Statistical Challenges in Proteomics

Making Sense of Two-Dimensional Electrophoretic Data

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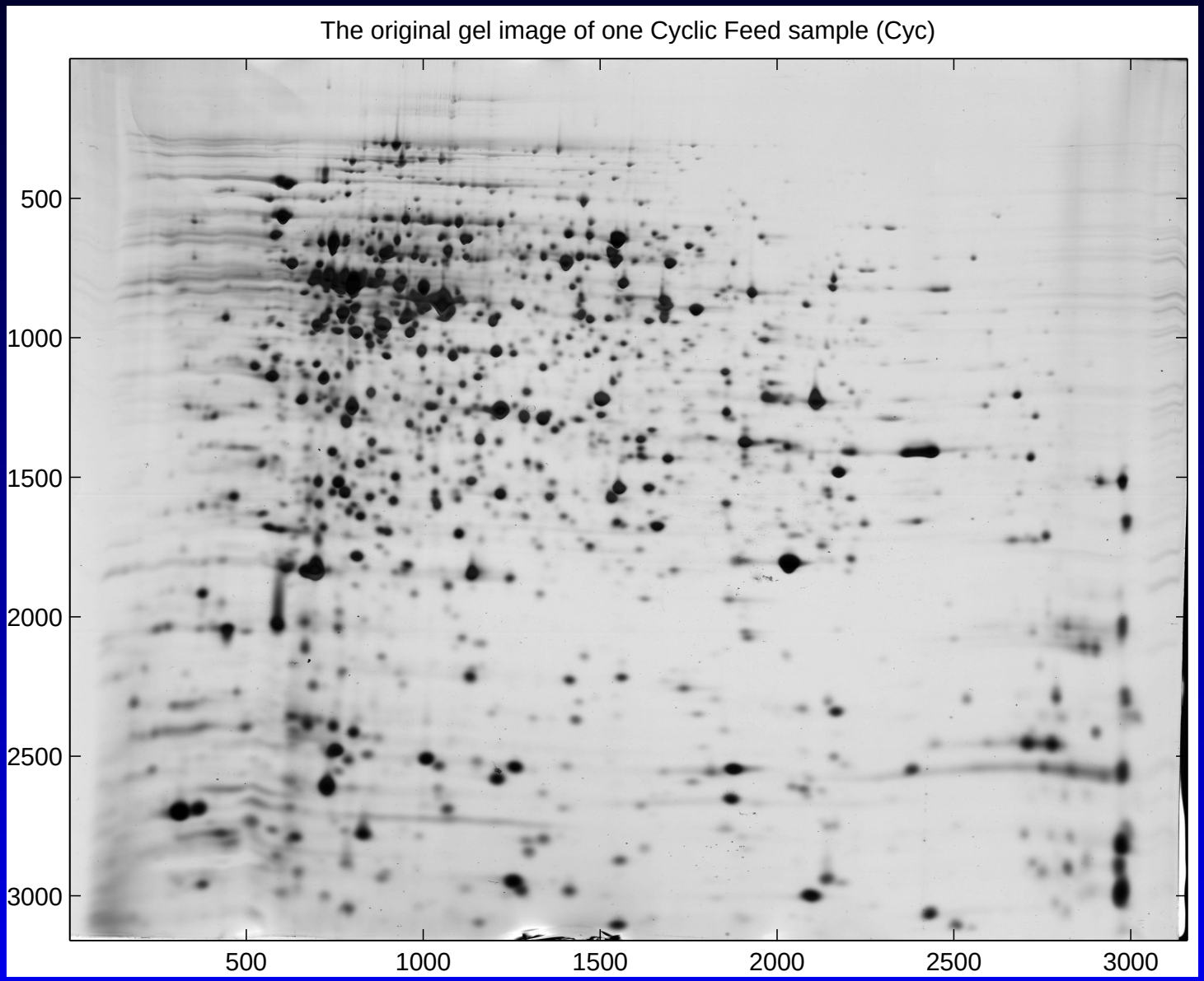
The original gel image of one Cyclic Feed sample (Cyc)
500
1000
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Outline

- Description of the Technology
  - 2-D polyacrylamide gel electrophoresis
    - separate thousands of proteins
    - (> 2,000 for mammalian cell sample)

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  - feature selection

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  - noise reduction
  - spot identification
  - feature selection
- Statistical Approaches
  - spike removal
  - streak removal
  - gel alignment
  - modeling

The Technology

First dimension isoelectric focusing

- proteins focused electrophoretically in pH gradient
- stop moving when at position with no net charge
  isoelectric point

Second dimension molecular mass

- proteins coated with sodium dodecylsulphate (SDS)
  $\longrightarrow$ same charge density
- separated orthogonally by electrophoresis on
  polyacrylamide gel

independent dimensions

The Technology (ctd)

separated proteins stained with fluorescent dyes

image of displayed proteins = proteome

digital scanning into database

Some Challenges

- gel reproducibility
  same quality from day to day and from lab to lab
- losses due to hydrophobic interactions between some proteins and gel
- removal of nucleic acids
  streaks, artifactual migration
- low abundance protein
  copy number for detection on gel?

