Trapping and Replicating DNA by Constant Heating

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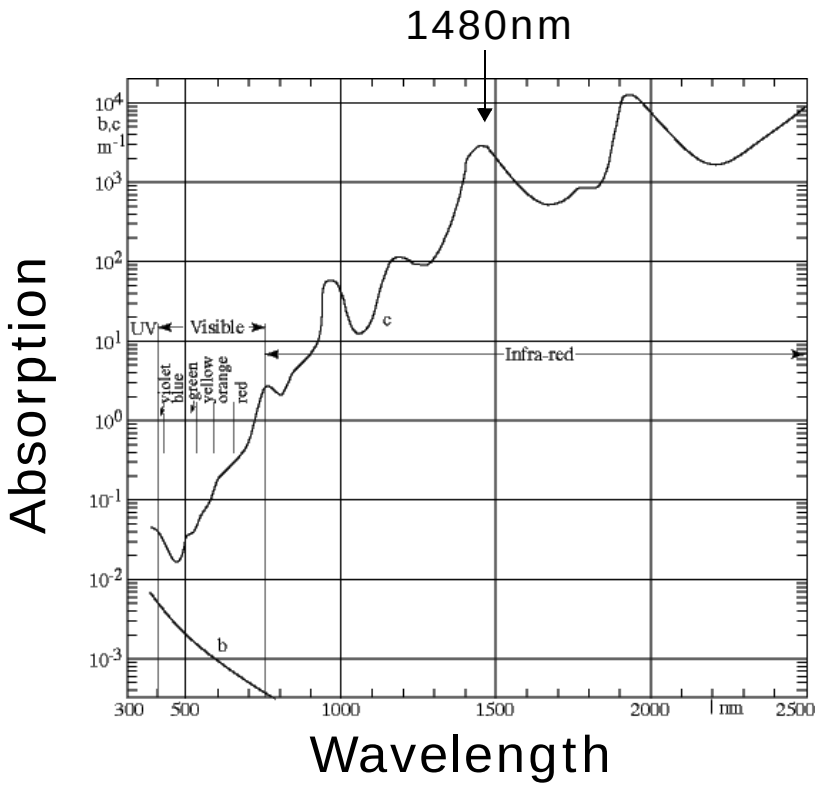
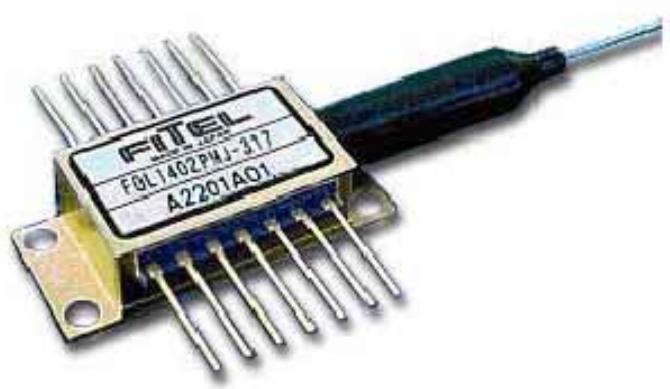
(University Munich starting Dec.)

Biophysics of Open Systems using Temperature Gradients

- Kinetics Measurements
- Thermophoresis
- Convective PCR
- Molecular Evolution

Infrared heating

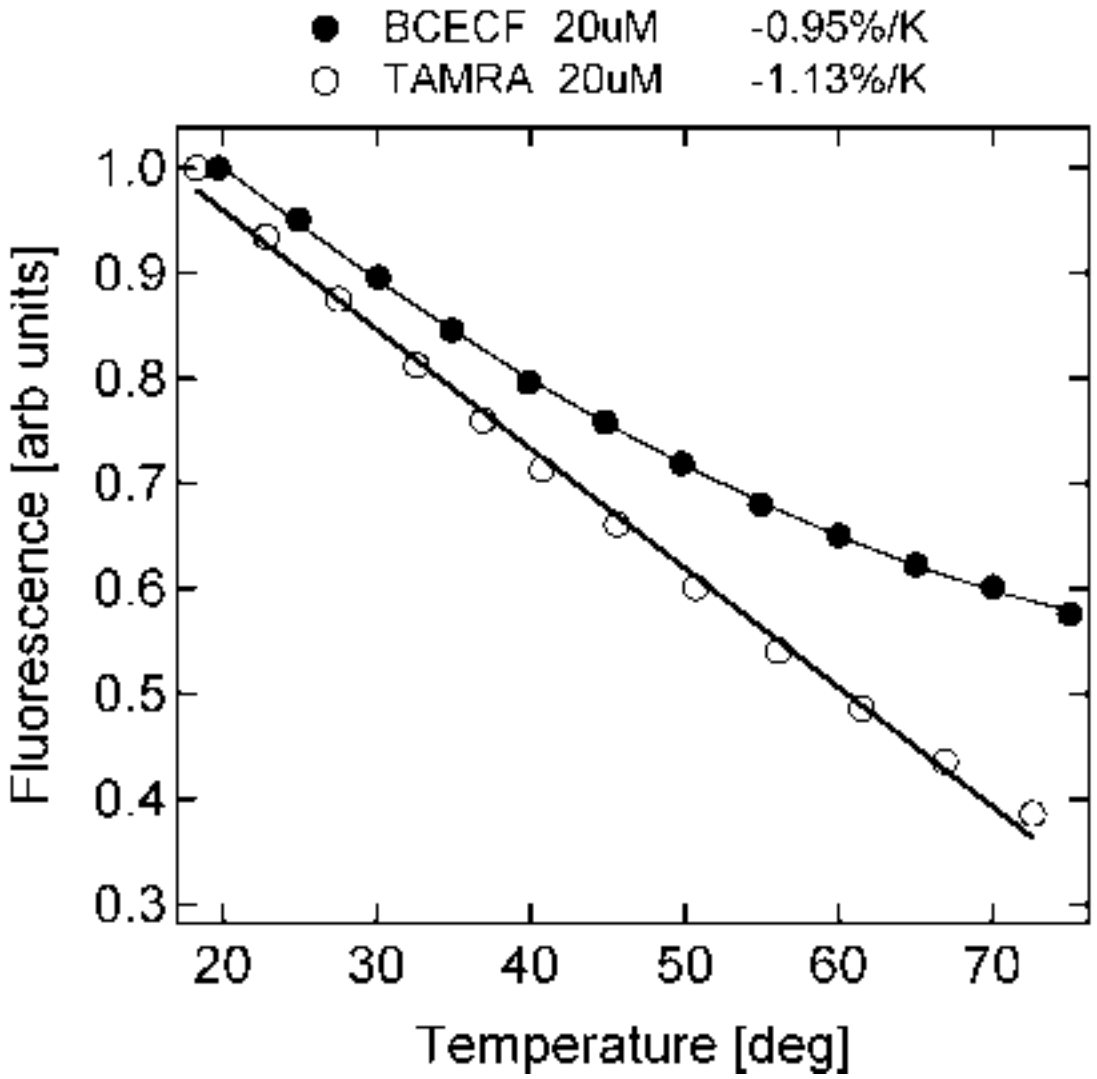




1480nm, 100mW

Using Dye Molecules as Thermometer

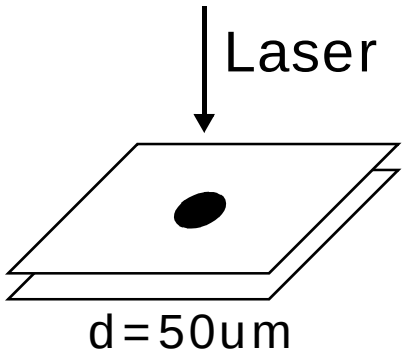
Temperature -> pH of TRIS -> pH Probe

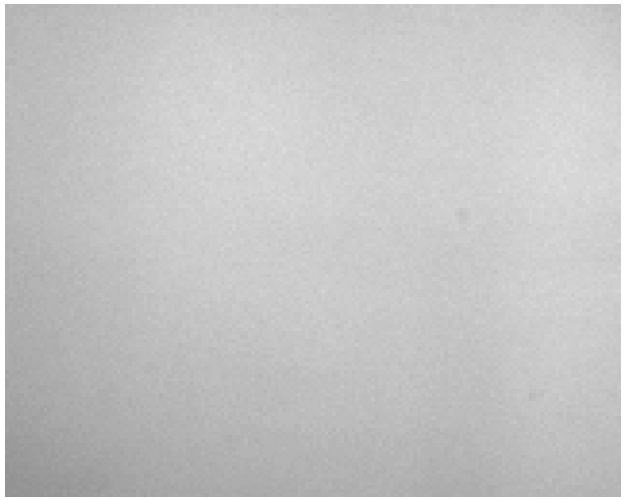


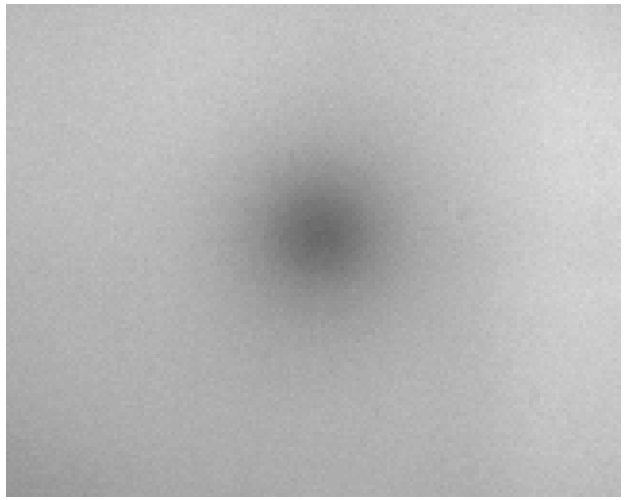
Imaging Temperature

Laser off: cold

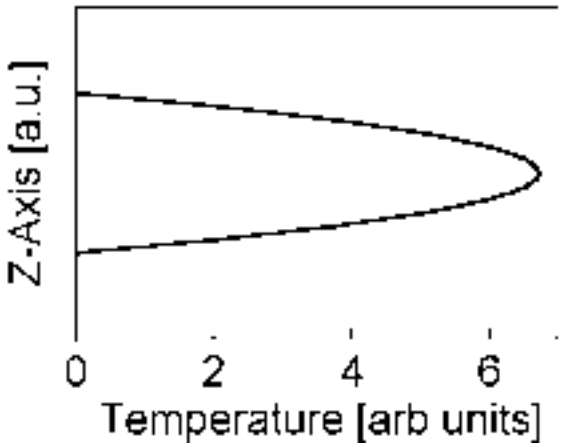
Laser on: hot





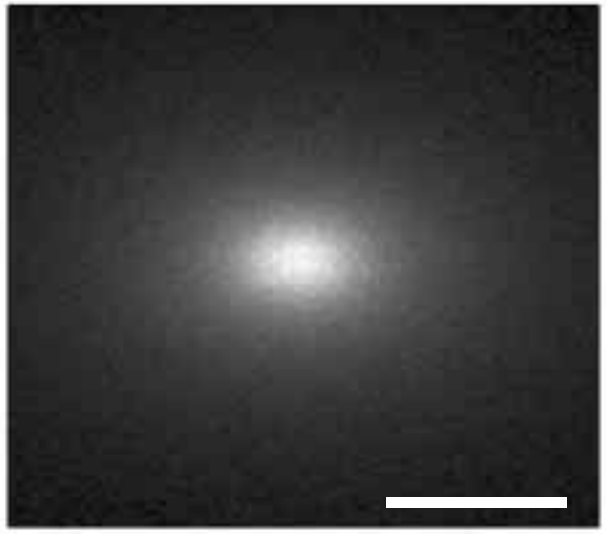


z-Profile



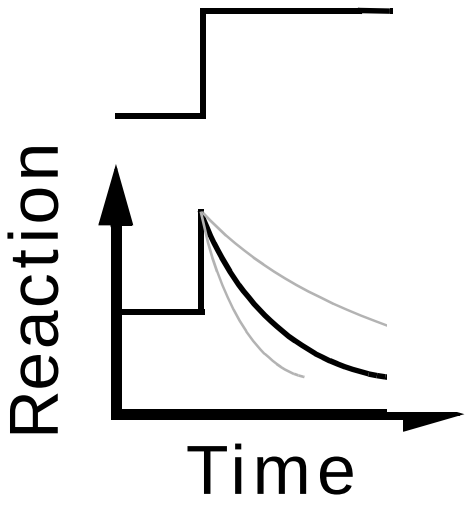
Temperature Image

(z-average)



