# Contents

# Particle-Laden Fluids: Introduction

- Particles suspended in fluids
  - Heterogeneous mixtures
  - Biological particles like cells
  - Contamination
  - Dust
  - Etc.
- Particles essential part of fluid
  - WBCs and leukocytes for blood
  - Coffee
  - Milk
- Unwanted particles
- Problems
  - Clogging
  - Removal
  - Selectivity
  - Etc.

# 13. Particle-Laden Fluids

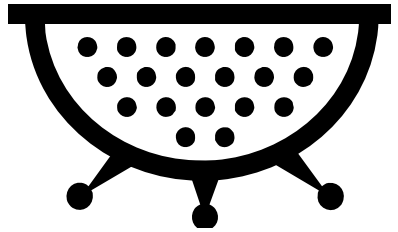
1. Diffusion Barriers
2. Manipulation of Suspended Particles
3. Particle Counting and Sorting
4. Blood Cell Counting

# 13. Particle-Laden Fluids

1. **Diffusion Barriers**
2. Manipulation of Suspended Particles
3. Particle Counting and Sorting
4. Blood Analysis

# 13.1. Diffusion Barriers

- Separation of components according to specific properties
- Various separating principles, e.g.
  - Mechanical separation
  - Diffusion
  - Adhesion



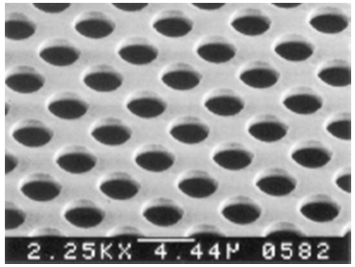
- Frequent purposes
  - Separation, e.g. for electrophoresis
  - Preconcentration
  - Removal of solid constituents

- Materials
  - Nylon
  - Porous silicon or aluminum oxide

# 13.1. Diffusion Barriers

1. **Microfilters**
2. Diffusion-Based Administration of Drugs
3. Diffusion-Based Particle Separation

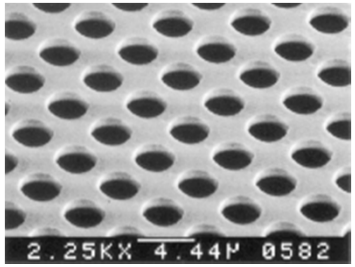
# 13.1. Microsieve by Aquamarijn





- Precision formed 0.1 to 100 micron pores
- 0.5 to 5 micron thick membrane plate
  - Surface roughness down to 10 nanometer
- Synthetic, ceramic and metallic *microsieve*(R)
  - Polyimide
  - Teflon
  - Aluminum
  - Silicon-nitride
  - Titanium
  - Chromium

# 13.1. Microsieve by Aquamarijn

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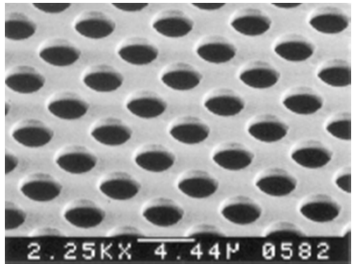
- Well defined pores
  - Controllable pore size and location in membrane layer
- Thickness in most cases smaller than pore size
  - Filter efficiency much higher than for other filters
- Applications
  - Biotechnology and medicine
  - Sterile filtration
  - Absolute filtration
  - Critical cell-cell separation
  - Cell deformability testing and cell harvesting
- Bio-compatibility
  - *microsieve*(R) material
  - *microsieve*(R) is coated with such material

$$R_{\mathrm{hd}} = C_{\mathrm{nc}} \frac{\eta l}{\varrho A^2}$$

# 13.1. Microsieve by Aquamarijn

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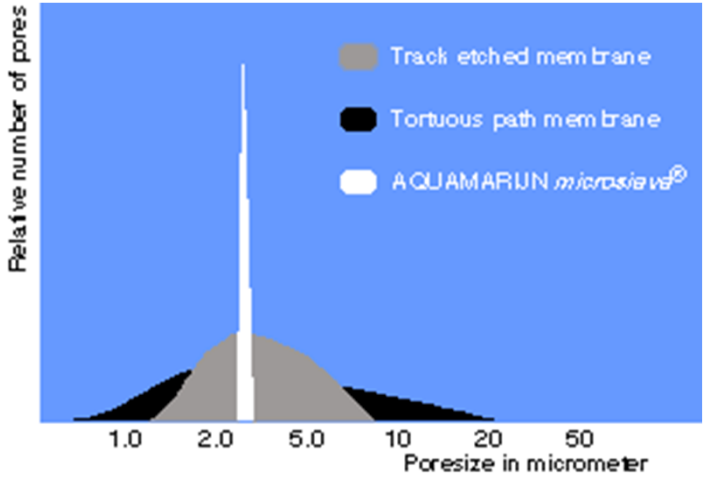
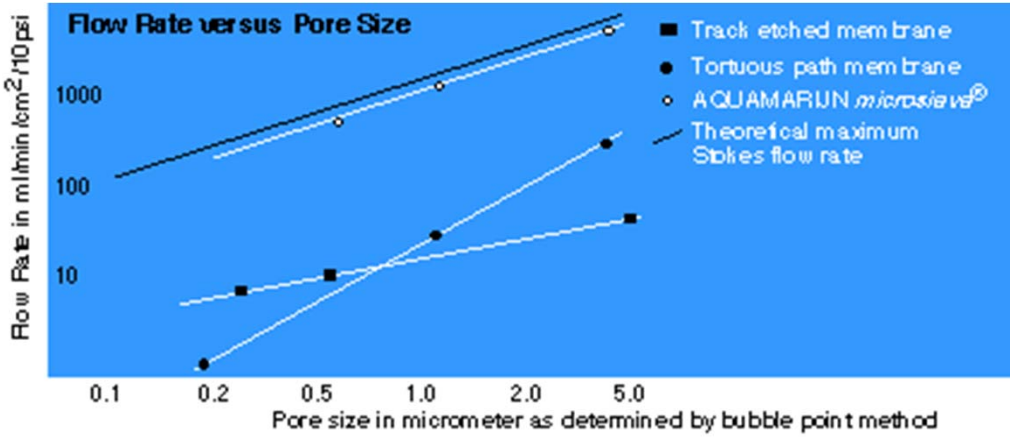


# 13.1. Diffusion Barriers

1. Microfilters
2. **Diffusion-Based Administration of Drugs**
3. Diffusion-Based Particle Separation

# 13.1. Diffusion-Based Administration of Drugs

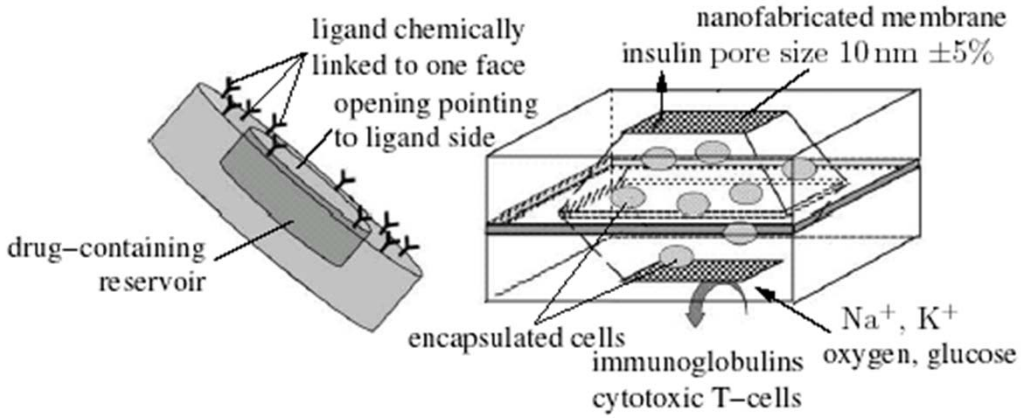



Fig. 17.14. Microparticle technology and nanopore technology [916, 308].

