Are Cells Robots?

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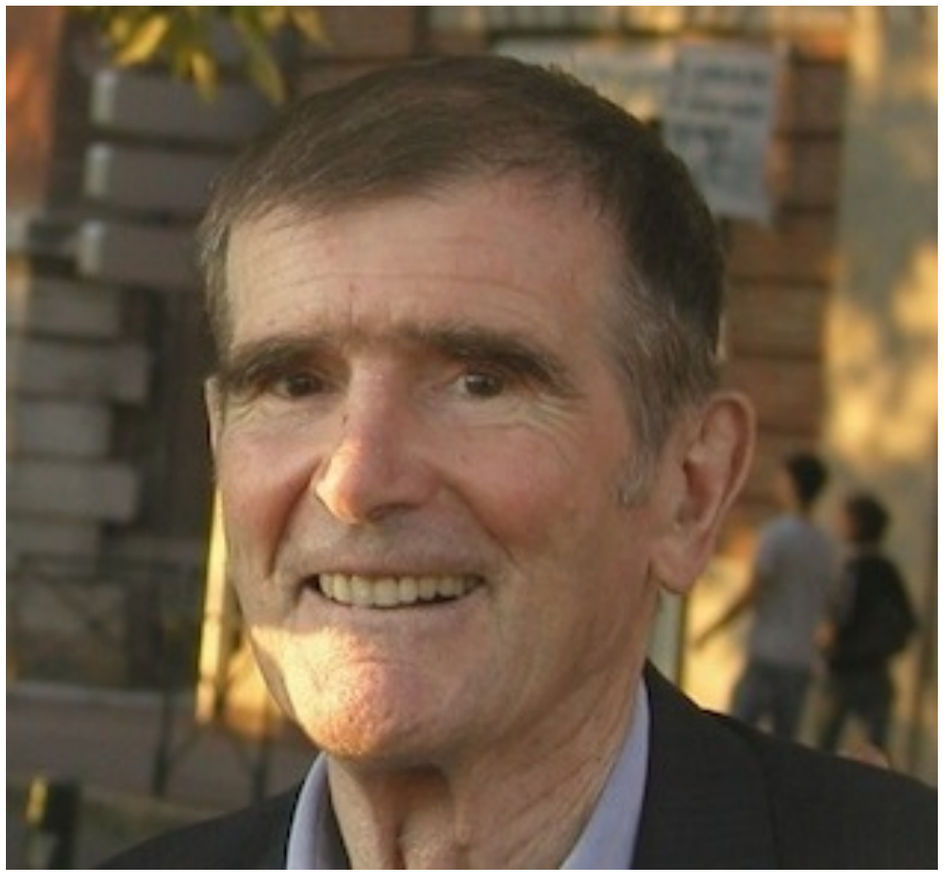
Richard Huang (Consultant)
Keith Morton (Canada NanoFab)
David Inglis (Australia)
Kevin Loutherback (UC Berkeley)
Juan Keymer (Chilean mathematical ecologist)
David Liao (now at UCSF)
Guillaume Lambert (now at U. Chicago)
Qiucen Zhang (now at UIUC)
Chih-Kuan Tung (now at Cornell)
Julia Bos (Princeton)

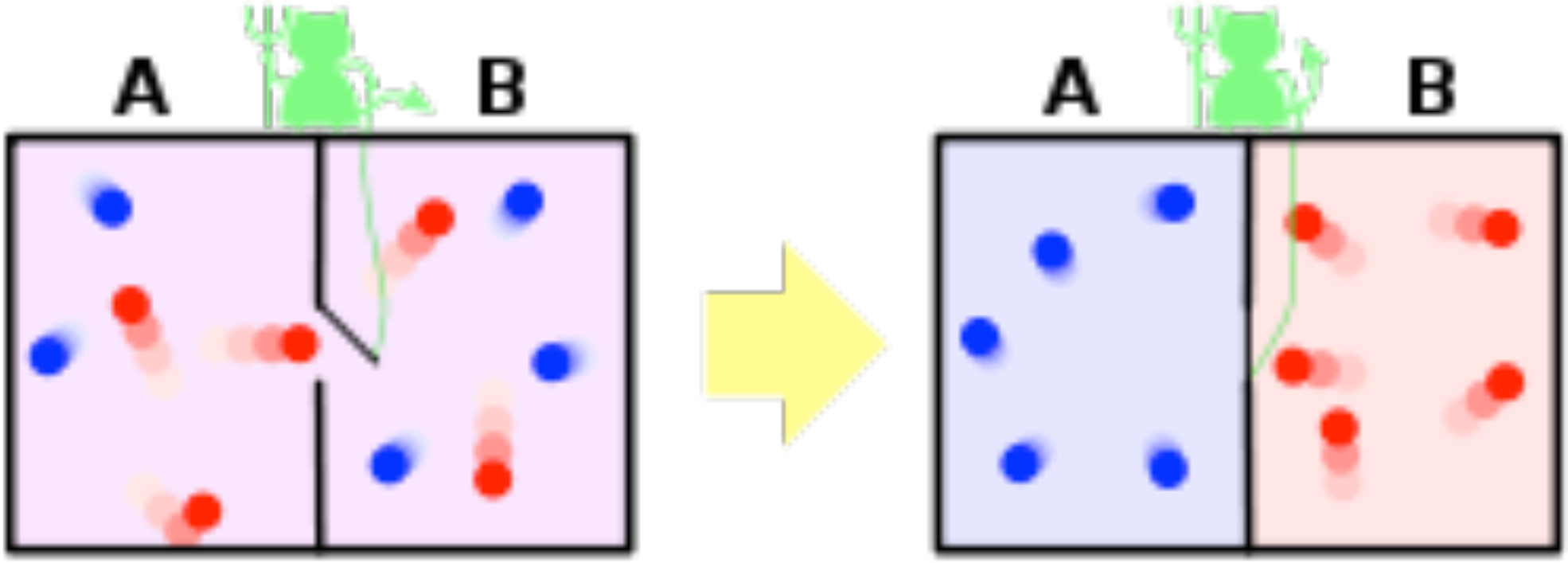
I want to explore a bit the question of "free will" in microorganisms.

I don't think Prof. Giralt would have thought that Robots have free will.

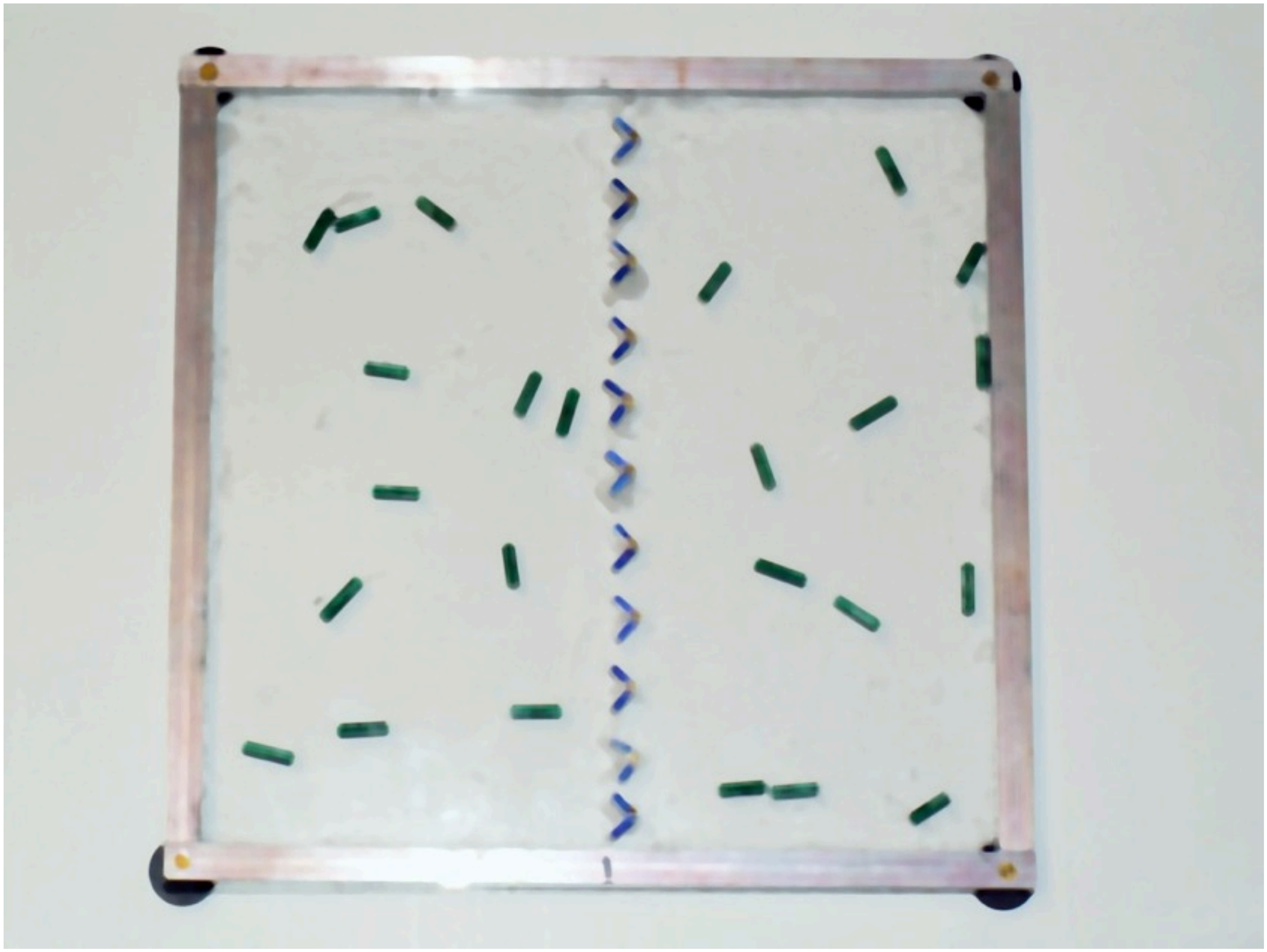


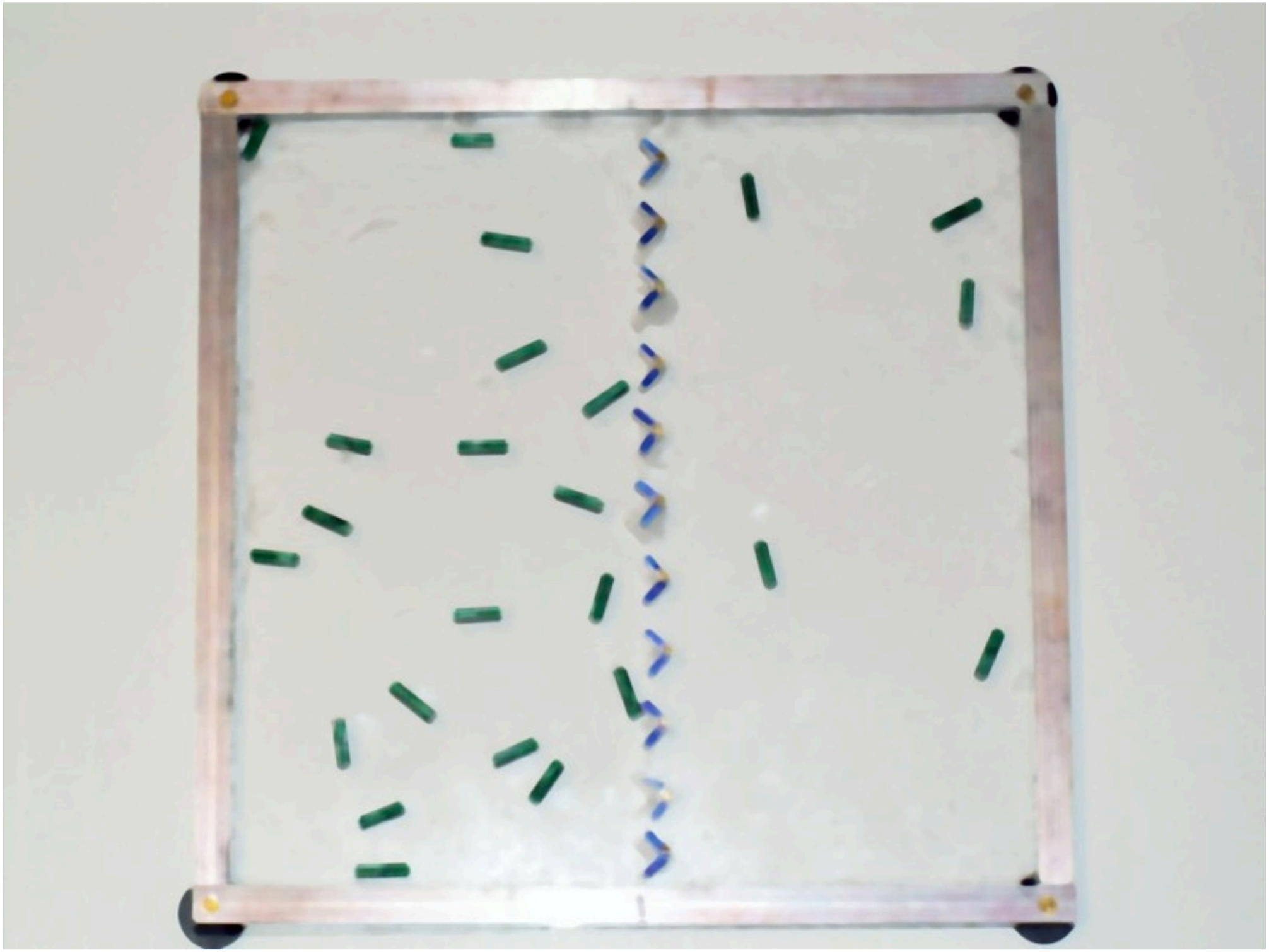
I think people do (maybe). How far down does this go?

1) Demon Bacteria!!



John Hopfield started all of this 35 years ago:



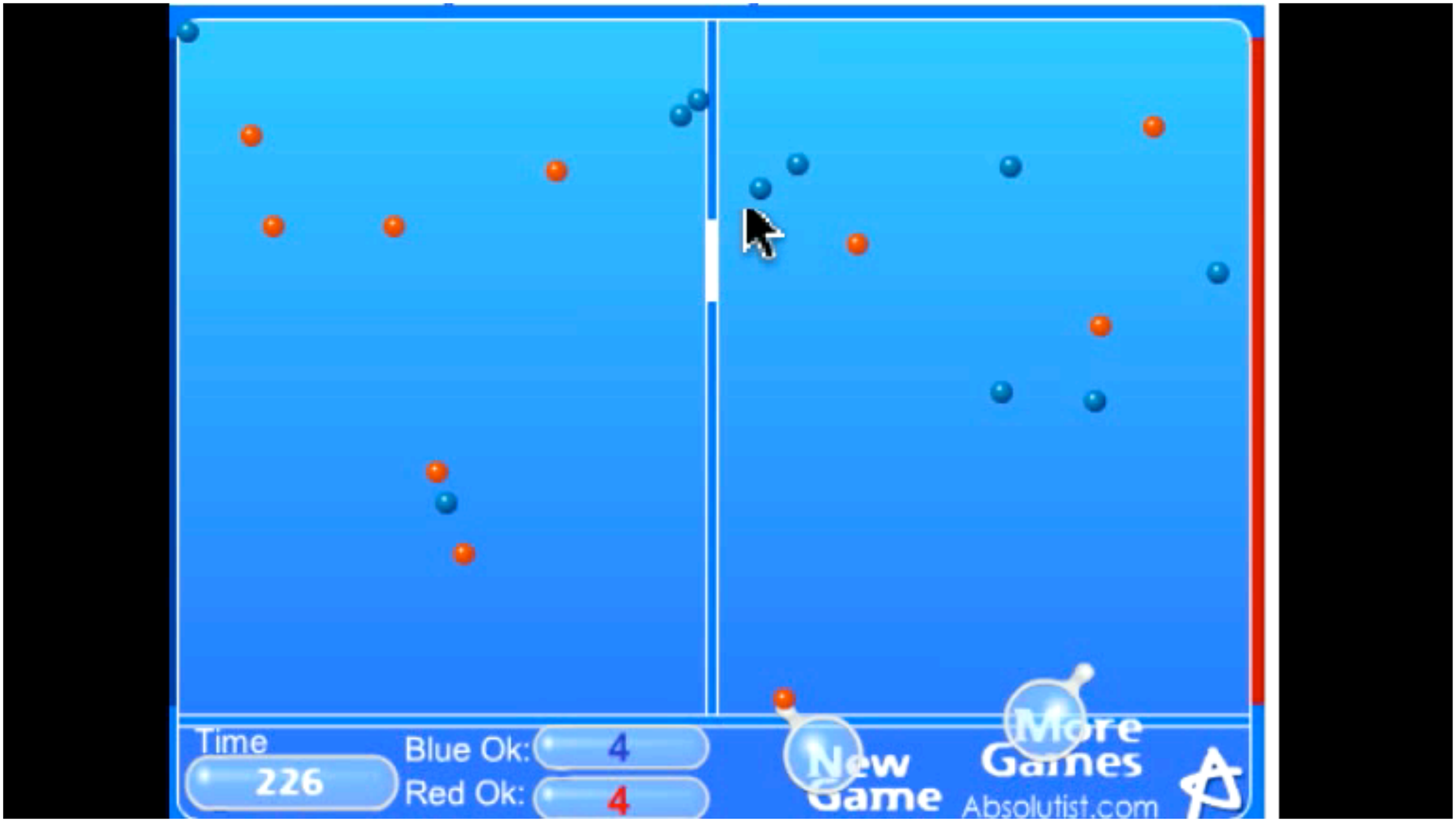


Maxwell's Demon at work?

Maxwell's demon is 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 between chambers containing a gas.

$$\Delta\rho = \rho_{left} - \rho_{right}$$


Time
226
Blue Ok:
4
Red Ok:
4
New Game
More Games
Absolutist.com

http://maxwells-demon.freeonlinegames.com/

You will find that if you are sufficiently fast (and young) that you CAN concentrate balls on one side, or separate red from blue.

This is a BIG problem for the 2nd Law of Thermodynamics, and the battles still rage.

The demon (or you) CAN separate hot from cold. The cost probably lies in the cost of erasing information, which increases entropy. Try not to think about it.

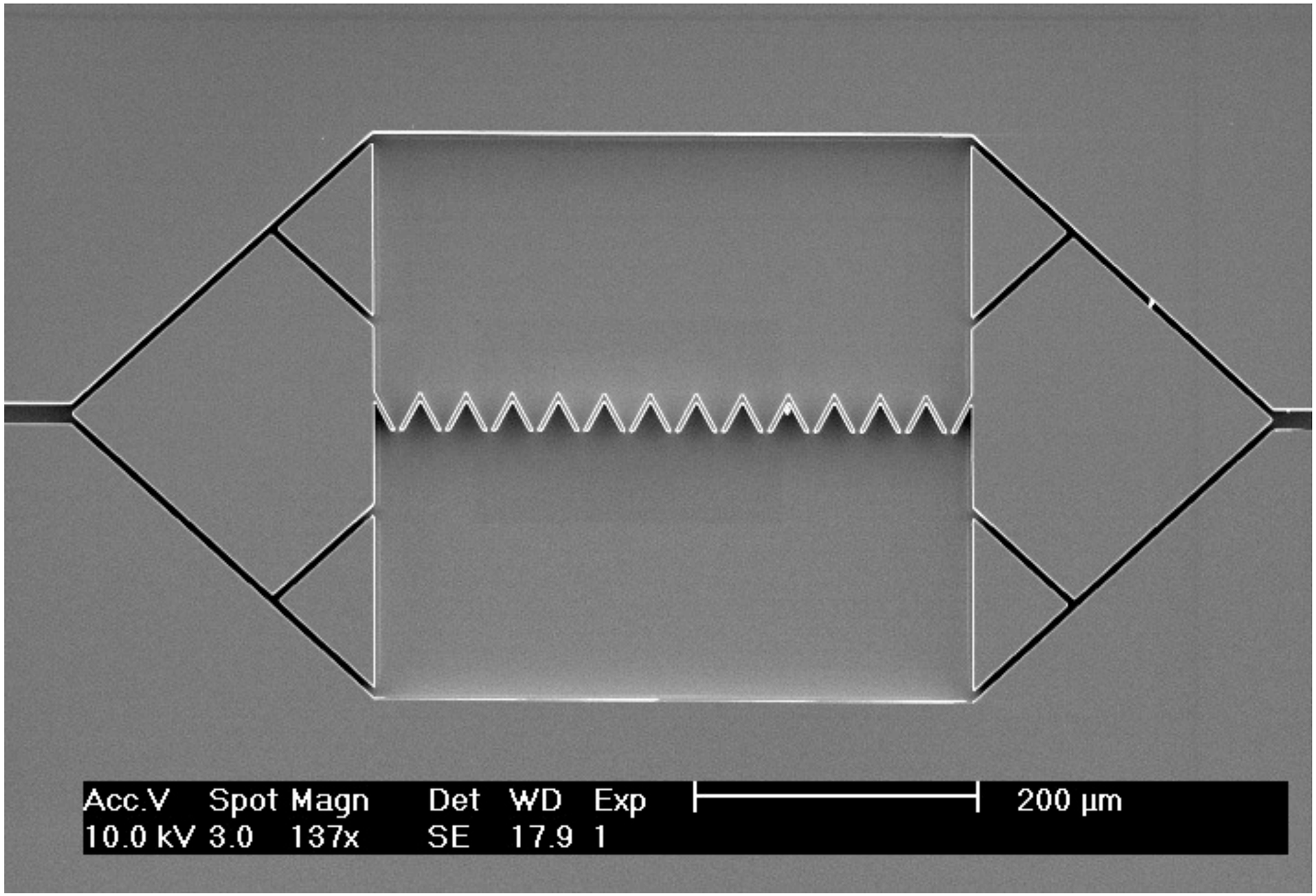
This number density results in a chemical potential difference

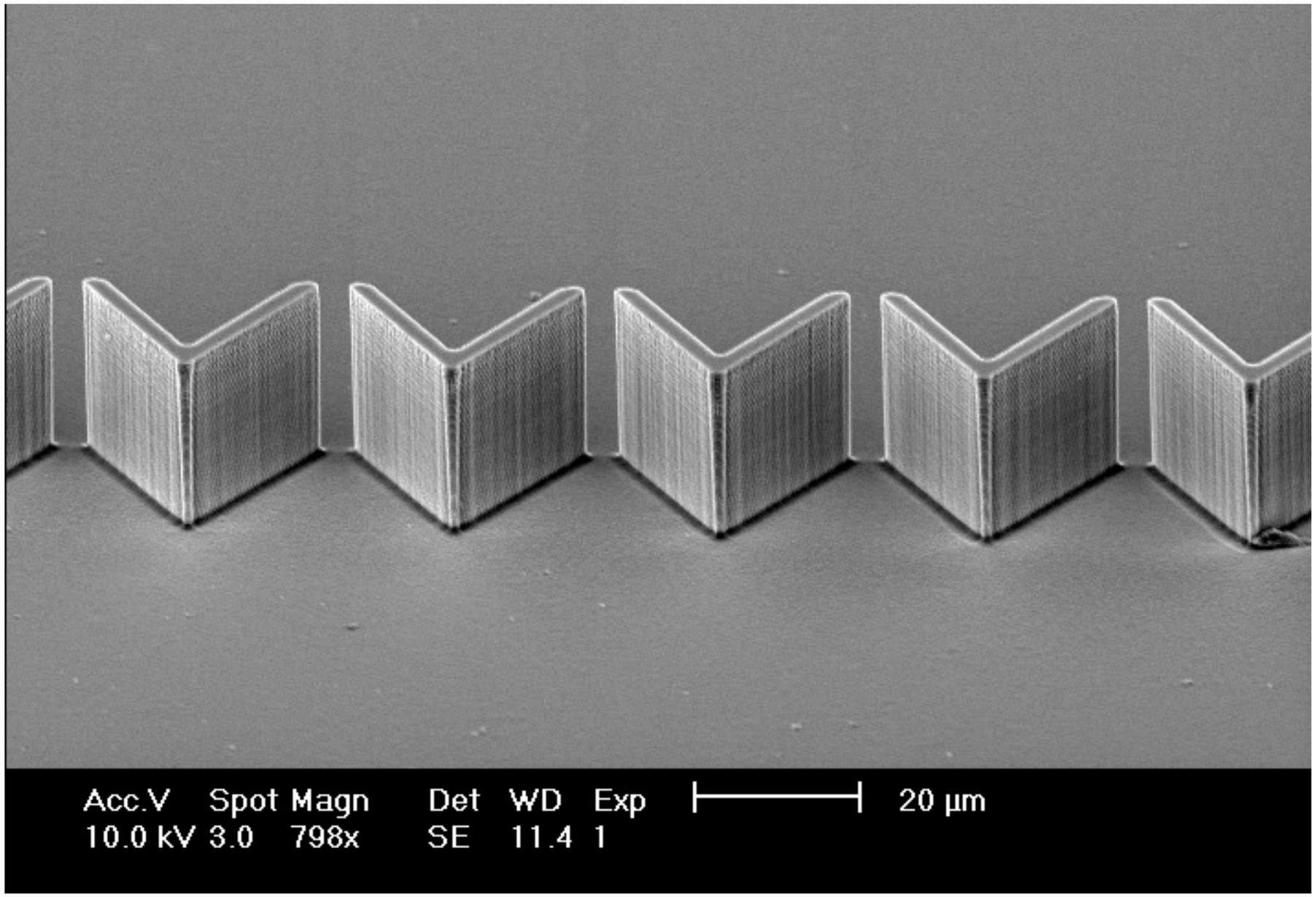
$$\Delta\mu = n_Q \log(\rho_{left}/\rho_{right})$$

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.

