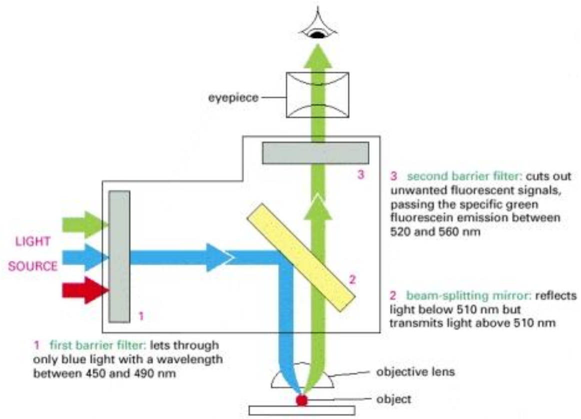
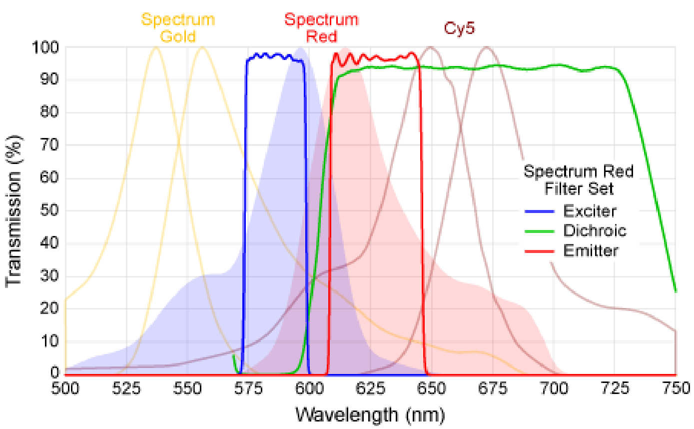
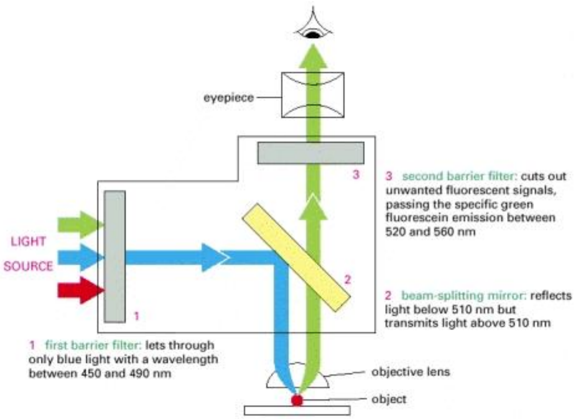
Single Molecule Bio-Physics

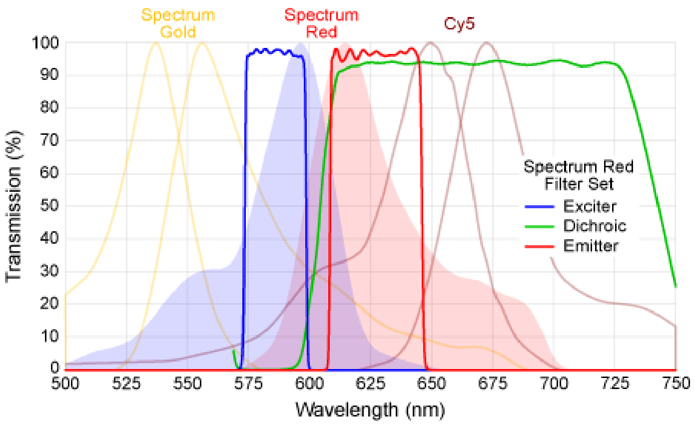
Single Molecule Fluorescence Techniques

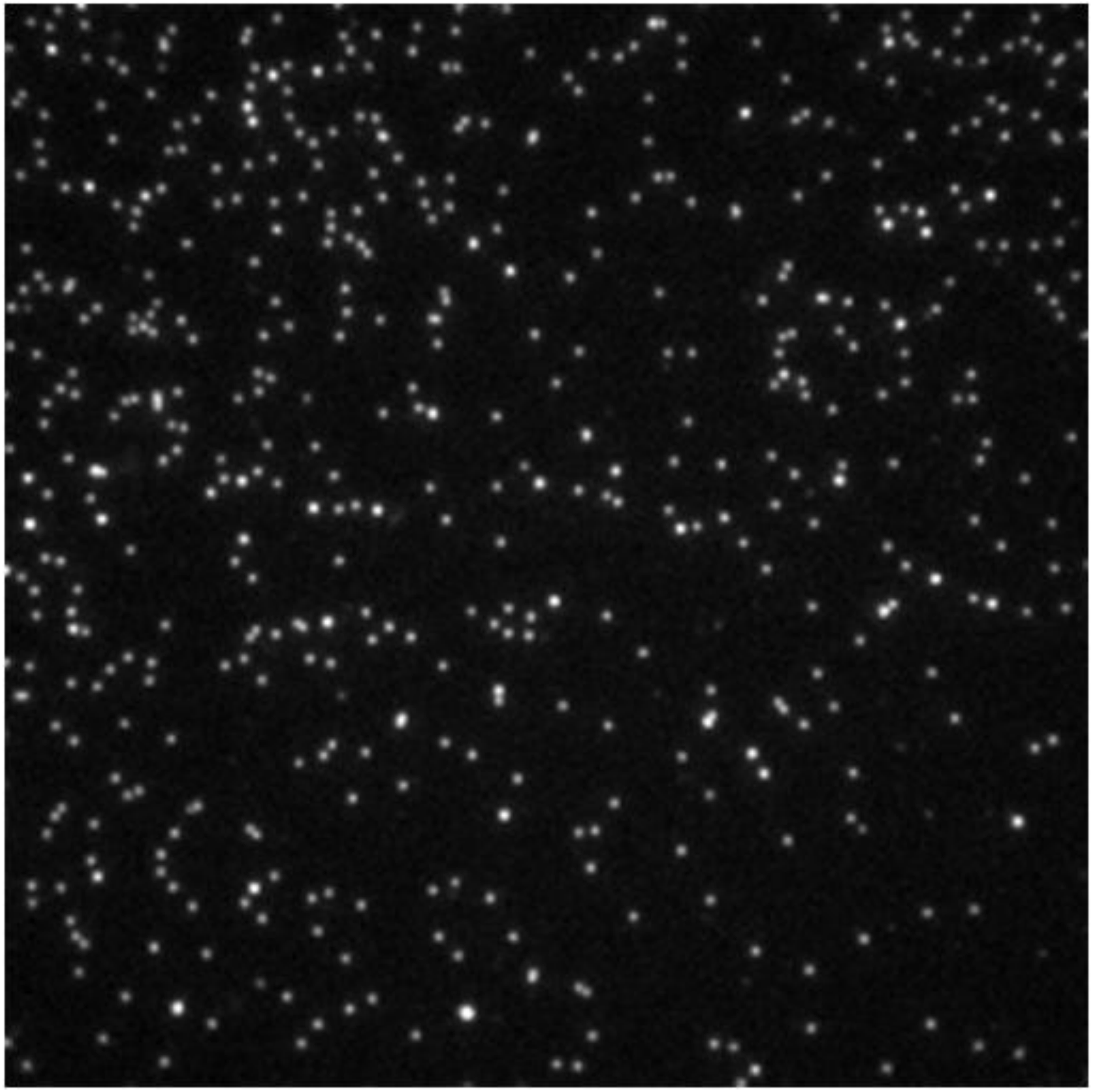




Single Molecule Fluorescence Techniques





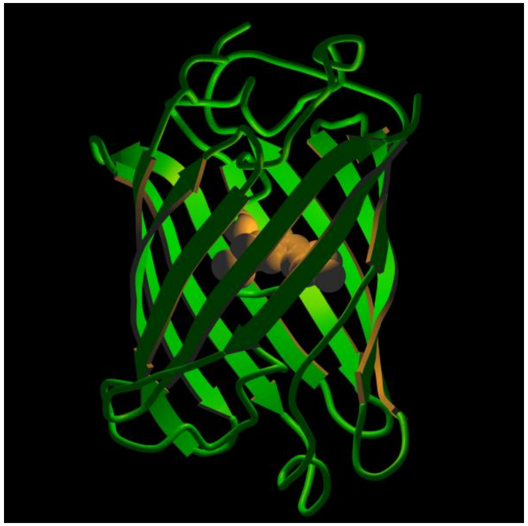


State of the Art imaging of single (immobilized) fluorescent Cy5 molecules

Pictures: Tinnefeld Lab

Fluorescence Techniques / GFP

Green Fluorescent Protein (GFP)
Discovered in Jelly Fish
Nobel Prize 2008

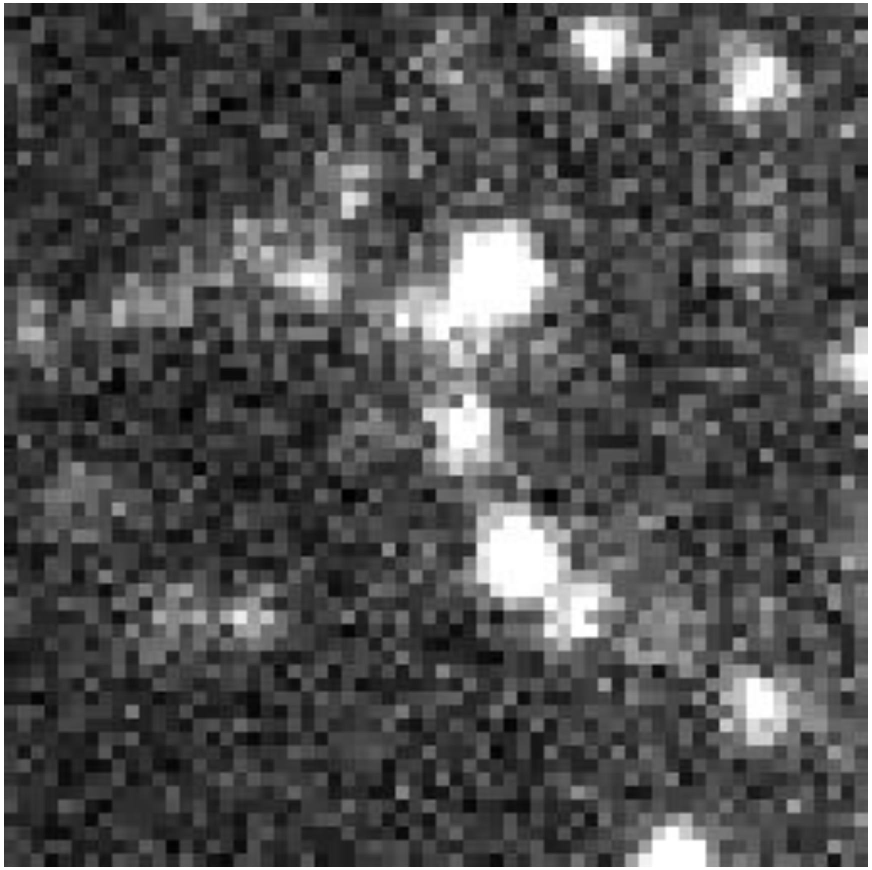


Super-Resolution Microscopy

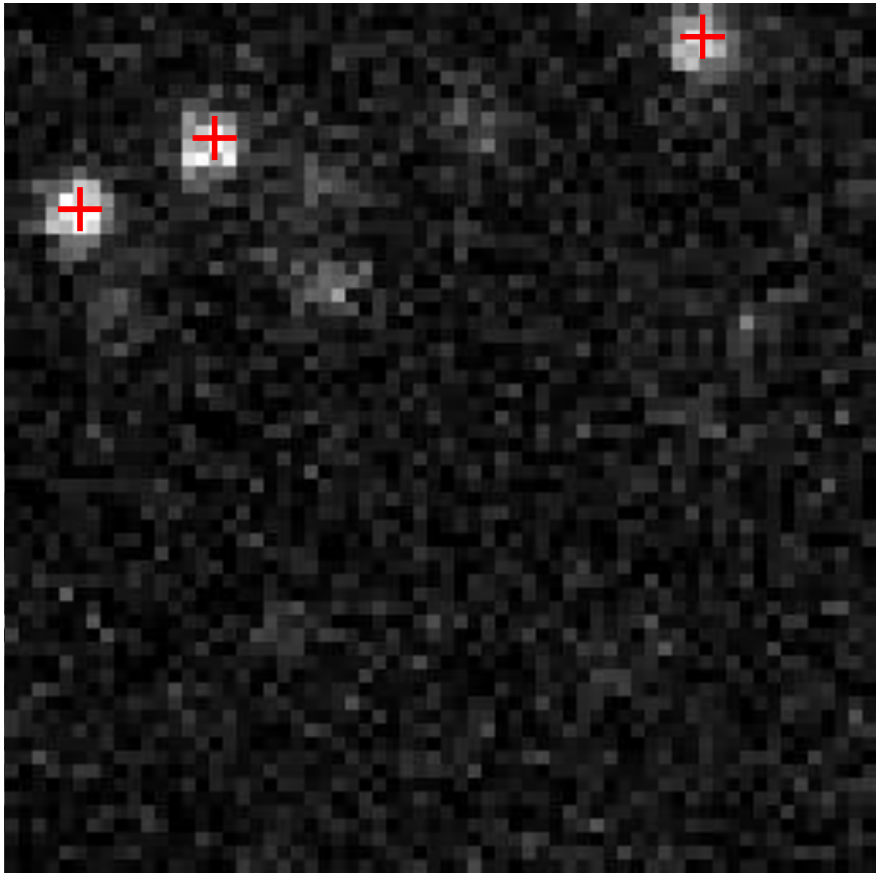
Super-Resolution Microscopy

4 s movie of actin labeled Cy5 molecules under 100 µM AA $–O_2$
1 ms integration time

