Analyzing Flow-Cytometry Count Data with Regression Mixtures

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University of Washington

Joint work with

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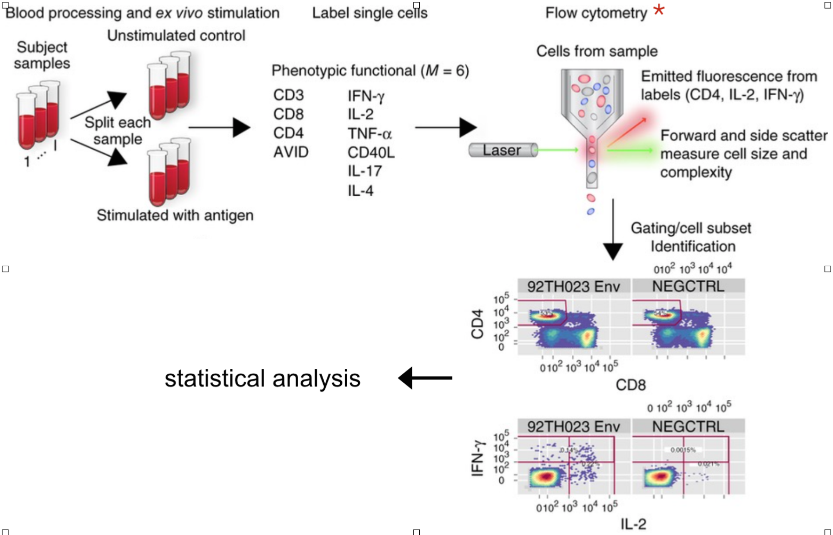
Hutchinson Cancer Research Center

May 8, 2017

Outline

1. **Introduction** to Flow-Cytometry
2. **Motivation**
   - Existing methods
   - Why a regression model?
3. **Models**:
   - A Marginal Model
   - A Joint HMRF model
4. **Data analysis**
   - RV144 HIV Vaccine Trial
   - Controlled Human Malaria Infection Study.
5. **Computation** (time permitting)

The RV144 HIV Vaccine Trial



statistical analysis

adapted from Lin et al, Nat. Biotech. 2015

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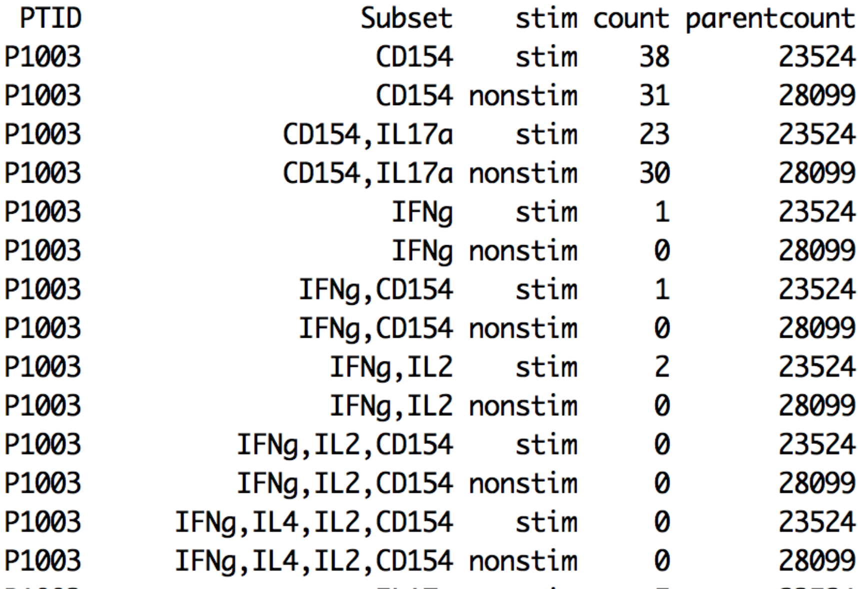

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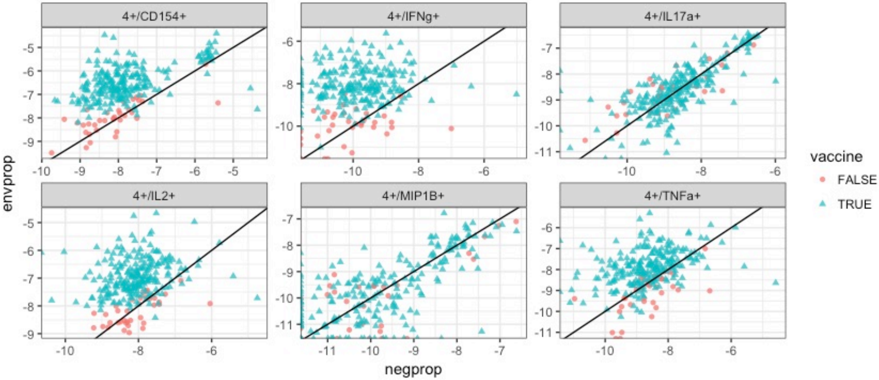
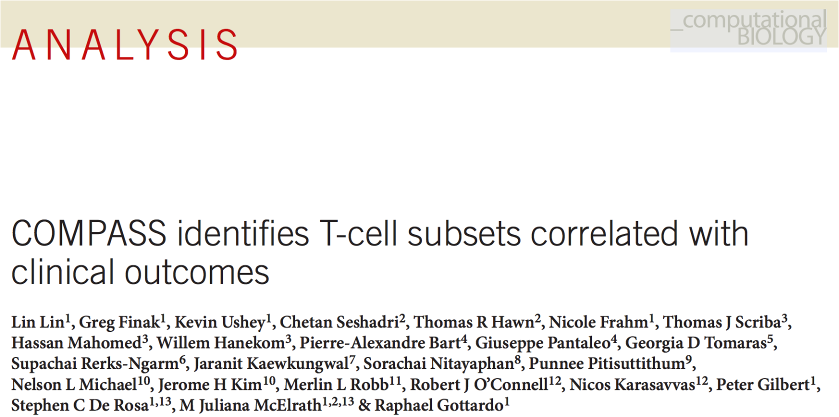
The RV144 HIV Vaccine Trial

