Cell Cycle Dynamics and Clustering for Yeast

New Directions in Dynamical Systems

In Honor of Henk Broer

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Acknowledgments

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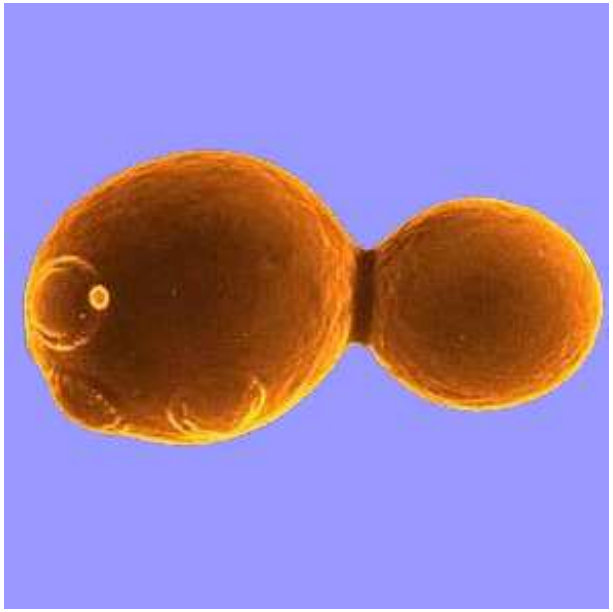
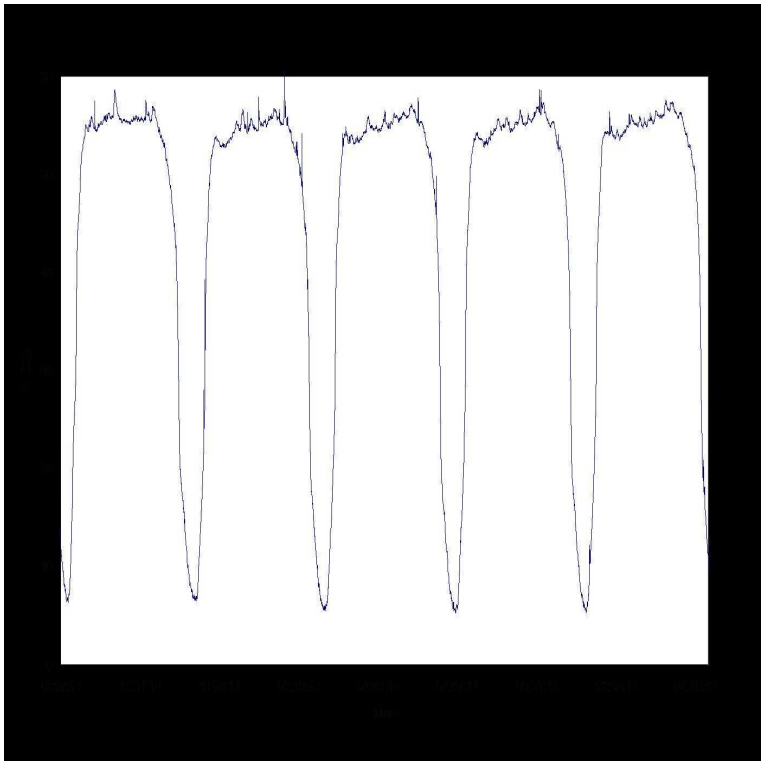
Saccharomyces cerevisiae Photo: Wikipedia



Sources: www.kaeberleinlab.org, www.alltech.com

Brewer's, Baker's or Ale Yeast.
Studied by biologists as a model eukaryotic organism.
Yeast are used in many bio-engineering processes.

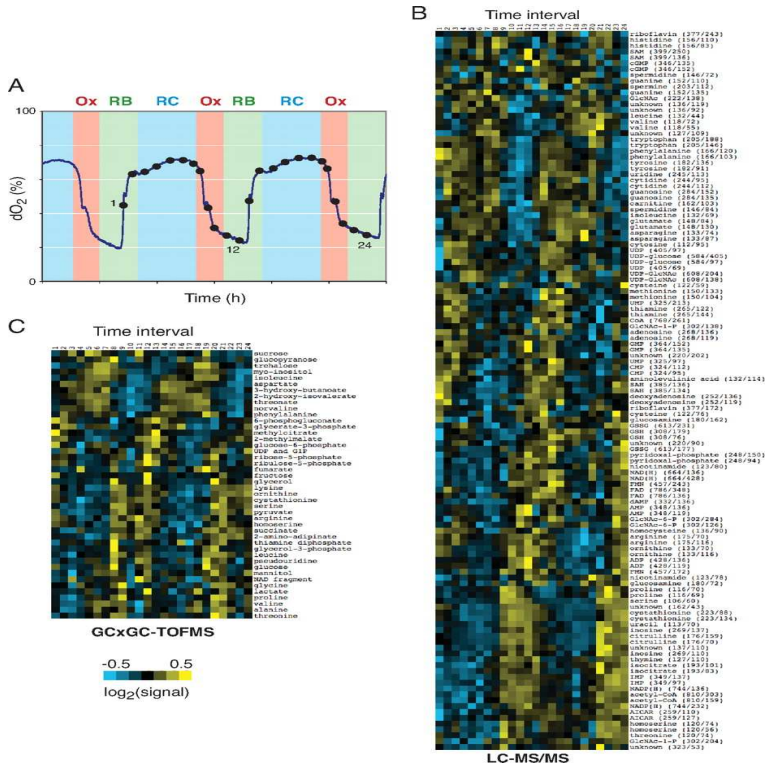
Oxygen oscillations



Fraction of Oxygen dilution in a culture of yeast.
Time scale 20 hours. Range 5% - 65%.

