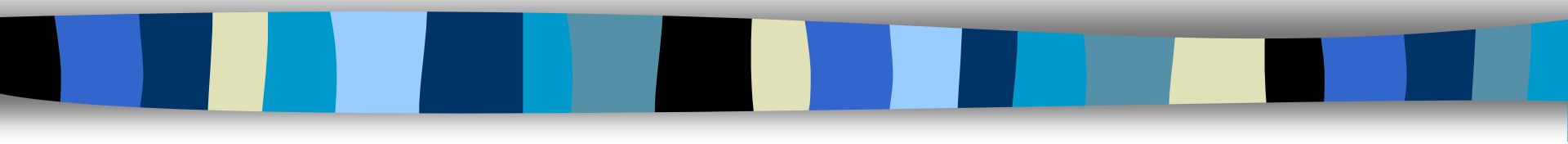
Bi9393e Analytical cytometry



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Cytometry

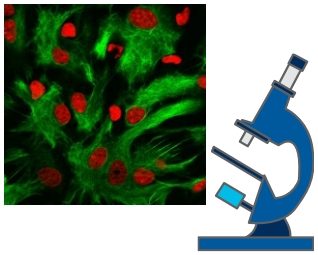
- Cytometry is the collective name for a group of methods used to measure various characteristics of cells. Variables that can be measured by cytometric methods include cell size, cell number, cell morphology (shape and structure), cell cycle phases, DNA content, and the presence or absence of specific proteins on the cell surface or in the cytoplasm. Cytometry is used to characterize and count blood cells in routine blood tests such as a complete blood count. Similarly, cytometry is also used in cell biology research and in medical diagnostics (for example, to detect cancer or AIDS).

There are different types of cytometry:

- Flow cytometry
- Spectral flow cytometry
- Hyperspectral cytometry
- Image cytometry
- Mass cytometry
- *In vivo* cytometry (non-invasive cytometry)

Ref. What is Cytometry?. International Society for Advancement of Cytometry. 2013-03-28

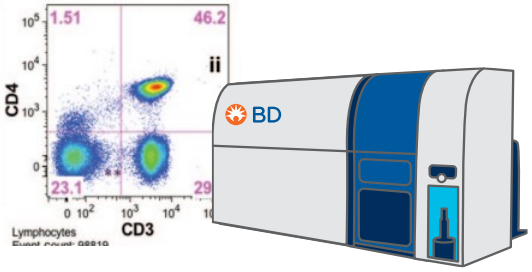
Two common ways to quantify the total number and type of cells in a sample



Microscopy

Provides details of cell morphology for tens or hundreds of cells. Can provide information about cellular interactions.

- Shape
- Distribution of components in the cell



Flow Cytometry

Quantifies a high number parameters of cells in suspension for hundreds or thousands of cells per second and is capable of sorting living cells.

- Size
- Granularity
- Surface and intracellular components

Article

The NK cell receptor NKp46 recognizes ecto-calreticulin on ER-stressed cells

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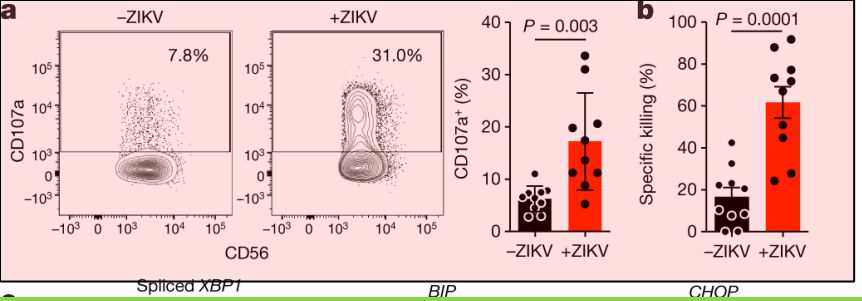
Check for updates

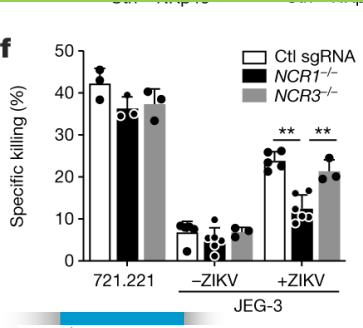
Sumit Sen Santara[1,2,3,9], Dian-Jang Lee[1,2,9], Ângela Crespo[1,2], Jun Jacob Hu[1,4], Caitlin Walker[1,4], Xiyu Ma[1,2], Ying Zhang[1,2], Sourav Chowdhury[5], Karla F. Meza-Sosa[1,2,6], Mercedes Lewandrowski[1,2], Haiwei Zhang[1,2], Marjorie Rowe[1,2], Arthur McClelland[7], Hao Wu[1,4], Caroline Junqueira[1,2,8]✉ & Judy Lieberman[1,2]✉

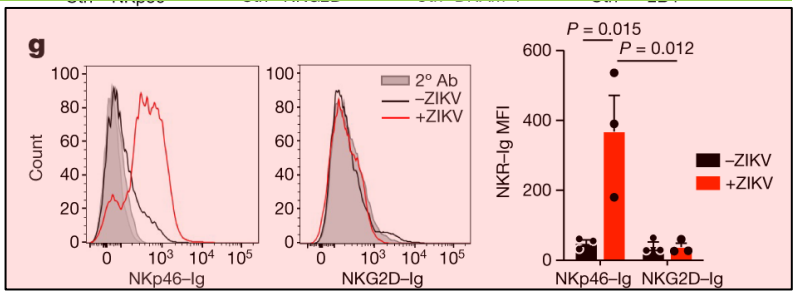
Natural killer (NK) cells kill infected, transformed and stressed cells when an activating NK cell receptor is triggered[1]. Most NK cells and some innate lymphoid cells express the activating receptor NKp46, encoded by *NCR1*, the most evolutionarily ancient NK cell receptor[2,3]. Blockage of NKp46 inhibits NK killing of many cancer targets[4]. Although a few infectious NKp46 ligands have been identified, the endogenous NKp46 cell surface ligand is unknown. Here we show that NKp46 recognizes externalized calreticulin (ecto-CRT), which translocates from the endoplasmic reticulum (ER) to the cell membrane during ER stress. ER stress and ecto-CRT are hallmarks of chemotherapy-induced immunogenic cell death[5,6], flavivirus infection and senescence. NKp46 recognition of the P domain of ecto-CRT triggers NK cell signalling and NKp46 caps with ecto-CRT in NK immune synapses. NKp46-mediated killing is inhibited by knockout or knockdown of *CALR*, the gene encoding CRT, or CRT antibodies, and is enhanced by ectopic expression of glycosylphosphatidylinositol-anchored CRT. *NCR1*-deficient human (and *Ncr1*-deficient mouse) NK cells are impaired in the killing of ZIKV-infected, ER-stressed and senescent cells and ecto-CRT-expressing cancer cells. Importantly, NKp46 recognition of ecto-CRT controls mouse B16 melanoma and RAS-driven lung cancers and enhances tumour-infiltrating NK cell degranulation and cytokine secretion. Thus, NKp46 recognition of ecto-CRT as a danger-associated molecular pattern eliminates ER-stressed cells.

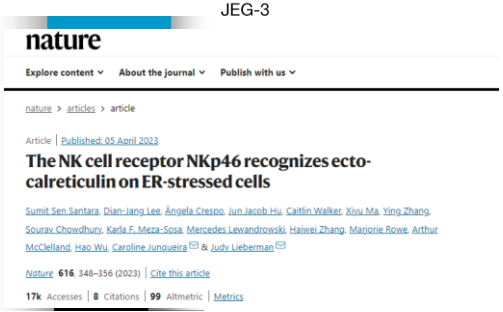


-CD56 is a single transmembrane glycoprotein also known as N-CAM (Neural Cell Adhesion Molecule), Leu-19, or NKH1. It is a member of the Ig superfamily. The 140 kD isoform is expressed on NK cells and NK-T cells. CD56 is also expressed in the brain (cerebellum and cortex) and at neuromuscular junctions.

-lysosomal-associated membrane protein-1 (LAMP-1 or CD107a) has been described as a marker of CD8+ T-cell degranulation following stimulation.



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Article | Published: 05 April 2023

The NK cell receptor NKp46 recognizes ecto-calreticulin on ER-stressed cells

Sumit Sen Santara, Dian-Jang Lee, Ángela Crespo, Jun Jacob Hu, Caitlin Walker, Xiyu Ma, Ying Zhang, Sourav Chowdhury, Karla F. Meza-Sosa, Mercedes Lewandrowski, Haiwei Zhang, Marjorie Rowe, Arthur McClelland, Hao Wu, Caroline Jungueira & Judy Lieberman

Nature **616**, 348–356 (2023) | Cite this article

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a, Representative flow cytometry plots (left) and percentage of degranulating NK cells isolated from the blood of ten healthy donors (right), as measured by surface CD107a, in response to uninfected and ZIKV-infected JEG-3 cells (8 h coculture, E:T ratio 1:3). **b**, NK cell-specific killing of uninfected and ZIKV-infected JEG-3 cells. **c**, ER stress, as assessed by *XBP1* splicing (left) and increases in *BIP* (middle) and *CHOP* (right) mRNA, in JEG-3 cells that were uninfected or infected with ZIKV, HSV-2 or human cytomegalovirus (HCMV) for 1–2 days or treated with tunicamycin (Tu) for 1 day. Indicated samples were pretreated with the ER stress inhibitor salubrinal ($n = 3$ samples). mRNA levels, as assayed by quantitative PCR with reverse transcription (RT–qPCR), were normalized to *ACTB*. **d**, Effect of salubrinal pretreatment of target cells on NK cell killing of ZIKV-infected (top) and tunicamycin-treated (bottom) JEG-3 cells ($n = 6$ samples). **e**, Effect of NKR-blocking antibodies (Ab) on NK cell killing of uninfected or ZIKV-infected JEG-3 cells ($n = 3$–7 samples). Ctrl, control. **f**, Specific killing of the classical NK cell target 722.221 cells, or of uninfected or ZIKV-infected JEG-3 cells by human NK cell line YT cells knocked out for *NCR1* or *NCR3* or treated with control single-guide RNAs ($n = 3$–6 samples). **g**, Representative flow cytometry histogram (left) and mean fluorescence intensity (MFI) of NKR–Ig fusion protein (NKp46–Ig and NKG2D–Ig) binding to uninfected or ZIKV-infected JEG-3 cells (right) ($n = 3$ samples). **b**,**d**–**f**, Specific killing assessed by 8 h ^{51}Cr release assay using an E:T ratio of 10:1 unless otherwise indicated. Data are mean ± s.e.m. of at least three independent experiments or technical replicates. Statistics were performed using two-tailed, nonparametric, unpaired *t*-test (**a**,**b**), one-way analysis of variance (ANOVA) (**c**), two-way ANOVA (**e**–**g**) or area under the curve followed by one-way ANOVA (**d**). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.


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The NK cell receptor NKp46 recognizes ecto-calreticulin on ER-stressed cells

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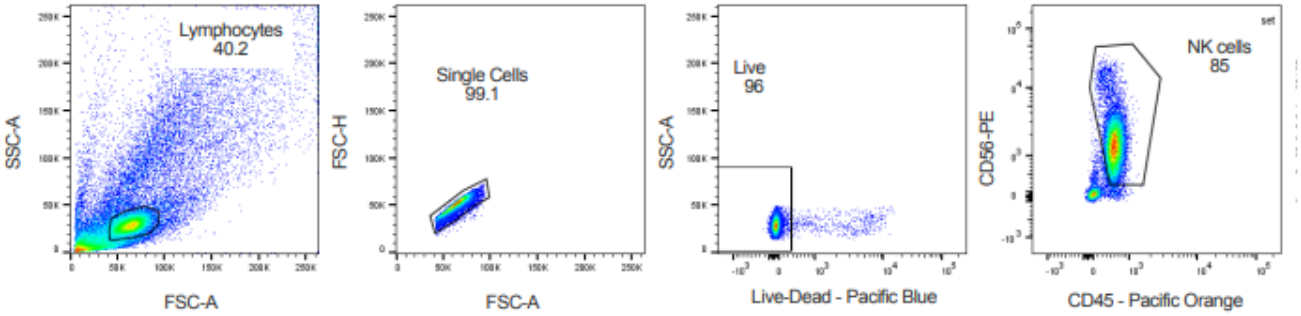
Flow cytometry

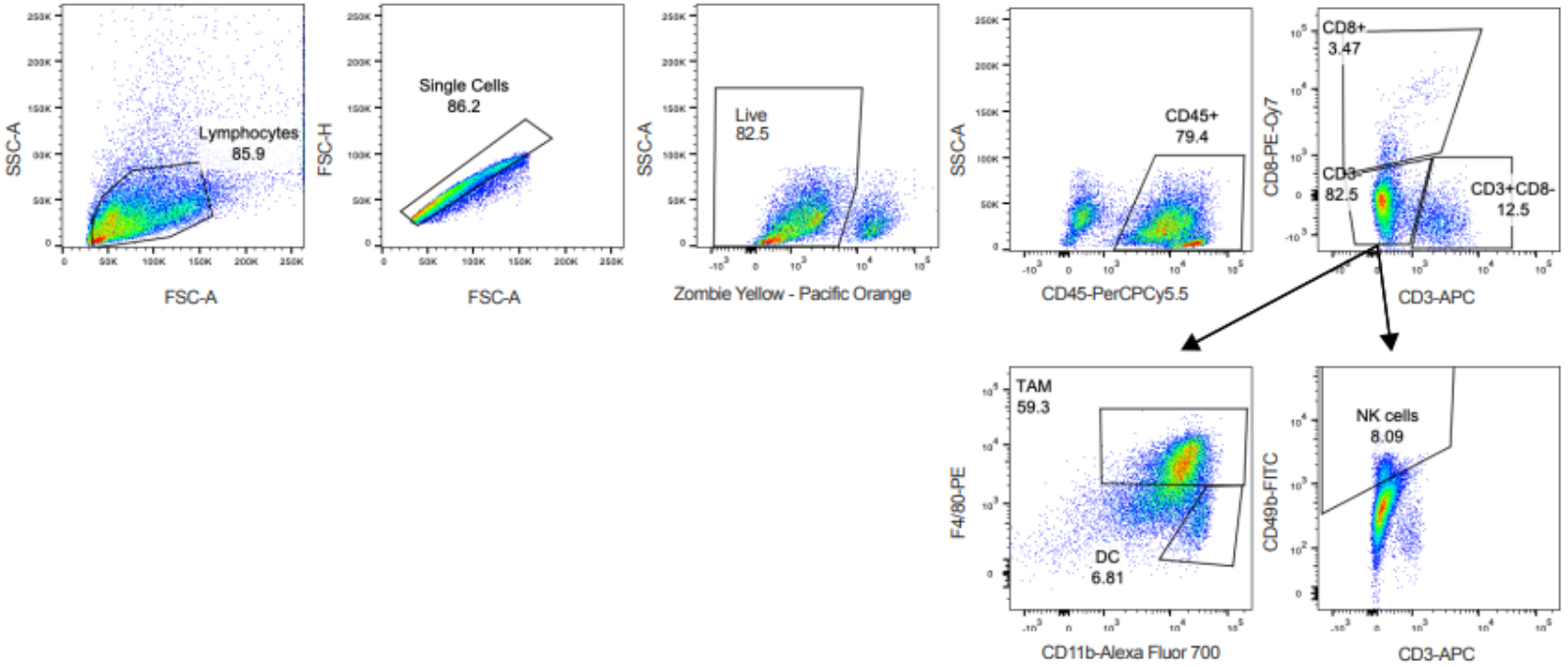
For surface staining, cells were stained for 30 min on ice in the dark with LIVE/DEAD-Violet stain (1:1,000) and then with primary antibodies for 15–30 min in PBS and 2% FCS (followed by secondary antibodies, when applicable, for 20 min). For protein–Ig staining, cells were incubated with 50 μg ml^{-1} fusion protein for 1 h at 4 °C and then stained with fluorescent-anti-human IgG for 1 h. Cells were fixed in 1% paraformaldehyde (Affymetrix) for 10 min before flow cytometry. Flow cytometry was assessed on gated live cells (Supplementary Fig. 1). For intracellular staining, cells were fixed and permeabilized using the CytoFix/CytoPerm kit. One of the treated samples was used for isotype staining, and MFI of staining with the isotype control antibody was subtracted from MFI of the specific antibody. Analysis was performed on a FACSCanto II (BD). BD FACSDiva 8.0 (BD) software was used for data collection, with analysis performed using FlowJo v.10.4.2 (TreeStar).

