Partial Differential Equation Modeling of Flow Cytometry Data from CFSE-based Proliferation Assays

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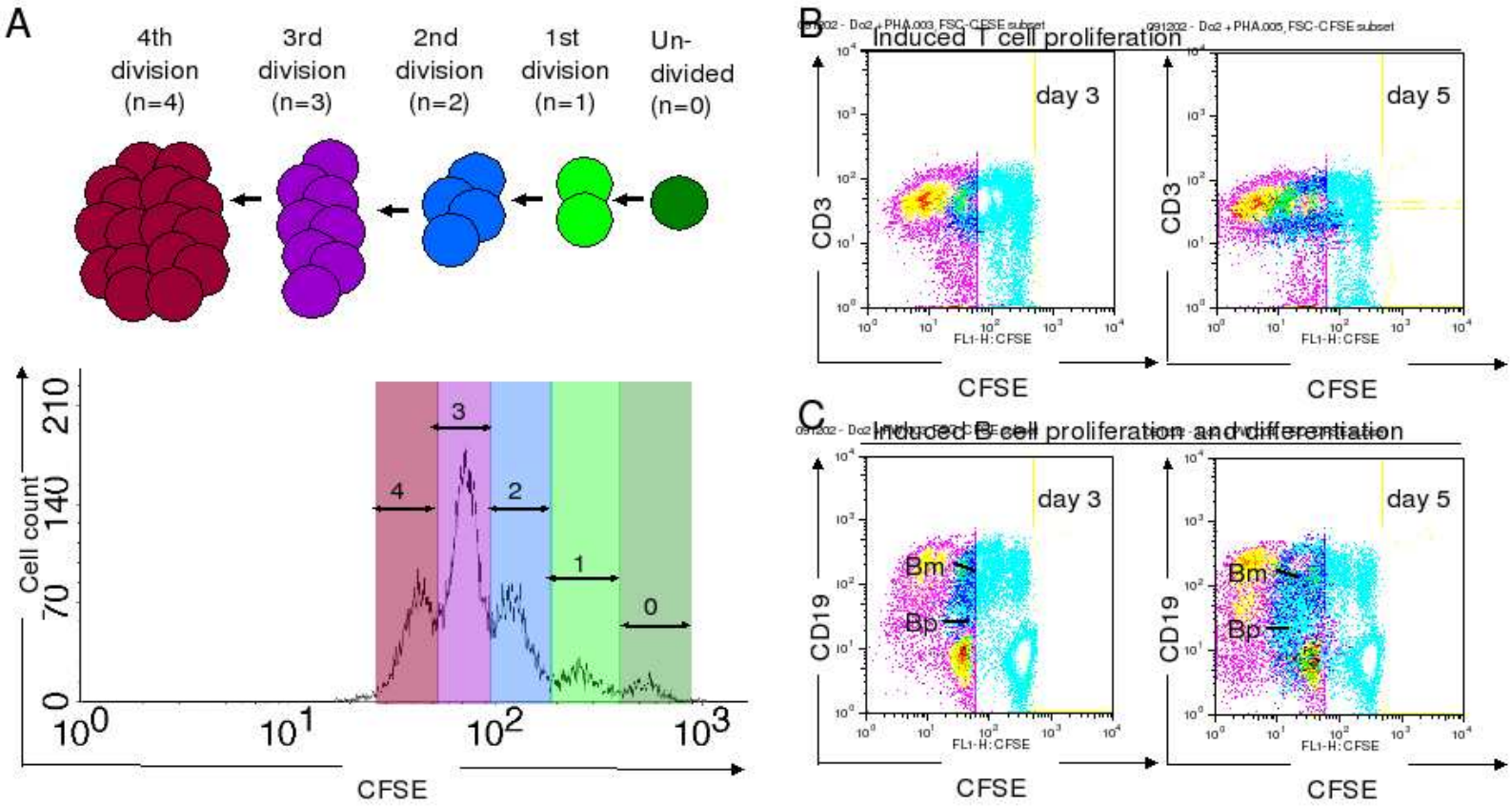
Data Overview

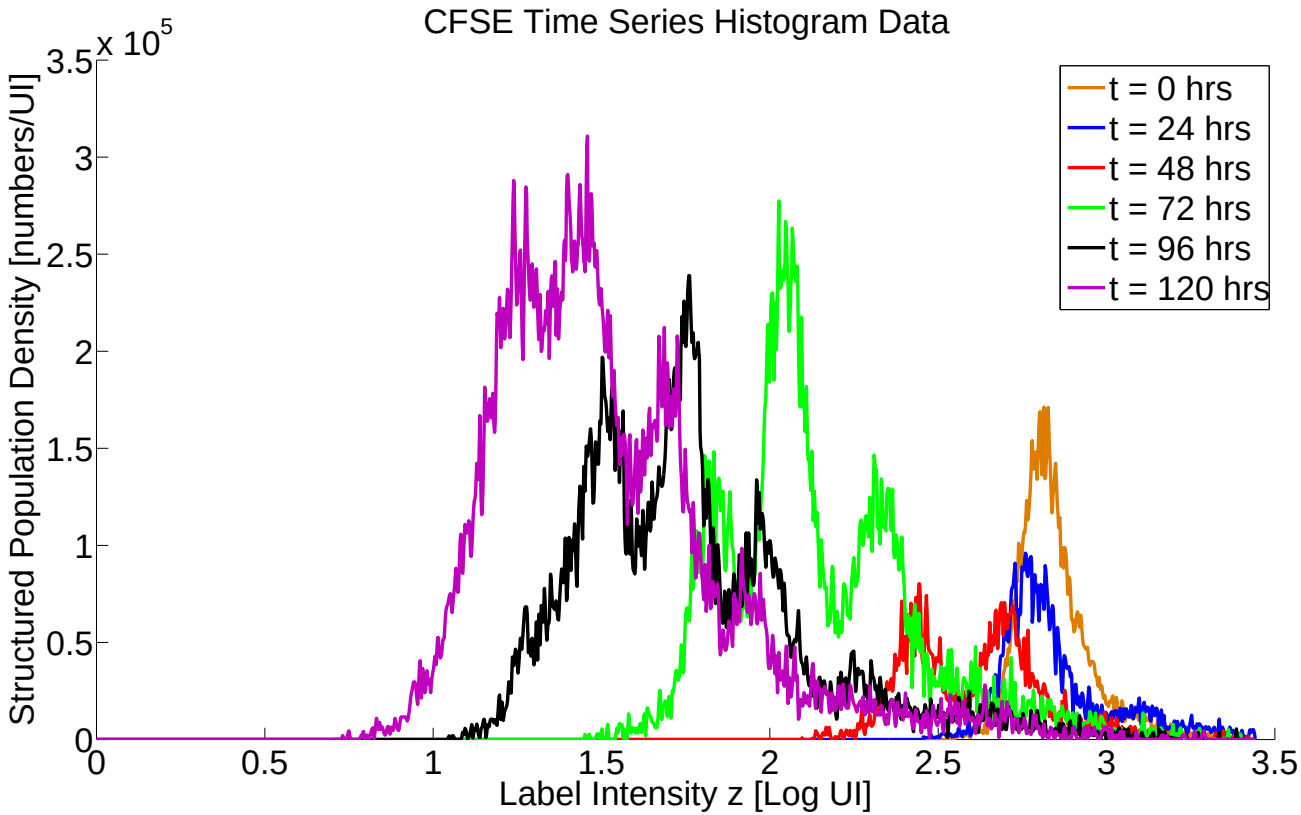


(A. Meyerhans)

CFSE Labeling (Lyons and Parish, 1994)

- Cells cultured with CFDA-SE then washed
- CFDA-SE becomes protein-bound and fluorescent CFSE
- Dye split between daughter cells at division
- Dye naturally turns over/degrades (very slowly)
- Fluorescence Intensity (FI) of CFSE measured via flow cytometry
- FI linear with dye concentration $\Rightarrow$ FI $\propto$ mass
- Several advantages over other dyes/techniques