Real-time movie

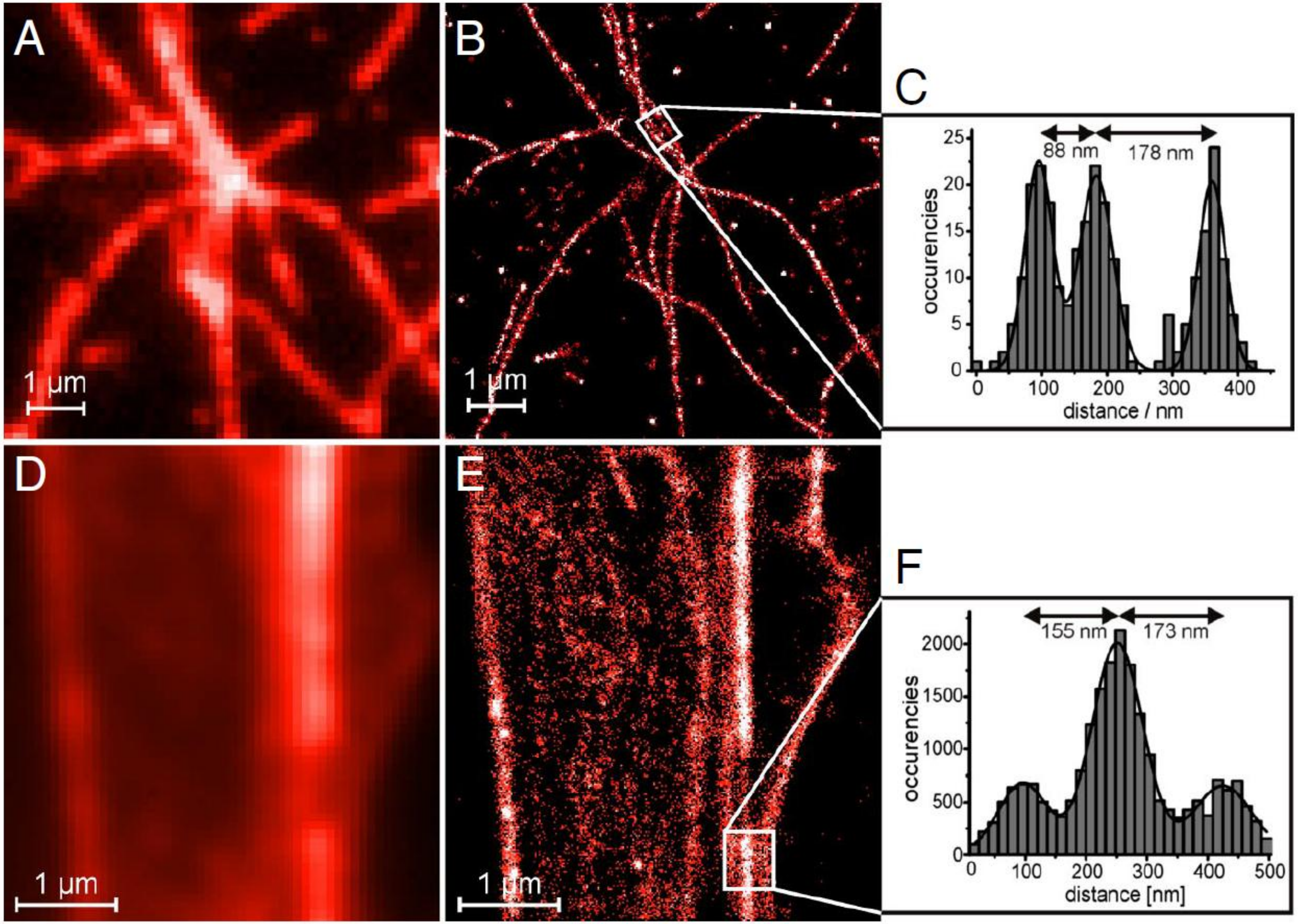


Analyzing frame by frame



Pictures: Tinnefeld Lab

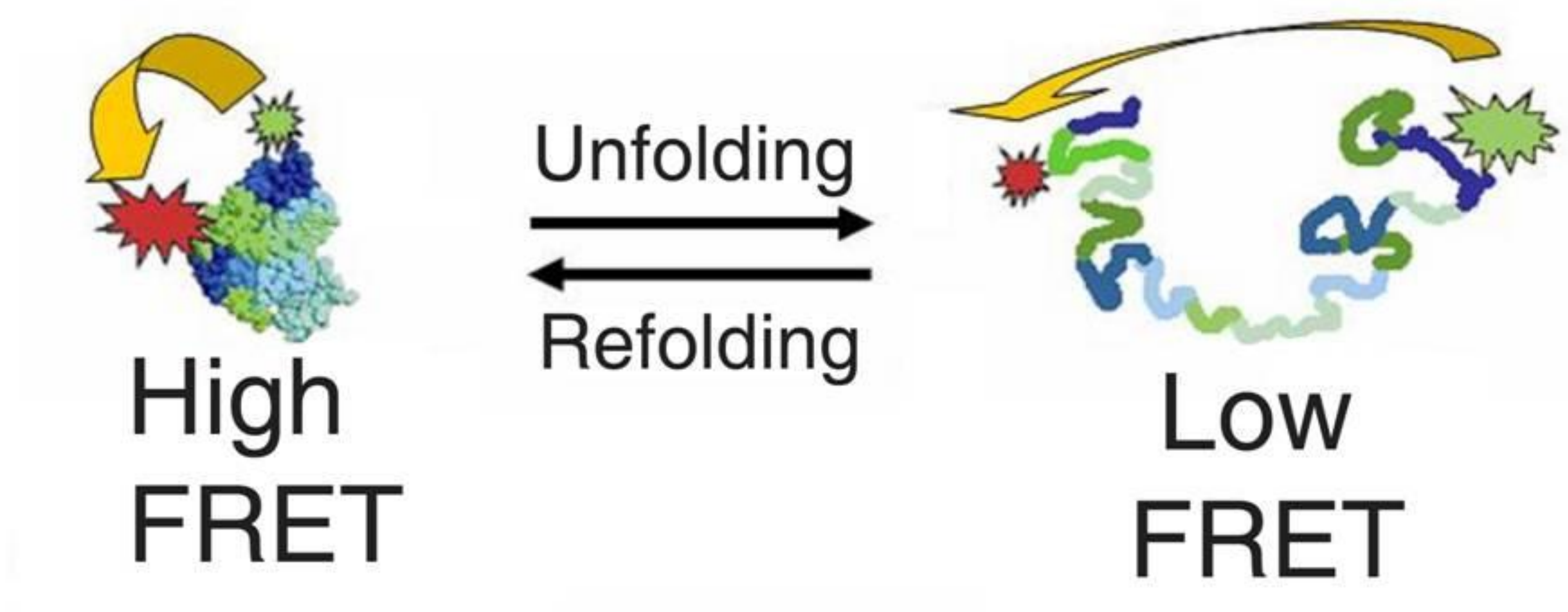
Super-Resolution Microscopy



Actin Fibers stained with ATTO647

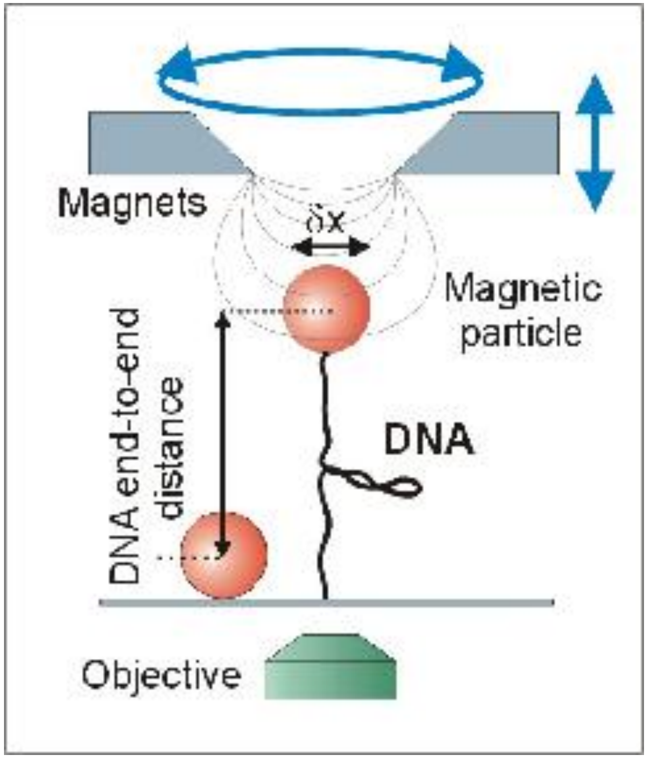
Pictures: Sauer Lab

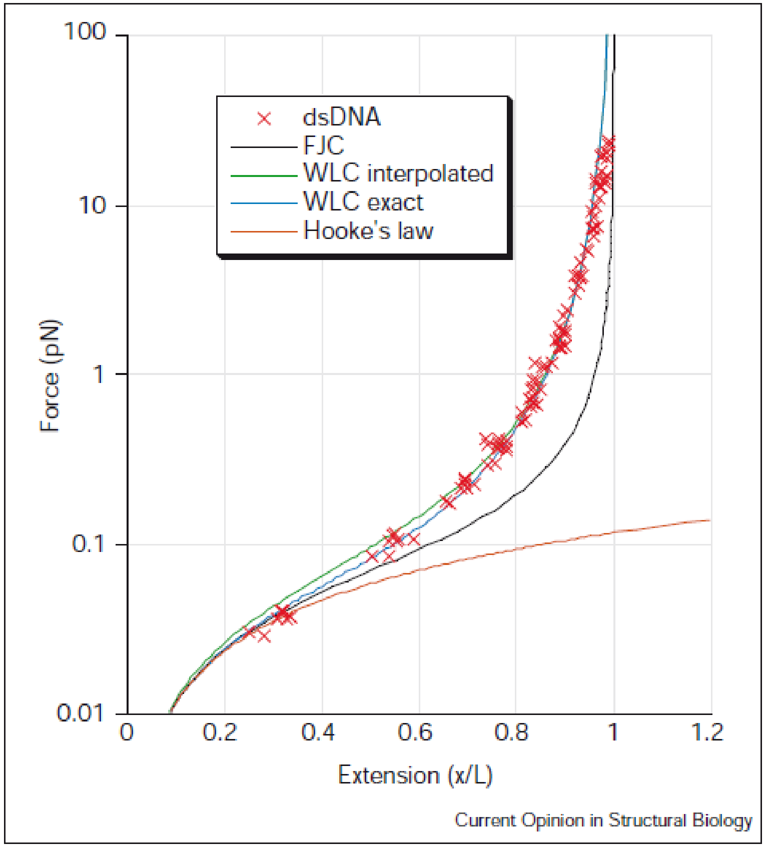
Fret / Quenching



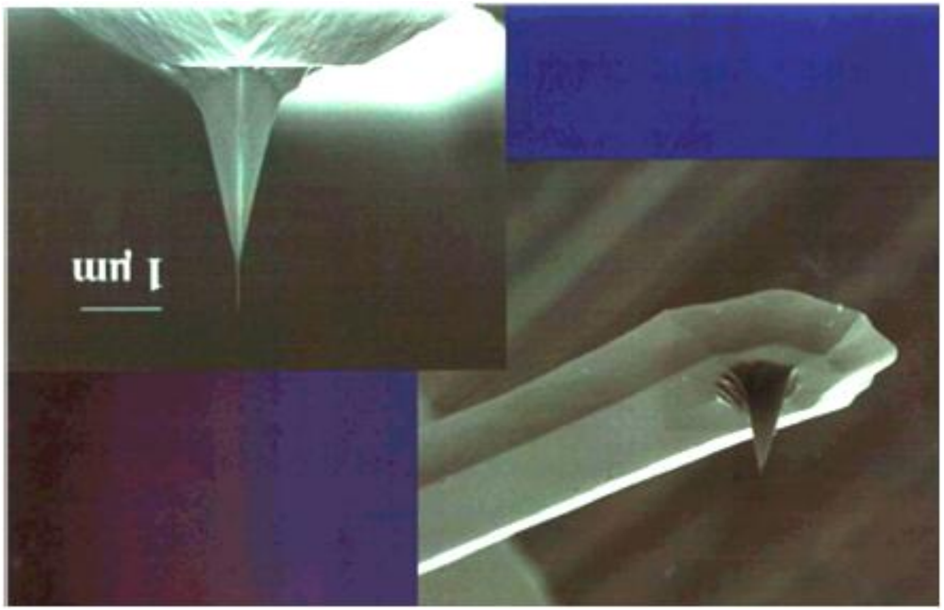
Force Spectroscopy

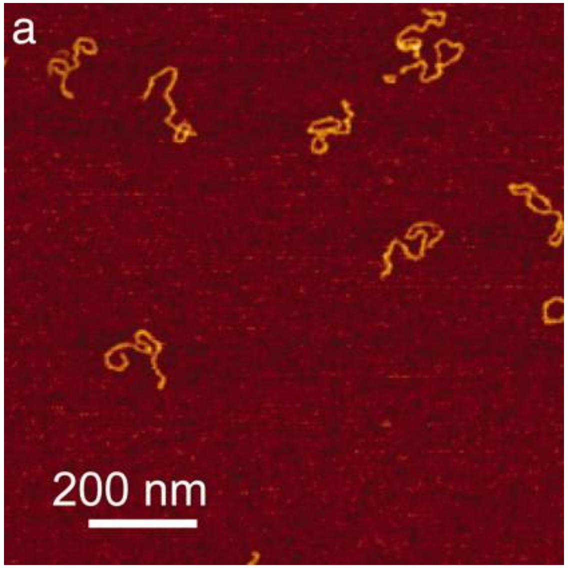
DNA Force Extension by Magnetic Tweezers

