# Bayesian Analysis of Mass Spectrometry Data Using Wavelet-Based Functional Mixed Models

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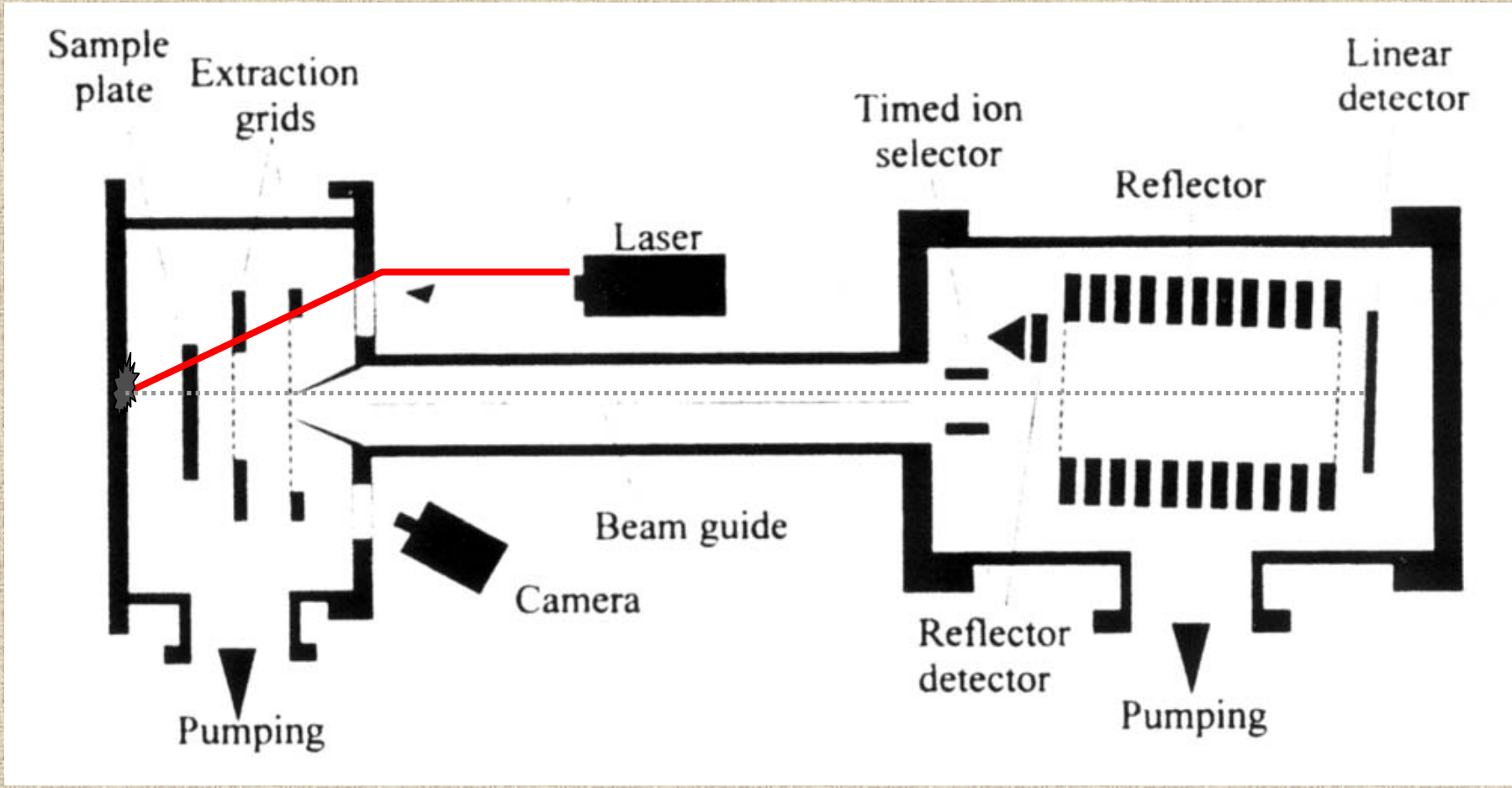
# Functional Data Analysis of Mass Spectrometry Data

- **Model as "functional data"**
  - **Idea: Model entire spectrum as single entity, not a collection of data points.**
- **Wavelet-based Functional Mixed Models**
  - **Peak detection**
  - **Identify differentially expressed peaks while controlling Bayesian FDR**
  - **Automatically account for block effects**
  - **Classify samples based on spectra, without having to search high dimensional model spaces**

# Outline

- **Introduction**
  - **Examples**
  - **Mixed Models/Functional Mixed Models**
  - **Wavelets**
- **Wavelet-Based Functional Mixed Models**
- **Bayesian Inference for Mass Spectrometry**
- **Apply to Examples**
- **Discussion**

# MALDI-TOF Schematic




Vestal and Juhasz. *JAS* **1998**, *9*, 892.

# Example: Pancreatic Cancer Study

- Koomen, et al. (2004)
- 256 blood serum samples – 141 pancreatic cancer, 115 normal controls
- 4 MALDI spectra/sample
  - Fractions: MYO25, MYO70, BSA25, BSA70
- Samples (all fractions) run in 4 blocks on 4 different dates
- Goals:
  - Identify differentially expressed protein peaks.
  - Classify samples as C/N based on spectra.
- Must adjust for block effects on spectra
- This talk: Focus on MYO25 fraction, 4kD-10kD

# Example:Organ-Cell Line Expt

- 16 nude mice had 1 of 2 cancer cell lines injected into 1 of 2 organs (lung or brain)
- Cell lines:
  - A375P: human melanoma, low metastatic potential
  - PC3MM2: human prostate, highly metastatic
- Blood Serum extracted from each mouse – placed on 2 SELDI chips
- Samples run at 2 different laser intensities (low/ high)
- Total of 32 spectra (observed functions), 2 per mouse

# Example: Organ-Cell Line Expt

- **Goal:**

  **Find proteins differentially expressed by:**

  – **Host organ site (lung/brain)**

  – **Donor cell line (A375P/PC3MM2)**

  – **Organ-by-cell line interaction**

- **Combine information across laser intensities:**

  **Requires us to include in modeling:**

  – **Functional laser intensity effect**

  – **Random effect functions to account for correlation between spectra from same mouse**

# Linear Mixed Models

## Linear Mixed Model (Laird and Ware, 1982):

$$\underbrace{Y}_{N\times 1} = \underbrace{X}_{N\times p}\overbrace{\beta}^{p\times 1} + \underbrace{Z}_{N\times m}\overbrace{u}^{m\times 1} + \underbrace{e}_{N\times 1}$$

$$u \sim N(0, \overbrace{P}^{m\times m})$$
$$e \sim N(0, \underbrace{R}_{N\times N})$$

- **Fixed effects part, $X\beta$, accommodate a broad class of mean structures, including main effects, interactions, and linear coefficients.**
- **Random effects part, $Zu$, provide a convenient mechanism for modeling correlation among the $N$ observations.**

# Functional Mixed Model (FMM)

**Suppose we observe a sample of *N* curves, $Y_i(t)$, $i$=1, …, $N$**

$$Y_i(t) = \sum_{j=1}^{p} X_{ij} B_j(t) + \sum_{k=1}^{m} Z_{ik} U_k(t) + E_i(t)$$

- $B_j(t)$ = **fixed effect functions**
- $U_k(t)$ = **random effect functions**
- $E_i(t)$ = **residual error processes**

# Pancreatic Cancer Example

**Let $Y_i(t)$ be MALDI spectrum from sample *i***

$$Y_i(t) = B_0(t) + \sum_{j=1}^{4} X_{ij} B_j(t) + E_i(t)$$

- $X_{i1}$ **=*1* if cancer, -*1* if normal**
  $X_{ij}$ **=*1* if block *j*, -*1* if block *1* for *j*=*2,3,4***
- $B_0(t)$ **= overall mean spectrum**
  $B_1(t)$ **= cancer effect function**
  $B_j(t)$ **= block effect function for *j*=*2,3,4***
- **No random effects necessary**

# Organ-by-Cell Line Example

**Let $Y_i(t)$ be the SELDI spectrum *i***

$$Y_i(t) = B_0(t) + \sum_{j=1}^{4} X_{ij} B_j(t) + \sum_{k=1}^{16} Z_{ik} U_k(t) + E_i(t)$$

- **$X_{i1}$=*1* for lung, *-1* brain. $X_{i2}$=*1* for A375P, *-1* for PC3MM2**

  **$X_{i3}$= $X_1$ * $X_2$ $X_{i4}$=*1* for low laser intensity, *-1* high.**

- **$B_0(t)$ = overall mean spectrum $B_1(t)$ = organ main effect function**

  **$B_2(t)$ = cell-line main effect $B_3(t)$ = org x cell-line int function**

  **$B_4(t)$ = laser intensity effect function**

- **$Z_{ik}$=*1* if spectrum *i* is from mouse *k* (*k=1, …, 16*)**
- **$U_k(t)$ is random effect function for mouse *k*.**

# Functional Mixed Models

- **Key feature of FMM: Does not require specification of parametric form for curves**
- **Methods based on kernels/fixed knot splines not well suited to spiky functional data**
- **Wavelet Regression: nonparametric regression technique that better preserves local features present in the curves.**

# Functional Mixed Model
## (Discrete version)

**Y= *N*-by-*T* matrix containing the observed spectra on sampling grid of size *T***

$$\underbrace{Y}_{N\times \mathrm{T}} = \overbrace{X}^{N\times p}\ \underbrace{B}_{p\times \mathrm{T}} + \overbrace{Z}^{N\times m}\ \underbrace{U}_{m\times \mathrm{T}} + \underbrace{E}_{N\times \mathrm{T}}$$

$$U_i \sim MVN(0,Q)$$
$$E_i \sim MVN(0,S)$$

- **$B_{ij}$ is the effect of covariate *i* at location $t_j$**
- **$Q$ and $S$ are covariance matrices (*T* x *T*)**
- **Note: Some structure must be assumed on form of Q and S (discussed later)**

# Introduction to Wavelets

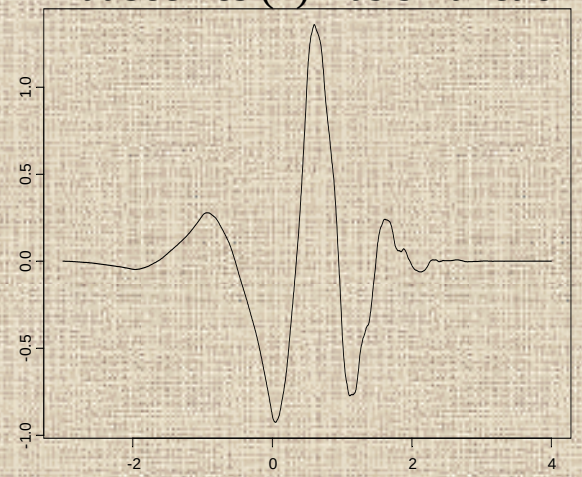
- **Wavelets: families of orthonormal basis functions**

$$g(t) = \sum_{j,k \in \Im} d_{jk} \psi_{jk}(t)$$

$$\psi_{jk}(t) = 2^{-j/2} \psi(2^{-j/2} t - k)$$

$$d_{jk} = \int g(t) \psi_{jk}(t) dt$$

Daubechies (4) Basis Function

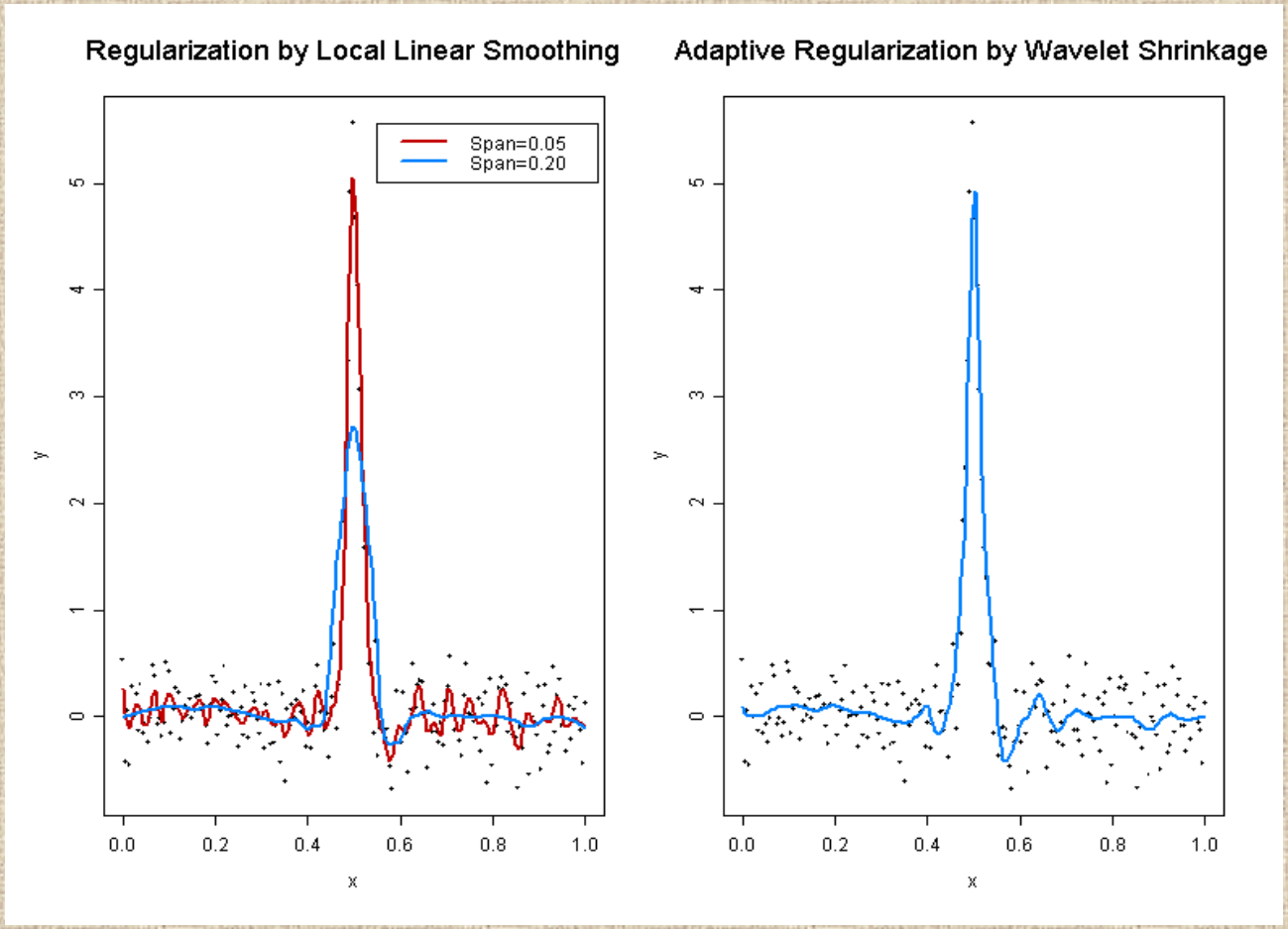



- **Discrete Wavelet Transform (DWT): fast algorithm {O(*T*)} for obtaining *T* empirical wavelet coefficients for curves sampled on equally-spaced grid of length *T*.**
- **Linear Representation:** $d = y\,W'$
  - ***W'* =*T*-by-*T* orthogonal projection matrix**
- **Inverse DWT (IDWT):** $y = d\,W$