- **262 Subjects**
  - 226 Cases
  - 36 Controls
- **2 Types of stimulus**
  - HIV antigen
  - Negative control
- **6 types of cytokines.**

Marginal Counts for RV144



Motivation: COMPASS



ANALYSIS

computational BIOLOGY

COMPASS identifies T-cell subsets correlated with clinical outcomes

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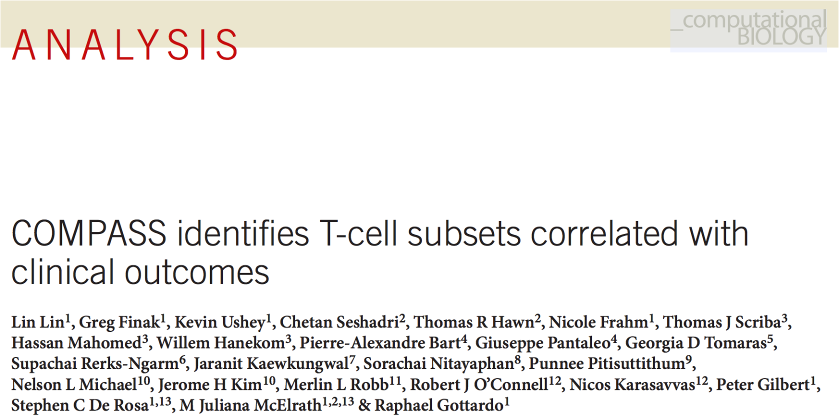

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Or in general, with immune response.

How do current methods work? (Approximately)

Current models are baseline/stimulation models.

- Unstimulated blood sample are compared stimulated ones.

For the unstimulated sample of the ith subject out of n, we sample a count proportion:

$$p_{i0} \sim \text{Dirichlet}(\alpha_0, \beta_0),$$

$$y_{i0} \sim \text{Multinomial}(N_{i0}, p_{i0}).$$

Let $k_i \in \{0, 1\}^p$ indicate in which subsets i responds:

$$k_{ij} \sim \text{Ber}(w_j),$$

$$p_{i1,\tau=0} \sim \delta(p_{i0,\tau=0}), \quad p_{i1,\tau=1} | p_{i0,\tau=0} \propto \text{Dirichlet}(\alpha_1, \beta_1)$$

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Controlled Human Malaria Infection Study

- 9 subjects were infected with Malaria.
  - +3 controls.
- Blood samples were collected at 6 time points.
  - Day 0, day 9, blood parasitemia, Day 28, Day 56, Day 168.
- Two types of stimulation:
  - Infected/uninfected blood-cells.
- 53 cell subsets.
  - (10 types of cytokines in 8 cell-types)

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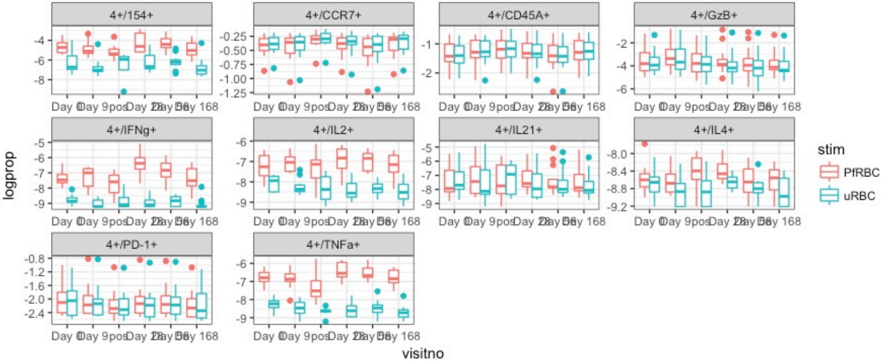

Motivation - A Regression Model

- We want to be able to include covariates:
  - Batch effects.
  - Other covariates such as age, gender...

- Longitudal data.

- More than one stimulation.

- Explicit dependence model:
  - For the observed proportions.
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Motivation - Unique Challanges

- **Dependence**
  - Within sample between cell subsets.
  - Within subject / across time.
- **Heterogenous treatment effect**
- **Over-dispersed Binomial counts**

A Marginal Model - Single Subset

Indexing: **i**-subject, **t**- stimulation/time-point.

