MATHEMATICAL MODELING AND OPTIMIZATION IN CRYOBIIOLOGY

James Benson, Ph.D.
NIST
Applied and Computational Math Division
OUTLINE

• Brief introduction to principles of Cryobiology
• Model development at three length scales
• Optimal control
• Current and future directions
WHY FREEZE BIOSPECIMENS?

- Colder temperatures mean longer storage: at least 100 years in LN
- Banking, distribution and testing of cells and tissues, maybe organs in the future
- Worldwide initiatives to preserve genetic samples
  - Millennium Seed Bank, Svalbard Seed Bank, UK Biobank (0.5M samples)
  - JAX Sperm bank (>10000 strains)
  - NCRR, MMRRC, MRRRC
  - NCI-Office of Biorepositories and Biospecimen Research
- Kill unwanted cells and tissues in living systems
HOW CELL FREEZING WORKS
HOW CELL FREEZING WORKS

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°C</td>
<td>No Ice</td>
</tr>
<tr>
<td>-10°C</td>
<td>Some Ice</td>
</tr>
<tr>
<td>-40°C</td>
<td>Lots of Ice</td>
</tr>
<tr>
<td>-80°C</td>
<td>Almost All Ice</td>
</tr>
</tbody>
</table>
To reduce the effects of high salt concentrations and to aid in “glass formation” we add cryoprotective agents (CPAs).

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Concentration</th>
<th>Salt Alone</th>
<th>Salt</th>
<th>CPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell at 22°C, No Ice</td>
<td>1%</td>
<td>1%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Cell at -10°C, Some Ice</td>
<td>10%</td>
<td>1.5%</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>Cell at -40°C, Lots of Ice</td>
<td>30%</td>
<td>3%</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>Cell at -80°C, Almost All Ice</td>
<td>-</td>
<td>4%</td>
<td>40%</td>
<td></td>
</tr>
</tbody>
</table>
THE TWO FACTOR HYPOTHESIS
CRITICAL CRYOBILOGICAL QUANTITIES

Above 0°C these quantities govern osmotically induced damage

Below 0°C these quantities govern the likelihood of intracellular ice
TRANSPORT PROBLEMS

Model Selection

Single Cell Suspensions

Multi Cell Tissues

Larger Tissues and Organs

<table>
<thead>
<tr>
<th>Mass</th>
<th>Single Cell Suspensions</th>
<th>Multi Cell Tissues</th>
<th>Larger Tissues and Organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODE System</td>
<td>Hybrid ODE/PDE System</td>
<td>PDE System</td>
<td></td>
</tr>
<tr>
<td>Stochastic ODE</td>
<td>Large Monte Carlo System</td>
<td>Nonlinear heat &amp; stefan problem</td>
<td></td>
</tr>
</tbody>
</table>
TRANSPORT PROBLEMS

Model Selection

All models in cryobiology are coupled systems!
THE SINGLE CELL PROBLEM
\[ \dot{n}_1 = P_1 (\mu_{1}^{ext} - \mu_{1}^{int}) \]
\[ \dot{n}_2 = P_2 (\mu_{2}^{ext} - \mu_{2}^{int}) \]

MASS TRANSFER
MASS TRANSFER

THE CHOICE OF $\mu$

\[
\Phi(T, P, N) = N_1\mu_0 + \sum_{i=2}^{n} N_i kT \ln \frac{N_i}{eN_1} + \sum_{i=2}^{n} N_i \psi_i + \frac{1}{2N_1} \sum_{i,j=2}^{n} \beta_{ij} N_i N_j
\]

Differentiating with respect to $N_1$ or $N_i$ and setting $\beta_{ij}/kT = (B_i + B_j)$

\[
\mu_1 = \mu_0 - kT \left( \sum_{i=2}^{n} m_i + \frac{1}{2} \sum_{i,j=2}^{n} (B_i + B_j)m_im_j \right)
\]

\[
\mu_i = kT \left( \ln m_i + \psi_i^* + \sum_{j=1}^{n} (B_i + B_j)m_j \right).
\]

Specific Model: set $B_i = 0$ and $M_i \approx x_2/x_1$.

