Un microlaboratoire électrophorétique pour l'étude du couplage entre transport et cinétique chimique : application à la réaction d'hybridation d'oligonucléotides

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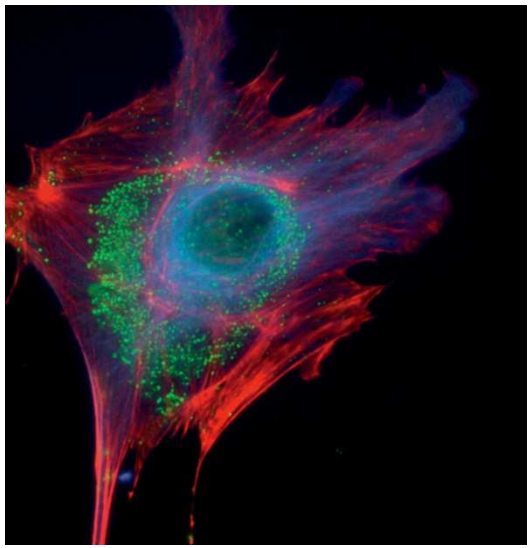
Goal



1 cell = 10^5 chemical species

Large concentration range

-miRNA 10-10^4 copies

- ATP 10^7 (1 mM)

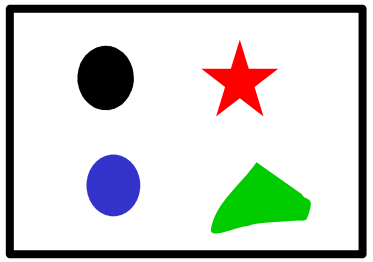
D.P. Bartel *Cell* **2004**

Need of powerful tools for Analytical Chemistry

selective and fast

Selectivity in analytical chemistry

Issue: select a species inside a mixture



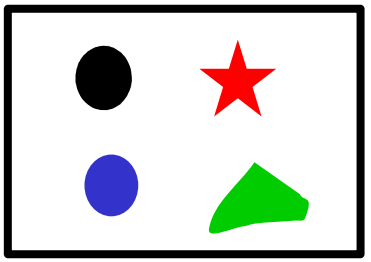
2 strategies:

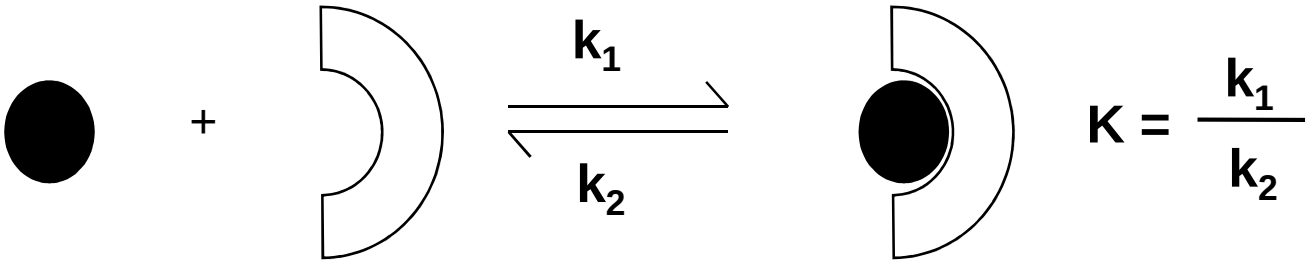
Improve existing strategies
- Spectroscopy
- Affinity separations (K)

Develop new selection strategies: reactivity (k_1, k_2)

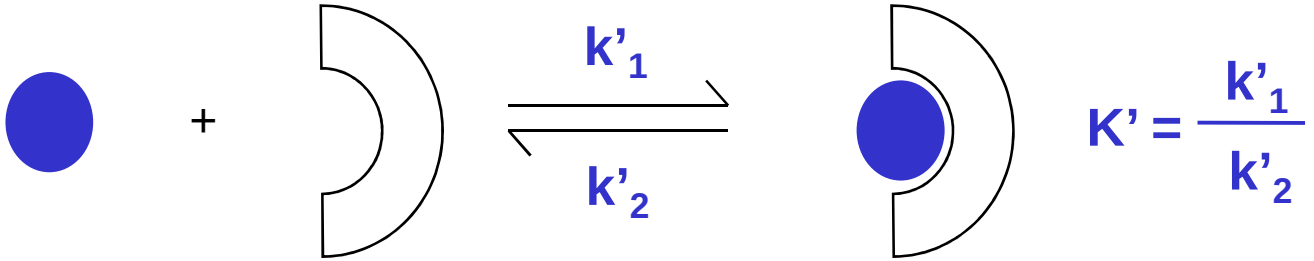
Selecting on kinetics

chemical species ⟶ reacting object





$$K = \frac{k_1}{k_2}$$



$$K' = \frac{k'_1}{k'_2}$$

Thermodynamics: **K**

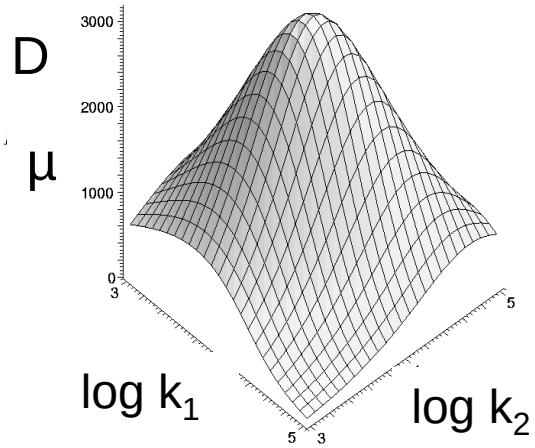
Kinetics: **k_1, k_2**

Resonance

A chemical reaction… $A_i + B \underset{k_2^i}{\overset{k_1^i}{\rightleftharpoons}} C_i$

… and a periodic excitation… $u(t) = u\cos(\omega t)$

… yield:



Response always maximum

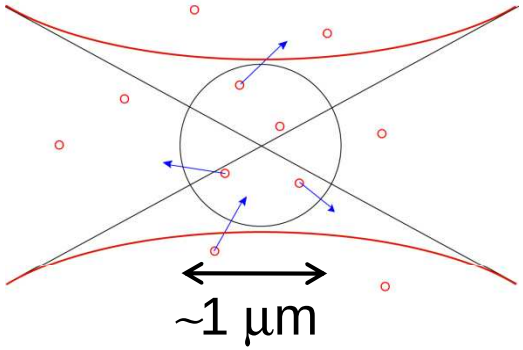
Focus on diffusion (D)

L. Jullien, A. Lemarchand, H. Lemarchand, *J. Chem. Phys.*, **2000**

Quantifying diffusion

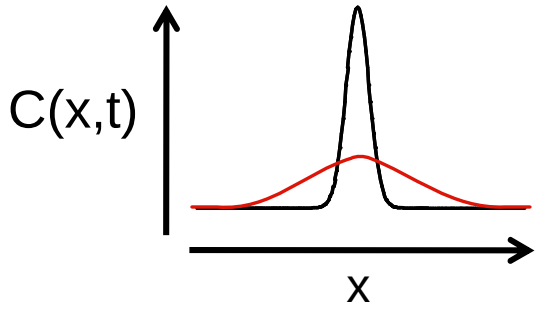
Two main approaches:

1. Fluctuations near equilibrium: FCS

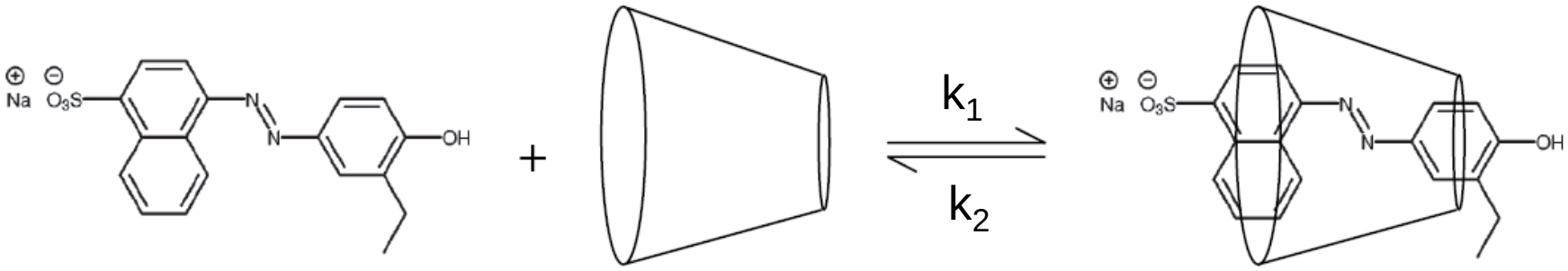


2. Relaxation of out-of-equilibrium concentration profile

- FRAP D. Axelrod *et al*, *Biophys. J.*, **1976**
- Injection



Controlling diffusion: electrodiffusion

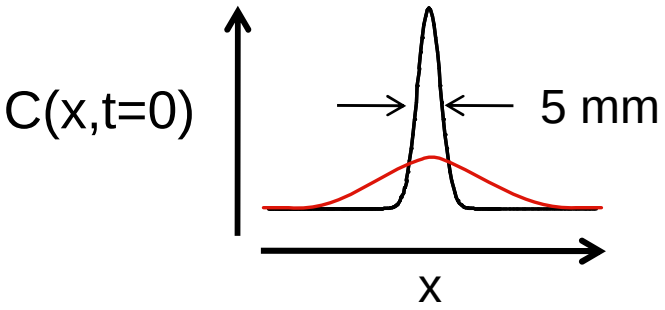


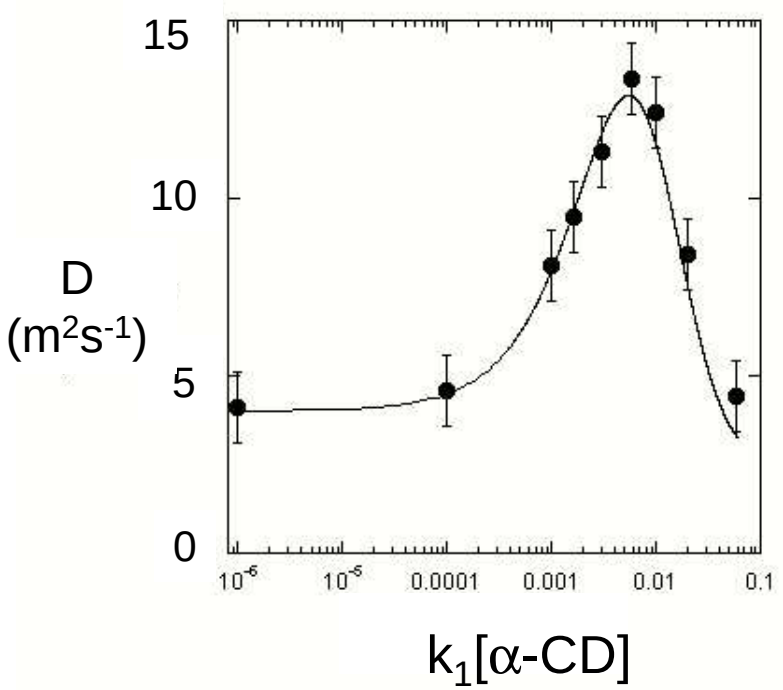
azo dye α-CD

D maximum when:

$$k_1[\alpha\text{-CD}] = k_2 = \frac{\omega}{2}$$

Initial condition:



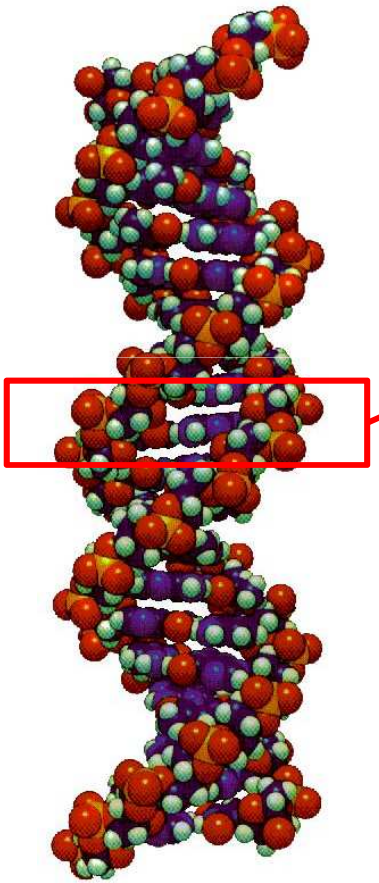


Alcor D, Croquette V, Jullien L, Lemarchand A, *PNAS,* **2004**

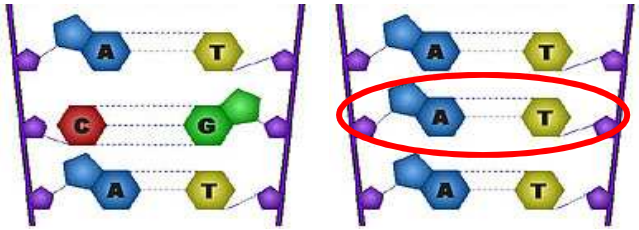
Outline

1. DNA hybridization reaction
2. A functional microlaboratory
3. A powerful tool to analyze dynamics

DNA point mutations (SNP)



What is a point mutation?

