Estimating AutoAntibody Signatures to Detect Autoimmune Disease Patient Subsets

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R package: spotgear

https://github.com/zhenkewu/spotgear

Common Questions on Individual and Population Health

1. a. What is the person's health state given health measurements?
   b. What is the population distribution of health states?
   (Wu et al., 2015, *JRSS-C*; Wu and Zeger, 2016a,b)
2. a. What is the person's health trajectory?
   b. What is the population's characteristics of health trajectory?
3. Does a particular intervention improve health - on average/for a particular person? (Wu et al., 2014, *Biometrics*; Frangakis, Qian, Wu, Diaz, 2015, *Biometrics*)
4. Are interventions being used optimally?

Example I

Pneumonia Etiology Research for Child Health (PERCH)

Background:

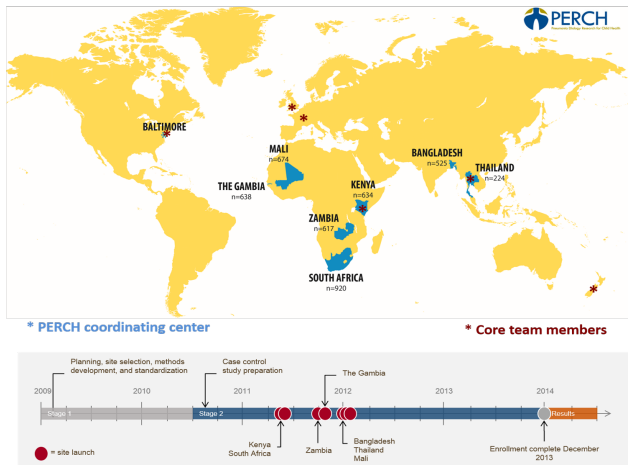
- $>$ 30 possible infectious causes
- Difficult to directly observe

Goal:

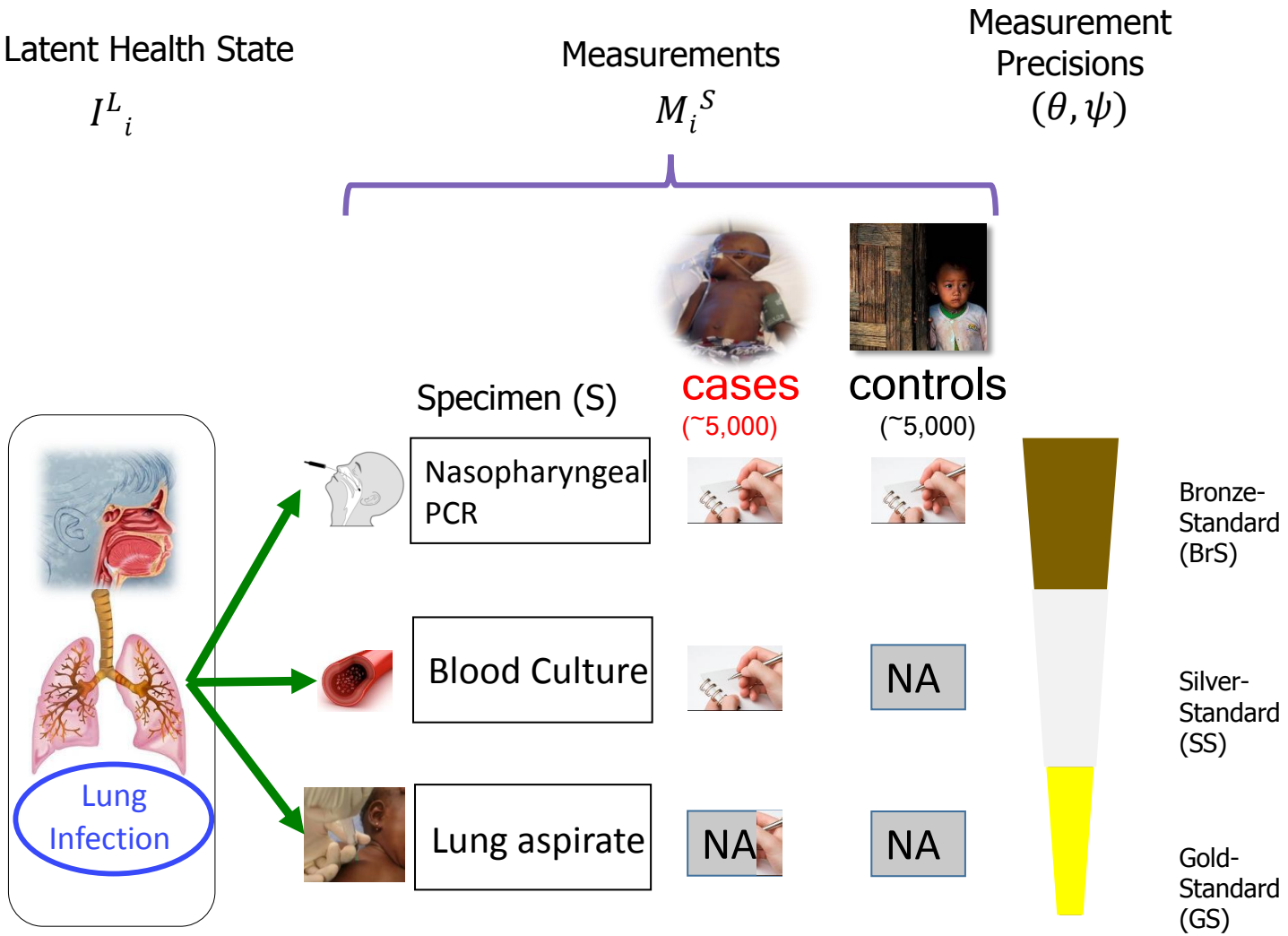
- Population disease etiology estimation
- Individual diagnosis

Study details:

- \$40-mil, Gates-funded 7-country study; Sites at Sub-Saharan Africa and South Asia
- Diverse measures; variable precisions
- $\sim$5,000 cases and $\sim$5,000 controls



Measurements of Different Quality



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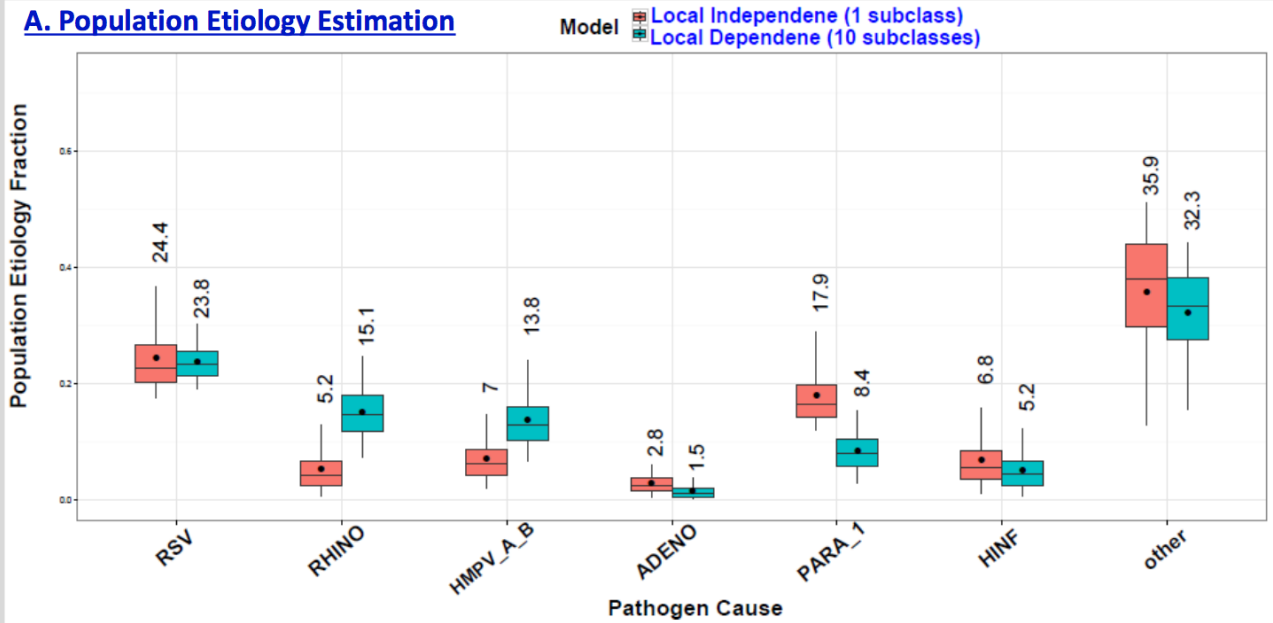
*NP: nasopharyngeal; PCR: polymerase chain reaction; LA: lung aspirate

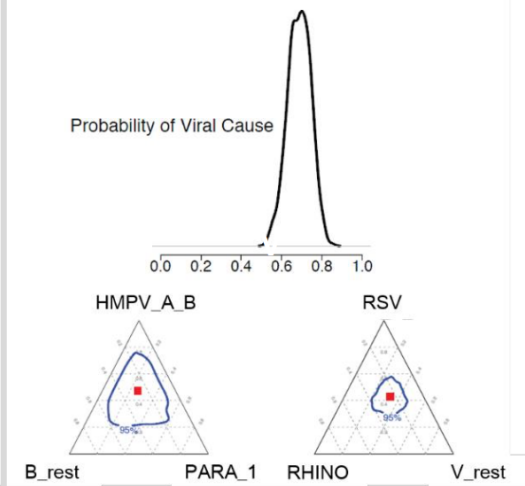
Nested Partially-Latent Class Models for Population and Individual Estimations

A. Population Etiology Estimation



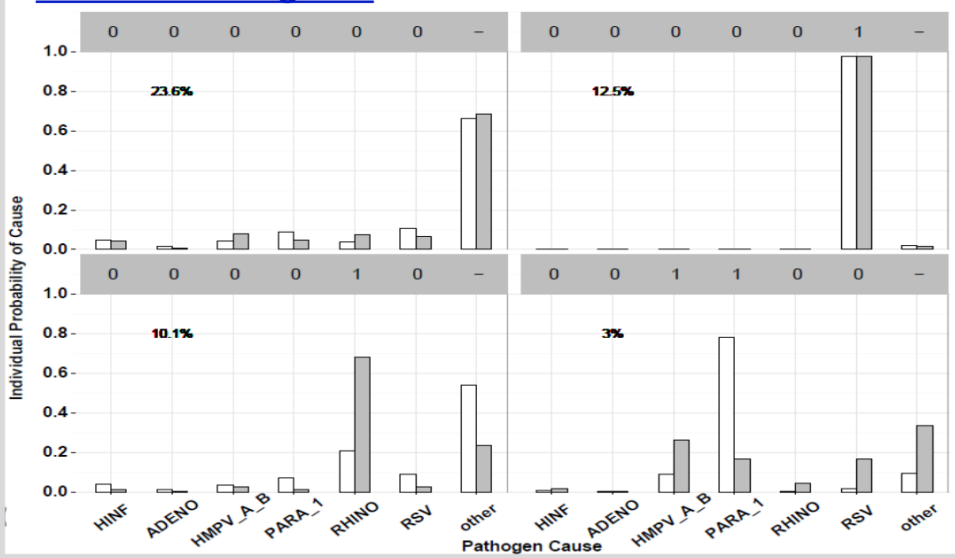
Marginal posterior distributions of the population etiology for 6 leading pathogens plus other. Results based on two models are shown.

B. Estimation by Pathogen Taxonomy



Posterior of viral and bacterial etiology

C. Individual Diagnosis

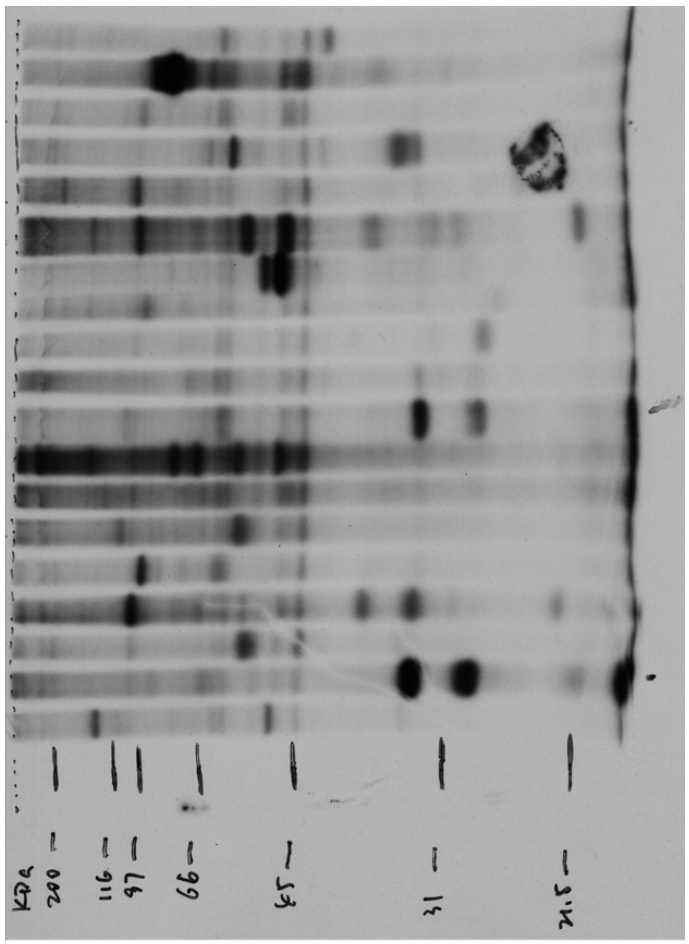


Individual predictions of the cause of pneumonia for 4 patterns of NPPCR data (binary codes in gray bands with its observed frequency shown below). Results from two models are shown. The top two have similar predictions; The bottom two differ, depending on how we correct for false positives.

