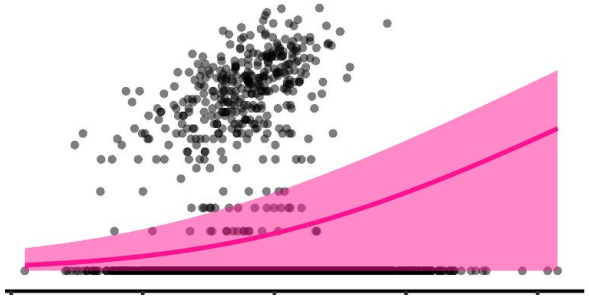
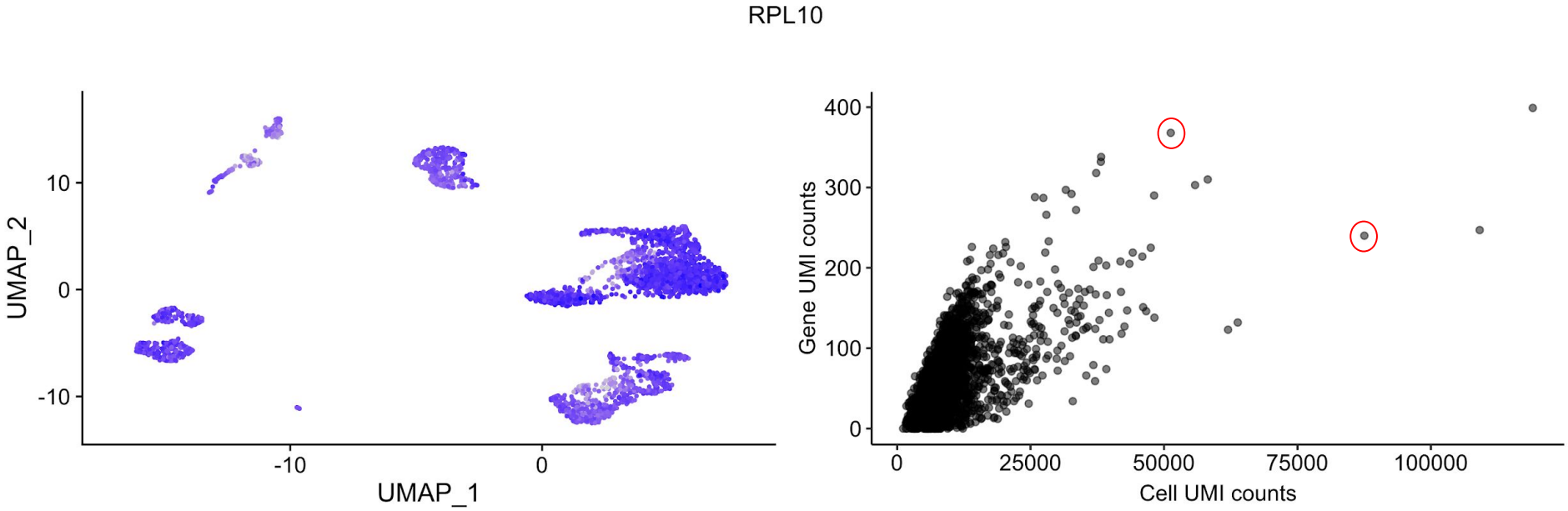
Count models for normalization of scRNA-seq data



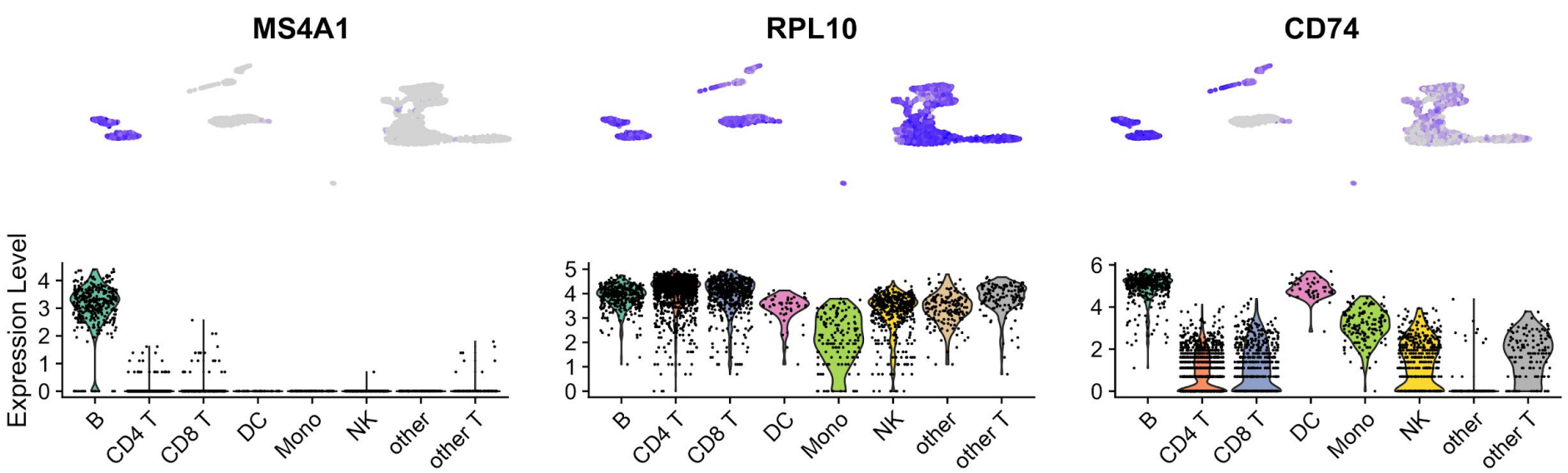
Saket Choudhary
Postdoc, Satija Lab
New York Genome Center