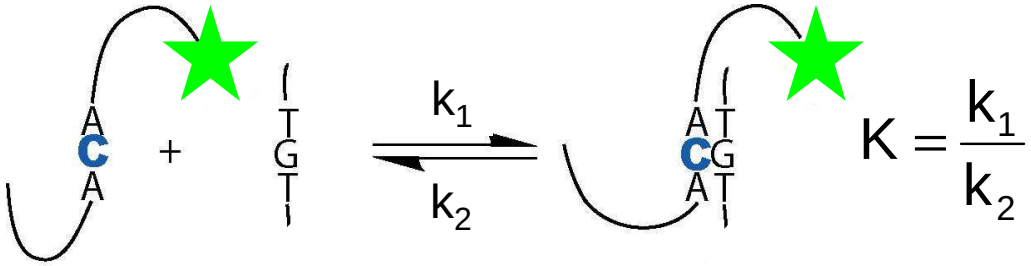


wild type mutated

Why detecting them?

- Related to important deseases
- Identification of bacterial strains
- 10^6 SNPs in genome

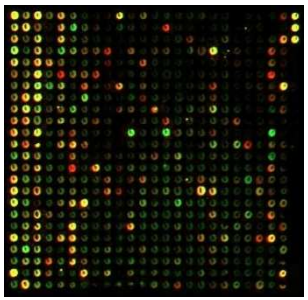
SNP detection



DNA chips rely on thermodynamics

Slow (10-1h) Liu, Quake, *Angew.Chem.*, **2006**

Weak observable (intensity)



Electrodiffusion rely on kinetics

Fast (seconds)

Reliable observable (distance)

Dynamics of electrodiffusion

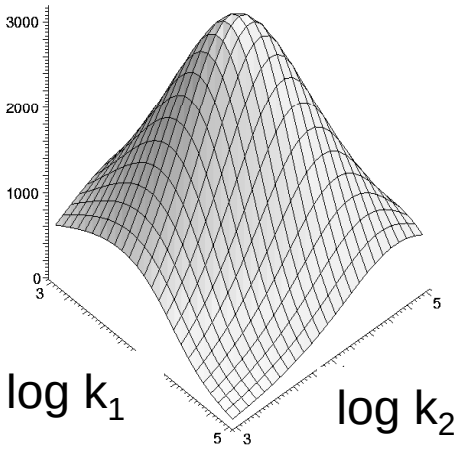
$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C$$

Important parameters:

Reaction dynamics: k_1, k_2

Transport dynamics: μ_A, μ_C, D_A, D_C

D_{disp}



$$D_{disp} = E^2 \left(\mu_A - \mu_C\right)^2 \frac{k_1 k_2}{(k_1 + k_2)^3} \left[\frac{1}{2(1 + \frac{\omega^2}{(k_1+k_2)^2})}\right]$$

Dynamics of DNA oligonucleotides

1. Easy predicted kinetics
   - K can be calculated — J. SantaLucia Jr. *PNAS* ***1998***
   - k_1 set by salt — A.P. Williams *et al Biochemistry* **1991**
   - $k_2 = k_1 / K$

2. Problem: k_1 independent of sequence (nucleation mechanism)
   D.Pörschke, M. Eigen *J. Mol. Biol* **1971**

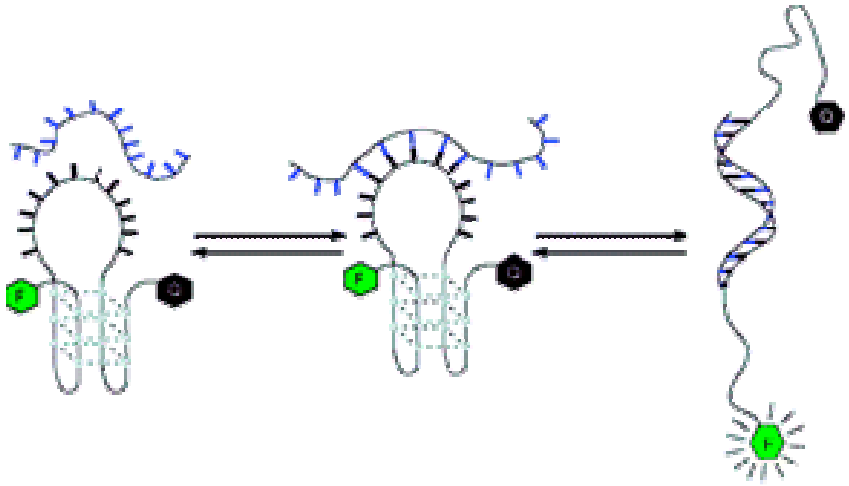
3. Problem: Electrophoretic mobility independent of length (free draining)
   N. Stellwagen *et al Biochemistry* **2003**

4. Easy to have $D_A \neq D_C$ (changing length)

Results: control of kinetics

1. An oligonucleotide bank with widespread kinetics $\begin{cases} k_1 \ (10^4 – 10^6\ M^{-1}\ s^{-1}) \\ k_2 \ (10^2 – 10^{-4}\ s^{-1}) \end{cases}$

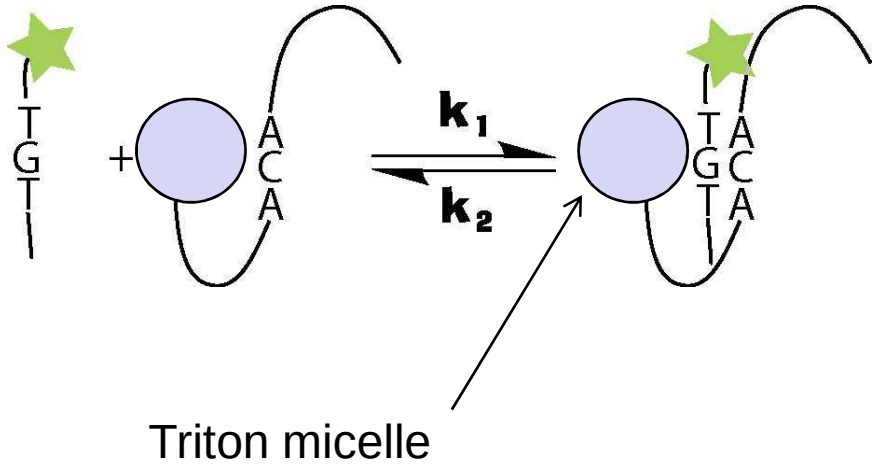
2. k_1 might depend on sequence but slow dynamics



A. Bourdoncle, A. Estévez-Torres et al. *JACS* **2006**

Results: mobility reduction

3. Electrophoretic mobility can be tuned $\mu_A \neq \mu_C$



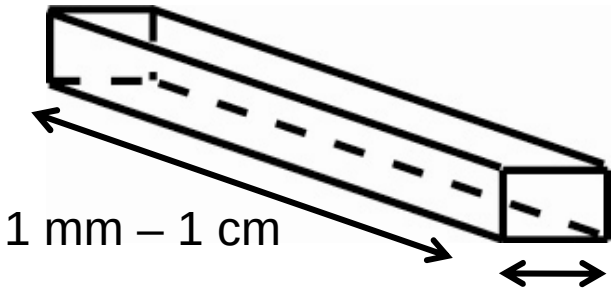
Very good dynamic model: k_1,k_2, D and μ can be modulated

