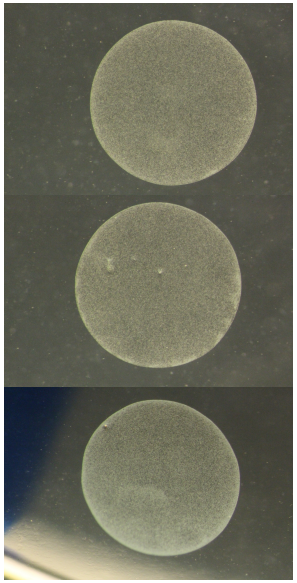
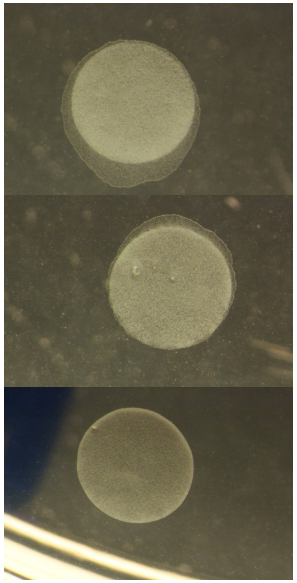
Compare red, blue, green, warm white LEDs with 1x and 0.5x cell density

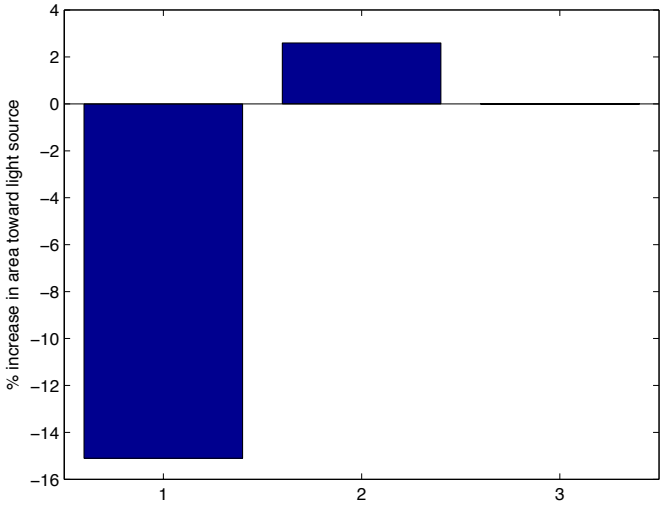
6 Oct
OD of culture: 2.732
2nd timestep is 17 hrs after inoculation
***Expt done on Devaki's old agarose plates

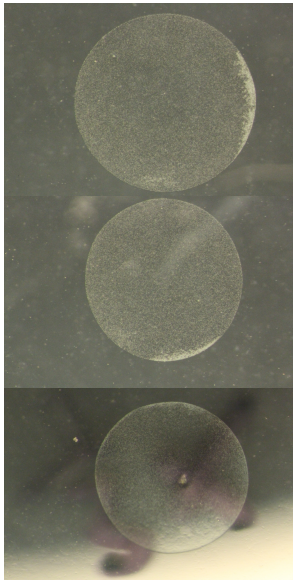
**did not take intensity readings for this expt. Estimated intensities taken in Nov:
(detector closest to LED ; detector furthest from LED)
Red 60 ; 10
Blue 380 ; 90
Green 140 ; 30
Warm white 220 ; 50
**** seriously estimates because I didn't note down the resistance in series with LED. These measurements were taken using a resistor of 330ohms

