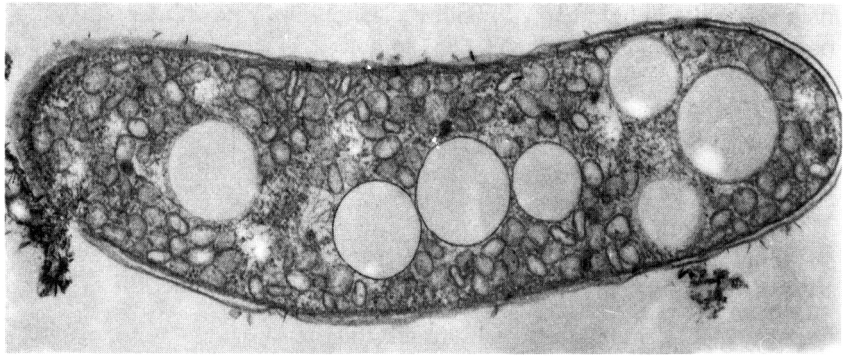
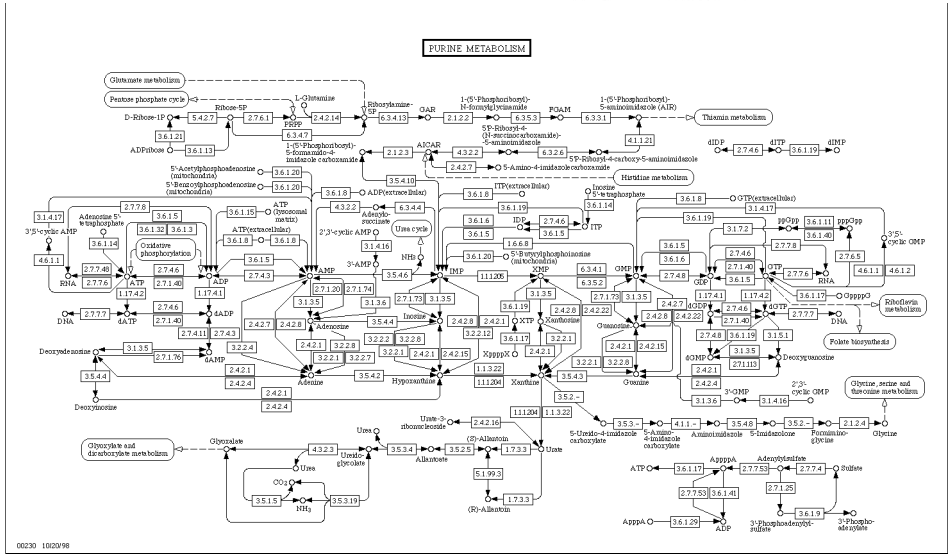
Lock-In approach to reaction kinetics

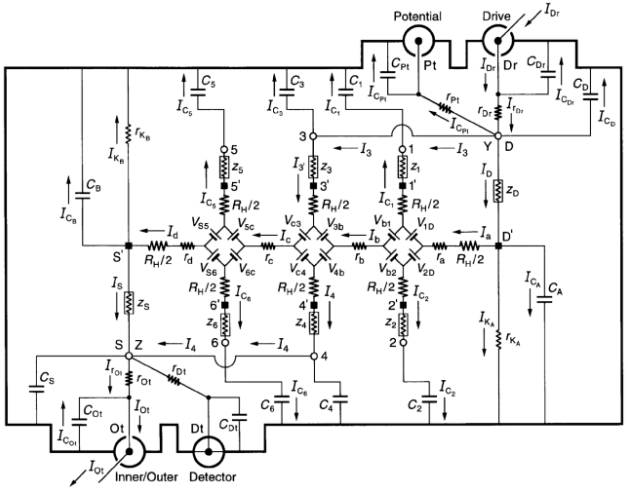
Dieter Braun

Reaction networks





Electric circuits



Signal

Concentrations,
Conformations

Potential
(Conc. of charge)

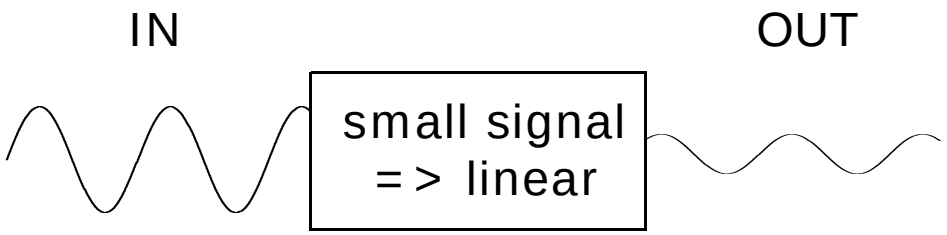
Propagation

Diffusion, Transport
between Compartments

Current, Diffusion

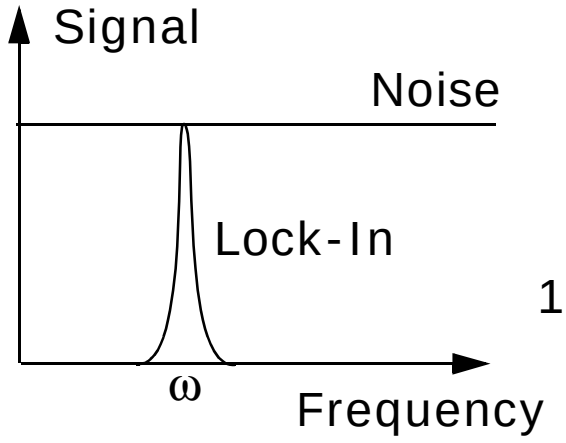
Transfer function
& Lock-In

Transfer function and Lock-In



$$\frac{\Delta OUT(\omega)}{\overline{OUT}} = \text{Transfer h}(\omega) \cdot \frac{\Delta IN(\omega)}{\overline{IN}}$$

Amplitude **Phase**
Delay



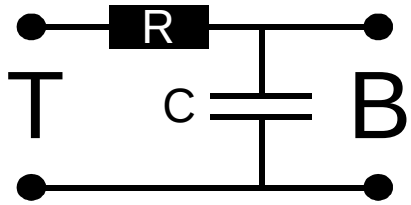
Enhance
S/N by
100..>1000

Transfer function of a reaction

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

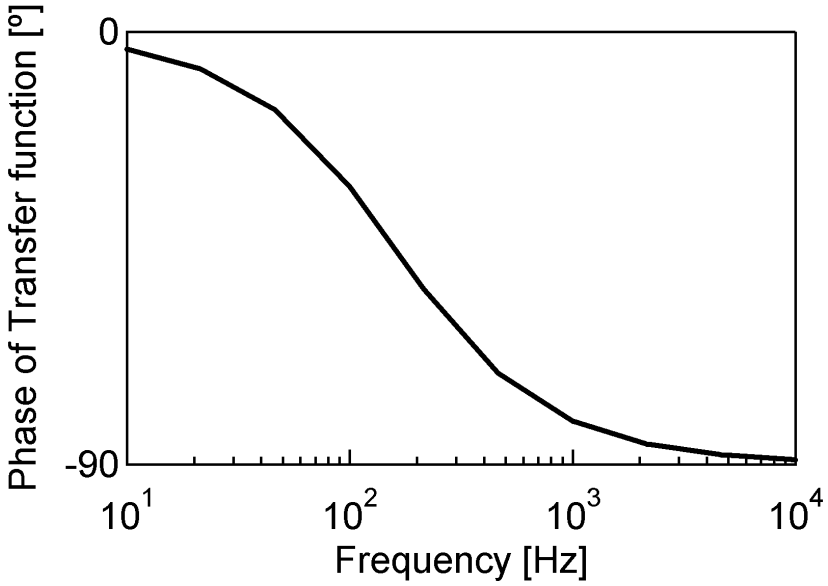
Temperature Oscillation ΔT/T

$$\frac{\Delta[B]}{[B]} = \underbrace{-\frac{\Delta H}{RT}\frac{1}{1+K}\frac{1}{1+i\omega\tau}}_{h(\omega)}\frac{\Delta T}{T} \qquad \tau = \frac{1}{k_1 + k_{-1}}$$



Voltage Oscillation ΔT/T

$$\frac{\Delta B}{B} = \underbrace{\frac{T}{B}\frac{1}{1+i\omega\tau}}_{h(\omega)}\frac{\Delta T}{T} \qquad \tau = RC$$