Overdispersion ⇒ Beta Binomial Model.

$$\text{logit}(\mu_{it}) = X_{it}\beta,$$

$$p_{it} \sim \text{Beta}(M\mu, M(1-\mu)),$$

$$y_{it} \sim \text{Binom}(N_{it}, p_{it})$$

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Dependence ⇒ ‘random’ subject baseline:

$$\nu_i \sim N(0, \sigma^2)$$

$$\text{logit}(\mu_{it}) = X_{it}\beta + \nu_i$$

$$p_{it} \sim \text{Beta}(M\mu, M(1-\mu)),$$

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A Marginal Model - Single Subset

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Non-response ⇒ Mixture-Model:

$$k \sim Ber(\theta),$$

$$\text{logit}(\mu_{itk}) = X_{it}\beta + T_{it}\tau_k + \nu_i,$$

- T a matrix of covariates related to the treatment.
- τ_k equals 0 if $k = 0$ or $\tau \neq 0$ if $k = 1$.

A Marginal Model - Recap

Indexing: **i**-subject, **t**- stimulation, **k**- cluster.

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$$\text{logit}(\mu_{itk}) = X_{it}\beta + T_{it}\tau_k + \nu_i,$$
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Model can be estimated via an EM algorithm

Wellness of Fit Evaluation

How do we evaluate the model?

- We fit the model without information regarding the true treatment allocation.
- The model should be able to discriminate between vaccinees and placebos.
- We use three type of figures:
  - Scatter plots w/classification information.
  - Receiver-Operator Curves.
  - False Detection Rates.

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Marginal Model - Results

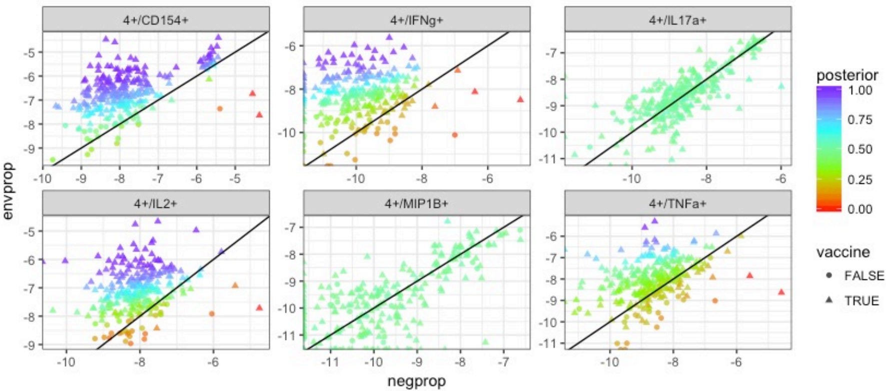

Figure: Posterior Probabilities for RV144 dataset - Independence Model

Comparison w/ MIMOSA - Finak et al. (2013)

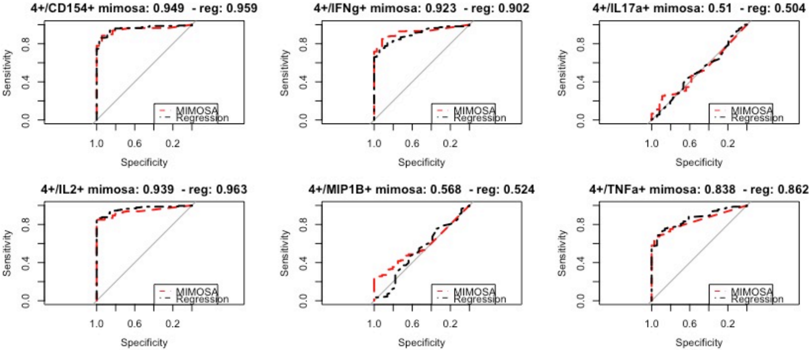

Figure: Comparison with MIMOSA (univariate COMPASS)

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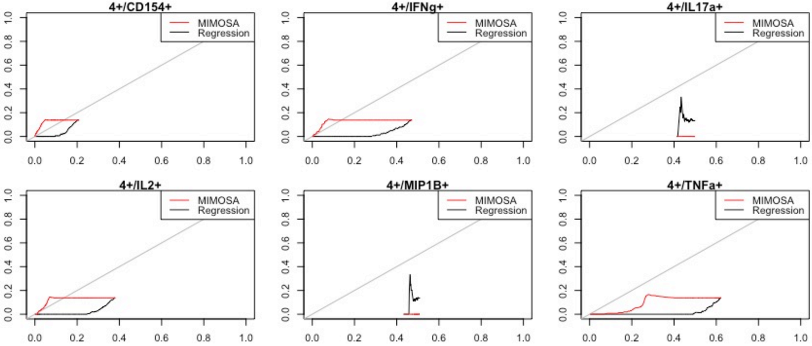

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Subject-Response Model

Performing analysis for each cell-subset at a time doesn't use all of the information available.

- Random Effects are correlated, can be estimated better simultaneously.
- Correlation structure might be of interest in itself.
- Response is probably not independent across cell-subsets.
- We might be able to improve classification of response by looking at several cell-subsets at once.

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A Hidden Markov Random Field Model

Indexing: **i**-subject, **t**- stimulation, **j**- subset, **k**- cluster.

Denote cluster (Response) by a $k \in \{0, 1\}^p$ vector with 1 indicating a responsive subset.

We assume an Ising model for the dependence structure between subsets:

$$P(k) \propto \sum_{j=1}^{p} k_j \theta_j + \sum_{s \neq t} k_t k_s \theta_{st},$$

$$P(k_j = 1 | k_{-j}) = \theta_j + \sum_{t \neq j} k_t \theta_{tj}.$$

We can induce sparsity through an ℓ_1 penalty.

