Wavelet-Based Nonparametric Modeling of Hierarchical Functions in Colon Carcinogenesis

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Outline

- Introduction
  - Colon Carcinogenesis Studies
  - Hierarchical Functional Data
  - Wavelets and Wavelet Regression
- Overview of Method
- Choice of Smoothing Parameters
- Application
- Conclusions

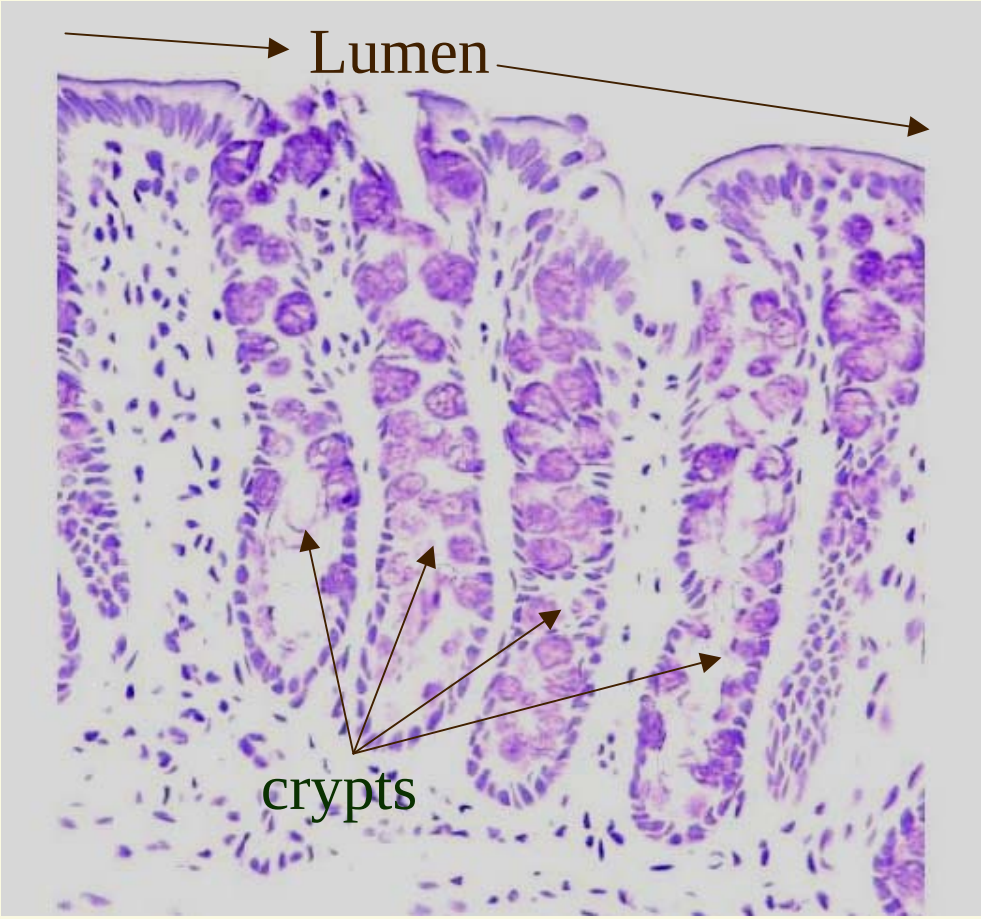
Colon Carcinogenesis Studies

- Colon cancer asymptomatic until advanced
  - Limited treatment options for advanced disease
- Preventive approaches crucial
  - Better understand carcinogenic mechanisms
  - Identify & understand risk factors
- Dietary factors key in colon cancer risk

Colon Carcinogenesis Studies

- "Carcinogen-induced colon cancer in rats" model used to study biological mechanisms *in vivo*
  - Rats given treatments of interest (diet) over time
  - Exposed to carcinogen
  - Animals euthanized, colon removed and examined.
- IHC staining used to quantify responses
  - Slides treated with chemical
  - Staining intensity indicates level of response.

