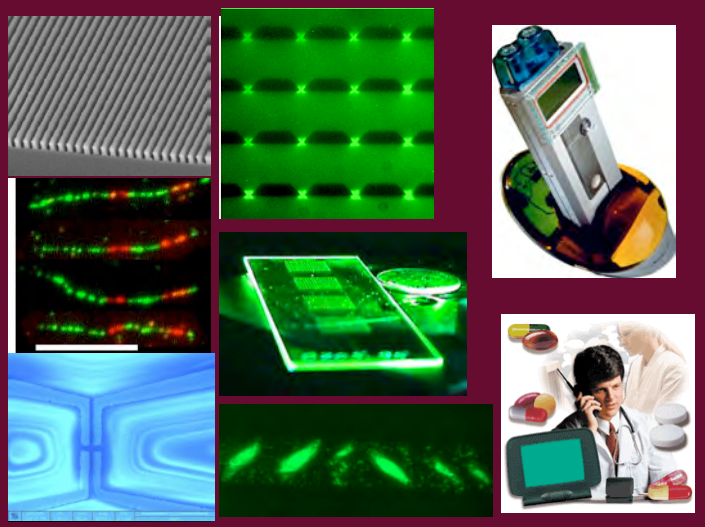
Introduction to Nanobiophotonics



Chia-Fu Chou（周家復）

Institute of Physics

Research Center for Applied Sciences

Genomics Research Center

Academia Sinica

(cfchou@phys.sinica.edu.tw)

TIGP, Fall 2007

Course outline

In this course, we will review the basics and recent developments of nanobiophotonics, an emerging field which is cross-displinary among nanoscience and nanotechnology, biology, and photonics. The course is comprised of lectures from several special topics given by experts in these areas, including micro/nanofluidics, single molecule biophysics, DNA microarray, polymer brush, optical tweezers, near-field optics, plasmonics (SPR, SERS), bioimaging techniques (fluorescence, confocal, TIRFM, FRET, higher harmonics, multiphoton, X-ray nanobeam, cryo-EM etc.) and image analysis. Grades will be evaluated on term project based on literature review or experimental setups.

Grade evaluation on term project:
Written report-50%
Presentation-50%

Office hours:
Mon.: 10-12 @ P612, IoP

Instructors and topics to be covered:

Chou, Chia-Fu 周家復 (6) – Introduction, micro/nanofluidics, SM biophys, bioimaging, Fluorescence microscopy, TIRFM, FRET, SMD, Term project assignment & evaluation

Lee, Chau-Hwang 李超煌 (1.5) – Confocal, NIWOP-cell membrane imaging, image processing

Wei, Pei-Kuen 魏培坤 (1.5) – Near-field optics/SNOM, SERS, Nanoscale optical control and biosensing

Cheng, Ji-Yen 鄭郅言 (1) – DNA microarrays-fabrication, detection, and analysis

Juan, Wen-Tau 阮文滔 (1) Polymer brush, optical tweezers

Hwu, Yeu-Kuang 胡宇光 (1) – X-ray nanobeams for bioimaging

Wei-Hau Chang 章為皓(1) – Nanoscale resolved CryoEM for structural biology

Dong, Chen-Yuan 董成淵 (1) (NTU) – Multiphoton imaging

Sun, Chi-Kuang 孫啟光 (1) (NTU) – Nonlinear optical bioimaging using higher harmonics

Kao, Fu-Jen 高甫仁 (1) (YMU) – Time-resolved fluorescence microscopy, TCSPC

Reference:

1 Optical microscopy in general- http://micro.magnet.fsu.edu/

2 Stefan A. Maier: Plasmonics: Fundamentals and Applications (Springer, 2007)

3 Lukas Novotny and Bert Hecht: Principles of Nano-Optics (Cambridge University Press, 2006)

4 Joseph R. Lakowicz: Principles of Fluorescence Spectroscopy (3rd Ed., Springer, 2006)

5 Alastair Smith, Christopher Gell, and David Brockwell: Handbook of Single Molecule Fluorescence Spectroscopy (Oxford University Press, 2006)

6 Katrin Kneipp, Martin Moskovits, and Harald Kneipp: Surface-Enhanced Raman Scattering: Physics and Applications (Springer, 2006)

7 Wolfgang Becker: Advanced Time-Correlated Single Photon Counting Techniques (Springer Series in Chemical Physics, 2005)

8 Christof M. Niemeyer and Chad A. Mirkin (Editors): Nanobiotechnology: Concepts, Applications and Perspectives (Hardcover, Wiley-VCH, 2004)

9 Roland Glaser: Biophysics (Springer, 2004)

10 Paras N. Prasad: Introduction to Biophotonics (Wiley-Interscience, 2003)

11 West JL, Halas NJ: Engineered nanomaterials for biophotonics applications: Improving sensing, imaging, and therapeutics. ANNUAL REVIEW OF BIOMEDICAL ENGINEERING 5: 285-292 2003.

12 H. Berg, The Random Walk in Biology, Princeton University Press, 1993.

Term project topics-Experimental

1. Optical tweezers (http://www.stanford.edu/group/blocklab/OpticalTweezers Introduction.htm)
2. Prism-based TIRFM (http://micro.magnet.fsu.edu/primer/techniques/fluorescence/tirf/tirfhome.html)
3. Nanometer Localization Analysis for Individual Fluorescent Probes. (Biophys. J. 82, 2775–2783, 2002)

Term project topics-Literature review

4 Sub-diffraction-limit imaging I:
- Toward fluorescence nanoscopy. Nature Biotechnology 21 (11): 1347-1355 NOV 2003.

5 Sub-diffraction-limit imaging II:
- Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. Science 313, 1642-1645, 2006.

6 Sub-diffraction-limit imaging III:
- Sub-Diffraction-Limited Optical Imaging with a Silver Superlens. Science 308, 534-537, 2005.
- Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nature Methods 3 (10): 793-795 OCT 2006.

7 Single-Molecule Analysis I:
- Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations. Science 299, 682-686, 2003.

8 Single-Molecule Analysis II:
- Single photons on demand from a single molecule at room temperature. Nature 407, 491-493, 2000.
- Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS). Physical Review Letters 78, 1667-1670, 1997

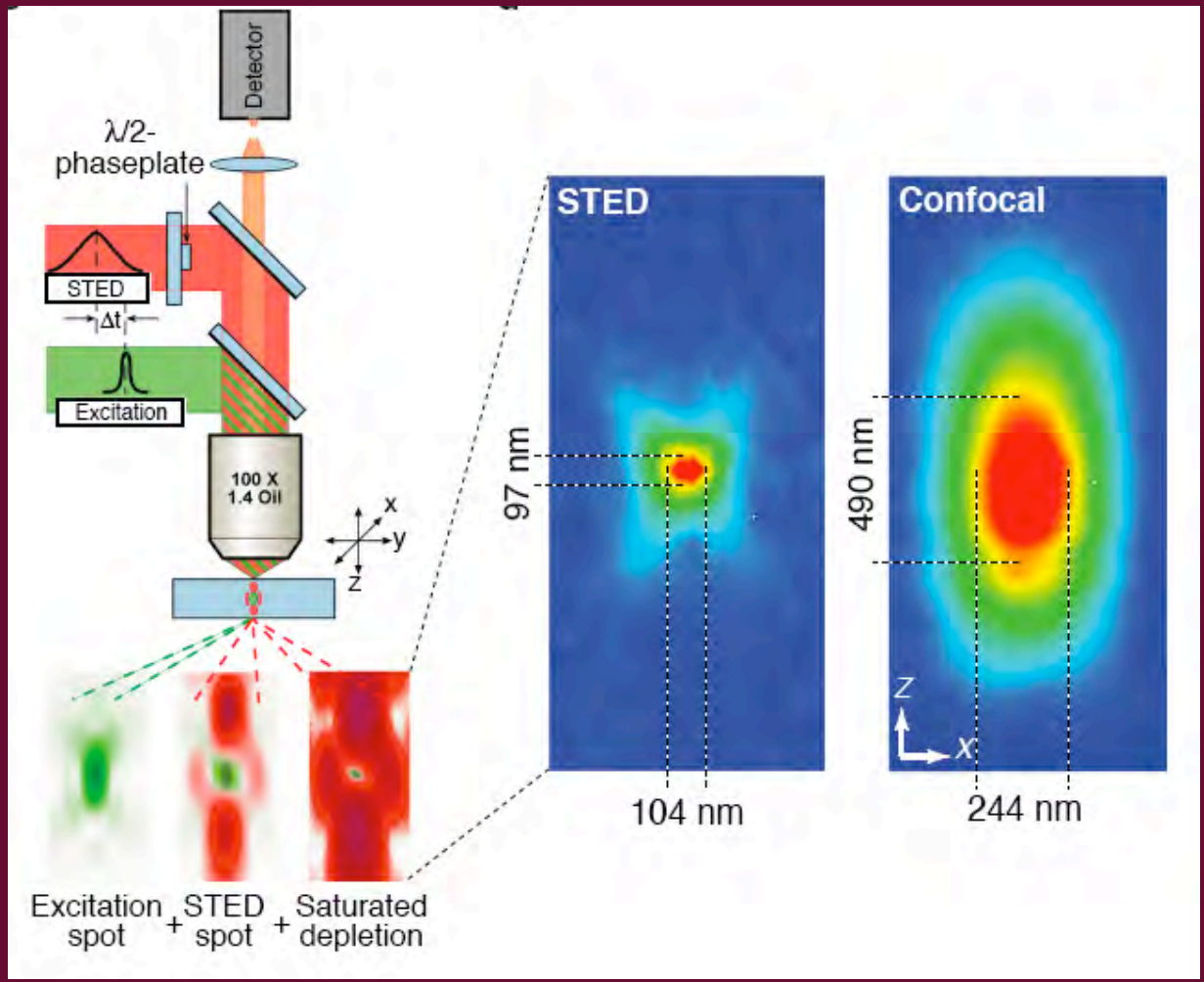
9 Stabilization of an optical microscope to 0.1 nm in three dimensions. Applied Optics 46, 421-427, 2007.

10 Million-fold Preconcentration of Proteins and Peptides by Nanofluidic Filter. Analytical Chemistry 77 (14), 4293-4299, 2005.

Term project topics-Literature review

Sub-diffraction-limit imaging I:

- Toward fluorescence nanoscopy. Nature Biotechnology 21 (11): 1347-1355 NOV 2003.



Sub-diffraction-limit imaging II:

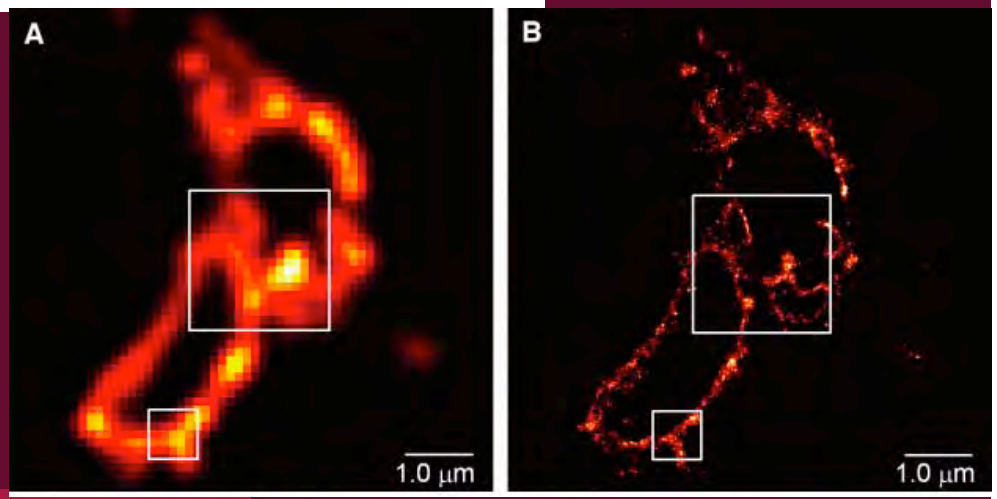
- Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. Science 313, 1642-1645, 2006.

Imaging Intracellular Fluorescent Proteins at Nanometer Resolution

Eric Betzig,[1,2*†] George H. Patterson,[3] Rachid Sougrat,[3] O. Wolf Lindwasser,[3] Scott Olenych,[4] Juan S. Bonifacino,[3] Michael W. Davidson,[4] Jennifer Lippincott-Schwartz,[3] Harald F. Hess[5*]

We introduce a method for optically imaging intracellular proteins at nanometer spatial resolution. Numerous sparse subsets of photoactivatable fluorescent protein molecules were activated, localized (to ~2 to 25 nanometers), and then bleached. The aggregate position information from all subsets was then assembled into a superresolution image. We used this method—termed photoactivated localization microscopy—to image specific target proteins in thin sections of lysosomes and mitochondria; in fixed whole cells, we imaged vinculin at focal adhesions, actin within a lamellipodium, and the distribution of the retroviral protein Gag at the plasma membrane.

PhotoActivated Localization Microscopy (PALM)



Sub-diffraction-limit imaging III:

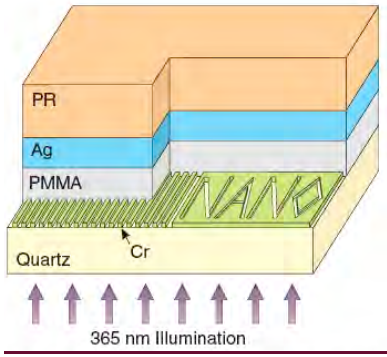
- Sub-Diffraction-Limited Optical Imaging with a Silver Superlens. Science 308, 534-537, 2005.
- Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nature Methods 3 (10): 793-795 OCT 2006.

REPORTS

Sub–Diffraction-Limited Optical Imaging with a Silver Superlens

Nicholas Fang, Hyesog Lee, Cheng Sun, Xiang Zhang*

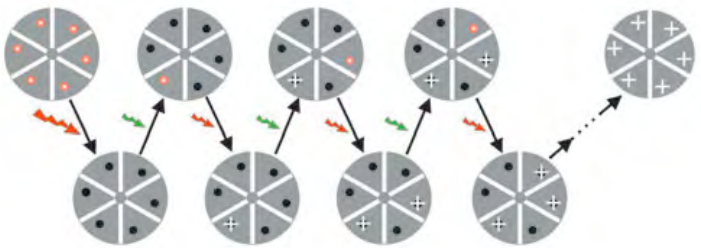
Recent theory has predicted a superlens that is capable of producing sub–diffraction-limited images. This superlens would allow the recovery of evanescent waves in an image via the excitation of surface plasmons. Using silver as a natural optical superlens, we demonstrated sub–diffraction-limited imaging with 60-nanometer half-pitch resolution, or one-sixth of the illumination wavelength. By proper design of the working wavelength and the thickness of silver that allows access to a broad spectrum of subwavelength features, we also showed that arbitrary nanostructures can be imaged with good fidelity. The optical superlens promises exciting avenues to nanoscale optical imaging and ultrasmall opto-electronic devices.



Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)

Michael J Rust[1,5], Mark Bates[2,5] & Xiaowei Zhuang[1,3,4]

We have developed a high-resolution fluorescence microscopy method based on high-accuracy localization of photoswitchable fluorophores. In each imaging cycle, only a fraction of the fluorophores were turned on, allowing their positions to be determined with nanometer accuracy. The fluorophore positions obtained from a series of imaging cycles were used to reconstruct the overall image. We demonstrated an imaging resolution of 20 nm. This technique can, in principle, reach molecular-scale resolution.



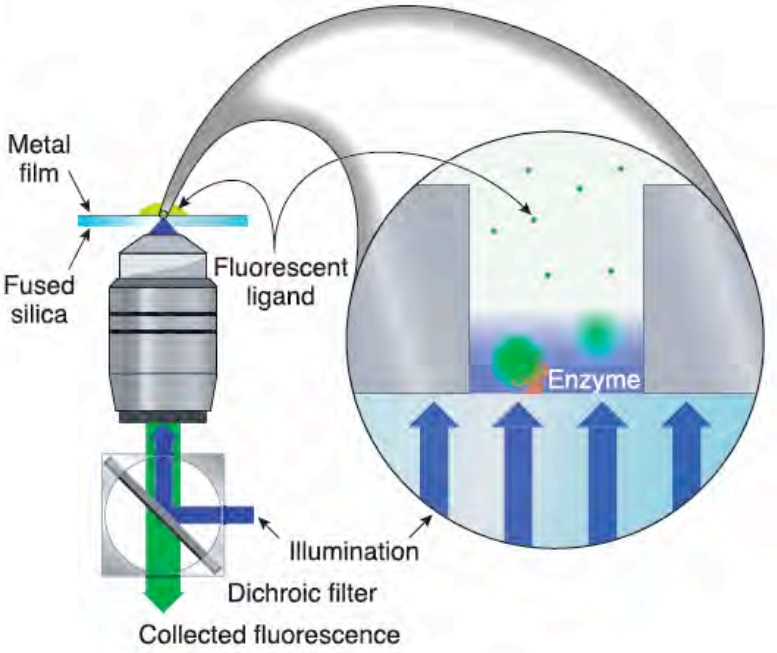
Single-Molecule Analysis I:

- Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations. Science 299, 682-686, 2003.

Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations

M. J. Levene,[1] J. Korlach,[1,2] S. W. Turner,[1]* M. Foquet,[1] H. G. Craighead,[1] W. W. Webb[1]†

Optical approaches for observing the dynamics of single molecules have required pico- to nanomolar concentrations of fluorophore in order to isolate individual molecules. However, many biologically relevant processes occur at micromolar ligand concentrations, necessitating a reduction in the conventional observation volume by three orders of magnitude. We show that arrays of zero-mode waveguides consisting of subwavelength holes in a metal film provide a simple and highly parallel means for studying single-molecule dynamics at micromolar concentrations with microsecond temporal resolution. We present observations of DNA polymerase activity as an example of the effectiveness of zero-mode waveguides for performing single-molecule experiments at high concentrations.



An apparatus for single-molecule enzymology using zeromode waveguides.

Single-Molecule Analysis II:

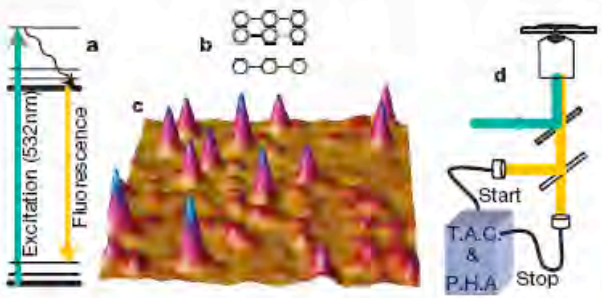
- Single photons on demand from a single molecule at room temperature. Nature 407, 491-493, 2000.
- Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS). Physical Review Letters 78, 1667-1670, 1997

Single photons on demand from a single molecule at room temperature

B. Lounis*† & W. E. Moerner*

** Department of Chemistry, Stanford University, Stanford, California 94305-5080, USA*
† CPMOH, Université Bordeaux I, 33405 Talence, France

The generation of non-classical states of light[1] is of fundamental scientific and technological interest. For example, 'squeezed' states[2] enable measurements to be performed at lower noise levels than possible using classical light. Deterministic (or triggered) single-photon sources exhibit non-classical behaviour in that they emit, with a high degree of certainty, just one photon at a user-specified time. (In contrast, a classical source such as an attenuated pulsed laser emits photons according to Poisson statistics.) A deterministic source of single photons could find applications in quantum information processing[3], quantum cryptography[4] and certain quantum computation problems[5]. Here we realize a controllable source of single photons using optical pumping of a single molecule in a solid. Triggered single photons are produced at a high rate, whereas the probability of simultaneous emission of two photons is nearly zero—a useful property for secure quantum cryptography. Our approach is



VOLUME 78, NUMBER 9 PHYSICAL REVIEW LETTERS 3 MARCH 1997

Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS)

Katrin Kneipp, Yang Wang,* Harald Kneipp,† Lev T. Perelman, Irving Itzkan, Ramachandra R. Dasari, and Michael S. Feld

George R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Department of Physics, Technical University of Berlin, D 10623 Berlin, Germany

(Received 6 November 1996)

By exploiting the extremely large effective cross sections (10^{-17}–10^{-16} cm^2/molecule) available from surface-enhanced Raman scattering (SERS), we achieved the first observation of single molecule Raman scattering. Measured spectra of a single crystal violet molecule in aqueous colloidal silver solution using one second collection time and about 2×10^5 W/cm^2 nonresonant near-infrared excitation show a clear "fingerprint" of its Raman features between 700 and 1700 cm^{-1}. Spectra observed in a time sequence for an average of 0.6 dye molecule in the probed volume exhibited the expected Poisson distribution for actually measuring 0, 1, 2, or 3 molecules. [S0031-9007(97)02524-6]

Stabilization of an optical microscope to 0.1 nm in three dimensions. Applied Optics 46, 421-427, 2007.

Stabilization of an optical microscope to 0.1 nm in three dimensions

Ashley R. Carter, Gavin M. King, Theresa A. Ulrich, Wayne Halsey, David Alchenberger, and Thomas T. Perkins

Mechanical drift is a long-standing problem in optical microscopy that occurs in all three dimensions. This drift increasingly limits the resolution of advanced surface-coupled, single-molecule experiments. We overcame this drift and achieved atomic-scale stabilization (0.1 nm) of an optical microscope in 3D. This was accomplished by measuring the position of a fiducial mark coupled to the microscope cover slip using back-focal-plane (BFP) detection and correcting for the drift using a piezoelectric stage. Several significant factors contributed to this experimental realization, including (i) dramatically reducing the low frequency noise in BFP detection, (ii) increasing the sensitivity of BFP detection to vertical motion, and (iii) fabricating a regular array of nanometer-sized fiducial marks that were firmly coupled to the cover slip. With these improvements, we achieved short-term (1 s) stabilities of 0.11, 0.10, and 0.09 nm (rms) and long-term (100 s) stabilities of 0.17, 0.12, and 0.35 nm (rms) in x, y, and z, respectively, as measured by an independent detection laser.

OCIS codes: 170.0180, 180.3170, 170.4520, 170.6900.

Million-fold Preconcentration of Proteins and Peptides by Nanofluidic Filter. Analytical Chemistry 77 (14), 4293-4299, 2005.

Anal. Chem. **2005,** *77,* 4293−4299

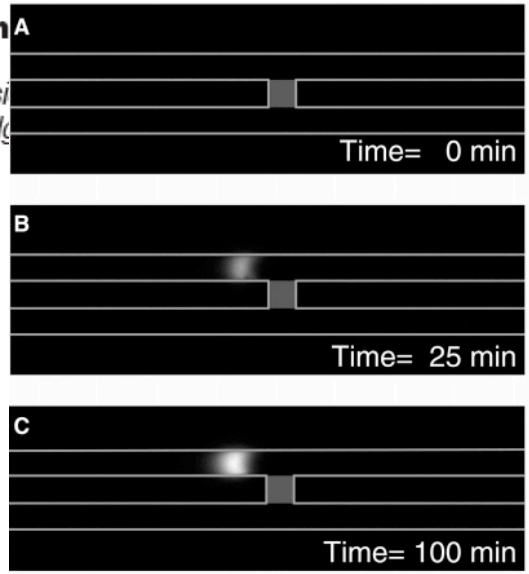
Accelerated Articles

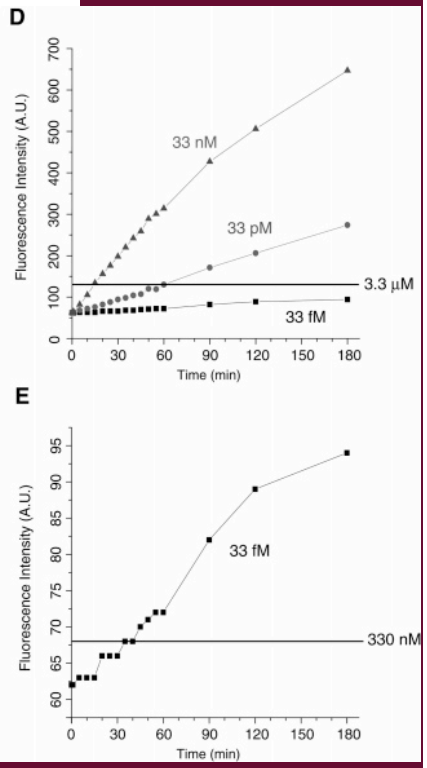
Million-fold Preconcentration of Proteins and Peptides by Nanofluidic Filter

Ying-Chih Wang,[†,‡] Anna L. Stevens,[§] and Jongyoon Han

Department of Mechanical Engineering, Biological Engineering Divisi
Computer Science, Massachusetts Institute of Technology, Cambrid

We have developed a highly efficient microfluidic sample preconcentration device based on the electrokinetic trapping mechanism enabled by nanofluidic filters. The device, fabricated by standard photolithography and etching techniques, generates an extended space charge region within a microchannel, which was used to both collect and trap the molecules efficiently. The electrokinetic trapping and collection can be maintained for several hours, and concentration factors as high as 10^6–10^8 have been demonstrated. This device could be useful in various bioanalysis microsystems, due to its simplicity, performance, robustness, and integrabilty to other separation and detection systems.

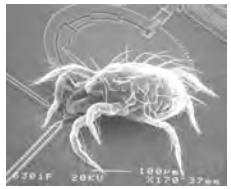




Introduction to nanoscience and nanotechnology

The Scale of Things – Nanometers and More

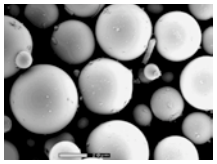
Things Natural



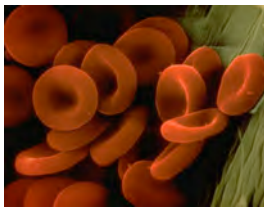
Dust mite
200 μm

Ant
~ 5 mm

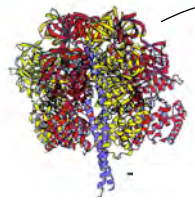
Human hair
~ 60-120 μm wide



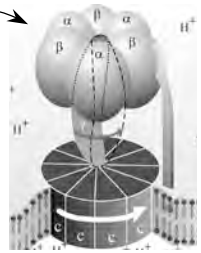
Fly ash
~ 10-20 μm



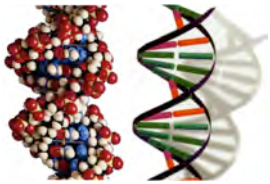
Red blood cells
(~7-8 μm)



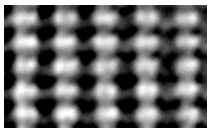
~10 nm diameter



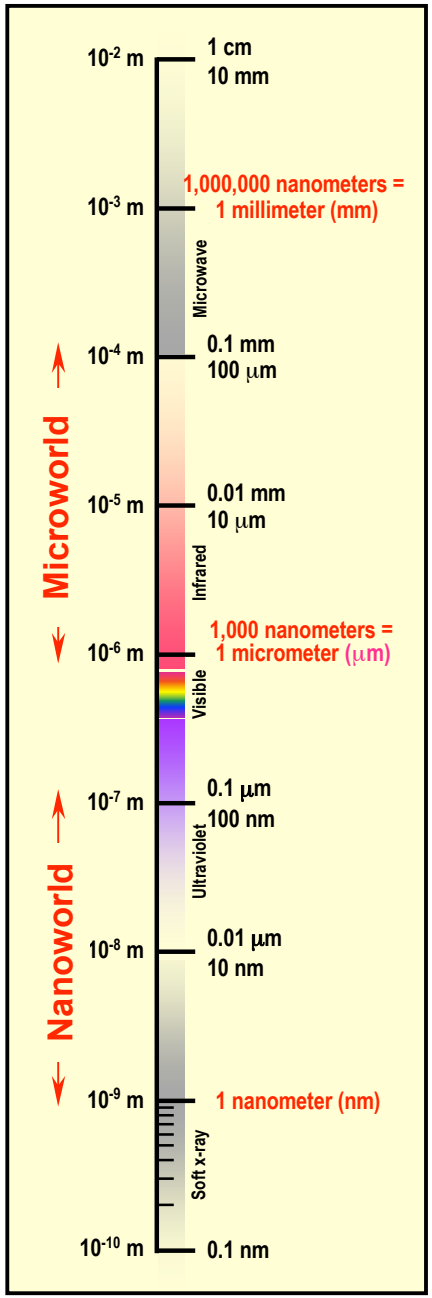
ATP synthase



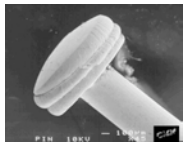
DNA
~2-1/2 nm diameter



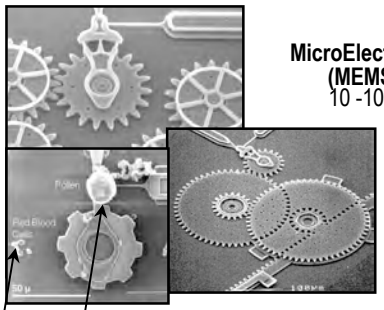
Atoms of silicon
spacing 0.078 nm



Things Manmade



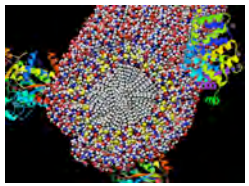
Head of a pin
1-2 mm



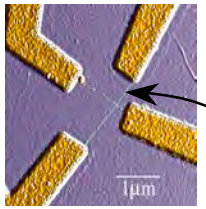
MicroElectroMechanical (MEMS) devices
10 -100 μm wide

Pollen grain
Red blood cells

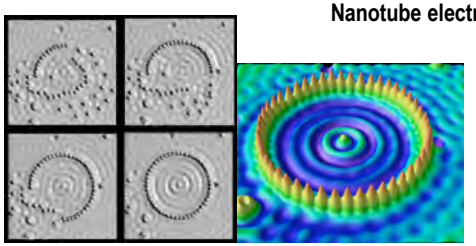
Zone plate x-ray "lens"
Outer ring spacing ~35 nm



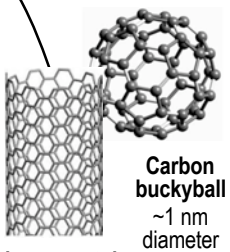
Self-assembled,
Nature-inspired structure
Many 10s of nm



Nanotube electrode



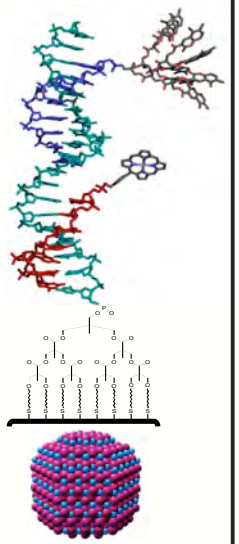
Quantum corral of 48 iron atoms on copper surface positioned one at a time with an STM tip
Corral diameter 14 nm



Carbon buckyball
~1 nm diameter

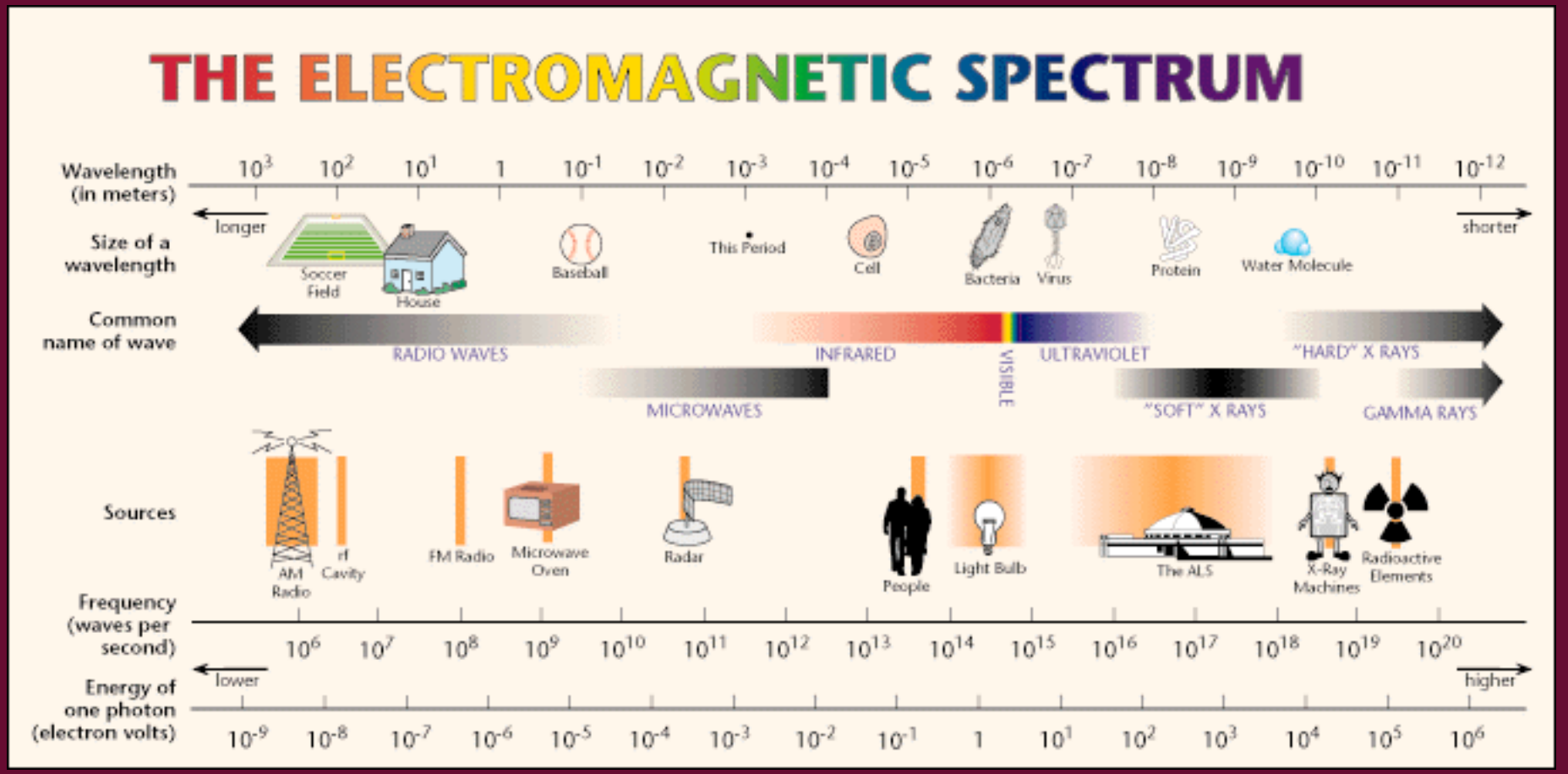
Carbon nanotube
~1.3 nm diameter

The Challenge



Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.