Possible Explanations for Oscillations

- Metabolic.
- Media feedback.
- Cell-cell signaling.
- Genetic.



Z.Chen et. al. *Science* **316** (2007).

Oxygen Oscillations

Oscillations occur under the following conditions:

- highly oxygenated media
- well-mixed, planktonic cultures.
- high cell density.
- The period of oscillation is nearly an integer fraction of the doubling time.

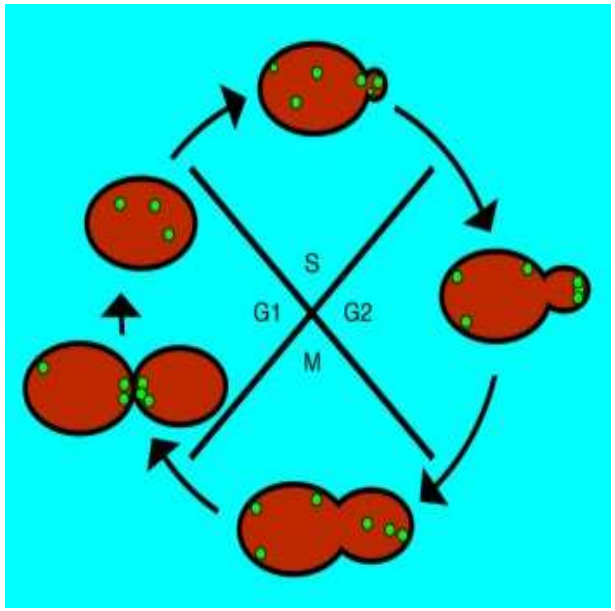
Cell Cycle of Budding Yeast

G1: gap phase, begins with cell division

S: replication phase, begins with budding

G2: second gap phase

M: narrowing or "necking", ends in cell division



Source: www.shef.ac.uk

A casual link between O_2 oscillations and the cell cycle was dismissed in one early paper without data.

Clustering

By *Clustering* we mean groups of cells traversing the cell cycle in near synchrony. (Not spatial clustering.)

Hypotheses:

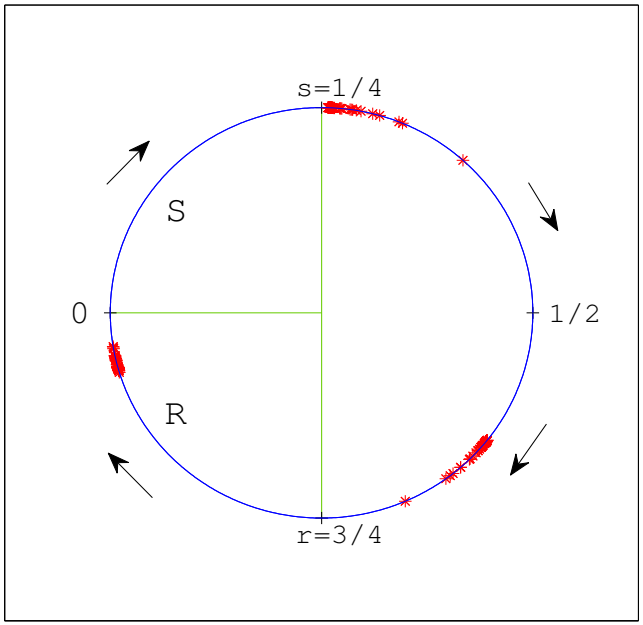
A large cluster of cells in one part of the cell cycle might influence the growth rate of cells in another part, e.g. a cluster of budded cells might inhibit the onset of budding in other cells.

A large cluster of cells at the G1-S boundary could consume enough oxygen to cause the observed dips in diluted O_2.

Hypothesized Feedback Mechanism and Clusters

S - Signaling region, R - Responsive region
Cells in S exert $+/-$ influence on the growth of cells in R

Simulation with negative feedback and diffusion:



Modeling of cell cycle dynamics

Onset of phases is marked by volume milestones. The volume v_i of a cell is a convenient variable representing location within the cell cycle.

For a given cell, indexed by i, let $v_i(t)$ denote its volume. It is observed that the volume growth of a cell in a culture is proportional to its volume, i.e.

$$\frac{dv_i}{dt} = c_i v_i.$$

Standard assumption: c_i depends on external factors.
Our assumption: c_i also depends on internal factors.

Normalized, Logarithmic Variables

Let $V_{b,i}$ denote the volume of a cell at the beginning of its cell cycle and $V_{d,i}$ its volume at division.
Consider:

$$x_i = \frac{\ln(v_i/V_{b,i})}{\ln(V_{d,i}/V_{b,i})}.$$

Then $x_i \in [0, 1)$ satisfies

$$\frac{dx_i}{dt} = c_i.$$

When $x_i(t)$ reaches 1 (division) it returns to 0 (birth), along with it daughter.

(Normalized variables also facilitate PDE models.)

Culture vs. Individual; Random D.E. model.

We distinguish influences on growth rate that are:

- common to all the cells in the culture: $a(t, x_i, \bar{x})$
  (but may depend on the position in cell cycle), and
- due to individual differences: $b_i(t)$

$$\frac{dx_i}{dt} = a(t, x_i, \bar{x}) + b_i(t). \qquad \text{(RDE)}$$

In most applications b_i will be relatively small and "random"; too complex to model.