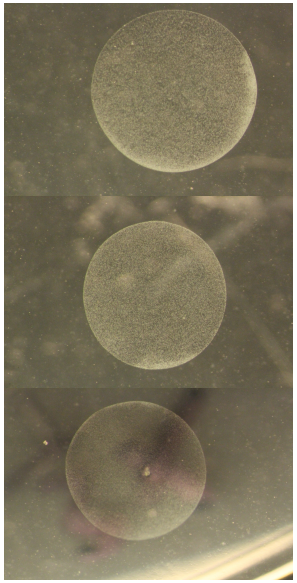
Blue



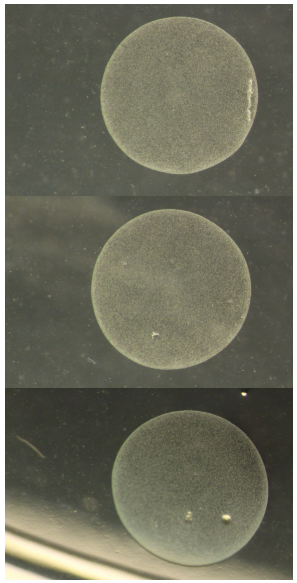


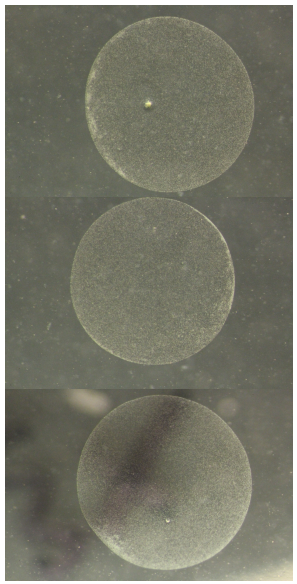


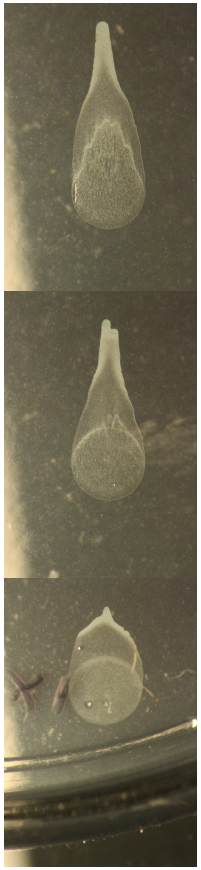


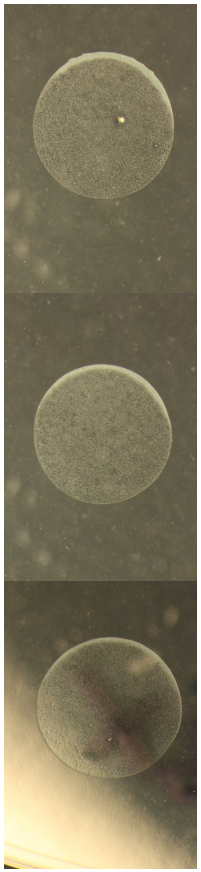


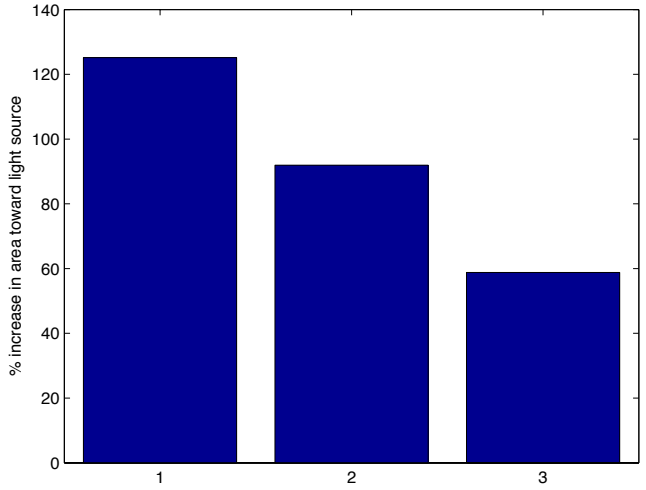
Green



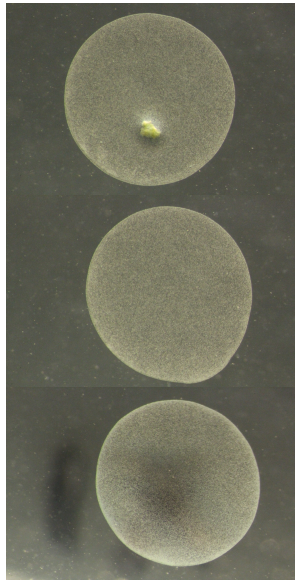


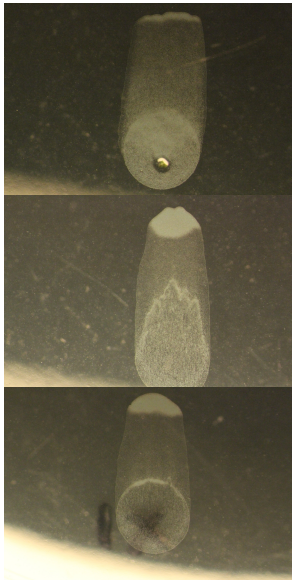


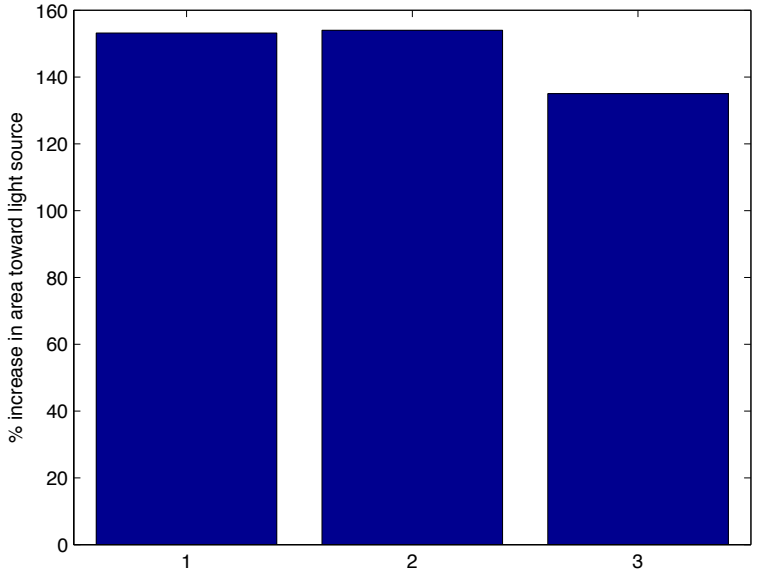


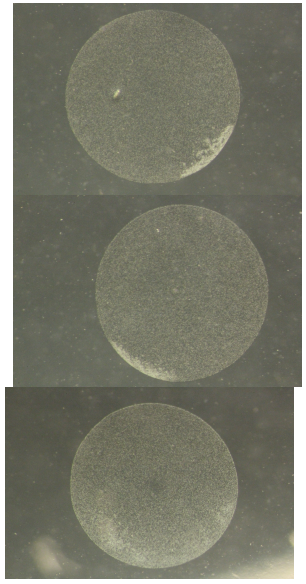

% increase in area toward light source
140
120
100
80
60
40
20
0
1
2
3

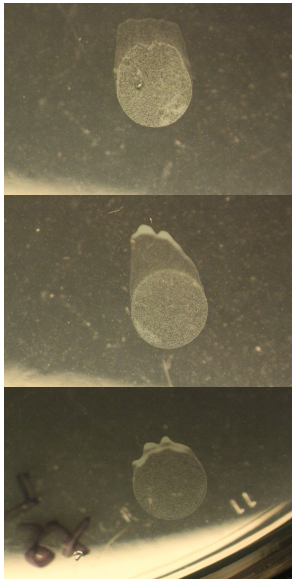
Red

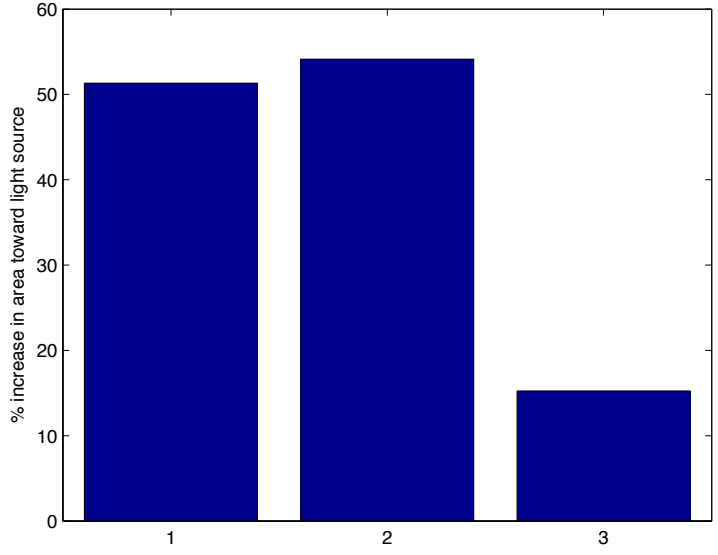




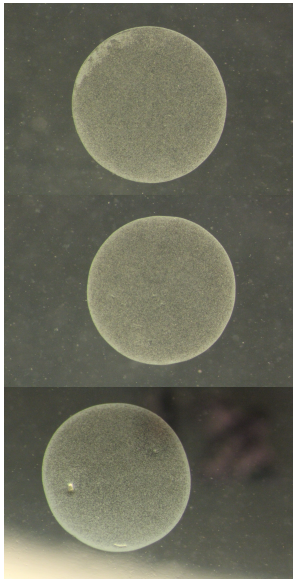

% increase in area toward light source
160
140
120
100
80
60
40
20
0
1
2
3

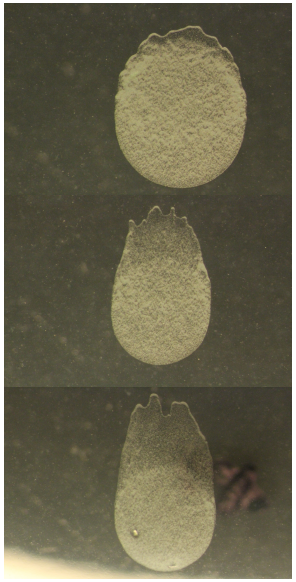


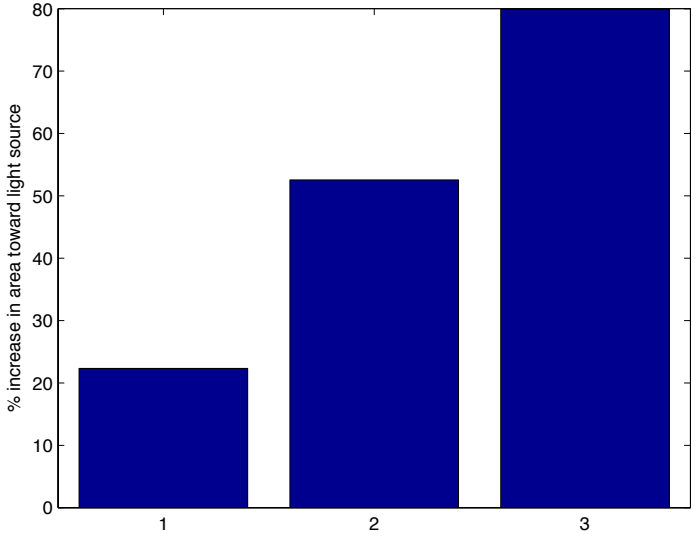


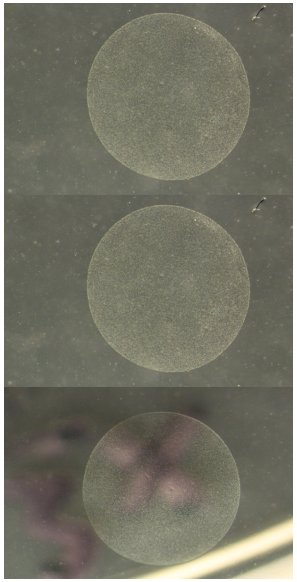

% increase in area toward light source
60
50
40
30
20
10
0
1
2
3

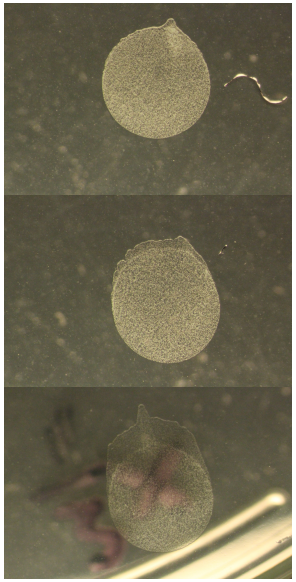
Warm white

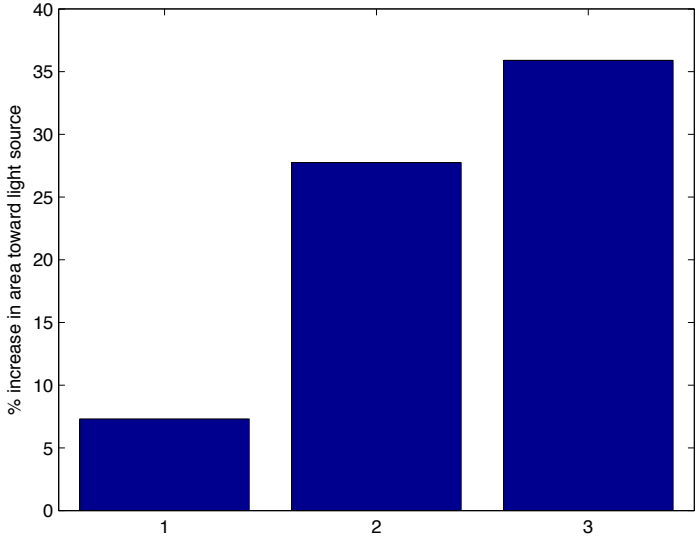





% increase in area toward light source
0
10
20
30
40
50
60
70
80
1
2
3






% increase in area toward light source
0
5
10
15
20
25
30
35
40
1
2
3

10 Oct comparing different colored LEDs

OD 2.064

Light coming from above

Each set of 3 rows are repeats of the same condition.