Office of Basic Energy Sciences
Office of Science, U.S. DOE
Version 05-26-06, pmd



http://www.lbl.gov/MicroWorlds/ALSTool/EMSpec/EMSpec2.html

Light used to "see" an object must have a wavelength about the same size as or smaller than the object.

$E = h\nu$

The Electromagnetic Spectrum

Wavelength in meters	Name	Uses
10^{-15} (size of a nucleus) -10^{-11}	Gamma Rays	Cancer Treatment
10^{-10} (size of an atom)	X-Rays	Materials testing Medical x-rays
10^{-8}	Ultraviolet	Germicidal, "black light", suntan
10^{-6} (diameter of a bacteria)	Visible Color	Optics
10^{-5} - 10^{-3}	Infrared	Human body radiation
10^{-2} (size of a mouse)	Microwave	Microwave ovens, atomic clocks
10^{0} (one meter, the size of a man)	-	Radar, Television, F.M. Radio, International Short-wave
10^{3} (size of a village)	Radio frequency (RF)	A.M. Radio
10^{6} (distance from Washington D.C. to Chicago)	Audio frequency	Long-wave broadcast
10^{8} (distance to the moon)	-	Brain waves

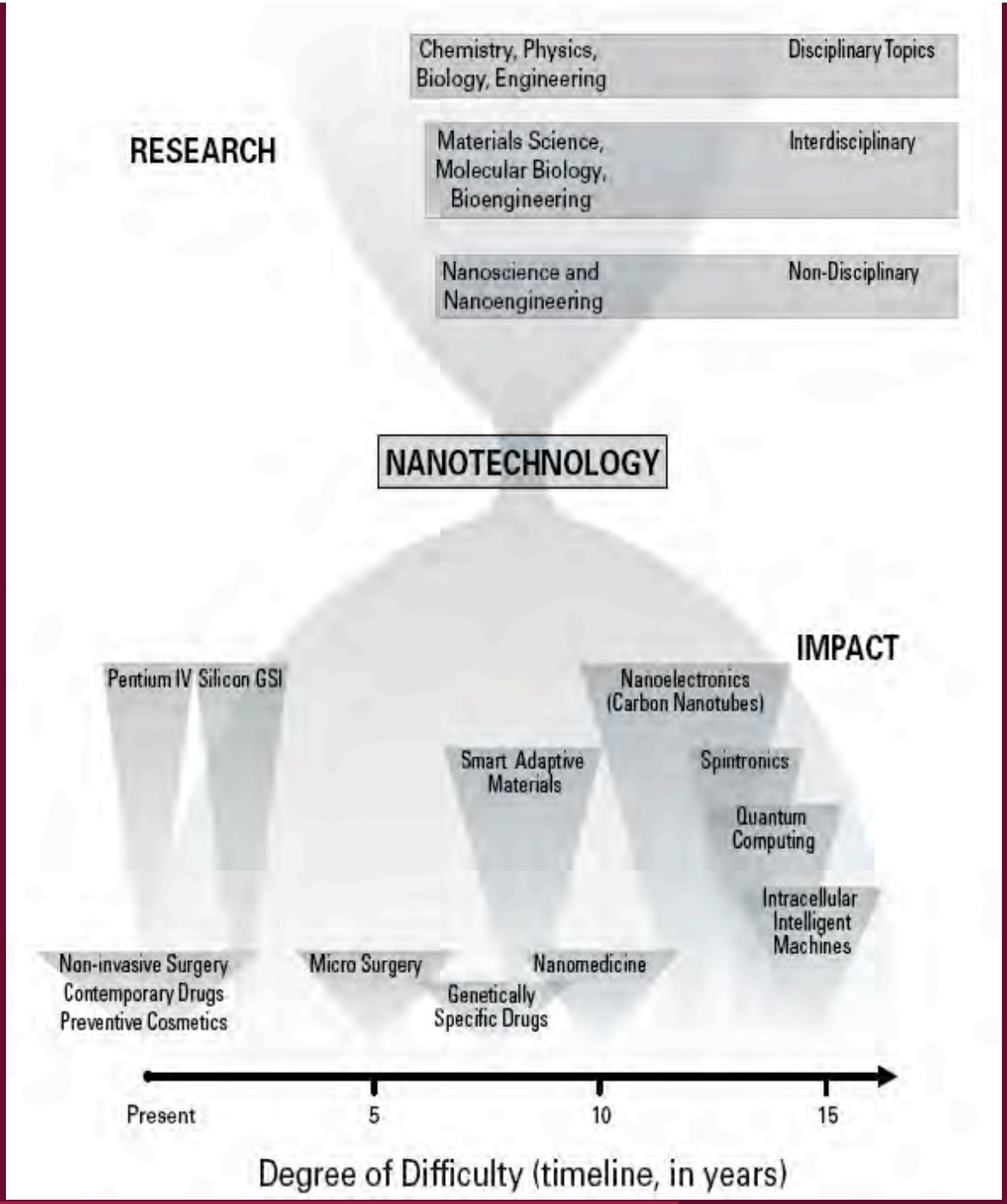
Specific Visible Colors

Color	Wavelength (nm)
Ultraviolet	380-280
Violet	430-390
Indigo	450-440
Blue	480-460
Green	530-490
Yellow	580-550
Orange	640-590
Red	750-650
Infrared	1000-750

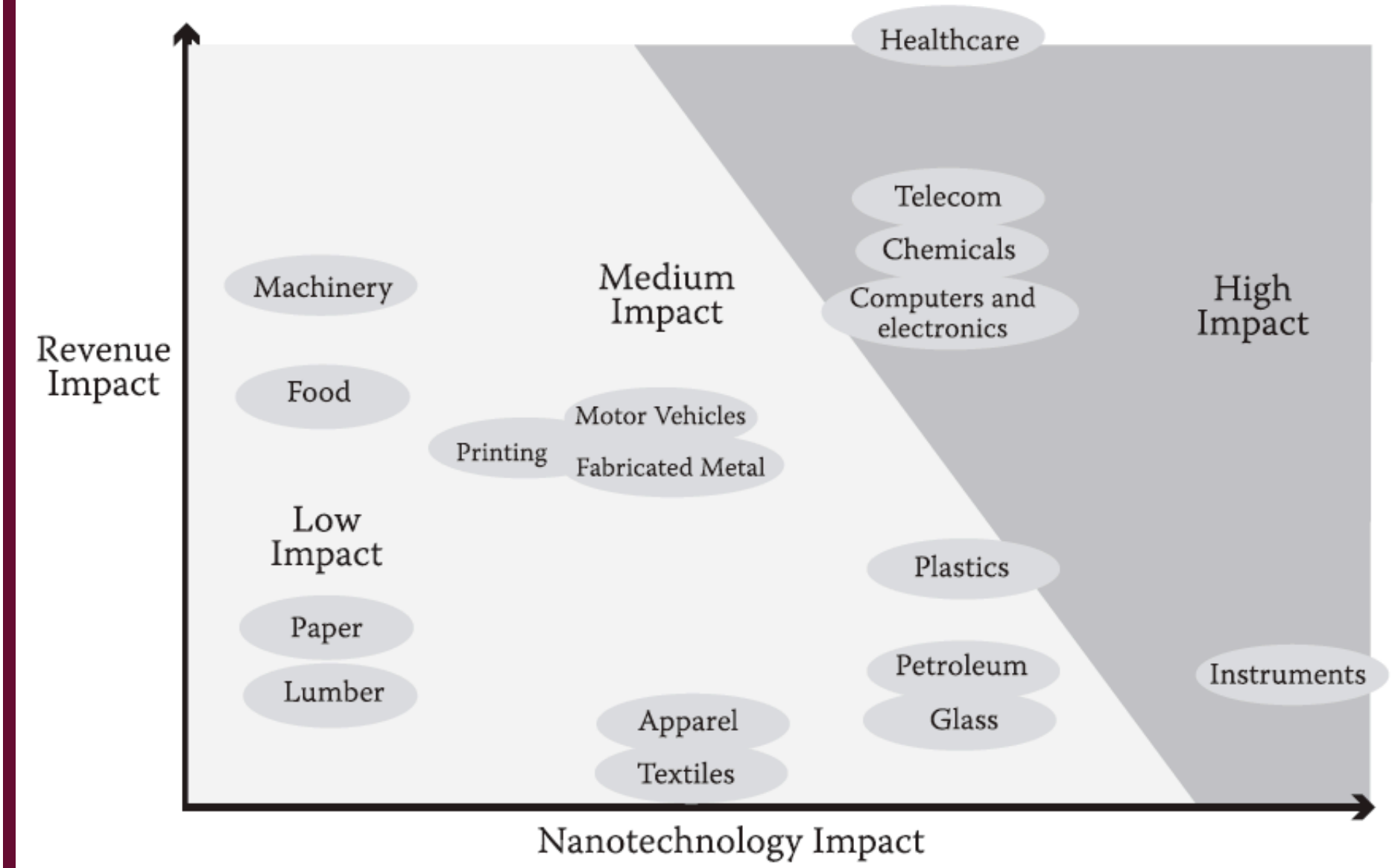
Note: Ultraviolet and infrared are given as a point of reference. They are not visible colors.

A glimpse of Research areas

- Nanophotonics: biophotonics, near-field optics (NSOM), plasmonics, photonic crystals, micro- and nanoscale photonic devices, optical MEMS, optical sensing,
- Nanomaterials: nanoparticles, nanowires, carbon nanotubes, catalyst, synthesis, polymer chemistry, biomotors, nanoscale metrology and sensors; nanodevices for biological, electrical, mechanical, photonic and hybrid systems, including low-dimensional quantum and molecular-scale devices
- Nanobio-mechanics: optical/magnetic tweezer, AFM
- Micro/nanofluidics
- Single molecule biophysics/biochemistry
- Biosensing: Micro/nano array, optical (SERS, TERS), bioelectronic, magnetic, mechanical, thermal.
- Advanced Bioimaging: Fluorescence spectroscopy, FRET, 2-photon, X-ray
- Molecular modeling: MD, QM/MM, Brownian Dynamics, Monte-Carlo
- Design, manufacturing, and integration of nanoscale devices and systems: Nanoimprint lithography, EBL, FIB, Dip-Pen Lithography, photonic nanolithography, macro-micro-nano interface/packaging, NEMS, device computation (CFD)


Chemistry, Physics, Biology, Engineering
Disciplinary Topics
RESEARCH
Materials Science, Molecular Biology, Bioengineering
Interdisciplinary
Nanoscience and Nanoengineering
Non-Disciplinary
NANOTECHNOLOGY
IMPACT
Pentium IV Silicon GSI
Nanoelectronics (Carbon Nanotubes)
Smart Adaptive Materials
Spintronics
Quantum Computing
Intracellular Intelligent Machines
Non-invasive Surgery
Contemporary Drugs
Preventive Cosmetics
Micro Surgery
Genetically Specific Drugs
Nanomedicine
Present
5
10
15
Degree of Difficulty (timeline, in years)

Nanotechnology's Probable Business Impact in 2007

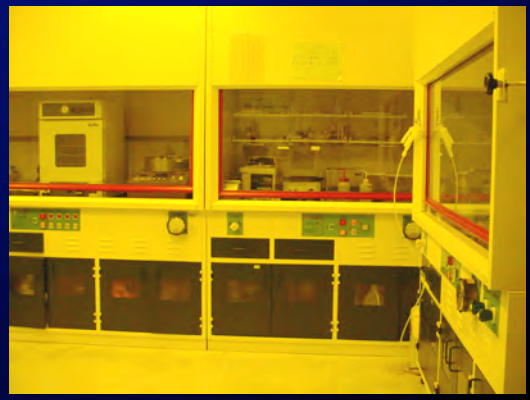


Device fabrication facilities in a class-1000 cleanroom

E-beam writer

Laser writer

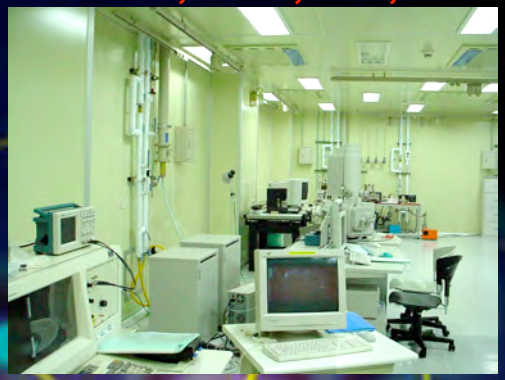
Photolithography yellow room



ICP Etcher

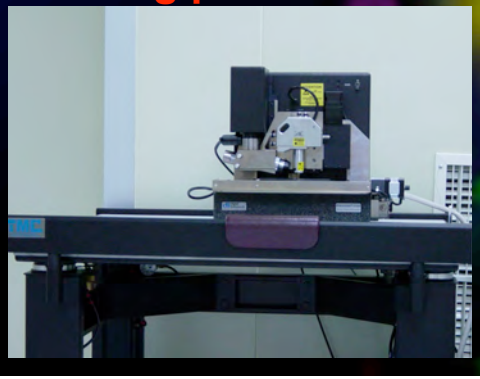
Evaporators

SEM, AFM, RIE, …



Dual beam FIB

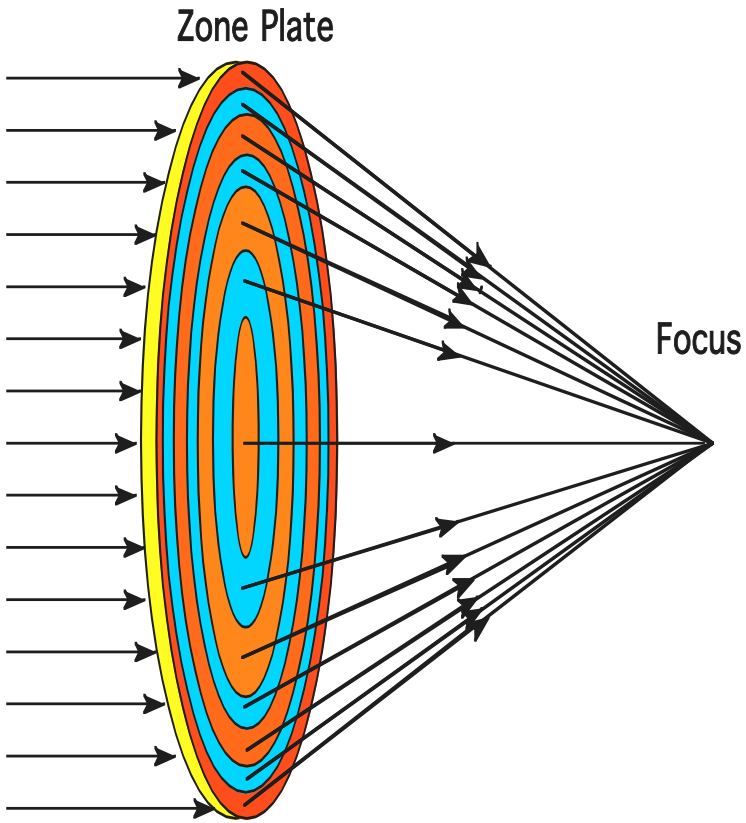
Scanning probe microscopy

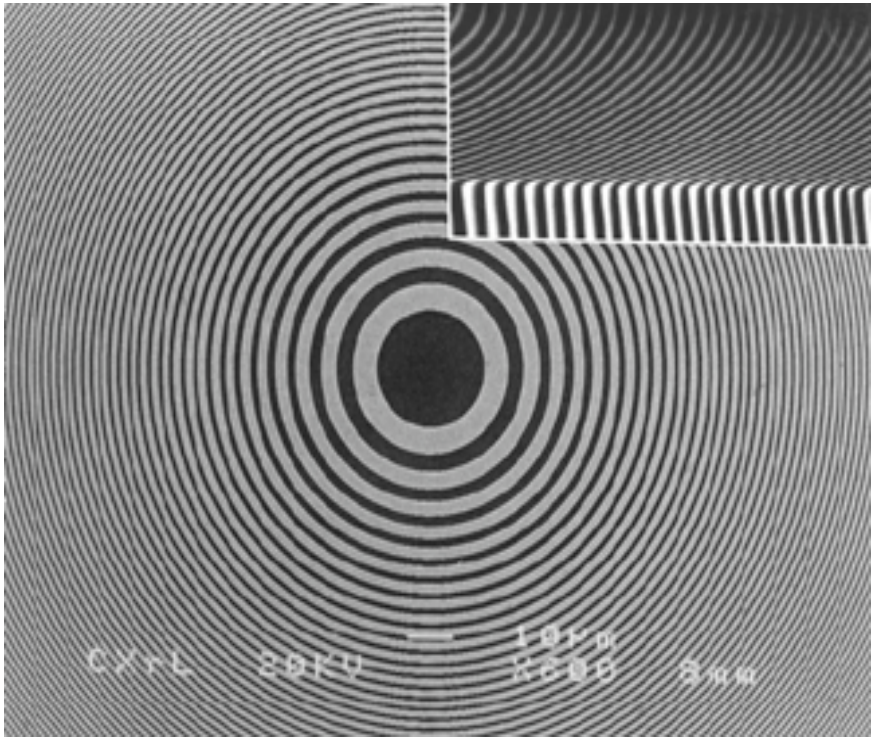


3D-FESEM

Zone Plate for X-ray Microscopy

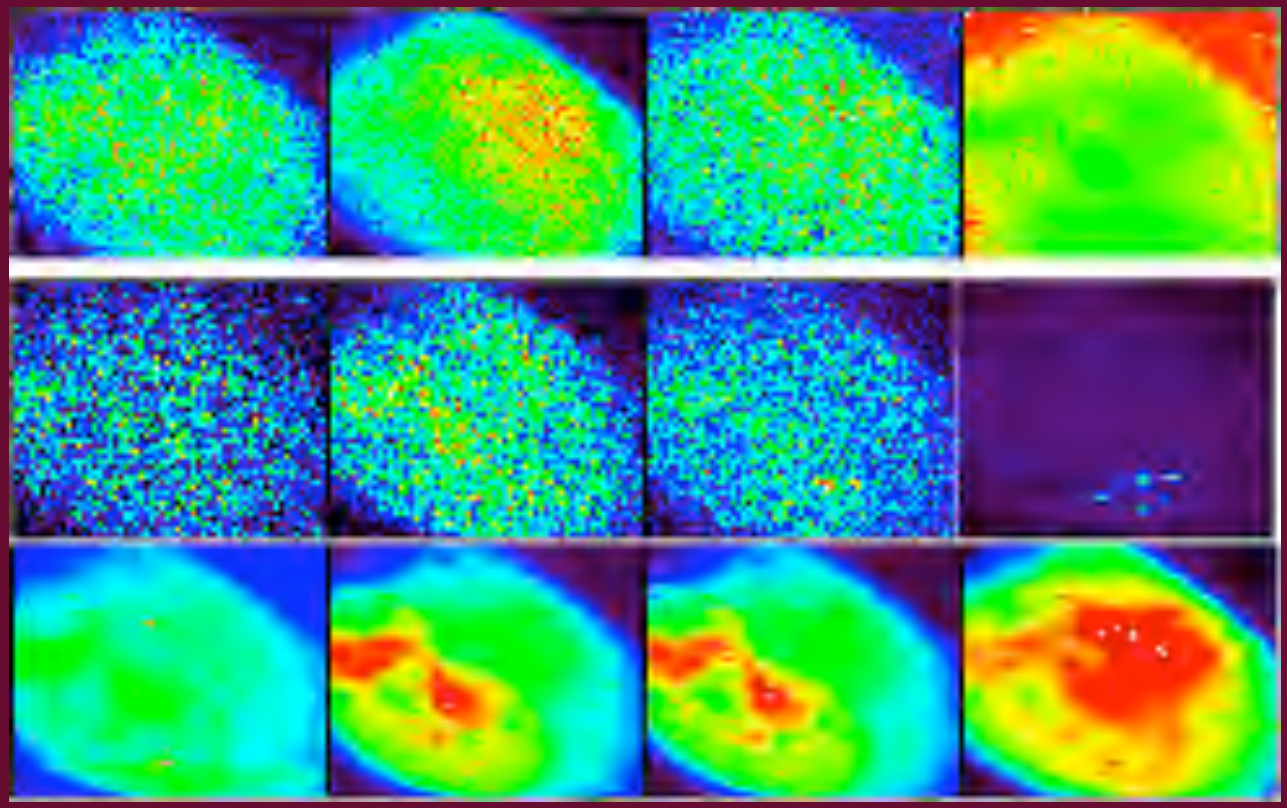
Yeukuang Hwu, IoP





When NA <<1, the ZP can be treated like an ordinary refractive lens, i.e., 1/q + 1/p = 1/f and M = p/q.

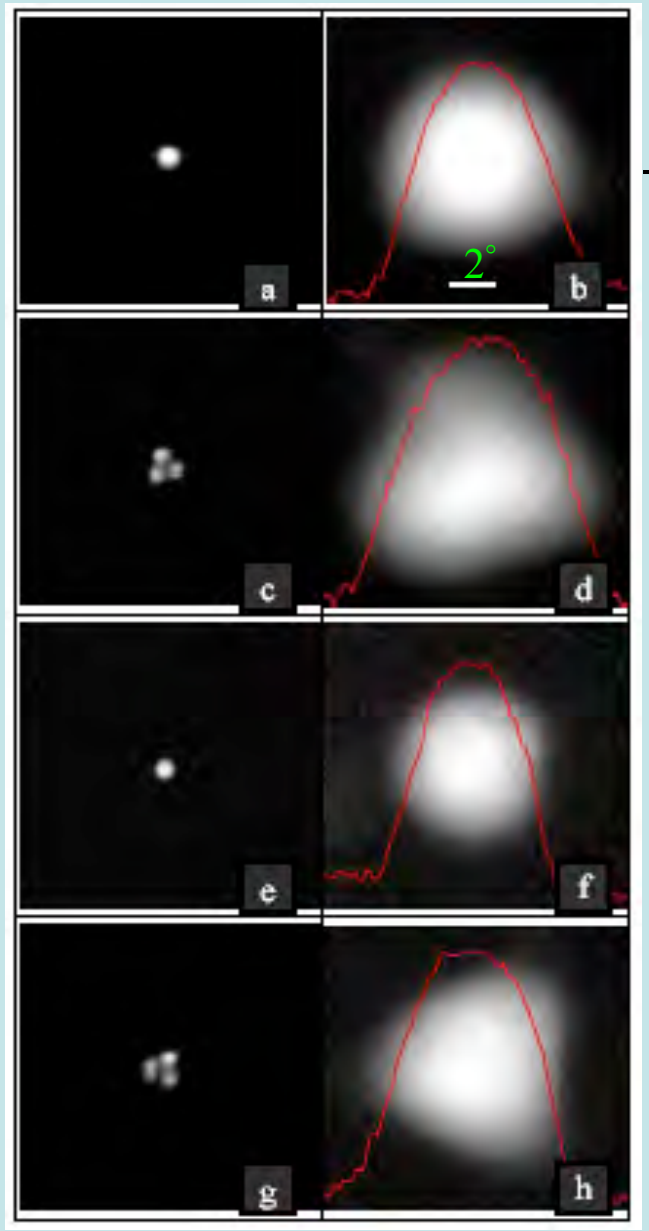
Fluorescence image of a single cell with 150 nm x-ray microbeam



Ca Os

Ca and Os distribution in a single cell

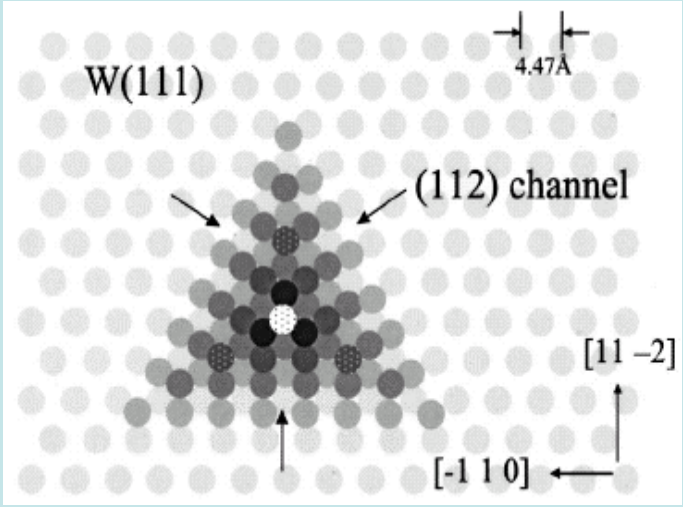
Electron Field Emission Pattern



Small opening angle

Pd/W(111) SAT: 6.6°

Pt/W(111) SAT: 5.6°



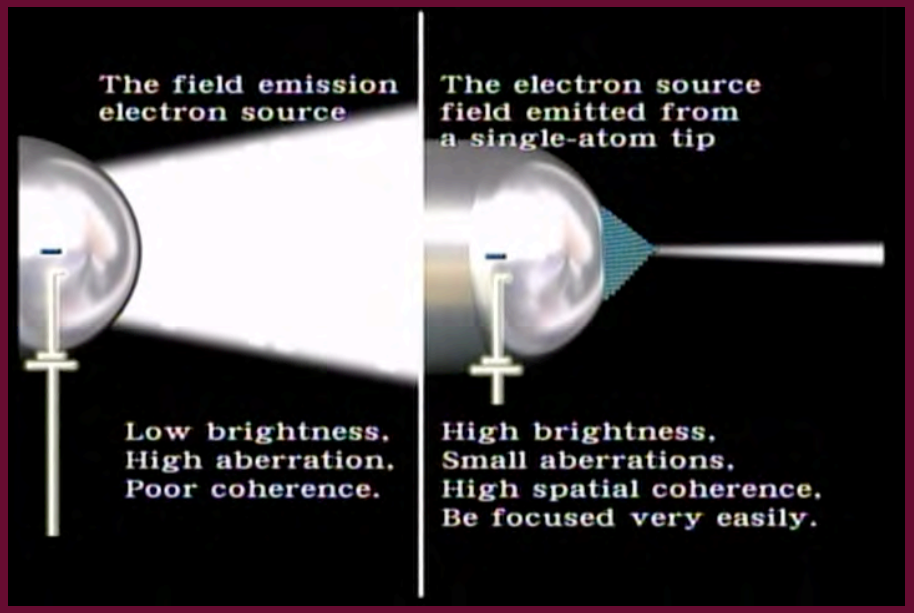
Fu, Cheng, Nien, Tsong,
Phys. Rev. B 64, 113401 (2001).

Noble Metal/W(111) Pyramidal Single-Atom Tips

Hong-Shi Kuo, Ing-Shouh Hwang, and T.T. Tsong

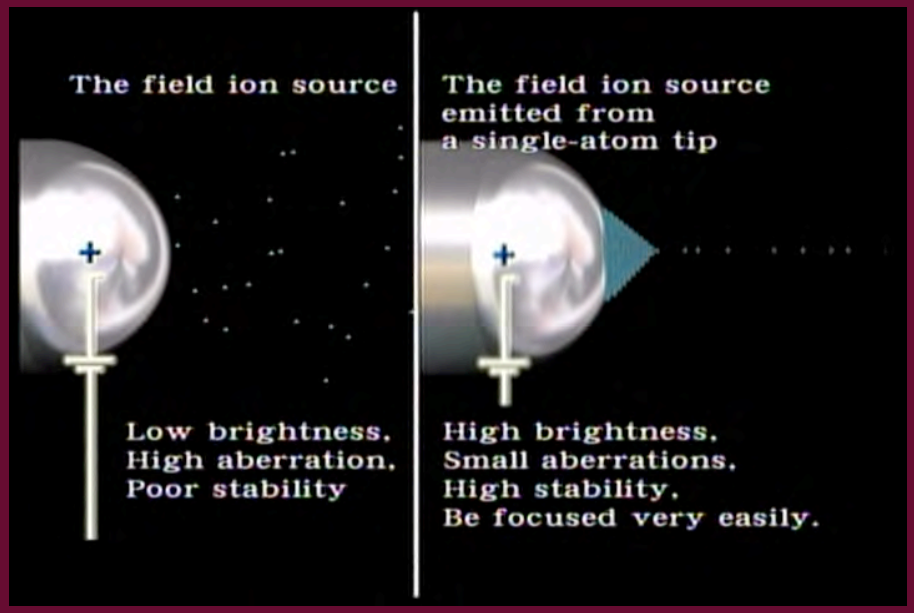
Traditional

Ideal electron point source



Traditional

Ideal ion point source



Peeling the nanotube from inside

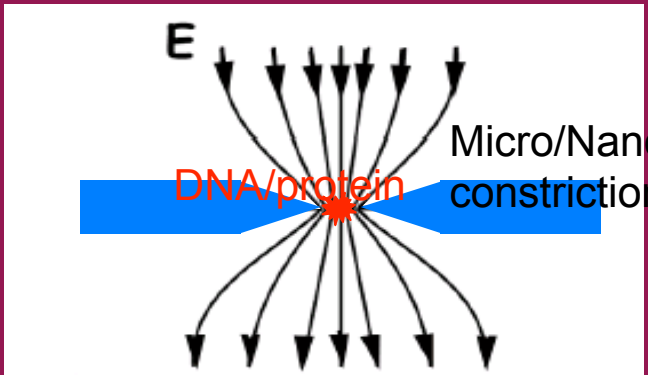
Micro/Nanoscale Molecular Traps

Chia-Fu Chou, IoP/RCAS

Insulator-based (electrodeless) dielectrophoresis (DEP)- use focused field to trap molecules

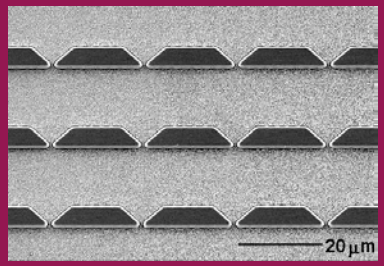
Dielectrophoretic force:

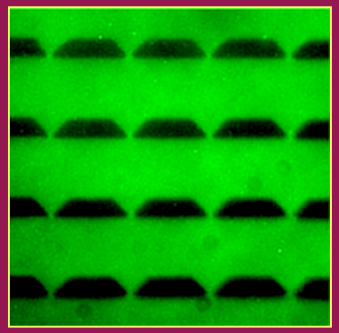
$$F \sim \nabla(E^2)$$



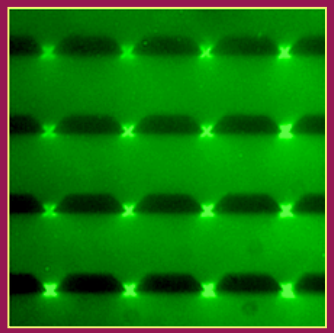
Electric field focused at the constriction

