Modeling & Simulation (Computational Immunology)

Steven H. Kleinstein

YALE Pathology Informatics

Departments of Pathology and Immunobiology
Yale University School of Medicine

steven.kleinstein@yale.edu

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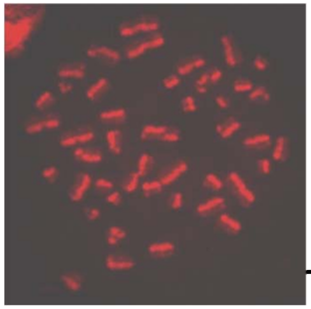
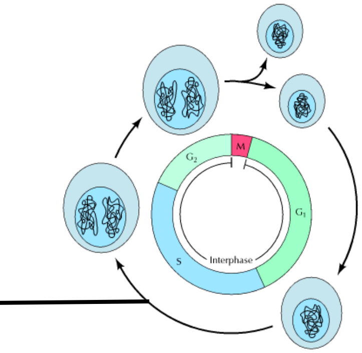
Understanding cell proliferation and death

BrdU (thymidine analog) incorporated into cell DNA during S-phase

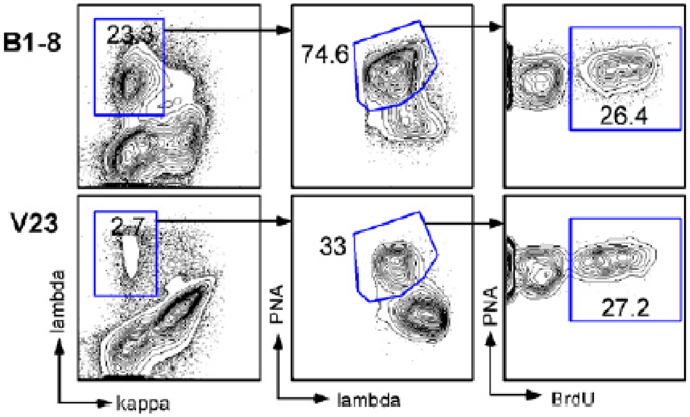
Flow cytometry to quantify antigen-specific germinal center B cells…

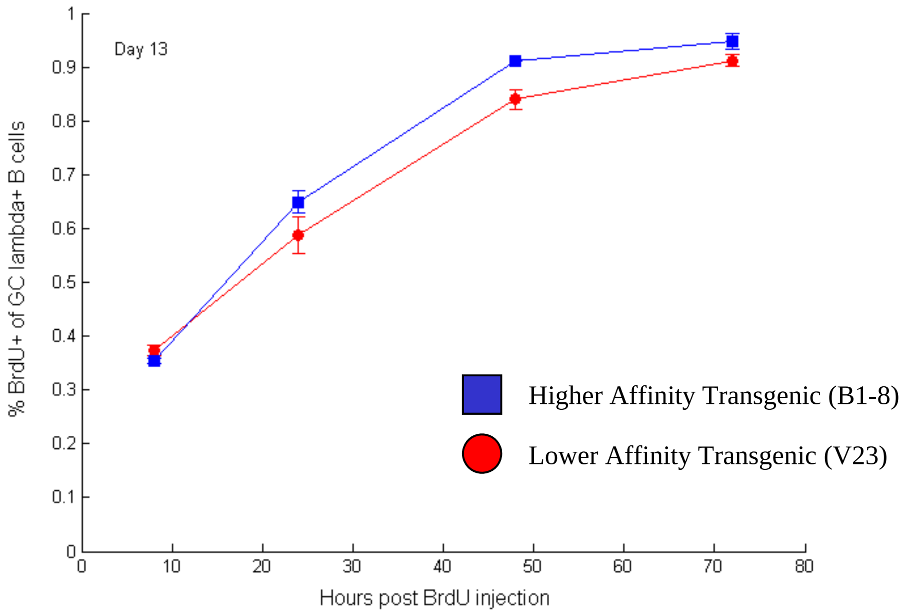


BrdU incorporated during S phase



science.csustan.edu/confocal/Images/SCE/index.SCE.htm

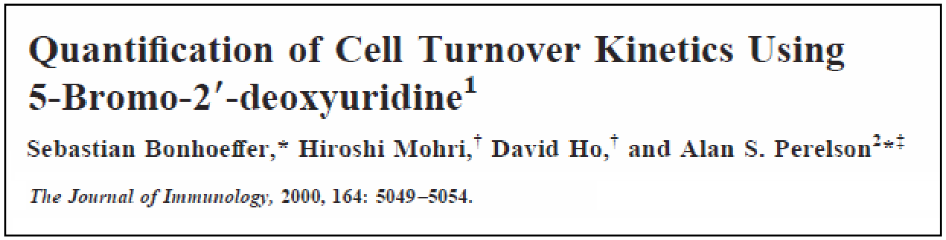




Labeling curves look similar – suggests same proliferation rate?

Understanding cell proliferation and death

At steady-state, rate at which the fraction of BrdU labeled cells increases is indicative of the sum of the per cell proliferation and death rates



Quantification of Cell Turnover Kinetics Using 5-Bromo-2′-deoxyuridine[1]

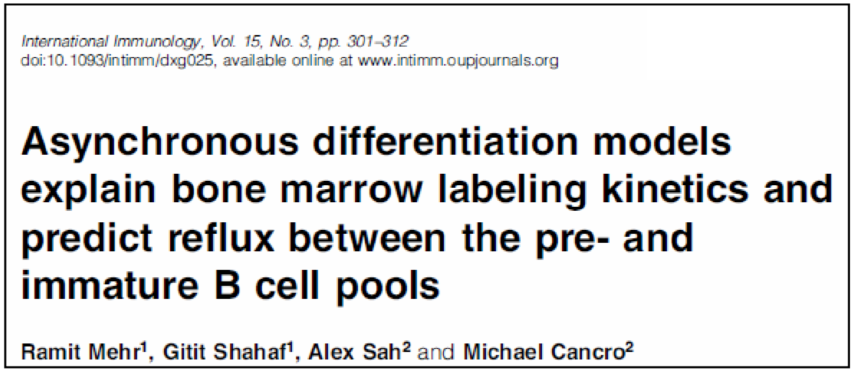
Sebastian Bonhoeffer,[*] Hiroshi Mohri,[†] David Ho,[†] and Alan S. Perelson[2*‡]

The Journal of Immunology, 2000, 164: 5049–5054.

Rapid Turnover of T Lymphocytes in SIV-Infected Rhesus Macaques

Hiroshi Mohri, Sebastian Bonhoeffer, Simon Monard, Alan S. Perelson, David D. Ho*

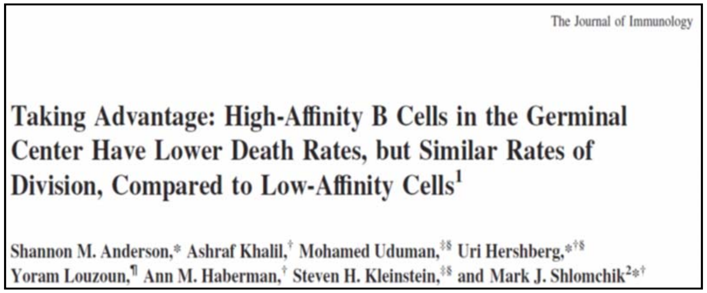
www.sciencemag.org • SCIENCE • VOL. 279 • 20 FEBRUARY 1998



International Immunology, Vol. 15, No. 3, pp. 301–312
doi:10.1093/intimm/dxg025, available online at www.intimm.oupjournals.org

Asynchronous differentiation models explain bone marrow labeling kinetics and predict reflux between the pre- and immature B cell pools

Ramit Mehr[1], Gitit Shahaf[1], Alex Sah[2] and Michael Cancro[2]



The Journal of Immunology

Taking Advantage: High-Affinity B Cells in the Germinal Center Have Lower Death Rates, but Similar Rates of Division, Compared to Low-Affinity Cells[1]

Shannon M. Anderson,[*] Ashraf Khalil,[†] Mohamed Uduman,[‡§] Uri Hershberg,[*†§] Yoram Louzoun,[¶] Ann M. Haberman,[†] Steven H. Kleinstein,[‡§] and Mark J. Shlomchik[2*†]

Oncogene (2005) 24, 7514–7523
© 2005 Nature Publishing Group All rights reserved 0950-9232/05 $30.00
www.nature.com/onc

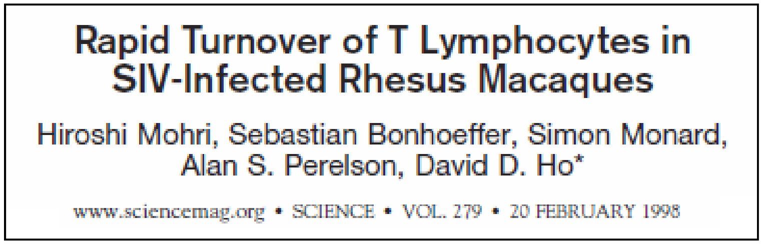
Reduced cell turnover in lymphocytic monkeys infected by human T-lymphotropic virus type 1

Christophe Debacq[1,5], Jean-Michel Héraud[2,5], Becca Asquith[3], Charles Bangham[3], Fabrice Merien[2], Vincent Moules[4], Franck Mortreux[4], Eric Wattel[4], Arsène Burny[1], Richard Kettmann[1], Mirdad Kazanji[2] and Luc Willems[*,1]