- Microparticle
  - Membrane encloses drug reservoir
  - Specific binding to site of drug release via ligands
  - Drug release upon electrical switching
- Nanopore
  - Insulin-producing cells encapsulated by nanofabricated membrane
  - Nutrients (small molecules) can traverse membrane
  - Antibodies (immunoglobulin protein) blocked

# 13.1. Diffusion Barriers

1. Microfilters
2. Diffusion-Based Administration of Drugs
3. **Diffusion-Based Particle Separation**

# 13.1. Diffusion-Based Particle Separations

- **Hydrodynamic Chromatography (HDC)**

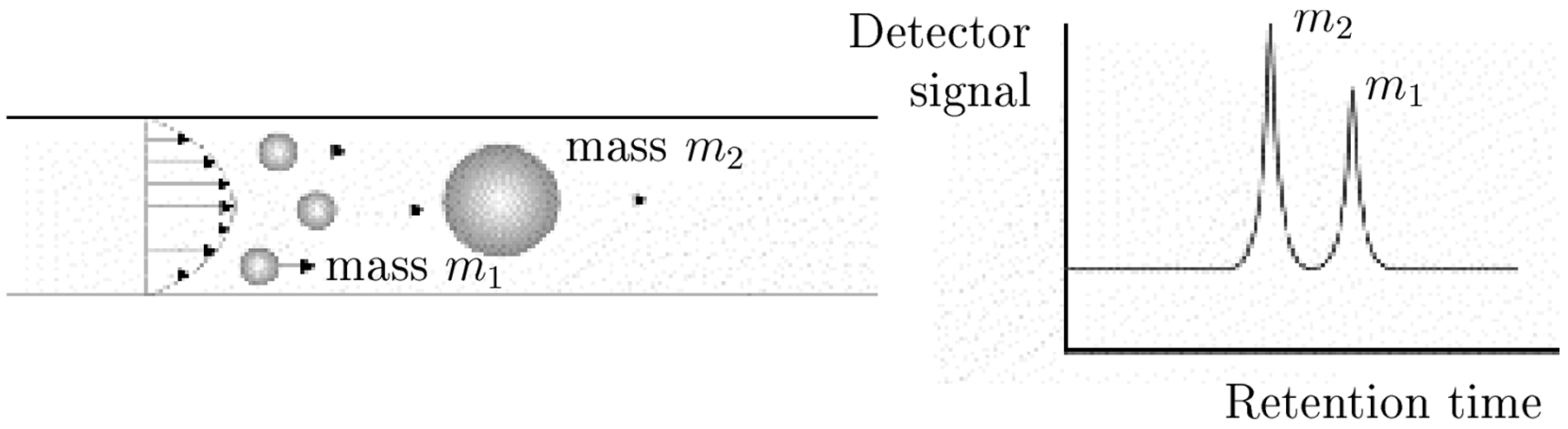
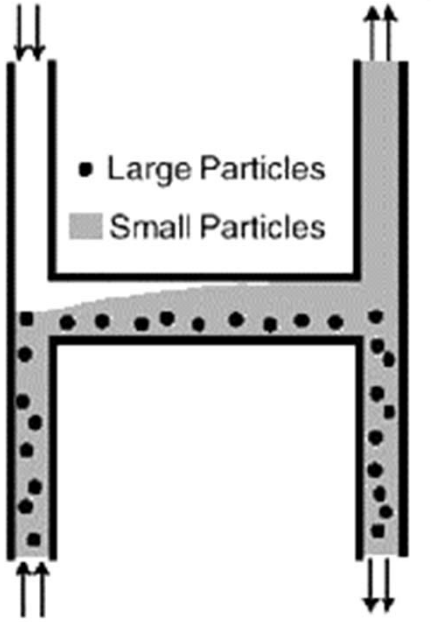



**Fig. 0.1.** Hydrodynamic chromatography in laminar, pressure-driven flow [**?**]. Due to the parabolic velocity profile, small particles (mass M1) display an increased probability for staying within the slow moving liquid layers near the wall. The larger particles (mass M2) always "touch" the central maximum velocity region and therefore possess a smaller retention time

# 13.1. Diffusion-Based Particle Separation

MICRONICS

H-Filter




- Laminar flow structure
- Two inlet streams
  - Pure liquid
  - Suspension of small and large particles
- Diffusion window
  - Small particles diffuse in pure liquid
  - Diffusion of large particles too slow
- Outlet
  - Suspension of small particles
  - Suspension of small and large particles

# 13.1. Diffusion-Based Particle Separation

- Diffusive filtering
  - Particle size sets mean flow velocity
  - Diffusion sets lateral motion

$$\boldsymbol{j}_N = -D\nabla \rho_N$$

$$D = \frac{1}{3} v_{\mathrm{th}} l_{\mathrm{mfp}}$$

$$v_{\mathrm{th}} = \sqrt{\bar{v^2}} = \sqrt{\frac{3k_{\mathrm{B}}T}{m}}$$

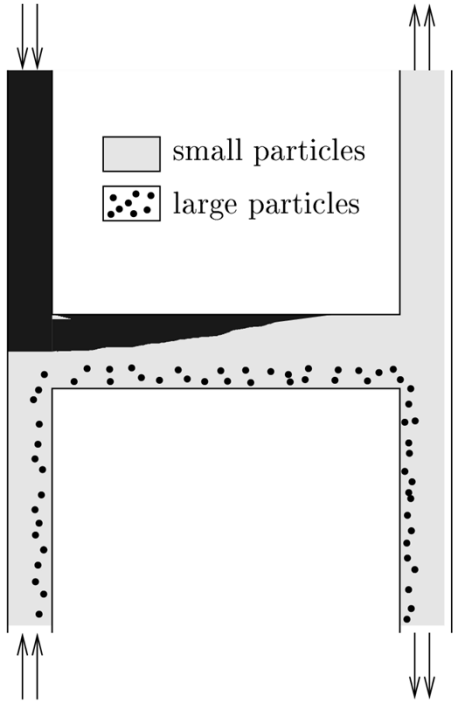



**Fig. 0.1.** The H-filter concept. Under laminar flow conditions, the two cocurrent streams in the central channel can only mix by diffusion. On a short distance, only the small particles have enough time to join the adjacent stream [?]

