Lecture #3

Case study FACS
Similarities to droplet microfluidics

Aims:

- Understanding of a fluorescence-activated cell sorter and how to present it as case study
- Analogy and differences to microfluidic droplet sorting (more fundamentals on microfluidics will be taught in Lecture #9)

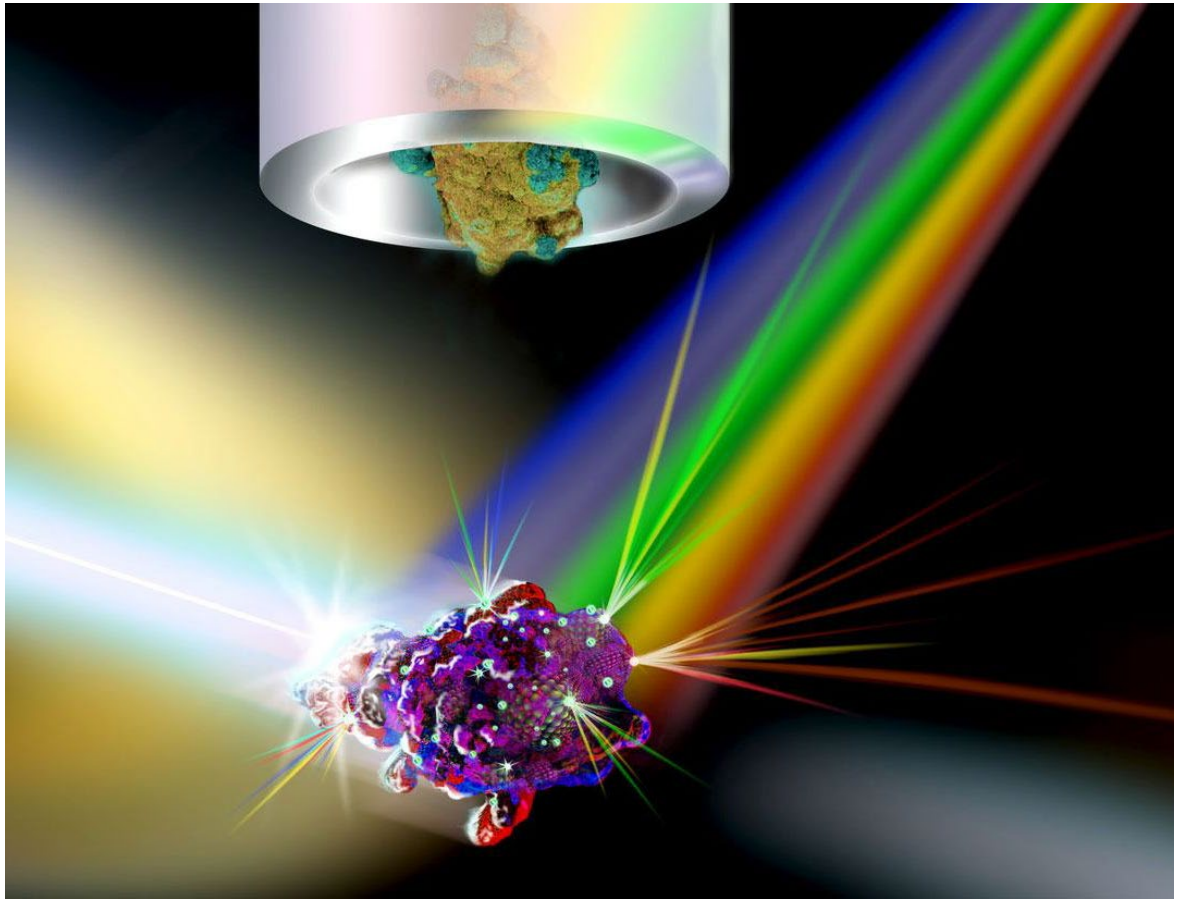
Group formation (urgent!)

Marco Francesco Luca Romano (EL)
Yasmina Jemili (EL)
Laura Worthington (SV)
Aidan Bedford (SV)

Lectures (PHH 331)	Date & Topic	Details	Practical
1	12.09 General Intro	Get to know teachers, TAs, students and aims of the course	16.09 Measure temperature using thermistor (using M&A explorer) TL
2	19.09 Lecture LabVIEW TL Group formation (4 students, each)	Some first basic steps in LabVIEW programming	23.09 Brief intro into LabVIEW thermistor program (input and output) TL
3	26.09 Case study FACS, similarities and differences to droplet microfluidics Selection of case study topics	1.) Property to measure? 2.) Device? 3.) Working principle? 4.) Alternatives?	30.09 Preparation of bioinstrument case study
4	03.10 Preparation of bioinstrument case study		07.10 Tour through LBMM workstation labs, intro into Nature Protocols (Groups A&B)
5	10.10 All groups presenting case study		14.10 Tour through LBMM workstation labs, intro into Nature Protocols (Groups C&D)
6	17.10 Lecture optics Homework: Students to prepare one laser/PMT blueprint FP	Mirrors, filters, microscope setup, lenses, etc.	21.10 Holidays
	25.10 Holidays		28.10 Build workstation optics 1
7	31.10 Lecture electronics	~~FPGA, PMTs, amplifier, function~~ generator	04.11 Build workstation 1 optics 2
8	07.11 Intro into enzyme concentration meas[illegible]ement experiment (kinetics, etc.) + task FP	Enzymes, kinetics, practical task	[illegible]11 Build workstation electronics
9	~~14.11 Intro to droplet analysis software (LabVIEW) TL~~	Software similar to Thermistor program, pdf on installation	18.11 Build workstation software: Add output LED (mimicking sorting trigger) into analysis software
10	21.11 Fundamentals of microfluidics and microfluidic chips	Flow at the microscale, microfluidic chips (manufacturing), droplet microfluidic modules	25.11 Run microfluidic experiments, e.g. determine concentration of MMP in droplets
11	28.11 Prepare presentation		2.12 Sorting Demo on LBMM workstation1 (Groups A&B)
12	05.12 Groups presenting results (A-C not present)		09.12 Sorting Demo on LBMM workstation1 (Groups C&D)
13	12.12 Groups presenting results (D-F not present) 15.12 Submit report (all!)		**16.12 – TUESDAY! - Individual Q & A sessions (10min, each)**

Green shading: Single seminar/practical with all 18 students
Red shading: Individual seminar/practical with only 6 students required (= 3 sequential 90min slots, 4.5h in total)

Case study (max 10min + Q&A)



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

Briefly present one bio-instrument of your choice, group task - 30% of your grade

What biological property is being measured? Is it measured directly or via “reporters”?

How does the instrument look like?

What is the working principle/ experimental setup?

What data is being processed (input/output in analogy to our temperature measurements)

