

**AN EXPLICIT SPATIAL MODEL
OF YEAST MICROCOLONY
GROWTH**

Advisor: Mark Alber
Multiscale modeling

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Thomas Apker
Jianfeng Zhu

1 Introduction

Here we present a single-cell-based model of cell-cell interactions in a growing yeast colony.

2 Biological background and assumptions

We assume that all the cells are round in shape and divide by budding; that is, they form a mother-daughter cell pair, with the daughter cell growing gradually from a small bud into a larger cell while attached to the mother cell throughout growth. At cell division, the nucleus of the mother cell divides, and one of the daughter cell, followed by the physical separation of the unevenly sized cells. The smaller size daughter cell then continues growing before reaching the size of the mother cell and undergoing its own cell division. The mother cell can continue dividing a multiple, although not an unlimited, number of times.

The location of the bud on the surface of the mother cell can be described by a vector χ connecting the center of the mother cell and the center of the bud. For a given mother cell, the direction of this vector for subsequent cell divisions is not random. There are several conventional types of division pattern, which we consider in the following:

1. The *axial* pattern(1): the vectors in the set $\chi_i, i = 1, 2, \dots$, are almost coincident; that is all the χ_i differ from one another by a small angle.
2. The *bipolar* pattern(1): the χ_i and χ_{i-1} for all i are collinear but opposite in direction. Thus, each successive cell division occurs at the opposite "pole" of the location of the mother cell.

Both axial and bipolar patterns also hold when a daughter cell becomes able to produce a bud and thus become a mother cell. Here, however, another distinction is sometimes made. If the axial pattern sets for a daughter cell, so that χ_i is collinear with χ_0 , the vector corresponding to the cell division leading to the formation of the daughter cell, then this pattern of division is termed unipolar. If the χ_i are opposite to χ_0 , the division pattern is still referred to as axial. It is a conventional wisdom among the yeast biologists that the axial division pattern leads to "bunching" of cells into tightly packed

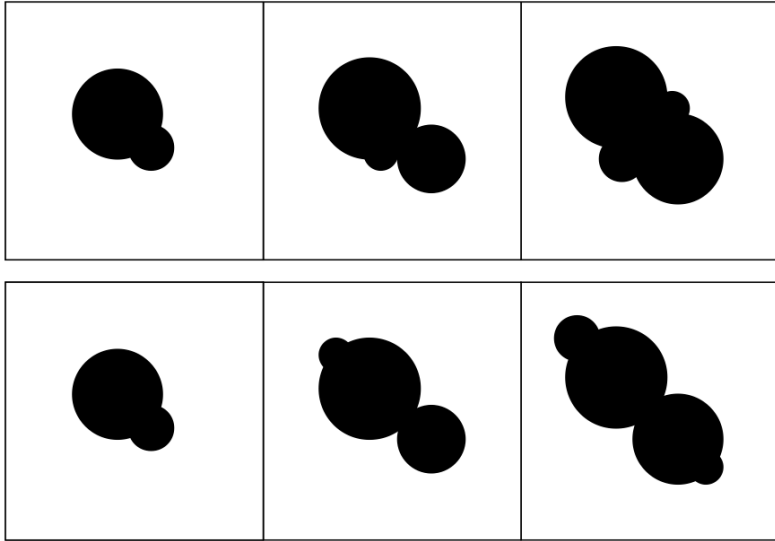


Figure 1: Top: axial cell divisions. Bottom: polar cell divisions

cell spheroids, whereas unipolar cell divisions can help expansion of a colony. The so-called dimorphic switch from axial to polar division patterns often occurs when cells find themselves starved for nutrients. Thus, if the polar pattern indeed can provide an opportunity for exploration of the cell's surroundings for food, the dimorphic switch can indeed be a good evolutionary strategy. In the following we will use the term polar to describe both bipolar and unipolar division-polarity patterns.

Another descriptor of yeast-cell division is the size threshold characterizing bud formation and mother-daughter cell separation. A sufficiently large yeast cell can initiate a cell-division cycle (the so-called G1 phase of the cell cycle) by selecting a bud site and forming a bud, that initially is just a semi-spherical projection present on the surface of a cell.

An additional important determinant of colony morphology is cell-cell adhesion. In fact, there are two types of cell adhesion. One is mother-daughter cell adhesion, and the other is cell adhesion between cells that localize by chance. The reason for this distinction is that mother-daughter cell adhesion can occur in a natural way following cell separation and that cell-cell separation is often incomplete. Here we suggest that cell-cell adhesion and the patterns of the cell division are the primary force-generating parameters.

Finally, an important fact of yeast-cell division and colony expansion is the dependence of cell growth on the nutrient supply. In general, as yeast cells begin to starve, their growth rate diminishes until they enter into the so-called stationary phase, when cell division stops.

3 Model description

3.1 Cell growth

Let the growth rate be a function of the radius $f(r)$ multiplied by a "slowing" factor $g(r, r_{max})$, where r_{max} is the maximal radius of the cell. The slowing factor serves to decrease the growth rate to zero as the maximal cell size is approached. The growth is implemented as

$$\frac{dr}{dt} = f(r)g(r, r_{max}), \quad (1)$$

where

$$f(r) = k_{g1}r + k_{g2} \quad (2)$$

and

$$g(r, r_{max}) = \left(1 - \frac{f(r)}{f(r_{max})}\right). \quad (3)$$

3.2 Cell-division cycle

The cell cycle in the model is driven by the growth of the cells, with no underlying molecular mechanisms accounted for. Two checkpoints are introduced to determine when a new bud begins to form (G1 phase) and when the mother and daughter cells separate (mitosis) as follows:

- G1 checkpoint: $r > k_{G1}r_{max}$ and no other bud present on the cell;
- mitosis checkpoint: $r_{bud} > k_{mitosis}r_{max}$.

3.3 Cell-division polarity

A cell polarity is defined for each cell, and new buds are formed according to this polarity. A spatial axis vector, χ_{cell} , is defined as being the direction between the mother and daughter at a division, and the next bud is formed in either a direction almost colinear to the vector or the opposite direction.

If the direction is defined from the cell positions at a division $\chi = (x_{daughter} - x_{mother})$, then axial polarization is defined by new buds forming close to

$$\chi_{mother} = \chi$$

and

$$\chi_{daughter} = -\chi,$$

leading to the new buds forming close to the old mother-daughter connection point.