# 13.1. Diffusive Filtering by Asymmetric Pathways

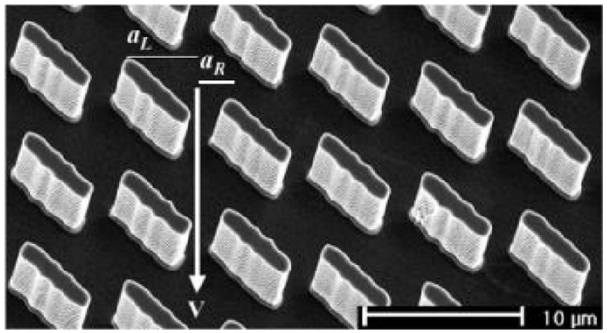



**Fig. 13.8.** A scanning electron micrograph of the obstacle course. The obstacles are 0.35 mm high and measure $1.5 \times 6.0\ \mu m^2$ with a gap between adjacent obstacles of 1.5 µm

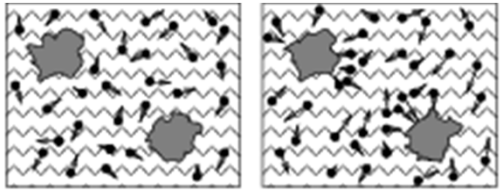
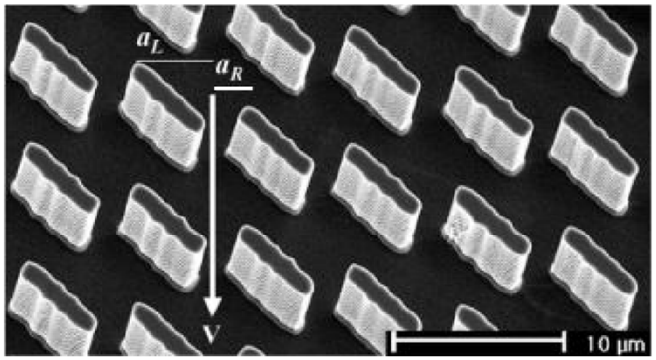


**Fig. .** Brownian motion resulting from random molecular pressure oscillations leads to wobbling motion of mesoscopic particles that can be observed under a microscope

- Molecules driven through microstructured silicon device
- Rectified Brownian motion
  - Array of 2-dimensional lattice of asymmetric obstacles
  - Propulsion of molecules by external electric field with velocity *v*
  - Gaps between adjacent obstacles measure 1.5 µm
  - Transverse Brownian motion may cause molecule to skip one channel
    - To the right, if it diffuses through displacement $a_R$
    - Or (very rarely), one channel to the left if it diffuses through $a_L$

# 13.1. Diffusive Filtering by Asymmetric Pathways

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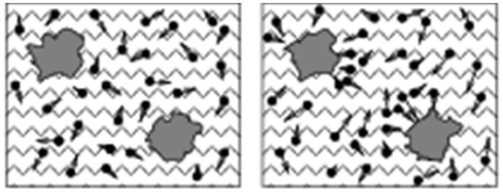


**Fig. .** Brownian motion resulting from random molecular pressure oscillations leads to wobbling motion of mesoscopic particles that can be observed under a microscope

Diffusive filtering by asymmetric pathways

- Microdevice allows to probe specific aspects of biological objects
- Micro-obstacles act on same length scale as Brownian motion
- Separation of DNA molecules of different size
  - Nominal resolution of 6% by length of DNA molecules
  - For size range 15 kbp

# 13.1. Diffusive Filtering by Asymmetric Pathways

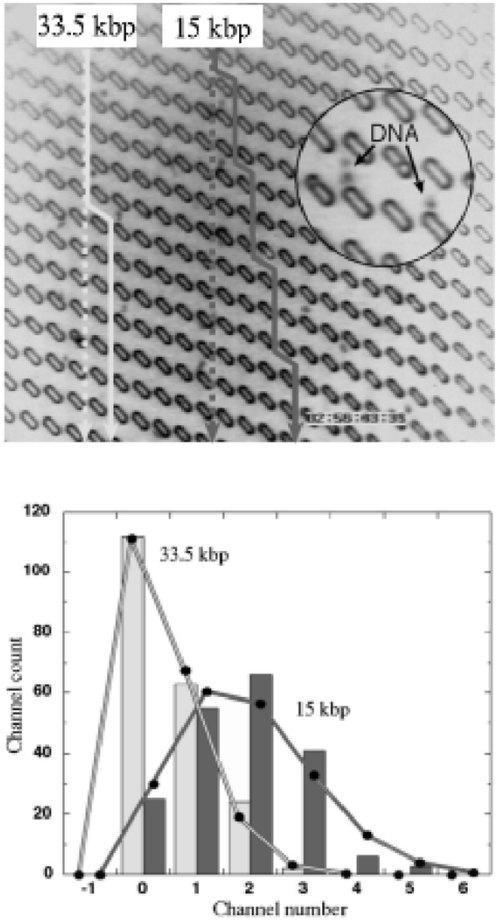



**Fig. 13.9.** Performance of the asymmetric obstacle separator in a $130 \times 114\,\mu m^2$ frame size and an electric field of $1.4\,V\,cm^{-1}$. (top) The trajectories of the 15-kbp DNA fragments deviate further to the right after 14 gates than the 35.5-kbp fragments since their size allows for increased transversal diffusion. (bottom) Accordingly, the smaller the molecule is registered at a higher mean channel number. The solid lines are binomial distributions with the same mean and area as the experimental data