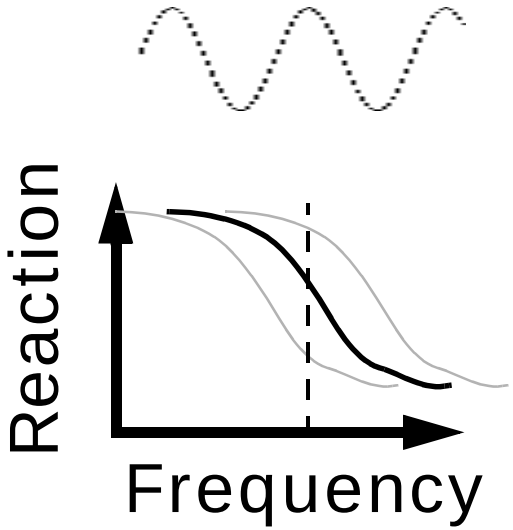
100um

Lock-In Approach to Kinetics A ⇌ B



Temperature Jump

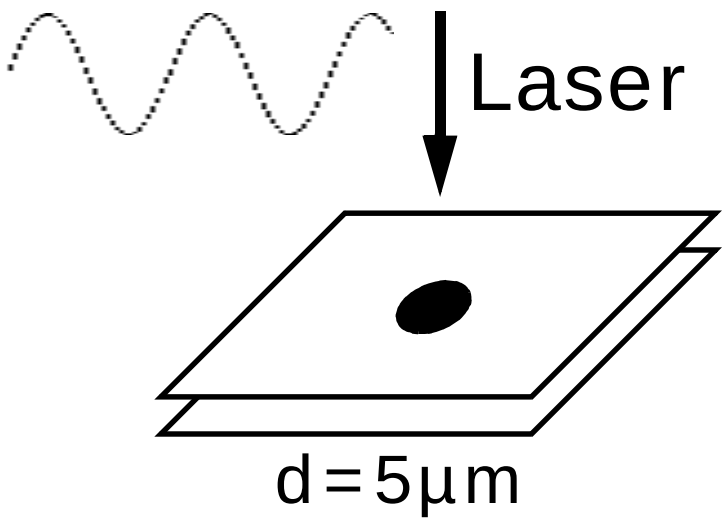
=> Low duty cycle
=> Incompatible with
Single Cell Biology & Microscopy

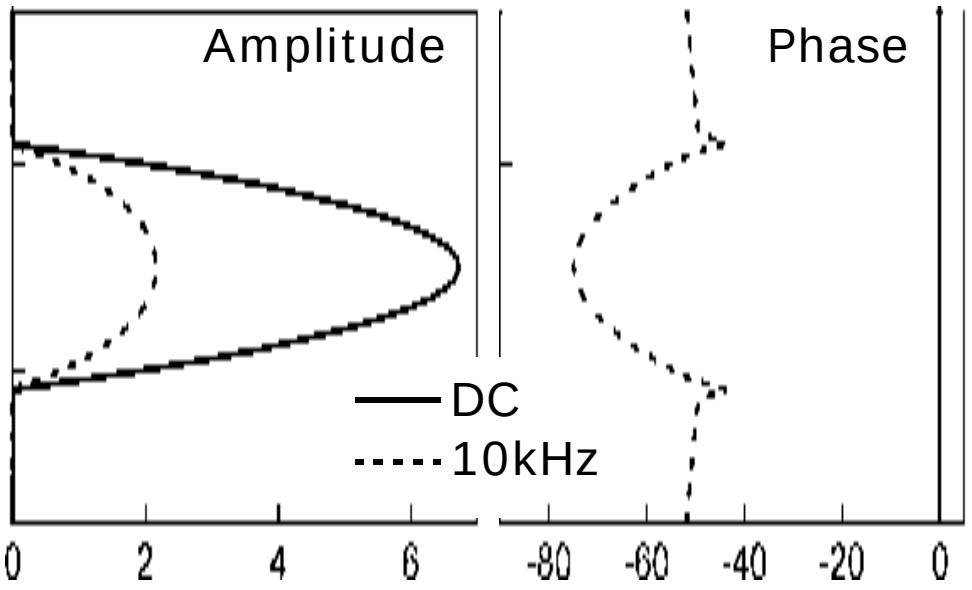


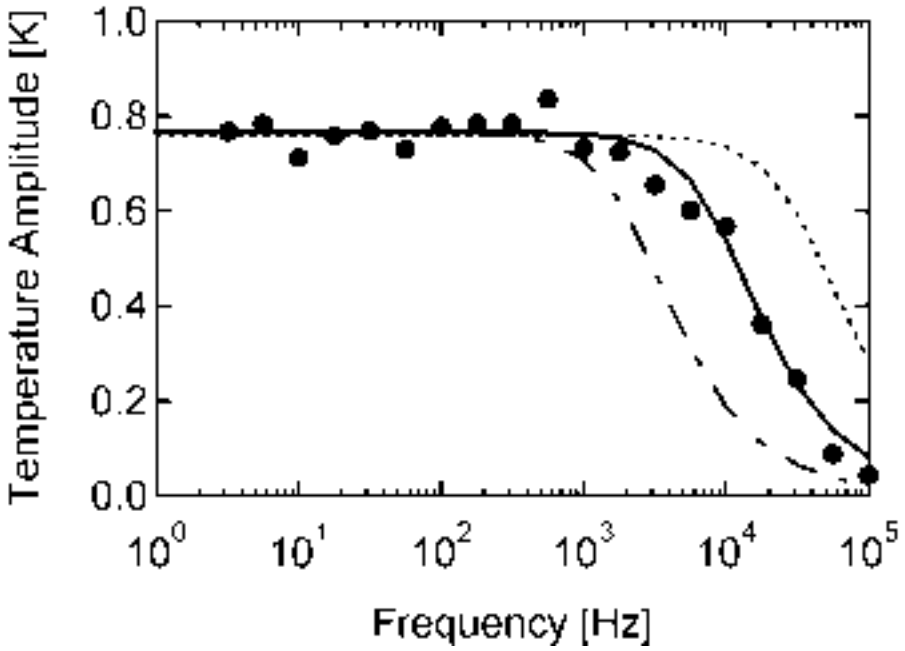
Temperature Oscillation ?

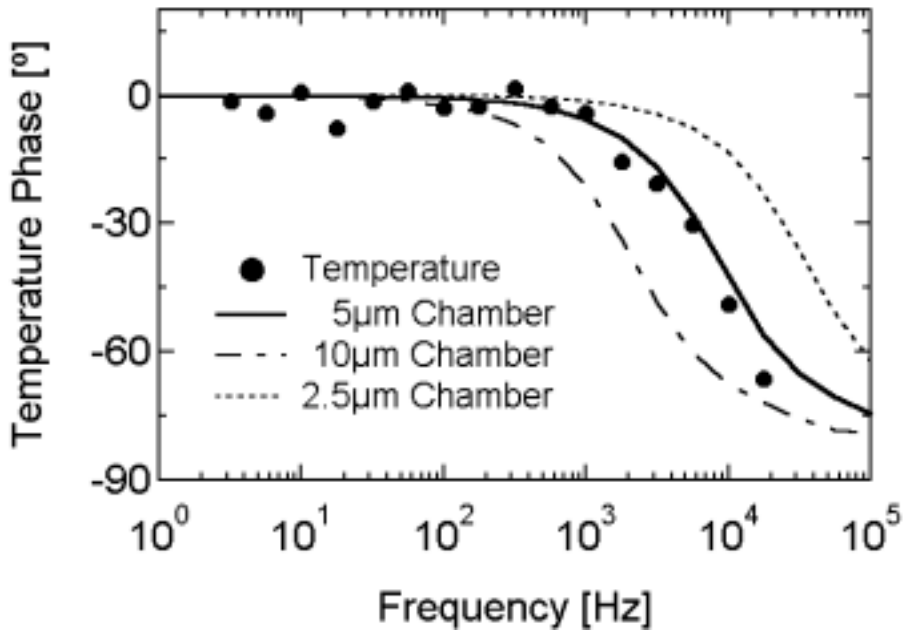
=> High duty cycle
=> Every Photon has Information
=> Measurements in Microscope
=> High S/N with Lock-In
=> High duty cycle

Fast Temperature Oscillations

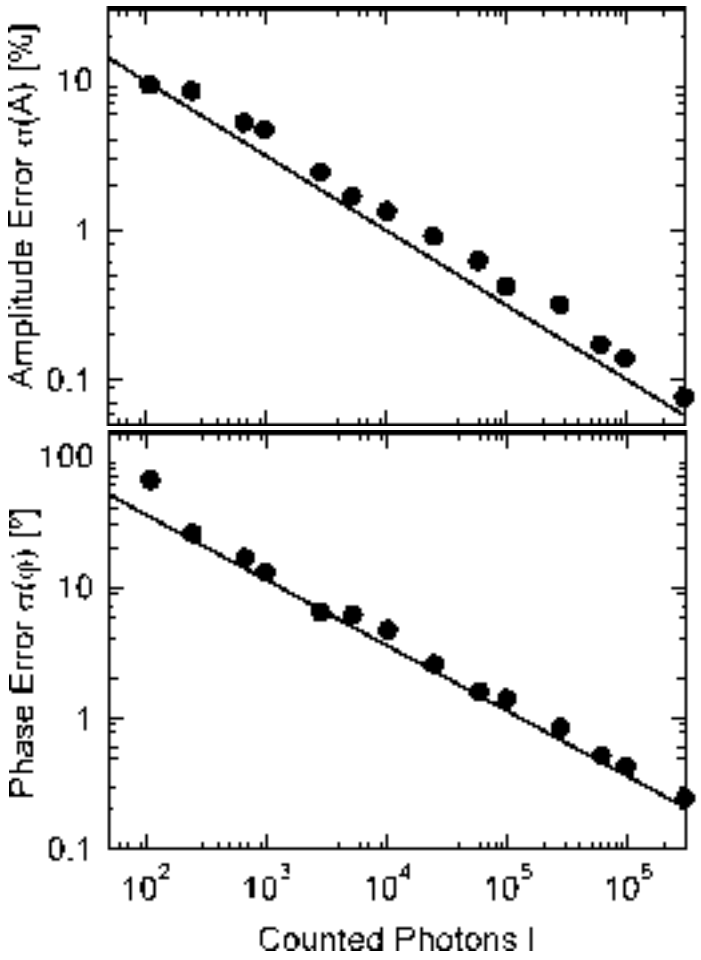








Photon Counting Lock-In

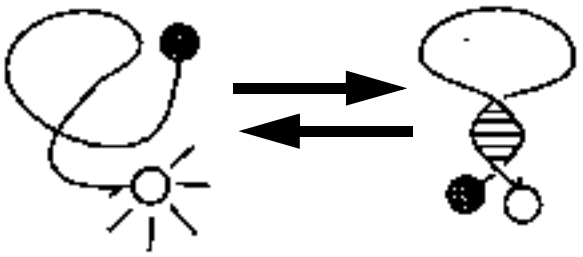


At Photonic Limit

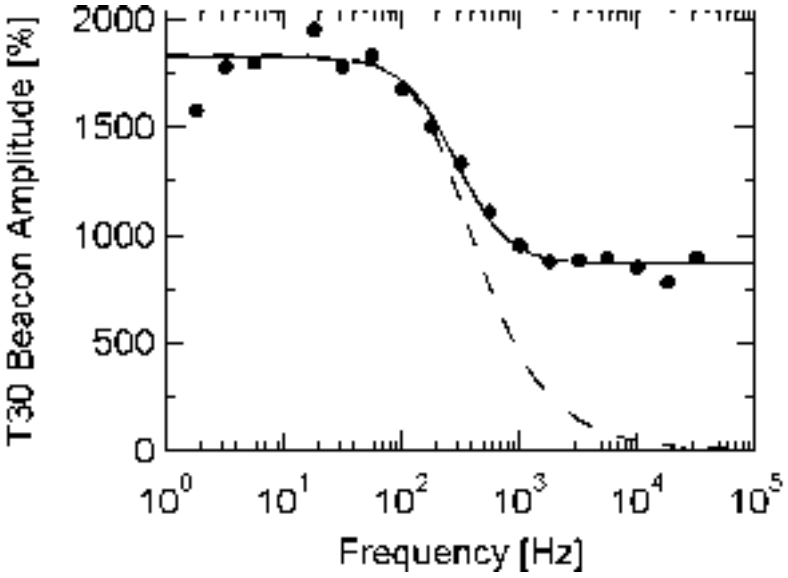
$$Error \propto \sqrt{\#\text{Photons}}$$

