1) Demon Bacteria

2) Darwin meets Nano (briefly)

Bob Austin

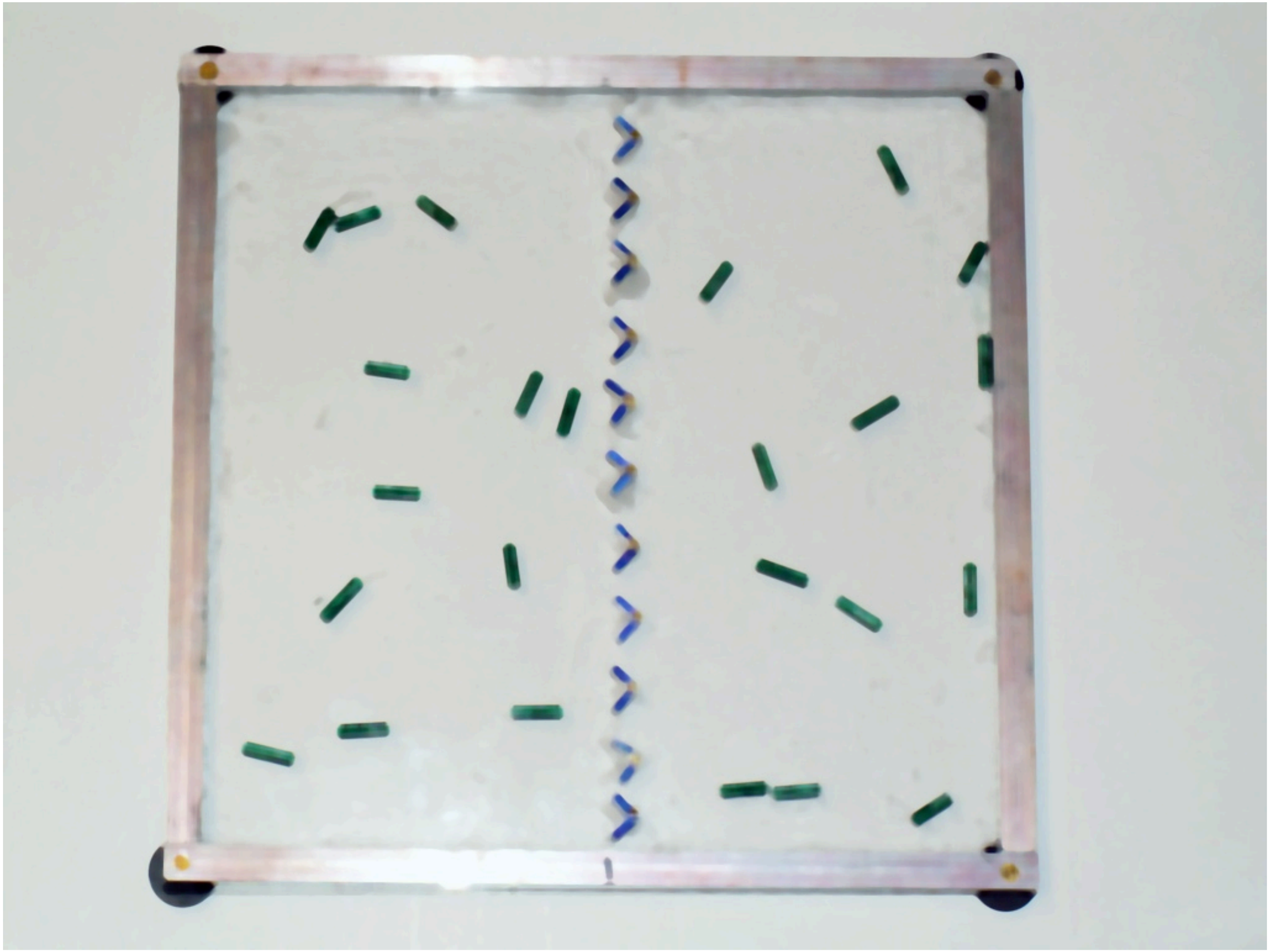
with:

Peter Galajda (Hungarian physicist)

Juan Keymer (Chilean mathematical ecologist)

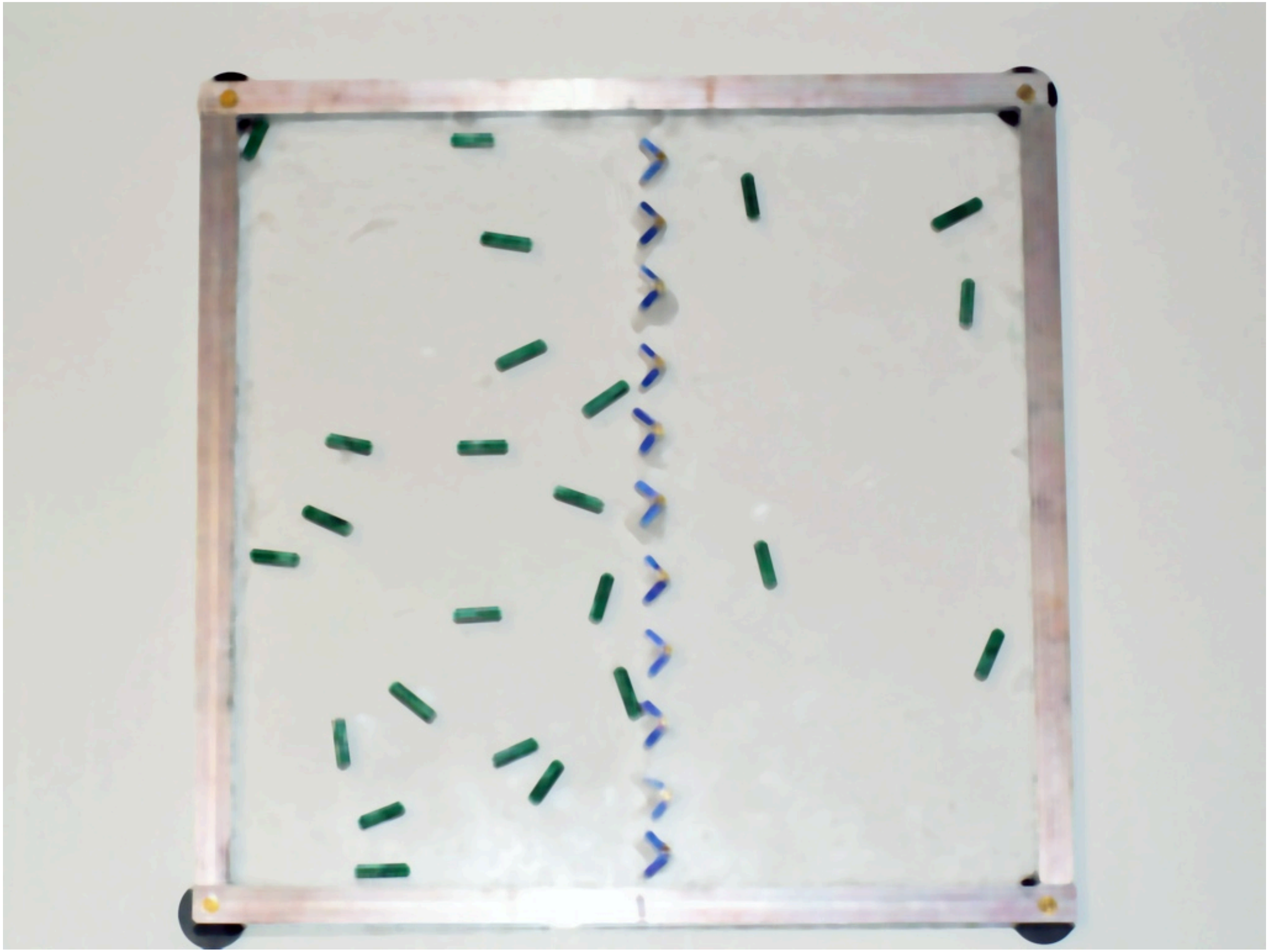
1) Demon Bacteria

John Hopfield started all of this 27 years ago:



Maxwell's demon was an agent with an infinitely fast gate and infinite information about the position and speed of gas molecules which are separated by the gate.

The demon agent uses information about the molecules in deciding whether to admit a particle. The pressure Maxwell demon is a form of Maxwell's demon which creates a number density difference $\Delta\rho = \rho_{left} - \rho_{right}$ between chambers containing a gas.

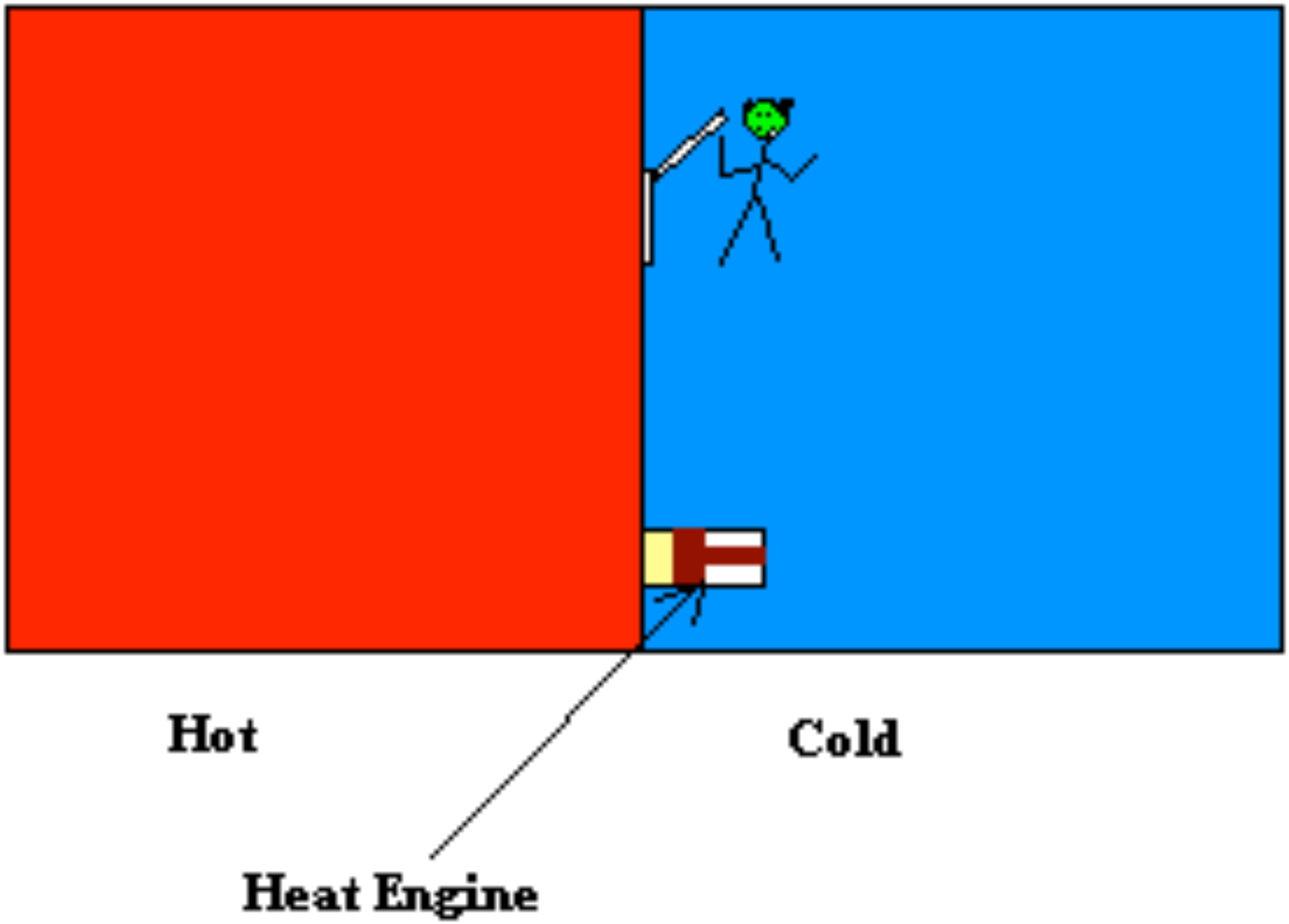


Maxwell's Demon at work?

