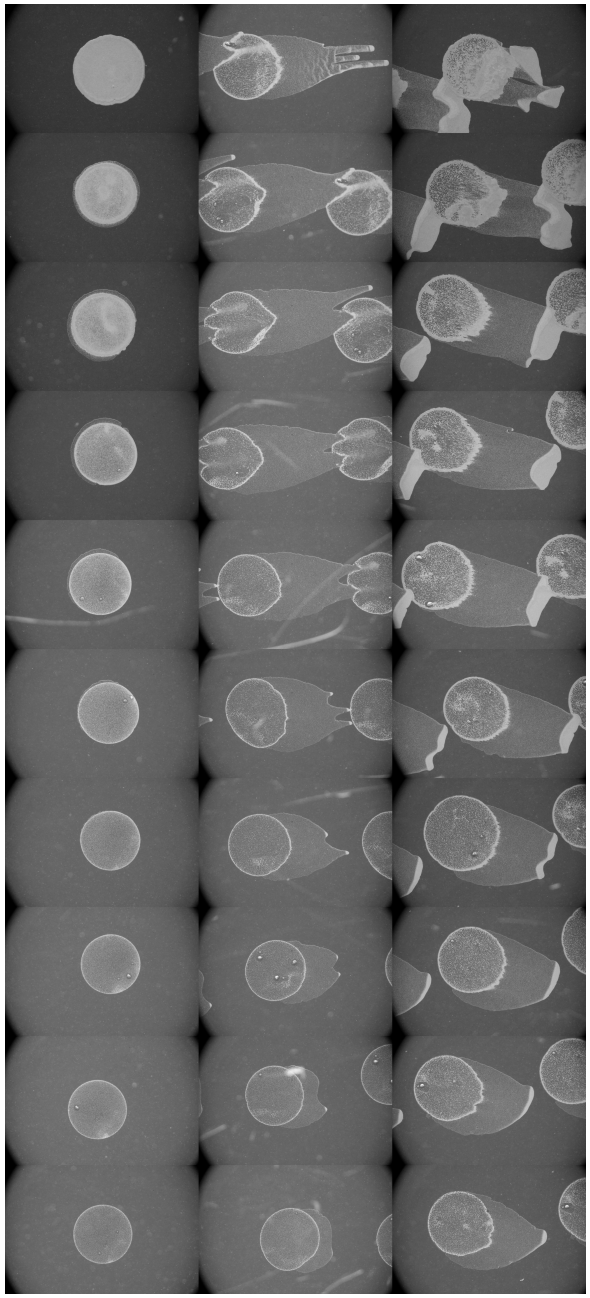
wavelengths

1Aug2013

Day2

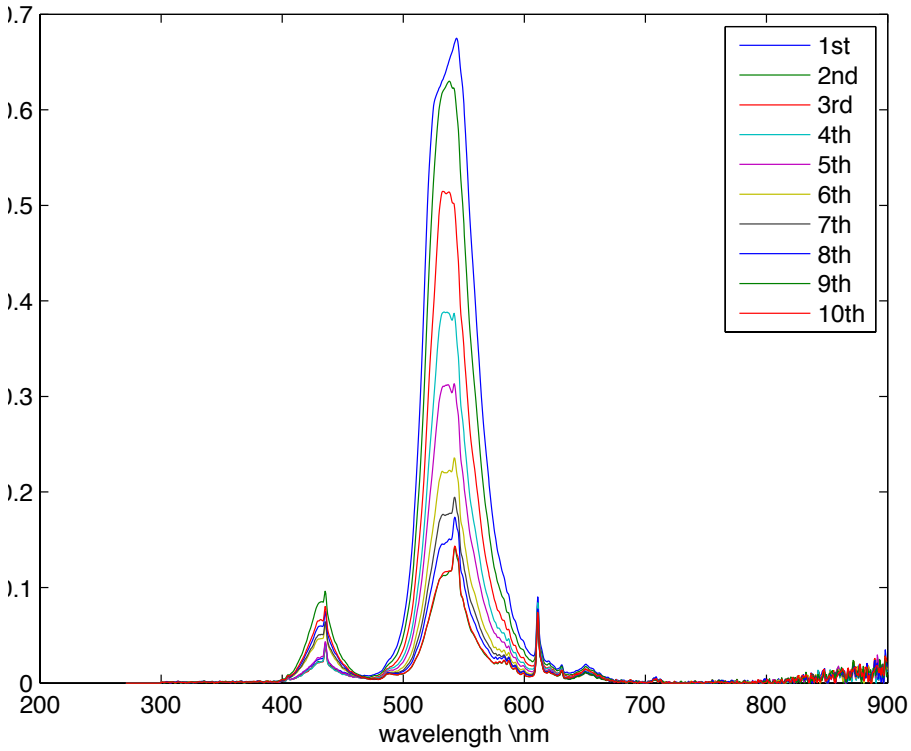
435nm 535nm 660nm

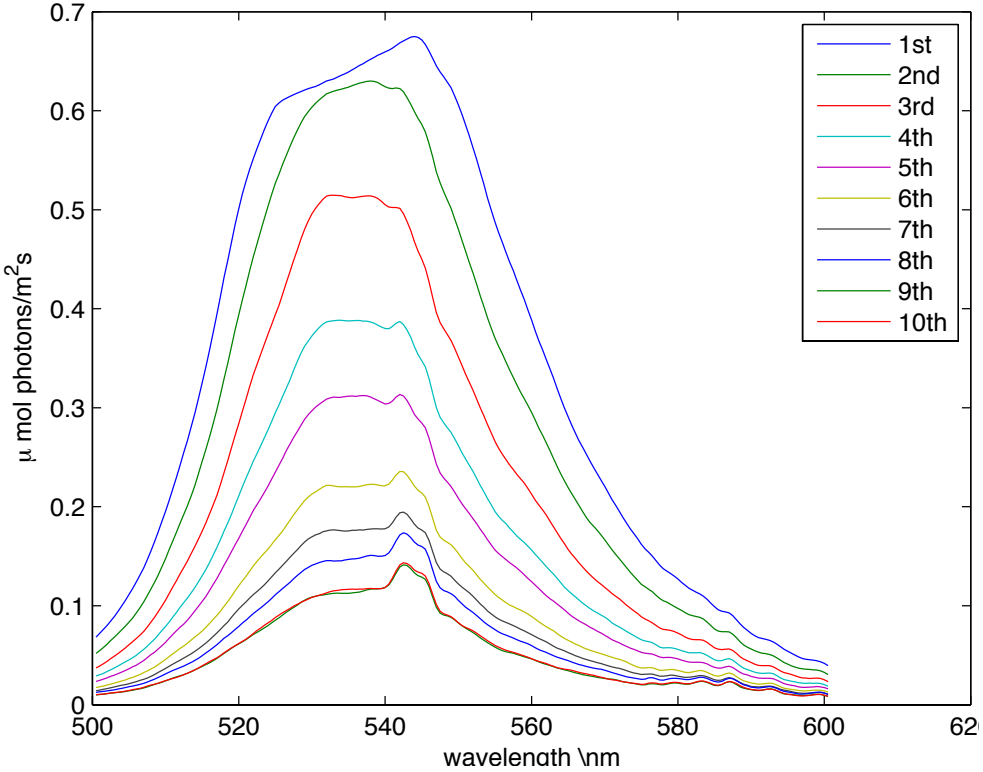


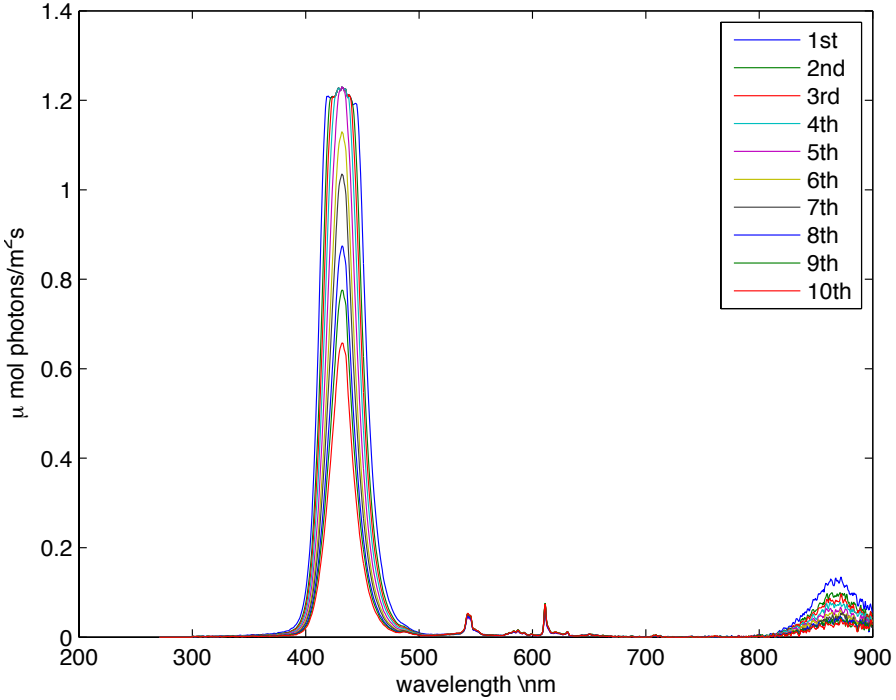
144	42	45
115	32	34
90	24	29
75	20	20
58	15	18
50	14	16
42	12	14
35	11	12
30	10	11
24	9	9

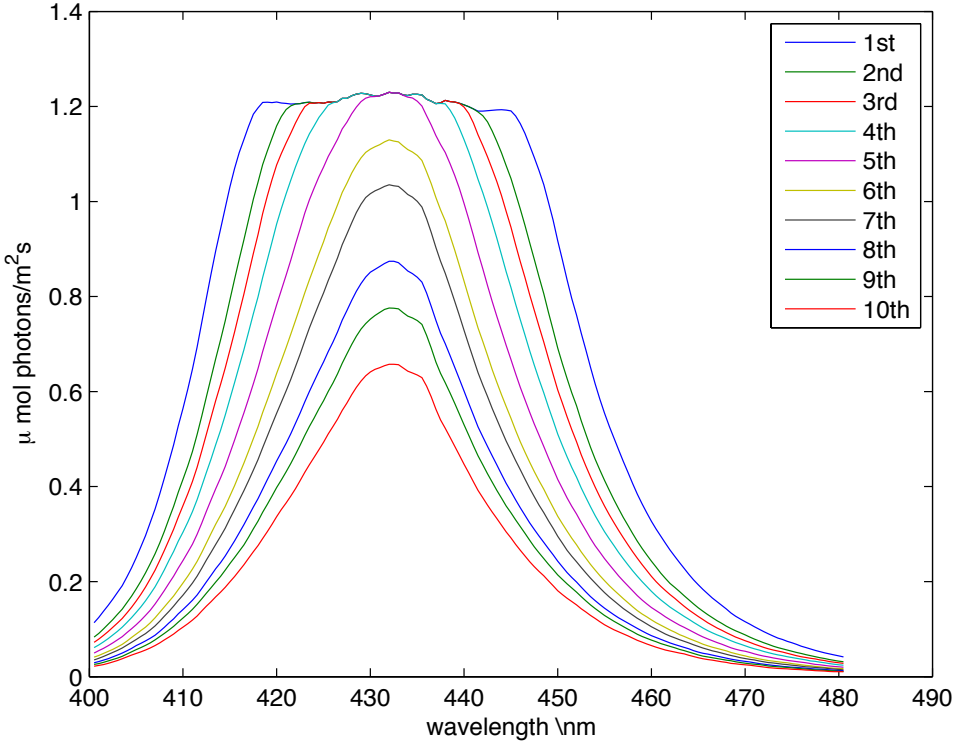
For the intensities tested, 535nm and 660nm show an intensity-dependent response. 435nm shows some forward movement after 3 days at the higher intensities.