Timesteps are: 0, 18, 43, 66 hrs

1 LED used for each condition, tried to match intensities by changing resistance in series to LED (umol photons/m^2s:

SuperbrightLED red 660nm 150

SuperbrightLED blue 470nm 190

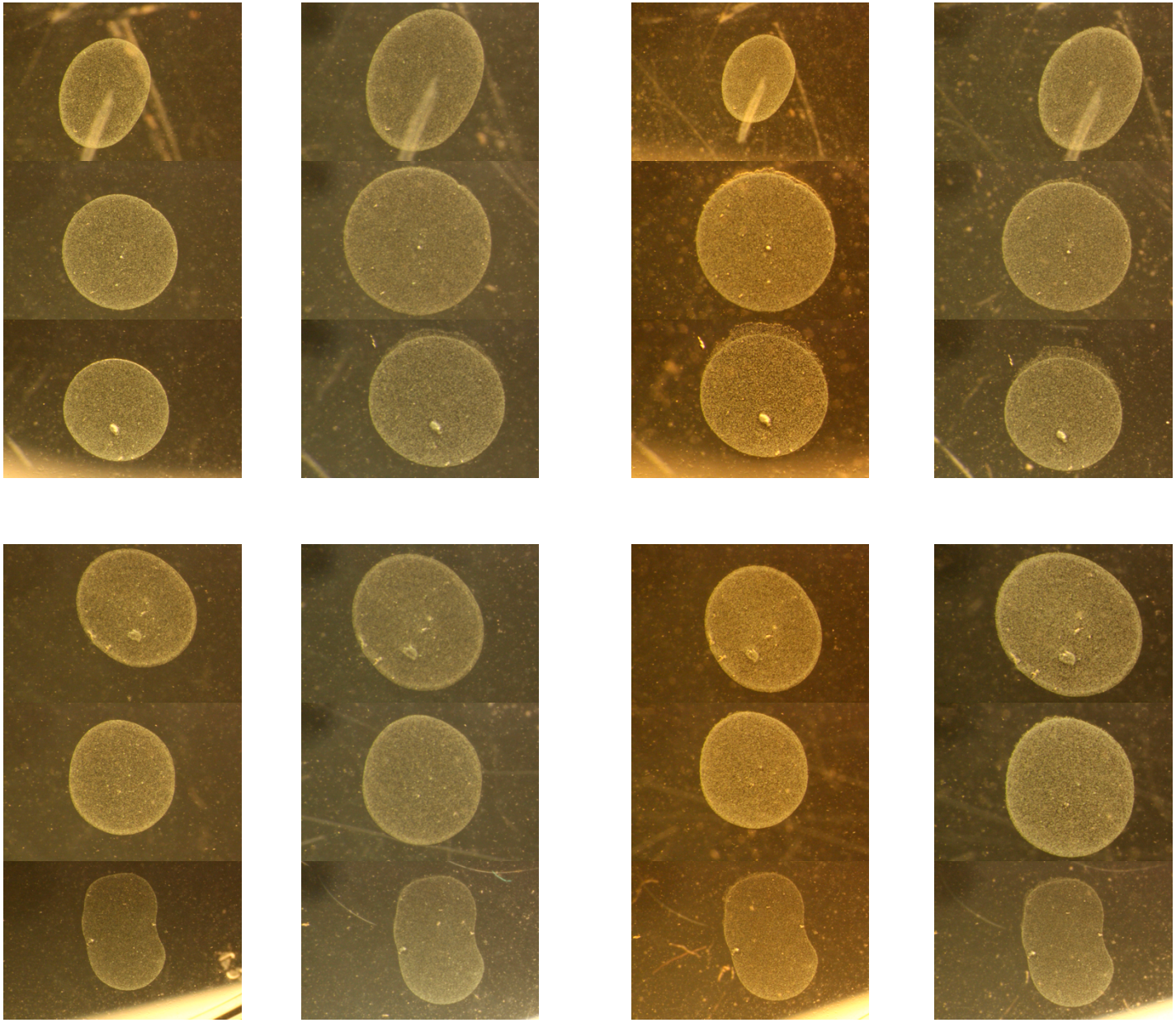
SuperbrightLED green 525nm 140

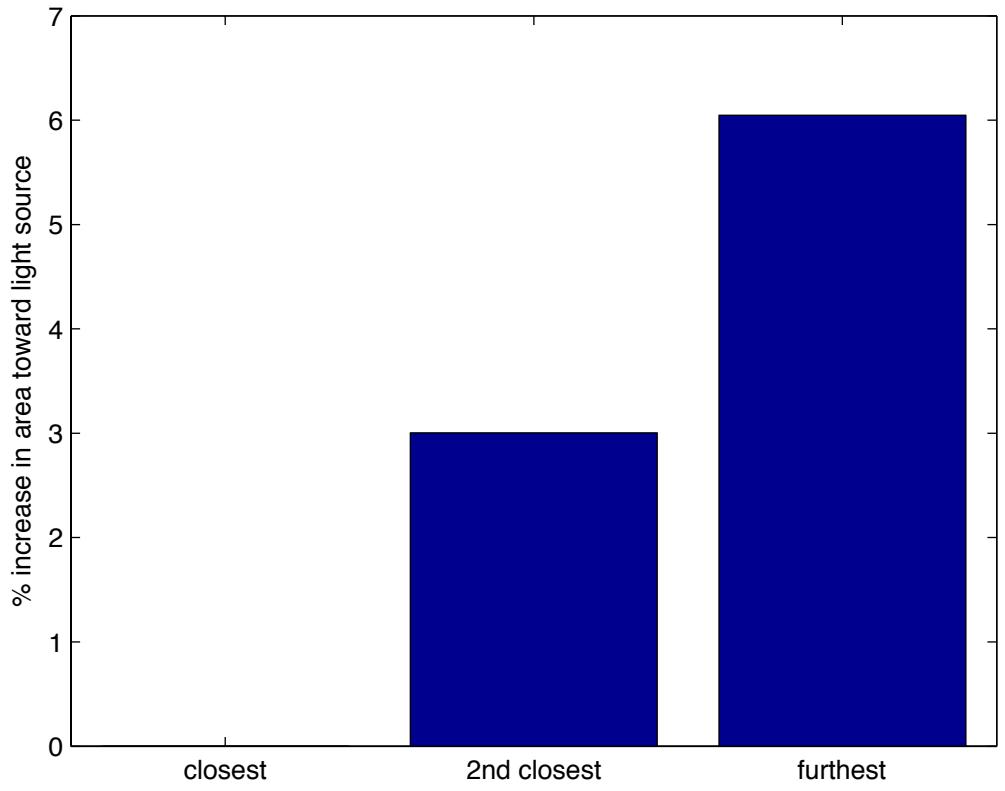
Roithner Green 150

Roithner Blue 200

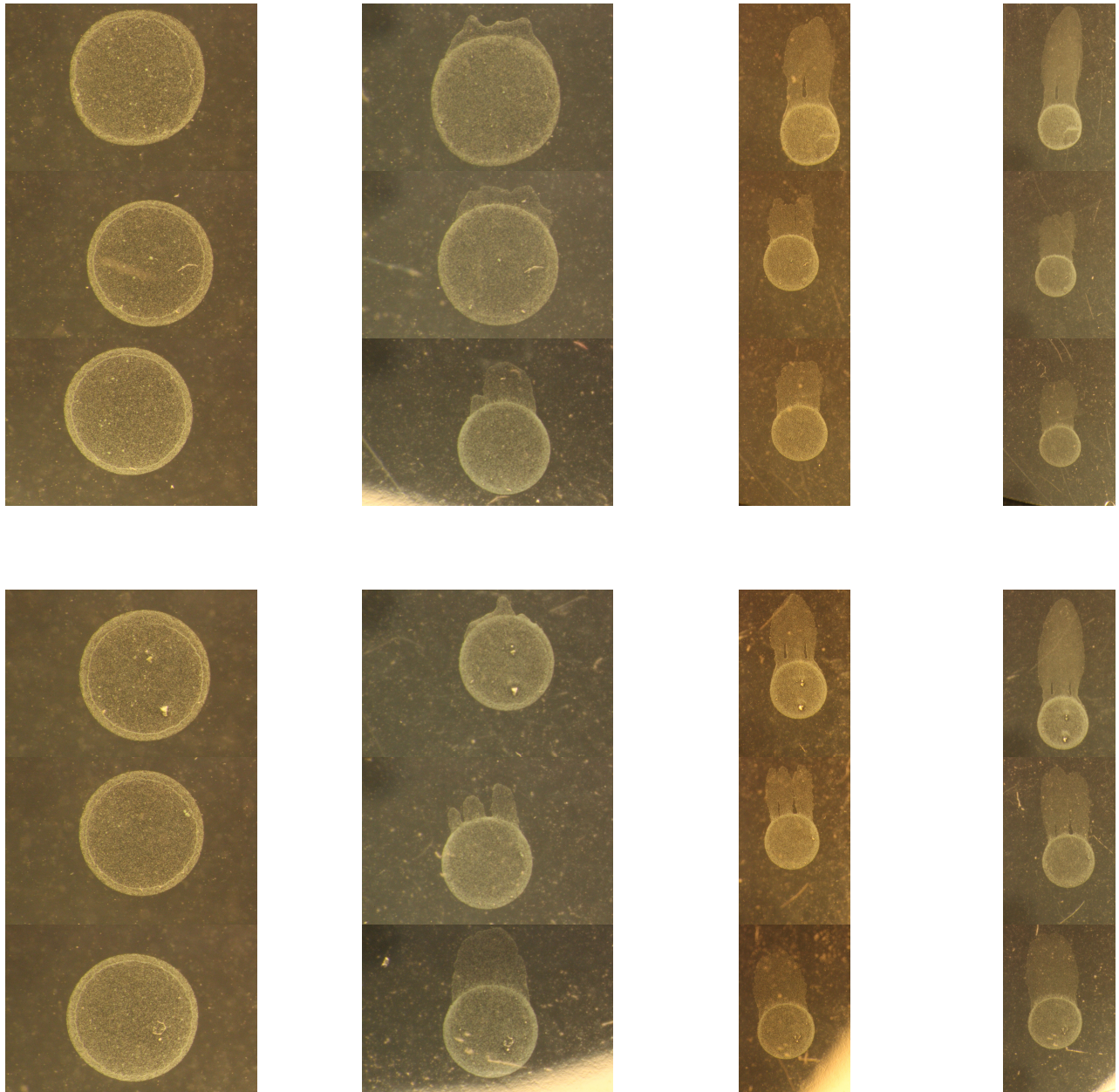
***Expt done on Devaki's old agarose plates. Note that intensity might not correspond to what is really incident on the cells, due to lightbox design (plastic partially blocks light)

SuperbrightLEDs blue (470nm)

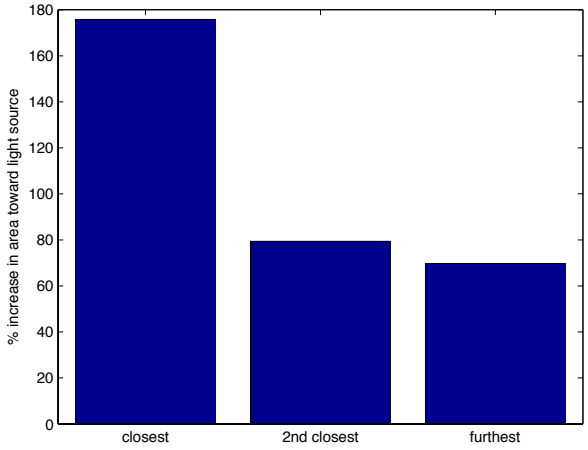



% increase in area toward light source
0
1
2
3
4
5
6
7
closest
2nd closest
furthest

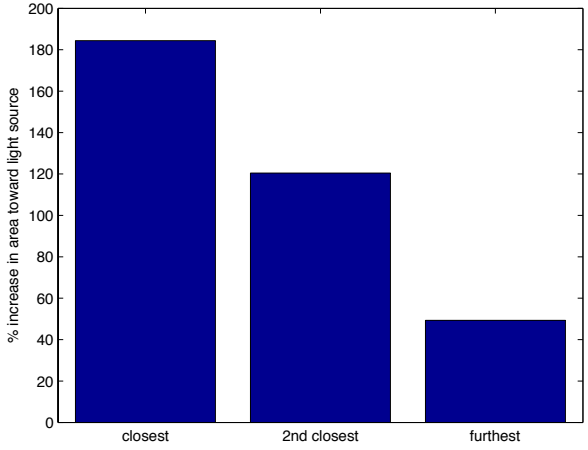
SuperbrightLEDs Green (525nm)

