ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

HSeT
Health Sciences
e-Training
Foundation

9. Methods in Immunology

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Web: http://ghi.epfl.ch

Outline

9.1 Antibody-based tools

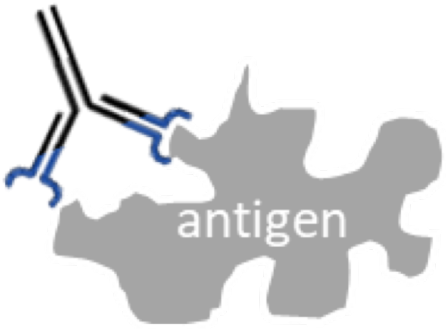
9.2 Identifying & quantifying antigens: **ELISA and Western Blot**

9.3 Cell-based assays to assess immune reactions: **cytotoxicity and proliferation**

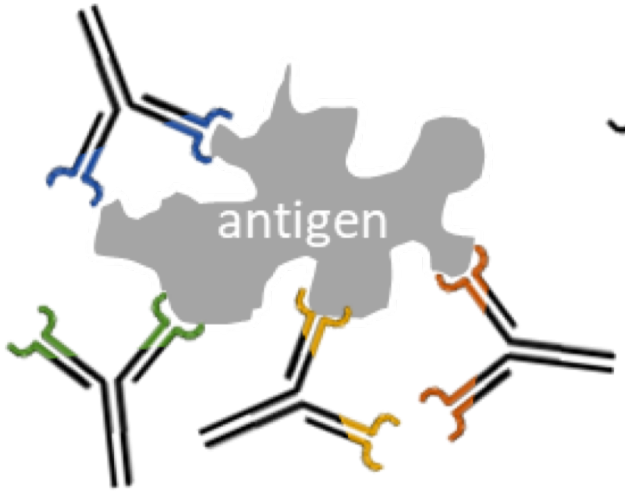
9.4 Identifying, characterizing and purifying cell types: **Flow cytometry and FACS**

Antibodies as tools to identify molecules

- Antibodies (predominantly **IgGs**) are used for a variety of applications in research, diagnostics and therapy.
- Antibodies can be **polyclonal** (recognize many epitopes of the same antigen) or **monoclonal** (recognize a single epitope of the same antigen)
- Antibodies can be raised in **different host species** (e.g. mouse, rabbit, donkey, chicken, goat)



Monoclonal antibody

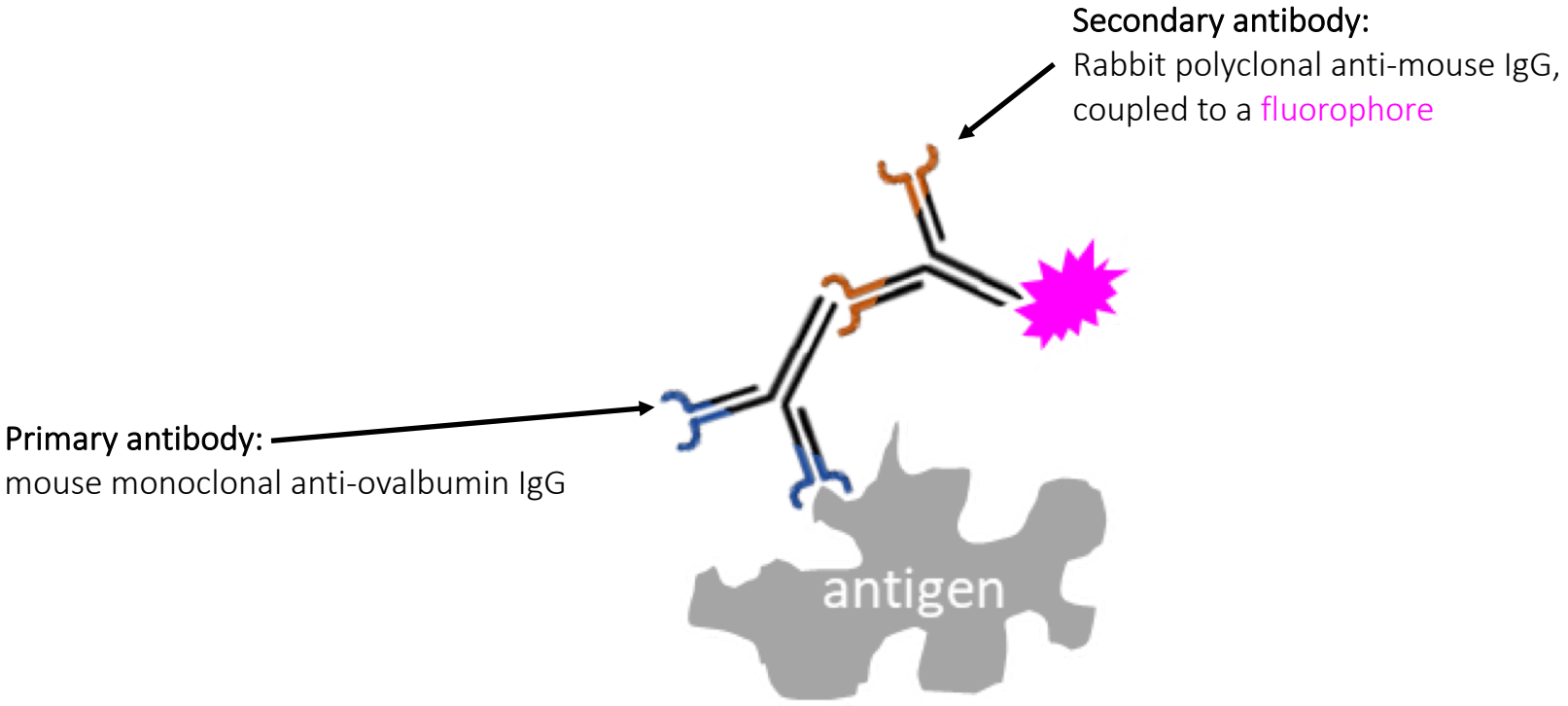


Polyclonal antibody

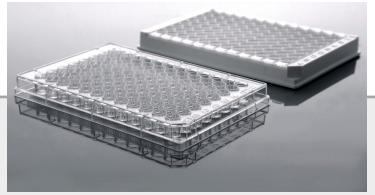
Antibodies from different species have species-specific Fc regions