The bipolar division pattern is defined by having the new buds form as follows:

$$\chi_{daughter} = \chi$$

and

$$\chi_{mother} = -\chi.$$

In the model, the new buds on the daughter and mother cell always form away from each other.

The implementation has a parameter, $k_{axialFrac}$, which sets the fraction of divisions that are axial and the fraction that are polar ($1 - k_{axialFrac}$).

3.4 Mechanical interaction

The mechanical interactions are implemented as spring forces between the centers of the spheres. The cells represent a highly viscous medium with friction, and, in the model, velocity (not acceleration) is proportional to force. Also an adhesion force is allowed for, but this is usually a fraction of the repulsive force, since high overlap between cells is highly not physical. The contribution from a neighboring cell pair to the positional update is a two-dimensional extension of the one-dimensional by

$$\frac{dx_i}{dt}_{repulsive} = k_{spring}(d - d_{relax}) = k_{spring}((x_j - x_i) - k_{rFrac}(r_i + r_j)) \quad (4)$$

and

$$\frac{dx_i}{dt}_{attractive} = k_{spring}k_{adFrac}(d - d_{relax}). \quad (5)$$

The parameter k_{rFrac} allows for a relaxing distance slightly different from the sum of the radii, and if it is a mother-bud pair, the bud has a relaxing distance equal to r_{mother} . Different values of the parameter k_{adFrac} are used if the pair is a mother-bud, a mother daughter, or an "unrelated" cell-cell pair. In addition to this, the adhesion part is only used at short distances. The contribution is truncated if the distance between two cells, i, j , is larger than $k_{neighbor}(r_i + r_j)$ to allow for cell-cell separation.

3.5 Growth inhibition by immediate neighbors

To test dependence on nutrients, in some simulations the growth is made dependent on the number of immediate cell neighbors in contracts with a given cell. It is assumed that cells with more neighbors within the colony compete more for nutrients, and hence grow slower. In the model, the growth parameter k_{g1} and k_{g2} in (2) are changed as follows:

$$k_{g(1,2)} \rightarrow \frac{k_{g(1,2)}}{k_{neighFrac}N_{neigh} + 1}, \quad (6)$$

where N_{neigh} is the number of the neighbors, and $k_{neighFac}$ is a factor setting the strength of the dependence. When $k_{neighFac}$ is zero, there is no dependence.

3.6 Different adhesion strength

In the simulations, the mother-daughter adhesion is twice that of other cell-cell adhesion ($2k_{adFac}^{cell-cell} = k_{adFac}^{mother-daughter}$).

4 Implementation

The primary unit in this simulation is the cell, which is modeled as having the components listed below:

1. **Location:** The x and y location of the cell are modeled as double precision floating point values.
2. **Radius:** The radius of the cell and its maximum radius are stored as double precision floating point values.
3. **Bud status:** An integer is stored with each instance of the cells that marks it as either being a bud, and so not yet born, a bud-free independent cell, or a cell with a bud.
4. **Bud direction:** The direction in which a bud grows is stored as both an angle and a unit vector in the direction pointed by the angle to save repeated calls to trigonometric functions.
5. **Bud radius:** If the cell has a bud, it's radius is stored as part of the mother cell. Once the bud reaches its critical size, this becomes the radius of the new cell.
6. **Cell ID:** This is an integer used to identify the cell to resolve whether it is interacting with its mother cell.
7. **Cell Mother ID:** This integer stores the Cell ID of the cell's mother.
8. **Number of Neighbors:** This in an integer used to determine growth inhibition due to proximity to other cells.

- 9. Number of daughter cells: An integer to determine if the cell as reproduced as many times as allowed by the current simulation rules.**

4.1 Bud simulation

The chief difference between the implementation here and in the paper cited is in the handling of buds. In the paper, the buds are treated as independent cells with a large d_{relax} relative to their mothers. This results in the repulsion and attraction rules being applied to them, implicitly changing χ as the bud was pushed around its mother. In the simulation presented here, bud motion must be explicitly modeled. Also, because “300 cells” in this simulation means three hundred independent, possibly bud-growing cells, the colonies shown in section ?? are significantly larger.

The easiest way to recover the original simulation is to create a space in the state vector for the bud angle of each cell, and then base the movement laws off of the combined geometry of the expanded mother cell. However, computing the forces and moments of this more complicated geometry is expensive, and biologically it is difficult to justify the idea of an undivided cell behaving as two separate ones.

The model used here assumes that a bud can repel other cells in a manner similar to an independent cell, and that its mother rotates slightly in response to contact with the bud. The speed and direction of this rotation are determined somewhat randomly, and motion continues until there is no contact with the bud. In some cases, this results in a cell that slowly rotates until mitosis. Also, buds do not interact with other buds in this version of the model.

This method of modeling bud behavior is that it allows for a wider variety of bud growth and position rules. For example, if cells with buds do not rotate away from other cells, then the resulting geometry changes from Fig. 7 to Fig. ?? for cells that . Similarly, if it is assumed that buds are “soft”, and form around their neighbors, one generally loses almost all cragginess, see Fig. 11, as the disruption caused by each mitosis tends to push cells towards the more stable circular geometry. It is possible that this explains the generally homogenous structure of baker’s yeast colonies. Alternatively, if cells are attracted to buds, such as if the default behavior is to protect nearby buds by pressing against them, the final colony ends up with a very craggy geometry, see Fig. 12.

4.2 Numerical integration

Another way in which this simulation may differ from the original authors is that cell status changes, such as mitosis and bud initiation, are handled at the end of each time-step, rather than in the derivative function. This results in cells and buds generally growing a tiny bit larger than they would otherwise, but as the average time between cell divisions roughly 1000 time-steps, that is probably negligible.

Also, the present work is integrated using a fixed time step fourth order Runge-Kutta integration scheme, as opposed to the adaptively stepping Runge-Kutta scheme used by the authors. This should have no substantial impact on the results, provided the fixed time step size is such that the system is well behaved. Empirically, this was found to be $< .1s$, as the cells tended to drift apart for larger time-steps.

5 Results

The most general observation that can be made from this simulation is that the more cells are able to move their neighbors, the rounder the overall shape of the colony. A corollary of this is that the longer a colony is allowed to grow, the rounder it will become past some threshold of maximum cragginess, generally occurring around 150 cells, see Fig. 5 and ???. In general, as shown in Fig. 1, polar cell division results in new cells that do not interact, but this was observed to be a small effect compared to cell size at mitosis and the strength of cell-bud interactions, see Fig. 2-4 and Fig. 6-8. In all of the figures below red circle indicates a budless cell, a blue dot indicates a mother cell, and a yellow dot indicates a bud.