Architecture of Colon



- ***Stem Cells***:
  Mother cells near bottom
- Depth in crypt ~ age of cells
- ***Relative Cell Position*** (Depth):
  $t \in (0,1)$

Colon Carcinogenesis Studies

- Data structure:
  - Multiple treatment groups (diets)
  - Multiple rats for each treatment
  - Multiple crypts sampled for each rat
  - Response quantified over fixed grid of *t* along side of each selected crypt
- Hierarchical functional data
  - Multi-level random effects model with functional observations

Hierarchical Functional Data

- **2-level HF model:**

$$\boxed{\begin{aligned} \mathbf{Y}_{abc} &= g_{abc}(t) + \mathbf{e}_{abc}, \\ g_{abc}(t) &= g_{ab}(t) + \eta_{abc}(t) \\ g_{ab}(t) &= g_{a}(t) + \xi_{ab}(t) \end{aligned}}$$

where $\mathbf{e}_{abc} \sim MVN(\mathbf{0}, \sigma_e^2 \mathbf{I})$,

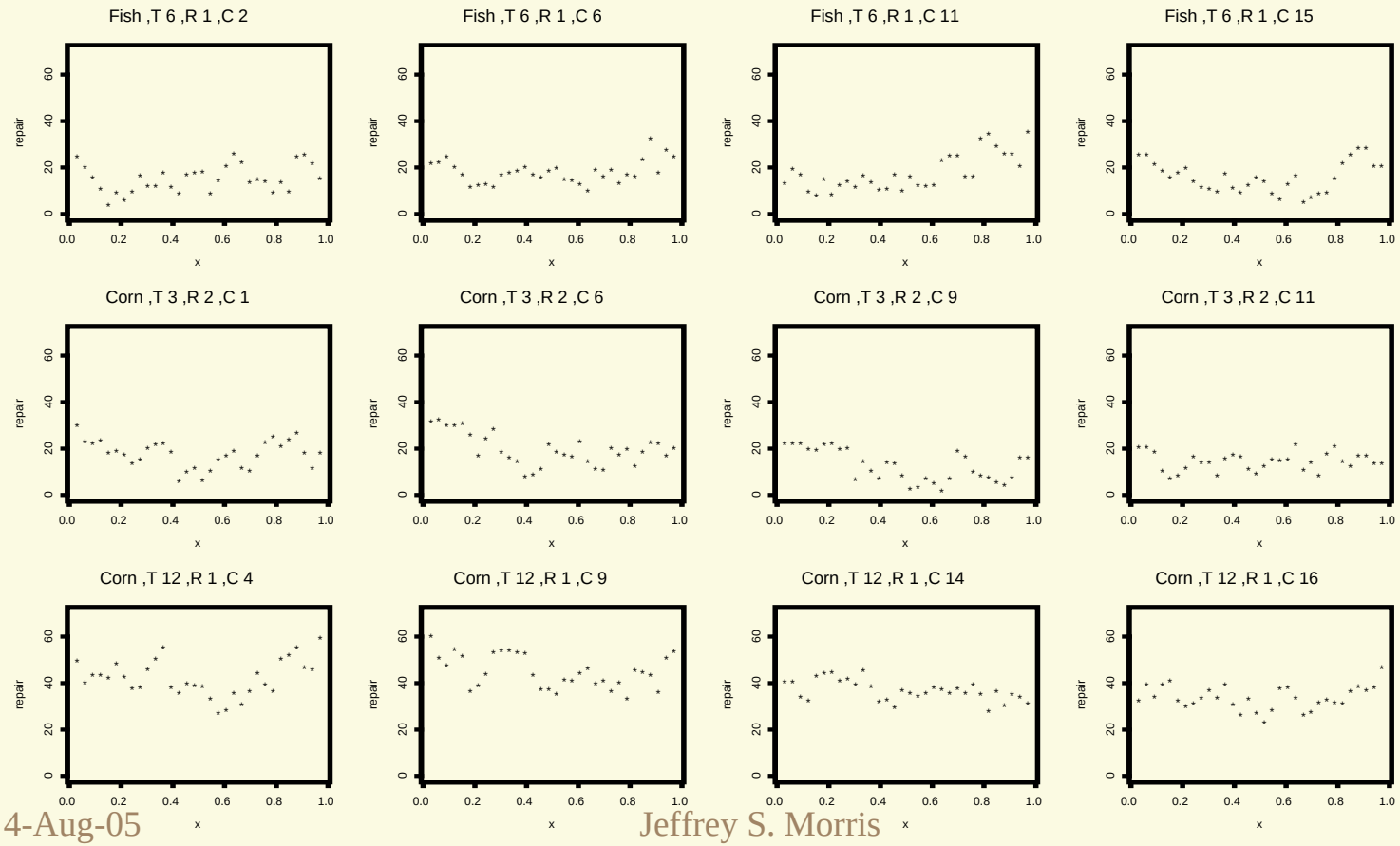
$\eta_{abc}(\bullet)$ and $\xi_{\mathrm{ab}}(\bullet)$: mean 0 with covariance matrices $\Sigma_1(t_1, t_2)$ and $\Sigma_2(t_1, t_2)$.