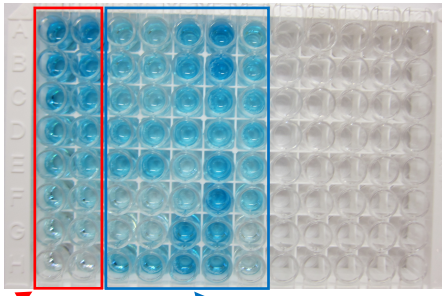
Antibodies as tools to identify molecules

- **Primary antibodies** recognize a given antigen (e.g. mouse anti-ovalbumin IgG)
- **Secondary antibodies** recognize the Fc portion of an antibody from a different species (e.g. goat anti-mouse IgG)
- Antibodies can be **coupled to fluorescent molecules** (e.g. FITC, PE) **or to effector molecules** (e.g. enzymes like peroxidase)



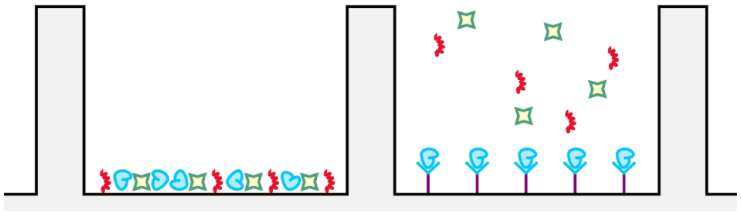
ELISA (**E**nzyme-**L**inked Immuno**S**orbent **A**ssay)



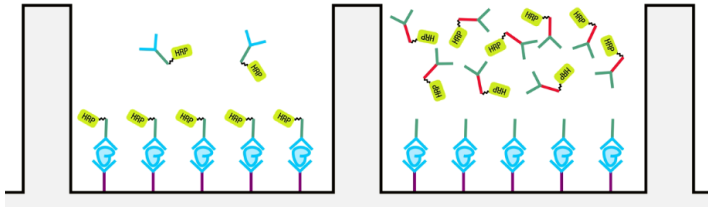
What is it?	Microplate-based assay
What can it do?	Detect and quantify molecules (peptides, proteins, hormones,…) from complex mixtures (e.g. serum, cell culture supernatant)
How does it work?	1. Antigen is immobilized on a solid surface 2. An antigen-specific antibody is added to detect the antigen 3. The enzyme coupled to the antibody catalyses a coloured substrate (dye) reaction that can be quantified in a spectrophotometer
What does an ELISA look like?	

ELISA (**E**nzyme-**L**inked Immuno**S**orbent **A**ssay)

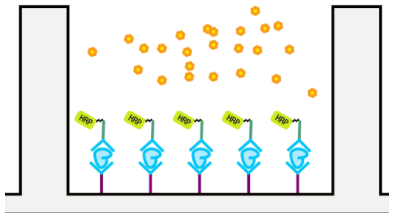
1. Direct versus indirect capture ('sandwich ELISA')

