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**

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):

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

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

$$e \sim N(0, \underbrace{R}_{N\times N})$$

- **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, $Y_i(t)$, $i=1, \ldots, N$

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

- $B_j(t)$ = 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*

$$\underset{N\times \mathrm{T}}{Y} = \overset{N\times p}{\overbrace{X}}\ \underset{p\times \mathrm{T}}{B} + \overset{N\times m}{\overbrace{Z}}\ \underset{m\times \mathrm{T}}{U} + \underset{N\times \mathrm{T}}{E}$$

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

- 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)

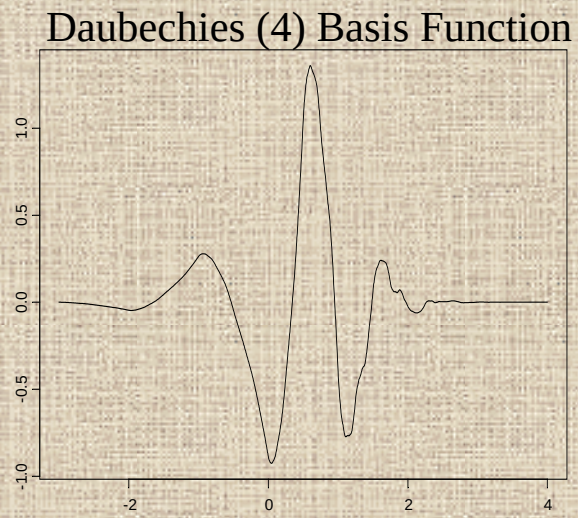
Introduction to Wavelets

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

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

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

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

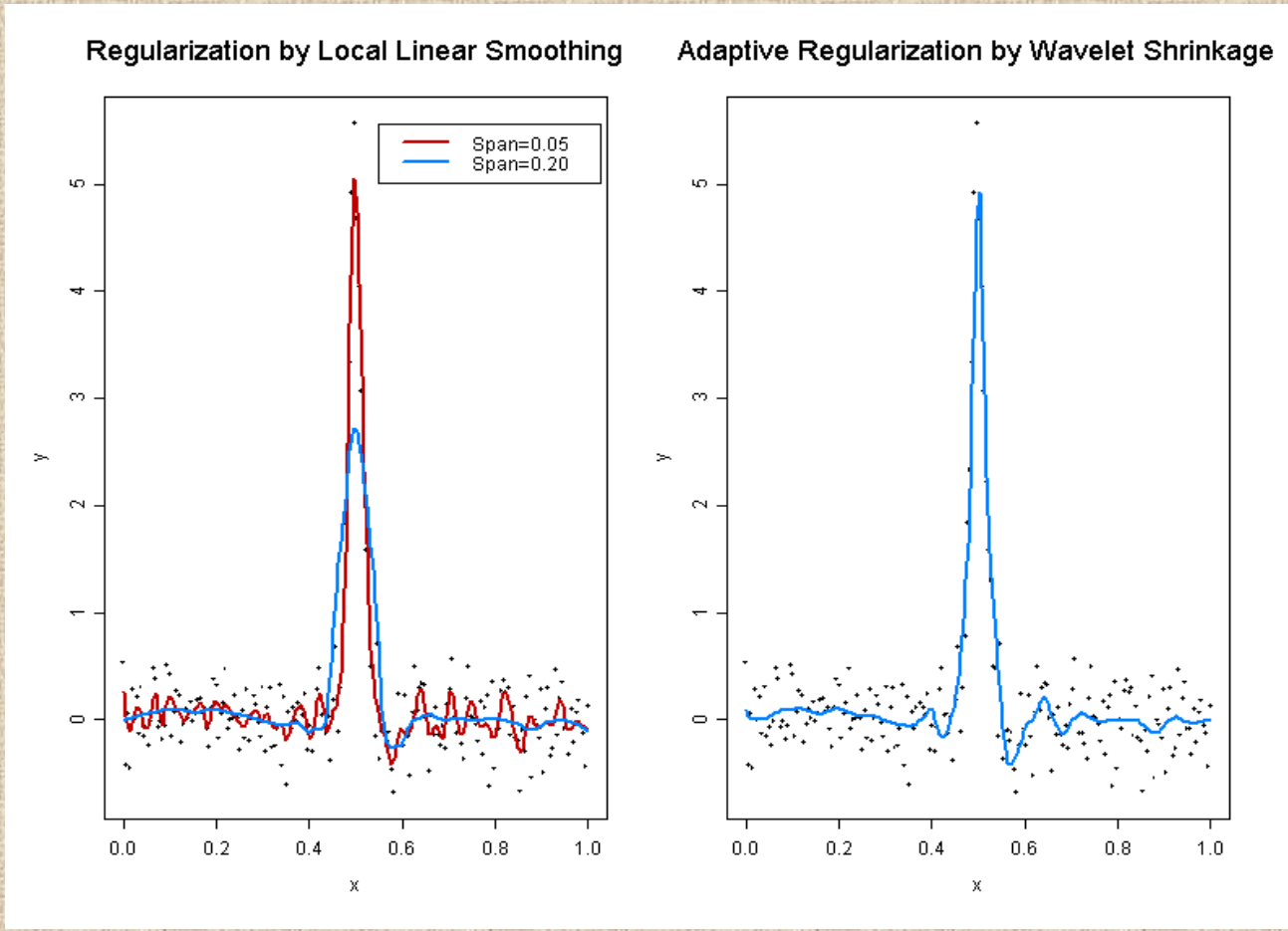


- **Discrete Wavelet Transform (DWT): fast algorithm {O(*T*)} for obtaining *T* empirical wavelet coefficients for curves sampled on equally-spaced grid of length *T*.**
- **Linear Representation:** $d = y\,W'$
  - ***W'* =*T*-by-*T* orthogonal projection matrix**
- **Inverse DWT (IDWT):** $y = d\,W$

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.**

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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.**

Wavelet-Based FMM:

General Approach

1. **Project** observed functions Y **into wavelet space.**
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   (Use MCMC to get posterior samples)

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

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

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

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

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Projecting FMM to Wavelet Space

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

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

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

Projecting FMM to Wavelet Space

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

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

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

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

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

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

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

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 $B_i(t)$.**
- **τ_{ij} & π_{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 Ω 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