The equation may be viewed as a Random D.E..

Stochastic D.E. and Ordinary D.E. Models

A reasonable *approximation* in some circumstances is to replace b_i by a stochastic term:

$$dx_i = a(t, x_i, \bar{x})\, dt + \sqrt{\sigma}\, dW_i, \qquad \text{(SDE)}$$

where dW_i is an independent white noise term.

It is also reasonable under certain circumstances to consider (RDE) and (SDE) as perturbations of:

$$\frac{dx_i}{dt} = a(t, x_i, \bar{x}). \qquad \text{(ODE)}$$

This model allows for variation of the growth rate on status of the cell within its cycle and the overall state of the system, but ignores differences between individual cells.

Assume a fixed number of cells

Mothers and daughters have the same trajectory under (ODE).

For a nearly-steady or nearly-periodic state: The *expected number* of cells descended from a given cell at the same point in a later cell cycle is ≈ 1.

Each variable $x_i(t)$ may be thought of as the state of the expected descendant at time t.

- A fixed # allows easy numerical investigation.

- A fixed # allows for rigorous analysis.

Advancing and blocking models of feedback

S – signalling region R – responsive region

$$\frac{dx_i}{dt} = \begin{cases} 1 & \text{if} \quad x_i \notin R \\ F(\#\{\text{cells in } S\}) & \text{if} \quad x_i \in R. \end{cases} \tag{1}$$

Limiting cases of $+/-$ feedback with a threshold τ:

Advancing: $F(N) = \begin{cases} 1 \text{ if } N < \tau, \\ +\infty \text{ if } N \geq \tau. \end{cases}$

Blocking: $F(N) = \begin{cases} 1 \text{ if } N < \tau, \\ 0 \text{ if } N \geq \tau. \end{cases}$

Results on Clustering

In the idealized advancing and blocking models:

- Cells will synchronize forming clusters.
- Clusters will remain synchronized forever.

- Given enough cells, clusters will form
- The number of stable clusters has explicit bounds.
- The number of clusters depends on $|S|$ and $|R|$.

$G = |R| + |S|$ minimum gap between non-interacting clusters.
$M = \lfloor G^{-1} \rfloor$ maximum number of isolated clusters.

Proofs by direct calculation.

Random perturbation of the idealized models

If we:
Assume a (explicit) bound on $|b_i|$
(small noise and individual differences)

Then we can prove, under certain conditions:
Existence of and bounds on clusters.

Proofs depend on careful, but simple estimates.

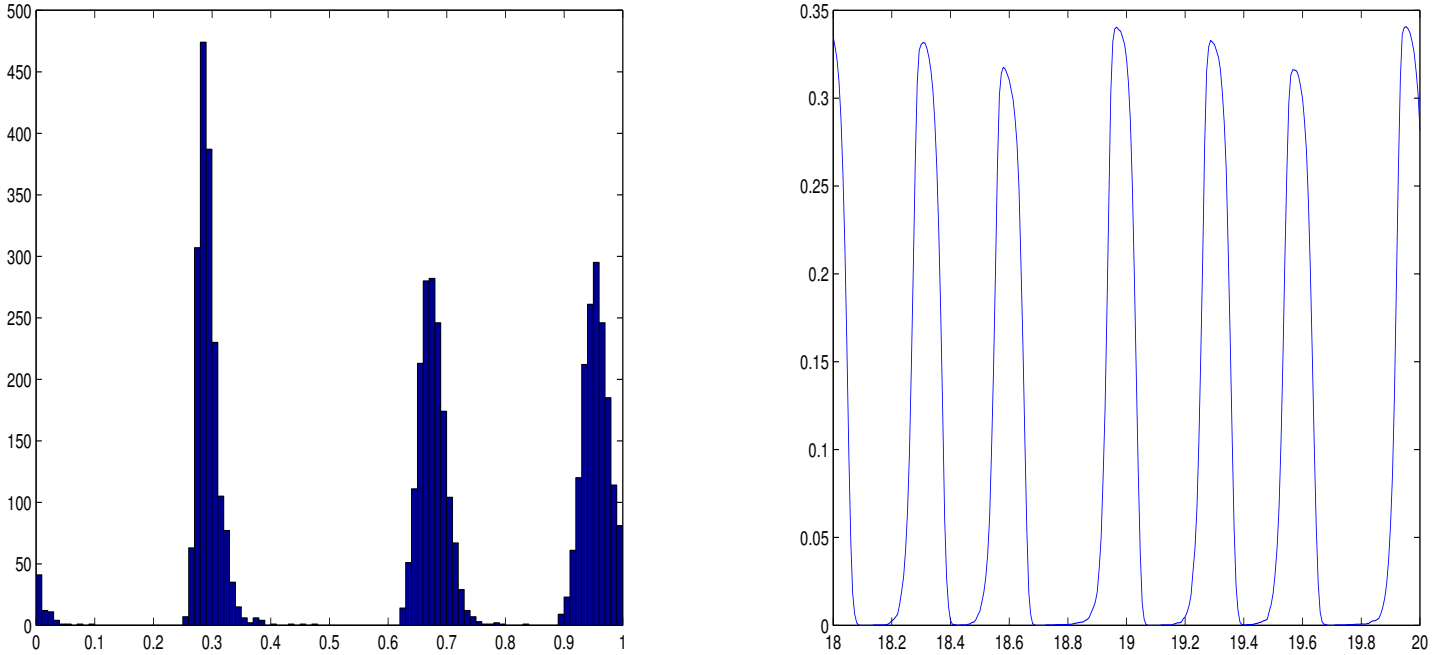
Simulations of SDE models with linear feedback

