

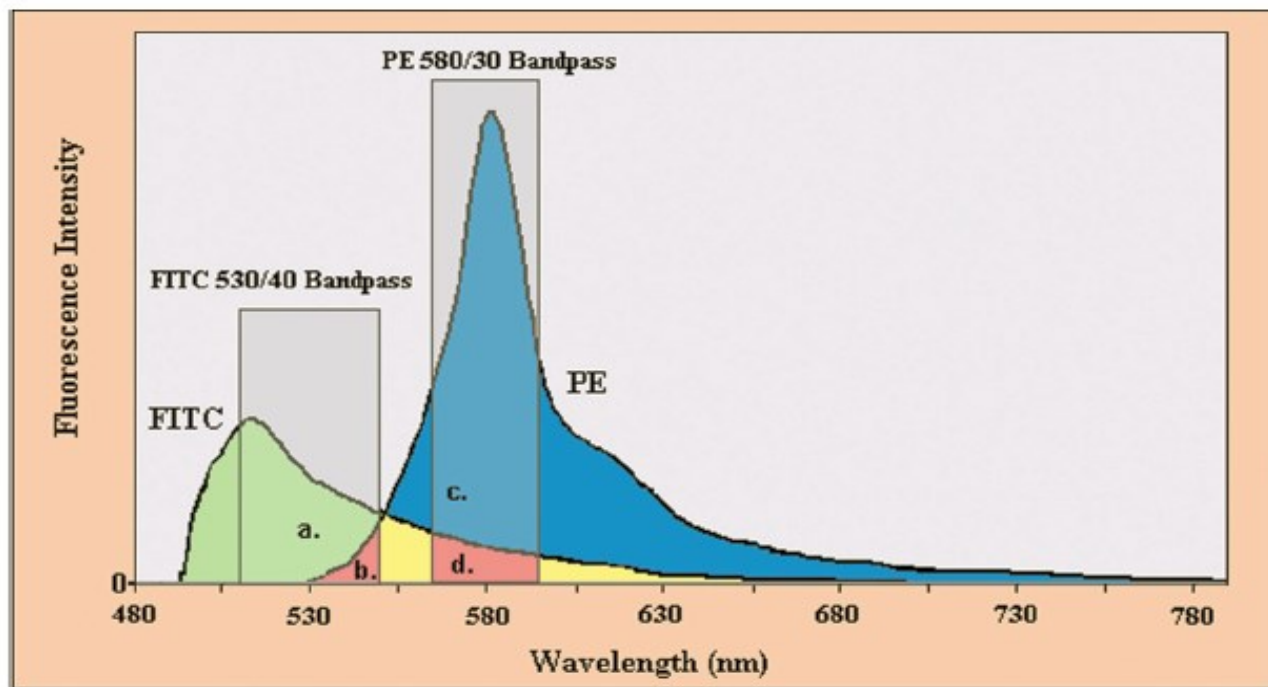
Bi9393 Analytická cytometrie

Karel Souček, Ph.D.

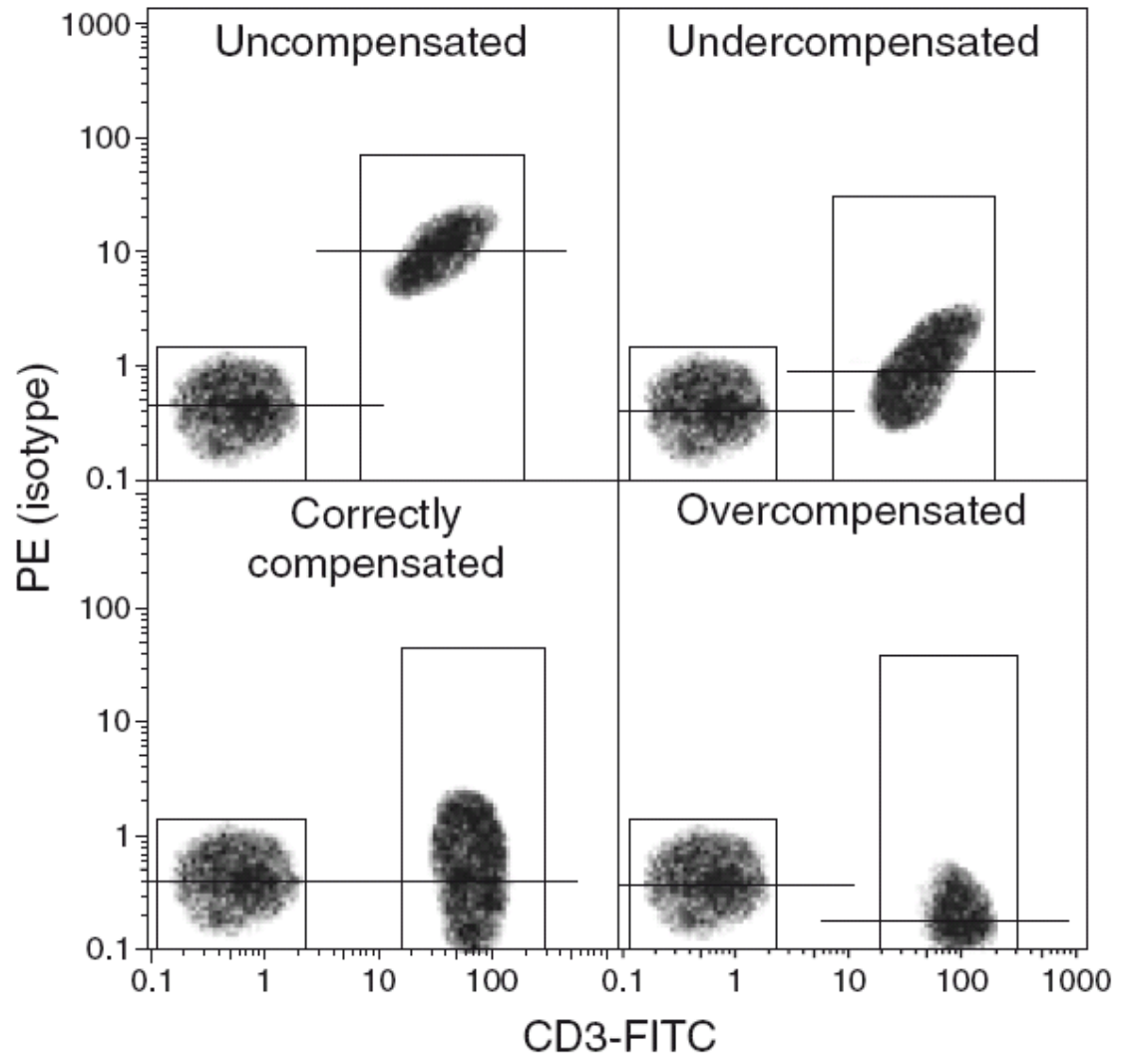
Oddělení cytokinetiky
Biofyzikální ústav AVČR, v.v.i.
Královopolská 135
612 65 Brno

e-mail: ksoucek@ibp.cz
tel.: 541 517 166



Co je problém při vícebarevné detekci?



Kompenzace fluorescenčního signálu



Spectral flow cytometry

SONY Sony Biotechnology Inc.   US

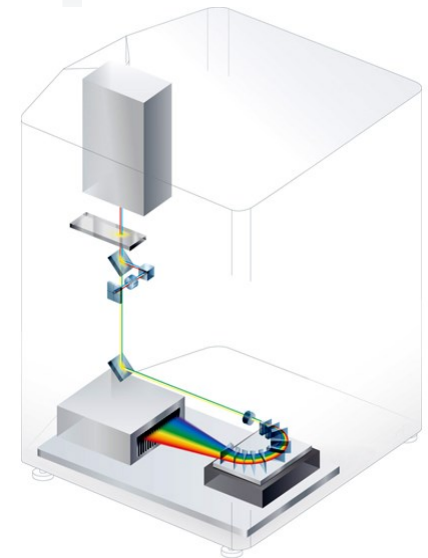
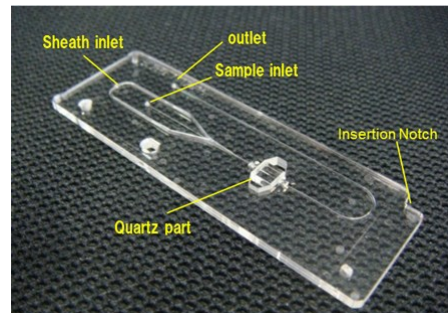
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Overview Features Applications Specifications Literature

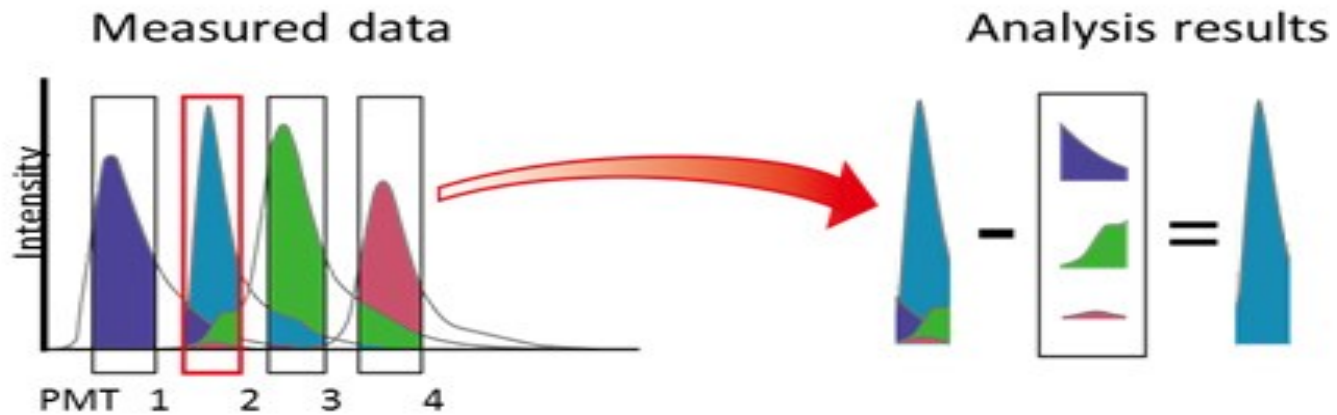
See Everything

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



Doporučení

- kombinujte fluorochromy s odpovídající svítivostí a nízkým dopadem na rozlišení dalších barev
- vyhněte se obtížně kombinovatelným fluorochromům
- posouzení dopadu na biologické rozlišení: větší není vždy lepší
- optimalizujte váš protocol: kontroly, kontroly, kontroly (**zkratky nefungují**)





Aplikace průtokové cytometrie



ANALÝZA NUKLEOVÝCH KYSELIN

buněčný cyklus a ploidyta

analýza zlomů DNA

inkorporace BrDU

exprese cyklinů

analýza denaturace DNA

ANALÝZA BUNĚČNÉHO FENOTYPU

imunofenotypizace pomocí CD antigenů

(detekce diferenciačních a nádorových markerů)

detekce cytokinových receptorů

CYTOGENETIKA

analýza chromozómů

STUDIUM BUNĚČNÝCH FUNKCÍ

viabilita

stanovení intracelulárního pH

analýza organel a cytoskeletu

stanovení membránového potenciálu

oxidativní vzplanutí

stanovení intracelulárního Ca²⁺

stanovení intracelulárních cytokinů

Natural Killer ligace značených buněk

analýza reportérových genů



Biologické aplikace průtokové cytometrie

- analýza proliferace
- fluorescenční proteiny

Buněčný cyklus

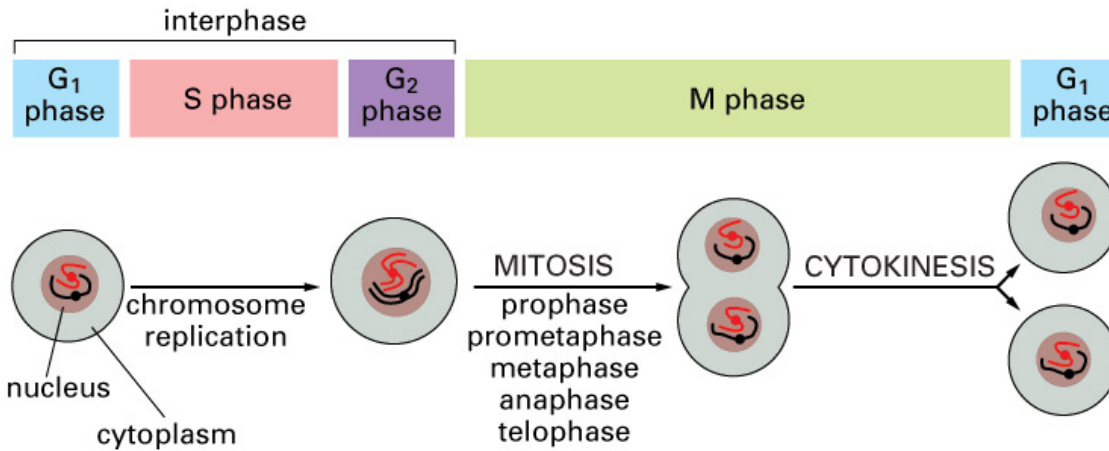


Figure 18-1. Molecular Biology of the Cell, 4th Edition.

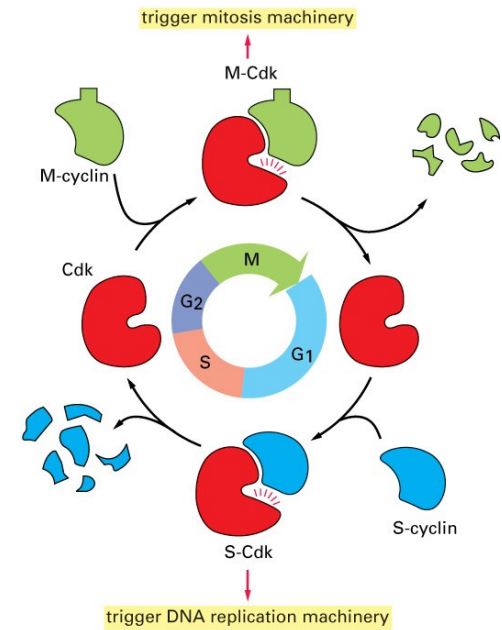
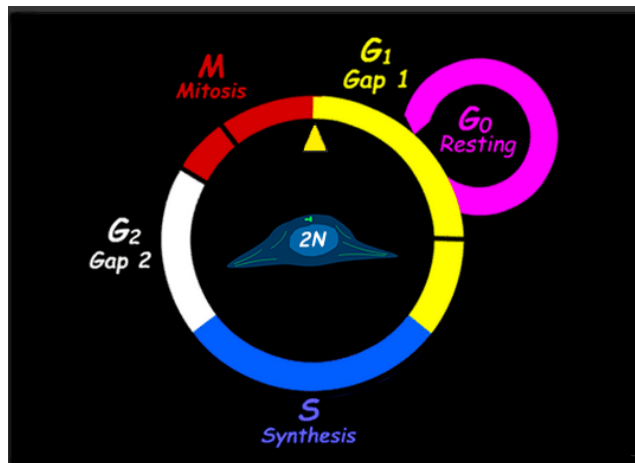


Figure 17-16. Molecular Biology of the Cell, 4th Edition.

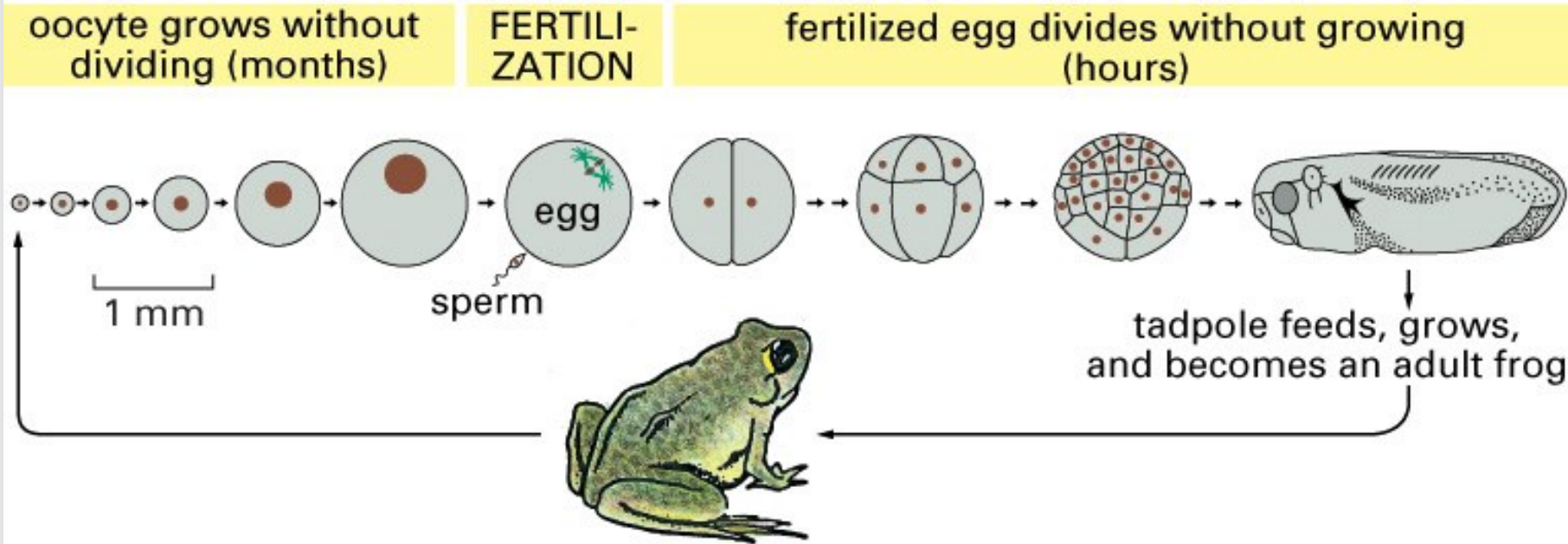
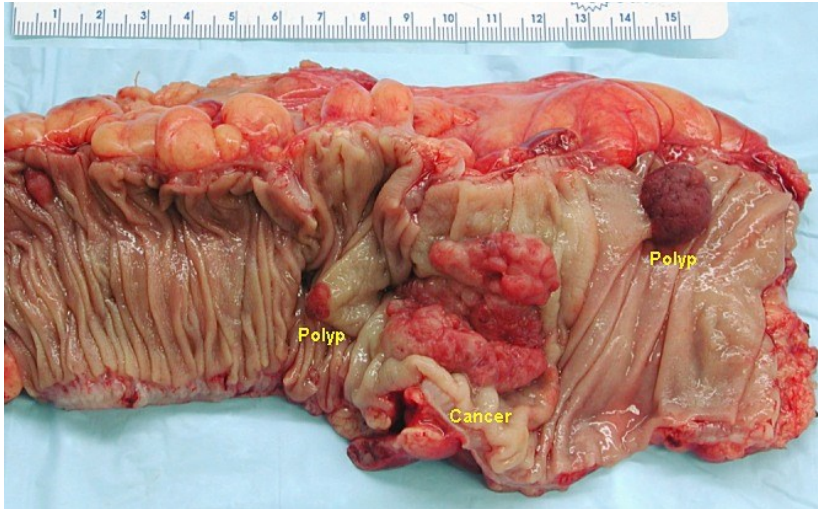


Figure 17-8. Molecular Biology of the Cell, 4th Edition.





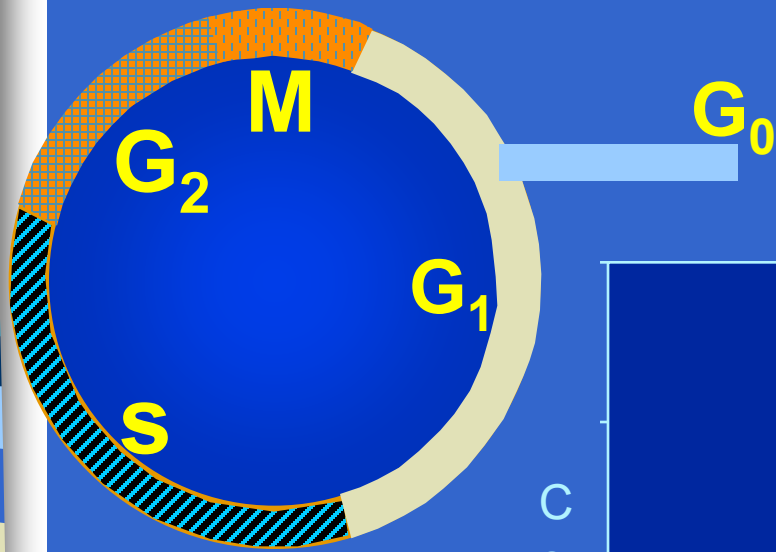
Co je důležité při přípravě vzorku a značení...

- Postup přípravy vzorku a značení nelze zobecnit – závisí na typu buněk a konkrétní analýze
 - suspenze jednotlivých buněk
 - vitální značení
 - fixace (etanol, formaldehyd)
 - permeabilizace (detergenty)
 - difúze
 - aktivní transport

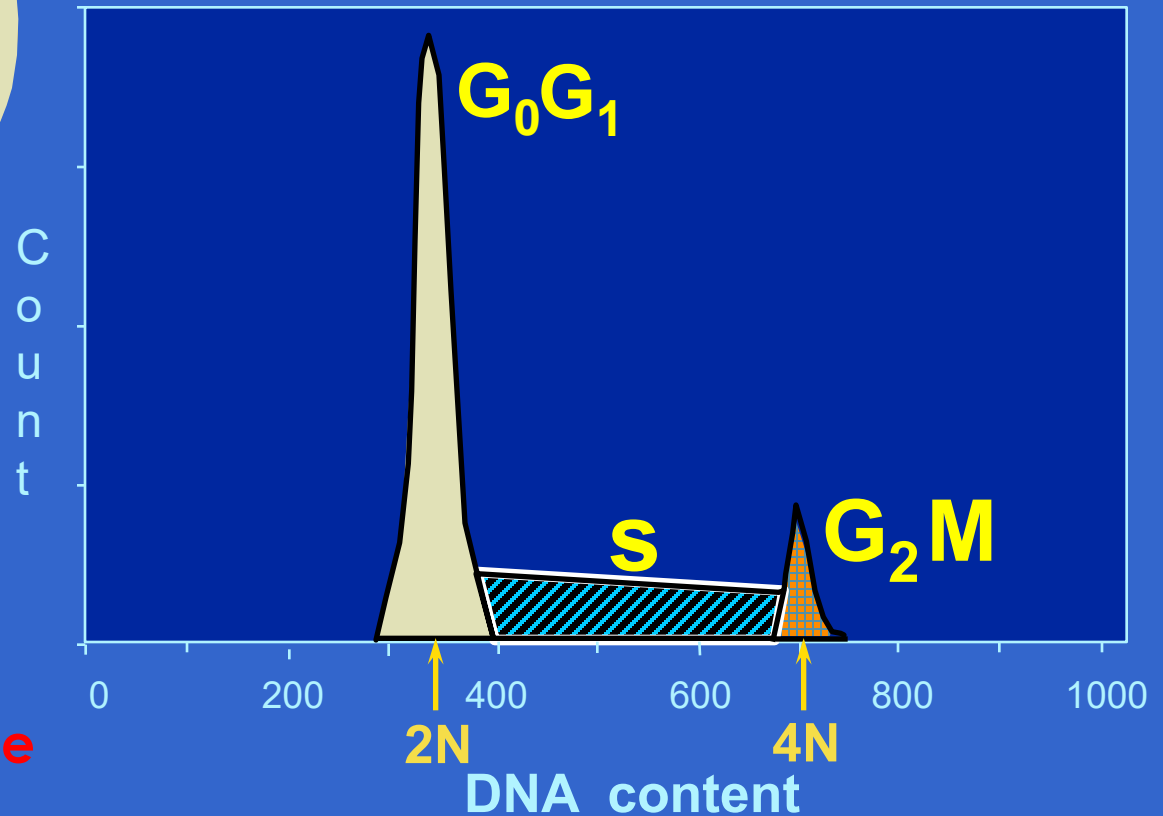
Analýza buněčného cyklu

- jedna z nejstarších aplikací flow cytometrie, stanovení fáze buněčného cyklu podle množství DNA
- průtoková cytometrie je vhodná metoda pro rychlou a přesnou determinaci buněčného cyklu
- jednoduchým způsobem je DNA obarvena fluorescenční barvou specifickou pro DNA.
- Propidium iodide
4',6-diamidino-2-phenylindole (DAPI)
 - dramaticky zvyšují fluorescenci po vazbě na DNA. Je nutná permeabilizace cytoplasmatické membrány .
- 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.
 - mohou být používány pro značení viabilních buněk.