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




Acc.V Spot Magn Det WD Exp
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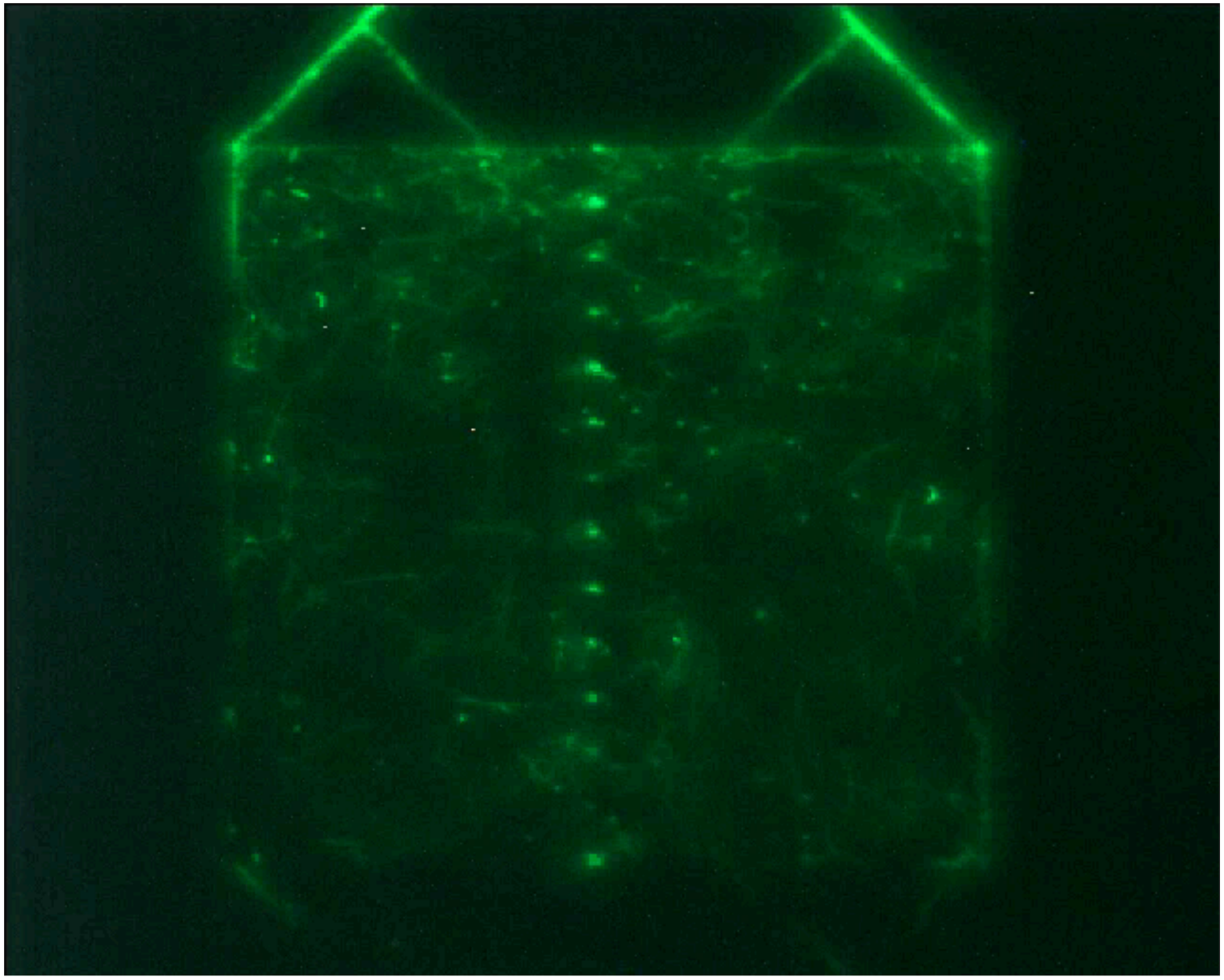
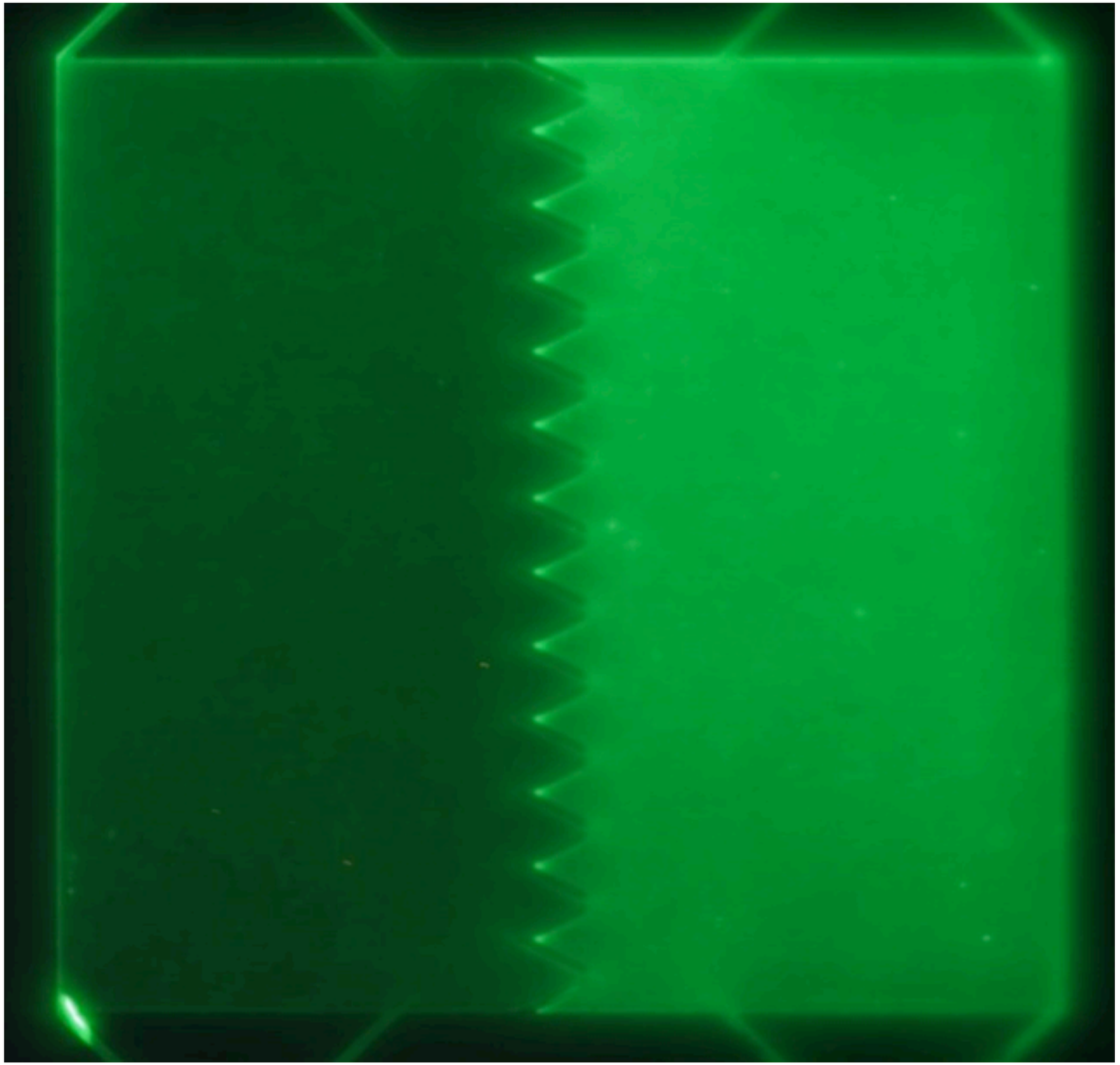
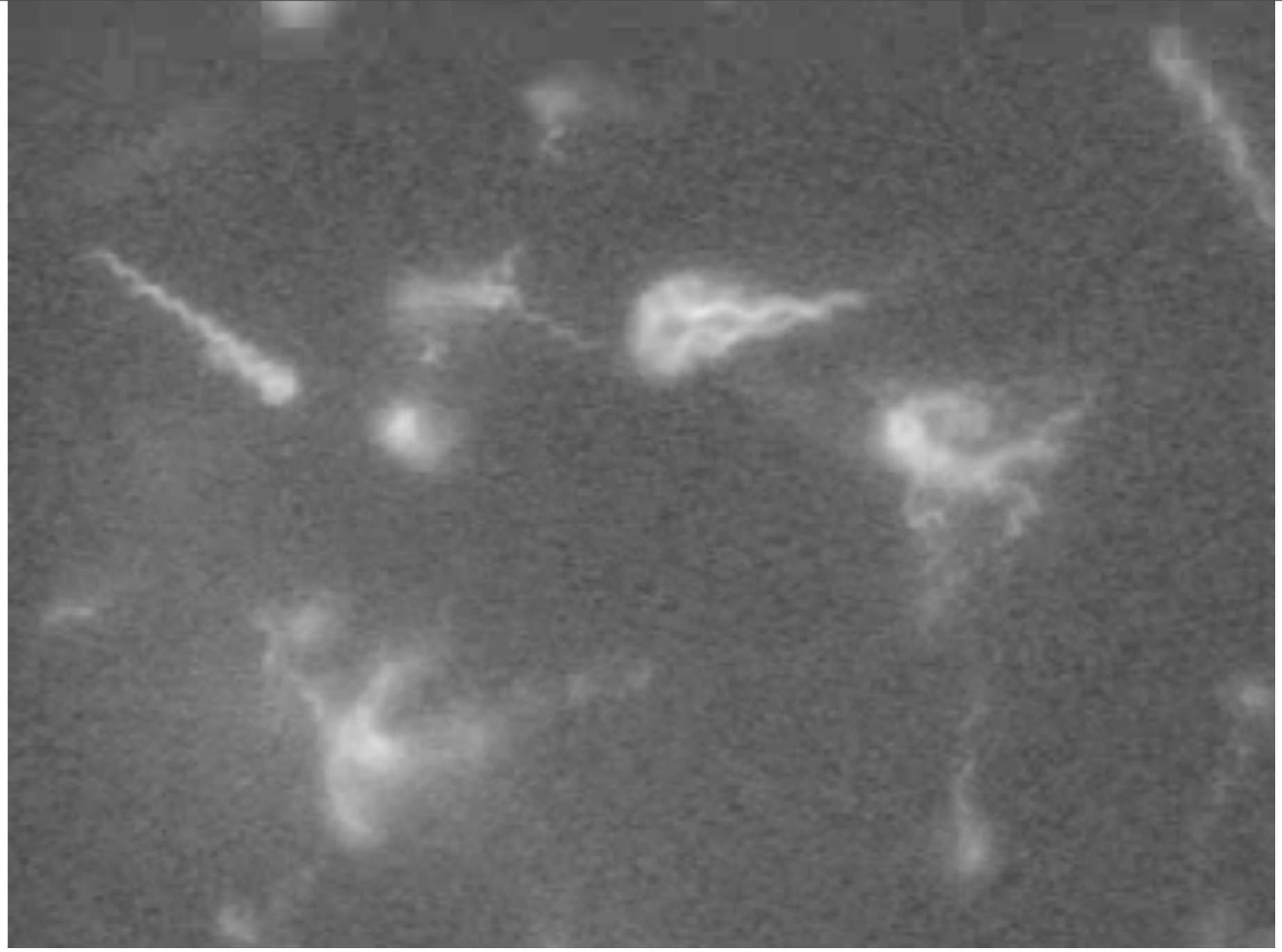
20 µm

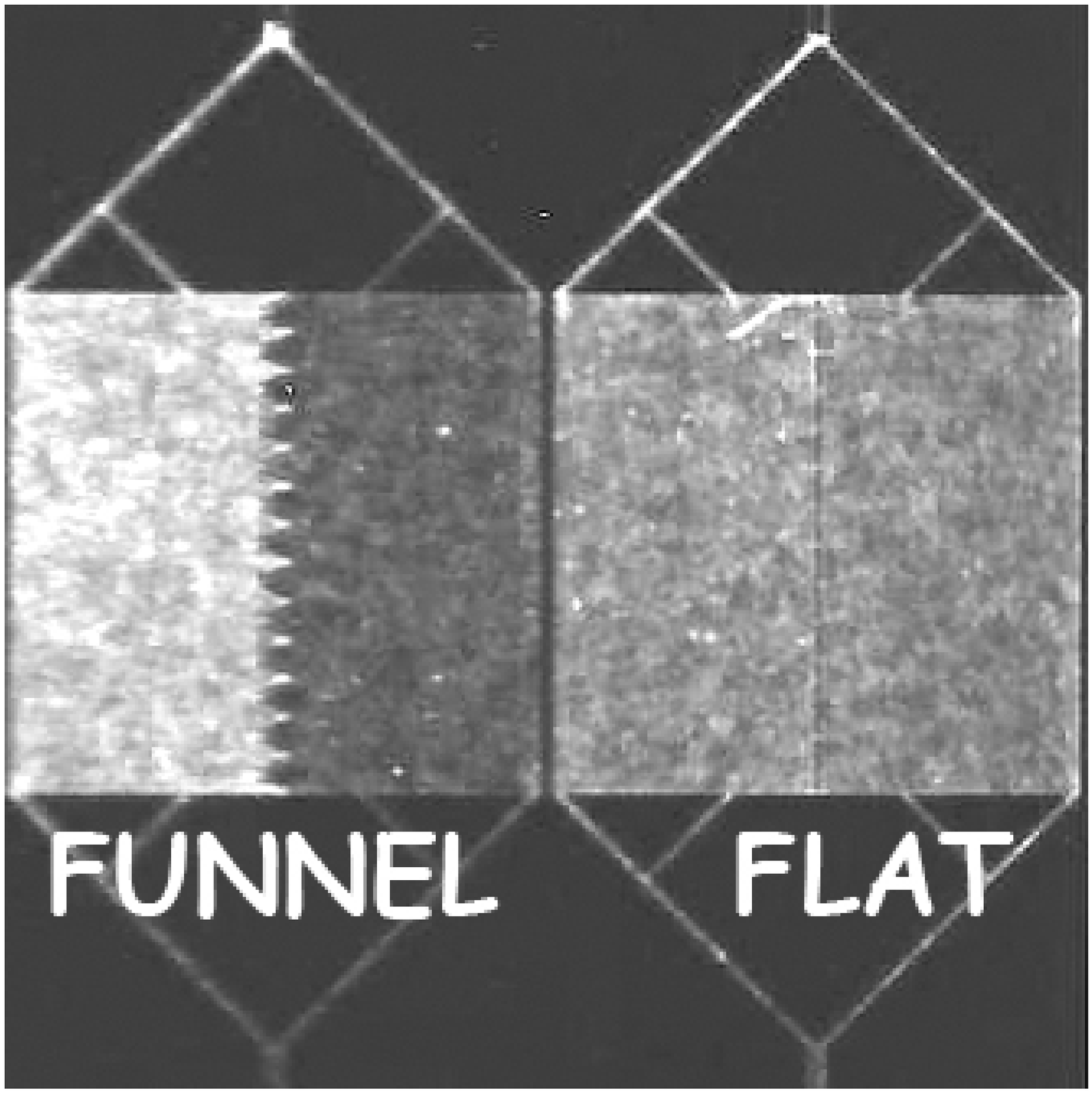


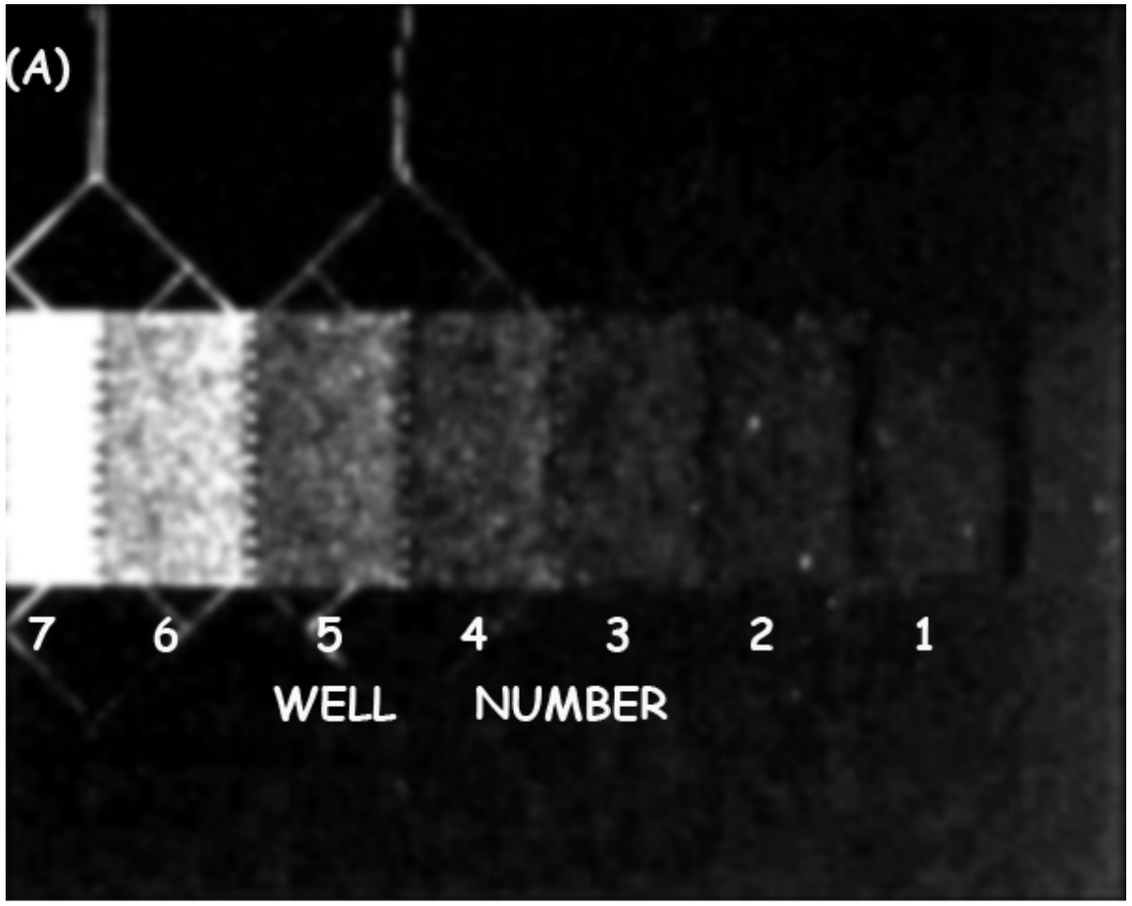


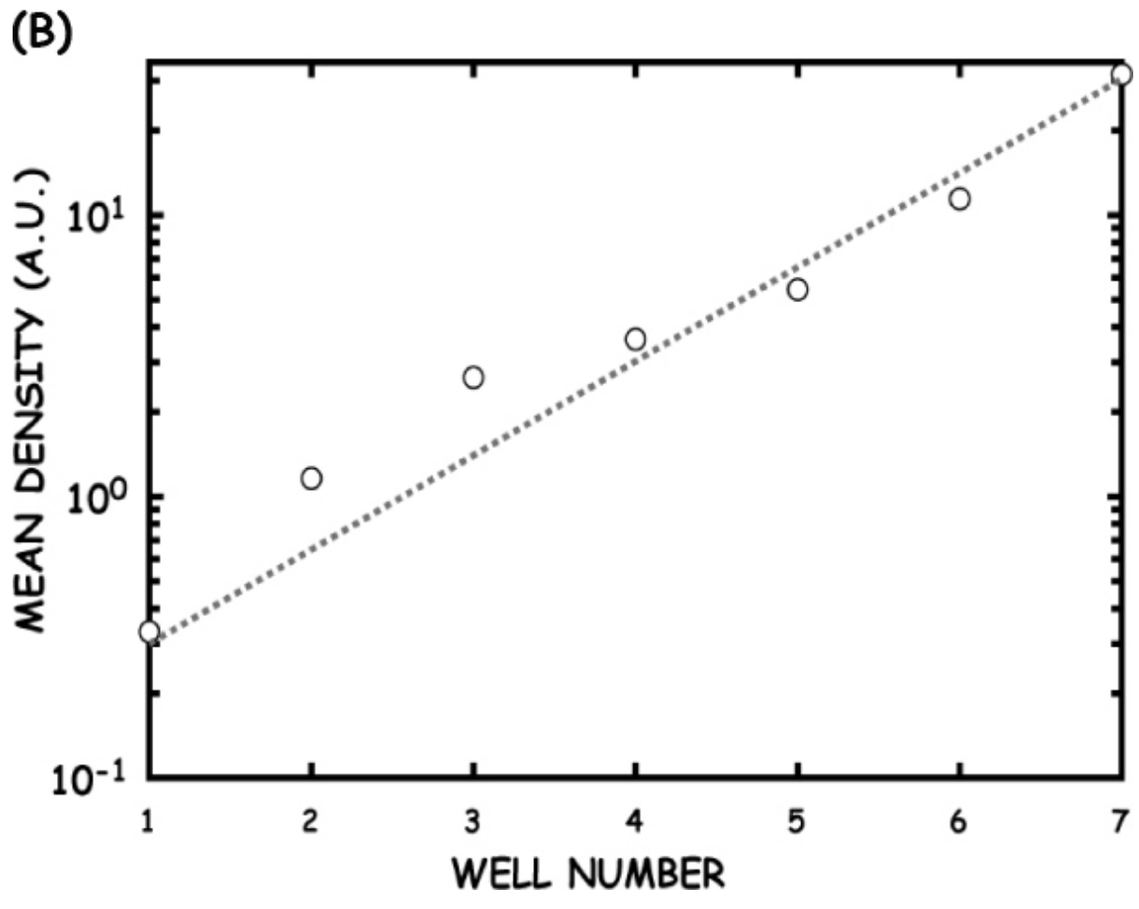


Howard Berg and Will Ryu
Motile bacteria do a random walk, sort of.
(don't confuse with Brownian motion!)

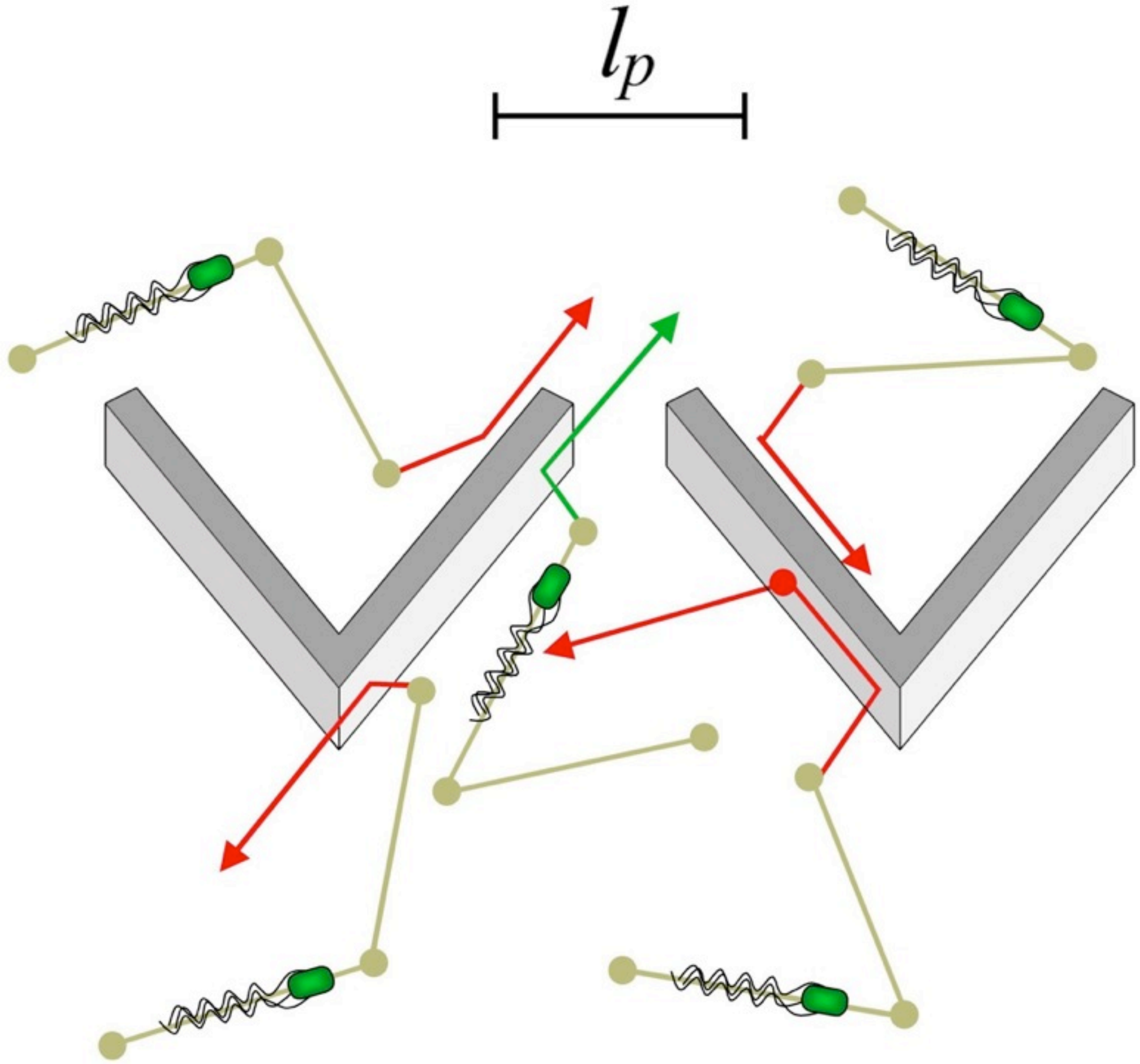
You need funnels: flat openings do nothing.







If you stack the funnels you can create a multi-stage kind of bacterial "pump".


l_p

This experiment has bothered Paul Chaikin, as most things do.

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 .