CFSE Data Set

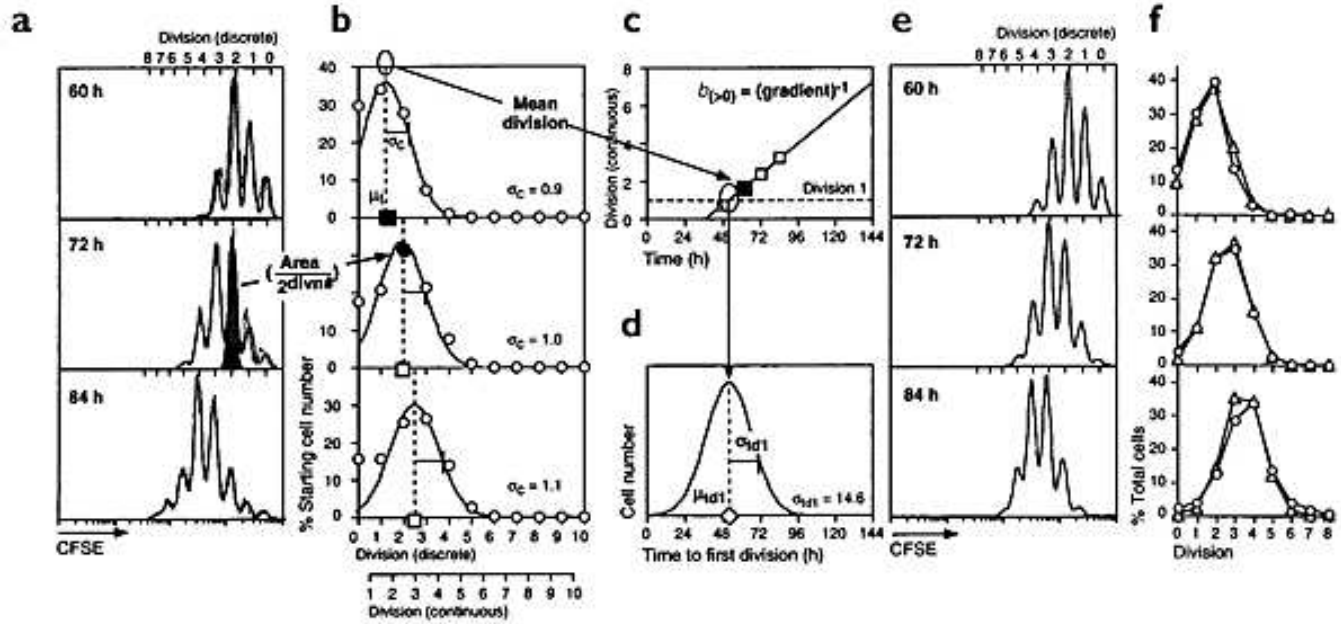


Goals of Modeling

- Cellular ‘Dynamic Responsiveness’
- Link cell counts with proliferation/death rates
  - Population doubling time
  - Cell viability
  - Biological descriptors (cell cycle time, etc.)
- Uncertainty Identification, Variability Quantification...
  - ... in the experimental procedure
  - ... for estimated rates/etc
- Analyze cell differentiation and division-linked changes
- Investigate immunospecific extracellular signaling pathways
- Comparison among donors/cell types/disease progression

Traditional Approach (curve fitting)

- Fit data with gaussian curves to determine approximate cells per generation
- Traditional ‘semi-quantitative analysis’ pioneered by Gett and Hodgkin et al. (2000)



(A.V. Gett and P.D. Hodgkin, A cellular calculus for signal integration by T cells, *Nature Immunology* **1** (2000), 239–244.)

Traditional Approach (cont'd)

- Gett-Hodgkin method quick, easy to implement, useful comparisons between data sets (e.g. stimulation conditions)
- Compatible with ODE, DDE models; 'indirect fitting' for parameter estimation
- Generalizations, extensions, and various other modeling efforts
  - Smith-Martin model (with generalizations)
  - Cyton model
  - Branching process models

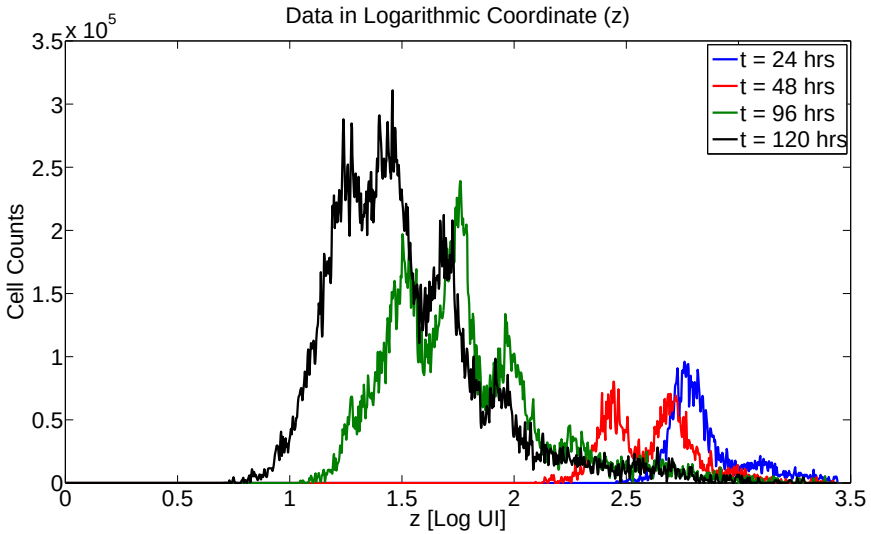
Label-Structured Model

- All previous work with *cell numbers* determined by deconvolution
- Alternatively, we propose to fit the CFSE histogram data directly
  - Capture full behavior of the population density
  - No assumption on the shape of CFSE uptake/distribution
- Histogram presentation of cytometry data makes structured population models a natural choice
  - Key ideas first formulated by Luzyanina et al., 2007
  - FI (or log FI) $\Leftrightarrow$ Division Number

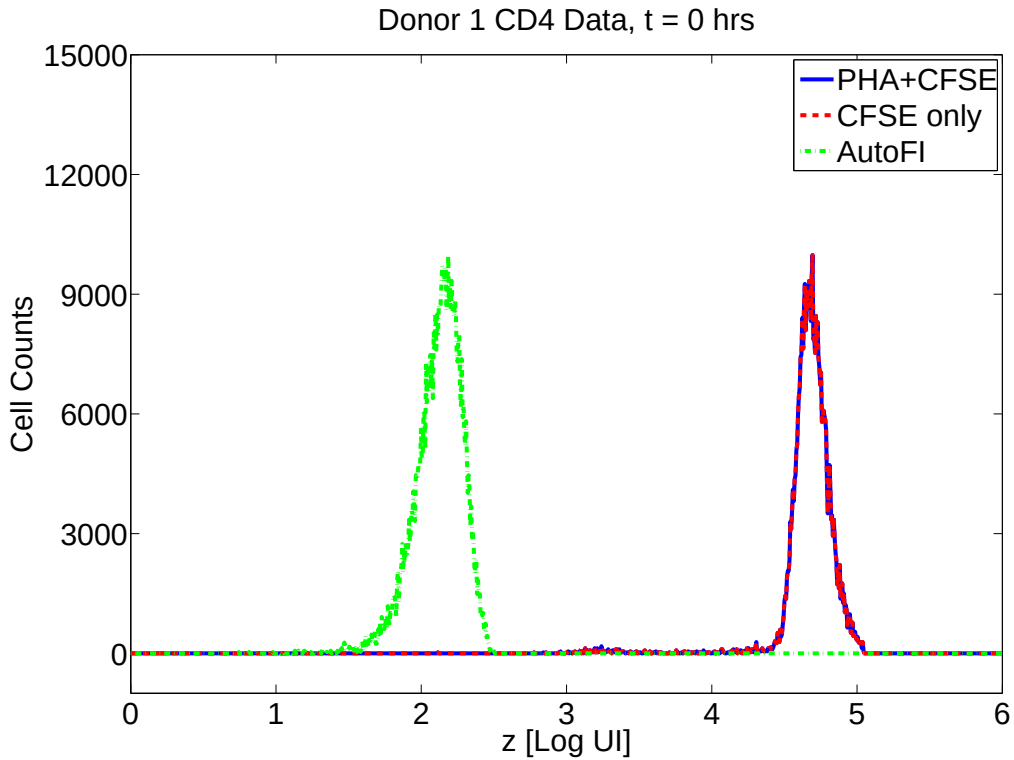
Label-Structured Model (cont'd)