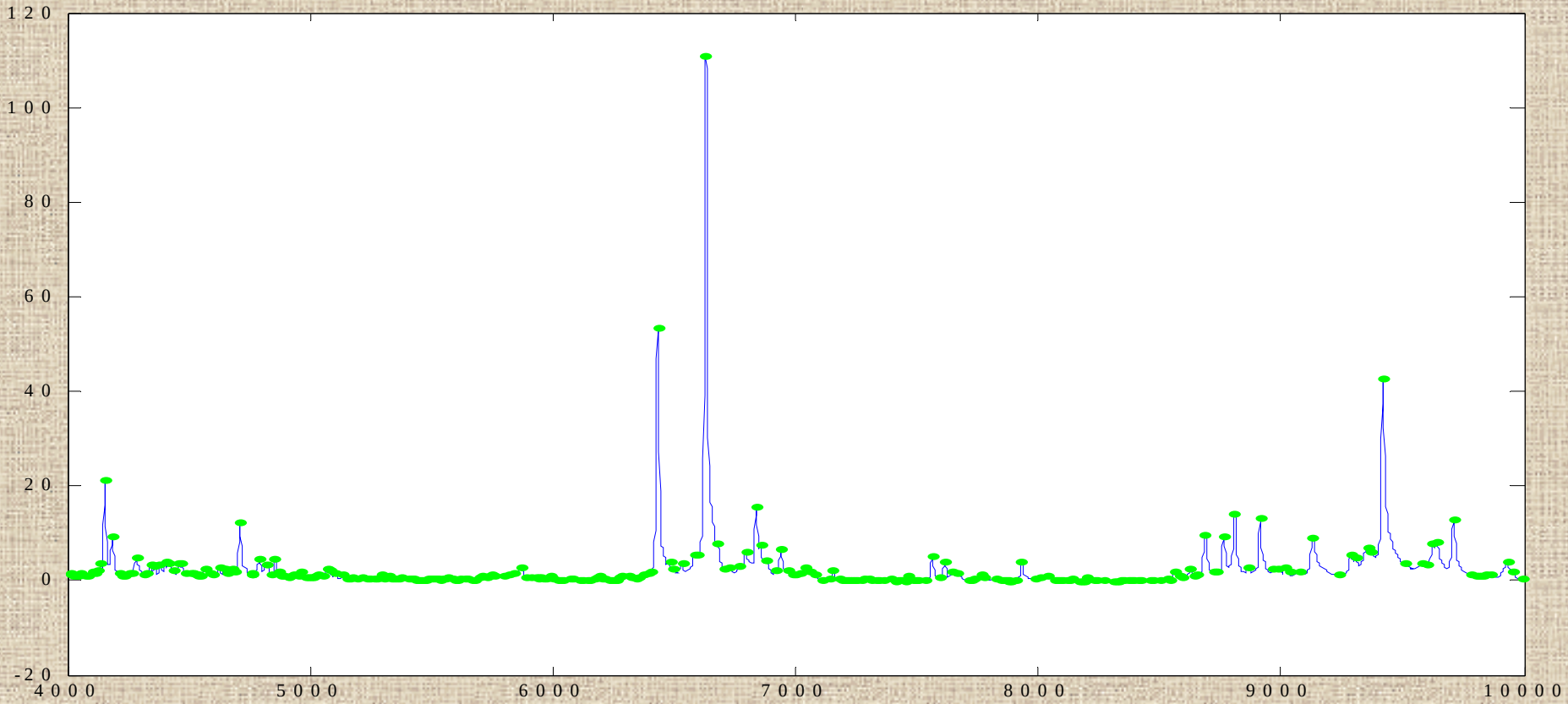
3. Project wavelet-space estimates (posterior samples) back to data space.

- **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

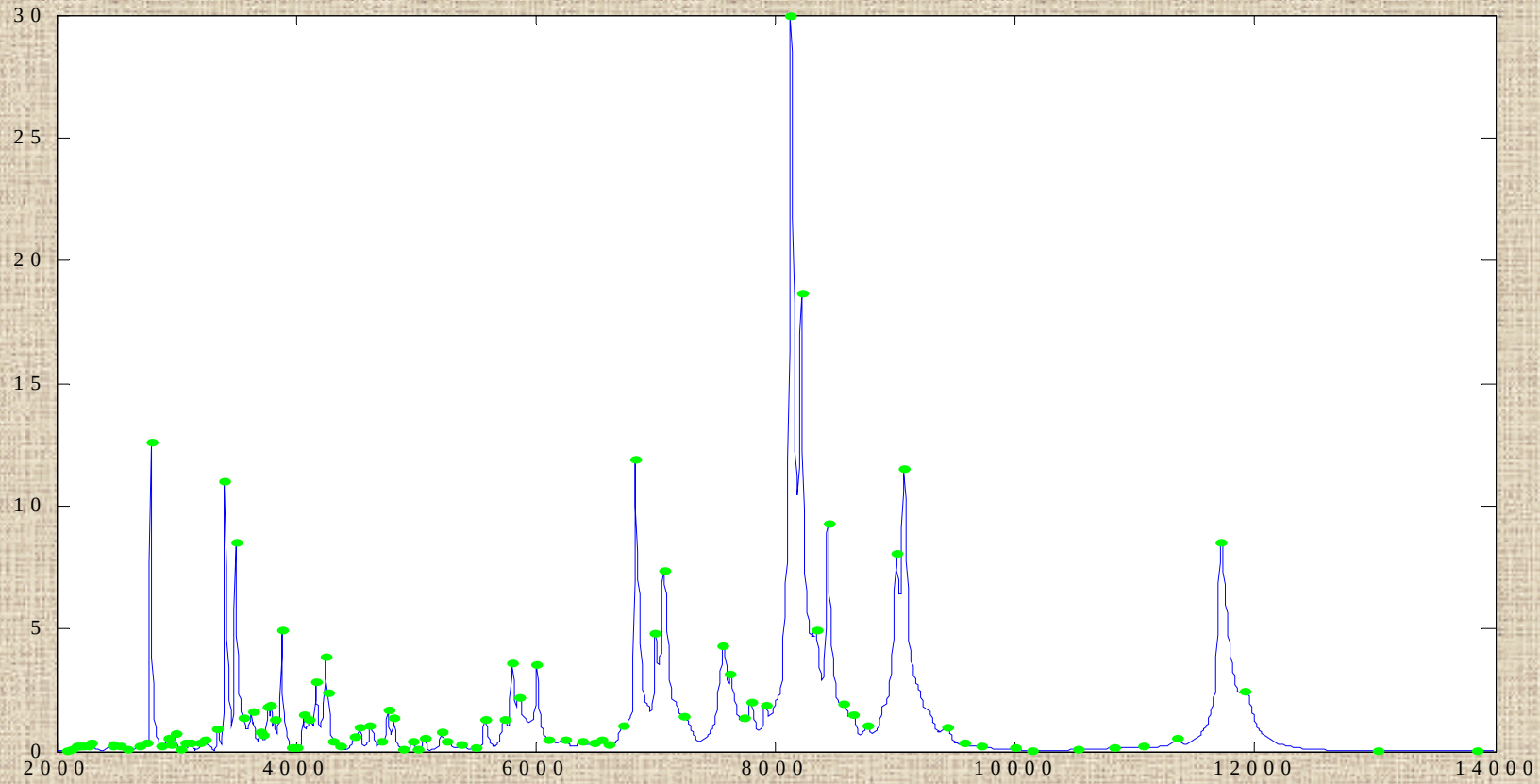
- **Focus specifically on peaks – locations in spectra likely to correspond to proteins/peptides**
- **Can use posterior mean estimate of overall mean spectrum for peak detection (Morris et al. 2005)**
- **All local maxima in (denoised) overall mean spectrum considered peaks, possibly subject to some 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.**