Supplementary Figure 1 | Flow cytometry gating strategy

a. Peripheral blood NK or YT NK cultured with JEG-3.



b. Tumor infiltrating lymphocytes (TILs) from tumor-bearing mice



Course structure

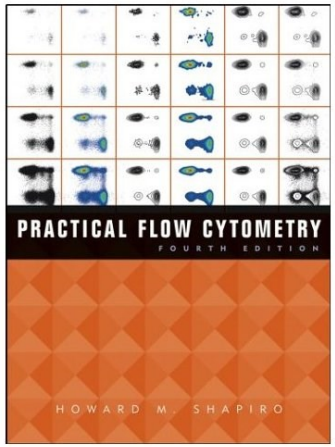
- Lectures
  - 8 lectures on flow cytometry and applications
  - 2 lectures on microscopy techniques and on the basics of image analysis
  - 2 lectures of student presentations
- Bi9393ec Analytical Cytometry- practical labs, 3 days in a block
  - Follows lectures on flow cytometry, in block groups
- Test
  - The course will conclude with an exam in the form of a test summarizing the material covered throughout the semester. The result of the test will constitute 75% of the overall grade.
  - Seminar
    - Each student will present a short seminar whose topic will be consulted with the lecturer and will be related to the focus of the course. Credit will be awarded on the basis of this presentation, and the seminar‘s grade will also be reflected in 25% of the overall grade.

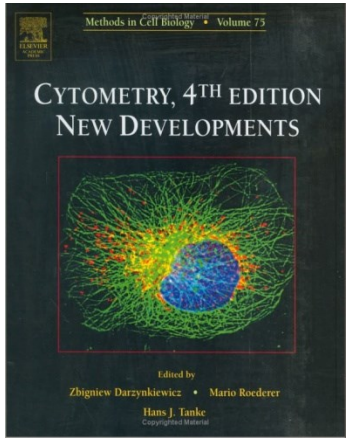
Seminar of students

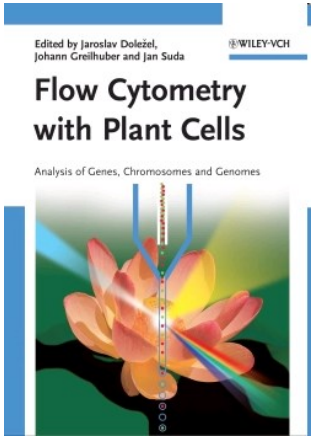
- Seminar topic: How I use/want to use/could use analytical cytometry methods in my DP/DSP.
- The aim is to demonstrate an understanding of the underlying principles and their application in biology.
- The presentation must be prepared e.g. in PowerPoint. It is recommended to submit the presentation in advance to the lecturer for consultation and review.
- The length of the presentation is 5 minutes (~2 min introduction of the essence of your experimental work) + discussion

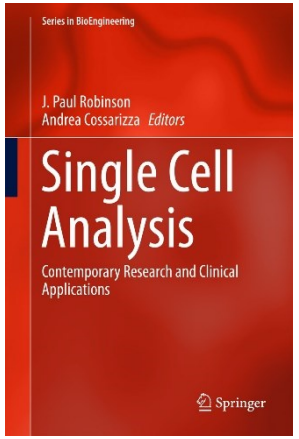
Information resources - flow cytometry

- Practical Flow Cytometry, Howard M. Shapiro, Wiley-Liss; 4th edition
- Cytometry: New Developments, Volume 75, Fourth Edition (Methods in Cell Biology), Zbigniew Darzynkiewicz, Academic Press; 4th edition
- Flow Cytometry with Plant Cells: Analysis of Genes, Chromosomes and Genomes; Jaroslav Dolezel (Editor), Johann Greilhuber (Editor), Jan Suda (Editor), February 2007
- Single Cell Analysis, J. Paul Robinson, Andrea Cossarizza, 2017



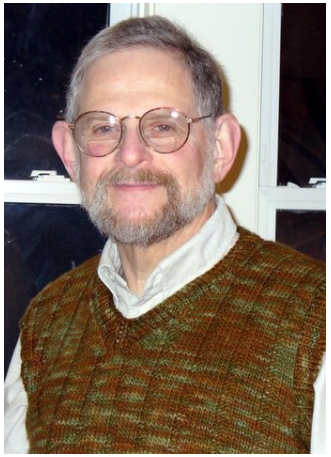






For more information on flow cytometry books, please visit:
http://www.cyto.purdue.edu/flowcyt/newbook.htm

https://www.beckman.com/resources/reading-material/ebooks/practical-flow-cytometry



Howard Shapiro

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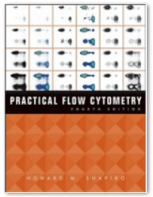

Advancing Flow Cytometry

Beckman Coulter is proud to make available Howard M. Shapiro, M.D., P.C.'s Practical Flow Cytometry, 4th Ed. This is the go to reference for anyone interested in developing expertise in flow cytometry applications. As part of our ongoing support for the advancement of flow cytometry applications we are proud to sponsor free digital downloads. Complete the form to activate your eBook and sign up for additional alerts related to flow cytometry innovations or educational content.

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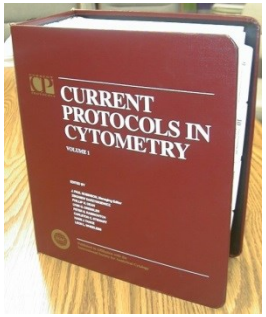
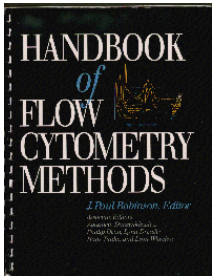
Information resources - flow cytometry (methods and protocols)

- The Handbook of Flow Cytometry Methods
- Current Protocols in Cytometry
- Company web pages, e.g.
  - https://www.thermofisher.com/cz/en/home/references/protocols.html
  - https://www.thermofisher.com/cz/en/home/references/protocols/cell-and-tissue-analysis/flow-cytometry-protocol.html
  - https://www.cellsignal.com/learn-and-support/protocols?_requestid=3668092





Information sources - cytometry (journals)

- Cytometry Part A

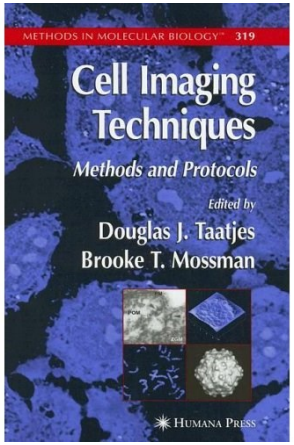
https://onlinelibrary.wiley.com/journal/15524930

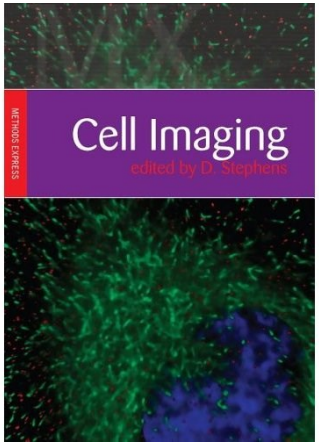
- Cytometry Part B: Clinical Cytometry

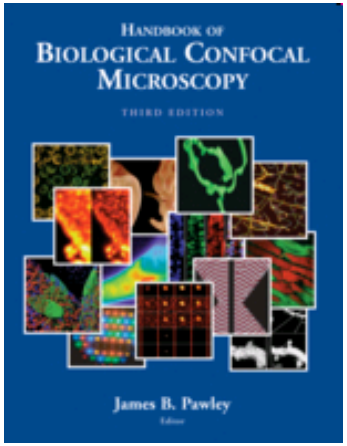
https://onlinelibrary.wiley.com/journal/15524957

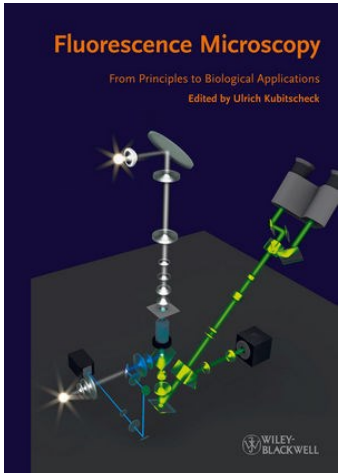
Information resources - microscopy

- Taatjes D. J. Cell Imaging Techniques, Methods and Protocols, Humana Press, Totowa, New Jersey, 2005
- Stephens D. Cell Imaging, Scion Publishing Ltd., 2006.
- Pawley, J. (Ed.), Handbook of Biological Confocal Microscopy, 3rd ed., 2006
- Fluorescence Microscopy: From Principles to Biological Applications
- Ulrich Kubitscheck (Editor), ISBN: 978-3-527-32922-9, 2013, Wiley-Blackwell









Information resources - (Internet)

- Purdue University, Cytometry Labs

http://www.cyto.purdue.edu/

- International Society for Advancement of Cytometry

http://www.isac-net.org/

- Excyte

https://expertcytometry.com

- https://x.com/ISAC_CYTO
- https://x.com/flowcytometryUK
- https://x.com/FlowJoNow
- https://x.com/CSAC_CZ

ČSAC

Česká společnost pro analytickou cytometrii, z. s.

www.csac.cz

ČSAC je malou organizací a žije jen aktivitou svých členů.

Co můžete udělat Vy pro ČSAC:

- svým členstvím podpořit aktivity ČSAC
- aktivně nabídnout spolupráci na tématech, jež se Vás týkají
- zorganizovat seminář na téma, které Vám chybí
- pomoci s obsahem webových stránek (doplnit odkazy, přeložit do angličtiny)

Proč být členem ČSAC?

ČSAC pro své členy:

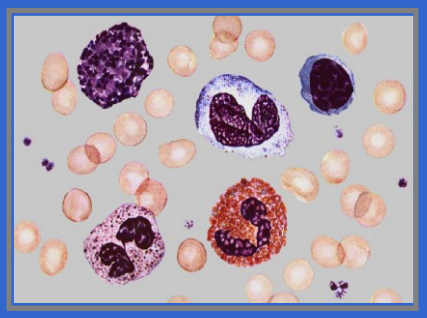
- organizuje konferenci Analytická cytometrie každé dva roky (s účastí vybraných zahraničních řečníků ze všech oblastí cytometrie)
- pořádá vzdělávací akce (např. Motolský Minikurz, B-klub a další)
- podporuje Vámi organizované cytometrické semináře (finančně, organizačně, odborně)
- uděluje ceny v soutěži o nejlepší publikaci s cytometrickou tématikou (cílem je zviditelnit zajímavé práce, poskytnout uznání kvalitním pracím)
- poskytuje cestovní granty ČSAC pro mladé členy na cytometrické akce
- informuje o aktivitách ISAC a ESCCA
- umožňuje kontakt s podobně zaměřenými kolegy a neformální výměnu zkušeností
- podporuje rozvoj cytometrie
- zprostředkovává výměnu zkušeností mezi členy a světovou cytometrickou komunitou

- https://www.csac.cz

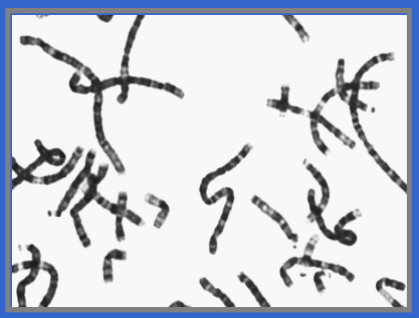
General introduction to flow cytometry

- Basic principles, possibilities of flow cytometry and its application
- History

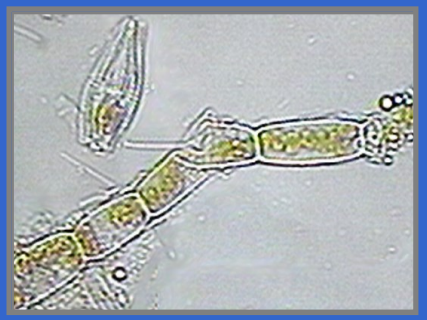
These particles have something in common...