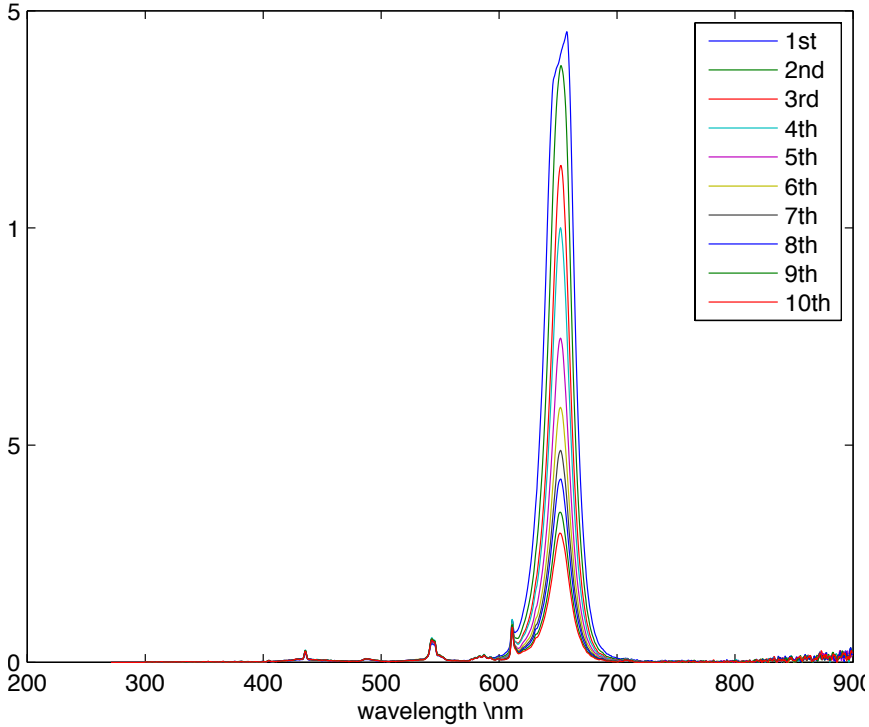
Done using square plate

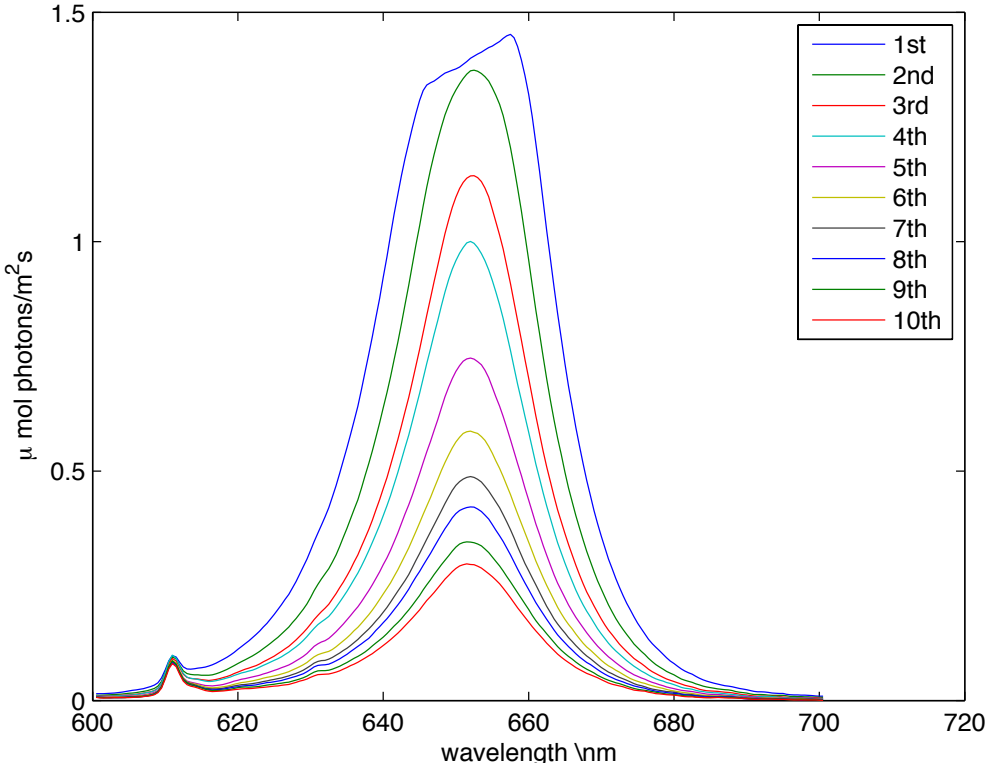

1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
wavelength \nm


1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
μ mol photons/m2s
wavelength \nm


1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
μ mol photons/m^2s
wavelength \nm
0
0.2
0.4
0.6
0.8
1
1.2
1.4
200
300
400
500
600
700
800
900


1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
μ mol photons/m^2s
wavelength \nm
0
0.2
0.4
0.6
0.8
1
1.2
1.4
400
410
420
430
440
450
460
470
480
490


1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
wavelength \nm


1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
μ mol photons/m2s
wavelength \nm

7Aug2013

435nm 535nm 660nm

For the intensities tested, 535nm and 660nm show an intensity-dependent response. 435nm shows some forward movement after 3 days at the higher intensities.