# 13.1. Entropic Trap

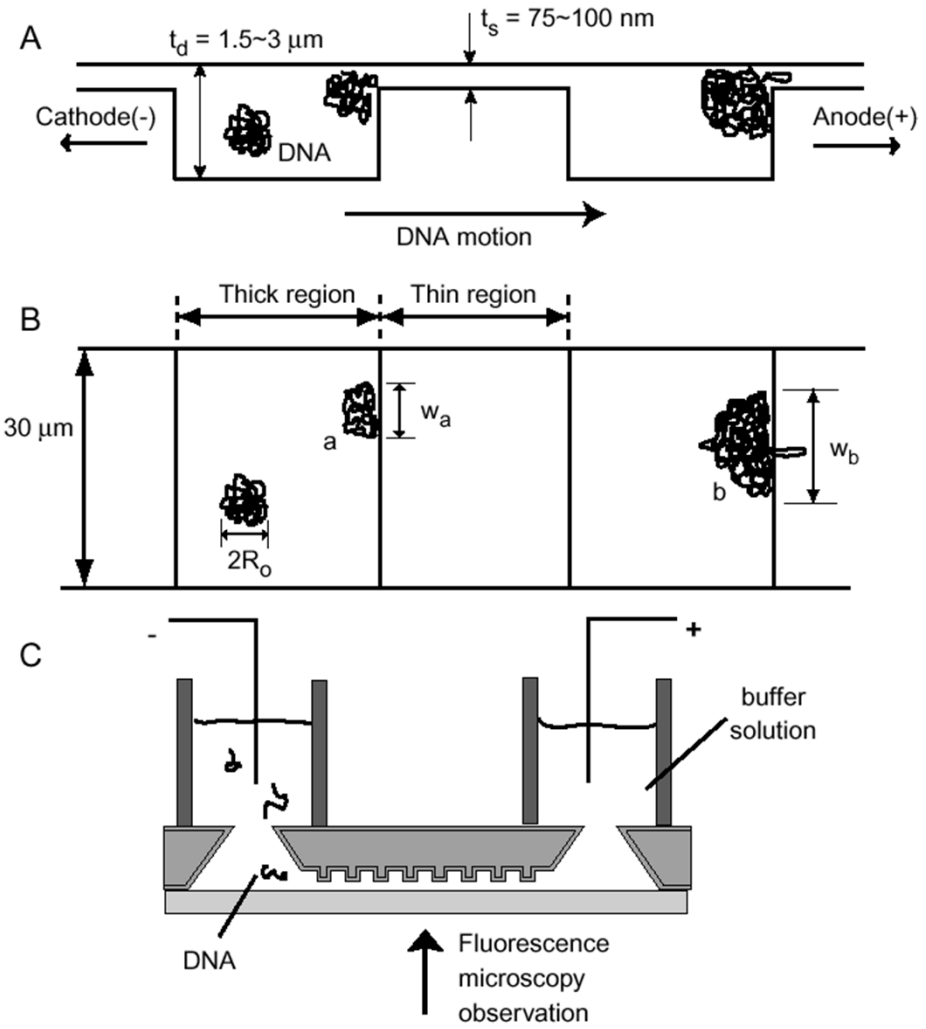
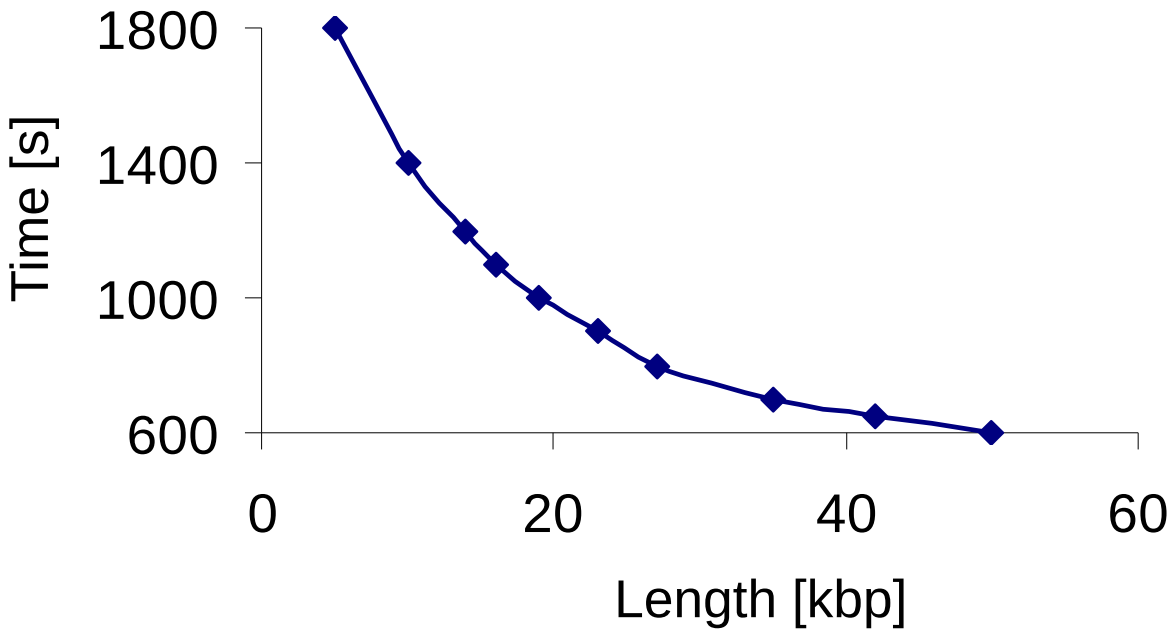



**Fig. 1.** Nanofluidic separation device with many entropic traps. (**A**) Cross-sectional schematic diagram of the device. Electrophoresed DNA molecules are trapped whenever they meet a thin region, because their radius of gyration ($R_o$) is much larger than the thin region depth (here, $t_d$ and $t_s$ are the thick and thin region depths, respectively). (**B**) Top view of the device in operation. Trapped DNA molecules eventually escape, with a probability of escape proportional to the length of the slit that the DNA molecule covers ($w_a$ and $w_b$). Larger molecules have a higher escape probability because they cover wider regions of the slit ($w_b > w_a$). (**C**) Experimental setup. Reservoirs are made at both ends of the channel and filled with DNA solution.

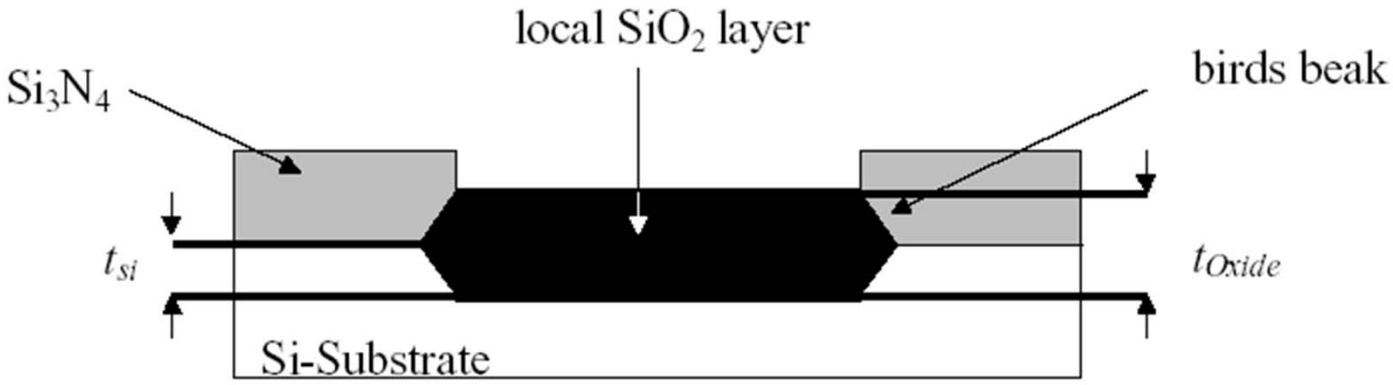
# 13.1. Entropic Trap




Craighead et al.
„Separation of Long DNA Molecules in a Microfabricated Entropic Trap Array“,
Science, **288**, 1026-1029, 2000