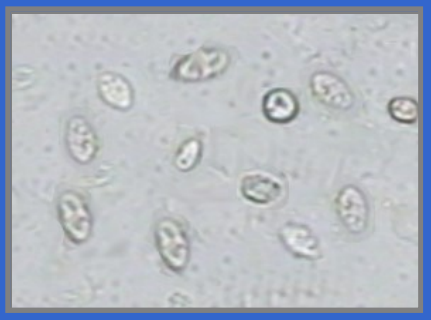
Blood cells



Chromosomes



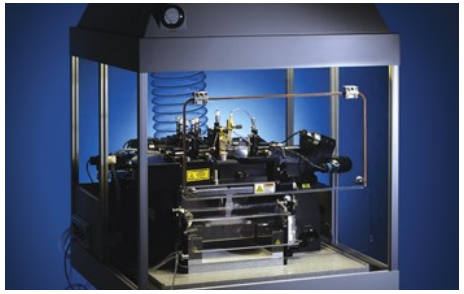
Algae

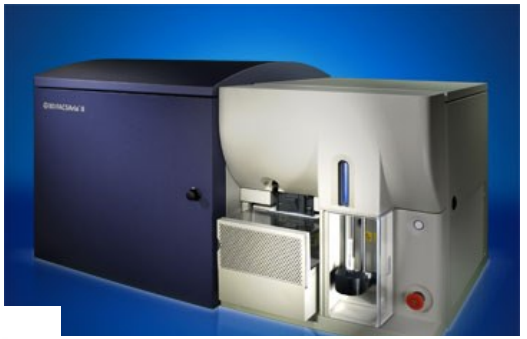


Protozoa

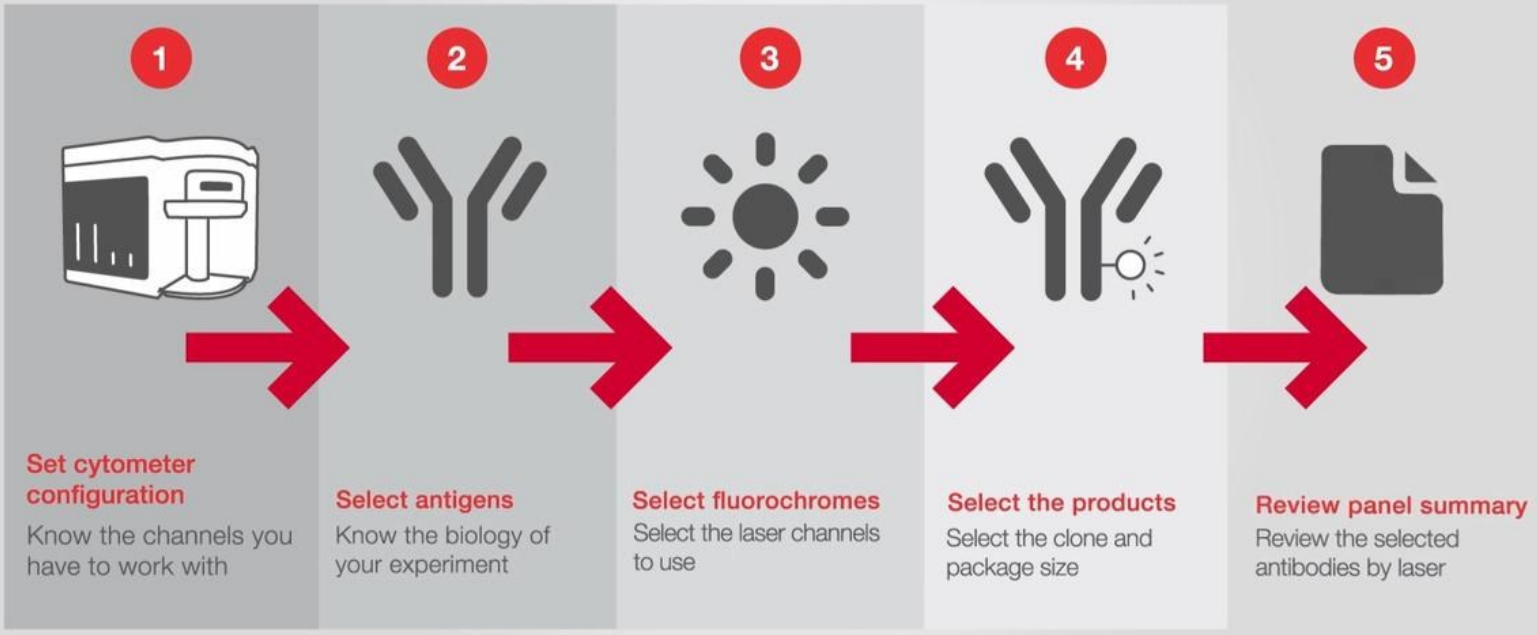
... certain parameters of these particles can be measured by flow cytometry.

Commercial equipment and development

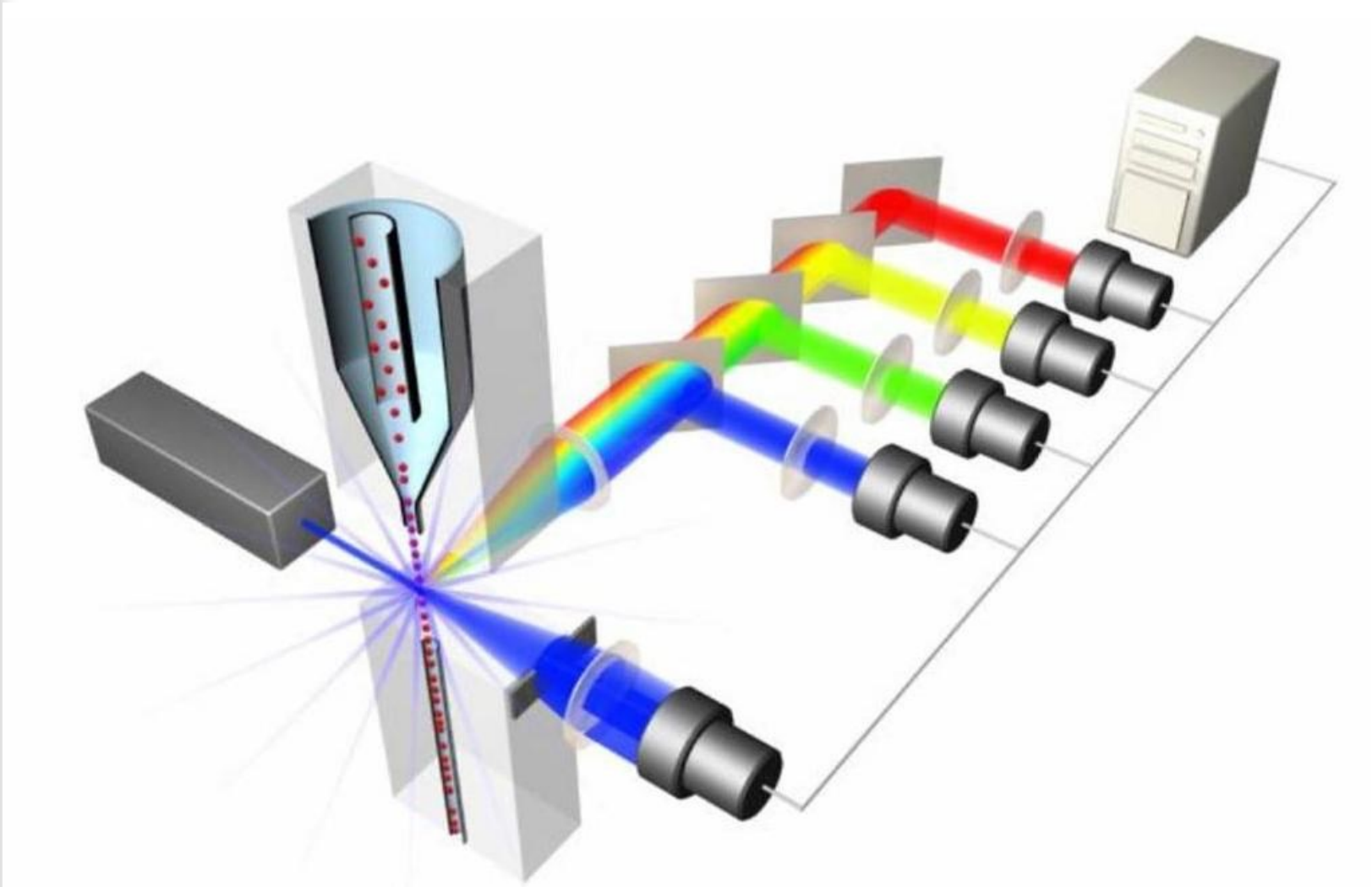




5 Steps to your flow cytometry panel



What is a flow cytometer?



What can we analyse with flow cytometry?

- Count particles in suspension
- Quantify light scattering, and **fluorescence intensity at the single cell level**
- Evaluate 10*5 to 10*6 particles in less than 1 minute
- Physically separate individual particles (defined populations and single cells) for further analysis

What are the principles?

- Light scattering (Light scatter) using a laser or UV lamp
- Specific fluorescence or full spectrum detection
- Hydrodynamically focused particle stream
- Electrostatic separation of particles
- Multivariate data analysis capability

Definition of

- **Flow cytometry**
  - Measuring the properties of flowing particles (cells)
  - also known as **Fluorescence-Activated Cell Sorting (FACS)**
- **Flow sorting**
  - physical separation of particles (cells) based on parameters measured by flow cytometry

Technical components

- Light sources
- Detection systems
- Fluid system
- Separation
- Data collection
- Data analysis

Technical components

- Detection systems

  Photomultiplier Tubes (PMTs)

  previously 1-2

  now > 8

  Diodes previously light scatters now also detect fluorescence

- Light sources

  Lasers (350-363, 420, 457, 488, 514, 532, 600, 633 nm)

  Argon ion, Krypton ion, HeNe, HeCd, Yag

  UV (Arc) Lamps

  Mercury, Mercury-Xenon

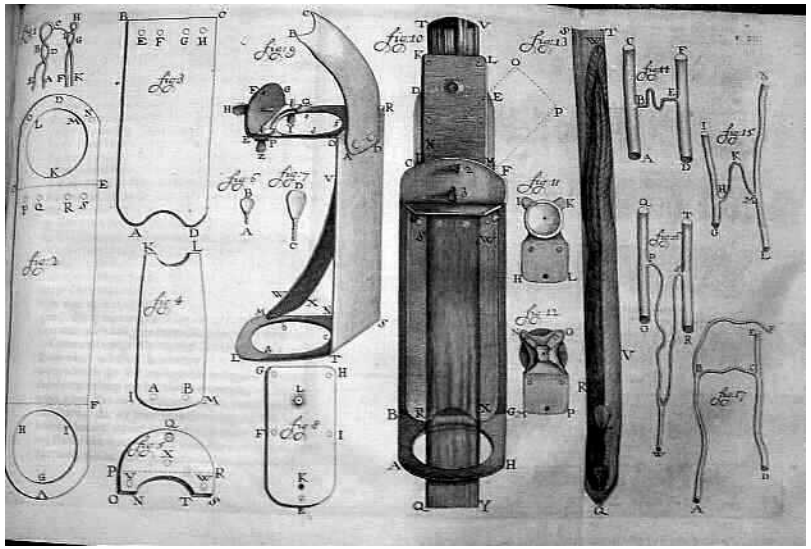
The non-technical part is equally important...

- (in)specific brands/sondas
- Antibodies
- biomarkers
- preparation, processing of material/samples/tissues
- ...

History of staining of biological materials

Until the mid-19th century - *only natural dyes were used*

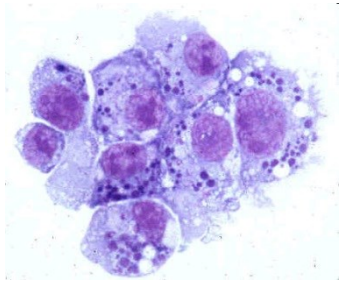
Anton van Leeuwenhoek used saffron to stain muscle cells in 1719



www.euronet.nl/users/warnar/leeuwenhoek.html

History of staining of biological materials

Paul Ehrlich - 1879 *used acidic and basic dyes to distinguish acidophilic, eosinophilic and neutrophilic leukocytes*



Clin Lab Med. 1993 Dec;13(4):759-71.

The Ehrlich-Chenzinsky-Plehn-Malachowski-Romanowsky-Nocht-Jenner-May-Grunwald-Leishman-Reuter-Wright-Giemsa-Lillie-Roe-Wilcox stain. The mystery unfolds.

Woronzoff-Dashkoff KP.

History of staining of biological materials

The Principle of the Fluorescence Microscope - August Köhler -

August Köhler
(1866-1948)

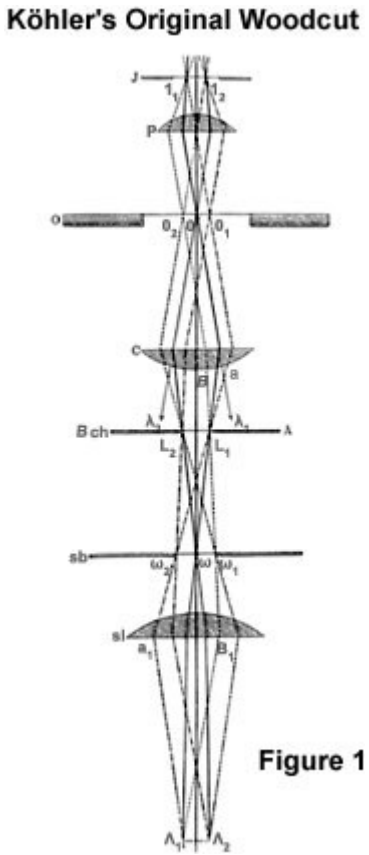
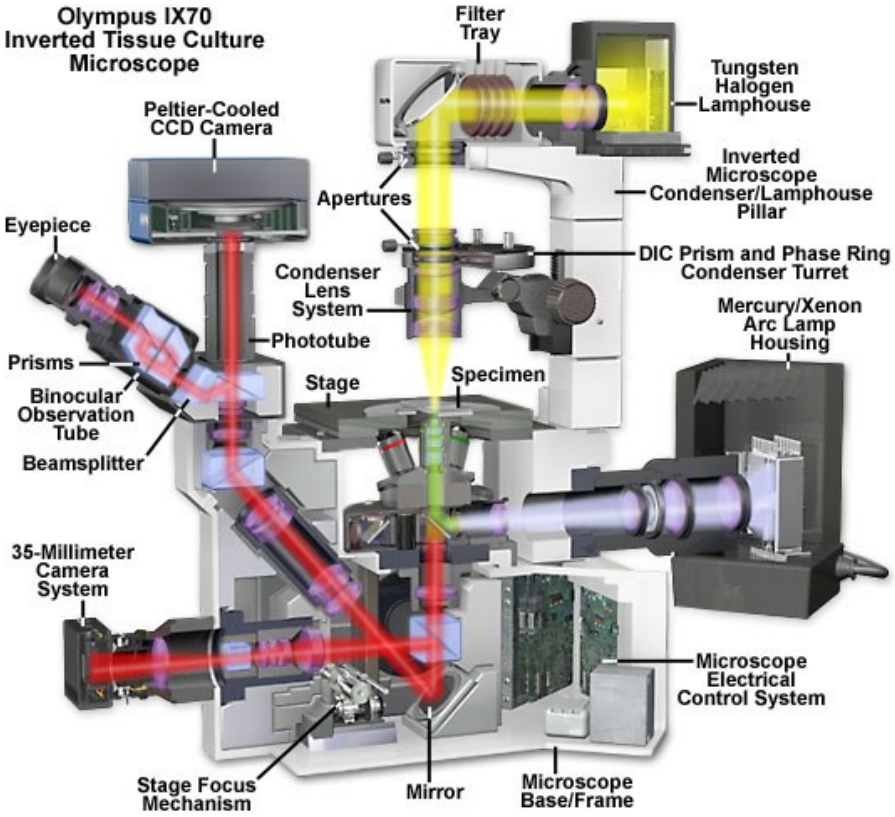