Day2

144

115

90

75

58

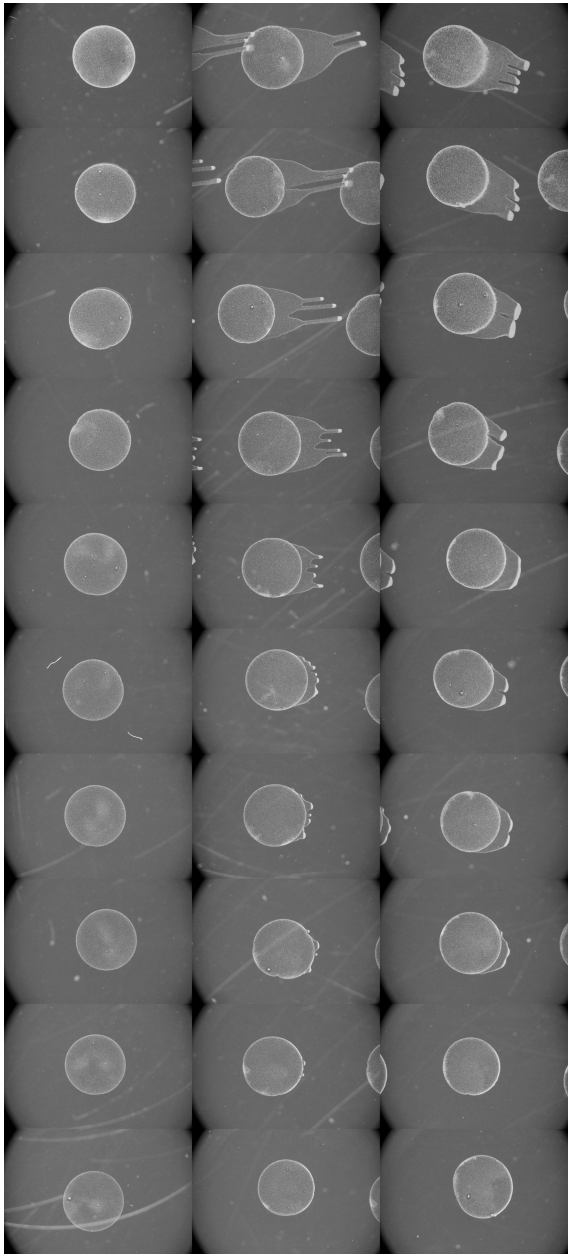
50

42

35

30

24



42 45

32 34

24 29

20 20

15 18

14 16

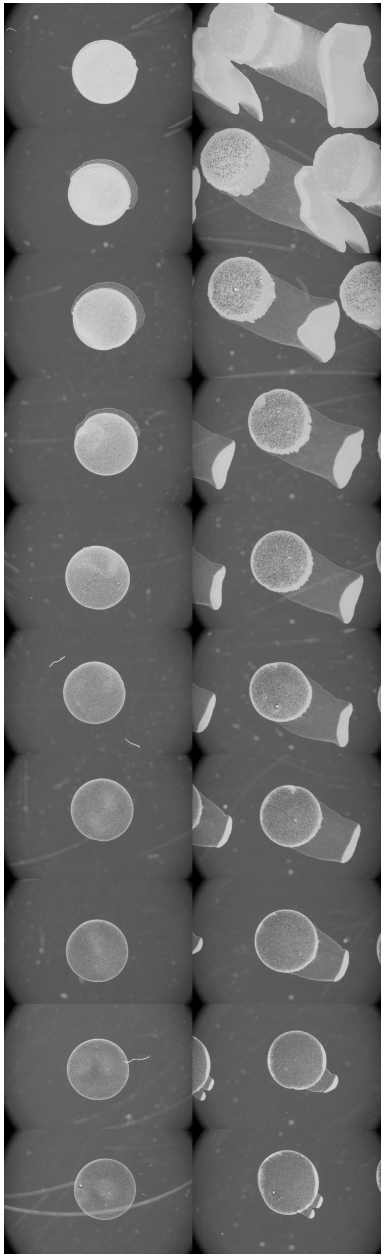
12 14

11 12

10 11

9 9

Day4



Done using square plate

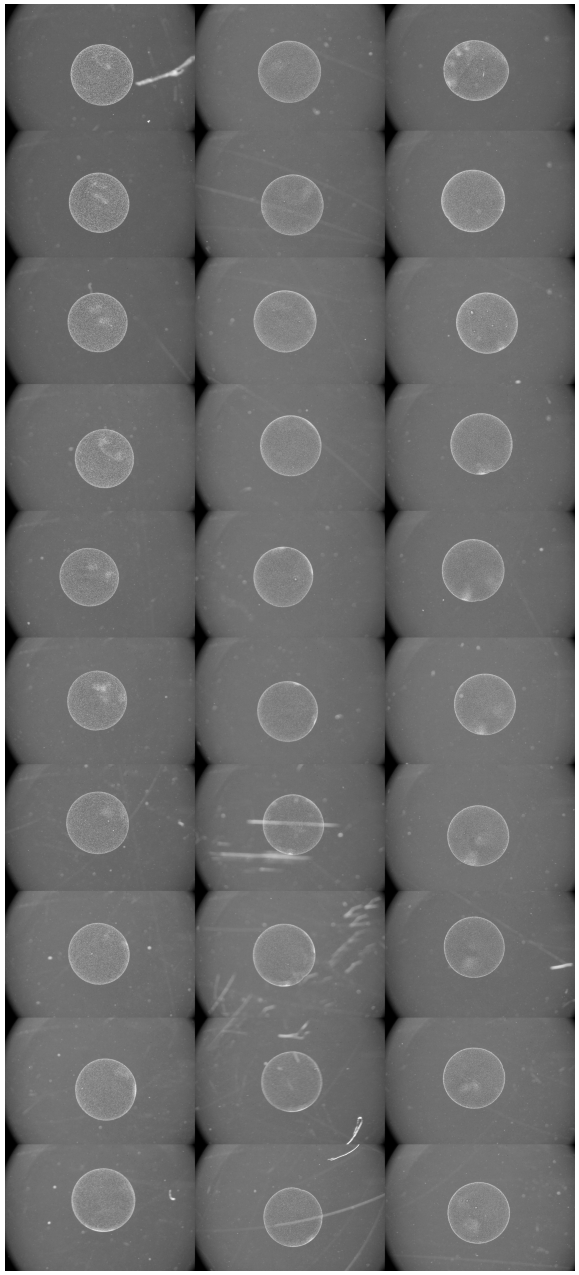
- At high 435nm intensities, the drop extends a single wide front, if any, toward light.
  - From what we know about TaxD1, the active blue-absorbing form converts to the inactive green-absorbing form after absorbing blue light.
  - From what we know from the taxD1 mutant, TaxD1 seems to suppress sideways noise in motility. Are we seeing the same effect?
  - But if TaxD1 is being inactivated, why are the cells not moving backward?
  - PixD (BLUF) also absorbs at 440nm, so are we seeing the effect of PixD as well? (pixD mutant is a backmover under 660nm)
- Under 535nm, the cells exhibit an intensity-dependent response
  - Is this a demonstration of TaxD1 converting to a higher ration of the active blue-absorbing form?
  - UiRs absorbs at 534nm, and the mutant is a forward mover under blue and green.

- Next steps: taxD1 mutant under the same intensities, to see if we are seeing the effect of inactivating TaxD1 under high blue intensities, and activating under high green.

13Aug2013

Day2

435nm	535nm	435nm
144	22.5	85
115	17	63
90	13.5	50
75	11	38
58	10	30
50	8.5	23
42	8	20
35	7	16
30	6.5	14
24	6	12

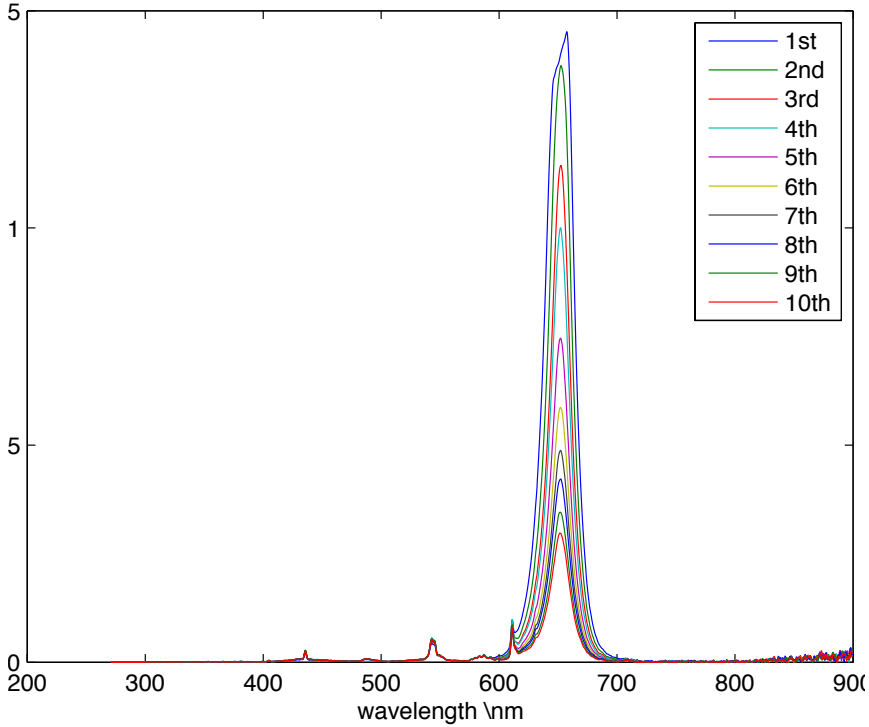


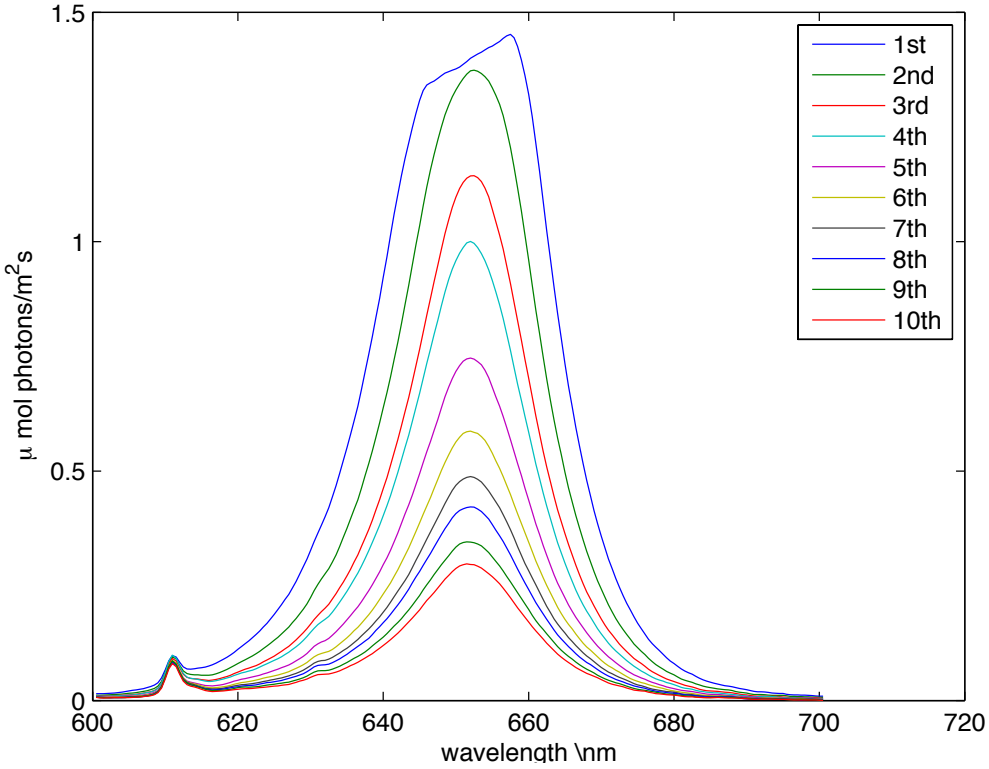
Testing low 535nm and 435nm intensities

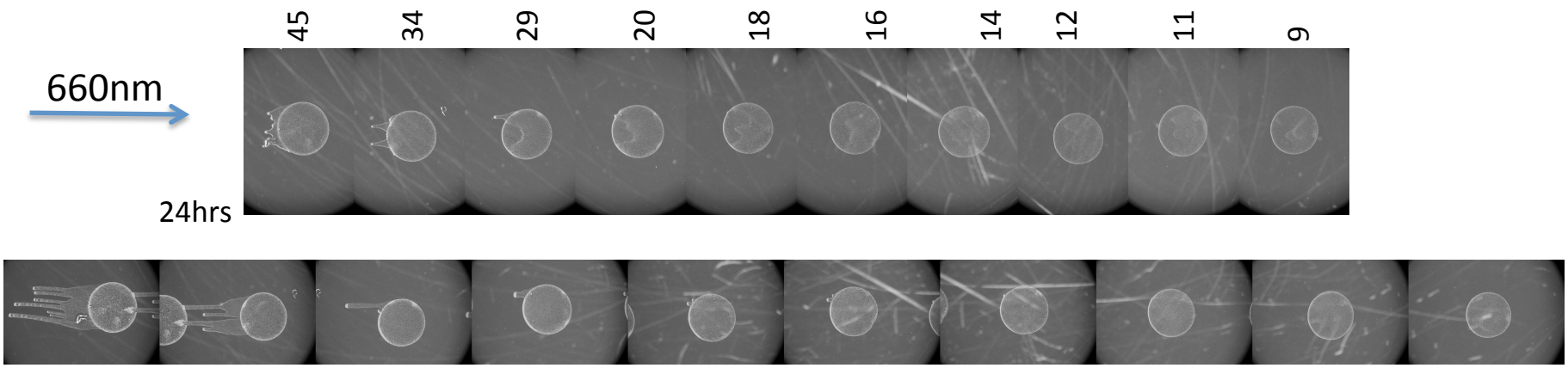
Done using square plate

Single-cell microscopy at early timepoints

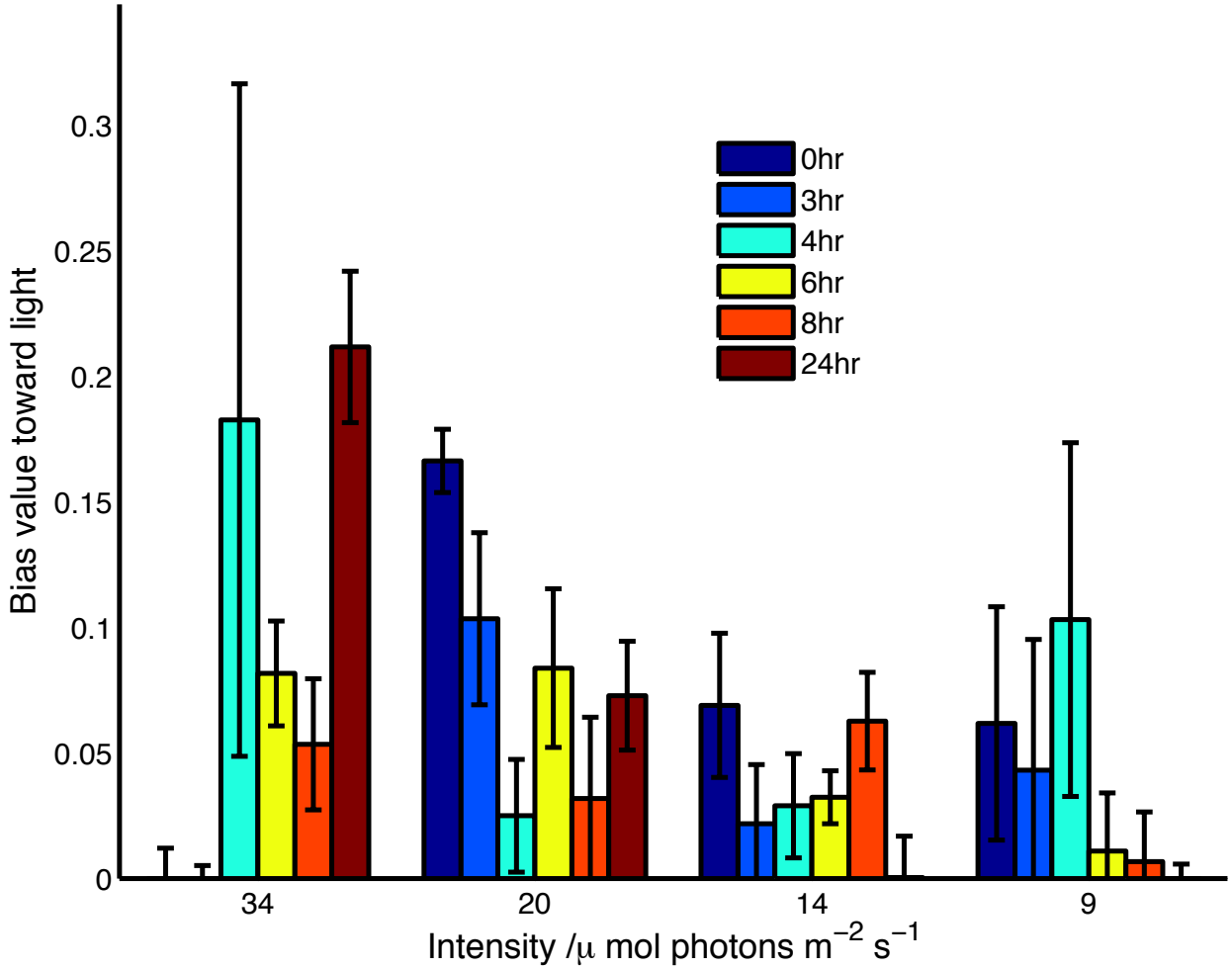
- To determine if the different observed fingering patterns are the result of intensity-dependent phototactic responses (and not higher cell density due to division at increasing intensities)
- 10-min videos taken every 2 hours, starting at 0-hr after light-on
  - At 0, 2, 4, 6, 8, 24 hrs
- 4 drops were imaged in each experiment

