You are mostly not human, and neither are other things

You were right about your parents – they're not human either.

Vernita Gordon

gordon@chaos.utexas.edu

You are mostly bacteria

- About 4 pounds of bacteria in & on you
- How much do you weigh?
  - 4 pounds is about 3% if you weigh 120 pounds
  - 3% is not “most”! What nonsense is this?

What is going on?

Possible answers:

(1) I am crazy.

(2) We haven’t asked the question the right way.

(these are not mutually exclusive)

Consider the cylindrical human…

- Are there more apples or more raisins?

- What about now?

You are mostly bacteria

- About 4 pounds of bacteria in & on you
- How much do you weigh?
  - 4 pounds is about 3% if you weigh 120 pounds
  - 3% is not “most”! What nonsense is this?

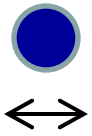
What is going on?

Possible answers:

Bacteria cells are much smaller than human cells

10 μm = 0.01 mm

bacterium



1 μm = 0.001 mm

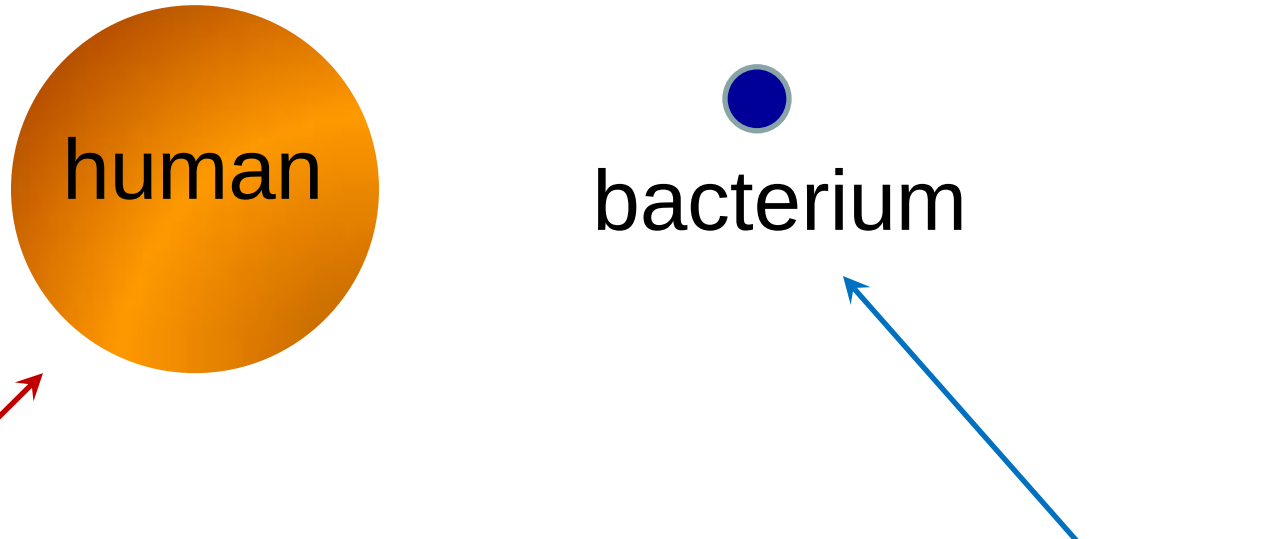
Volume = length x length x length

1000 μm^3 = 0.000001 mm^3

1 μm^3 = 0.000000001 mm^3

Weight = density x volume

Density is the same for bacteria and human cells



This is 1000x heavier than this.

You are mostly bacteria by number

10x more bacteria cells
than human cells

Lesson #1: Question authority!

Lesson #2: Sometimes the answer depends on the way you ask the question.

Lesson #3: Numbers can be manipulated.

So what about these bacteria?

- Where are they?
  - Mostly in your guts
    - Also on your skin and other surfaces in your body
- What do they do?
  - They make you fart.
- So bacteria are evil.
  - No! Farting is good!

How is farting good?

- There are some things you can’t digest.
  - Mostly large fibers.
    - These mostly come from vegetables.

How is farting good?

- There are some things you can't digest.
  - Mostly large fibers.
    - These mostly come from vegetables.

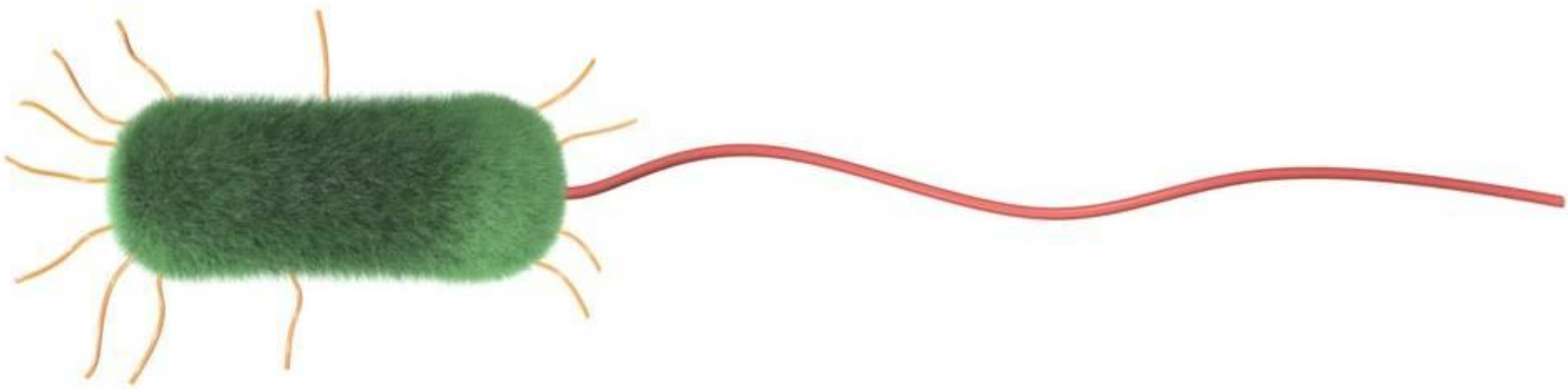
- Bacteria can digest those things.
  - They break them down and make nutrients you can use.
  - During this digestion, they produce gas.
    - Like you produce carbon dioxide gas
  - This gas is what you fart.

Lesson #4: Farting isn't bad.

So are vegetables evil?

Lesson #5: ummm…. No.

So what are bacteria like?



What do bacteria do?

- They can be loners, and swim around as isolated individuals.
- They can talk to each other, and learn what’s going on.
- They can hang out with their friends.

When you are with your friends:

- Do you do things you don’t do when you’re by yourself?

How many of you have played Truth or Dare?

What did you do?

Teachers and grownups, cover your ears.

How many of you have played Truth or Dare?

What did you do?

When bacteria are together:

- They can talk to each other, and learn what's going on.
  - **They find out when it makes sense to behave cooperatively**
- They can hang out with their friends.

How many of you have played on a sports team, or in a band, or sung in a choir, or acted in a play?

Could you have done this by yourself?

Are you one cell, or many?

- **Lesson #6: Some things require cooperation to happen.**

Bacteria talk to each other through a process called quorum sensing

- Bacteria make chemicals called autoinducers
- They sense the concentration of autoinducers
- When there are a lot of autoinducers, they know there are lots of bacteria nearby
  - Now it makes sense to behave cooperatively!

Bacteria that sense a quorum change their gene expression

- They still have the same genes

- But the ways they use these genes change.
  - Some genes are used more
    - We say they are “expressed” more, or “upregulated”
  - Some genes are used less
    - We say they are “expressed” less, or “downregulated”

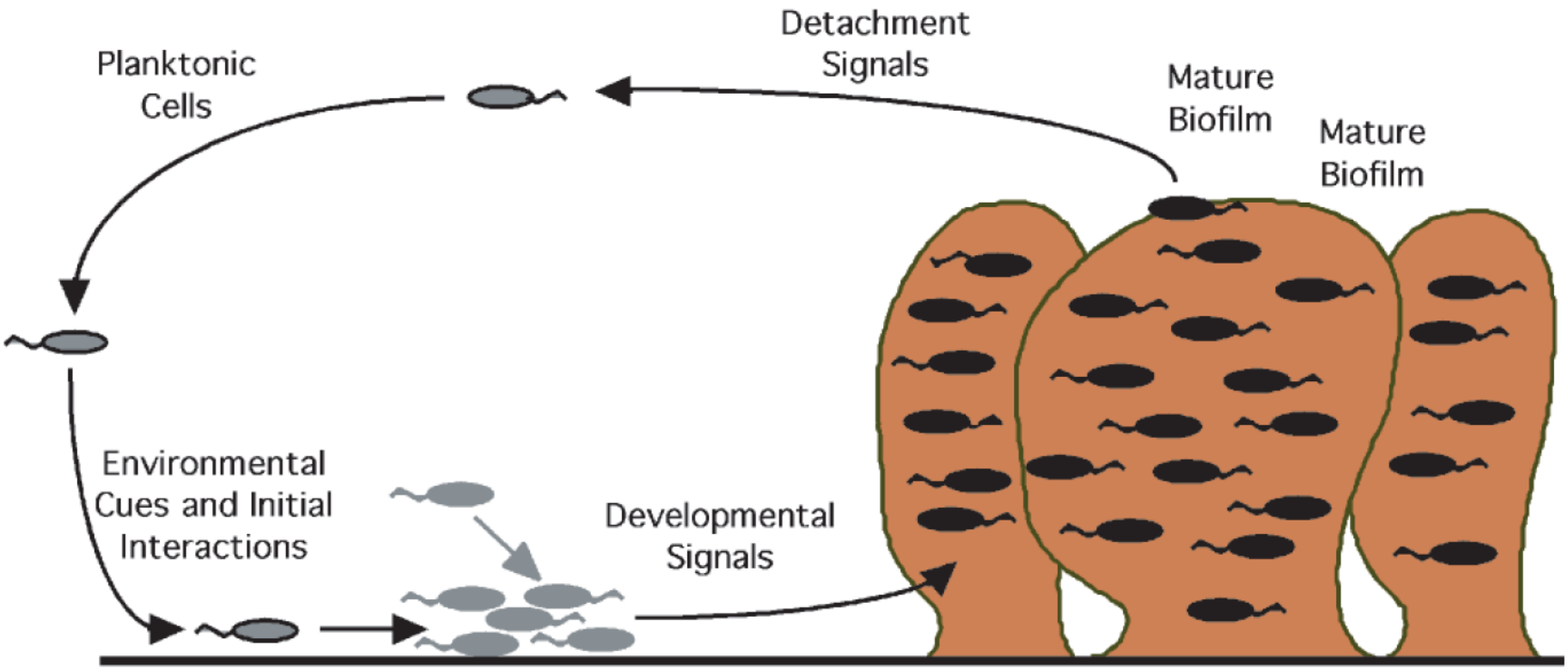
Bacteria that sense a quorum change their gene expression

- Lesson #7: Bacteria can cooperate.
- Lesson #8: Bacteria cooperate when they sense a lot of similar bacteria around them.
- Lesson #8: Bacteria cooperate by changing their gene expression.

When bacteria are together:

- They can talk to each other, and learn what's going on.
  - **They find out when it makes sense to behave cooperatively**
- They can hang out with their friends.
  - **They form a biofilm.**

Life cycle of a Biofilm



Biofilm Formation as Microbial Development. O'Toole, Kaplan, and Kolter. Annu. Rev. Microbiol. 2000. 54:49–79. Modified.