We consider the SDE model with cells in S influencing cells in R by a linear relation:

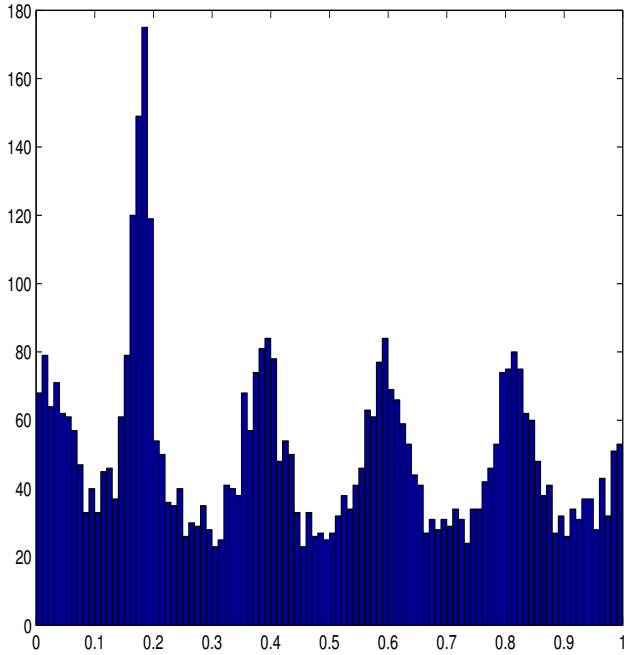
$$dx_i = \begin{cases} dt + \sqrt{\sigma}\, dW & \text{if} \quad x_i \notin R \\ dt + f(\#\{\text{cells in } S\})\, dt + \sqrt{\sigma}\, dW & \text{if} \quad x_i \in R. \end{cases} \tag{2}$$

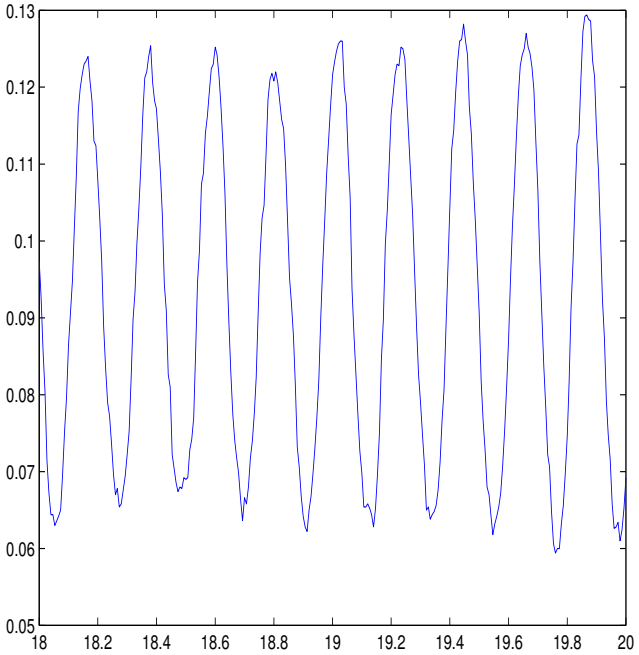
with f linear.

We integrate a fixed set of 5,000 cells.