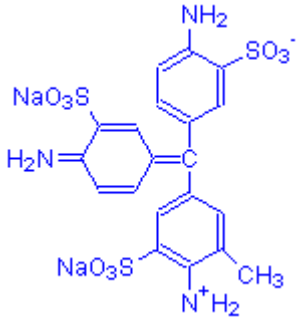
Figure 1



http://micro.magnet.fsu.edu/primer/anatomy/anatomy.html

Friedman

Friedman (1950) - combined acid fuchsin, acridine yellow and berberine for the detection of uterine tumour cells using a fluorescence microscope



Acid Fuchsin

Acid magenta
Acid ruby
Acid roseine

Absorption Max 540-545

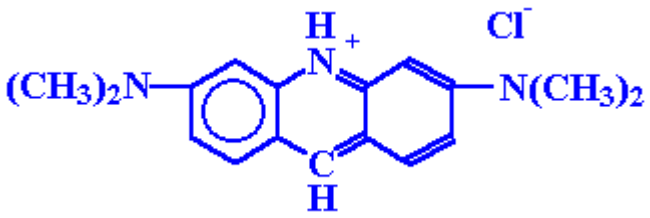
von Bertalanffy & Bickis

Ludwig von Bertalanffy (1901-1972)

von Bertalanffy & Bickis (1956)

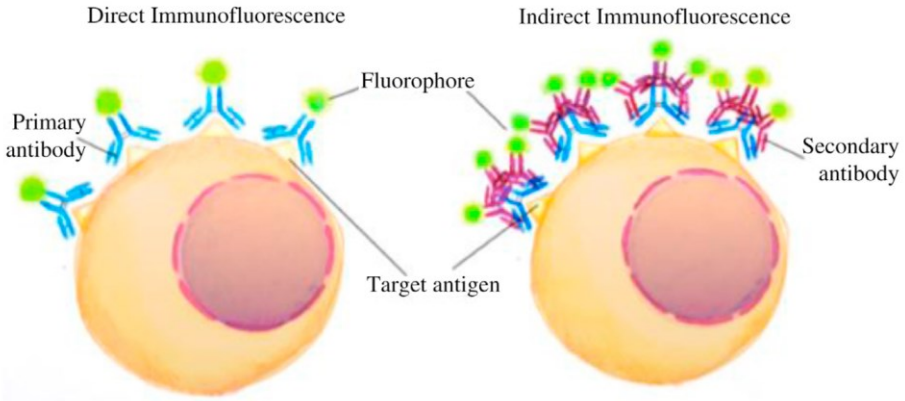
- Acridine orange metachromatic fluorescence was used to detect RNA in tissue

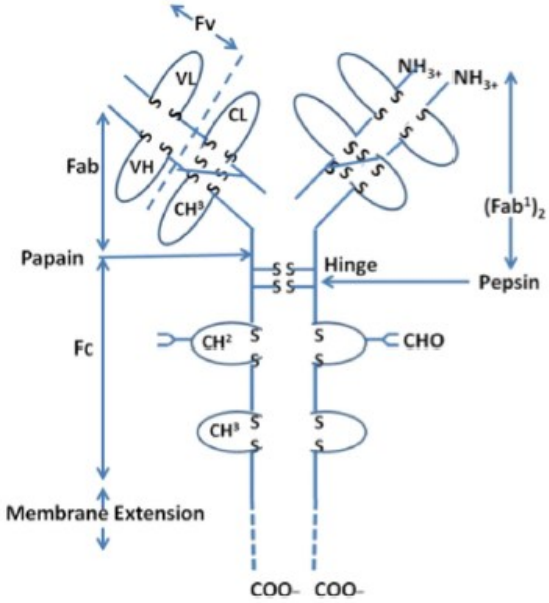
- they also used it to distinguish between normal and tumor cells



Absorption Max 467 nm

Immunodetection





History:

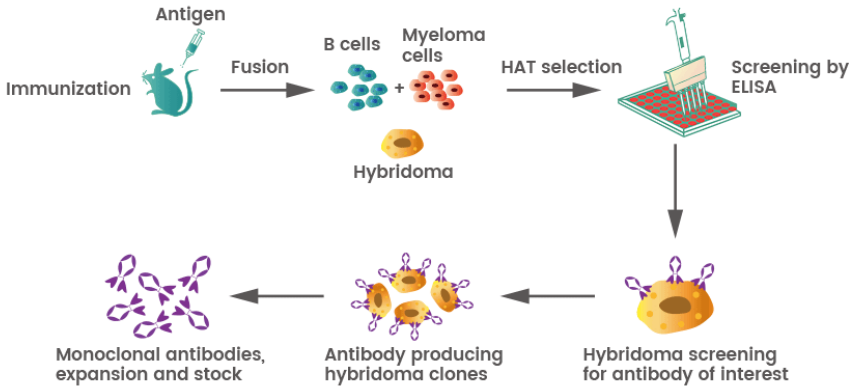
1940 - Conns, immunofluorescent labeling of cryosections

1959 - Singer, development of the method of conjugating antibodies with a marker

1966 - Graham&Karnovsky, the enzyme labelling method (HRP)

1974 - Taylor& Burns - routine immunohistochemistry

1975 - Kohler& Milstein - production of monoclonal antibodies using hybridoma



antibodypedia

Explore | Validate | Learn

Search h

Search for

Application

Any

Search

Advanced search

e.g. Her2, Transcription factors, Chromosome X

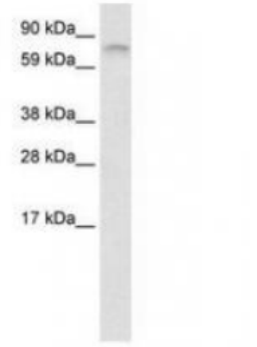
A portal for validated antibodies

Antibodypedia scores antibodies to guide researchers to choose an appropriate antibody for a particular application. The resource contains information about more than four million publicly available research antibodies towards over 19,000 human protein targets from more than 85 providers.

Use "Search for" to find validated antibodies against your target protein for a particular application! The antibodies are scored using the validation principles outlined by the International Working Group for Antibody Validation and we encourage feedback from researcher by submitting validation data for a particular antibody.

Content updated 2021-09-09
4480670 reviewed antibodies from 95 providers,
covering gene-products encoded by **19109** genes
(approximately **95%** of all human genes).
Primary data available for **2181179** experiments.

Featured Validations



LS-C433002
Fetal Brain Lysate. This image was taken for the unconjugated form of this product. Other forms have not been tested.
More info

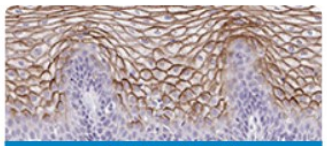
THE HUMAN PROTEIN ATLAS

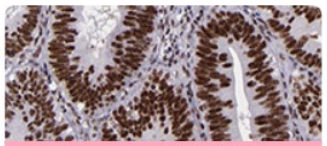
MENU HELP NEWS

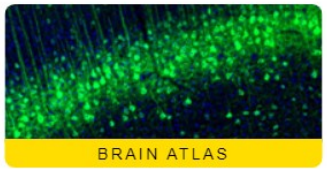
SEARCH[i]

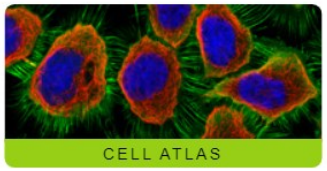
dsg1 Search Fields »

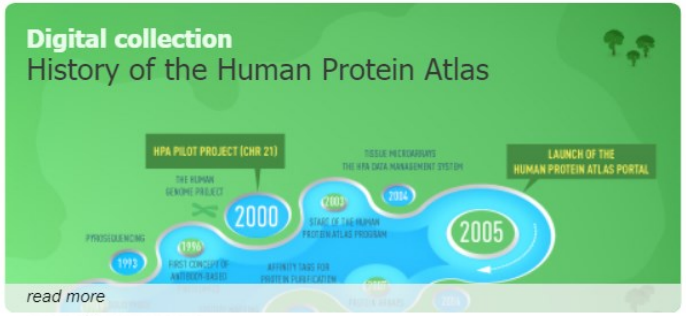
e.g. ACE2, GFAP, EGFR











Recent news

Wed, 28 Jul 2021
A single cell type map of human tissues

Thu, 22 Jul 2021
Movie of the month: The Fatty Liver

Tue, 15 Jun 2021
Movie of the month: The Spleen

all news articles

PRESS ROOM

contact@proteinatlas.org

INTRODUCTION
PUBLICATIONS
LICENCE & CITATION
DOWNLOADABLE DATA

Version: **20.1**
Atlas updated: 2021-02-24
release history

Proteome analysis based on **26941** antibodies targeting **17165** unique proteins

Knut and Alice Wallenberg Foundation
The Human Protein Atlas project is funded by the Knut & Alice Wallenberg foundation.
elixir Core Data Resource
SciLifeLab
UPPSALA UNIVERSITET
Karolinska Institutet
KTH

Stain Your Own Cell

ThermoFisher SCIENTIFIC

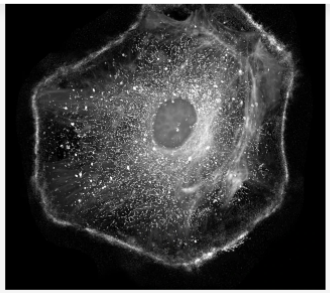
Home › Life Sciences › Lab Data Management & Analysis Software › Lab Apps › Cell Staining Tool

Cell Staining Tool

Stain your own cell

Stain your own cell using our cell staining tool, for reproducible results with many of our signature fluorescent dyes. Create your perfectly labeled fluorescent cell and share it with your colleagues using the email or print function. If you have any questions, simply click the help button to send an email to our technical support group.

sher FIC | Search All | Search by catalog number, product name, keyword, application

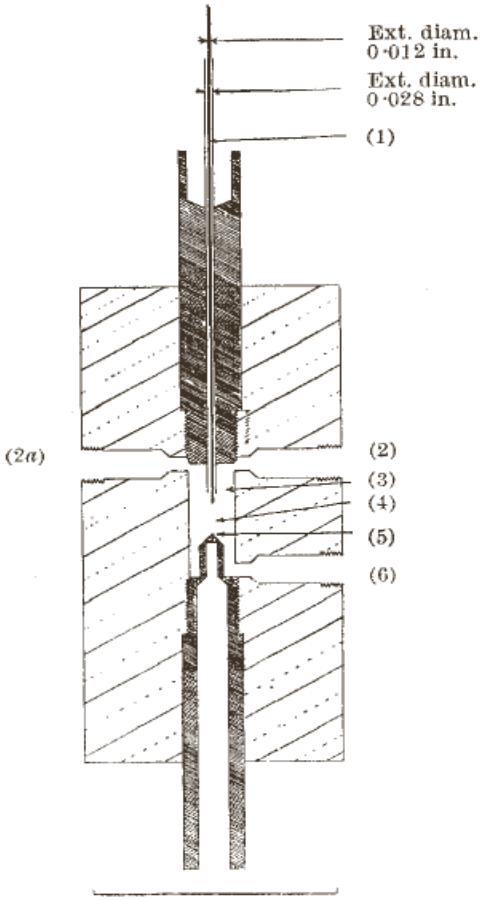


select a cell structure to get started

1 SELECT A STRUCTURE	2 SELECT A COLOR	3 SELECT A STAIN
Autophagosomes		PRODUCT LIVE FIXED
Cytoskeleton-Actin		
Cytoskeleton-Tubulin		
Cytoskeleton-Talin		
Endoplasmic Reticulum		
Endosomes		
Golgi		
Lysosomes		
Mitochondria		
Nucleus		
Peroxisomes		
Plasma Membrane		

P.J. Crossland-Taylor

"Sheath Flow" principle



(1) Needle in holder; (2) and (2a) inflow tubes; (3) wide-bore tube; (4) observation area for (3); (5) vortex; (6) flushing tube

"Provided there is no turbulence, the wide column of particles will then be accelerated to form a narrow column surrounded by fluid of the same refractive index which in turn is enclosed in a tube which will not interfere with observation of its axial content."

A Device for Counting Small Particles suspended in a Fluid through a Tube

ATTEMPTS to count small particles suspended in fluid flowing through a tube have not hitherto been very successful. With particles such as red blood cells the experimenter must choose between a wide tube which allows particles to pass two or more abreast across a particular section, or a narrow tube

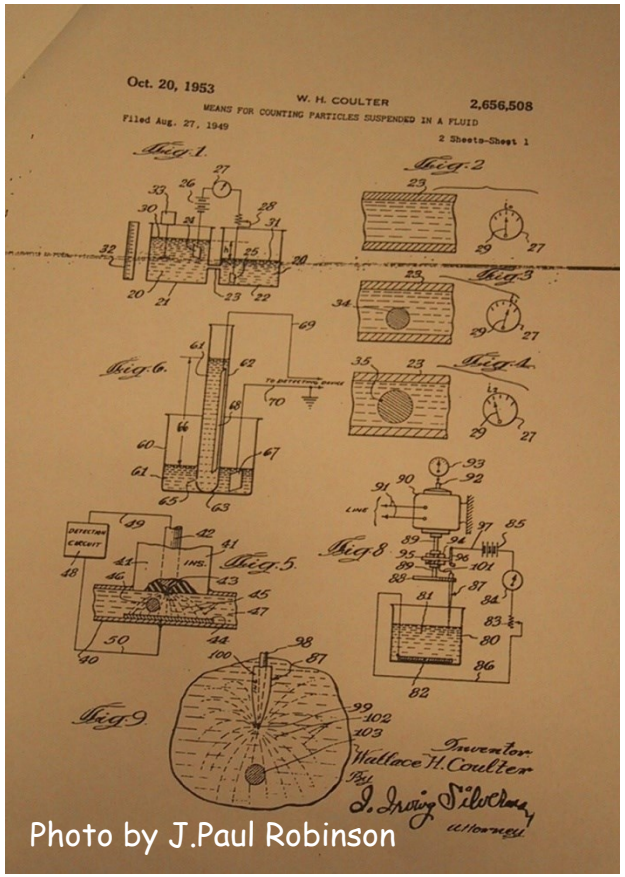
P. J. CROSLAND-TAYLOR*

Bland–Sutton Institute of Pathology,
Middlesex Hospital,
London, W.1.
June 17.

