

Micro and Nano-Fabrication: Using Asymmetric Structures to sort biological objects

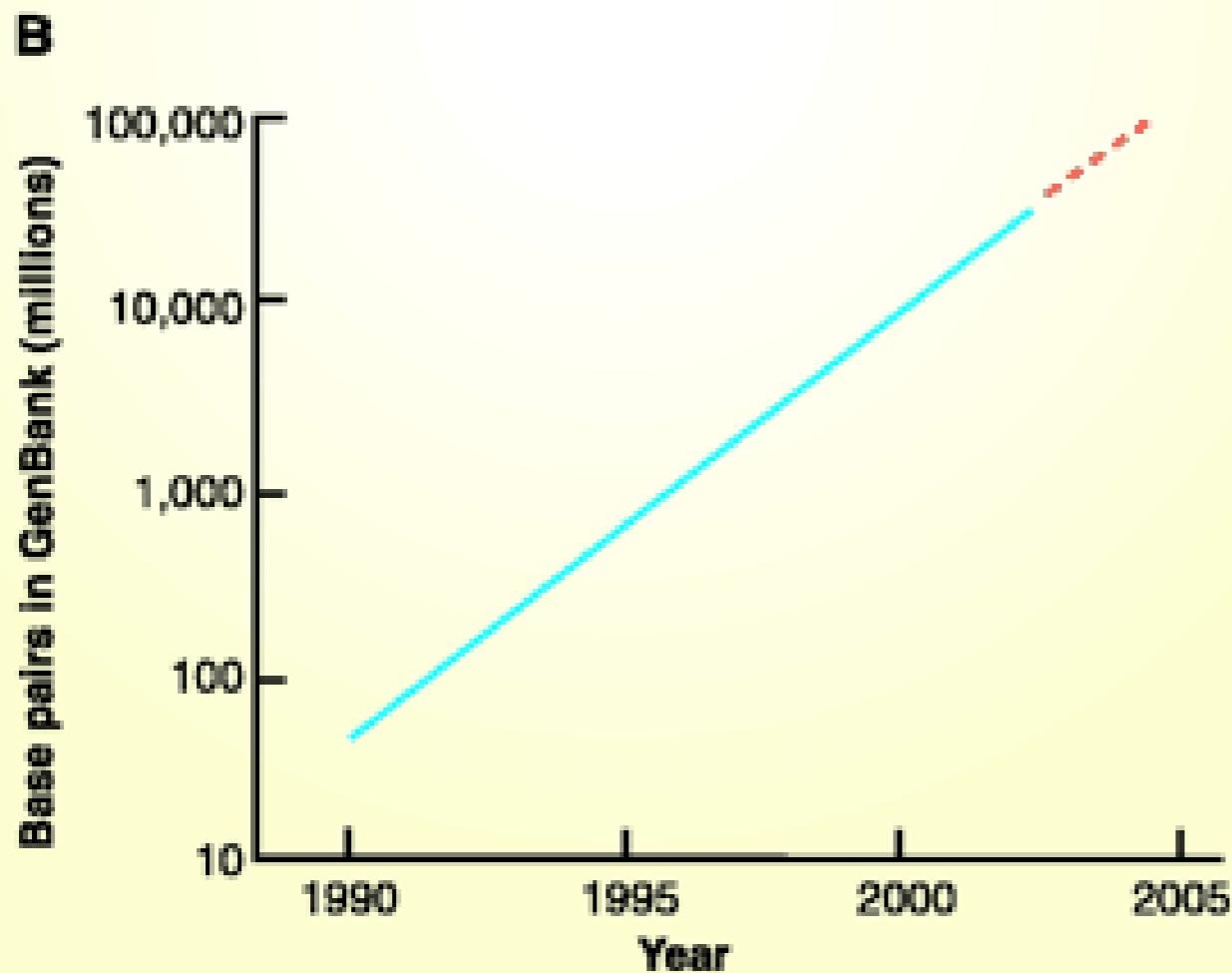
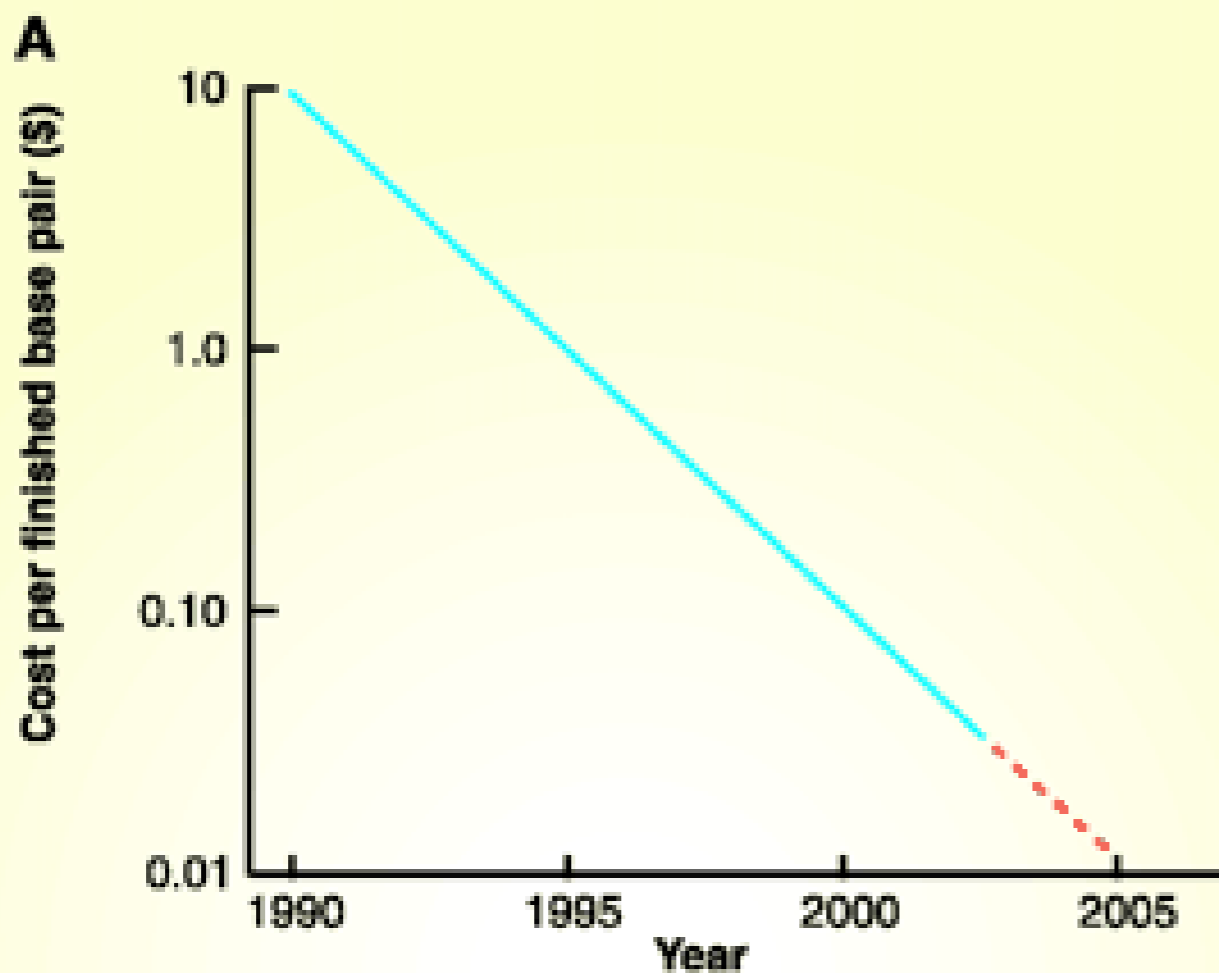
**Bob Austin
Princeton University**

with: Jim Sturm, Keith Morton, John Davis, David Inglis

Note Well: I am basically a total fraud, not in the lab near as much as I would like to me (but $\neq 0$), spend a lot of time in my damn office typing away.

This work is the product of hard-working students and postdocs, not me.

I also don't believe in Robotics, I think it is the wrong approach to the problems we are facing in biology.



**Molecular Biology
may face about the
same Moore's Law as
semiconductor
companies face: cost/
base has been
decreasing by factor
of 2 every 1.5 years**

Collins et al., Science

We won't get there via robotics, robotics doesn't change the face of biology.

The UNIVAC was 25 feet by 50 feet in length, contained 5,600 tubes, 18,000 crystal diodes, and 300 relays. It utilized serial circuitry, 2.25 MHz bit rate, and had an internal storage capacity 1,000 words or 12,000 characters.

It utilized a Mercury delay line, magnetic tape, and typewriter output. The UNIVAC was used for general purpose computing with large amounts of input and output.

Power consumption was about 120 kwatts. Its reported processing speed was 0.525 milliseconds for arithmetic functions, 2.15 milliseconds for multiplication and 3.9 Milliseconds for division.

The UNIVAC was also the first computer to come equipped with a magnetic tape unit and was the first computer to use buffer memory.

“Pure Hydrodynamics” has no diffusion,

just advective transport.

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

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

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.)

I'm going to talk about pure hydrodynamics in micro and nanofabricated structures today.

The ugly non-linear convective term:

$$(\mathbf{v} \cdot \nabla) \mathbf{v}$$

fortunately is small compared to the viscous drag term in the N-S equation if the following inequality is satisfied:

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

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

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 ($\ll 1$) forget Freshman Physics.

We will ALWAYS be in $Re \ll 1$ here.

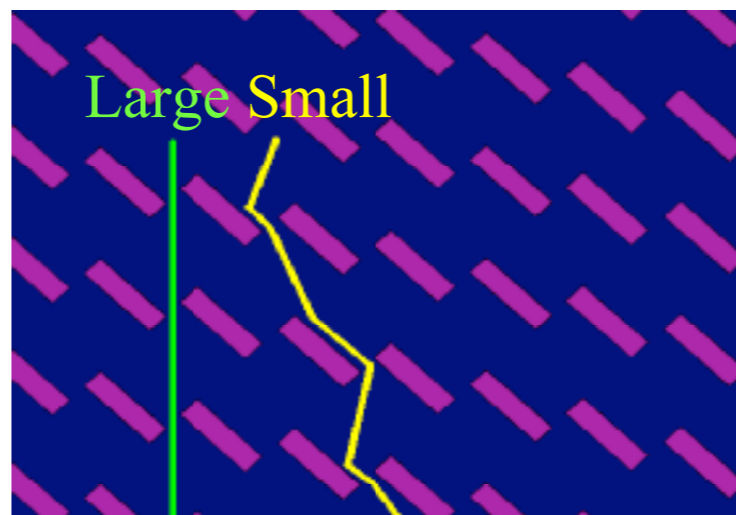
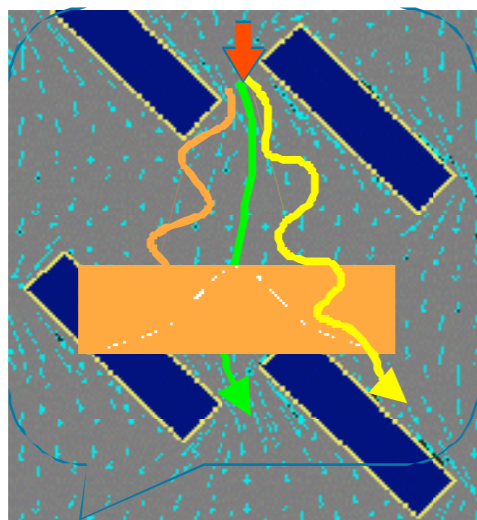
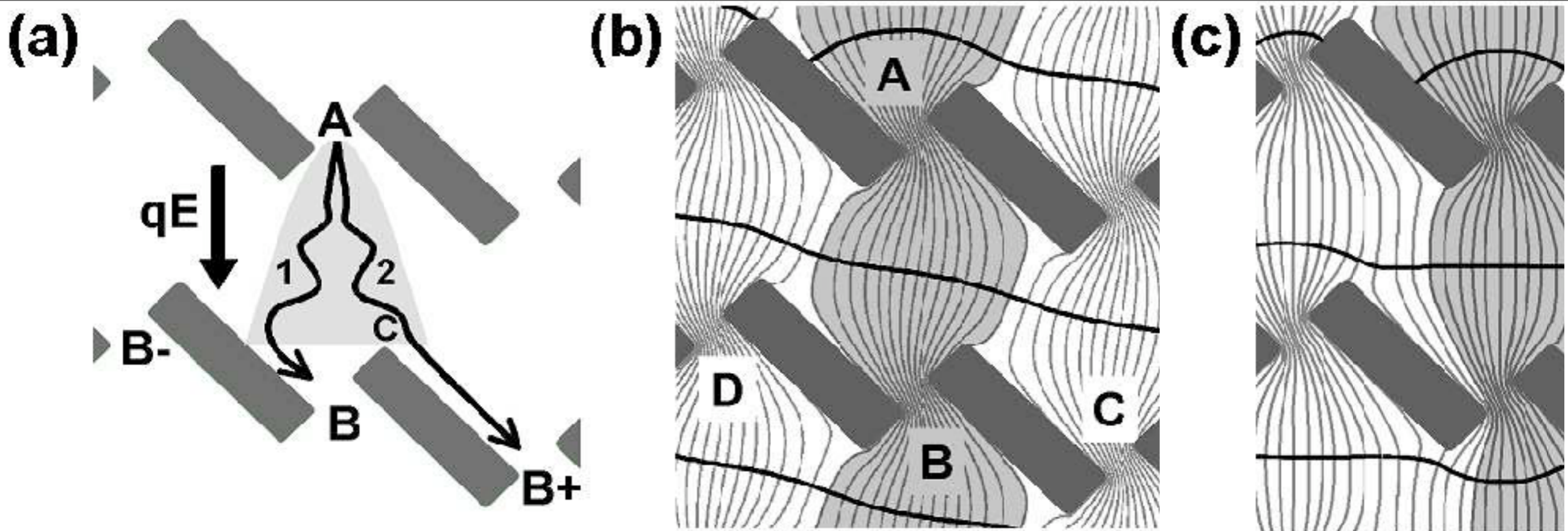
Re is of course known even by your parents and they are NOT impressed by you using it to describe mixing your coffee by advection to justify the outrageous cost of your Stanford education.

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?