Experimental constraints

$k_2 \sim 1\ s^{-1}$

$$x \sim \sqrt{\frac{D}{k_2}} \sim 100\mu\mathrm{m}$$

shorter times → shorter lengths



better heat dissipation

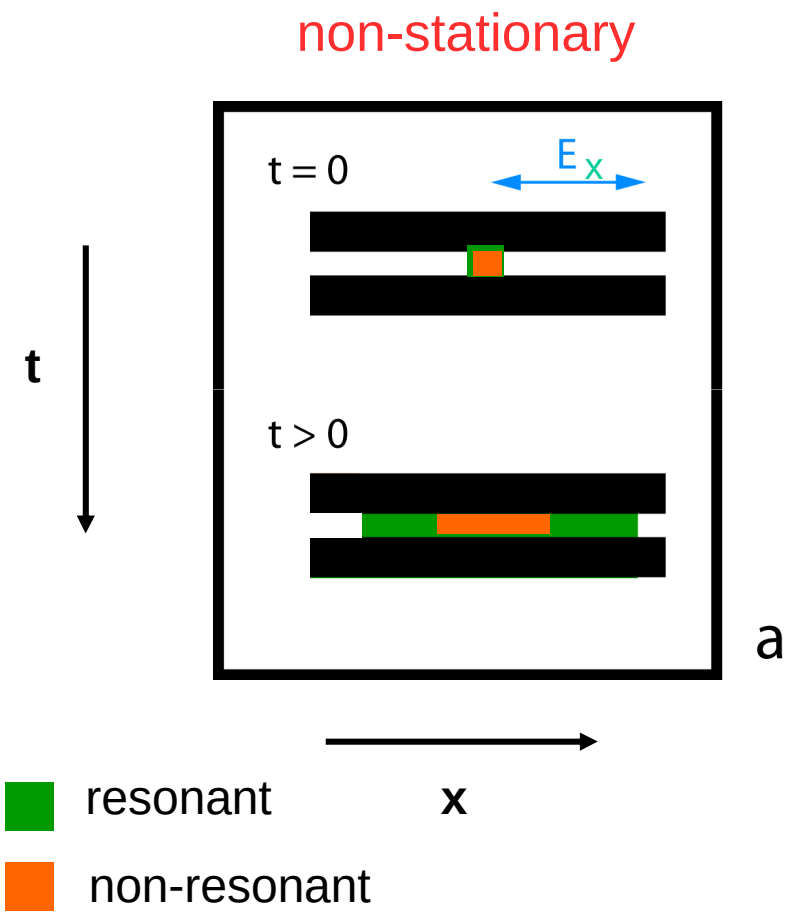
$E = 10^4 - 10^5\ V\ m^{-1}$

Need of microfluidics

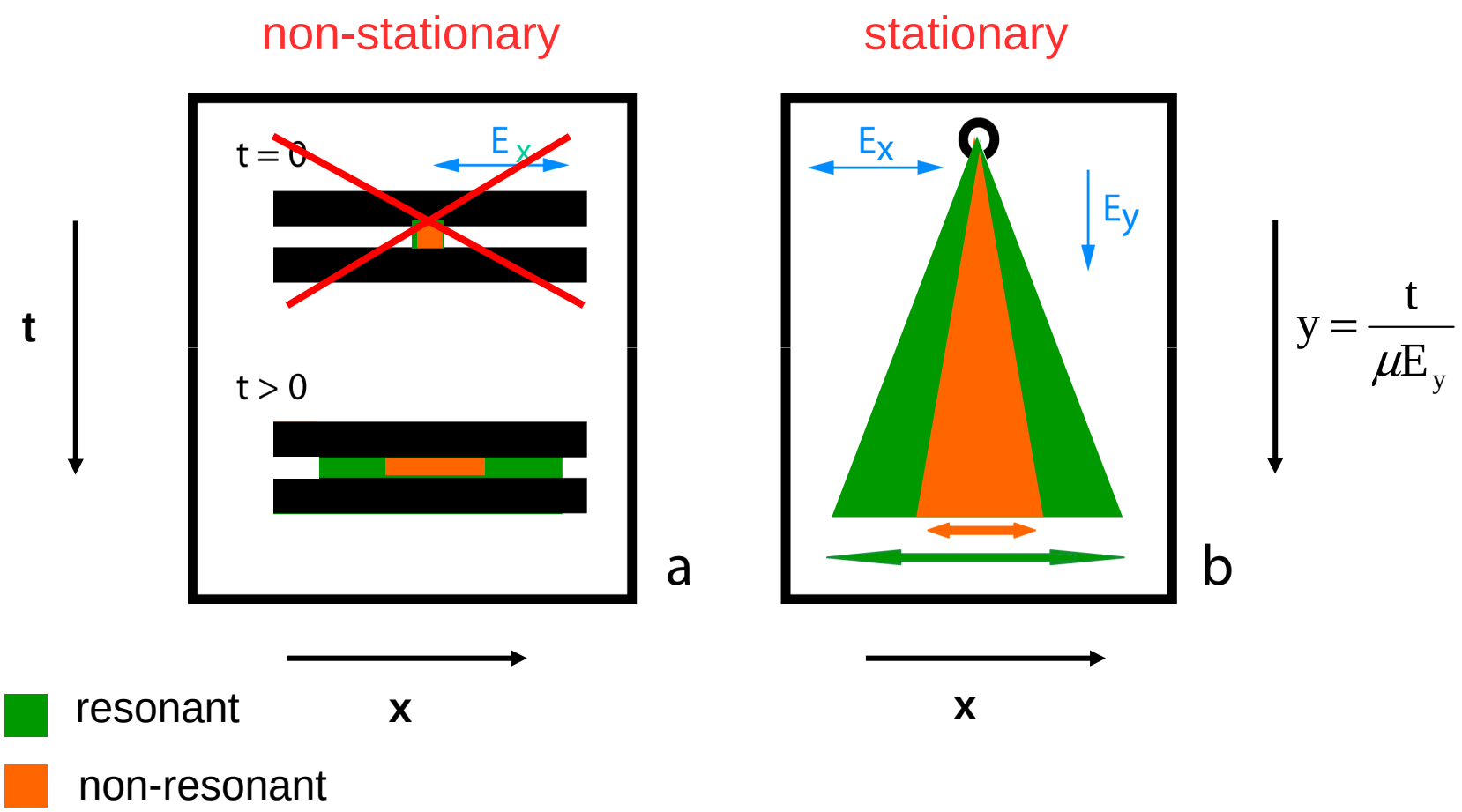
Outline

1. DNA hybridization reaction
2. A functional microlaboratory
3. A powerful tool to analyze dynamics

Initial condition: 1D

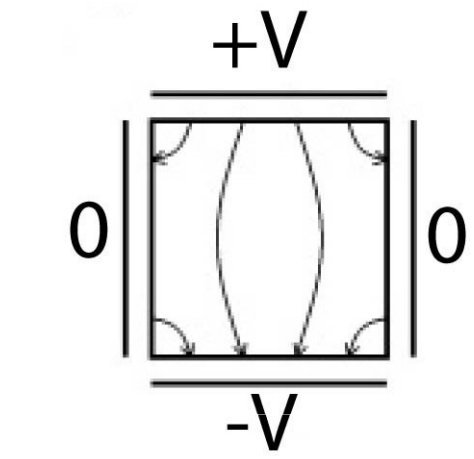


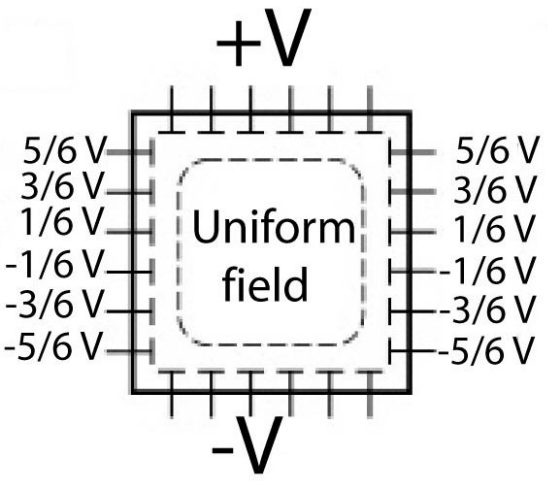
Initial condition: 2D



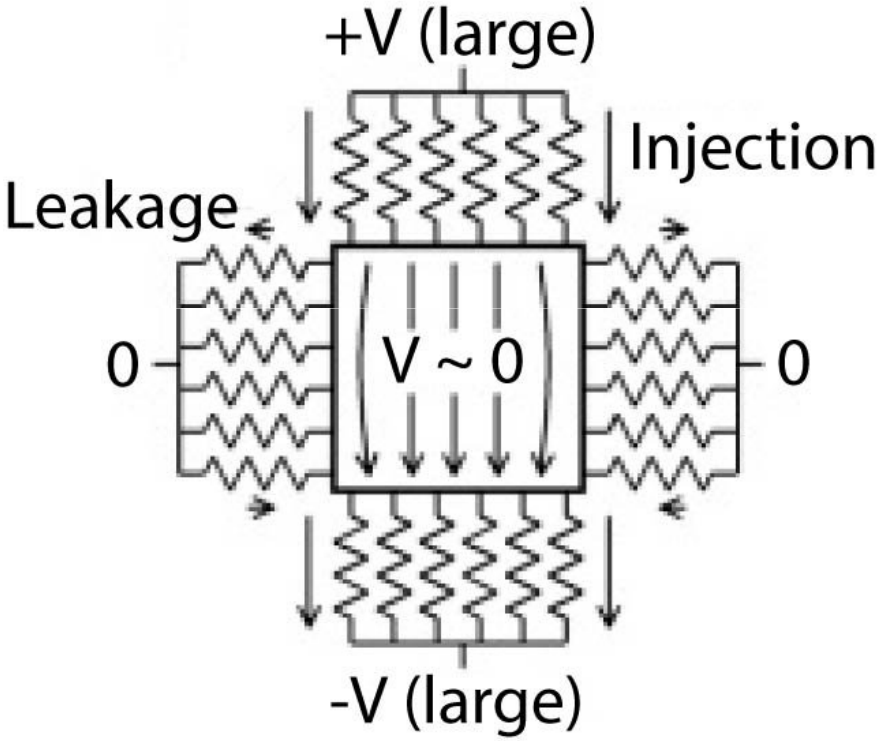
Uniform velocity?

Cross-field generation



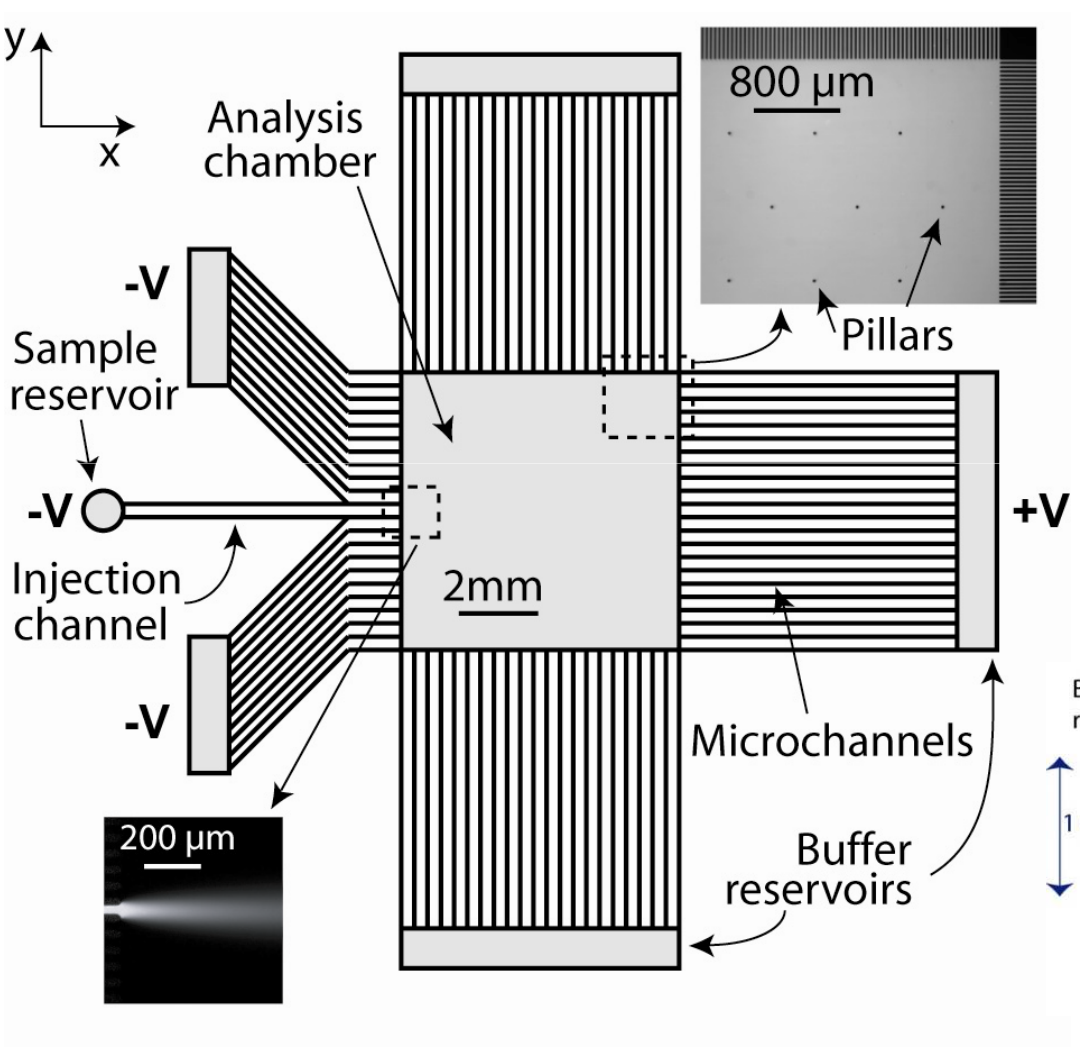


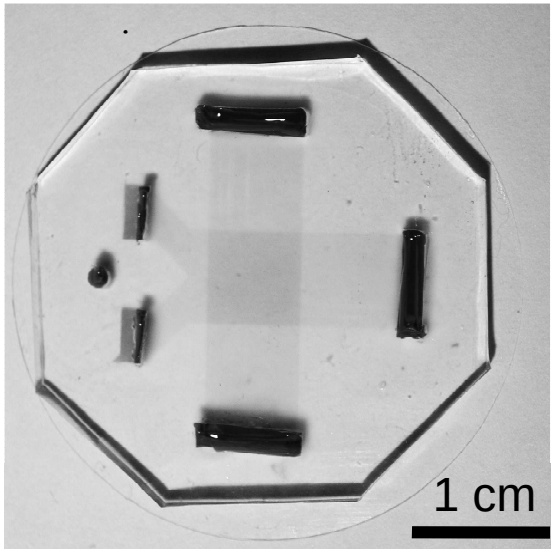
D. C. Schwartz C.R. Cantor *Cell* **1984**

