Nanobiology, Nanochannels and Nanofluidics: The Unholy Nano-Trinity

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NBTC
Nanobiotechnology Center

Comment: People have been coming up to me continually about their students that have worked or are working in my lab, it is amazing to me how many connections I have to Asia!

To all of you:

谢谢!

Outline of this talk:

I. A little biology about the importance of both gene sequence and transcription factors in evolution.

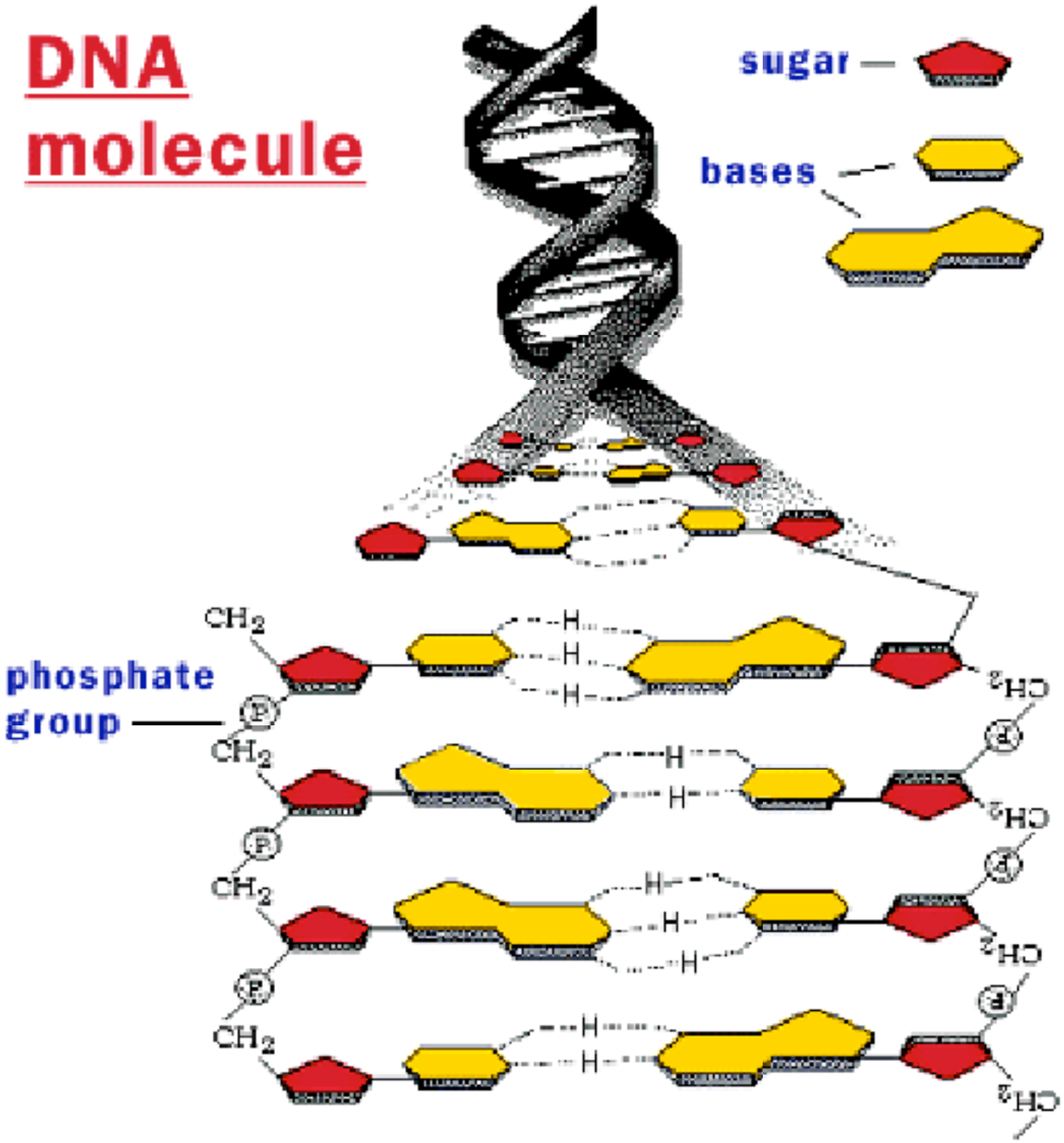
II. A little polymer physics about dsDNA

III. Some recent work in our lab to use nanochannels to analyze dsDNA at high spatial resolution.

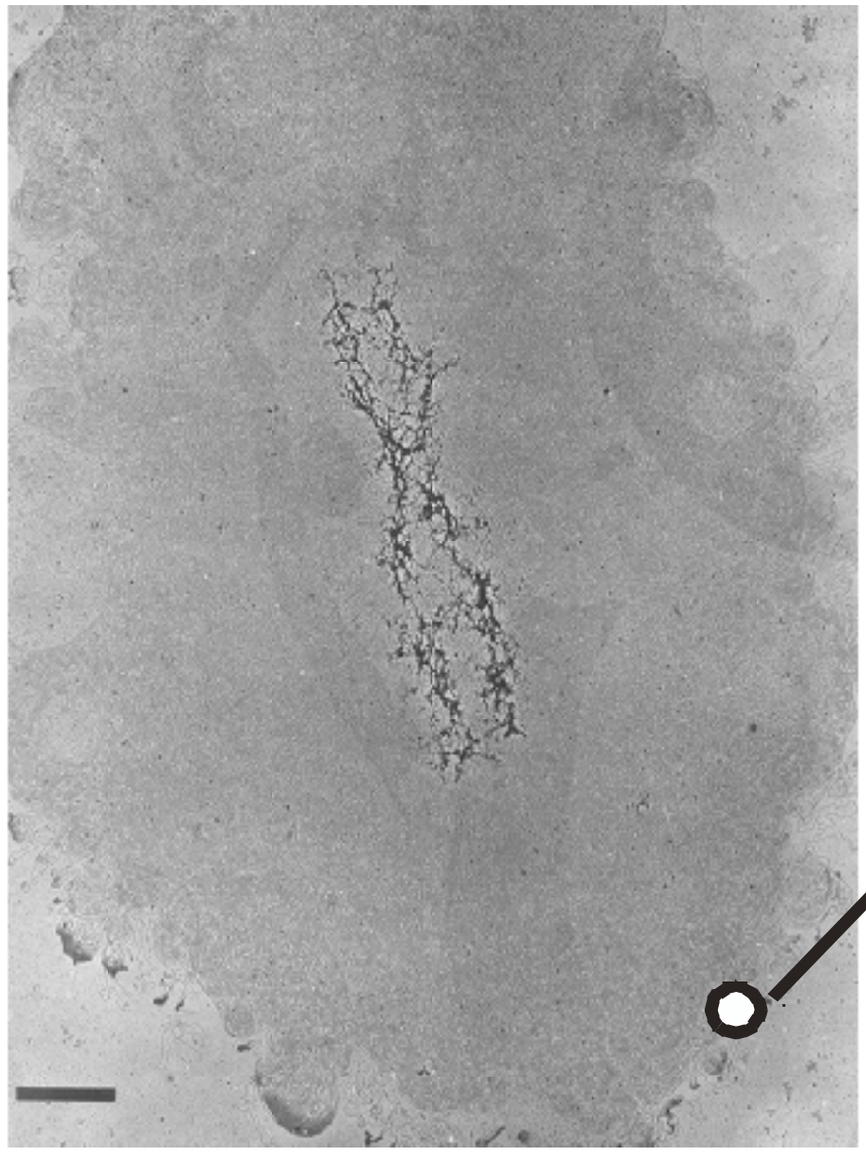
IV. The Anti-Lotus Leaf Effect.

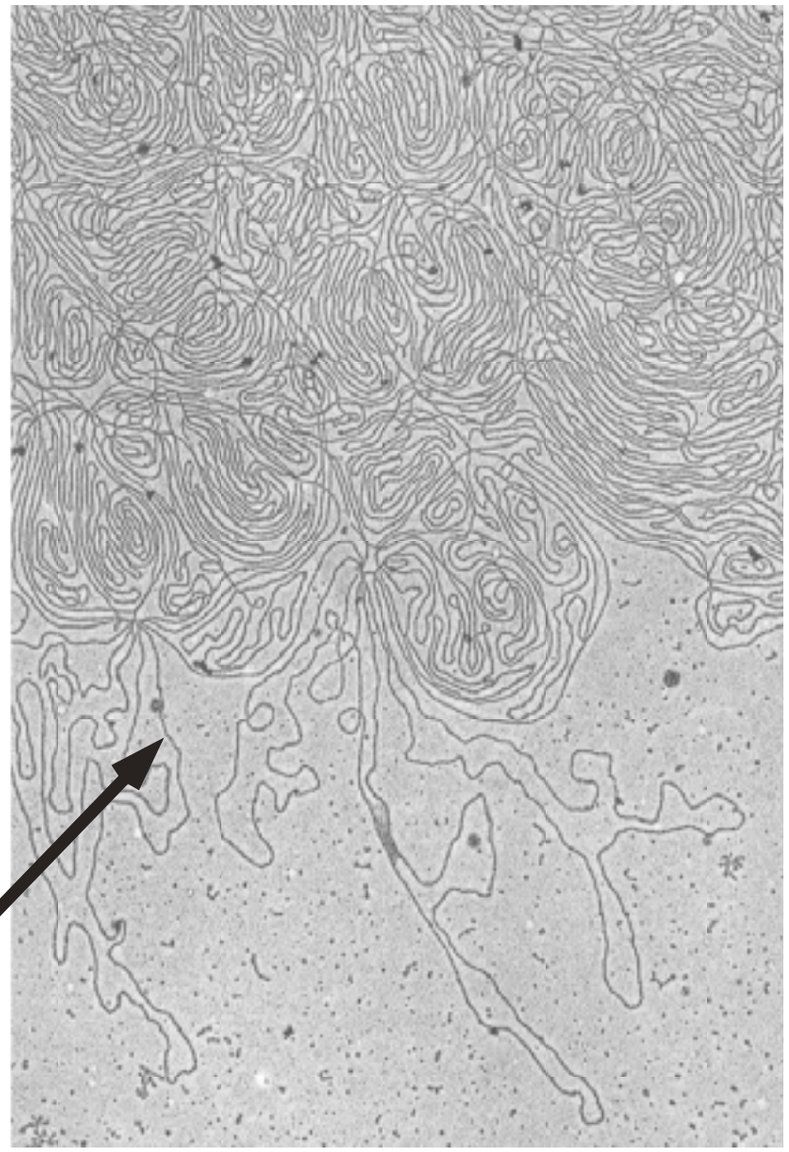
I. A little biology about the importance of both gene sequence and transcription factors.

DNA molecule



DNA contains the code for you. There are about 3 billion basepairs in the human genome, or about 1 meter of DNA.





Sequencing the human genome was an absolutely stupendous problem.

earth

country

state

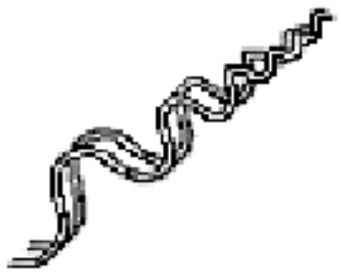
city

people

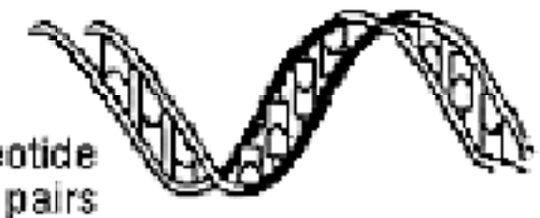
cell

chromosome

chromosome fragment



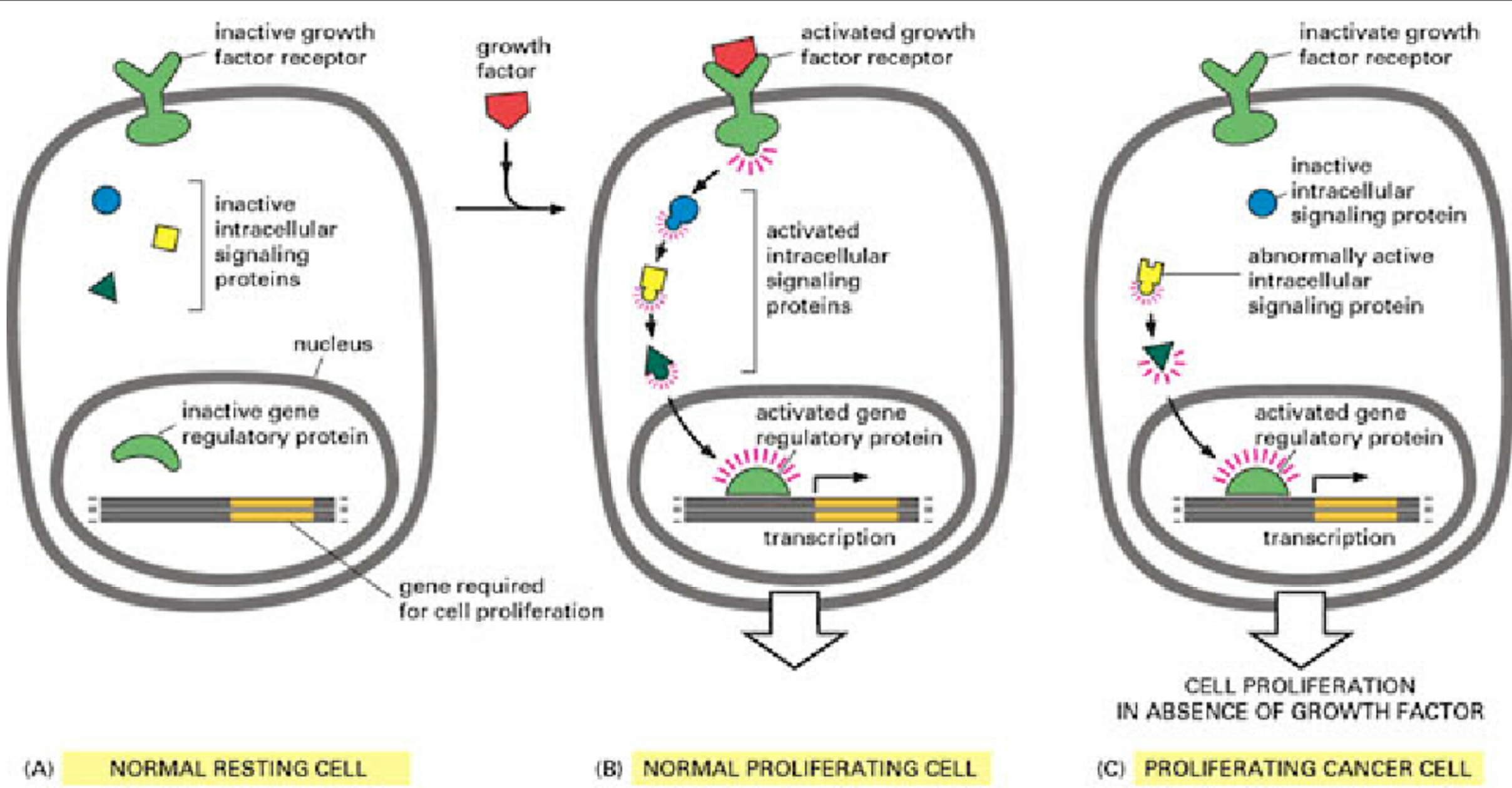
gene



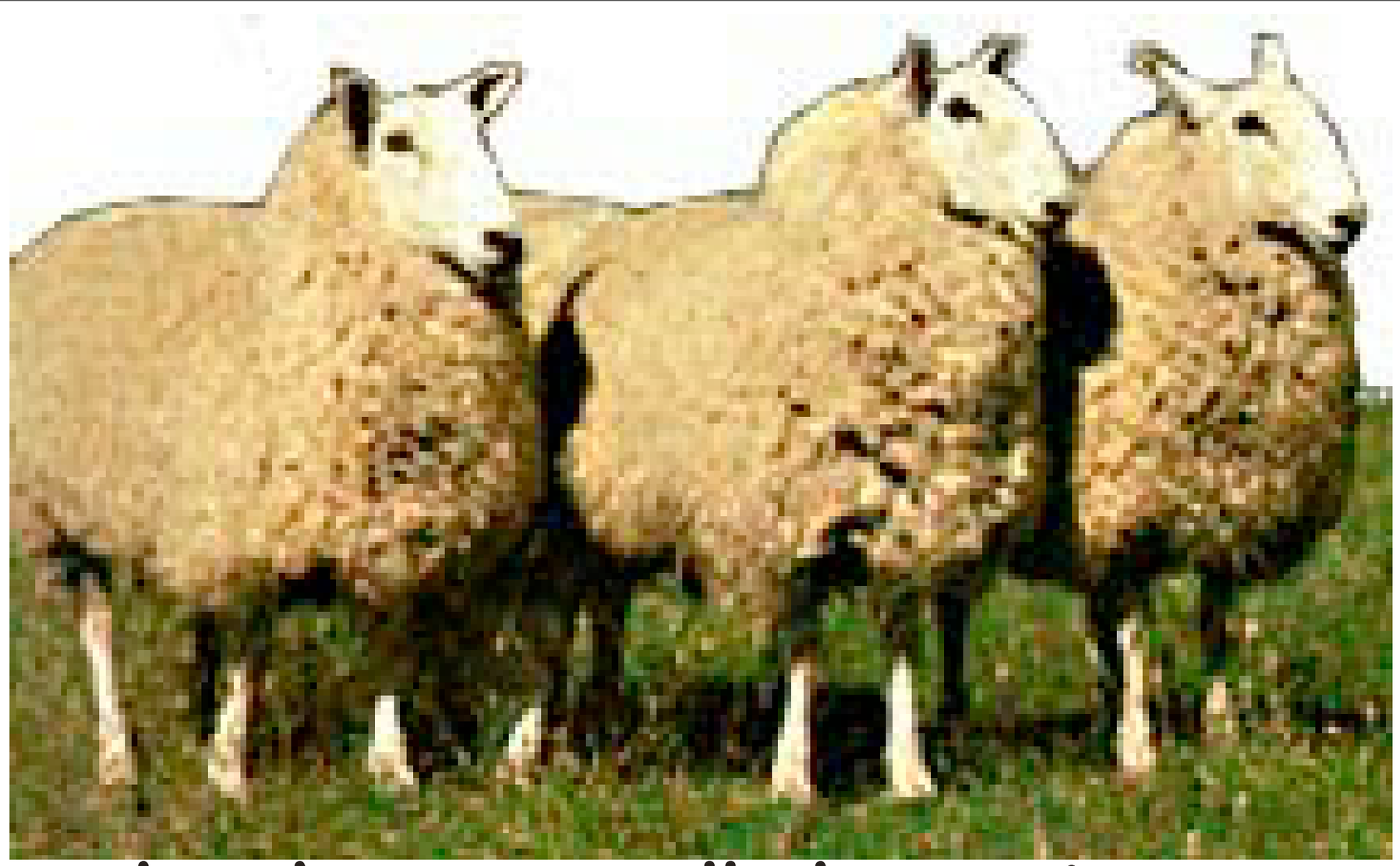
nucleotide base pairs

Comparative Scale of Mapping

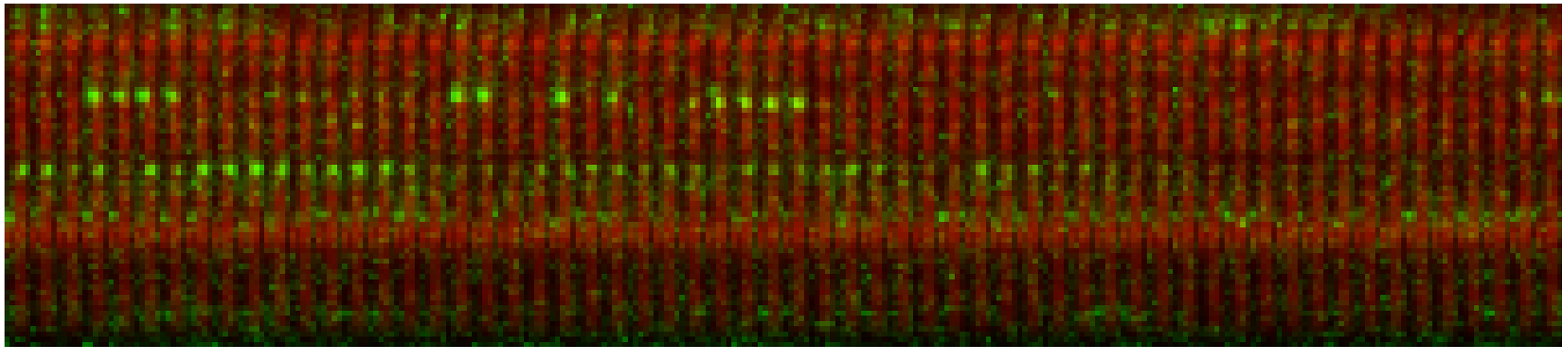
Nearly all the 10^{14} cells in your body contain the SAME genome. Probably each cell has a slightly different genetic sequence. But, it isn't the genome that makes each cell different.

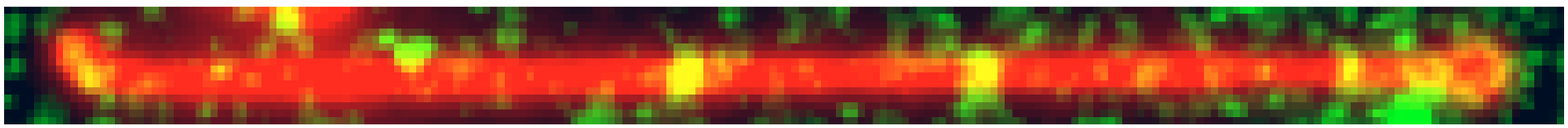


Promoter and repressor proteins, which bind to specific parts of the genome, control expression. The CYTOPLASM of the cells contains the DYNAMIC control information. The DNA is the ROM, the proteins are the OS that makes a liver cell a liver cell.



That's why there was all the excitement about Dolly: they took the NUCLEUS from a fully differentiated cell in the udder, put it into the egg cell of another sheep which presumably had the right protein content to reset the clock, and transformed a mature cell into a "fertilized" egg.





Yan Mei Wang: single transcription factors binding to non-specific sites

What then is our Quest? (our favorite color is black)

0) FIND unusual cells, maybe amongst billions

1) take the entire genome from a single cell.

2) do precision cuts on the genome at known restriction sites.

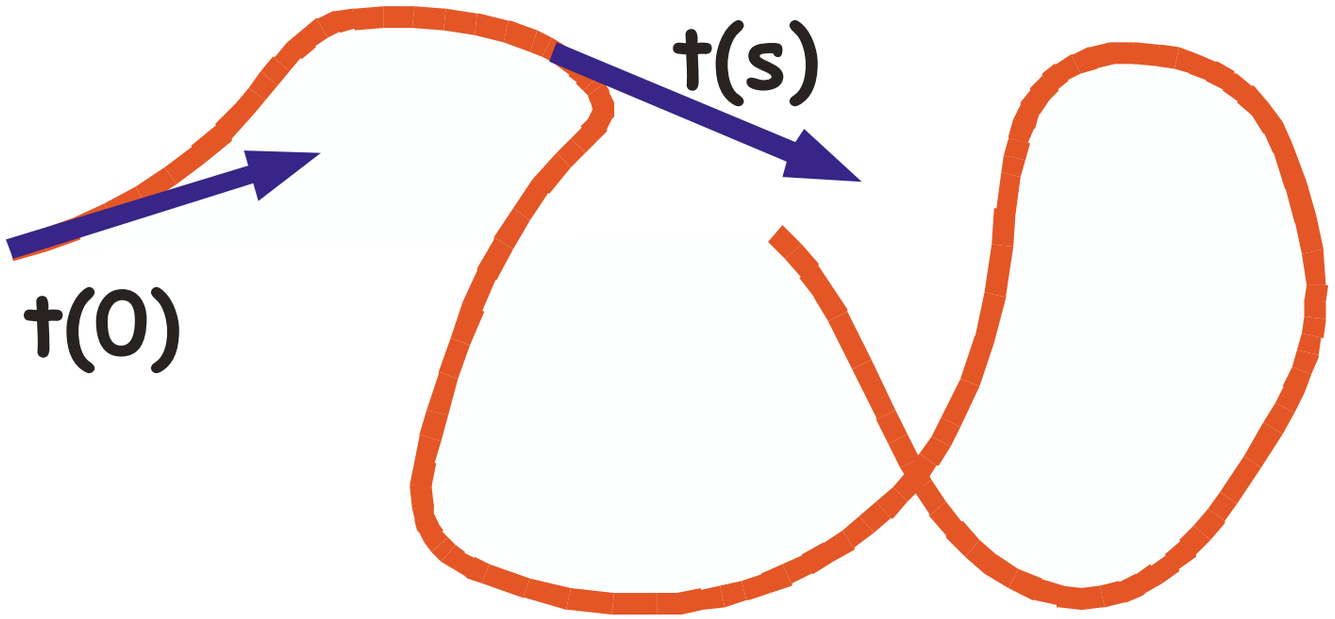
3) determine lengths to 10 nm (30 bases) in a "few" minutes optically (haplotype mapping), to determine basic changes in genome

4) Map the change in control sequences

5) Convert Francis Collins into an agnostic again.

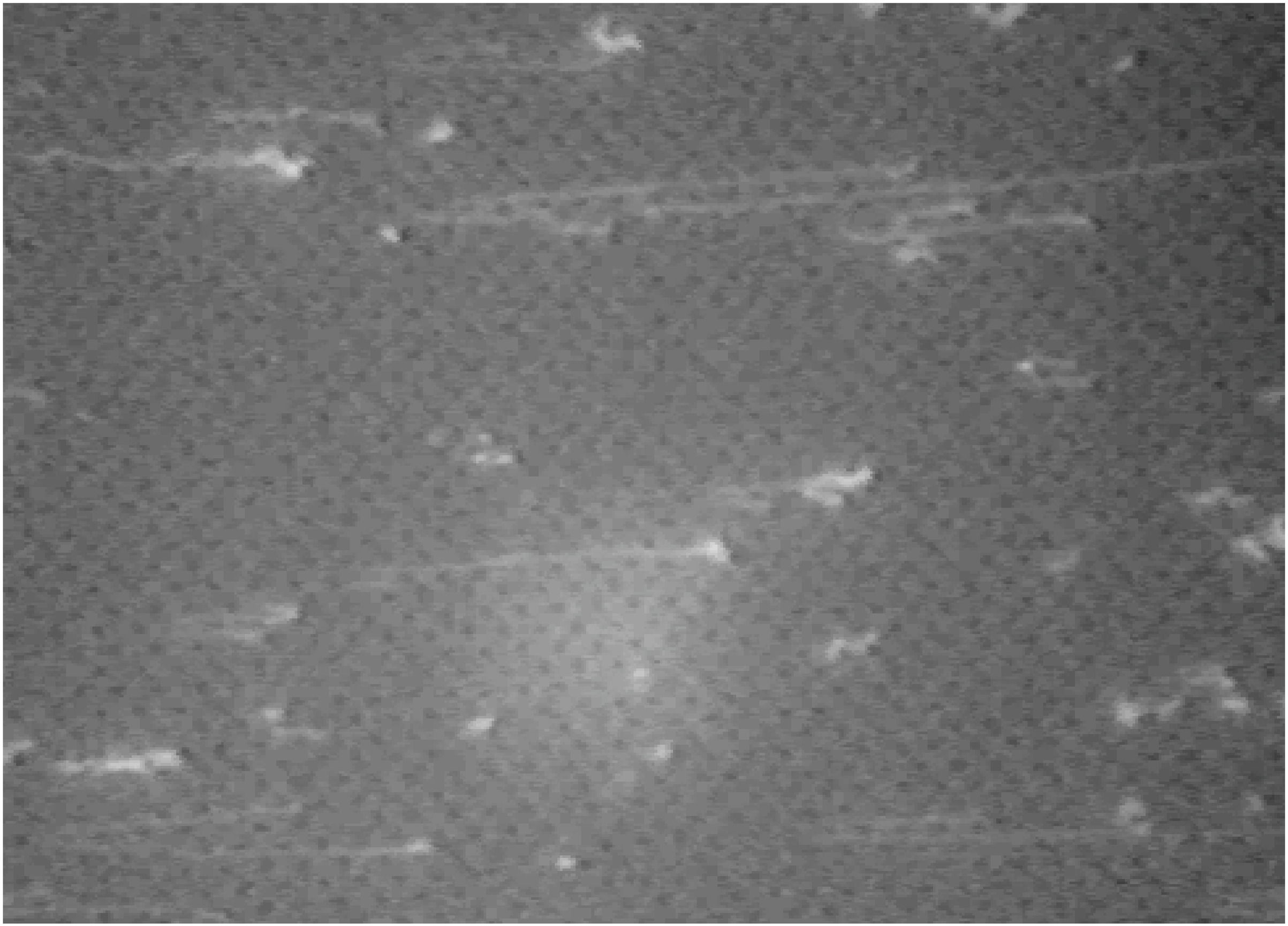
II. A little polymer physics about dsDNA

A very important concept here: the persistence length "p" of a flexible polymer. Basically, it is a measure of how far you move along an arc before thermal energy bends the polymer randomly.



movie:wayne

<t(0) t(s)> = exp(-s/p) p = 600 Angstroms = 200 bp (double-strand DNA)



What's moving these molecules here? Although we are using an applied electric field E, it isn't F= qE, a common misconception.

