Inferring
single cell metabolic fluxes

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Synopsis

1)Introduction

Bayes Theorem with example

2) Inferring single cell fluxes

- Growth rates from imaging
- Uptakes/turnover from nano-sims

3)Perspectives: inverse modeling

- Intercellular exchange from Nanofibers sensing

The Monty Hall problem

1) There are three closed doors and only one hides a prize

2) You can choose one (that will not be opened yet)

3) The game-show host (that knows where the prize is)
opens one of the other two in such a way as not to reveal the prize and offers you to swap your door for the remaining closed door

Is it convenient to swap?

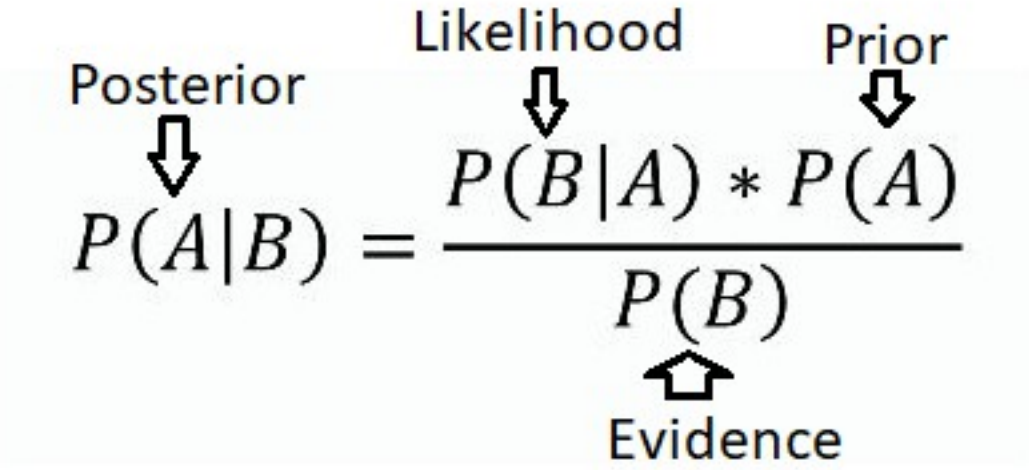
Eg you choose door 1, the host opens 3
 showing it is empty, will you swap to 2?

Bayes Theorem

$$P(A|B)P(B) = P(B|A)P(A)$$

$$P(A|B) = \frac{P(A \cap B)}{P(B)}$$

Demonstration: symmetry of conditional probability by definition



Solution

Solution to exercise 3.8 (p.57). Let $\mathcal{H}_i$ denote the hypothesis that the prize is behind door i. We make the following assumptions: the three hypotheses $\mathcal{H}_1$, $\mathcal{H}_2$ and $\mathcal{H}_3$ are equiprobable *a priori*, i.e.,

$$P(\mathcal{H}_1) = P(\mathcal{H}_2) = P(\mathcal{H}_3) = \frac{1}{3}. \tag{3.36}$$

The datum we receive, after choosing door 1, is one of $D=3$ and $D=2$ (meaning door 3 or 2 is opened, respectively). We assume that these two possible outcomes have the following probabilities. If the prize is behind door 1 then the host has a free choice; in this case we assume that the host selects at random between $D=2$ and $D=3$. Otherwise the choice of the host is forced and the probabilities are 0 and 1.

$$\begin{array}{|c|c|c|} P(D=2\,|\,\mathcal{H}_1)=1/2 & P(D=2\,|\,\mathcal{H}_2)=0 & P(D=2\,|\,\mathcal{H}_3)=1 \\ P(D=3\,|\,\mathcal{H}_1)=1/2 & P(D=3\,|\,\mathcal{H}_2)=1 & P(D=3\,|\,\mathcal{H}_3)=0 \end{array} \tag{3.37}$$

Now, using Bayes' theorem, we evaluate the posterior probabilities of the hypotheses:

$$P(\mathcal{H}_i\,|\,D=3) = \frac{P(D=3\,|\,\mathcal{H}_i)P(\mathcal{H}_i)}{P(D=3)} \tag{3.38}$$

$$\left|\; P(\mathcal{H}_1\,|\,D=3) = \frac{(1/2)(1/3)}{P(D=3)} \;\right|\; P(\mathcal{H}_2\,|\,D=3) = \frac{(1)(1/3)}{P(D=3)} \;\left|\; P(\mathcal{H}_3\,|\,D=3) = \frac{(0)(1/3)}{P(D=3)} \;\right| \tag{3.39}$$

