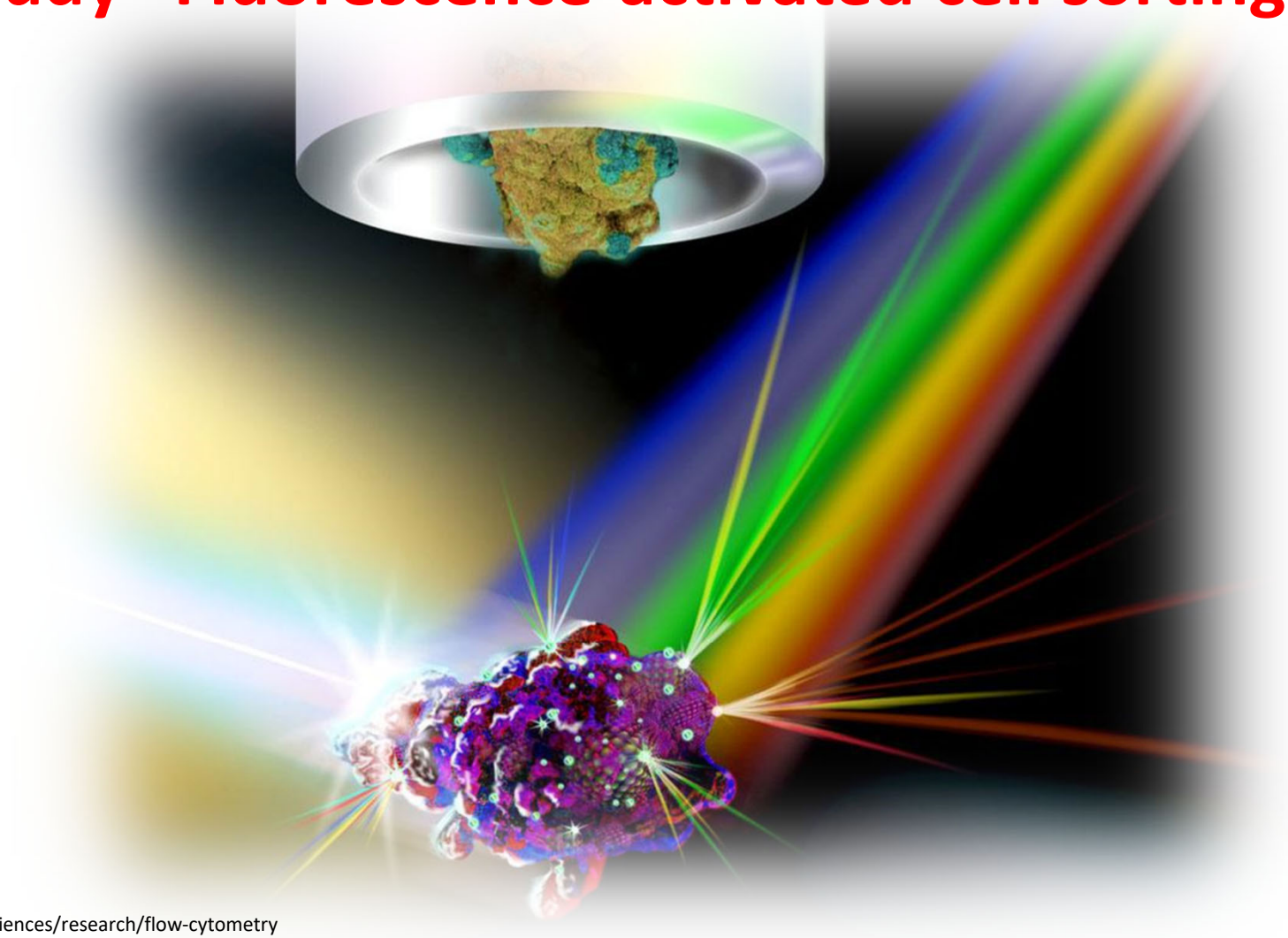
Case study “Fluorescence-activated cell sorting (FACS)”



https://umanitoba.ca/health-sciences/research/flow-cytometry

What property is being measured (analytical), what function provided (preparative)?