This model must account for (Luzyanina et al., 2007):

- Dilution of CFSE as cells divide (AutoFI)
- Slow decay of FI over time (CFSE turnover)
- Asynchronous division times



Cellular Autofluorescence

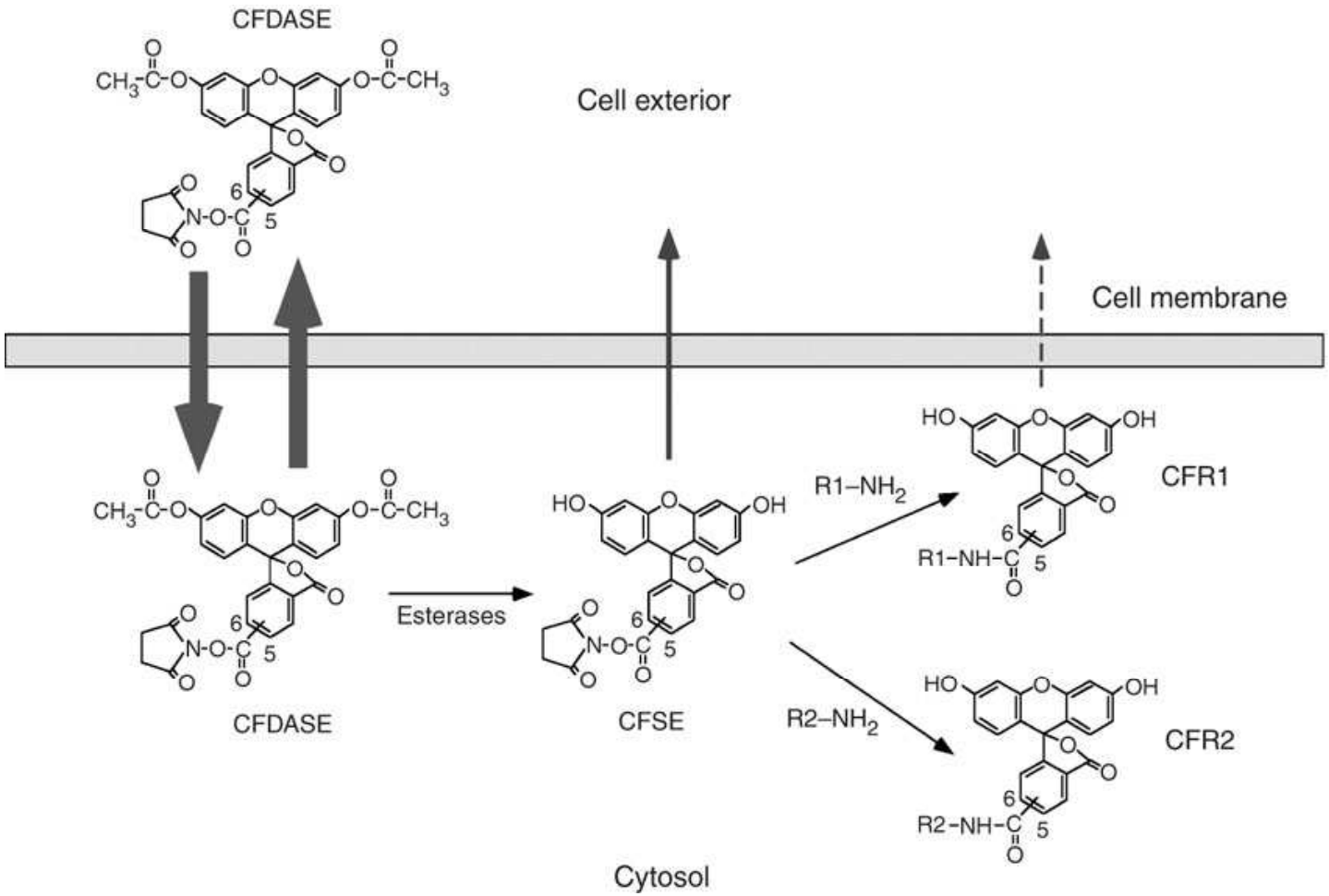


$$X_i = X_i^{\text{CFSE}} + X^{\text{Auto}}$$

$$\Downarrow$$

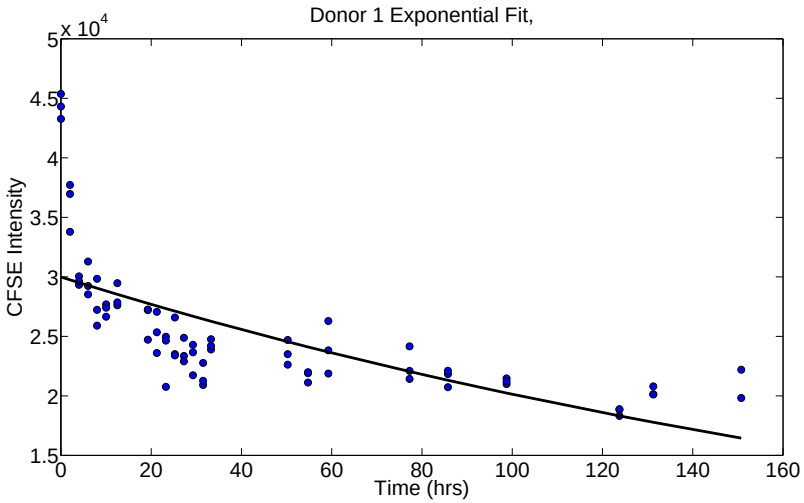
$$X_{i+1} = X_i^{\text{CFSE}}/2 + X^{\text{Auto}}$$

CFSE Turnover



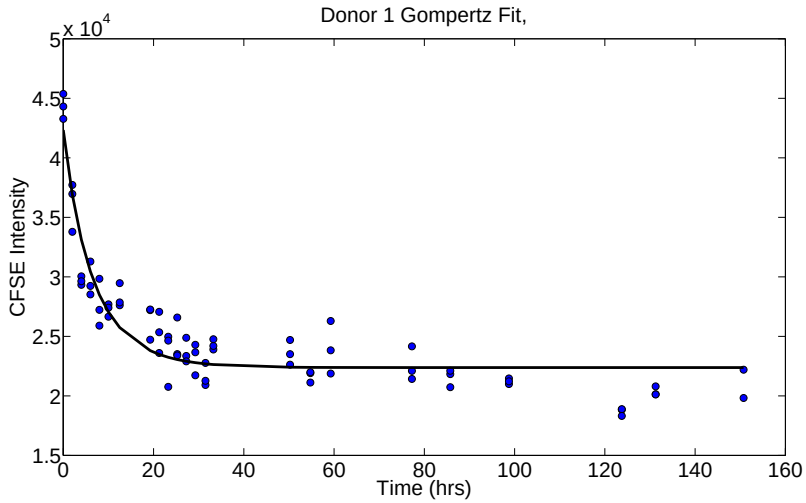
(C. Parish, Fluorescent dyes for lymphocyte migration and proliferation studies, *Immunology and Cell Biol.* **77** (1999), 499–508.)