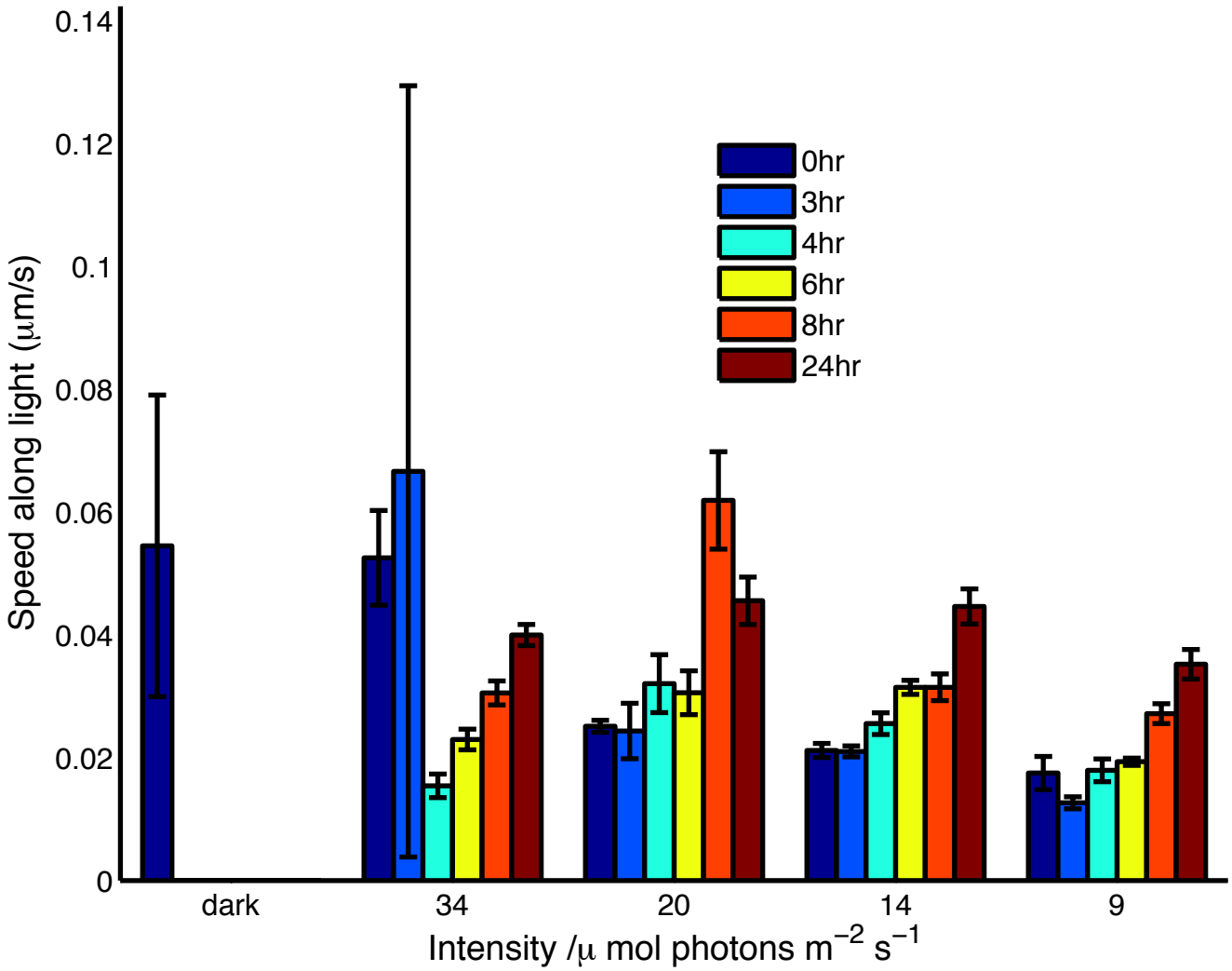

1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
wavelength \nm


1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
μ mol photons/m2s
wavelength \nm

