The Voice of Ignorance (Robert Austin) Speaks:

Darwin Meets Nano

JUAN KEYMER taught me everything I have to say here! He has opened my eyes.

Peter Galajda, gifted experimenter

Cici Muldoon, Sungsu Park

The incredible staff of the NBTC and the CNF at Cornell University.

Here is what I have learned in life:

(0) Publish or perish. What don't you understand about that?

1) Nobody is THAT smart -Feynman.

2). Follow your instincts, and remember that being boring is worse than being wrong ("it isn't even wrong" -Pauli)

3) There are no stupid questions..... (well.... that's Dean-speak)

4) Experiment trumps theory every time.

5) Ignore the pithy sayings of Einstein about the violent opposition of little minds. You aren't Einstein.

6) If you talk to a molecular biologist, do exactly the OPPOSITE of what he says: you can't go wrong.

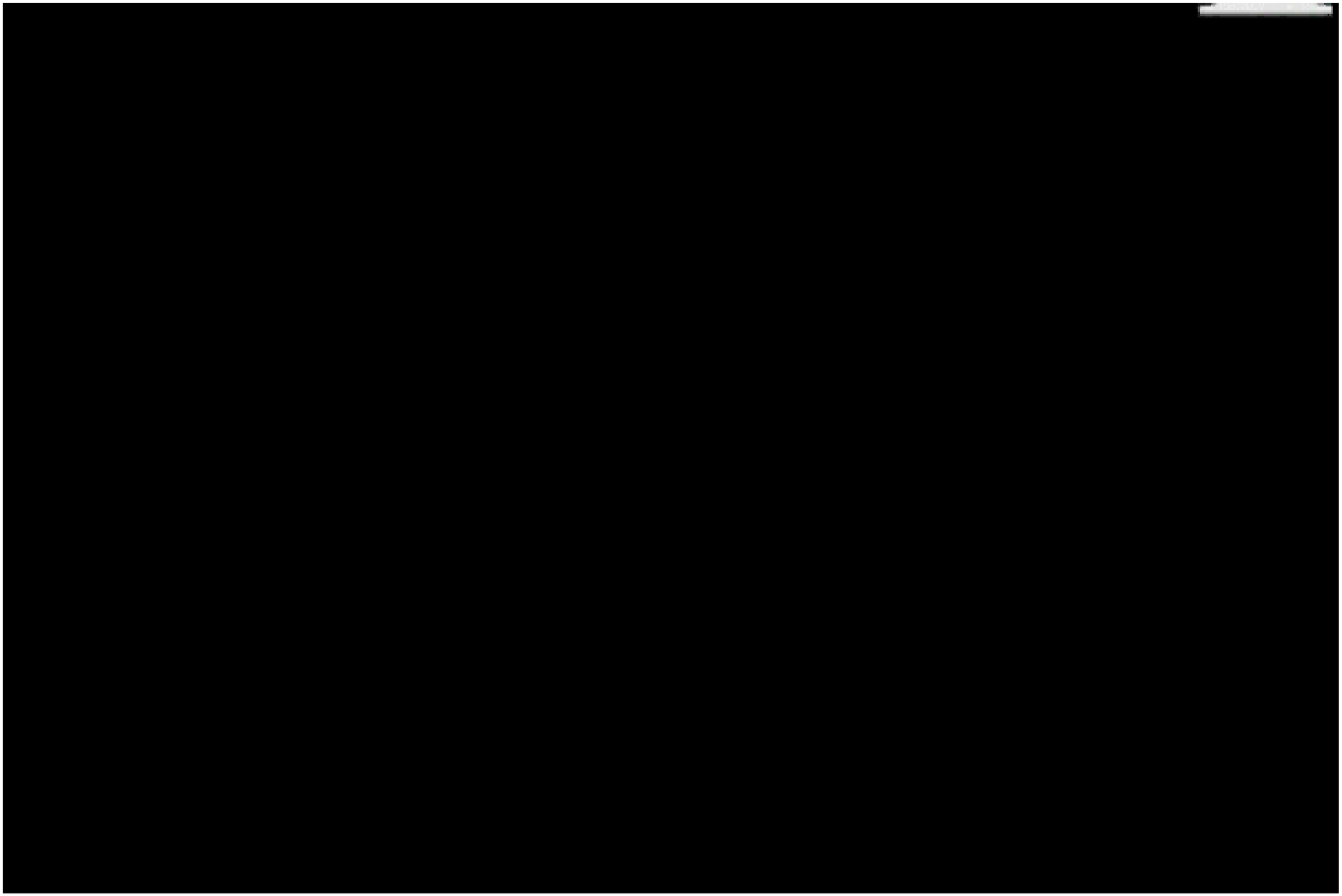
If you know a biologist, do exactly the OPPOSITE of what he says: you can't go wrong.

Example 1: Howard Berg et al have stressed the random walk nature of bacterial motion.

I myself think that bacteria are not only BMW M5s, but also quite possibly sentient beings that use us as spaceships.

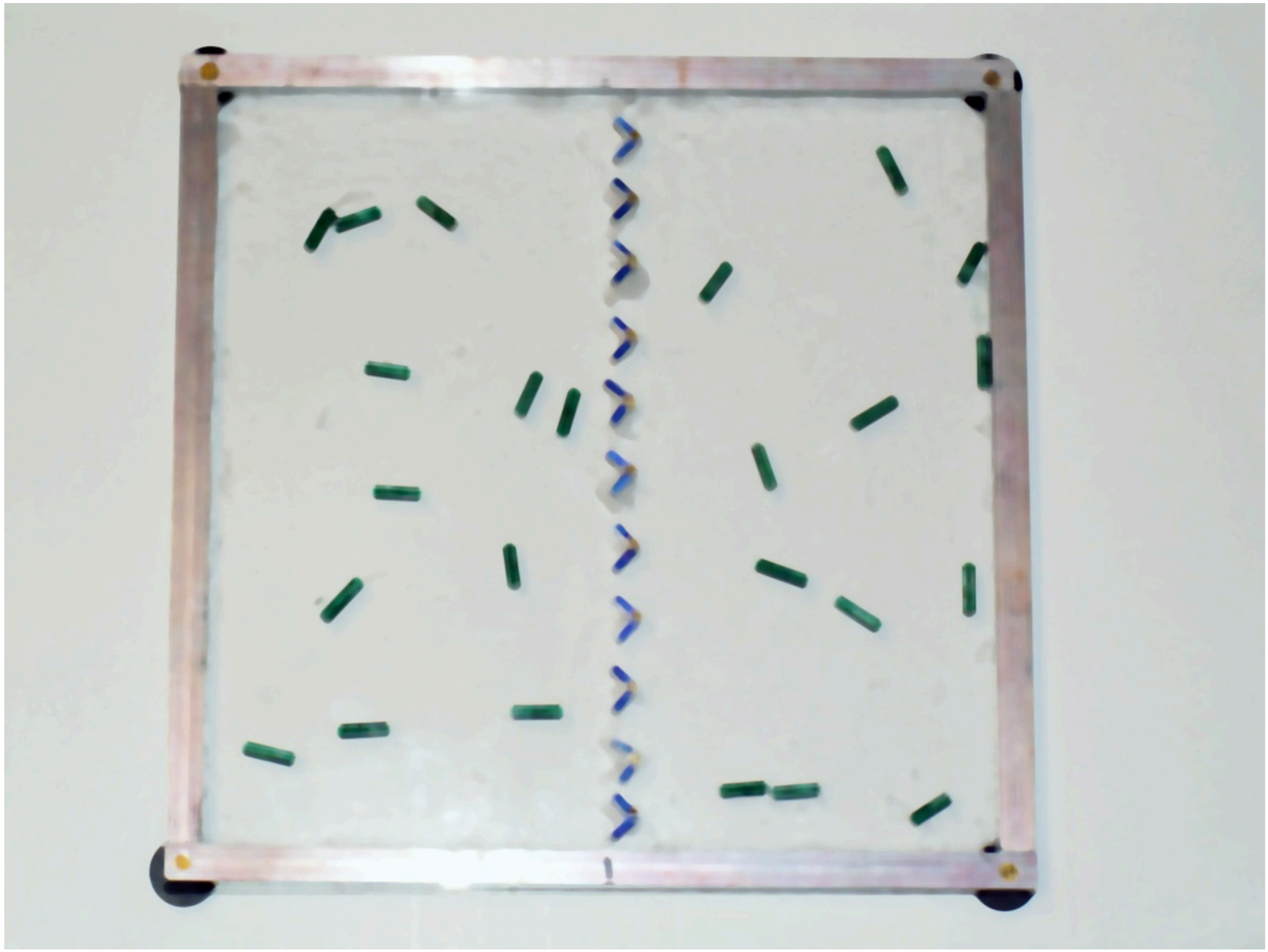
Anyway, about 15 years ago I tried to do an experiment where E. coli would wander through a maze, like the Dicty poster here, I I thought that maybe E. coli, as sentient beings, could find an efficient path through the maze. 4 years ago I finally found a student (string theorist!) brave enough to do the experiment.

Why brave? This is a stupid experiment, random walk in a maze, so what?

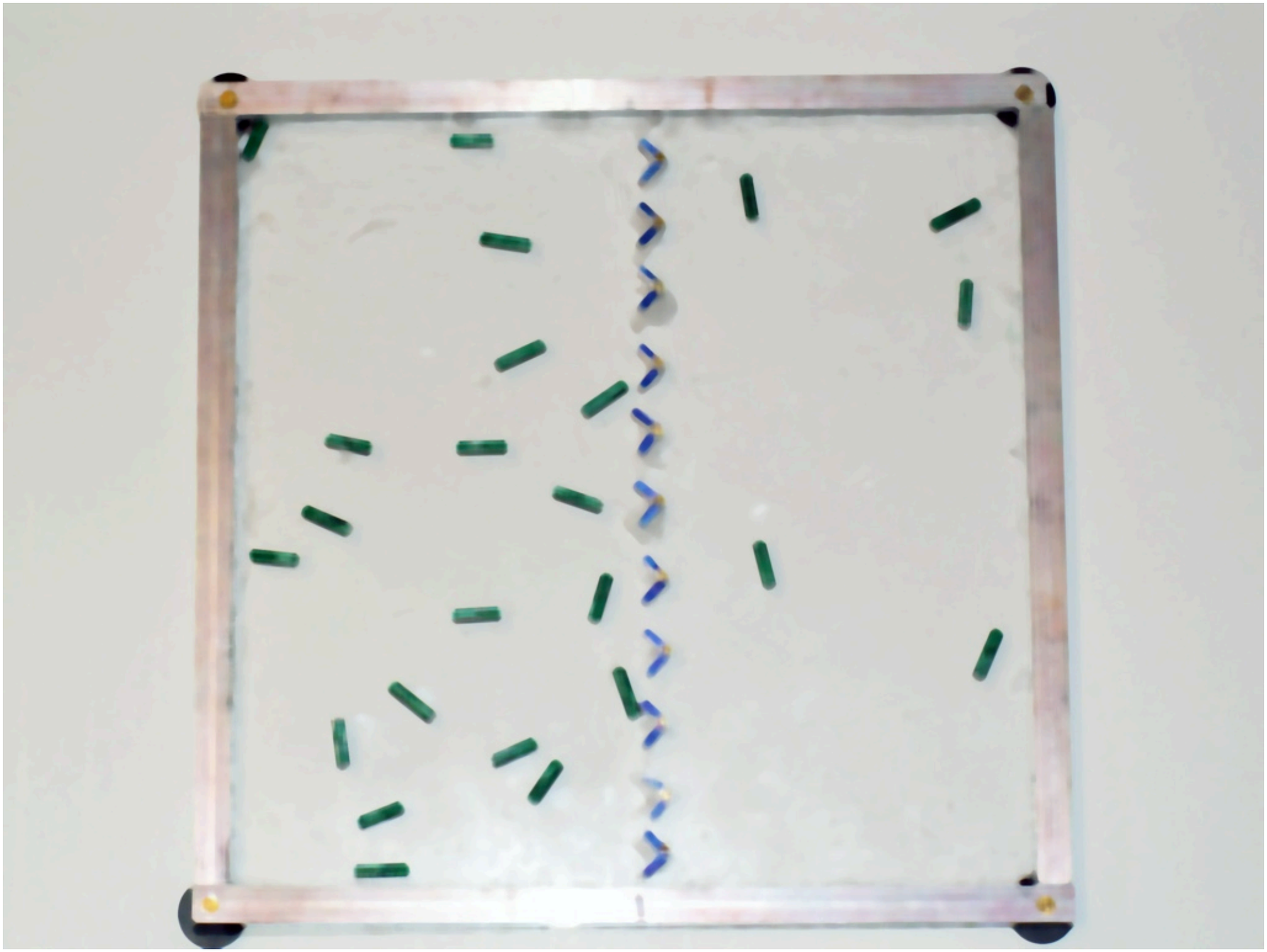


QUORUM SENSING AND BIOFILMS!!! NO BACTERIA IS AN ISLAND!!!!!

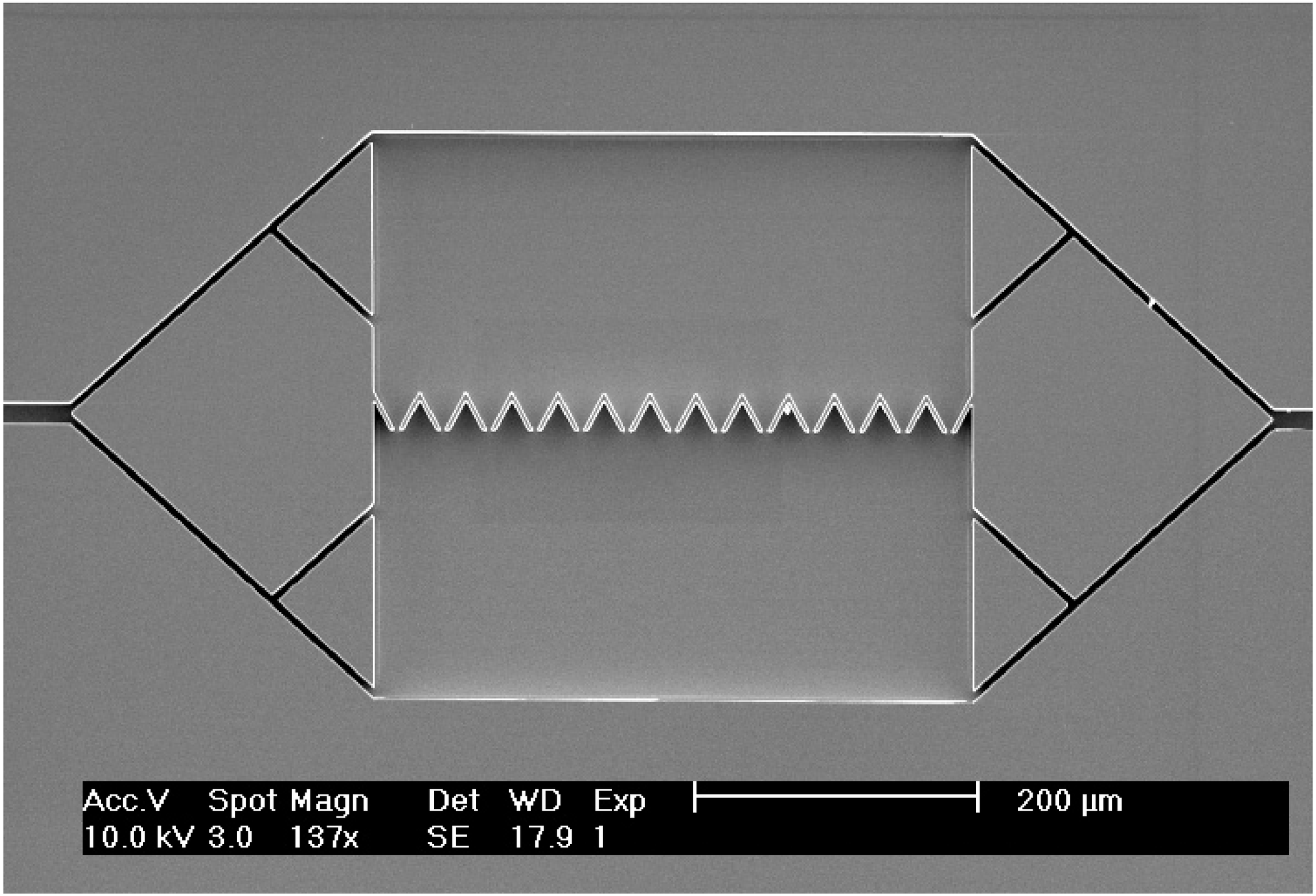
If you know a biologist, do exactly the OPPOSITE of what he says: you can't go wrong.