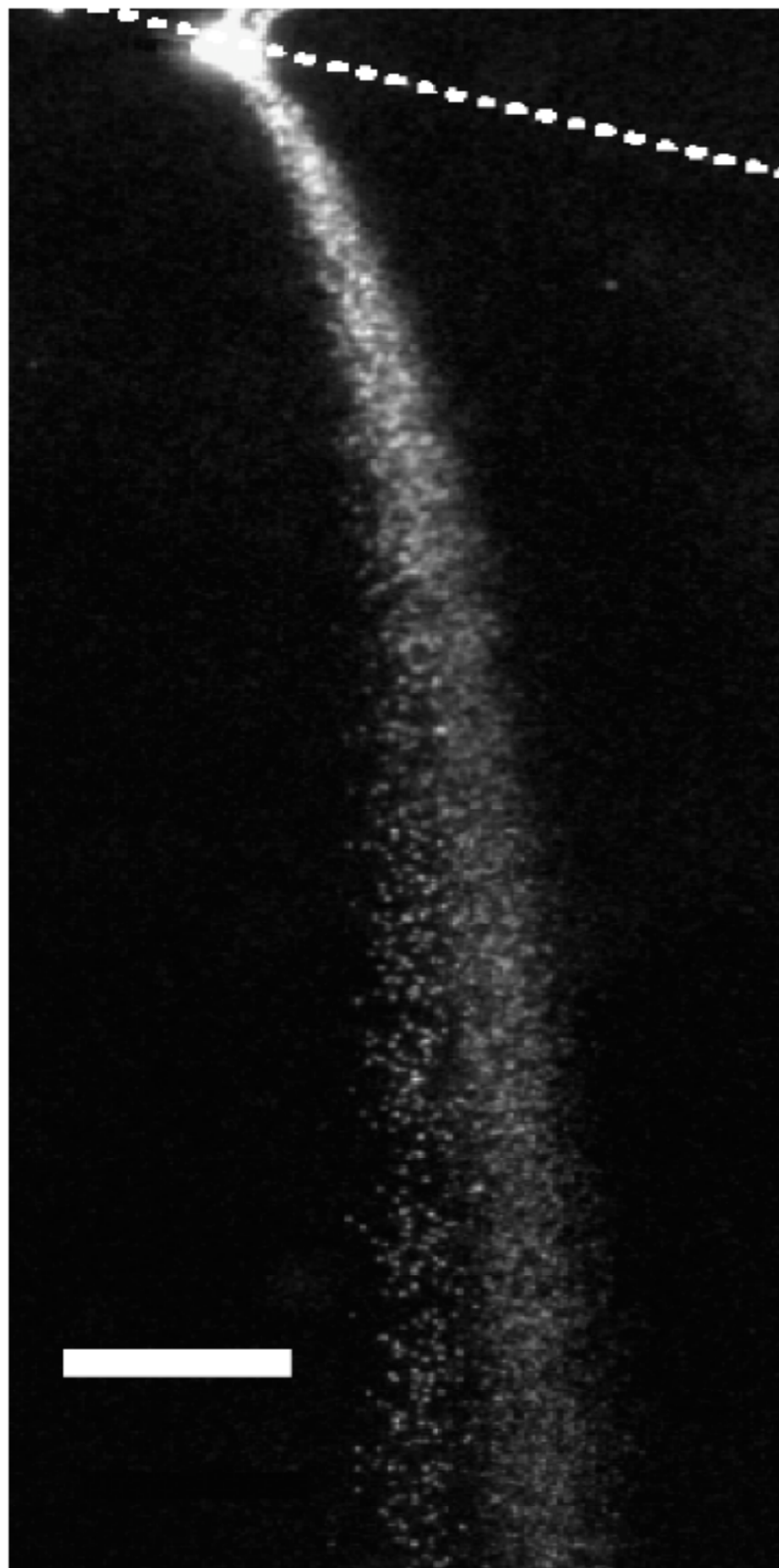
The Peclet number Pe is the ratio of the TIME to diffuse distance “a” to the time to advect that distance:

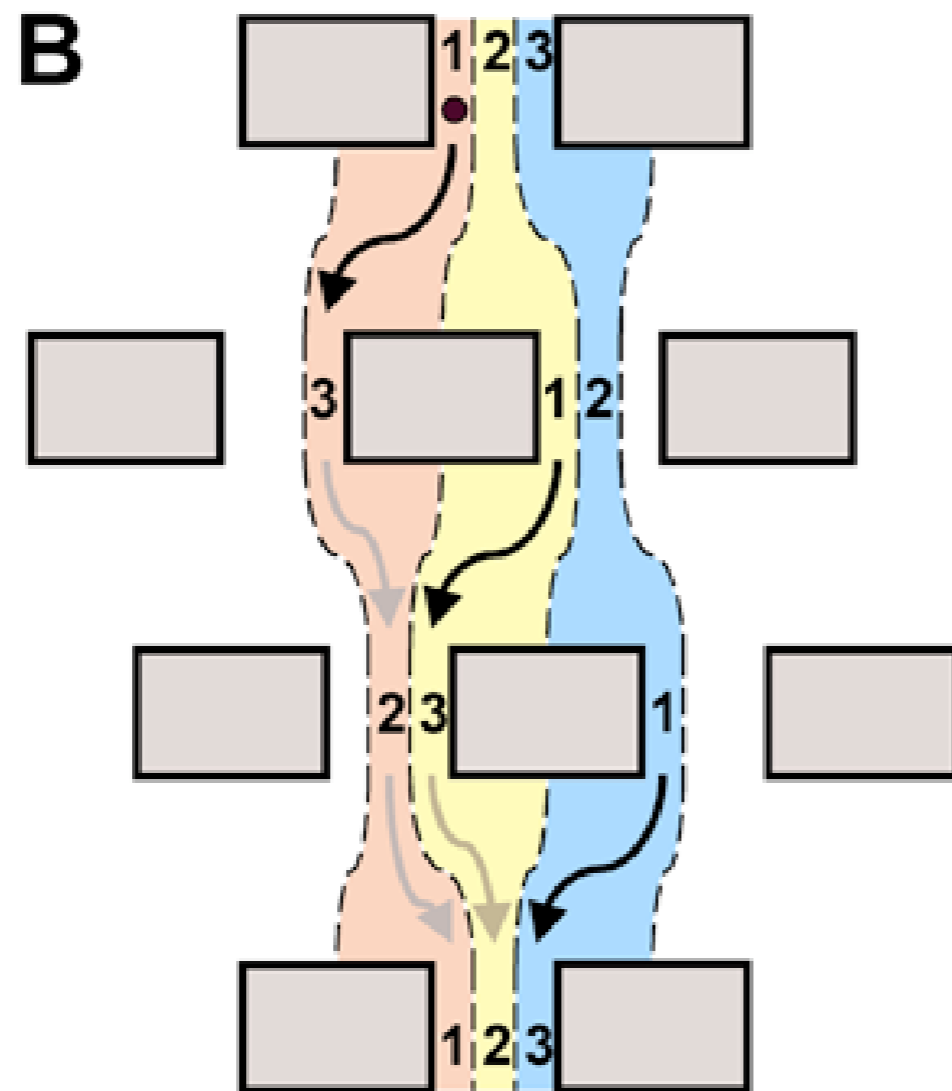
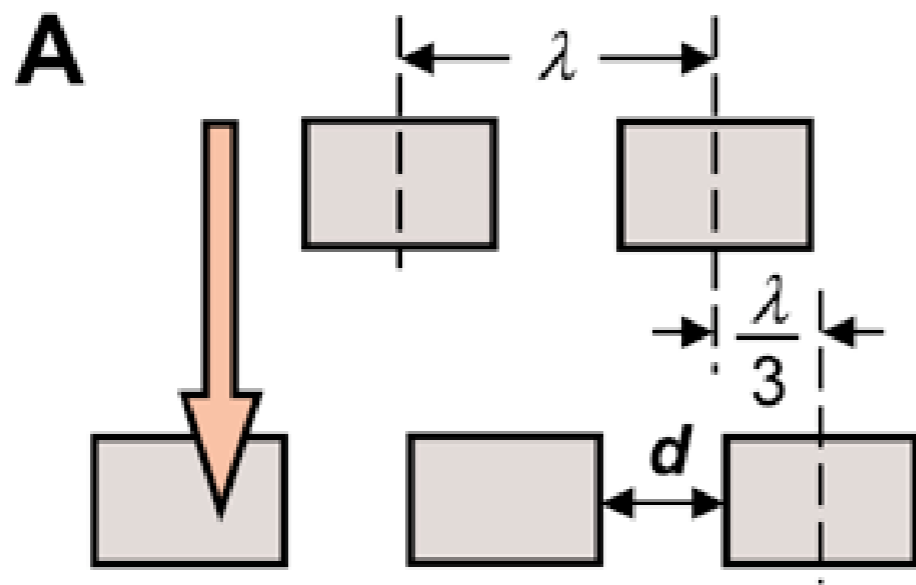
$$P_e = \frac{a^2/D}{(a/v)} = \frac{va}{D}$$

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



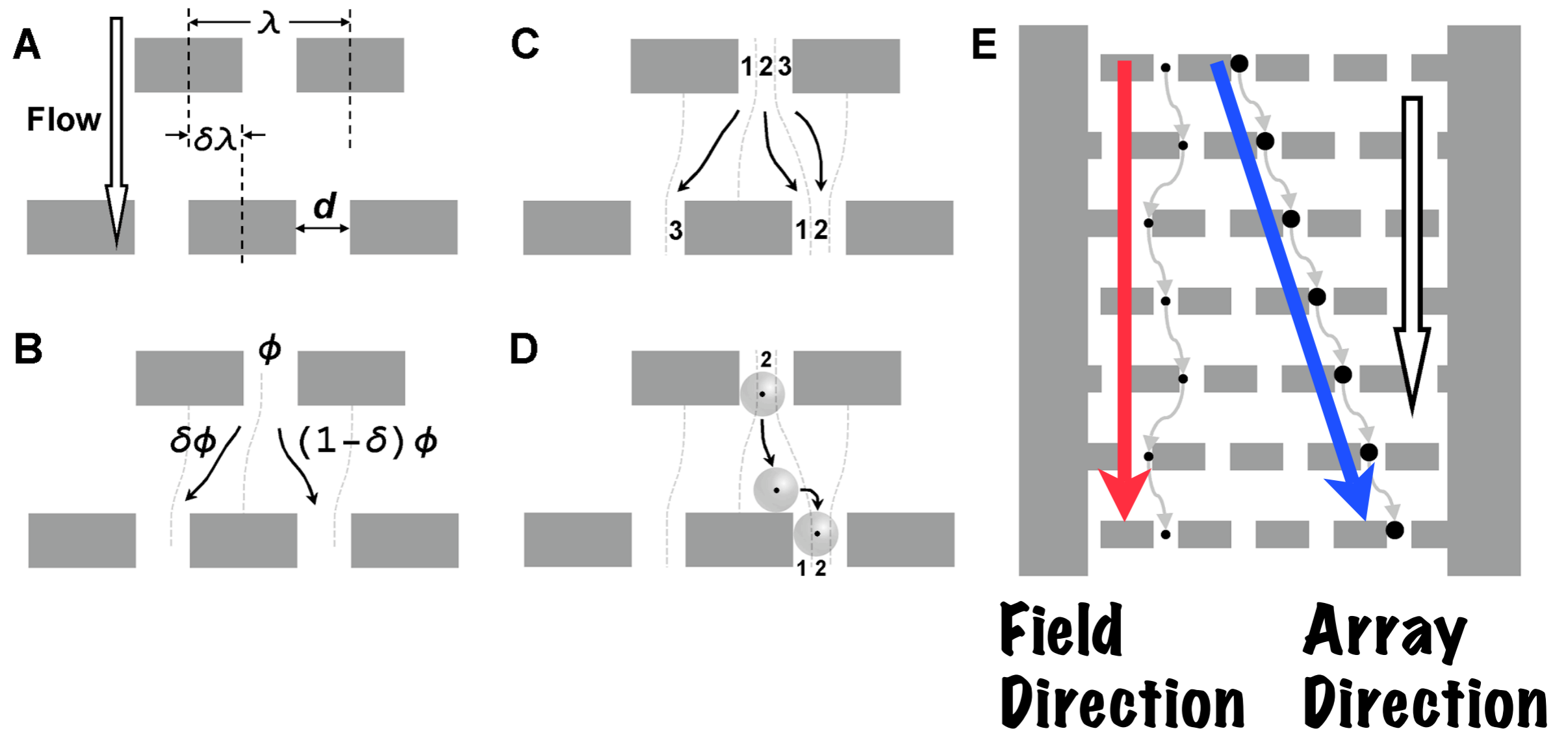
This device WORKS, but only for $Pe = 1$, transverse diffusion makes it go.