Models of BrdU incorporation integral part of many studies

BrdU labeling of CD4+ and CD8+ T lymphocytes

SIV-infected and an uninfected macaque. Data are from Mohri et al., Science (1998)



Rapid Turnover of T Lymphocytes in SIV-Infected Rhesus Macaques

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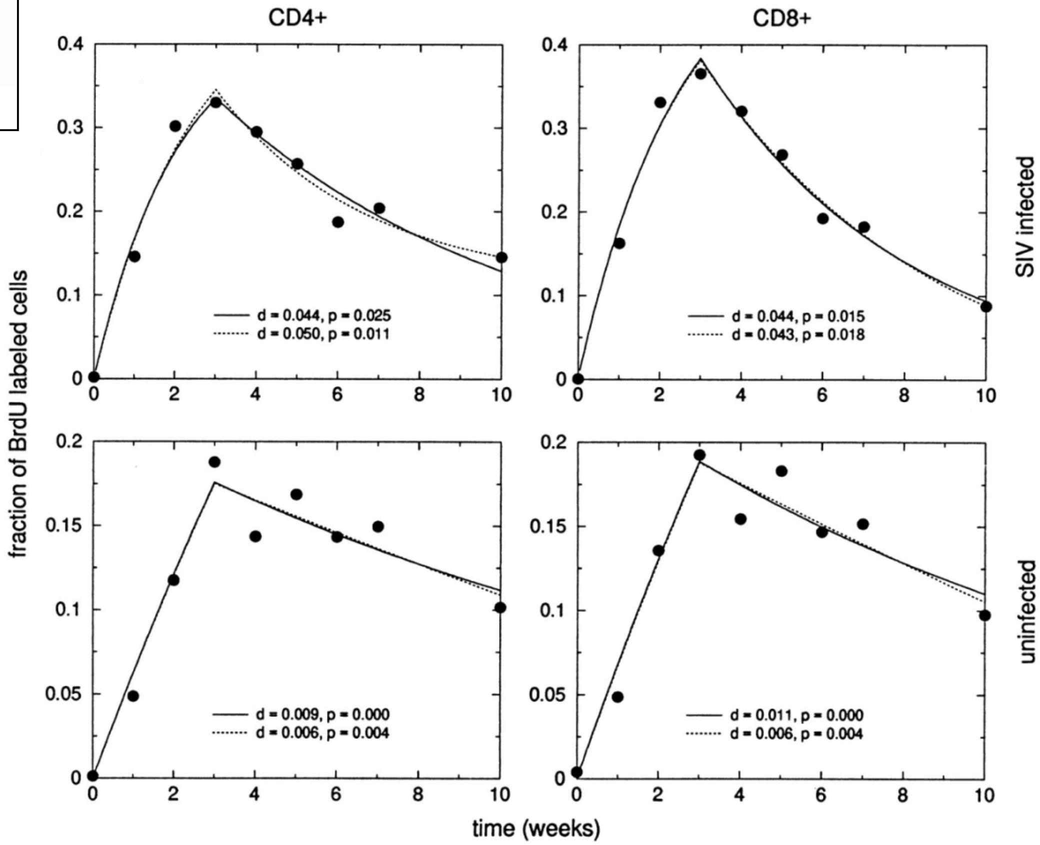
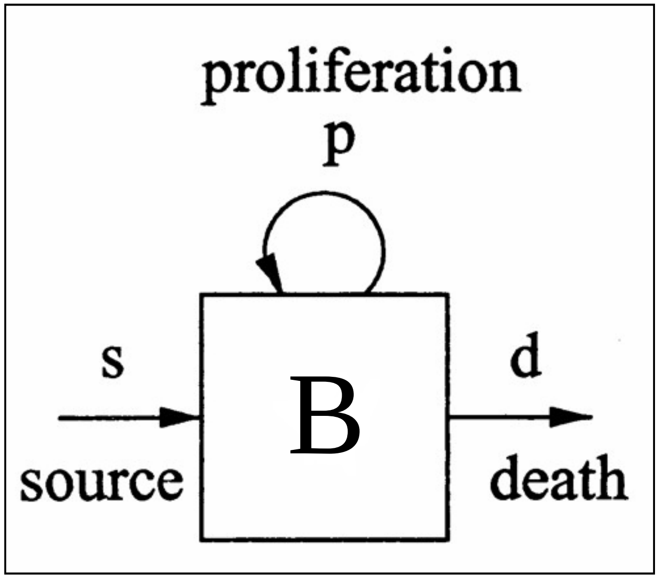


Is there a difference in cell turnover?

Model of BrdU Labeling

Start with a basic model of cell population dynamics…



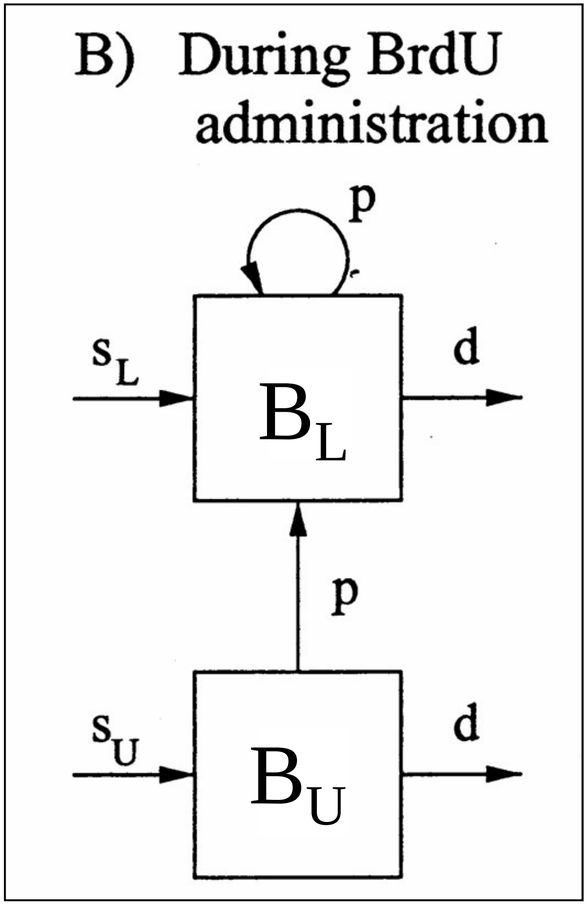
Rate of change in B cell population

$$\frac{dB}{dt} = s + pB - dB$$

Often can often assume population in steady-state (i.e., constant)

Model of BrdU Labeling

Split the B cell population into Labeled (B_L) and Unlabeled (B_U) subsets



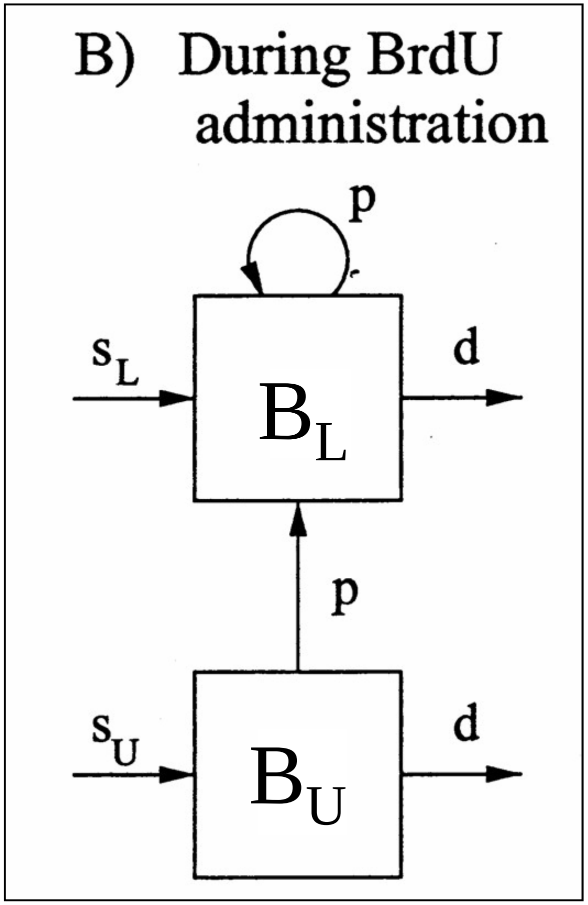
$$\frac{dB_U}{dt} = s_u - pB_U - dB_U$$

$$\frac{dB_L}{dt} = s_l + 2pB_U + pB_L - dB_L$$

Do data contain enough information to estimate parameters?