Q&A

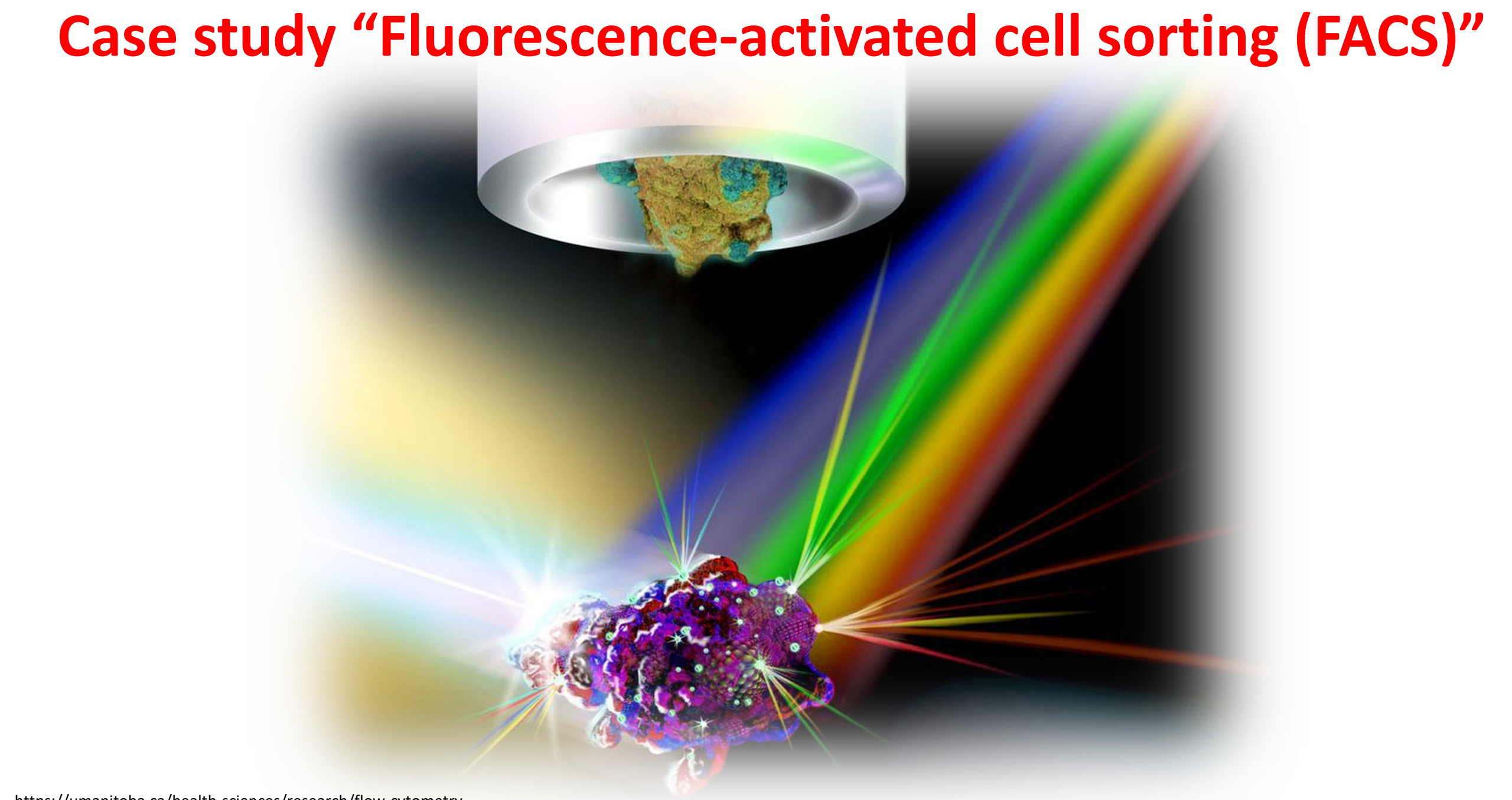
Useful online tutorials

https://www.youtube.com/watch?v=TDRhCWaYRsg
(12:09, pretty good!)

https://www.youtube.com/watch?v=EQXPJ7eeesQ
(4:35, good animation, but obscuration bar in front of FSC not shown, antibody staining neither)

https://www.youtube.com/watch?v=W1BFeiDwqnk
(33:54 excellent, but quite long)

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

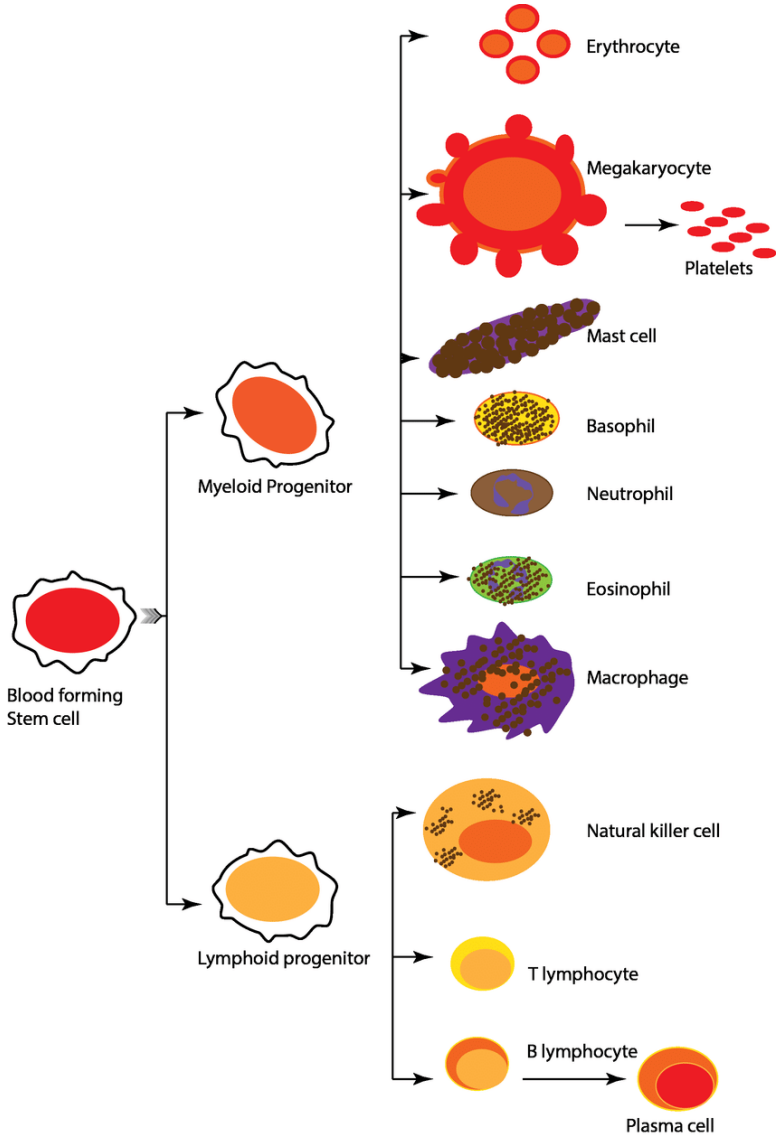
Preparative function

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

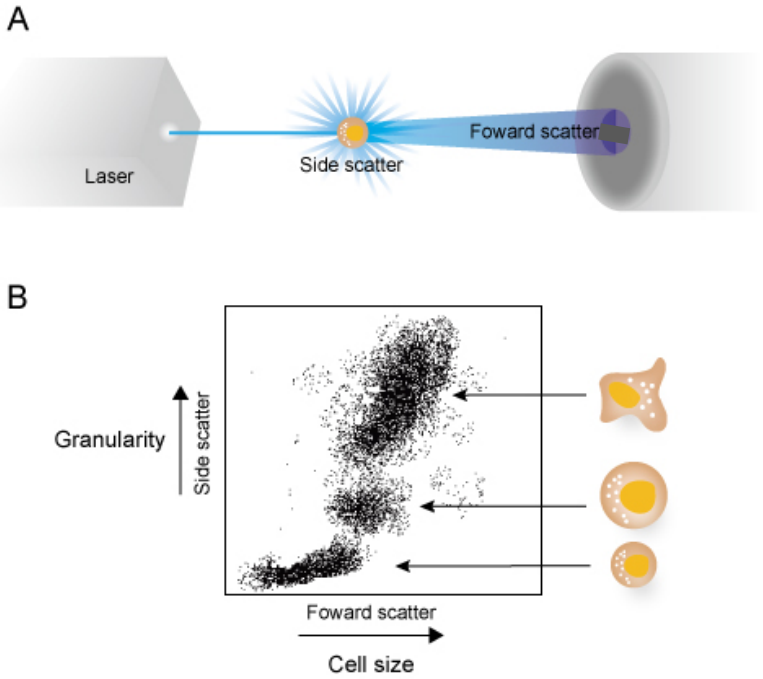
  AND
- No or only low expression of surface receptor Y