Biofilms are important problems in biomedicine and industry

Pseudomonas aeruginosa in biofilms: – **resistance to antibiotics** – **virulence**

- Lung infections in cystic fibrosis

  most common genetic disease in US

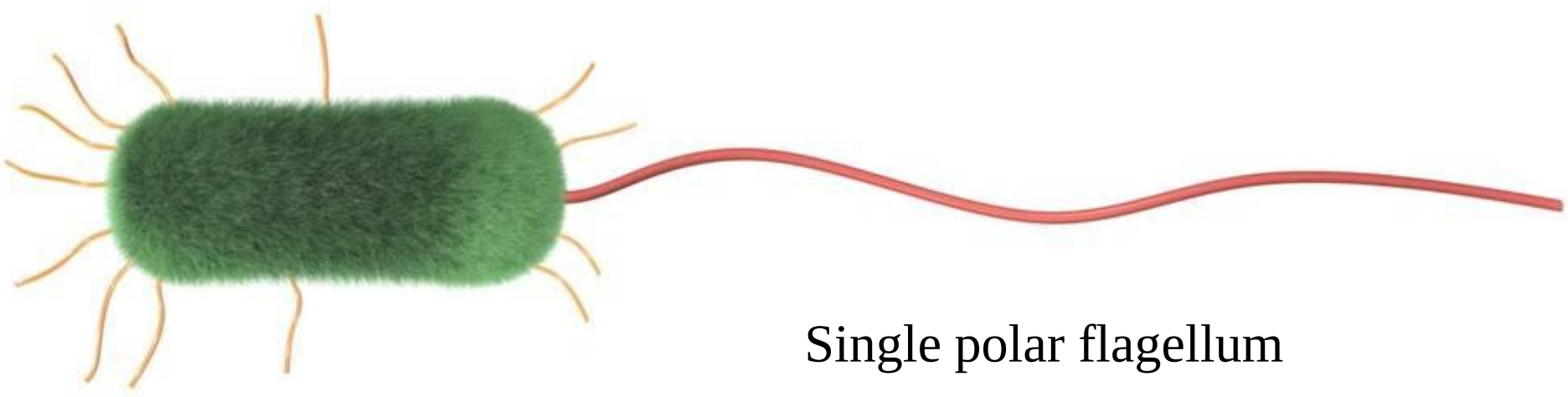
  life expectancy of ~30 years

- Acute ear infections; bacterial endocarditis; dental plaque
- Infections on medical implants
- Industrial biofouling

  nearly every industrial water-based process

  pipe plugging and corrosion; water contamination

Physics tools probe the interplay between motility, matrix production, and signaling networks



Single polar flagellum

Type IV pili

Open question:

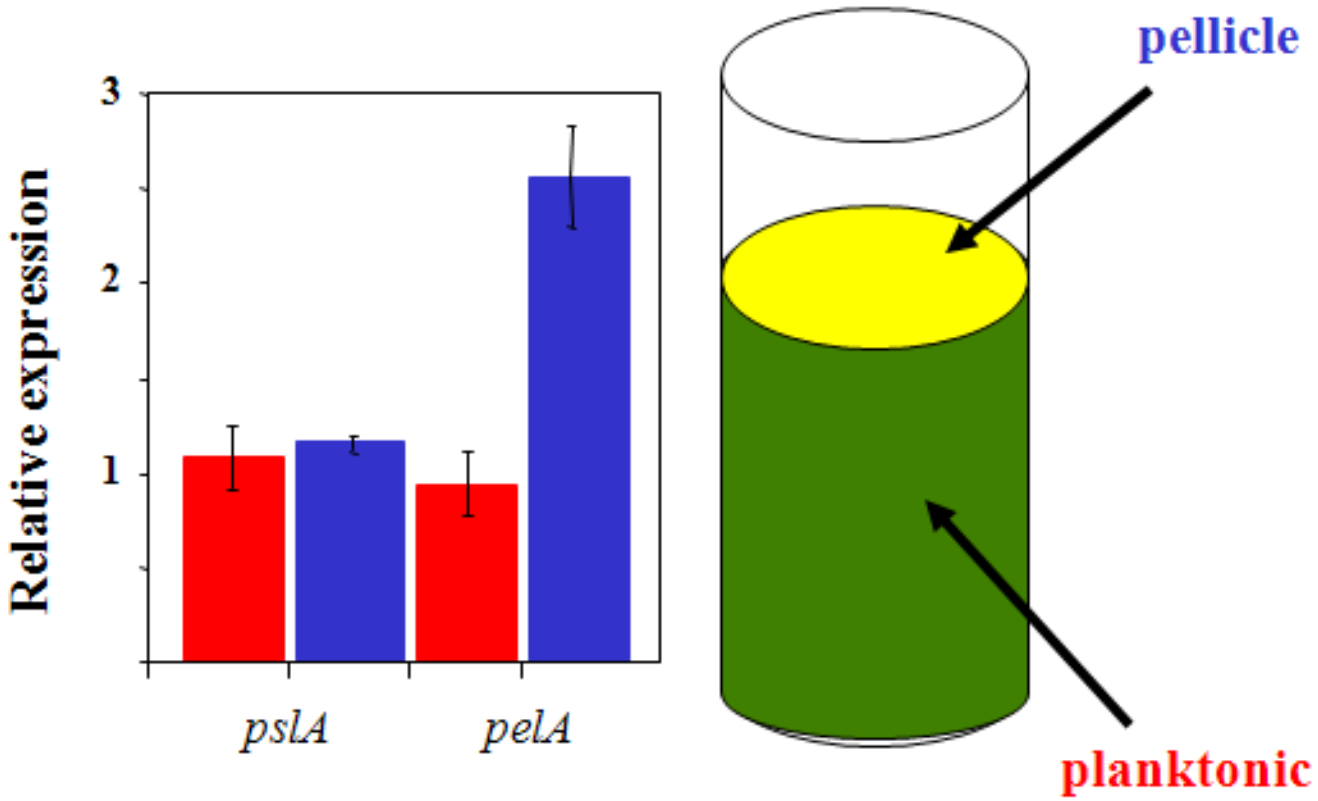
What are the key initial steps for microcolony formation and biofilm initiation?

Bacteria must

- **sense they are at a surface**
- **initiate production of some EPS**

  many possible candidates
- **interact specifically with other bacteria**

pel expression is induced in pellicles formed in standing liquid cultures



Data from Borlee and Parsek, University of Washington, Seattle

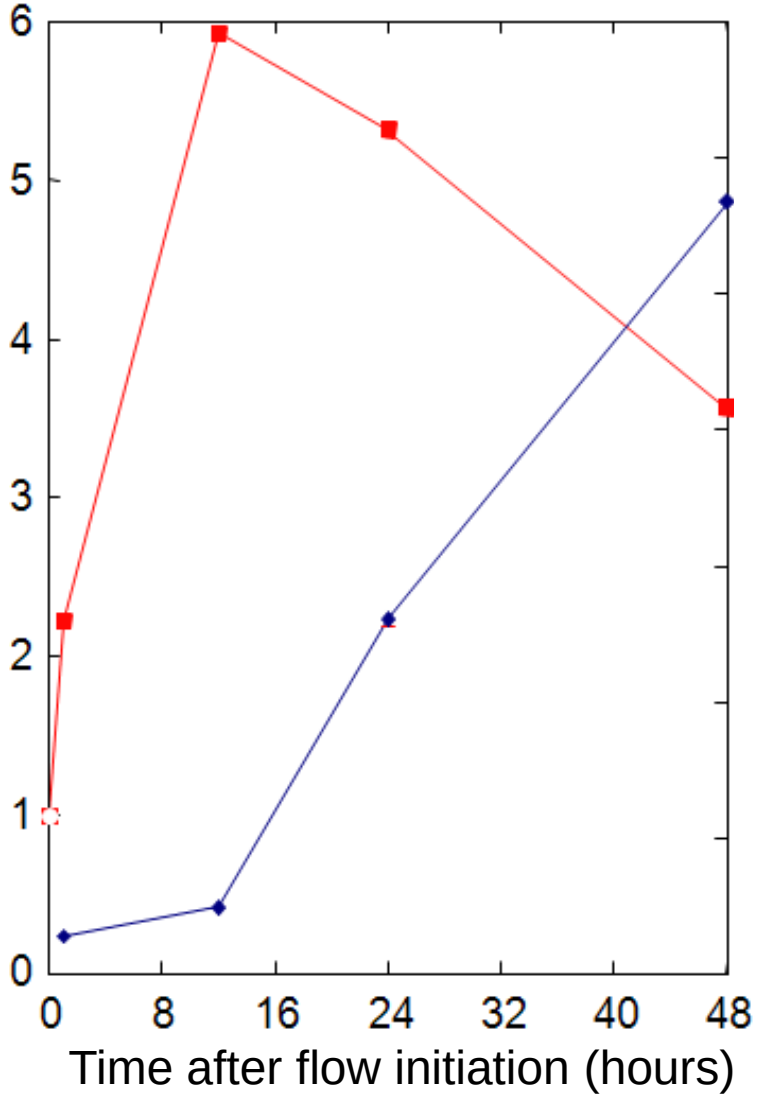
pelA expression induced after surface adhesion

P. Aeruginosa biofilm grown in a silicone tube:

- incubate statically for 30 min
- begin flowing fresh medium
- adherent cells harvested off surface

monitor gene transcription levels and viability of cells in biofilm:

Pel turns on early in biofilm development, but turns off as the biofilm matures.

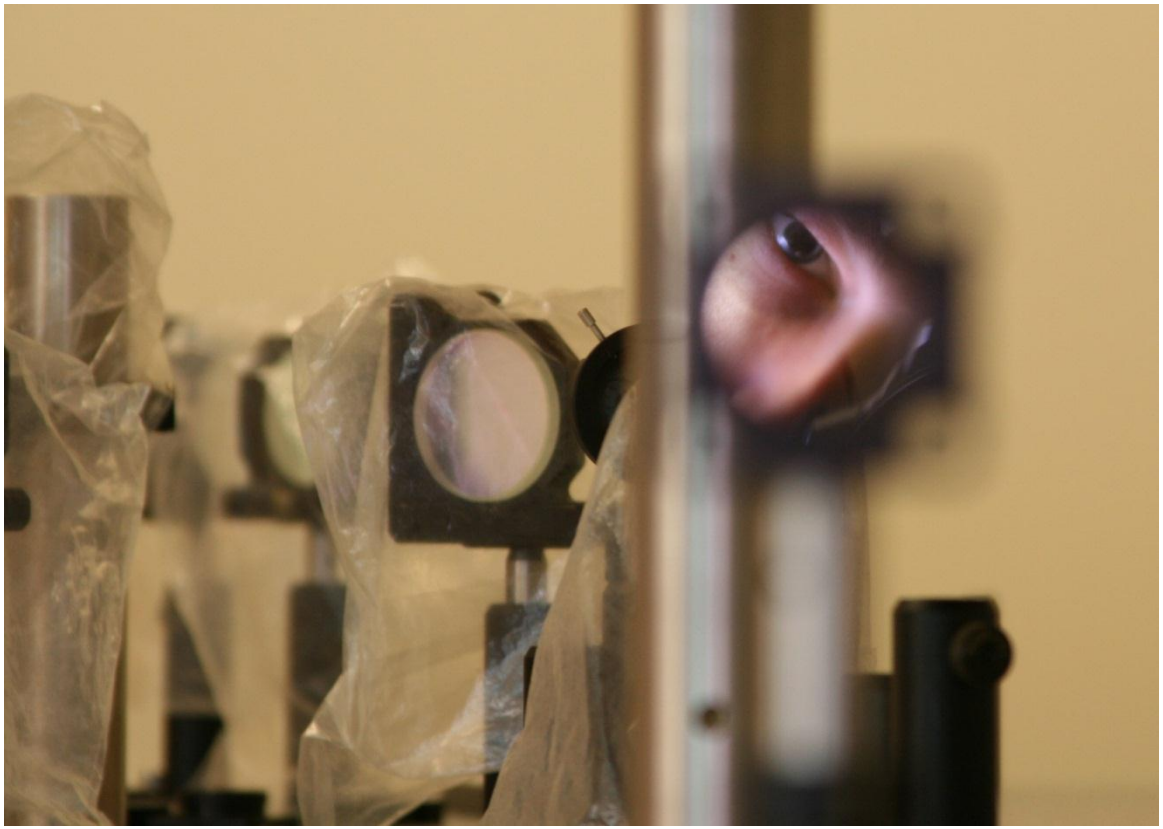


Data from Borlee and Parsek, University of Washington, Seattle

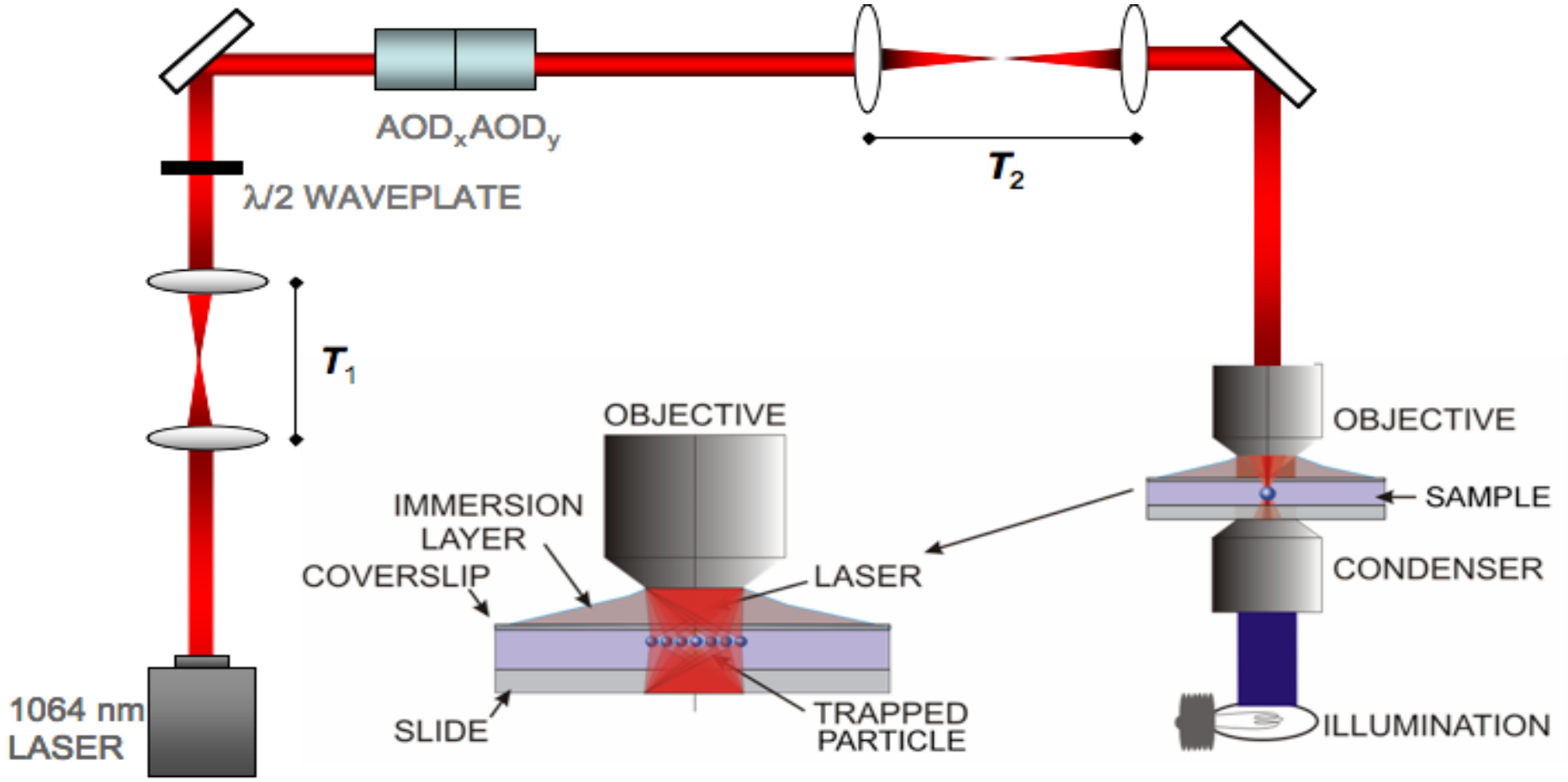
CFU = colony-forming unit (typically 1 cell)