‘Biphasic Decay’



$$\frac{dx}{dt} = v(x) = c(x - x_a)$$

Exponential



$$\frac{dx}{dt} = v(t, x) = c(x - x_a)e^{-kt}$$

Gompertz

Fragmentation Mathematical Model

- Structured density $n(t,x)$ (cells/UI)
- (Exponential) Proliferation rate $\alpha(t,x)$
- (Exponential) Death rate $\beta(x)$
- Gompertz decay rate, $v(t,x) = c(x - x_a)e^{-kt}$

$$\frac{\partial n(t,x)}{\partial t} + \frac{\partial [v(t,x)n(t,x)]}{\partial x} = -(\alpha(t,x) + \beta(x))n(t,x) + \chi_{[x_a,x^*]} 4\alpha(t, 2x - x_a)n(t, 2x - x_a)$$

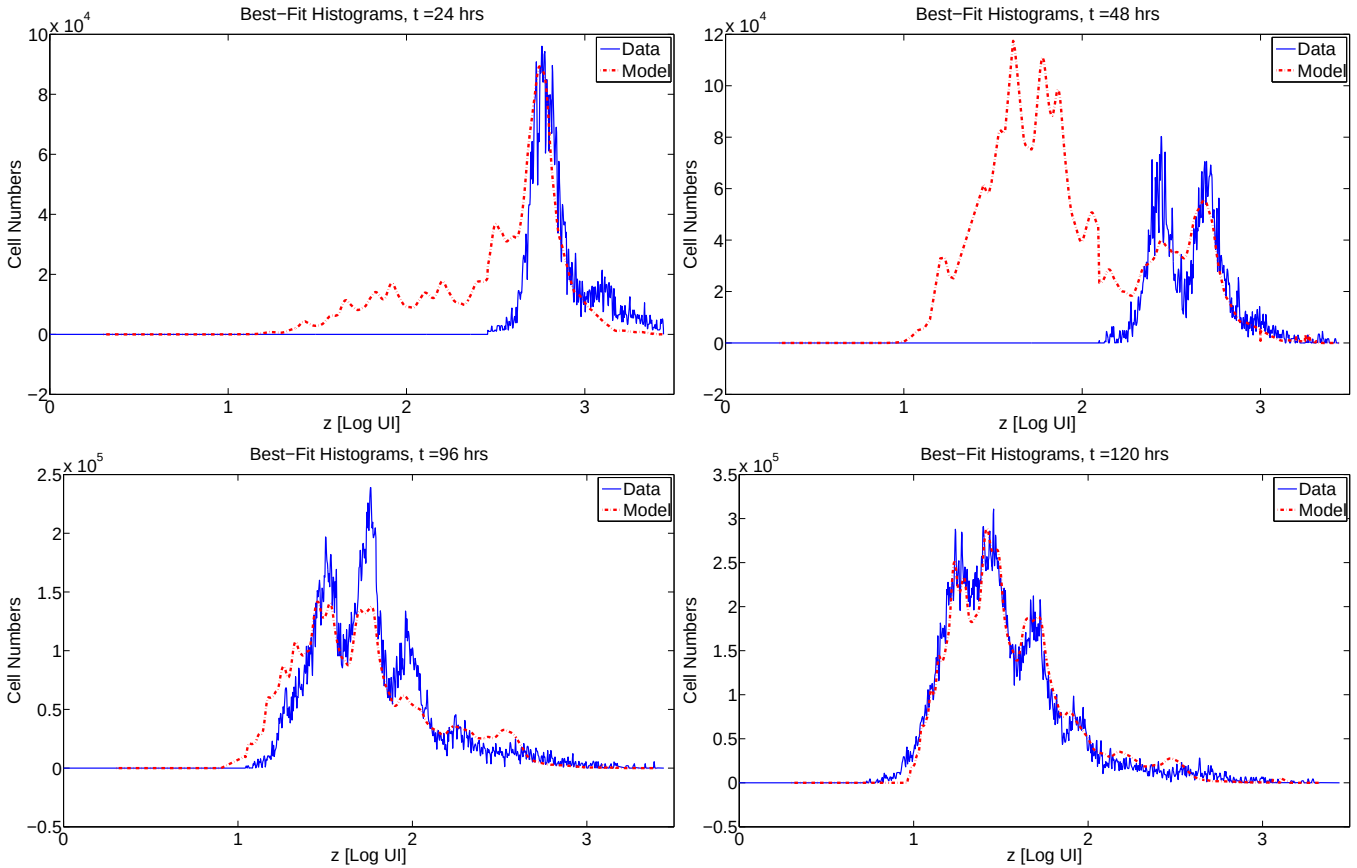
Inverse Problem

- Parameters x_a, c, k, $\alpha(t,y)$, $\beta(y)$ to be determined by fitting to data.
- Need (finite-dimensional) parameterization of α and β.
  - Piecewise linear functions
- Statistical properties of error currently unknown
- Use OLS (independent, identically distributed, constant variance error) for proof of concept

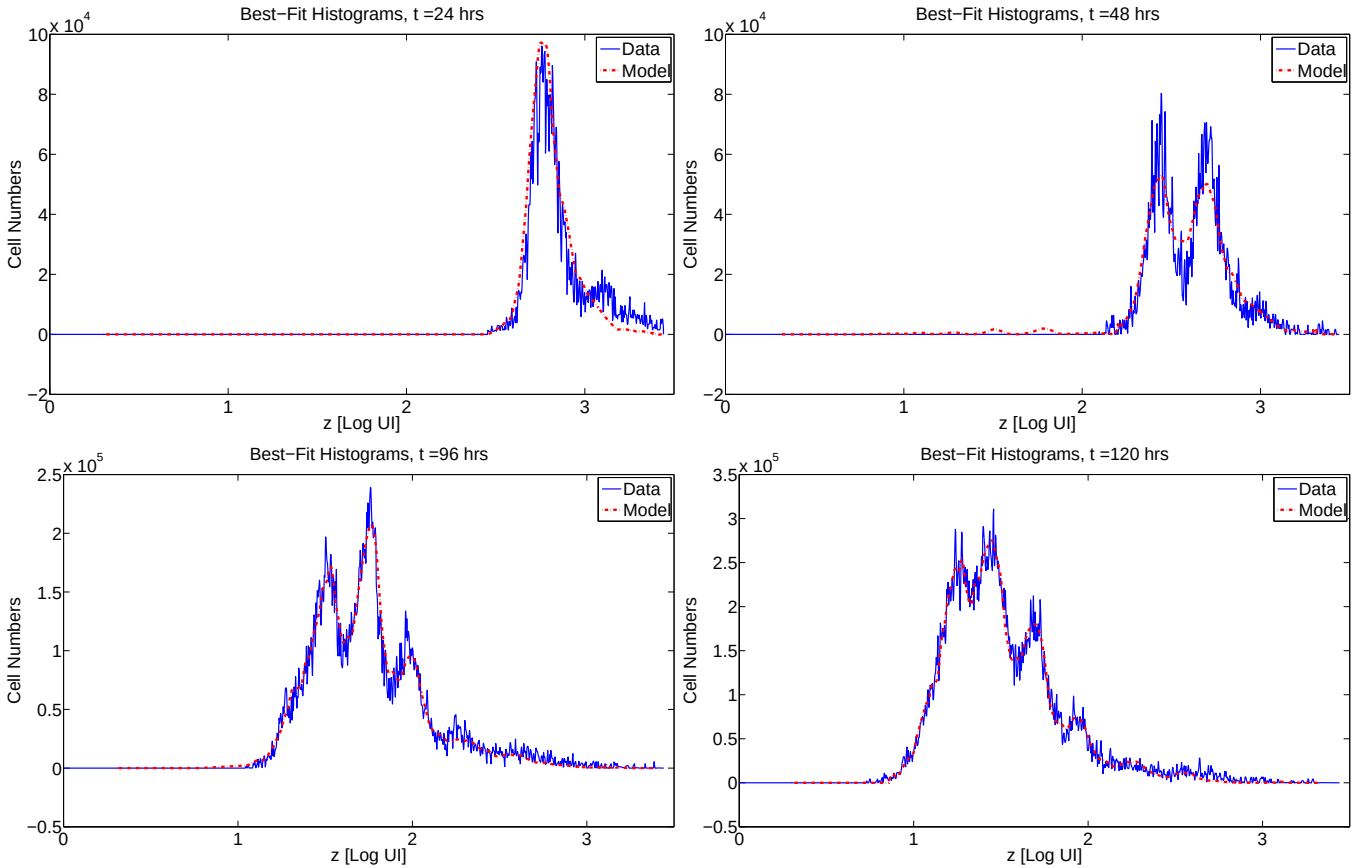
$$\hat{\theta}_{\mathrm{OLS}} = \arg\min_{\theta\in\Theta} \sum_{i=1}^{I}\sum_{j=1}^{J(i)} (I[\hat{n}](t_i, z_j; \theta) - N_i^j)^2 = \arg\min J(\theta),$$