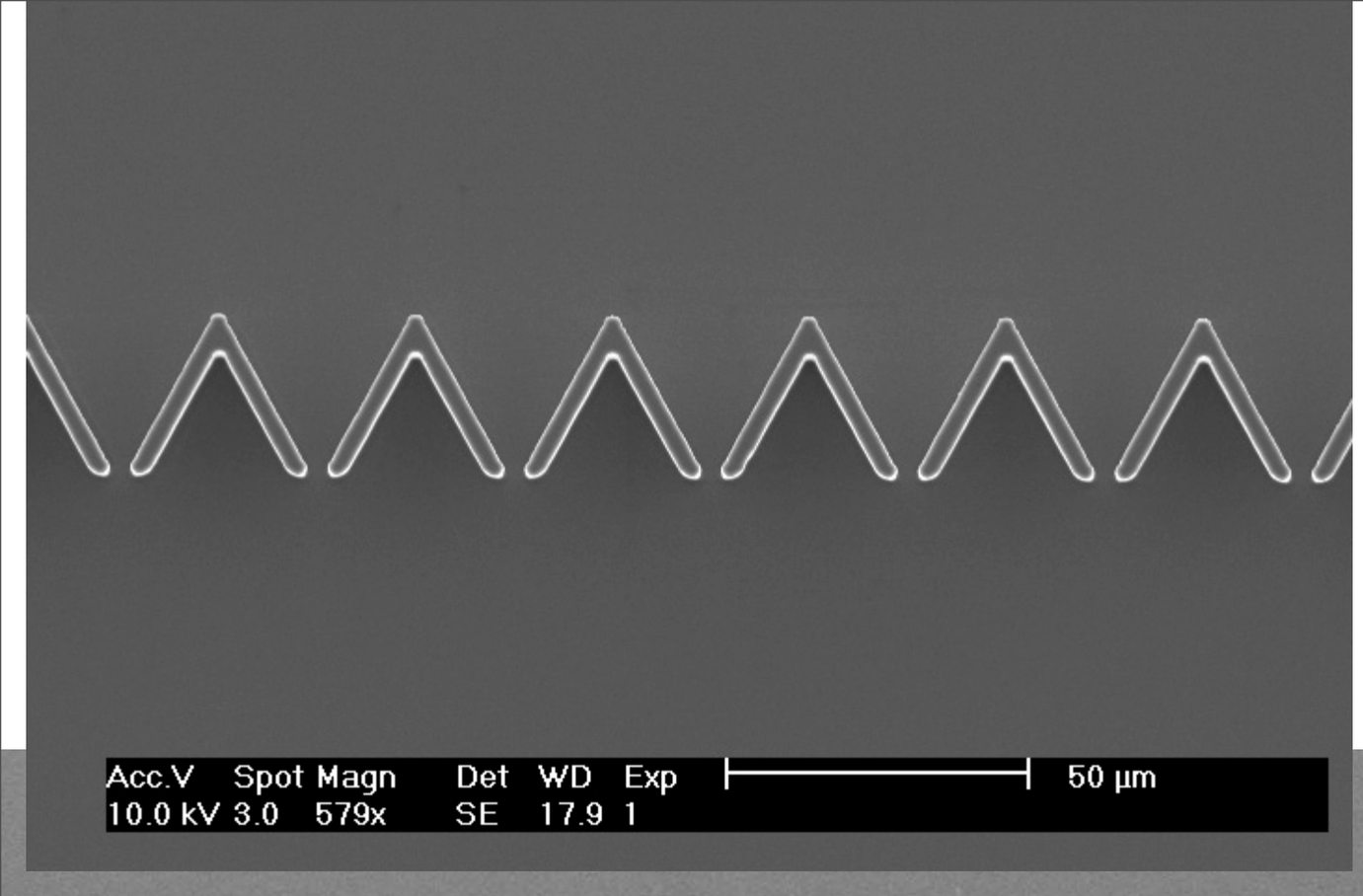


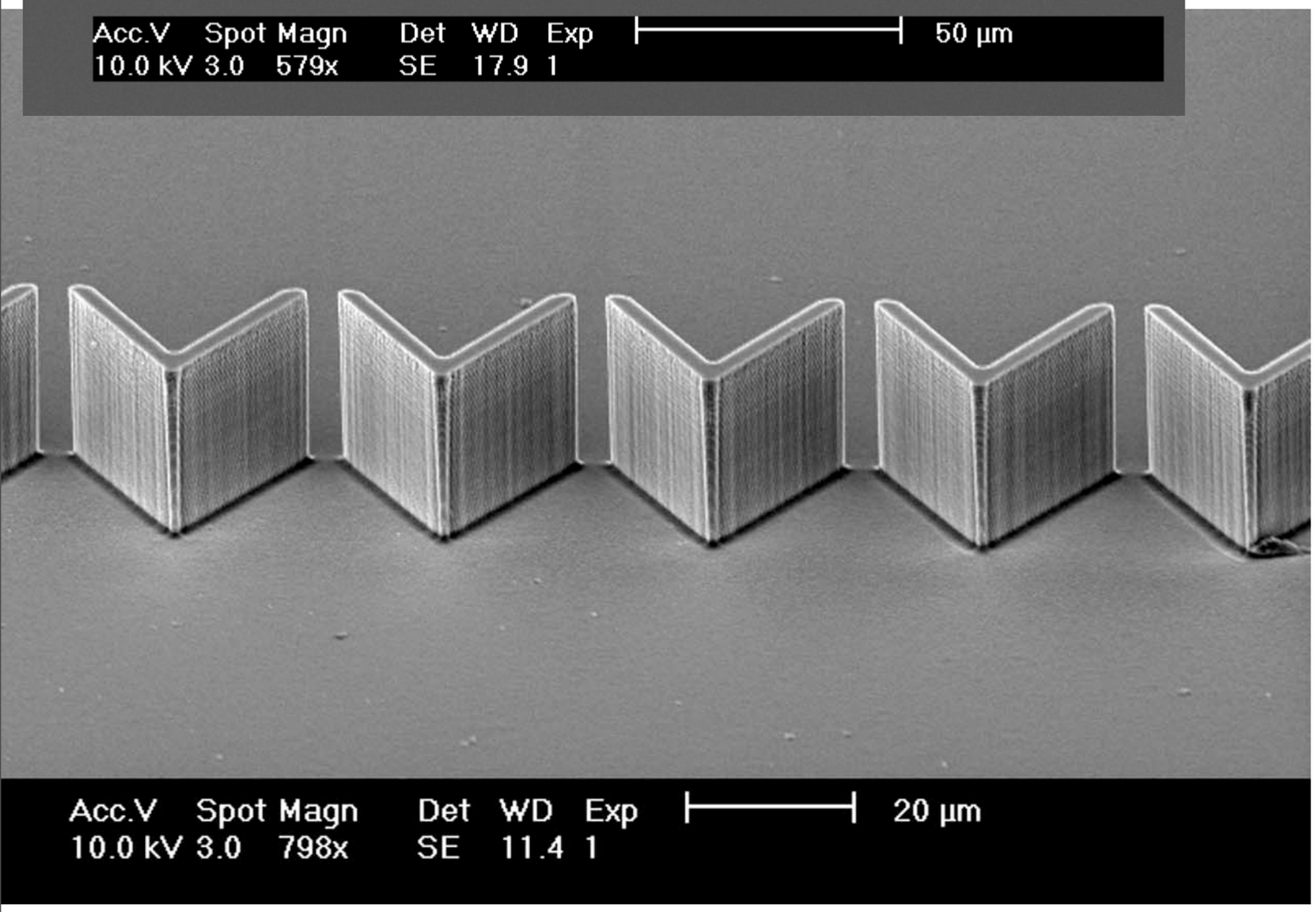
Example 2: The Maxwell Demon on a tabletop.



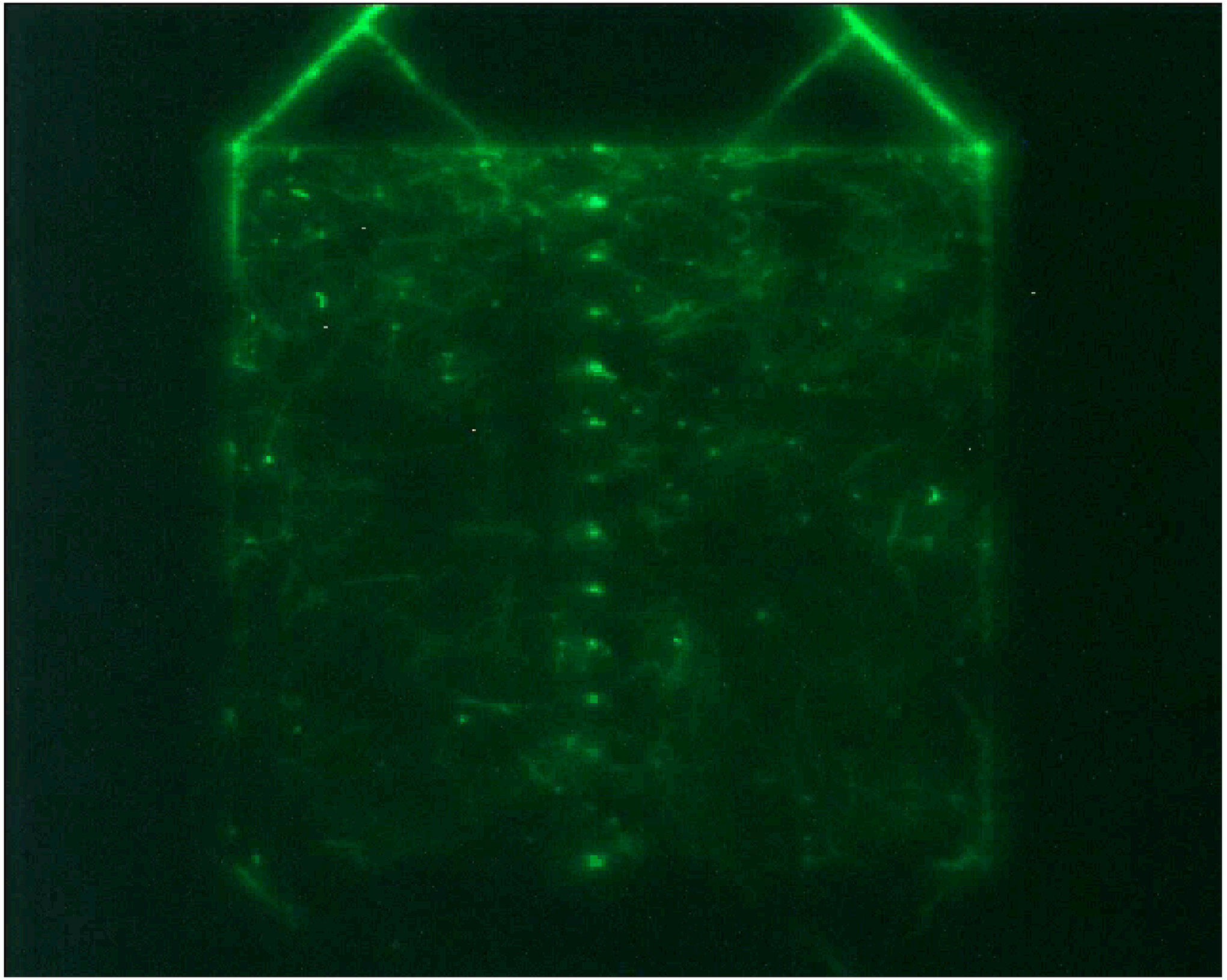
Are you disturbed by this?

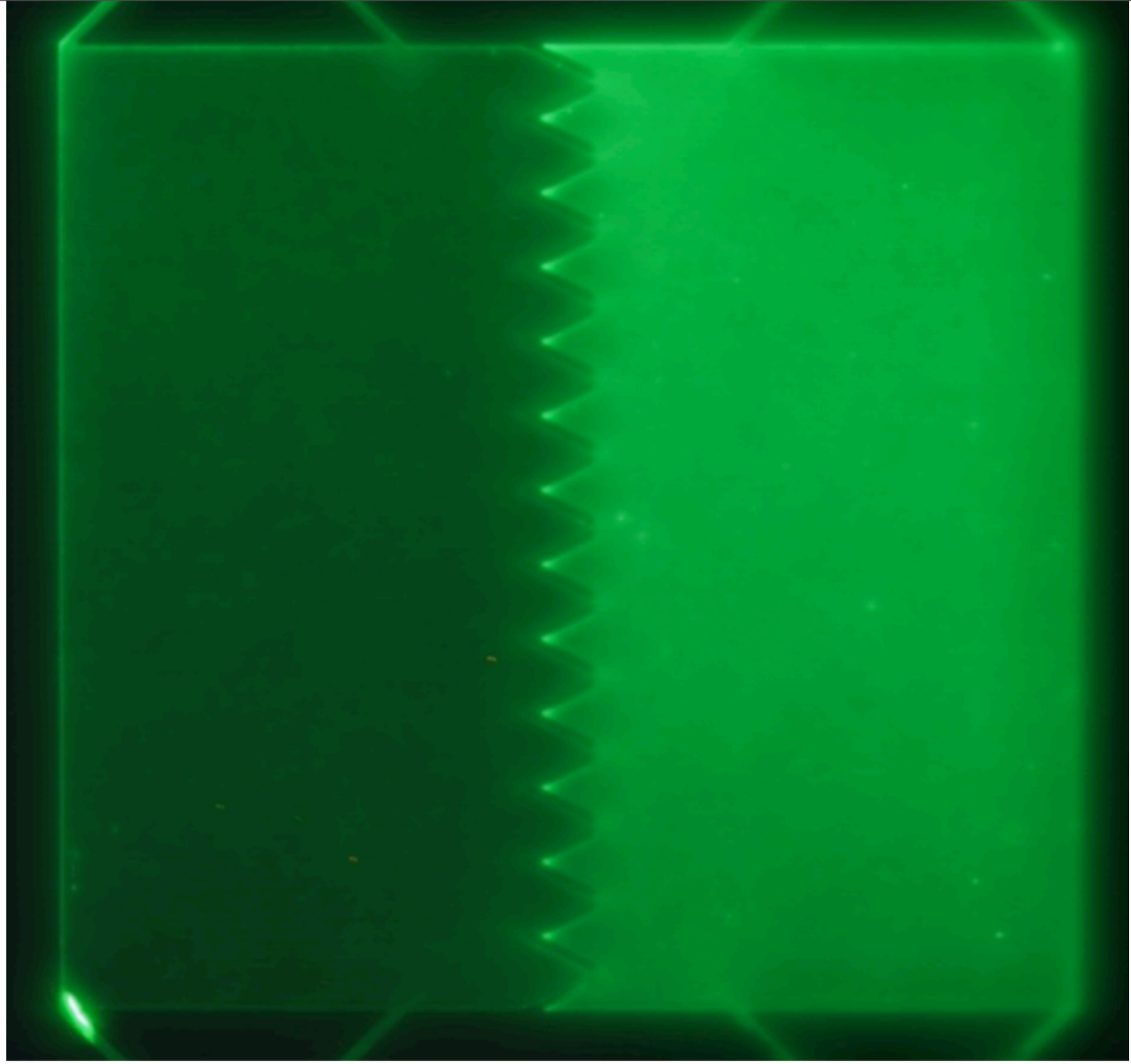

Acc.V Spot Magn Det WD Exp
10.0 kV 3.0 137x SE 17.9 1
200 µm



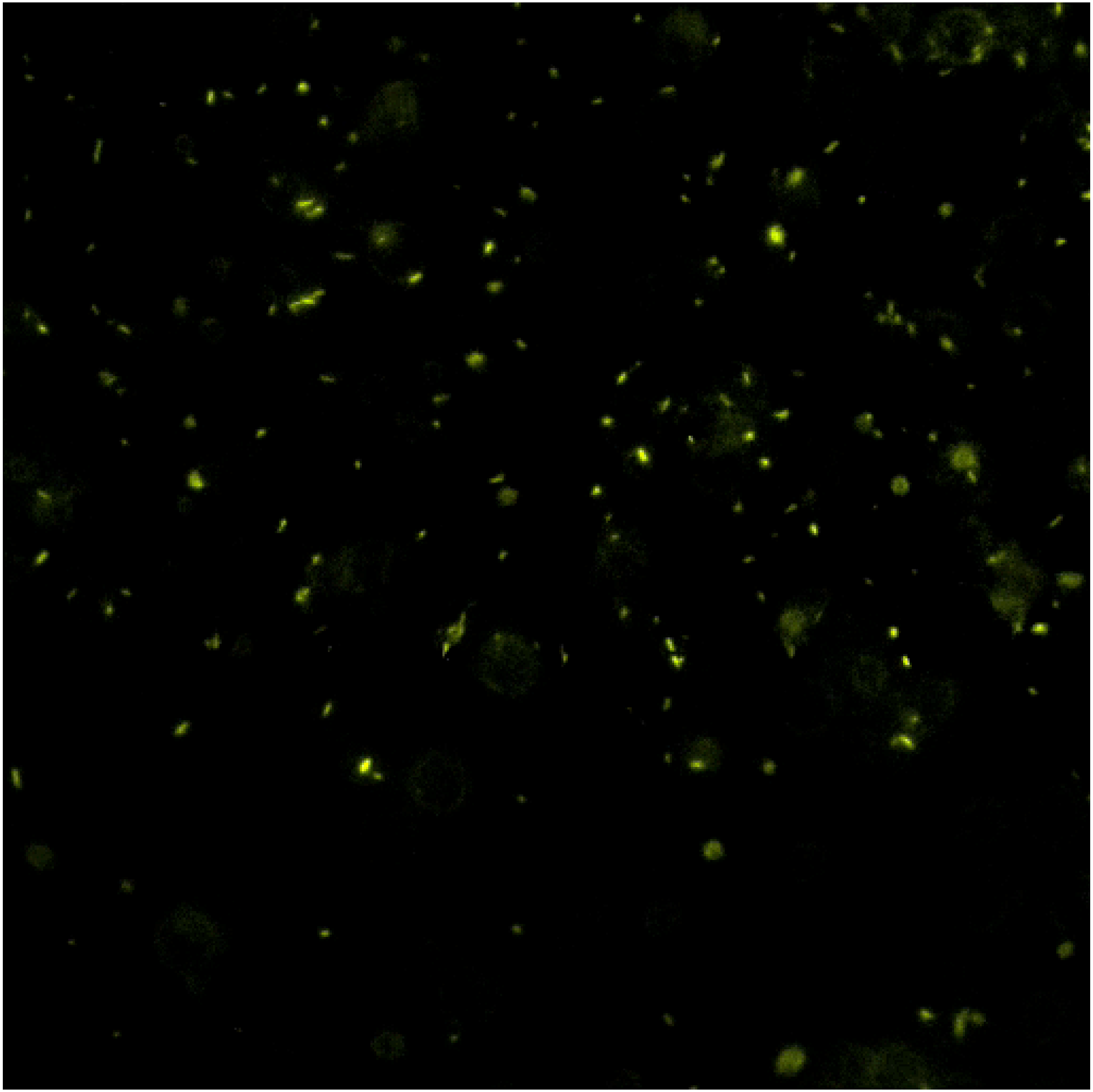


You have to love it. The technological power of micro/nanofabrication is amazing in the right hands (Peter Galajda).





So much for the either the random walk of bacteria or the Second Law of Thermodynamics. Your choice. Can you see where the random walk breaks down?



Could E. coli be sentient beings we all should worship? I think so. They certainly seem more intelligent that Geo. Bush.

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

Example 3. 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.