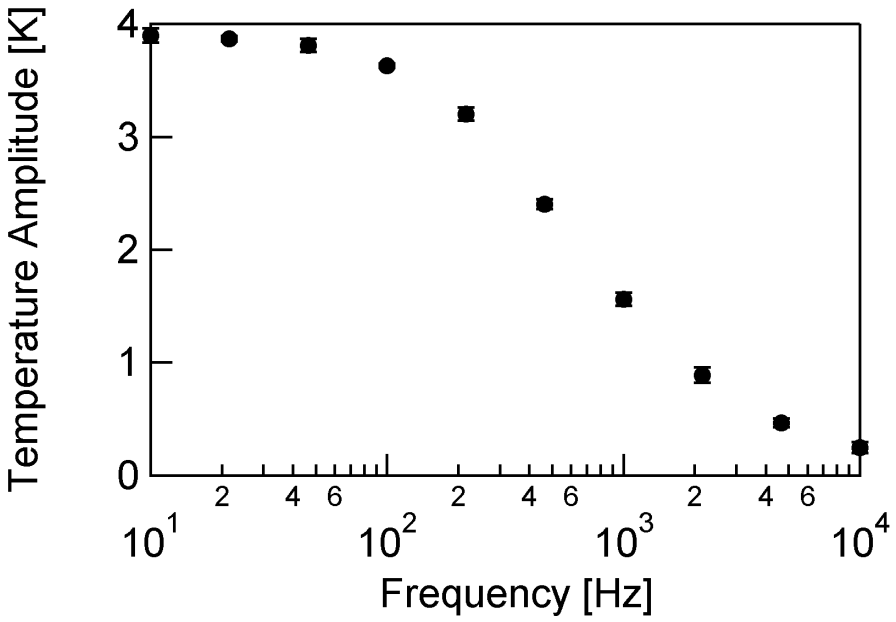
Relaxation time from Phase

$$\tau = \frac{\tan(\varphi)}{\omega} = 1ms$$

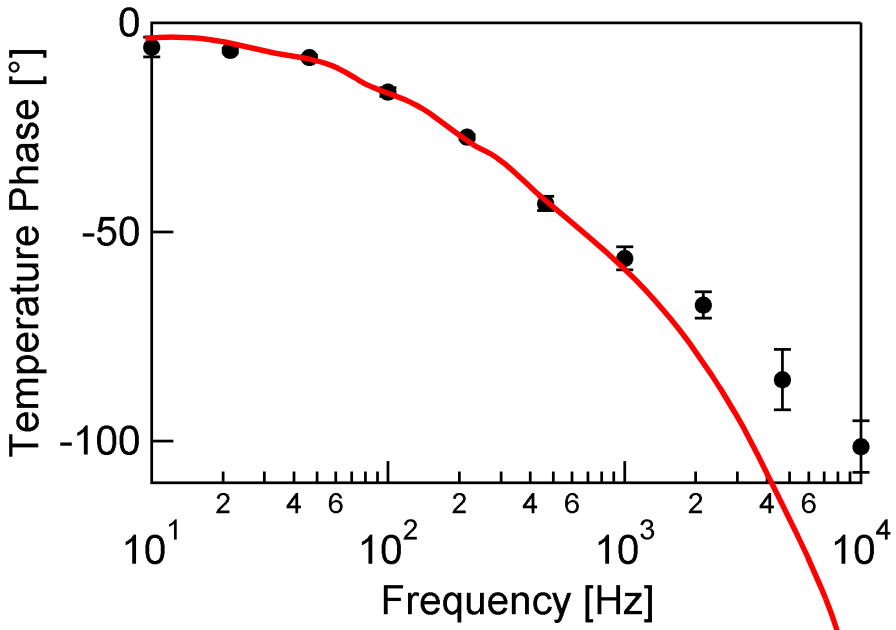
’Fast’ Temperature Oscillations

Laser heating in small volume, measured by temperature dependent fluorescence

Amplitude



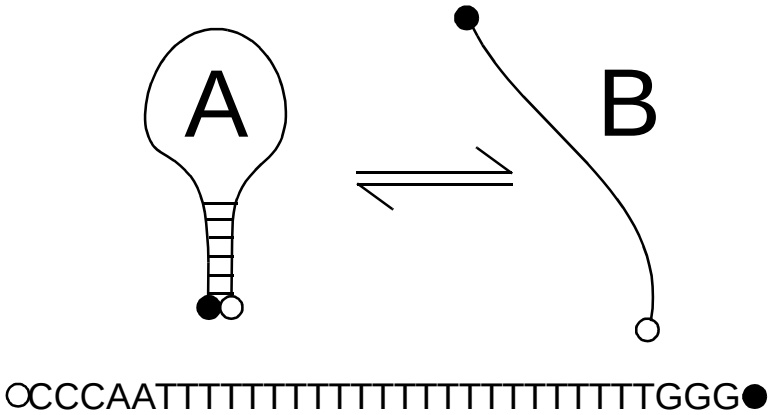
Phase

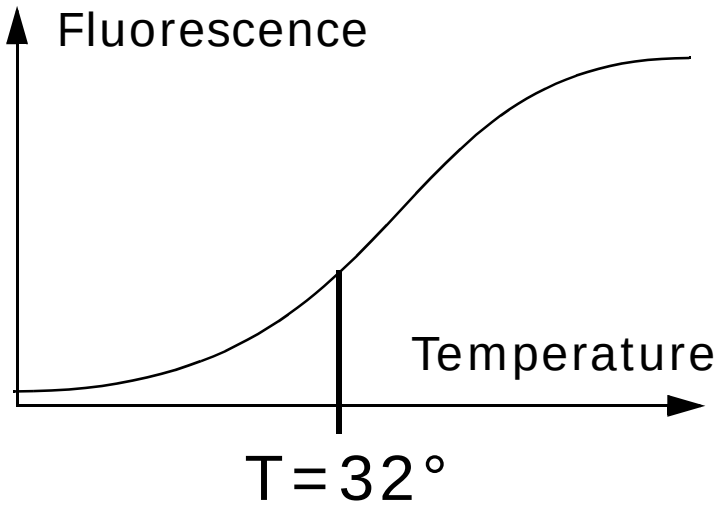


Concentration

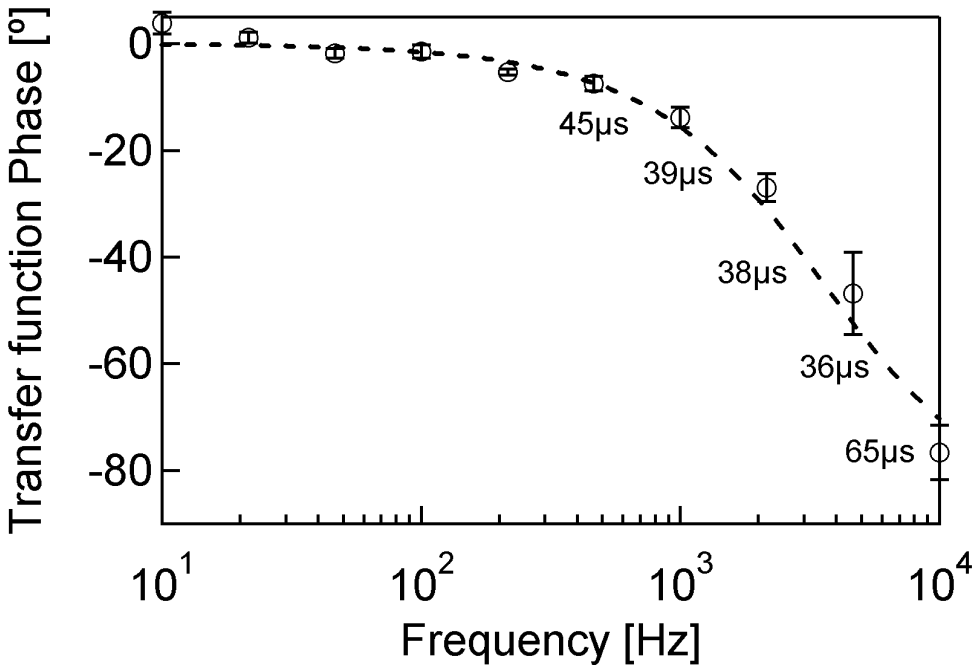
Opening/Closing of a DNA hairpin

Molecular Beacon





$$\frac{\Delta[B]}{[B]} = \underbrace{-\frac{\Delta H}{RT}\frac{1}{1+K}\frac{1}{1+i\omega\tau}}_{h(\omega)}\frac{\Delta T}{T}$$



