DNA separation

- Introduction to separation science
- Conventional gel electrophoresis
- Conventional pulsed-field gel electrophoresis
- Capillary electrophoresis
- Advanced DNA separation techniques

(On-chip electrophoresis and pulsed-field electrophoresis, DNA prism, etc.)

References:

- JC Giddings, *"Unified Separation Science"* (Wiley-Interscience, New York, 1991)
- *Electrophoresis* (Journal)

The big column for separation…

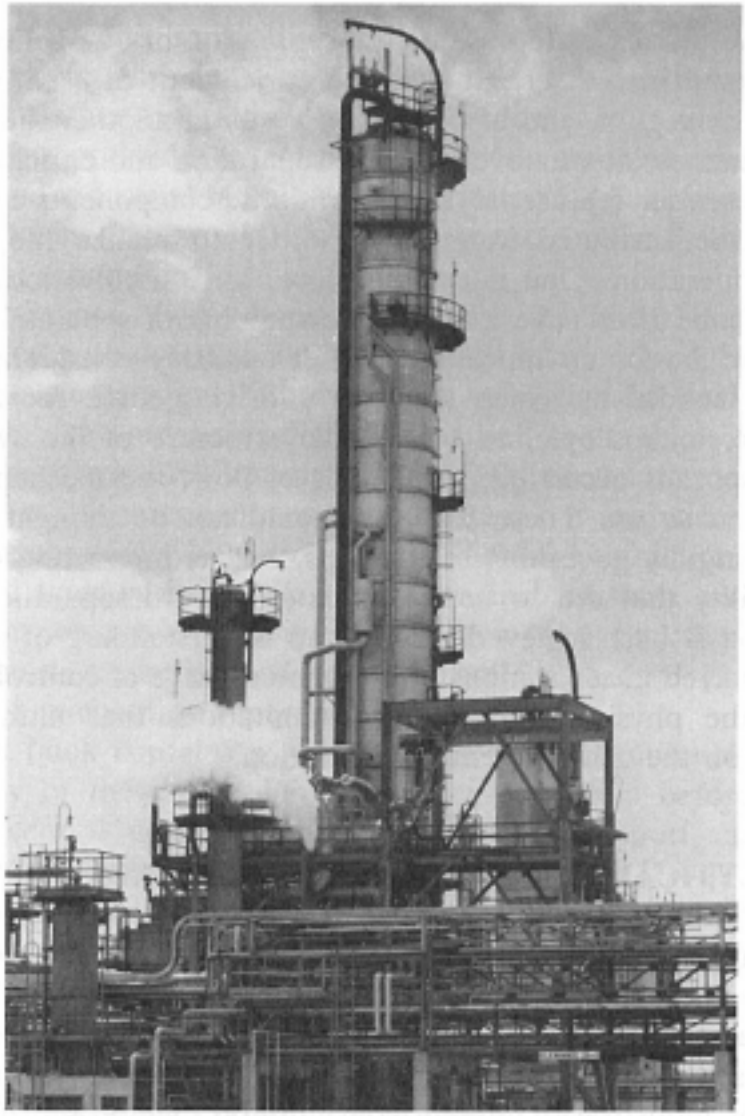


Figure 1.1. Crude fractionation tower at the Phillips Oil Refinery in Woods Cross, Utah. In this crude fractionator, 25,000 barrels per day of incoming crude oil is divided into six fractions by distillation. (Photo by Alexis Kelner.)

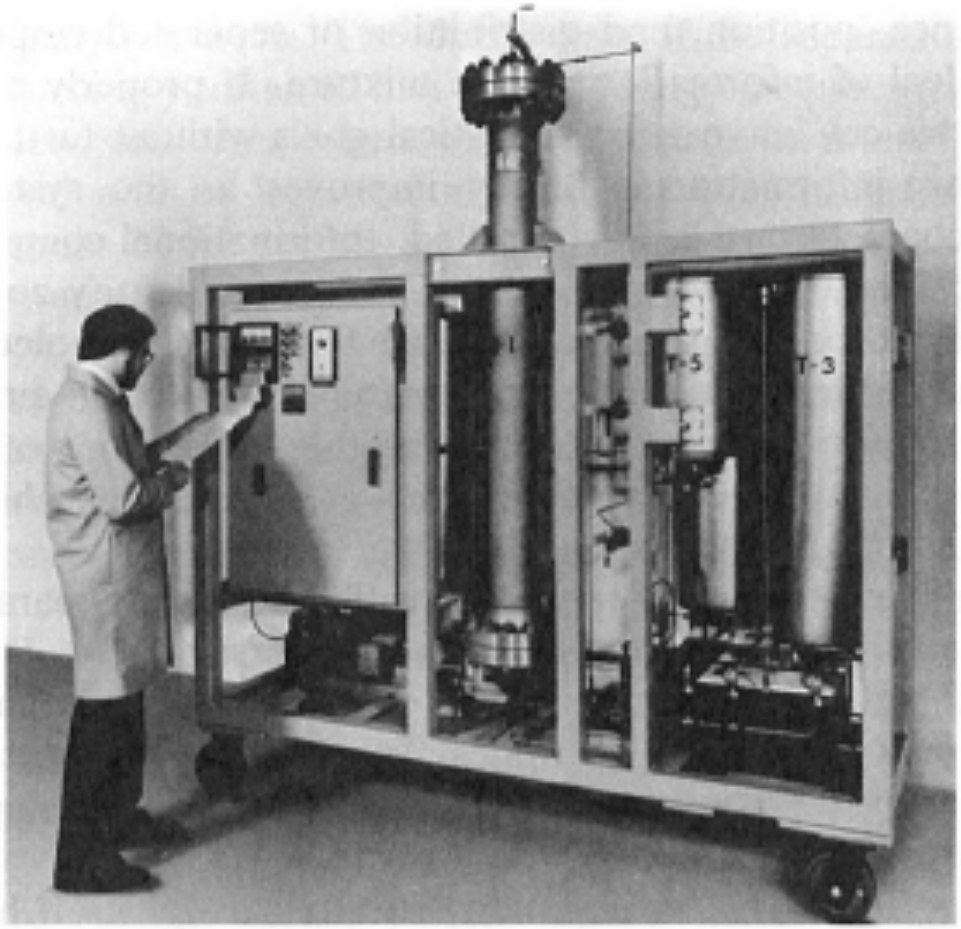
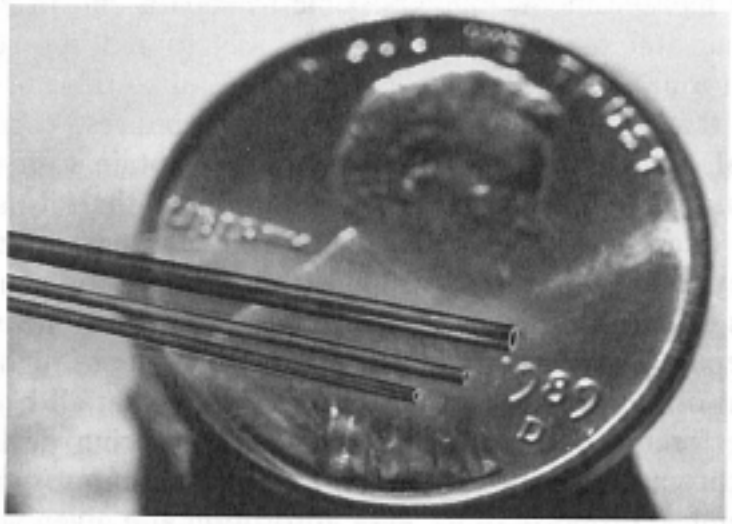


Figure 1.2. Preparative scale liquid chromatography unit with 6 ft (1.8 m) long column (in center) having an inner diameter of 6 in (15 cm). Up to 2 L of sample solution can be injected and processed in a single 90-min run. (Courtesy Thomas J. Filipi and Whatman Chemical Separation Division.)



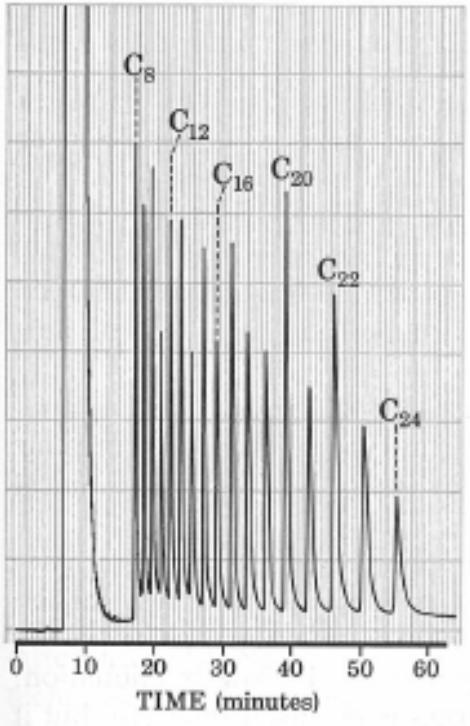
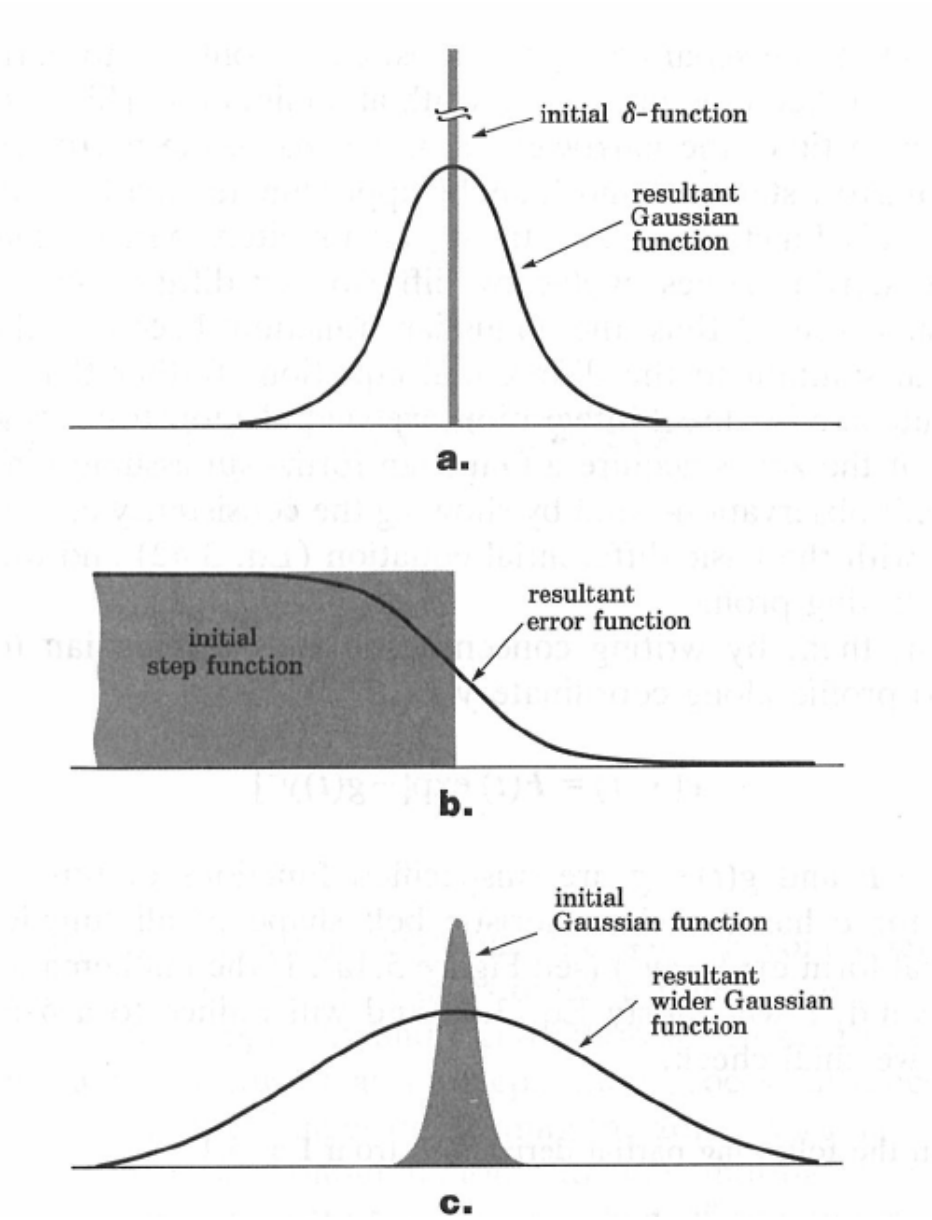


Figure 1.4. High-resolution separation of free fatty acids of indicated carbon numbers by supercritical fluid chromatography using a compressed CO_2 mobile phase at 170°C. Column is 50 cm long fused silica capillary tube of 250 μm internal diameter. (Courtesy of Frank J. Yang.)