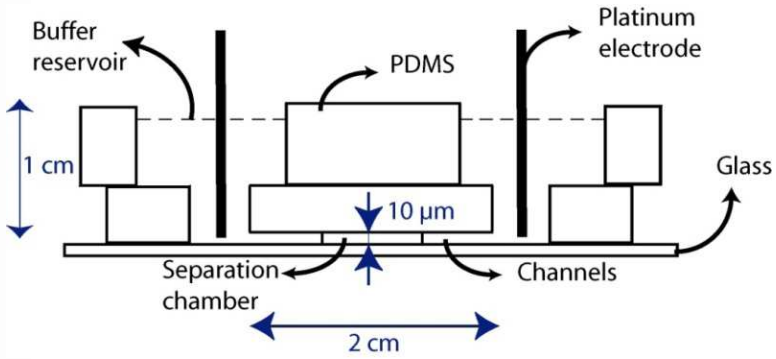


L. R. Huang et al. *Int. Elect. Dev. Meet. Tech. Digest*, **2001**

A versatile 2D electrophoretic device



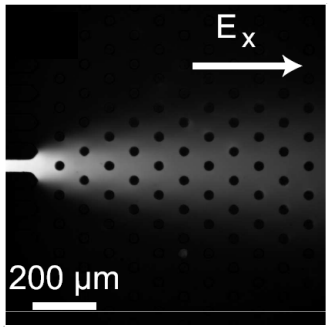




A. Estévez-Torres *et al Anal. Chem.* submitted

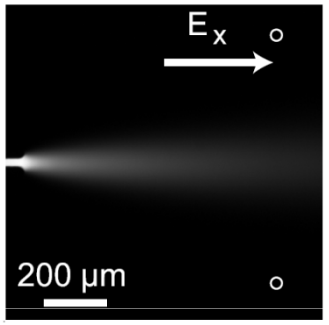
Solved issues

Reduction of pillar density



D = 150 ± 10 $\mu m^2 s^{-1}$

Pillars induce dispersion

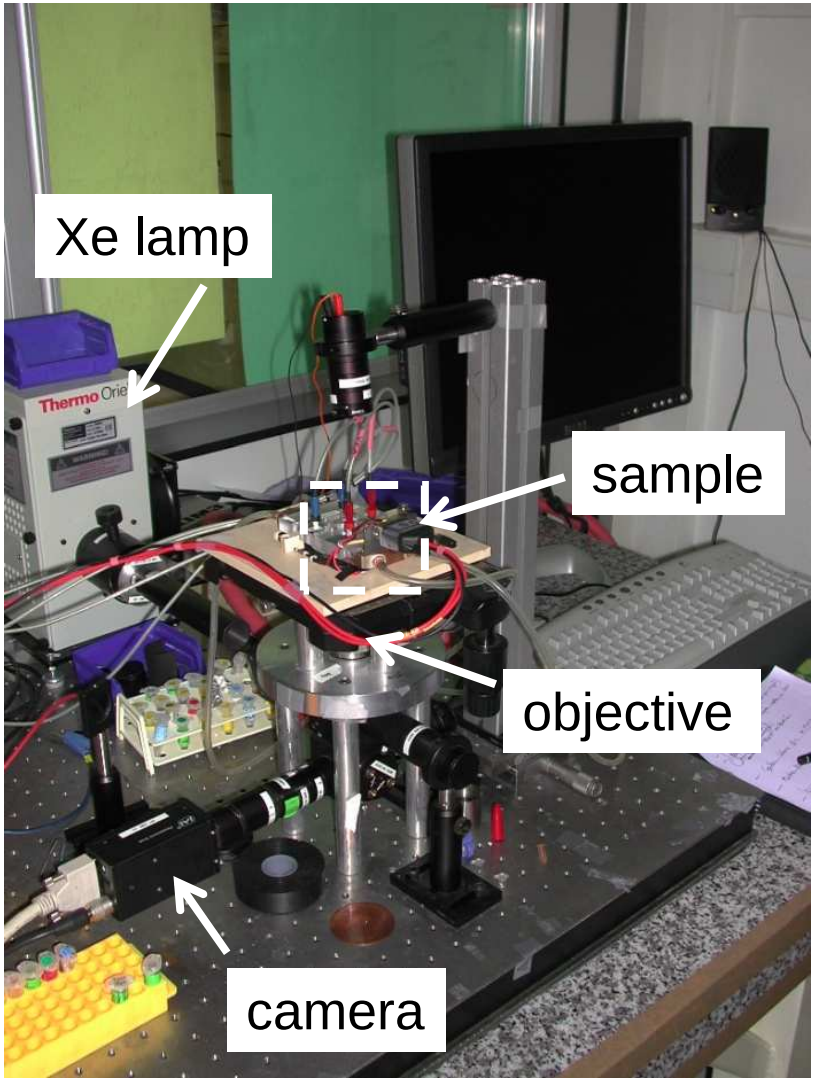


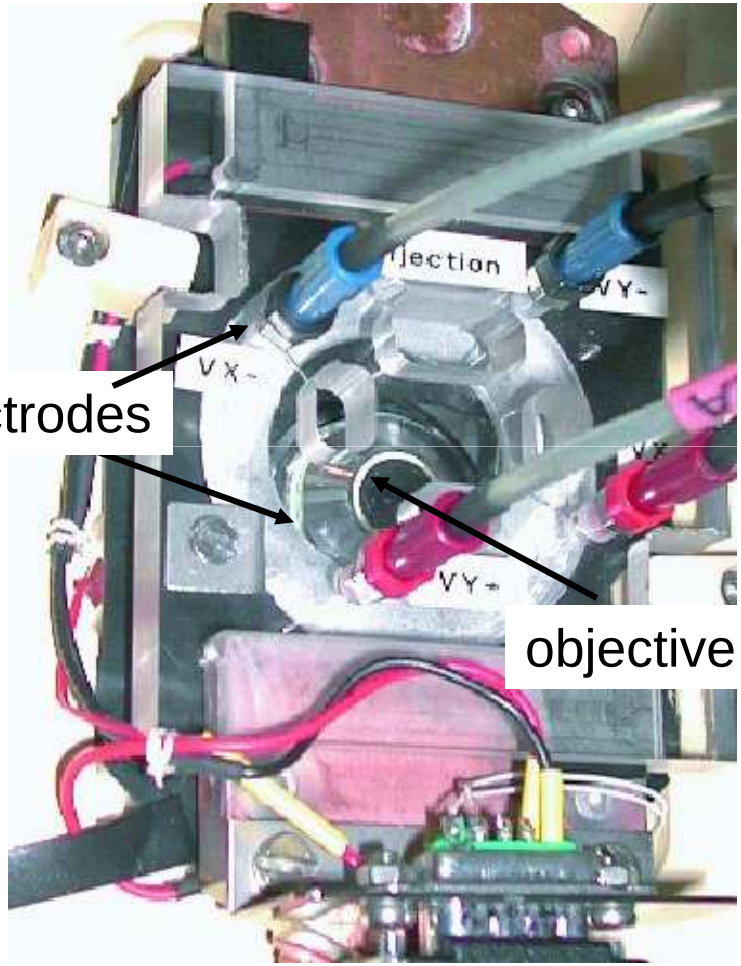
D = 100 ± 10 $\mu m^2 s^{-1}$

Filling protocol avoid collapsing:

1. Plasma treatment
2. Chip heating for capillary force reduction
3. Vacuum pumping

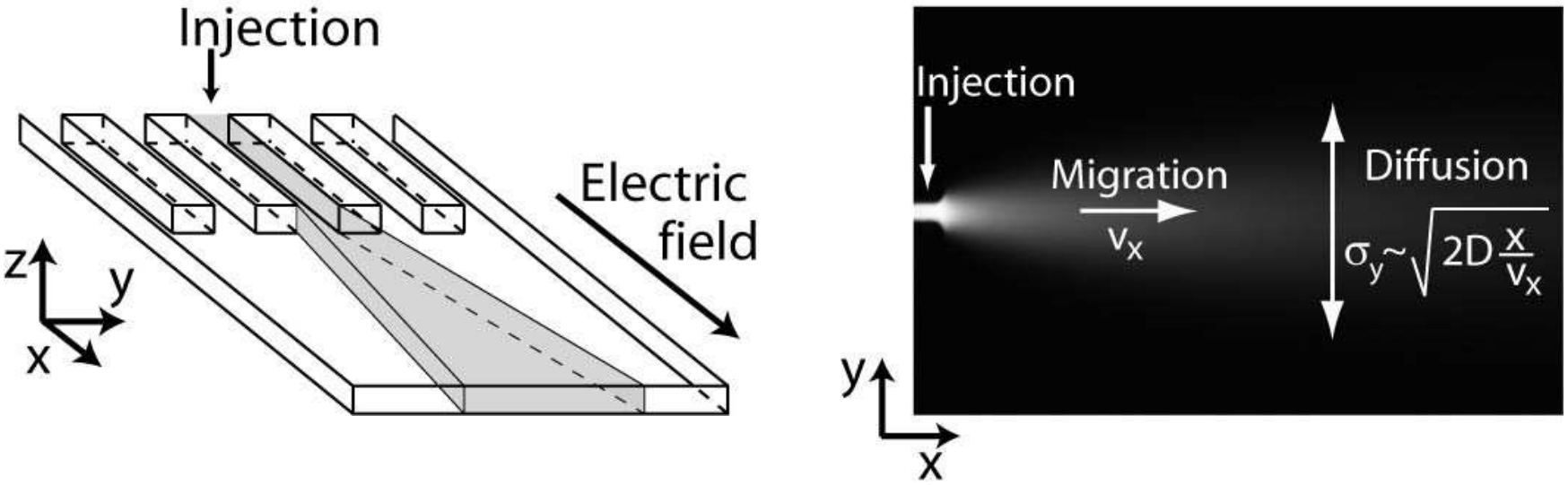
Observation set-up





Chip mounted on a thermostat

Stationary electrophoretic device

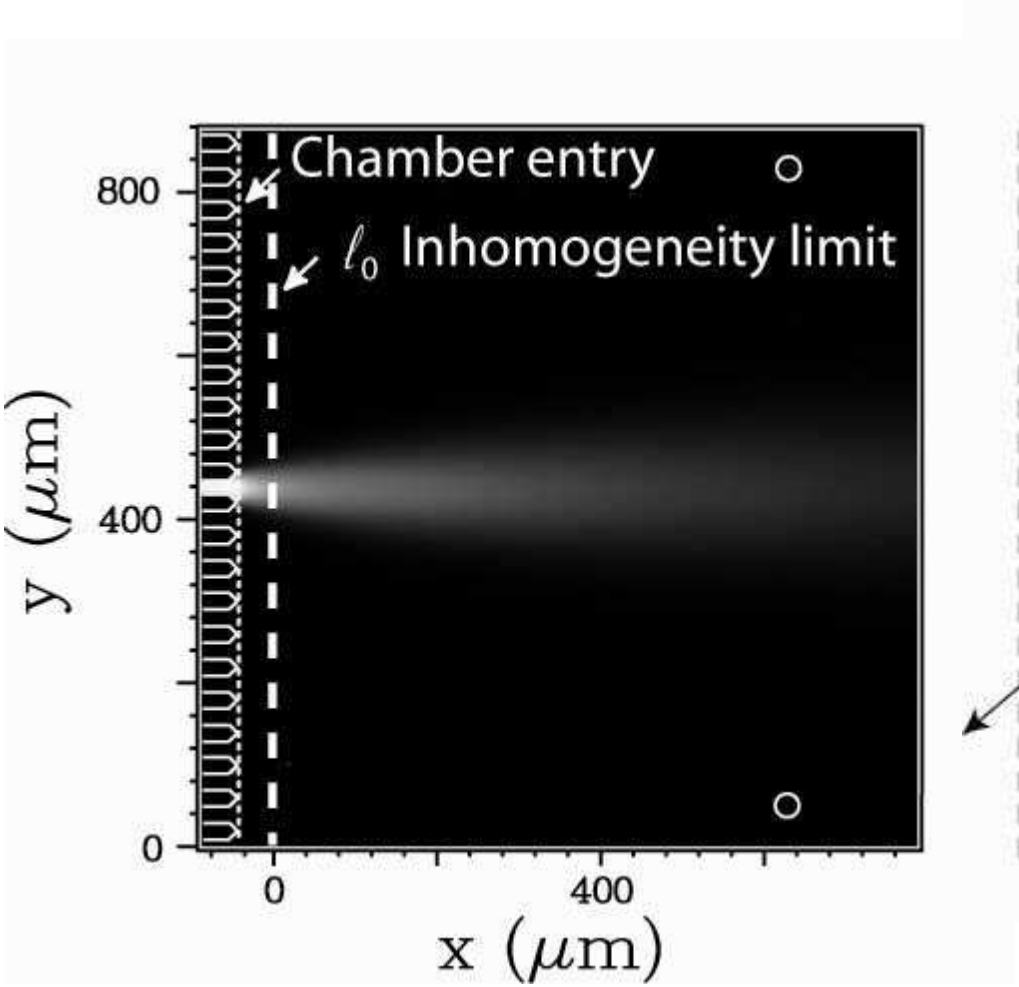


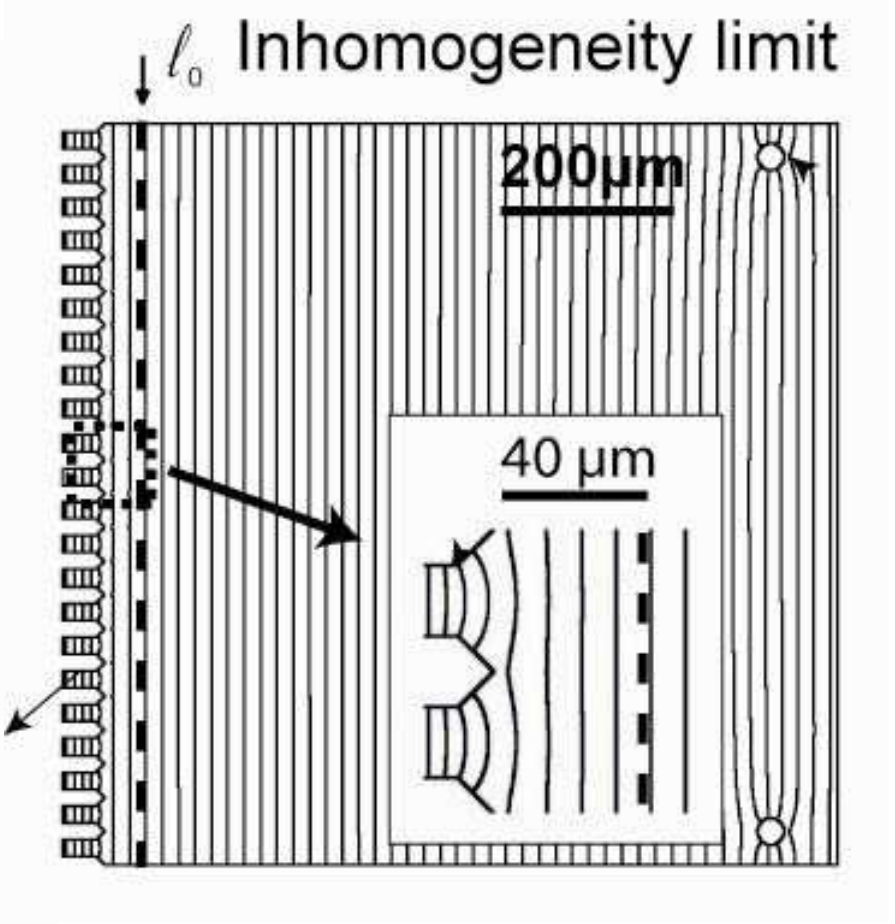
Velocity field homogeneity

$$\vec{v} = (\mu + \mu_{EO}(x, y, z))\vec{E}(x, y, z)$$

Electric field must be uniform

Electric field homogeneity



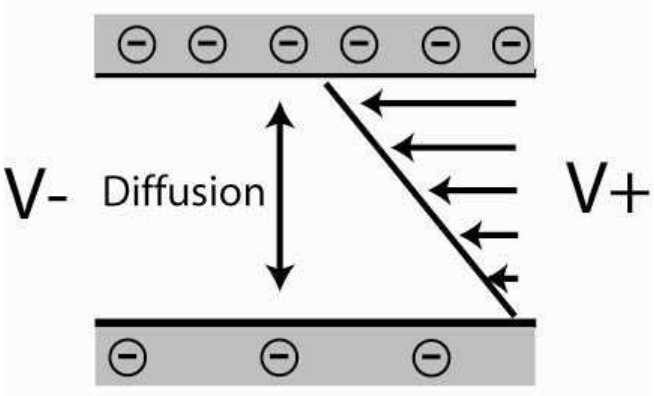


Simulations with Finite Element Methods

Electroosmosis homogeneity

$$\vec{v} = (\mu + \underbrace{\mu_{EO}(x, y, z)})\vec{E}$$

Heterogenous electroosmosis :

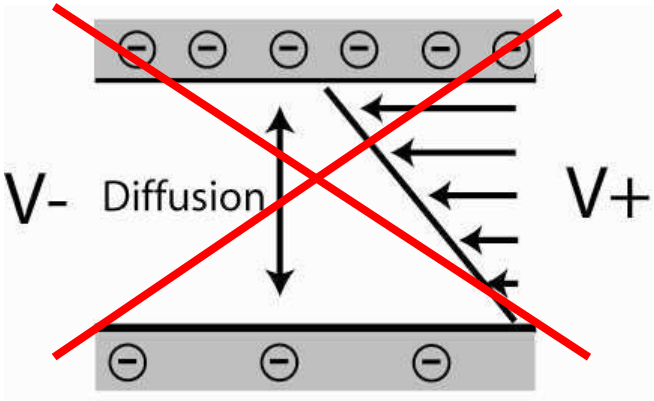


→Taylor dispersion

Electroosmosis homogeneity

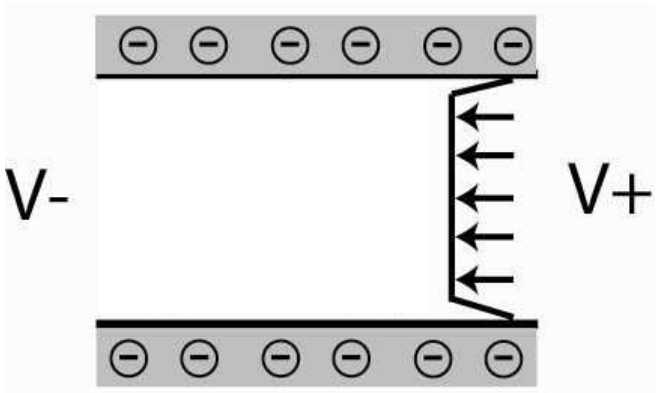
$$\vec{v} = (\mu + \underbrace{\mu_{EO}(x, y, z)}_{\downarrow})\vec{E}$$

Heterogenous electroosmosis :



→Taylor dispersion

Homogenous electroosmosis :



Electroosmosis must be controlled

Electroosmosis control medium

- 1% Agarose
  - Null hydrodynamic flow
  - But limited lifetime of the chip

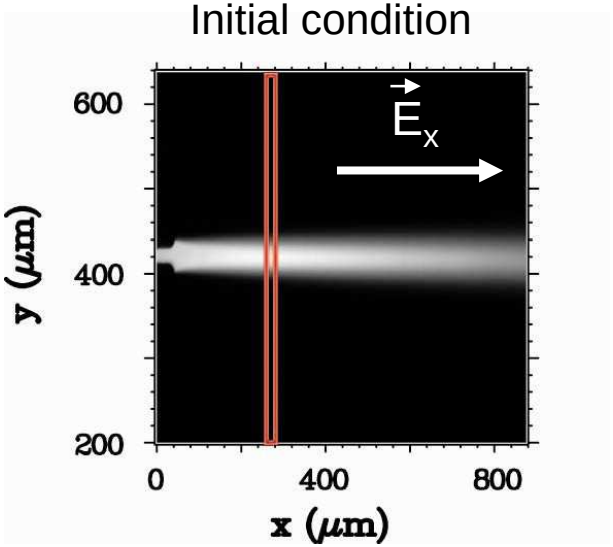
- 0.1% PDMA (polydimethylacrylamide):
  - Dynamic coating
  - Controlled electroosmosis

May allow to work with uncharged species

Taylor dispersion?

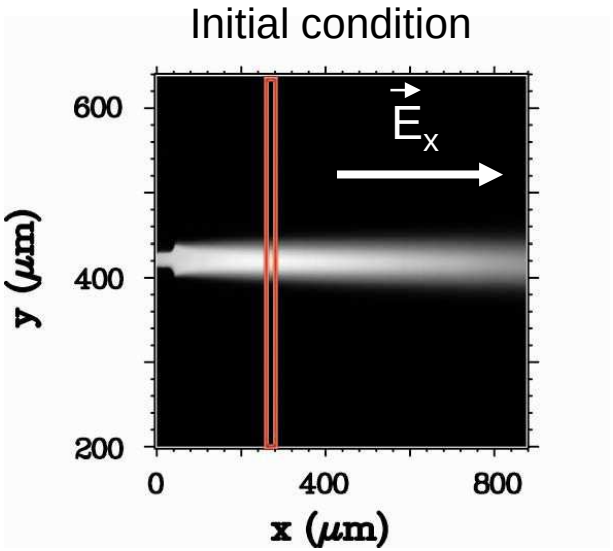
We designed a time-based control experiment:

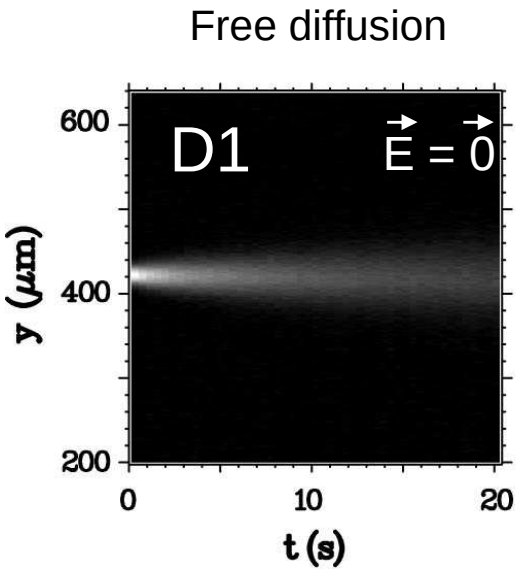
Initial condition

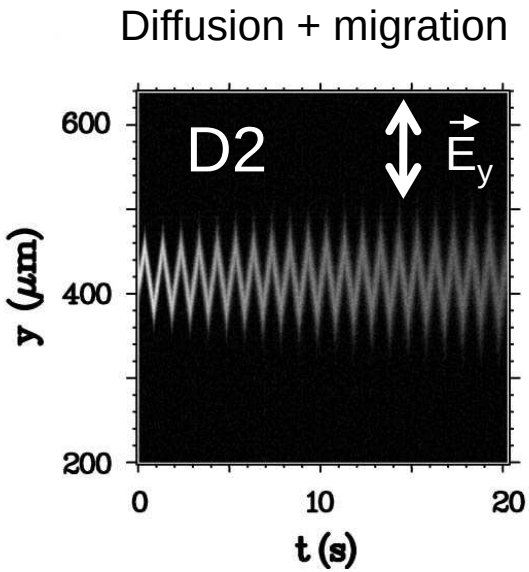


Taylor dispersion?