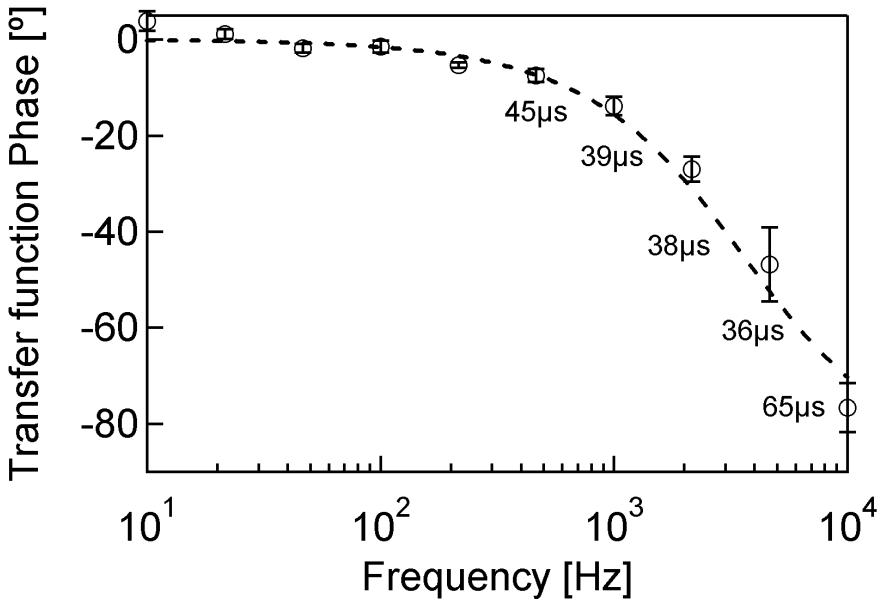
$\tau = 44 \pm 3\mu s$

G. Bonnet,
N. Goddard 90µs

Phase and Amplitude

$$\frac{\Delta[B]}{[B]} = -\frac{\Delta H}{RT}\frac{1}{1+K}\frac{1}{1+i\omega\tau}\frac{\Delta T}{T}$$

Phase

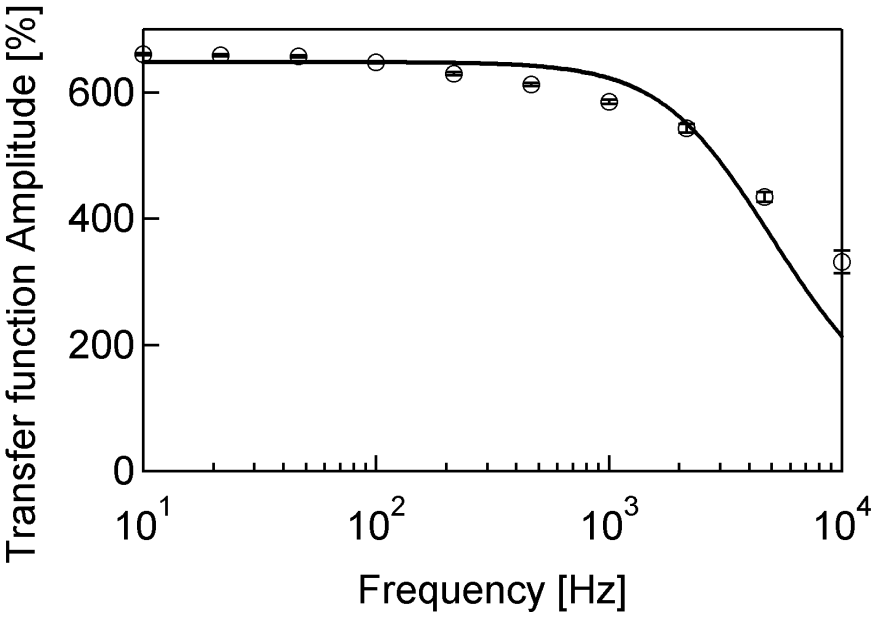


$\tau = 44 \pm 3\mu s$

K = 0.2

$$\frac{1}{k_1} = 200\mu s, \frac{1}{k_{-1}} = 41\mu s$$

Amplitude



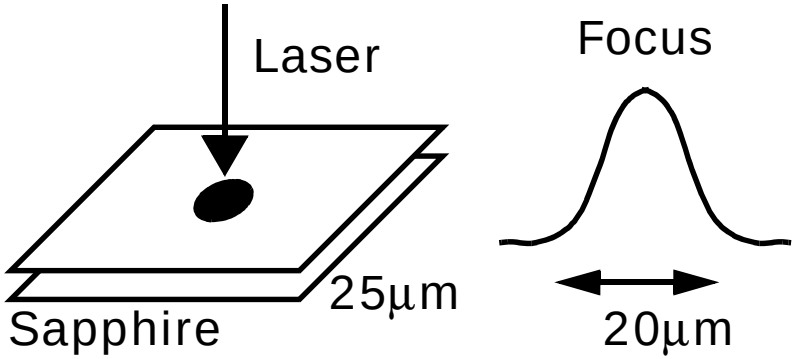
$\tau = 40 \pm 2\mu s$

K = 0.2

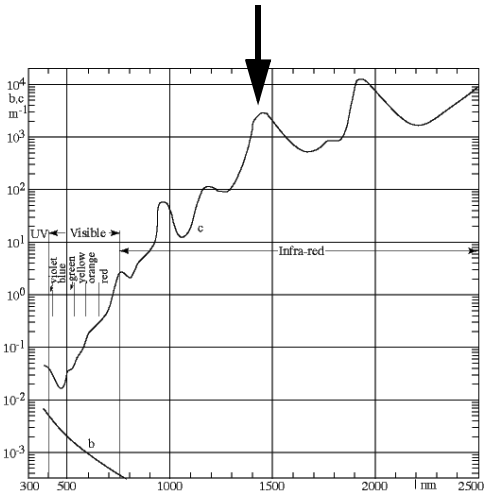
$$\Delta H = -4.7\frac{kcal}{mol} \quad (??)$$