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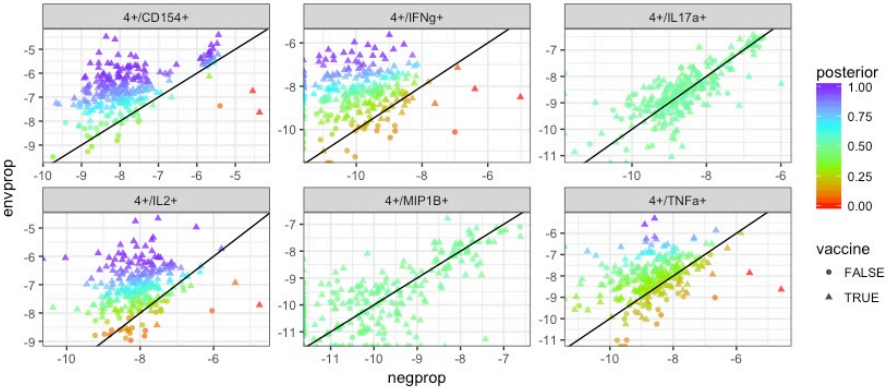

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HMRF Model - Results

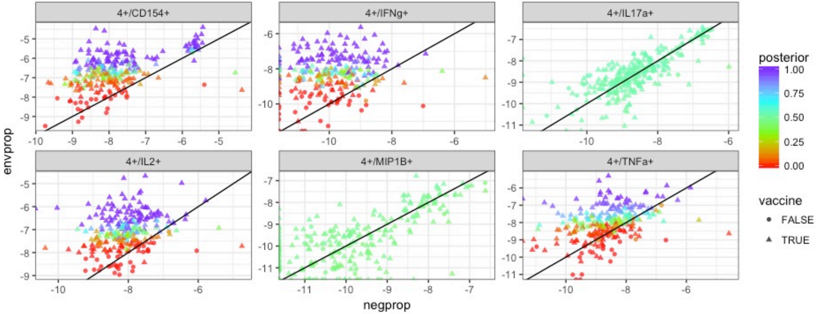

Figure: Scatter Plot for HMRF Modle Model

Subset-Response Model - Results

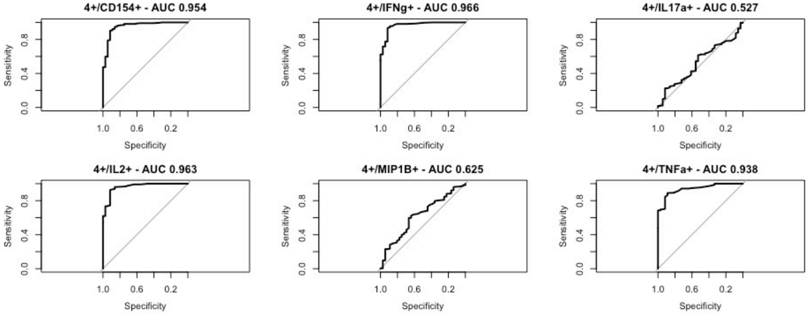

Figure: ROC for HMRF Modle

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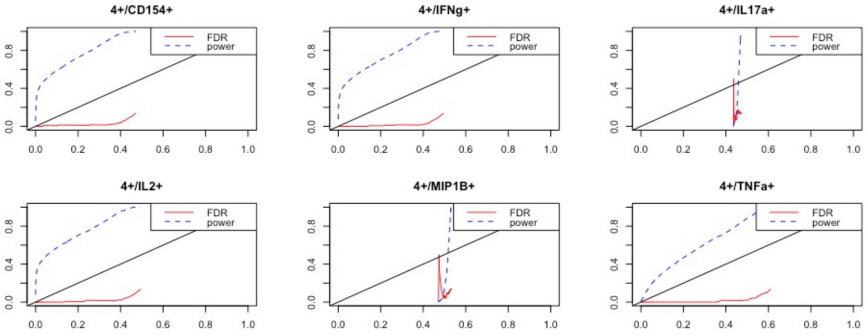