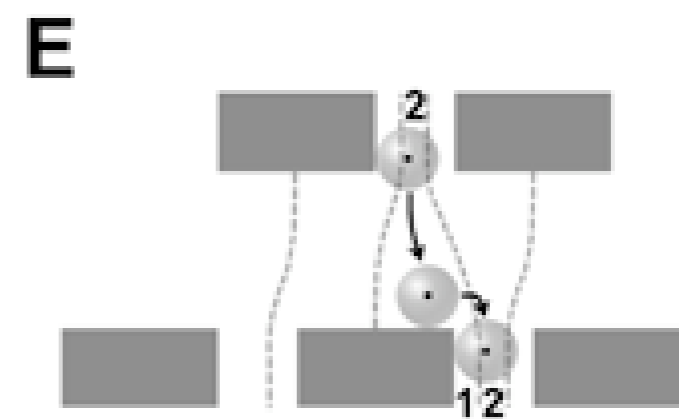
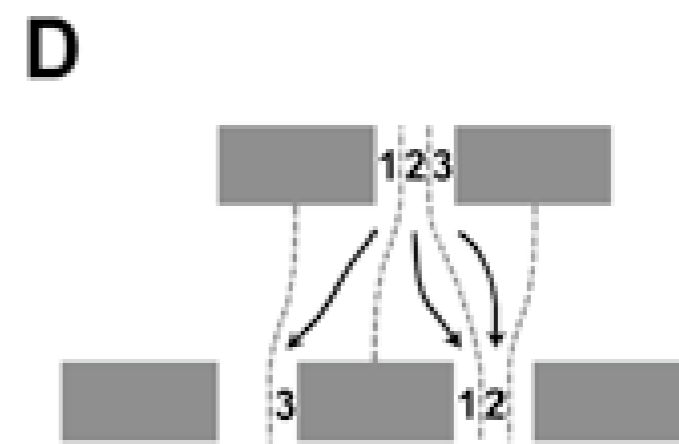
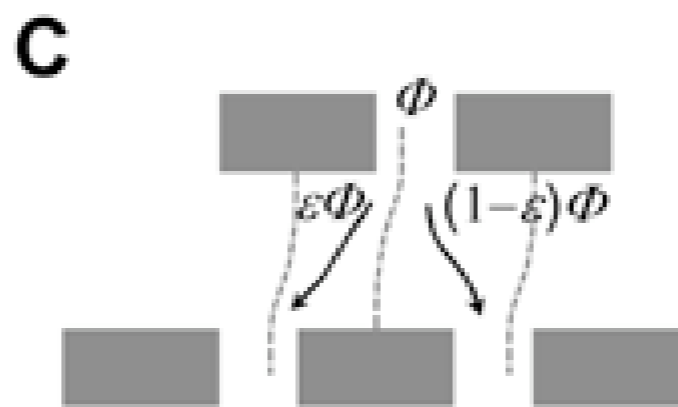
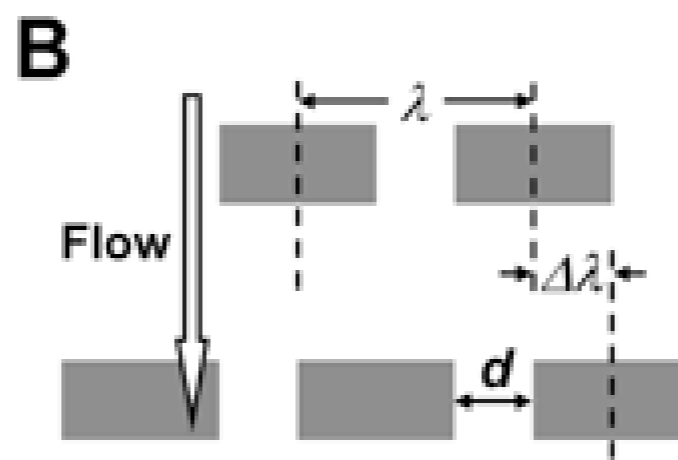
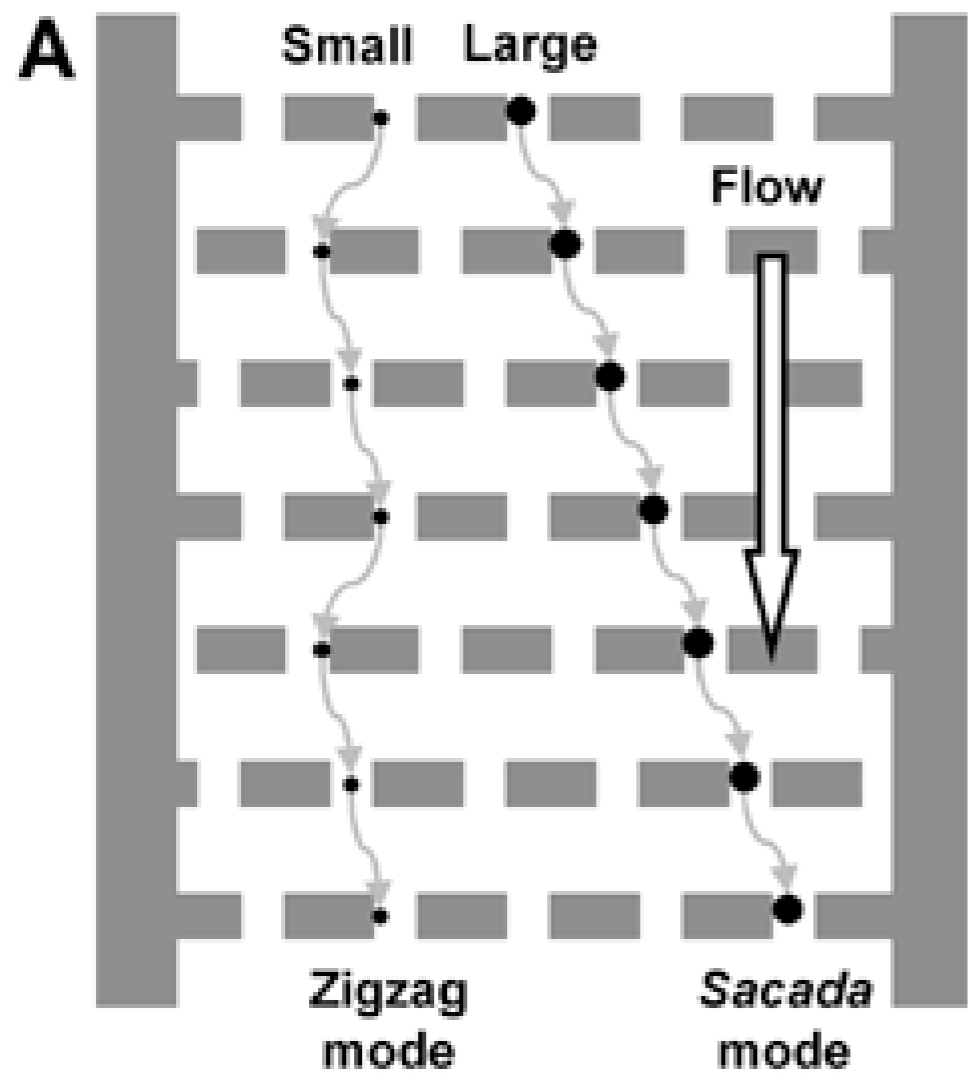


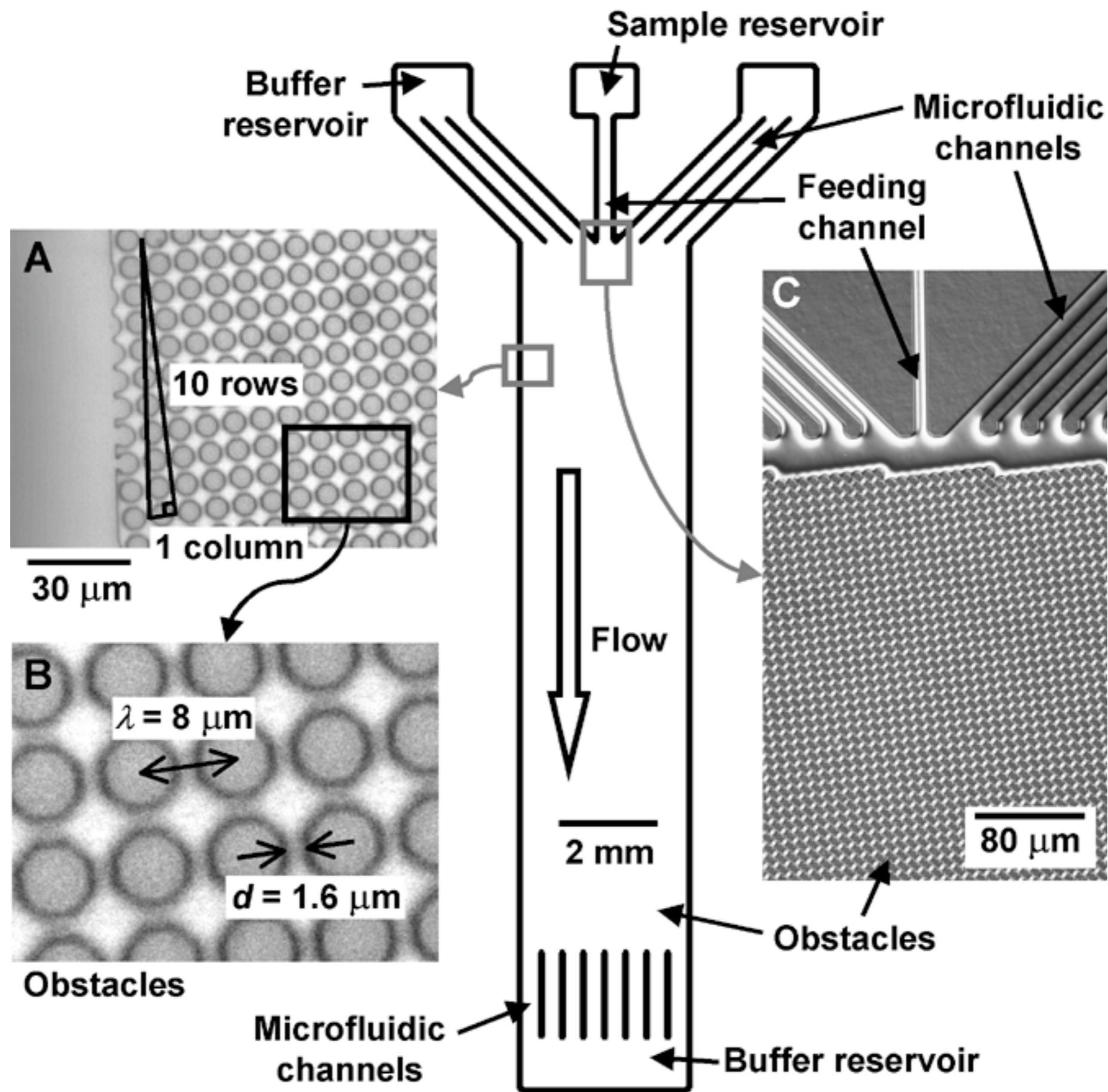
This is an array created by Richard Huang, it is **NOT** a brownian ratchet array, there is no direct path straight down the array as we had for the brownian array.

You have to dance step down the array, 1-3-2-1, etc. A step in the tango. This is tricky. If you get it, you are doing well.

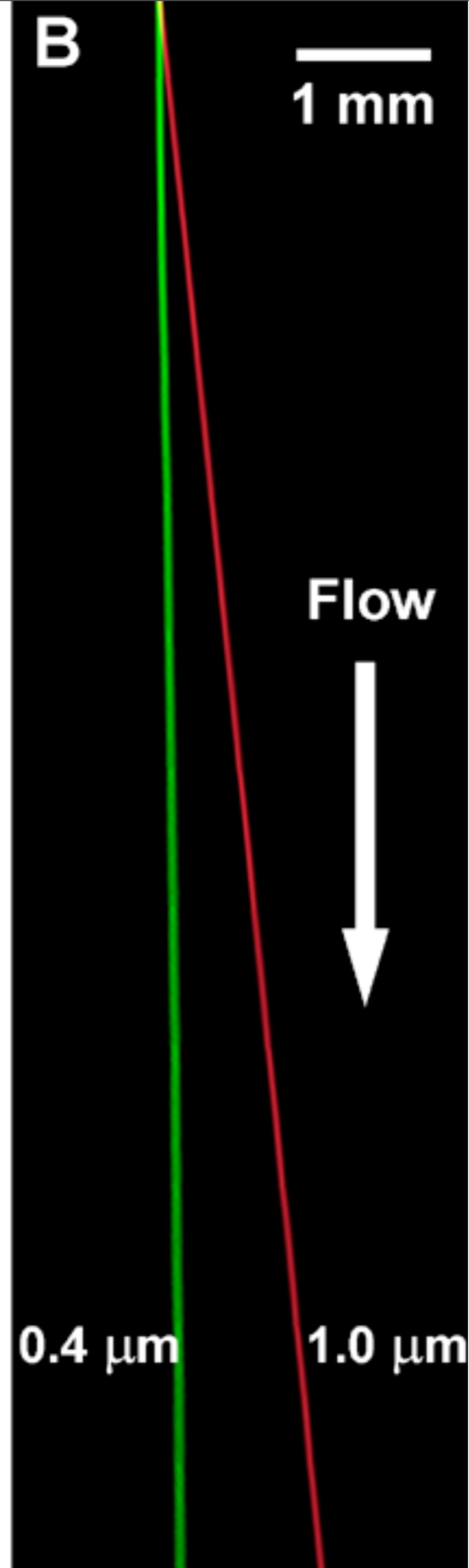
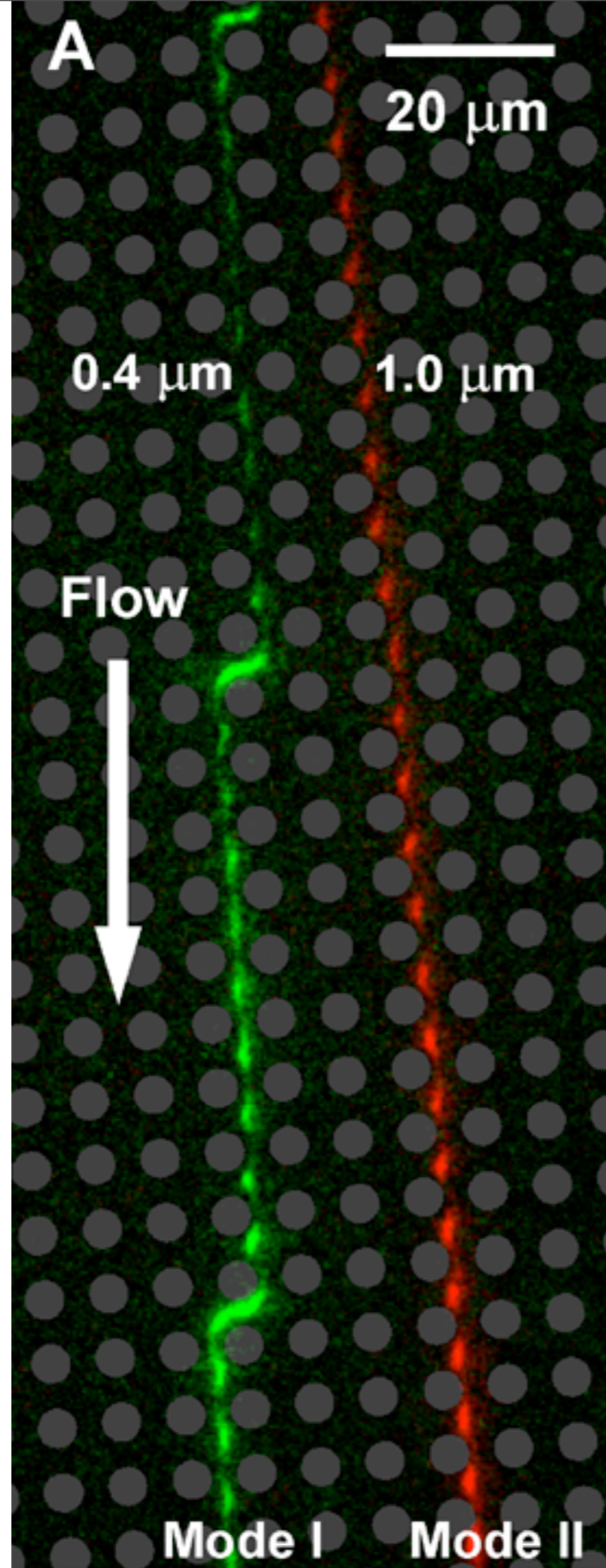