6 Small mitosis size

For $k_{mitosis}$ of .5, there was almost no distinguishable effect of varying the fraction of cells that divided axially, see Fig. 2-4. This is most likely because as soon as a new cell is formed it can find a nice little gap into which it can fall, and then grow and disturb its neighbors as it grows.

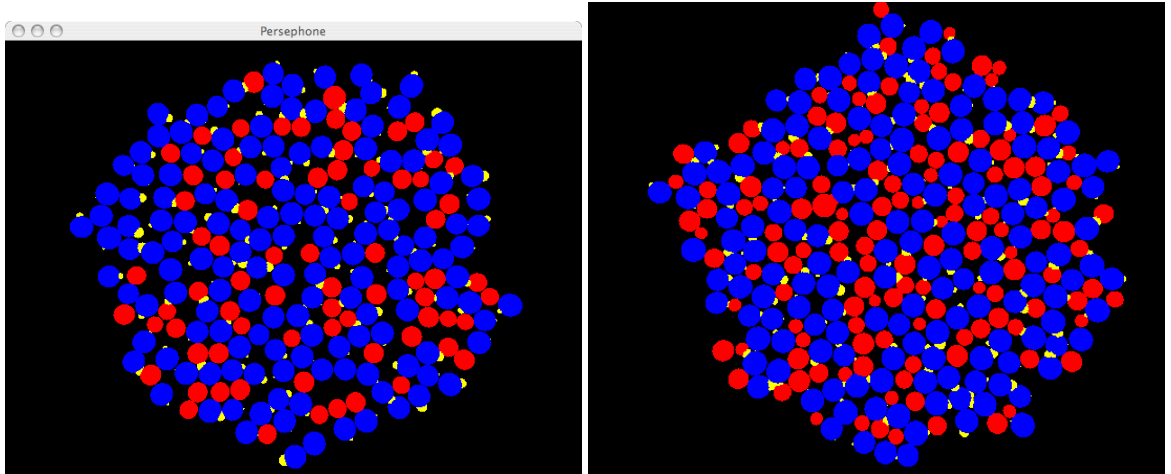


Figure 2: Right: $k_{mitosis} = .5$; $k_{axialFrac} = .25$

Figure 3: Left: $k_{mitosis} = .5$; $k_{axialFrac} = .5$

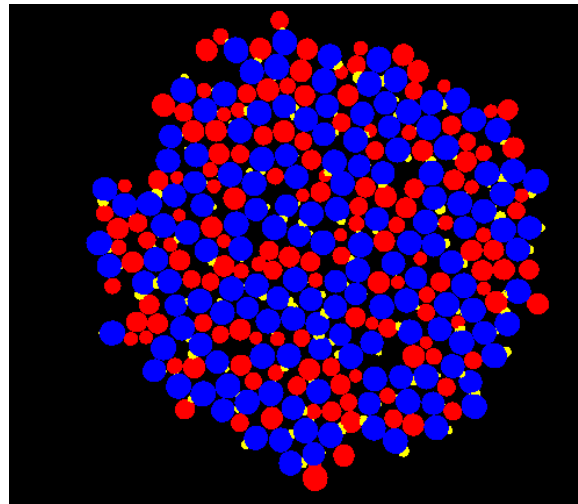


Figure 4: $k_{mitosis} = .5$; $k_{axialFrac} = .75$

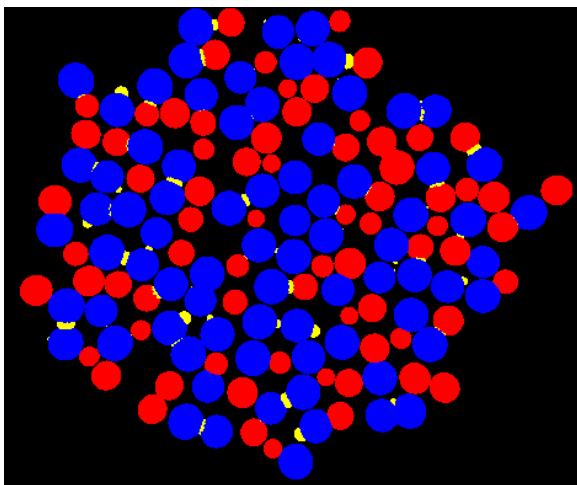


Figure 5: $k_{mitosis} = .5$; $k_{axialFrac} = .50$; 150 cells

However, if the simulation exits at 150 cells, roughly the point at which the original author's simulations quit, there are some distinct peaks that seem to grow from the center of the colony. Figure ?? shows this, suggesting that there is a critical value for the stability of colony extrusions.

7 Large mitosis size

Figures 6 through ?? show the effects of changing $k_{axialFrac}$ more clearly, as the larger cells have spent longer as buds pushing away other cells, and have relatively little growth left to do once they can move freely. However, it is worth noting that whether there are 150 or 300 cells, the largest protrusion is roughly three cell diameters across, compare Fig. 8 and ??.

8 Bud-rule driven geometry

Macroscopically, physically realistic rules for cell-bud interactions and cell division do not allow for complicated colony geometries of any realistic size.