Physics gives new optical techniques

- Manipulation of bacterial cells
  - In groups
  - Singly



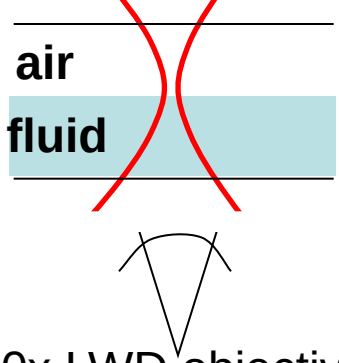
Laser-trapping setup



- Built on inverted microscope
- Simultaneous trapping and imaging in brightfield transmission or fluorescence

Laser-directed aggregation

Making Pel is essential for bacteria aggregation on short timescales!

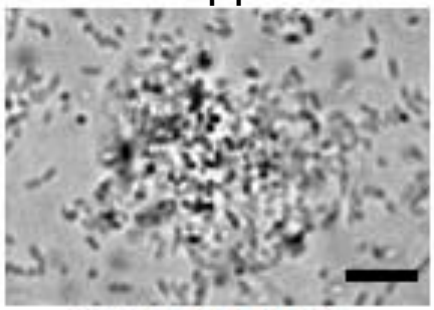


40x LWD objective

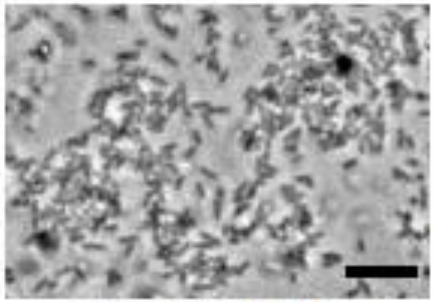
trapped

released

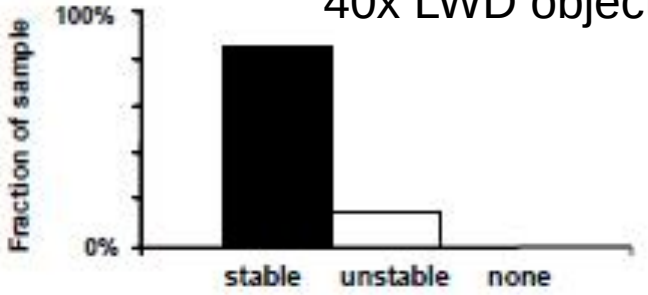
Pel



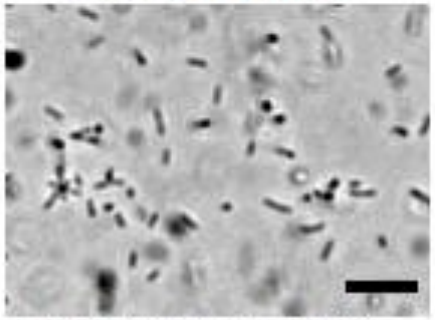
PA14 trapped 20 min



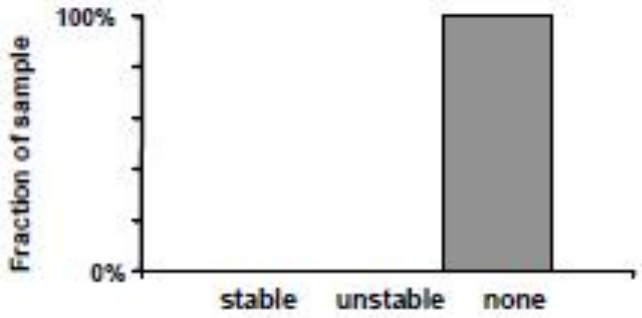
PA14 released 5 min



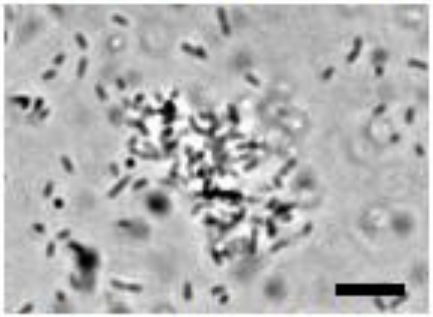
No Pel



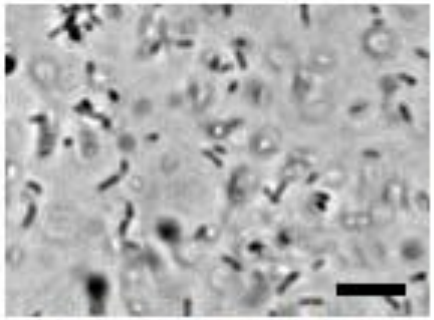
PA14Δ*pelB* trapped 20 min



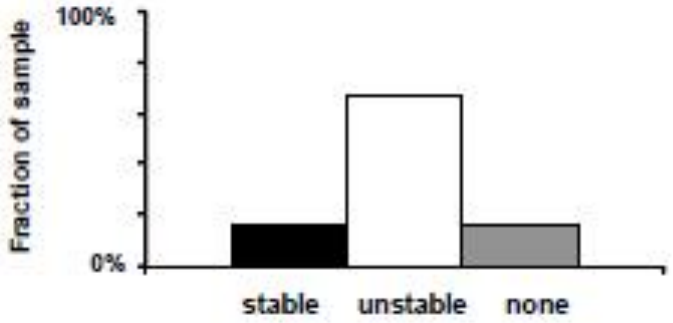
No Pel



PA14Δ*pelB* trapped 45 min



PA14Δ*pelB* released 5 min



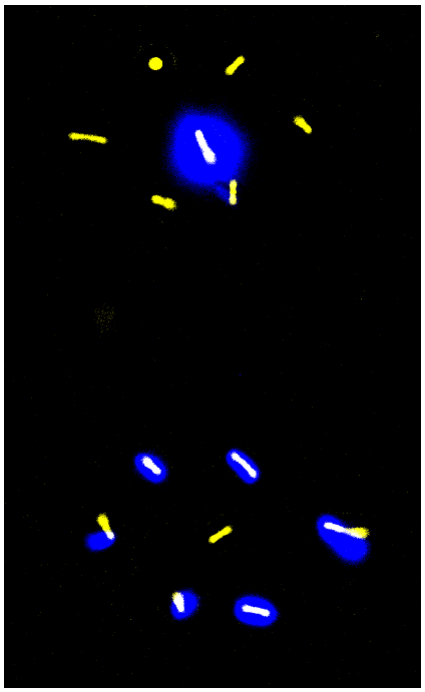
What we've learned:

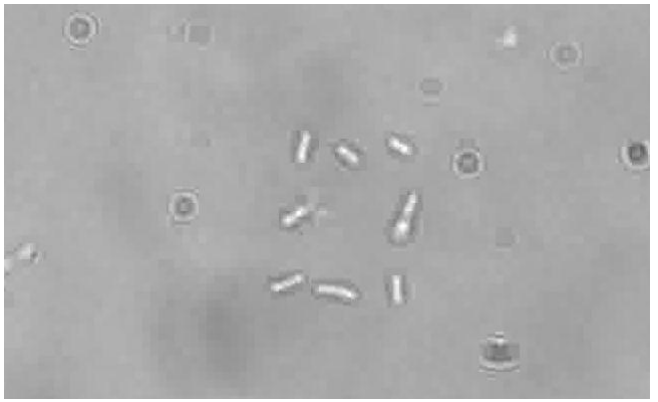
- *pel* is the molecular glue first activated when bacteria sense a surface or each other
- *pel* is responsible for inter-bacterial adhesion early in biofilm development

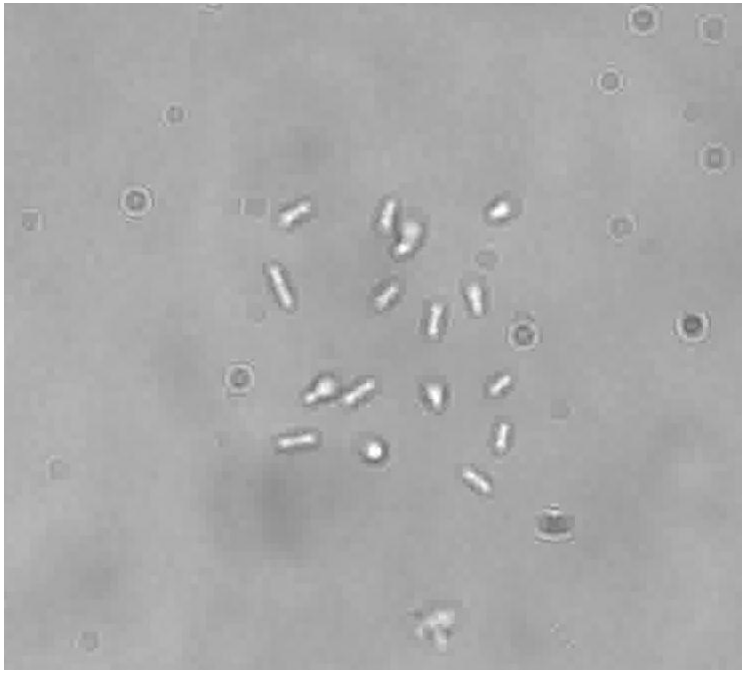
K. M. Colvin, V. D. Gordon, *et al*, in submission to PLOS Pathogens

Future Work

- How do interbacterial interactions depend on spatial structure?



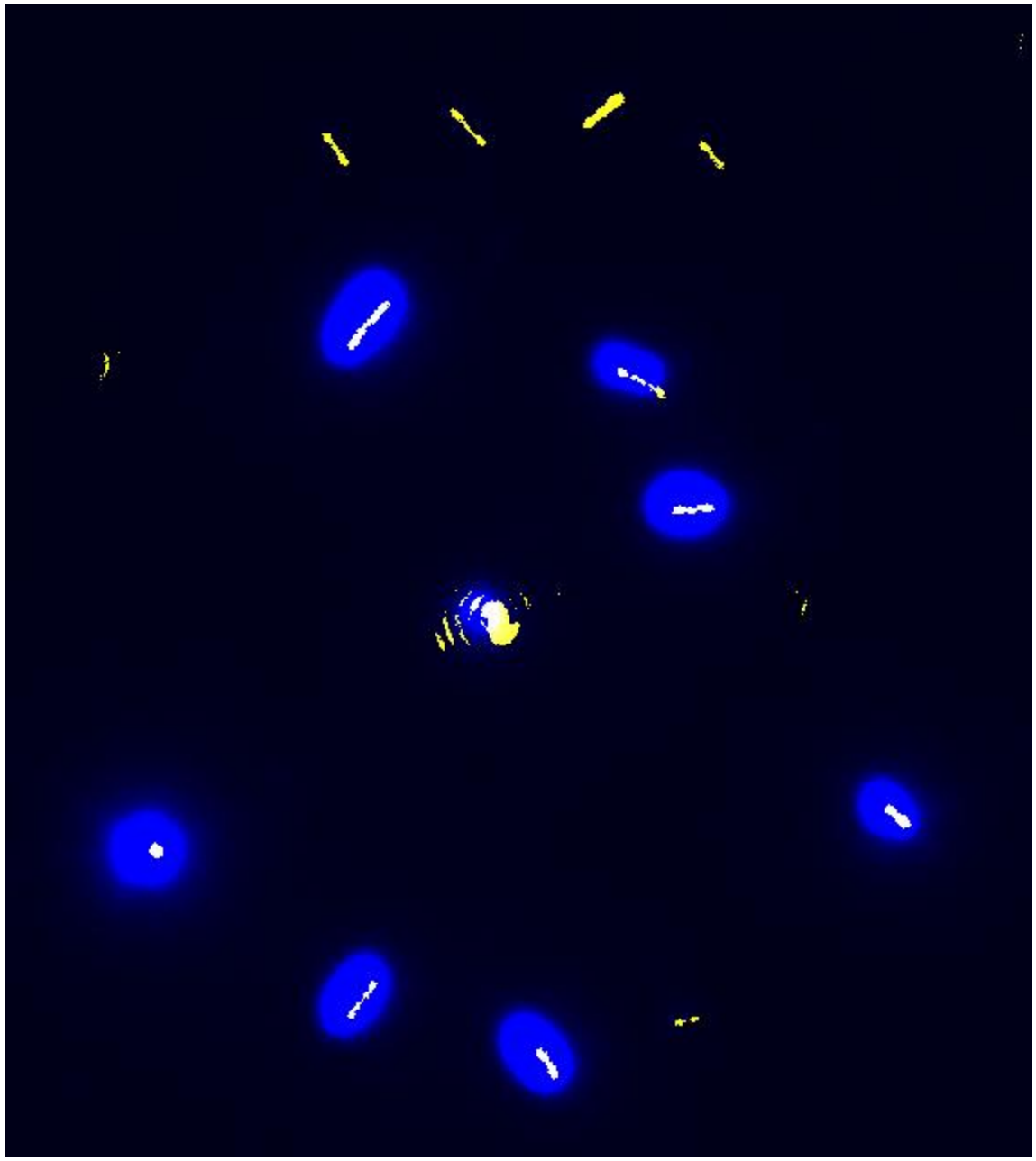




Lessons from today:

- **Question authority!**
- **Sometimes the answer depends on the way you ask the question.**
- **Numbers can be manipulated.**
- **Some things require cooperation to happen**
- **Bacteria can cooperate**
  - **When they sense lots of nearby bacteria**
  - **By changing their gene expression**

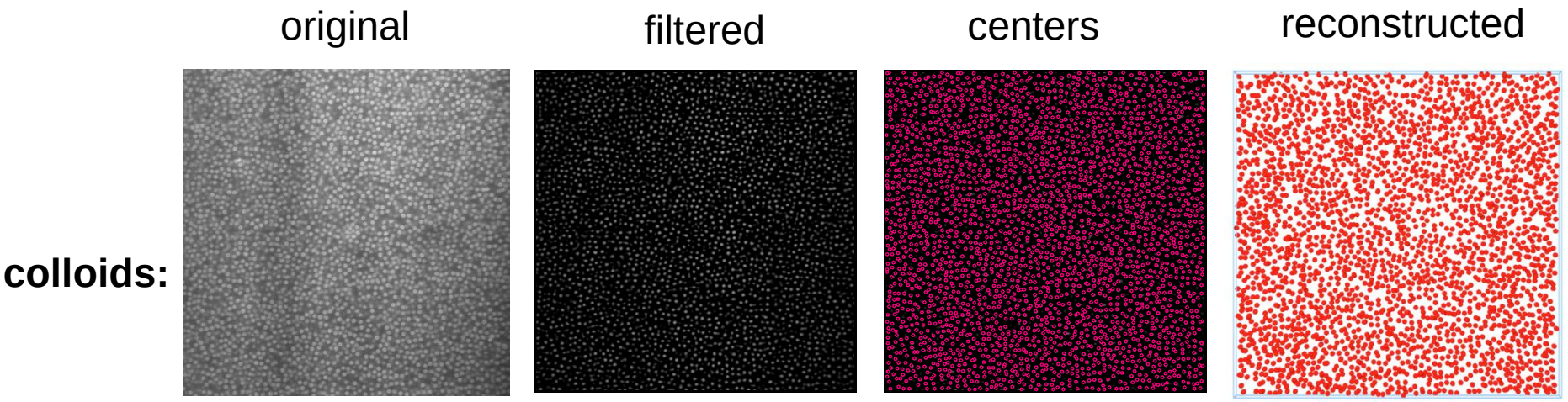
Thank you!



High throughput tracking and analysis of bacterial surface motility

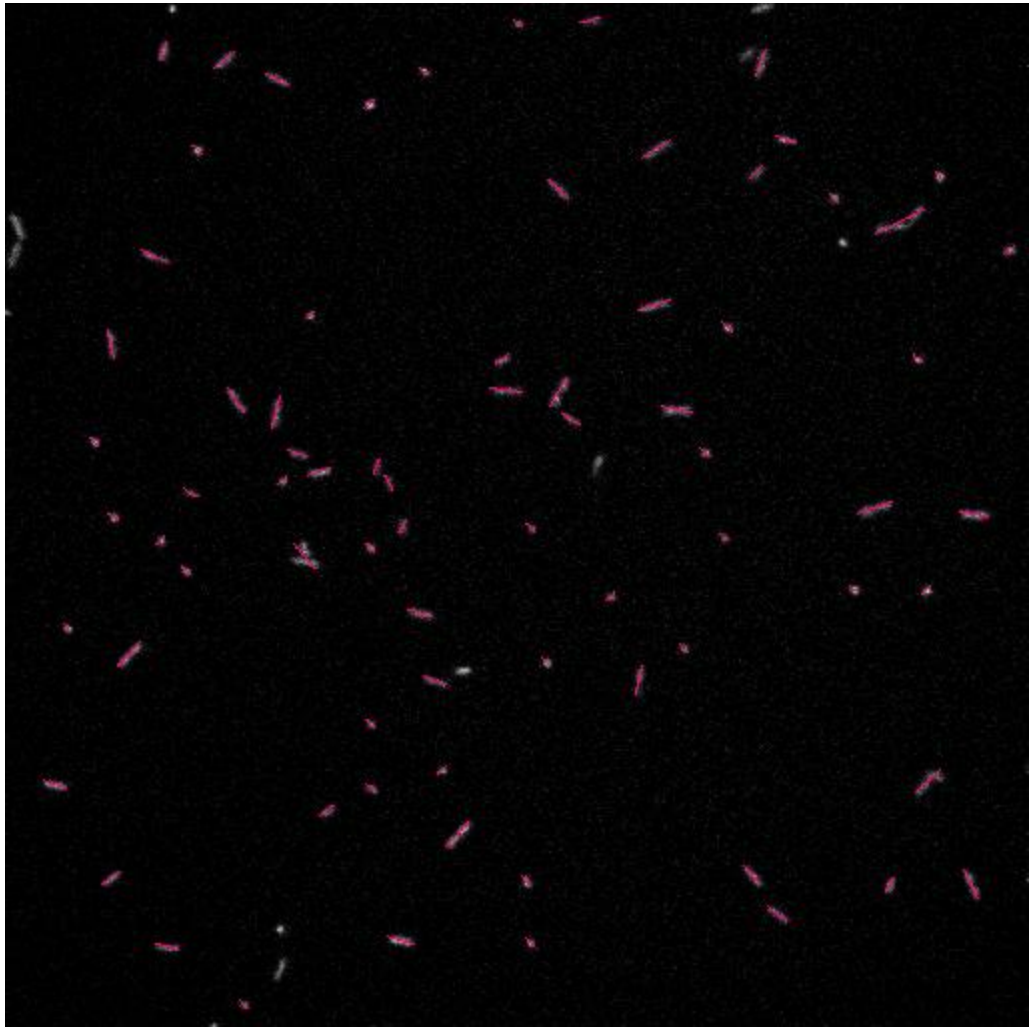
- Codes developed for colloid physics :

- Find centers (& characteristics - orientation, aspect ratio, etc.)
- Link coordinates and characteristics to form trajectories.
- Trajectories reconstruct the original movie's moving bacteria

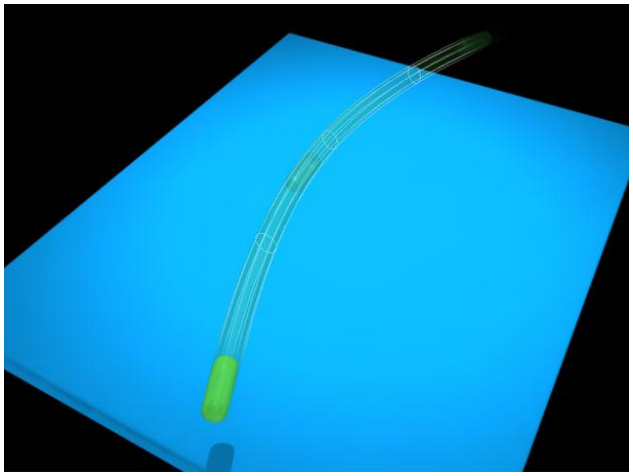


Particle-tracking reference: J. C. Crocker and D. G. Grier, J. Colloid Interface Sci. **179**, 298 (1996)

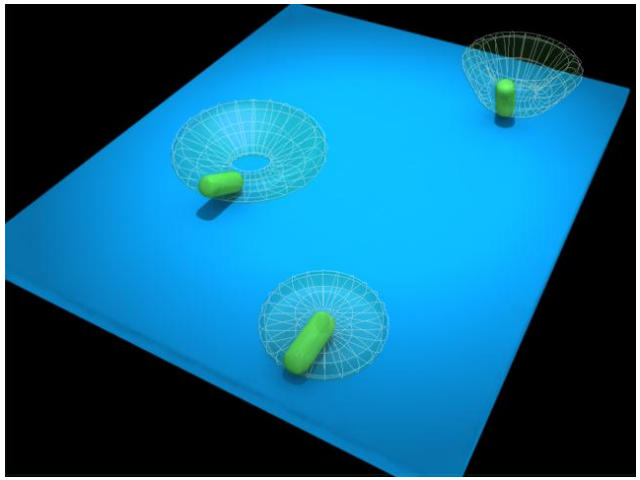
Moving bacteria overlaid with tracked info



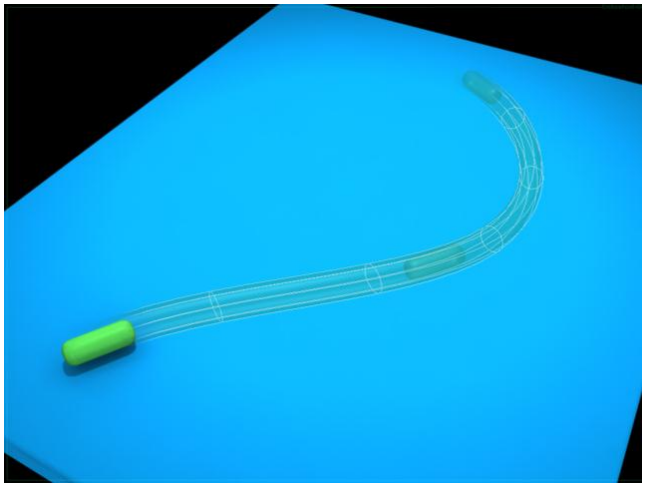
Tracking identifies distinct motility modes



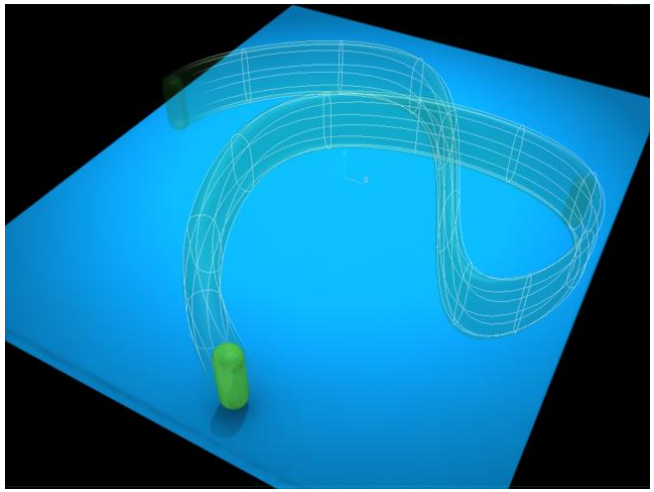
Flagellum-based "skimming"



Flagellum-based "Spinning"