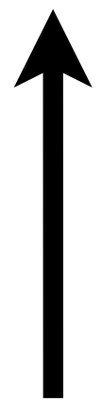
Now instead I start with a random set of swimmers on the back side of the funnel I run the movie and they should go back to the up side of the funnels. They don't. So that's what bugged me. What's the answer?"- Paul Chaikin

$$\rho \frac{\partial \vec{v}}{\partial t} \sim \nabla P + \eta \nabla^2 \vec{v}$$

The SIMPLE answer is that when a swimmer comes "in contact" with a wall that local interaction is NOT part of the N-S low R_e the motion becomes non-time reversible.

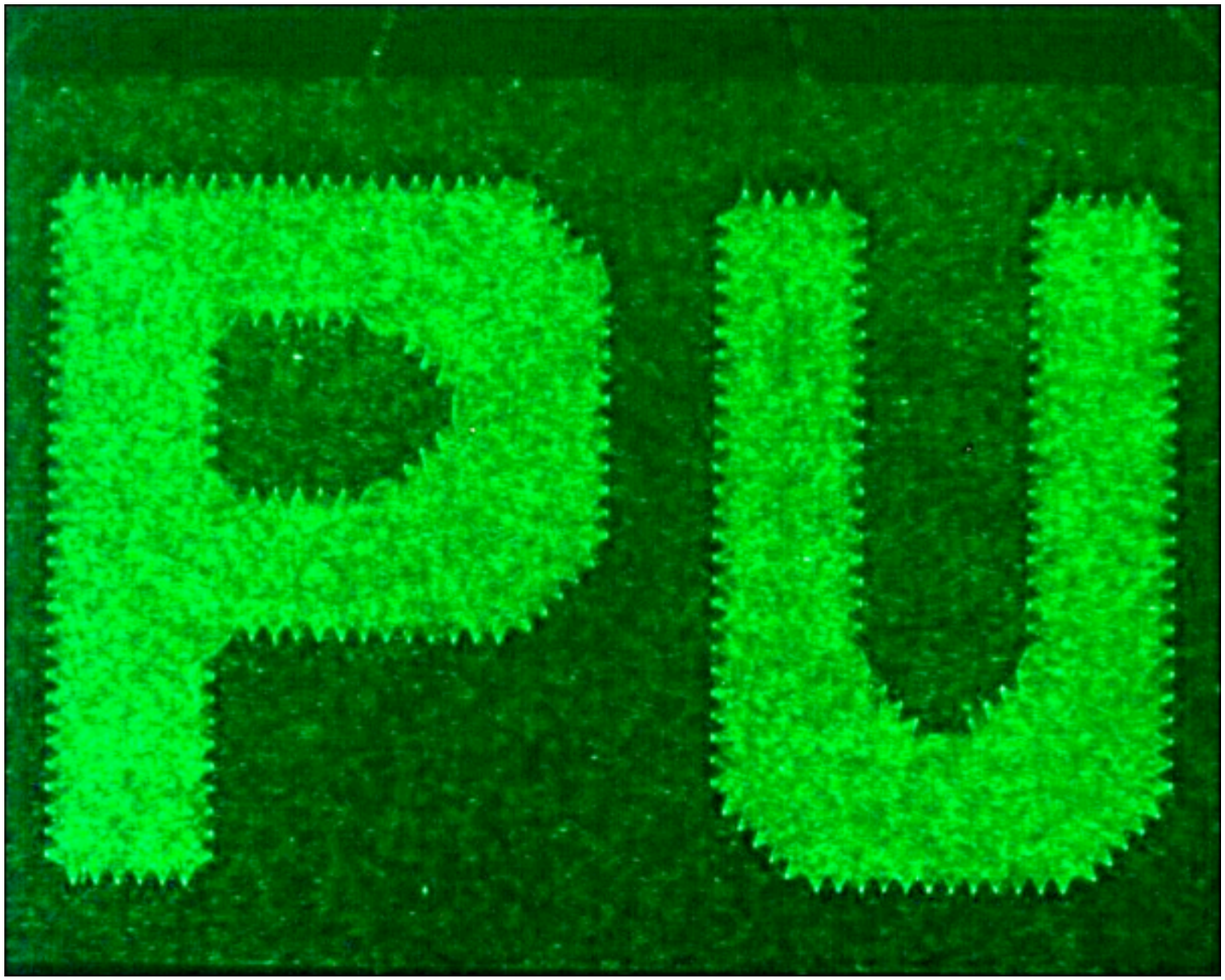
I think that means that inertial terms become important in the full N-S equation. Opinions differ.

$$\rho[\frac{\partial \vec{v}}{\partial t} + (\vec{v} \bullet \nabla)\vec{v}] = \eta \nabla^2 \vec{v} - \nabla P$$



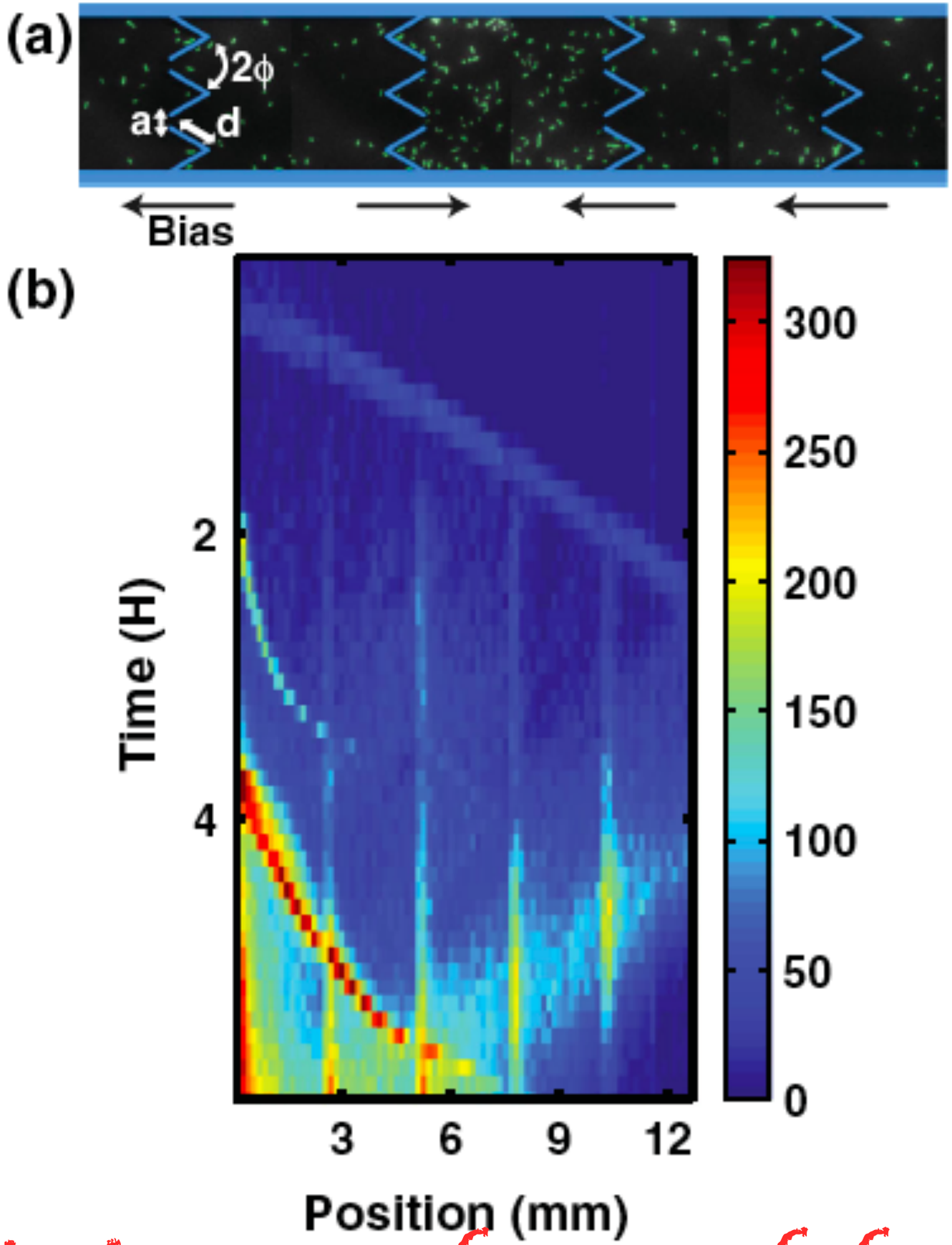
This isn't TOTALLY useless.

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



2) 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?



Is that some form of free will?

2) How to Make a Time Machine

The Time Machine issue comes into play simply with the value of the rate at which mutations occur, since it sets the rate at which evolution occurs stochastically.

My narrow view of neoclassical (Fisher) evolution modeling:

1) Successful mutations are random: $\Delta N = suN$

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

$$u << 1$$

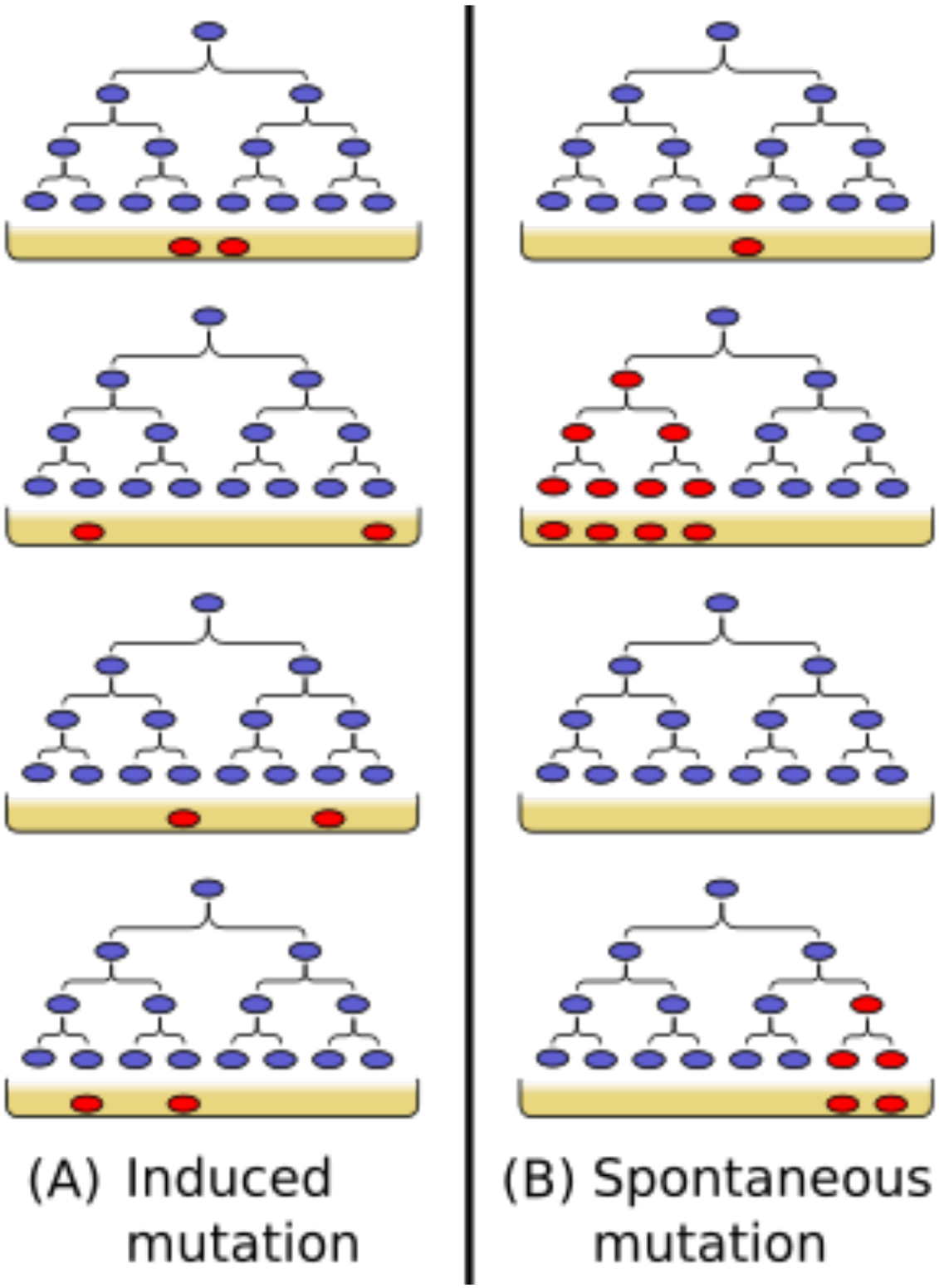
3) Most mutations are deleterious (reduce fitness). Selection coefficient very small:

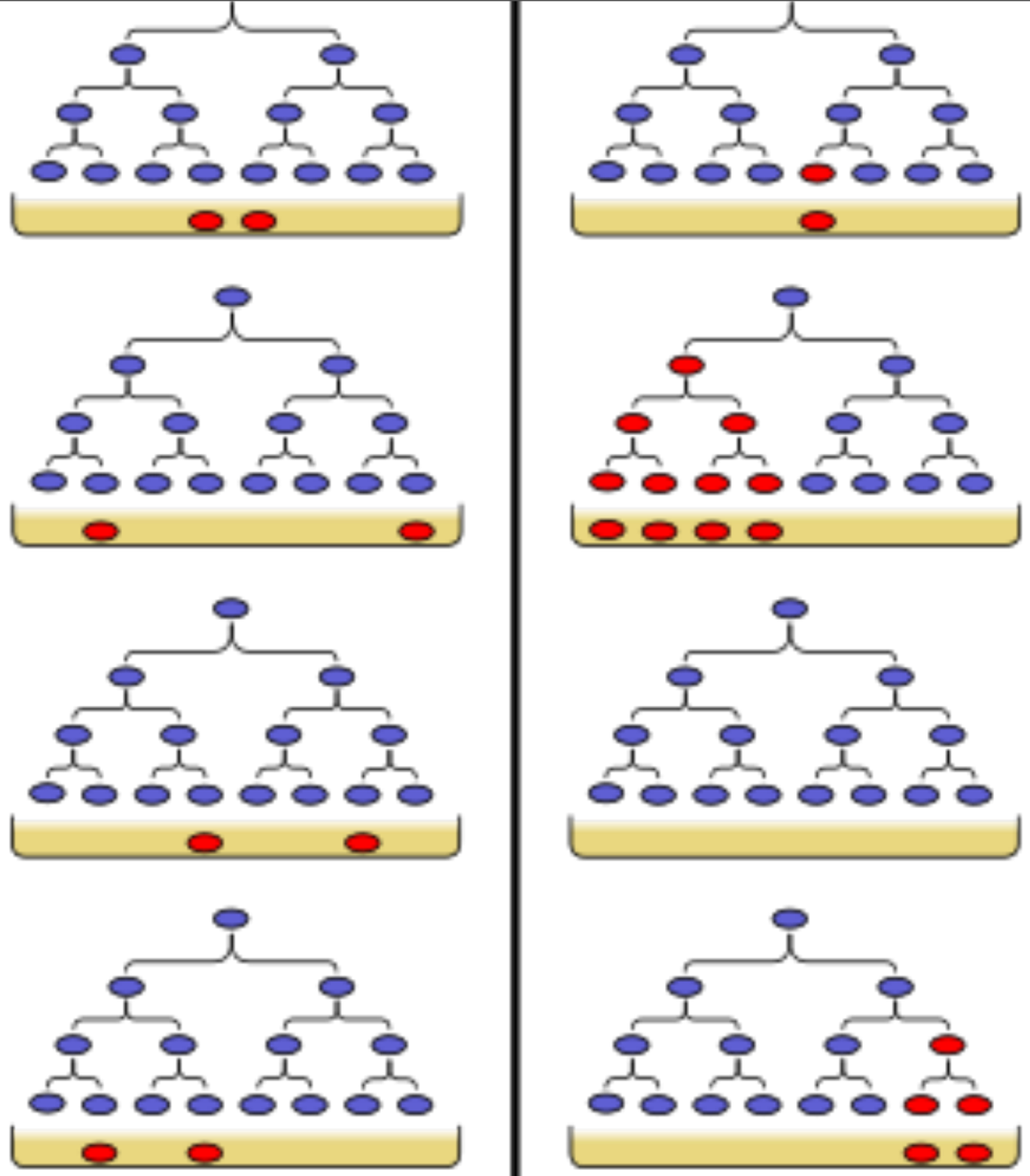
$$s << 1$$

4) Evolution best studied in large numbers N in big buckets, because of the low mutation rates and small selection coefficients.

"Mutations are very rare"

Max Delbruck (physicist): the question of when mutations occur. Dogma for for many.





This is a fundamental question: do mutations occur BEFORE stress is imposed (ie, an antibiotic), or in RESPONSE to the stress?

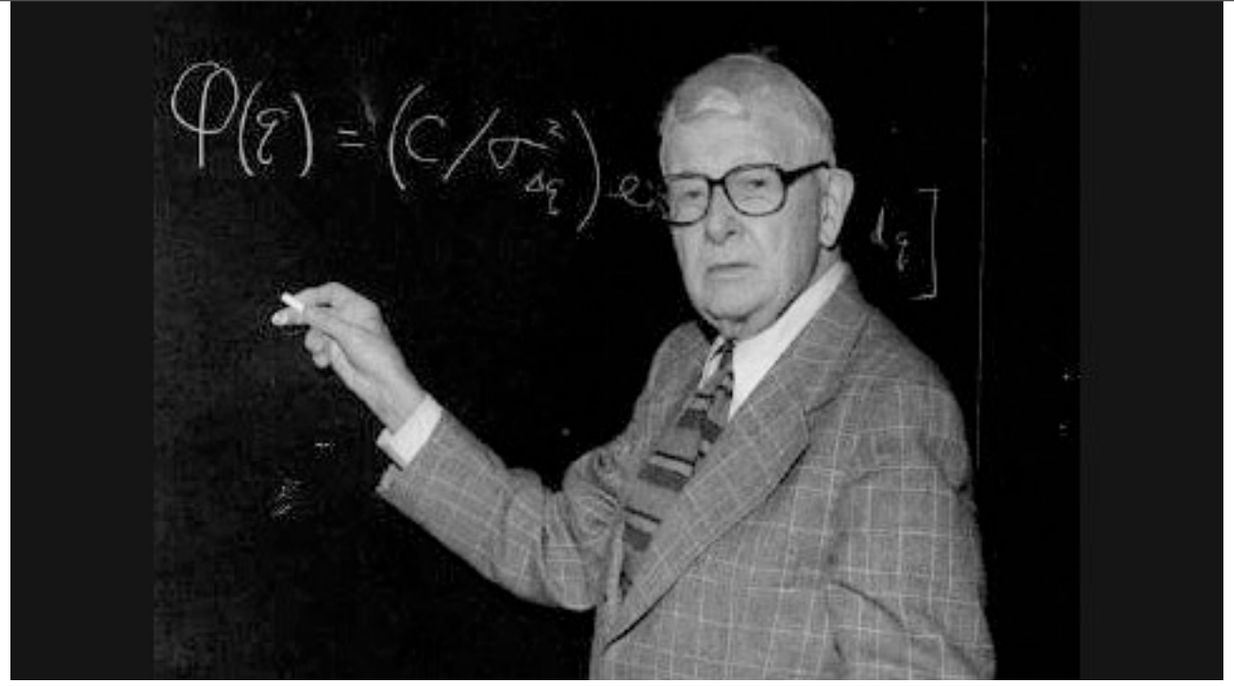
"RESPONSE to the stress?"

This is a loaded and controversial question. "RESPONSE" indicates if you will some sort of a free will: the system decides what it will do in response to a stress, rather than simple use what is already there (pre-existing random mutations).

Delbruck did an experiment where there was no time for evolution after phage infection: you either have resistance and survive, or you dont, and die.

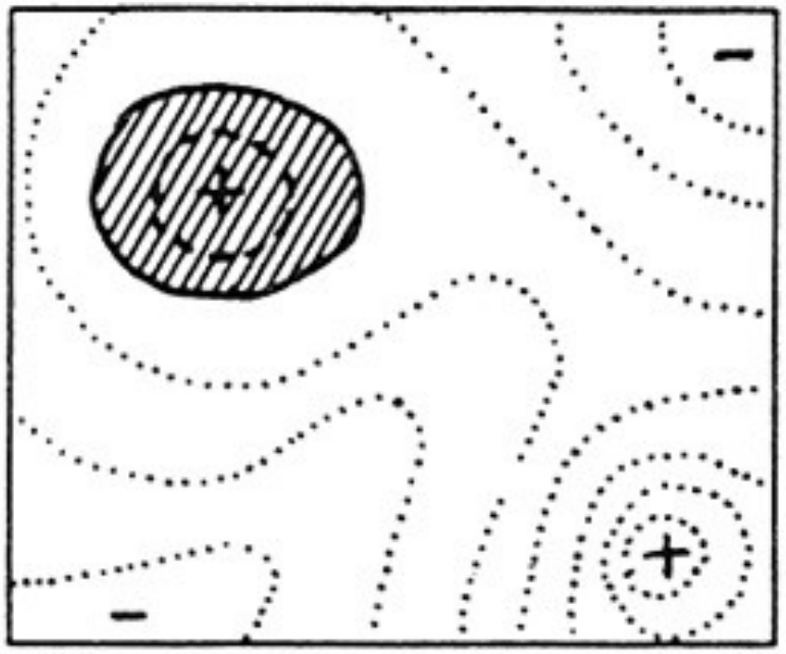
But certainly resistance CAN evolve, the question is: how long does this take?

Delbruck did not address this question.

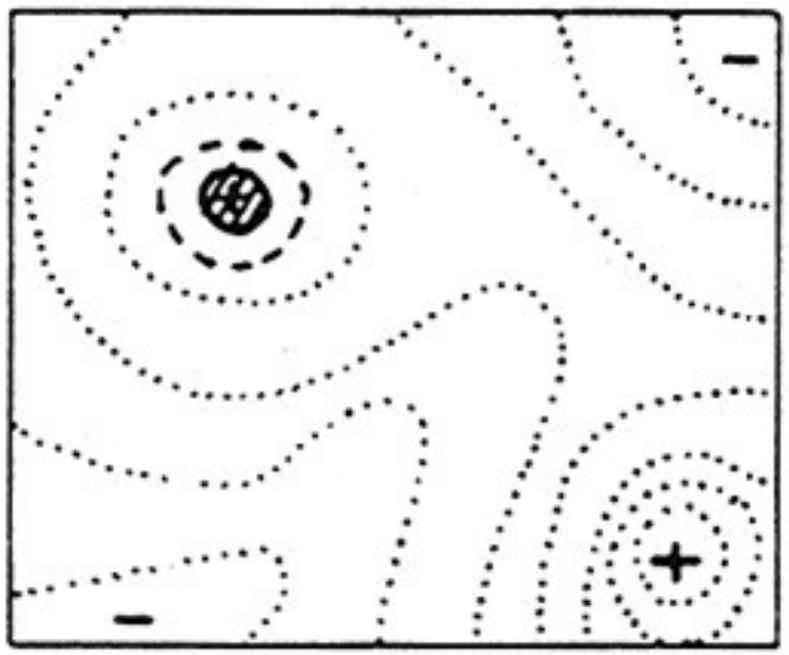


Seawall Wright had the breakthrough idea in the early 30's of viewing "fitness" as a form of free energy which was maximized under selective pressure.

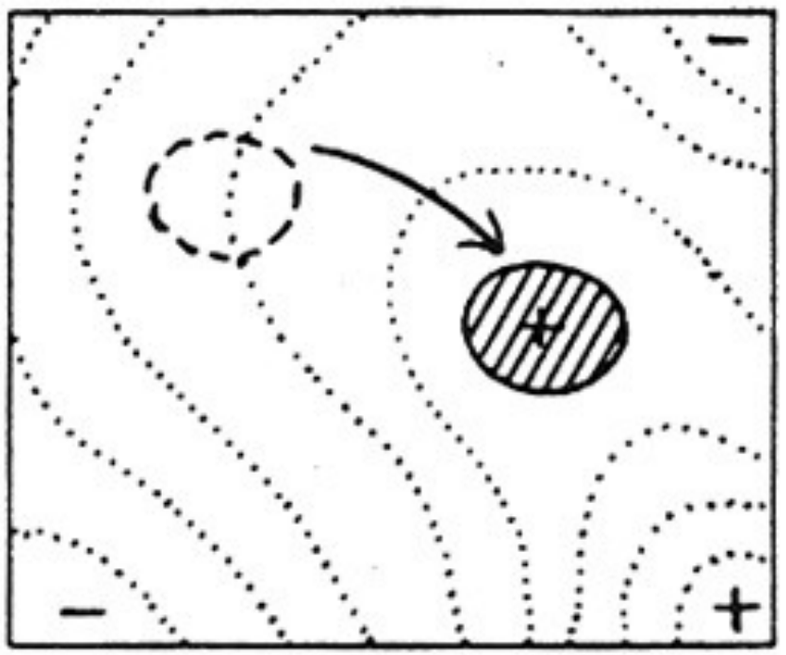
He viewed evolution as movement on a fitness surface to peaks of fitness: a fitness landscape is a free energy landscape.



