Micro Electro Mechanical Systems for mechanical engineering applications

Lecture 14:
Device examples (2): Microfluidic Cell Sorter and Manipulation

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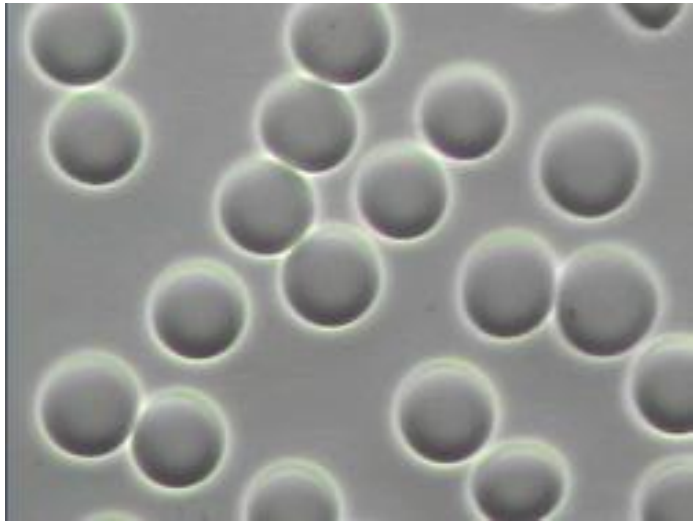
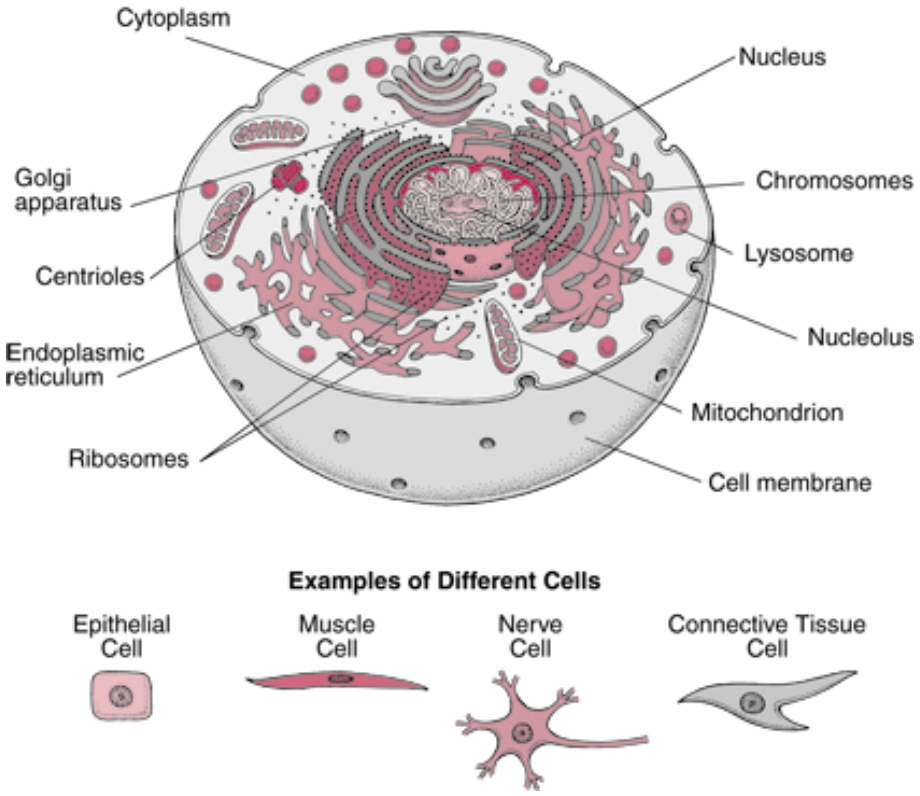
Why Cell Separation?





The cell is the basic functional unit of all organisms. Therefore, it is important to start with exactly the right cell for performing good cell-biology experiments

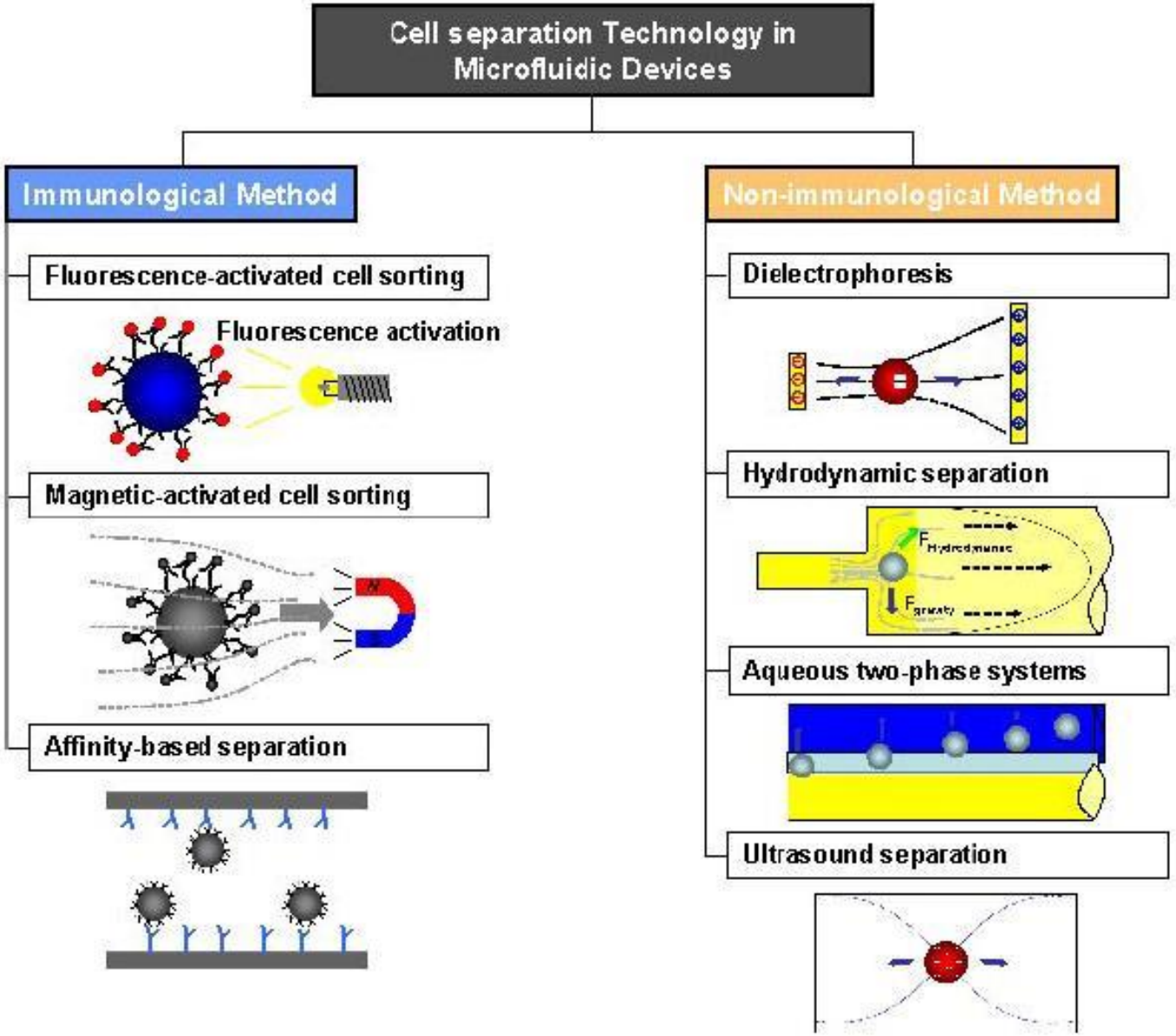
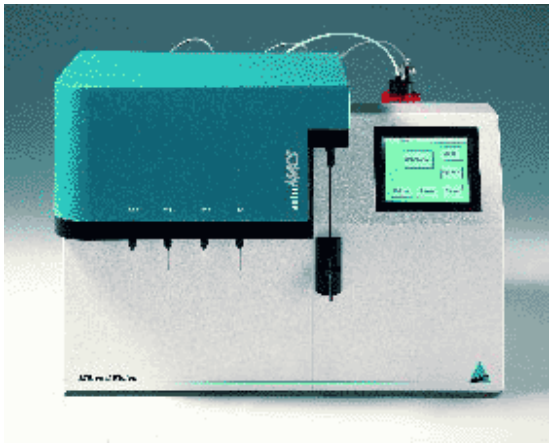


Fig 1. Schematics of microfluidic cell separation techniques.

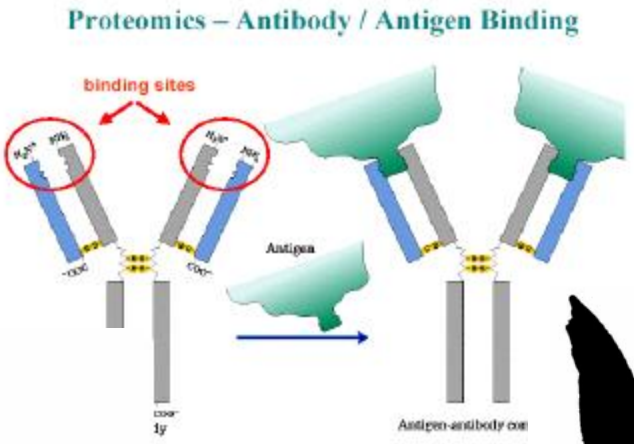
Immunological separation: Use of a biomarker

The BD FACSAria uses a sorting flow-cell.