Figure: FDR for HMRF Modle

Simulated Data Experiment

- Posterior probabilities are not well calibrated.
  - Might be due to true non-response.
- Is the optimization algorithm estimating the model properly?
- Does the model fit the data well?
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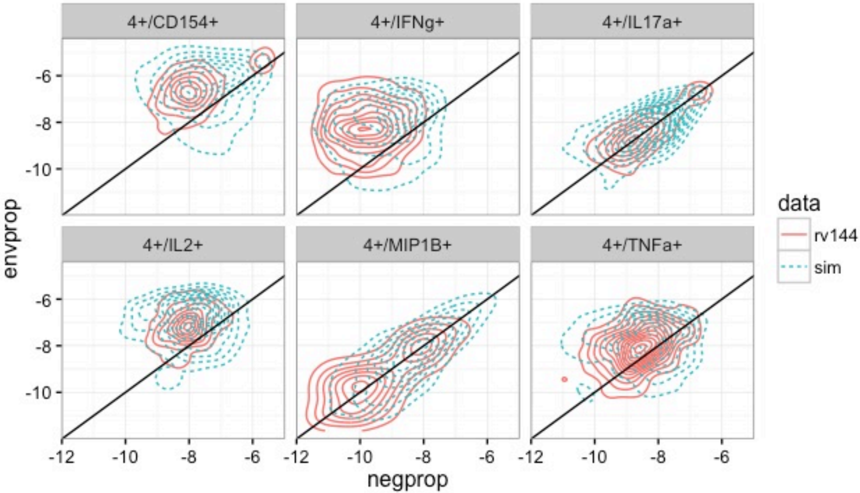
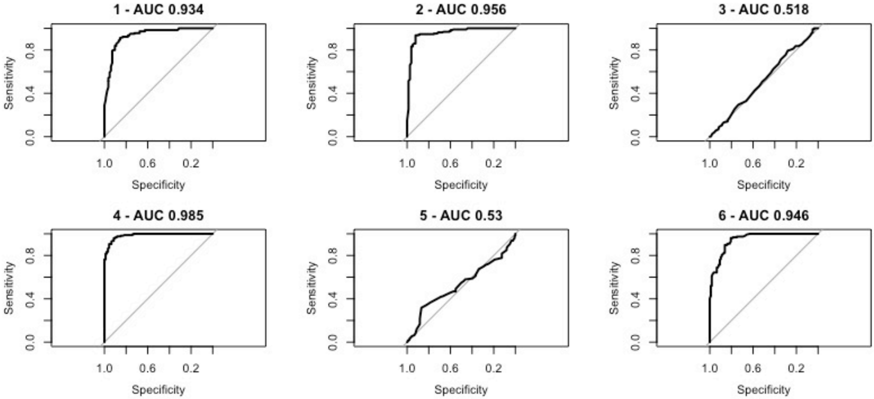
How close are we to the distribution of the data?



Results for Simulated Data



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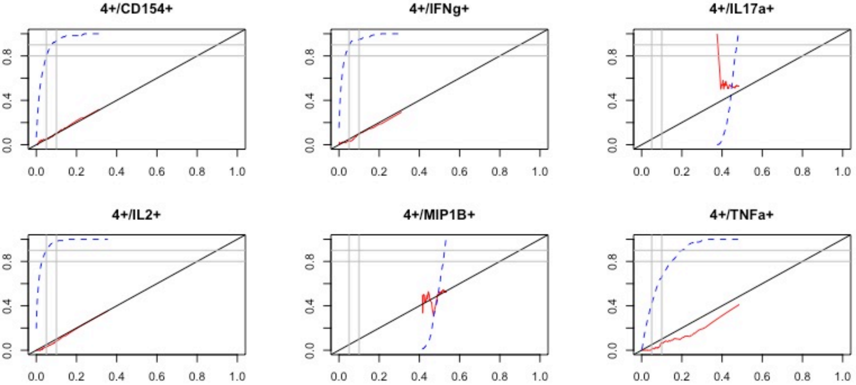
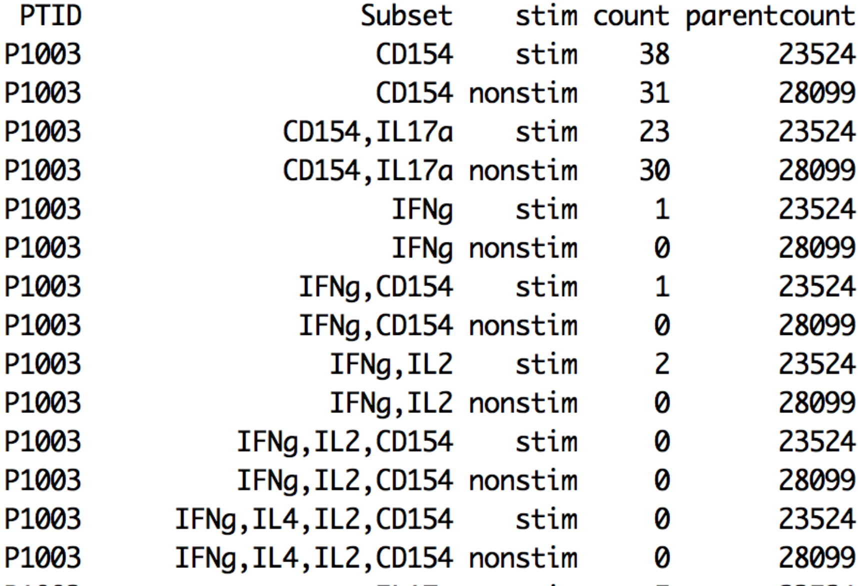


RV144 Booleans Dataset



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- single-cell measurements enable a more comprehensive understanding of cellular functions.
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  - 226 Cases
  - 36 Controls
- **2 Types of stimulus**
  - HIV antigen
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- **23 types of cells with non-negligible counts.**
  - (At least two instances of $count \geq 5$)

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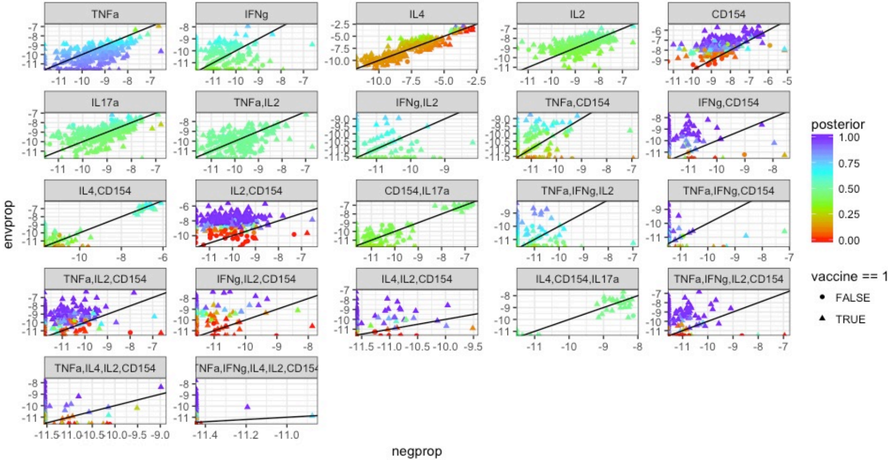