Example II: Raw Data

Gel Electrophoresis Autoradiography; 20 Samples

Raw Image



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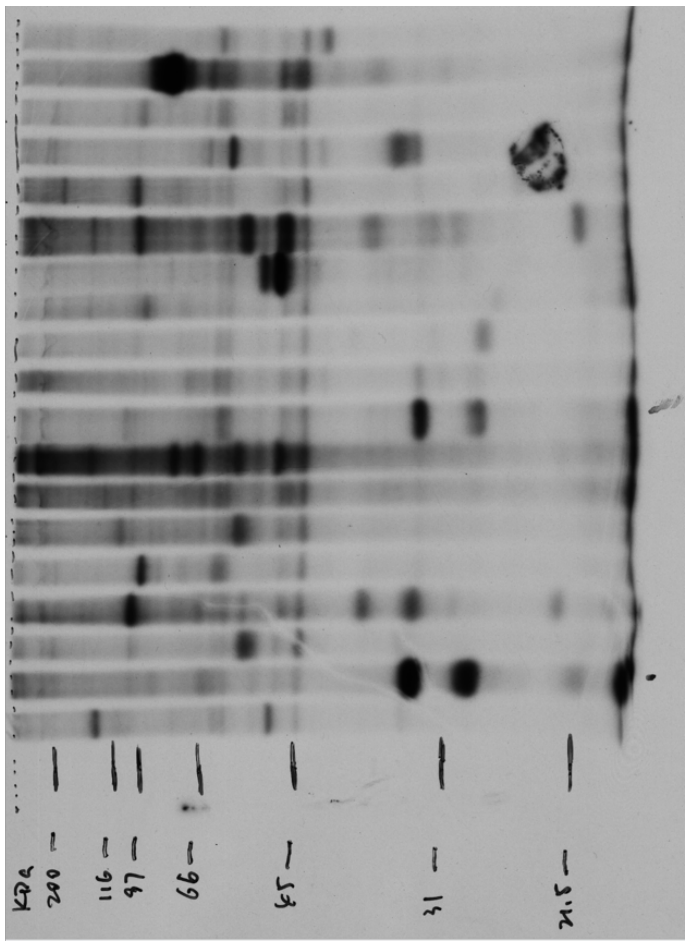

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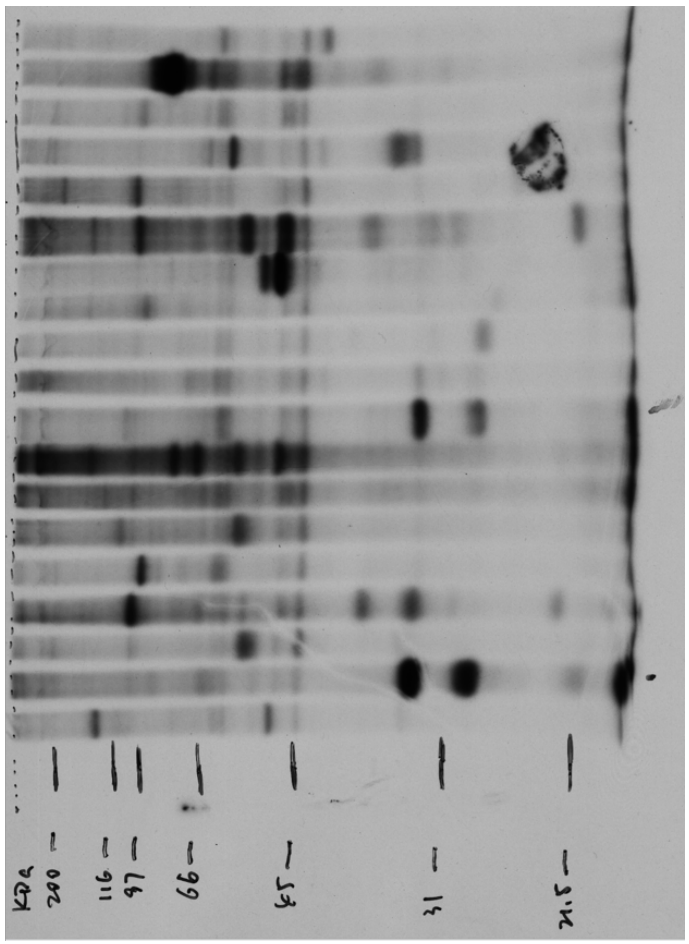

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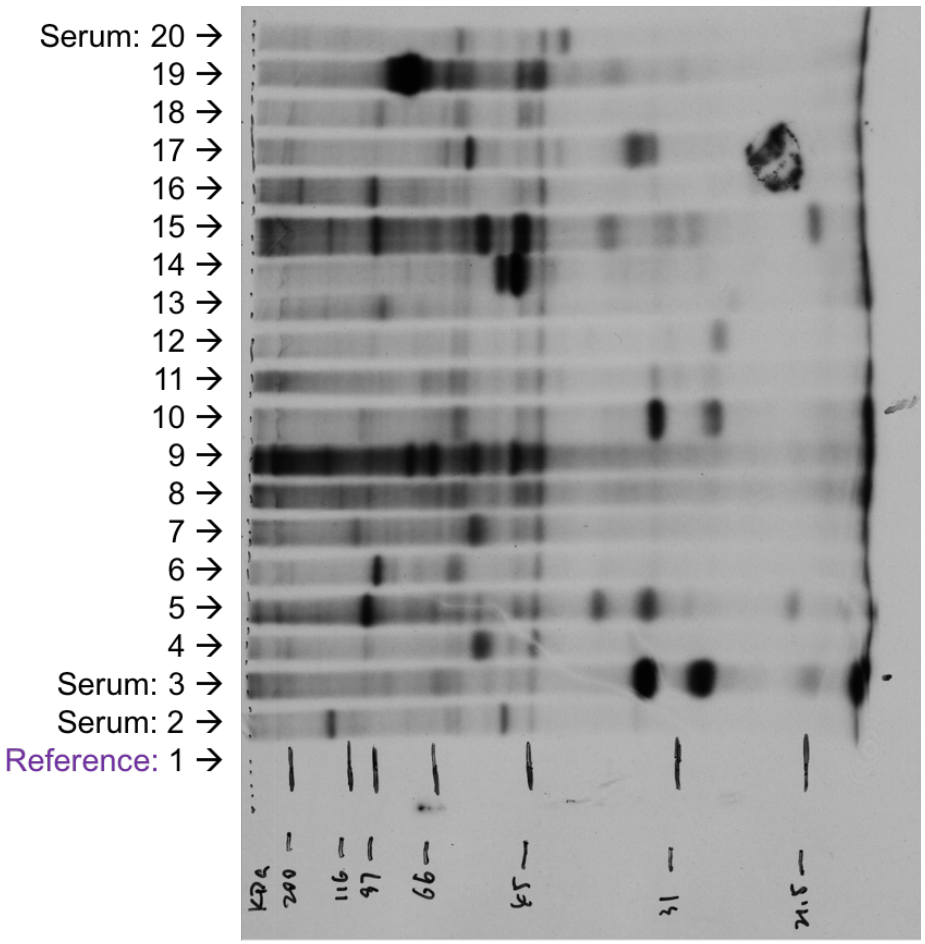

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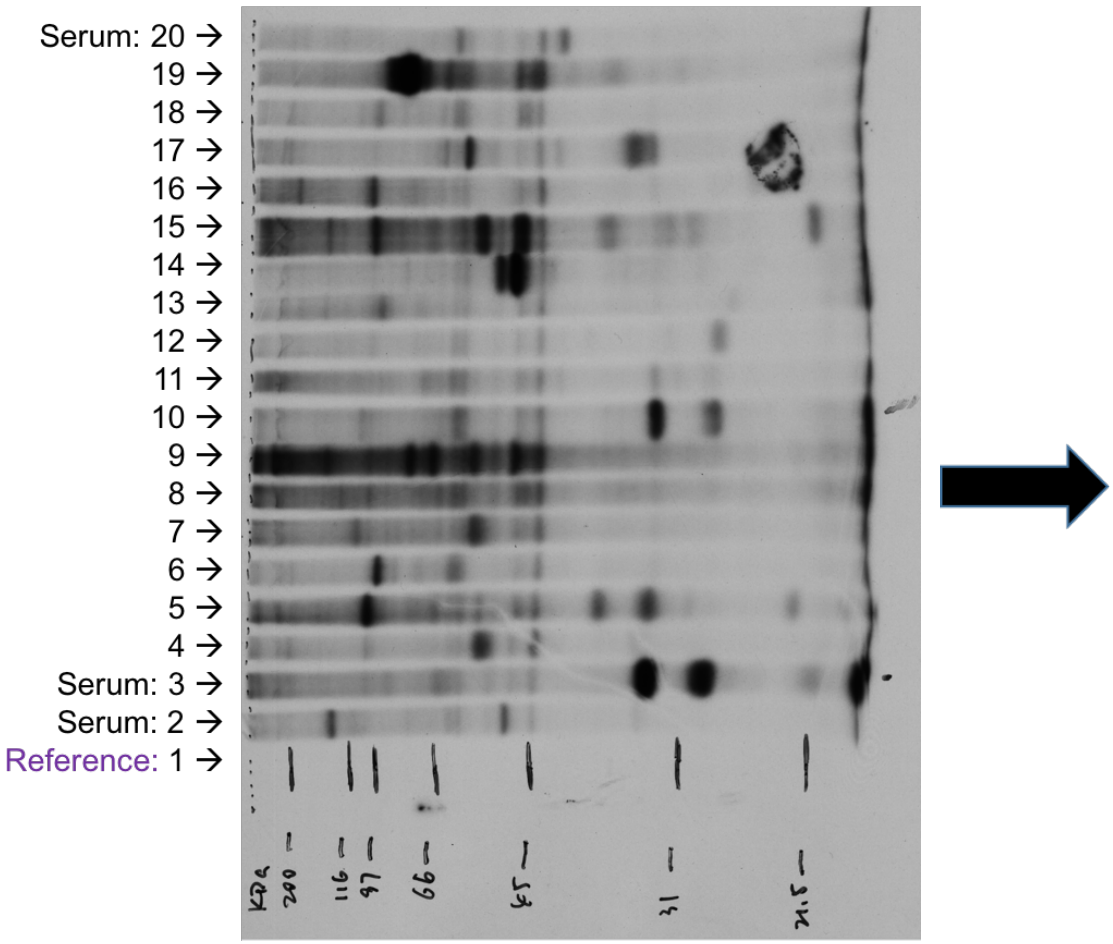

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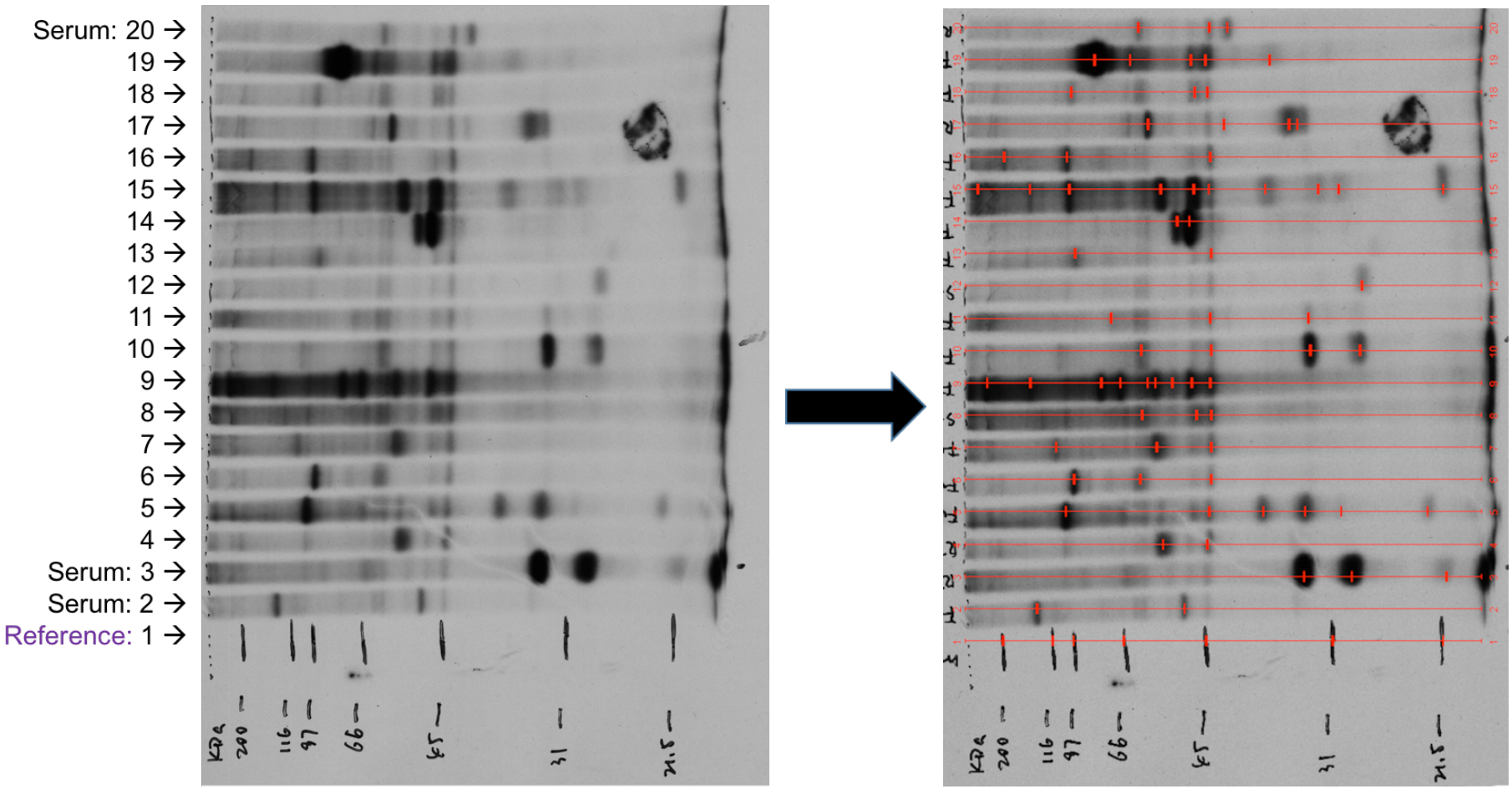
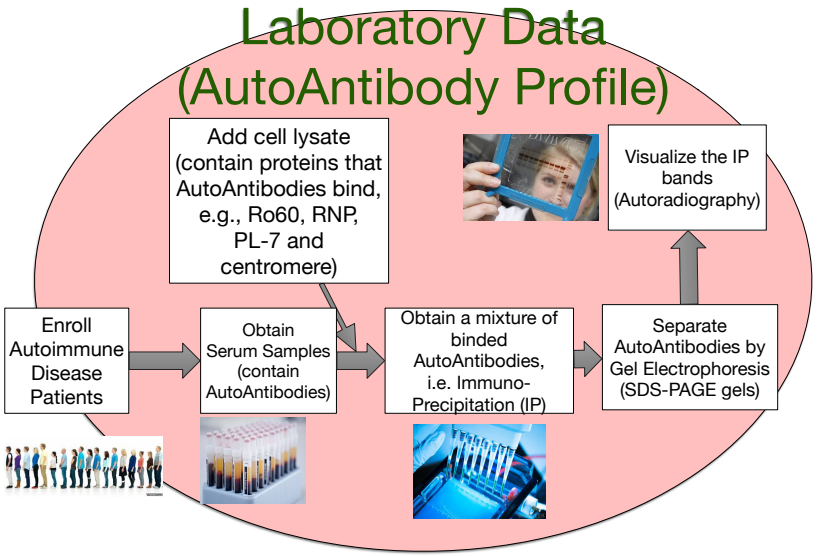
Hand-picked Bands "|"