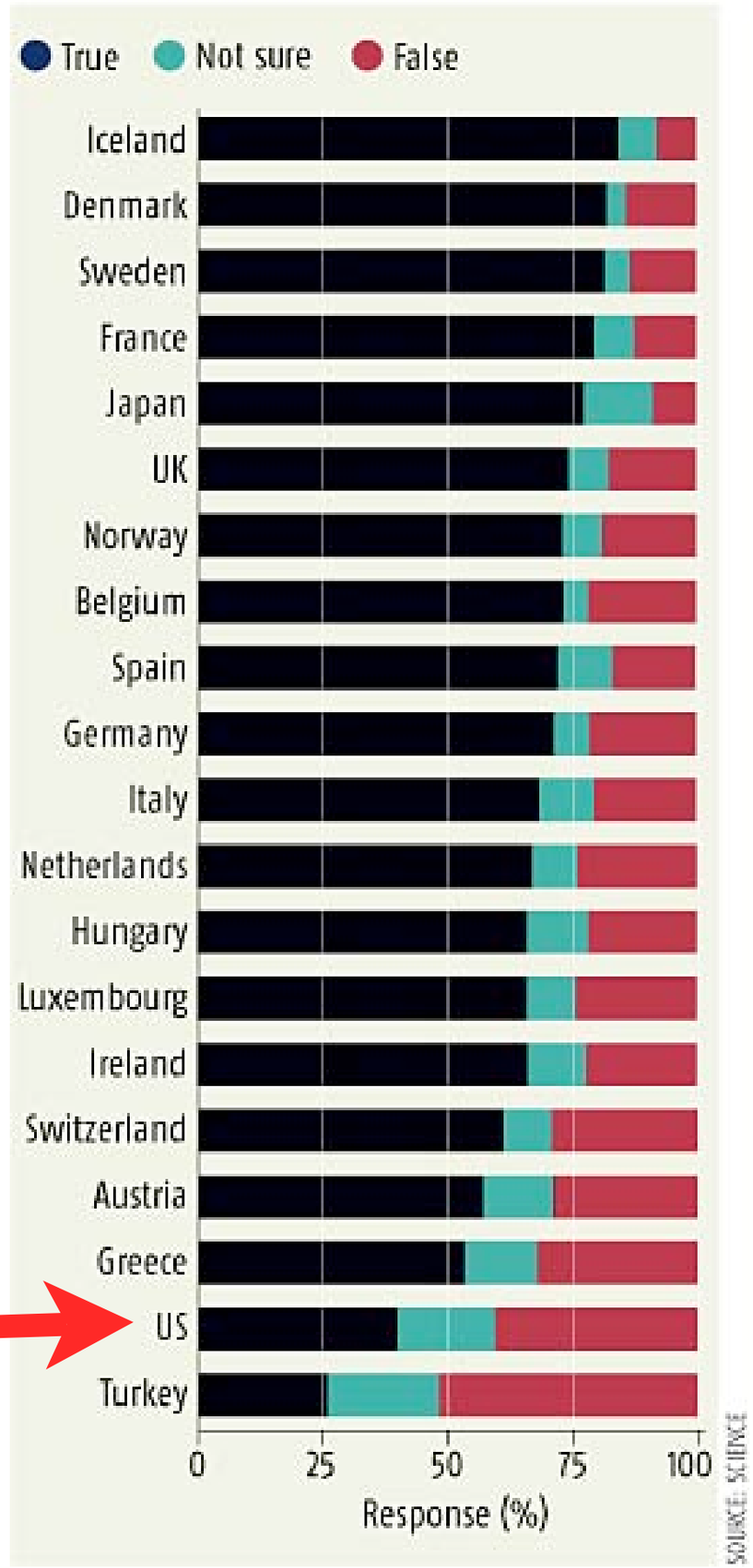
Some biologists became ecological and evolutionary biologists because they felt phenotype was more important that genotype. Ecologists got the crappy old buildings with steam heat, mo bio types got Italian designed palaces of light with artsy sculptures built of lead in the plaza.

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:

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

So: waste of time.



"The percentage of people in the country who accept the idea of evolution has declined from 45 in 1985 to 40 in 2005 (Science, vol 313, p 765). That's despite a series of widely publicised advances in genetics, including genetic sequencing, which shows strong overlap of the human genome with those of chimpanzees and mice. "We don't seem to be going in the right direction," Miller says."

That is, the more we learn, the less the US public believes us.

Most American's don't dispute Newton's Laws of motion, why don't most American's accept evolution as the origin of the species?

It isn't because they are stupid, I would remind you that Einstein of the pithy wisdom never accepted Quantum Mechanics.

Here is an interesting 1973 statement from Jacques Monod of Nobel Prize fame and "Chance and Necessity", which has really done a lot of damage.

..."The other great difficulty about the theory of evolution is that it is what one might call a second-order theory. Second-order, because it is a theory aimed at accounting for a phenomenon *that has never been observed*, **and that never will be observed**, namely evolution itself. In the laboratory, we are able to set-up conditions so that we may be able to isolate mutations of a given bacterial strain, for instance; but to observe a mutation is a very far cry from observing actual evolution. That has never been observed even in its simplest form–the one which is required by modern theorists to account for evolution, namely the simple differentiation of one species from another. This is a phenomenon that has never been seen. I would not say it never will be, but it seems extremely doubtful."

Now, why would he say that? (A) Because of Neoclassical Evolution Dogma accepted by most biologists themselves.

B) Because he was dead wrong, 3 years before he wrote it.

Speciation: lack of interbreeding due to genome changes in the transcription factors which control gene expression.

1) Dobzhansky, Th., and O. Pavlovsky, 1971. "An experimentally created incipient species of Drosophilia", Nature 23:289-292 (in 2 years before Monod)

2) Formation of five new species of cichlid fishes which formed since they were isolated less than 4000 years ago from the parent stock, Lake Nagubago.

Mayr, E., 1970. Populations, Species, and Evolution, Massachusetts, Harvard University Press. p. 348

And so on, and so on! Even the biologists don't believe in evolution!

My narrow view of the neoclassical evolution dogma:

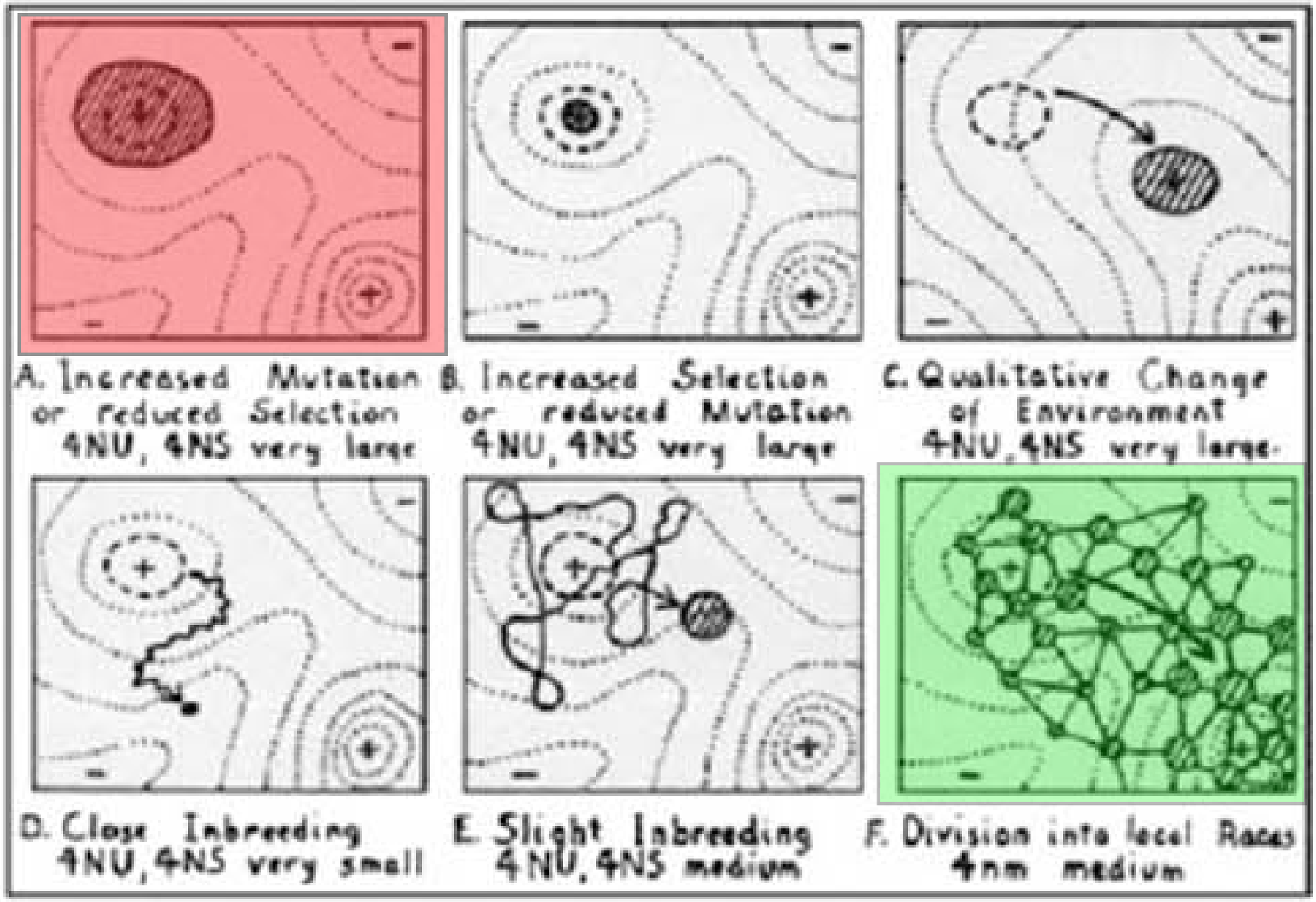
1) Mutations are random on the genome.

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. We have heard this here this week ad infinitum...

5) 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. You won't see evolution on a flat landscape. You will get Kansas.



In this view, evolution at the species level is a very slow, very infrequent and basically passive process that is also rather stupid: faster wins, slower loses, end of story. Zzzzzzz

But Seawall Wright in 1932 knew better.

There is a newer view that is emerging:

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 opinons, quite firm.

I'm not a big fan any more of stochastic models of organism response, as if that explains everything. There is a great deal more to biology than stochastic response and blind statistics, but random analysis has a nice physics air to it and you don't have to know any biology to do it, so why not?

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.

Would biologists be upset?

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

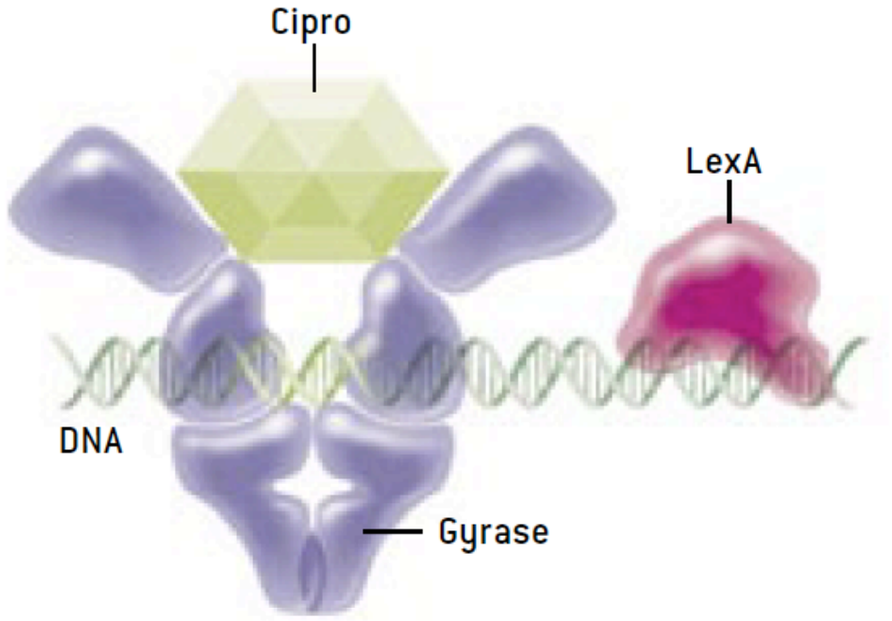
Can in fact evolution be directed by the organism itself? This statement seems to really frighten many biologists, since it seems to smack of some sort of "design". Biologists will defend Darwin even more fiercely than physicists defend Einstein.

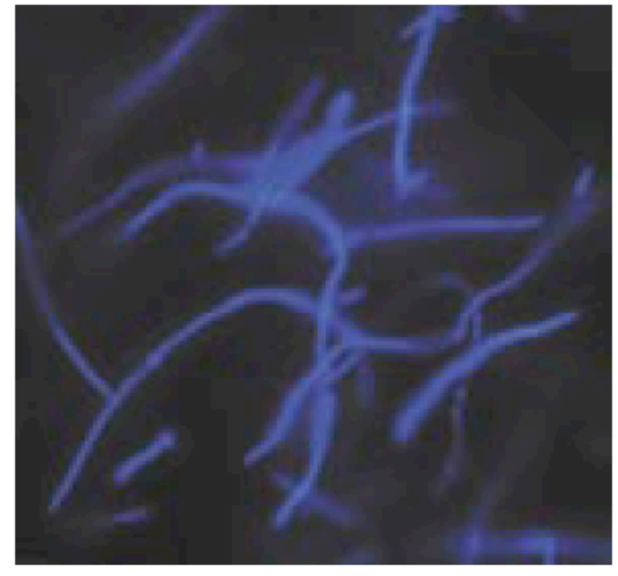
Bacteria are able to evade certain antibiotics like ciprofoxacin USING the "SOS response" and directed mutations! So much for the "War on Cancer" in the 70's!!!

Rapid-fire mutations in *Escherichia coli* bacteria can undermine the effectiveness of ciprofloxacin (cipro), an antibiotic that is increasingly being prescribed by physicians.

Cipro's Action

Cipro usually harms bacteria by binding to an enzyme called gyrase and preventing it from functioning properly

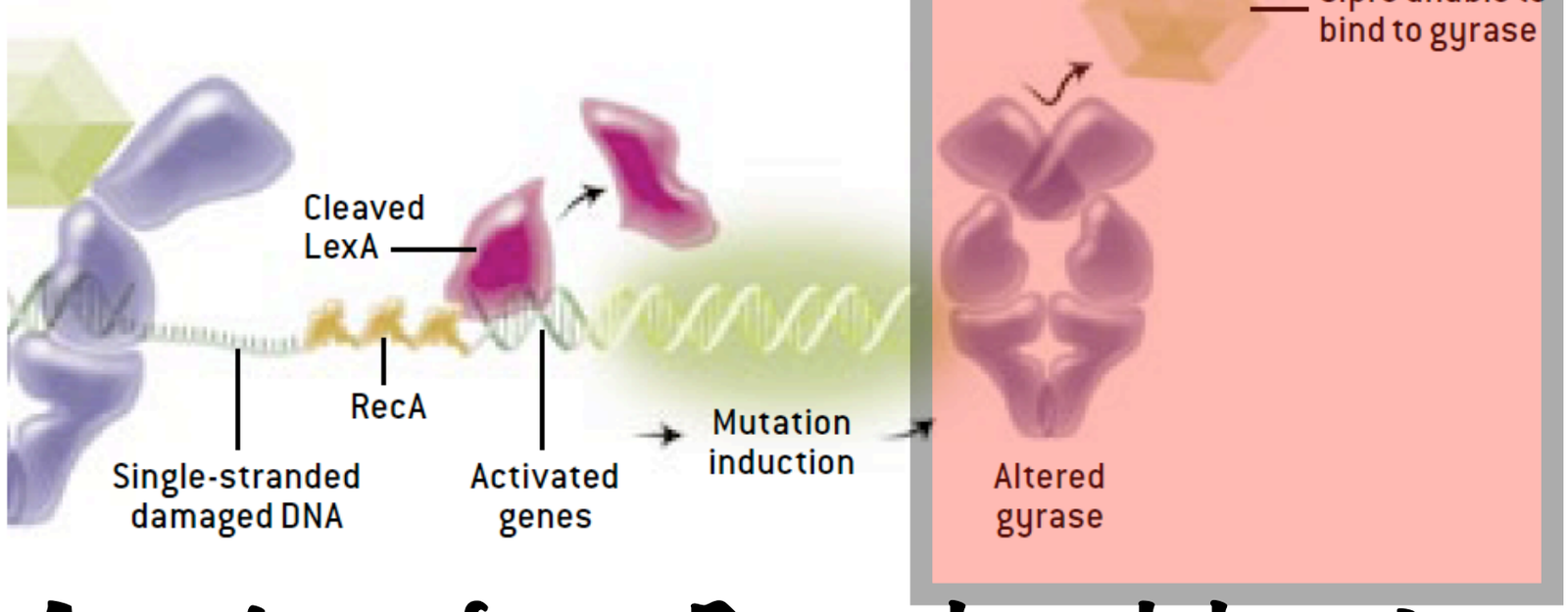




DNA of *E. coli* (*shown above*) cannot replicate when it is exposed to cipro.

How Resistance Arises

Resistance is initiated when *E. coli* responds by generating single-stranded DNA. Individual molecules of another protein, RecA, then line up in a chain and attach to the single-stranded DNA. RecA facilitates cleavage of a regulatory protein, LexA. This change frees a set of formerly repressed genes to induce mutations elsewhere. The mutations end up blocking cipro's binding to gyrase, thereby preventing the drug from working



April 2006 Scientific American, from Romesberg laboratory

Lesson learned from SOS :

"Mutations are traditionally thought of as happening as a random process and as a liability to the organism. Many strategies exist in a cell to curb the rate of mutations.

Mutations on the other hand can also be part of a survival strategy. For the bacteria under attack from an antibiotic, mutations help to develop the right biochemistry needed for defense. It is found that certain polymerases in the SOS response to DNA damage actually are assigned the task of promoting mutations in the genes that code for the topoisomerases. As in evolution a larger variety of topoisomerases improve the survival chances." - Wikipedia

Mutation rates can be under network control and can be turned up and down by the organism at directed gene targets, and I suspect the "evolution clock" is no clock at all, but rather an integral over time of a wildly varying process.

So, it is a Cruche de Merde to think that mutation rates are fixed and random.

SOS response is about radiation damage, what about NON-radiation damage, or simple metabolic stress. Not all of us live in Hanford WA. Is there a similar turn-on of mutation rates for other forms of stress?

The non-radiation variant of this is called Stress-Induced Mutagenesis (SIM). A related form of this is called MAC: Mutagenesis in Aging Colonies. That is, as bacterial colonies age mutation rates go up.

Most biologists avoid MAC like the plague by always keeping their bacteria in the exponential growth phase, which means they basically miss everything about adaptation and evolution. Say no to exponential growth.