Normal Cell Cycle



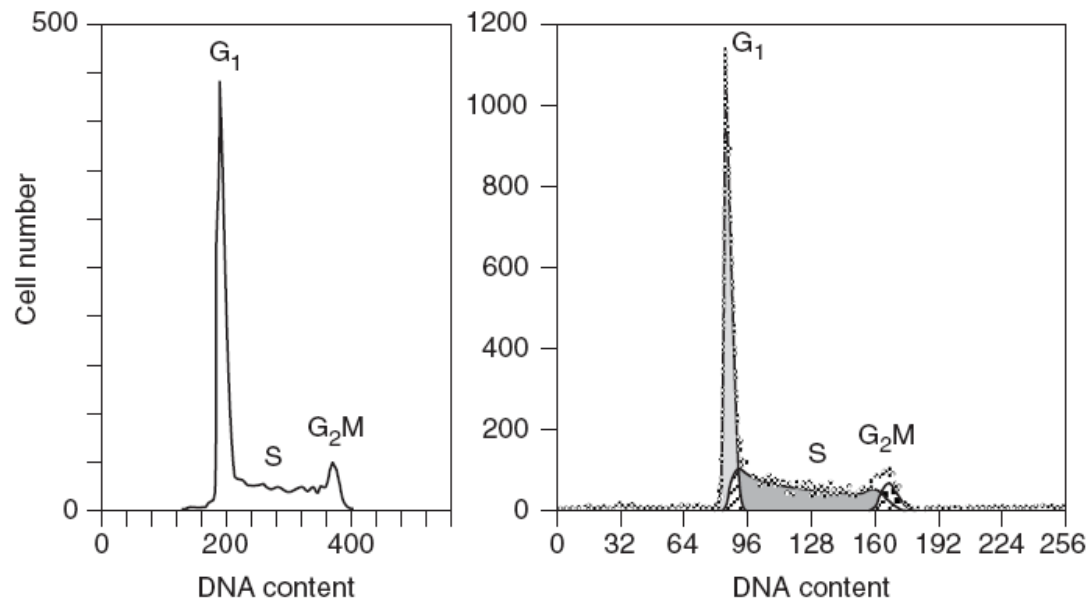
DNA Analysis



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

Analýza histogramu buněčného cyklu

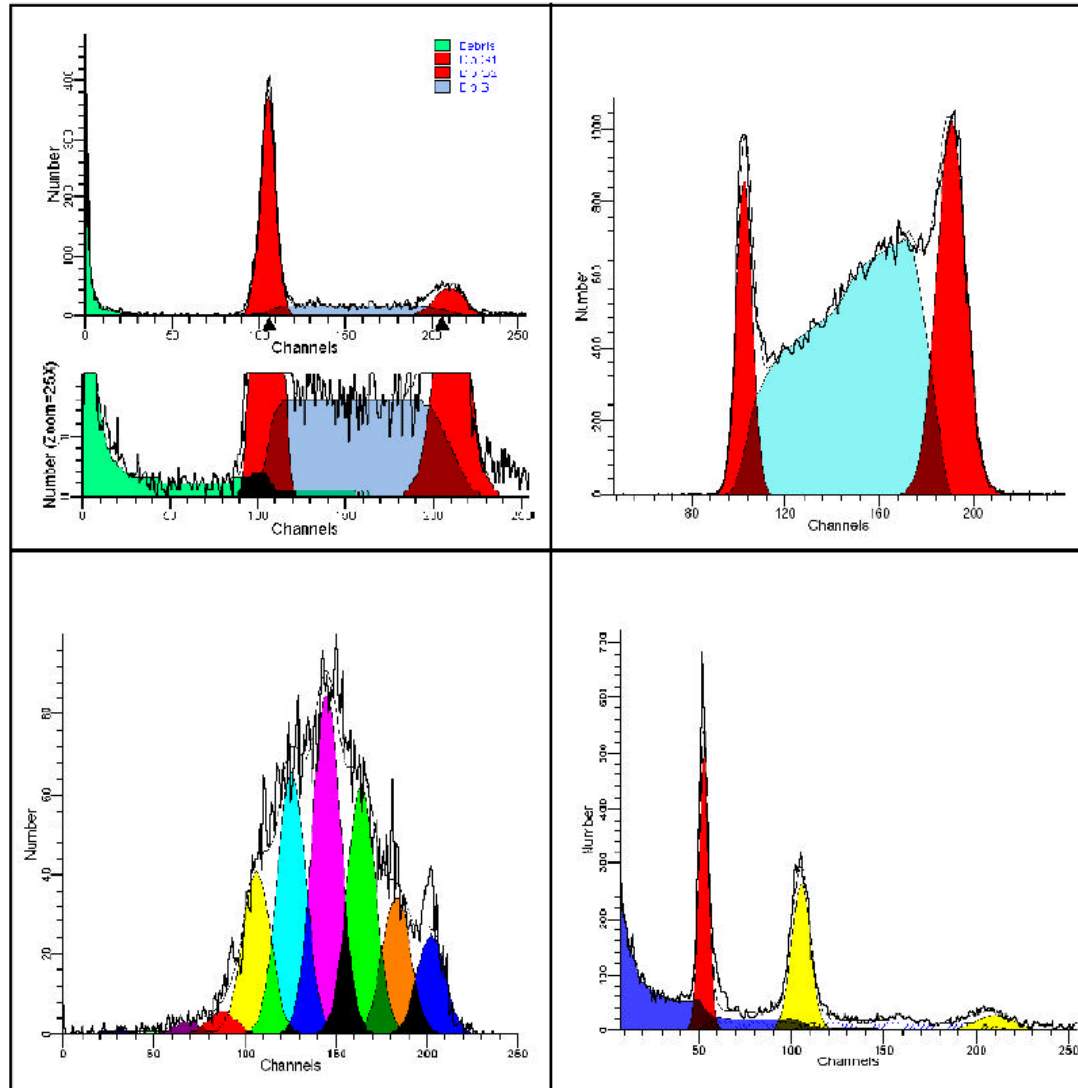
- **nepoužívá** se běžná analýza pomocí úseček (regionů) v histogramu
- **je nutné** používat speciální software pro modelování analýzu distribuce jednotlivých fází



ModFit LT™



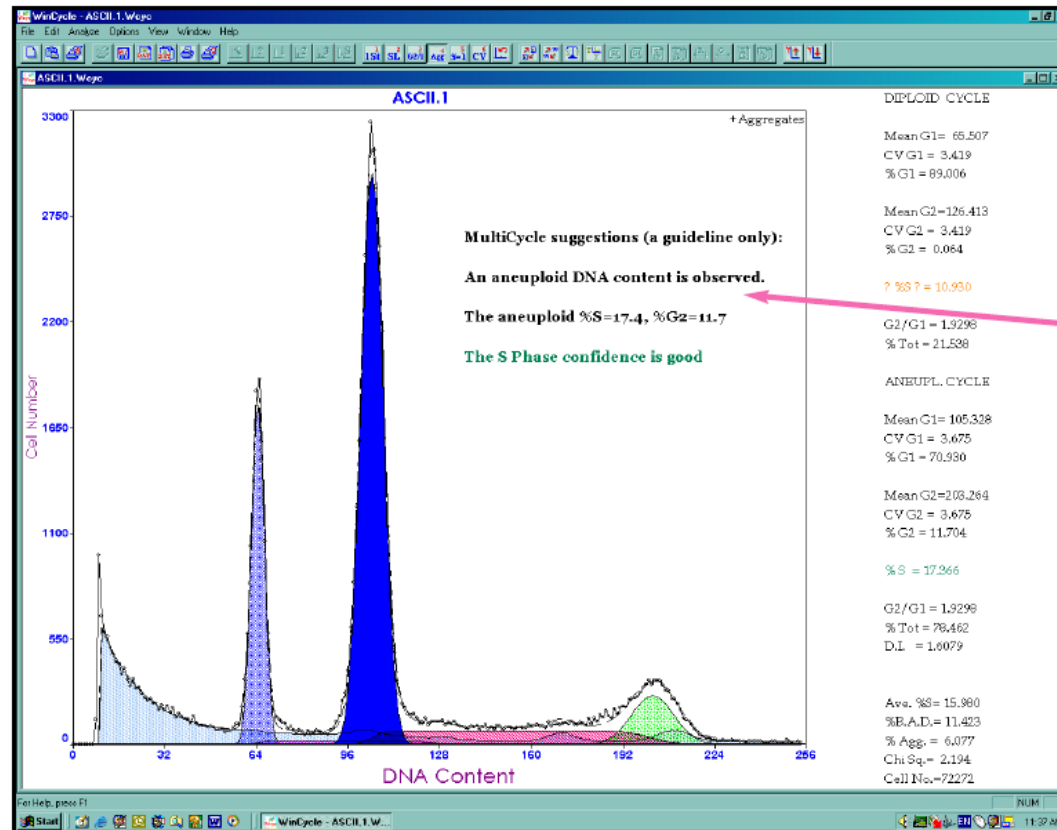
An impressive new version of the industry standard.



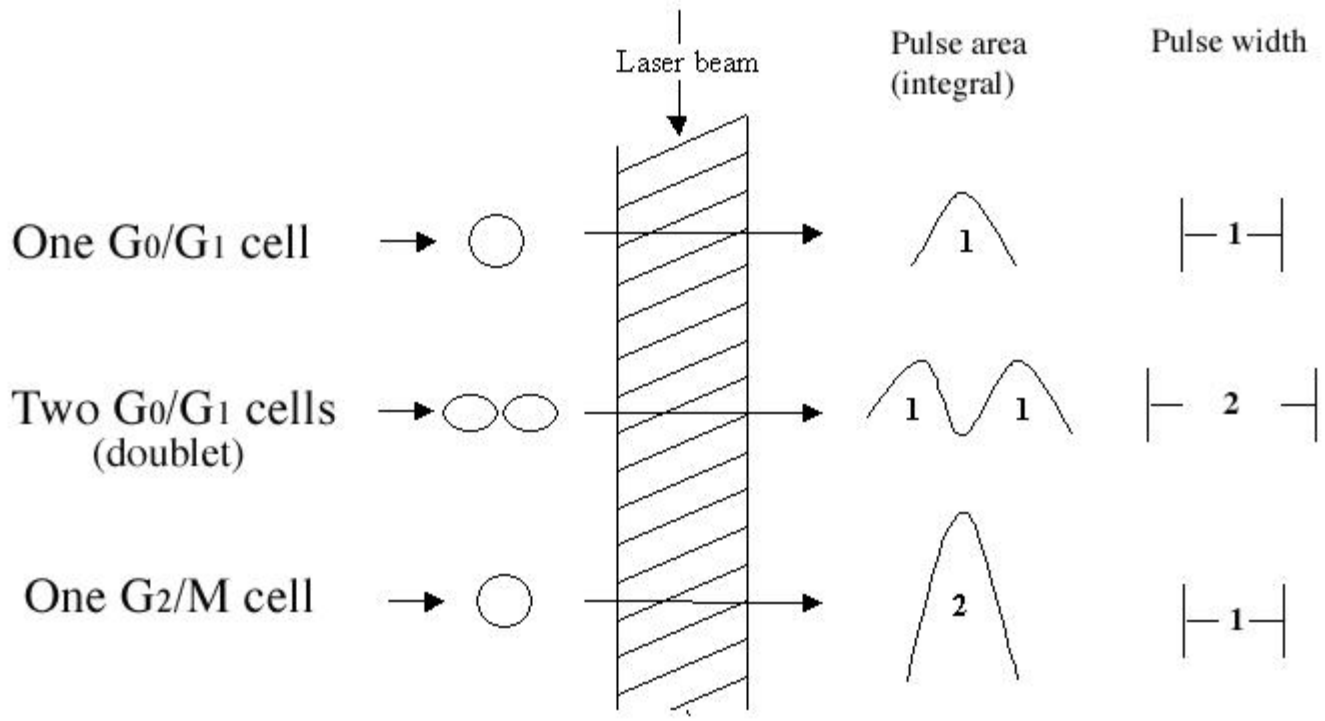
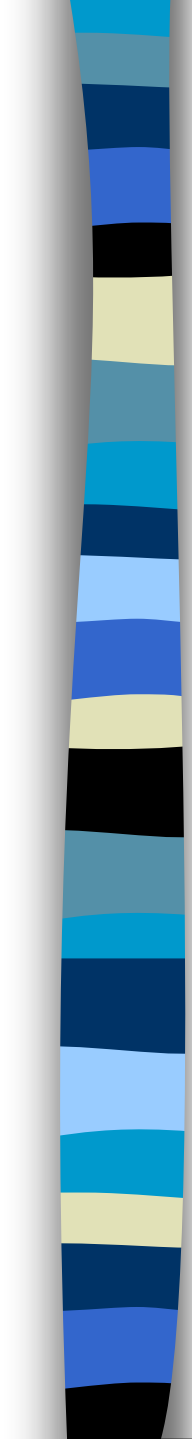
MultiCycle for Windows

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.



Cell cycle histogram: gating strategy

■ Dip G1
■ Dip G2
▨ Dip S

File analyzed: Worklist_A_Tube_001_012_20170425_124644.fcs
Date analyzed: 5-Aug-2017
Model: 1nn0A_DSF
Analysis type: Manual analysis

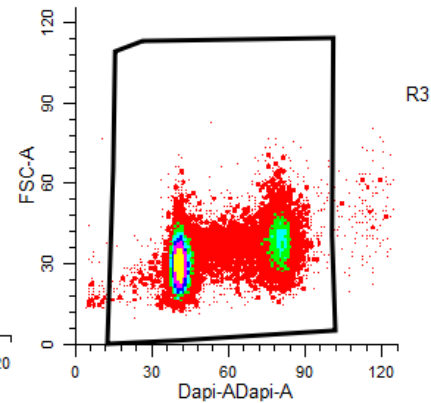
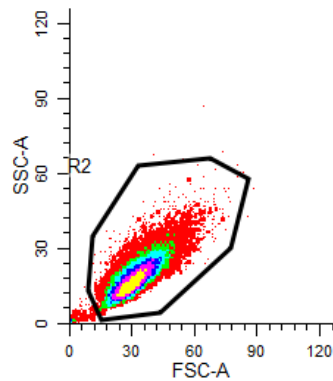
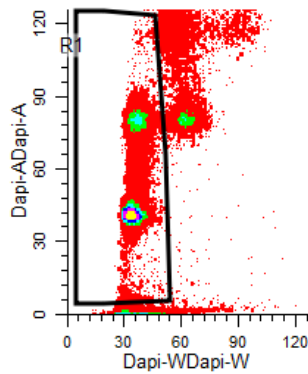
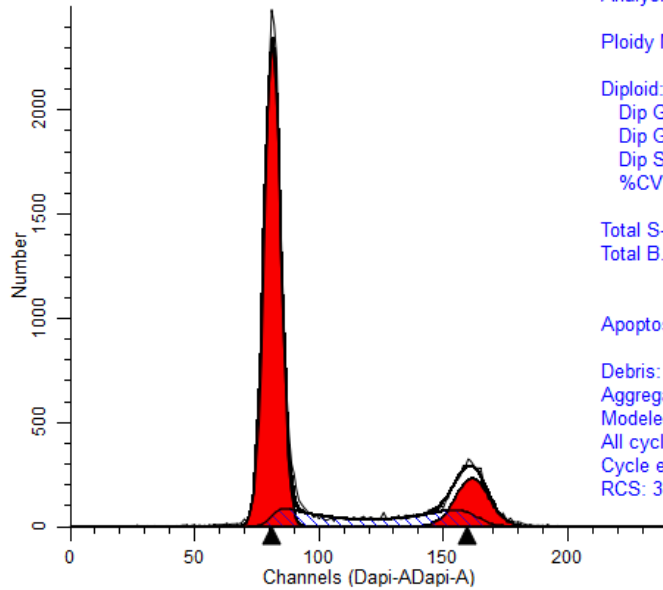
Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 68.57 % at 81.54
Dip G2: 13.67 % at 161.99
Dip S: 17.76 % G2/G1: 1.99
%CV: 4.05

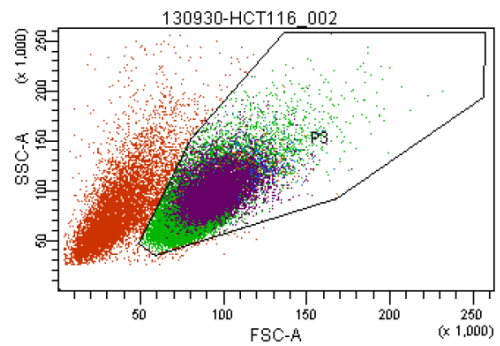
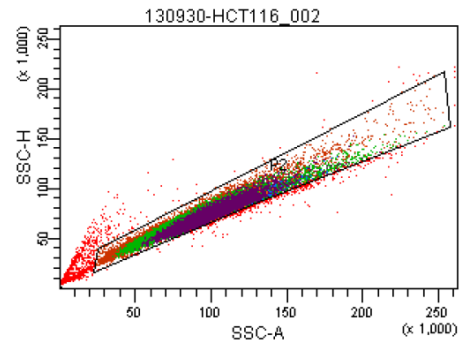
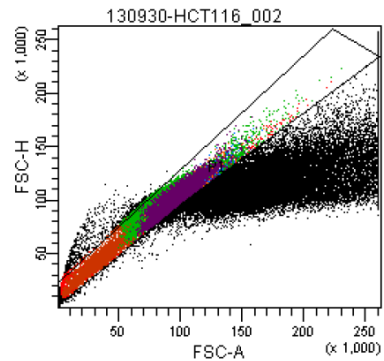
Total S-Phase: 17.76 %
Total B.A.D.: 0.00 % no debris no aggs

Apoptosis: % Mean:

Debris: %
Aggregates: 0.00 %
Modeled events: 27982
All cycle events: 27982
Cycle events per channel: 344
RCS: 3.026



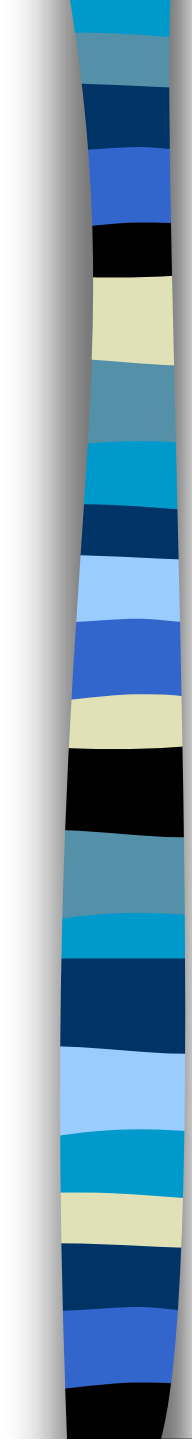
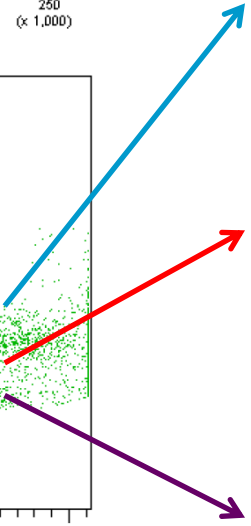
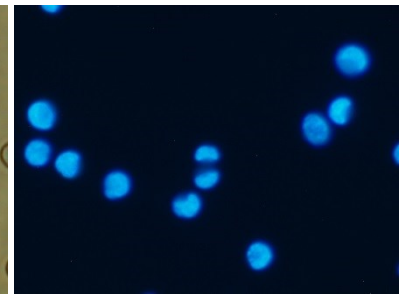
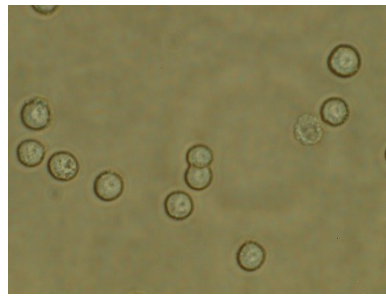
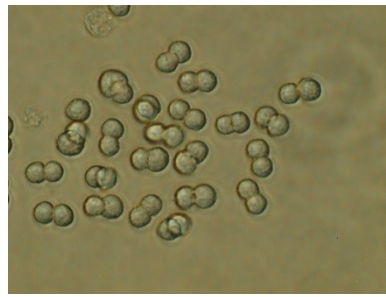
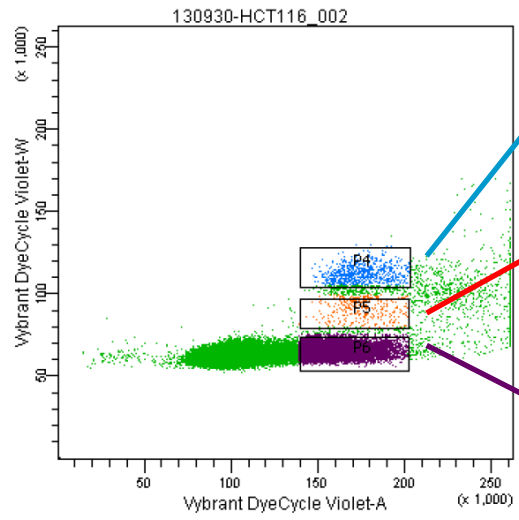
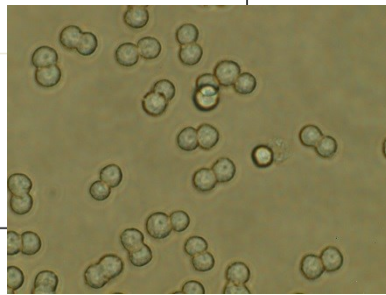
VybrantDCV_CellCycleSorting