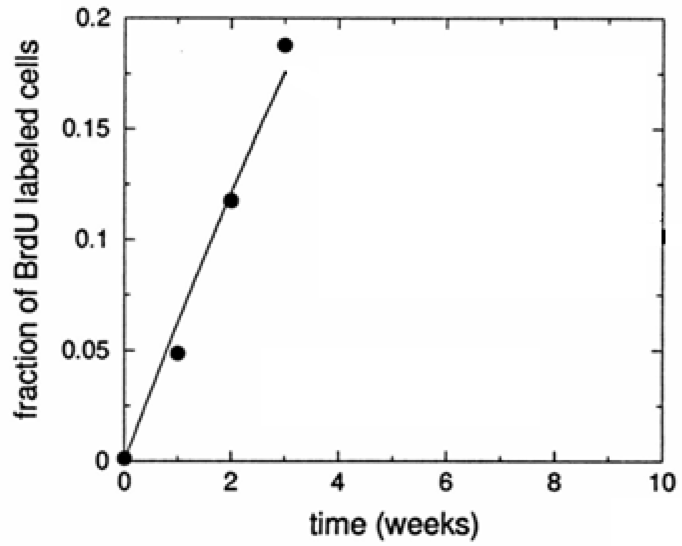
Model of BrdU Labeling

Label is administered continuously over some time-period



$$f_L(t) = A_1(1 - e^{-(d+p)t})$$

$$A_1 = 1 - \frac{s_U}{(s_U + s_L)} \times \frac{(d - p)}{(d + p)}$$



Labeling curve reflects both proliferation AND death

Model Identifiability

A model is identifiable if possible to learn true value of underlying parameter after obtaining enough observations

Identifiable parameters are those which effect the value of the data and can be estimated with some degree of certainty.

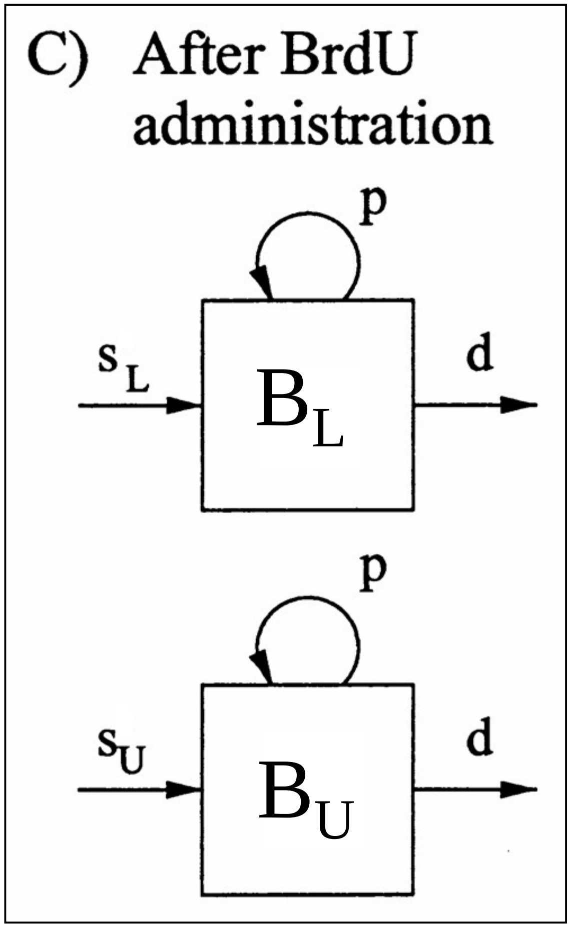
Non-identifiable parameters are those which effect the value of the data but which cannot be estimated accurately

Non-observable parameters are those which don't have an effect on the data.

Cannot estimate both proliferation AND death

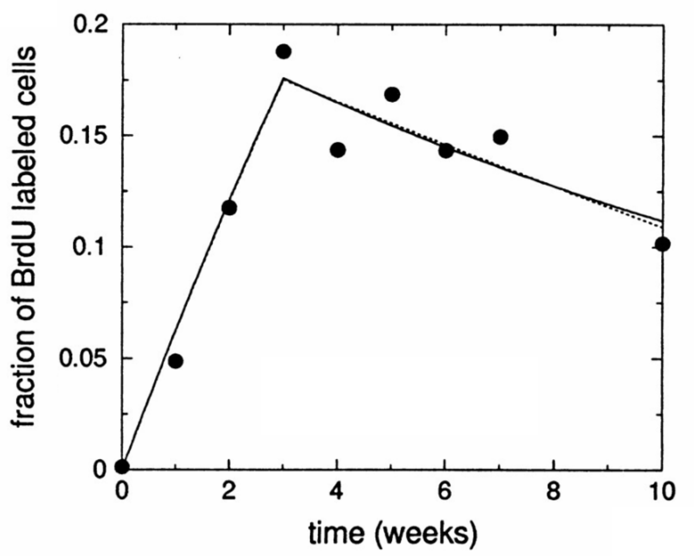
Model of BrdU DE-Labeling

Stop administering label after some time (t_e)



$$f_L\left(t\right) = A_2 + A_3 e^{-(d-p)(t-t_e)})$$

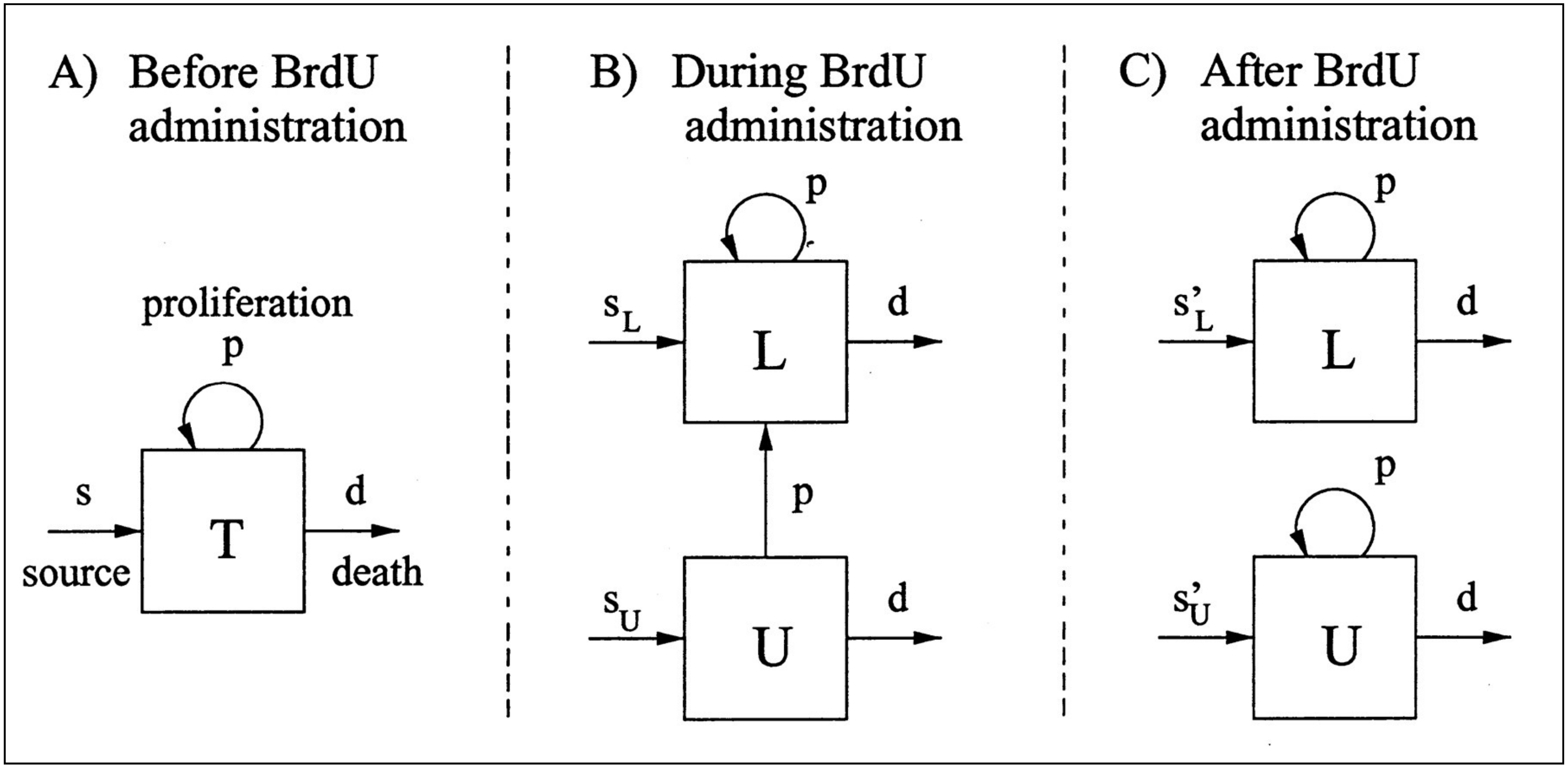
$$A_2 = \frac{s_L}{(s_U + s_L)}, A_3 = f_L(t_e) - A_2$$



Now, we can estimate BOTH proliferation AND death

Model of BrdU Labeling

Model changes with experiment


A) Before BrdU administration
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death
B) During BrdU administration
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U
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C) After BrdU administration
p
s'_L
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We can express these as sets of ordinary differential equations

Characteristics of a Good Inverse Model

- Fit is good—model should be able to adequately describe a relatively noise-free data set (of course a poor fit provides some insight also)
- Model parameters are unique
  - Theoretically identifiable for noise-free data
  - Well-determined model parameters in presence of measurement noise
- Values of parameter estimates are consistent with hypothesized physical or physiologic meanings and change appropriately in response to alterations in the actual system

