Clustering in Cell Cycle Dynamics with General Forms of Feedback or, How a Dynamical Systems Viewpoint can be Useful for Biology.

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New Professor Lecture Series

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Todd Young
Department of Mathematics
Ohio University

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External Collaborators:
E. Bozcko, Vanderbilt Medical Center
B. Fernandez, CNRS Luminy, Marseille
T. Gedeon, Mathematics, Montana State
C. Stowers, Dow Chemical, Indianapolis
R. Klevecz, City of Hope Medical Center

At Ohio:
Faculty: S. Aizicovici, W. Just
Graduate Students: R. Buckalew, B. Elbert, G. Moses, B. Oduro, M. Smith, D. Sturgill
Undergraduate Students: N. Burk, M. Korb,

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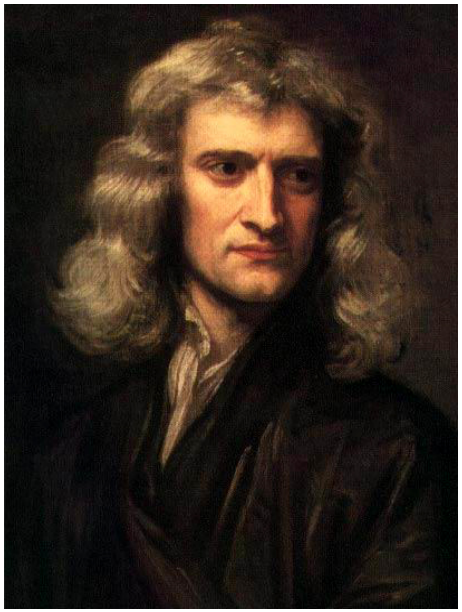
A Brief History of Science

Ques: Was Newton a Mathematician or Physicist?



A brief history, continued ...

Ans: Both, as were many prominent scientists.

1665 - 1900: Math and Physics developed together

Physics - Propose physical laws
Math - Find analytic solutions (formulas)

$\approx$ 1900: The Divorce

1900 - present: Mathematics developed separately from Physics

The Divorce

$\approx$ 1900 - Henri Poincaré discovered:
In a system as simple a three celestial bodies moving under gravitational force, solutions may be chaotic and analytic solutions may be impossible.

He invented *TOPOLOGY* as a tool to understand properties of solutions that he could not find analytically.

This was the beginning of *Dynamical Systems Theory*.

Traditional Science Dichotomy

Physics:
Few Types of Particles +
Few interactions $\Rightarrow$
Precise laws (Diff. Eqns.)
Analytic Solutions
Math answers the questions.

Biology:
Many types of objects +
Complex interactions $\Rightarrow$
No precise laws
Qualitative Description
Math is of little use.

"Mathematical Biology"

In the last 30 years:
Mathematical modeling in biology has become very common.

But still:
Many types of objects +
Complex interactions +
Multiple scales +
Parameters are not precisely known $\Rightarrow$
Differential Equations that are not precise.

Is Math of any use?

What is a Dynamical Systems Viewpoint?

Study *Possible Types of Solutions* rather than exact formulas.

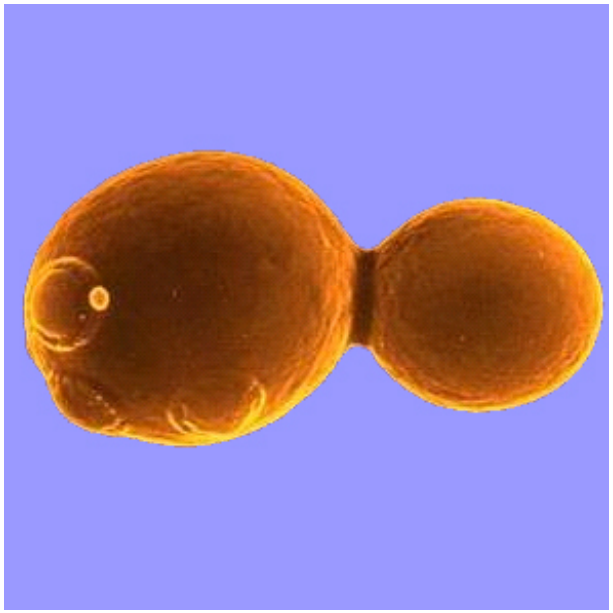
Focus on *Classes of Equations* rather than specific equations.