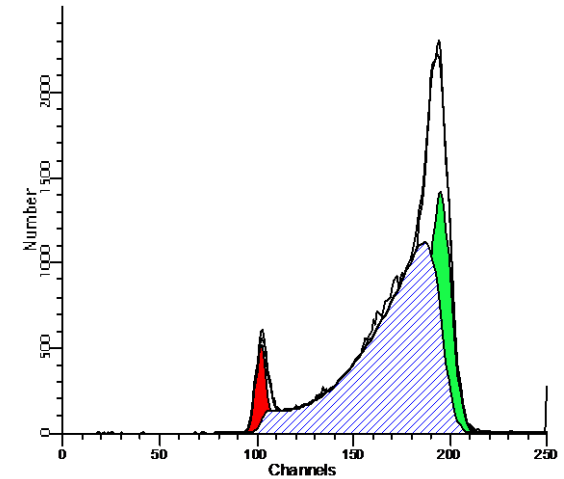
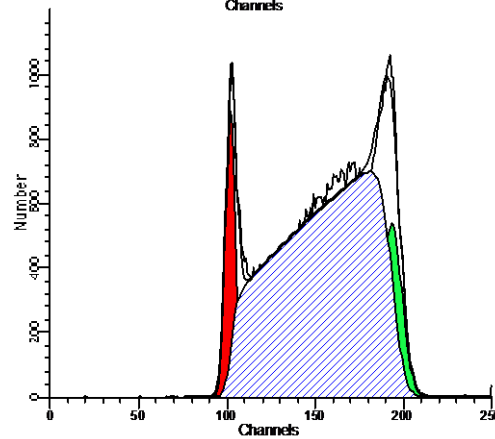
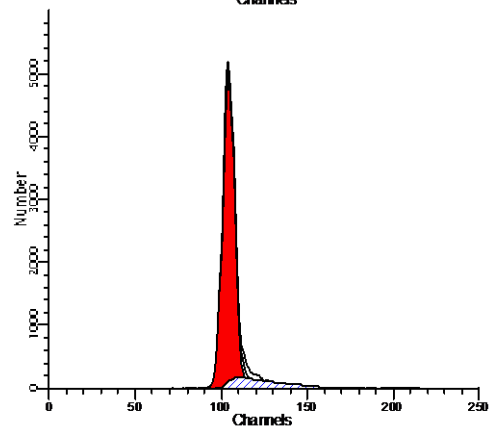
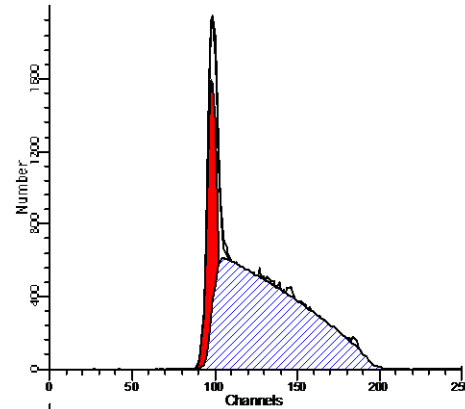
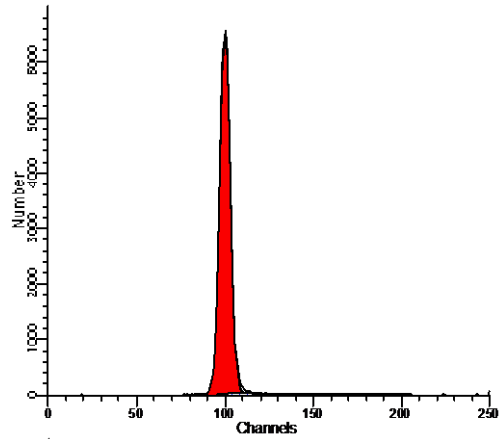
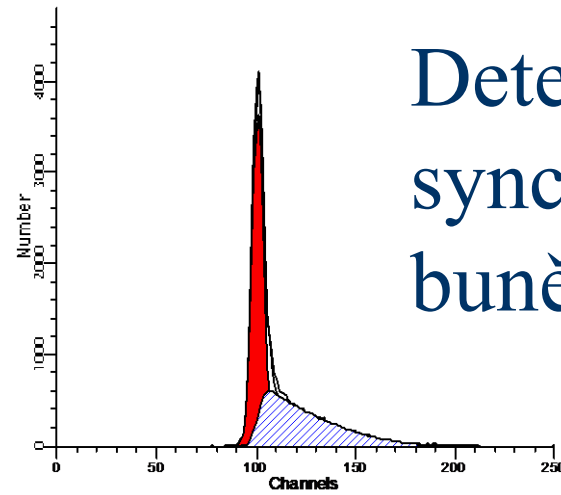
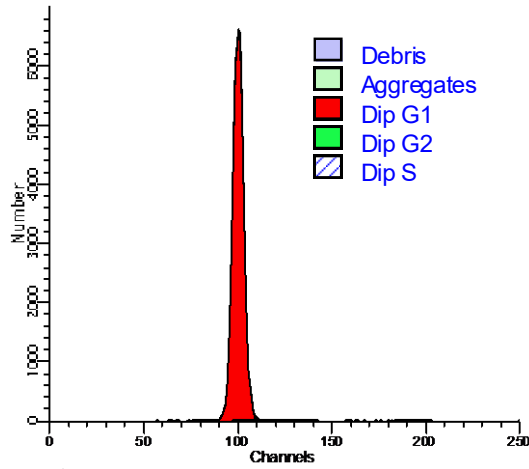
Tube: HCT116_002

Population

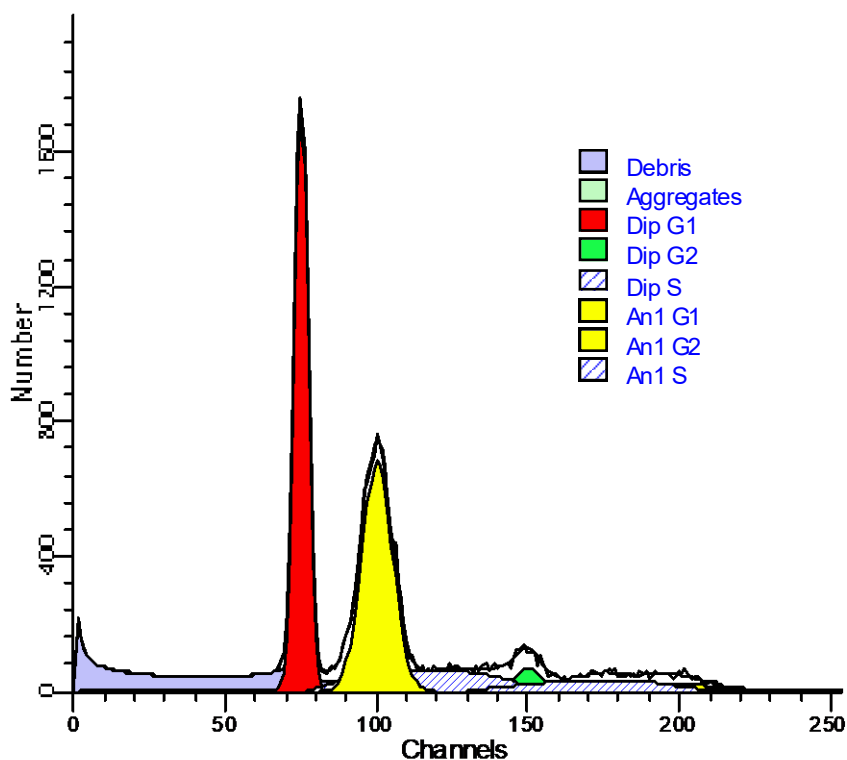
- All Events
- P1
- P2
- P3
- F
- F
- F



Detekce buněk v synchronizovaném buněčném cyklu



Aneuploidie je významný diagnostický marker



File analyzed: SAMPLE2.FCS
Date analyzed: 16-Oct-2006
Model: 2DA0n_DSD_ASD
Analysis type: Automatic analysis

Diploid: 57.22 %
Dip G1: 70.35 % at 75.05
Dip G2: 5.60 % at 150.10
Dip S: 24.05 % G2/G1: 2.00
%CV: 3.02

Aneuploid 1: 42.78 %
An1 G1: 83.63 % at 100.15
An1 G2: 5.87 % at 200.30
An1 S: 10.50 % G2/G1: 2.00
%CV: 5.02 DI: 1.33

Total Aneuploid S-Phase: 10.50 %
Total S-Phase: 18.25 %
Total B.A.D.: 11.22 %

Debris: 19.13 %
Aggregates: 3.96 %
Modeled events: 31253
All cycle events: 24037
Cycle events per channel: 190
RCS: 0.842

Analýza ploidity u vyšších rostlin

Nicotiana tabacum

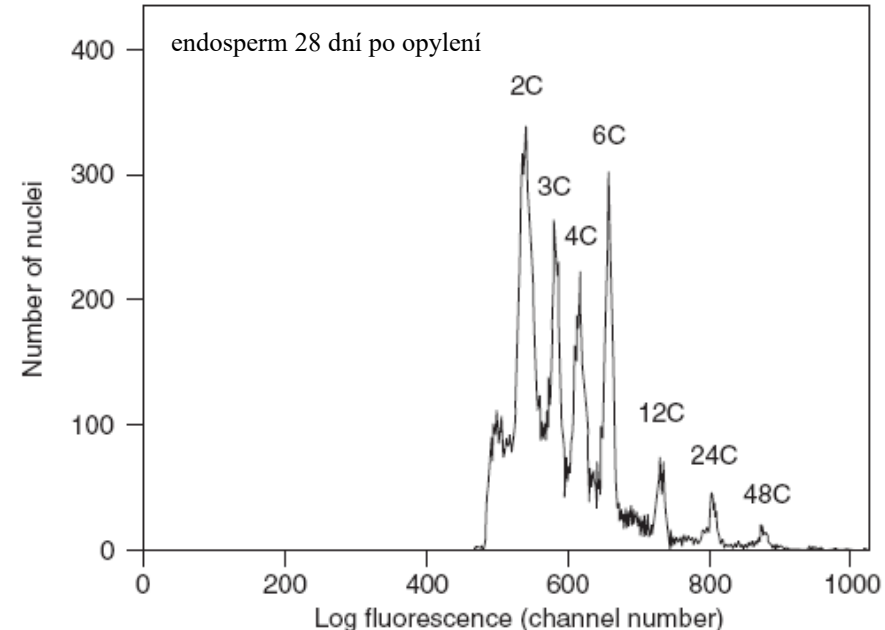
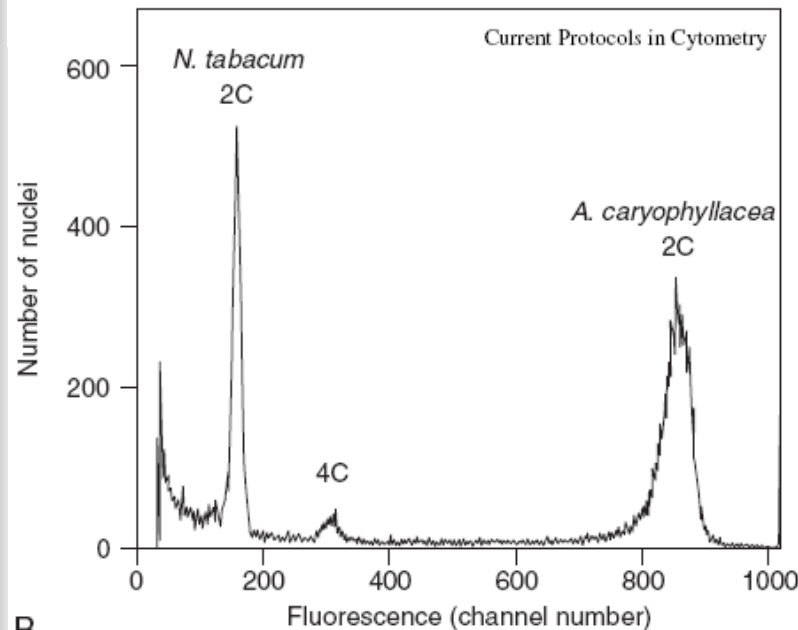


Alstroemeria caryophyllacea

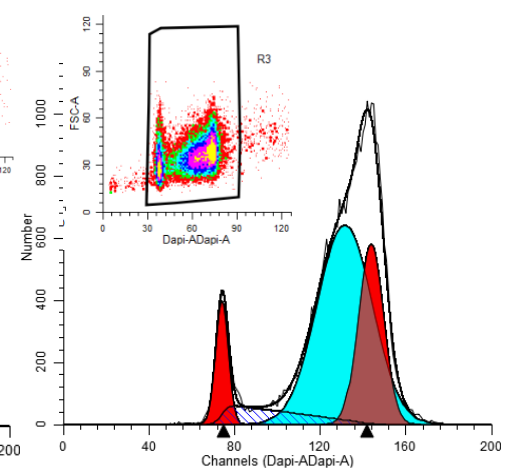
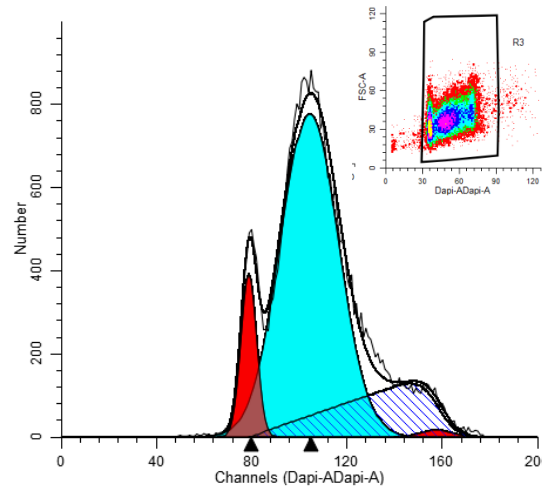
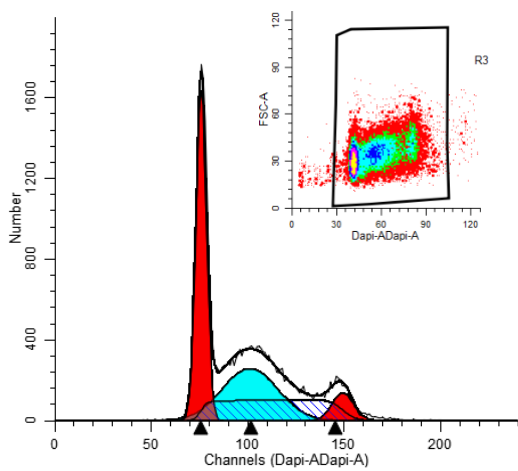
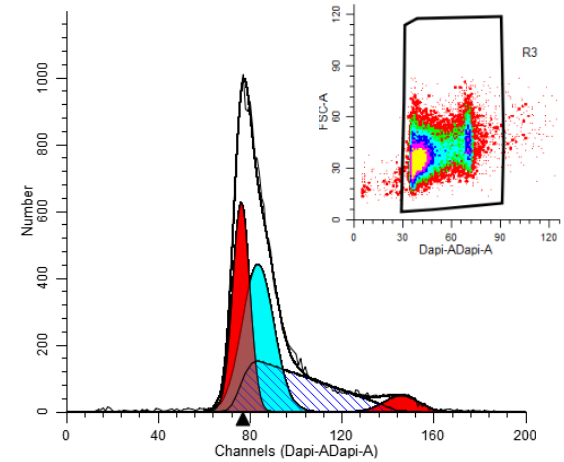
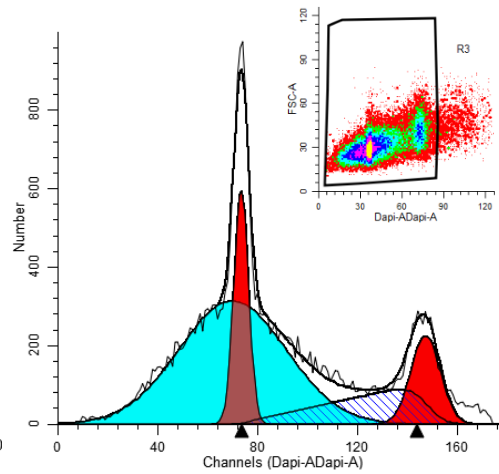
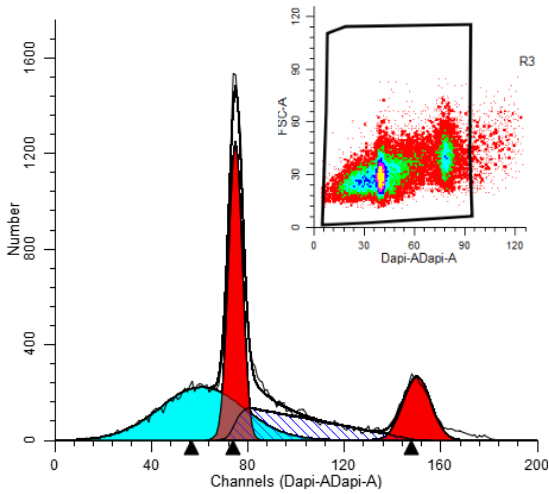


Zea mays

CyFlow® Ploidy Analyser



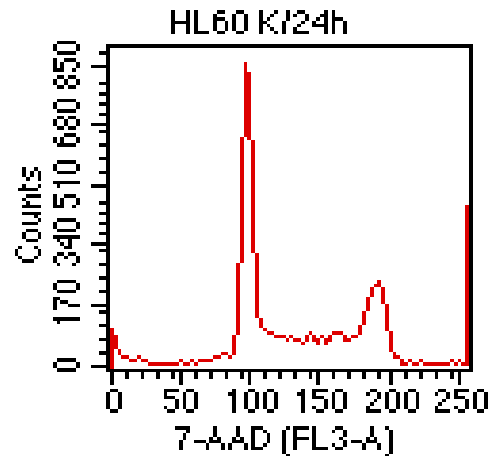
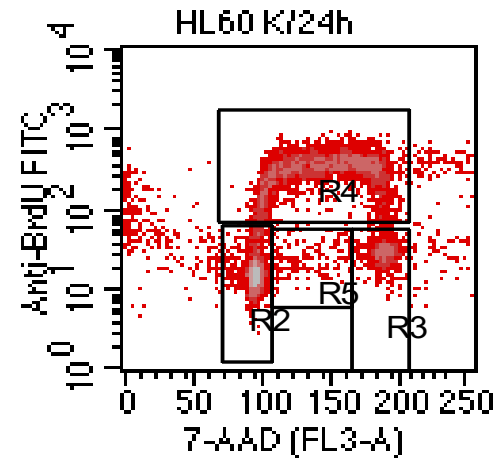
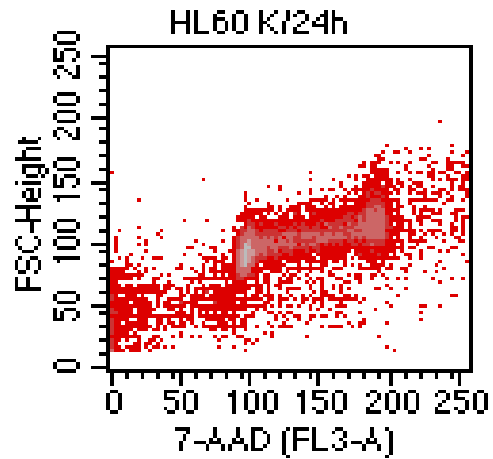
Cell cycle analysis- limitations



Analýza inkorporace BrdU

- bromodeoxyuridin se inkorporuje do DNA namísto tymidinu během S-fáze
- po fixaci a částečné denaturaci DNA je možné BrdU detekovat pomocí specifické protilátky značené fluorochromem
- v posledním kroku můžeme obarvit DNA

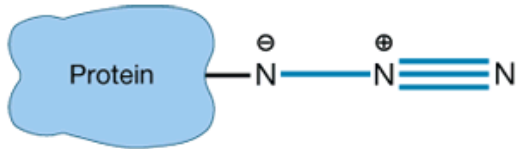
Analýza inkorporace BrdU



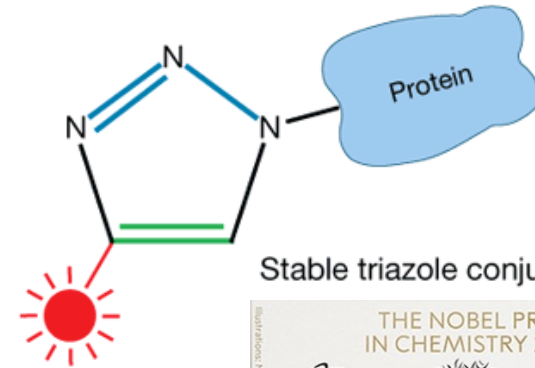
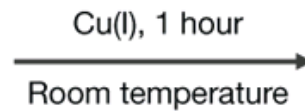
File: HL60 K/24h

Region	% Gated
R1	100.00
R2	35.48
R3	10.25
R4	47.87
R5	1.32

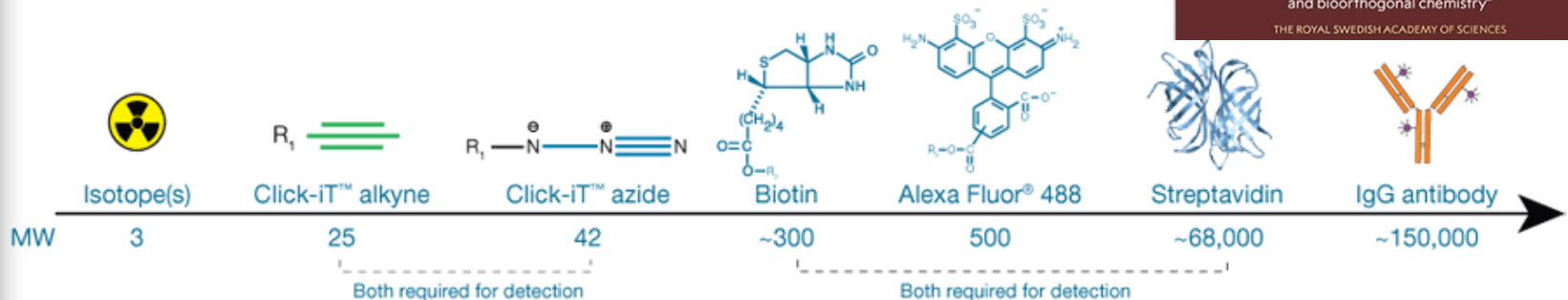
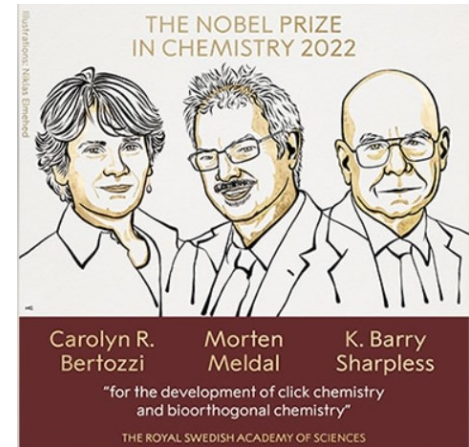
Click azide/alkyne reaction



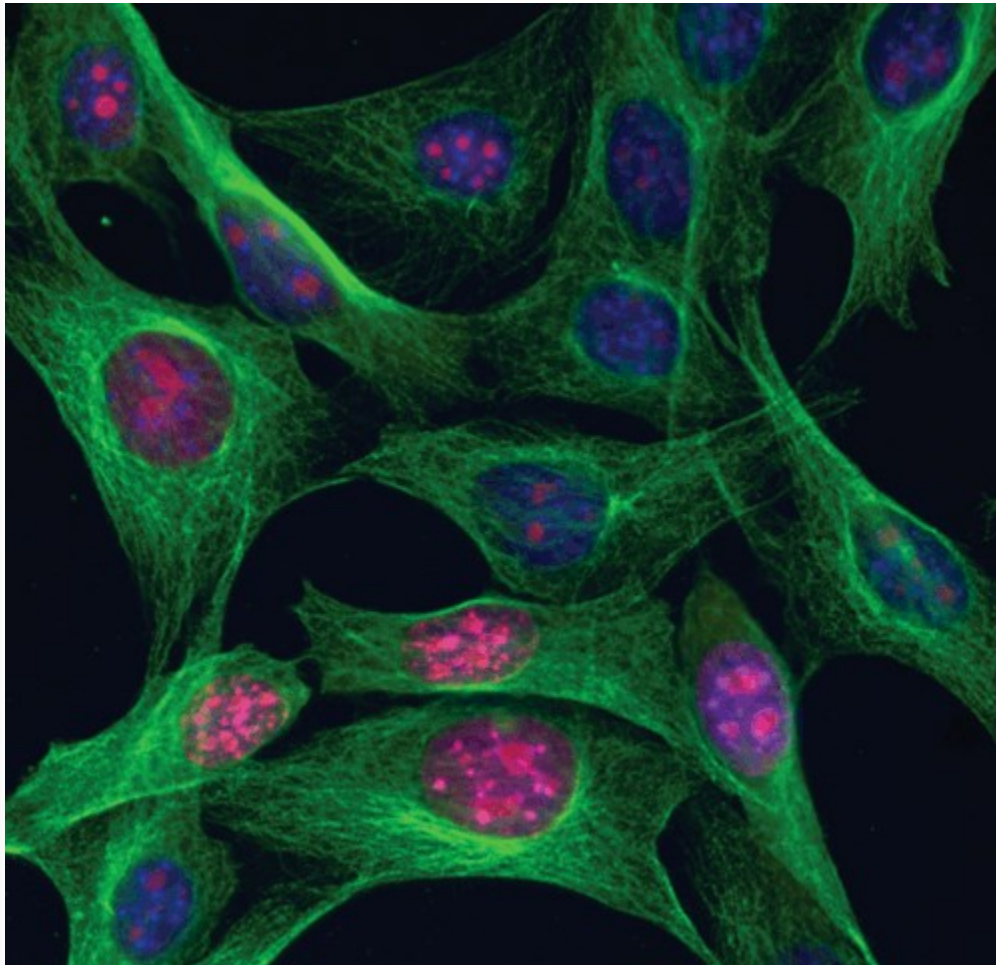
Metabolically or enzymatically azide-modified protein



TAMRA, Dapoxyl®, or biotin alkyne



Aplikace Click-IT (Invitrogen)



Multiplex imaging with Click-iT® RNA assays.