Roadmap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Add cell lysate
(contain proteins that
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Visualize the IP
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(Autoradiography)
Enroll
Autoimmune
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Obtain
Serum Samples
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Obtain a mixture of
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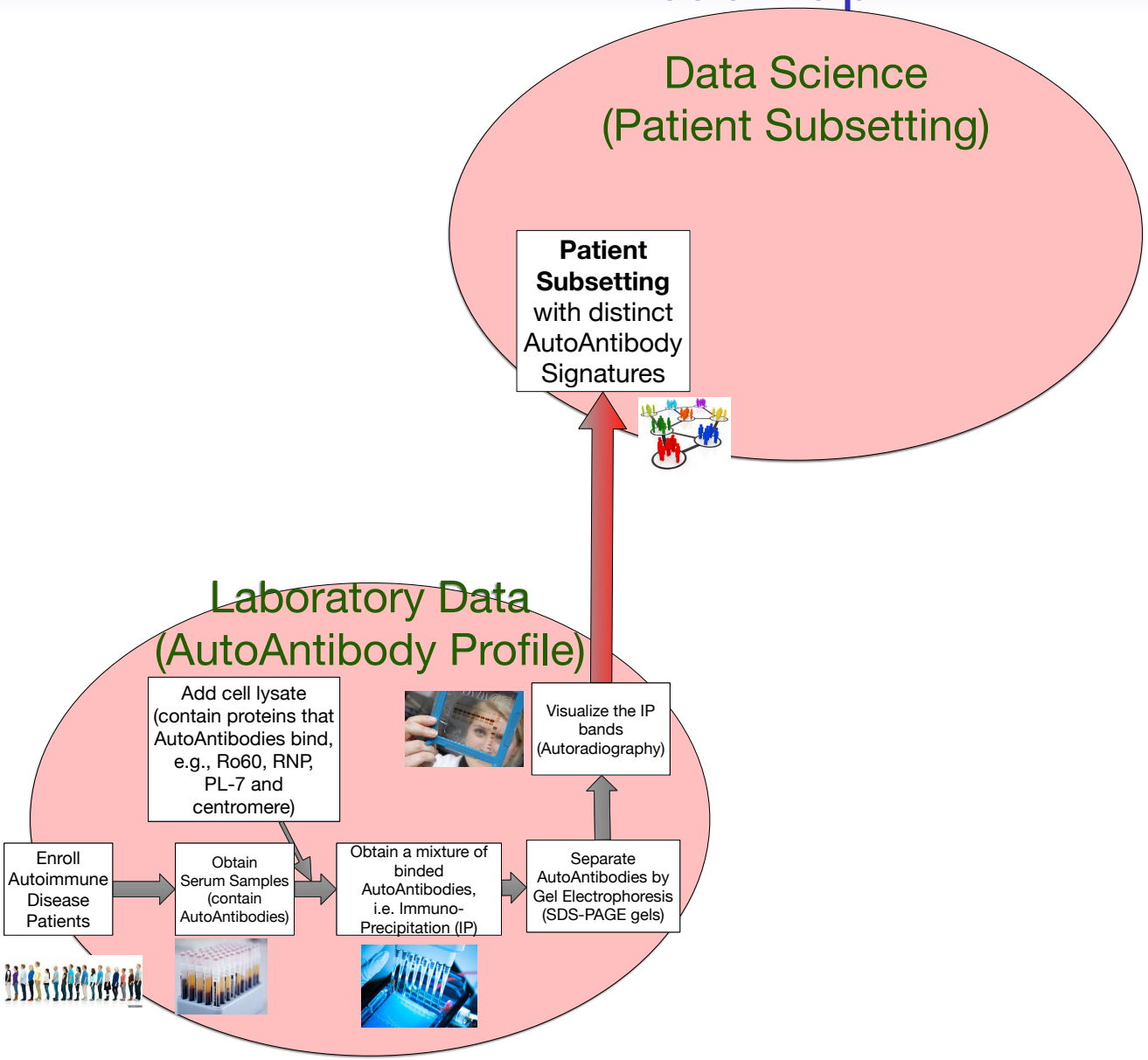
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Obtain Serum Samples (contain AutoAntibodies)
Obtain a mixture of binded AutoAntibodies, i.e. Immuno-Precipitation (IP)
Separate AutoAntibodies by Gel Electrophoresis (SDS-PAGE gels)

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2) Population Cancer Registry Data as reference (Surveillance, Epidemiology and End Results - SEER)

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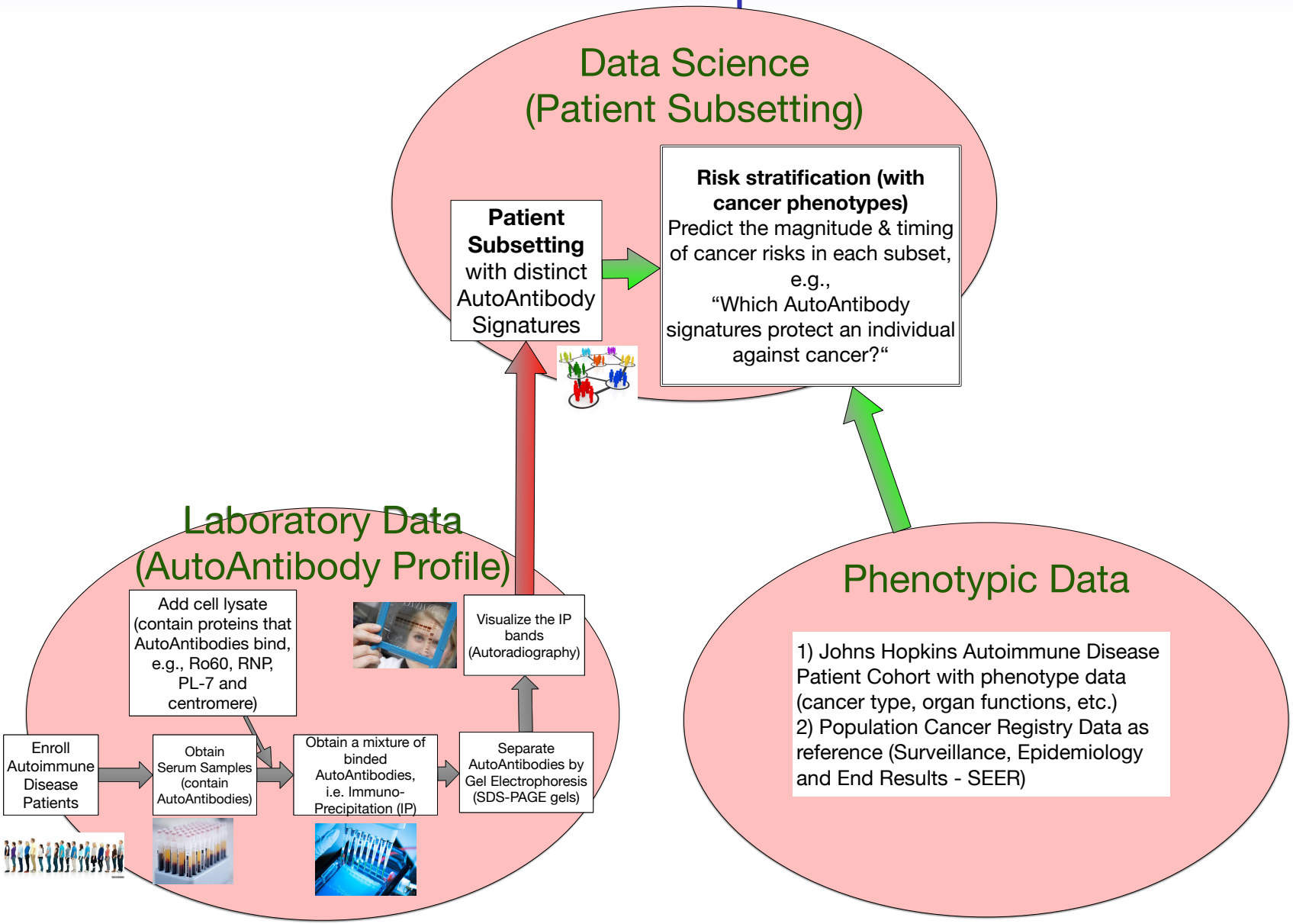
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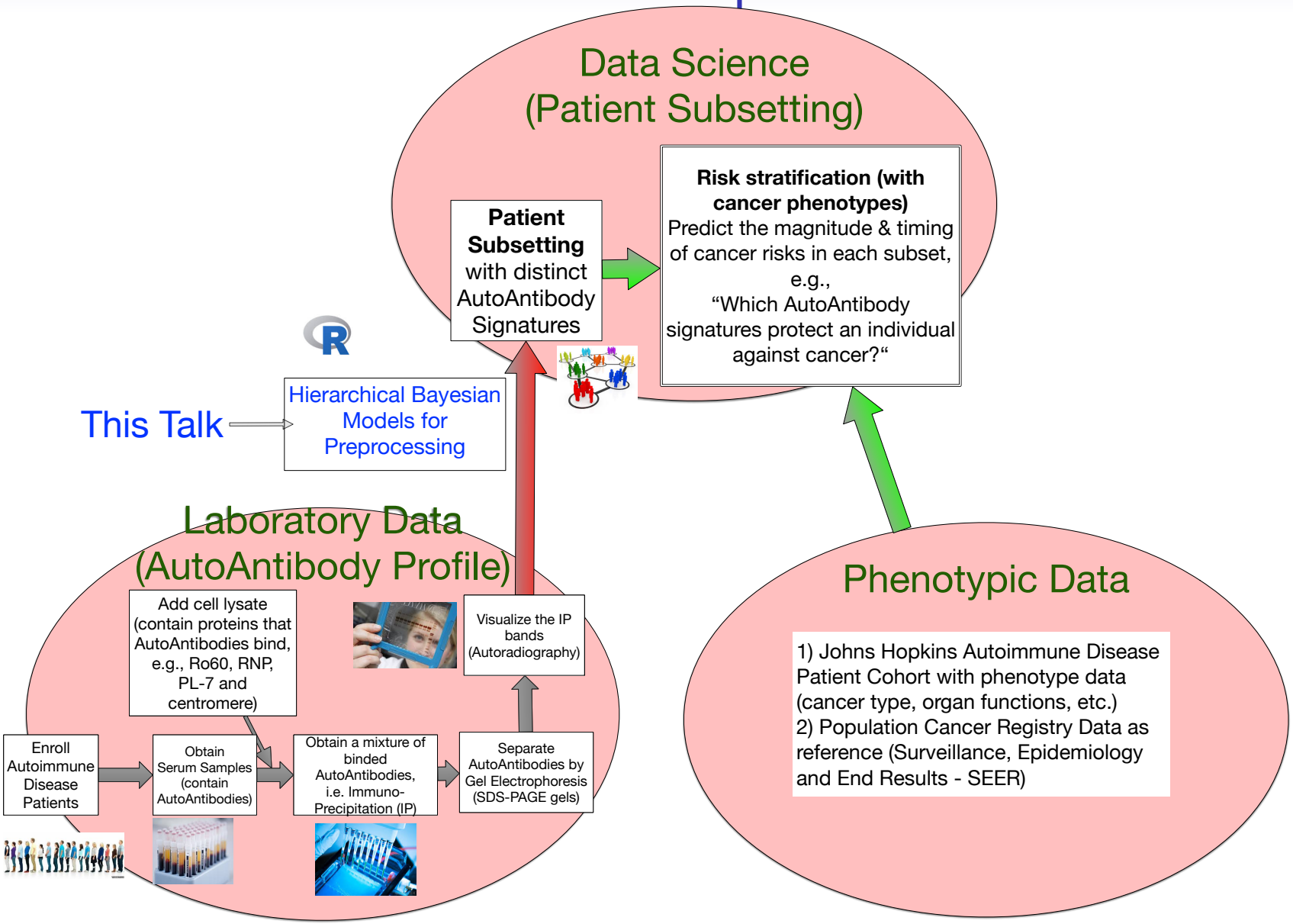
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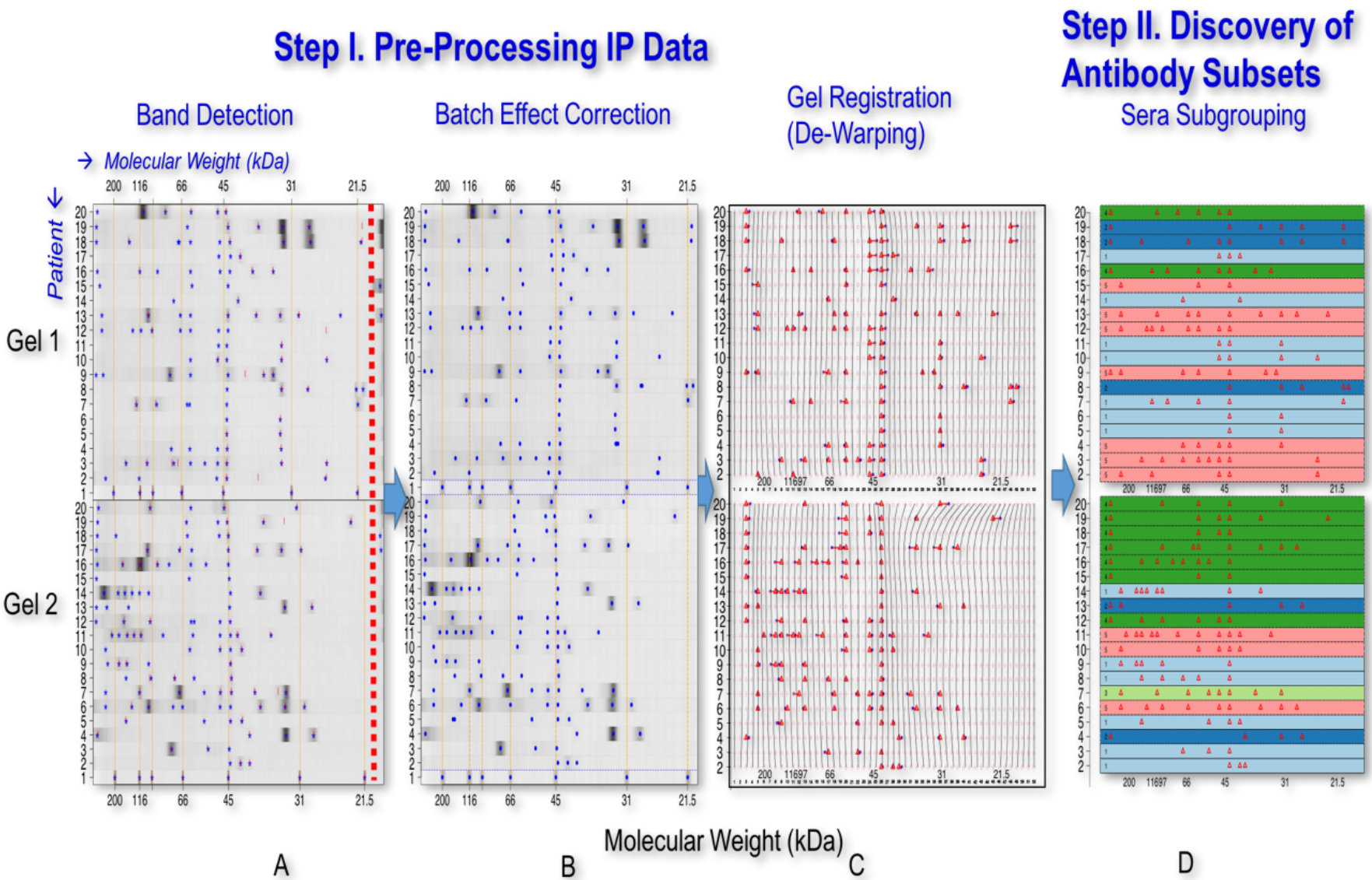
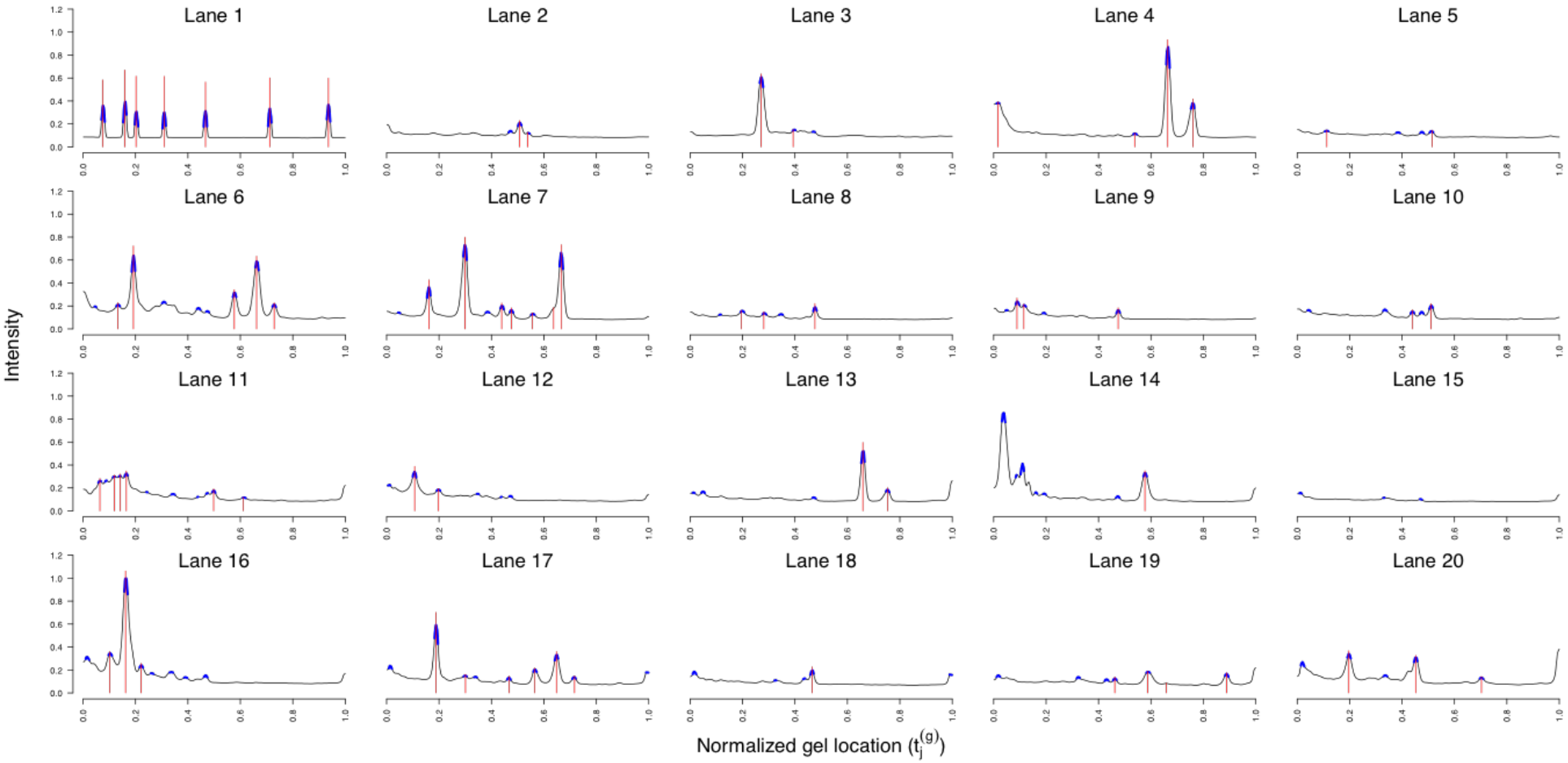
- Solution: Pre-filtering to define subgroups with similar specificities based on the bands observed by GEA