Focus on *Stability* and *Eventual or Long-term Behavior.*

Study the *Periodic Solutions* as a frame for all solutions.

Study which types of solutions are *Likely to be Observed.*

A Biological Problem: *Saccharomyces cerevisiae*

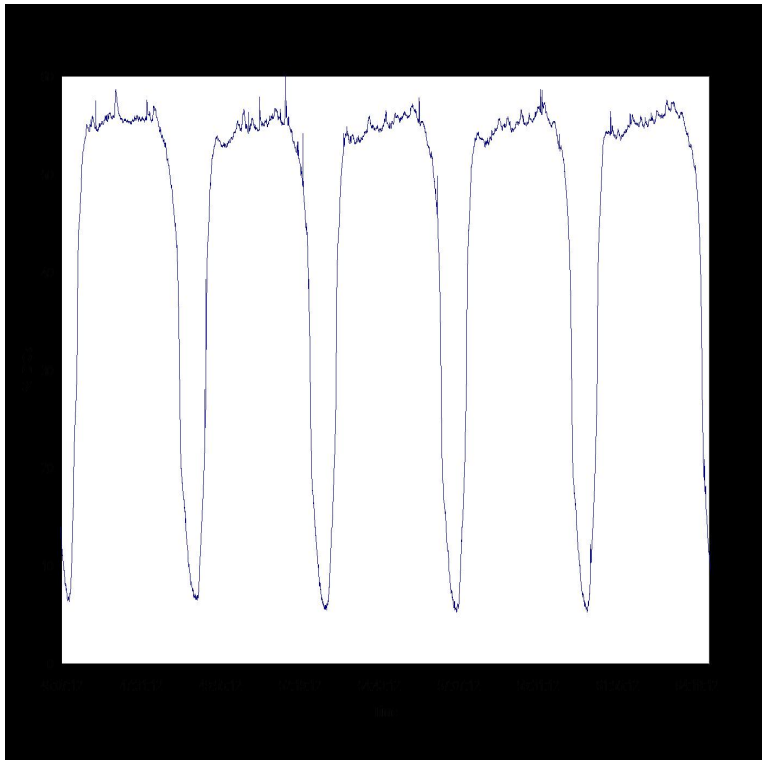


Photos: Wikipedia, www.kaeberleinlab.org, www.alltech.com

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

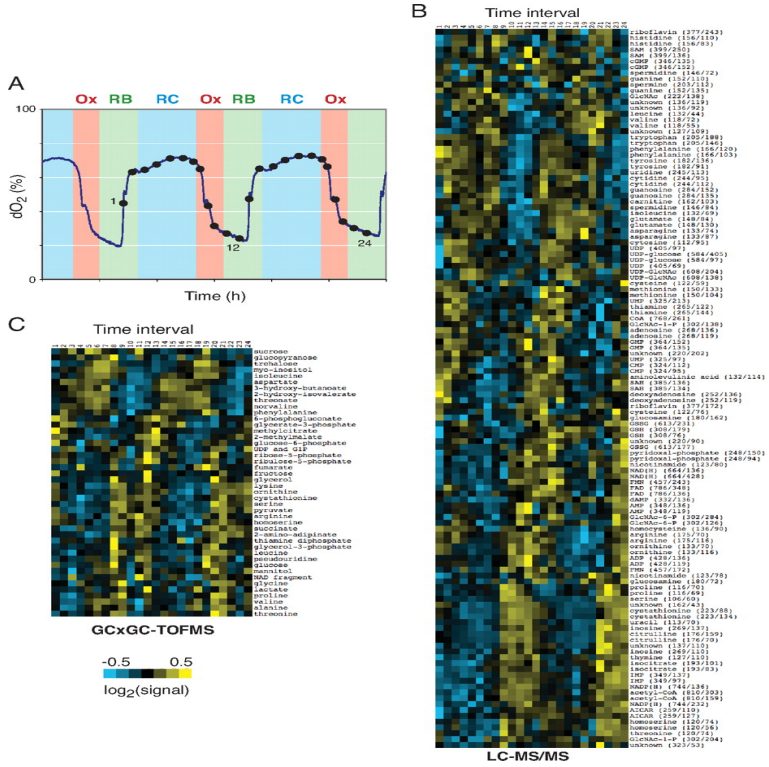
Oxygen oscillations

Dissolved O_2 vs. time:



20 hrs. Range 5% - 65%.

Microarray time-series:



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

Oxygen Oscillations

Oscillations occur under the following conditions:

- high cell density, low nutrient density.

- highly oxygenated media

- well-mixed, planktonic cultures.

Experiments are done in a stirred bioreactor with slow input/output.

- The period of oscillation is always nearly an integer fraction of the doubling time.

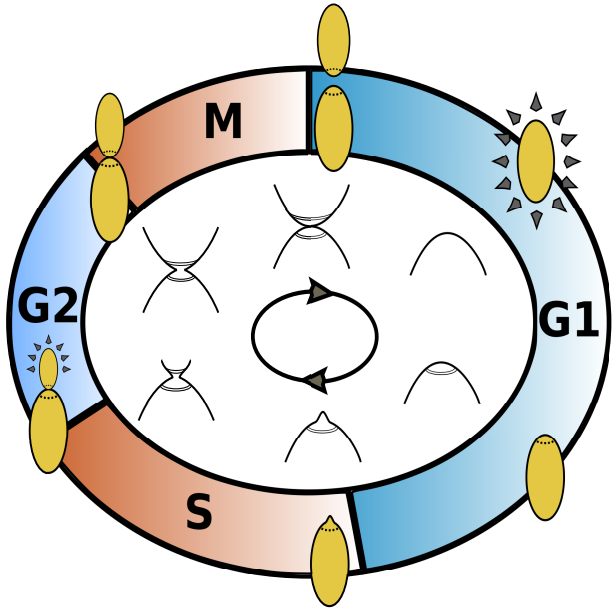
Cell Cycle of Budding Yeast

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