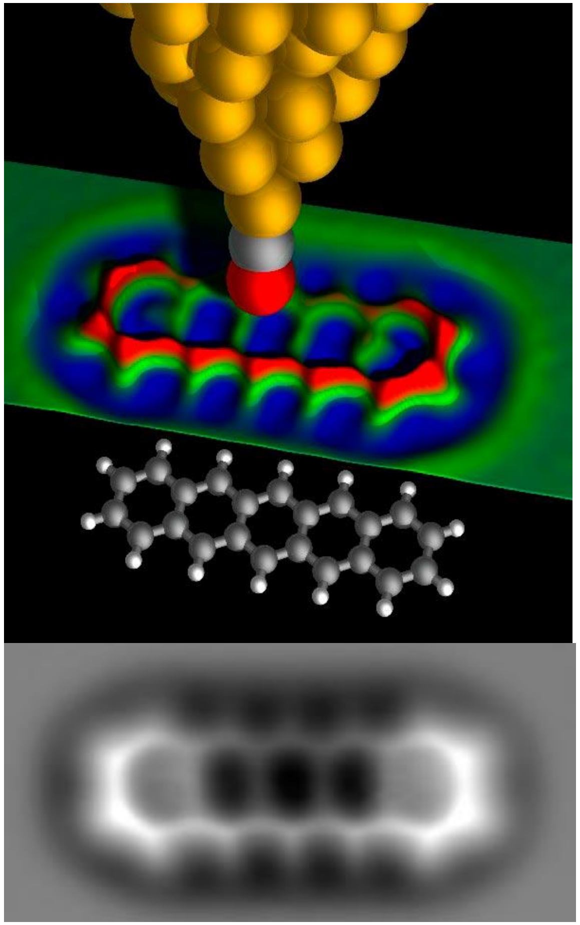




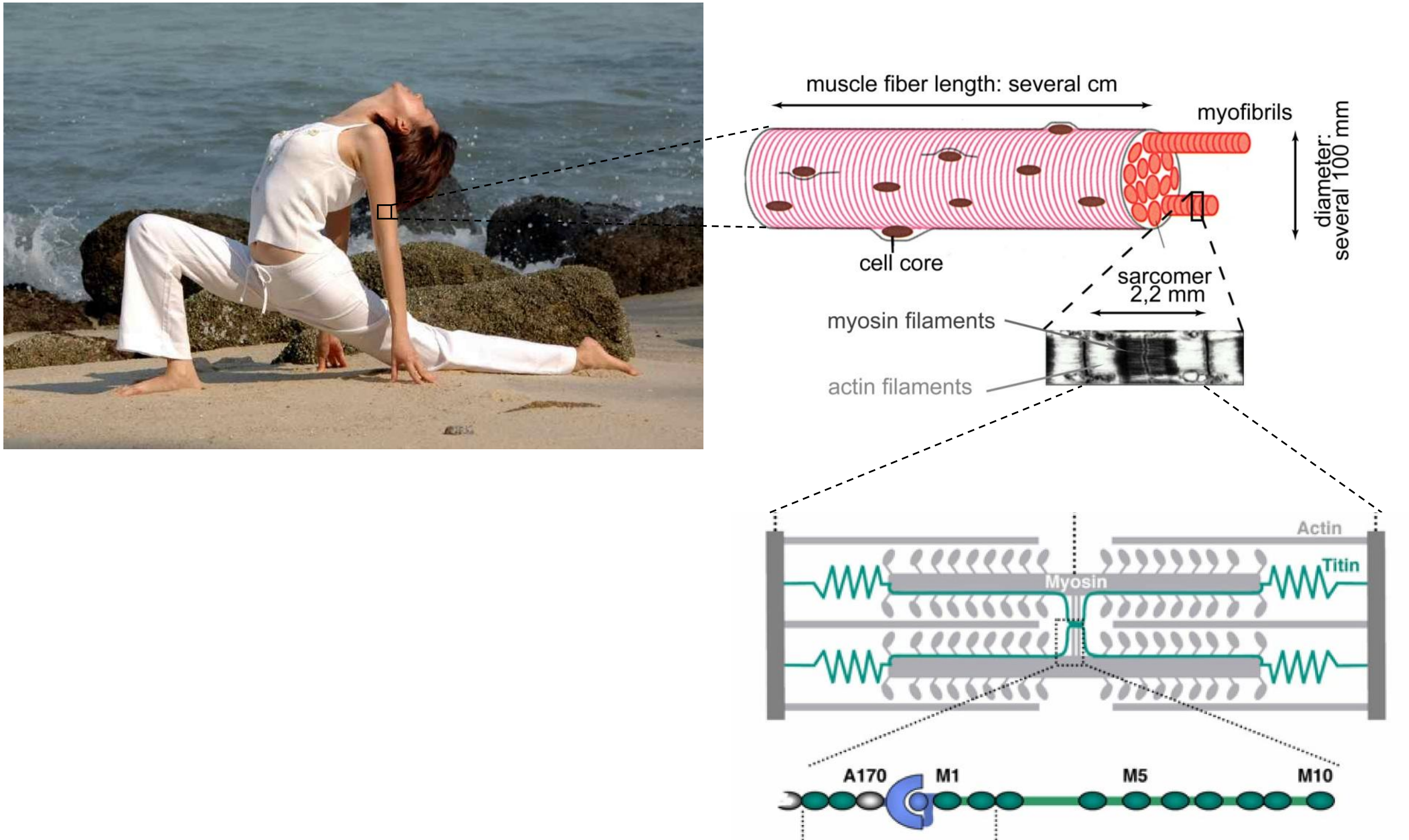
Applying force to single molecules





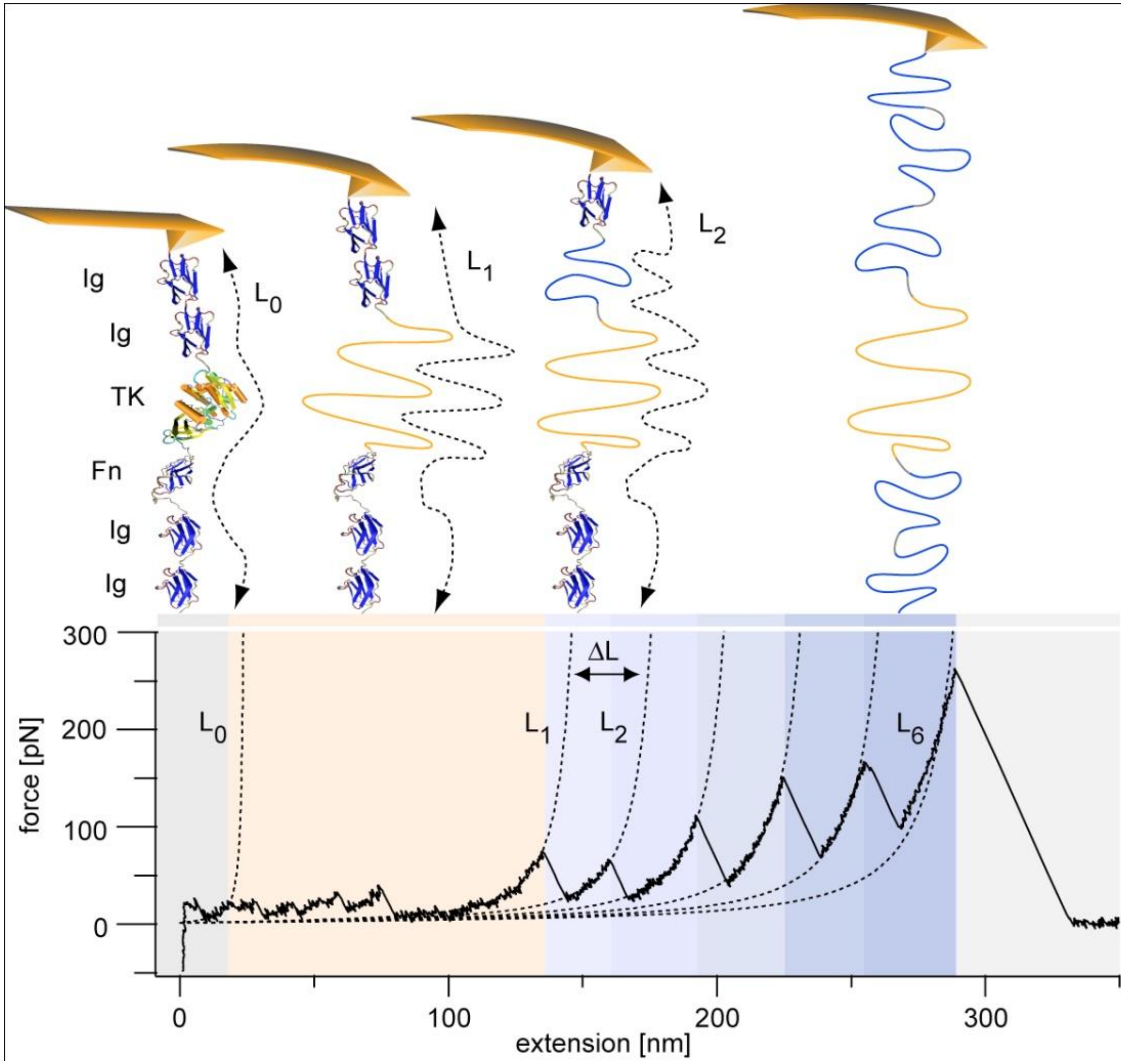


Molecular function of muscle

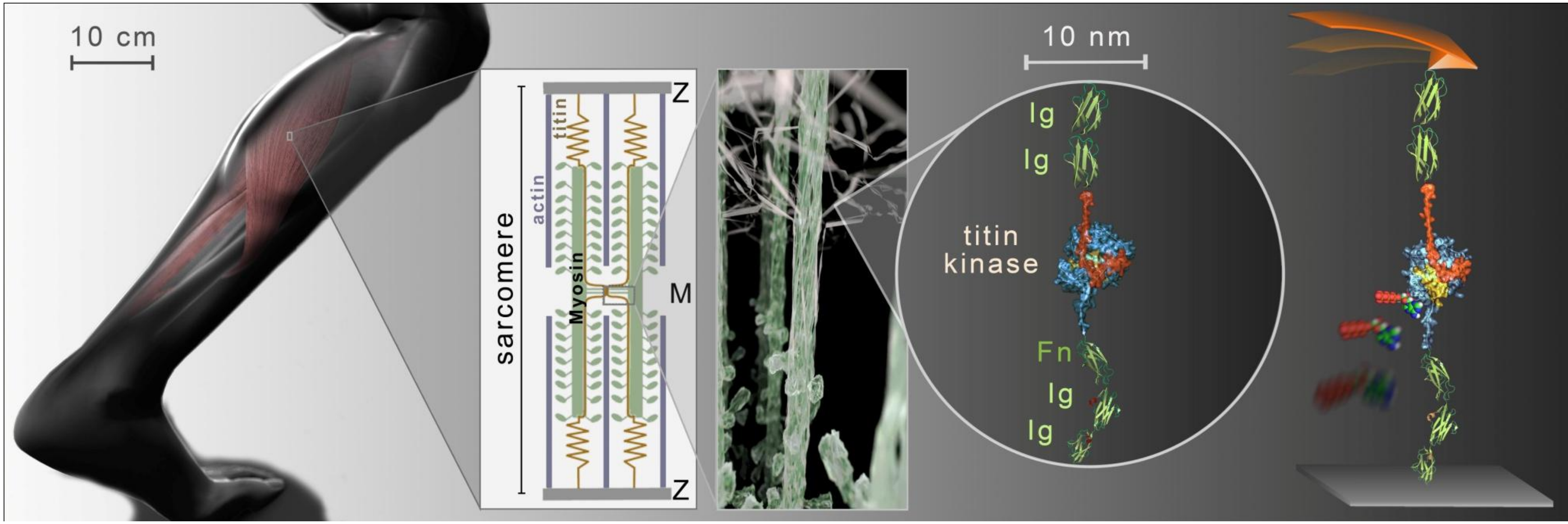


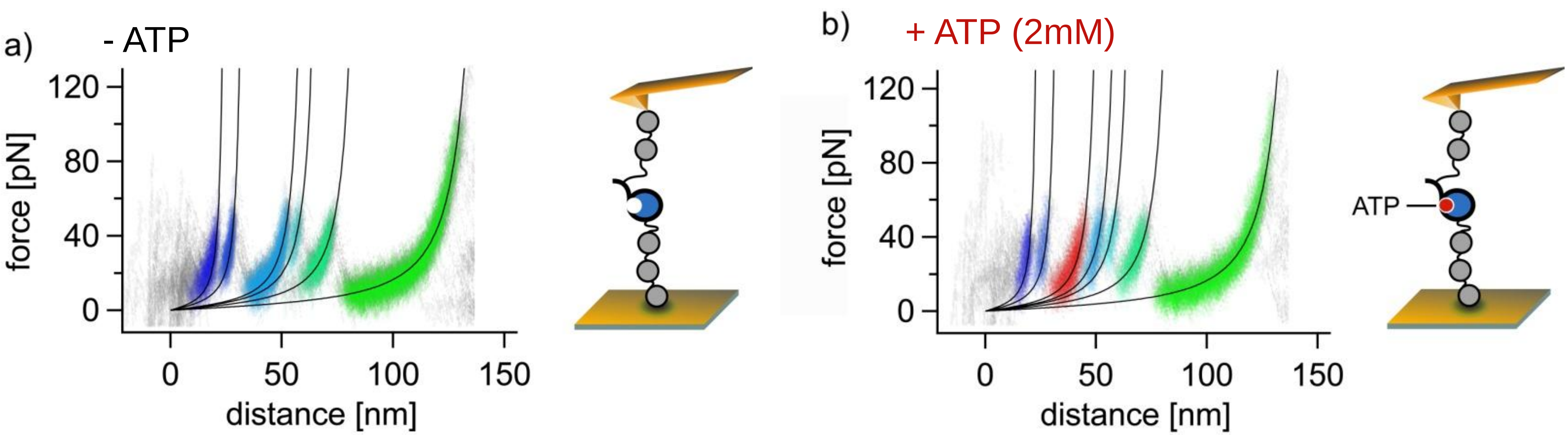
Pictures: Gaub Lab

Estimation of entropic forces on a polymer



Pictures: Gaub Lab





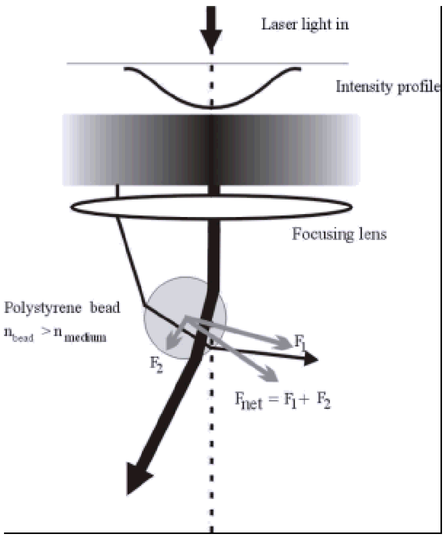
Pictures: Gaub Lab

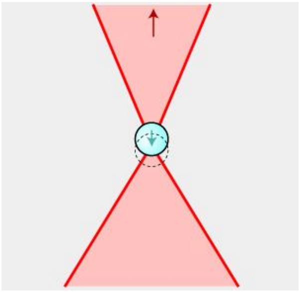
Force Spectroscopy with Optical Tweezers

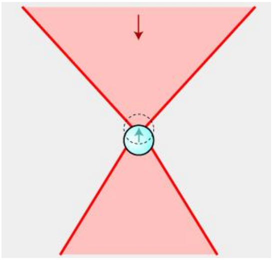
Optical Tweezers

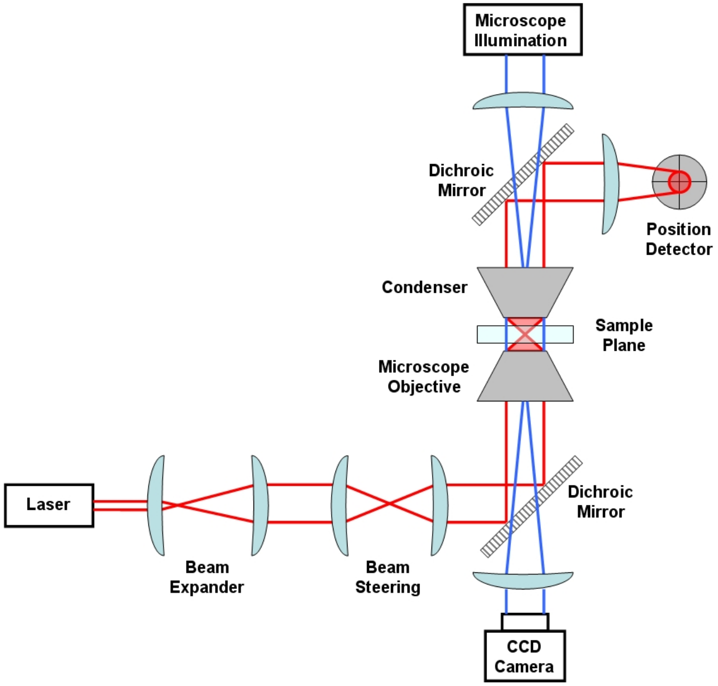
→ A. Ashkin et al., *Opt. Lett. 11, 288 (1986)*