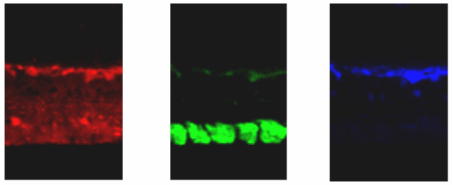


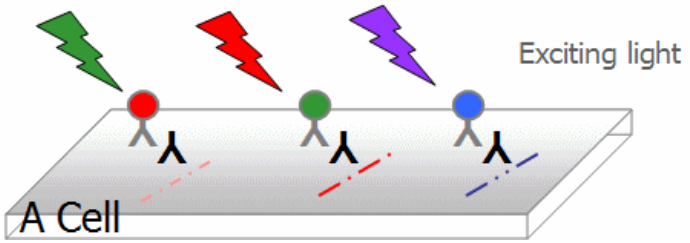
Immunological technique is a mainstay of commercialized cell separation methods such as fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS)

Immunological separation - Advantage



Fluorescence-labeling





magnetism-labeling

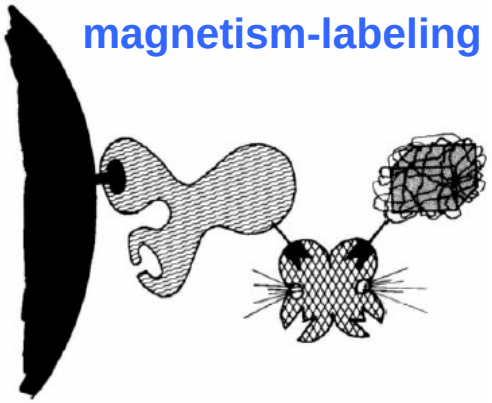


FIG. 1. Magnetofluorescence staining for MACS. Labelling of cell surface structures with biotinylated antibody, fluorochrome-conjugated streptavidin, and biotinylated superparamagnetic ferrit-dextran beads.

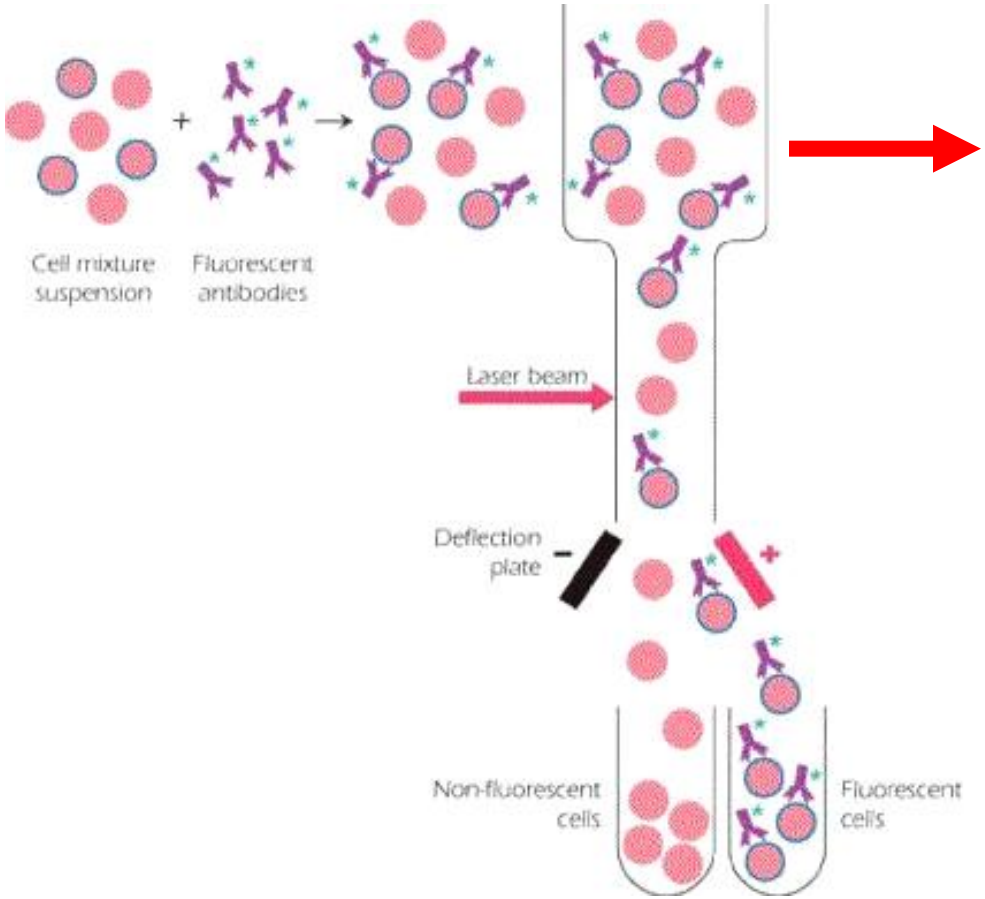
High specificity and selectivity :

Because this approach stems from the highly specific immunoreaction between the membrane marker proteins and labeling antibodies

Immunological separation - Disadvantages

- Bulky volume
- Expensive price
- Complicated operation
- Annoying sample preparation
  cell staining (fluorescence-labeling)

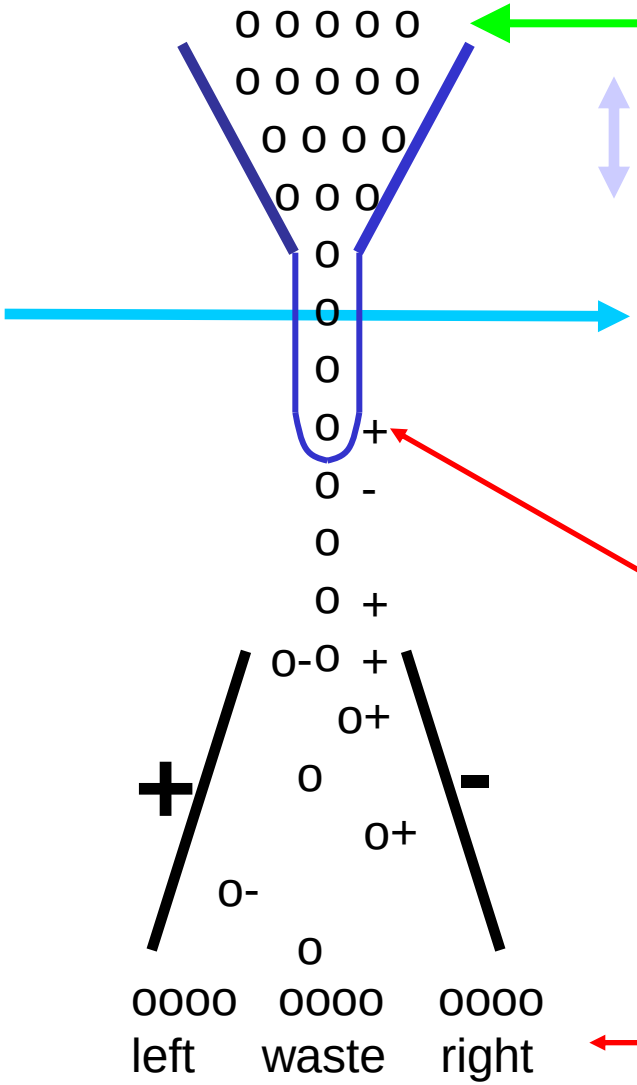
Fluorescence - Activated Cell Sorting



antibodies proteins are "tagged" or labeled with fluorescent dyes.

- Fluorescence-activated cell sorter (FACS) is one of the common methods to evaluate cell population

FACS Principle



Hydrodynamic focusing in a nozzle vibrated by a transducer produces a stream breaking into droplets.

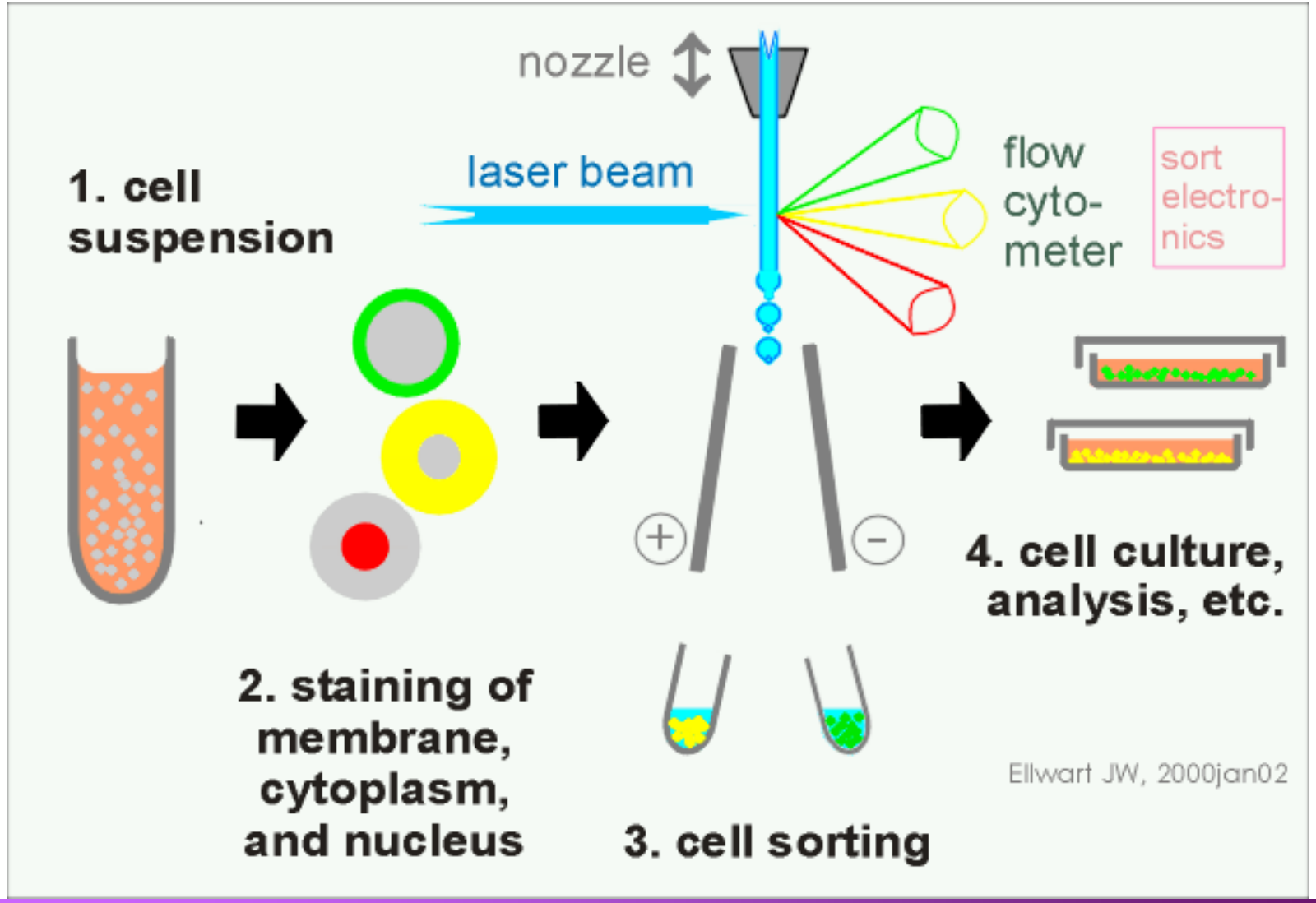
Laser interrogation and signal processing followed by sort decision : black sort right, blue sort left, green no sort.