# Wavelet Regression

- **Wavelet Regression – 3 step process**
  1. **Project data into wavelet space**
  2. **Threshold/shrink coefficients**
  3. **Project back to data space**
- **Yields *adaptively regularized* (plot) nonparametric estimates of function**
- **Morris, et al. (2003) extended to nested functional model (Bayesian)**
- **Morris and Carroll (2004) extended to general functional mixed model framework (Wavelet-based FMM)**

# Adaptive Regularization



# Wavelet-Based FMM:

## General Approach

1. **Project observed functions** Y **into wavelet space.**
2. **Fit FMM in wavelet space.**

   **(Use MCMC to get posterior samples)**
3. **Project wavelet-space estimates (posterior samples) back to data space.**

# Wavelet-Based FMM:

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# Wavelet-Based FMM

**1. Project observed functions Y to wavelet space**

- **Apply DWT to rows of Y to get wavelet coefficients corresponding to each observed function**

$$\underbrace{D}_{N\times T} = \underbrace{Y}_{N\times T}\ \underbrace{W'}_{T\times T}$$

- **Projects the observed curves into the space spanned by the wavelet bases.**

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## General Approach

1. **Project observed functions Y into wavelet space.**
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# Projecting FMM to Wavelet Space

$$\underbrace{Y}_{N\times \mathrm{T}} = \overbrace{X}^{N\times p}\ \underbrace{B}_{p\times \mathrm{T}} + \overbrace{Z}^{N\times m}\ \underbrace{U}_{m\times \mathrm{T}} + \underbrace{E}_{N\times \mathrm{T}}$$

$$U_i \sim MVN(0, Q)$$

$$E_i \sim MVN(0, S)$$

# Projecting FMM to Wavelet Space

$$\underbrace{Y}_{N\times \mathrm{T}}\ \overbrace{\mathbf{W'}}^{T\times T} = \overbrace{X}^{N\times p}\ \underbrace{B}_{p\times \mathrm{T}} + \overbrace{Z}^{N\times m}\ \underbrace{U}_{m\times \mathrm{T}} + \underbrace{E}_{N\times \mathrm{T}}$$

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# Projecting FMM to Wavelet Space

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$$U_i \sim MVN(0, Q)$$

$$E_i \sim MVN(0, S)$$

# Projecting FMM to Wavelet Space

$$\underbrace{Y}_{N\times \mathrm{T}}\overbrace{\mathbf{W'}}^{\mathbf{T}\times\mathbf{T}} = \overbrace{X}^{N\times p}\underbrace{B}_{p\times \mathrm{T}}\overbrace{\mathbf{W'}}^{T\times T} + \overbrace{Z}^{N\times m}\underbrace{U}_{m\times \mathrm{T}}\overbrace{\mathbf{W'}}^{T\times T} + \underbrace{E}_{N\times \mathrm{T}}\overbrace{\mathbf{W'}}^{T\times T}$$

$$U_i\mathbf{W'} \sim MVN(0, \mathbf{W}Q\mathbf{W'})$$

$$E_i\mathbf{W'} \sim MVN(0, \mathbf{W}S\mathbf{W'})$$

# Projecting FMM to Wavelet Space

$$\underbrace{\mathbf{D}}_{N\times \mathrm{T}} = \overbrace{X}^{N\times p}\ \underbrace{\mathbf{B}^*}_{p\times \mathrm{T}} + \overbrace{Z}^{N\times m}\ \underbrace{\mathbf{U}^*}_{m\times \mathrm{T}} + \underbrace{\mathbf{E}^*}_{N\times \mathrm{T}}$$

$$\mathbf{U}_i^* \sim MVN(0, \mathbf{Q}^*)$$

$$\mathbf{E}_i^* \sim MVN(0, \mathbf{S}^*)$$

# Model Each Column Separately

$$\underbrace{d_{jk}}_{N\times 1} = \overbrace{X}^{N\times p}\underbrace{\beta^{*}_{jk}}_{p\times 1} + \overbrace{Z}^{N\times m}\underbrace{u^{*}_{jk}}_{m\times 1} + \underbrace{e^{*}_{jk}}_{N\times 1}$$

$$\boldsymbol{u^{*}_{jk} \sim N(0, q^{*}_{jk})}$$

$$\boldsymbol{e^{*}_{jk} \sim N(0, s^{*}_{jk})}$$

# Prior Assumptions

**Mixture prior on $B_{ijk}^*$:**

$$B_{ijk}^* = \gamma_{ijk}^* N(0, \tau_{ij}) + (1 - \gamma_{ijk}^*)\delta_0$$

$$\gamma_{ijk}^* = \text{Bernoulli}(\pi_{ij})$$

- **Nonlinearly shrinks $B_{ijk}^*$ towards 0, leading to adaptively regularized estimates of $B_i(t)$.**
- **$\tau_{ij}$ & $\pi_{ij}$ are regularization parameters**
  - **Can be estimated from the data using empirical Bayes**
  - **Extend Clyde&George (1999) to functional mixed model**

# Model Fitting

- **MCMC to obtain posterior samples of model quantities**
  - **Work with marginal likelihood; U* integrated out;**
- **Let $\Omega$ be a vector containing ALL covariance parameters (i.e. for *P*, *Q**, *R*, and *S**).**

## MCMC Steps

1. **Sample from $f(B^*|D,\Omega)$:**

   **Mixture of normals and point masses at 0 for each *i,j,k*.**

2. **Sample from $f(\Omega|D,B^*)$:**

   **Metropolis-Hastings steps for each *j,k***

3. **If desired, sample from $f(U^*|D,B^*,\Omega)$:**

   **Multivariate normals**

# Wavelet-Based FMM:

## General Approach

1. **Project observed functions Y into wavelet space.**
2. **Fit FMM in wavelet space**
   **(Use MCMC to get posterior samples)**
3. **Project wavelet-space estimates (posterior samples) back to data space.**

# Wavelet-Based FMM

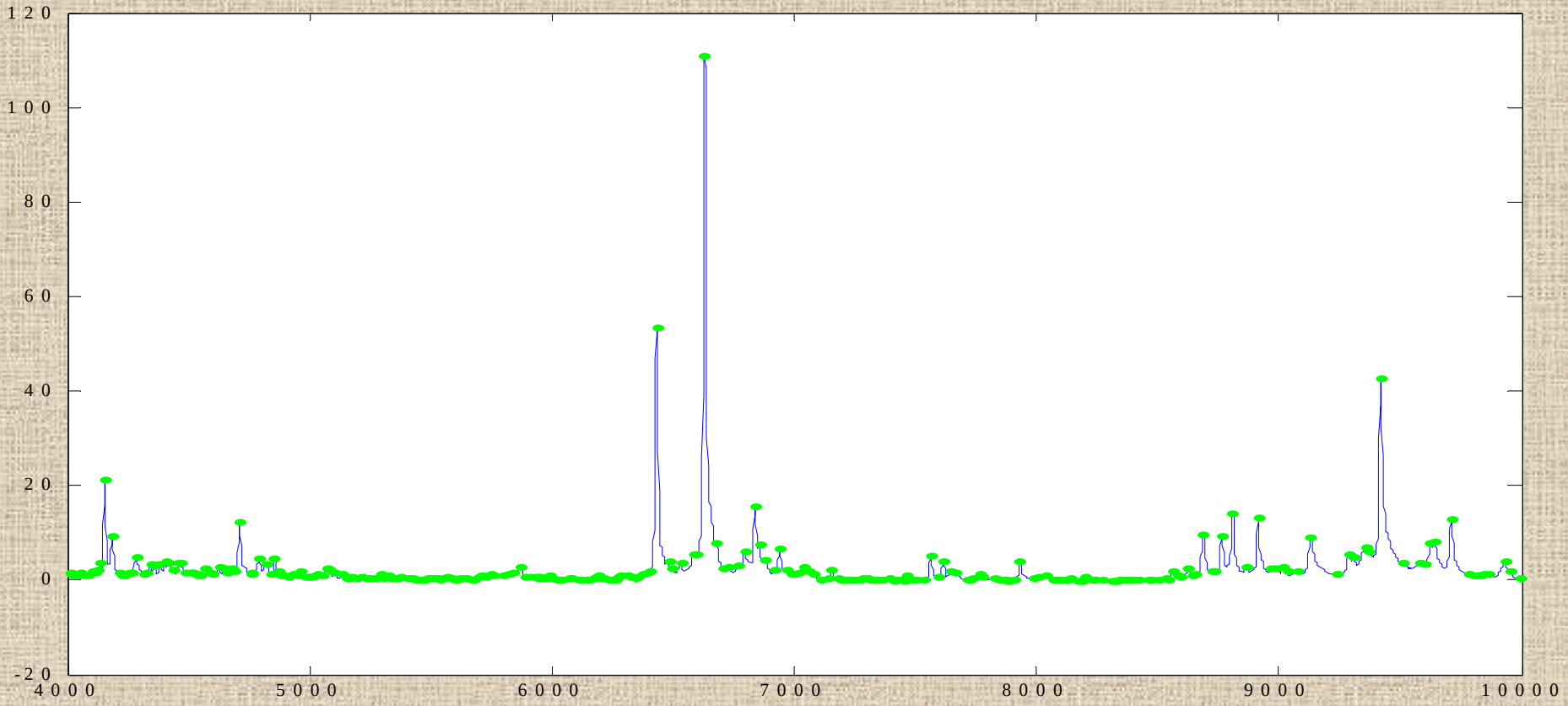
**3. Project wavelet-space estimates (posterior samples) back to data space.**

- **Apply IDWT to posterior samples of $B^*$ to get posterior samples of fixed effect functions $B_j(t)$ for $j=1,\ldots,p$, on grid $t$.**
  - **$B=B^*W$**
- **Posterior samples of $U_k(t)$, $Q$, and $S$ are also available, if desired.**
- **Can be used for Bayesian inference/prediction**

# Bayesian Inference: Peak Detection

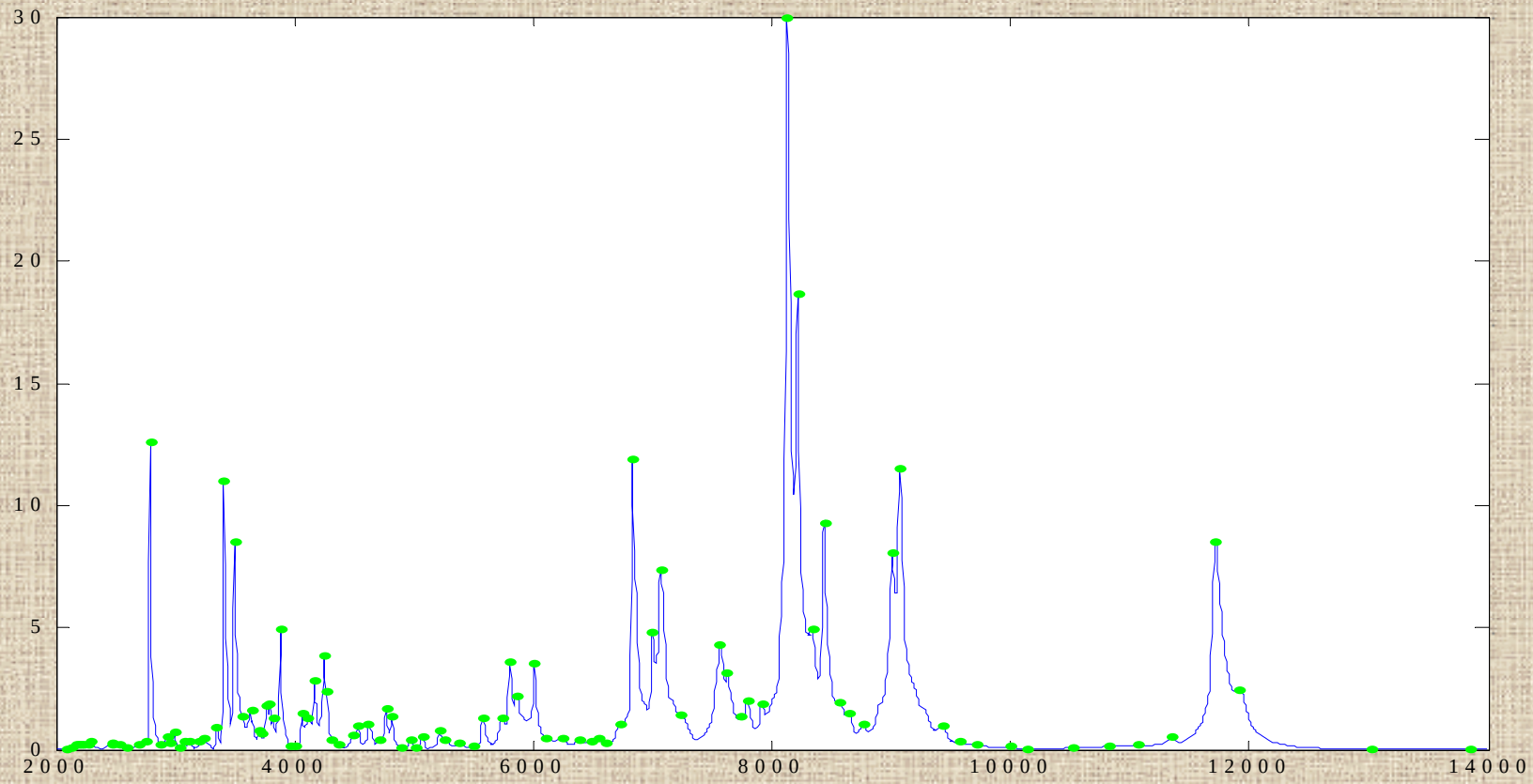
- **Focus specifically on peaks – locations in spectra likely to correspond to proteins/peptides**
- **Can use posterior mean estimate of overall mean spectrum for peak detection (Morris et al. 2005)**
- **All local maxima in (denoised) overall mean spectrum considered peaks, possibly subject to some threshold on Signal-to-Noise ratio (S/N>$\delta$)**
- **Let *K*=# of peaks found**

# Pancreatic Cancer:
# Peak Detection




- ***K=370* peaks detected**

# Organ-by-Cell Line:
# Peak Detection




- **Found *K=102* peaks (*58* with *S/N*>2)**

# Bayesian Inference:

## Identifying Differentially Expressed Peaks

- **Identify which peaks are related to clinical factors of interest (cancer/normal, organ, cell line, interaction)**

**Procedure:**

1. **Compute posterior probability of differential expression for each peak using posterior samples for suitable fixed effect function (2-sided)**