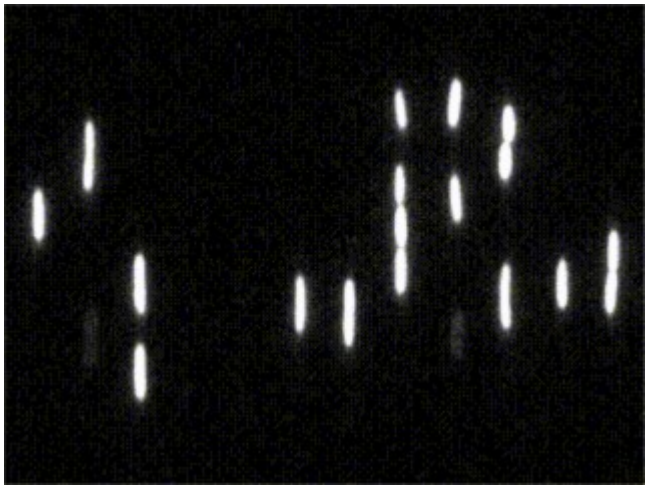
The denominator $P(D=3)$ is $(1/2)$ because it is the normalizing constant for this posterior distribution. So

$$\left|\; P(\mathcal{H}_1\,|\,D=3) \;=\; 1/3 \;\right|\; P(\mathcal{H}_2\,|\,D=3) \;=\; 2/3 \;\left|\; P(\mathcal{H}_3\,|\,D=3) \;=\; 0. \;\right| \tag{3.40}$$

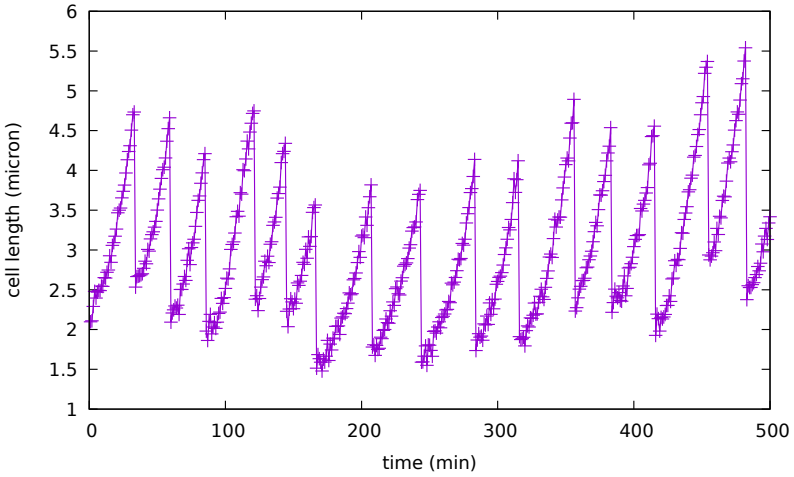
So the contestant should switch to door 2 in order to have the biggest chance of getting the prize.

MacKay, D. J. (2003). Information theory, inference and learning algorithms.

Inferring single cell growth rates



Mother machine movie



Time traces length vs time

Data from:
Tanouchi, Y., Pai, A., Park, H., Huang, S., Buchler, N. E., & You, L. (2017).
Long-term growth data of Escherichia coli at a single-cell level. Scientific data, 4(1), 1-5.

The Linear fit, revisited (I)

$$l(t) = l_0 e^{\lambda t}$$

Hypothesis 1: exponential growth!

$$\log l(t_i)/l_0 = \lambda t_i + \mathrm{noise}(t_i)$$

Let us linearize taking the log!
i runs over data points

Hyopthesis 2: noise terms are Gaussian random variables independent and identically distributed with stdv sigma

$$p(data|\lambda)p(\lambda) = p(\lambda|data)p(data)$$

Bayes theorem

$$p(\lambda) \sim \mathrm{const.}$$

Hypothesis 3: approx. uniform prior

$$p(data) = \int p(data|\lambda)p(\lambda)d\lambda$$

The “evidence” is just a constant

Linear fit revisited (II)

$$P(\lambda|data) \propto P(data|\lambda) \propto \prod_i e^{-\frac{(\log l(t_i)/l_0 - \lambda t_i)^2}{2\sigma^2}}$$

We have the full posterior!

$$\mathcal{L} = -\frac{1}{2\sigma^2}\sum_i (\log l(t_i)/l_0 - \lambda t_i)^2 - N \log\sqrt{2\pi\sigma^2}$$

Log-likelihood

$$\frac{\partial \mathcal{L}}{\partial \lambda} = 0$$

$$\lambda^* = \frac{\sum_i t_i (\log l(t_i)/l_0)}{\sum_i t_i^2}$$

Max likelihood sol. coincides with Chi^2 min..

Constant rate or constant speed?

$$l(t) = l_0 + vt$$

Alternative model: constant elongation speed!

$$l(t_i) = l_0 + vt_i + \text{noise}(t_i) \qquad p(v) \sim \text{const.}$$

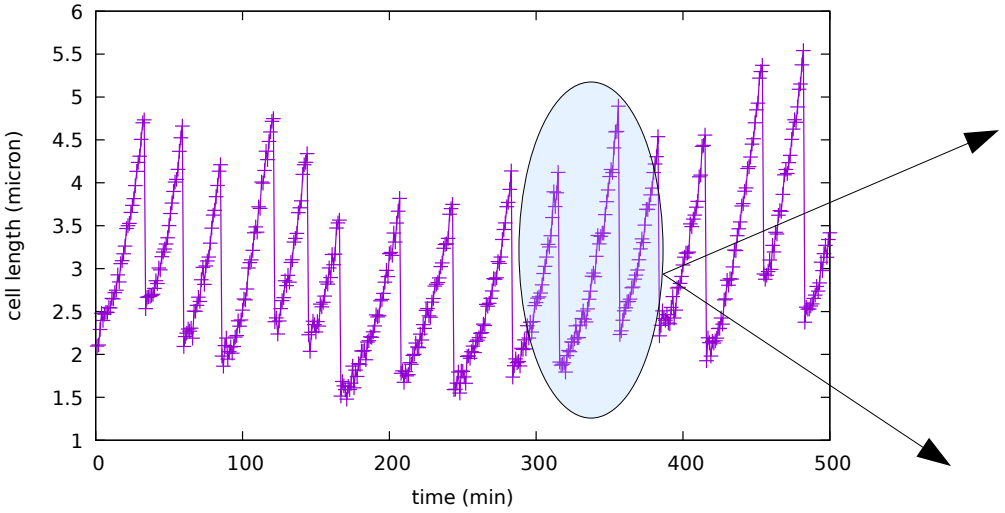
$$P(v|data) \propto P(data|v) \propto \prod_i e^{-\frac{(l(t_i)-l_0-vt_i)^2}{2\sigma^2}}$$