Optics Letters, 27:1418-1420 (2002)
Source-Code: www.dieterb.de

Kinetics of DNA Hairpins

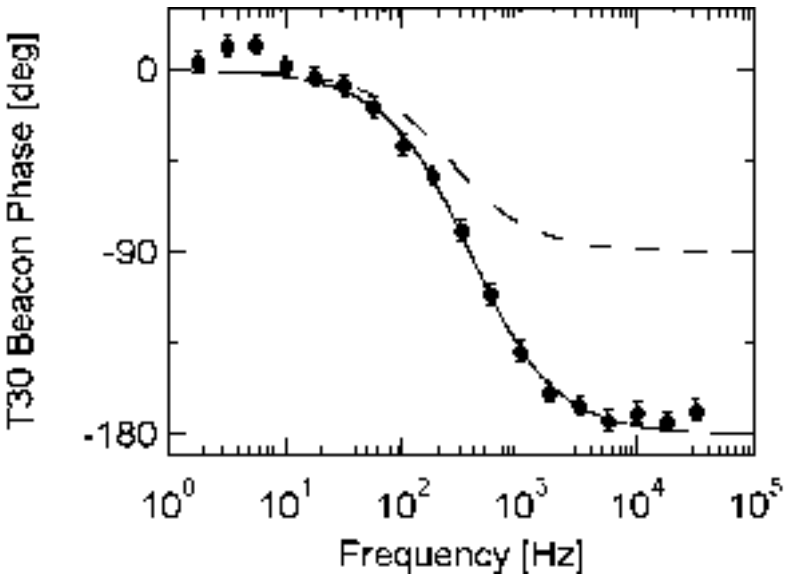


Temperature
Dye



$\tau = 620\mu s$

5% Error

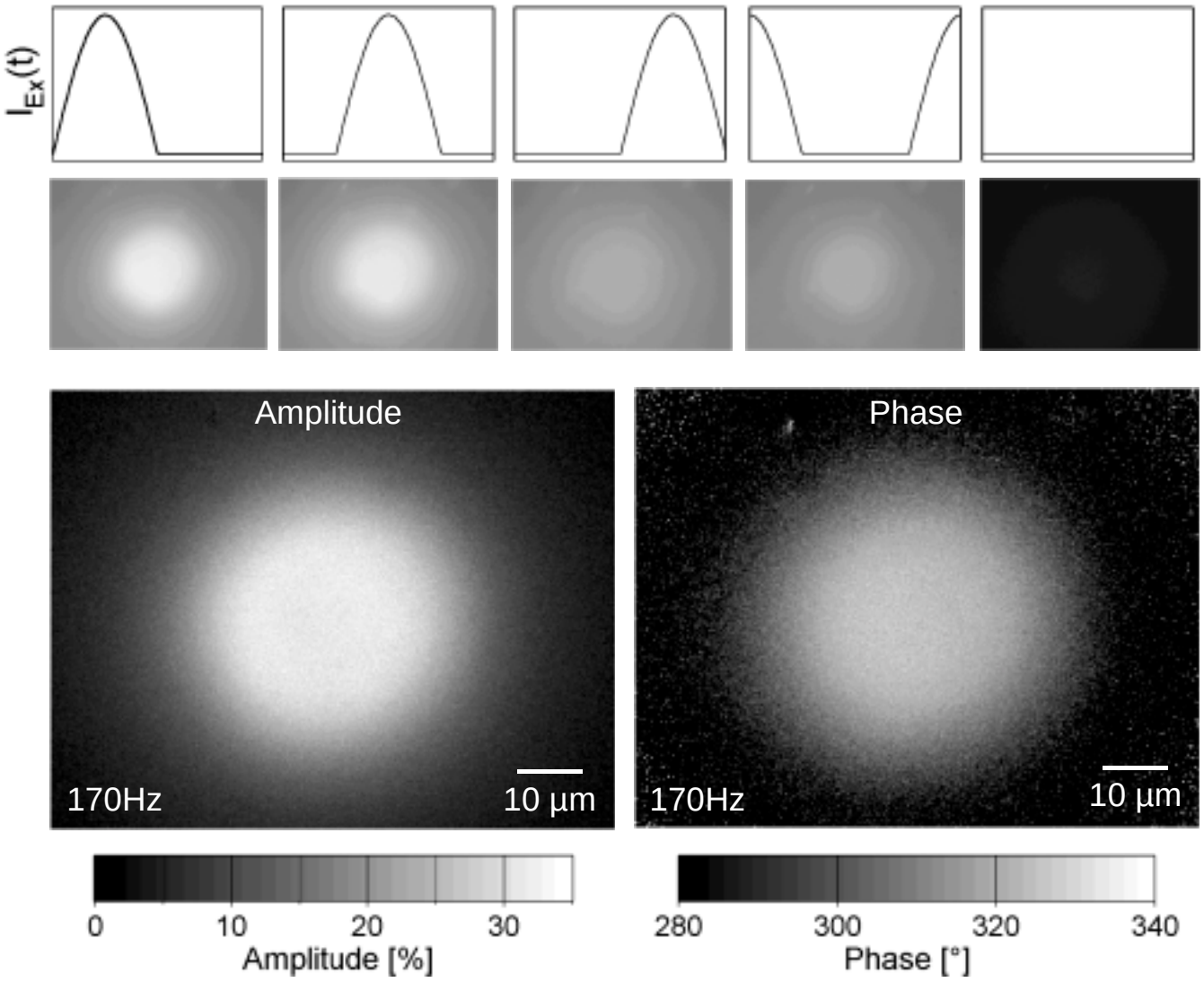


Res. 5µs
with
Phase

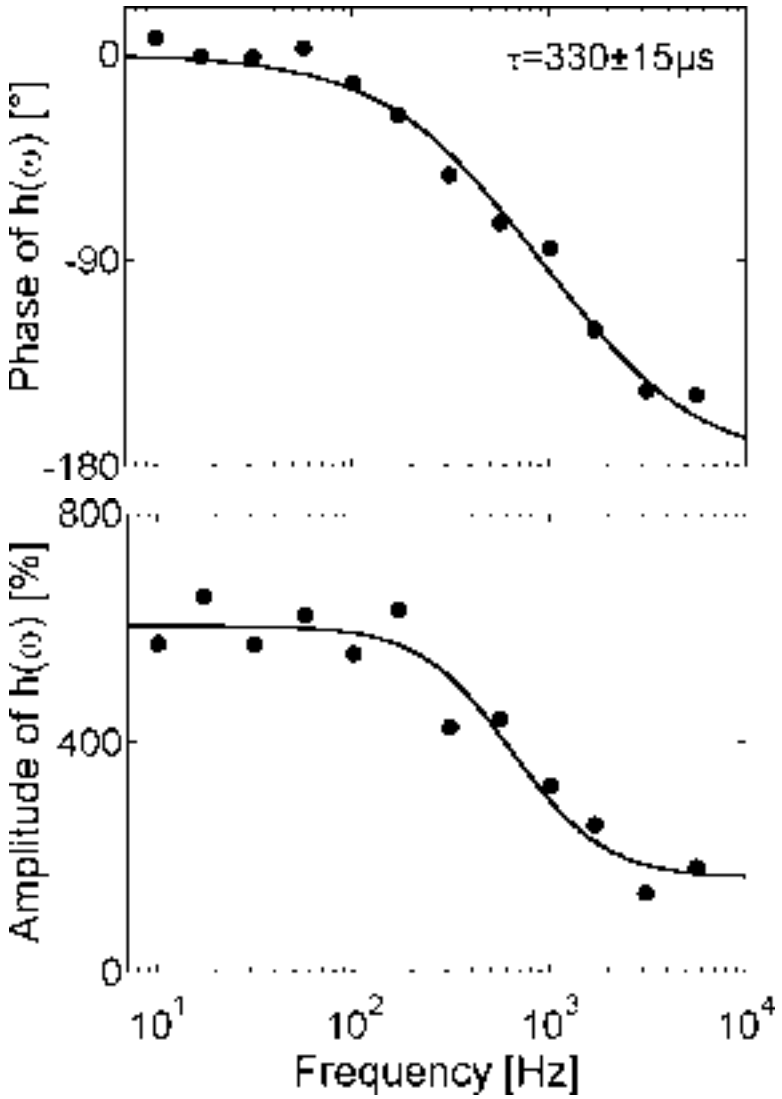
“Molecular” LockIn with a CCD

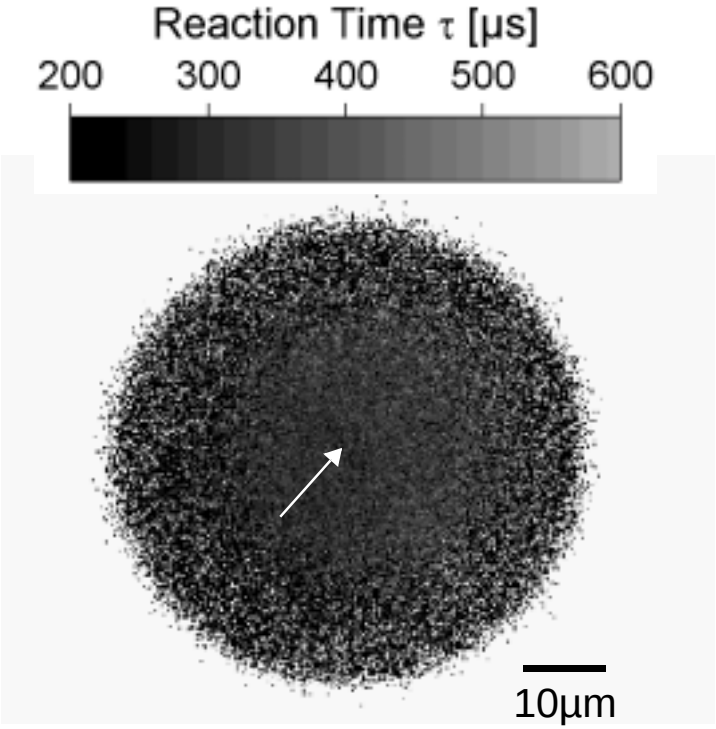
$$I = \int q(E) \times I_{Ex} dt$$

Fluorescent Dye
and slow detection



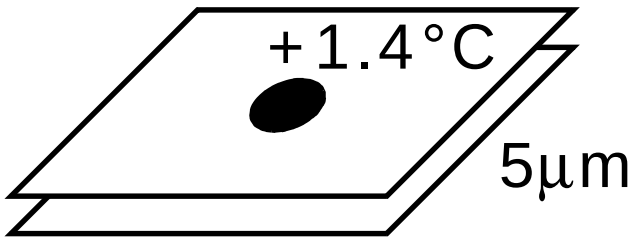
Kinetics for Each Pixel



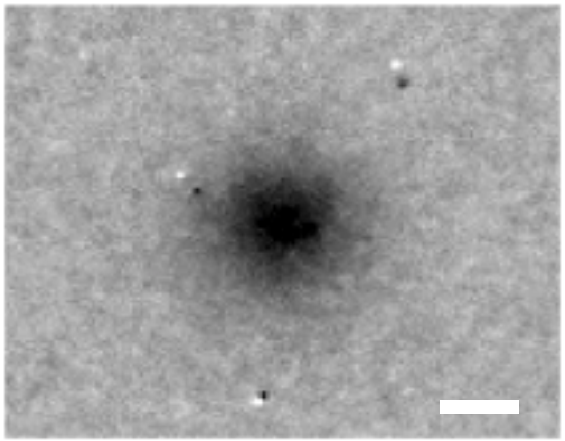


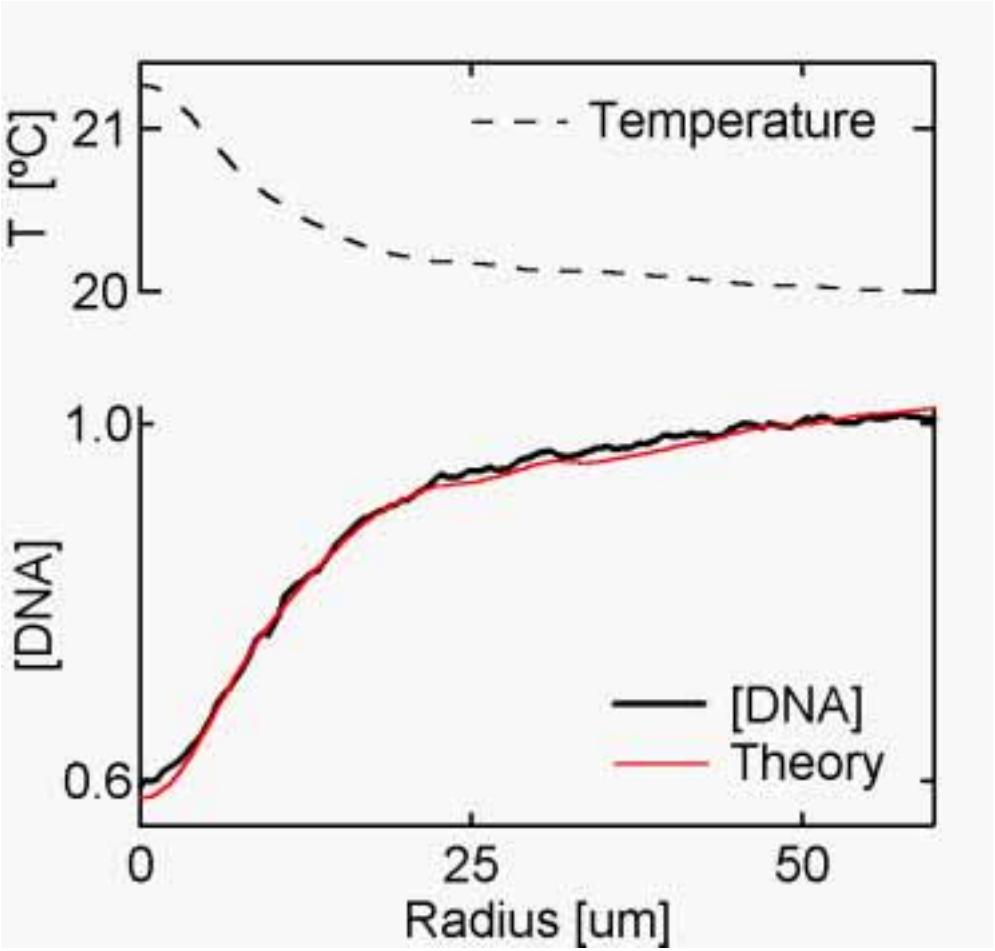
Thermophoresis
(Thermal diffusion, Soret Effect)

Thermophoresis of DNA



5000bp DNA





$$\frac{c_0}{c} = e^{\frac{D_T}{D}(T-T_0)}$$

$D_T/D = 0.38K^{-1}$

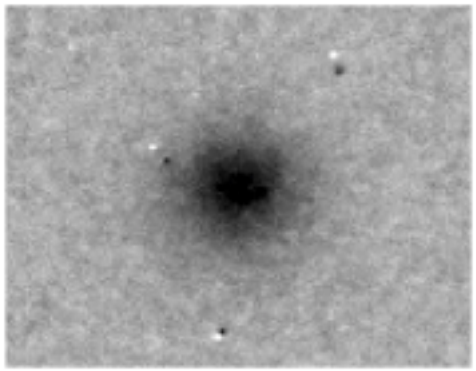
Backdiffusion

$D = 1.0 \times 10^{-8} cm^2/s$

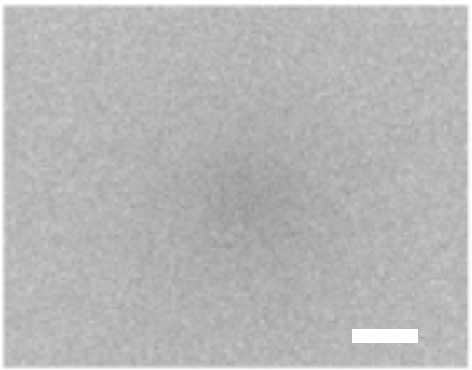
$D_T = 0.4 \times 10^{-8}\ cm^2/(sK)$

Exponential Dependence on Length and Temperature ?