Many genes exhibit technical variation

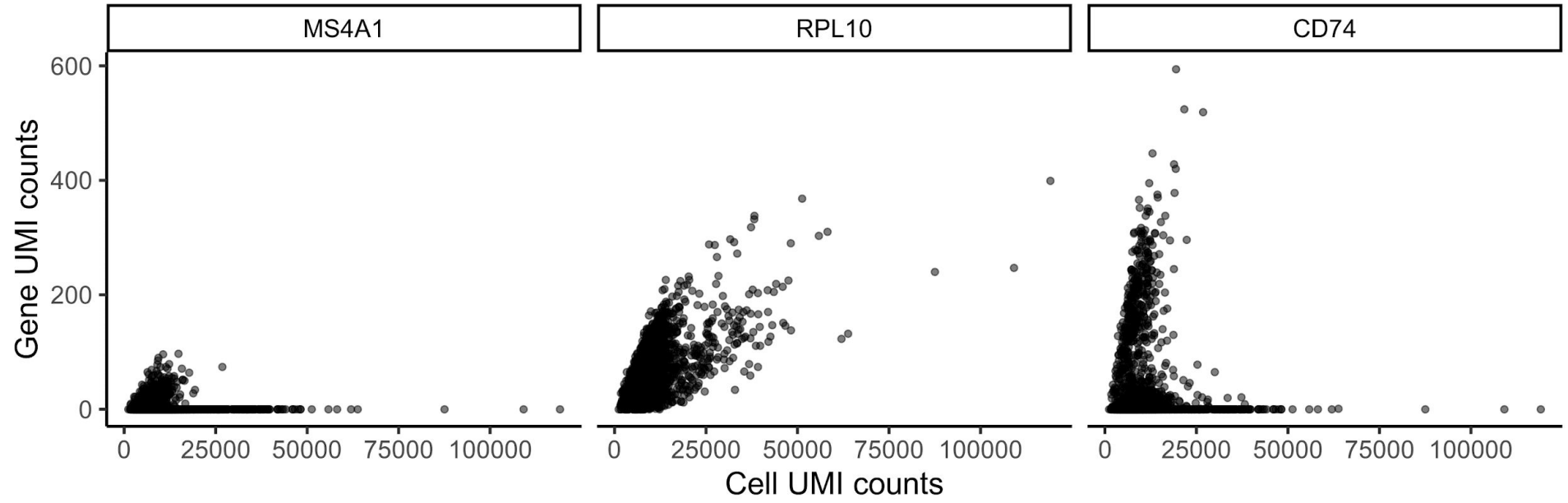


Data: Human PBMC Smart-seq3, 3k cells from Hagemann-Jensen et al., NBT 2020

Some genes exhibit both technical and biological variation



Some genes exhibit both technical and biological variation



Goal: Remove technical variation while retaining biological variation

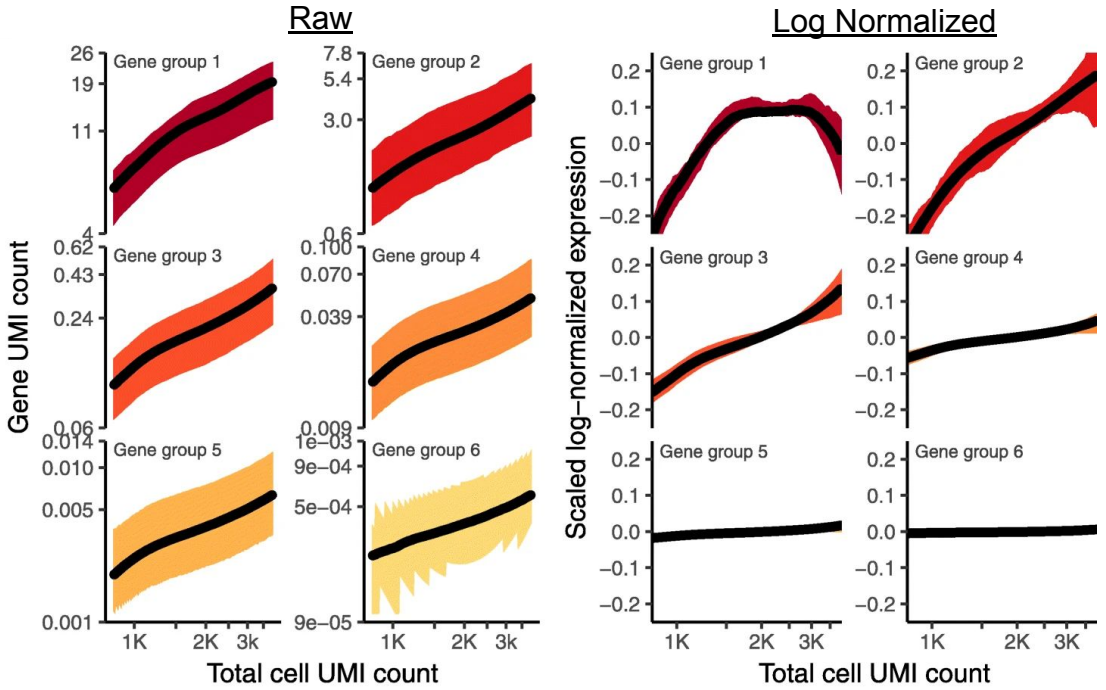
Standard Log Normalization

Step 1 (Size factor normalization):

- Goal: Correct for different sequencing depth
- Solution: Rescale cells to have same total UMI
- Dampens biological difference between cells

Step 2 (Log transformation):

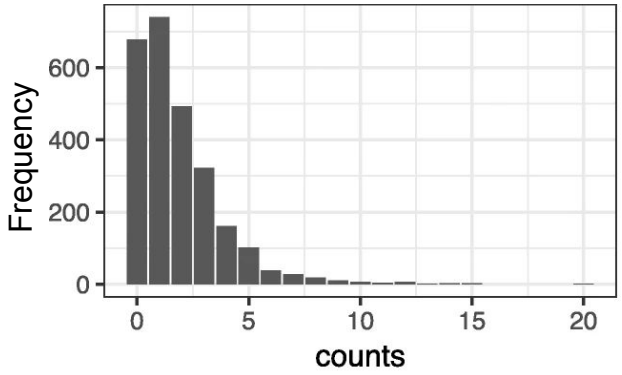
- Goal: Reduce impact of expression outliers
- Solution: Add pseudocount (+1) to avoid negative values
- Dampens both technical and biological variance