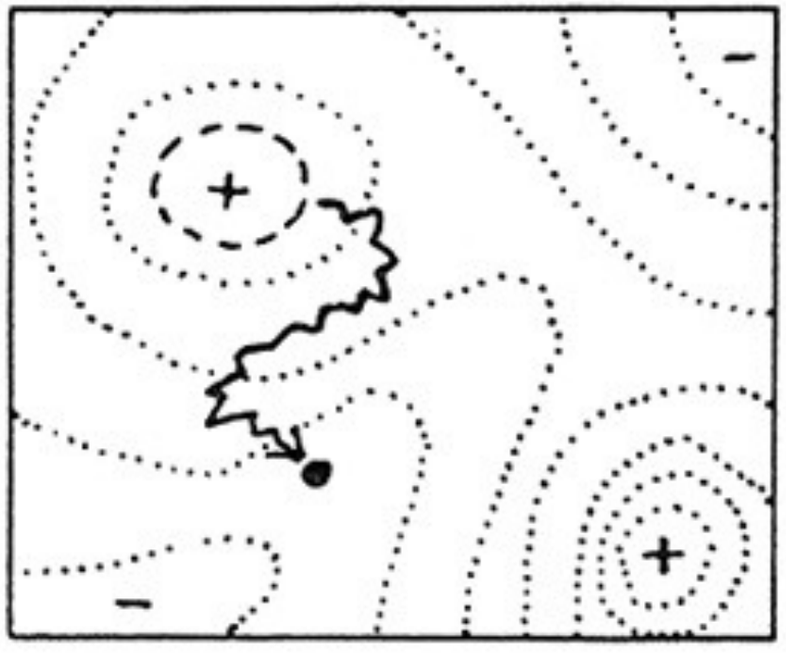
Increased Mutation
or reduced Selection
4NU, 4NS very large



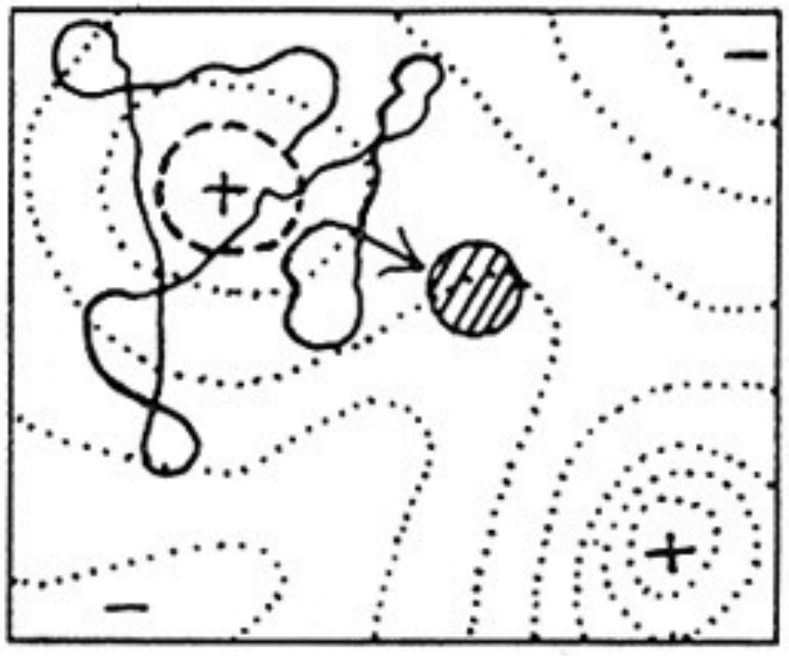
Increased Selection
or reduced Mutation
4NU, 4NS very large



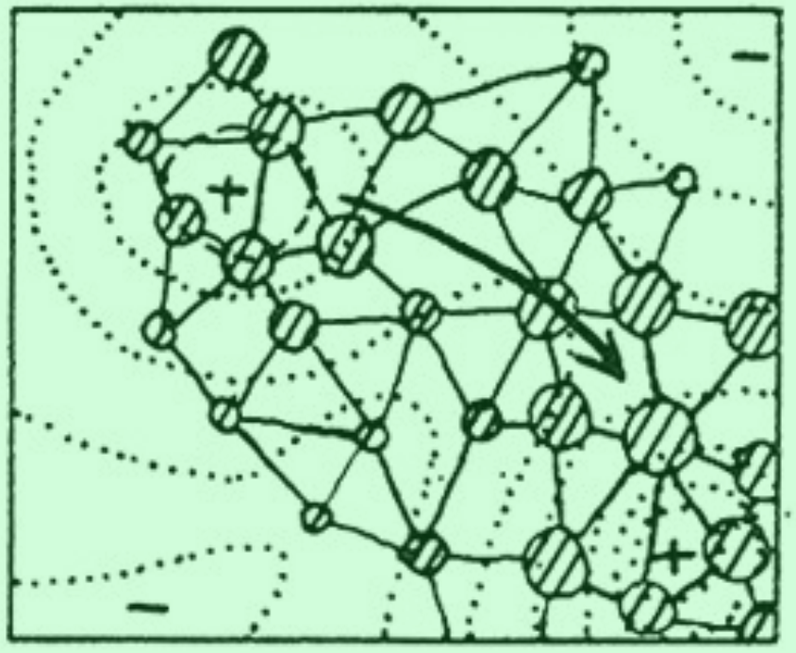
Qualitative Change
of Environment
4NU, 4NS very large



Close Inbreeding
4NU, 4NS very small

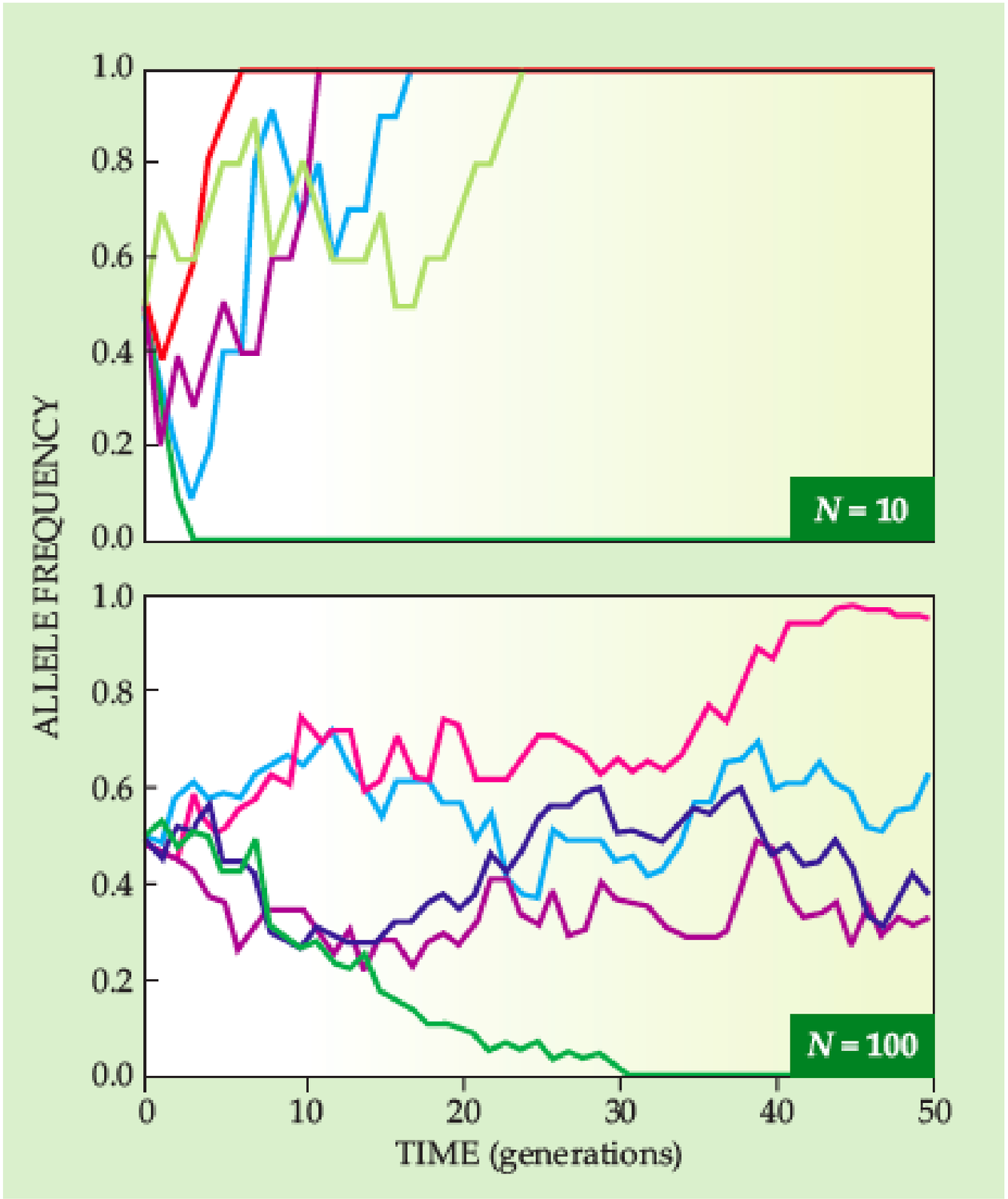


Slight Inbreeding
4NU, 4NS medium



Division into local Races
4nm medium

Sewall Wright, 1932



$$< t > \sim \frac{1}{s} ln(N)$$

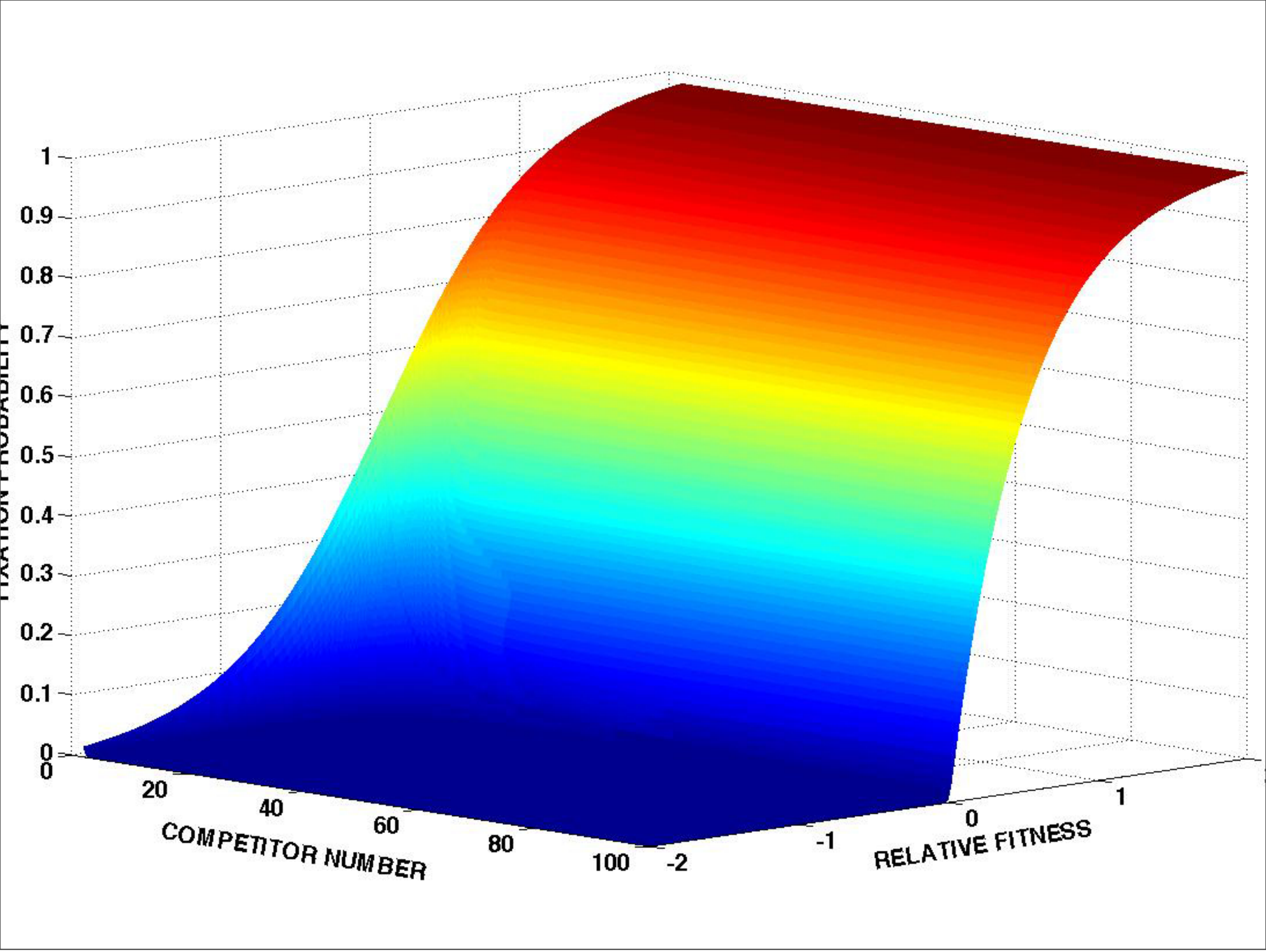
Hallatschek and D. Nelson, Physics Today, July, 2009

The probability of fixation decreases with increasing population size N for fixed s:

$$p_f \sim \frac{2s}{1 - \exp(-4Ns)}$$

1)The time to fix scales as 2N (big)

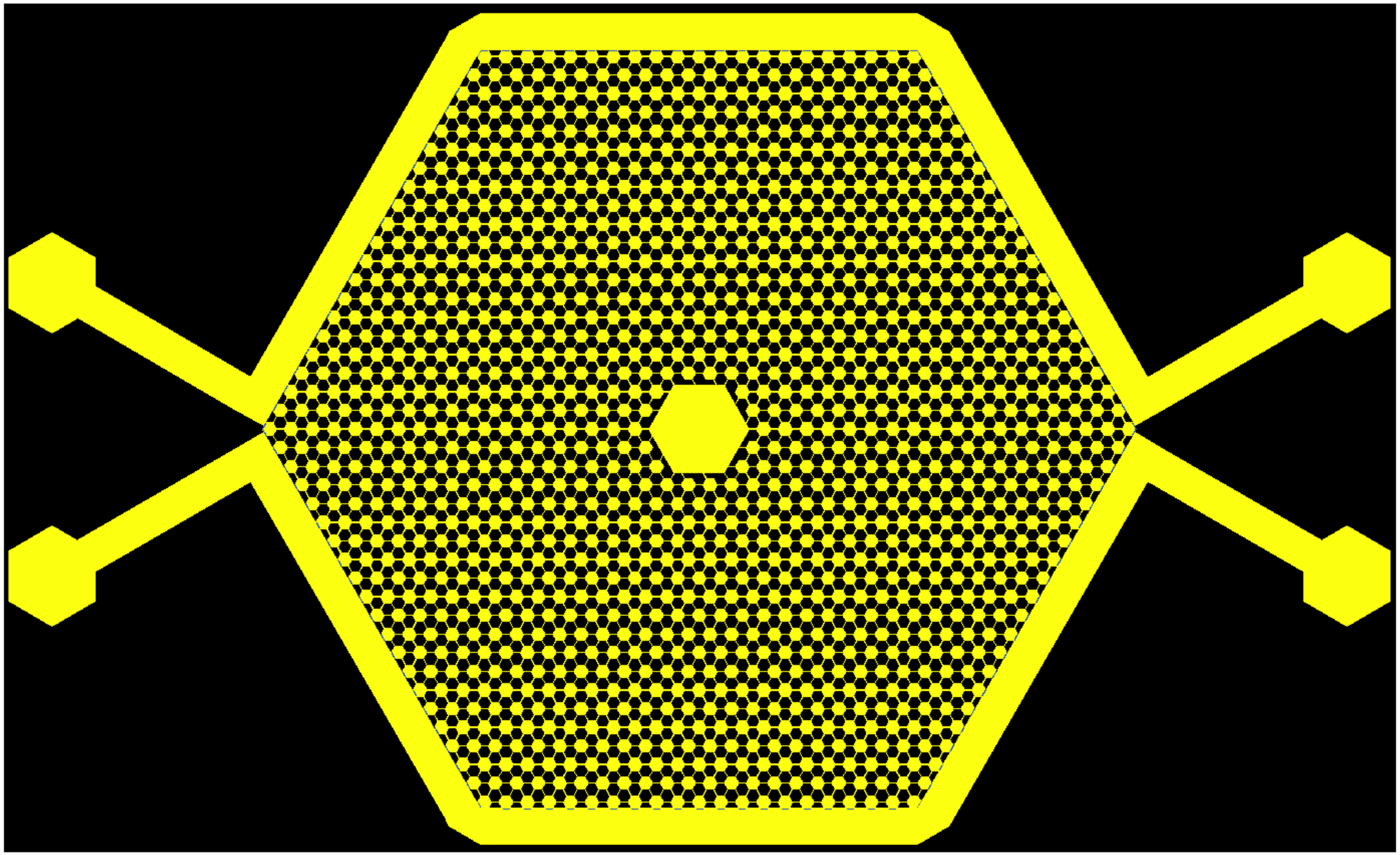
2) The time to lose scales as ln(N) (small).


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
COMPETITOR NUMBER
-2
-1
0
1
RELATIVE FITNESS

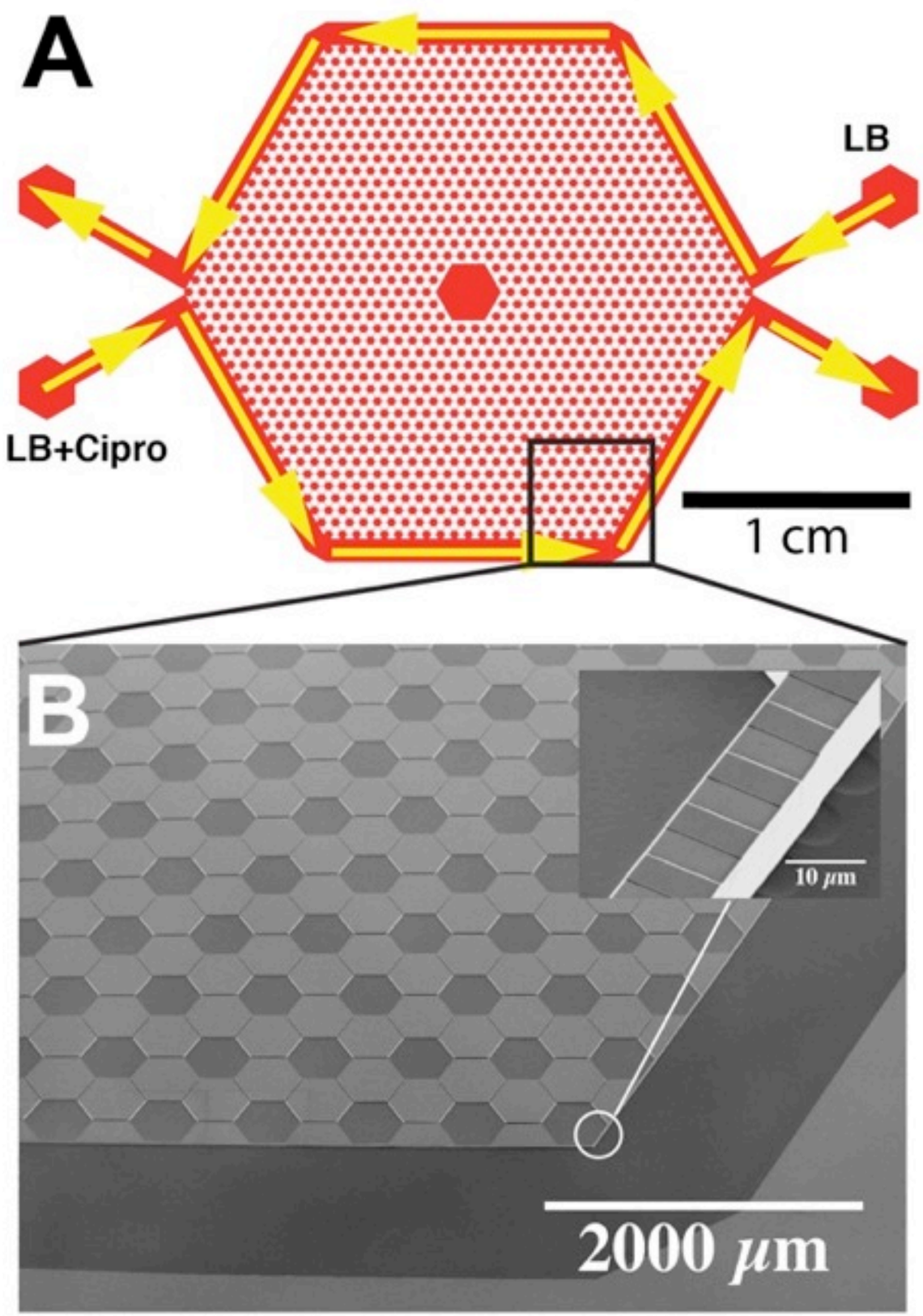
So, if you want to build a Time Machine to move forward rapidly in evolution, throw away your agar plates!!! They are not designed for Sewall Wright's model for robust evolution.

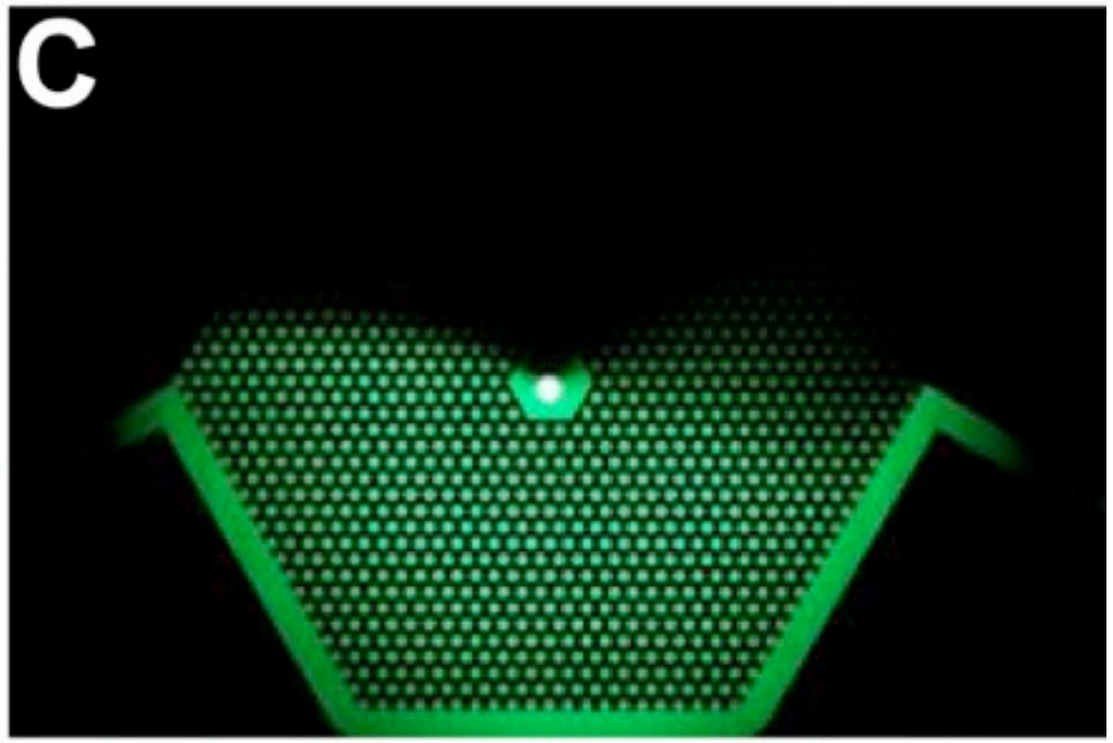
This should make Robot People happy!