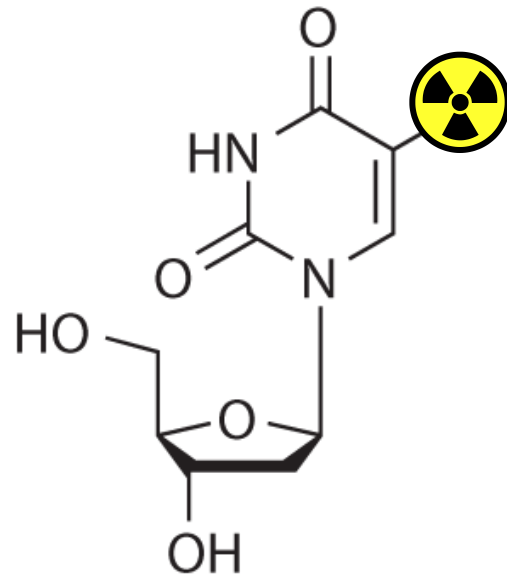
NIH3T3 cells were incubated with 1 mM EU, formaldehyde-fixed, and permeabilized with Triton® X-100. EU incorporated into newly synthesized RNA (red) in some cells was detected using the Click-iT® RNA Alexa Fluor® 594 Imaging Kit. Tubulin (green) was detected with anti-tubulin mouse IgG9 and visualized with Alexa Fluor® 488 goat anti-mouse IgG. Nuclei (blue) were stained with Hoechst 33342.



Aplikace Click-IT (Invitrogen)

analýza syntézy DNA
(proliferace)

^3H -thymidine



Tritiated (^3H) thymidine

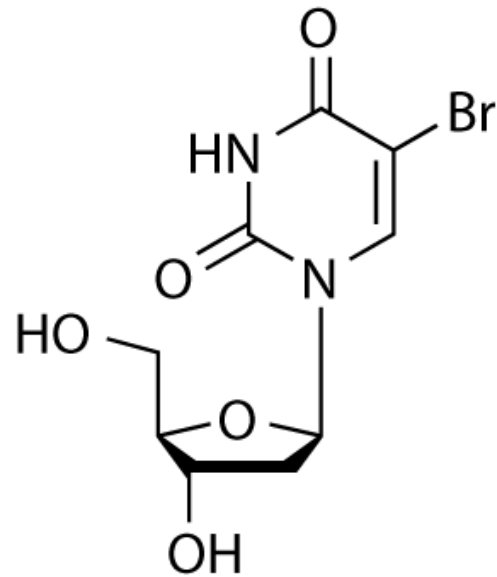


^3H -thymidine

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



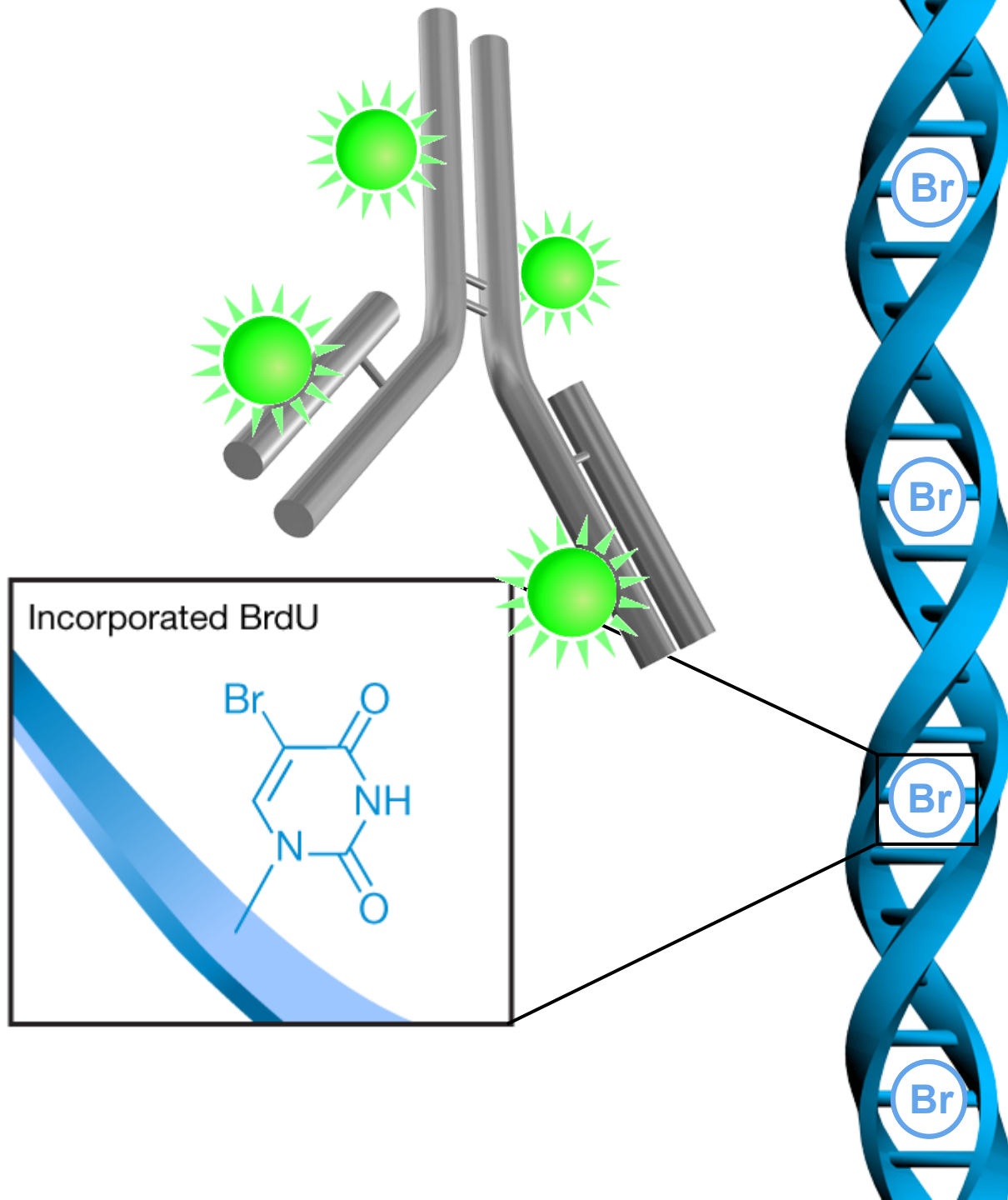
BrdU



BrdU (5-bromo-2'-deoxyuridine)

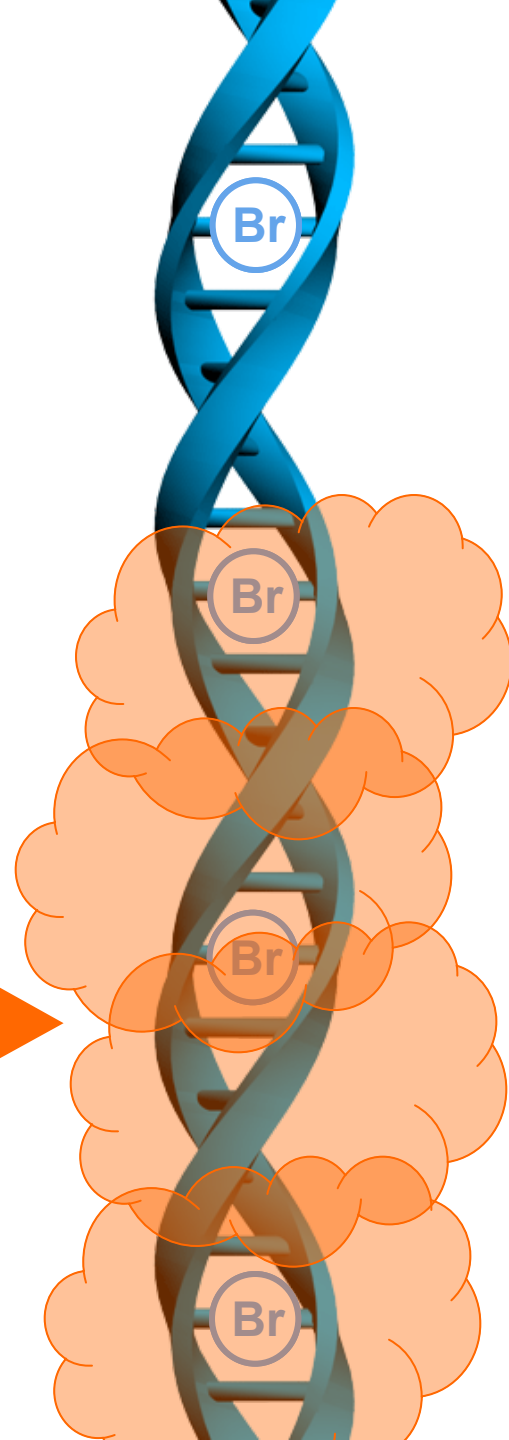


BrdU

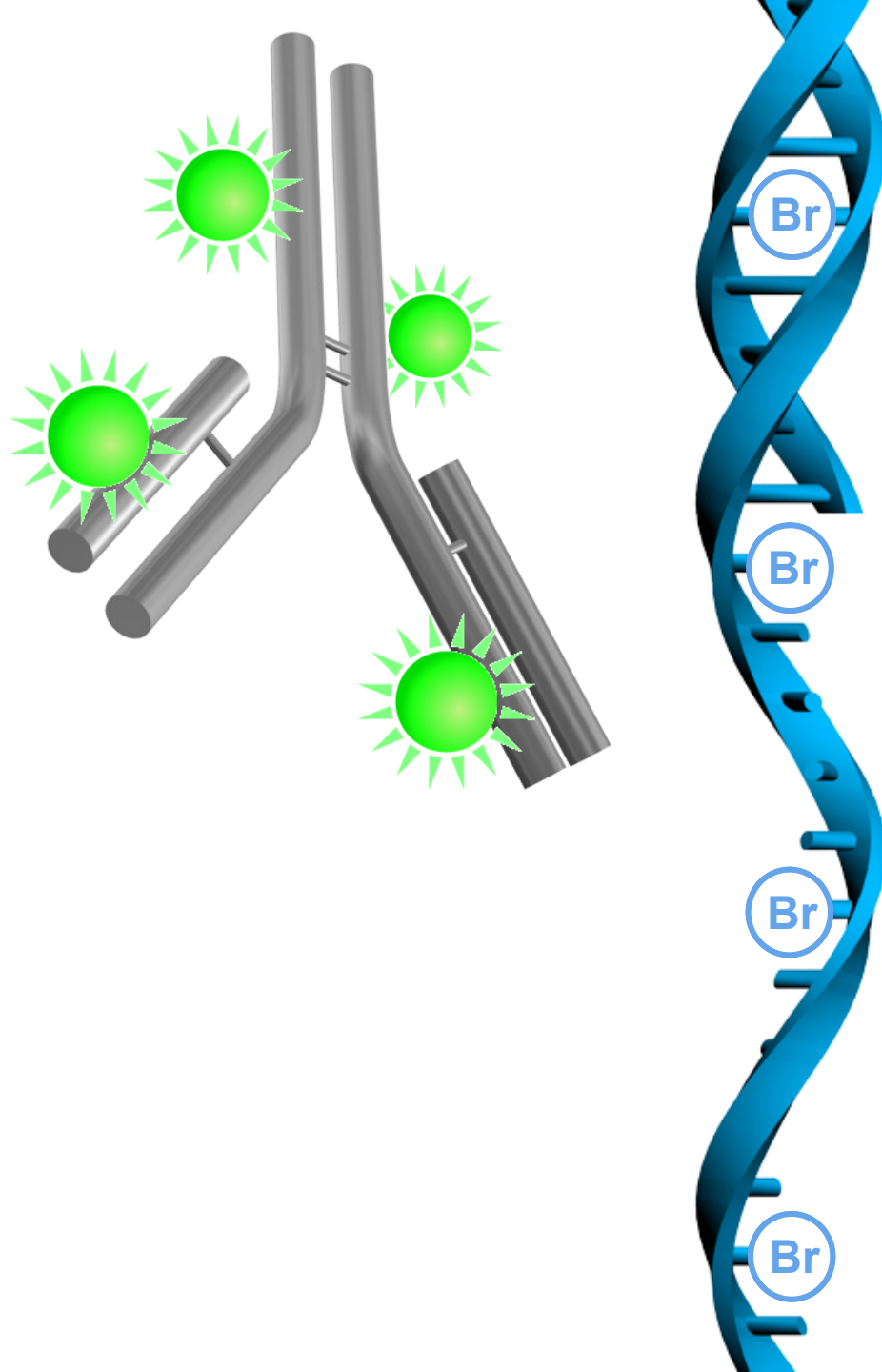


BrdU

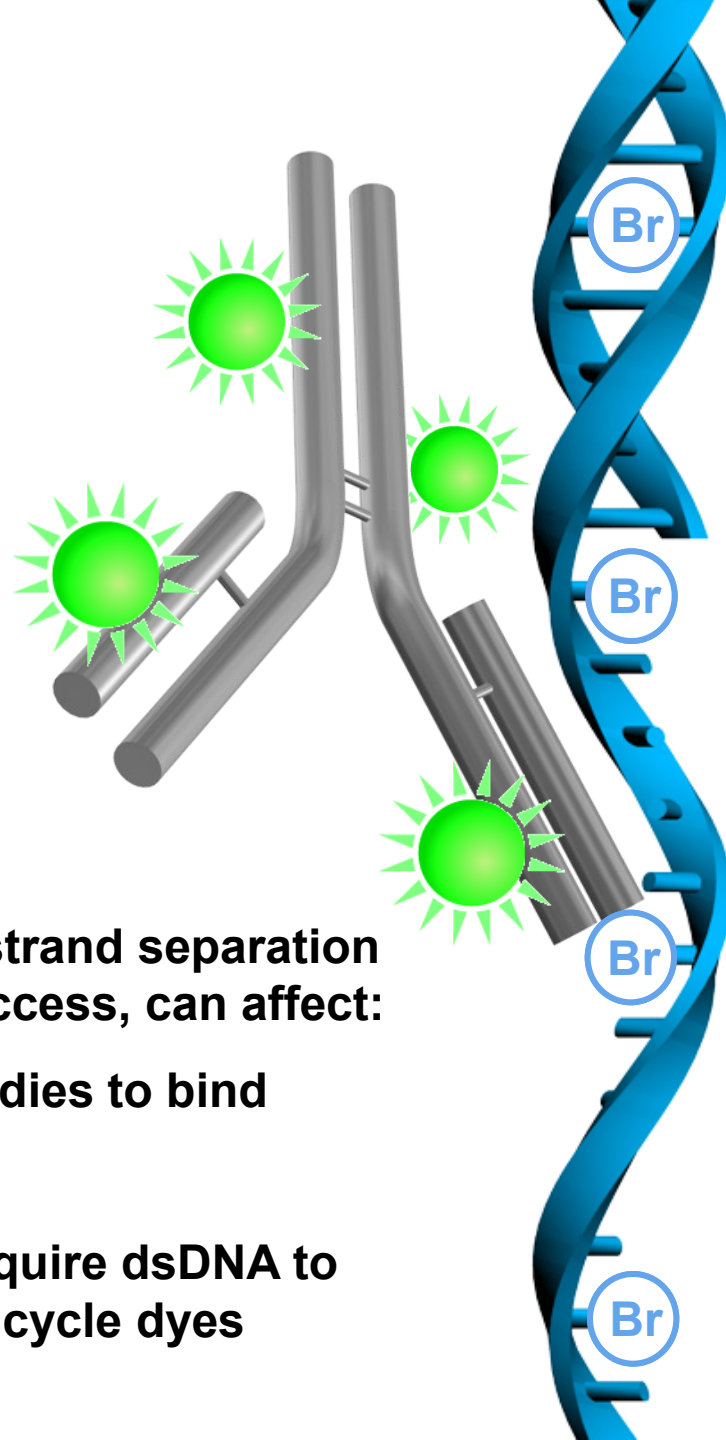
Acid or DNase 



BrdU

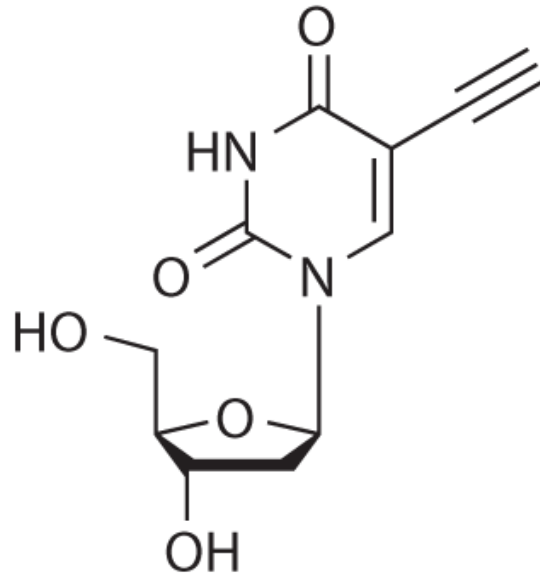


BrdU



- **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**

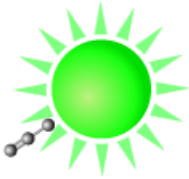
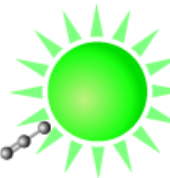
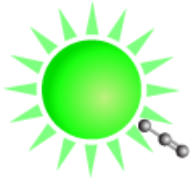
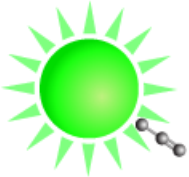
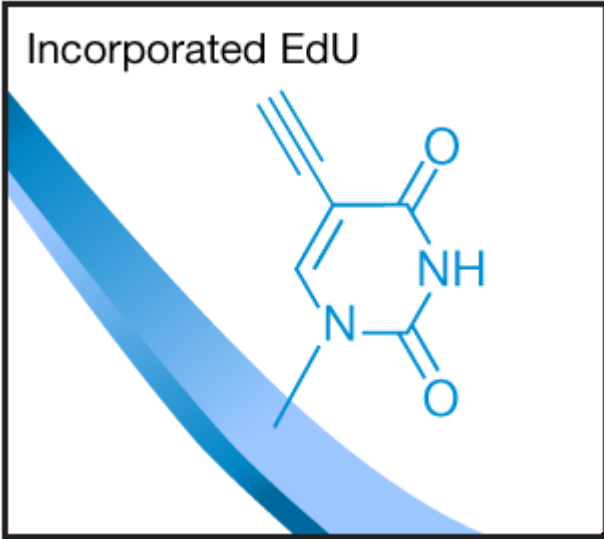
Click-iT™ EdU



EdU (5-ethynyl-2'-deoxyuridine)