Concentration (band) broadening by diffusion



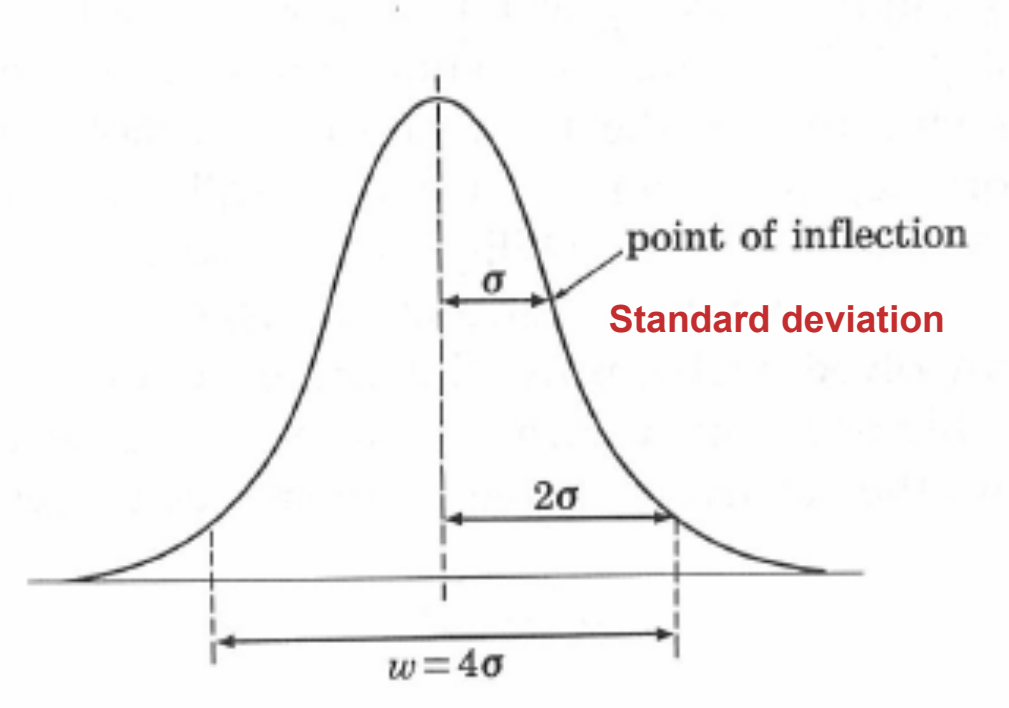
Normalized Gaussian Profile

$$c = \frac{1}{(4\pi Dt)^{1/2}} \exp\left(\frac{-y^2}{4Dt}\right)$$

$$c = \frac{n}{(4\pi Dt)^{1/2}} \exp\left(-\frac{y^2}{4Dt}\right)$$

$$\sigma^2 = 2Dt$$

$$\sigma = (2Dt)^{1/2}$$



Effective zone width

$$X = v\,t$$

$$\sigma^2 = (2D/v)\,X$$

Plate height: H

$$\sigma^2 = H\,X$$

$$H = \sigma^2 / X$$

Theoretical plates: N = X / H

Resolution

$$R_s = \frac{\Delta X}{4\sigma} \qquad \sigma = (HX)^{1/2}$$

$$R_s = \frac{\Delta v}{v}\left(\frac{X}{16H}\right)^{1/2} = \frac{\Delta v}{v}\left(\frac{N}{16}\right)^{1/2}$$

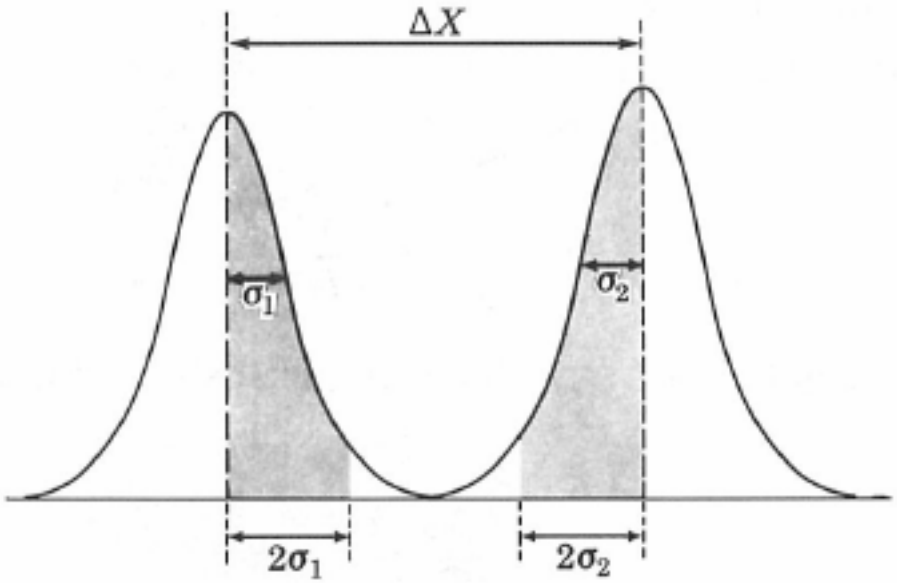


Figure 5.7. The resolution of two peaks, defined as $R_s = \Delta X/(2\sigma_1 + 2\sigma_2)$, measures peak separation ΔX relative to the zone dispersion ($2\sigma_1$ and $2\sigma_2$) leading to overlap and contamination.

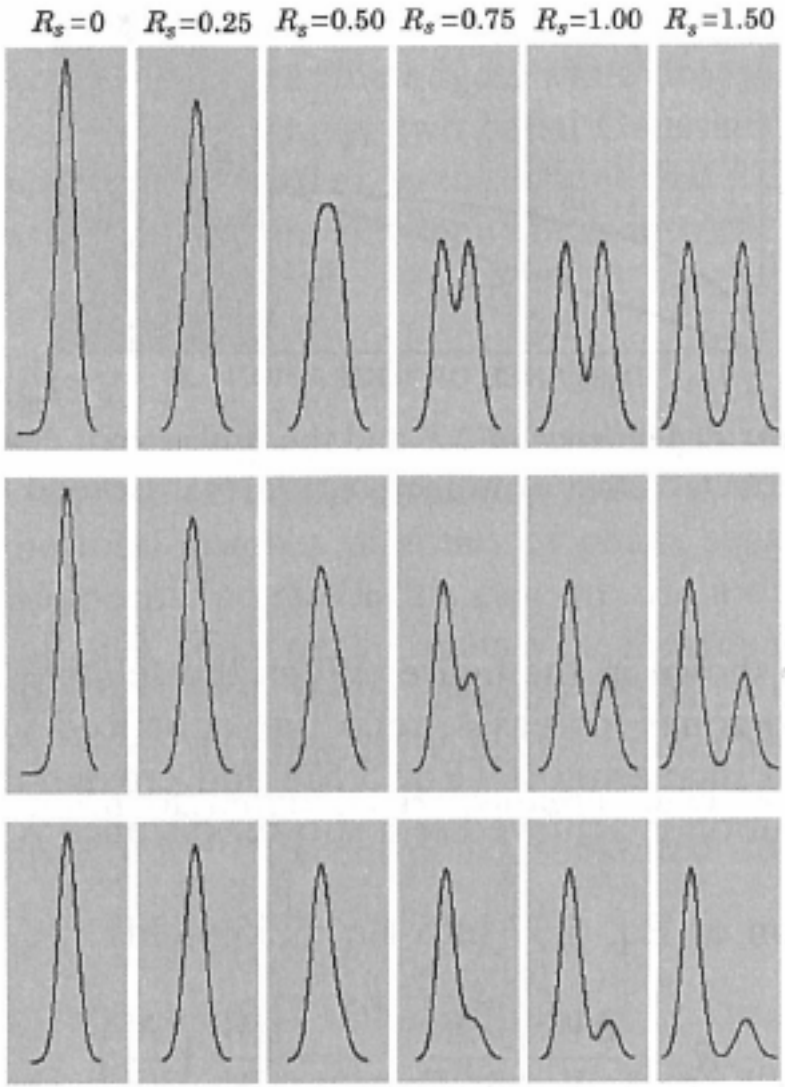
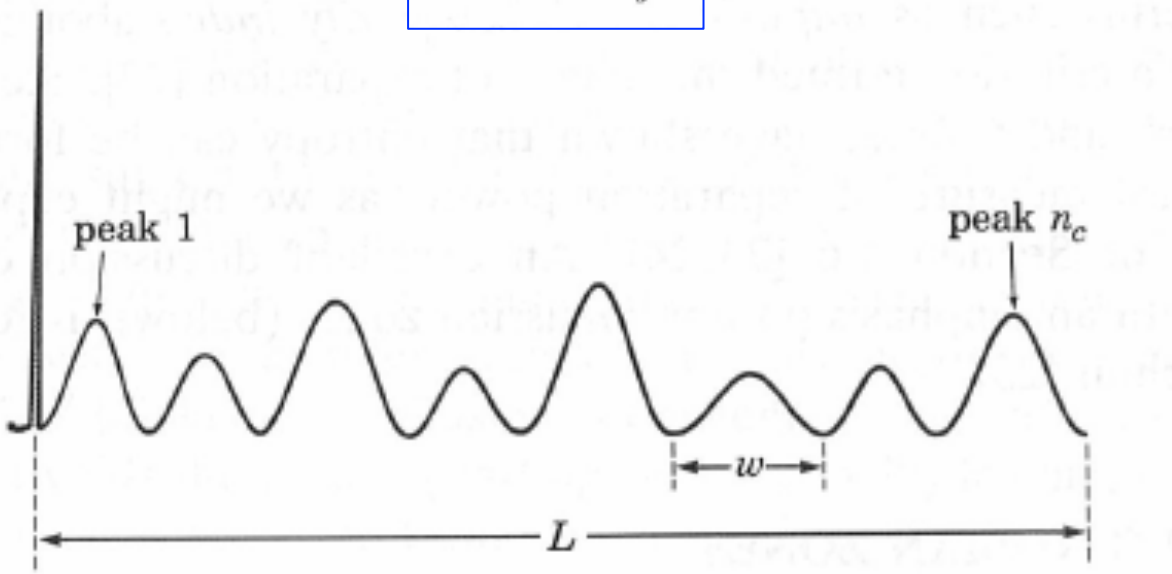


Figure 5.8. Left to right sequence gives the profile of a pair of Gaussian zones of equal σ at increasing levels of resolution, as shown. Top row shows this sequence for two zones of equal peak height; middle and bottom rows show the sequence for 2:1 and 5:1 peak height ratios (profiles courtesy of Joe M. Davis).

Peak capacity: $n_c = L/w = L/4\sigma$ (at Rs = 1)

the max number of separated peaks that can be fit (with adjacent peaks at some specified Rs value) into the path length.

$$n_c = \frac{L}{4\sigma R_s}$$



Theoretical plates: N = L / H

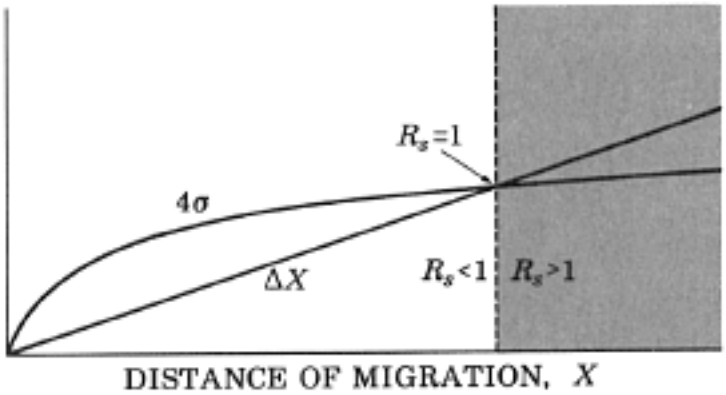


Figure 5.9. The linear dependence of ΔX and the square root dependence of 4σ on X lead to their inevitable crossing, at which point $R_s = 1$. Beyond the crossing (shaded area), $R_s > 1$.

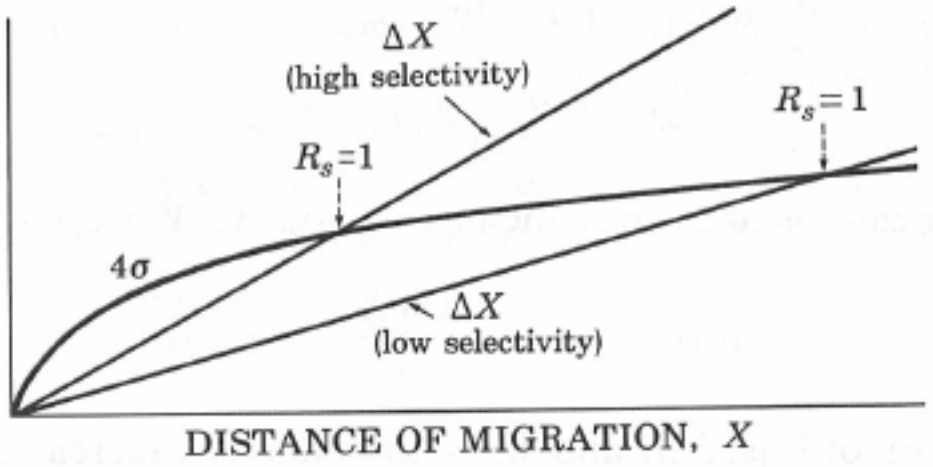
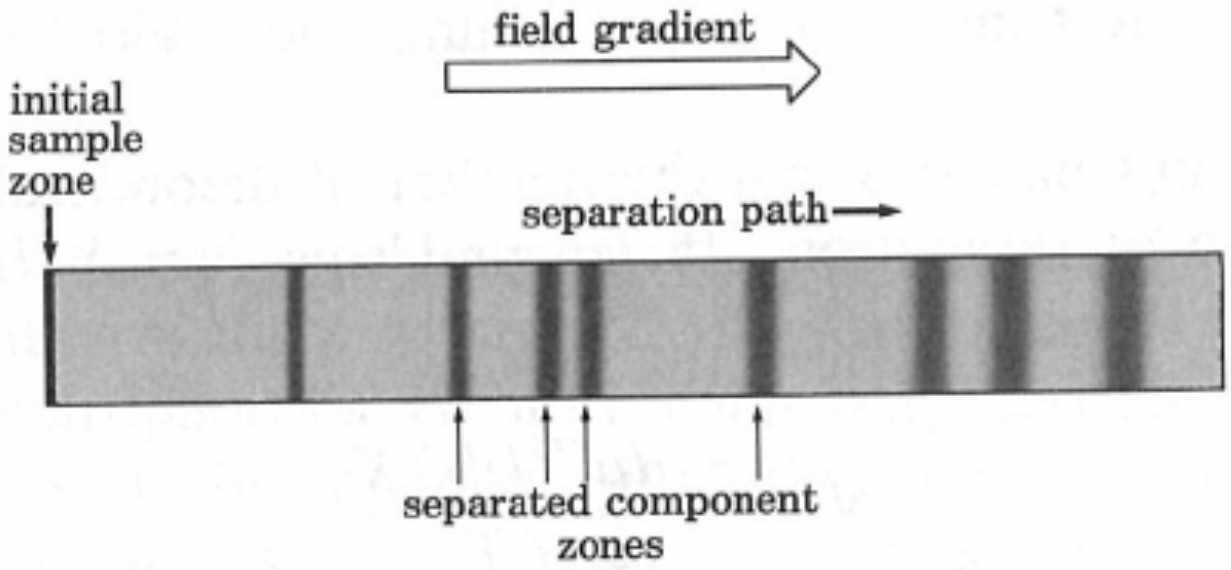


TABLE 8.1 Types of "Fields" that Cause Relative Displacement and thus (potentially at least) Some Degree of Relative Separation[a]