Biomolecules like DNA have to be in a saline buffer, a plasma of neutral net charge. The ions cancel out the negative charge of the DNA backbone.

Famous equation: Poisson-Boltzmann:

$$\nabla^2\psi = \frac{zen_o}{\varepsilon\varepsilon_o}\left[e^{\frac{ze\psi}{k_bT}} - e^{-\frac{ze\psi}{k_bT}}\right] \quad (1)$$

$$\nabla^2\psi = D^2\psi \quad (2)$$

$$D = \left[\frac{2z^2e^2n_o}{\varepsilon\varepsilon_o k_BT}\right]^{1/2} \quad (3)$$

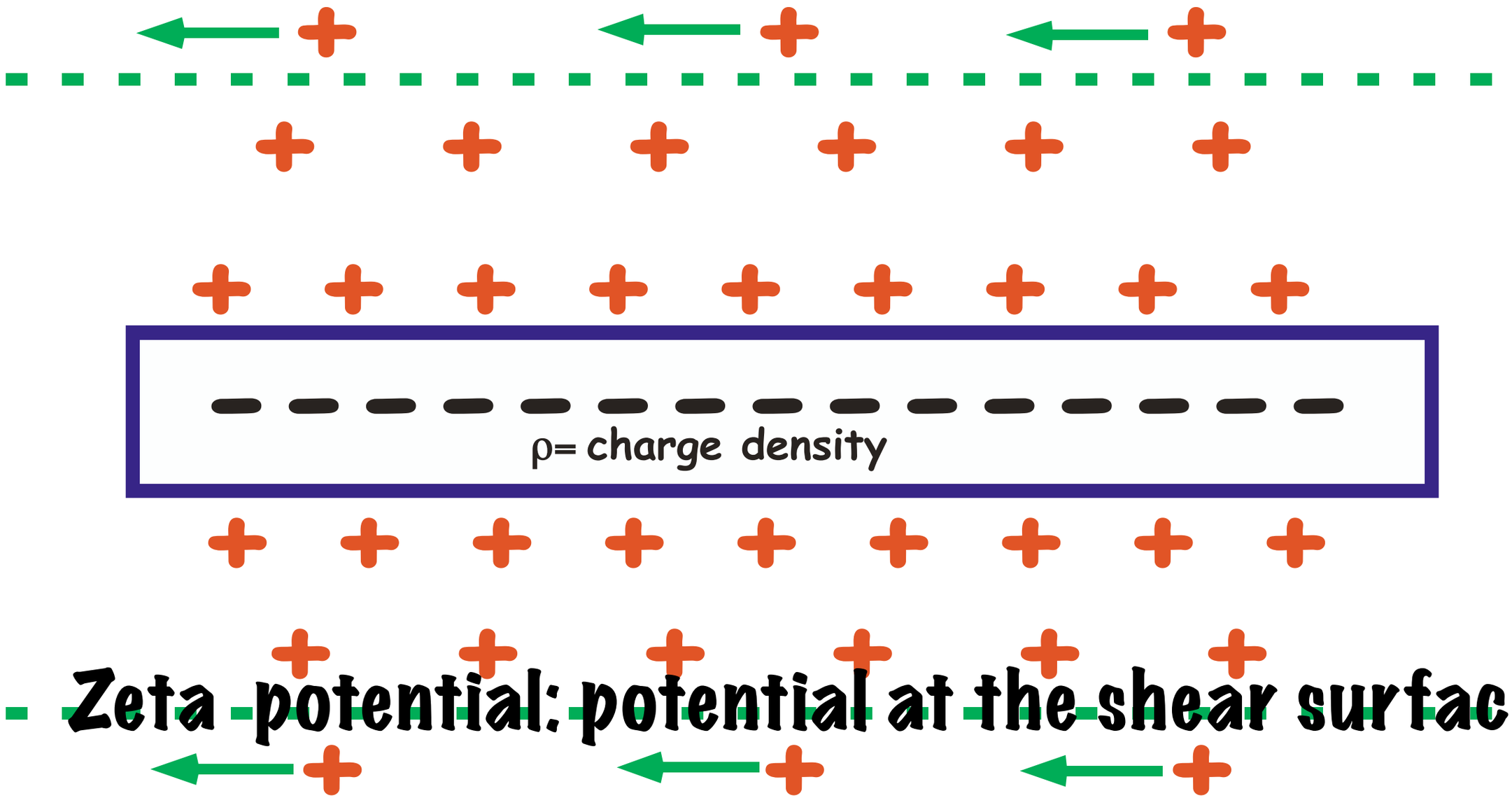
For typical biological salt concentrations, n_o is about 100 mM and D is about 0.2 nm, so the shielding range is quite short: molecules look neutral. Even in the presence of an electric field, the transport is strongly influenced by hydrodynamics.

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

$$m\frac{d\vec{v}}{dt} = \vec{F}$$

The infamous Navier-Stokes Equation, a lifetime of work can easily be invested in studying this.

Electrophoresis: really hydrodynamics because water is an isulator. The moving ions pull molecules along via hydrodynamic coupling.



- Zeta potential: potential at the shear surface

The result is that the polymer is "free draining" and the parts are hydrodynamically decoupled from each other.

Free-draining is a BIG DEAL if you want to fractionate polymers. In the NS Equation if you move an object the hydrodynamic fluid flow reaches out to very large distances, but NOT if free-draining happens, as in electrophoresis.

This is why gels were invented: in the free draining approximation all polymers have the same electrophoretic mobilities and you can't separate as a function of length in bulk solution. You need to rub up against something and add a length dependent force.

This is why I first started making obstacle arrays using microfabrication, although I didn't know it at the time since no physicist knows hydrodynamics.

There is another problem however.

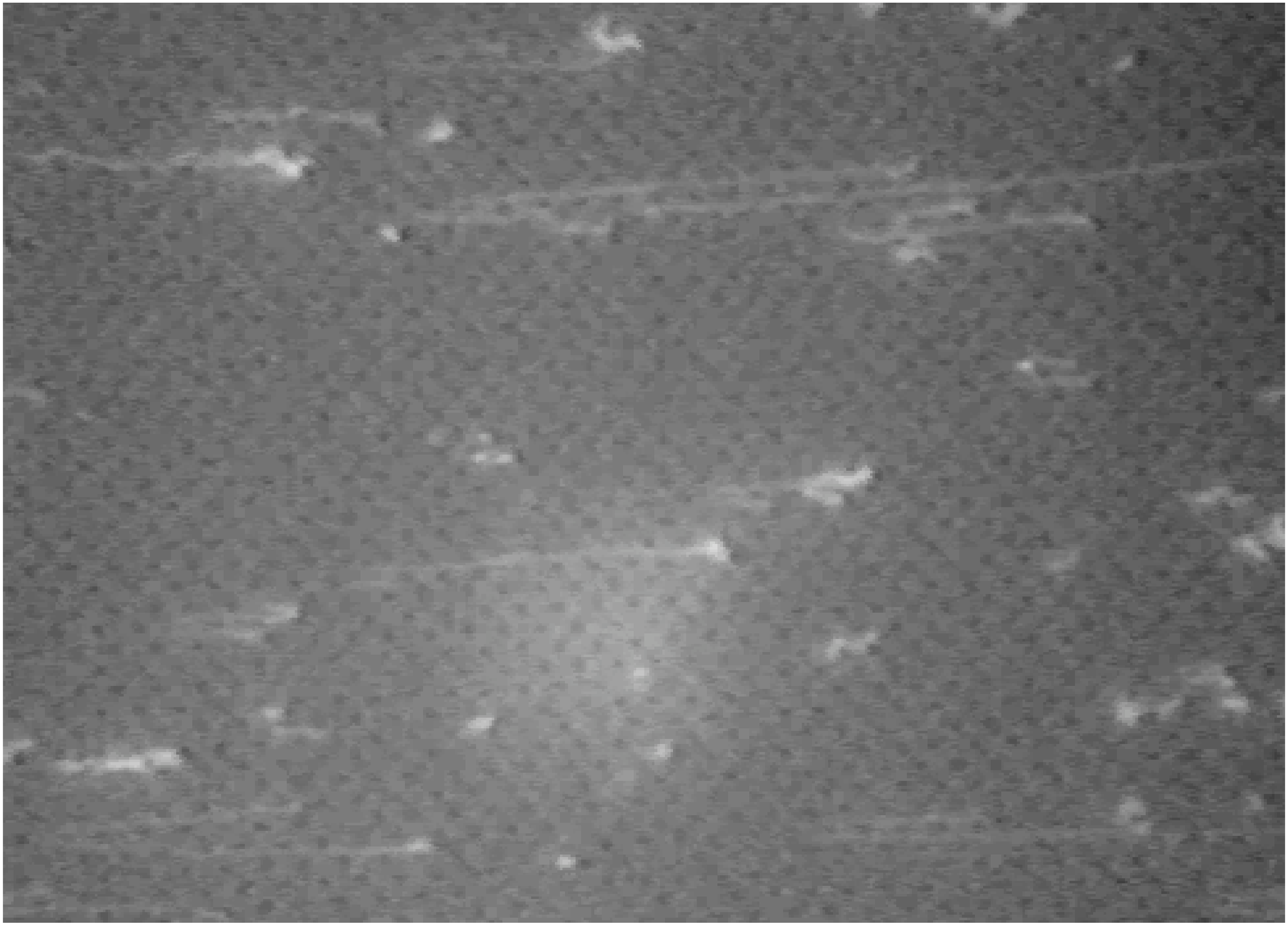
Genomic length DNA gets really stretched out in weak fields because the persistence length is actually pretty big! Young's modulus about the same as nylon.

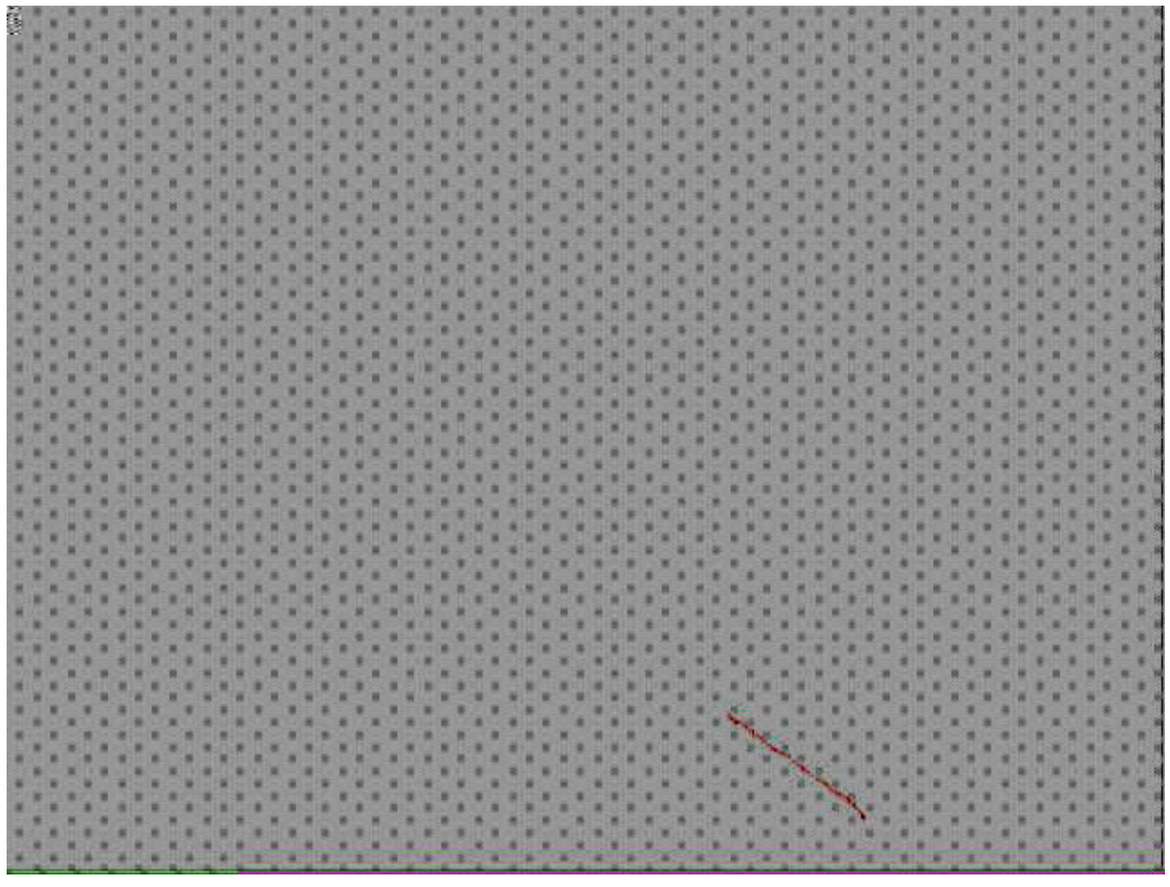
$$Rz = \kappa \ln[\sinh(L/\kappa)/(L/\kappa)]$$

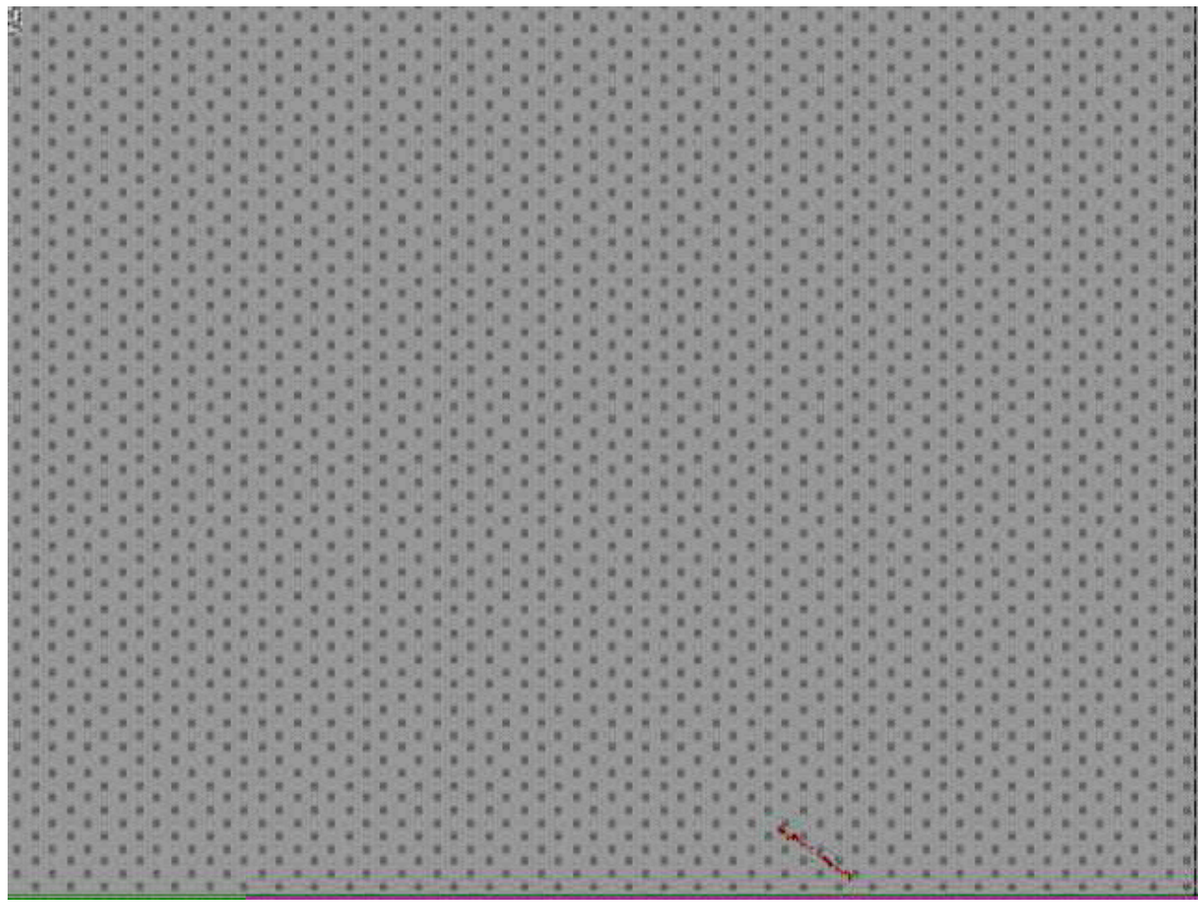
Where L= total length of the polymer, made of N pieces of length 2p (p=persistence length of the polymer) and:

$$\kappa = 2\lambda pE/kbT$$

λ = charge/length of the polyelectrolyte.







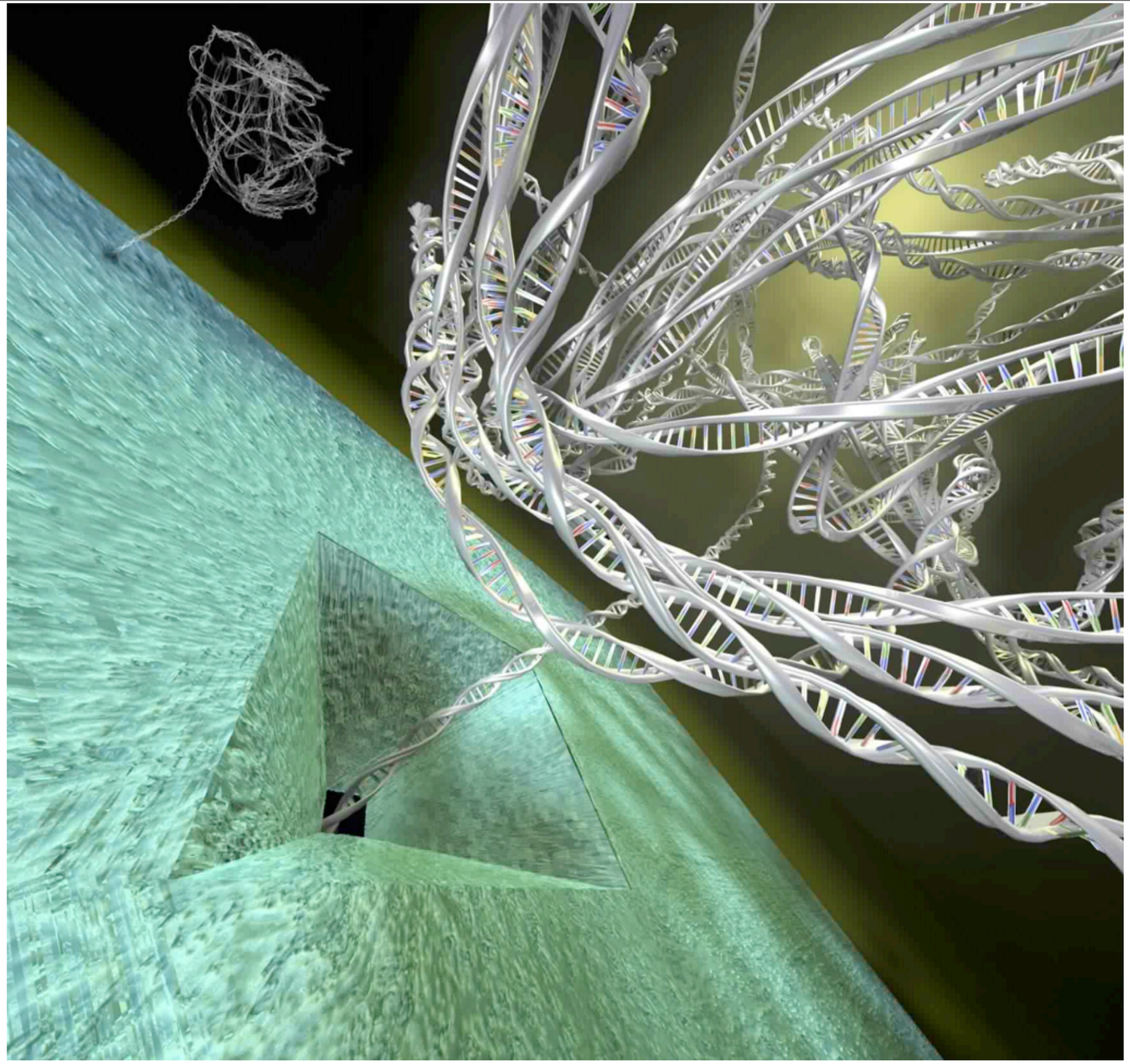
I have worked with some very smart people about ways around this fundamental problem, but I won't talk about that today. The point here is: the physics you don't know always bites you in the ass.

III. Some recent work in our lab to use nanochannels to analyze dsDNA at high spatial resolution.

"SEQUENCING AND RESEQUENCING THE HUMAN GENOME": A RECENT MEETING AT THE NHGRI?.

THERE WAS A CLEAR CONCENSUS (WELL DUH!): WE NEED TO DROP THE PRICE OF SEQUENCING A LARGE GENOME FROM ABOUT $100,000,000 TO $1,000: A DROP IN 5 ORDERS OF MAGNITUDE.

HOW CAN WE DO IT? WILL THE NHGRI SCREW UP?



http://www.mb.tn.tudelft.nl/image_gallery/big/dnatranslocatie_big.jpg

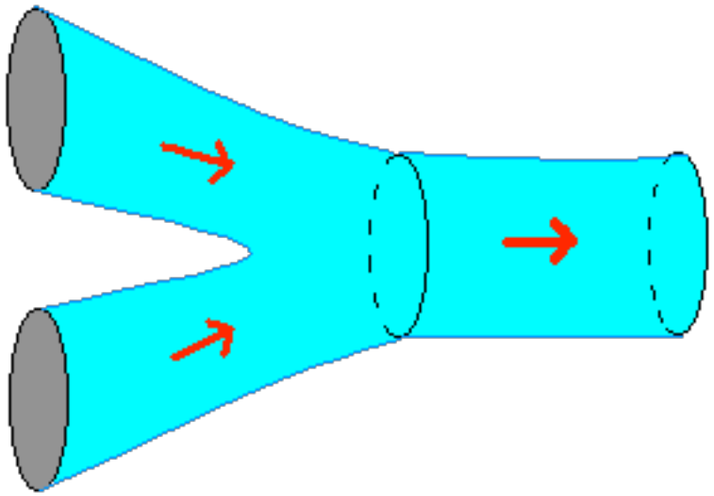
We decided to make nanochannels to align genomic length DNA molecules to their "full" contour length.

"Full" elongation requires nanofabrication of channels whose width is less than the persistence length of dsDNA, which is about 50 nanometers.

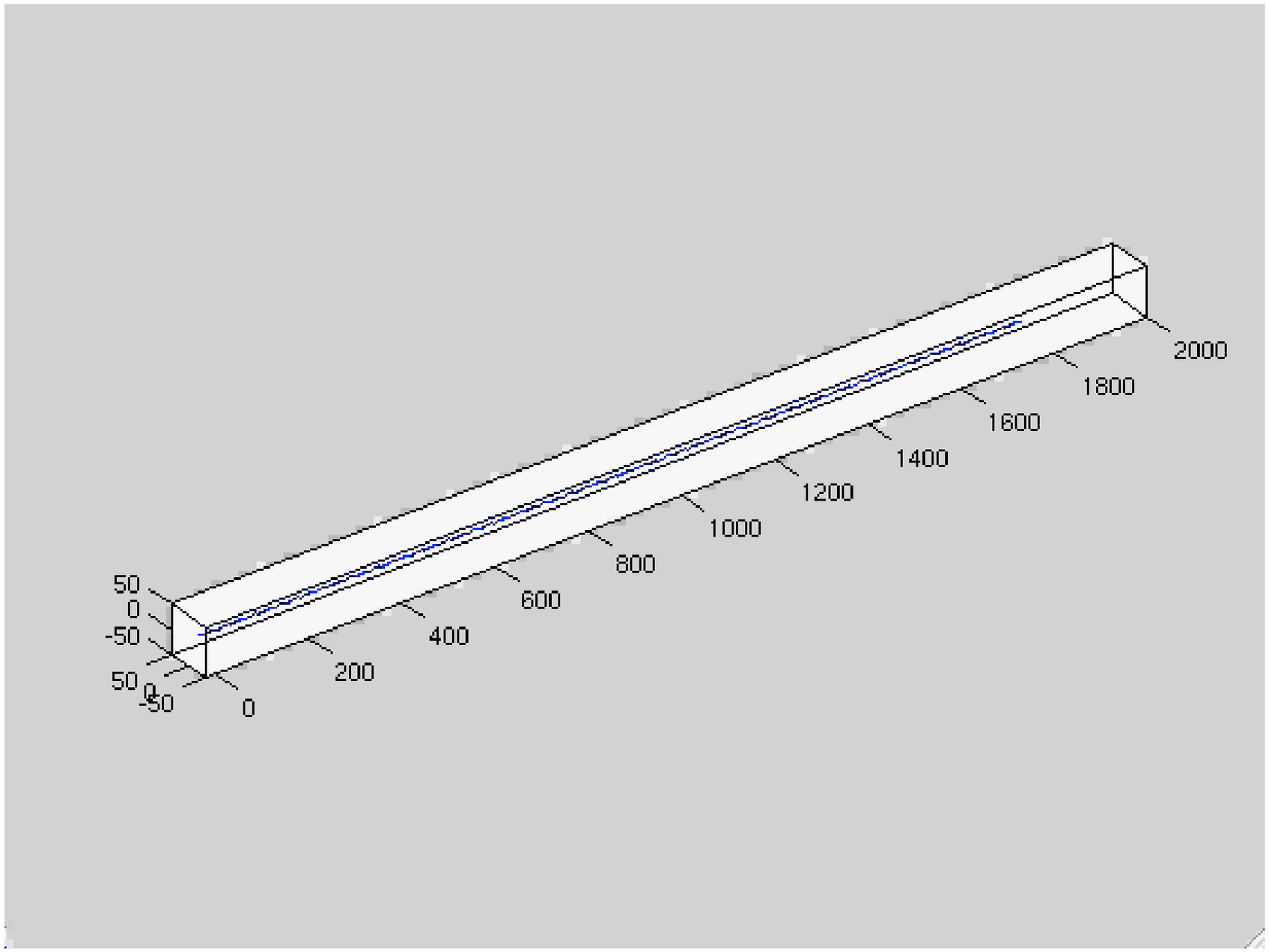
E-beam lithography: Yes, but very expensive, time consuming, hard to make massively parallel. E-beam is bankrupting me in fact.