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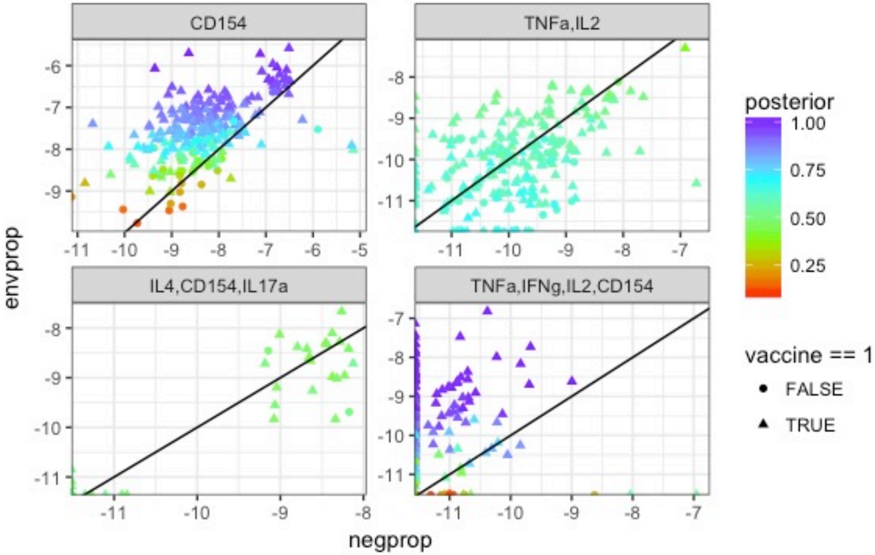

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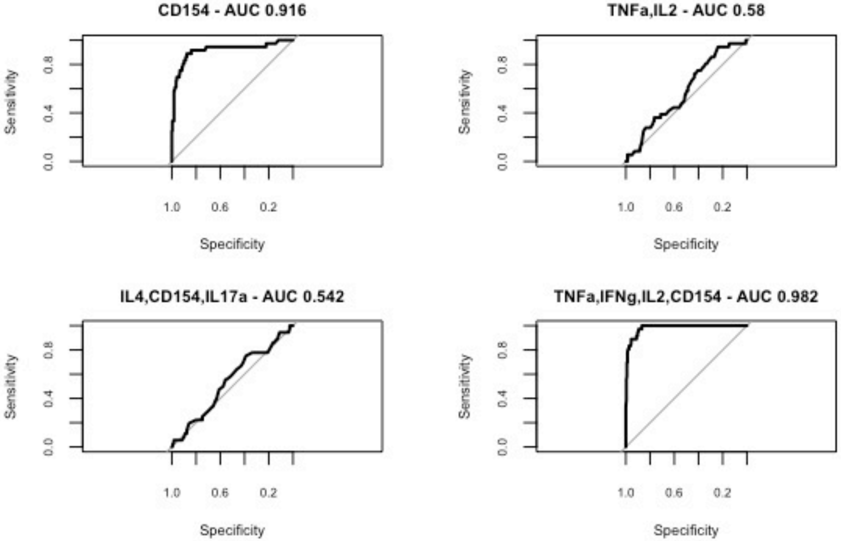

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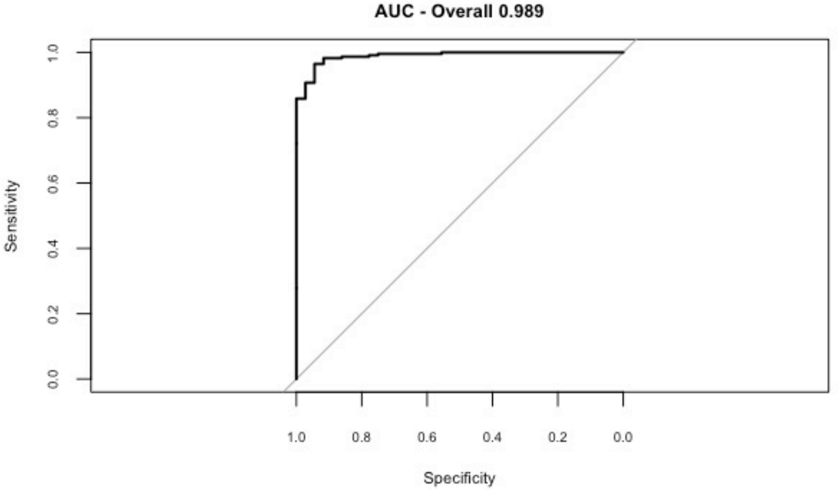