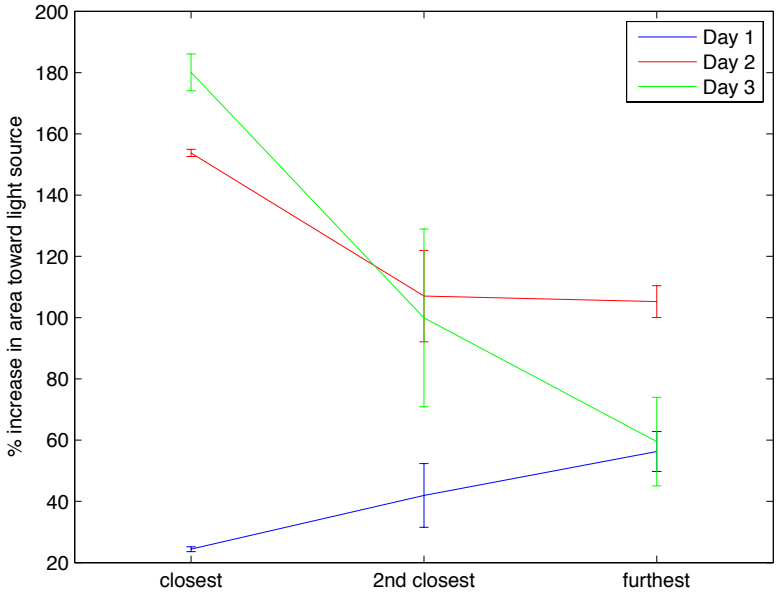


Repeat 1

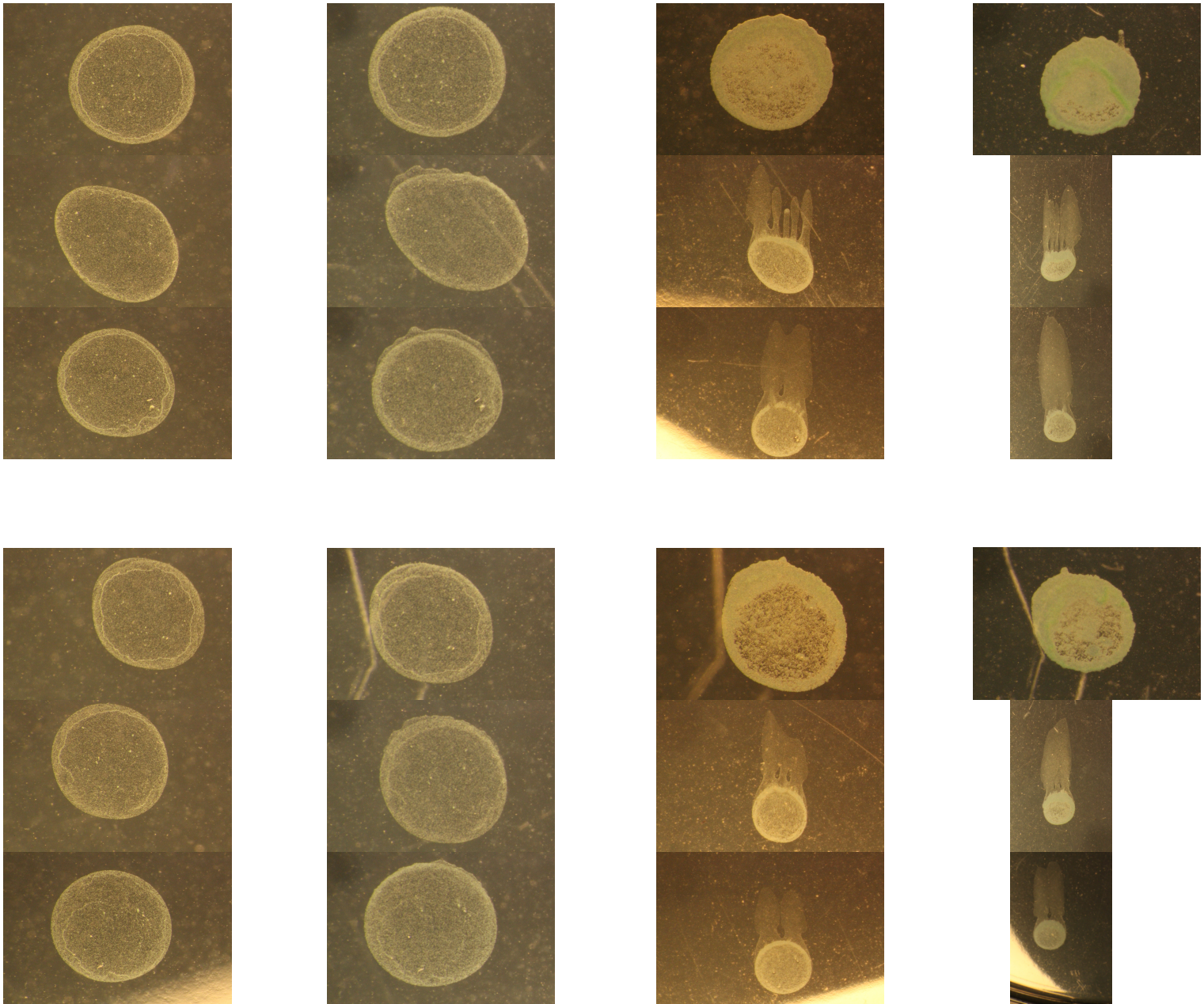


Repeat 2

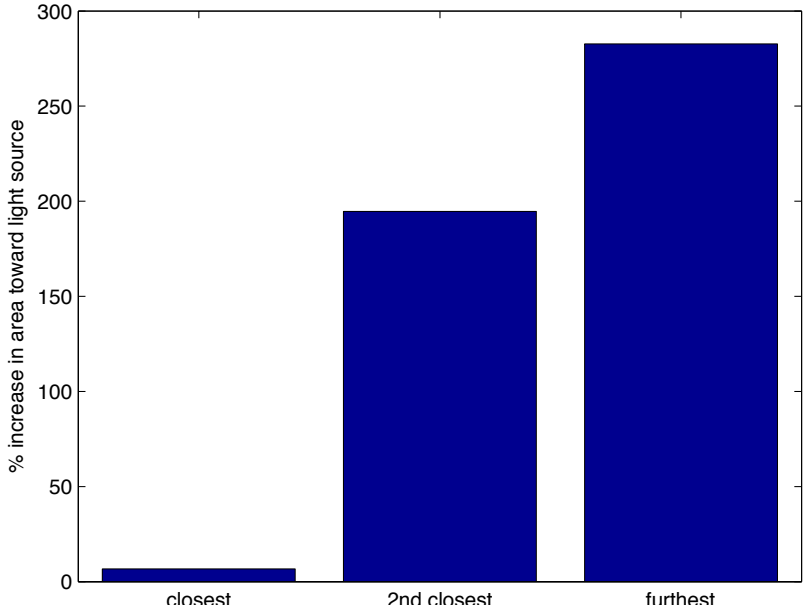




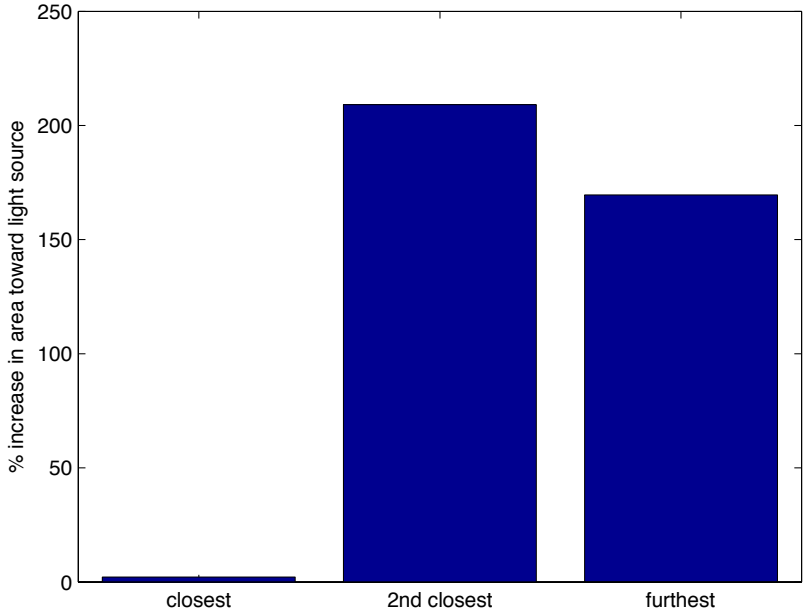
SuperbrightLEDs Red (660nm)



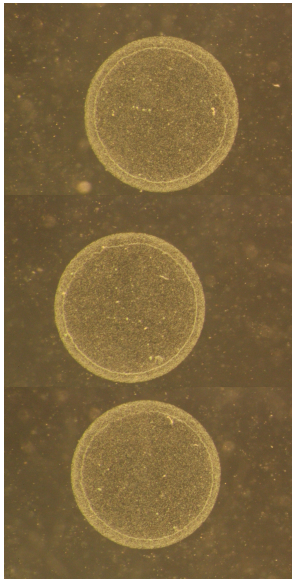
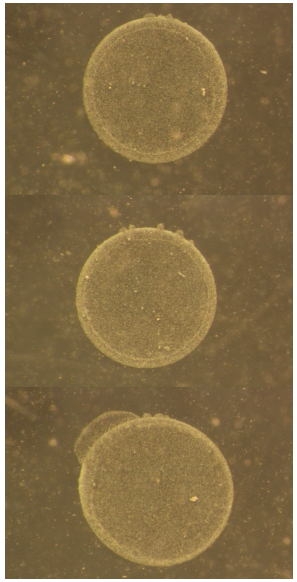
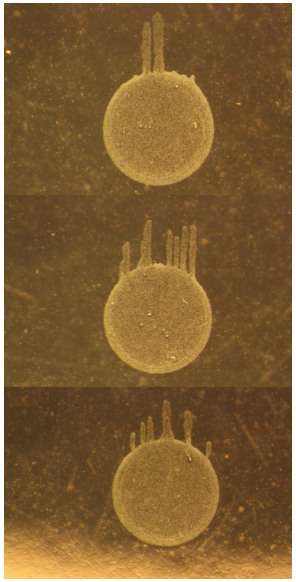
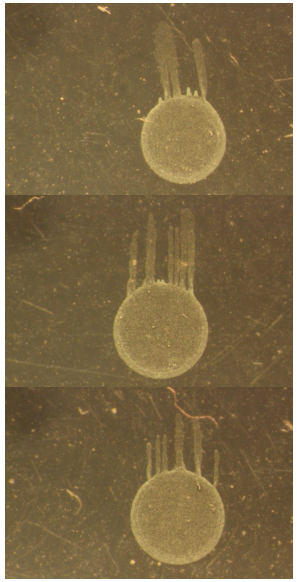
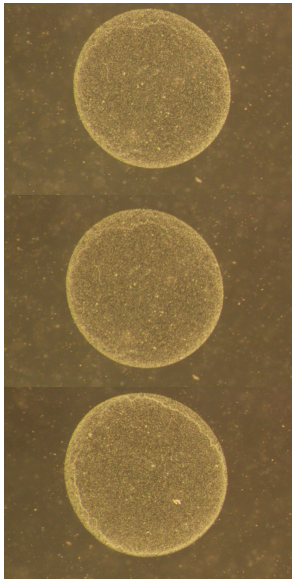
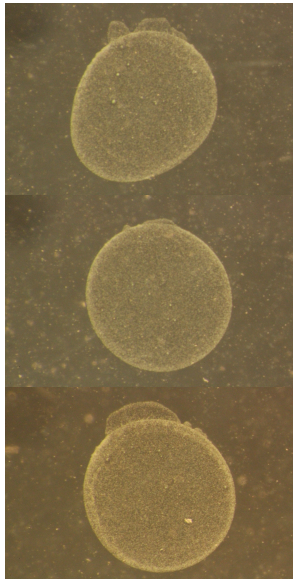
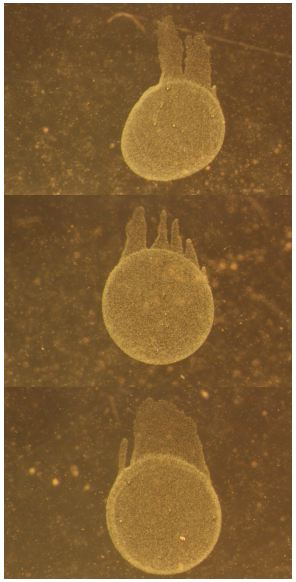
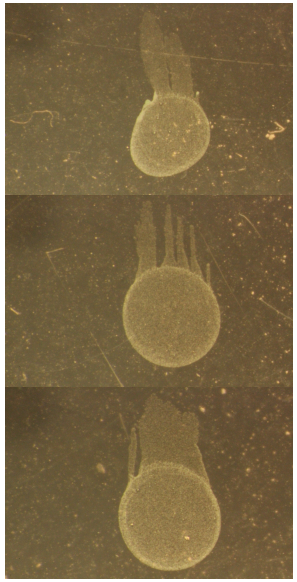
Repeat 1