Six Steps for Inverse-Modeling of Data

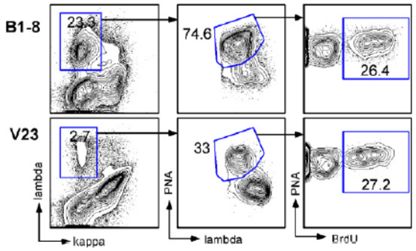
1. Select an appropriate mathematical model
   - Polynomial or other functional form
   - Based on underlying theoretical equations
2. Define a “figure of merit” function
   - Measures agreement between data & model for given parameters
3. Adjust model parameters to get a “best fit”
   - Typically involves minimizing the figure of merit function
4. Examine “goodness of fit” to data
   - Never perfect due to measurement noise
5. Determine whether a much better fit is possible
   - Tricky due to possible local minima vs. global minimum
   - F-test for comparing models of different complexity
6. Evaluate accuracy of best-fit parameter values
   - Provide confidence limits and determine uniqueness
   - Assess physical reasonability of estimated parameter values

(Costa, Kleinstein and Hershberg, Sci Signal. 2011)

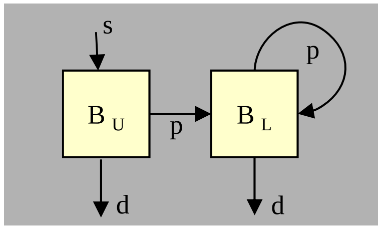
Interaction of Computation & Experiment

Compare simulation and experiment using least-squares objective

Experimental Observations



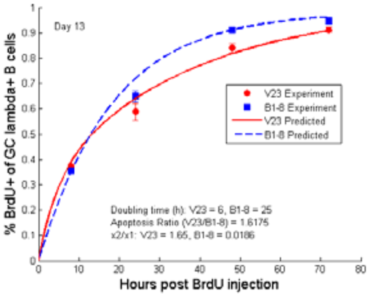
Computational Model



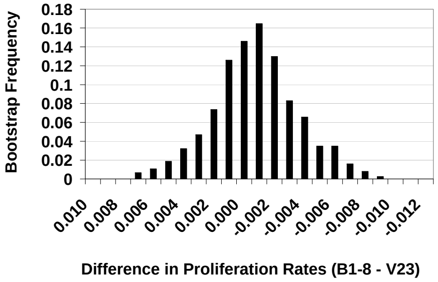
Least-squares objective function

$$E = \sum_i \frac{(y_i - \hat{y}_i)^2}{VAR(y_i)}$$

Fit Model to Data



Model Predictions



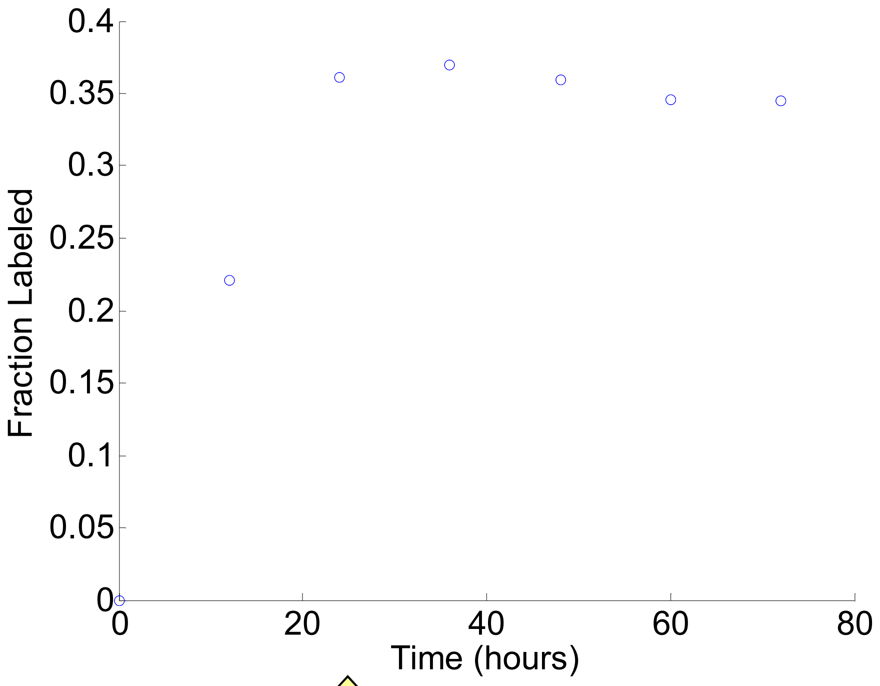
Bootstrapping Confidence Intervals

New Experiments

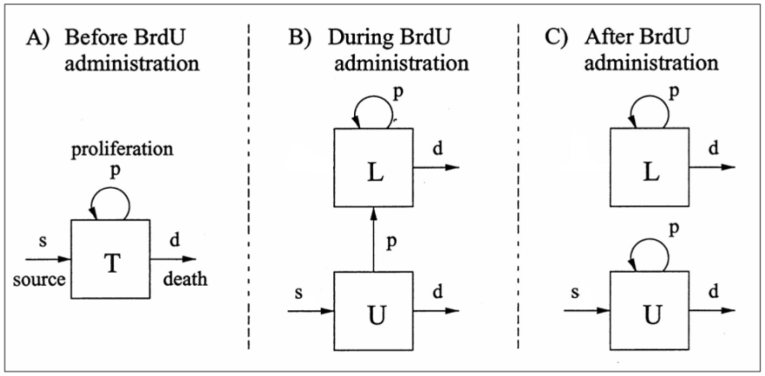
Continuous cycle of modeling and experimentation

Simulated Experiment

Demonstrate full cycle of fitting model to data to estimate parameters



BrdU withdrawn


A) Before BrdU administration
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B) During BrdU administration
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C) After BrdU administration
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Parameters used to create synthetic data

s = 0.003 per hour

p = 0.01 per hour

d = p + s (to achieve steady state)

Random noise added to each data point

How can we estimate flow/proliferation/death rates?

Numerical solution to ODEs

Euler's Method

$$y'(t) = f(t, y(t)), \qquad y(t_0) = y_0,$$

$$y'(t) \approx \frac{y(t+h) - y(t)}{h},$$

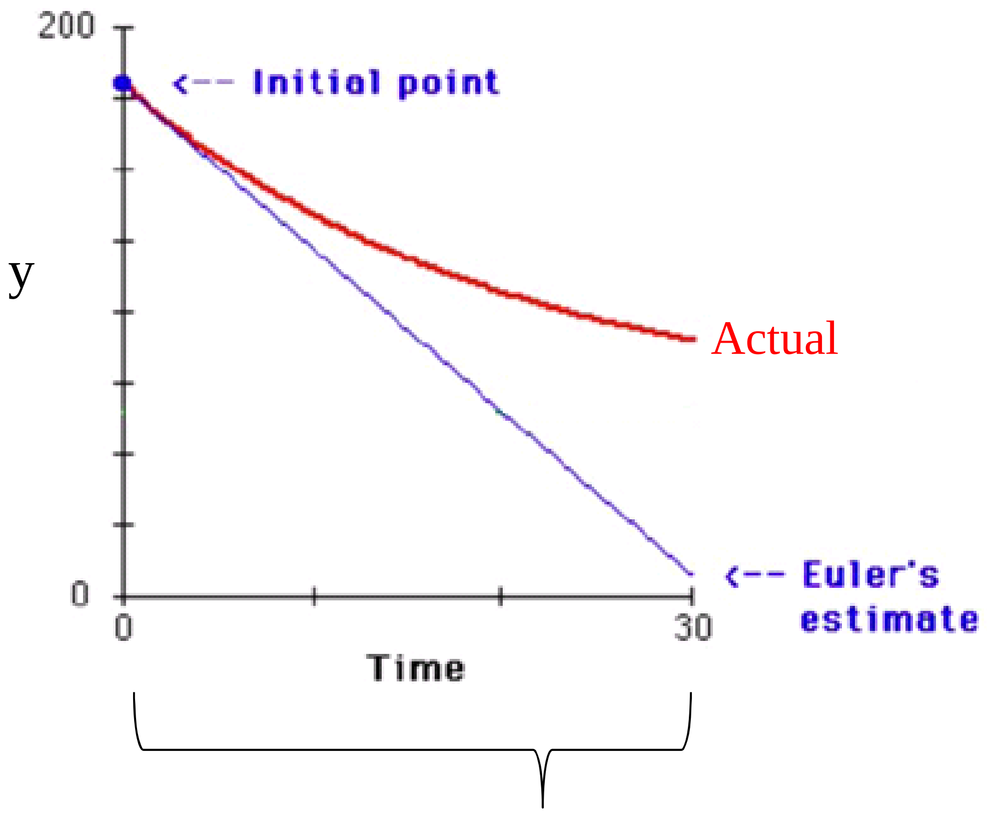
$$y(t+h) \approx y(t) + hf(t, y(t)).$$

Leonhard Euler
(1707-1783)