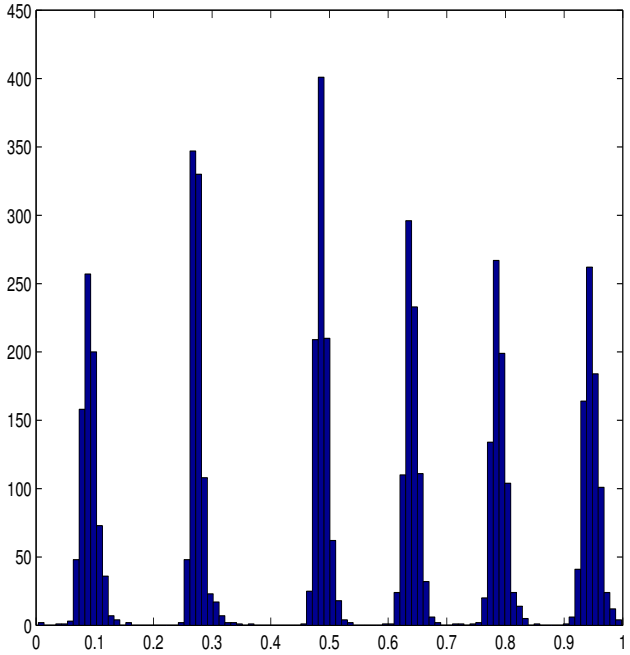


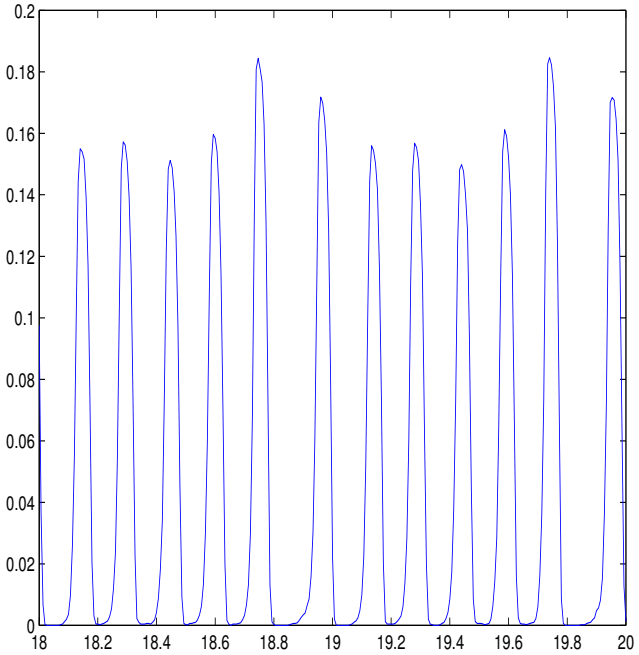
Simulations of the SDE model with linear acceleration. Here $R = [.1, .2]$, $S = [.2, .3]$, $\sigma = .02$. $M = 3$. (a) Histogram of the final distribution of cells within the cell cycles. (b) Time-series of the final two time frames. One unit of time corresponds to one unperturbed cell cycle.



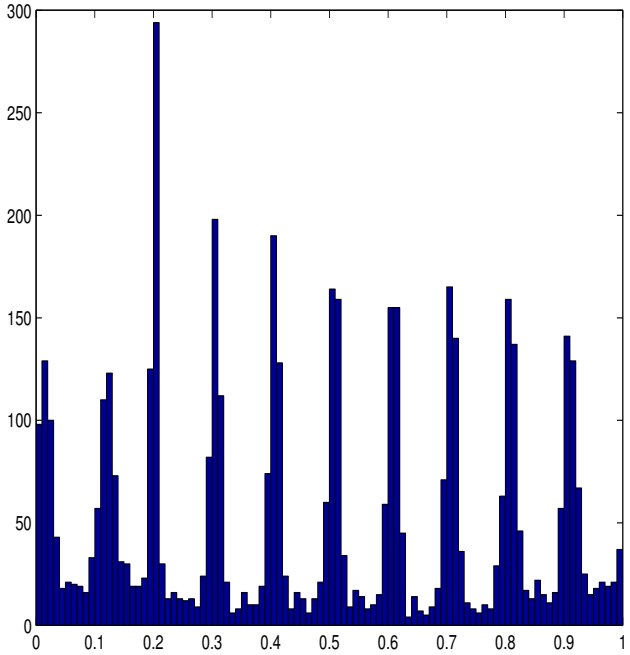


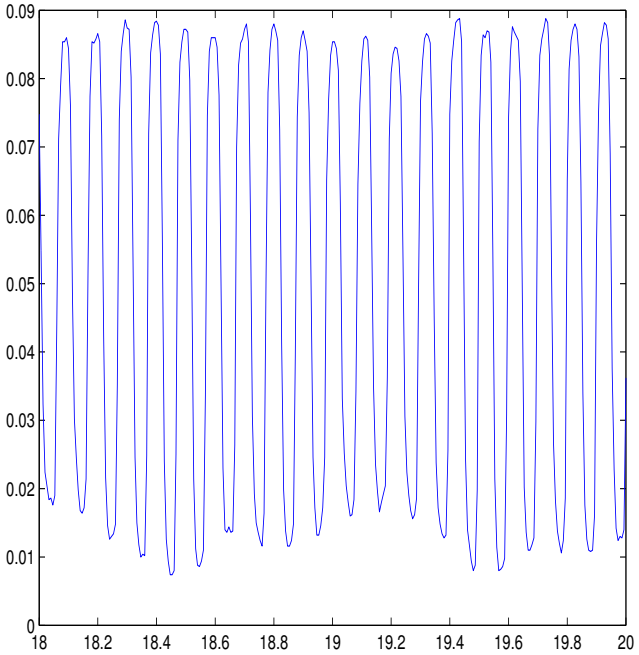
Simulations of the SDE model with linear inhibition. Here $R = [.1, .2]$, $S = [.2, .3]$, $\sigma = .02$. $M = 3$. (a) Histogram of the final distribution of cells. (b) Time-series of the final two time frames.





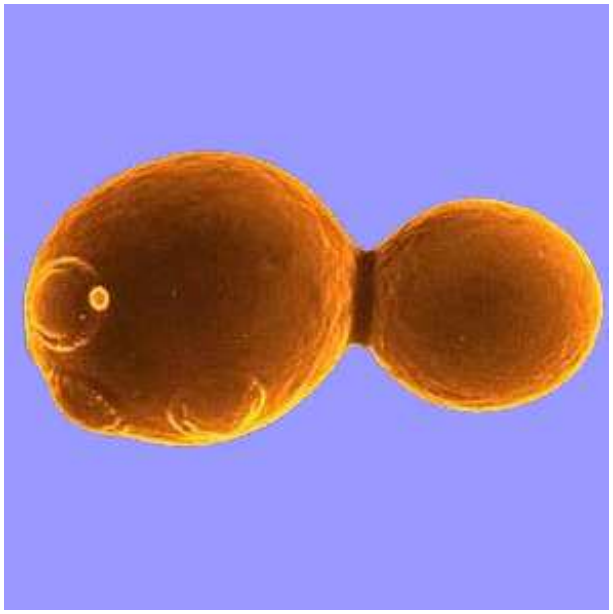
Simulations of the SDE model with linear acceleration. Here $R = [.14, .2]$, $S = [.2, .26]$, $\sigma = .01$. $M = 9$ (a) Histogram of the final distribution of cells. (b) Time-series of the final two time frames.