DNA trapping



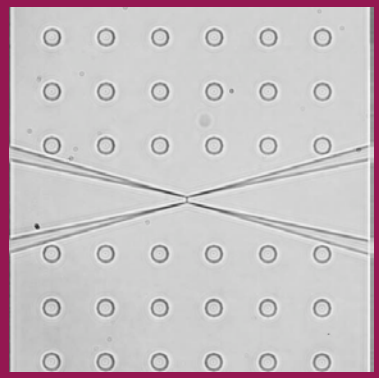


AC field off- no trapping



AC field on- trapping

Protein trapping



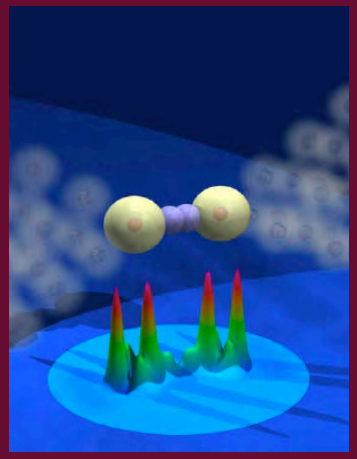
50 nm gap/250 nm deep

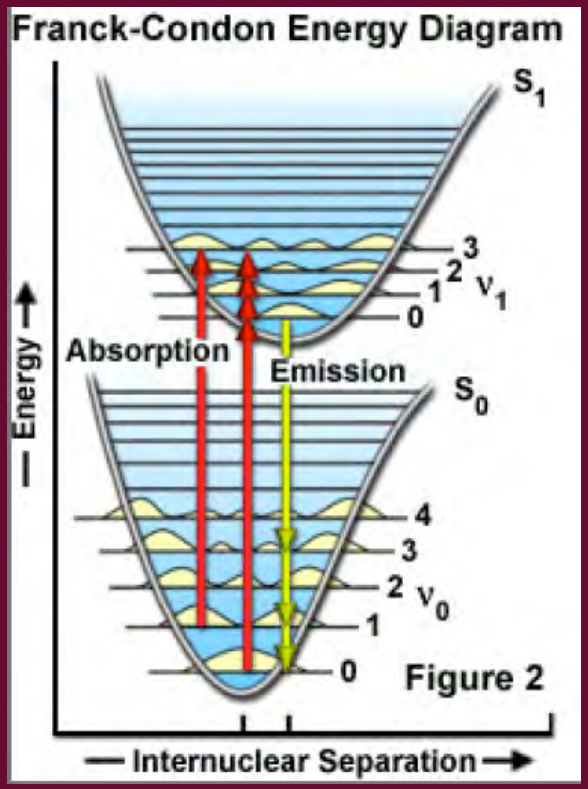
~ 1000x enrichment

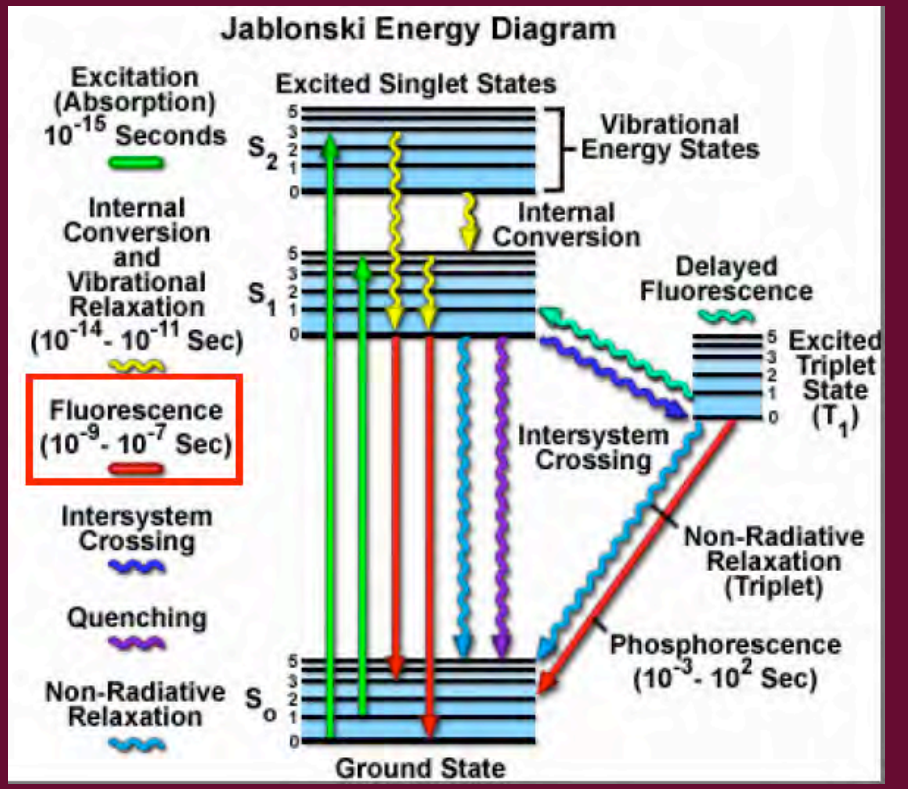
Alexa-488 labeled Streptavidin

Introduction to Fluorescence Microscopy

Franck-Condon and Jablonski Energy Diagram

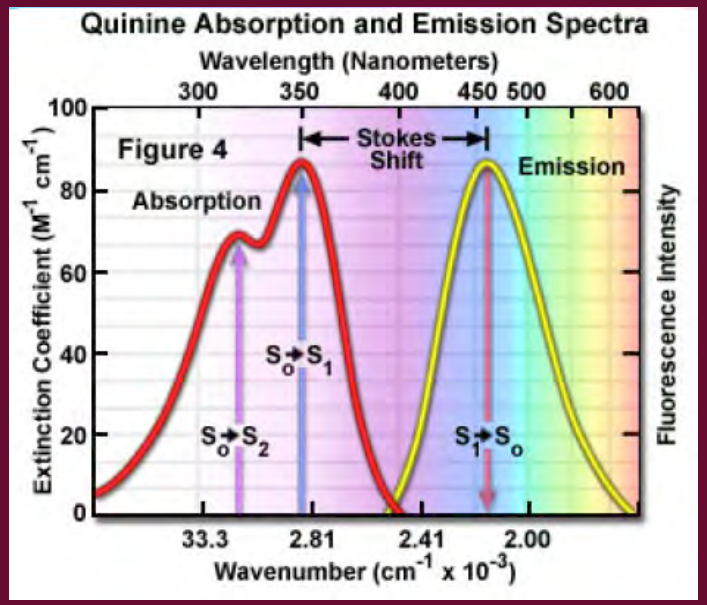


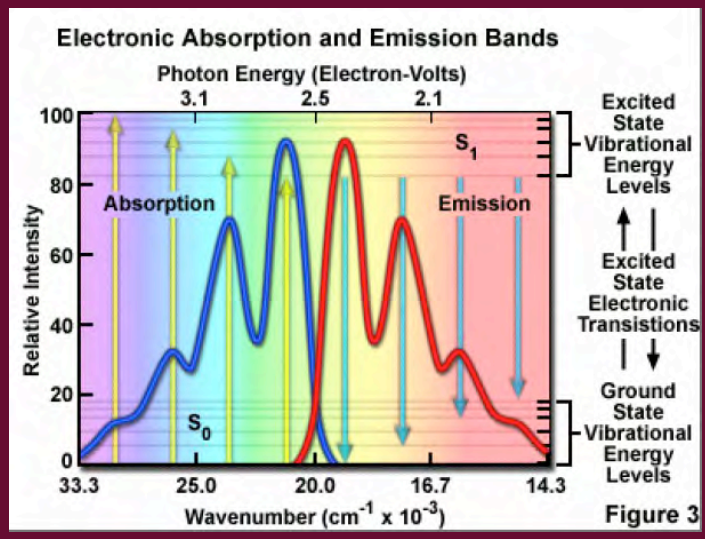




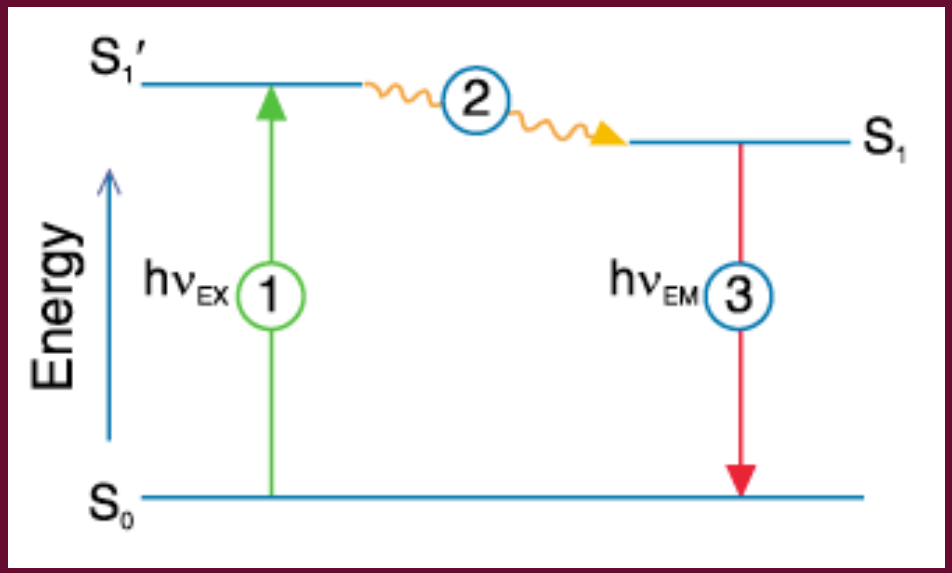
Golden Rules of Fluorescence

1. Stoke Shift: the emission is at longer wavelength (lower energy) than the excitation.
2. Internal Conversion: the shape of the emission spectrum does not vary when the excitation wavelength is changed
3. For a single emitting species, the excitation spectrum is the same as the absorption spectrum.
4. Mirror Symmetry: In many cases, the emission spectrum is a mirror image of the absorption/excitation spectrum



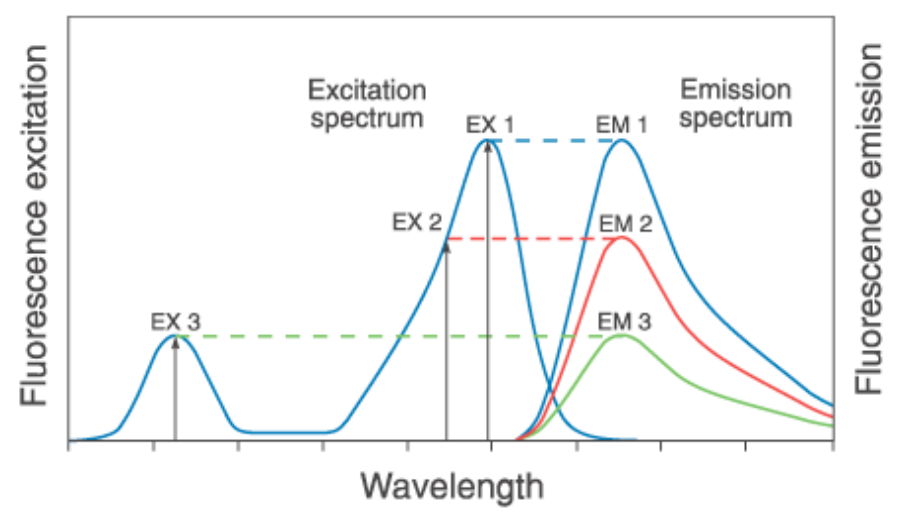


Quantum Efficiency

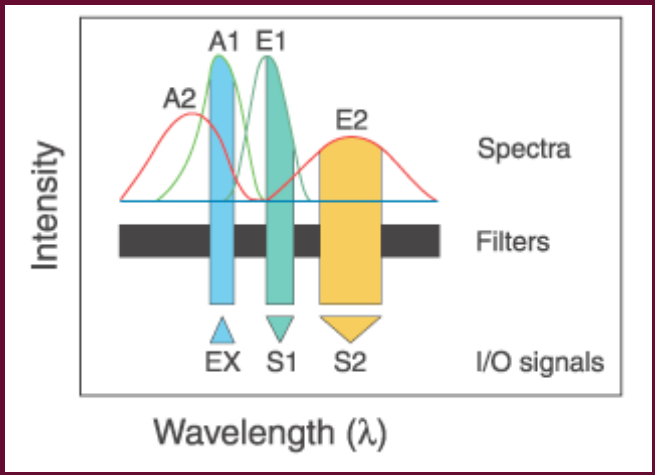


quantum efficiency (QE): light energy absorbed/ fluorescence emitted

QE determines how much of this absorbed light energy will be converted to fluorescence. The most efficient common fluorophores have a QE of ~0.3, but the actual value can be reduced by processes known as quenching, one of which is photobleaching.



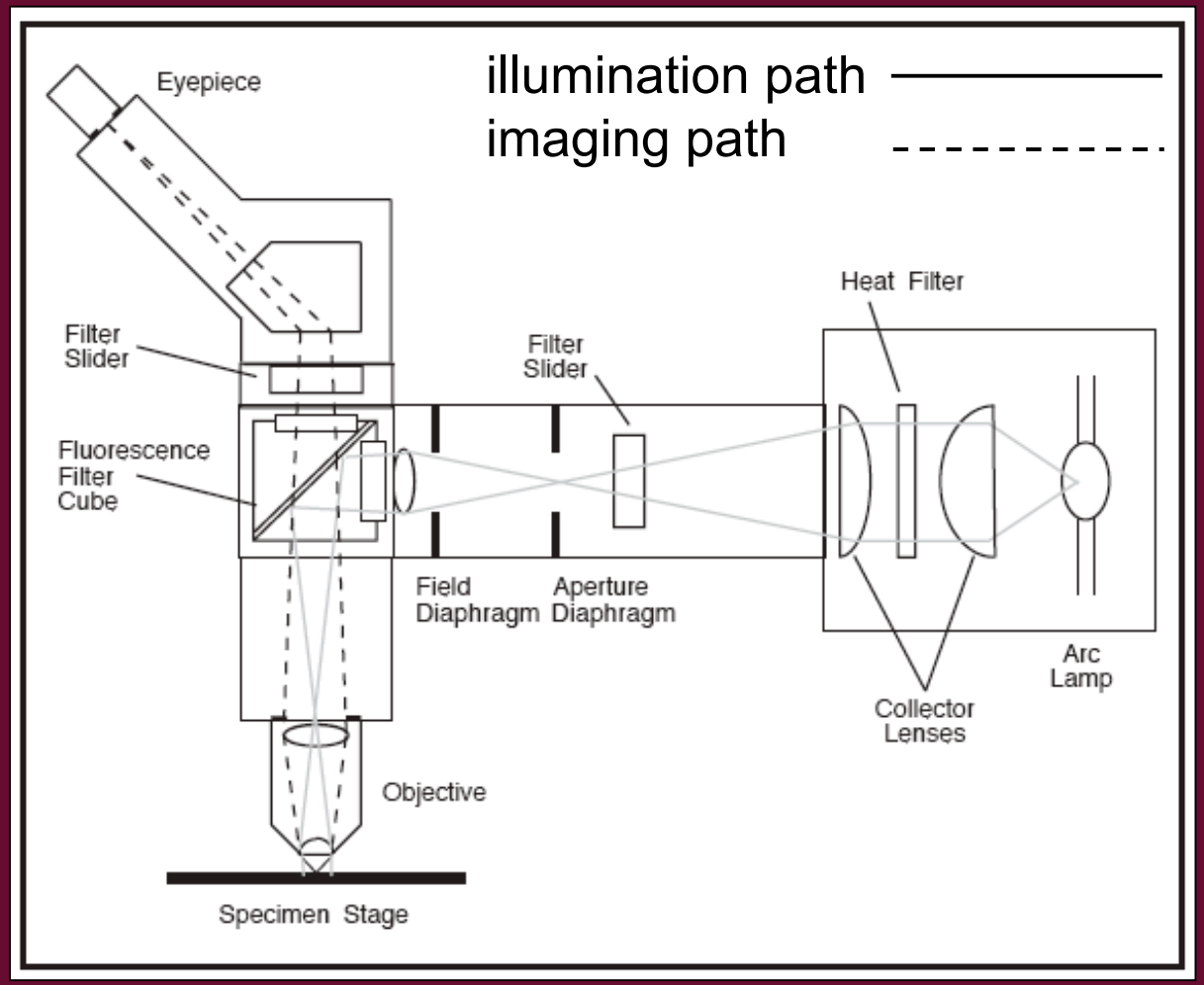
Excitation of a fluorophore at three different wavelengths (EX 1, EX 2, EX 3) does not change the emission profile but does produce variations in fluorescence emission intensity (EM 1, EM 2, EM 3) that correspond to the amplitude of the excitation spectrum.



Fluorescence detection of mixed species. Excitation (EX) in overlapping absorption bands A1 and A2 produces two fluorescent species with spectra E1 and E2. Optical filters isolate quantitative emission signals S1 and S2.

http://probes.invitrogen.com/handbook/

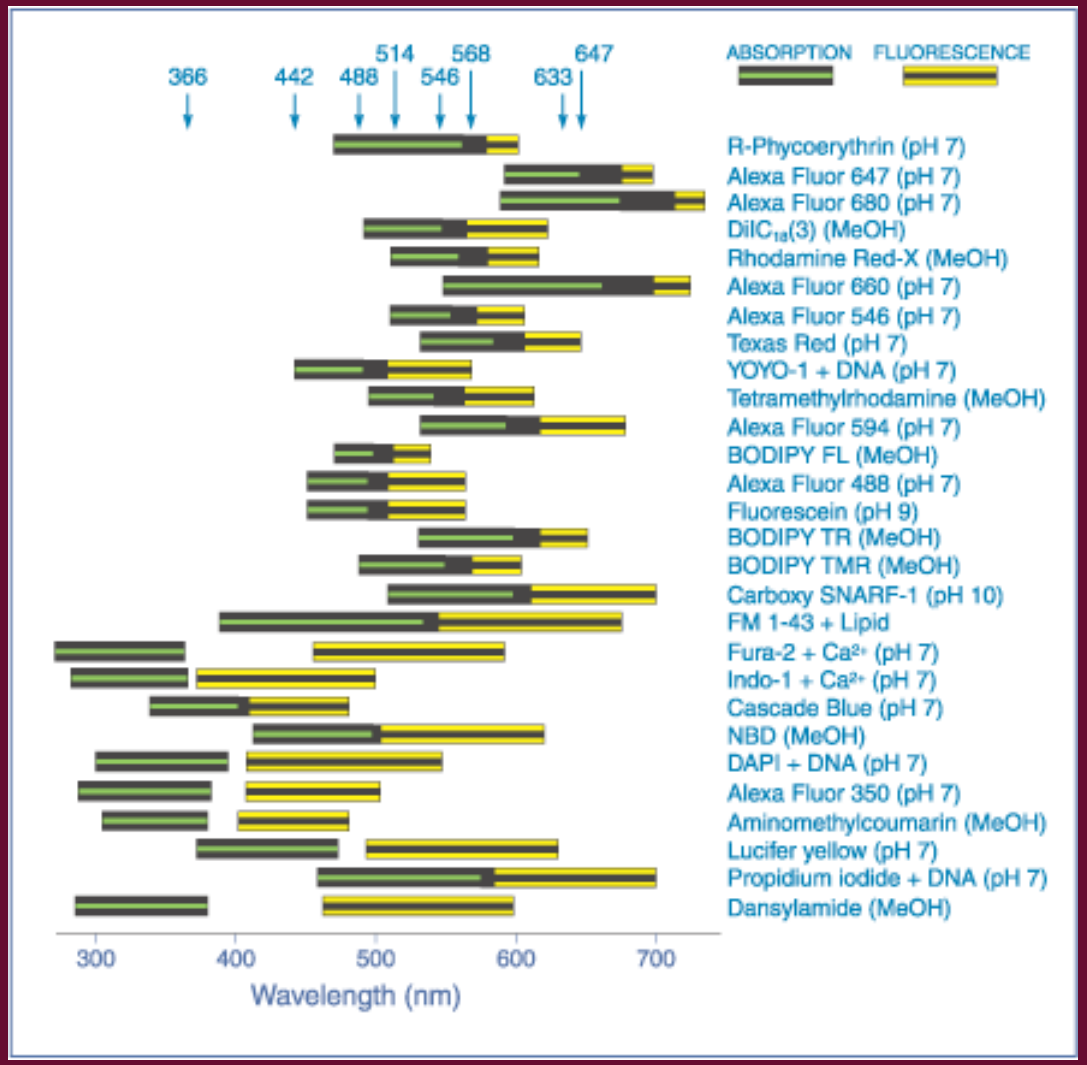
Schematic of a wide-field epifluorescence microscope



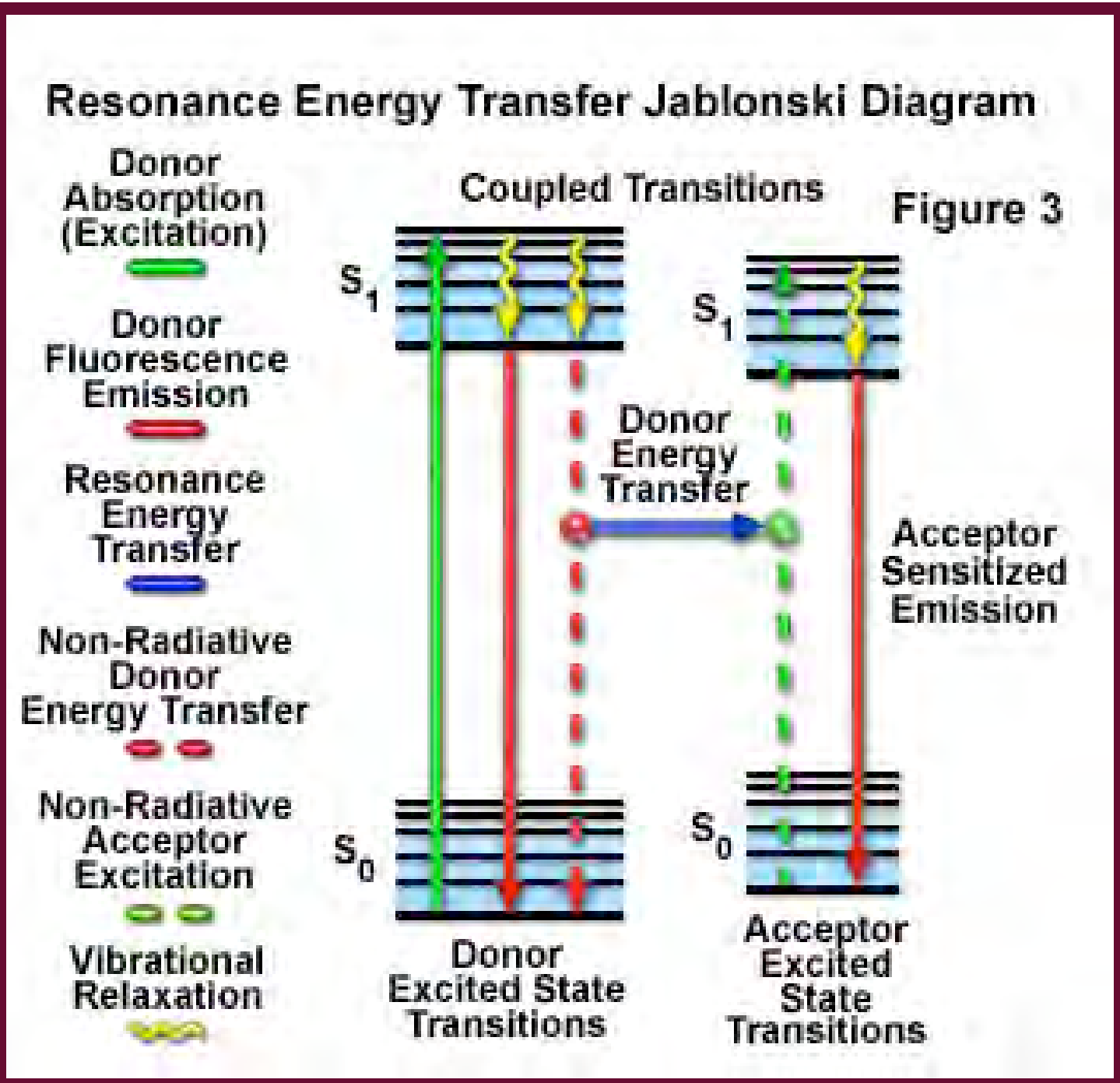
Chroma Technology Corp.: "Handbook of Optical Filters for Fluorescence Microscopy" (http://www.chroma.com/)

Schematic of a fluorescence filter cube

Know your friends….

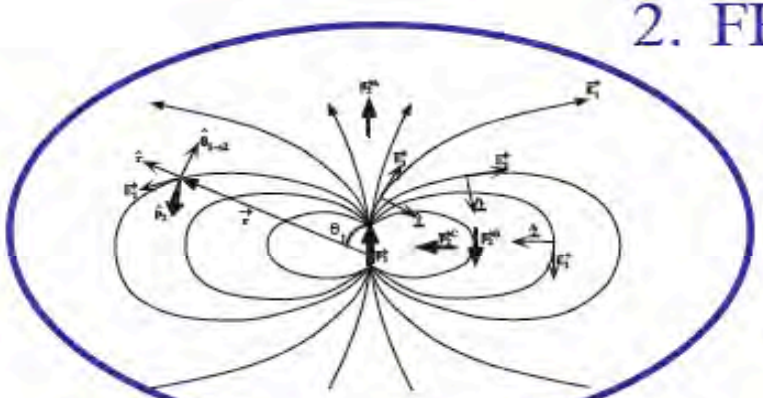


Absorption and fluorescence spectral ranges for 28 fluorophores of current practical importance. The range encompasses only those values of the absorbance or the fluorescence emission that are >25% of the maximum value. Fluorophores are arranged vertically in rank order of the maximum molar extinction coefficient (epsilonmax), in either methanol or aqueous buffer as specified. Some important excitation source lines are indicated on the upper horizontal axis.


Resonance Energy Transfer Jablonski Diagram
Donor Absorption (Excitation)
Donor Fluorescence Emission
Resonance Energy Transfer
Non-Radiative Donor Energy Transfer
Non-Radiative Acceptor Excitation
Vibrational Relaxation
Coupled Transitions
Figure 3
S1
S0
Donor Energy Transfer
Acceptor Sensitized Emission
Donor Excited State Transitions
Acceptor Excited State Transitions

Dipole-dipole interaction

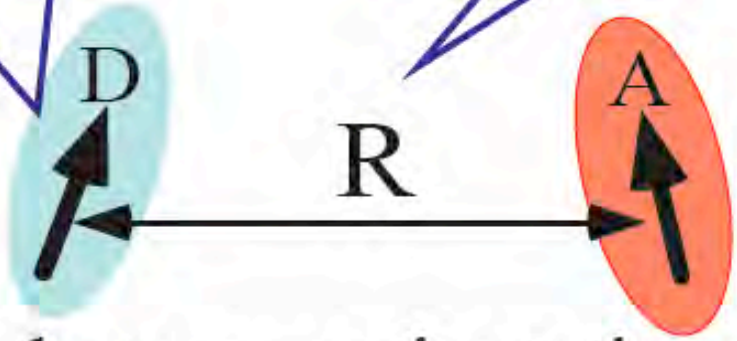
1. Van der Waal force
2. FRET



Rate of energy transfer

$$K_T = (1/\tau_D) \cdot [R_0/r]^6$$

R_0 is the Förster critical distance, τ_D is the donor lifetime in the absence of the acceptor, and r is the distance separating the donor and acceptor chromophores.



$$R_0 = 2.11 \times 10^{-2} \cdot [\kappa^2 \cdot J(\lambda) \cdot \eta^{-4} \cdot Q_D]^{1/6}$$

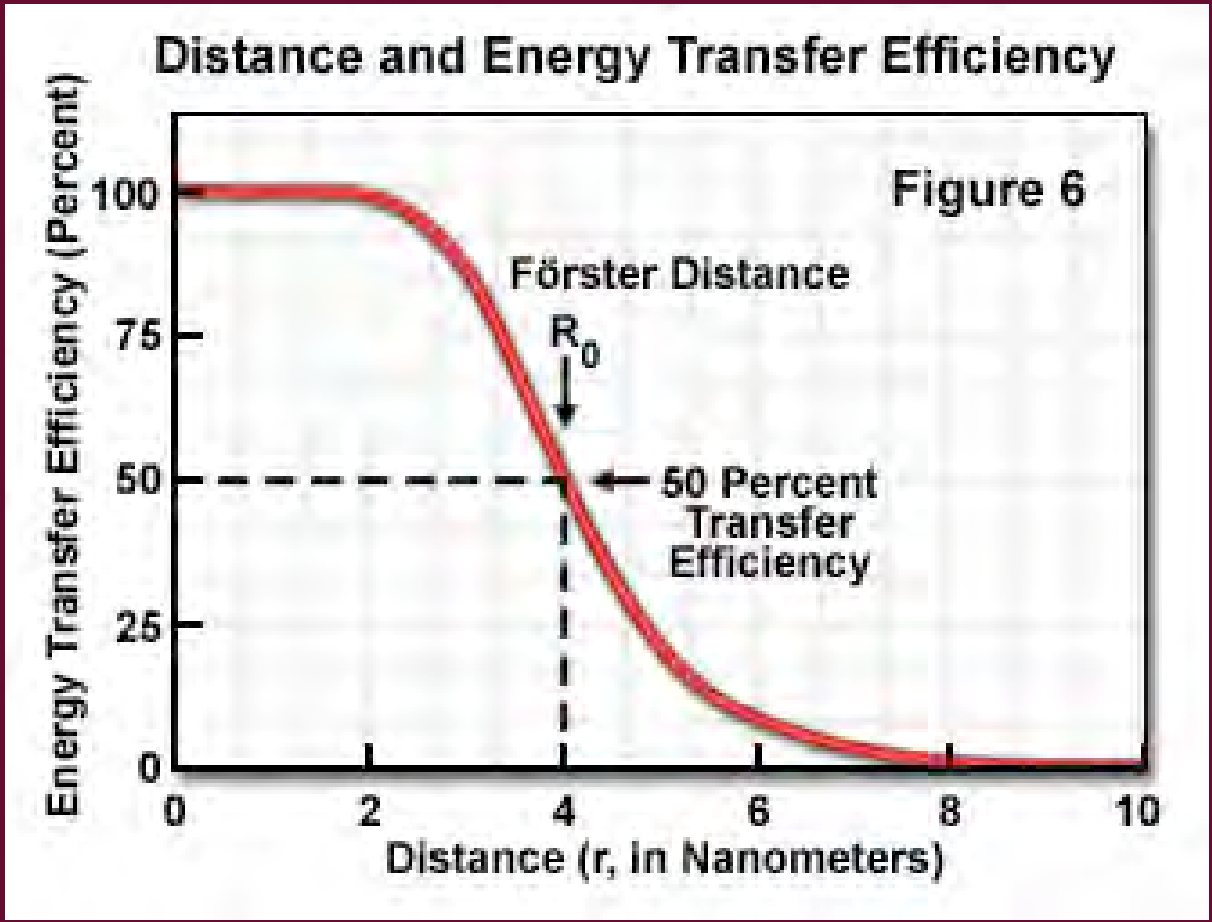
κ = kappa = orientation factor $\quad \kappa^2 \approx 2/3$

The efficiency of energy transfer, E(T), is a measure of the fraction of photons absorbed by the donor that are transferred to the acceptor, and is related to the donor-acceptor separation distance, r, by the equation:

$$r = R_0 \cdot [(1/E_T) - 1]^{1/6}$$

$$E_T = 1 - (\tau_{DA}/\tau_D)$$

where t(DA) is the donor lifetime in the presence of the acceptor and t(D) is the donor lifetime in the absence of the acceptor.



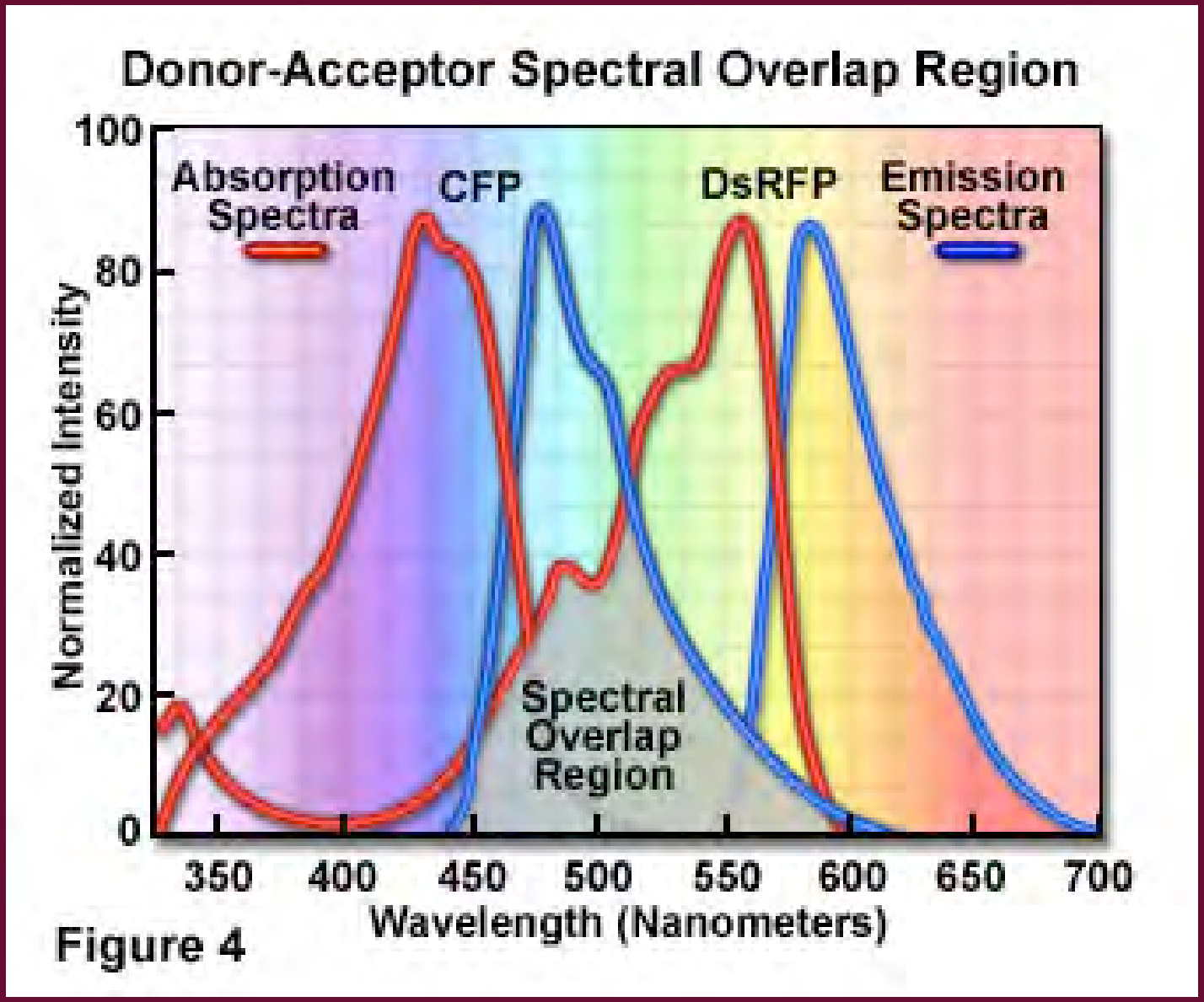
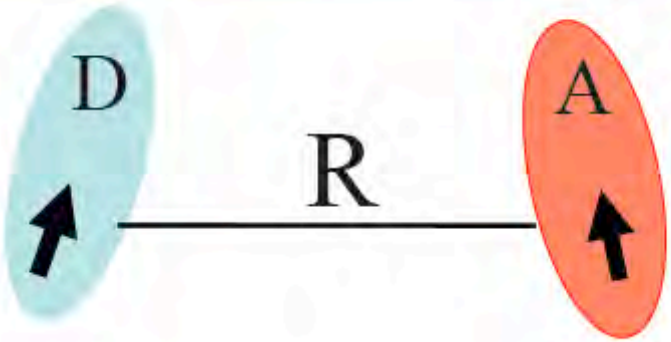

Donor-Acceptor Spectral Overlap Region
Absorption Spectra
CFP
DsRFP
Emission Spectra
Spectral Overlap Region
Normalized Intensity
100
80
60
40
20
0
350
400
450
500
550
600
650
700
Wavelength (Nanometers)

Figure 4

Conditions for FRET



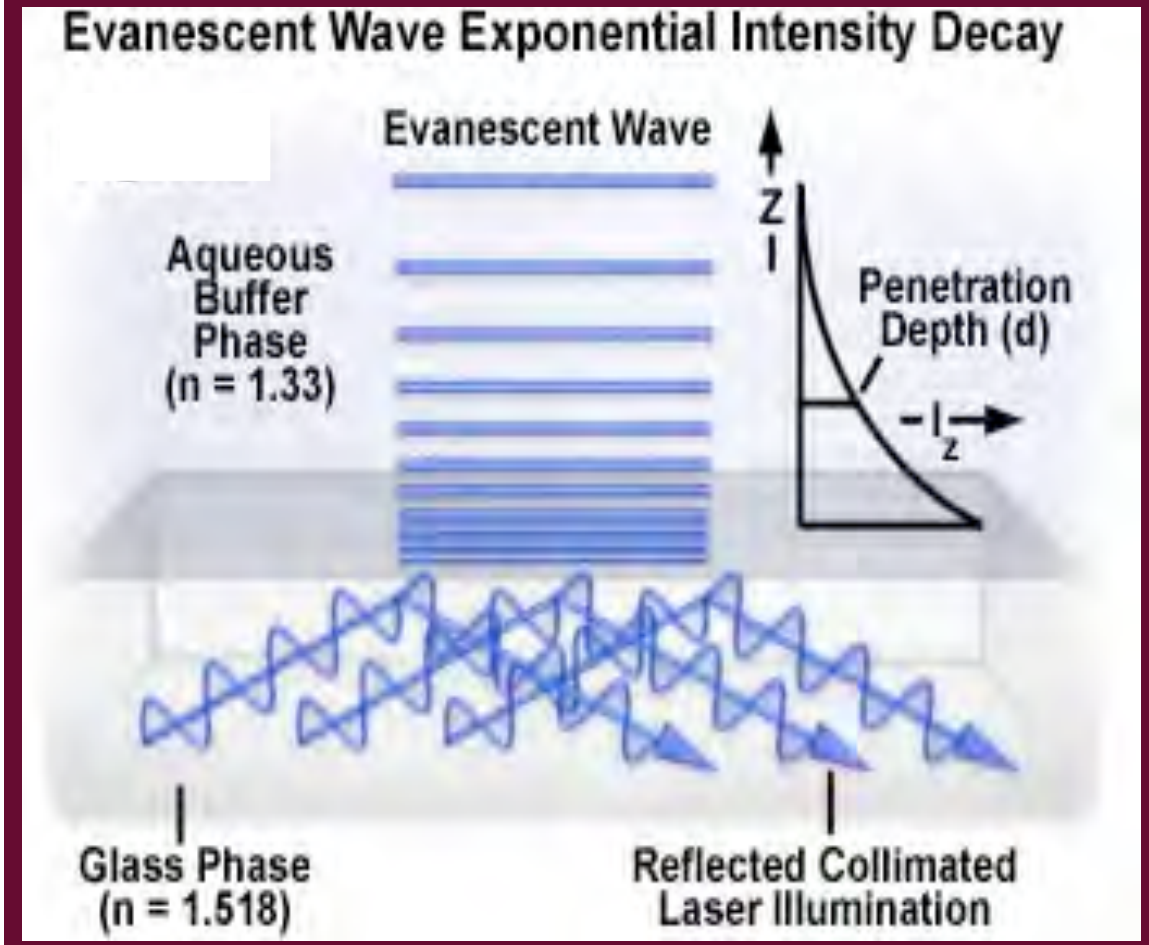
(1) the emission spectrum of D has to overlap the absorption spectrum of A by a considerable percentage (30%).