This number density results in a chemical potential difference

$$\Delta\mu = n_Q \log(\rho_{left}/\rho_{right})$$

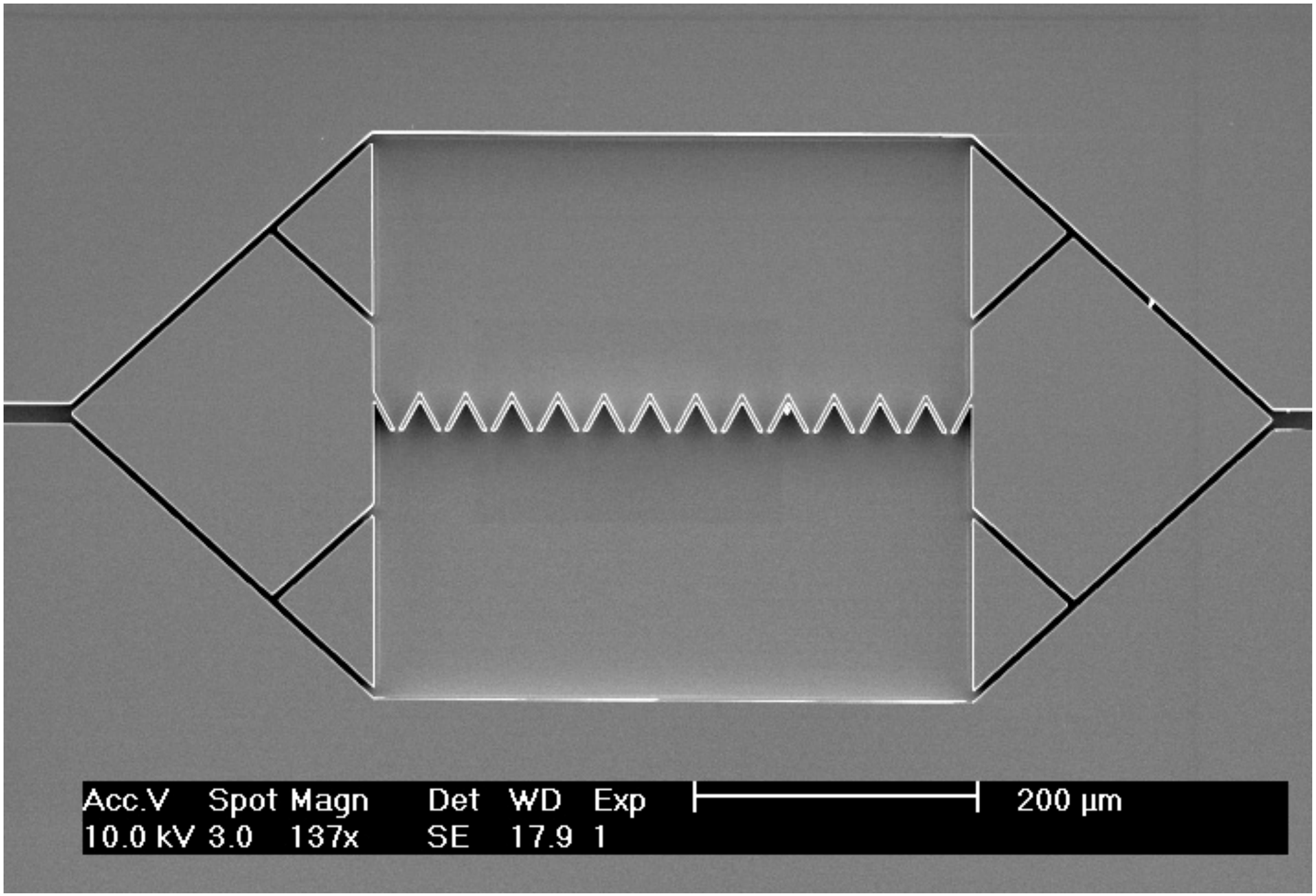
which can be used as a source of free energy, but such a chemical energy created by a population difference must ultimately have a energy source, either informational or mechanical.

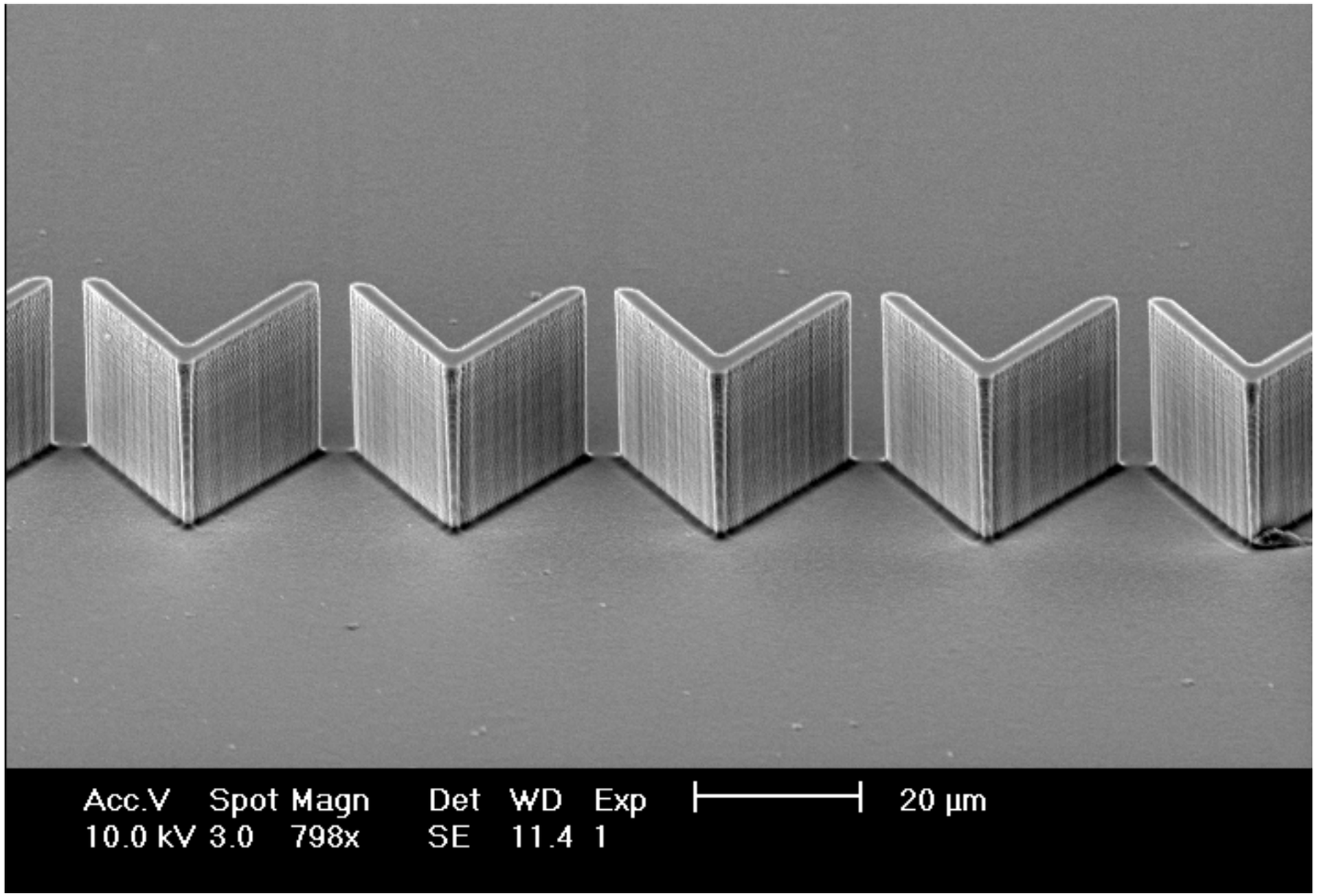


In this house Lisa, we OBEY the laws of thermodynamics!- Homer Simpson

Well, this device had better not work at the molecular level without a computational agent (a Demon, perhaps Bill Gates), or we are all in trouble.

In a probable career-ending move, my post-doc Peter Galajda saw my toy and wondered what bacteria would do....so he made a micro one for bacteria at Cornell CNF.