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

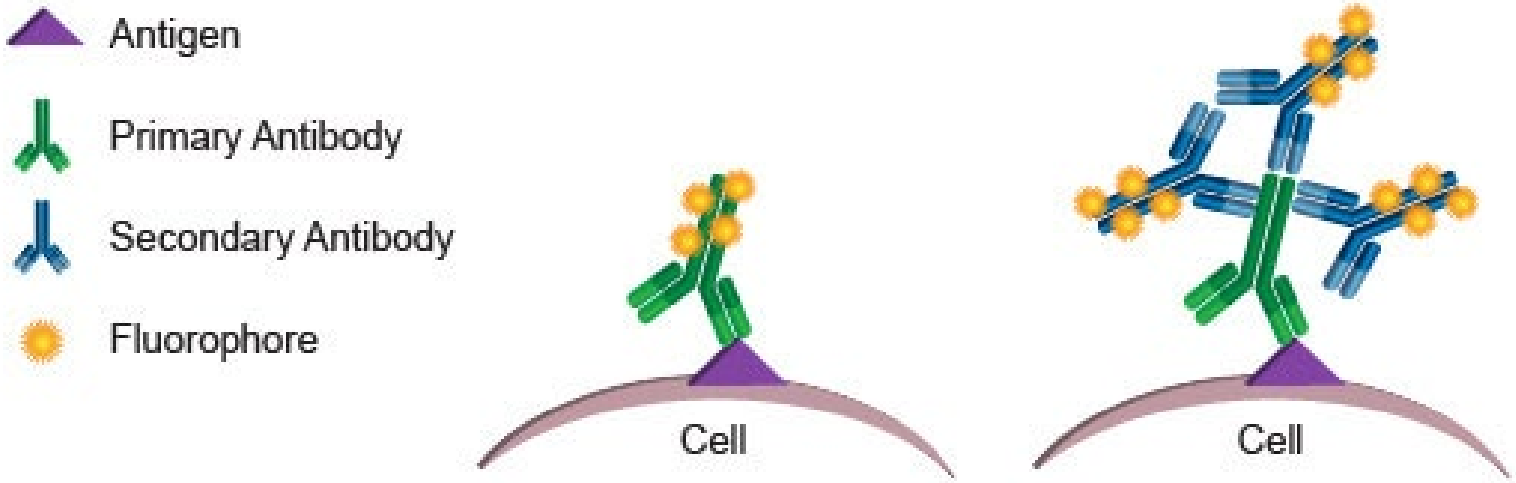
How are the cellular properties measured?

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



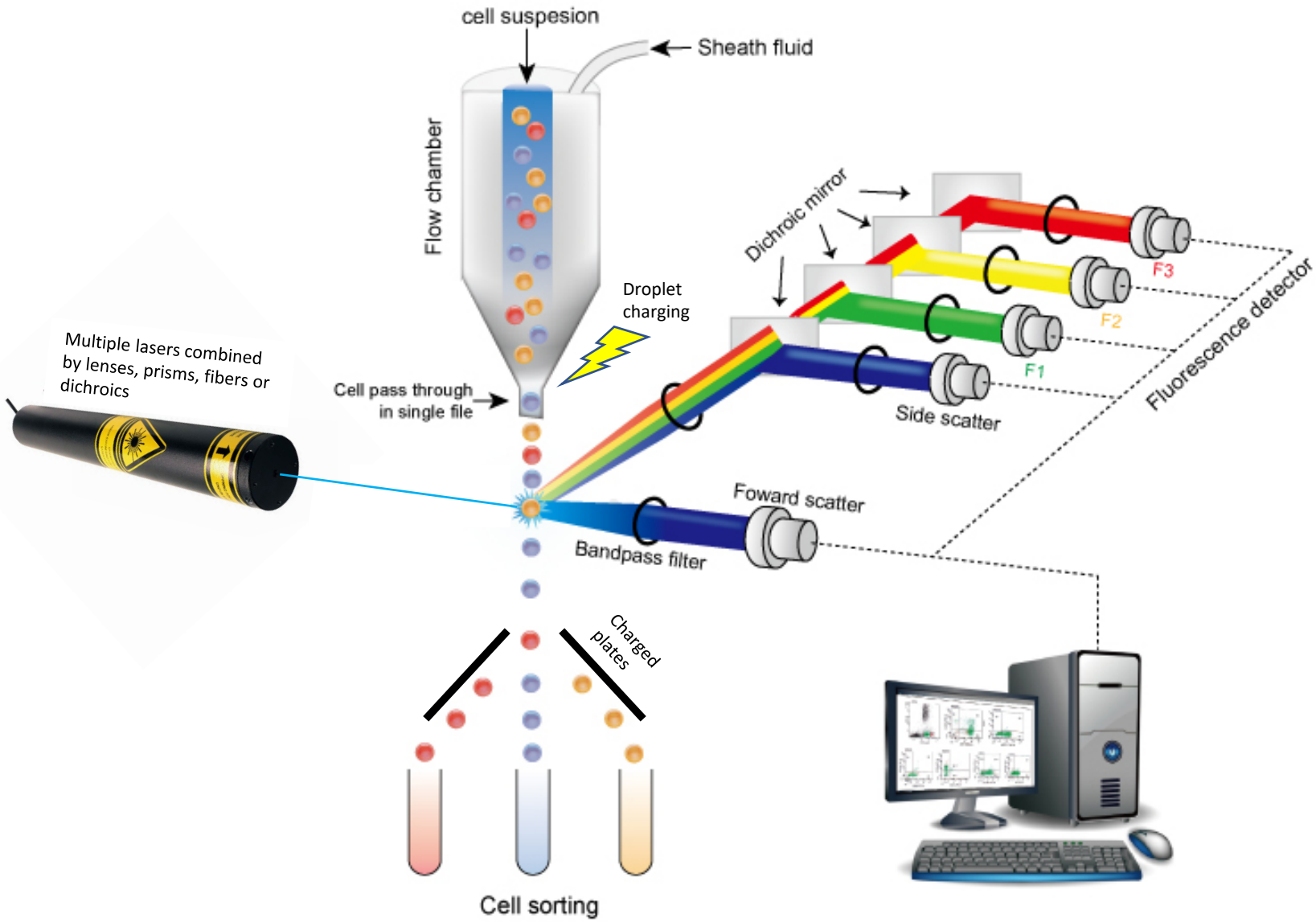
https://onelab.andrewalliance.com/library/intracellular-flow-cytometry-9jn2Gwav

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)

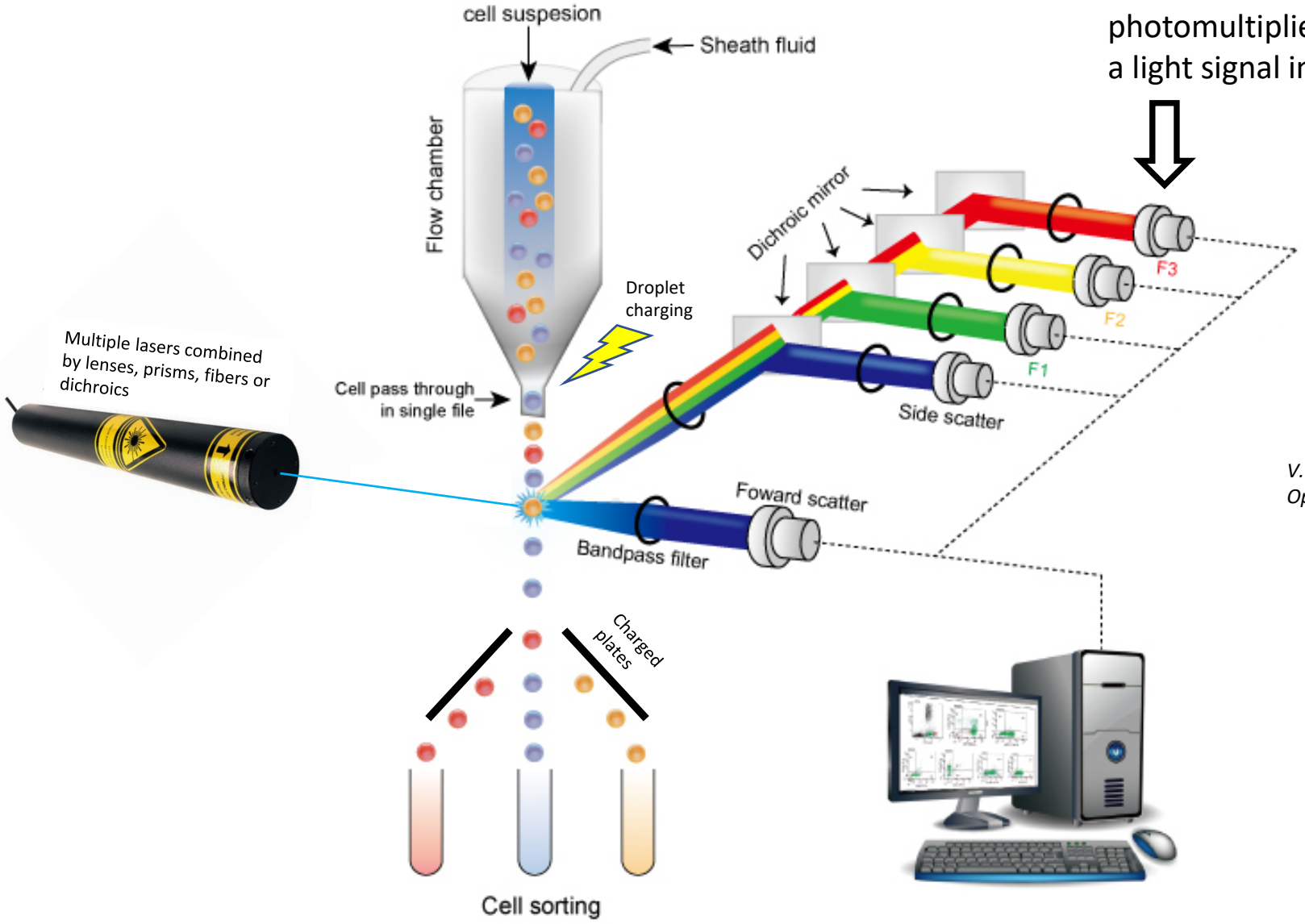
https://www.bdbiosciences.com/en-us/products/instruments/flow-cytometers/research-cell-sorters/bd-facsymphony-s6