Simulations of the SDE model with linear inhibition. Here $R = [.15, .2]$, $S = [.2, .26]$, $\sigma = .01$. $M = 9$. (a) Histogram of the final distribution of cells. (b) Time-series of the final two time frames.

Budded Yeast

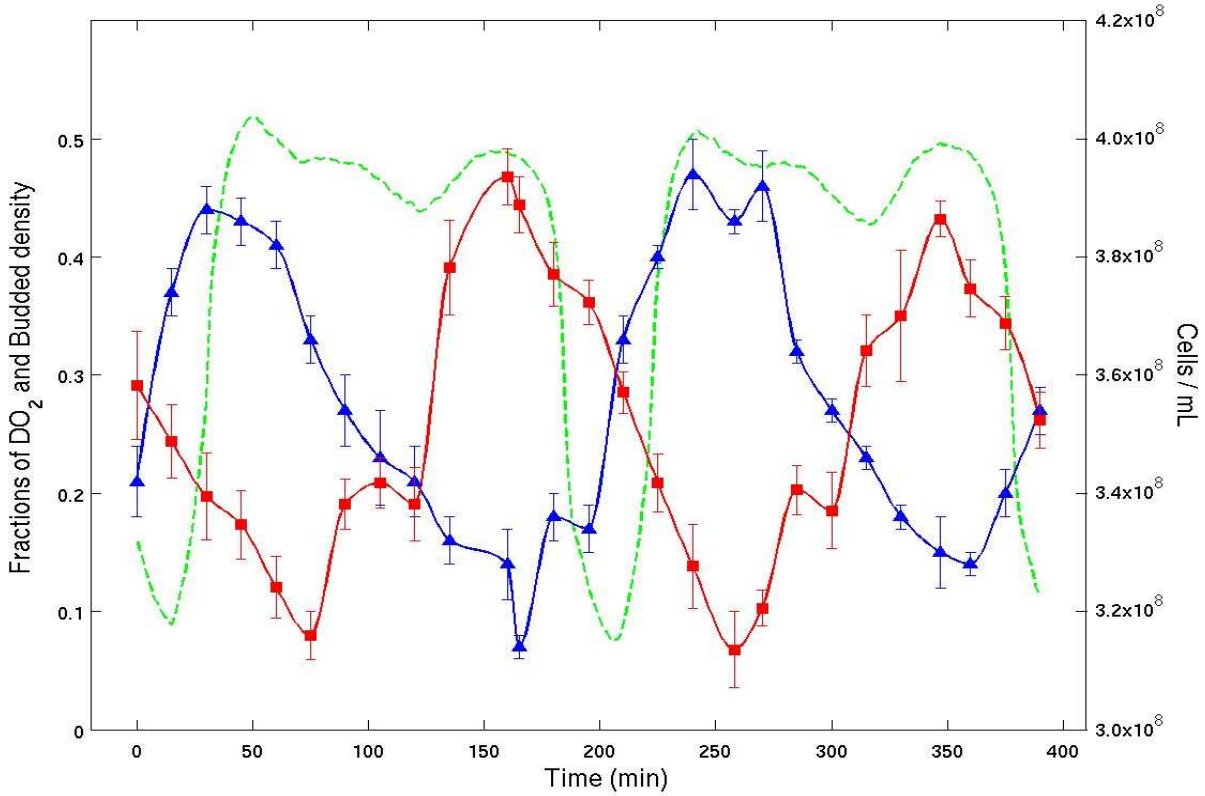


Sources: www.kaeberleinlab.org, www.alltech.com

The fraction of budded yeast in a sample may be determined.