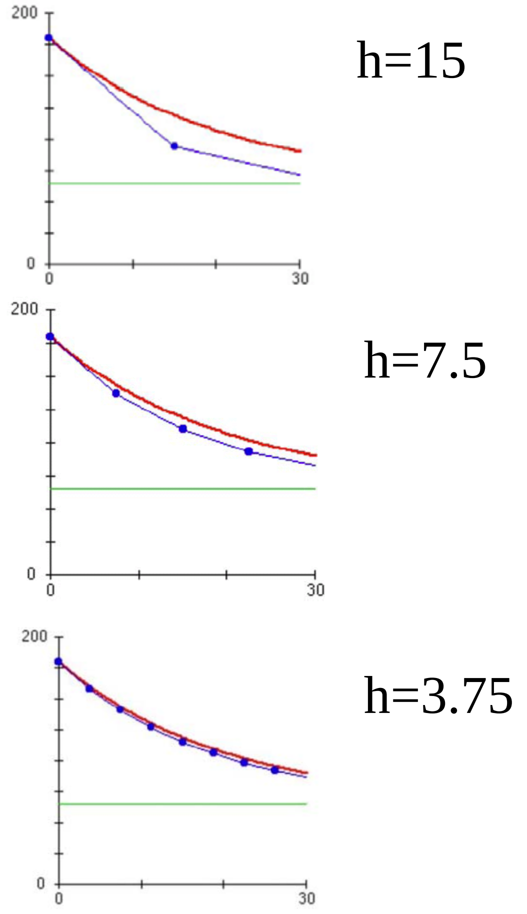
From any point on curve, find approximation of nearby point on curve by moving a short distance along a line tangent to the curve

Numerical solution to ODEs: Euler Method

From any point on curve, find approximation of nearby point on curve by moving a short distance along a line tangent to the curve



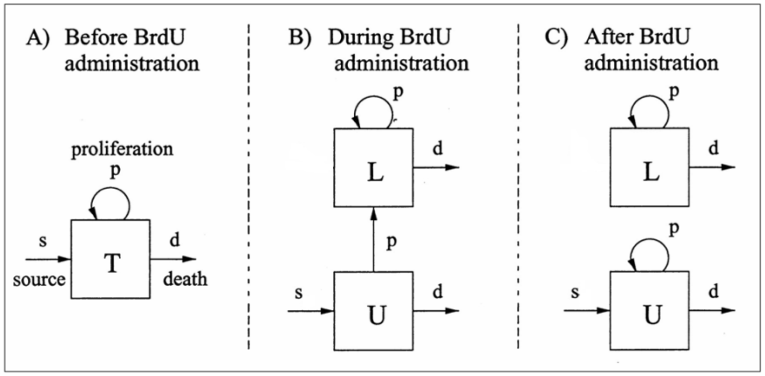
$$y(t+h) \approx y(t) + hf(t, y(t)).$$



Much better ways to do this in practice. Eg, Runge-Kutta

Simulating the BrdU Labeling Model

Use integration functions (e.g., ode45 in MATLAB)


A) Before BrdU administration
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C) After BrdU administration
p
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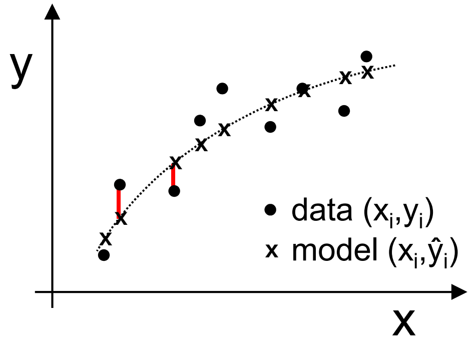
d

```
Yin = [1 0];        % Initial Conditions [unlabeled labeled]
pr = [s p d tau];  % Model Parameters
t = [0,12,24,36,48,60,72];   % Times to evaluate
[T,Y] = ode45(@fode,t,Yin,opts,pr);
fl = Y(:,2) ./ sum(Y,2);  % Fraction labeled
```

```
function dy = fode(t, y, pr)
s = pr(1); p = pr(2); d = pr(3); tau = pr(4);
U = y(1); L = y(2);
dy = zeros(2,1); % Vector of derivatives
if (t<tau)  % During BrdU Administration (B)
   dy(1) =  s - p.*U - d.*U;                  % dbU/dt
   dy(2) =  2.*p.*U + p.*L - d.*L;        % dbL/dt
else        % After BrdU Administration (C)
   dy(1) =  s + p.*U - d.*U;                  %dbU/dt
   dy(2) =        p.*L - d.*L;                %dbL/dt
end
```

Simple models can be solved analytically -- faster

Least-Squares Error Minimization



- Goal is to fit *N* data points (x_i, y_i) i=1..N
- The model is a function with *M* adjustable parameters a_k, k=1..M used to generate *N* model points (x_i, $\hat{y}_i$)

$$\hat{y}_i = \hat{y}(x_i, a_1..a_M)$$

- The residual measures the difference between a data point and the corresponding model estimate

$$y_i - \hat{y}(x_i, a_1..a_M)$$

- Since residuals can be positive or negative, a sum of residuals is not a good measure of overall error in the fit

$$\sum_{i=1}^{N}[y_i - \hat{y}(x_i, a_1..a_M)]$$

- A better measure is the sum of squared residuals, *E*, which is only zero if each and every residual is zero

$$E = \sum_{i=1}^{N}[y_i - \hat{y}(x_i, a_1..a_M)]^2$$

(Costa, Kleinstein and Hershberg, Sci Signal. 2011)

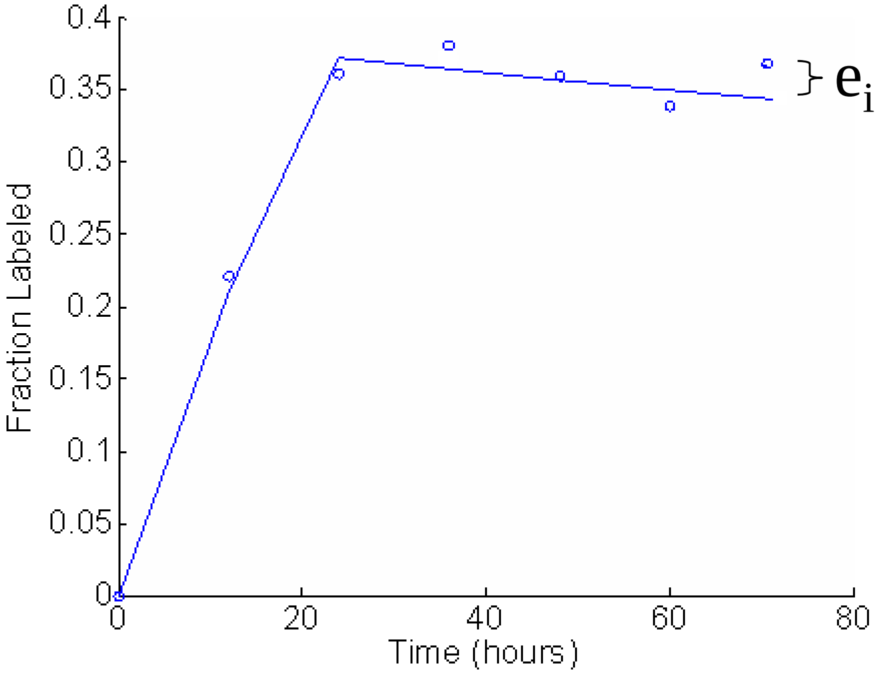
Maximum Likelihood Estimation

- Not meaningful to ask “What is the probability that my set of model parameters is correct?”
  - Only one correct parameter set ➔ Mother Nature!
- Better to ask “Given my set of model parameters, what is the probability that this data set could be obtained?”
  - What is the likelihood of the parameters given the data?
- Inverse modeling is also known as “maximum likelihood estimation”.

(Costa, Kleinstein and Hershberg, Sci Signal. 2011)

Fitting the Model to Experimental Data

Compare simulation and experiment using least-squares objective



Least-squares objective function

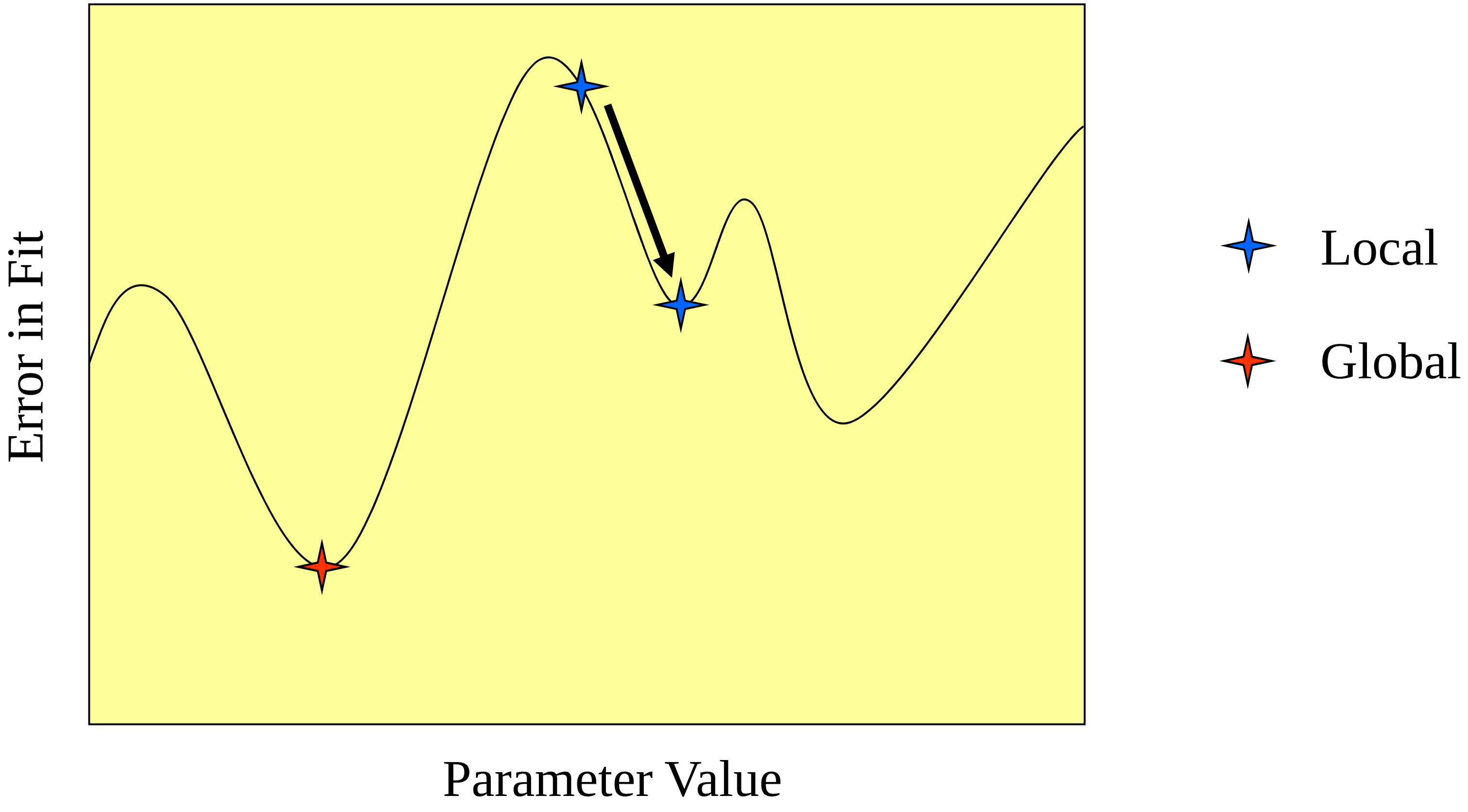
$$E = \sum_i \frac{(y_i - \hat{y}_i)^2}{VAR(y_i)}$$