Hierarchical Functional Data

- Parametric model (linear):
  - $g(\mathbf{t}) = X(\mathbf{t})\,\beta$ at each level of hierarchy:
    - e.g. line or parabola at each level.
  - i.e. $g_{abc}(\mathbf{t}) = X(\mathbf{t})\,\beta_{abc}$, $\mathrm{Cov}(\beta_{abc}|\beta_{ab}) = \Sigma_{\beta 1}$
    $g_{ab}(\mathbf{t}) = X(\mathbf{t})\,\beta_{ab}$, $\mathrm{Cov}(\beta_{ab}|\beta_{a}) = \Sigma_{\beta 2}$
    $g_{a}(\mathbf{t}) = X(\mathbf{t})\,\beta_{a}$
- Can be fit using standard mixed models software.
- Efficient and easy IF you can find a parametric form for the g(•) functions.

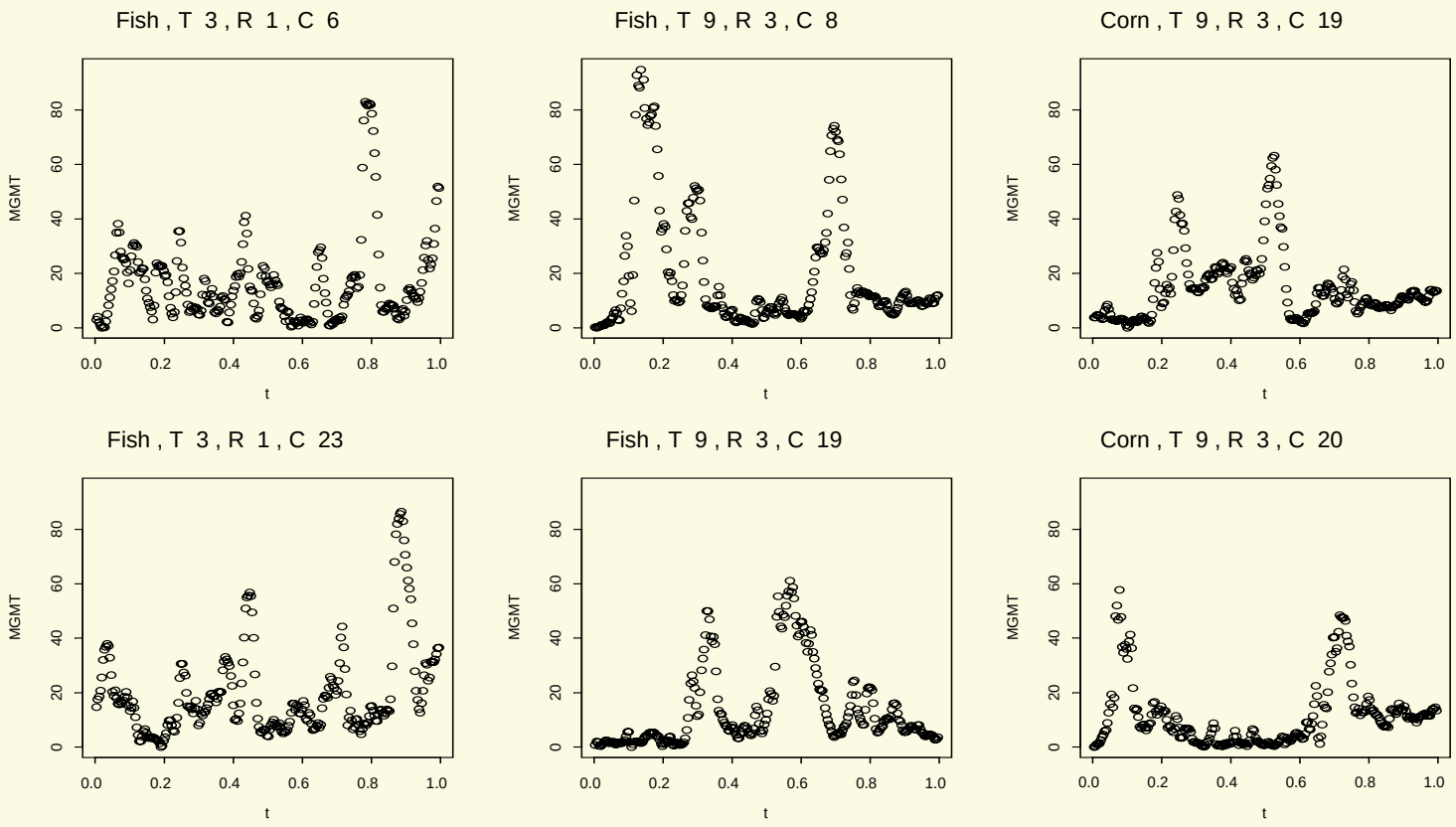
Hierarchical Functional Data

DNA Adduct Level (Damage)



Hierarchical Functional Data

MGMT Level (DNA Repair Enzyme)



Goals

- Develop method to analyze H.F.D.
  - Est. population-level functions $g_a(t)$ (nonparametrically)
  - Perform Bayesian inference
  - Uncover relative variability at each hierarchical level.
  - Work for hierarchical models with 1 or 2 levels
  - Adjust for possibly unequal sample sizes
  - Model spike-like data at lowest hierarchical level
- Our method first to do all these
- Apply to colon carcinogenesis data set
- Use of wavelets fundamental to our approach

Wavelets & Wavelet Regression

- Wavelets: families of orthonormal basis functions.
- Wavelet series for $f(t)$: $f(t) = \sum_{j,k \in \Im} d^{j,k} \psi^{j,k}(t)$
- Wavelet **basis functions**: $\psi^{j,k}(t) = 2^{j/2} \psi(2^j t - k)$
