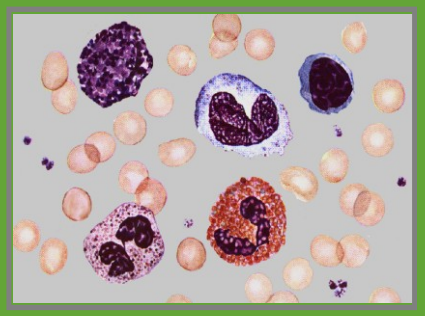
CYTOKINETIKA

Průtoková cytometrie a sorting

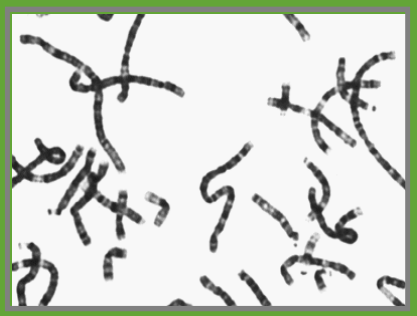
Karel Souček & Radek Fedr

Biofyzikální ústav AV ČR, Masarykova Univerzita, FNUSA-ICRC

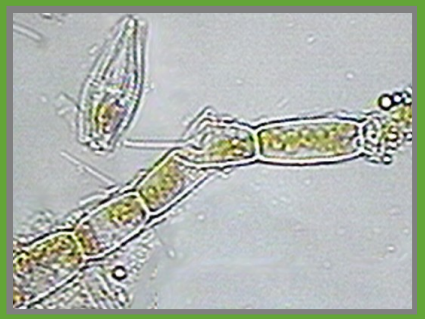
Tyto částice mají něco společného …



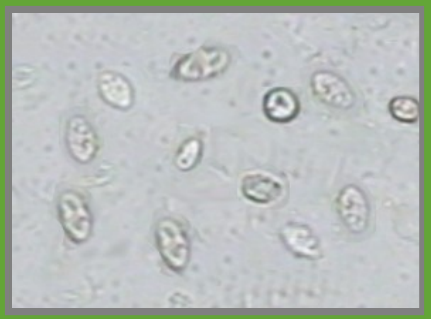
Blood cells



Chromosomes



Algae



Protozoa

… určité parametry těchto částic mohou být měřeny pomocí průtokové cytometrie.

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BD

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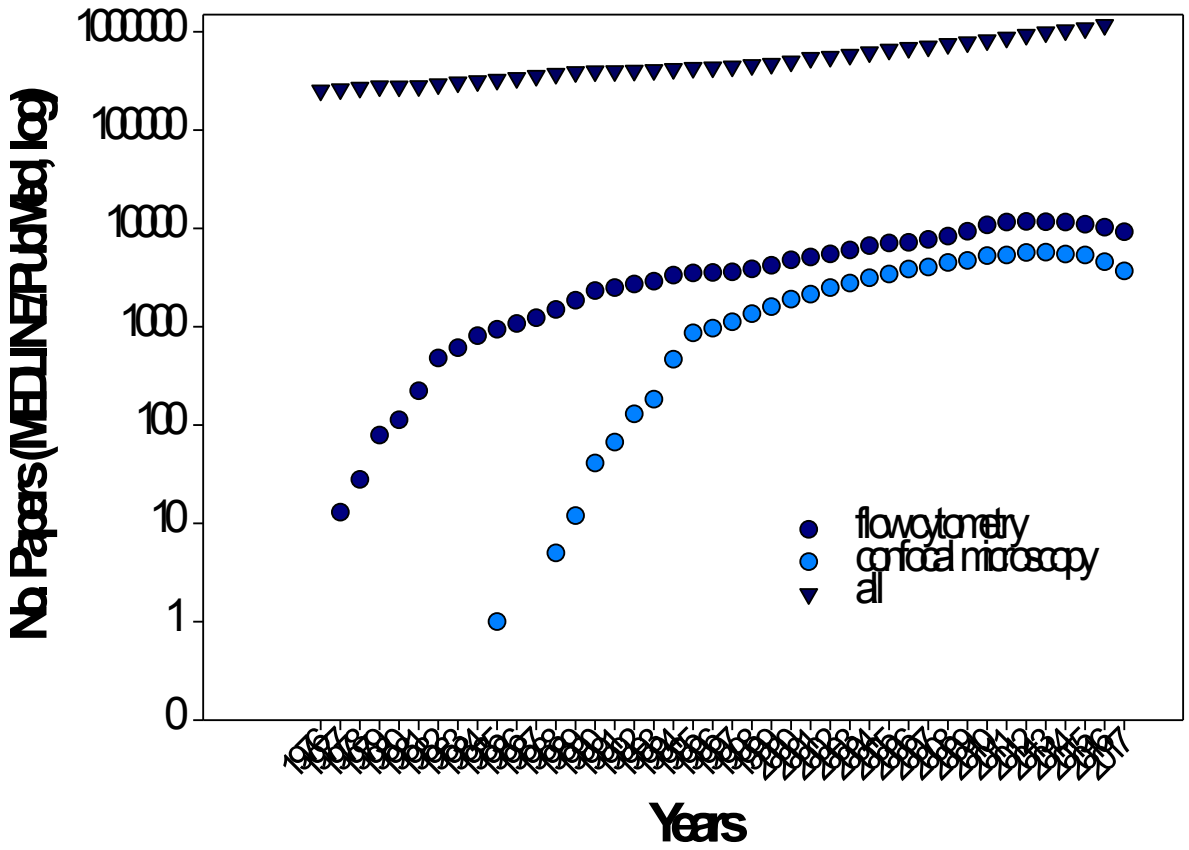
Komerční zařízení a vývoj

Cytometry Publications/Year



Data taken from PubMed

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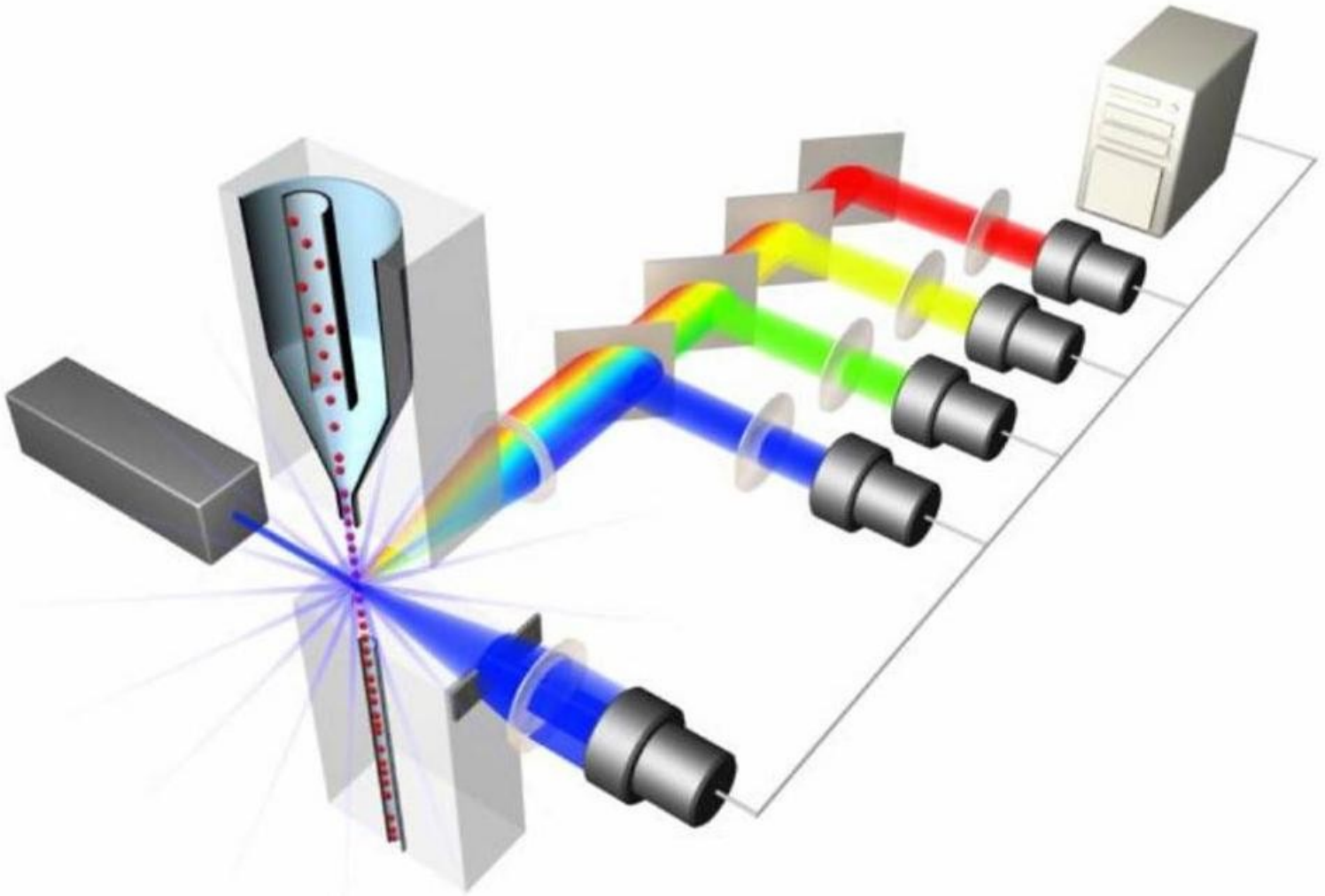
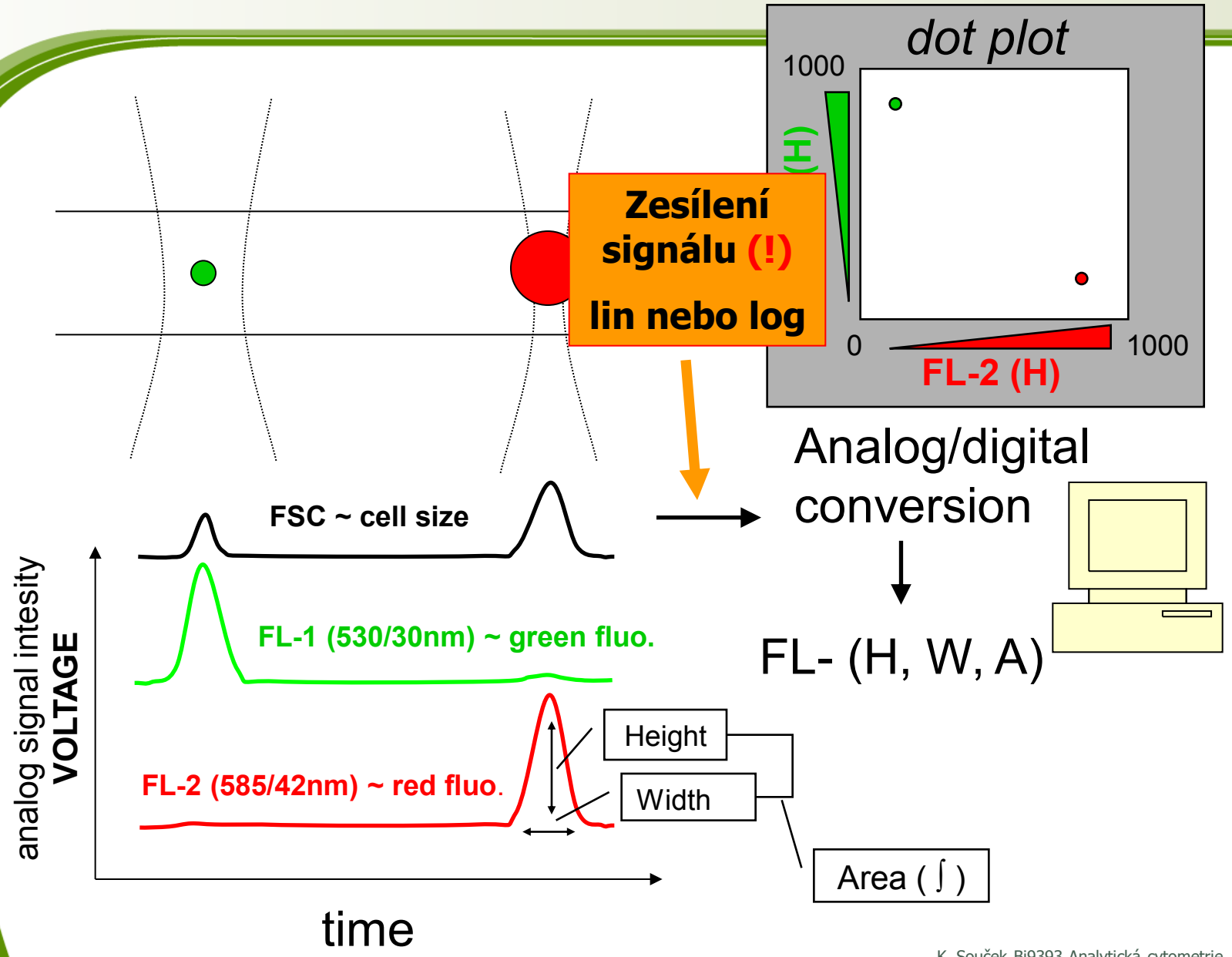
Co je průtokový cytometr?



Signal processing



Co můžeme analyzovat pomocí průtokové cytometrie?

- Počítat částice v suspenzi
- Oddělit živé částice od neživých
- Hodnotit 10*5 až 10*6 částic za méně než 1 minutu
- Kvantifikovat rozptyl světla, a intenzitu fluorescence pro jednotlivé buňky (částice)
- Fyzicky separovat jednotlivé částice (populace) pro další analýzu

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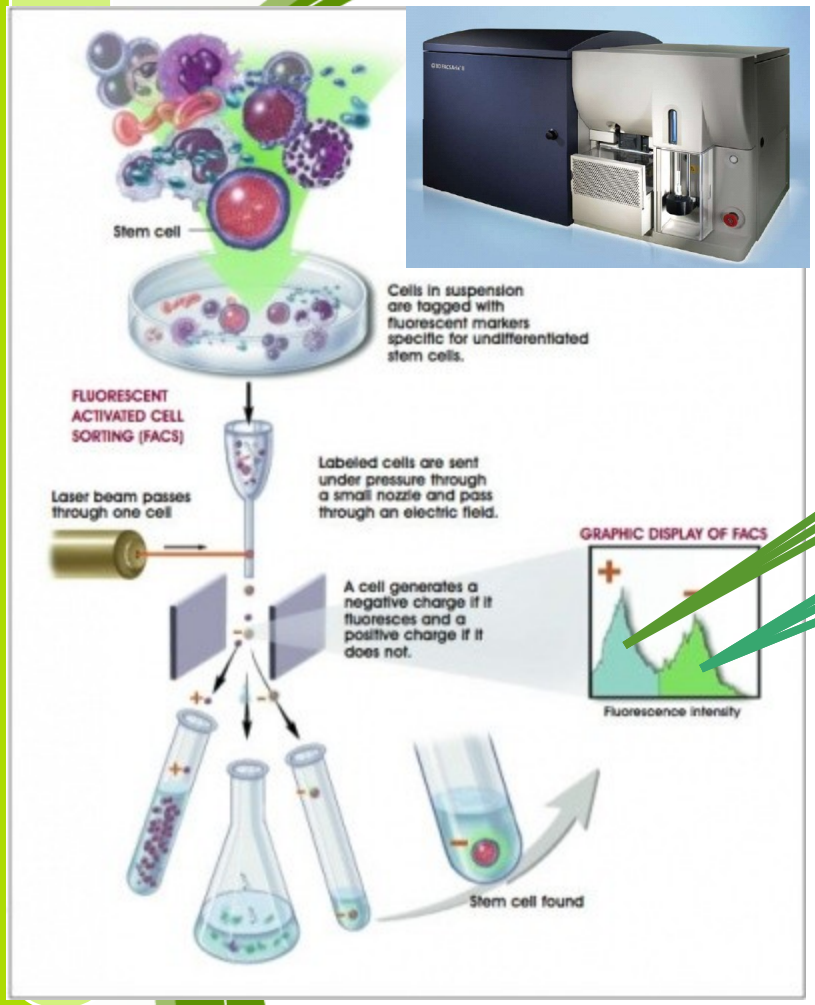
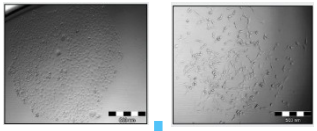
new automatic cell cloning assay (ACCA) for determination of clonogenic capacity of CSCs

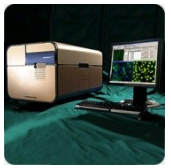


single cell/well
up to 384 well plate

re-culture after sorting (2D, 3D)



analysis: CyQuant, ATP, xCelligence, microscopy



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Jaké jsou principy?

- Rozptyl světla (Light scatter) pomocí laseru nebo UV lampy
- Detekce specifické flurescence
- Hydrodynamicky zaostřený proud částic
- Elektrostatická separace částic
- Možnost multivariační analýzy dat

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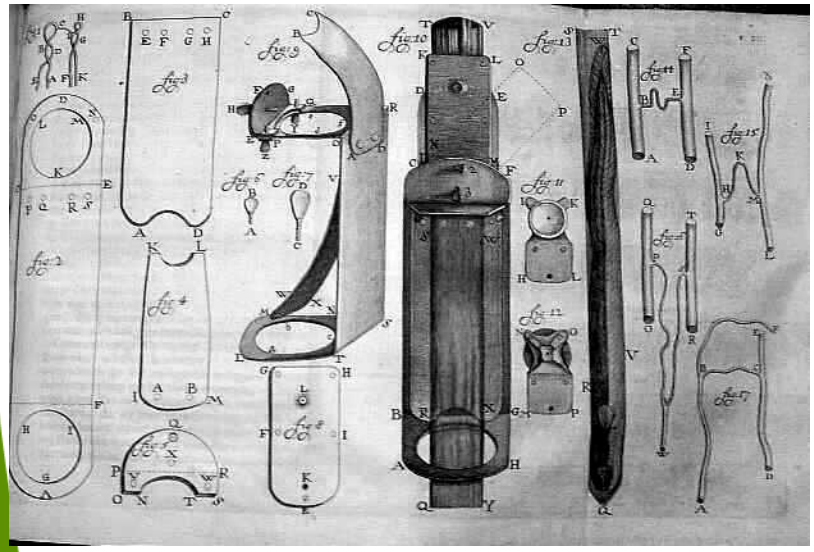
Stain Your Own Cell

life technologies™

Historie barvení biologických materiálů

Až do poloviny 19. století – *byly používány pouze přírodní barviva*

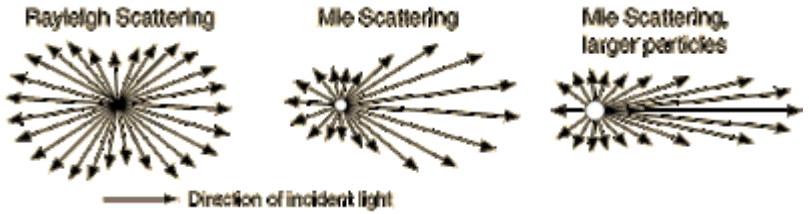
Anton van Leeuwenhoek použil v roce 1719 šafrán na obarvení svalových buněk



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

Rozptyl světla

- Hmota rozptyluje světlo vlnových délek které není schopna absorbovat
- Viditelné spektrum je 350-850 nm proto malé částice a molekuly (< 1/10 λ) spíše viditelné světlo rozptylují
- Pro malé částice byl popsán tzv. Rayleightův rozptyl (scatter) jehož intenzita je ~ stejná všemi směry
- Rozptyl větších částic charakterizuje tzv. Mieův rozptyl. Jeho množství je větší ve směru v jakém dopadá světlo na ozářenou částici ⇨ *na tomto principu je založeno měření velikosti částic pomocí průtokového cytometru*



http://hyperphysics.phy-astr.gsu.edu/hbase/atmos/blusky.html

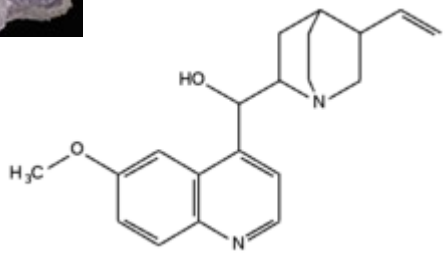
Shapiro p.106

George Gabriel Stokes (1819 – 1903)

Anglický fyzik a matematik
působící na univerzitě v Cambridge

http://www.nndb.com/people/131/000097837/

1852 – popsal fluorescenci
Název vznikl z anglického slova *fluospar* (fluorit, kazivec = nerost CaF_2)
- ke svému pozorování použil roztok chininu, jako zdroj světla sluneční paprsky, jako excitační filtr sloužilo tmavě modré okenní sklo a jako emisní filtr byla použita sklenice bílého vína



G. C. Stokes „*On the Change of Refrangibility of Light*“ Philosophical Transactions of the Royal Society of London, 1852, vol. 142, p. 463.)

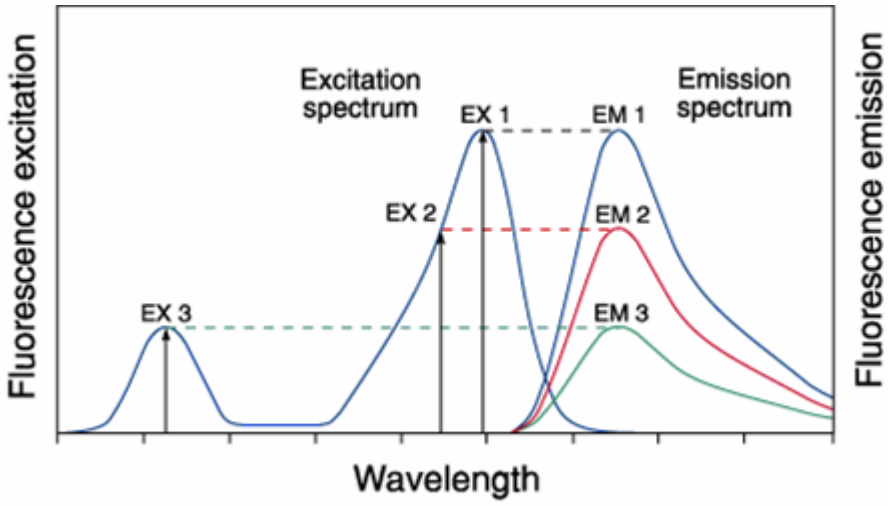
[463]

XXX. *On the Change of Refrangibility of Light.* By G. G. Stokes, *M.A., F.R.S., Fellow of Pembroke College, and Lucasian Professor of Mathematics in the University of Cambridge.*

Received May 11,—Read May 27, 1852.

Fluorescenční spektra

Fluorescenční proces je cyklický.
Kromě fluorochromu nevratně zničeného (photobleaching - „vysvícení“) může být opakovaně excitován.



Excitation of a fluorophore at three different wavelengths (EX 1, EX 2, EX 3) does not change the emission profile but does produce variations in fluorescenceemission intensity (EM 1, EM 2, EM 3) that correspond to the amplitude of the excitation spectrum.

Detekce fluorescence

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Vybavení pro fluorescenci

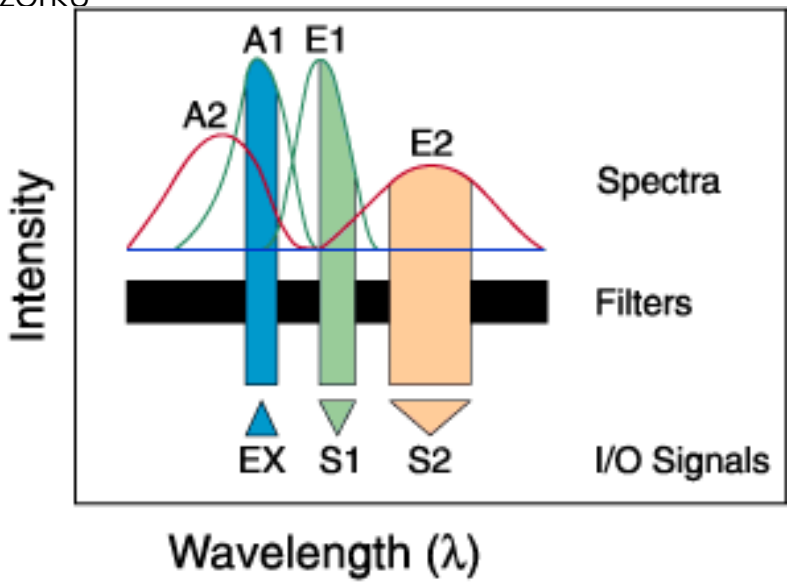
(1) zdroj excitace
(2) fluorochrom
(3) vlnové filtry pro izolaci emitovaných fotonů od excitovaných
(4) detektory pro registraci emitovaných fotonů

Fluorescenční přístroje

- spektrofluorometer měří průměrné vlastnosti objemu vzorku v kyvetě.
- fluorescenční mikroskop popisuje fluorescenci jako jev v prostorovém systému souřadnic
- flow cytometer měří fluorescenci v proudícím toku, umožňuje detekovat a kvantivikovat subpopulace uvnitř velkého vzorku

Fluorescenční signál

- spektrofluorometer je flexibilní, umožňuje m
v kontinuálním spektru excitačních a emisní
vlnových délek
- flow cytometr potřebuje fluorescenční zna
excitovatelné
určitou vlnovou délkou.