   $p_{ij}=\min[\Pr\{B_j(t_i)>0\},\Pr\{B_j(t_i)<0\}]$

   $i=1, \ldots, K \qquad j=1, \ldots, p$

2. **Rank peaks based on $p_{ij}$**

# Bayesian Inference:

## Identifying Differentially Expressed Peaks

**Procedure:**

1. **Rank peaks in ascending order of their 2-sided posterior probabilities of differential expression.**

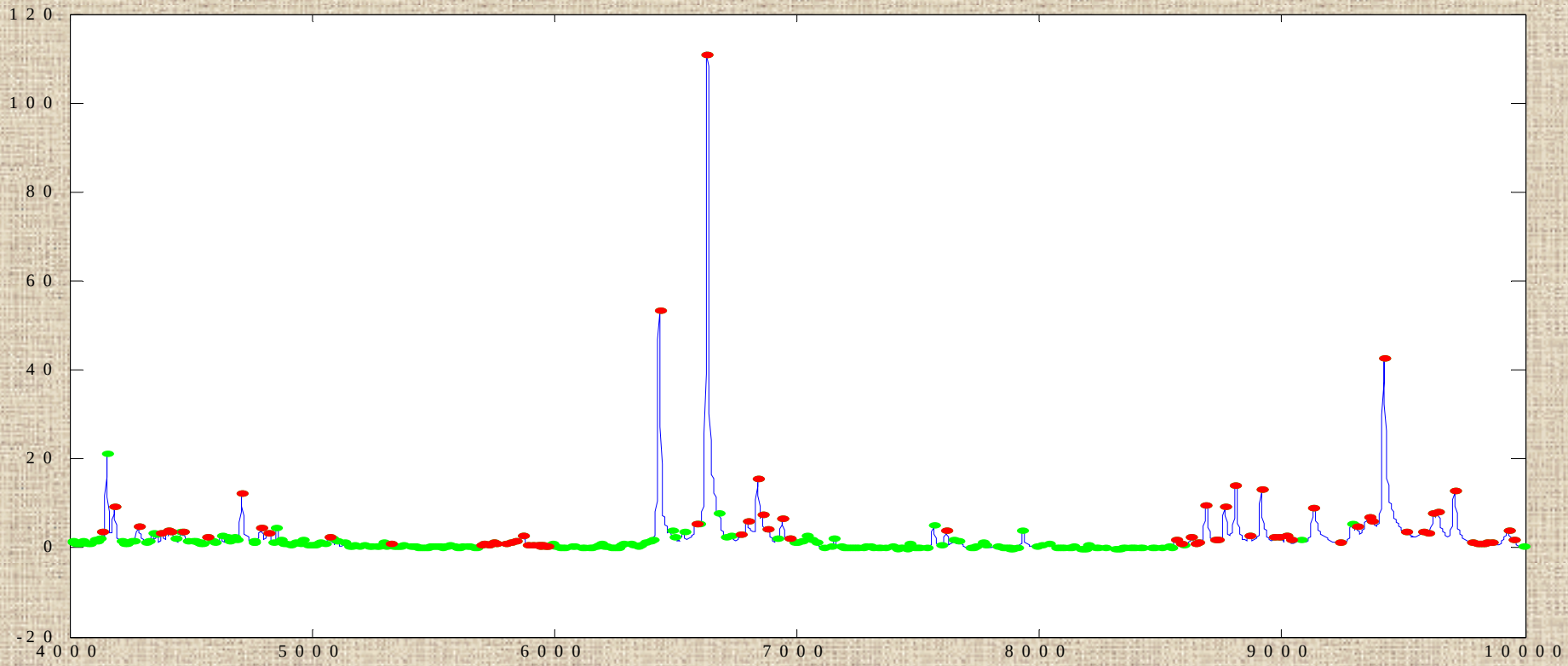
   $p_{(1)}, p_{(2)}, \cdots, p_{(pK)}$

2. **Find $K^*$ such that:**

$$(K^*)^{-1}\sum_{k=1}^{K^*} p_{(k)} < \alpha / 2$$

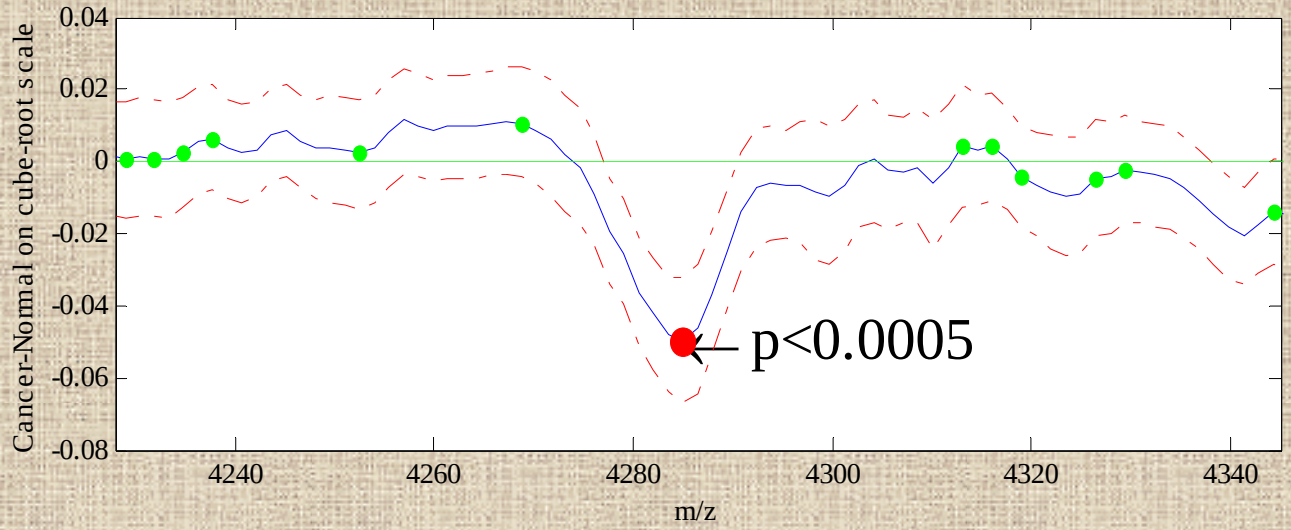
3. **Let $\psi = p_{(K^*)}$. Any peak $i$ with $p_{ij} < \psi$ is called "differentially expressed" for outcome $j$**

# Pancreatic Cancer:
# Differentially Expressed Peaks

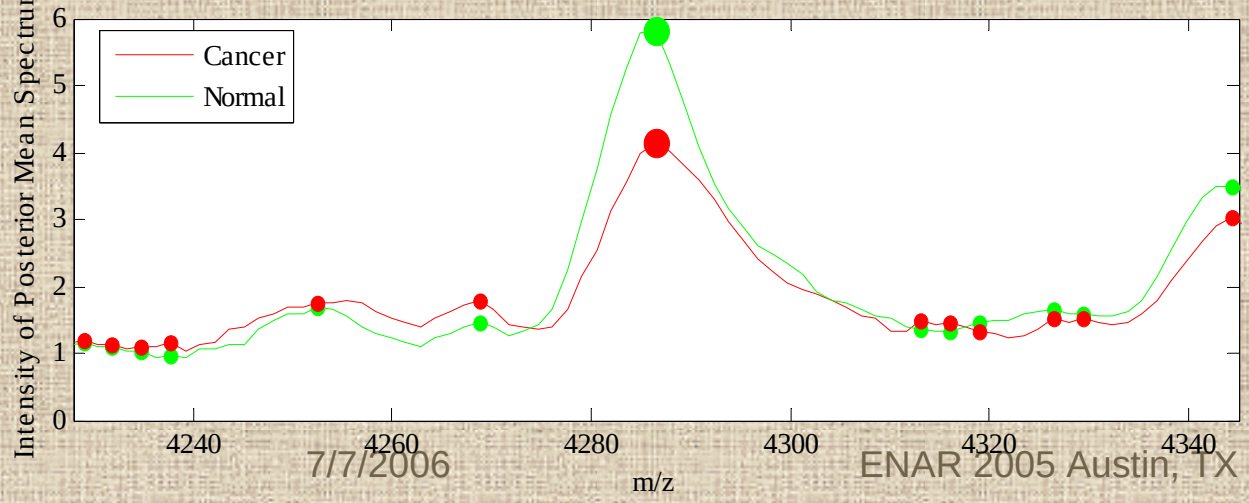



- **370 peaks detected**
- **83 differentially expressed using $\alpha$=0.01**

# Pancreatic Cancer: Results

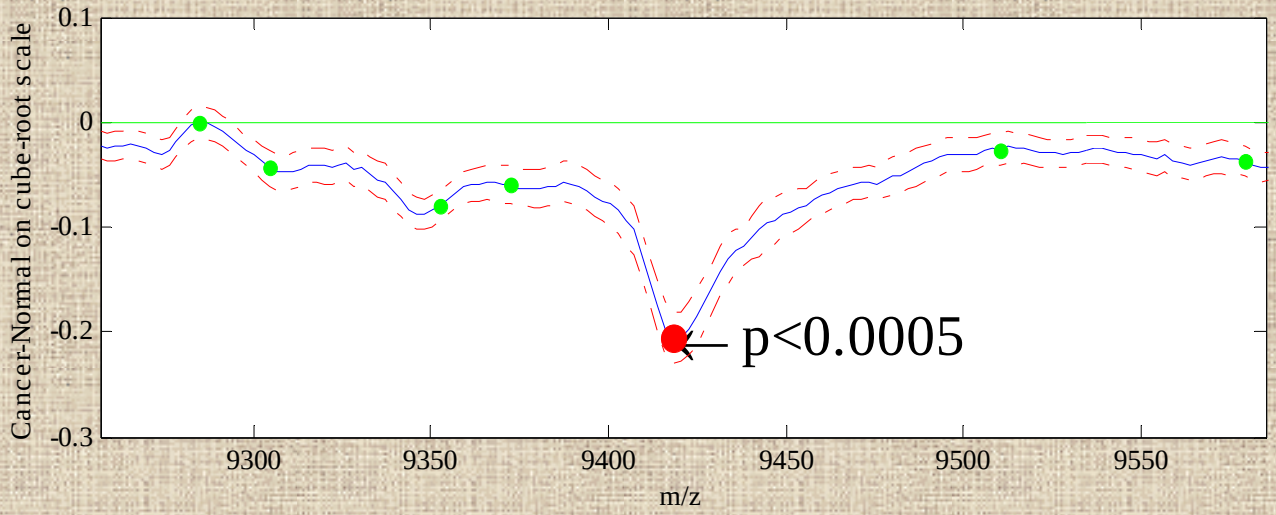



Inter-α trypsin inhibitor (4284 D)

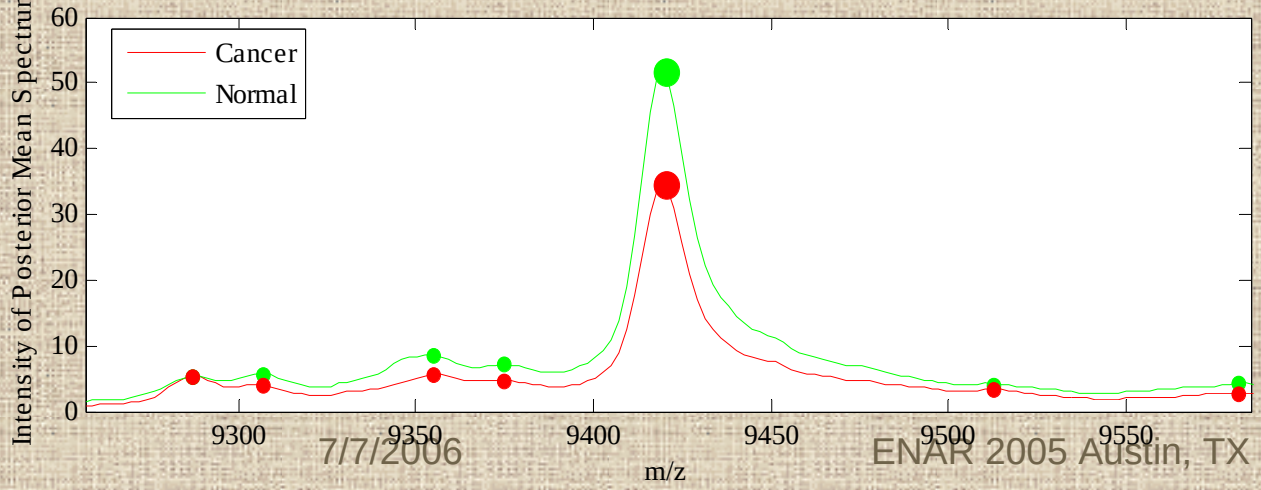



- **Known to be related to pancreatic cancer**
- **Under-expressed in serum of cancerous patients**
- **May not be specific to pancreatic cancer**

# Pancreatic Cancer: Results

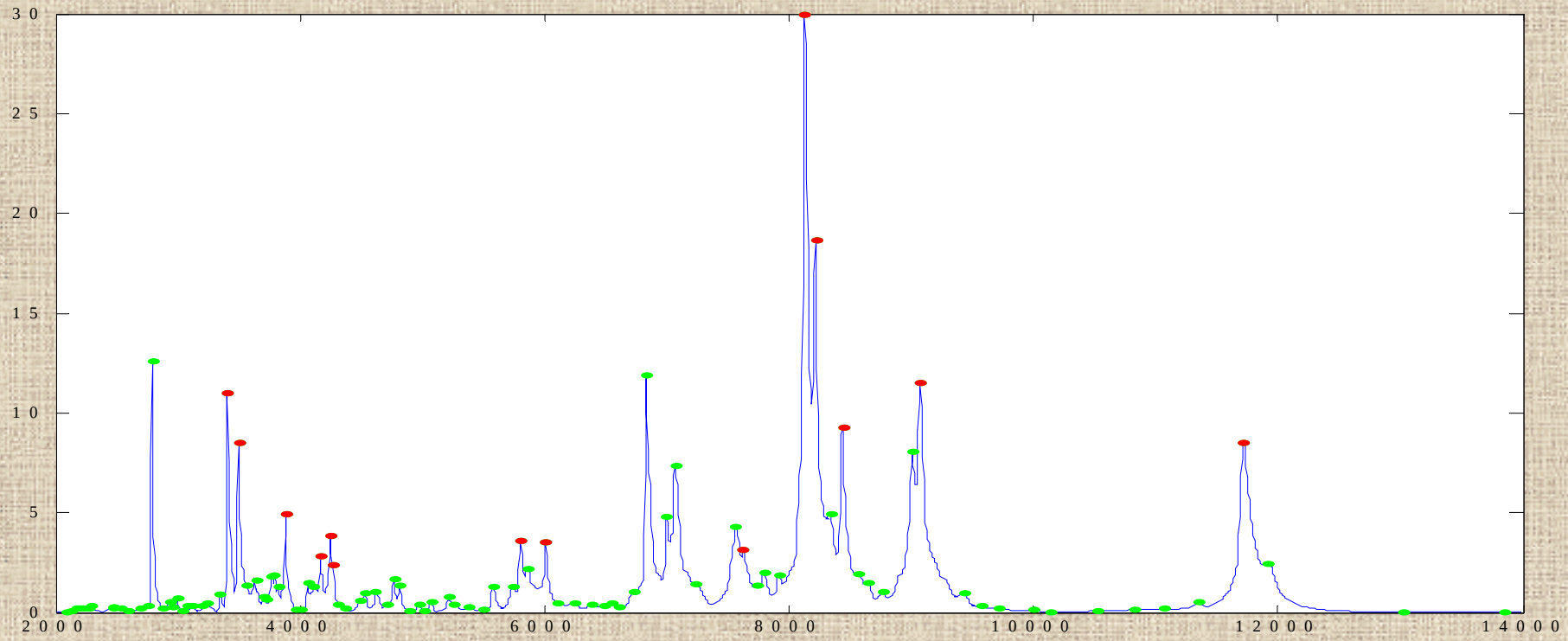



Small inducible cytokine B14 precursor (9419 D)