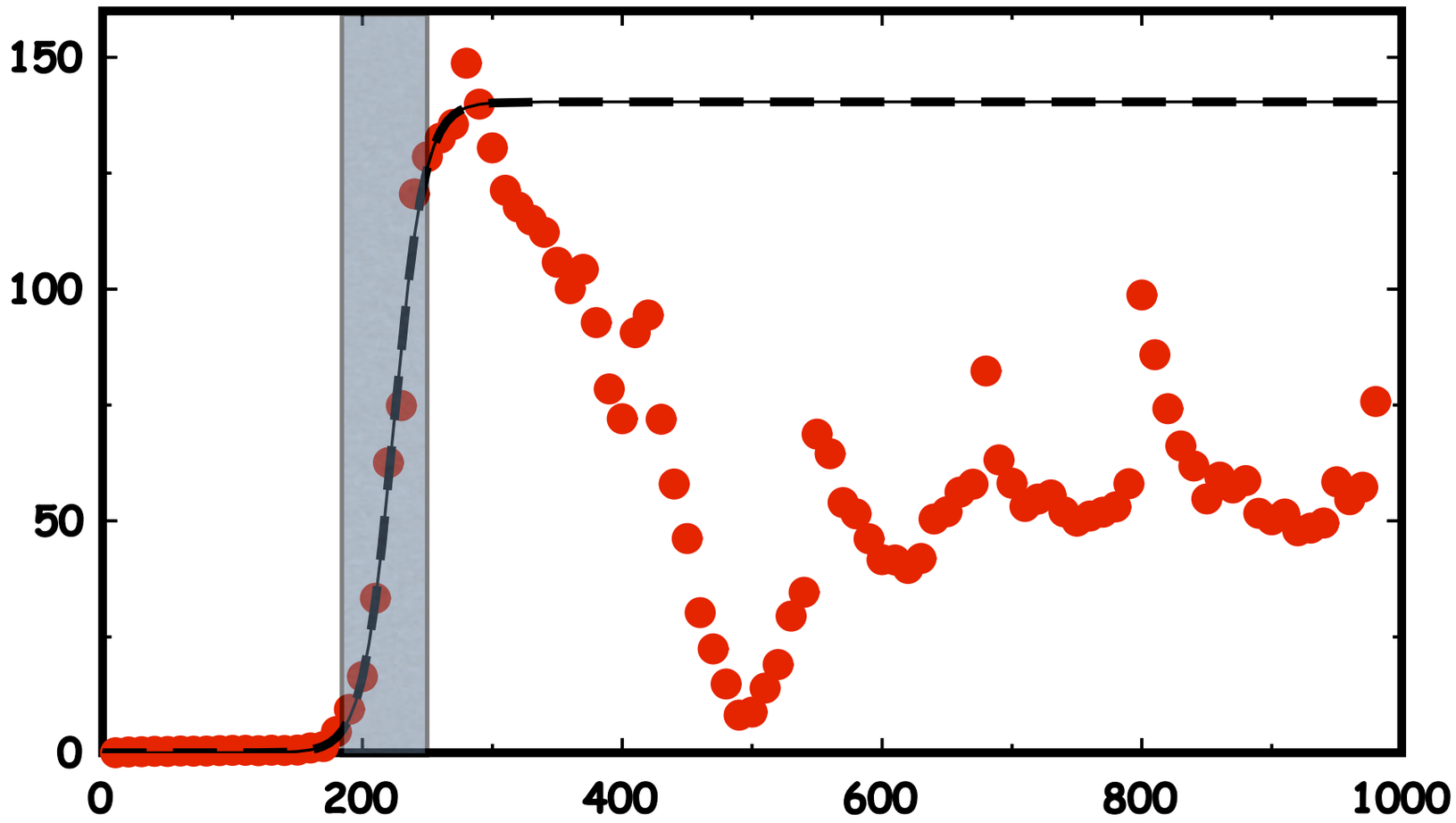
A brief note about the lives of bacteria : the exponential ("log") phase is only a very small part of the life of a bacteria. Keeping bacteria in the log phase is like only studying humans in kindergartens. Amusing, but infantile.

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:

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

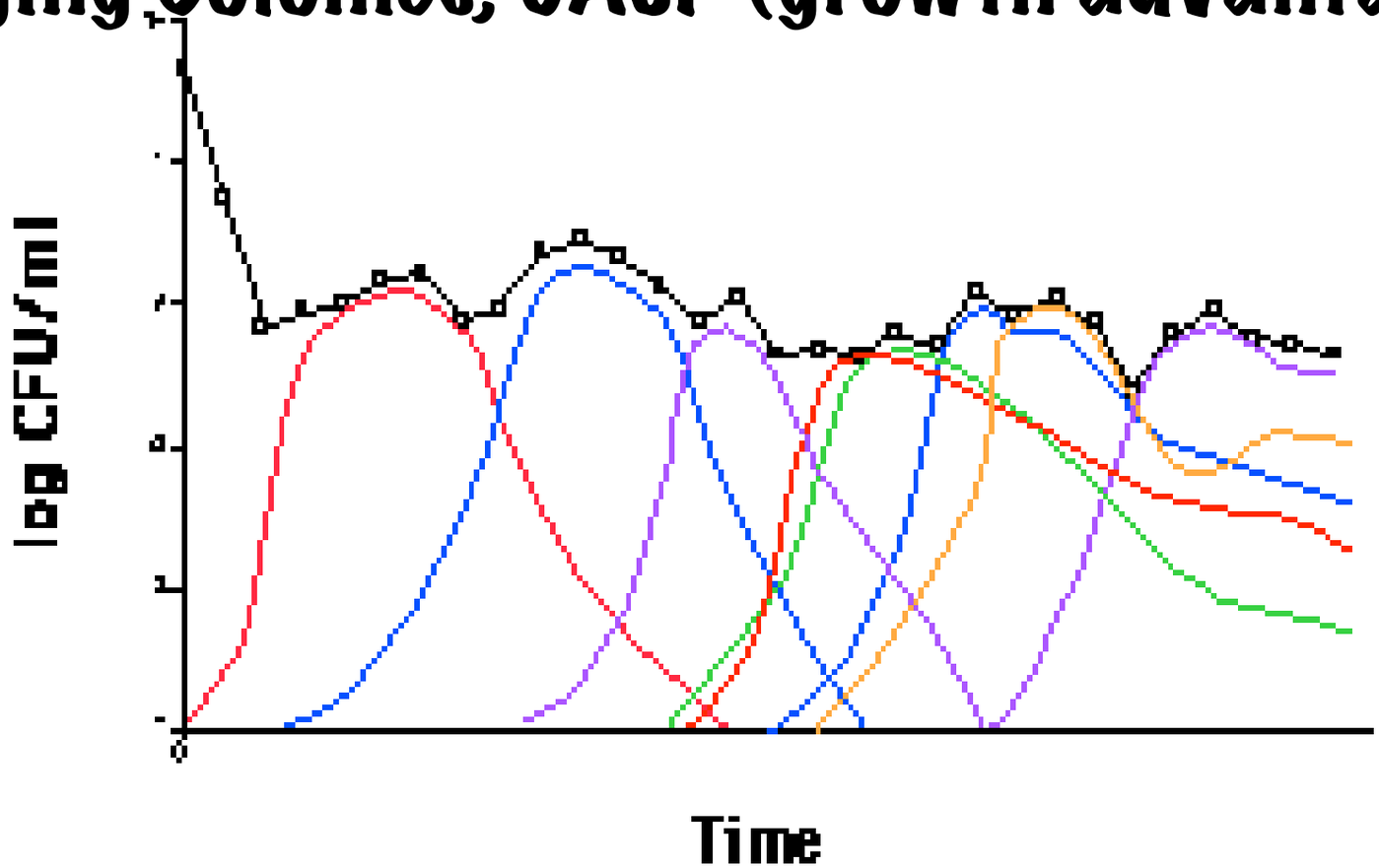
"R" selection: advantage through numbers (fish eggs)

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



Mutagenesis in Aging Colonies, GASP (growth advantage in stationary phase)

Roberto Kolter,
Harvard



"The backbone of modern genetics and the neo-Darwinian theory of evolution by natural selection is that gene mutations occur at random, independently of the environment in which the organisms find themselves".

The idea that genes do not mutate at random, but 'adaptively', as though 'directed' by the environment in which the organisms find themselves, is so heretical that most biologists simply dismiss it out of hand." I have found this to be the case.

Microbiologist(!!!) (originally physicist) Max Delbrück first used the term 'adaptive mutations' in 1946 (!!) to refer to mutations formed in response to an environment in which the mutations are selected." So, physicists have a history being heretics.

http://www.i-sis.org.uk/TMONTM.php

So.....what is indeed the connection with nanotechnology, physics, biology and myself?

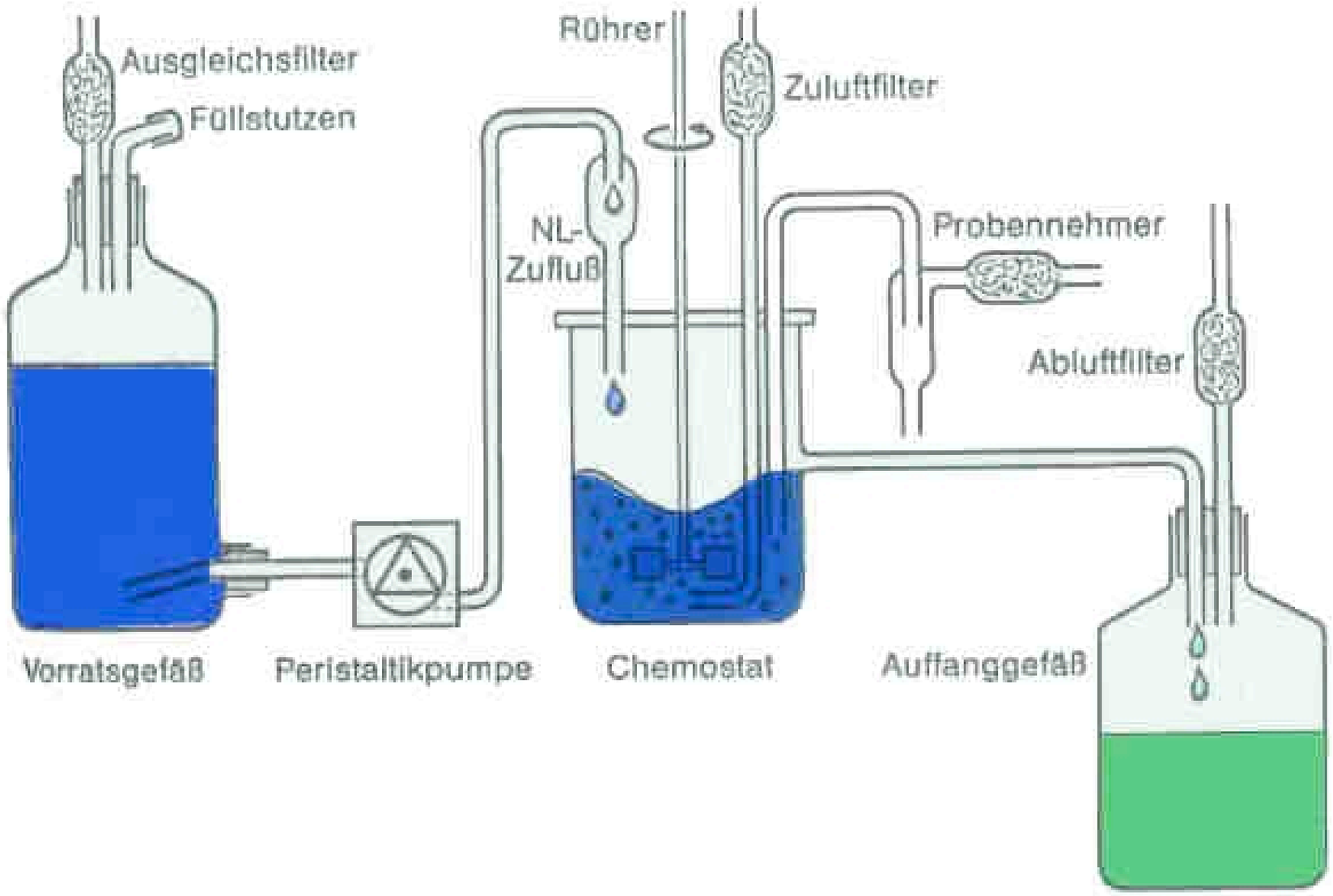
Juan Keymer is a Mathematical Evolutionary Ecologist (from Chile), who stumbled on our Quorum Sensing paper.

Through Juan we have been conducting evolution experiments in a Mutagenesis in Aging Colonies environment.

This came from earlier work I did on Quorum Sensing, Bonnie Bassler's groundbreaking work on cell-cell communication in bacteria that was also heretical in its' time and now is turning into a dreaded paradigm.

Molecular biologists (but not ecologists mostly study evolution in a chemostat.

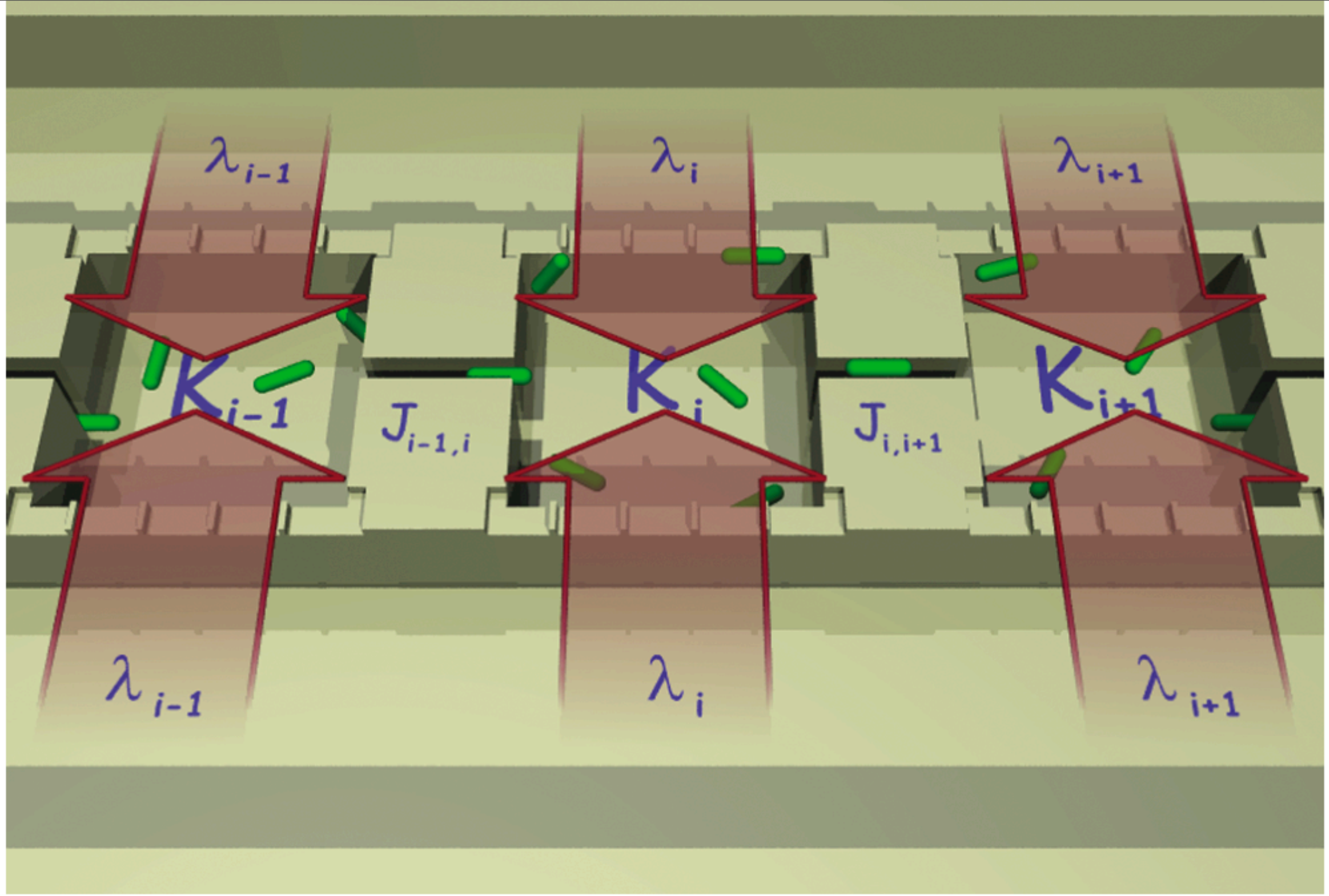
A chemostat is a continuous flow evolution reactor, invented by Leo Szilard, the great physicist after he went into biology from bomb building, and never had a good bath again.



A chemostat is designed to keep organisms in exponential growth, has about 10^{10} organisms, is homogenous and looks at "R" selection only: far, far from the real world. You learn little about fitness landscape dynamics in such a device.

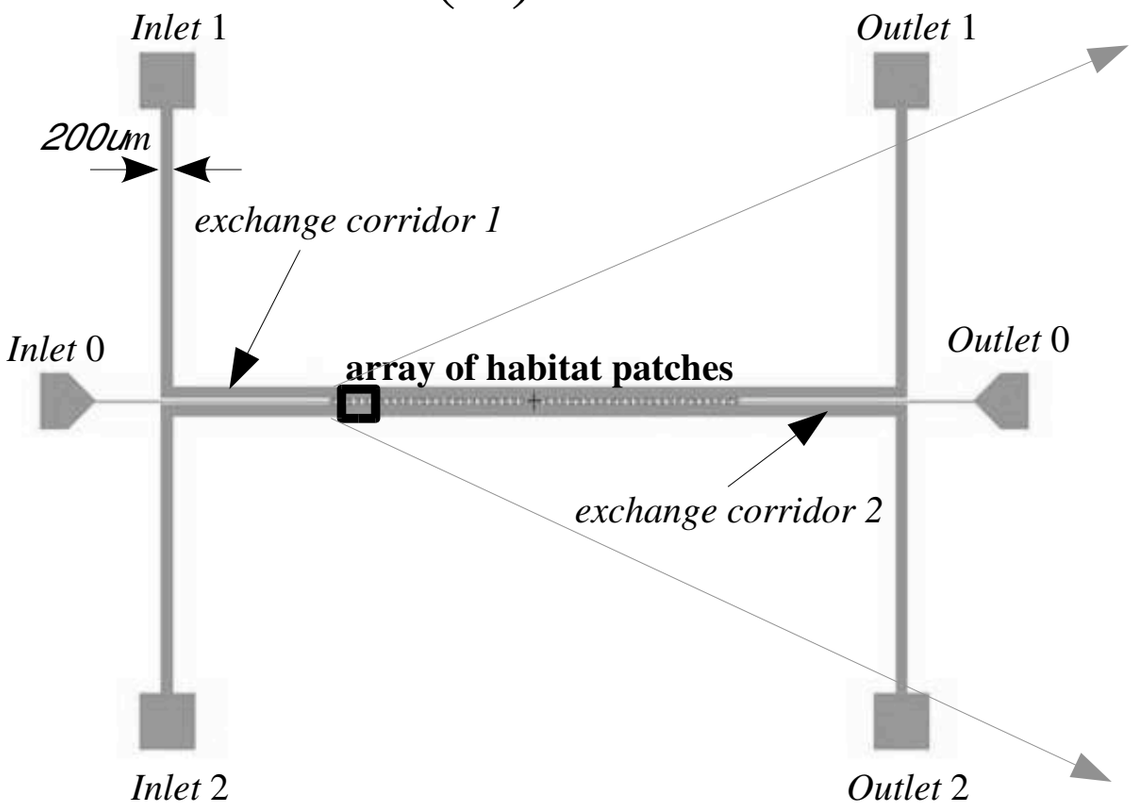
Here is our idea is a nutshell: Create a series of very small microhabitats in which bacteria are kept in stationary phase under highly stressed conditions, and make the microhabitats different from each other but allow the bacteria to move around: that is, create a complex network of metapopulations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

How do the bacteria evolve and adapt in this stressed, heterogenous environment? Do the metapopulations communicate with each other?