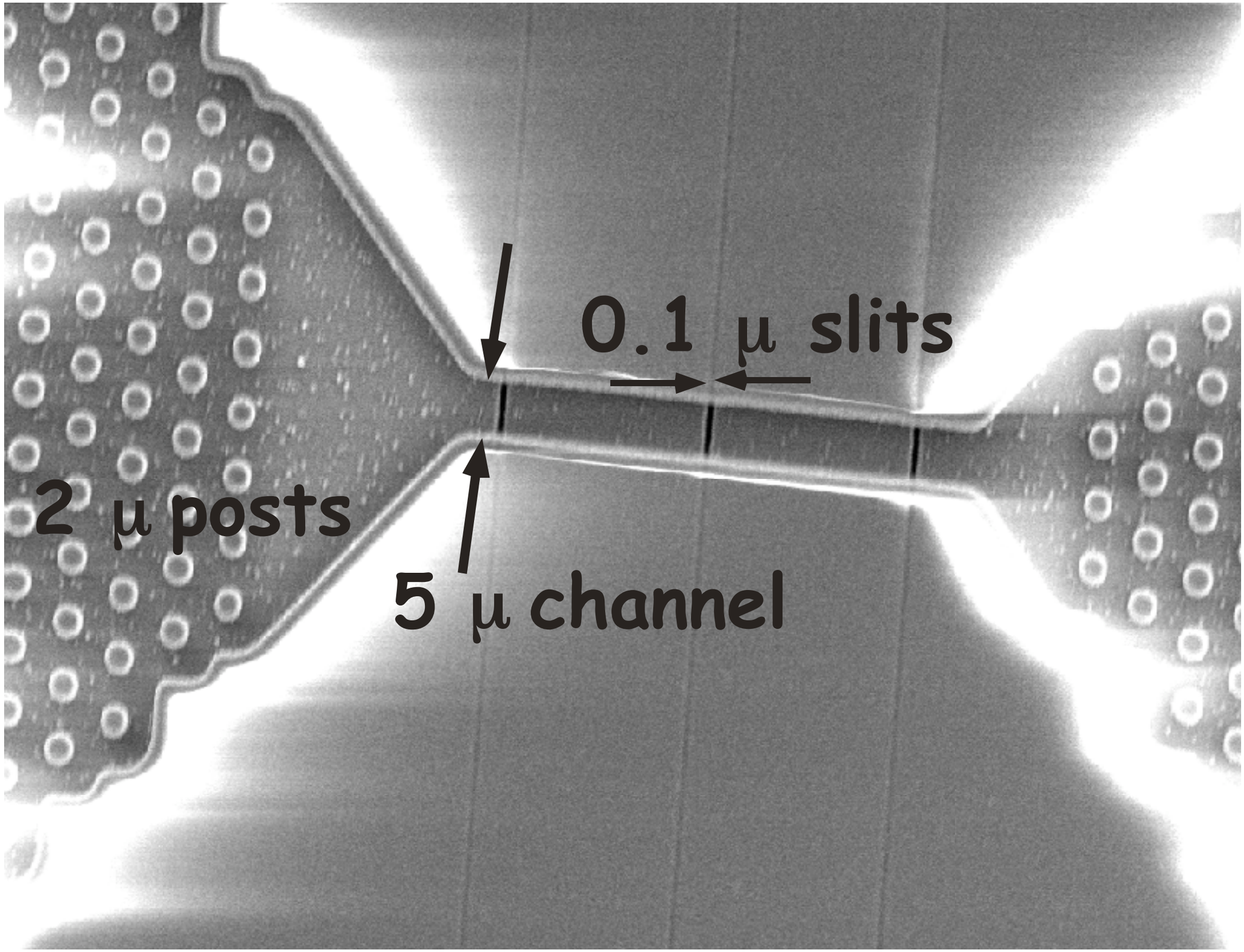
Focused Ion Beam milling: Hard to get below 50 nm

Nanoimprint interference lithography (Chou)

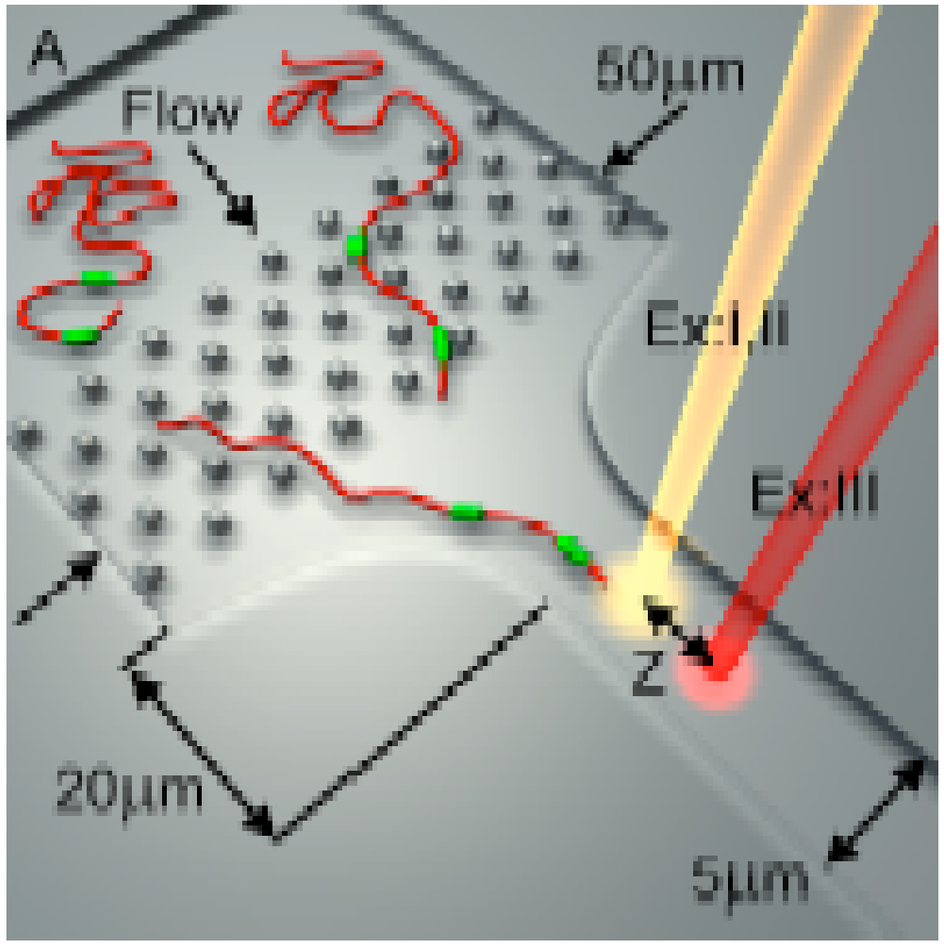


String theory spreads interactions out in another dimension, kills the singularities.





Jonas Tegenfeldt, great post-doc



US Genomics, commerical scanner. My experience with US Genomics has taught me that Universities should stay the hell out of patenting and launching companies, or hire better lawyers.

There are 3 companies based on my ideas right now, they are rich and I am poor. Where is the justice?

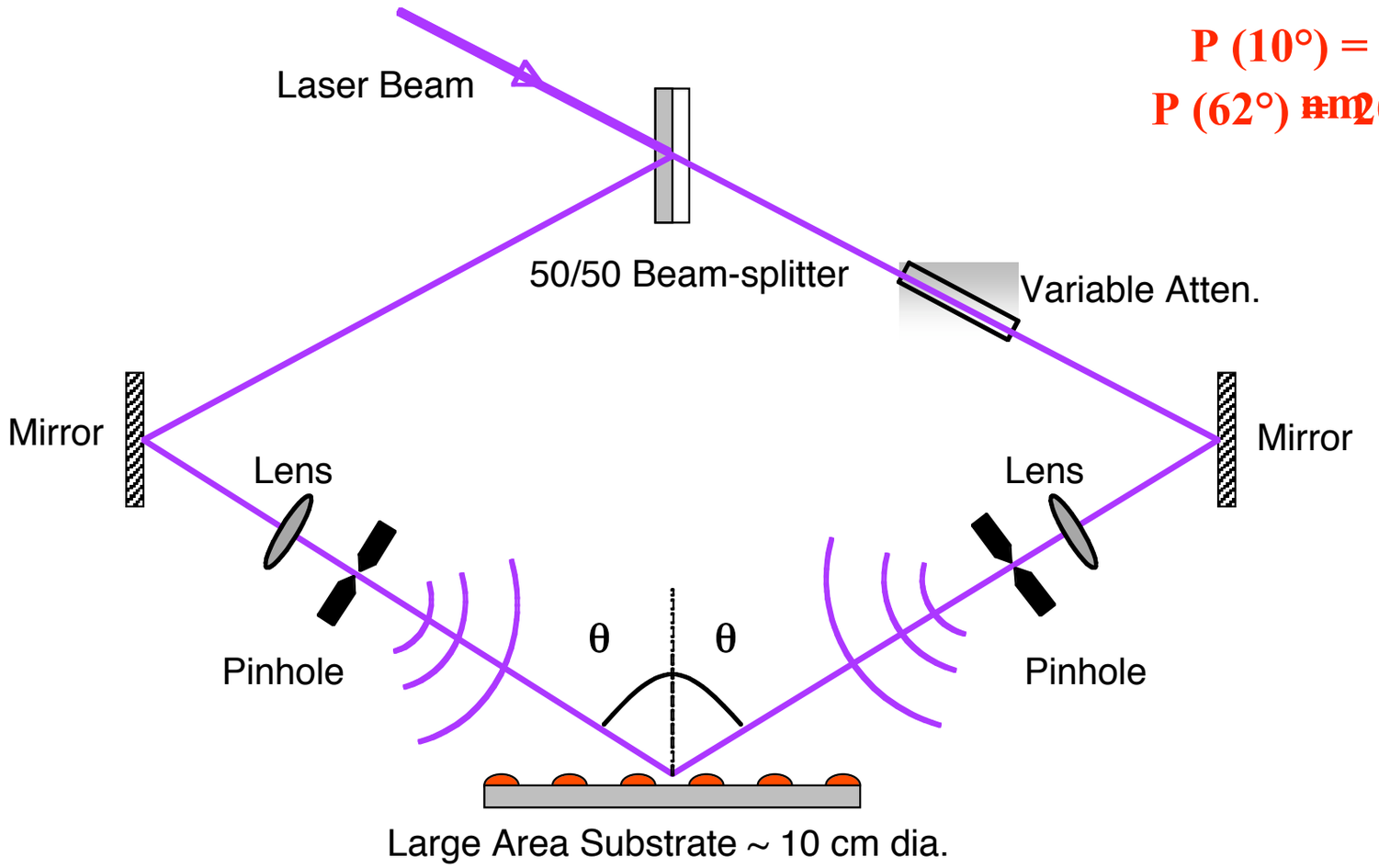
Nanoimprint Molds: Interference Lithography

Argon Ion Laser (λ = 351 nm)

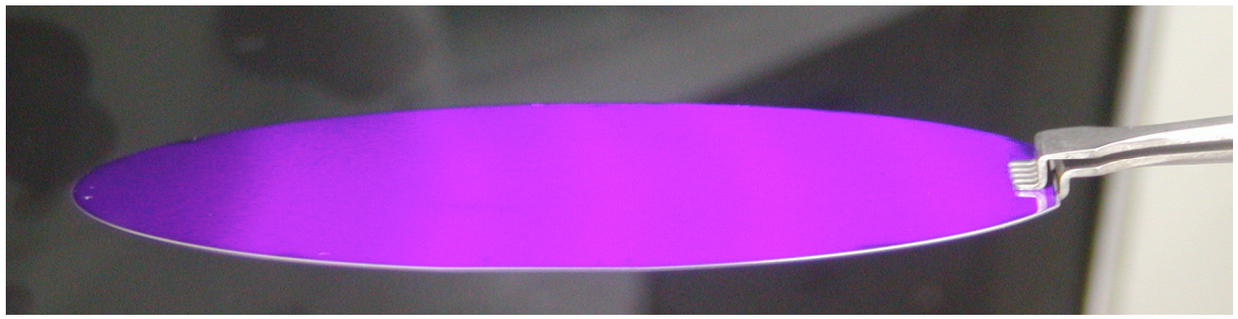
$$P(\theta) = \frac{\lambda}{2 \sin \theta}$$

P (10°) = 1000 nm

P (62°) ~~nm~~ 200 nm

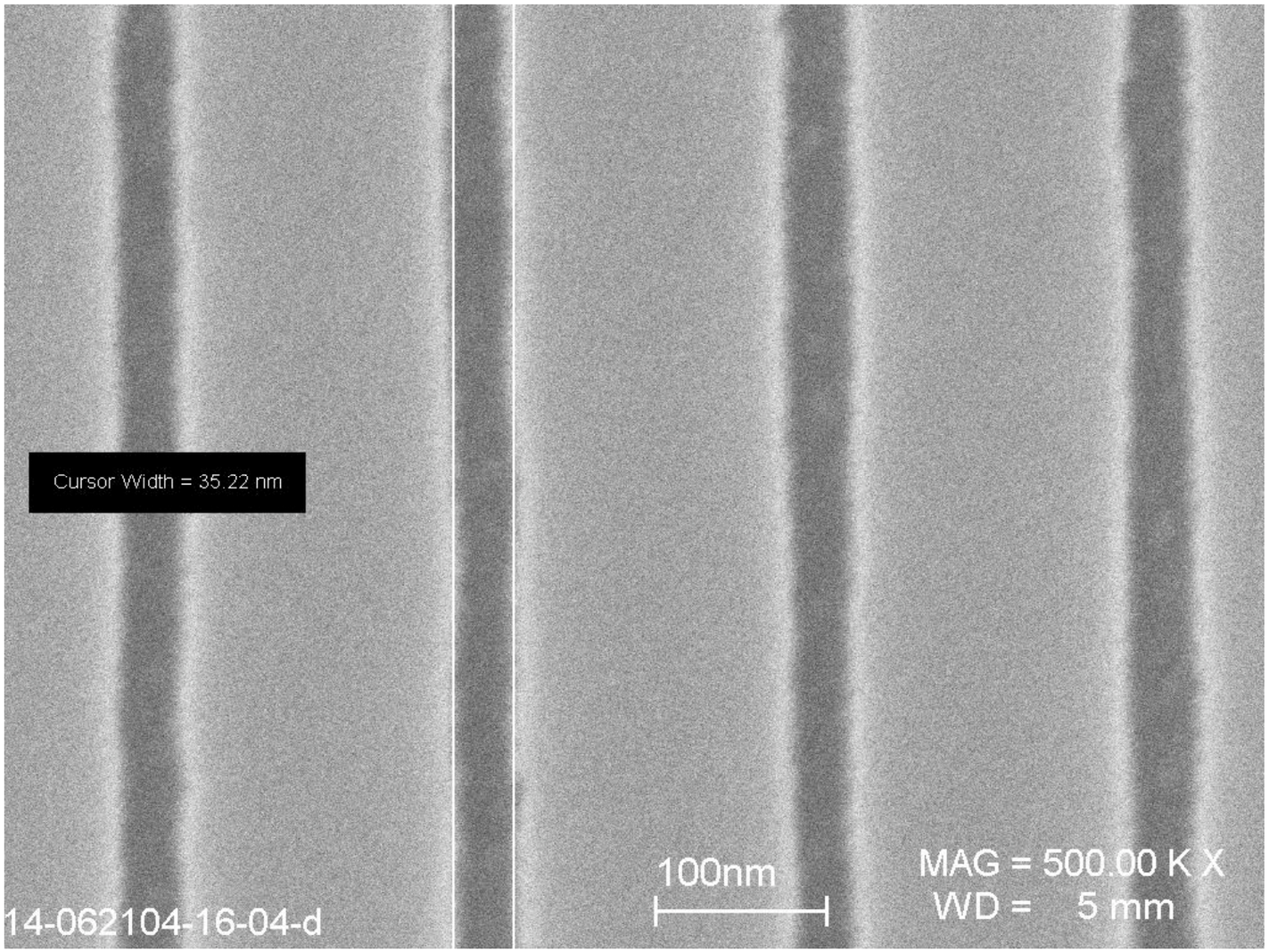


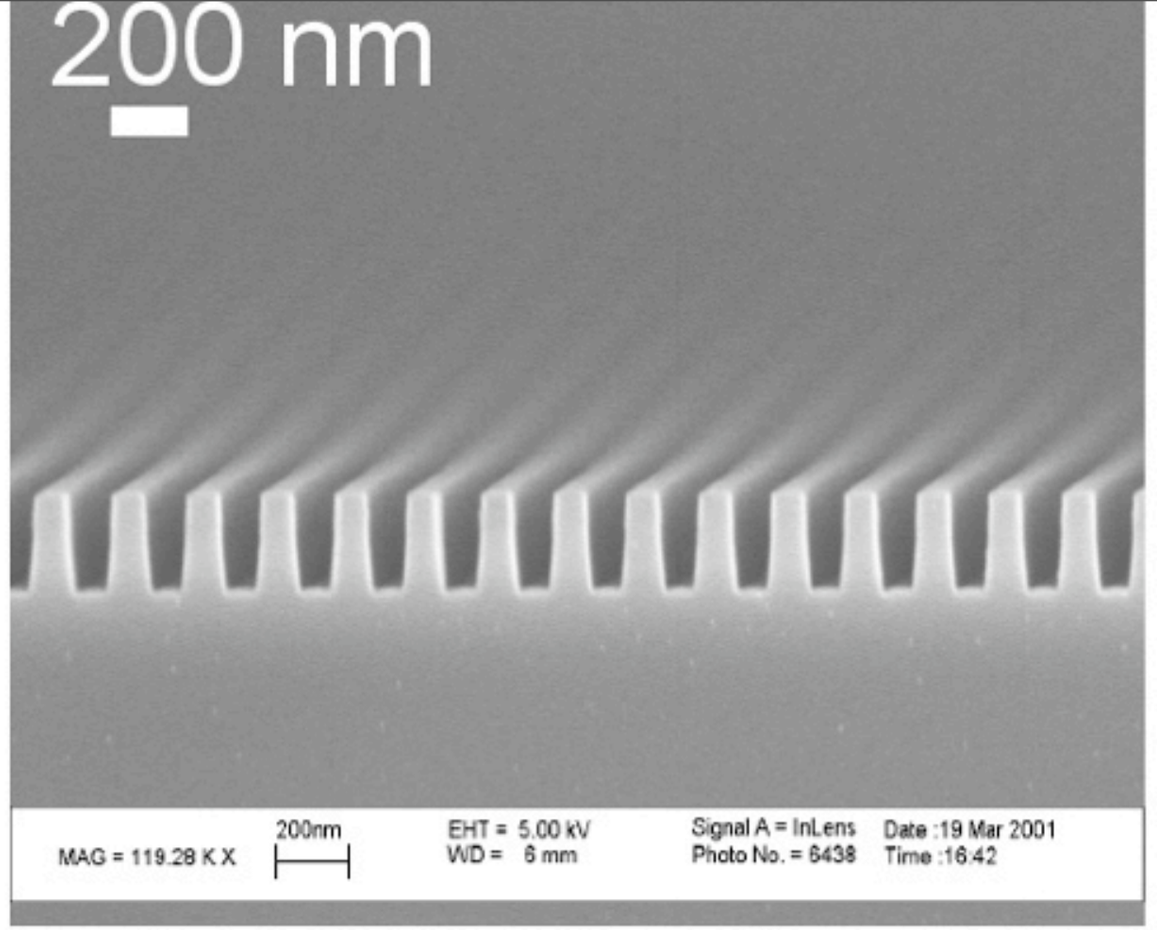
Steve Chou

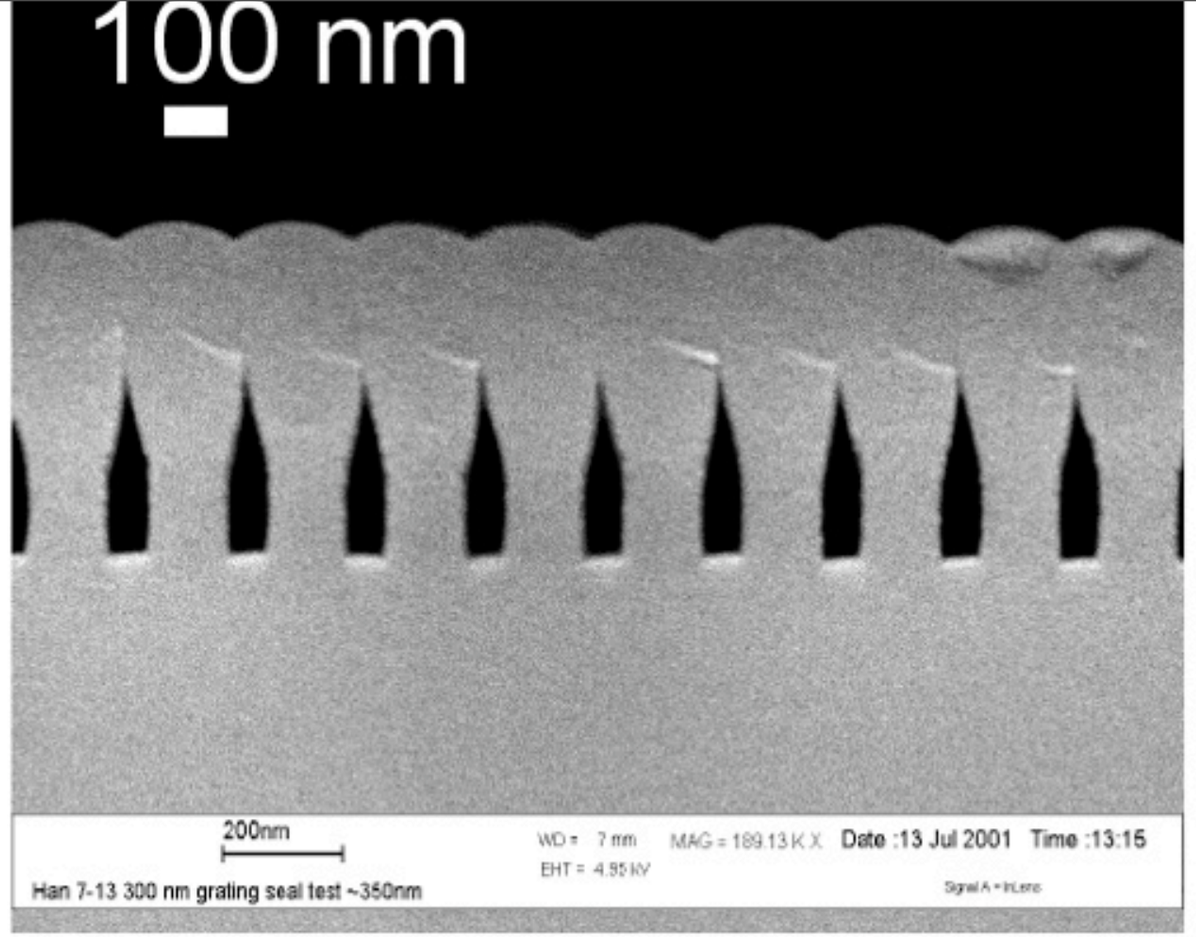


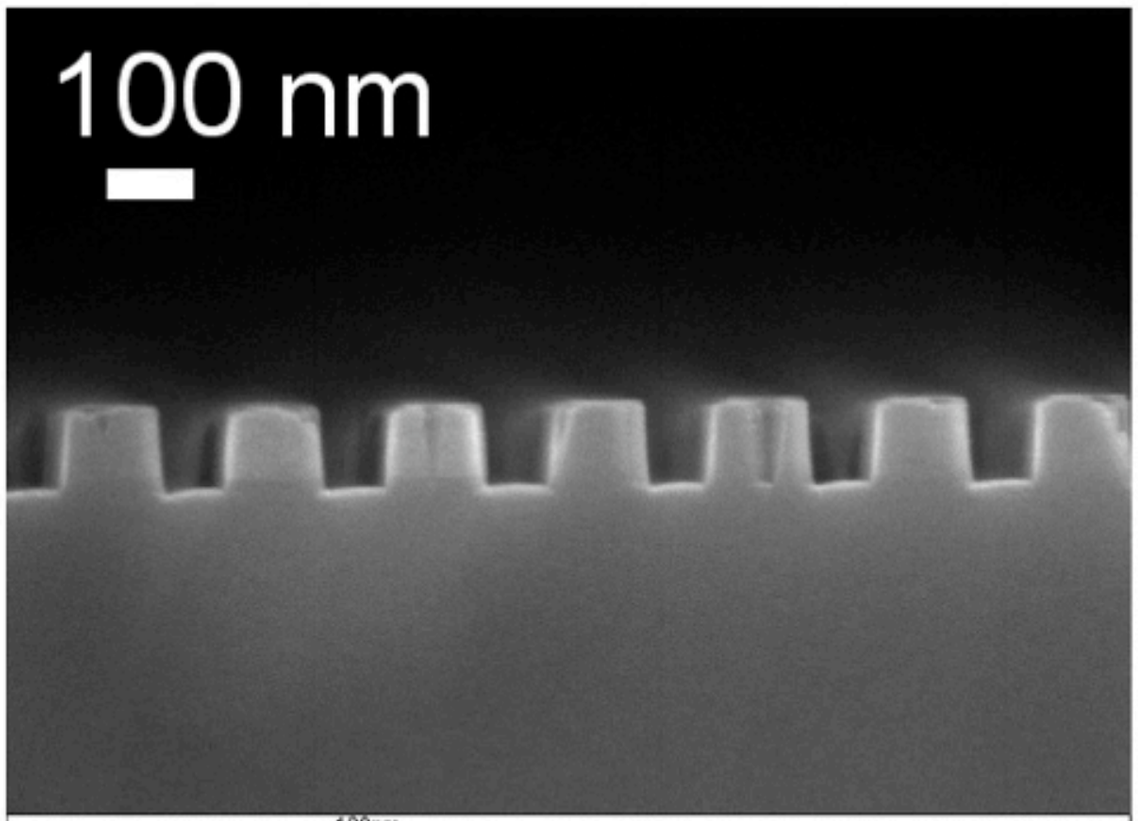
35 nm Channels

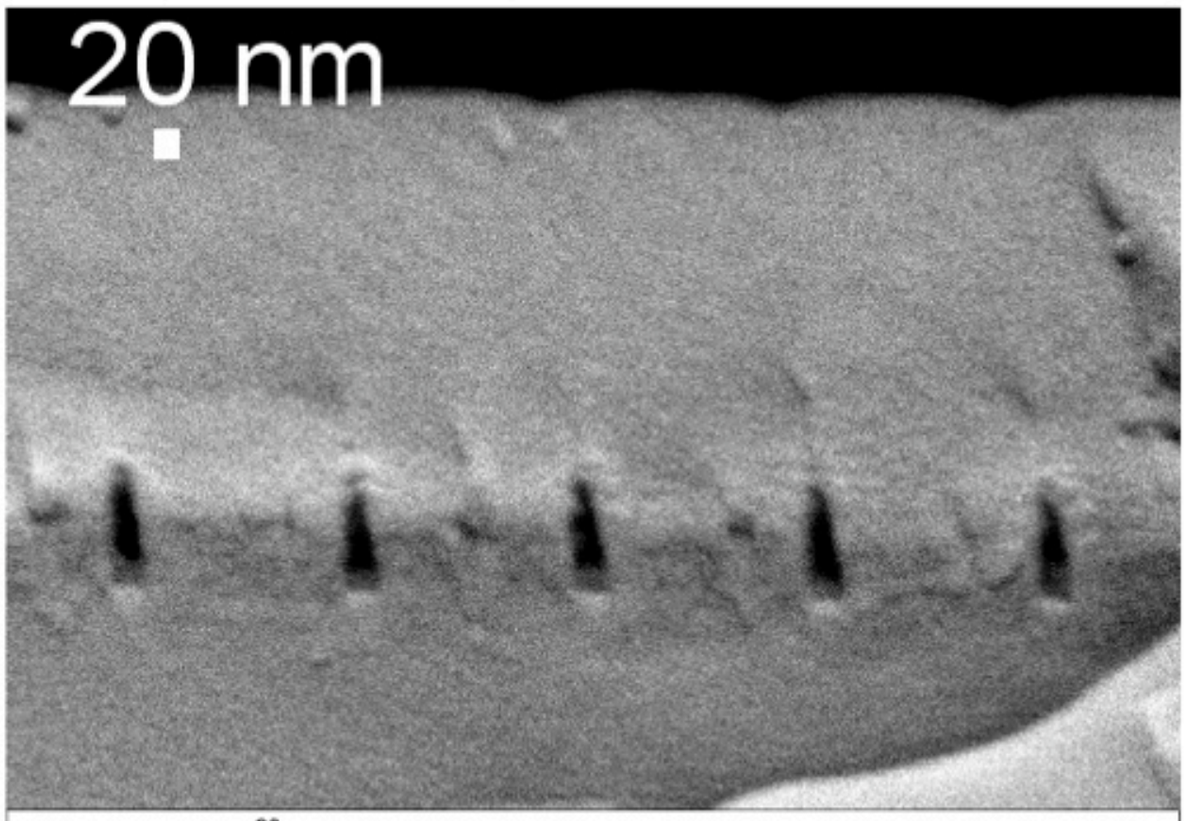
Tolerances: ~ 10 nm




200 nm
MAG = 119.28 K X
200nm
EHT = 5.00 kV
WD = 6 mm
Signal A = InLens
Photo No. = 6438
Date :19 Mar 2001
Time :16:42


100 nm
200nm
WD = 7 mm
EHT = 4.95 kV
MAG = 189.13 K X
Date :13 Jul 2001
Time :13:15
Han 7-13 300 nm grating seal test ~350nm


100 nm
MAG = 265.53 K X
100nm
WD = 6 mm
EHT = 4.20 kV
Signal A = InLens
Date :11 Jul 2001
Time :15:16


20 nm
20nm
WD = 10 mm
EHT = 4.70 kV
MAG = 364.35 K X
Date :16 Jul 2001
Time :15:37
Han 7-16 200 nm grating seal test 80 nm deep~350nm

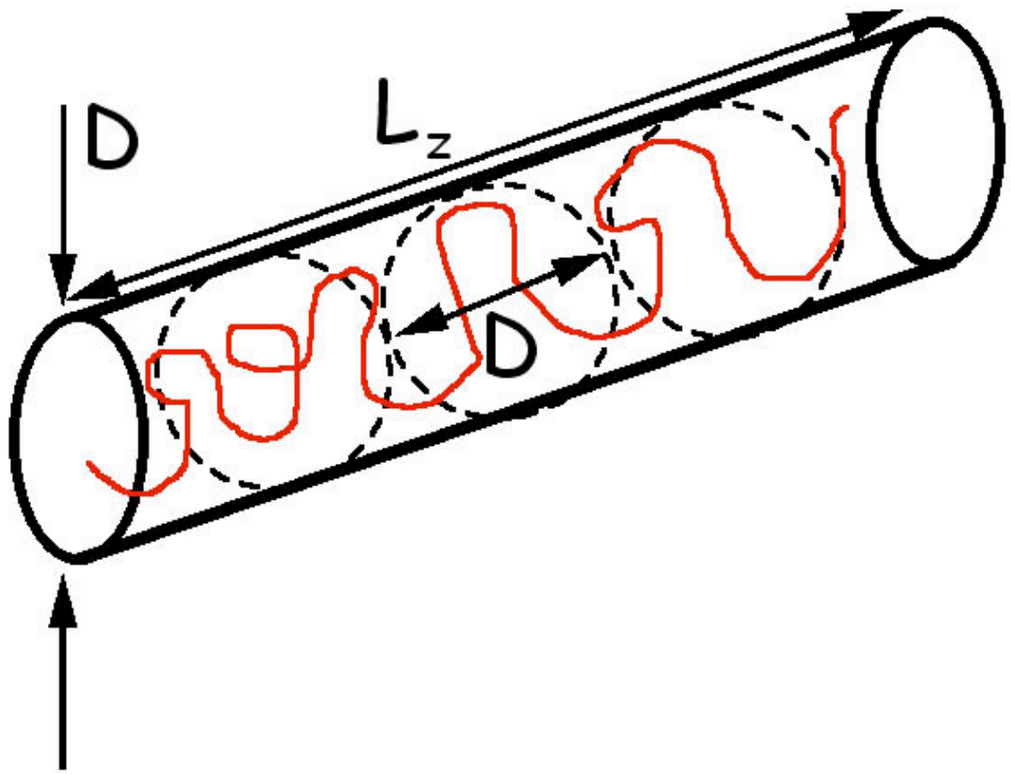
Here's an interesting polymer problem: what happens when you put a long polymer of persistence length p in a nanochannel? Lot's of surprises.