No. 4340 January 3, 1953 NATURE

Wallace Coulter

- **Wallace Coulter - Coulter orifice - 1956 -**
- (patent 1953) - measurement of conductivity change during passage of cells in suspension through a small hole

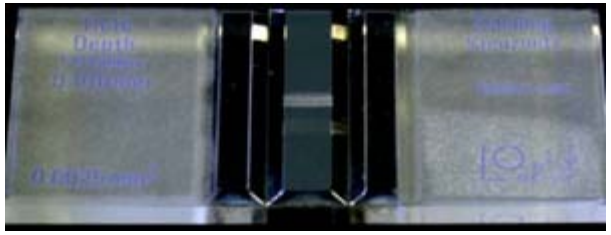


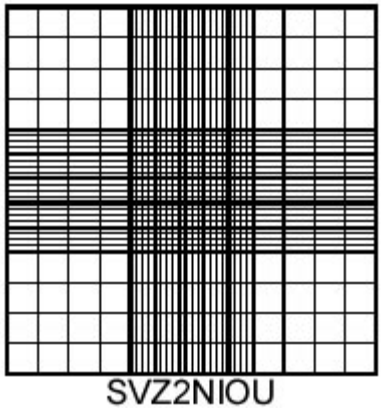
Original patent application of W. Coulter 1953

Photo by J.Paul Robinson

How to count cells?

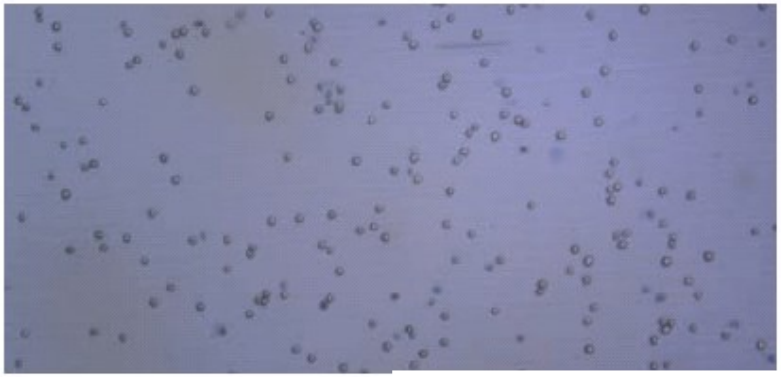
- Hemocytometer (Bürker chamber) was the standard for cell counting until ~1950
- Dimensions are 3x3x0.1 mm. Usually red blood cells (1×10^6 /mm^3) are counted after dilution 1:200
- Leukocytes (5×10^3 /mm^3) are diluted 1:10 in red blood cell lysing solution
- Statistical error:
  - coefficient of variance (CV) is 4.4% with 500 cells counted
  - pipetting and dilution error is ~10%



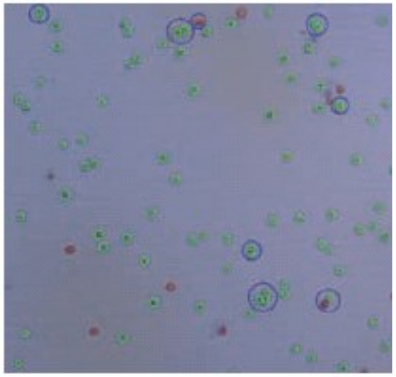


Adapted from J.P. Robinson Purdue University BMS 631

Roche Innovatis Cedex

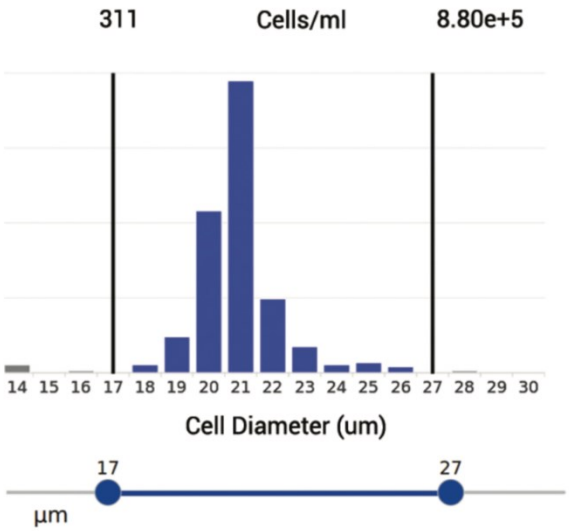


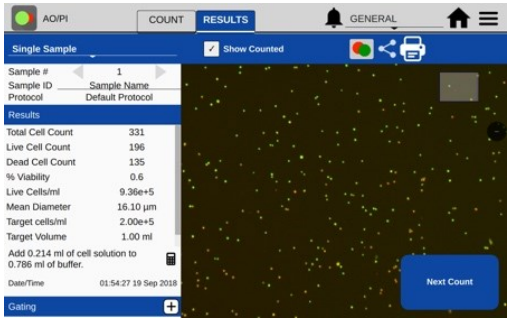
High Resolution Color Image

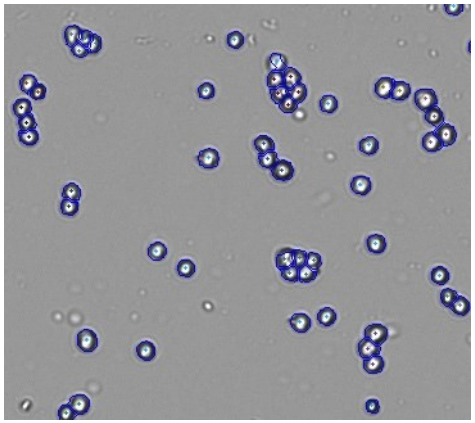


Visual Labeling

CellDrop - Denovix



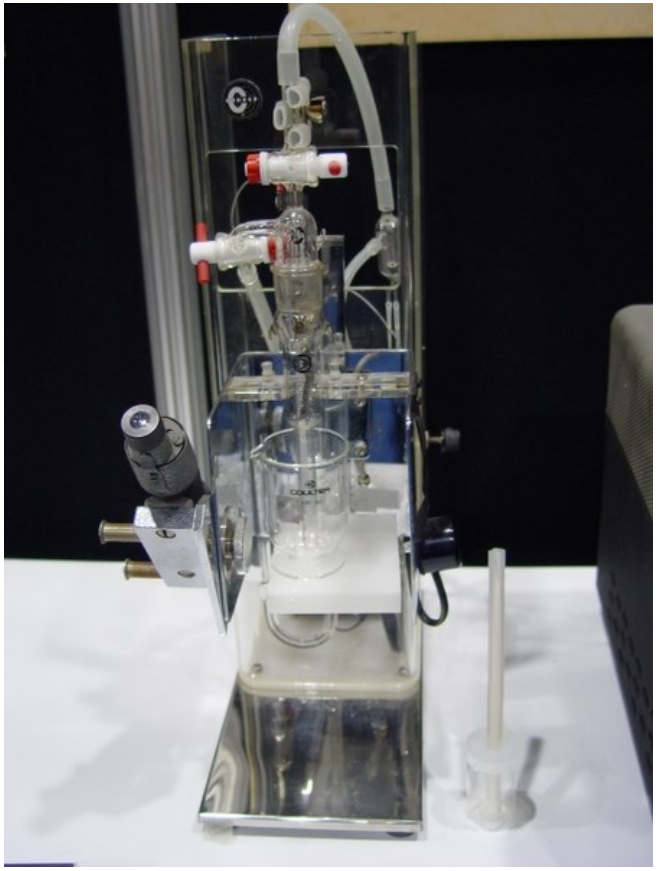




https://www.denovix.com/celldrop/

Coulter Counter

First commercial version of CC



Coulter Counter

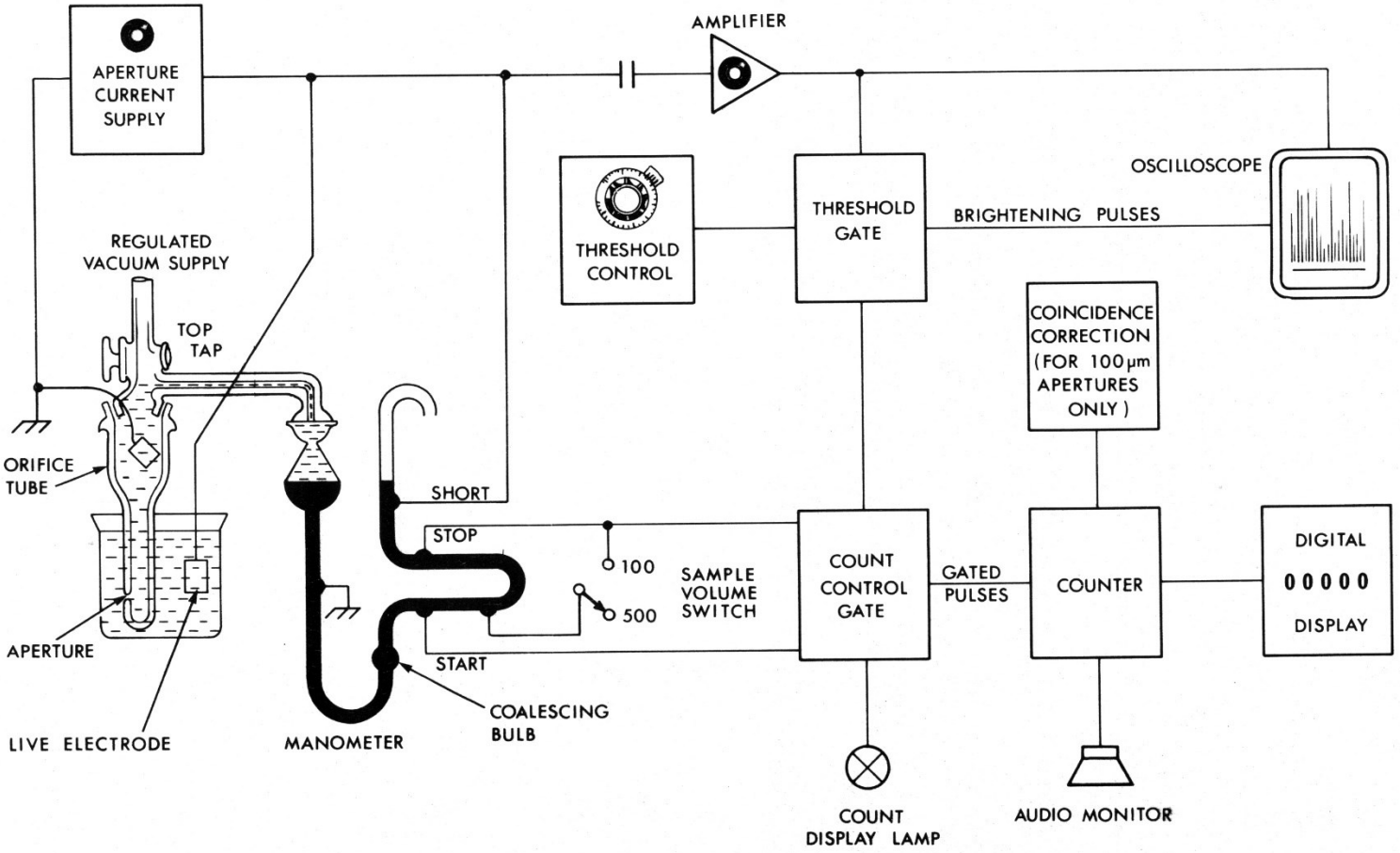
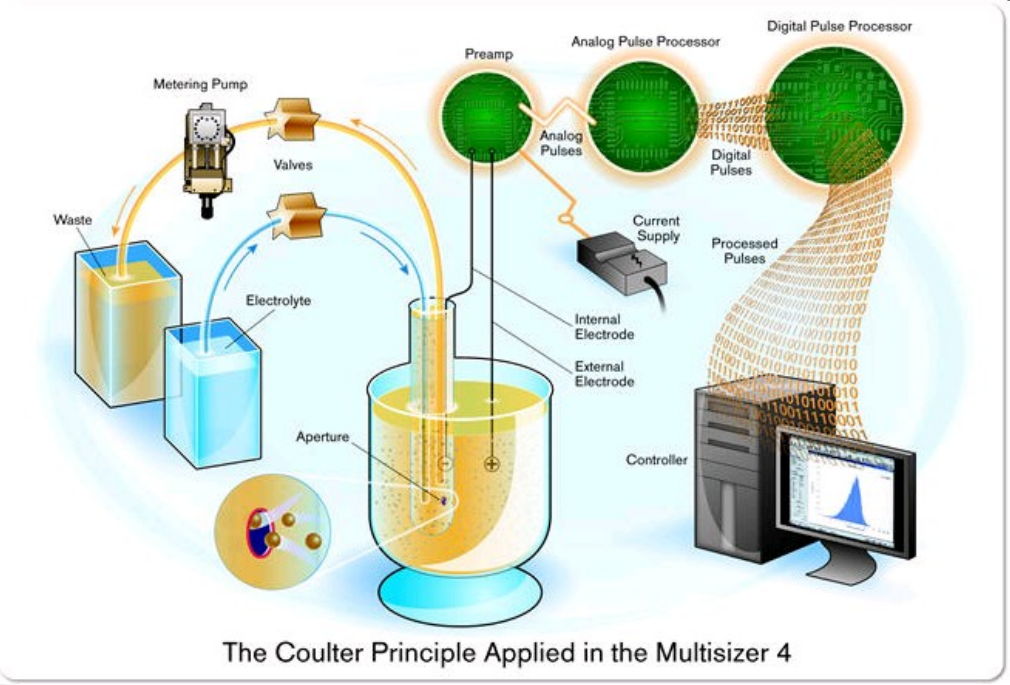