5000bp DNA

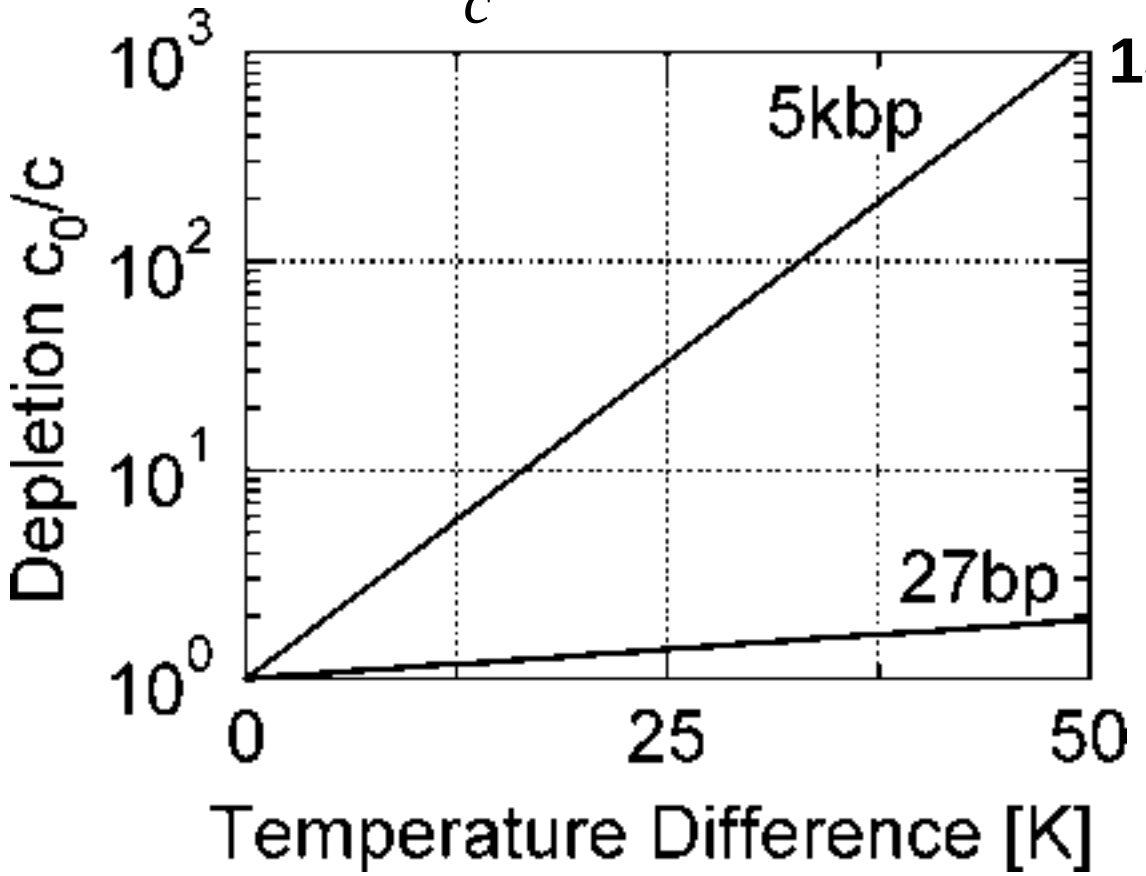


27bp DNA



50µm

$$\frac{c_0}{c} = e^{\frac{D_T}{D}(T - T_0)}$$



Origin of Thermophoresis?

Phenomenological approach

$$j = -D\nabla c - D_T c(1-c)\nabla T \overset{!}{=} 0 \xrightarrow[D_T = \text{const.}]{} \frac{c}{c_0} = e^{-\frac{D_T}{D}(T-T_0)}$$

Interfacial Free Energy argument for ionic thermophoresis (Piazza 2002, Ruckenstein 1981)

Debye Hückel Length $\lambda_{DH} = \sqrt{(\varepsilon k_B T)/[2 c_S e^2]}$

Interfacial Free Energy $\gamma = -\varepsilon \psi_S^2 / 8\pi\lambda_{DH}$

Drift velocity (Ruckenstein) $v = -l\nabla\gamma/\eta = -\frac{\varepsilon\psi_S^2}{32\pi\eta T}\nabla T$

$$\psi_S^2 = Z_{eff} e \lambda_{DH}/\varepsilon a^2$$

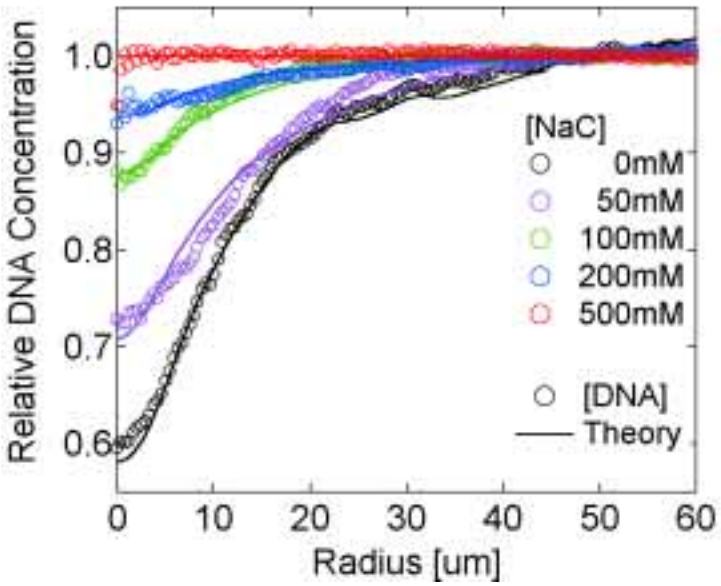
$$l_B = e^2/4\pi\varepsilon k_B T$$

$$f_0 = 6\pi\eta a$$

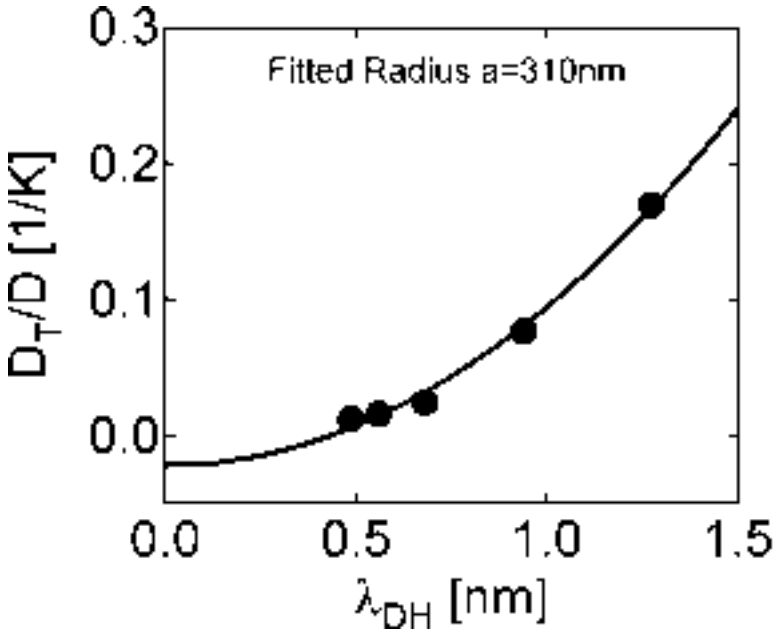
$$D_0 = k_B T/f_0$$

$$\left.\frac{D_T}{D_0}\right|_{ionic} = \frac{3\pi l_B Z_{eff}^2}{4Ta^3}\lambda_{DH}^2 \sim \lambda_{DH}^2$$

Scaling with Salt Concentration

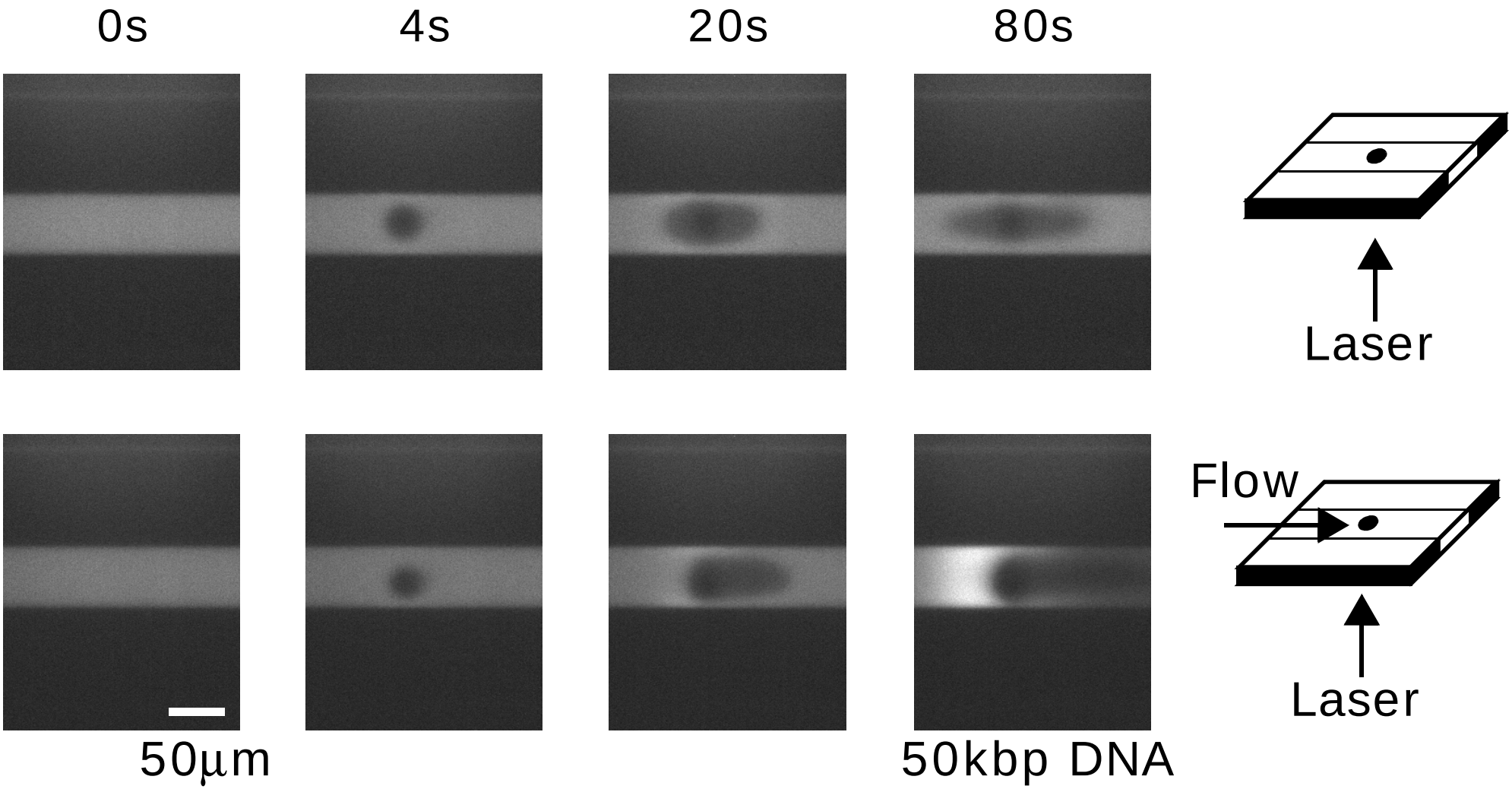


[Similar for 40nm Beads]