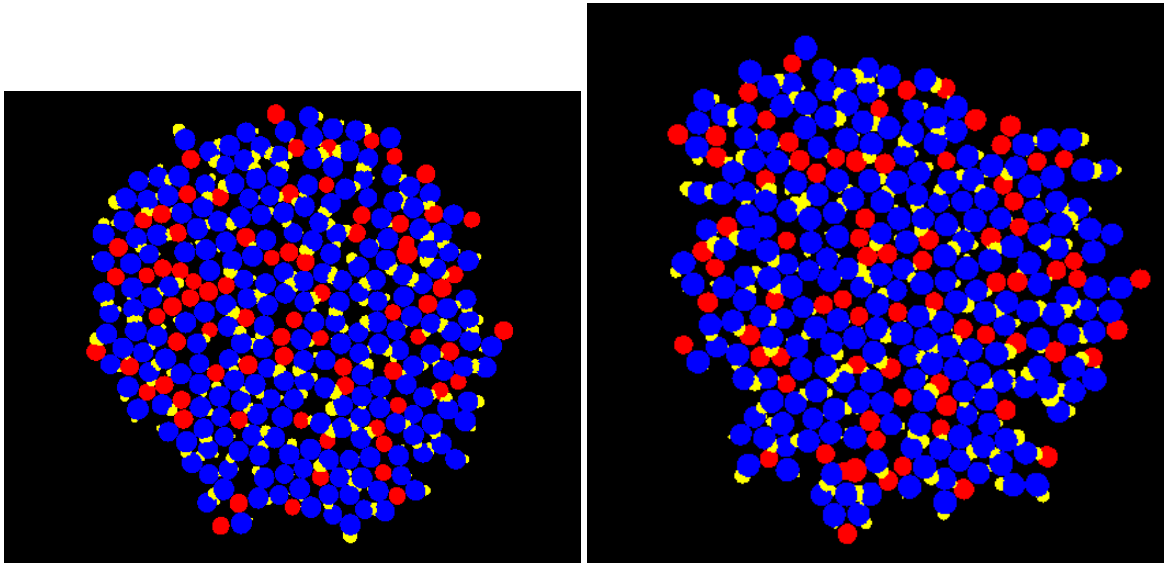


Figure 6: Right: $k_{mitosis} = .75$; $k_{axialFrac} = .15$

Figure 7: Left: $k_{mitosis} = .75$; $k_{axialFrac} = .5$

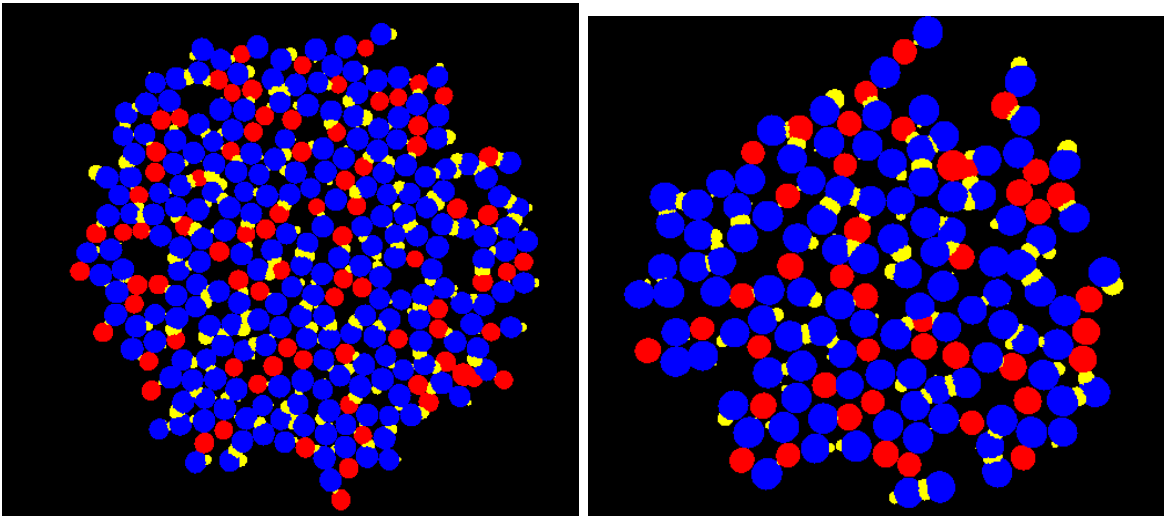


Figure 8: Left: $k_{mitosis} = .75$; $k_{axialFrac} = .75$

Figure 9: Right: $k_{mitosis} = .75$; $k_{axialFrac} = .5$; 150 cells

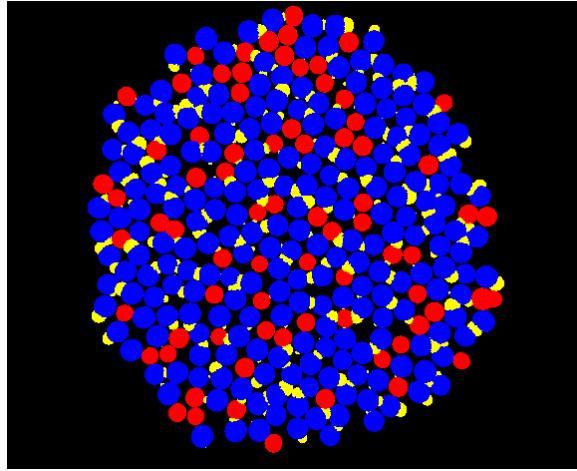


Figure 10: $k_{mitosis} = .75$; $k_{axialFrac} = .75$ “Soft buds”

However, as is suggested by the original author, the geometry of the colony is determined by the rules governing bud interactions with their environment.

For example, if one assumes that buds are essentially “soft”, and so conform to the shape of any cells pressed up against them, then the only shape possible at 300 cells is a circle. The separation of each cell from its mother disrupts any non-circular structures in the immediate area, and with this happening all over the colony, no structures and few gaps can form, as seen in Fig. 11, compare to Fig. 8.

If, instead, the mother cells do not rotate in response to other cells impinging on their mother cells, there is a slight increase in the size of the largest protrusion from the colony, and in general more elongation. Figure ?? shows this effect with a colony of 300 cells, which is more square-shaped and has a much rougher surface overall.

If one makes the assumption that buds are in fact adhesive, then craggy structures have more inherent support, as cells are able to actively bond to each other rather than simply move around each other’s edges. The result, shown in Fig. 12, is a much more widely spaced colony, with “strings” of

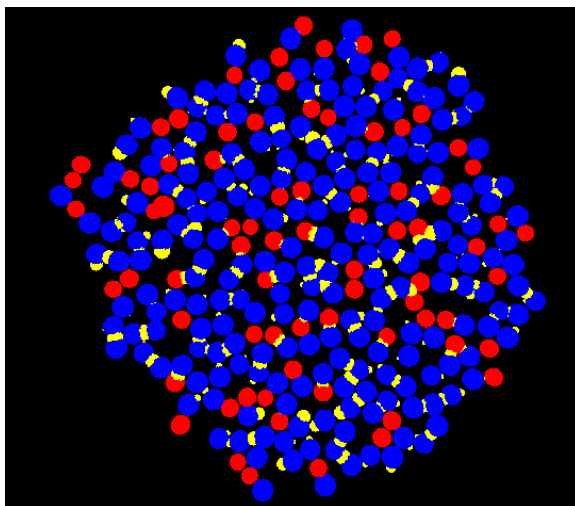


Figure 11: $k_{mitosis} = .75$; $k_{axialFrac} = .5$ “No rotation”

yeast cells growing mostly parallel to each other. However, even in this case, by 300 cells, it is fairly obvious that the eventual colony geometry will be circular if no environmental factor intervenes.