FIG.1-1 FUNCTIONAL BLOCK DIAGRAM FOR MODEL ZF COUNTER

Beckman Coulter

- Multisizer™ 3&4 COULTER COUNTER®



The Coulter Principle Applied in the Multisizer 4

Roche Innovatis
CASY TT

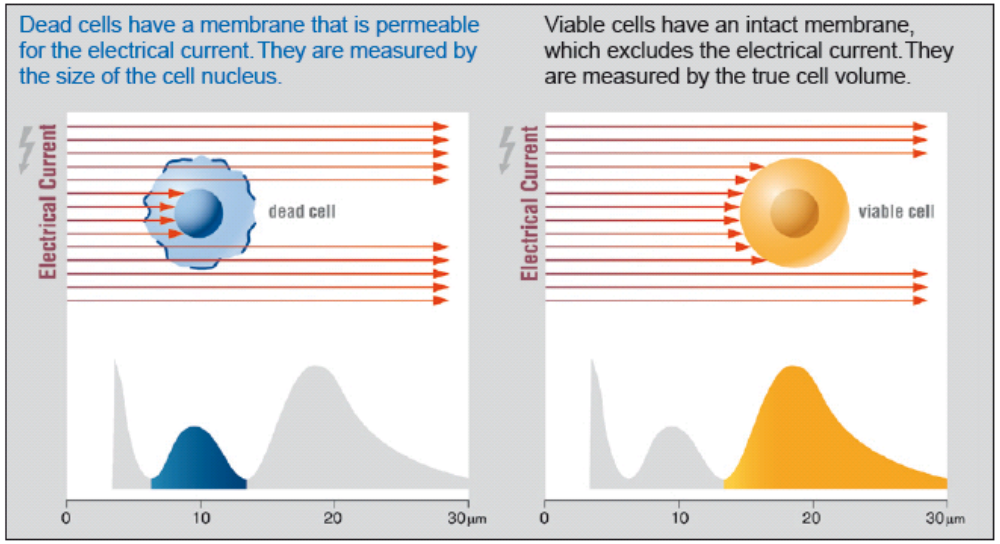


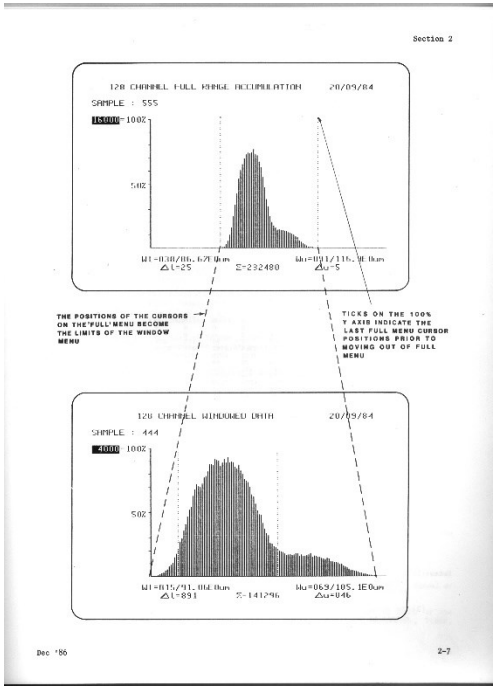
Figure 1: Viability Measurement by Electrical Current Exclusion. The status of the cell membrane distinctively affects the electrical signal generated when a cell is passing the measuring pore.

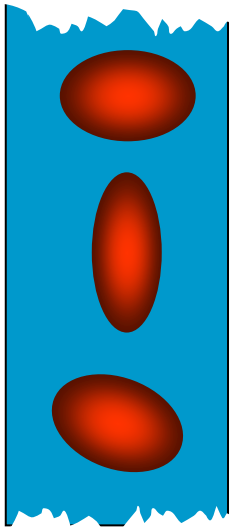
Cytograph. A benchtop instrument capable of measuring the light scattering of a HeNe laser (1970).

Mack Fulwyler- sorter

Mack Fulwyler - sorter 1965 - Los Alamos National Labs - his sorter separated particles based on electronically measured volume (same principle as Coulter counter) and separated by electrostatic deflection.

The aim was to sort the red blood cells, because a bimodal distribution of cell volume was measured. The principle of separation was based on the inkjet printer principle of Richard Sweet of Stanford (1965).





After it was clarified that red cell bimodality is an artifact, this group was able to separate neutrophils and lymphocytes from blood.

Richard Sweet

Richard Sweet developed an electrostatic inkjet printer whose principle was used by Mack Fulwyler for his cell sorter

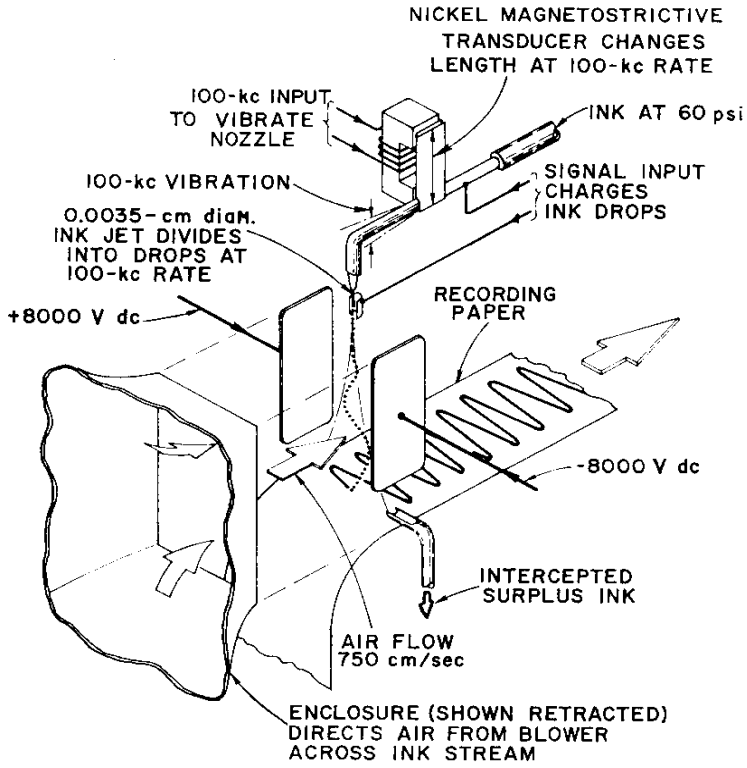


FIG. 1. Ink-jet oscillograph.

THE REVIEW OF SCIENTIFIC INSTRUMENTS

VOLUME 36, NUMBER 2 FEBRUARY 1965

High Frequency Recording with Electrostatically Deflected Ink Jets*

RICHARD G. SWEET

Systems Techniques Laboratory, Stanford Electronics Laboratories, Stanford University, Stanford, California

(Received 28 September 1964)

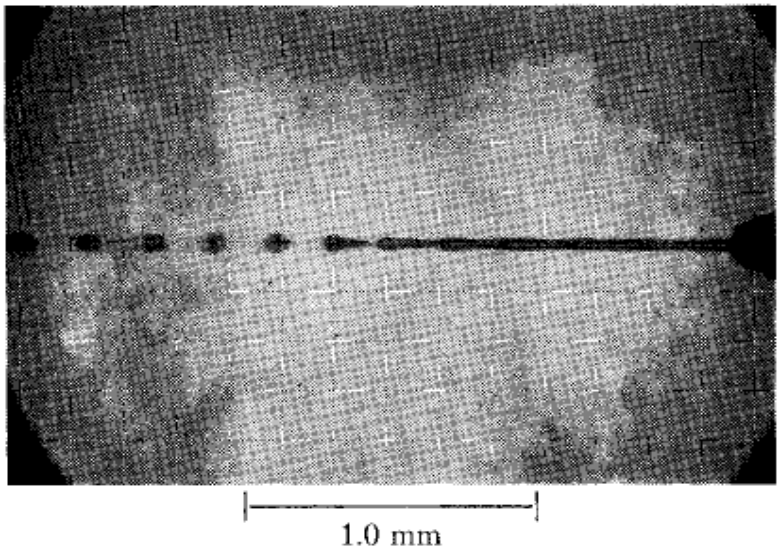
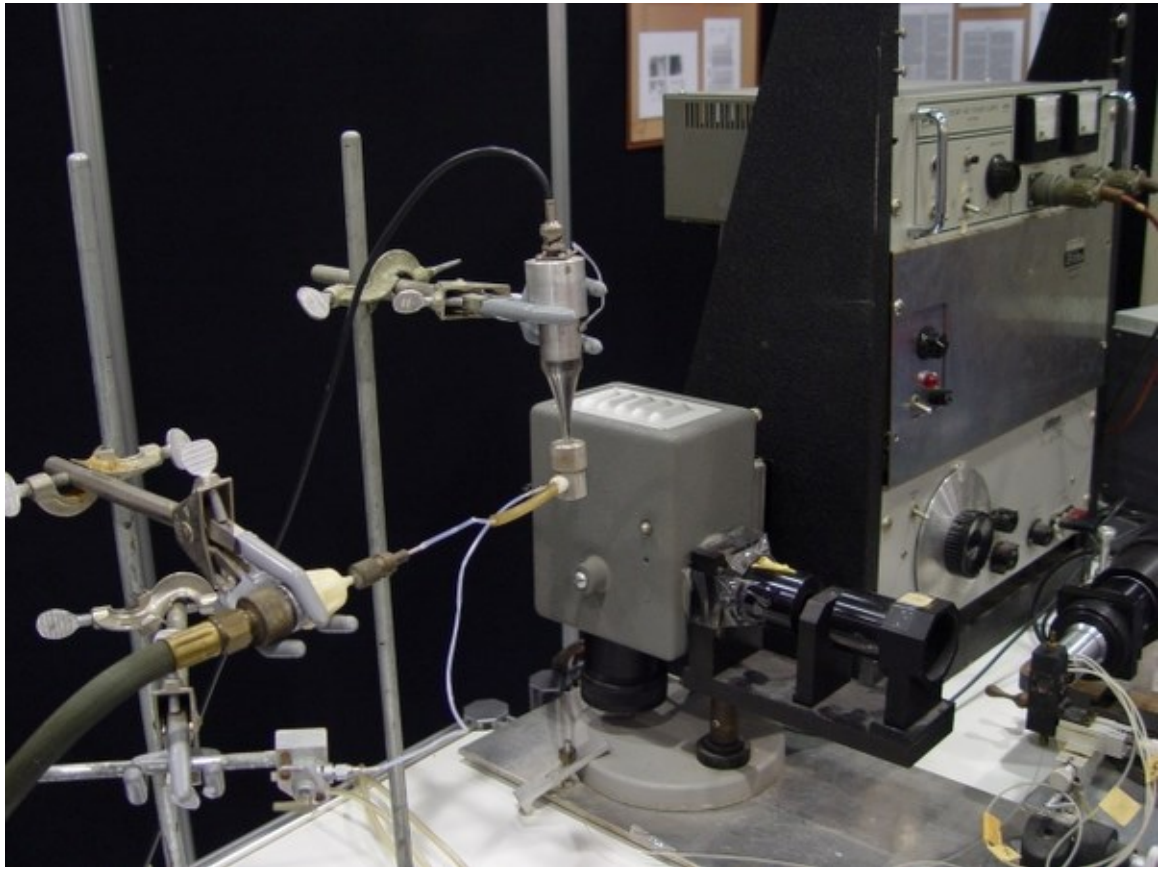
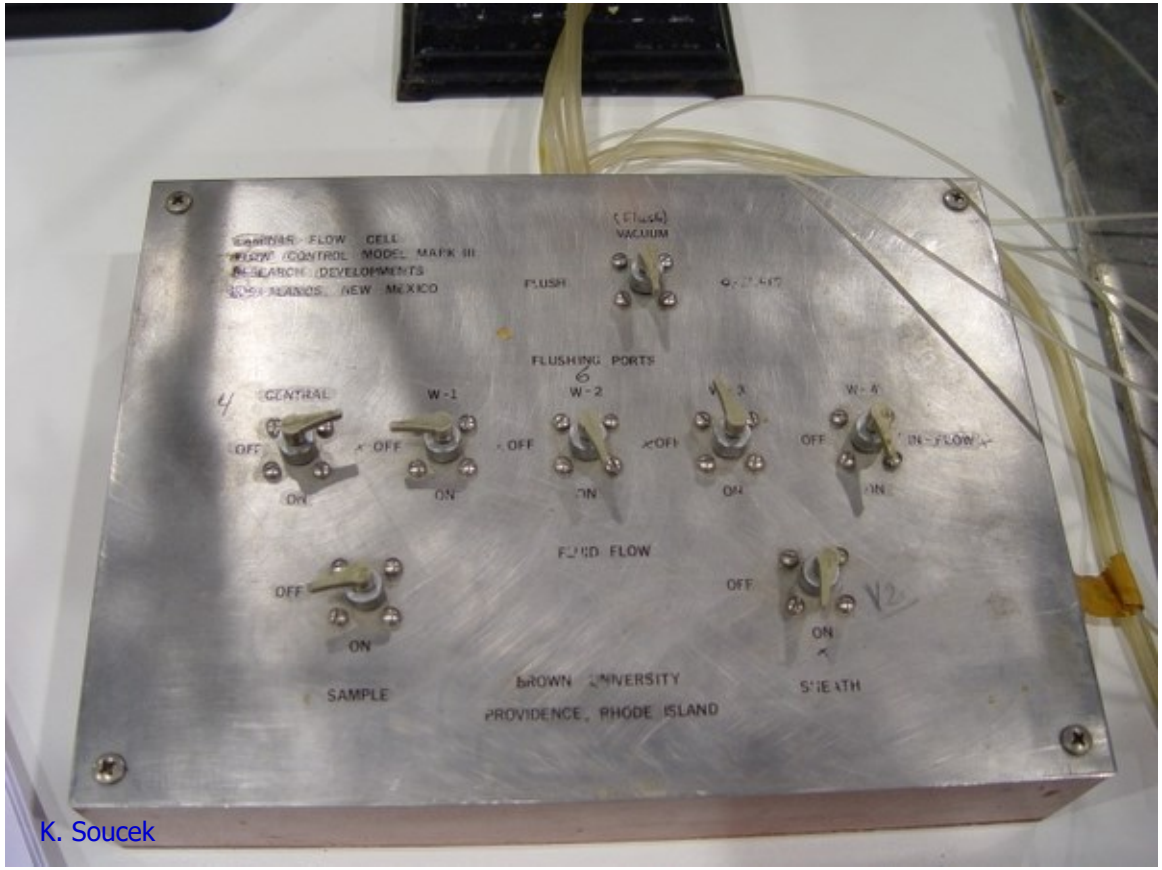


FIG. 3. Ink-drop formation.