Suppose the channel is say 200 nm wide, and the polymer has a persistence length of 50 nm. The diameter of the dsDNA molecule is only about 2 nm, so most of the volume of the channel is water, since the diameter of the polymer is much less than the persistence length or the channel dimension.

You might think that the self-avoiding random walk would be an unnecessary complication.

That's wrong! Without self avoidance, the radius of gyration of a long polymer in a channel is independent of the channel diameter.

As usual, P. de Gennes worked this out long ago. The idea is very simple: a self-avoiding polymer forms incompressible "blobs" in a channel, each "blob" has a diameter equal to the diameter of the channel.

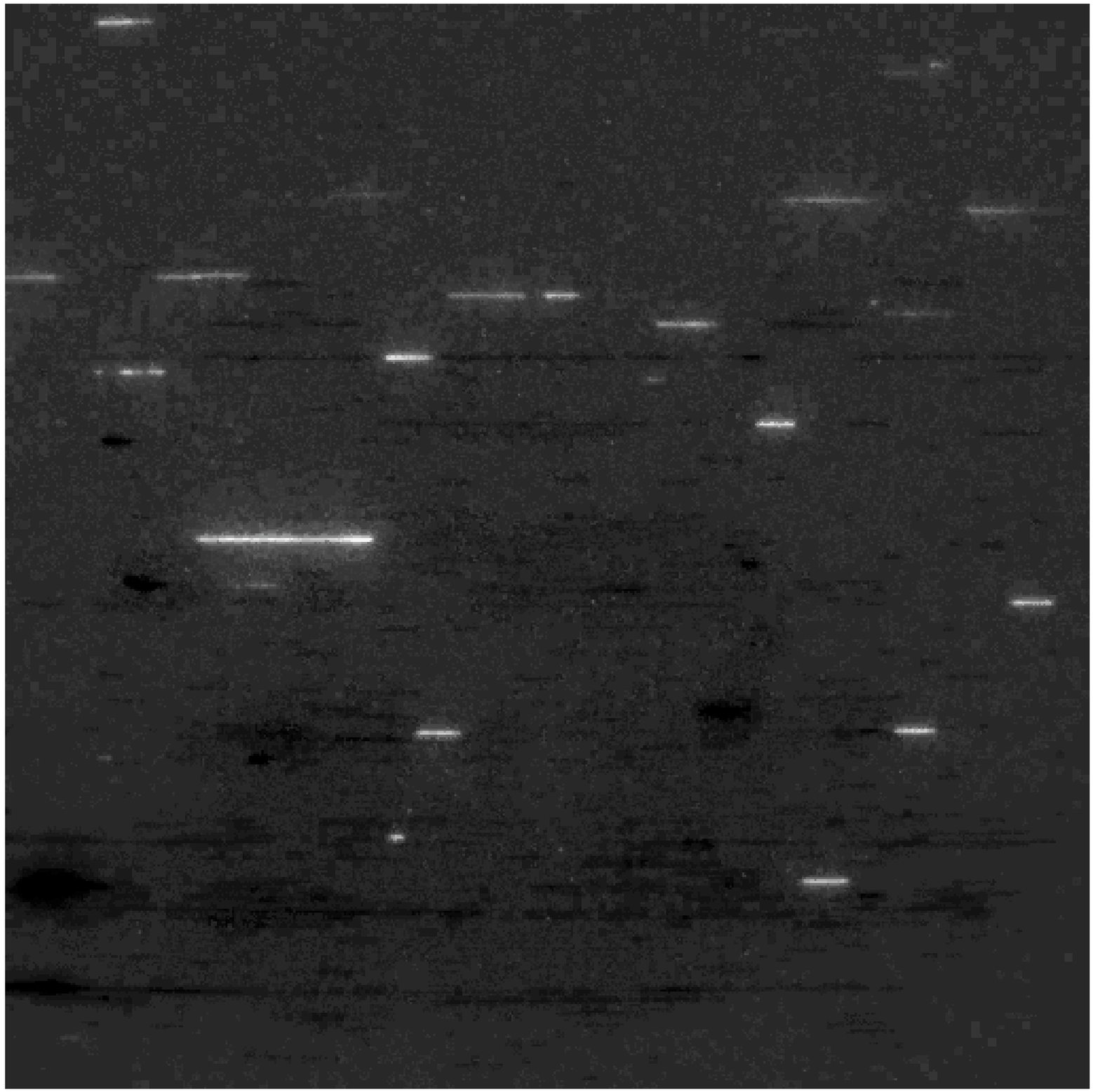


If the polymer has contour length L, the end-end length L_z in a tube of diameter D for a polymer of width w doing a DeGennes SAW is (not hard to calculate if you cheat but you won't get this equation):

$$L_z = L\frac{(pw)^{1/3}}{D^{2/3}}$$

Note that L_z is proportional to the Length , not the square root of the length for an unconfined polymer, somewhat unexpected.

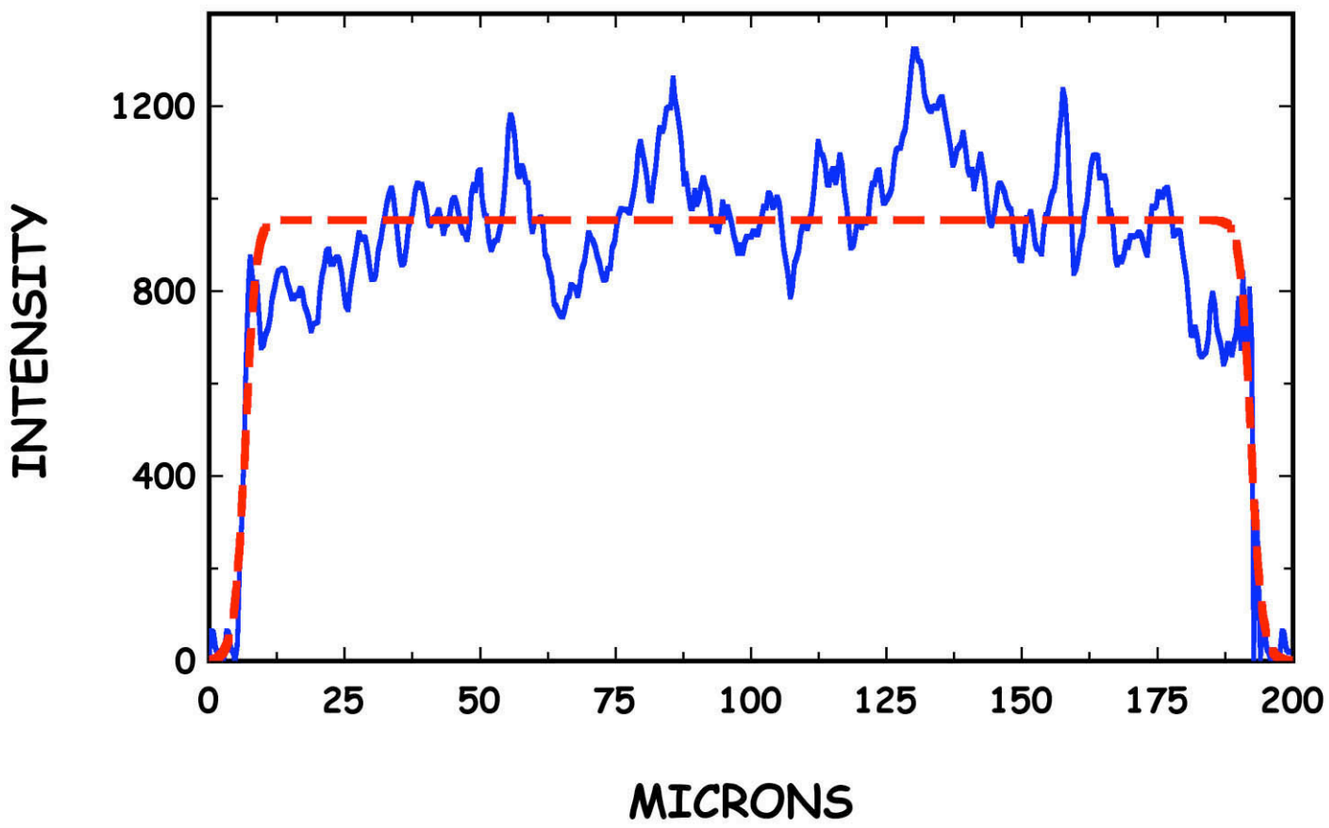
This is a Big Deal, if you stare at L_z you know L! And, as D gets smaller L_z approaches L (sort of...)



The idea is to run the molecules in, stare, get the length, put new ones in, and build histograms quickly.

The dynamics of confined polymers is extremely important. Why? Because, the longer you stare at the dynamics of these molecules, the more you know about the standard deviation of the mean and hence the length, to much better than the wavelength of the observing light. This is a Big Deal.

$$I(z) = \frac{I_o}{2}\left[Erf\left(\frac{z}{\sigma_o\sqrt{2}}\right) - Erf\left(\frac{z-L_z}{\sigma_o\sqrt{2}}\right)\right],$$



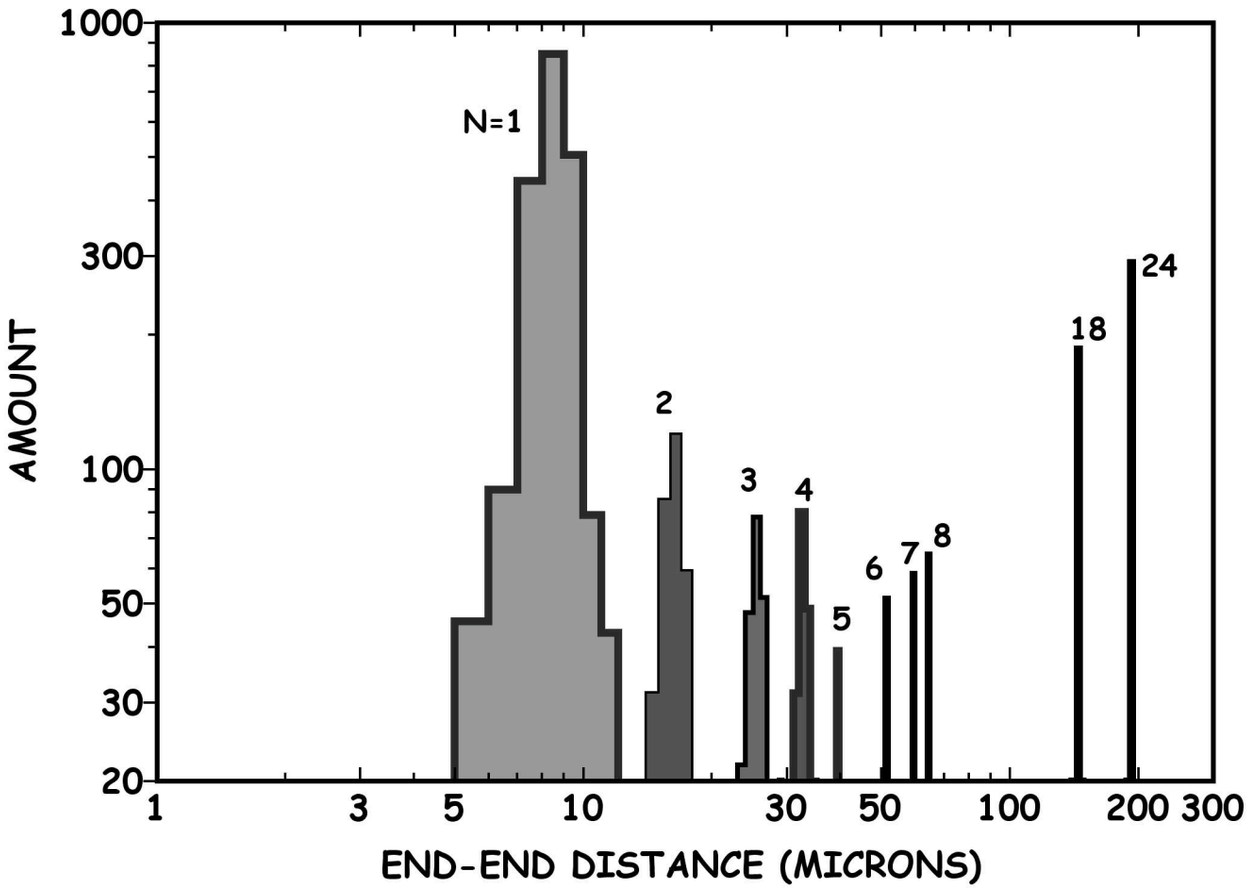
The entropic spring constant k of this confined polymer is thus really important to calculate. Unless you really get below the persistence length in channel width the spring constant is all entropic and can be calculated from the free energy of a confined, self-avoiding spring. This sounds horrible but if you have smart grad students (Reisner) they can figure this out for you:

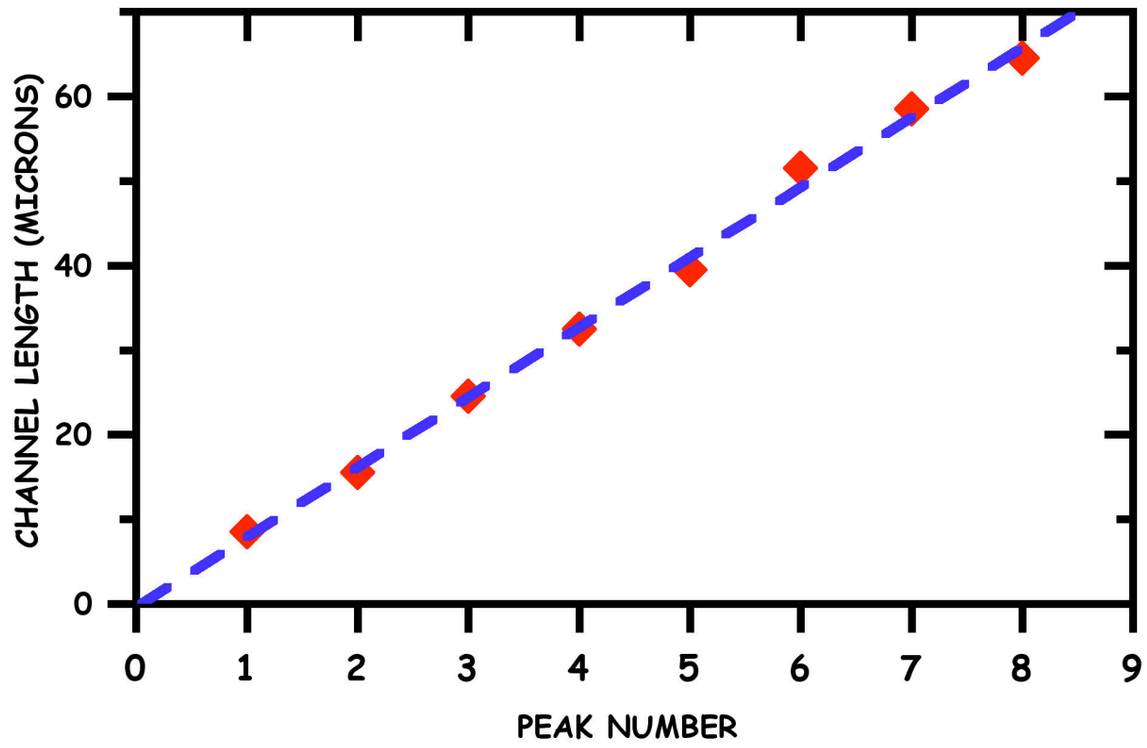
$$F \sim k_B T \times (Blobs) = k_B T \times \frac{L}{L_z}$$

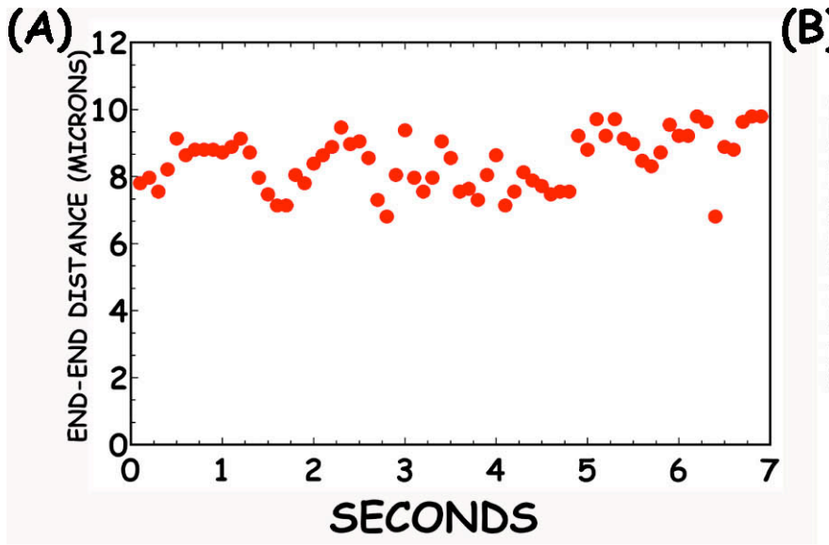
Take the grad of the free energy and you are done!

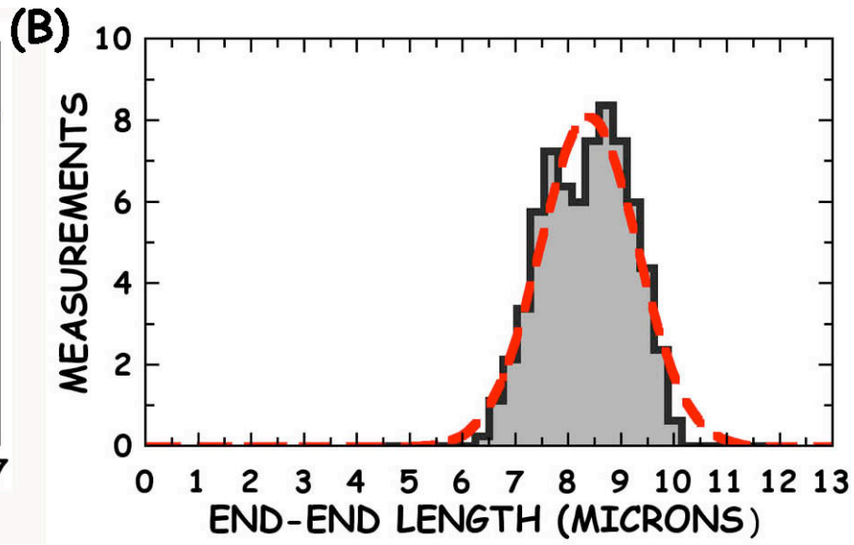
$$k \simeq \frac{15}{4}\frac{k_B T}{L}\left[\frac{1}{pwD}\right]^{1/3}$$

Interesting result! As you confine DNA in a channel the stiffness gets bigger the narrower the channel!

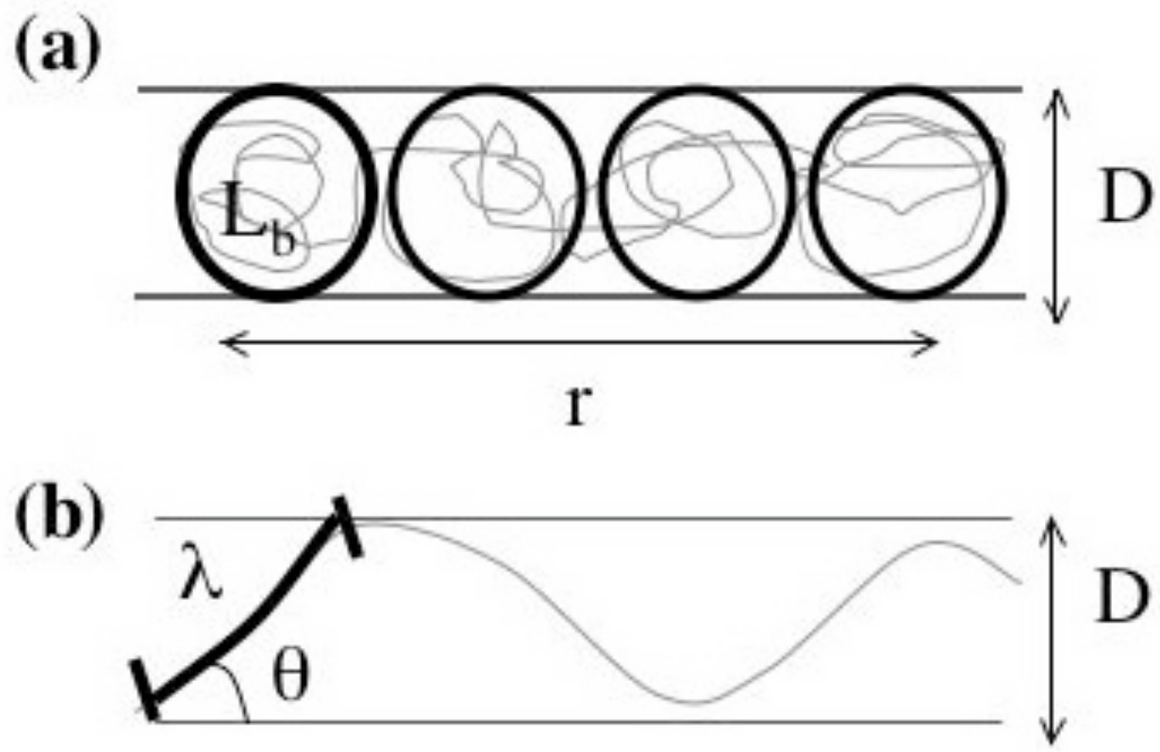

1000
300
100
50
30
20
AMOUNT
N=1
2
3
4
5
6
7
8
18
24
1
3
5
10
30
50
100
200 300
END-END DISTANCE (MICRONS)


CHANNEL LENGTH (MICRONS)
60
40
20
0
0 1 2 3 4 5 6 7 8 9
PEAK NUMBER


(A)
END-END DISTANCE (MICRONS)
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7
SECONDS

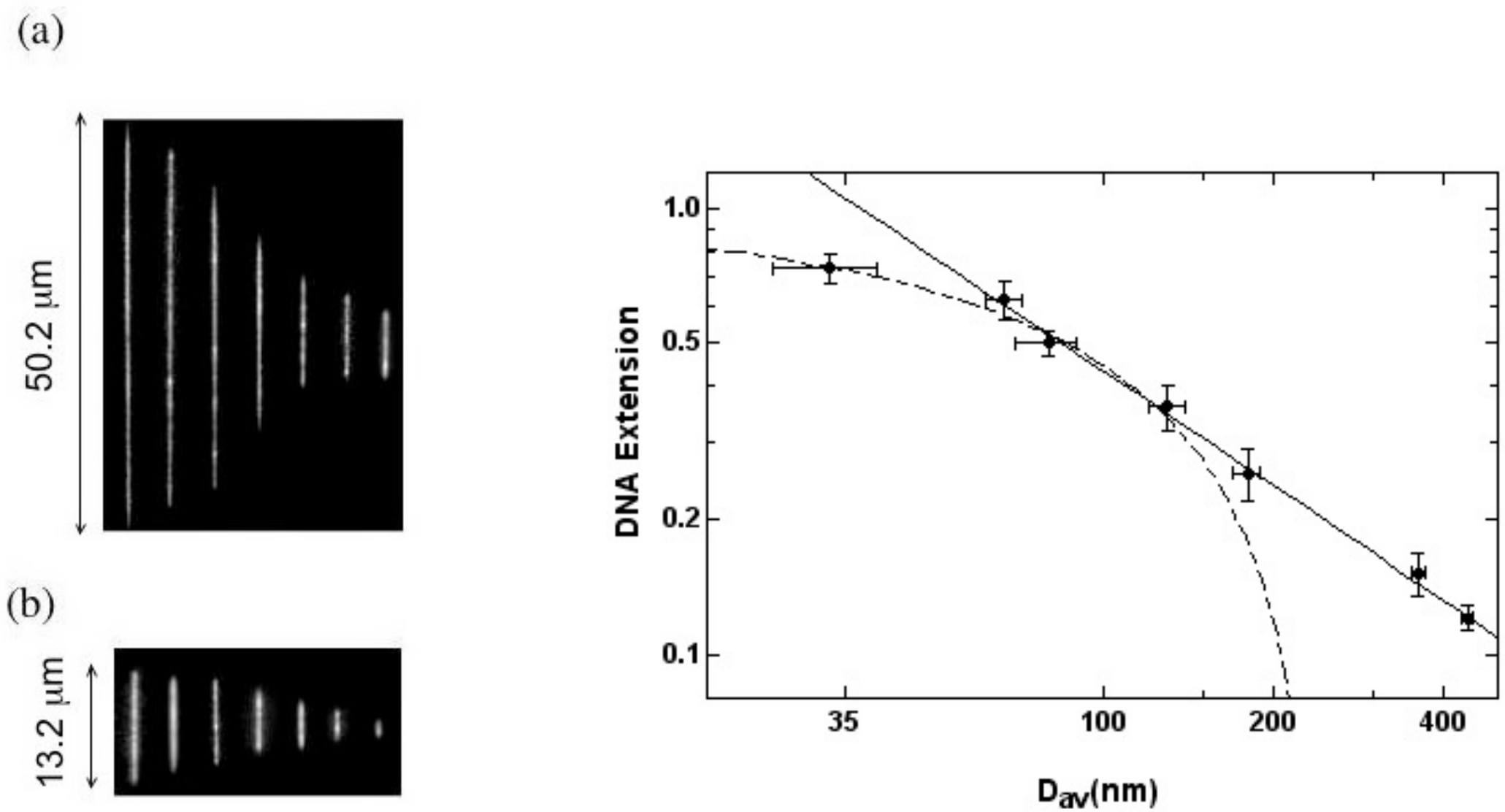

(B)
MEASUREMENTS
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
END-END LENGTH (MICRONS)