\[
\begin{align*}
\dot{x}_1 &= \frac{x_{np}}{x_1} + \sum_{j=2}^{k} \frac{x_j}{x_1} - \sum_{i=1}^{n} M_i, \\
\dot{x}_2 &= b_2 \left( M_2 - \frac{x_2}{x_1} \right), \\
\vdots \\
\dot{x}_n &= b_n \left( M_n - \frac{x_n}{x_1} \right),
\end{align*}
\]

**Cellular Quantities**
- $x_1 =$ Water Volume
- $x_2,\ldots,n =$ Moles of permeating solute
- $x_{np} =$ Moles of nonpermeating solute
- $b_2,\ldots,n =$ Relative permeability

**Extracellular Quantities**
- $M_1 =$ Nonpermeating solute molality
- $M_2,\ldots,n =$ Permeating solute molality
- $\bar{M}_i =$ Maximal $i$th solute molality

SINGLE CELLS

Specific Model

\[
\begin{align*}
\dot{x}_1 &= \frac{1}{x_1} \left( x_{np} + \sum_{j=2}^{k} x_j - \sum_{i=1}^{n} M_i x_1 \right), \\
\dot{x}_2 &= \frac{b_2}{x_1} (M_2 x_1 - x_2), \\
\vdots \quad & \quad \vdots \quad \vdots \\
\dot{x}_n &= \frac{b_n}{x_1} (M_n x_1 - x_n),
\end{align*}
\]

Cellular Quantities

\[
\begin{align*}
x_1 &= \text{Water Volume} \\
x_2, \ldots, n &= \text{Moles of permeating solute} \\
x_{np} &= \text{Moles of nonpermeating solute} \\
b_2, \ldots, n &= \text{Relative permeability}
\end{align*}
\]

Extracellular Quantities

\[
\begin{align*}
M_1 &= \text{Nonpermeating solute molality} \\
M_2, \ldots, n &= \text{Permeating solute molality} \\
\bar{M}_i &= \text{Maximal } i\text{th solute molality}
\end{align*}
\]

We have a system of the form \( \dot{x}(t) = \lambda(x(t)) f(x(t)) \), where \( \lambda(x(t)) = 1/x_1(t) \) is a positive scalar function. In this case, we can define an invertible transformation

\[
q(\tau) = \int_{0}^{\tau} \frac{1}{\lambda(x(s))} ds = \int_{0}^{\tau} x_1(s) ds
\]

and a new system \( w'(\tau) = f(w(\tau)) \) such that

\[
w(\tau) = x(q(\tau))
\]

meaning that we may, without any penalty, linearize the system by removing the \( 1/x_1 \) term.
\begin{align*}
x'_1(\tau) &= x_{np} + \sum_{j=2}^{n} x_j - \sum_{i=1}^{n} M_i(\tau)x_1, \\
x'_2(\tau) &= b_2 (M_2(\tau)x_1 - x_2), \\
&\quad \vdots \\
x'_n(\tau) &= b_n (M_n(\tau)x_1 - x_n).
\end{align*}

or

\[ x' = f(x, M) := A(M)x + x_{np}e_1, \]

where

\[ A(M) = \begin{pmatrix}
-\sum_{i=1}^{n} M_i & 1 & 1 & \cdots & 1 \\
b_2M_2(t) & -b_2 & 0 & \cdots & 0 \\
b_3M_3(t) & 0 & -b_3 & \cdots & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
b_nM_n(t) & 0 & 0 & \cdots & -b_n
\end{pmatrix}. \]
Define

\[ D := \text{diag}(1, (b_2 M_2)^{-1/2}, \ldots, (b_n M_n)^{-1/2}) \]

Then:

\[
DA(M)D^{-1} = \begin{pmatrix}
-\sum_i M_i & \sqrt{b_2 M_2} & \sqrt{b_3 M_3} & \ldots & \sqrt{b_n M_n} \\
\sqrt{b_2 M_2} & -b_2 & 0 & \ldots & 0 \\
\sqrt{b_3 M_3} & 0 & -b_3 & \ldots & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
\sqrt{b_n M_n} & 0 & \ldots & 0 & -b_n
\end{pmatrix}
\]

is symmetric, negative definite, and our original \( n \)-dimensional nonlinear system is globally asymptotically stable.