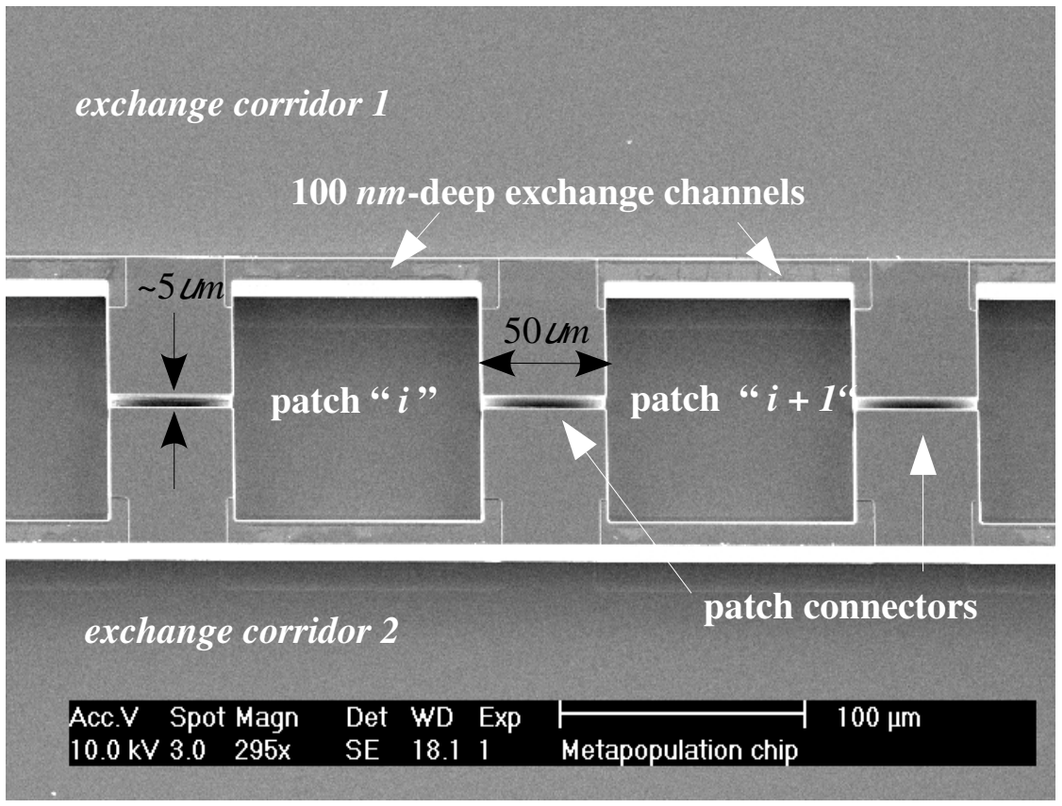


A nanofabricated habitat landscape.

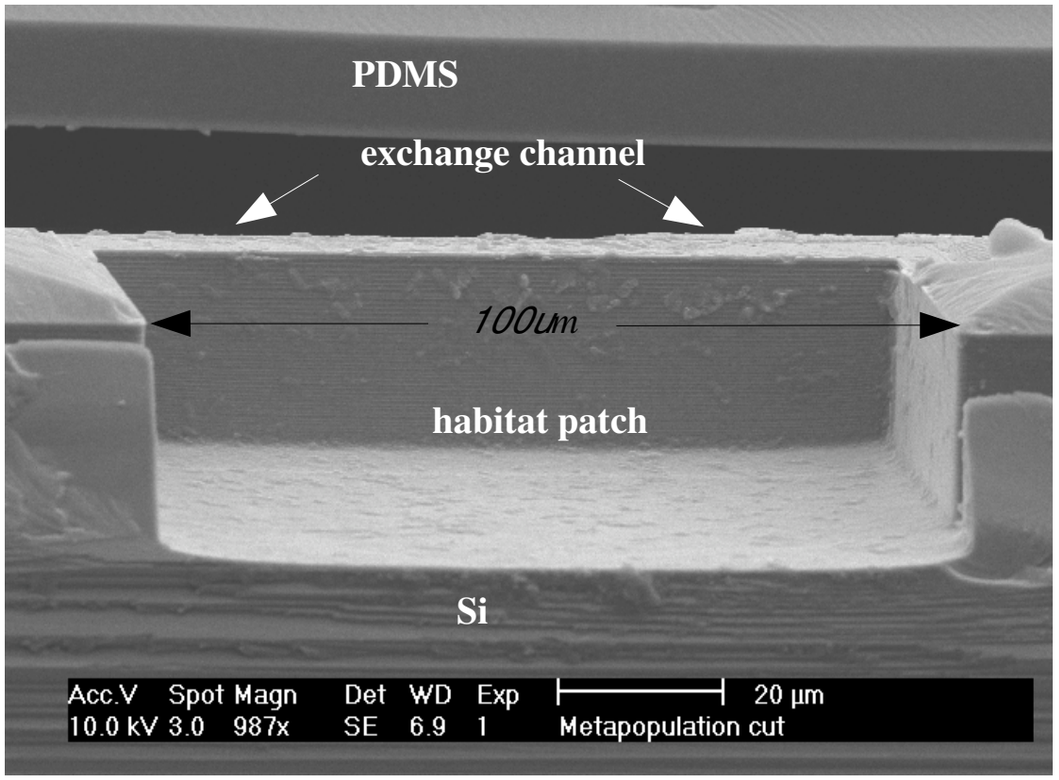
(A)


Inlet 1
Outlet 1
200um
exchange corridor 1
Inlet 0
array of habitat patches
Outlet 0
exchange corridor 2
Inlet 2
Outlet 2

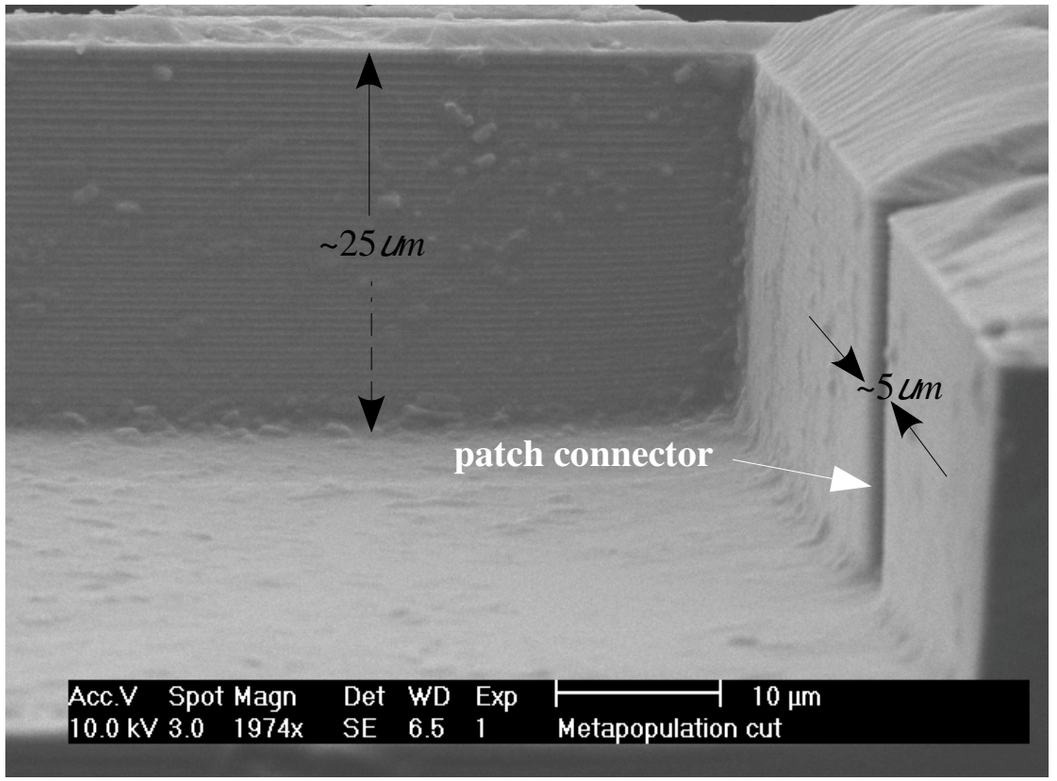
(B)


exchange corridor 1
100 nm-deep exchange channels
~5 um
50 um
patch " i "
patch " i + 1"
patch connectors
exchange corridor 2
Acc.V Spot Magn Det WD Exp 100 µm
10.0 kV 3.0 295x SE 18.1 1 Metapopulation chip

(C)


PDMS
exchange channel
100um
habitat patch
Si
Acc.V Spot Magn Det WD Exp 20 µm
10.0 kV 3.0 987x SE 6.9 1 Metapopulation cut

(D)


~25 um
~5 um
patch connector
Acc.V Spot Magn Det WD Exp 10 µm
10.0 kV 3.0 1974x SE 6.5 1 Metapopulation cut

Why this is not a series of microchemostats:

1) Dead bacteria do not flow out but simply lyse. You live here, you die here.

2) The holding capacity "K" is small and diffusion limited, the organism strongly influences the local holding capacity.

3) You don't have to live in Paris. There are connecting channels to the provinces where life may be better or worse.

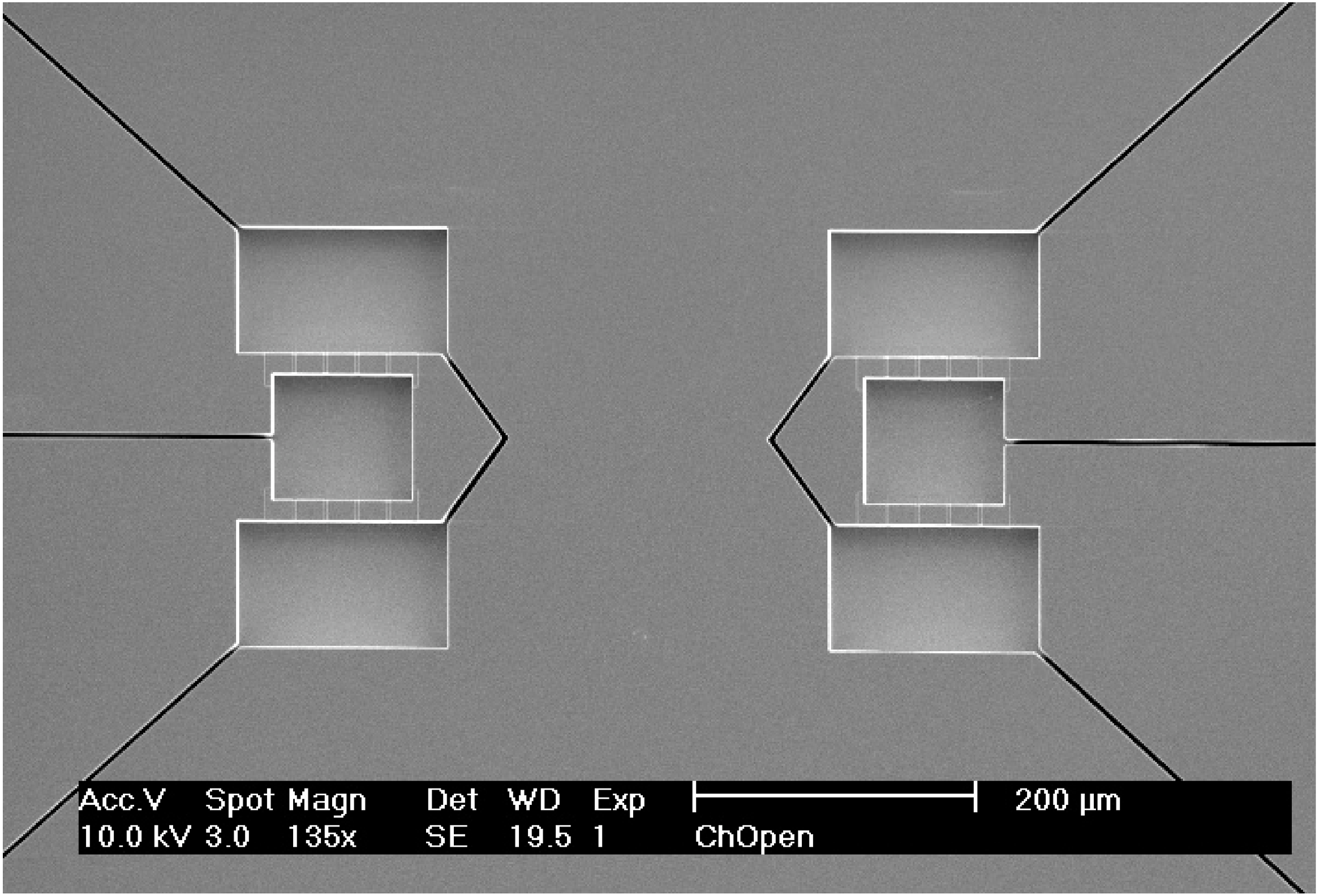
4) The quality of the landscape from chamber to chamber can be varied: we can design a habitat landscape which is not flat.

5) Like our world, the resources of our microfabricated string of islands is finite if no pumps run on the feeder channels. You WILL run out of oil!

We call these chambers "micro-habitat patches",
or MHPs, and get very annoyed if they are referred to as microchemostats, not just because of Steve Quake's dominance of the field of microfabrication.

I am aware (and am reminded by a biologist every time I give this talk) that I don't know what I am talking about and that in fact bacterial behavior at high densities is complex (yes Virginia, bacteria have sex). I'll just briefly review some of the things we are seeing in this microworld we made that are perhaps a bit unexpected, but it is just the tip of the iceberg, not an ice cube.

0-D MHP (a single MHP): Hong Kong

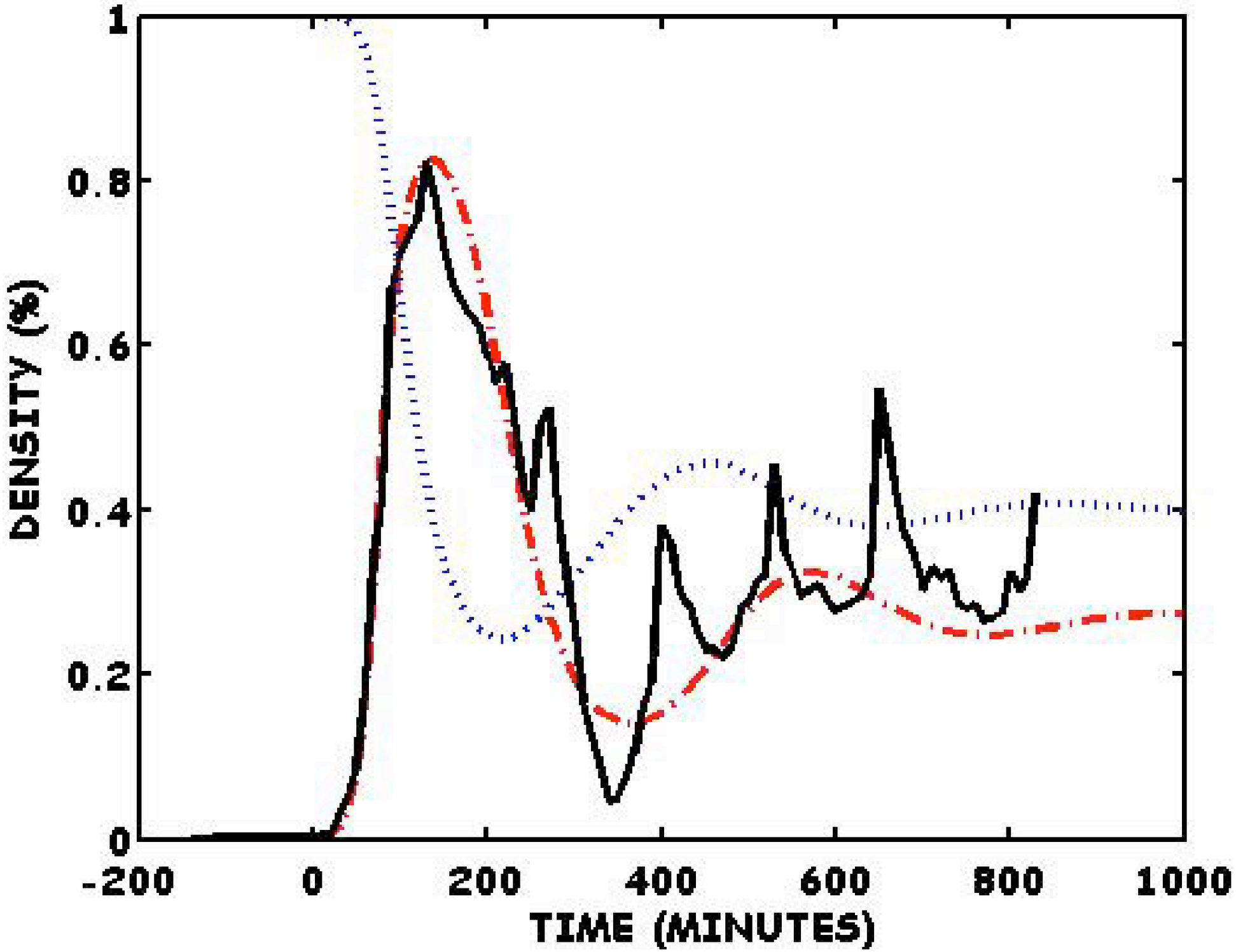


In the logistic eq., the growth rate R is a function of time to replicate, time to die, the food concentration, your metabolic rate