Analytical properties

A FACS instrument can measure cellular properties such as:

- Cell size
- Cell granularity
- Expression of (surface) proteins

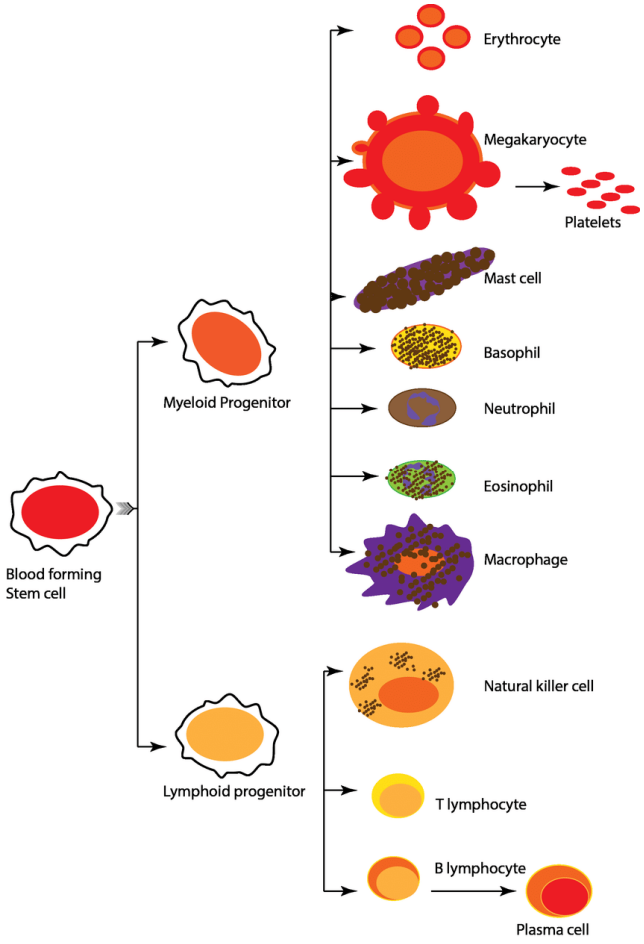
Preparative function

Single-cell sorting at very high throughput (up to more than 10kHz), according to thresholds for each measured parameter. This can be done in a multiplexed fashion e.g., sort all cells that are:

- Larger than 20µm in diameter

  AND
- Expressing high levels of surface receptor X

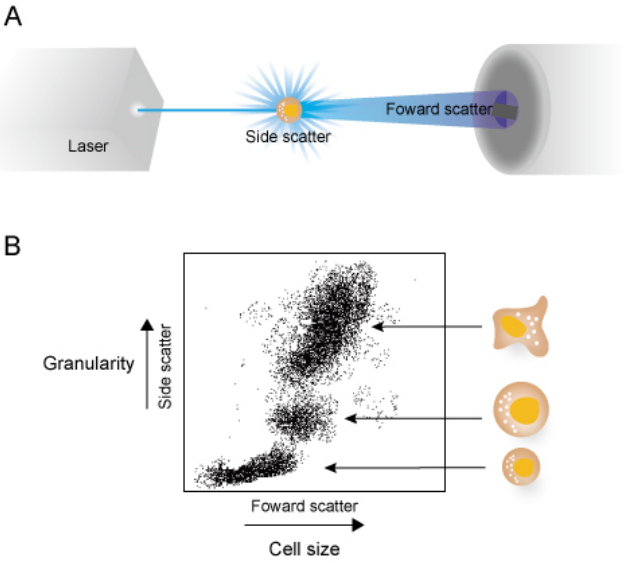
  AND
- No or only low expression of surface receptor Y



https://www.researchgate.net/figure/Different-blood-cell-types-and-their-origin_fig1_331791259