# 13.1. Entropic Trap

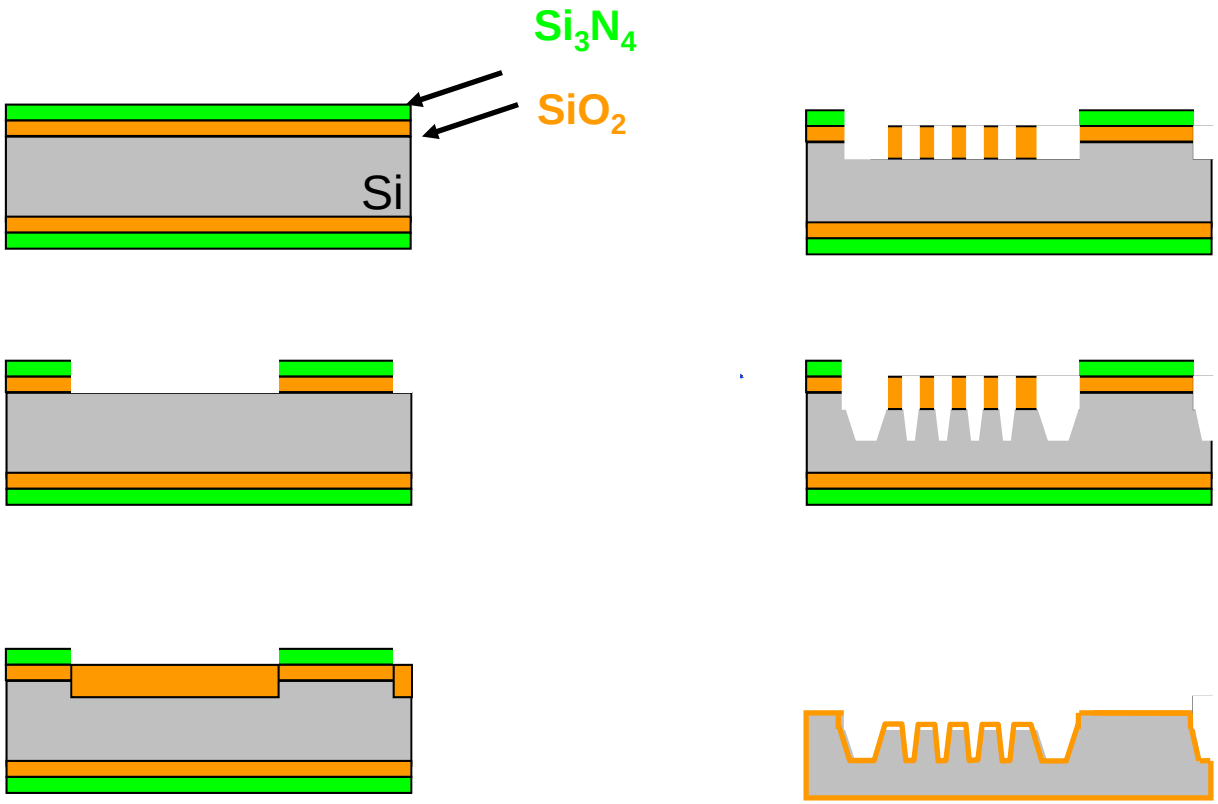
Fabrication by LOCOS process




$$t_{Si} = 0.44 \bullet t_{oxide}$$

# 13.1. Entropic Trap

Fabrication by LOCOS process



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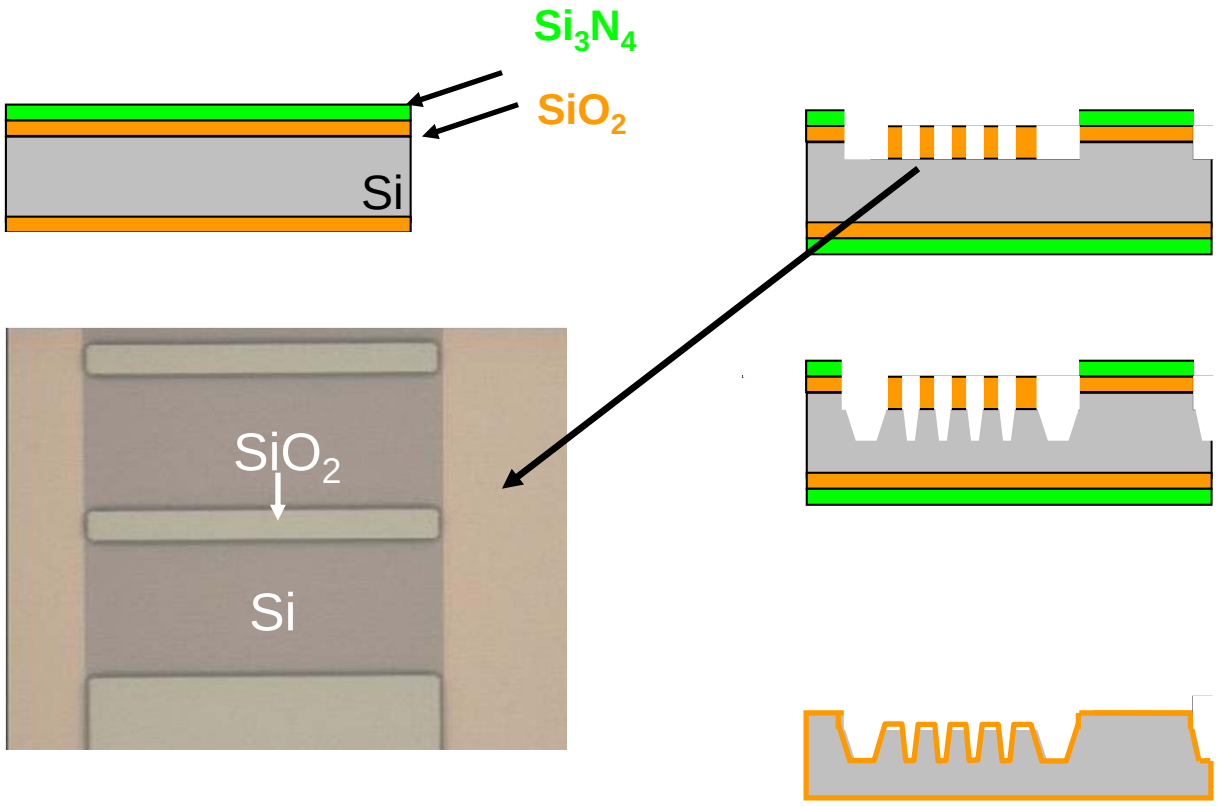
Fabrication by LOCOS process