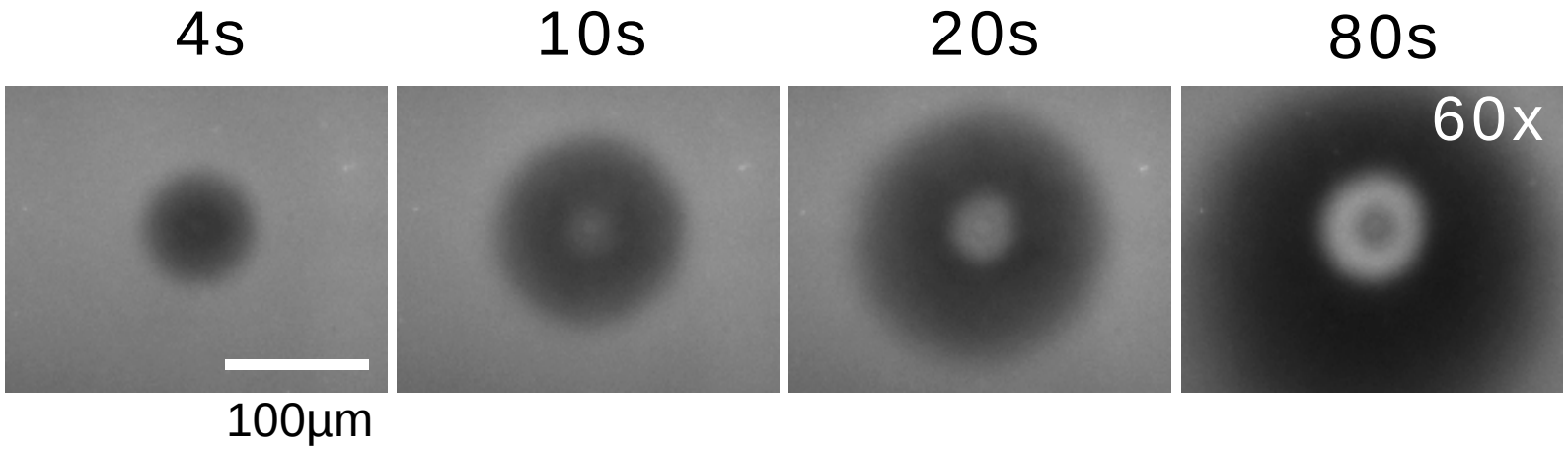


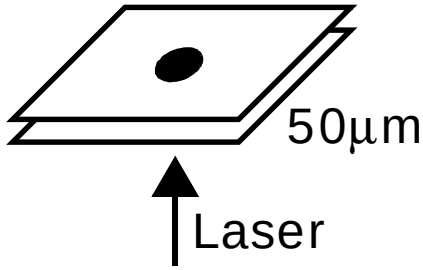
$$\left.\frac{D_T}{D_0}\right|_{ionic} = \frac{3\pi l_B Z_{eff}^2}{4Ta^3}\lambda_{DH}^2 \sim \lambda_{DH}^2$$

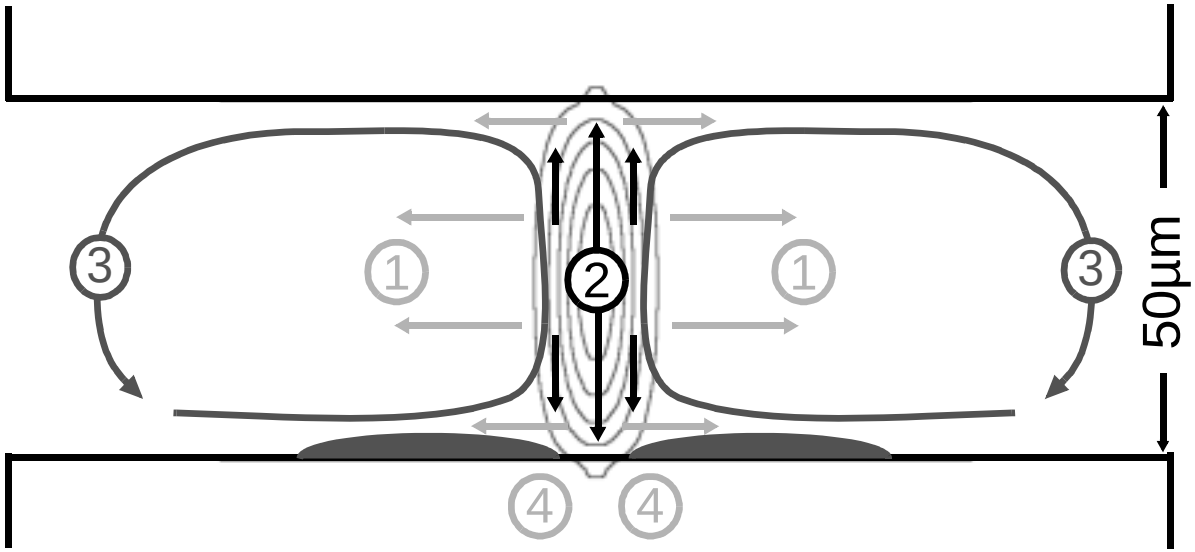
Thermophoresis in Microfluidics: Flow turns depletion into accumulation