- Forward solve with `hpde` by L.Shampine (Lax-Wendroff)
- Use `fmincon` (BGFS + active set) for optimization

Time-Independent Proliferation is Insufficient



Time-Dependent Proliferation is Sufficient



Fragmentation Model Summary

- Model is capable of precisely fitting the observed data
- c, k, x_a estimated consistently (as α and β nodes change), though subject to high experimental variability
- Time-dependence of the proliferation rate is an essential feature of the model
- Biologically relevant average values of proliferation and death (in terms of number of divisions undergone) are easily computable.
- But...
  - Still cannot compute cell numbers
  - Data overlap affecting estimated rates (?)
  - Large number of parameters necessary

Fragmentation Model Summary (cont'd)

$$\frac{\partial n(t,x)}{\partial t} + \frac{\partial [v(t,x)n(t,x)]}{\partial x} = -(\alpha(t,x) + \beta(x))n(t,x) + \chi_{[x_a,x^*]} 4\alpha(t, 2x - x_a)n(t, 2x - x_a)$$

- Applications to protein fragmentation and aggregation
- Possible generalizations to size/volume structure

Division Structure: The Compartmental Model

- Use compartments (on division number) to eliminate fragmentation terms
- No need for structure dependence of estimated rates

$$\frac{\partial n_0}{\partial t} + \frac{\partial [v(t,x)n_0(t,x)]}{\partial x} = -(\alpha_0(t) + \beta_0(t))n_0(t,x)$$
$$\frac{\partial n_1}{\partial t} + \frac{\partial [v(t,x)n_1(t,x)]}{\partial x} = -(\alpha_1(t) + \beta_1(t))n_1(t,x) + R_1(t,x)$$
$$\vdots$$
$$\frac{\partial n_{i_{\max}}}{\partial t} + \frac{\partial [v(t,x)n_{i_{\max}}(t,x)]}{\partial x} = -\beta_{i_{\max}}(t)n_{i_{\max}}(t,x) + R_{i_{\max}}(t,x)$$

where $R_i(t,x) = 4\alpha_{i-1}(t)n_{i-1}(t, 2x - x_a)$ for $1 \leq i \leq i_{\max}$

Method of Characteristics Solution

$$n_0(t, x(t;s)) = \Phi_0(s)\exp\left(-\int_0^t f_0(\tau)d\tau\right)$$

$$n_i(t, x(t;s)) = \Phi_i(s)\exp\left(-\int_0^t f_i(\tau)d\tau\right) + \int_0^t R_i(\tau, x(\tau;s))\exp\left(-\int_\tau^t f_i(\xi)d\xi\right)d\tau$$

where $f_i(t) = \alpha_i(t) + \beta_i(t) - ce^{-kt}$

The cell numbers can be easily computed $N_i(t) = \int n_i(t,x)dx$

Parameterizations

B1 $\beta_i(t) = 0$ for all i and for all t

B2 $\beta_i(t) = \beta$ for all i and for all t

B3 $\beta_0(t) = \beta_0$, $\beta_i(t) = 0$ for $i \geq 1$

B4 $\beta_0(t) = \beta_0$, $\beta_i(t) = \beta$ for $i \geq 1$

B5 $\beta_i(t) = \beta_i$ for each i

A1 $\alpha_0(t) = \alpha_0$; $\alpha_i(t) = \alpha$ for all i

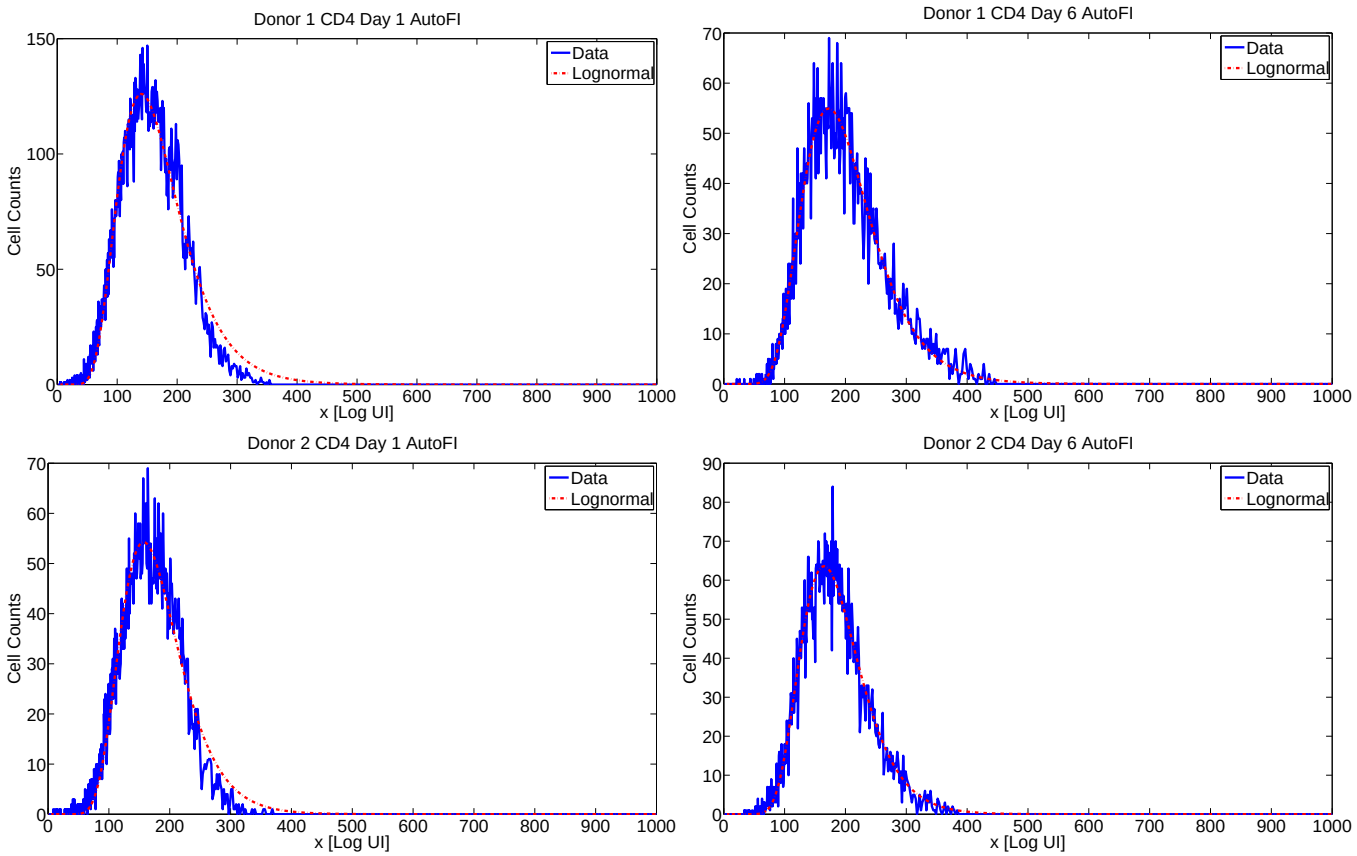
A2 $\alpha_i(t) = \alpha_i$ for all t

A3 $\alpha_0(t) = \alpha_0 \chi_{[t>t^*]}$; $\alpha_i(t) = \alpha$ for all i

A4 $\alpha_0(t) = \alpha_0 \chi_{[t>t^*]}$; $\alpha_i(t) = \alpha_i$

A5 piecewise linear functions of time (see below)