  - **Mother** wavelet: $\psi(t)$
- Wavelet **coefficients**: $d^{j,k} = \int f(t) \psi^{j,k}(t)\, dt$

Wavelets & Wavelet Regression

- Wavelet coefficients calculated using Discrete Wavelet Transform (DWT)
- Efficient -- $O(n)$
- n data points $\boldsymbol{y} \Rightarrow n$ wavelet coefficients $\boldsymbol{d}$
  - Indexed by: resolution level j *(scale)*, wavelet number k *(location)*.
- Can be written: $\boldsymbol{d} = \mathrm{W}\,\boldsymbol{y}$ (W orthogonal)
- Inverse Transform (IDWT): $\boldsymbol{y} = \mathrm{W}^t\,\boldsymbol{d}$

Wavelets & Wavelet Regression

- Key Properties:
  - Compact support -- model local features in time (spikes)
  - Whitening property -- less correlation in wavelet space, allowing parsimonious modeling
  - Sparsity -- most wavelet coefficients 0 for given function
- Can be used to perform Nonparametric Regression (Donoho & Johnstone, 1994, many others)

Wavelets & Wavelet Regression

- Data space model: $\boldsymbol{y} = f(\boldsymbol{t}) + \boldsymbol{e}$
  - $\boldsymbol{t}$ = equally spaced grid, length $n=2^J$, on (0,1)
  - $\boldsymbol{e} \sim \text{MVN}(\mathbf{0},\sigma^2 \text{ I})$
- In wavelet space: $\boldsymbol{d} = \boldsymbol{W}\boldsymbol{y} = \boldsymbol{\theta} + \boldsymbol{e}^*$
- $\boldsymbol{d}$ = 'empirical' wavelet coefficients
  $\boldsymbol{\theta}$ = 'true' wavelet coefficients
- By orthogonality, $\boldsymbol{e}^* \sim \text{MVN}(\mathbf{0},\sigma^2 \text{ I})$