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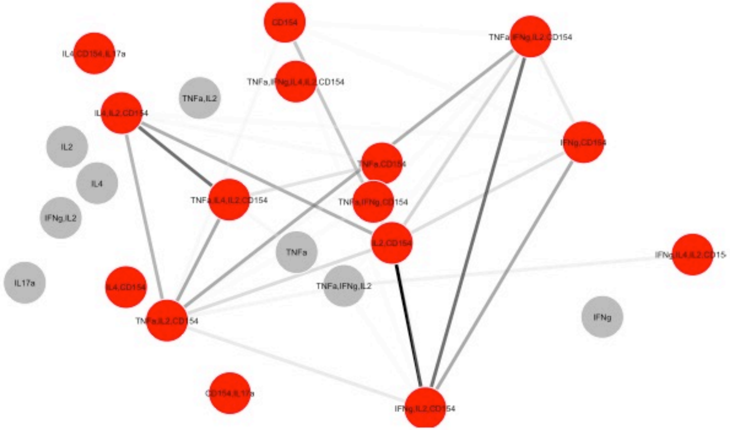

Figure: Estimated Ising Model - Red marks CD154

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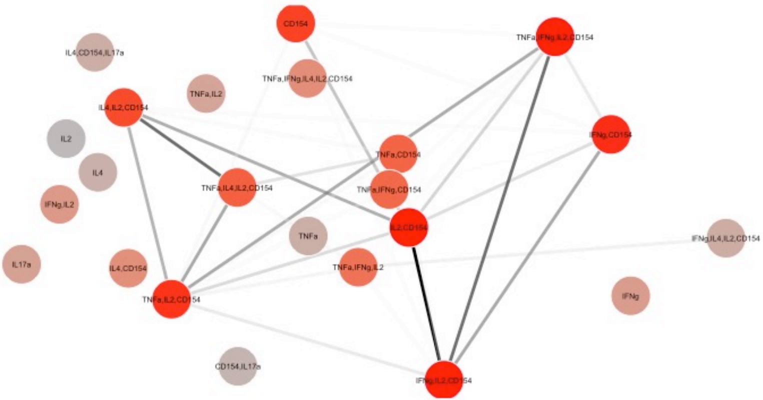
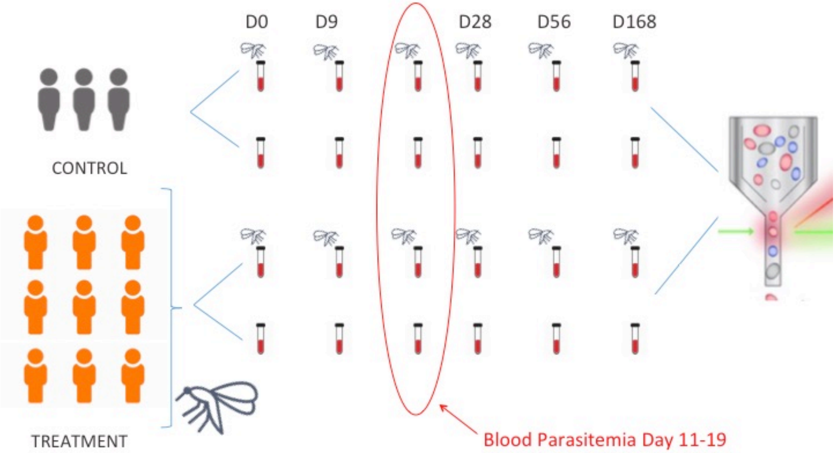


Figure: Estimated Ising Model - Red marks AUC

Controlled Human Malaria Infection Study



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- Individuals who experience malaria infections develop immunity.
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  - Even at day 0!
  - What is the profile of the immune response?

- The immunity is not long lived.
  - We might expect to see a rise in response during experiment.
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malaraFourPlusSmoothed

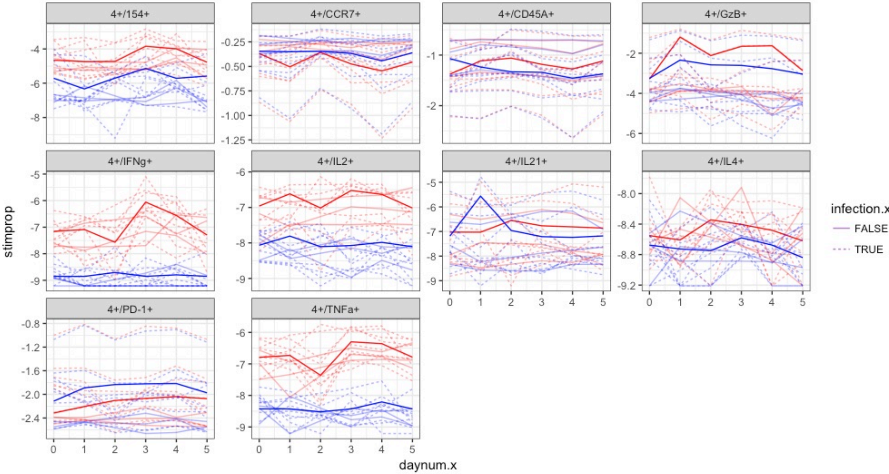

Figure: CD4 Helper Cells

FDR Adjusted p-values for CHMI Study

Standard errors for significance tests computed using Jackknife.