MASS TRANSPORT IN SMALL TISSUES

\[ c_t = D^{-2}(c_{rr} + 2c_r/r) \]

Layer 1 2 ... n

Cells

Channel/Virtual Cells

Discretization

Water Volume (µm³)

Time (s)

JDB, C Benson, J Critser. Submitted to J. Biomech Eng.
SOLIDIFICATION DURING COOLING, SMALL TISSUES:

Monte Carlo Simulation of IIF

\[ p_j(\delta \tau) \approx p^i_j + p^p_j \]
\[ \approx (1 + k_j \alpha) \delta t \]

- \( p_j \) is the probability of ice
- \( p^i_j \) is the probability of ice forming spontaneously
- \( p^p_j \) is the probability of ice propagating from neighbor
- \( k_j \) is the number of icy neighbors

MASS TRANSFER IN LARGE TISSUES

\\[
\frac{\partial c}{\partial t} = \nabla \cdot (D \nabla c)
\]

Fig. 2. The proton MR spectrum at 7 T from the sample holder loaded with two ovaries and 40% (w/w) EG solution. The excitation frequency was centered at the resonance frequency for the \(-\text{CH}_2\) group in EG molecules.

Fig. 5. Two sample MR images, with water signal saturated, showing the increasing EG concentration in ovaries during perfusion.

Fig. 6. The experimental data with their fitted curve for the average EG concentration change on the centric cross-section of an ovary with 1.1 mm as its identical radius.
MASS TRANSFER IN LARGE TISSUES

\[ \frac{\partial c}{\partial t} = \nabla \cdot (D(T) \nabla c) \]

\[ D(T) = \exp\left(-\frac{E_a}{RT}\right) \]

X Han, L Ma, A Brown, JDB, J Critser. In review: IJHMT
WHAT CAN WE CONCLUDE FROM THE ABOVE MODELS?
HEAT AND MASS TRANSFER LIMIT THE SIZE OF FREEZABLE TISSUE!
An improved cryopreservation method for a mouse embryonic stem cell line

Corinna M. Kashuba Benson, James D. Benson, John K. Critser *

Comparative Medicine Center, Research Animal Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, 1600 East Rollins Street, Columbia, MO 65211, USA

Received 15 May 2007; accepted 3 December 2007
Available online 14 January 2008

- Previous best protocol: 31% recovery
- "Optimally" defined new best protocol: 64% recovery
OPTIMAL CONTROL IN CRYOBIOLOGY:

- control quantity to minimize cost $J$ (e.g. time, energy, stress, $P_{\text{IF}}$ or combinations.)

- subject to exact and inequality constraints:
  - exact constraints: governing physical system, (e.g. 2P model, heat equation, diffusion, etc).
  - inequality constraints: state or control constraints, (e.g. cell volume $> 0$).
min \left\{ s_f \right\} \quad \text{subject to}
\begin{align*}
\dot{w}_1 &= \frac{x_{np}}{w_1} + \frac{w_2}{w_1} - M_1 - M_2, \\
\dot{w}_2 &= b_2 \left( M_2 - \frac{w_2}{w_1} \right),
\end{align*}
and
\begin{align*}
w_1 + \gamma w_2 - k^* &\leq 0, \\
k^* - w_1 - \gamma w_2 &\leq 0.
\end{align*}

FIRST CONTROL PROBLEM
\[ \min_{M \in A} s_f = q(t_f) = \int_0^{t_f} x_1(\tau) \, d\tau \]

subject to

\[ \begin{align*}
\dot{x}_1 &= -(M_1 + M_2)x_1 + x_2 + x_{np} \\
\dot{x}_2 &= bM_2x_1 - bx_2
\end{align*} \]

and

\[ \begin{align*}
x_1 + \gamma x_2 - k^* &\leq 0, \\
k^* - x_1 - \gamma x_2 &\leq 0.
\end{align*} \]

Bilinear state equation (in controls and state) give:

Existence \checkmark Controllability \checkmark
OPTIMAL CONTROL

\[ H(x^*, p^*, M^*) = \max_{M \in CP} \left( A(M)x + x_1 e_1 \right) \cdot p - x_1 \]

\[ = \max_{M \in CP} \left( -M_1 x_1 p_1 + x_1 \sum_{i=2}^{n} M_i (b_i p_i - p_1) \right) \]

+ terms with no \( M \)

\[ M_1(t) = \begin{cases} 0, & p_1 > 0 \\ \bar{M}_1, & p_1 \leq 0 \end{cases} \]

\[ M_i(t) = \begin{cases} 0, & b_i p_i - p_1 < 0 \\ \bar{M}_i, & b_i p_i - p_1 \geq 0 \end{cases} \]

Optimal controls maximize the Hamiltonian
WHY GEOMETRIC OPTIMAL CONTROL?
WHY GEOMETRIC OPTIMAL CONTROL?
WHY GEOMETRIC OPTIMAL CONTROL?
Boltayanskii sufficiency theorem: a “regular, distinguished” trajectory defined by a state dependent control function $v(x)$ is optimal.