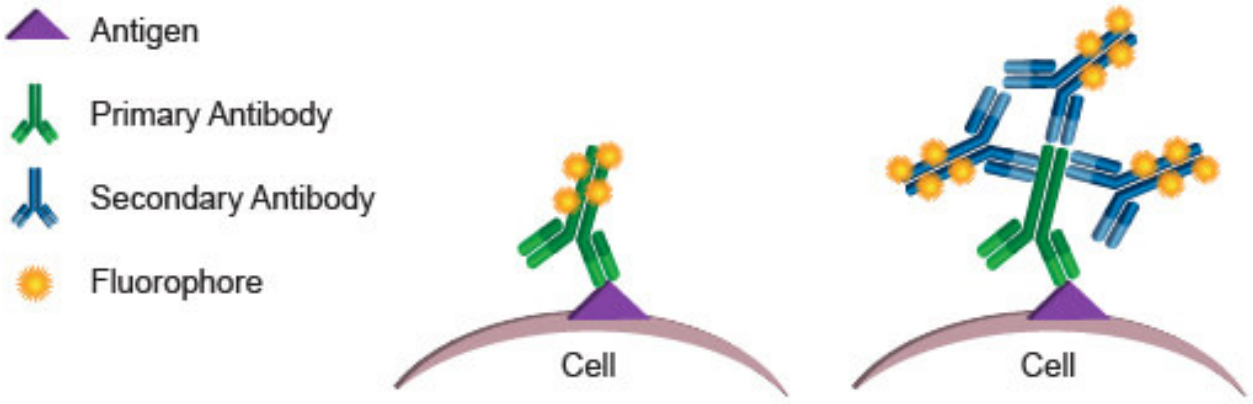
How are the cellular properties measured?

Measurement of size and granularity:
Directly, based on light-scattering



https://www.creative-diagnostics.com/flow-cytometry-guide.htm
and modified

Measurement of surface protein expression: Indirectly via fluorescently labelled antibodies specifically recognizing the protein of interest



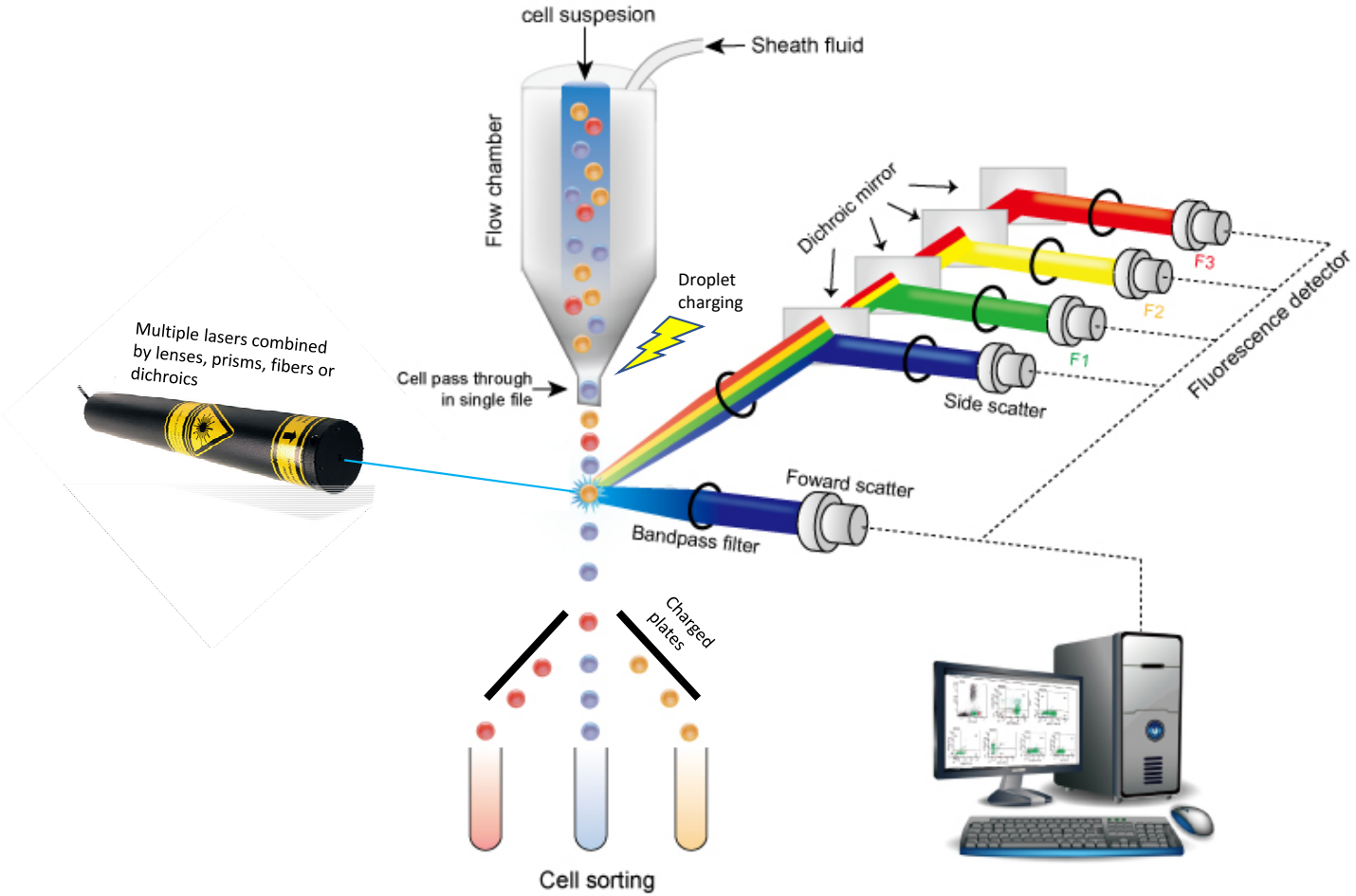
https://onelab.andrewalliance.com/library/intracellular-flow-cytometry-9jn2Gwav

