Uncertainty Quantification in Multivariate Mixed Models for Mass Cytometry Data

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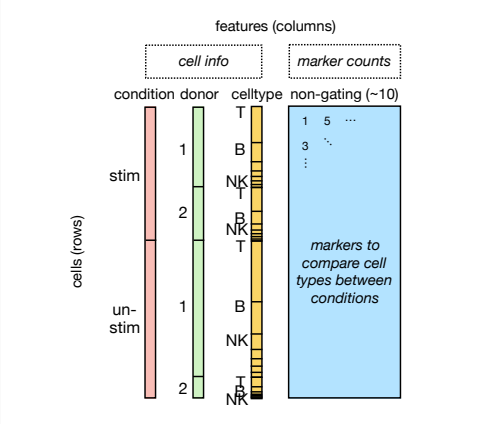
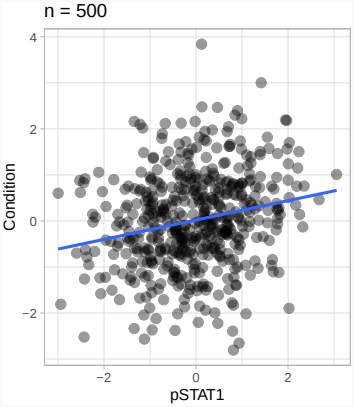
Mass Cytometry Data

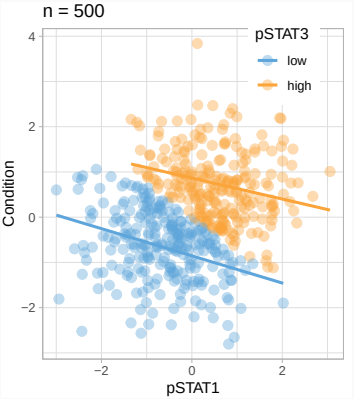


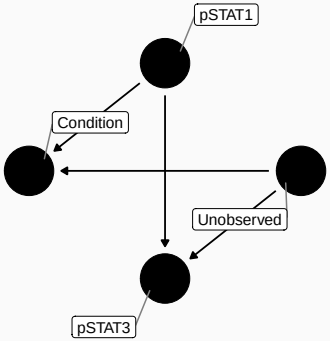
- experimental condition → non-gating markers or
- non-gating markers → experimental condition?




```
lm(formula = Condition ~ pSTAT1, data = cytof_data) %>% tidy
```

```
## # A tibble: 2 x 5
##   term        estimate std.error statistic    p.value
##   <chr>          <dbl>     <dbl>     <dbl>      <dbl>
## 1 (Intercept)   0.0208    0.0453     0.460 0.646
## 2 pSTAT1        0.211     0.0429     4.91  0.00000122
```

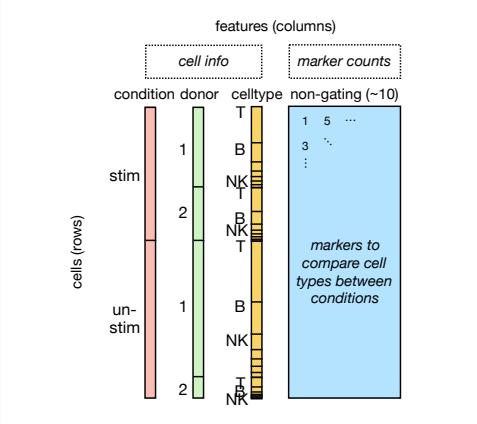





```
lm(formula = Condition ~ pSTAT1 + pSTAT3, data = cytof_data) %>% tidy
```

```
## # A tibble: 3 x 5
##   term        estimate std.error statistic  p.value
##   <chr>          <dbl>     <dbl>     <dbl>    <dbl>
## 1 (Intercept)   -0.834    0.0521    -16.0  8.78e-47
## 2 pSTAT1        -0.269    0.0385     -6.99 8.90e-12
## 3 pSTAT3high     1.72     0.0814     21.1  3.01e-71
```

Mass Cytometry Data



- Rows are **clustered** and columns are **correlated**

Clustered Data

⋮

Robust methods (Huber 1964, 1973)

Varying coefficients (Hastie and Tibshirani 1993)

Mixed effects (Pinheiro and Bates 2000)

Maximin effects (Meinshausen and Bühlmann 2015)