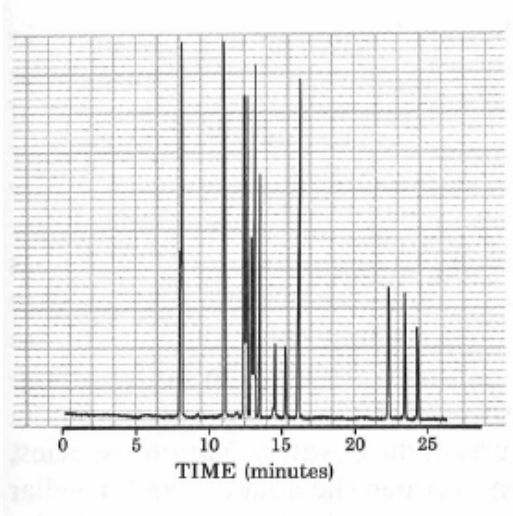
Field or Gradient	Static (*Sc*) Separation Methods or Phenomena	Other (non-*Sc*) Separation Methods
1. Electrical	Electrophoresis, isoelectric focusing, isotachophoresis	Electrodialysis, electrodeposition, electrostatic precipitation, countercurrent electrophoresis, electrical FFF[b]
2. Sedimentation	Rate-zonal sedimentation, isopycnic sedimentation	Equilibrium sedimentation, centrifugal elutriation, sedimentation FFF[b]
3. Temperature gradient	Thermophoresis	Thermogravitational methods, thermal FFF[b]
4. Electrical (nonuniform)	Dielectrophoresis	
5. Magnetic (nonuniform)	Magnetophoresis	Mass spectroscopy[c], magnetic separations, magnetic FFF[a]
6. em radiation	Photophoresis	
7. Concentration gradient	Diffusophoresis	

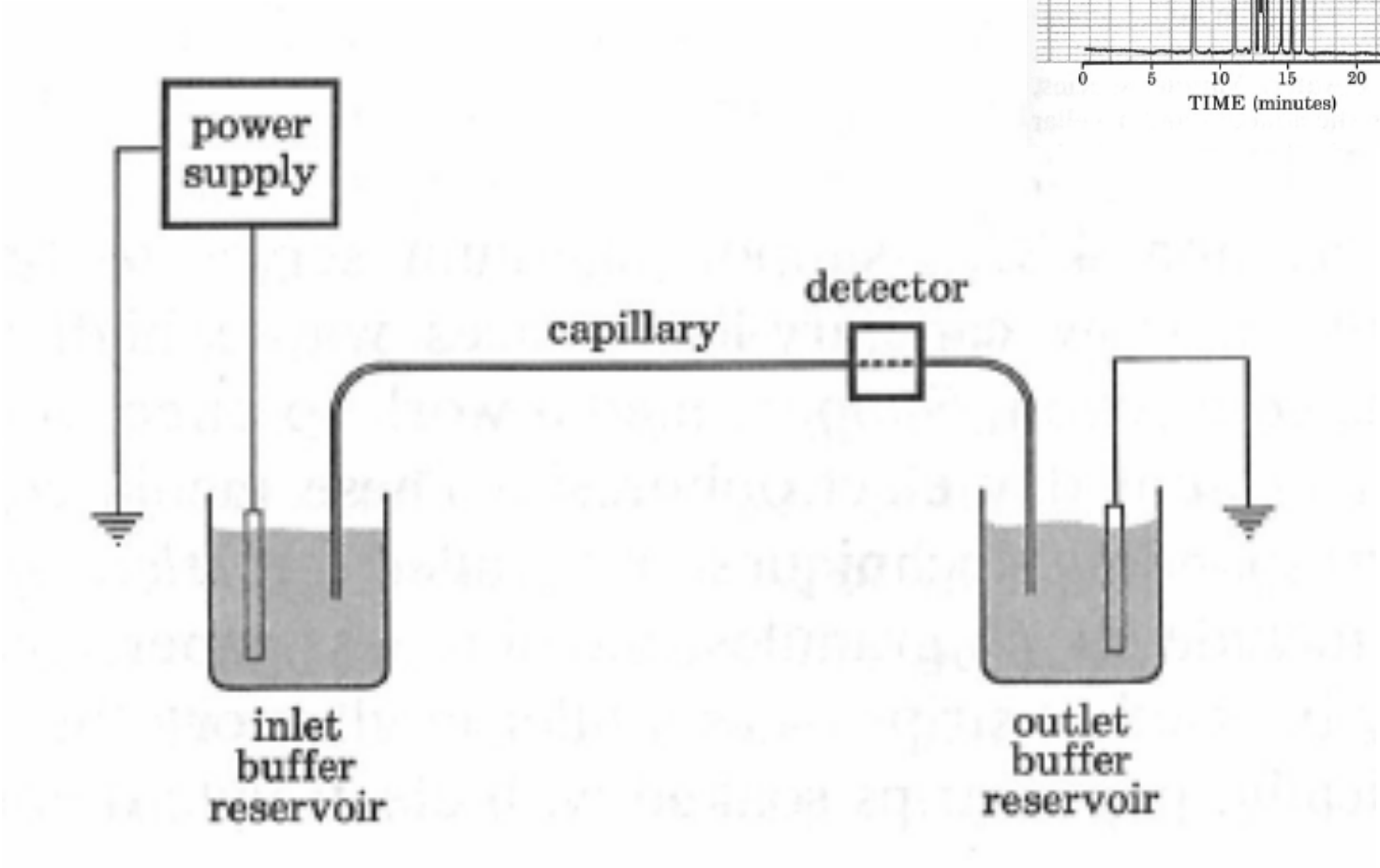
[a]Principal separation techniques, if they exist, are listed for each field.
[b]FFF = field-flow fractionation
[c]Inertial transport term important

Electrophoresis

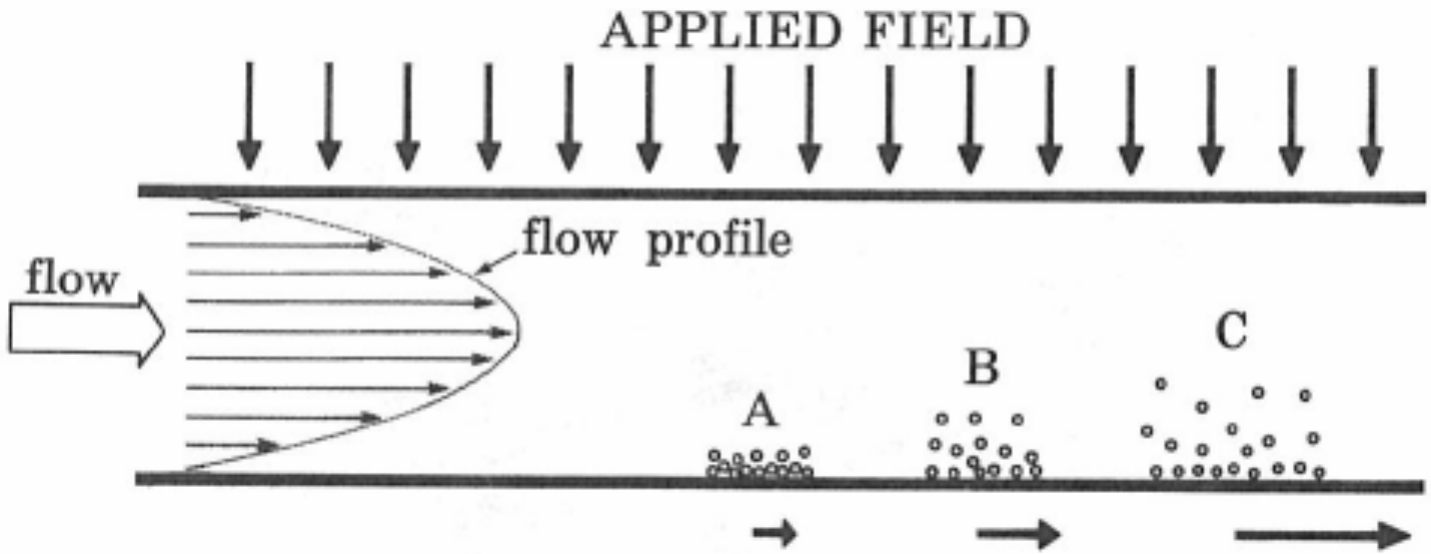


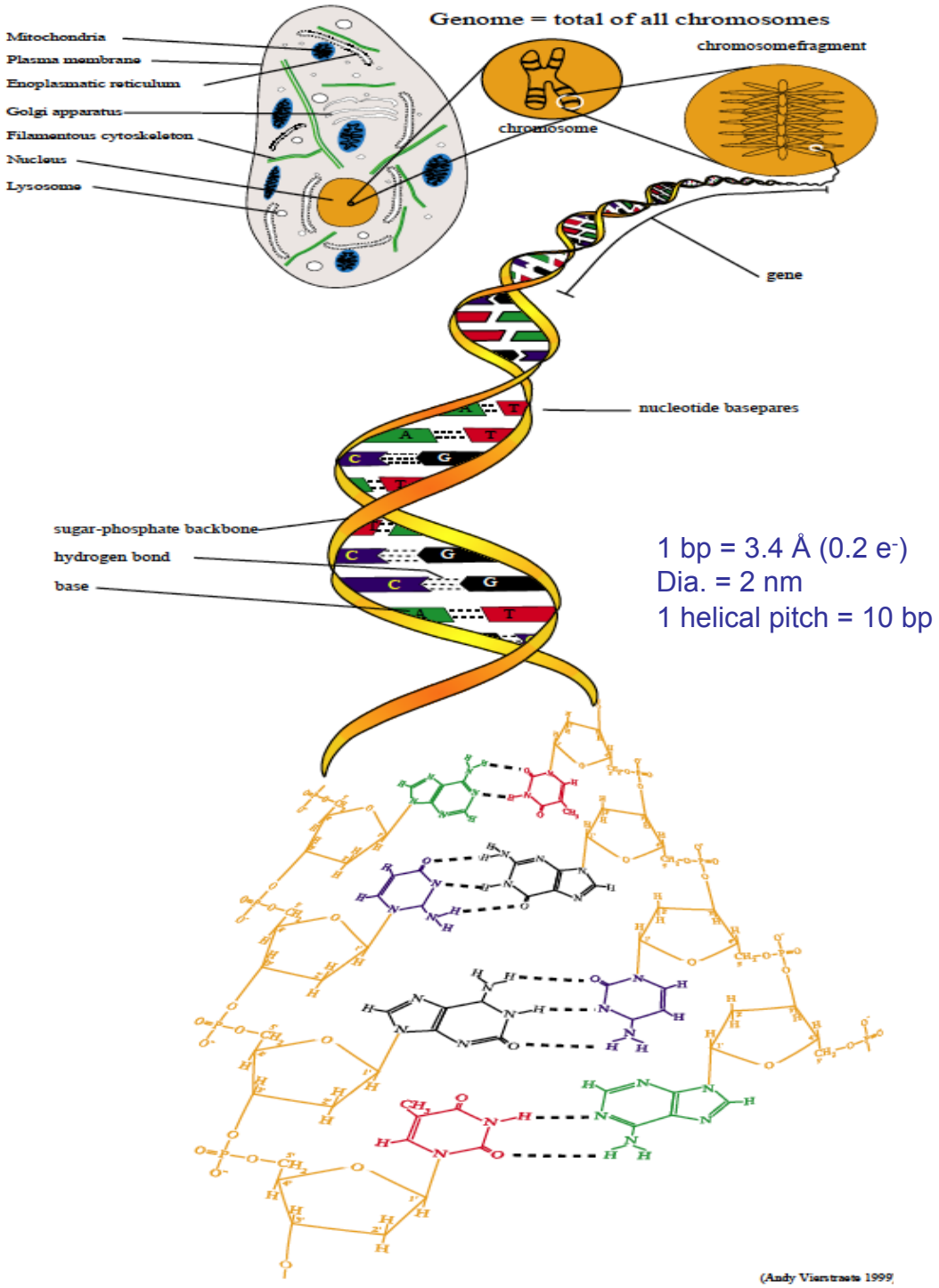
Capillary electrophoresis



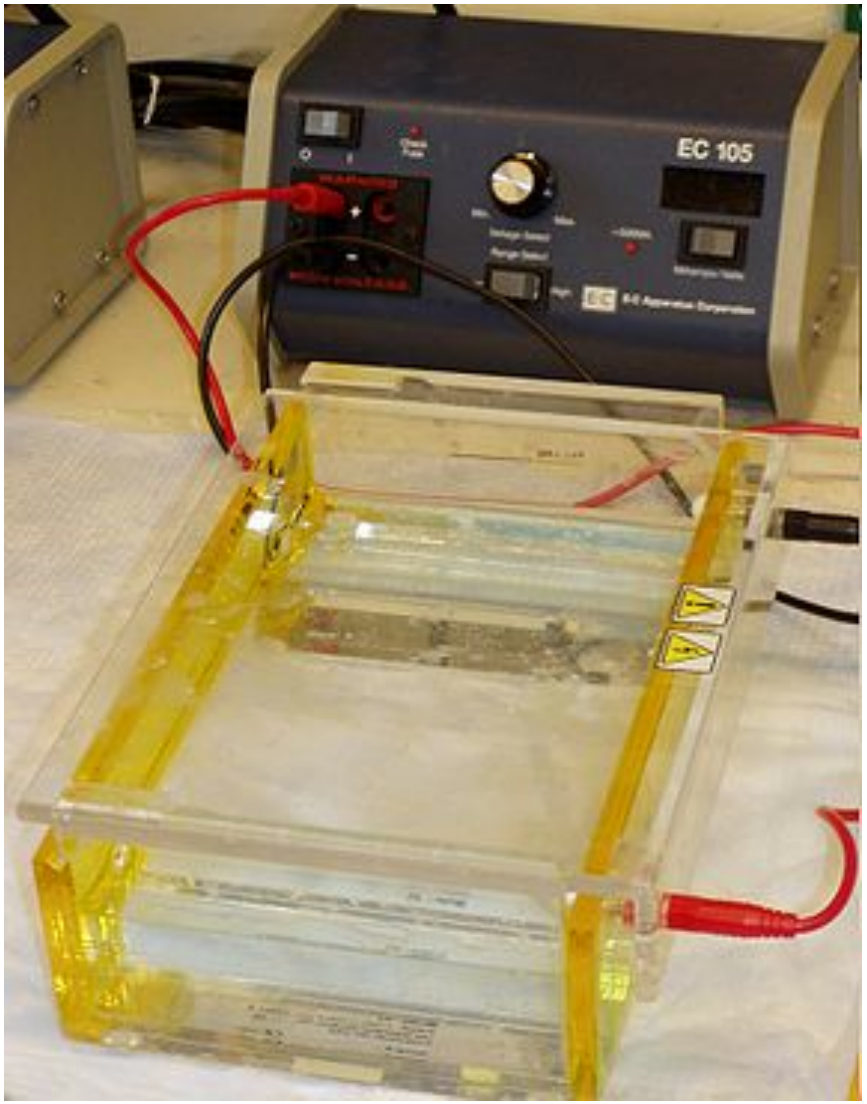


Field flow fractionation (FFF)