Fluorescence pozadí

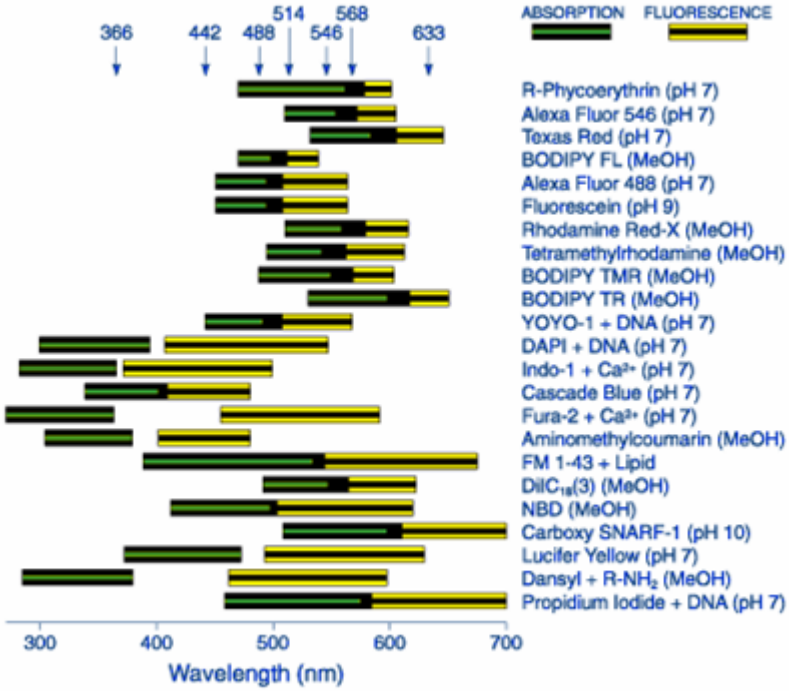
- endogení složky - autofluorescence
- nenávazané nebo nespecificky vázané zr
= reagenční pozadí

Vícebarevné značení

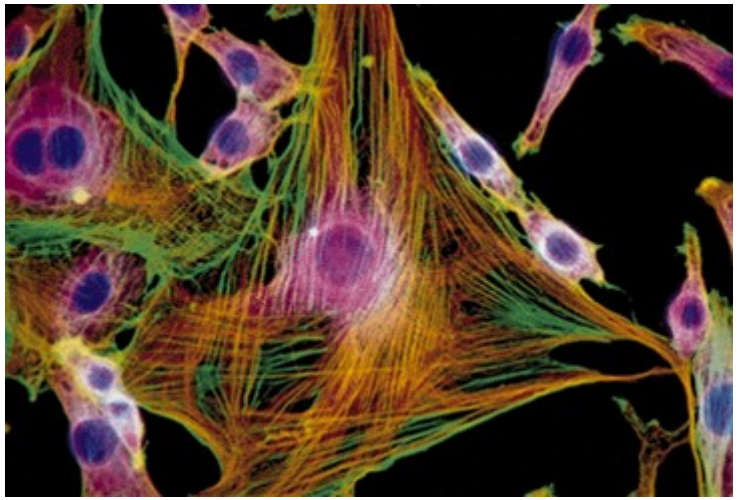
- dvě a více značek, zároveň monitoruje různé funkce
- nutné: vhodně zvolit značky zdroj excitace a separační filtry

Fluorescence Output of Fluorophores

Comparing Different Dyes



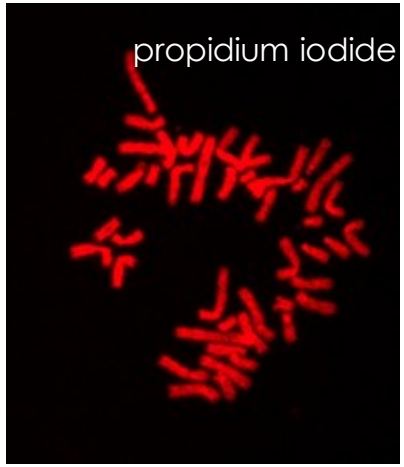
Mouse 3T3

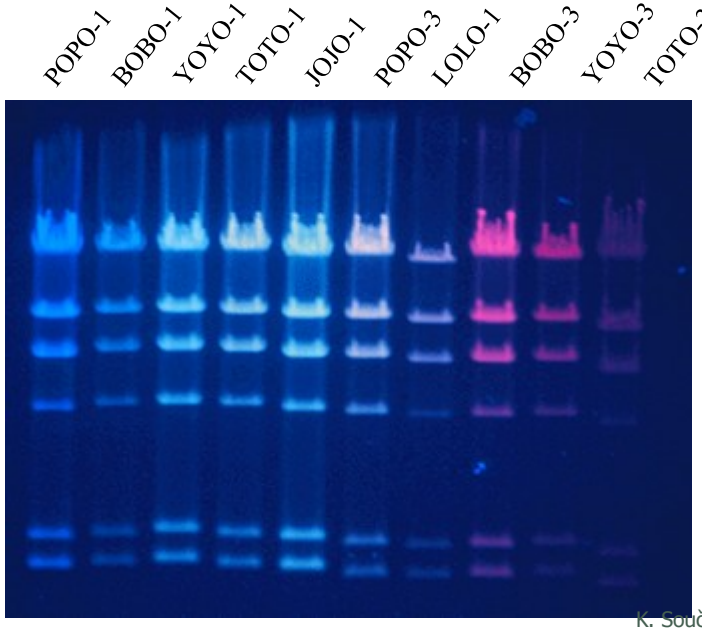


F-actin ~
BODIPY FL phallacidin

anti-ß tubulin ~
Texas Red
goat anti–mouse IgG

DNA ~
DAPI





λ Hind III

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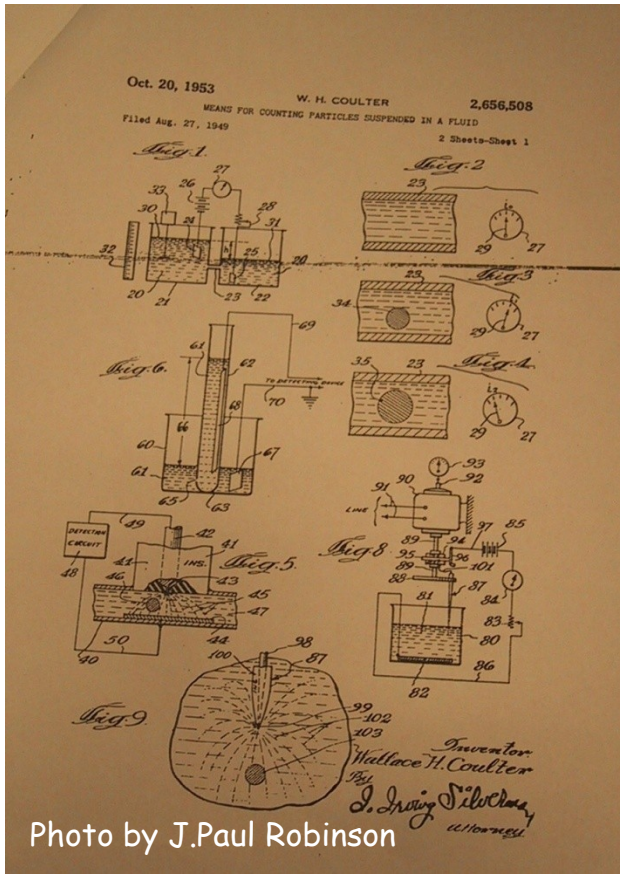
Technické součásti

- Zdroje světla
- Detekční systémy
- Fluidní systém
- Separace
- Sběr dat
- Analýza dat

Wallace Coulter

- **Wallace Coulter - Coulter orifice - 1956 -**
- (patent 1953) – měření změny vodivosti během průchodu buněk v suspenzi malým otvorem

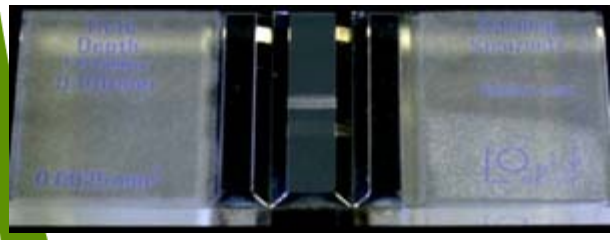


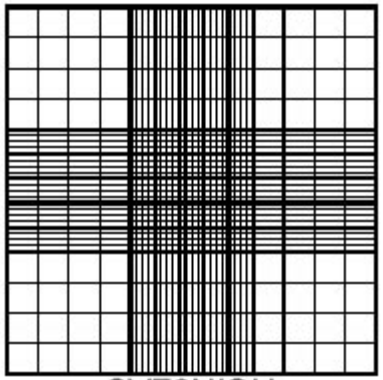
Originální patentová aplikace W.Coultera 1953

Photo by J.Paul Robinson

Jak počítat buňky?

- Hemocytometer (Bürkerova komůrka) byla standardem pro počítání buněk do ~ 1950
- Rozměry jsou 3x3x0.1 mm. Obvykle jsou červené krvinky (1 x $10^6/mm^3$)počítány po naředění 1:200
- Leukocyty (5x$10^3/mm^3$) jsou ředěny 1:10 v roztoku lyzujícím červené krvinky
- Statistická chyba:
  - koeficient variance (CV) je při 500 spočítaných buňkách 4.4%
  - chyba pipetování a ředění je ~ 10%





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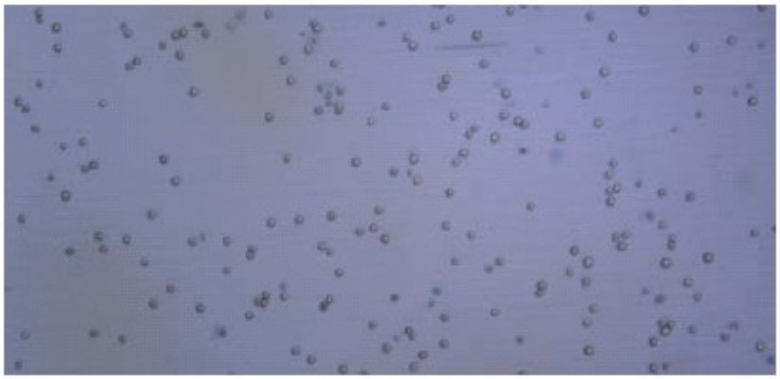
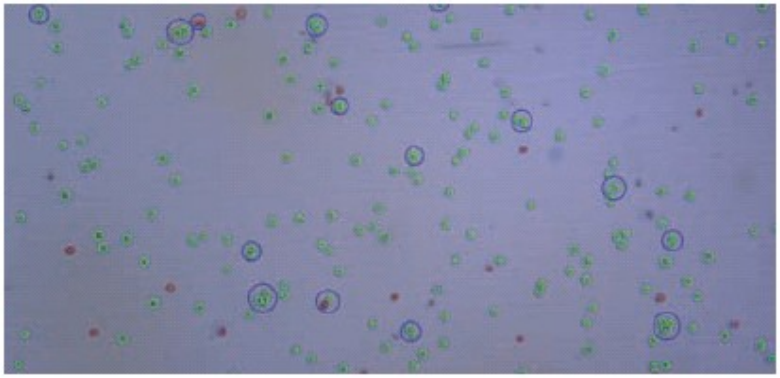
Roche Innovatis Cedex

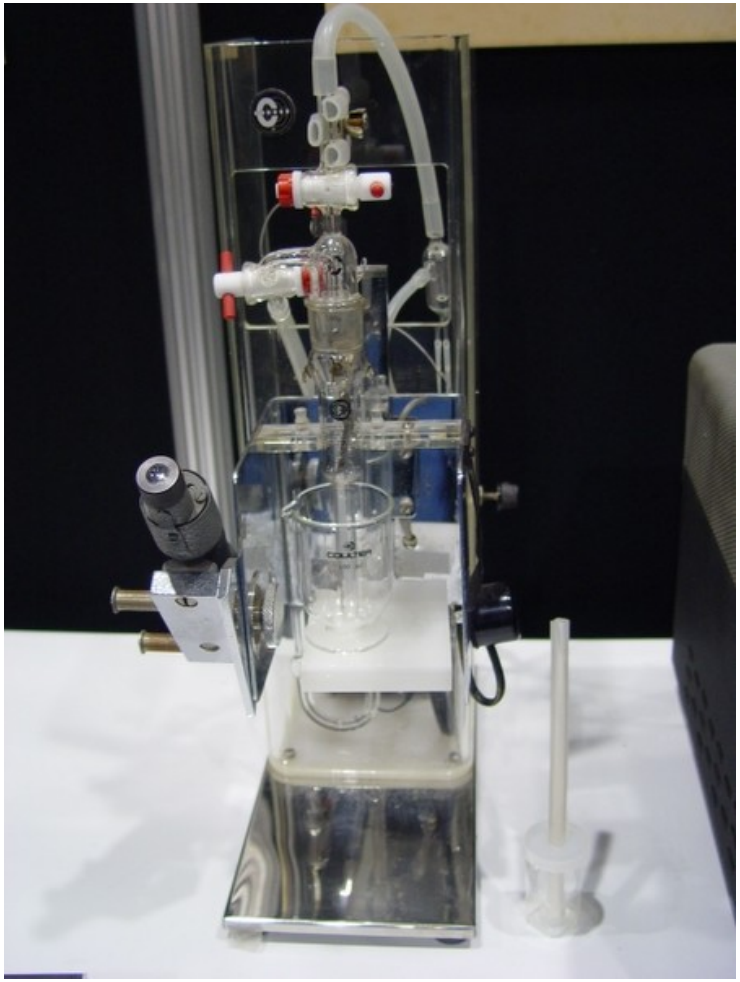


High Resolution Color Image



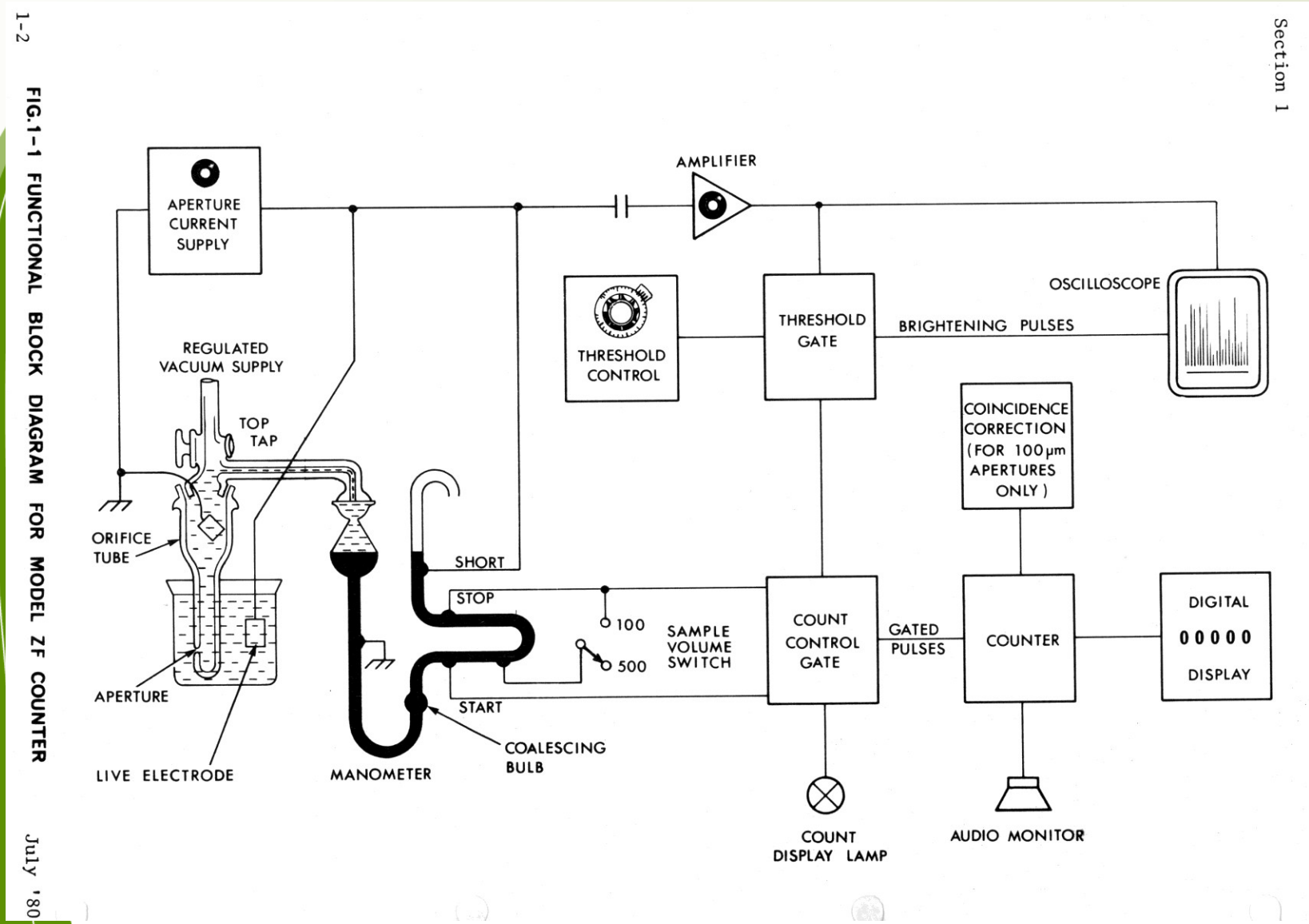
Visual Labeling

Coulter Counter



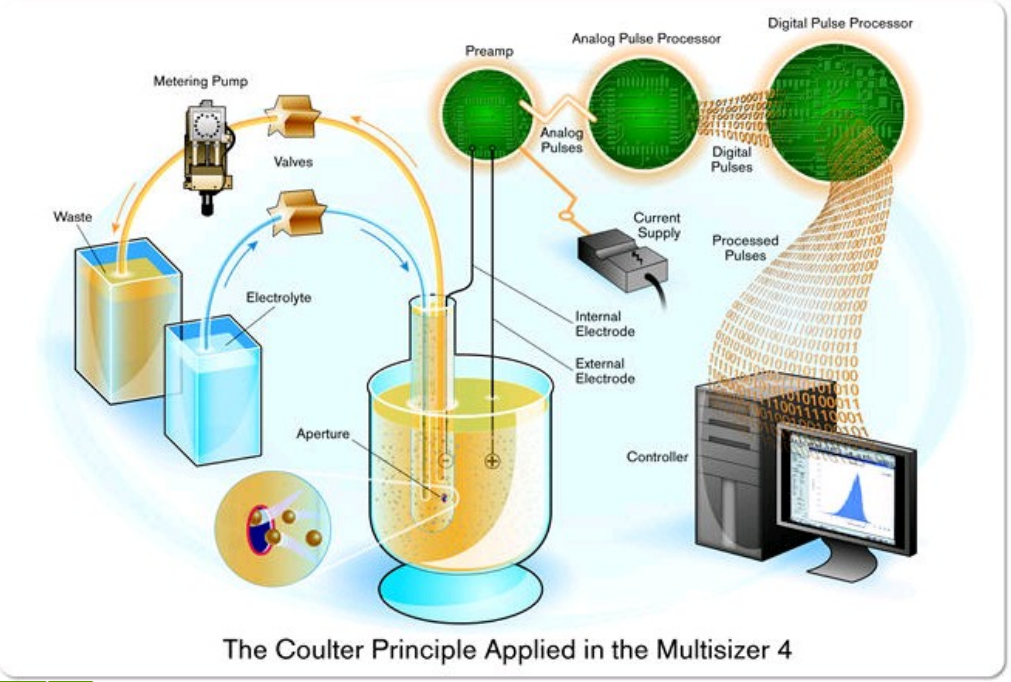
První komerční verze CC

Coulter Counter



Beckman Coulter

- Multisizer™ 3&4 COULTER COUNTER®



The Coulter Principle Applied in the Multisizer 4

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Roche Innovatis
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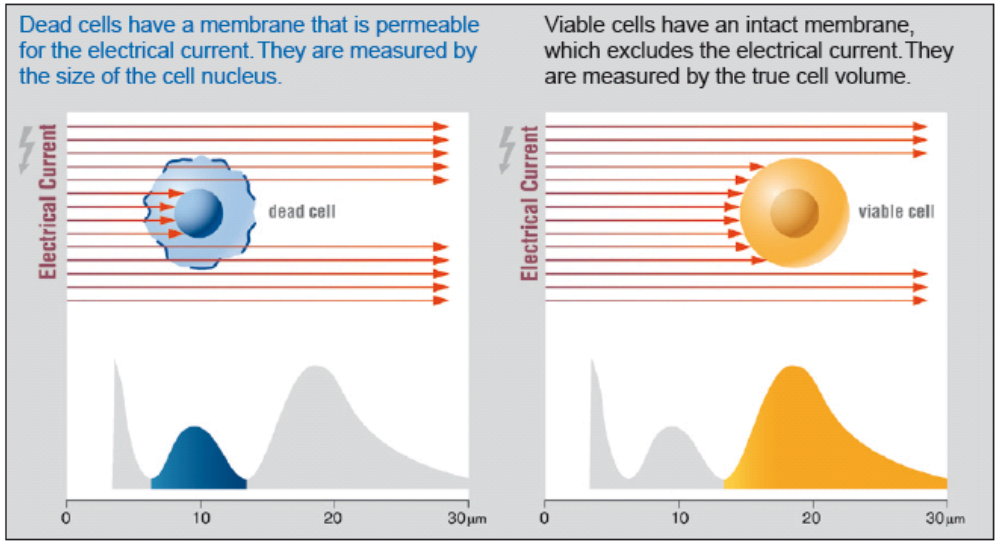


Figure 1: Viability Measurement by Electrical Current Exclusion. The status of the cell membrane distinctively affects the electrical signal generated when a cell is passing the measuring pore.

Cytograph. Stolní přístroj schopný měřit rozptyl světla He-Ne laseru (1970).

Technické součásti

Detekční systémy

Fotonásobiče (Photomultiplier Tubes (PMTs))

dříve 1-2

nyní 4-8 (12-18)

Diody

detekce rozptylu světla (light scatters)

Zdroje světla

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

Argon ion, Krypton ion, HeNe, HeCd, Yag

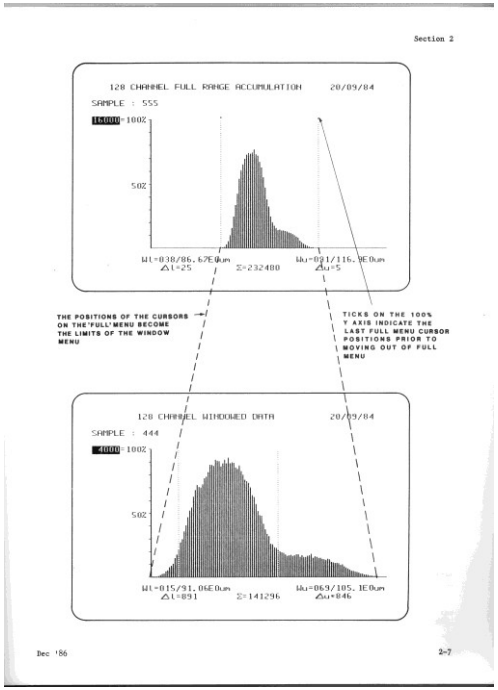
UV (Arc) Lampy

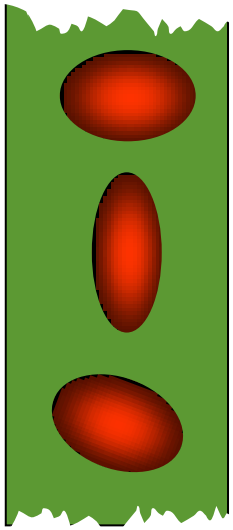
Mercury, Mercury-Xenon

Mack Fulwyler- sorter

Mack Fulwyler - sorter 1965 - Los Alamos National Labs – jeho sorter separoval částice na základě elektronicky měřeného objemu (stejný princip jako Coulter counter) a separoval pomocí elektrostatického vychýlení.

Cílem bylo sortrovat červené krvinky,protože u nich byla naměřena bimodální distribuce buněčného objemu. Princip separace byl založen na principu inkoustové tiskárny Richarda Sweeta ze Stanfordu (1965)





Po té co bylo objasněno, že bimodalita červených krvinek je artefakt byla tato skupina schopna separovat neutrofily a lymfocyty z krve.

Richard Sweet

Richard Sweet vyvinul elektrostatickou inkoustovou tiskárnu jejíž princip využil Mack Fulwyler pro svůj buněčný sorter.

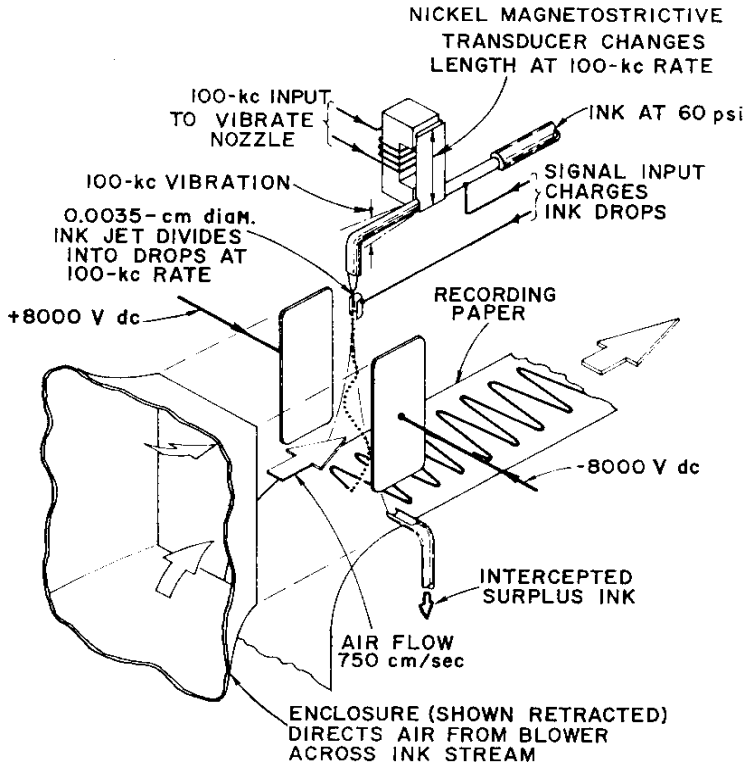


FIG. 1. Ink-jet oscillograph.

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)

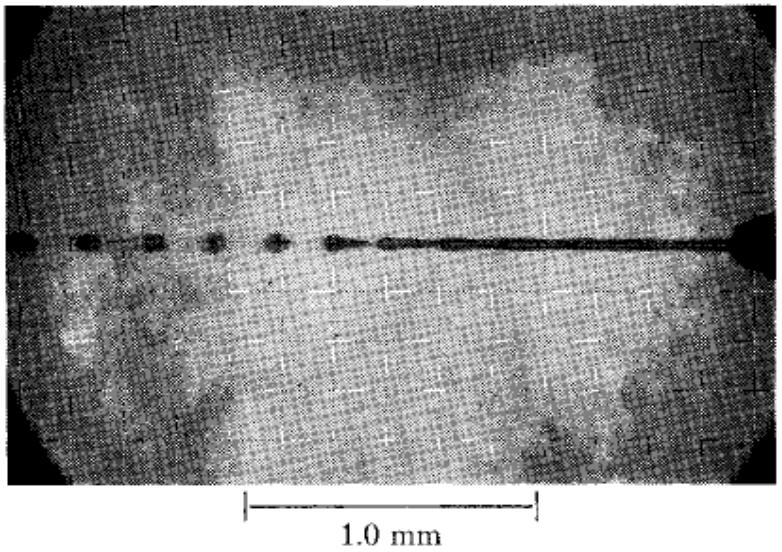


FIG. 3. Ink-drop formation.