Working principle and experimental setup



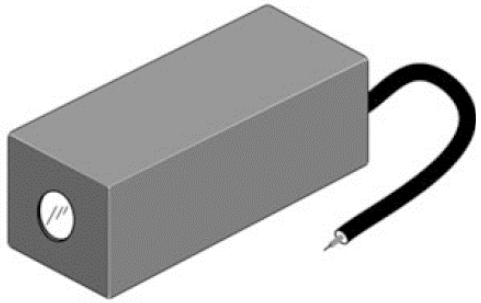
https://www.creative-diagnostics.com/flow-cytometry-guide.htm & www.excelitas.com, modified

Working principle and experimental setup

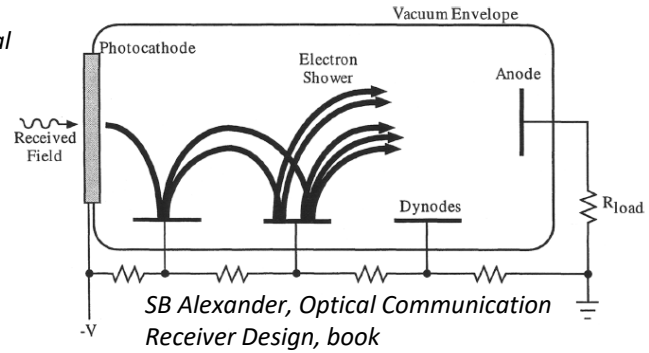


https://www.creative-diagnostics.com/flow-cytometry-guide.htm & www.excelitas.com, modified

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



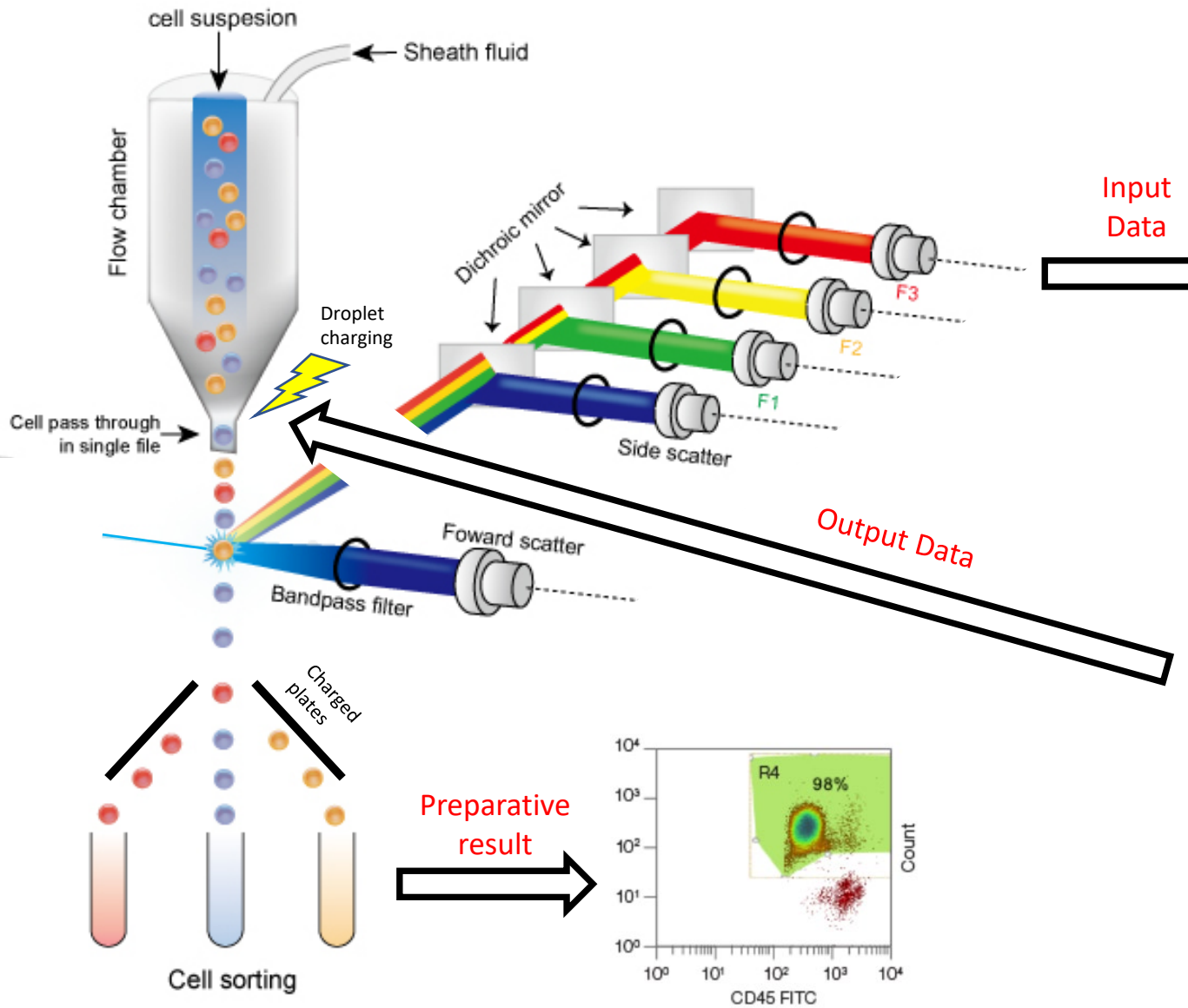
V. Protopopov, Practical Opto Electronics, book



SB Alexander, Optical Communication Receiver Design, book

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

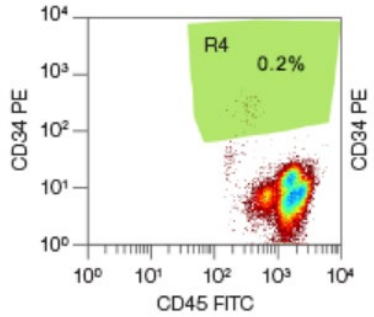
What input/output data being is processed during operation of the instrument?