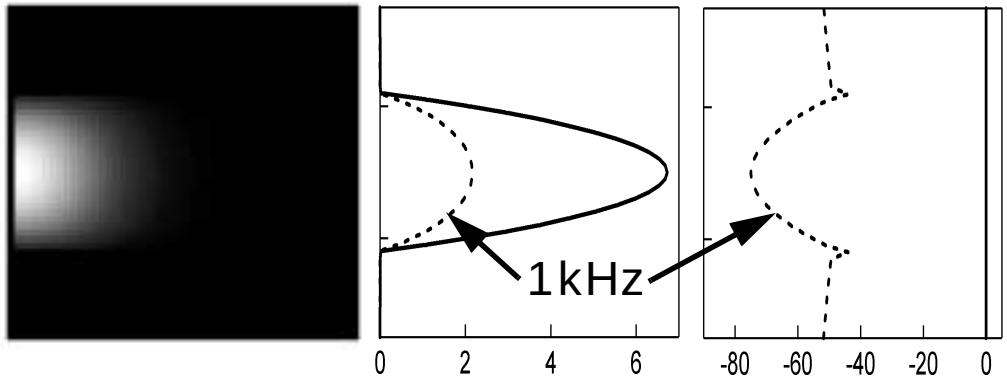
Sensitive to background

Heating / Cooling

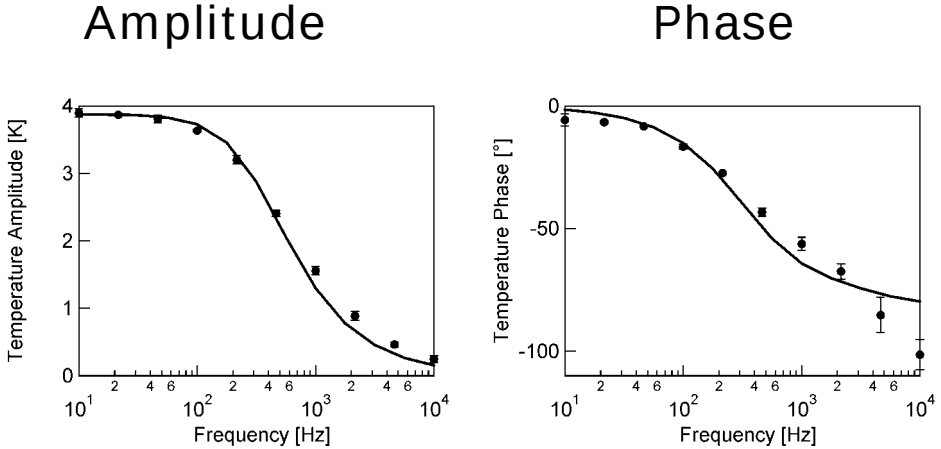


1480nm & <250mW



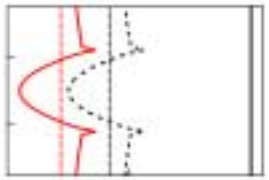


Temperature ΔT

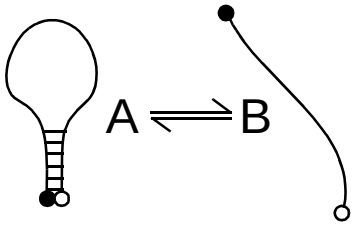


Potential error sources

o Amplitude, Phase profile => no effect



o Bad quenching and background
=> no effect on phase



$F=\alpha[A]+\beta[B]+\gamma$

$\mathbf{\Delta F=(\beta-\alpha)\Delta[B]}$

o Lensing effects => No difference between NA=0.75 and NA=0.4 in Temperature profile measurements

o Movement in phase gradient:
Diffusion, Convection

$$\Delta\frac{1}{k} = \sqrt{D/f}\cdot\frac{1^\circ}{1\mu m}\cdot\frac{1/f}{360^\circ} = 0.5\mu s$$

But: phase gradient has different signs!

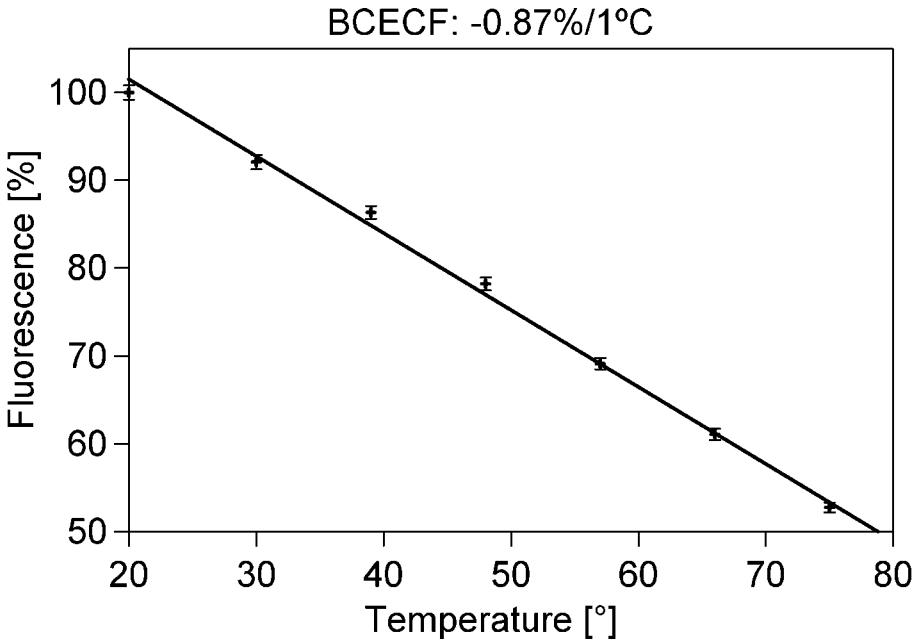
In 1% agarose gel: $\tau=42\pm2\mu s$

Corrections to do:

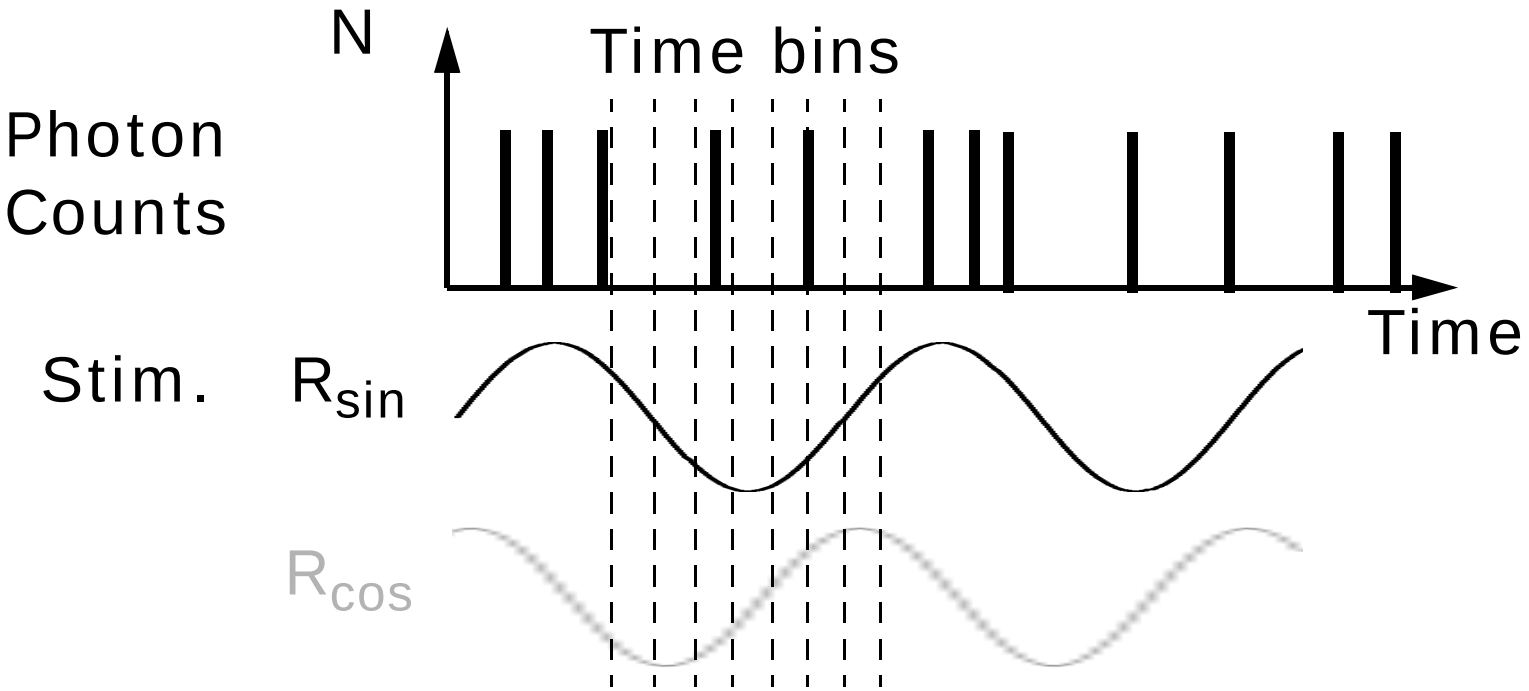
- We average over DC Temperature profile 20..38° with phase profile
- Dependence of beacon dye on Temperature $h \rightarrow h-\alpha T$