9 Conclusion and Future Work

Perhaps the most interesting find on of this work is that a peak in cragginess occurs for most combinations of parameters somewhere between 100 – 200 cells. After this, the extrusions tend to get pushed toward one another by the expanding colony behind them, leaving the occasional gap, but not displaying the sort of “foraging” behavior observed in certain strands of yeast. Thus, the mechanism driving yeast colony geometry in the wild is more likely to be chemotactic than stochastic.

Alternatively, stable, non-round structures are possible if buds, or some other part of the yeast cell, are themselves adhesive. This tends to cause filaments of various lengths, although in the absence of external stimuli they tend to wrap up on each other and, given enough time, would probably produce as round a structure as any of the other stochastic models presented.

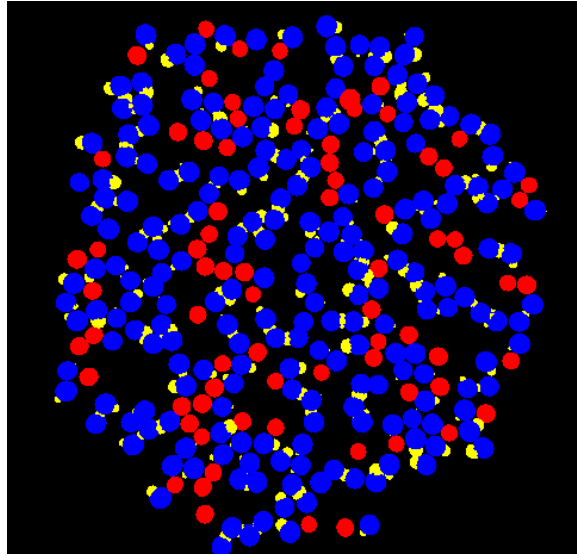


Figure 12: Left: $k_{mitosis} = .75$; $k_{axialFrac} = .5$ “Sticky buds”

At the micro scale, the most fertile ground for further study is the nature of stable, non-round clumps of yeast cells, such as possible binding sites as shown in Fig. 12. Once these are known, it should be possible to explore the mechanisms for colony expansion. Alternatively, it is possible that the growth is entirely chemotactic or phototactic, and individual cells, rather than groups of them, orient their χ vector towards the maximum local gradient of either food or some tracer chemical emitted by their peers, and so the colony simply grows in the right direction.

10 Acknowledge

All the assumptions, the model descriptions and the value of the parameters used in the simulations can be found in the paper *An explicit spatial model of yeast microcolony growth* by H. Jonson and A. Levchenko.

An explicit spatial model of yeast colony growth

Multiscale modeling

Thomas Apker

Jianfeng Zhu

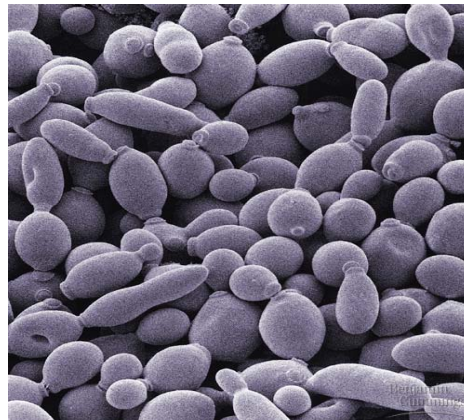
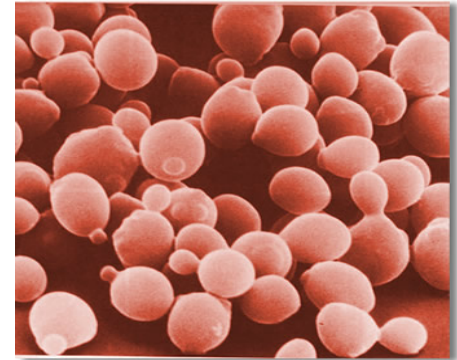
Introductions

- The fungi (singular fungus) are a kingdom of eukaryotic organisms. They digest their food externally, absorbing nutrient molecules into their cells.
- Fungal infections remain an important cause of disease.



Introductions

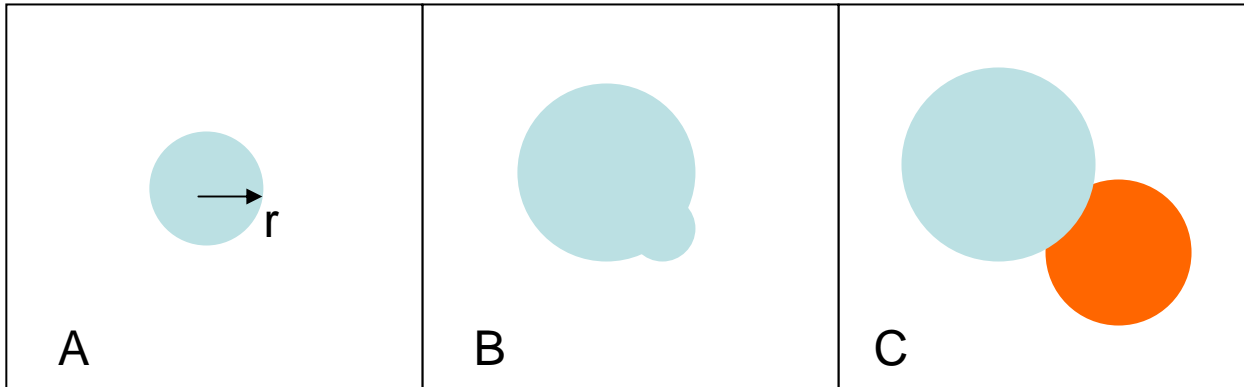
- Yeasts are single-celled (unicellular) fungi.



Different scale modeling

- The scale of the whole colony: growth-front instabilities.
- The scale of large subparts of a colony: structures as “spokes” and “cables”.
- The scale of smaller subparts of a colony: granular structures.
- The scale of single cells.