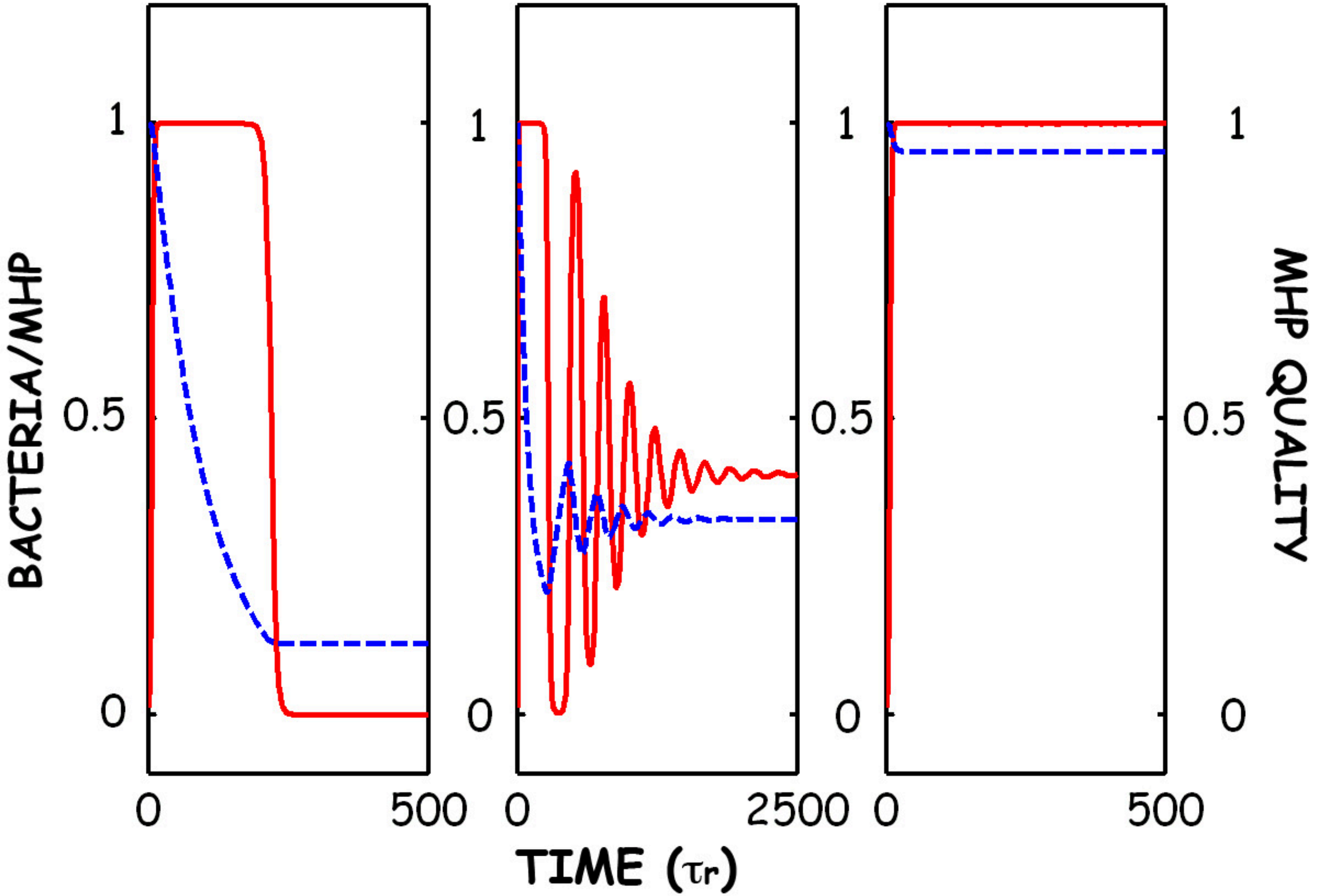
$$R(w) = \left[\frac{\varepsilon w_{MEP}}{\tau_r} - \frac{1}{\tau_m} \right]$$

The food in the MEP is a function of how fast it diffuses in, the difference in food concentration in the feed and the MEP, and how fast the food is consumed.

$$\frac{dw_{MEP}}{dt} = \lambda \left(w_r - w_{MEP} \right) - \frac{\varepsilon w_{MEP}}{\tau_r}$$



Bacterial populations overshoot, crash, and recover as food diffuses in. Spikes are.....new strains?

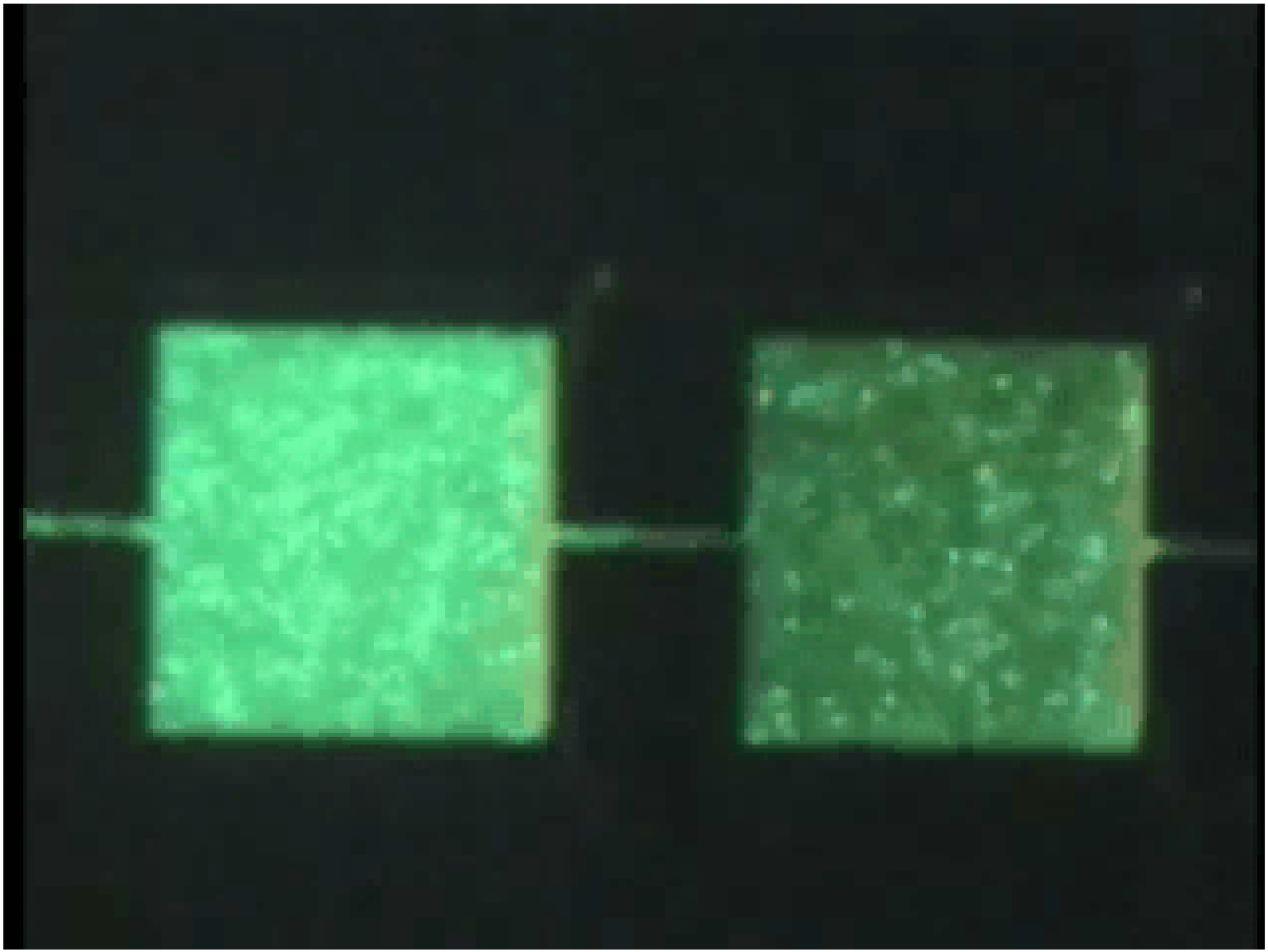


Depending on parameters, you can move from extinction with food left over (!) to steady-state with no oscillations.

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..

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

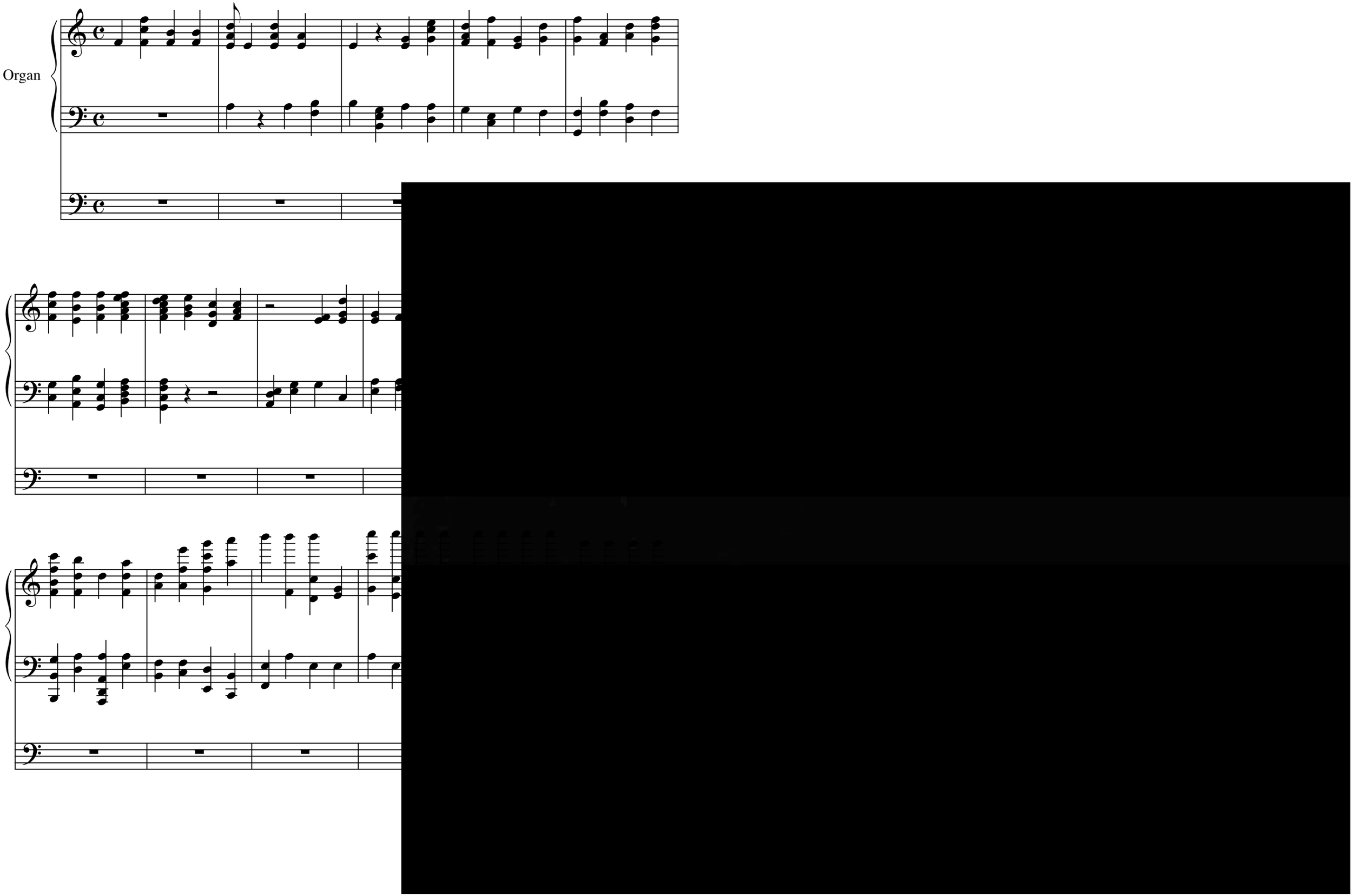


Bacterial colonies are social organisms!

bug_music_2

e. coli

Organ

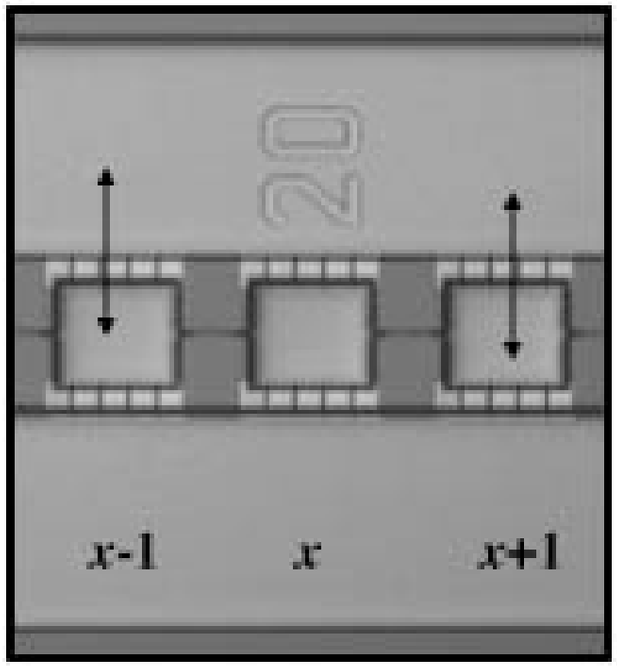


You can guess a LOWER bound to the number of generations that have existed in Habitat Flatland by calibrating the camera to bug number density and doing a little bit of tricky math:

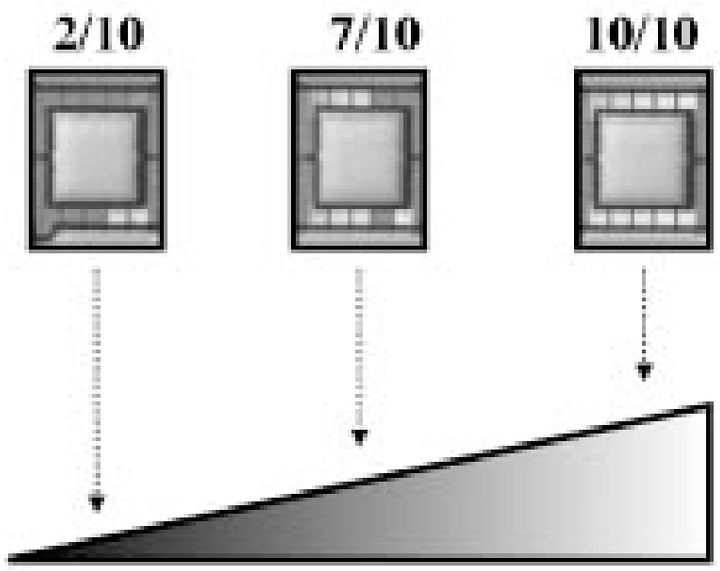
$$N(T) = \int_0^T \frac{d[log_2 \rho(t)]}{dt}|_+ dt = log(e/2) \int \frac{d\rho}{\rho}|_+$$

We only take positive derivatives (death does not concern us here) and we are careful to only look at fractional changes, many generations can occur at very low absolute population densities!

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.

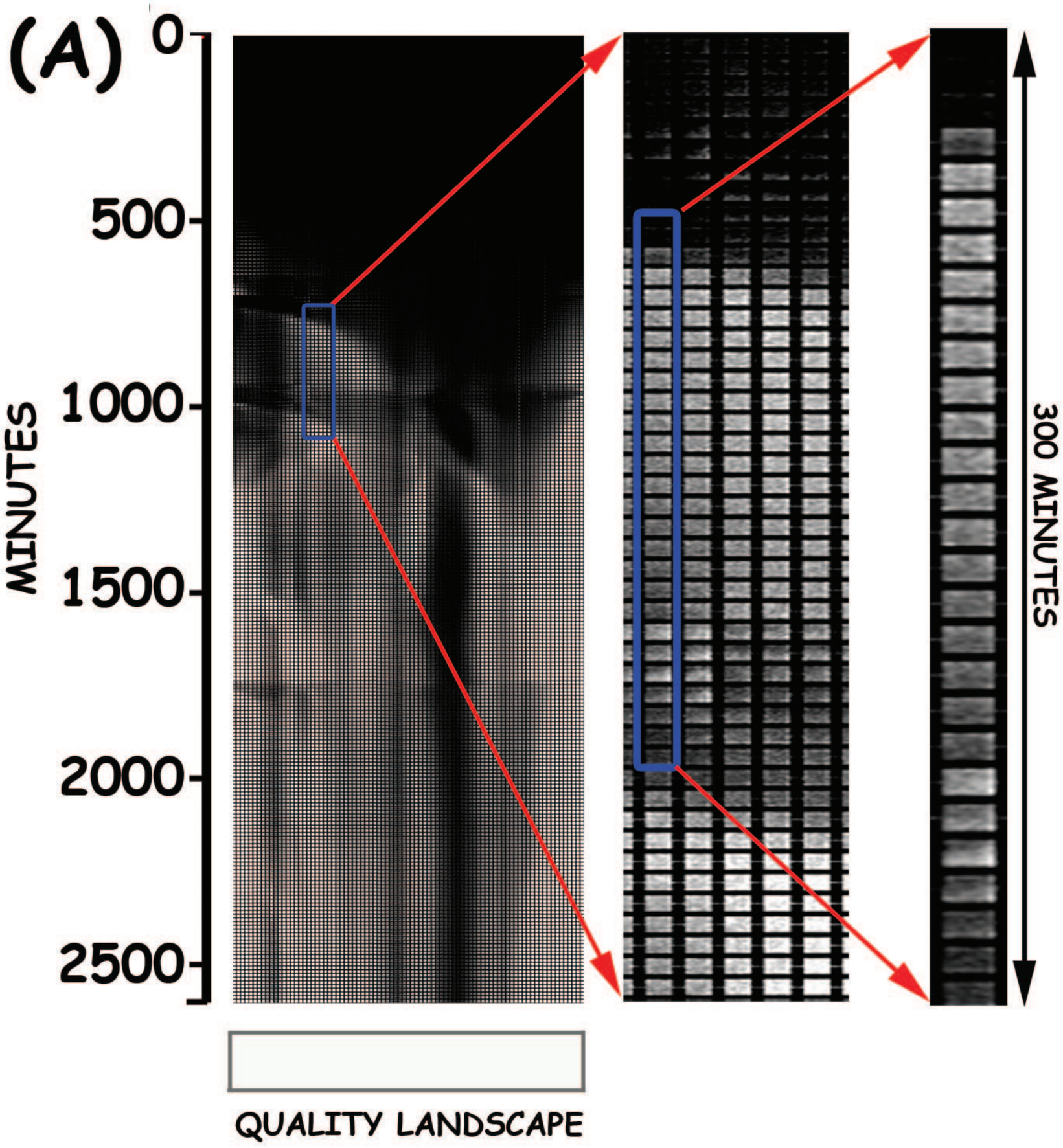


patch quality gradients

Life in our microhabitat landscape becomes a game of survival by change and movement. We are realizing Seawall's 1932 idea.