Electronic delay until cell reaches break off point. Then the stream is charged :
+ if black - if blue.

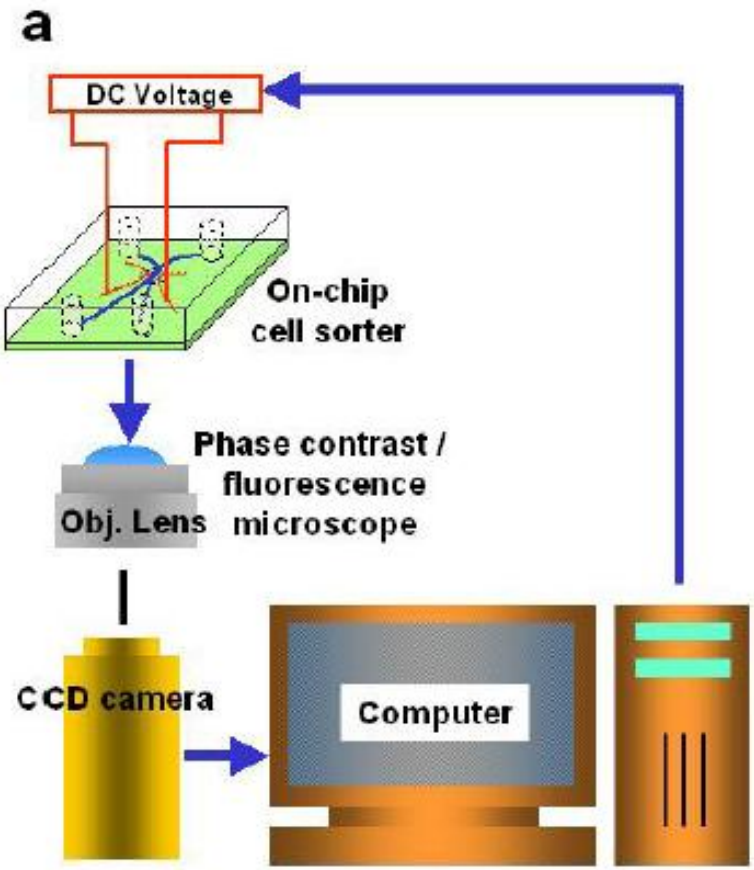
Charged droplets deflect by electrostatic field from plates held at high voltage (+/- 3000 volts).

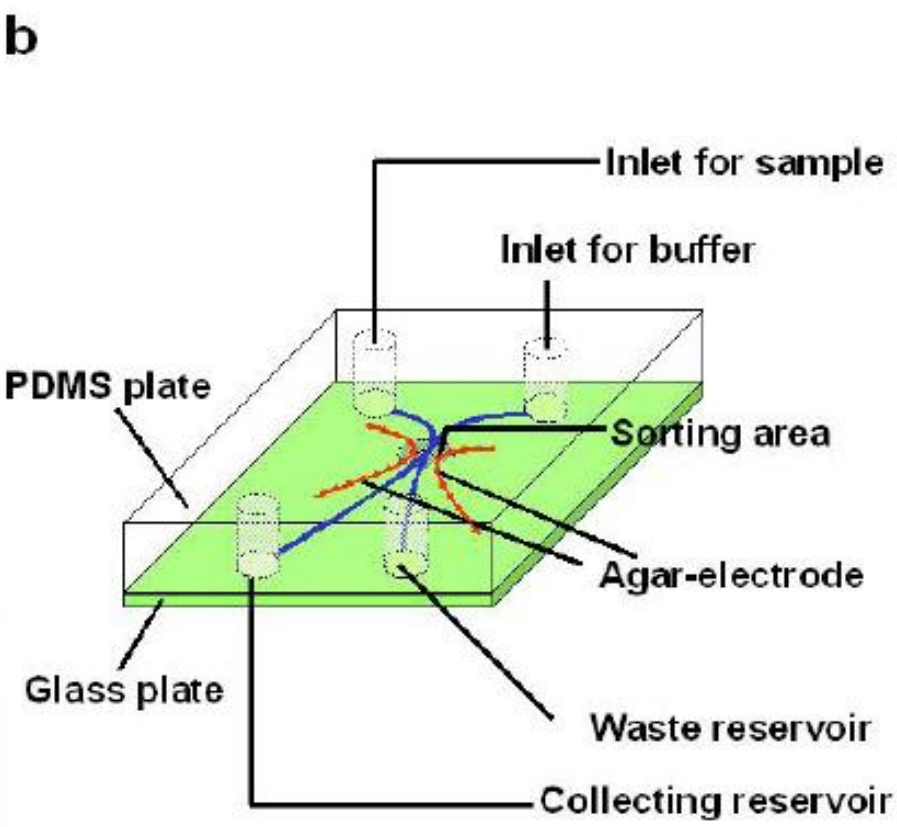
Various collection devices can be attached : tubes, slides, multi-well plates.

Fluorescence - activated cell sorting



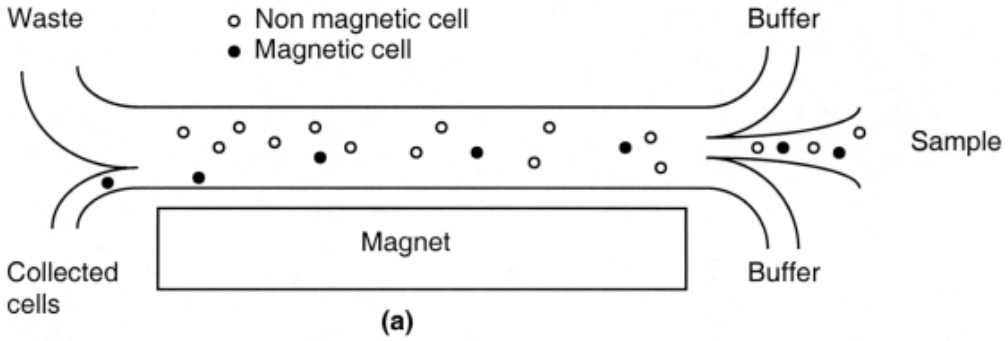
Microfabricated FACS

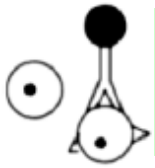




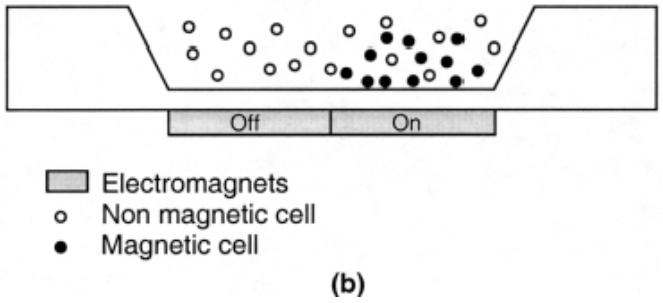
Nature 1999

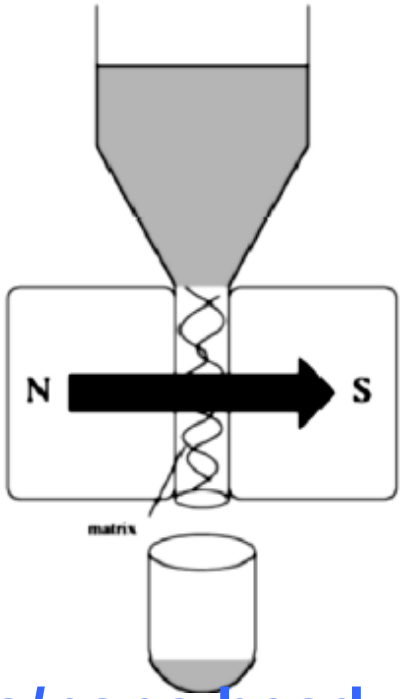
Magnetic-activated cell sorting - Schematics