Analytical Methods

implemented in MELANIE package

- Spot detection

  non-parametric method based on Laplacian and second derivatives

  $I(x, y)$ = 2-D image

  $\mathbf{p} = (x, y)$ = point on image

  S_i = spot

  T = saturation threshold

  $\Delta I(\mathbf{p})$ = Laplacian

$$= -\left(\frac{\partial^2}{\partial x^2} I(\mathbf{p}) + \frac{\partial^2}{\partial y^2} I(\mathbf{p})\right)$$

Analytical Methods (ctd)

Is **p** part of a spot?
l, r, c small positive thresholds

- $I(\mathbf{p}) < T$

$$\mathbf{p} \in S_i \iff \min\left(\frac{\partial^2}{\partial x^2} I(\mathbf{p}) - r, \frac{\partial^2}{\partial y^2} I(\mathbf{p}) - c\right) > 0$$

when $-\Delta I(\mathbf{p}) - l \geq 0$

- $I(\mathbf{p}) > T$

$$\mathbf{p} \in S_i \iff \min\left(\frac{\partial^2}{\partial x^2} I(\mathbf{p}), \frac{\partial^2}{\partial y^2} I(\mathbf{p})\right) > 0$$

Analytical Methods (ctd)

- Spot quantification

Direct method

spot area = number of pixels $\times$ pixel area

spot optical density = $\max_{x,y \in \text{spot}} I(x,y)$

spot volume = $\sum_{x,y \in \text{spot}} I(x,y)$

Gaussian curve fitting

Analytical Methods (ctd)

- Image alignment

  polynomial image warping

$$(x, y) \rightarrow (u(x), v(y))$$

  (x, y) = pixel coordinates in original image
  (u, v) = pixel coordinates in warped image
  = 1st-, 2nd- or 3rd-order polynomials estimated via least-squares criterion
  – select landmarks on each image
  – choose one gel as reference
  – estimate parameters by summing over landmarks

Analytical Methods (ctd)

- Spot matching
  - for each spot, select cluster of neighbor spots
    central spot = primary spot
    surrounding spots = secondary spots
    spot $\in$ cluster if centroid in circle of fixed radius
    radius depends on image dimension, number of spots, minimum number of spots in cluster
  - match highest-intensity clusters
    primary spots
  - compare clusters
    probabilistic similarity measure:
    probability of next random hit within a cluster where $m-1$ spots have been matched

Analytical Methods (ctd)

- Spot matching (ctd)

  – consistency check for possible mismatching

$$\begin{pmatrix} u \\ v \end{pmatrix} = \begin{pmatrix} t_x \\ t_y \end{pmatrix} + \begin{pmatrix} A & B \\ C & D \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix}$$

  check $L = AD - BC \approx 1$ rotation
  for each primary cluster parameters estimated from 3 matched spots

  $L = 1.0 \pm 0.25$ and $\theta \pm 10$ degrees

  $\rightarrow$ *good match*

  – transformation to match remaining spots
  from good matches in 2 clusters estimate
  $A, \cdots, D$ by least-square method

Analytical Methods (ctd)

- Creating synthetic gels
  merge gel images to get master gel
  – select reference gel
  spot positions in reference gel
  = spot position in synthetic gel
  – check spots on reference gel well matched to spots on 2 other gels
  → triangles of matched spots (= starting groups)
  – extend starting groups by adding spots
  connectivity test:
  spot matched with at least one other spot in initial group

Analytical Methods (ctd)

- Creating synthetic gels (ctd)

  – when all spots on reference gel considered, create additional groups with spots on 2nd gel which are not part of group and repeat with other gels

  – synthetic gel contains same number of spots as determined groups

  representative spots

  position: from reference gel if group has spot on reference gel
  closest spot + translation otherwise

  intensity: average for spots in group

  shape: shape of spot in group closest in area to average of group

Analytical Methods (ctd)