$$\mathcal{L} = -\frac{1}{2\sigma^2}\sum_i (l(t_i) - l_0 - vt_i)^2 - N\log\sqrt{2\pi\sigma^2}$$

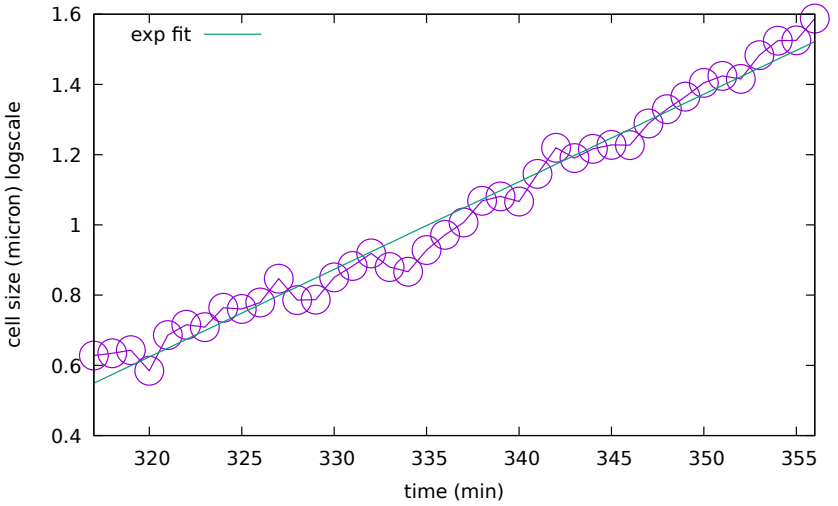
$$\frac{\partial \mathcal{L}}{\partial v} = 0$$

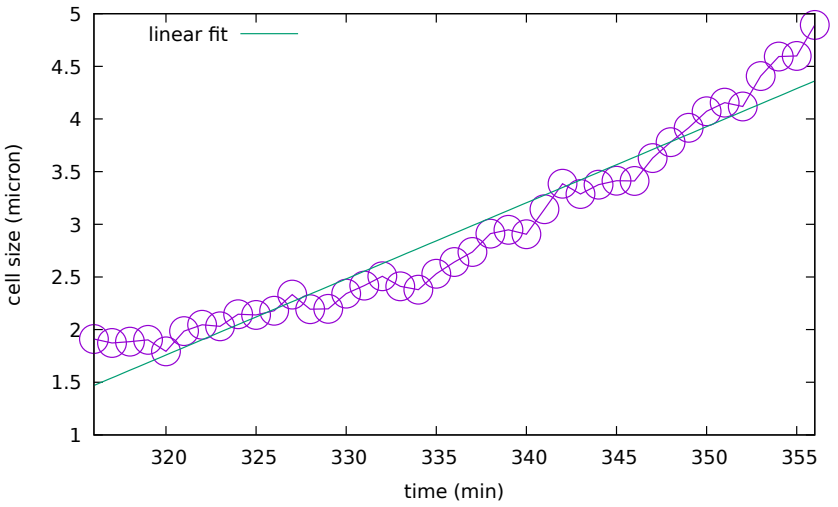
$$v^* = \frac{\sum_i t_i(l(t_i) - l_0)}{\sum_i t_i^2}$$

Let us test them!