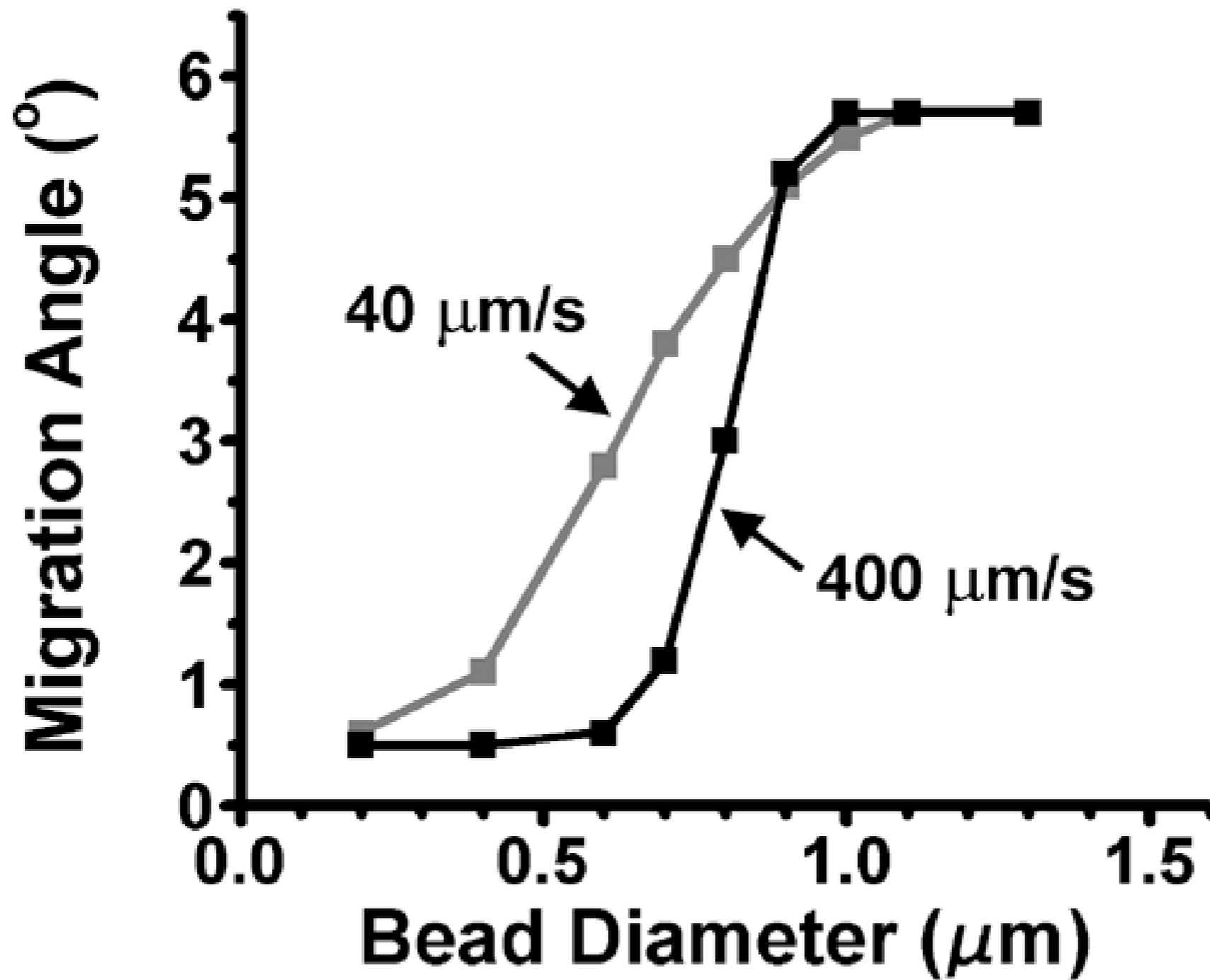
Now, here is the really tricky part. Large particles are moved by a sum of streamlines, like a sailboat or a woman surrounded by men (or visa versa) and if the net drag is big enough the particle can move not 3-1-2-3 etc. but rather 1-1-1-1 etc.: along the clear axis, at an angle to the flow direction. We call that process “bumping”.



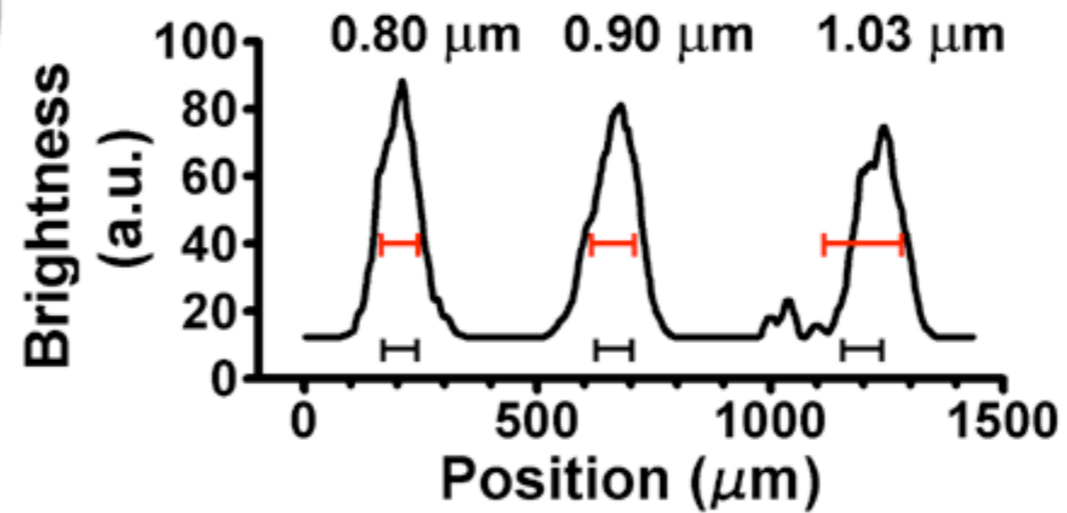
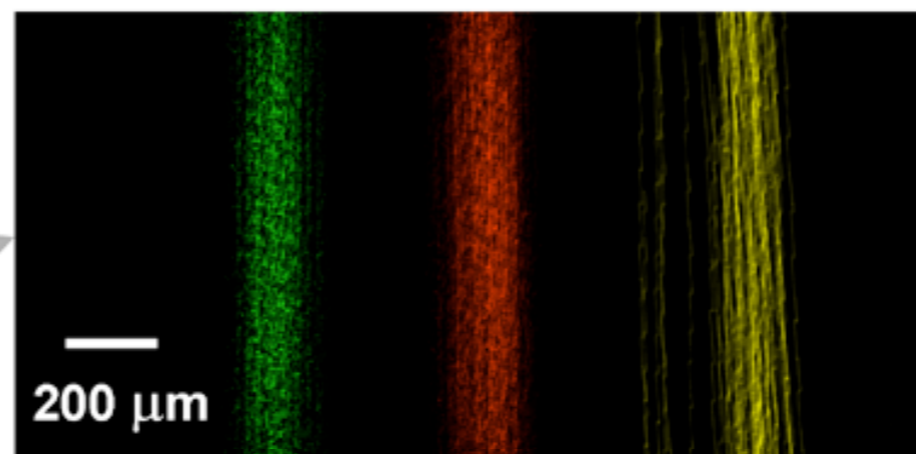
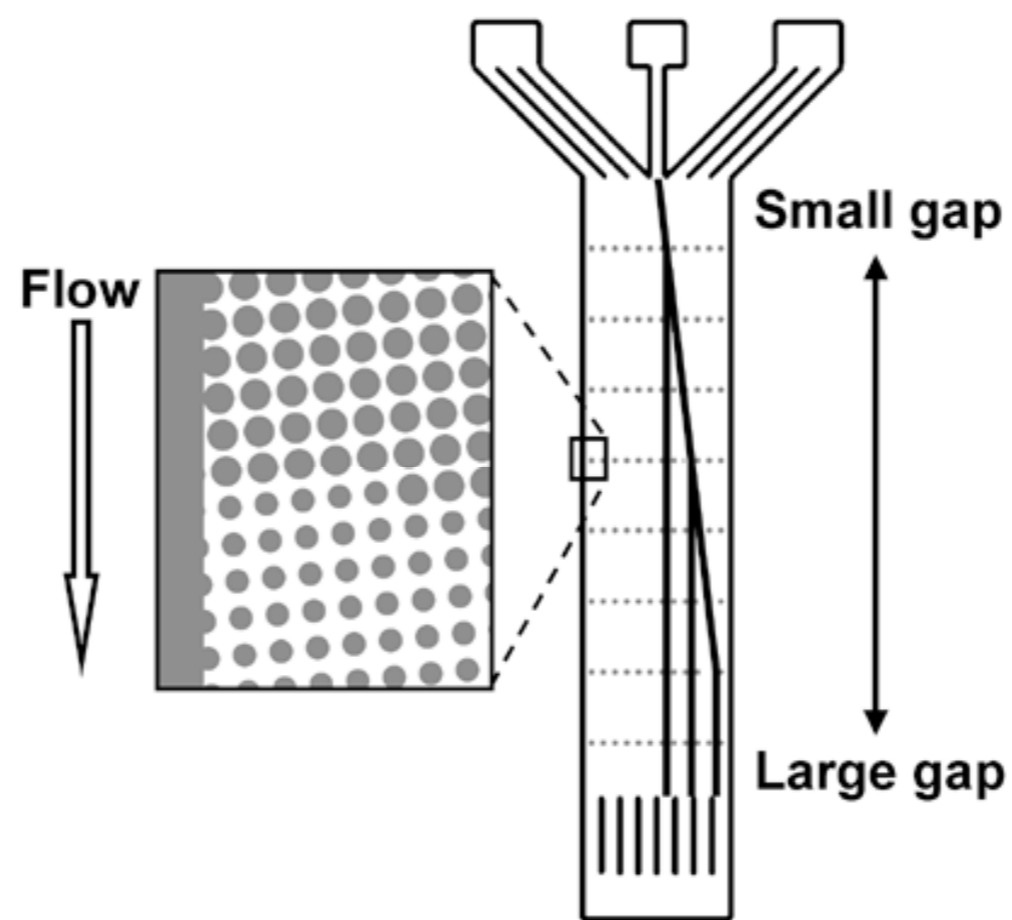
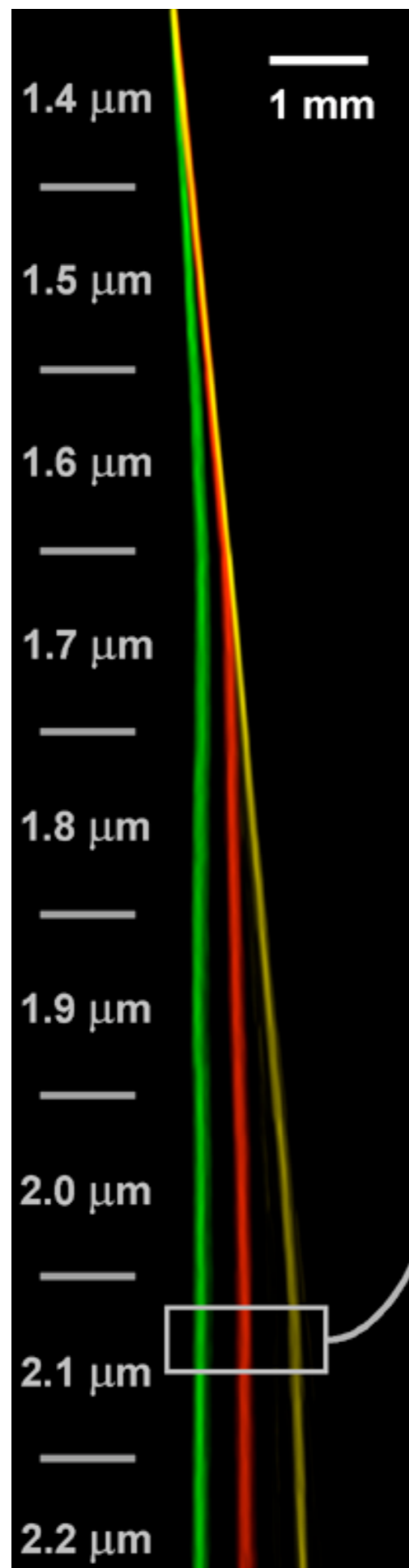


$$\varepsilon = \frac{\delta\lambda}{\lambda} = 1/10$$





The FASTER the flow, the SHARPER the edge. This is the opposite of a diffusion array. This device wants Pe to be infinity.



