

# Uncertainty Quantification in Multivariate Mixed Models for Mass Cytometry Data

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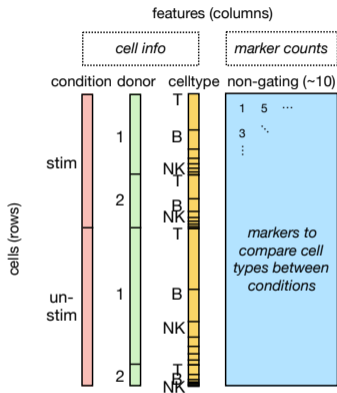
and Knowledge Engineering

Maastricht University, The Netherlands

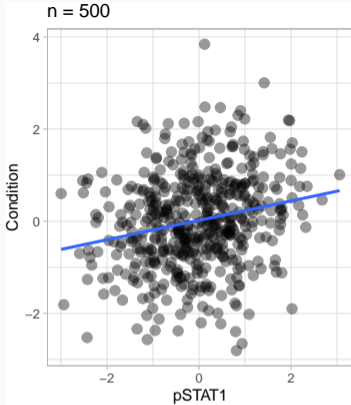
<http://christofseiler.github.io>

Second Dutch Stan Meetup 2019, Utrecht

# Mass Cytometry Data



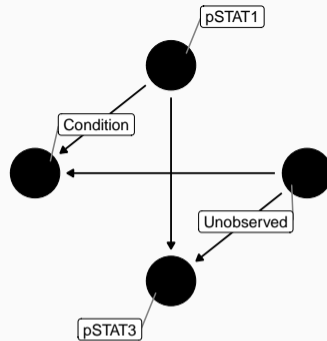
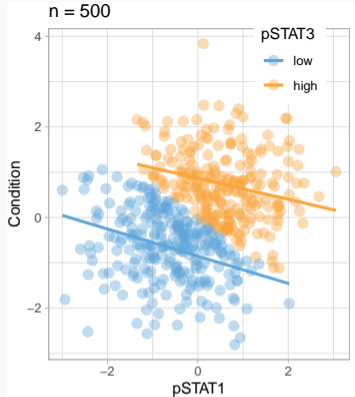
- **experimental condition** → **non-gating markers** or
- **non-gating markers** → **experimental condition**?



```
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
```