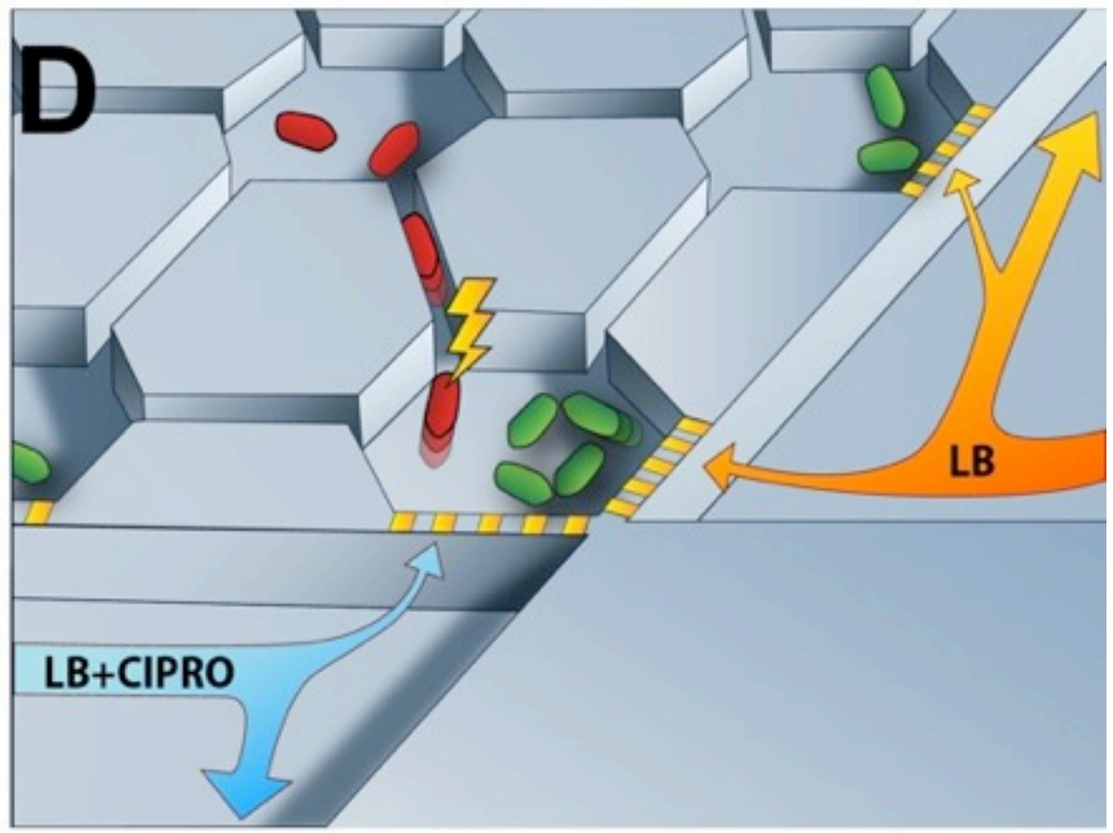
My Time Machine



Bio-Technology for the 21st Century







Following then in Wright's footsteps,
I decided that by:

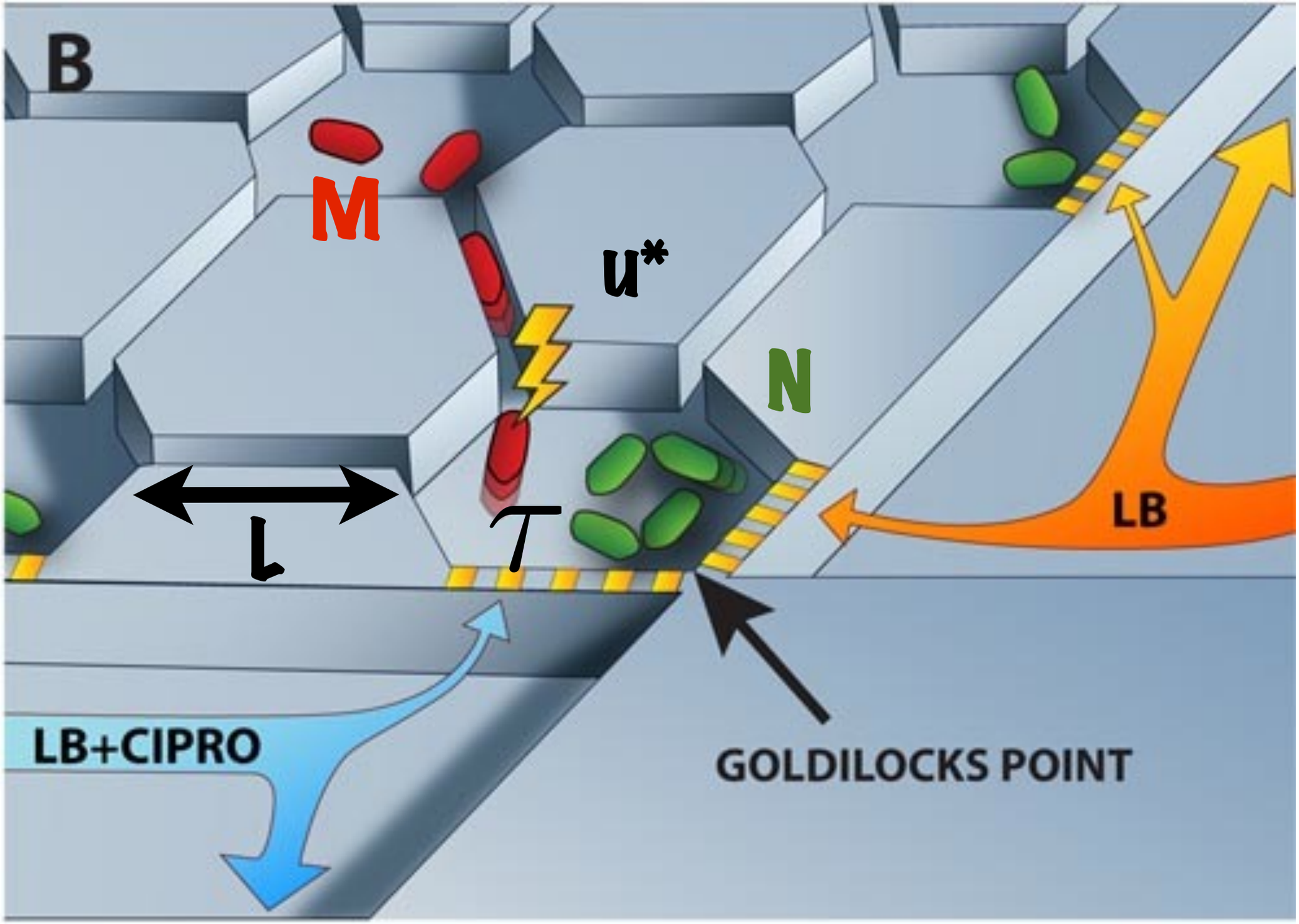
1) Breaking the population into a metapopulation of small n individuals

2) Allow individuals to move on a fitness landscape

3) Use a mutagenic stress

I could rapidly accelerate evolution, in my case resistance to antibiotics by E. coli.

Goldilocks Points: Being at the Right Time at the Right Place



Evolution flow along the free energy gradient here should consist of 3 steps:

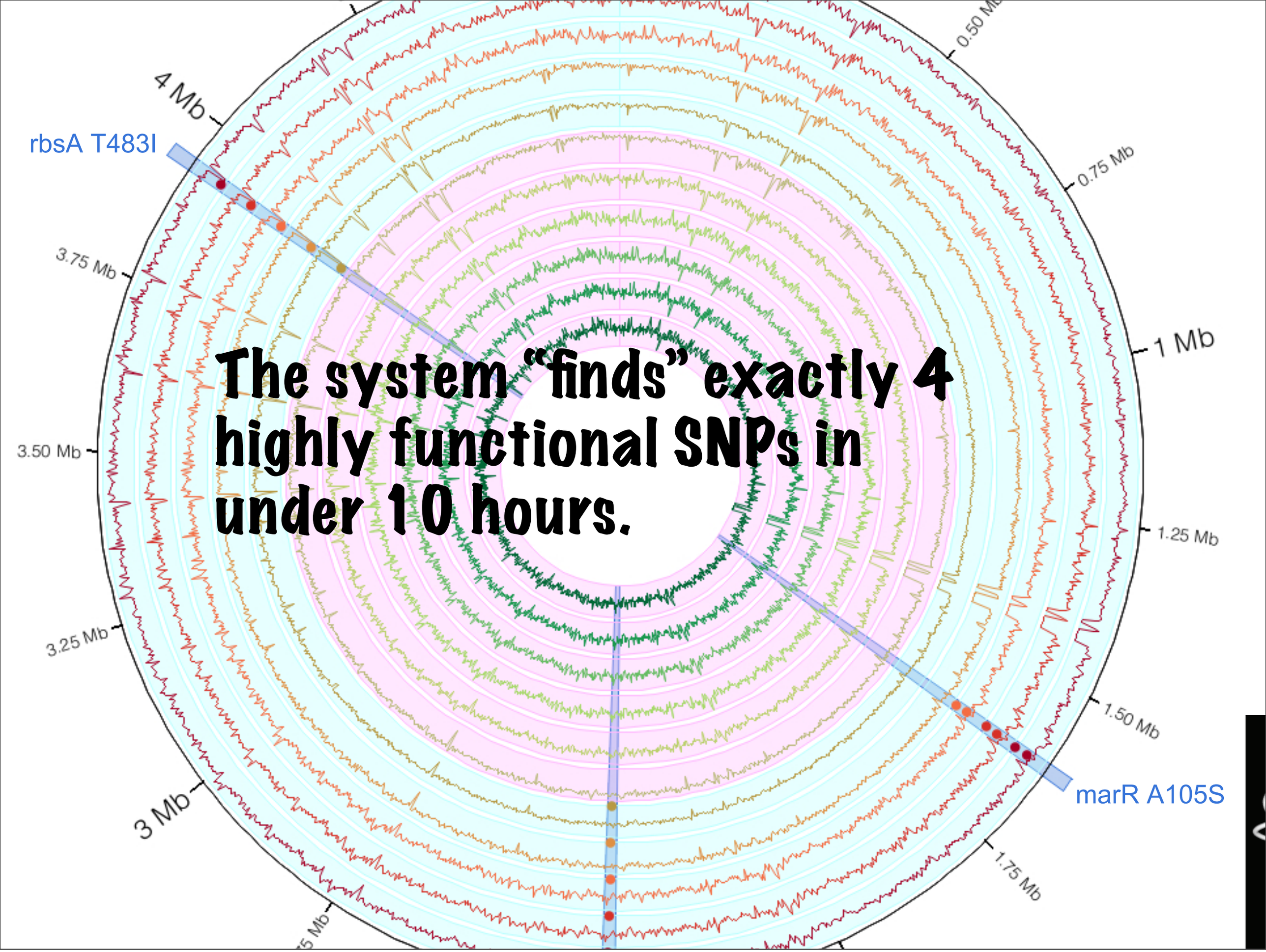
1) The emergence of a mutant (M) in the presence of stress on wild-type N in a region where mutations are common.

2) The flow of the emergent mutant M to a region with less competition (smaller N_i).

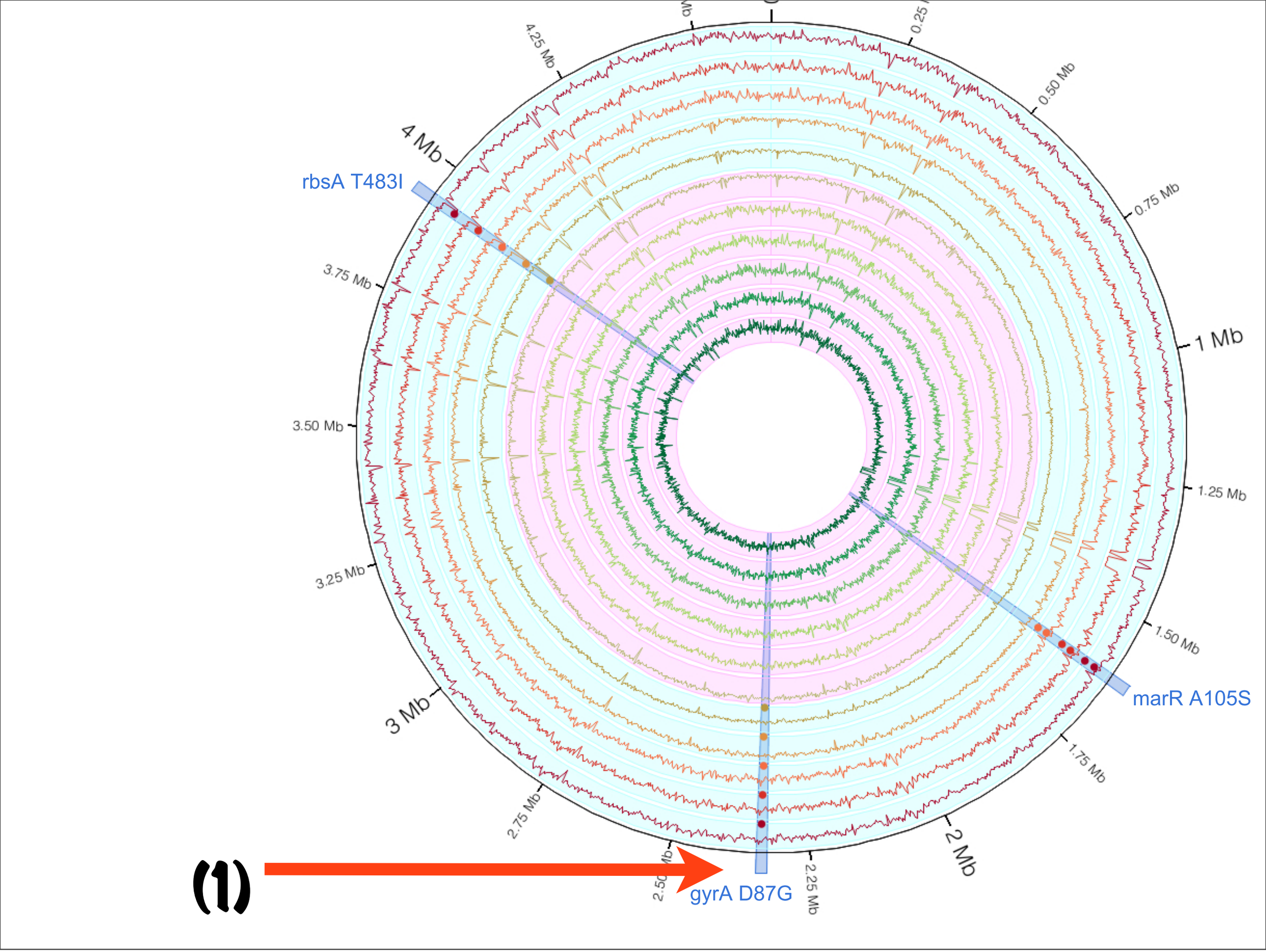
3) Fixing of the new mutant in the region as a new strain.

Stressor: mutagenic Cipro; 10^6 bacteria.

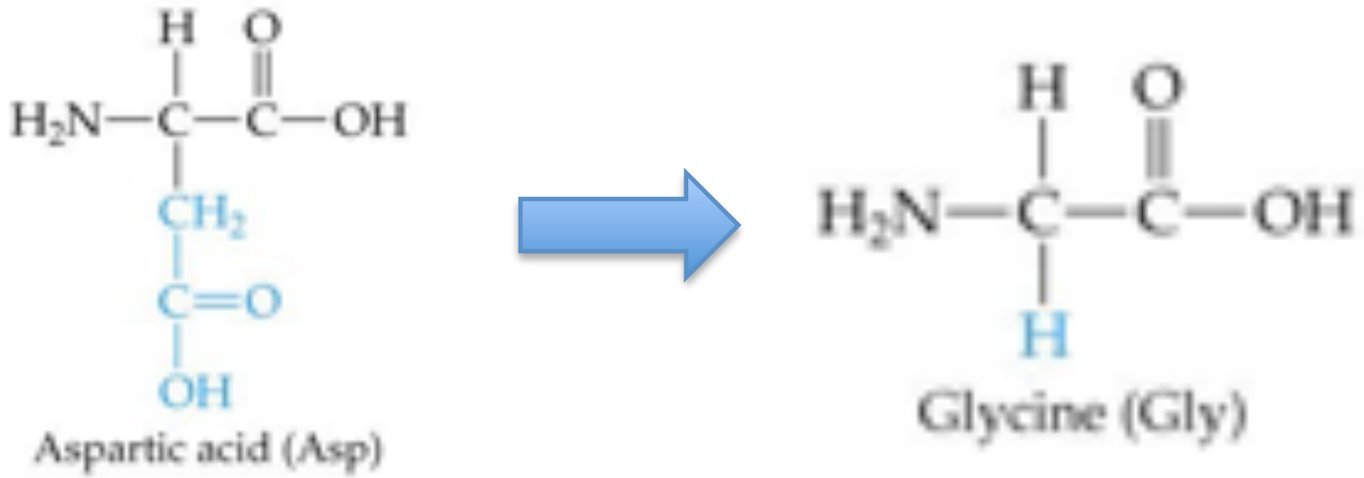
x200 MIC around bottom perimeter



The system "finds" exactly 4 highly functional SNPs in under 10 hours.

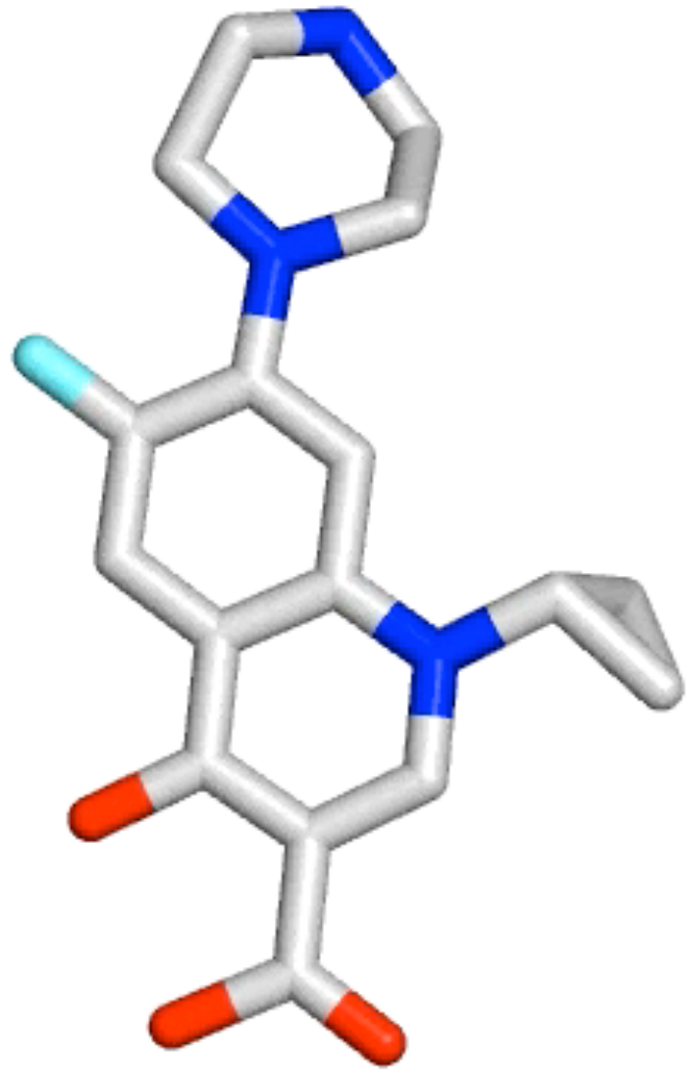

rbsA T483I
marR A105S
gyrA D87G
(1)
4 Mb
3.75 Mb
3.50 Mb
3.25 Mb
3 Mb
2.75 Mb
2 Mb
1.75 Mb
1.50 Mb
1.25 Mb
1 Mb
0.75 Mb
0.50 Mb
0.25 Mb
4.25 Mb
2.25 Mb

Missense Mutation in gyrA



- Mutations in gyrA have been shown to impart cipro-resistance to e. coli
- Previous studies show a D87N also imparts resistance
- Mutations that impart resistance most often occur in active binding site

How did the cell find "the" solution so fast?



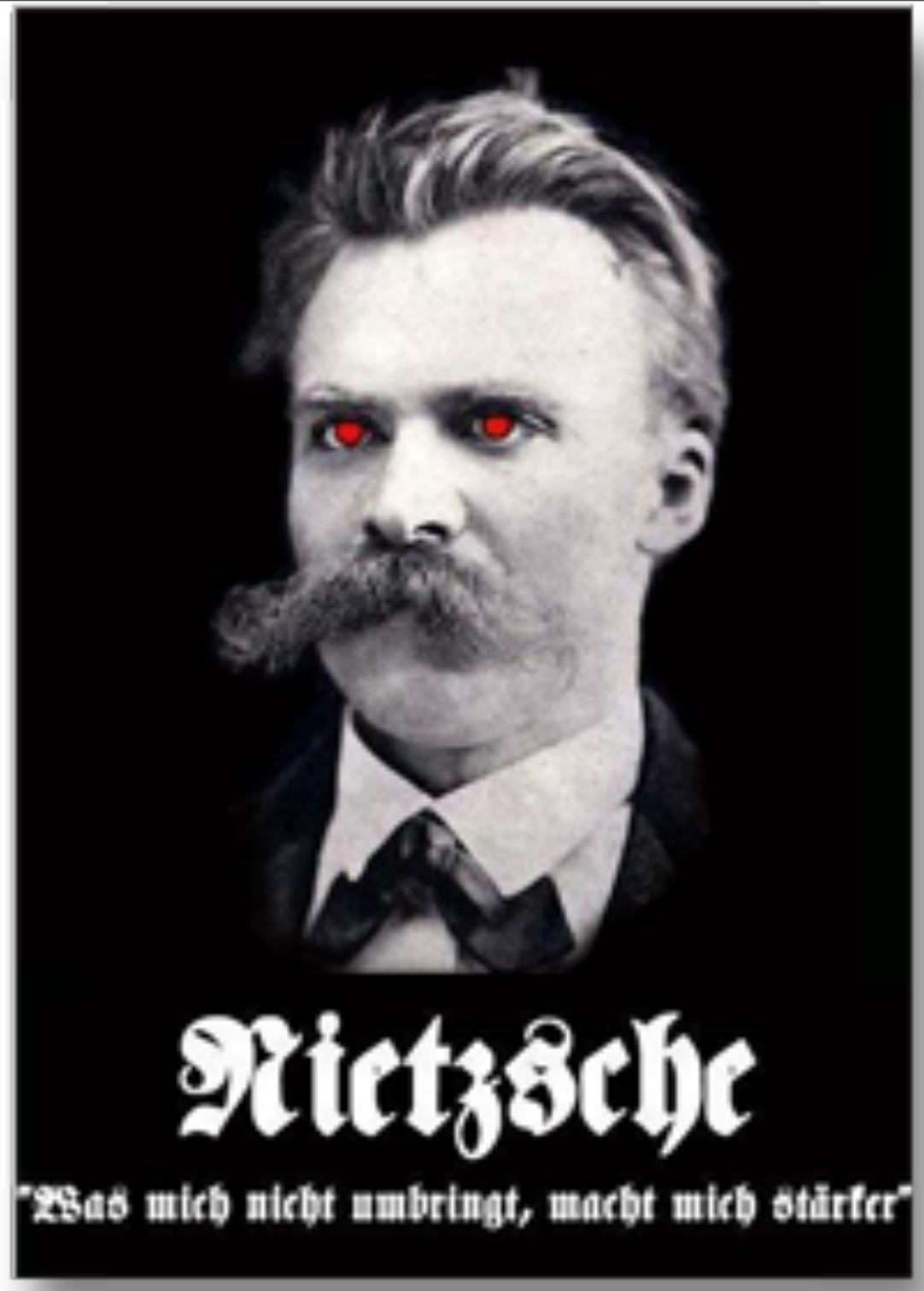
Personally, I find this pretty shocking:

Not only are we finding rapid emergence of antibiotic resistance in bacteria scaling down to very small numbers of bacteria, but also we see rapid and innovative finding of ways to bypass the antibiotics.

These mutations occur rapidly and in highly specific places that are highly functional.

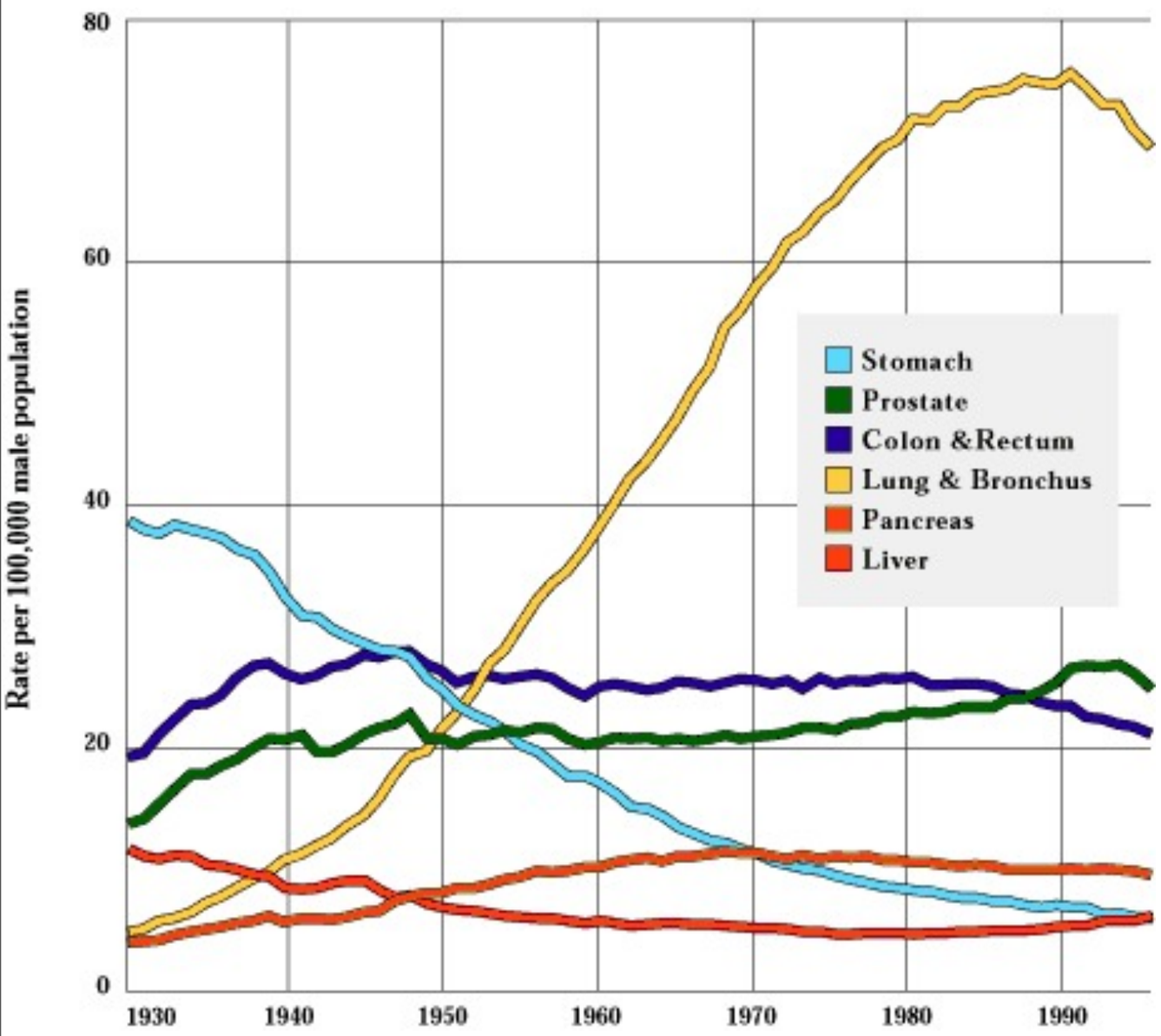
I think the system knows what it is doing, but I didn't say that.

