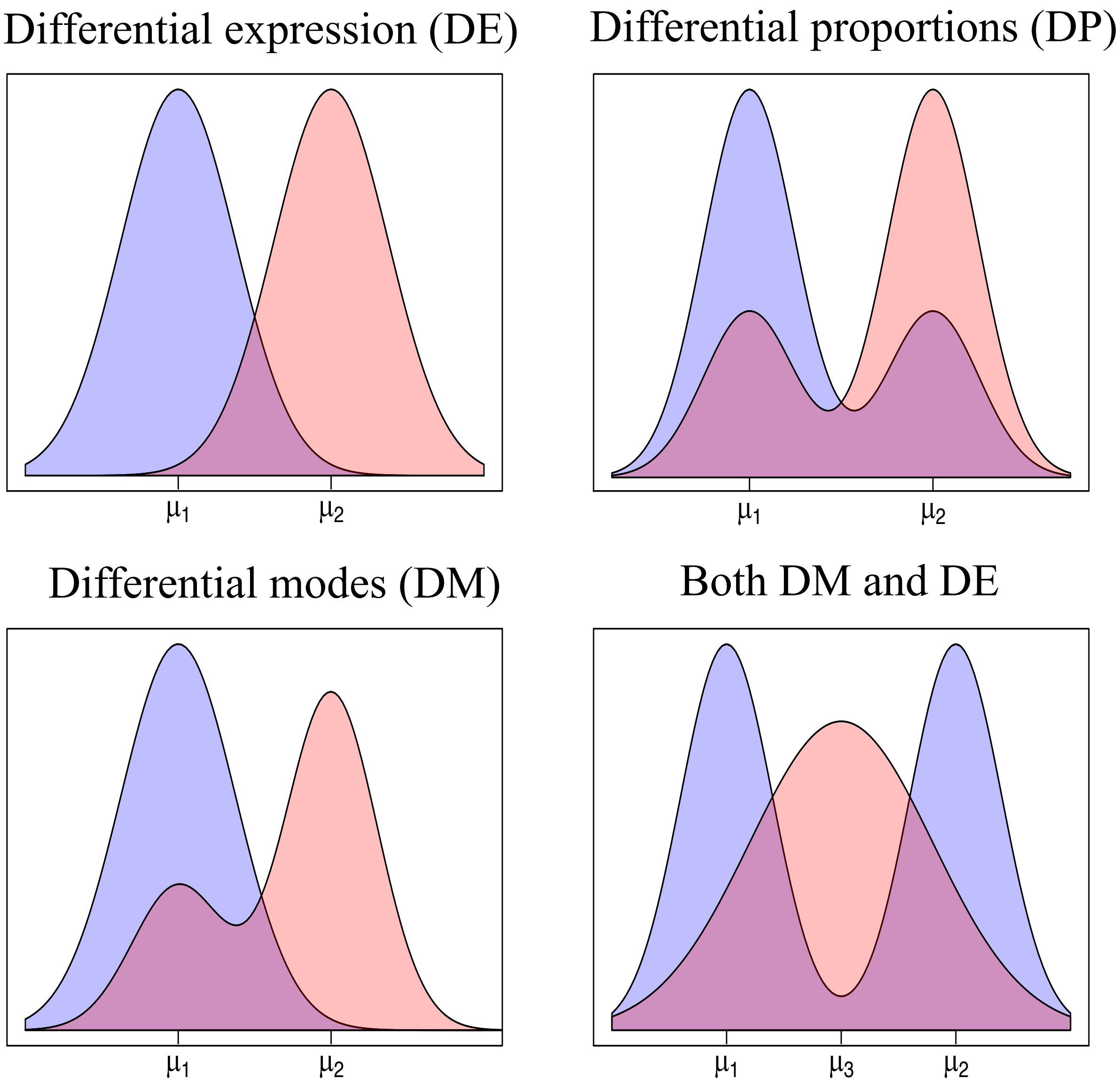
Histogram of Observed Expression Level of Gene X

Many genes show multi-modal expression distributions





Opportunity to identify differences beyond traditional DE



Differential expression (DE)

Differential proportions (DP)

Differential modes (DM)

Both DM and DE

scRNA-seq DE Analysis

- Recent methods use mixture modeling to account for ‘on’ and ‘off’ components
  - Shalek et al. (2014)
  - SCDE (Kharchenko *et al.*, 2014)
  - MAST (Finak *et al.*, 2015)

- When detected, each gene has a latent level of expression within a biological condition, and measurements fluctuate around that level due to biological and technical sources of variability

scDD: Goal

- Model expression profiles while accommodating the often multimodal distributions in the detected cells