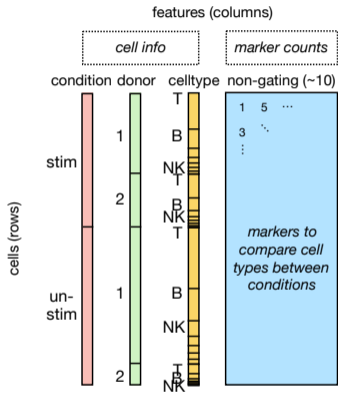


```
lm(formula = Condition ~ pSTAT1 + pSTAT3, data = cytof_data) %>% tidy
```

```
## # 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**

⋮

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)

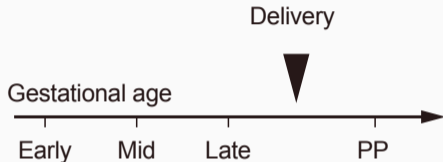
Tree-structured (Berger and Tutz 2018)

Anchor regression (Rothenhäusler et al. 2018)

⋮

## Pregnancy Study by Aghaeepour et al. (2017)

(n = 18)



- 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  $j$
- Flip a biased coin which lands Heads with probability  $\theta$
- 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):

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

- $b$  cell mixed effect and  $u$  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):

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

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

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

- **Condition** regression coefficients:

Weakly informative prior (typical counts range from  $10^{-4}$  to 1096)

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

- **Cell-to-cell** variability:

Full rank covariance matrix distribution (Lewandowski, Kurowicka, and Joe 2009)

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

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

- **Donor-to-donor** variability:

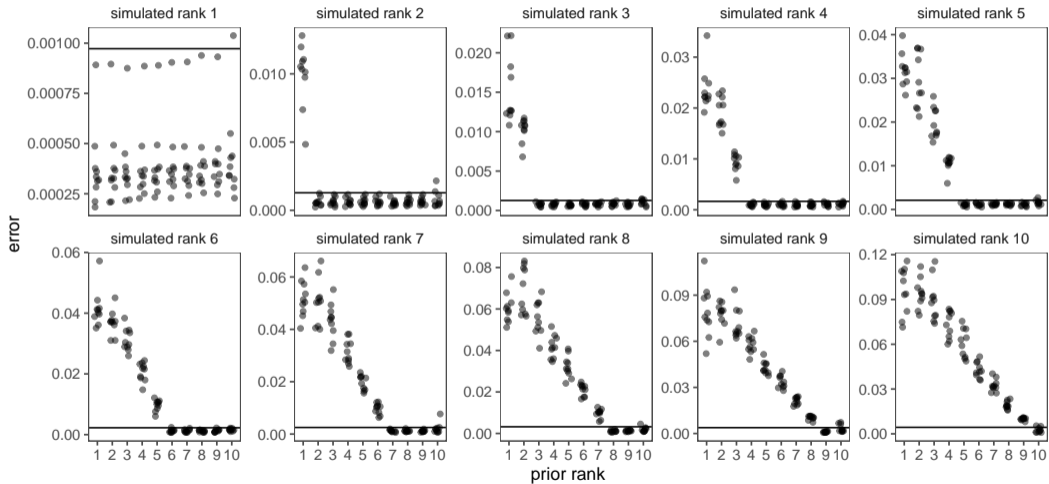