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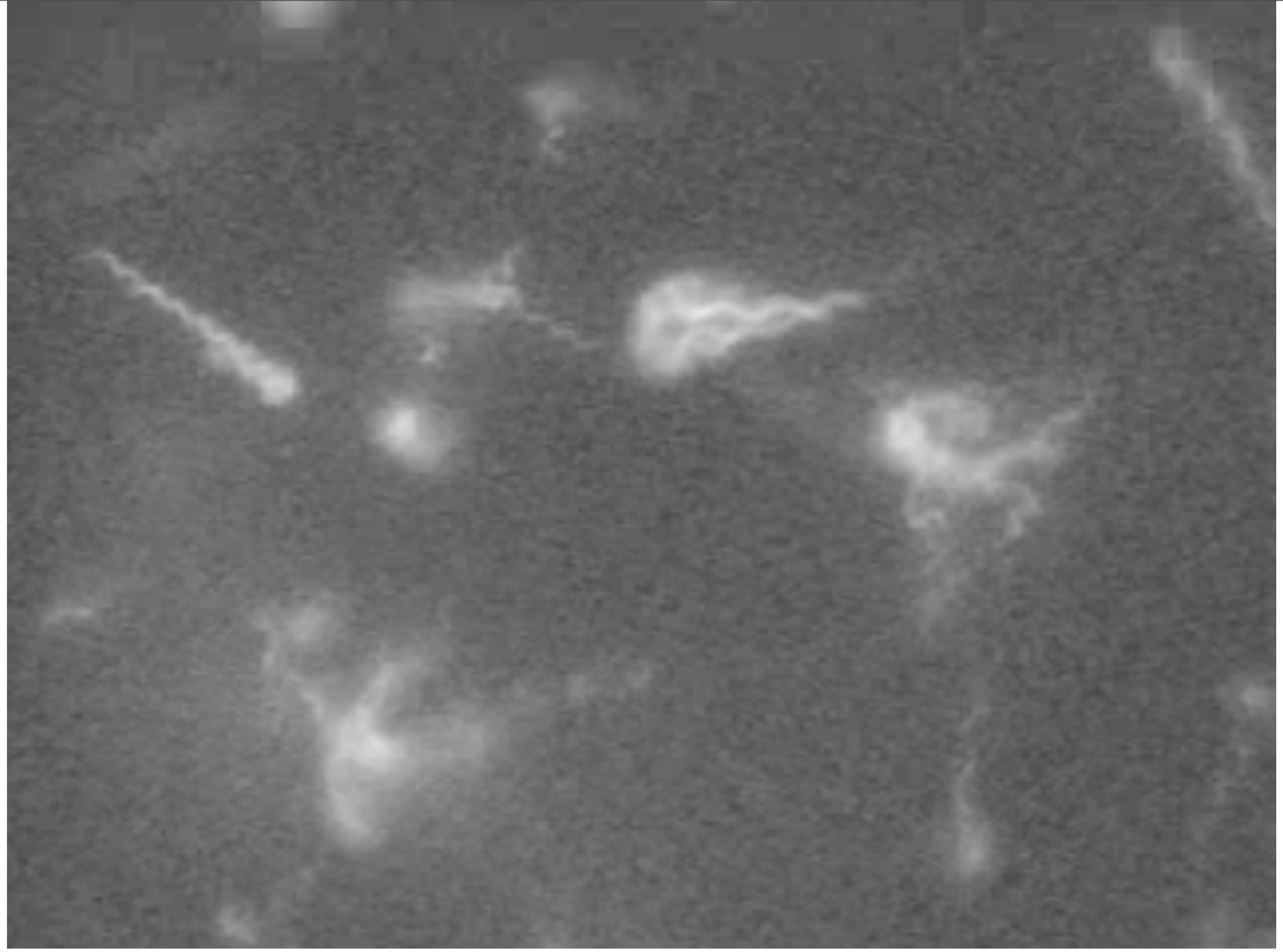
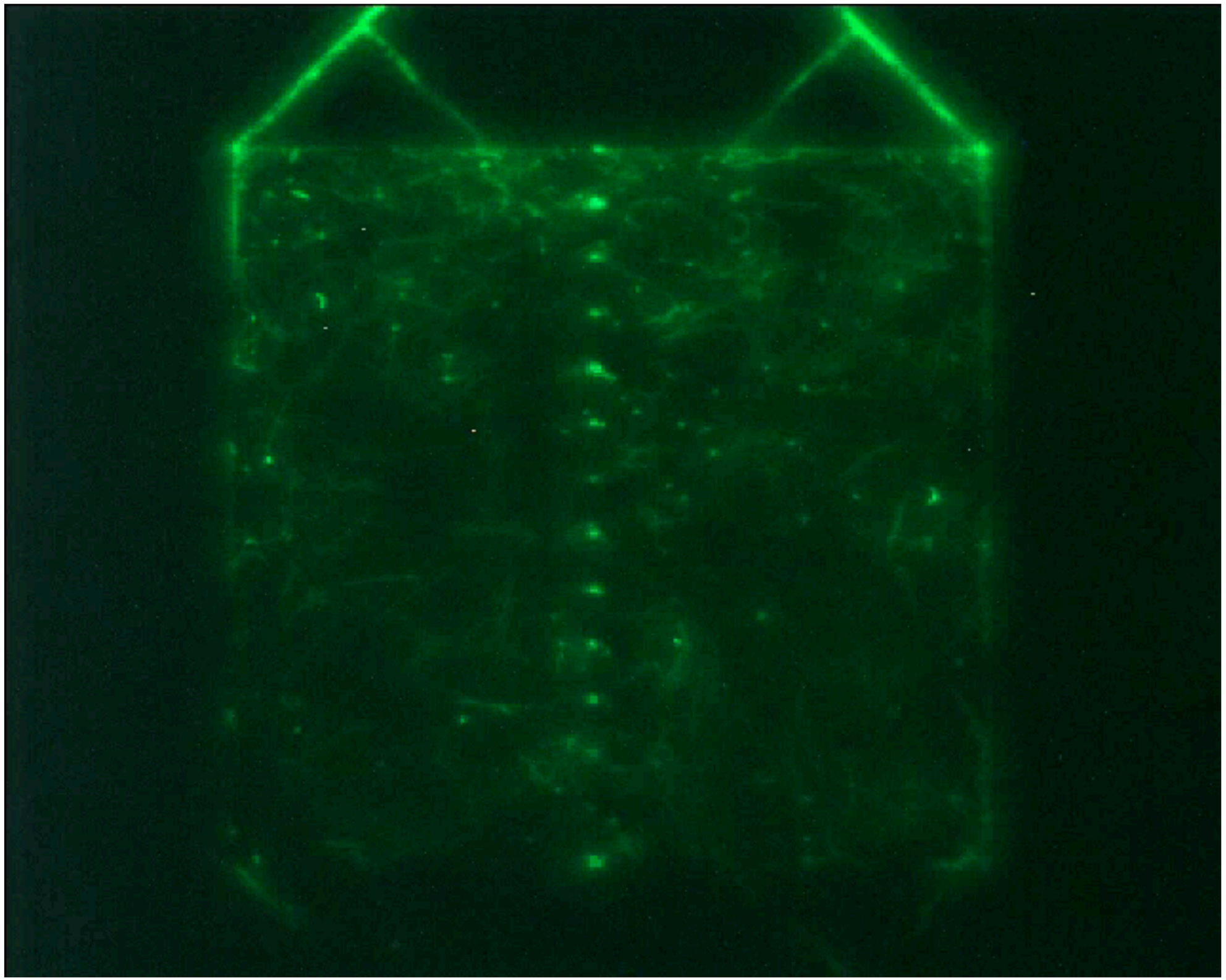
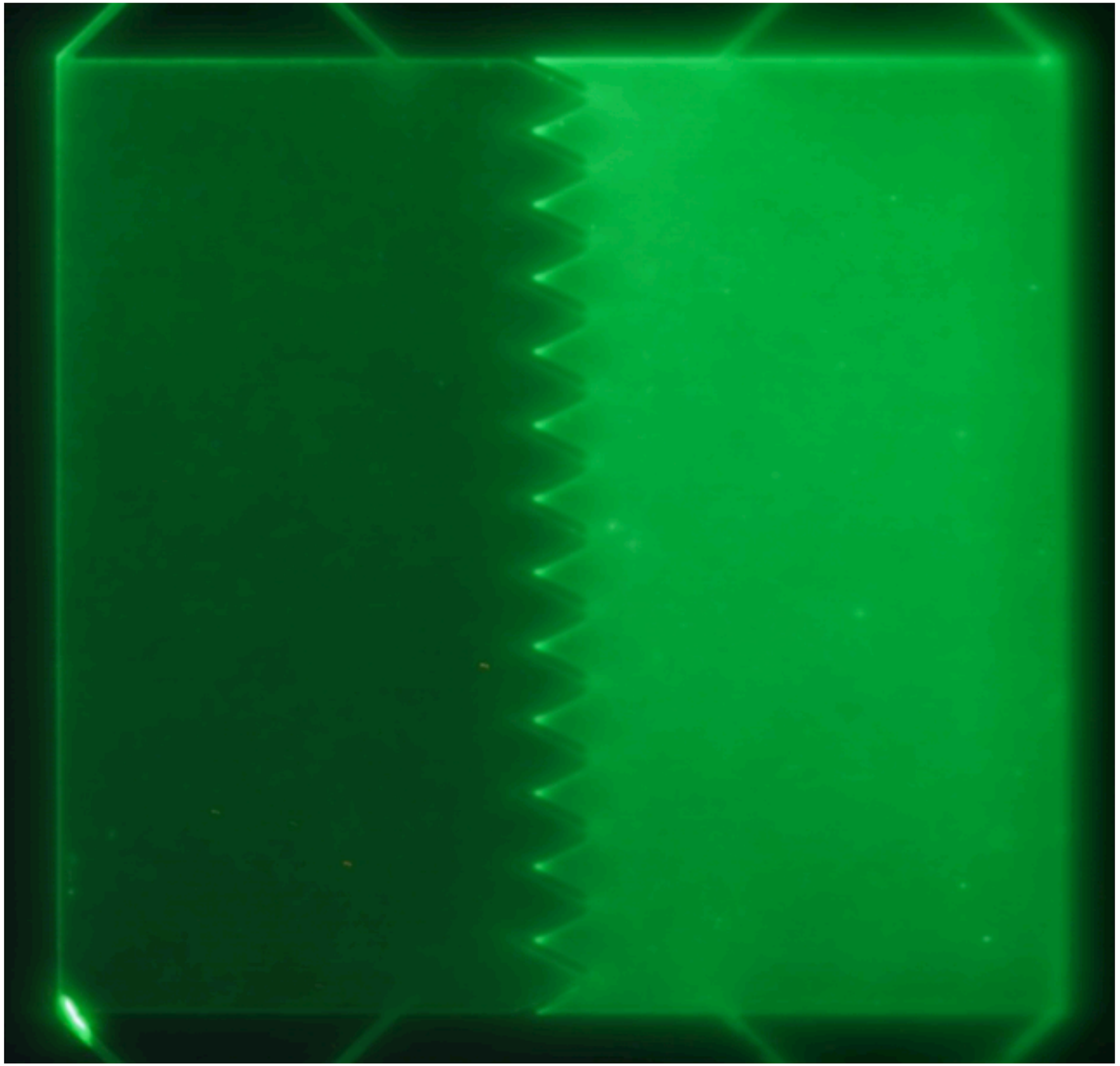
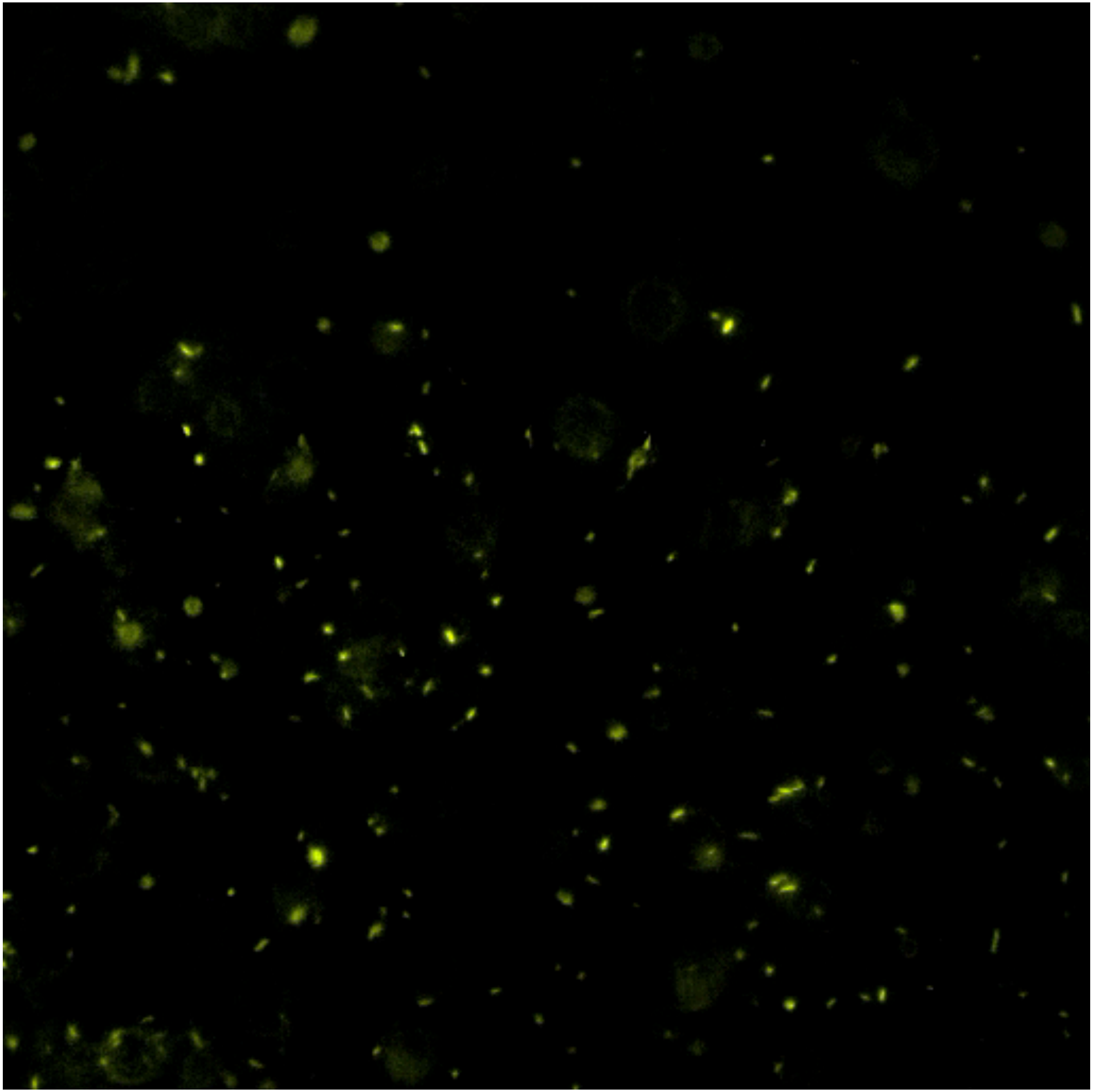


Howard Berg and Will Ryu
Motile bacteria do a random walk, sort of.
(don't confuse with Brownian motion!)

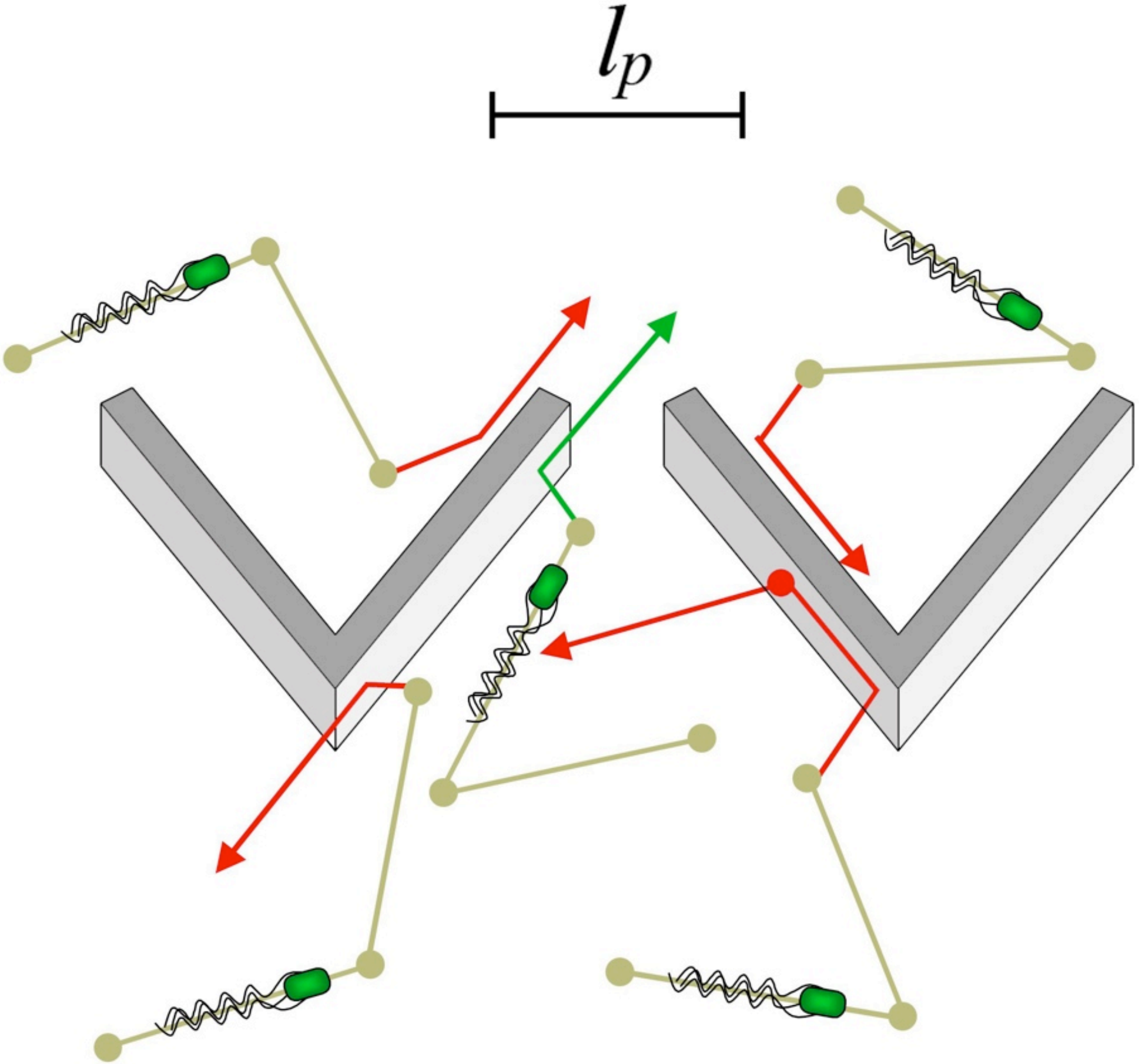






The motion of swimming bacteria such as E. coli is not the brownian motion of a particle which cannot swim, but rather in the absence of chemotaxis (computation) is a random walk characterized by a finite run length l_p between randomizing tumbles.