(2) the two fluorophores are within 1 to 10 nm of each other.

(3) when the D emission dipole moment, the A absorption dipole moment and their separation vectors are in favorable mutual orientation.

(4) when the emission of D has a reasonably high quantum yield.

Total Internal Reflection Fluorescence Microscopy (TIRFM)



$I(z) = I_o \exp(-z/d)$

$d = \lambda_o / 4\pi \cdot (n_1^2 \sin^2\theta_1 - n_2^2)^{-1/2}$

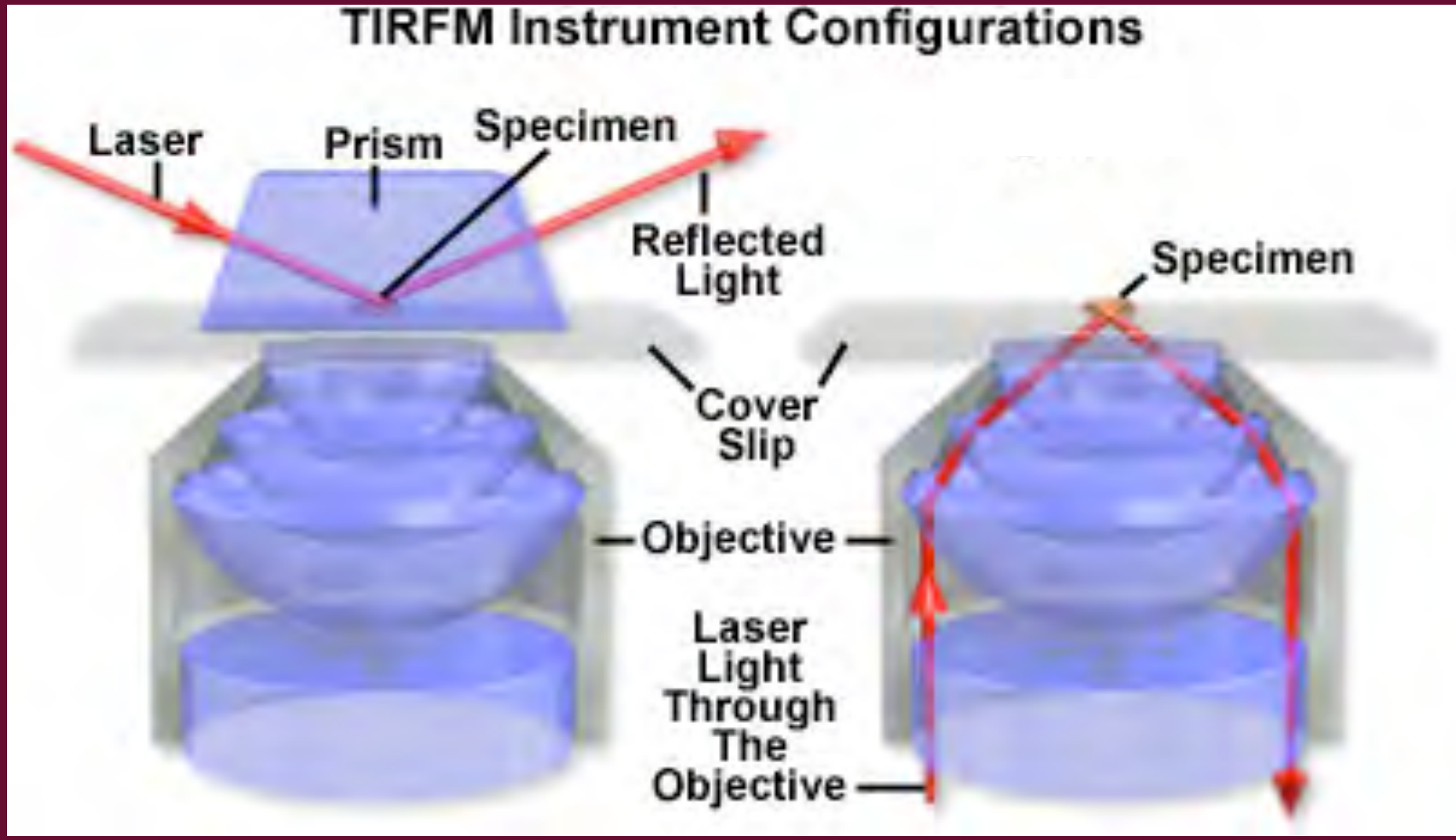
I_o: intensity at the interface

λ_o: the wavelength of incident light in a vacuum

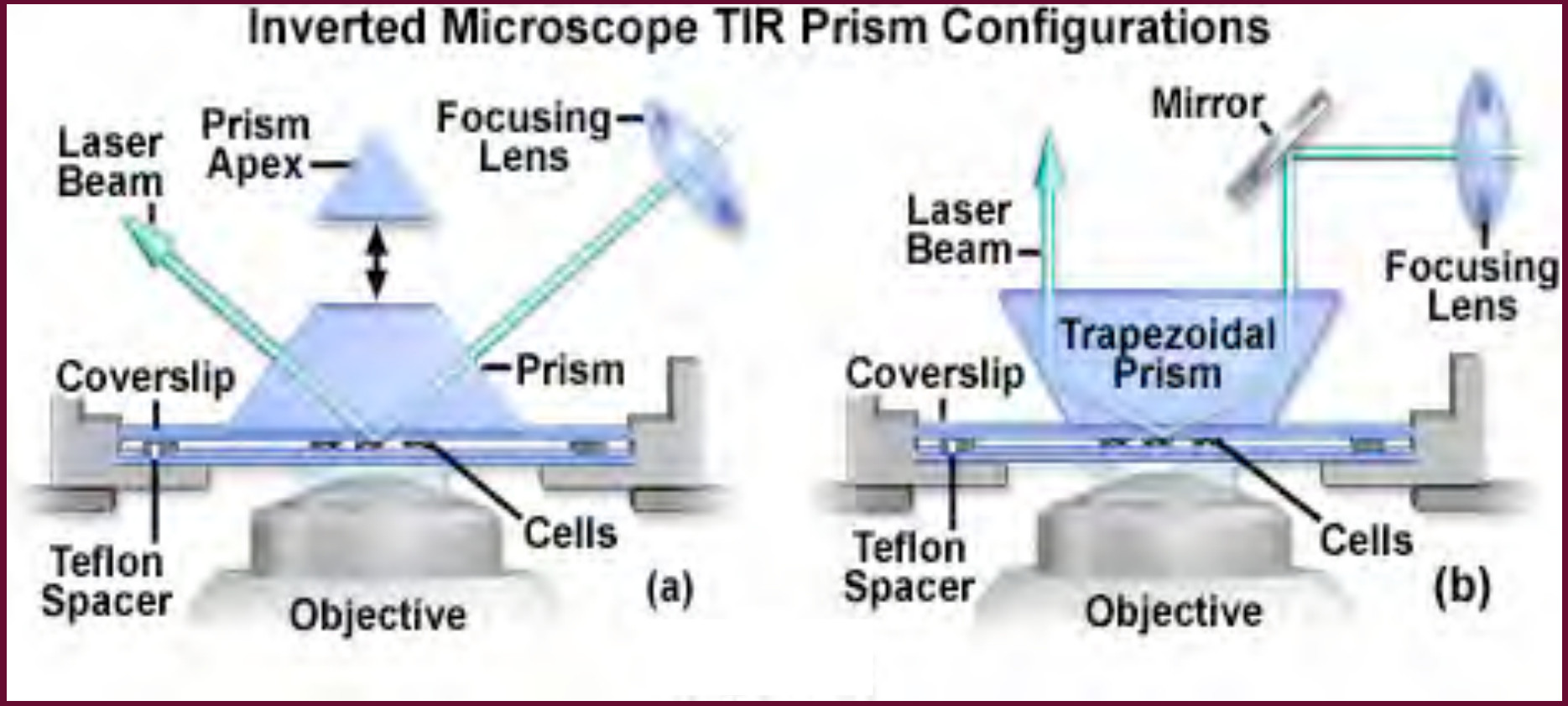
http://micro.magnet.fsu.edu

Fluorescence Intensity versus Penetration Depth

Distance (Nanometers)	Relative Intensity
1	0.99
10	0.92
100	0.43
1000	0.0002

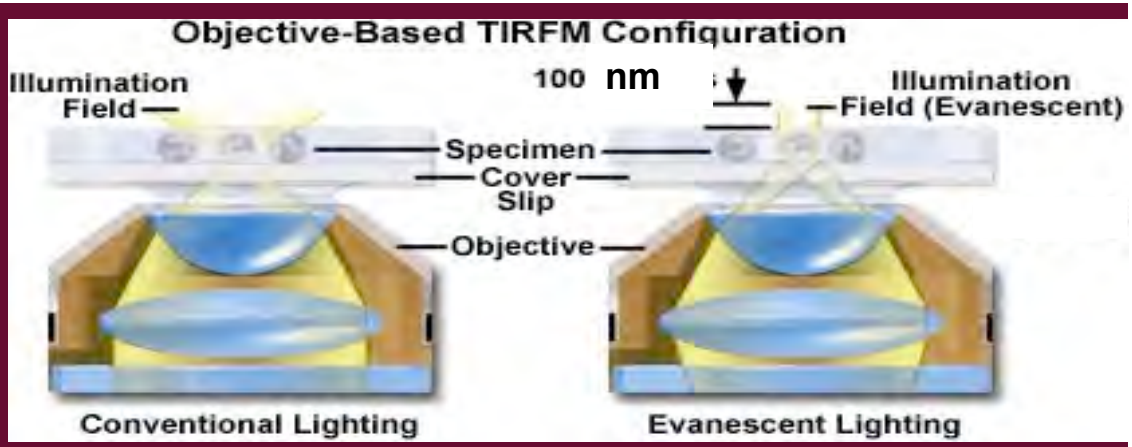

TIRFM Instrument Configurations
Laser
Prism
Specimen
Reflected Light
Specimen
Cover Slip
Objective
Laser Light Through The Objective

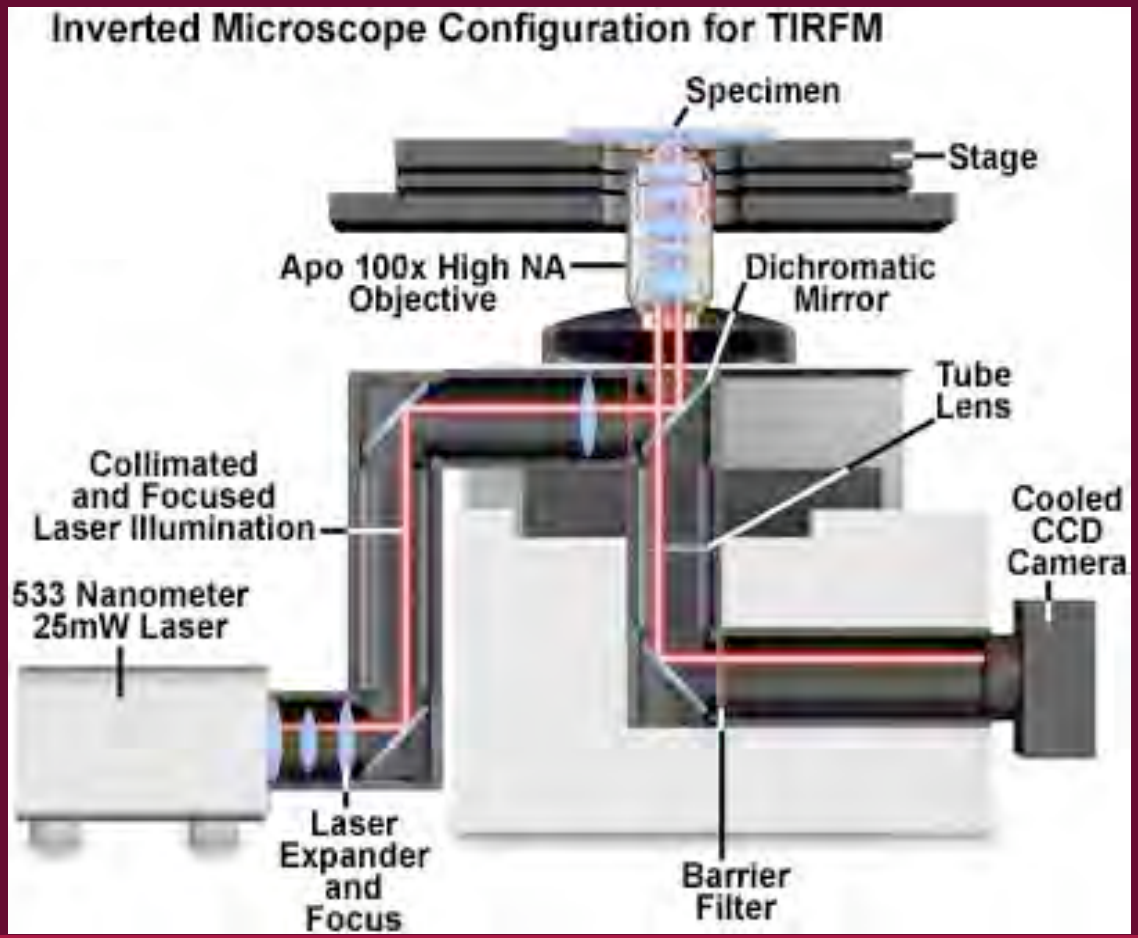
Prism-based TIRFM



http://micro.magnet.fsu.edu

Objective-based TIRFM

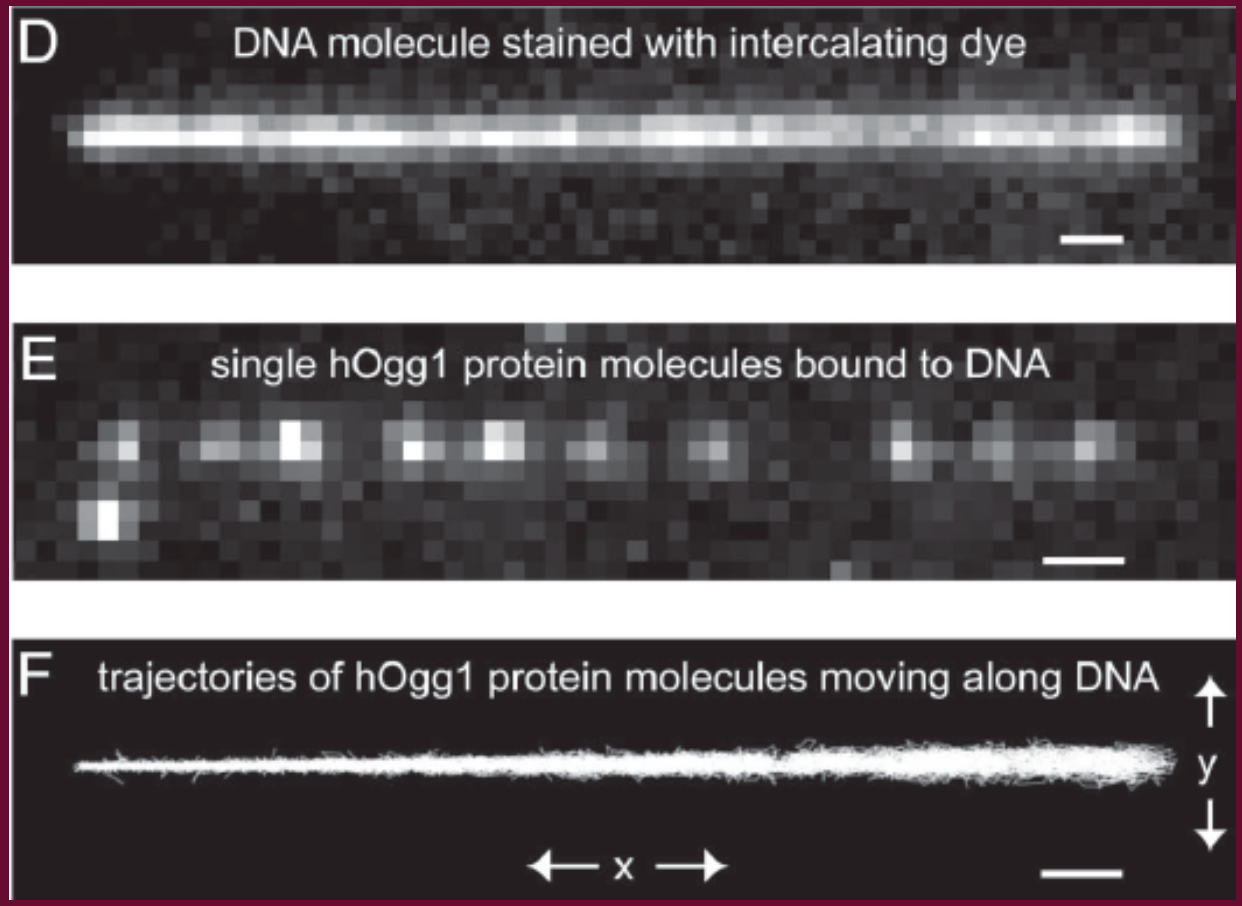




http://micro.magnet.fsu.edu

TIRFM application

DNA-protein interaction



Sunney Xie group, PNAS 2006

TIRFM REVIEW ARTICLES

Evanescent-wave microscopy: A new tool to gain insight into the control of transmitter release., Oheim, M., Loerke, D., Chow, R., and Stühmer, W., Philosophical Transactions of the Royal Society of London, Series B: 354, 307-318 (1999).

Surface fluorescence microscopy with evanescent illumination., Axelrod, D., Light Microscopy in Biology, Lacey, A. (ed), Oxford University Press, New York, 399-423 (1999).

Total reflection., Born, M. and Wolf, E., Principles of Optics, 7th Edition, Cambridge University Press, Cambridge, United Kingdom, 49-53 (1999).

Total internal reflection and the evanescent wave., Hecht, E., Optics, Addison Wesley Longman, Incorporated, New York, 121-127 (1998).

Total internal reflectance fluorescence microscopy., Tamm, L., Optical Microscopy: Emerging Methods and Applications, Herman, B. and Lemasters, J. (eds), Academic Press, New York, 295-337 (1993).

Total internal reflection fluorescence., Axelrod, D., Hellen, E., and Fulbright, R., Topics in Fluorescence Spectroscopy, Volume 3: Biochemical Applications, Lakowicz, J. (ed), Plenum Press, New York, 289-343 (1992).

Introduction to Micro/Nanofluidics

Navier-Stokes Equation for Newtonian fluid:

$$\rho \left[\frac{\partial \mathbf{v}}{\partial t} + (\mathbf{v} \cdot \nabla)\mathbf{v} \right] = \eta \nabla^2 \mathbf{v} - \nabla p.$$

$$Re = \frac{\rho v_s^2 / L}{\mu v_s / L^2} = \frac{\rho v_s L}{\mu} = \frac{v_s L}{\nu} = \frac{\text{Inertial forces}}{\text{Viscous forces}}$$

For water, $\mu = 0.01$ cm^2/s

D_h (μm)	U (cm/s)	Re ($D_h v/\mu$)
100	0.1	0.1
	100	100
200	0.1	0.2
	100	200
500	0.1	0.5
	100	500

Typical values of Reynolds number

* Spermatozoa ~ 1×10^{-2}
* Blood flow in brain ~ 1×10^{2}
* Blood flow in aorta ~ 1×10^{3}

Onset of turbulent flow ~ 2.3×10^{3} for pipe flow to 10^{6} for boundary layers

* Typical pitch in Major League Baseball ~ 2×10^{5}
* Person swimming ~ 4×10^{6}
* Blue Whale ~ 3×10^{8}
* A large ship (RMS Queen Elizabeth 2) ~ 5×10^{9}

Poiseuille flow

We'll start with the flow of a viscous fluid in a channel. The channel has a width in the *y*-direction of *a*, a length in the *z*-direction of l_z , and a length in the *x*-direction, the direction of flow, of . There is a pressure drop along the length of the channel, so that the constant pressure gradient is (such a pressure gradient could be supplied by gravity, for instance). Assuming the flow to be steady, $\partial \mathbf{v}/\partial t = 0$. Also, we'll assume that the flow is of the form ; then . The no-slip boundary condition at the top and bottom edges of the channel reads $v_x(y = \pm a/2) = 0$. The Navier-Stokes equation then becomes

$$\eta \frac{\partial^2 v_x}{\partial y^2} + \frac{\Delta p}{l_x} = 0. \qquad (3.14)$$

Integrating twice, we obtain

$$v_x(y) = -\frac{1}{2\eta}\frac{\Delta p}{l_x} y^2 + C_1 y + C_2, \qquad (3.15)$$

where C_1 and C_2 are integration constants. To determine these, we impose the boundary conditions to obtain

$$v_x(y) = \frac{1}{2\eta}\frac{\Delta p}{l_x}\left[(a/2)^2 - y^2\right]. \qquad (3.16)$$

We see that the velocity profile is a parabola, with the fluid in the center of the channel having the greatest speed. Once we know the velocity profile we can determine the flow rate *Q*, defined as the volume of fluid which passes a cross section of the channel per unit time. This is obtained by integrating the velocity profile over the cross sectional area of the channel:

$$\begin{aligned} Q &= \int_0^{l_z} dz \int_{-a/2}^{a/2} dy\, v_x(y) \\ &= \frac{l_z a^3}{12\eta}\frac{\Delta p}{l_x}. \end{aligned} \qquad (3.17)$$

The analogous result for flow through a pipe of radius *a* and length *l* in the presence of a uniform pressure gradient $\Delta p/l$ is

$$Q = \frac{\pi a^4 \Delta p}{8\eta l}. \qquad (3.18)$$

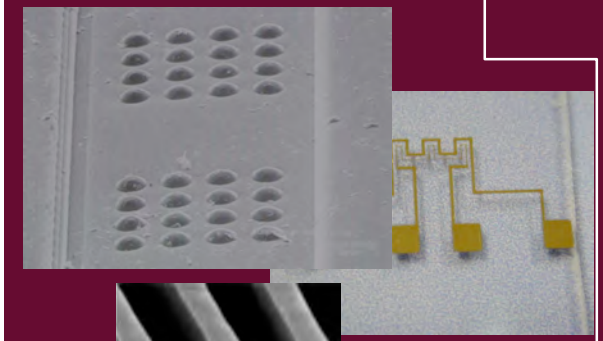
The important feature of both of these results is the sensitive dependence upon either the channel width *a* or the pipe radius *a*. For instance, for a pipe with a fixed pressure gradient, a 20% reduction in the pipe radius leads to a 60% reduction of the flow rate! This clearly has important physiological implications -- small amounts of plaque accumulation in arteries can lead to very large reductions in the rate of blood flow.

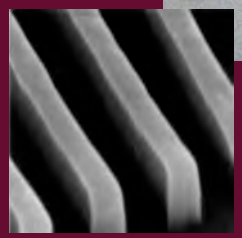

ASU
ANSE

Manufacturing and prototyping

Fabrication methods

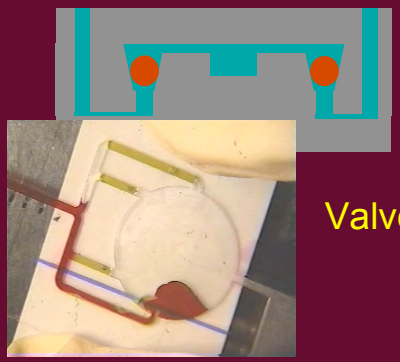
Molding, embossing, NanoImprinting



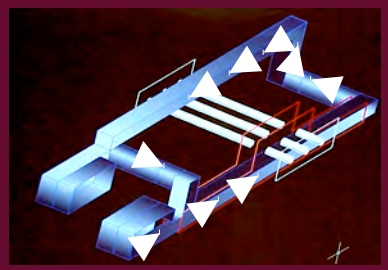


NIL Mask, 70 nm

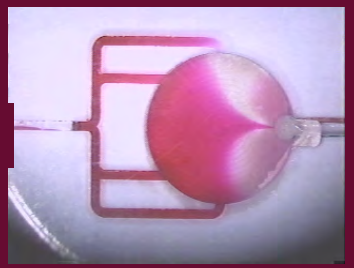
Device components



Valves

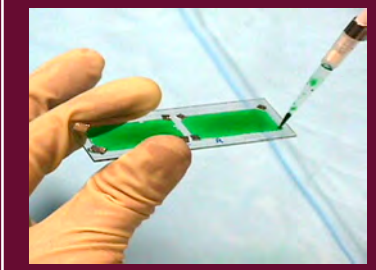


Pumps

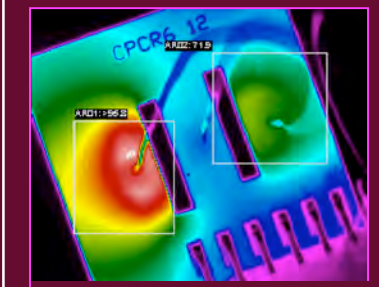


Mixers

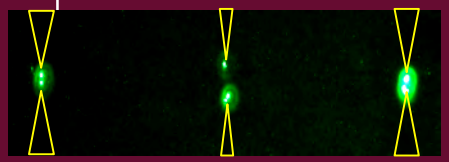
Assays



Channel hyb

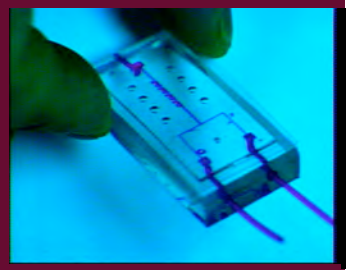


Amplification - PCR

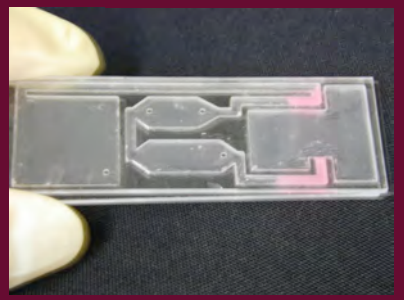


Cell and DNA capture

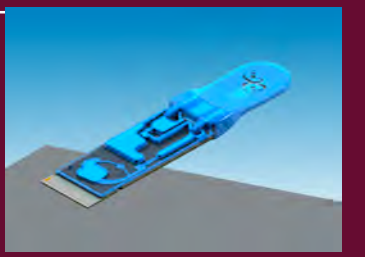
Integrated solutions



Multi-layer devices



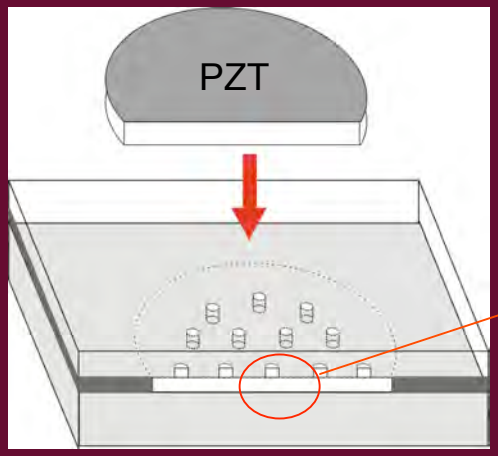
Multi-functional cartridges

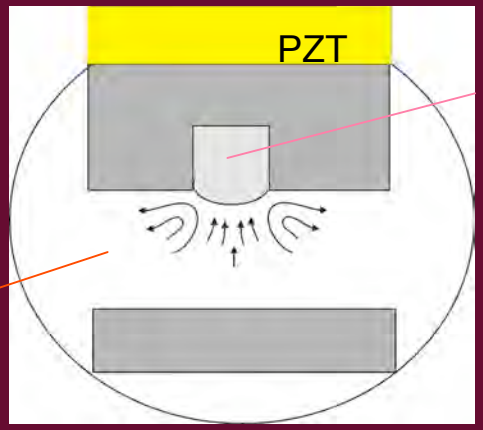


Amplification-
Detection
On Chip

Cavitation Microstreaming

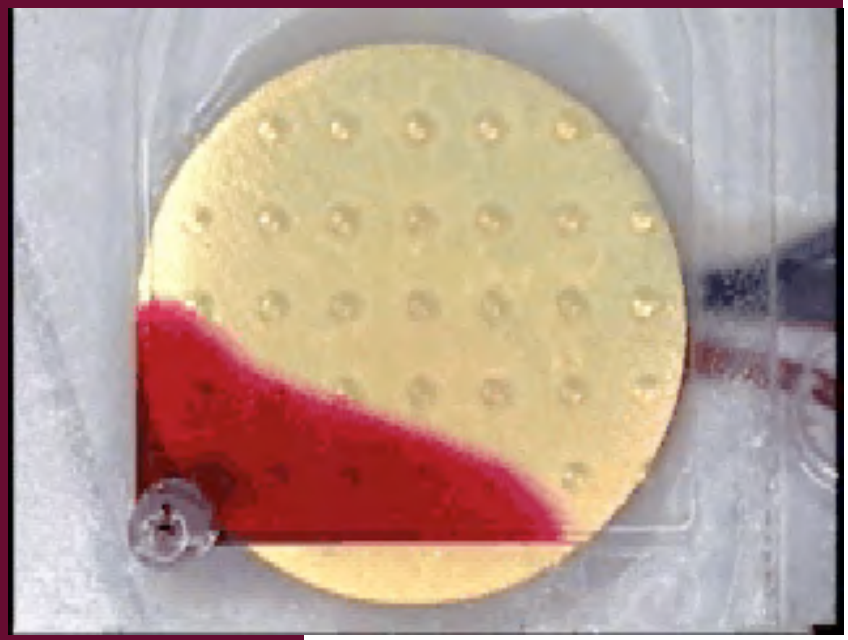
- Flow streaming around bubbles in an acoustic field
- Optimized mixing conditions (waveform, amplitude, etc.)





$$f = \frac{\sqrt{3\gamma P_o / \rho}}{2\pi a}$$

R. Liu et al., Anal. Chem. 2003



Now (10 sec for 100 uL)
5 kHz, 40 Vpp, square wave

Integrated cartridge for low abundance bacteria detection

Crude Sample

Target cell capture /enrichment

Cell purification

Cell Lysis

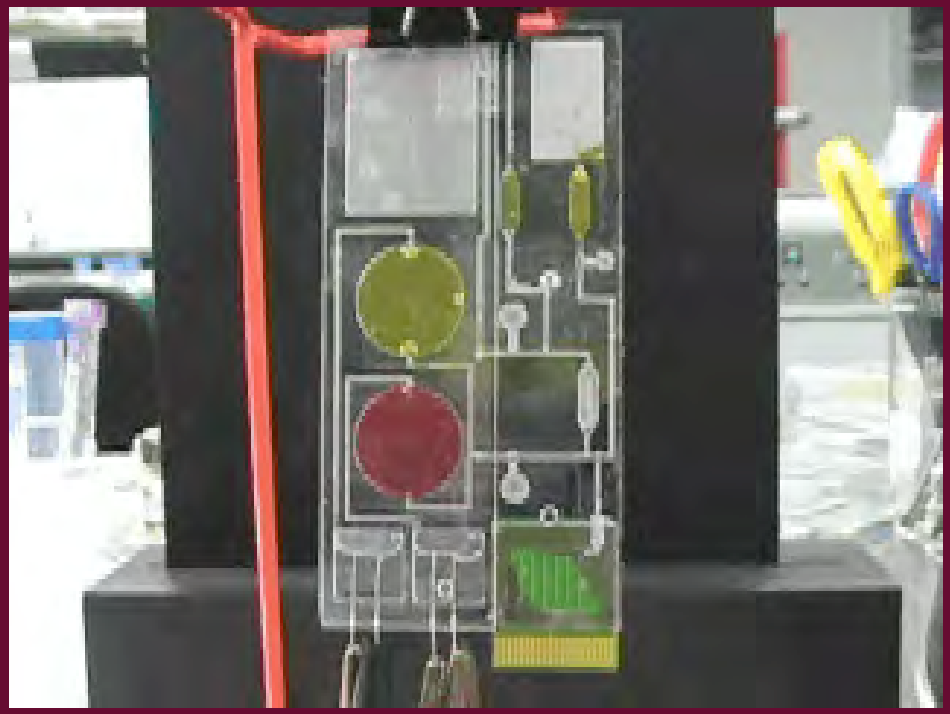
PCR

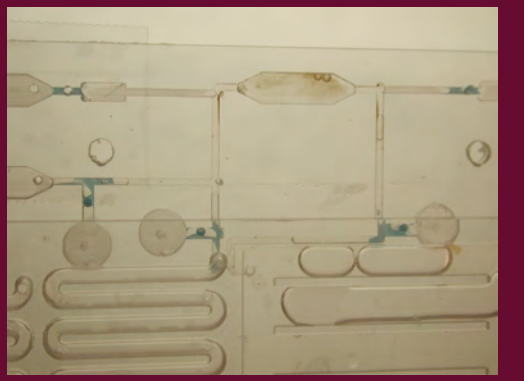
Hybridization

Detection

Answer

Objective: Integrate whole sample prep with microarray for low abundance bacteria detection from blood (1 mL)

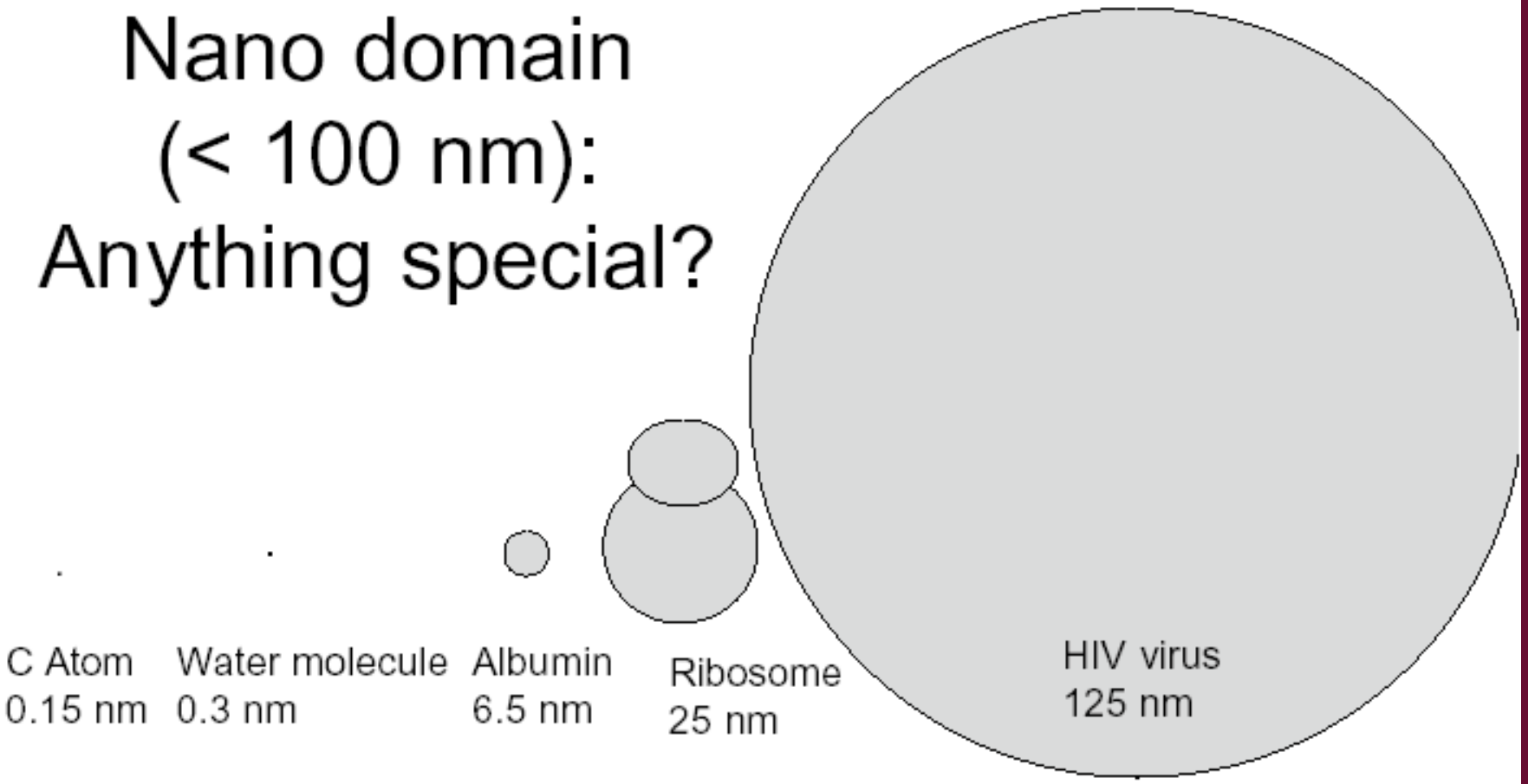




Successfully demonstrated cell capture + purification + lysis + PCR + detection of 1000 E. coli K12 cells / 1mL sheep blood

Liu RH, Yang JN, et al. ANAL. CHEM. 76, 1824 (2004)

Nano domain (< 100 nm): Anything special?