- Find genes with Differential Distributions (DD) of expression across two conditions:
  - differential means
  - differential proportion within modes
  - differential modality (number of modes)
  - combination thereof
  - differential zeroes (detection rate)

scDD: Overview

- Assume that log non-zero normalized, de-noised, expression measurements $Y_g = (y_{g1},...,y_{gJ})$ for gene g in J cells arise from a conjugate Dirichlet Process Mixture (DPM) of normals model:

$$\begin{aligned} y_j &\sim N(\mu_j, \tau_j) \\ \mu_j, \tau_j &\sim G \\ G &\sim DP(\alpha, G_0) \\ G_0 &= NG(m_0, s_0, a_0/2, 2/b_0) \end{aligned}$$

- Let K denote the number of components (unique values in $\{\mu_{j,} \tau_j\}, j=1,.., J$). Of primary interest is the posterior of (μ,τ), which is intractable for moderate sample sizes.

- Let $Z = (z_1, \ldots, z_J)$ denote component memberships. Then $f(Y \mid Z)$ is a PPM.

$$\begin{aligned} f(Y|Z) &= \prod_{k=1}^{K} f(y^{(k)}) \\ &\propto \prod_{k=1}^{K} \frac{\Gamma(a_k/2)}{(b_k/2)^{a_k/2}} s_k^{-1/2} \end{aligned}$$

scDD: Overview (continued)

- To quantify the evidence of DD for gene *g*, obtain MAP partition estimate, $\hat{Z}$, and evaluate $f\left(Y,\hat{Z}\right)$ under competing hypotheses:
  - ignoring condition ($\mathcal{M}_{ED}$: equivalent distributions)
  - separately within condition ($\mathcal{M}_{DD}$: differential distributions)

- Evaluate $\mathcal{M}_{DD}$ using a pseudo-Bayes Factor score:

$$Score_g = \log\left(\frac{f\left(Y_g,\hat{Z}_g|M_{DD}\right)}{f\left(Y_g,\hat{Z}_g|M_{ED}\right)}\right)$$

- Assess significance via permutation.

scDD: Evaluation via simulation studies

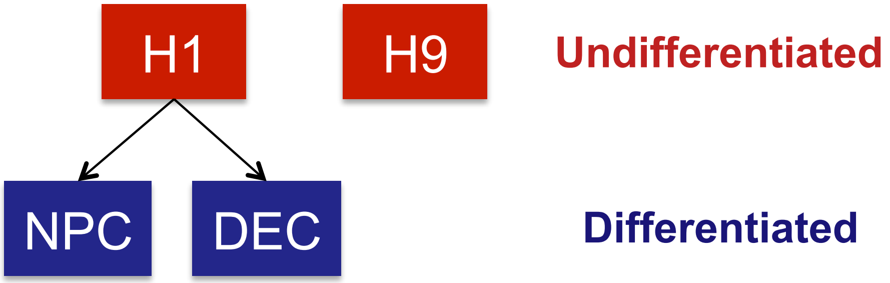
- 8000 ED genes:
  - 4000 from single Negative Binomial component
  - 4000 from two component mixture of Negative Binomial
- 2000 DD genes:
  - 500 DE genes
  - 500 DP genes (0.33/0.66 proportion difference)
  - 500 DM genes (0.50 belong to second mode)
  - 500 DB genes (mean in second condition is average of means in the first)
- Sample sizes varied $\in \{50, 75, 100\}$
- Component distances Δ_μ for multimodal conditions varied $\in \{2, 3, 4, 5, 6\}$ SDs
- Means, variances, and detection rates sampled empirically

Evaluate: Power to identify DD genes
Rate at which DD genes are correctly classified
Rate at which correct # components are identified

scDD: Power to detect DD genes within each category

		True Gene Category				
Sample Size	Method	DE	DP	DM	DB	Overall (FDR)
50	scDD	0.893	**0.418**	**0.898**	**0.572**	**0.695** (0.030)
	SCDE	0.872	0.026	0.816	0.260	0.494 (0.004)
	MAST	**0.908**	0.400	0.871	0.019	0.550 (0.026)
75	scDD	0.951	0.590	**0.960**	**0.668**	**0.792** (0.031)
	SCDE	0.948	0.070	0.903	0.387	0.577 (0.003)
	MAST	**0.956**	**0.632**	0.942	0.036	0.642 (0.022)
100	scDD	0.972	0.717	**0.982**	**0.727**	**0.850** (0.033)
	SCDE	0.975	0.125	0.946	0.478	0.631 (0.003)
	MAST	**0.977**	**0.752**	0.970	0.045	0.686 (0.022)
500	scDD	**1.000**	0.985	**1.00**	**0.903**	**0.972** (0.034)
	SCDE	**1.000**	0.858	0.998	0.785	0.910 (0.004)
	MAST	**1.000**	**0.992**	**1.00**	0.174	0.792 (0.021)