Input Data

Voltage of PMTs

Data processing



apply sorting gates

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

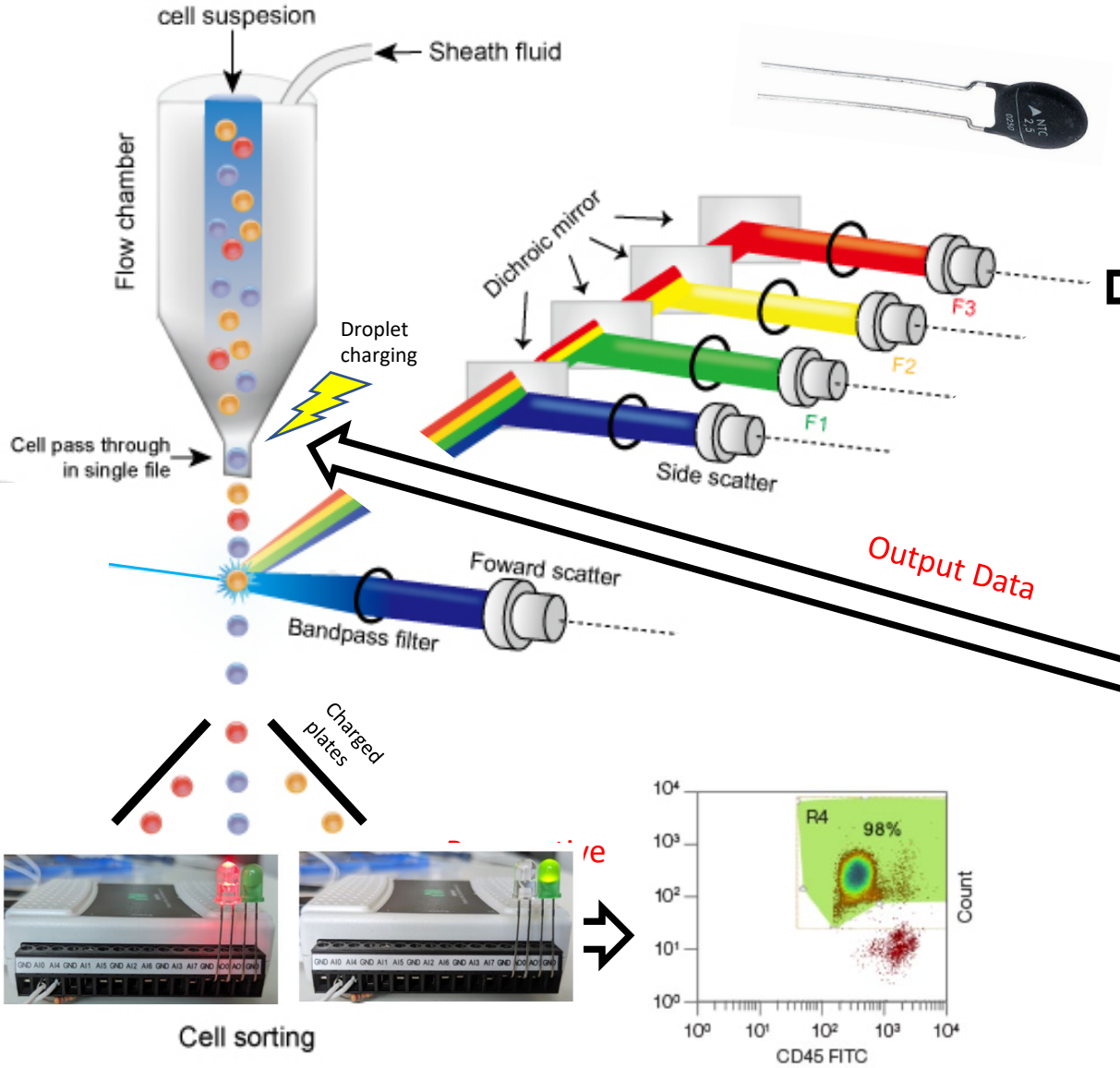
Processing

Output Data

Preparative result

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

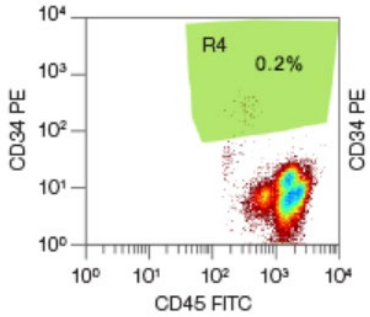
What input/output data being is processed during operation of the instrument?



Input Data

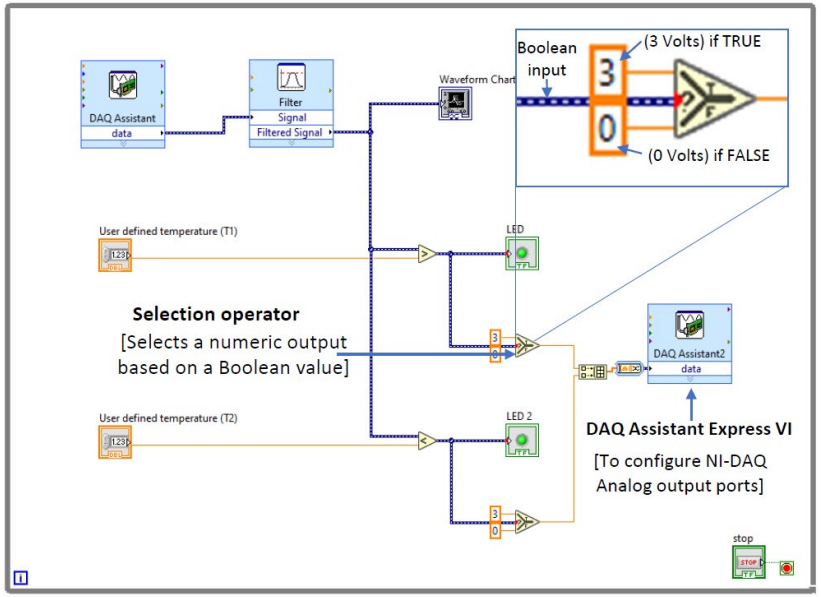
Voltage of PMTs

Data processing



apply sorting gates

Output Data



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

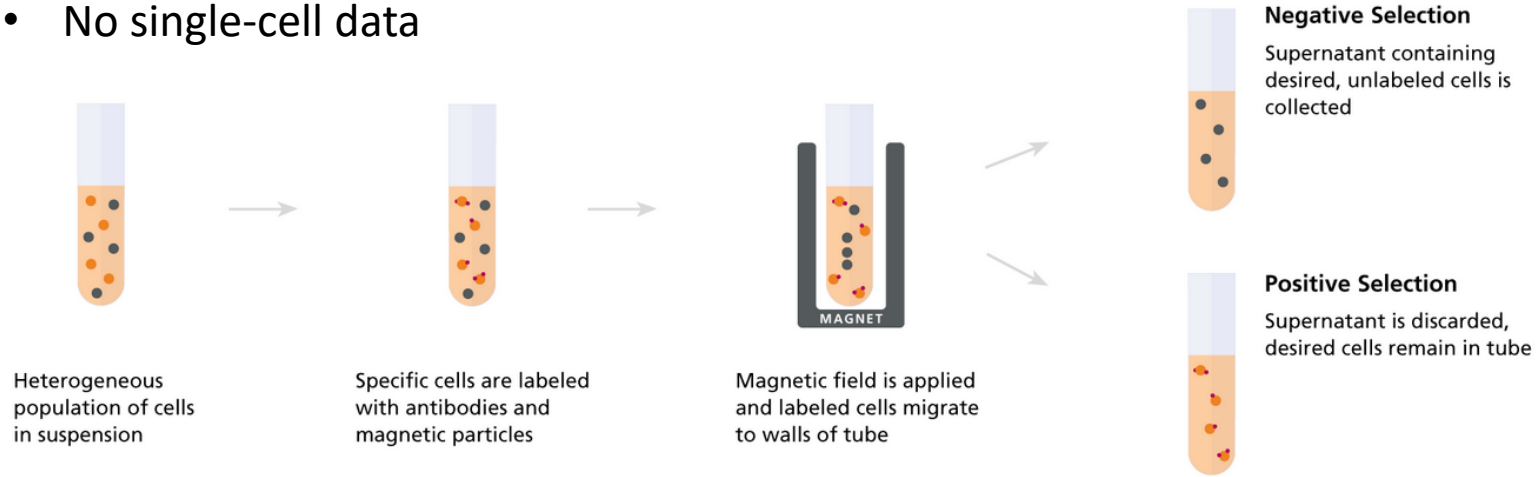
What are alternative approaches for measuring the same biological property or providing the same function (if available) and how do they compare to FACS?

- 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)
    - No single-cell data



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

Typical pitfalls and recommendations for preparing a case study

- Not all questions addressed
- Use of incorrect figures from the internet, e.g. FACS nozzles without coaxial sheath fluid, mentioning of only one laser, no electrode shown, etc.
- Always try to find and point out analogies to setups, modules and workflows that have already been discussed in this course

How to chose a bioinstrument for your own case study?