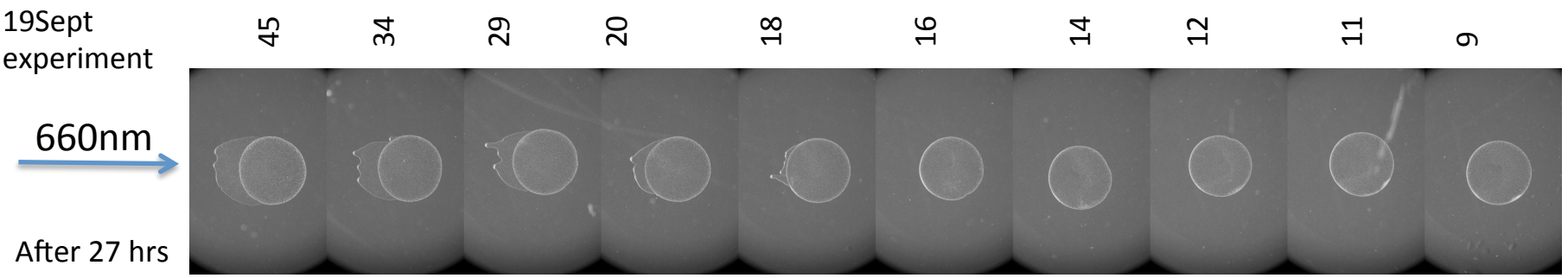


Plotting the mean bias value across 6 100-sec intervals in each case

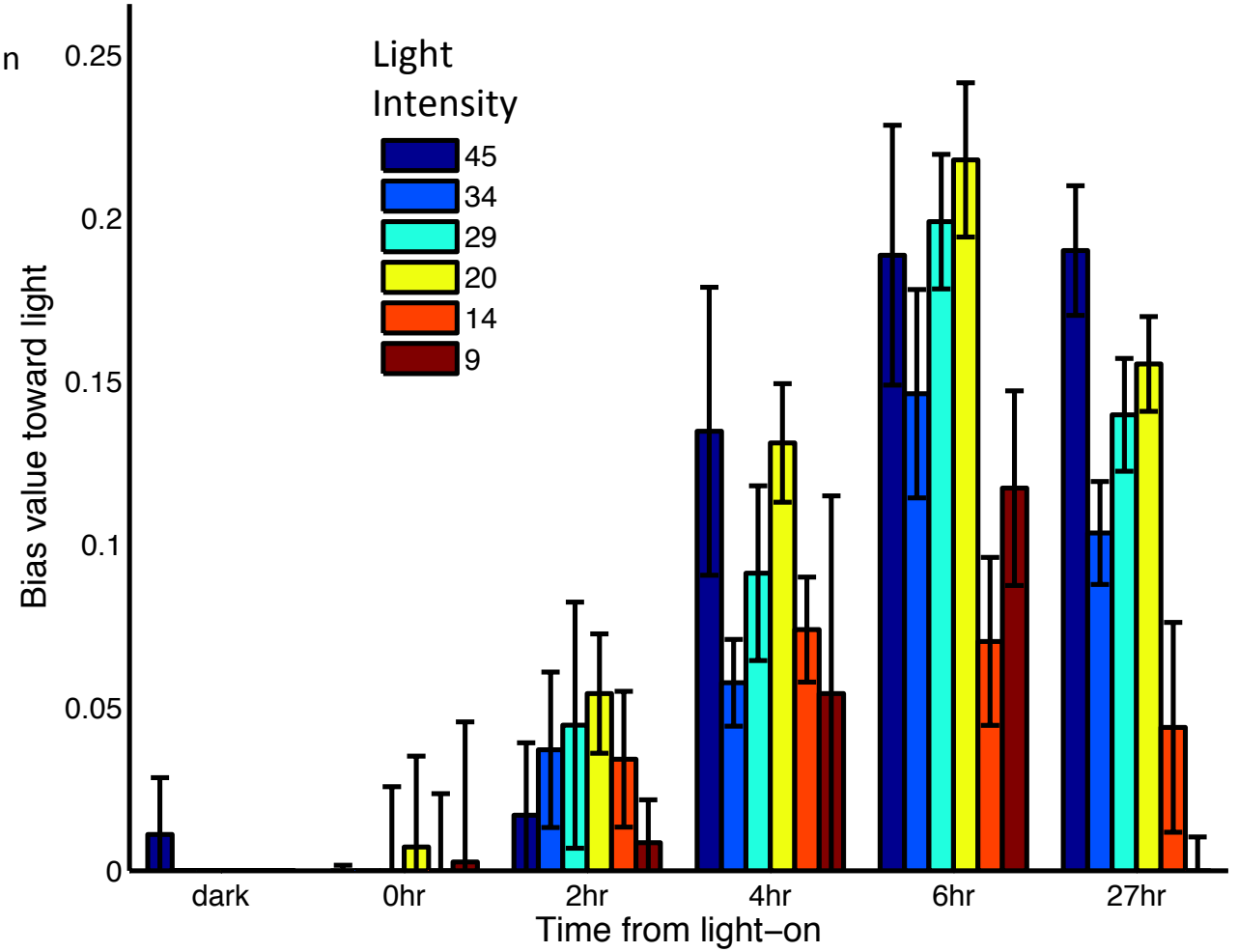


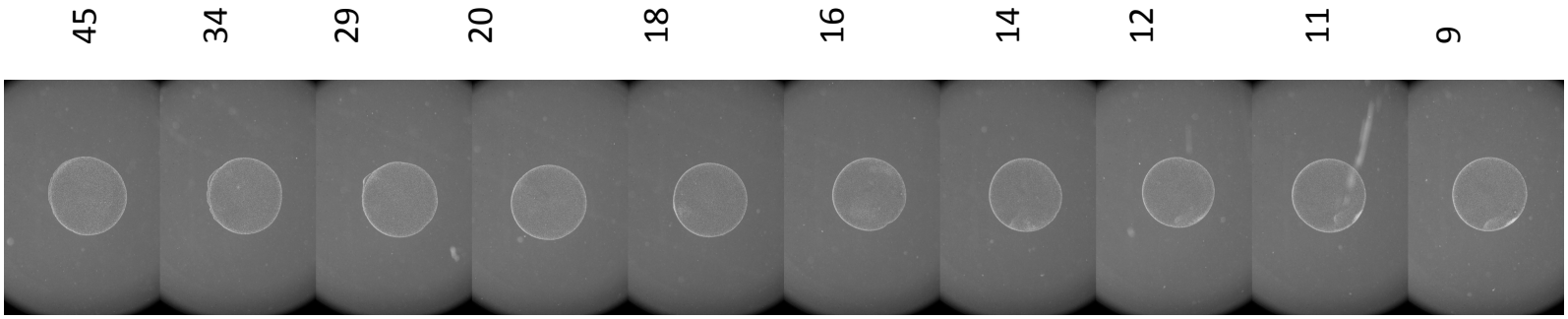

0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Speed along light (μm/s)
0hr
3hr
4hr
6hr
8hr
24hr
dark
34
20
14
9
Intensity /μ mol photons m−2 s−1

19Sept experiment



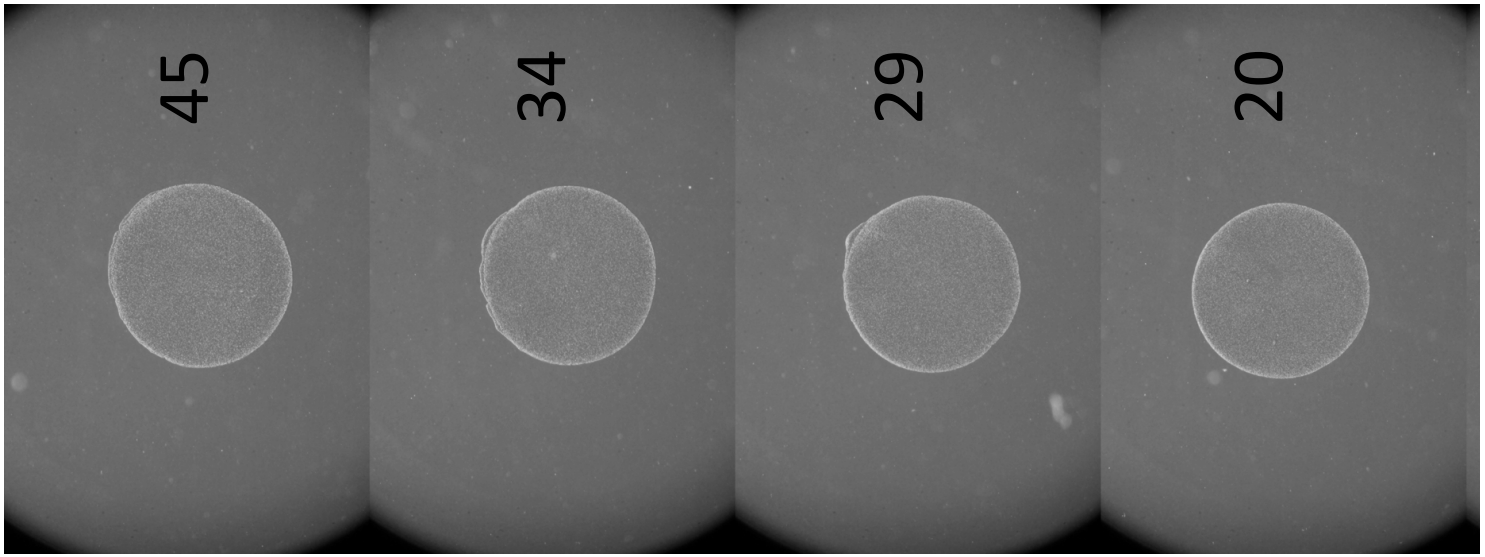
In this expt, I measured the bias in 6 drops.





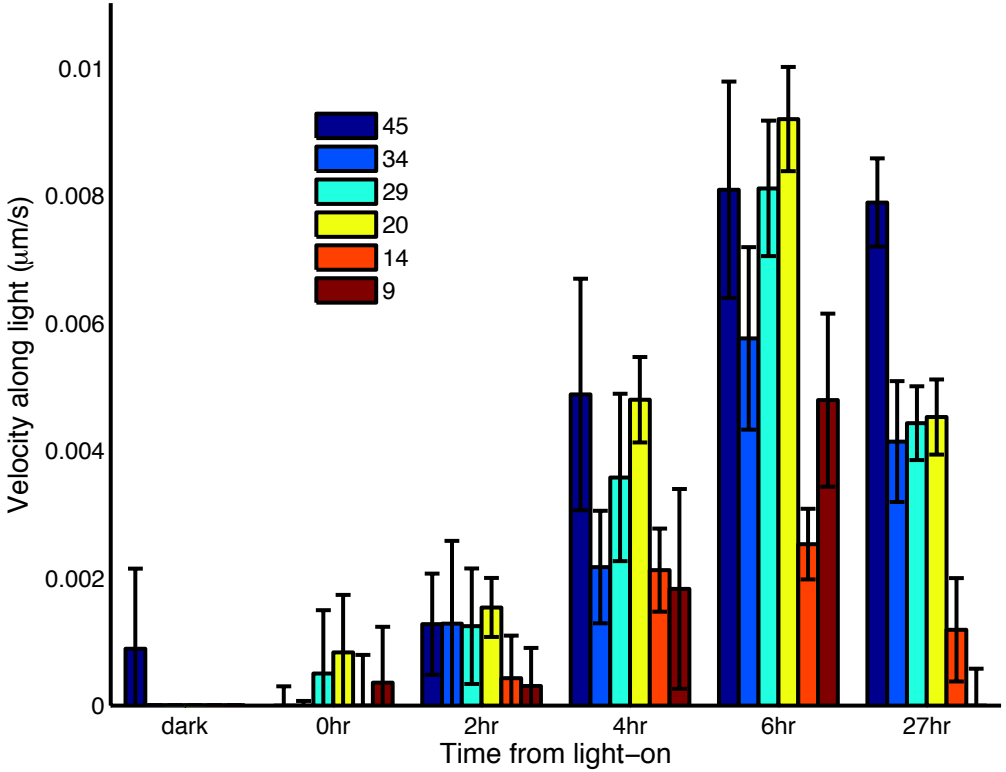
After 6 hrs

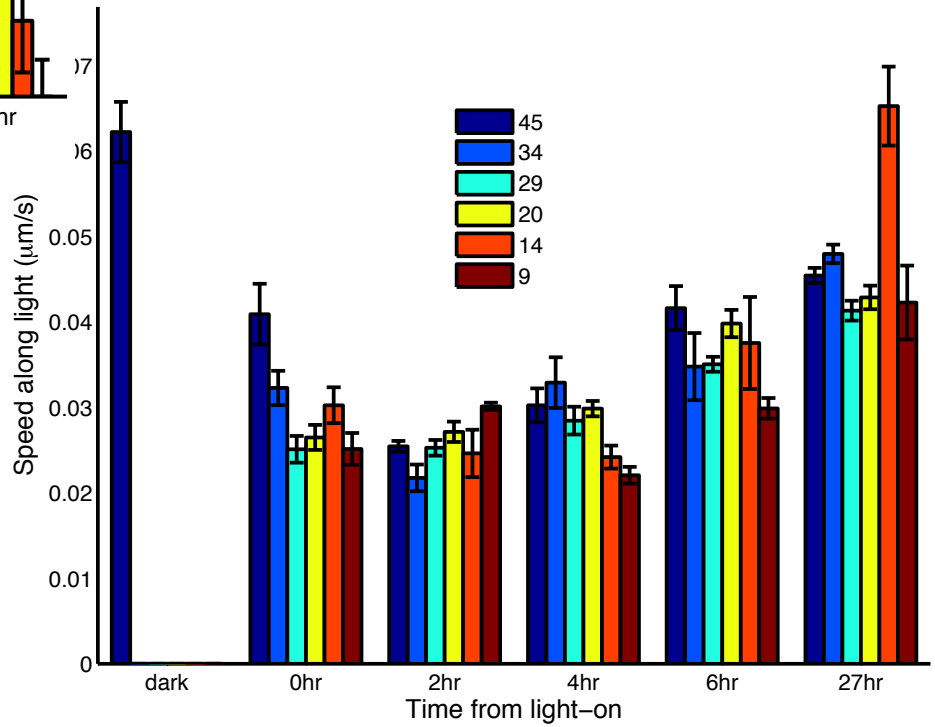
- I was surprised that there wasn't a clear trend in the bias values at 6-hrs, esp. from the drops experiencing 45, 34, 29, 20 uE, because cells had already clearly moved out of the respective drops by then.



First four drops magnified

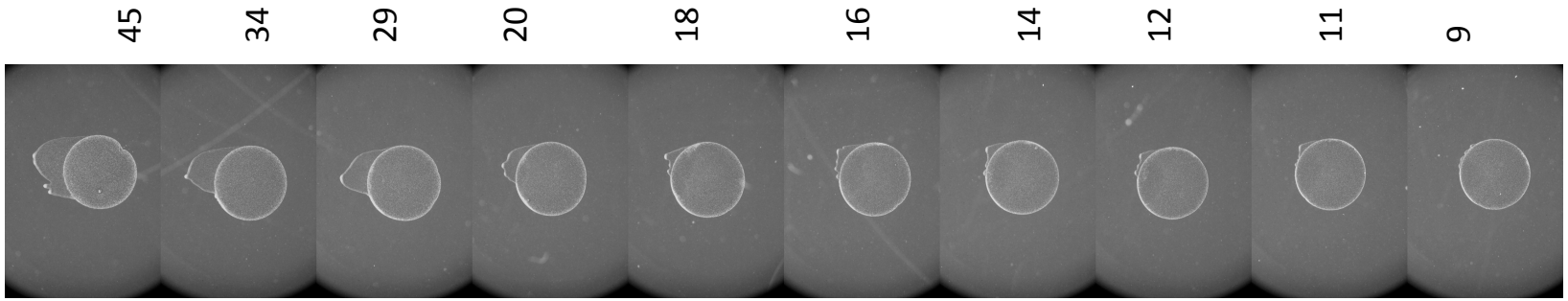
Couldn’t see a good trend in the velocities or speeds either