Wavelets & Wavelet Regression

- Wavelet Regression procedure:
  - (1) Transform to wavelet space
  - (2) Threshold/shrink coefficients $\rightarrow 0$
  - (3) Transform back to data space (IDWT)
- Thresholding/shrinkage filters out noise, performs regularization.
- Shrinkage can be done by placing a certain prior structure on the θ.

Method: Overview

1. Convert data $\boldsymbol{y_{abc}}$ to wavelet space $\boldsymbol{d_{abc}}$
   - Involves 1 DWT for each crypt

2. Fit hierarchical model in wavelet space to obtain
   * Posterior distribution of 'true' wavelet coefficients $\boldsymbol{\theta_a}$ corresponding to $g_a(t)$
   * Variance component estimates to assess relative variability

3. Use IDWT to obtain posterior distributions of $g_a(t)$ for estimation and inference

Method: Model in Wavelet Space

- $\mathbf{d}_{abc} = \{ d_{abc}^{j,k} \} = \mathrm{W}\ \mathbf{y}_{abc}$

$$\boxed{\begin{aligned} d_{abc}^{j,k} &\sim iid\ N(\theta_{abc}^{j,k}, \sigma_e^2) \\ \theta_{abc}^{j,k} &\sim iid\ N(\theta_{ab}^{j,k}, \sigma_{1,j}^2) \\ \theta_{ab}^{j,k} &\sim iid\ N(\theta_{a}^{j,k}, \sigma_{2,j}^2) \end{aligned}}$$

  - σ_e^2 assumed known, in practice estimated by $\mathrm{Var}(d_{abc}^{1,k})$
  - IG priors on $\sigma_{1,j}^2$ and $\sigma_{2,j}^2$ when unknown

Method; "Shrinkage" Prior

- Prior on $\theta_a^{j,k}$ $\Rightarrow$ thresholding/shrinkage
- Mixture of point mass at 0 and Normal

$$\theta_a^{j,k} \sim N(0, \gamma_a^{j,k} \tau_j^2)$$

$$\gamma_a^{j,k} \sim \text{Bernoulli}(p_j)$$

- p_j and τ_{j}^2 : smoothing parameters

Method: Estimating $g_a(t)$

- If VC known $\Rightarrow$ closed form posterior

$$(\theta_a^{j,k} \mid \mathbf{d}, \sigma_{1,j}^2, \sigma_{2,j}^2) \sim \text{Normal}(\mu, \sigma^2)$$

$$\mu = \hat{\Theta}_{a,NS}^{j,k} \times h\left(\tau_j^2, p_j, \hat{\Theta}_{a,NS}^{j,k}\right)$$

$$\sigma^2 = \text{Var}\left(\hat{\Theta}_{a,NS}^{j,k}\right) \times h\left(\tau_j^2, p_j, \hat{\Theta}_{a,NS}^{j,k}\right)$$

$\hat{\Theta}_{a,NS}^{j,k} = MLE$ of $\theta_a^{j,k}$ assuming **no shrinkage**

$h(.)$ = "shrinkage" function

- Using inverse wavelet transform, we get entire posterior distribution of $g_a(t)$.

Method: Estimating $\theta_a^{j,k}$

- VC Unknown -- no closed form posterior
- Metropolis-within-Gibbs Sampler used on marginalized model.
- Sampling scheme: Let $\Omega^j = \{\sigma_{1,j}^2, \sigma_{2,j}^2\}$

  and $\mathbf{\Theta}_a^j = \left\{\Theta_a^{j,k}, k = 1, \ldots, K_j\right\}$

  (1) Sample from $\left(\Theta_a^{j,k} \mid \mathbf{d}, \Omega^j\right)$ for each j, k

  (2) Sample from $\left(\Omega^j \mid \mathbf{d}, \mathbf{\Theta}_a^j\right)$ for each j