Mie-Regime: Particle >> λ : ray-optics









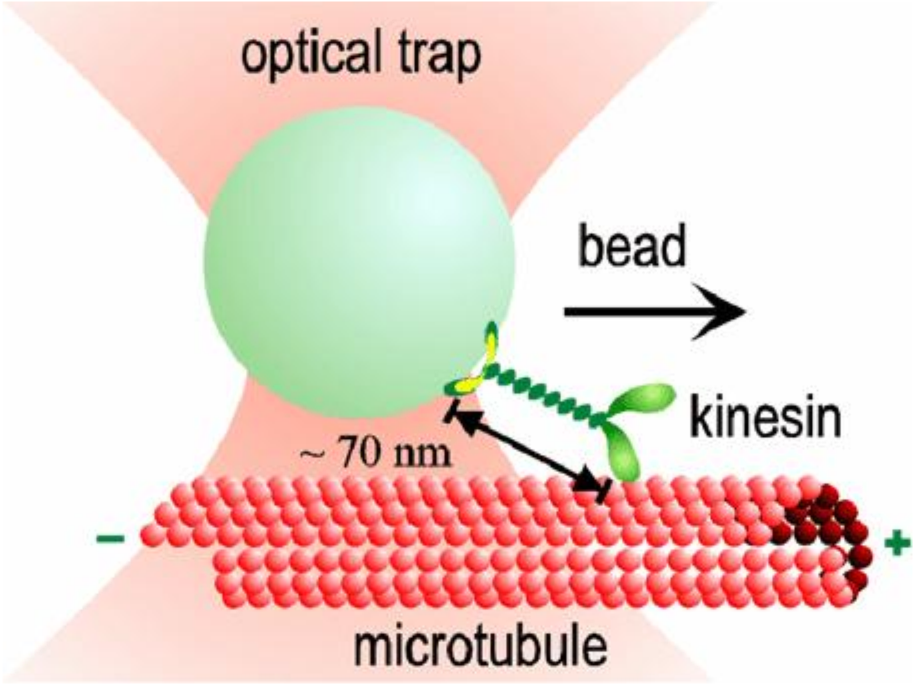
Typical Trapping wavelength: 1064 nm

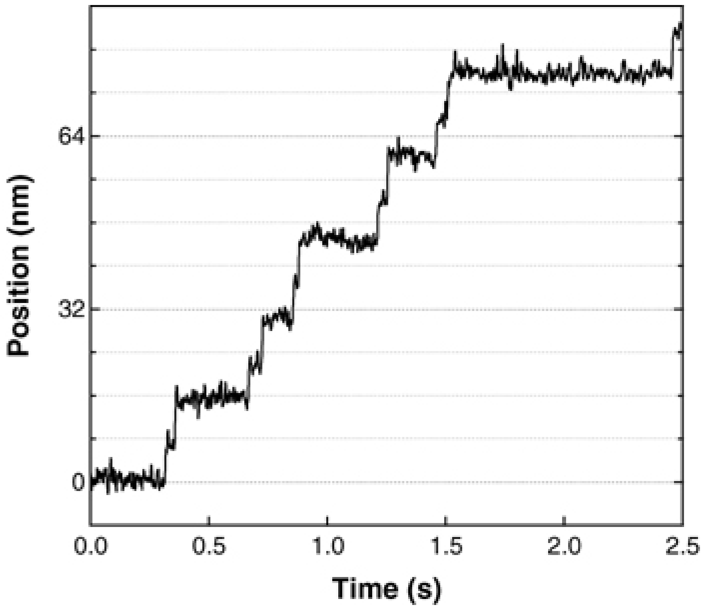
Rayleigh-Regime

Particle diameter << lambda

Consider particle as electric dipole

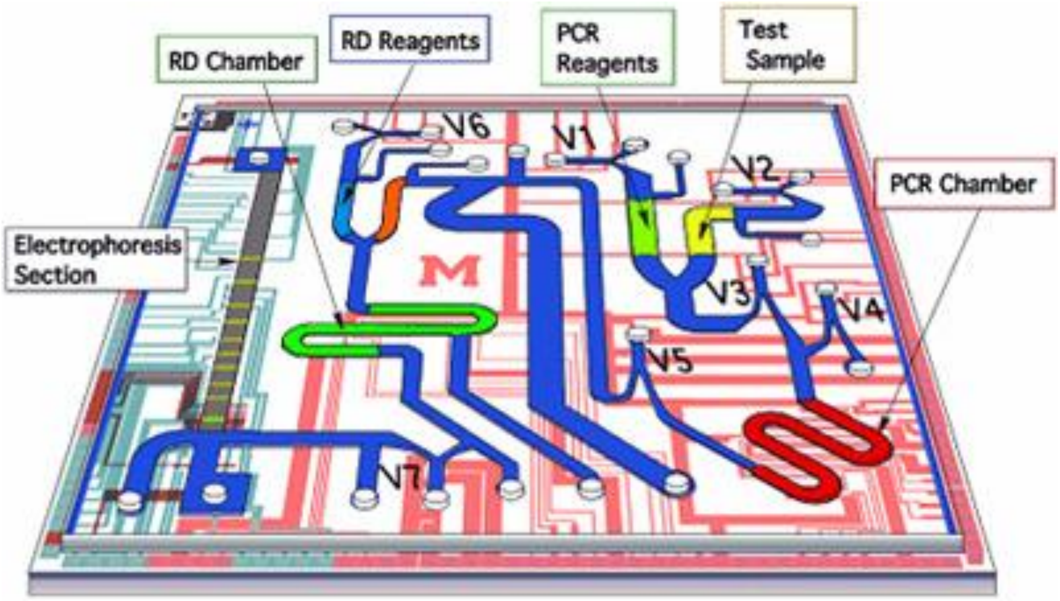
Investigation of Kinesin





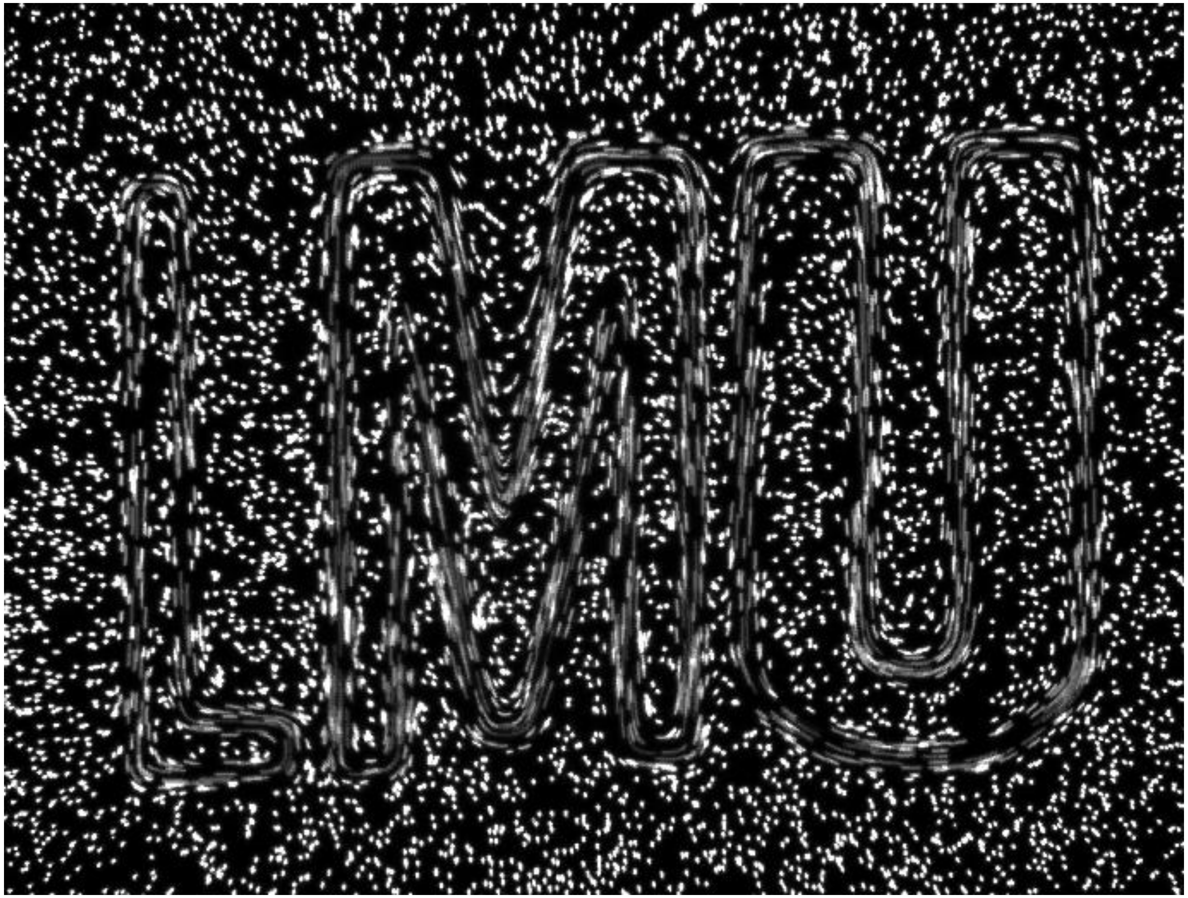
Light Driven Microfluidics

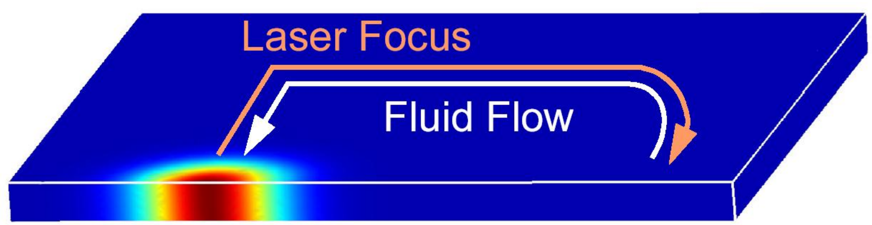
Lab-on-a-Chip



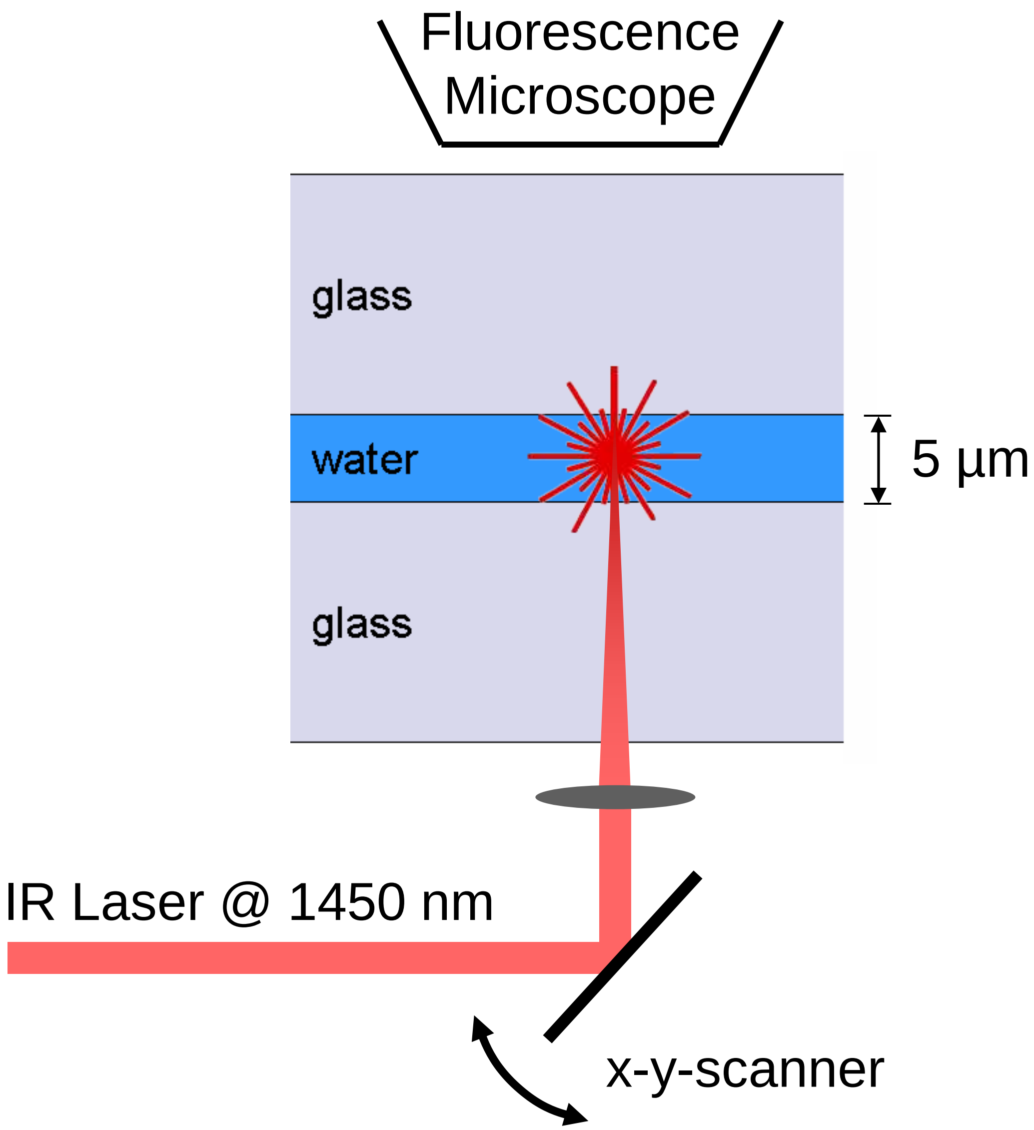
Controlled Fluid Flow without channels?

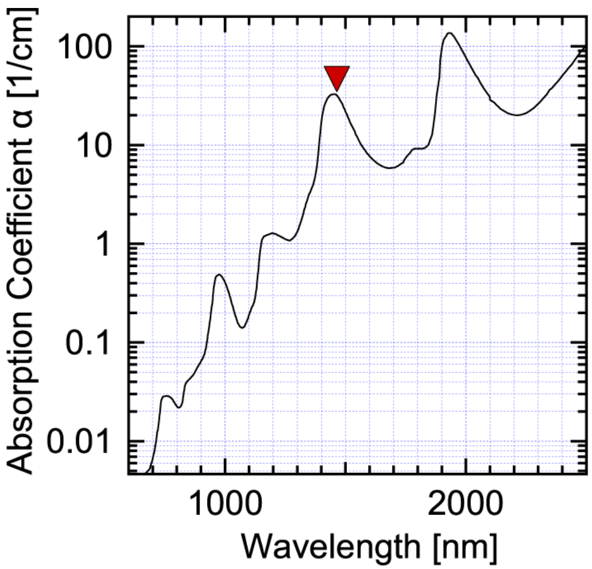
Full Fluid Control





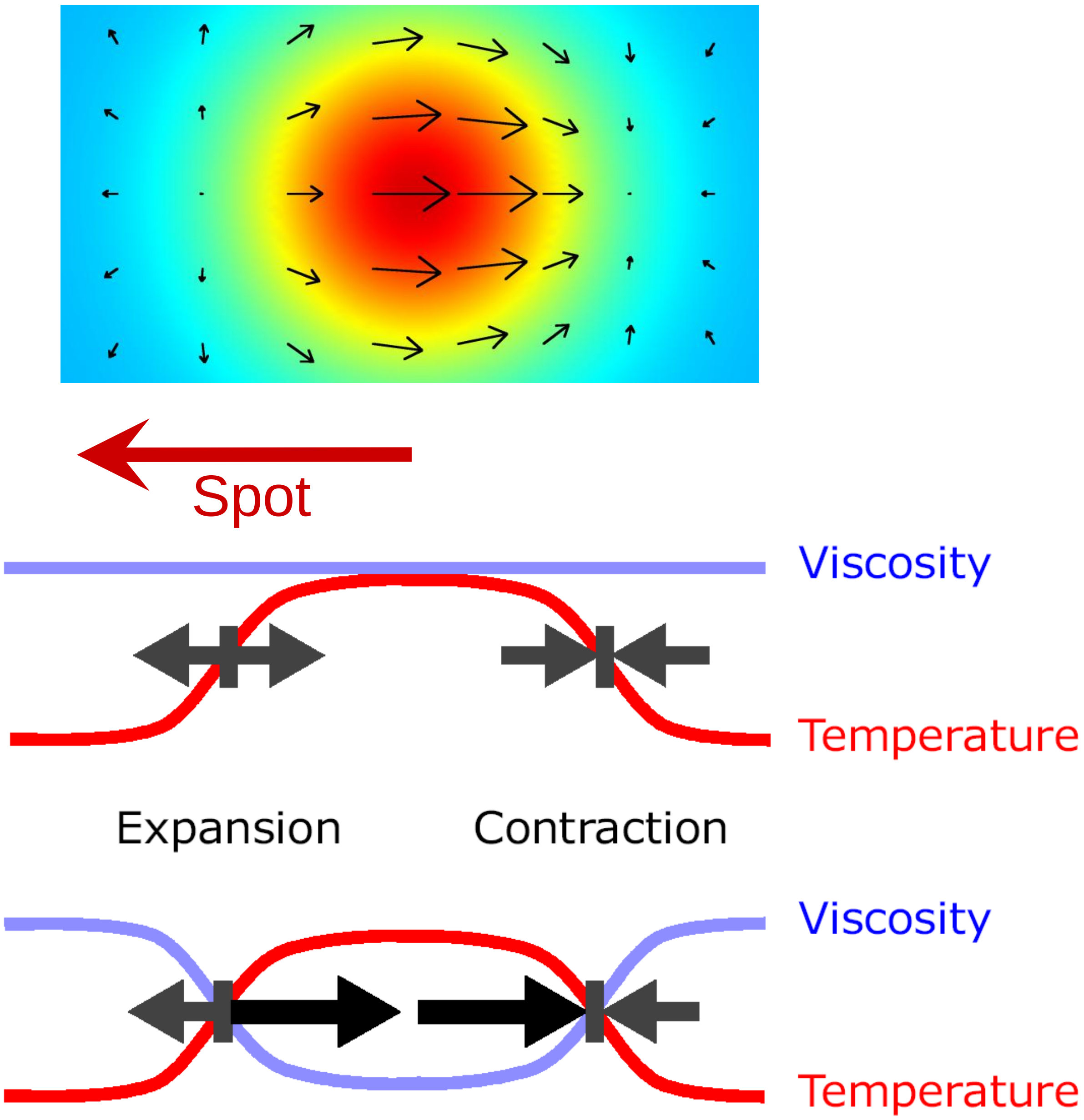
Setup


Fluorescence
Microscope
glass
water
5 µm
glass
IR Laser @ 1450 nm
x-y-scanner

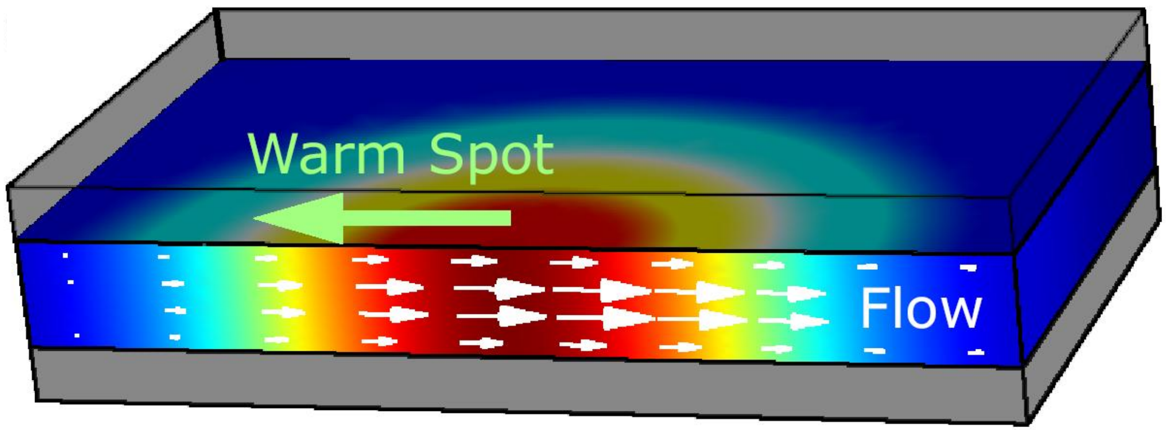

Absorption Coefficient α [1/cm]
100
10
1
0.1
0.01
1000
2000
Wavelength [nm]

What is the driving mechanism?

Moving warm spot



Finite Element Analysis



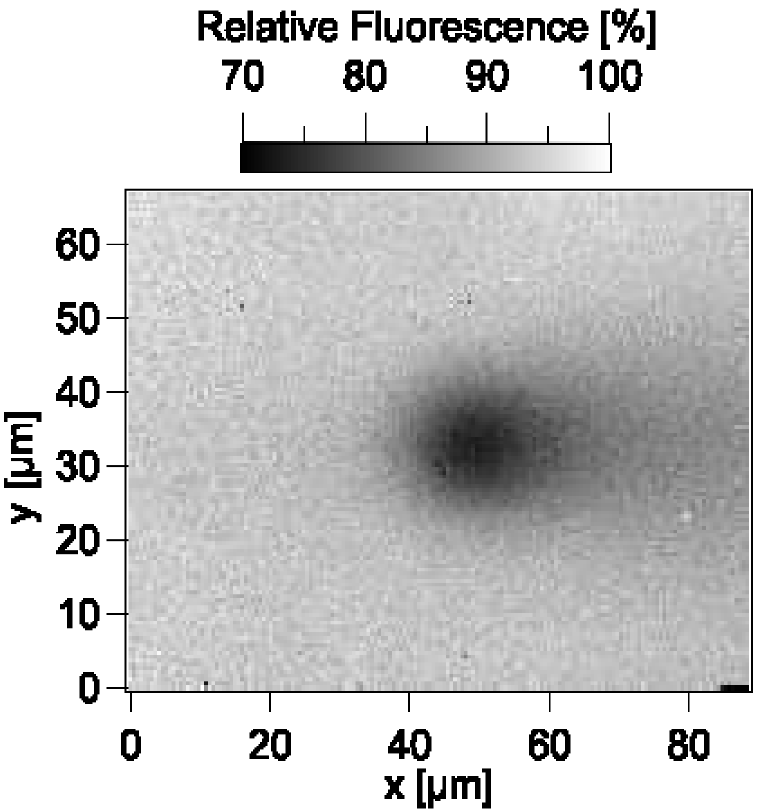
$$-\rho\frac{\partial u}{\partial t} - \rho(uu_x + vu_y + wu_z) = \frac{\partial}{\partial x}[p - 2\eta u_x] - \frac{\partial}{\partial y}[\eta(u_y + v_x)] - \frac{\partial}{\partial z}[\eta(u_z + w_x)]$$

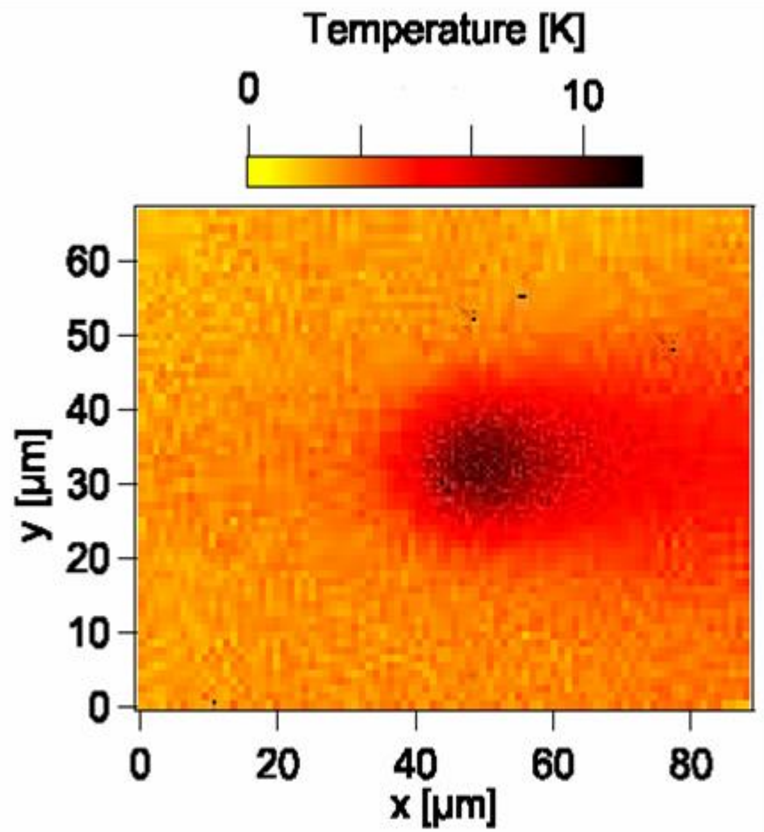
$$-\rho\frac{\partial v}{\partial t} - \rho(uv_x + vv_y + wv_z) = -\frac{\partial}{\partial x}[\eta(v_x + u_y)] + \frac{\partial}{\partial y}[p - 2\eta v_y] - \frac{\partial}{\partial z}[\eta(v_z + w_y)]$$

$$-\rho\frac{\partial w}{\partial t} - \rho(uw_x + vw_y + ww_z) = -\frac{\partial}{\partial x}[\eta(w_x + u_z)] - \frac{\partial}{\partial y}[\eta(w_y + v_z)] + \frac{\partial}{\partial z}[p - 2\eta w_z]$$

$$\frac{\partial\rho}{\partial t} + \rho(u_x + v_y + w_z) + \rho_x u + \rho_y v + \rho_z w = 0$$