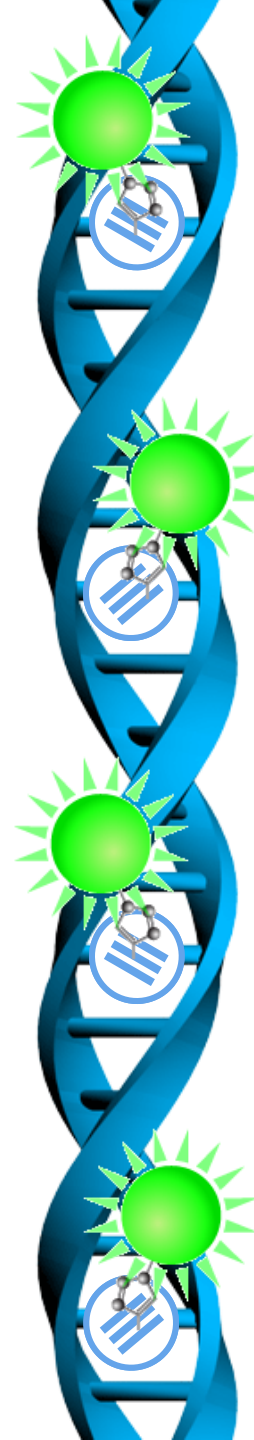


Click-iT™ EdU



Click-iT™ Edu

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



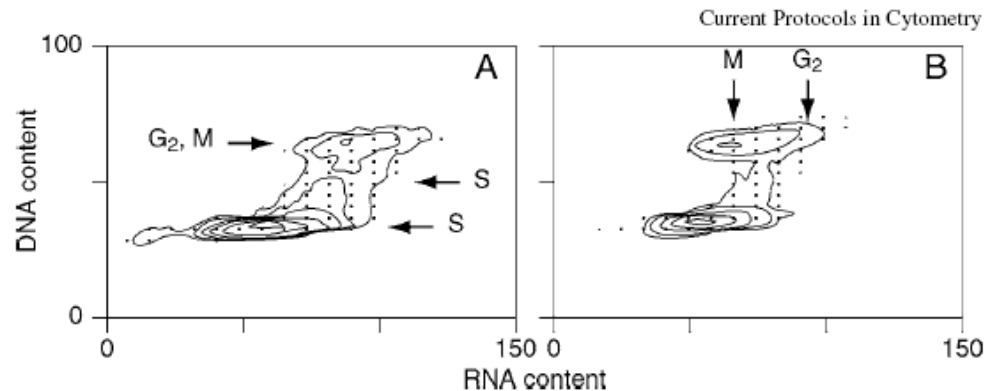
Analýza DNA a RNA

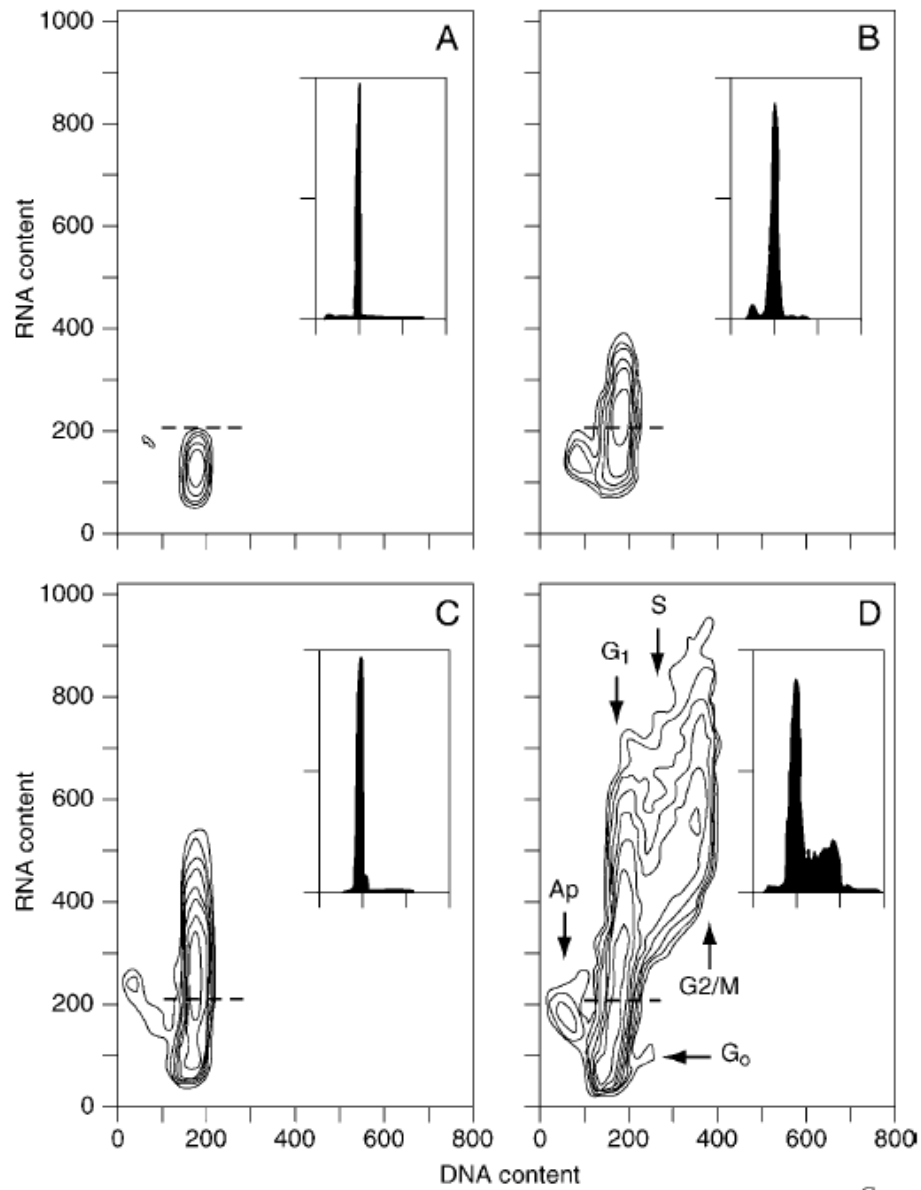
Pyronin Y vs. Hoechst 33342

- Pyronin interaguje s ds RNA a DNA ale jeho vazba na DNA je inhibována přítomností Hoechst 33342

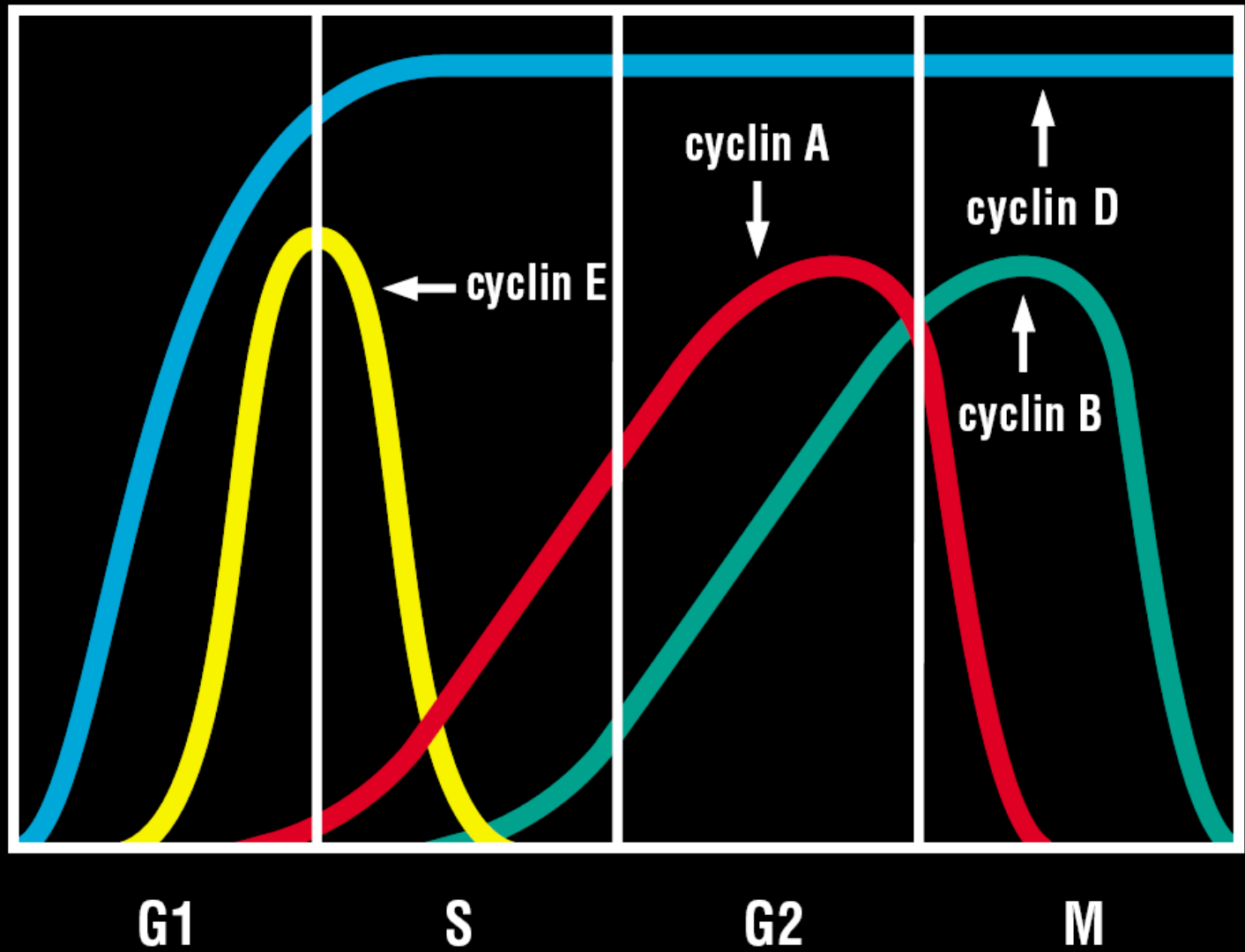
- Acridine orange

- při interakci s RNA emituje červené světlo a při interakci s DNA zelené

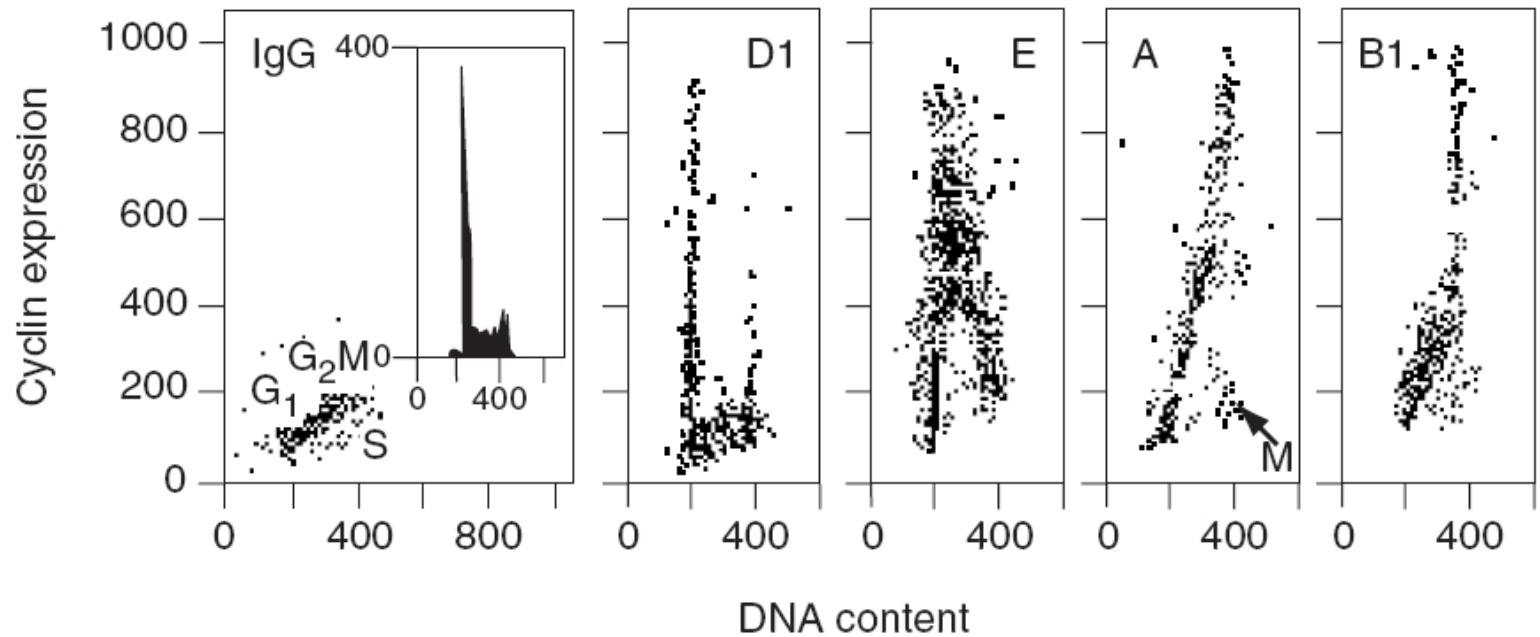




Cyclin Expression: Periodicity

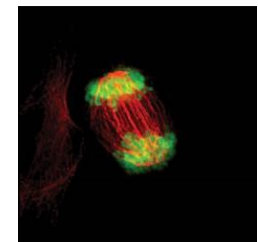
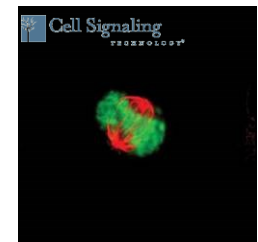
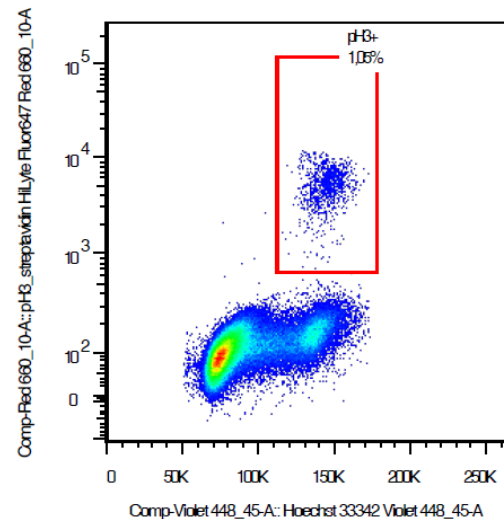
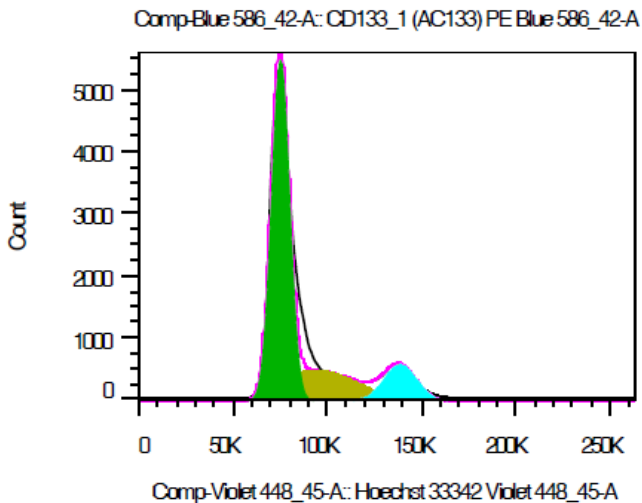


Detekce intracelulárních proteinů v kombinaci s detekcí DNA



Detekce mitotických buněk

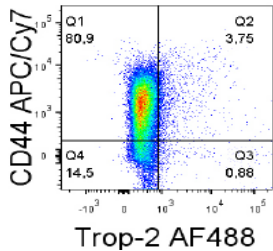
- Histone H3 je specificky fosforylován během mitózy (Ser10, Ser28, Thr11)
- dvojité značení DNA vs. H3-P identifikuje populaci buněk v M-fázi



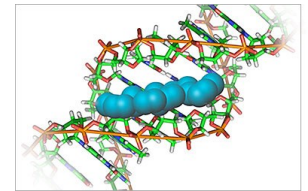
Flow cytometry

most common applications

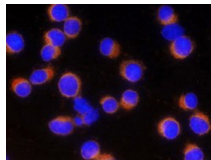
Immunophenotype characterisation of the cells
(CSCs markers, differentiation, ...)



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



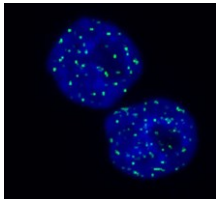
Cell Death analysis
(AnnexinV, Cleaved Caspase3, ...)



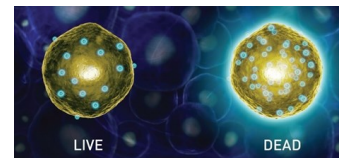
Proliferation (BrdU, EdU, mitosis - pH3)



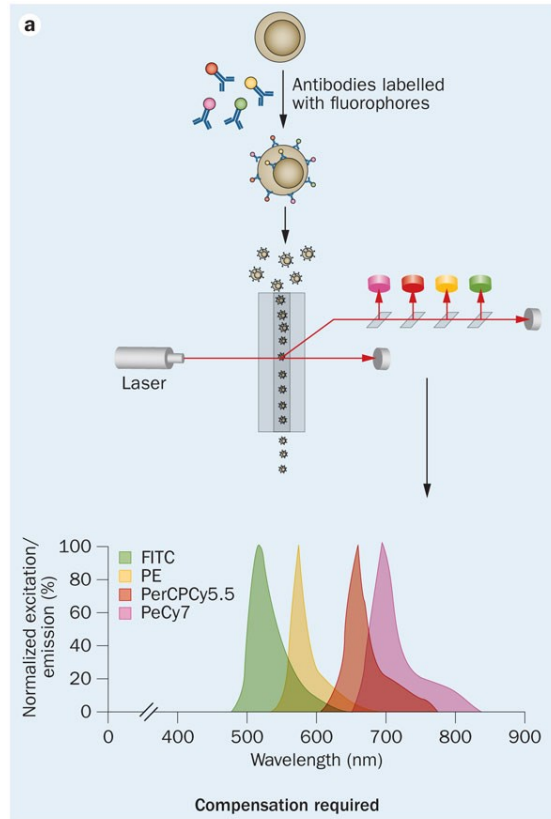
DNA damage (γ H2AX,...)



Viability assays (propidium iodid, Calcein AM, ...)



IMMUNOPHENOTYPING



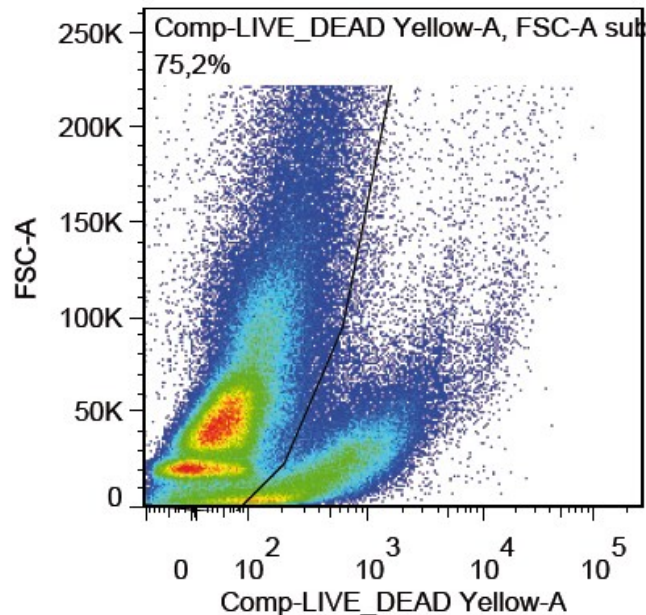
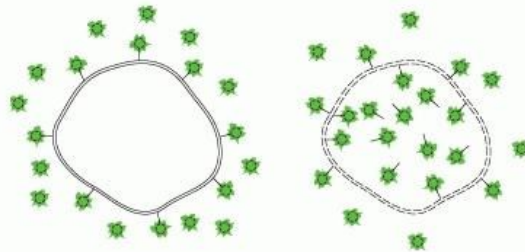
Ermann, J. *et al.* (2015) Immune cell profiling to guide therapeutic decisions in rheumatic diseases
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2015.71

Principle: cells are stained with monoclonal antibodies conjugated to various fluorescent dyes and analyzed with using flow cytometry

Pros: simple, standard, broad spectrum of tested reagents, multiplexing

Cons: not every epitope is fixable, compensation, possible artefacts from dying cells, dissociation of solid tissue may affect results

VIABILITY using LIVE/DEAD fixable stains

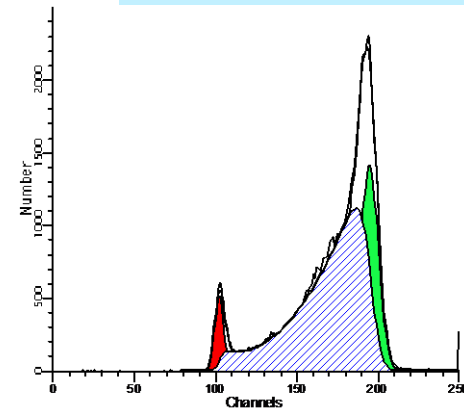
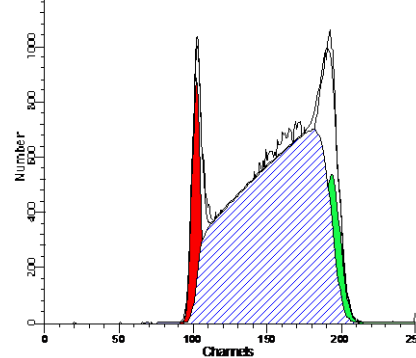
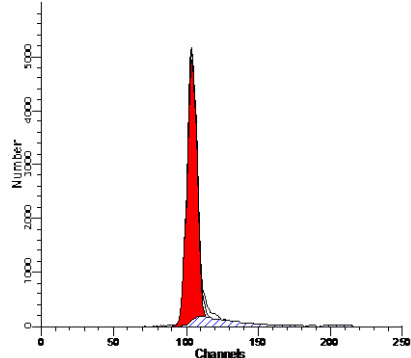
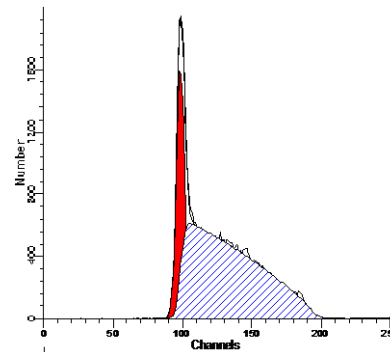
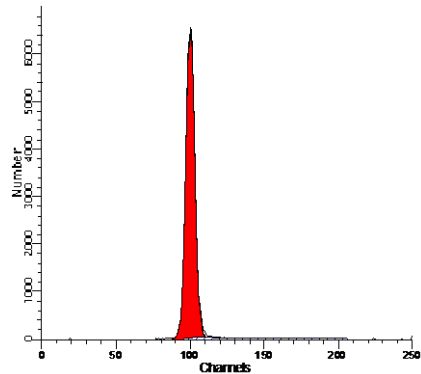
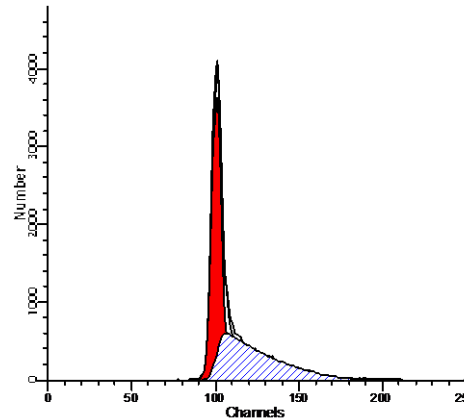
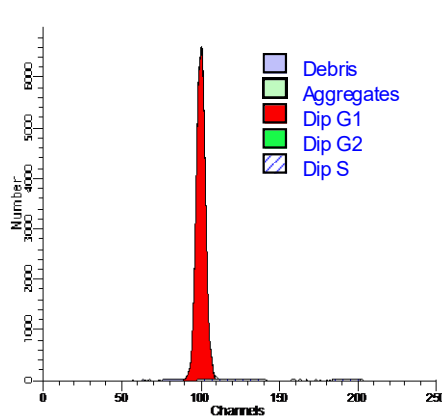


Principle: reaction of a fluorescent reactive dye with cellular amines, in necrotic cells react with free amines both in the interior and on the cell = intense staining, live cells stained on surface only = dim signal

Pros: simple, wide spectrum of dyes, fixable, The ArC™ Amine Reactive Compensation Bead Kit

Cons: live cells have signal, stain only in buffers w/o BSA or serum, Tris or azide