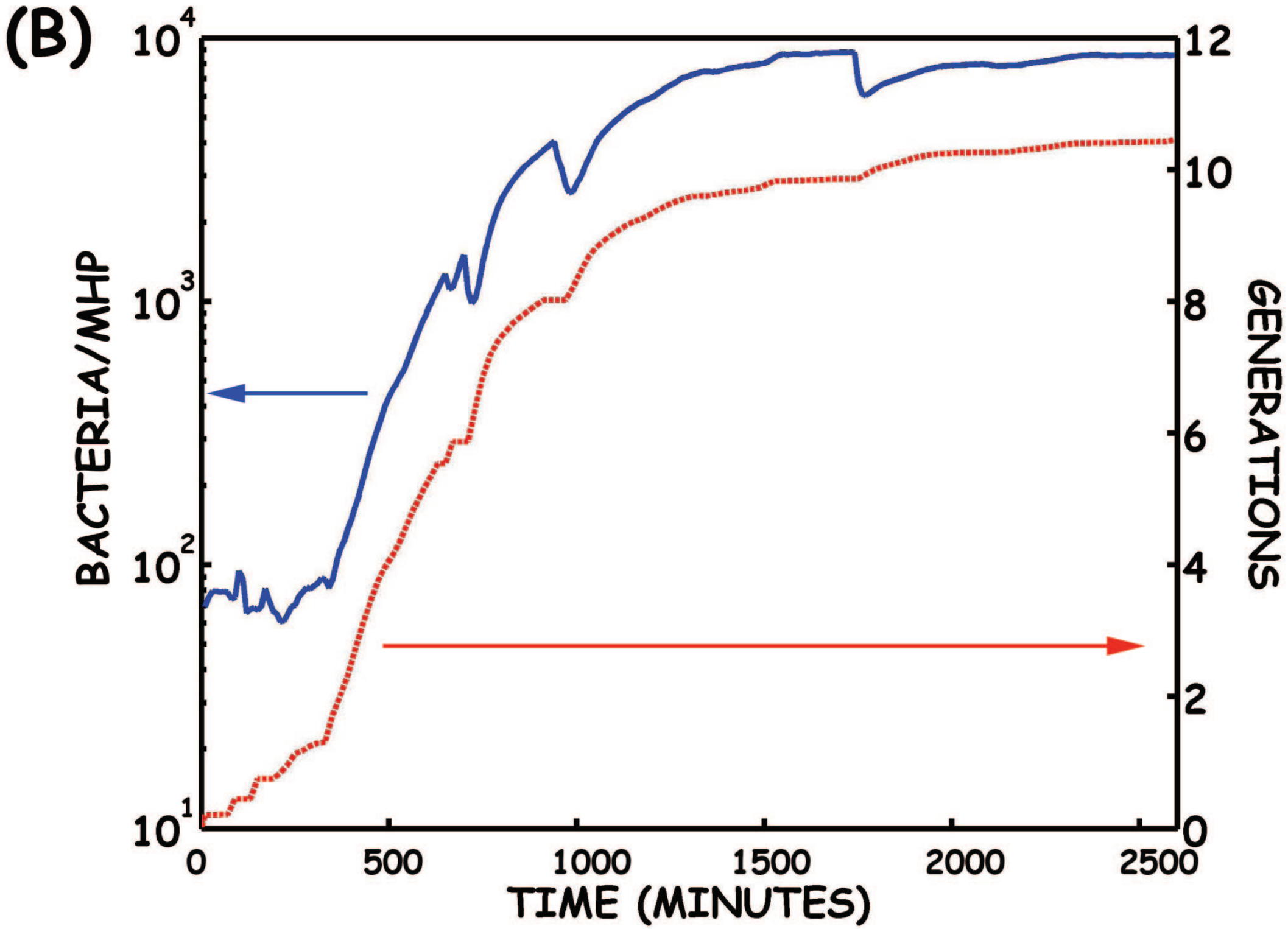
Midwest Flat Landscape: all MHPs are same

(A)



Complicated local dynamics but ultimately the bacteria spread out

Gradual colonization of the heartland of America by conservative bacteria who have family values.



We've let the bacteria play two more interesting evolutionary games:

1) Coastal China/Mongolia: where there is a dividing line in resource supply between good (Coast) and bad (Mongolia)

2) Peking-Hong Kong: There are a few pseudo-random islands of good resource supply surround by large regions of poor supply.

In the Mutation of Aging Colonies scenario, we would expect that after rapid growth in the good regions and dying off a few mutant species will figure out how to survive by K selection in the poorer regions of Keymerland (Juan is from Chile, a linear country of course).

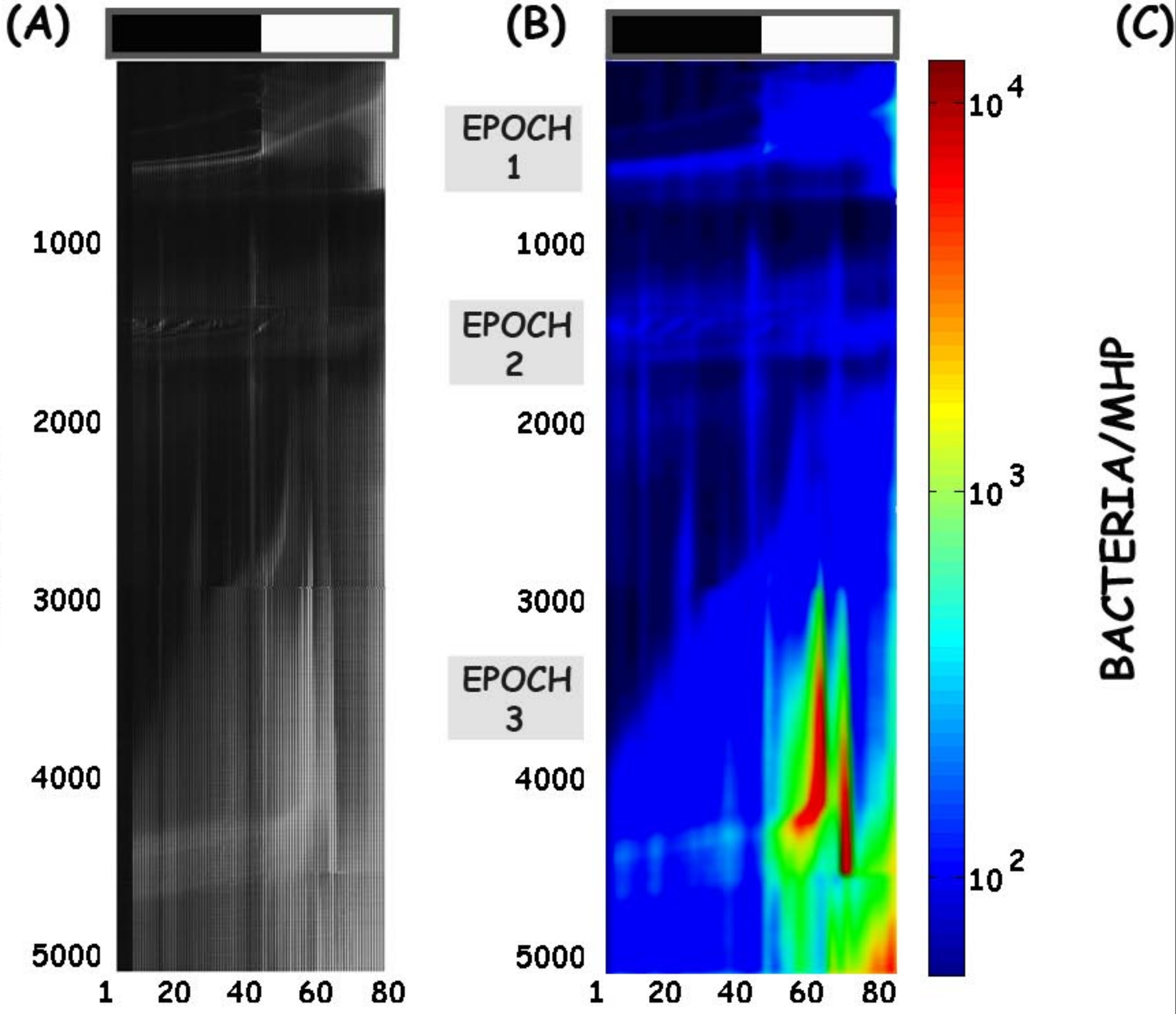
1) East Coast/Midwest

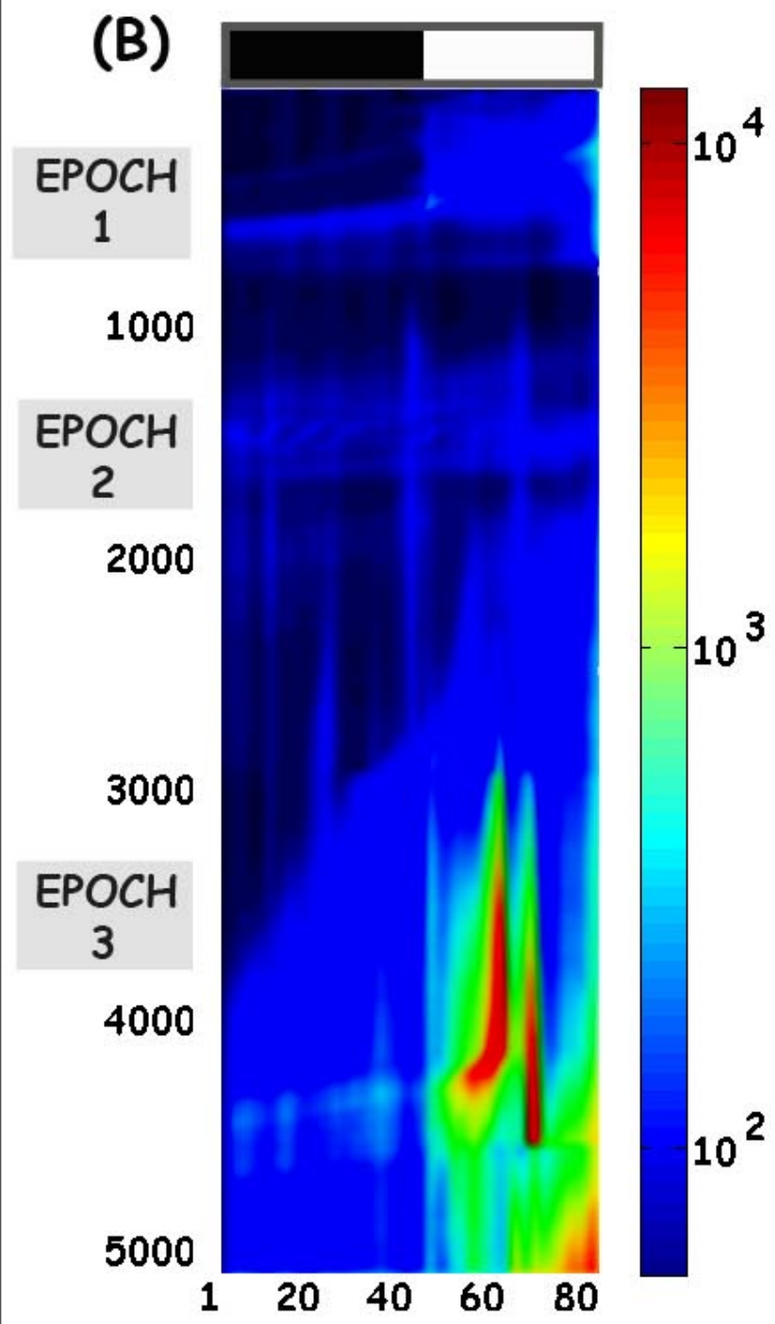
There seems to be a pattern in more complex landscapes of three basic phases or Epochs:

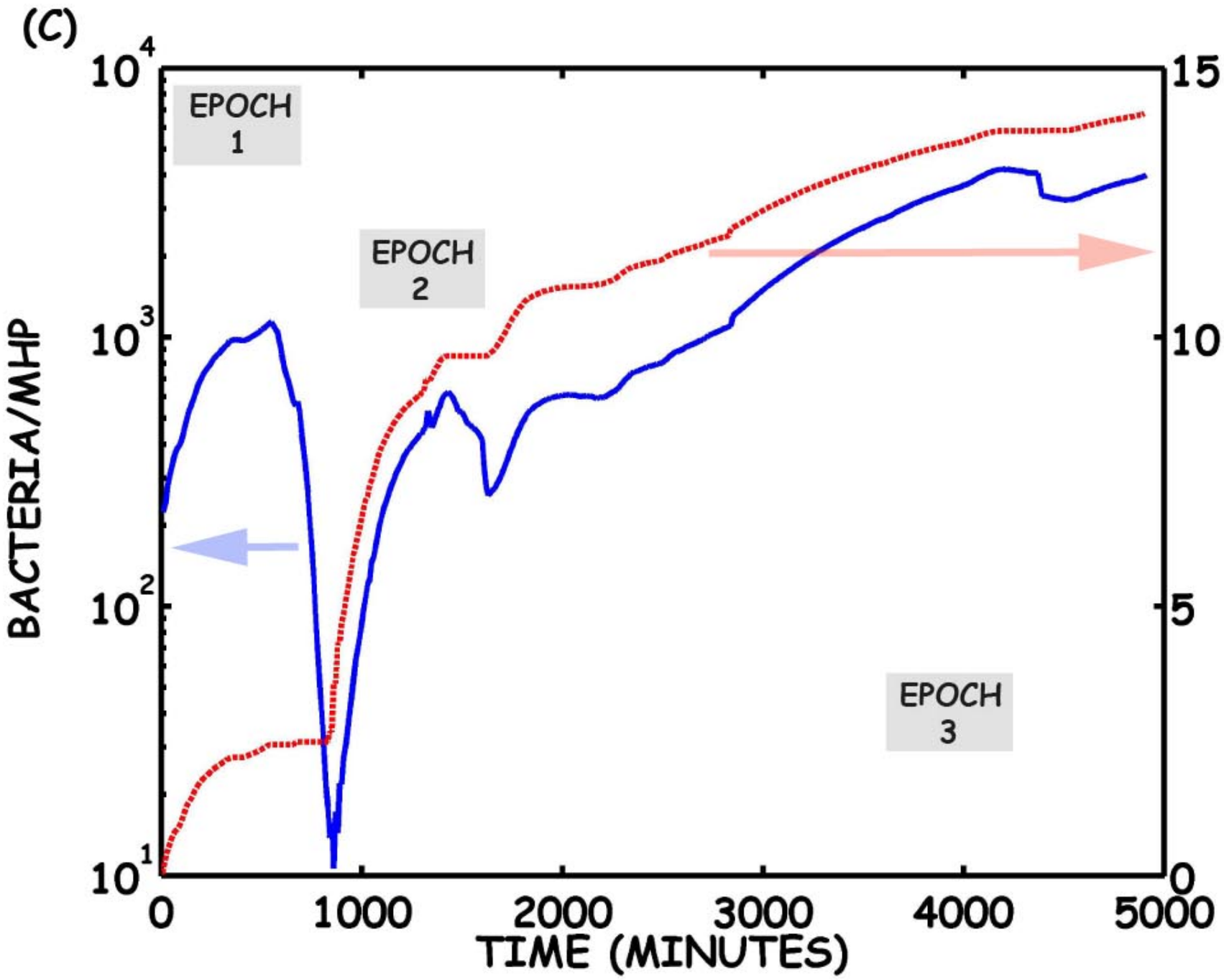
1) Rapid growth.

2) Shuffle

3) New growth



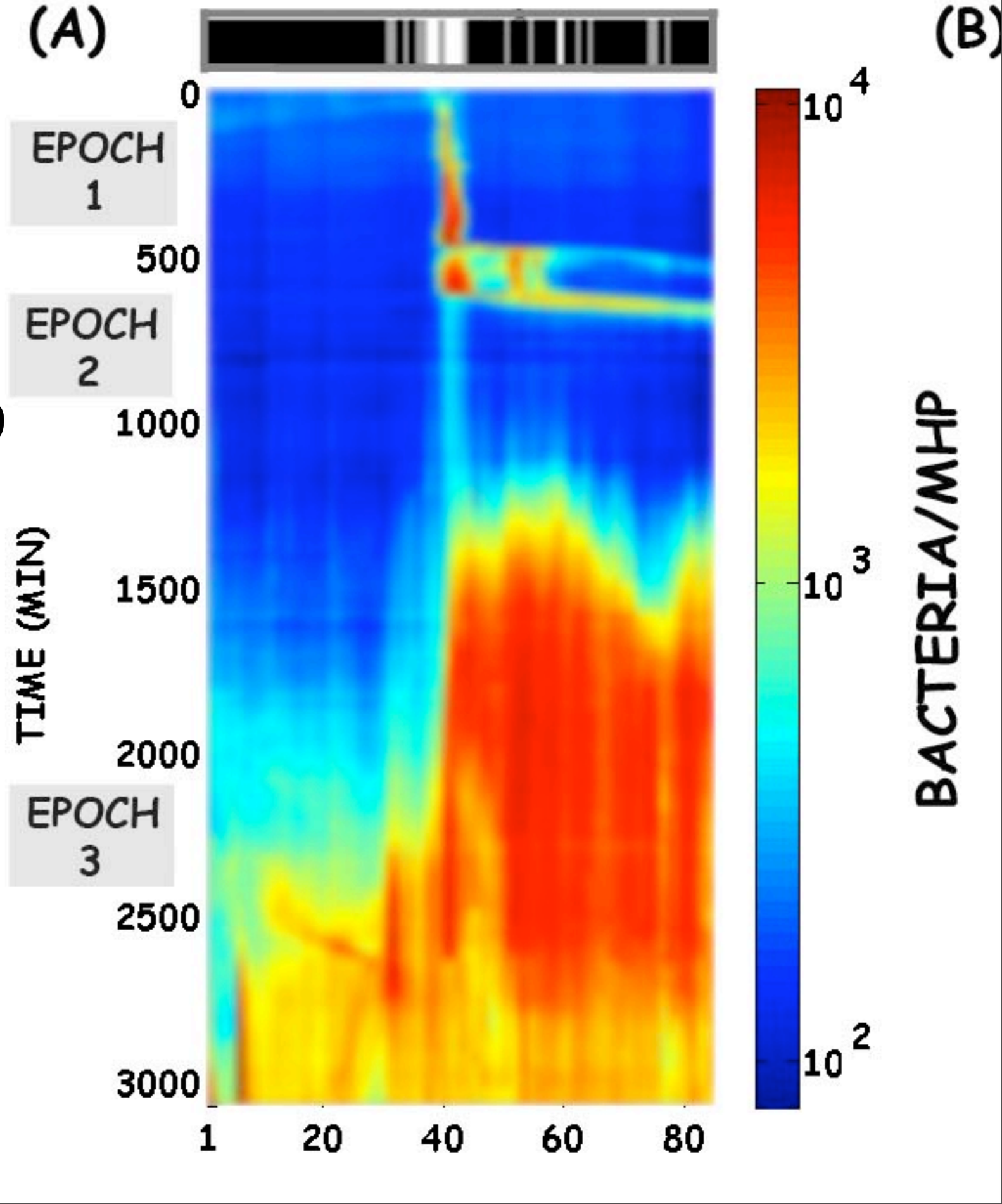




Bacteria in Mongolia have learned to grow more slowly and use their resources more sparingly. It isn't clear if we have a new species that replaced the original one, or if there is now a gradient in phenotype across the chip.....or.....or

2) Coastal China :

Same basic pattern as Black-White: quick 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.