We designed a time-based control experiment:



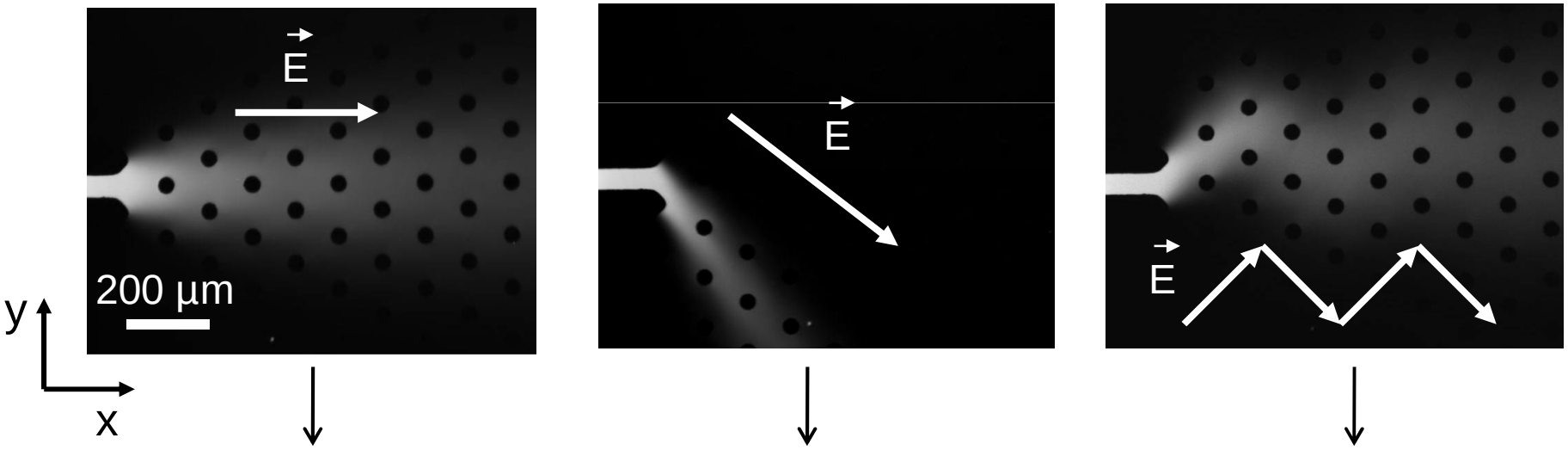




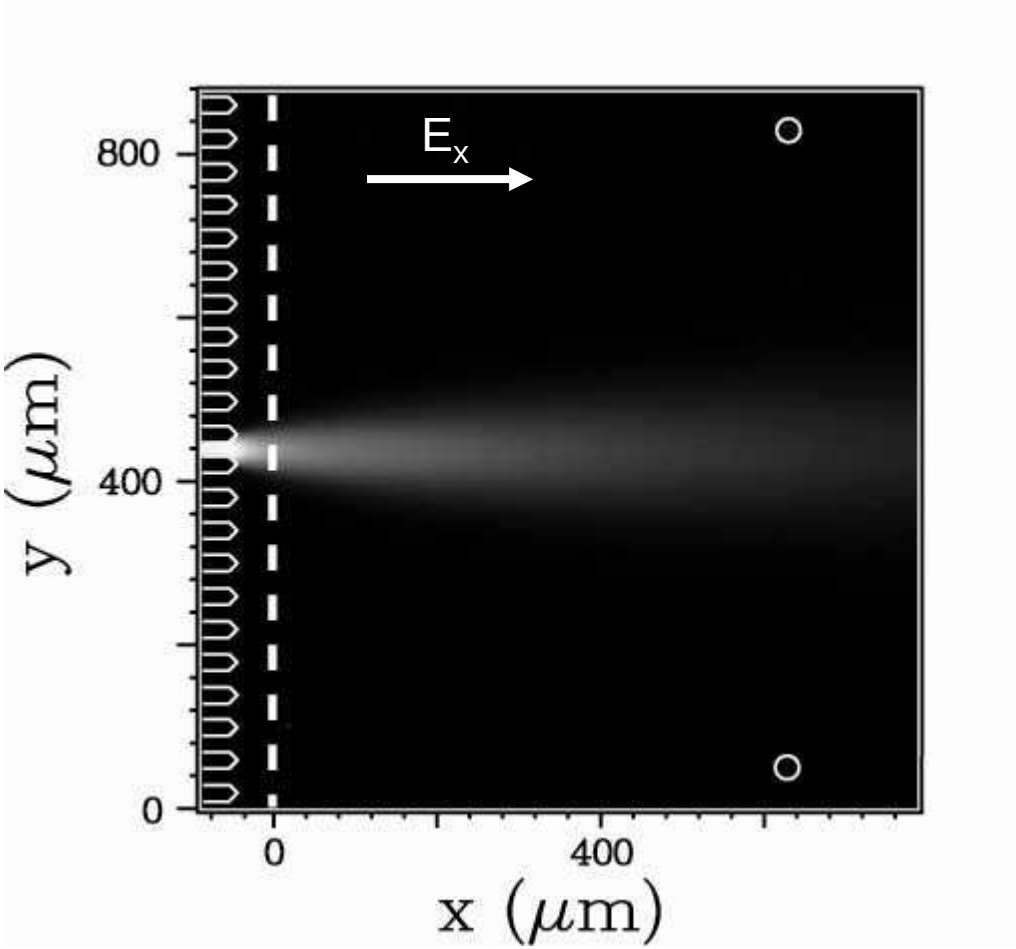
D1 = D2 ⟶ Total absence of Taylor dispersion

A functional 2D electrophoresis chip

Constant and alternative electric field in 2D



Diffusion analysis by Fourier Transform



$$\frac{\partial A}{\partial t} = D\frac{\partial^2 A}{\partial y^2}$$

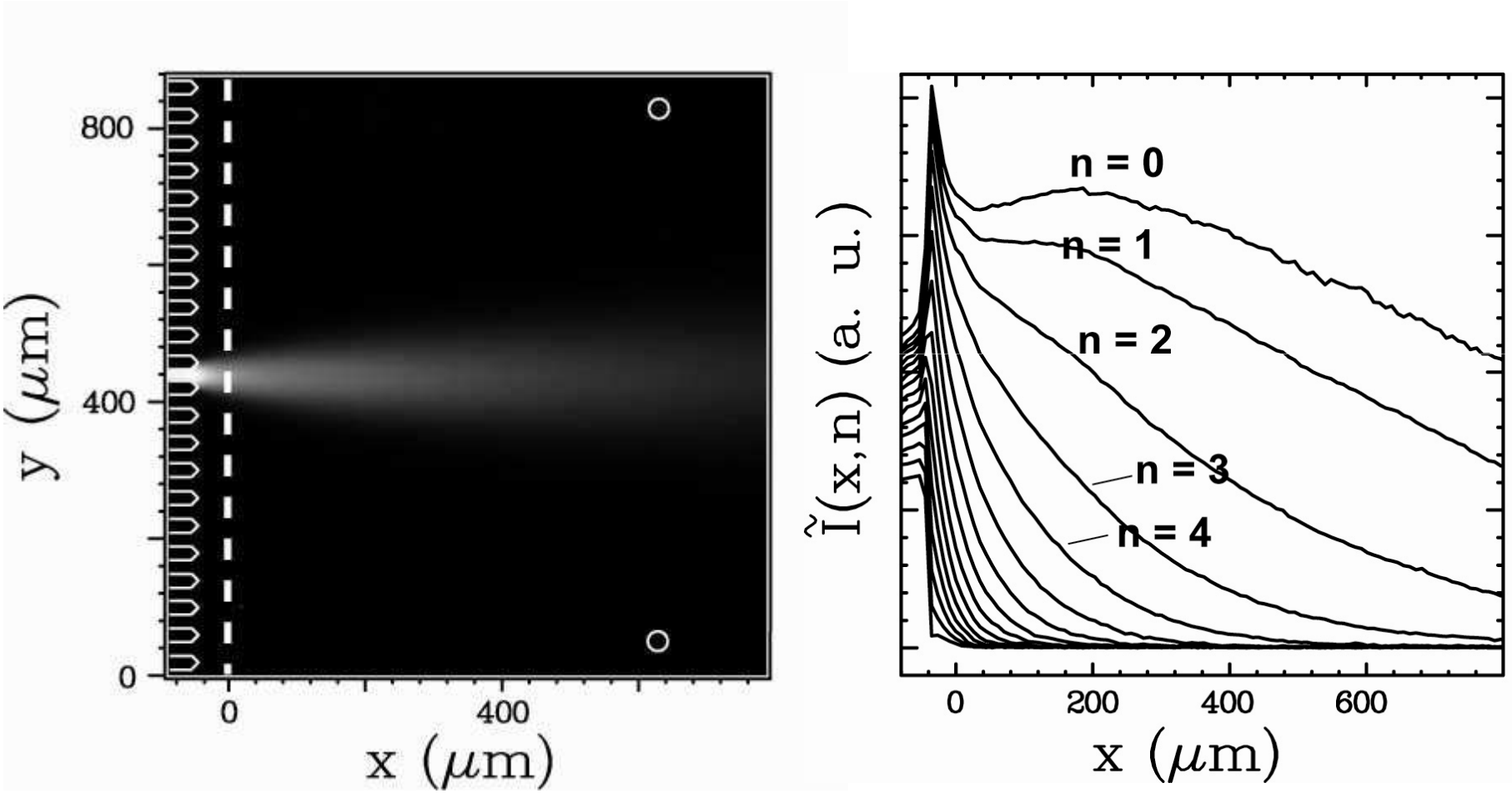
$$\frac{\partial \tilde{A}}{\partial t} = -Dq_n^2\tilde{A}$$