Exponential growth





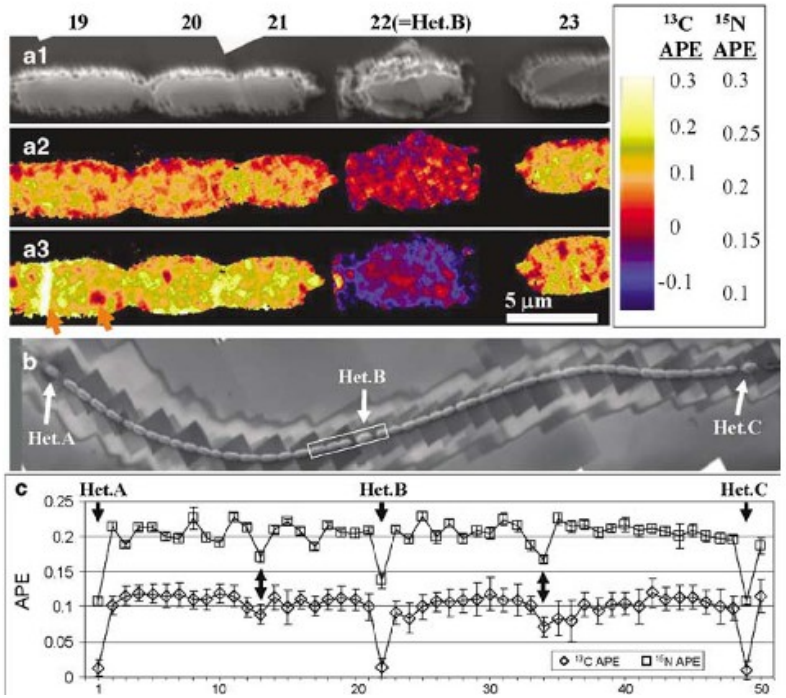
Linear growth

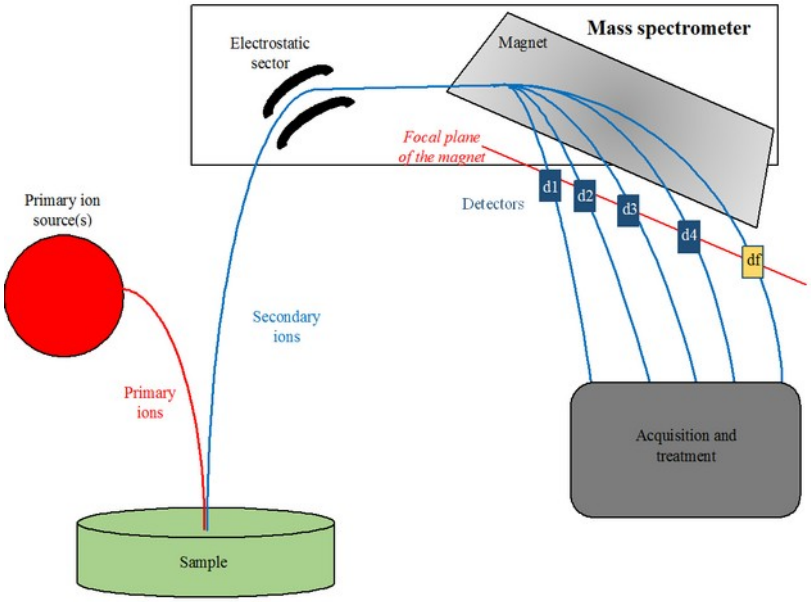
log-likelihood(exp)/log-likelihood(lin)>20
Single cell exponential growth!
(this is non-trivial no?)

Inferring single cells uptakes/turnover/growth from nanosims data

Mass spectrometry + isotope labeling is the most widespread technique for flux analysis → extension to single cells?

“Nanoscale secondary ion mass spectrometry”
+ stable isotope labeling





https://en.wikipedia.org/

Radu et al. "Carbon and nitrogen fixation and metabolite exchange in and between individual cells of Anabaena oscillarioides." The ISME journal 1.4 (2007): 354-360.

From ratios to rates

$$C(t) = C_0 + (C_1 - C_0)(1 - e^{-rt})$$

Main hypothesis:
linear kinetics for concentrations

$$x_0 = \frac{C_0^H}{C_0^L} \qquad x(t) = \frac{C^H}{C^L} = \frac{C_0^H + (C_1^H - C_0^H)(1 - e^{-rt})}{C_0^L + (C_1^L - C_0^L)(1 - e^{-rt})}$$

$$x_1 = \frac{C_1^H}{C_1^L} \qquad C_0^H + C_0^L = C_1^H + C_1^L$$

$$x(t) = \frac{x_1(x_0 + 1) + (x_0 - x_1)e^{-rt}}{1 + x_0 + (x_1 - x_0)e^{-rt}}$$

$$r = -\frac{1}{T} \log \left(\frac{(x_1 - x)(x_0 + 1)}{(x_1 - x_0)(x + 1)} \right)$$

T: incubation time
x:observed ratio
x_1: ratio, labeled
x_0: ratio, natural
r: rate

But what is really r?