CELL CYCLE

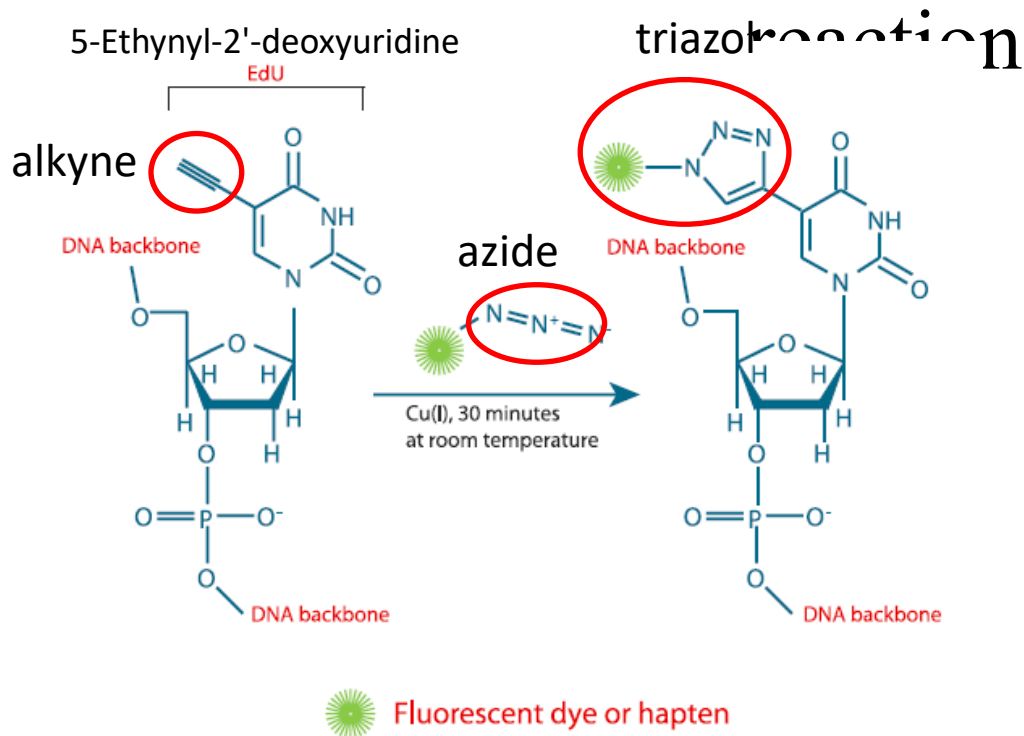


Principle: DNA content measurement by fluorescent nucleic-acid-binding dyes

Pros: simple, wide spectrum of dyes, in both native and fixed samples

Cons: doublets > G2/M, single parameter ≠ DNA synthesis, > CV if not fixed by organic solvents

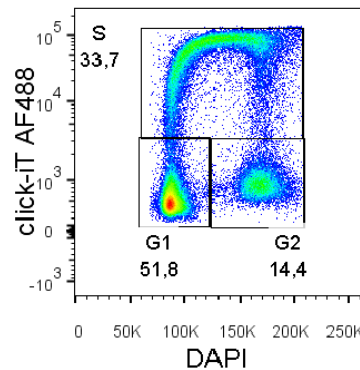
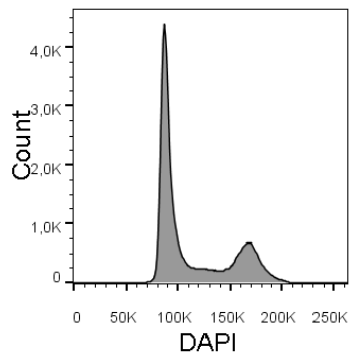
DNA SYNTHESIS using click azide/alkyne



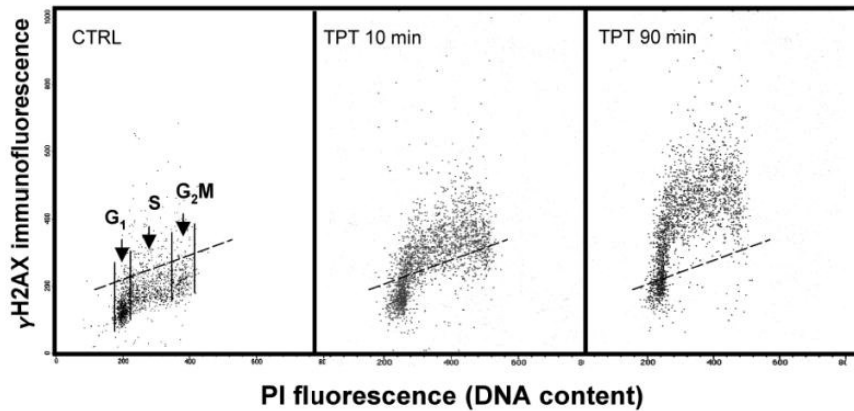
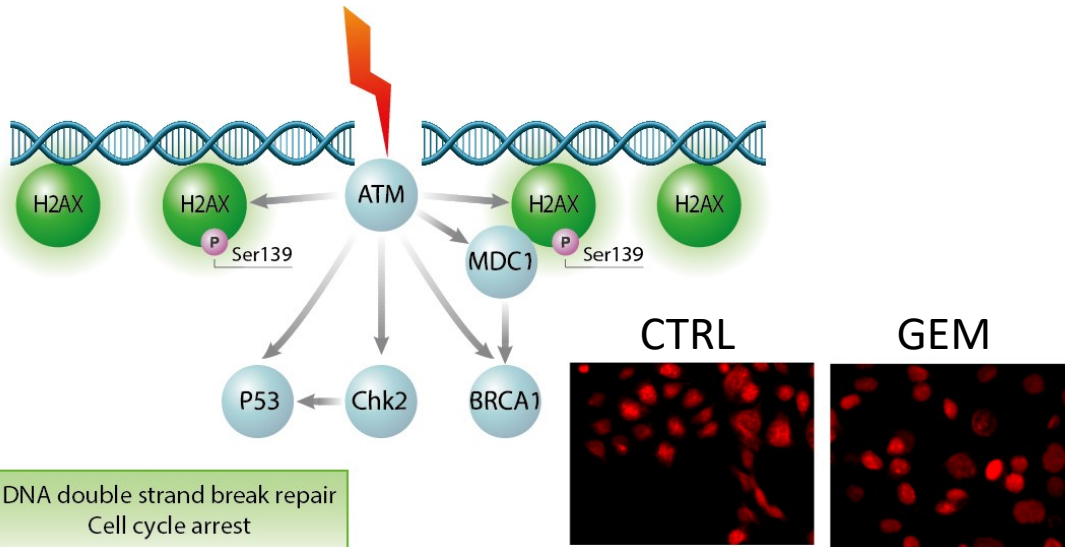
Principle: direct measurement of DNA synthesis via visualization of incorporation of nucleoside analogue

Pros: no DNA denaturation required, simplified protocol, small molecule detection, multiplex compatible

Cons: high concentration of Cu in reaction = not compatible with all fluorochromes



DNA DAMAGE using γ H2A.X



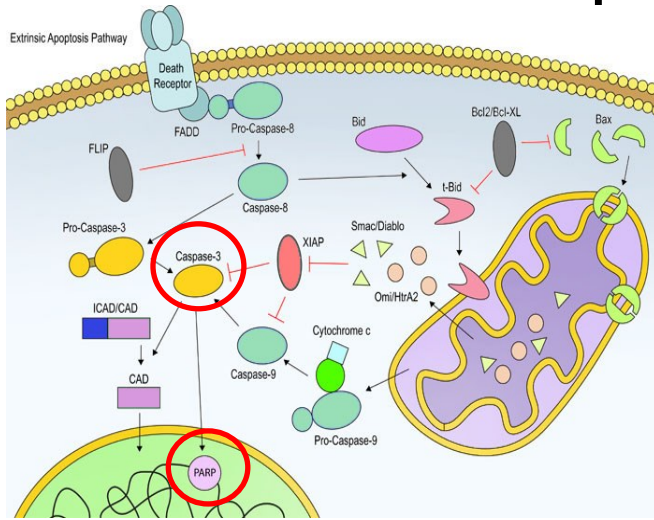
Huang X, Darzynkiewicz Z: **Cytometric Assessment of Histone H2AX Phosphorylation.** In *DNA Repair Protocols: Mammalian Systems.* Edited by Henderson DS. Totowa, NJ: Humana Press; 2006: 73-80

Principle: Phosphorylation of the Ser-139 residue of the histone variant H2A.X, forming γ H2A.X, is an early cellular response to the induction of DNA double-strand breaks

Pros: in theory simple immuno-staining after fix&perm

Cons: DSBs can also be intrinsic, occurring in healthy, nontreated cells, DSBs are formed in the course of DNA fragmentation in apoptotic cells

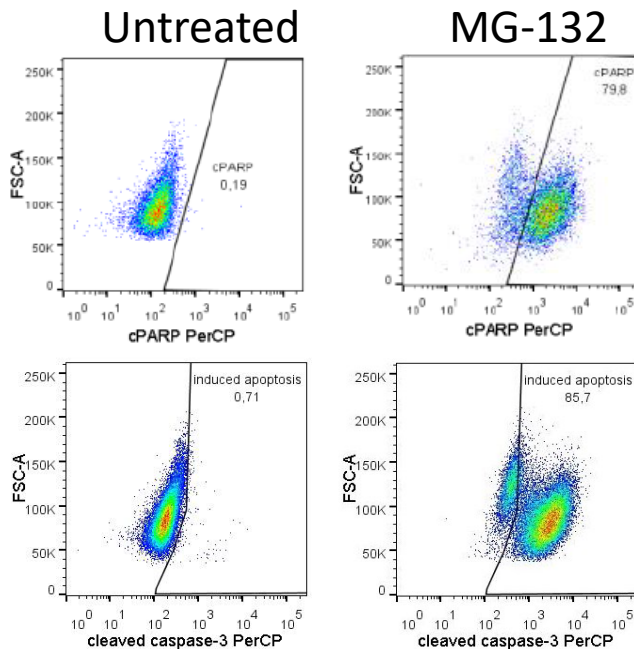
APOPTOSIS detected via PARP cleavage or caspase-3 activation



Principle: Cleaved Caspase-3 (Asp175) Antibody detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3. Cleaved PARP (Asp214) detects endogenous levels of the large fragment (89 kDa) PARP1 protein produced by caspase cleavage.

Pros: simple immunostaining after fix&perm, validated antibodies available

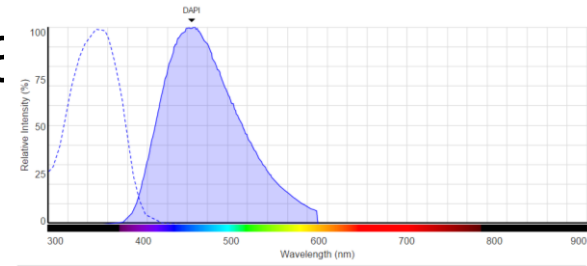
Cons: not every cell type or signal necessary activates cp-3 or leads to PARP cleavage, timing



DNA stain

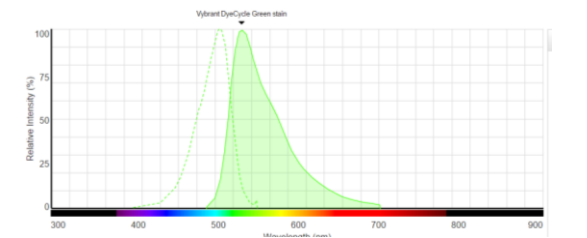
■ Violet laser

DAPI, Hoechst 33342
FxCycle Violet, ...



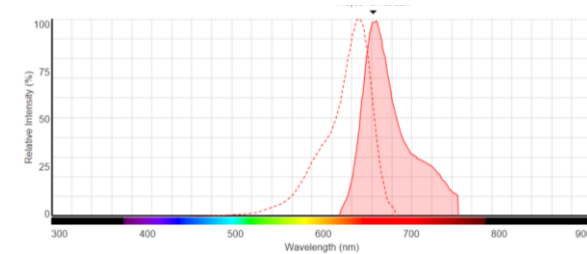
■ Blue laser

Vybrant Dyes, PI, ...



■ Red laser

FxCycle Far Red
7-AAD



Broad spectrum of the dyes

Problems:

High concentration of dye, no wash
Spillover & Compensations

Compensation

Antibody conjugates:

- anti-rat and anti-hamster Igk/negative control compensation beads (BD Biosciences),
- Sphero™ Biotin Polystyrene Particles (Spherotech, Lake Forest, IL, USA)

Live/Dead fixable dyes:

- Arc™ Amine Reactive Compensation Bead Kit beads (Thermo Fisher Scientific)

DNA stain:

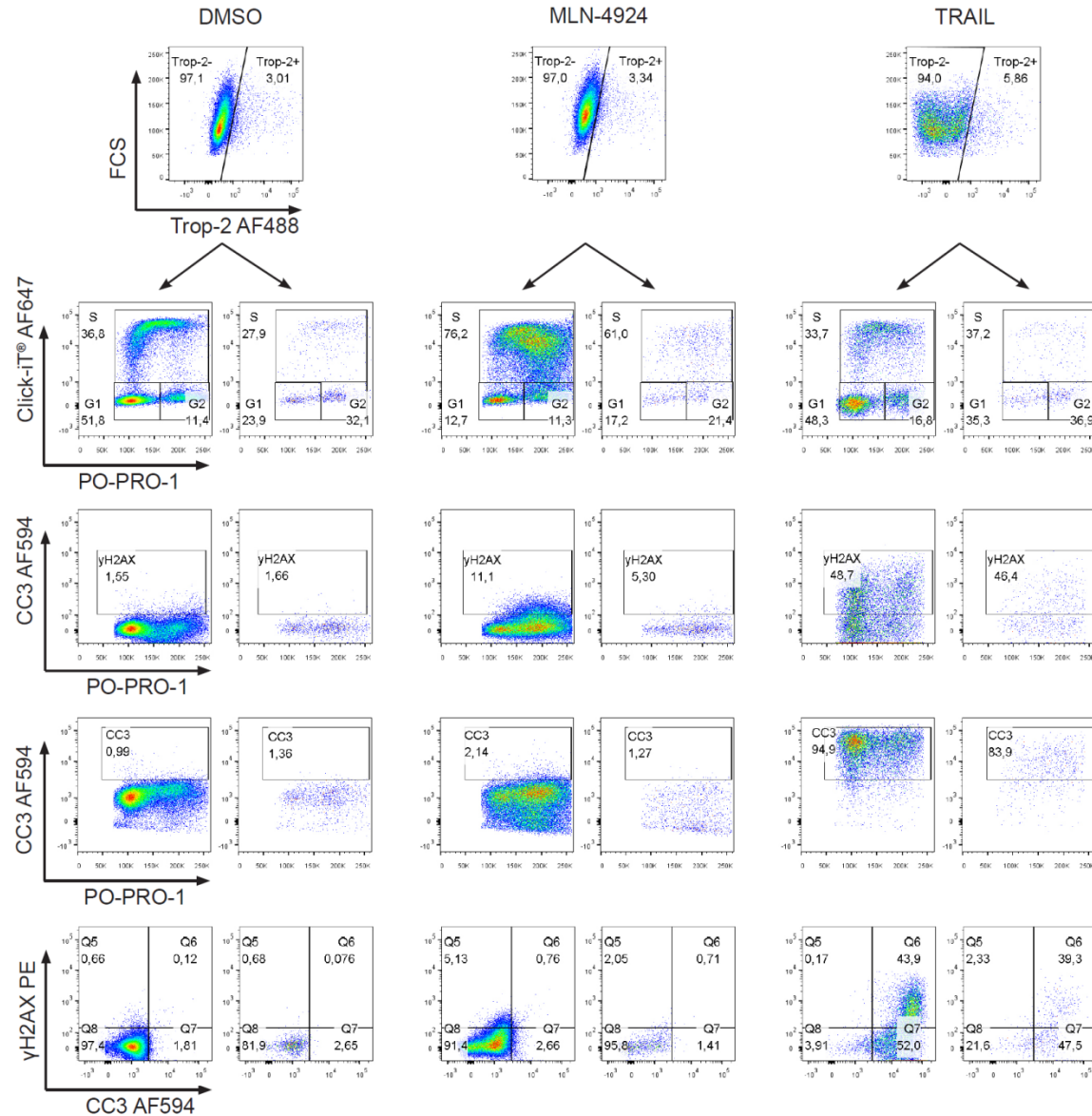
- fixed and permeabilized cells with/without appropriately diluted DNA probe

Isotype controls were recorded for all samples. Gates were set according to isotype controls and control untreated cells (for γ H2AX and cleaved caspase-3)

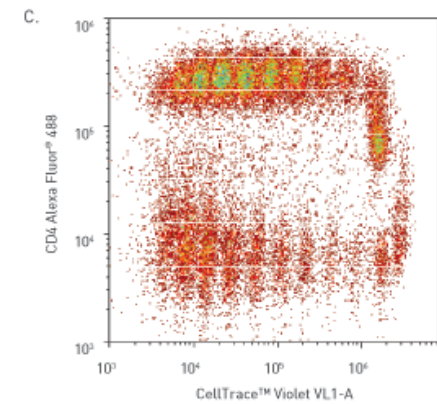
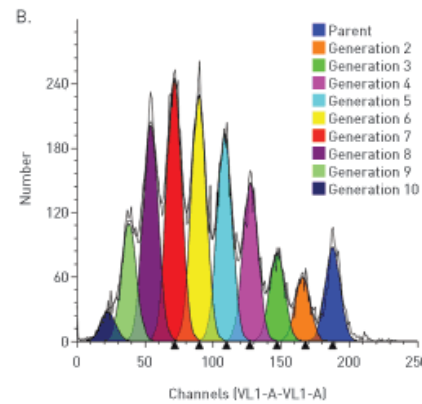
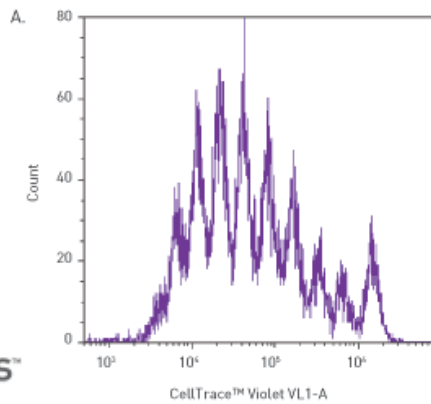
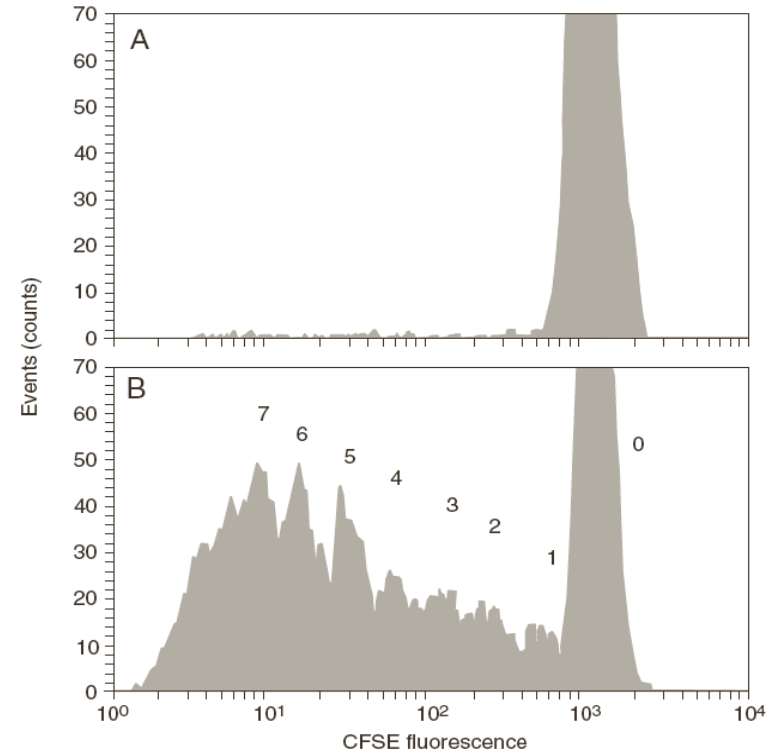
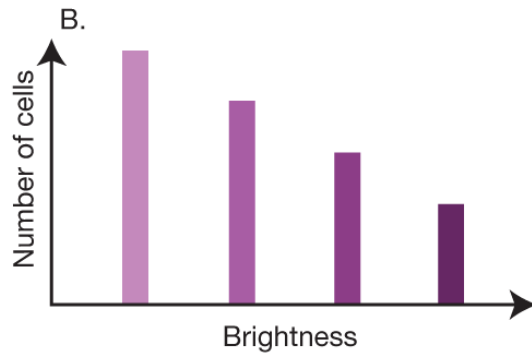
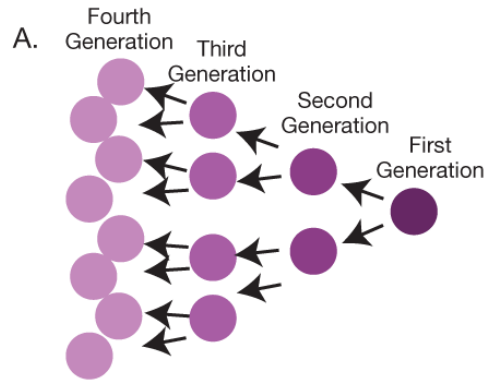
Gating strategy included viability, discrimination of doublets (FSC-H vs. FSC-A) and debris (FSC vs. SSC). In samples with DNA marker, doublets we further discriminated using DNA marker (PO-PRO-1 A vs. PO-PRO-1 W) .

In the process of protocol optimization, FMO controls were measured and revealed DNA dye spillover.

Examination of small subpopulation (Trop-2⁺) in response to experimental treatment



Detekce počtu buněčného dělení





The Nobel Prize in Chemistry 2008

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

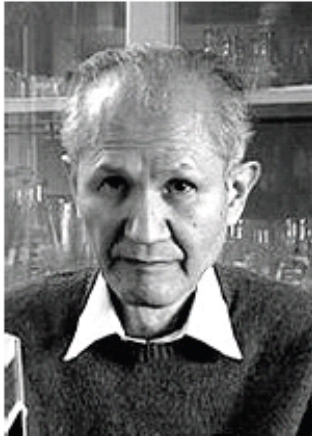


Photo: J. Henriksson/SCANPIX

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

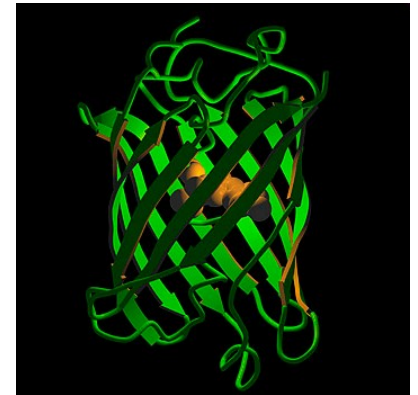
Roger Y. Tsien

🕒 1/3 of the prize

USA

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

b. 1952



Fluorescenční proteiny

■ bioluminescence resonance energy transfer (BRET)

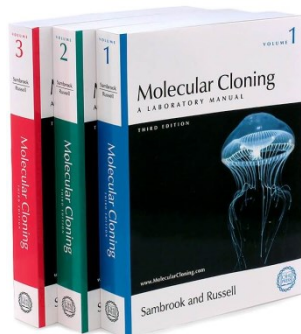
Aequorea victoria - medúza žijící ve vodách na pobřeží Severní Ameriky.

- je schopna modře světélkovat (bioluminescence). Ca^{2+} interaguje s fotoproteinem aequorinem.
- modré světlo excituje **green fluorescent protein**.

Renilla reniformis – korál žijící ve vodách na severním pobřeží Floridy.