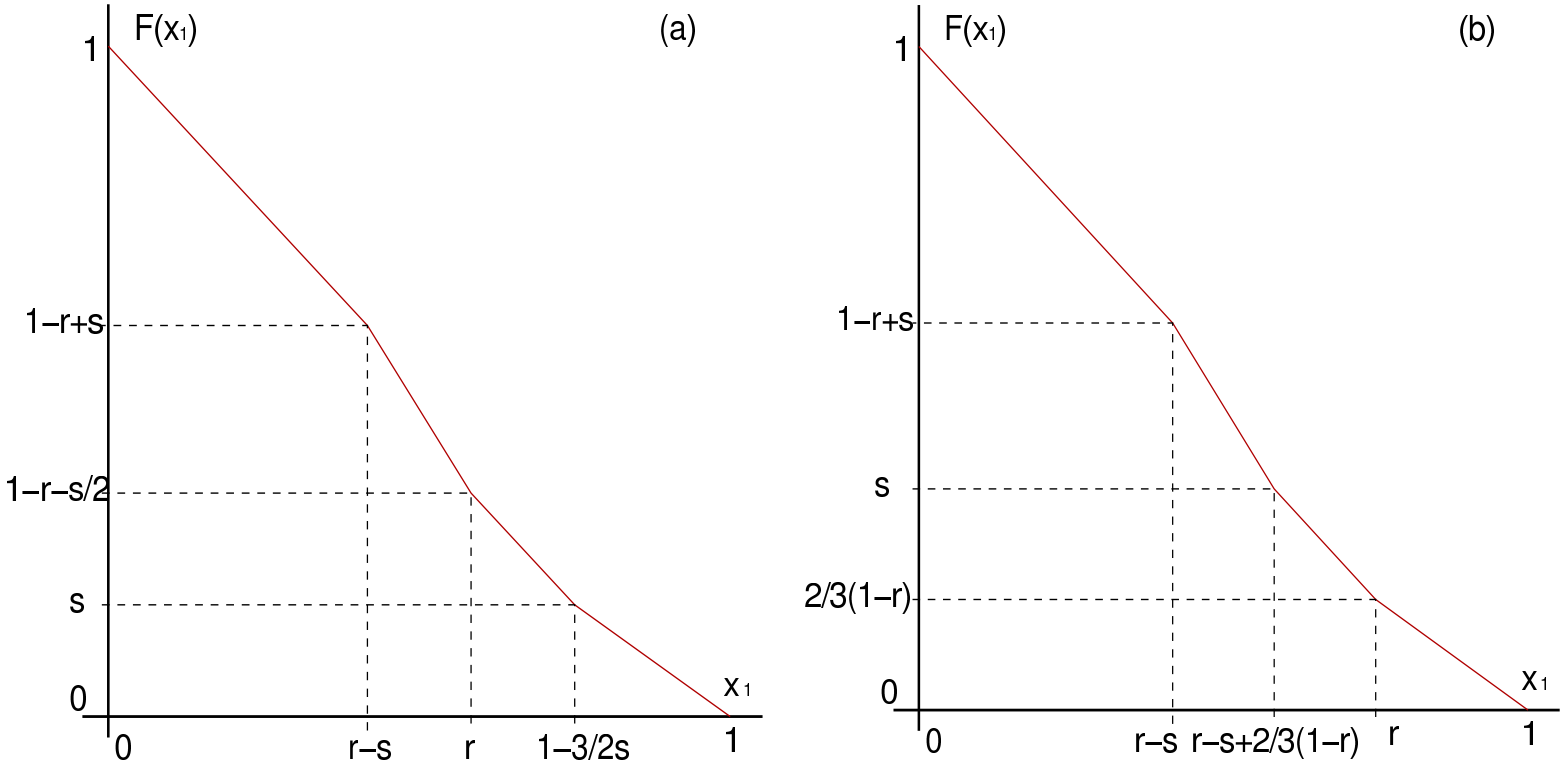
The replicative ages of yeast cells in a sample may be determined (harder).

Experimental evidence for Clustering



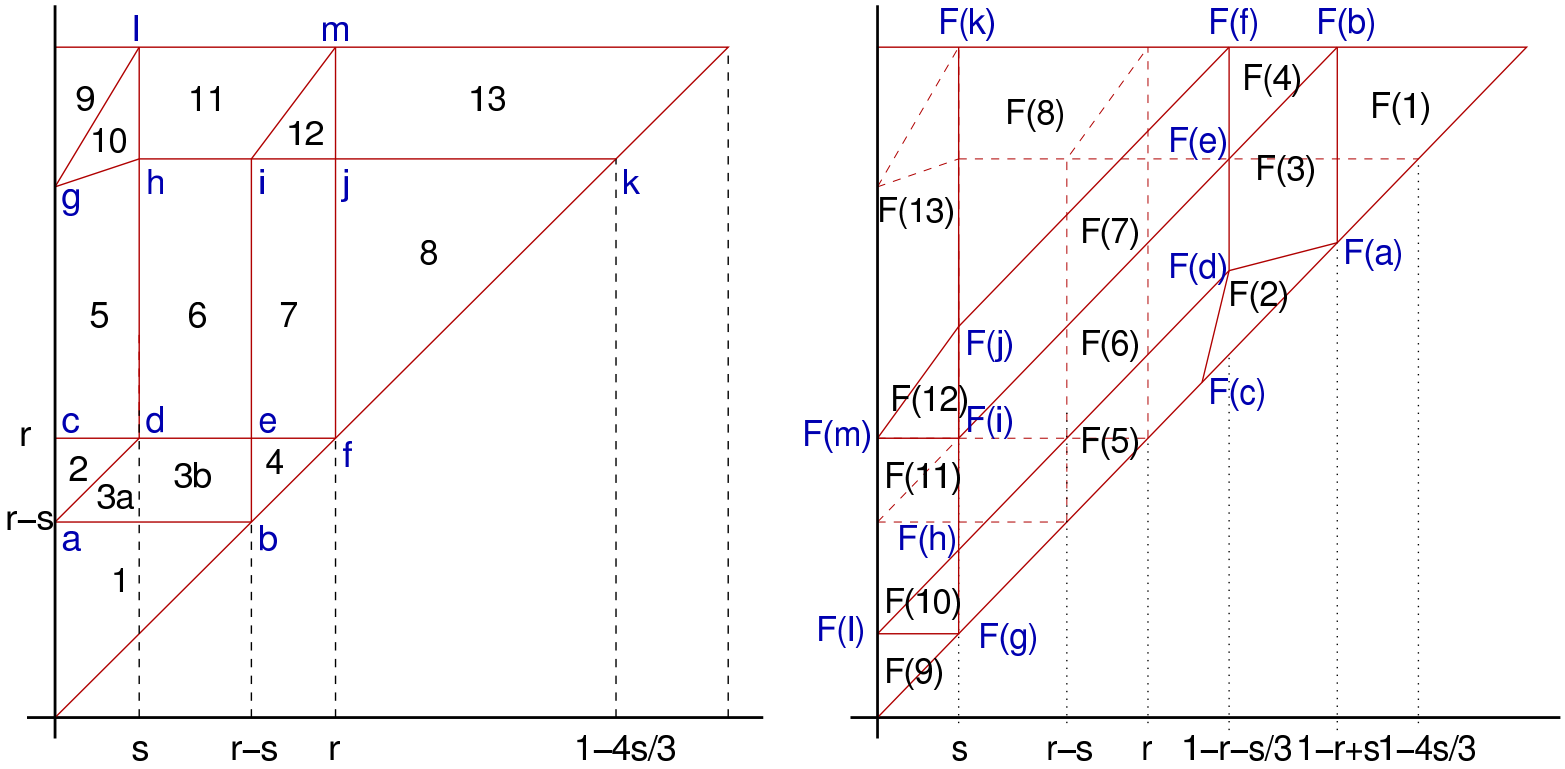
Oxygen dilution (green), bud index (blue) and cell density (red) over one cell cycle period. There are 2 clusters.