Cell cycle synchrony is impossible to sustain in the lab. Initially synchronized cultures quickly de-synch.

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

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.

$$\frac{dv_i}{dt} = c_i v_i, \qquad c_i > 0.$$

Standard assumption: c_i is a constant that depends only on external factors, e.g. resources.

Consequences of assumptions.

Traditional approach:
c_i is a constant implies:

$$x_i(t) = x_i(0)e^{c_i t}, \qquad c_i > 0$$

and we are done – we have found a solution.

For this solution Statistics $\Rightarrow$ cells are generally uniformly distributed throughout the cell cycle at any given time.

Our assumption: c_i depends on location in the cycle and feedback from other cells implies:

Cell-Cycle Clustering

Clustering

By *Cluster* 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.

This feedback can reinforce the formation of clusters.

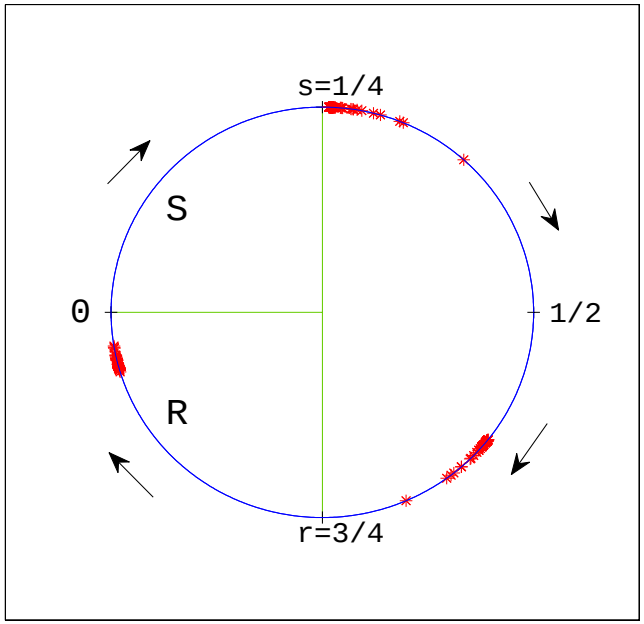
Clustering and Oscillations are intrinsically linked.