A simple model for the linear kinetics: a growing rod fed by diffusion

$$\dot{N} = \alpha(c_e - c)S$$

Simple diffusion through surface S

$$\dot{c} = \frac{\dot{N}V - \dot{V}N}{V^2}$$

Derivative of a ratio

$$\lambda = \dot{V}/V$$

Growth rate

$$S/V = \theta$$

Rod hypothesis

$$\dot{c} = u - (\gamma + \lambda)c$$

$$u = \alpha\theta c_e$$

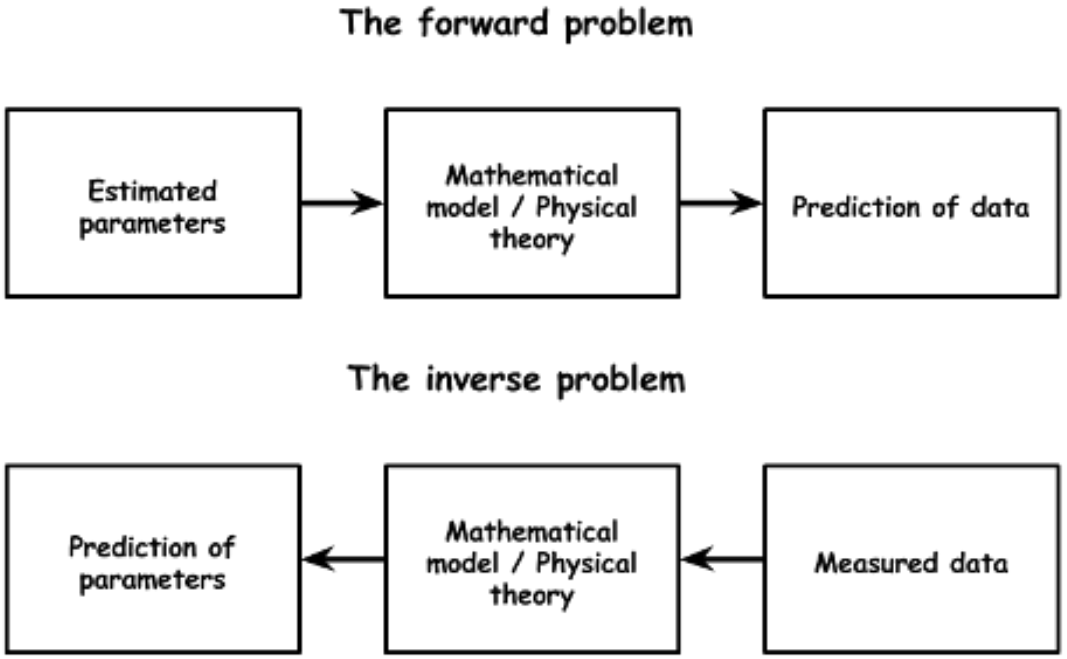
$$\gamma = \alpha\theta$$

Linear kinetics!

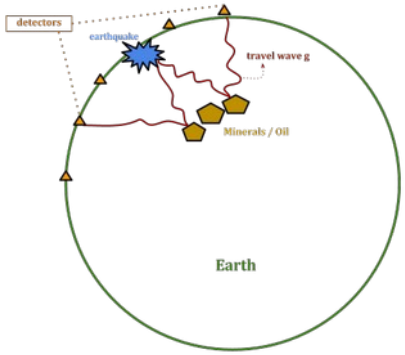
$$r = \gamma + \lambda$$

Incorporation rate = growth rate+turnover
r is an upper bound for the growth rate!

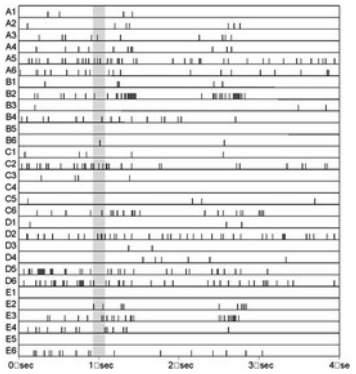
More in general: Inverse modeling



e.g. geophysics
Inverting wave equations
to reconstruct earth density profiles



Neuroscience: Inferring neural interactions from spike data
Schneidman et al (2006), Nguyen, Zecchina & Berg (2017)



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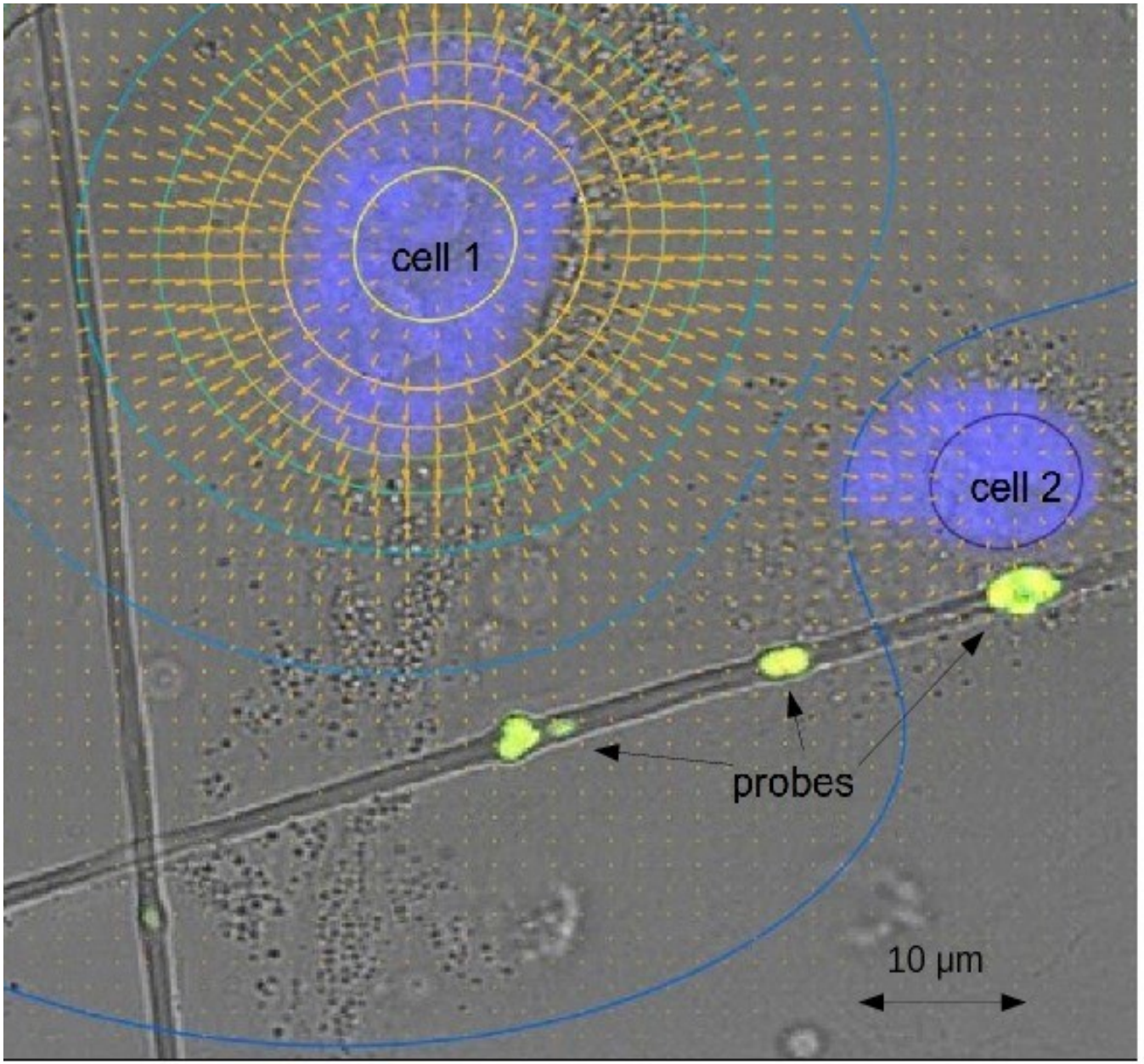
Flux inference & gradient reconstruction