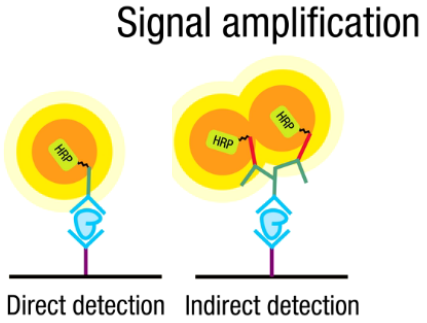


2. Direct versus indirect detection



3. Substrate reaction

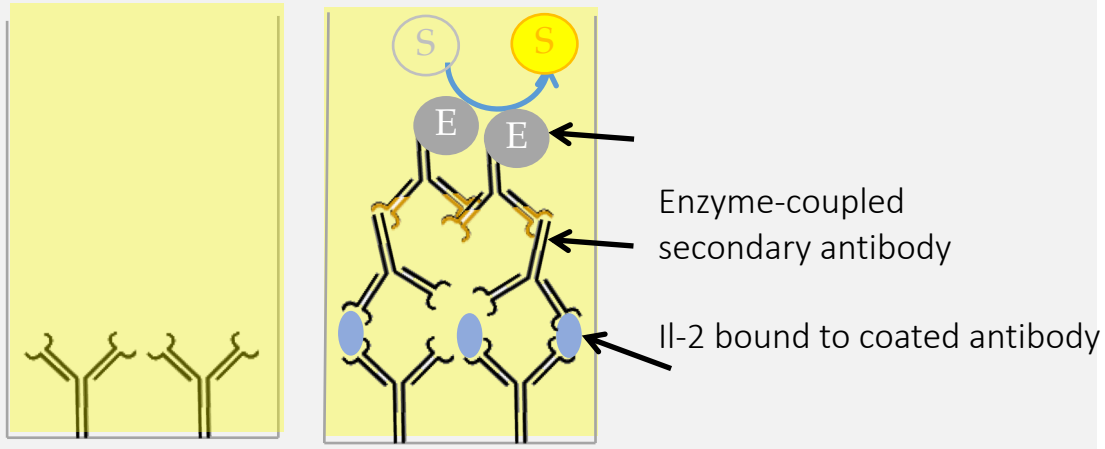


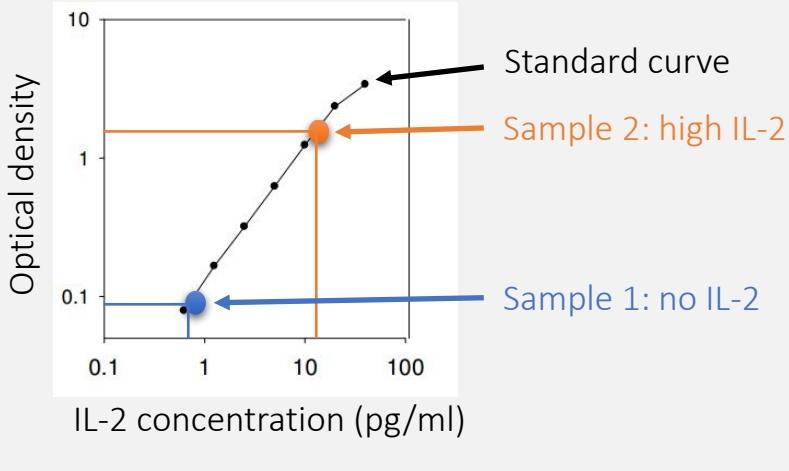


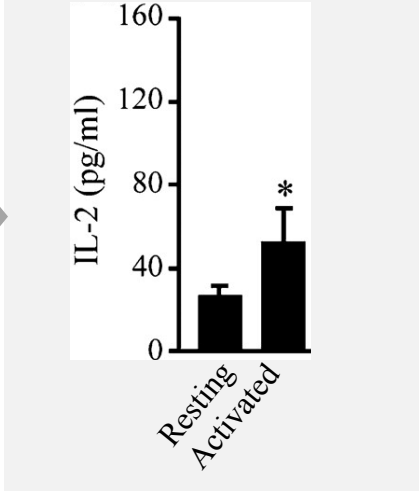
Watch video of ELISA here

Example: Cytokine levels in serum or tissue culture

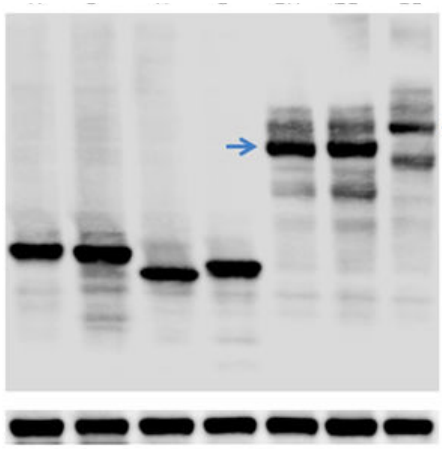
Il-2 sandwich ELISA



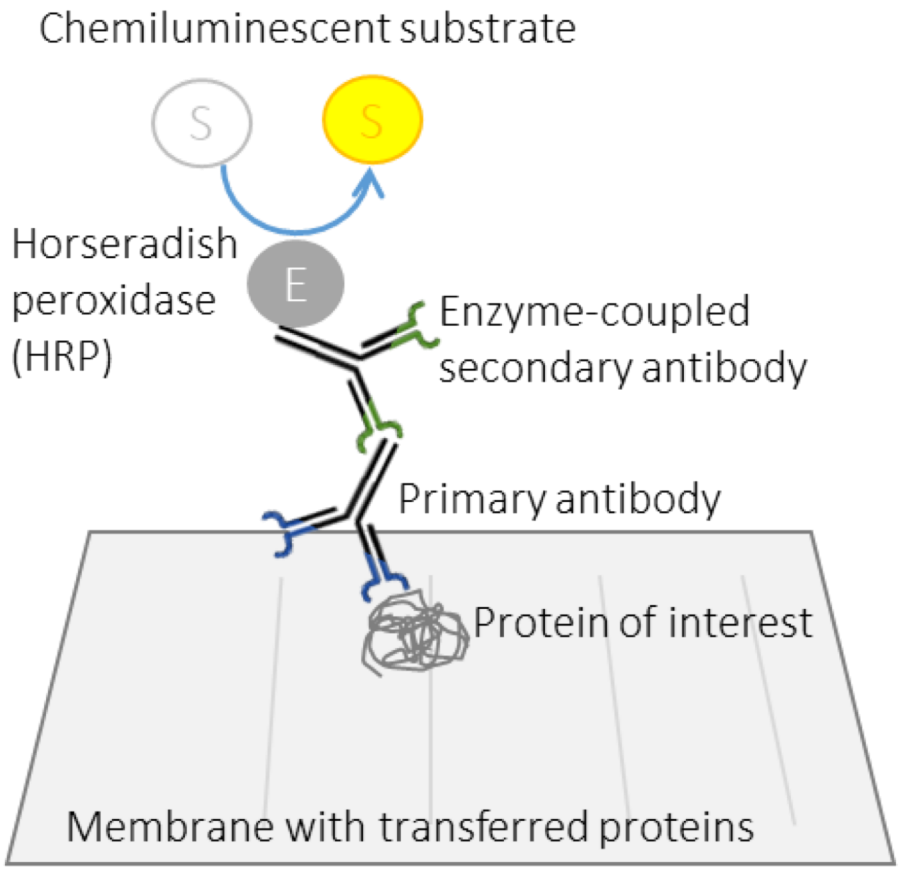




Western Blot

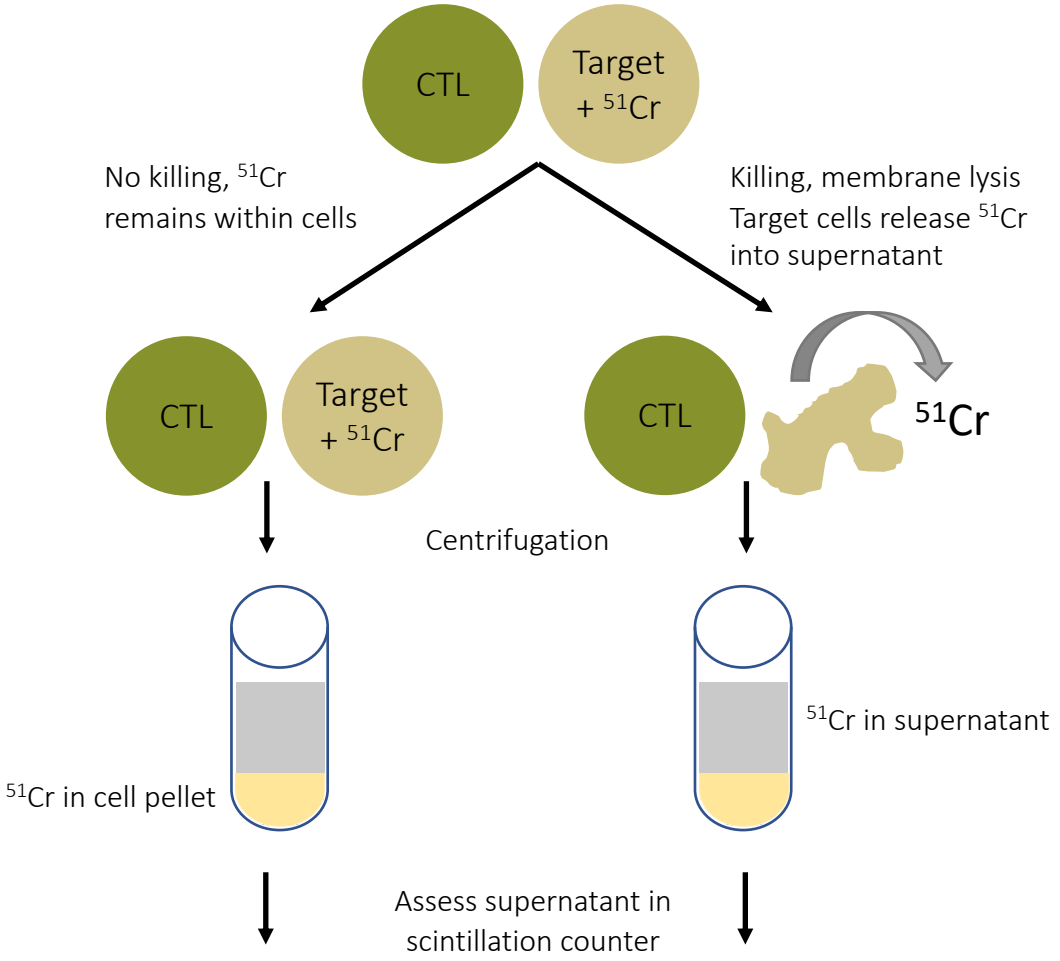
What is it?	Solid-phase protein detection assay
What can it do?	Detect, quantify and characterize proteins and post-translational modifications (e.g. phosphorylation)
How does it work?	1. A protein mix is separated by gel electrophoresis and transferred to a nitrocellulose membrane (blot) 2. An antigen-specific antibody is added to detect the antigen 3. The enzyme coupled to the antibody catalyses a chemiluminescent reaction that can be visualized on film