Cell growth and division

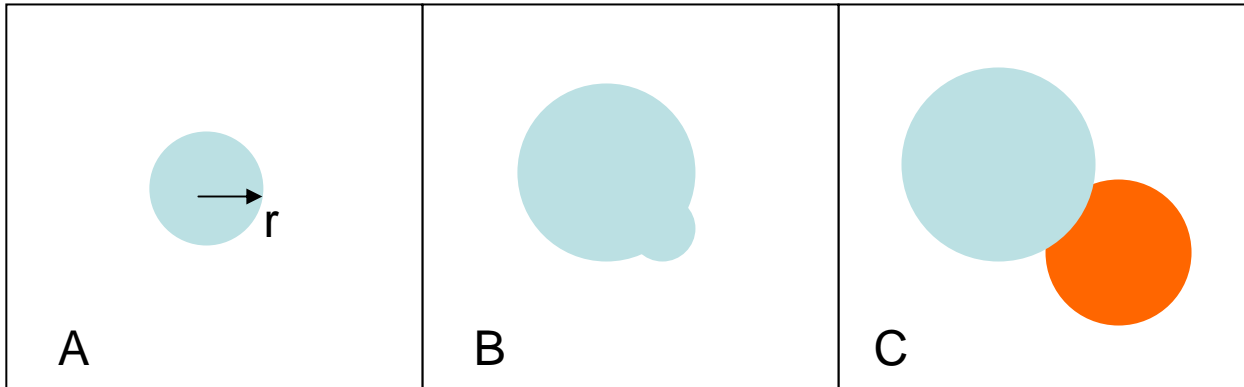


A: Cell growth

where

Growth inhibition by neighbors

Cell growth and division



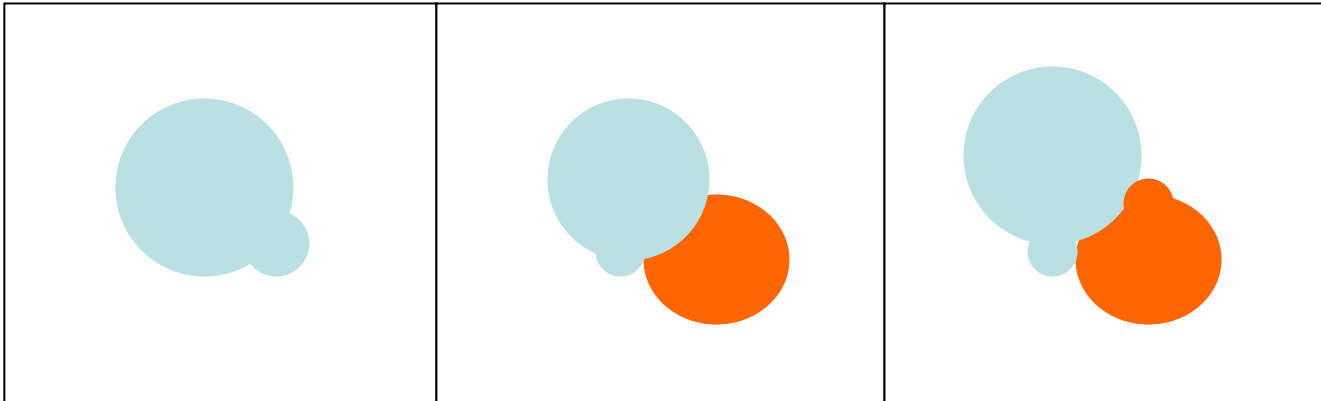
B: A new bud begins to form (G1 check)

and no other bud present in the cell

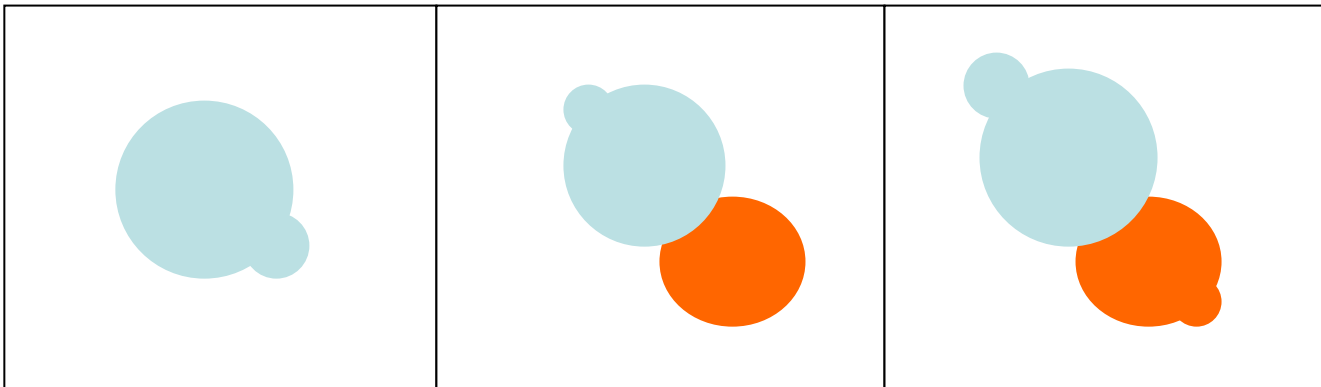
C: Mother and daughter cell separate (mitosis check)