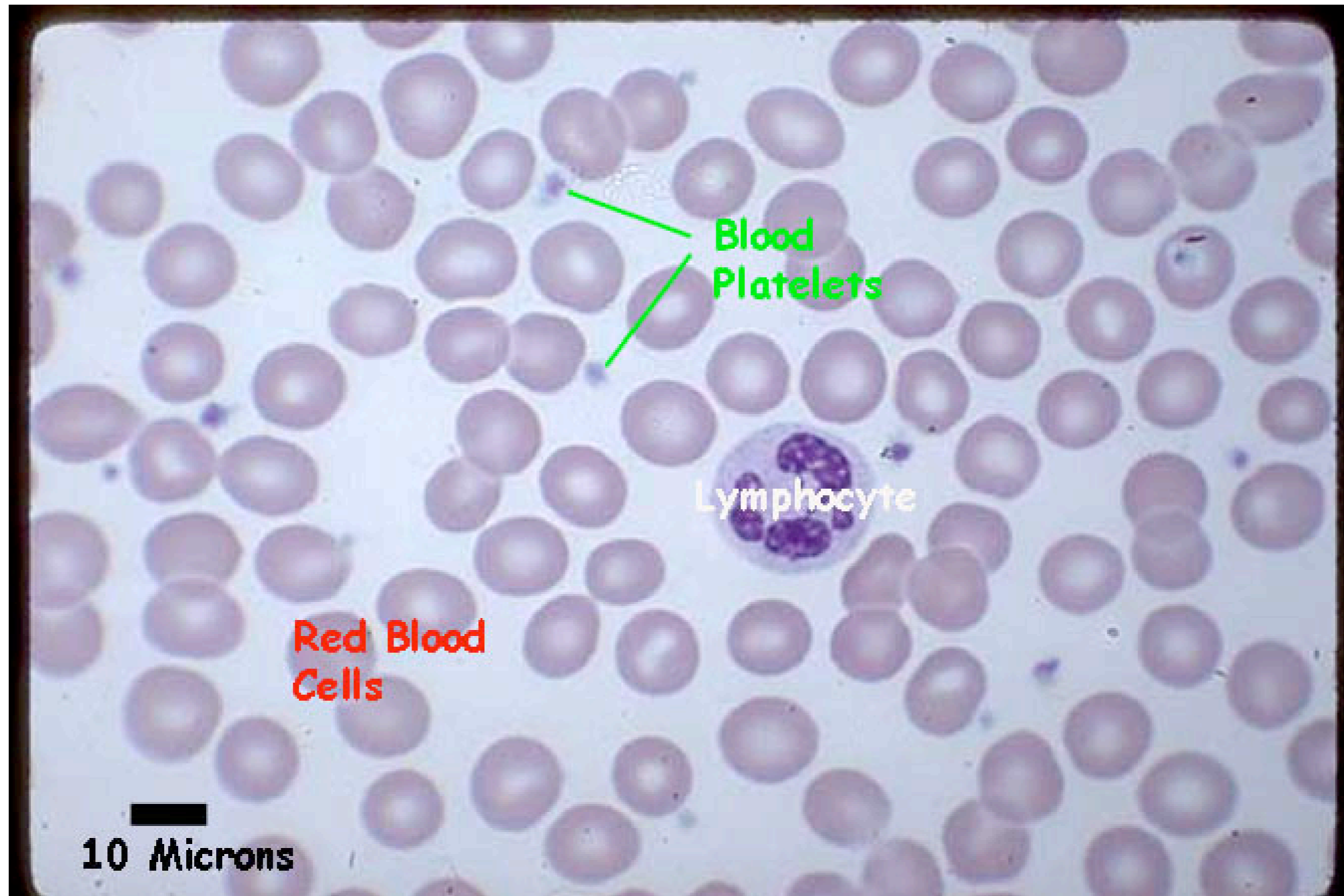
But...it is just DAMN boring and anal to sort micron beads to less than 1% (10 nm!) resolution.

We have two projects right now trying to exploit this “hydrodynamic microscope”:

1) Sorting activated blood and blood cell platelets, at the micron level (pretty easy)

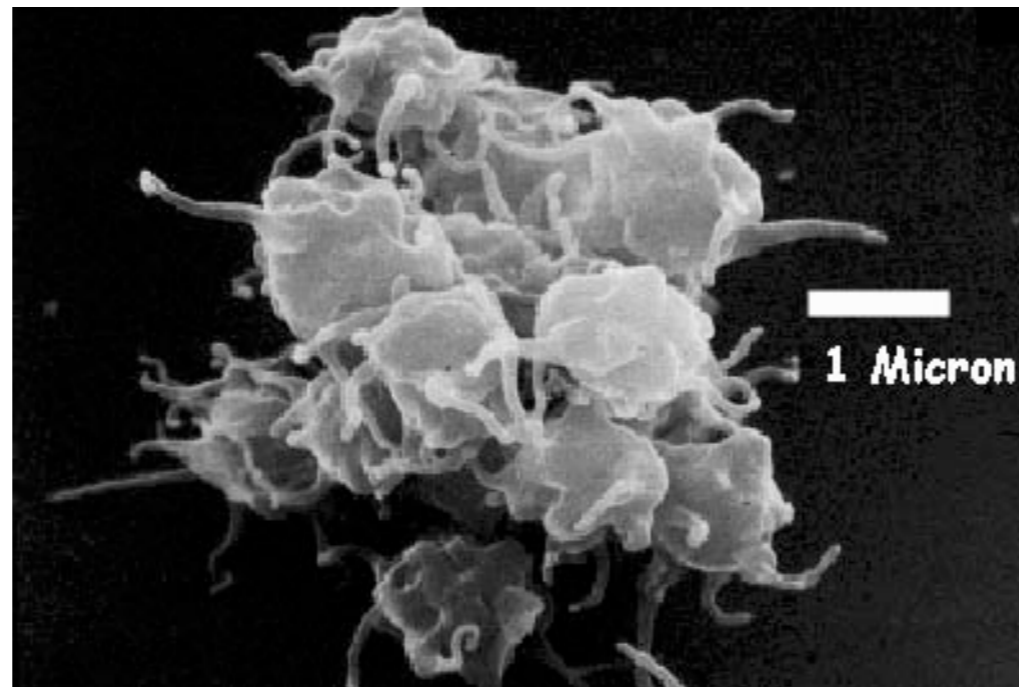
2) Sort single molecules (proteins or protein-DNA complexes) at the 10 nm (100 Å) hydrodynamic scale with 1 Å resolution (really, really hard).

We are machines, and blood is the super-complex fluid that keeps us feed, immune and not leaky.

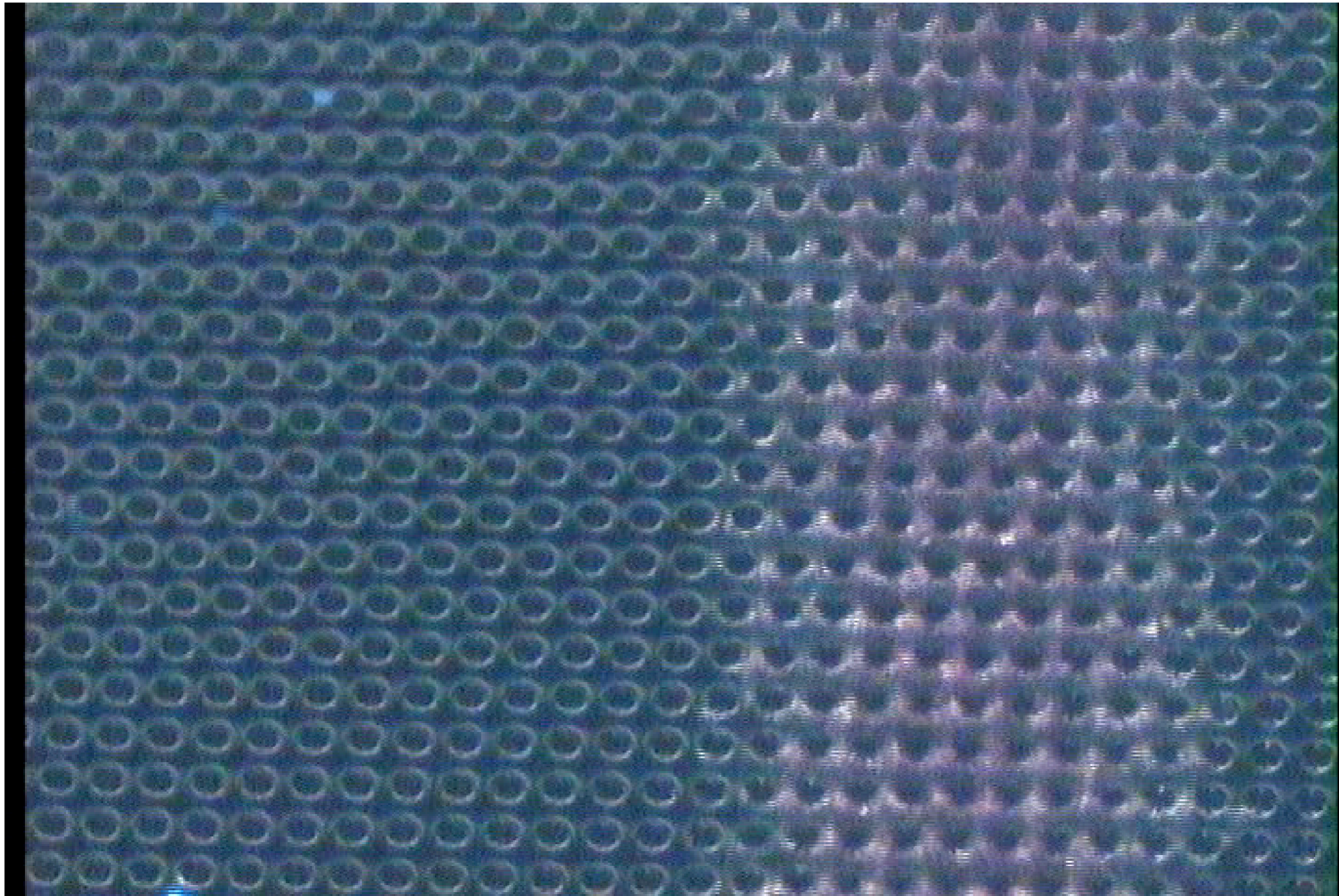




“resting” platelet, fragile state



Activated platelet, triggers clotting response.



**John Davis data on Red Blood Cell/White Blood Cell
separation**



**2) Scaling down to the nm resolution level.
Harder problem but of great importance : Can
we scale down to getting critical radius to 10
nm, that is separation of proteins or
measuring protein-protein interactions at the
single molecule scale, or from a single cell?**

It is all in the Peclet number and how it scales with a and D :

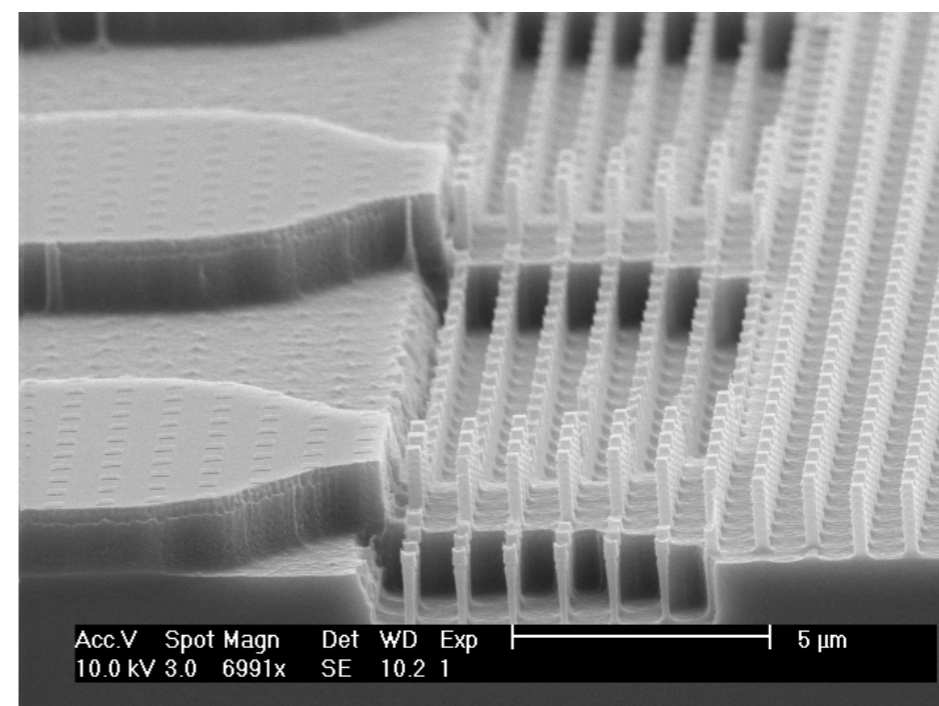
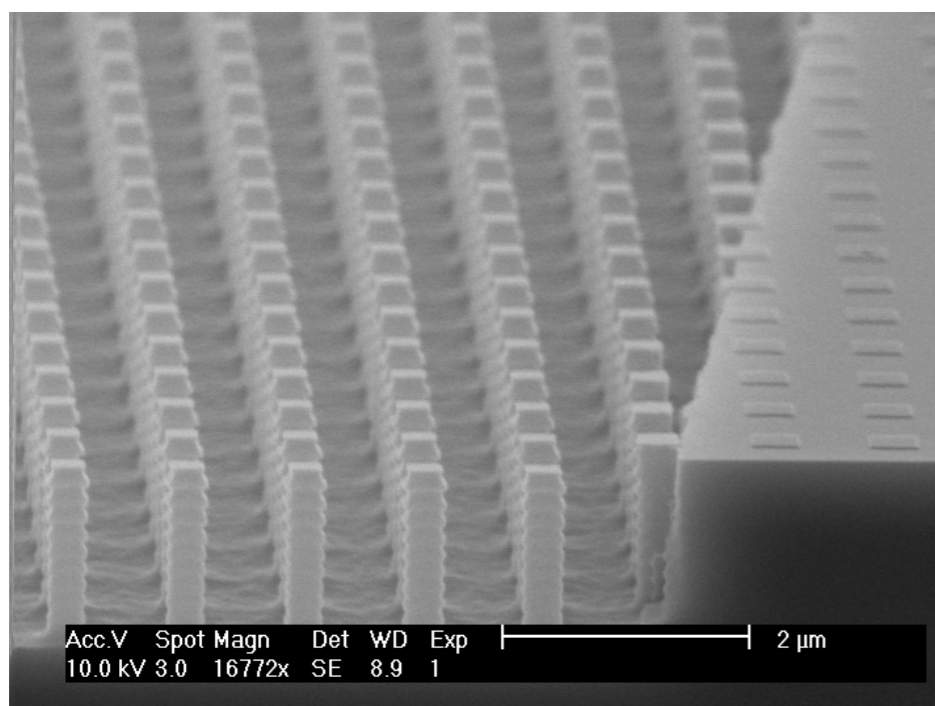
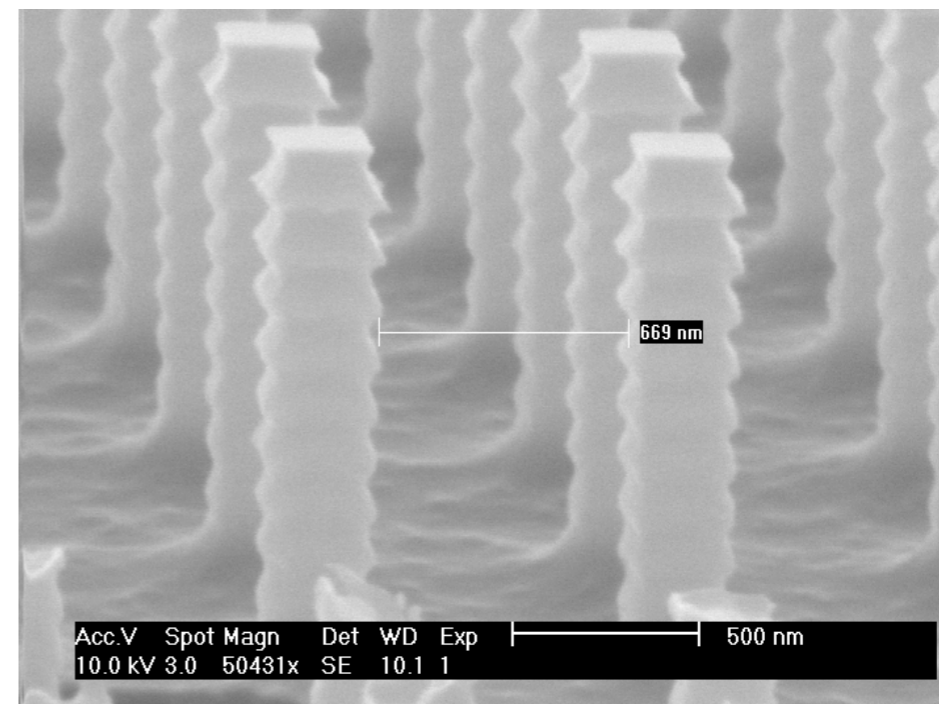
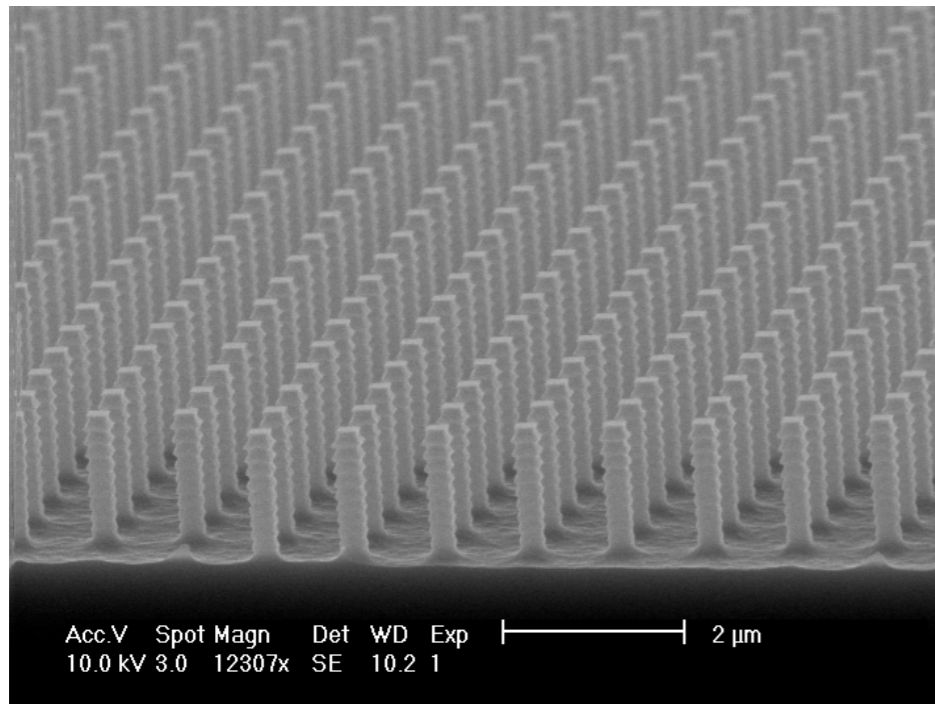
$$P_e \sim \frac{va}{D} \sim \frac{6\pi\eta v^2 a^2}{k_B T}$$