Method: Estimating Variability

- Relative variability at different hierarchical levels can help guide design of future experiments
- Assessed using the trace: $\mathrm{tr}\{\Sigma_1\}$, $\mathrm{tr}\{\Sigma_2\}$, and $\mathrm{tr}\{\sigma_e^2 I\}$
- Orthogonality $\Rightarrow$ Compute trace in wavelet space

$$V_0 = \mathrm{tr}\{\sigma_e^2 I\} = n\sigma_e^2$$

$$V_1 = \mathrm{tr}\{\Sigma_1\} = \sum_{\mathrm{j}} K_j \sigma_{1,j}^2$$

$$V_2 = \mathrm{tr}\{\Sigma_2\} = \sum_{\mathrm{j}} K_j \sigma_{2,j}^2$$

Relative variability at level i:

$$V_i / (V_0 + V_1 + V_2)$$

Choice of Smoothing Parameters

Shrinkage Function:

$$h(Z, T_j^2, p_j) = \underbrace{\left(\frac{T_j^2}{T_j^2+1}\right)}_{\text{Linear Shrinkage}} \underbrace{\Pr\left\{\gamma_a^{j,k} = 1 \mid \mathbf{d}_a^{j,k}\right\}}_{\text{Nonlinear Shrinkage}}$$

$$\Pr\left(\gamma_a^{j,k} = 1 \mid \mathbf{d}_a^{j,k}\right) = \frac{BF}{BF+1}, \qquad BF = \text{Bayes Factor}$$

$$\underbrace{BF}_{\text{Bayes Factor}} = \underbrace{\left(\frac{p_j}{1-p_j}\right)}_{\text{Prior Odds}} \underbrace{\left(1+T_j^2\right)^{-1} \exp\left\{\frac{Z^2}{2}\left(\frac{T_j^2}{T_j^2+1}\right)\right\}}_{\text{Likelihood Ratio}}$$

$$Z = \hat{\theta}_{a,NS}^{j,k} / \sqrt{\mathrm{Var}(\hat{\theta}_{a,NS}^{j,k})} \qquad T_j^2 = \tau_j^2 / \mathrm{Var}(\hat{\theta}_{a,NS}^{j,k})$$

Choice of Smoothing Parameters

- p_j : Expected proportion of non-negligible wavelet coefficients at resolution j
  - $p_j \downarrow \Rightarrow$ Belief that many coeffs. are essentially noise.
  - Results in more ‘smoothing’ of features at scale j
- Generally -- expect p to decrease in j
  - Higher frequency behavior mostly noise

Choice of Smoothing Parameters

- τ_j^2 : Variation of nonzero wavelet coeffs.

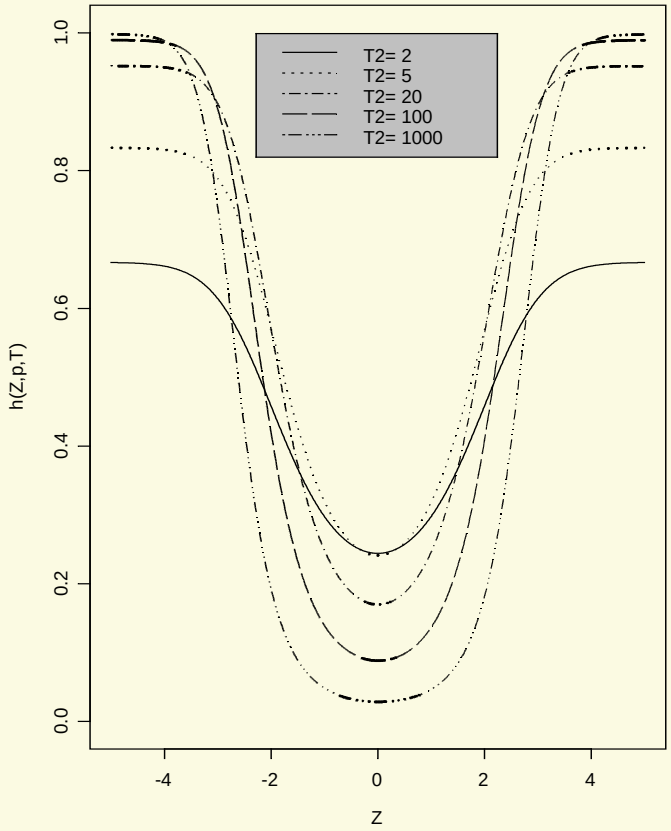
$$T_j^2 = \tau_j^2 / \mathrm{Var}\left(\hat{\Theta}_{a,NS}^{j,k}\right)$$