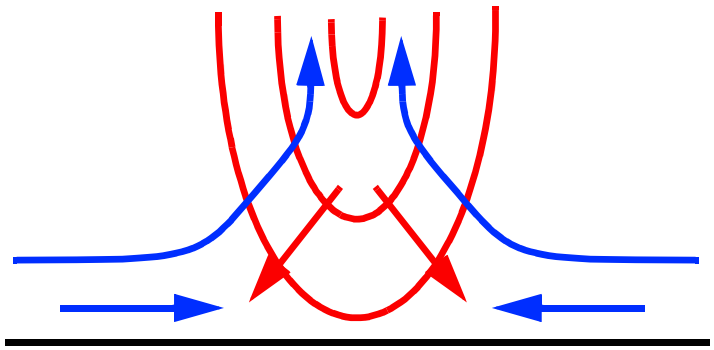


Thermophoretic Trapping by Convection



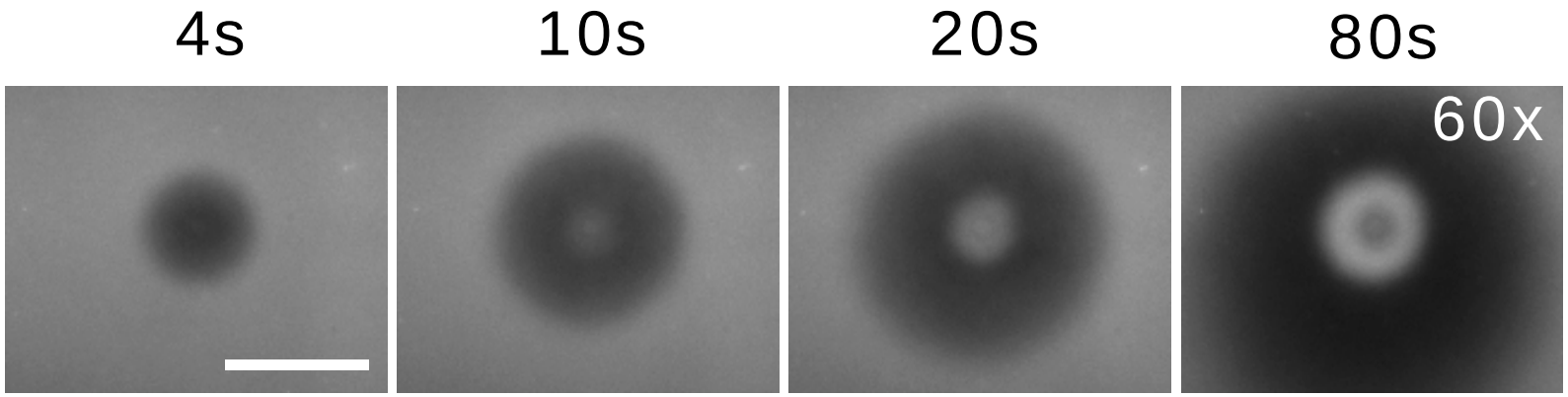


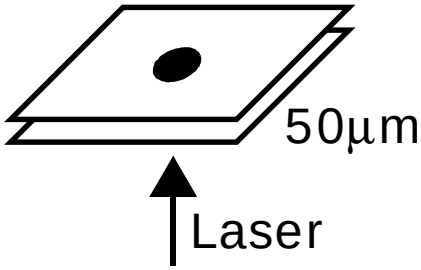


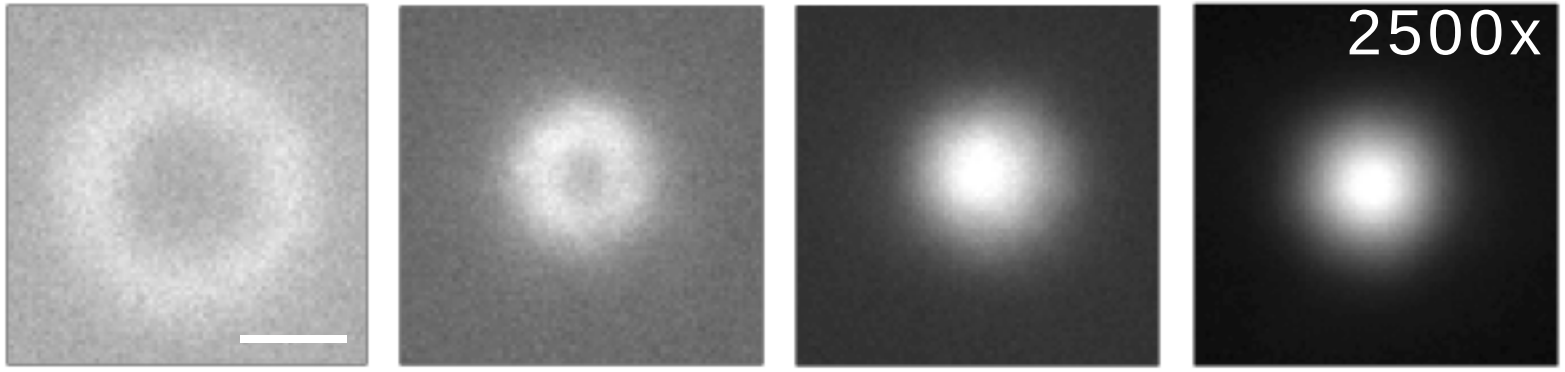


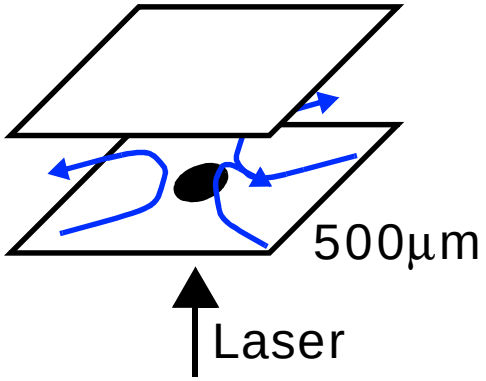
Heat
-attracts convection
-repels DNA

Thermophoretic Trapping by Convection



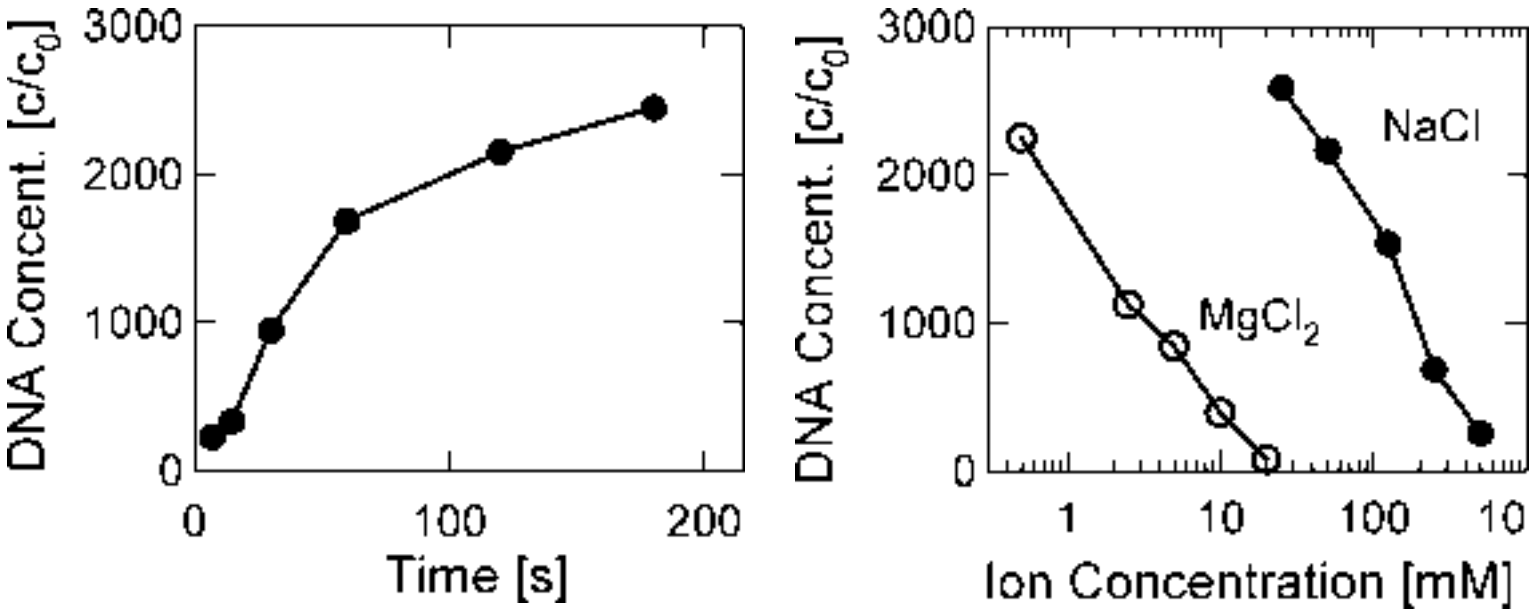




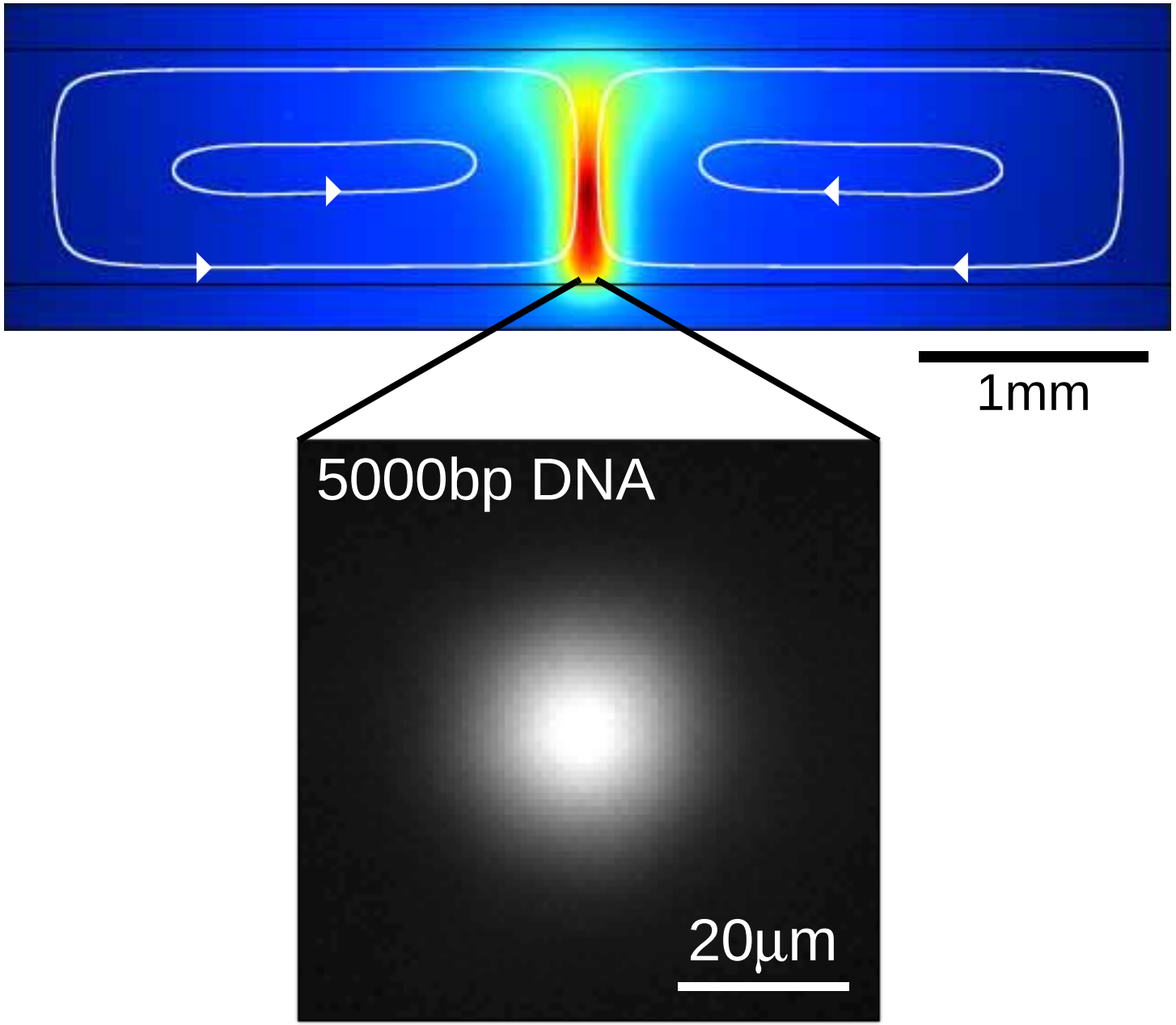


PRL 89:188103 (2002)
Physics Today Feb. 2003

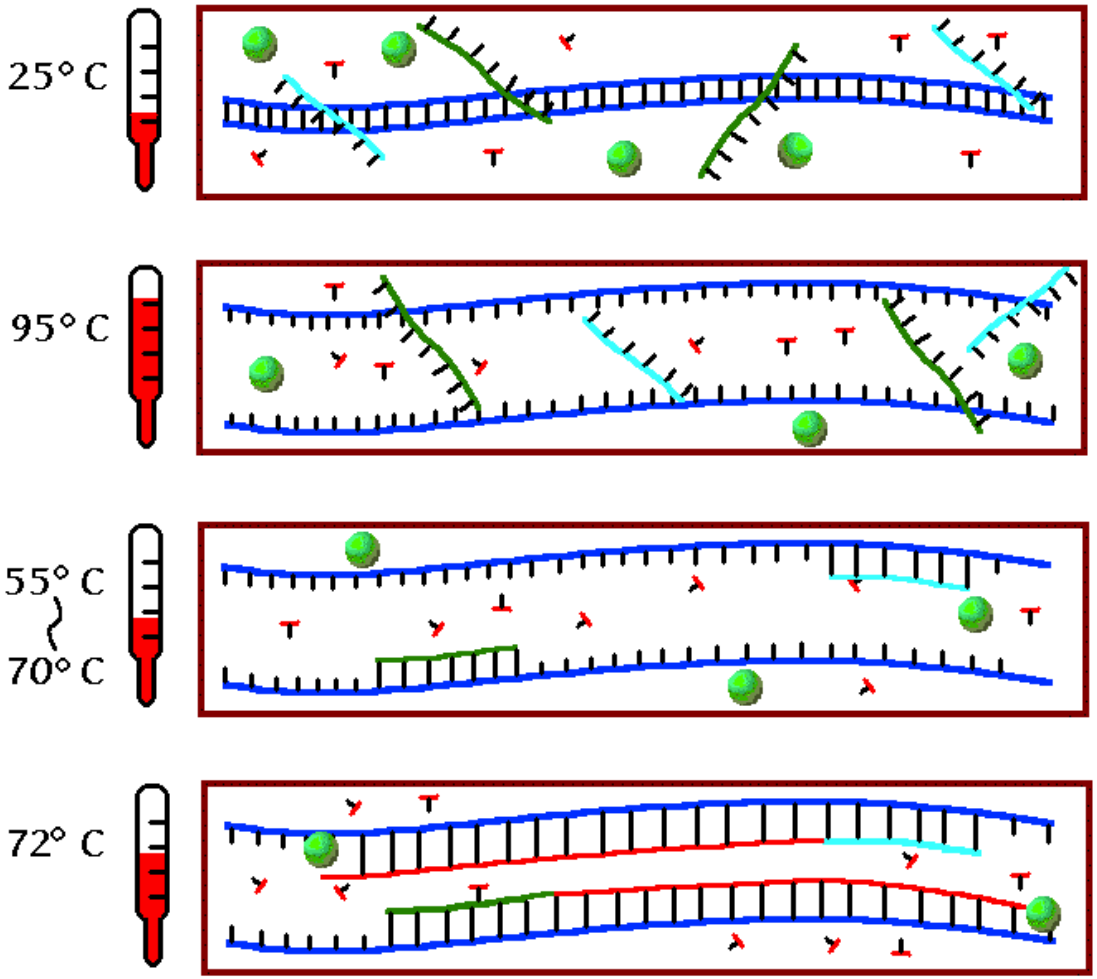
Strong accumulation



Thermophoretic Trapping by Convection



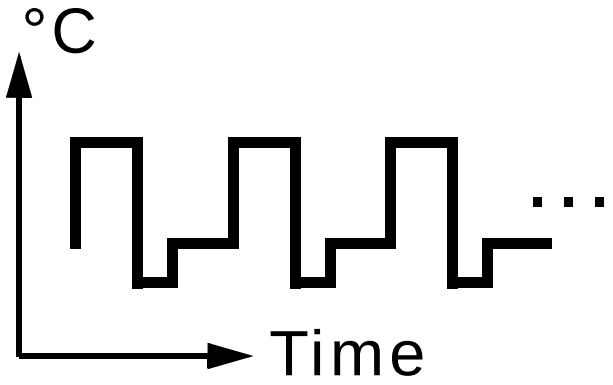
Polymerase Chain Reaction (PCR)



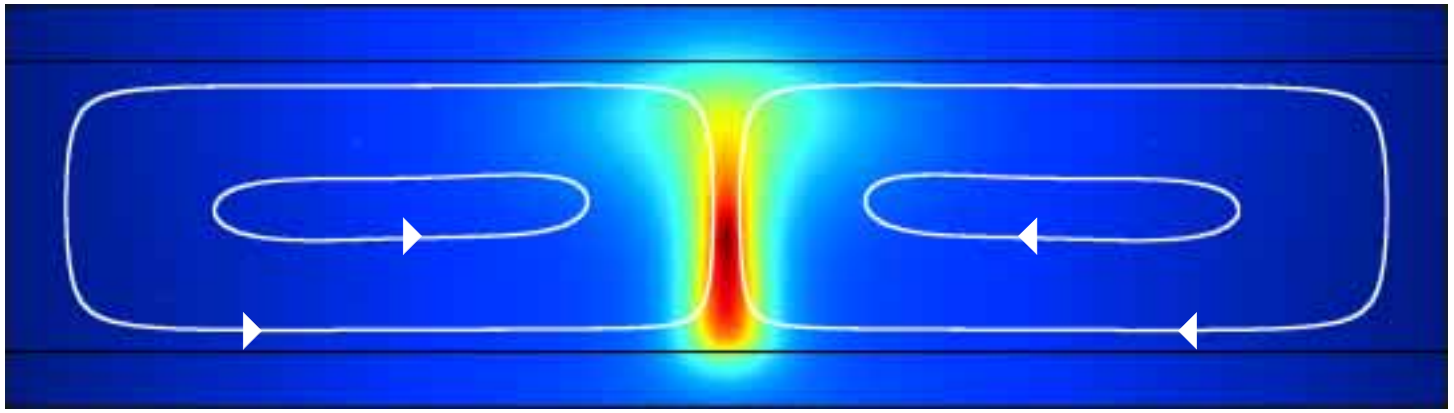
DNA Detection down to single Molecules

AIDS-Test

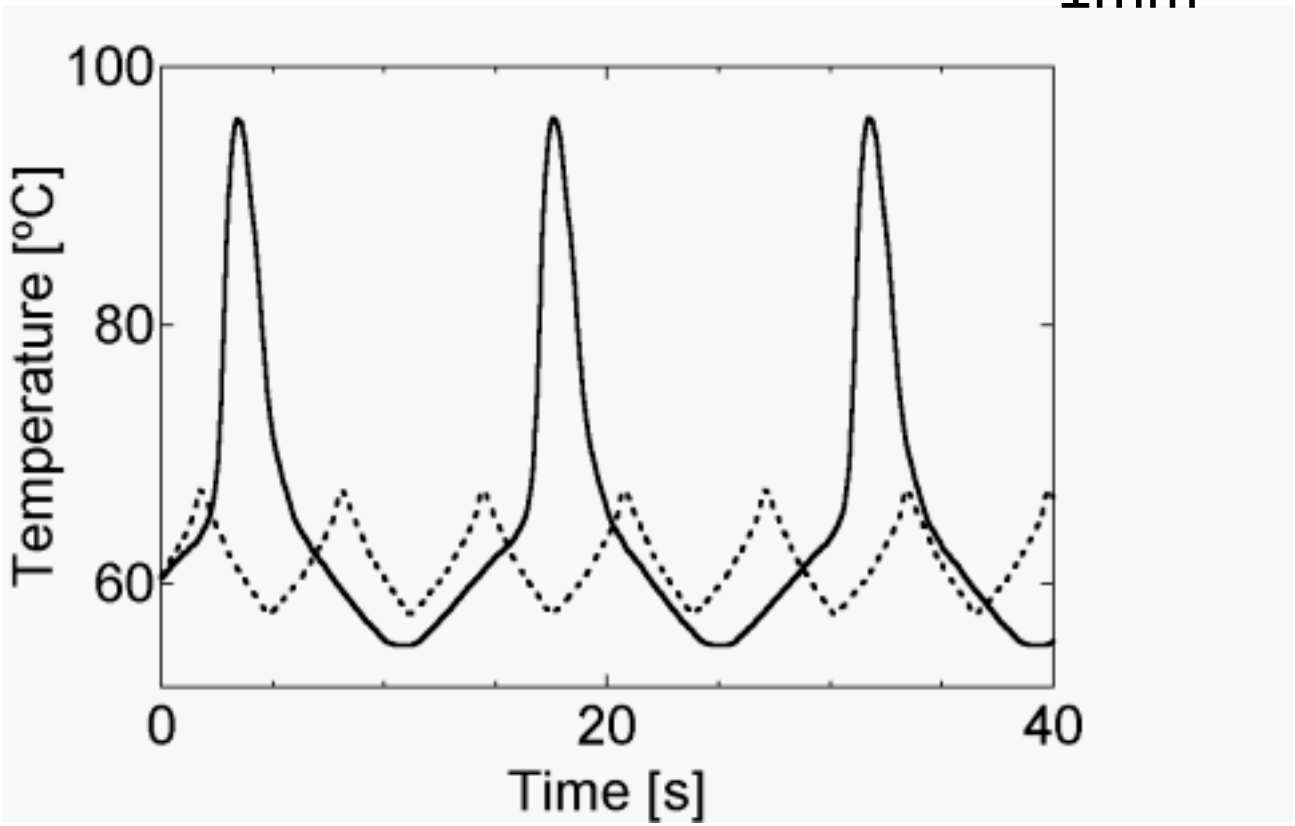
Future of DNA Chips ?!