Find parameters to minimize objective

Many options for how to optimize the fit

Local and Global Optimization

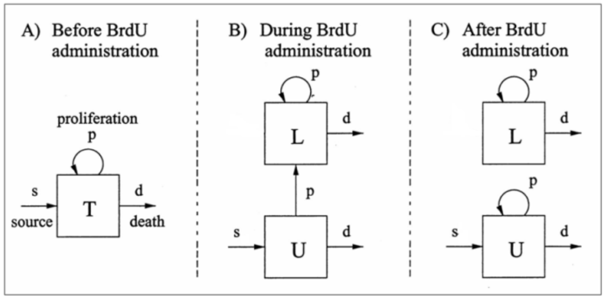
The error function depends on M model parameters, and can be thought of as an M-dimensional "surface" of which we seek the minimum



Local optimization techniques find optimal fit around given starting point
Global optimization attempts to avoid local minima

Fitting Models to Data in MATLAB

Several optimization functions available in many programming languages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```
pri = [.01 .01];  %Initial guess for parameter values to be fitted [s p]
[pr,fval,exitflag] = lsqnonlin (@efun,pri,[],[],options,fl_observed,t,tau);
s = pr(1); p = pr(2); % Optimal parameter values
```

Optional parameters

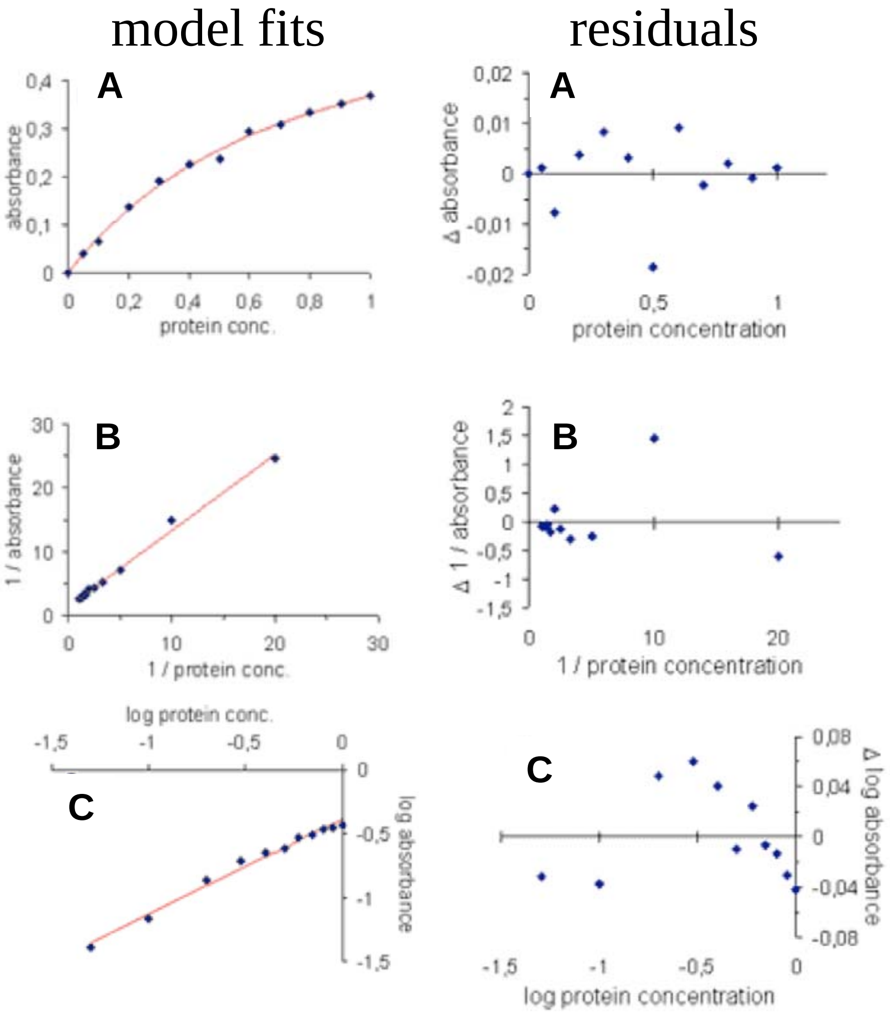
```
function error = efun (pr,fl_observed,t,tau)
  s = pr(1); p = pr(2); d = s+p;                    % Assume steady-state
  [fl_predicted] = labelBrdU(s,p,d,tau,t);          % Function that simulates model
  error = sum((fl_predicted-fl_observed).^2);       % Least-squares objective
```

lsqnonlin, fminsearch, fmincon, fminbnd

Goodness of Fit and the Residuals Plot

- A high correlation can exist even for a model that systematically differs from the data (all 3 examples have $r^2 > 0.99$)

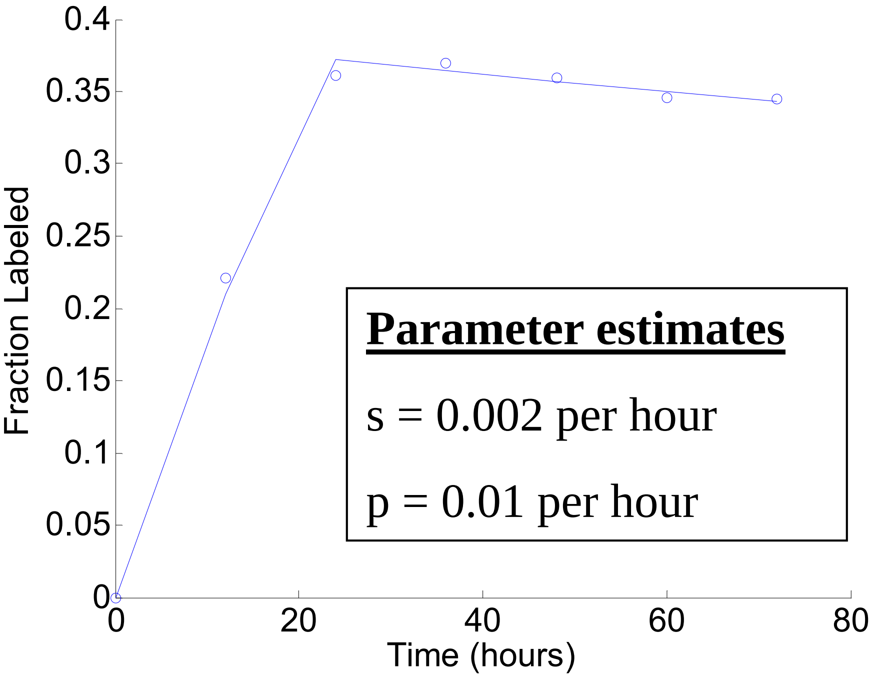
- One must also examine the distribution of residuals—a good model fit should yield residuals equally distributed along *x* and normally distributed around zero with no systematic trends, as in A rather than B or C



adapted from Lobemeier, 2000

Optimal Parameter Estimates

Least-squares fit using lsqnonlin in MATLAB

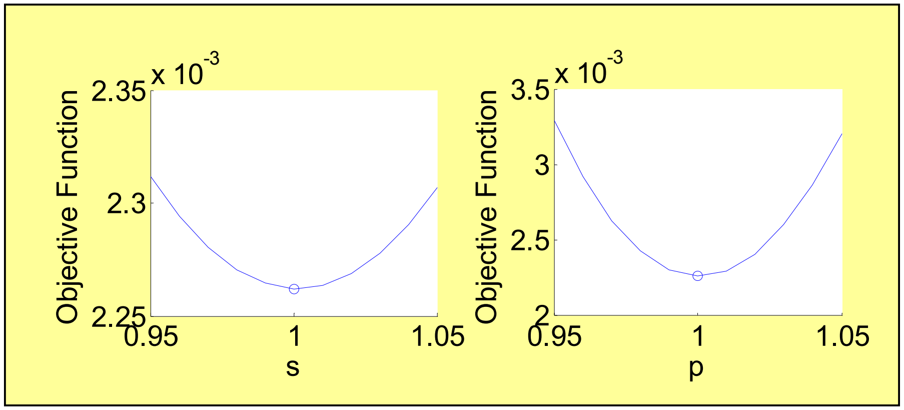


Parameter estimates

s = 0.002 per hour

p = 0.01 per hour

Plot local curvature to check minimization…



Recall, parameters used to create data:

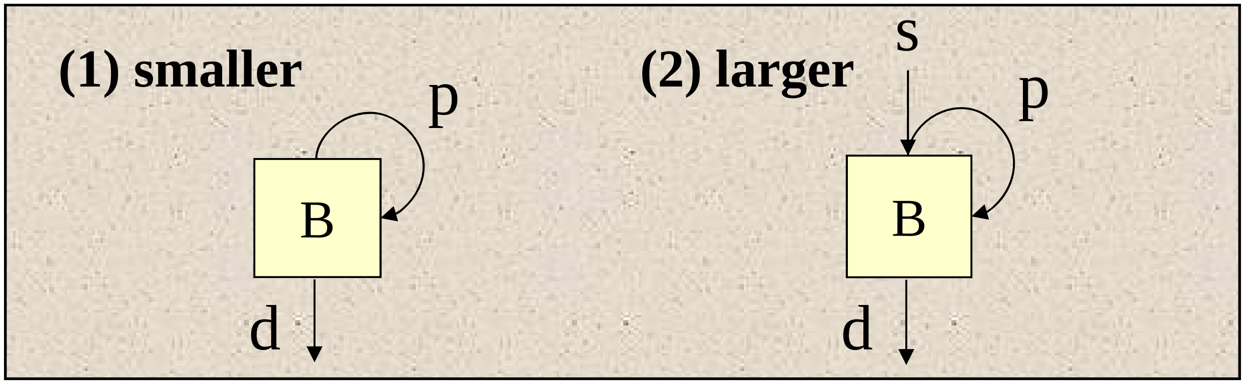
s = 0.003 per hour

p = 0.01 per hour

d = p + s (to achieve steady state)

Is inflow necessary to fit the data? Can we use simpler model?

Is inflow (s) significant?



Residual Sum of Squares $\boxed{RSS = \sum_i (y_i - \hat{y}_i)^2}$

$$F = \frac{\left(\text{RSS}_{\text{smaller}} - \text{RSS}_{\text{larger}}\right) / \left(df_{\text{smaller}} - df_{\text{larger}}\right)}{\text{RSS}_{\text{larger}} / df_{\text{larger}}}$$

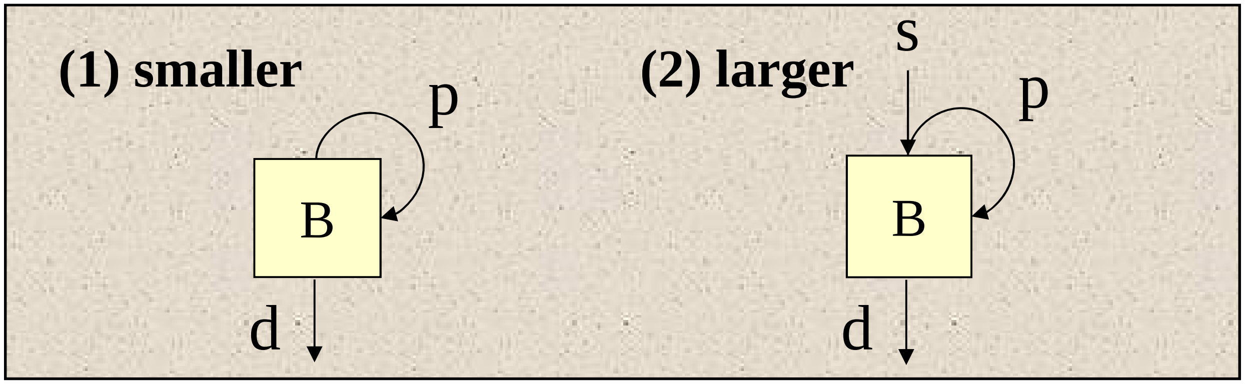