Temperature Imaging

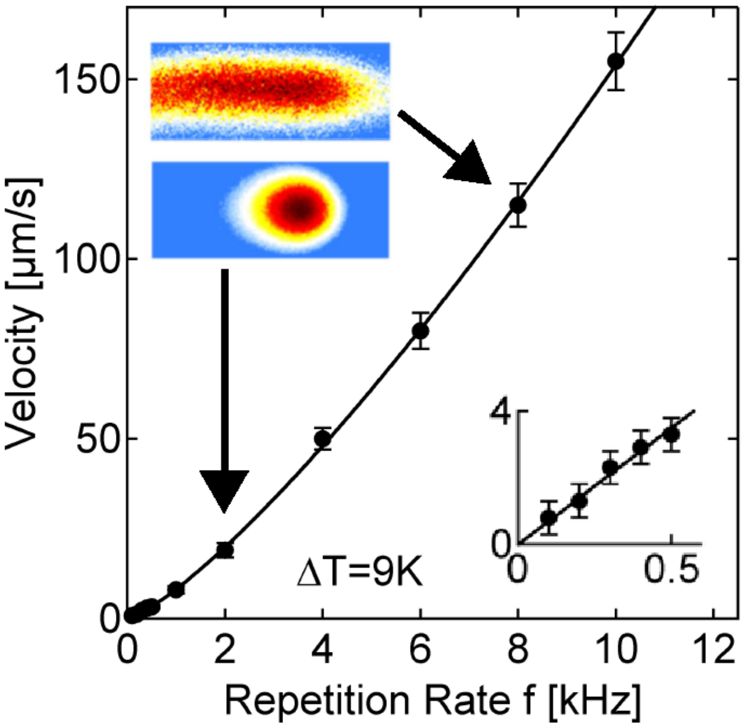


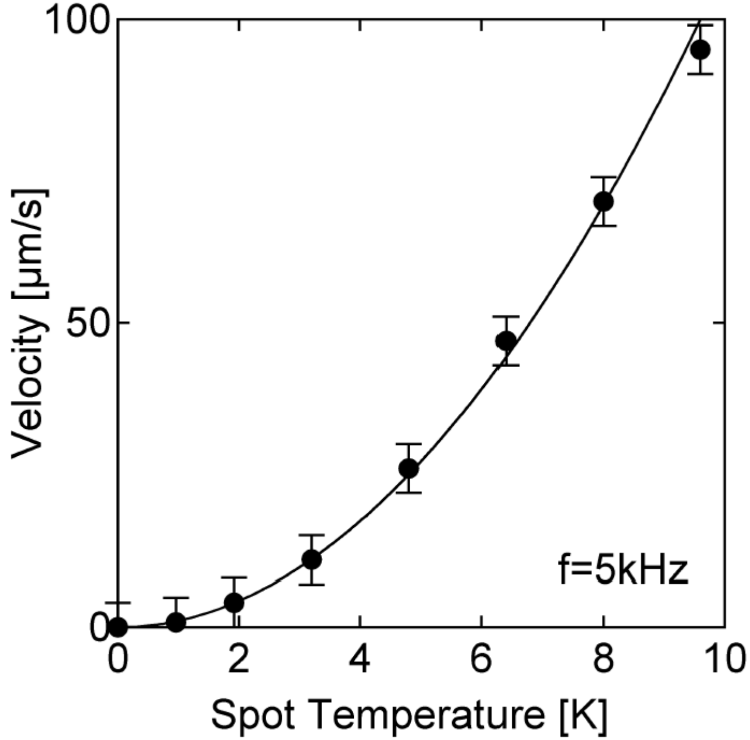


Dependencies

$$v \propto f$$

$$v \propto \alpha \Delta T \times \beta \Delta T \propto \Delta T^2$$

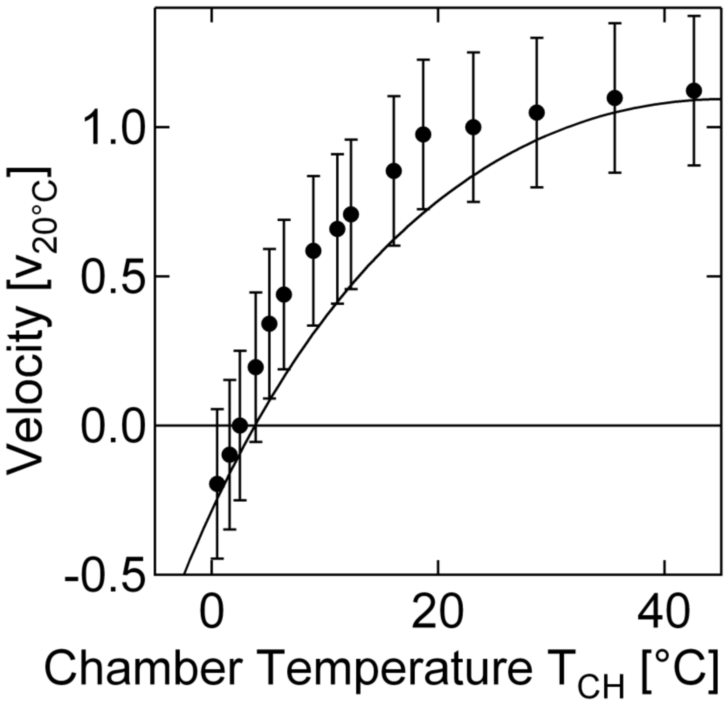


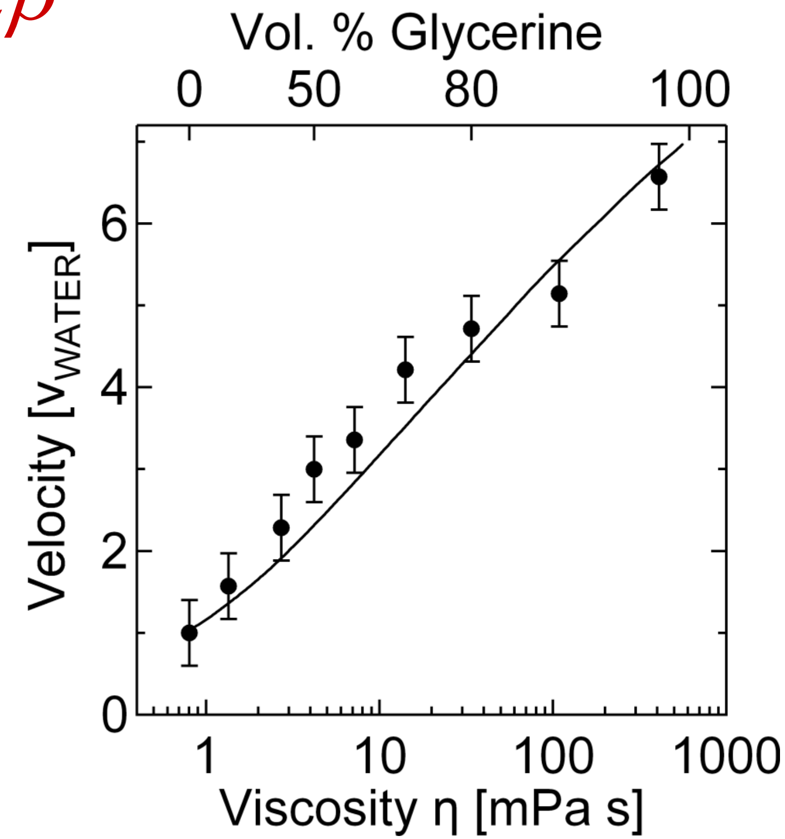


$$v_{\mathrm{pump}} = \frac{-3\sqrt{\pi}}{4} f \alpha \beta b \Delta T^2$$

Expansion coefficient and viscosity

$$v \propto \alpha\beta$$

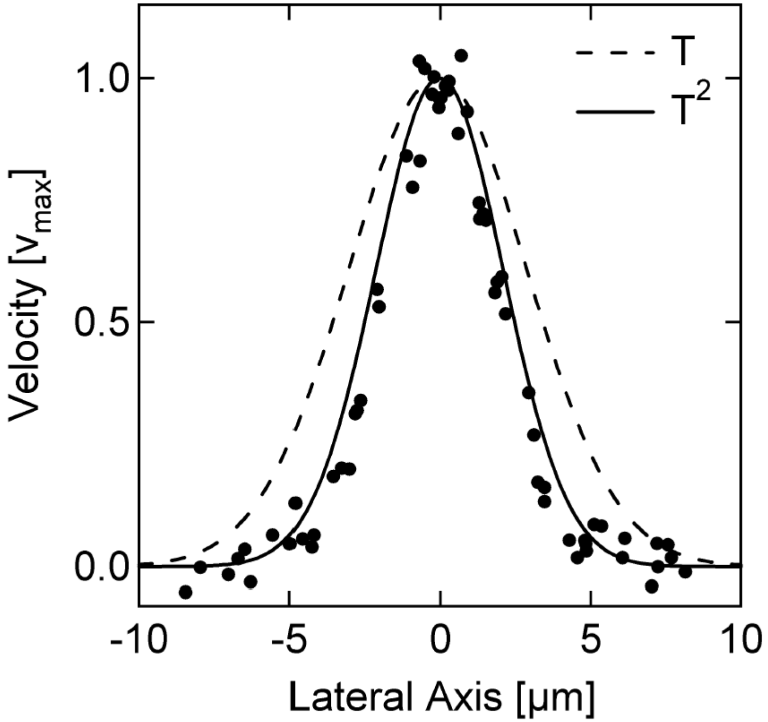


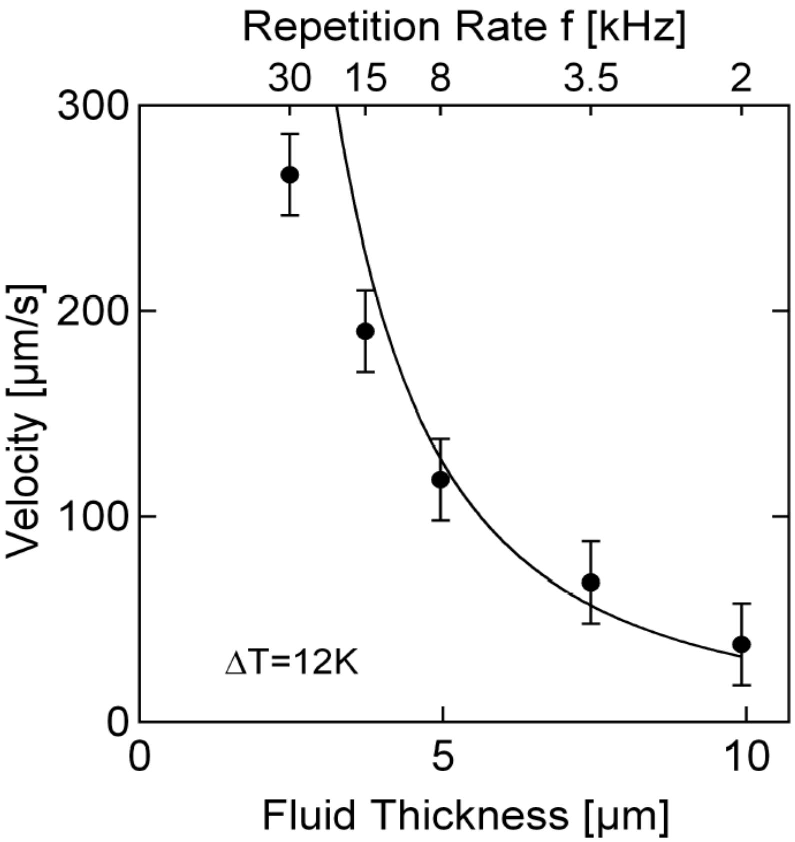


$$v_{\text{pump}} = \frac{-3\sqrt{\pi}}{4} f \alpha \beta b \Delta T^2$$

More Efficient towards Nanofluidics

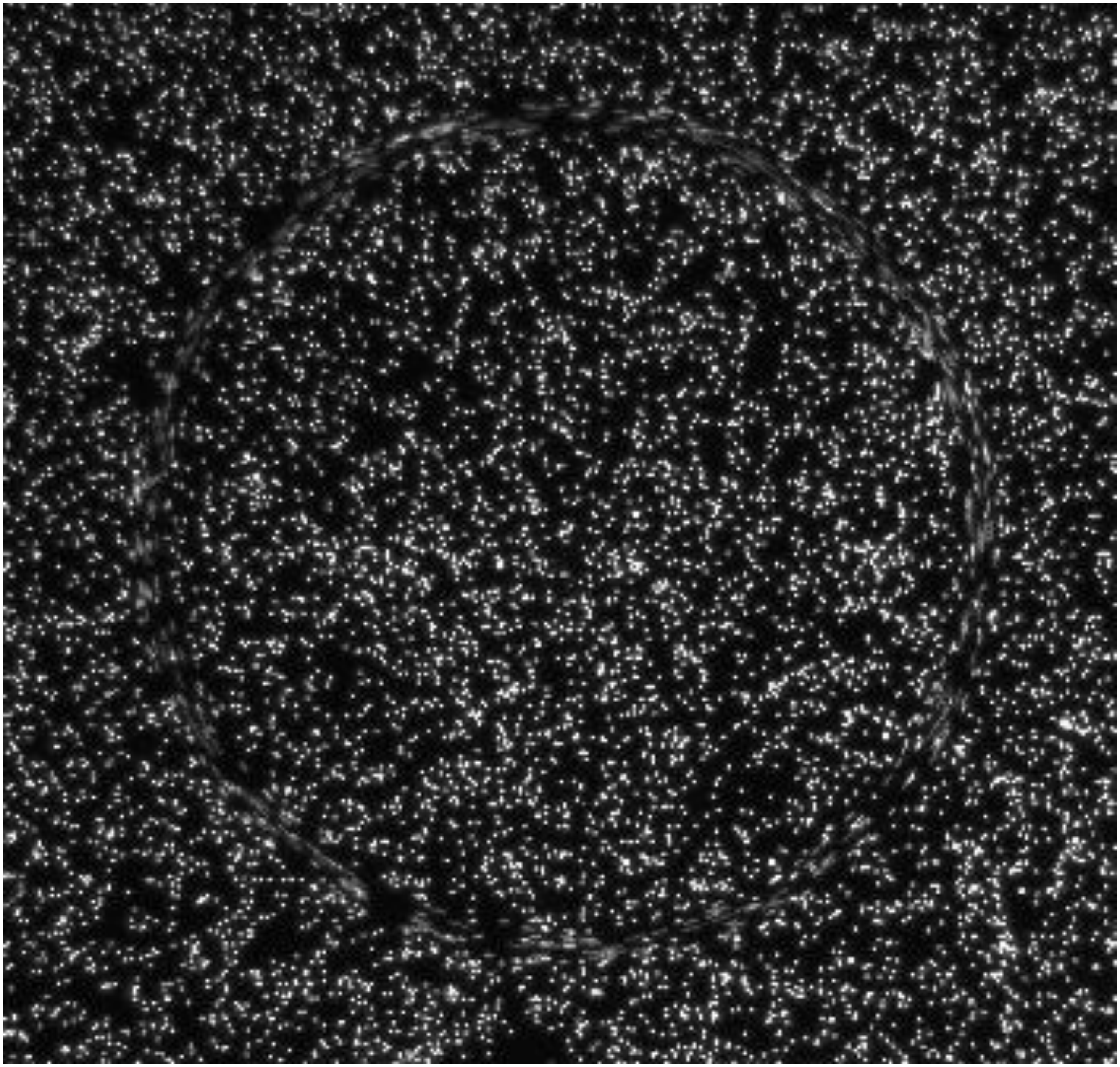
$$v \propto 1/d^2$$



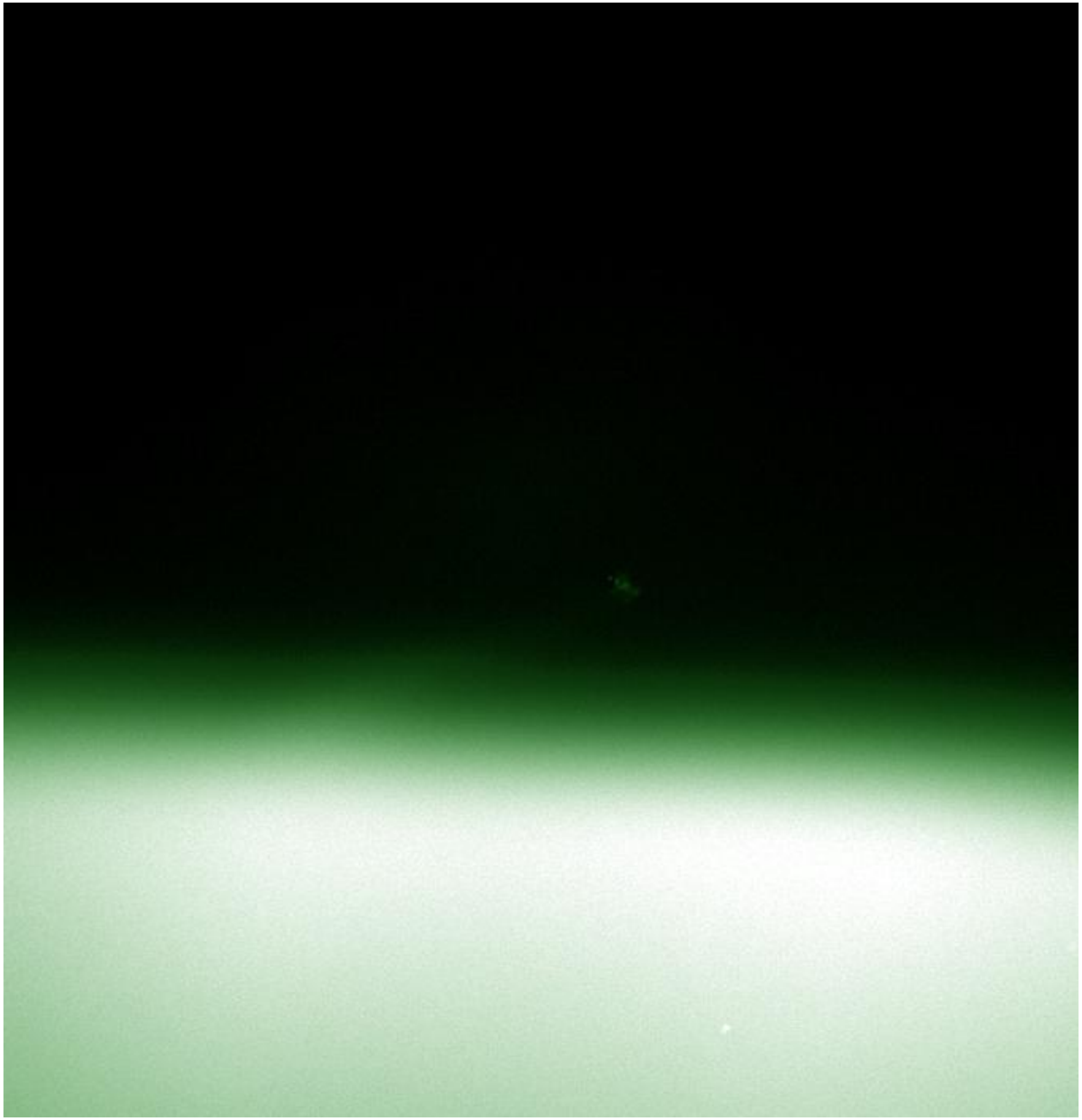


F. M. Weinert and D. Braun, **J. Appl. Phys.** 104, 104701 (2008).

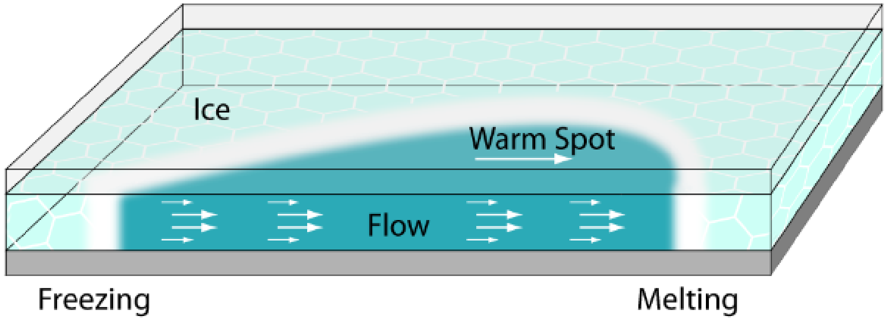
Full Fluid Control



Microfluidics in Gels

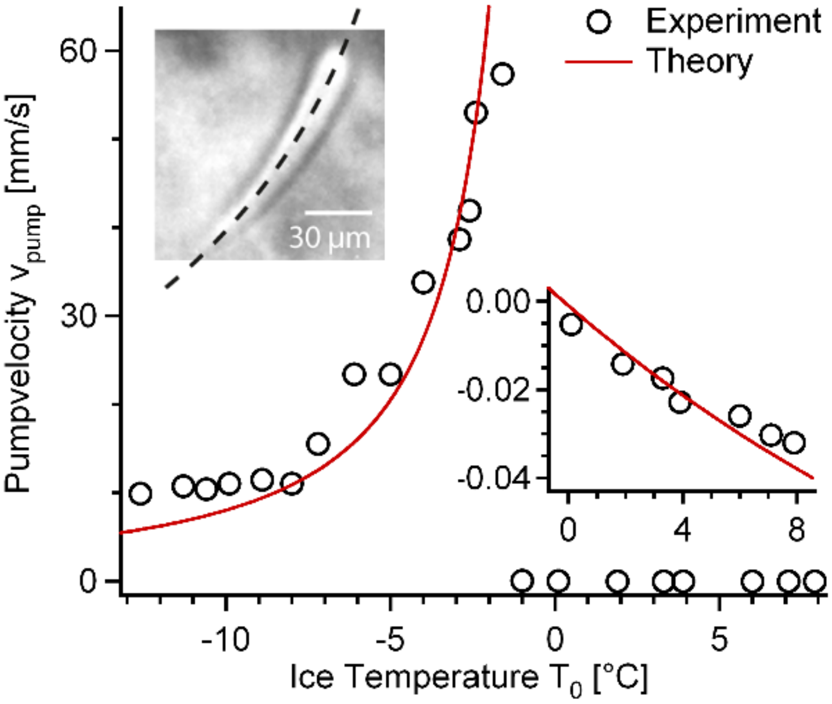


Pumping in Ice