Distributed Autofluorescence



- AutoFI appears approximately lognormally distributed
- Dynamic properties ignored (for now)
- Can study effective design of intracellular dyes

Distributed Autofluorescence (cont'd)

$$\eta(t,x) = E[n(t,x;x_a)|P] = \int_{x_a^{min}}^{x_a^{max}} n(t,x;x_a)dP(x_a)$$

$$\frac{dP}{dx_a} = p(x_a) = \frac{1}{x_a\sigma\sqrt{2\pi}}\exp\left(-\frac{(\log x-\mu)^2}{2\sigma^2}\right)$$

where

$$\mu = \log(E[x_a]) - \frac{1}{2}\log\left(1+\frac{Var(x_a)}{E[x_a]^2}\right)$$

$$\sigma^2 = \log\left(1+\frac{Var(x_a)}{E[x_a]^2}\right)$$

Another Inverse Problem

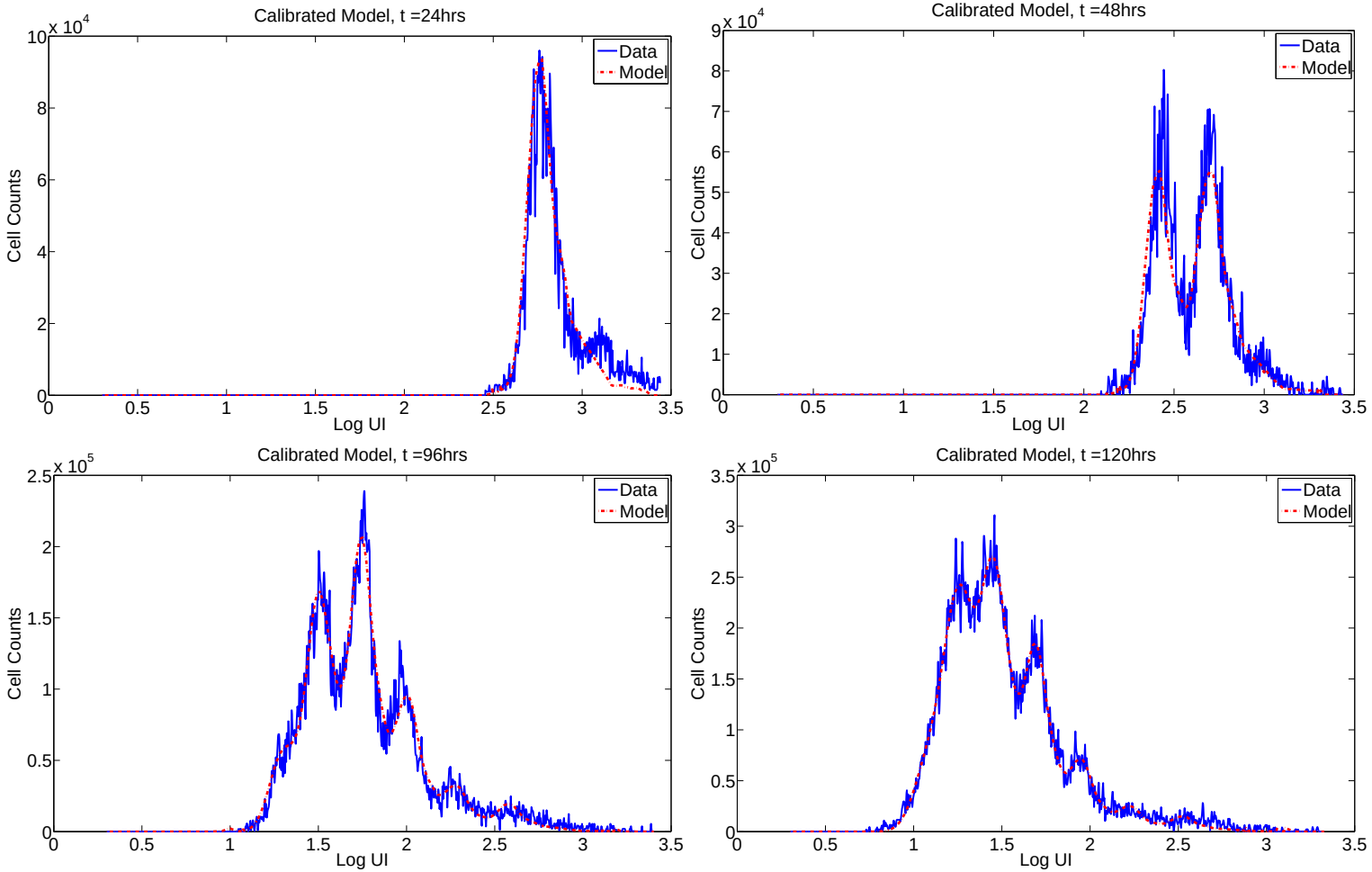
- Population density $n(t,x) = \sum_{i=0}^{i_{\max}} n_i(t,x)$
- Use OLS framework again–assume constant variance error

$$\begin{aligned}\hat{\theta}_{OLS}(n_k^j) &= \arg\min_{\theta\in\Theta} J(\theta|n_k^j) \\ &= \arg\min_{\theta\in\Theta} \sum_{k,j} \left(I[\tilde{n}](t_j, z_k^j; \theta) - n_k^j\right)^2\end{aligned}$$

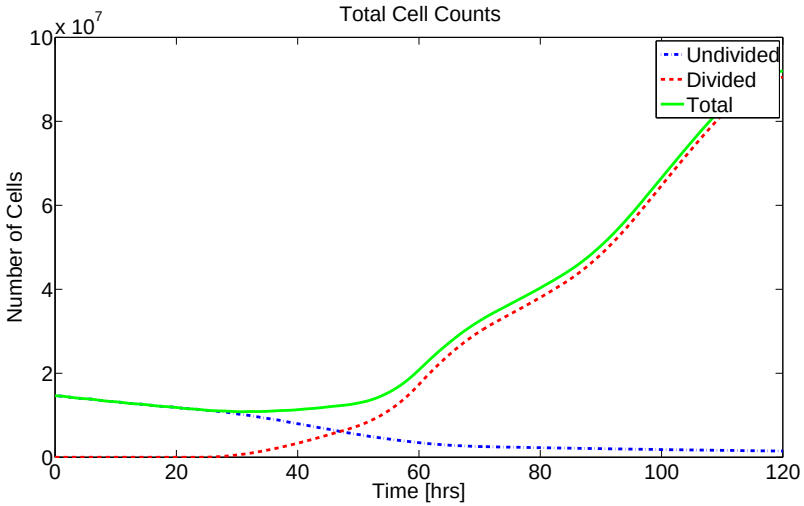
Need to compare different parameterizations (model comparison)–Akaike Information Criterion