Well…yes!

- Size of 100 nm structures comparable to macromolecules
- Size of 1 nm structures comparable to water molecules

Surfaces – work of the devil?

There is an old legend that solids are the work of God and that surfaces are the work of the Devil. Solids, especially crystalline solids, have a structure that reflects the harmony of creation, while surfaces often have a form that represents disharmony, fracture, and the end of order. People who investigate surface phenomena (including, presumably, cell adhesion) are studying the works of the Devil; and in the past, societies used to punish those attracted to study the works of the Devil. In more modern and enlightened times...it is...recognized that such studies are punishment enough themselves.

P. D. RICHARDSON - *Priciples of Cell Adhesion* (1995)

Some of nature's nanostructures

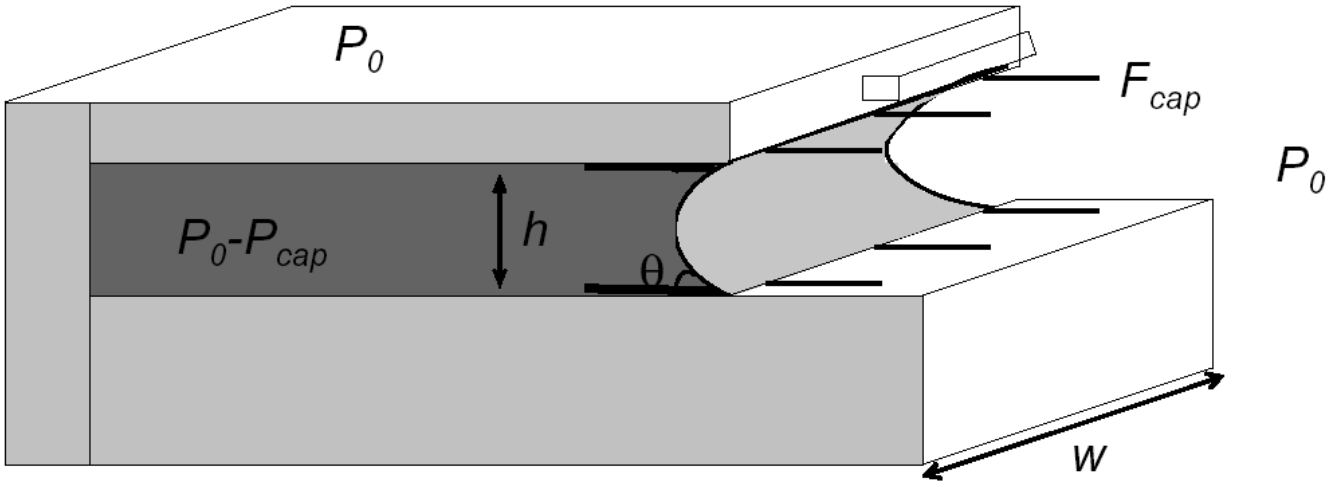
- 0.3 nm: Aquaporin pores in cell walls
- 1.5 nm: spacing between clay layers
- 7 nm: basement membrane pores in kidney (filter albumin)
- 100 nm: canaliculi ("small channels") in bone
- 3-4 μm: smallest blood capillaries (red blood cells just fit in)

Some technological ('man-made') nanostructures

- 0.3 nm: 6,6 carbon nanotubes
- < 1 nm: pores in reverse osmosis membranes
- 1-10 nm: nanofiltration membrane pores
- 10-100 nm: agarose gel pores
- 10-100 nm: ultrafiltration membrane pores
- 50 nm: transistor gate length in Intel Dothan and Prescott processors

Capillary Forces

Capillary pressure



$$F_{cap} = 2w\gamma\cos\theta$$

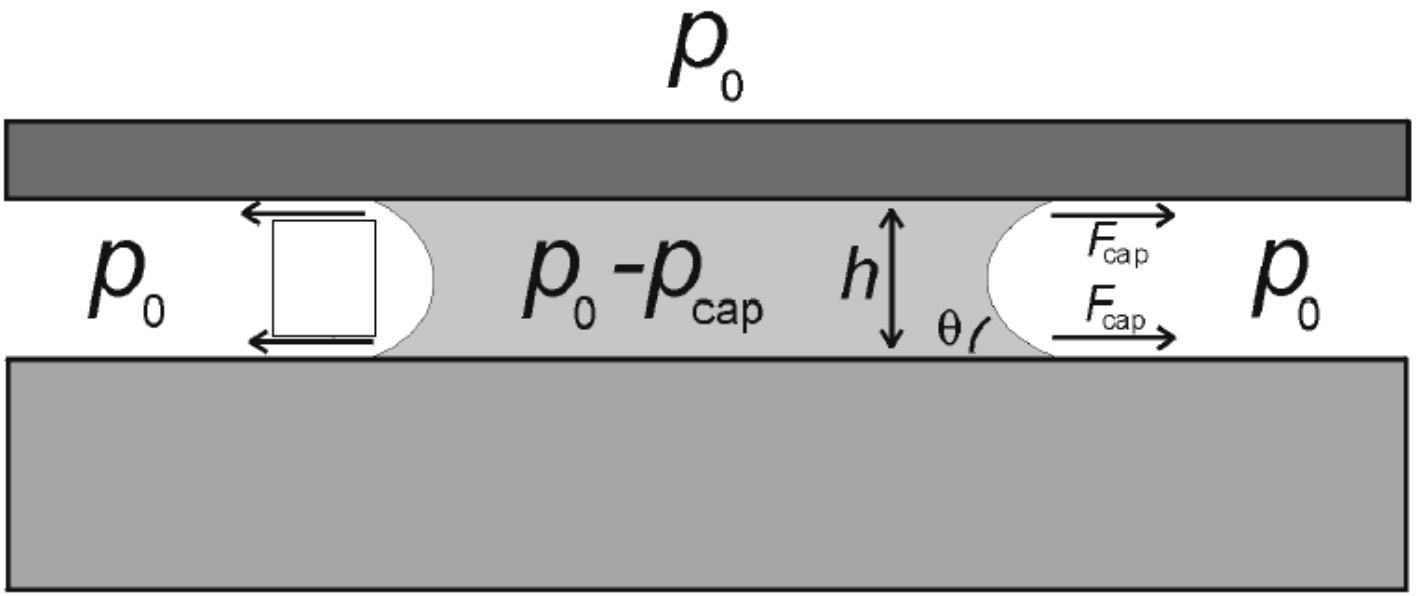
$$P_{cap} = \frac{2\gamma\cos\theta}{h}$$

Pressure increases with *1/h* !!

γ : surface tension (Nm^{-1})

θ : contact angle

Capillarity induced negative pressure

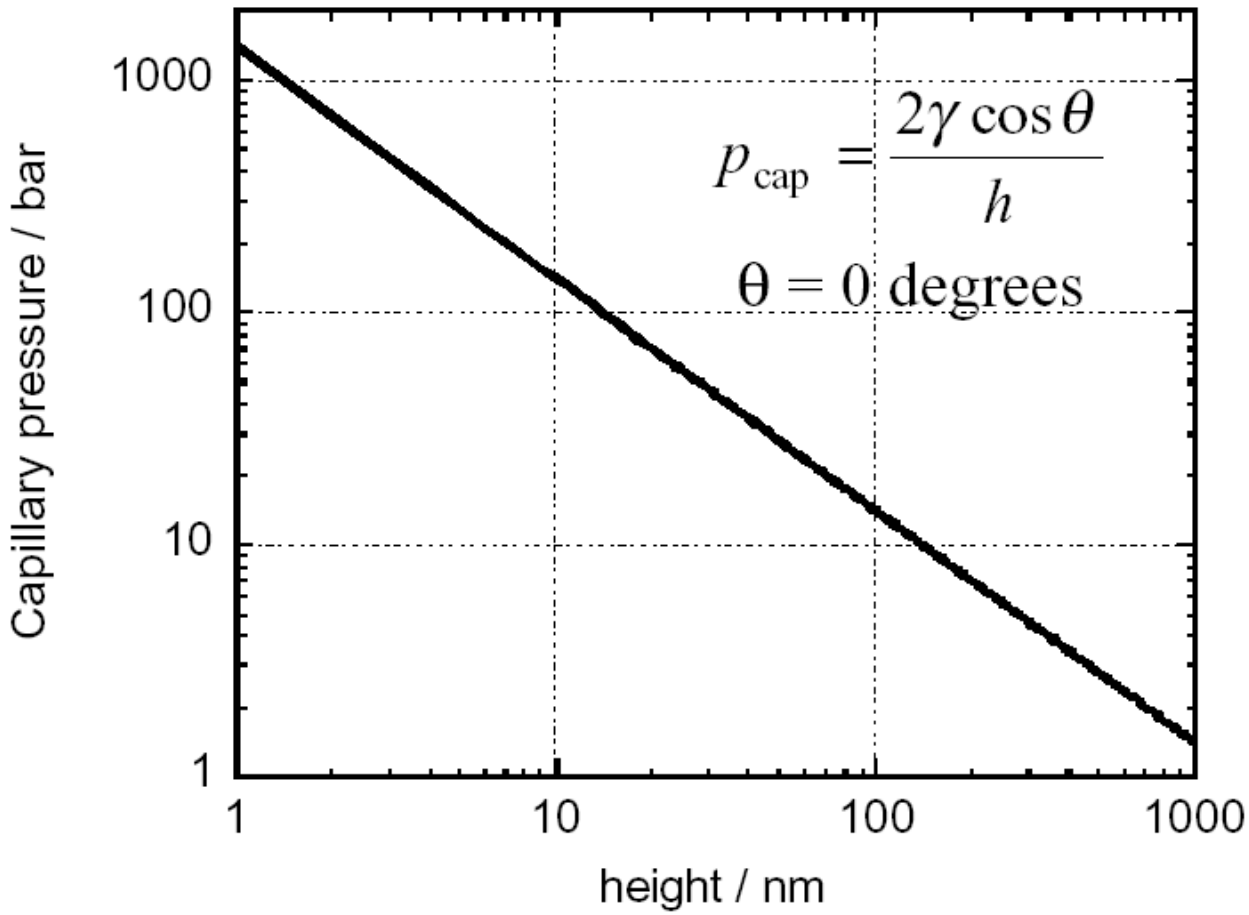


$$p_{cap} = \frac{2\gamma \cos\theta}{h}$$

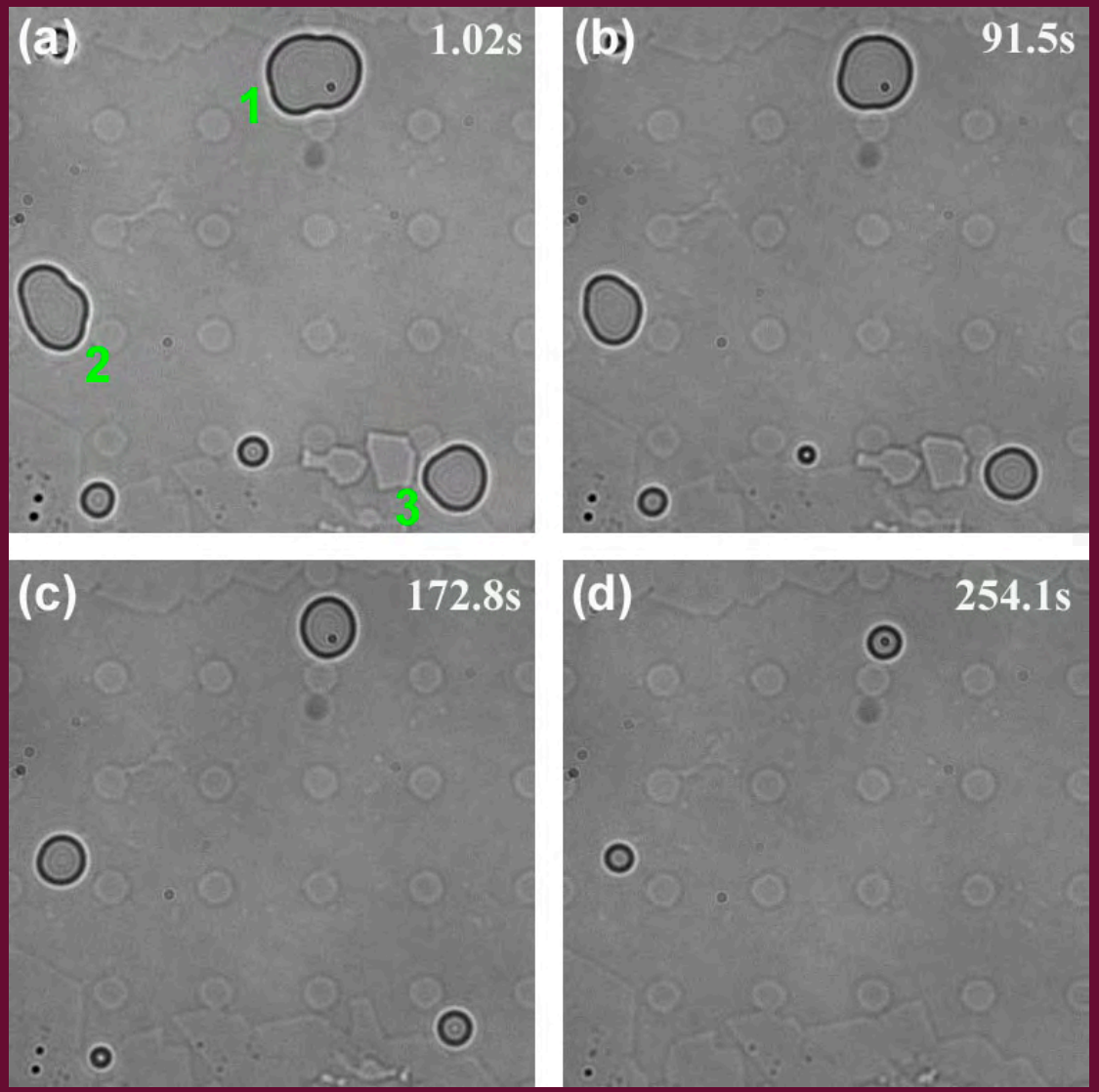
h = 108 nm, γ = 0.07 Nm^{-1} θ = 18°

$\rightarrow p_0 - p_{cap}$ = -12 bar

Scaling

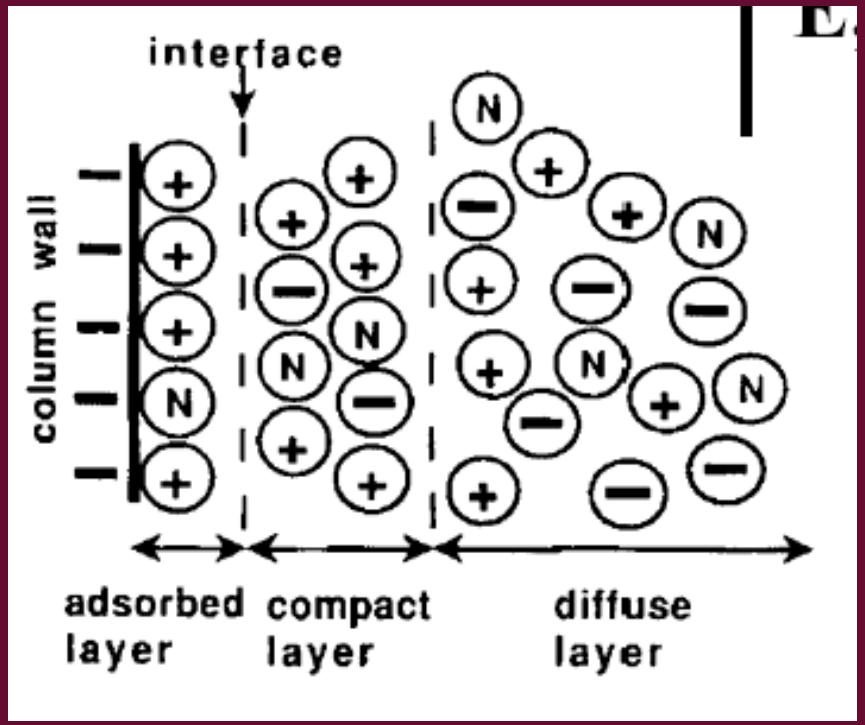

Capillary pressure / bar
1000
100
10
1
1
10
100
1000
height / nm
$p_{cap} = \frac{2\gamma \cos\theta}{h}$
$\theta = 0$ degrees

Microbubble shrinking in nanofluidic channels



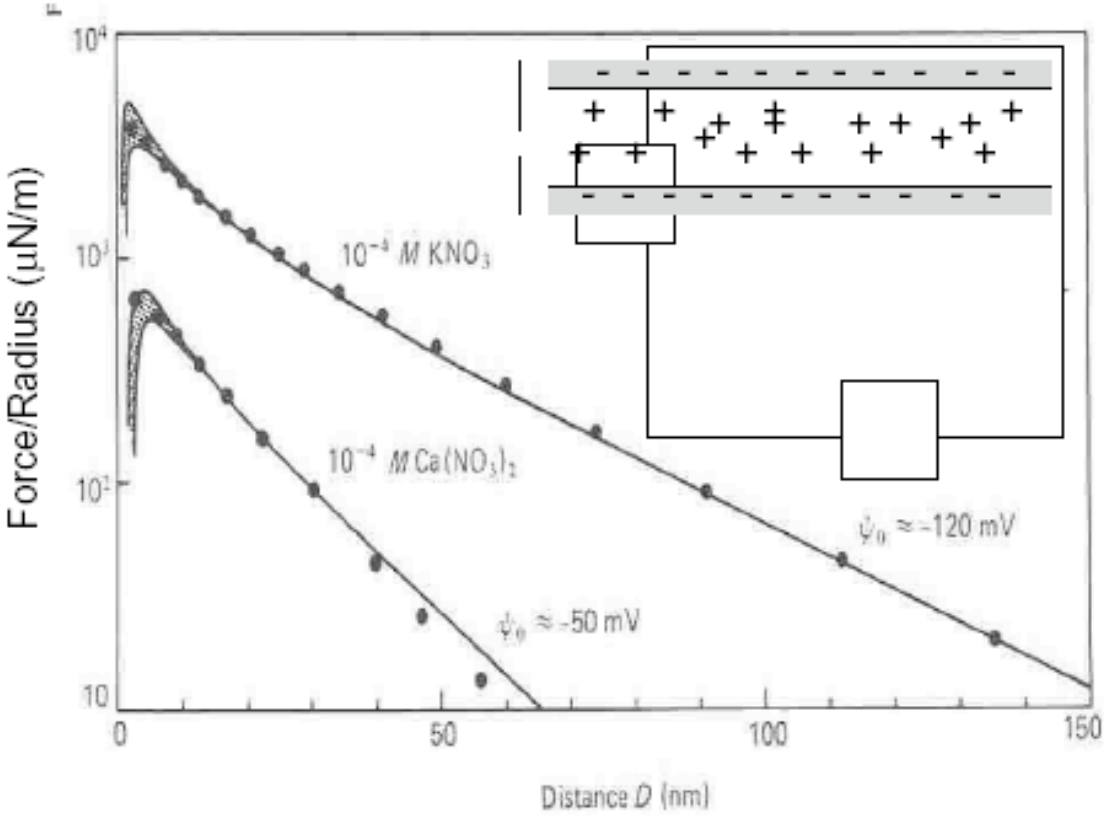
Wei, Chou et al., submitted.

Double Layer Model

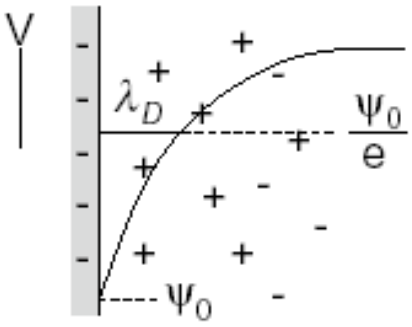


- Maxwell-Poisson Equation
- Debye-Hükel length
- Interaction form
- Constant charge assumption
- Constant potential assumption

Electrostatic forces



Points: measured double layer and van der Waals forces between two curved mica surfaces of radius R in water; exponential decay; continuous curves: DLVO theory (Derjaguin-Landau-Verwey-Overbeek)



Thickness of double layer is characterized by Debye screening length λ_D; for a 1:1 electrolyte of c M, λ_D in nm is:

$$\lambda_D = \sqrt{\frac{\varepsilon RT}{2F^2 c}}$$

Conc / M	λ_D / nm
10^{-5}	100
10^{-4}	30
10^{-3}	10
10^{-2}	3
10^{-1}	1

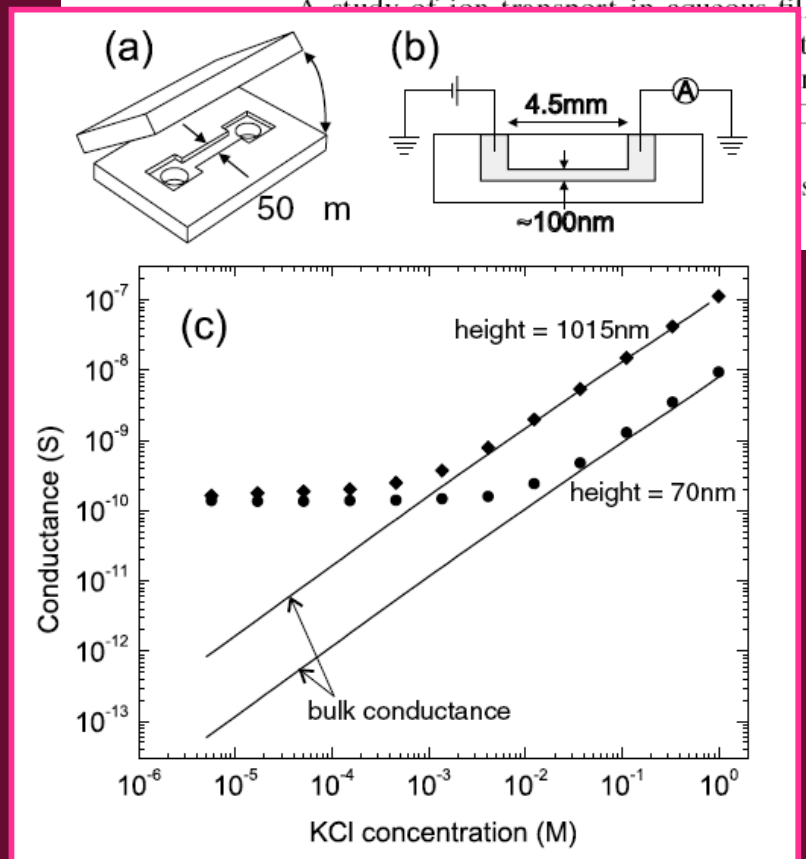
Recent experiments

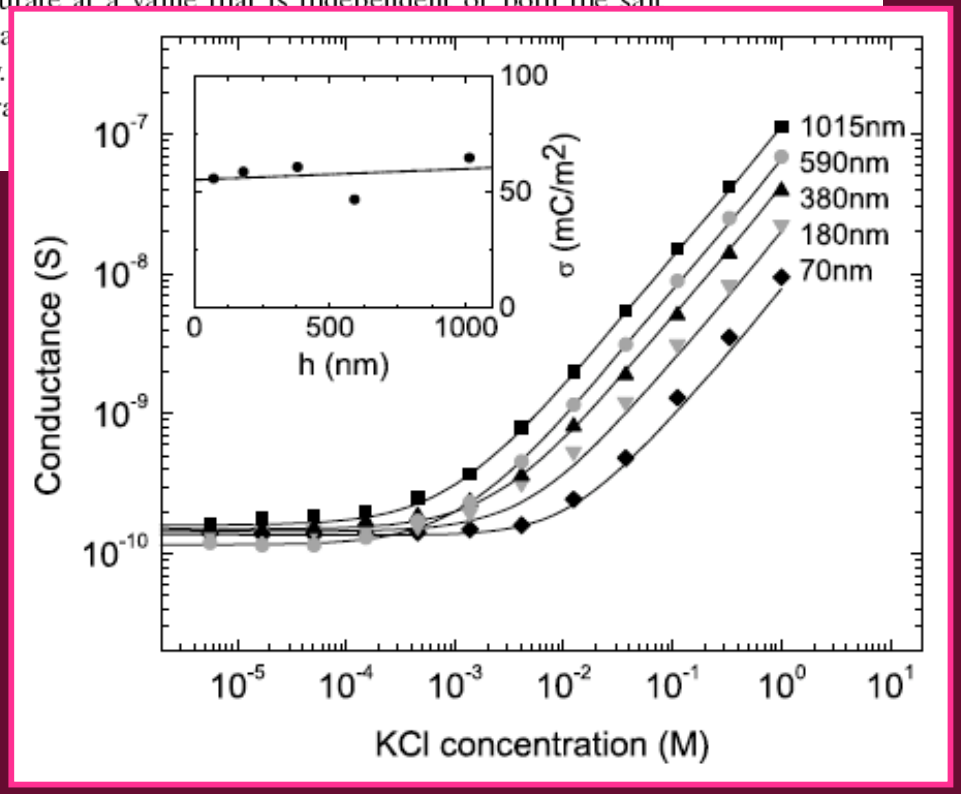
VOLUME 93, NUMBER 3 PHYSICAL REVIEW LETTERS week ending 16 JULY 2004

Surface-Charge-Governed Ion Transport in Nanofluidic Channels

Derek Stein, Maarten Kruithof, and Cees Dekker
Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands
(Received 15 April 2004; published 15 July 2004)

...filled silica channels as thin as 70 nm reveals a remarkable ...tions that departs strongly from bulk behavior: In the dilute ...nels saturate at a value that is independent of both the salt ...Our data ... density. ...s, ion tra...





NANO
LETTERS

2004
Vol. 4, No. 6
1099−1103

Ion-Enrichment and Ion-Depletion Effect of Nanochannel Structures

Qiaosheng Pu,[†] Jongsin Yun,[‡] Henryk Temkin,[‡] and Shaorong Liu*[,†]

Department of Chemistry and Biochemistry and Department of Electric Engineering and Computer Science, Texas Tech University, Lubbock, Texas 79409

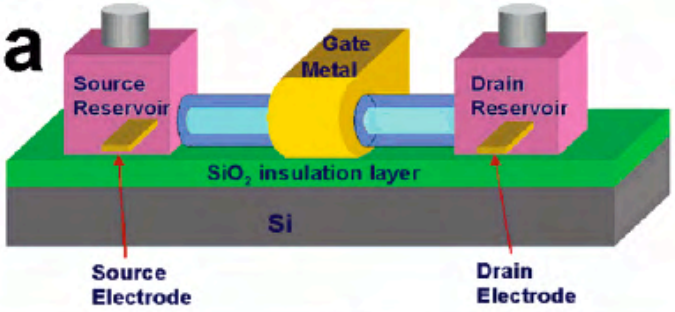
Received April 6, 2004; Revised Manuscript Received April 29, 2004