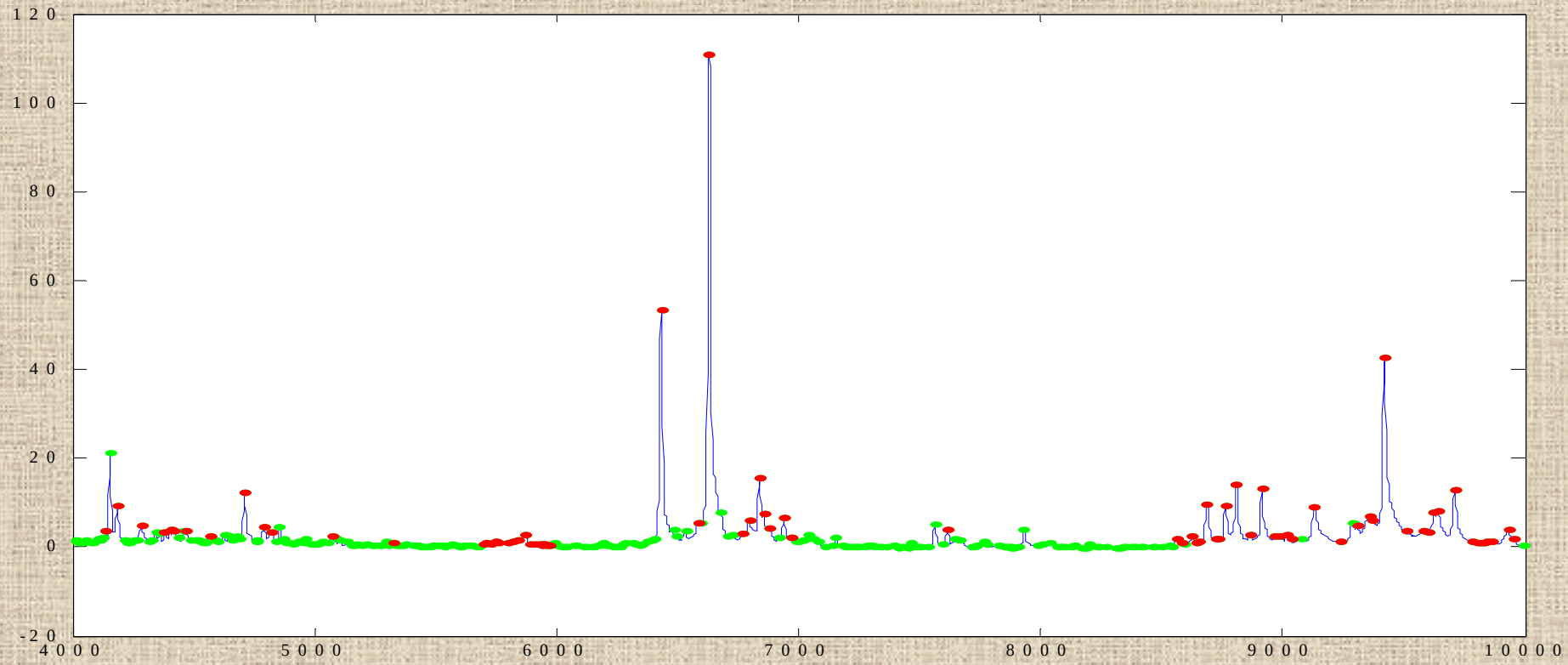
   $p_{(1)}, p_{(2)}, \cdots, p_{(pK)}$

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

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

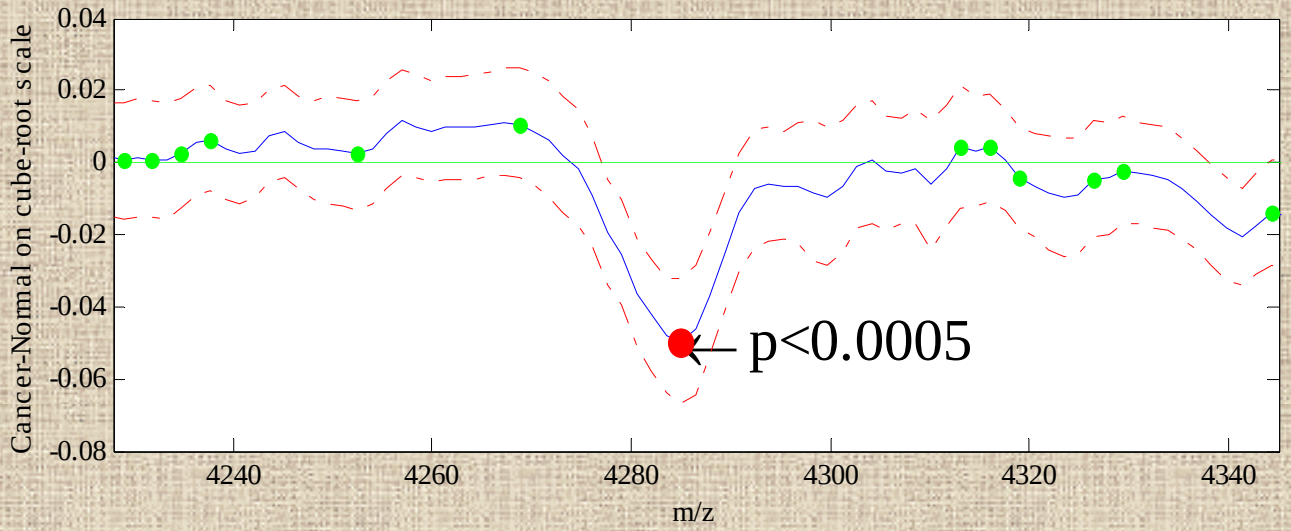
3. **Let $\psi = p_{(K^*)}$. Any peak i with $p_{ij} < \psi$ is called "differentially expressed" for outcome j**