Possible instruments for case studies:

- PCR cycler/ qPCR cycler
- Cell counter
- Spectrophotometer/ Plate reader
- Sequencer (NGS)
- Mass spectrometer
- HPLC Chromatograph
- Bioanalyzer, Electrophoresis systems (Gel)
- NMR Spectrometer
- Different type of microscopes (including e.g. AFM)
- Calorimeter
- Surface plasmon resonance device
- Scintillation counter

Possible questions to be answered with novel bioinstruments:

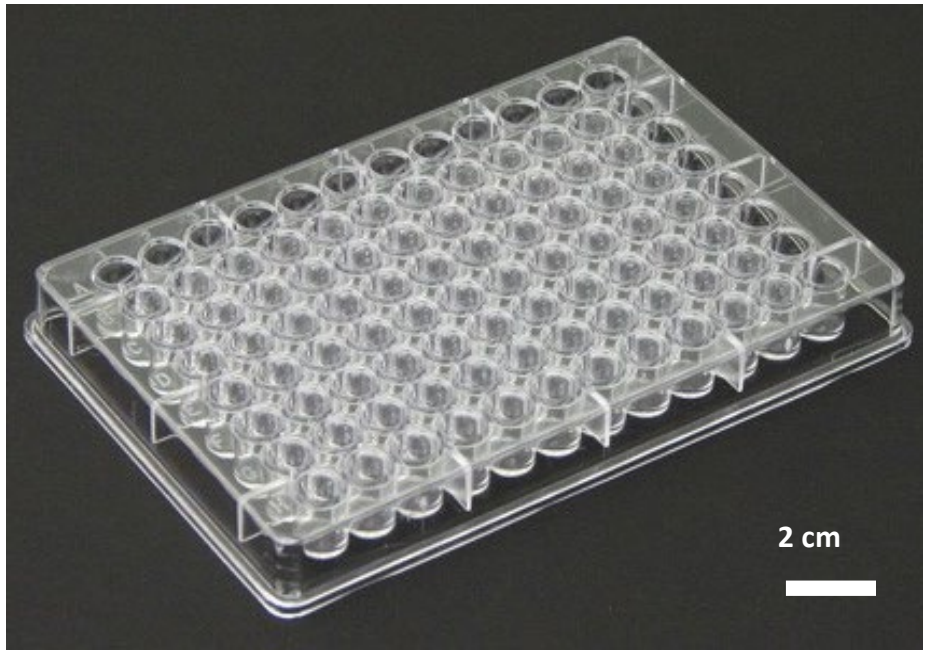
- How to measure the mass & volume of a cell?
- How to measure cell mechanics/ deformability?
- How to measure cell conductivity?

Feel strongly encouraged to expand these lists based on your own ideas and preferences!

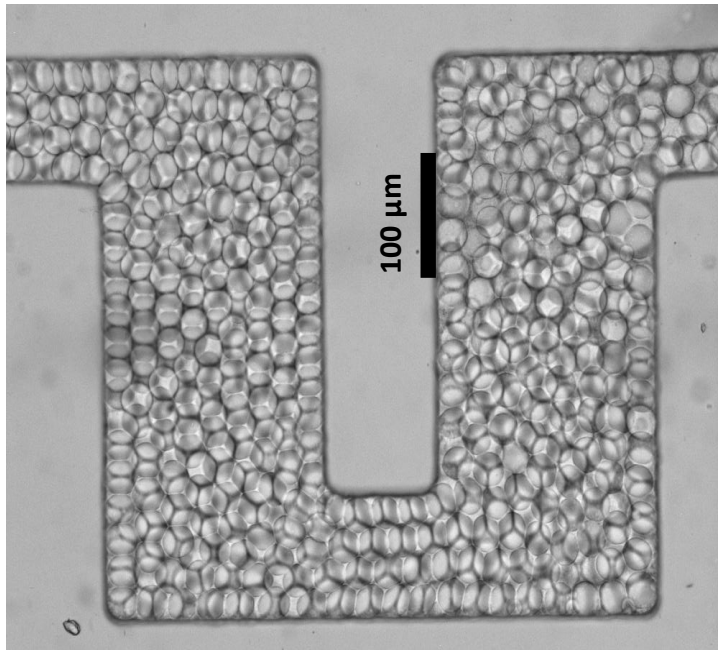
From cytometry and FACS to microfluidic droplet analysis and sorting

The basic setup of a microfluidic droplet analyzer/sorter is almost identical, with just a few conceptual differences:

- Instead of sorting solid particles such as cells, droplet microfluidics can be used to run and sort assays in a miniaturized high throughput fashion:



96 wells



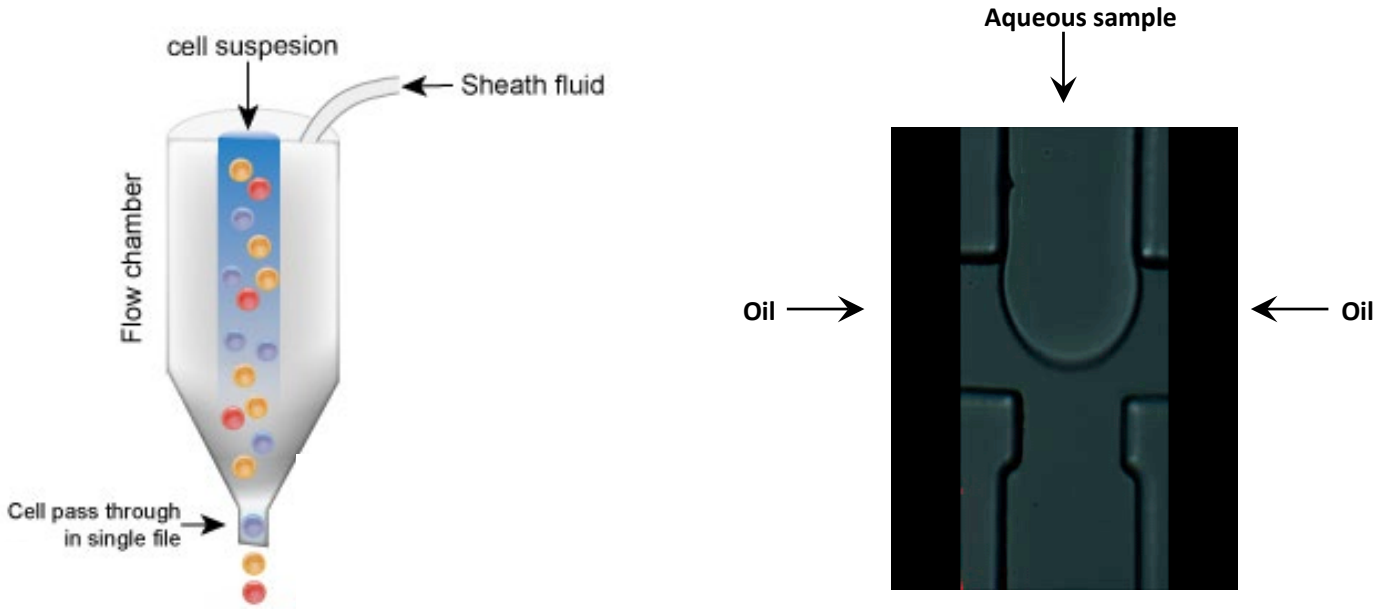
Millions of droplets in a single experiment

Droplets surrounded by oil and stabilized by surfactants can serve as miniaturized test tubes

From cytometry and FACS to microfluidic droplet analysis and sorting

The basic setup of a microfluidic droplet analyzer/sorter is almost identical, with just a few conceptual differences:

- Instead of having an aqueous solution as carrier phase, oils are used to separate specimen



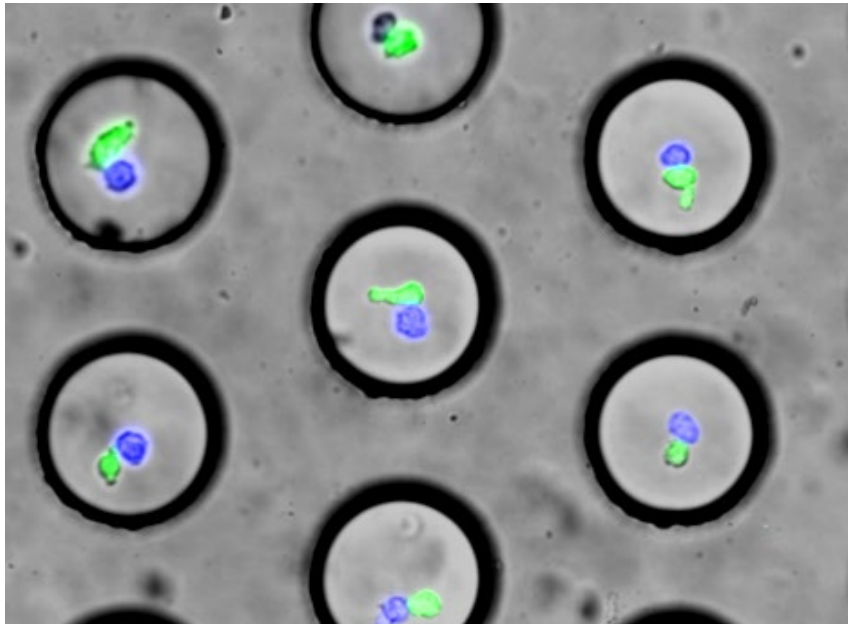
Flow focussing FACS nozzle versus droplet maker

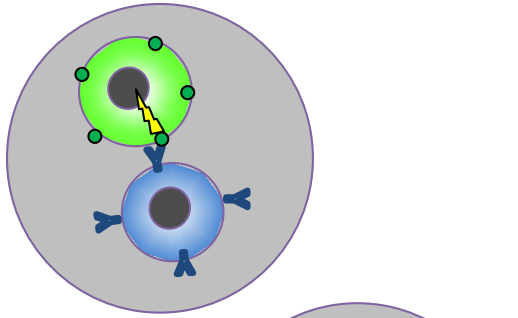
Why droplet microfluidics?