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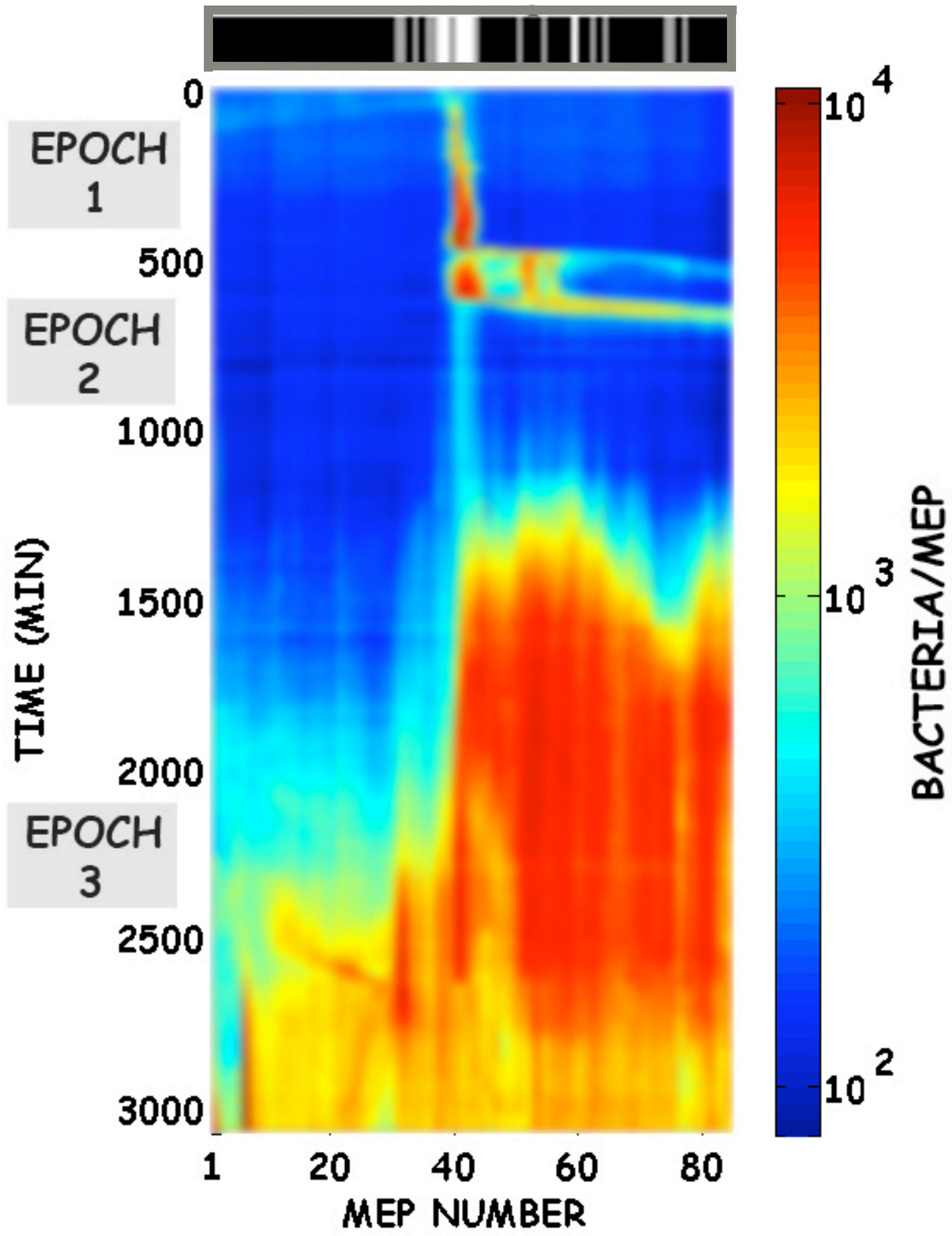
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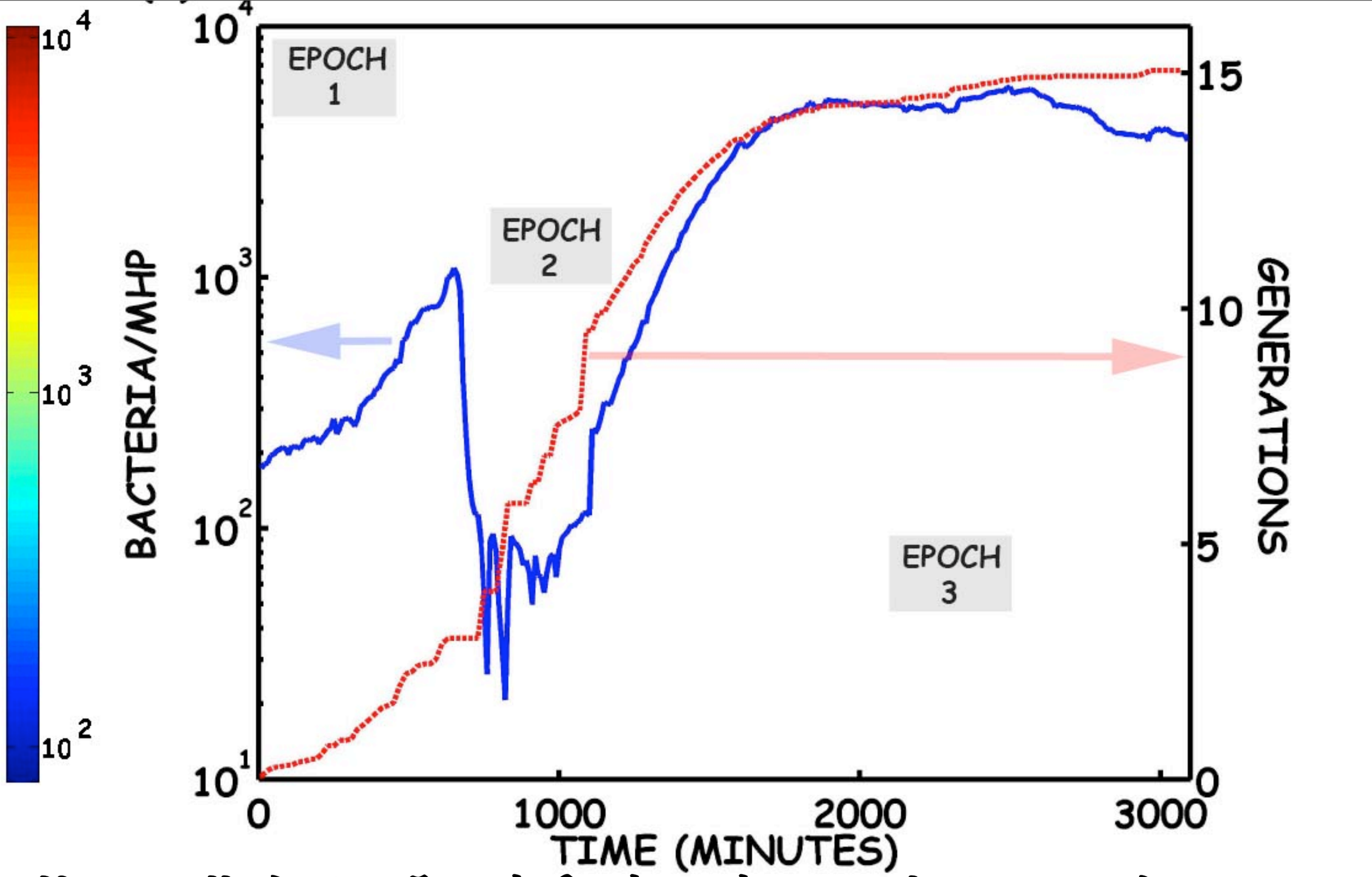


Note well: during Epoch 2 when the population number is small the actual generation rate is at a maximum, as if the organism is rapidly trying to solve a problem of survival.

Are we seeing Mutagenesis in Aging Colony-directed evolution at accelerated rates?

Or are we seeing "simple" adaptation via biological networks like the lac operon switch?

As a physicist with biology friends who just cross their arms with a smile and wait for me to make the project worth doing, I can only say I don't know right now.

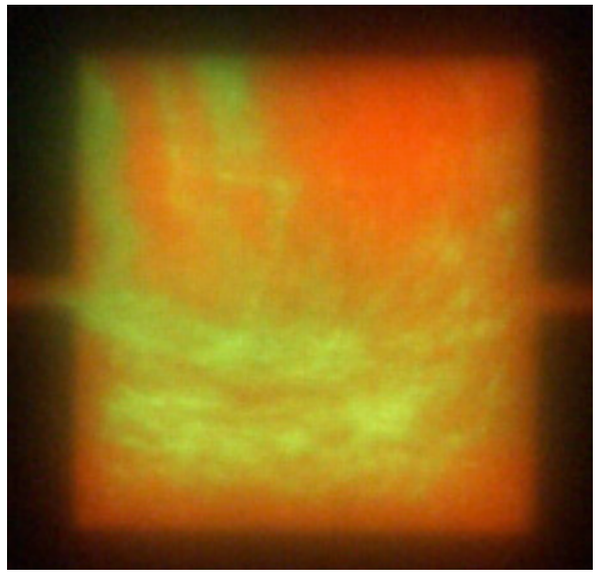
I do know we have a huge array of work that has to be done (at the single cell level) to get at what is happening at the genomic and network level as bacteria explore and adapt to complex environments with mimic reality as best we can.

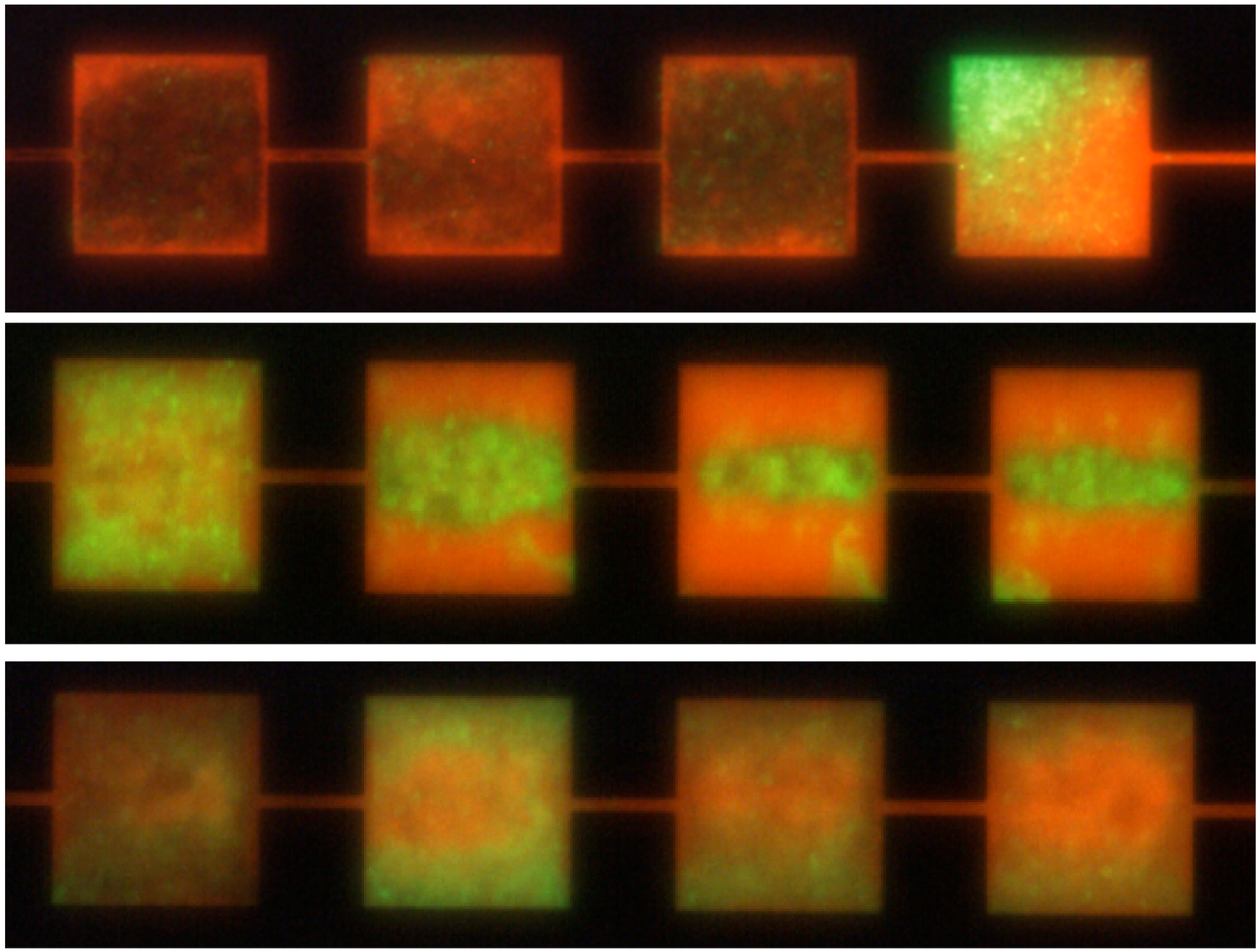
But I suspect one thing is true:

Classical NeoDarwinism is...a cruche de merde.

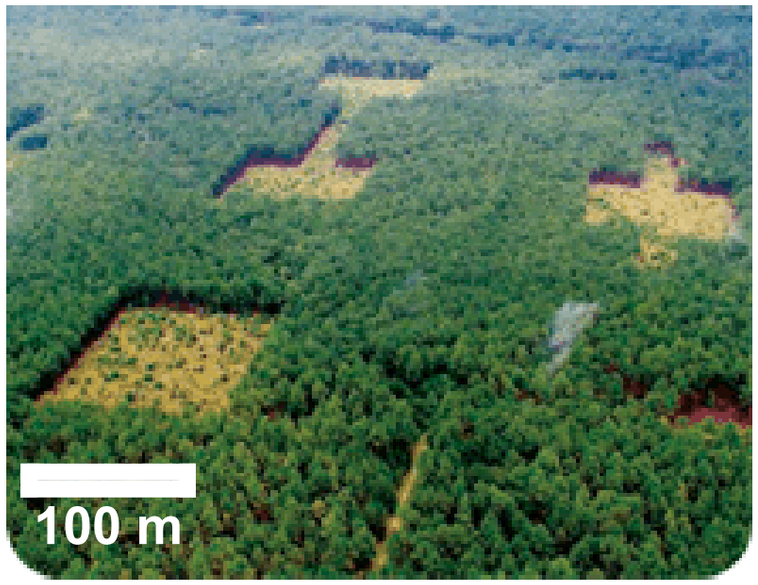
Ongoing & Future Work

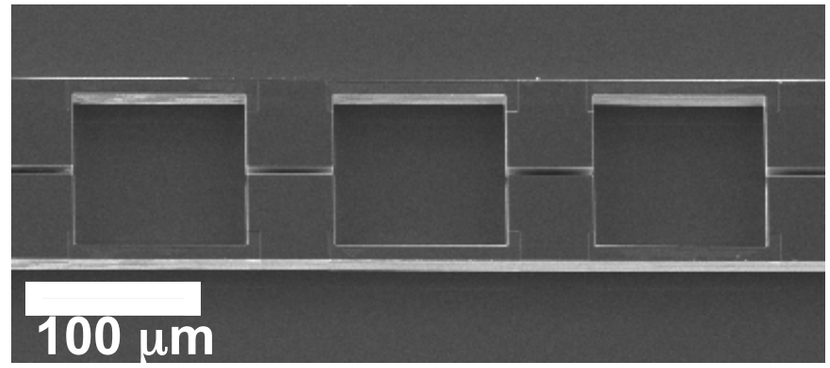
Competitive assay,
genetic analysis



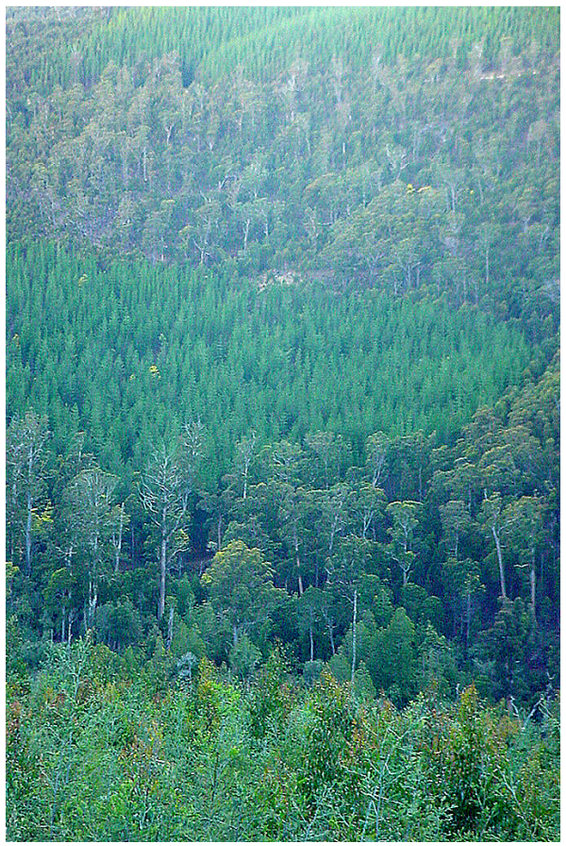


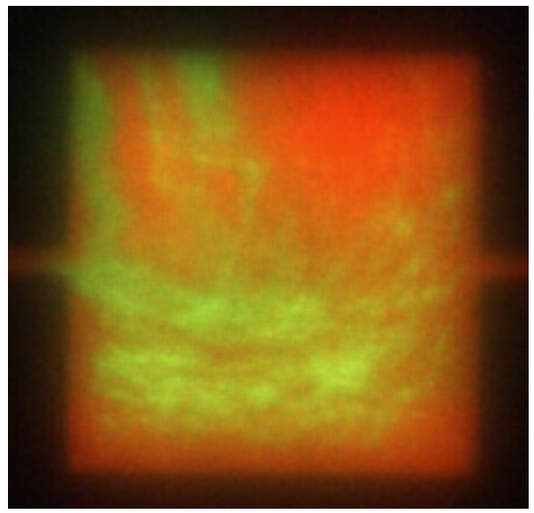
What can nanotechnology bring to ecology?





What can ecology bring to nanotechnology ?





I am getting more and haunted, or bothered, by how well we really understand the dynamics of evolution, and how poorly understood are the experimental foundations of the subject.

To some extent, we scientists can only blame ourselves for our inability to project to the American (and world) public the struggle we all have in understanding the dynamics of life and evolution, which is by no means trivial or obvious

So this is my aim: to use our habitat landscapes and drive a new species to form.....a new species, through directed evolution and the evolvability of organisms under stress due to enhanced mutation rates. I think it can be done and understood quantitatively.

THANKS!