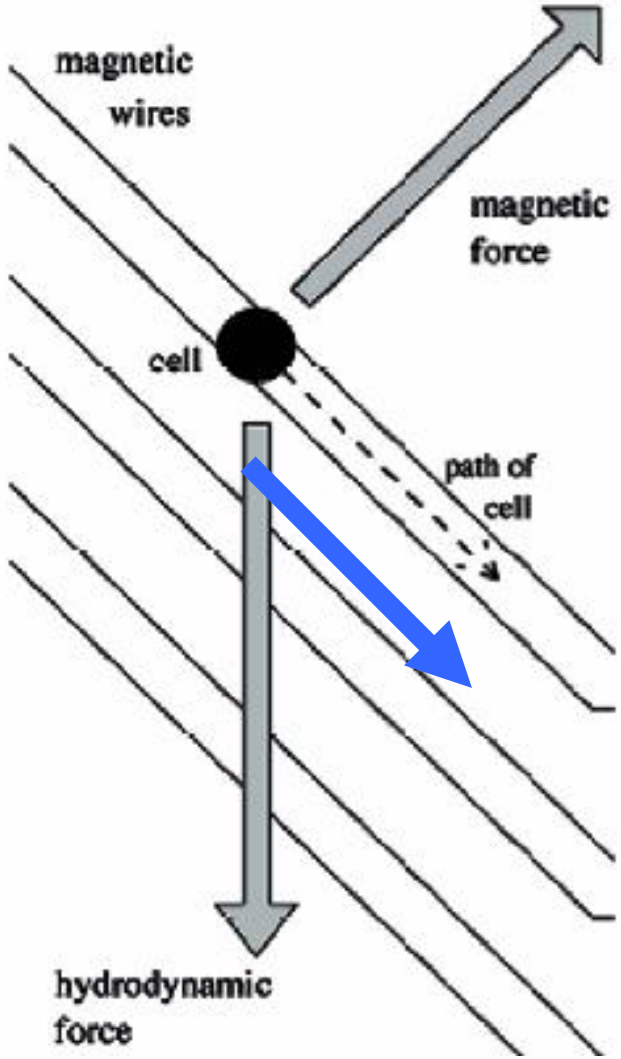
Magnetically labeled cells





The MACS uses magnetic micro/nano bead conjugated with antibody proteins that are specific to the cell membrane

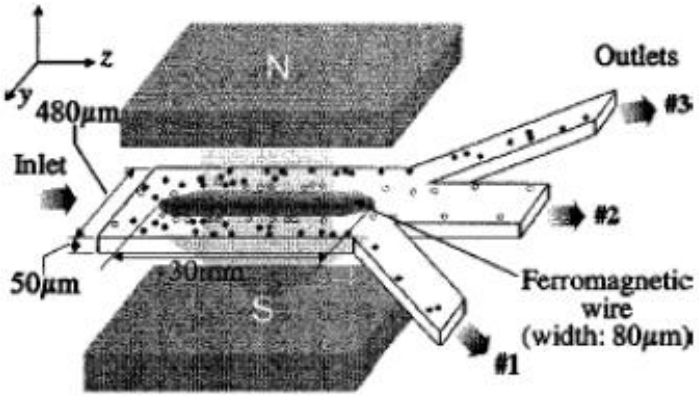
Magnetic-activated cell sorting - Basic Principle

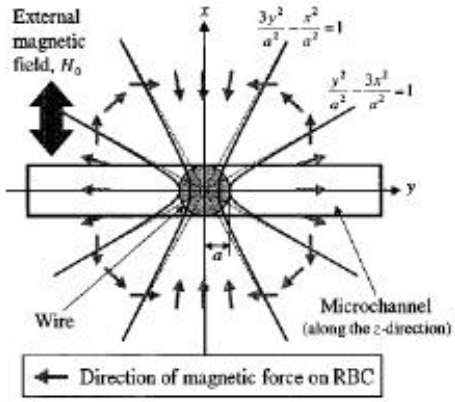


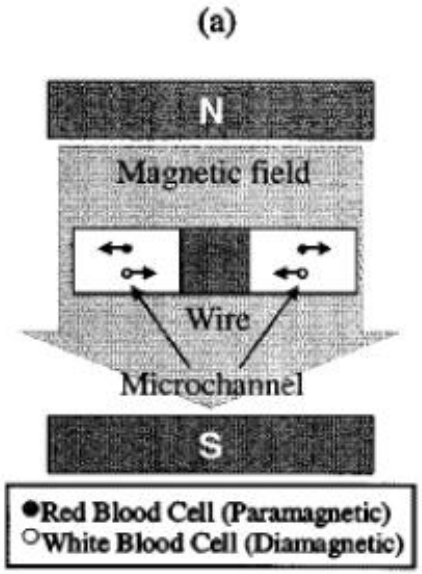
Magnetic force separation Idea:

High magnetic field gradients provide force at an angle to the flow of magnetic bead-bound cells

Magnetic-activated cell sorting







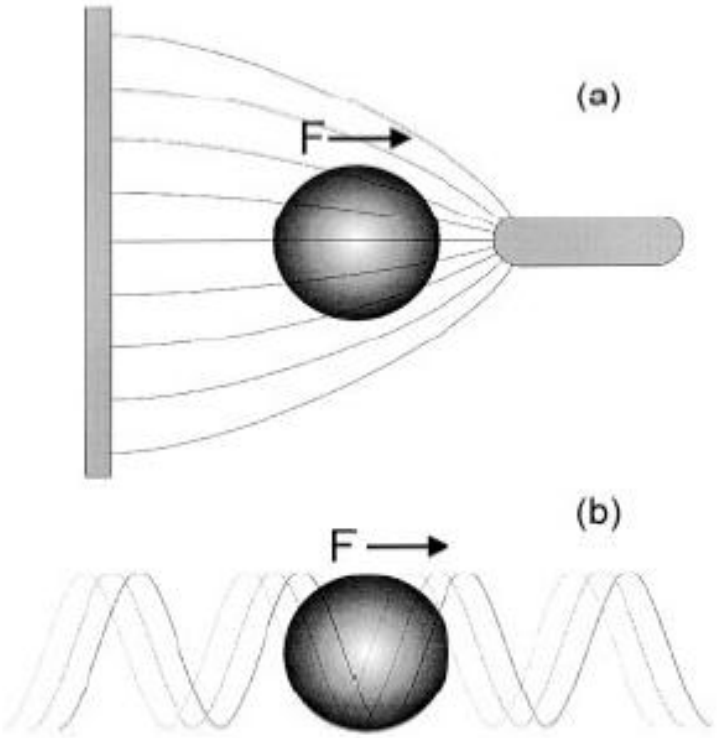
APL 2004

When an external magnetic field was applied normal to the microchannel, the red blood cells were forced away from the ferromagnetic wire and the white blood cells were drawn closer

Therefore the blood cells can be separated continuously as the whole blood passes through the microchannel of the magnetophoretic separator.

-RBCs are forced into #1 and #3
-WBCs are forced into #2

Dielectrophoresis (DEP) Separation



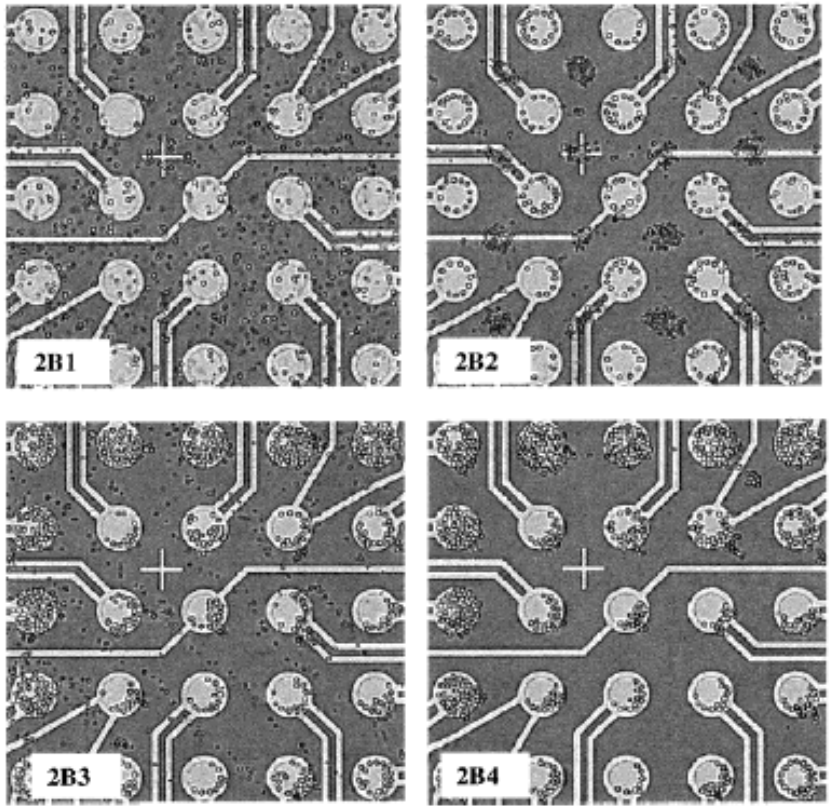
$$F_{elec} = qE + (m \bullet \nabla)E + \frac{1}{6}\nabla(Q : \nabla E) + \ldots$$

q: net charge of the particle
E: electrical field

When a particle is suspended in an alternating electric field, a force is induced on the particle