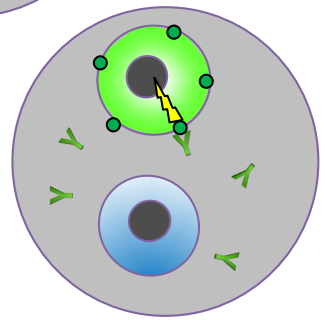
- Small assay volumes enable to obtain **detectable concentrations of DNA, RNA and proteins from single cells** (Fluidigm, DropSeq, 10X genomics, etc)

- Small assay volumes enable **large scale screens on very limited patient material**

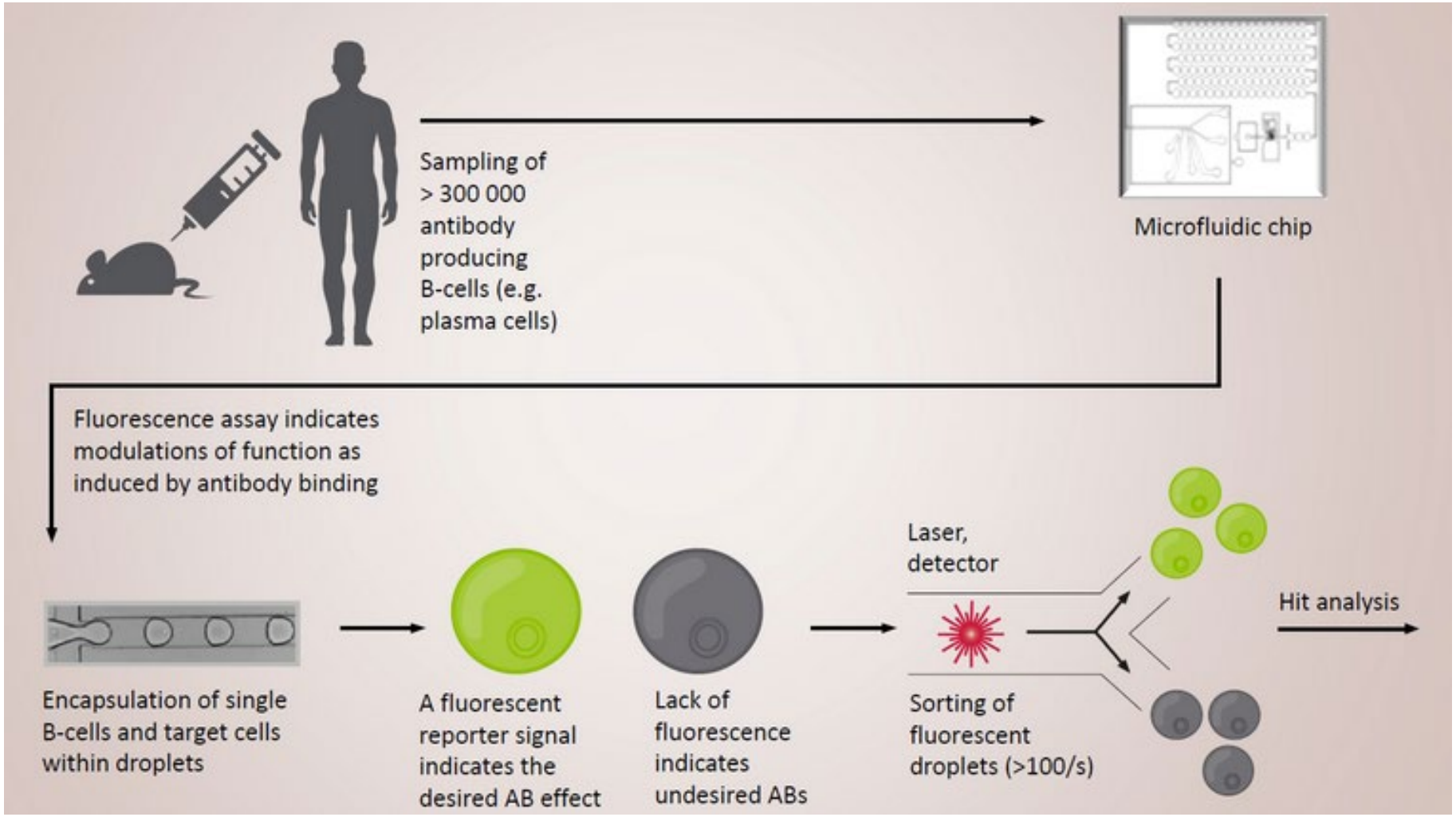
- Sorting of droplets enables the identification of cells causing a particular assay signal (e.g. due to the **secretion of specific factors** such as antibodies or **cell-cell interactions**)







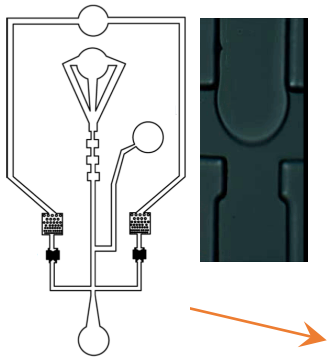
Screening of antibody-expressing cells using microfluidic droplet sorting



…similar approaches can be applied for screening pairs of T-cells and APCs!