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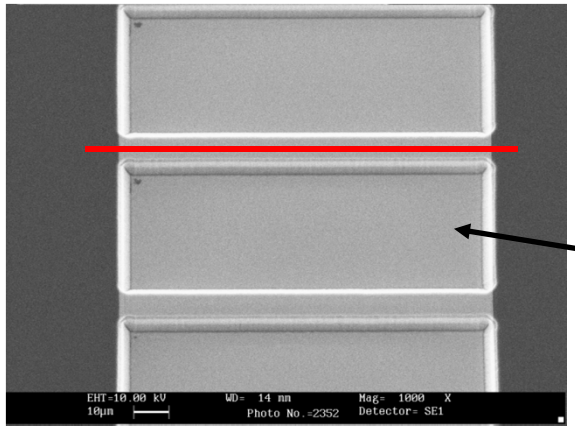
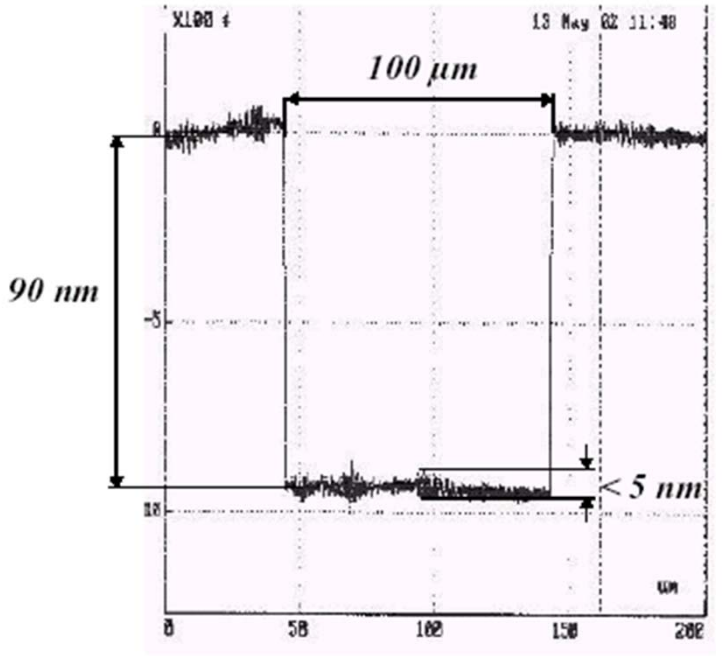
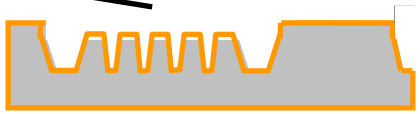
Fabrication by LOCOS process



# 13. Particle-Laden Fluids

1. Diffusion Barriers
2. **Manipulation of Suspended Particles**
3. Particle Counting and Sorting
4. Blood Analysis

# 13.2. Manipulation of Suspended Particles

1. **Trapping by Electromagnetic Fields**
2. Manipulation by Pressure Waves
3. Trapping by Multiple Forces

# 13.2. Manipulation by Traveling Electric Fields

- Electrically charged or polarizable particles trapped at certain locations by oscillating fields in special electrode configurations
- Propagation of guiding waves
  - Transport of  trapped particles
- Examples for particles of interest
  - DNA molecules in solution
    - Normally negatively charged
  - Dielectric beads or cells
    - Polarizable by external field

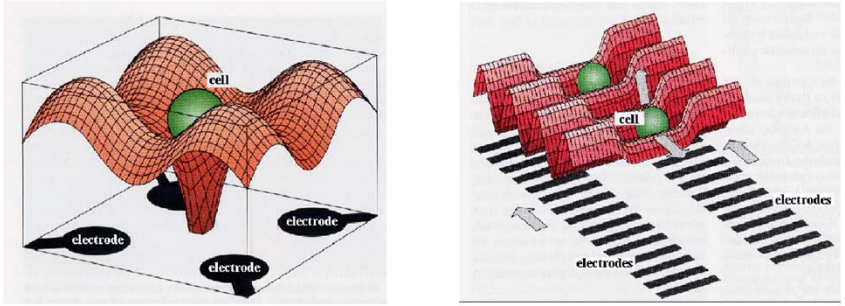



**Fig. 0.1.** Transports of individual polarized cells by traveling wave principles. An electrode configuration for linear transport is shown in Fig. **??** (JD: ask Roland for appropriate reference to Prof. Fuhr)

# 13.2. Dielectrophoresis

- Inhomogeneous alternating electric field ***E***
- Electrically polarizable particles
- Induced dipole moment $\boldsymbol{p}_q(\nu)$
- Force

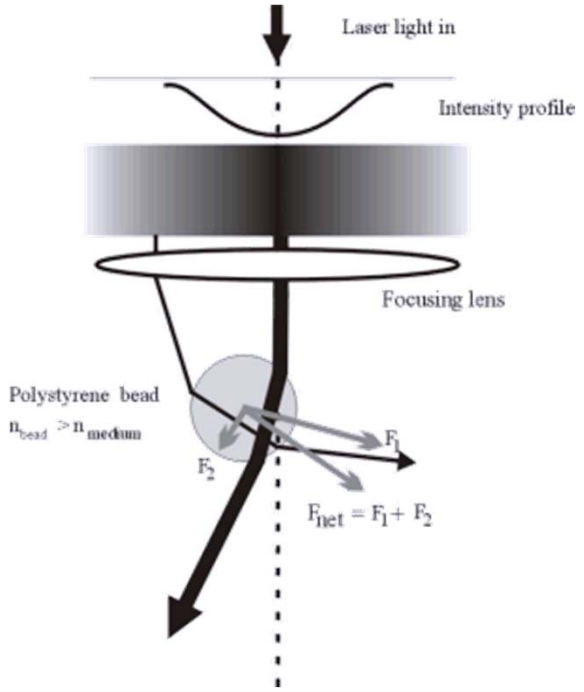
$$\boldsymbol{F}_{\mathrm{DEP}} = \frac{\mathrm{Re}[\boldsymbol{p}_q]}{2|\boldsymbol{E}|}\nabla\left(|\boldsymbol{E}|^2\right)$$

$$\boldsymbol{p}_q(\nu) = 4\pi\varepsilon_0\varepsilon_{\mathrm{fluid}} f(\varepsilon^*_{\mathrm{part}}, \varepsilon^*_{\mathrm{fluid}}) r_0^3 \boldsymbol{E} = \alpha_\varepsilon(\nu)\boldsymbol{E}$$

$$f(\varepsilon^*_{\mathrm{part}}(\nu), \varepsilon^*_{\mathrm{fluid}}(\nu)) = \frac{\varepsilon^*_{\mathrm{part}}(\nu) - \varepsilon^*_{\mathrm{fluid}}(\nu)}{\varepsilon^*_{\mathrm{part}}(\nu) + 2\varepsilon^*_{\mathrm{fluid}}(\nu)}$$

# 13.2. Manipulation

- Laser tweezers
  - Optical frequencies for small particles like DNA



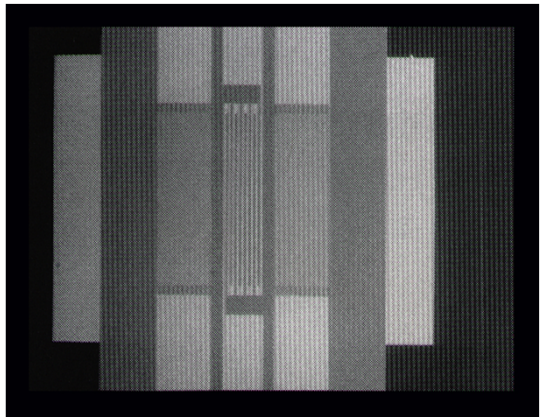

http://www.nbi.dk

# 13.2. Manipulation of Suspended Particles

1. Trapping by Electromagnetic Fields
2. **Manipulation by Pressure Waves**
3. Trapping by Multiple Forces

# 13.2. Manipulation by Pressure Waves

- Electro-acoustic piezo-transducers
- Concentrating
  - Standing acoustic in microchannel
    - Nodes and anti-nodes
  - Acoustic force dependent on
    - Particle size
    - Particle density
    - Acoustic energy
  - Separation according to material properties
  - Ultrasonic sedimentation
    - Demonstrated for DNA and cells
- Particle transport by acoustic pressure
  - Traveling wave
  - Flexural plate wave (FPW) based on PZT film
  - FPW as sensor for measuring concentration of cells