Automated Pipeline for Autoimmune Disease Subsetting

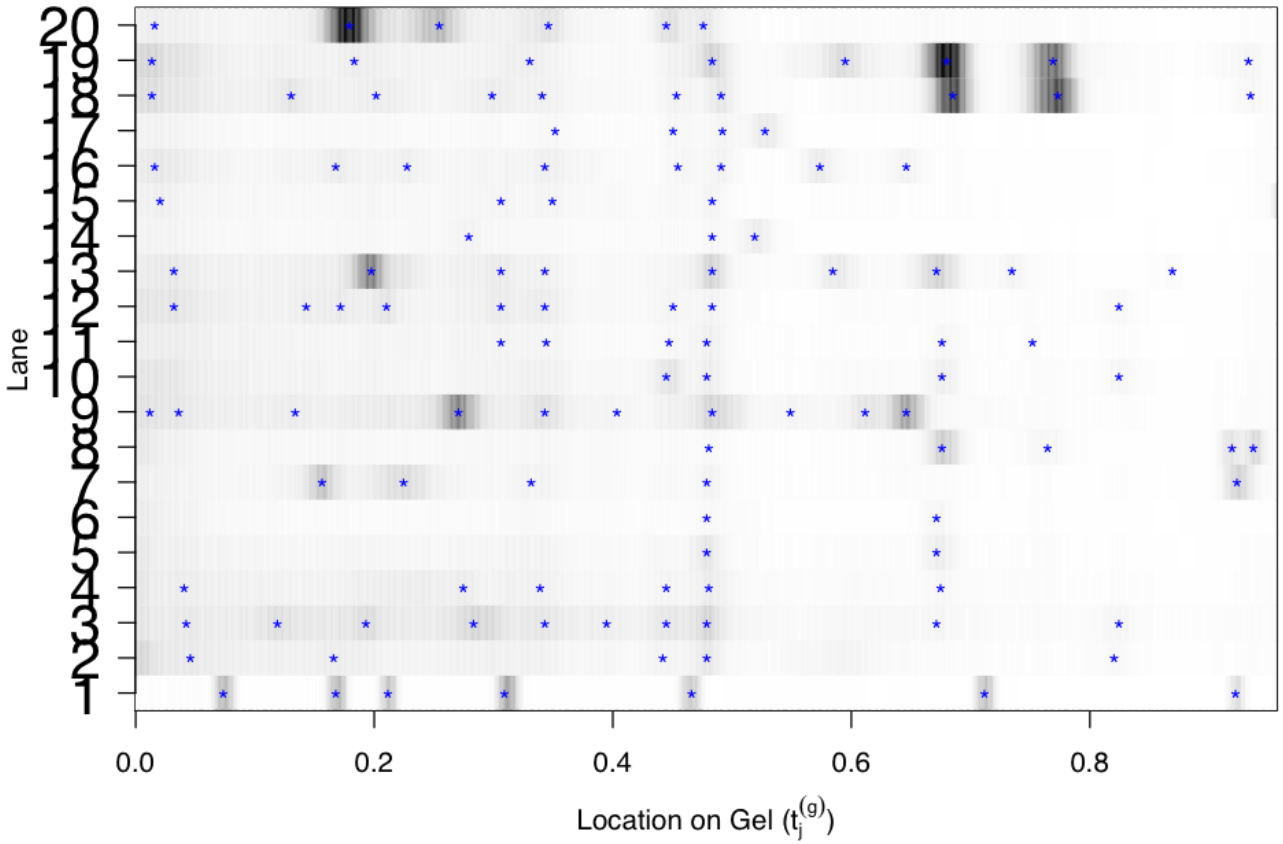


Step I-A: Automated Peak Detection



Step I-A: Automated Peak Detection

Overlaid against gel image; "$*$" for detected peaks



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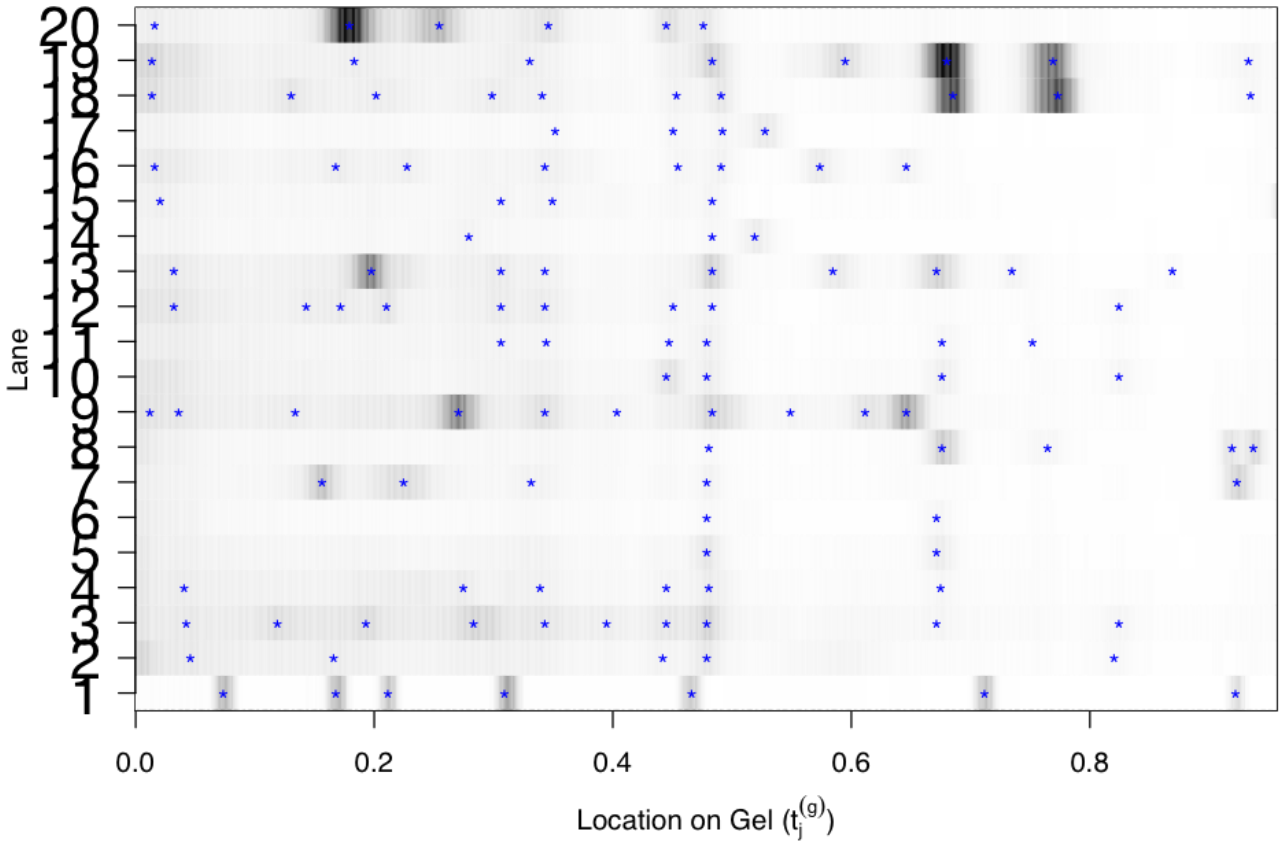
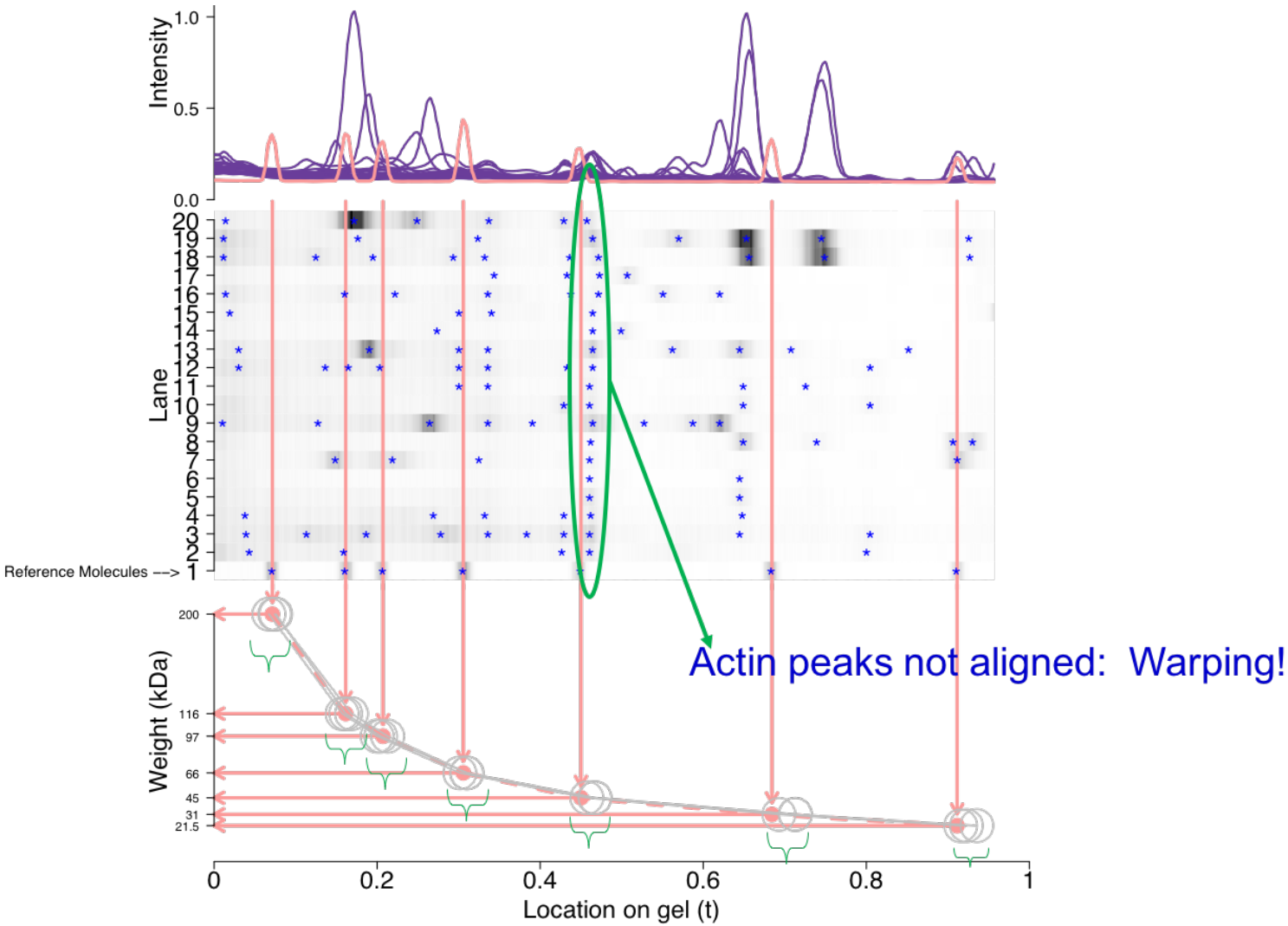