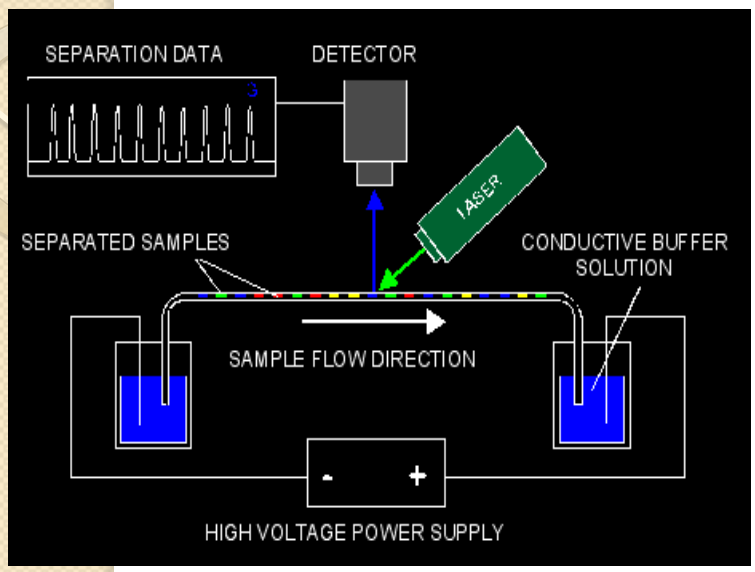


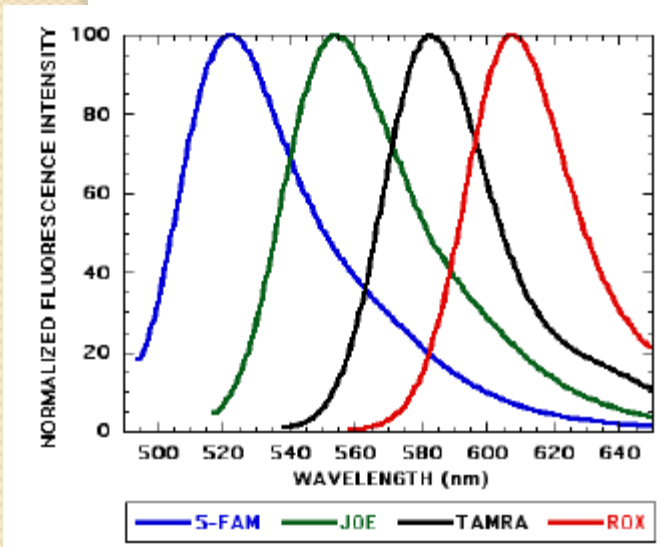

Genome = total of all chromosomes
Mitochondria
Plasma membrane
Enoplasmatic reticulum
Golgi apparatus
Filamentous cytoskeleton
Nucleus
Lysosome
chromosome
chromosomefragment
gene
nucleotide basepares
sugar-phosphate backbone
hydrogen bond
base
1 bp = 3.4 Å (0.2 e-)
Dia. = 2 nm
1 helical pitch = 10 bp
(Andy Vierstraete 1999)

Gel electrophoresis apparatus



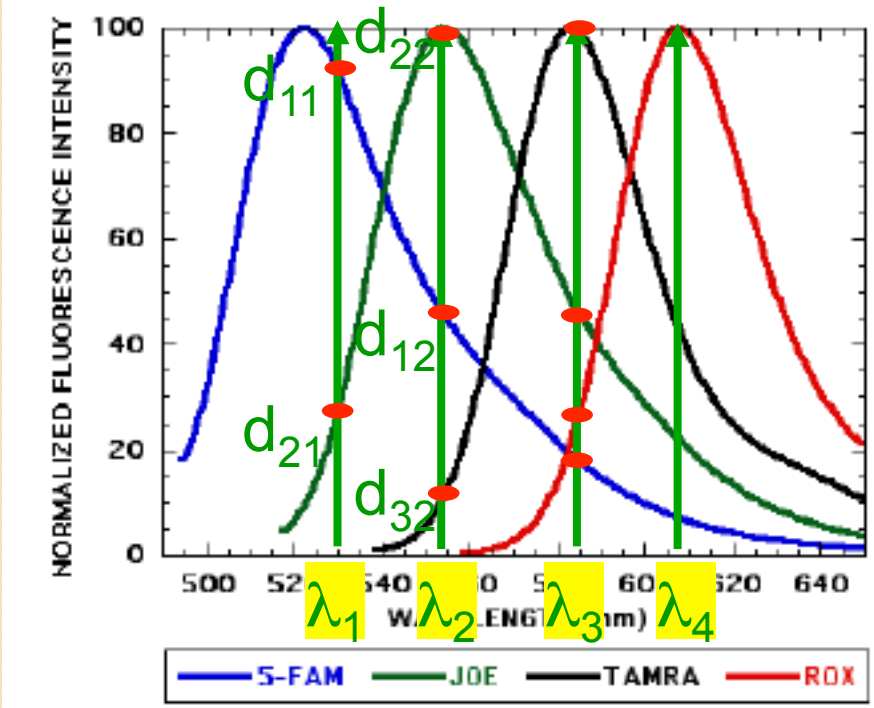
Capillary (Array) Electrophoresis





CE DNA Readout→Least-square fitting

What is needed: optical spectrum of 4 channels vs. time



Calibrated Coefficient:
d_{11}, d_{21}, d_{31}, d_{41}.....

$I(\lambda_1)=d_{11}c_1+d_{21}c_2+d_{31}c_3+d_{41}c_4$

$I(\lambda_2)=d_{12}c_1+d_{22}c_2+d_{32}c_3+d_{42}c_4$

$I(\lambda_3)=d_{13}c_1+d_{23}c_2+d_{33}c_3+d_{43}c_4$

$I(\lambda_4)=d_{14}c_1+d_{24}c_2+d_{34}c_3+d_{44}c_4$

$I(\lambda_5)=d_{15}c_1+d_{25}c_2+d_{35}c_3+d_{45}c_4$

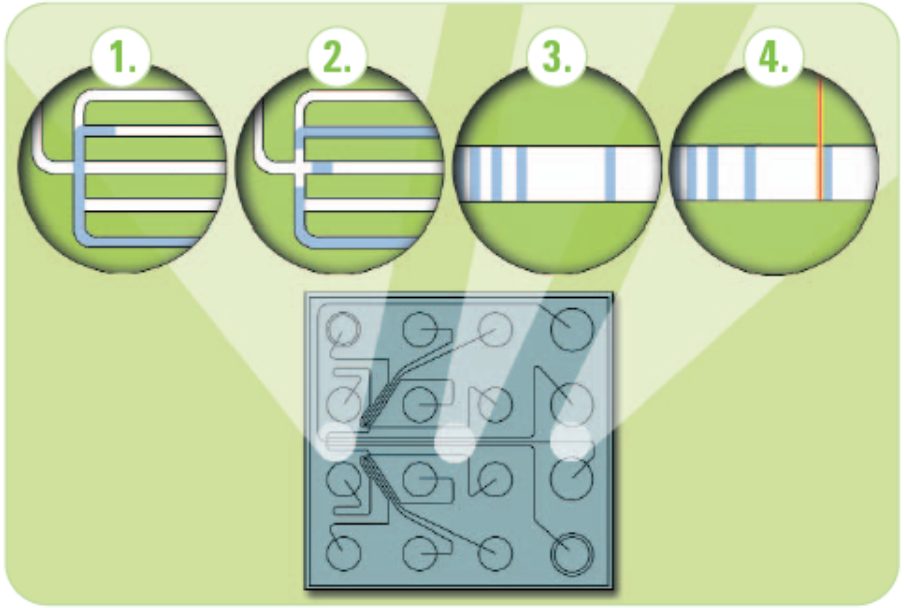
---

c_1, c_2, c_3, c_4 are the dye (DNA) concentration

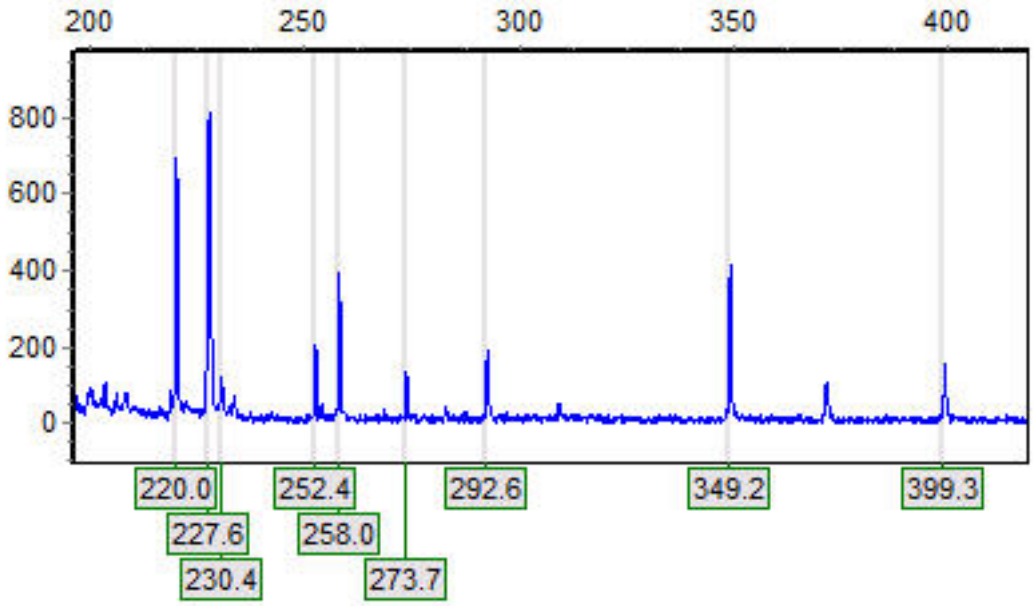
Agilent 2100 Bioanalyzer

Applied microfluidic lab-on-a-chip technology

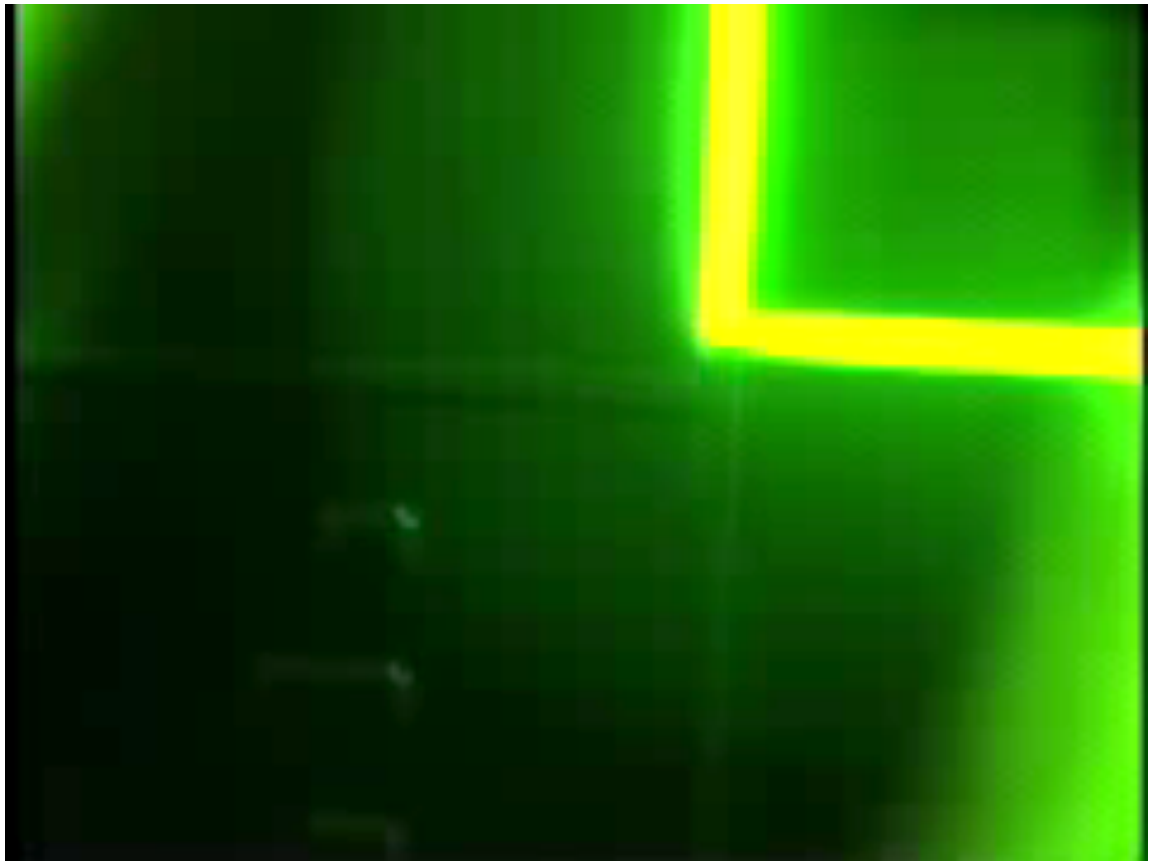
Principle of lab-on-a-chip operation



1. The sample moves through the microchannels from the sample well.
2. The sample is injected into the separation channel.
3. Sample components are electrophoretically separated.
4. Components are detected by their fluorescence and translated into gel-like images (bands) and electropherograms (peaks).