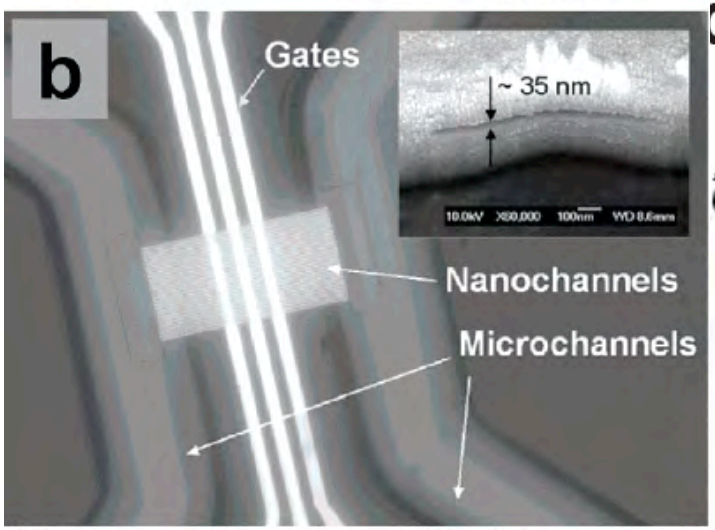
NANO
LETTERS

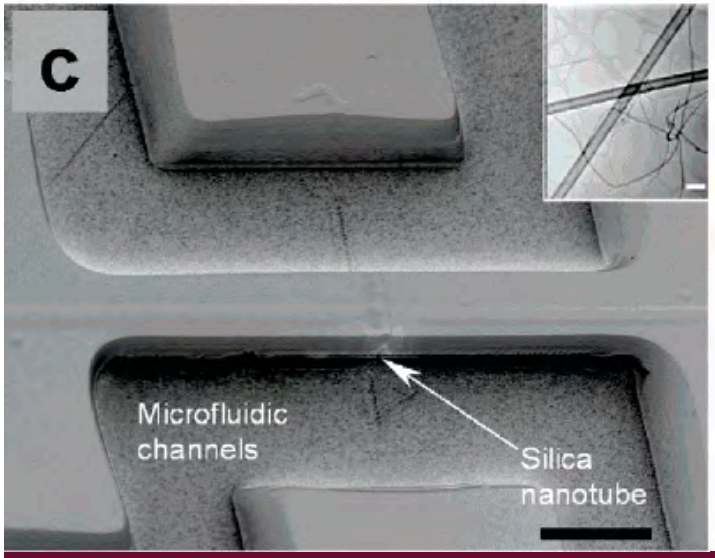
2005
Vol. 5, No. 5
943−948

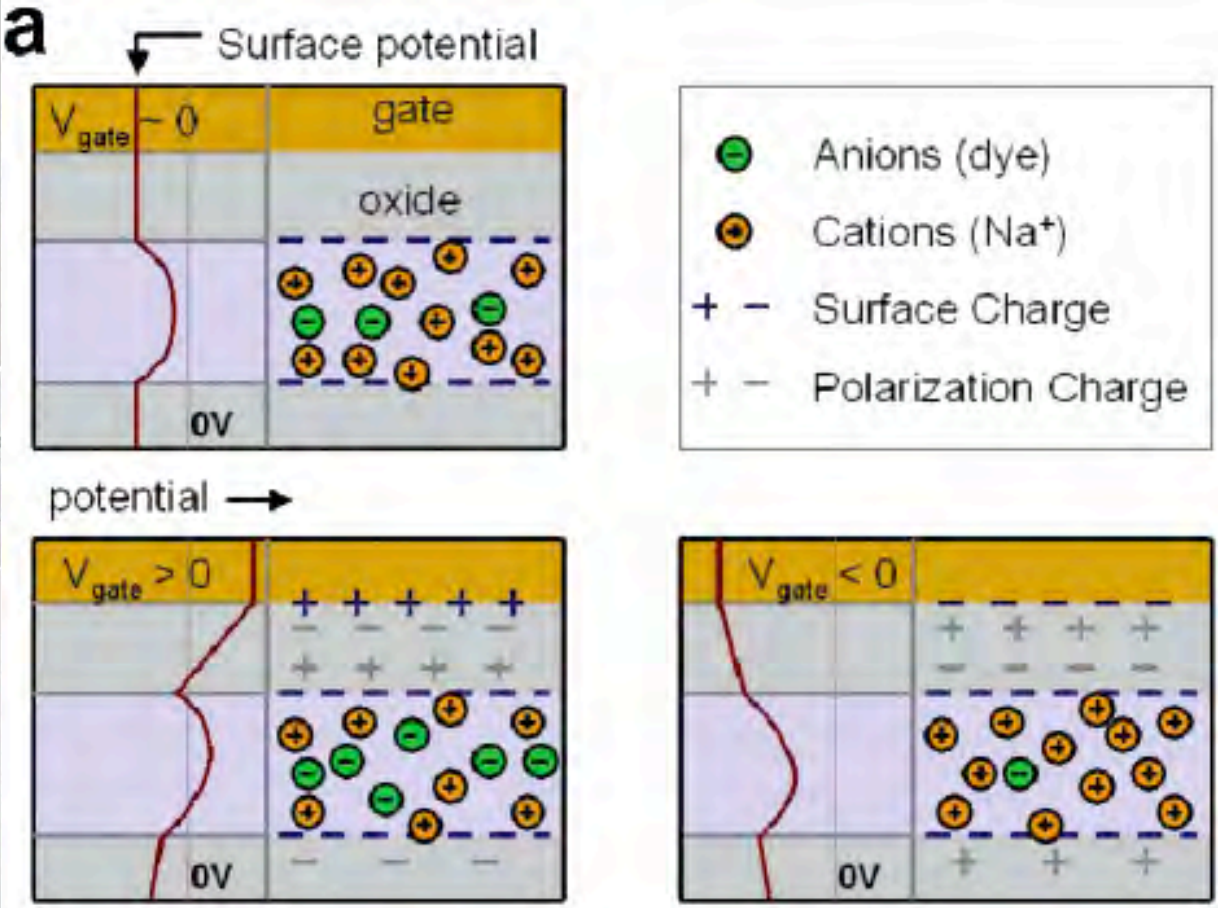
# of Ions and
dic Transistors

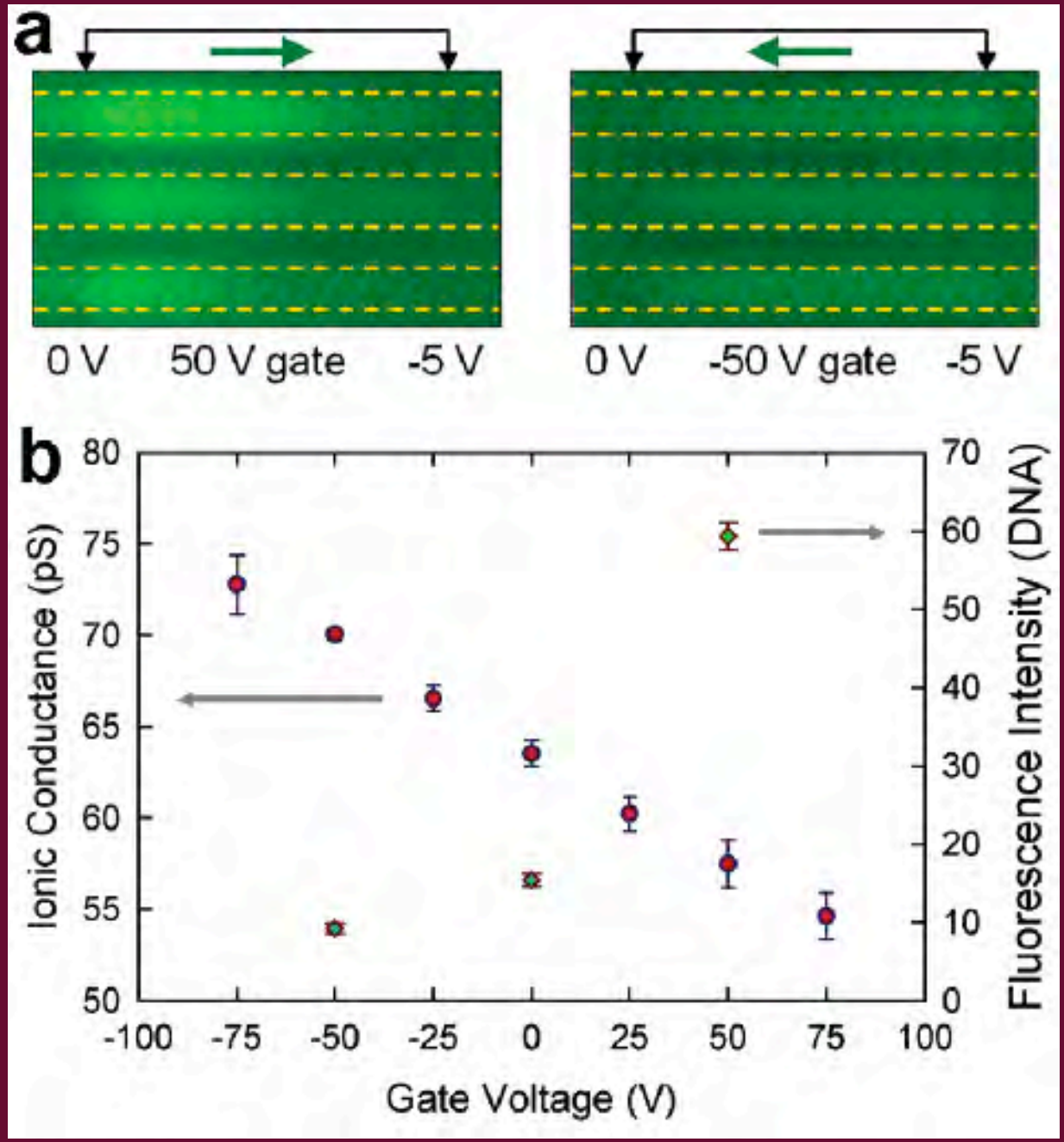
Li,† Peidong Yang,*,‡,§ and





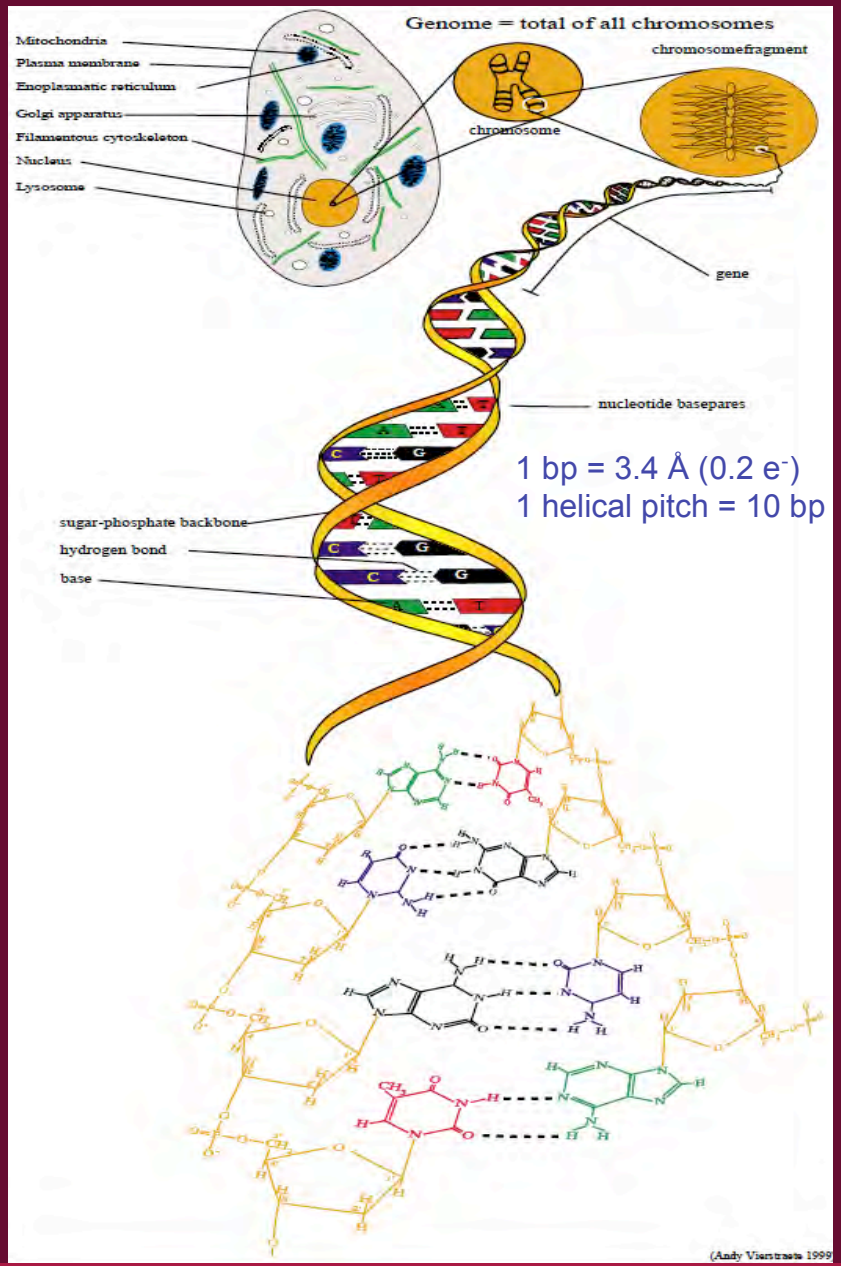


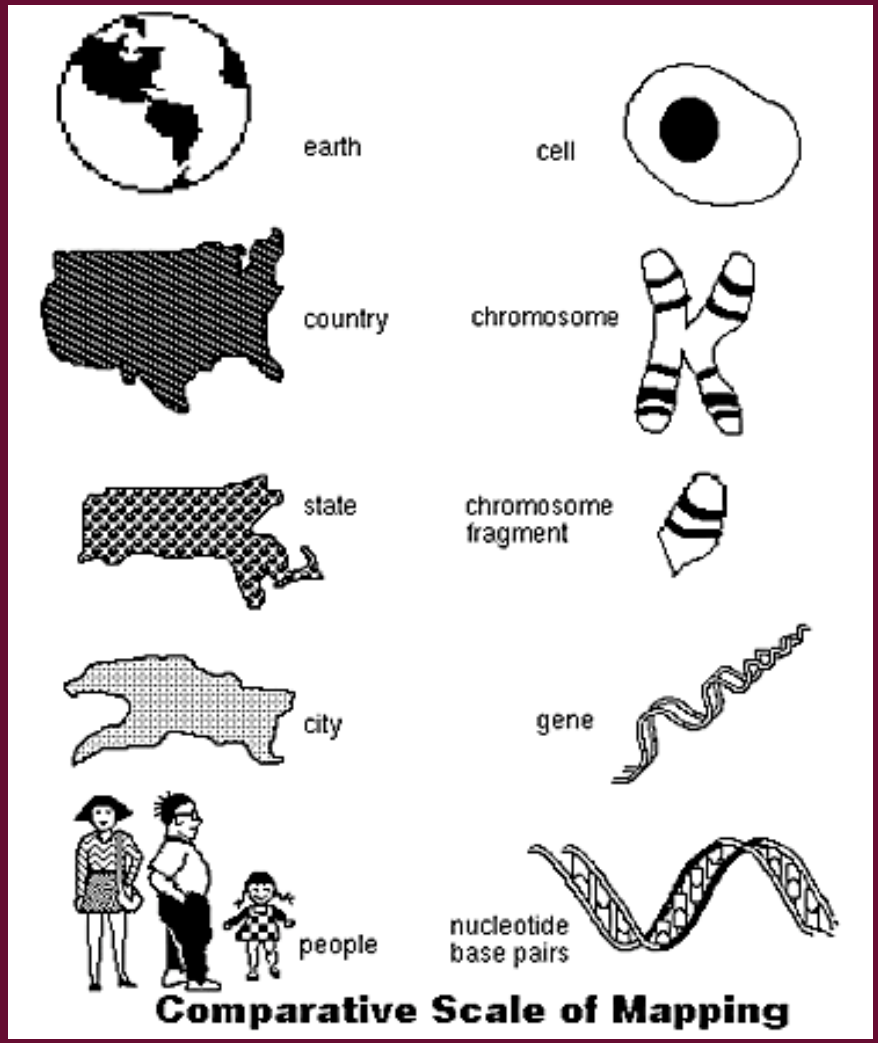



a
0 V
50 V gate
-5 V
0 V
-50 V gate
-5 V
b
Ionic Conductance (pS)
Fluorescence Intensity (DNA)
Gate Voltage (V)
80
75
70
65
60
55
50
70
60
50
40
30
20
10
0
-100
-75
-50
-25
0
25
50
75
100

Introduction to biomolecular analysis

Cell and Molecules





http://accessexcellence.org/RC/VL/GG/comparative.html

On-chip Molecular analysis and diagnostics

Molecular analysis– understand the behavior, events, information flow, etc., based on the knowledge down to individual molecules

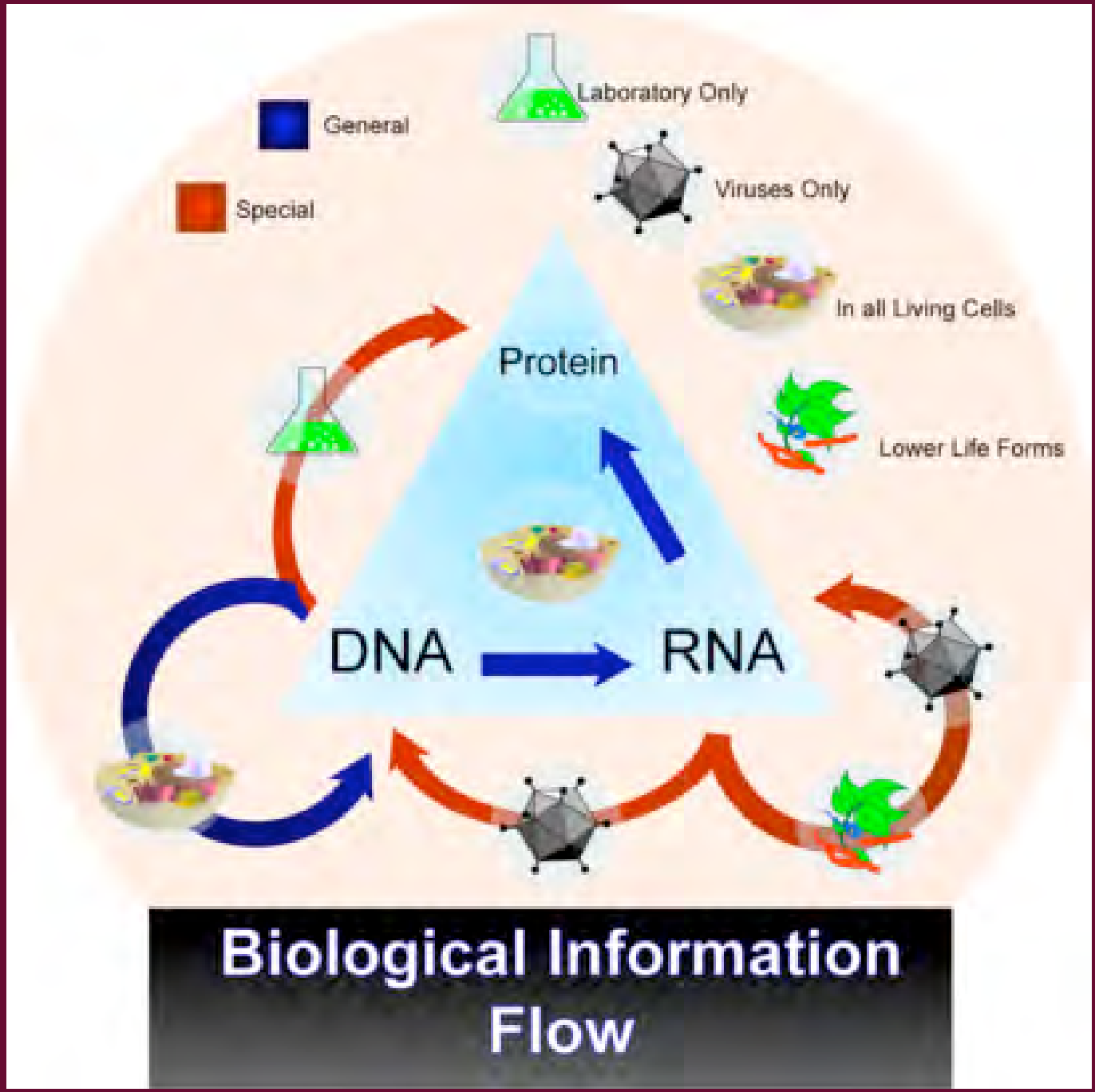
Behavior: polymer dynamics, protein folding…

Events: binding, hybridization…

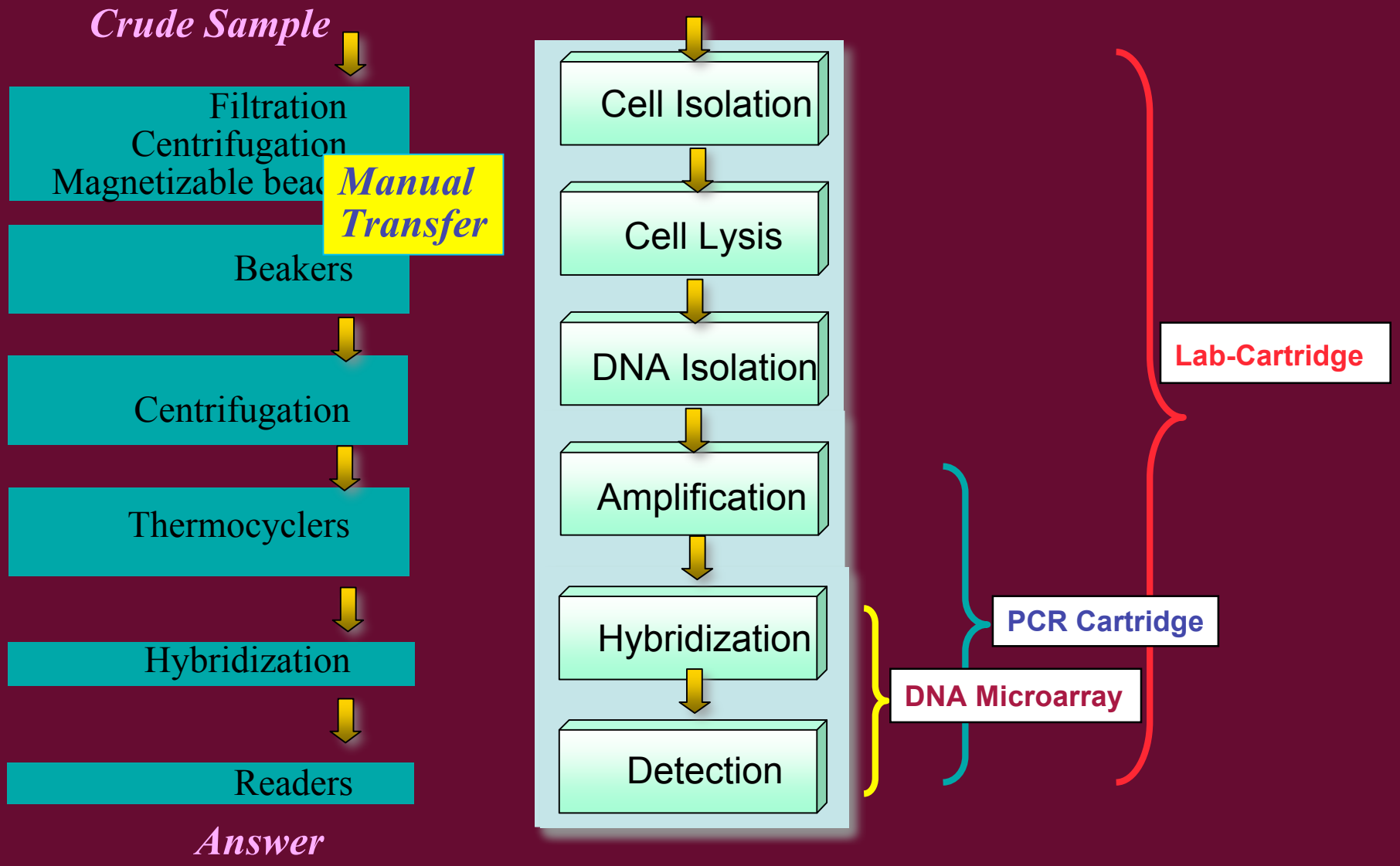
Information flow: DNA----> RNA----> Protein (Central dogma!)

Molecular diagnostics– apply the knowledge from molecular analysis to serve a diagnostic purpose (genetic screening)

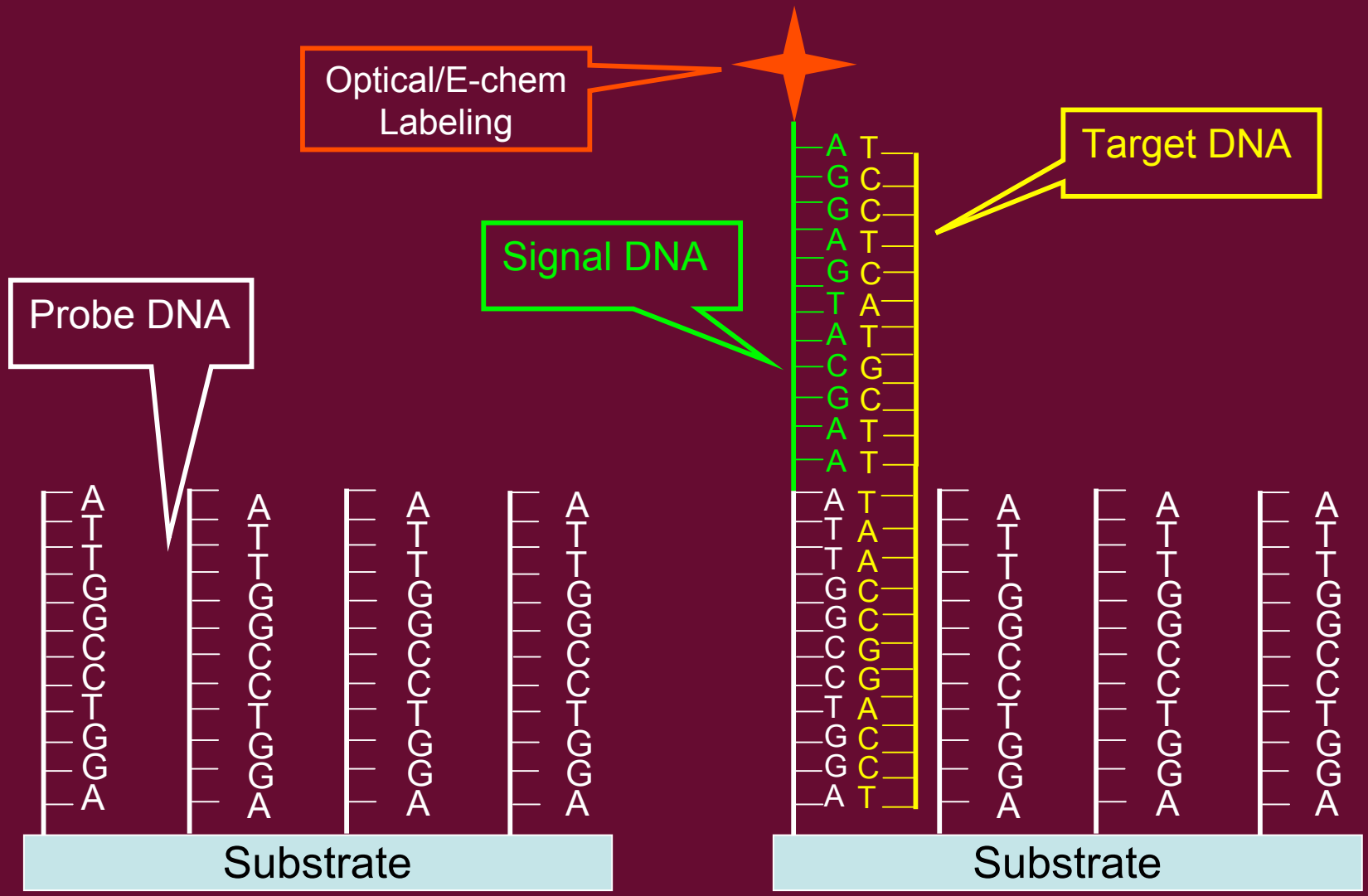
Both Molecular analysis and diagnostics can be performed on *ensemble* of or *single* molecules


General
Special
Laboratory Only
Viruses Only
In all Living Cells
Lower Life Forms
Protein
DNA
RNA
Biological Information Flow

Array-based Genetic Analysis

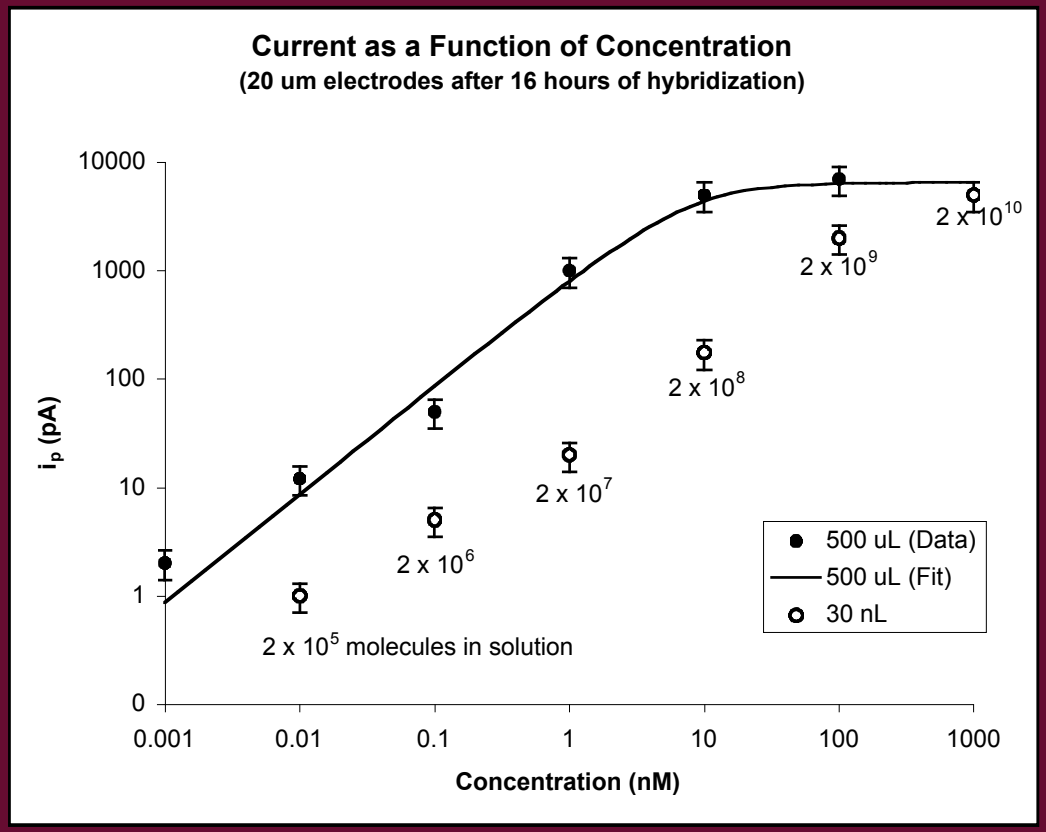


Principles of DNA Hybridization & Detection



Why molecular traps?

Titration of detection limit with microelectrodes and microfluidics



N. Swami

This data set demonstrates a chief bottleneck for *all* miniaturized sensing methods in general, and surface binding assays in particular. This is associated with the chemical kinetic limits to sensitivity upon miniaturization. → Solution: sample pre-concentration!!

DEP for molecular focusing and sorting

Dielectrophoresis (DEP) is a technique which can be used to separate cells or molecules based on the difference in their polarizability

Brief Theory

Potential energy of a dielectric object in an electric field:

$$U = -p \cdot E = -\alpha V E^2$$

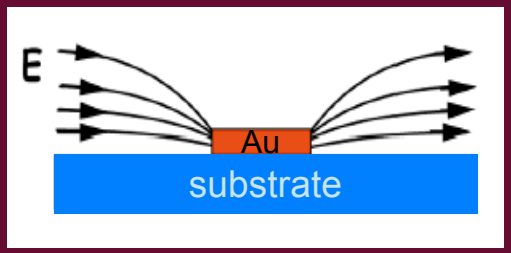
Dielectrophoretic force:

$$F = -\nabla U \sim E(dE/dy)$$

$$F = 2\pi a^3 \varepsilon_m \,\mathrm{Re}\left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right)\nabla(E^2)$$

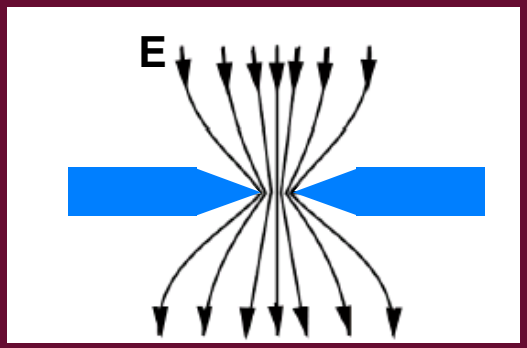
Sphere of radius a, ε_m = 80 for water

a. Metallic trap



(Side view)

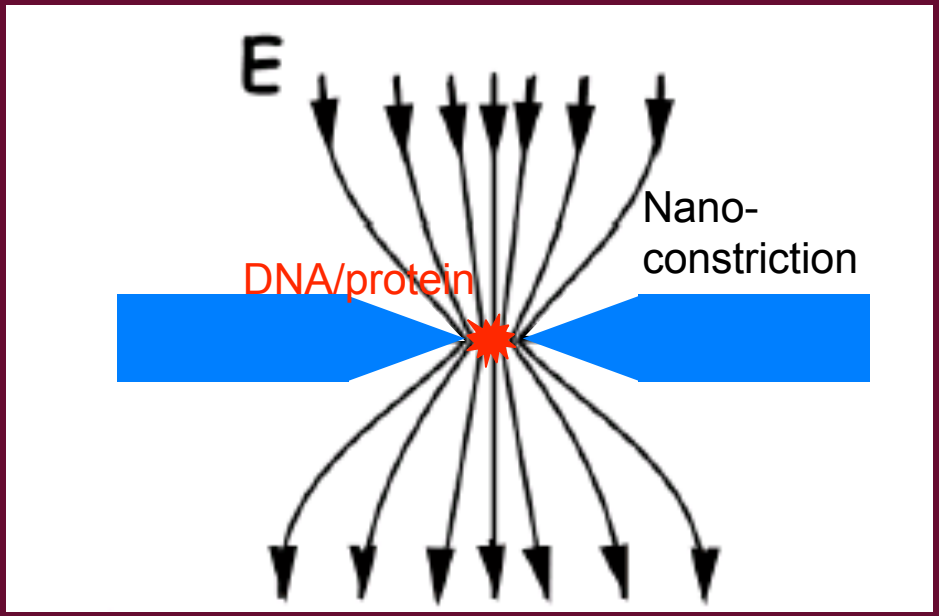
b. Electrodeless trap (EDEP)



(top view)

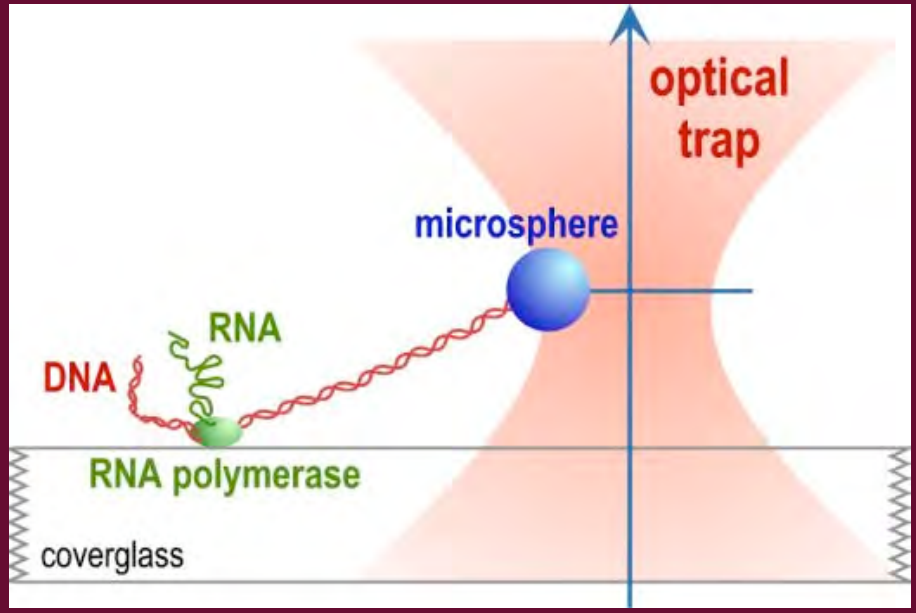
EDEP Molecular Trap vs. Optical Trap

EDEP molecular trap



Electric field focused
at the constriction

Optical trap

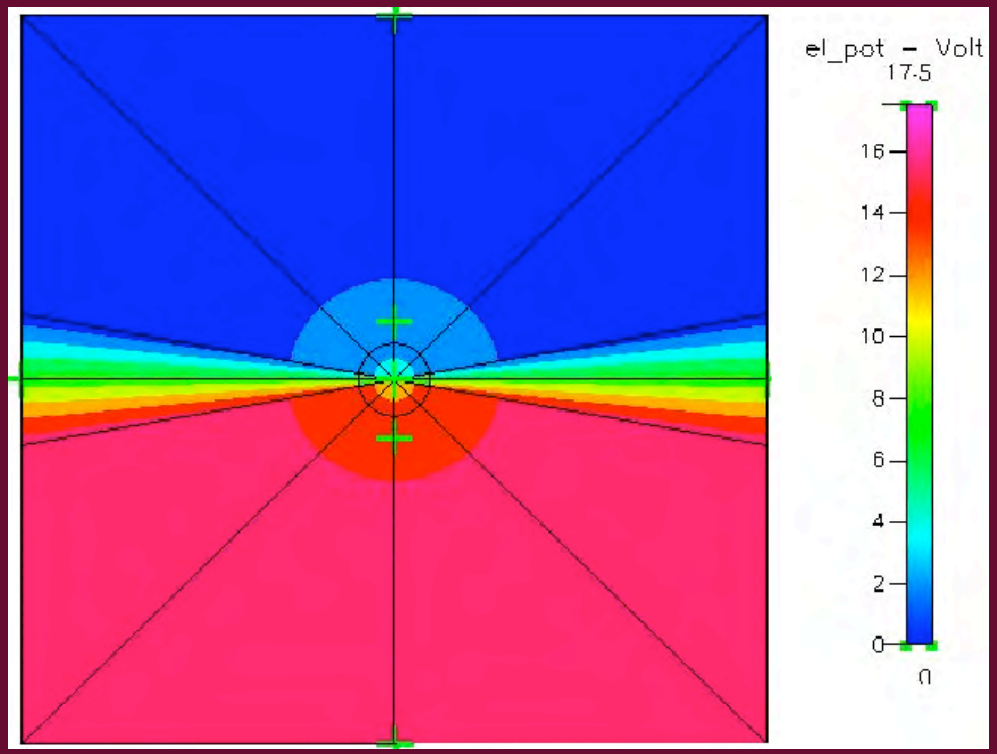


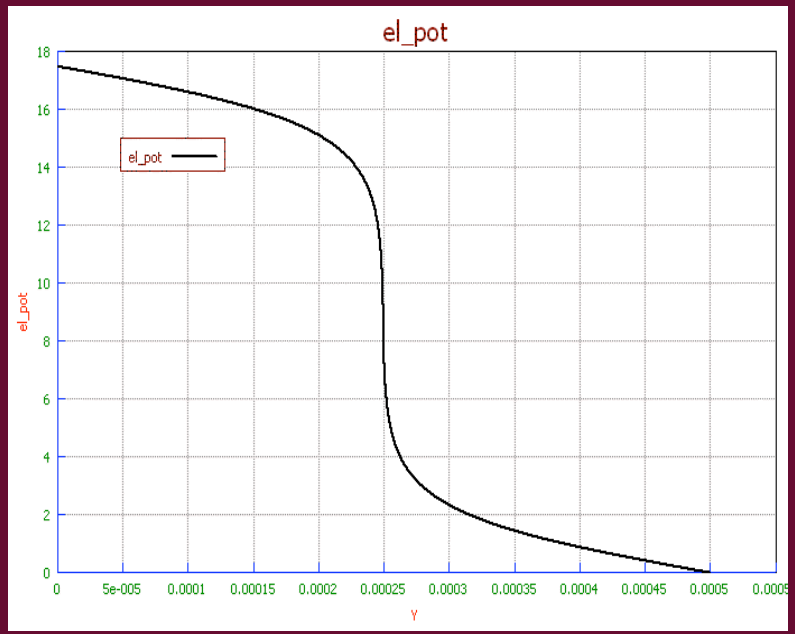
Electric field focused
at the focal point

Application of DEP in Biology and ...