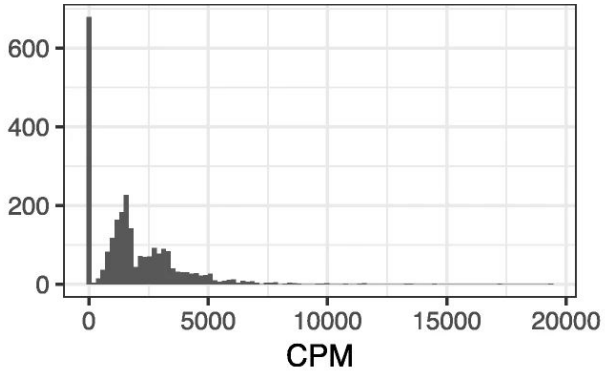
Hafemeister & Satija, Genome Biol. 2019

Differentially normalizes only low/medium abundance genes

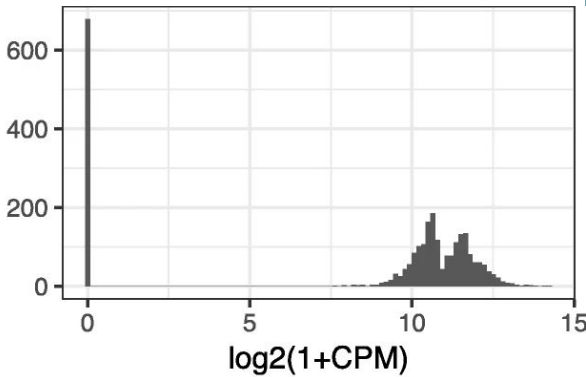
Standard Log Normalization



UMI counts



Step 1 LogNorm

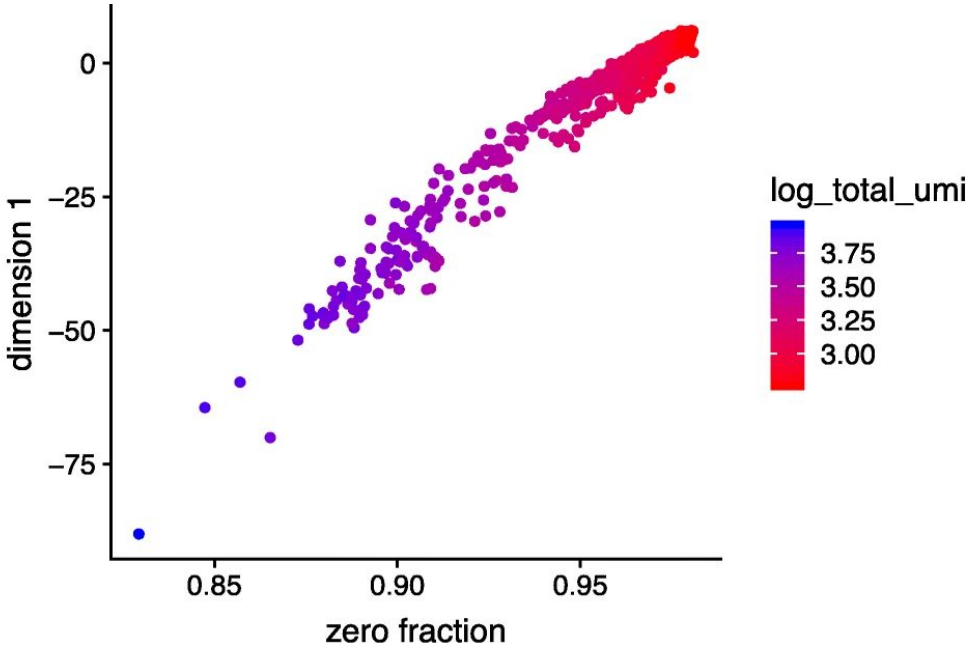


Step 2 LogNorm

Townes et al., Genome Biol. 2019

Distorts difference between zero and non-zero counts

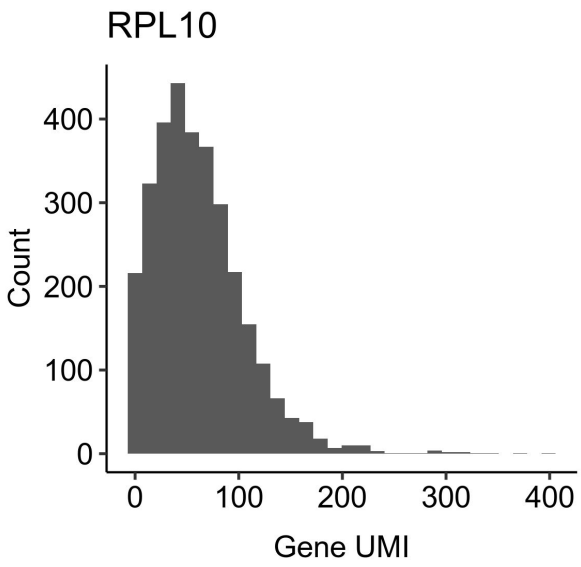
Standard Log Normalization

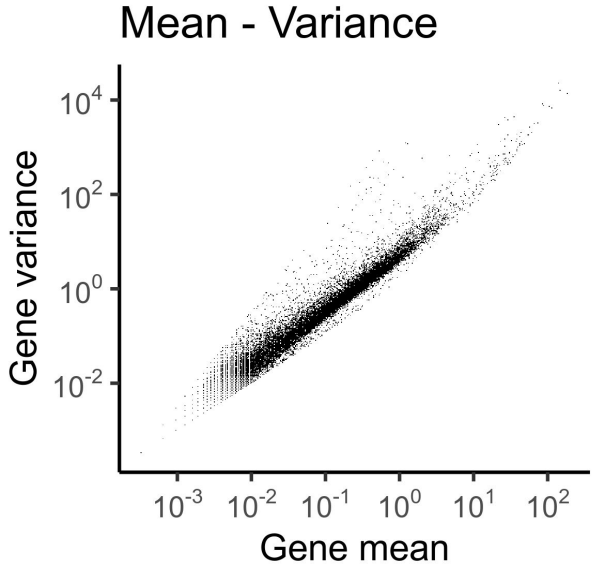


Townes et al., Genome Biol. 2019

A lot of variance even post normalization is explained by difference in sequencing depth

Count models for normalizing UMI data





- UMI counts are not normally distributed
- Variance depends on mean

Goal: Remove the effect of technical variation using an appropriate error model

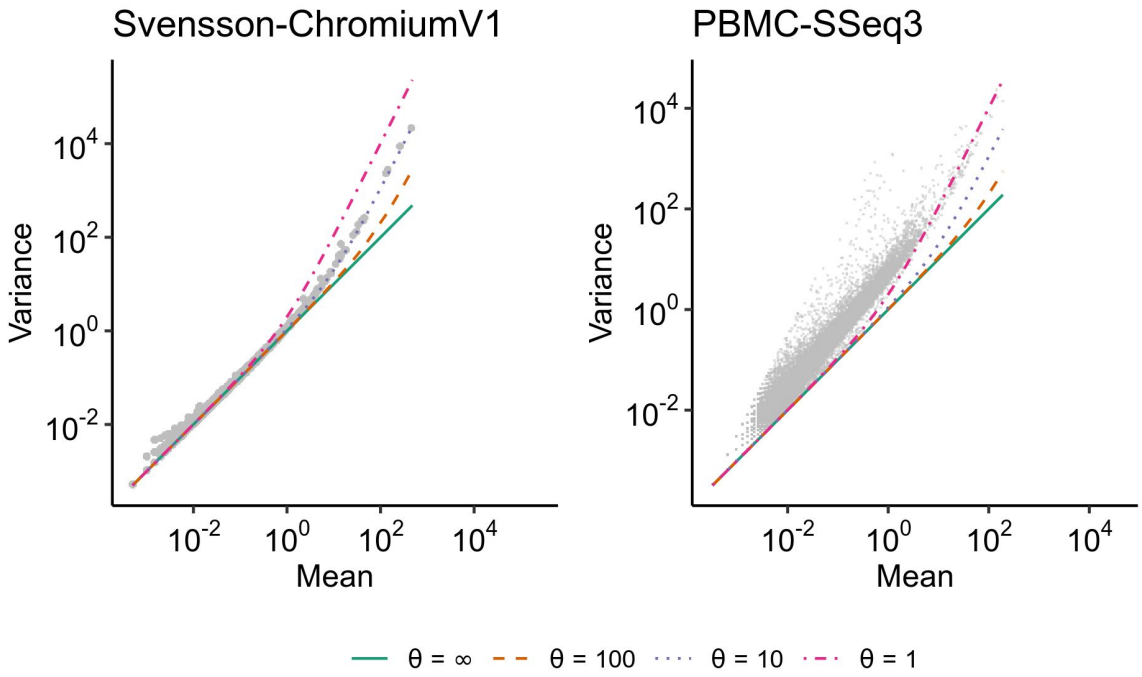
Negative Binomial distribution for modeling UMIs

Poisson:

Mean = μ
Variance = μ

Negative Binomial:

Mean = μ
Variance = $\mu + \mu^2/\theta$
Inverse-dispersion parameter = θ



Modeling technical noise

Goal

Learn a model that describes the **technical variance** in the data

Approach

- An “ideal” model should fit *perfectly* to genes showing only **technical variance**
- Biologically **‘interesting’** genes should **deviate** from the model
- Use deviations (residuals) from the model for downstream analysis

Generalized Linear Models: SCTransform

x_{gc} = Observed UMI count of gene g in cell c,

μ_{gc} = Expected UMI count of gene g in cell c,

n_c = Observed total UMI count of cell c,

$$x_{gc} \sim \text{NB}(\mu_{gc}, \boldsymbol{\theta_{gc}}),$$

$$\log \mu_{gc} = \boldsymbol{\beta_{g0}} + \boldsymbol{\beta_{g1}} n_c.$$

inverse-dispersion

Should fit the "uninteresting" genes *perfectly*

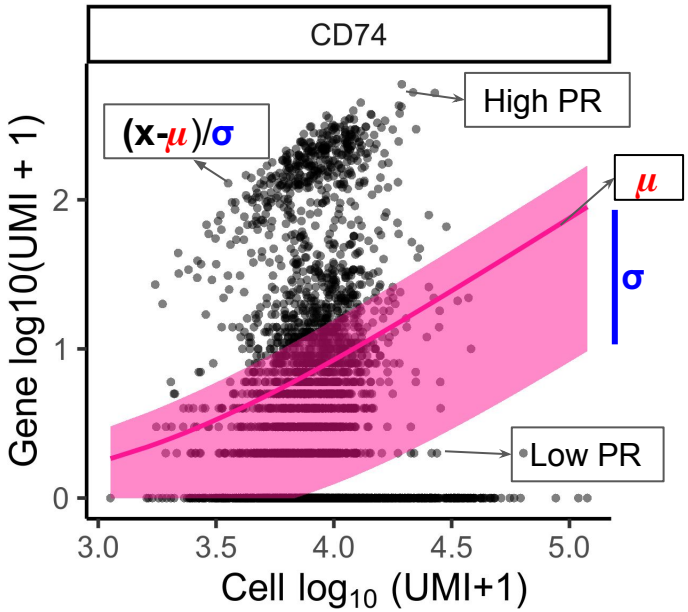
Intercept

Slope

$$\sigma_{gc} = \sqrt{\mu_{gc} + \frac{\mu_{gc}^2}{\theta_{gc}}},$$

Pearson residual

$$z_{gc} = \frac{x_{gc} - \mu_{gc}}{\sigma_{gc}}.$$



Idea: Learn a model of technical noise from the data

Generalized Linear Models: GLM-PCA

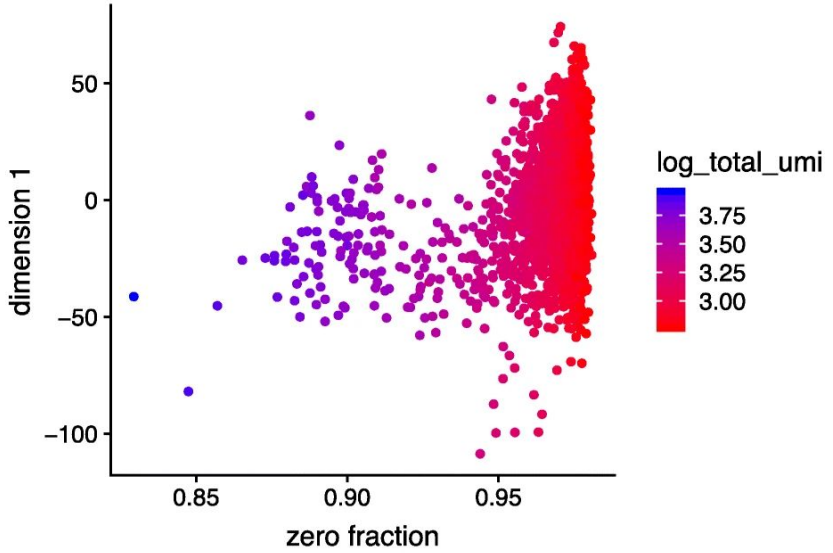
$$x_{gc} \sim \text{NB}(\mu_{gc}, \theta_{gc}) \text{ or Poisson}(\mu_{gc}),$$

$$\log \mu_{gc} = \log n_c + \sum_{l=0}^{k} U_{cl} V_{lg},$$

Loadings

PCs

$$D = \sum_{g,c} x_{gc} \log \frac{x_{gc}}{\mu_{gc}} - (x_{gc} - \mu_{gc})$$



Townes et al., Genome Biol. 2019

Idea: GLM residuals are asymptotically normal

How should we determine inverse-dispersion (θ)?