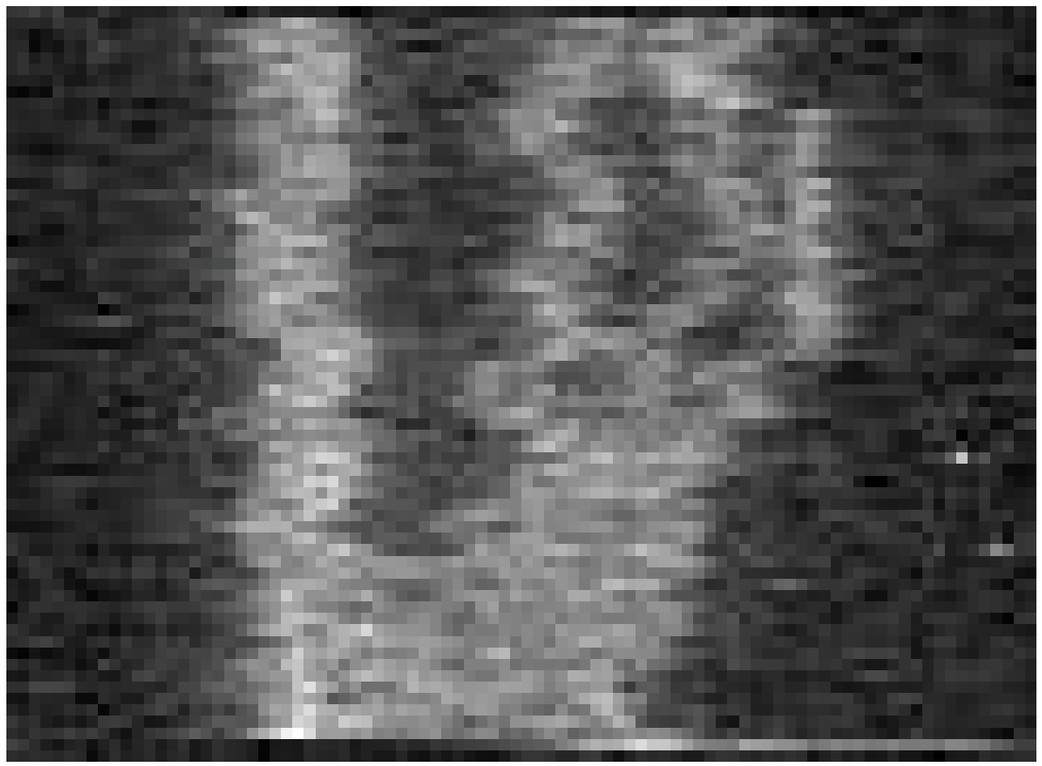
That work came from 100 nm channels, 2 persistence lengths wide. We have pushed down since them to channels of diameter D below 1 persistence length. At this scale, de Gennes scaling breaks down, and we enter a new area, the Odijk limit where now it is NOT entropy but elastic deformation that determines the statistics.



We enter the regime where even French Nobelists fail, only the Dutch are strong enough:

$$L_z = Lcos(\theta) = L\left[1 - A\left(\frac{D}{P}\right)^{2/3}\right].$$



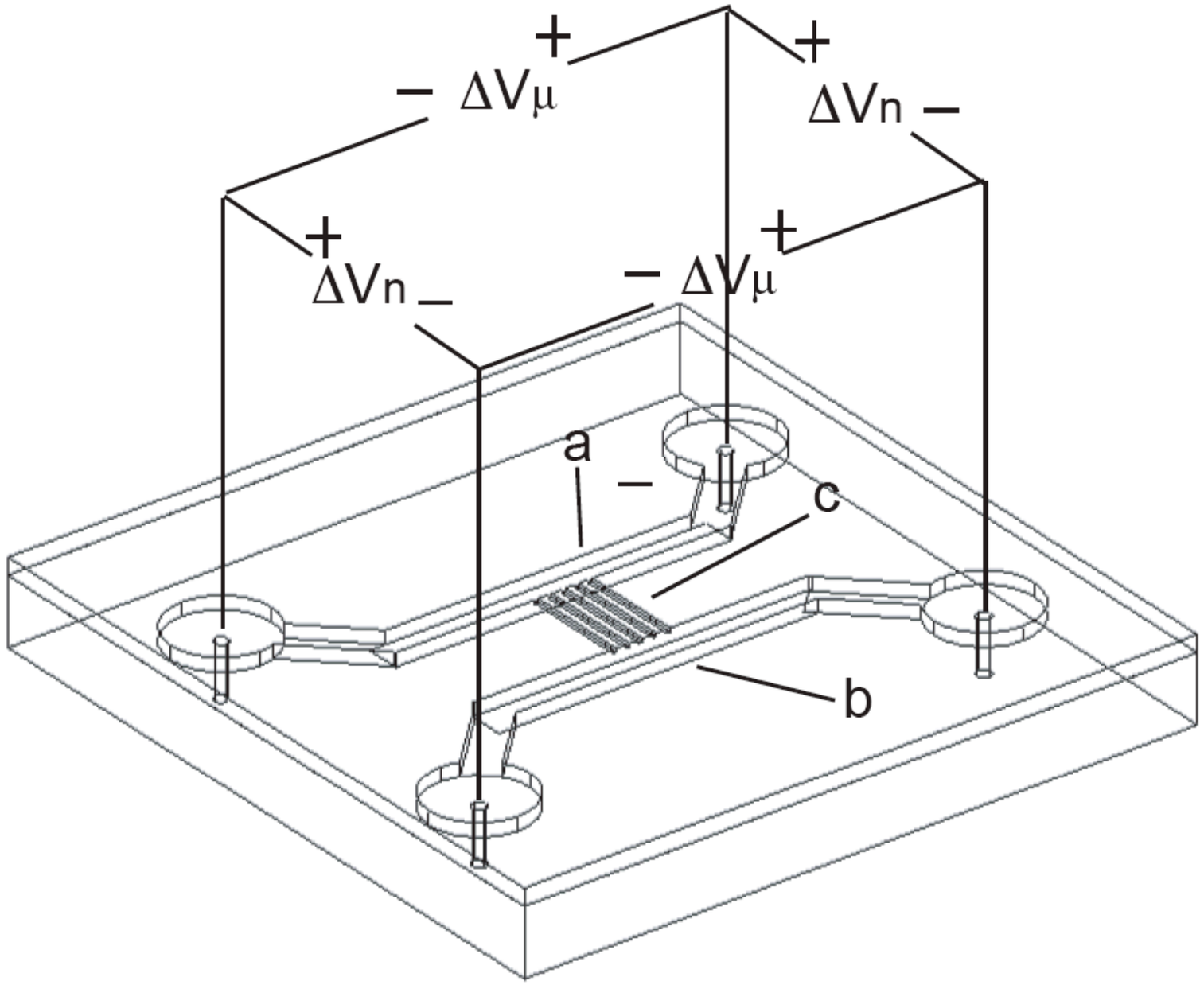


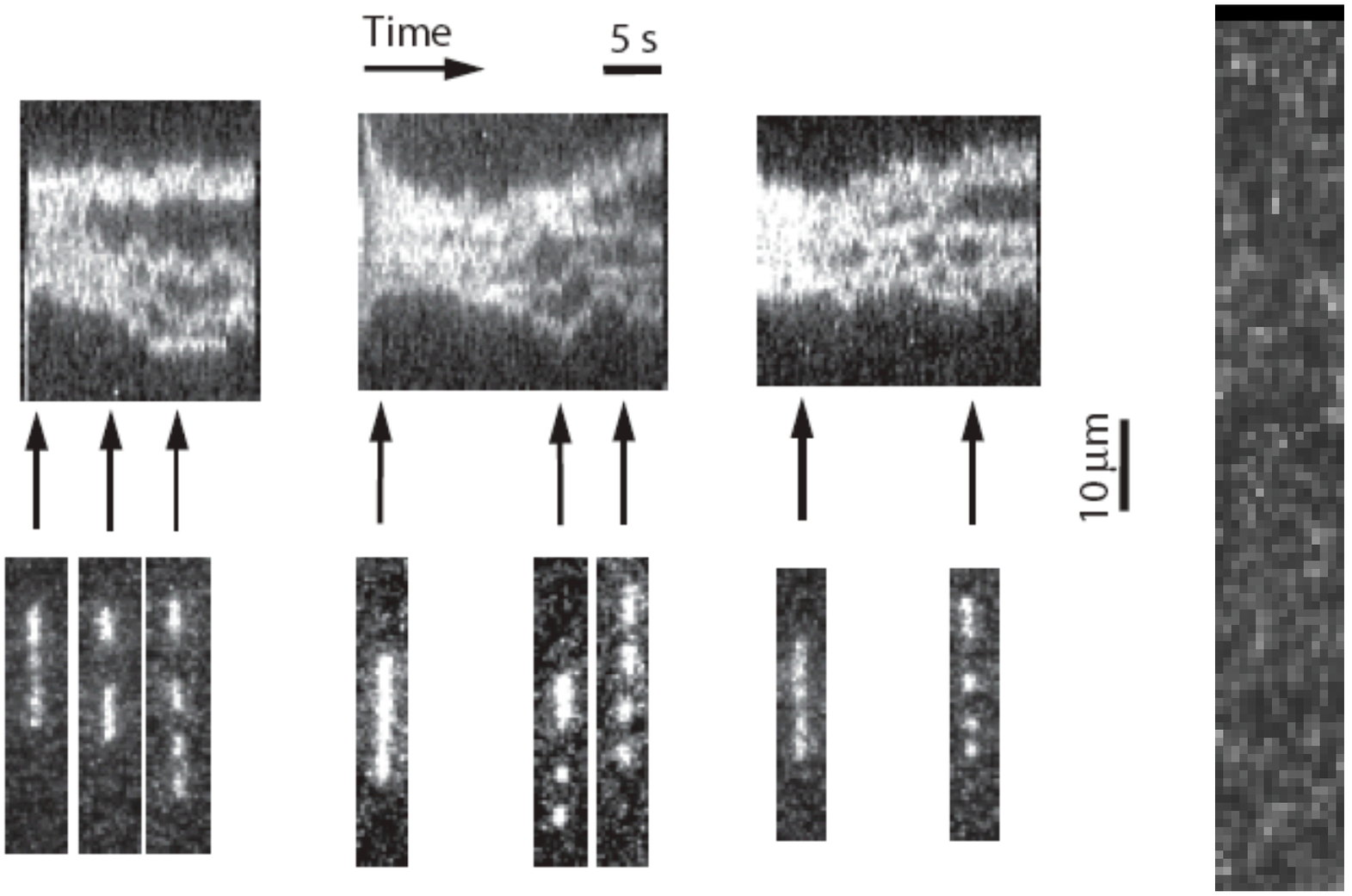
My post-doc Robert Riehn with help from a molecular biologist Manchun Lu has even started watching the time-resolved cutting of genomic length DNA by restriction enzymes, in nanochannels.

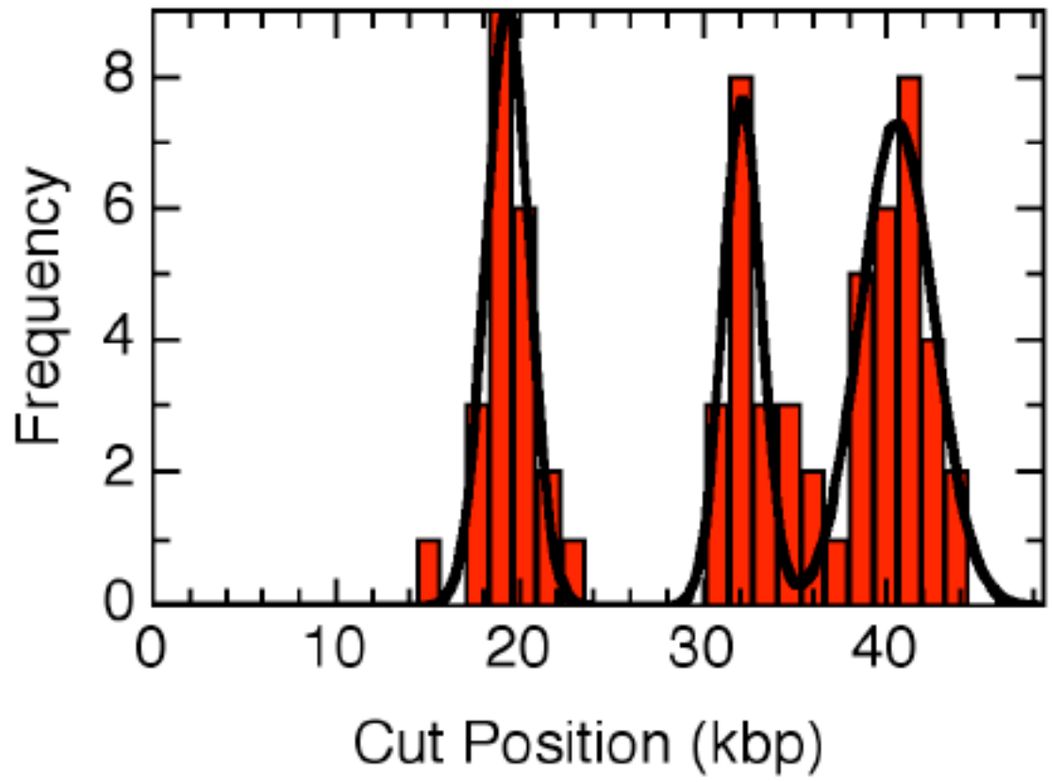
Restriction mapping of DNA with endonucleases is a central method of modern molecular biology. It is based on the measurement of fragment lengths after digestion, while possibly maintaining the respective order.

We decided that perhaps we could bring restriction enzymes into these nanochannels and cut genomic length DNA molecules at precise sites. Since we would observe the cutting directly, there would be no scrambling of the order of the cut sites, and so we could do a direct physical map of a DNA of genomic length.

This has been a hard road to go down!


+
− ΔVμ
+
ΔVn −
+
ΔVn −
+
− ΔVμ
a
−
c
b


Time
5 s
10 µm



Sequence	19.4	31.6	39.9
Histogram	19.3 ± 1.2	32.1 ± 1.0	40.6 ± 2.0
Weighted Average	19.9 ± 1.3	32.8 ± 1.3	40.7 ± 1.7

Hydraulic Optics: The Anti-Lotus Leaf Effect

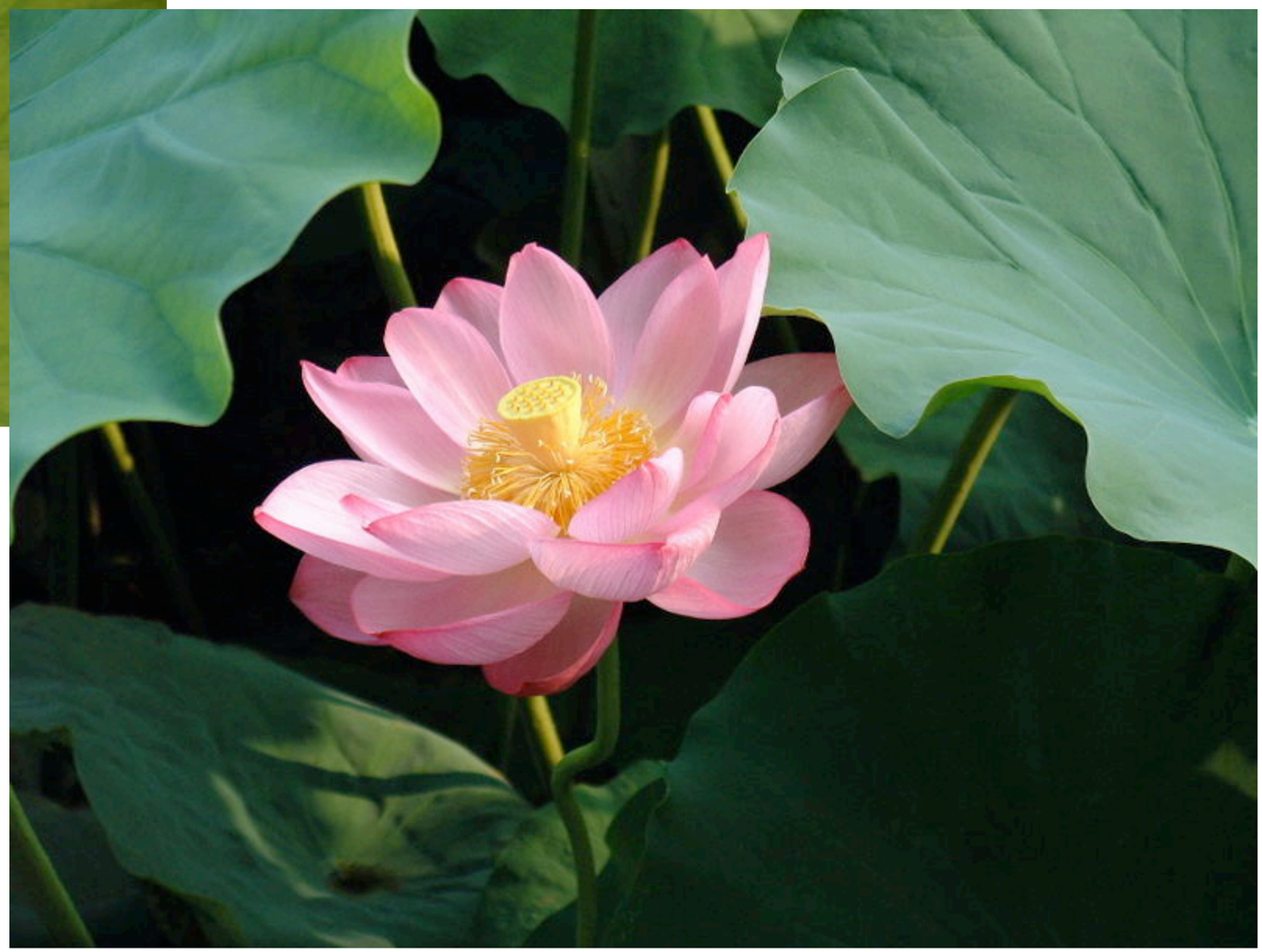
Prof. Ophelia Tsui (Hong Kong Univ. Science Technology)
Keith Morton (EE, Princeton Univ.)
Ho Cheuk Ting (Cherry) (HKUST)

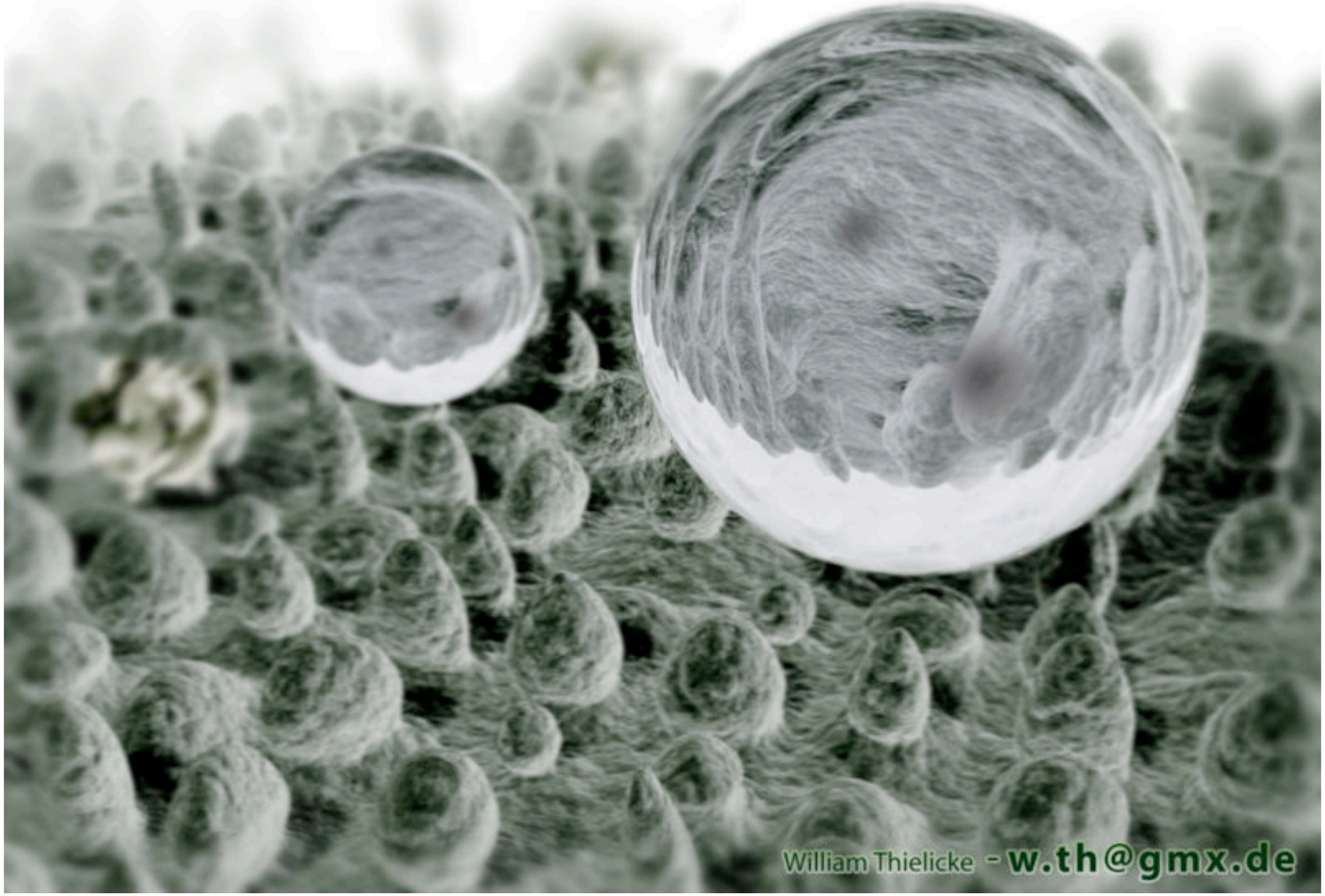
Prof. Stephen Chou (EE, Princeton Univ.)

David Inglis (EE, Princeton University)
John Davis (EE, Princeton University)
Prof. Jim Sturm (EE, Princeton University)

Reported by: Robert Austin (Physics, Princeton Unversity)

The lotus plant is a symbol of cleanliness in an unclean and dirty world, esp. the world of big time academic egos. Although lotuses grow in the muddy rivers and lakes of modern day China, the leaves remain clean, like my own soul.





The lotus leaf is a nanostructured metamaterial, not simply hydrophobic!

Hand waving way to understand the effect:

$$P = \frac{2\gamma}{R}$$

As R decreases, the pressure increases. If the relative surface tension is (-), metamaterial becomes "super-hydrophobic" (Lotus Leaf). If the relative surface tension is (+), material becomes "superhydrophilic" (anti-Lotus Leaf).

Effective pressure is huge: for water at R=100 nm, P = 20 Atmospheres!

MUCH of the flexibility and power of ray optics, surprisingly, can be realized in hydraulic flows on surfaces that are properly nanopatterned metamaterials. We ourselves have only recently become aware of what our previous discoveries have been leading to: a whole new way of transporting objects on surfaces.

"Pure Hydrodynamics" has no diffusion,

just advective transport.

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

$$m\frac{d\vec{v}}{dt} = \vec{F}$$

There is a hell of a lot of physics in the Navier-Stokes Eq., the key to sailing AND flying is in it (not in Bernoulli's Eq.)

The ugly non-linear convective term:

$$(v \bullet \nabla) v$$

fortunately is small compared to the viscous drag term in the N-S equation if the Reynold's number is <1:

$$R_e = \frac{\rho v L}{\eta}$$

$$R_e = \frac{\rho v L}{\eta}$$

Basically, the Re is a measure the rate at which kinetic energy (inertial energy) is sucked out of a system due to viscous drag. L is some length over which the velocity vector v changes, and the smaller v is and the bigger the viscous drag is the smaller a distance you coast. At low Re (<<1) forget Freshman Physics. Aristotle was right!

We will ALWAYS be in Re<<1 here.

Re is of course known even by your parents and they are NOT impressed by you using it to describe mixing your coffee by advection to justify the outrageous cost of your college education.

Actually, there is a related number which your parents probably do NOT know. Mixing of course ultimately must happen by diffusion, and the question is how can diffusion compete with advection?

The Peclet number Pe is the ratio of the TIME to diffuse distance "a" to the time to advect that distance:

$$\mathrm{P}_e = \frac{a^2/D}{(a/v)} = \frac{va}{D}$$

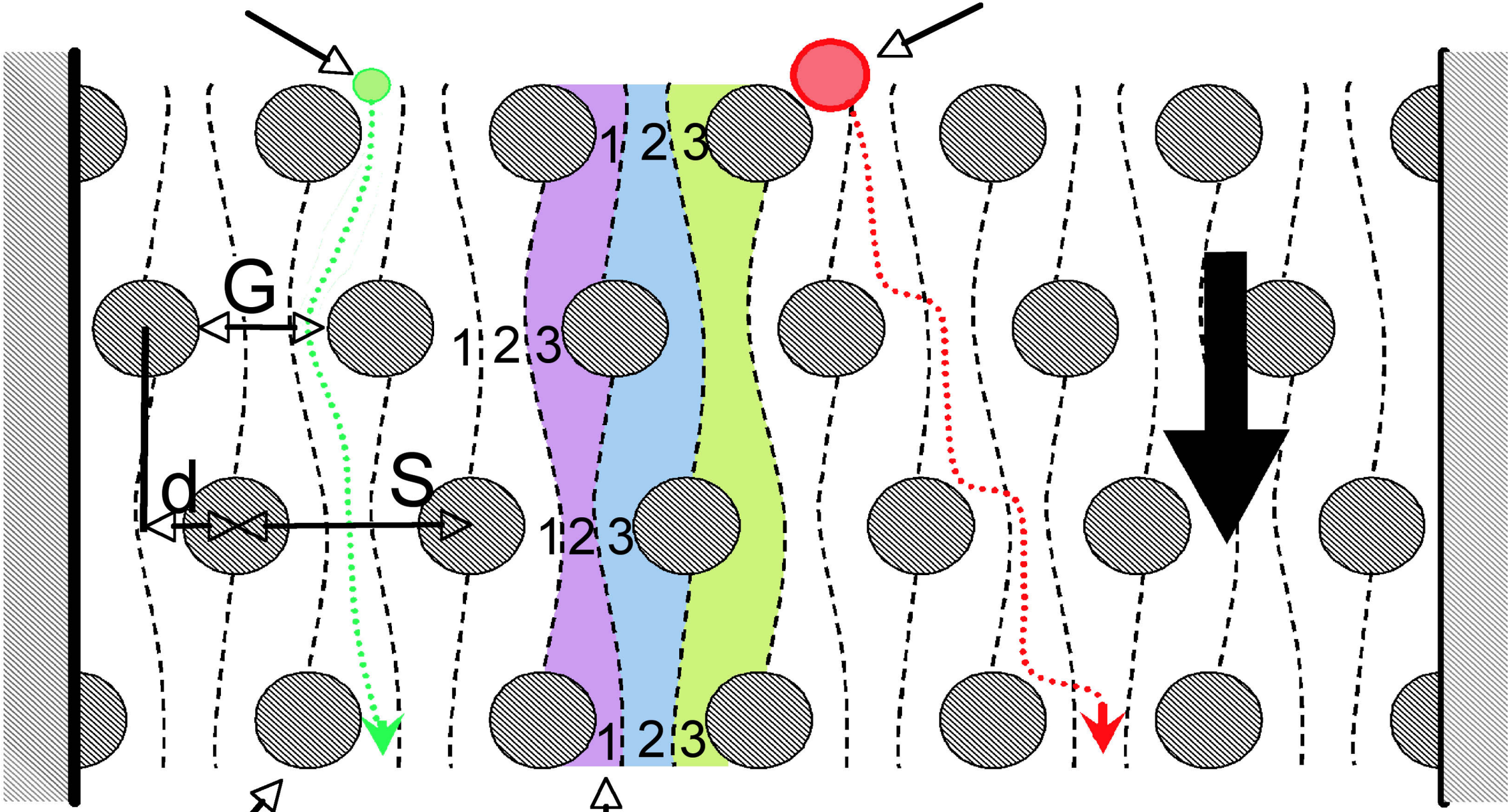
At large Pe you can ignore diffusion. You can be simultaneously at LARGE Pe and SMALL Re!