SUFFICIENCY
RESULTS

CPA Addition

CPA Removal
Cost function: \[ J = \int_0^T C_{\text{cell}}(t)^2 \, dt \]

Solved with a direct method: parametrize system with piecewise linear controls, minimize constrained system with a truncated-Newton approach to the augmented Lagrangian.
OPTIMAL CONTROL

System Coupling

All models in cryobiology are coupled systems!

<table>
<thead>
<tr>
<th>Before cooling</th>
<th>During cooling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass &amp; Heat</td>
<td>Thermal convection/mushy layers/etc...</td>
</tr>
<tr>
<td>Parabolic System</td>
<td></td>
</tr>
</tbody>
</table>
EXTENSION TO TISSUES, SYSTEMS

- Find $c^e(t)$ such that $f(t)$ approximates the desired independent control $u_D(t)$, known a priori.

- Can use “inverse problem” techniques to solve analytically.

- This gives a tool to develop numerical schemes for completely novel optimal control problems.

Cell or tissue

External media: diffusion or heat

\[ c_t = D \left( c_{rr} + \frac{2}{r} c_r \right) \quad (r, t) \in (a, 1) \times [0, \infty), \]

\[ \frac{\partial c}{\partial r} = k(c^c - c), \quad (r, t) \in \{a\} \times [0, \infty), \]

\[ c = c^e, \quad (r, t) \in \{1\} \times [0, \infty), \]

\[ c = c_0, \quad (r, t) \in [0, 1] \times \{a\}, \]

In Media

\[ \dot{x} = h(x, c(a, t)) \]

\[ c^c = \mathcal{M} c(a, t) \]

\[ \mathcal{M} = M_1 M_2, \quad M_1 : (x, y) \rightarrow x/y, \]

\[ \frac{d(M_2 c(a, t))}{dt} = h(M_2 c(a, t), c(a, t)) \]
After Laplace transform, we may solve for

\[ \tilde{f}(s) = \tilde{h}_1(s)\tilde{c}^e(s) + \tilde{h}_2(s)\tilde{c}^c(s) \]

where \( \tilde{h}_1(s), \tilde{h}_2(s) \) are modified spherical Bessel functions*. Thus,

\[ f(t) = \int_0^t c^e(\tau)h_1(t - \tau) + c^c(\tau)h_2(t - \tau) d\tau, \]

\[ := K_1 c^e + K_2 c^c. \]

Define \( K = K_1[I - K_2 M_2]^{-1} \).

Lemma: \( \mathcal{M}, M_2 \) exist and are bounded.

Lemma*: \( K_1 \) and \( K_2 \) are compact linear operators with zero spectral radius and unbounded inverse.

Formally:
\[
c^e = K_1^{-1} K_2 c^e + K_1^{-1} f = K_1^{-1}(K_2 \mathcal{M} + I)f
\]
which exists for \( f \) with sufficient decay.

Problem

Define \( J_1(v) = \{T : |(M_2v)(T) - x^d| = 0\} \).

Define the cost

\[
J(c^e) := T + \epsilon_1 |(M_2Kc^e)(T) - x^d|^2 + \epsilon_2 \|c^e\|^2.
\]

Find \( \min_{c^e \in A} J(c^e) \) subject to above PDE-ODE system and with state constraints \( \Gamma \cdot x \leq 0, \Gamma \in \mathbb{R}^2 \).

Proposition: Let \( \epsilon_2 = 0 \). Then there exists an \( \epsilon_1 \) such that

\[
c_j^e(t) = K_1^{-1}(K_2M + I)f := K^{-1}f = \arg\min J(c^e)
\]
From above, we recall that $f$ has step changes, and thus the frequency spectrum will not exponentially decay.

Theorem: The PDE-ODE system has no exact optimal controls.