- Cells
  - separation of yeast (Pethig et al, 1994)
  - cell fission of sea urchin eggs (Marszalek & Tsong, 1995)
  - cell fussion (Matsuda et al., 1979)
- Viruses
  - separation of tobacco mosaic virus and herpes simplex virus (Morgan et al, 1999)
- Latex beads
  - DEP ratchet (Silberzan et al, 1998)
- DNA
  - Increase resolution of DNA fractionatoin or sequencing
  - Free-flow electrophoresis in 2D slab
  - Micro-PCR
  - Enhance in-situ hybridization

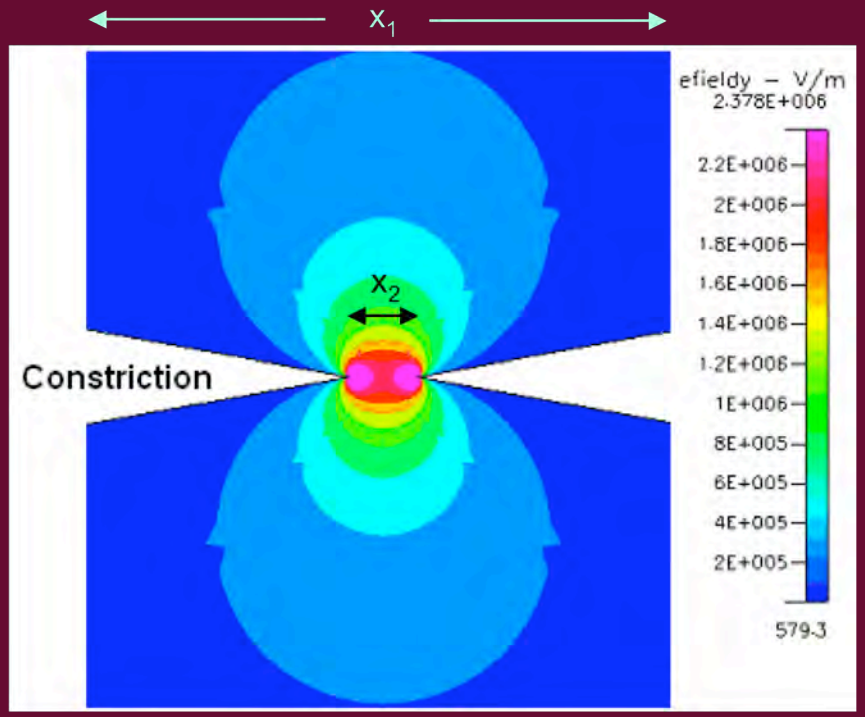
Electric Potential Distribution

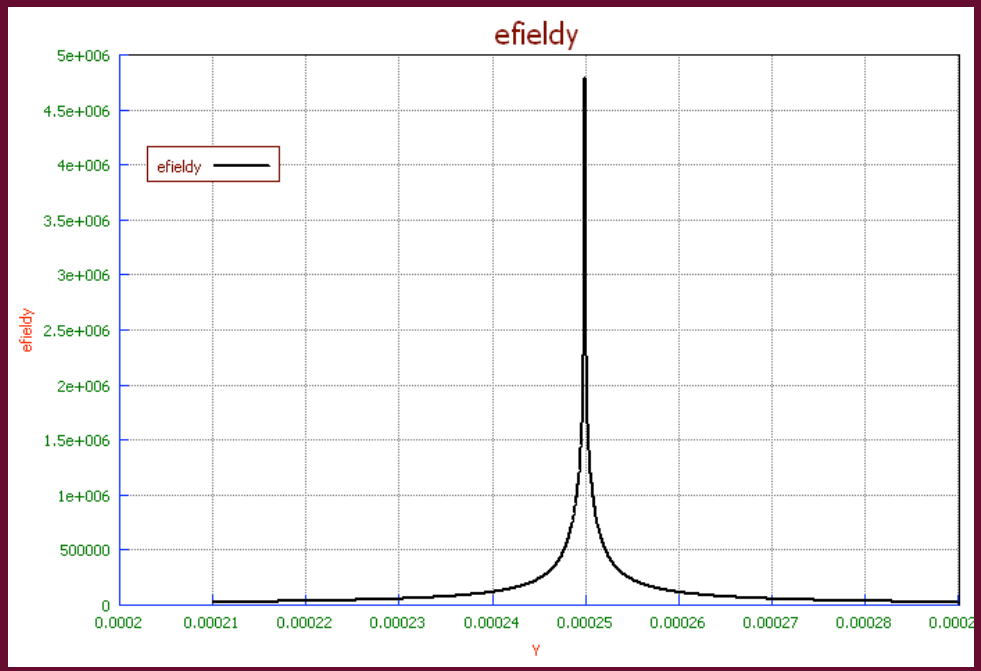




Potential drop mostly occurs at the constriction

Electric Field Enhancement





Field focusing factor: x_1/x_2

For 50 μm/50 nm:

$E \rightarrow 10^3x$

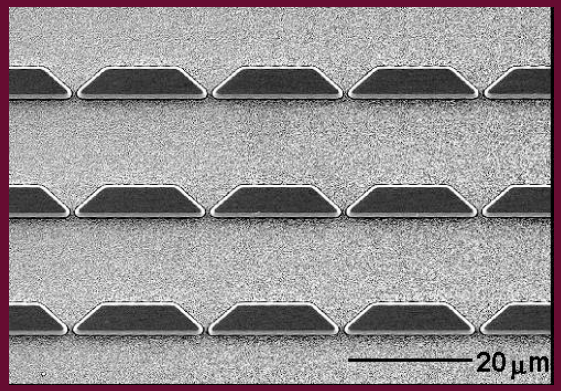
$\nabla(E^2) \rightarrow 10^6x$

Field focusing mostly occurs at the (tips of) constriction

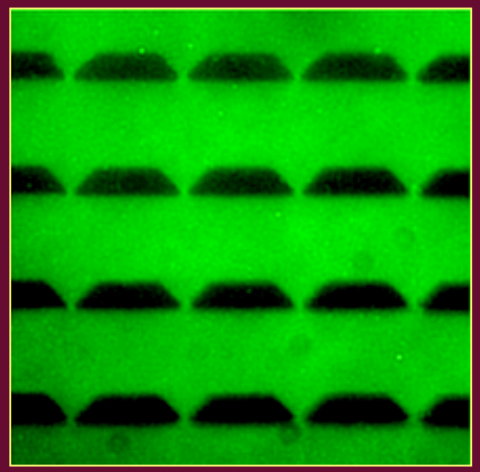
EDEP for DNA trapping

368 bp DNA

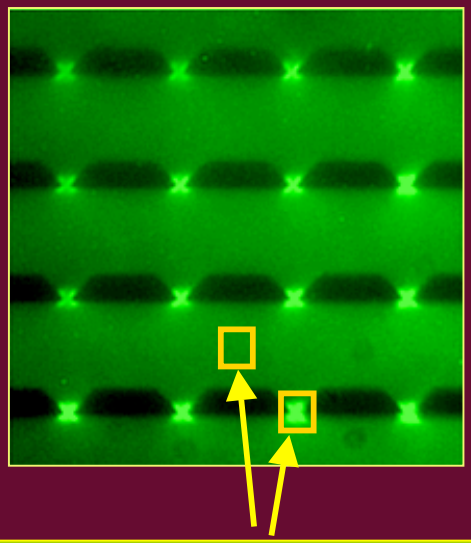
EDEP devicce



AC field off



AC field on



DNA concentration enhancement by this EDEP device: ~ 100x

Conc. estimated by light intensity of TOTO-1 dye

C.F. Chou et al., Biophys. J. 83: 2170-2179 (2002);
US Patent # 6,824,664 (2004)

Fick's equation for diffusion with drift

Flux: $J(x,t) = v\, n(x,t) - D\, \mathrm{grad}[n(x,t)]$

$= [DF(x,t)/kT]\, n(x,t) - D\, \mathrm{grad}[n(x,t)]$

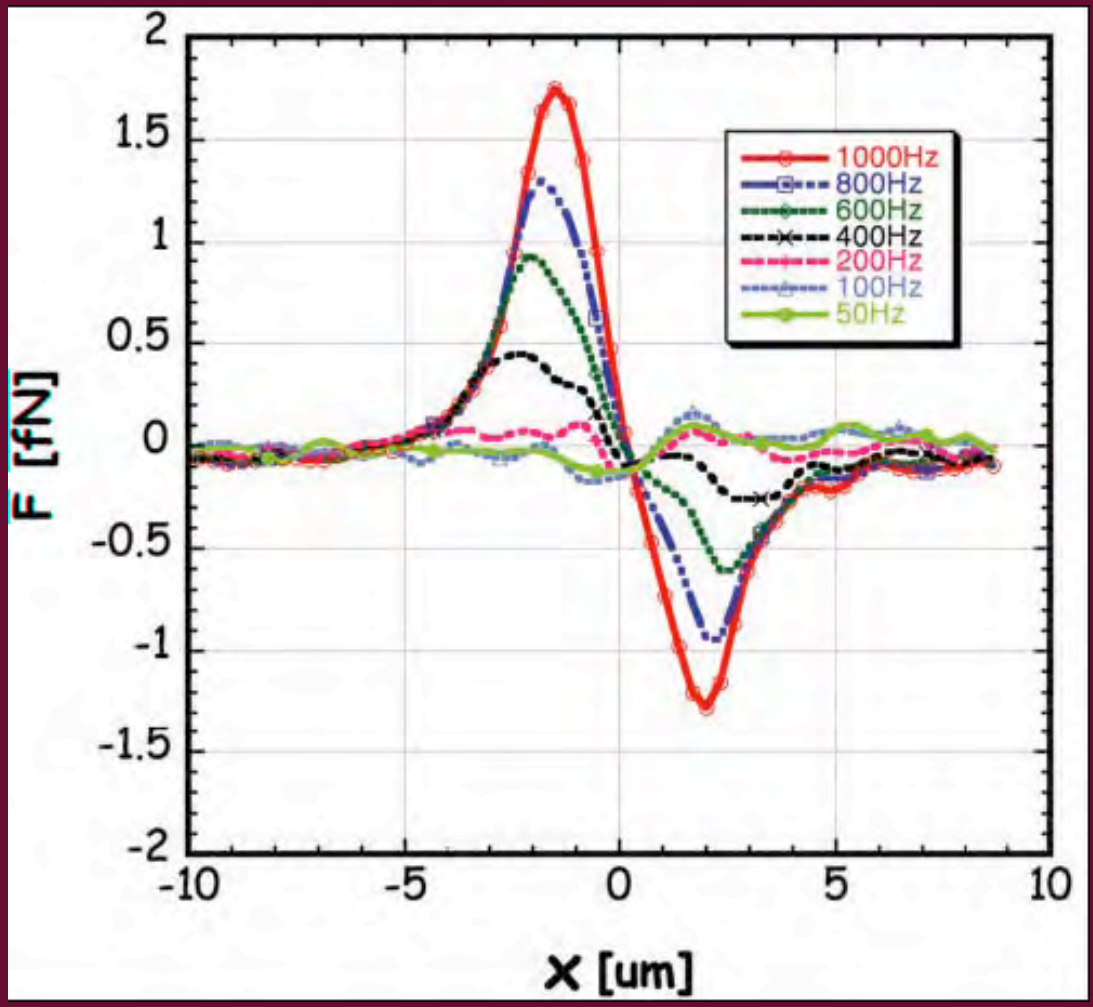
At equilibrium: $J(x,t) = 0$

$$F(x) = kT\, \mathrm{grad}[n(x)]/n(x)$$

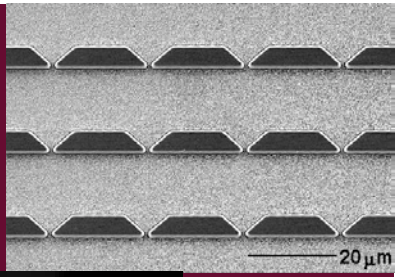
$kT = 4$ pN·nm
$= 4$ fN·μm

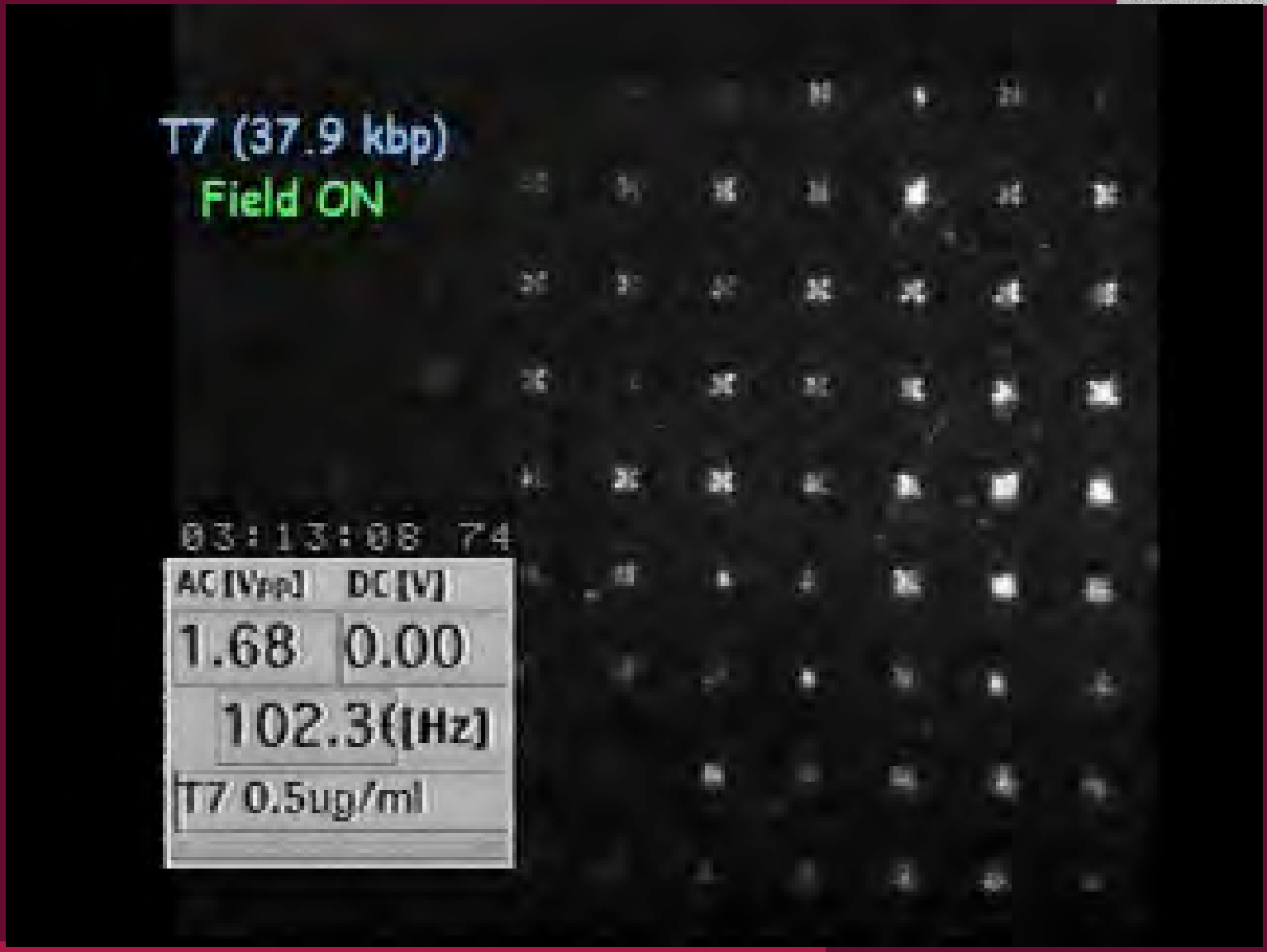
Frequency response at a fixed field

368 bp@1000Vpp/cm (5Vpp/cell)

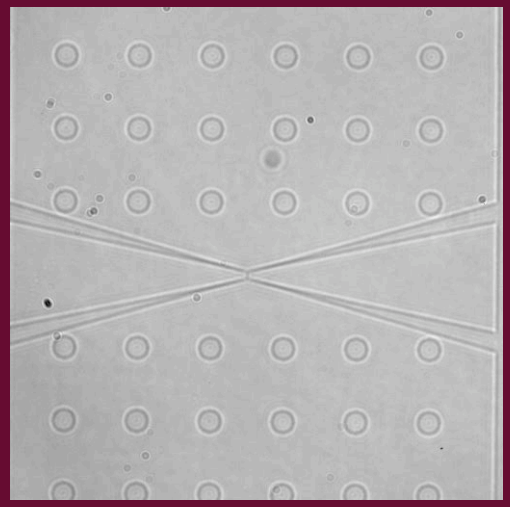


EDEP-Trapping of DNA-movie

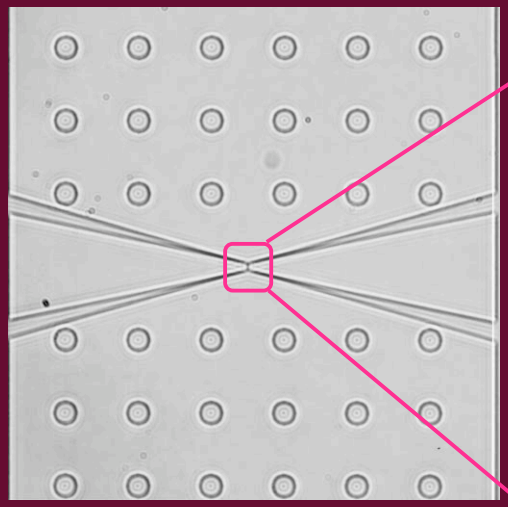




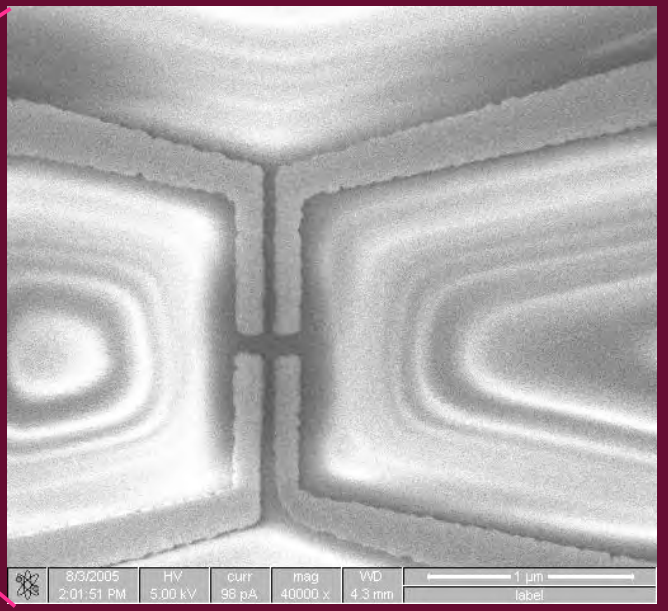
Nanoscale Molecular Trap

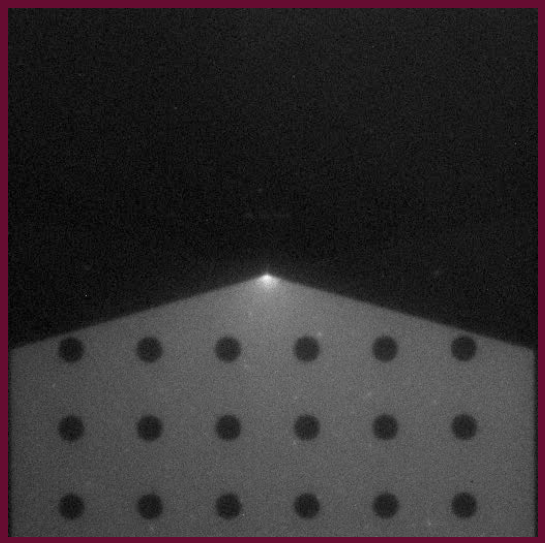


50 nm gap/70 nm deep



70 nm gap/250 nm deep





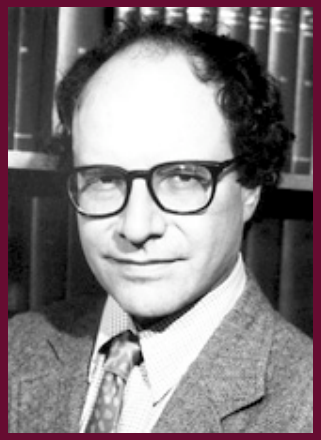
Alexa-488 Streptavidin
(0.5x TBE)

Unpublished

~1000x enrichment

Organism	Genome size	Size relative to human	Status
HIV-1	9,750	0.0003%	complete 1985, Wain-Hobson,et.al.
Mycoplasma genitalium	580,070	0.0171%	complete 1995, TIGR
Mycoplasma pneumoniae	816,394	0.0240%	complete 1995, Univ. of Heidelberg
Lyme disease spirochete	946,000	0.0278%	complete 1997, TIGR
Methanococcus jannaschii	1,664,974	0.0490%	complete 1996, TIGR
H. influenzae	1,830,137	0.0538%	complete 1995, TIGR
Mycobacterium tuberculosis	4,397,000	0.1293%	complete 1998, Sanger Centre
Escherichia coli	4,639,221	0.1364%	complete 1997,Univ. of Wisc. + others
Yeast, Baker's	12,067,280	0.3549%	complete, 1996, Stanford Genome Center
C. elegans	100,000,000	3%	complete, 1999 Washington Univ. and Sanger Ctr.
Fruit fly	180,000,000	5%	complete, 2000 European and US groups
Pufferfish, Japanese (Takifugu rubripes)	400,000,000	12%	90% complete, 2005
Rice	400,000,000	12%	complete, 2002 International Rice Genome Seq. Proj.
Pig	2,700,000,000	79%	Mapping complete - sequencing mid stages
Rat	2,900,000,000	85%	Draft assembly
Cattle	3,000,000,000	88%	Begun in April, 2003, draft assembly
Mouse	3,000,000,000	88%	Complete, 2002
Rabbit	3,000,000,000	88%	
Human	3,400,000,000	1.0	Complete, 2001,2002,2003
Onion	18,000,000,000	5.3	
Marbled lungfish	139,000,000,000	40.9	
Fern	160,000,000,000	47.1	
Amoeba proteus	290,000,000,000	85.3	

History

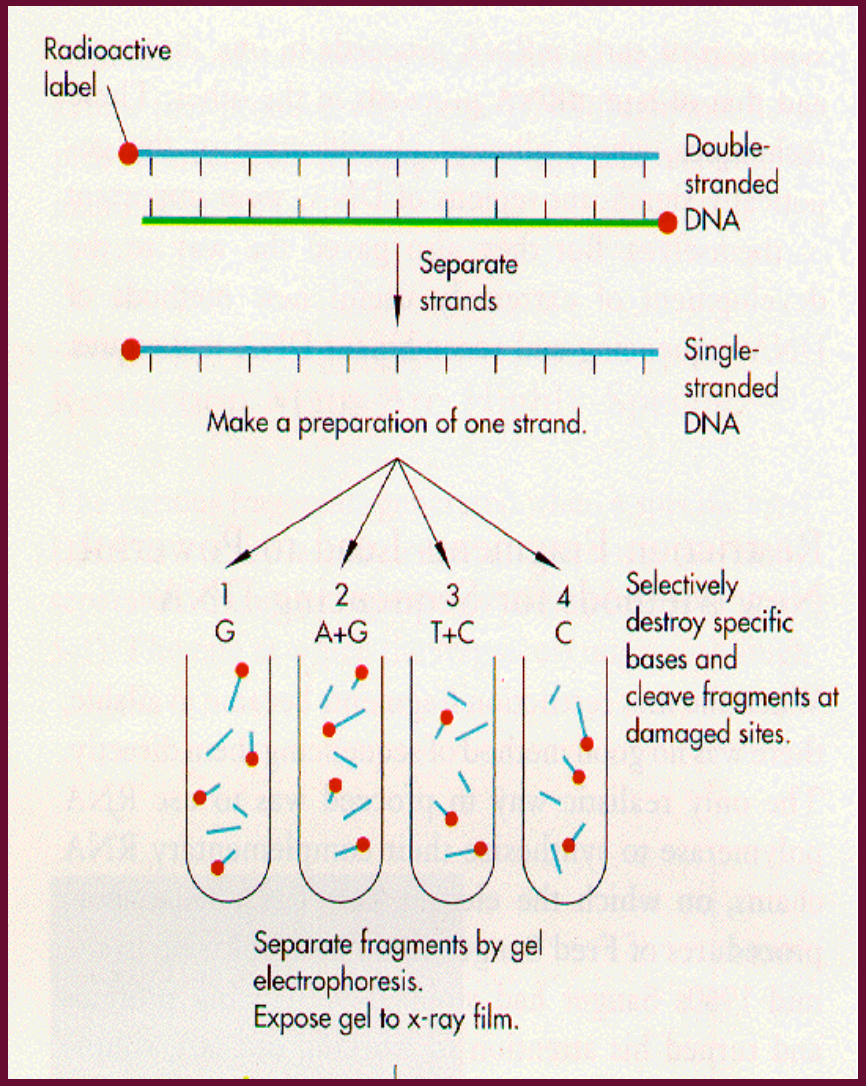


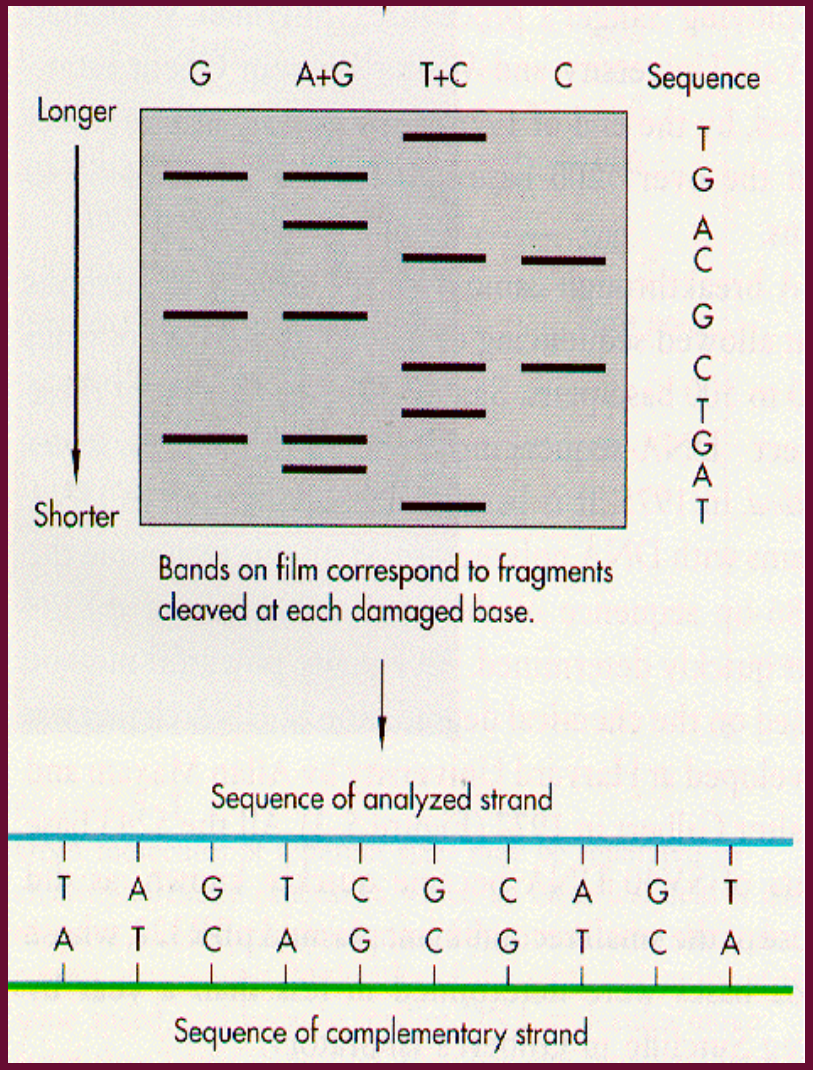
Walter Gilbert

Frederick Sanger

- Year 1977
- Maxem and Gilbert – “Chemical Cleavage Protocol”
- Sanger – Dideoxy sequencing or chain termination method.
- Gilbert and Sanger shared 1/2 Nobel Prize in 1980.
- Sanger method is used widely because of its practicality.

Maxem-Gilbert Sequencing





Sanger Method

- Based on the use of ddNTP's in addition to the normal nucleotides found in DNA.
- ddNTP's are same as NTP's except they contain a Hydrogen group on the 3' carbon instead of –OH group.
- ddNTP's when integrated into a sequence, prevent the addition of further nucleotides.

Sanger Method (Cont.)

- This is because a Phosphodiester bond can’t form between ddNTP and the next incoming nucleotide.
- Thus, DNA chain is terminated.
- Automated Sequencer Method.
- Human Genome Project (1990) by US Dept. of Energy and National Institute of Health.