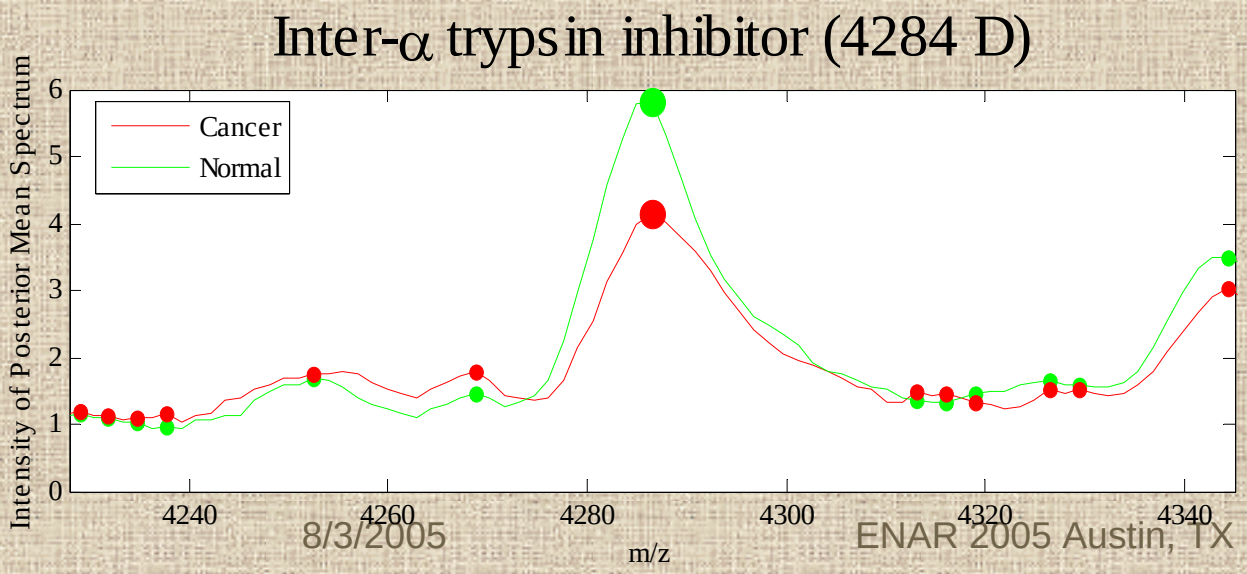
Pancreatic Cancer:
Differentially Expressed Peaks



- **370 peaks detected**
- **83 differentially expressed using α=0.01**

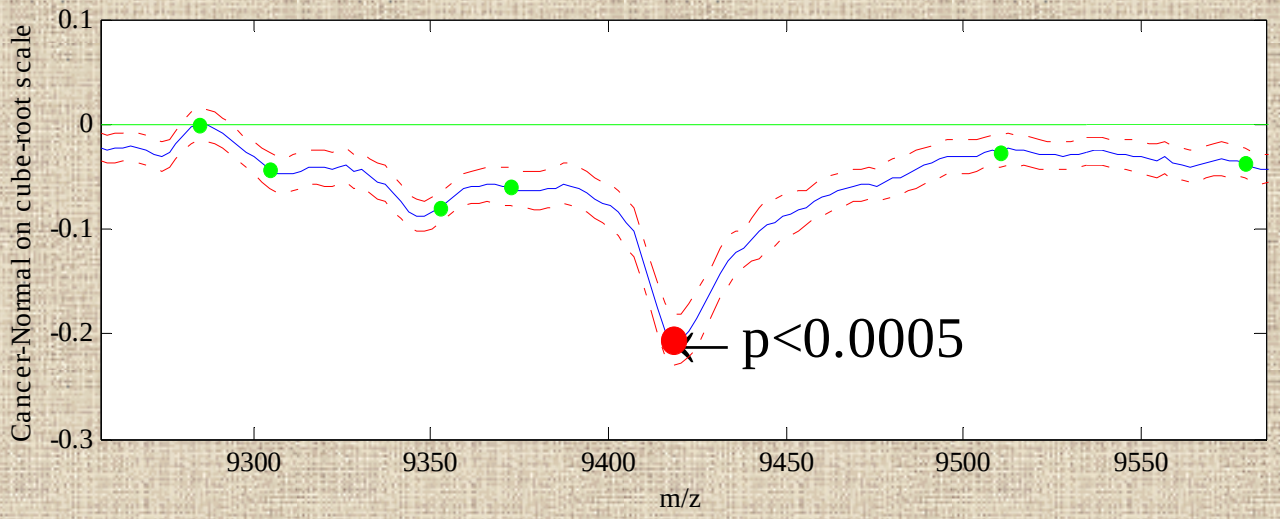
Pancreatic Cancer: Results



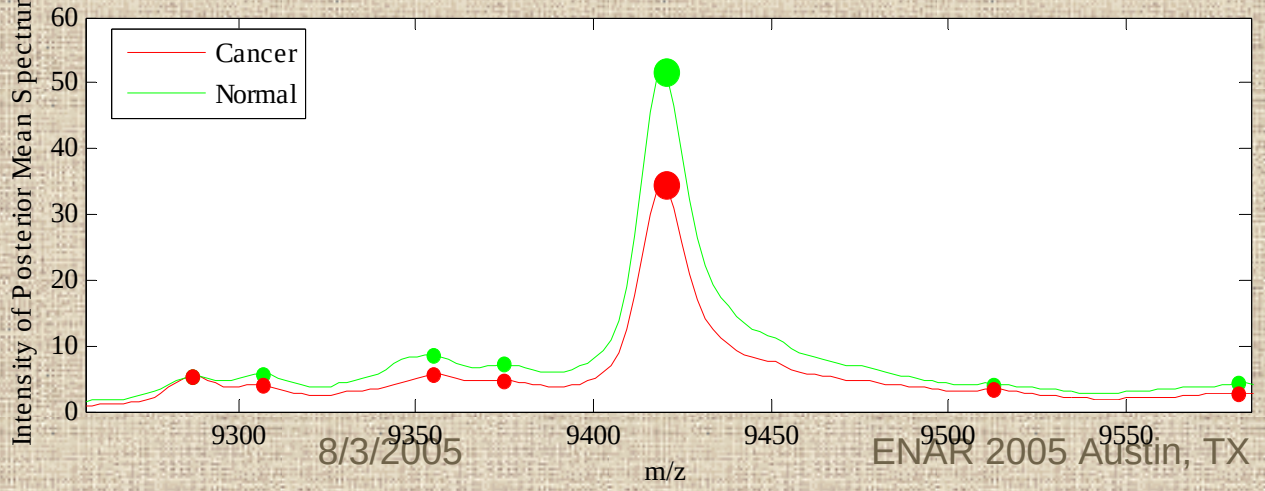


- Known to be related to pancreatic cancer
- Under-expressed in serum of cancerous patients
- May not be specific to pancreatic cancer