Micro-CE injection



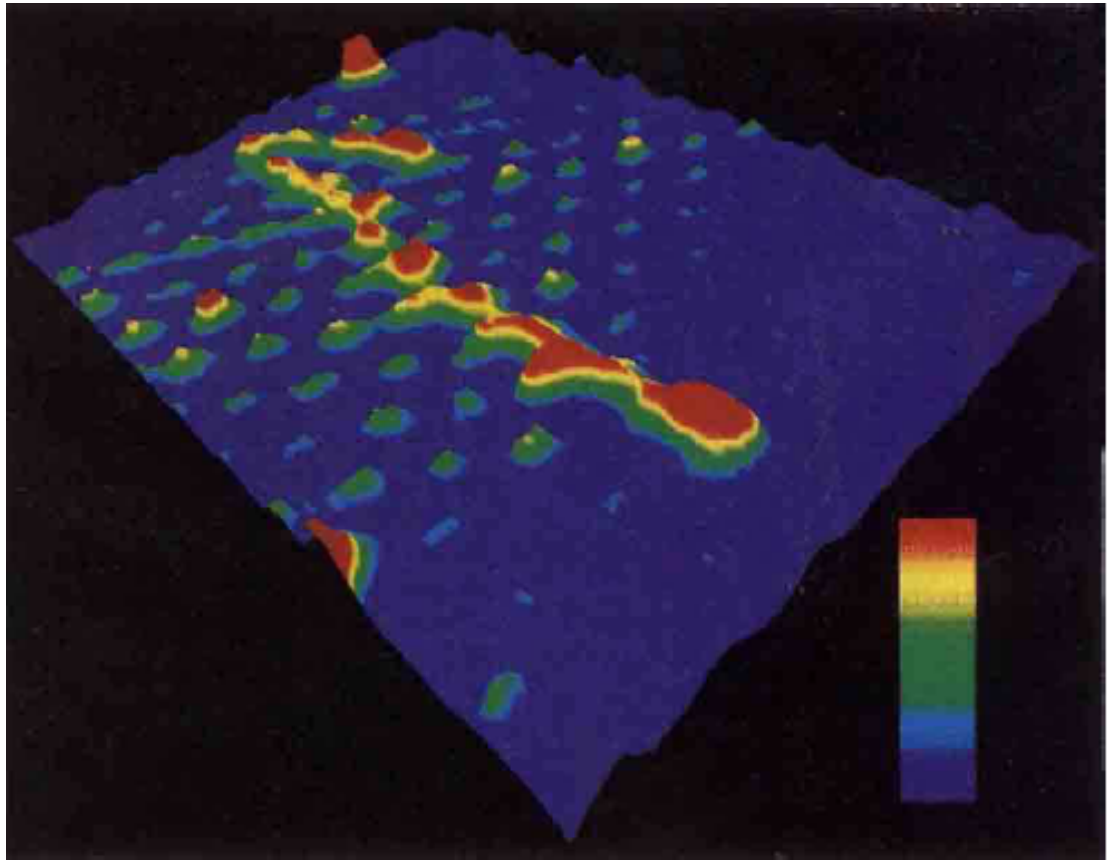
LETTERS TO NATURE

Nature 358, 600 (1992)

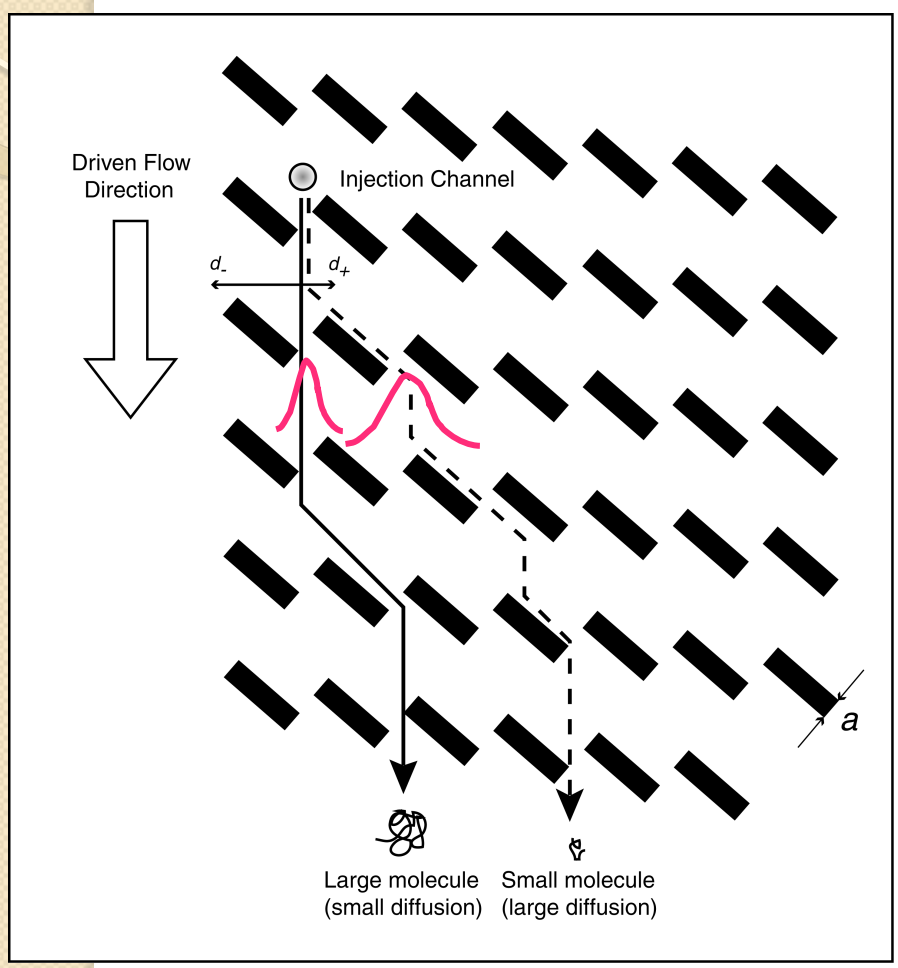
DNA electrophoresis in microlithographic arrays

W. D. Volkmuth & R. H. Austin

Department of Physics, Princeton University, Princeton, New Jersey 08544, USA



Separation based on Rectified Brownian Motion



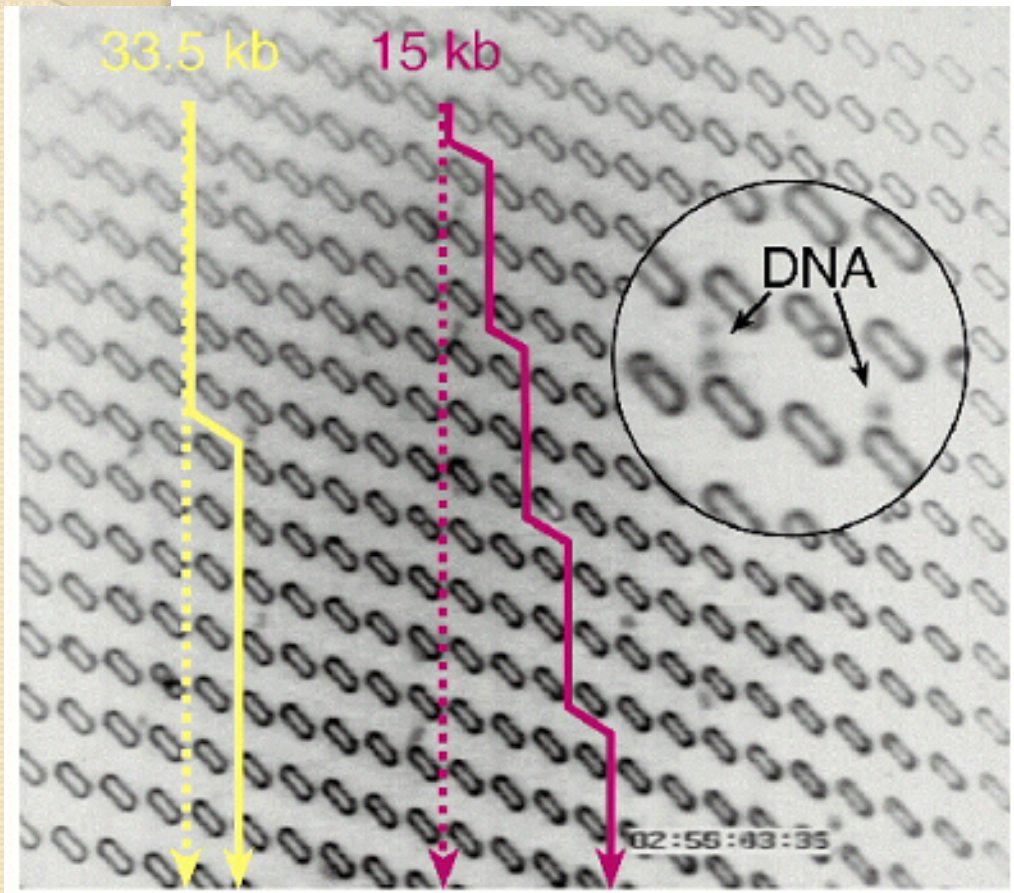
A simple idea: let the altered probabilities effectively move molecules to one side

— Rectified Brownian motion

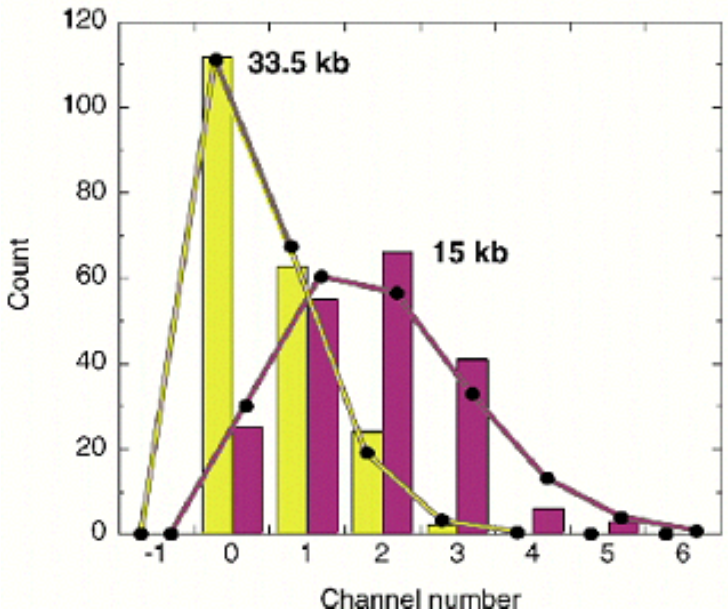
Duke and Austin, *PRL (*1998)
Ertas, *PRL (*1998)

Continuous molecular sorting by rectified Brownian motion

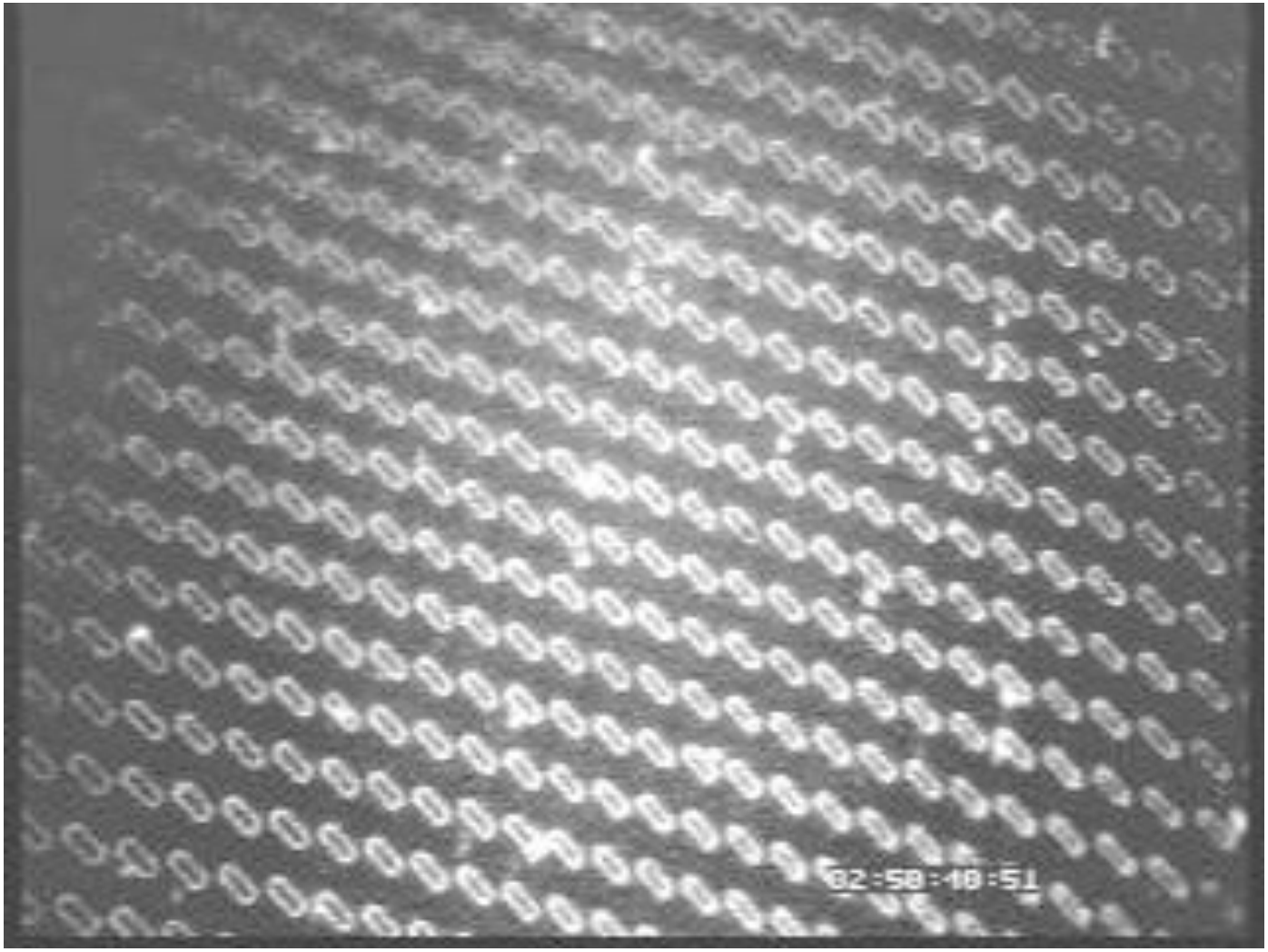
Continuous Lateral Separation



Chou et al, *PNAS* (1999)