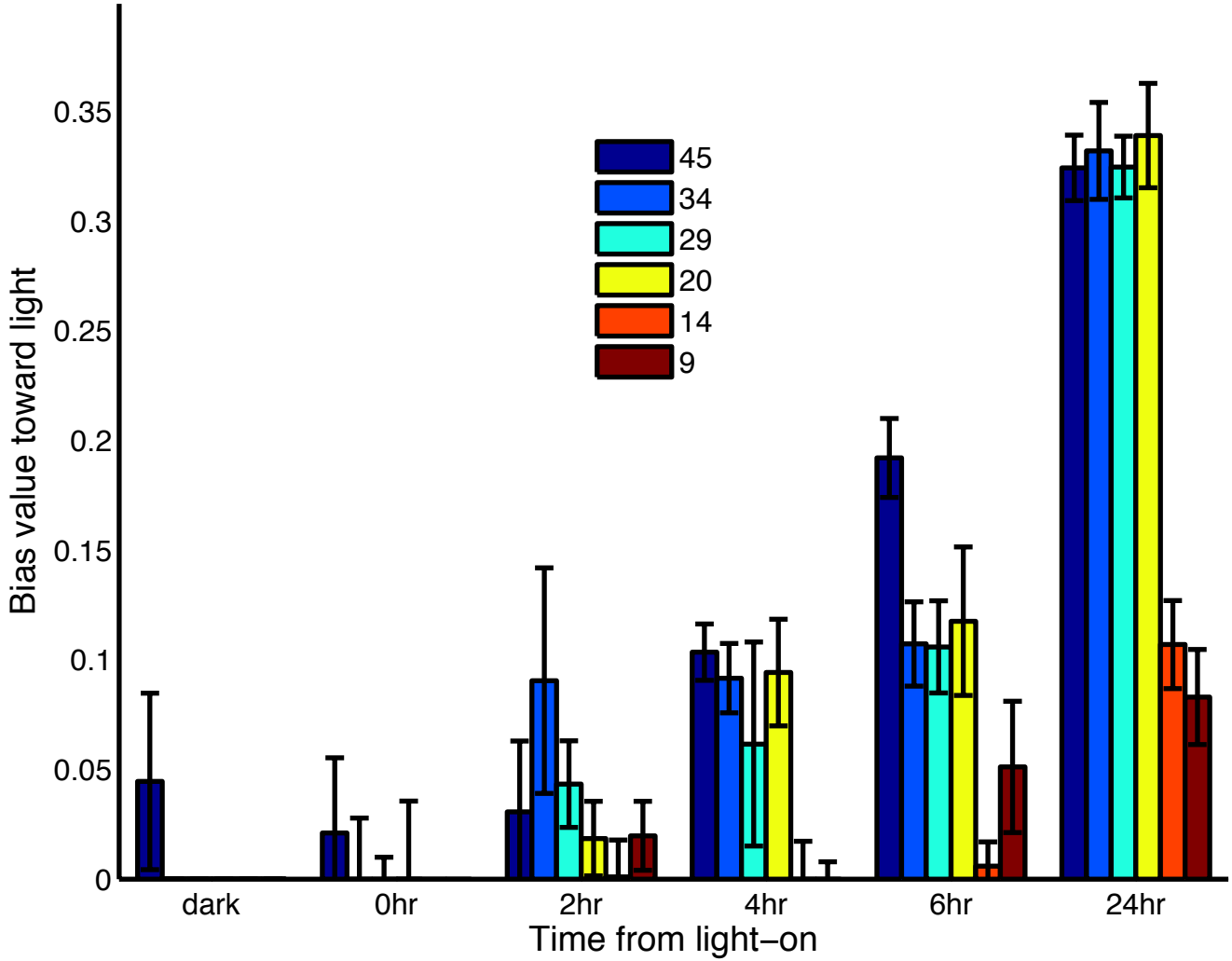


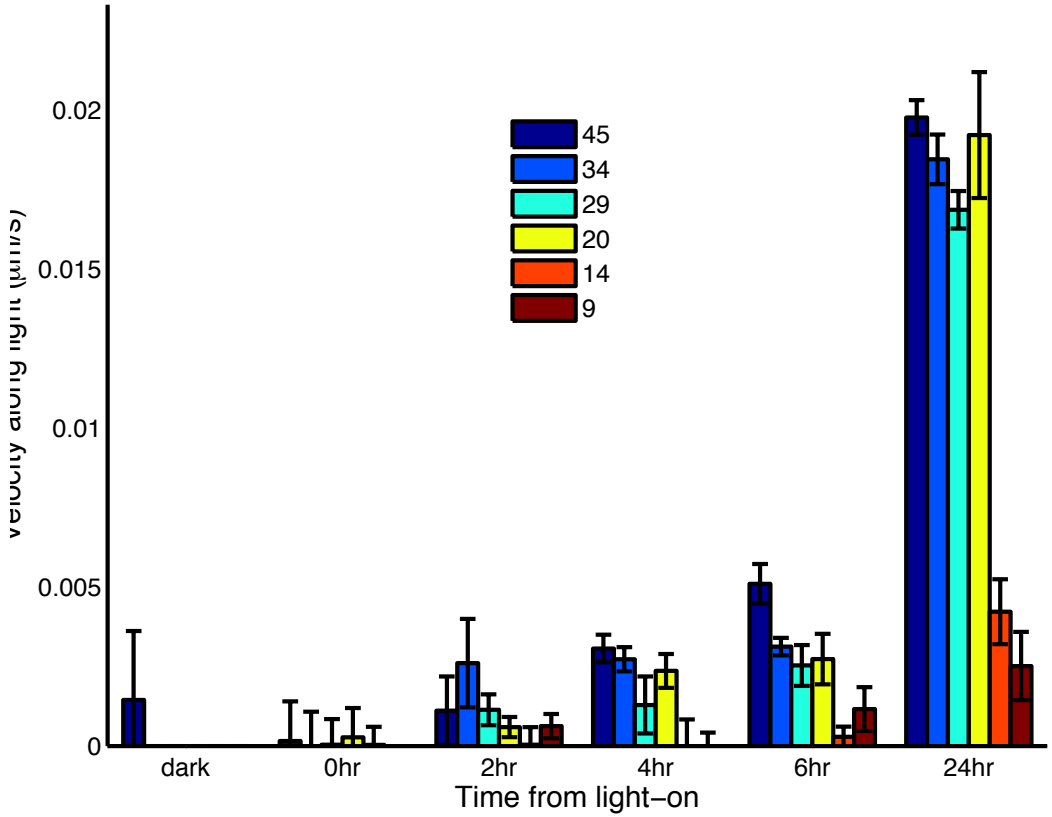
So I tried to repeat the experiment, to see if I can get the same quick finger formation and image the cells in the initial fingers..

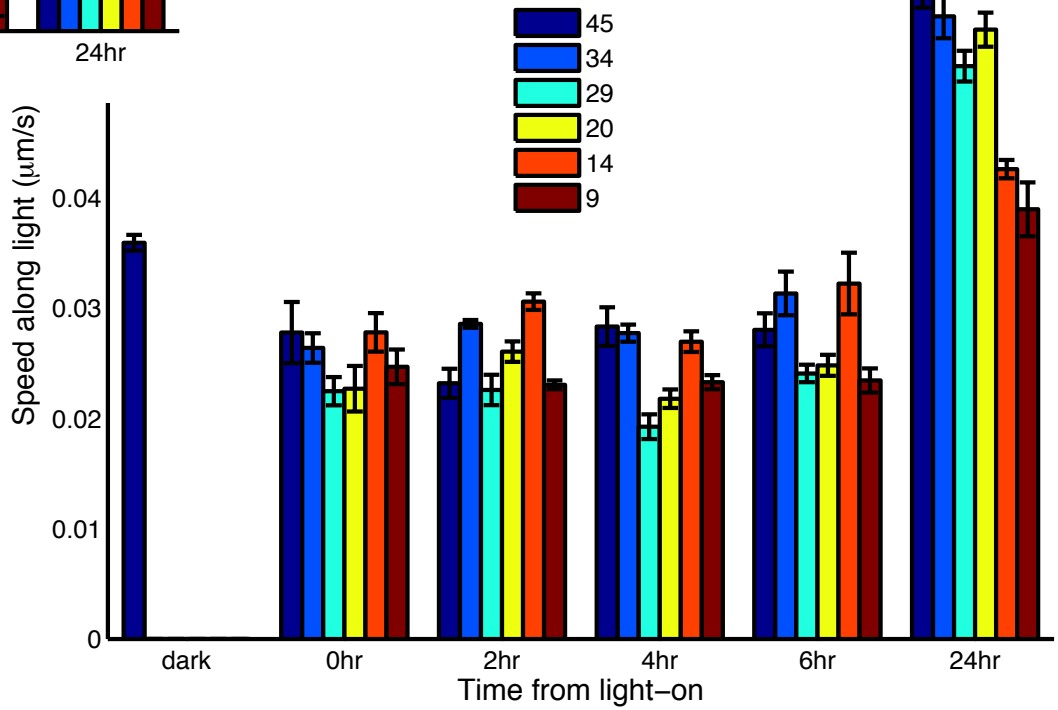
27Sept expt



But the cells didn't move out of the drop till many hrs after, so I was only able to image the front of the drop