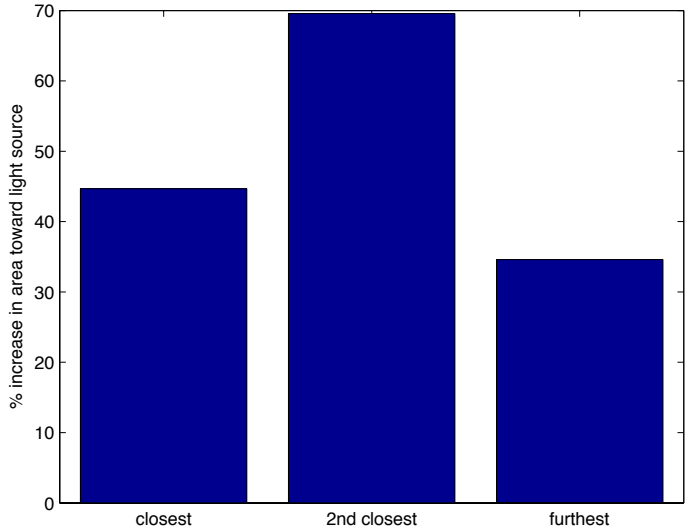
Repeat 2



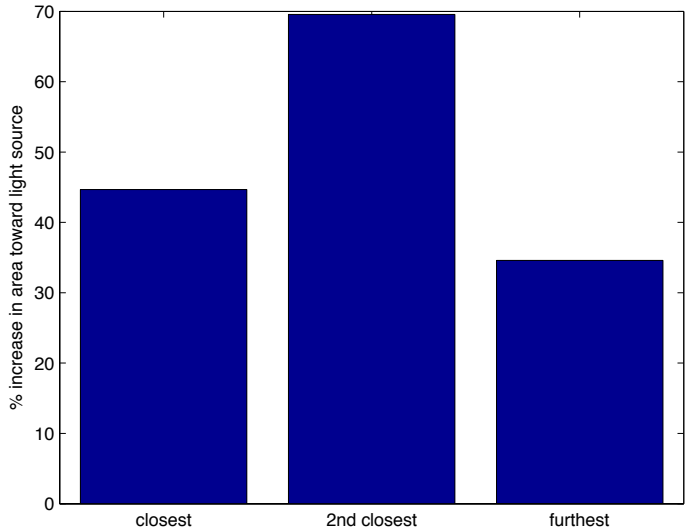
Roithner Green (535nm)

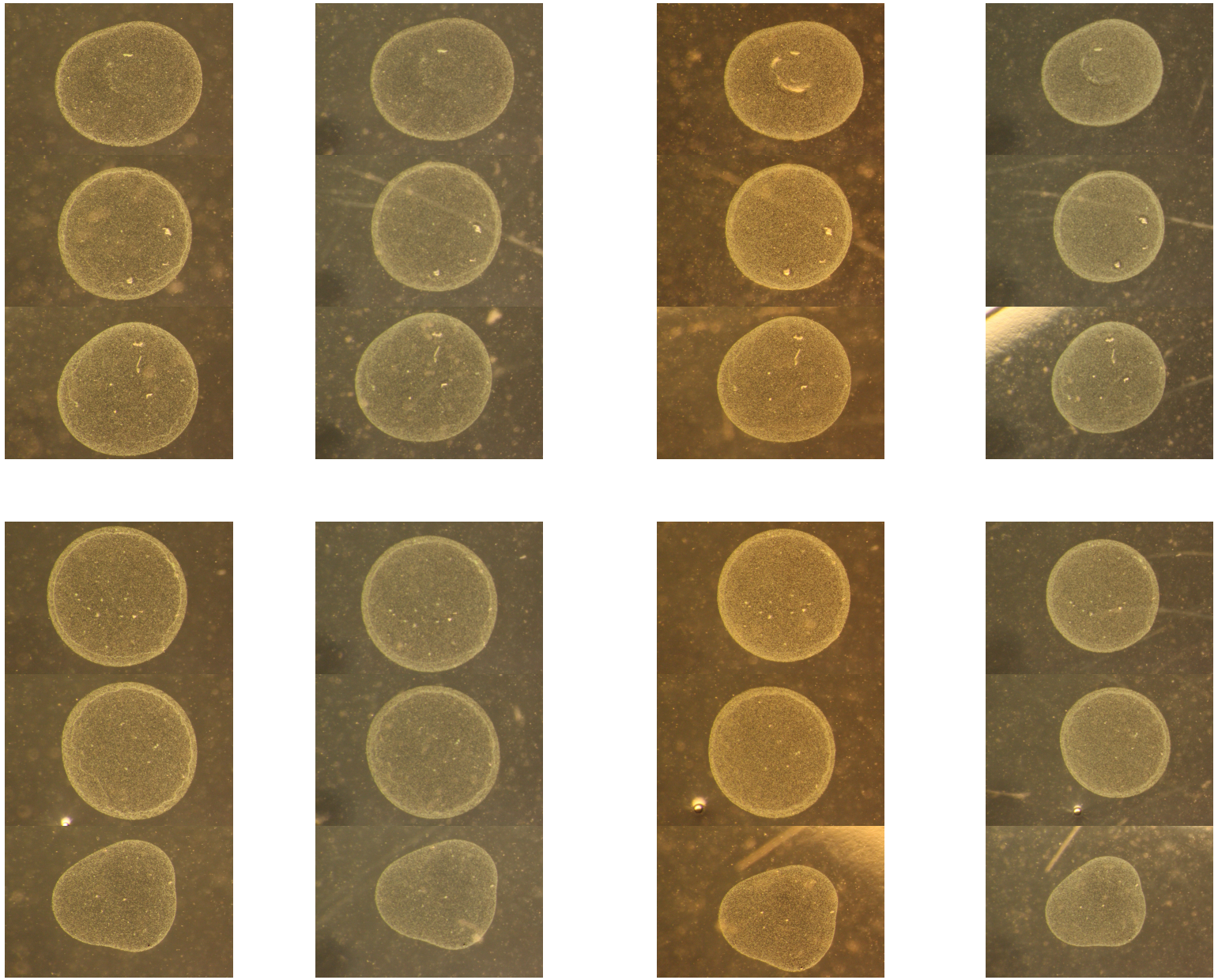
Repeat 1



Repeat 2



Roithner Blue (435nm)
no movement observed

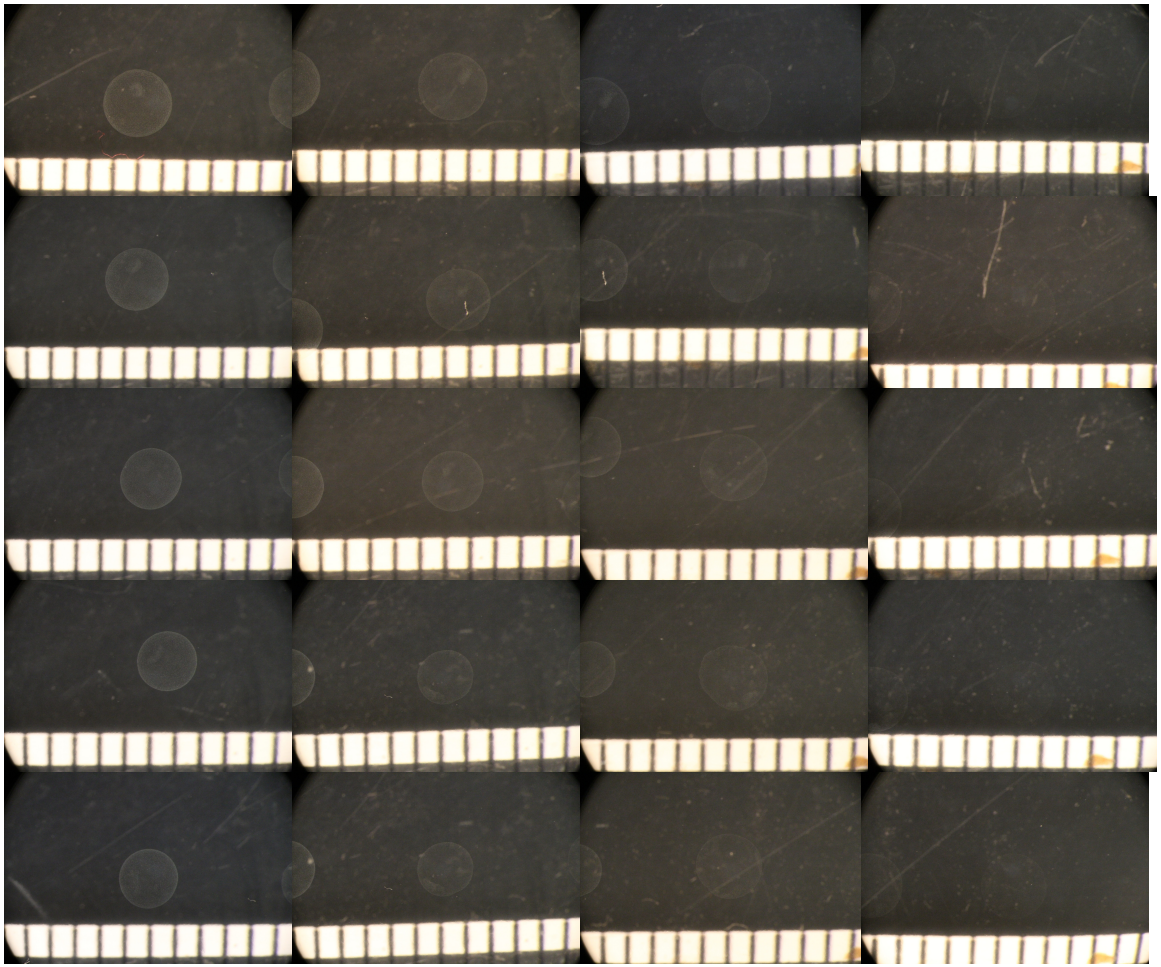


- This experiment did not yield the same results as the 6 Oct experiment, for red and blue
- No backward movement in blue for the closer drops, possibly because the intensity was not high enough, but one can predict that if I used a slightly higher intensity than 190 umolphotons/m^2s, I can start getting photophobic behavior
- Can start observing retardation of movement at higher intensities of red, while for the 6 Oct experiment, cells were more motile at the higher intensity (~60 umolphotons/m^2s).
- For all wavelengths, I can expect to see a curve, along decreasing light intensity, where cells go from being photophobic to increasingly attracted to light and then to be less attracted