- Must take care in choosing
  - Too small $\Rightarrow$ too much linear shrinkage
  - Too large $\Rightarrow$ *Lindley's paradox*
    - Too much nonlinear shrinkage for some Z

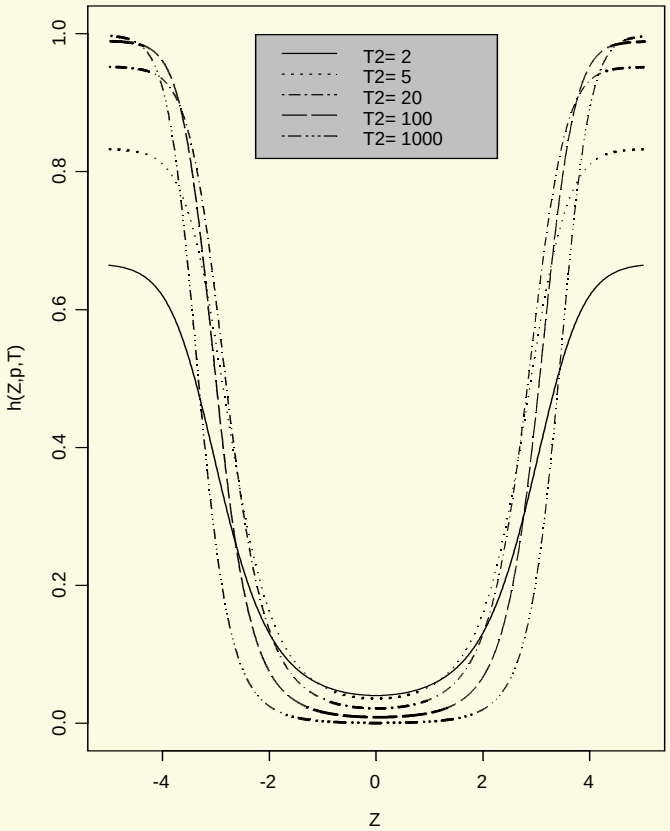
$T_j^2 \in (10,100)$ seem to work quite well.