The basic idea of the Tango, or Bump, .

Genius idea of Lotien (Richard) Huang (PU EE *05)

Small Particle

Large Particle

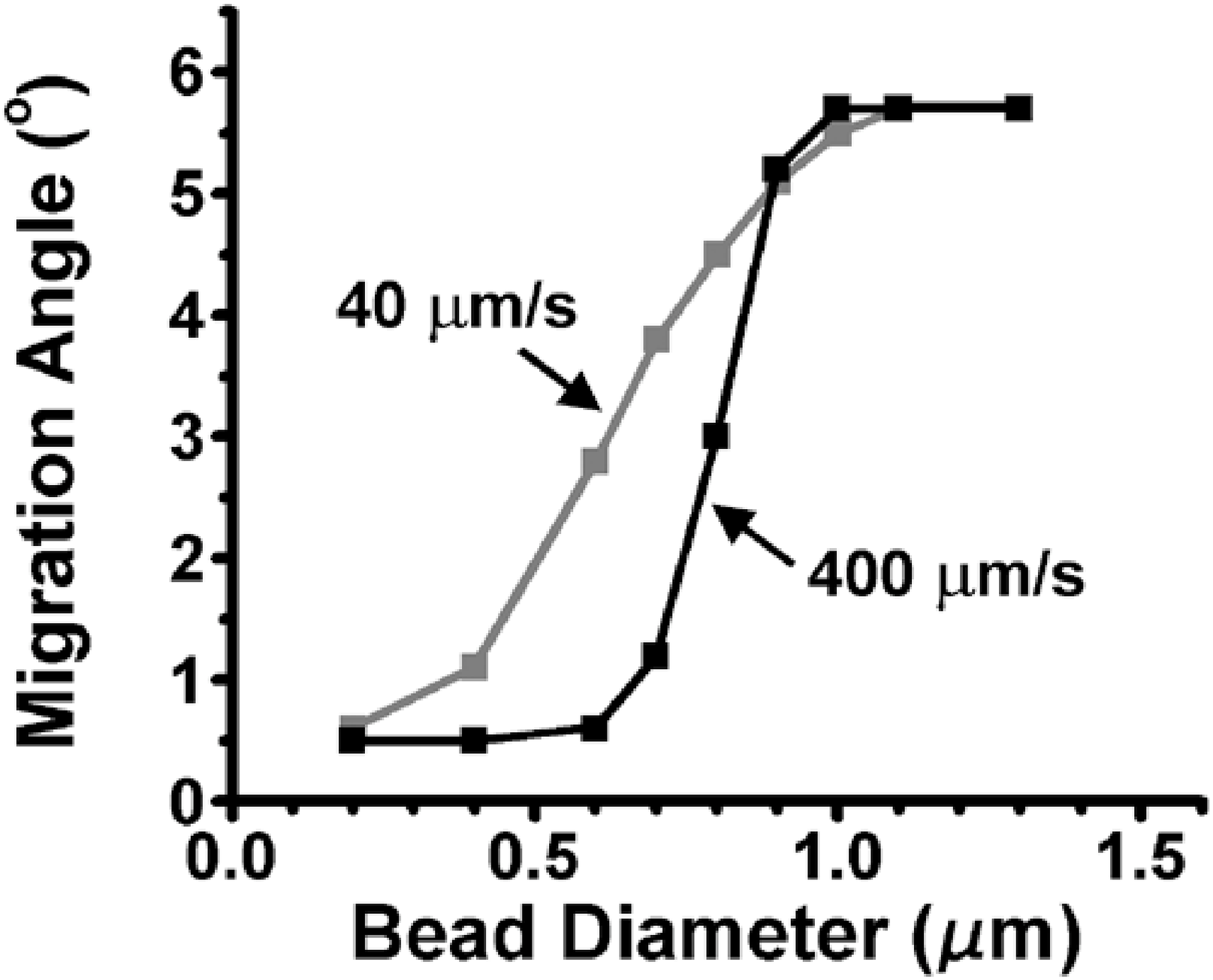


Posts

Streamlines

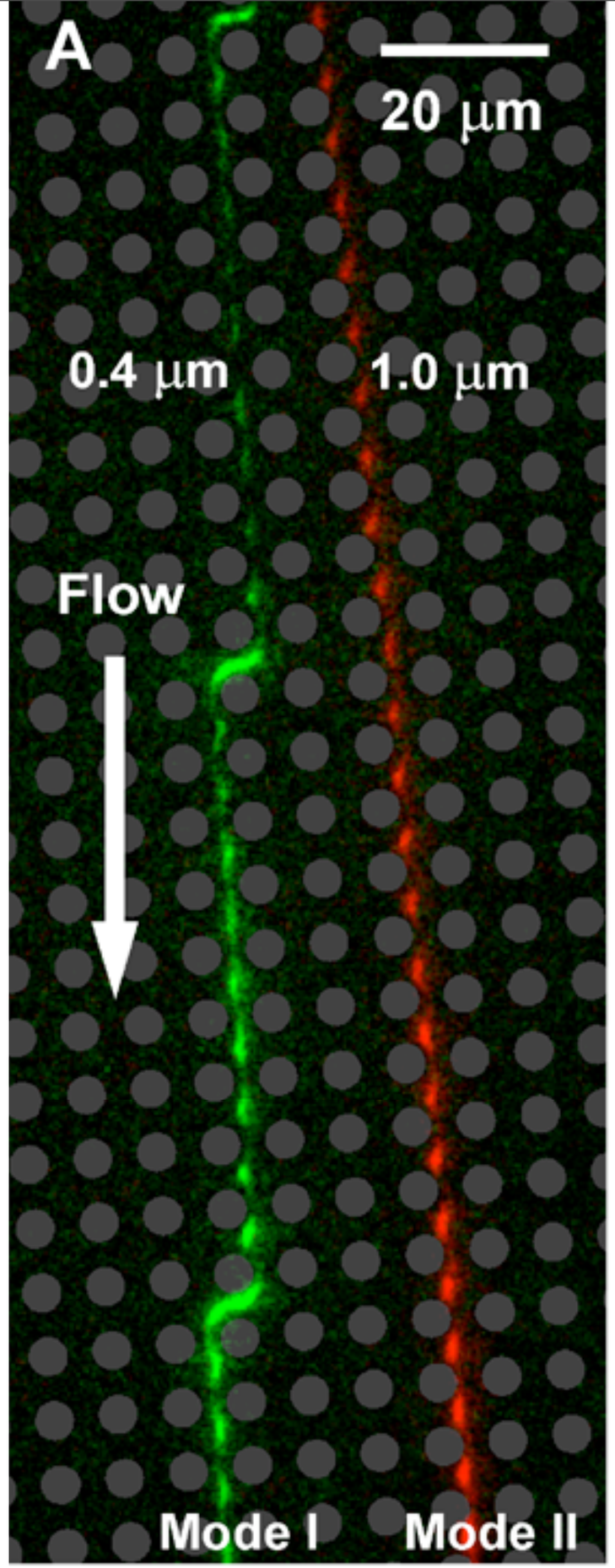
$$\varepsilon = \frac{d}{S}$$

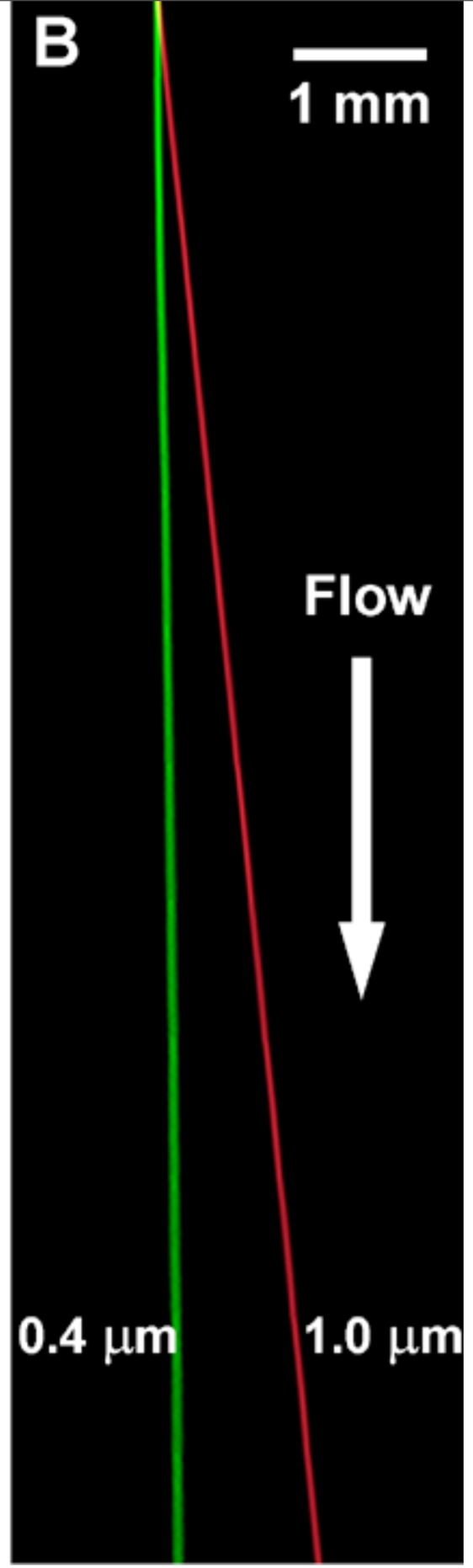
G= gap= minum size to clog

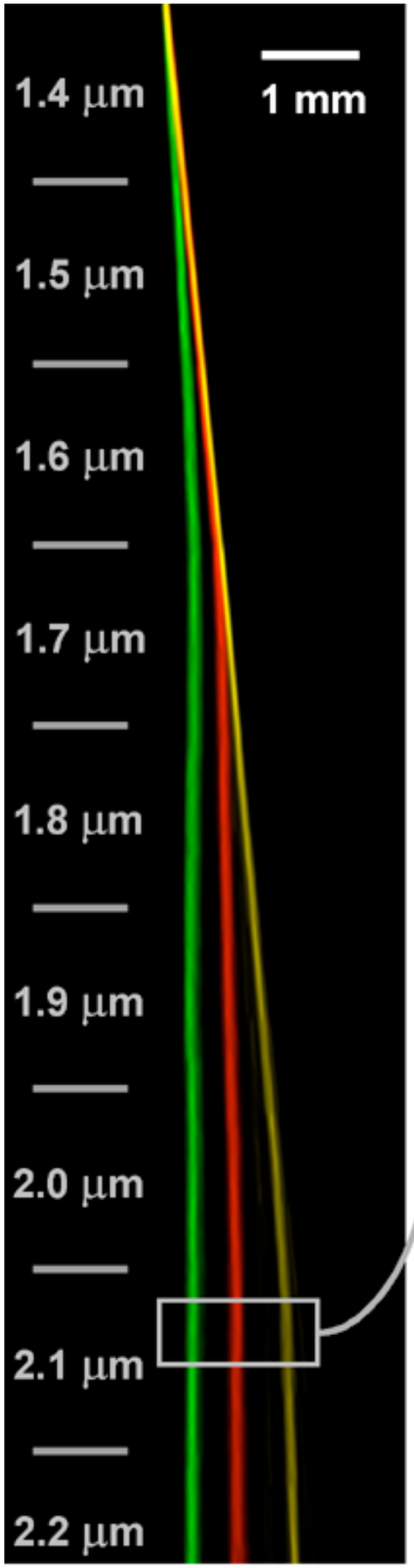


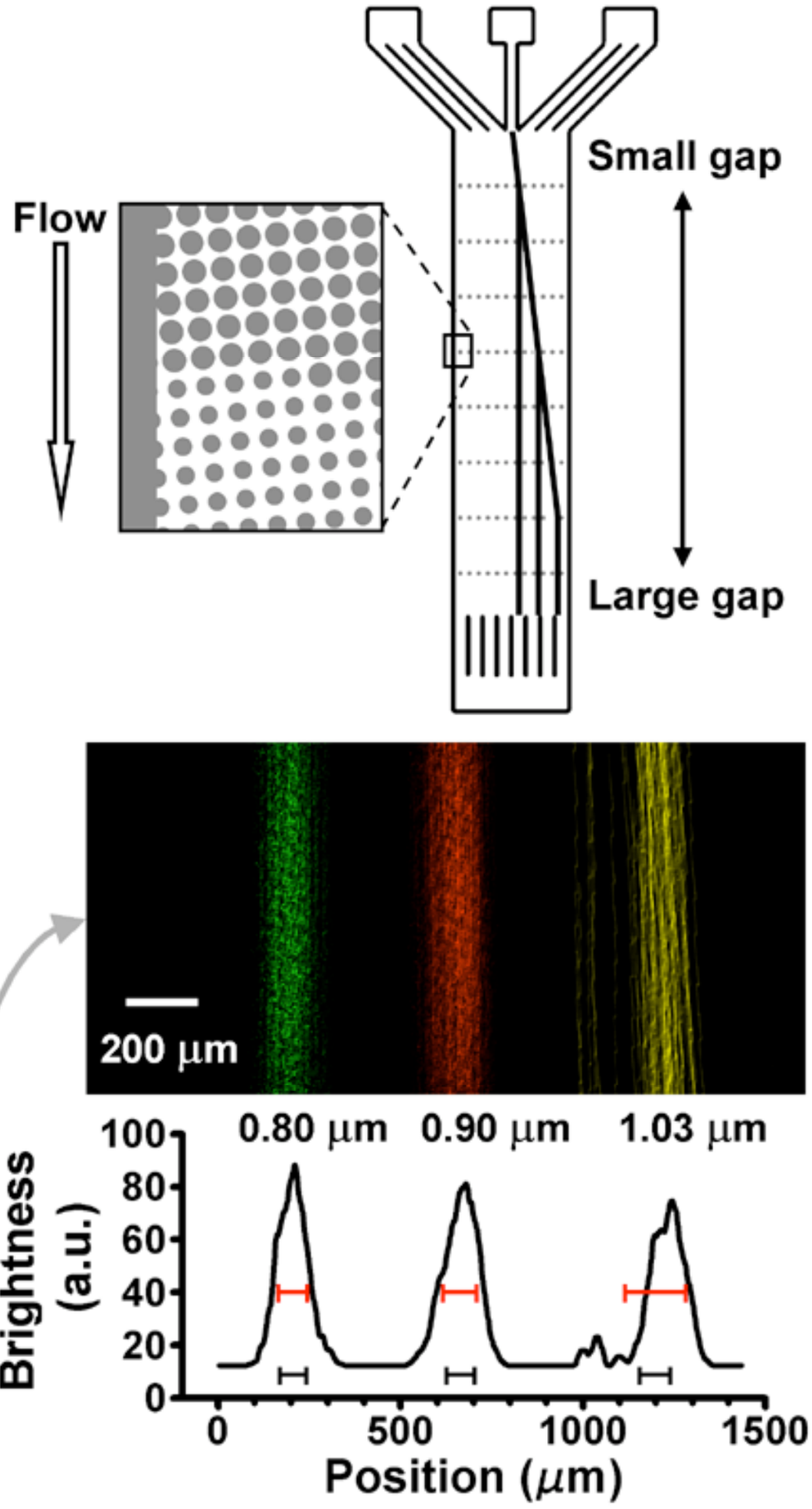
The FASTER the flow, the SHARPER the edge. This is the OPPOSITE many other techniques. The Peclet number, va/D, has to be >>1.

$$\varepsilon = \frac{\delta\lambda}{\lambda} = 1/10$$






1.4 μm
1 mm
1.5 μm
1.6 μm
1.7 μm
1.8 μm
1.9 μm
2.0 μm
2.1 μm
2.2 μm


Flow
Small gap
Large gap
200 μm
0.80 μm
0.90 μm
1.03 μm
Brightness (a.u.)
100
80
60
40
20
0
0
500
1000
1500
Position (μm)

Beautiful, careful work by David Inglis and John Davis

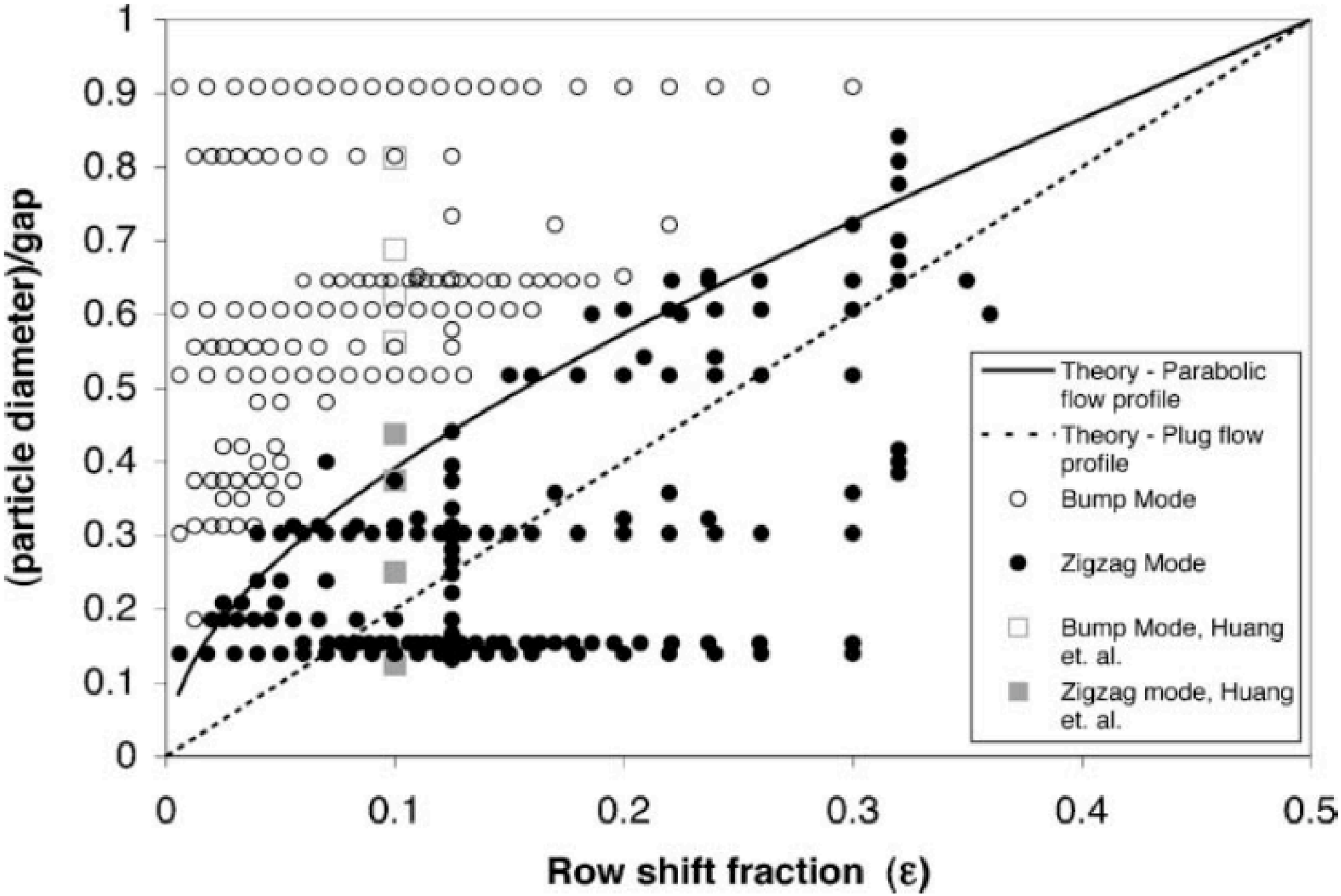
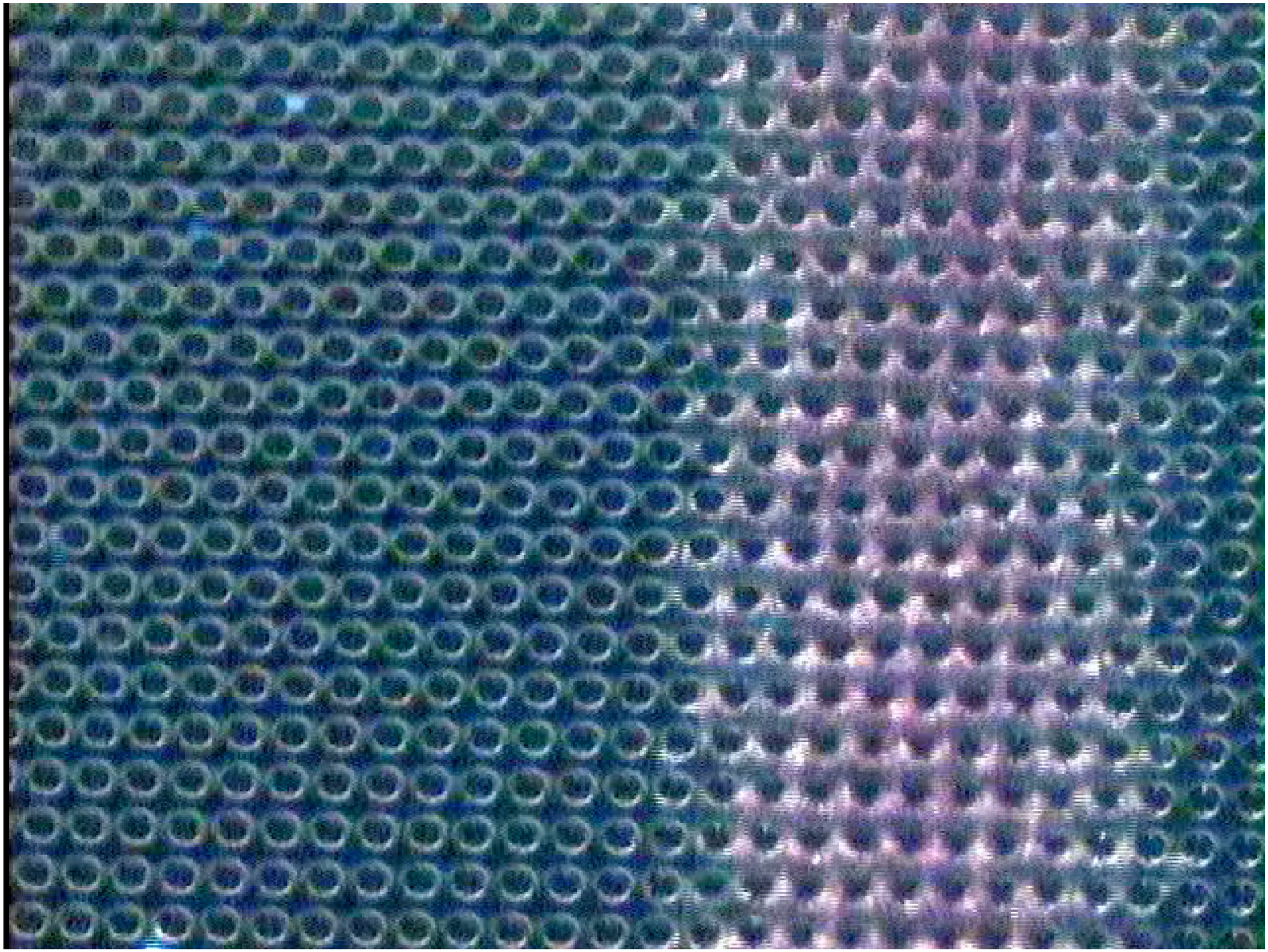


Table I: Design Principles of Bump Arrays

Design Principle	Mode	Non-clogging Dynamic Range
Fixed ϵ, G	Binary	Low
Chirped ϵ, fixed G	Stepped	Medium, good at large end
Fixed ϵ, chirped G	Stepped	Medium, good at small end
Cascade: chirped ϵ, G	Stepped	High at both ends

Table II: Operation Modes of Bump Arrays

Operation Mode	Injector Width
Analytical	Narrow, on order of G
High Throughput	Wide, on order array width



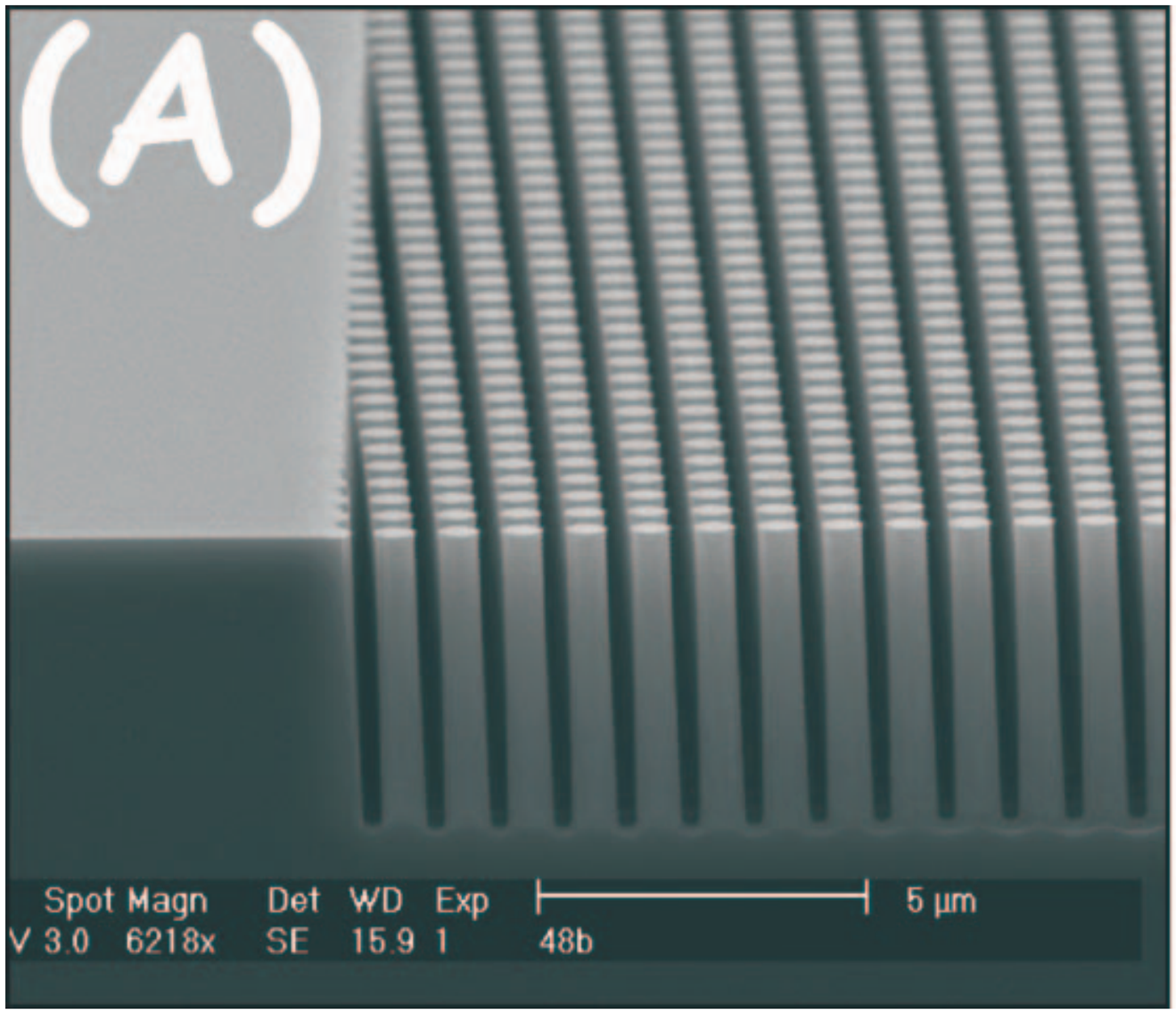
I have shown how the simple linear shifting of a row of obstacles from the previous row of obstacles resulted in the non-intuitive movement of particles at angles to the overall movement of the fluid carrying the objects, rather like a sailboat can move at an angle to the wind and can even move up against the wind to a certain degree.