Under the conditions of our experiment the observed distance l_p between randomizing tumbles was about 50 microns, and l_p sets the scale at which our funnels can work.


l_p

$$\frac{d\rho_L}{dt} = -c_{LR}\rho_L + c_{RL}\rho_R$$

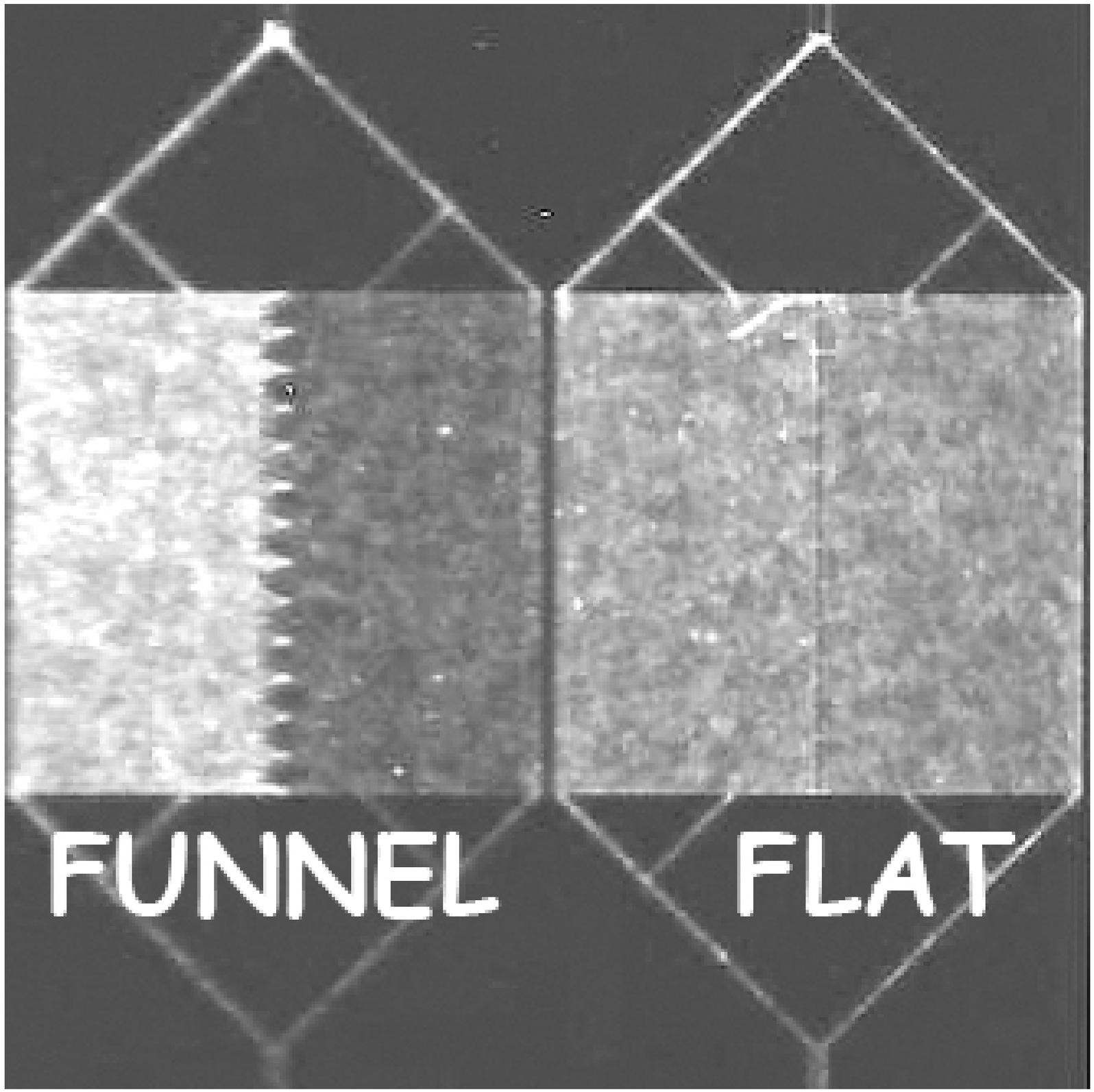
$$\frac{d\rho_R}{dt} = c_{LR}\rho_L - c_{RL}\rho_R$$

$$\frac{\rho_L}{\rho_R} = A(t) = \frac{c_{RL} - \frac{c_{RL}-c_{LR}}{2} \cdot e^{-(c_{RL}+c_{LR})\cdot t}}{c_{LR} + \frac{c_{RL}-c_{LR}}{2} \cdot e^{-(c_{RL}+c_{LR})\cdot t}}$$

$$\frac{c_{RL}}{c_{LR}} = 2.9$$

This doesn't explain anything, it just puts numbers in.

You need funnels: flat openings do nothing.



This experiment has bothered Paul Chaikin.

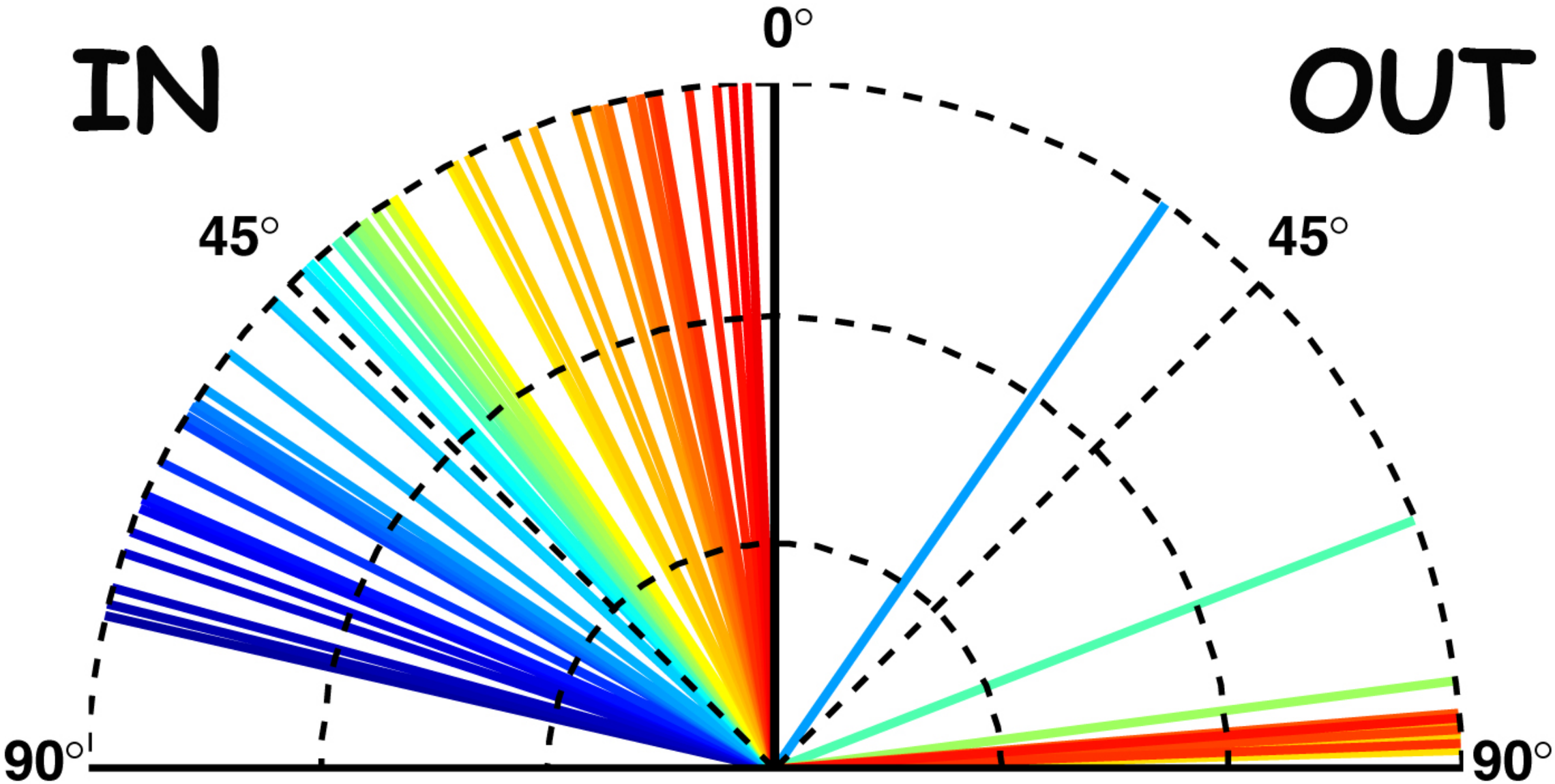
In light of Purcell's gorgeous "Life at Low Reynold's Number", one can ask where the time-irreversibility comes from, since the Navier-Stokes Eq. at low Re is even under time reversal if you change the sign of the forces:

$$\rho \frac{\partial v}{\partial t} = -\eta \nabla^2 v$$

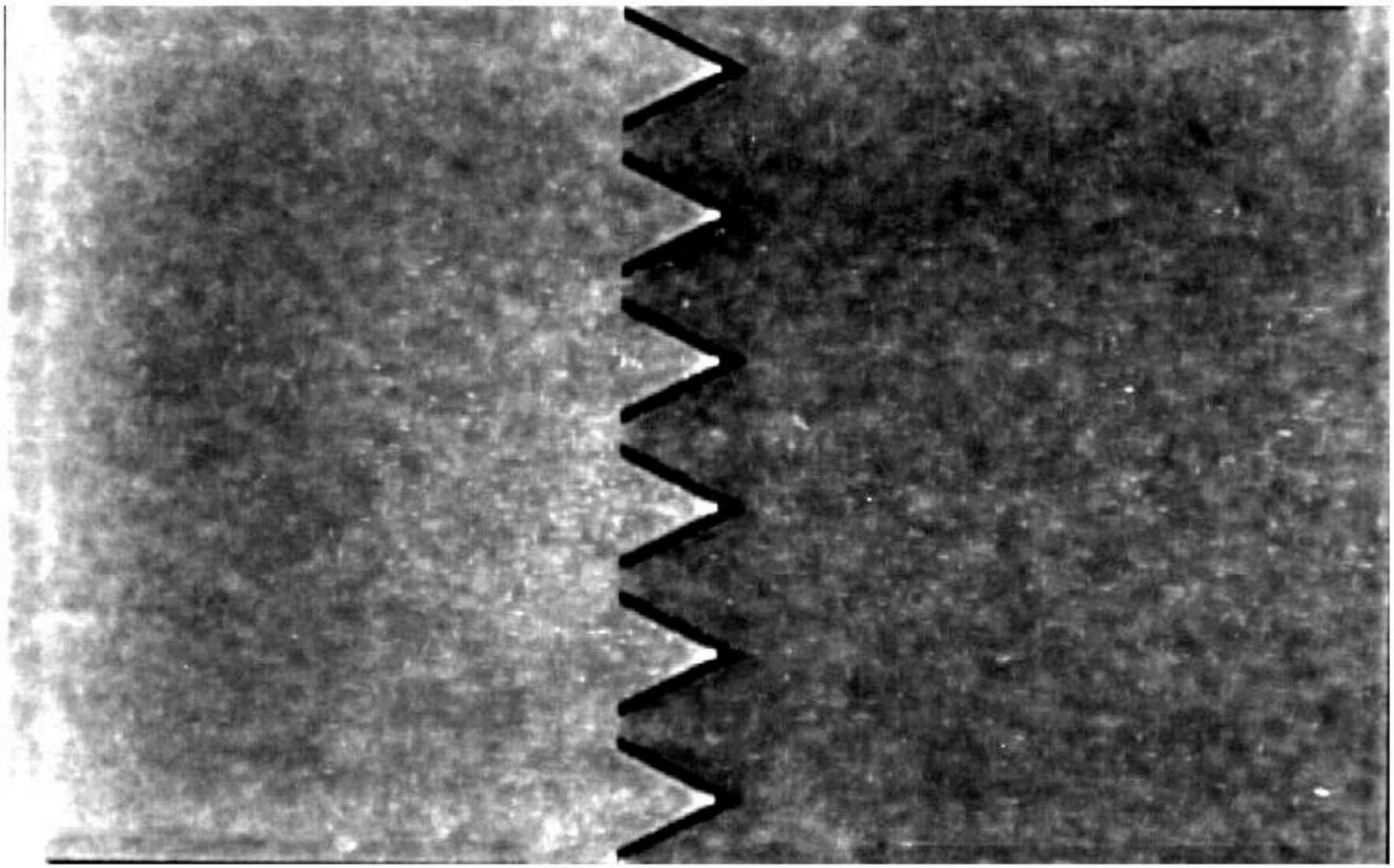
"Suppose I run your movie forward - the bugs are evenly distributed, then go to one side and then assume a random array of swimmers .

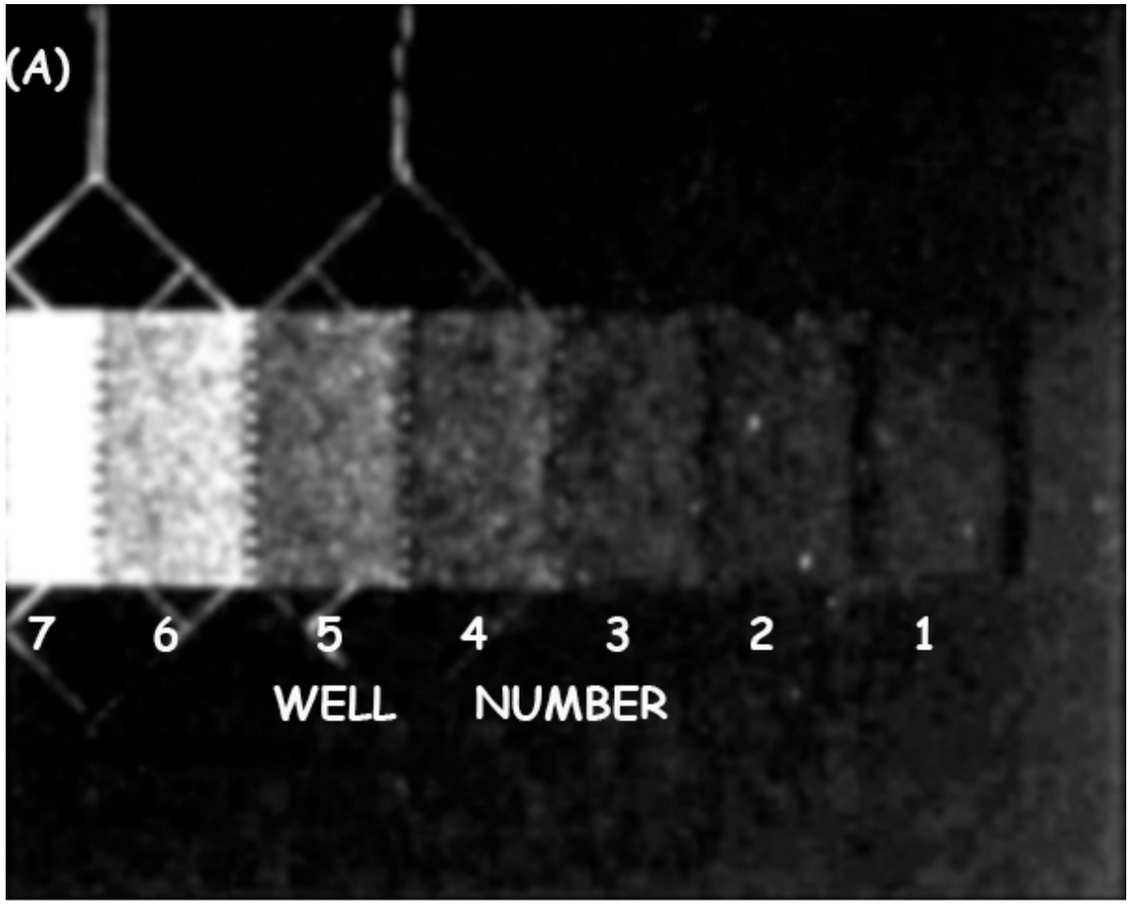
Now instead I start with a random set of swimmers on the back side of the funnel I run the movie and they should go back to the up side of the funnels. They don't. So that's what bugged me. What's the answer?" Paul Chaikin