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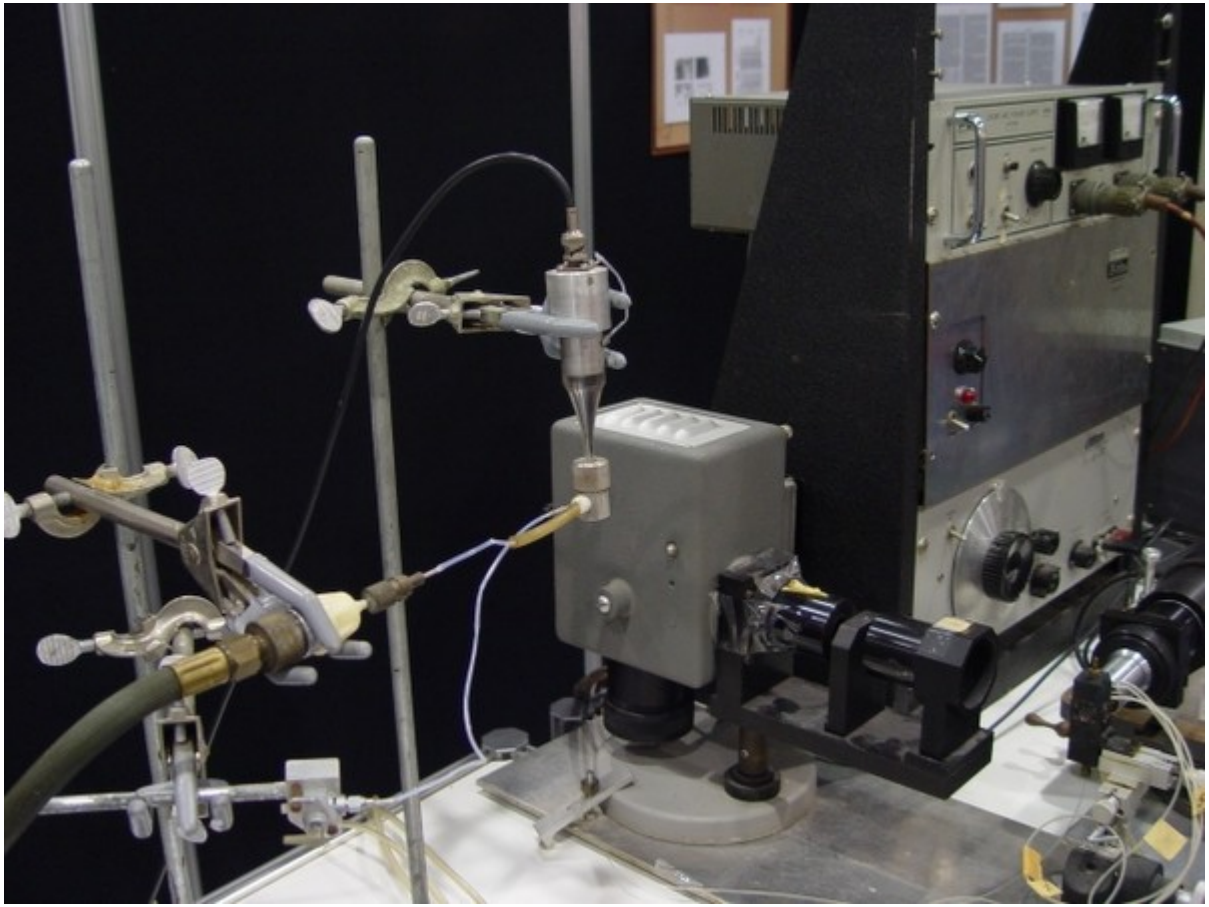
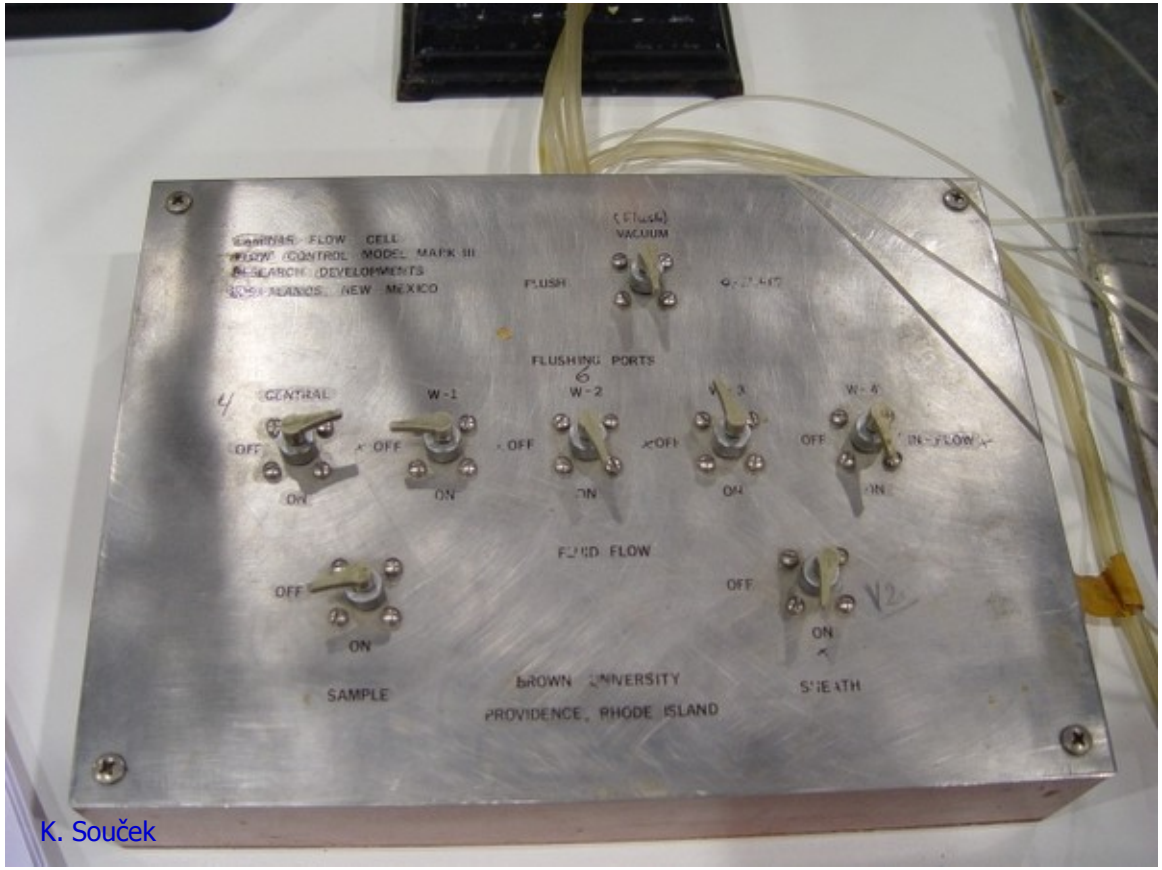
Mack Fulwyler- sorter



Mack Fulwyler- sorter



K. Souček

Cytometry Part A 67A:61–67 (2005)

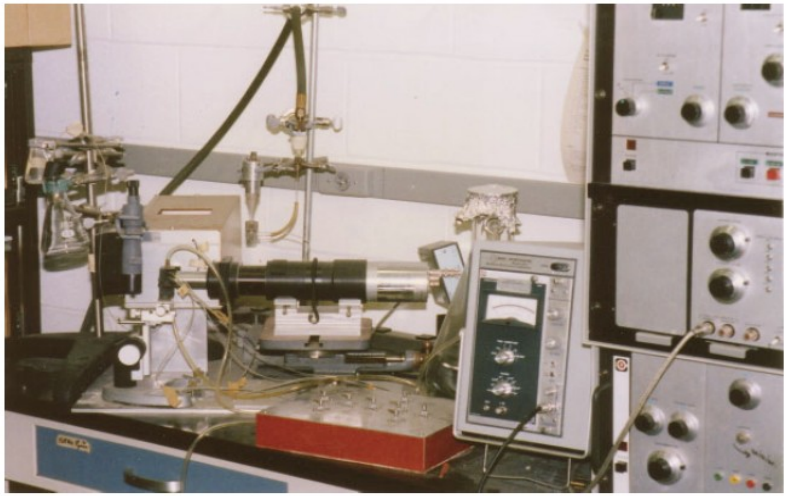
Mack Fulwyler in His Own Words

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

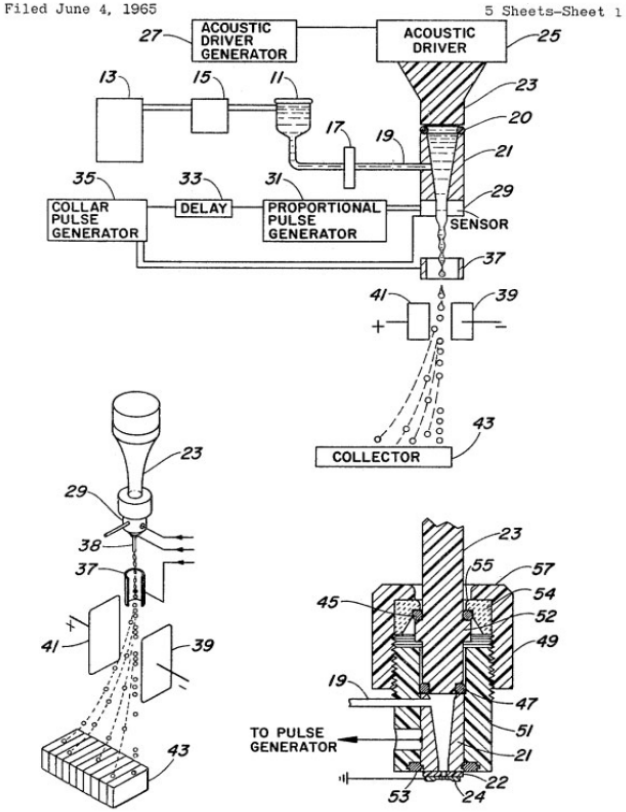


FIG. 4. A page from Fulwyler's patent on the cell separation technology patent #3,380,584 showing the fundamental components of the invention of the cell sorter.

INVENTOR.
Mack J. Fulwyler
BY

K čemu to všechno je… například…

ASLO

Association for the Sciences of Limnology and Oceanography

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Position Announcements

Flow Cytometer Research Technician

Flow Cytometer - Research Technician

The Ocean Biogeochemistry Laboratory at Bigelow Laboratory of Ocean Sciences is seeking an energetic and motivated technician to join our research group. The primary responsibility of the successful candidate will be operation of our high-speed flow cytometer/ cell sorter in support of our oceanographic research. This technical position requires extensive knowledge of cytometric principles, an ability to troubleshoot and maintain analytical instrumentation, prior experience in method development, a willingness and capability of going to sea to operate the flow cytometer (cruises from 1 to several weeks), and a high degree of self-confidence and independence. A wide diversity of sample types are analyzed ranging from enumerating and sorting single cells from oceanic samples to quantification of cellular rate processes employing fluorogenic assays to combining isotopic methods with flow sorting.

The successful applicant must have at least a B.Sc. degree and 2+ years of demonstrated experience with flow cytometric cell sorters as a primary operator. The successful applicant must also be highly organized, have a strong ability to multi-task, be self-confident and independent. This position will initially be for one year with continuation for additional years based upon successful job performance.

Send CV, cover letter, and contact information for 3 references to jobs@bigelow.org. Please reference (RT-2012-4) in the subject line. Review of applicants will begin immediately and continue as applications are received until the position is filled. The preferred starting date is no later than 1 January 2013, but this may be negotiated. Salary will be commensurate with prior experience. Bigelow Laboratory is an Equal Opportunity Employer.

Submission Forms
- Submit Job Announcements
- Submit Student Opportunities

Employment and Student Opportunities
- Positions Offered
- Student Opportunities
- Job-Related Links

Career Information
- Aquatic Science Careers
- Careers in Public Policy
- Early Career Scientists

Programs and Opportunities
- Programs for Recent PhDs

Moss Landing Marine Laboratories

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- Contact Information
- Map & Directions

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- Events
- News
- Seminars

4/9/12 - Job Opportunity: Biological Oceanography Technician

Date: April 9, 2012 - 8:00am
Biological Oceanography – Job Opportunity

The Biological Oceanography Lab is seeking assistance (half-time or more) on Dr. Welschmeyer's ballast treatment testing project associated with the California Maritime Academy (CMA). The duties will include training/execution

many routine chemical and biological oceanographic analyses (e.g., DOC/POC, chlorophyll a, live organism microscopy, epifluorescence microscopy, flow cytometry, bacteria culturing assays, etc.). The employee will travel often between MLML and CMA.

Requirements: the employee must be available for two consecutive 48 hour periods (or more) each week; full time work is ideal. (Student schedules with M-W or T-Th classes will not work). A willingness to work long hours (odd hours) is mandatory; excellent pipeting skills, notetaking, and spreadsheet skills would be appreciated (state drivers license is helpful). Pay is $20/h immediately; work will proceed through summer with possible sampling trips to southern California and South America.
Please contact Dr. Nick Welschmeyer by email (Welschmeyer@mlml.calstate.edu) for information.

Position Available

FLOW CYTOMETRY TECHNICIAN

Oceanography, MIT

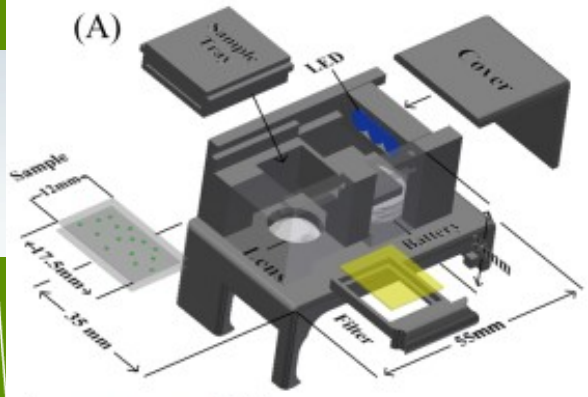
The Chisholm Laboratory at MIT (http://web.mit.edu/chisholm/www/) is seeking a full-time flow cytometry technician to participate in research involving oceanic cyanobacteria. The position requires a Bachelors degree in science or engineering and two years experience. Applicants must have a solid background in and experience with flow cytometry, including extensive knowledge of hardware, data analysis, and experimentation. Duties include maintenance of flow cytometry instruments, experimentation, and assisting other lab members with flow cytometry as needed. Must be able to work as a member of a multidisciplinar[redacted]

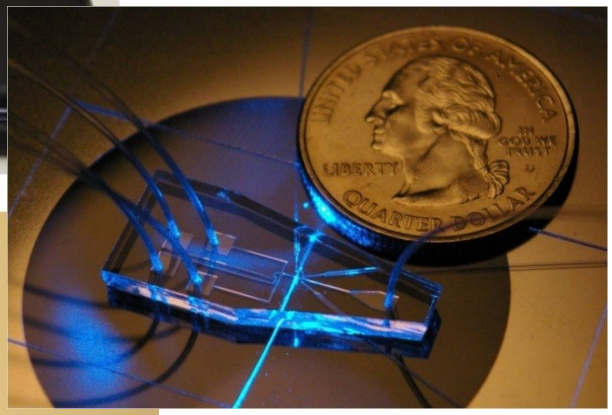
Please send a resume and 3 letters of recommendation to Dr. Marcia Osburne (mosburne@mit.edu), or Dr. Marcia Osburne, MIT, 15 Vassar St. rm 48-336B, Cambridge, MA 02139

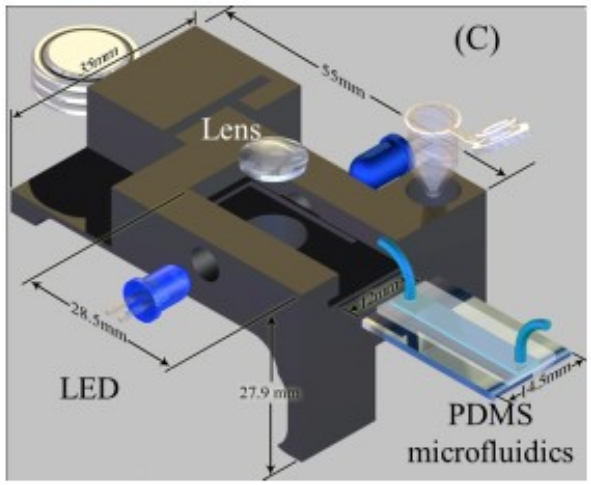
CYTOKINETIKA

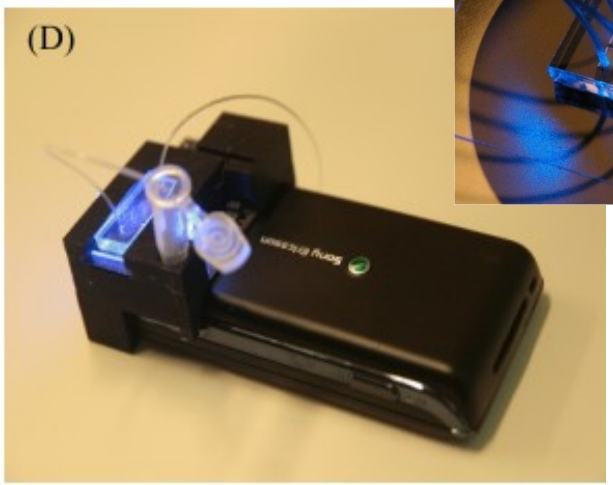
K čemu to všechno je… například…

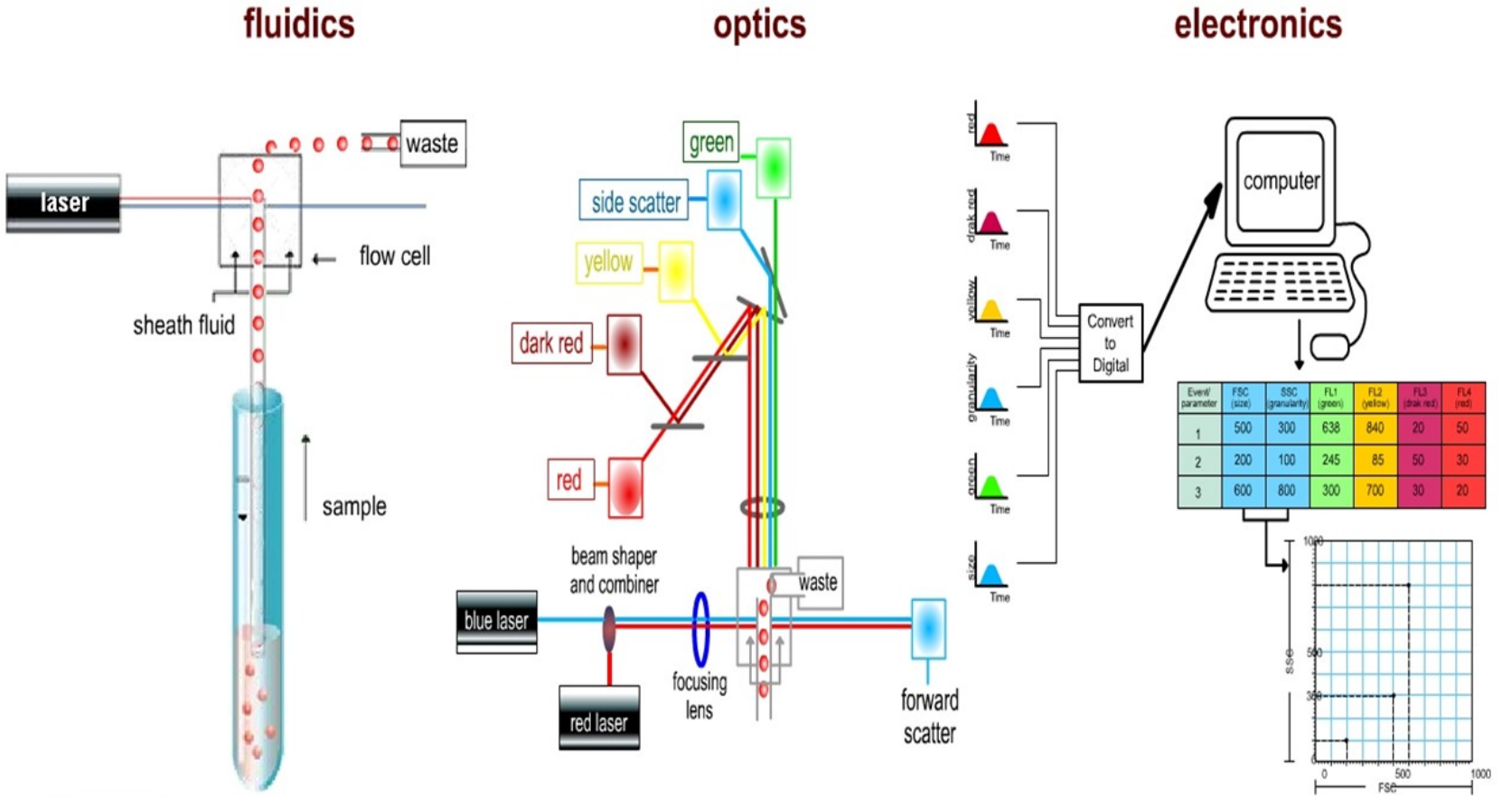
- 43 miliónů lidí na světě je infikováno virem HIV (WHO)
- ročně zemře ~ 2 miliónu lidí na HIV/AIDS (v Africe je ~ 11 miliónu AIDS sirotků)
- kvantifikace CD4 T lymfocytů je klíčový parametr při monitorování léčby
- Průtoková cytometrie je „zlatý standard“
- Optimalizované postupy a zařízení pro levné (< 3 EUR / vzorek) a rychlé detekce (250 vzorků / den)
- Aydogan Ozcan: „Kill the cost. safe live“

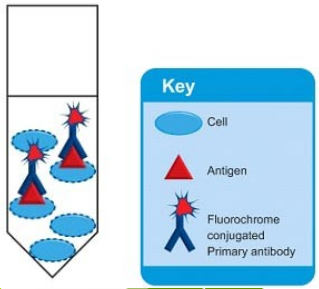








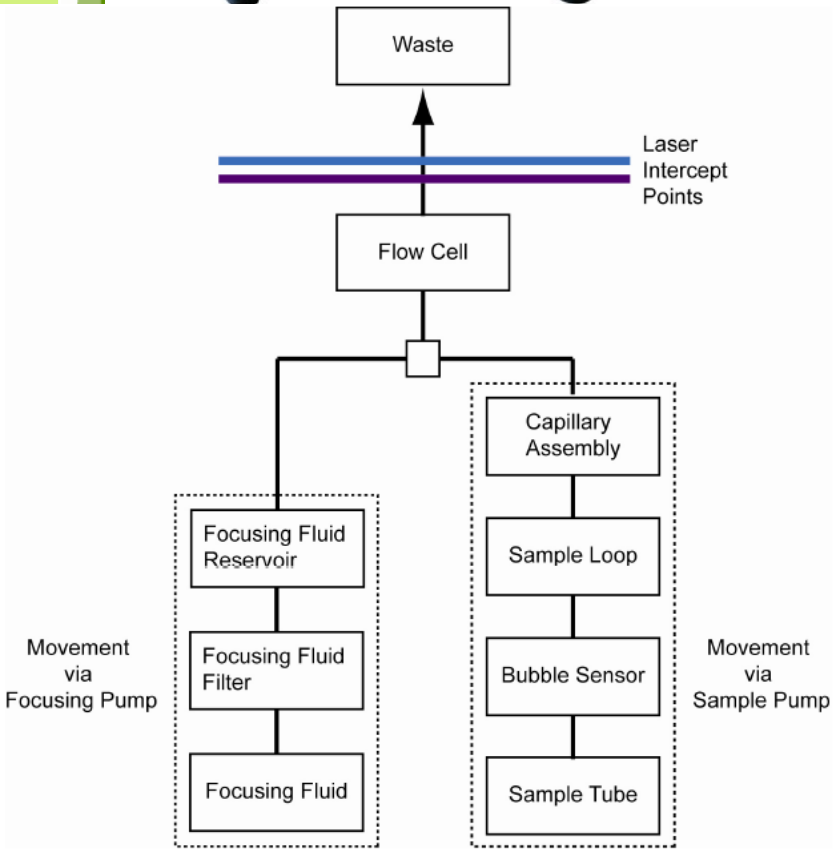

fluidics
optics
electronics
waste
laser
flow cell
sheath fluid
sample
green
side scatter
yellow
dark red
red
beam shaper
and combiner
blue laser
red laser
focusing
lens
waste
forward
scatter
Convert
to
Digital
computer
Event/
parameter
FSC
(size)
SSC
(granularity)
FL1
(green)
FL2
(yellow)
FL3
(drak red)
FL4
(red)
1
500
300
638
840
20
50
2
200
100
245
85
50
30
3
600
800
300
700
30
20
SSC
FSC
0
500
1000