What does a Western Blot look like?	

Western Blot



Cell-based assays: Cr51 release assay to assess cytotoxicity

- Used to assess **CTL or NK cell function**
- CTLs or NK cells kill by releasing granzyme and perforin, which disrupts target cell membrane
- Target cells are loaded with radioactive chromium



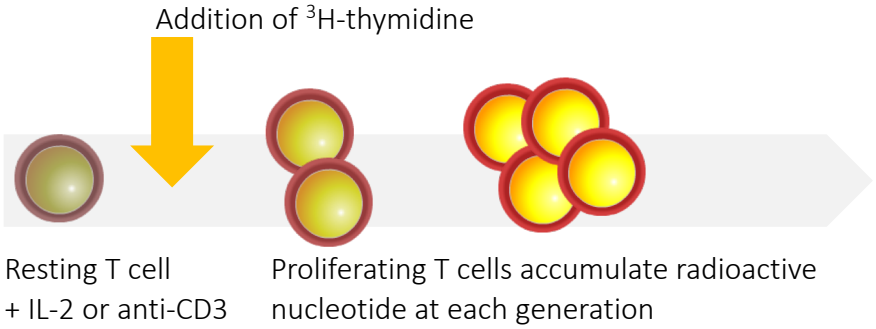
Cell-based assays: proliferation

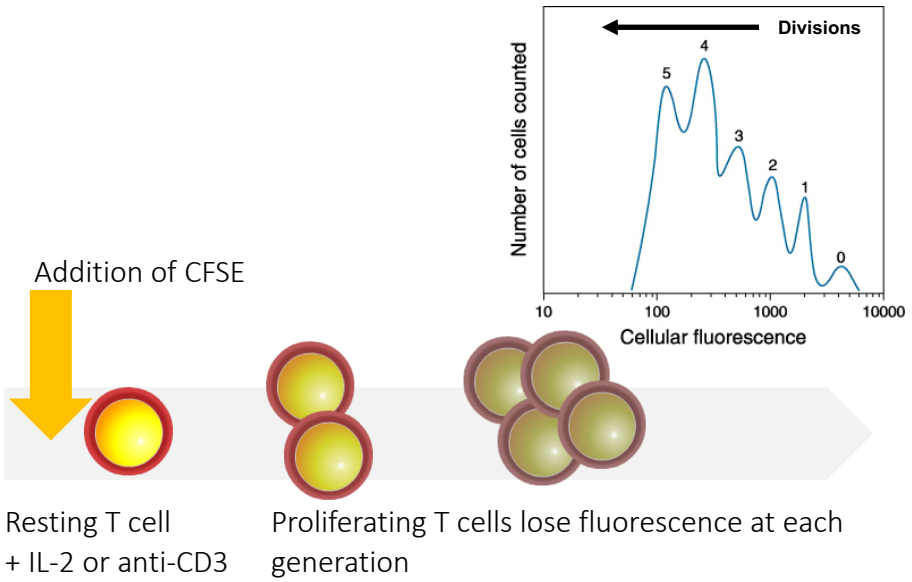
Radioactive labelling

- Cells are stimulated to induce proliferation
- Radiolabelled nucleotides are added to the medium
- Cells incorporate ^{3}H-thymidine into their DNA during each round of replication
- After several days, cells are harvested, lysed, and radioactivity is counted