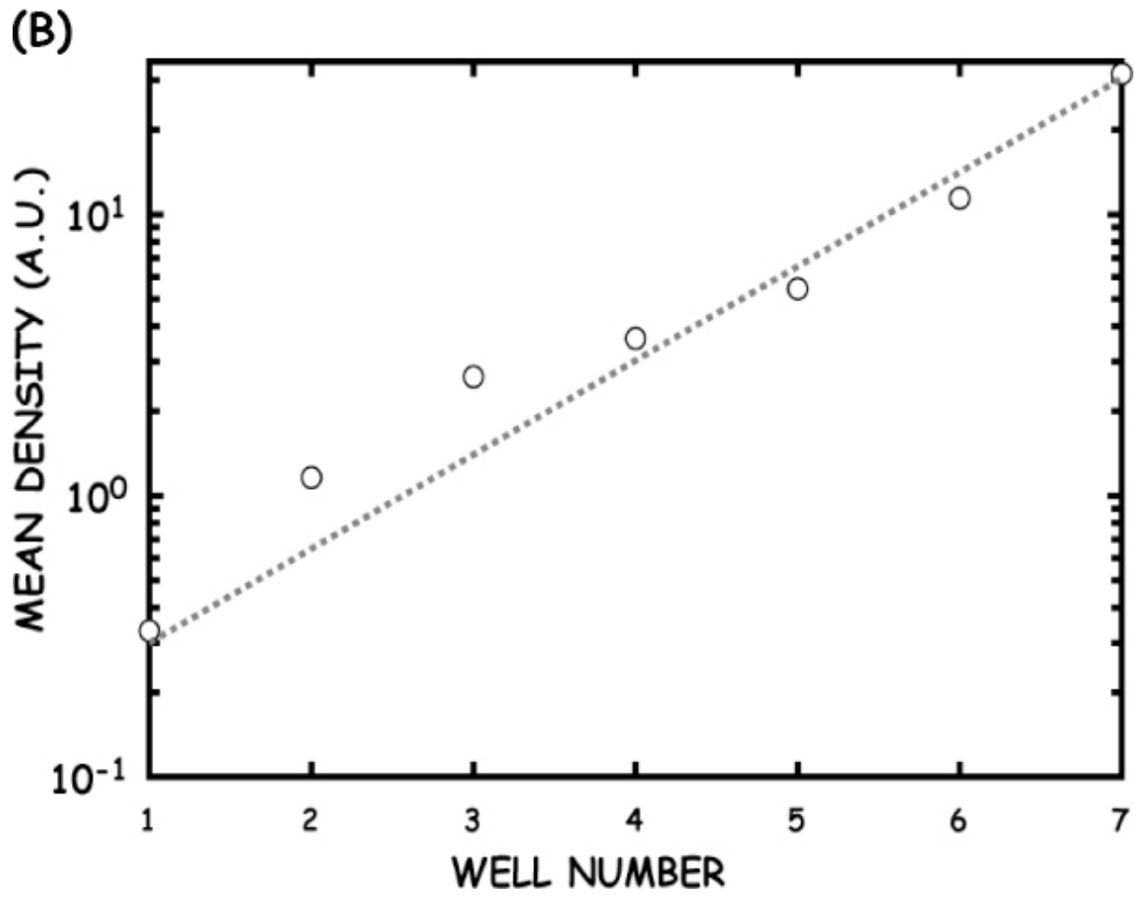
Swimming bacteria follow walls, presumably a hydrodynamic effect. Not reversible, 10-fold phase space compression.



The answer to Paul's question is that in fact and of course the particle distribution on the concentrated side is NOT random, and cannot be. It must have imprinted in it the asymmetry of the interaction which caused the effect, in effect imprinted information, but rather cryptically...



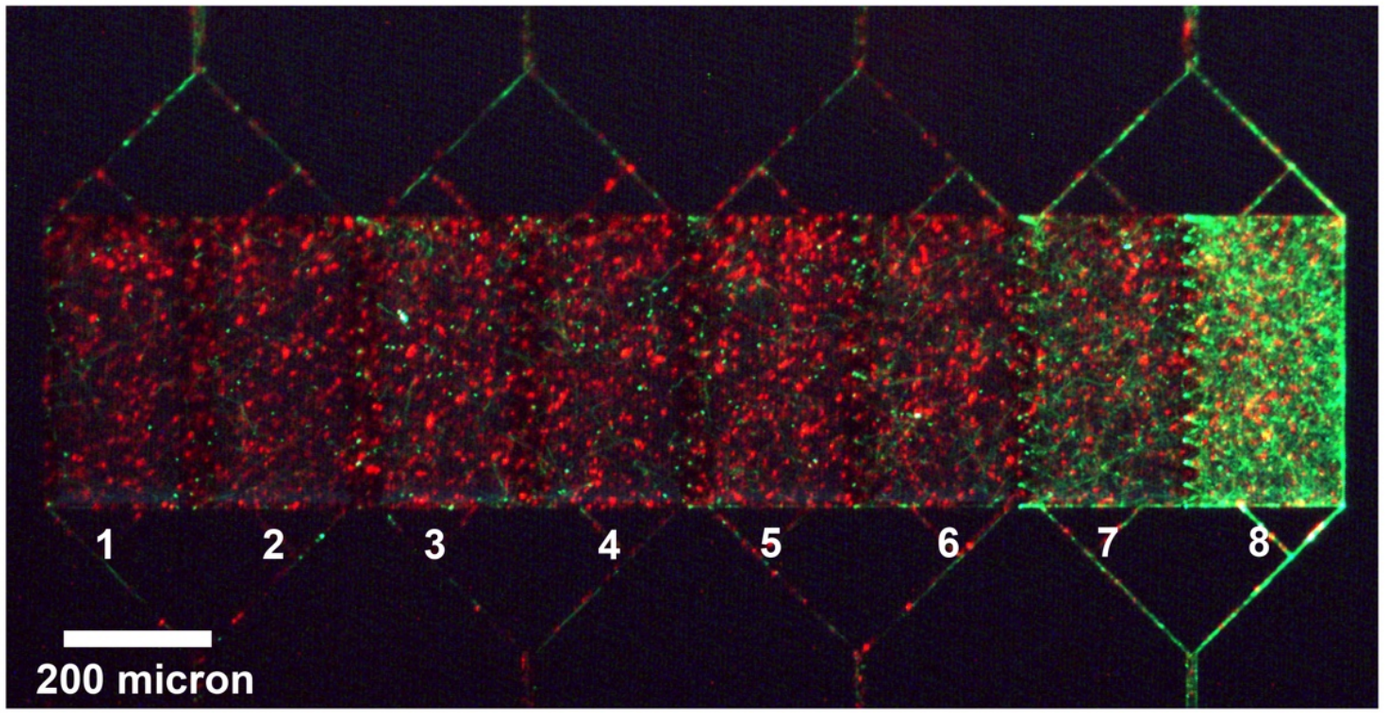




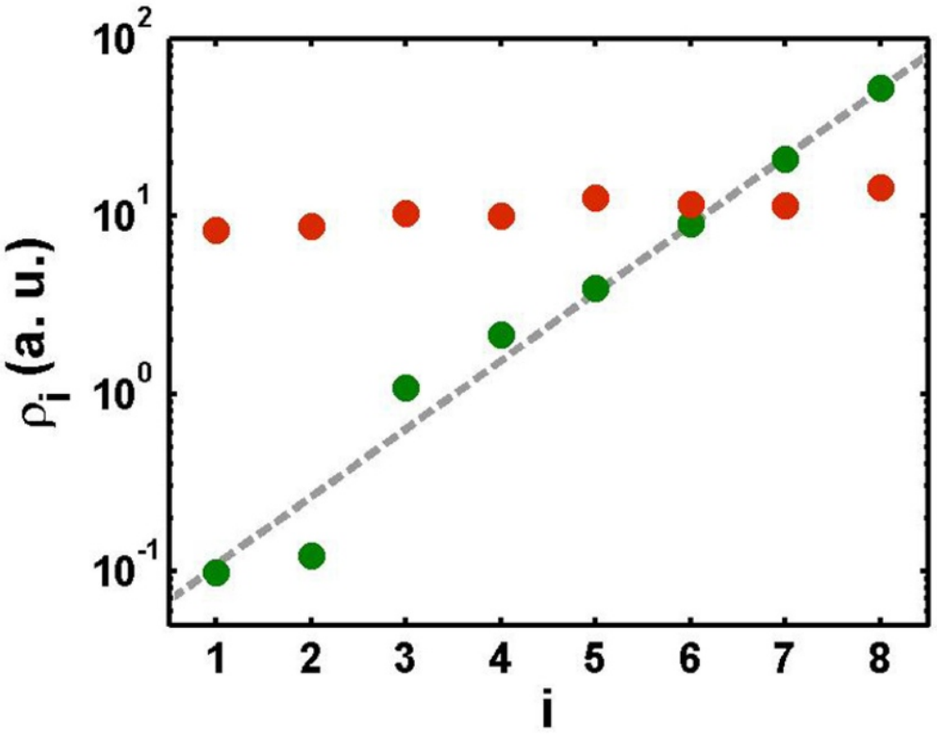
The concentration is an exponential process, roughly a factor of 3 per funnel wall with this design, and roughly what you would expect from an entropy calculation of the wall compression, 3 is about log(10).

You have to be a swimmer to be selected by the demon: the red bacteria are non motile (zombies), green are swimmers. The effect is driven by motors, the bacteria have to swim.

(A)

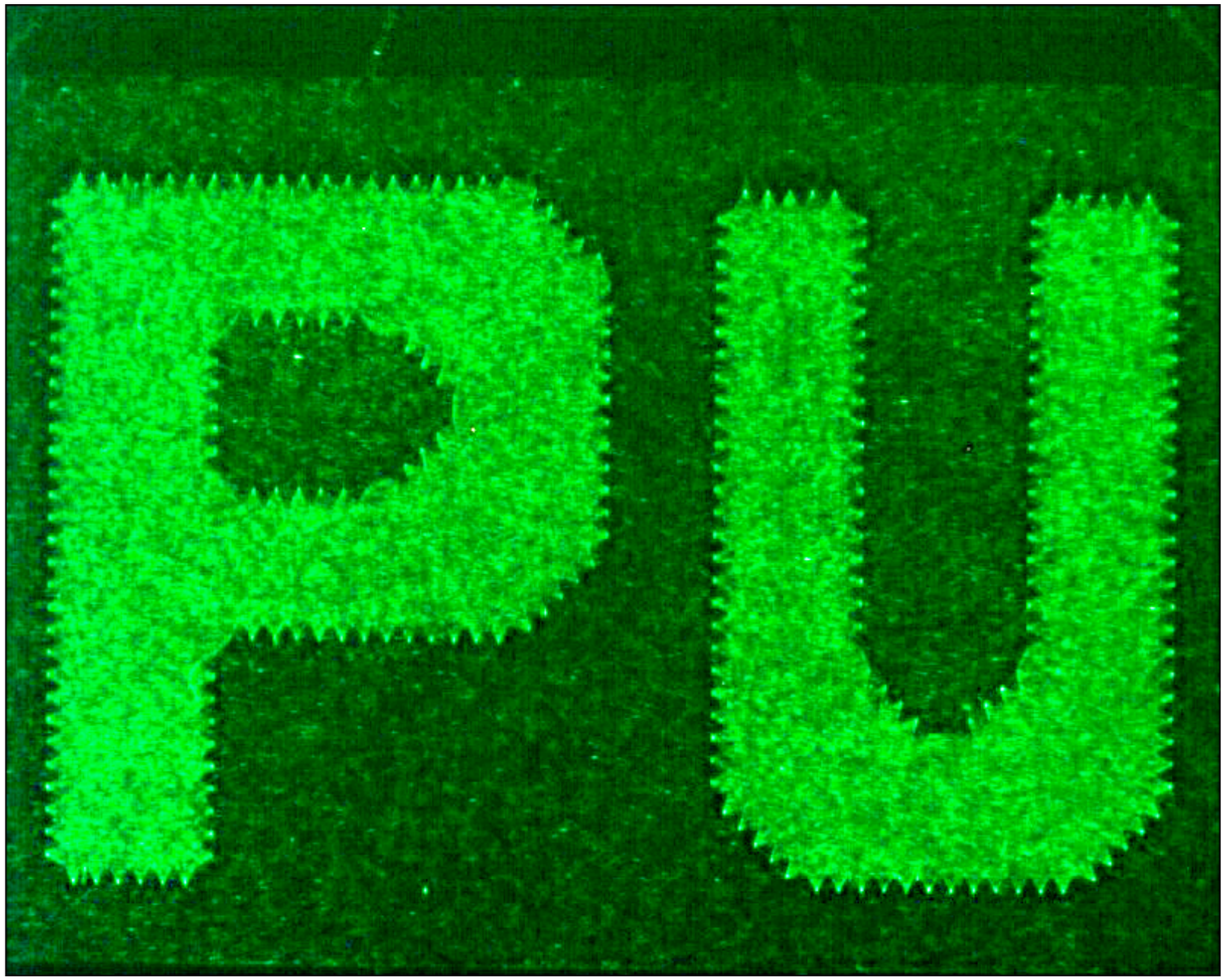


(B)



This isn't TOTALLY useless.