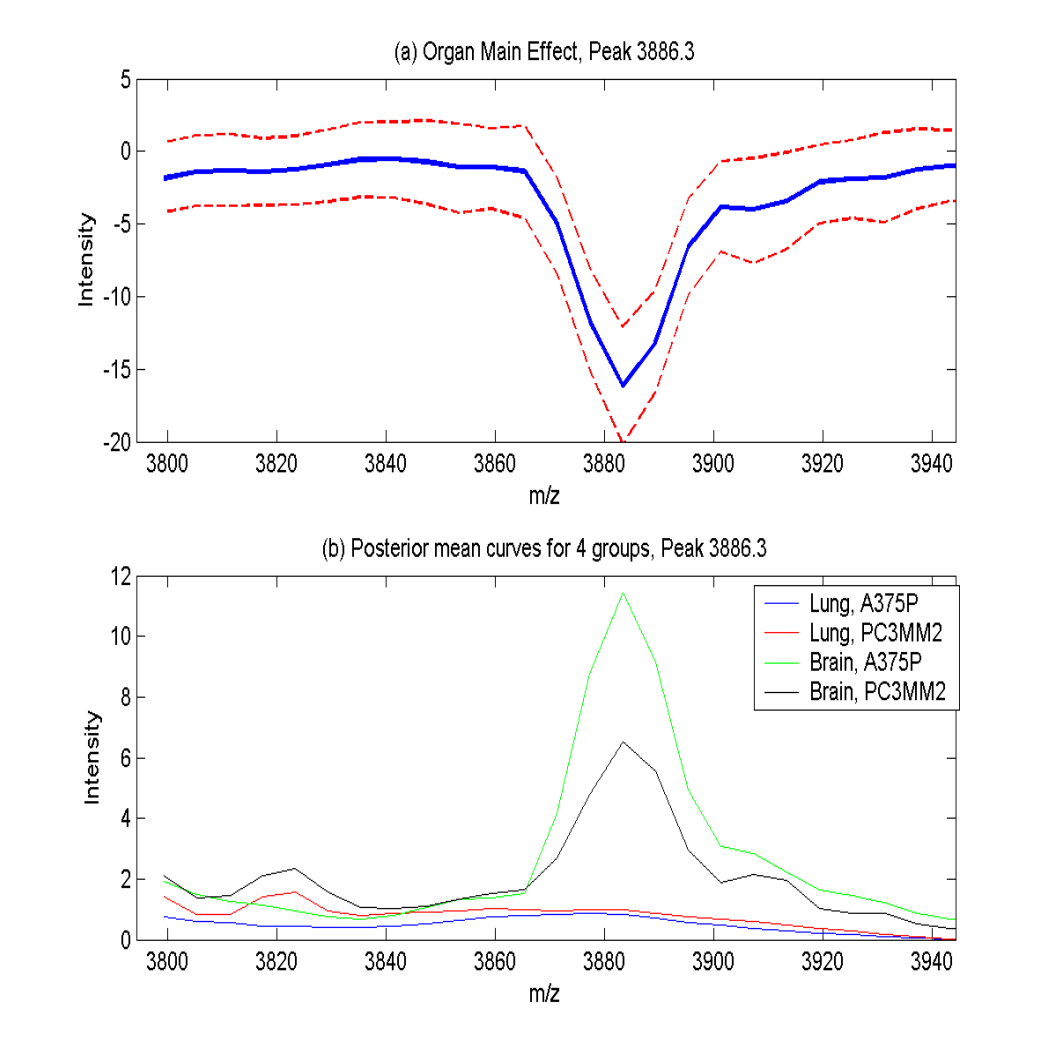

- Secreted from various organs, including pancreas
- Highly expressed in normal tissue with no inflammatory response
- Low expression in cancer cell lines

# Organ-by-Cell Line:
# Differentially Expressed Peaks



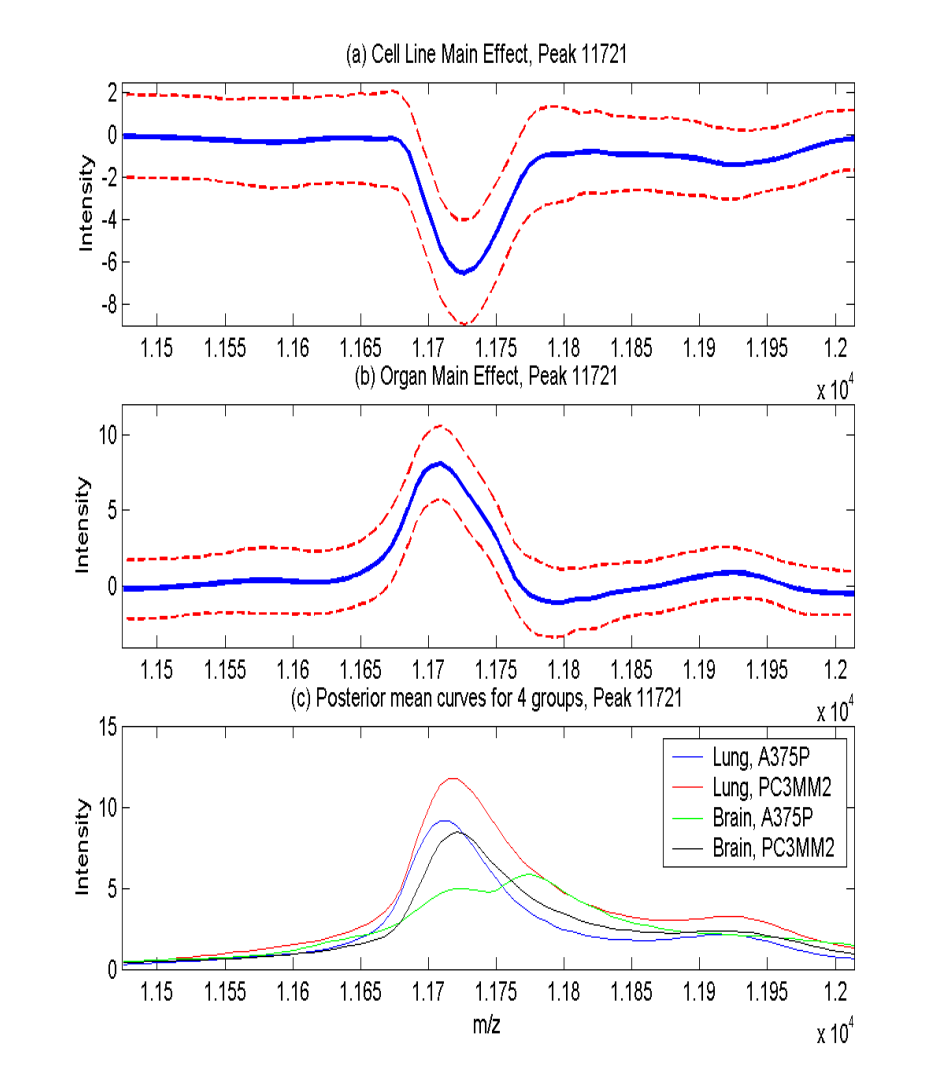

- **14/102 differentially expressed using $\alpha$ =0.01**
- **5 interaction, 2 organ, 3 cell line, 4 organ+cell line**

# Organ-by-Cell Line: Results




- Specific to brain-injected mice
- May be CGRP-II (3882.34 Dal), peptide in mouse proteome that dilates blood vessels in brain
- Host response to tumor implanted in brain?

# Organ-by-Cell Line: Results

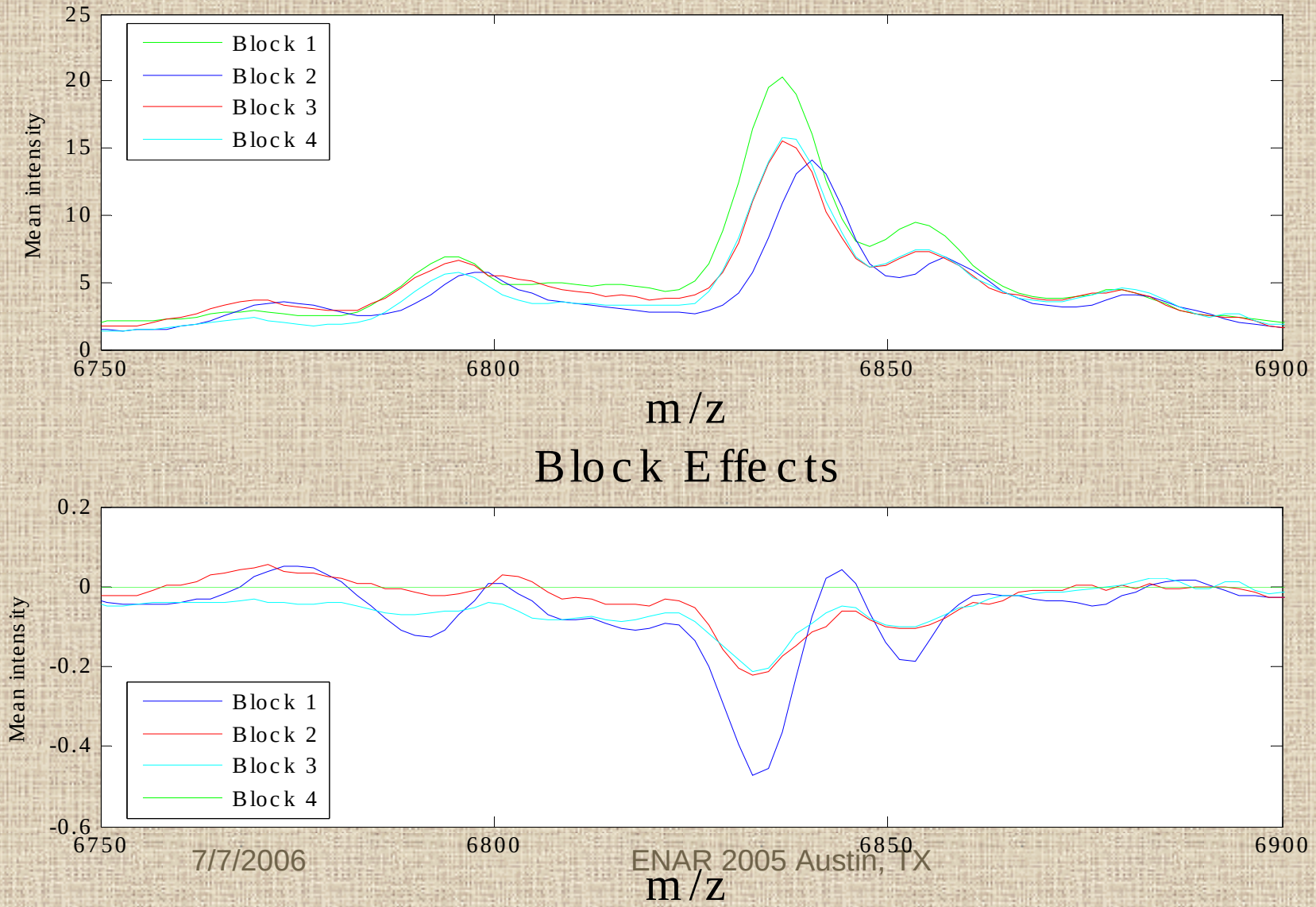



- **Higher in mice injected with metastatic (PC3-MM2) cell line**
- **May be MTS1 (11721.43 Dalt), metastatic cell protein in mouse proteome.**
- **Also higher in lung-injected mice than brain-injected mice**

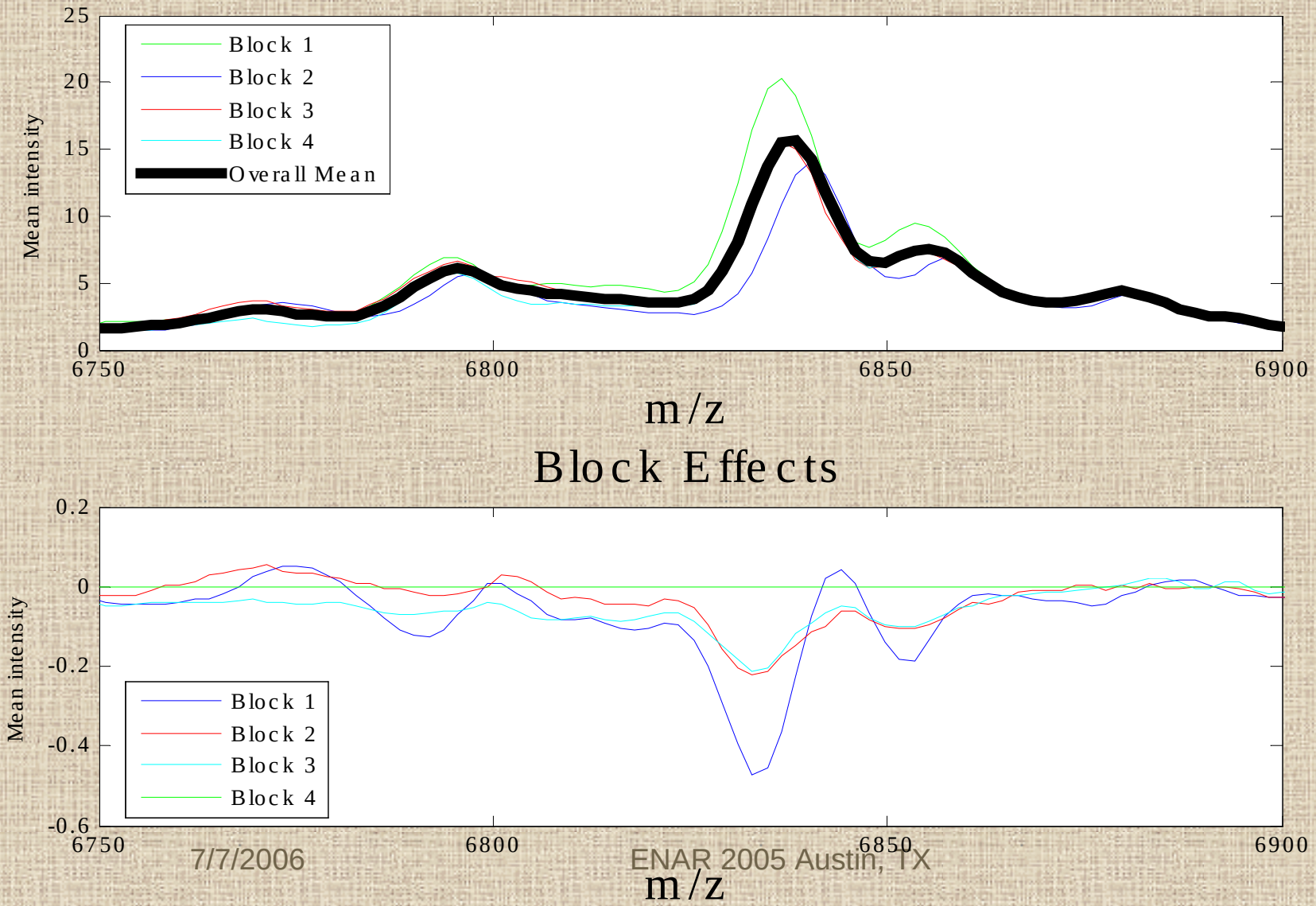
# Bayesian Inference:
## Investigating Block Effects