Mack Fulwyler- sorter



Mack Fulwyler- sorter



K. Soucek

Cytometry Part A 67A:61–67 (2005)

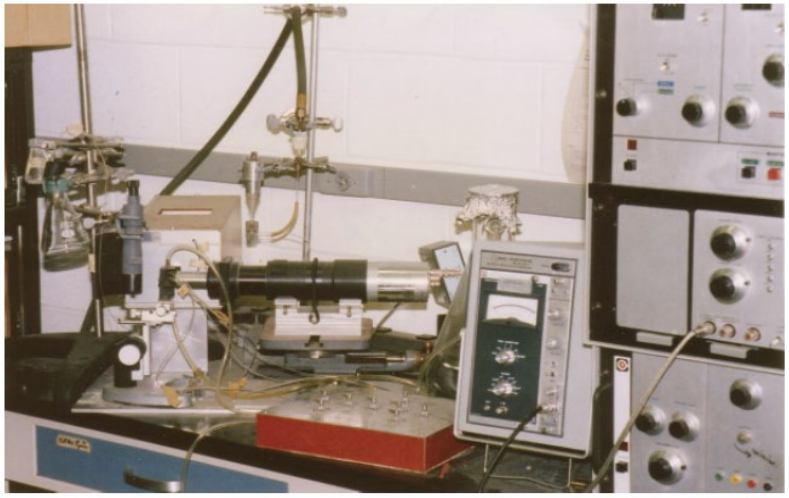
Mack Fulwyler in His Own Words

J. Paul Robinson

Purdue University Cytometry Laboratories, Bindley Biosciences Center, Purdue University, West Lafayette, Indiana

Received 12 July 2005; Revision 15 July 2005; Accepted 15 July 2005

FIG. 1. The Fulwyler instrument as installed in Dr. Boris Rotman's Laboratory in Brown University, immediately prior to disassembly in March 2005. The instrument had not been altered or moved since installation in 1967, except for the addition of a laser instead of the UV lamp.



April 30, 1968 M. J. FULWYLER 3,380,584

PARTICLE SEPARATOR

Filed June 4, 1965 5 Sheets-Sheet 1

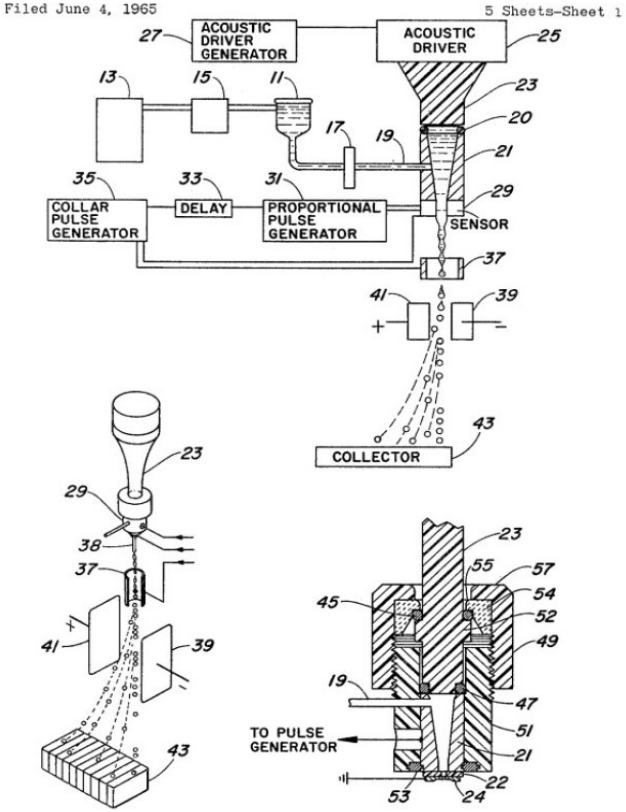


FIG. 4. A page from Fulwyler's patent on the cell separation technology patent #3,380,584 showing the fundamental components of the invention of the cell sorter.

INVENTOR.
Mack J. Fulwyler

BY

Leonard Arthur "Len" Herzenberg

From Hulett, HR, Bonner, WA, Barrett, J, and Herzenberg, LA. Cell Sorting: Automated Separation of Mammalian Cells as a Function of Intracellular Fluorescence. Science 1969; 166: 747-749. Reprinted with permission from AAAS.

Cell Sorting: Automated Separation of Mammalian Cells as a Function of Intracellular Fluorescence

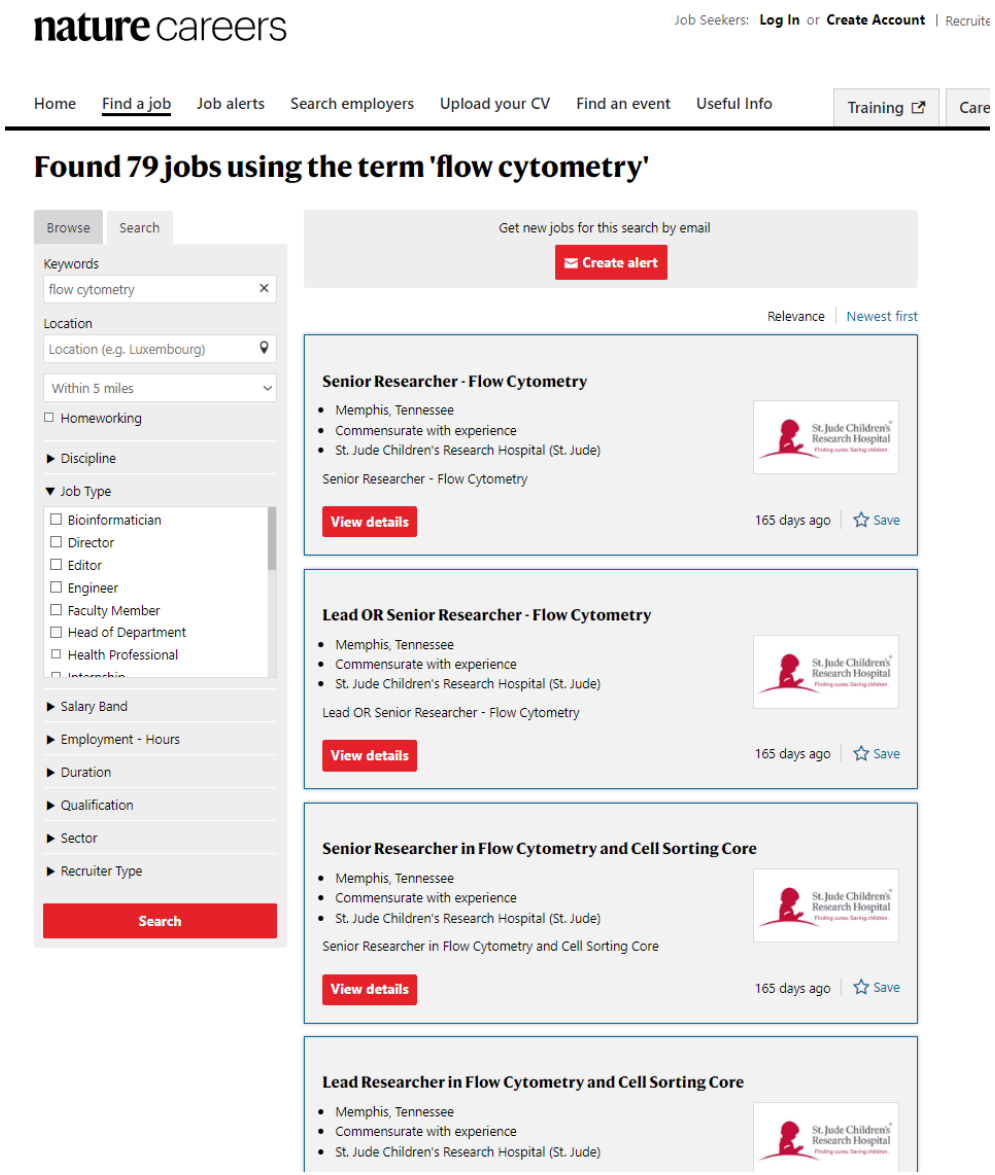
Abstract. *A system for high-speed sorting of fluorescent cells was able to sort mouse spleen cells from Chinese hamster ovarian cells after development of fluorochromasia. Highly fluorescent fractions separated after similar treatment from mouse spleen cells immunized to sheep erythrocytes were enriched in antibody-producing cells by factors of 4 to 10.*

Key "cytometric" publications

- 1934: Moldovan - Photoelectric measurement of cells in a capillary
- 1947: Gucker - photoelectric cell counting
- 1949: Coulter's particle computer
- 1961: Rotman uses fluorescence for the first time to quantify an enzymatic reaction
- 1964: Sweet - electrostatic inkjet printer
- 1965: Fulwyler - May 1965 - patent for electrostatic sorter
- 1965: Kanetsky - spectrophotometric measurement of cells
- 1965: Fulwyler - November 1965 - publication on cell separation in Science magazine
- 1968: Gohde - first article on fluorescence flow cytometry (in German)
- 1969: Gohde - patent
- 1969: Van Dilla - second paper on fluorescence flow cytometry
- 1969: Mullaney - first paper on the description of light scattering as a cytometric parameter
- 1969: Heryenberg - third paper on fluorescence flow cytometry
- 1973: Gohde - double marking patent
- 1977: Gohde - description of signal compensation in double marking
- 1978: Kachel - flow imaging - combination of flow cytometry and image analysis
- 1983: isolation and detection of nuclei (DNA) from paraffin-embedded tissues
- 1984: congress on DNA cytometry nomenclature
- 1987: Graz - high-speed chromosome sorting
- 1991: Robinson - automation of clinical systems - flow cytometer and barcode reader

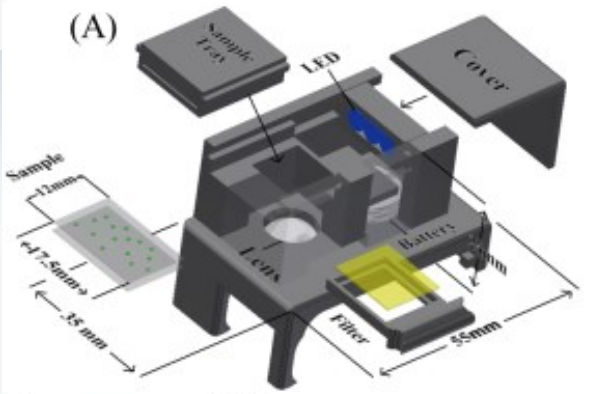
Source - ISAC 2006 - The Wall of History

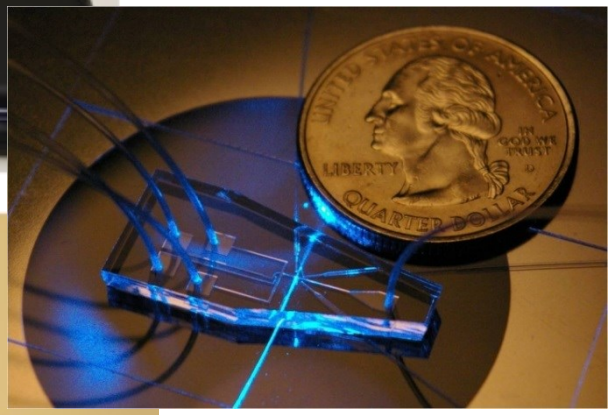
What's it all for... like...

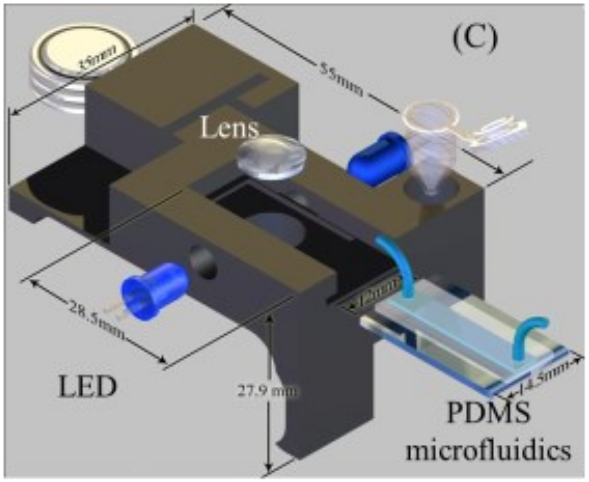


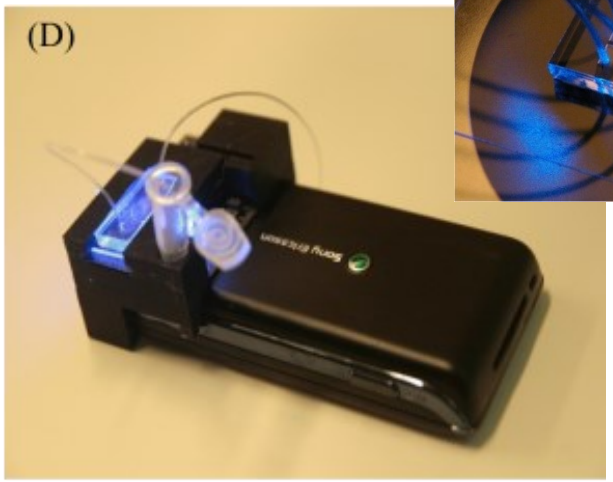
What's it all for... like...

- 38 million people worldwide are infected with HIV (WHO, 2019)
- ~0.7 million people die annually from HIV/AIDS, 1.7 million newly infected (there are ~11 million AIDS orphans in Africa)
- CD4 T cell quantification was/is a key parameter in disease/treatment monitoring, since ~1985
- Since 2016, WHO recommended that all people living with HIV be provided with lifelong ART, including children, adolescents and adults, and pregnant and breastfeeding women, regardless of clinical status or CD4 cell count. By the end of 2019, 185 countries had already adopted this recommendation, covering 99% of all people living with HIV globally.
- Flow cytometry is the "gold standard"
- Optimized procedures and equipment for low-cost (<3 EUR/sample) and fast detection (250 samples/day)
- Aydogan Ozcan: "Kill the cost, safe life"









TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

Flow Cytometry On-a-Chip

MAGNETIC COUNTING

- **RESEARCHER:** Hakho Lee, Assistant Professor of Radiology, Harvard Medical School
  PROJECT: Enumerating and characterizing circulating tumor cells
  PROBLEM: Circulating tumor cells (CTCs) are incredibly rare, with just a handful per milliliter of human blood.
  SOLUTION: Lee's group fabricated a hybrid microfluidic device out of polydimethylsiloxane (PDMS) that can count CTCs in real time using tiny sensors called micro-Hall detectors. (*Sci Transl Med*, 4:141ra92, 2012)

PCR-ACTIVATED SORTING

- **RESEARCHER:** Adam Abate, Assistant Professor of Bioengineering and Therapeutic Sciences, University of California, San Francisco
  PROJECT: Analysis of rare, uncultivable microbes
  PROBLEM: Developing specific antibodies for bacteria that cannot be cultured
  SOLUTION: As a postdoc in the Harvard University lab of droplet-based microfluidics pioneer David Weitz, Abate codeveloped a device that could sort droplets according to their fluorescence intensity. Unlike traditional microfluidics, in which molecules and cells flow naked through channels, droplet-based devices encapsulate molecules or cells in uniform, picoliter-scale aqueous reaction chambers encased in oil.