**MBBNet Gallery**

# 13.2. Manipulation of Suspended Particles

1. Trapping by Electromagnetic Fields
2. Manipulation by Pressure Waves
3. **Trapping by Multiple Forces**

# 13.2. Trapping by Multiple Forces

“Multidimensional” Interaction

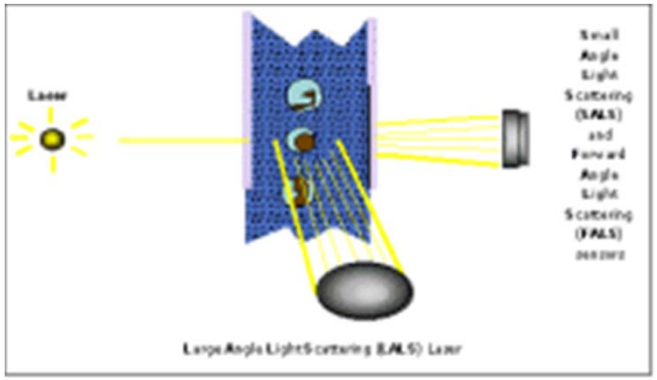
- Acoustic
- Dielectrophoretic
- Laser tweezers
- Sheath flow (hydrodynamic)

# 13. Particle-Laden Fluids

1. Diffusion Barriers
2. Manipulation of Suspended Particles
3. **Particle Counting and Sorting**
4. Blood Cell Counting

# 13.3. Flow Cytometry

- Technique to analyze individual biological cells
- Particles suspended in small volumes of fluid, typically water or buffer solution
- Viewed superficially
  - Process of sorting, counting, and / or sizing of cells or tissue sections
- Broadly classified into two categories based on medium through which sample is analyzed
  - Image cytometry
    - Sample on microscope
  - Slide flow cytometry
    - Sample immersed in a stream or flow
- Flow cytometer draws particles from sample delivery tube into flow
- Cells pass the microscope objective in single file




*Microsystems™ – Micro Flow Cytometer*

# 13.3. Flow Cytometry - Sheath Flow Principle

- Laminar domain, no turbulence
- Wide column of particles
  - Accelerated to form narrow column
  - Surrounded by fluid of same refractive index
- Sheath fluid in turn enclosed in tube
  - Not interfering with observation of its axial content
- Alignment of particles in single file
  - Hydrodynamic focusing
- Injection of aqueous sample suspension
  - Injected into faster flowing sheath fluid
  - Providing sheath for alignment of particles
  - Sample delivery fluid entrained in sheath fluid by velocity gradient

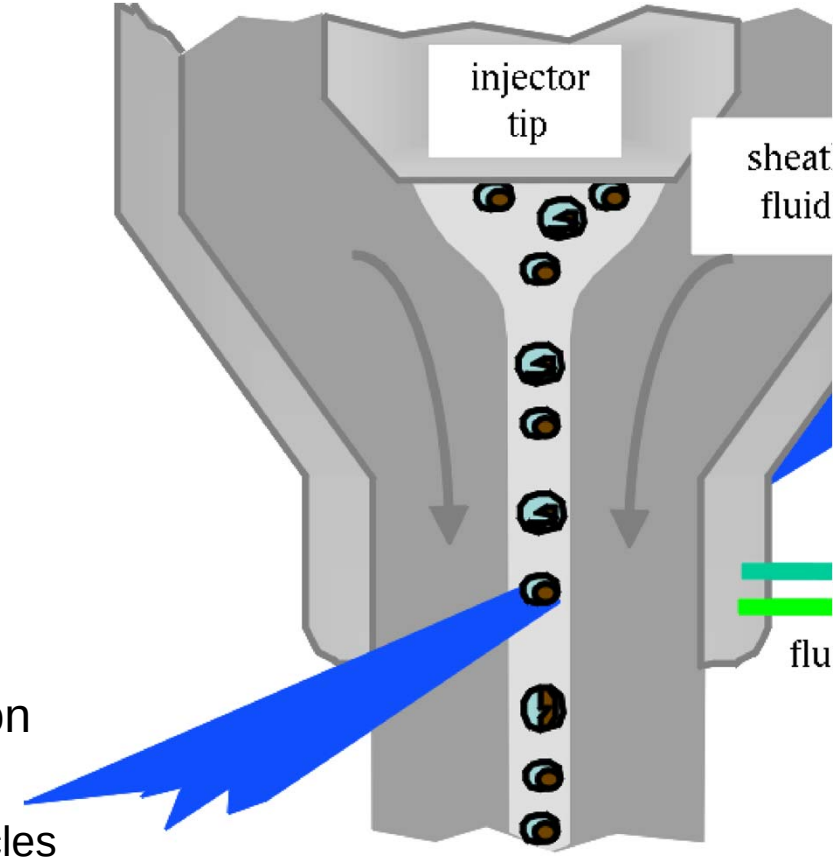



**Fig. 0.1.** Micro flow cytometer operated with a sheath fluid. Suspended particles suspended in a fluid are aligned in a single file while they pass the laser excitation. Labelled cells are subsequently detected by fluorescence

# 13.3. Particle Counters

- Cells usually illuminated individually by focused beam of laser light
- High speed analysis of intrinsic and extrinsic cell (or nuclear) parameters
- Measurements on single cells
- Characterization of heterogeneity that would be masked by bulk fluorimetry