Temperature Fluorescence

Temperature -> pH of TRIS -> pH Probe

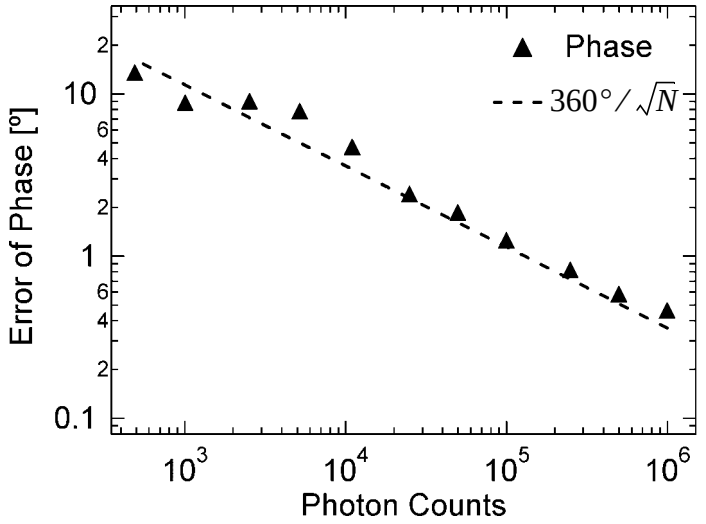


Single Photon Lock-In

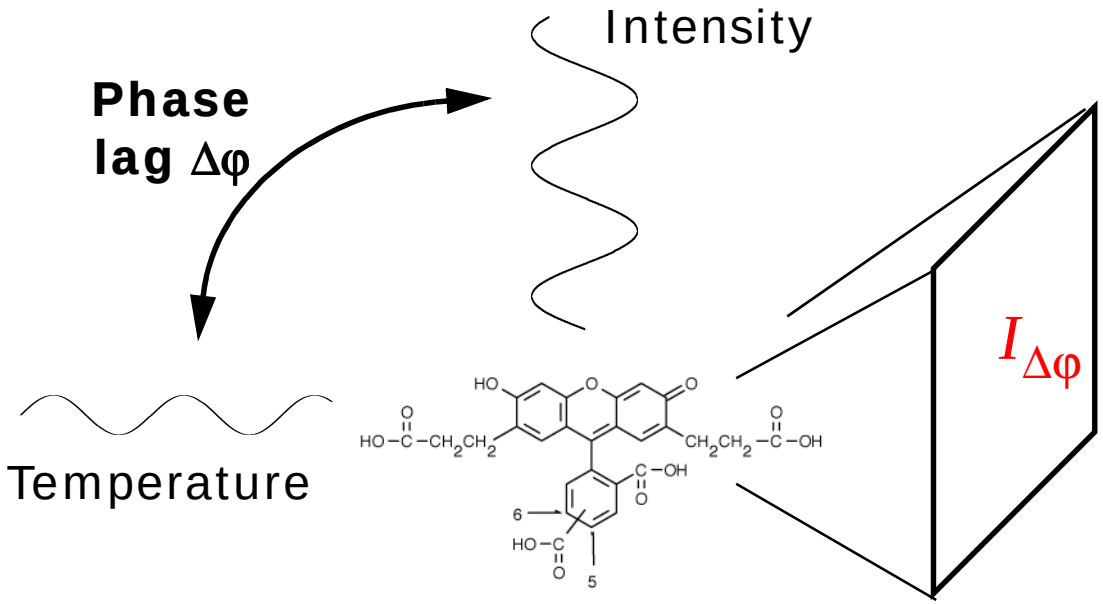


$$\frac{\Delta I}{I} = \frac{\sqrt{2}}{\langle N \rangle}\left[\frac{\langle NR_{\sin}\rangle}{\sqrt{\langle R_{\sin}^2\rangle}} + i\frac{\langle NR_{\cos}\rangle}{\sqrt{\langle R_{\cos}^2\rangle}}\right]$$

Phase error=$360°/\sqrt{N}$

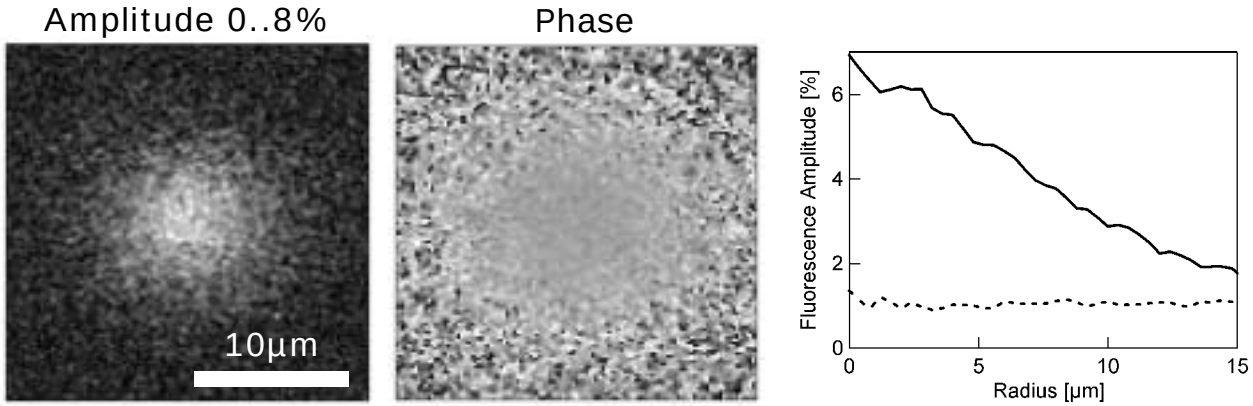


Imaging-Lock-In: Principle



$$\frac{\Delta I}{I} = \frac{2}{\pi}\left[\frac{I_{0°} - I_{180°}}{I_{0°} + I_{180°} - 2I_B} + i\frac{I_{270°} - I_{90°}}{I_{270°} + I_{90°} - 2I_B}\right]$$

First images@2kHz



12bit camera
350ms/pic
Laser noise 3.5%

Advantages

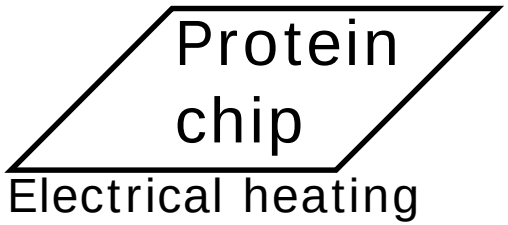
+ Reduction of noise by Lock-In
+ Sensitivity of photon counting
+ Low Temperature modulation (1K)
+ Fast measurement (4s)
+ Potential to image kinetics in cells
+ Fit reaction transfer functions
+ Setup compatible to single molecule detection, cell biology and microfluidics

- No faster than µs

Prospects

Measure DNA concentration
from beacon-target kinetics

Repetitive uncaging



Screen for (binding) kinetics
with imaging Lock-In

Kinetics within cells/bacteria:
probing reaction networks

Accumulation of Biopolymers by Convection + Thermophoresis:

Role in prebiotic evolution?

Dieter Braun

Thermophoresis...