Reduction in RSS per extra parameter

Measure of ‘noise’ in model

Degrees of Freedom $\boxed{df = \#observations - \#parameters}$

F distribution with (df_{smaller}-df_{larger}, df_{larger}) degrees of freedom

Is inflow (s) significant?



$$F = \frac{\left(\mathrm{RSS}_{\text{smaller}} - \mathrm{RSS}_{\text{larger}}\right) / \left(df_{\text{smaller}} - df_{\text{larger}}\right)}{\mathrm{RSS}_{\text{larger}} / df_{\text{larger}}}$$

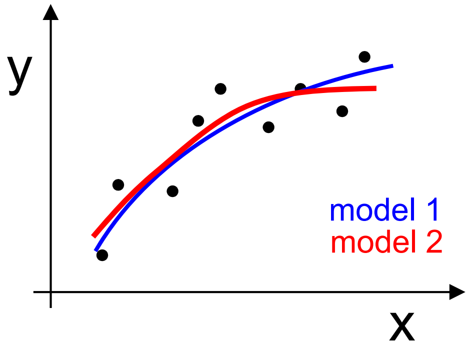
Reduction in RSS per extra parameter

Measure of ‘noise’ in model

	Observations	Parameters	RSS	F test (1-fcdf in MATLAB)
(1) No flow (s=0)	6	1	9.38e-7	
(2) Including flow	6	2	0.95e-7	**53.1 (p<0.0004)**

Inflow (s) is important to explain observations

Comparing Two Model Fits



- The number of data points, *N*, must exceed the number of model parameters, *M*, yielding the degrees of freedom (*DOF* = *N*-*M*)

$$M \leq N - 1$$

- Increasing *M* using a more complex model will generally improve the quality of fit and reduce RSS
- Increasing *MSE* with decreasing RSS can reveal an over-parameterized model

$$MSE = \frac{RSS}{N-M} = \frac{RSS}{DOF}$$

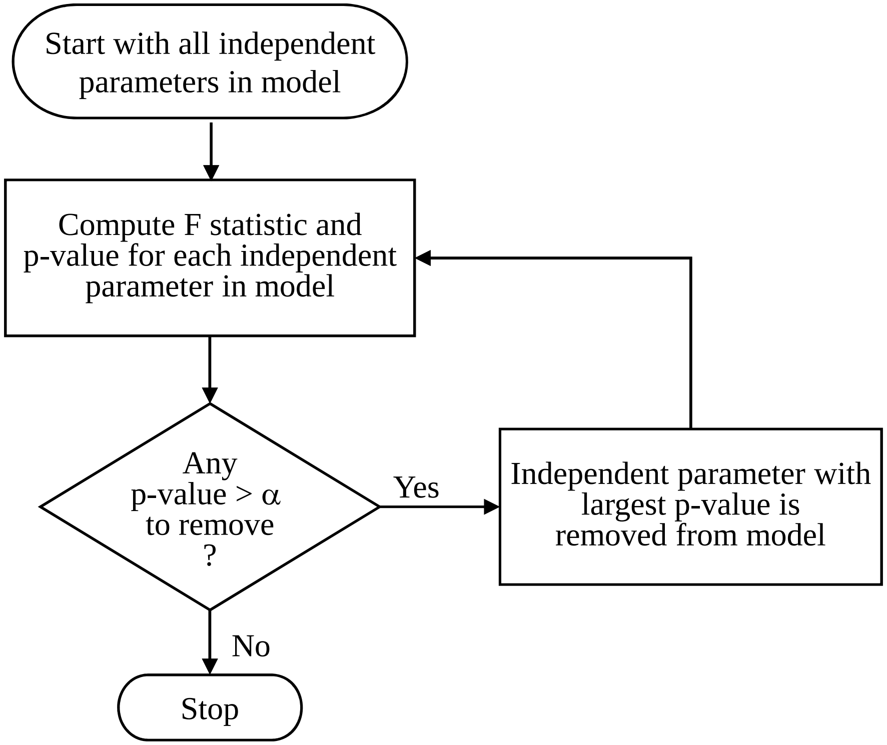
- An F-statistic can be computed to compare the results of two model fits
  - F ~ 1, the simpler model is adequate
  - F > 1, the more complex model is better, or random error led to a better fit with the complex model
  - P-value defines the probability of such a "false positive" result (lookup in F table)

(Costa, Kleinstein and Hershberg, Sci Signal. 2011)

Building models with variable selection

F statistic determines if variable added or deleted from model

Backward Elimination


Start with all independent parameters in model
Compute F statistic and p-value for each independent parameter in model
Any p-value > α to remove ?
Yes
Independent parameter with largest p-value is removed from model
No
Stop

Other Variations:

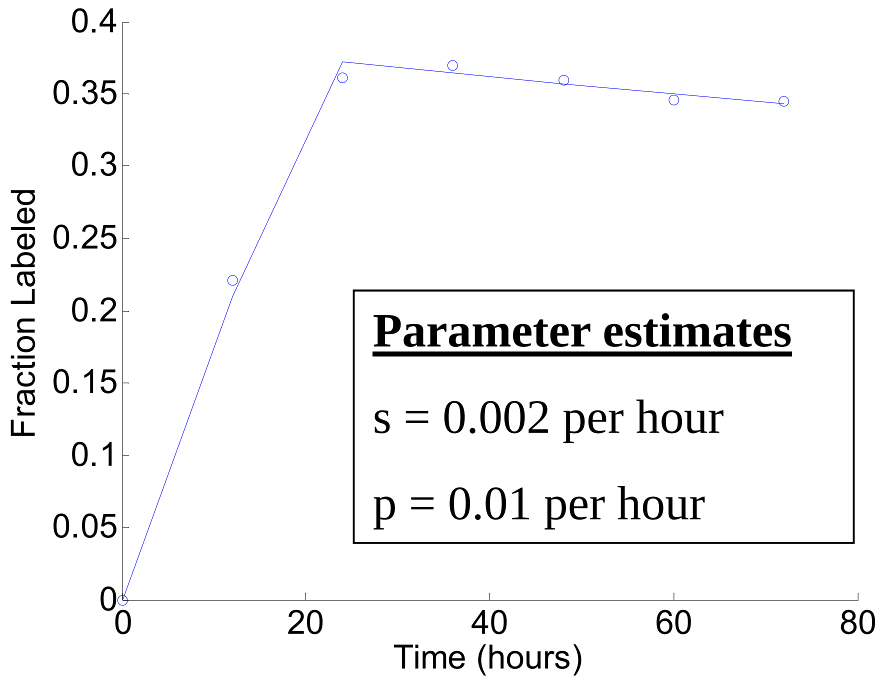
Forward selection: adds variables one at a time as long as significant F test.

Stepwise procedure: allows for removal of a parameter at each step

No guarantee that globally optimal model with be found (need all subsets, but prohibitive for large parameter space)

How much confidence to put in estimate?

Construct confidence intervals for model parameters



Parameter estimates

s = 0.002 per hour

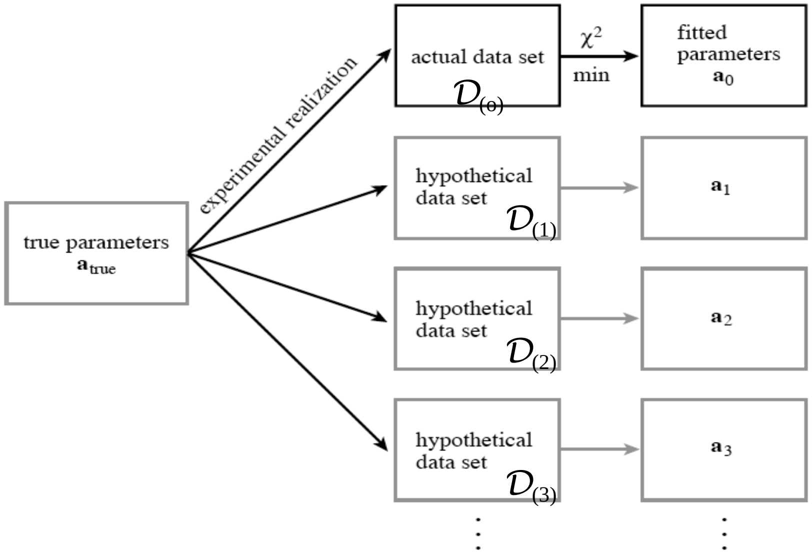