Pancreatic Cancer: Results

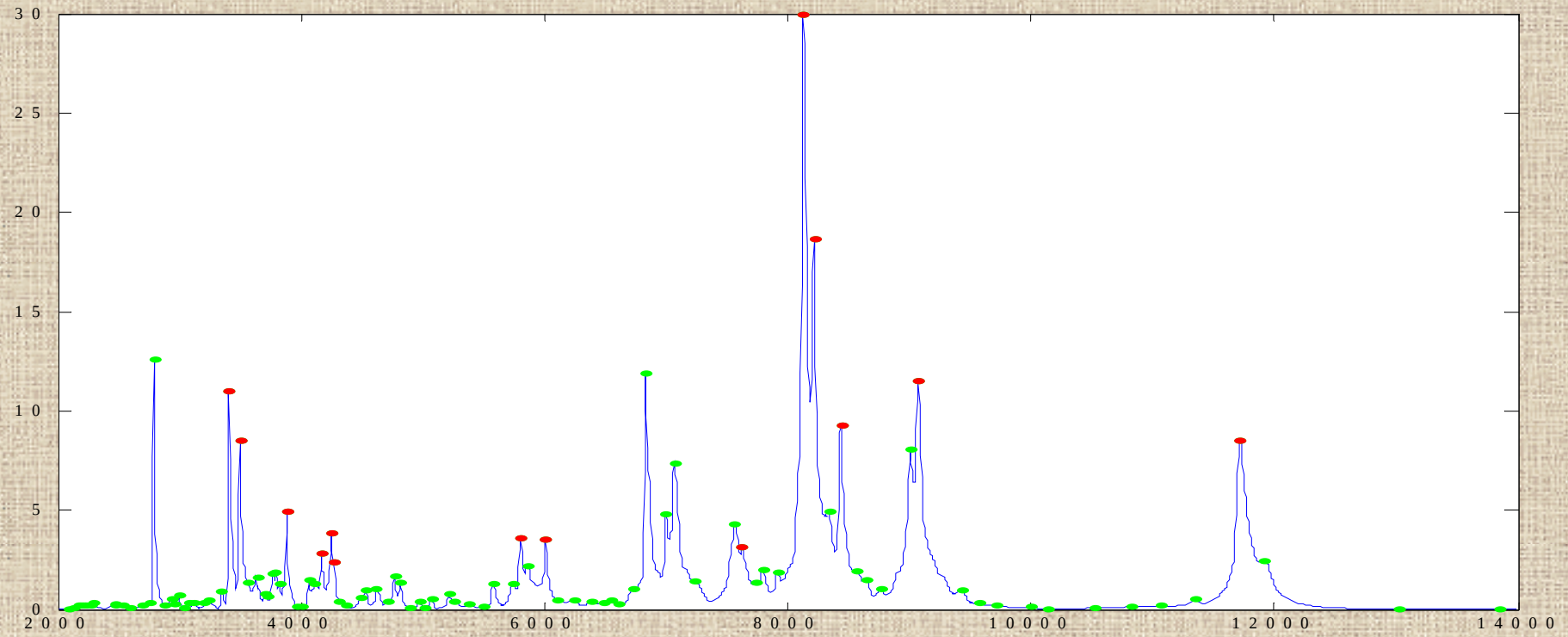


Small inducible cytokine B14 precursor (9419 D)



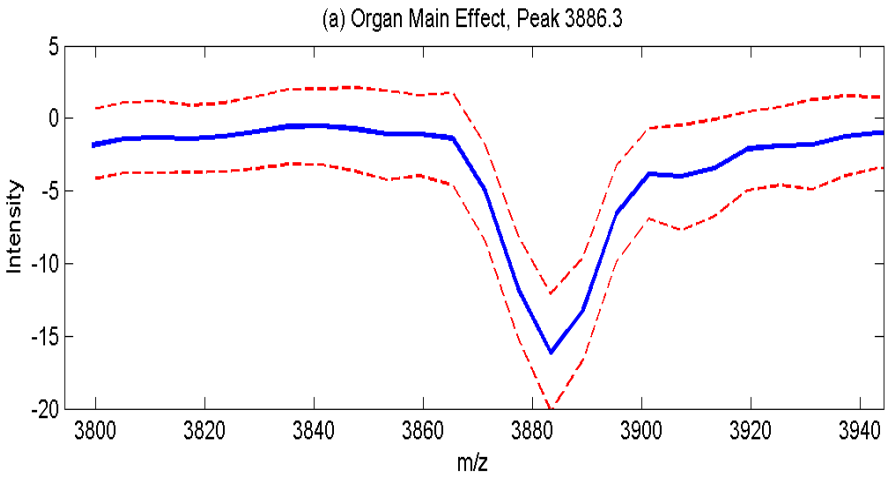
- Secreted from various organs, including pancreas
- Highly expressed in normal tissue with no inflammatory response
- Low expression in cancer cell lines

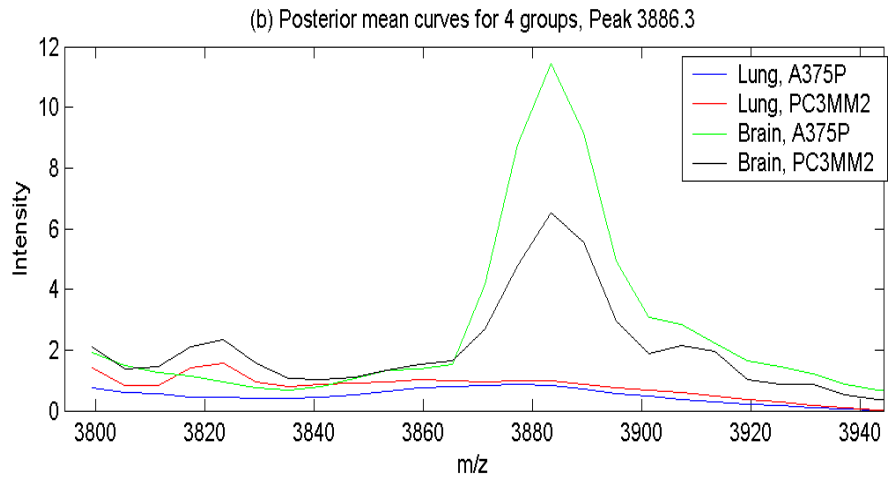
Organ-by-Cell Line:
Differentially Expressed Peaks



