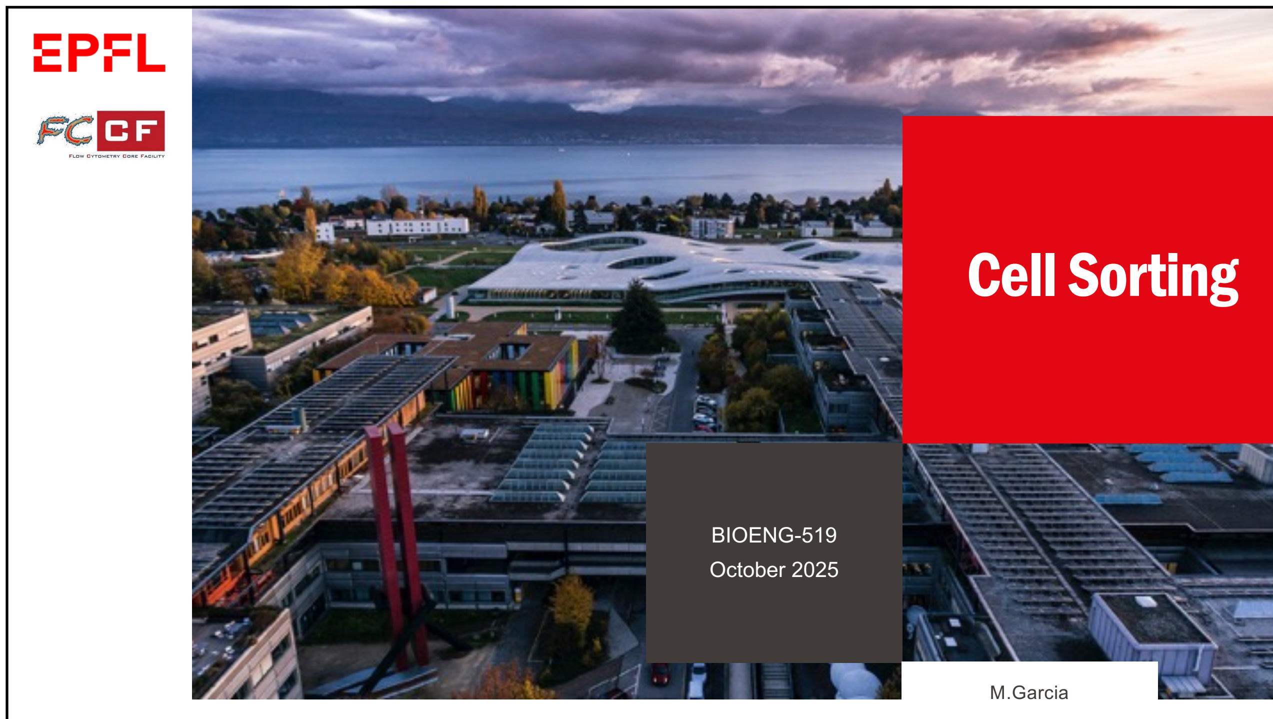

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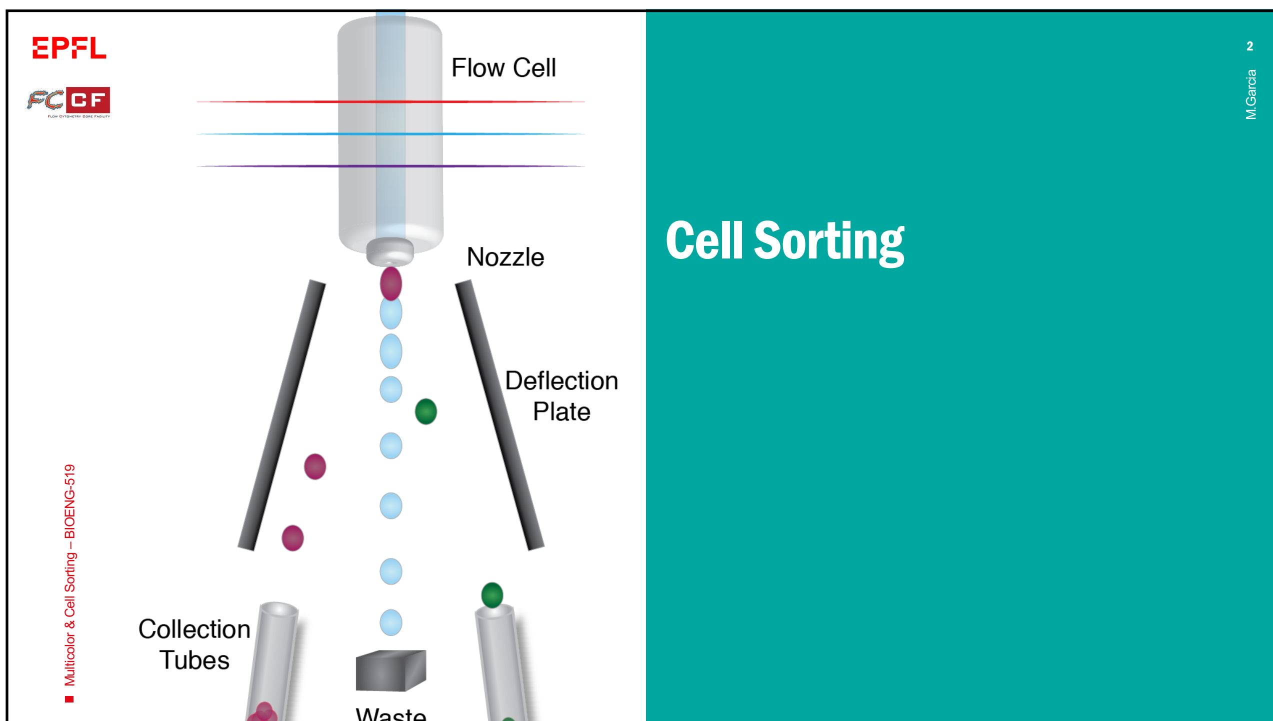

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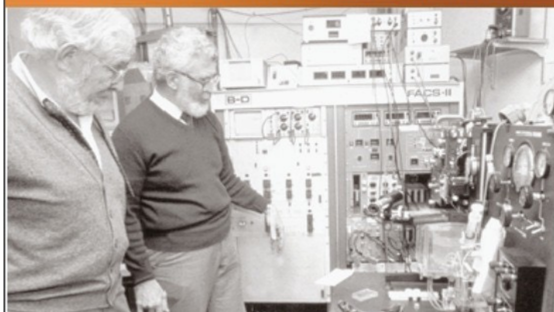