Cold **Hot**

Particles ←

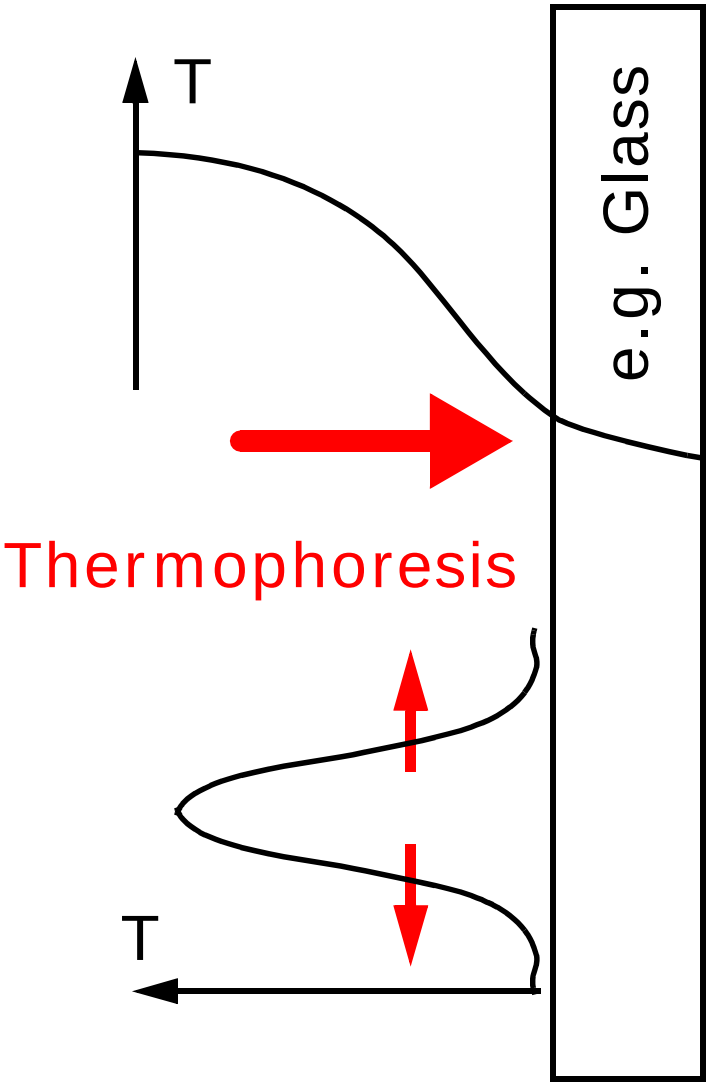
DNA ←

Cl^- ← $\vec{E}$

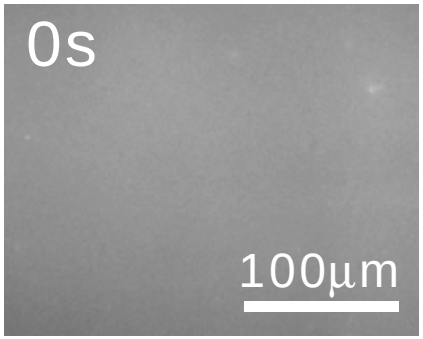
K^+

↑ Lower density convection ↑

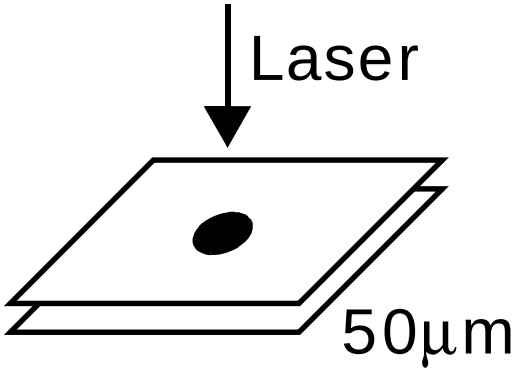
...at interface

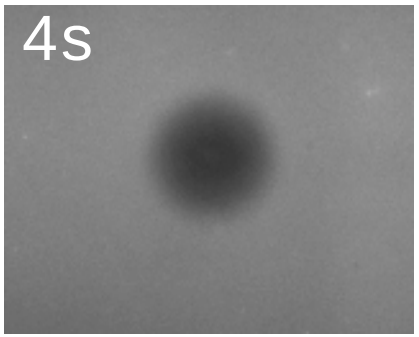


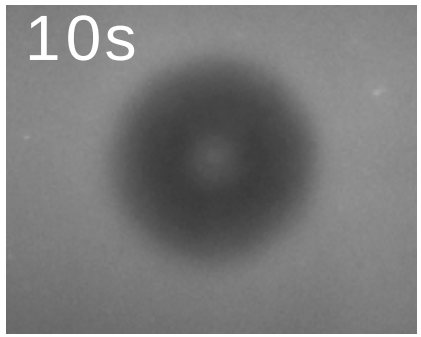
Microconvective Accumulation

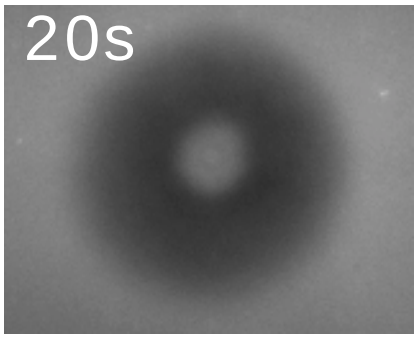


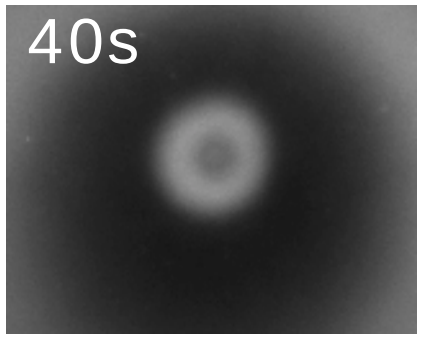
5kBp DNA



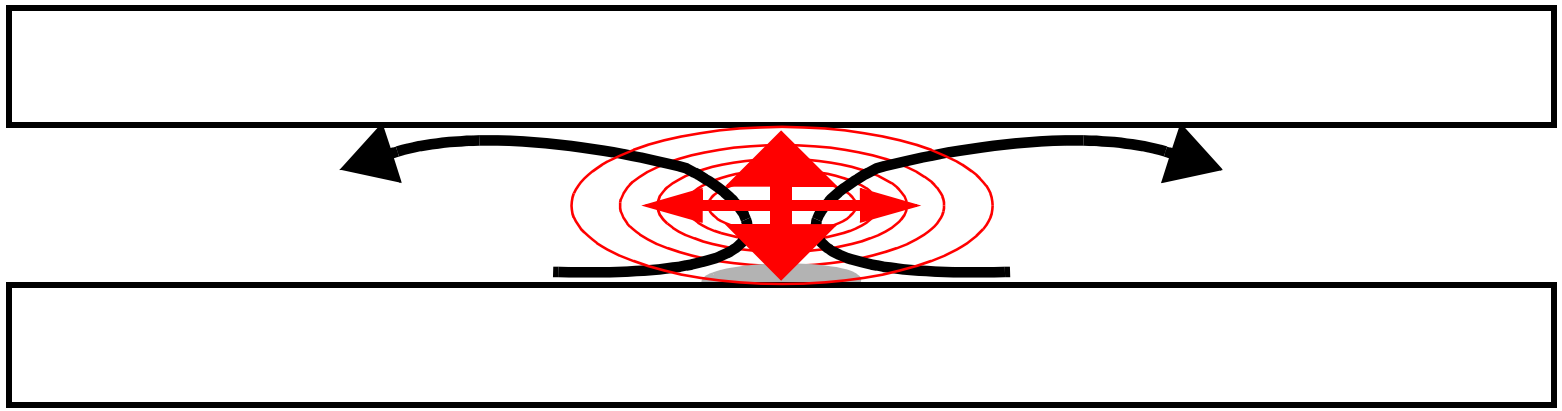




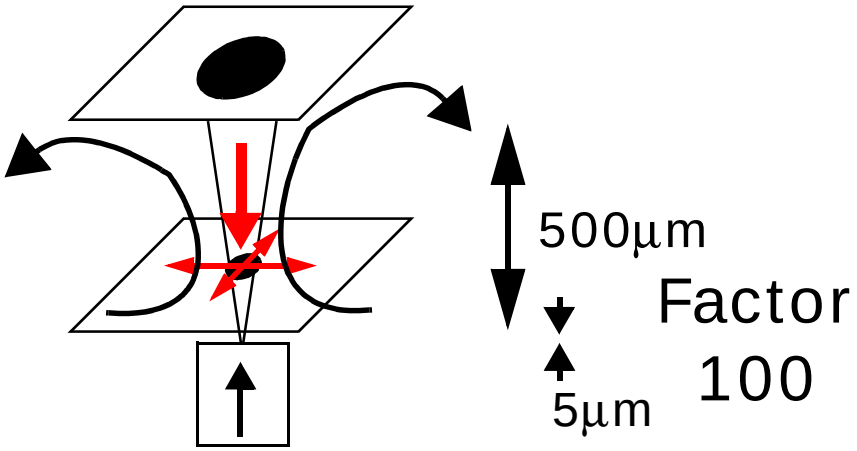


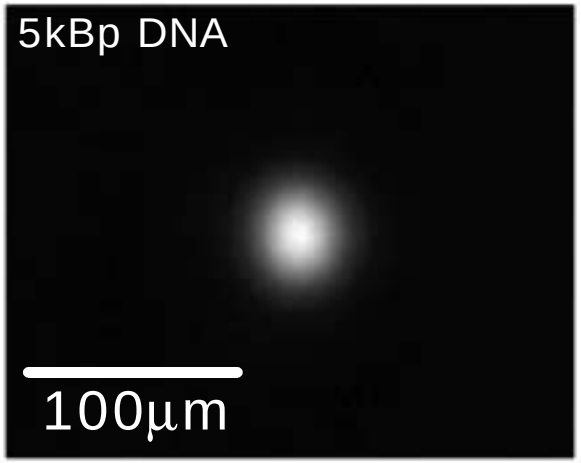


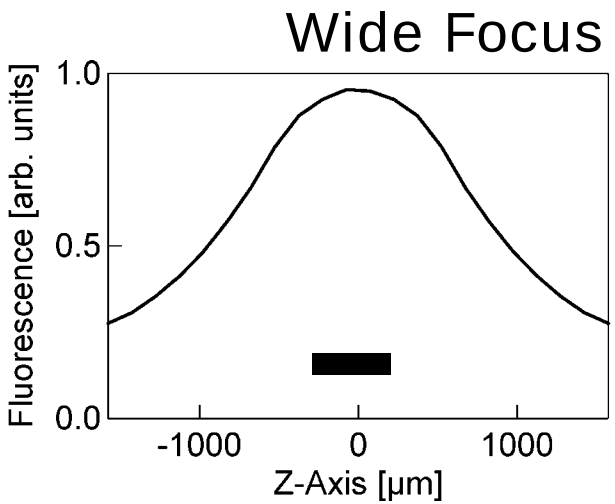
Mechanism

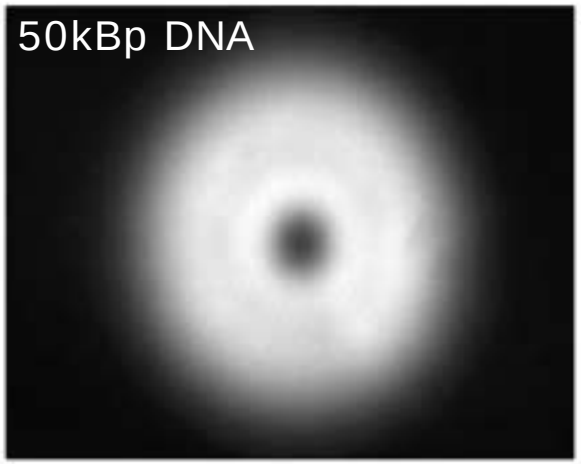


Convective Concentration of DNA