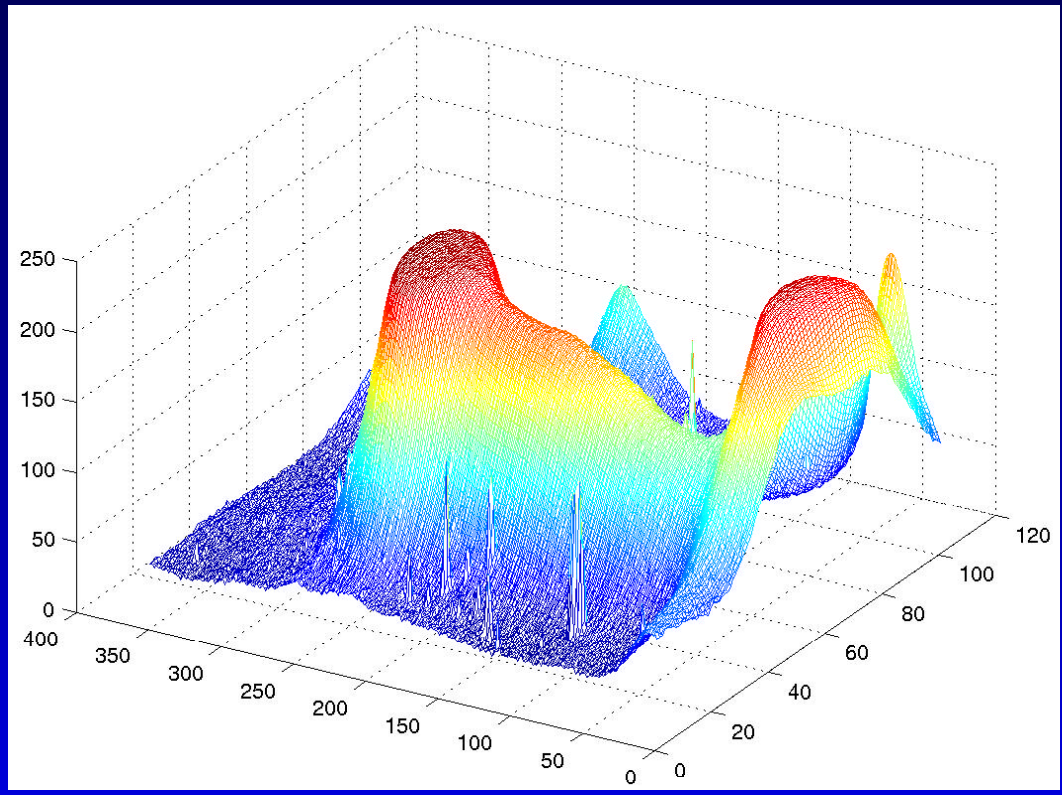
- Filtering gel images
  - Gaussian smoothing
  - diffusion smoothing
  - polynomial smoothing
  - daptive smoothing
    preserves significant discontinuities

Analytical Methods (ctd)

- Background filtering
  - global minimum pixel value
  - estimate background outside spots with 3rd-order polynomial

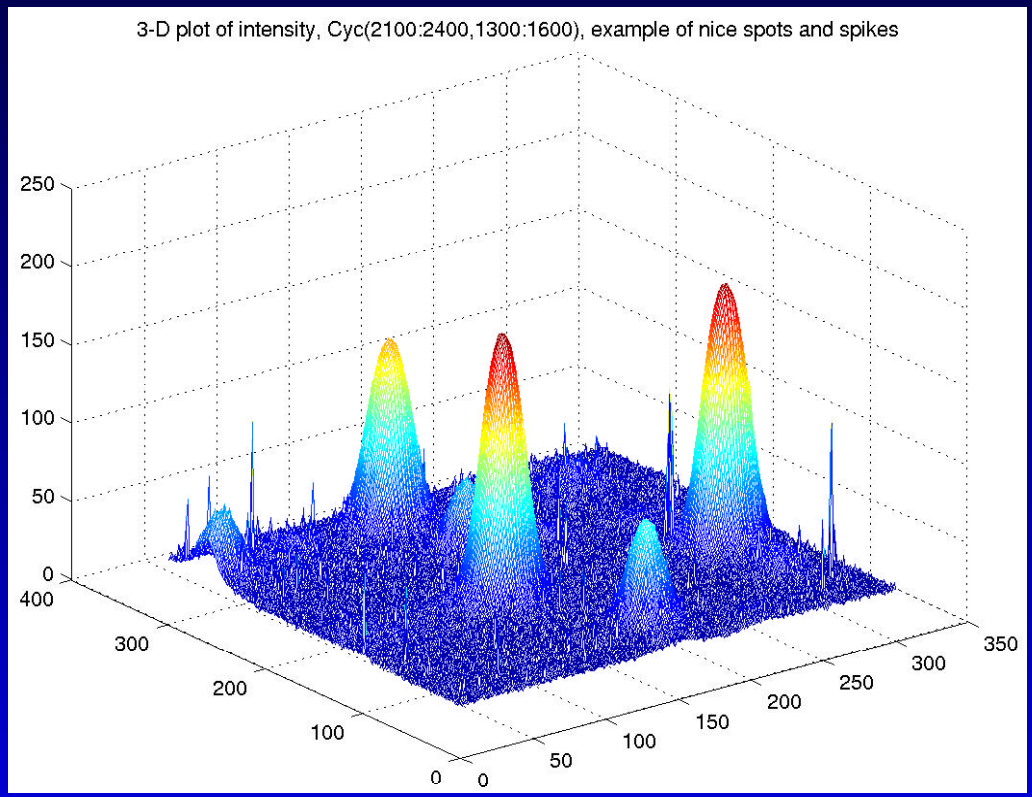
Statistical Approaches

Gaussian?