Cell Sorting

FACS: Fluorescence-Activated Cell Sorter

Developed by Prof. Leonard Herzenberg, Stanford University, 1974



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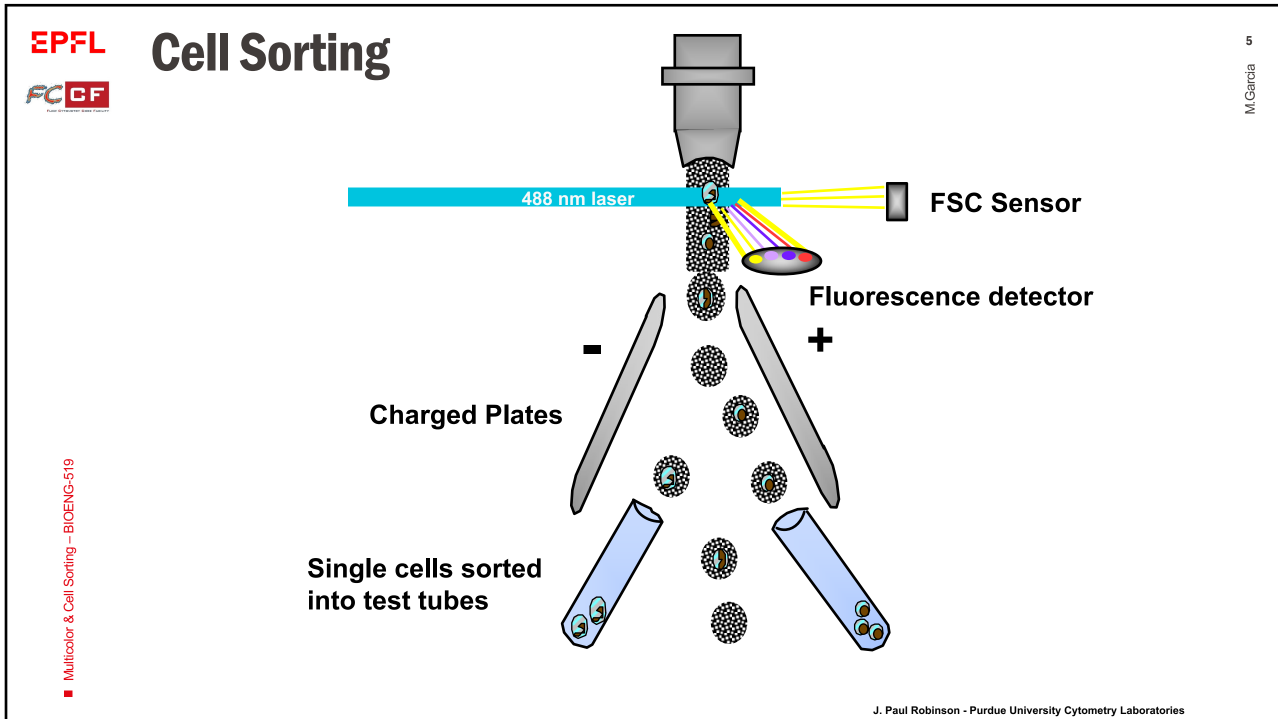
Cell Sorting