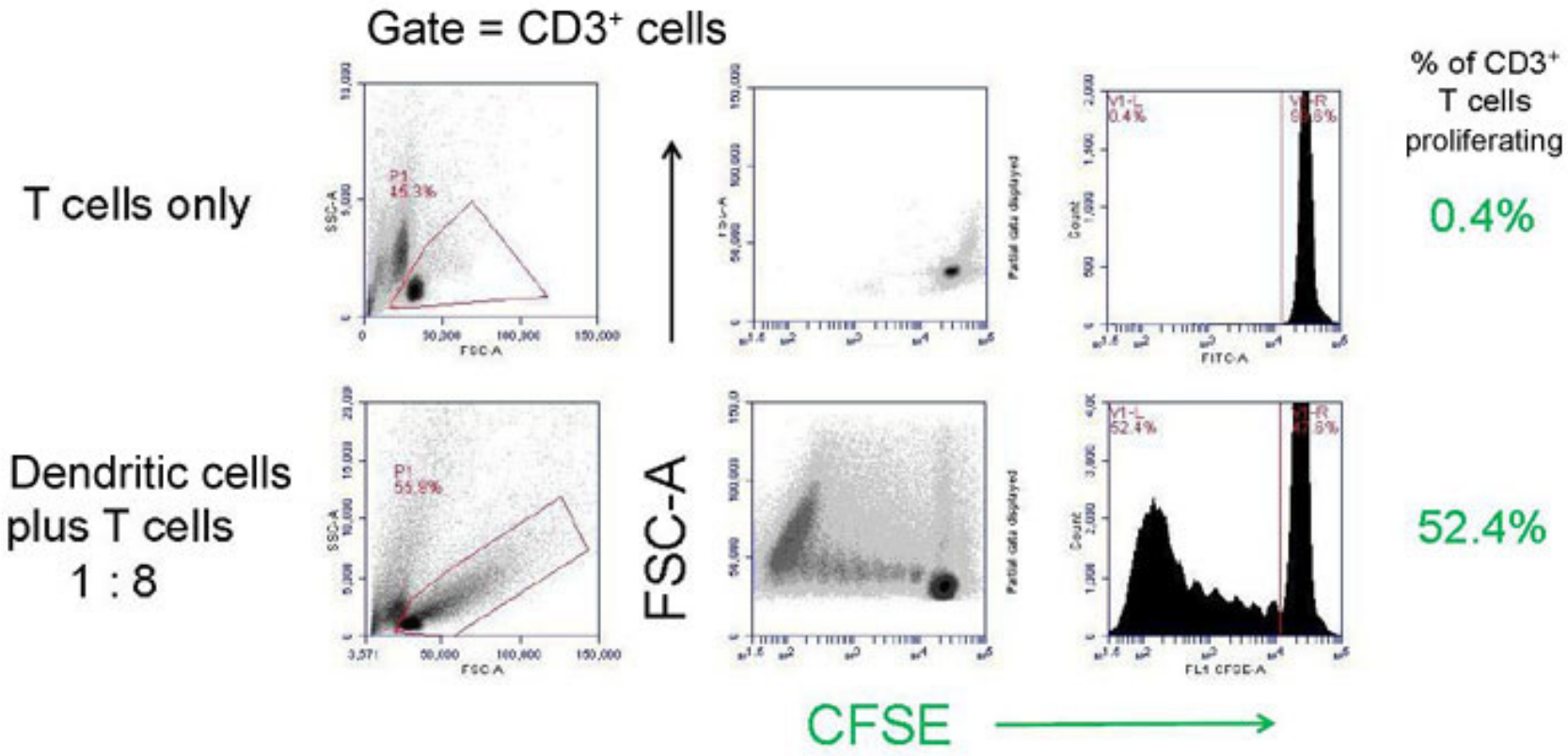
Fluorescent labelling

- Cells are labelled with a cytoplasmic fluorophore (e.g. CFSE)
- Cells are stimulated to induce proliferation
- At each division, daughter cells receive half the amount of the fluorophore
- After several days, cells are fixed and their fluorescence measured by flow cytometry