Choice of Smoothing Parameters

(a) Shrinkage functions for p= 0.5



(b) Shrinkage functions for p= 0.1

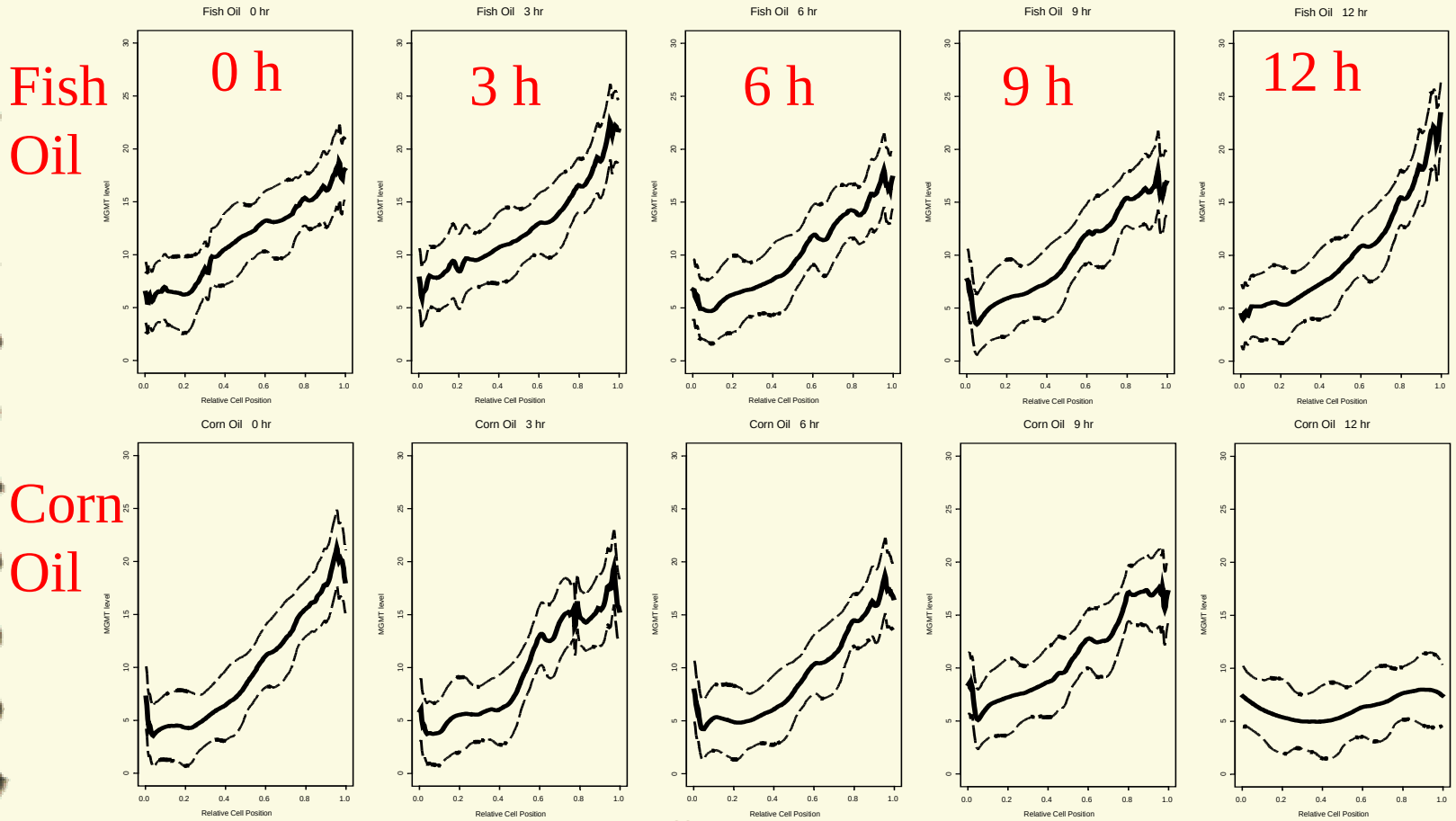


Example: Data

- MGMT expression (DNA repair enzyme)
  - 2 diets (fish/corn) x 5 times (0h,3h,6h,9h,12h)
  - 3 rats per diet x time combination
  - ~ 25 crypts per rat,
  - function sampled at $256=2^8$ equally spaced t
- Daubechies-8 wavelet chosen
  - $\boldsymbol{p}=\{1,.20,.07,.03,.015,.01,0.005\}$, $T_j^2 = 20 \ \forall j$
  - MCMC 5000 iterations; Burn-in=5000

Example: Results

Estimates & 90% posterior bounds by diet/time



Conclusion: Biological Results

- More MGMT at top of crypts
- Diet difference at 12 h near luminal surface ($t \sim 1$)
  - Fish oil-fed rats -- MGMT ↓ from 9 h to 12 h
  - Corn oil-fed rats -- MGMT ↑ from 9h to 12 h
  - Fish MGMT > Corn MGMT
- Crypt-level dominates variability
  - 90.0% at crypt level, 9.6% at rat level, 0.4% residual
  - Need lots of crypts!!
- MGMT operates on cell-by-cell basis

Conclusion: Summary

- Method to fit hierarchical functional data
  - nonparametrically estimate mean functions w/ bounds
  - evaluate relative variability at hierarchical levels
  - perform Bayesian inference
  - can handle 1 or 2 hierarchical levels & unbalanced designs
  - appropriate when functions at base level irregular
- Fitting done in wavelet space
  - Whitening property allows parsimonious modeling of Σ_i
  - Can represent spatially heterogeneous functions well
- Motivated by colon carc., but applicable elsewhere
- Possible extensions of method