Definition :

The ability to select any population defined by a logical combination of regions (a “gate”) and isolate this population from the sample.

How Do You Sort Cells?

- Preliminary processes are the same as for analysis :
- 1) Hydrodynamic focusing of a mixture of cells or particles to form a central core within a fluid sheath.
- 2) Interrogation of the cells by a laser source with subsequent analysis of scatter and fluorescence signals.
- 3) Application of regions and gates to define sub-populations.



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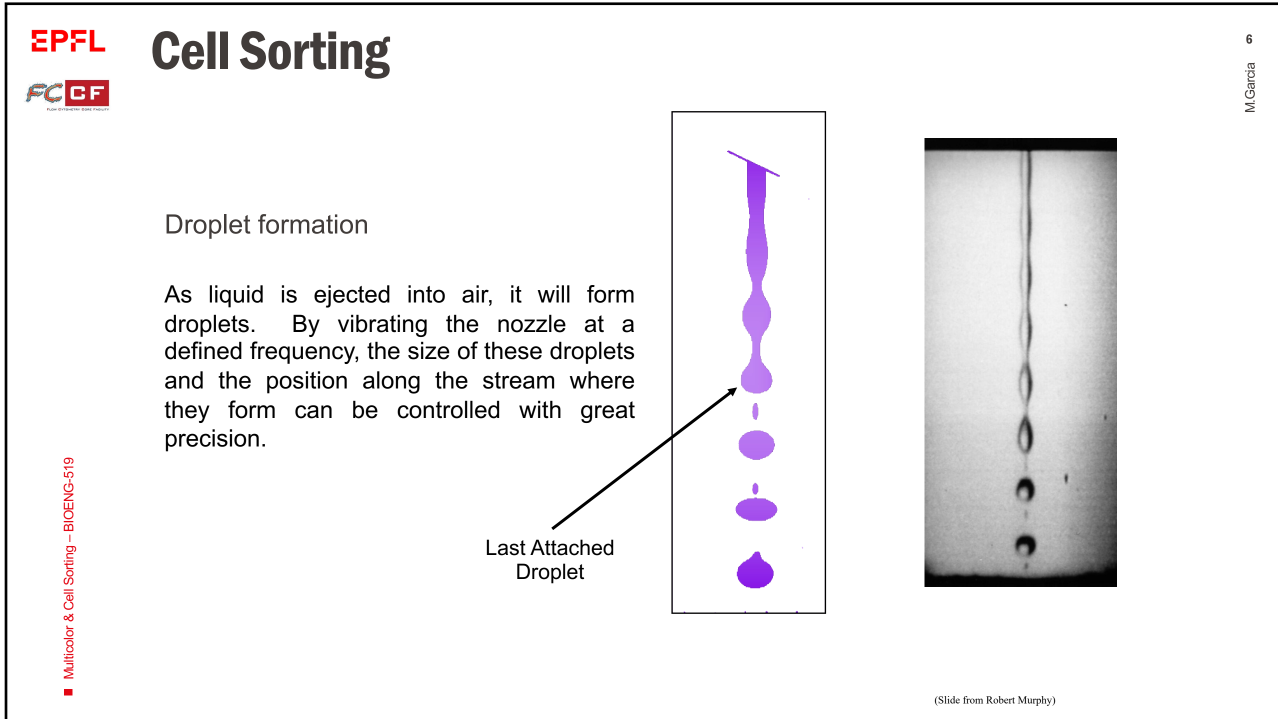