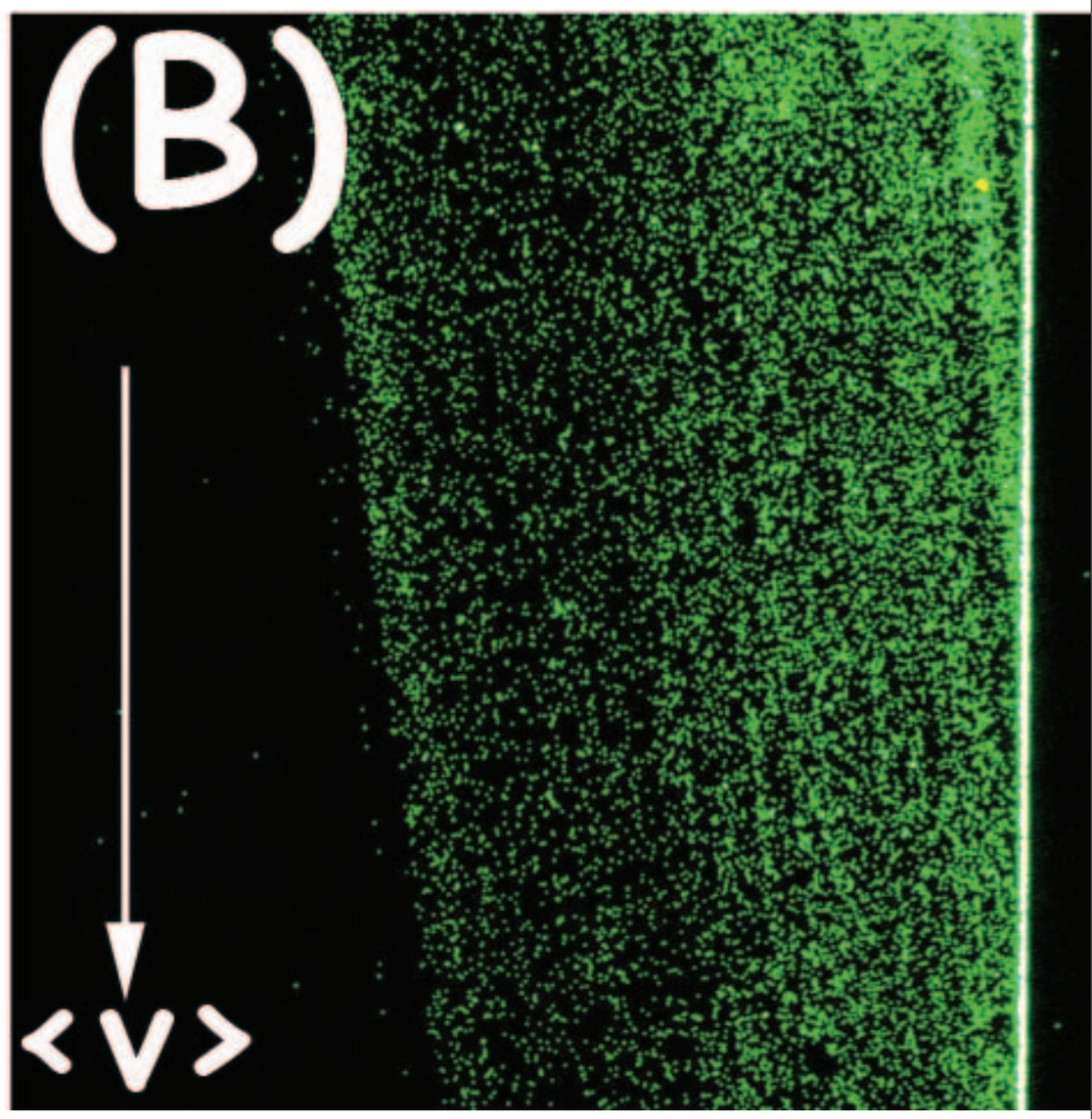
Ray optics: photons which move in straight lines and refracted into media where the speed of light is a function of color.

(don't like viewing optical tweezers as ray optics problem!)

Particles moving in laminar flow at high Peclet number in structured surfaces resemble that early but useful view of light and can be effectively refracted using a trick of making the media structured and anisotropic.

Our "refractive metamedia": particle size replaces wavelength: particles greater than a critical size can be made to move at an angle to the flow axis (in analogy to the extraordinary ray in birefringent media), while particles below a critical size move straight ahead. Big particle: short wavelength in normal dispersion relationship.


(A)
Spot Magn Det WD Exp
V 3.0 6218x SE 15.9 1 48b
5 µm


(B)
<V>

A hydraulic lens can be formed!

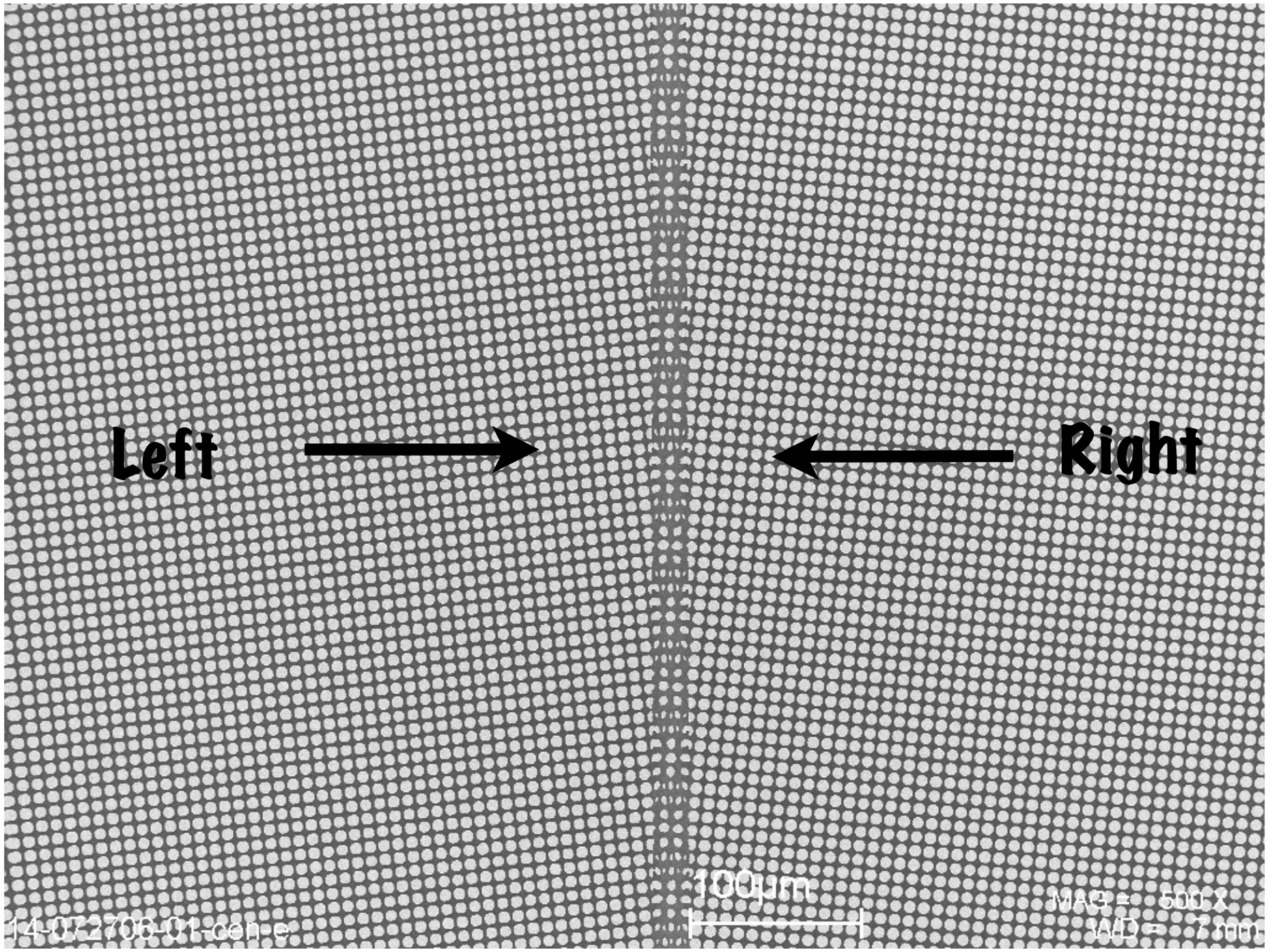
(1) Split into metamaterial two regions of different symmetry.

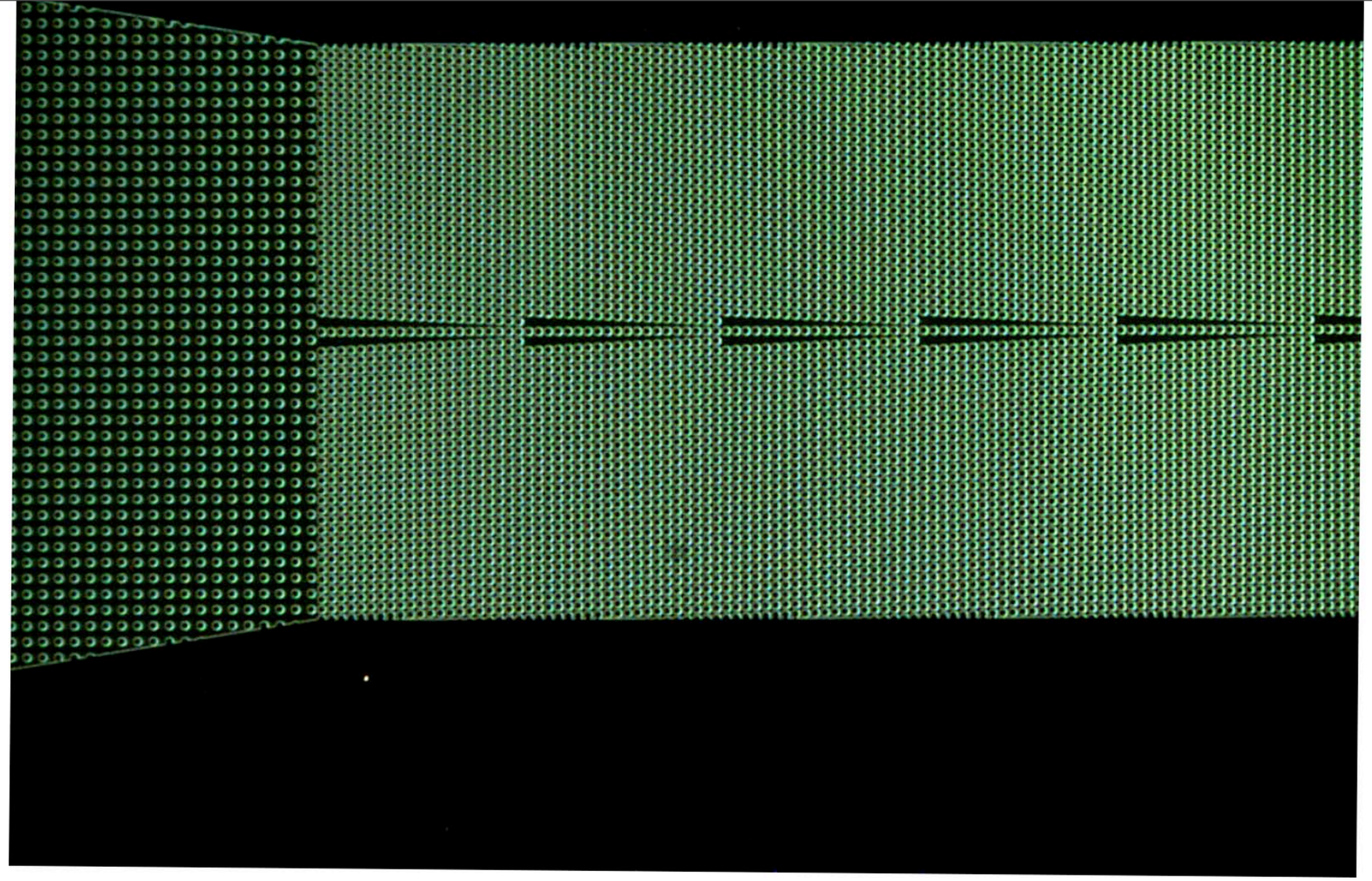
(2) L region on the right hand side of the flow channel which moves large objects to the left

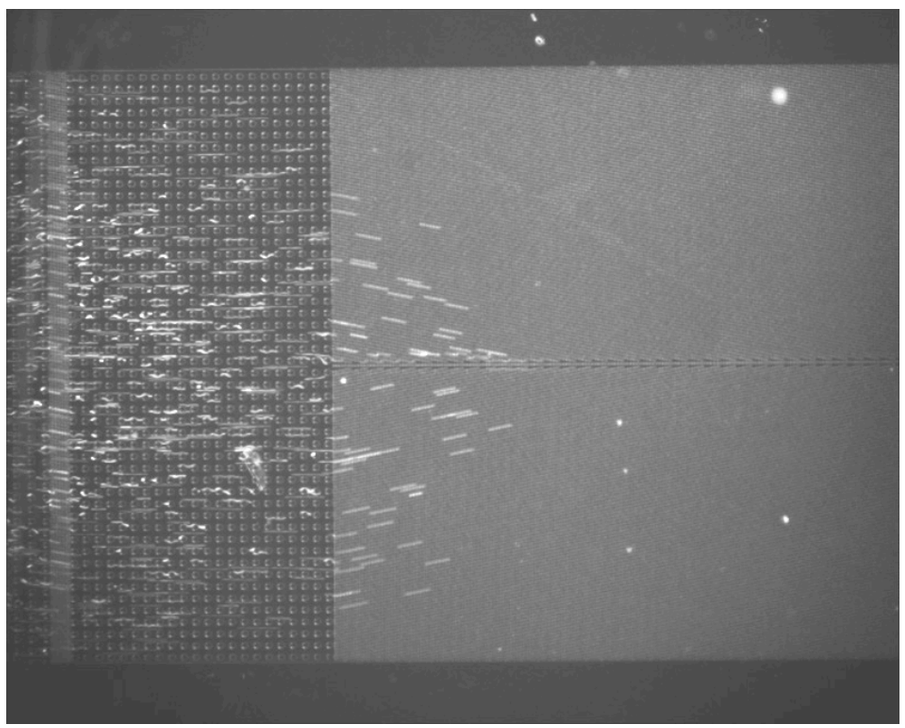
(3) R region on the left hand side of the flow channel.

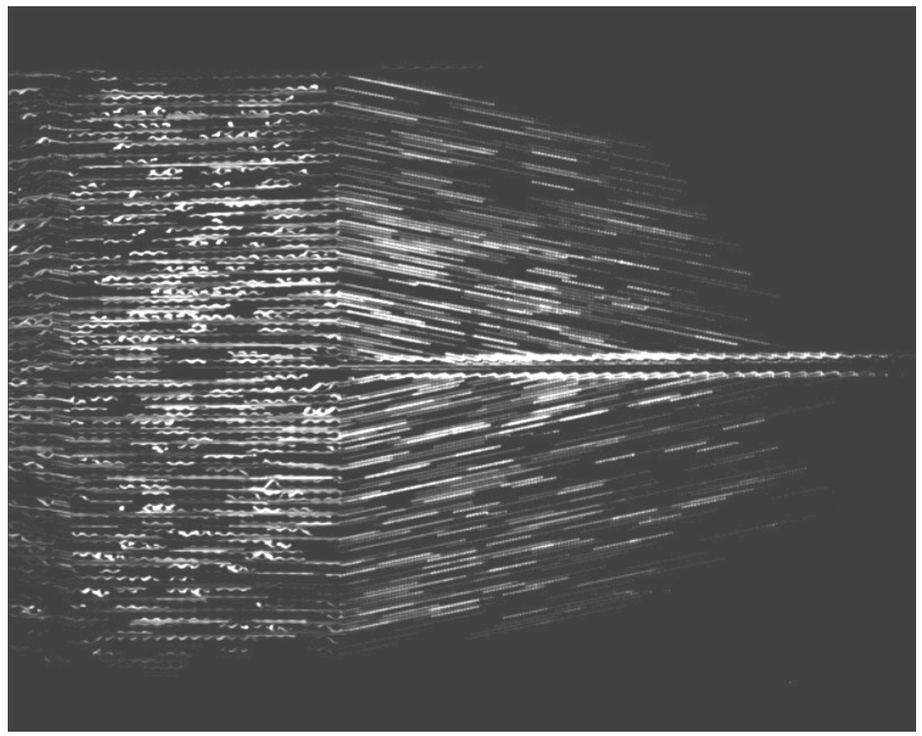
Particles above a critical radius will thus be actively focused to a line as they move down the channel, while those below a critical size will simply move unfocussed. point and which has a binary dispersion vs. particle size.

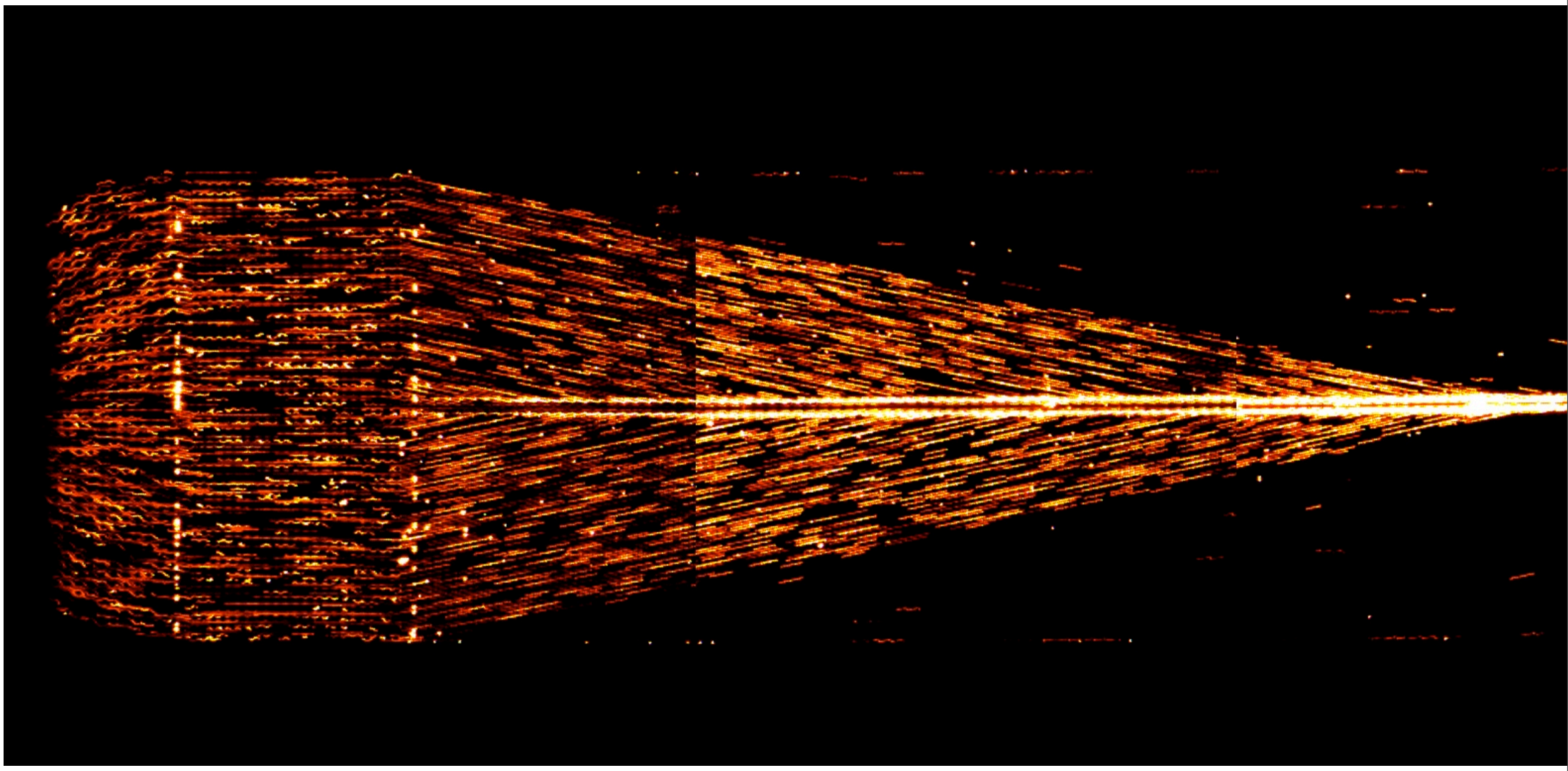
One can view this structure as representing a dispersive positive (+) lens, of a rather strange sort which focusses to a line rather than a point, like cylindrical optics (Bessel beams).