Pili "Crawling" motility



Pili "Walking" motility

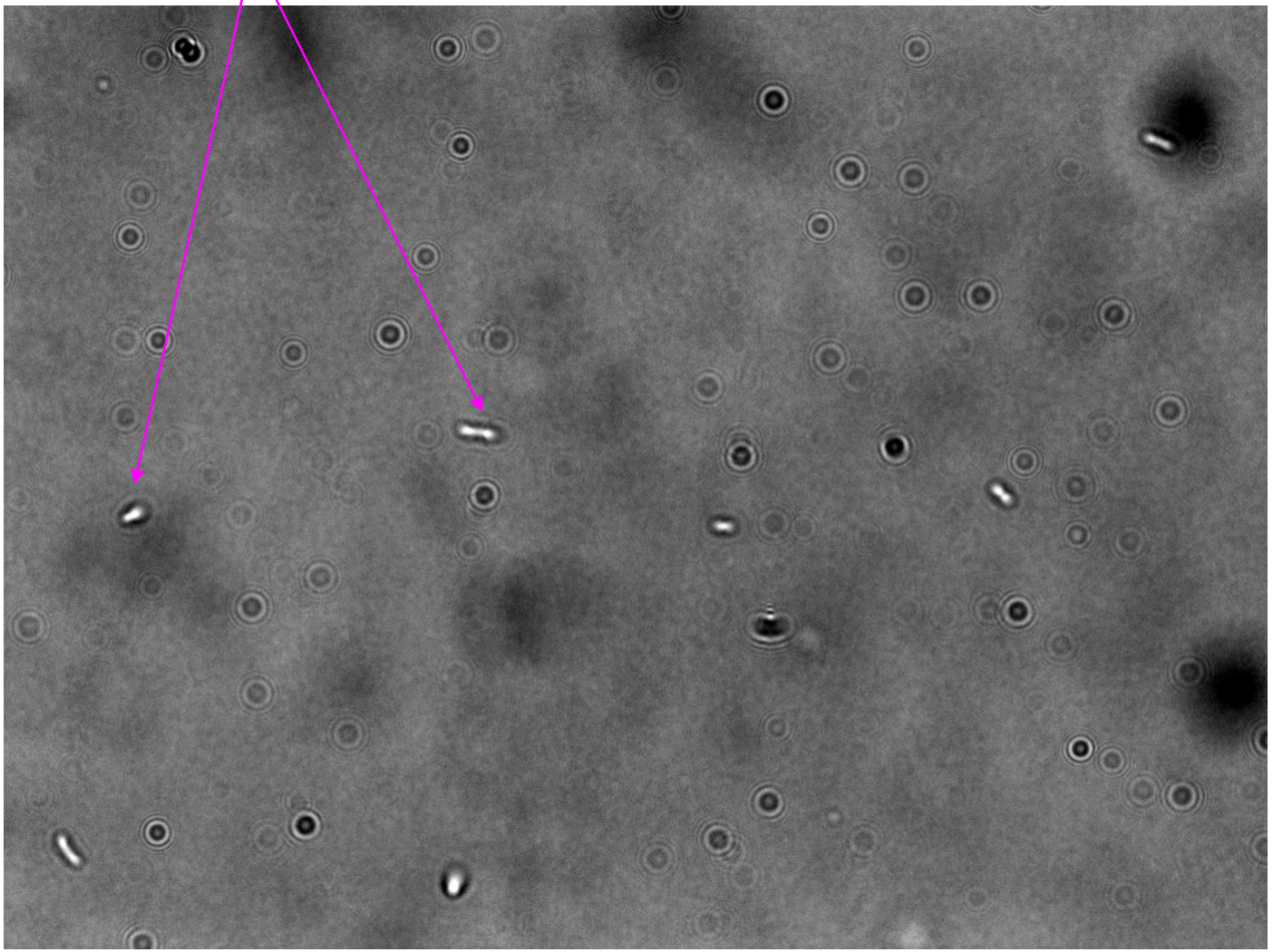
M. Gibiansky, J. Conrad, et al., accepted in Science

Bacterial associations result from movement and division

P. aeruginosa initially attach randomly to glass coverslip.

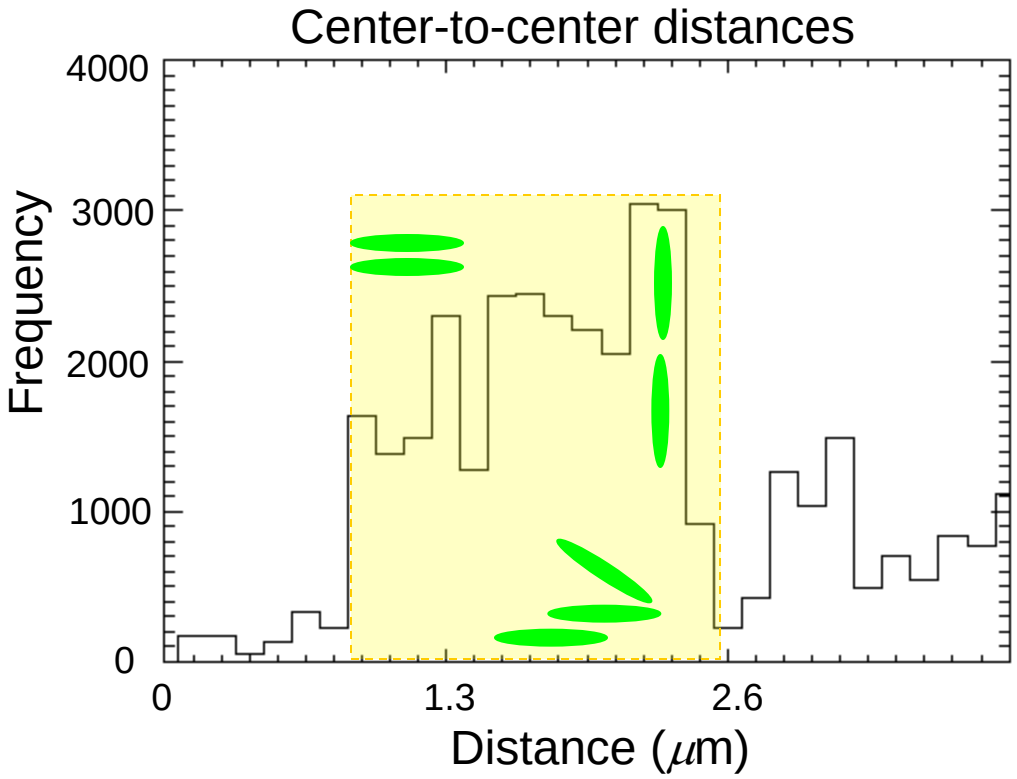
10 μm

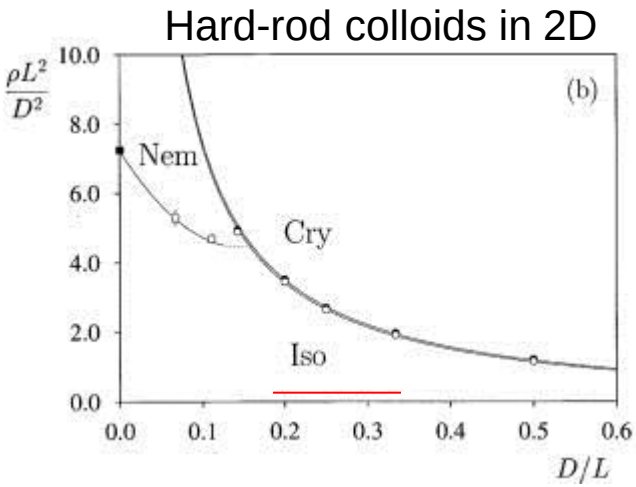
1min/frame



Widefield microscopy segments adjacent *Pseudomonas* using Fraunhoffer diffraction.

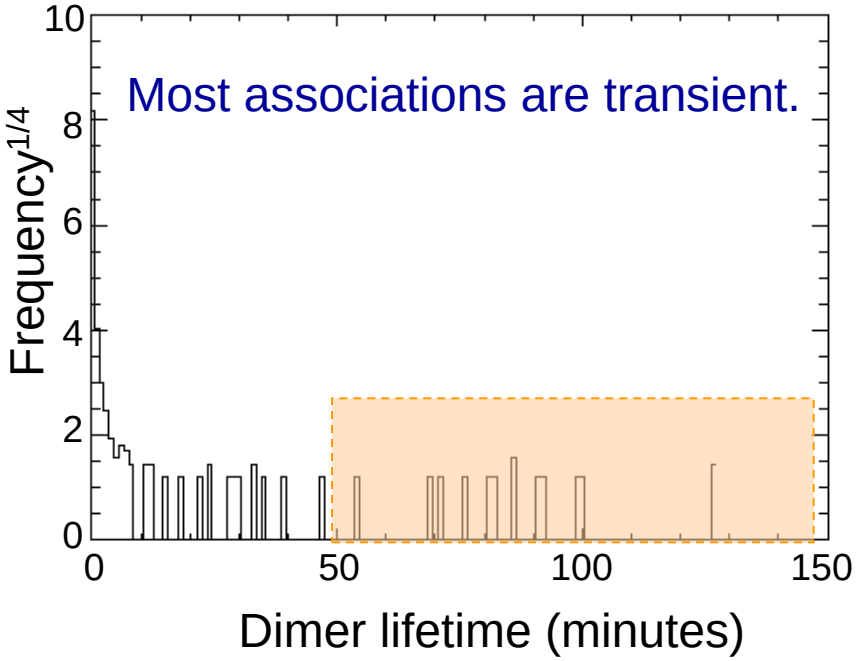
Bacteria cluster at surprisingly-low concentrations





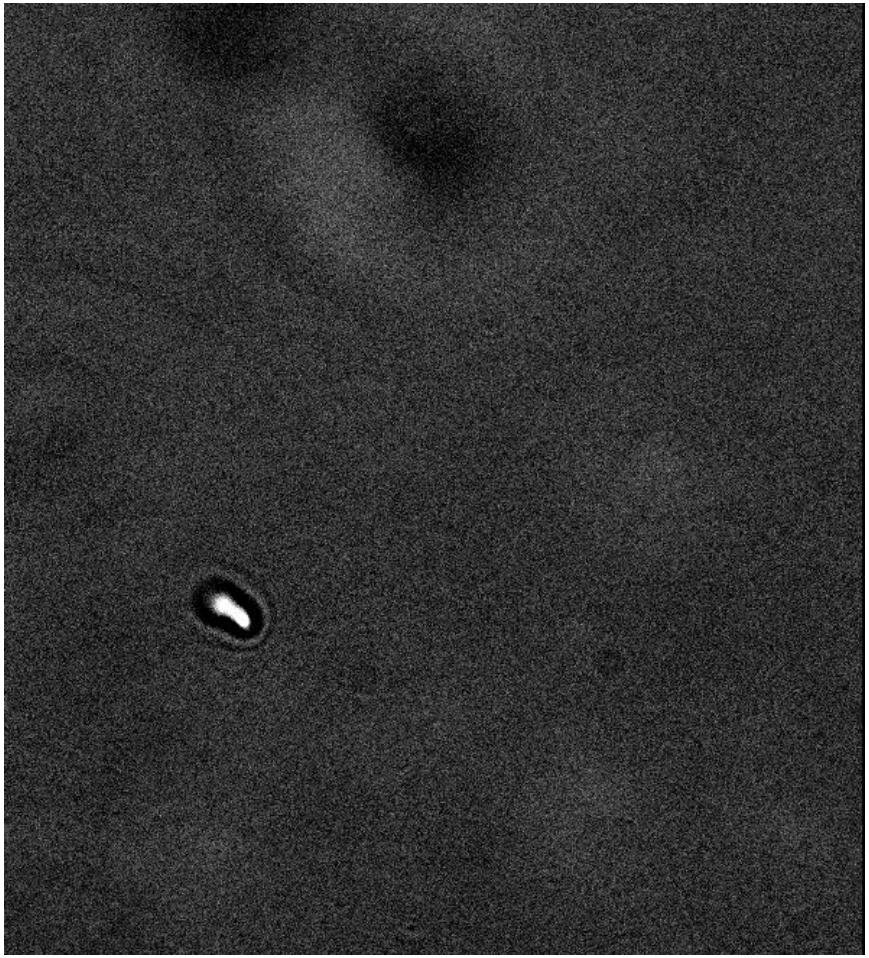
Area fraction ~0.006

Bates & Frenkel (2000) JCP 110:10017–10033



What causes long-lived associations (and thus leads to biofilm formation)?

Likelyhood of forming permanent associations increases with time (and time around neighbors?)



Other things change over time:

- **Attaching permanently to surface**

  - entropy cost paid for not being throughout system volume

  - EPS *psl* gene is important

- **Surface population**

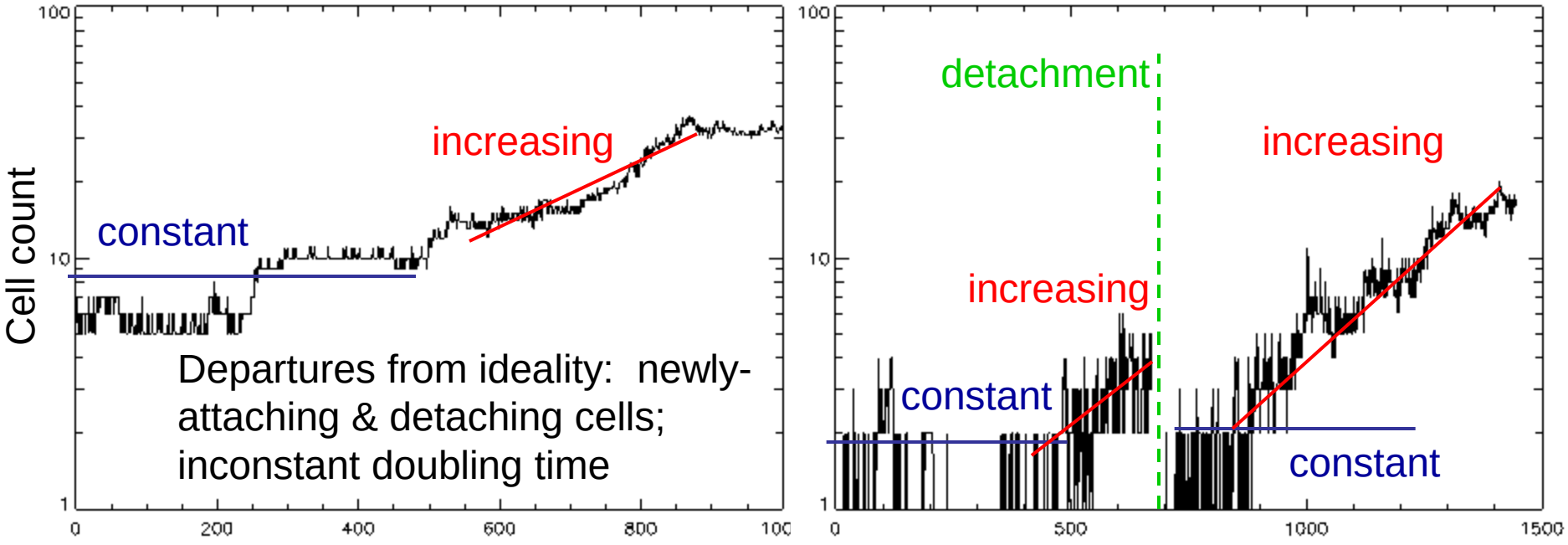
  – stop detatching post-division

Trackable transitions in early biofilm formation

- Attachment → Permanent Attachment
- Permanent Attachment → Surface Population
- Onset of Permanent Aggregation