(a) A nonuniform electric field (magnetic gradient)
(b) A traveling electric field (phase gradient)

Dielectrophoresis (DEP) Separation

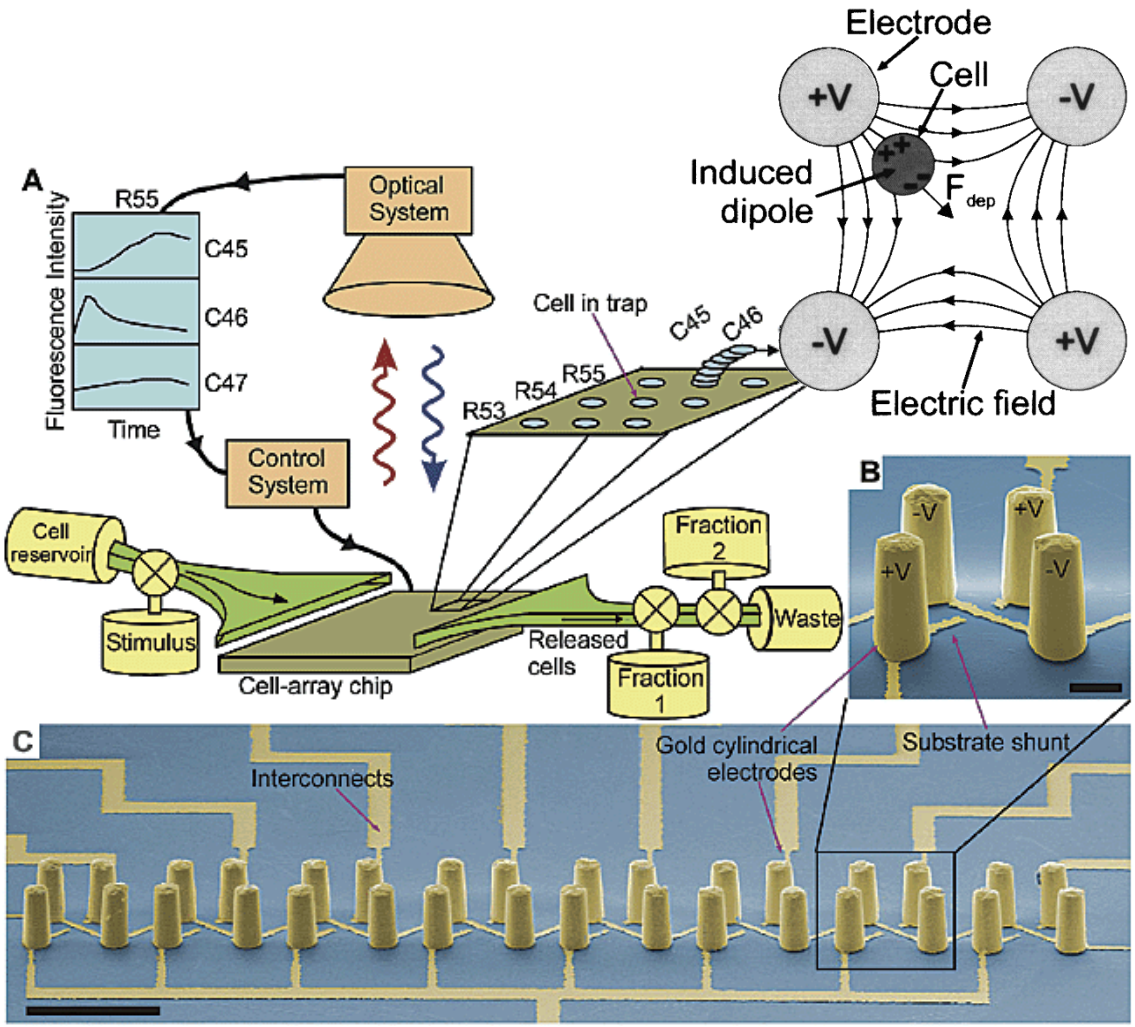


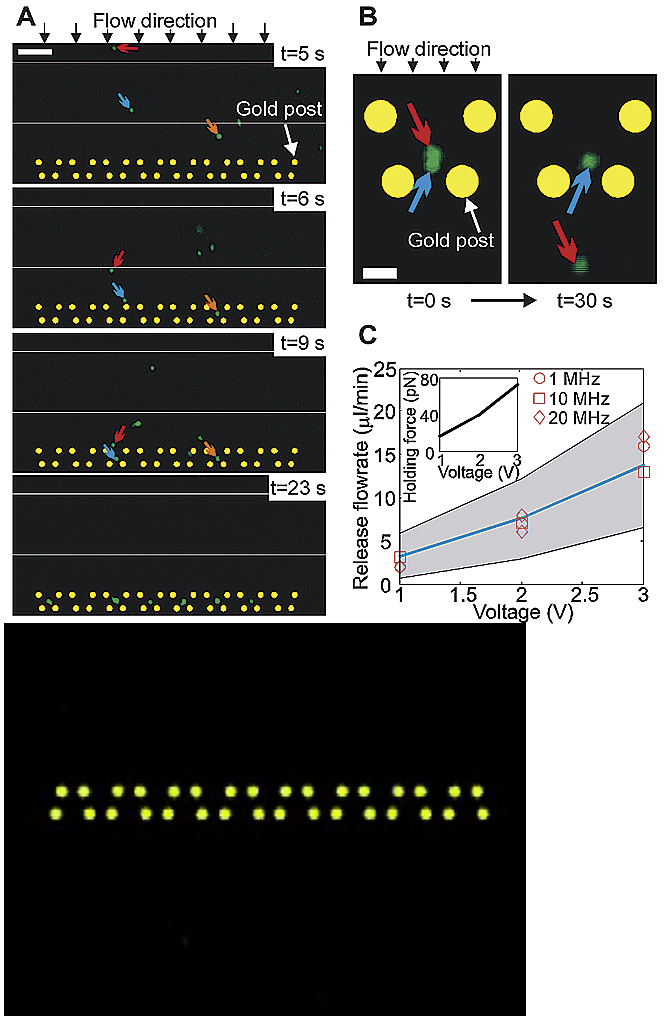
Anal. Chem. 2002

Cell separation on the microelectronic chip array based on intrinsic cell dielectric properties

1. **Cell mixture were introduced to the chip**
2. **The flow was stopped and an ac voltage was applied to the microelectrodes**
3. **E field minimums are located in the areas between the electrodes**
4. **Cells experiencing DEP forces accumulated in the space between electrodes, the minimal field region**

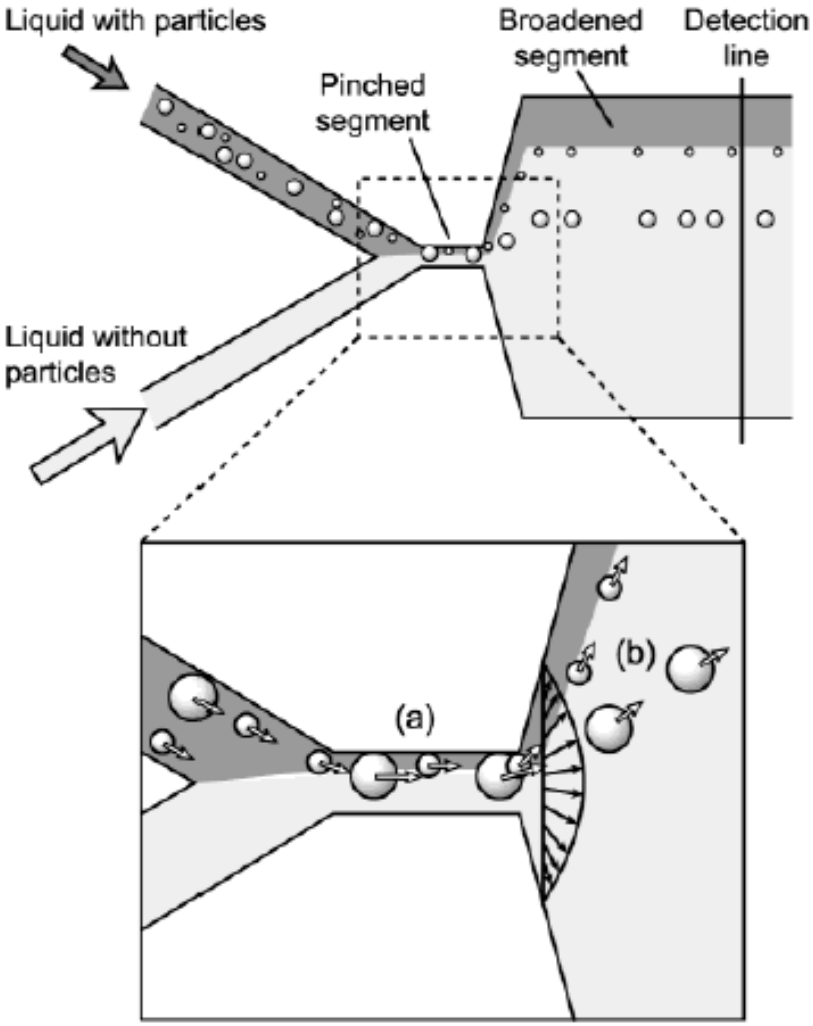
Dielectrophoresis





Joel Voldman et al., *Anal. Chem.* **74**, 3984, 2002.