- u_{gi}: lane number for lane $i = 1, \ldots, N_g$, gel $g = 1, \ldots, G$
- T_{gij}: location for the j-th peak ("$*$"), $j = 1, \ldots, J_{gi}$, for lane i, gel g

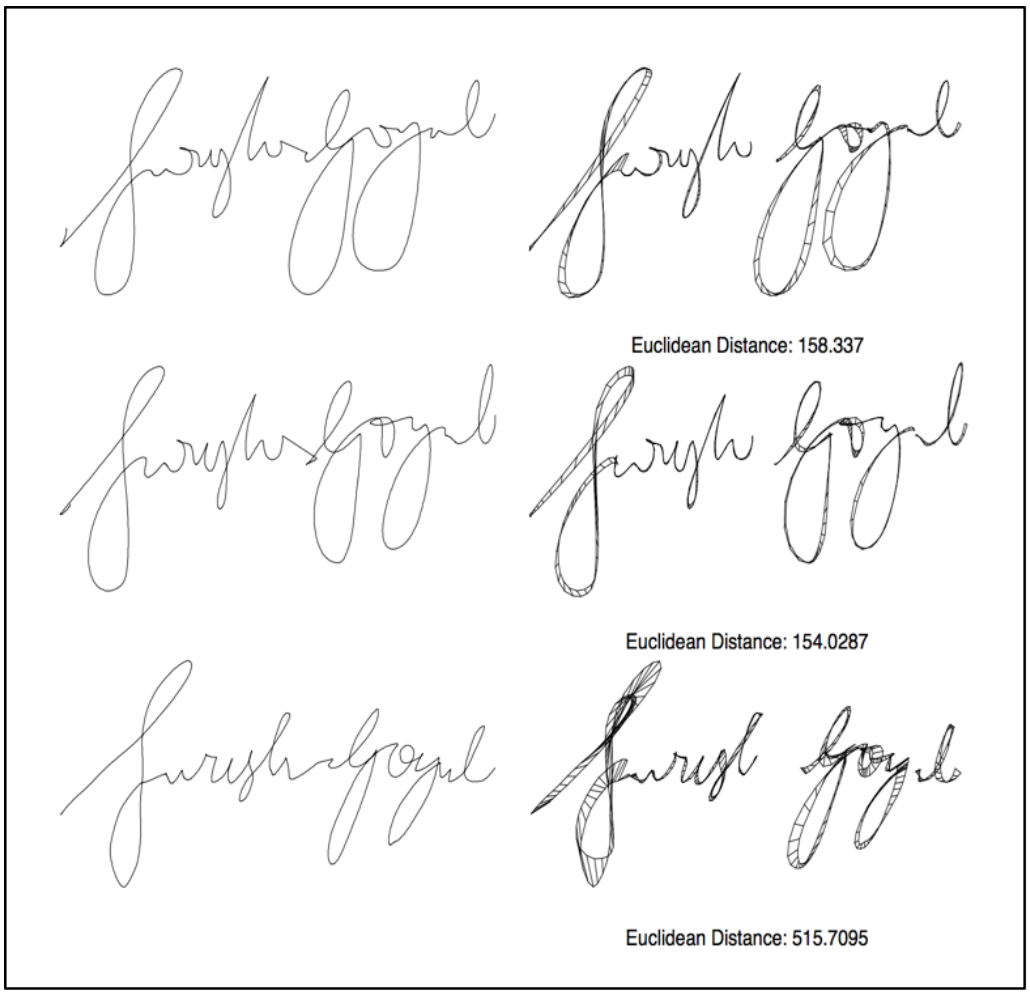
Step I-B: Batch Effect Correction

Must address before meaningful subgrouping

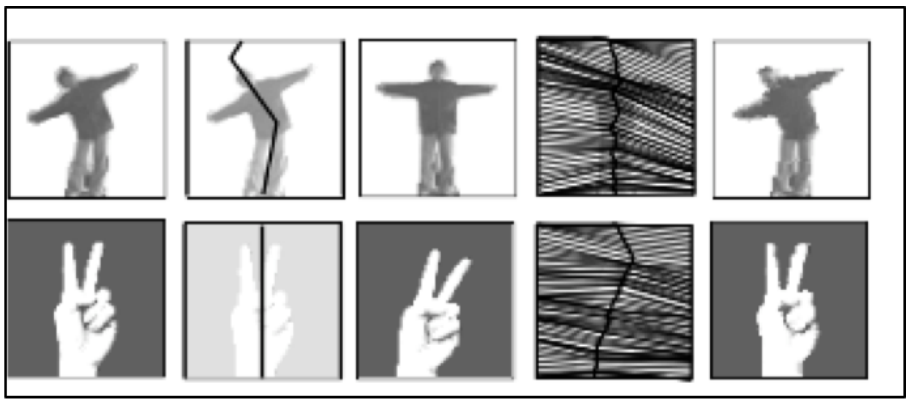


Reference proteins not aligned: Batch effects!

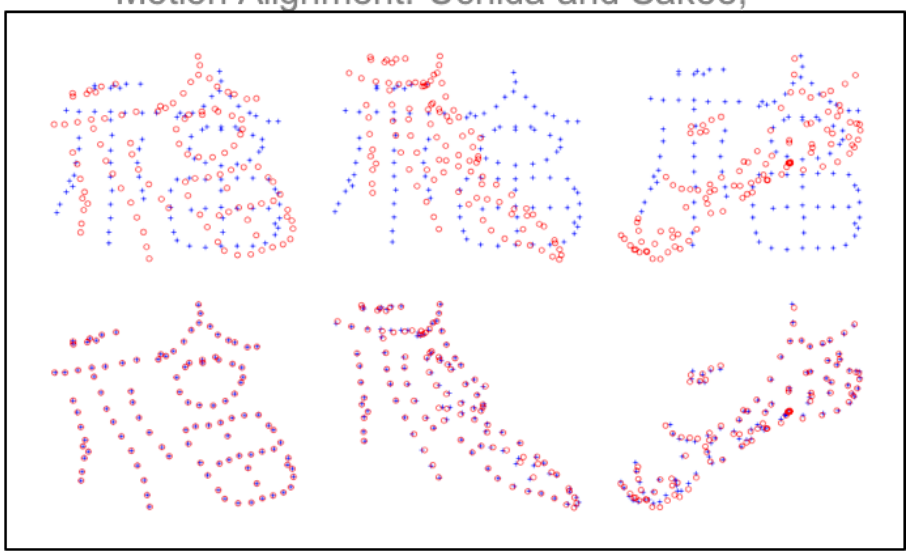
Warping Examples



Signature Verification: Hastie et al. 1991



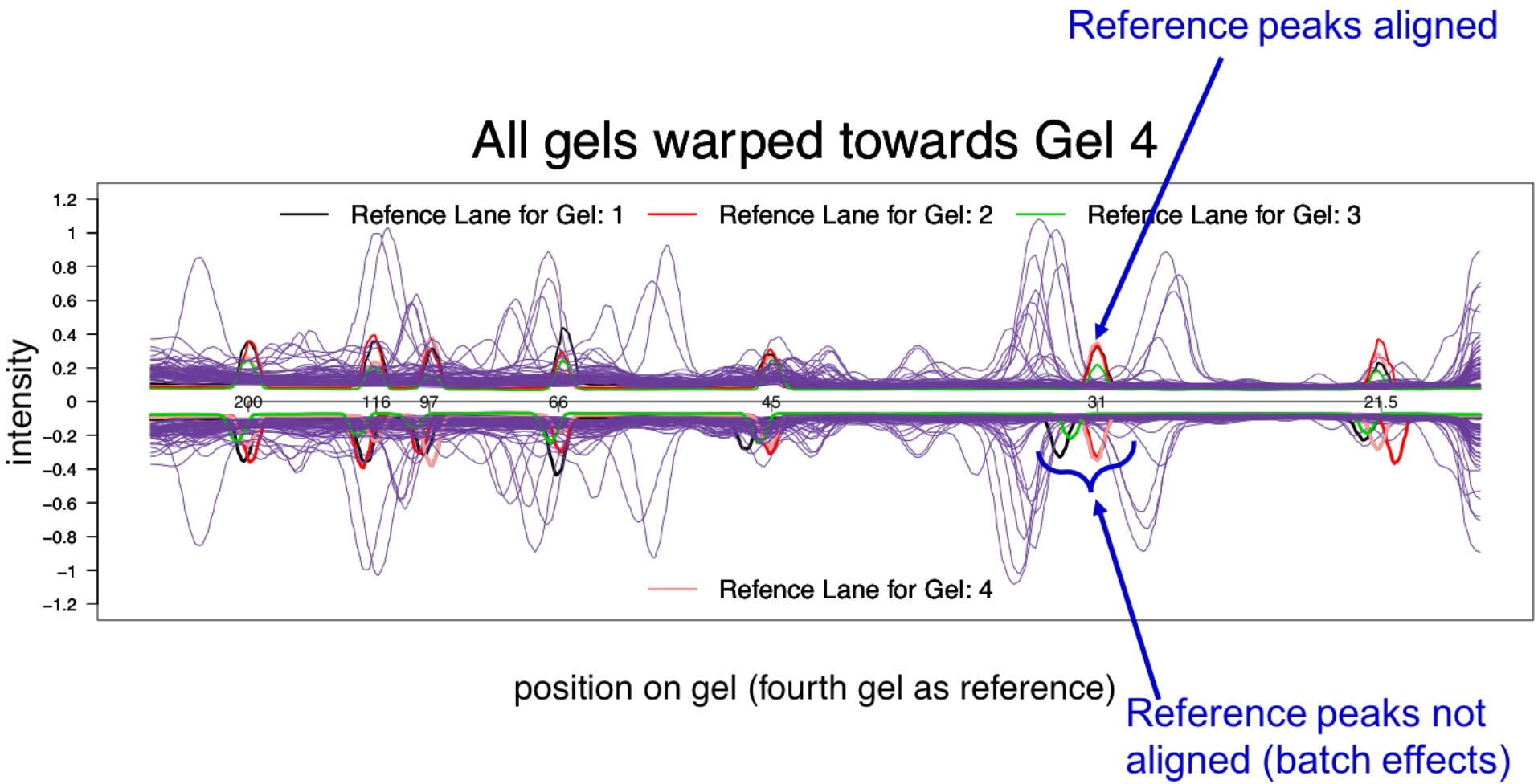
Motion Alignment: Uchida and Sakoe,



Handwritten Chinese: Ma and Zhao (2015)