“Permanent Attachment → Surface Population” Signature 1

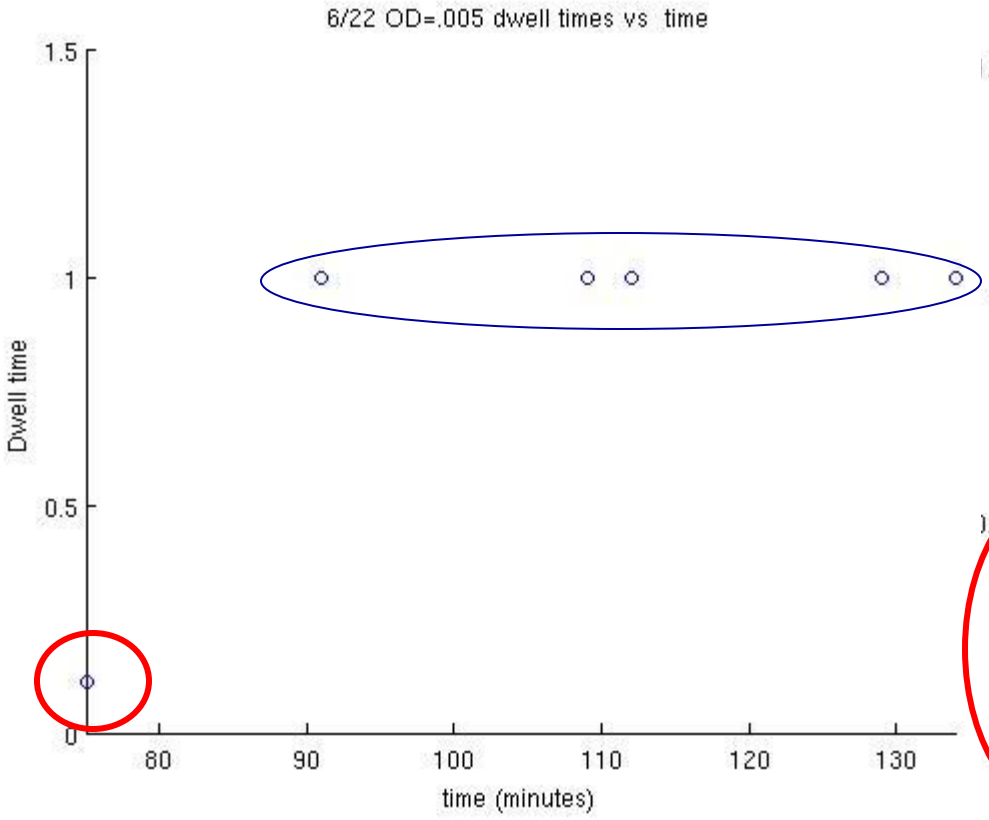
Cell count: constant → ~ exponentially increasing

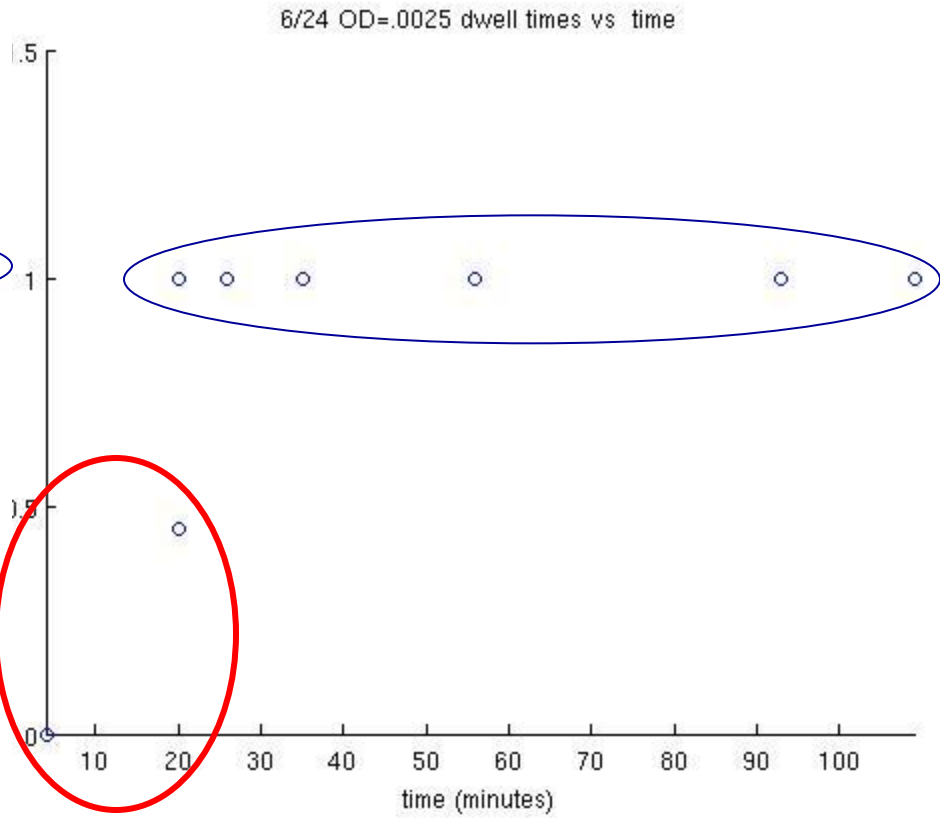


Time in experiment (15 sec)

“Permanent Attachment → Surface Population” Signature 2?

- Post-division dwell time of detaching daughter





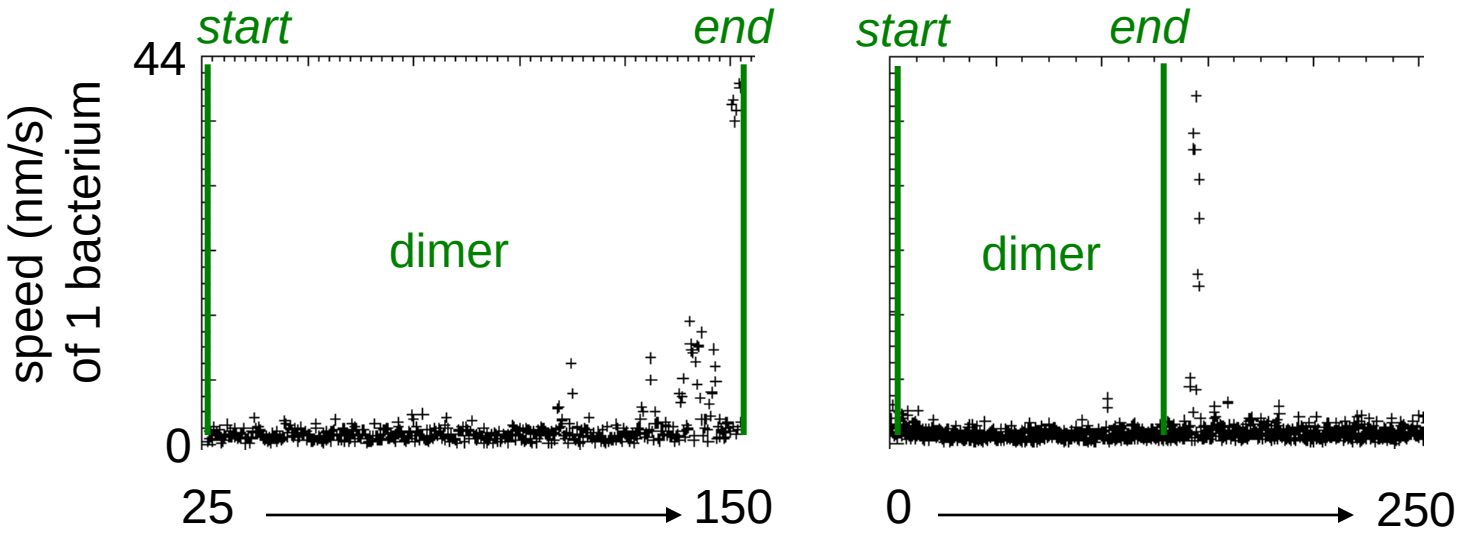
Work in progress

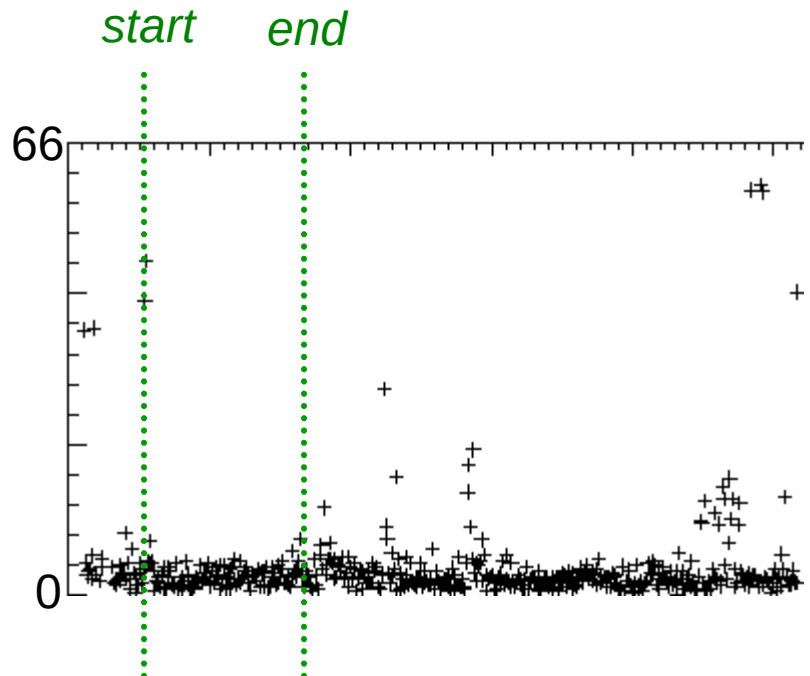
Current work on this

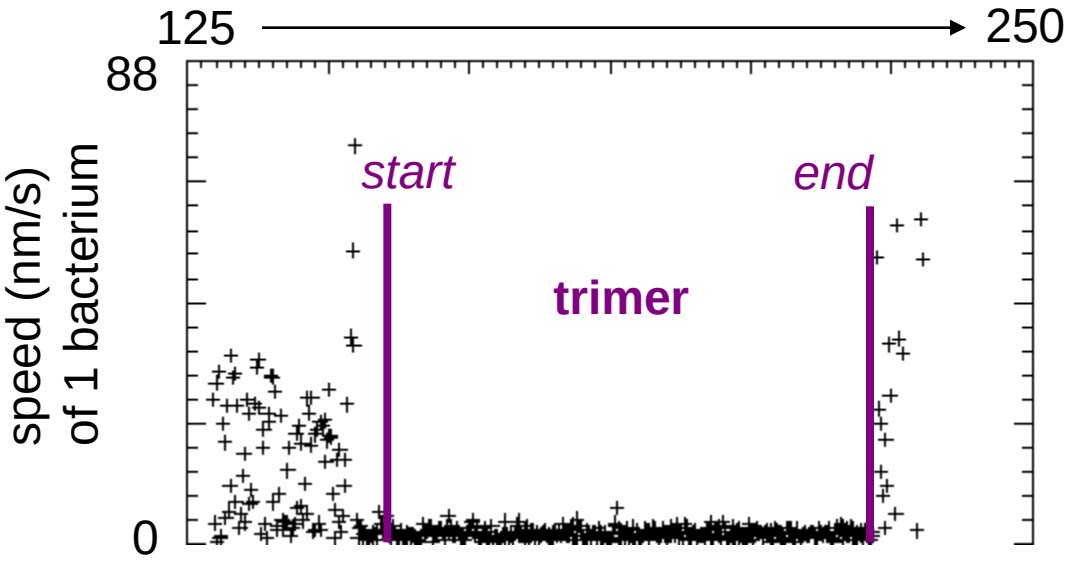
Develop and implement code for identifying and quantifying key transitions in early biofilm formation (and effects of chemotaxis)

Travis Thatcher - Physics undergrad

Bacteria jump *into* (50-60%) and *out of* (~33%) associations





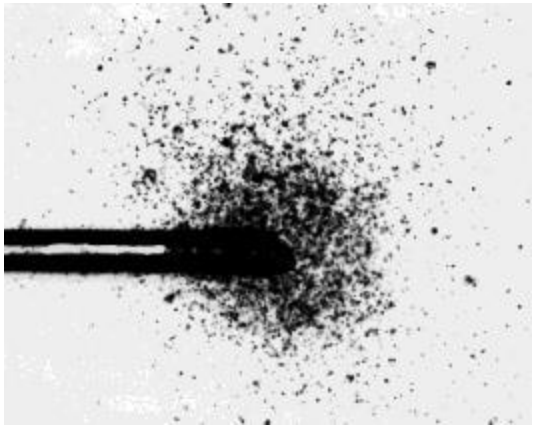


Do “jumps” result from chemotaxis?