Continuous DNA sorting by rectified Brownian motion



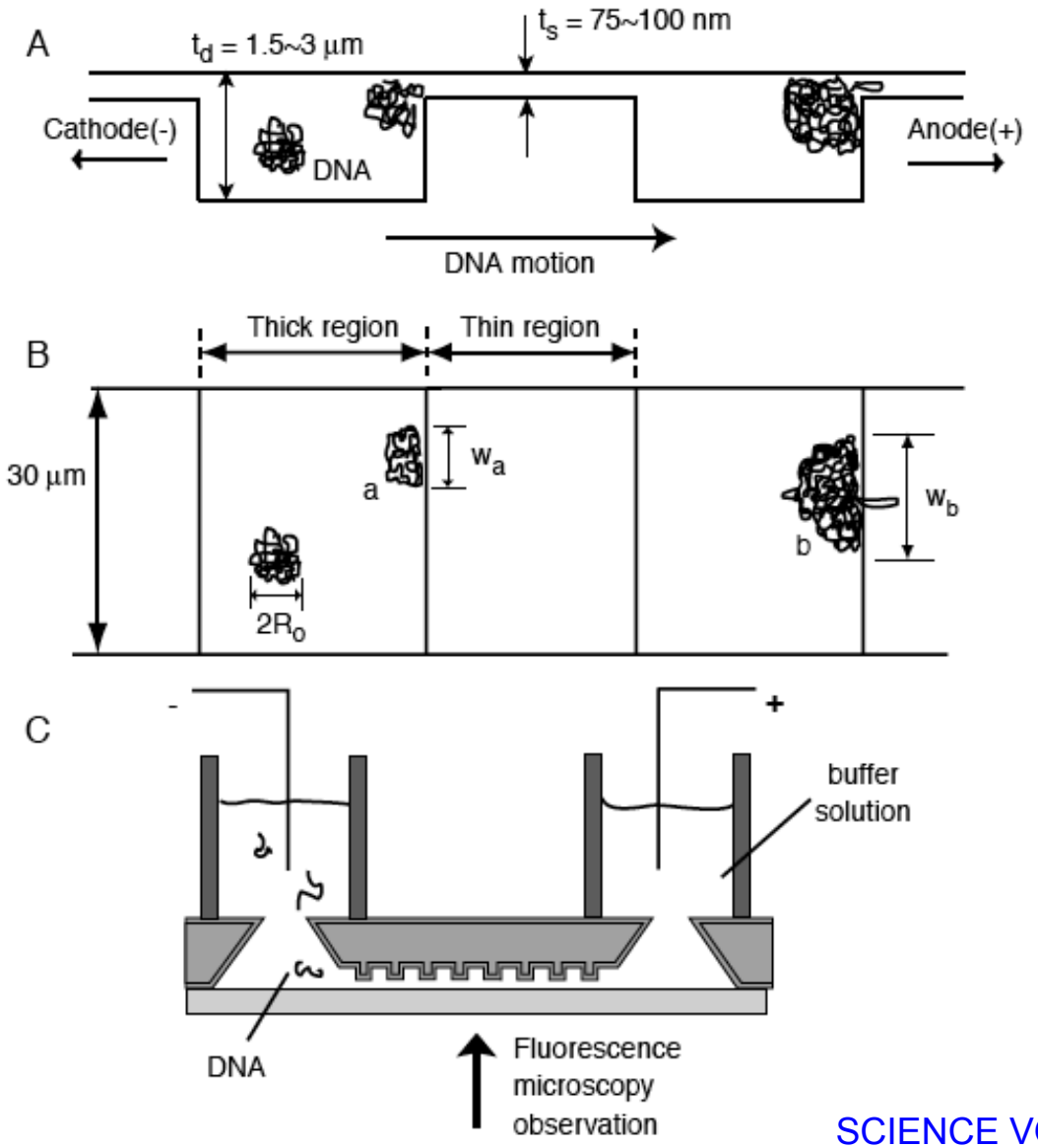
SCIENCE VOL 288 12 MAY 2000 1026-1029

Separation of Long DNA Molecules in a Microfabricated Entropic Trap Array

J. Han and H. G. Craighead*

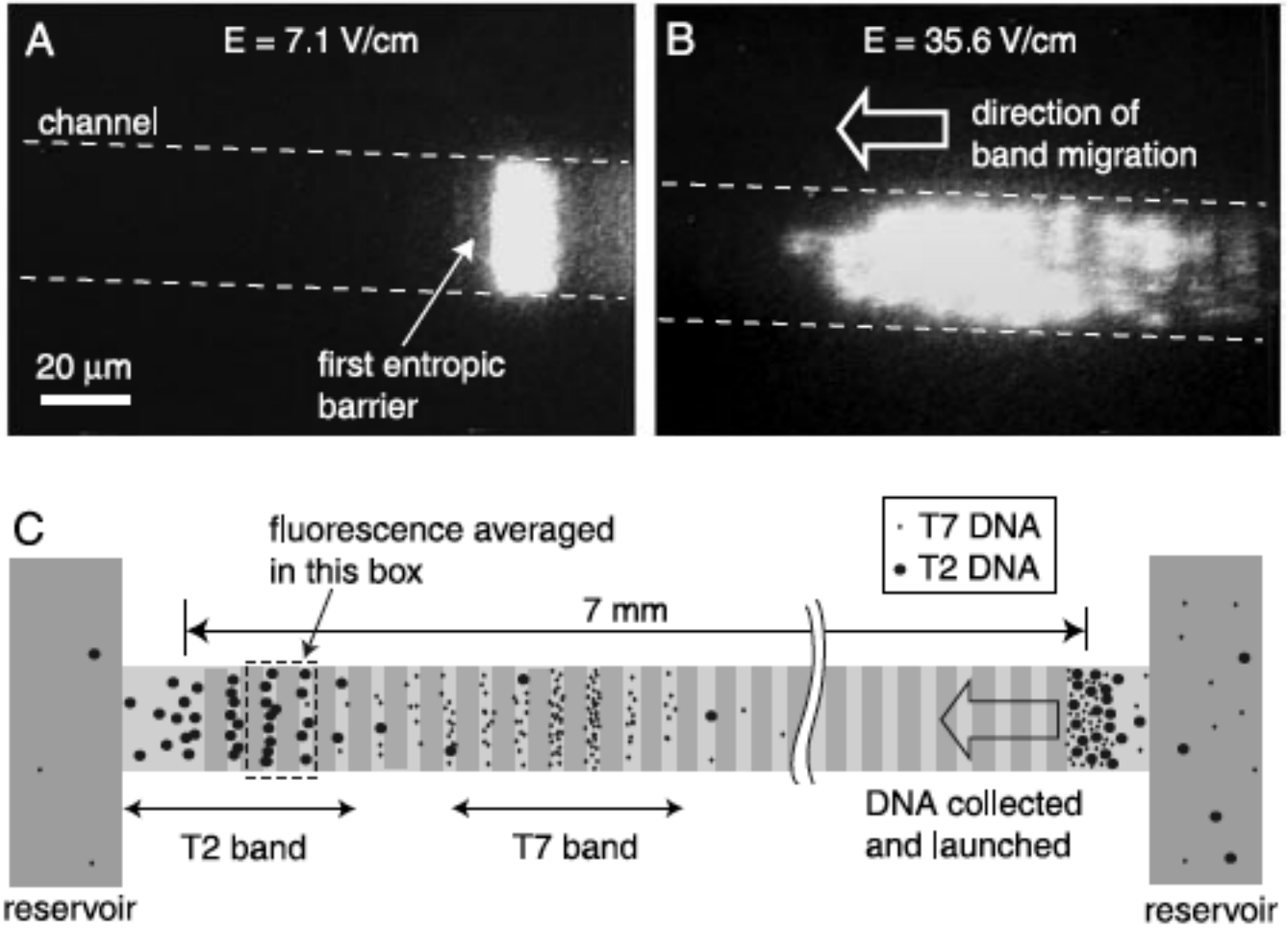
A nanofluidic channel device, consisting of many entropic traps, was designed and fabricated for the separation of long DNA molecules. The channel comprises narrow constrictions and wider regions that cause size-dependent trapping of DNA at the onset of a constriction. This process creates electrophoretic mobility differences, thus enabling efficient separation without the use of a gel matrix or pulsed electric fields. Samples of long DNA molecules (5000 to ~160,000 base pairs) were efficiently separated into bands in 15-millimeter-long channels. Multiple-channel devices operating in parallel were demonstrated. The efficiency, compactness, and ease of fabrication of the device suggest the possibility of more practical integrated DNA analysis systems.

Nanofluidic DNA separation device with entropic trapping array



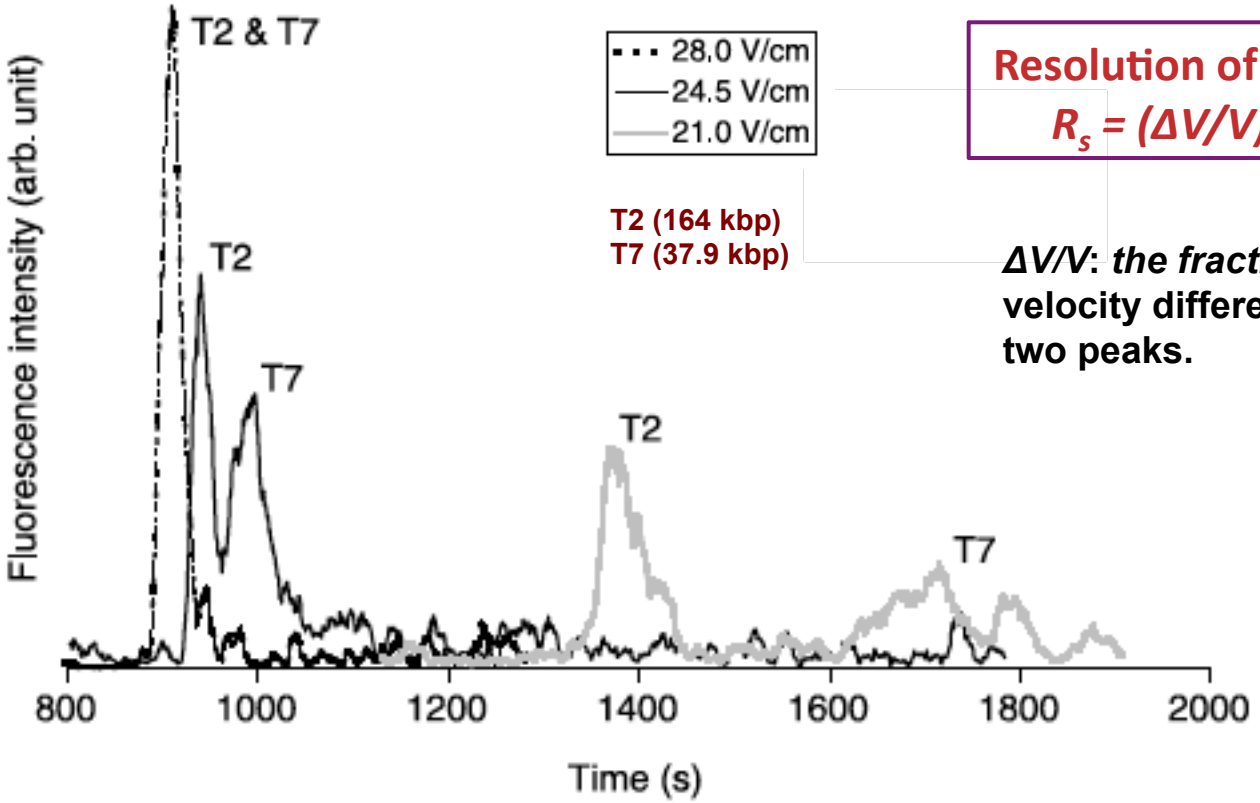
SCIENCE VOL 288 (2000) 1026

Band launching and analysis of bands



SCIENCE VOL 288 (2000) 1026

Electric field dependence of the separation of T2-T7 DNA mixture



Resolution of separation:

$R_s = (\Delta V/V)\sqrt{(N/16)}$

***ΔV/V*: *the fractional band* velocity difference between two peaks.**

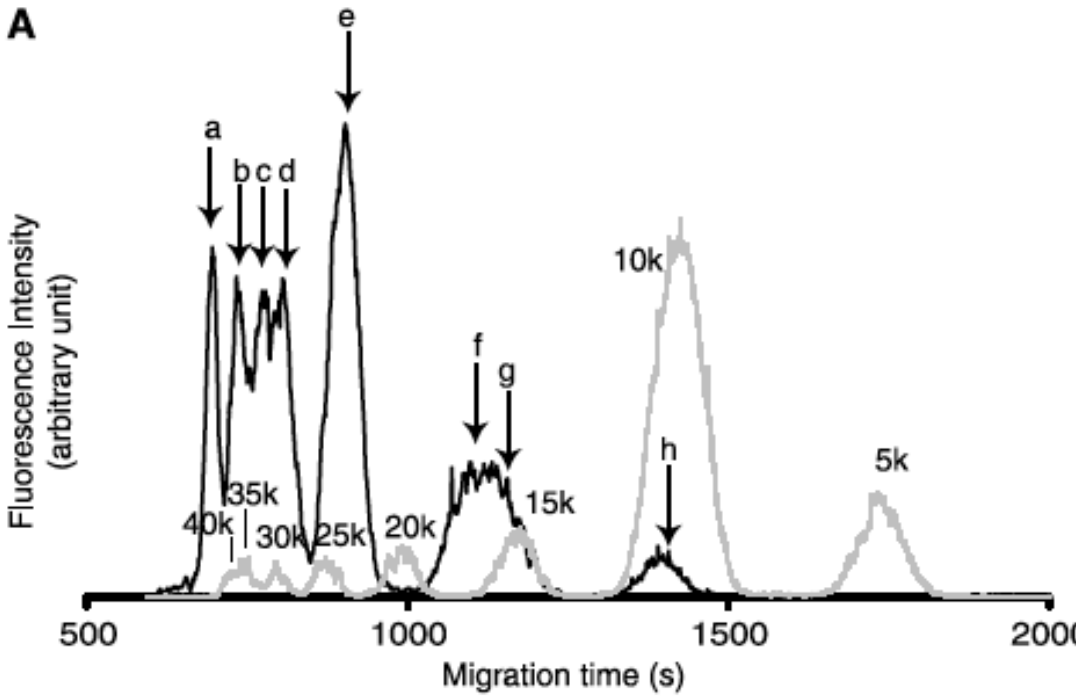
At 21.0 V/cm (gray line), the theoretical plate number (*N*) *was* 4900 for the T2 peak and 970 for the T7 peak, and the resolution was 1.95.

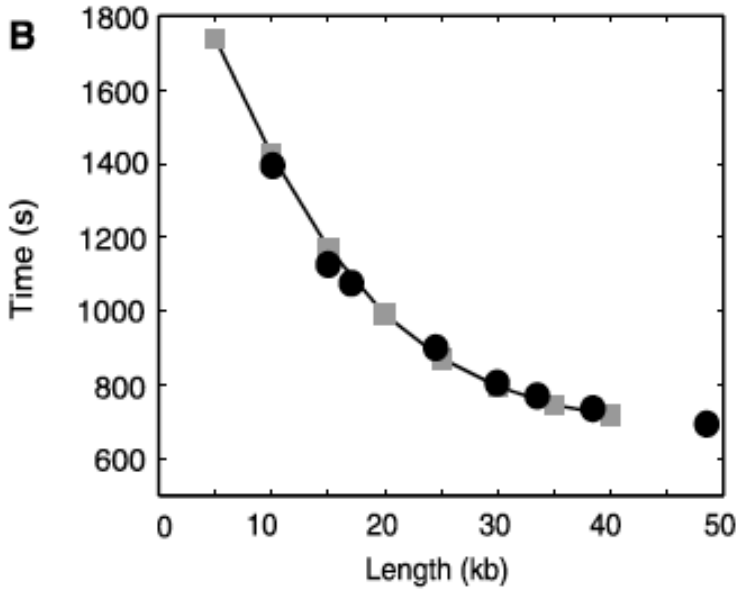
At 24.5 V/cm (black line), *N = 8500 for the T2 peak* and 3400 for the T7 peak, and the resolution was 0.89.