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

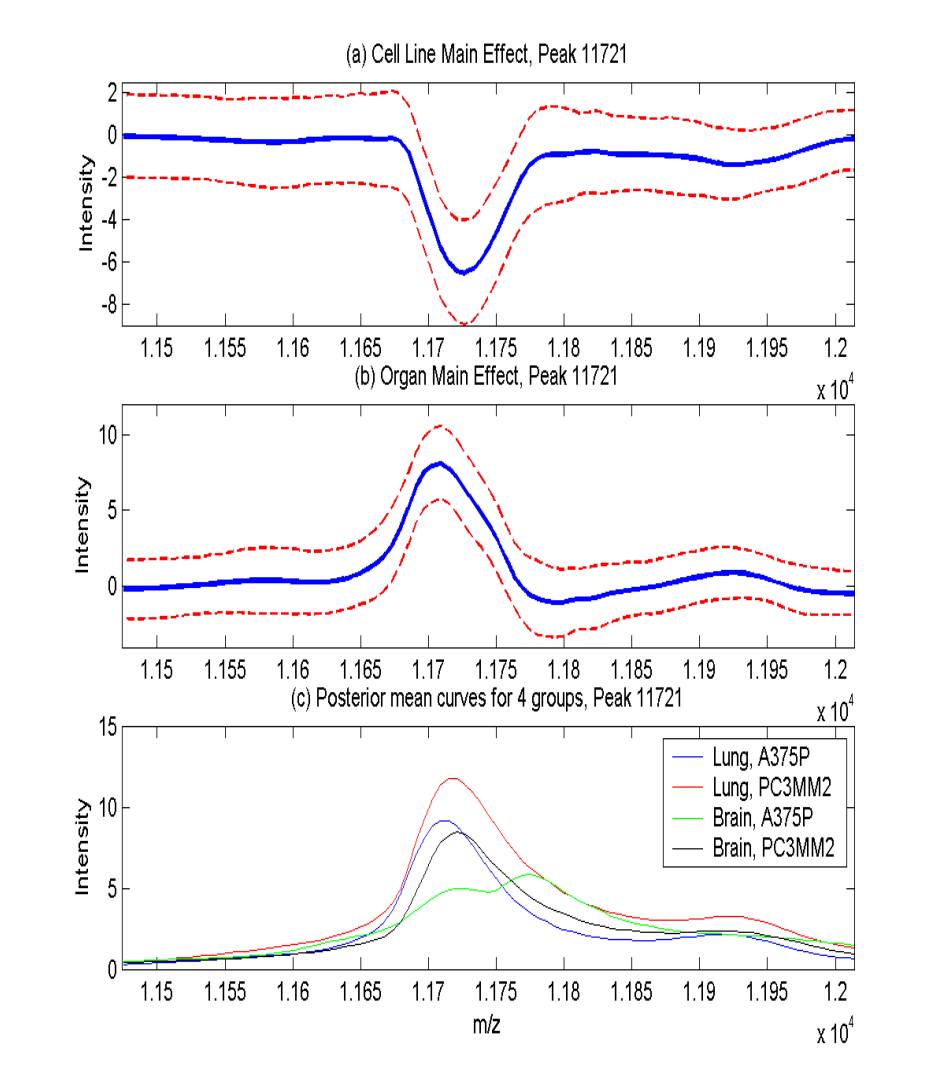
Organ-by-Cell Line: Results





- 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

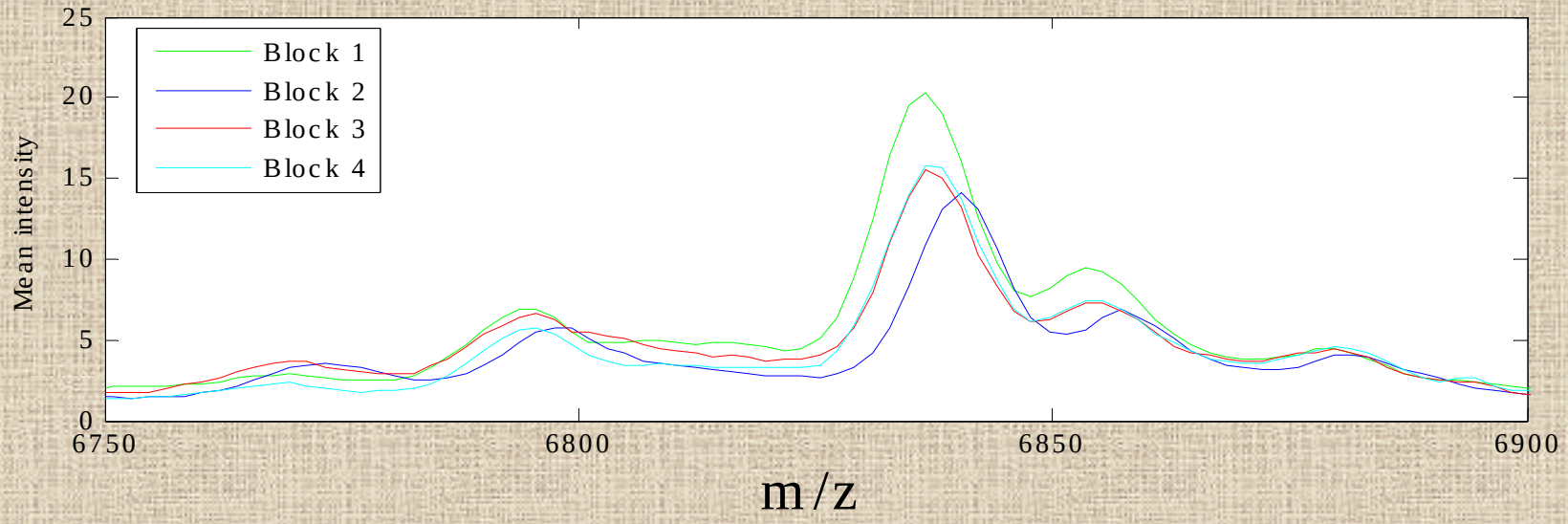


- **Higher in mice injected with metastatic (PC3-MM2) cell line**
- **May be MTS1 (11721.43 Dalt), metastatic cell protein in mouse proteome.**
- **Also higher in lung-injected mice than brain-injected mice**

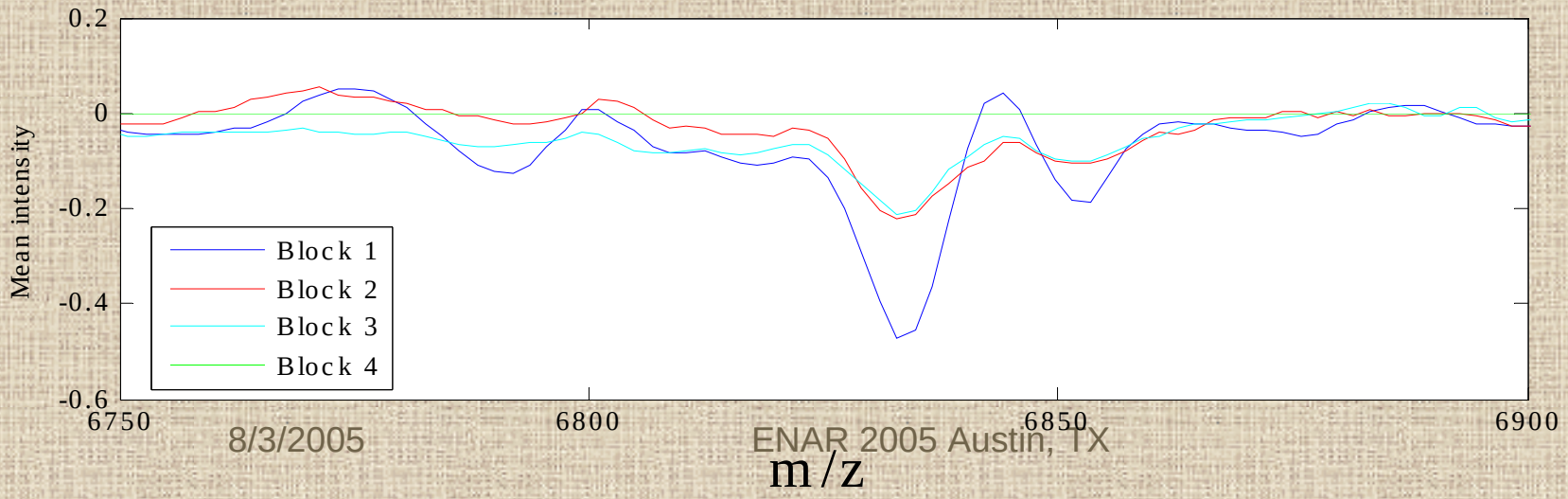
Bayesian Inference:
Investigating Block Effects