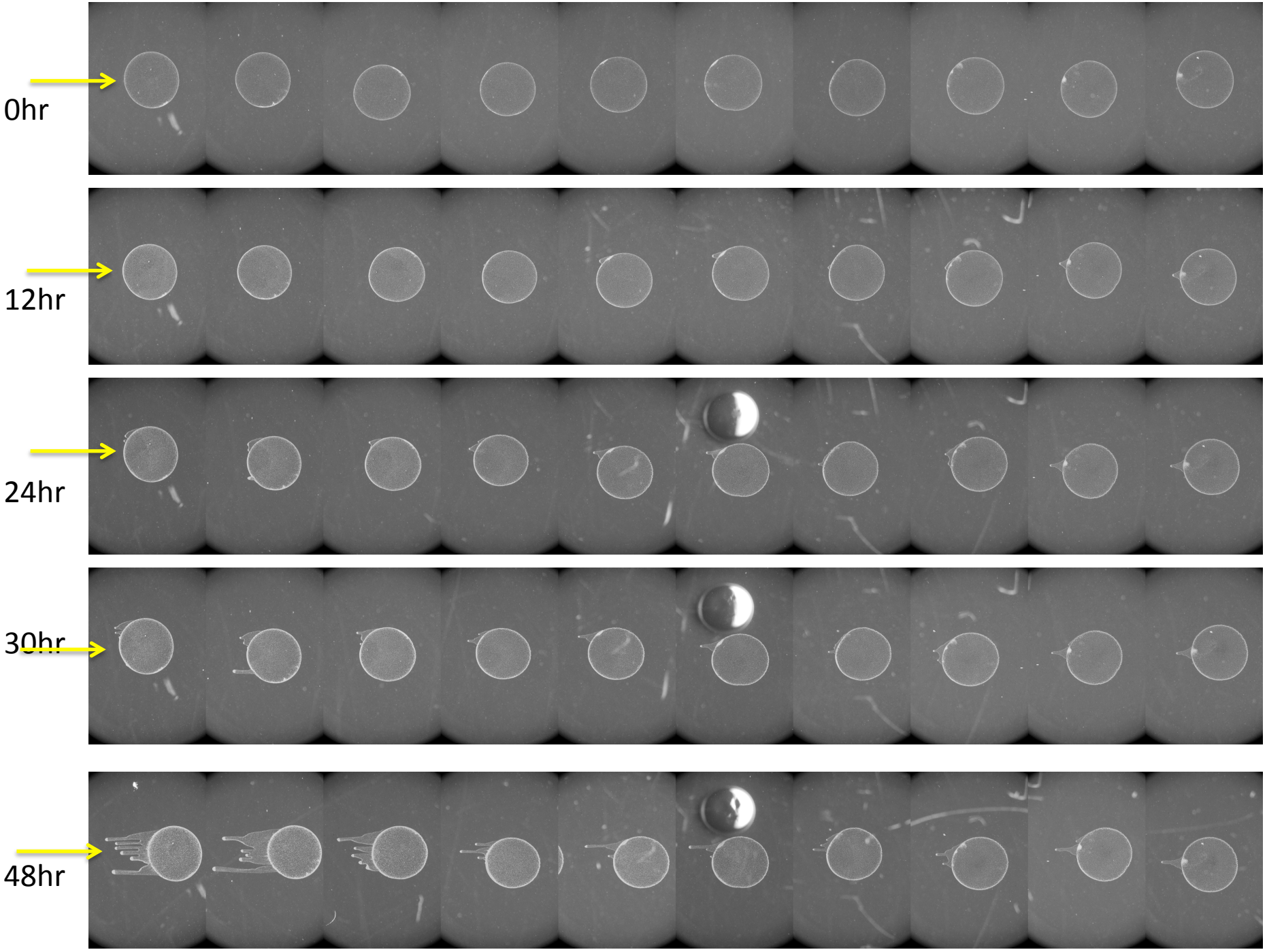


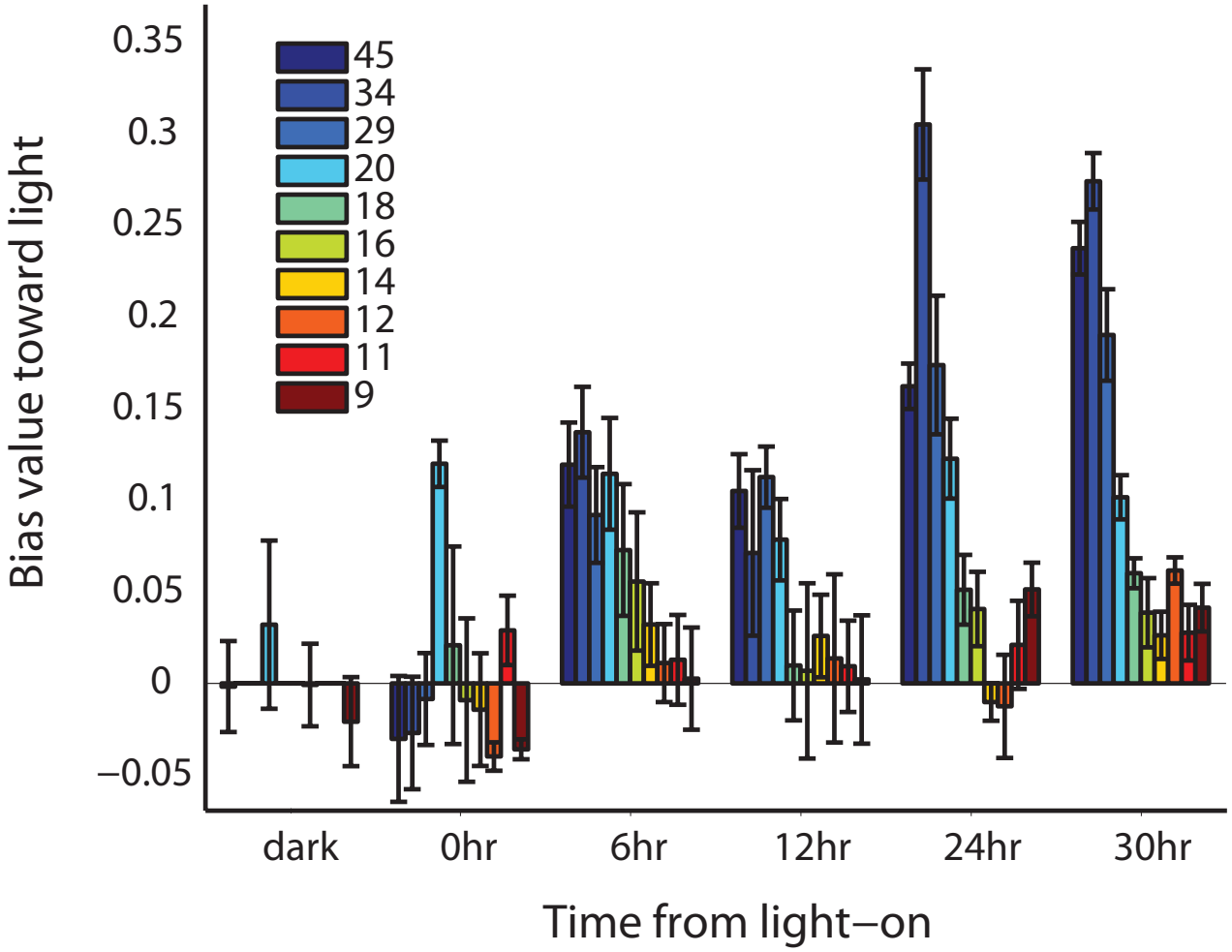

0.02
0.015
0.01
0.005
0
45
34
29
20
14
9
dark
0hr
2hr
4hr
6hr
24hr
Time from light−on

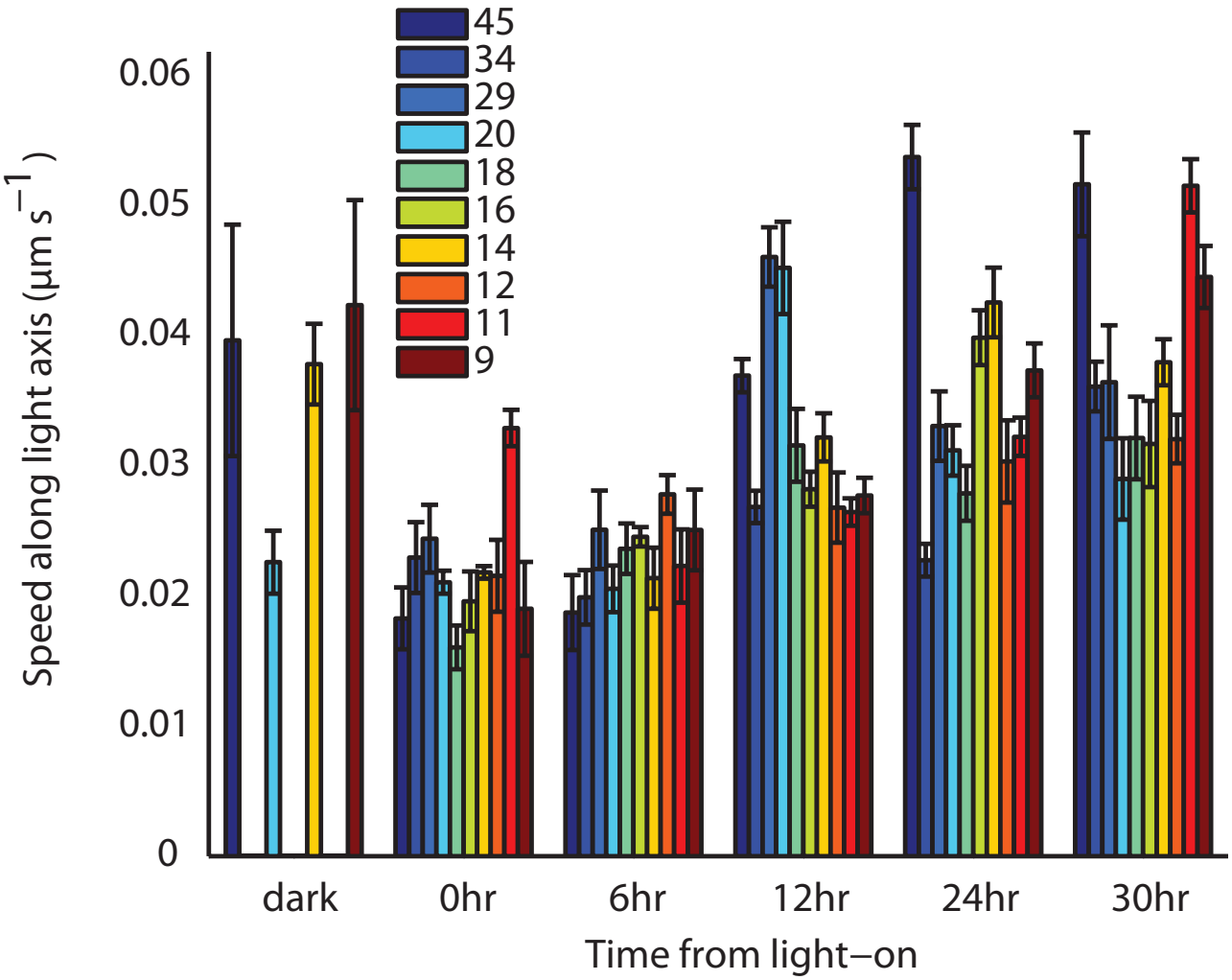

Speed along light (μm/s)
0.04
0.03
0.02
0.01
0
45
34
29
20
14
9
dark
0hr
2hr
4hr
6hr
24hr
Time from light−on

- Experiment done on 20 Nov
- 660nm tested at 10 intensities
  - 45, 34, 29, 20, 18, 16, 14, 12, 11, 9
- Cells were imaged at the front of each drop every 6 hrs, skipping the 18th hour