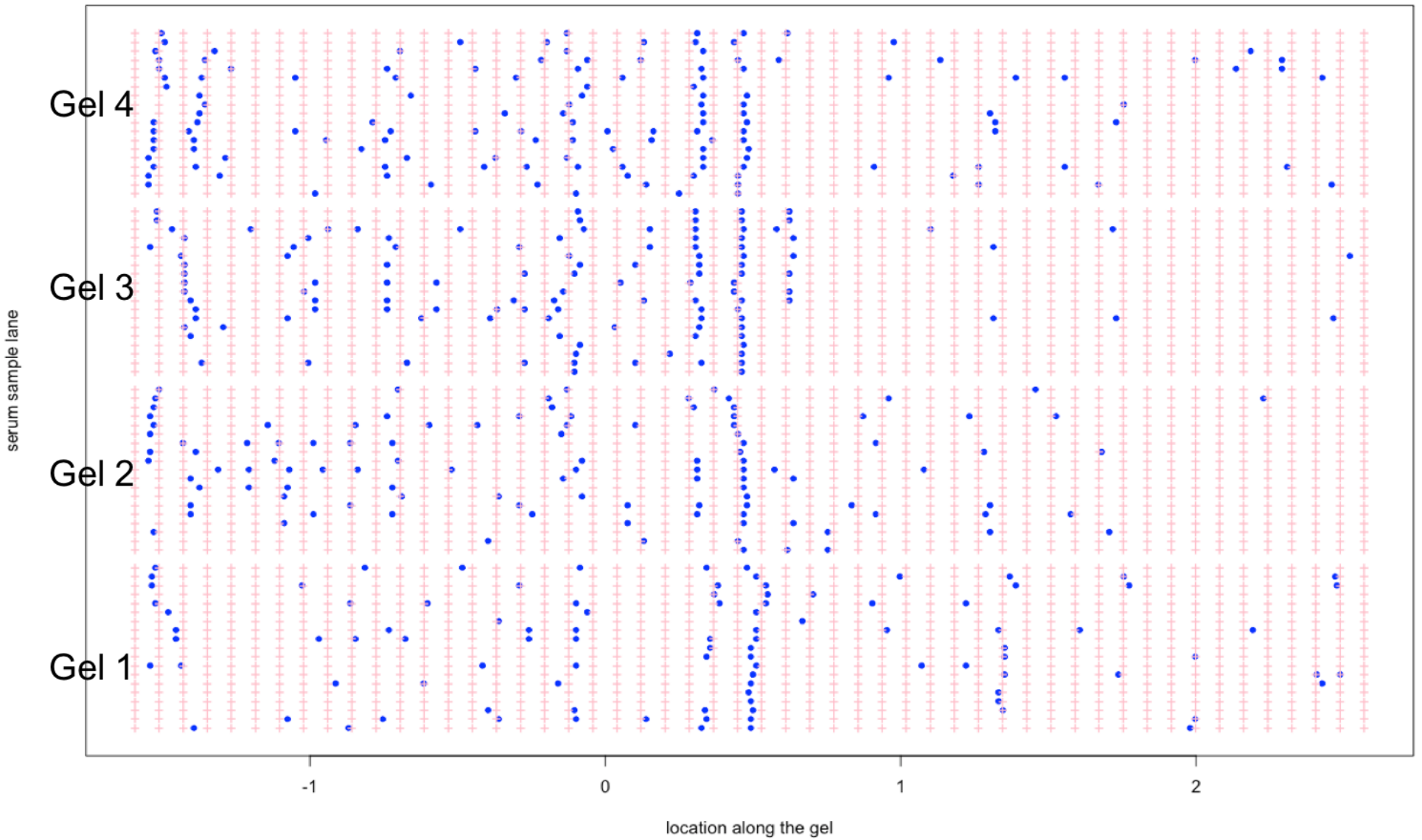
Step I-B: Batch Effect Correction

Piecewise Linear Warping by Reference Marker Molecules



Step I-C: Align the peaks

"Which "+" do the peaks "•" belong?"



Step I-C: Two-Dimensional De-Warping

- The physical process of autoradiography could cause image deformation
- Challenges
  - In general, few light-weight proteins on the right side of the image; If we don't see bands, how to align?
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  - The observed peak locations are noisy.
    Solution: Gaussian noise around the true location

Step I-C: Model for 2-Dimensional Image Dewarping

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  - Number of observed peaks in lane i, gel g:
    $J_{gi} \overset{d}{\sim}$ Poisson(Λ)
    Λ: Cumulative intensity; Controls the total number of peaks
  - Peak-to-landmark indicators:
    $(Z_{gi1}, \ldots, Z_{giJ_{gi}}) =$ increasing sort $\{Z^*_{gi1}, \ldots, Z^*_{giJ_{gi}}\}$
  - $Z^*_{gij} \overset{iid}{\sim}$ Categorical $\left(\{\lambda^*_\ell\}_{\ell=1}^{L}\right)$
    λ^*_ℓ: Landmark-specific intensity; Independent of g; Hence, when possible, encourages nearby peaks to be aligned to an identical landmark

Step I-C: 2-Dimensional Image Dewarping

Gaussian Mixture Model for Noisy Peak Locations "$*$"

- Model the observed peaks T_{gij} as observations from a L-component Gaussian mixture, for each candidate landmark ℓ

Step I-C: 2-Dimensional Image Dewarping

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$$p\left\{ (\underbrace{T_{gij}=t}_{\substack{\text{peak}\\ \text{location}}}, \underbrace{u_{gi}}_{\substack{\text{lane}\\ \text{number}}}) \mid \underbrace{Z_{gij}=\ell}_{\substack{\text{matched to}\\ \text{landmark } \ell}}, \underbrace{T_{gi,j-1}}_{\substack{\text{nearest left}\\ \text{peak location}}}, \underbrace{\mathcal{S}_g}_{\substack{\text{warping}\\ \text{function}}}, \underbrace{\sigma_\epsilon}_{\substack{\text{noise}\\ \text{level}}} \right\}$$

$$= \begin{cases} \phi\left(t; \mathcal{S}_g(\nu_\ell, u_{gi}), \sigma_\epsilon\right), & t \in \mathcal{I}_{gij}(\nu_\ell, A_0); \\ 0, & \text{otherwise}, \end{cases}$$

$\ell = 1, \ldots, L$, peak $j = 1, \ldots, J_{gi}$, lane $i = 1, \ldots, N_g$, gel $g = 1, \ldots, G$.

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- $\phi(\cdot; a, b)$: Gaussian density with mean a and standard deviation b.

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$\ell = 1, \ldots, L$, peak $j = 1, \ldots, J_{gi}$, lane $i = 1, \ldots, N_g$, gel $g = 1, \ldots, G$.

- $\mathcal{S}_g$: $(\nu_\ell, u_{gi}) \mapsto \mathcal{S}_g(\nu_\ell, u_i)$, *unknown*, smooth bivariate function for the spatial deformation

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$\ell = 1, \ldots, L$, peak $j = 1, \ldots, J_{gi}$, lane $i = 1, \ldots, N_g$, gel $g = 1, \ldots, G$.

- The set $\mathcal{I}_{gij}(\nu_\ell, A_0) \triangleq \{t : |t - \nu_\ell| < A_0 \text{ and } t > T_{gi,j-1}\}$ assumes a peak appears within distance A_0 from its true landmark

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Step I-C: 2-Dimensional Image Dewarping

Warping Function by Tensor Product Basis Expansion

- We assume the warping function

$$\mathcal{S}_g(\nu, u) = \sum_{s=1}^{T_\nu} \sum_{t=1}^{T_u} \beta_{gst} B_{g1s}(\nu) B_{g2t}(u),$$

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  - Boundary constraint: $\mathcal{S}_g(\nu_0, u) = \nu_0, \mathcal{S}_g(\nu_{L+1}, u) = \nu_{L+1}$

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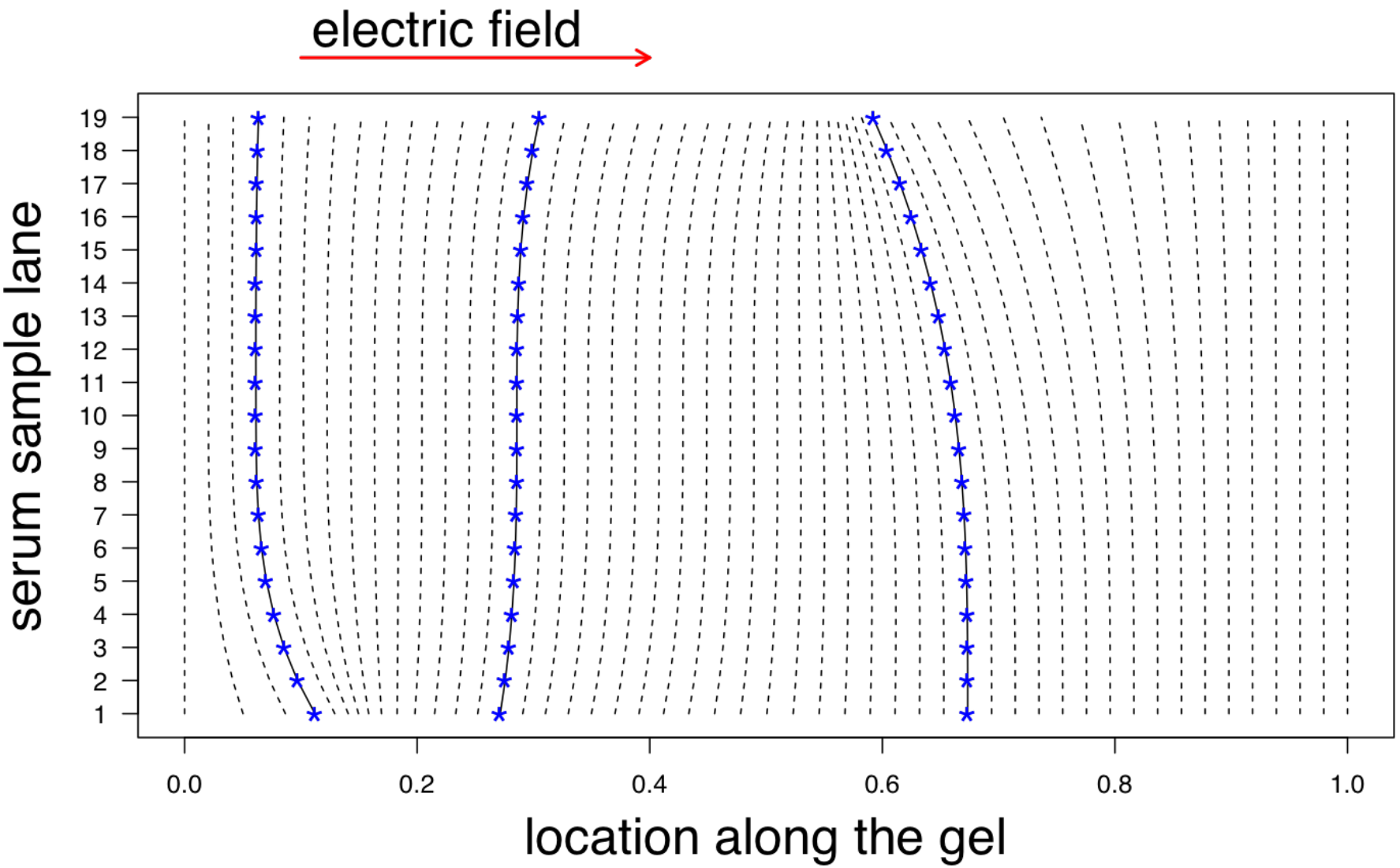
  - Both constraints above can be implemented via constraints on $\{\beta_{gst}\}$
  - Smoothness: Bayesian penalized-splines to make adjacent $\{\beta_{gst}\}$ similar
  - Vary by gel: $\mathcal{S}_g(\nu_\ell, u)$

Step I-C: A Mathematical Model for Warping

Estimate the warping, then reverse



Step I-C: Goal of 2-Dimensional Image De-warping

The posterior distribution $[\mathbf{Z} \mid \mathcal{P}]$

Recall:

- $\mathbf{Z}$: the collection of peak-to-landmark indicators

Step I-C: Goal of 2-Dimensional Image De-warping

The posterior distribution $[\mathbf{Z} \mid \mathcal{P}]$

Recall:

- $\mathbf{Z}$: the collection of peak-to-landmark indicators
- $\mathcal{P}$: the collection of all the observed peaks

Step I-C: Posterior Inference of the De-Warping

Joint distribution $[\mathcal{P}, \mathbf{Z}]$:

$$\prod_{g=1}^{G}\Bigg\{\underbrace{\prod_{i=1}^{N_g}\Big[\prod_{j=1}^{J_{gi}} N\left(T_{gij}; \boldsymbol{B}_{g1}(\nu_{Z_{gij}})'\boldsymbol{\beta}_g\boldsymbol{B}_{g2}(u_{gi}), \sigma_\epsilon^{-2}\right)\mathbf{1}\{T_{gij}\in\mathcal{I}_{gij}(\nu_{Z_{gij}}, A_0)\}}_{\text{likelihood (2.2)}}$$

$$\times\underbrace{J_{gi}!\prod_{j=1}^{J_{gi}}\text{Categorical}(Z_{gij};\boldsymbol{\lambda})\mathbf{1}\{Z_{gij}\le Z_{gi,j+1}, j=1,\ldots,J_{gi}-1\}\Big]}_{\text{prior of }\mathbf{Z}}$$

$$\times\underbrace{N_{T_\nu-1}\left(\{\beta_{gs1}\}_{s=1}^{T_\nu-1}; \boldsymbol{\beta}^{\text{id}}_{[-T_\nu]}, \sigma_{g1}^{-2}\Delta_1'\Delta_1\right)\mathbf{1}\{\nu_0=\beta_{g11}<\ldots<\beta_{gs1}<\ldots<\beta_{g,T_\nu-1,1}<\nu_{L+1}\}\cdot p(\sigma_{g1}^2)}_{\text{prior (2.6) and hyperprior of the smoothing parameter}}$$

$$\times\underbrace{\prod_{s=2}^{T_\nu-1}\left[N_{T_u}\left(\{\beta_{gst}\}_{t=1}^{T_u}; \mathbf{0}, \sigma_{gs}^{-2}\Delta_2'\Delta_2\right)\cdot p(\sigma_{gs}^2, \rho_g)\right]\Bigg\}}_{\text{prior (2.7) and hyperpriors of the smoothing parameters}}\times\underbrace{p(\boldsymbol{\lambda})}_{\text{hyperprior for }\mathbf{Z}}, \qquad (2.9)$$

- Goal: Joint distribution $[\mathcal{P}, \mathbf{Z}]$(data+unknowns) $\rightarrow$ Posterior distribution $[\mathbf{Z} \mid \mathcal{P}]$ (unknown given data)