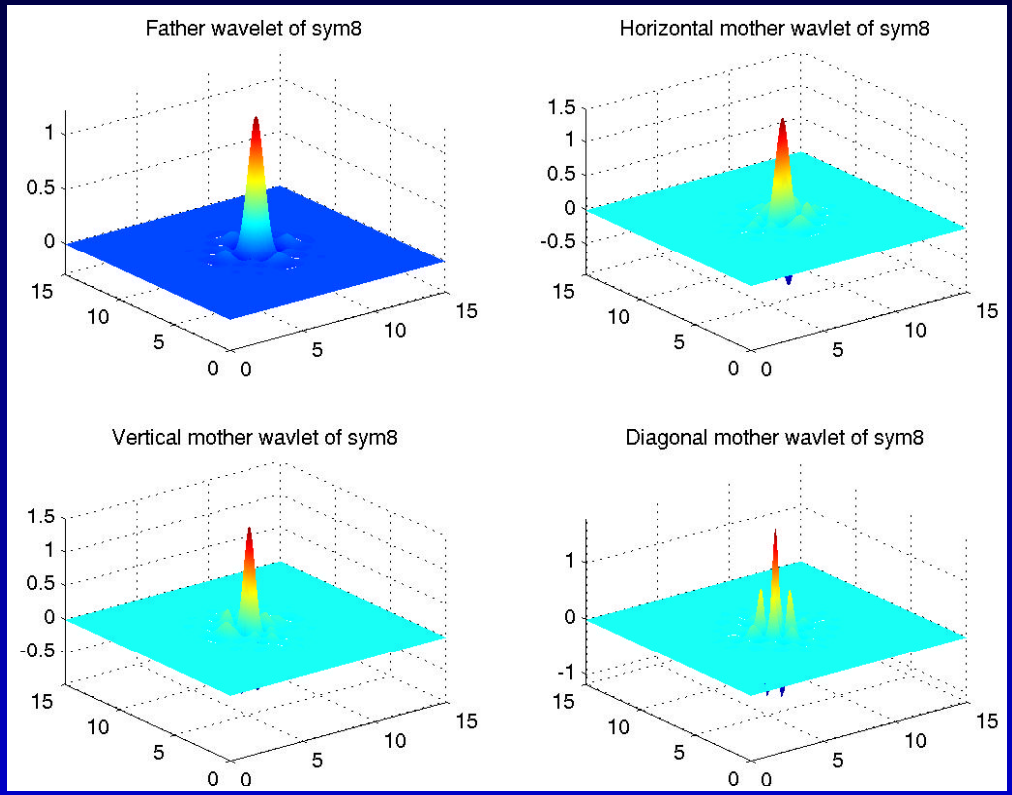
Statistical Approaches (ctd)

- Spike Removal



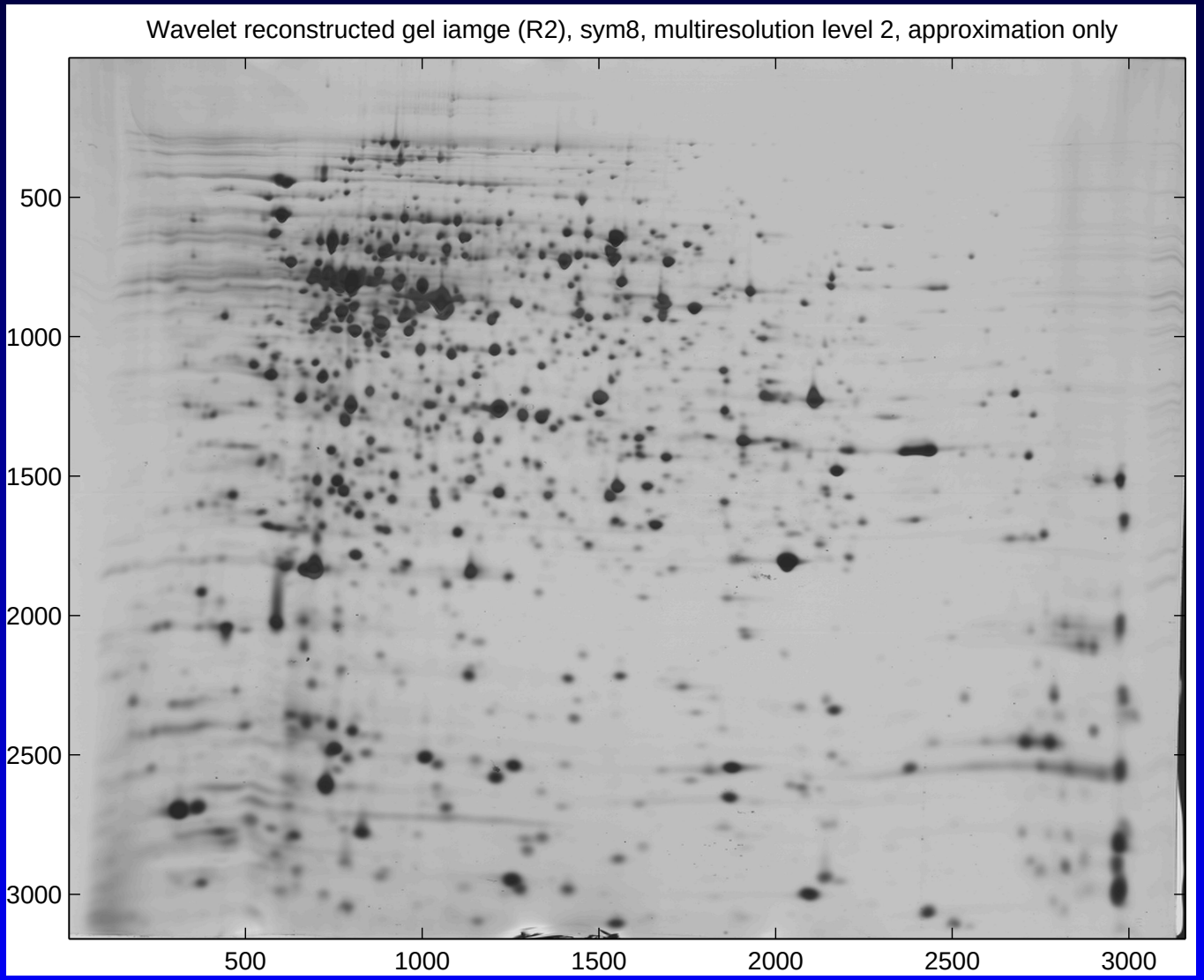
Statistical Approaches (ctd)