- 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

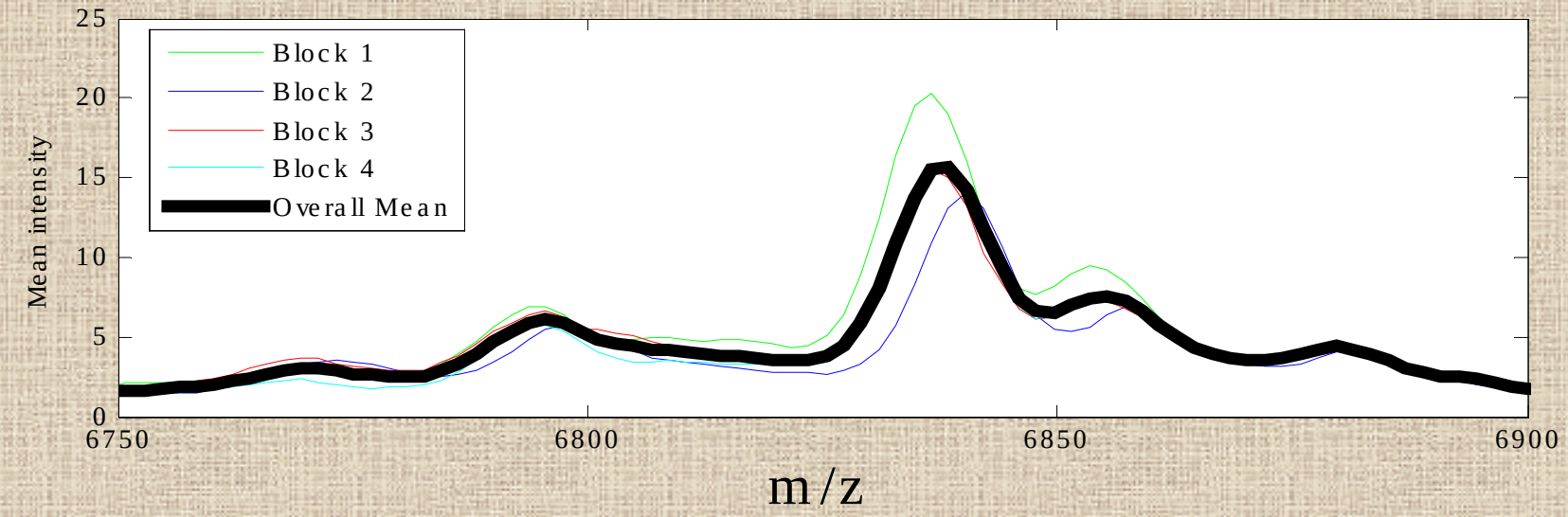
Pancreatic Cancer: Block Effects



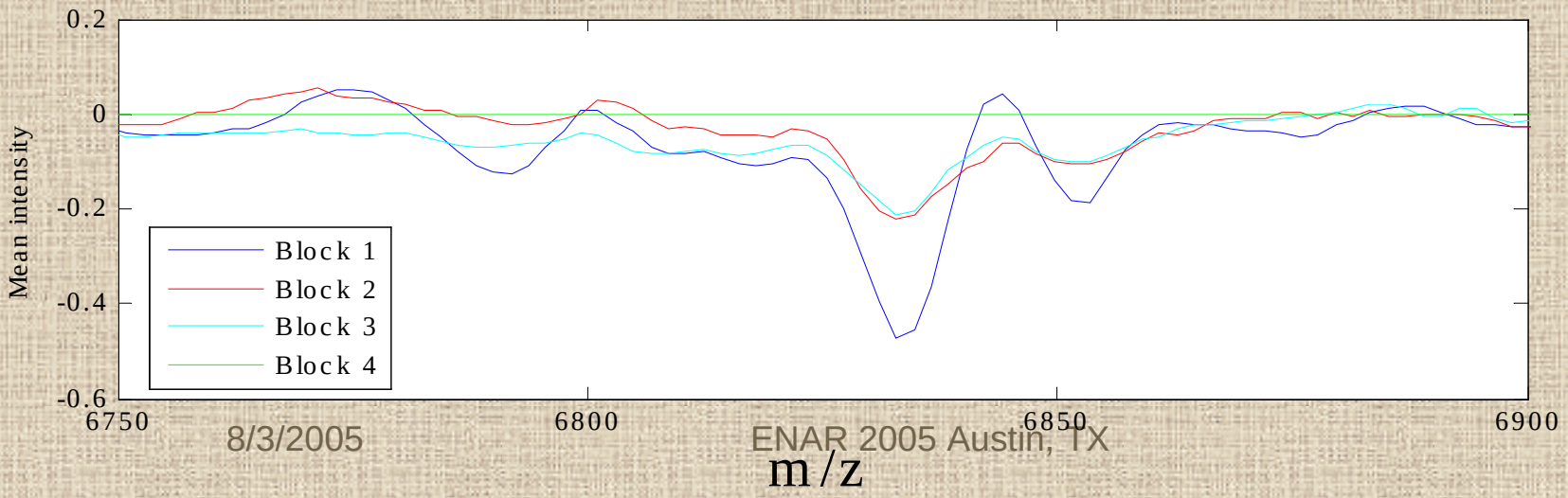
Block Effects



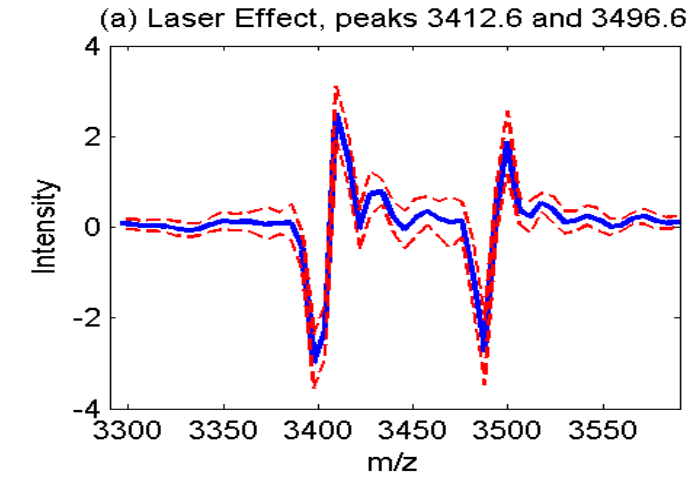
Pancreatic Cancer: Block Effects

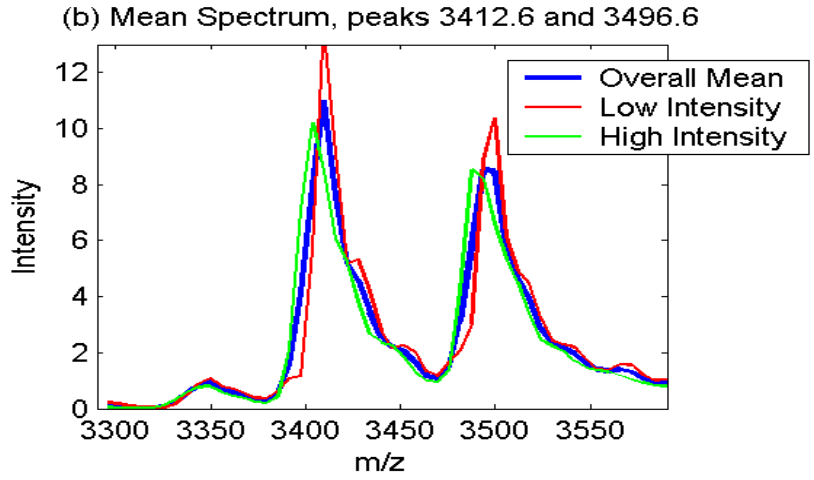


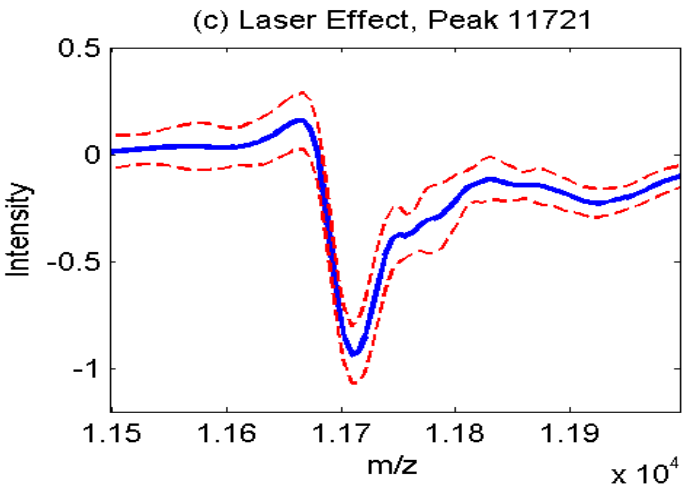
Block Effects

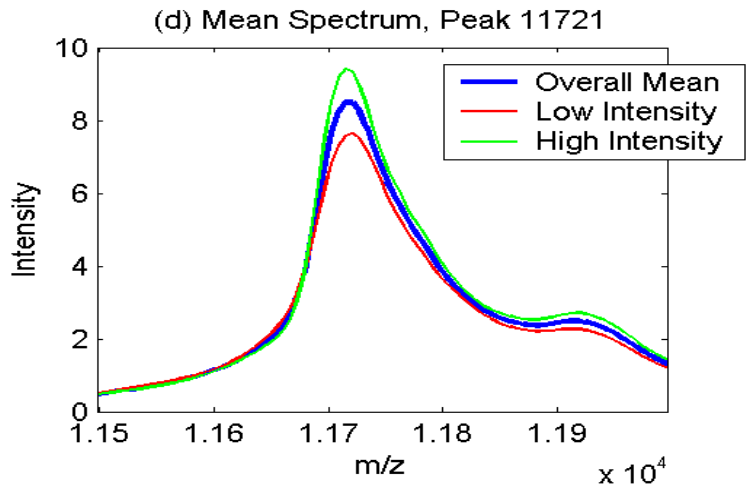