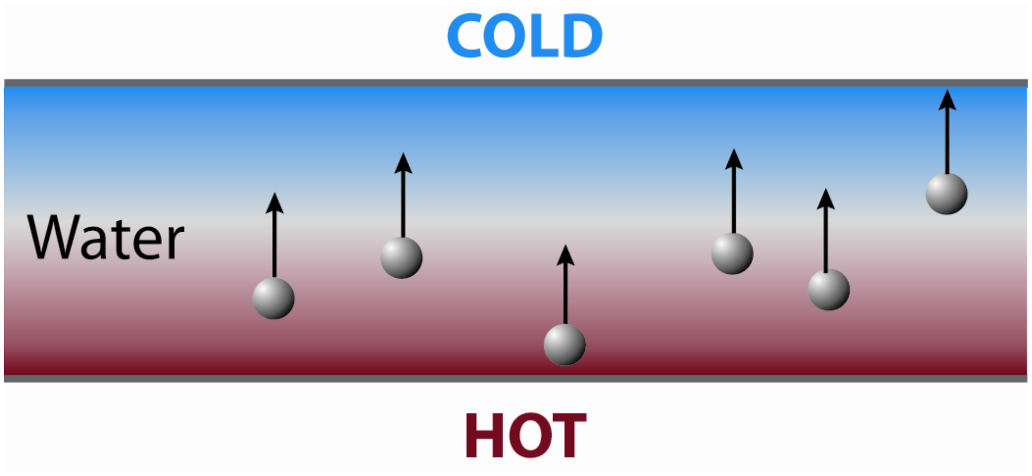
Pumping in Ice

$$v_{\mathrm{pump}} \approx 0.14\, d_{\mathrm{spot}}\, f$$



F. M. Weinert, M. Wühr and D. Braun, **Appl. Phys. Lett.** 94, 113901 (2009)

Thermophoresis



$$v = -D_T \nabla T$$

D_T: Thermodiffusion Coefficient

$$\frac{c}{c_0} = \exp(-S_T \Delta T)$$

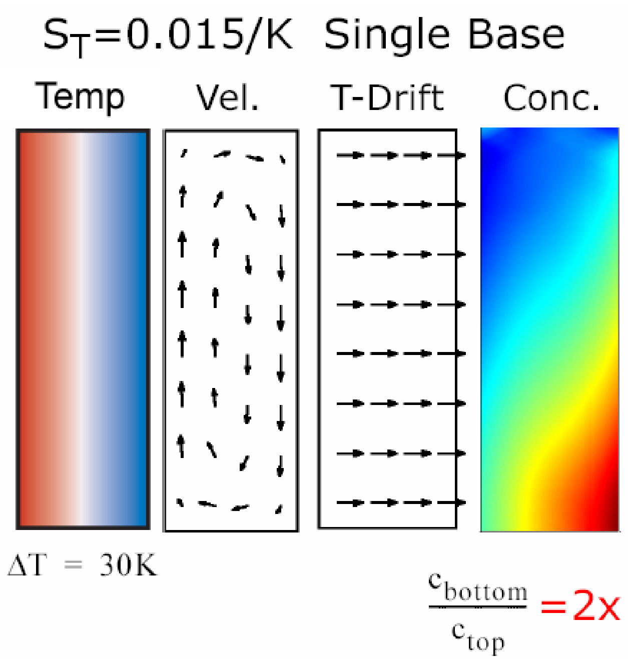
$S_T = \frac{D_T}{D}$: Soret Coefficient

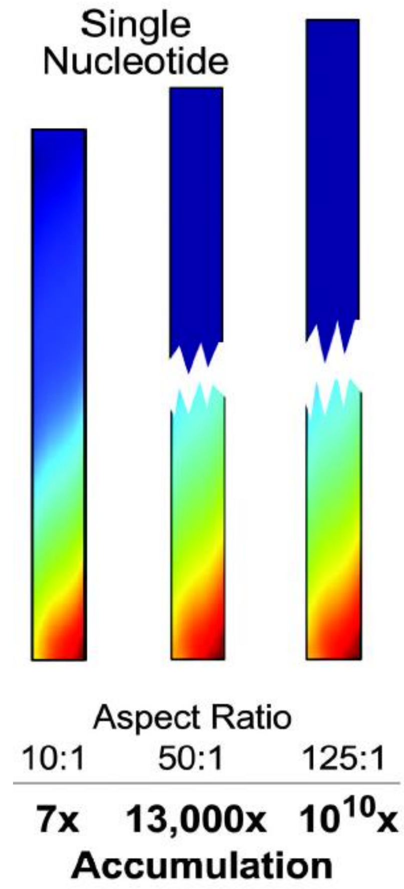
Towards a Molecule Trap

[Paternoster]

Towards Accumulation

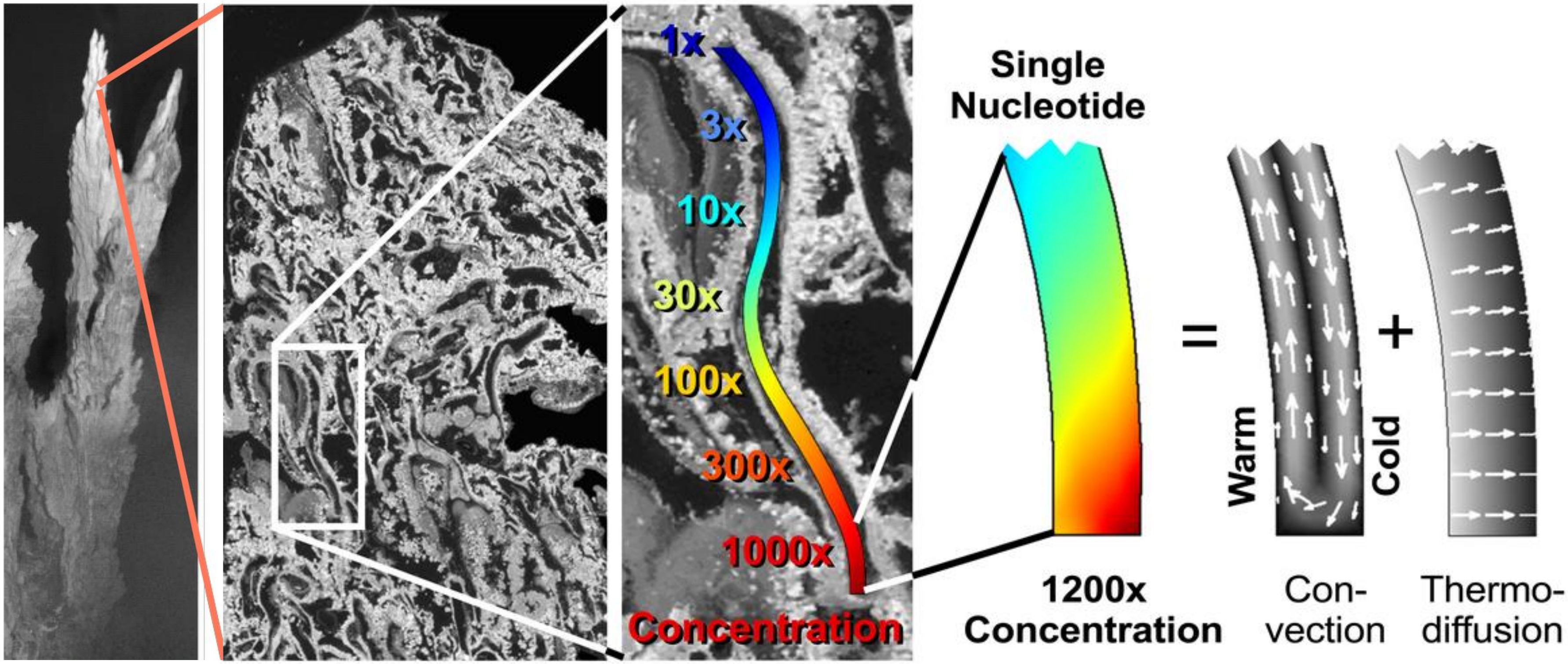
Thermogravitational Separation Column





$$\frac{c_{\mathrm{BOTTOM}}}{c_{\mathrm{TOP}}} = \exp\left[0.42 \times S_{\mathrm{T}} \times \Delta T \times \frac{\text{length}}{\text{width}}\right]$$

Concentration Problem at the Origin of Life

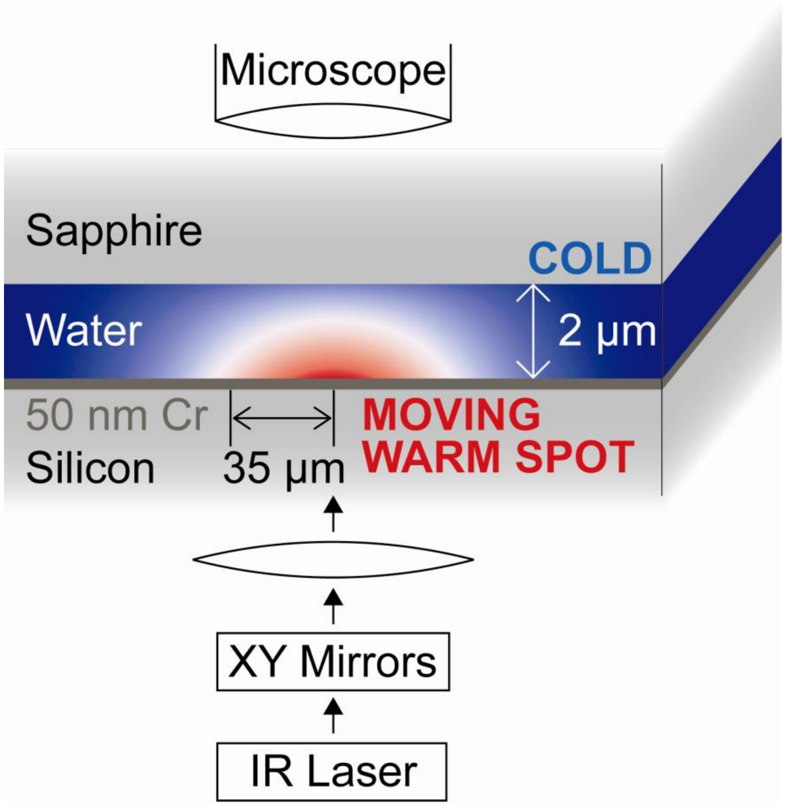


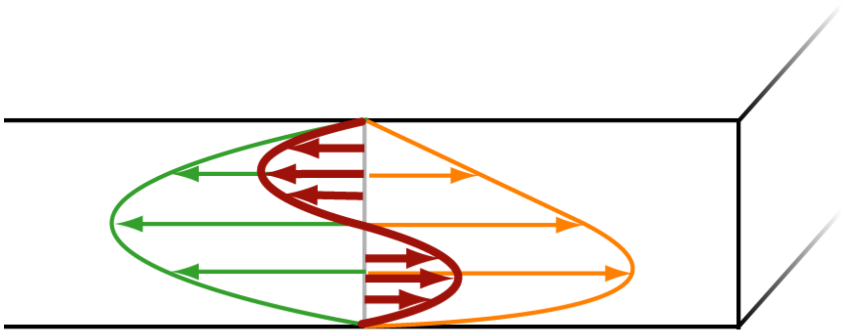
P. Baaske, F. M. Weinert, S. Duhr, K. H. Lemke, M. J. Russell and D. Braun **PNAS** 104, 9346 (2007)