Wavelet methods



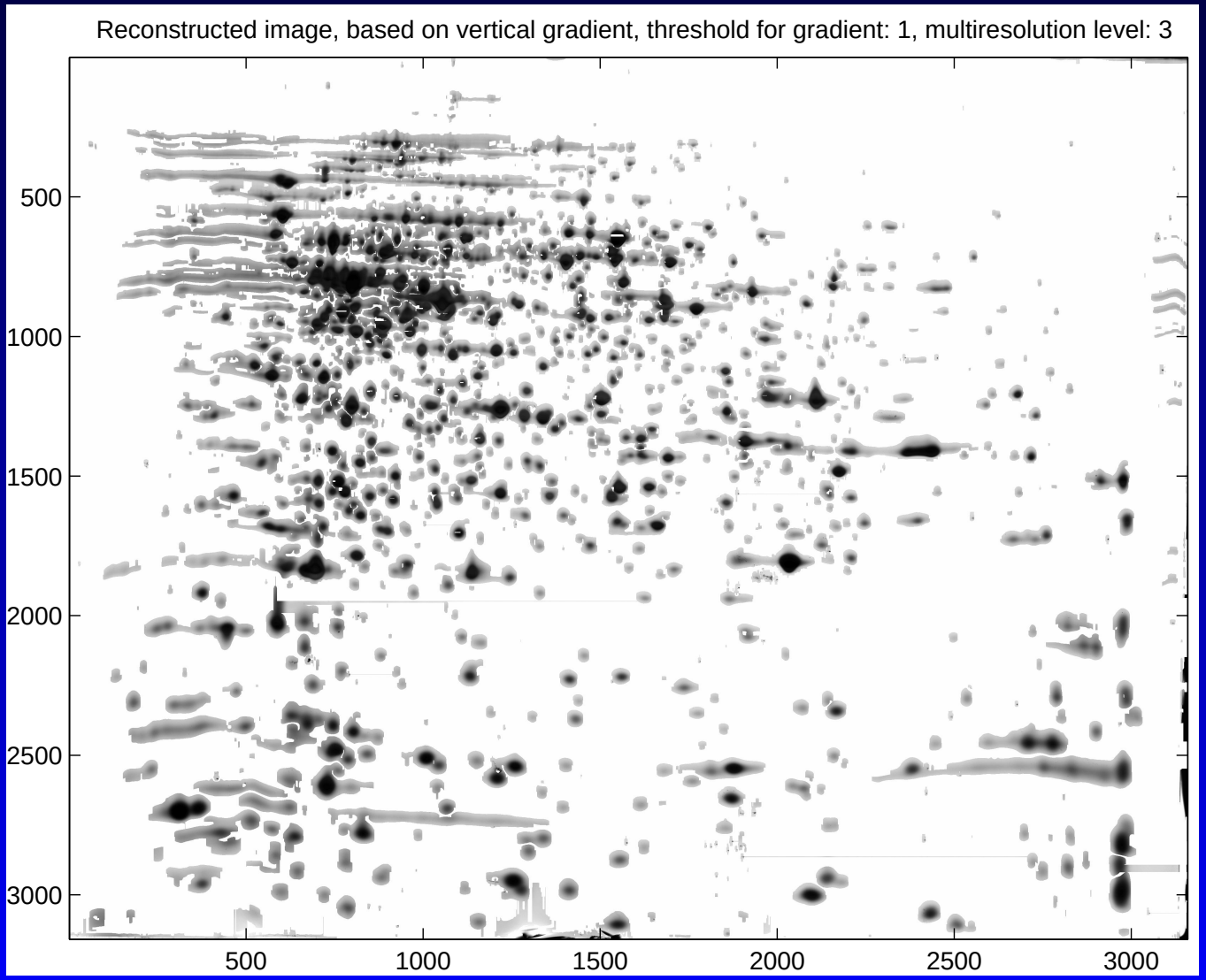
Statistical Approaches (ctd)

- Streak Removal



Statistical Approaches (ctd)

- Streak Removal (ctd)



Statistical Approaches (ctd)