1) Motile, live, microorganisms can be separated, concentrated from suspensions, and they can be 'guided' by the Demon into specific, enclosed chambers.



2). Ongoing project: what happens if we shrink this device to nanoscale and apply a driving AC force (electric field, for example). Can this device concentrate molecules, acting like a molecular diode?

3) Use such structures in evolution on a chip experiments, where motile bacteria face food gradients in the "wrong" funnel direction, providing a selection pressure against the Demon's wishes.

Can bacteria can learn to swim in different ways than the the Demon wants to force them to move? Chaikin says yes, I say no, Physics laws rule even the Demon.

3) Bacteria as Sentient Beings and not as Slot Machines.

There is a movement afoot across the land and in certain Institutes and in certain Courses that try to emphasize the influence of statistics in biology. Life as one big statistical Boltzmann Equation.

I sort of hate that, hence my fall from grace to Zombie Land in Physics at Princeton, and the unwarranted seizure of my kick scooters by The Man.

If you know a Princeton biologist, do exactly the OPPOSITE of what s/he says: you can't go wrong.

Last year Juan Keymer wandered into my office.

He is an mathematical ecologist. About 30 years ago the biologists split into 2 species under selective pressure.

Some biologists turned into molecular biologists because they took the reductionist approach that by studying genes we could understand life, and besides that was where the money was.

Some biologists became ecological and evolutionary biologists because they felt phenotype was more important that genotype. Ecologists got the crappy old buildings with steam heat, mo bio types got Italian designed palaces of light with artsy sculptures built of lead in the plaza.

Juan for some reason had read my papers on bacterial density instabilities under chemo-attractive self-generated gradients and wanted to use my chips to do evolution dynamics from an ecology prospective.

I was told by the biologists this was a waste of time because evolution is very, very slow, like erosion:

1 bp mutation/10^9 bp/generation, random process, and most mutations are neutral or bad.

So: waste of time.

Instantly, I knew this was a good project!

My narrow view of the neoclassical evolution dogma:

1) Mutations are random on the genome.

2) Mutation rates are low: rate of about $1/10^9$ mutations/basepair/generation.

3) Most mutations are deleterious, so species are marooned on the peaks of fitness landscapes with large valleys that are difficult to cross.

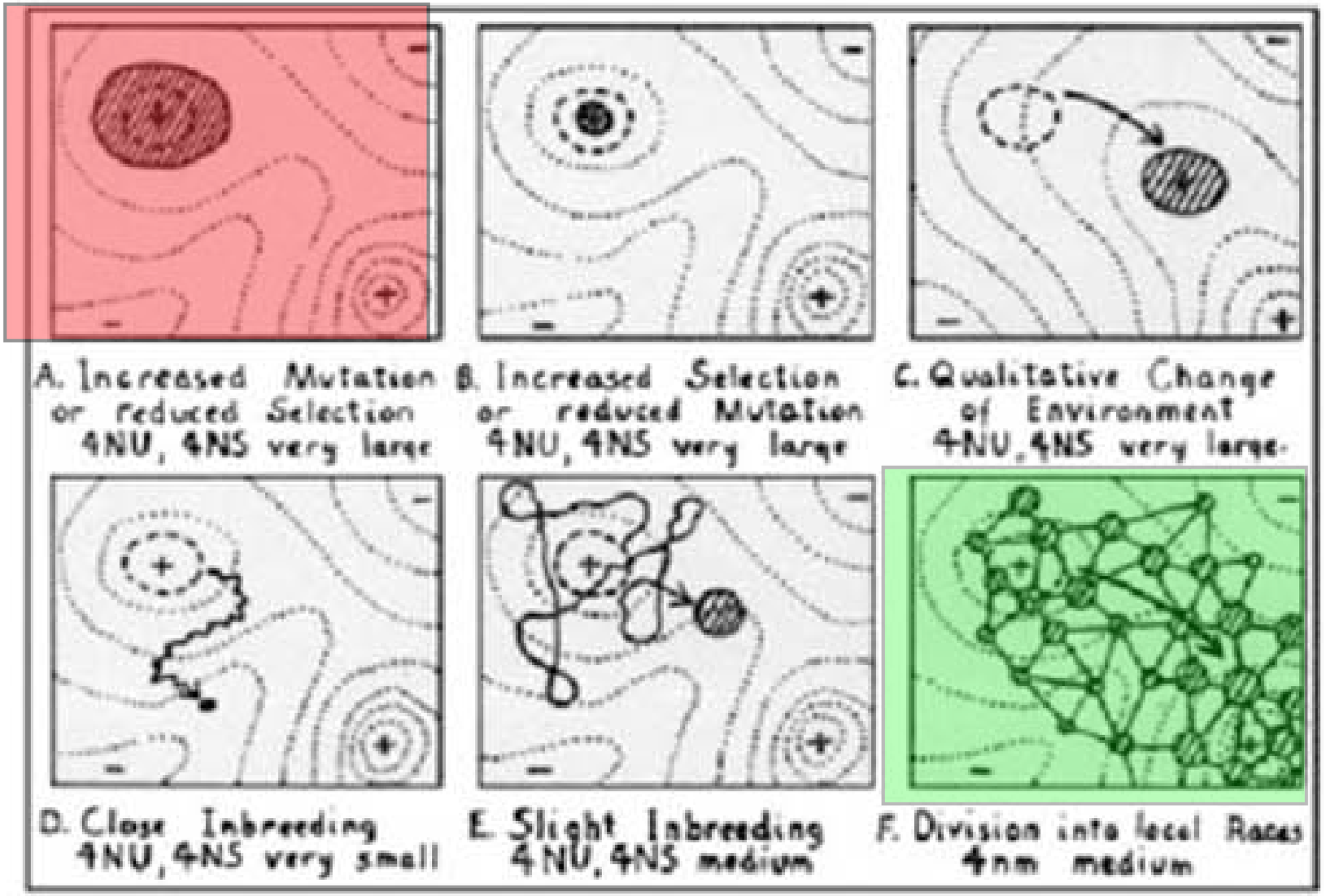
4) Most evolution occurs through "R selection": faster grower wins.

5) Thus: Evolution best studied in large numbers in big buckets, because of the low mutation rates (point 2 above). But, the thermodynamic chemical potential of a rare species is very small. They can't compete in a flat landscape against a large population. You won't see evolution on a flat landscape. You will get Kansas, and it will be as interesting as Kansas.

Here is another important thing to realize: it isn't a good thing to keep a population of organisms genetically "pure". The problem with that plan is that the population cannot respond quickly to a change in the environment, and if the death rate exceeds the birth rate the population goes extinct.

A better survival plan is to maintain a population of populations that are weakly linked with each other: a metapopulation is the name coined by Levins in 1969.

A metapopulation is several distinct populations together with areas of suitable habitat which are currently unoccupied.



Sewall Wright in 1932 understood the inherent stability and evolutionary adaptibility of Metapopulations.

There is also a newer view that is emerging not only of the importance of metapopulation dynamics, and but also the use of adaptive mutation rates.

Nature uses evolution (mutagenesis) in a directed way to rescue organisms from critical situations, she is willing to take a risk of bad proteins if the genome is sufficiently damaged or the environment sufficiently poor that the present genotype cannot survive.

I have received a fair amount of misinformation about how Nature uses mutation to respond to stress in a collective way. Everybody seems to have different opinions, quite firm, even dogmatic.

Dan Fisher (Harvard) has a good question:

Suppose we discovered that we had made a mistake in the calculation of the age of the earth via radioactive dating, and the earth was only 100 million years old, not 4 billion years or so but everything else we see around us in life is true.

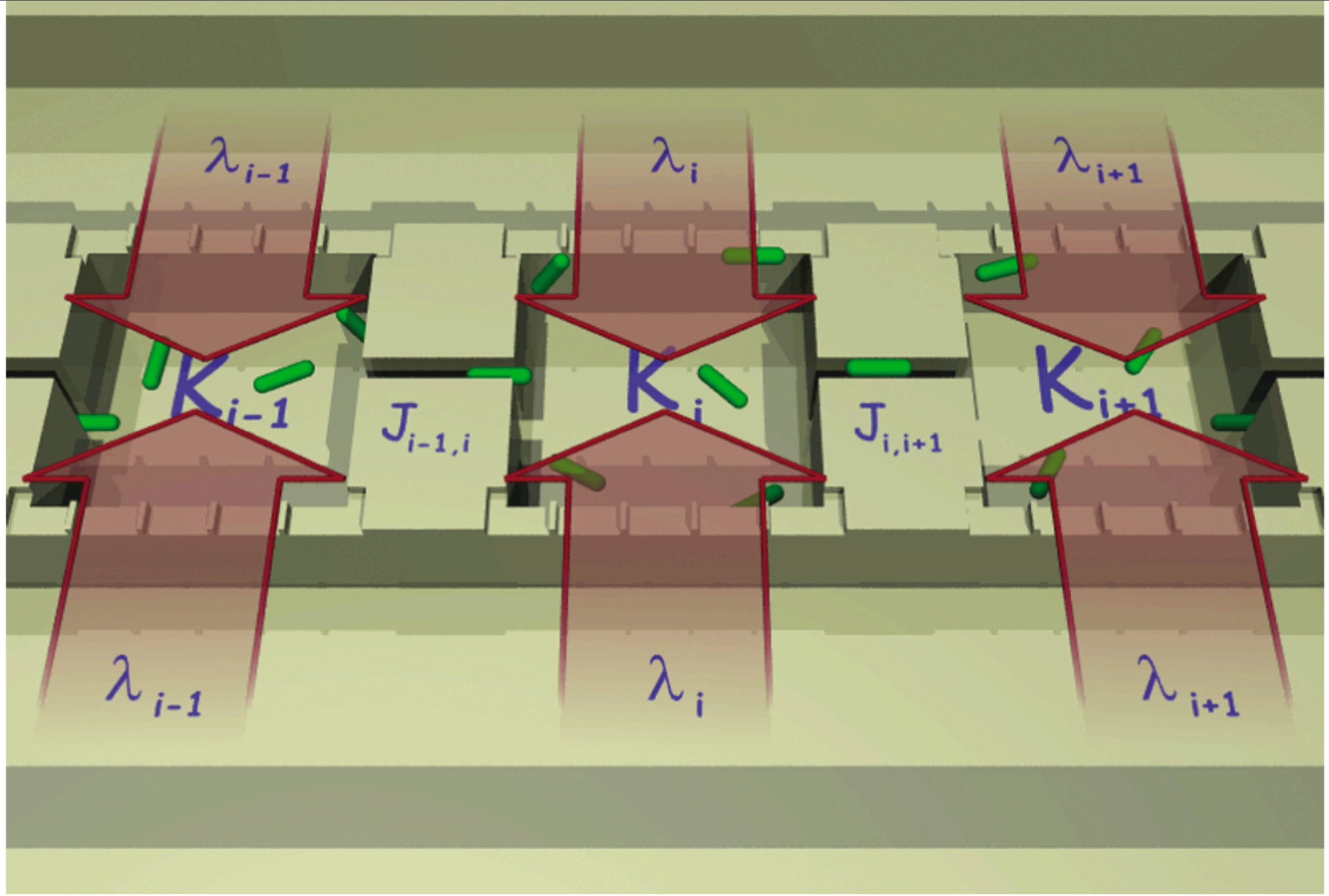
Would biologists be upset? That is, could evolution have kept up with climate changes?

What sets the clock of evolution: stress or mutation rates? Do we understand the clock quantitatively?

Can in fact evolution be directed by the organism itself? This statement seems to really frighten many biologists, since it seems to smack of some sort of "design". Biologists will defend Darwin even more fiercely than physicists defend Einstein.

Here is our idea is a nutshell: Create a series of very small microhabitat patches (MHPs) in which bacteria are kept in stationary phase under highly stressed conditions, and make the microhabitats different from each other but allow the bacteria to move around: that is, create a complex network of metapopulations under differential stress, like a complex natural environment.

How do the bacteria evolve and adapt in this stressed, heterogeneous environment? Does a complex, connected network of metapopulations evolve faster than a large homogenous one? Do the metapopulations communicate with each other in a evolutionary way?



A nanofabricated habitat landscape.

Why this device is all wrong and nuts:

1) It's too small: only at most 10^4 bacteria/MHP.

2) It's too complicated: you don't have homogenous conditions, and the bacteria can greatly influence the quality of the MHP.

3) You don't know what is going on: the medium is changing, the bacteria are moving around, they are exchanging DNA.

4) You don't know what is going on genetically: you have a heterogeneous (possibly) population, and you can't tell adaptation from evolution.

5) It isn't reductionist: you are starting too high.