Hydrodynamic separation

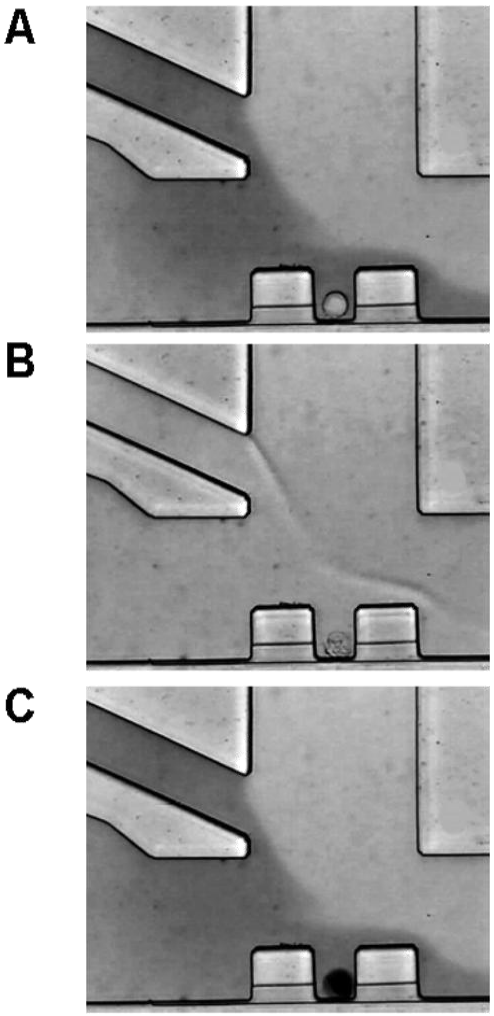


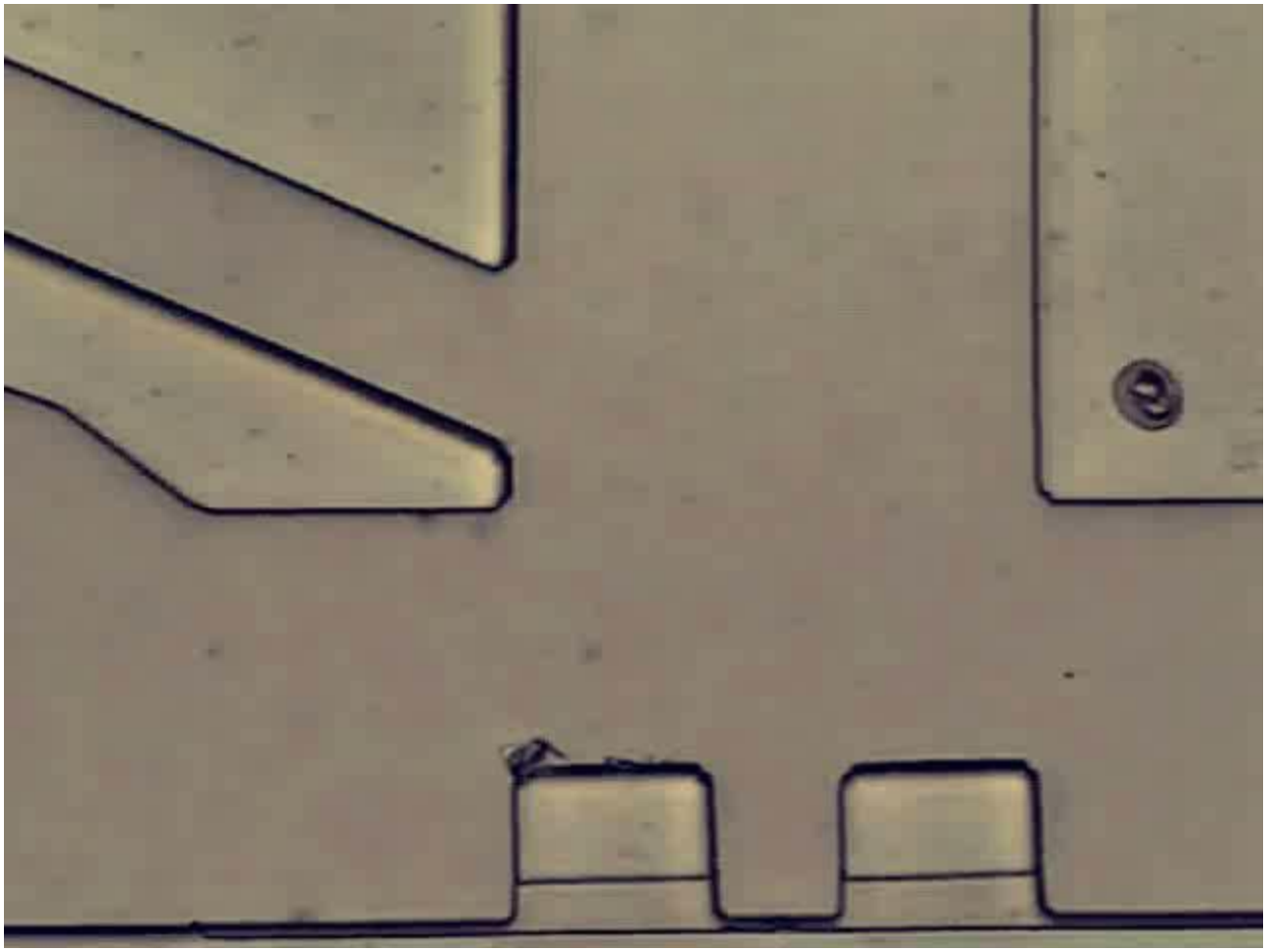
(a) **In the pinched segment, particles are aligned to one sidewall regardless of their sizes by controlling the flow rates from two inlets**

(b) **Particles are separated according to their sizes by the spreading flow profile at the boundary of the pinched and broadened segments**

Anal. Chem. 2004

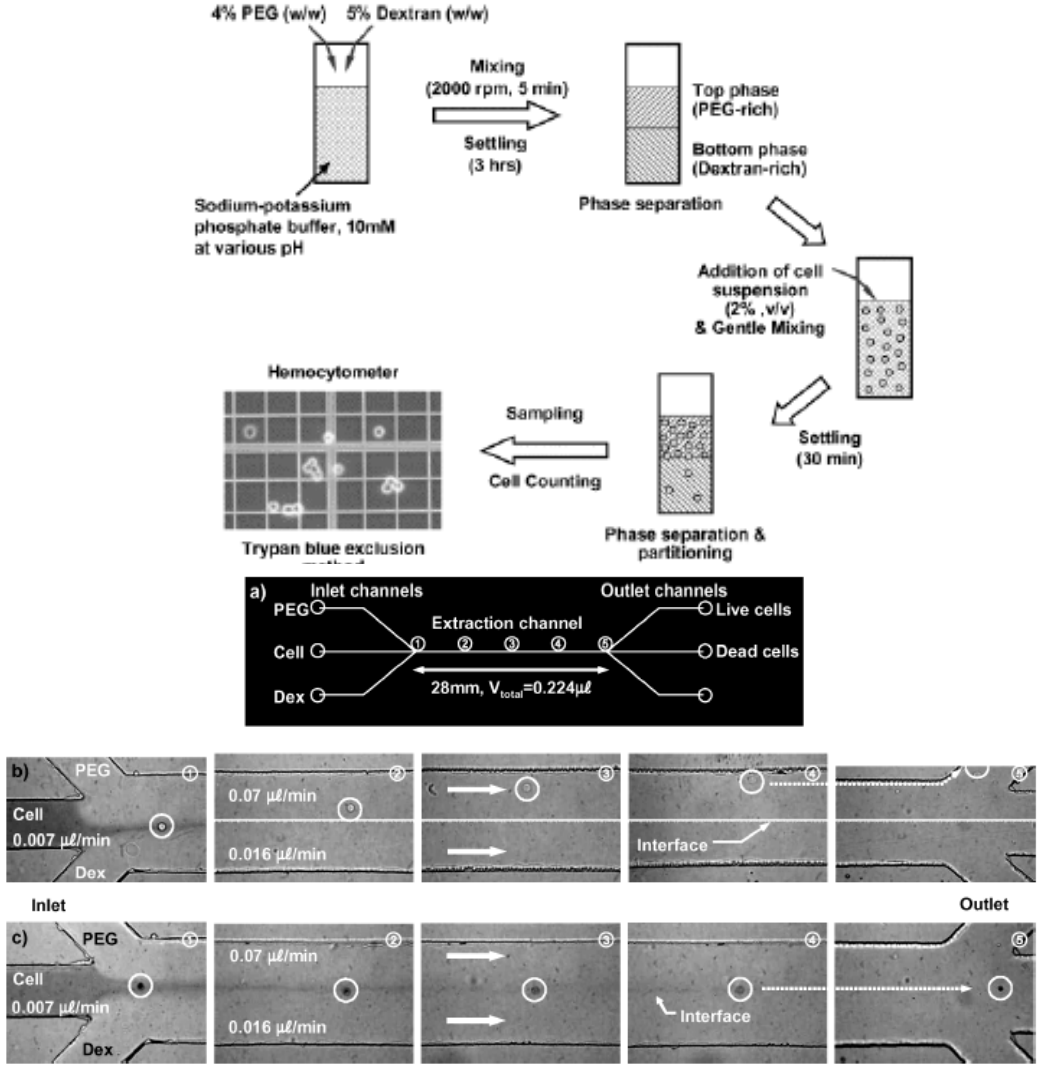
Hydrodynamics





Aaron R. Wheeler et al., *Anal. Chem.* **75**, 3581, 2003.

Aqueous two-phase separation



In two phase system of PEG and dextran, plant cells and CHO cells are separated.