We must use approximate controls.
Define

\[ J_2(v) := \|c(a, t) - Kv\|^2 + \left( \frac{\epsilon}{M} \right)^2 \|v\|^2. \]

Theorem: The unique minimizer of \( J_2 \) is

\[ v(t) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} e^{i\xi t} \frac{\hat{h}_1^{-1}(\xi) \left( \hat{h}_2(\xi) \hat{M} v(\xi) + \hat{v}(\xi) \right)}{1 + \omega^2 \hat{h}_1^{-1}(\xi) \left( \hat{h}_2(\xi) \hat{M} \hat{v}(\xi) \right)} \, d\xi \]

Pf: Solve the overdetermined system

\[ Kv = c^\rho, \quad \omega v := \frac{\epsilon}{M} v = 0, \]

in the frequency domain and take inverse FT.
Define

\[ J(c^e, t) := t + \epsilon_1 |(M_2 K c^e)(t) - x^d|^2 + \epsilon_2 \|c^e\|^2. \]

Theorem: Fix \( t^f = J_1(f) \). Then there exist \( \epsilon_1 \) and \( \epsilon_2 \) and \( \omega(\epsilon_1, \epsilon_2, M_2, t^f) \)

\[ K^{-1}_\omega f = \text{argmin } J(c^e, t^f). \]
Pf:

\[ |(M_2 K c^e)(t) - x^d|^2 = |(M_2 K c^e)(t) - (M_2 K K^{-1} f)(t)|^2, \]

\[ = |(M_2 K c^e)(t) - (M_2 f)(t)|^2, \]

Since \( T \) is fixed, and \( M_2 \) and \( K \) are bounded, there exists \( \epsilon_3 > 0 \) (depending on \( T, M_2 \)), such that

\[ |M_2 K c^e \ t - M_2 f \ t|^2 \geq \epsilon_3 \|K c^e - f\|_{L^2}^2, \]

and

\[ J(c^e, t^f) - t^f \geq \epsilon_1 \epsilon_3 \left( \|K c^e - f\|^2 + \omega_2 \|c^e\|^2 \right) > 0. \]

Thus, \( \text{argmin } J(c^e, t^f) = \text{argmin } \|K c^e - f\|^2 + \omega_2 \|c^e\|^2 \]

\[ = K_{\omega_2}^{-1} f. \]
Now note that
\[ \|M_2\|^2 \|Kc^e - f\|_{L^2}^2 \geq |(M_2Kc^e)(t) - (M_2f)(t)|^2, \]
and thus
\[ \|M_2\|^2 \|(KK^{-1}_\omega - I)\|^2 \|f\|_{L^2}^2 \geq \|M_2\|^2 \|(KK^{-1}_\omega - I)f\|_{L^2}^2 \]
\[ \geq \|M_2\|^2 \|KK^{-1}_\omega f - f\|_{L^2}^2 \]
\[ \geq |(M_2KK^{-1}_\omega)(t) - (M_2f)(t)|^2, \]
and with \( \|M_2\| \|f\| < k < \infty \), given \( \delta(\omega) > 0 \) there exists an \( \omega > 0 \) such that
\[ \delta \geq \|KK^{-1}_\omega - I\| \]
\[ \geq k^{-2} |(M_2KK^{-1}_\omega)(t) - (M_2f)(t)|^2. \]
NUMERICS

• PHAML: hp-adaptive multilevel elliptic solver

• Implicit-Filtering minimization algorithm: adaptive secant approximation to gradient

Model scaling shows where future work lies:

<table>
<thead>
<tr>
<th>MODELS</th>
<th>ODE System</th>
<th>Hybrid ODE/PDE System</th>
<th>PDE System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Heat</td>
<td>✓</td>
<td>✓ Large Monte Carlo System</td>
<td>nonlinear heat equation</td>
</tr>
<tr>
<td>Mass</td>
<td>✓</td>
<td>Hybrid ODE/PDE System</td>
<td>PDE System</td>
</tr>
<tr>
<td>Heat</td>
<td>Stochastic ODE</td>
<td>Large Monte Carlo System</td>
<td>nonlinear heat equation</td>
</tr>
</tbody>
</table>

Tuesday, October 19, 2010
CURRENT AND FUTURE PROBLEMS

• Develop cost functions for entire cryo-protocol

• Extend ‘inverse’ approach to 2D and 3D systems

• Model multiphase ternary solidification and interaction with biomaterials

• Iterative optimization of freezing protocols

• Optimal design of counter-current dialysis devices
QUESTIONS?