A brief note about the lives of bacteria : the exponential ("log") phase is only a very small part of the life of a bacteria. Keeping bacteria in the log phase is like only studying humans in kindergartens. Amusing, but infantile.

The logistic equation, a famous expression coming from ecology with a lot of tricks in it, is a much better description the full life of a bacterial colony:

$$\frac{d\rho(t,w)}{dt} = R(w)\rho(t,w) \times \left[1 - \frac{\rho(t,w)}{K}\right]$$

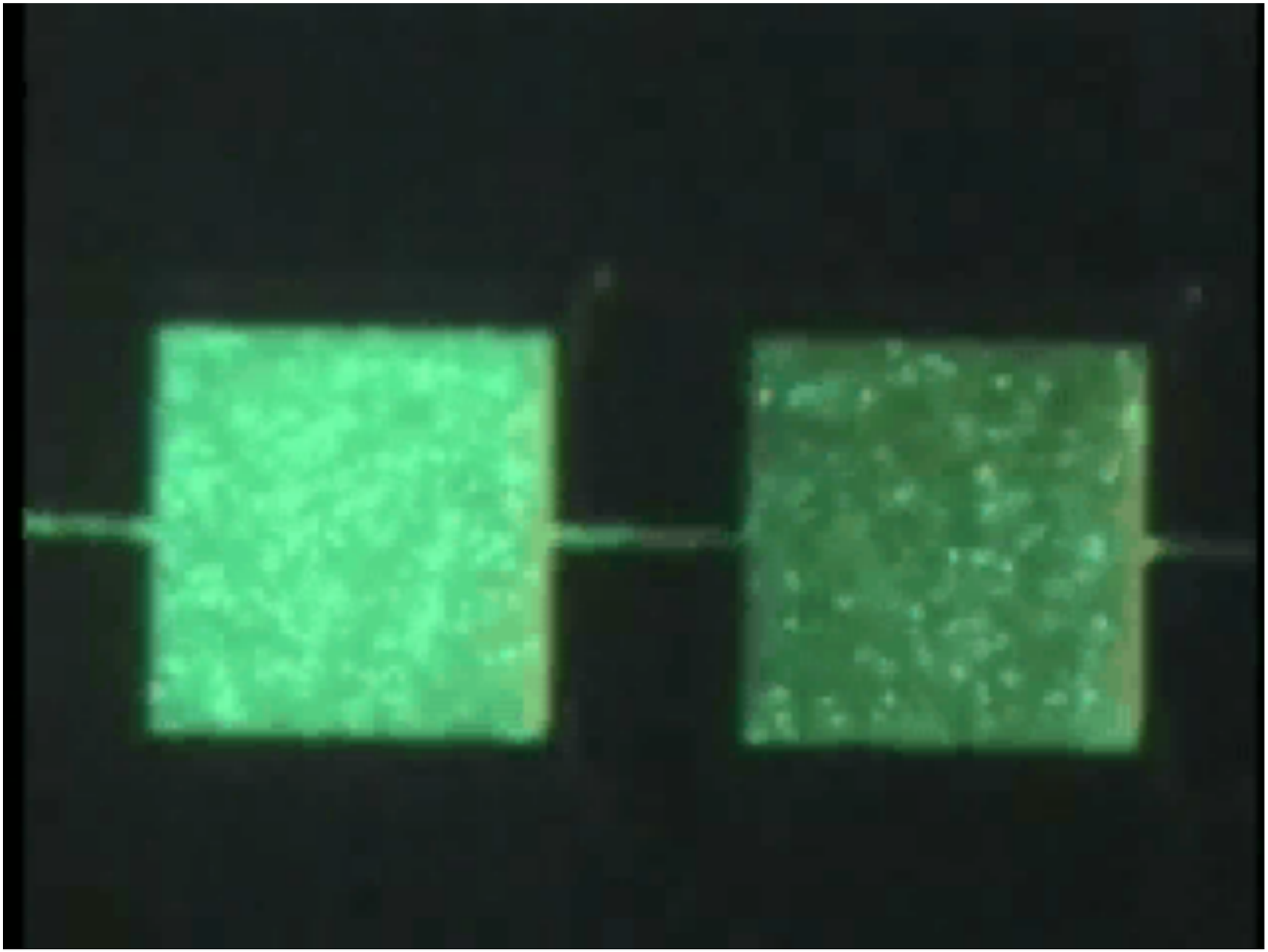
"R" selection: advantage through numbers (fish eggs)

"K" selection: advantage through environment (Ivy League)

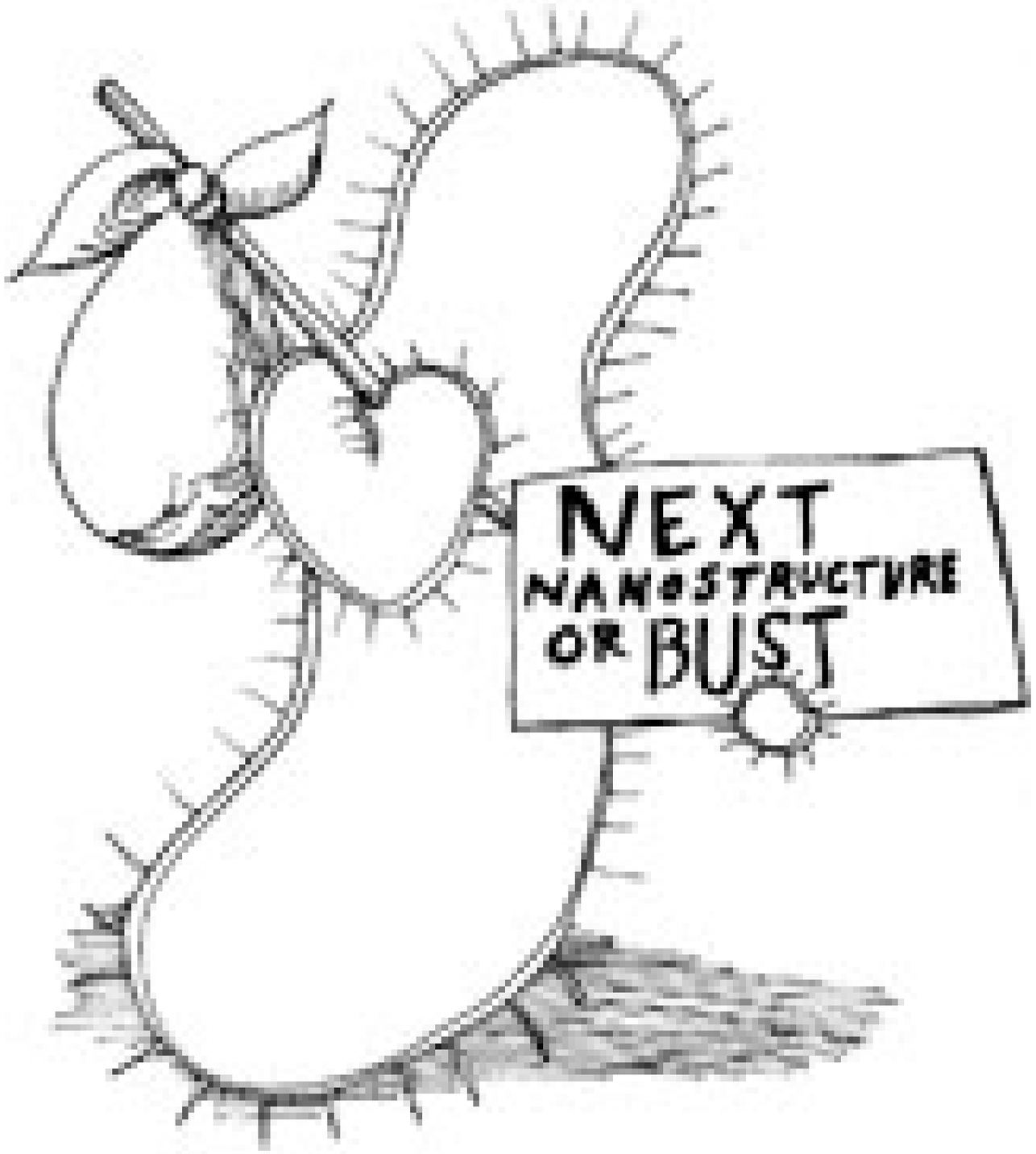
In a 1-D MHP array, the dynamics are much more complicated but much more interesting: the bacteria not only can grow in a MHP, they can move around from MHP to MHP. The dynamics are driven by many things:

1) Chemotaxis, both driven by food gradients but also by the attraction of bacteria to each other. Bacteria are very social as a rule. Keller-Segel equations are basic start for this..

$$\partial \rho / \partial t = D_b \nabla^2 \rho - \nabla \bullet [\kappa \rho \nabla c] + \alpha \rho$$
$$\partial c / \partial t = D_c \nabla^2 c + \beta f \rho$$
$$\partial f / \partial t = D_f \nabla^2 f - \gamma \rho$$



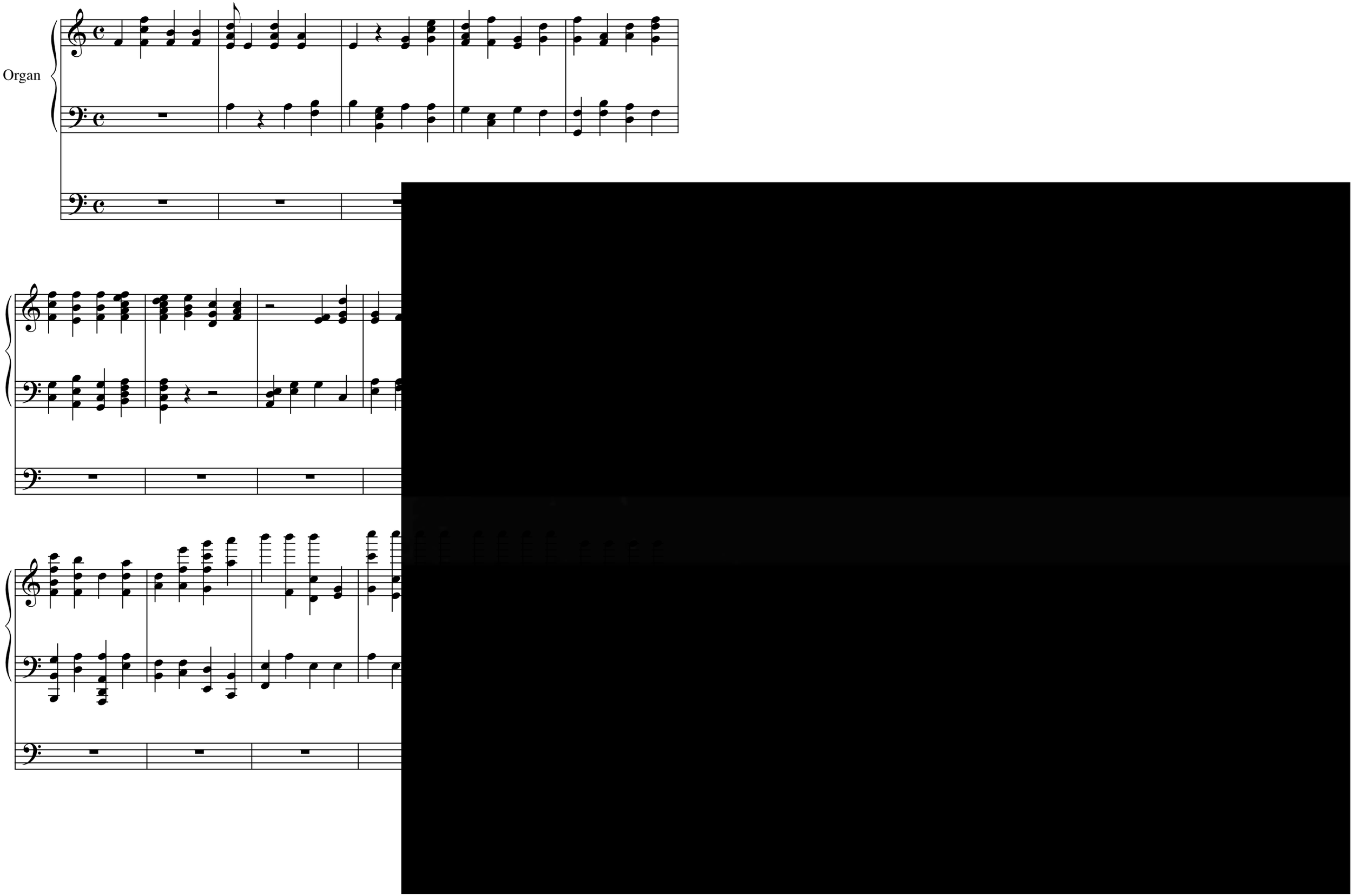
Bacterial colonies are social organisms!