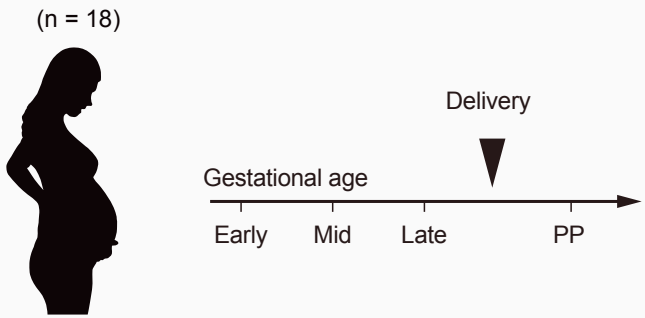
Mixtures (Tutz and Oelker 2017)

Tree-structured (Berger and Tutz 2018)

Anchor regression (Rothenhäusler et al. 2018)

⋮

Pregnancy Study by Aghaeepour et al. (2017)



- Blood samples collected during and after **pregnancy**
- **Protein expression** measured using mass cytometry (INF$-\alpha$ stimulated)
- **Differential analysis** between first and third trimester

Multivariate Poisson Log-Normal Model with Zero Inflation

Zero inflation:

- $y_{i,j}$ are counts in cell i of protein j
- For zero counts, flip a biased coin which lands Heads with probability θ
- If it comes up Heads, then set $y_{i,j} = 0$, otherwise

$$y_{i,j} \sim \text{Poisson}(\lambda_{i,j})$$

Multivariate Poisson (Chib and Winkelmann 2001):

$$\log(\lambda_{i,j}) = \beta_{\text{cond}[i],j} + b_{i,j} + u_{\text{donor}[i],j}$$

- $\boldsymbol{b}$ cell mixed effect and $\boldsymbol{u}$ donor mixed effect
- $\text{cond}[i] = 1$: first trimester and $\text{cond}[i] = 2$: third trimester

Model for Correlations

- **Cell-to-cell** variability (J total number of protein markers):

$$\begin{pmatrix} b_{i,1} \\ \vdots \\ b_{i,J} \end{pmatrix} \sim \text{Multivariate Normal}\left(\mathbf{0}, \text{diag}(\boldsymbol{\sigma})\, \boldsymbol{\Omega}\, \text{diag}(\boldsymbol{\sigma})\right)$$

- **Donor-to-donor** variability ($\boldsymbol{V}$ is a orthogonal matrix):

$$\begin{pmatrix} u_{k,1} \\ \vdots \\ u_{k,J} \end{pmatrix} \sim \text{Multivariate Normal}\left(\mathbf{0}, \boldsymbol{V}\boldsymbol{D}\boldsymbol{V}^T\right)$$

Priors

- **Condition** regression coefficients:
  Weakly informative prior (typical counts range from 10^{-4} to 1096)

$$\boldsymbol{\beta} \sim \text{Normal}(0, 7^2)$$

- **Cell-to-cell** variability:
  Full rank covariance matrix distribution (Lewandowski, Kurowicka, and Joe 2009)

$$\boldsymbol{\sigma} \sim \text{Half-Cauchy}(0, 2.5)$$

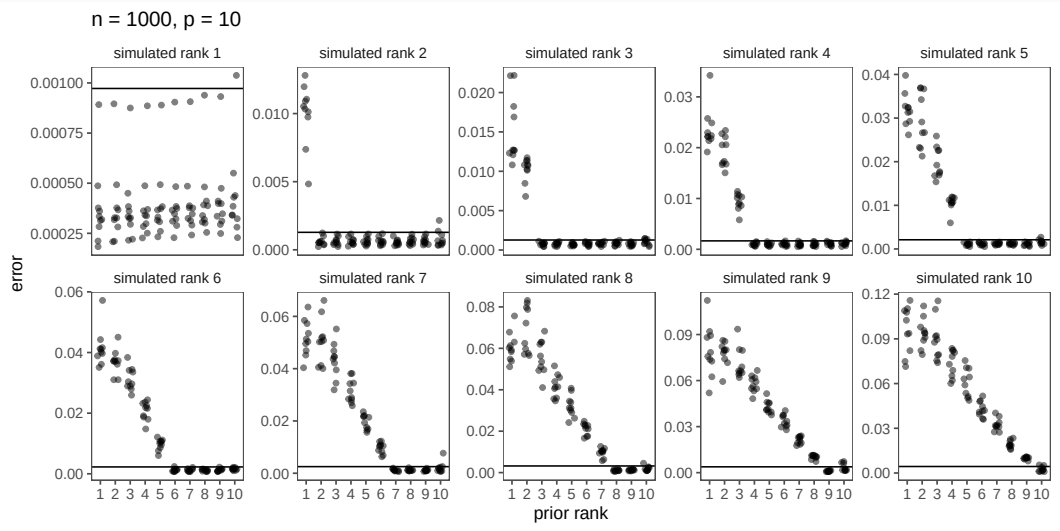
$$\boldsymbol{\Omega} \sim \text{Uniform correlation matrix}$$