Step I-C: Posterior Inference of the De-Warping

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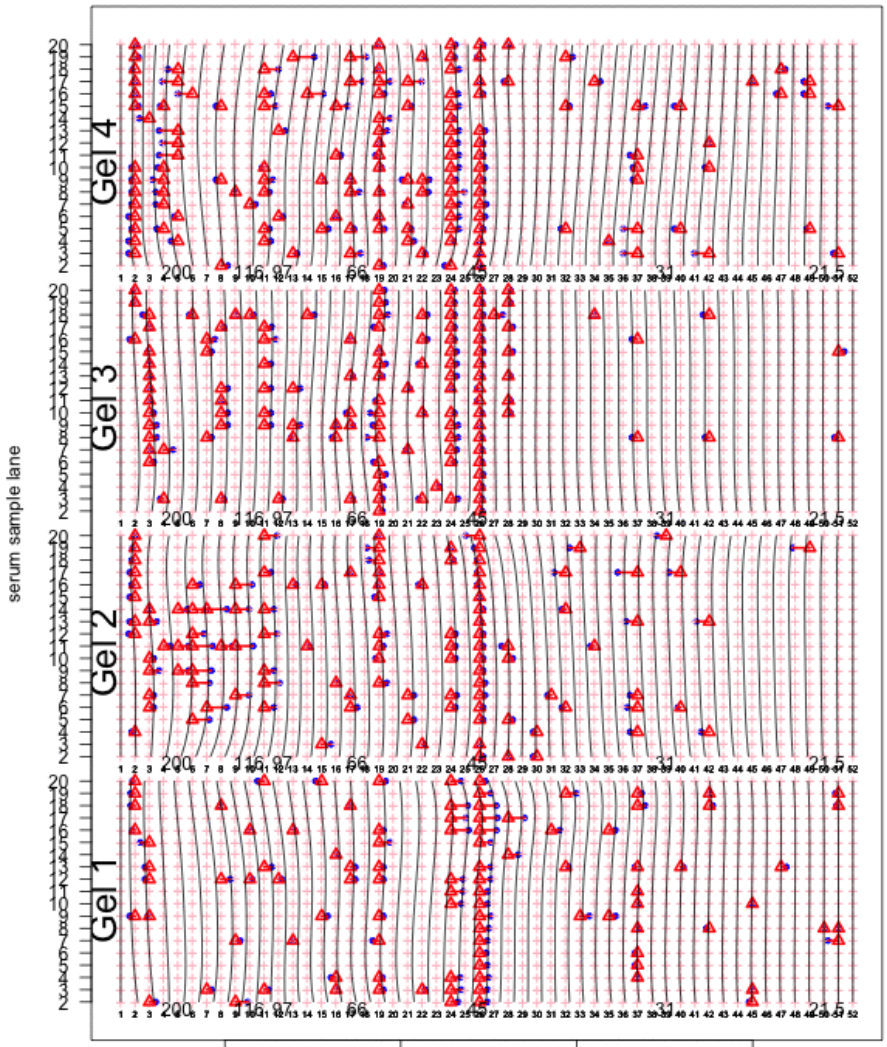
- Tool: Markov chain Monte Carlo (MCMC)
- Idea: Simulate samples from the joint posterior distribution of the unknowns given the data; Then use the samples to do posterior inference for any functions of the unknowns

Step I-C: Align the peaks – Result

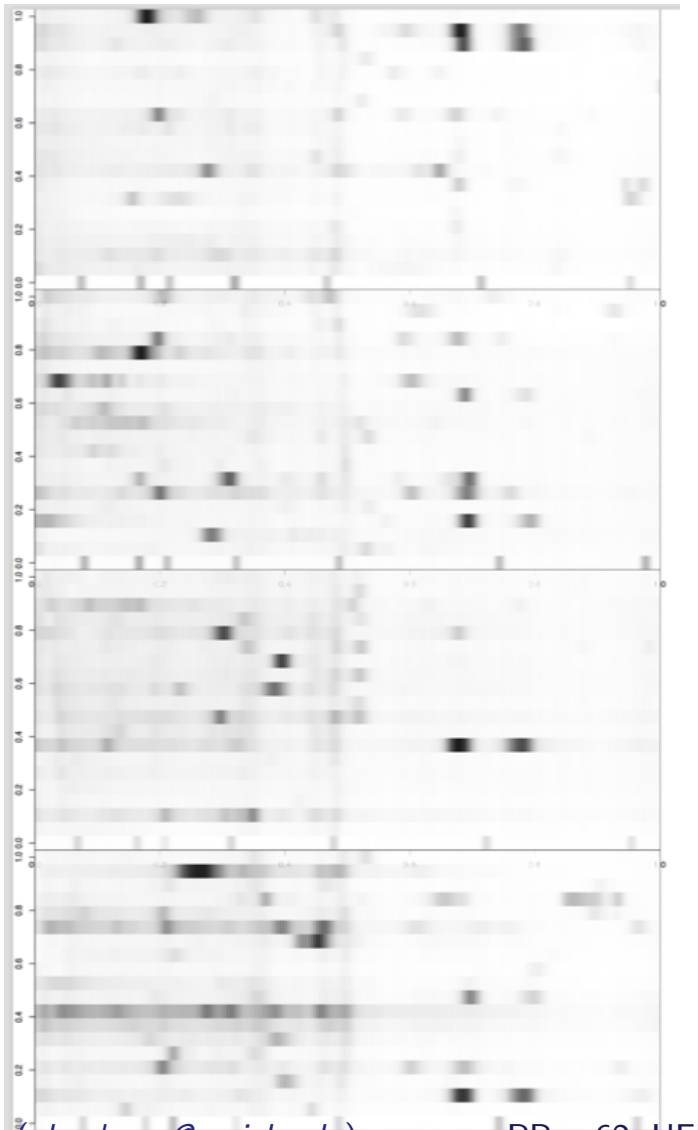
Animation; "Δ" for signature; "•" for the observed peaks

(Please Click the Image for Animation)



Step I-C: Aligned High-Frequency Intensity Data

Before



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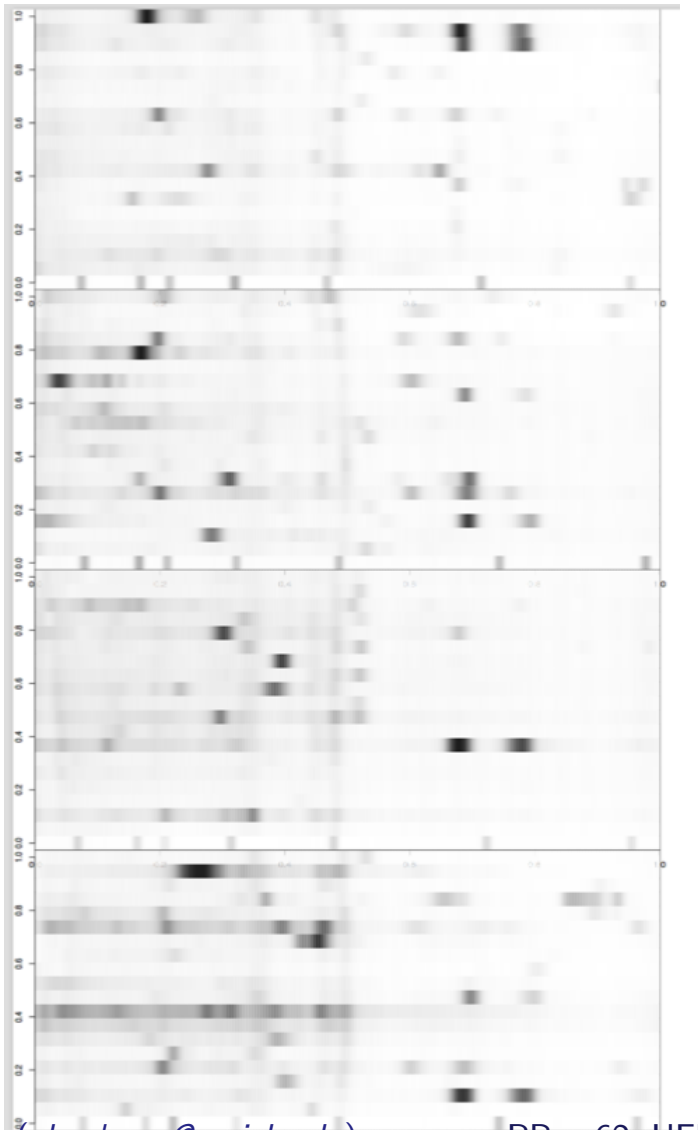
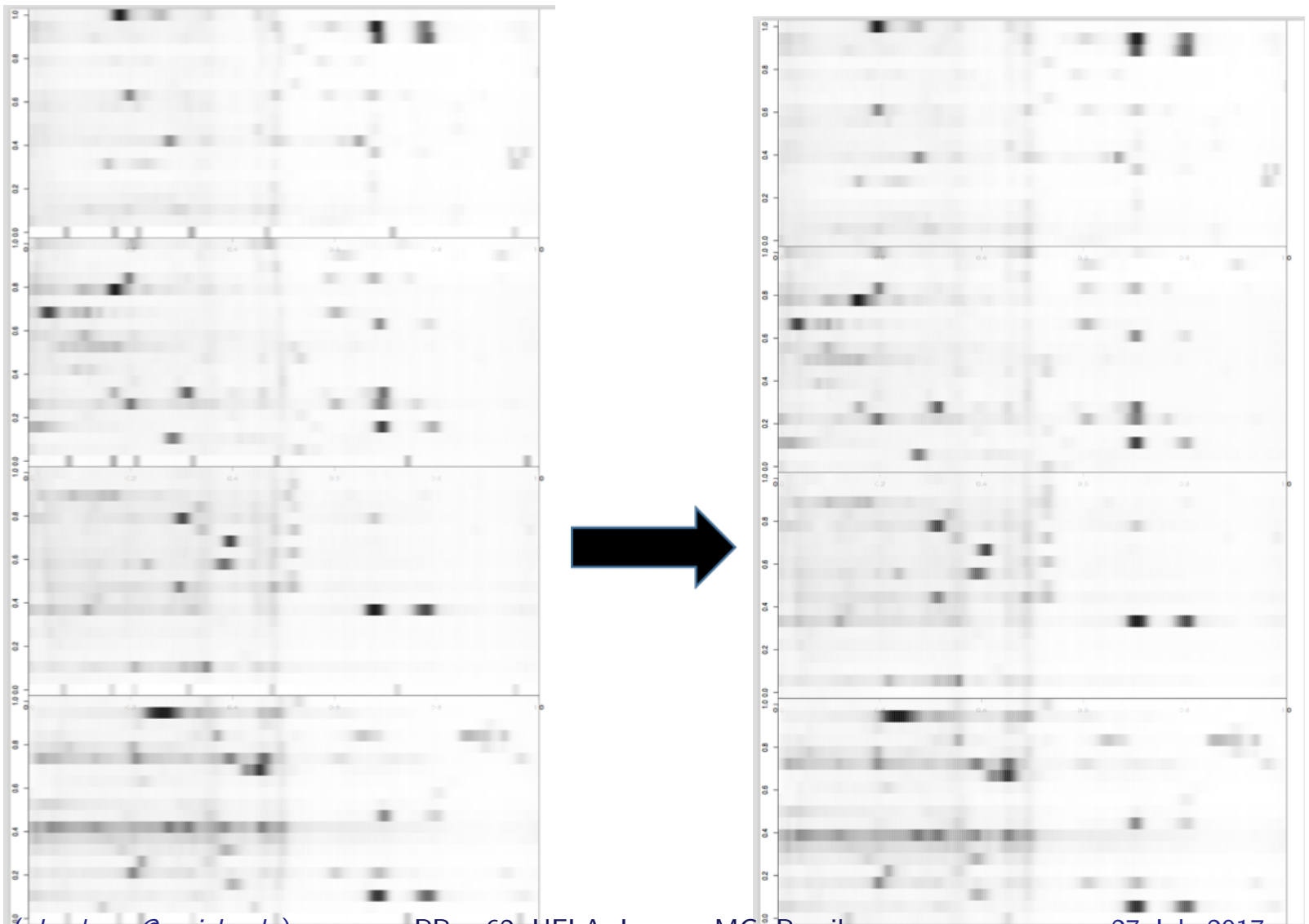
Before