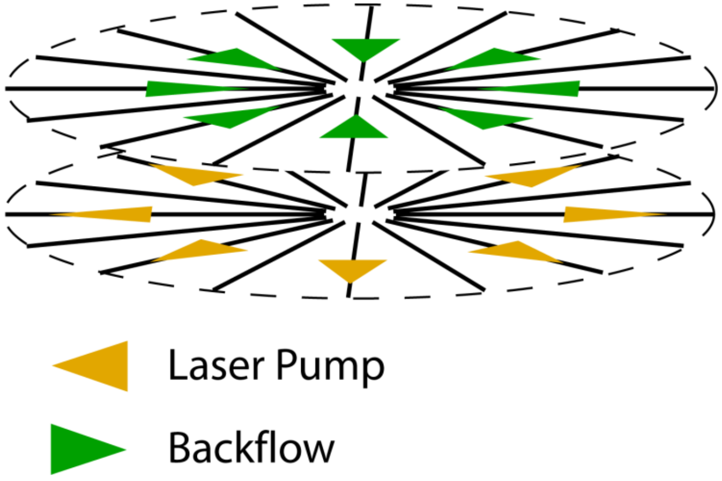
Problem for Applications: long equilibration times ~ hours/days

Linear Clusius Tube

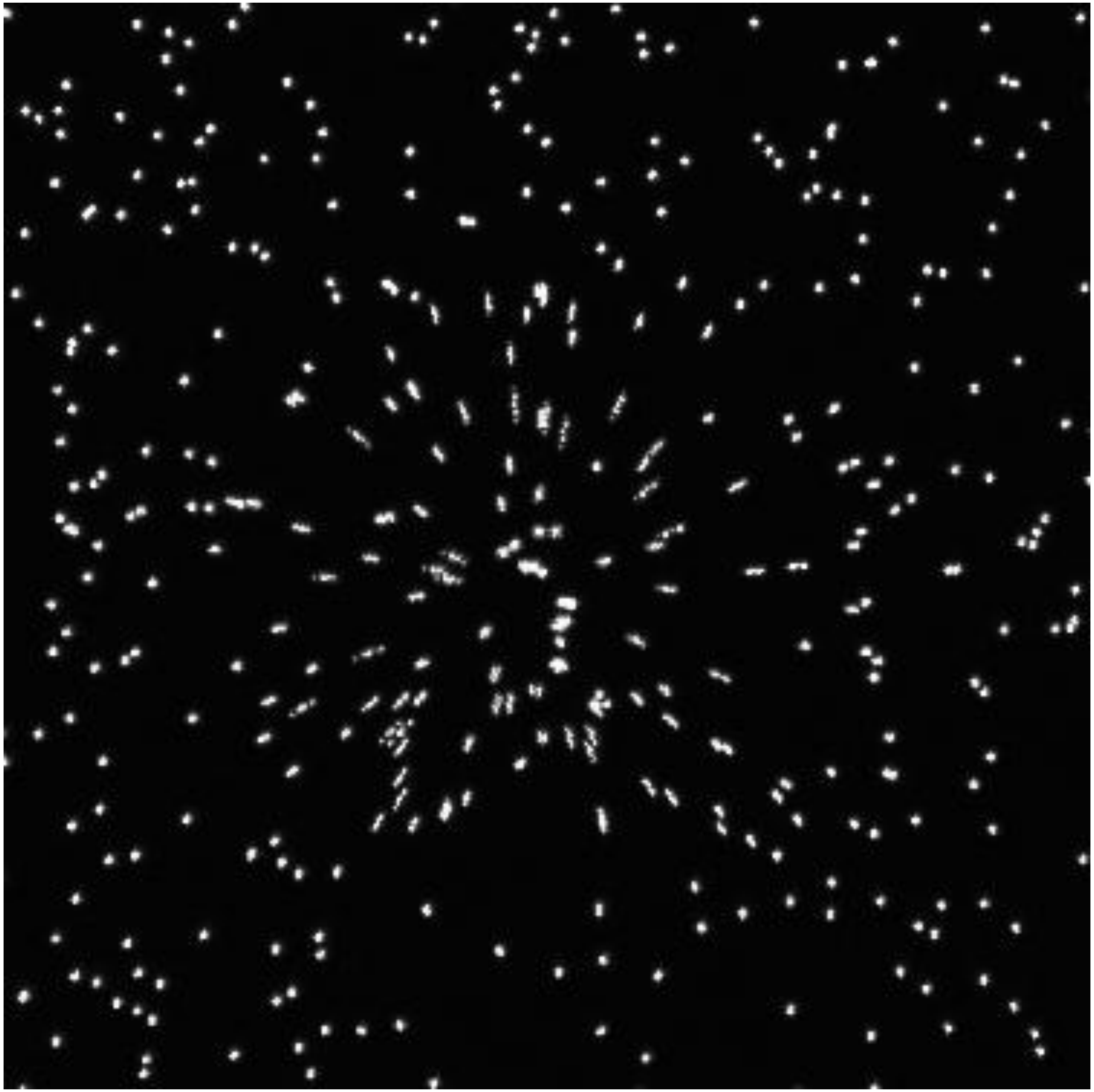
Temperature Gradient & Bidirectional Flow



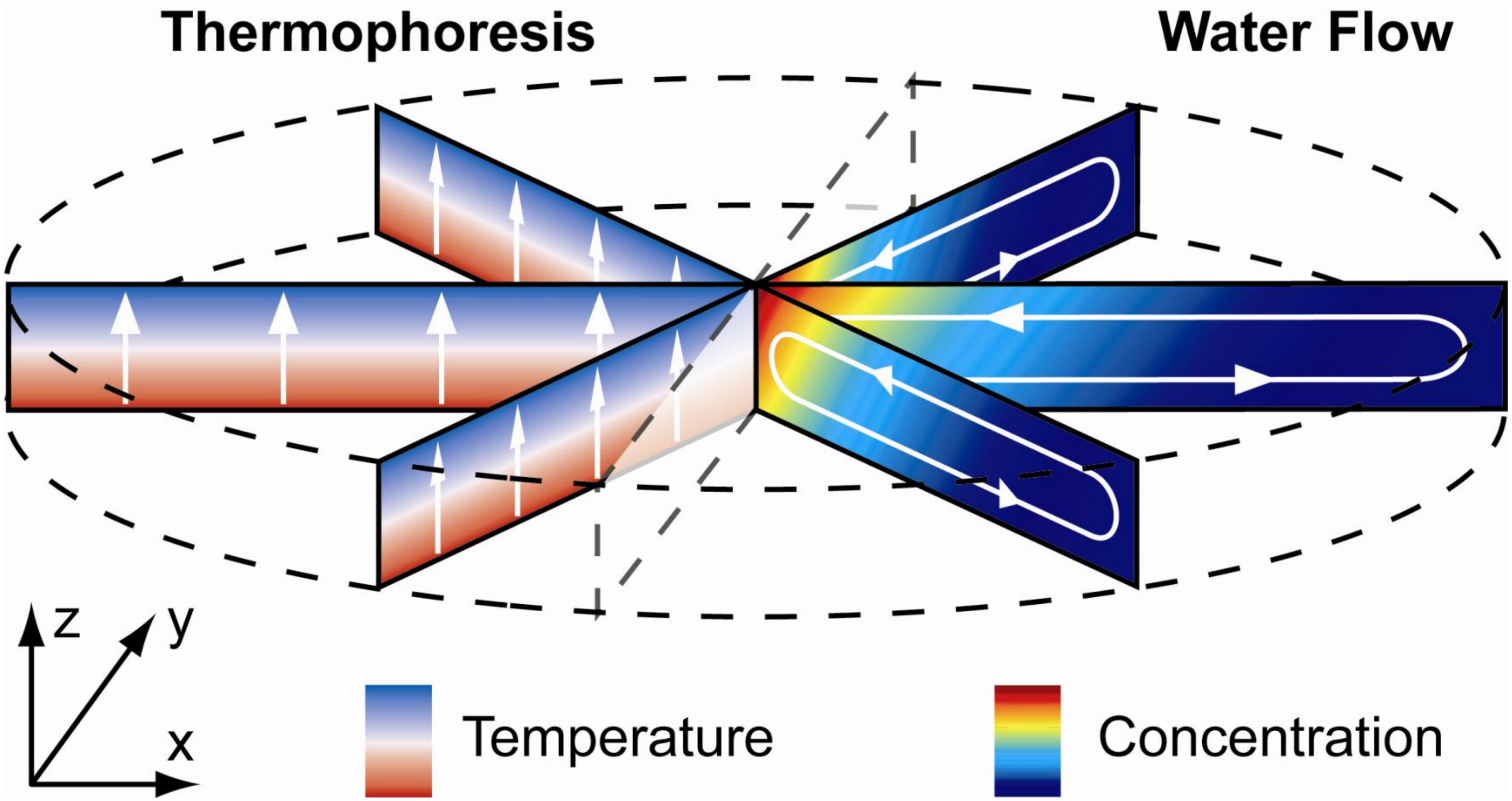




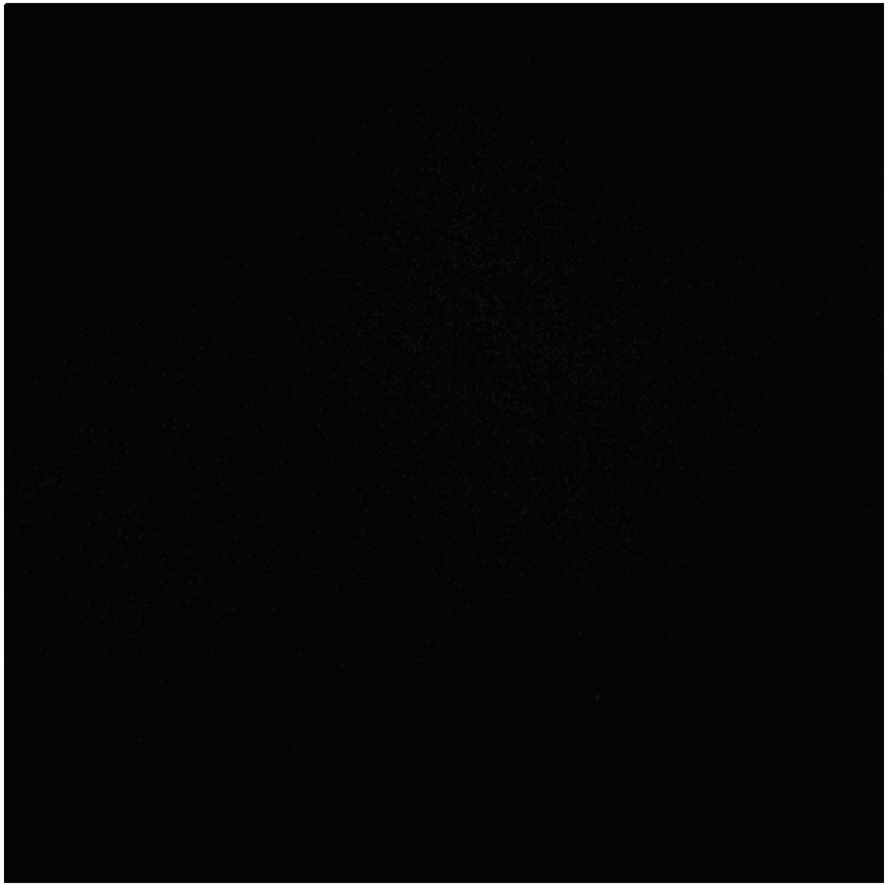
Biderectional Flow

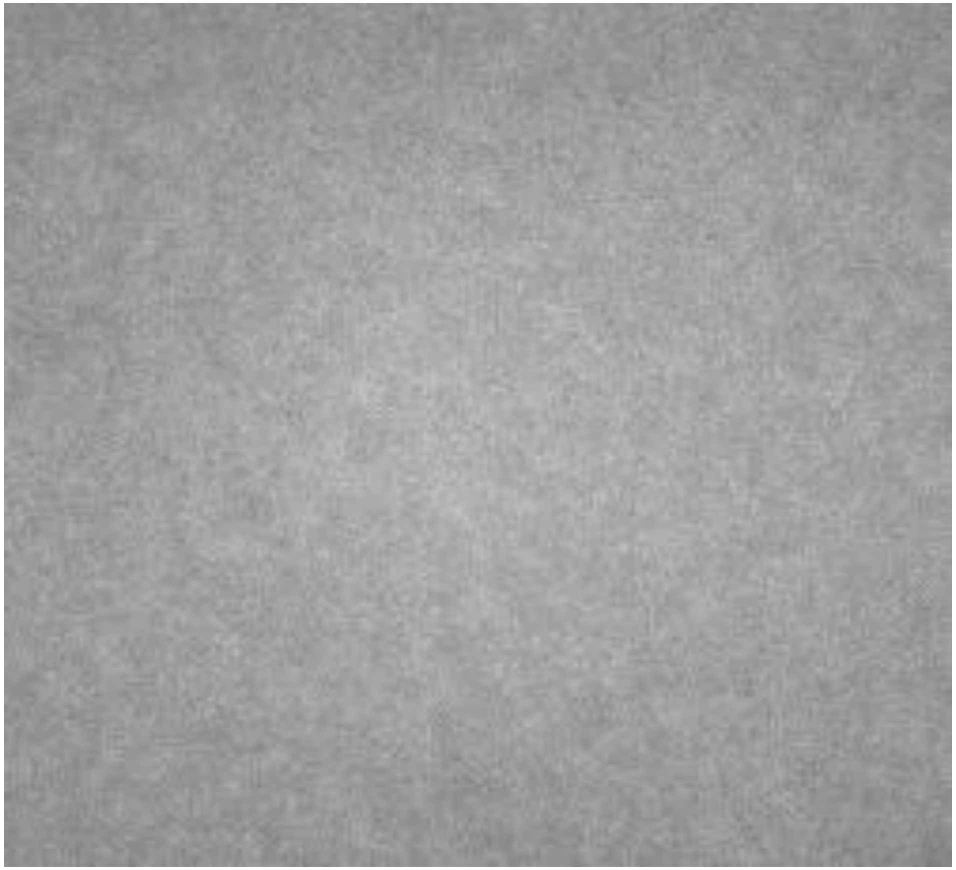


Thermophoresis + Bidirectional Flow = Accumulation

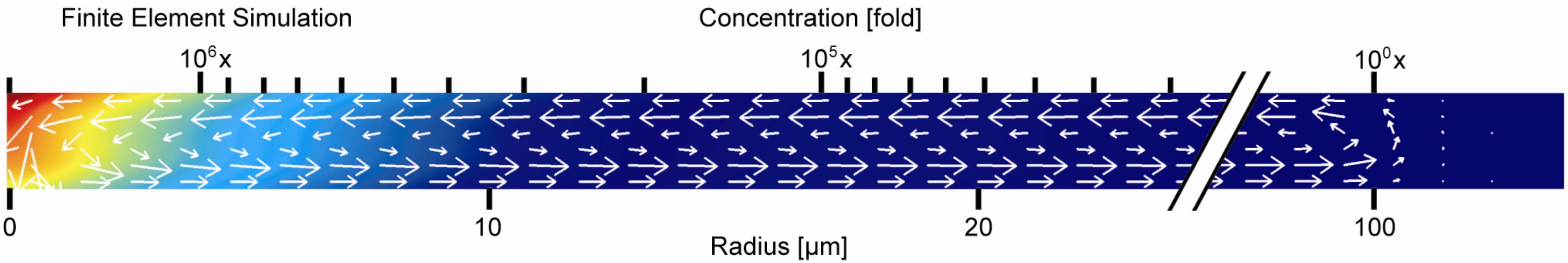


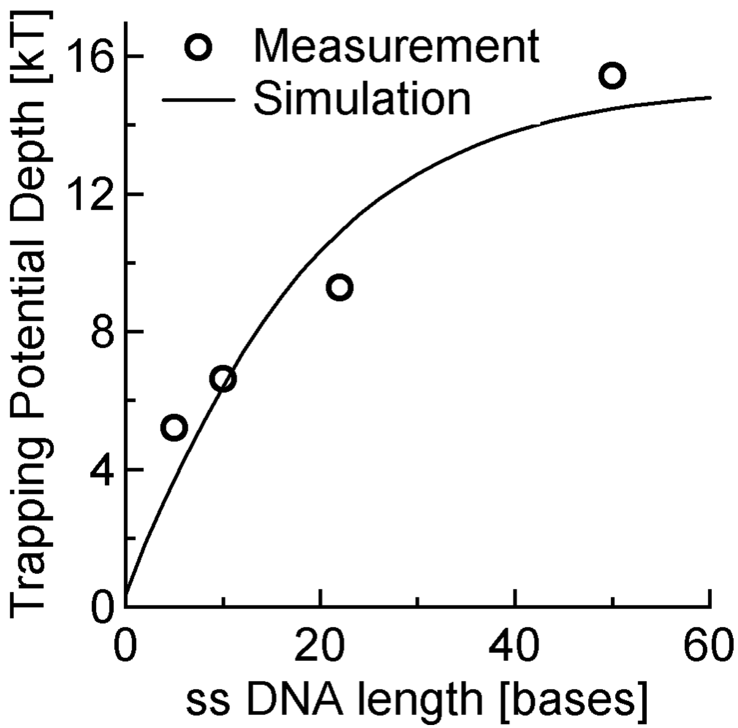
Accumulation of 5 base single stranded DNA