Example - Metabolic products from a large cluster of recently 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 linear feedback and diffusion:



Ordinary D.E. Model

After a logarithmic, normalizing change of coordinates, we consider the following model:

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

$x_i \in [0, 1]$ represents the position of the i-cell in the cell cycle.
$0 \sim$ birth, $1 \sim$ division.
$\bar{x}$ represents the state of all the cells.

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.

This is the simplest model with cell-cycle dependent feedback.

General Models of RS Feedback

Signalling region $S = [0, s)$. Responsive region $R = [r, 1)$.
$I = \#\{\text{cells in } S\}/n$.
z - "signaling" agent (metabolite?)

Mediated feedback:

$$\dot{x}_i = \begin{cases} 1, & \text{if} \quad x_i \notin R \\ 1 + f(z), & \text{if} \quad x_i \in R, \end{cases} \qquad \dot{z} = \alpha I - \mu z. \tag{1}$$

Instant feedback:

$$\dot{x}_i = \begin{cases} 1, & \text{if} \quad x_i \notin R \\ 1 + g(I), & \text{if} \quad x_i \in R. \end{cases} \tag{2}$$

$f(z)$, g(I) are increasing or decreasing "response" functions.

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.

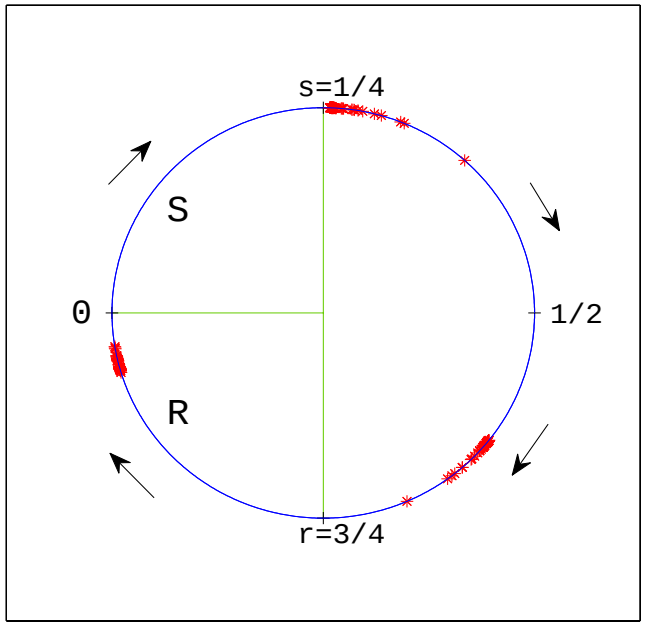
- A fixed # allows easy numerical investigation.

- A fixed # allows for rigorous analysis.

Initial Results - Simple Response Functions

S - Signaling, R - Responsive, $g(I)$ linear or step function.

Simulation, 500 cells,
Neg. feedback & noise:

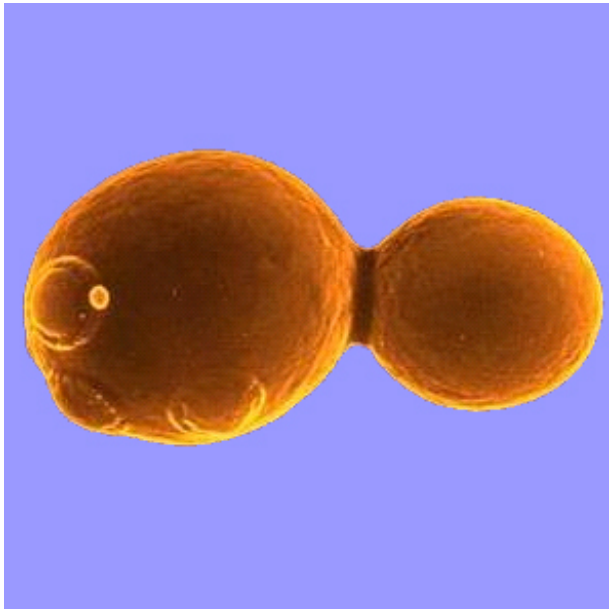


- Simulations with RS feedback almost always form clusters.