- luminescence vzniká degradací coelenterazinu za katalytického působení luciferázy.
- modré světlo excituje **green fluorescent protein**.

Aequorea victoria “Crystal jelly “



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

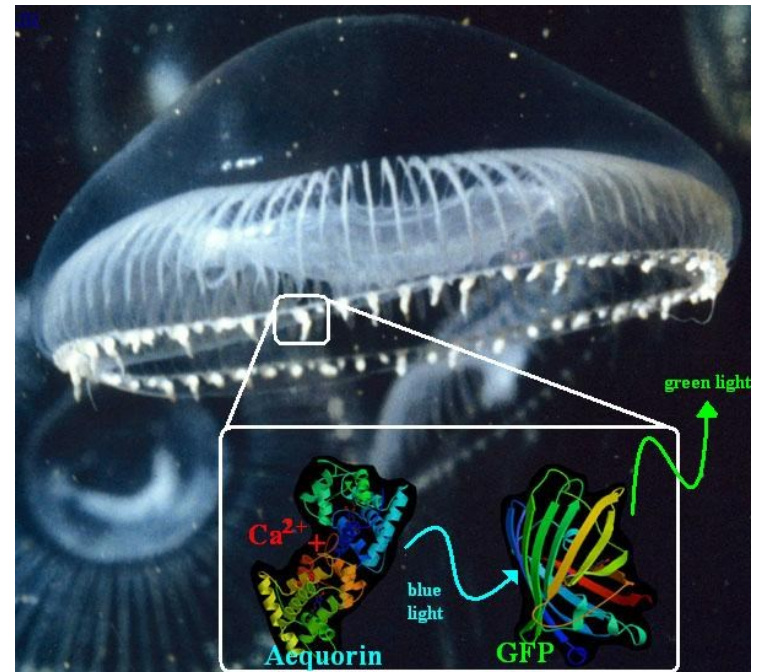
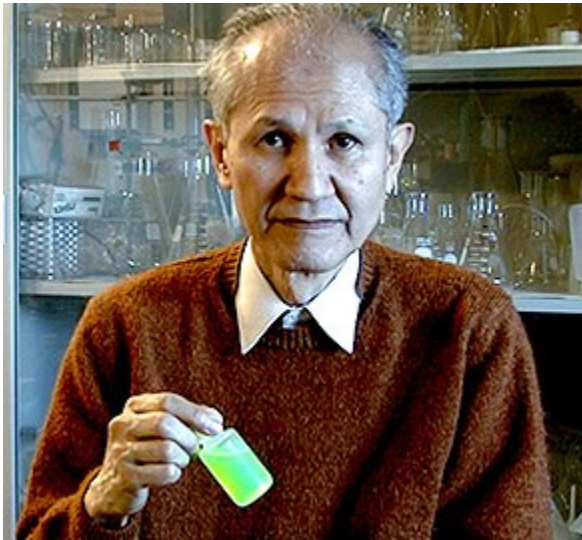
Renilla reniformis "Sea Pansy"



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

Fluorescenční proteiny

- **Osamu Shimomura**
– 1961 objevil GFP a aequorin



Fluorescenční proteiny

■ Douglas Prasher

■ Martin Chalfie

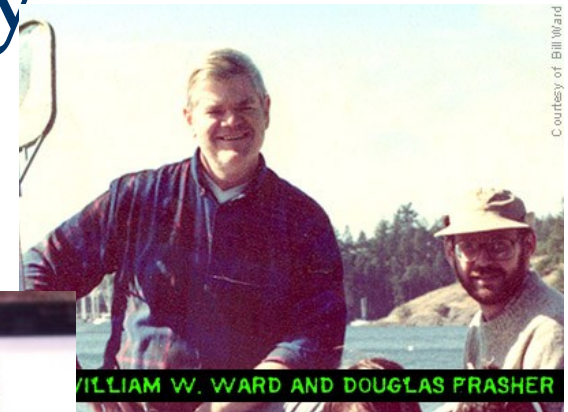
Science. 1994 Feb 11;263(5148):

Green fluorescent protein as a marker for gene expression.

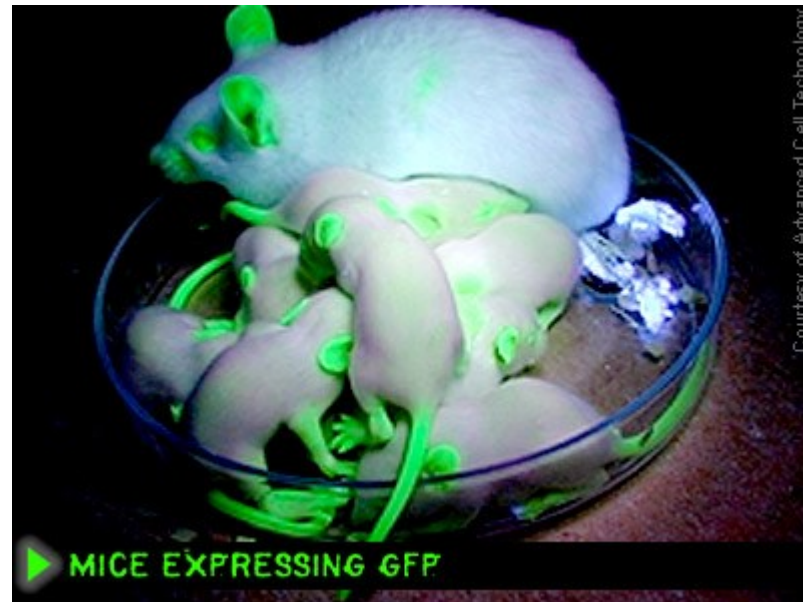
Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC.

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.

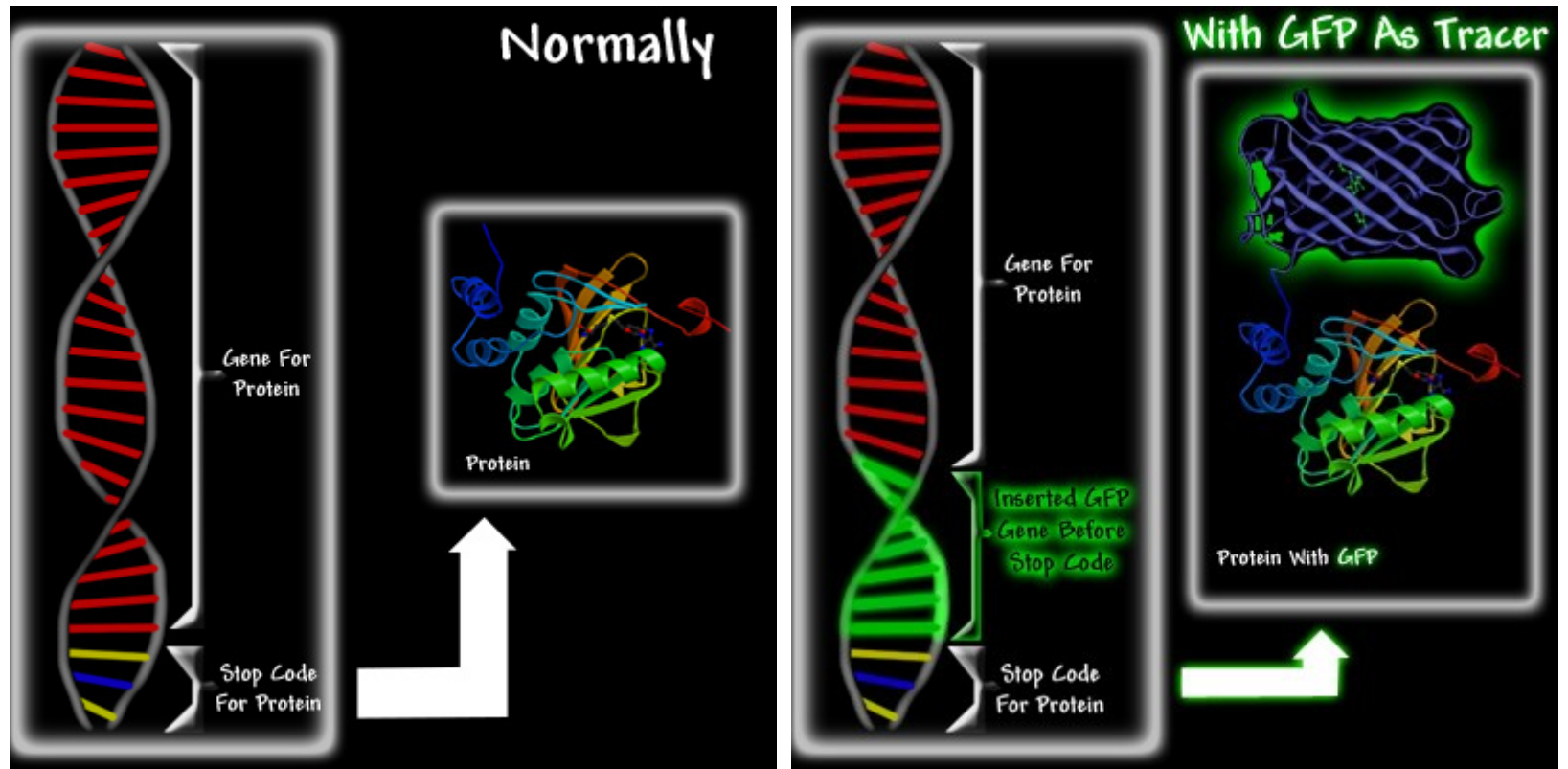


Courtesy of Bill Ward



Courtesy of Advanced Cell Technology

Fluorescenční proteiny

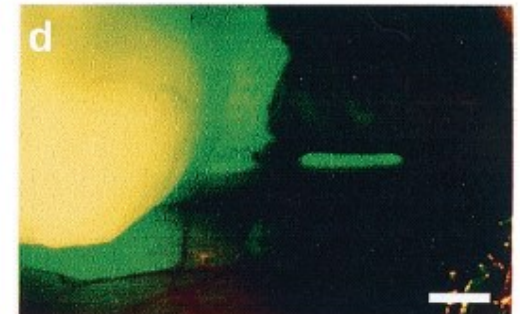
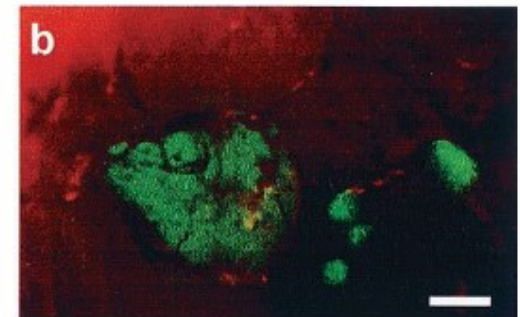
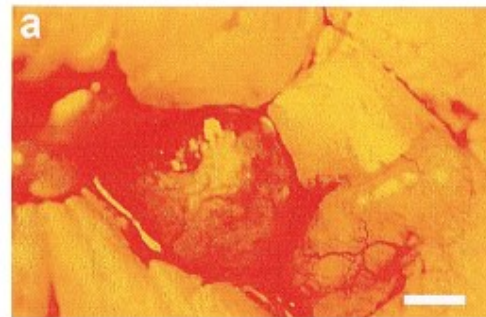
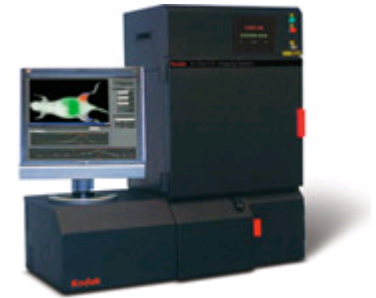
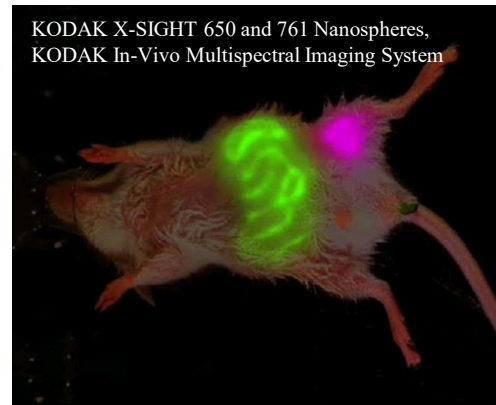


<http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm>

in vivo molekulární vizualizace



KODAK X-SIGHT 640 LSS Dyes *in vivo* with x-ray overlay



Hasegawa, S., Yang, M., Chishima, T., Miyagi, Y., Shimada, H., Moossa, A. R., and Hoffman, R. M. In vivo tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. *Cancer Gene Ther*, 7: 1336-1340, 2000.

Fluorescenční proteiny

■ Sergey A. Lukyanov

– Objevil „GFP-like“ proteiny u nesvětélkujících korálů



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RESEARCH

Fluorescent proteins from nonbioluminescent Anthozoa species

Mikhail V. Matz, Arkady F. Fradkov, Yulii A. Labas¹, Aleksandr P. Savitsky², Andrey G. Zaraisky, Mikhail L. Markelov, and Sergey A. Lukyanov*

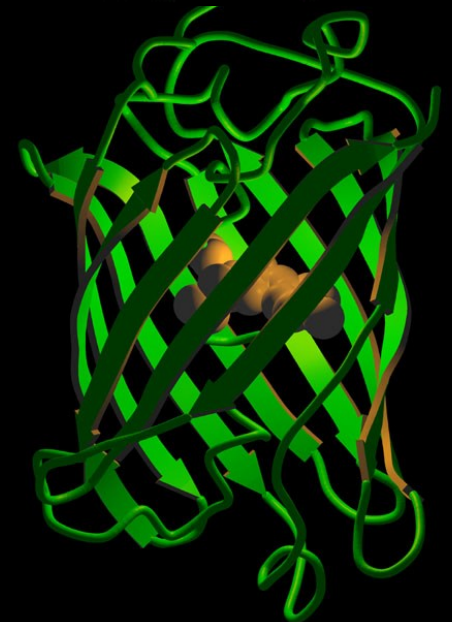
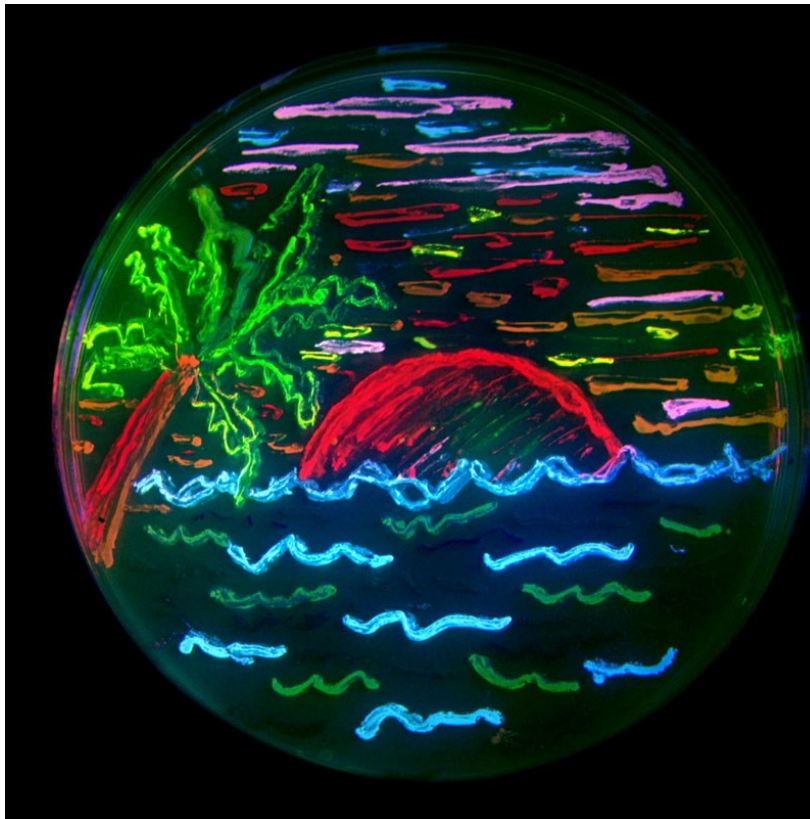
*Institute of Bioorganic Chemistry, Russian Academy of Science, 117871 Moscow, Russia. ¹Institute of Ecology and Evolution, and ²Institute of Biochemistry Russian Academy of Science, 17071 Moscow, Russia. *Corresponding author (e-mail: luk@ibch.siobc.ras.ru).*

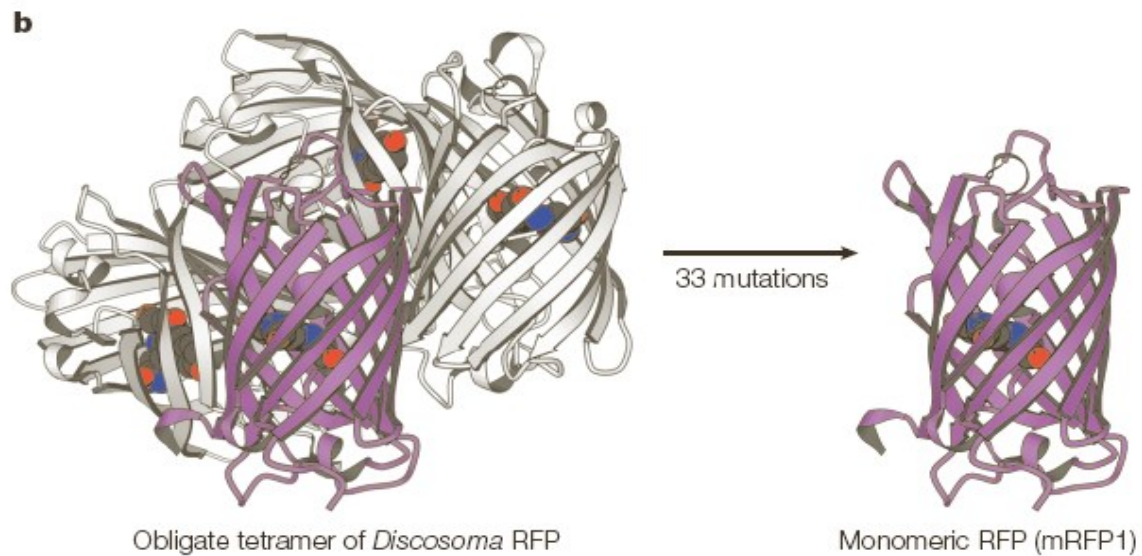
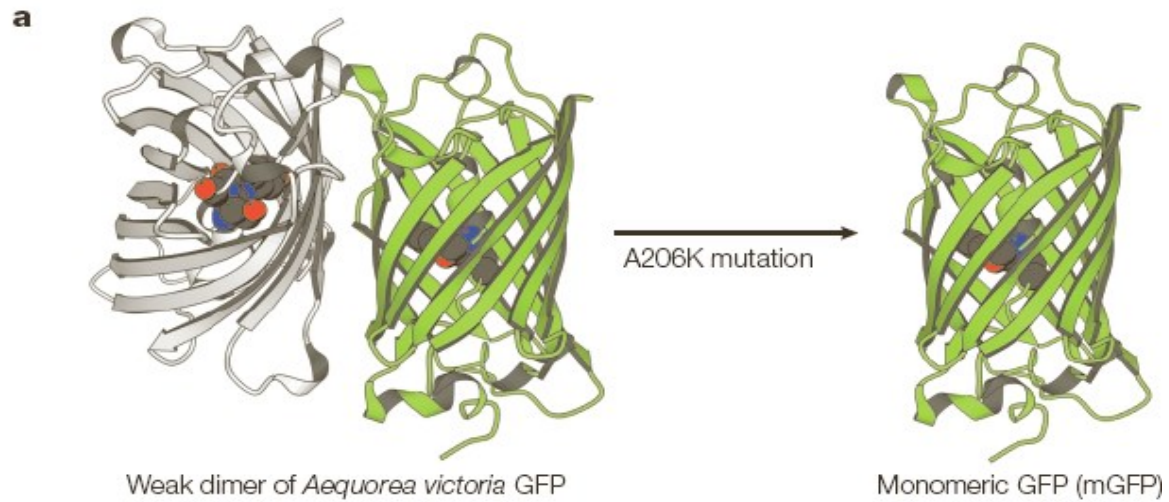
Received 28 May 1999; accepted 18 July 1999

Roger Tsien

- ~ 2002 – mutace FP = barevné spektrum

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





CREATING NEW FLUORESCENT PROBES FOR CELL BIOLOGY

Jin Zhang*, Robert E. Campbell*, Alice Y. Ting*[‡] and Roger Y. Tsien*[‡]