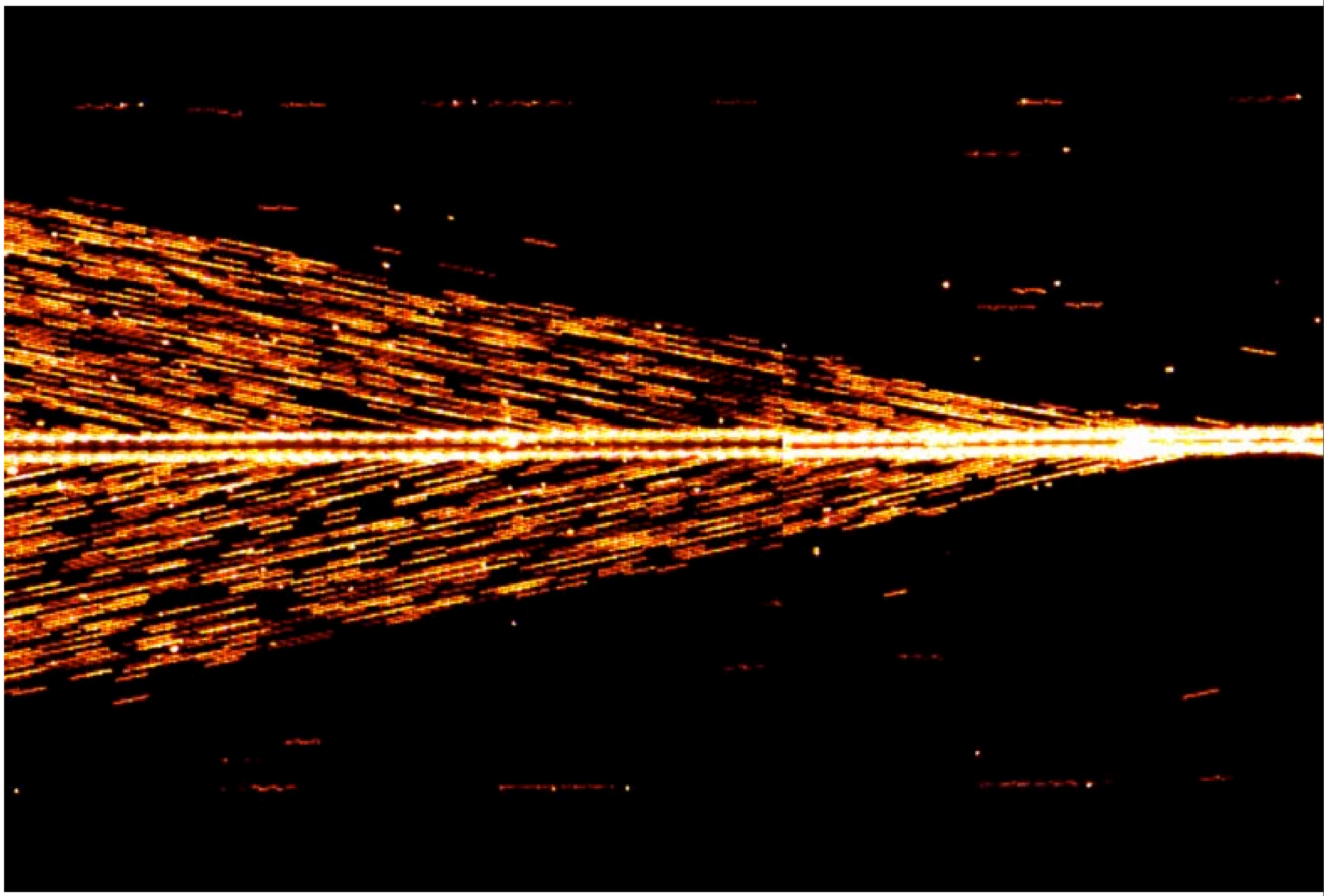






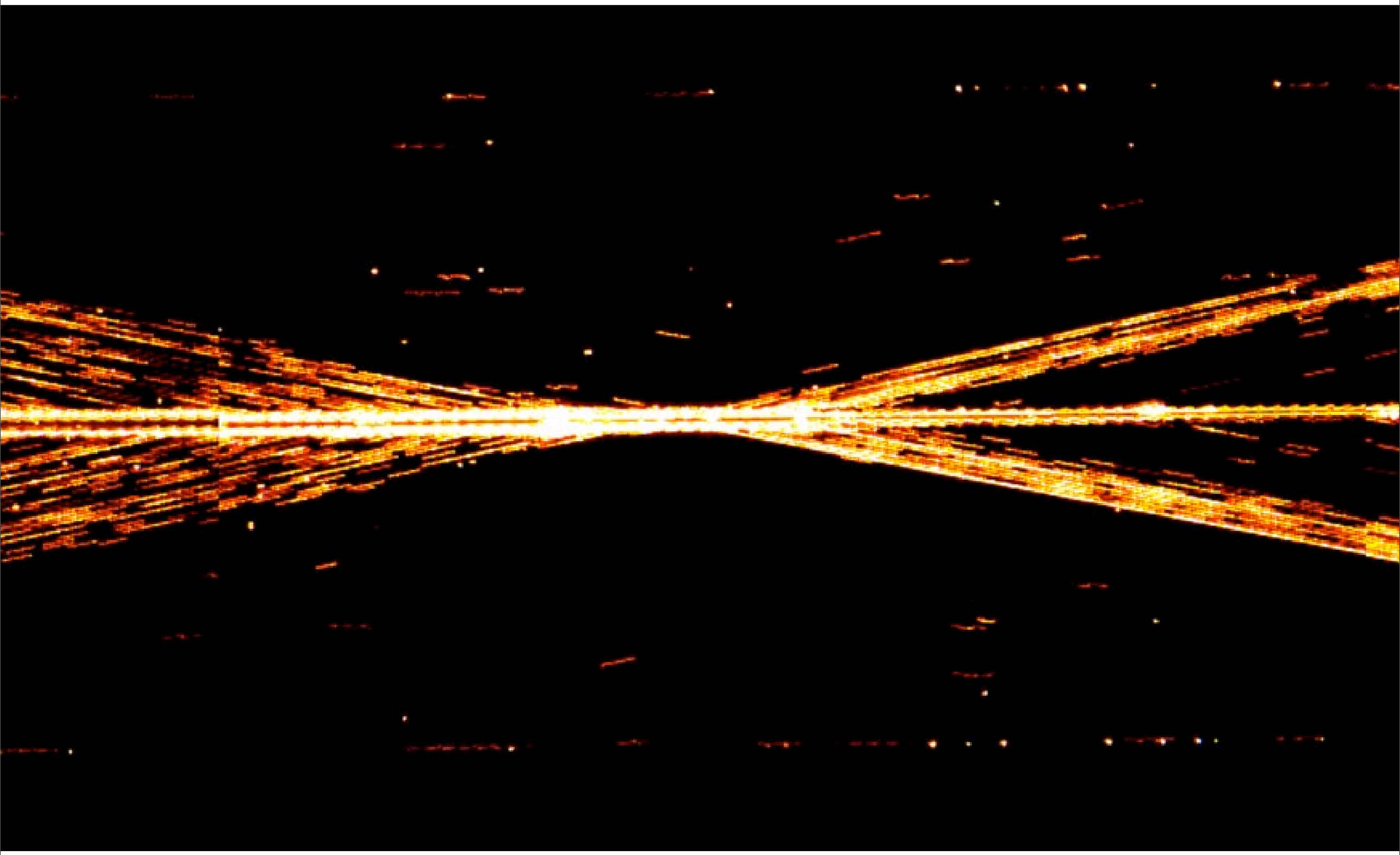


+ lens focuses to a line, not a point

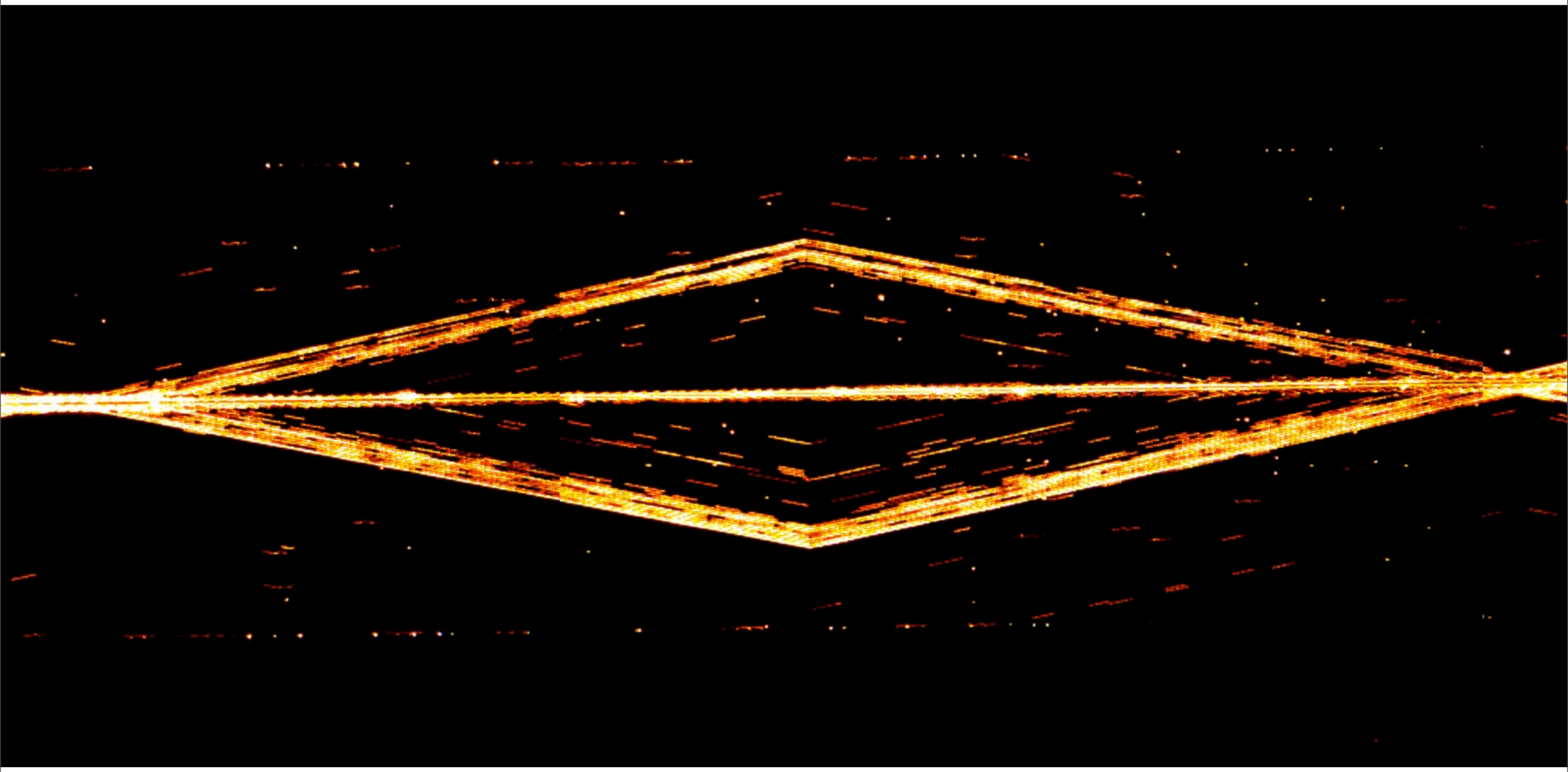


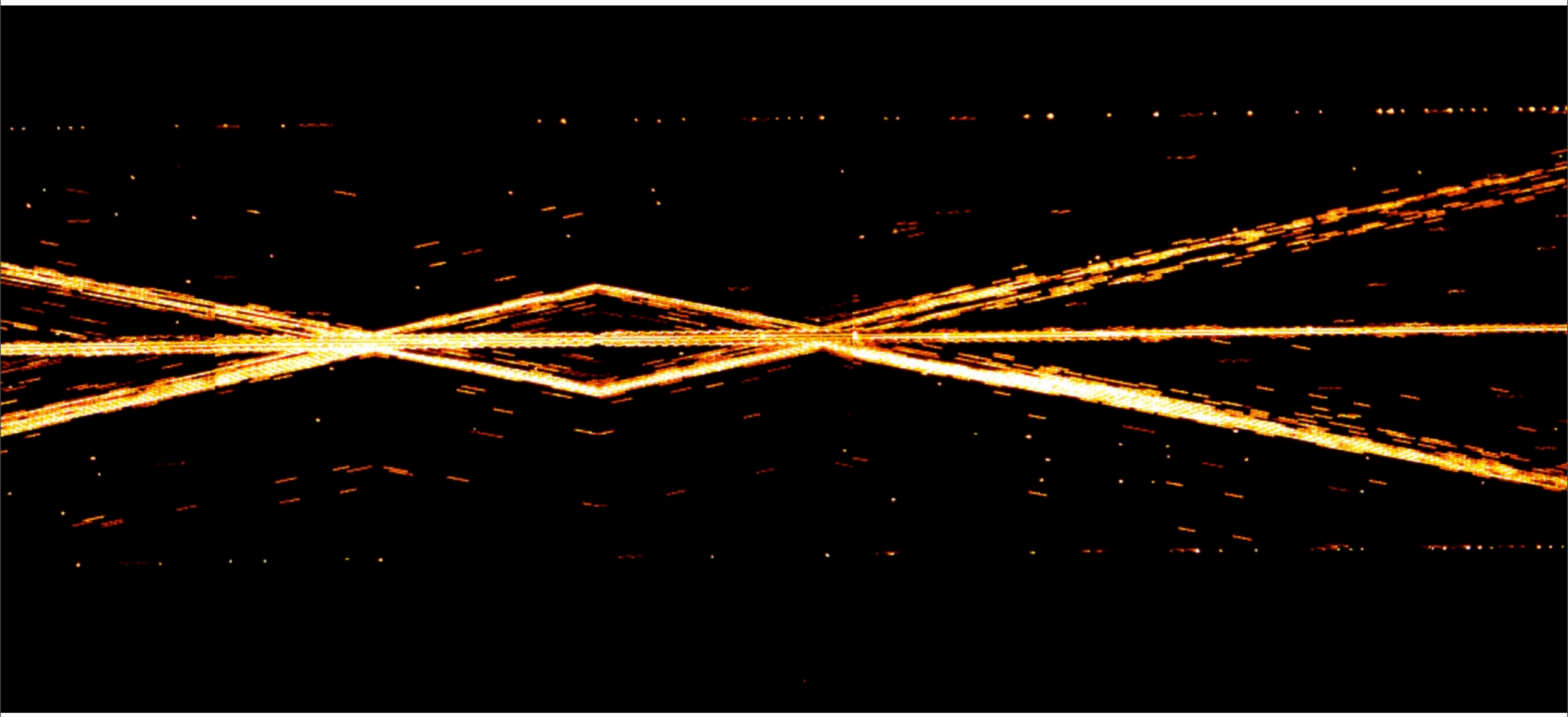
A -F metamaterial would simply be the mirror image of the +F material, so that objects above a critical size will move away from the focal line rather than towards it, defocusing.

Rather strange appearance!



If the sign of the matamaterial is flipped to a -F hydronic region, the balls will now be dispersed but as cones along the clear axis: that is, they do no re-disperse into the original stream. In that sense our hydronic optics are irreversible in their influence on the particles and change the phase space of the particles, in violation of the brightness theorem of regular optics.





Playing games with the focal lengths.

The implications of this discovery for nanobiotechnology are up to your imagination.

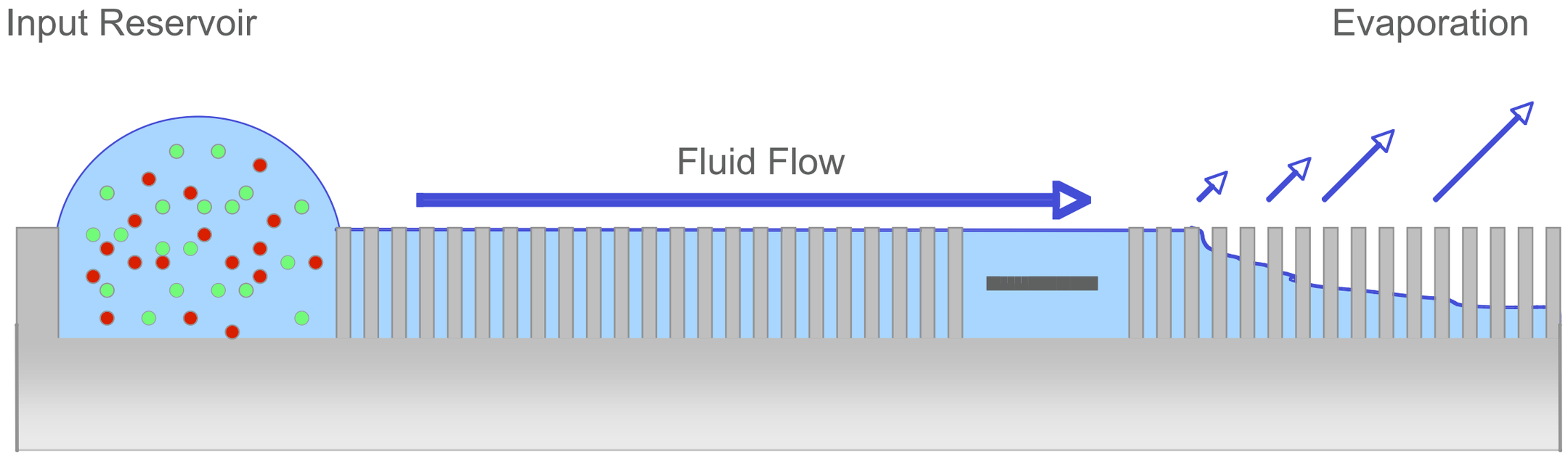
For example: Since we can now treat particles as light rays and design arrays of obstacles as optical elements, we can bring objects together using our optics, we can mix them, we can move them apart, and since our hydraulic optics are sensitive to the size of the object, we can separate particles that interact from particles that don't interact with each other.

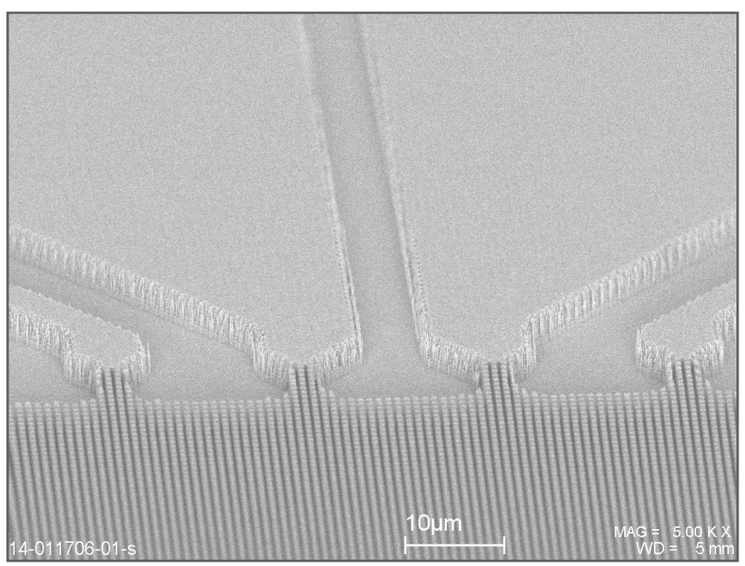
This could address a fundamental problem in biology: measuring protein-protein interactions.

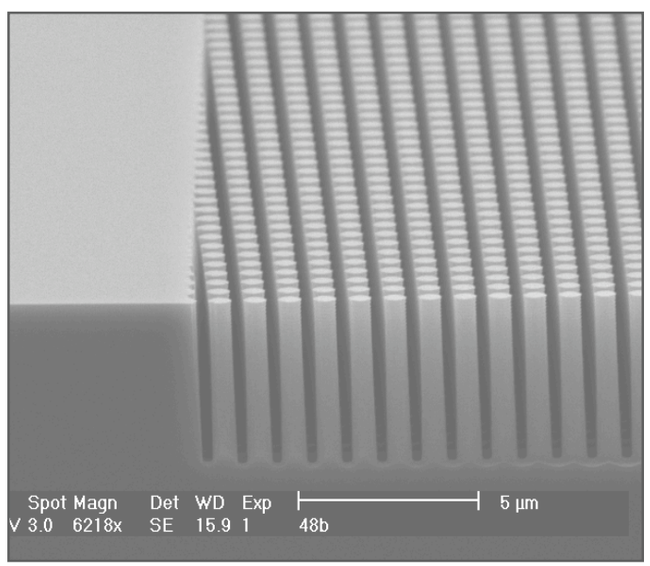
Connection with the Lotus-Leaf effect: what happens if I leave the top on these nanometa-surfaces open rather than sealing them? Can you get directed flows on open surfaces?

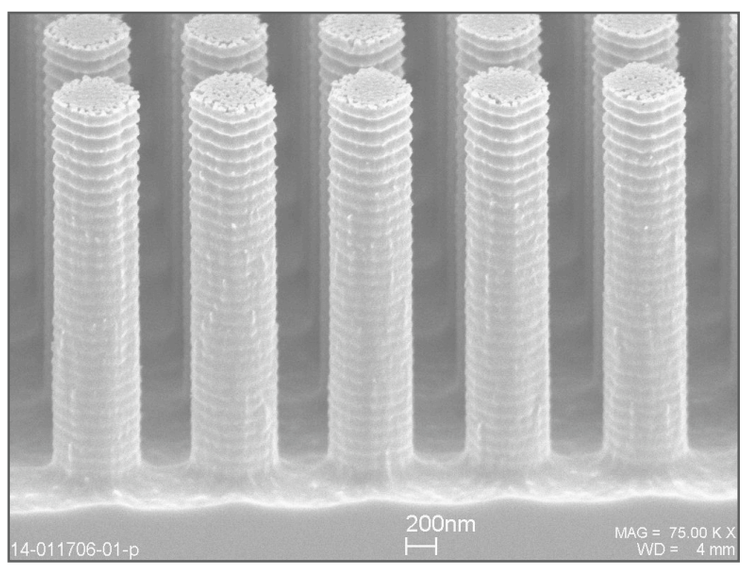
This is an important question for me, since as you go to nanoetched surfaces the applied pressure gradients needed to keep the flow velocities high is rather daunting (10's of bar/cm).

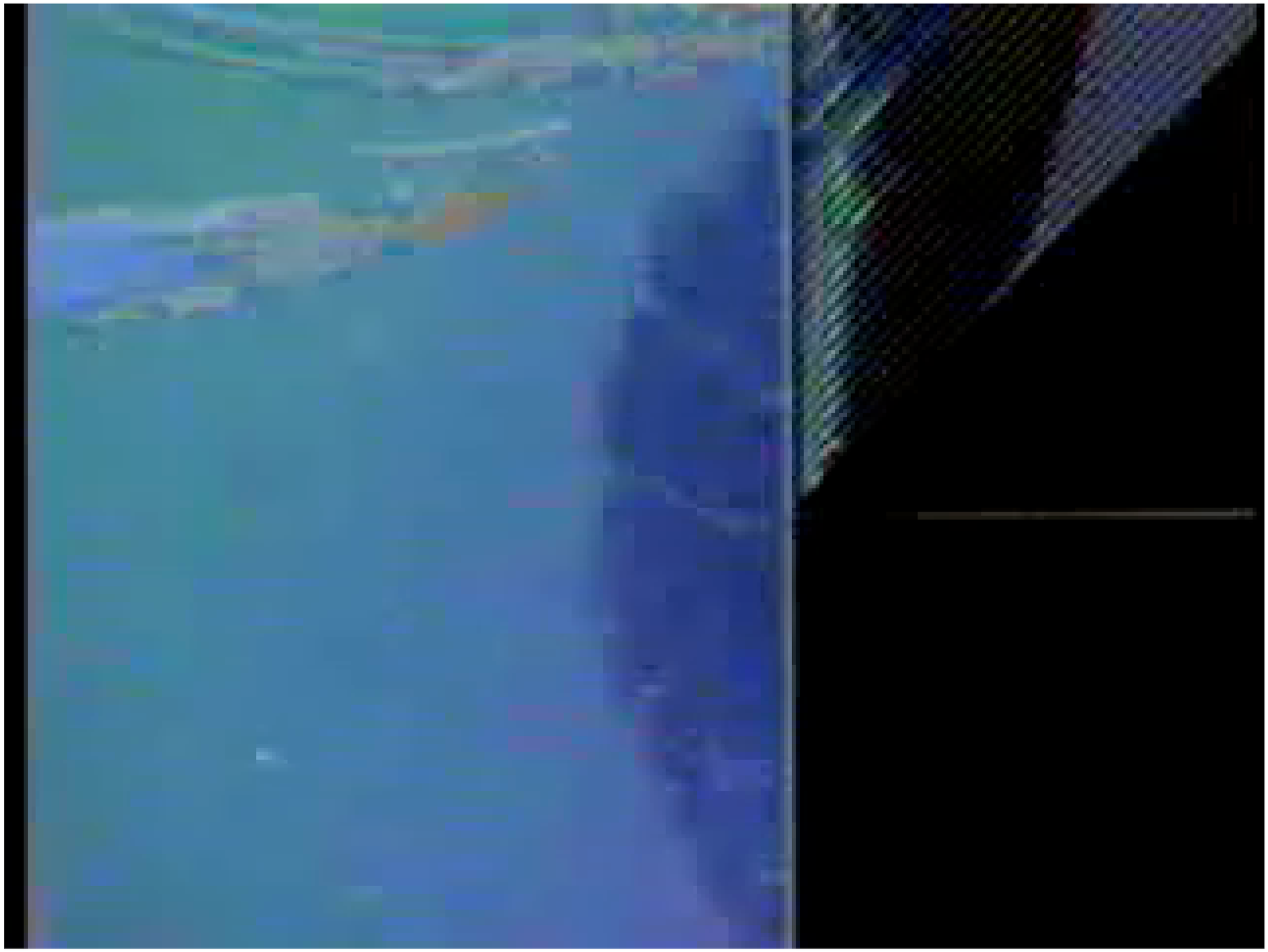
Schematic: Open Channel Flow



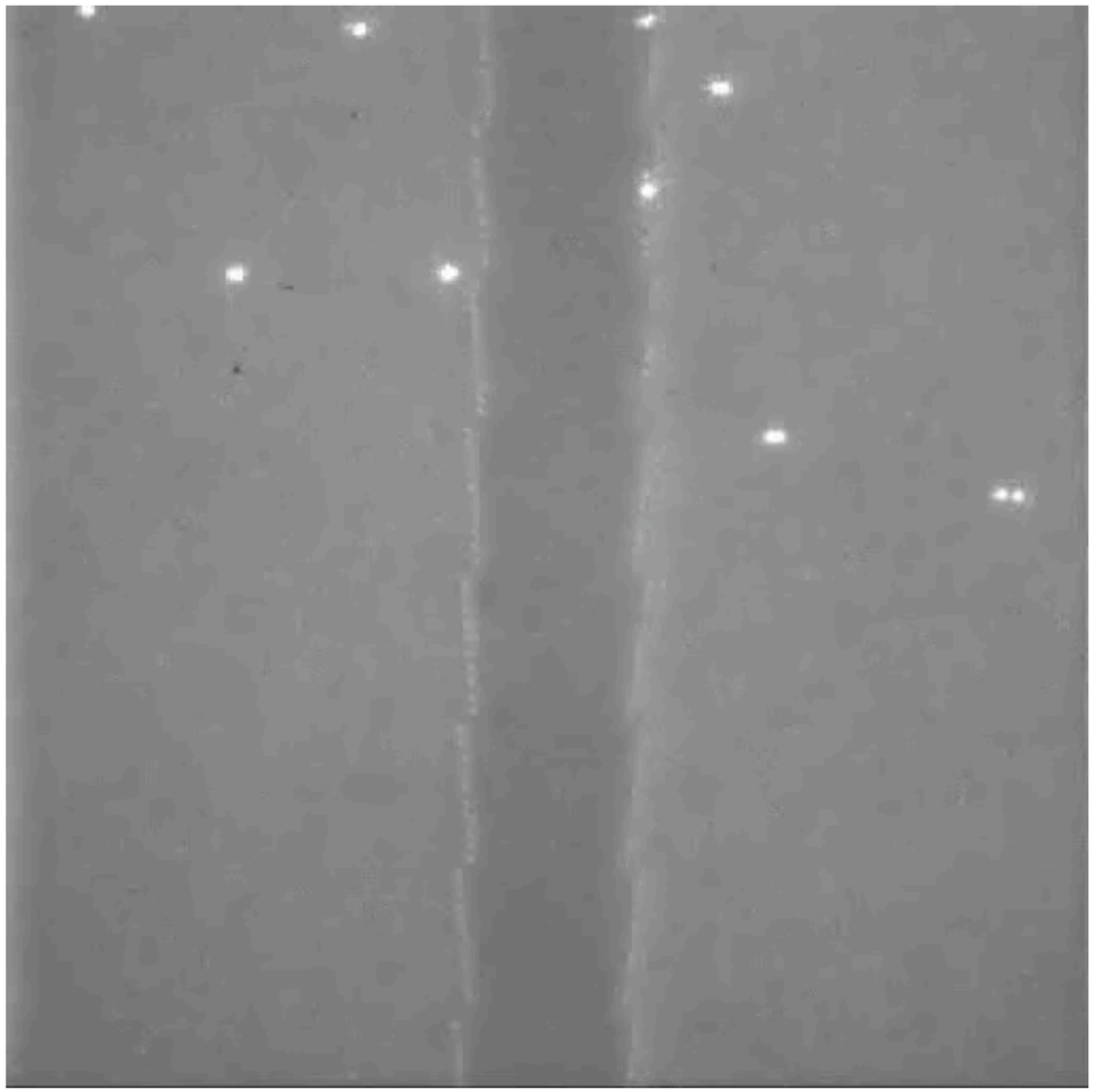


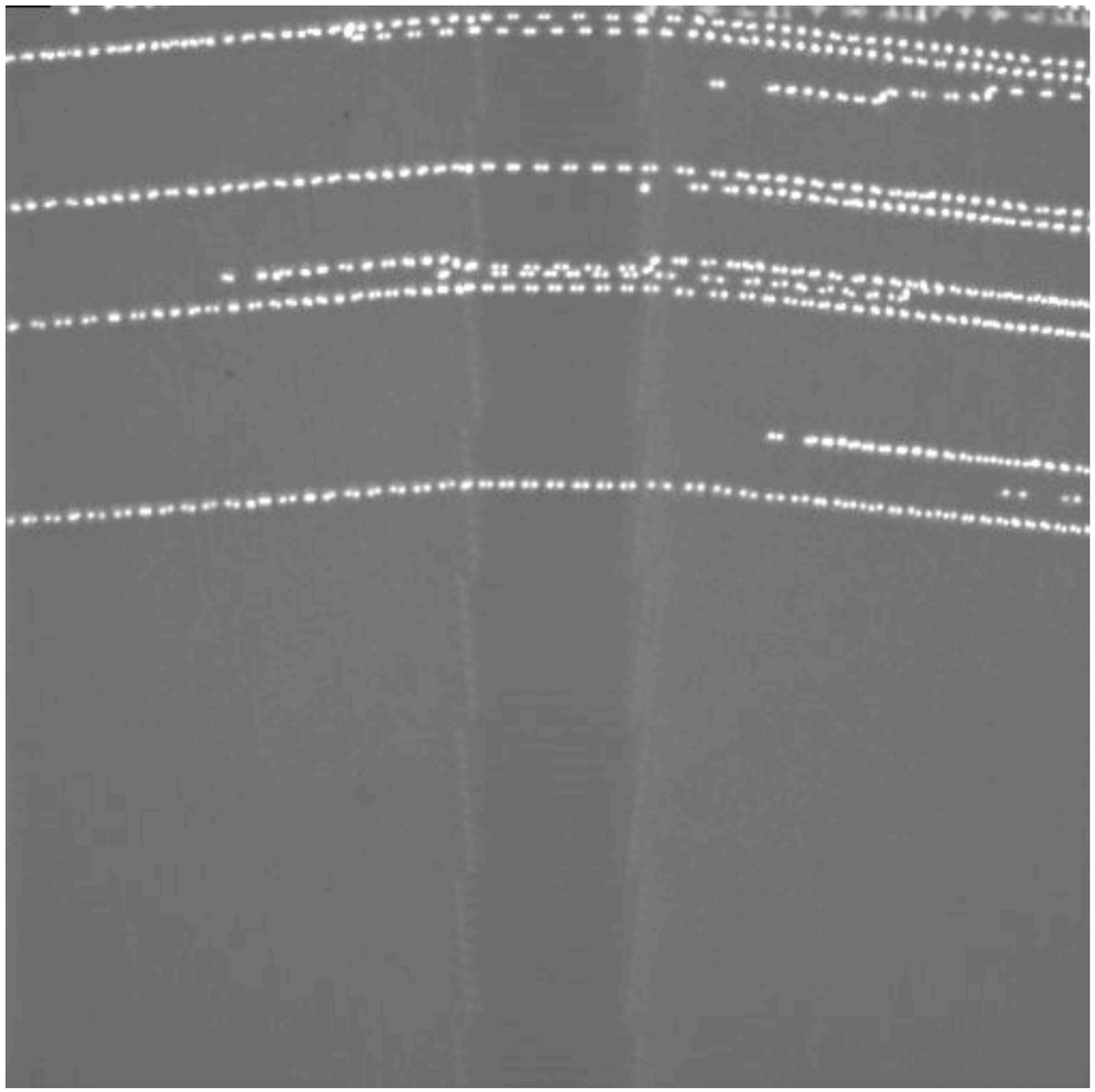






Works!





Directed motion on the surface of a chip with no top.

Well, I actually know a lot about nano/ microfluidics and there are a lot of things I can do with such an open top anti-Lotus Leaf nanometamaterial surfaces, but there no time left.....

Your ideas are welcome!!

谢谢!