One θ for all datasets

New Results

Comment on this paper

Analytic Pearson residuals for normalization of single-cell RNA-seq UMI data

Jan Lause, Philipp Berens, Dmitry Kobak

doi: https://doi.org/10.1101/2020.12.01.405886

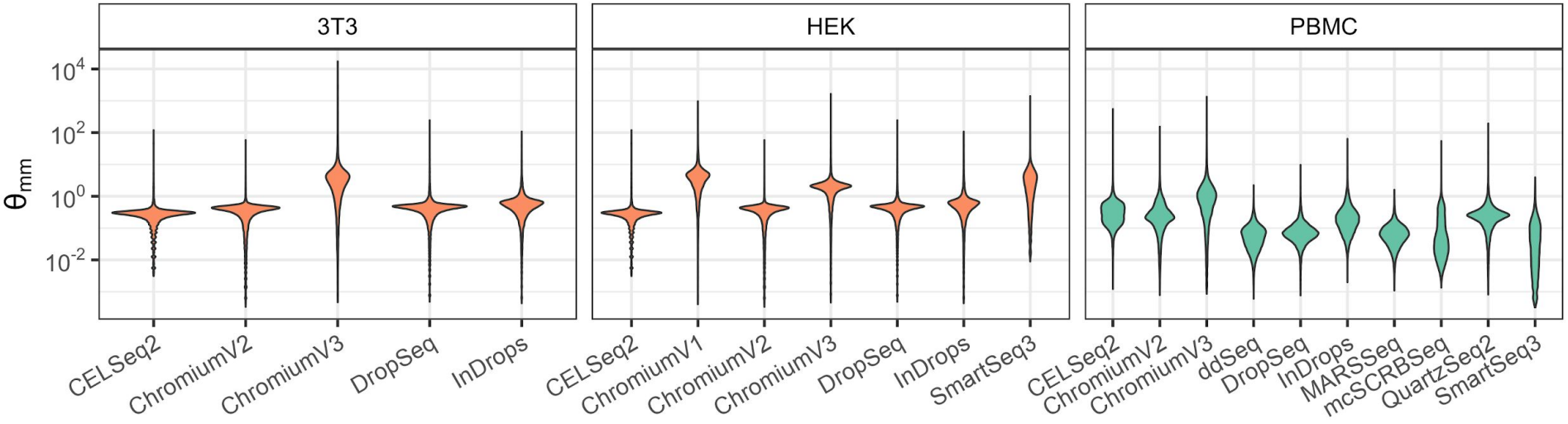
$$\beta_{g0} = \log \frac{1}{N} \sum_c x_{c,g} - \log \frac{1}{N} \sum_c n_c$$

$$\beta_{g1} = 1$$

$$\theta_{gc} = \{10, 100\}$$

Question: Can we fix dispersion estimates across all datasets?

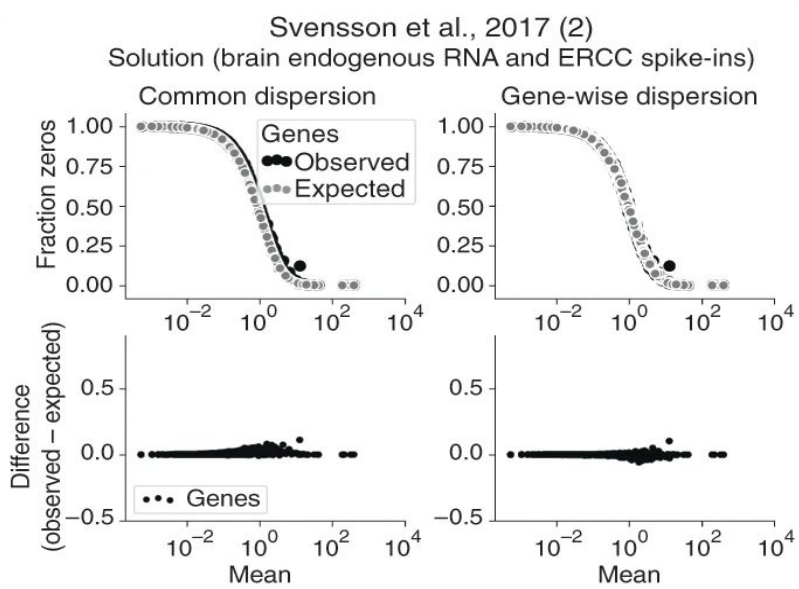
Dispersion varies across technologies

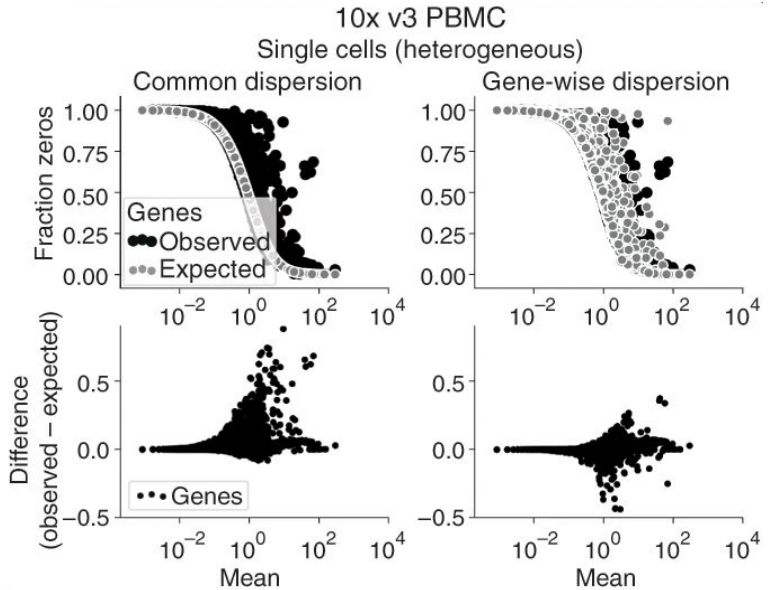


Data from: Mereu et al., NBT 2020; Ding et al., NBT 2020; Hagemann-Jensen et al., NBT 2020

Question: Can we estimate dispersion per gene per dataset?