- Analysis of simple RS feedback confirms that clustering is robust.

- We began looking for clustering in yeast experiments.

Budded Yeast

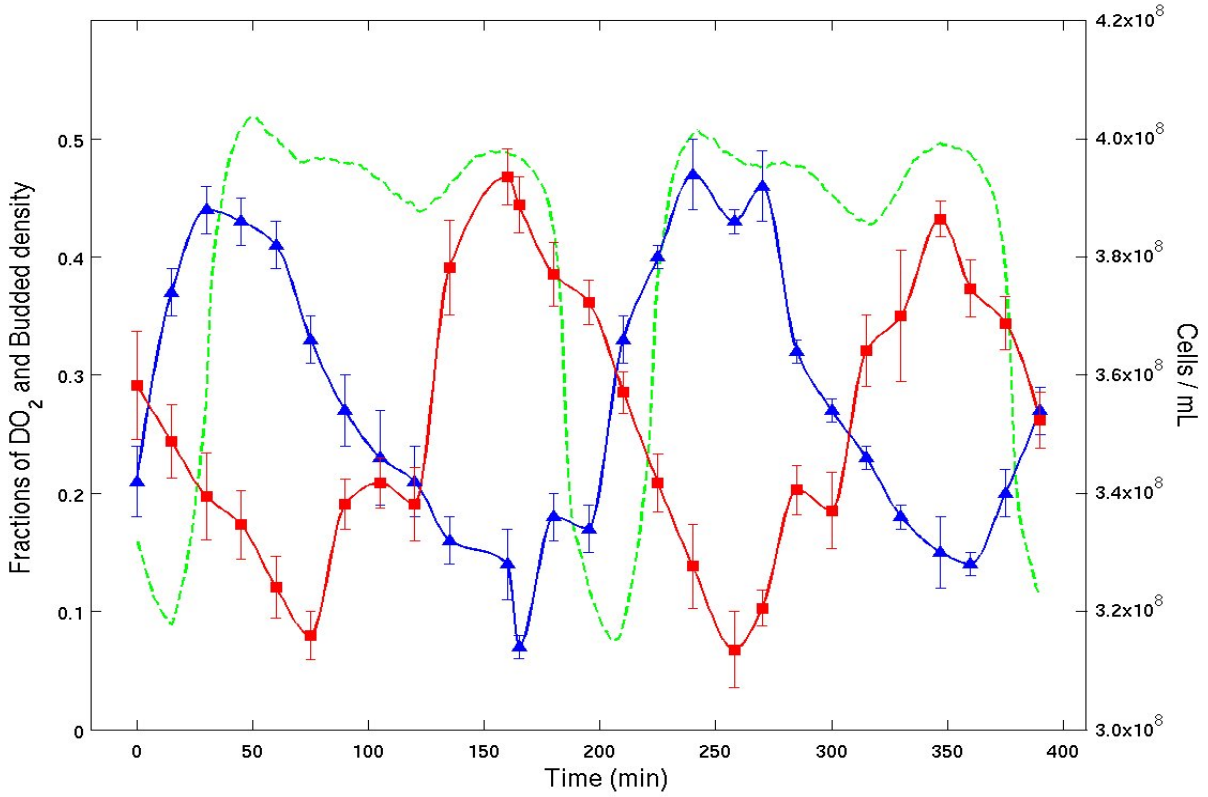


Sources: www.kaeberleinlab.org, www.alltech.com

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

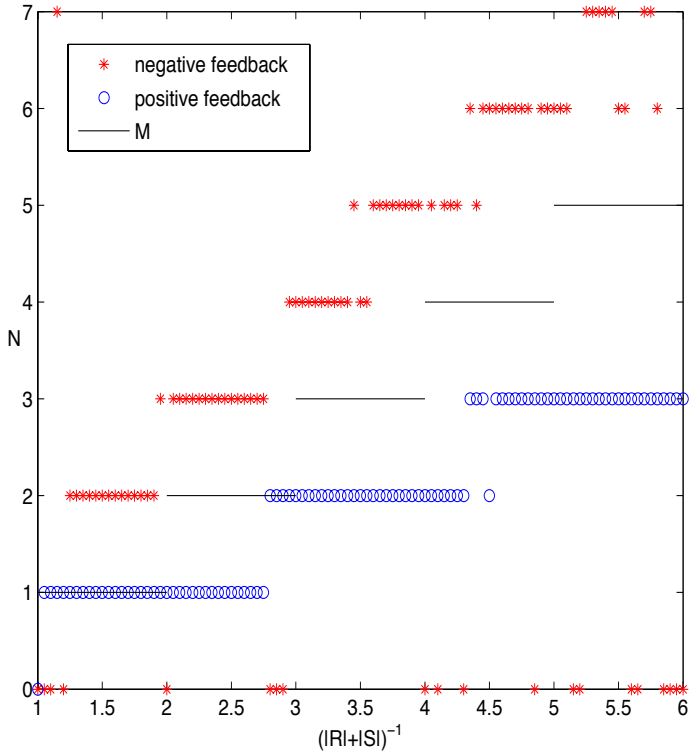
The replicative ages of yeast cells in a sample may be determined (harder).

Experimental Evidence for Clustering



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

Negative vs. Positive Feedback



The number of clusters that form in simulations compared with $M = \lfloor (|R| + |S|)^{-1} \rfloor$. M is the number of clusters that can exist without interactions.

Isolated Clusters and the Geometric Constant M

If the distance between two clusters is more that $|R| + |S|$ then those two clusters will not interact.

$M = \lfloor (|R| + |S|)^{-1} \rfloor$.

M - max # of clusters that can exist without interactions.