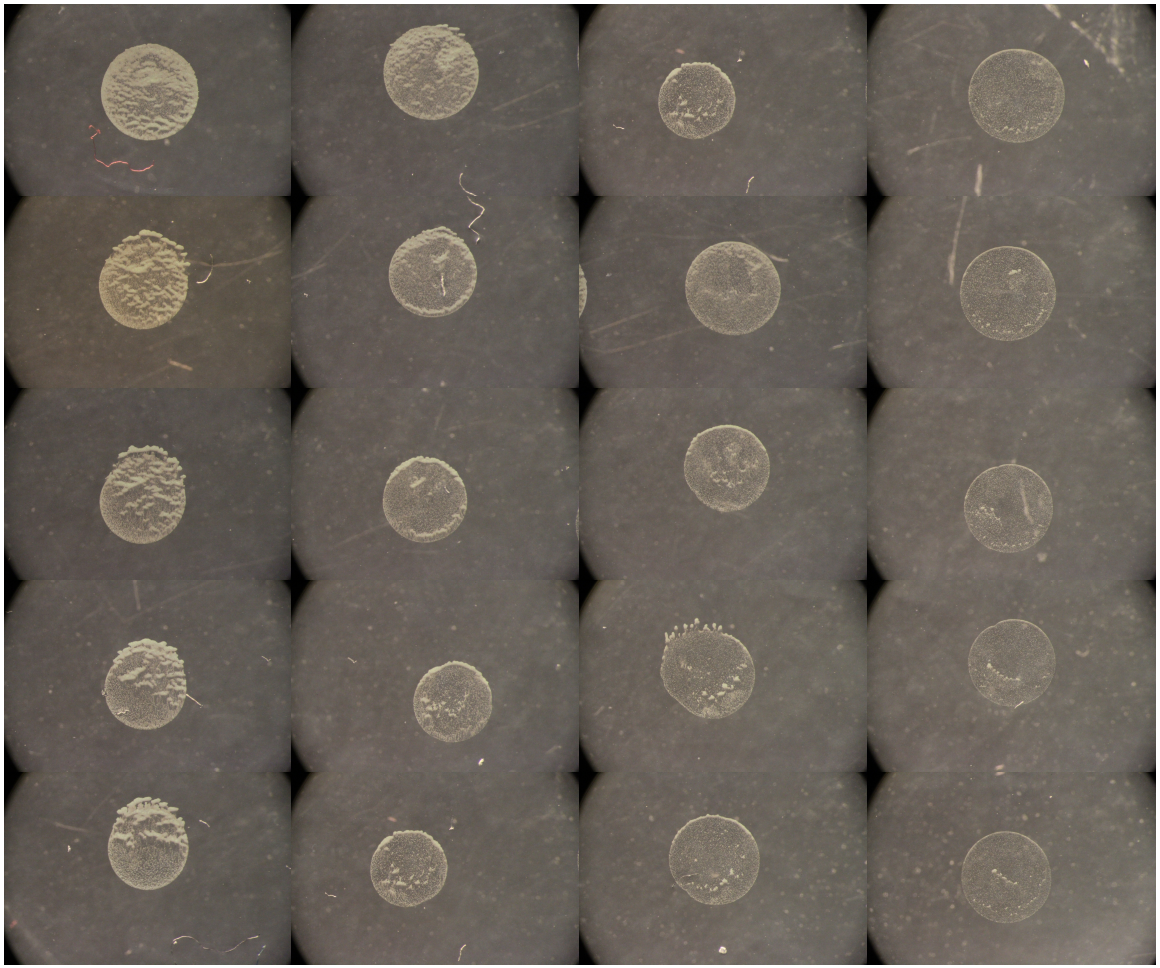
- ** will note down resistance and intensities for all experiments in future

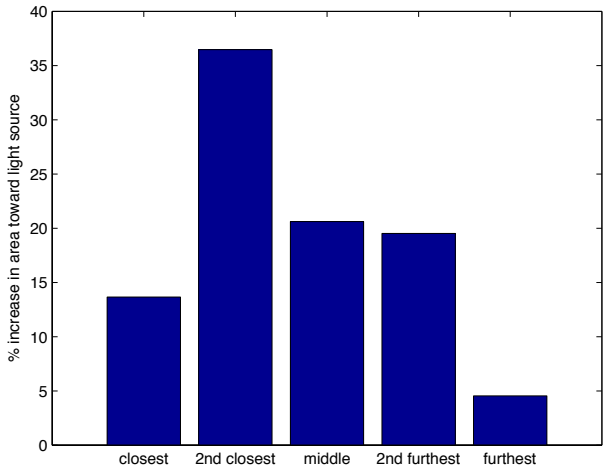
15 Nov (OD 1.632), 4 dilutions (1x,0.5x,0.25x,0.125x), 5 distances away from light. Done with new lightbox, where there is no obstacle between LED and plate.

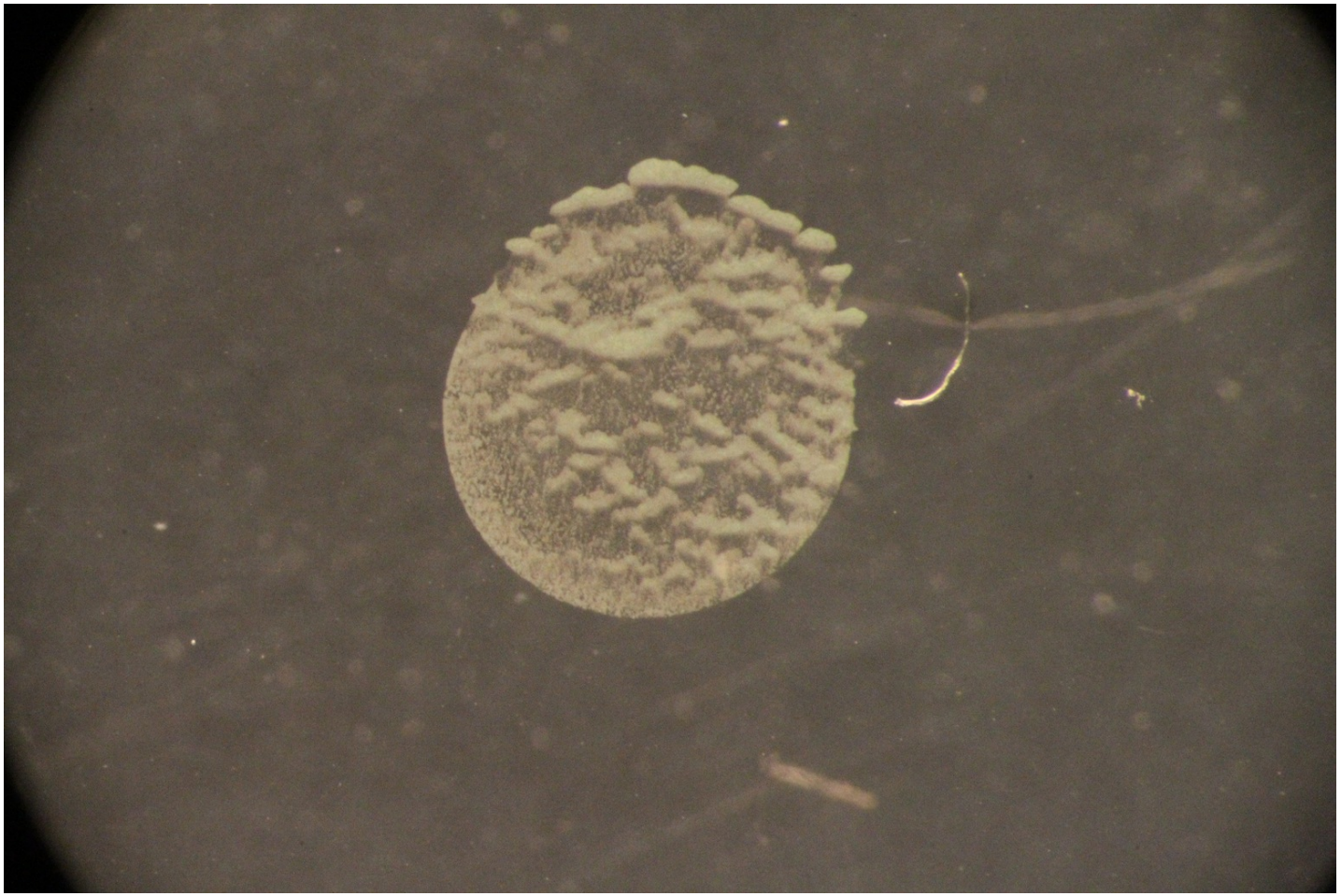
0 hrs



120 hrs




% increase in area toward light source
0
5
10
15
20
25
30
35
40
closest
2nd closest
middle
2nd furthest
furthest



48 hrs before visible fingerbumps formed. Fingers might have taken much longer to form because I used cells that were not in mid-exponential phase (OD 1.632, close to stationary phase which sets in around OD2. I used this density because this was originally a test to make sure I could get fingering behavior with a more dense culture.)

96 hrs before finger formation is observed, and multiple fingering fronts can be seen. Supports the hypothesis that slime is degraded over time, and the age of slime is important for whether cells can follow the “slime trail” or not.