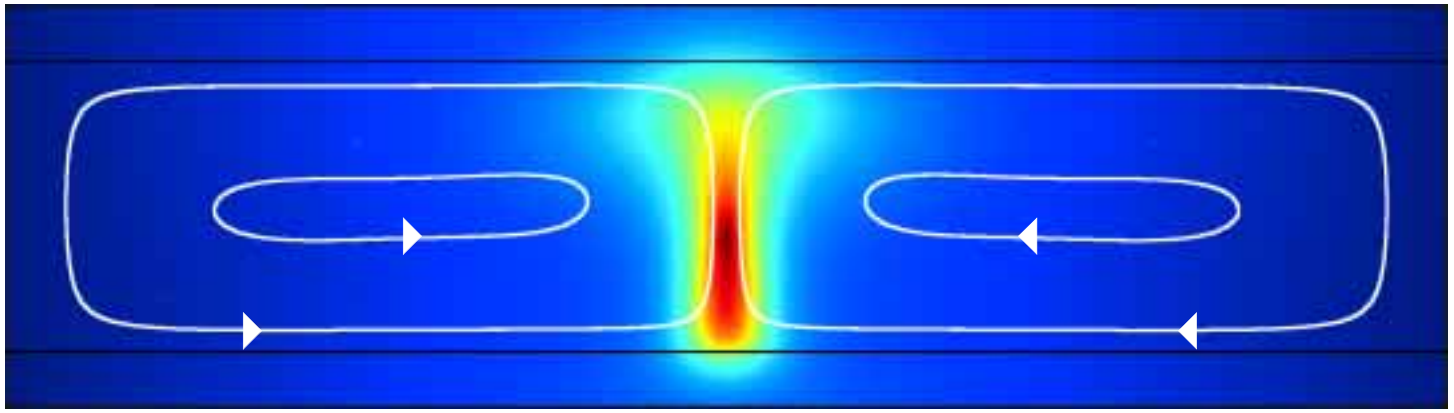
PCR by Convection



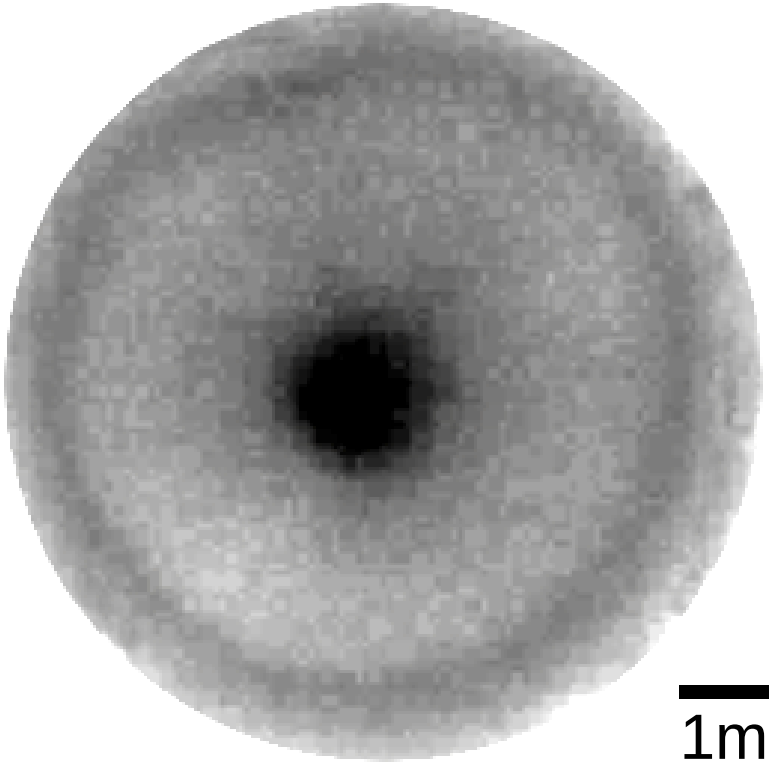
1mm



PCR by Convection

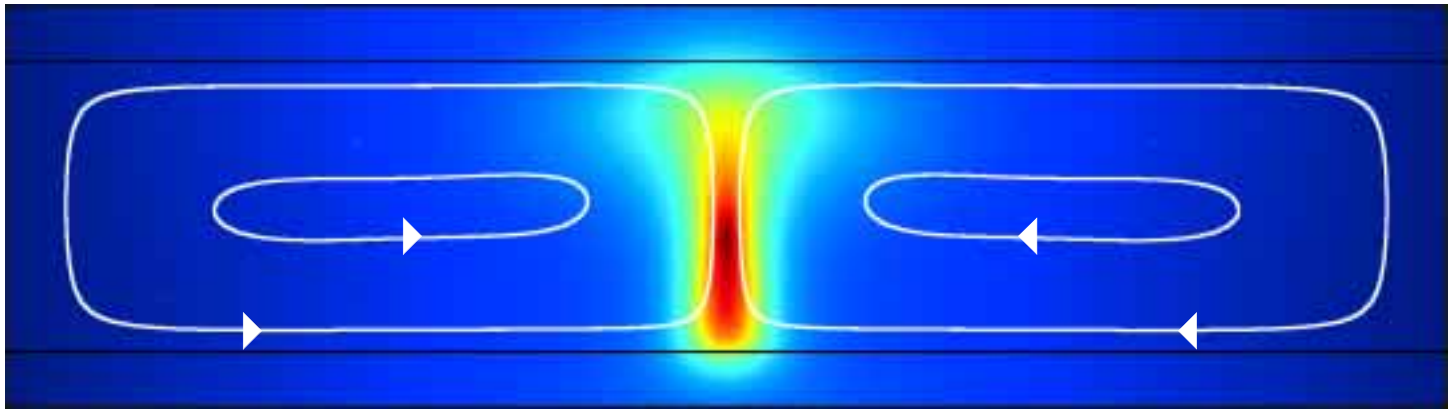


1mm



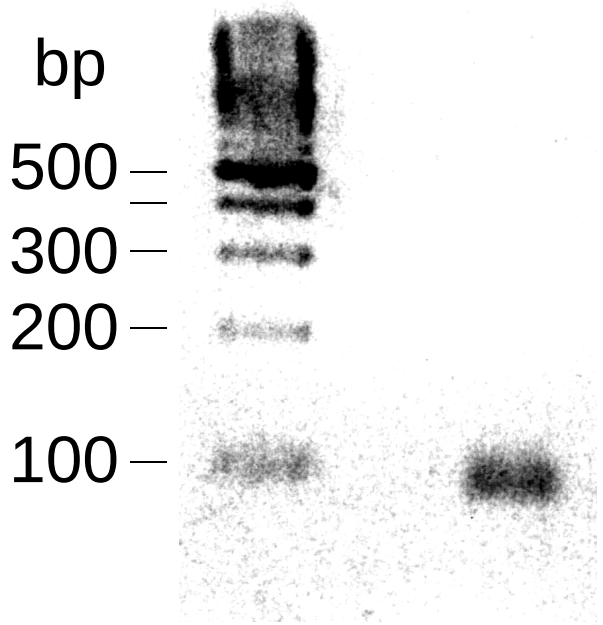
1mm

PCR by Convection



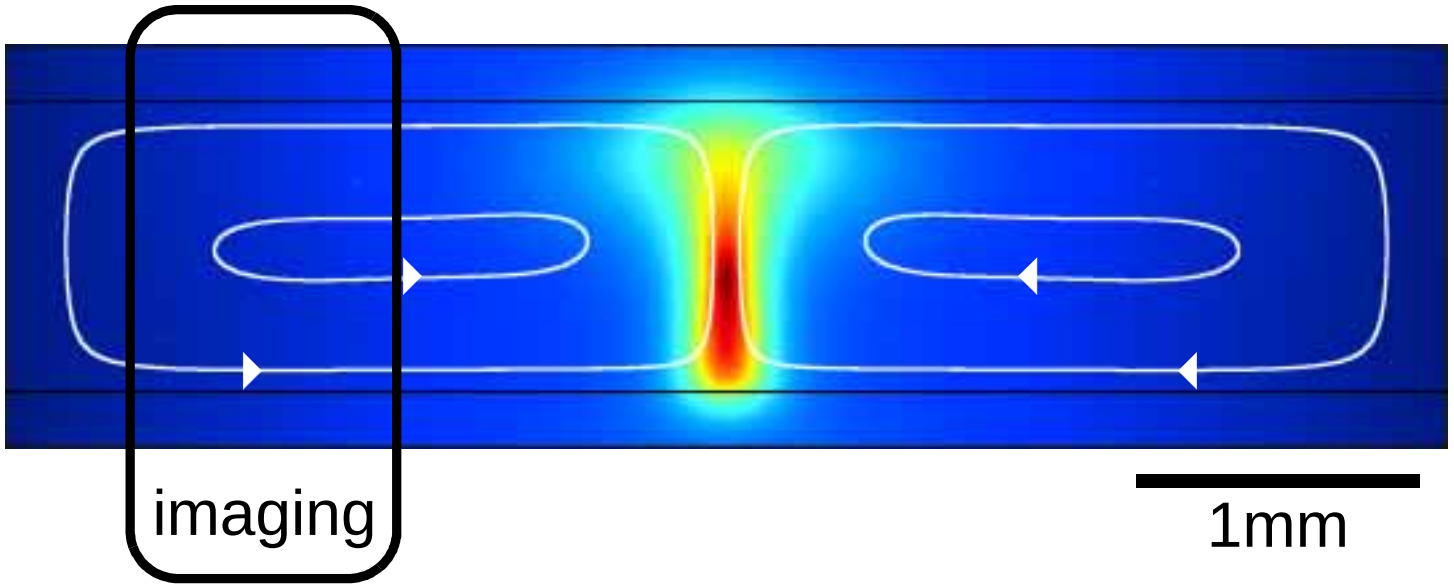
1mm

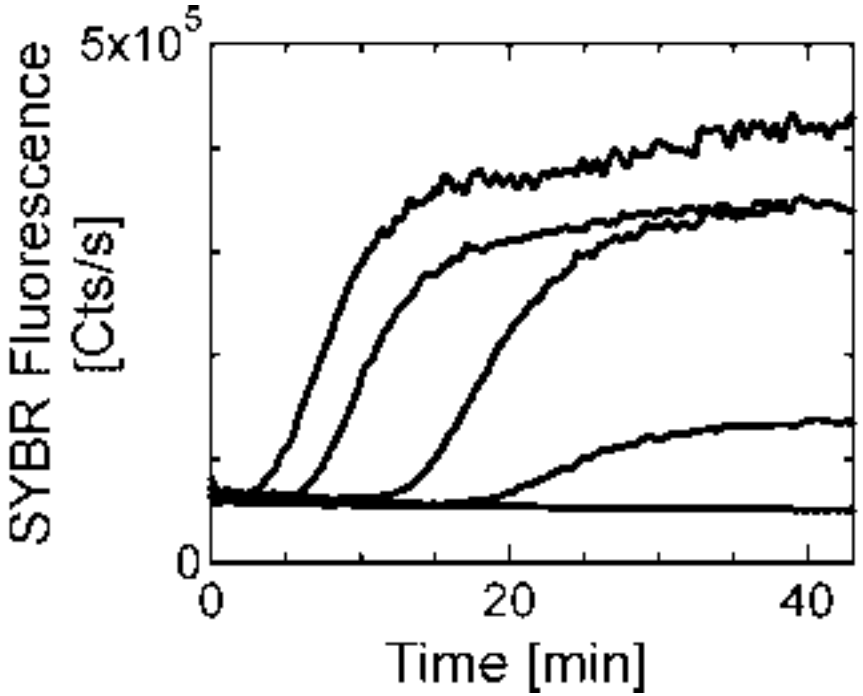
Amplification 100.000x
in 25 Minutes

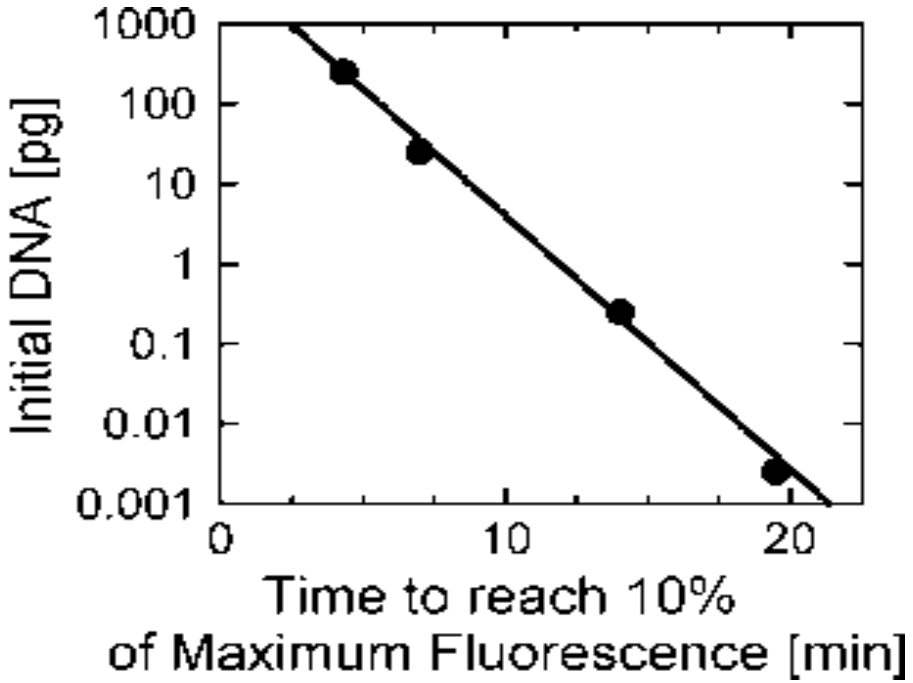


100bp out
of λ-DNA

Quantitative PCR by Convection

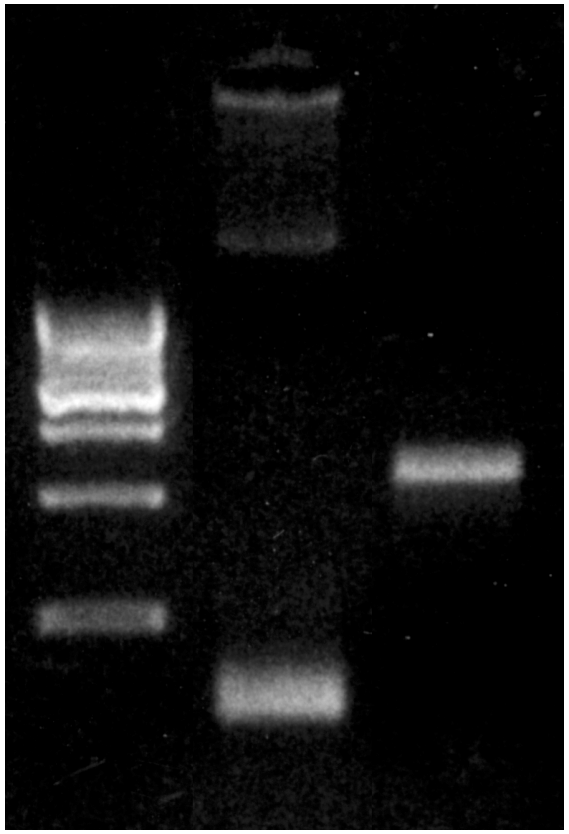






Standard PCR by Convection

Chamber size **10x0.5mm** (before: 5x1mm)