$$\tilde{A} = \tilde{A}_0 e^{-Dq_n^2 t}$$

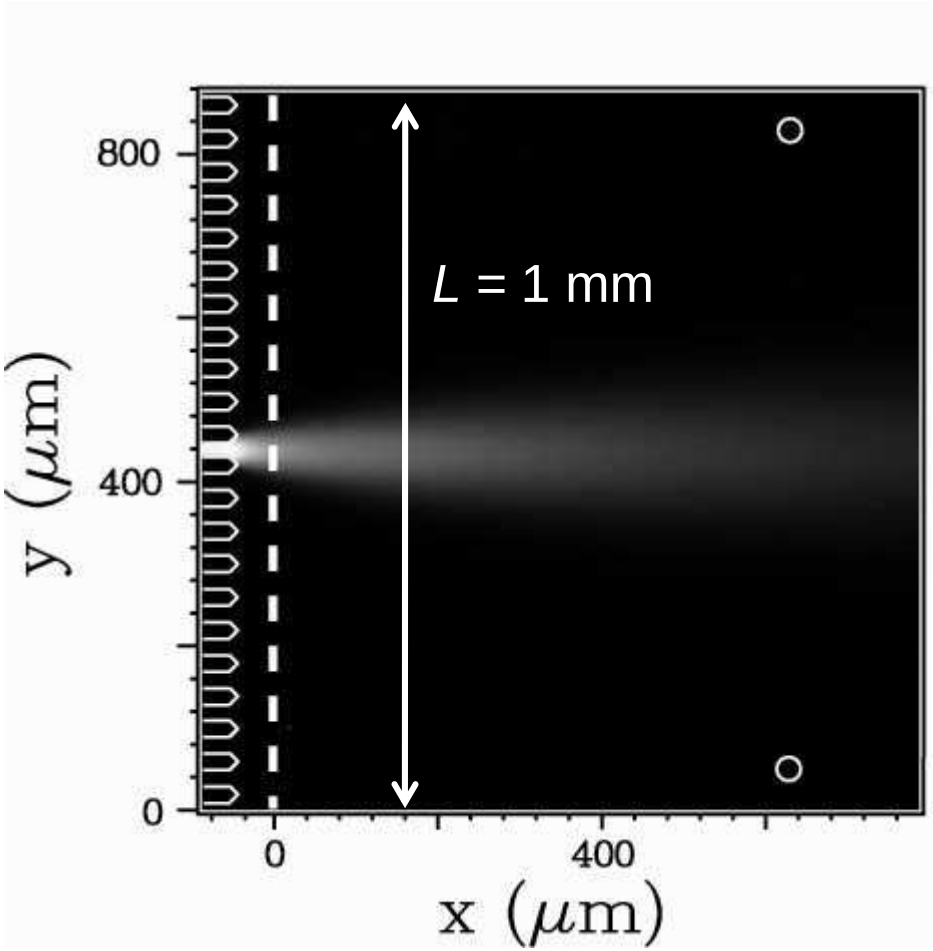
$$\tilde{A} = \tilde{A}_0 e^{-\frac{D}{vx}q_n^2 x}$$

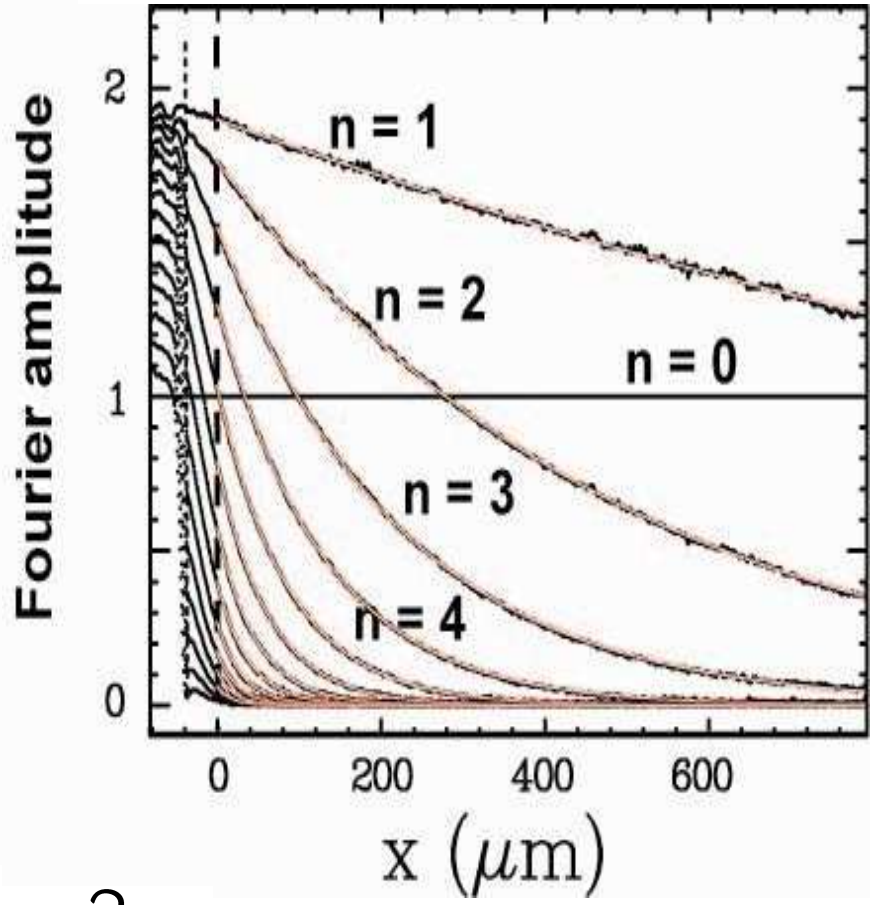
- Independent of initial condition
- Monoexponential fits

Before illumination correction



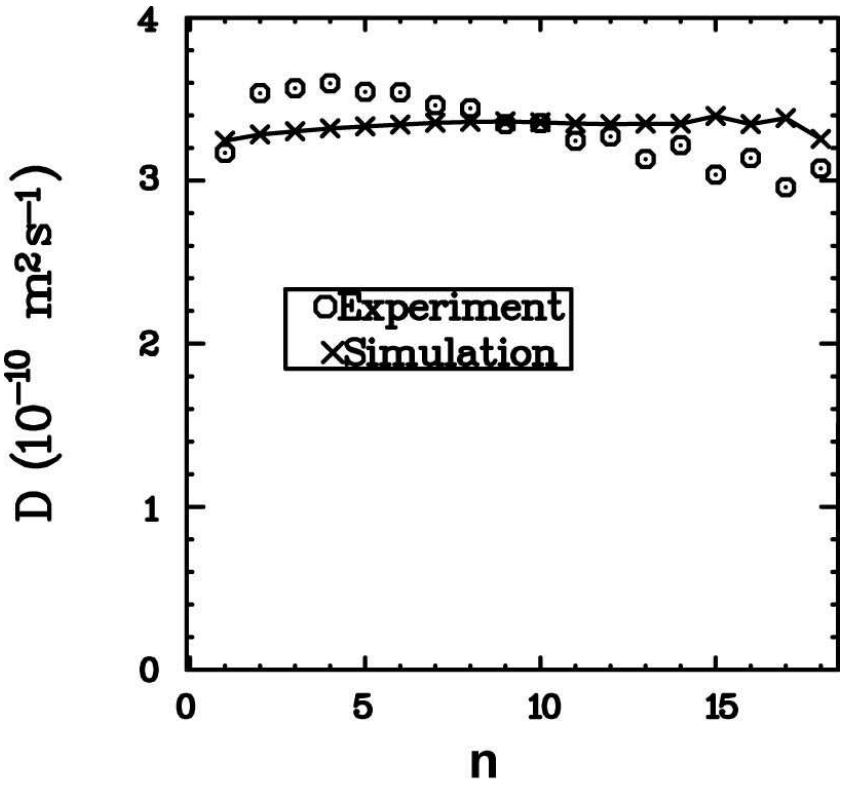
After illumination correction





spatial frequency — $q_n = \frac{2\pi n}{L}$ — Fourier mode

D independent of Fourier mode



Fourier mode is $[\mathrm{m}^{-1}]$

$$q_n = \frac{2\pi n}{L}$$

n	$(q_n)^{-1}$ $(\mu\mathrm{m})$
1	140
20	7

Fourier analysis is multiscale

Validation of D measurement

D (10^{-12} m^2 s^{-1})

	Fluorescein	ssDNA (105 bases)	dsDNA (1200 bp)
Stationary	310 ± 24	39 ± 4	3.8 ± 0.7
Non-stationary	320 ± 20	40 ± 2	4.2 ± 0.3
FCS	350 ± 70	44 ± 5	–