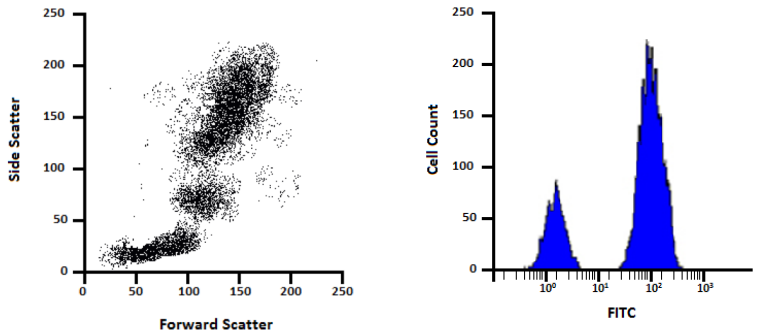
Example: T cell proliferation assay

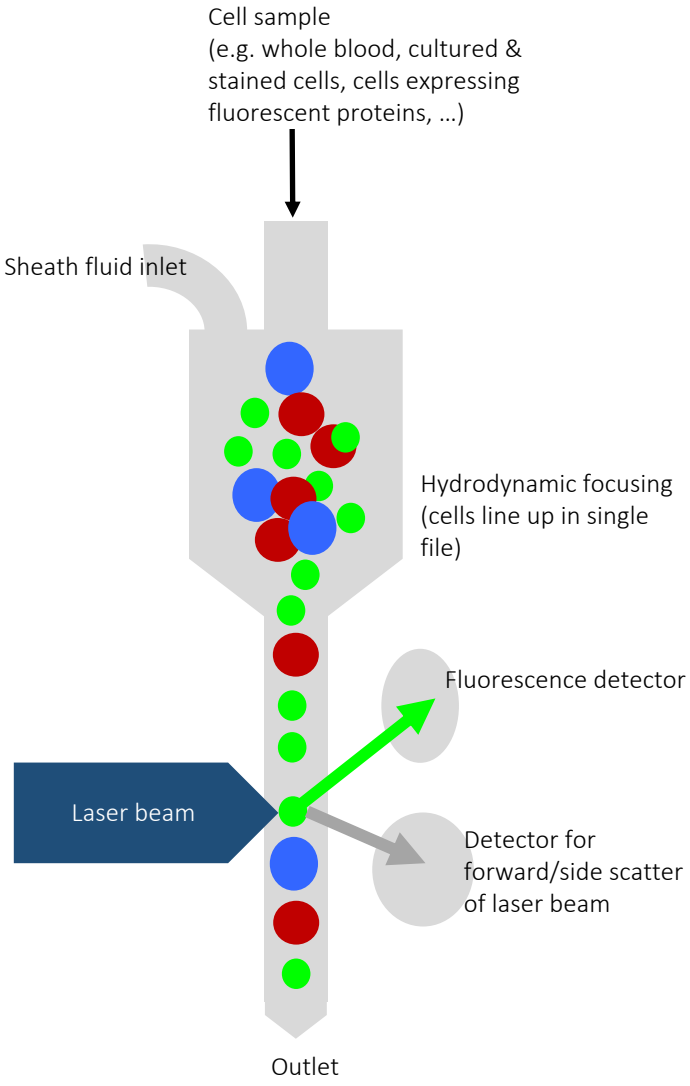


Source: http://m.bdbiosciences.com/kr/instruments/accuri/sample_data/index.jsp

Flow cytometry: principle

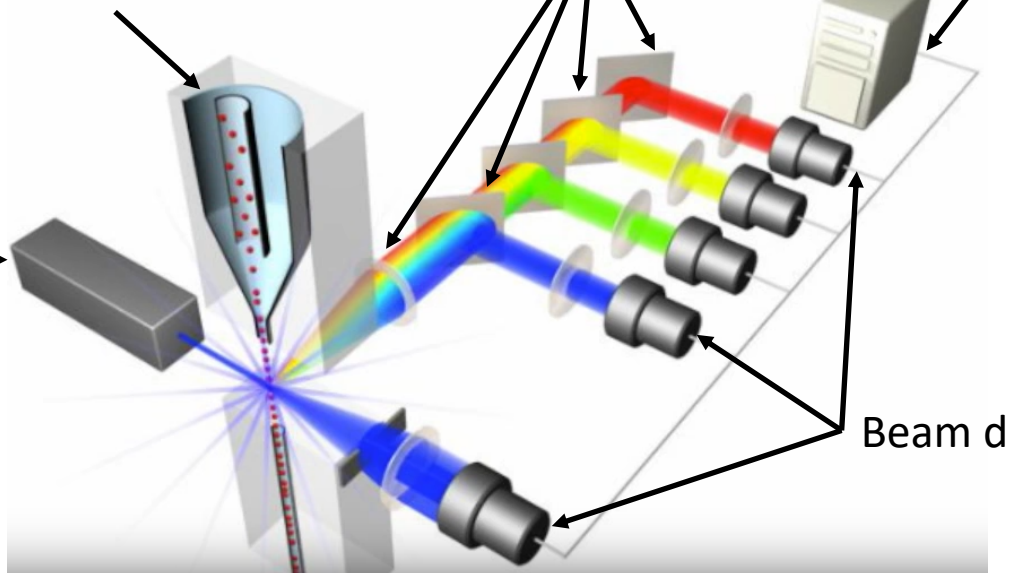
Developed in 1954 by Walter Coulter

What is it?	Laser-based single-cell fluidics technology
What can it do?	Characterize cells or particles
How does it work?	1. A single-cell suspension is passed in front of a laser 2. Detectors measure • how each cell scatters the laser beam • fluorescence emitted from each cell
What does flow cytometry data look like?	



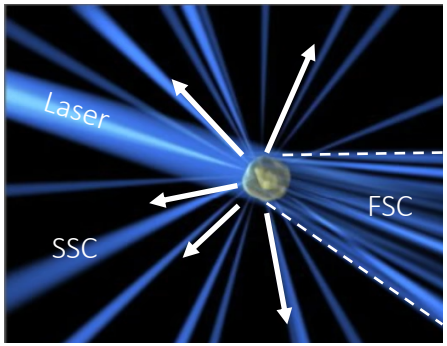
Watch video of flow cytometry principles here