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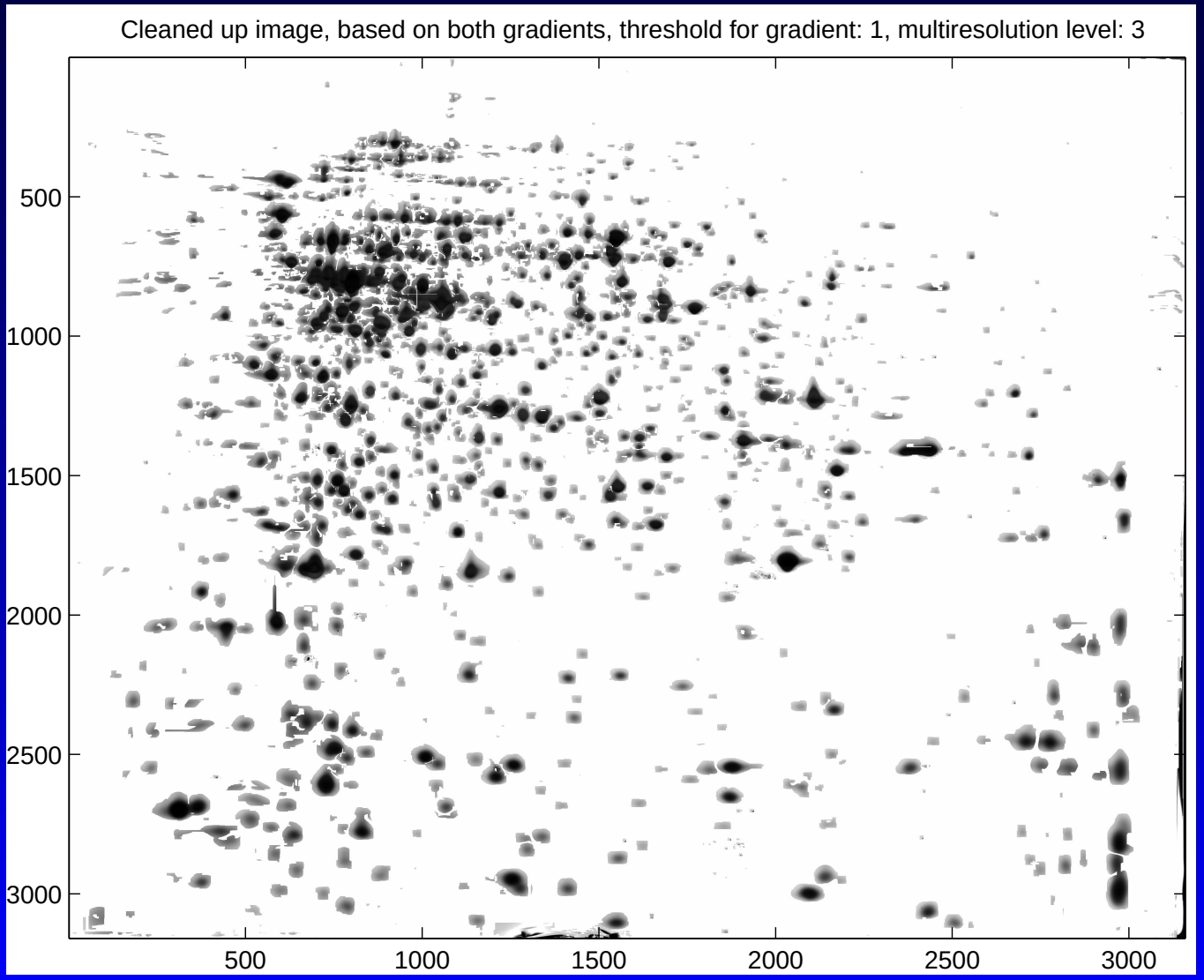


Statistical Approaches (ctd)

- Gel Alignment

N gels with M points on each gel
Find transformations
$T_i : R^2 \rightarrow R^2$, for gel i ($i = 1, 2, \ldots, N$)
describing changes for all spots on the gel.
Linear transformations for every gel:
$T_i(x) = A_i x + b_i$, where

$$A_i = \begin{pmatrix} \alpha_i & \beta_i \\ \gamma_i & \delta_i \end{pmatrix}, b_i = \begin{pmatrix} \varphi_i \\ \psi_i \end{pmatrix}, i = 1, 2, \ldots, N$$

Statistical Approaches (ctd)

- Gel Alignment (ctd)

  Position of center of spot j on gel i :
  $l_{ij}, i = 1, 2, \ldots, N, j = 1, 2, \ldots, M$, i.e.

  $$l_{ij} = \begin{pmatrix} l_{ij}^1 \\ l_{ij}^2 \end{pmatrix}$$

  Transformed l_{ij}

  $$\begin{pmatrix} \theta_{ij}^1 \\ \theta_{ij}^2 \end{pmatrix} = \theta_{ij} = T_i(l_{ij})$$

  $$\theta_{ij}^1 = \alpha_i l_{ij}^1 + \beta_i l_{ij}^2 + \varphi_i$$

  $$\theta_{ij}^2 = \gamma_i l_{ij}^1 + \delta_i l_{ij}^2 + \psi_i$$

  True location of spot j : l_j

Statistical Approaches (ctd)

- Gel Alignment (ctd)

  Restriction on transformed points:
  – not be too far away from the true location
  $\|\theta_{ij} - l_i\|_\infty \leq \epsilon_{ij}$, i.e.