Solutions with $k \leq M$ noninteracting clusters will be periodic.

Theorem - For instant positive feedback (2) solutions with non-interacting clusters solutions are stable. For negative feedback they are unstable.

Cluster Systems

In the models (1) and (2), a fully synchronized (sharp) cluster of cells will remain synchronized.

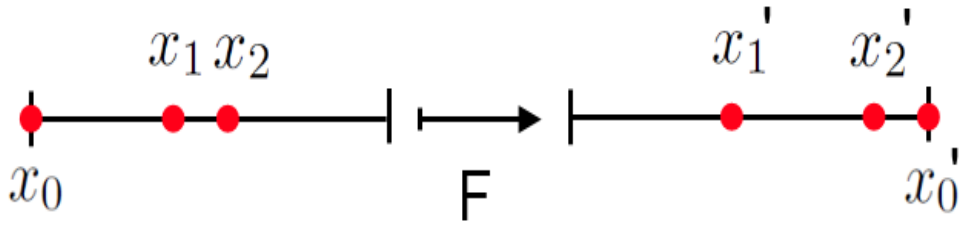
Strategy: Study the existence and stability of periodic solutions consisting of k sharp clusters.

We can show, for any form of feedback:
• Existence of k-cluster, periodic solutions for any k, $k|n$.
• Existence of "Steady-state" solutions where all n cells are nearly evenly-distributed. They appear to be unstable.

How to study a system without knowing solutions?

Poincaré: study "return maps".

Example: Three clusters ($k = 3$)



$F(x_1, x_2) = (x_1', x_2')$ is a continuous, two dimensional function.

Studying F we discover many thing about the dynamics of the system.

How to study a system without a solution, continued ...