- **Donor-to-donor** variability:
  Low rank covariance matrix distribution (Easton 1989; Jauch, Hoff, and Dunson 2019)

$$\text{diag}(\boldsymbol{D}) \sim \text{Half-Cauchy}(0, 2.5)$$

$$\boldsymbol{V} \sim \text{Uniform orthogonal matrix}$$

Priors: Choosing the Rank



Posterior Inference

- Parameters of interest:

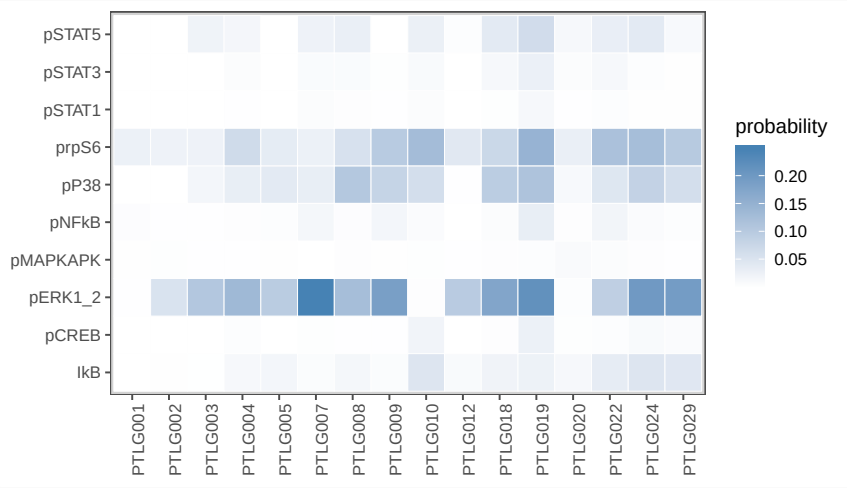
$$\textbf{pars} = \{\boldsymbol{\theta}, \boldsymbol{\beta}, \boldsymbol{\sigma}, \boldsymbol{\Omega}, \boldsymbol{V}, \boldsymbol{D}\}$$

- Sample from **posterior distribution** using Stan (Carpenter et al. 2017):

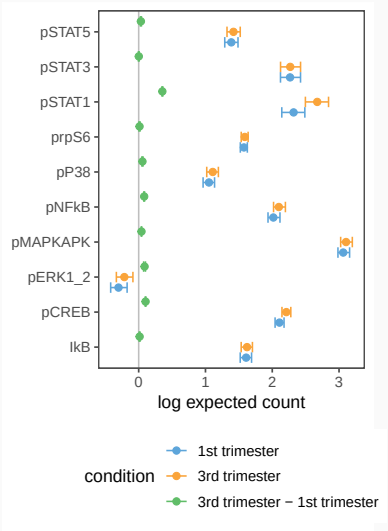
$$p(\textbf{pars}|\boldsymbol{y}) = \frac{p(\boldsymbol{y}|\textbf{pars})\, p(\textbf{pars})}{p(\boldsymbol{y})}$$

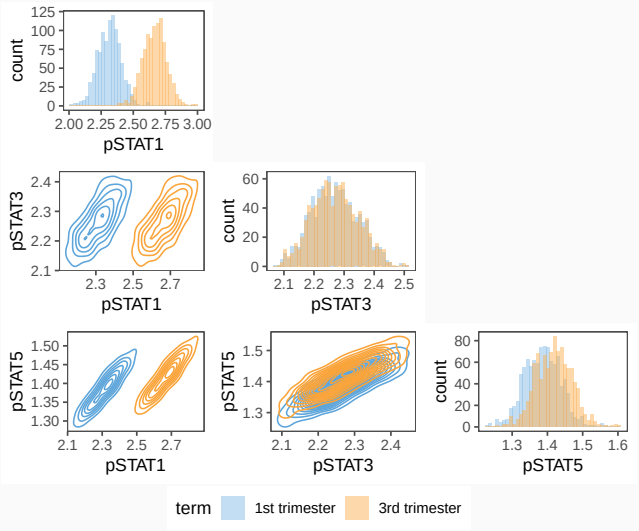
- Complete Stan model available on GitHub: poisson.stan
- **Summarize** posterior samples (e.g. median, credible intervals, MDS)