2 Cluster Systems - Detailed Analysis



Plots of the return mapping piecewise constant Positive Feedback for $K = 2$. (a) $r + \frac{3}{2}s < 1$. (b) $r + \frac{3}{2}s \geq 1$.

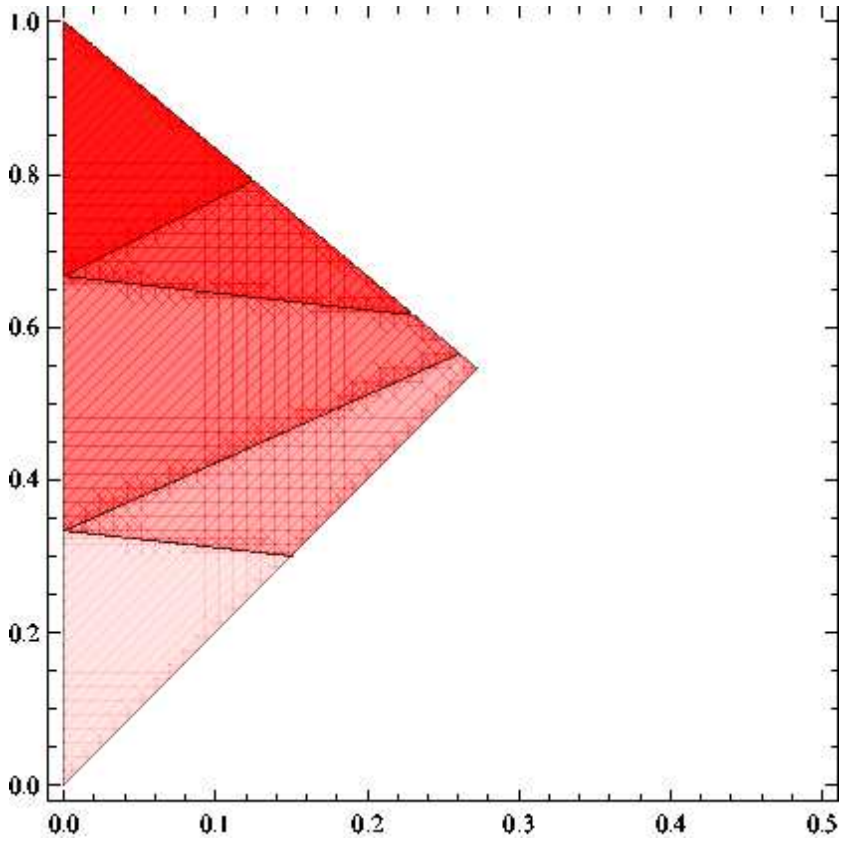
3 Cluster Systems - An Example



$K = 3$. Partition of $0 \leq x_1 \leq x_2 \leq 1$ and its image for $(s, r) = (\frac{1}{9}, \frac{5}{12})$.

3 Cluster Systems - Detailed Analysis in Progress

Parameter domains for three clusters ($K = 3$).



General Feedback

1. For positive feedback, isolated clusters are stable and most solutions tend to one of $1, 2, 3, ...,$ or M isolated clusters.

2. For negative feedback, cluster-cluster interaction is necessary to enforce coherence and most solutions tend to N non-isolated clusters, where N is a fixed number with $N = M + i$ and i is a small positive integer. In particular $N \geq 2$.

3. "Evenly-distributed", steady-state solutions exist for any form of feedback. They appear to be completely unstable.

Flow on $\mathbb{T}^n$

The state space is $\mathbb{T}^n$.

On the covering space, cells coordinates satisfy:

$$x_1(t) \leq x_2(t) \leq \ldots \leq x_n(t) \leq x_1(t) + 1$$

Rotation vectors are along the diagonal.

Two different rotation vectors are attained.

$\exists$ example with no intermediate rotation vectors.

Conclusions

• Models of cell cycle dynamics can be accessible to analytic investigation.

• Clustering is a robust phenomenon:
  - Either positive or negative feedback.
  - Not dependent on functional form of feedback.
  - It occurs for many parameter values.

• Clustering is experimentally verified in oscillating cultures.

• The biological mechanism driving Clustering is still unknown.

• Detailed modeling of cellular processes must include cell cycle effects.

Directions for Further Study

• Clustering seems to depend heavily on *geometry*, of S and R. Develop a bifurcation picture based on this observation.

• Study clustering and steady-states in PDE models of cell cycle.

• Study multiple generation models.

• Use analytical results to inform experiments to determine the precise nature of feedback.

• Combine with cellular process studies to discover feedback mechanisms.

• Autonomous oscillation also to occur in dense bacterial colonies.