At 28.0 V/cm, no separation was achieved (broken line).

SCIENCE VOL 288 (2000) 1026

Simultaneous separation of the Mono Cut Mix sample (black line) and 5-kbp ladder sample (gray line) by the entropic trap array, run at 80 V/cm.





Large molecules move faster!!

SCIENCE VOL 288 (2000) 1026

On-chip pulsed-field electrophoresis

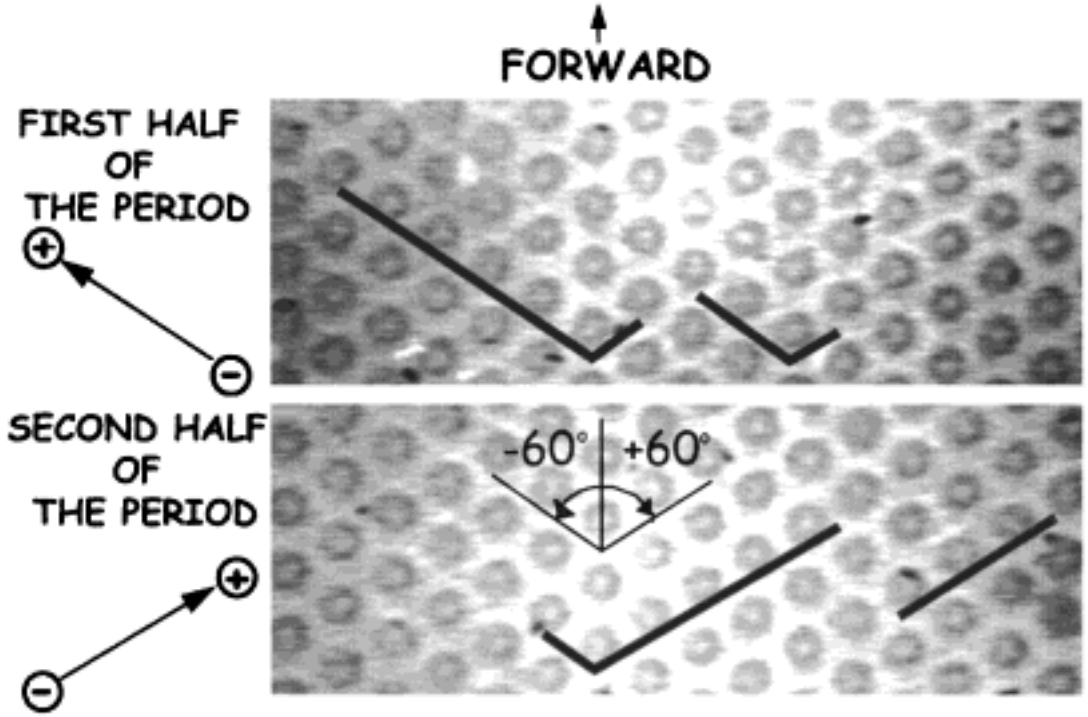
Separation of 100-Kilobase DNA Molecules in 10 Seconds

Anal. Chem. 2001, 73, 6053-6056

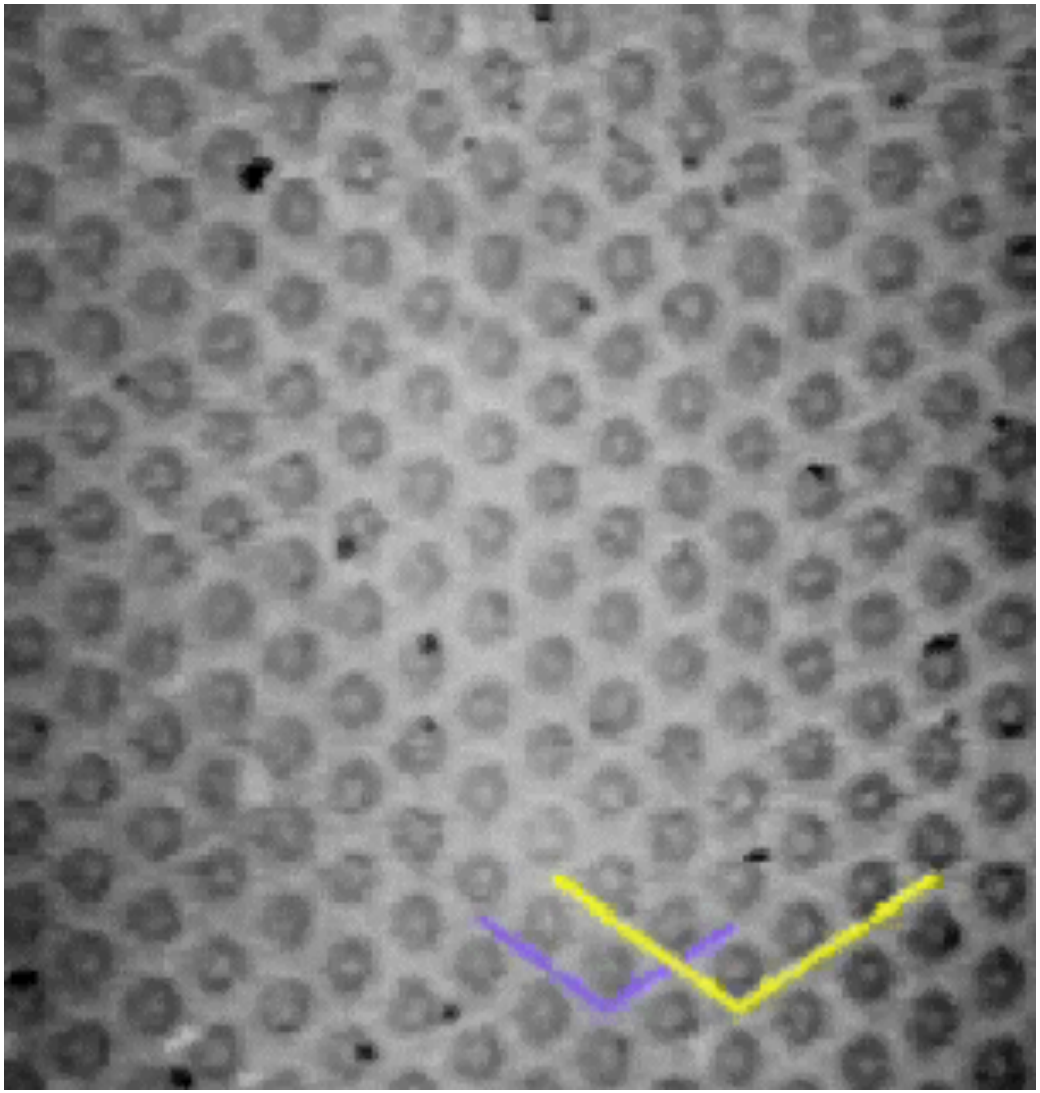
Olgica Bakajin,[†,||] Thomas A. J. Duke,[‡] Jonas Tegenfeldt,[†] Chia-Fu Chou,[†] Shirley S. Chan,[†] Robert H. Austin,[*,†] and Edward C. Cox[§]

Physics Department, Princeton University, Princeton, New Jersey 08544, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, U.K., and Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

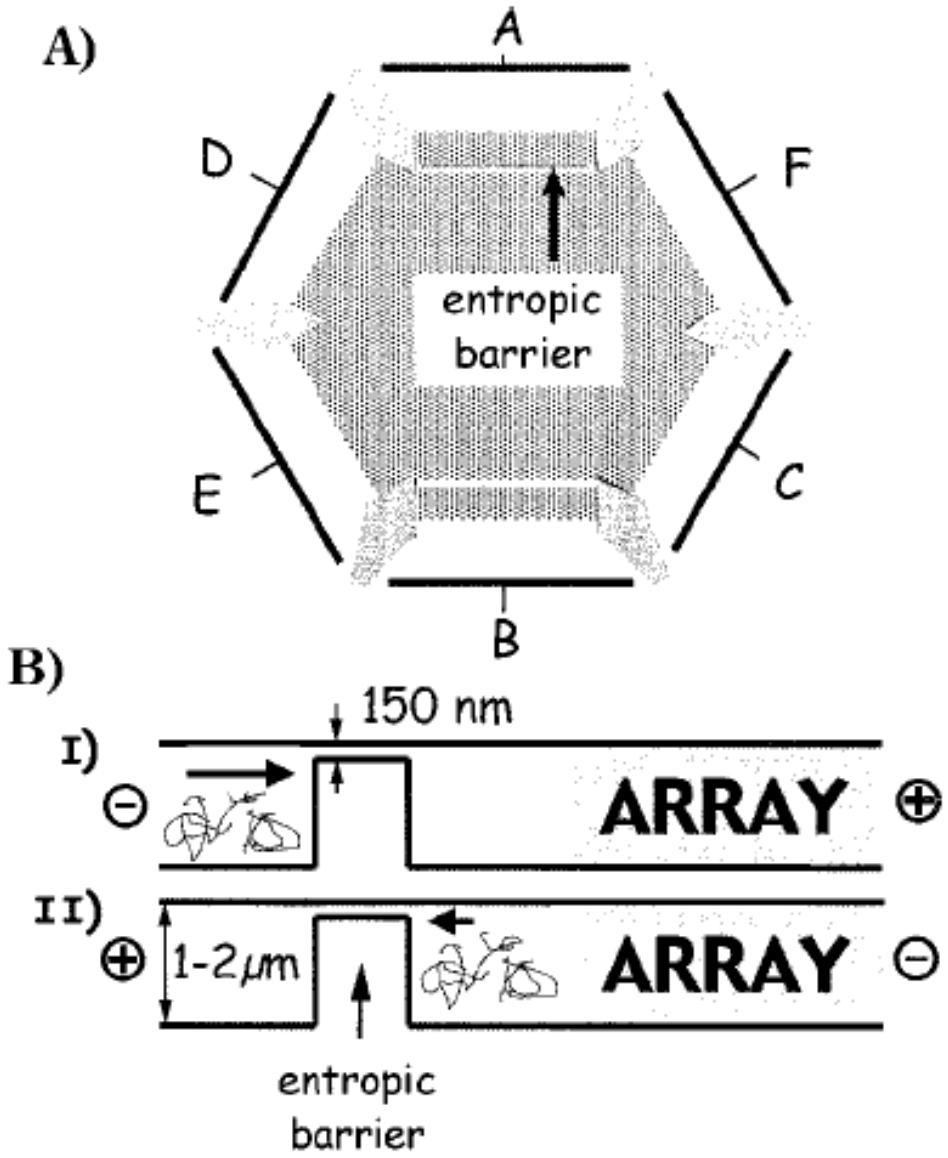
Microscopic view of the pulsed hexagonal array



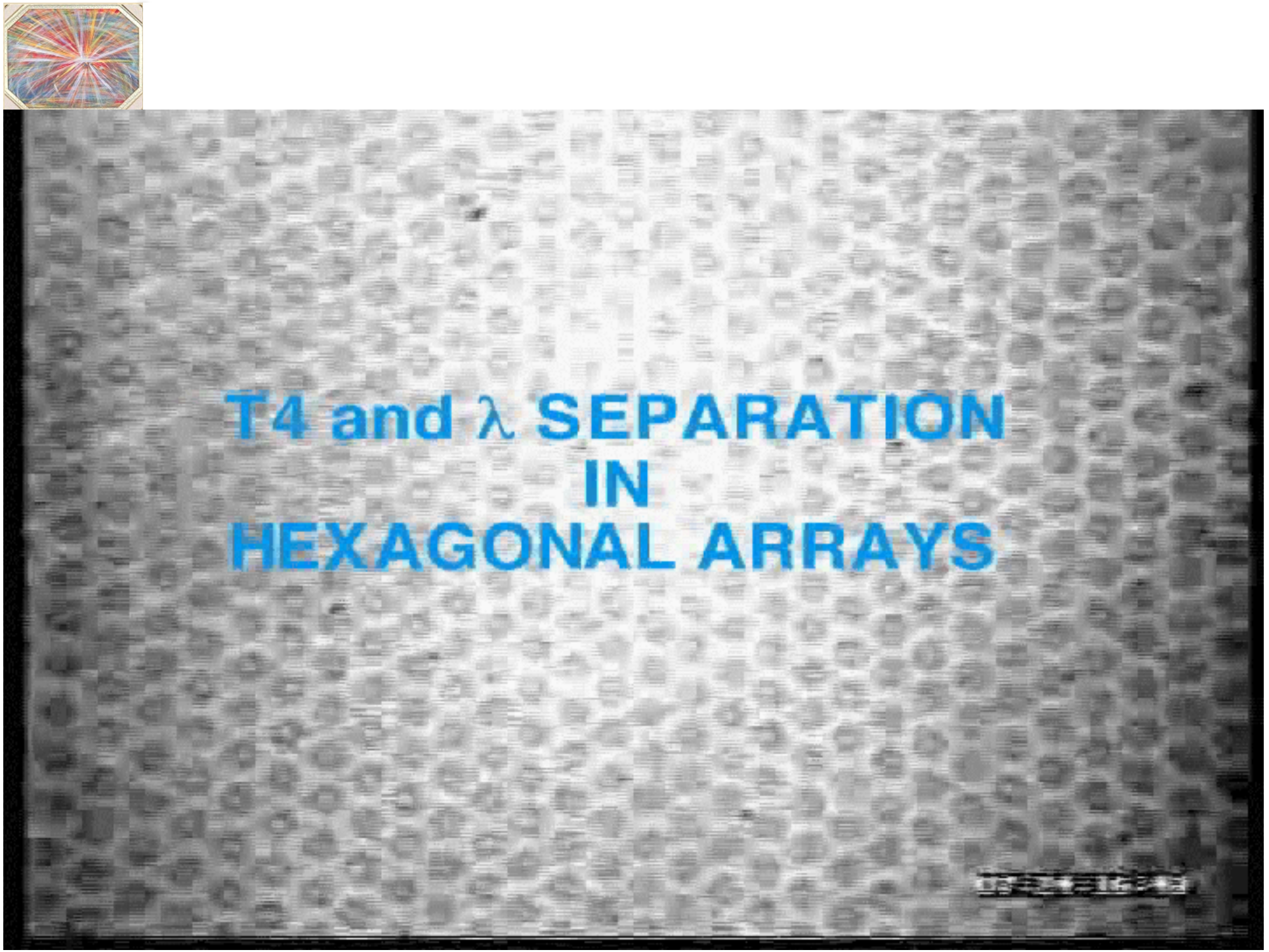
On-chip pulsed-field electrophoresis (animation)