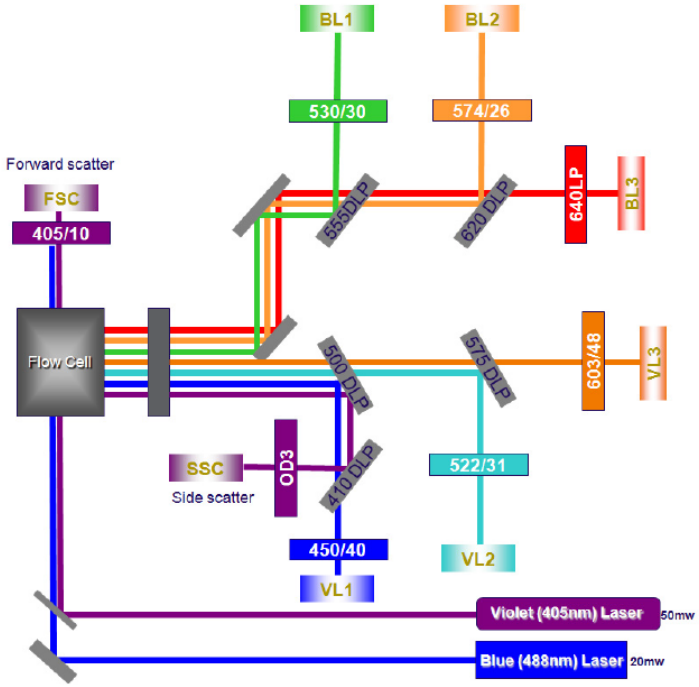

Key
Cell
Antigen
Fluorochrome
conjugated
Primary antibody

Fluidika - Laminární vs. turbulentní proudění

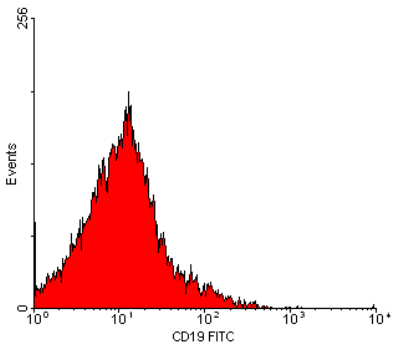
- **Turbulentní** proudění je charakteristické chaotickými (stochastickými) změnami
- **Laminární** proudění – kapalina proudí v paralelních vrstvách které se vzájemně nemísí

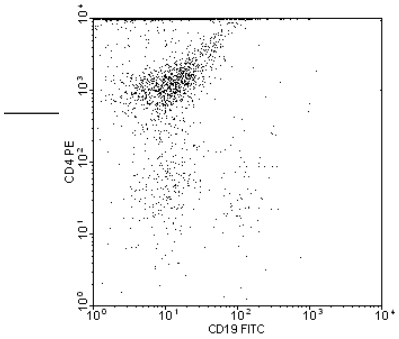
life technologies

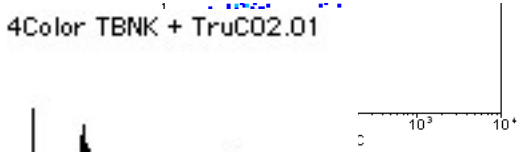


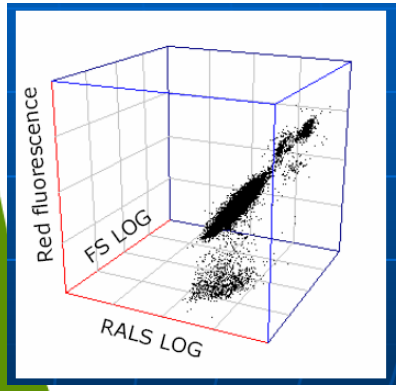


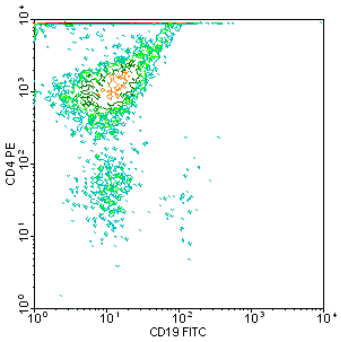
Způsoby pro zobrazení dat

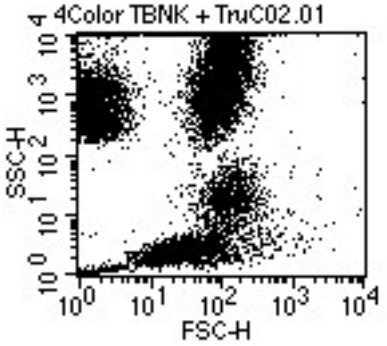


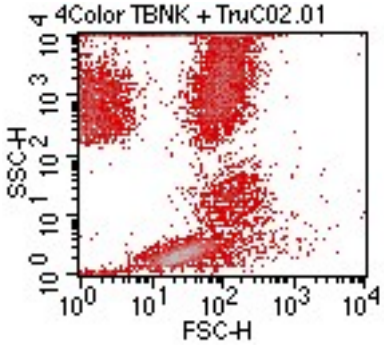


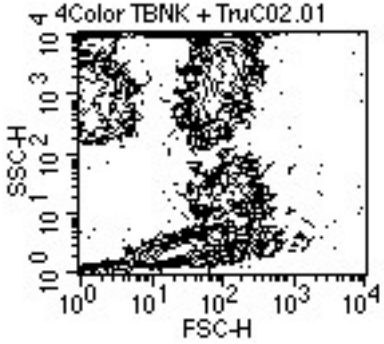




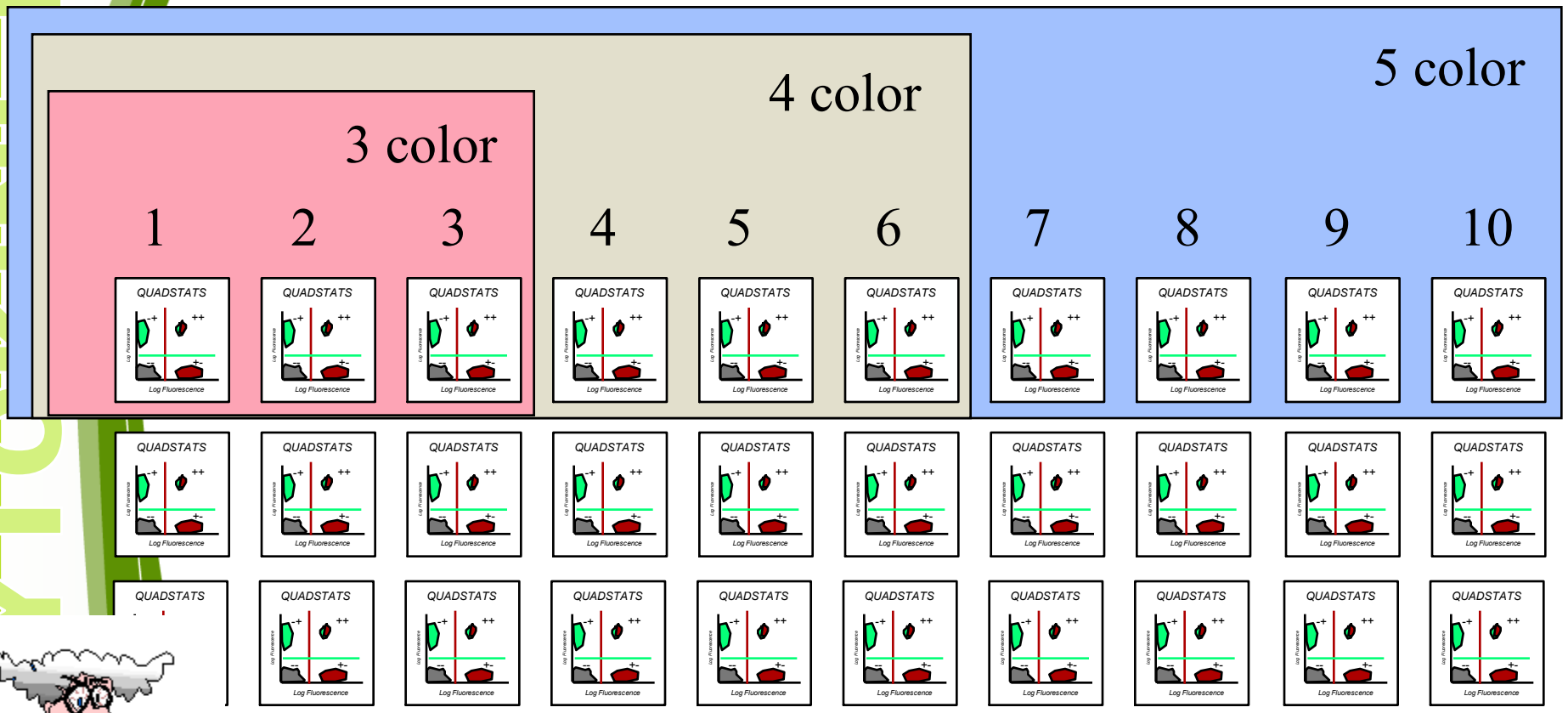








Vícebarevné analýzy generují mnoho dat…



upraveno podle J.P.Robinson

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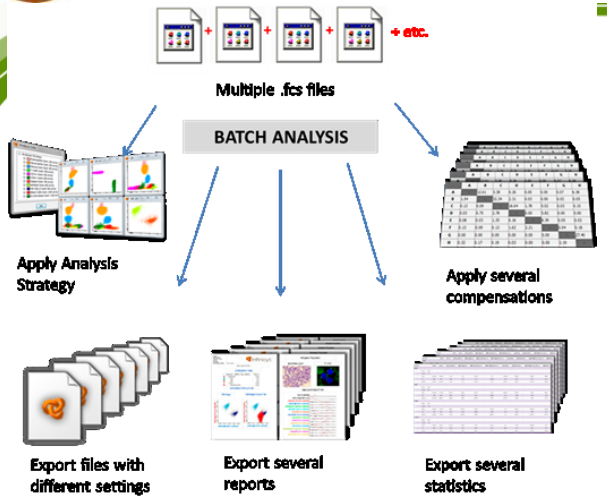
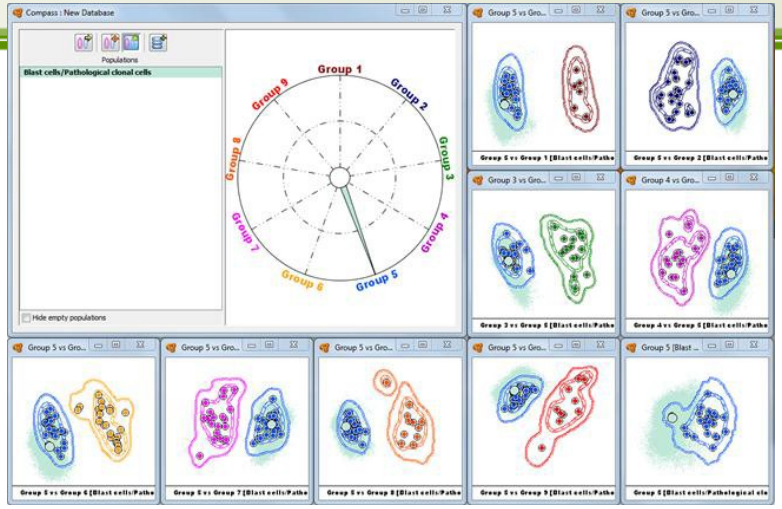
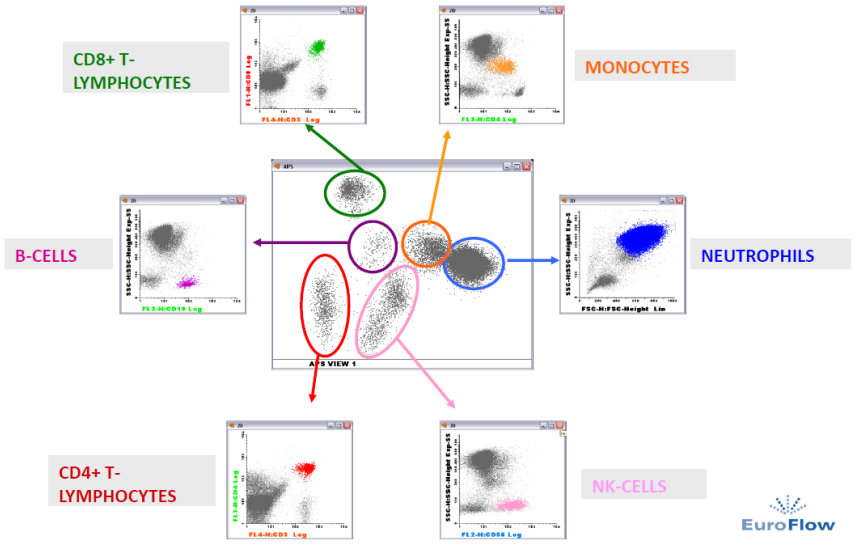
Infinicyt™



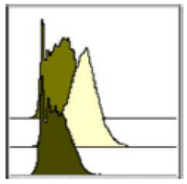
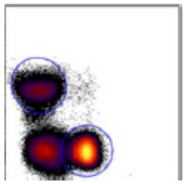
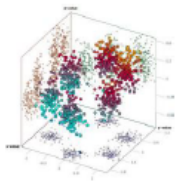
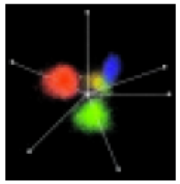
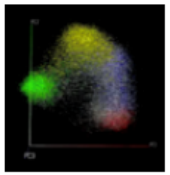
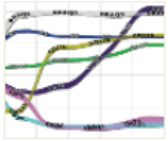
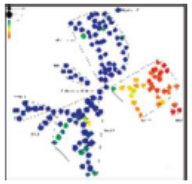
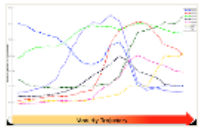
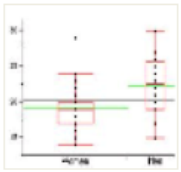
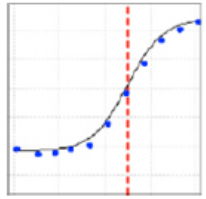


Automatic Population Separator



EuroFlow

Analyze: Cytobank

Plot raw data	 Histogram	 Biaxial plot	 3D plot	 Radar	
Reduce dimensionality	 PCA	 Gemstone	 SPADE	 viSNE	 Wanderlust
Summarize statistics	 Box plot	 Heat map	 Network	 Sunburst	 Dose curve

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Cell tracking and proliferation

CYTOKINETIKA

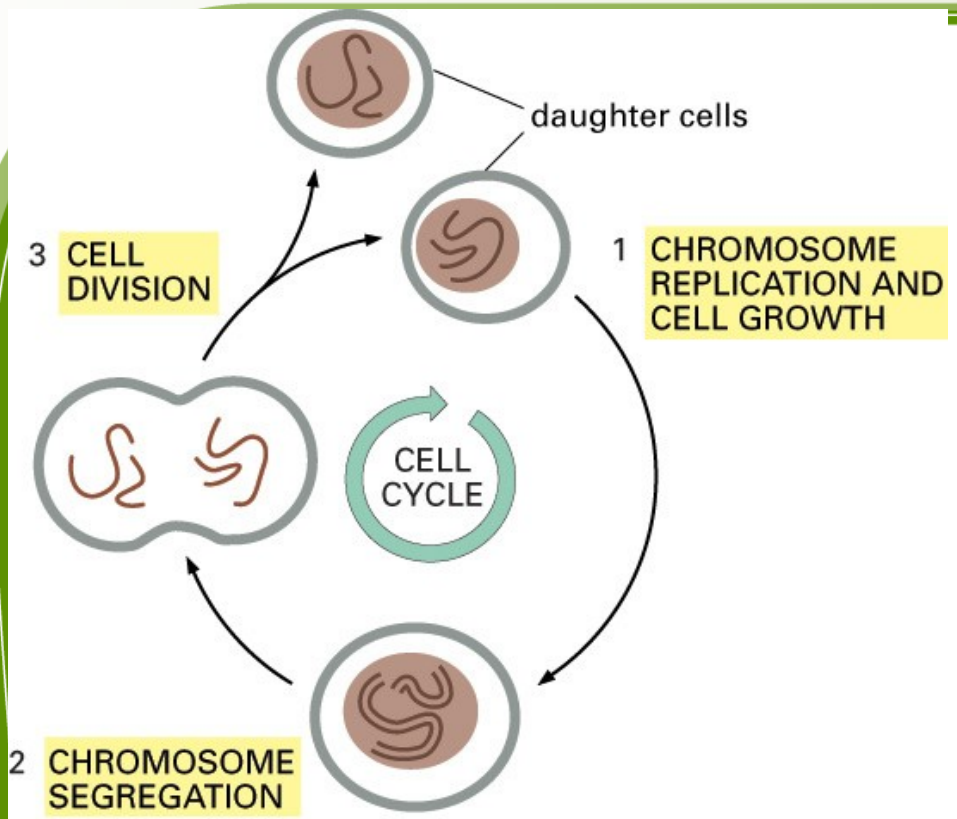
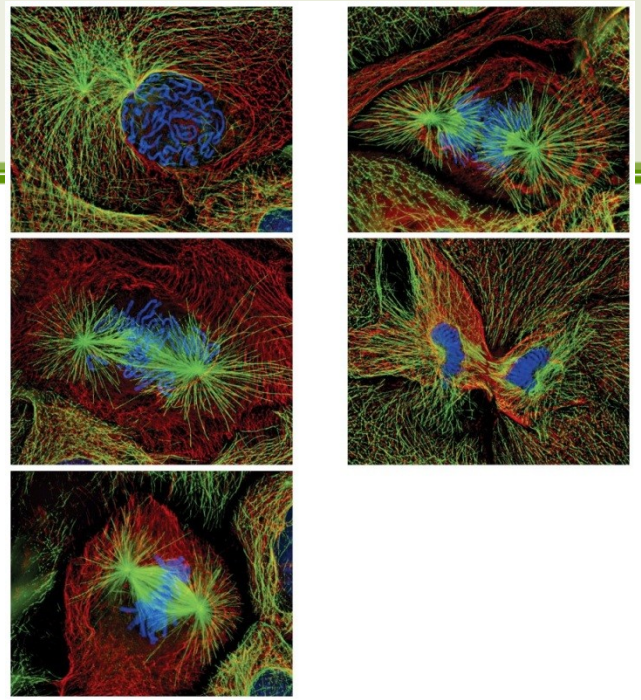
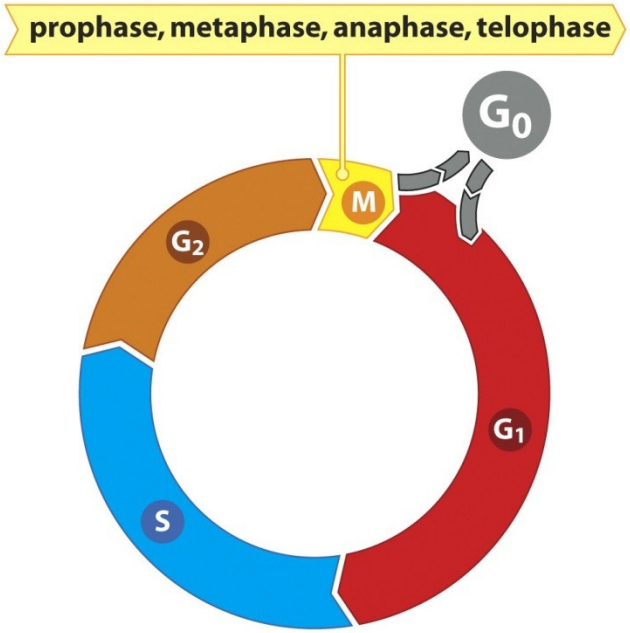


Figure 17–1. Molecular Biology of the Cell, 4th Edition.





Approaches

- Cell cycle analysis
- DNA synthesis analysis
- Cell tracking

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Buněčný cyklus

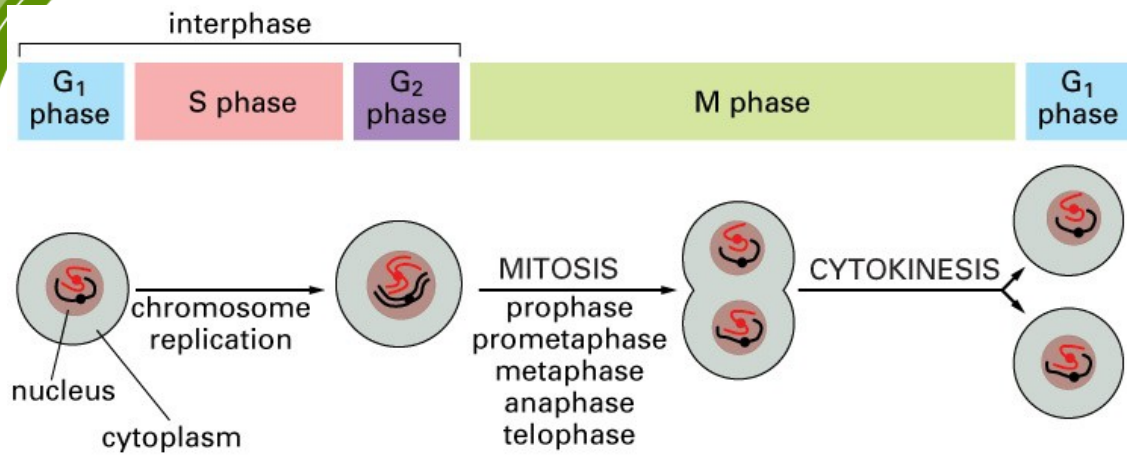
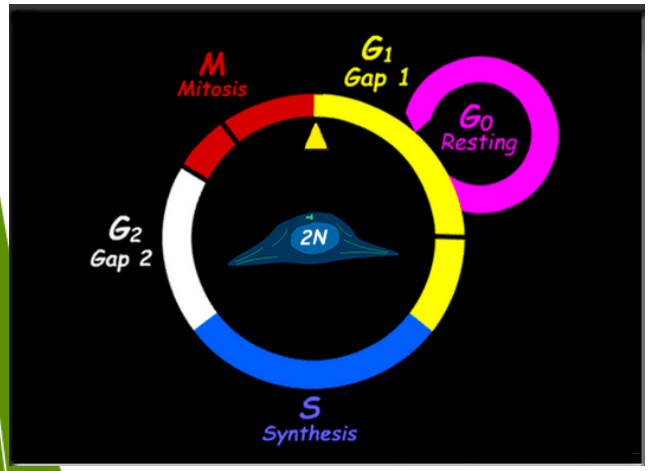


Figure 18–1. Molecular Biology of the Cell, 4th Edition.



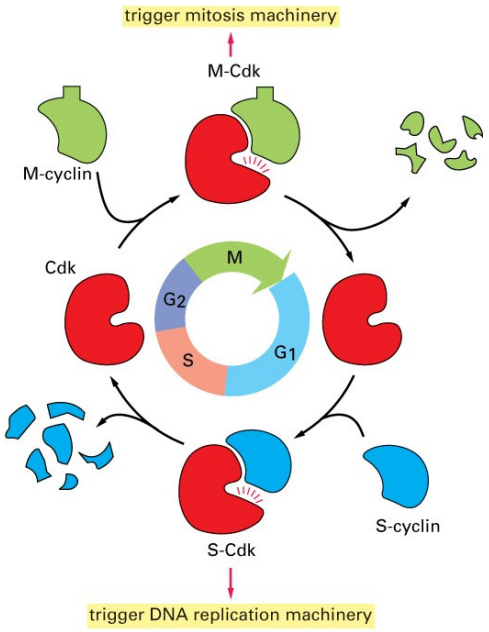


Figure 17–16. Molecular Biology of the Cell, 4th Edition.

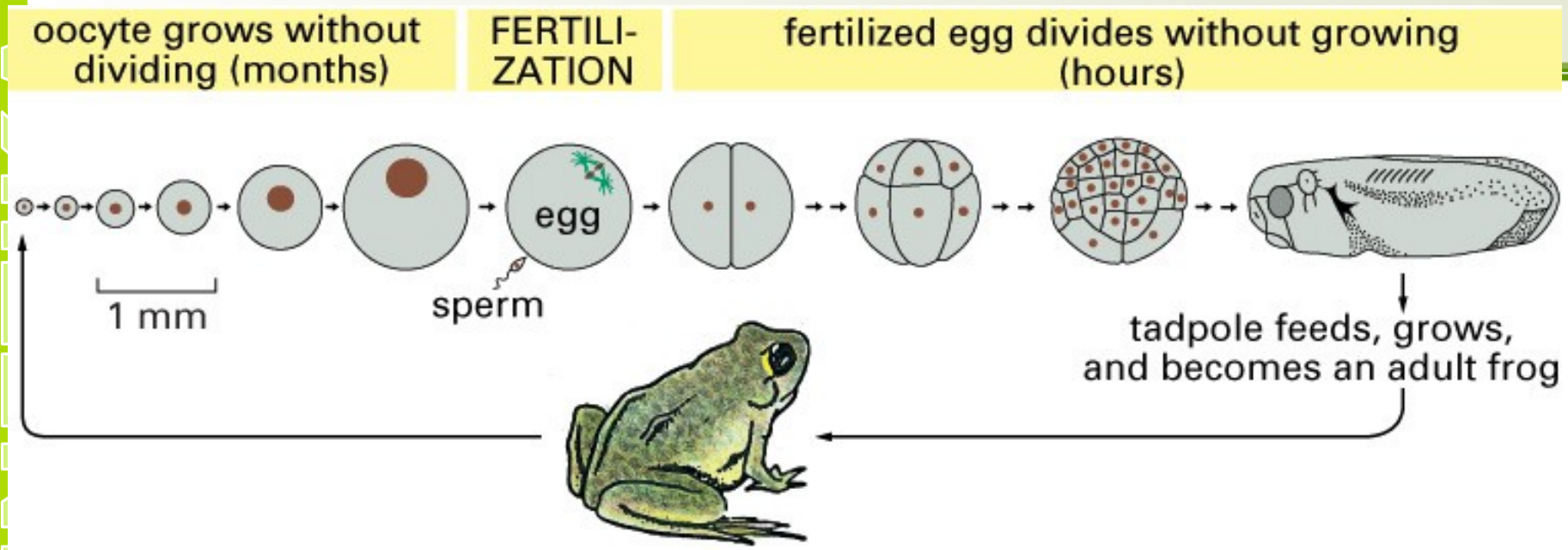
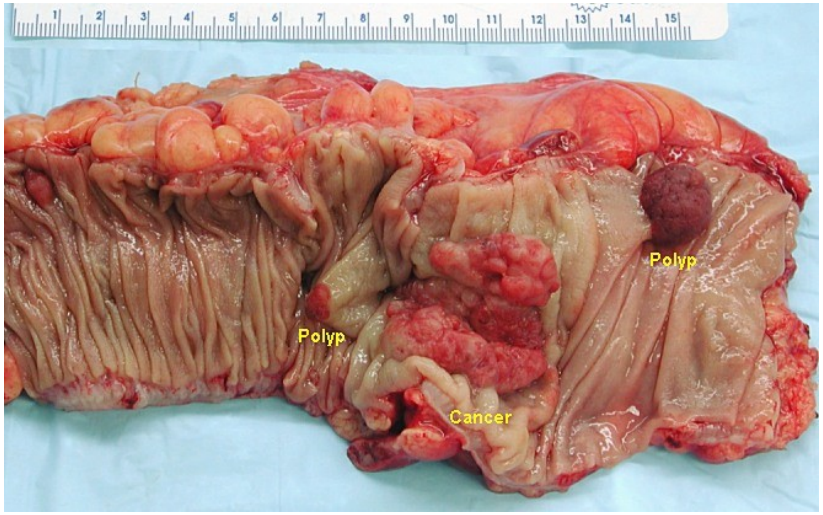


Figure 17–8. Molecular Biology of the Cell, 4th Edition.