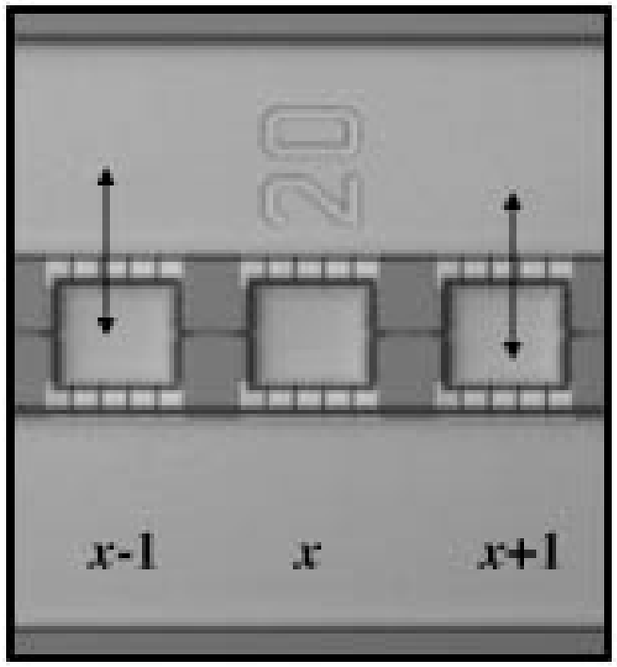
Illustrations by Matilda Luk

bug_music_2

e. coli

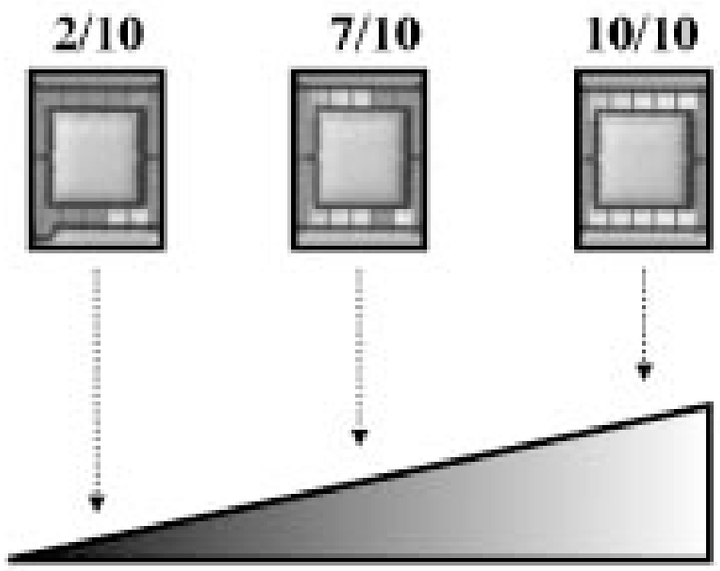


patch quality



2) We can close various fractions of the nanoslits which feed the MHPs. In this way we can develop a habitat landscape, and in response to the habitat landscape organism adapts/evolves and generates a fitness landscape, and moves into good or bad regions.

Life in our habitat landscape becomes a game of survival by change and movement.

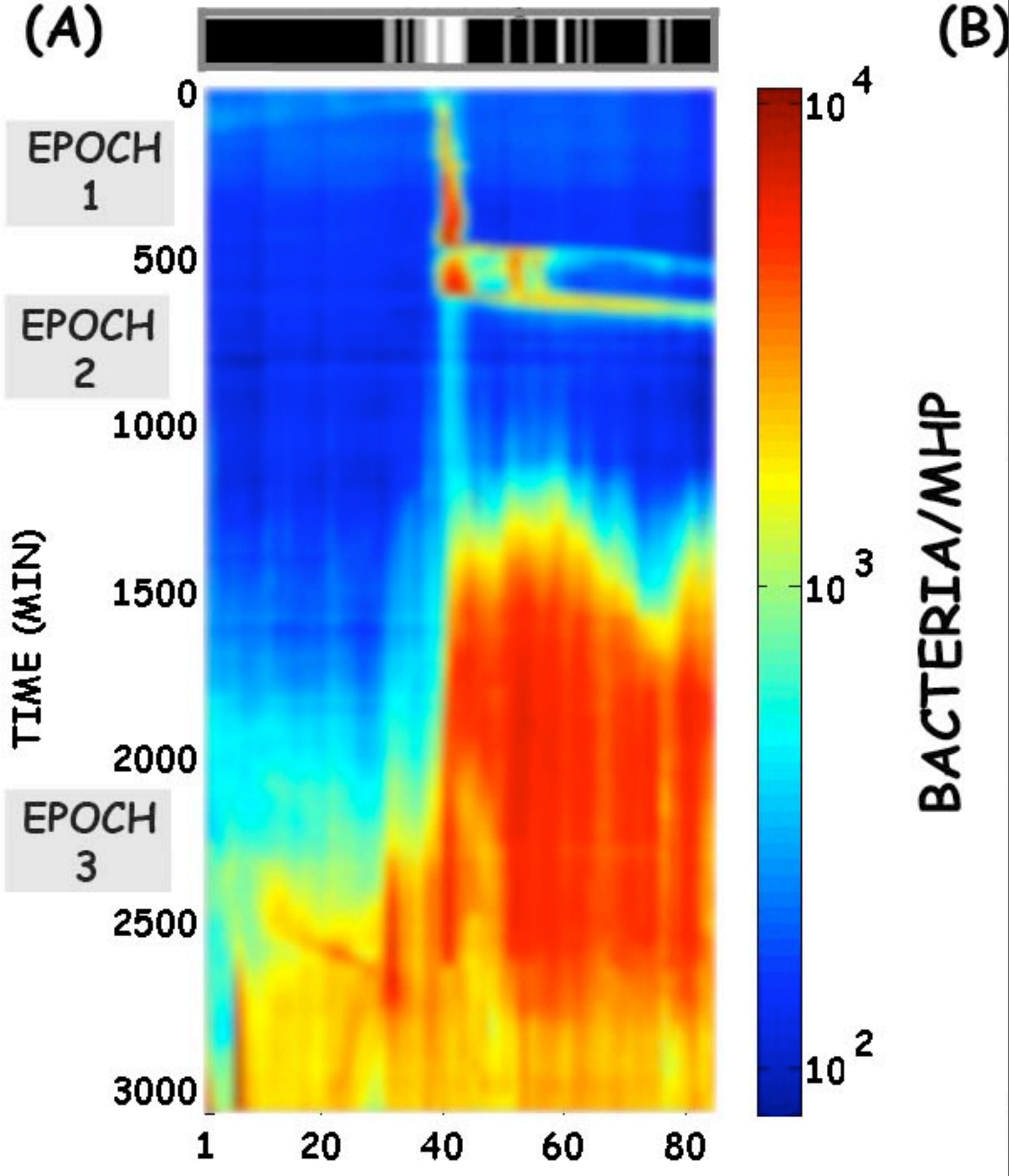


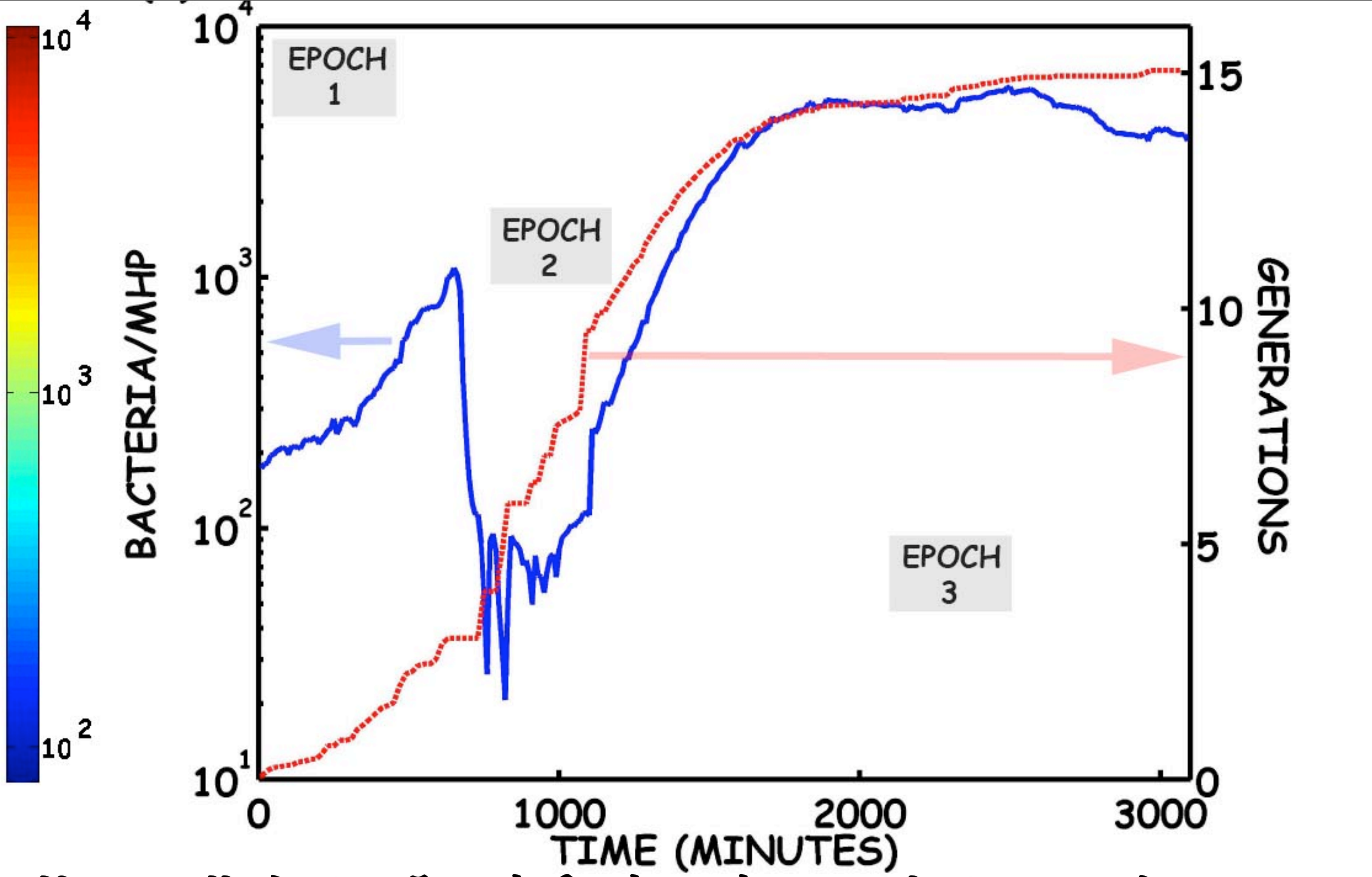
patch quality gradients

Coastal China:

Adaptation and change in a week by a metapopulation.

Too rapid growth in the good region, a fast probe into the (island rich) side of the habitat landscape, a quiet period when it probes the entire chip, then a regrowth, first in the richer region, than across the entire chip.



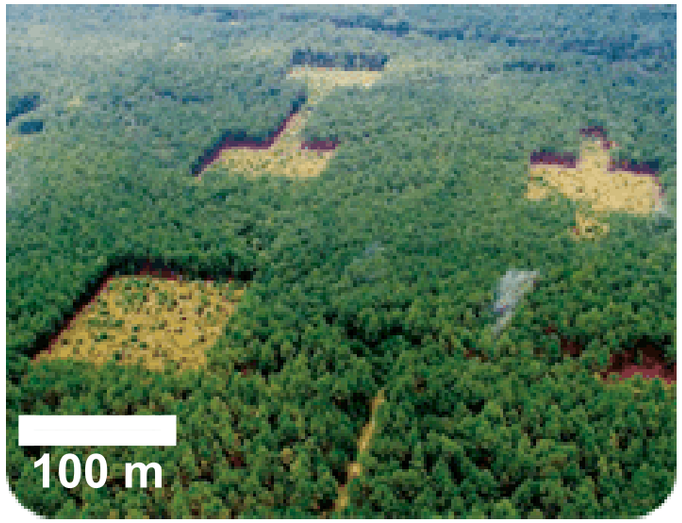


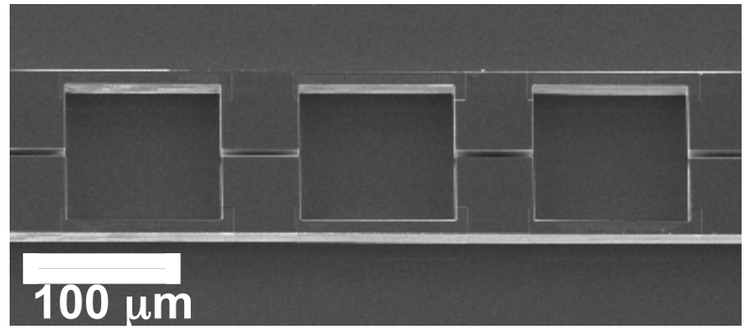
Note well: during Epoch 2 when the population number is small the actual generation rate is at a maximum, as if the organism is rapidly trying to solve a problem of survival.

Illustrations by Matilda Luk

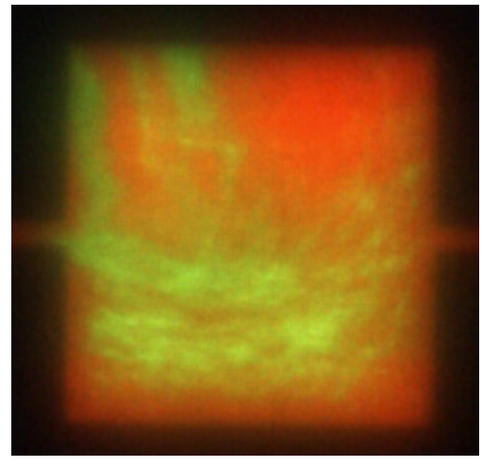
This not news to ecologists, or to Darwin in fact.

What can nanotechnology bring to ecology?





What can ecology bring to nanotechnology ?



There is a connection with this evo-devo work and the nanochannel work I have been doing with Robert Riehn and my other students and post-docs that I talked about yesterday.

The key concept here is Horizontal Gene Transfer, A.K.A. Sex in Bacteria. Bacteria with the F (fertility) plasmid are stud muffins.

Bacteria, being the Demons that they are, exchange genetic information that is useful to the population....they have a collective intelligence.

But, how the bacteria compete with one another in a game of life is another story for another day, it deeply connects with game theory and doesn't have much to do with nanofluidics.

Finally: Next Year Algeria!

Thanks!