- **By including fixed effect for blocks, we can adjust for systematic differences in spectra from different blocks (time blocks, laser intensity)**
  - **Systematic shifts in spectral intensities (y)**
  - **Systematic shifts in peak locations (x)**
- **These adjustments are done automatically by the model-fitting.**
  - **Flexibility of nonparametric fixed effects allows us to adjust for arbitrarily nonlinear misalignments**

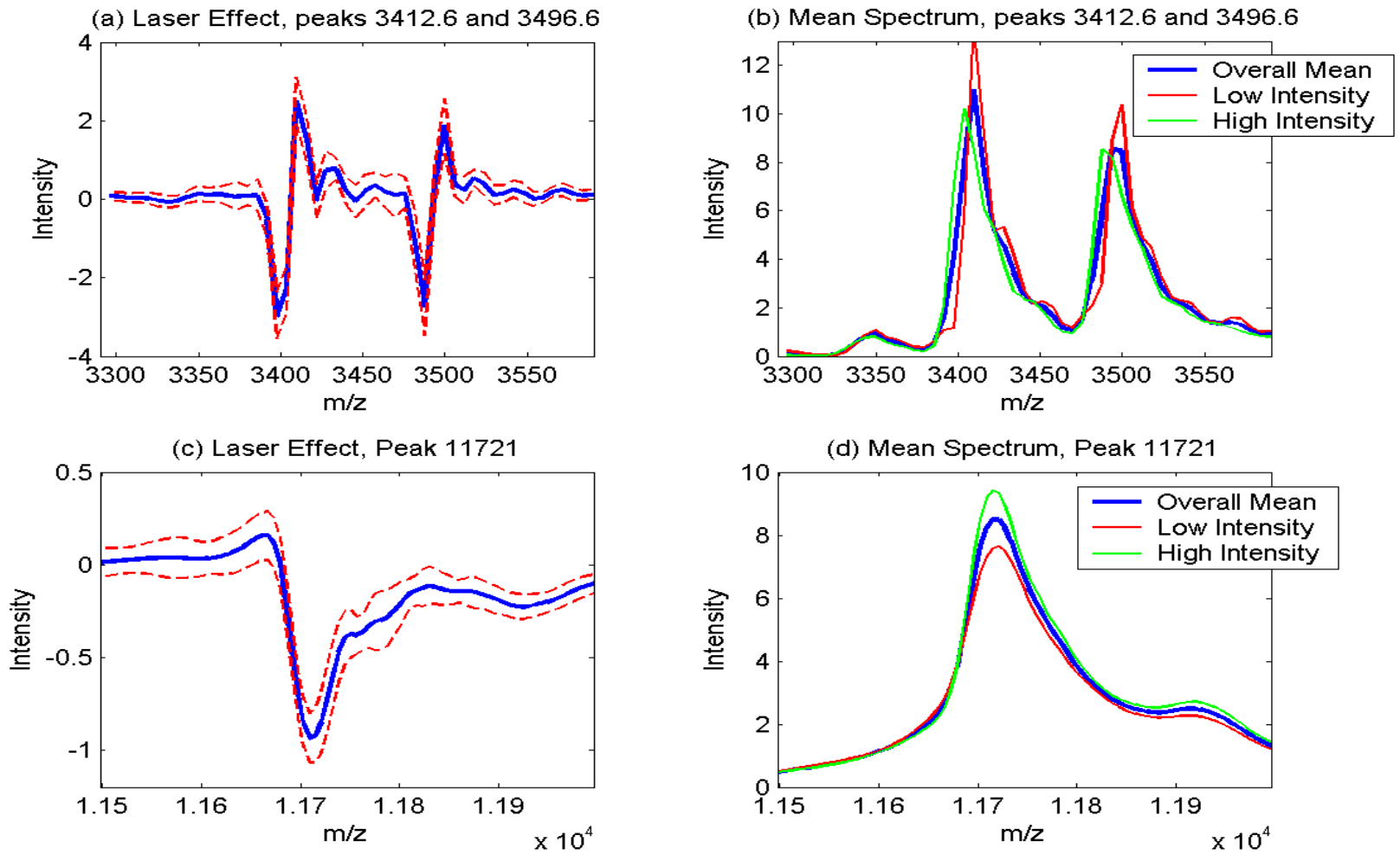
# Pancreatic Cancer: Block Effects



# Pancreatic Cancer: Block Effects



# Organ-by-Cell Line: Block Effects



# Bayesian Inference: Discrimination/Classification

- New samples can be classified as Cancer/Normal based on their spectra using posterior predictive probabilities
  - $X$=cancer status of test sample (1=cancer, -1=not)
  - $y$=test spectrum, $Y^t$=training spectra
  - Classify as cancer if $Pr(X=1|y,Y^t)>0.50$
- Straightforward to compute given posterior samples of model parameters
- Can be used to perform classification without having to first do feature selection

# Bayesian Inference: Discrimination/Classification

$$\Pr(X=1 \mid y, Y^t) = O/(O+1)$$

$$O = \overbrace{\frac{\Pr(X=1)}{1-\Pr(X=1)}}^{\text{prior odds}} \times \overbrace{BF}^{\text{Bayes Factor}}$$

$$BF = \frac{f(y \mid X=1, Y^t)}{f(y \mid X=-1, Y^t)}$$

$$f(y \mid X=1, Y^t) = \int f(y \mid X=1, \Theta)\, f(\Theta \mid Y^t)\, d\Theta$$

$$\approx B^{-1} \sum_{b=1}^{B} f(y \mid X=1, \Theta^{(b)})$$

# Bayesian Inference: Discrimination/Classification

$$f(y \mid X = 1, \Theta^{(b)}) = f(d \mid X = 1, \Theta^{*(b)})$$
$$= \prod_{j,k} f(d_{jk} \mid X = 1, \Theta_{jk}^{*(b)})$$

$$BF = \prod_{j,k} BF_{jk}$$

# Pancreatic Cancer: Classification Accuracy

| | Accuracy | Sensitivity | Specificity |
|---|---|---|---|
| Training Data | 81% | 78% | 83% |
| Test Data (8-fold CV) | 70% | 73% | 66% |

- Koomen, et al. 2004: 90% sensitivity, 77% specificity
- Used entire spectrum and all 4 fractions
- We only used small region of 1 fraction – doing others

# Pancreatic Cancer: Classification Accuracy

- Performance improved by not using all wavelet coeffs
  - Leave out those likely to be unrelated to peaks
  - Lowest frequencies removed (j=1,2,3,4): baseline
  - Highest frequency removed (j=16): noise

| | Accuracy | Sensitivity | Specificity |
|---|---|---|---|
| Training Data | 83% | 78% | 89% |
| Test Data (8-fold CV) | 74% | 75% | 73% |

# Discussion

- **Flexible method for modeling mass spectrometry data**
  - **Multiple fixed effects**
  - **Block effects**
  - **Random effects**
- **Various types of inference possible**
  - **Peak detection, differentially expressed peaks, control FDR, classification without feature selection**
- **Easy-to-use code being developed**
  - **Only necessary inputs: Y, X, Z matrices**
  - **Available by end of Summer 2005.**
- **Method also applies to other types of functional data.**

# Acknowledgements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- **Thanks to Dick Herrick for assistance in optimizing the code for the method, and for converting the Matlab code to C++.**

# Wavelet-Based Hierarchical Functional Models

- **Most existing wavelet regression methods are for single function case**
- **Morris, Vannucci, Brown, and Carroll (2003)**
  - **Bayesian wavelet-based method for estimating mean function for functional data from nested design.**
  - **Extended wavelet regression to hierarchical functional context.**
- **Morris and Carroll (2004)**
  - **Extended to functional mixed model framework**
  - **Allowed nonstationary covariance structures**

# Example: Model Fitting

- **Daubechies 8 wavelet basis, $J$=11 levels**
- **Empirical Bayes procedure used to estimate regularization parameters $\pi_{ij}$ and $\tau_{ij}$ from data.**
- **Burn-in 1000; 20,000 MCMC samples; thin=10**
- **Took 7hr 53min on Win2000 P-IV 2.8GHz 2GB RAM**
  - **That is Matlab code; C++ code takes ~2 hours.**
- **Trace plots indicated good convergence properties**
- **Metropolis Hastings acceptance probabilities good:**
  - **Range of (0.04, 0.53)**
  - **($10^{th}$,$50^{th}$,$90^{th}$) percentiles of (0.20, 0.29, 0.50)**

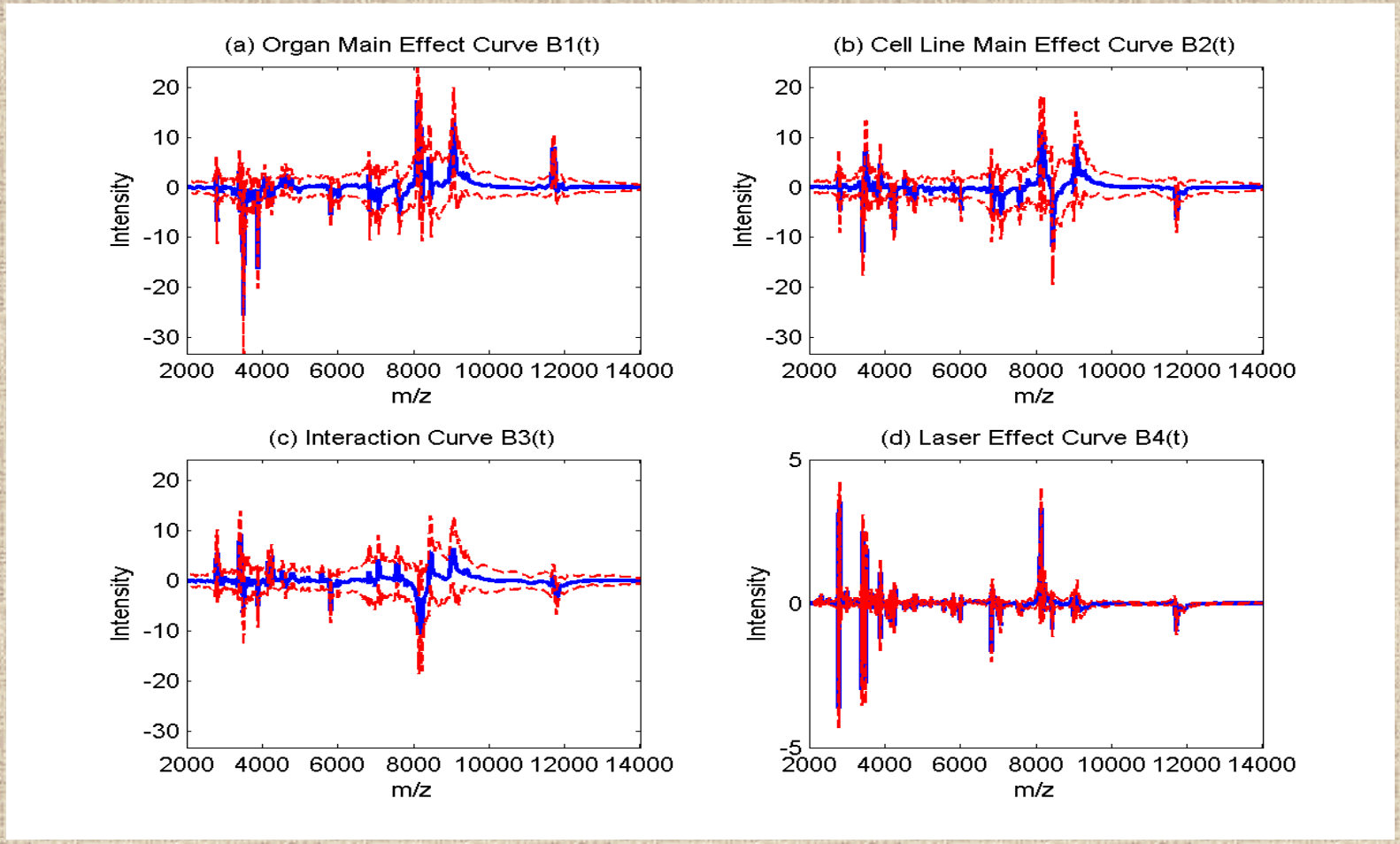
# Discussion

- **Introduced unified modeling approach for FDA**
  - **Applied here to MALDI-TOF, but method is general.**
- **Method based on mixed models; is FLEXIBLE**
  - **Accommodates a wide range of experimental designs**
  - **Addresses large number of research questions**
- **Posterior samples allow Bayesian inference and prediction**
  - **Posterior credible intervals; pointwise or joint**
  - **Predictive distributions for future sampled curves**
  - **Predictive probabilities for group membership of new curves**
  - **Bayesian functional inference can be done via Bayes Factors**
- **Since a unified modeling approach is used, all sources of variability in the model propagated throughout inference.**

# Discussion

- **Since functions adaptively regularized using wavelet shrinkage, the method is appropriate for spatially heterogeneous functional data.**
- **Approach is Bayesian. The only informative priors to elicit are regularization parameters, which can be estimated from data using empirical Bayes.**
- **Method generalizes to higher dimensional functions, e.g. image data, space/time (fixed domain) data.**
- **We used wavelet bases, but approach can be generalized to other orthogonal basis functions.**
- **Major challenges in developing unified statistical modeling approach for replicated functional data, but worth the effort.**