- My guess is “no, jumps are caused by nonspecific attraction”

So, what role *does* chemotaxis play in biofilm development?

- *P. aeruginosa* shows chemotaxis towards components of CF mucin
  - This has mostly been studied in bulk, fluid situations

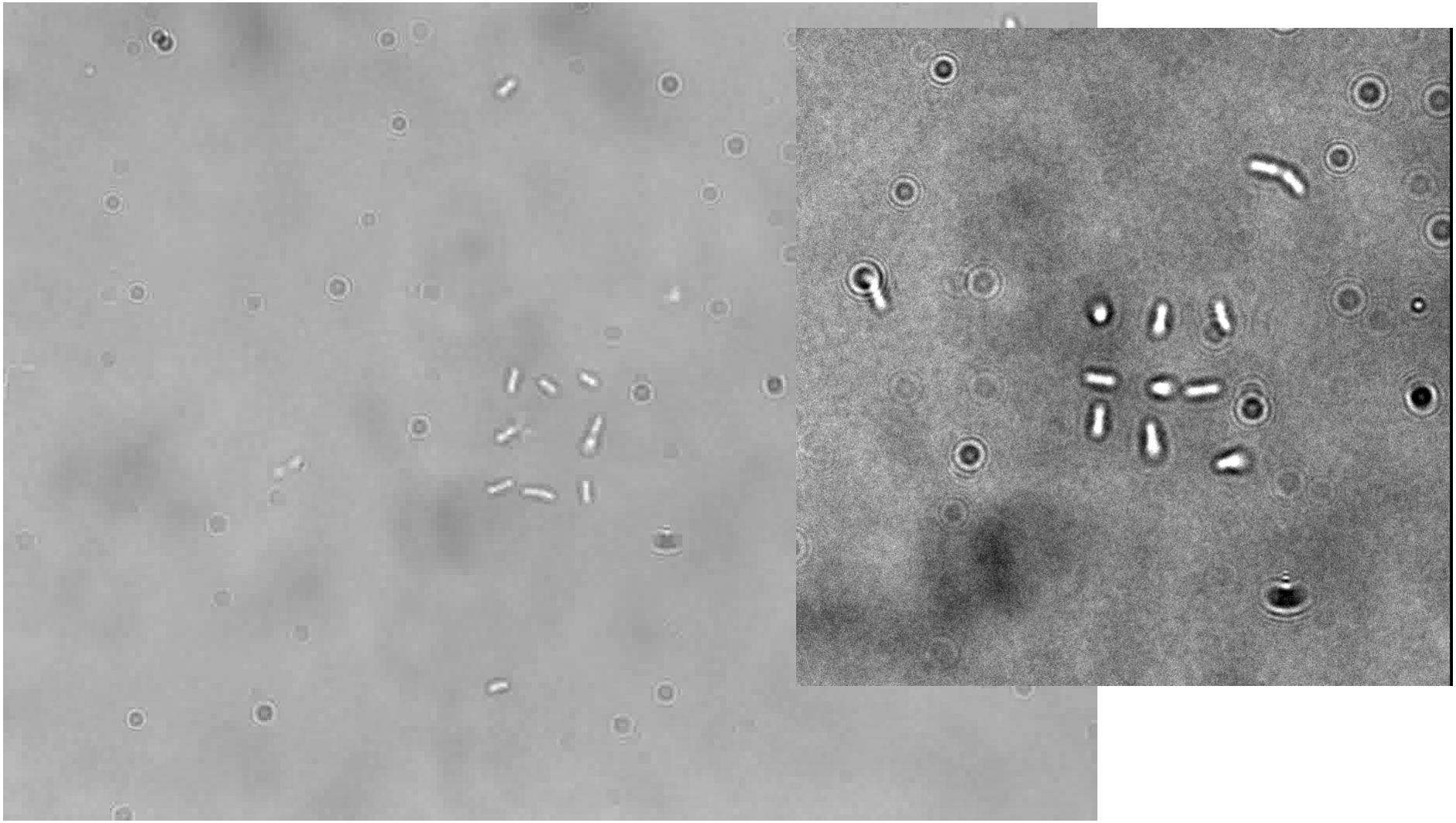


We are in a unique position to quantitatively study surface-motility chemotaxis at very low cell numbers

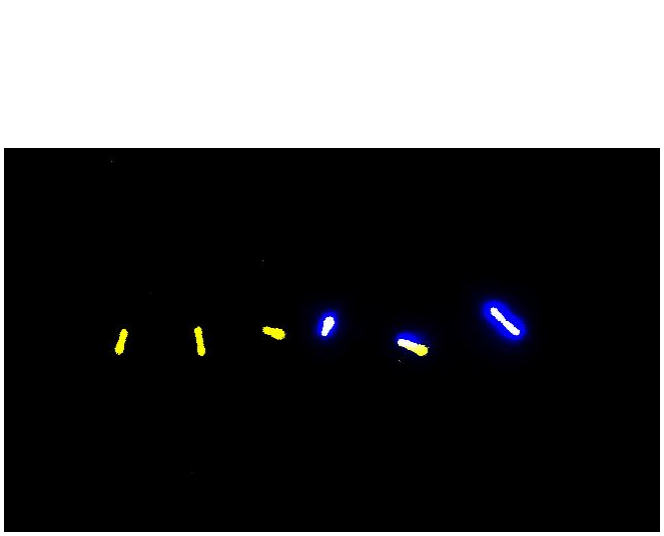
The conditions most relevant for biofilm initiation!

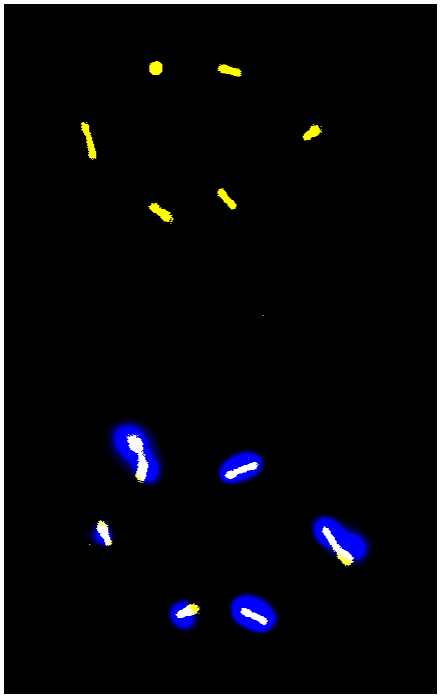
Sam Bienvenu and Shinji Strain – physics undergrads

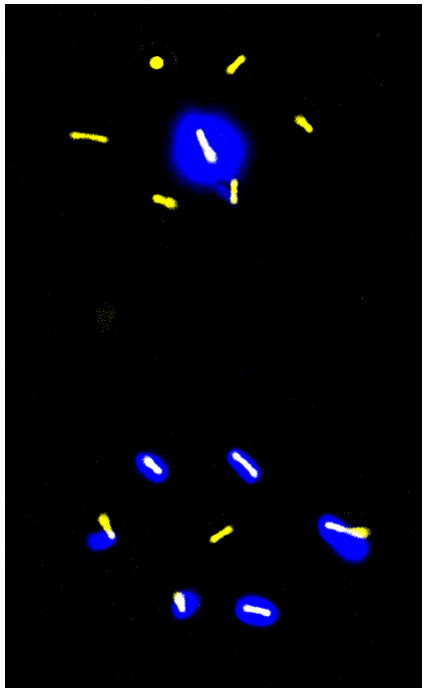
Laser tweezers “stamping” bacteria will allow study of autotaxis as a function of neighbor spatial distribution