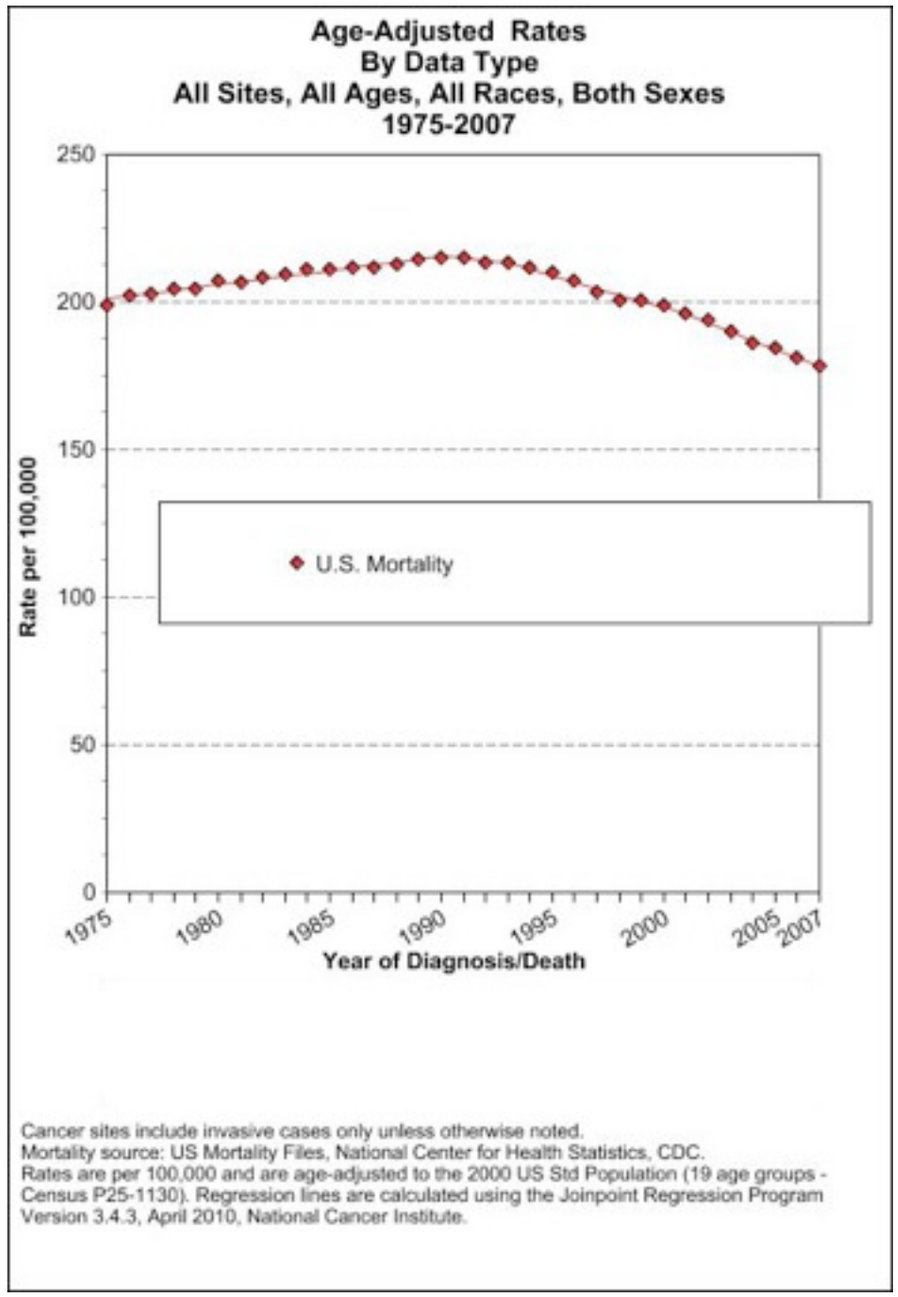
III. This gizmo, what good is it?



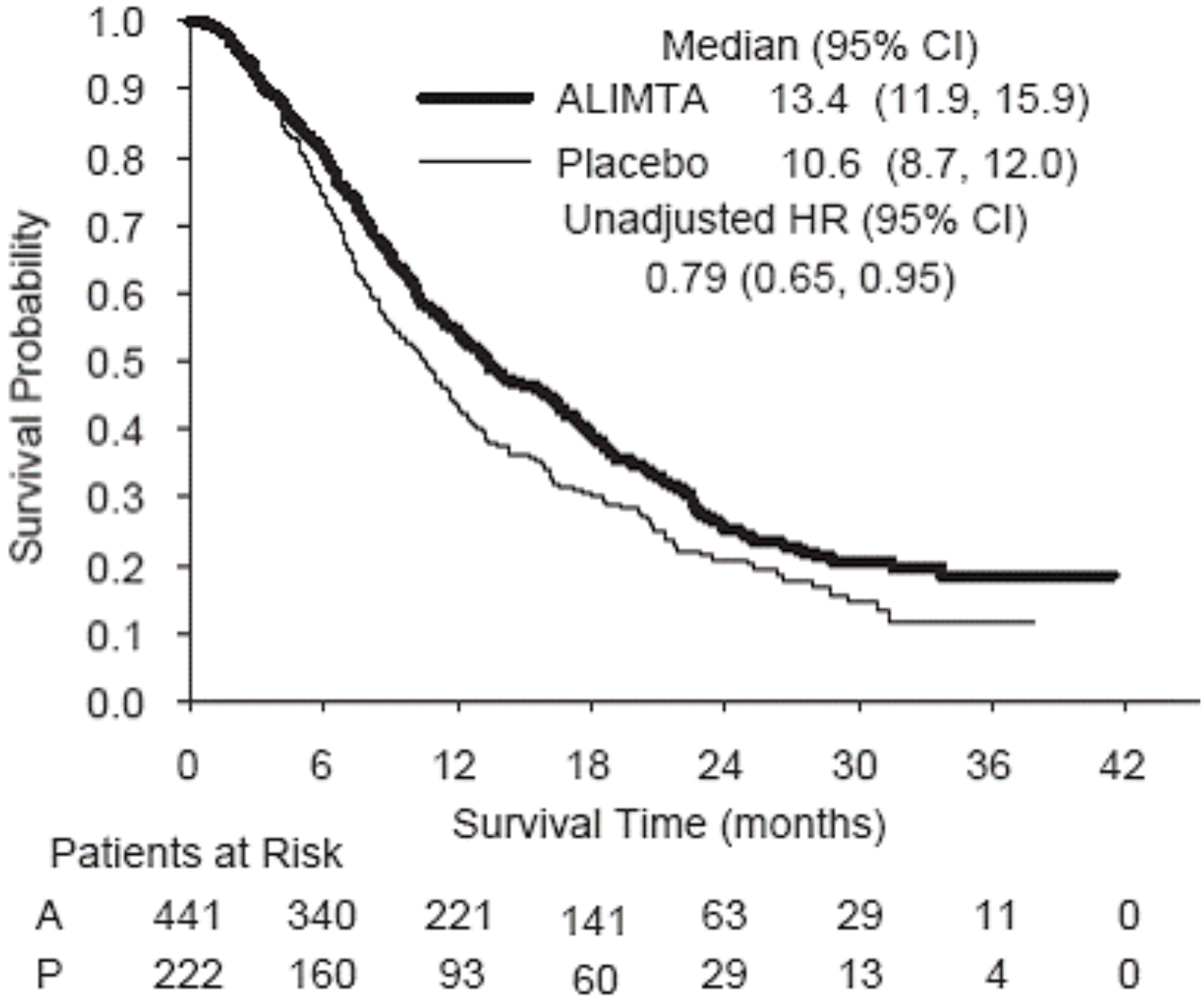
Cancer: What does not kill me makes me stronger.

We seem to be not winning the "wars" against Cancer.



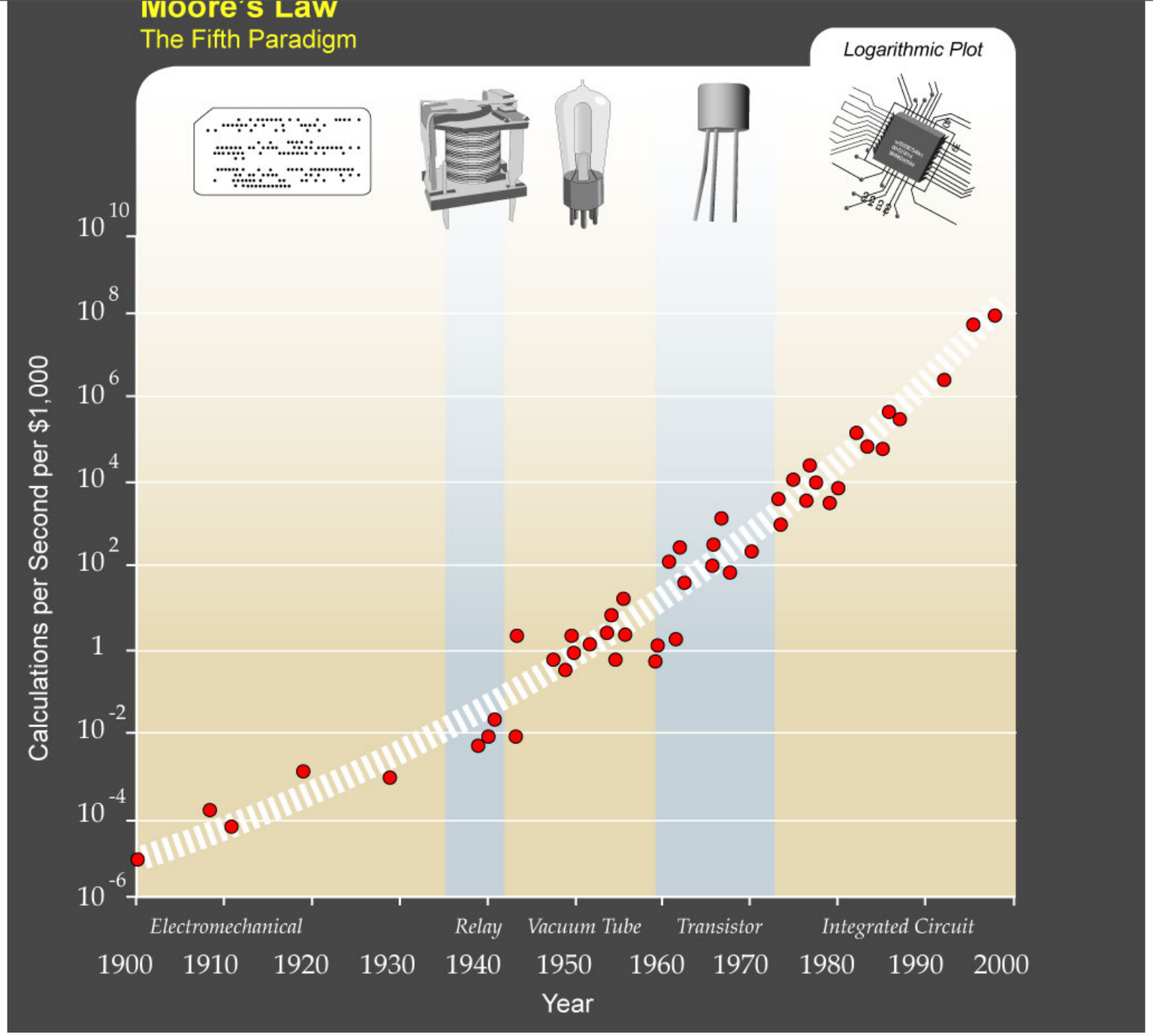


Except for a few remarkable exceptions (childhood leukemias, I think I know why) , cancer mortality rates have been basically flat for 40 years. Most the drops are due to prevention.

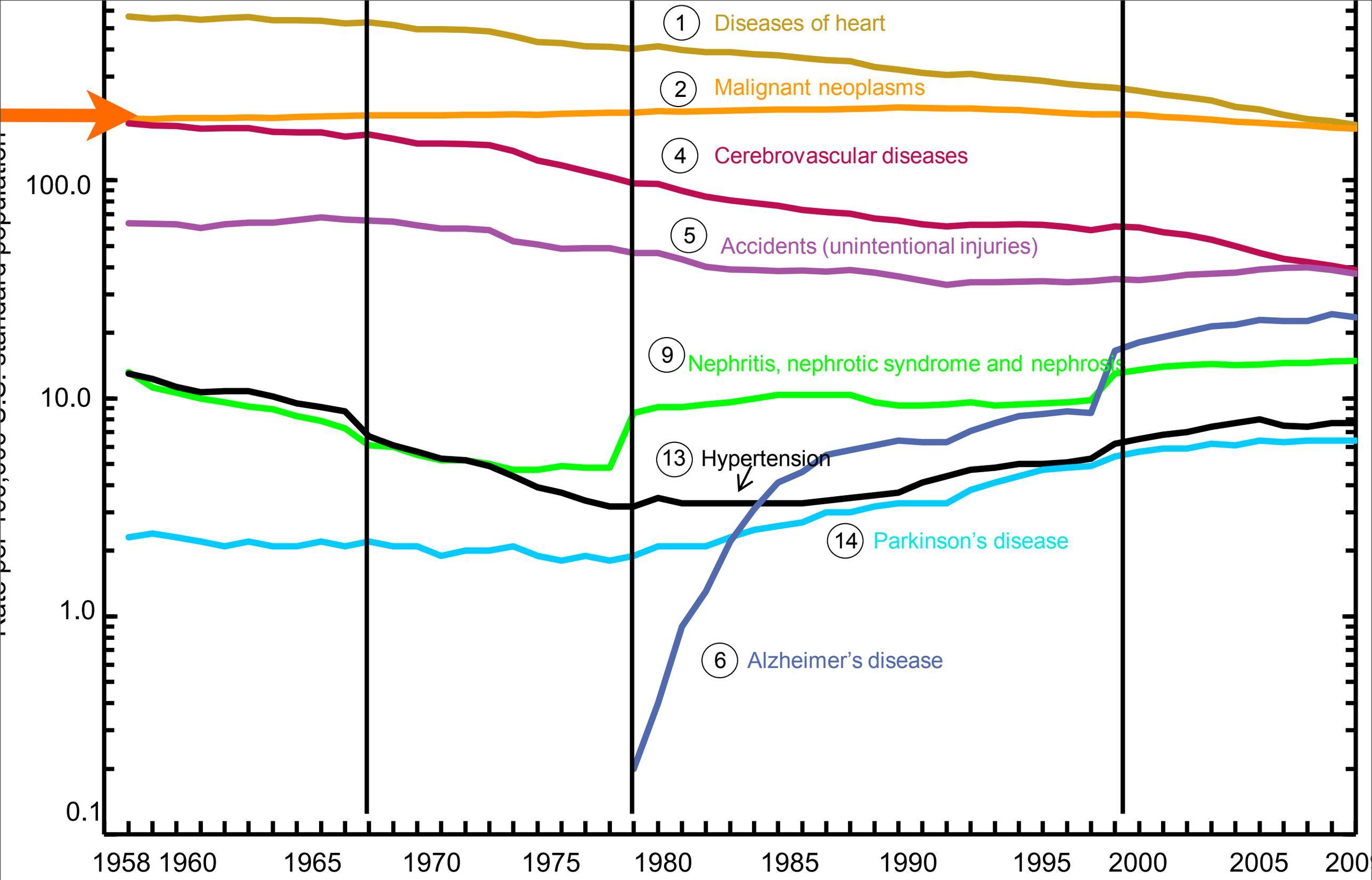


Two major problems: evolution of drug resistance, and failure to predict metastasis (90% of deaths?).

Warning: 80% of oncology papers cannot be reproduced.



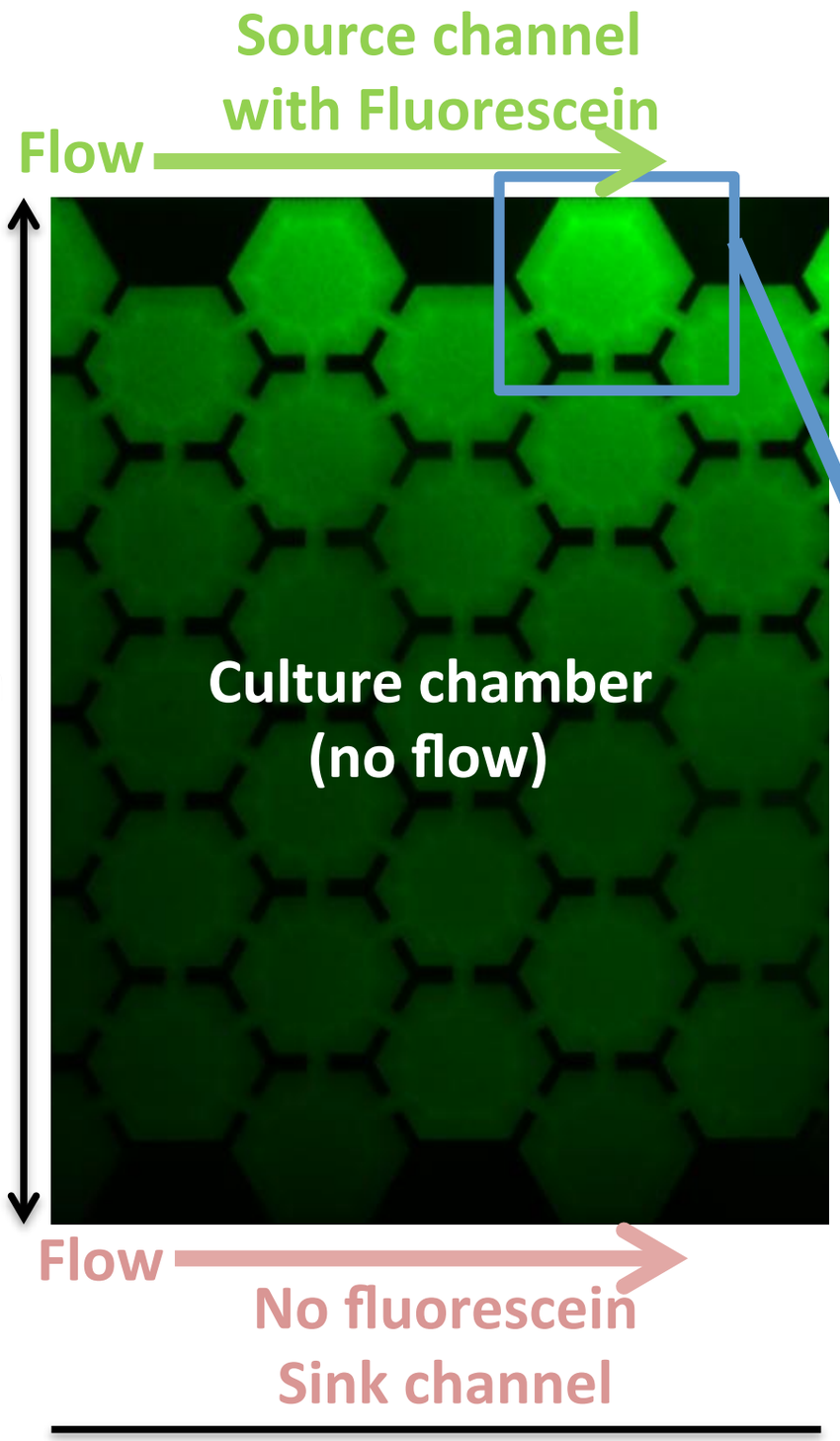
16 orders of magnitude improvement in 100 years! Robotics has been made a real field because of this incredible surge in technology.

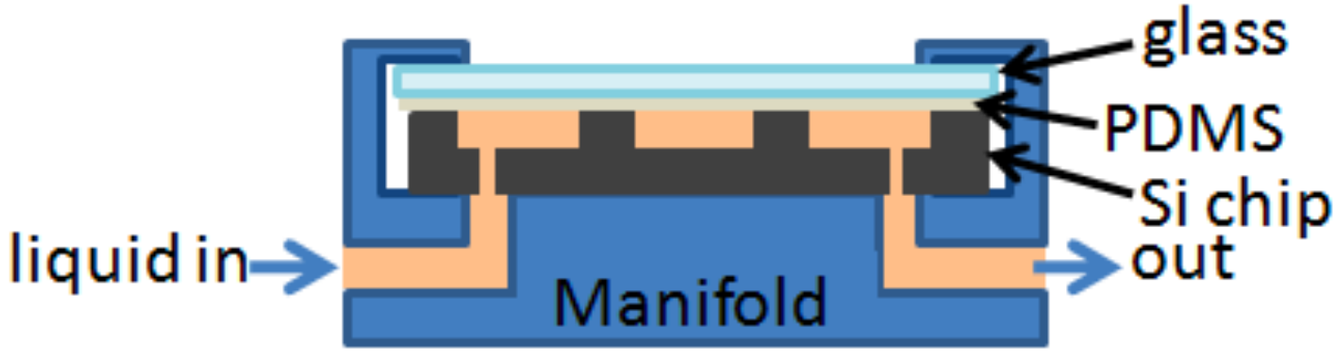


NOTE: ICD is the *International Classification of Diseases.* Circled numbers indicate ranking of conditions as leading causes of death in 2009.
Age-adjusted death rates per 100,000 U.S. standard population; see "Technical Notes."
SOURCE: CDC/NCHS, National Vital Statistics System, Mortality.

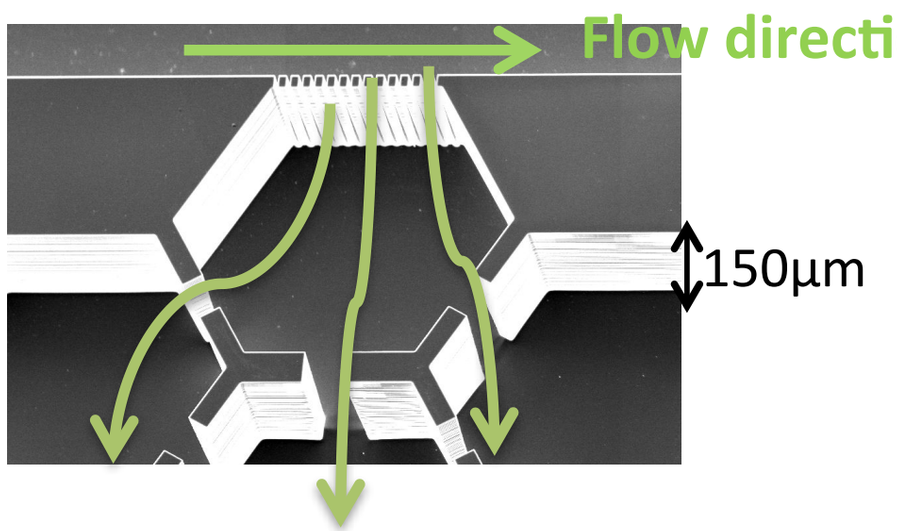
There has been NO paradigm shift in cancer!