# Organ-by-Cell Line: Results



# Organ-by-Cell Line: Flagged peaks

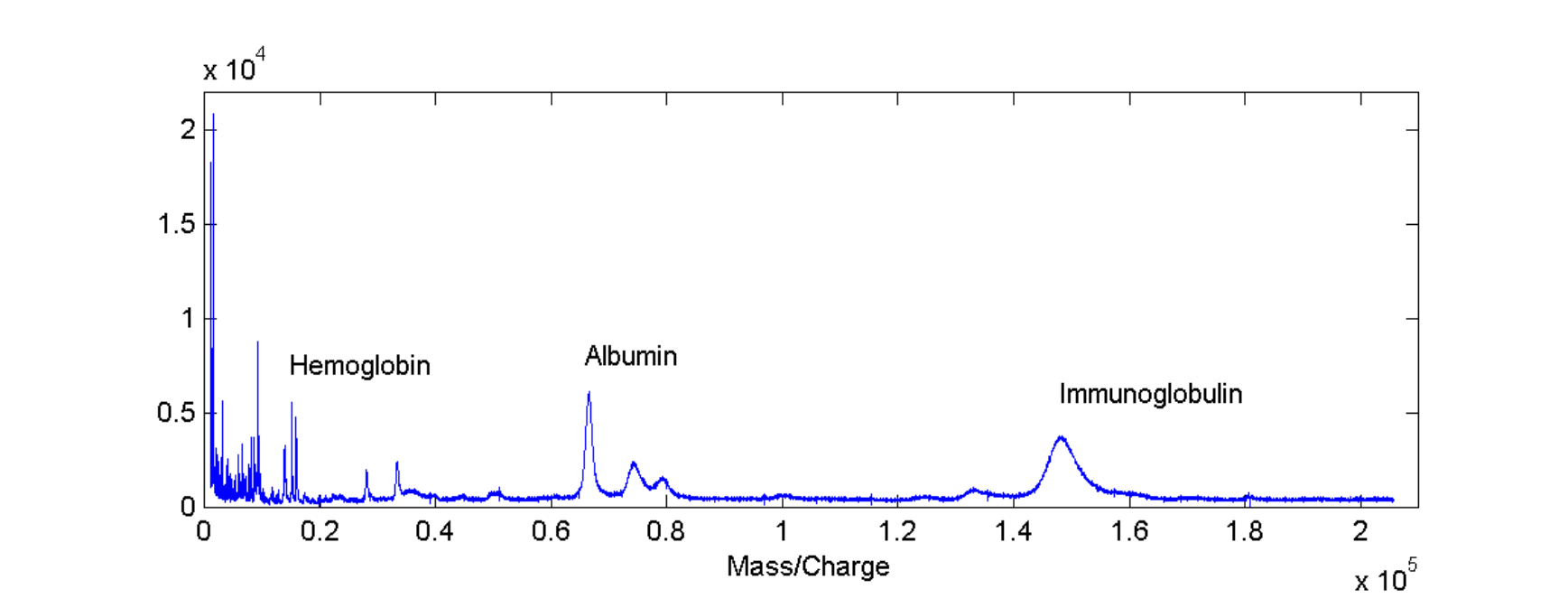
**Detecting 'significant' peaks: Top 9 peaks**

| m/z | Effect | *p* | Comment |
|---|---|---|---|
| 3412.6 | int. | <0.0005 | PC3MM2>A375P for brain-injected only |
| 3496.6 | organ | <0.0005 | Only expressed in brain-injected mice |
| 3886.3 | organ | <0.0005 | Only expressed in brain-injected mice |
| 4168.2 | int. | 0.0005 | PC3MM2>A375P in brain-injected only |
| 4252.1 | int. | <0.0005 | PC3MM2>A375P in brain-injected only |
| 4270.1 | cell line | <0.0005 | PC3MM2>A375P |
| 5805.3 | int. | <0.0005 | brain>lung only for mice given A375P cell-line |
| 6015.2 | cell line | <0.0005 | PC3MM2>A375P |
| 11721 | cell line | <0.0005 | PC3MM2>A375P |
| 11721 | organ | <0.0005 | lung>brain |

# Example: Mass Spectrometry Proteomics

- **Central dogma: DNA → mRNA → protein**
- **Microarrays: measure expression levels of 10,000s of genes in sample (amount of mRNA)**
- **Proteomics: look at proteins in sample.**
  - **Gaining increased attention in research**
    - **Proteins more biologically relevant than mRNA**
    - **Can use readily available fluids (e.g. blood, urine)**
- **MALDI-TOF: mass spectrometry instrument that can see 100s or 1000s of proteins in sample**

# Sample MALDI-TOF Spectrum




- **MALDI-TOF Spectrum: observed function**
- **g(*t*) = intensity of spectrum at m/z value *t***
- **Intensity at peak (roughly) estimates the abundance of some protein with molecular weight of *t* Daltons**

# Example: Mouse proteomics study

- 16 mice had 1 of 2 cancer cell lines injected into 1 of 2 organs (lung or brain)
- Cell lines:
  - A375P: human melanoma, low metastatic potential
  - PC3MM2: human prostate, highly metastatic
- Blood serum extracted and placed on SELDI chip
- Run at 2 different laser intensities (low/ high)
- Total of 32 spectra (observed functions), 2 per mouse
- Observations on equally-spaced grid of 7985

# Example: Mouse proteomics study

- **Goal:** **Find proteins differentially expressed by:**
  - **Host organ site (lung/brain)**
  - **Donor cell line (A375P/PC3MM2)**
  - **Organ-by-cell line interaction**
- **Combine information across laser intensities:**

**Requires us to include in modeling:**

- **Functional laser intensity effect**
- **Random effect functions to account for correlation between spectra from same mouse**

# Model: SELDI Example

**Let $Y_i(t)$ be the SELDI spectrum $i$**

$$\log_2\{Y_i(t)\} = B_0(t) + \sum_{j=1}^{4} X_{ij} B_j(t) + \sum_{k=1}^{16} Z_{ik} U_k(t) + E_i(t)$$

- **$X_{i1}=1$ for lung, $-1$ brain. $X_{i2}=1$ for A375P, $-1$ for PC3MM2**

  **$X_{i3} = X_1 * X_2$ $X_{i4}=1$ for low laser intensity, $-1$ high.**

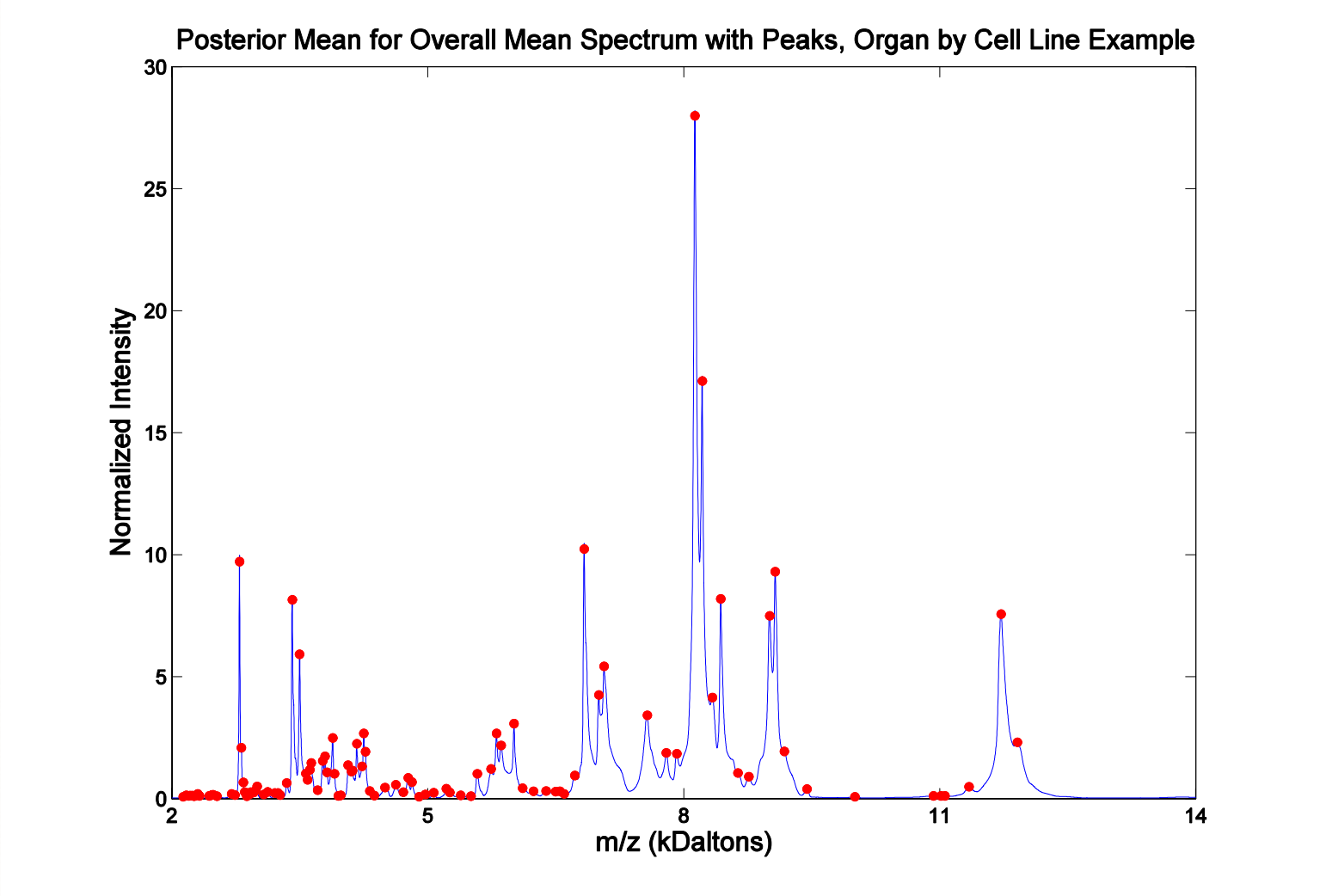
- **$B_0(t)$ = overall mean spectrum $B_1(t)$ = organ main effect function**

  **$B_2(t)$ = cell-line main effect $B_3(t)$ = org x cell-line int function**

  **$B_4(t)$ = laser intensity effect function**

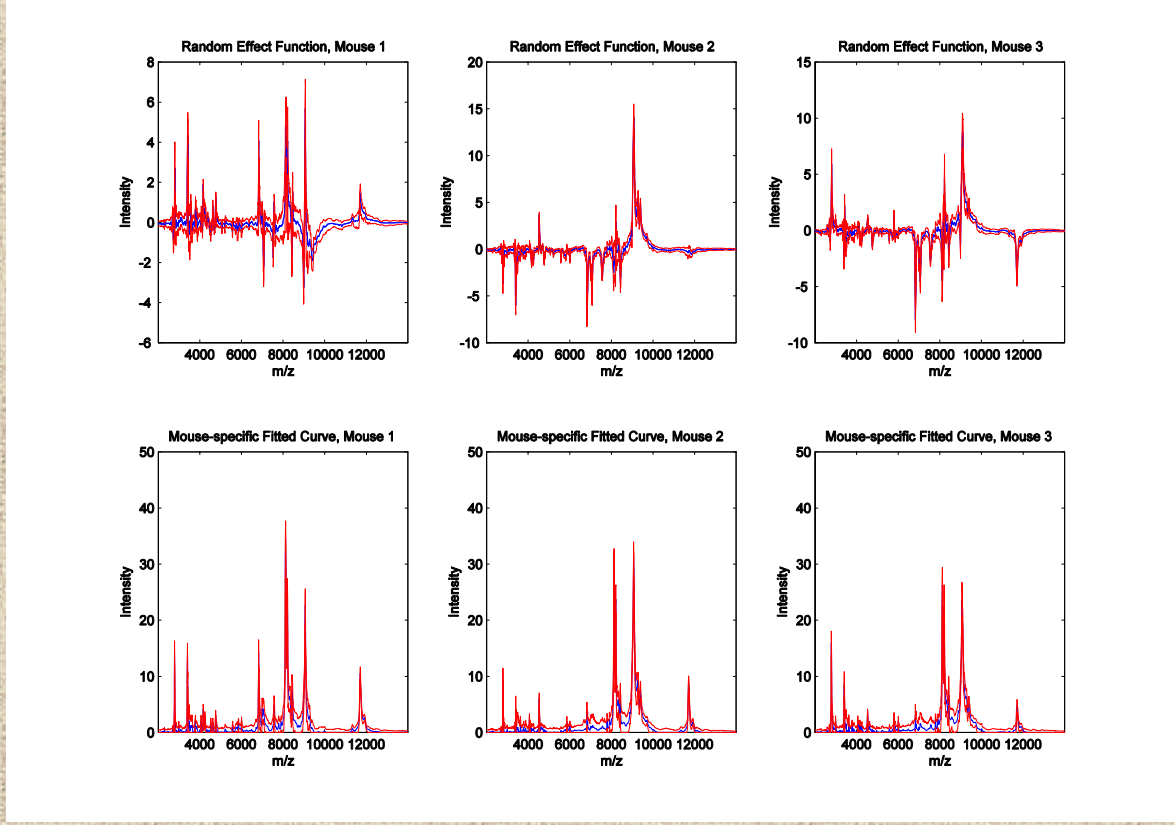
- **$Z_{ik}=1$ if spectrum $i$ is from mouse $k$ ($k=1, \ldots, 16$)**
- **$U_k(t)$ is random effect function for mouse $k$.**

# Adaptive Regularization



# Adaptive Regularization

- **Posterior samples/estimates of random effect functions $U_j(t)$ are also *adaptively regularized* from Gaussian prior, since each wavelet coefficient has its own random effect & residual variance**




- **Able to preserve spikes in random effect functions, as well**
- **Important for estimation of random effect functions AND for valid inference on fixed effect functions.**

# Bayesian Inference

**Given posterior samples of all model quantities, we can perform any desired Bayesian inference or prediction:**

1. **Pointwise posterior credible intervals for funct. effects**
2. **Posterior probabilities of interest – either pointwise, joint, or aggregating across locations within the curve.**
3. **Can account for multiple testing in identifying significant regions of curves by controlling the expected Bayesian FDR**
4. **Can compute posterior predictive distributions, which can be used for model-checking or other purposes.**