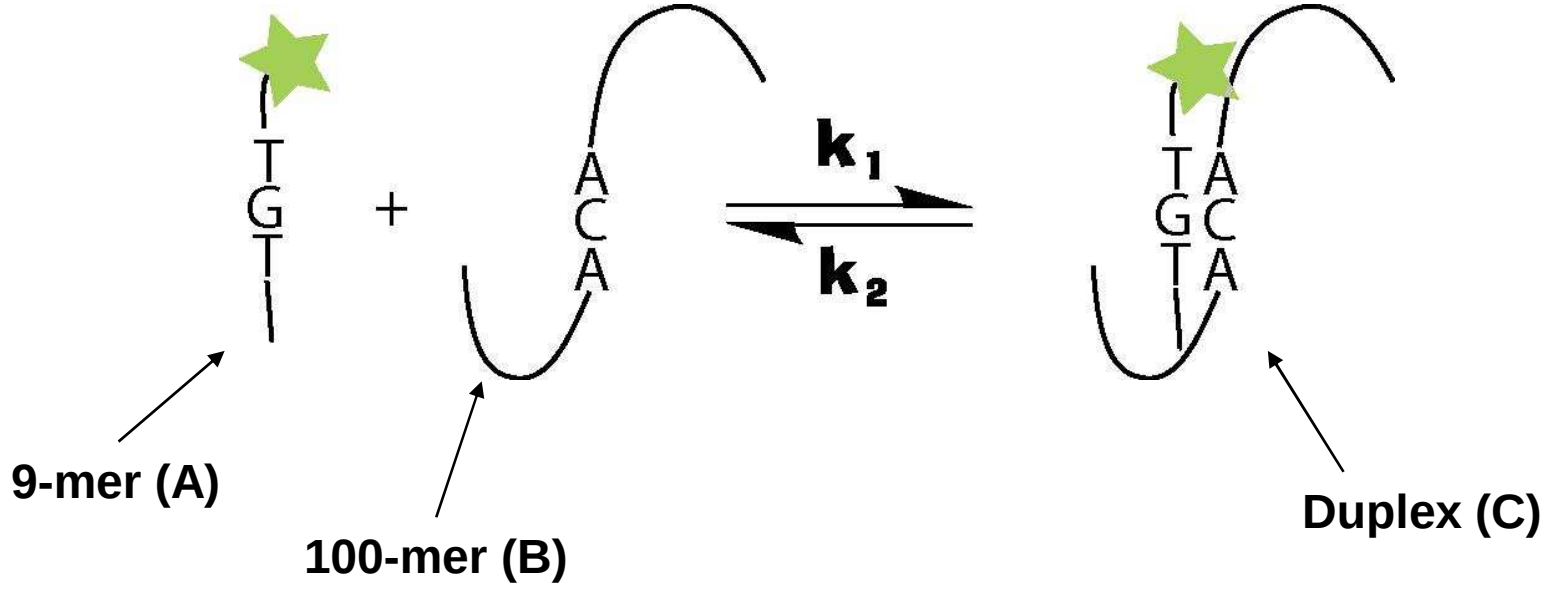
Large M_w range: 10^2-10^6 g mol^{-1}

A. Estévez-Torres *et al Anal. Chem.* submitted

Outline

1. DNA hybridization reaction
2. A functional microlaboratory
3. A powerful tool to analyze dynamics

Dynamics of a reacting mixture

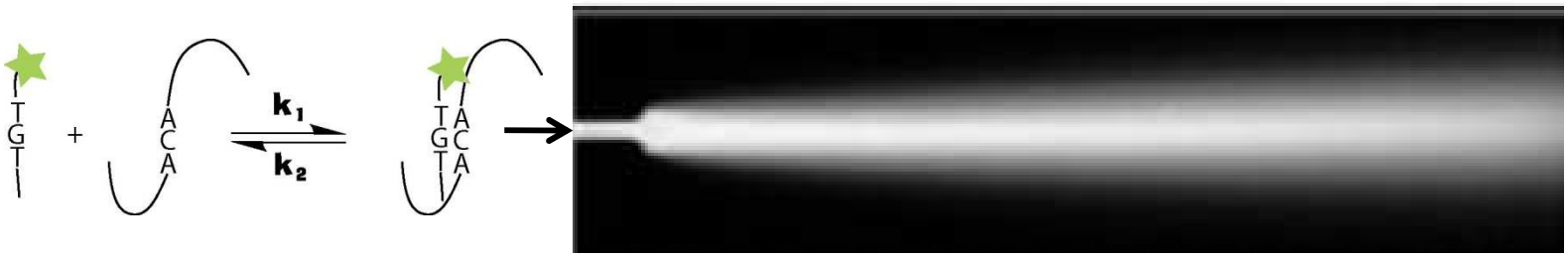


$$\tau = \frac{1}{k_1[B] + k_2}$$

characteristic time
of the reaction

Finding the good time window

$$t = x/v_x$$



$t \ll \tau$

2 non-reactive species : A and C

THERMODYNAMICS

$t \sim \tau$

2 reactive species : A ⇆ C

KINETICS

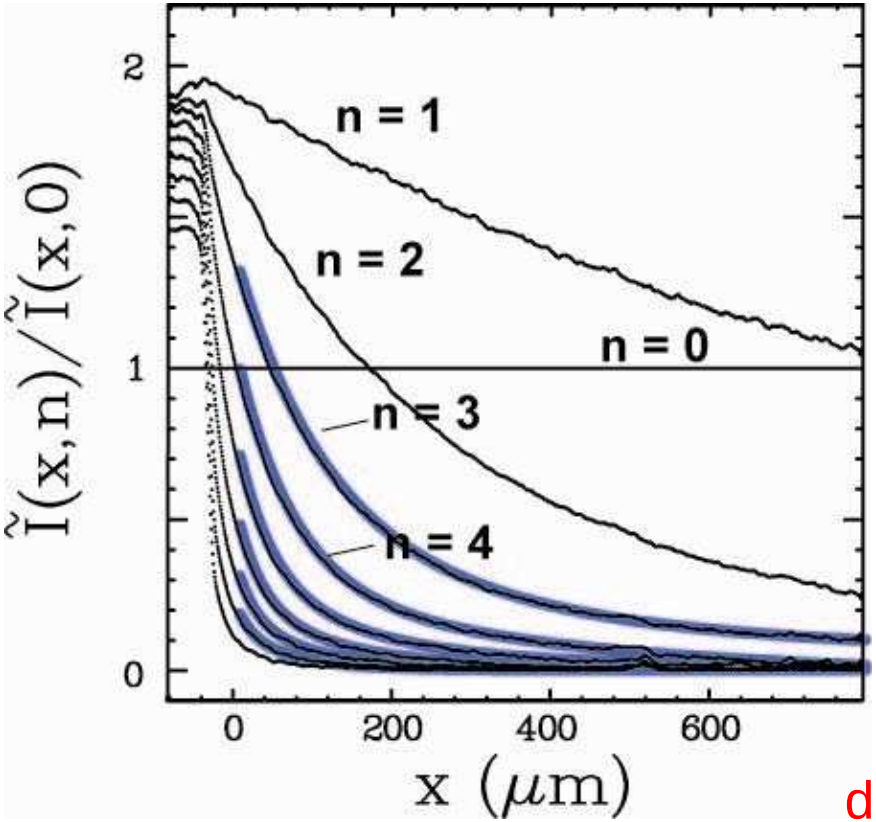
$t \gg \tau$

1 average species : {A, C}

THERMODYNAMICS

Analysis of a binary mixture

$$\frac{\tilde{I}(x,n)}{\tilde{I}(x,0)} = a_1(n)e^{-l_1(n)x} + a_2(n)e^{-l_2(n)x}$$

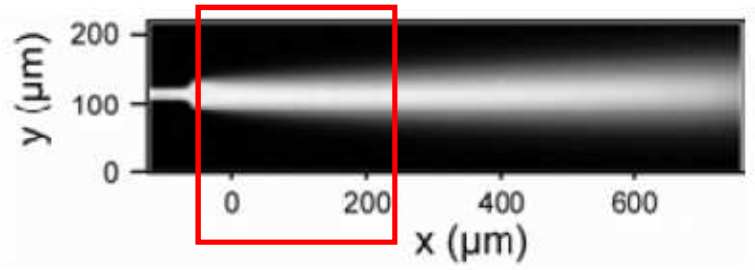


$l_1(n)$
$l_2(n)$
→ dynamics (diffusion + kinetics)

$a_1(n)$
$a_2(n)$
→ concentrations

Fourier analysis + microfluidics
decouples diffusion/kinetics/concentrations

Thermodynamic library screening

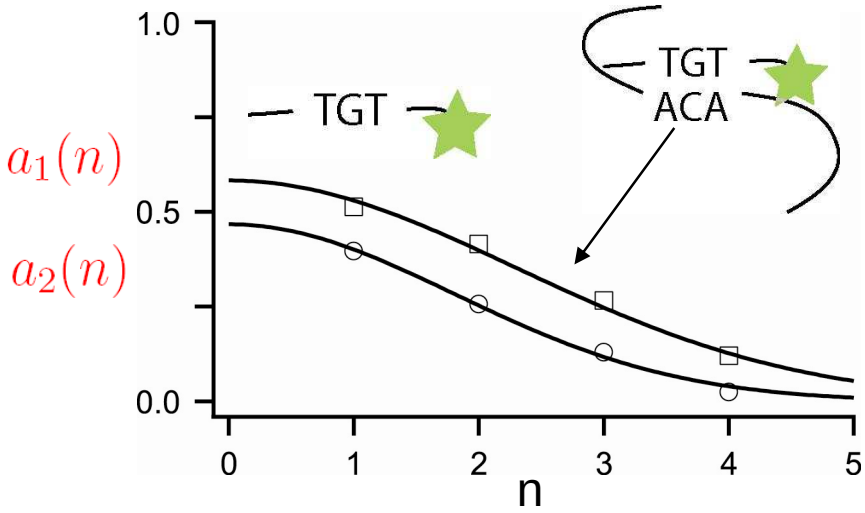


$t \ll \tau$

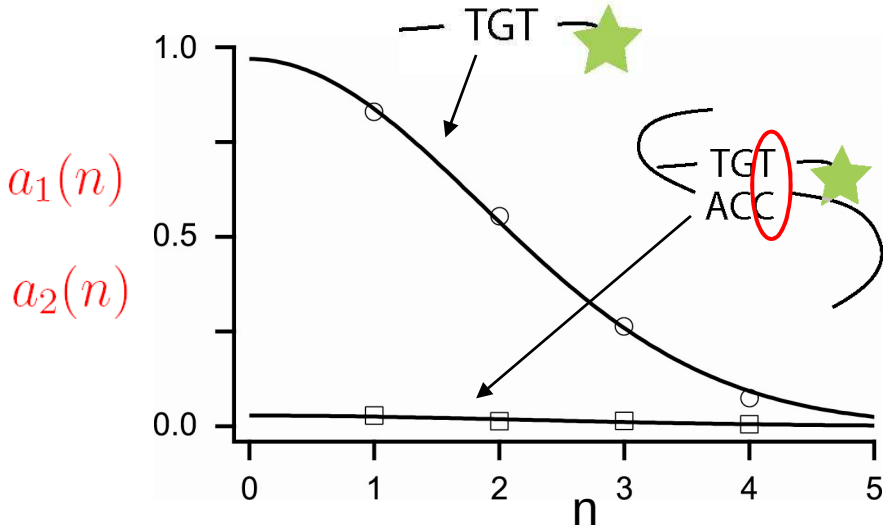
SNP detection

-20 seconds
-1 pmol
- Without matrix

Wild type



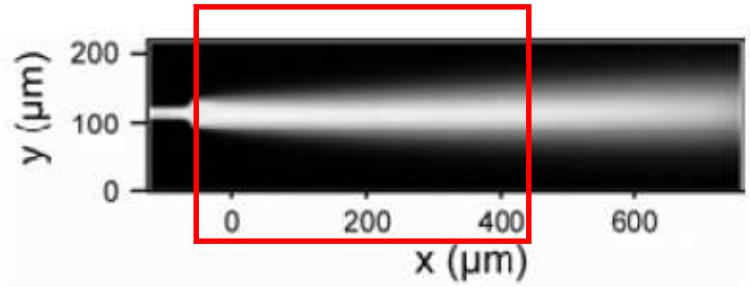
Mutant



Thermodynamic selectivity but very robust observable

A. Estévez-Torres *et al*. *Proc. µTAS* **2007**

Measuring kinetics



$$t \sim \tau$$

	k_1 (10^5 $M^{-1}s^{-1}$)	k_2 (s^{-1})	K (10^5)
On chip measurements	1.2 ± 0.3	0.33 ± 0.05	4 ± 1
Independent measurements	1.9 ± 0.1	0.38 ± 0.01	3.4 ± 0.6

Good understanding of the physical phenomenon

A. Estévez-Torres *et al*. *JACS*, submitted

Conclusion

An interesting concept

Electrodiffusion

+

A biotechnological issue

SNP detection in DNA

1. A chemical system with controllable dynamics

- An oligonucleotide database with controlled k_1, k_2
- An easy-to-use mobility reduction strategy (cholesteryl-triton)
- A quadruplex molecular beacon tunes k_1 with sequence

Conclusion

An interesting concept

Electrodiffusion

+

A biotechnological issue

SNP detection in DNA

1. A chemical system with controllable dynamics

2. A versatile microlaboratory

- Electric fields in 2D
- Thermostated
- Electroosmosis control

Conclusion

An interesting concept

Electrodiffusion

+

A biotechnological issue

SNP detection in DNA

1. A chemical system with controllable dynamics

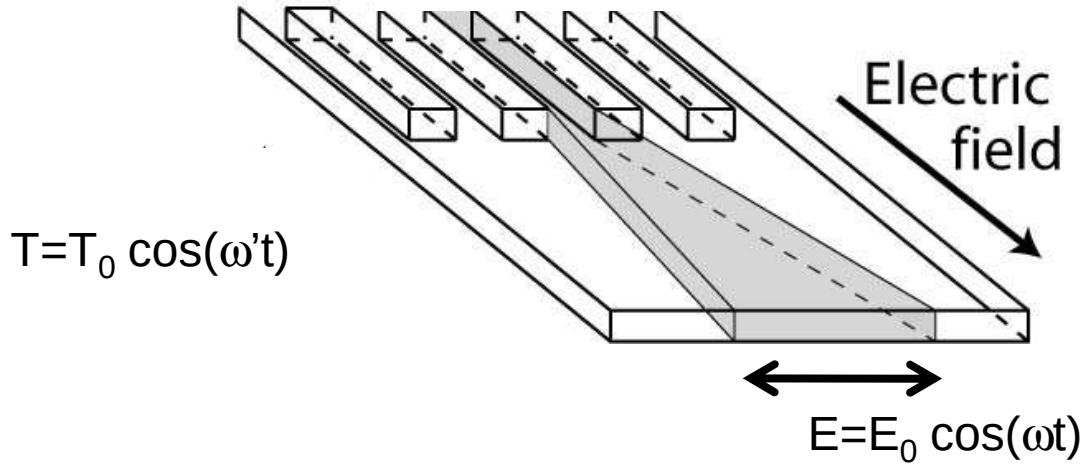
2. A versatile microlaboratory

3. A powerful analysis to measure dynamics: Fourier transform

- D and μ
- k_1, k_2 and K

Perspectives

- Electrodiffusion experiment ready to be performed



- Electric field + temperature modulation ⟶ oriented motion

(Thomas Barilero)

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