So, to keep P_e high as “ a ” shrinks v has to go up.

At $a = 10$ nm and 0.1 nm resolution, v becomes 1 meter/second. This is possible, but requires 1000 bar/cm pressure gradient.

Quite spectacular “real nano” bump structures (not, “it’s micro but we want to call it nano”), by Keith Morton in Steve Chou’s lab.

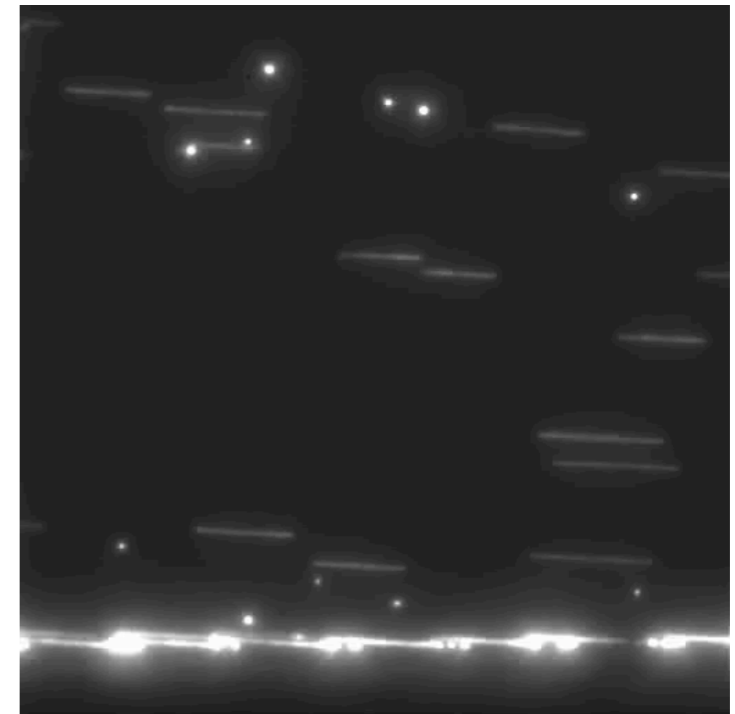
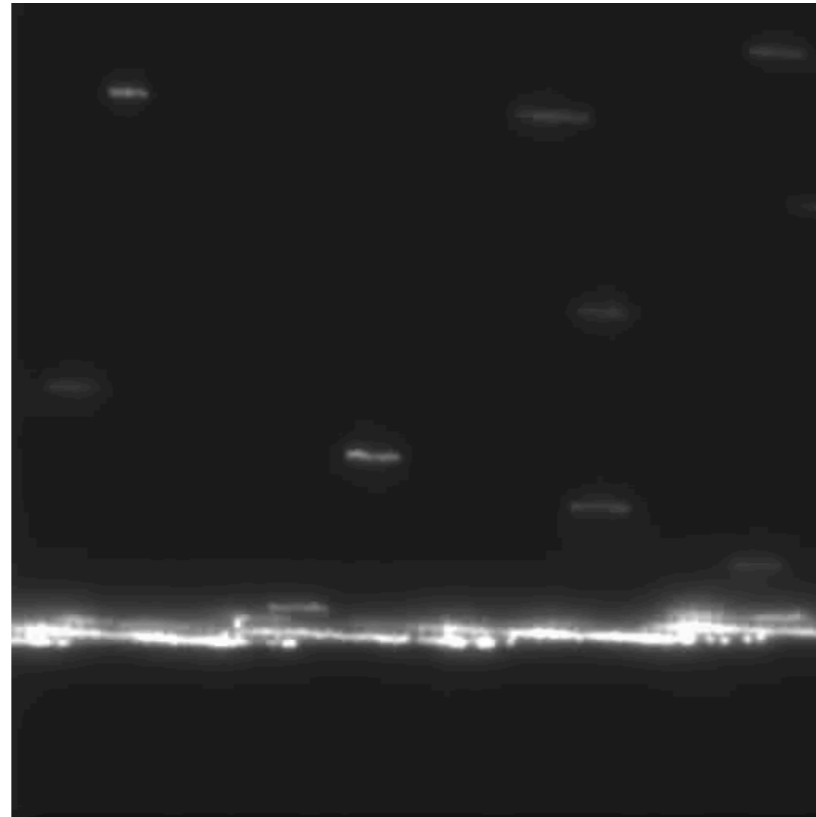
Keith Morton
Nanostructures Lab
09.26.05



**These are 400 nm beads, above the critical bumping threshold.
At low pressure gradients, the Pa is small and
diffusion competes quite well with bumping.**

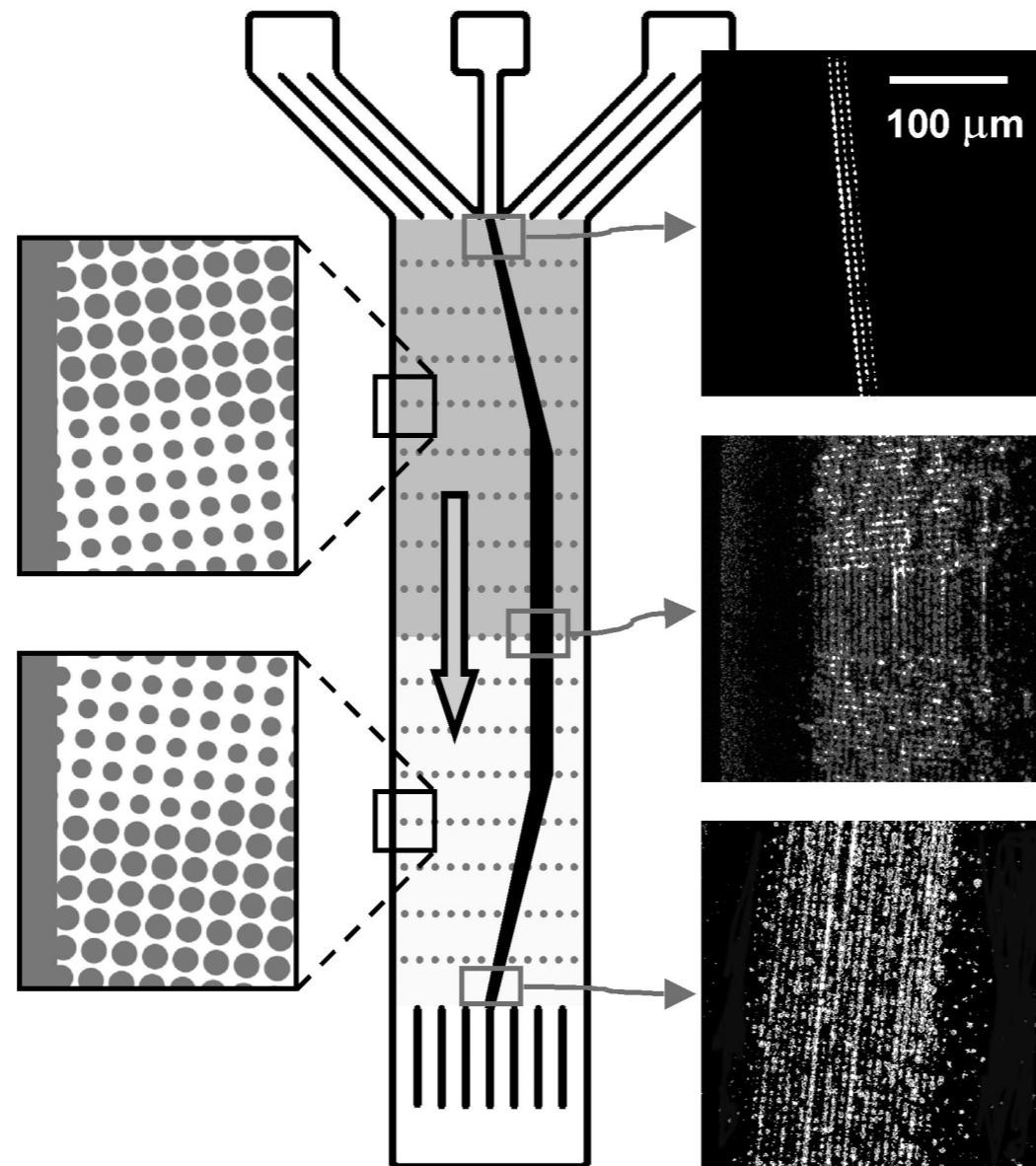


As we increase the pressure gradient, bumping is increasingly more effective against diffusional smearing.



This is Salt Lake City, think of the adiabatic lapse rate as you drive up to Alta tomorrow (as you should).

An interesting thing about this bump technology is that by changing the shift of the posts from right to left you change the sign of the particle displacement (if they have greater than the critical size). It is like optics, where you have positive and negative indices of refraction.



Pressure gradients are an interesting aspect of this technology as we go nano. High pressures and high pressure gradients and nanofeatures require that these structures be made not out of PDMS, which is too soft, but of hard materials such as quartz (fused quartz actually) or silicon.