The Death Galaxy for Cancer Cells

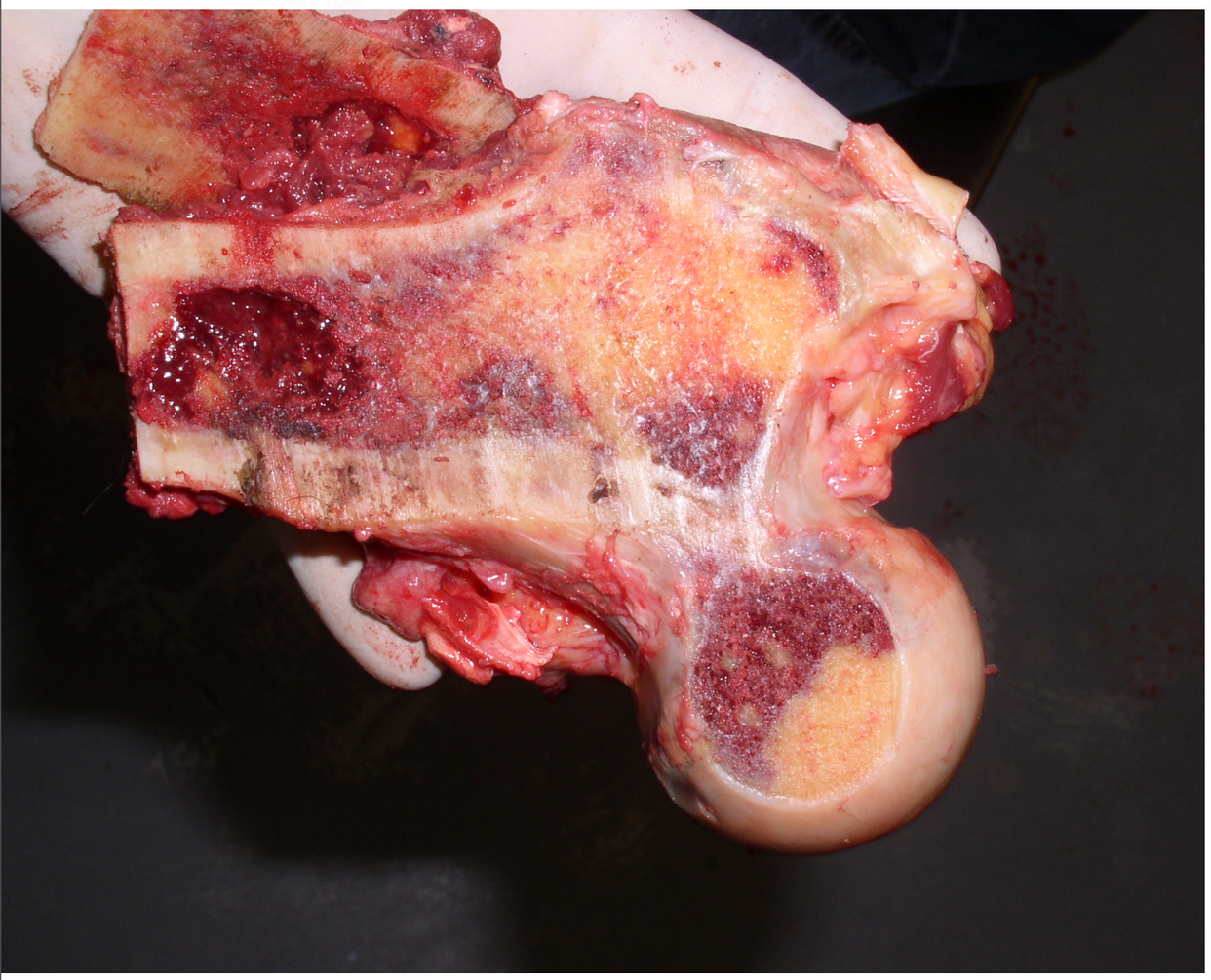


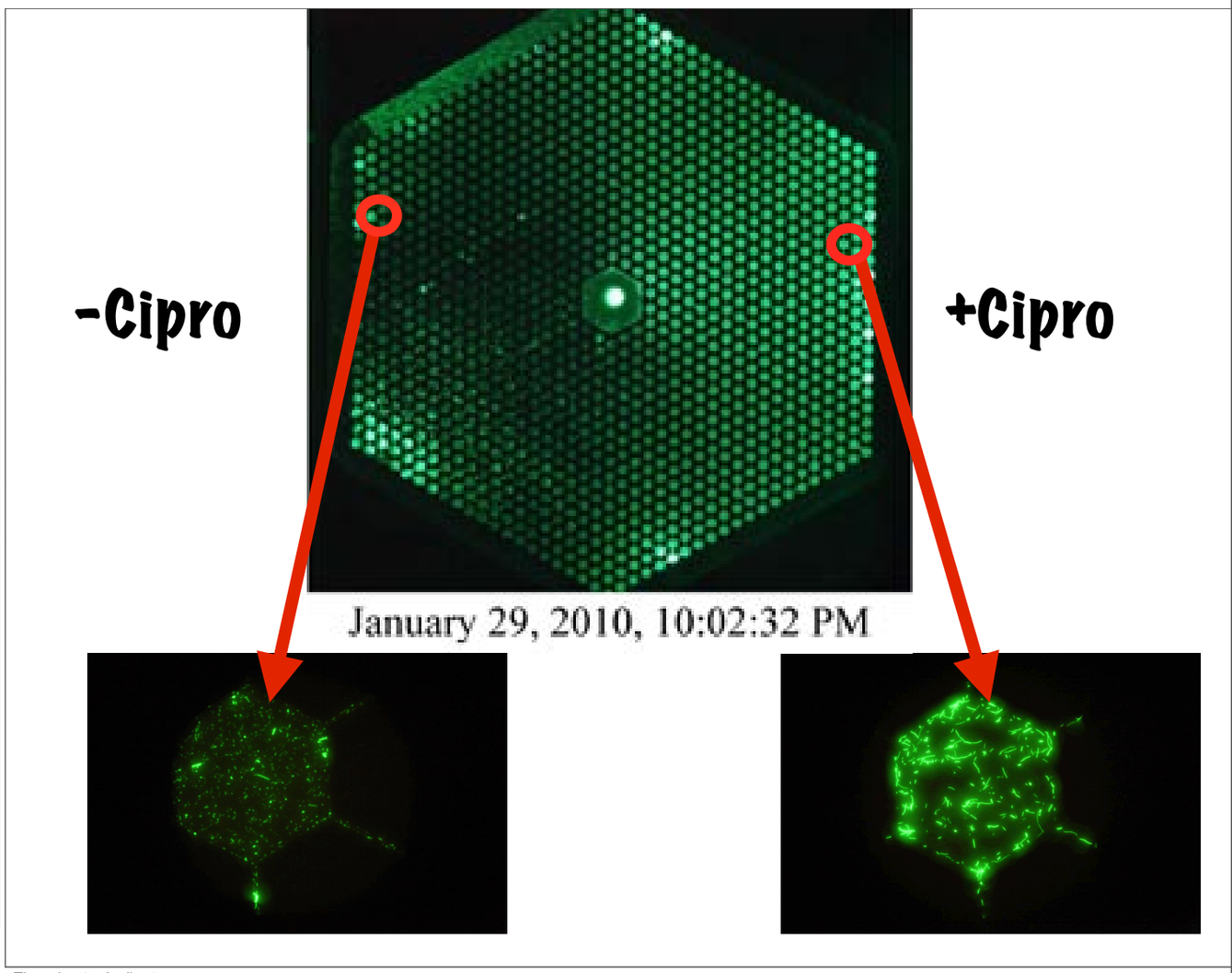


Microposts allow diffusion of biomolecules



- Reconstruct tumor microenvironment
  - Stable drug gradient
  - Connected microhabitats
  - Extracellular matrix (matrigel)





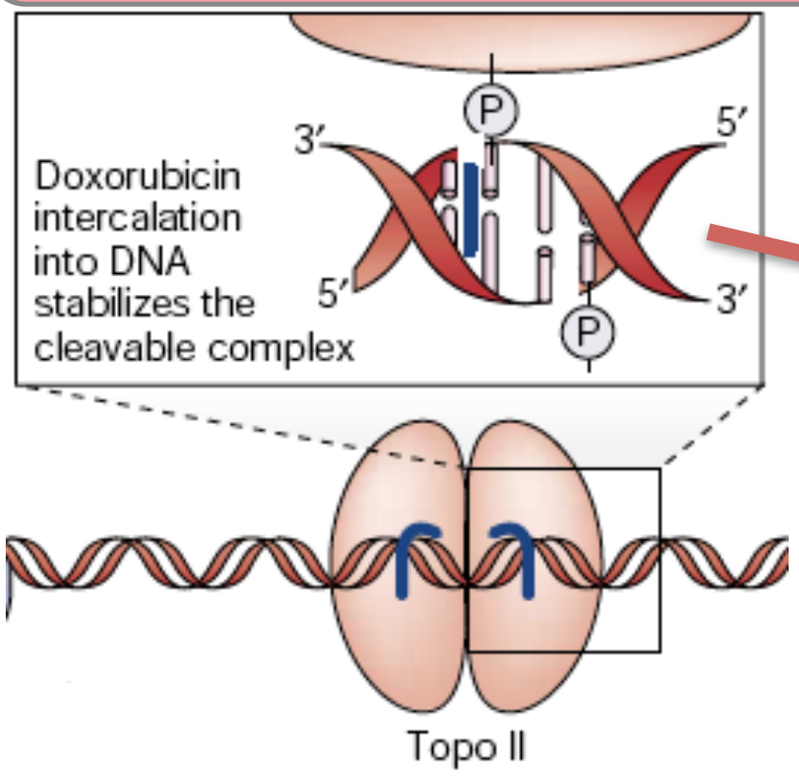
You can't recapitulate the evolution of cancer in a homogeneous environment.

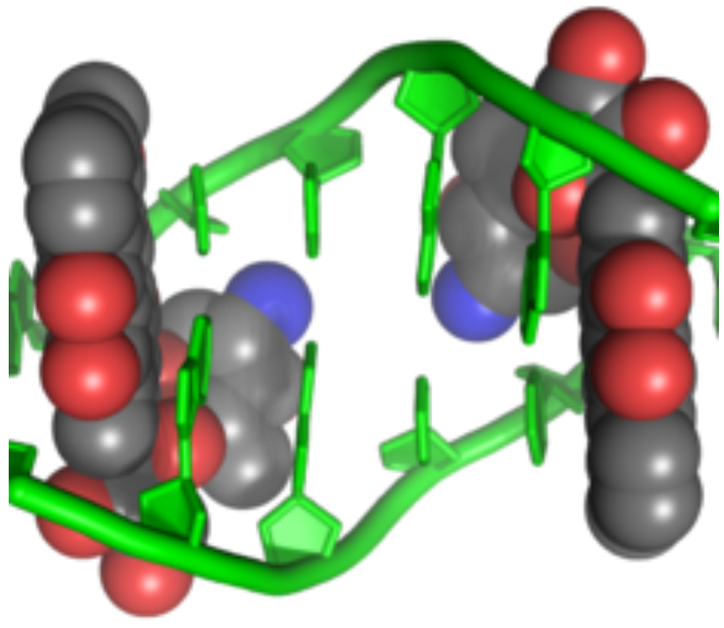
Princeton Physical Sciences - Oncology Center
NIH Site Visit – 9/7/2012

The Bottom Line:

What is the stress we applied?

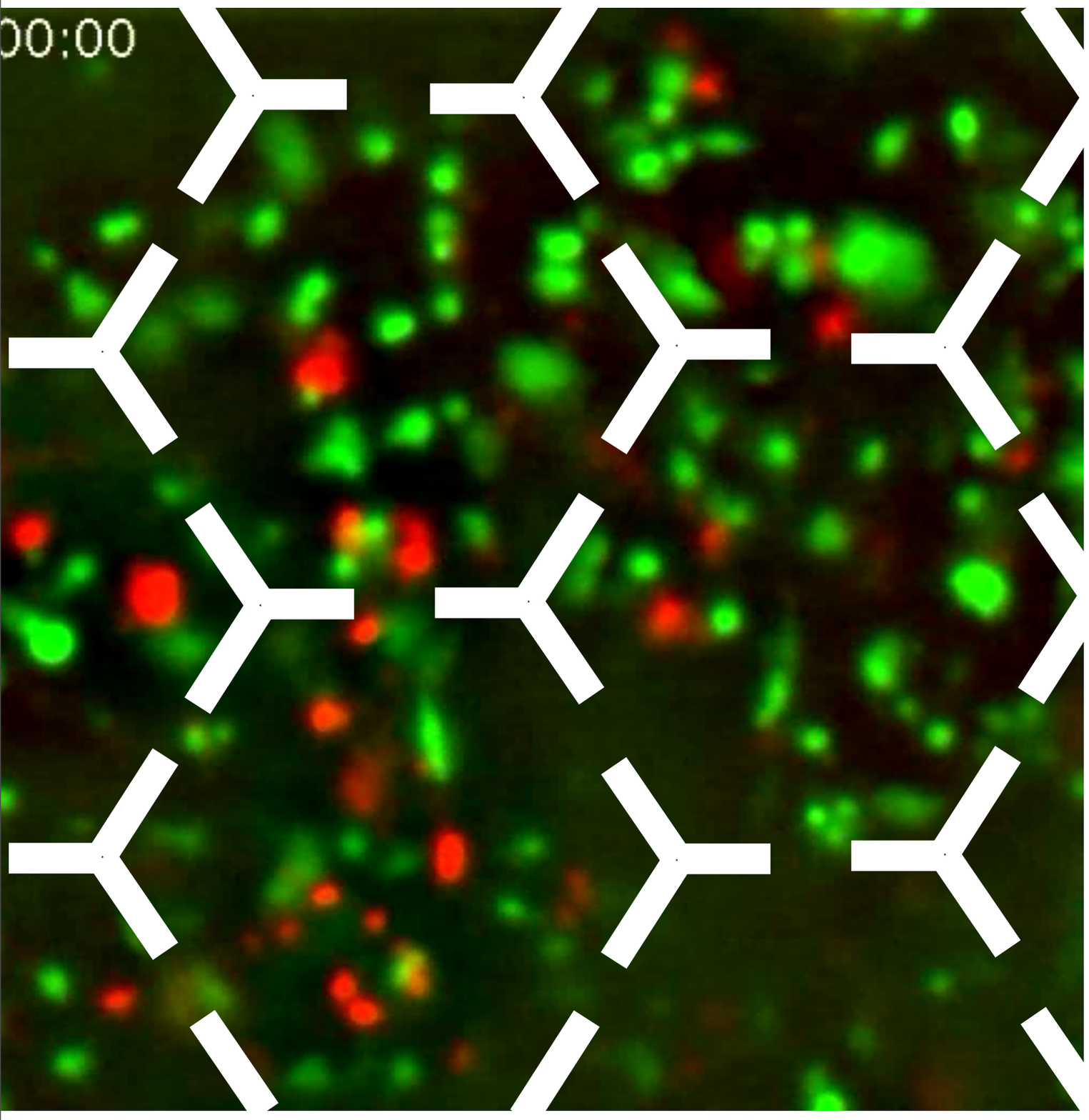
- Doxorubicin
  - Chemotherapeutic drug
  - Genotoxic, blocks DNA replication
  - 20 nM Kills 100% myeloma cells within 144 hours





Protein Data Bank

Hurley, Nat Rev Cancer 2002



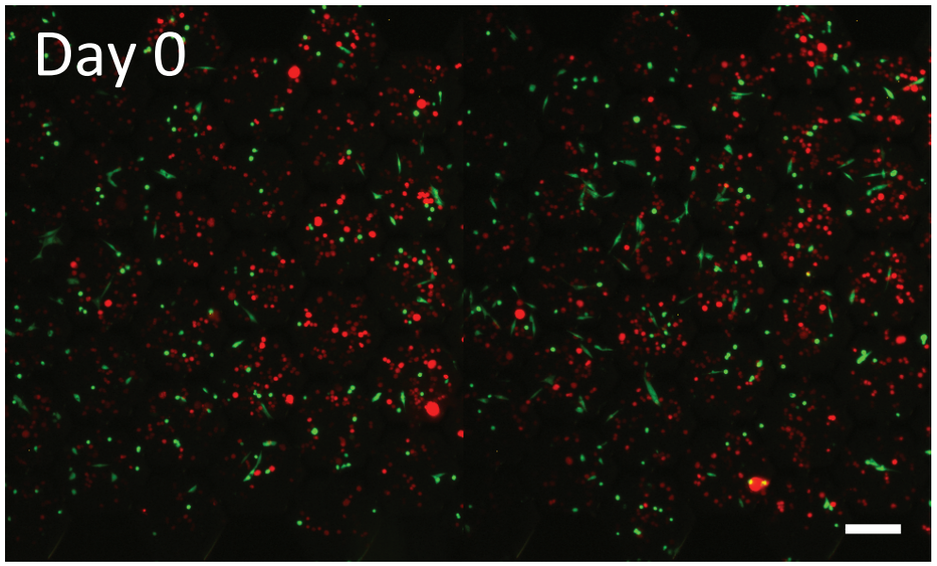
Red: myeloma (8226/RFP)
Green: stroma (HS-5/GFP)

You need 2 "players" when you try to simulate a tumor. The cells act in a communal fashion.

2012: DOX gradient (0-200nM/2mm)

DOX 200nM

DOX 0nM

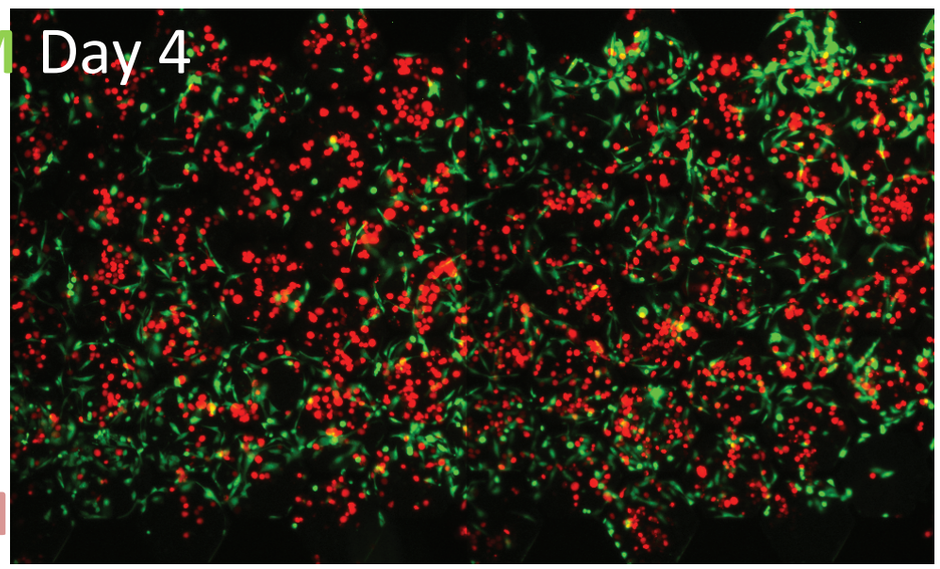


Myeloma
Stroma

White line: 200µm

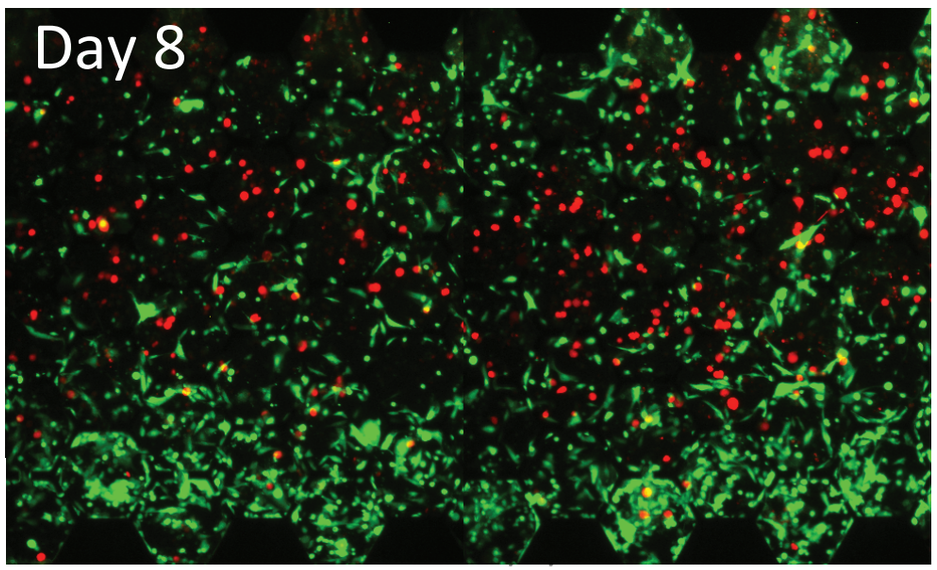
DOX 200nM

DOX 0nM



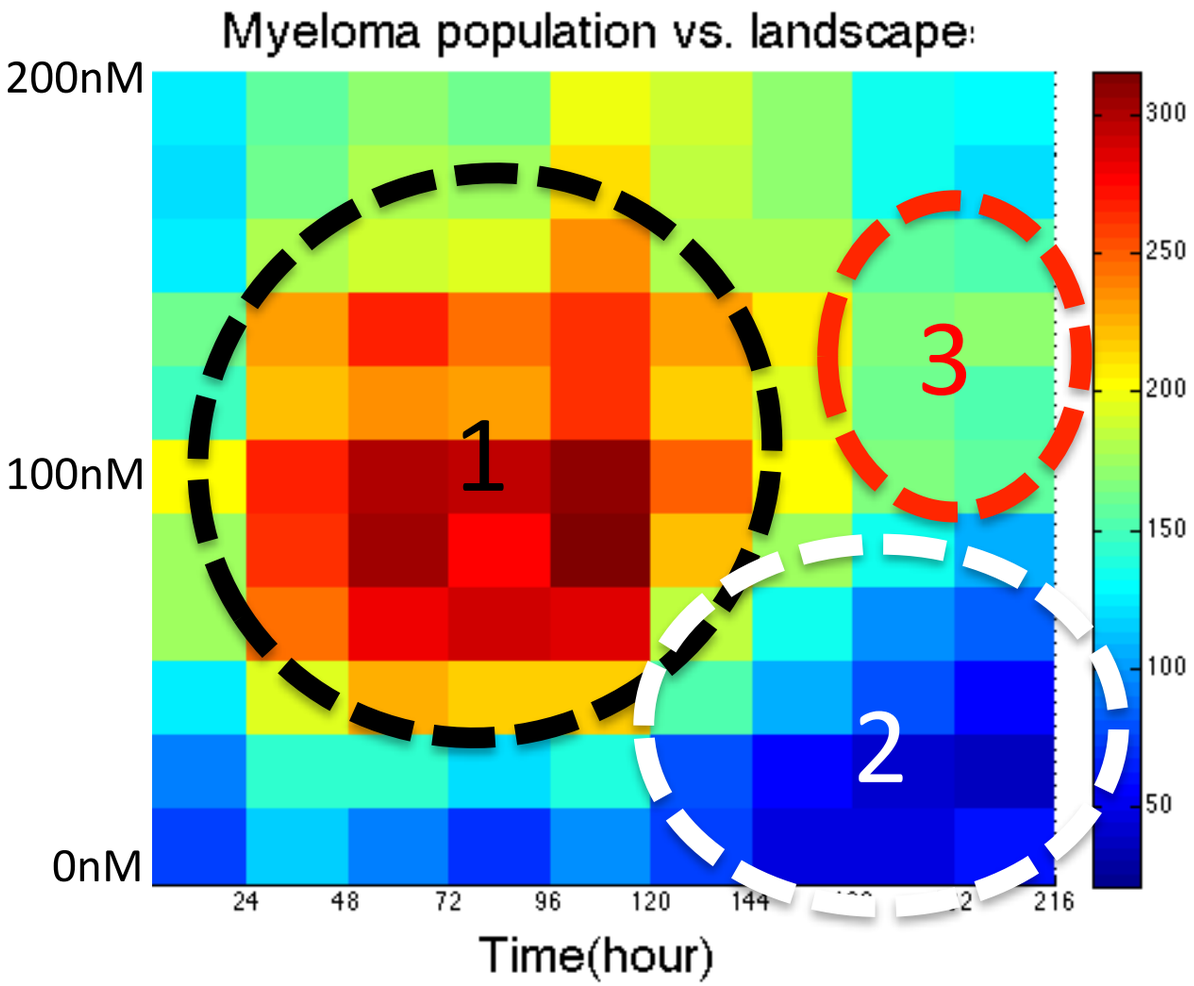
DOX 200nM

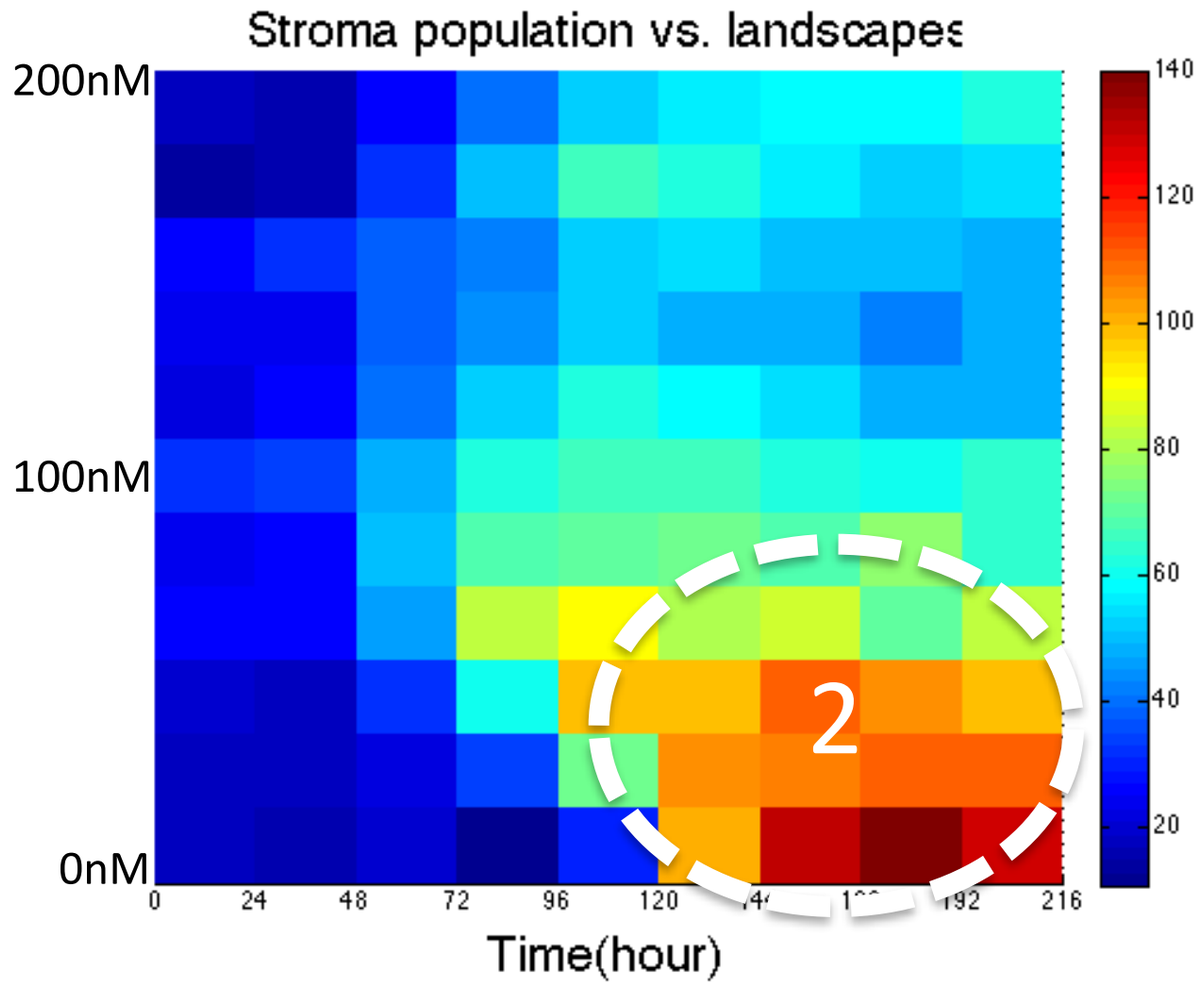
DOX 0nM



Lots of myeloma can bare >100nM drug for > 1 week

DOX gradient (0-200nM/2mm)





1) Myeloma first grew
->stroma "pain-killer" transient effect

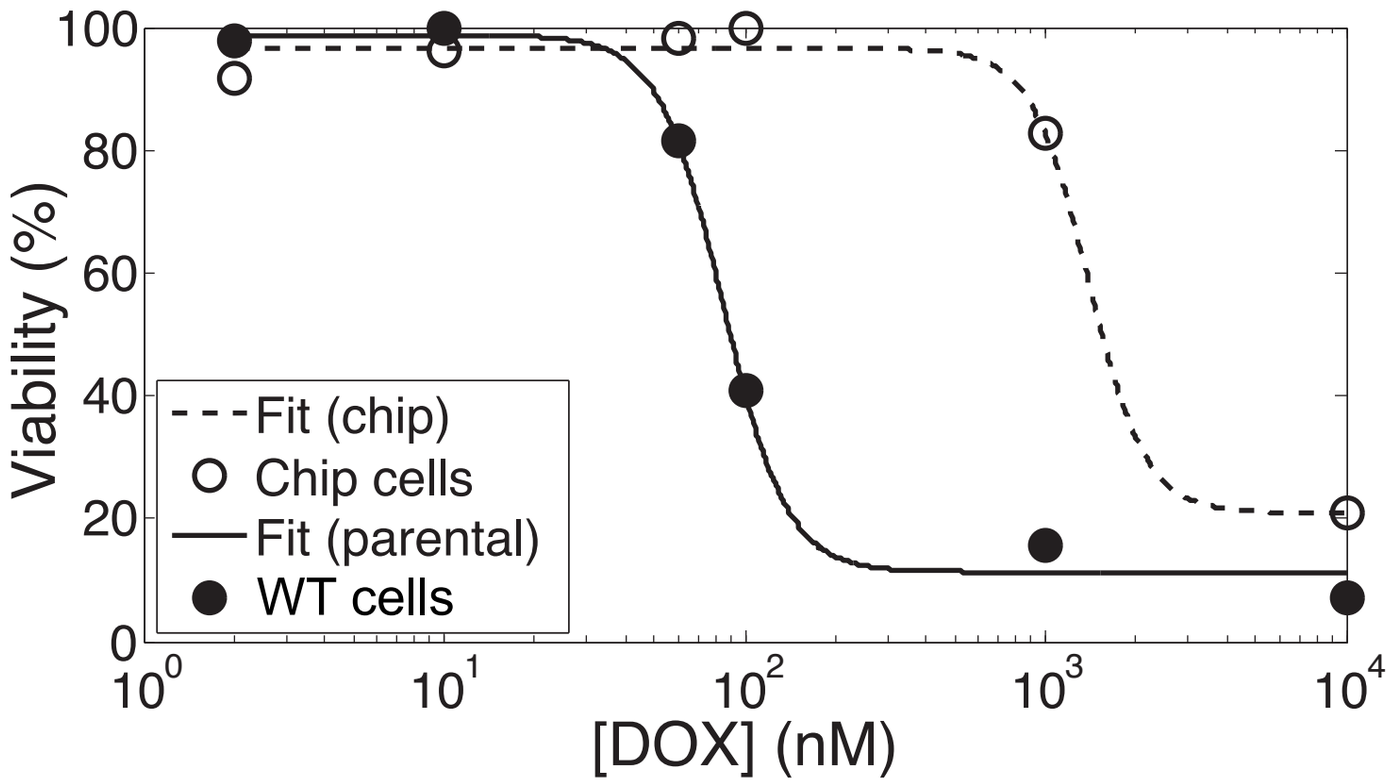
Stroma adhesion inhibits apoptosis signals in myeloma
Ref: Hazlehurst et al, Oncogene 2003

2) Stroma crowds out the myeloma

3) Myeloma is more resistant than the case without stroma

How resistant the cells have become? How fast?