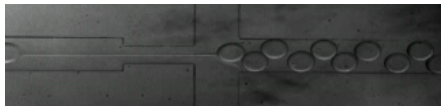
Droplet microfluidic screening

I- Encapsulation



II- 6h off-chip incubation

III- Reinjection



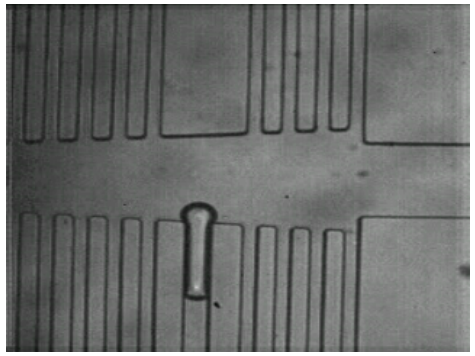
VII- Sorting

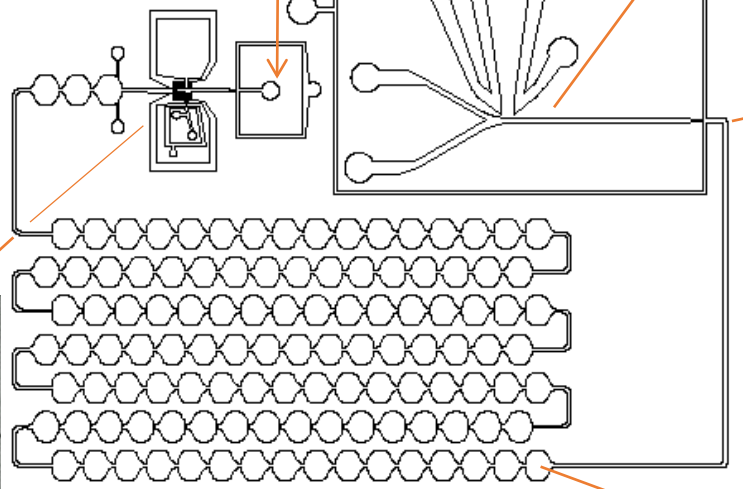
VI- Spacing

IV- Substrate addition

active

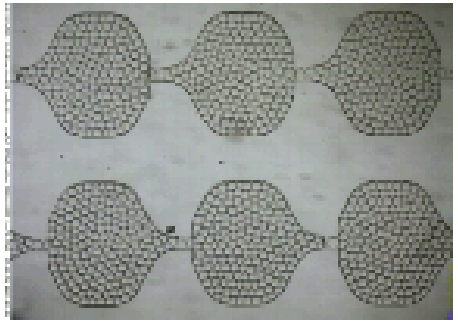
inhibited



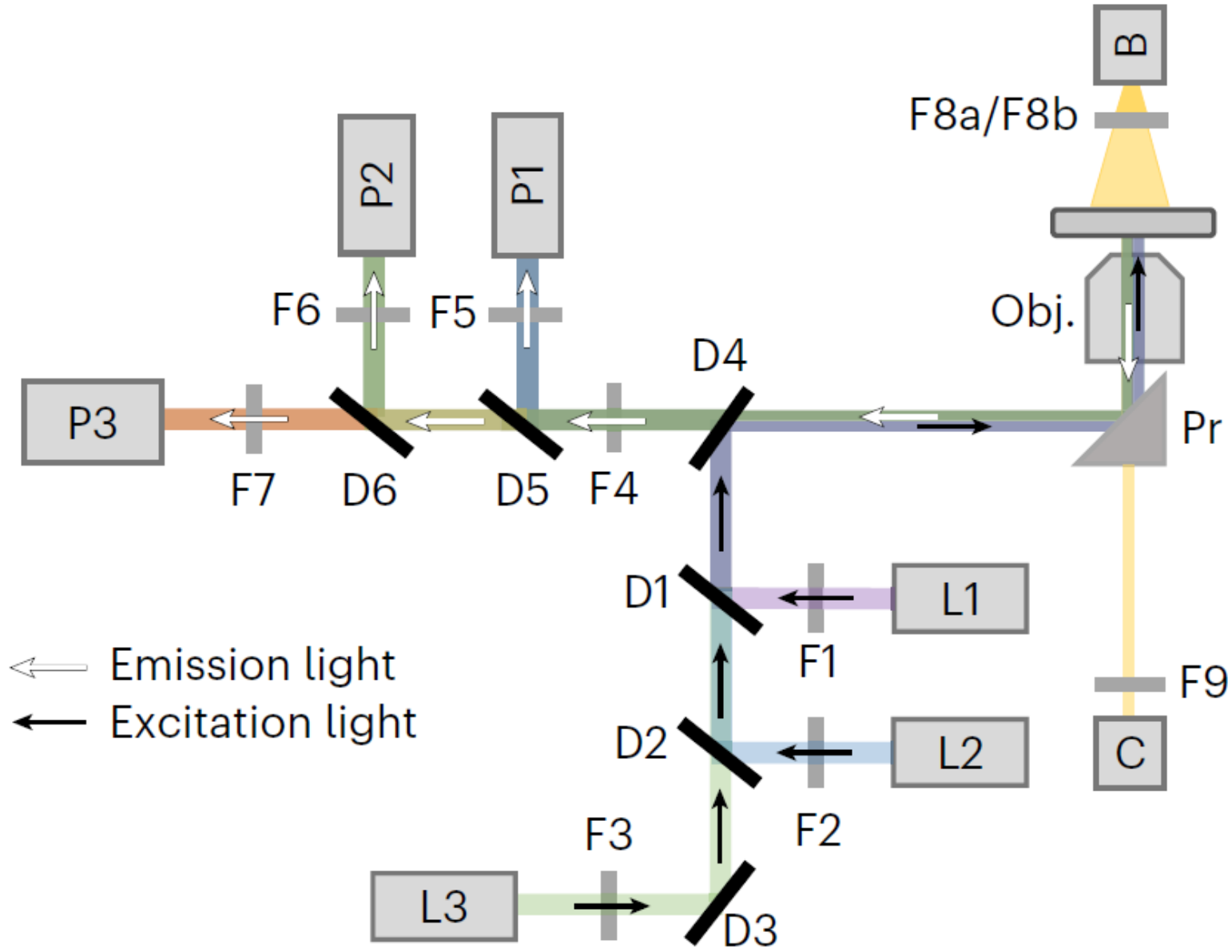


Throughput = 300,000 cells/exp.
Sorting enrichment = 9,400-fold

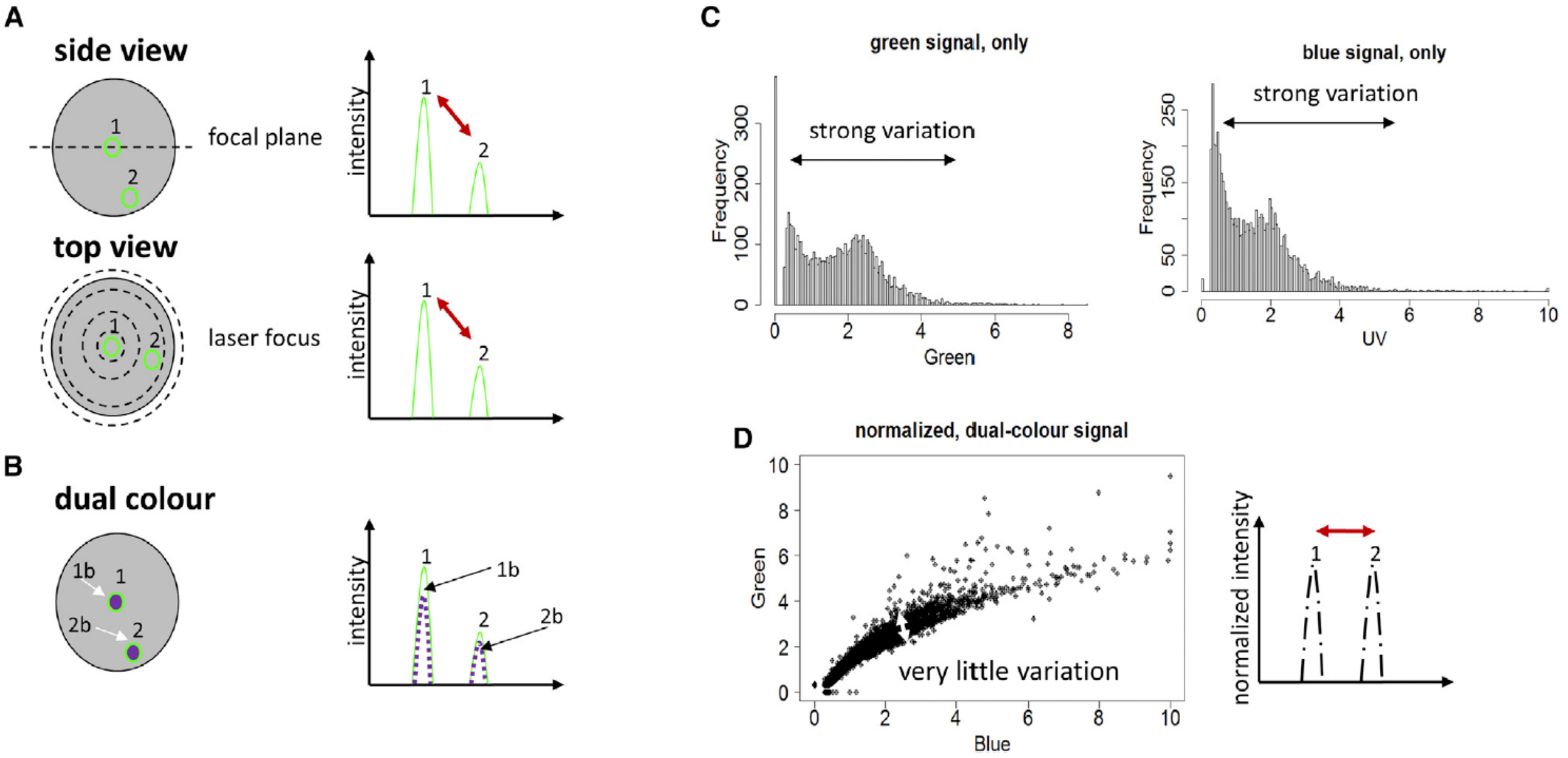
V- Incubation



Optical setup of a microfluidic workstation is very similar to FACS



Detection of cellular fluorescence within droplets is challenging



Tasks until next lecture

Form groups

Select a bioinstrument to present (to be approved by teachers).

Questions?