  $$-\epsilon_{ij} \leq \theta^1_{ij} - l^1_i \leq \epsilon_{ij}, i = 1, 2, \ldots, N, j = 1, 2, \ldots, M$$

  $$-\epsilon_{ij} \leq \theta^2_{ij} - l^2_i \leq \epsilon_{ij}, i = 1, 2, \ldots, N, j = 1, 2, \ldots, M$$

  where ϵ_{ij} is a given error bound.
  – transformation as close as possible to identity transformation

Statistical Approaches (ctd)

- Gel Alignment (ctd)

  Objective function:

$$\sum_{i=1}^{N}(\|A_i - I\|^2 + \|b_i\|^2)$$

$$+ \text{ penalty term}$$

  weighted sum distance between true location and average spot centers

  Constraint: weighted sum of distances between true location and transform points

Statistical Approaches (ctd)

- Gel Alignment (ctd)

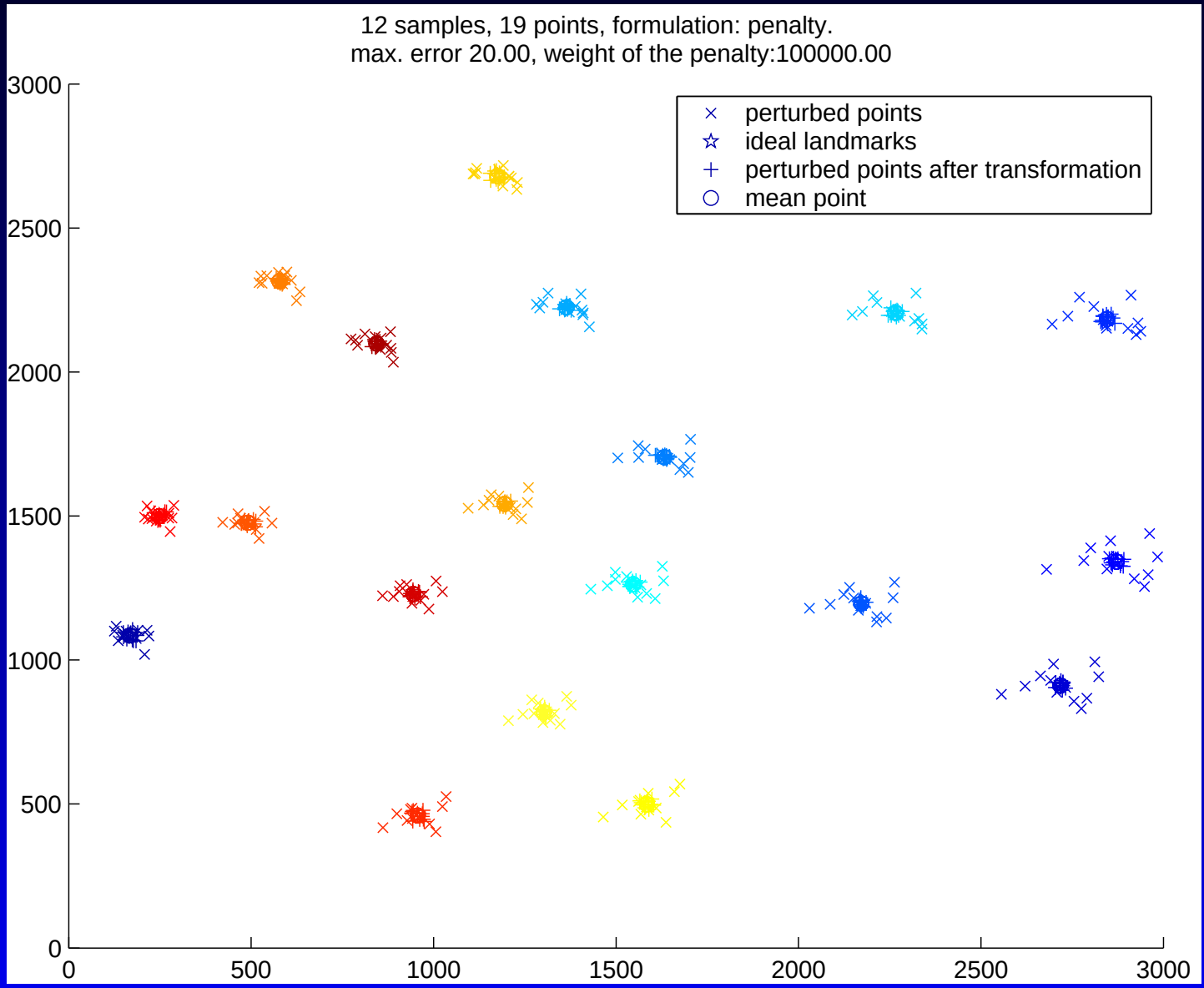
  Quadratic Programming

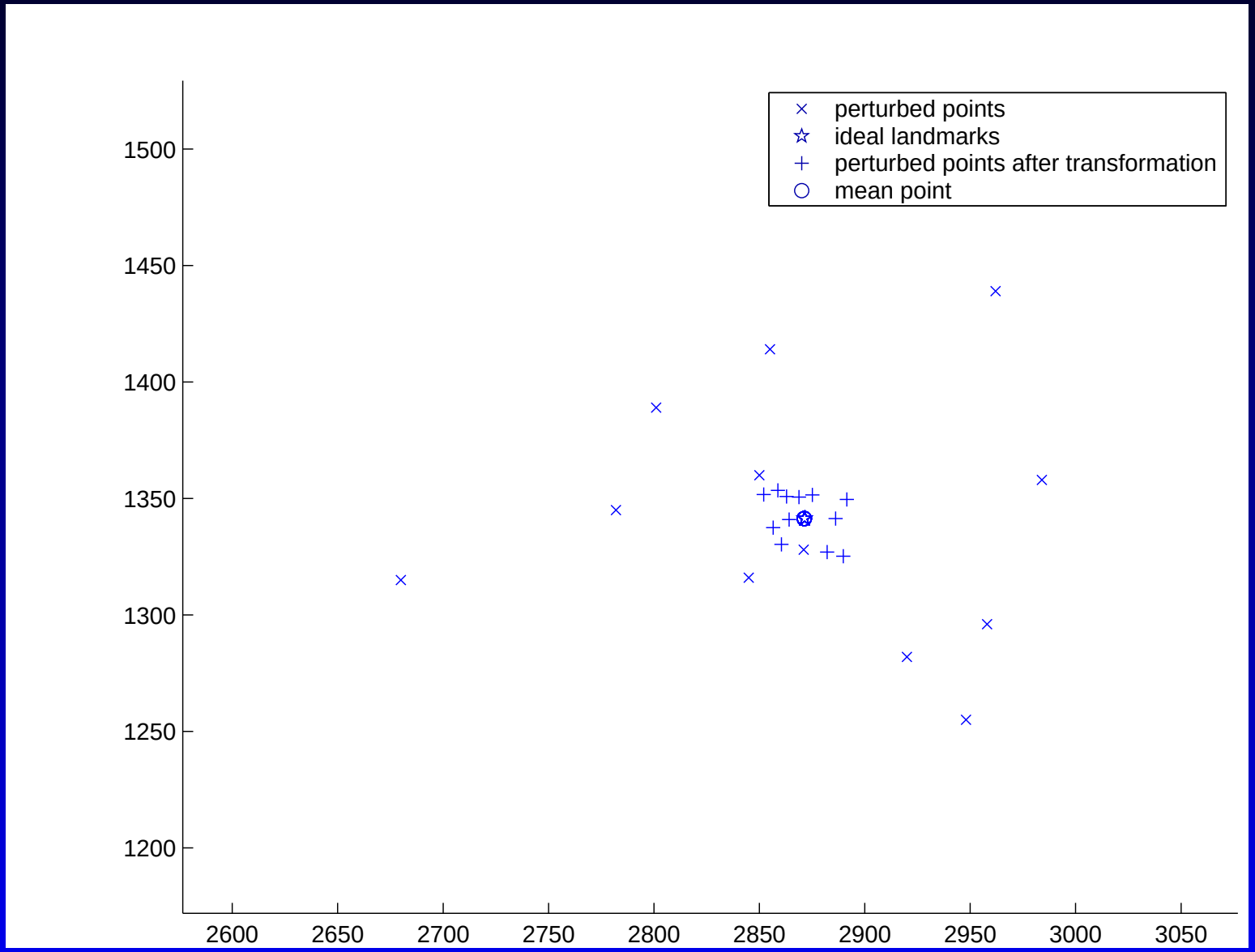
  Given data l_{ij} ($i = 1, 2, \ldots, N, j = 1, 2, \ldots, M$), maximum allowable error ϵ_{ij}, and a weight w, first compute the mean of each spot center on different images ml_j, then solve

$$\min_{\alpha_i,\beta_i,\gamma_i,\delta_i,\varphi_i,\psi_i,l_j} \sum_{i=1}^{N} \left[(\alpha_i - 1)^2 + \beta_i^2 + \gamma_i^2 + (\delta_i - 1)^2 \right.$$