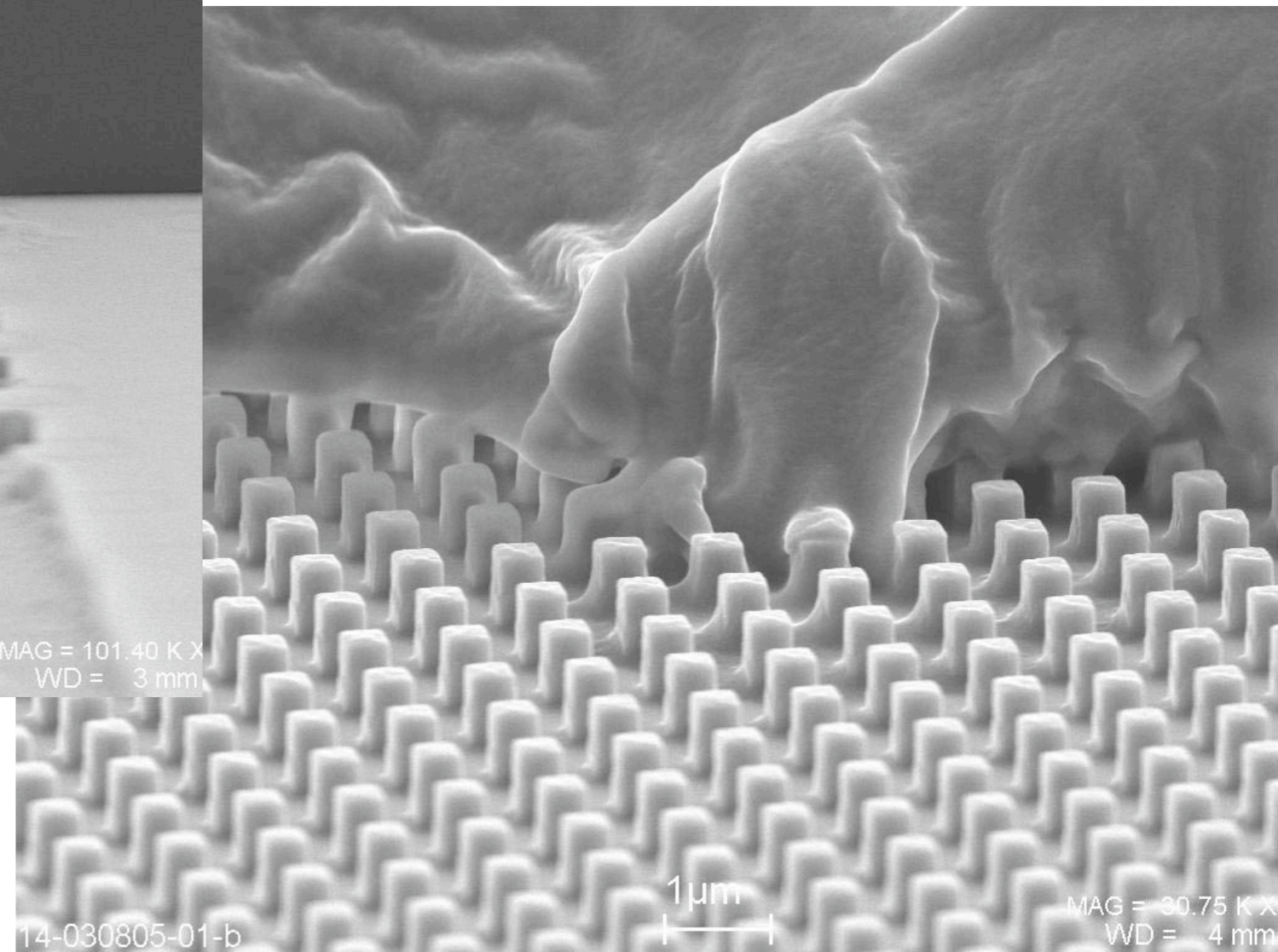
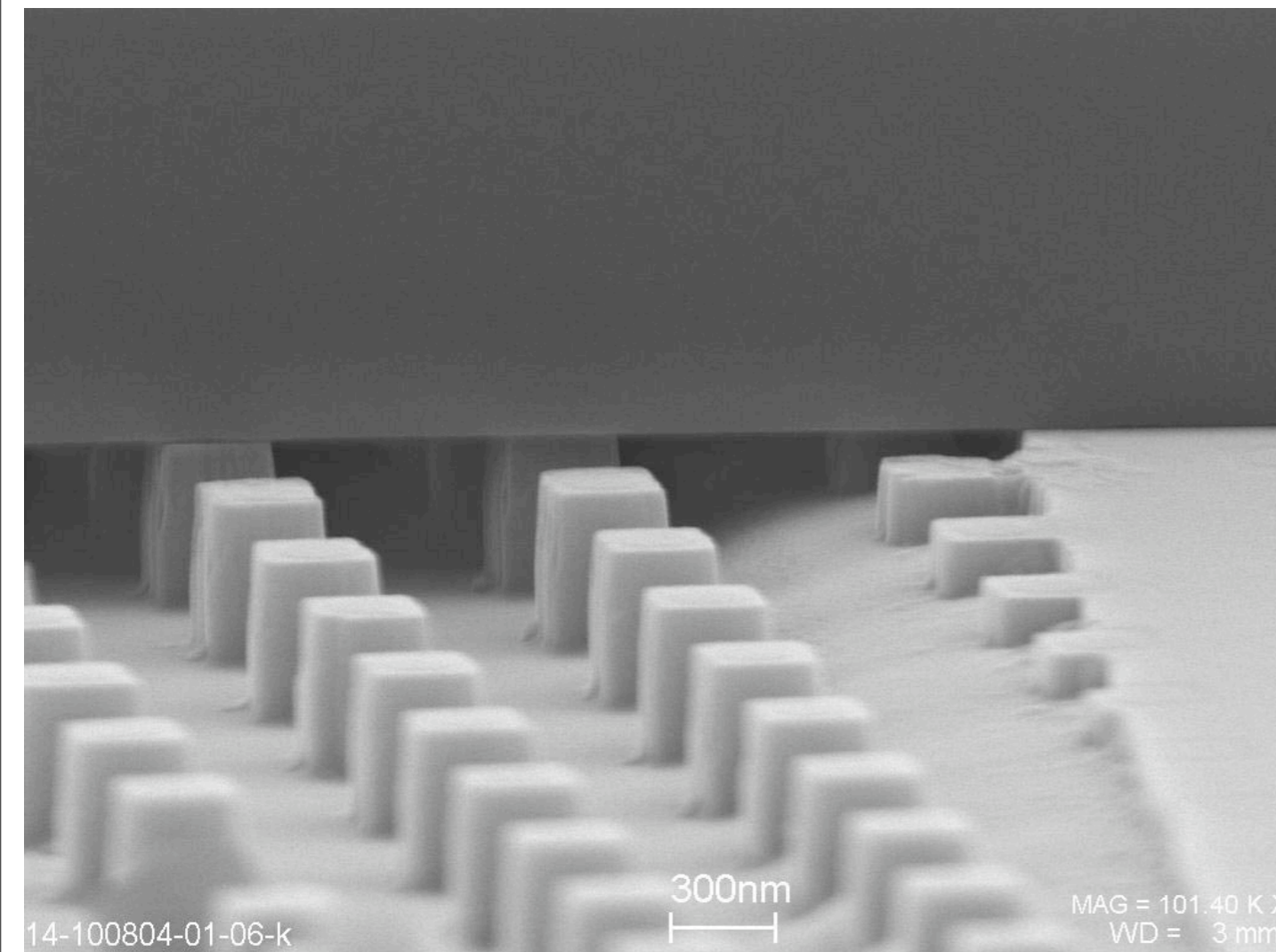
Several problems arise:

- 1) SEALING such structures to maintain high pressures**
- 2) Wetting nanostructured sealed devices, and the heartbreak of bubbles.**

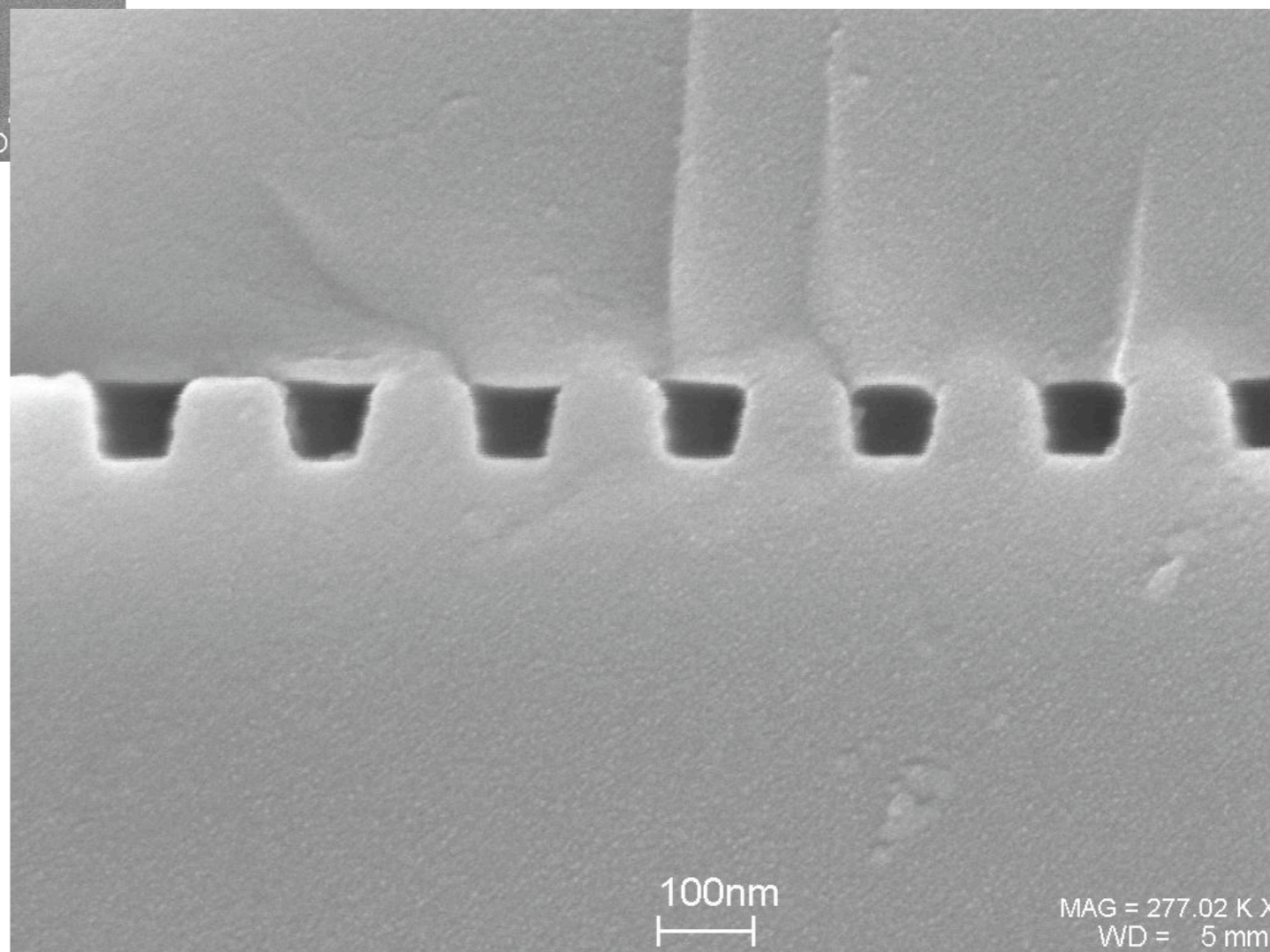
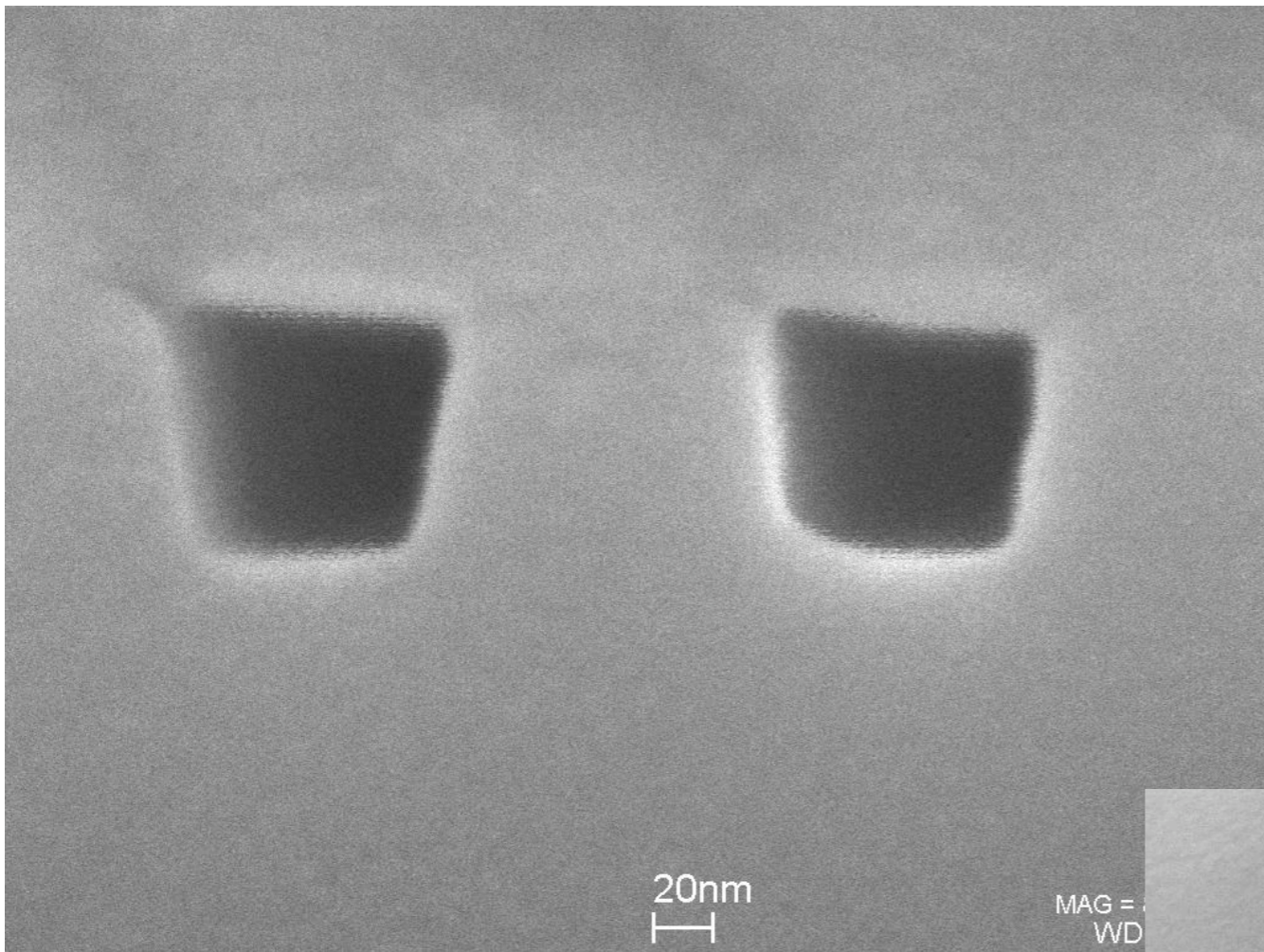
1) SEALING such structures to maintain high pressures.