$$AIC = m \log\left(\frac{J(\hat{\theta}_{OLS})}{m}\right) + 2p$$

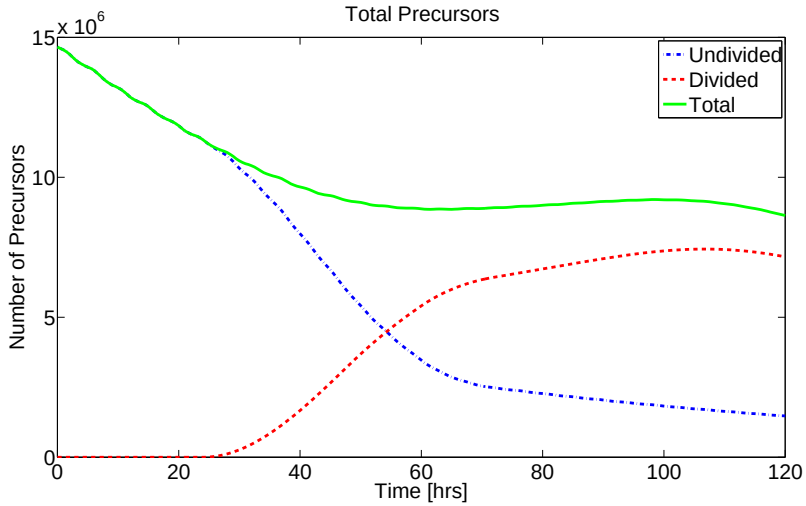
Best-fit, AIC-selected results



Cell Numbers



$$N_i(t) = \int n_i(t,x)$$



$$P_i(t) = N_i(t)/2^i$$

Population doubling time and precursor viability easily computable

Model Results and Conclusions

- Cell/precursor numbers (per generation) easy to compute
- More complex models receive highest ranking
  - Highly time-dependent proliferation rates (A5)
  - Heterogeneous death rates (B5)
  - Distributed AutoFI is an important modeling feature
- But...
  - AIC may be biased by statistical model
  - ‘Time-dependence’ possibly a byproduct of Malthusian form
  - Cell counts between data points biased by model form

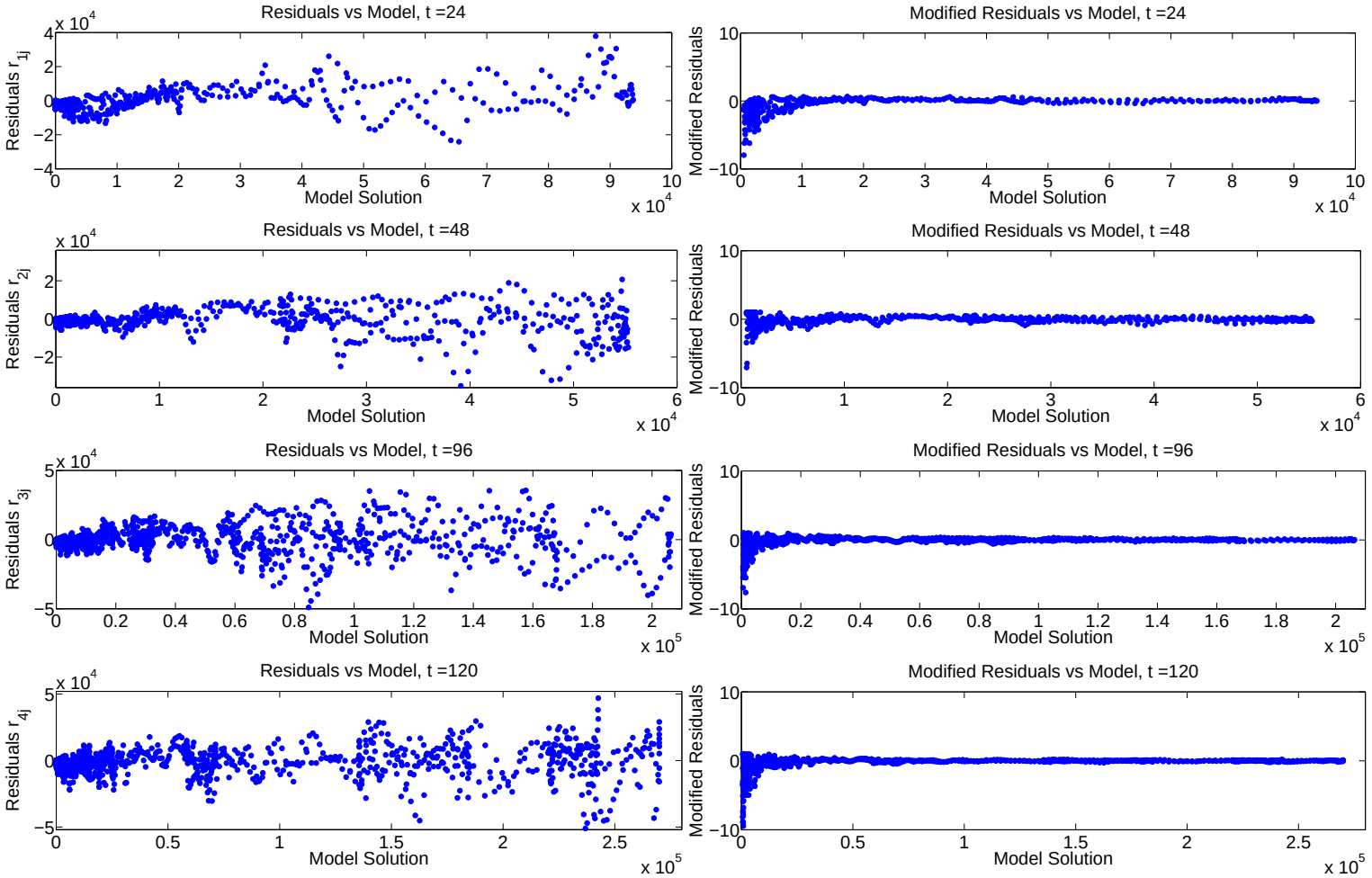
The Statistical Model

- Links the mathematical model to the data
- Implications for estimation procedure

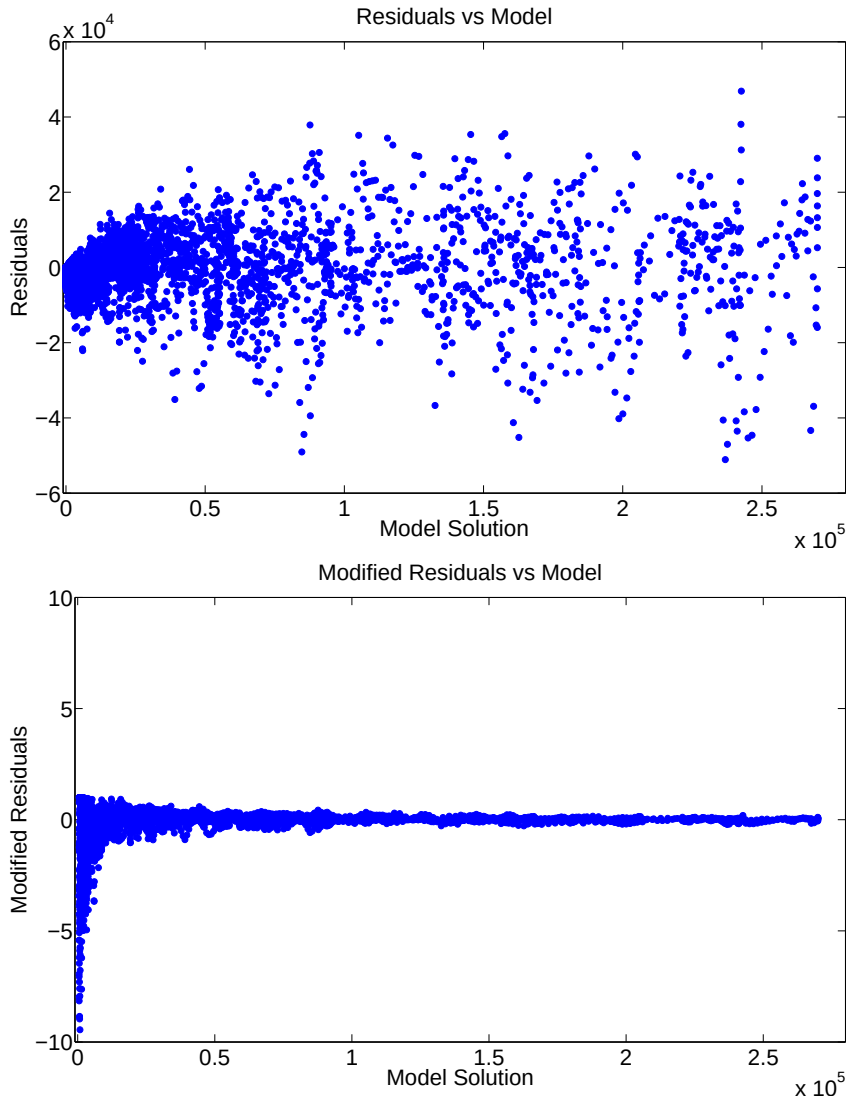
$$N_k^j = I[\tilde{n}](t_j, z_k^j; \theta_0) + \mathcal{E}_{kj}$$