Cell separation depends on cell surface properties such as surface charge

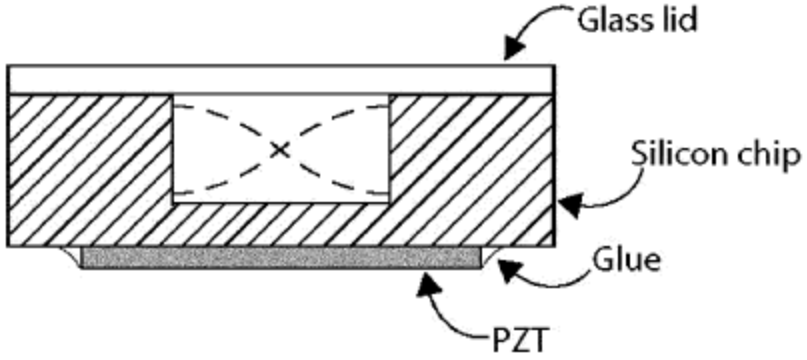
(a) Structure of system

(b) Movement of live cell

(c) Movement of dead cell

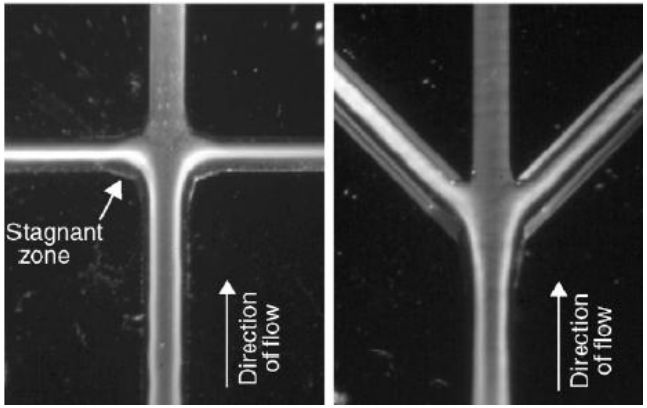
Biomed. Microdevices, 2005

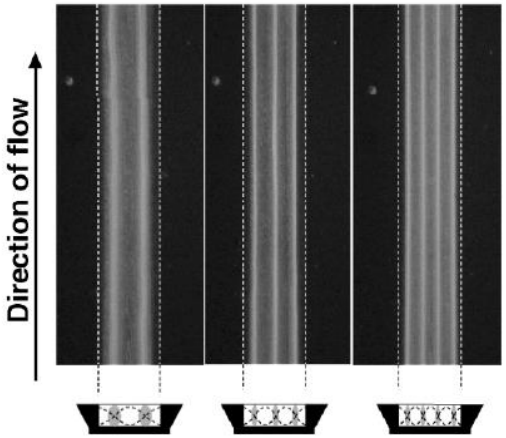
Acoustic separation



The separation channel with the piezoceramic element glued to the rear side.

The acoustic signal is tuned to fit the resonance criterion defined by the channel width, generating an acoustic standing wave in plane, orthogonal to the flow channel

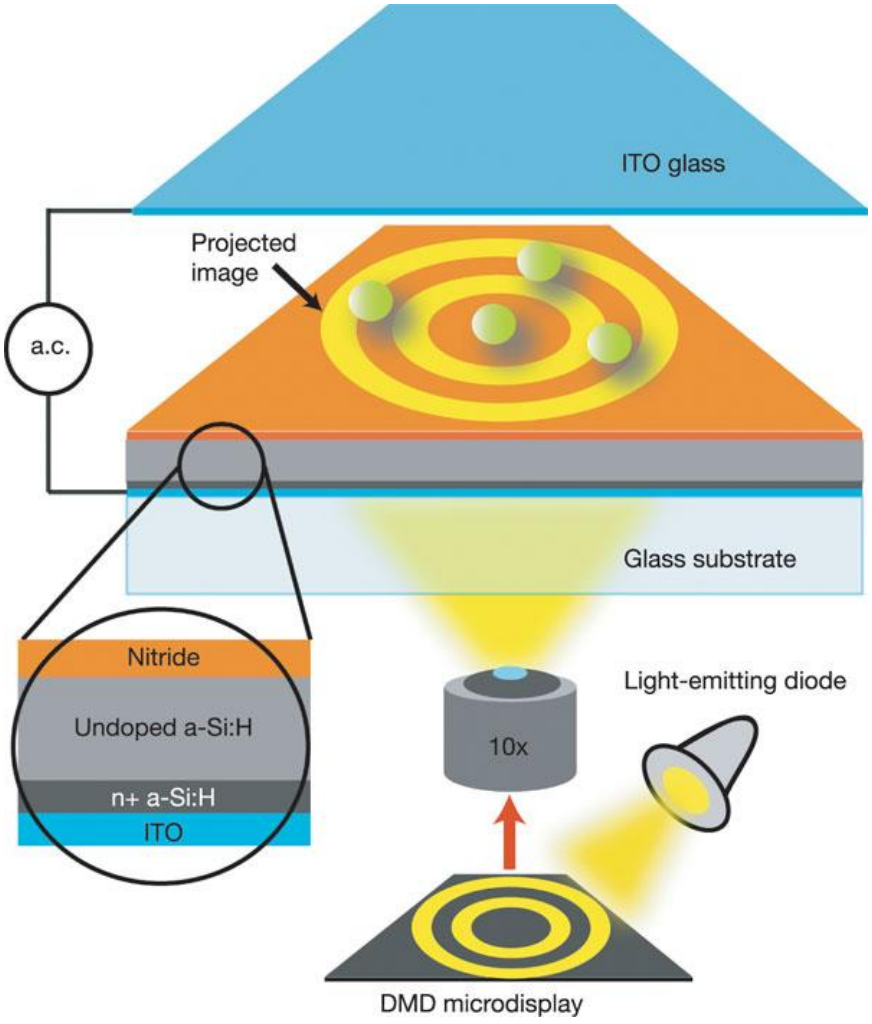


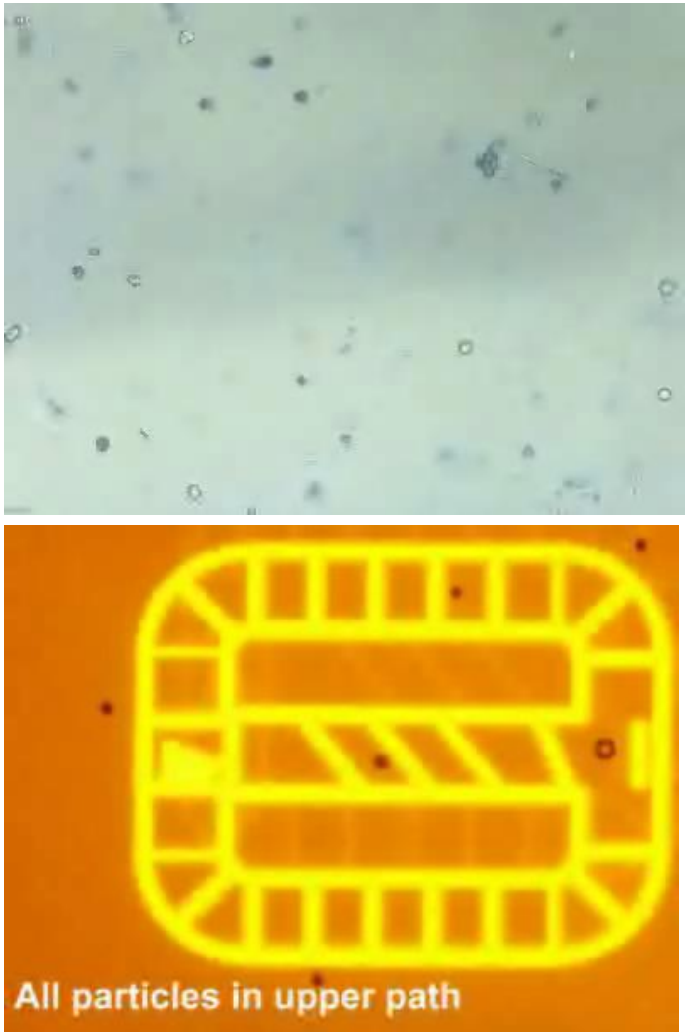


Particle enrichment in microchannel. The bands show the enriched particles in resonance mode

Lab chip, 2004

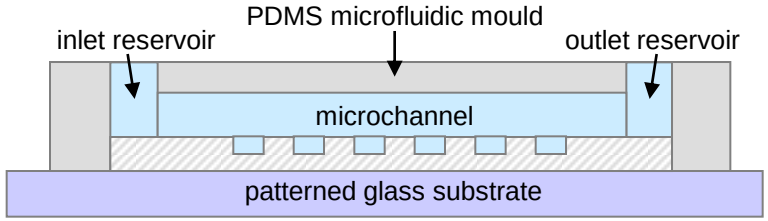
Optoelectronic tweezers

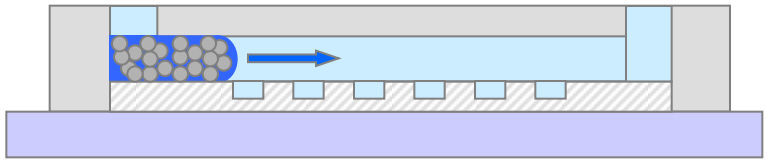




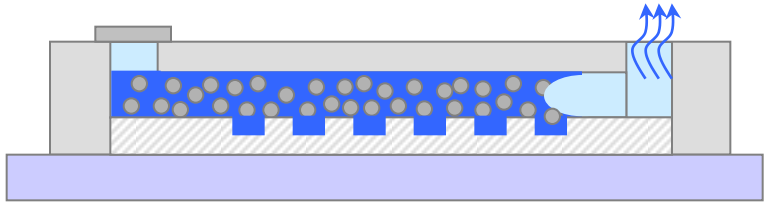
Pei Yu Chiou et al., *Nature* **436**, 370 (2005).