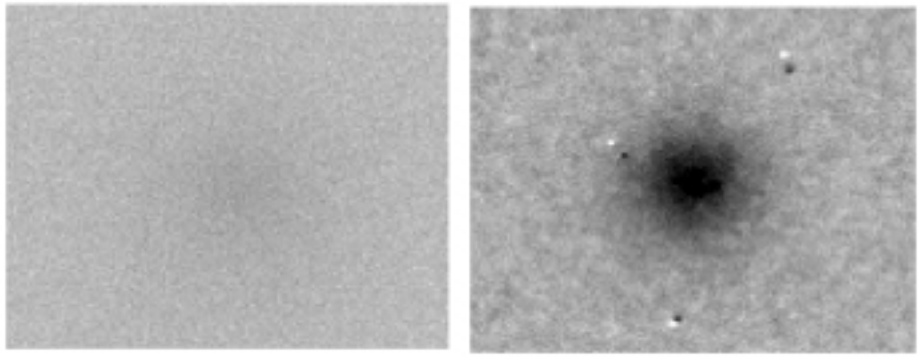


350bp of β-Actin
out of human DNA

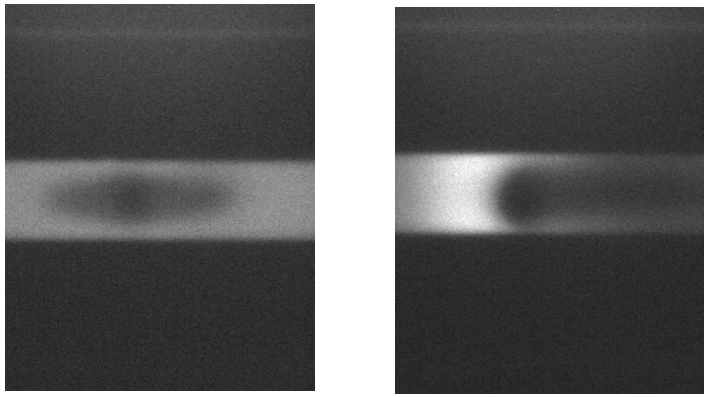
1100bp out
of λ-DNA

Localized Heating

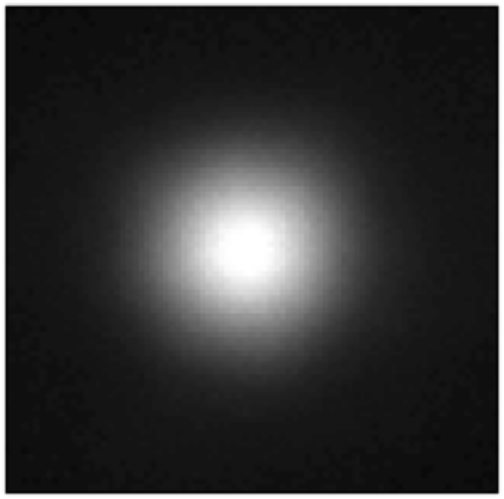
Depletion is highly length dependent



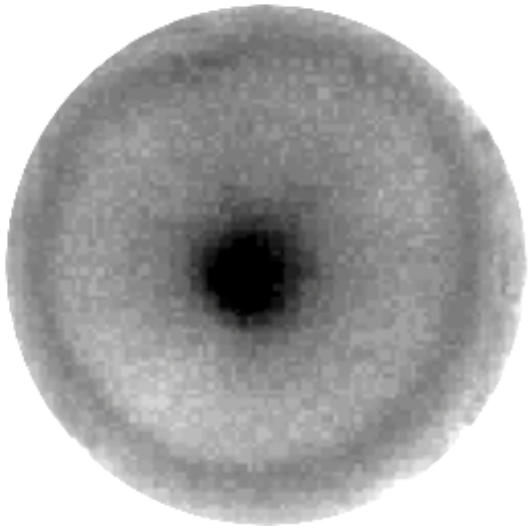
IR heating compatible with microfluidics



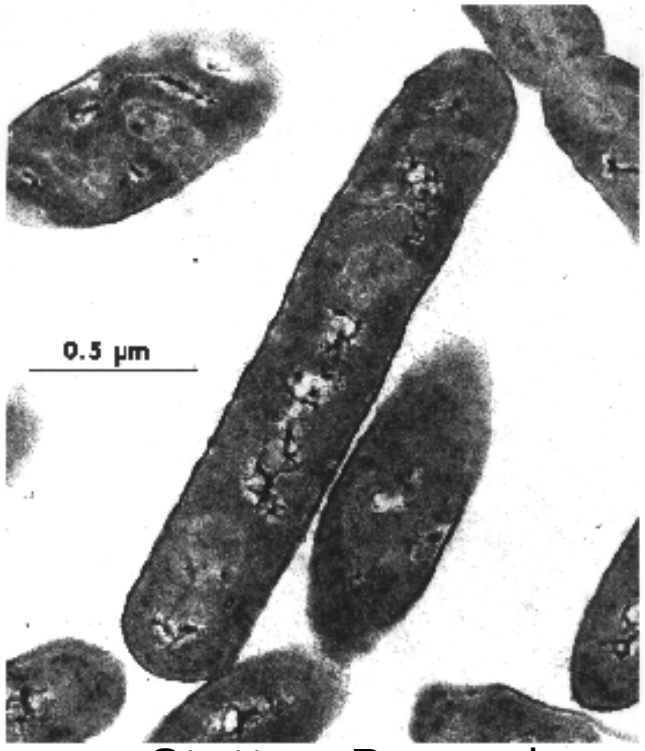
With Convection leads to strong accumulation



Laminar Convection can drive PCR fast



Archaea



Stetter, Regensburg

“The unseen majority”

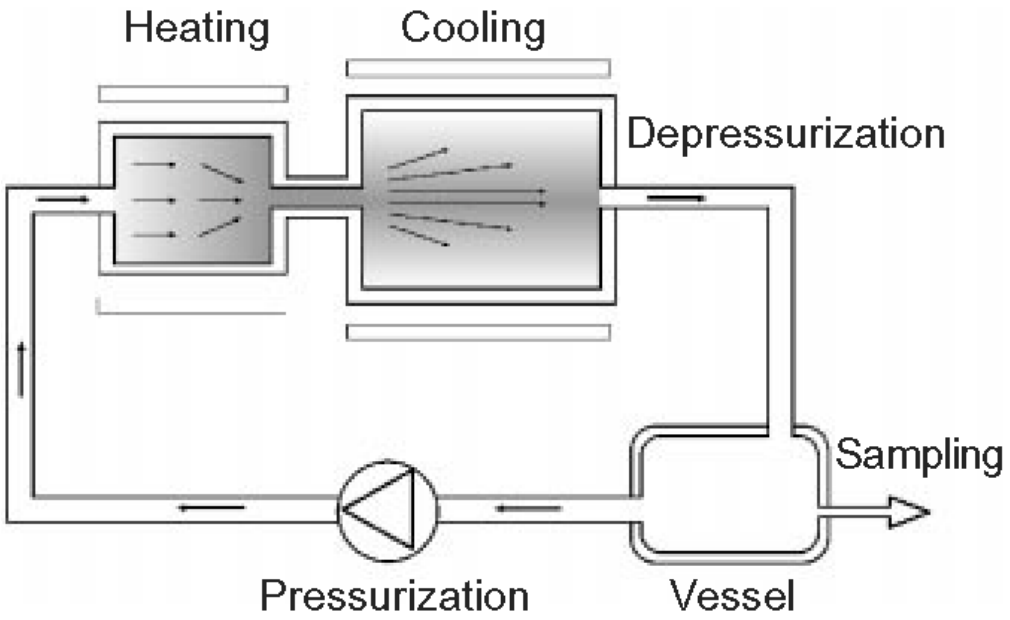
(Whitman, PNAS, 95:6578, 1998)

Table 5. Number of prokaryotes in the world

Environment	No. of prokaryotic cells, $\times 10^{28}$
Aquatic habitats	12
Oceanic subsurface	355
Soil	26
Terrestrial subsurface	25–250
Total	415–640

Peptide Synthesis from quenched Heating

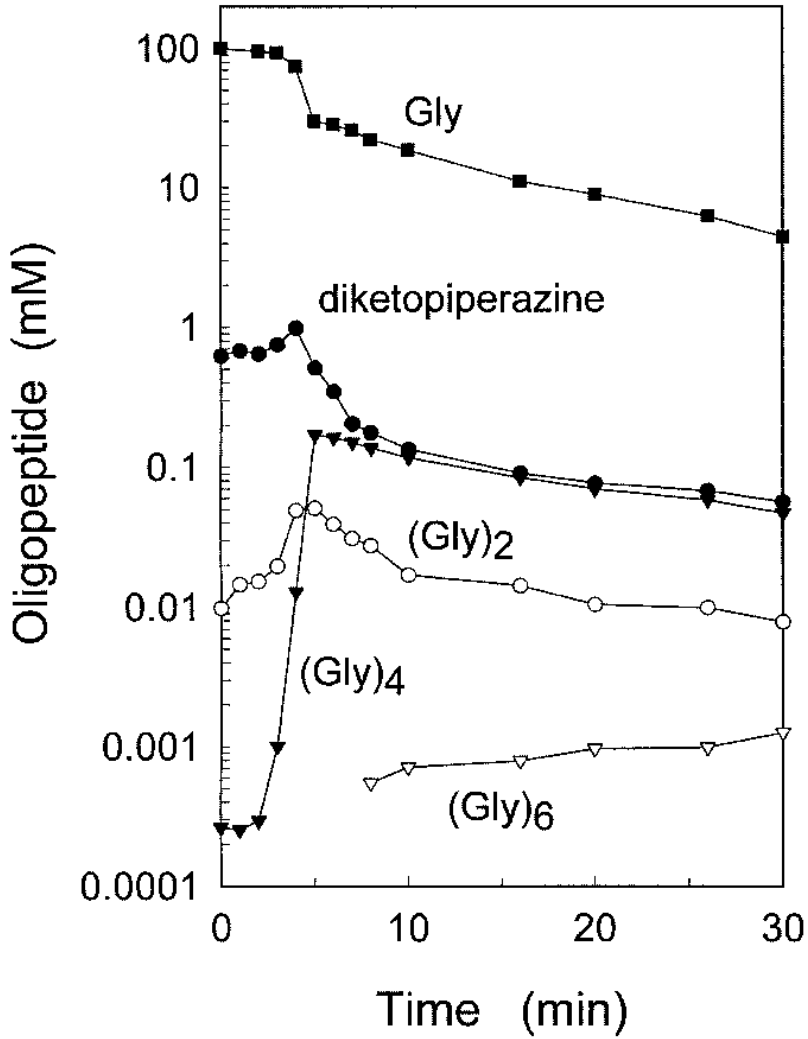
Koichiro Matsuno et.al. Science 283:831 (1999)



250bar, 220°C

↓ 10ms

4°C

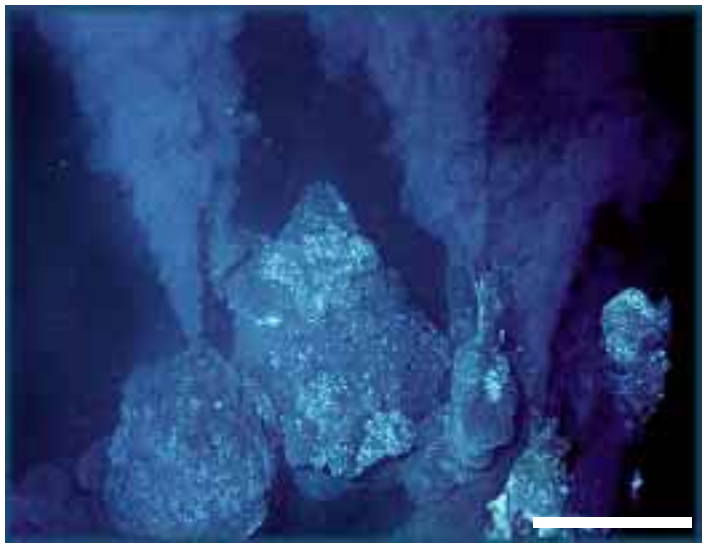


Temperature Gradients

Accumulate DNA
Induce DNA Replication
Synthesize Peptides (?)

=> A convective Protocell ?

Origin of Life in pores near Hydrothermal Vents?



2m

2m