Organ-by-Cell Line: Block Effects









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.**

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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 π_{ij} and τ_{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)

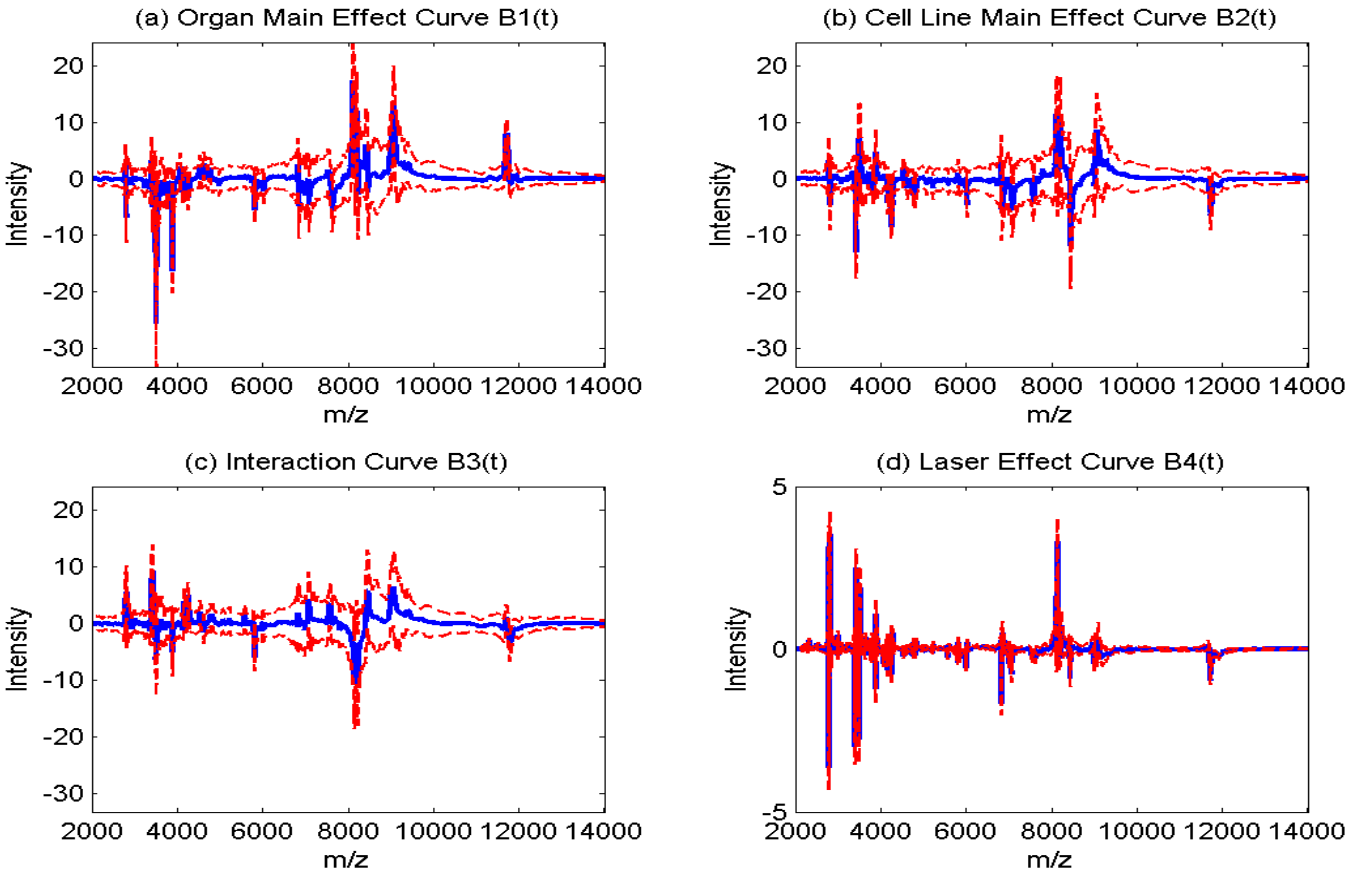
Discussion

- **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