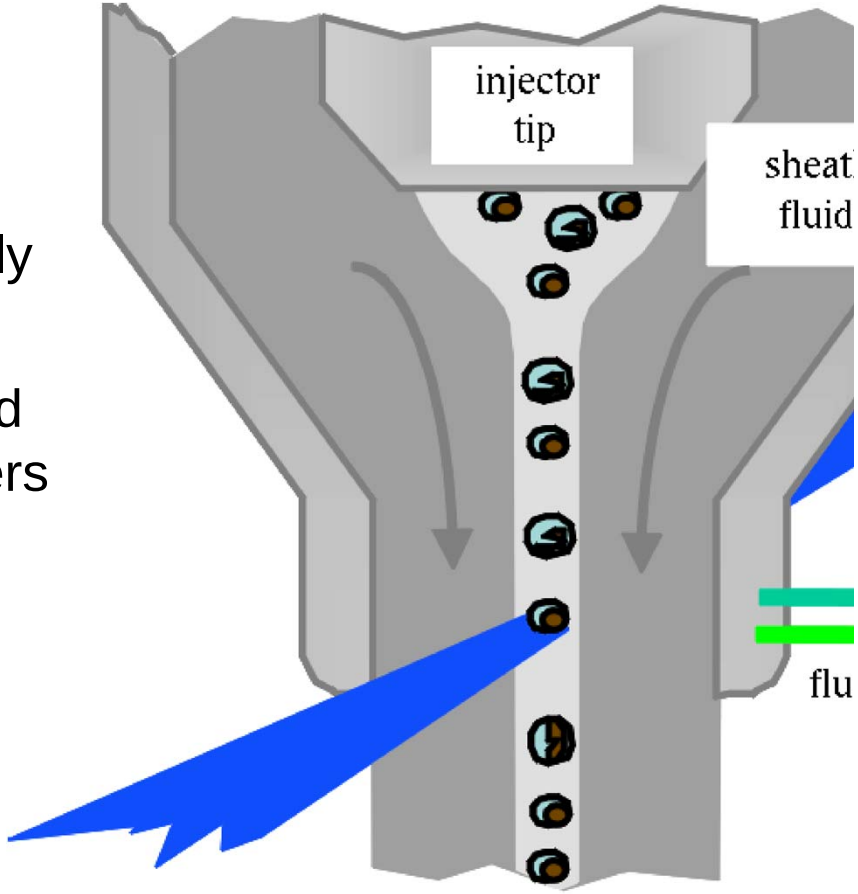



**Fig. 0.1.** Micro flow cytometer operated with a sheath fluid. Suspended particles suspended in a fluid are aligned in a single file while they pass the laser excitation. Labelled cells are subsequently detected by fluorescence

# 13.3. Coulter Counter

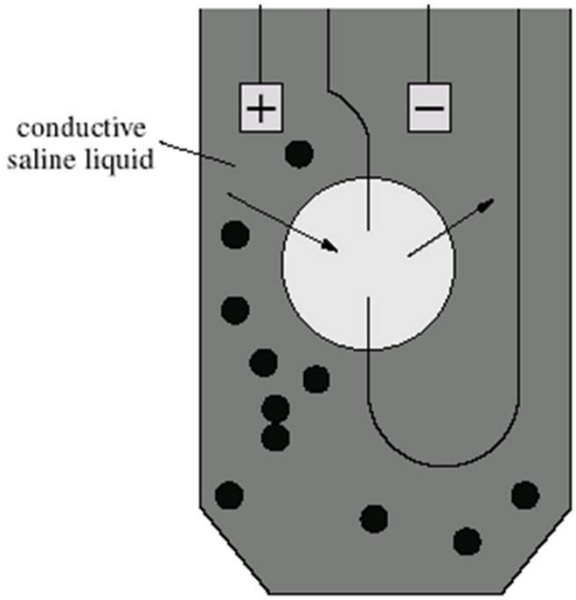



Fig. 17.8. Principle of a Coulter counter. Two chamber are filled with a conductive saline liquid separated by a small orifice exhibiting a diameter below 100 µm. The voltage drop between the two electrodes occurs almost entirely along the orifice as the resistance scales with the square of the inverse of the cross-section (6.26). A poorly conducting cell traversing the orifice thus shifts the resistance or impedance between the electrodes.

- Cells traverse electrode gap to displace conductive liquid
- Counting by change in impedance between electrodes

# 13. Particle-Laden Fluids

1. Diffusion Barriers
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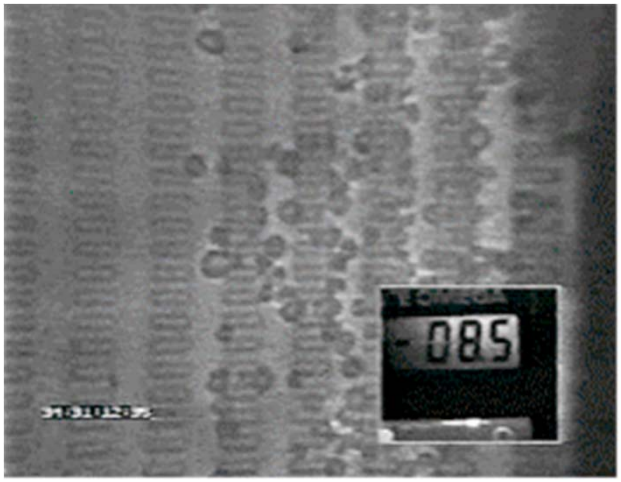
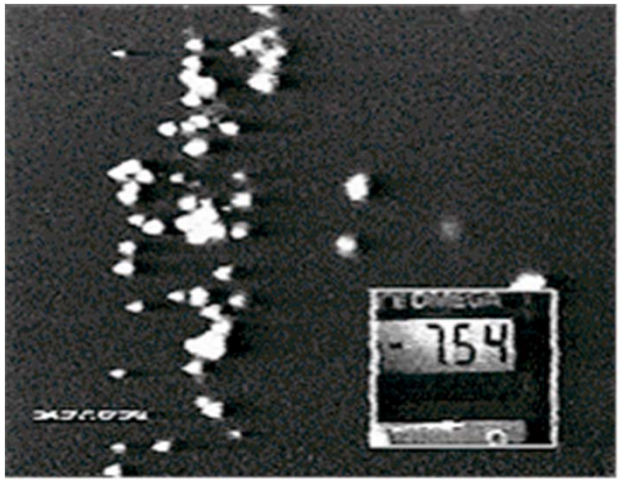
4. **Blood Cell Counting**

# 13.4. Separation of White and Red Blood Cells

- Blood pumped from right to left
- Array of obstacles spaced at 2 to 4 µm
- White blood cells get stuck
  - ➢ Larger and less flexible



# 13.4. Separation of White and Red Blood Cells

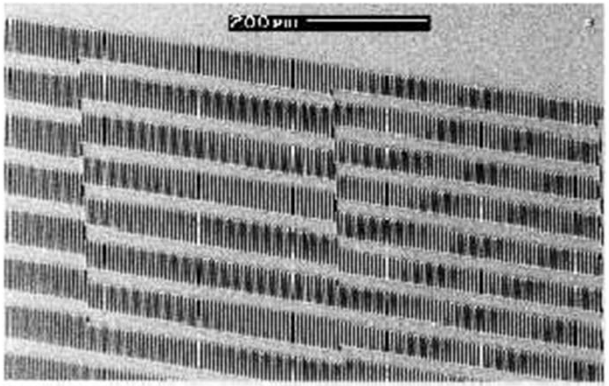


Fig. 17.12. SEM image of a small section of the variable length array [205].

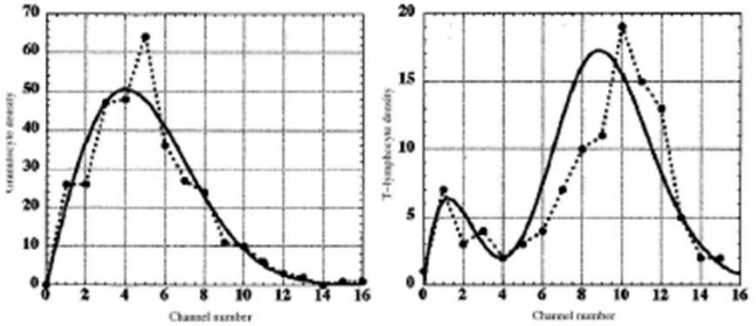



Fig. 17.13. Observed (solid line) granulocyte (left) and T-lymphocyte densities (right) compared to fits (dashed line) of the model function taking into account lattice and, for T-lymphocytes, also intercellular interactions [205].