Cell-division polarity

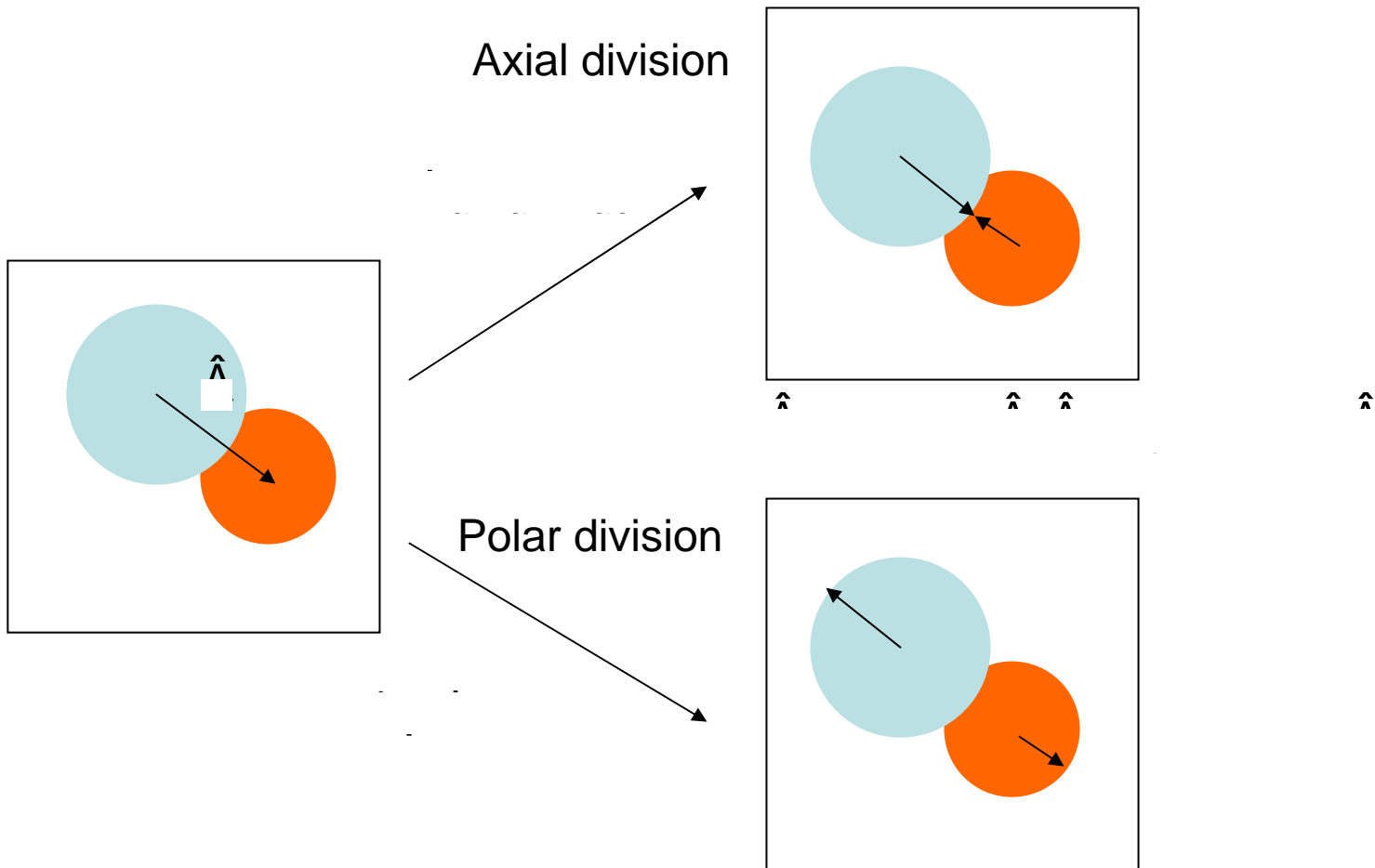
Axial cell division



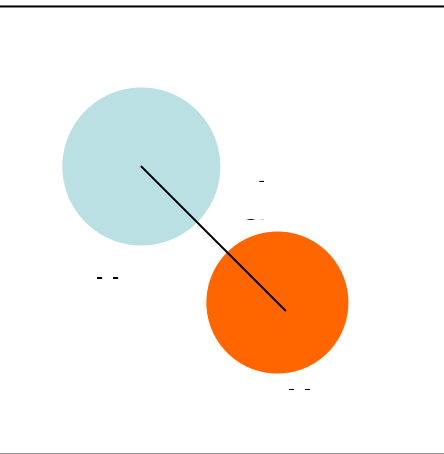
Polar cell division



Cell-division polarity



Cell-cell interaction



Different adhesion strength:

Model Implementation

Our Simulation

Buds are part of the mother cell

- We grow more cells

fixed time-step

K4

mitosis handled at the end of each

time-step

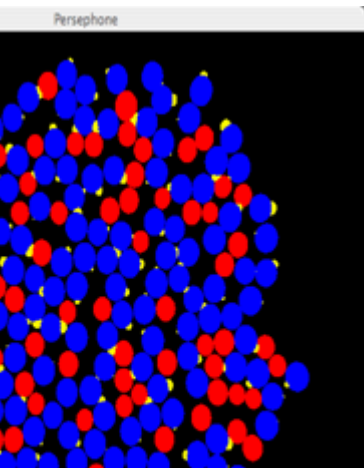
Jonsson and Levchenko

- Buds are independent cells with special rules
 - Buds count towards cell population
- Adaptive time-step RK

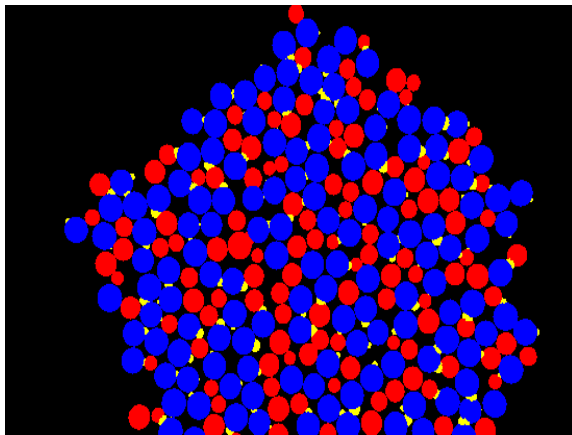
Simulation Results

Small mitosis size leads to less stable structures
No significant effect of axial vs. polar division

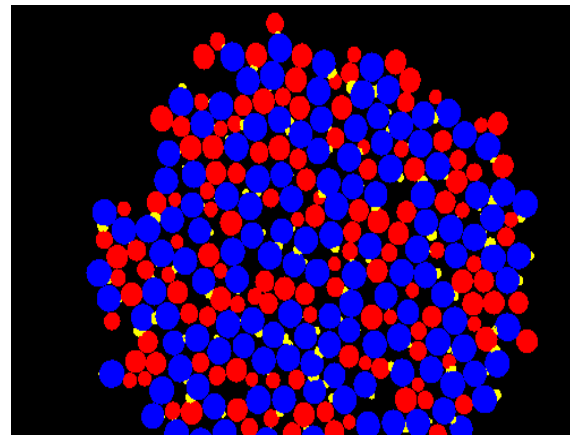
axial fraction= .25



Axial fraction= .5



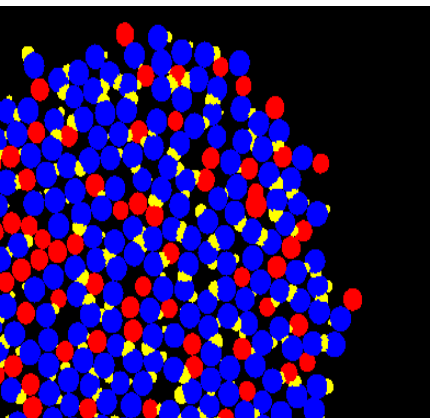
Axial fraction= .75



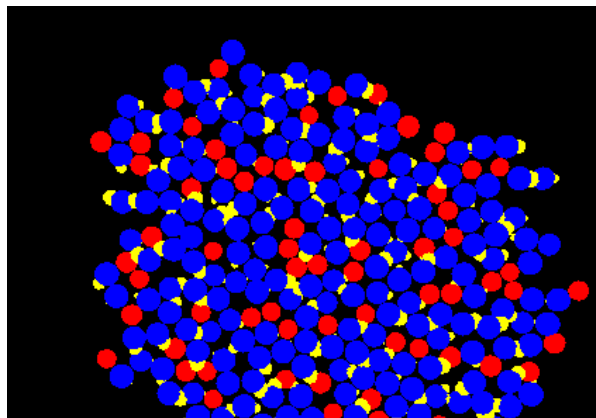
Simulation Results (2)