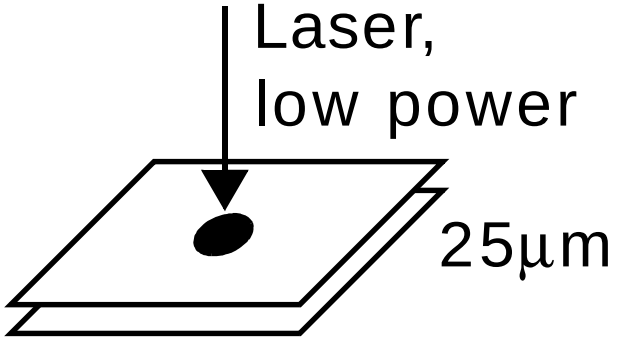


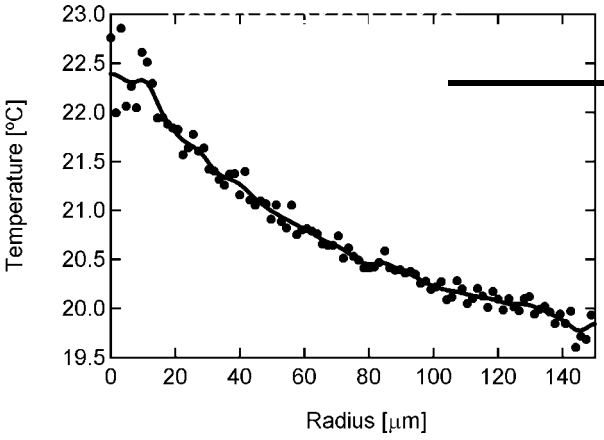


30fold Fluorescence enhancement

=> 3000fold Accumulation (within 180s)

Measure S/D and D => S

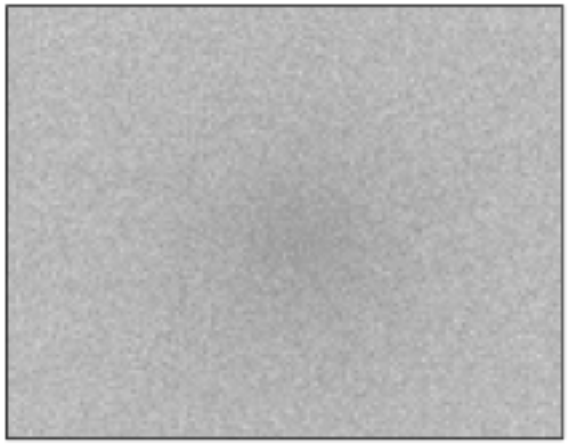




$$\frac{c}{c_0} = \left(\frac{T}{T_0}\right)^{-S/D}$$

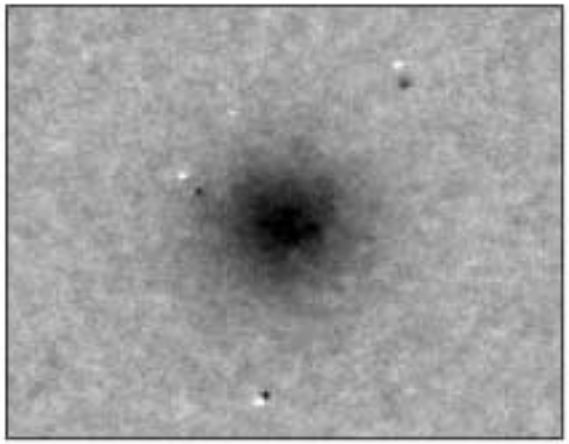
and D from Backdiffusion

27Bp DNA

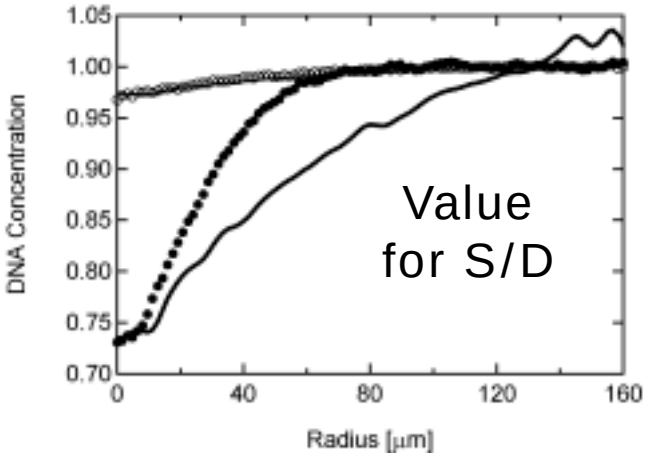


S=120µm²/s

5000Bp DNA



S=130µm²/s



40nm Beads: **S=45µm²/s**

S undetecteable for 500mM NaCl

Implications for Prebiotic Evolution?

Concentration problem of early evolution
e.g. for basic polymerisation reactions

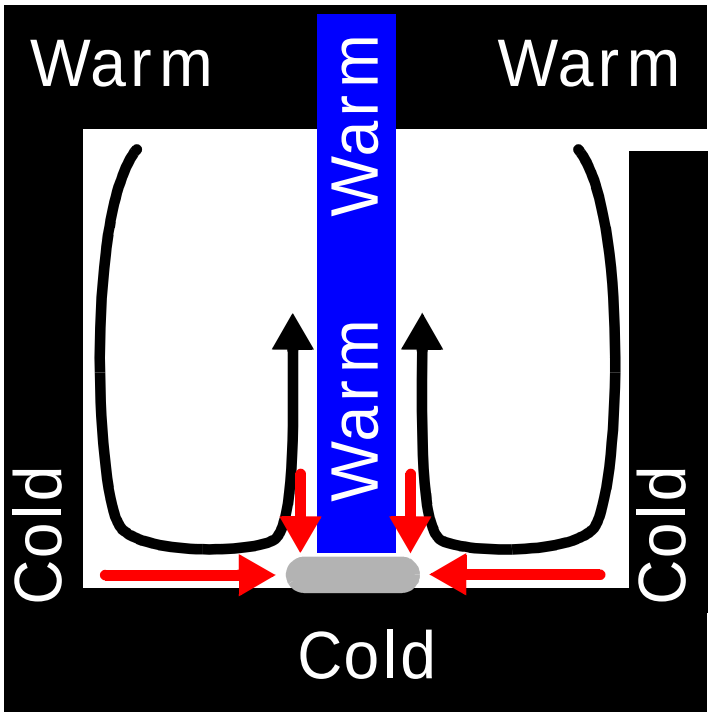
Vesicles no solution on this (?)
Evaporation schemes not stable enough