Posterior Summaries: θ

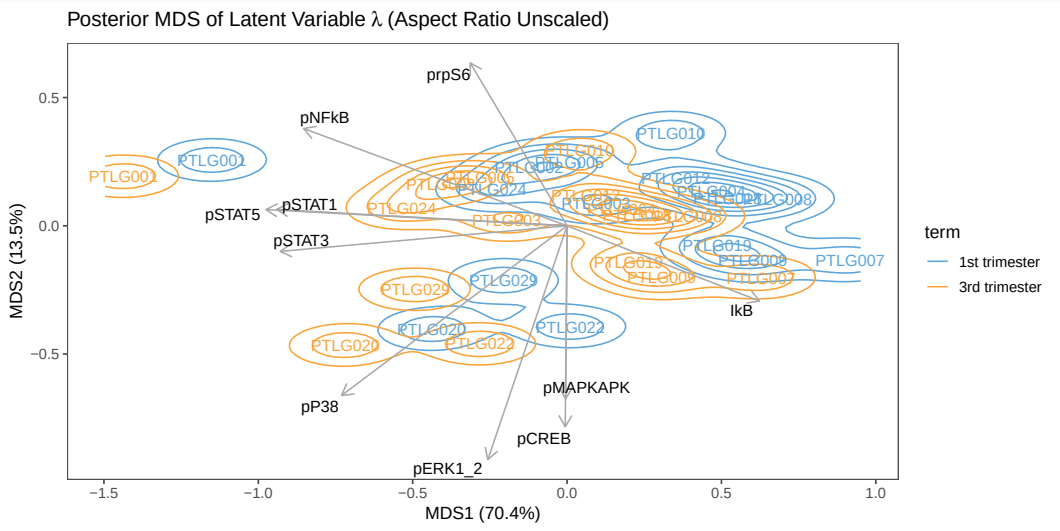


Posterior Summaries: β

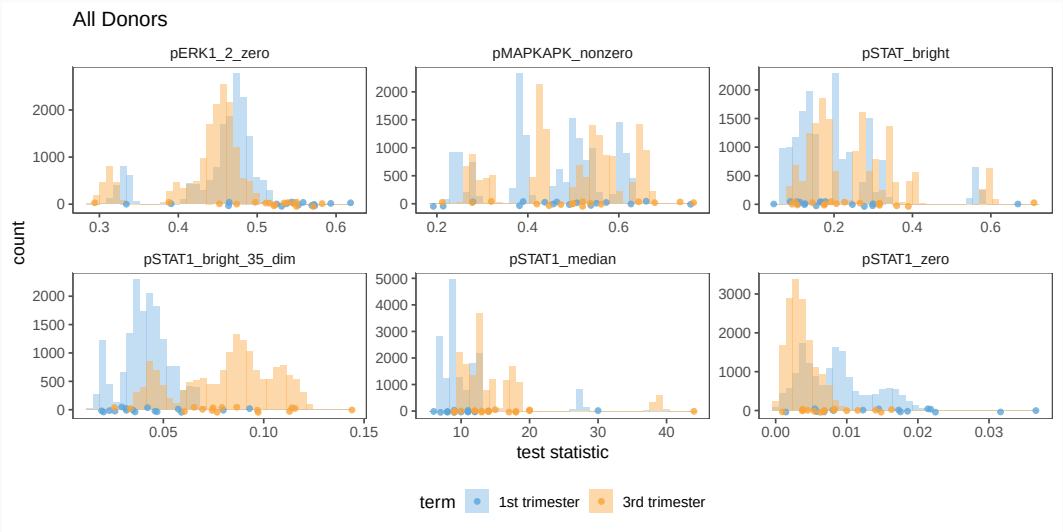




Posterior Summaries: λ



Goodness of Fit: Posterior Predictive Checks



Related Workflows/Packages

1. **Response variable**: Mean counts and cell type abundance
   - R package **diffcyt** (Weber et al. 2018):
     - High-resolution clustering and empirical Bayes moderated tests adapted from transcriptomics
   - F1000 CyTOF Workflow (Nowicka et al. 2017):
     - Manual gating and univariate analyses
2. **Response variable**: experimental condition
   - R package **Citrus** (Bruggner et al. 2014):
     - Hierarchical clustering and regularized regression to select predictive features
   - Python package **CellCnn** (Arvaniti and Claassen 2017):
     - Convolutional neural networks to detect rare cell populations

Take Home

- **Multivariate models**
  - describe marker correlations
  - avoid biases
- **Mixed models**
  - describe individual donor effects
  - avoid reporting overconfident results
- R package **cytoeffect** with vignettes:
  https://christofseiler.github.io/cytoeffect/
- **Preprint**: Seiler et al. (2019)

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