How does the instrument look like?

A variety of FACS sorters and cytometers (purely analytical) are available at the EPFL Flow Cytometry Core Facility (FCCF)

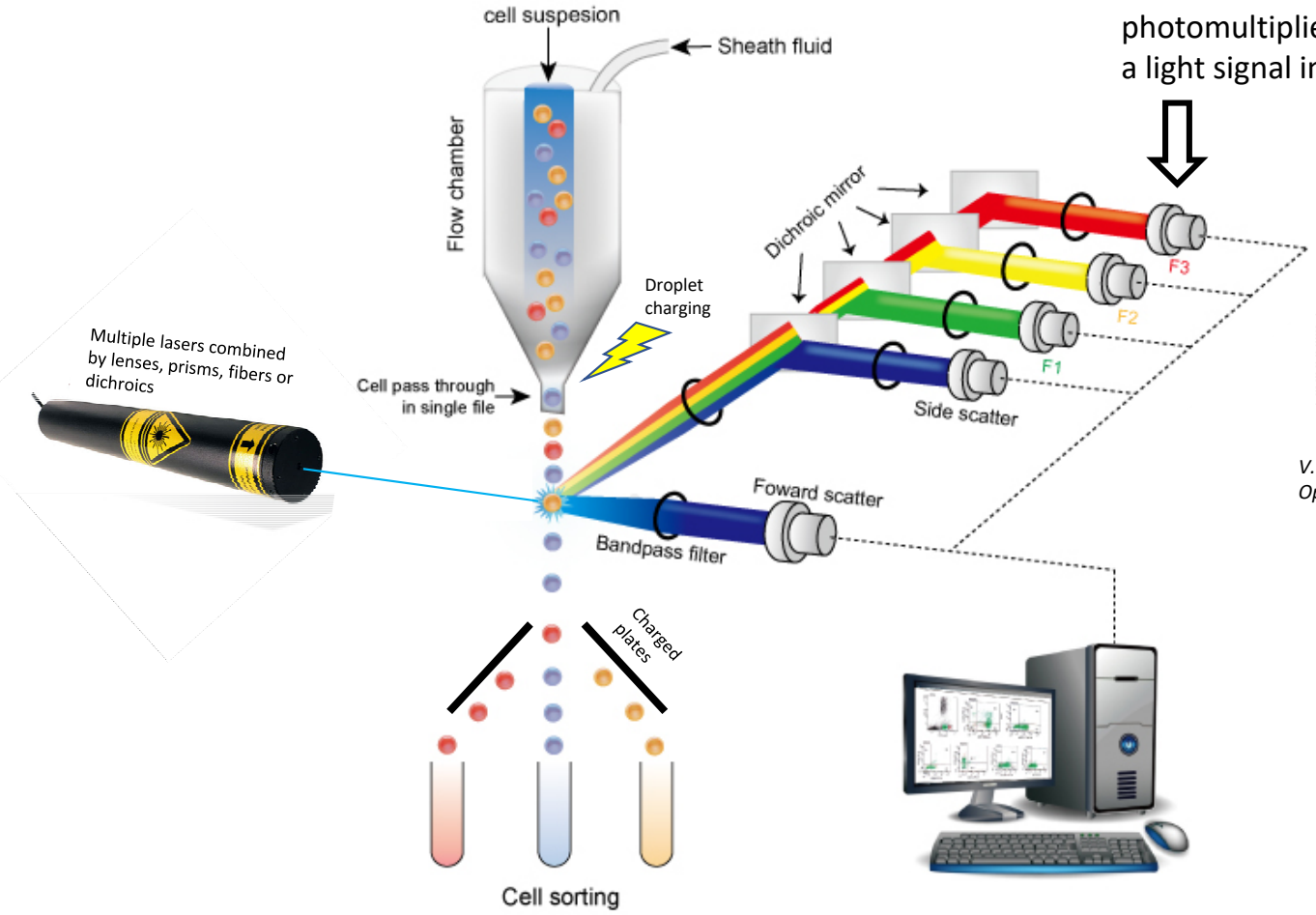
https://www.bdbiosciences.com/en-us/products/instruments/flow-cytometers/research-cell-sorters/bd-facsymphony-s6