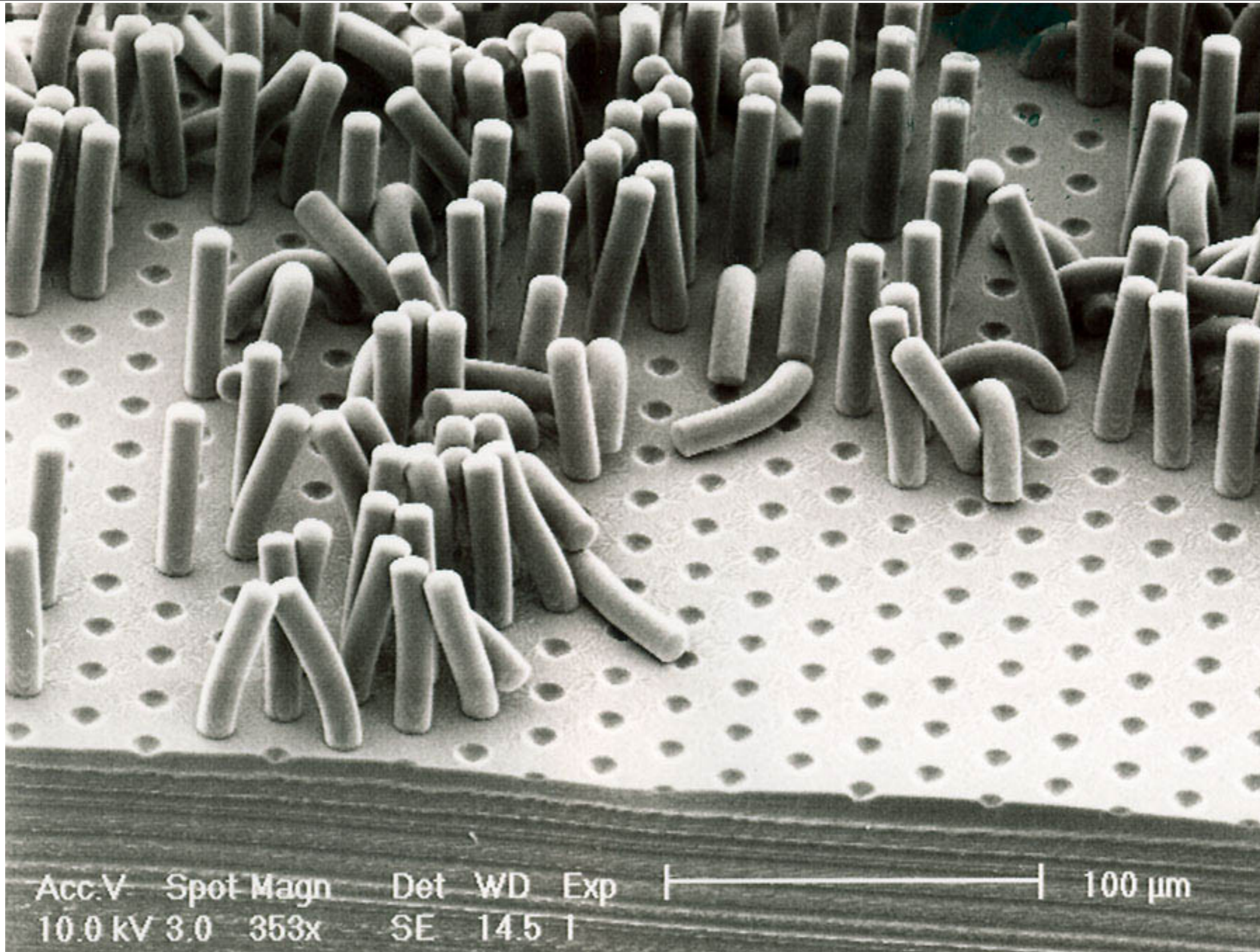
Fusion bonding of fused silica requires two bonding steps: prebonding after "reverse RCA" treatment and then the fusing step at 1000° C.

Plasma treatments in an ICP etcher can be used lower the temperatures required to bond fused silica structures to each other. Keith Morton has developed recently a new bonding technique for fused silica structures. The surface is modified by O₂ + H₂O ICP plasma preparation followed by mild (200° C) heat treatment to eliminate the water and form a covalent ester bond.



SEM image of a fused silica-fused silica bonded nanoimprinted array. The hydroxyl groups have been activated to form a hermetic seal. “The Blob” shows what PDMS looks like on the same array. We can easily go to 10 atmospheres.





PDMS is for the weak and soft, Steve.

2) Wetting nanostructured sealed devices, and the heartbreak of bubbles.

The heartbreak of bubbles comes from Laplace's expression for the internal pressure P inside a bubble of radius R within a medium with surface tension s :

$$P = 2s/R$$

**Well, for air/water $s = 0.03$ N/m, so if $R = 100$ nm
 $P = 14$ atmospheres!**

You will never push that bubble out.

Of course, one of the reasons many of us went into physics is we like serious danger and explosions. It is one of the many things that make physicists so very, very cool. Join the APS Division of Biological Physics and be a real Woman or Man, enough of this **foo-foo** biophysics.



Anyway, Pascal's formula breaks down if γ , the relative surface tension between water and steam, is zero. Then there are no bubbles. This occurs at the critical point of water, which as any nuclear plant operator (except those running Three Mile Island) knows, is at **370 C and 4000 PSI**. My great post-doc Robert Riehn (peace be upon him) suggested: why not bloody cycle a sealed nanodevice through the critical point of water?

This sounds frightening, but if like me you hang around high vacuum equipment you know about the wonders of the Conflat Flange, designed to take 300 C bakeout at 10^{-9} torr. The geniuses at Varian have done our engineering for us.



Like Han Solo, I made a few modifications: drilled out the bolt holes for thicker bolts, those are 180,000 PSI high strength alloy bolts, heavy duty deep thread nuts.

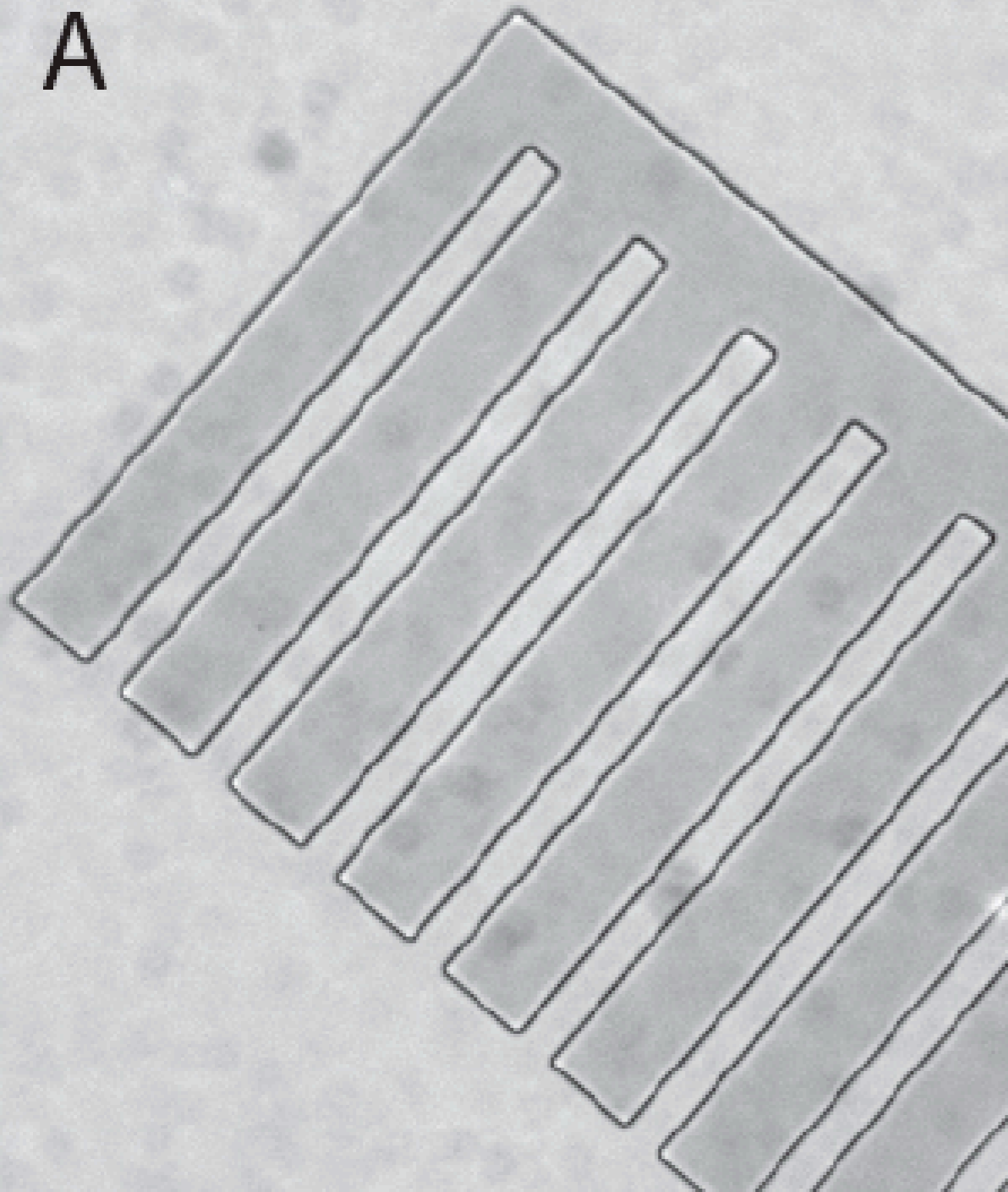
You can see the ring that the conflat knife edge presses into the soft copper gasket. New gasket for every cycle.

You just put your sample to be wet between two Conflat flanges, immerse the flanges in water and bolt the beast together under water, put in a furnace and heat to 400 C, albeit a bit nervously. Have an unfavorite Dean of Whatever in the room and tell him/her what you are doing to scare the bejesus out of them and keep them out of your hair.

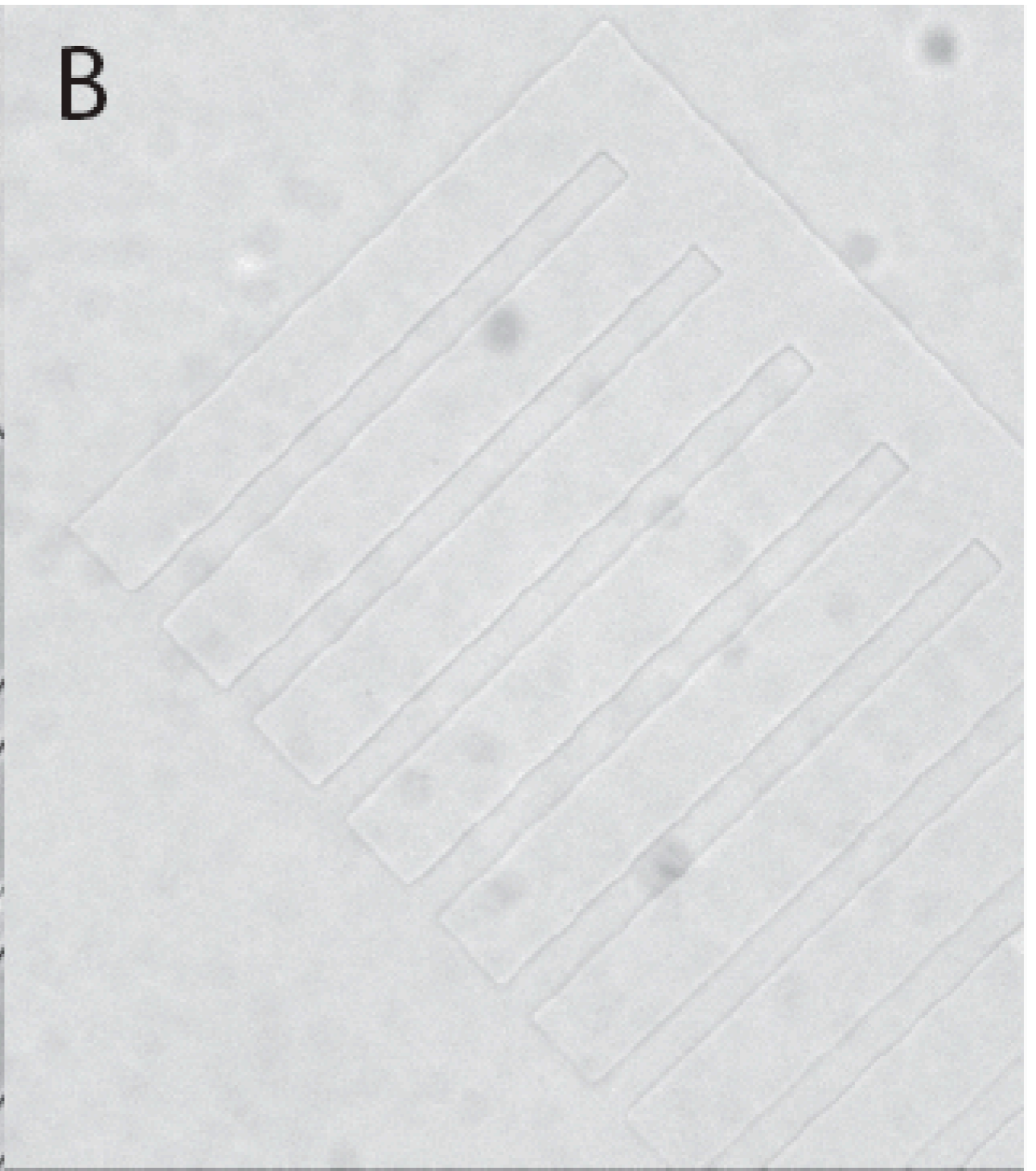
The total force acting acting on the flange at 400 C is about 80,000 lbs, or the weight of 20 cars. Stored energy is “small”.

Every time I have done this, after I cool down and open the beast, all the water is there and the nanostructure is wet with no bubbles at all. Tis a miracle.

A



B



Dead-end critical point wetting of a 100 nm deep etched quartz-sealed structure, picture in darkfield.

I hope I wasn't too engineering based here.

I figured that in a workshop you would want to hear some of the knotty technical fixes we have come across in order to move particle separation into the nanoscale regime.

There are other things we have done I haven't told you about, perhaps you can guess what they are.

If so, then my job here has been accomplished.

Thanks.