p = 0.01 per hour

Estimate uncertainty given limited number of experimental observations

Accuracy of Estimated Model Parameters

Underlying true set of model parameters ($\mathbf{a}_{\text{true}}$) known to Mother Nature but hidden from the experimenter

- True parameters are statistically realized as measured data set $\mathcal{D}_{(o)}$

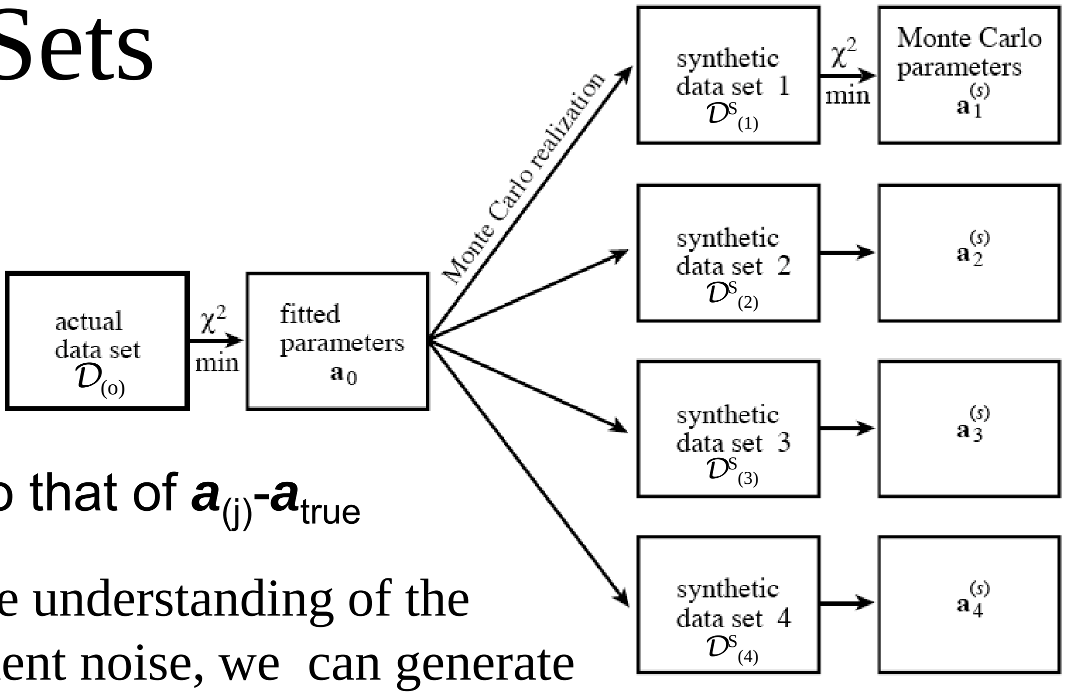


from Numerical Recipes online

- Fitting $\mathcal{D}_{(o)}$ yields estimated model parameters $\mathbf{a}_{(o)}$
- Other experiments could have resulted in data sets $\mathcal{D}_{(1)}$, $\mathcal{D}_{(2)}$, etc. which would have yielded model parameters $\mathbf{a}_{(1)}$, $\mathbf{a}_{(2)}$, etc.

Estimate probability distribution of $\mathbf{a}_{(i)}$ - $\mathbf{a}_{\text{true}}$ without knowing $\mathbf{a}_{\text{true}}$

Monte Carlo Simulation of Synthetic Data Sets

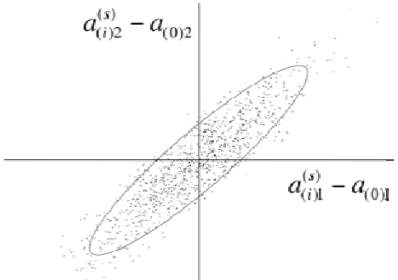

actual data set $\mathcal{D}_{(o)}$
χ^2 min
fitted parameters $\mathbf{a}_0$
Monte Carlo realization
synthetic data set 1 $\mathcal{D}^S_{(1)}$
synthetic data set 2 $\mathcal{D}^S_{(2)}$
synthetic data set 3 $\mathcal{D}^S_{(3)}$
synthetic data set 4 $\mathcal{D}^S_{(4)}$
χ^2 min
Monte Carlo parameters $\mathbf{a}^{(s)}_1$
$\mathbf{a}^{(s)}_2$
$\mathbf{a}^{(s)}_3$
$\mathbf{a}^{(s)}_4$

from Numerical Recipes online

- Assume that if $\boldsymbol{a}_{(0)}$ is a reasonable estimate of $\boldsymbol{a}_{\text{true}}$, then the distribution of $\boldsymbol{a}_{(j)}$-$\boldsymbol{a}_{(0)}$ should be similar to that of $\boldsymbol{a}_{(j)}$-$\boldsymbol{a}_{\text{true}}$

- With the assumed $\boldsymbol{a}_{(0)}$, and some understanding of the characteristics of the measurement noise, we can generate "synthetic data sets" $\mathcal{D}^S_{(1)}$, $\mathcal{D}^S_{(2)}$,… at the same x_i values as the actual data set, $\mathcal{D}_{(0)}$, that have the same relationship to $\boldsymbol{a}_{(0)}$ as $\mathcal{D}_{(0)}$ has to $\boldsymbol{a}_{\text{true}}$

- For each $\mathcal{D}^S_{(j)}$, perform a model fit to obtain corresponding $\boldsymbol{a}^S_{(j)}$, yielding one point $\boldsymbol{a}^S_{(j)}$- $\boldsymbol{a}_{(0)}$ for simulating the desired M-dimensional probability distribution. **This is a very powerful technique!!**



2-parameter probability distribution for 1,000 Monte Carlo simulations

(Costa, Kleinstein and Hershberg, Sci Signal. 2011)