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Cell Sorting

Droplet formation is governed by
- transducer frequency
- transducer amplitude
- nozzle diameter
- sheath pressure
- phase of the charging pulse

- Fixed parameters
  - transducer frequency (minor adjustment)
  - nozzle diameter
  - sheath pressure
- Adjustable parameters
  - transducer amplitude
  - phase of the charging pulse
  - drop delay position

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Cell Sorting

- Fixed parameters
  - transducer frequency (minor adjustment)
  - nozzle diameter
  - sheath pressure

Problems with droplet formation
- temperature
  - viscosity of sheath fluid is temperature dependent
- dirt in/on the nozzle
  - proper sample preparation
- complete or partial blockage of the flow cell

Cell Sorting

As the frequency is known the time taken for a cell to traverse L can be calculated. This time determines when to charge the stream as the required cell breaks off in a drop.

The stream has to be charged when the cell of interest arrives at the last droplet. This „delay“ in charging the stream is called the drop delay.

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Cell Sorting

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Cell Sorting

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Cell Sorting

Cells are randomly distributed throughout the stream

As droplets form, they can contain wanted cells as well as unwanted cells. If all droplets containing a wanted cell are sorted (regardless of whether they also contain unwanted cells), the **purity** of the sorted sample will be reduced.

The purity can be improved by checking for coincidence events and not sorting any wanted cell that occurs too close to an unwanted cell.

This causes an increase in **purity** but a reduction in sorting **efficiency**.

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Cell Sorting

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Sorting mask

- A region of the stream monitored for the presence of cells
- Determines how droplets will be deflected if a sorting conflict occurs
- Measured in 1/32 drop increments

4 4 8 8 16 16

Mask = 0 Mask = 8 Mask = 16 Mask = 32

Multicolor & Cell Sorting – BIOENG-519

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Cell Sorting

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Cells could be sorted into:

- 1.5 ml / tubes
- 5 ml FACS tubes
- 15 ml tubes
- 6 to 384-well plates
- Microscopy slides
- Chambers slides

four-way 12 x 75 mm

two-way 15 mL

four way 1 mL

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Cell Sorting

What can you do with the cells ? :

- Culture
- Bone marrow and peripheral blood
- Single cells (cloning)
  - Transcriptomics
  - Epigenomics
- Cells transfected with marker genes.
- Chromosomes.
- Cell depletion (negative sorting)
- DNA/RNA studies – PCR, RNAseq
- Protein studies – Mass spectroscopy
- Imaging – Cellular structure and fine detail

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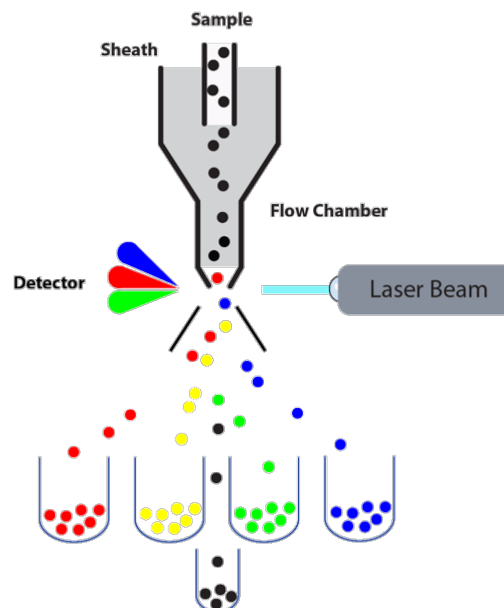
Cell Sorting



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