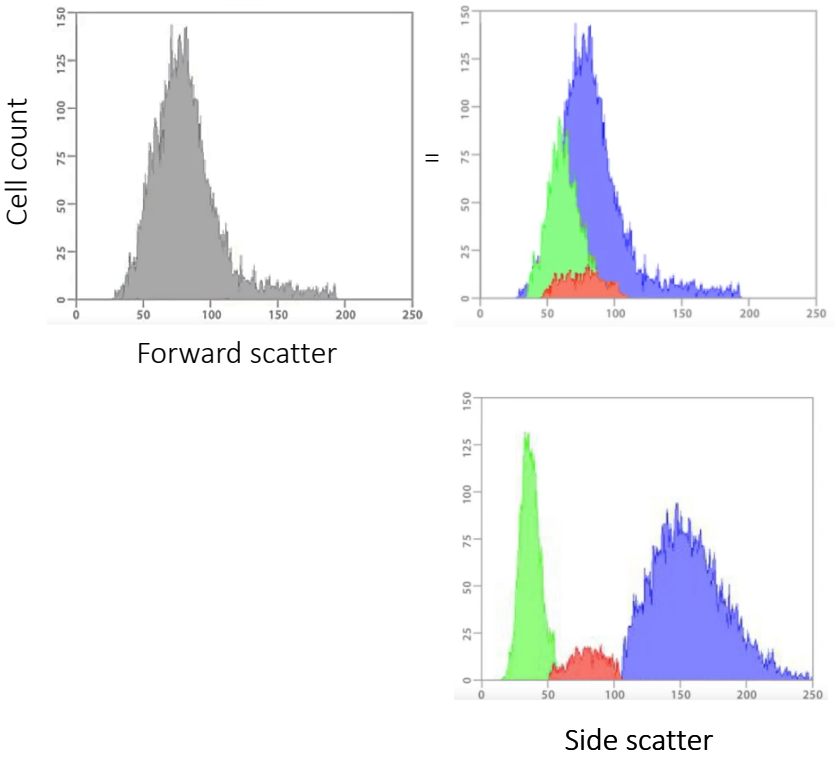
Flow cytometry: equipment



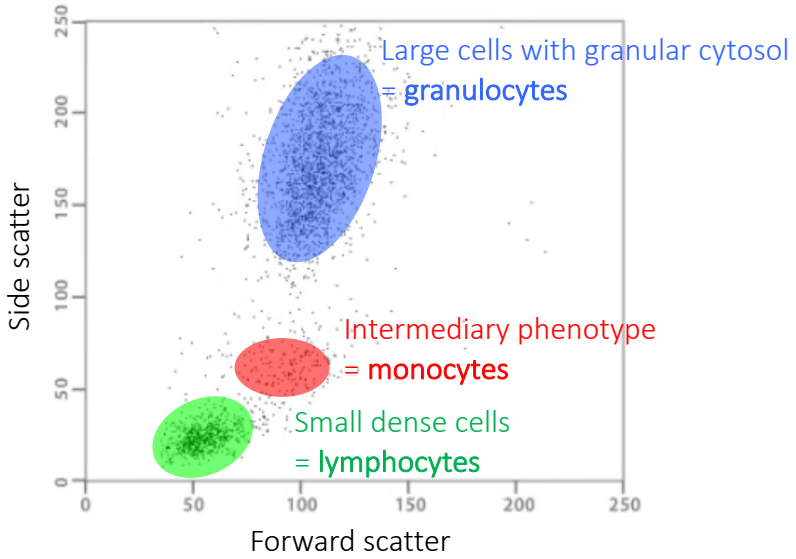
Flow cytometry with unstained cells

- A cell passing through the laser scatters the beam
  - **Forward scatter (FSC)** depends on cell size
  - **Side scatter (SSC)** depends on cell granularity
- Each event is counted and plotted on a **scatter histogram**
  - Histograms show single-dimensional data of a cell population
  - **Scatter plots show multi-dimensional data**



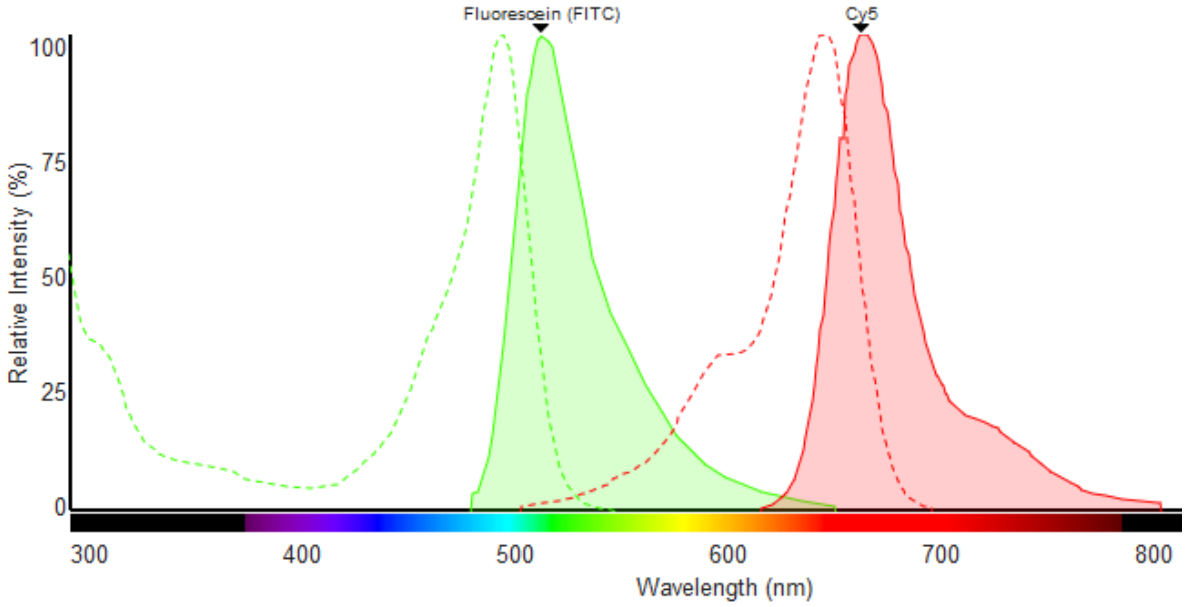


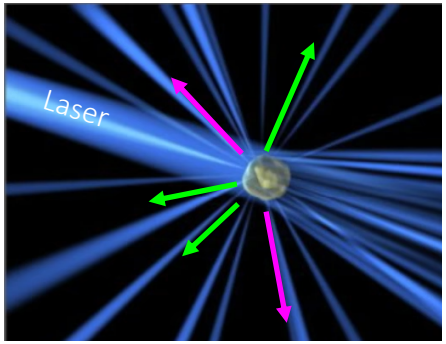
Whole blood scatter plot



Flow cytometry with fluorescence markers

- Cells can be labelled with **fluorophore-coupled antibodies**
- Fluorophores are chosen so as to have **distinct emission spectra** (e.g. ‘green’ and ‘red’ like FITC and Cy5)
- A **cell expressing** the corresponding **antigen(s)** will bind fluorophore-antibody conjugates and **emit fluorescence** upon passing through the laser beam.