Working principle and experimental setup

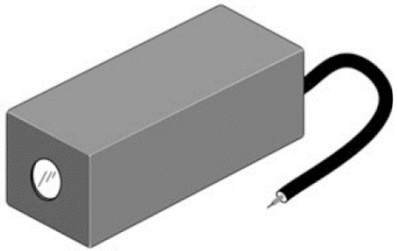


https://www.creative-diagnostics.com/flow-cytometry-guide.htm & www.excelitas.com, modified

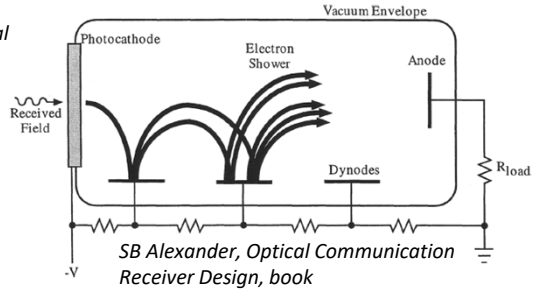
Working principle and experimental setup



photomultiplier tube (PMT), converting a light signal into a voltage signal



V. Protopopov, Practical Opto Electronics, book

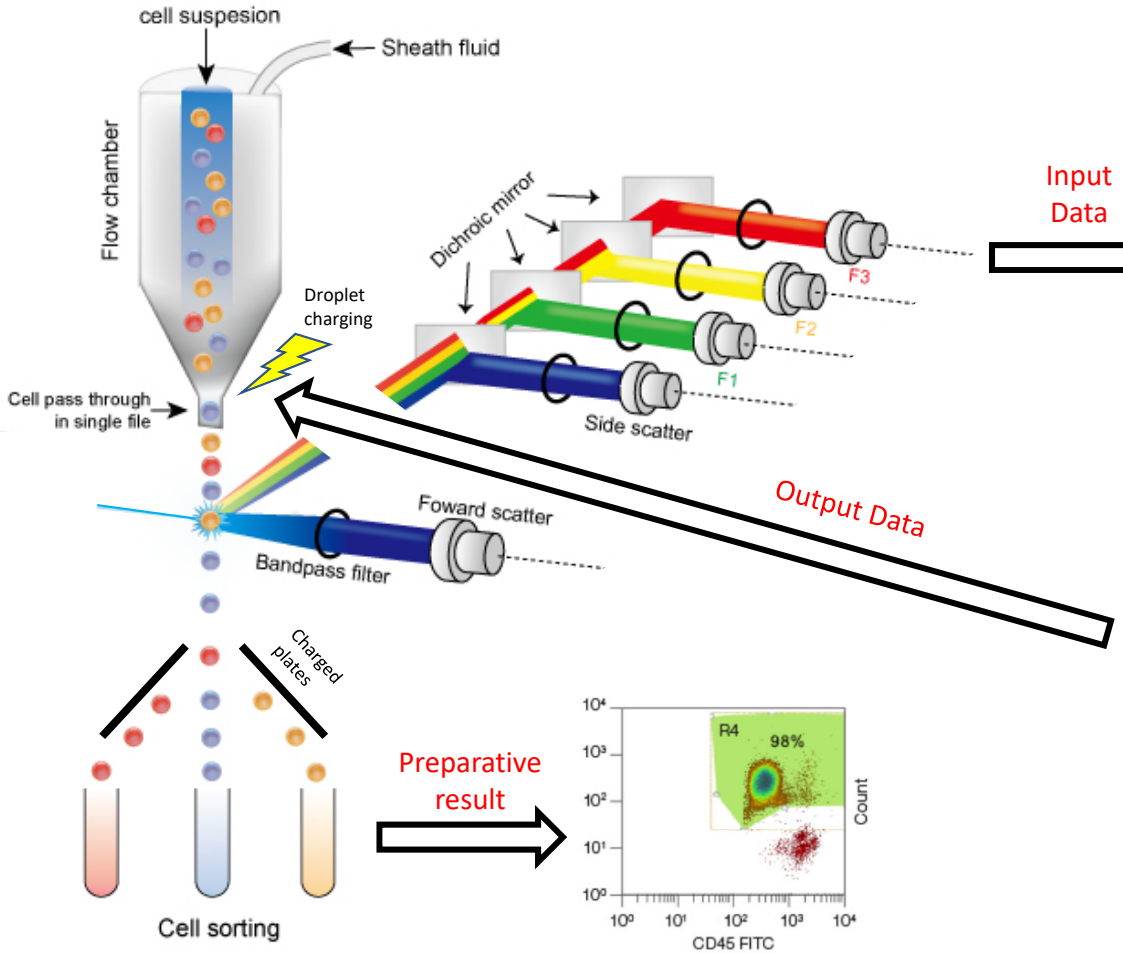


SB Alexander, Optical Communication Receiver Design, book

Analogy to thermistor experiment, just converting light instead of temperature into a voltage signal

https://www.creative-diagnostics.com/flow-cytometry-guide.htm & www.excelitas.com, modified

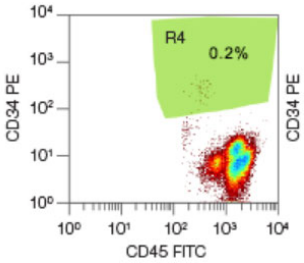
What input/output data being is processed during operation of the instrument?



Input Data

Voltage of PMTs

Data processing