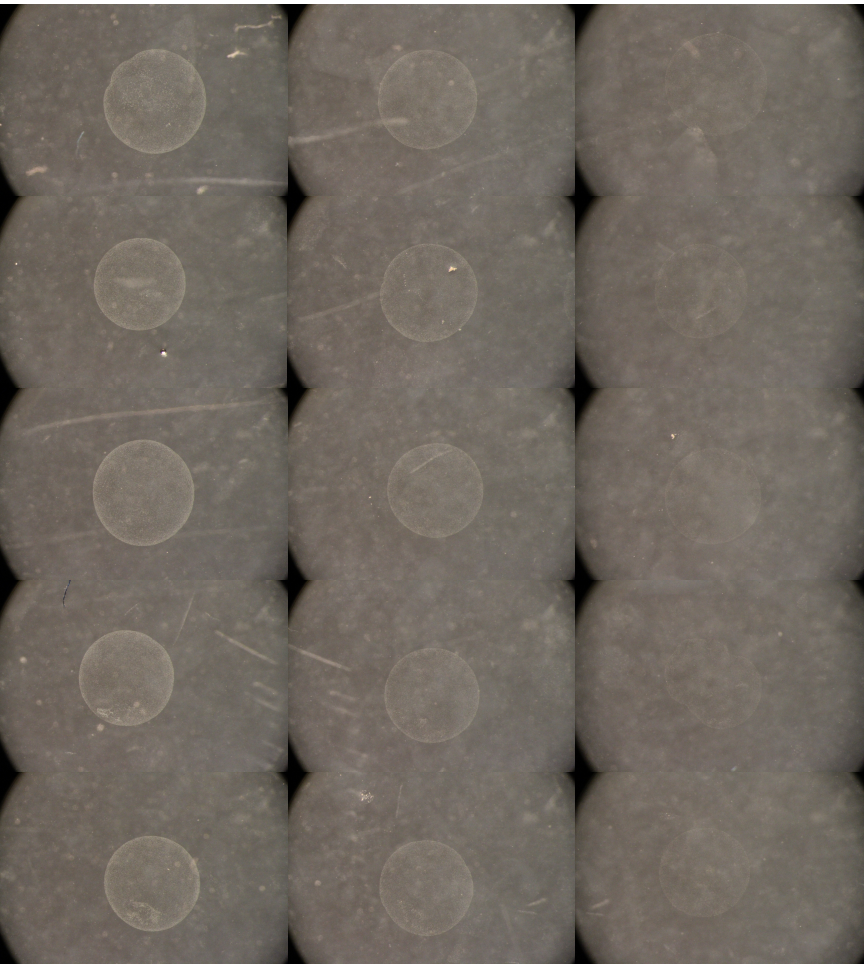
20Nov

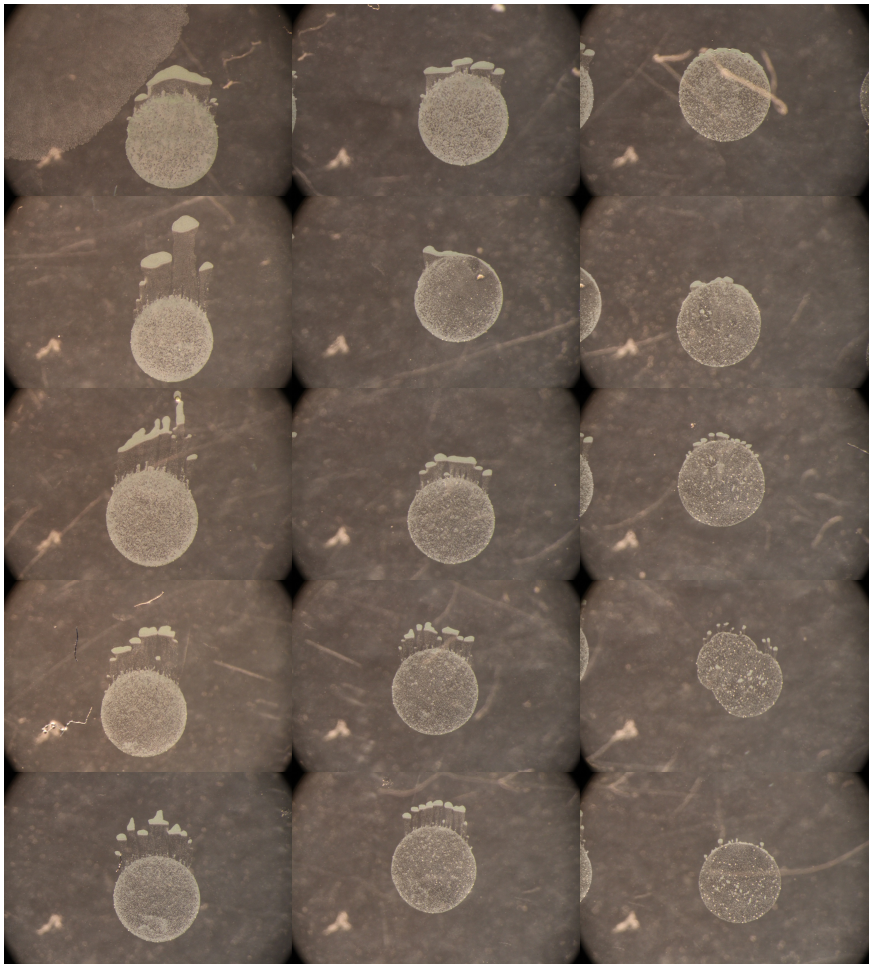
- OD of cells:1.02
- 3 dilutions: 1, 0.5x, 0.25x
- 5 distances away from light, with intensities [26.7; 22.6; 18.4; 14.2; 10];