Receding meniscus

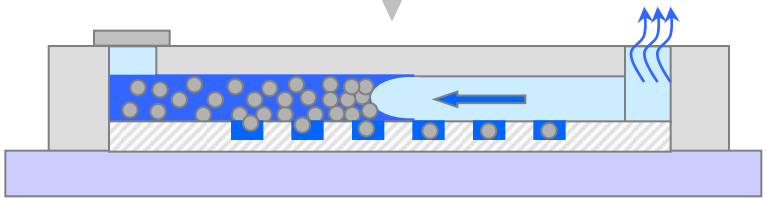




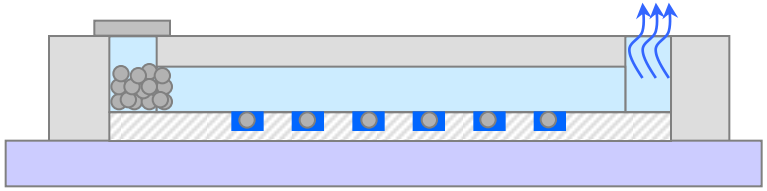
Introducing a yeast cell suspension by capillary filling



Generating a receding meniscus by evaporation



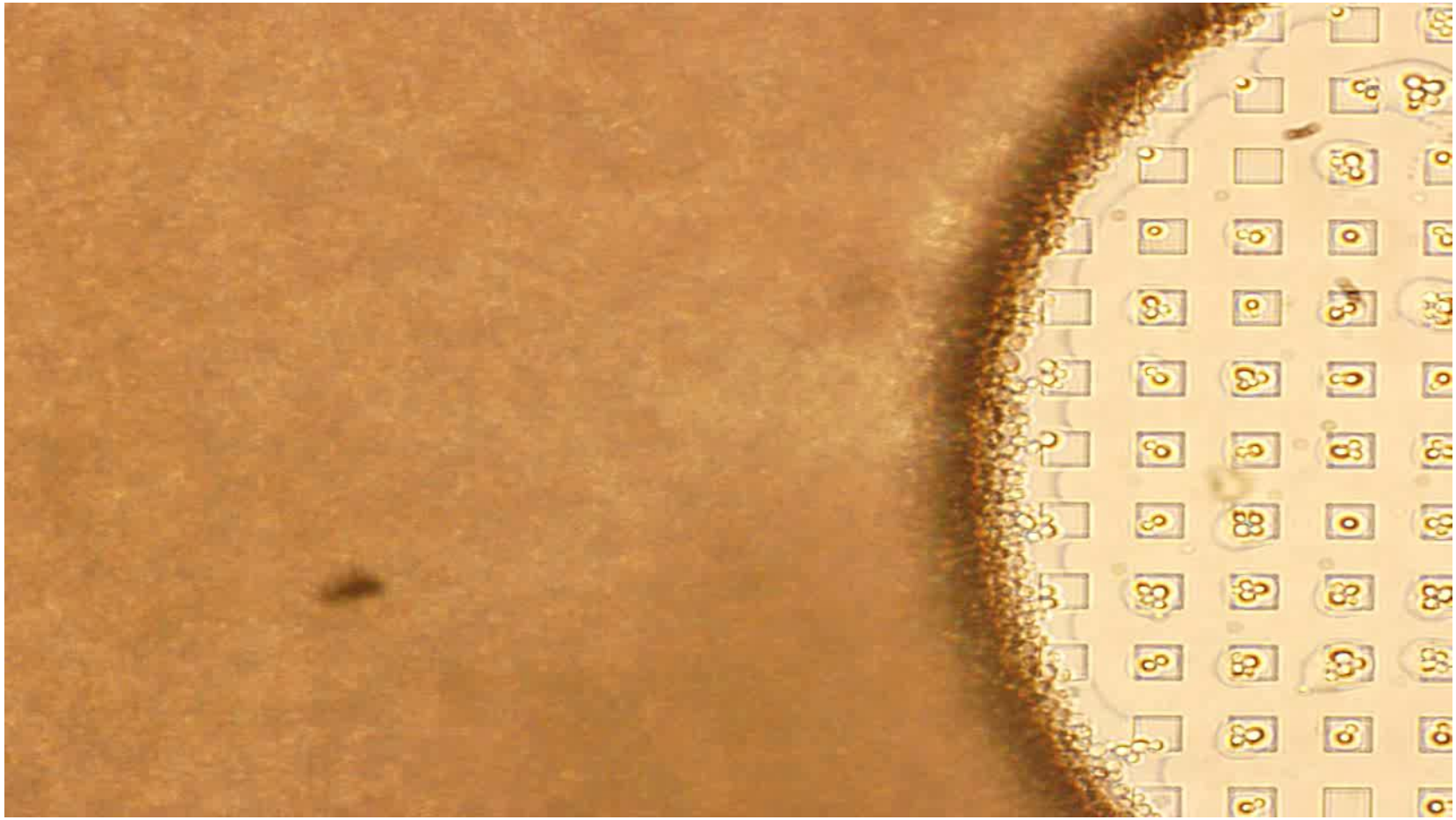
Capturing the cells by lateral capillary force



Receding meniscus induced docking

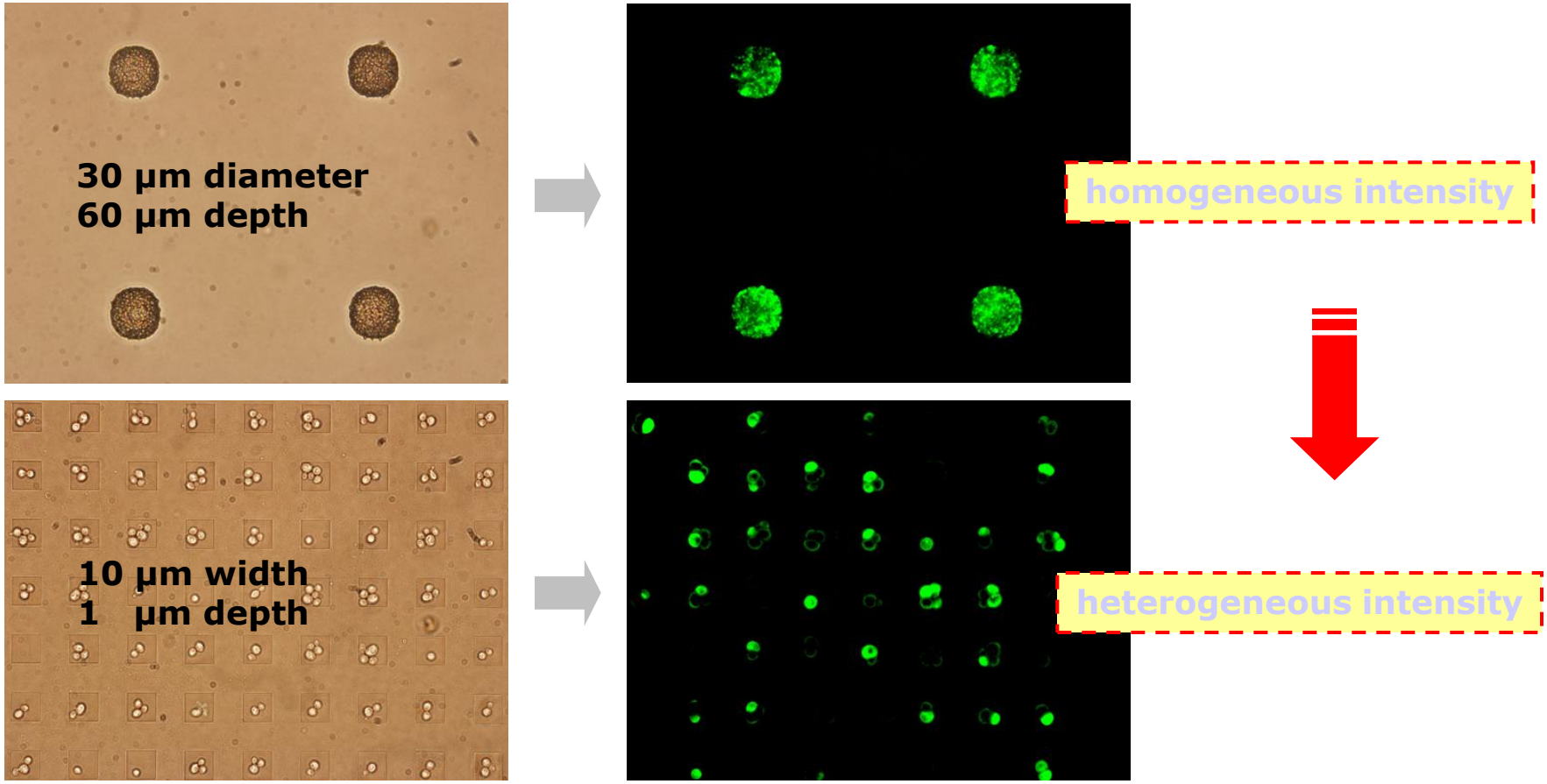
Receding meniscus

- In case of 10 μm width, 1 μm depth microwells



Importance of single cell analysis

- GFP of captured yeast cells (autofluorescent)



Increasing Options

- The longevity of the fluorescent cell sorter is a clear testament to its power, but subsequent years have also shown a need for complementary methods that can be applied for more specialized experiment

- Meanwhile, growing interest in stem-cell isolation, clinical cell sorting and single-cell analysis are fuelling the drive to develop microscale cell sorters such as microfluidic cell sorting devices