apply sorting gates

If cells are within sorting gate, **then** power electrode & plates

Output Data

Analogy to LED in LabVIEW thermistor program

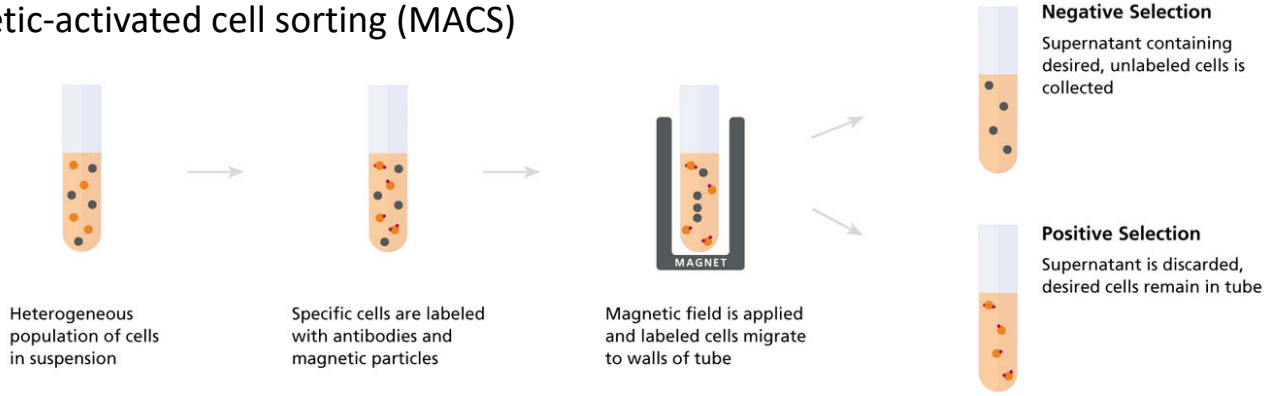
Preparative result

https://www.creative-diagnostics.com/flow-cytometry-guide.htm & www.excelitas.com, modified

What are alternative approaches for measuring the same biological property or providing the same function (if available) and how do they compare to FACS?

- Imaging flow cytometry (IFC, using spatially resolved images of cell populations rather than fluorescence intensities)
  - Higher content, lower throughput
- Microfluidic cell sorters e.g. using valves to sort cells rather than applying electric fields.
  - Lower throughput

  www.youtube.com/watch?v=5ZTdspVOhiU
- Magnetic-activated cell sorting (MACS)



www.stemcell.com/cell-separation/magnetic-cell-isolation