We can use abstraction:

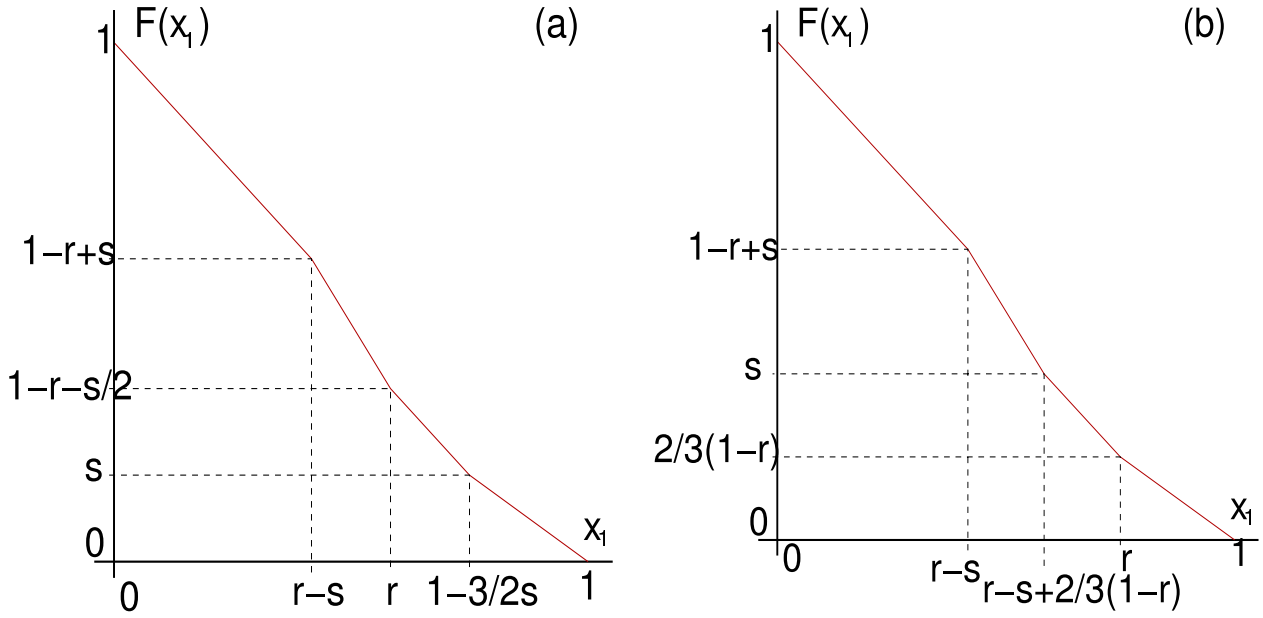
For any $k \geq 2$, F is a continuous function from the set S given by $0 \leq x_1 \leq \ldots \leq x_{k-1} \leq 1$ into itself.

Brouwer's Fixed Point: Any continuous function from a convex, compact subset S of a Euclidean space into S itself has a fixed point.

$\Rightarrow$ For any k there is a periodic solution consisting of k equal clusters, including the case $k = n$.