Estimating dispersion per gene



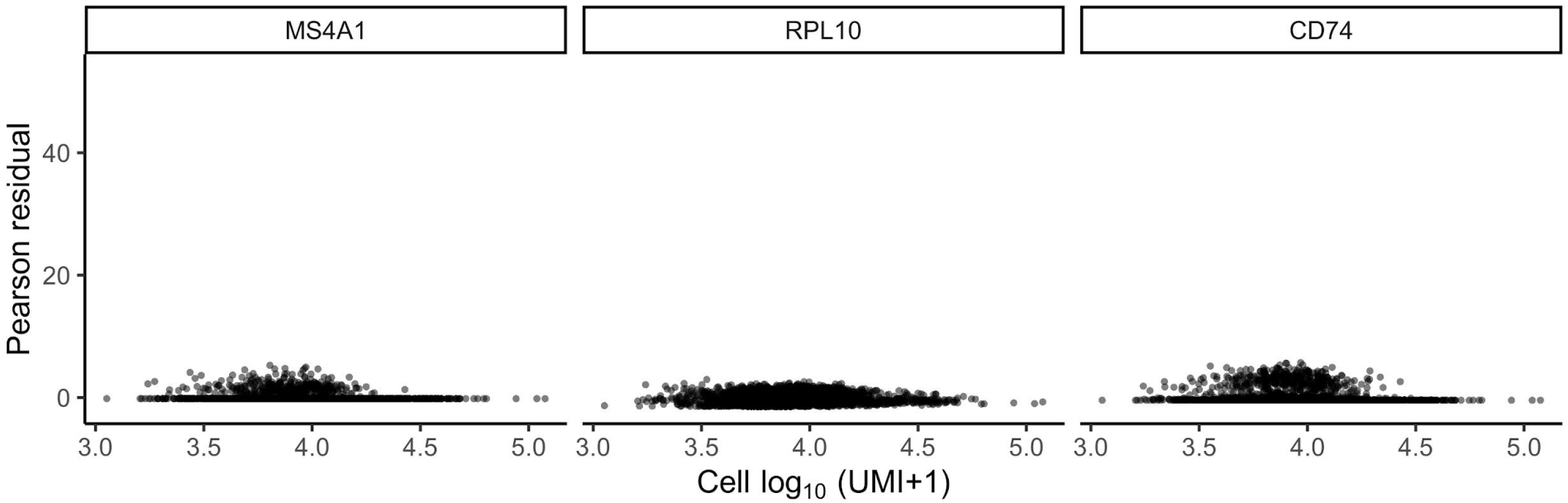


% deviation	4.7%	0.49%	9.65%	3.82%

Svensson, NBT 2020

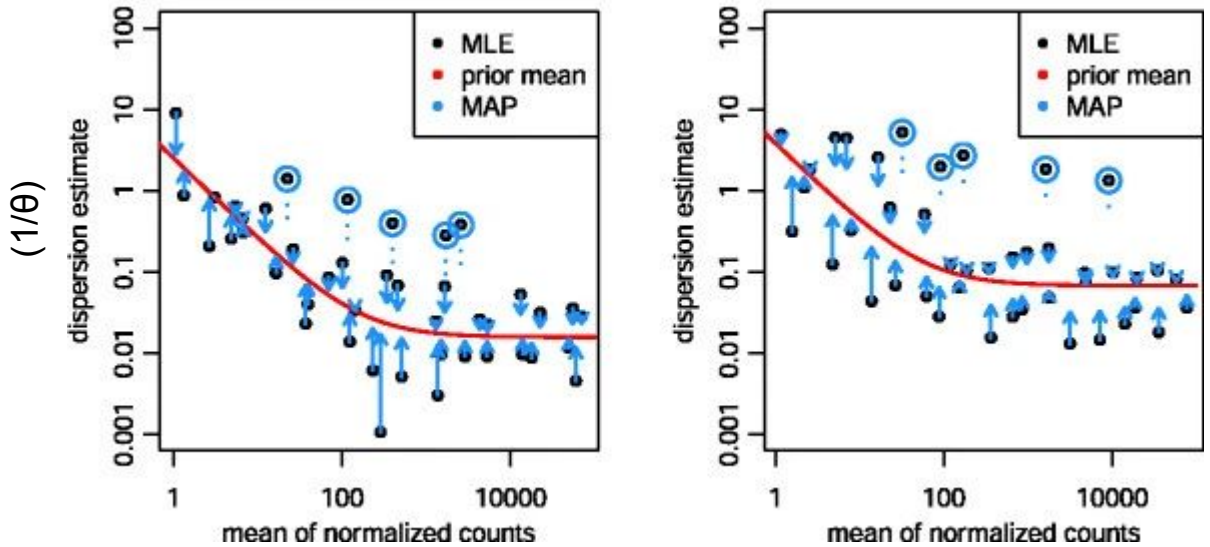
Genewise dispersion estimates lead to better fit

Learning per gene estimate dampens both biological and technical variance



Question: Is there a middle ground between learning per gene estimates and fixing dispersion estimates?

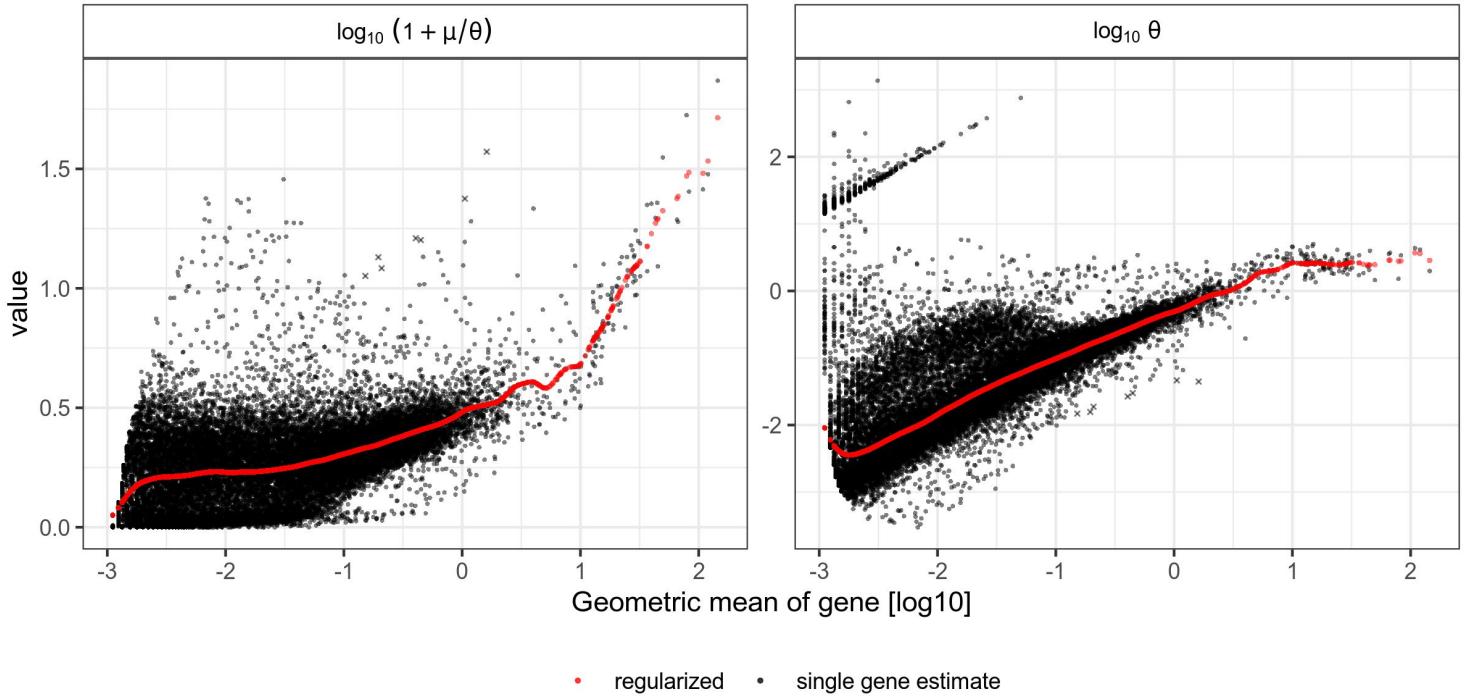
Sharing information across genes



Love et al., Genome Biol. 2014

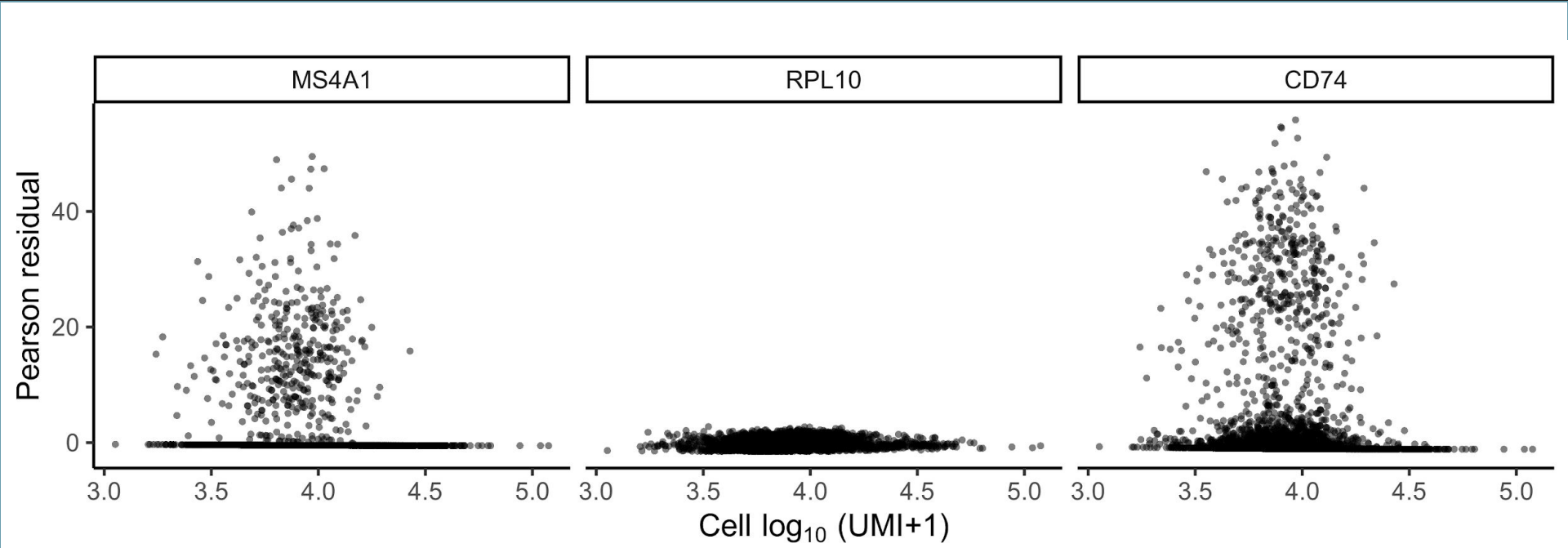
Genes with <u>similar mean</u> have <u>similar dispersion</u>