# Bayesian Inference:

## Identifying Significant Regions of Curves

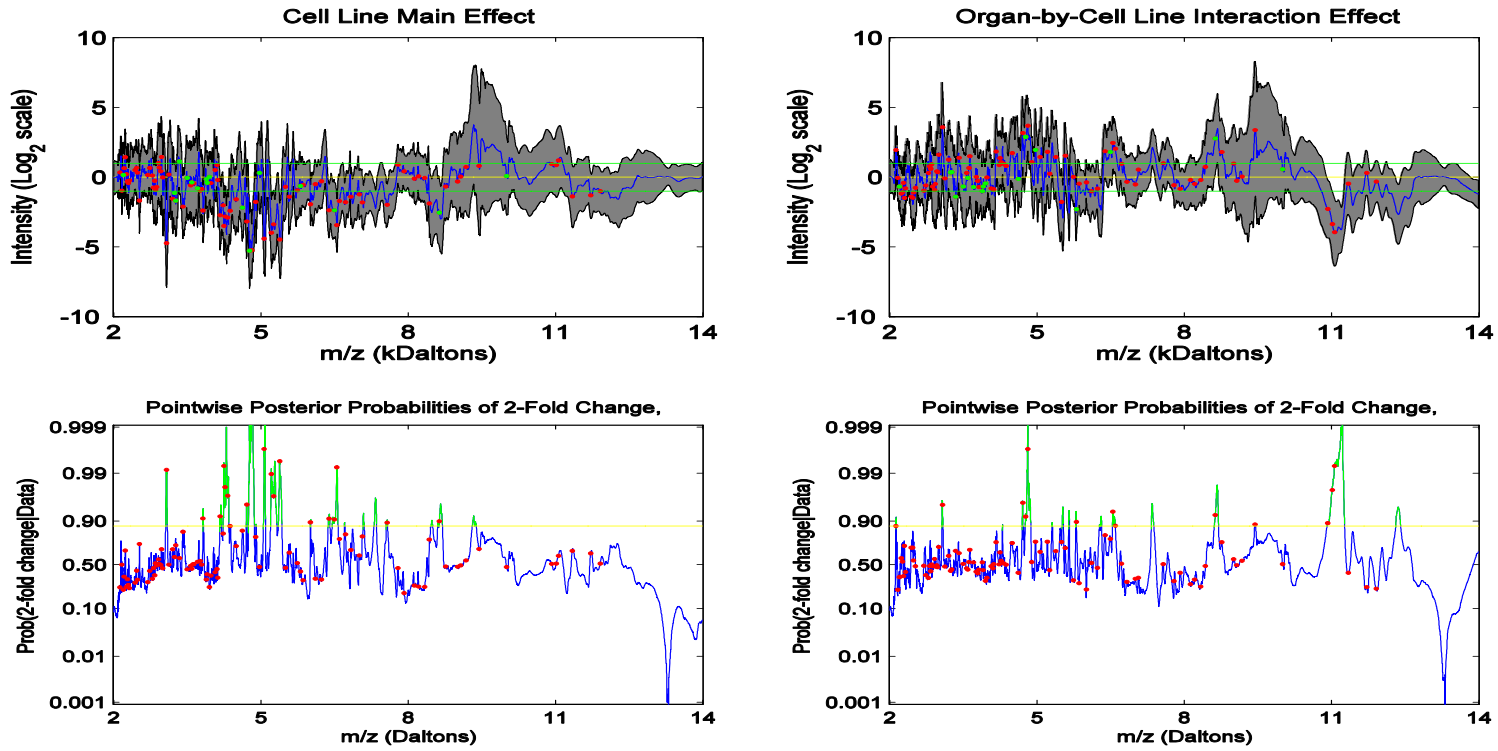
**Procedure (desired effect size $\geq\delta$, FDR $\alpha$)**

1. **Compute pointwise posterior probabilities of effect size of interest being at least $\delta$ for $l=1, \ldots, T$** $\quad p_{il}=\Pr\{|B_j(t_l)|>\delta|Y\}$
2. **Sort peaks in descending order of $p_{il}$ $\{p_{i(l)}, l=1, \ldots, T\}$**
3. **Identify cutpoint $\varphi_\alpha$ on posterior probabilities that controls expected Bayesian FDR to be $\leq \alpha$** $\quad \varphi_\alpha=p_{i(\lambda)}$, **where**

$$\lambda = \max\left[ l^* : \sum_{l=1}^{l^*} \{1 - p_{i(l)}\} \leq \alpha \right]$$

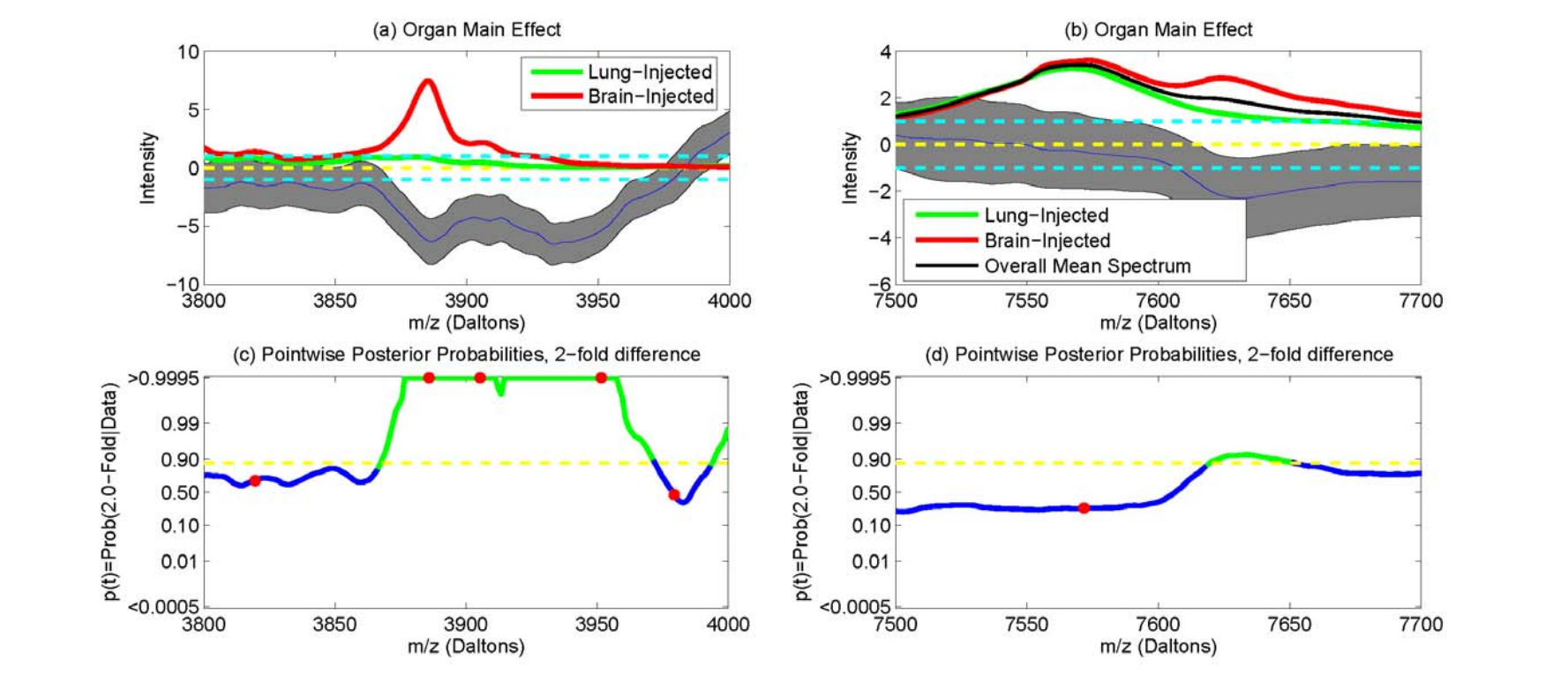
4. **Flag the set of locations $\{t_l : p_{il} \leq \varphi_\alpha\}$ as significant (According to model, expect only $\alpha$ to be false pos.)**

# Results: SELDI Example

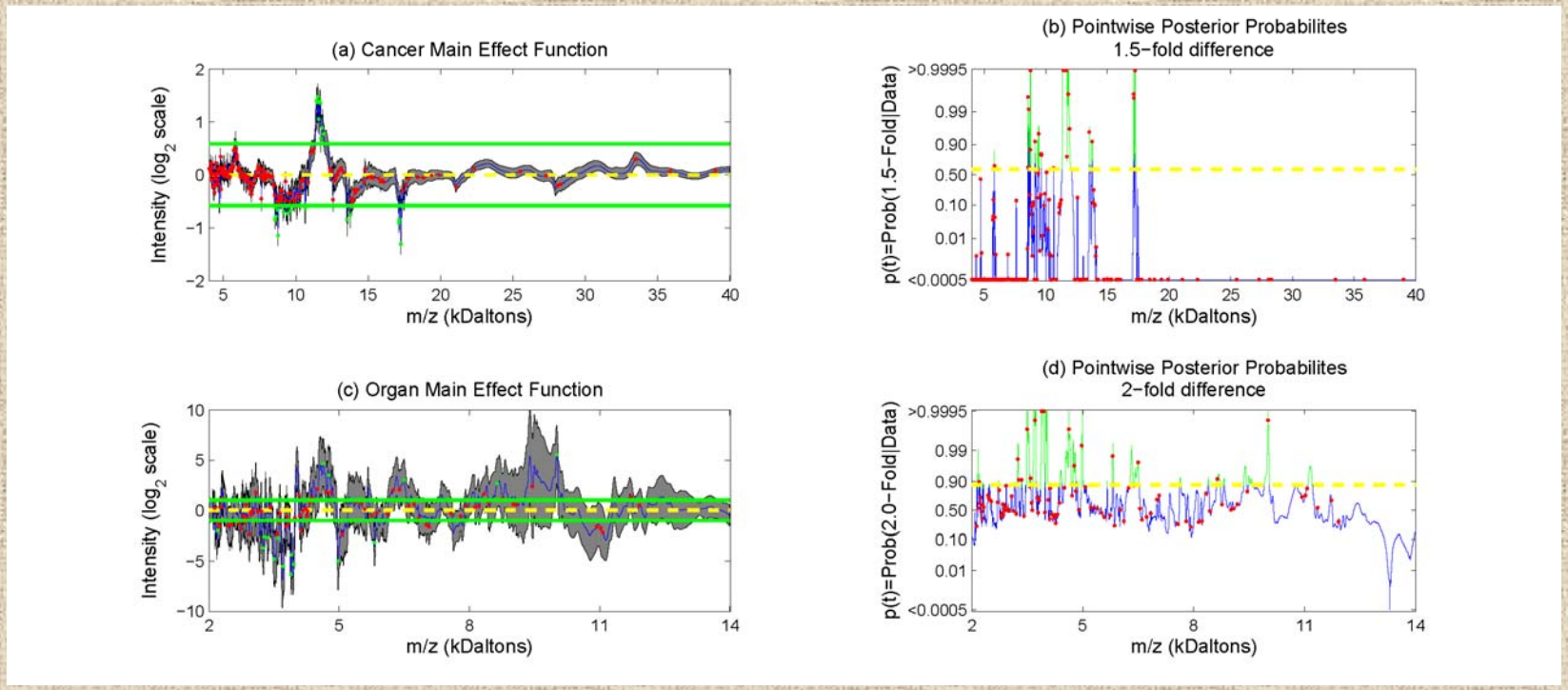


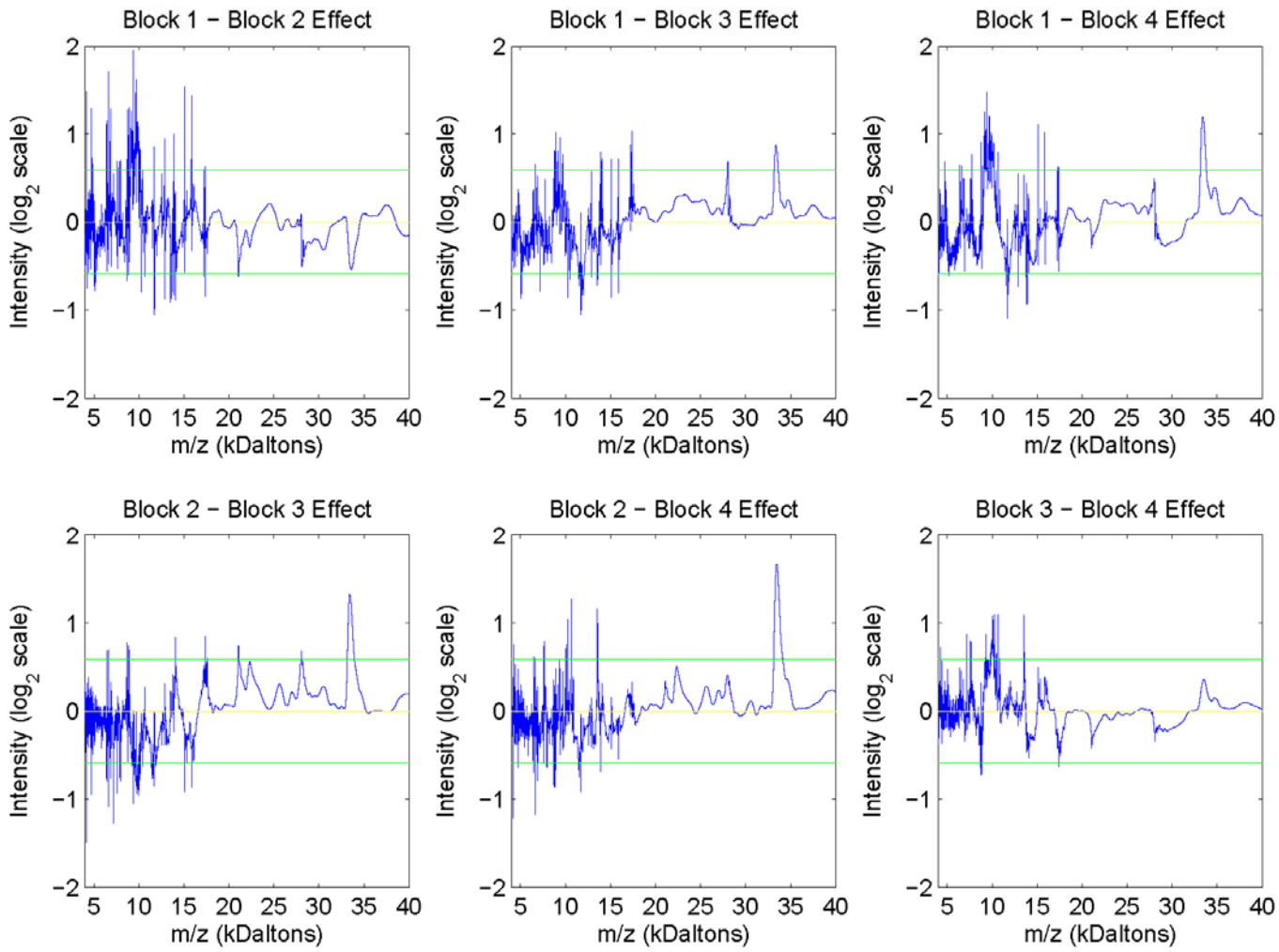

- **Using $\alpha$=0.05, $\delta$=1 (2-fold expression on $\log_2$ scale), we flag a number of spectral regions.**