- Currently using constant variance (CV) model, $Var(\mathcal{E}_{kj}) = \sigma_0^2$ ($\Rightarrow$ Absolute Error)
- Could use constant coefficient of variance (CCV), $Var(\mathcal{E}_{kj}) = \sigma_0^2 I[\tilde{n}](t_j, z_k^j; \theta_0)^2$ ($\Rightarrow$ Relative Error)

Residual Plots



Residual Plots (cont'd)



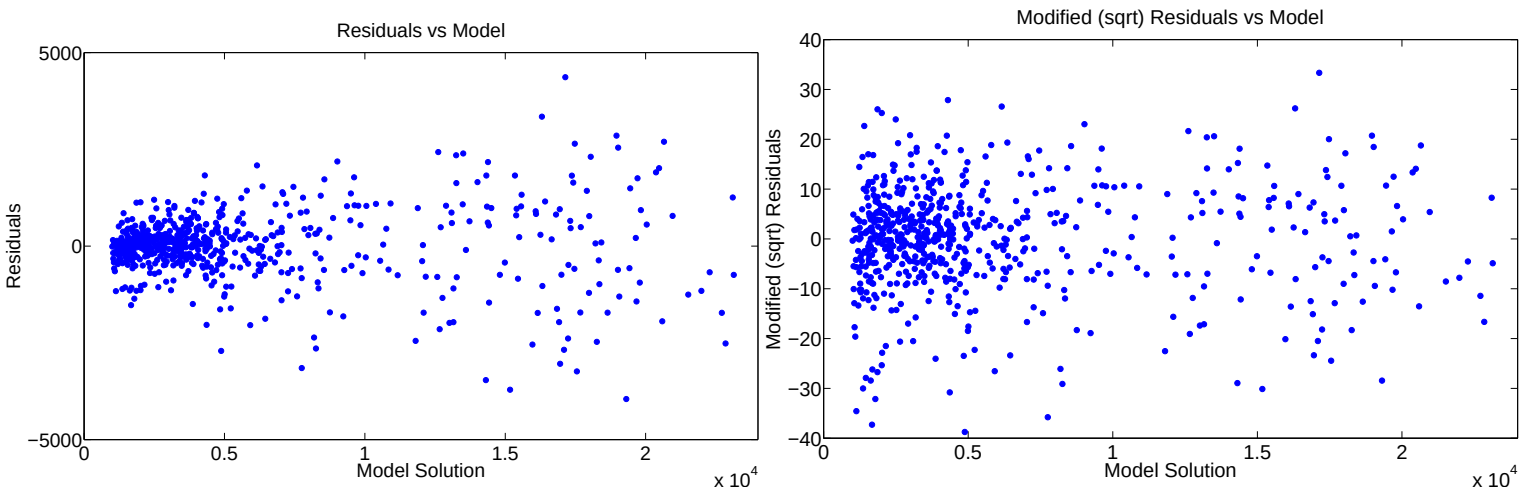
New Statistical Model

$$N_k^j \sim \mathcal{N}\left(\lambda_j I[\tilde{n}](t_j, z_k), \lambda_j \frac{B}{\hat{b}_j} I[\tilde{n}](t_j, z_k)\right)$$

- $\lambda_j = b_j / \hat{b}_j$
- b_j is the 'true' number of beads counted at time t_j
- $\hat{b}_j$ is the actual number of beads counted
- B is the total number of beads original placed into each well
- 'Sampling without replacement'

New Statistical Model (cont'd)

- Can be derived from counting arguments (ignoring interdependence)
- Additional parameters b_j to be estimated
- Explains residual variance, 'precursor cohort problem'
- Implications for estimation procedure, model comparison



Model Generalizations

- Examination of AutoFI distribution
  - Cell division as a fission process
  - Activation and/or time-dependence (machine calibration issues?)
  - Nonparametric estimation?
  - ... or not even estimate it at all?
- (Improved) biologically meaningful prolf/death rates
  - Smith-Martin, probabilistic mechanisms
  - Include stimulation/signaling mechanisms

Allgöwer et al.

- Dynamics for cell division, CFSE quantity, and measured FI can be decoupled
- Allows for fast computational solution

$$n_i(t,x) = N_i(t,x)\bar{n}_i(t,x)$$

where

$$\frac{dN_i}{dt} = -(\alpha_i(t) + \beta_i(t))N_i(t) + 2\alpha_{i-1}(t)N_{i-1}(t)$$

$$N_0(0) = N_0, N_i(0) = 0$$

and

$$\frac{\partial \bar{n}_i}{\partial t} - \frac{\partial[v(t,x)\bar{n}(t,x)]}{\partial x} = 0$$

$$\bar{n}_i(0,x) = 2^i \Phi(2^i x)/N_0$$

- Convolution operator to link CFSE content with measured FI (hence AutoFI)

Experimental Extensions

- Account for multiple cell cultures present in PBMC culture
- Antigen-specific stimulation
- Division-linked changes, differentiated subsets
- Extracellular signaling, knockout experiments
- In vitro vs in vivo differences
- Linking to immune/pathogenesis models
- **Analyze Proliferation in Diseased vs Healthy cells**

Selected Sources

D. Schittler, J. Hasenauer, and F. Allgower, A generalized population model for cell proliferation: integrating division numbers and label dynamics, *Proc. 8th Intl. Workshop on Computational Systems Biology*, June 2011, Zurich, Switzerland.

H.T. Banks, Karyn L. Sutton, W. Clayton Thompson, G. Bocharov, Marie Doumic, Tim Schenkel, Jordi Argilaguet, Sandra Giest, Cristina Peligero, and Andreas Meyerhans, A New Model for the Estimation of Cell Proliferation Dynamics Using CFSE Data, CRSC-TR11-05, North Carolina State University, Revised July 2011; *J. Immunological Methods* **343** (2011), 143–160.

H.T. Banks, W. Clayton Thompson, Cristina Peligero, Sandra Giest, Jordi Argilaguet, and Andreas Meyerhans, A Division-Dependent Compartmental Model for Computing Cell Numbers in CFSE-based Lymphocyte Proliferation Assays, CRSC-TR12-03, North Carolina State University, February 2012; *Math Biosci. Eng.* (submitted).

T. Luzyanina, D. Roose, T. Schenkel, M. Sester, S. Ehl, A. Meyerhans, and G. Bocharov, Numerical modelling of label-structured cell population growth using CFSE distribution data, *Theoretical Biology and Medical Modelling* **4** (2007), Published Online.

W. Clayton Thompson, Partial Differential Equation Modeling of Flow Cytometry Data from CFSE-based Proliferation Assays, Ph.D. Dissertation, Dept. Mathematics, North Carolina State University (2012).