Laser “stamping” allows study of other intercellular interactions



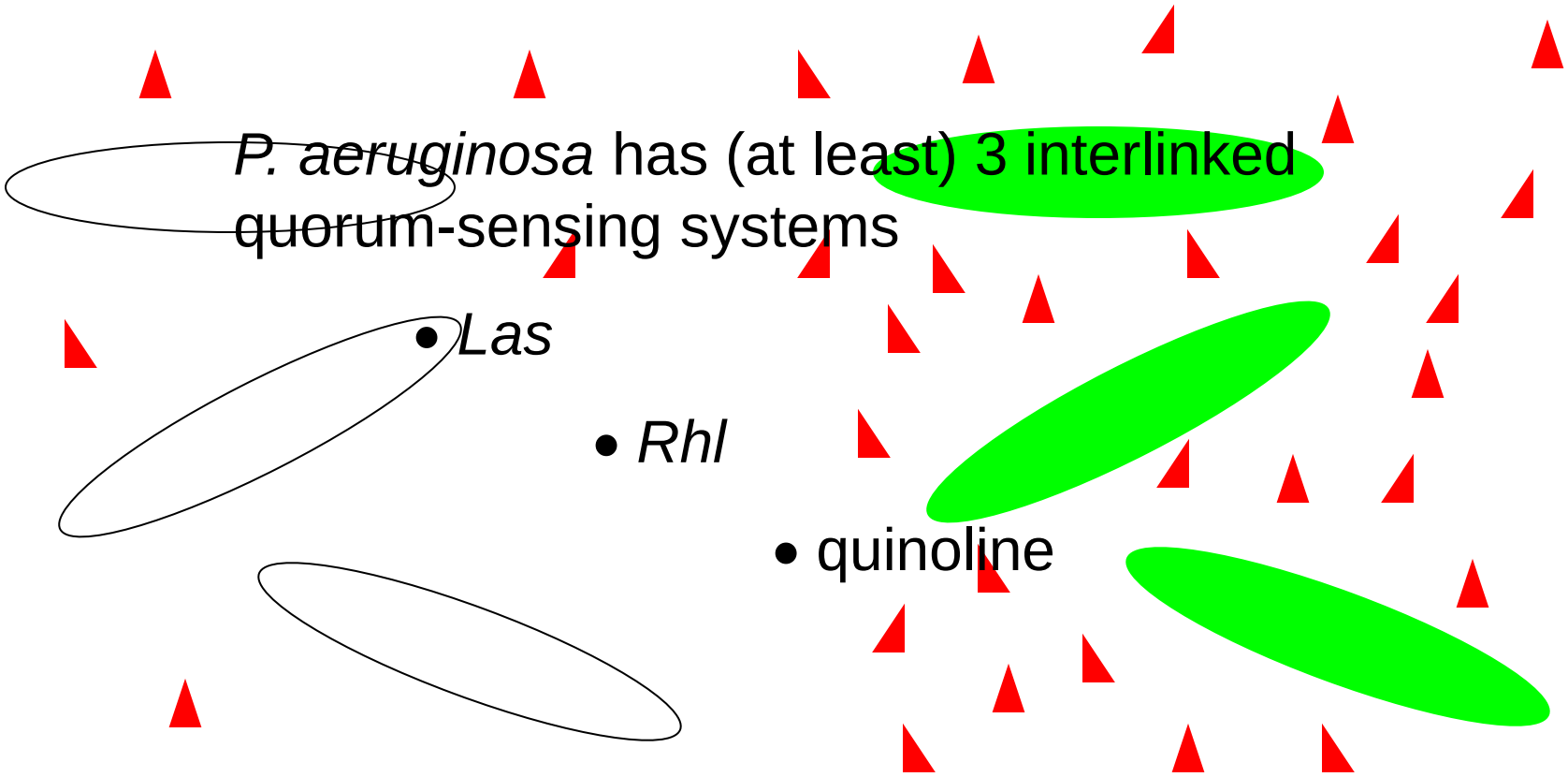




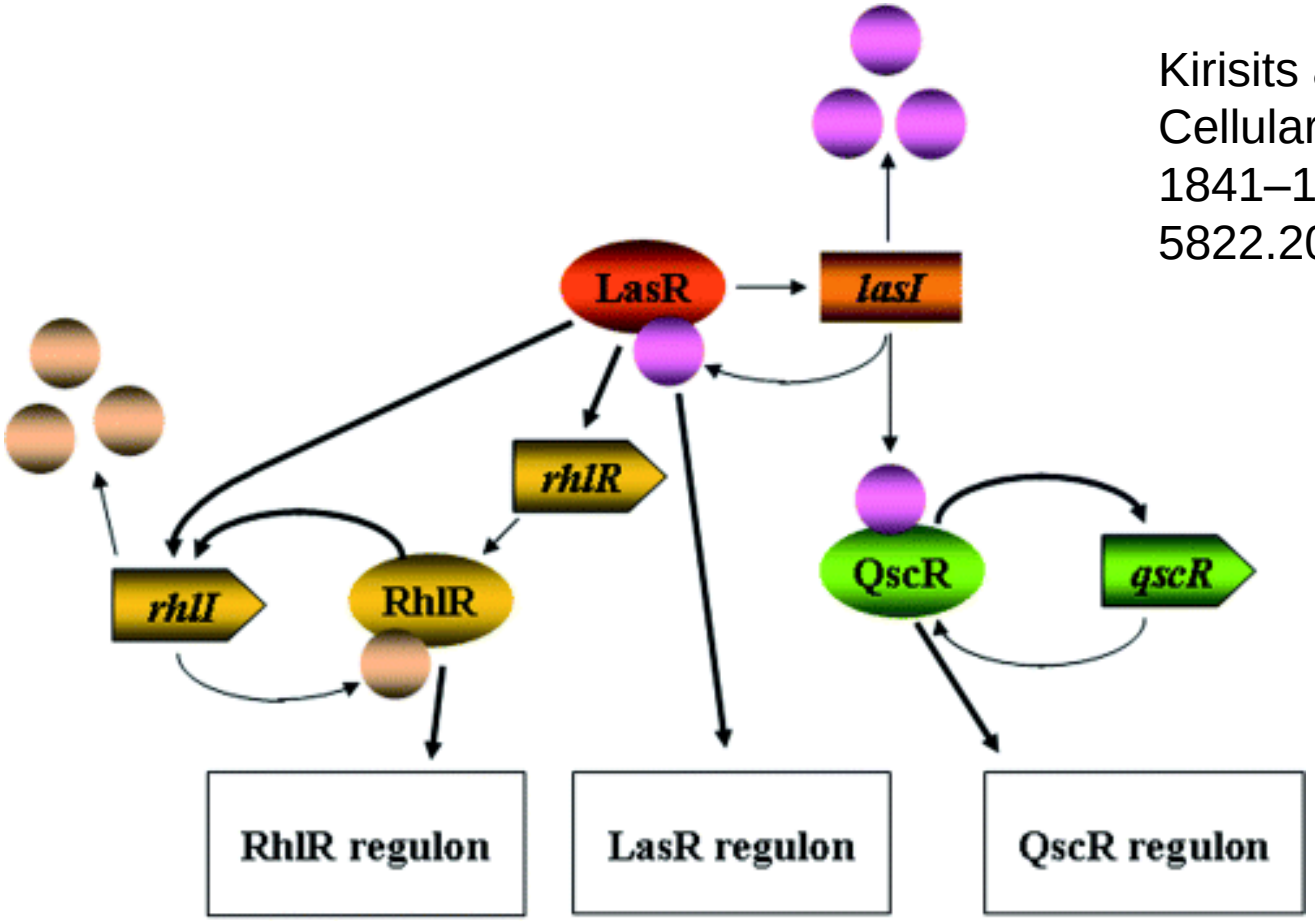
Quorum Sensing

Bacteria produce and release chemical *autoinducers* and sense their concentration.

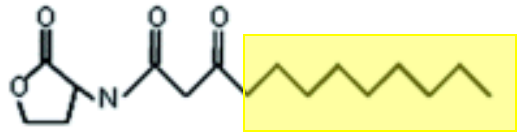
When the concentration of autoinducers is high enough, *quorum sensing* is initiated and gene expression alters.



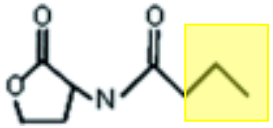
Kirisits and Parsek (2006)
Cellular Microbiology 8 (12) ,
1841–1849 doi:10.1111/j.1462-5822.2006.00817.x



= 3-oxododecanoyl homoserine lactone



= butyryl homoserine lactone



The major structural difference is in the length of the hydrophobic tails!

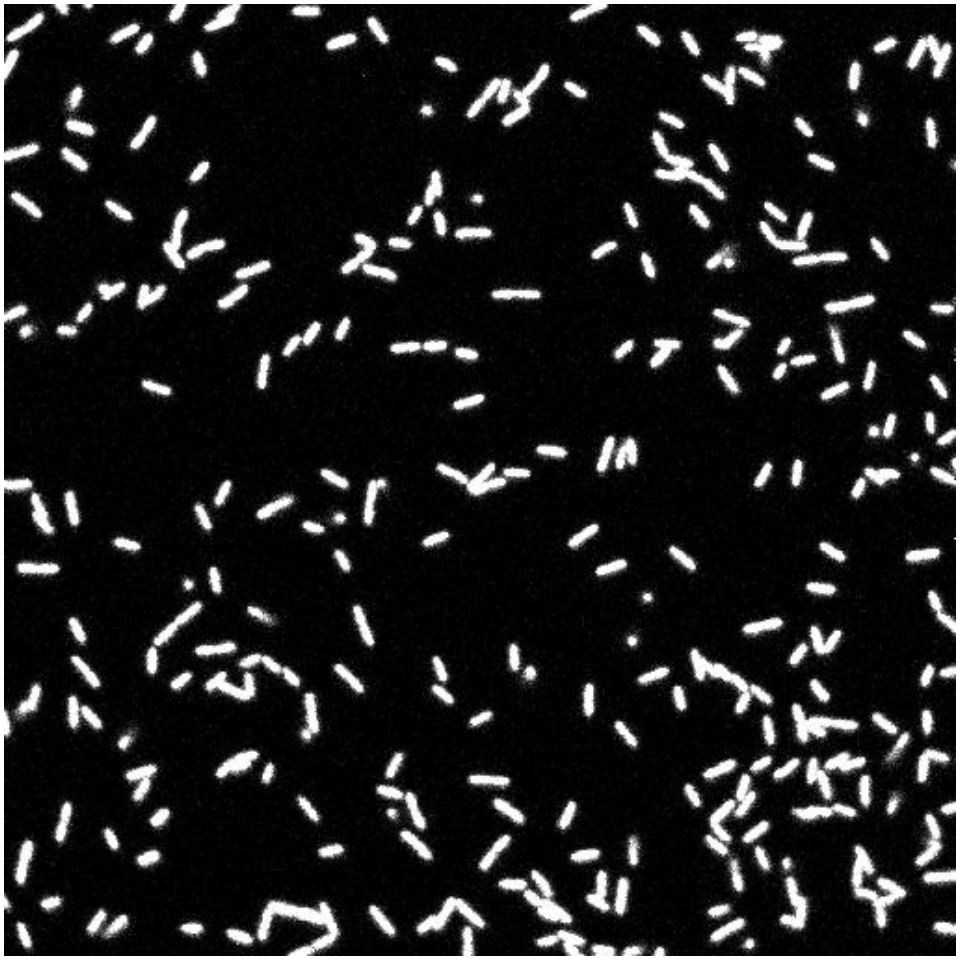
What role does autoinducer physical chemistry (amphiphilicity) play in quorum-sensing signal delivery, localization, and persistence?

Becky Morrison – ICMB graduate student

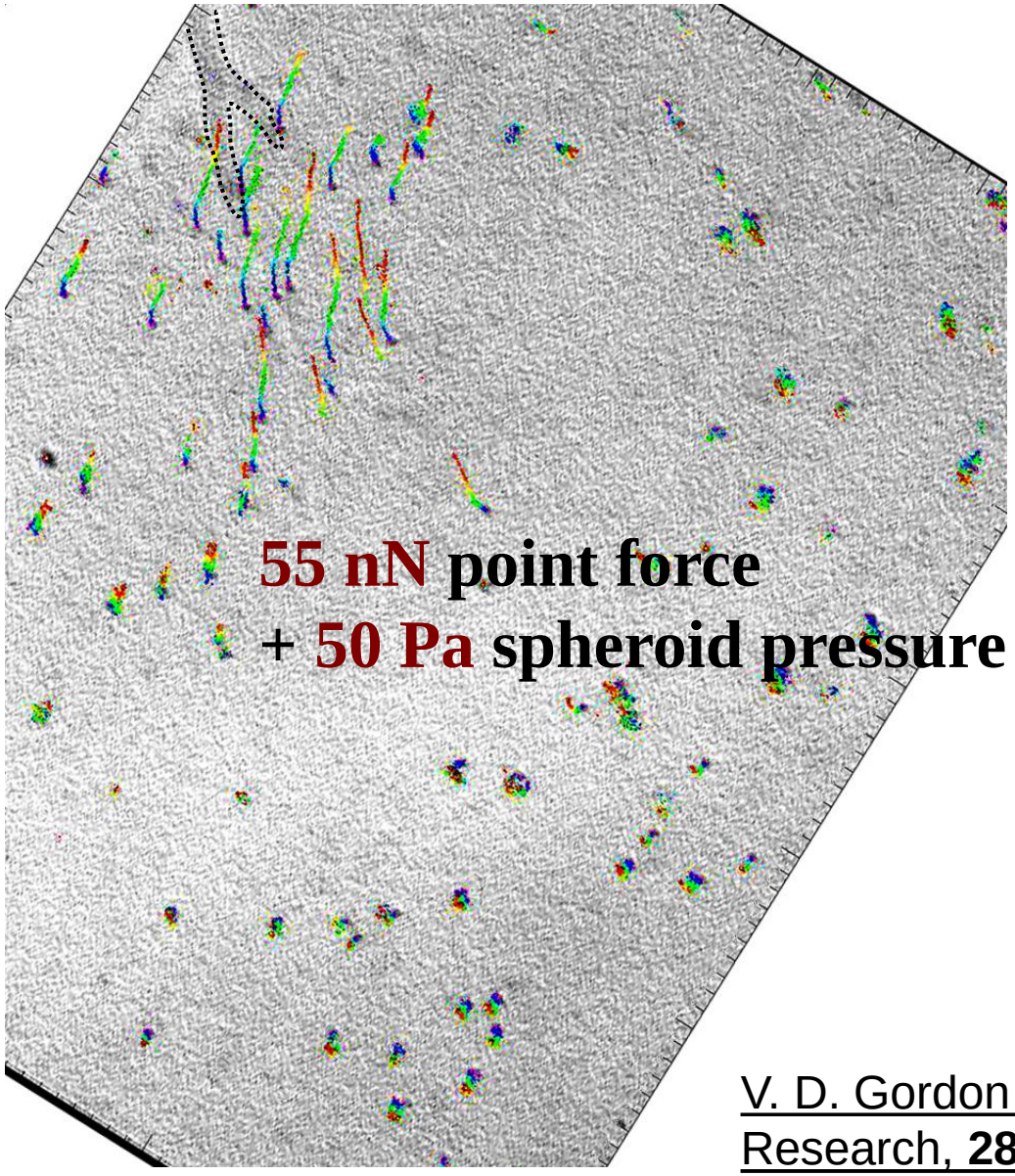
Other questions:

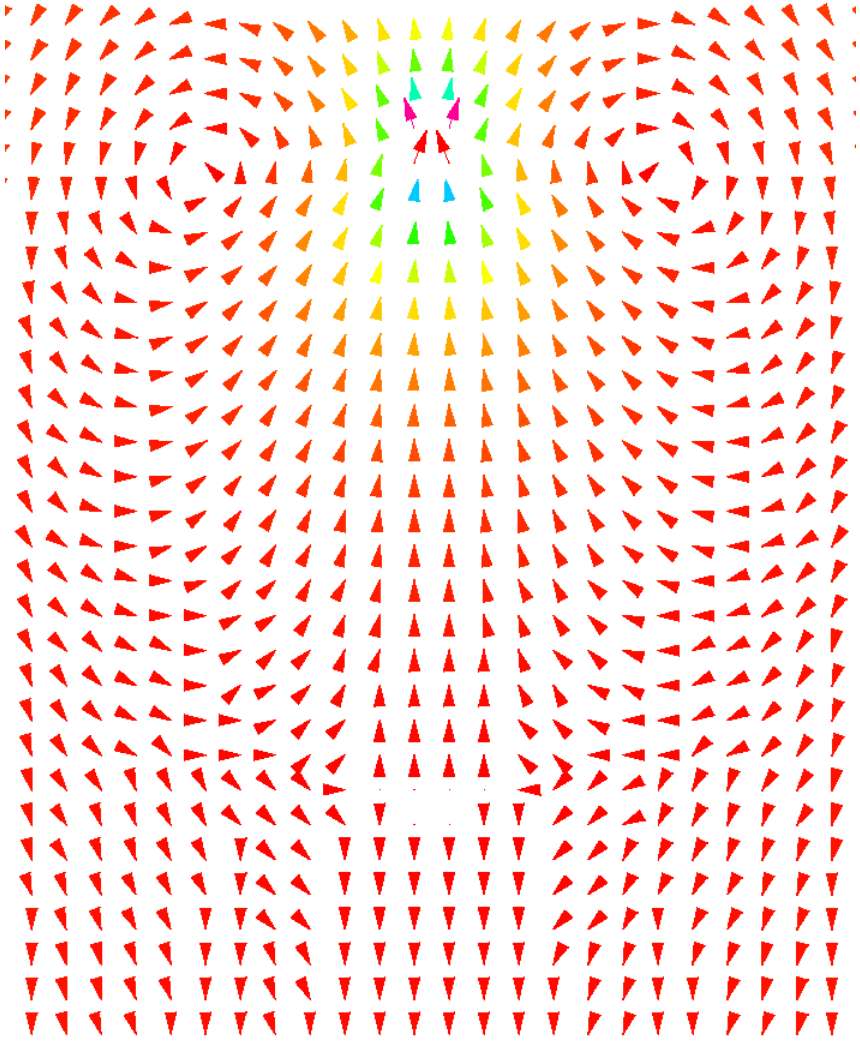
B.J. Cooley – Physics postdoc

Is the process of microcolony formation analogous to a condensation transition?



What are the mechanics of biofilm development?





V. D. Gordon *et al*, 2003 Experimental Cell Research, **289**, p. 58