Low rank covariance matrix distribution (Easton 1989; Jauch, Hoff, and Dunson 2019)

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

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

# Priors: Choosing the Rank

$n = 1000, p = 10$



- Parameters of interest:

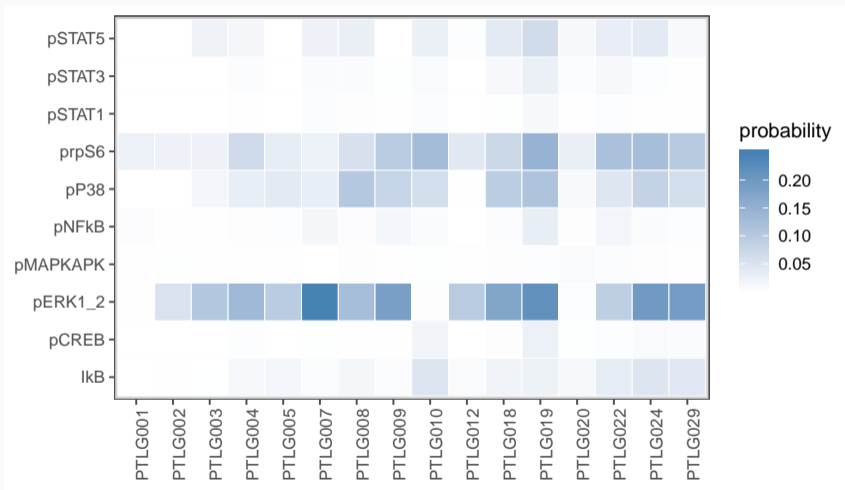
$$\mathbf{pars} = \{\theta, \beta, \sigma, \Omega, \mathbf{V}, \mathbf{D}\}$$

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

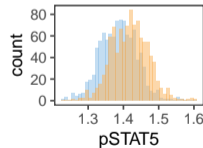
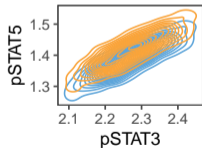
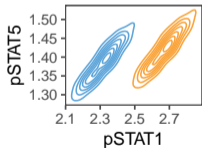
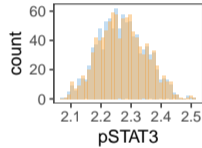
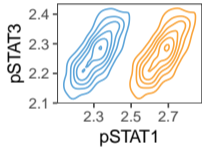
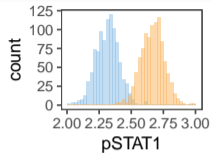
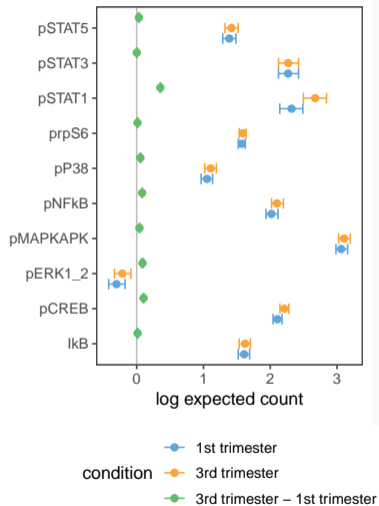
$$p(\mathbf{pars}|\mathbf{y}) = \frac{p(\mathbf{y}|\mathbf{pars}) p(\mathbf{pars})}{p(\mathbf{y})}$$

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

# Posterior Summaries: $\theta$



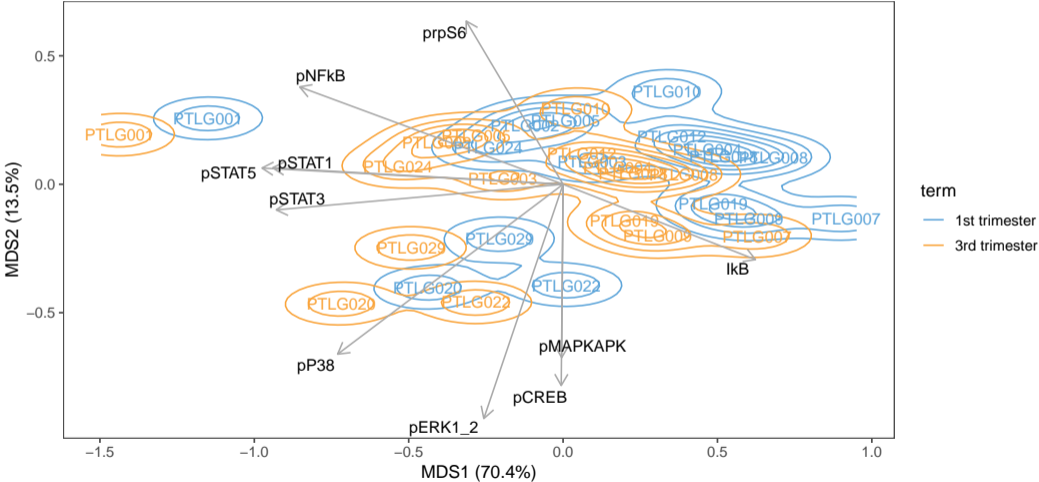
# Posterior Summaries: $\beta$



term 1st trimester 3rd trimester

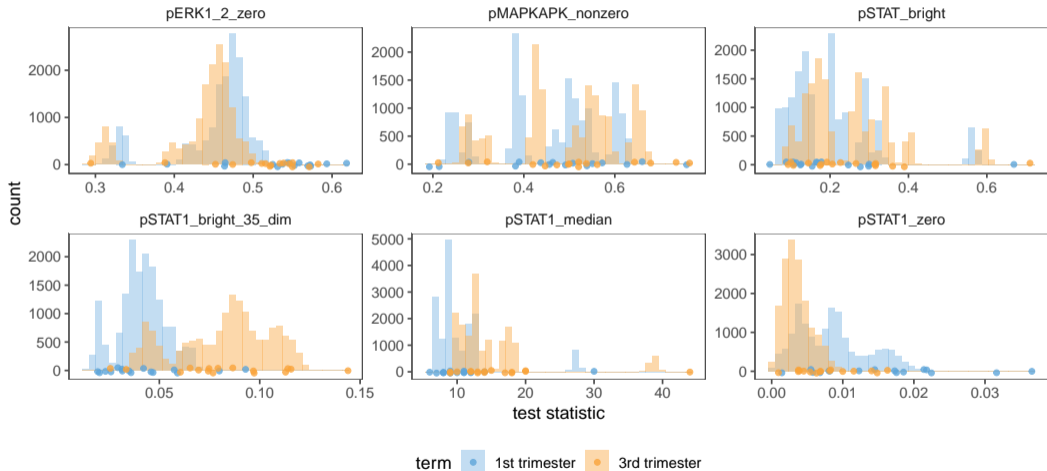
# Posterior Summaries: $\lambda$

Posterior MDS of Latent Variable  $\lambda$  (Aspect Ratio Unscaled)



# Goodness of Fit: Posterior Predictive Checks

All Donors





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

- **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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