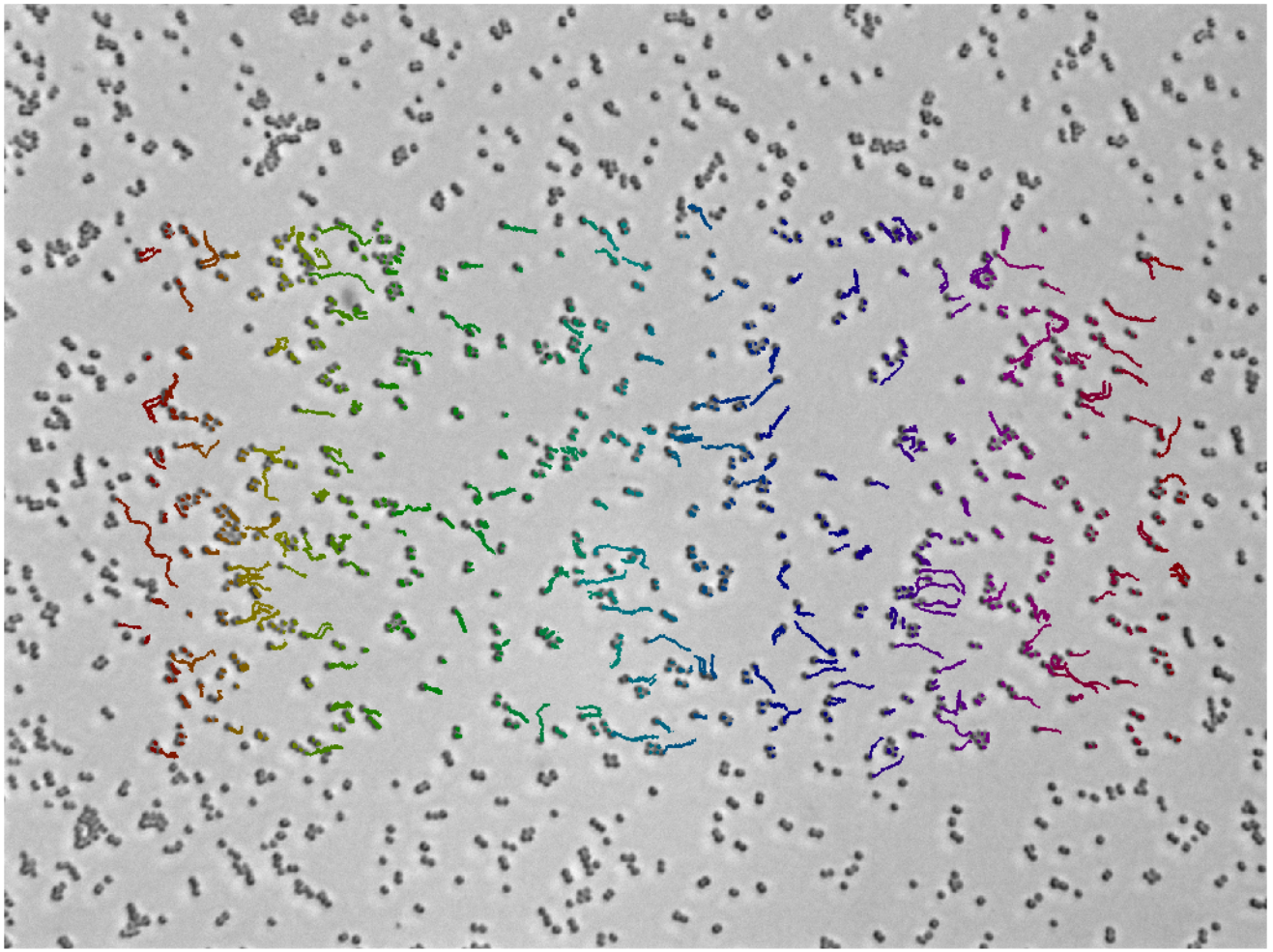
** Some drops started with a heterogeneous distribution of cells (5th, 6th, 8th, 9th, 10th distances away from the LED), hence the extent of fingering from the cell-dense regions of these drops should not be considered when viewing the macroscopic data. The results from 19th Sept and 27th Sept can be used as a reference instead.


0hr
12hr
24hr
30hr
48hr

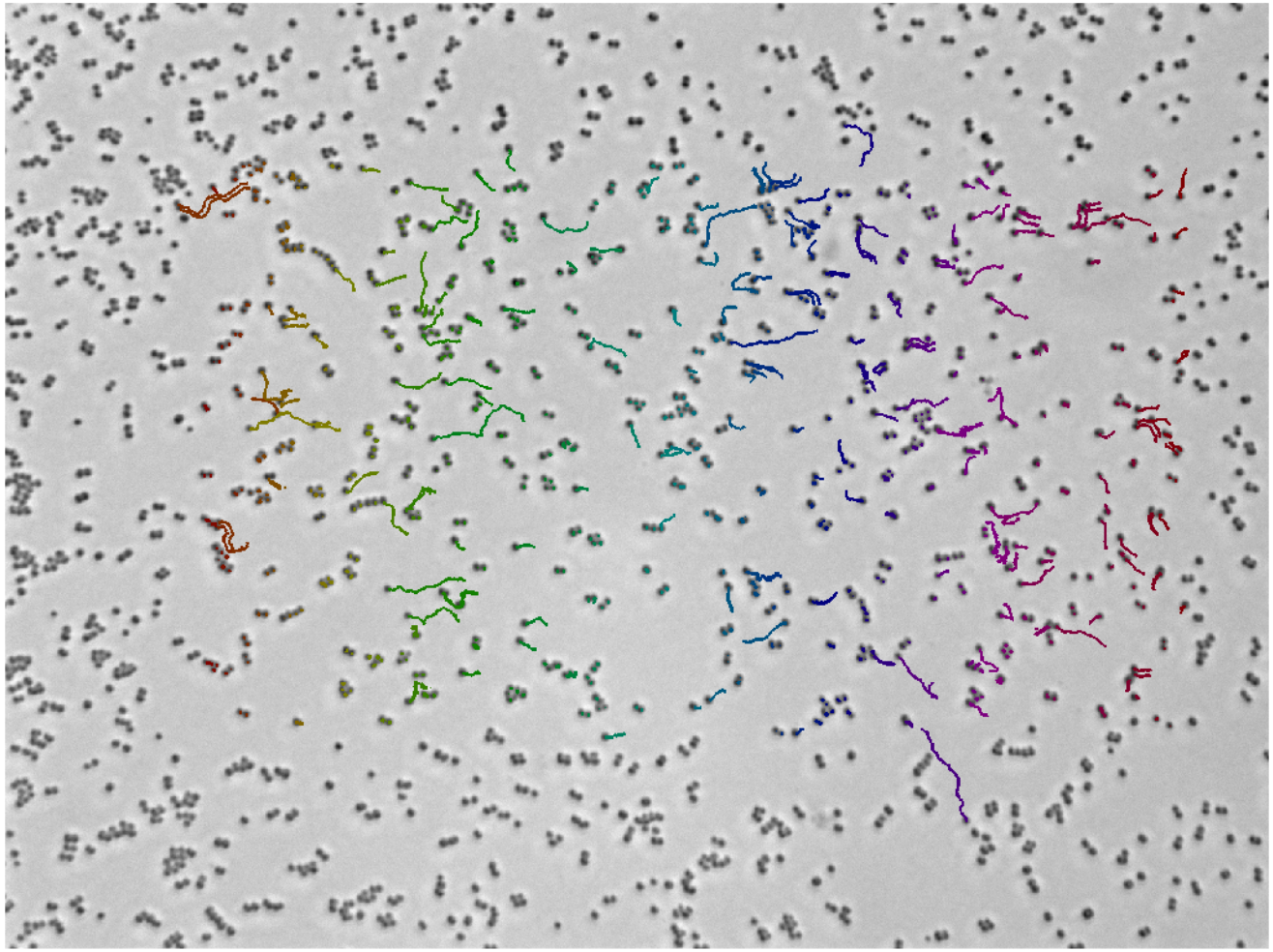

0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
−0.05
Bias value toward light
45
34
29
20
18
16
14
12
11
9
dark
0hr
6hr
12hr
24hr
30hr
Time from light−on


45
34
29
20
18
16
14
12
11
9
0.06
0.05
0.04
0.03
0.02
0.01
0
Speed along light axis (μm s−1)
dark
0hr
6hr
12hr
24hr
30hr
Time from light−on

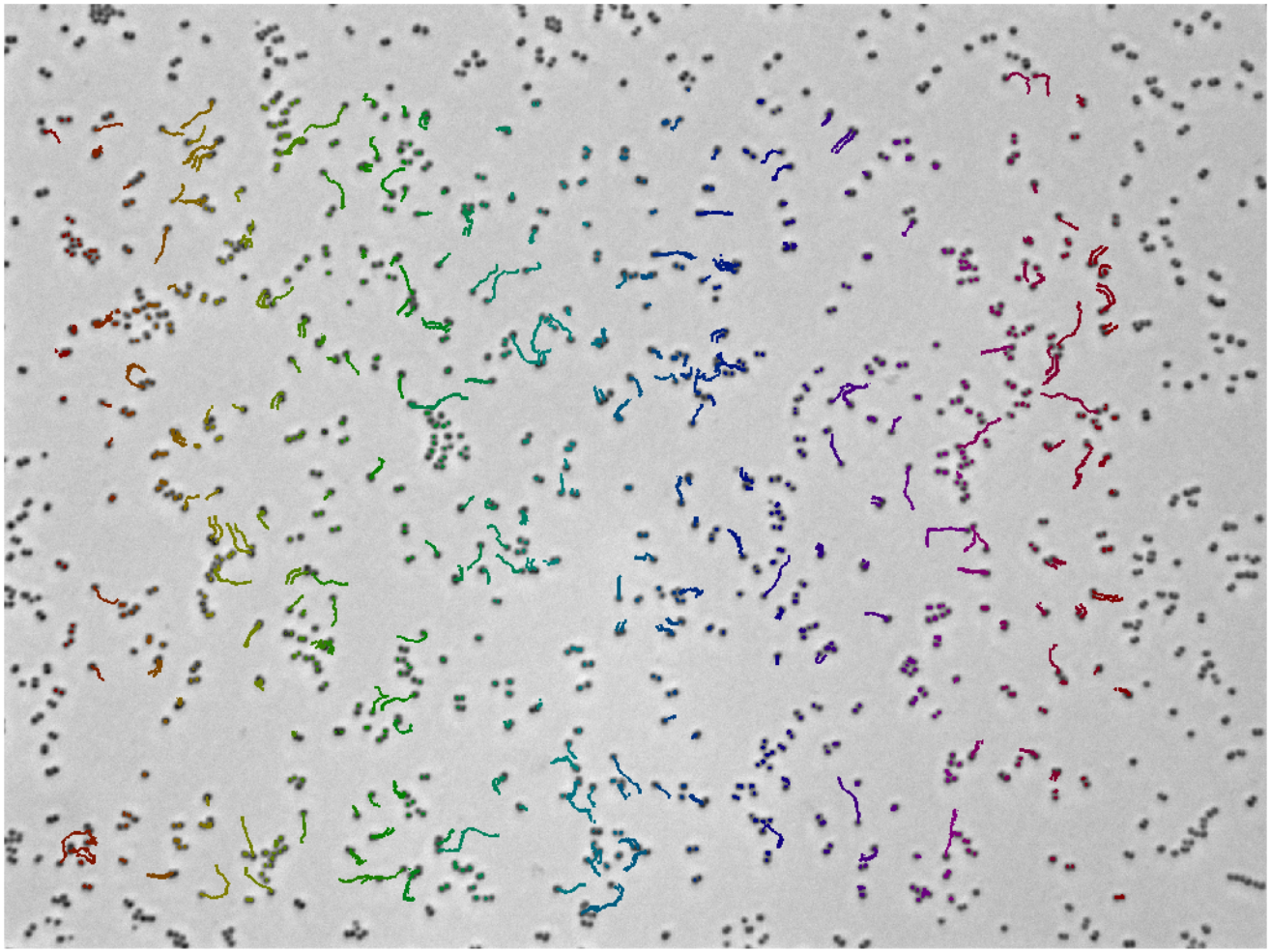
45uE @ 24 hrs



34uE @ 24 hrs



14uE @ 24 hrs



12uE @ 24 hrs