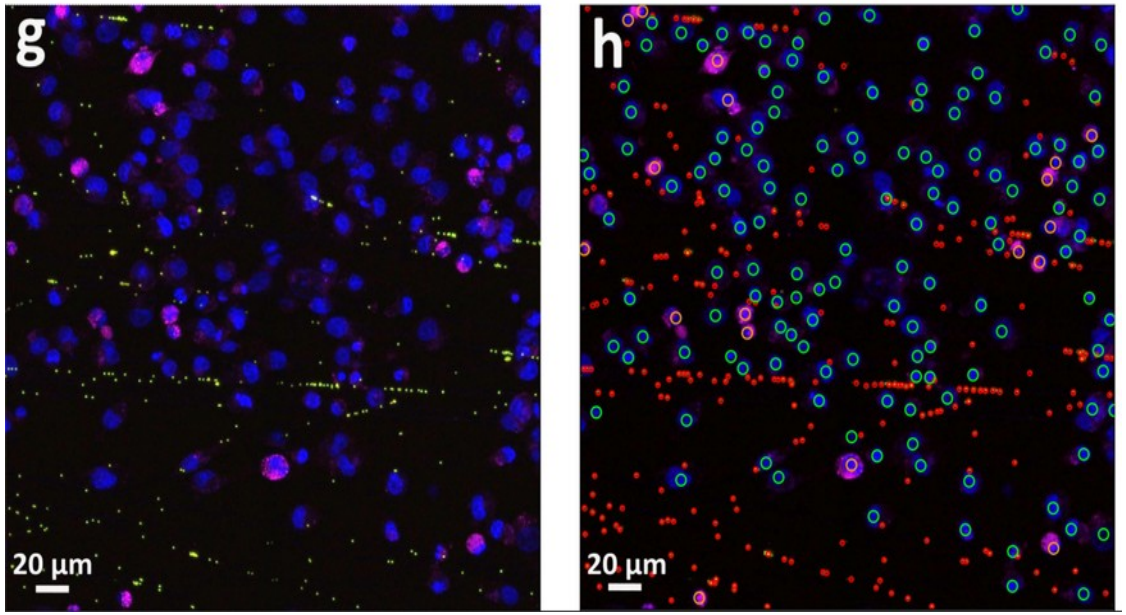


Inverse modeling of the Laplace equation

$$\nabla^2 c(\mathbf{r}) = 0$$

Given (noisy) measurements of C find the (complex) Boundary conditions

A single cell picture of tumor acidification (Warburg)