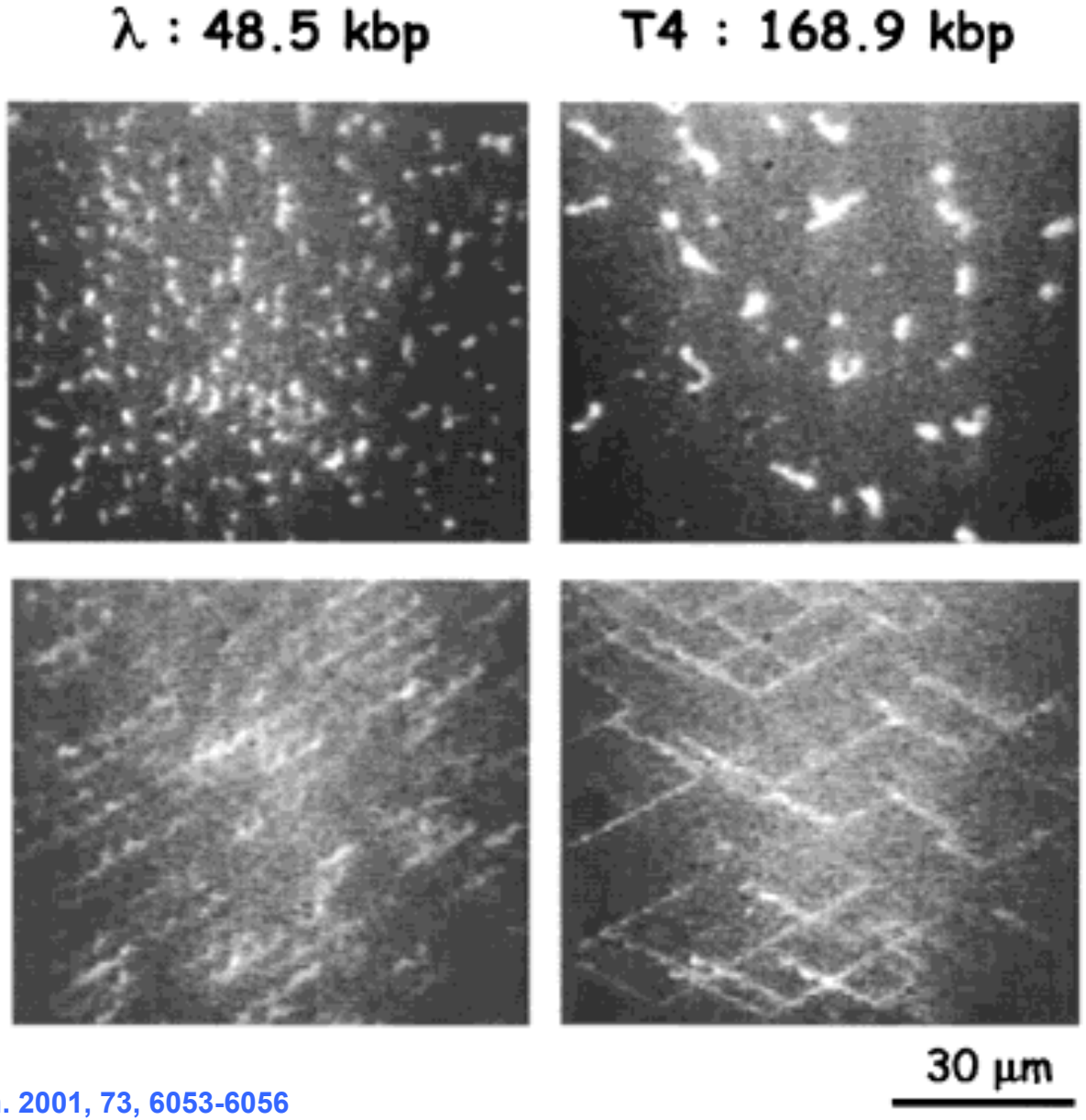
Device configuration of a pulsed hexagonal array



Anal. Chem. 2001, 73, 6053-6056


T4 and λ SEPARATION
IN
HEXAGONAL ARRAYS

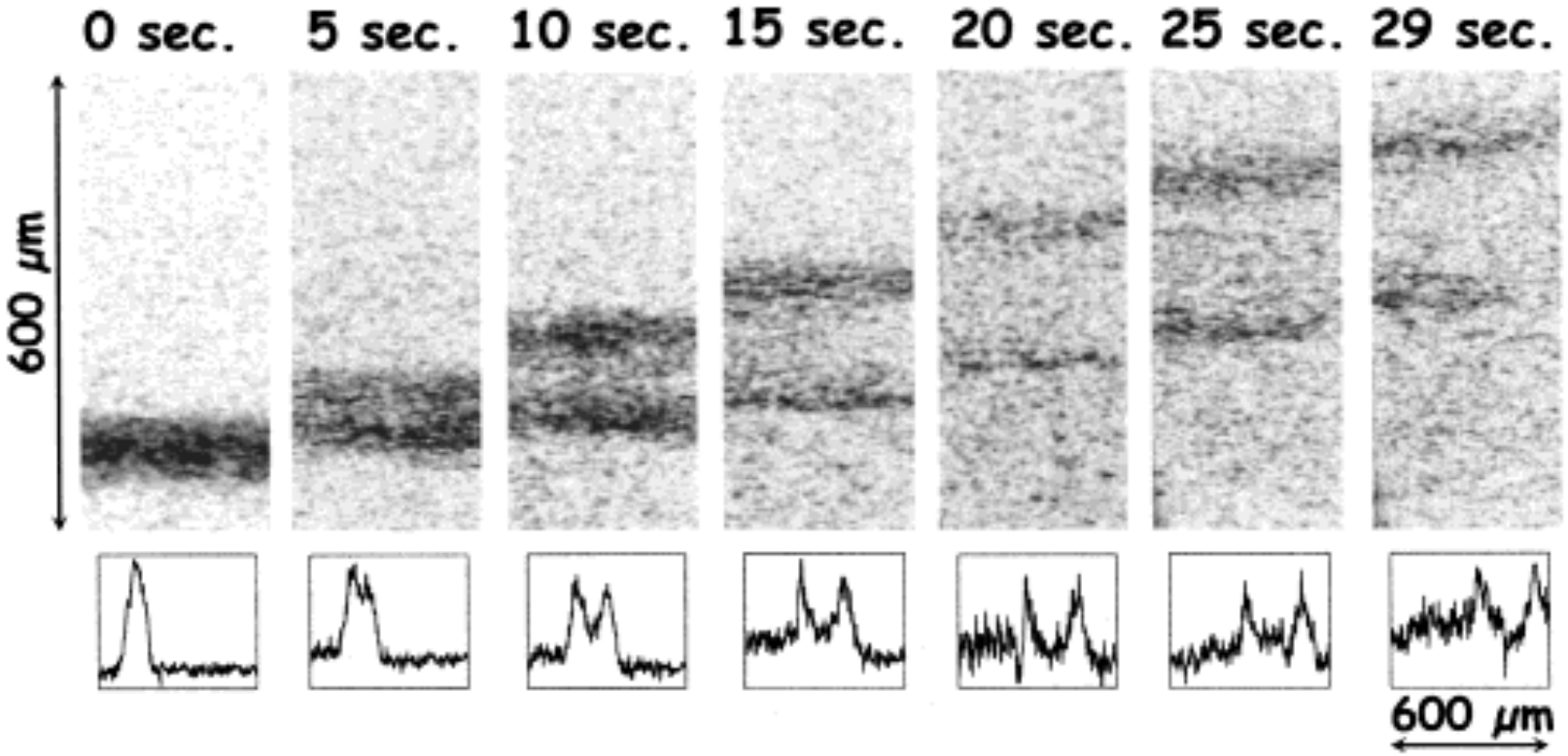
Single-molecule images



Anal. Chem. 2001, 73, 6053-6056

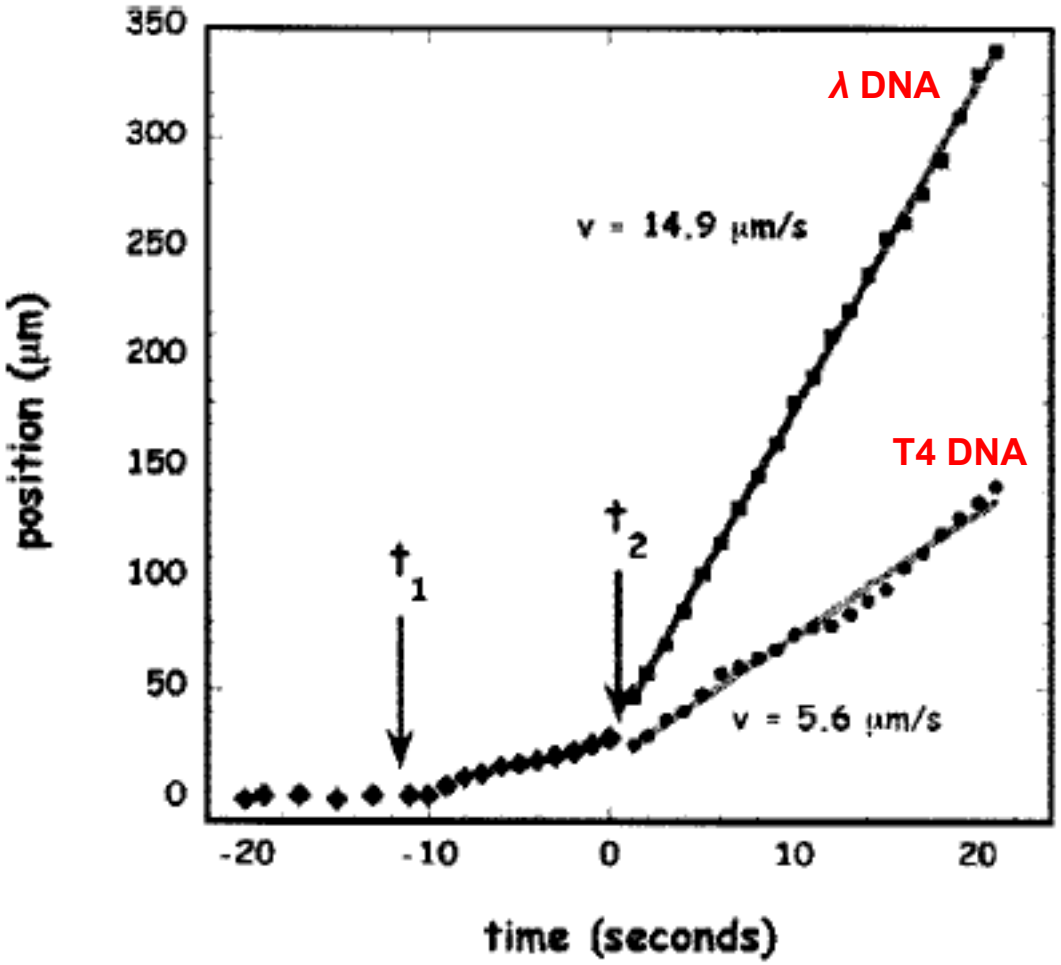
λ and T4 DNA separation

***λ and* T4 DNA pulsed at 244 V and with period T = 1 s after release from the entropic trap**



Anal. Chem. 2001, 73, 6053-6056

DNA position as a function of time

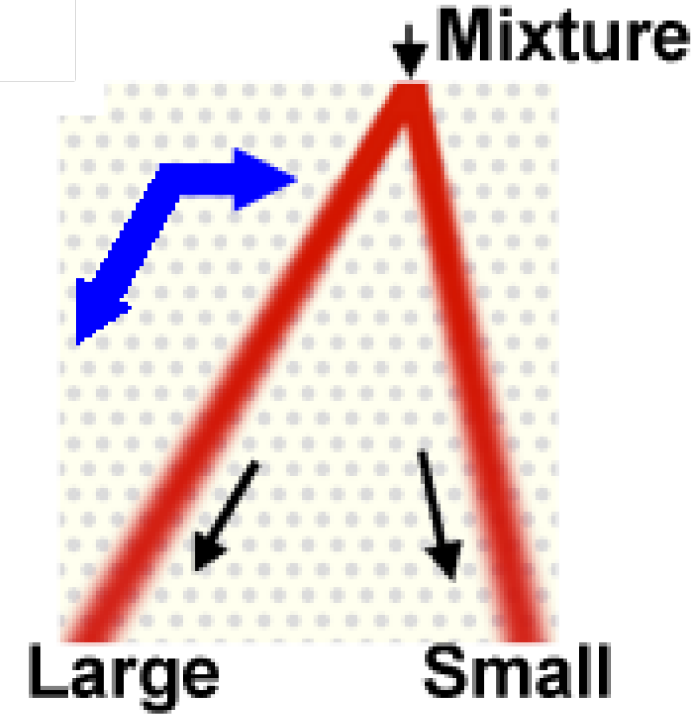


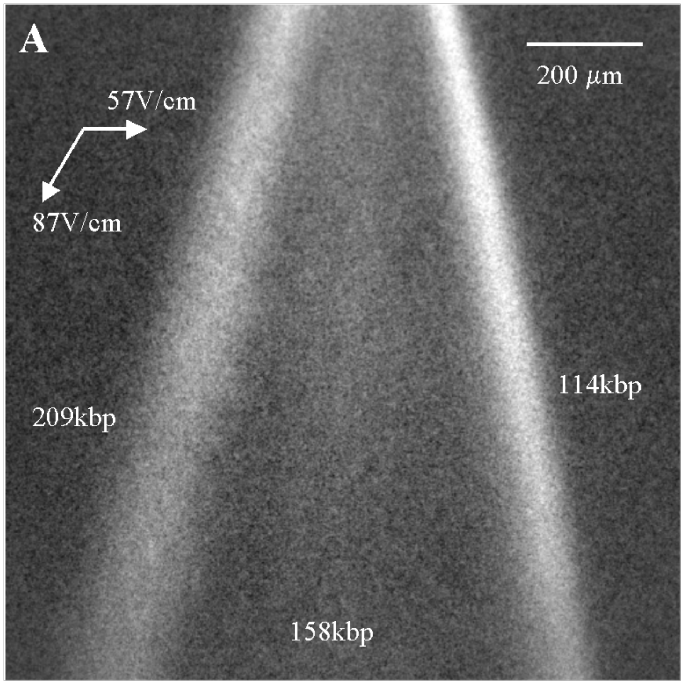
Anal. Chem. 2001, 73, 6053-6056

DNA Prism

Asymmetric pulsed fields:

The direction of the net flow motion of the DNA is angle-dependent.





Continuous separation of BAC/PAC mixture of 114, 158, and 209 kbp fragments (Huang *et al.*, Nat. Biotech. 2001)