Sharing information across genes



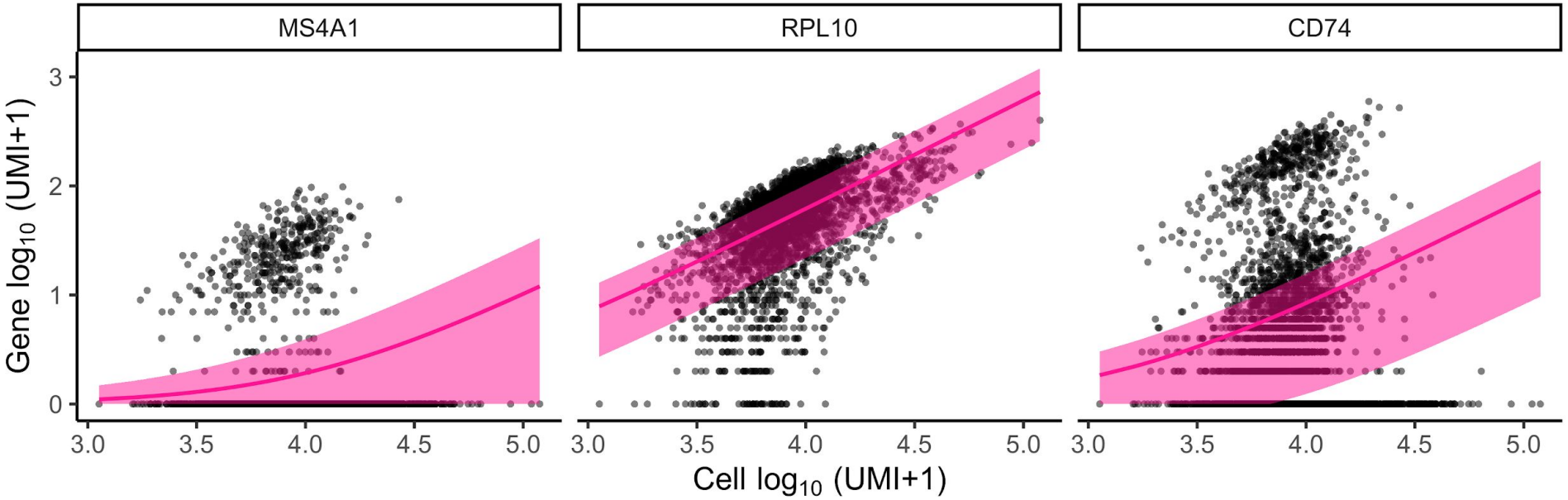
Model captures expected variation for a given gene mean by regularizing dispersion

SCTransform learns a model of technical noise from each dataset



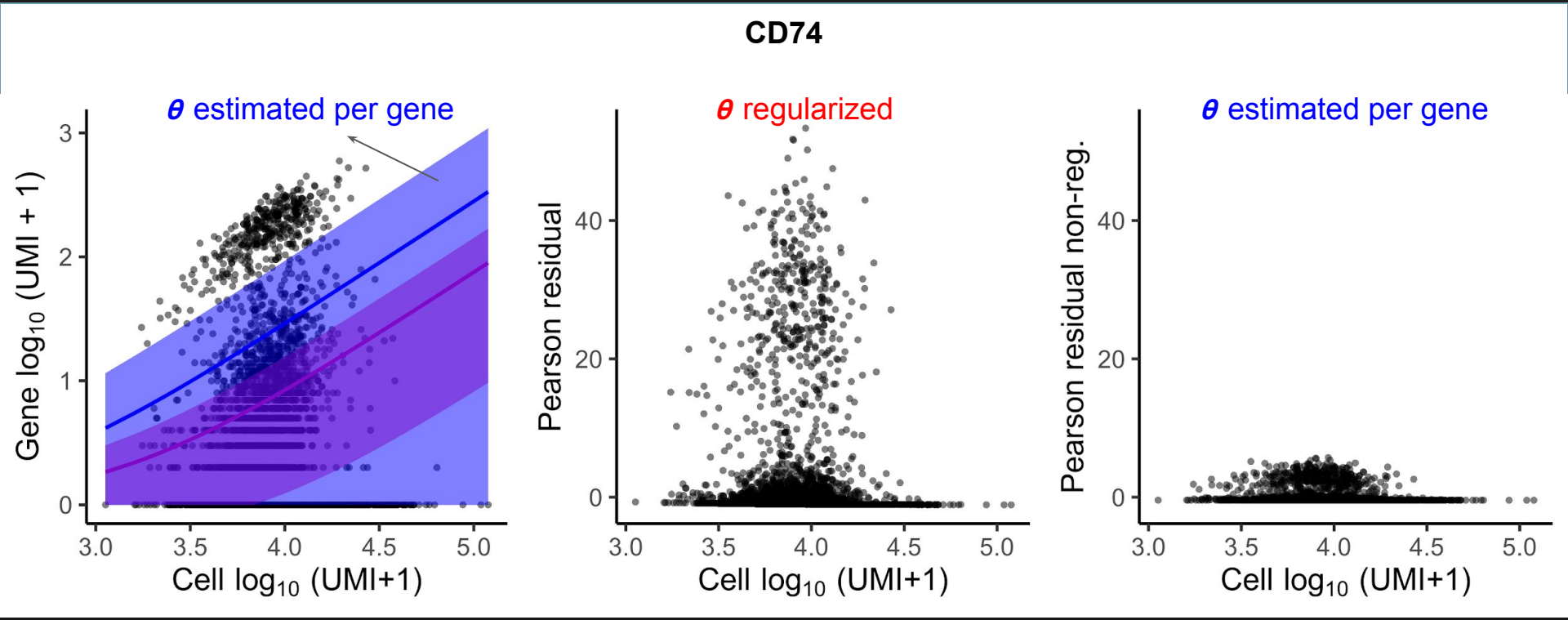
Variability that cannot be explained by the fit is <u>biological</u>