Cell cycle analysis

- One of the oldes application of flow cytometry, analysis of the cells in cell cycle phases based on the quantification of DNA
- flow cytometry is convenient method for quick and relatively precise determination of cell cycle
- DNA is simply labeled using fluorescent dye specific for DNA
  - Propidium iodide
  - 4',6-diamidino-2-phenylindole (DAPI)

    - fluorescence increases after binding to DNA. Membranes have to be permeabilized.
  - Hoechst 33342
  - Vybrant® DyeCycle™
  - DRAQ5
  - Quaternary benzo[c]phenanthridine alkaloids (QBAs)

I. Slaninova, J. Slanina and E. Taborska, "Quaternary benzo[c]phenanthridine alkaloids--novel cell permeant and red fluorescing DNA probes," *Cytometry A*, vol. 71, no. 9, pp. 700-708, 2007.

- labeling of live cells (possible cytotoxicity)

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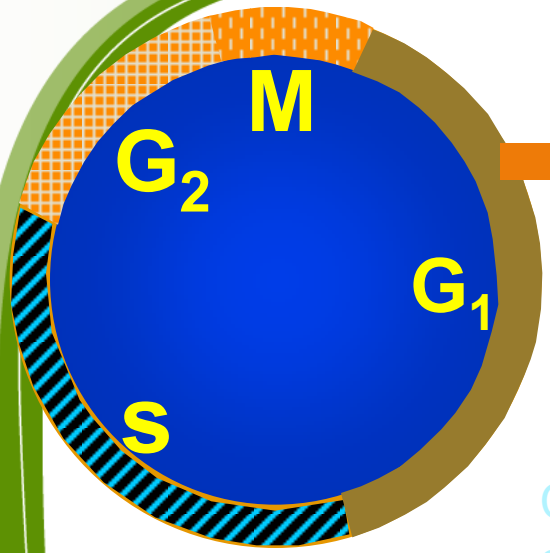
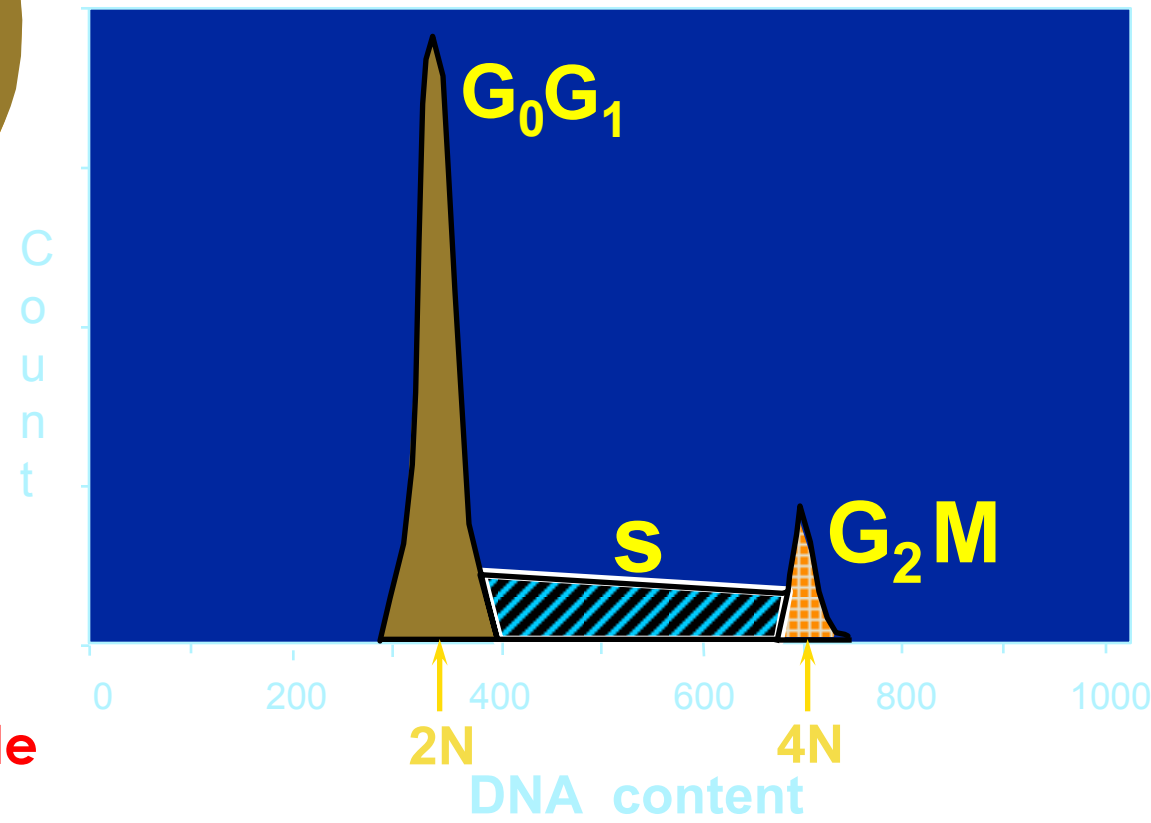
Normal Cell Cycle



G_0

DNA Analysis

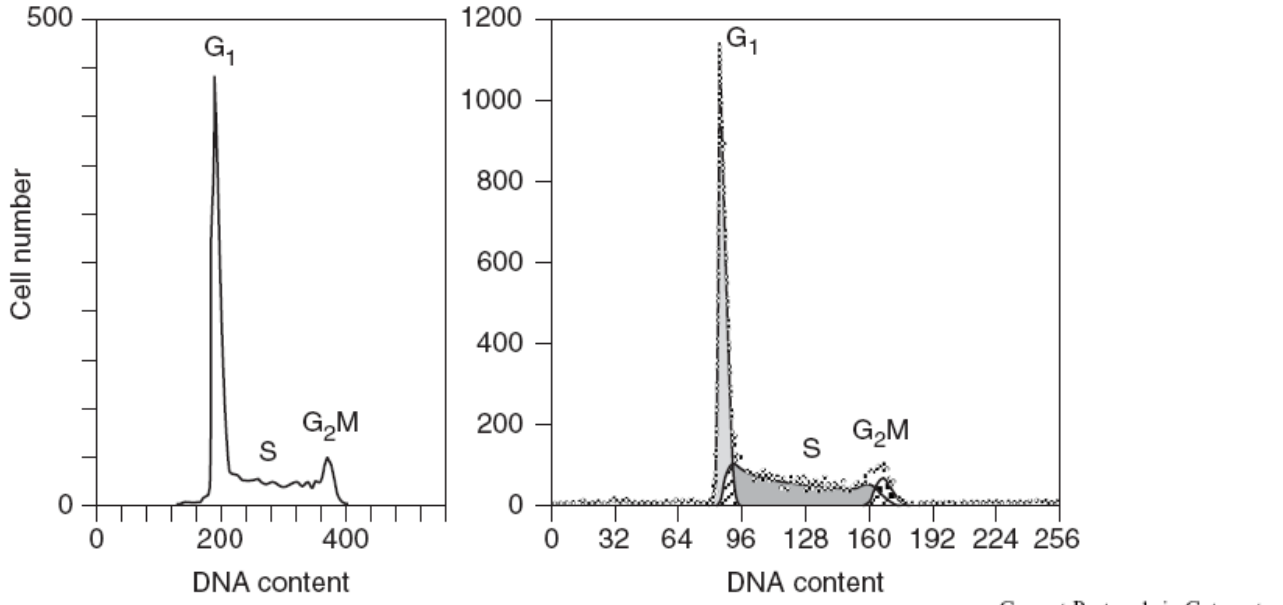


- propidium iodide
- DAPI
- Hoechst 33342
- 7-AAD

Purdue University Cytometry Laboratories

Cell cycle histogram: how to analyze?

- It is not recommended to statistically analyze it using simple gating in the histogram
- It is necessary to use software tolls for modeling of distribution of cell cycle phases



Current Protocols in Cytometry

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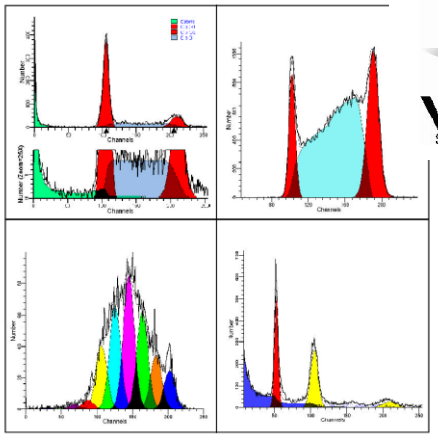
ModFit LT™

An impressive new version of the industry standard.



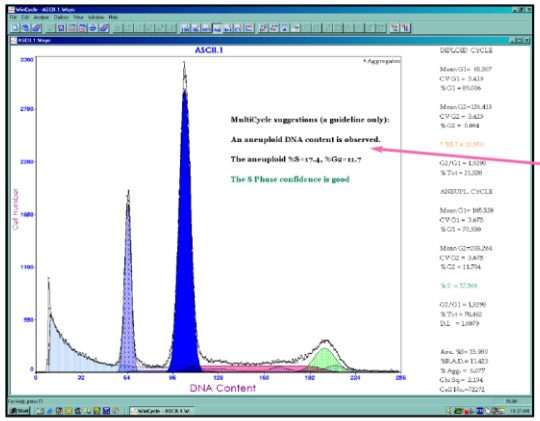
Verity Software House

PHOENIX FLOW SYSTEMS

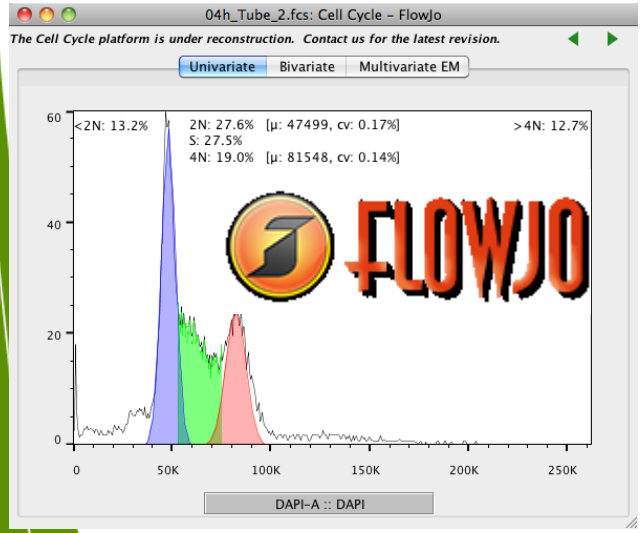
MultiCycle for Windows

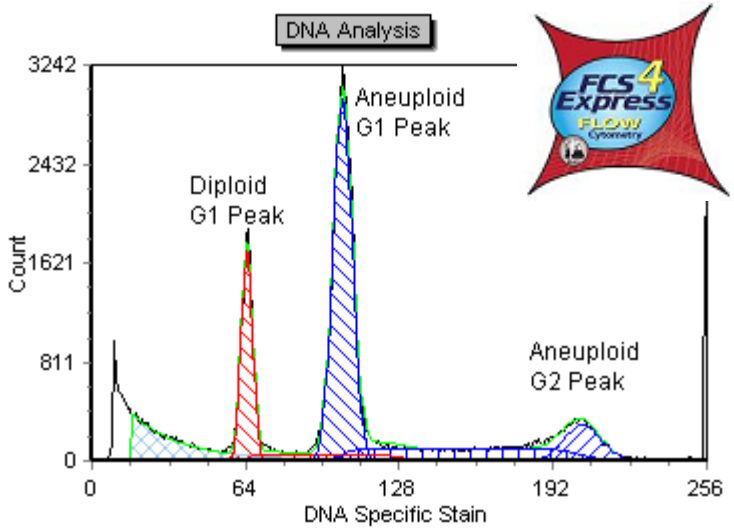
Advanced DNA Cell Cycle Analysis Program

MultiCycle AV fits 6 different cell cycle models automatically. The variability in results is one aid to assessing confidence in S and G2 phase estimates. Display of statistics is optional.



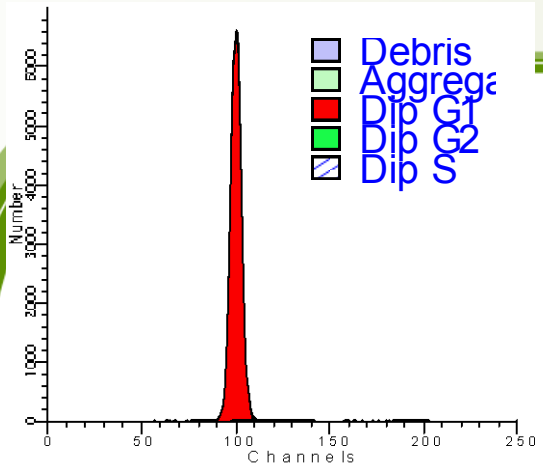
A **summary interpretation** clearly states results. A built-in decision tree helps take the guesswork out of evaluating the quality of the cell cycle analysis.

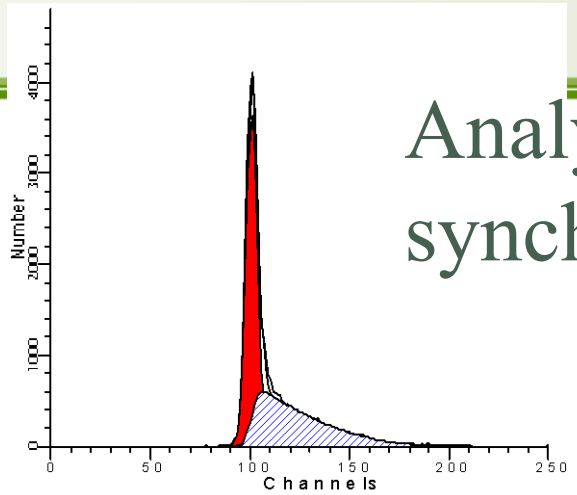


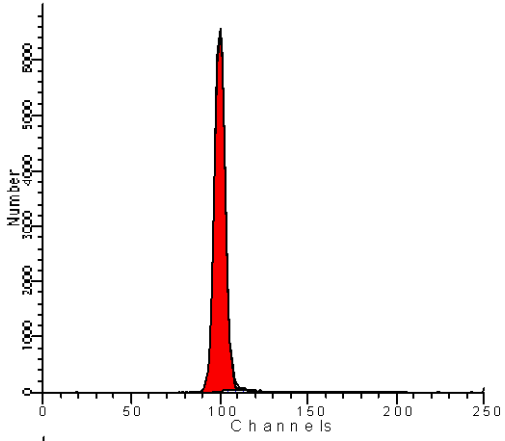


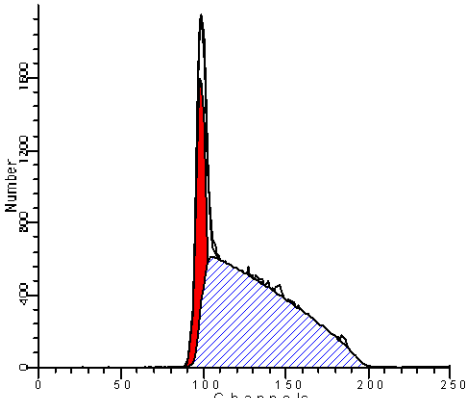
CYTOKINETIKA

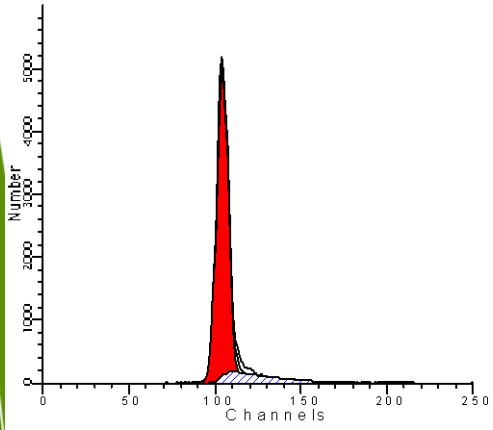
Analysis of synchronized cells

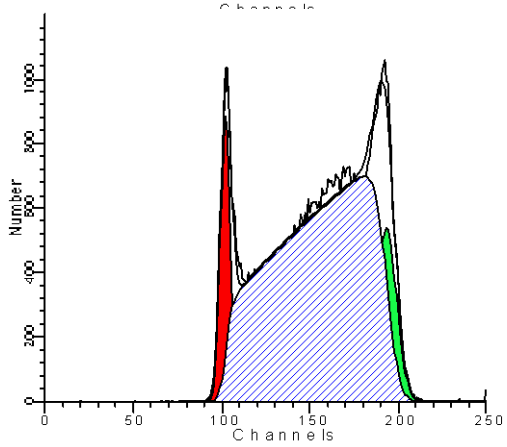


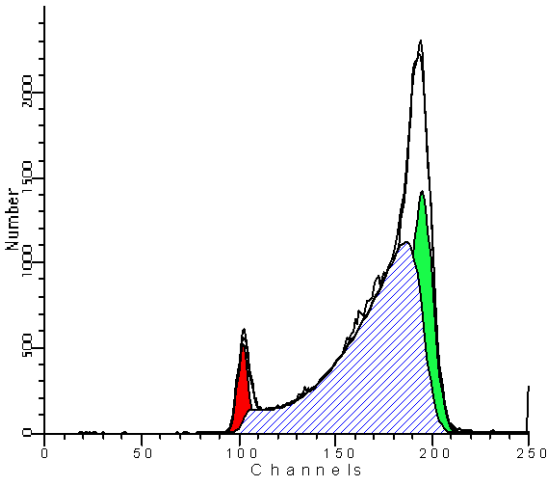








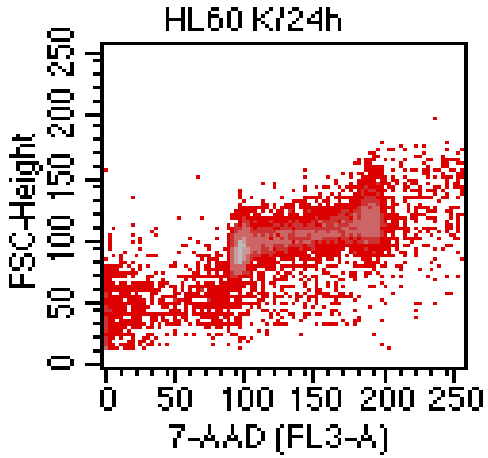


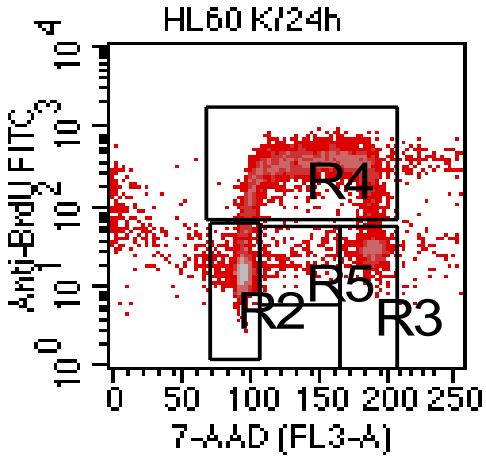


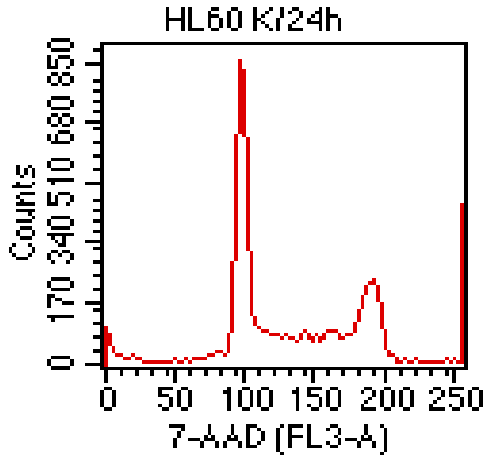
Analysis if BrdU incorporation

- Bromodeoxyuridin (BrdU) is incorporated into DNA instead of thymidine during S-phase
- BrdU is detected using specific antibody after the fixation and partial denaturation of DNA (acid, DNAse)
- DNA can be stained in the last step

Analysis if BrdU incorporation



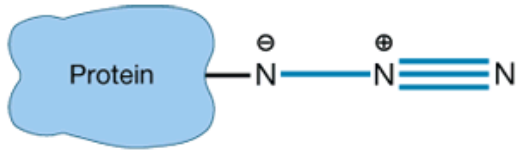




File: HL60 K/24

Regi	% Ga
R	100.(
R	35.4
R	10.2
R	47.8
R	1.3

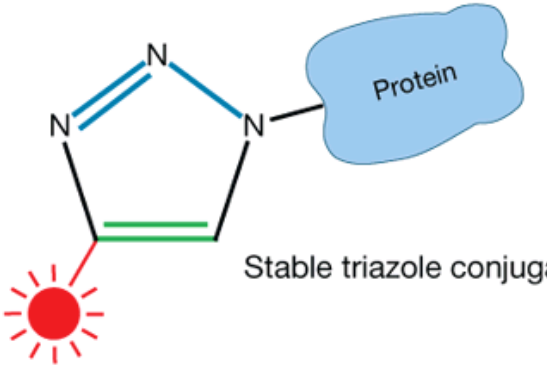
Click azide/alkyne reaction



Metabolically or enzymatically azide-modified protein

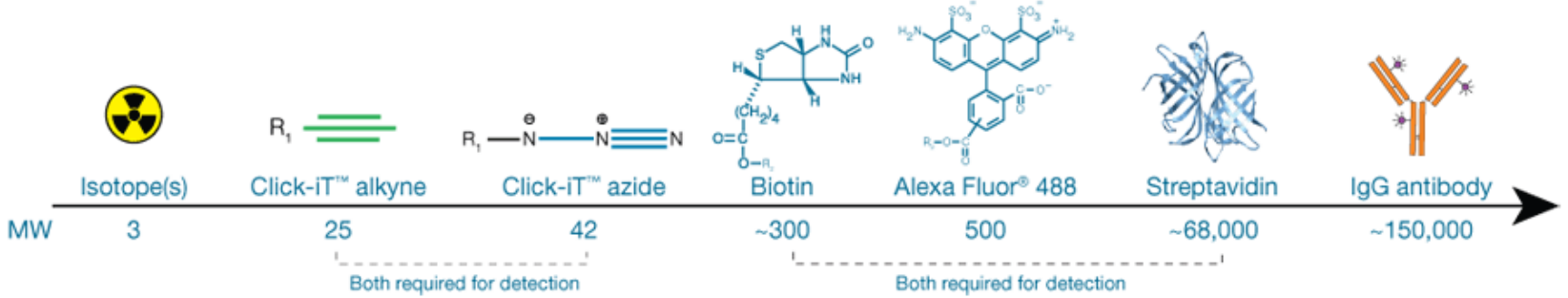
Cu(I), 1 hour

Room temperature



Stable triazole conjugate

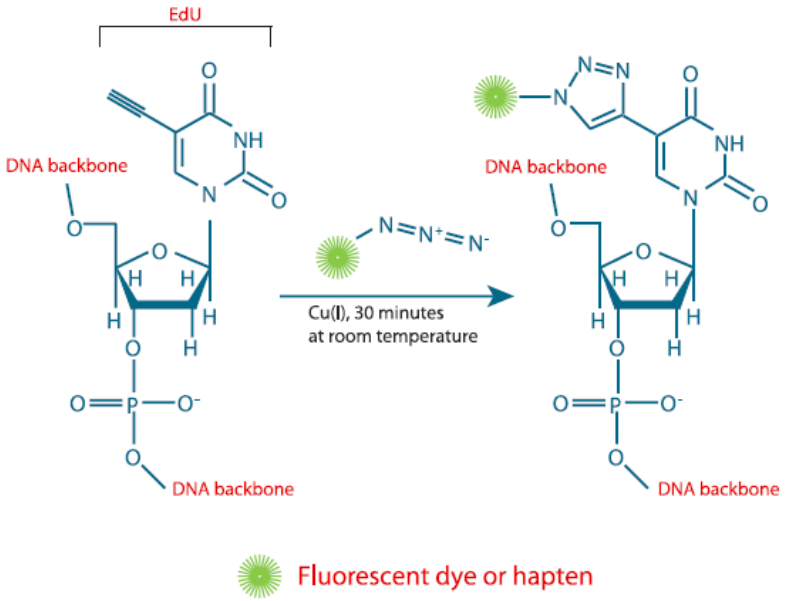
TAMRA, Dapoxyl®, or biotin alkyne



CYTOKINETIKA

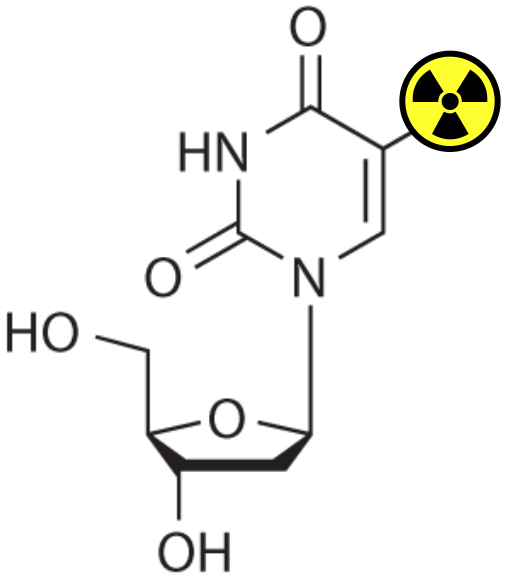
Click-IT (Invitrogen) applications

analysis of DNA synthesis (EdU - 5-Ethynyl-2'-deoxyuridine)