$$\left. + \varphi_i^2 + \psi_i^2 \right] + w \sum_{j=1}^{M} \left[(l_j^1 - m l_j^1)^2 + (l_j^2 - m l_j^2)^2\right]$$

$$\text{s.t.} \quad -\epsilon_{ij} \leq \alpha_i l_{ij}^1 + \beta_i l_{ij}^2 + \varphi_i - l_j^1 \leq \epsilon_{ij}$$
$$-\epsilon_{ij} \leq \gamma_i l_{ij}^1 + \delta_i l_{ij}^2 + \psi_i - l_j^2 \leq \epsilon_{ij}$$


12 samples, 19 points, formulation: penalty.
max. error 20.00, weight of the penalty:100000.00
perturbed points
ideal landmarks
perturbed points after transformation
mean point
0
500
1000
1500
2000
2500
3000


perturbed points
ideal landmarks
perturbed points after transformation
mean point
1500
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1350
1300
1250
1200
2600
2650
2700
2750
2800
2850
2900
2950
3000
3050

Statistical Approaches (ctd)

- Statistical Modeling

  – Analysis of variance for spot volumes

  Mixed-effect model:

  fixed group effect
  random individual effect
  spatial correlation structure

  – Feature selection based on hypothesis testing of groups of wavelet coefficients