OD of cells: 1.020

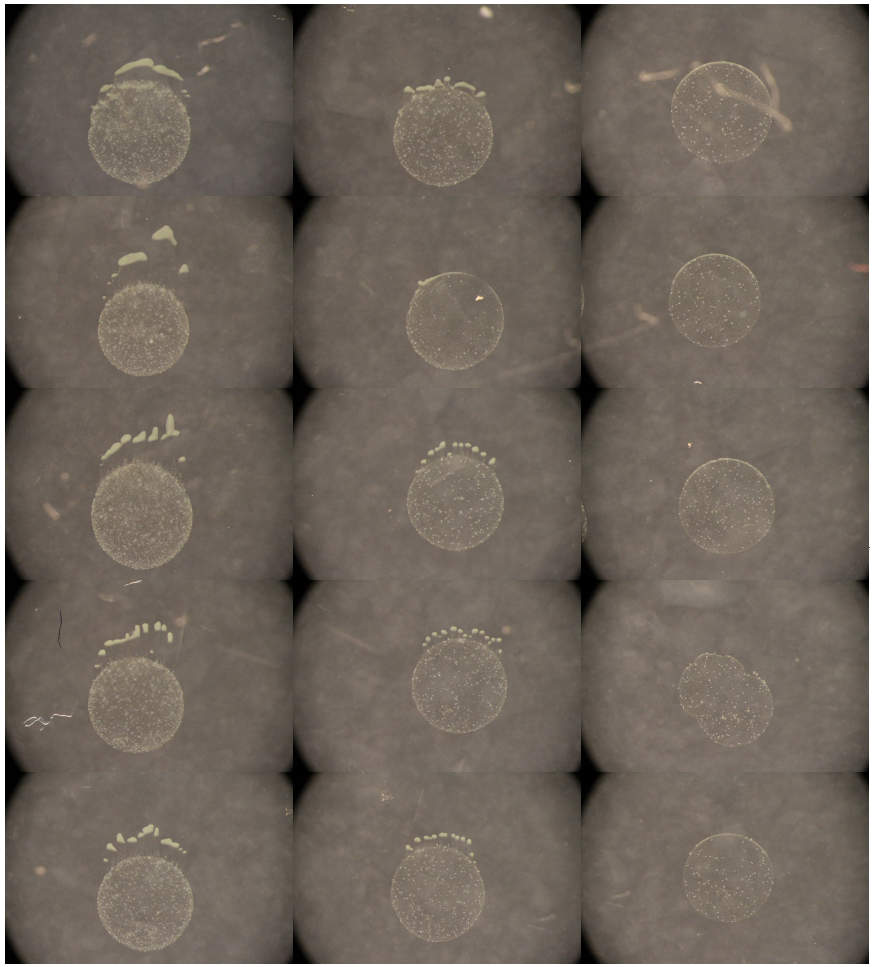
After 92 hrs

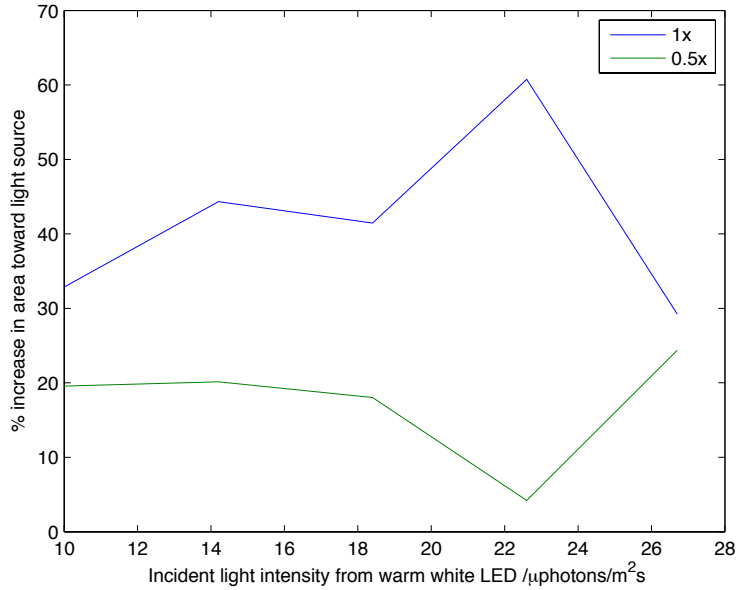
1x 0.5x 0.25x

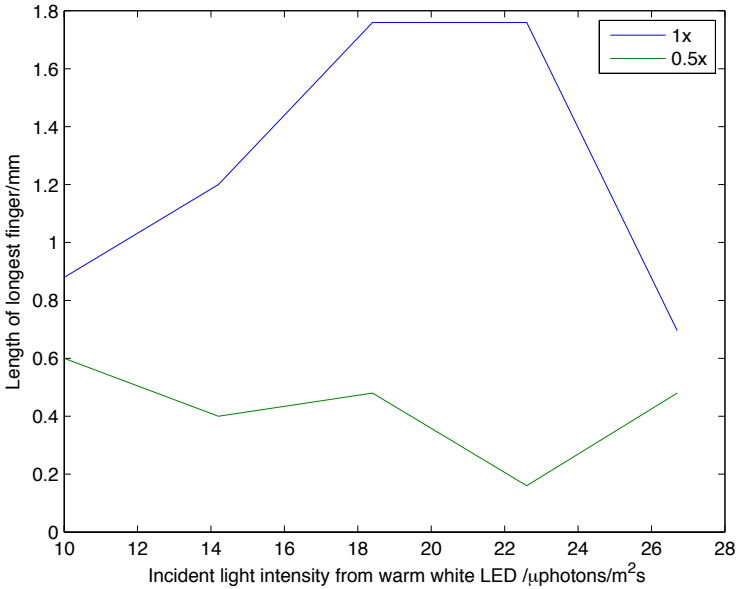




After 70 hrs

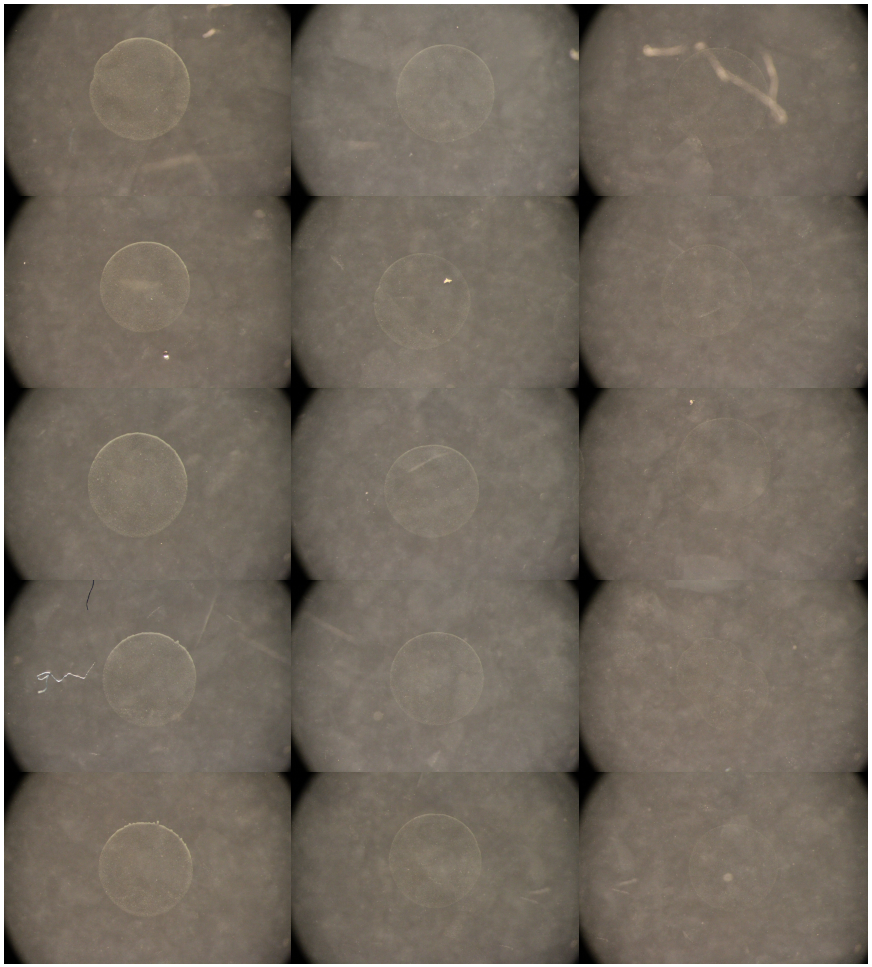




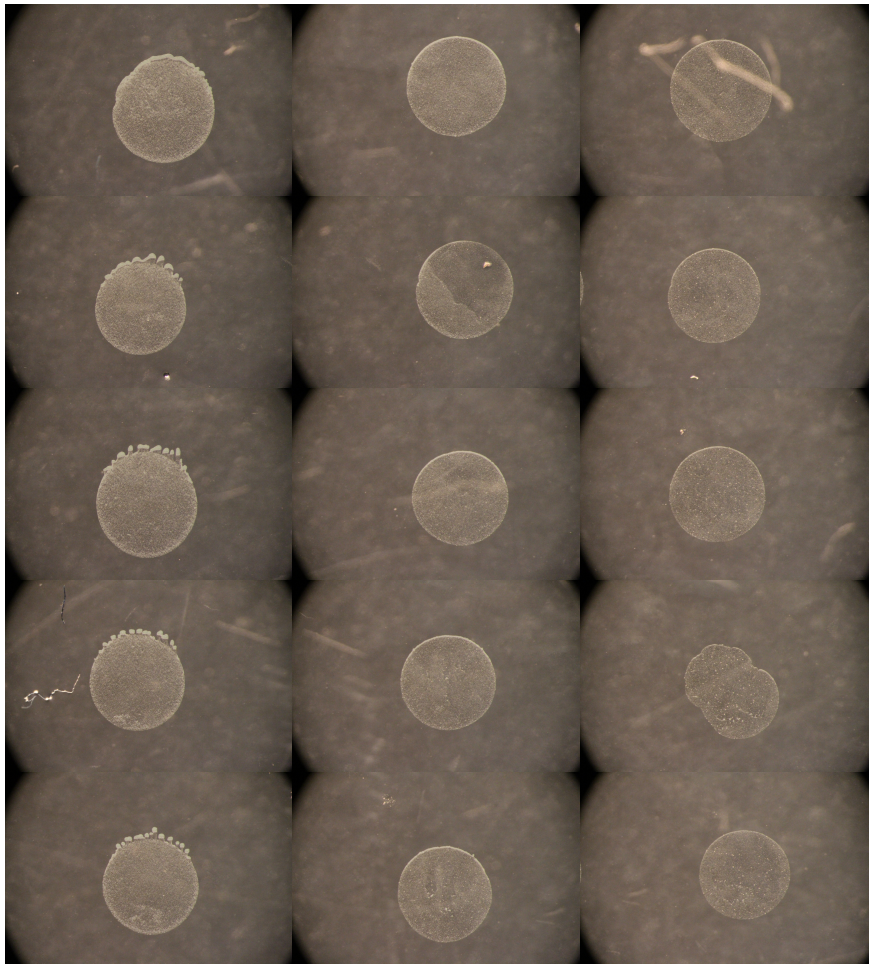


There seems to be a “sweet spot” for maximal finger formation, at ~22.6 umol photons/m^2s for the 1x inoculations. More area is “invaded” by the cells, and the length of the longest finger is also longest.

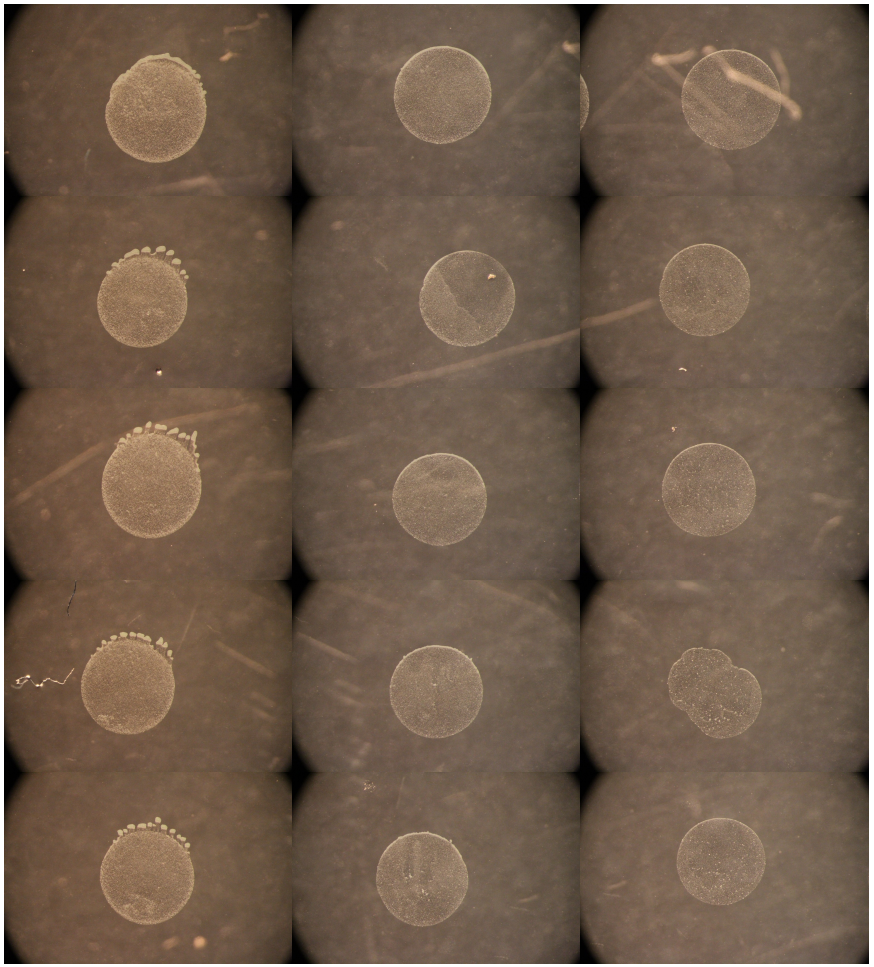
24 hrs



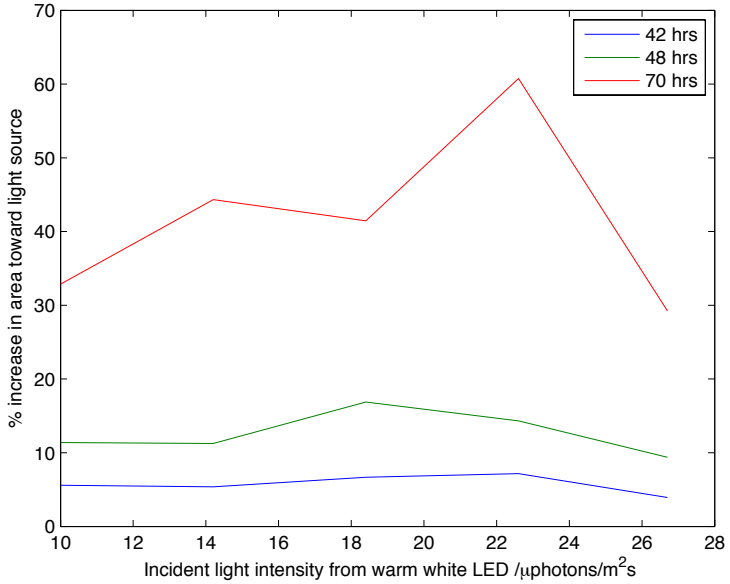
42 hrs



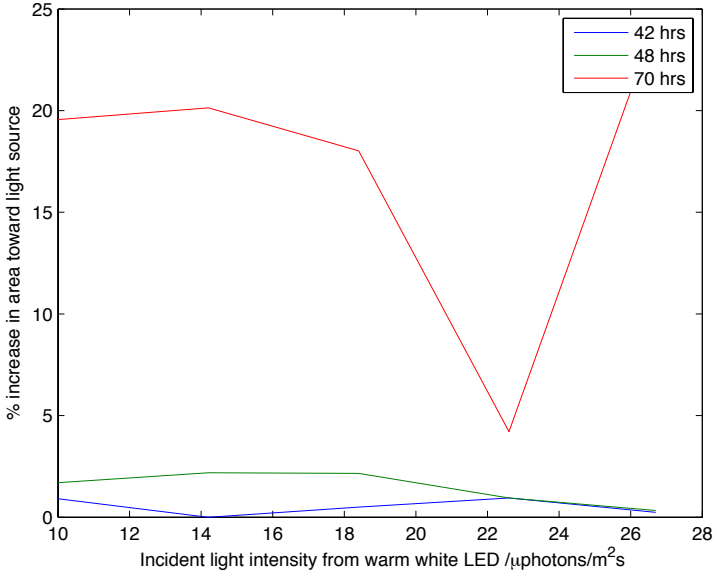
48 hrs



1x


42 hrs
48 hrs
70 hrs
% increase in area toward light source
Incident light intensity from warm white LED /μphotons/m2s

0.5x


42 hrs
48 hrs
70 hrs
% increase in area toward light source
Incident light intensity from warm white LED /μphotons/m2s

- Can observe a greater lag in the lower concentrations before finger movement occurred.
- There seems to be a switch in phototactic motility in the range between 14-26 umolphotons/m^2s, where cells start moving less toward the highest intensity
  - It will be interesting to test a more fine range of intensities within this range
  - Also can test higher intensities to see if there will be an crossing point into entirely photophobic movement
- As warm white consists of many wavelengths together, can start testing individual wavelengths corresponding to the various photoreceptors
  - 660nm (PixD, cph1), 700nm (cph1), 535nm, 435nm (for TaxD1), 360nm (cph2)
- finally, not sure why the second closest drop for 0.5x didn't move.
  - Should have done a replicate experiment alongside
- ** update from 30 Nov: the drops looked like they were contaminated (can see contamination spreading at 92nd hr from the top left of petri dish). Not sure if this was from culture or picked up during inoculation (eppendorf tube etc). Doing contamination check now, but will repeat this experiment for sanity check

Plan for next few weeks

1. Repeat the warm white experiment for paper
2. Experiments where the light is incident overhead, and also when there is no incident light
    1. To be done alongside experiment 1, once I ensure that my cultures are clean (estimate to do this during week of 5 Dec)
3. Finger-crashing experiment (after expt 1)
4. Run wavelength and intensity experiments on WT cells (OD 0.8-1.2)
    1. 660nm , 535nm, 525nm, 470nm, 435nm
    2. Intensities will range from <10 to >50 umol photons/m^2s.
        1. As mentioned earlier, I expect the incident intensities from the Oct expts to be lower than measured with the probe (placed level with LED). This will be a good range to start with, as it is observed that cells start moving less toward light at ~26umol photons/m^2s under warm white.
    3. This experiment is to get an idea of the motility behavior of the cells under different intensities. Once interesting behavior is observed, I will run repeats for the corresponding conditions to obtain single-cell data.
    4. Analysis done on this set will be measuring total area progressed beyond drop , average finger length and longest finger formed.