CYTOKINETIKA

^{3}H-thymidine



Tritiated (3H) thymidine

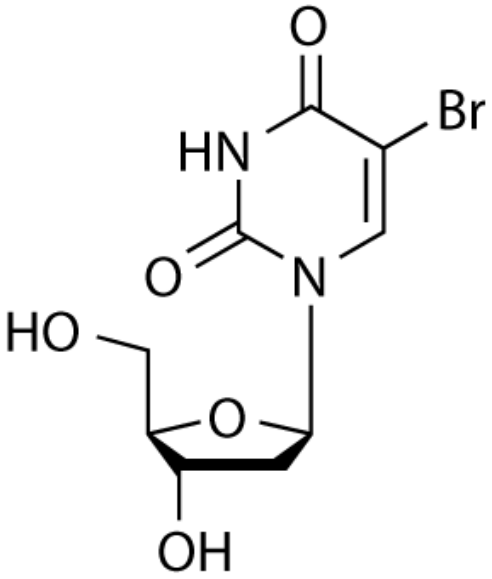
CYTOKINETIKA

^{3}H-thymidine

- Original method for measuring cell proliferation
- Radioactive
- Not compatible for multiplexed analyses

CYTOKINETIKA

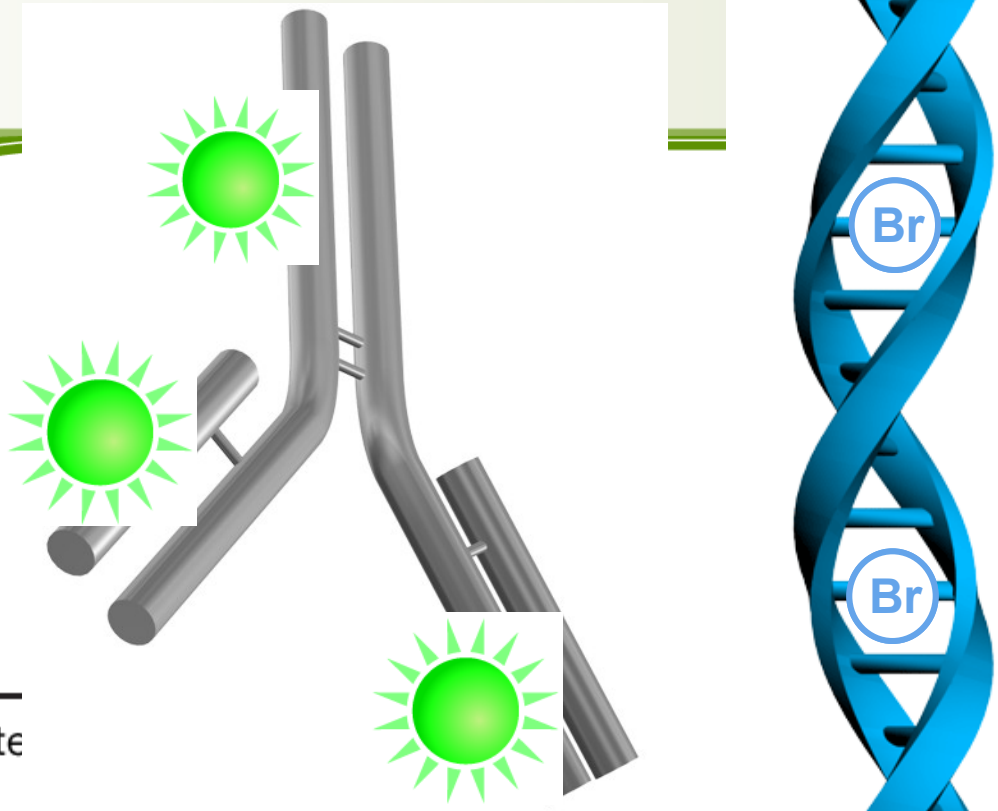
BrdU

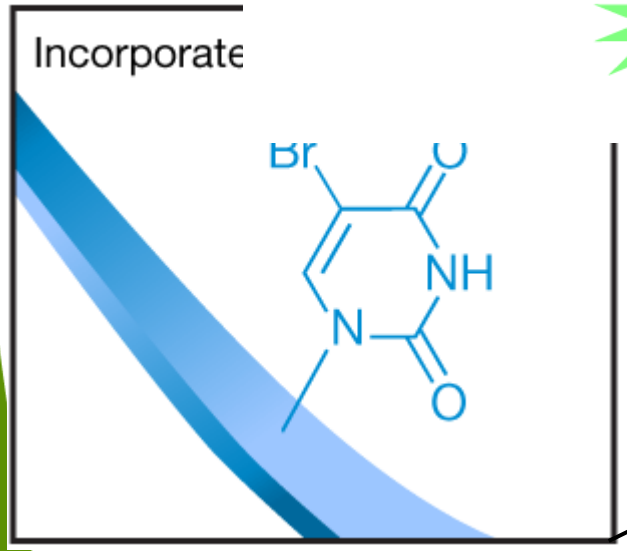


BrdU (5-bromo-2'-deoxyuridine)

CYTOKINETIKA

BrdU



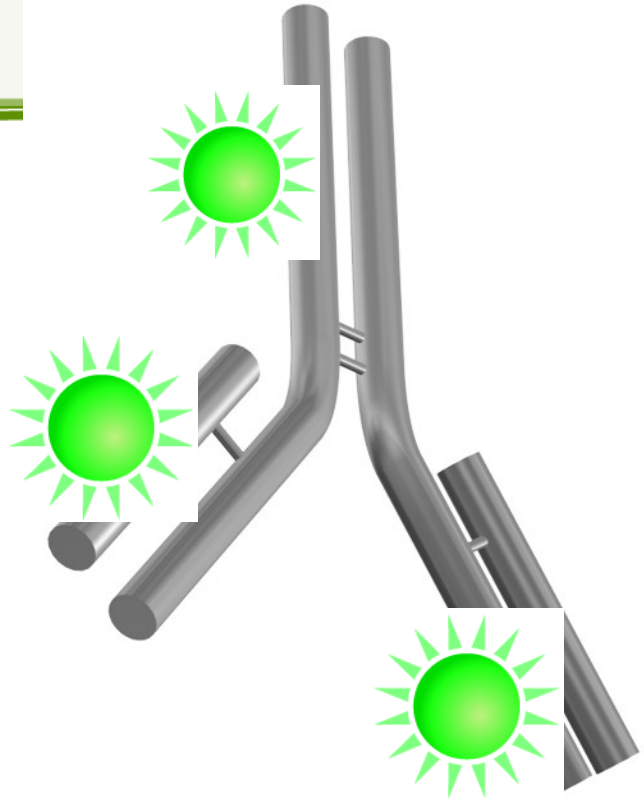


BrdU

Acid or DNase

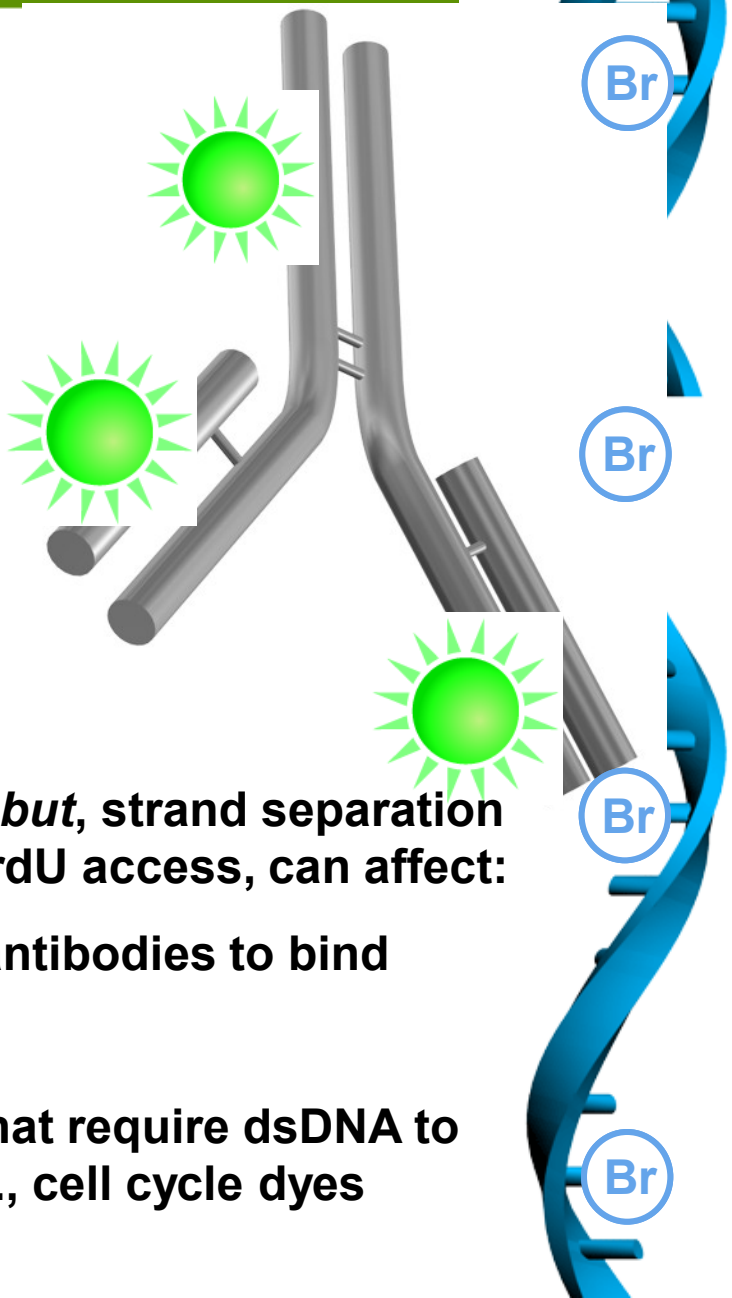
CYTOKINETIKA

BrdU



CYTOKINETIKA

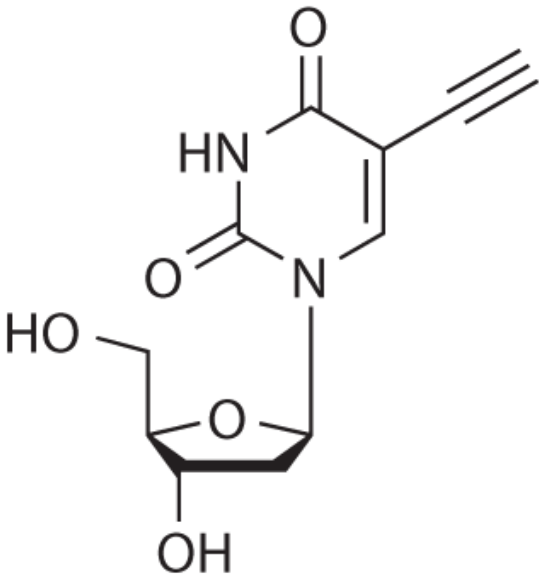
BrdU



- Non-radioactive
- Multiplex compatible *but*, strand separation requirement for anti-BrdU access, can affect:
  - Ability for other antibodies to bind
  - Morphology
  - Ability for dyes that require dsDNA to bind efficiently, i.e., cell cycle dyes

CYTOKINETIKA

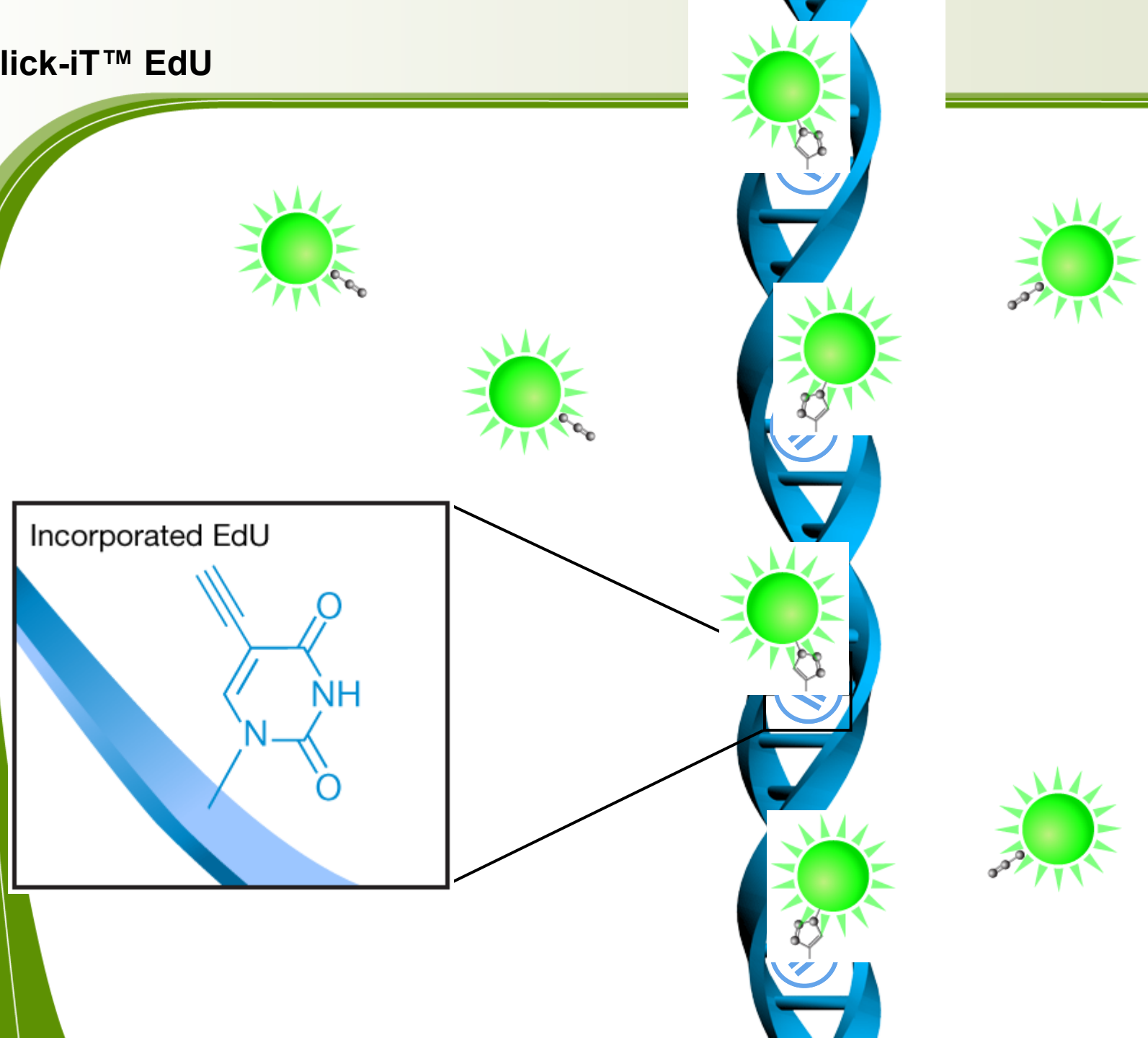
Click-iT™ EdU



EdU (5-ethynyl-2'-deoxyuridine)

CYTOKINETIKA

Click-iT™ EdU



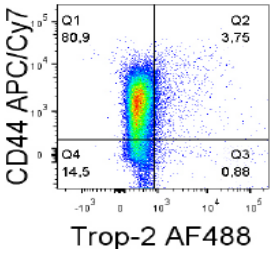
CYTOKINETIKA

Click-iT™ Edu

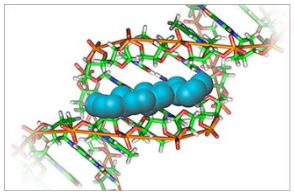
- Non-radioactive
- No DNA denaturation required
- Simplified protocol
- Small molecule detection
- Multiplex compatible, including
  - Other antibodies
  - Dyes for cell cycle analysis

Flow cytometry
most common application

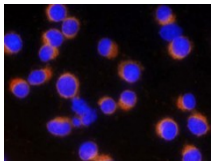
Immunophenotype characterisation of the cells
(CSCs markers, differentiation, …)



Cell Cylcle (DNA content, Cell cycle modulation after treatment)

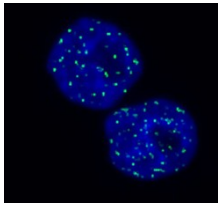


Cell Death analysis
(AnnexinV, Cleaved Caspase3, …)

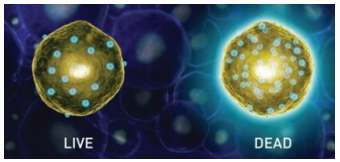


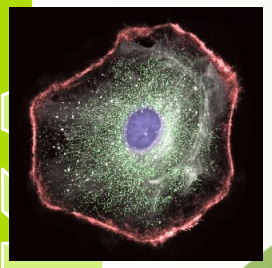
Proliferation (BrdU, EdU, mitosis - pH3)

DNA damage (yH2AX,…)

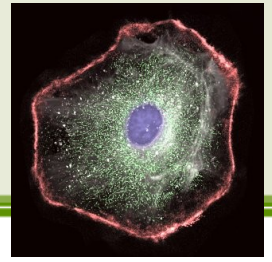


Viability assays (propidium iodid, CalceinAM, …)

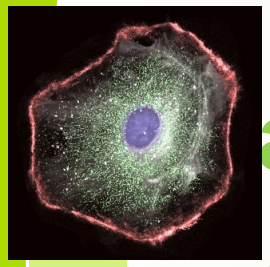




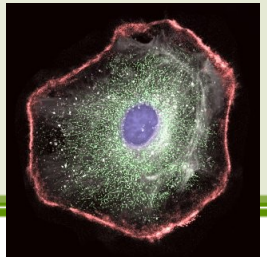
Example of final set-up



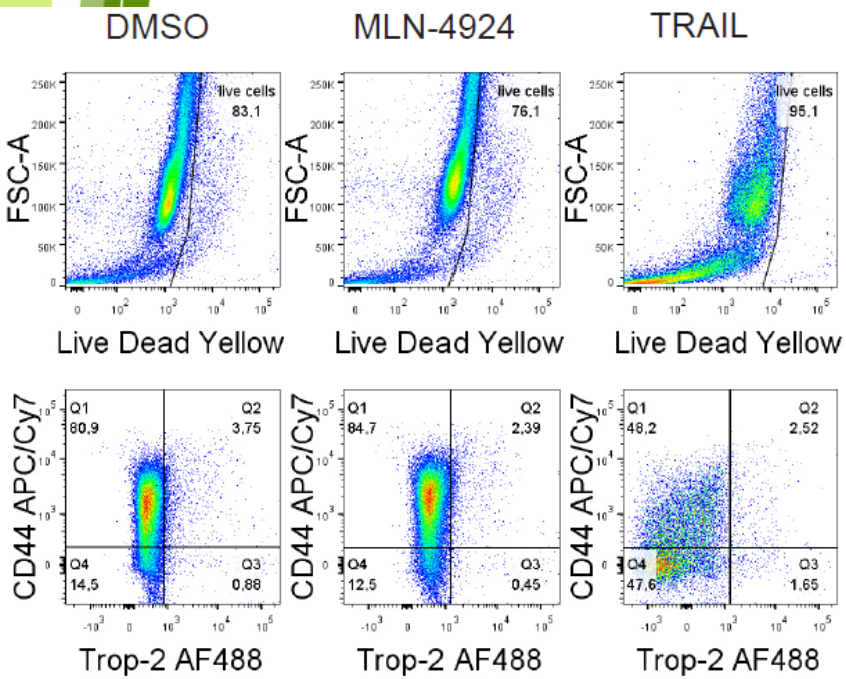
Parametr	Marker	Fluorochrome
Cell Surface Marker	CD44	APC/Cy7
Cell Surface Marker	Trop-2	AF488
Viability	LIVE/DEAD kit	Yellow
DNA synthesis	Click-iT EdU	AF647
Cell Cycle	DNA content	PO-PRO-1
DNA damage	yH2AX	PE
Apoptosis	Cleaved Caspase 3	AF494



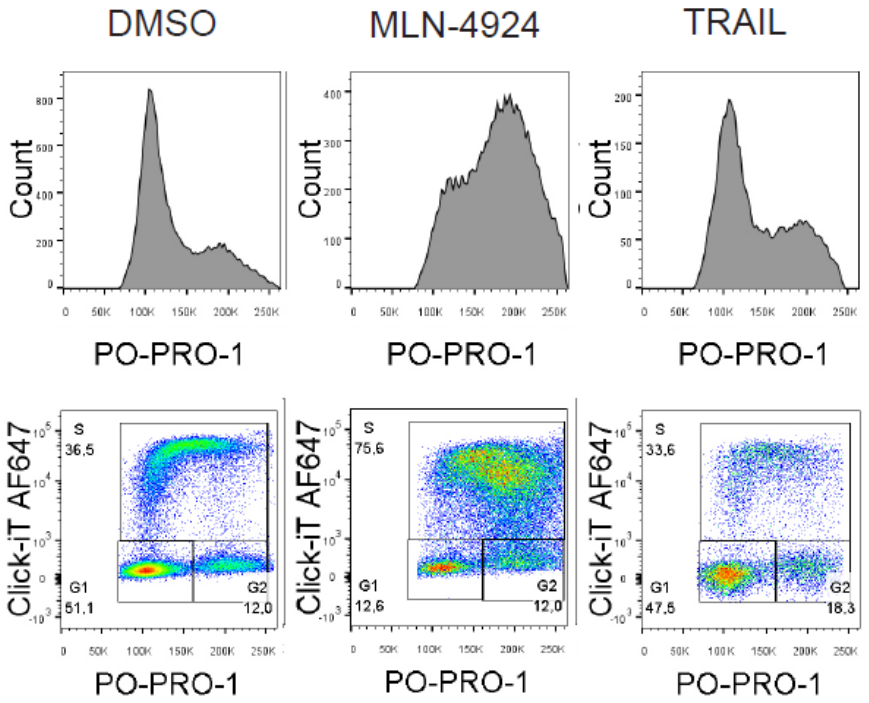
ample of final results (DU-145)



Viability and Immunophenotype

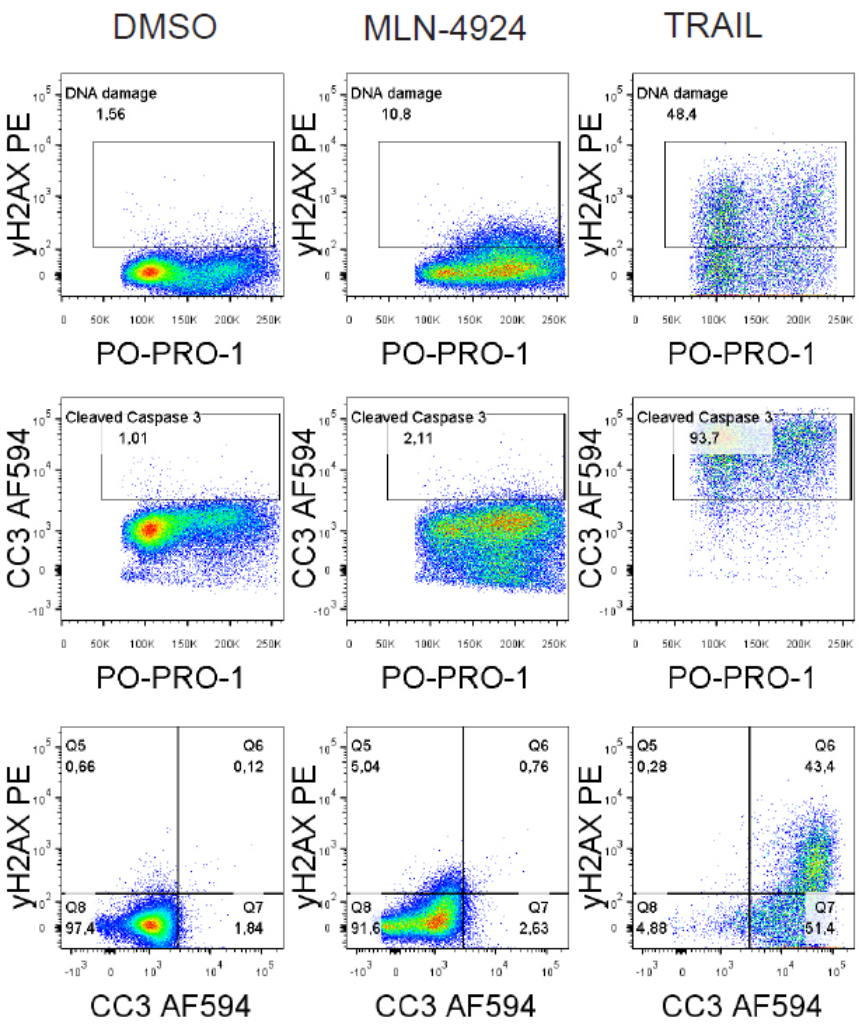


Cell cycle and Proliferation



ample of final results

DNA damage and Apoptosis



The Nobel Prize in Chemistry 2008

- "for the discovery and development of the green fluorescent protein, GFP"

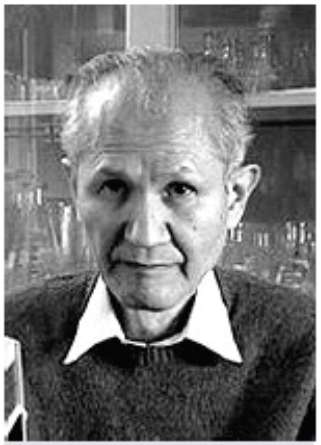


Photo: J. Henriksson/SCANPIX

Osamu Shimomura

1/3 of the prize

USA

Marine Biological Laboratory (MBL)
Woods Hole, MA, USA;
Boston University Medical School
Massachusetts, MA, USA

b. 1928
(in Kyoto, Japan)

Photo: J. Henriksson/SCANPIX

Martin Chalfie

1/3 of the prize

USA

Columbia University
New York, NY, USA

b. 1947

Photo: UCSD

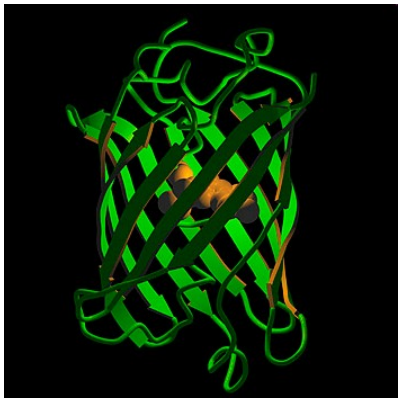
Roger Y. Tsien

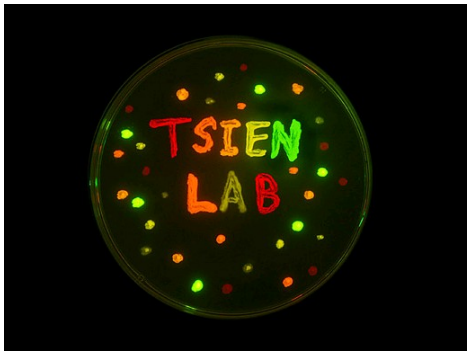
1/3 of the prize

USA

University of California
San Diego, CA, USA;
Howard Hughes Medical Institute

b. 1952





http://nobelprize.org/nobel_prizes/chemistry/laureates/2008/

Fluorescent proteins

- **bioluminescence resonance energy transfer (BRET)**

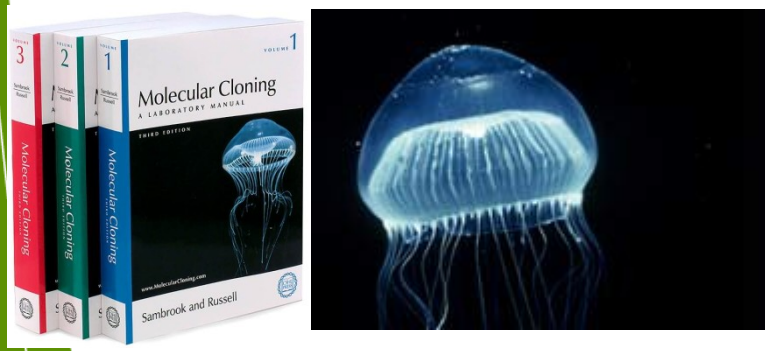
Aequorea victoria **-** jellyfish

- Blue bioluminescence. Ca^{2+} interacts with aequorin photoprotein.
- Blue light excites **green fluorescent protein**.