Step I-C: Aligned High-Frequency Intensity Data

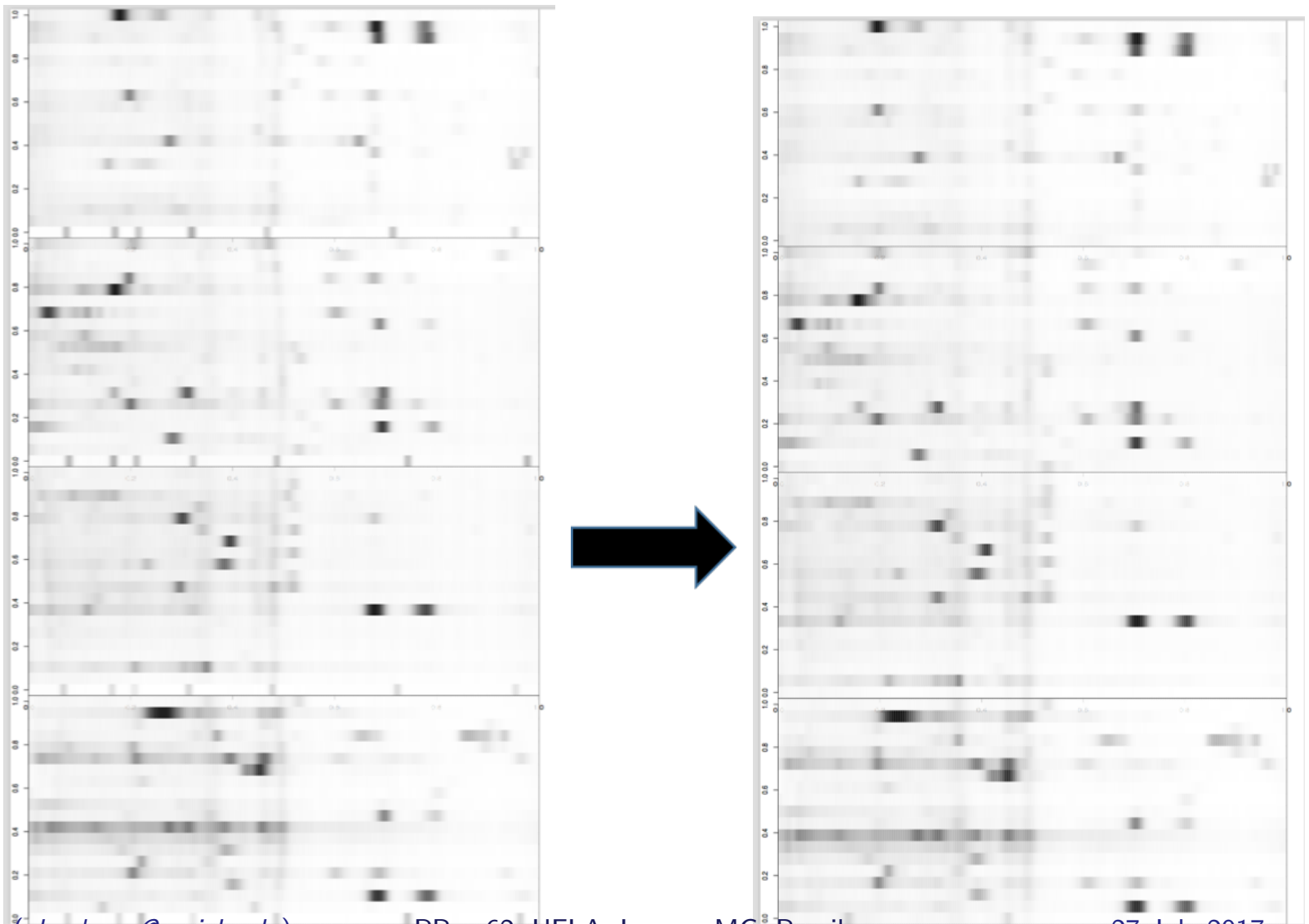
Before

After



Step I-C: Aligned High-Frequency Intensity Data

Before → note the curvatures are removed → After



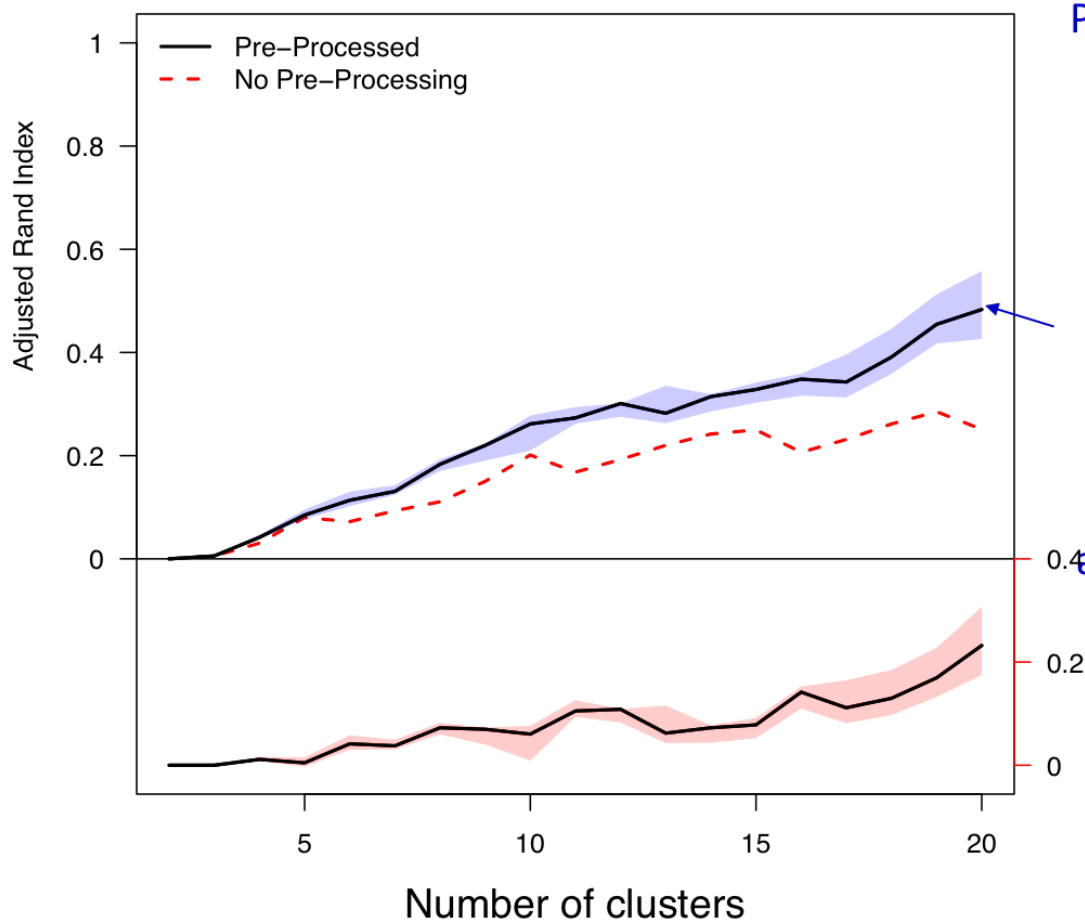
Data

Scleroderma

- Long-term clinical objective: find autoantibody signature that subsets autoimmune disease patients into groups with more homogeneous phenotypes and trajectories
- Sera from well-characterized patients with scleroderma and an associated cancer from Johns Hopkins Scleroderma Center database
- Data
  1. Known clustering: two replicate GEA experiments on 20 samples
  2. Unknown clustering: non-replicate GEA experiment on 80 samples
- Steps:
  1. Pre-processing
  2. Clustering (into 2, 3, . . .,N groups) based on the pre-processed high-frequency intensity data (hierarchical clustering here)
  3. Evaluate the separation of the obtained clusters and compare them to the truth (known in the replicate experiment)

Pre-processing Improves the Accuracy of Cluster Estimation

Data with technical replicates; 20 samples, long- and short- exposures



Posterior mean of adjusted Rand index (95% credible interval)

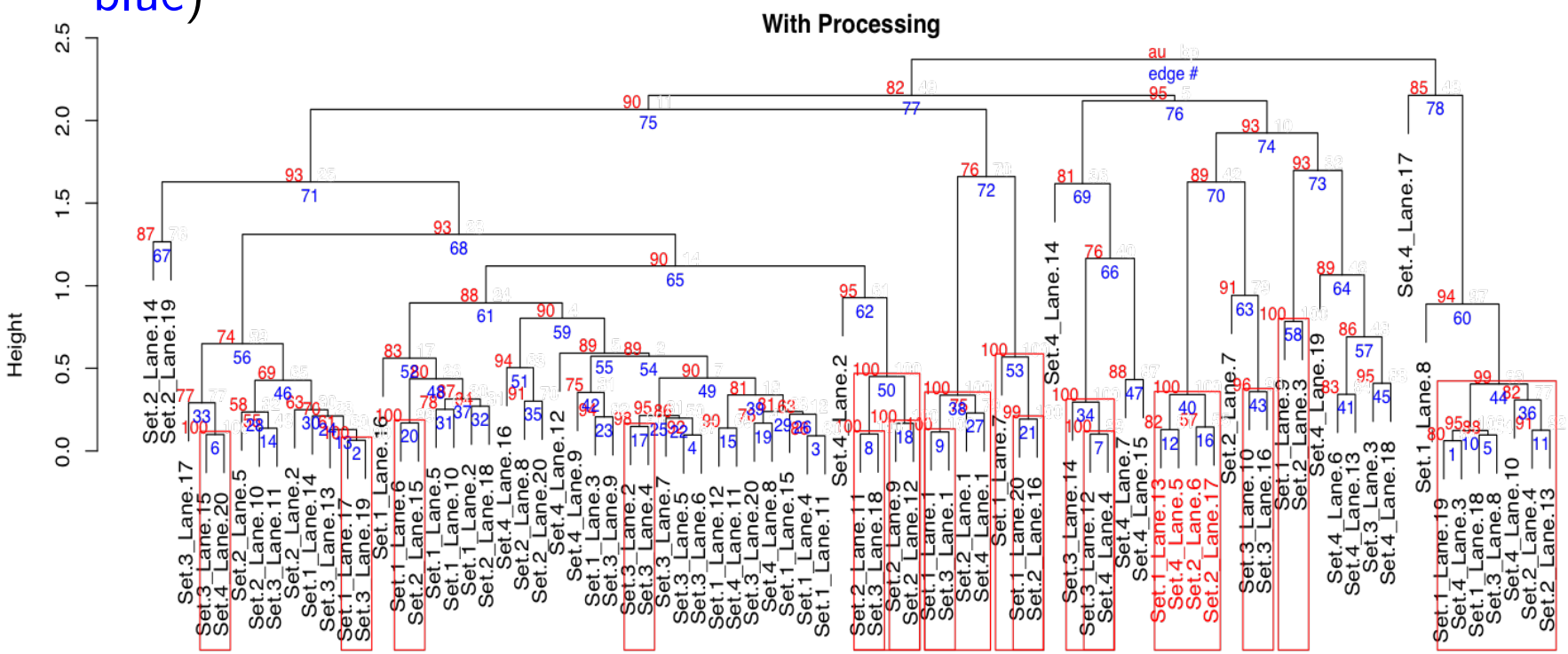
Recall that we obtain the posterior distribution of alignment $[\mathbf{Z} \mid P]$

* Adjusted Rand index: assess the similarity of two ways of clustering the same set of observations; the higher the better

Pre-processing Improves the Separation of Clusters

Data without Replicates; Hierarchical Clustering; Pre-processed vs Non-Pre-processed

- Distance: Correlation-based distance; complete linkage
- Interpretation: adjacent terminal nodes in the tree $\rightarrow$ similar in AutoAntibody signatures
- Uncertainty: confidence levels by multiscale boostrapping (red numbers; ones > 95 are shown in red boxes; a numbering of the subtrees is shown in blue)



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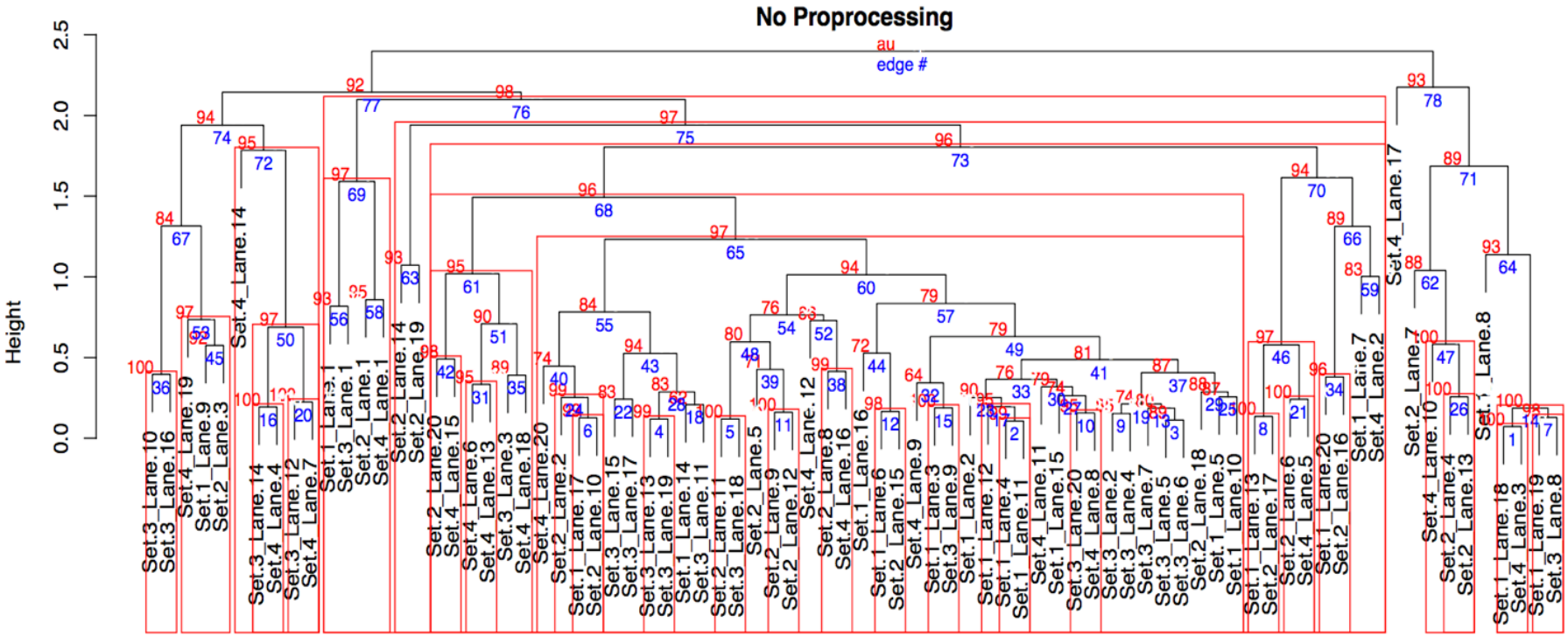
- Interpretation: adjacent terminal nodes in the tree $\rightarrow$ similar in AutoAntibody signatures
- Uncertainty: confidence levels by multiscale boostrapping (red numbers; ones > 95 are shown in red boxes; a numbering of the subtrees is shown in blue)



Summary

- Problem: Human recognition of autoantibody patterns and hence clustering becomes more difficult when patterns are composite and on multiple gels
- Method: Novel automated algorithms that
  1. Estimate autoantibody signatures
  2. The pre-processed data (Step I) can be the input of many subgroup discovery methods (Step II) including hierarchical clustering, latent class models and factor analyses
  3. Improves the accuracy of subgroup discovery
- Free publicly available open-source software: `https://github.com/zhenkewu/spotgear`
- Manuscript: Wu, Casciola-Rosen, Shah, Rosen, Zeger (2017). `http://biorxiv.org/content/early/2017/04/21/128199`
- Ongoing work: novel Bayesian clustering model to find disease subsets; Based on the biology that autoantibodies recognize protein complexes.

Thank You!

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Some References (More at: `zhenkewu.com`)

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