COMPUTATIONAL ANALYSIS OF CELL-TO-CELL HETEROGENEITY IN SINGLE-CELL RNA-SEQUENCING DATA REVEALS HIDDEN SUBPOPULATIONS OF CELLS

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Saket Choudhary

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BISC 542

OUTLINE

- Motivation
- Methods[Overview]
- Results
- Challenges & Possibilities
- Conclusion

MOTIVATION

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- Profiling hundreds of cells: How to identify subpopulations?
- Identify regulatory landscape of each cell population
- **Account for hidden confounding factors that might explain cell heterogeneity**

WHAT WAS IT ALL ABOUT?

- Single cell transcriptomics heterogeneity: many single cells at the same time
- Accounting for technical noise was a solved problem; how do you account for *other* sources of variability: cell cycle, differentiation state etc.
- Given expression levels, how do you infer the effect of *latent* variables

Key focus: How does cell cycle affect expression levels? Given the apriori nature of genes(association with cell-cycle), is it possible to remove the effect of cell cycles?

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- Discovering previously unidentified cell types?!

METHODS

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- Decompose the variability of expression levels *across* single cells: expression = mean effect + random effect
- Regress out effects of hidden factors
- Corrected gene expression level

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RESULTS

scLVM: single Cell Latent variable Models

CELL CYCLE AFFECTS GLOBAL GENE EXPRESSION

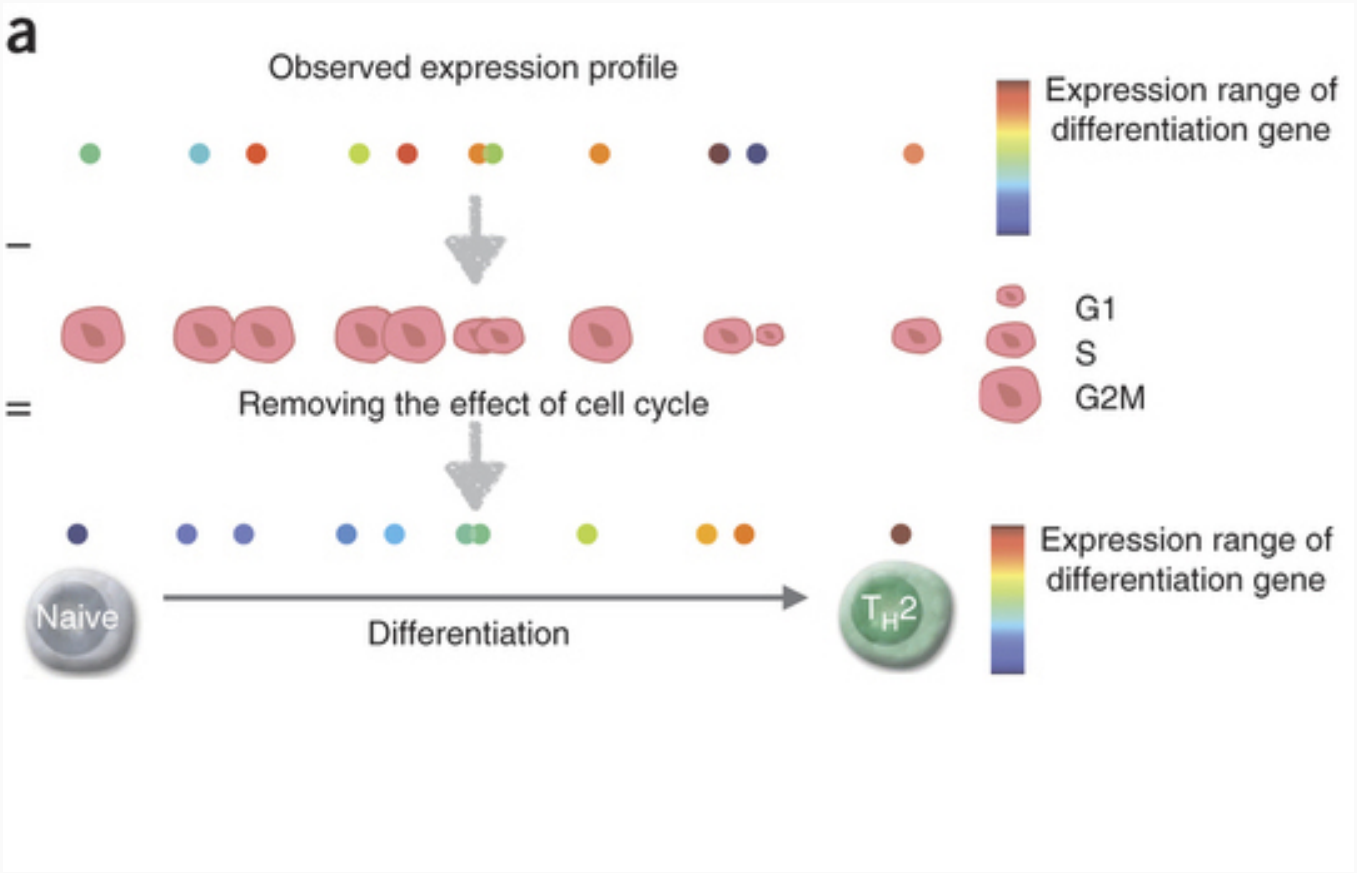

Figure: Observed Expression = Effect of differentiation + Effect of state of cell($G1, S, G2$)

VARIANCE MATRIX

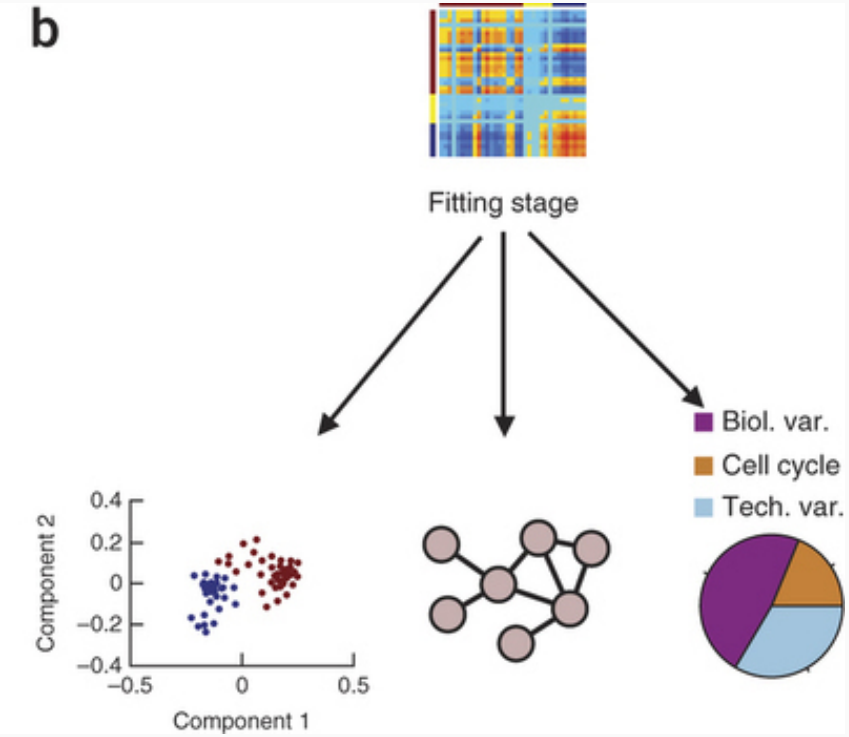

Figure: Inferring the cell-cell covariance matrix using hidden factors such as the cell cycle. This is then used to calculate adjusted gene expression values for downstream analysis