Renilla reniformis – coral

- luminescence appears after degradation of coelenterazine in the presence of luciferase enzyme.
- Blue light excites **green fluorescent protein**

Aequorea victoria "Crystal jelly "



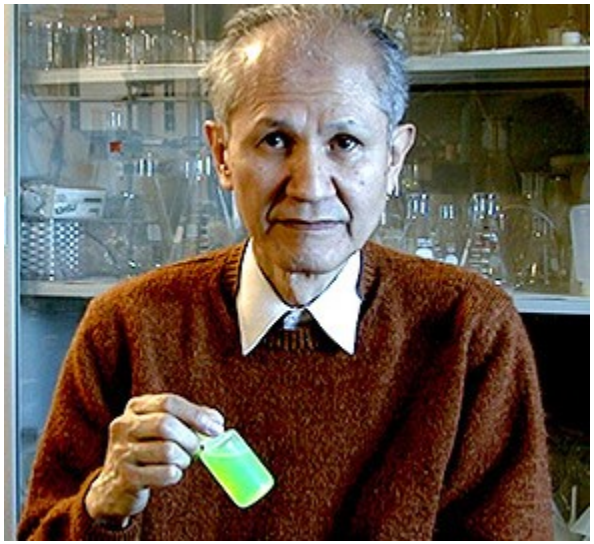
http://www.mbayaq.org/efc/living_species/default.asp?hOri=1&inhab=440

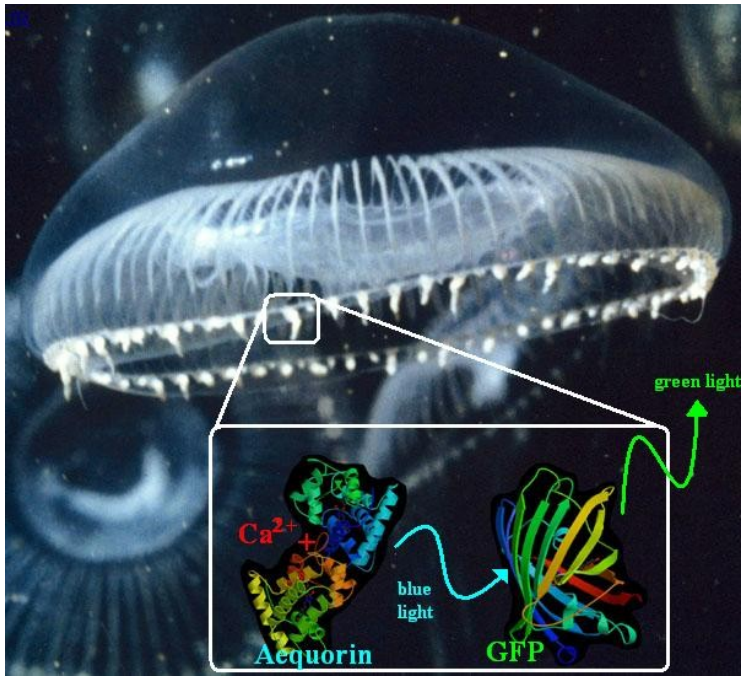
Renilla reniformis "Sea Pansy"

http://www.whitney.ufl.edu/species/seapansy.htm

Fluorescent proteins

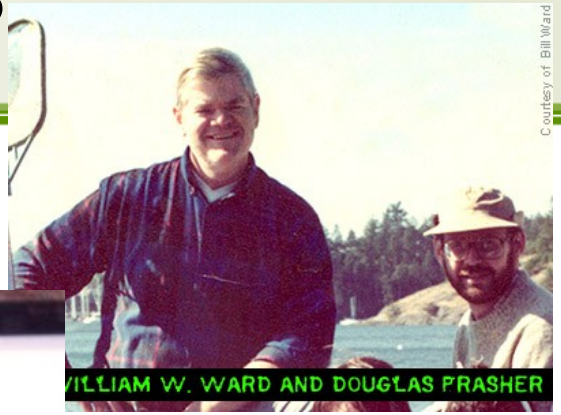
- **Osamu Shimomura**
  - **1961** discovered GFP and aequorin



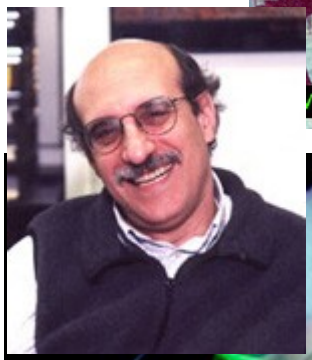


http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm

Fluorescent proteins



- **Douglas Prasher**
- **Martin Chalfie**



Science. 1994 Feb 11;263(5148):

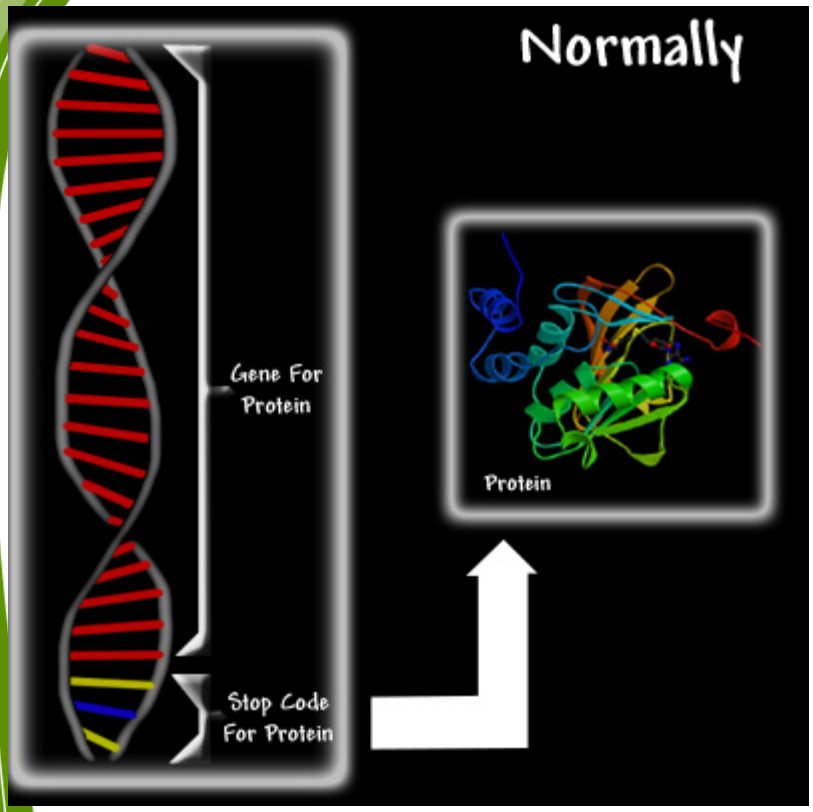
Green fluorescent protein as a marker for gene expression.

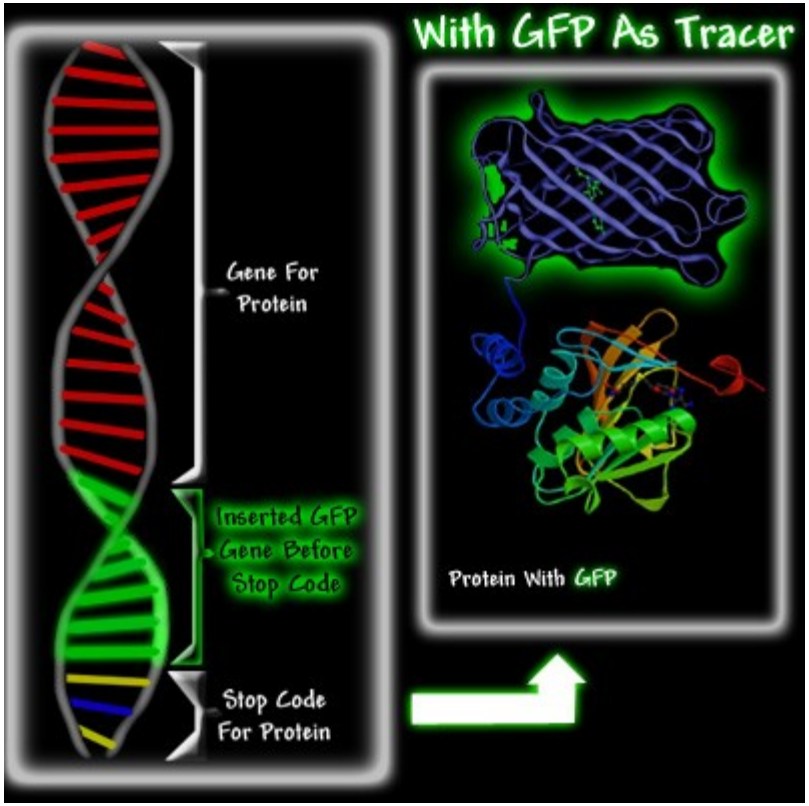
Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC.

Department of Biological Sciences, Columbia University, New York, NY 10027.

- A complementary DNA for the Aequorea victoria green fluorescent protein (GFP) produces a fluorescent product when expressed in prokaryotic (Escherichia coli) or eukaryotic (Caenorhabditis elegans) cells. Because exogenous substrates and cofactors are not required for this fluorescence, GFP expression can be used to monitor gene expression and protein localization in living organisms.

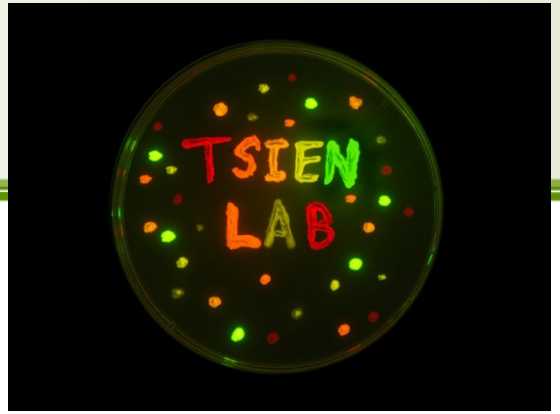
Fluorescent proteins





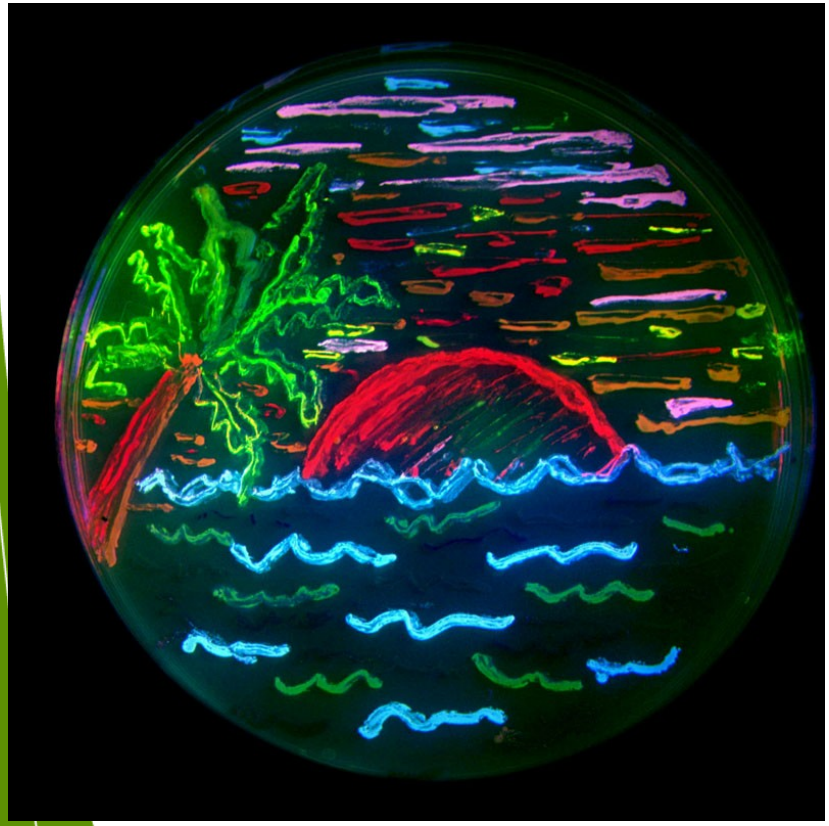
http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm

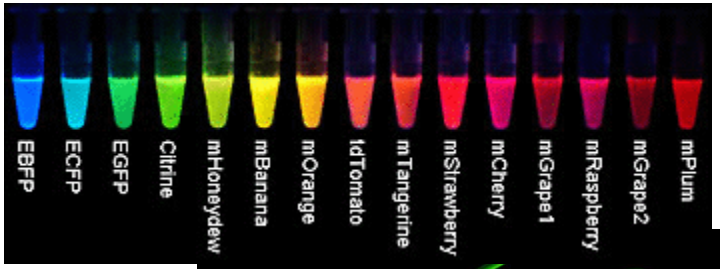
Roger Tsien

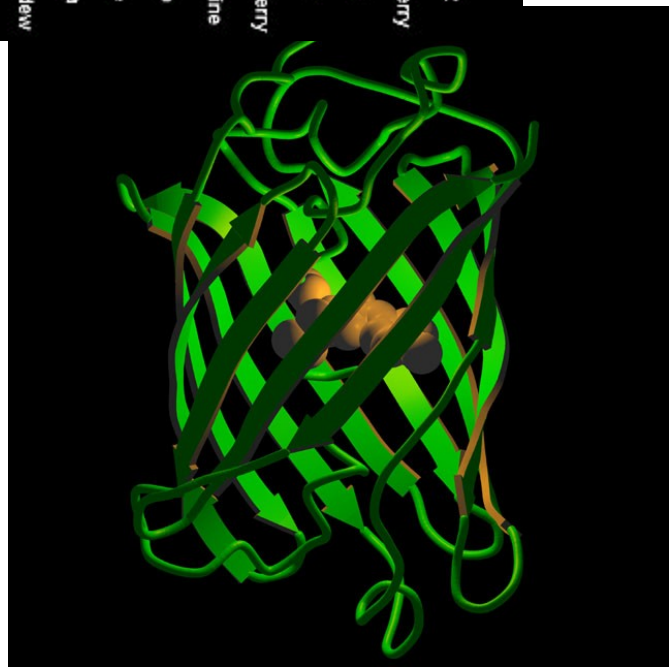


- ~ 2002 – mutated FP = wide spectrum of colors

http://www.tsienlab.ucsd.edu/

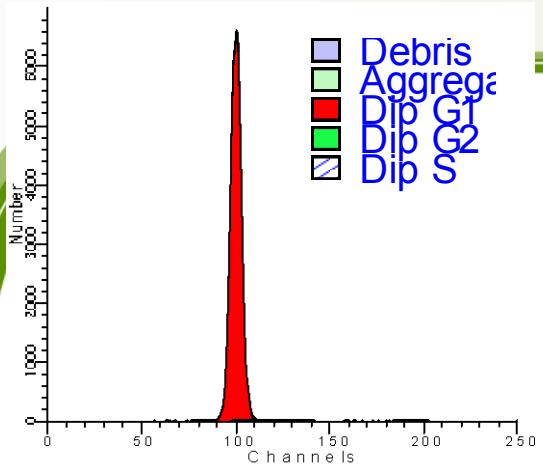


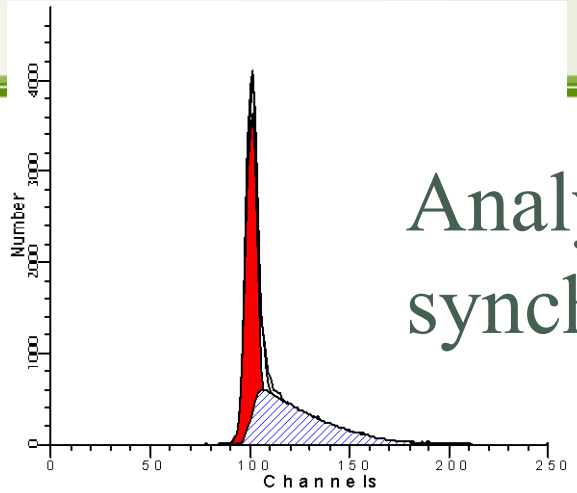


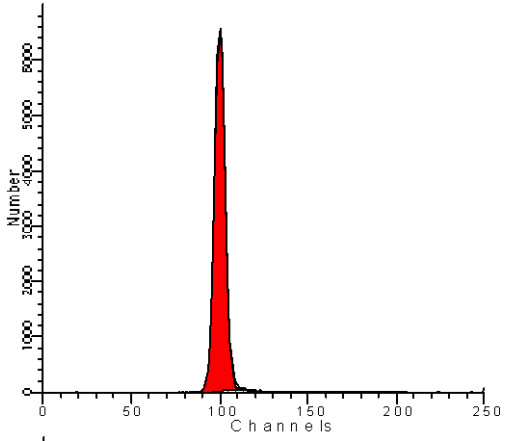


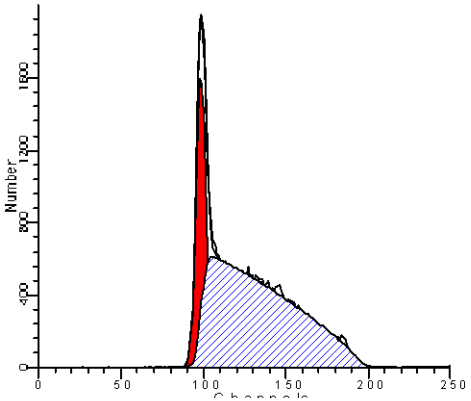
CYTOKINETIKA

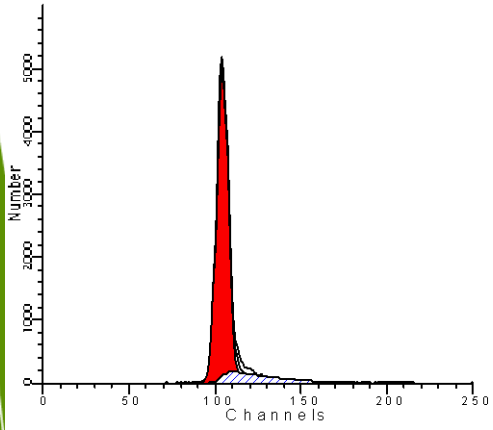
Analysis of synchronized cells

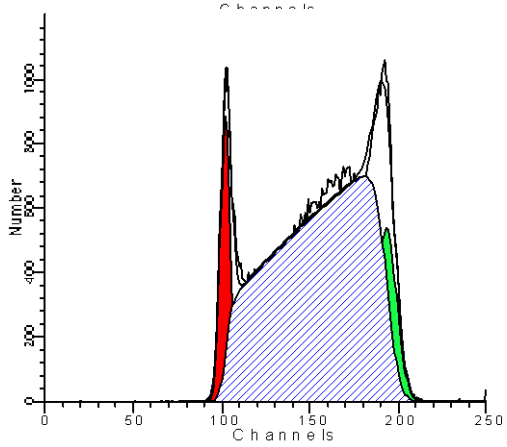


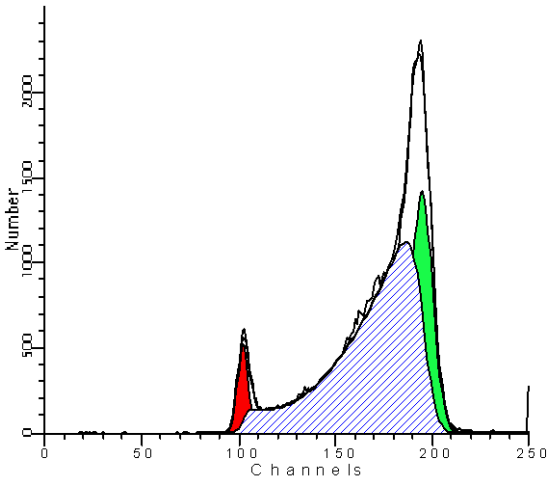




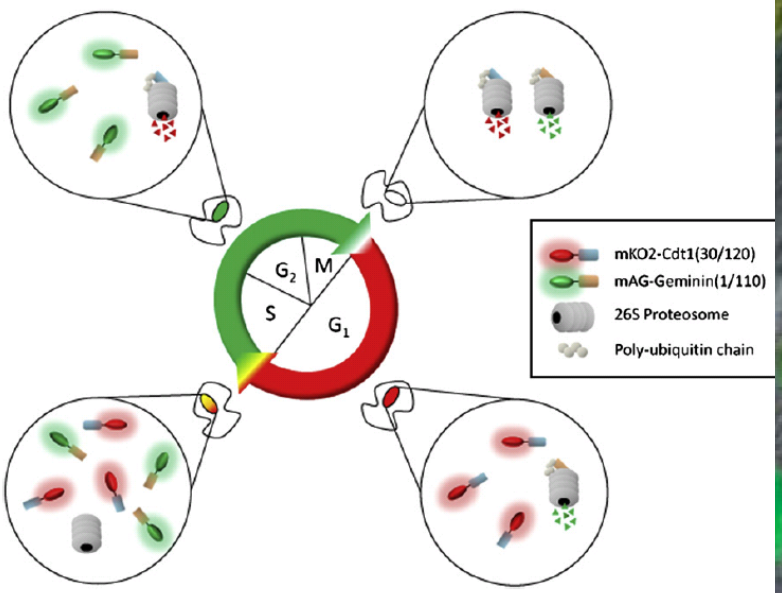




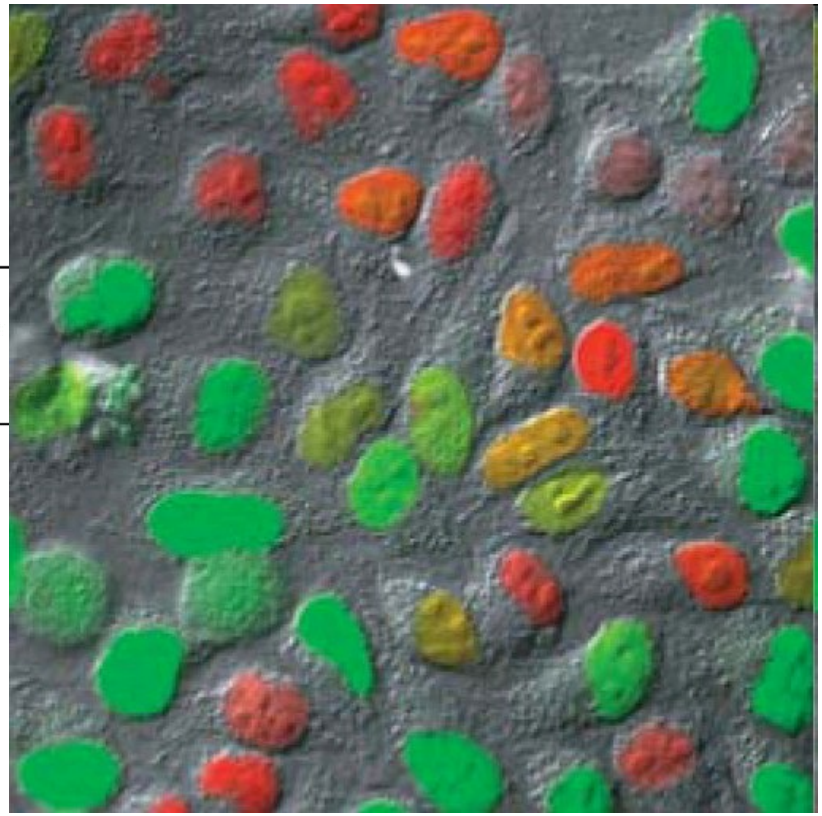




Fucci
(fluorescent ubiquitination-based cell cycle indicator) cells



Chemistry & Biology *15*, February 2008 ©2008 Elsevier Ltd



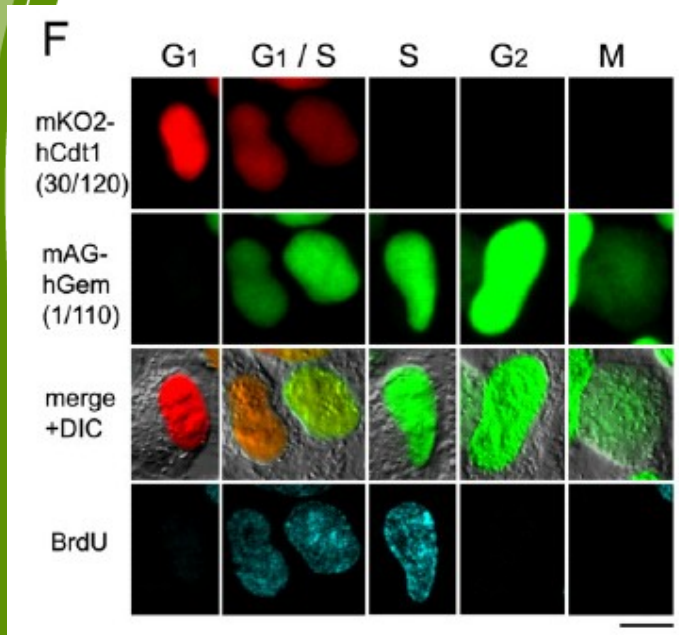
Ubiquitin E3 ligase complexes

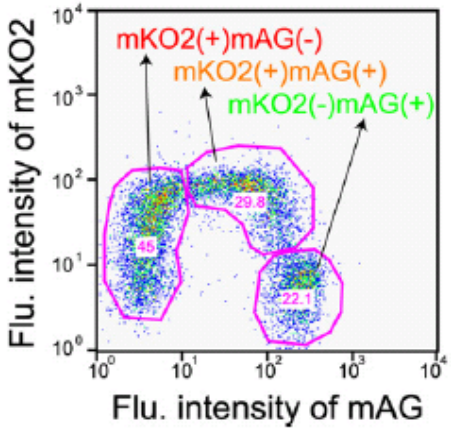
G1 - APC^{Cdh1}

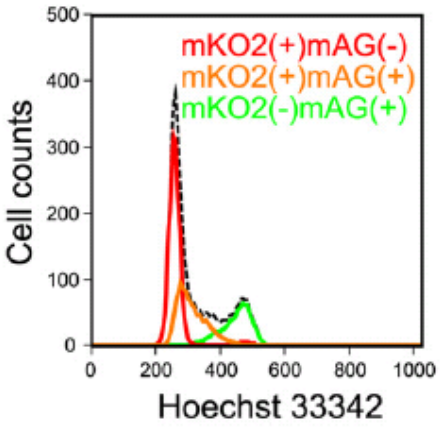
S, G2, M- SCF^{Skp2}

Nature Methods - **5, 283 (2008)**

Fucci







http://cfds.brain.riken.jp/Fucci.html

Resource

Cell

Visualizing Spatiotemporal Dynamics of Multicellular Cell-Cycle Progression

Asako Sakaue-Sawano,[1,3] Hiroshi Kurokawa,[1,4] Toshifumi Morimura,[2] Aki Hanyu,[5] Hiroshi Hama,[1] Hatsuki Osawa,[1] Saori Kashiwagi,[2] Kiyoko Fukami,[4] Takaki Miyata,[6] Hiroyuki Miyoshi,[7] Takeshi Imamura,[5] Masaharu Ogawa,[2] Hisao Masai,[8] and Atsushi Miyawaki[1,3,*]