SCTransform learns a model of technical noise from each dataset



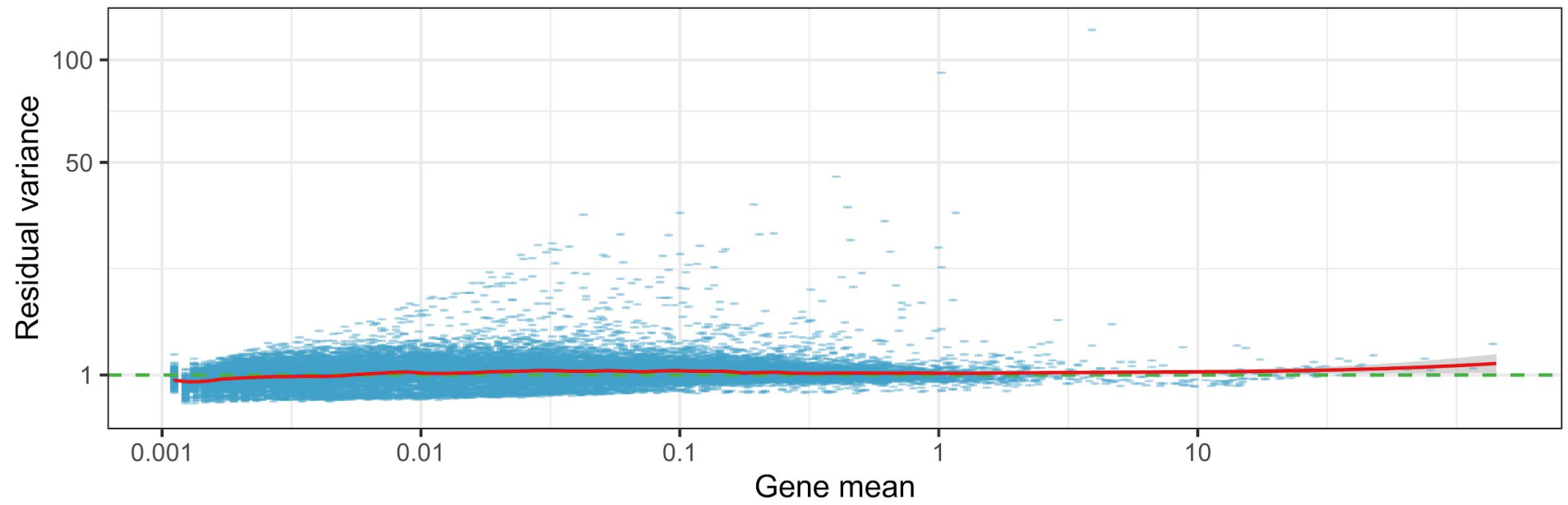
Biologically interesting genes show deviation from the model while uninteresting ones fit *perfectly*

SCTransform learns a model of technical noise from each dataset



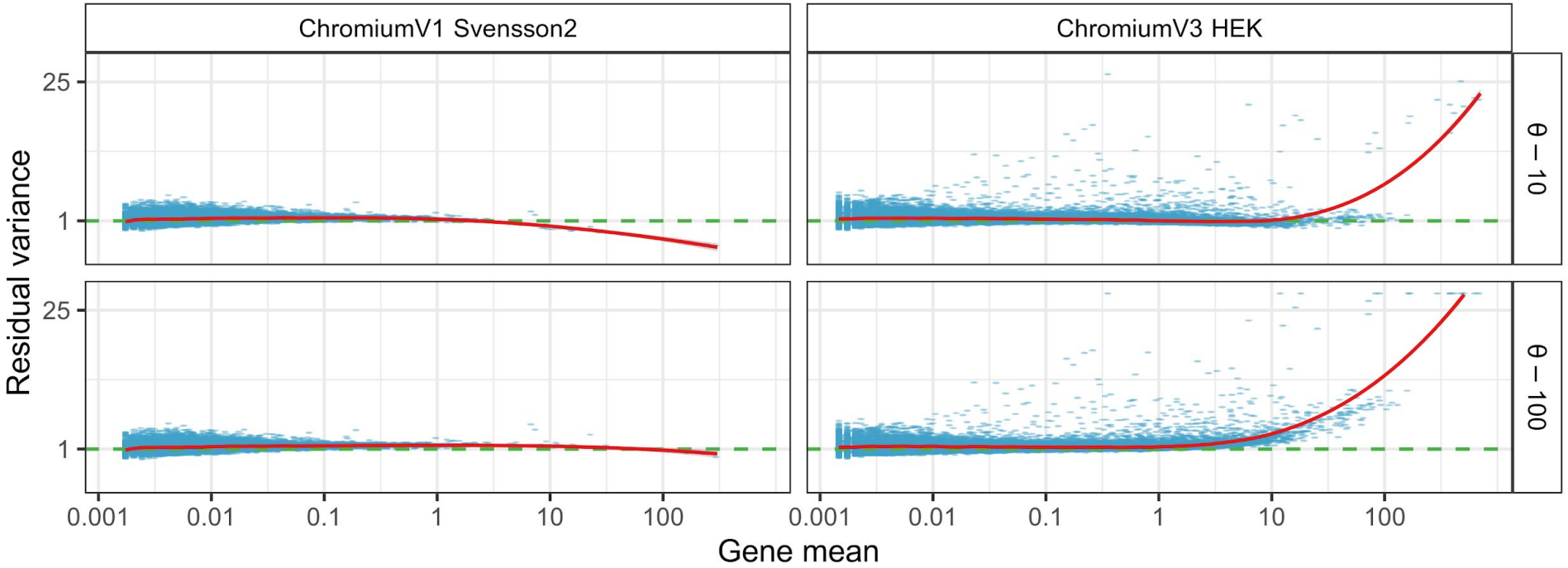
Per gene estimates dampen biological variability

SCTransform achieves variance stabilization



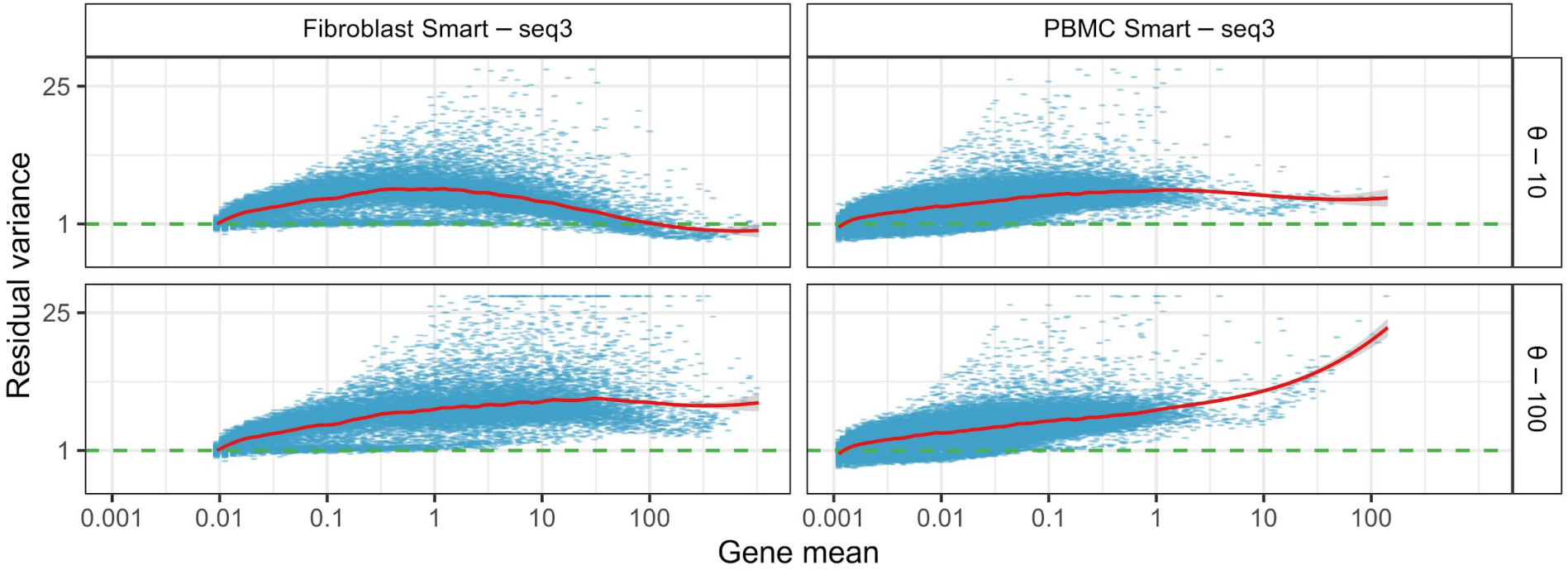
Residual variance is <u>uncorrelated</u> with gene mean