Large cell size at mitosis means larger structures
Effect of cell division geometry is more obvious, if
counterintuitive
Largest protrusion roughly 3-4 cell diameters

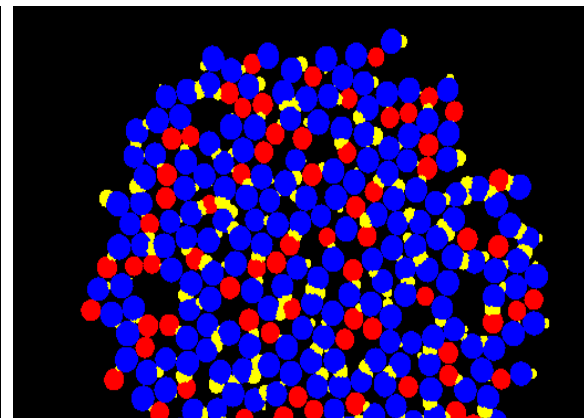
Axial fraction= .25



Axial fraction= .5



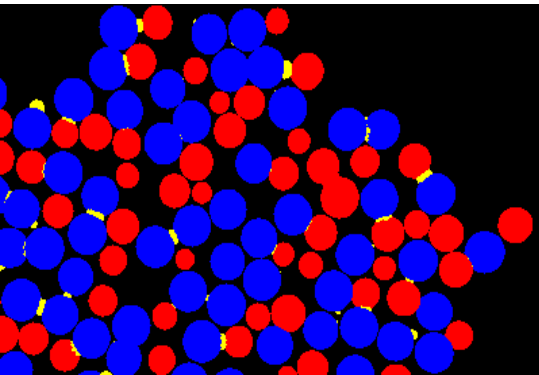
Axial fraction= .75



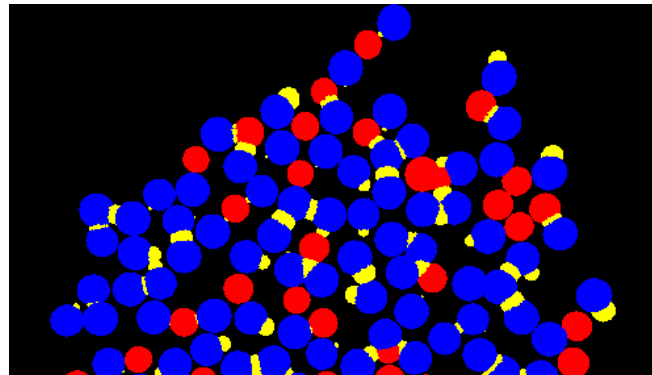
Simulation Results (3)

As noted above, we effectively ran our simulation longer
at Jonsson and Levchanko
When run to 150 cells (roughly 300 cells and buds),
structures are more prominent

Small mitosis size



Large mitosis size



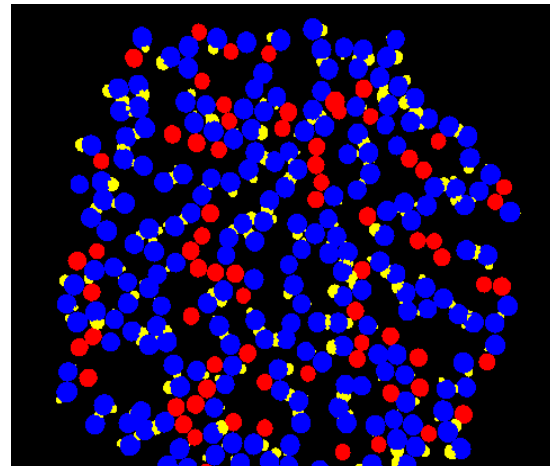
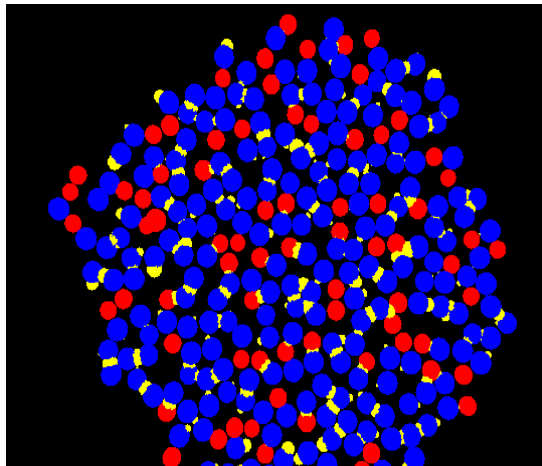
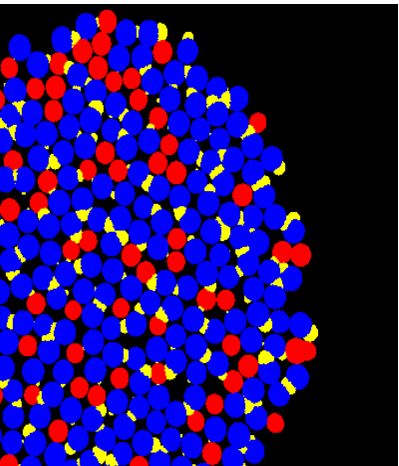
Simulation Results (4)

bud rules important if mitosis size is large

“soft” buds eliminate all structure

non-rotating cells mean large crags

“sticky” buds result in stringy colonies



Conclusions

Stability of non-round shapes depends on
limiting cells' ability to move other cells

- "Harder" buds, large cells at mitosis, less
rotation

No repulsive rules allow structures larger
than three or four cell diameters

Attractive sites create stringy structures

- In long time, fronts still become circular

Acknowledgement and reference

All the assumptions and model descriptions can be found in the paper:

.Jonsson and A Levchenko, *An explicit spatial model of yeast microcolony growth*, 2005.