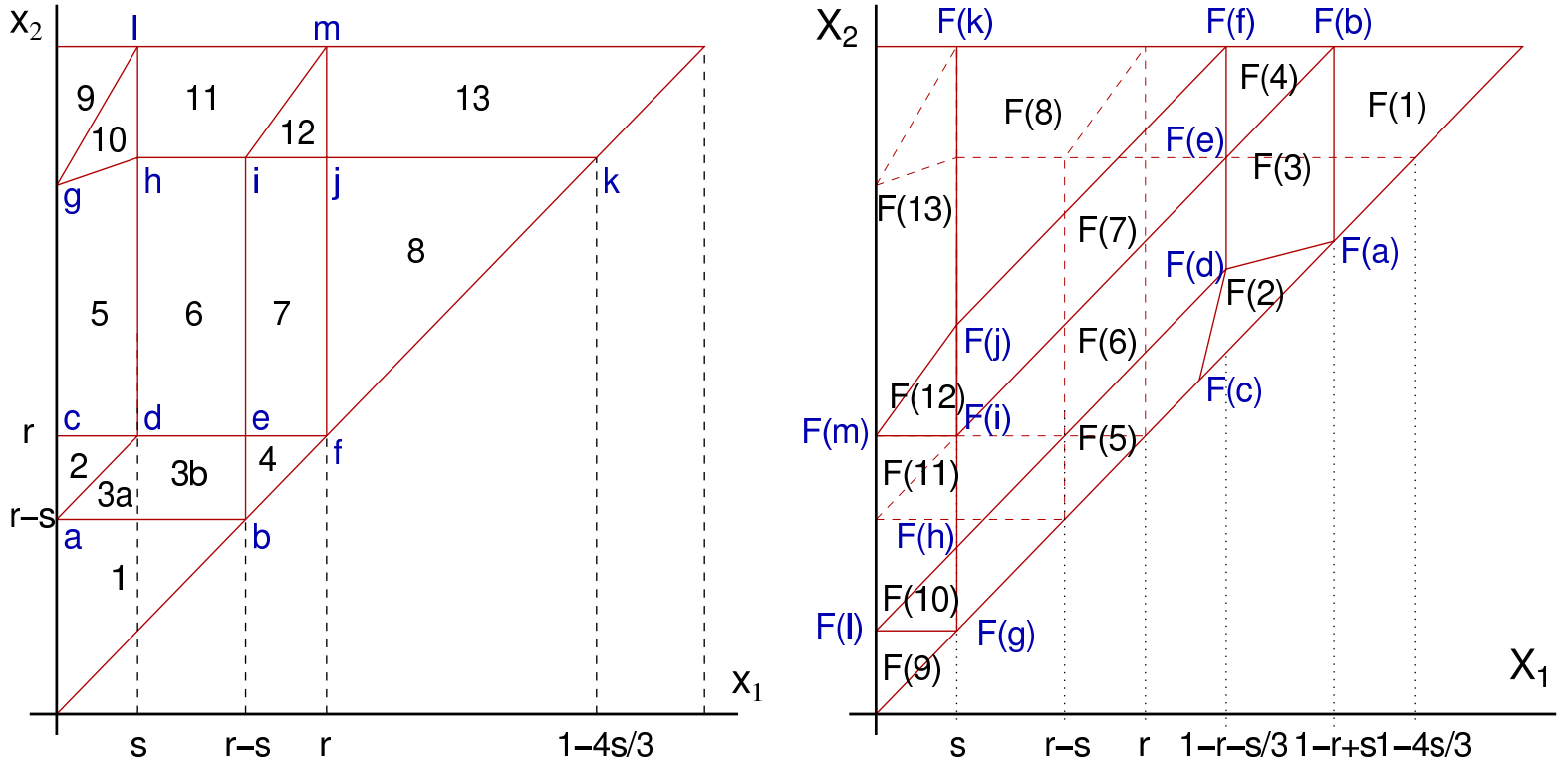
How to study a system without a solution, continued ...

For some cases, we can solve the equations analytically for a short time $\Rightarrow$ we can calculate F.



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 - Detailed Analysis - Example



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

General Instant Feedback

1. For positive feedback, isolated clusters are stable and in simulations solutions tend to one of $k \leq M$ isolated clusters, including $k = 1$.

2. For negative feedback, cluster-cluster interaction is necessary to enforce coherence. Most solutions tend to k non-isolated clusters, where $k = M + i$ and i is a small positive integer. $k \neq 1$!

$k = 1$ "synchronization" is never observed

Theorem: The $k = M + 1$ cluster periodic solution is:

- *Stable* for negative feedback, and
- *Unstable* for positive feedback.

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 large open sets of parameter values.

• Clustering seems to depend heavily on *geometry* of S and R.

• Clustering is experimentally verified in oscillating cultures.

• The biological mechanism driving clustering is still unknown, but **negative feedback** is more likely.

• Modeling of cellular processes should consider cell cycle effects.

Directions for Further Study

- Study Mediated Model (1) - underway.

- Study clustering in PDE models with feedback.

- 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.

- Oscillations also occur in dense bacterial colonies.