Sequencing Methods

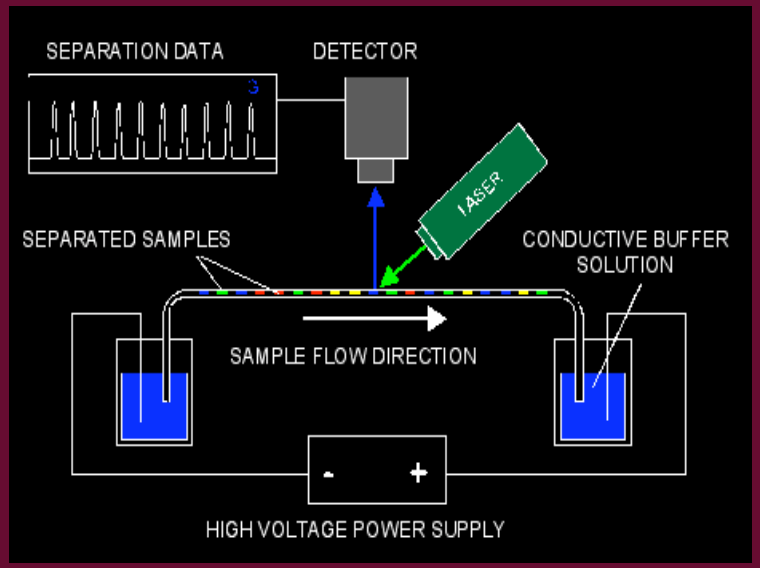
Electrophoretic Methods:

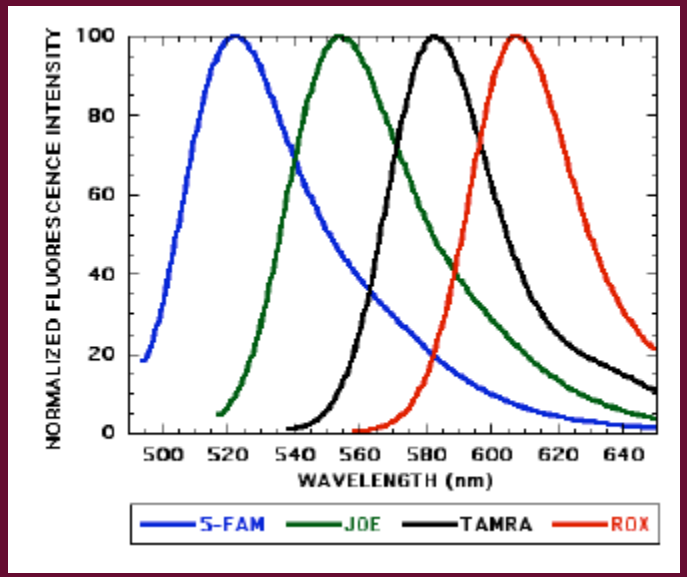
- Slab Gel Electrophoresis
- Capillary (Array) Electrophoresis
- Microfabricated Capillary Arrays
- Free Solution Electrophoresis

Non-Electrophoretic Methods:

- Pyrosequencing
- Sequencing by Hybridization
- Massively Parallel Signature Sequencing
- MALDI-TOF Mass Spectrometry
- Single Molecule Methods

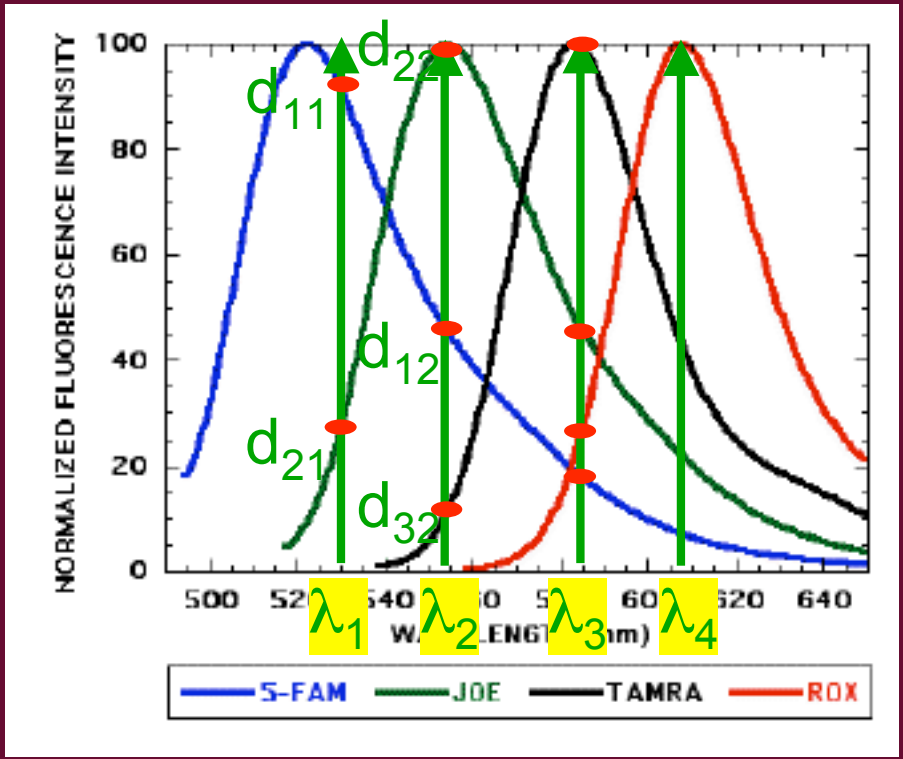
Capillary (Array) Electrophoresis





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

Cost:
~ $ 1M/genome
Time: months

Cost:
$1,000/genome
Time: hours

"$100,000" Genome Grants-Near-Term Development for Genome Sequencing

Stevan B. Jovanovich, Ph.D., Microchip Biotechnologies Inc., Fremont, Calif.
$6.1 million (3 years) "Microbead INtegrated DNA Sequencer (MINDS) System"

Gina L. Costa, Ph.D., Agencourt Bioscience Corp., Beverly, Mass.
$5.4 million (3 years) "Bead-based Polony Sequencing"

Kenton Lohman, Ph.D., 454 Life Sciences Corp., Branford, Conn.
$2 million (2 years) "Massively Parallel High Throughput, Low Cost Sequencing"
and
Marcel Margulies, Ph.D., 454 Life Sciences Corp., Branford, Conn.
$5 million (3 years) "454 Life Sciences Massively Parallel System DNA Sequencing"

John Williams, Ph.D., LI-COR Inc., Lincoln, Neb.
$2.5 million (3 years) "Single-Molecule DNA Sequencing Using Charge-Switch dNTPs"

Michael L. Metzker, Ph.D., Human Genome Sequencing Center, Baylor College of Medicine, Houston
$2 million (3 years) "Ultrafast SBS (Sequencing by Synthesis) Method for Large-Scale Human Resequencing"

Stephen R. Quake, Ph.D., Stanford University, Palo Alto, Calif.
$1.8 million (3 years) "High-Throughput, Single-Molecule DNA Sequencing"

Mostafa Ronaghi, Ph.D., Stanford Genome Technology Center, Palo Alto, Calif.
$1.8 million (3 years) "Pyrosequencing Array for DNA Sequencing"

Jingyue Ju, Ph.D., Columbia University, New York
$1.8 million (3 years) "An Integrated System for DNA Sequencing by Synthesis"

Peter Williams, Ph.D., Arizona State University, Tempe
$1.7 million (3 years) "Multiplexed Reactive Sequencing of DNA"

Steven A. Benner, Ph.D., University of Florida, Gainesville
$800,000 (3 years) "Polymerases for Sequencing by Synthesis"

Amit Meller, Ph.D., Rowland Institute at Harvard, Harvard University, Cambridge, Mass.
$600,000 (2 years) "Ultra-fast Nanopore Readout Platform for Designed DNA's"

"$1,000 Genome" Grants-Revolutionary Genome Sequencing Technologies

J. Michael Ramsey, Ph.D., University of North Carolina, Chapel Hill
$2 million (2 years) "Nanotechnology for the Structural Interrogation of DNA"

James Weifu Lee, Ph.D., Oak Ridge National Laboratory, Oak Ridge, Tenn.
Two grants: $700,000 (3 years); $750,000 (3 years)
"Computational Research & Development for Rapid Sequencing Nanotechnology" "Experimental Research & Development for Rapid Sequencing Nanotechnology"

Scott D. Collins, Ph.D., University of Maine, Orono
$850,000 (2 years) "High-speed Nanopore Gene Sequencing"

Steven A. Benner, Ph.D., University of Florida, Gainesville
$800,000 (3 years) "DNA Sequencing Using Nanopores"

Andre Marziali, Ph.D., University of British Columbia, Vancouver
$650,000 (3 years) "Nanopores for Trans-Membrane Bio-Molecule Detection"

Stuart Lindsay, Ph.D., Arizona State University, Tempe, Ariz.
$550,000 (3 years) "Molecular Reading Head for Single-Molecule DNA Sequencing"

Ronald W. Davis, Ph.D., Stanford University, Stanford, Calif.
$450,000 (2 years) "Single Molecule Nucleic Acid Detection with Nanopipettes"

http://www.genome.gov/12513210

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 13770–13773, November 1996
Biophysics

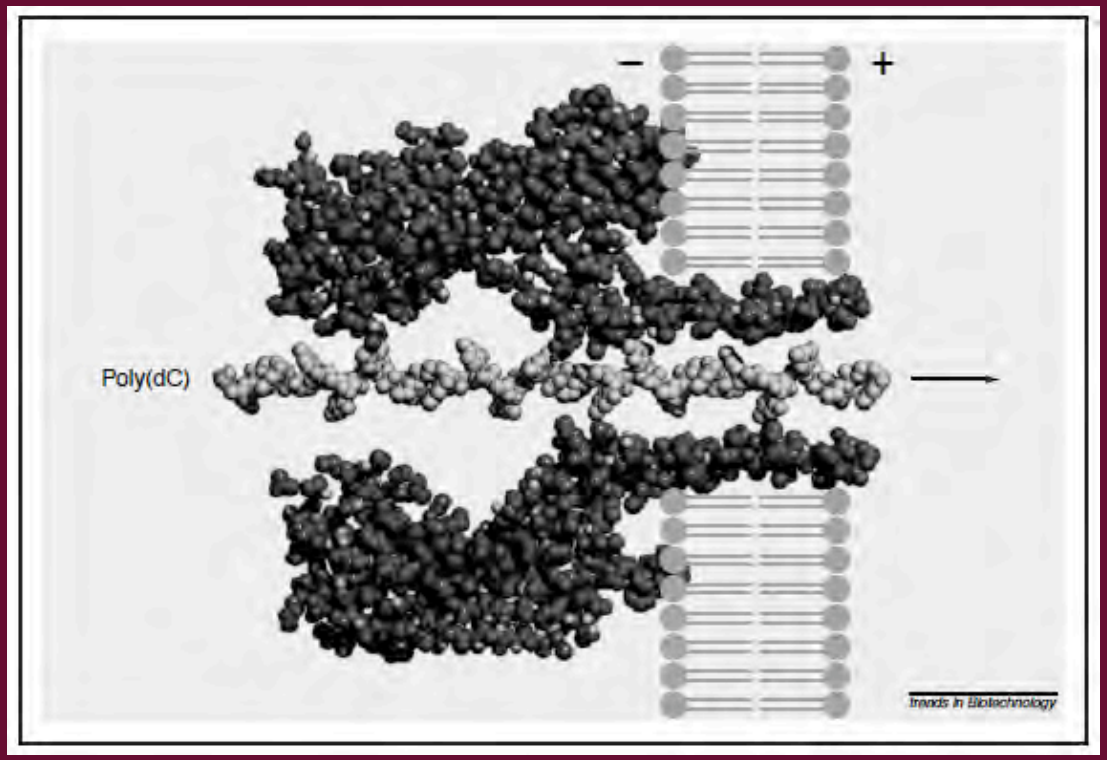
Characterization of individual polynucleotide molecules using a membrane channel

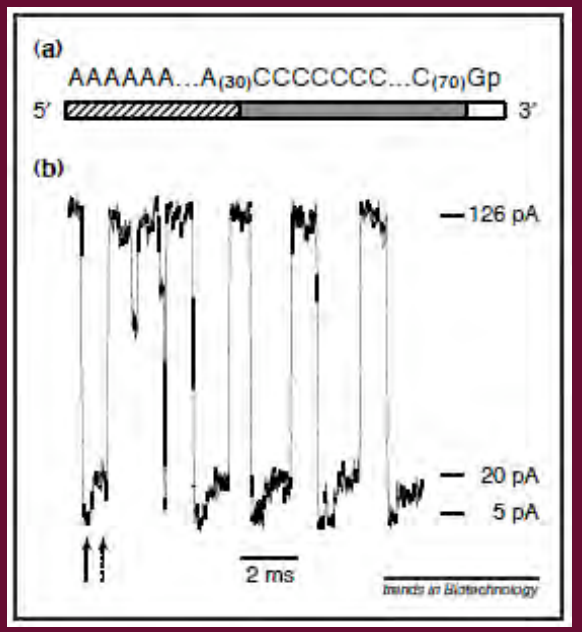
JOHN J. KASIANOWICZ*, ERIC BRANDIN†, DANIEL BRANTON†‡, AND DAVID W. DEAMER§

*Biotechnology Division, National Institute of Science and Technology, 222/A353, Gaithersburg, MD 20899; †Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138; and §Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064

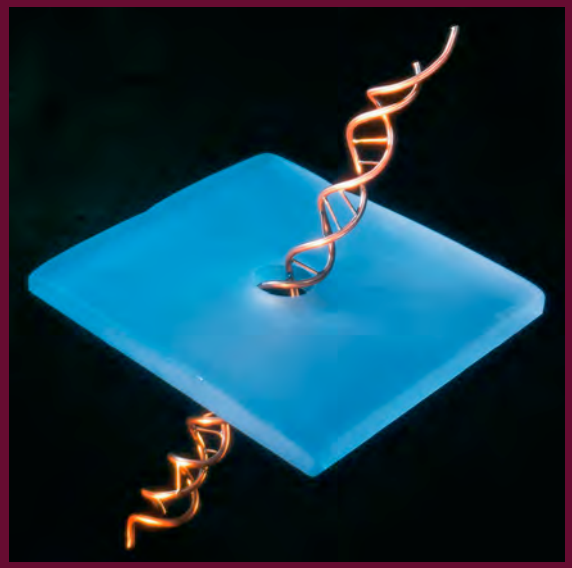
Contributed by Daniel Branton, September 5, 1996

A single α-hemolysin channel (Ø =1.5 nm) embedded in a lipid bilayer

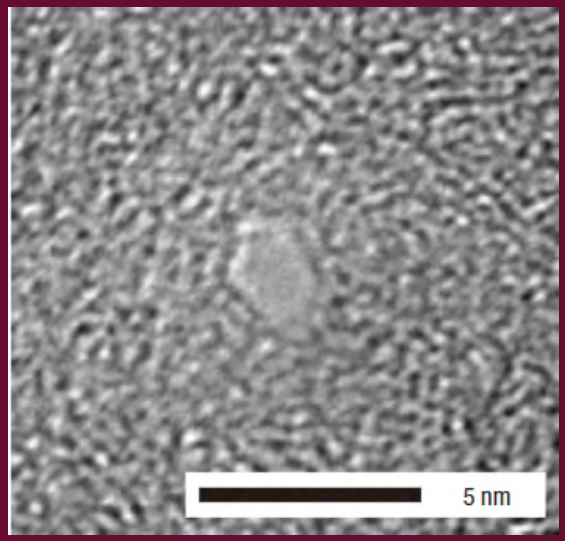


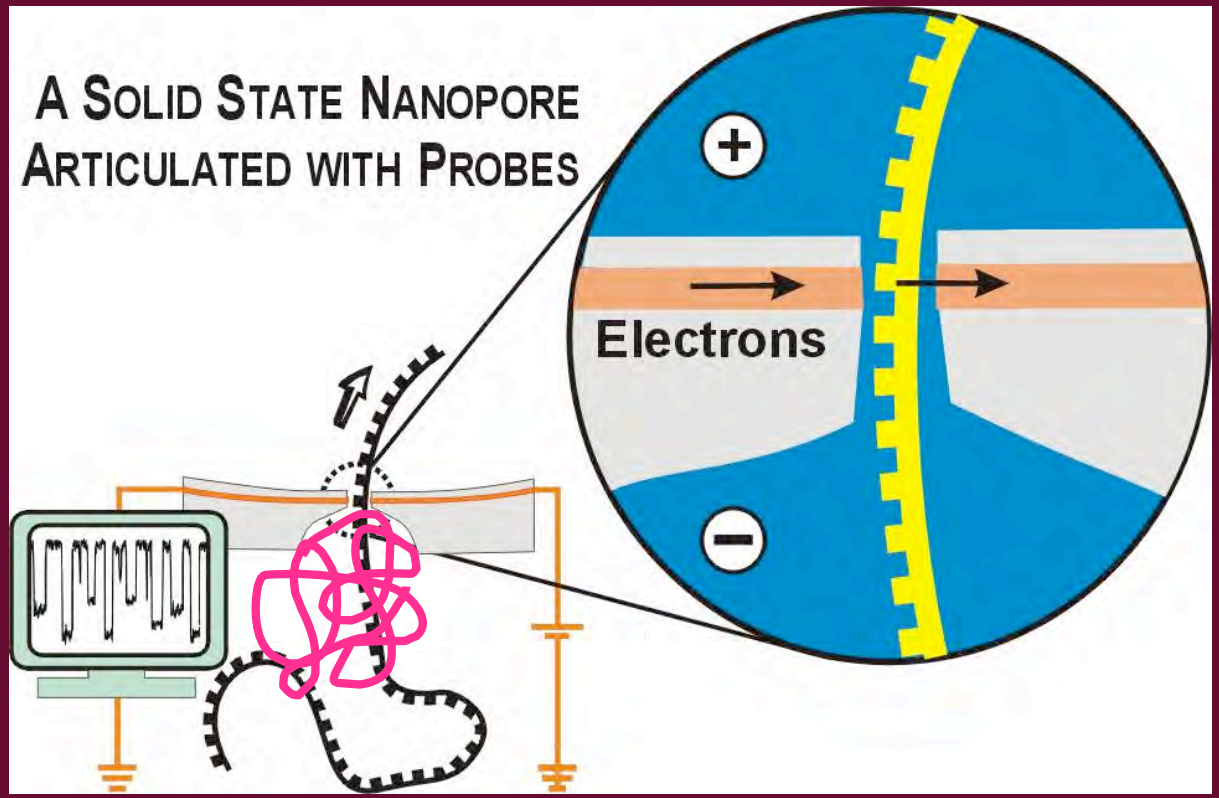


Solid State Nanopore



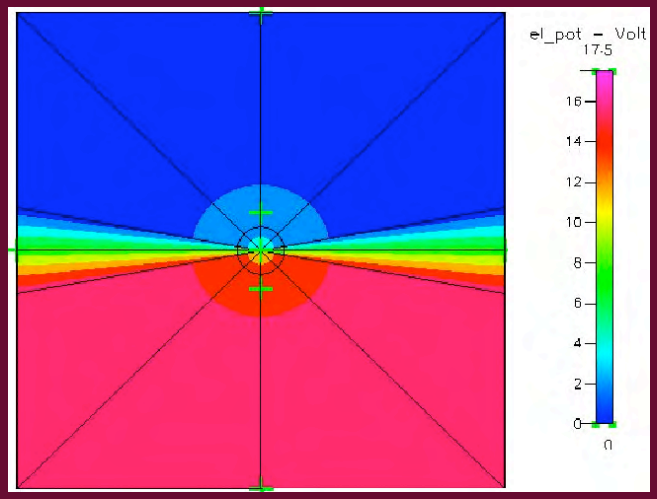
Dekker group, Nat. Mater. 2003

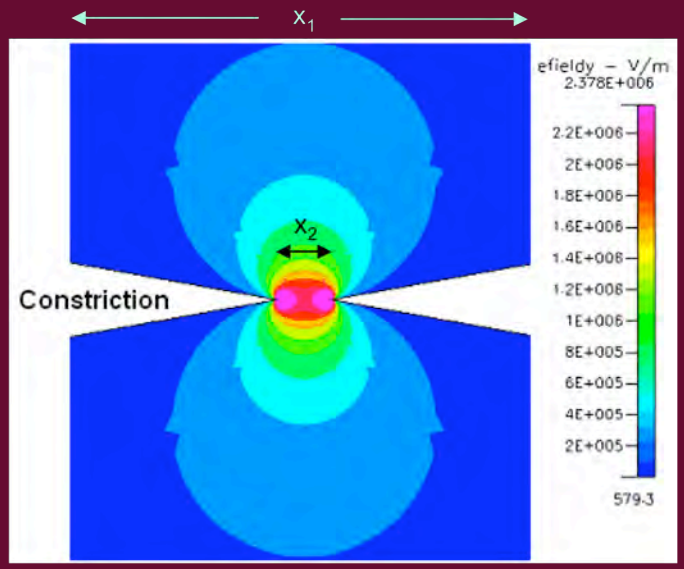


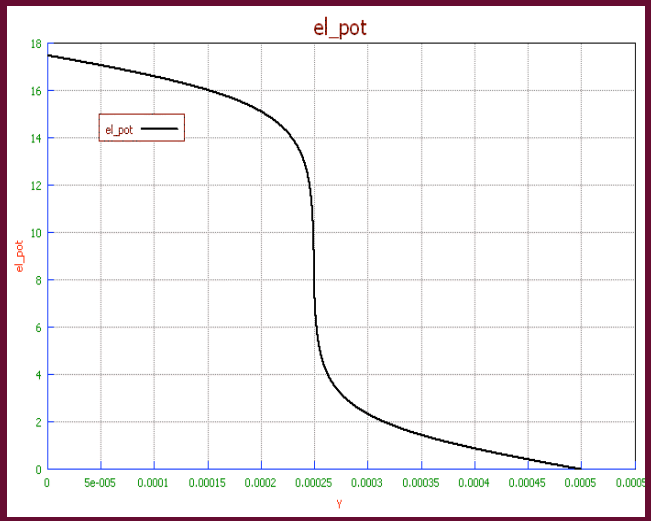


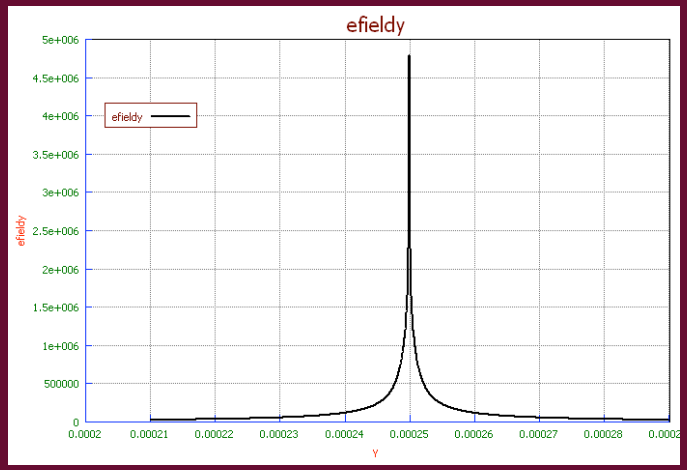
D. Branton, J. Golovchenko, Harvard

Electric Field Distribution









Potential drop and field focusing mostly occurs at the nanopore

Nanopore single molecule sequencing

Current issues:

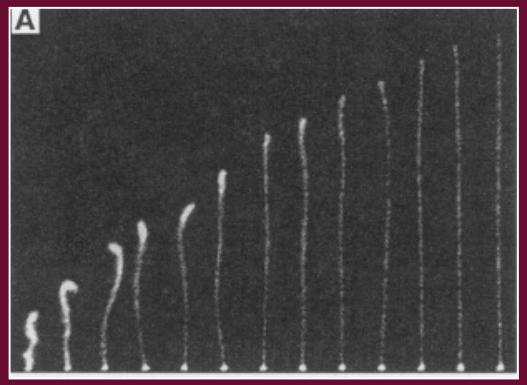
1. Resolution: 15-20 nucleotides
2. Speed: 1 base/μs → 1 base/ms to reduce electronic noise
3. World-Nano interface too abrupt
4. Needle pulling thread

Potential solutions–alternative approach:

1. Build a smooth World-Micro-Nano interface (smoother potential drop and better controlled speed)
2. Pre-stretch DNA
3. Planar nanopores (tunneling current & SERS measurements)
4. Resolution?

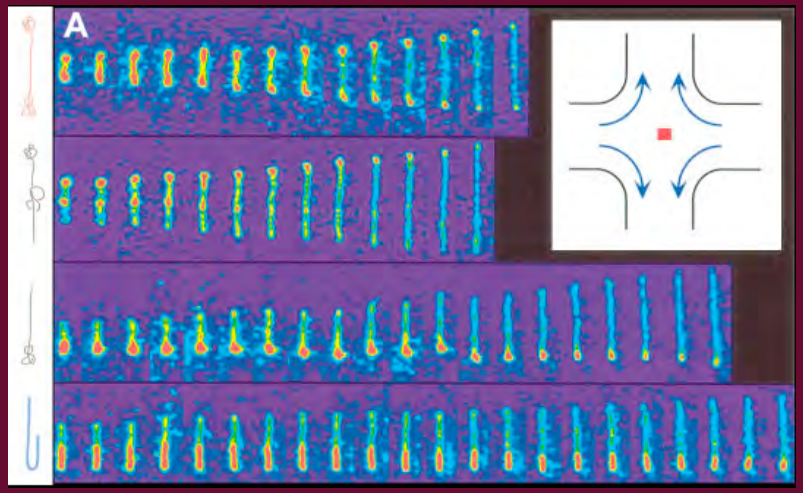
DNA stretching

Hydrodynamic flow



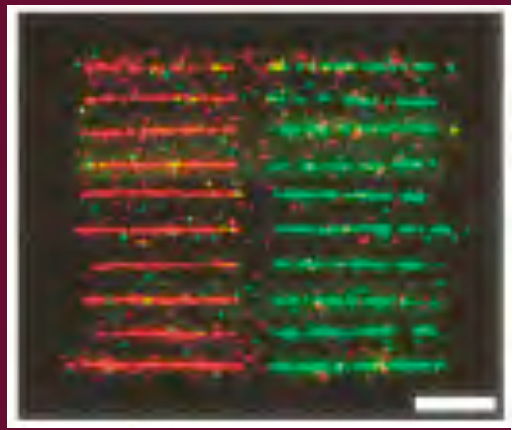
Perkins et al. Science (1995)

Elongation flow



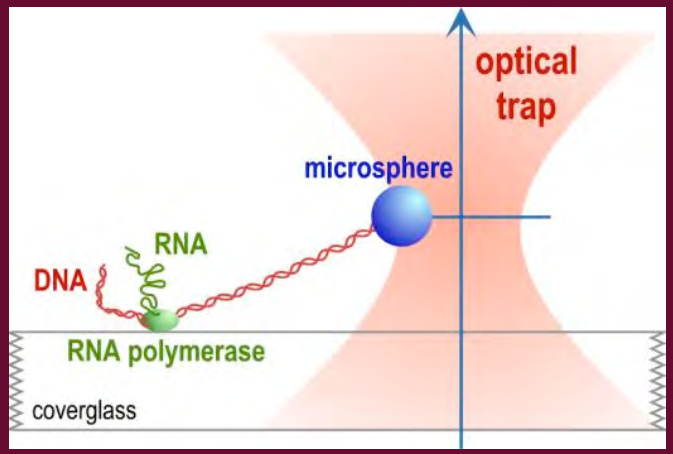
Perkins et al. Science (1997)

Molecular combing



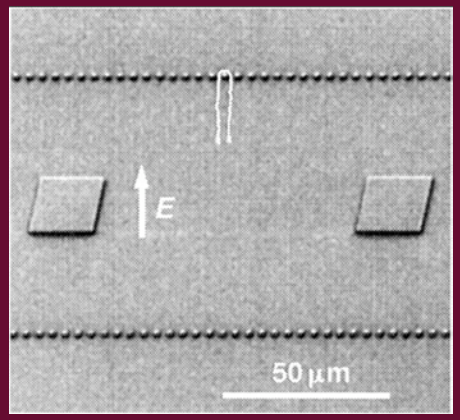
Bensimon, Science (1997)

Optical tweezer



S. Chu; S. Block (1994-5)

Electrohydrodynamics

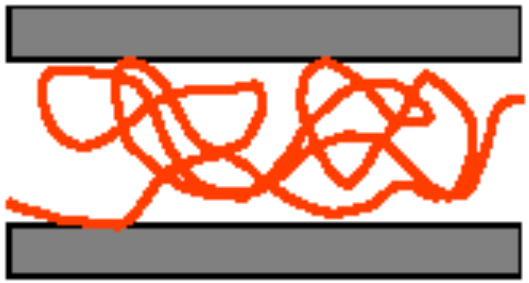


Bakajin et al. PRL (1998)

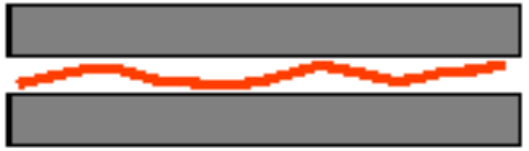
Polymer dynamics in confined nanoenvironment

DNA is stretched in small channels because the energy to form a loop is greater than kT

$$W_{loop} = \pi k_B T \frac{L_p}{R}$$



$2R = 2\mu m$ $\quad W_{loop} \approx 0.15 k_B T < k_B T$



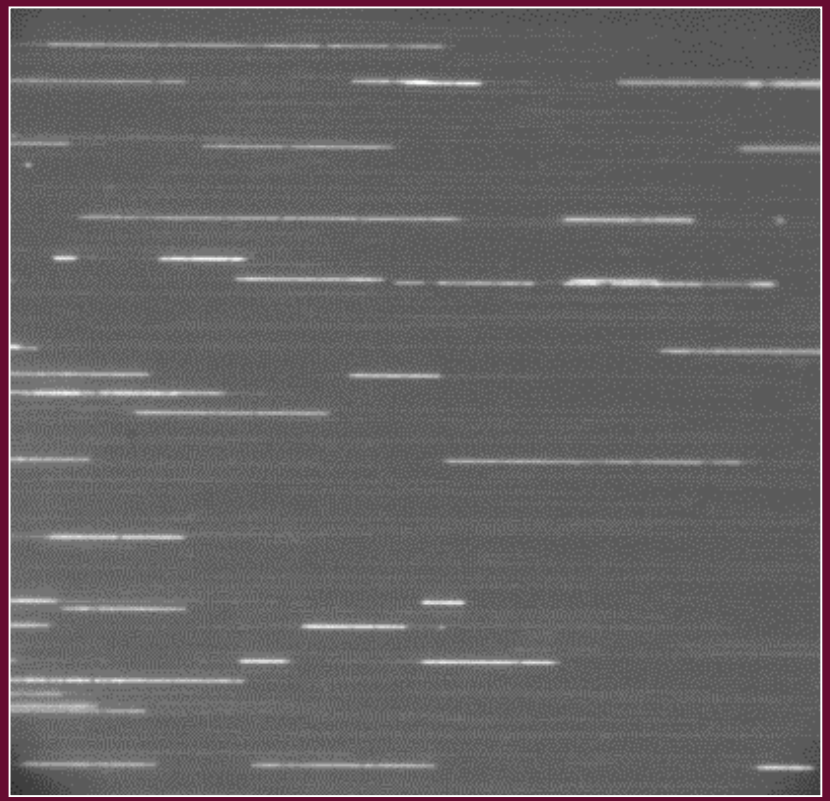
$2R = 0.1\mu m$ $\quad W_{loop} \approx 3 k_B T > k_B T$

For DNA the persistence length L_p=50nm

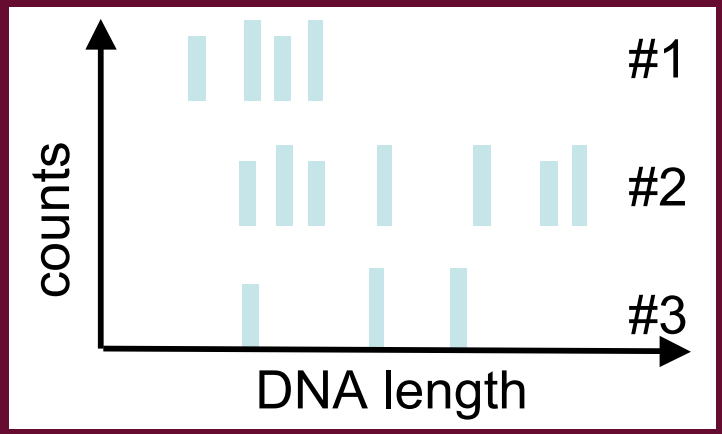
J. Tegenfeldt

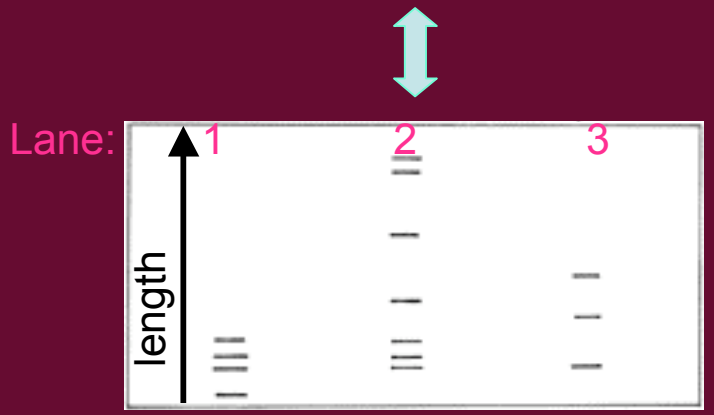
Nanofluidic Channels for DNA Mapping

The idea: to perform RFLP on-chip at the molecular level



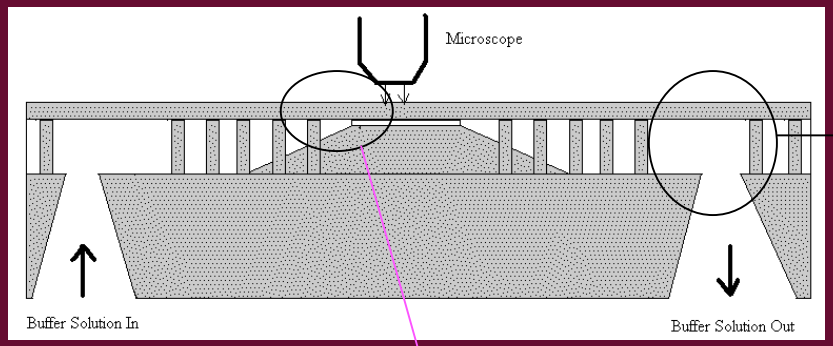
Fully stretched DNA in nanochannels



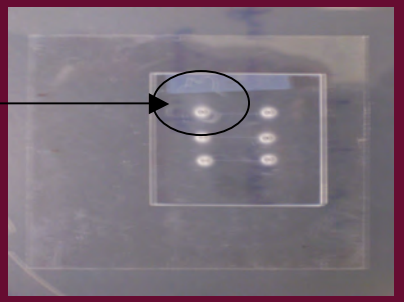


RFLP on gel

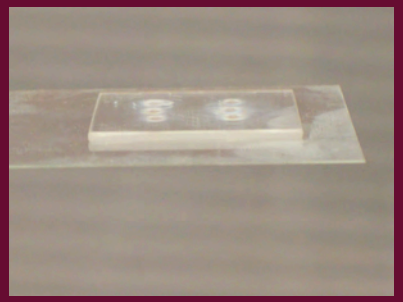
Final Packaged Device



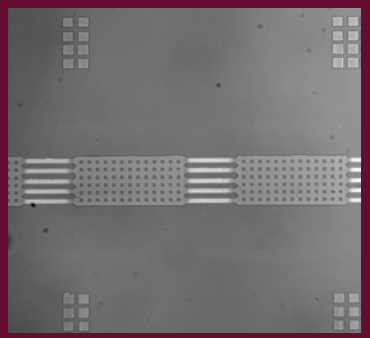
a) Schematic

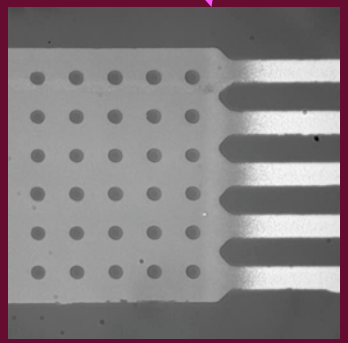


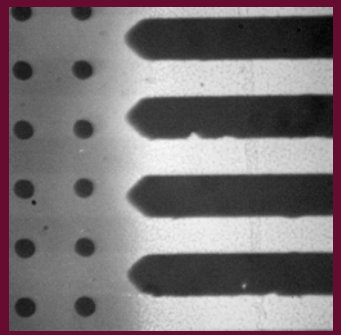
b) Top View



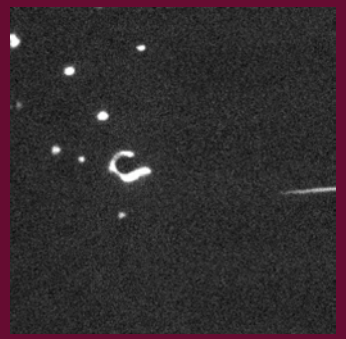
c) Side View

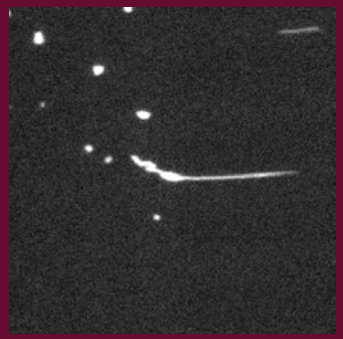






Magnified View@ 20X, 64X, 100X





DNA strands going up the ramp in the interfaced area

Gu, Gupta, Chou, Wei, *Lab Chip*, 2007, DOI: 10.1039/b704851c