Comparison of hESCs


H1

H9

NPC

DEC

Undifferentiated

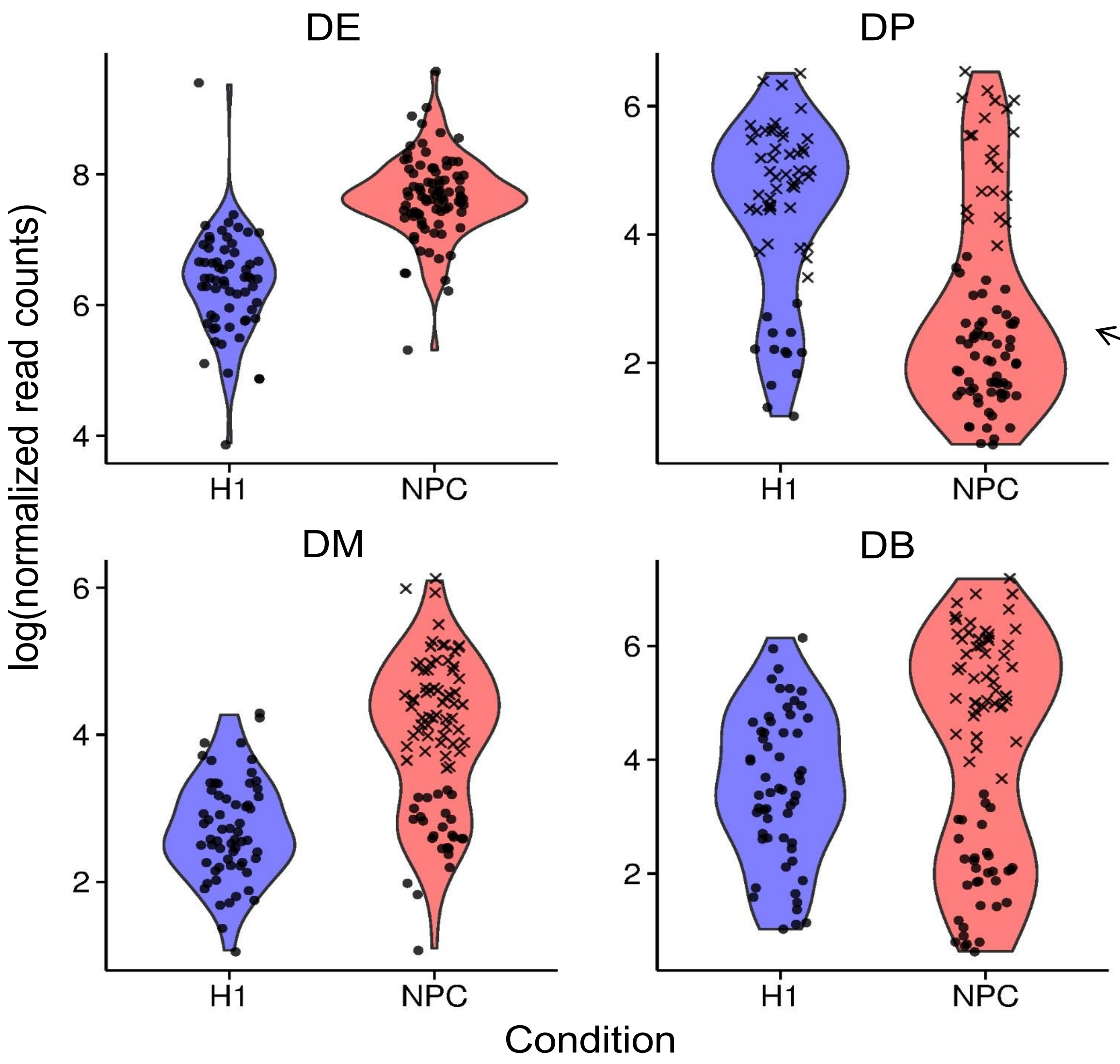
Differentiated

Number of DD genes identified in each cell type comparison

	scDD							
Comparison	DE	DP	DM	DB	DZ	Total	SCDE	MAST
H1 vs NPC	1342	429	739	406	1590	4506	2938	5729
H1 vs DEC	1408	404	939	345	880	3976	1581	3523
NPC vs DEC	1245	449	700	298	2052	4744	1881	5383
H1 vs H9	194	84	55	32	145	510	102	1091

scDD only: 2% 21% 38% 24% 15%

Genes identified in H1 vs. NPC comparison



Not DE by SCDE or MAST

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