Variance stabilization: Fixed dispersion



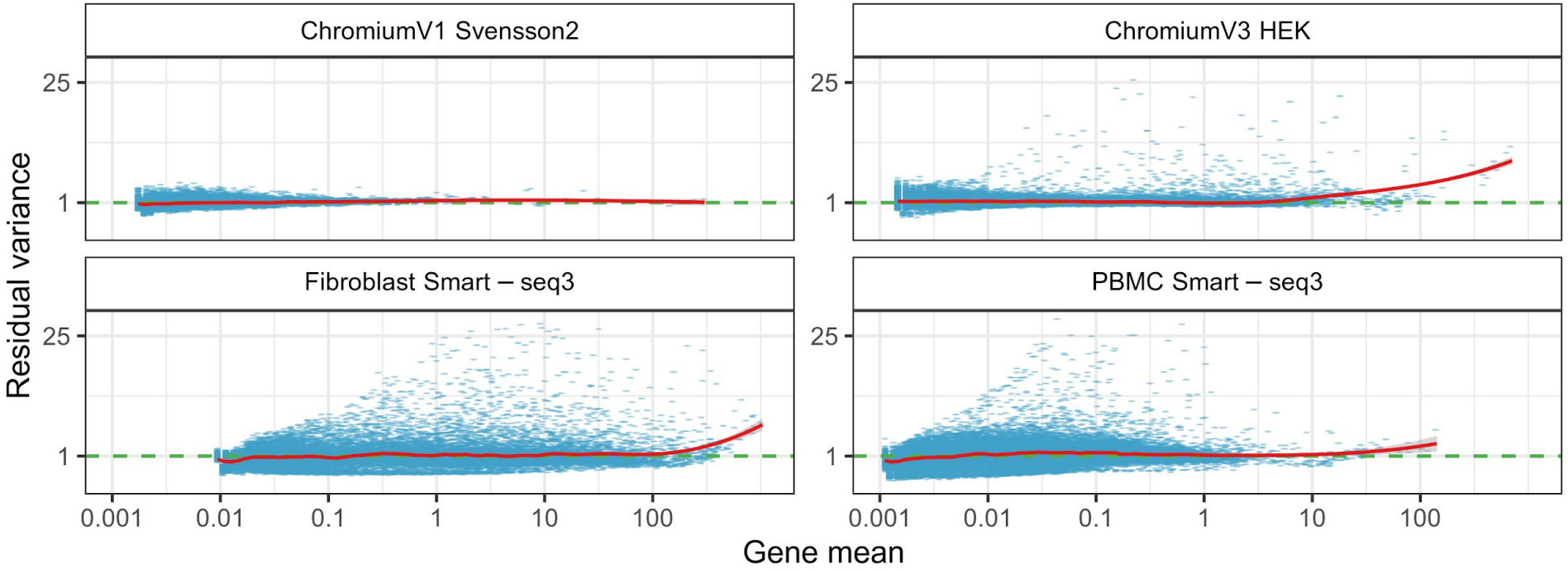
Residual variance is expected to be uncorrelated with gene mean

Variance stabilization: Fixed dispersion



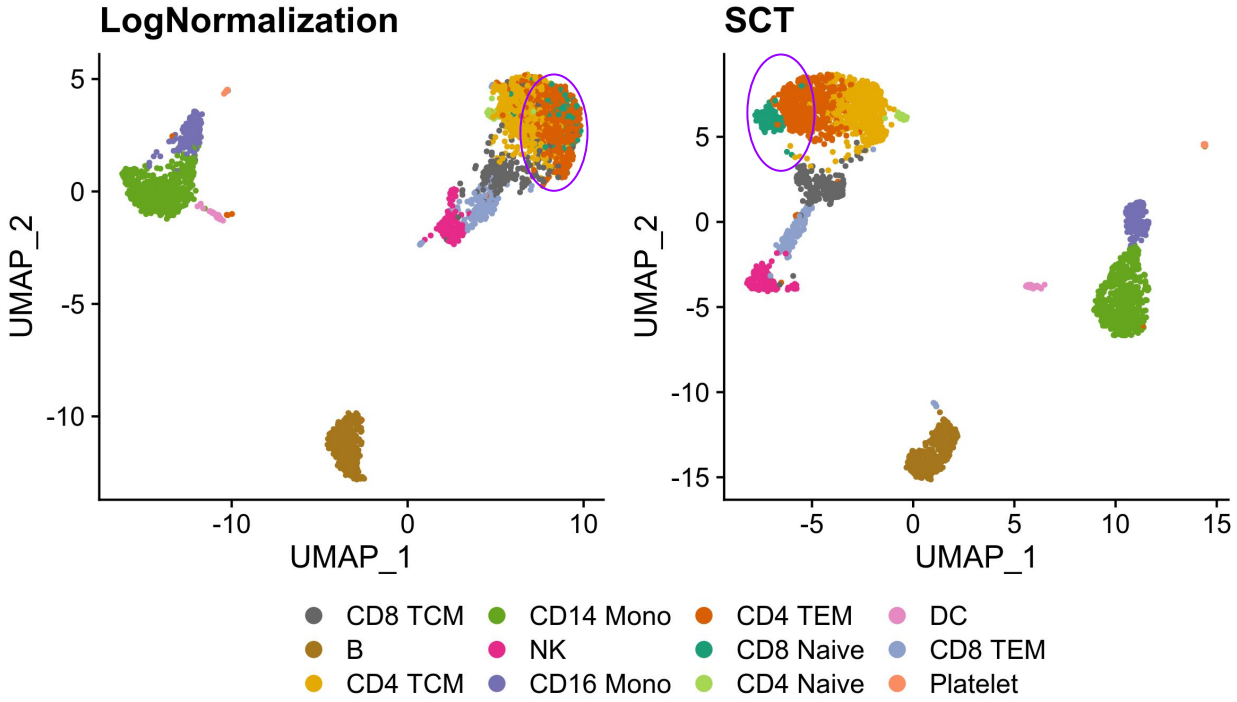
Residual variance is expected to be uncorrelated with gene mean

Variance stabilization: Regularized dispersion



Residual variance is <u>uncorrelated</u> with gene mean

SCTransform reveals more biological distinction



Data: PBMC3k (Chromium), 10X Genomics

Summary

- Standard Log Normalization introduces systematic errors
- GLM approach improves single-cell normalization
- Negative binomial dispersion (θ) can **vary substantially** across datasets and technologies
- We recommend learning and regularizing θ **separately** for each dataset

Other count models

[1] A. K. Sarkar and M. Stephens, “Separating measurement and expression models clarifies confusion in single cell RNA-seq analysis,” *BioRxiv*, 2020 [Online].

[2] K. Choi, Y. Chen, D. A. Skelly, and G. A. Churchill, “Publisher Correction: Bayesian model selection reveals biological origins of zero inflation in single-cell transcriptomics,” *Genome Biol.*, vol. 21, no. 1, p. 270, Nov. 2020, doi: 10.1186/s13059-020-02182-1. [Online].

[3] R. Lopez, J. Regier, M. B. Cole, M. I. Jordan, and N. Yosef, “Deep generative modeling for single-cell transcriptomics,” *Nat. Methods*, vol. 15, no. 12, pp. 1053–1058, Dec. 2018, doi: 10.1038/s41592-018-0229-2. [Online].

[4] T. H. Kim, X. Zhou, and M. Chen, “Demystifying ‘drop-outs’ in single-cell UMI data,” *Genome Biol.*, vol. 21, no. 1, p. 196, Aug. 2020, doi: 10.1186/s13059-020-02096-y. [Online].

[5] F. W. Townes, S. C. Hicks, M. J. Aryee, and R. A. Irizarry, “Author Correction: Feature selection and dimension reduction for single-cell RNA-Seq based on a multinomial model,” *Genome Biol.*, vol. 21, no. 1, p. 179, Jul. 2020, doi: 10.1186/s13059-020-02109-w. [Online].

[6] J. Lause, P. Berens, and D. Kobak, “Analytic Pearson residuals for normalization of single-cell RNA-seq UMI data,” *bioRxiv*, 2020 [Online].

[7] D. Risso, F. Perraudeau, S. Gribkova, S. Dudoit, and J. P. Vert, “ZINB-WaVE: A general and flexible method for signal extraction from single-cell RNA-seq data,” *BioRxiv*, 2017 [Online].

[8] G. Eraslan, L. M. Simon, M. Mircea, N. S. Mueller, and F. J. Theis, “Single-cell RNA-seq denoising using a deep count autoencoder,” *Nat. Commun.*, vol. 10, no. 1, pp. 1–14, 2019 [Online].

[9] S. Sun, Y. Chen, Y. Liu, and X. Shang, “A fast and efficient count-based matrix factorization method for detecting cell types from single-cell RNAseq data,” *BMC Syst. Biol.*, vol. 13, no. Suppl 2, p. 28, Apr. 2019. [Online].

[10] M. Huang *et al.*, “SAVER: gene expression recovery for single-cell RNA sequencing,” *Nat. Methods*, vol. 15, no. 7, pp. 539–542, Jul. 2018, doi: 10.1038/s41592-018-0033-z. [Online].

[11] W. Tang *et al.*, “bayNorm: Bayesian gene expression recovery, imputation and normalization for single-cell RNA-sequencing data,” *Bioinformatics*, vol. 36, no. 4, pp. 1174–1181, Feb. 2020, doi: 10.1093/bioinformatics/btz726. [Online].

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