Take cells out of chip (Chip cells) after 288 hours and compare the dose response with that of the parental cells (WT cells)



- Degree of cross-resistance (48-hour exposure) $= \frac{IC_{50}(Chip)}{IC_{50}(WT)} = \frac{1390}{85} = 16.3$
- Using the traditional protocol, it takes several months in tissue culture flasks to develop resistant cell lines (Dalton et al, Cancer Research 1986)

We have built a cancer time machine.

Free Will: Game Theory

There are many classical games, the standard one is the Prisoner's Dilemma.

Just-so Story: Science mag accuses 2 collaborators of falsifying an oncology paper, but doesn't know enough to convict. They offer a deal for future submissions:

0) You defect, your colleague is silent: you get 0 year wait (Temptation, T), he gets -10 years (Sucker, S)

1) Both remain silent (Cooperate): both no submissions for -2 years (Reward, R)

3) Both defect: both get -3 years (Punishment, P)

What is your strategy?

Player 1 / Player 2	Cooperation	Defection
Cooperation	win,win	win++, lose--
Defection	lose--, win ++	lose,lose

Temptation (win++) > Reward (win) > Punishment (lose) >Sucker (lose--)

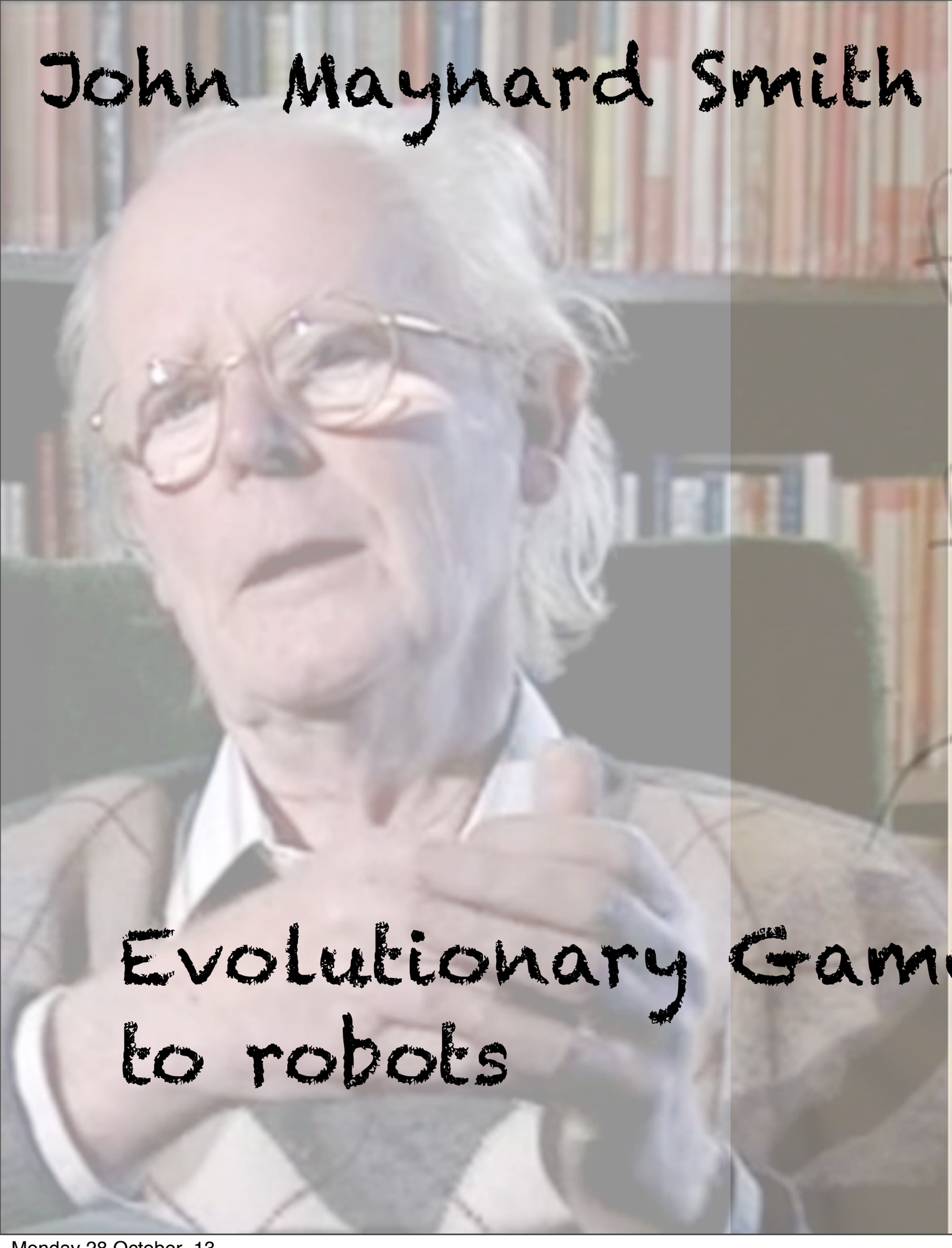
(0 > -2 > -3 > -10)

(this order defines a particular game)

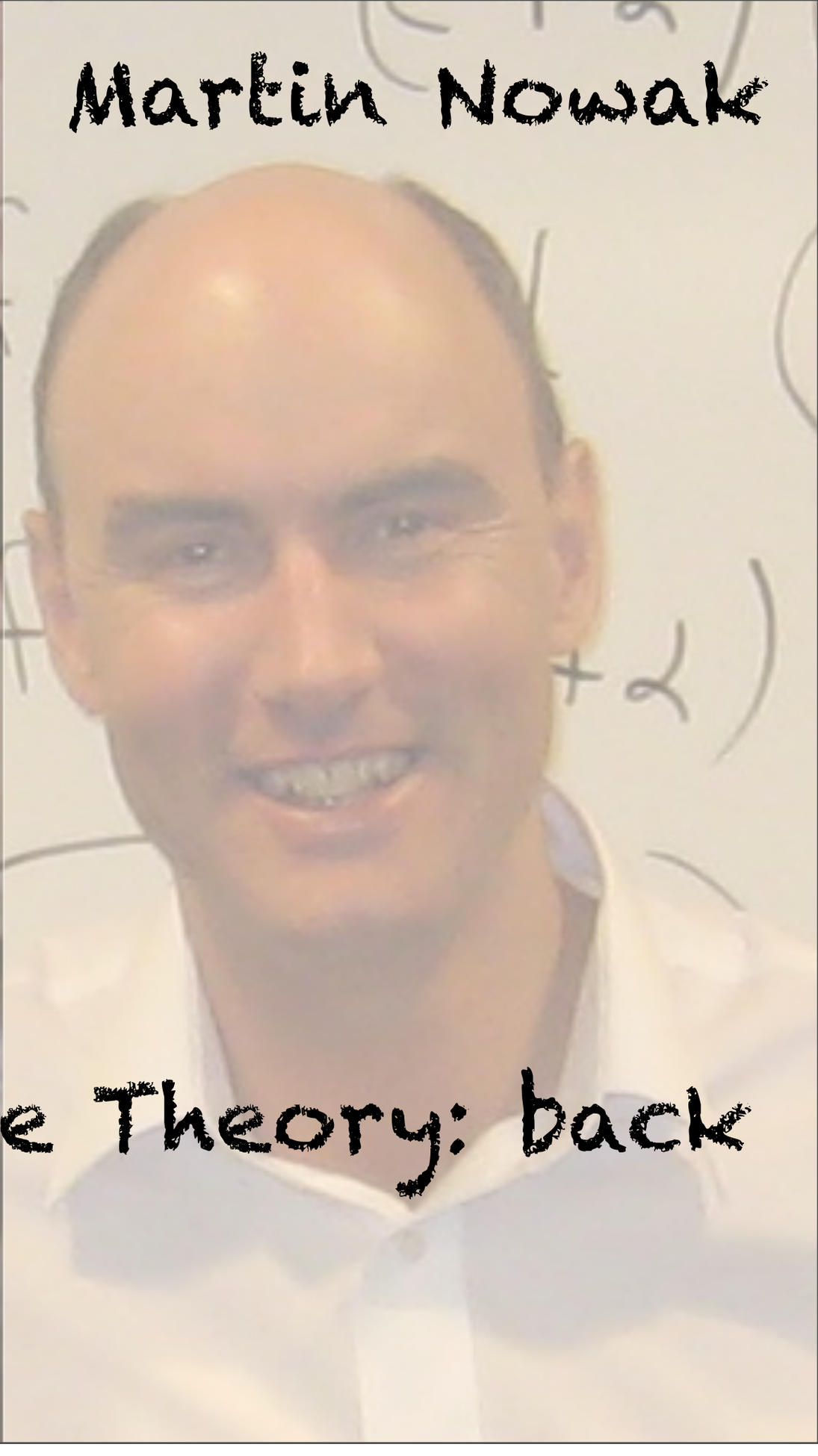
The NASH equilibrium is that point where a change in your strategy only makes things worse for YOU, so both parties defect, even though that is not the rational thing to do. No altruism.

But....it seems to imply sentient beings.

John Maynard Smith



Martin Nowak



Evolutionary Game Theory: back to robots

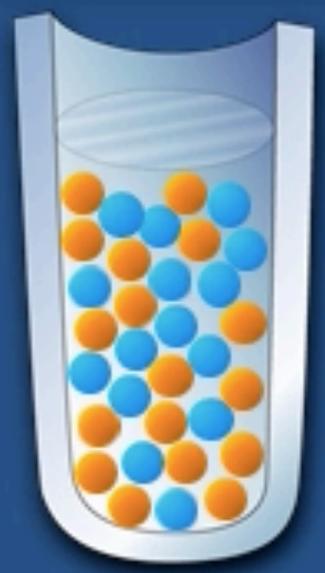
"Classical" game theory implies rational agents with strategies that can change based on their perception of what the competitor will do.

Evolutionary game theory is based at its simplest level on rates of growth (fitness) and how other populations can change that rate.

One need not have sentient beings.

And we finally have a set of coupled non-linear equations to solve.

Collisional population events



$$C \xrightarrow[f_0]{R_C} 2C \qquad 2C \xrightarrow[R/[N]]{R_R} 3C \qquad C + D \xrightarrow[S/[N]]{R_S} 2C + D$$

$$D \xrightarrow[f_0]{R_D} 2D \qquad C + D \xrightarrow[T/[N]]{R_T} C + 2D \qquad 2D \xrightarrow[P/[N]]{R_P} 3D$$

$$\frac{dC}{dt} = \textbf{+1}\ f_0 C + \textbf{+1}\ \frac{R}{[N]}[C]C + \textbf{+1}\ \frac{S}{[N]}[D]C$$

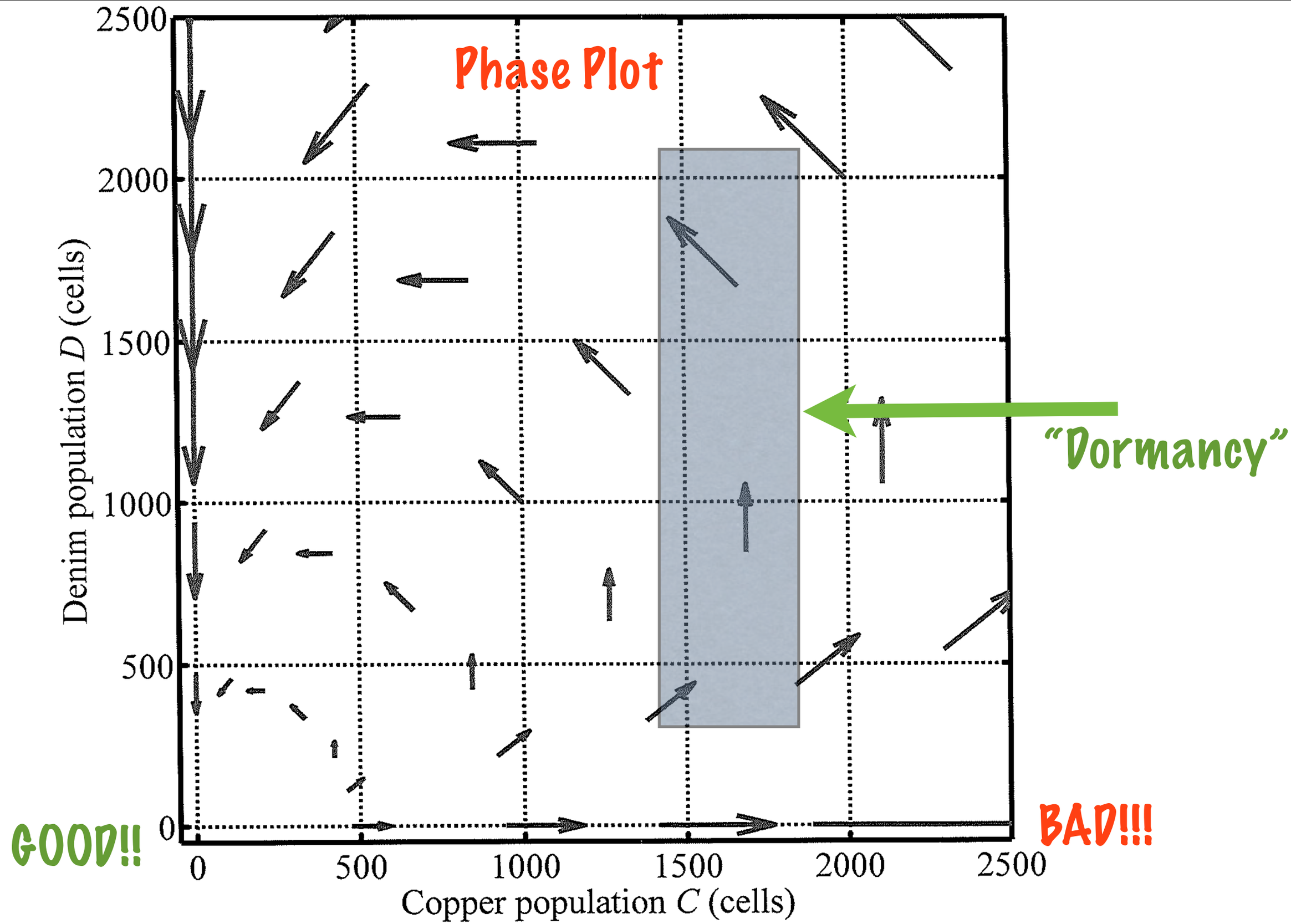
$$\frac{dC}{dt} = (f_0 + Rp_C + Sp_D)C$$

$$\frac{dD}{dt} = \textbf{+1}\ f_0 D + \textbf{+1}\ \frac{T}{[N]}[C]D + \textbf{+1}\ \frac{P}{[N]}[D]D$$

It is possible to construct a phase map of this game (David Liao+ Amy Wu), using Evolutionary Game Theory, but averaged over space right now.

$$\frac{dC}{dt} = (Rp_C + Sp_D)C \qquad \frac{dD}{dt} = (Tp_C + Pp_D)D$$

This is written in the terminology again of the Prisoner's Dilemma, but that's really of no meaning.



There are mathematical tricks to numerically solving these equations.

Smith:

"(1) Evolutionary version of Game Theory not really a requirement that players be rational - it is only required that they have a strategy. The results of the game will test how good that strategy is.

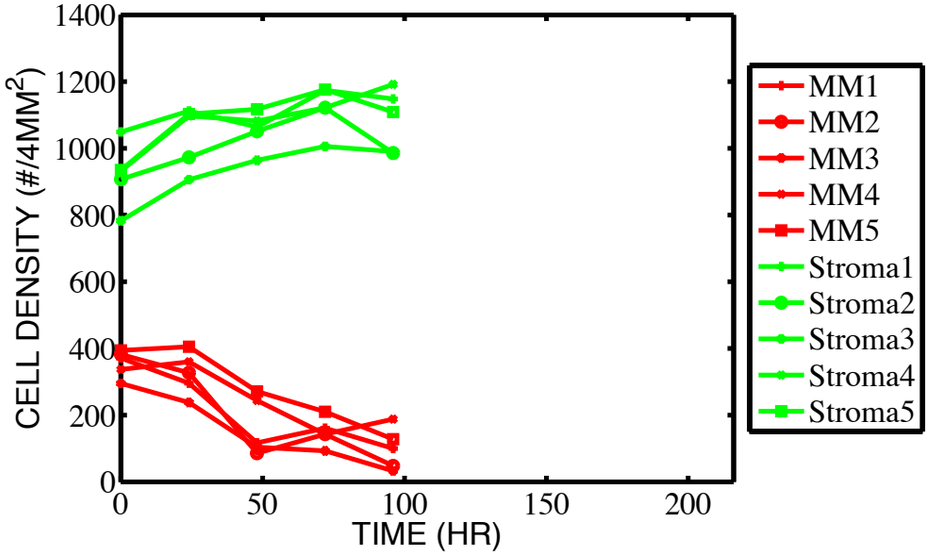
(2) That is what Evolution does - it tests alternative strategies for the ability to survive and reproduce.

(3) Strategies are algorithmic - just like computer programs. (Yes, mathematics and physics).

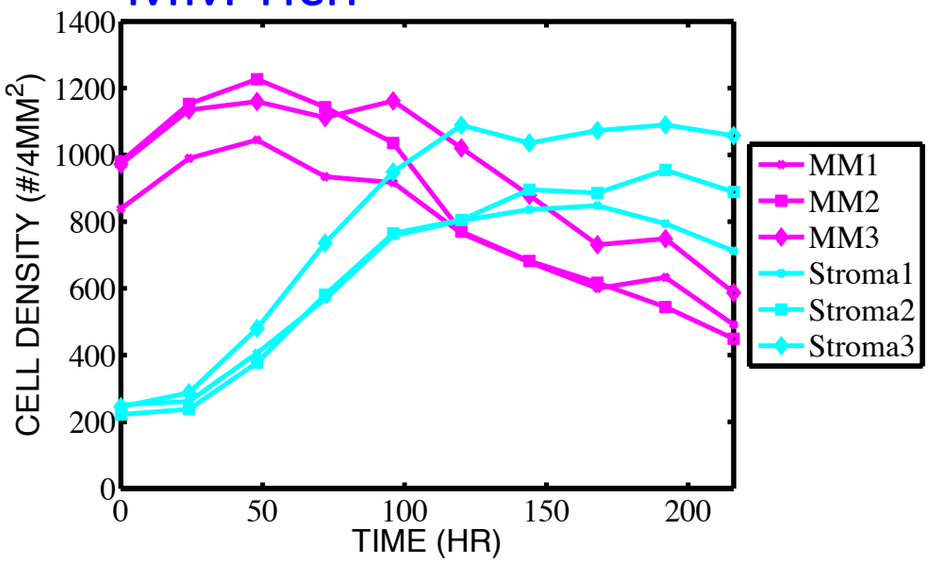
(4) The key point in the Evolutionary Game Theory model is that the success of a strategy is not just determined by how good the strategy is in itself, it is a question of how good the strategy is in the presence of other alternative strategies."

DOX gradient (0-200nM/2mm)

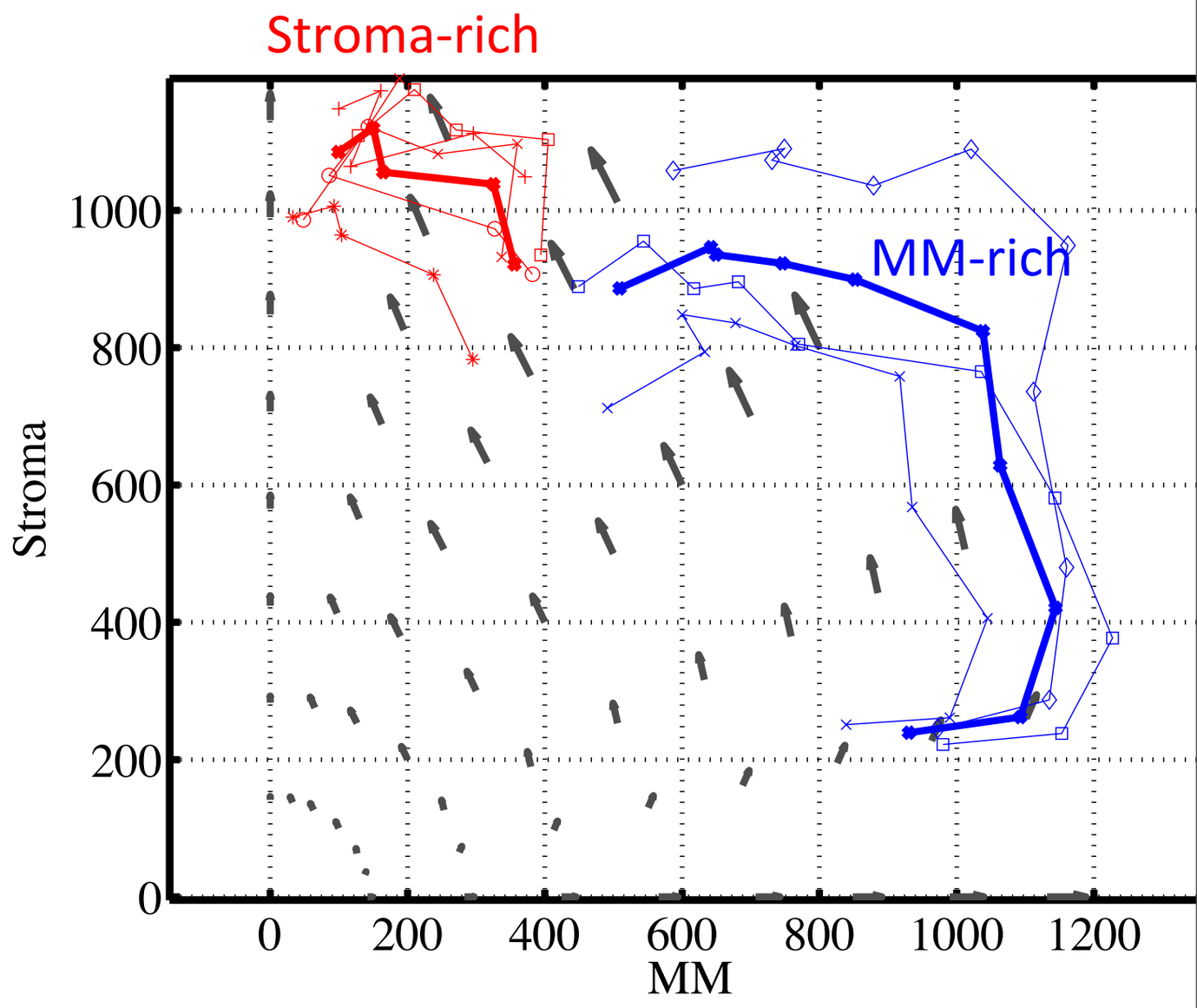
Stroma-rich



MM-rich



Phase portrait



The vector field of arrows is the prediction of the game theory model, the solid lines are the data.

IV. Conclusions

1. Living systems seem to have more behind them than a purely robotic
system, but we don't understand where that line is drawn.

2. Seemingly rational behavior at the microscopic level can be reduced to mathematical understanding of the behavior.

3. Yet, some microscopic phenomena seem to elude a purely mathematical formalism, such as the emergence of cancer.

4. Many mysteries lie between today's robots and how living systems operate.


THANKS!