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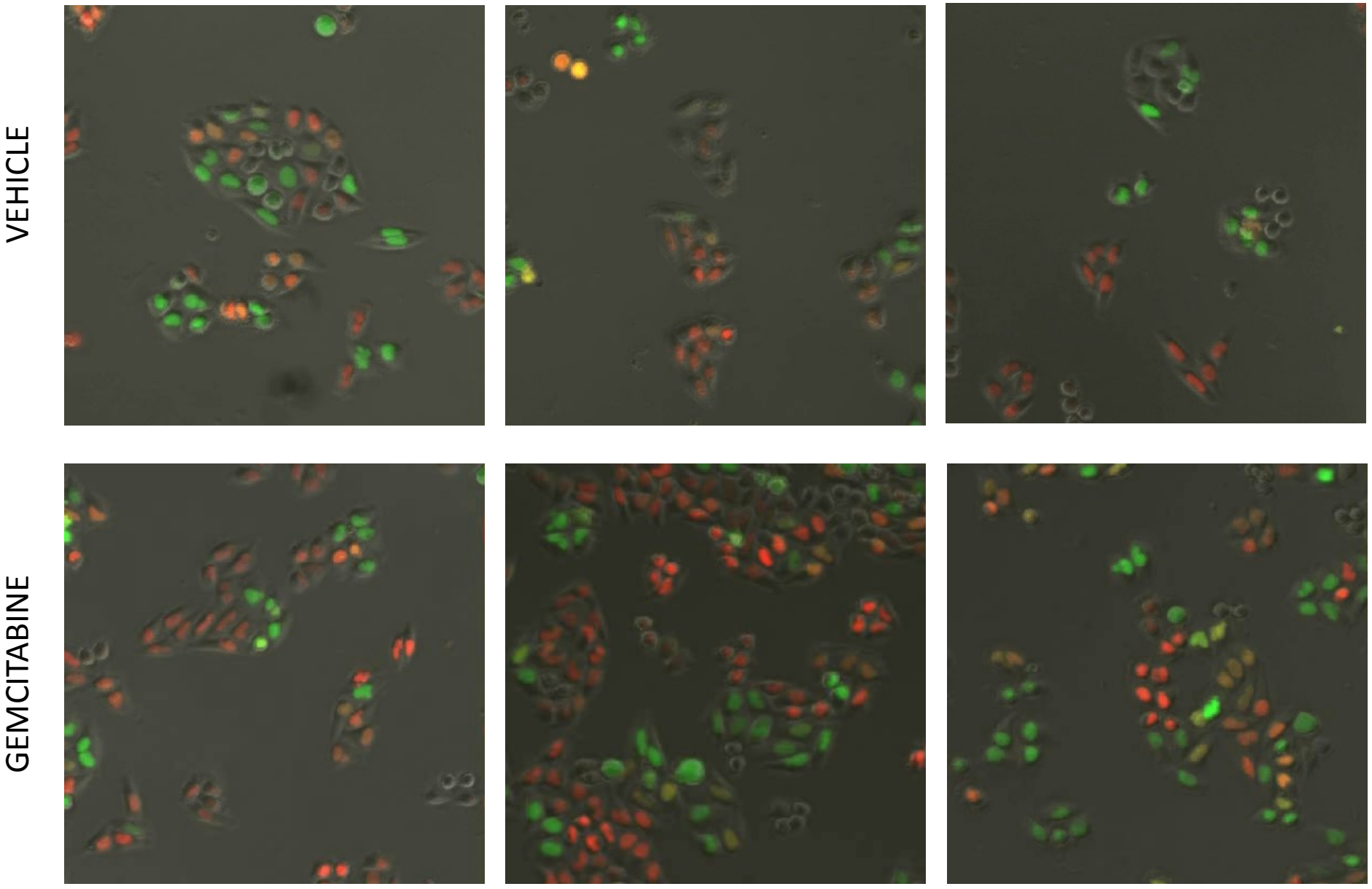
Advanced Technology Development Group, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan
[3]Life Function and Dynamics, ERATO, JST, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan
[4]School of Life Science, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan
[5]Departments of Biochemistry, The Cancer Institute of the Japanese Foundation for Cancer Research, 3-10-6 Ariake, Koto-ku, Tokyo 135-8550, Japan
[6]Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Syowa-ku, Nagoya, Aichi 466-8550, Japan
[7]Subteam for Manipulation of Cell Fate, BioResource Center, RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan
[8]Genome Dynamics Project, Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan
*Correspondence: matsushi@brain.riken.jp
DOI 10.1016/j.cell.2007.12.033


CONTROL
SCH900776
MU380
VEHICLE
GEMCITABINE

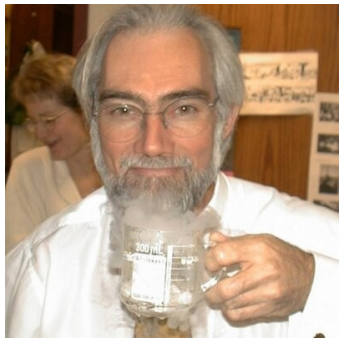
Summary

- **DNA analysis**
  - Require fine sample preparation, debris elimination, sw tool for precise analysis of histograms
  - It is possible to combine with analysis of other parameters e.g. DNA synthesis
- **Cell division enumeration**
  - Mostly for synchronized populations
- **Fluorescent proteins**
  - Fucci – elegant tool for *in vitro* a *in vivo* experiments

Trendy: instrumentace

Spectral flow cytometry

J.P. Robinson, Purdue University



Cytometry Part A • 81A: 35–44, 2012

ORIGINAL ARTICLE

Cytometry PART A Journal of the International Society for Advancement of Cytometry

MIFlowCyt

Hyperspectral Cytometry at the Single-Cell Level Using a 32-Channel Photodetector

Gérald Grégori,[1,2] Valery Patsekin,[1,3] Bartek Rajwa,[1,3] James Jones,[4] Kathy Ragheb,[1,3] Cheryl Holdman,[1,3] J. Paul Robinson[1,3,4*]

2
DOI: 10.1017/S1431927605510328

Microsc Microanal 11(Suppl 2), 2005

Multispectral Flow Cytometry: Next Generation Tools For Automated Classification

J. Paul Robinson[a,b], Valery Patsekin[a] , Gerald Gregori[a], Bartek Rajwa[a,b], and James Jones[a,b]

[a]Department of Basic Medical Science, School of Veterinary Medicine, and [b]Weldon Department of Biomedical Engineering, Purdue University, West Lafayette, IN, 47907, USA

US007280204B2

(12) **United States Patent**
Robinson et al.

(10) **Patent No.: US 7,280,204 B2**
(45) **Date of Patent: Oct. 9, 2007**

(54) **MULTI-SPECTRAL DETECTOR AND ANALYSIS SYSTEM**

(75) Inventors: **Joseph Paul Robinson**, West Lafayette, IN (US); **Bartlomiej Rajwa**, West Lafayette, IN (US); **Gérald Grégori**, Marseille (FR); **Valery Patsekin**, West Lafayette, IN (US)

(73) Assignee: **Purdue Research Foundation**, West Lafayette, IN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 139 days.

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7,057,712 B2 *	6/2006	Beck et al.	356/72

(Continued)

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(Continued)

Spectral flow cytometry

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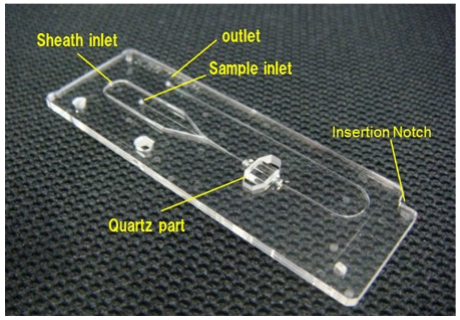
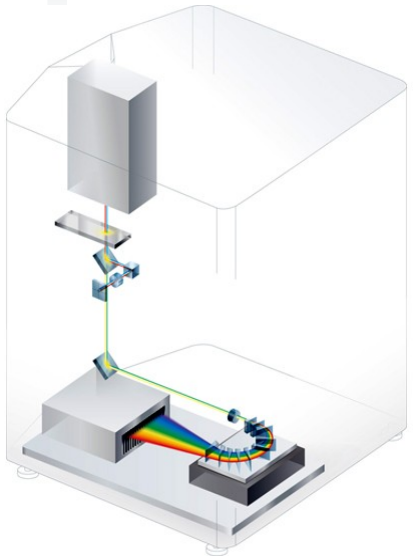
Overview Features Applications Specifications Literature

See Everything

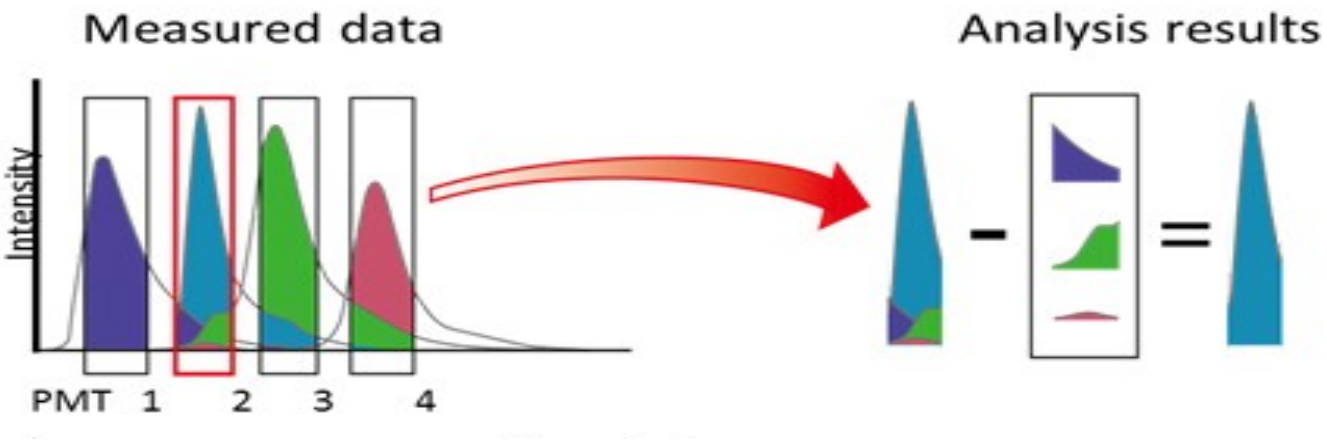
The SP6800 Spectral Analyzer is Sony Biotechnology Inc.'s newest innovative life science system fundamentally expanding the way cell and biomarker analysis can be performed. This system incorporates a unique optical bench, Blu-ray™ disc technology, and advanced algorithms to deliver some of the most accurate and precise data available.

The SP6800 Spectral Analyzer also introduces new Flow Point technology to analyze core stream and sample event location within the flow cell. To improve accuracy of data, this system also provides unique functions to display and analyze cellular autofluorescence and allows the user to easily automatically remove.



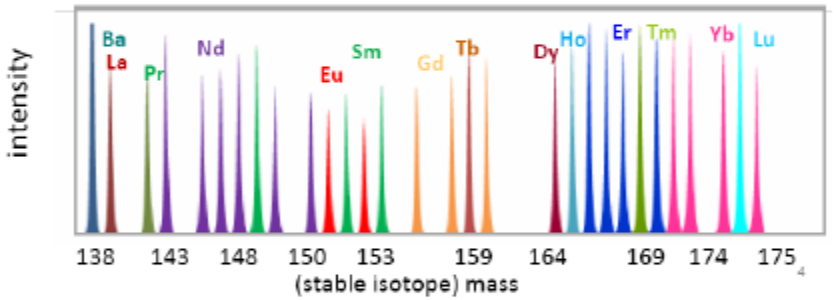


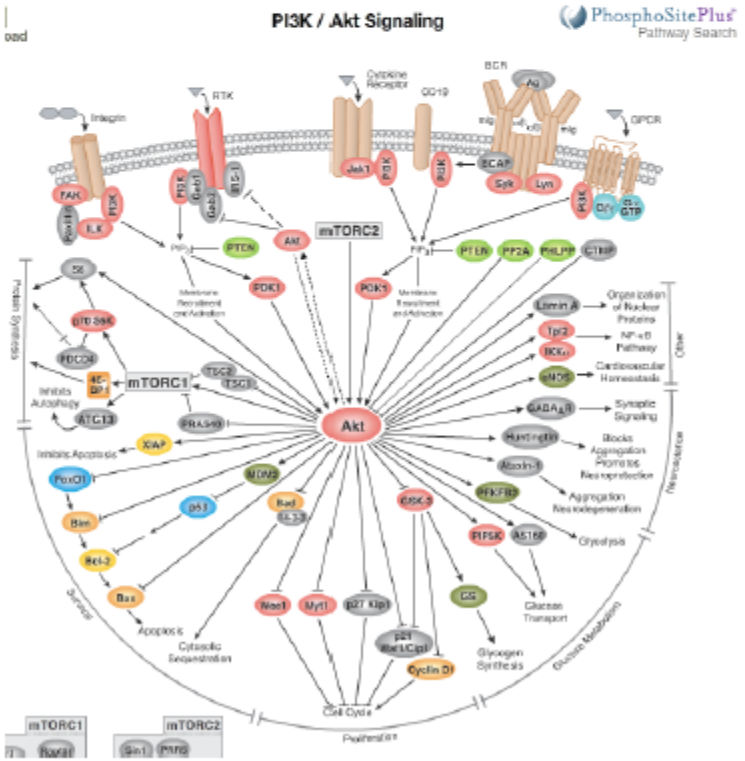
Conventional vs. spectral analysis



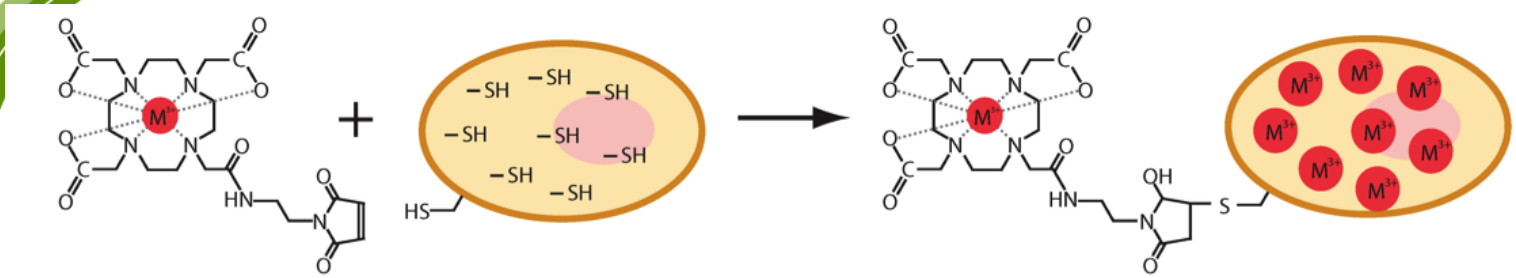
Why Mass Cytometry?

- Highly multi-parametric, on a single cell basis
- Facilitates exploration of complex pathways
- Enables discovery of cellular relationships, responses, and developmental pathways
- Allows deep-profiling of your cell system of interest





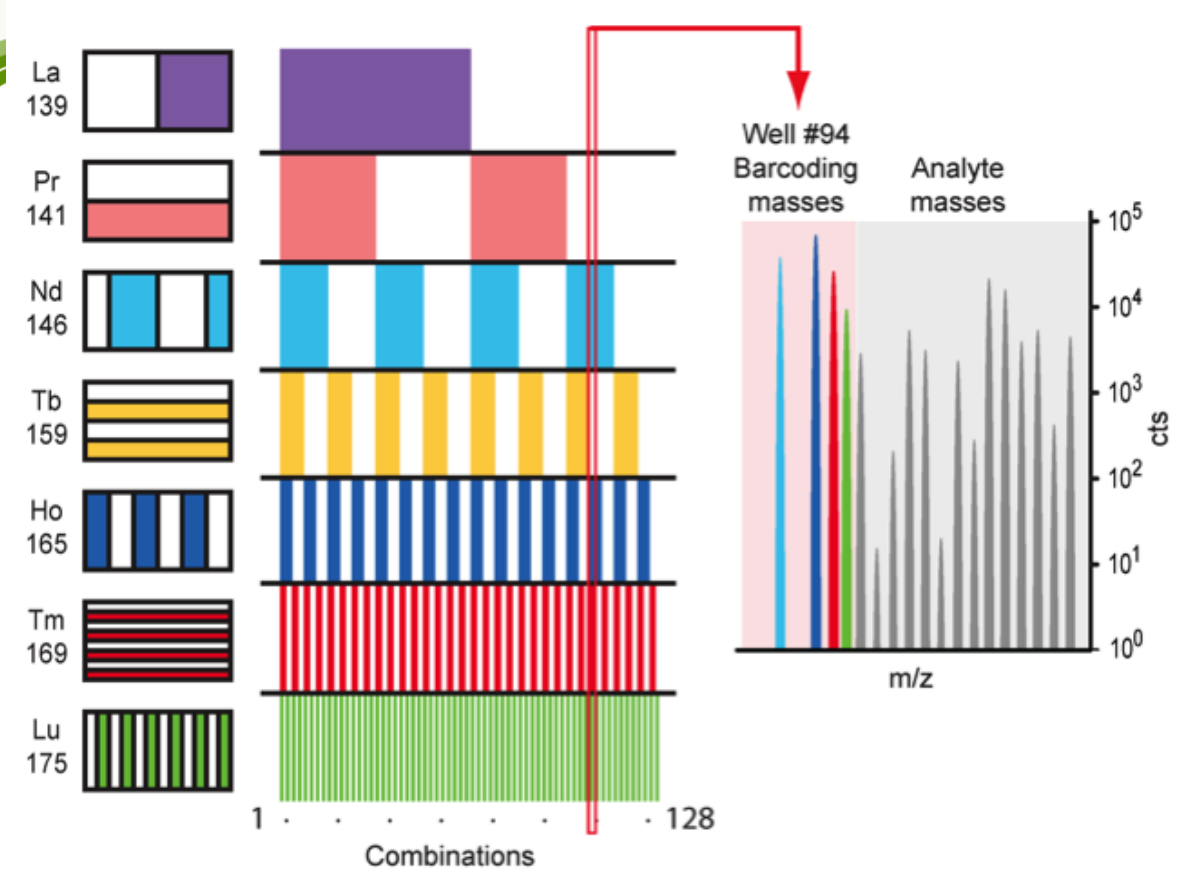
Single Cell Mass Cytometry



Cells were covalently labeled with a bifunctional compound, maleimido-mono-amide-DOTA (mDOTA). This compound can be loaded with a lanthanide(III) isotope ion, and reacts covalently with cellular thiol groups through the maleimide moiety.

Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum
Sean C. Bendall, *et al.*
Science **332**, 687 (2011);

Single Cell Mass Cytometry



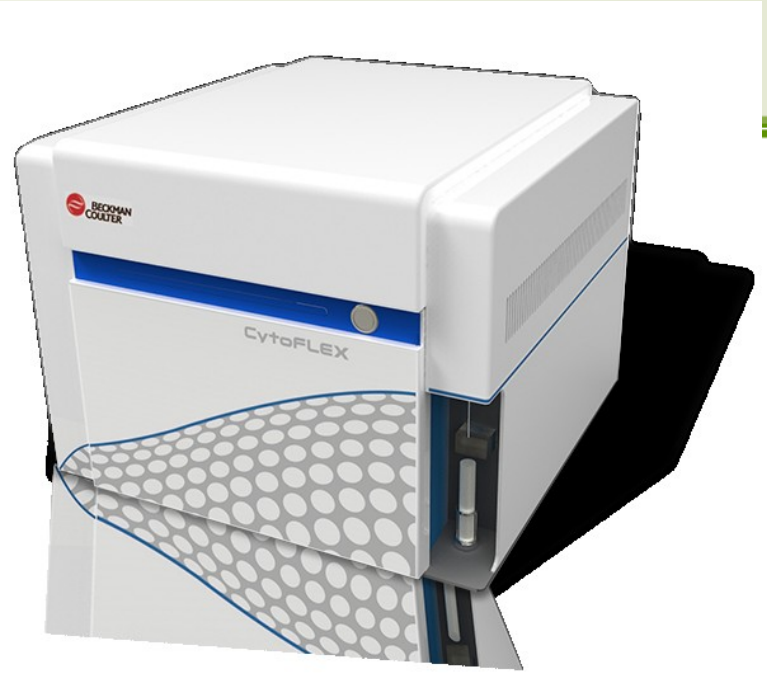
Seven unique lanthanide isotopes were used to generate 128 combinations, enough to barcode each sample in a 96-well plate. The seven lanthanide isotopes, their masses and their locations on the 96-well plate are shown.

Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum
Sean C. Bendall, *et al.*
Science **332**, 687 (2011);

CYTOKINETIKA

Personální systémy

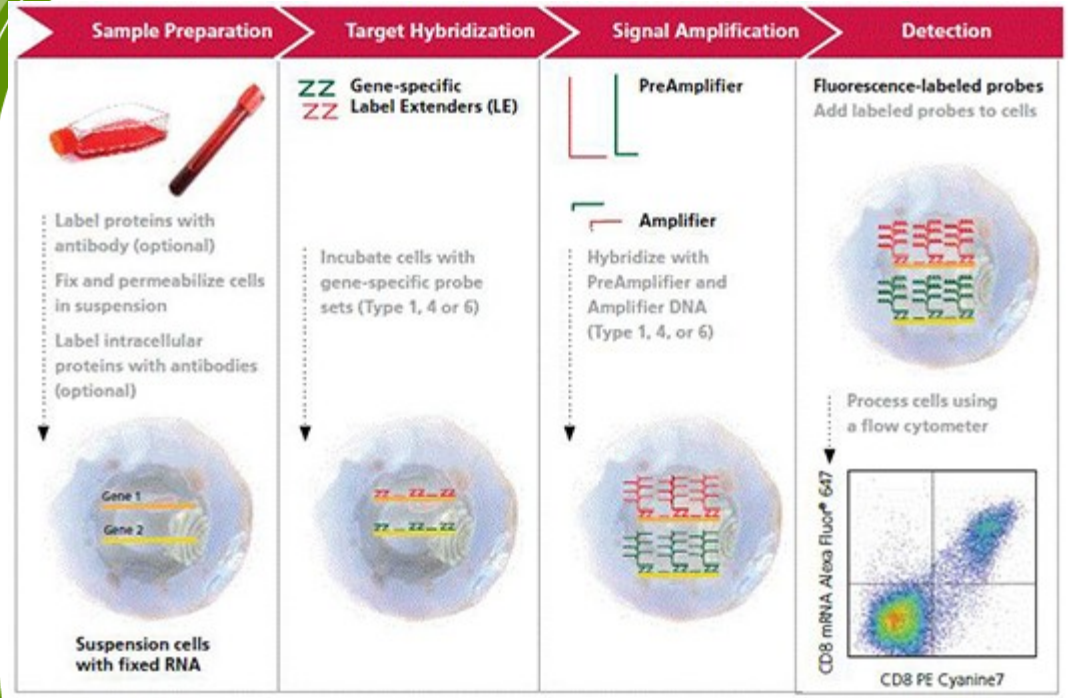
Cytonome Viva™ G1 Desktop System



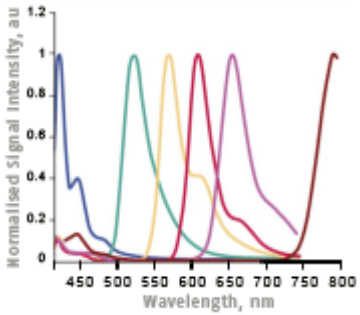
Trendy: Reagencie

CYTOKINETIKA

PrimeFlow™ RNA Assay



Briliant Violet polymers



Shrnutí přednášky

průtoková cytometrie:

- nabízí široké spektrum aplikací;
- rychlý způsob analýzy a separace heterogenních populací;
- separace populací;
- multiparametrové analýzy