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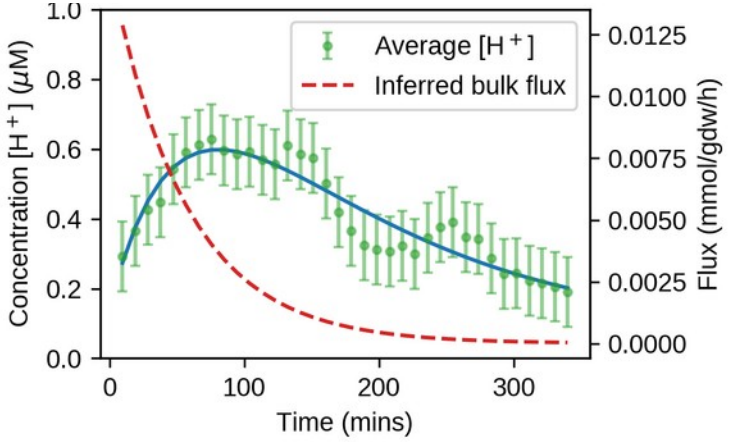
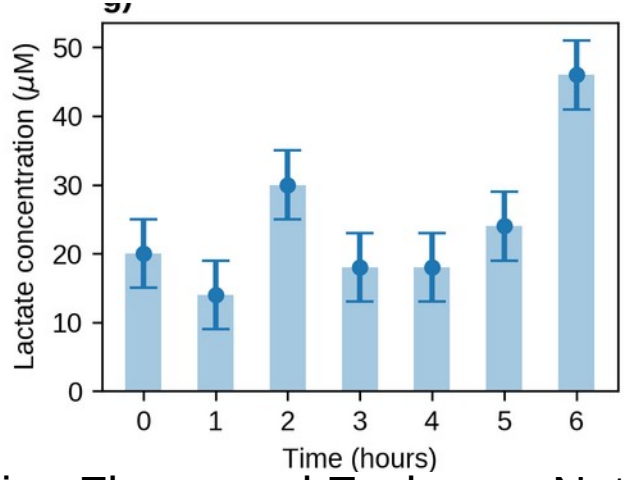
Non-trivial segmentation and tracking problem





Onesto et al. "Probing Single-Cell Fermentation Fluxes and Exchange Networks via pH-Sensing Hybrid Nanofibers." ACS nano (2022).

Some equations..

$$c(\mathbf{r}) = \sum_{i=1}^{N} \frac{u_i}{D|\mathbf{r} - \mathbf{r}_i|} + U \int_B \frac{ds}{D|\mathbf{r} - \mathbf{r}(s)|}$$

Multipolar expansion truncated to the first term

$$\chi^2_L(\mathbf{u}) = \sum_{\mu} \frac{(c_\mu - \sum_i A_{\mu i} u_i)^2}{2c_\mu^2 \sigma_\mu^2}$$

Find parameters from maximum likelihood
+ sampling the full posterior
(to estimate the errors!)