	4+	4+/CXCR5+	56+dim	56+hi	8+	8+/CXCR5+	NK T cells	PD-1+
154+	0.029	0.004			0.103	0.75	0.006	0.024
CCR7+	0.649	0.996			0.596	0.51		
CD45A+	0.575	0.307			0.543	0.54		
IFNg+	0.001	0.006	0.065	0.146	0.001		0.052	0.097
IL2+	0	0.005			0.119	0.56	0.321	0.052
IL21+	0.676	0.649	0.751	0.589	0.649		0.71	
IL4+	0.12	0.543		0.751	0.649		0.583	
TNFa+	0	0.001	0.261	0.309	0.276		0.053	0.09
GzB+	0.583		0.511	0.001	0.589		0.596	
PD-1+	0.751				0.596	0.83		

Controlled Human Malaria Infection Study

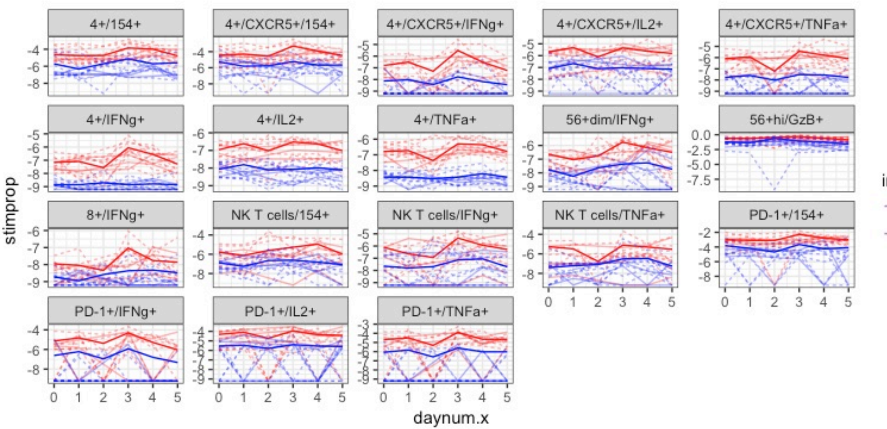

Figure: Significant Subsets

Controlled Human Malaria Infection Study

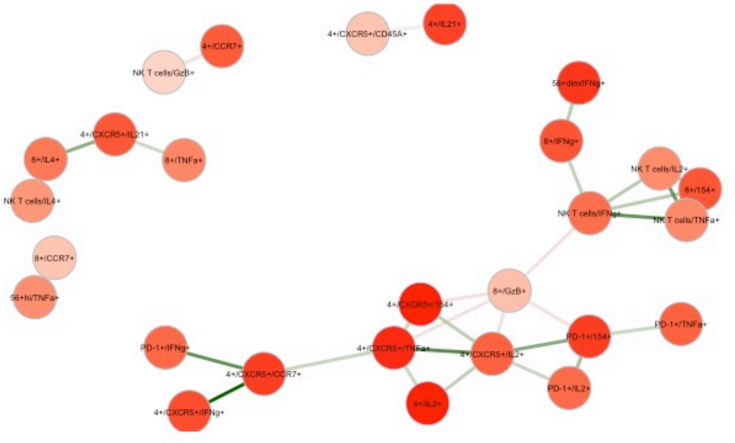

Figure: Estimated Graph - Red marks probability of response

Computation - A Difficult Likelihood

With cluster assignments and random effects known:

$$f(y_i|\nu_i, k_i) = \prod_j \prod_t f(y_{ijt}|\nu_{ij}, k_{ij})$$

The log-likelihood of the data is given by:

$$\ell(\beta, \tau, \theta, \Sigma) = \sum_{i=1}^{n} \log \left(\sum_{k \in \{0,1\}^J} P_\theta(k) \int_{\mathbb{R}^J} f_{\beta,\tau}(y_i|\nu_i, k_i) \varphi(\nu_i; 0, \Sigma) d\nu_i \right)$$

The log-likelihood is intractable for large J.

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Computation - How About EM?

The integrals are replaced with conditional expectations:

$$\sum_{i=1}^{n} \log \left(\sum_{k \in \{0,1\}^J} P_\theta(k|y_i) \int_{\mathbb{R}^J} f_{\beta,\tau}(y_i|\nu_i, k_i) f_\Sigma(\nu_i|y_i, k) d\nu_i \right)$$

We can use sampling to approximate the intractable integrals:

$$k^*_{i1}, ..., k^*_{iM} \sim P_\theta(k|y_i)$$

$$\nu^*_{i1} \sim f(\nu_i|y_i, k_{i1}), ..., \nu^*_{iM} \sim f(\nu_i|y_i, k_{iM})$$

$$(\beta, \tau, \theta, \Sigma) = \arg\max \sum_{i=1}^{n} \frac{1}{M} \sum_{m=1}^{M} \sum_{j} \sum_{t} \log f(y_{ijt}|\nu^*_{im}, k^*_{im})$$

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Computation - Stochastic EM Algorithm

We alternate between a stochastic E-step and an M-step.

S - Step

- $\boldsymbol{k}^*$ - Gibbs sampler.
- $\nu^*|\boldsymbol{k}^*$ - component-wise MH algorithm.

M - Step

- β, τ - **glm, glmnet** for sparsity or **gamlss** for BB.
- $\boldsymbol{\theta}$ - Pseudo-likelihood or **isingFit** for sparsity .
- $\boldsymbol{\Sigma}$ - Option for sparse estimation via **PSDE** package.

Thank you!

Questions?

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