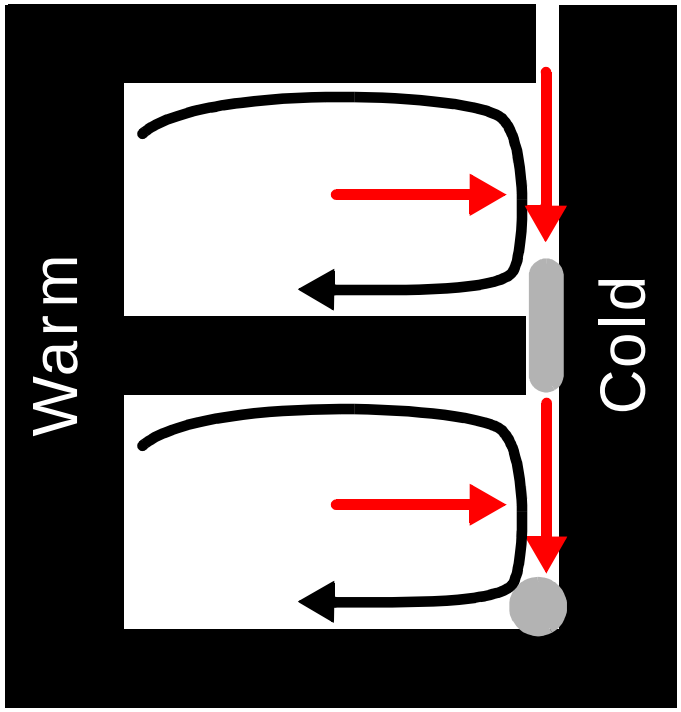
Proposition of mechanism:

Hot ocean bed applies temperature gradients,
e.g. in pores of stones

- Accumulation of particles and molcules
- 'Bioreactor' with convective temperature cycling
- Concentration feeding first metabolisms
- Catalysis at stone surface (Wächtershäuser)

Hypothetic Geometries





=> Need for experimental tests

Thanks to

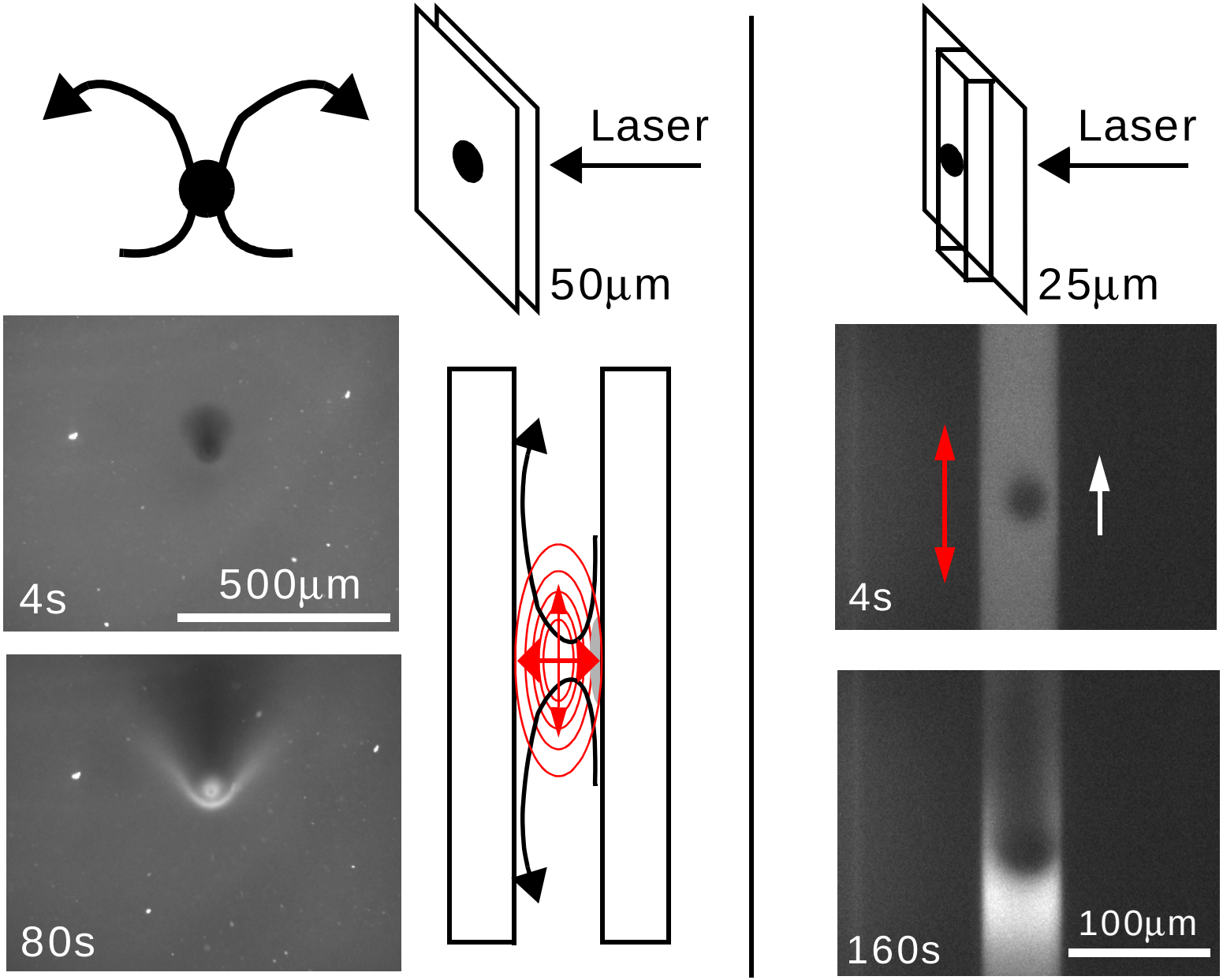
Libchaber Lab

LabView

Emmy Noether Program

mail@dieterb.de

Same pattern in other geometries



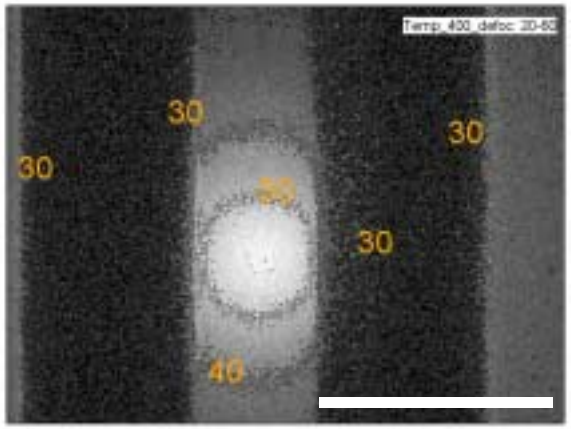
Measurement strategy for S/D

Fick's law

$$j = -D\nabla c - Sc\frac{\nabla T}{T} \stackrel{!}{=} 0 \quad \Rightarrow \quad \frac{c}{c_0} = \left(\frac{T}{T_0}\right)^{-S/D}$$

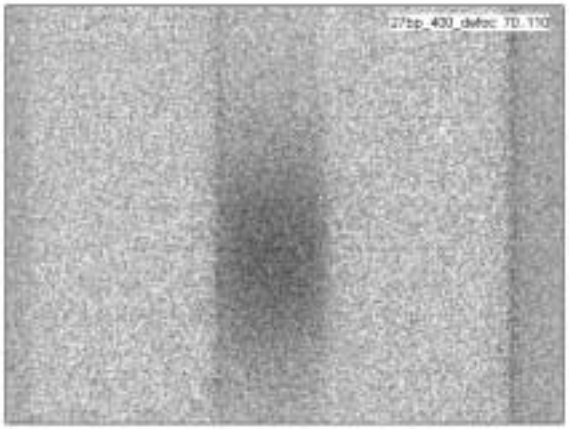
Soret coefficient

Temperature T

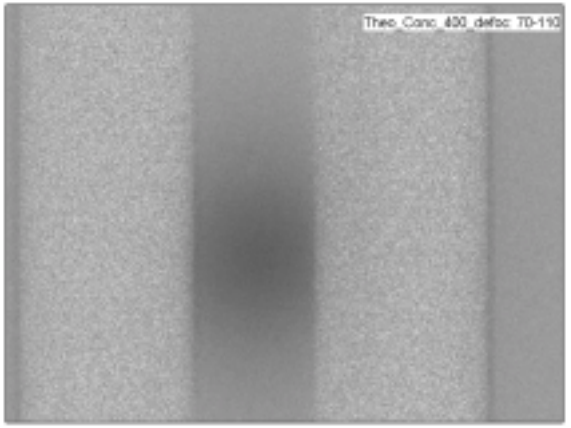


100µm

Concentration c



S/D=1.3



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