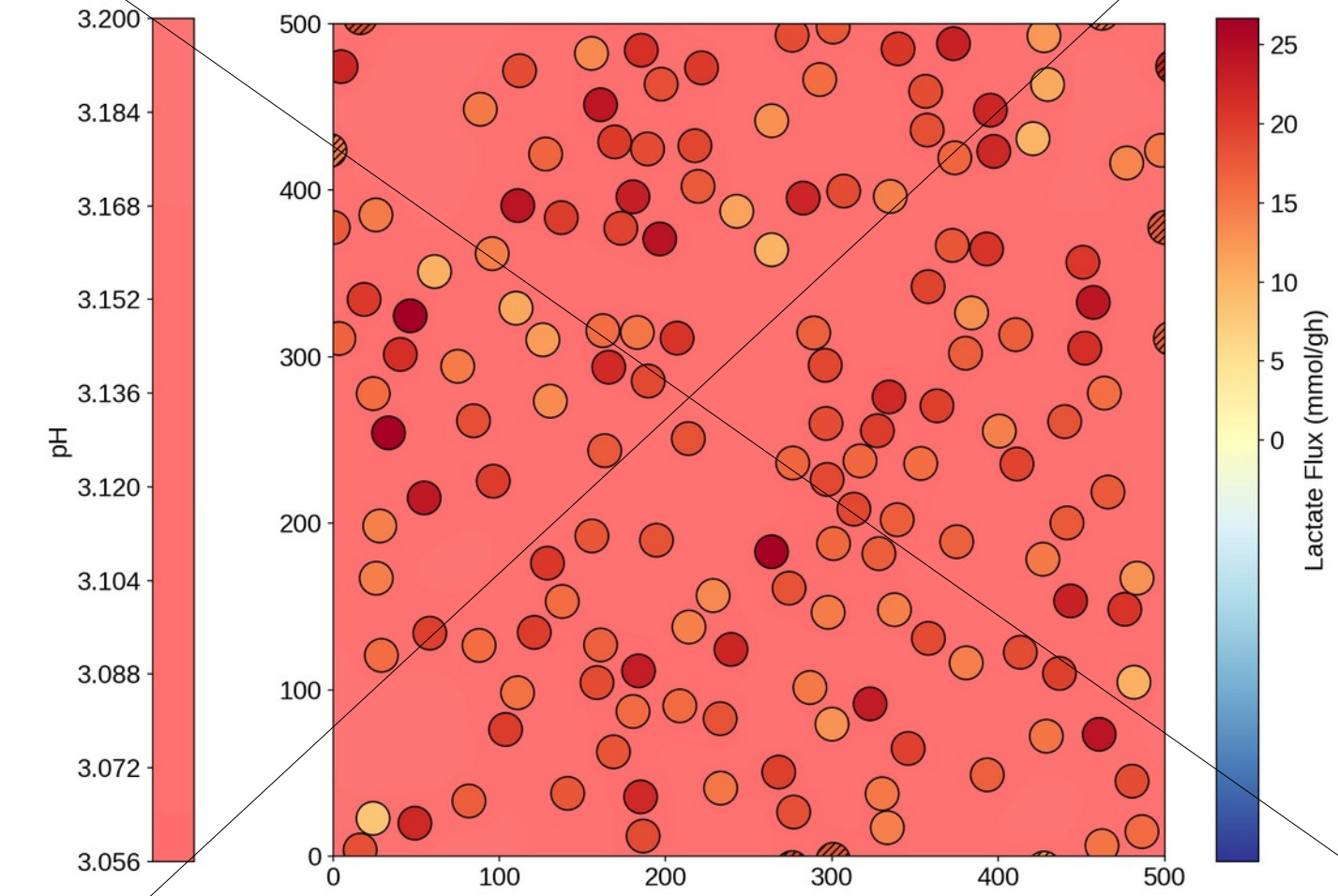
$$u_j^* = \sum_i (B_{ij})^{-1} b_i$$

$$b_i = \sum_{\mu} \frac{A_{\mu i}}{c_\mu \sigma_\mu^2}$$

$$B_{ij} = \sum_{\mu} \frac{A_{\mu i} A_{\mu j}}{c_\mu^2 \sigma_\mu^2}.$$

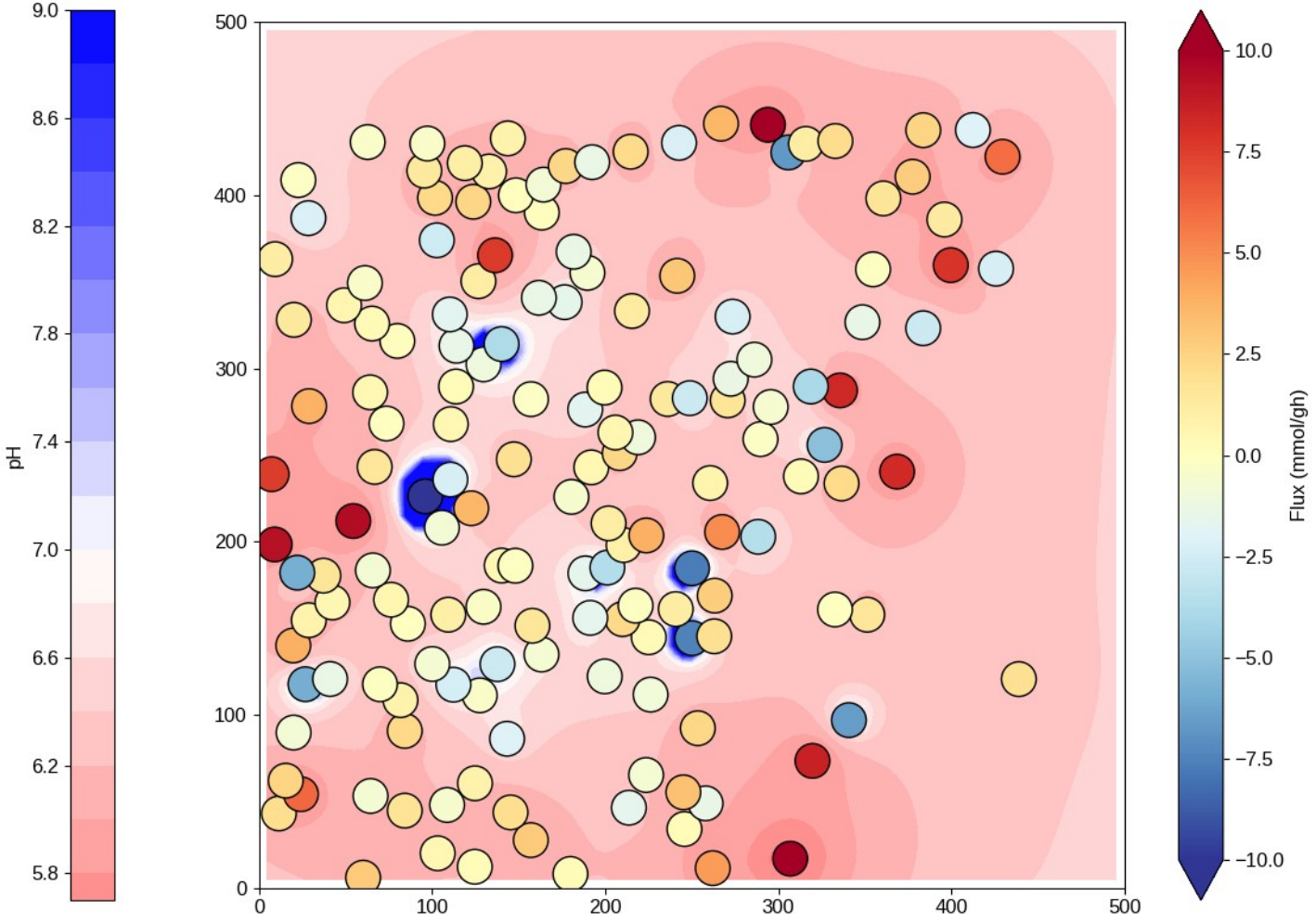
Many assumptions:
stationarity, spherical cows, etc

We do not obtain this!



FBA Simulations of cells maximizing growth/ATP

We obtain this!



Tumor acidification as a spillover from an unbalanced exchange network

Summary & references

> you can't do inference – or data compression – without making assumptions.

MacKay, D. J. (2003). Information theory, inference and learning algorithms.

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