SORTING BY CELL DEFORMABILITY

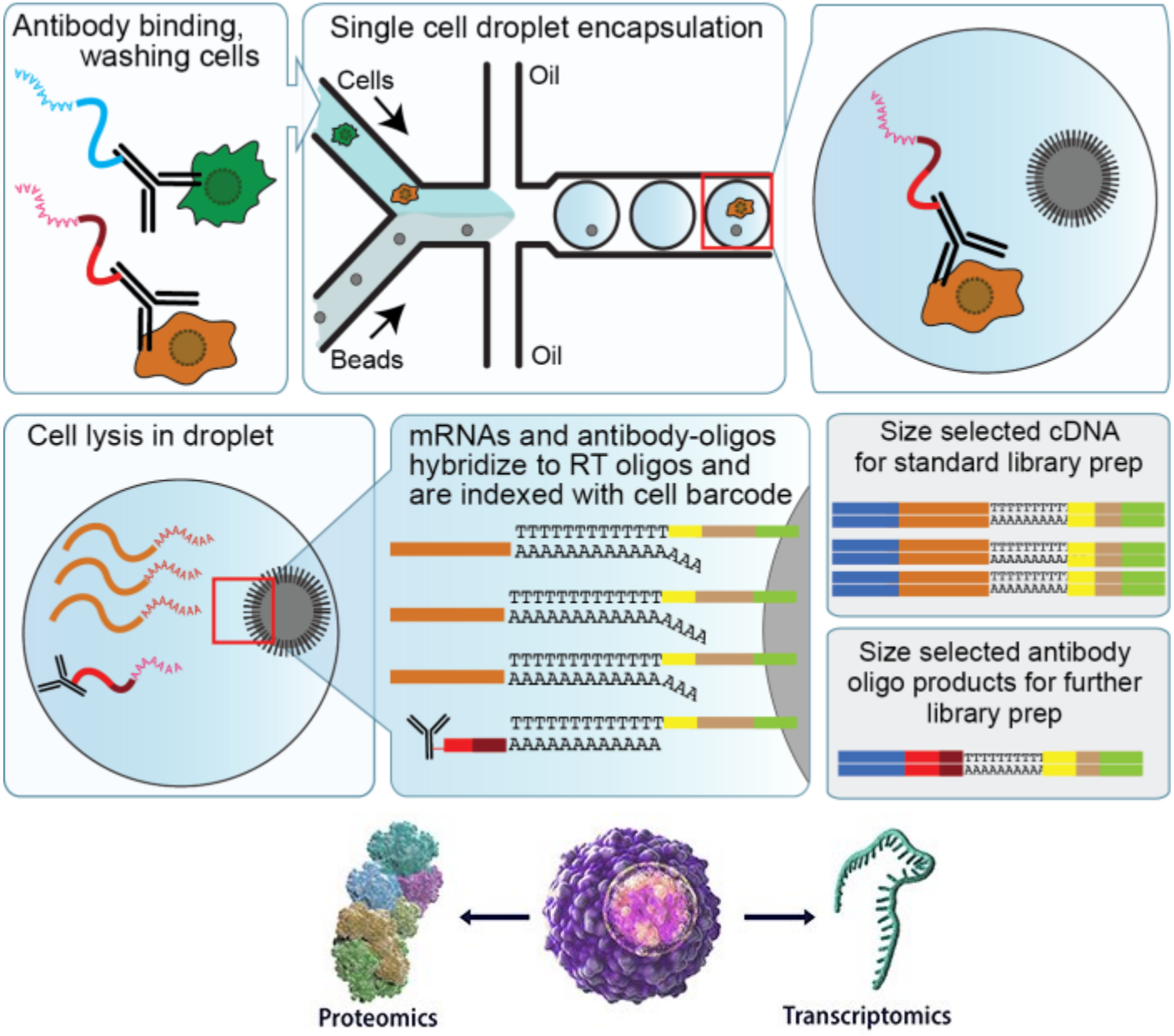
- **PROJECT:** Cancer cell phenotyping
  PROBLEM: Not every cell type has a known antigen that defines it. Plus, antibody binding may activate receptors, potentially changing the cell's behaviour.
  SOLUTION: A physicist by training, Guck used laser beams in his graduate work to study the physical properties of cells. Not all cells are equally squishy, he found: while normal cells are relatively rigid, cancer cells are more pliable. "The more aggressive the cell, the softer it is, and that may be necessary for it to pass into tissues," Guck explains.

RAMAN-ACTIVATED CELL SORTING

- **RESEARCHER:** Jian Xu, Professor and Director, and Bo Ma, Group Lead of Microfluidics, Single-Cell Center, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences
  PROJECT: Microbial biofuels development
  PROBLEM: Biofuels R&D requires identifying cells capable of specific carbon chemistries. But as these cells have yet to be cultured and studied, researchers have few if any molecular hooks for identifying and sorting them.
  SOLUTION: The team turned to a label-free method of single-cell interrogation known as Raman-activated cell sorting (RACS) (*Anal Chem*, 87:2282-89, 2015).

http://www.the-scientist.com/?articles.view/articleNo/43034/title/Flow-Cytometry-On-a-Chip/

Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq)



What preceded it...and what we are part of now...

- Development of techniques for rapid and reproducible detection of cytometric parameters.
- New scientific knowledge leading to the definition of appropriate diagnostic markers.

ISAC presents: Mack Fulwyler - Innovator, Inventor & Pioneer

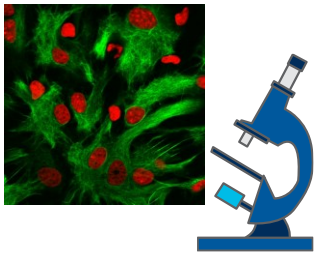
http://www.cyto.purdue.edu/cdroms/gh/HTML/start.htm?loc=http://www.cyto.purdue.edu/cdroms/gh/HTML/video/video.html?v=Flowtheinvention.wmv

http://www.cyto.purdue.edu/cdroms/cyto10a/seminalcontributions/fulwyler.html

https://www.youtube.com/watch?v=3s5l2mepKkY

State-of-the-Art

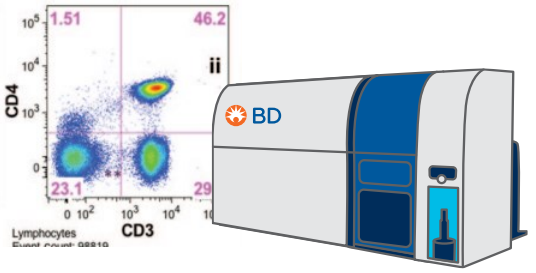
Two common ways to quantify the total number and type of cells in a sample



Microscopy

Provides details of cell morphology for tens or hundreds of cells. Can provide information about cellular interactions.

- Shape
- Distribution of components in the cell



Flow Cytometry

Quantifies a high number parameters of cells in suspension for hundreds or thousands of cells per second and is capable of sorting living cells.

- Size
- Granularity
- Surface and intracellular components

BD CellView™ Image Technology, provides the features of both and more

- High speed, high-parameter flow cytometry
- High-content images providing cell morphology and cellular interactions
- Rapid analysis and sorting of cell populations defined by traditional flow parameters combined with new image parameters

January 21st, 2022

RESEARCH TECHNOLOGY

High-speed fluorescence image–enabled cell sorting

Daniel Schraivogel[1], Terra M. Kuhn[2]†, Benedikt Rauscher[1]†, Marta Rodríguez-Martínez[1]†, Malte Paulsen[3]‡, Keegan Owsley[4], Aaron Middlebrook[4], Christian Tischer[5], Beáta Ramasz[3], Diana Ordoñez-Rueda[3], Martina Dees[2], Sara Cuylen-Haering[2]*, Eric Diebold[4]*, Lars M. Steinmetz[1,6,7]*

Fast and selective isolation of single cells with unique spatial and morphological traits remains a technical challenge. Here, we address this by establishing high-speed image-enabled cell sorting (ICS), which records multicolor fluorescence images and sorts cells based on measurements from image data at speeds up to 15,000 events per second. We show that ICS quantifies cell morphology and localization of labeled proteins and increases the resolution of cell cycle analyses by separating mitotic stages. We combine ICS with CRISPR-pooled screens to identify regulators of the nuclear factor κB (NF-κB) pathway, enabling the completion of genome-wide image-based screens in about 9 hours of run time. By assessing complex cellular phenotypes, ICS substantially expands the phenotypic space accessible to cell-sorting applications and pooled genetic screening.

Schraivogel *et al.*, *Science* **375**, 315–320 (2022)

Real-time cell images

Mouse over any data point and see the cell image pop-up

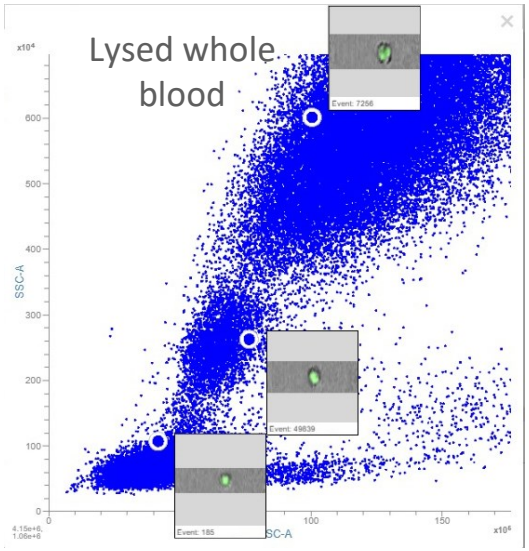
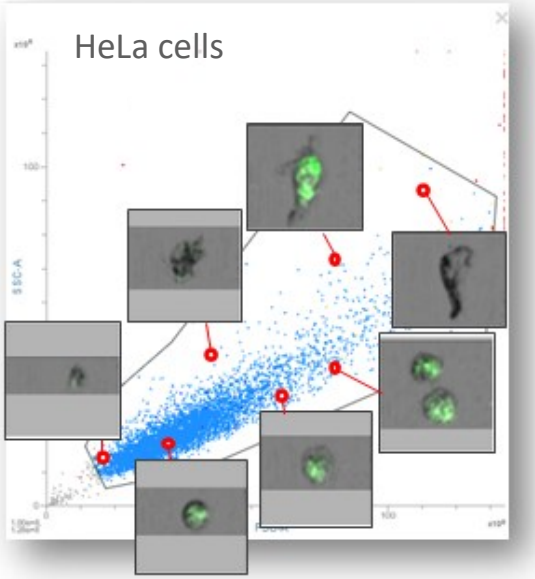
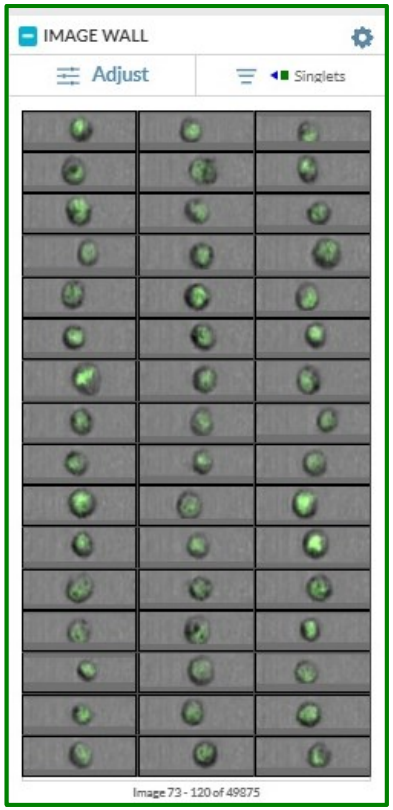


Image data and all flow data are merged and correlate one to one



Real time display of gated events on image wall



Real-time image-enabled sorting

See what you sort. Sort what you see.

The BD FACSDiscover™ S8 Cell Sorter leverages BD CellView™ Image Technology, a novel high-speed imaging technology that facilitates analysis and sorting through real-time integration of image and flow data.

BD CellView™ Image Technology

CELL MORPHOLOGY

Explore cell morphology with internal and external spatial characteristics

SAMPLE QC

Visualize and confirm images in real time to obtain sample data for flow cytometry and downstream assays

IMAGING DETECTORS

Image detectors with fluorescent, scatter and light loss to visualize events in real time and at high speed

3-color fluorescence imaging

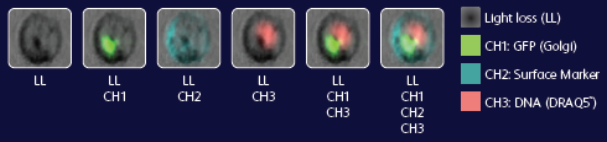
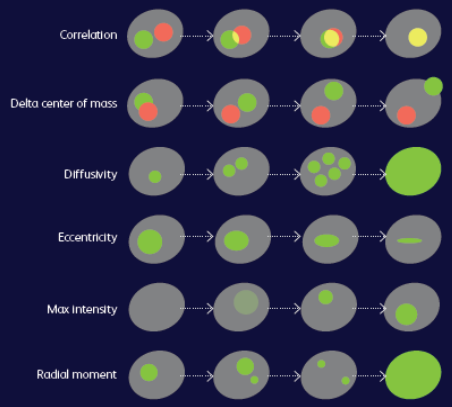


IMAGE FEATURES

Image features combined with traditional flow parameters open the door to new dimensions in single-cell analysis



ADDITIONAL IMAGE FEATURES

- Center of mass (X)
- Center of mass (Y)
- Forward scatter (FSC)
- Light loss (blue)
- Long moment
- Short moment
- Size
- Side scatter (SSC)
- Total intensity

NEW INSIGHTS

New insights and image-based applications help to address previously impossible-to-answer questions

Summary of the lecture

- Introduction to the course
- Sources of literature
- History of flow cytometry
- Basic principles

At the end of today's lecture, you should:

1. know what the requirements are for this course
2. know the basic sources of information
3. have a brief overview of the history of flow cytometry
4. be familiar with some basic principles of flow cytometry