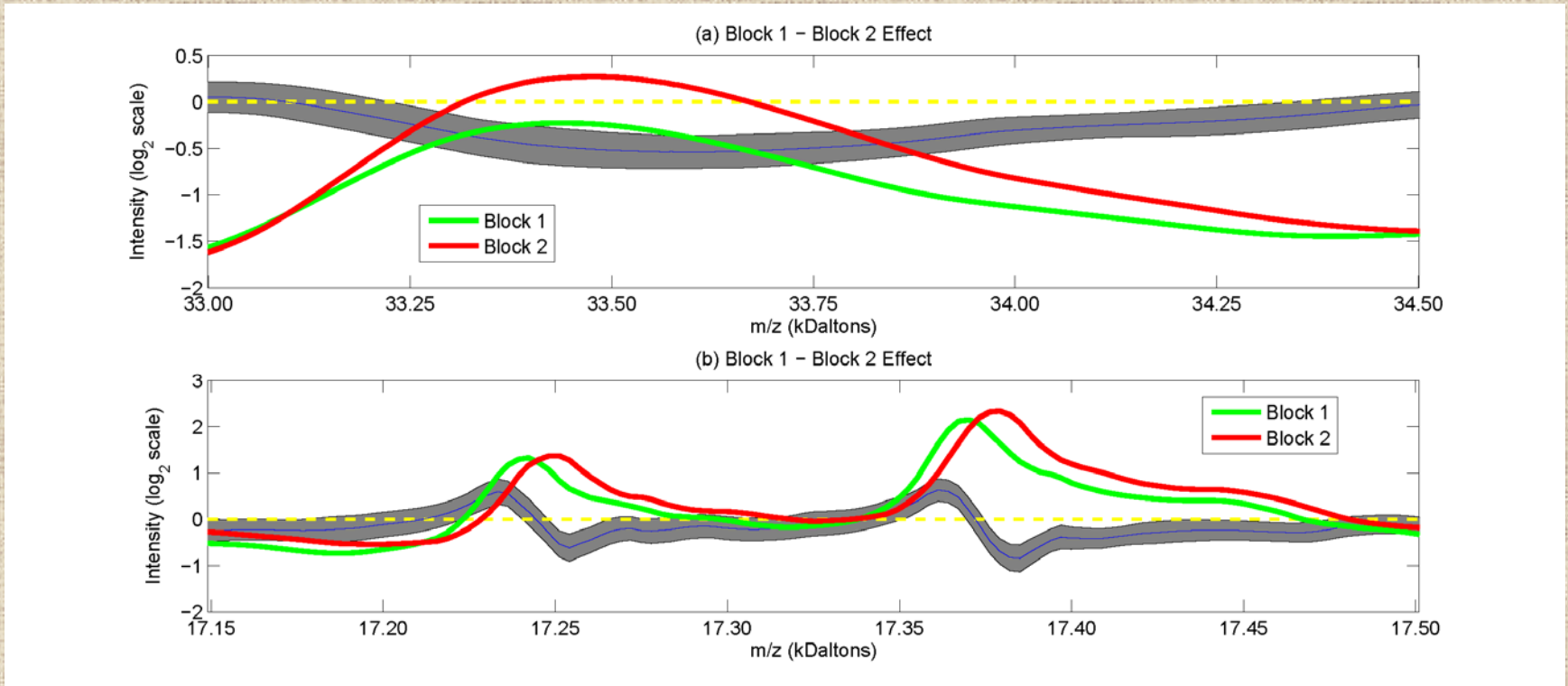
# Results: SELDI Example



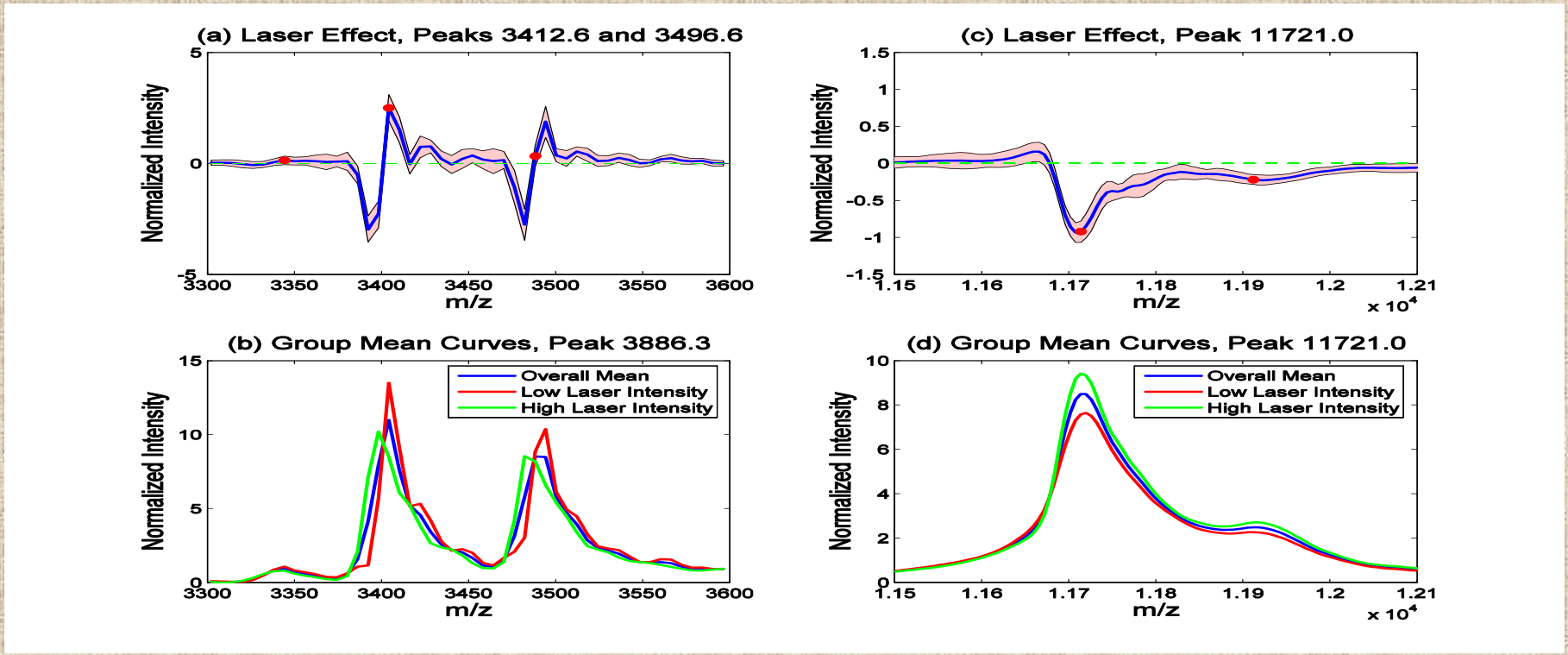

- **3900 D (CGRP-II): dilates blood vessels in brain**
- **7620 D (nerogranin): active in synaptic modeling in brain (Not detected as peak)**


(a) Cancer Main Effect Function
Intensity (log2 scale)
m/z (kDaltons)
(b) Pointwise Posterior Probabilites
1.5−fold difference
p(t)=Prob(1.5−Fold|Data)
>0.9995
0.99
0.90
0.50
0.10
0.01
<0.0005
m/z (kDaltons)
(c) Organ Main Effect Function
Intensity (log2 scale)
m/z (kDaltons)
(d) Pointwise Posterior Probabilites
2−fold difference
p(t)=Prob(2.0−Fold|Data)
>0.9995
0.99
0.90
0.50
0.10
0.01
<0.0005
m/z (kDaltons)


Block 1 − Block 2 Effect
Block 1 − Block 3 Effect
Block 1 − Block 4 Effect
Block 2 − Block 3 Effect
Block 2 − Block 4 Effect
Block 3 − Block 4 Effect
Intensity (log2 scale)
m/z (kDaltons)
2
1
0
−1
−2
5 10 15 20 25 30 35 40

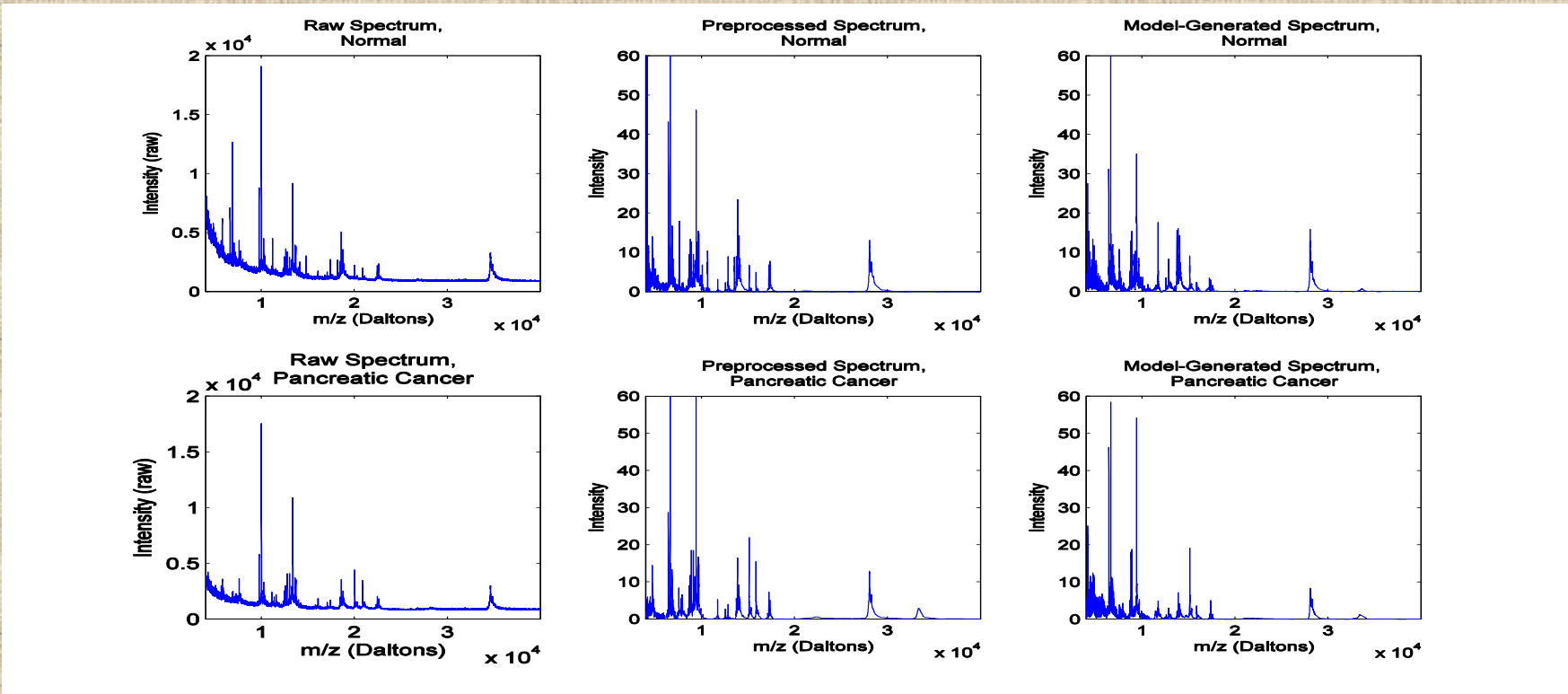

(a) Block 1 − Block 2 Effect
Intensity (log2 scale)
0.5
0
−0.5
−1
−1.5
−2
33.00
33.25
33.50
33.75
34.00
34.25
34.50
m/z (kDaltons)
Block 1
Block 2
(b) Block 1 − Block 2 Effect
Intensity (log2 scale)
3
2
1
0
−1
−2
17.15
17.20
17.25
17.30
17.35
17.40
17.45
17.50
m/z (kDaltons)
Block 1
Block 2

# Results: SELDI Example

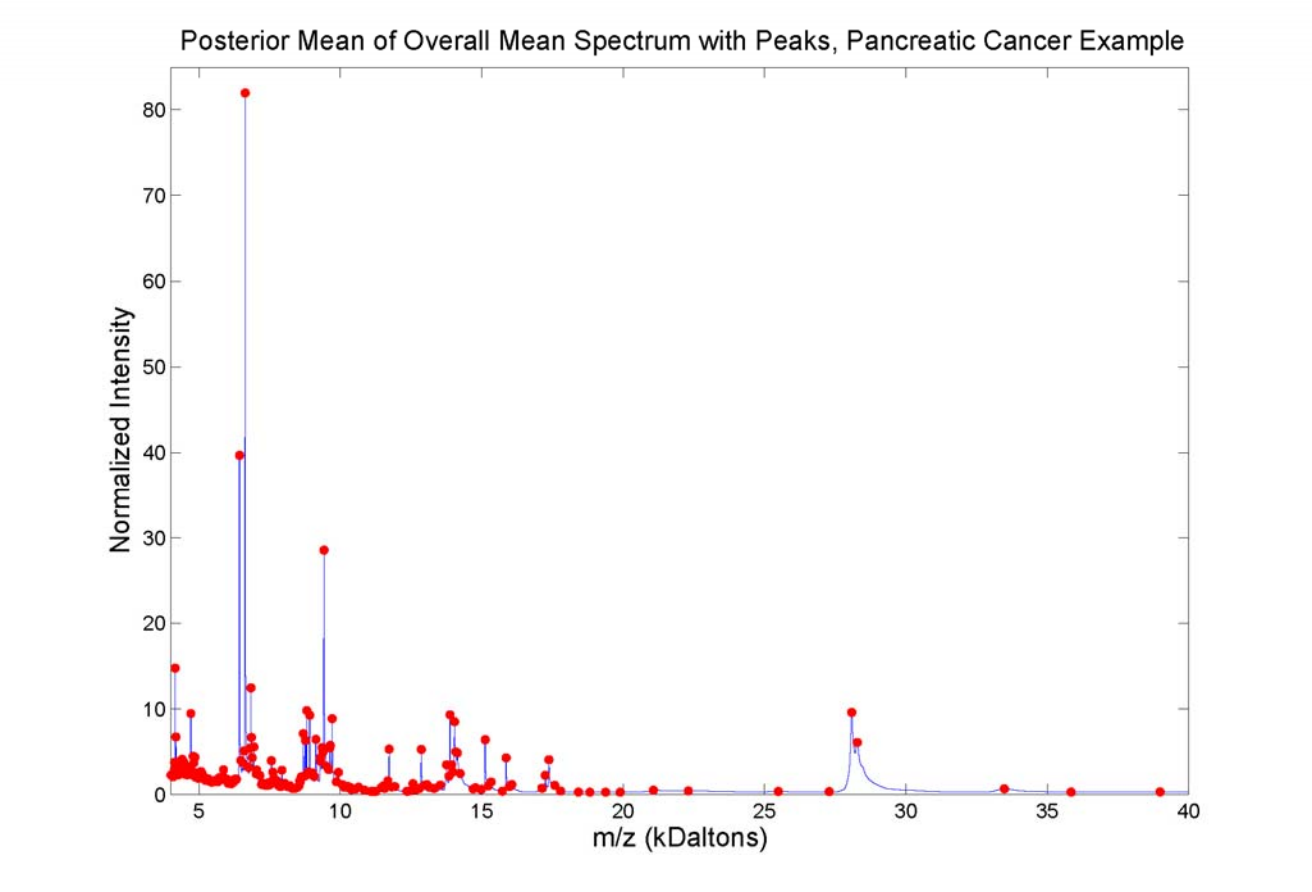



- **Inclusion of nonparametric functional laser intensity effect is able to adjust for systematic differences in the *x* and *y* axes between laser intensity scans**

# Results: SELDI Example




- **Draws of spectra from posterior predictive distribution yield data that looks like real SELDI data (3rd column), indicating reasonable model fit.**


Posterior Mean of Overall Mean Spectrum with Peaks, Pancreatic Cancer Example
Normalized Intensity
m/z (kDaltons)
0
10
20
30
40
50
60
70
80
5
10
15
20
25
30
35
40