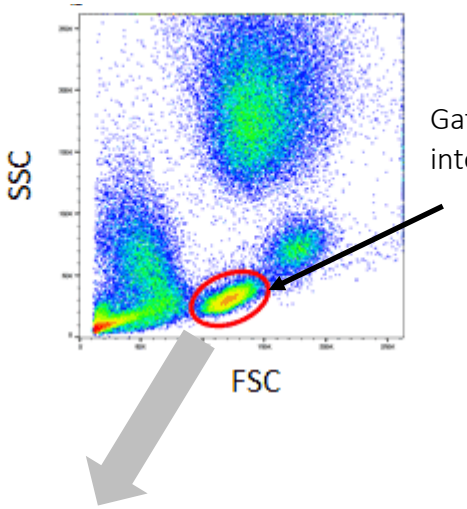


Example: quantifying T cell populations from thymus

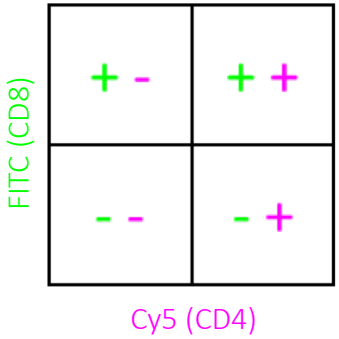
Label cells with antibodies → Quantify cell-associated fluorescence → Plot cell counts

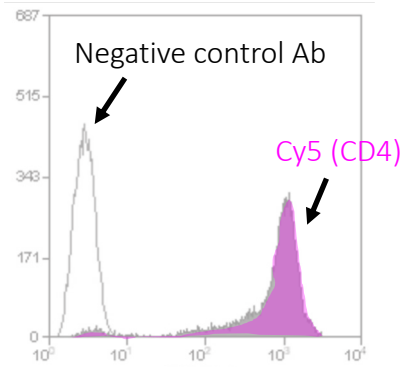
FITC-coupled anti-CD8 antibody

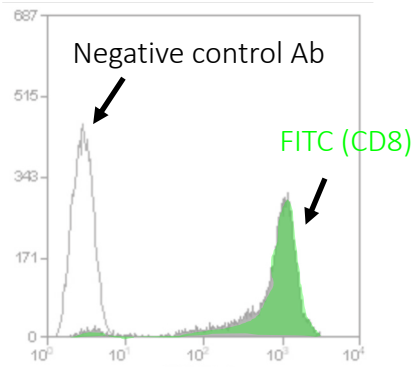
Cy5-coupled anti-CD4 antibody

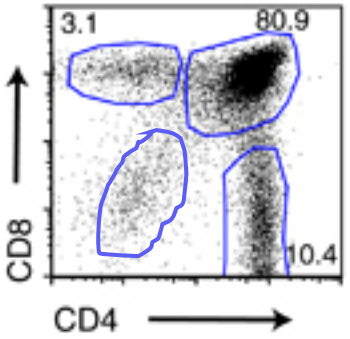


Gate on population of interest (T cells)

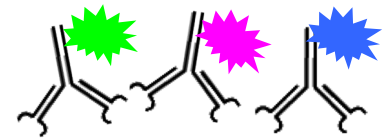




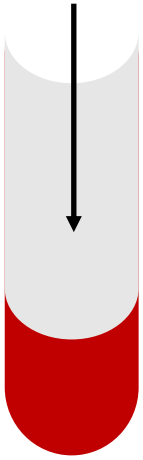




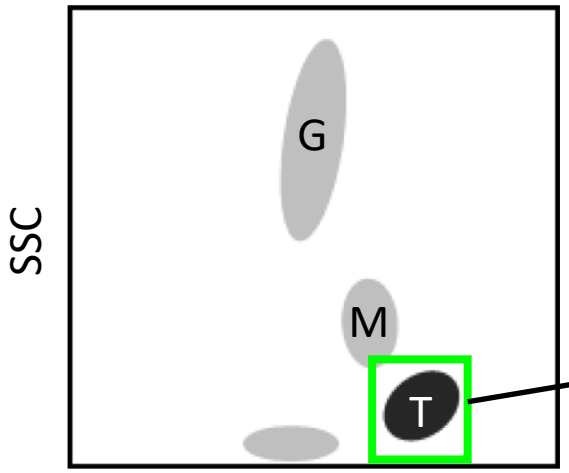
Example: CD4+ T cell counts in HIV infection



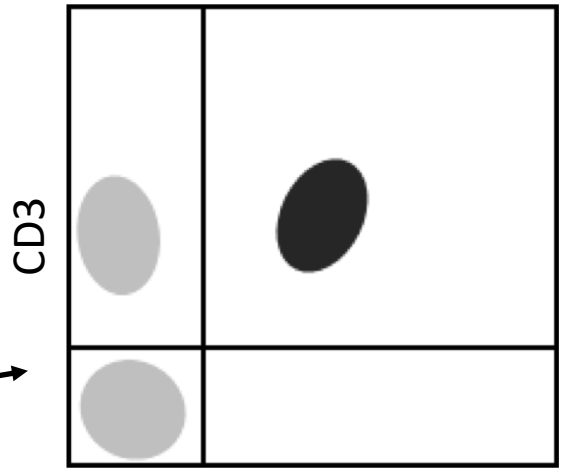
Anti-CD45/CD3/CD4
antibody panel



Whole blood



'gate' on lymphocytes



CD4+ T cells

FACS (**F**luorescence-**A**ctivated **C**ell **S**orting)

- As cells pass through the laser beam, their characteristics are analysed
- Cells are given a charge based on their fluorescence profile
- Charged cells are deviated between deflector plates
- FACS yields highly purified cell populations
- Applications:
  - purify cells expressing a GFP-tagged protein
  - purify subpopulations of lymphocytes from blood
  - purify bone marrow stem cells for transplantation
  - …