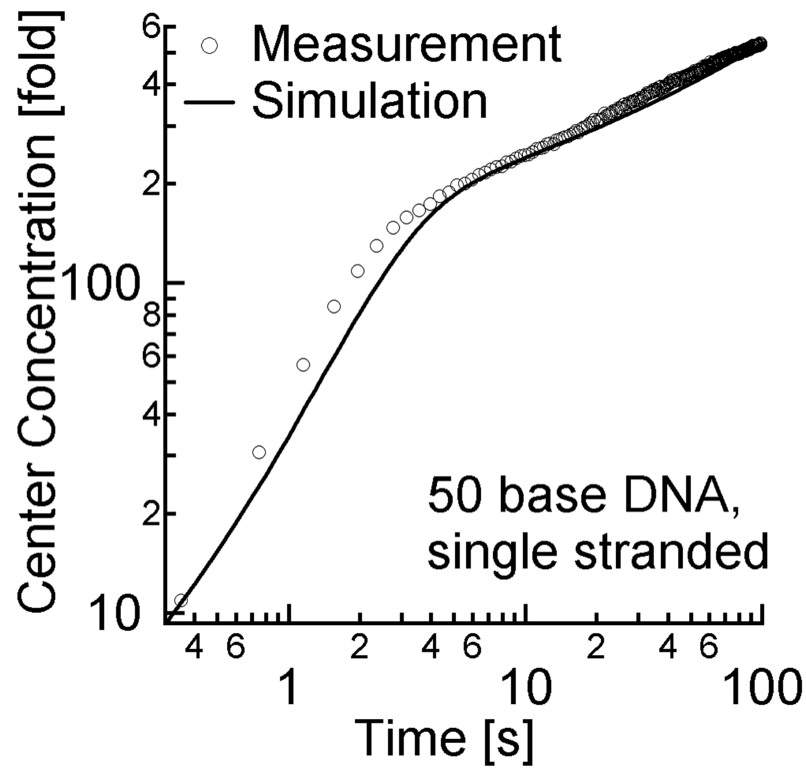




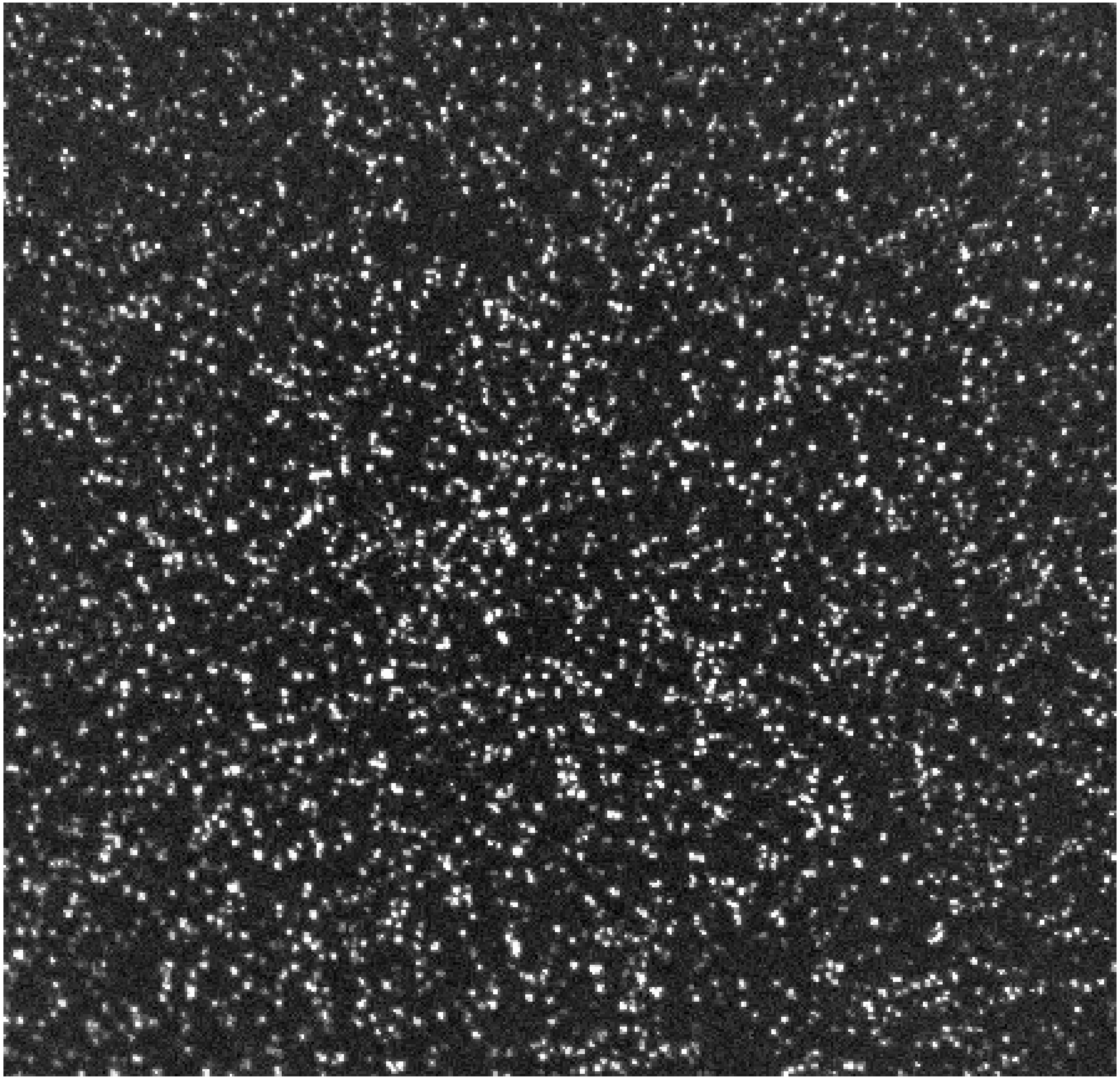
Simulation of 50 base ss DNA





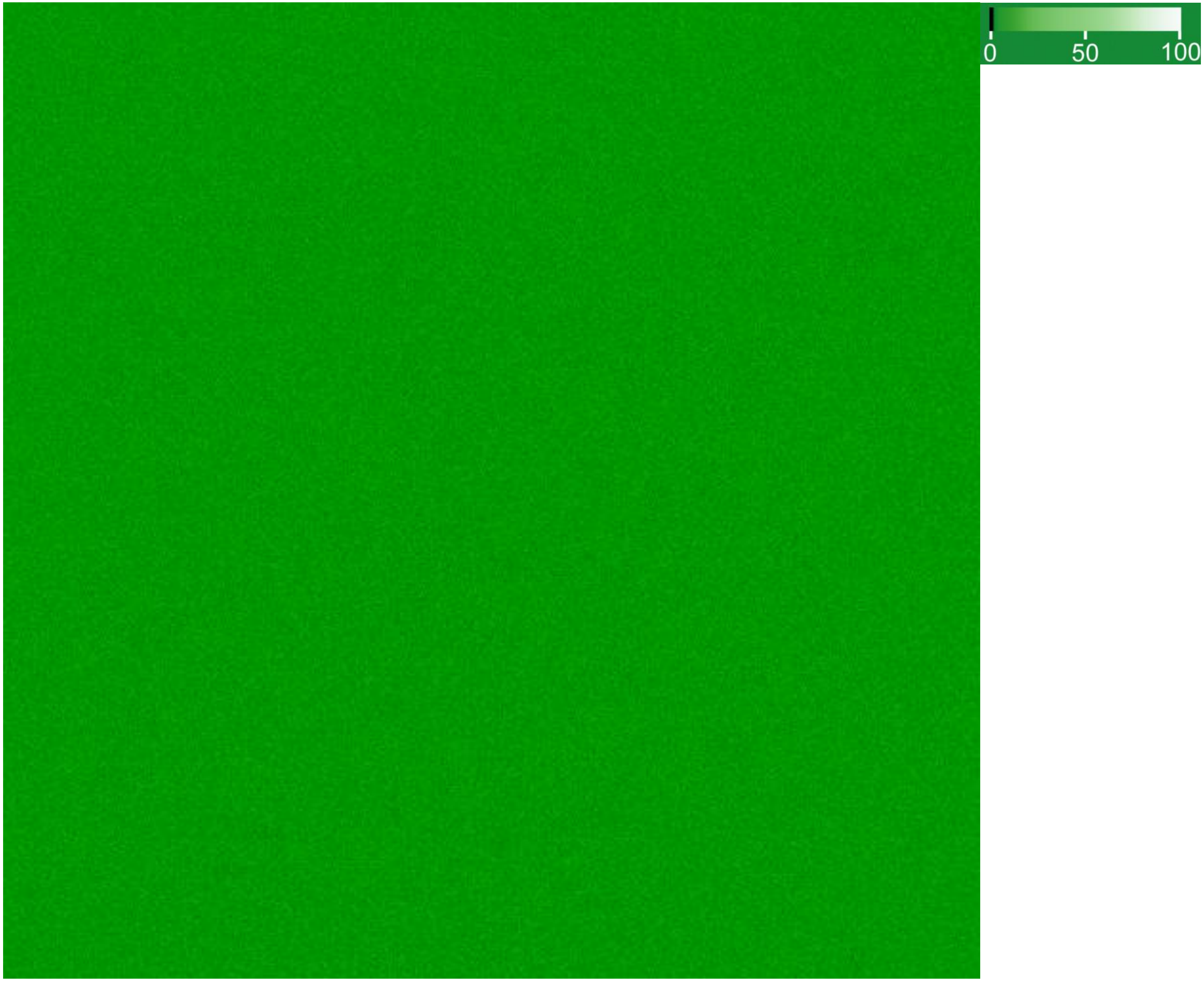


Vacuum Cleaner for 40nm beads

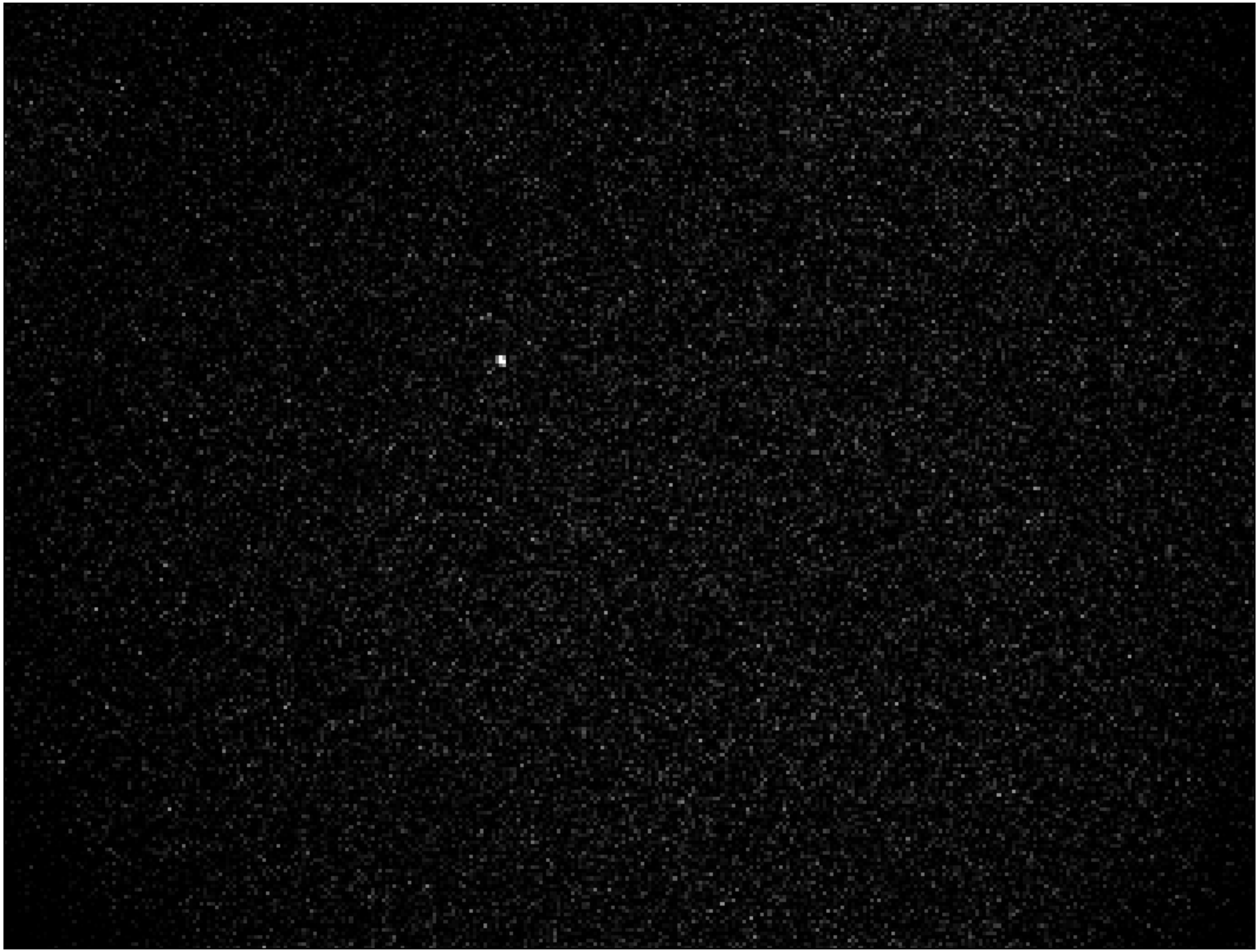


(real time)

Vacuum Cleaner for ss 50 base DNA

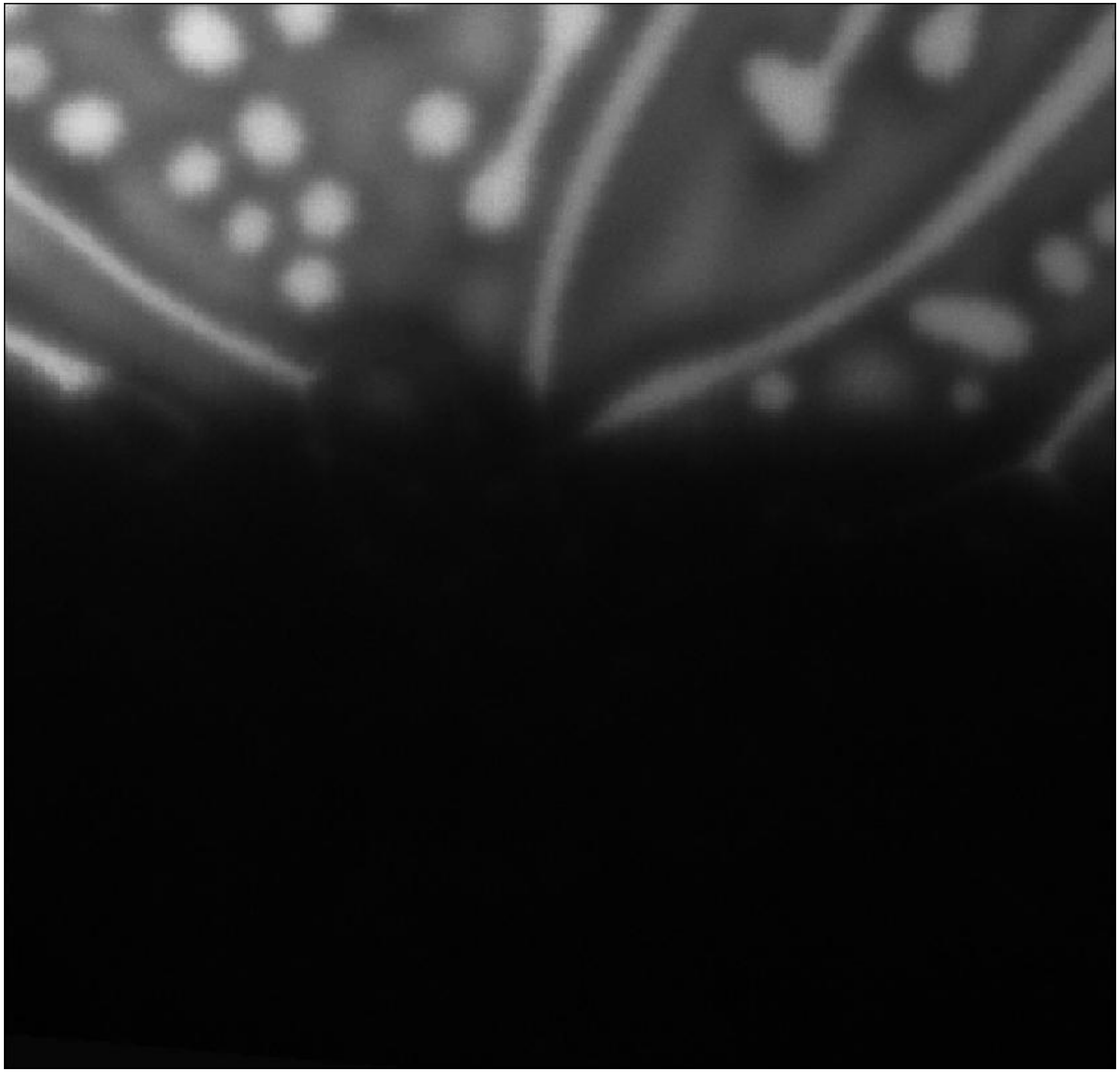


40nm bead trap



Polystyrene Spheres D = 40 nm, S_T = 0.04 1/K

Microfluidics in Ice



Parabolic Backflow