VALIDATION

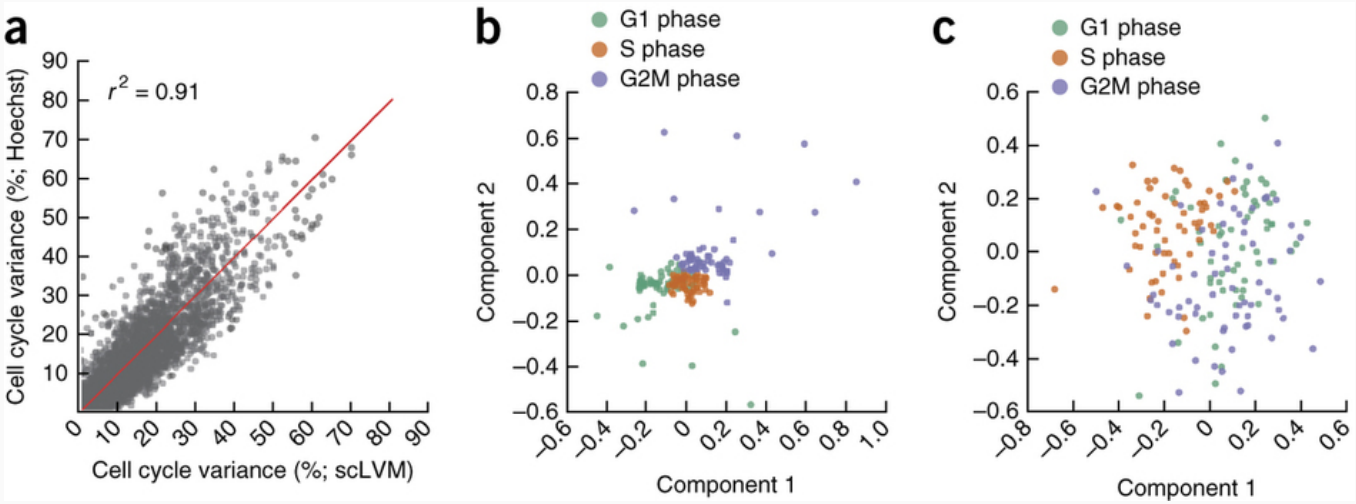

Figure: a. Gold standard v/s scLVM agreement b.Non Linear PCA on scLVM corrected data: no separation c.nonlinear PCA on unorrected data: separation by cell cycle!

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- The results were consistent even if the size of annotated genes was reduced to 10
- The gold standard and scLVM results are in sync

EFFICACY: HOW ABOUT JUST THROWING OUT THOSE KNOWN GENES?

If the genes are known to be associated with cell cycle, why not simple throw them out to rule out the effect of cell cycle?

A non-linear PCA of datasets with cell-cyclee associated genes thrown out gave a clear separation, and this separation was later validated to be two different cell cycles $=>$ *scLVM* accounts for the latent factors which cannot be simply accounted by throwing away those *informative* genes.

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- To further validate: non linear PCA with and without scLVM correction. Without correction: no obvious subgroups
- One of the two sub-populations post scLVM correction were found to have genes that marked completion of differentiation

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- Before applying *scLVM* the cells looked like a variable population
- scLVM corrected expression data showed there existed two sub-populations
- These two sub-populations were infact found to be associated with T-cell differentiation stages
- The method is not about single cell transcriptomics. It is a general approach to isolate, model and understand sources of variability

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- When multiple confounding factors are considered, in order to ensure robust analysis it is important to ensure the statistical significance if the factors are weak or nonindependent
- No formal tests exist for testing the presence of a particular factor

THE UNDERLYING MODEL

Let N = Number of cells
G = Number of variable genes(determined using a T-test on pre-processed count data) G_h = Set of marker genes(cell-cycle related)
Y_h = $[y_1, y_2, ...y_h]$ = Vector of gene expressions where y_g represents gene g's expression acorss cells

$$Y_h = \mu + CU + XW + \psi \tag{1}$$

U, W = Weight of linear covariance model where C models Q known covariates and W models unknown covariates.

ψ models the rest of noise

C, X are determined using a bayesian approach assuming both U,W as gaussians prior.

$$P(Y_h|\sigma_u^2, \nu^2, X, C) = \Pi_{g=1}^{G} N(y_g|\mu_g, \sigma_u^2 CC^T + XX^T + \nu^2) \tag{2}$$

The parameters are then estimated using maximum likelihood approach. Once XX^T is modeled, the genes are modeled as sume of mean and random effect:

$$y_g = \mu + \sum_{h=1}^{H} u_h + \psi_e + \psi_t \tag{3}$$

where $P(u_h) = N(\mu_h|0, \sigma_{gh}^2 \sigma_h)$, the last two terms accouting for residual and technical noise

and hence:

$y_{corrected} = y_g - y_g^{(hidden)}$ where $y_g^{(hidden)}$ is the posterior estimation:
$y_g^{hidden} = \sigma[\sigma + \nu_g]^{-1}(y_g - \mu_g)$

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- Heterogeneity in gene expression in single cells can be compromised by factors such as cell-cycle which are often ignored
- scLVM provides a bayesian approach towards filtering out the effect of confounding variables
- Counter-intuitively it is easier to cope with *lots* of data that is homogeneous than with limited data that is heterogeneous
- Apriori knowledge of confounding factor association can help decompose the variance, possibly raveling underlying undiscovered biology