Table 1 | Properties of the best FP variants^{a,b}

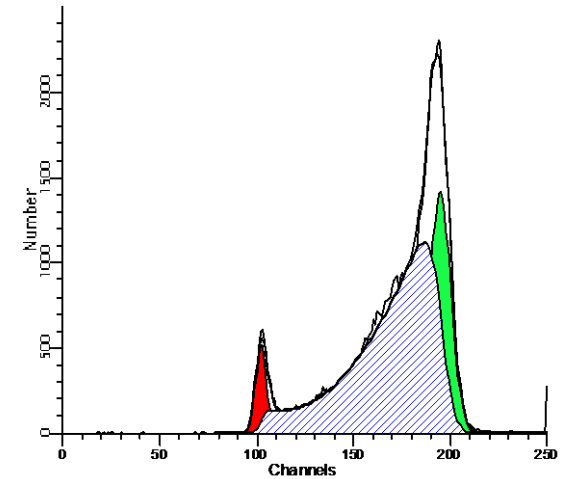
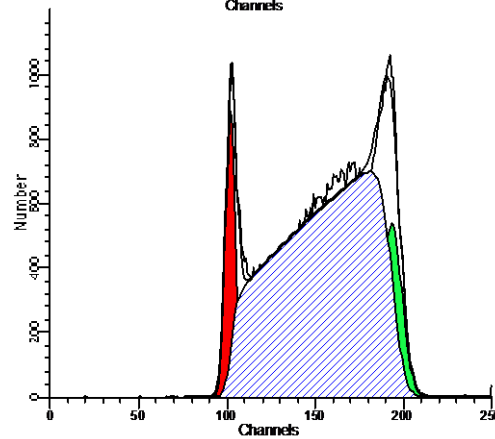
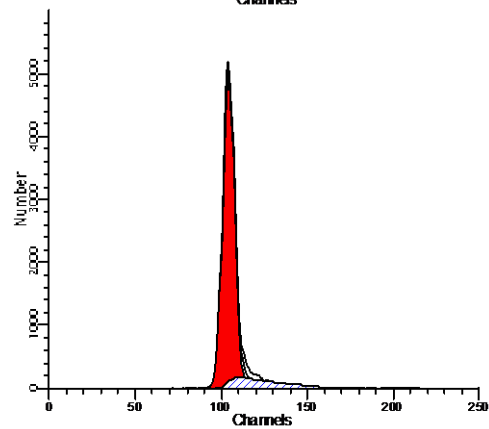
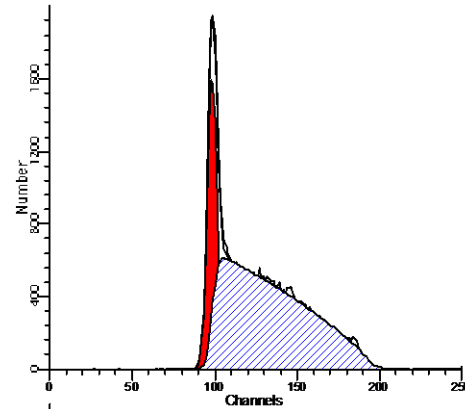
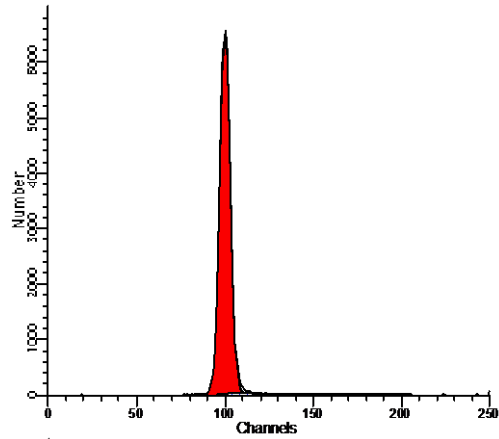
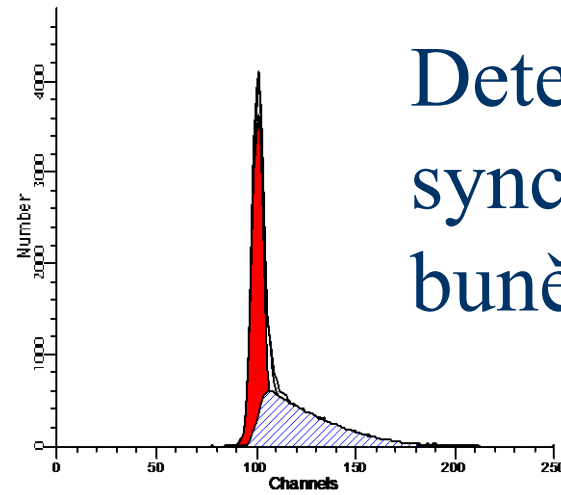
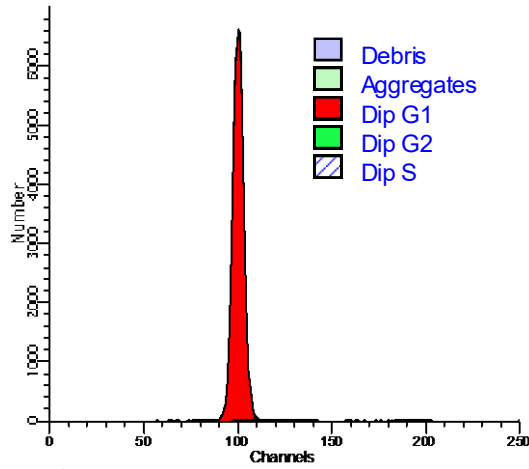
Class	Protein	Source laboratory (references)	Excitation ^c (nm)	Emission ^d (nm)	Brightness ^e	Photostability ^f	pKa	Oligomerization
Far-red	mPlum ^g	Tsien (5)	590	649	4.1	53	<4.5	Monomer
Red	mCherry ^g	Tsien (4)	587	610	16	96	<4.5	Monomer
	tdTomato ^g	Tsien (4)	554	581	95	98	4.7	Tandem dimer
	mStrawberry ^g	Tsien (4)	574	596	26	15	<4.5	Monomer
	J-Red ^h	Evrogen	584	610	8.8*	13	5.0	Dimer
	DsRed-monomer ^h	Clontech	556	586	3.5	16	4.5	Monomer
Orange	mOrange ^g	Tsien (4)	548	562	49	9.0	6.5	Monomer
	mKO	MBL Intl. (10)	548	559	31*	122	5.0	Monomer
Yellow-green	mCitrine ⁱ	Tsien (16,23)	516	529	59	49	5.7	Monomer
	Venus	Miyawaki (1)	515	528	53*	15	6.0	Weak dimer ^j
	YPet ^g	Daugherty (2)	517	530	80*	49	5.6	Weak dimer ^j
	EYFP	Invitrogen (18)	514	527	51	60	6.9	Weak dimer ^j
Green	Emerald ^g	Invitrogen (18)	487	509	39	0.69 ^k	6.0	Weak dimer ^j
	EGFP	Clontech ^l	488	507	34	174	6.0	Weak dimer ^j
Cyan	CyPet	Daugherty (2)	435	477	18*	59	5.0	Weak dimer ^j
	mCFPm ^m	Tsien (23)	433	475	13	64	4.7	Monomer
	Cerulean ^g	Piston (3)	433	475	27*	36	4.7	Weak dimer ^j
UV-excitable green	T-Sapphire ^g	Griesbeck (6)	399	511	26*	25	4.9	Weak dimer ^j

^aAn expanded version of this table, including a list of other commercially available FPs, is available as **Supplementary Table 1**. ^bThe mutations of all common AFPs relative to the wild-type protein are available in **Supplementary Table 3**. ^cMajor excitation peak. ^dMajor emission peak. ^eProduct of extinction coefficient and quantum yield at pH 7.4 measured or confirmed (indicated by *) in our laboratory under ideal maturation conditions, in (mM • cm)⁻¹ (for comparison, free fluorescein at pH 7.4 has a brightness of about 69 (mM • cm)⁻¹). ^fTime for bleaching from an initial emission rate of 1,000 photons/s down to 500 photons/s (t_{1/2}; for comparison, fluorescein at pH 8.4 has t_{1/2} of 5.2 s); data are not indicative of photostability under focused laser illumination. ^gBrightest in spectral class. ^hNot recommended (dim with poor folding at 37 °C). ⁱCitrine YFP with A206K mutation; spectroscopic properties equivalent to Citrine. ^jCan be made monomeric with A206K mutation. ^kEmerald has a pronounced fast bleaching component that leads to a very short time to 50% bleach. Its photostability after the initial few seconds, however, is comparable to that of EGFP. ^lFormerly sold by Clontech, no longer commercially available. ^mmCFPm with A206K mutation; spectroscopic properties equivalent to ECFP.

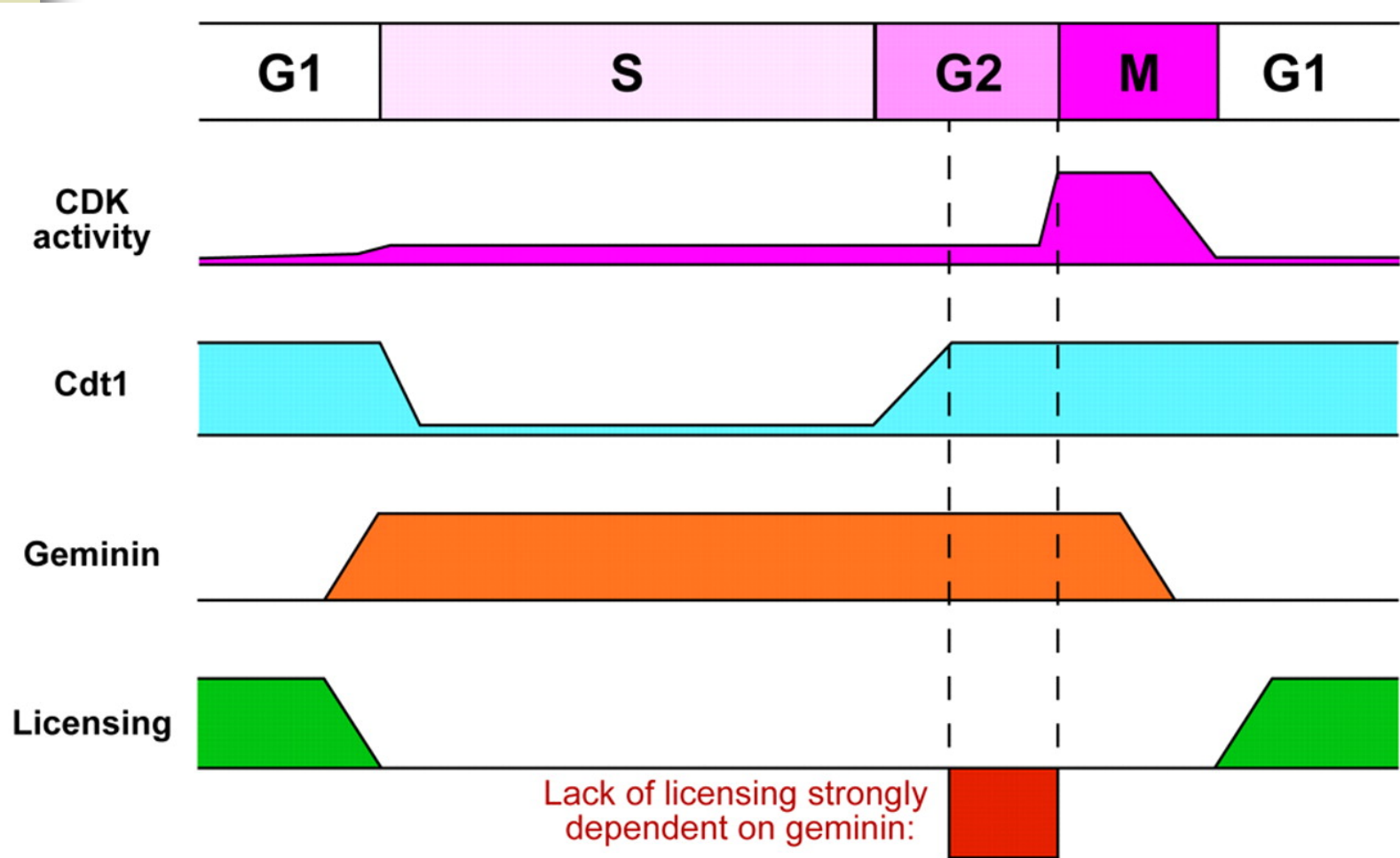
A guide to choosing fluorescent proteins

Nathan C Shaner^{1,2}, Paul A Steinbach^{1,3} & Roger Y Tsien^{1,3,4}

Detekce buněk v synchronizovaném buněčném cyklu

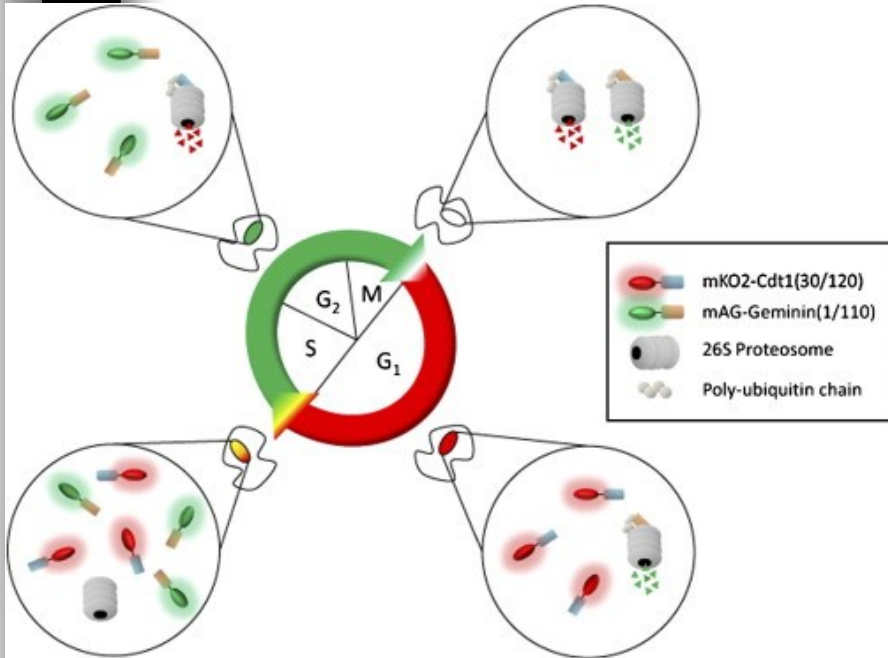


Licensing control by Cdt1 and geminin

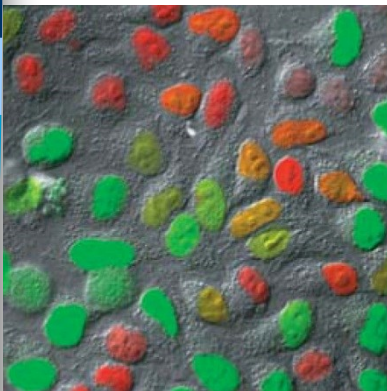


Fucci

(fluorescent ubiquitination-based cell cycle indicator) cells



Chemistry & Biology 15, February 2008 ©2008 Elsevier Ltd



Ubiquitin E3 ligase complexes

G₁ - APC^{Cdh1}

substrate: **Geminin**, inhibitor of DNA replication
inhibits Cdt1

S, G₂, M- SCF^{Skp2}

substrate: DNA replication factor **Cdt1** – key
licensing factor

Fucci sensors - 1st generation, coral FP

monomeric Kusabira orange 2 – hCdt1 (30/120)

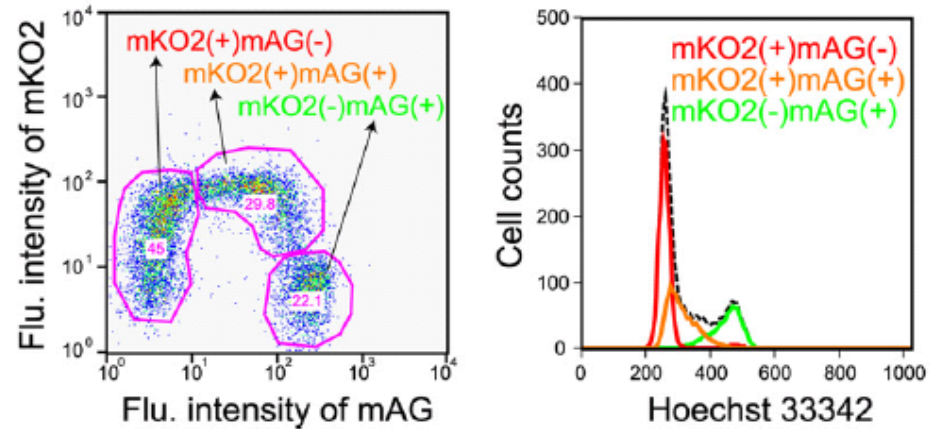
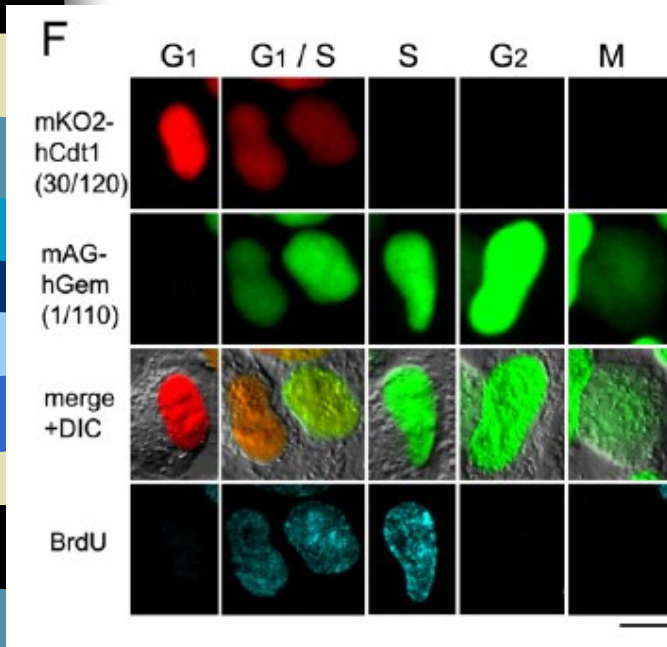
Monomeric Azami-Green – hGeminin (1/110)

Fucci sensors – 2nd generation, *Aequorea* FP

red monomeric fluorescent protein - mCherry -
hCdt1 (30/120)

yellowish green monomeric variant of GFP –
mVenus – hGeminin (1/110)

Fucci



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,*}

¹Laboratory for Cell Function and Dynamics

²Laboratory for Cell Culture Development

³Advanced Technology Development Group, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan

⁴Life Function and Dynamics, ERATO, JST, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan

⁵School of Life Science, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

⁶Departments of Biochemistry, The Cancer Institute of the Japanese Foundation for Cancer Research, 3-10-6 Ariake, Koto-ku, Tokyo 135-8550, Japan

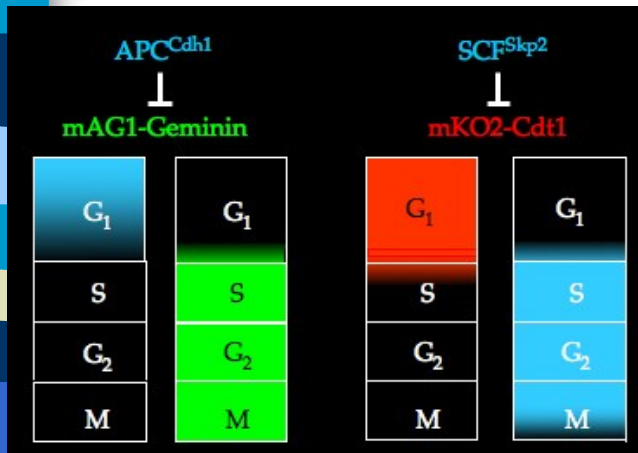
⁷Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Syowa-ku, Nagoya, Aichi 466-8550, Japan

⁸Subteam for Manipulation of Cell Fate, BioResource Center, RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

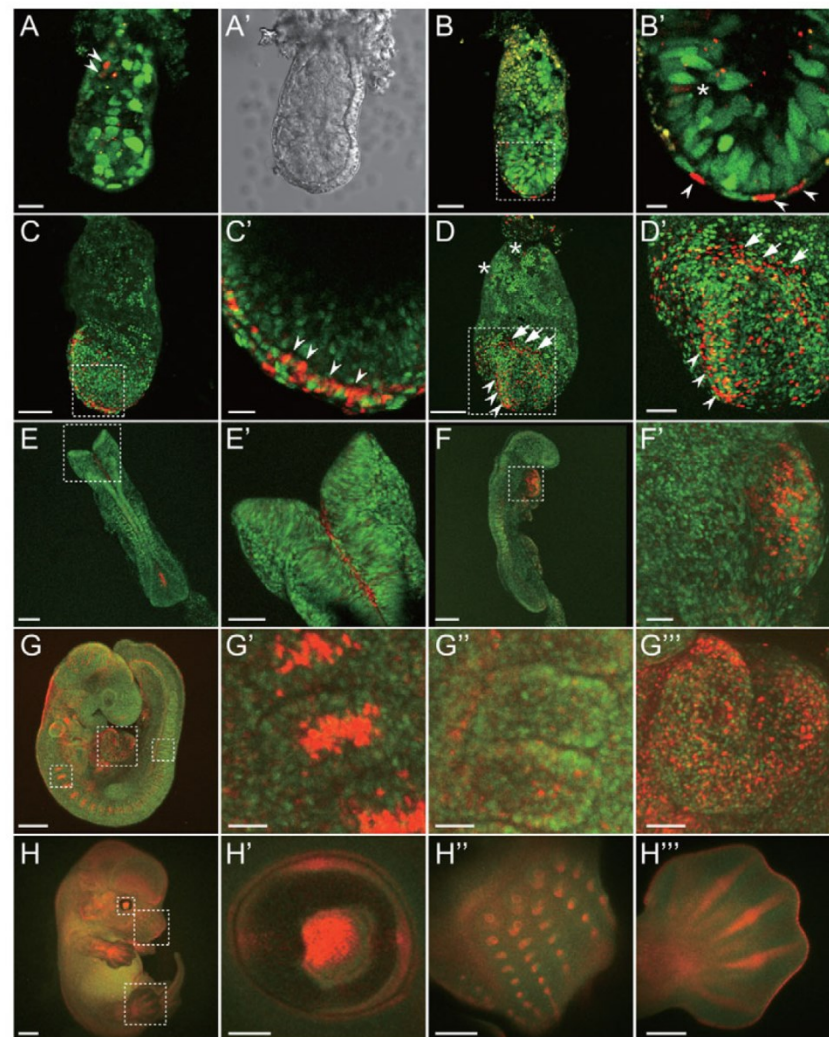
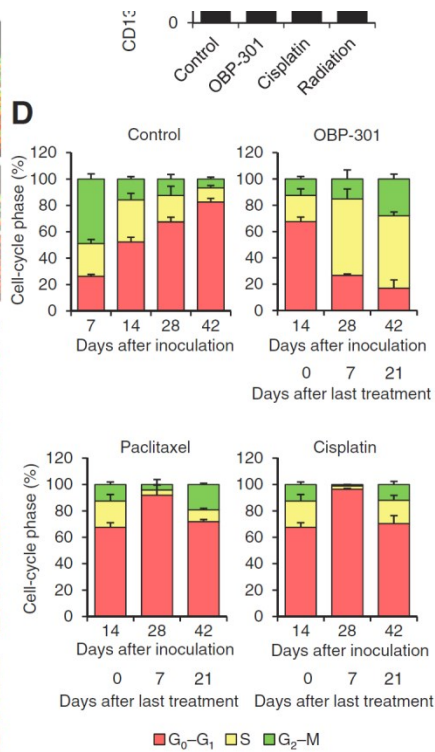
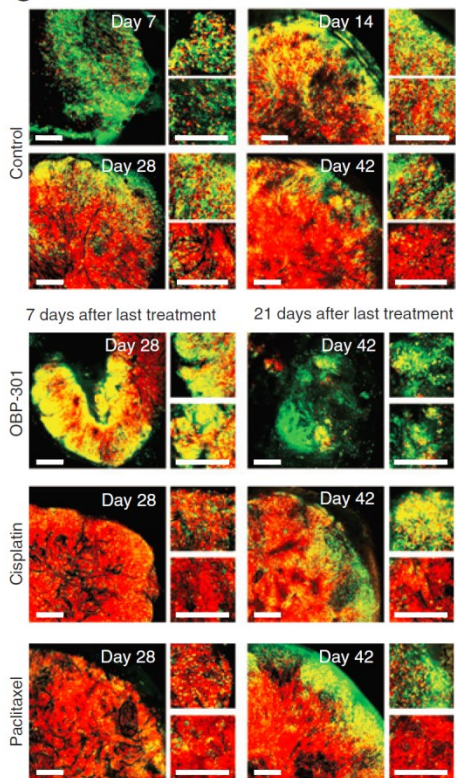
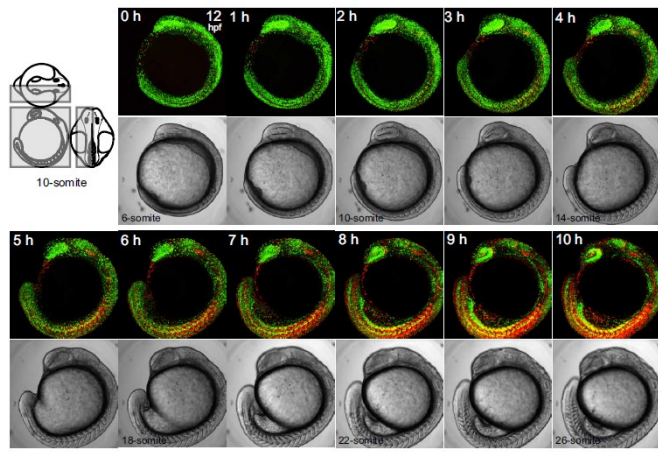
*Genome Dynamics Project, Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan

*Correspondence: matsushi@brain.riken.jp

DOI 10.1016/j.cell.2007.12.033



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

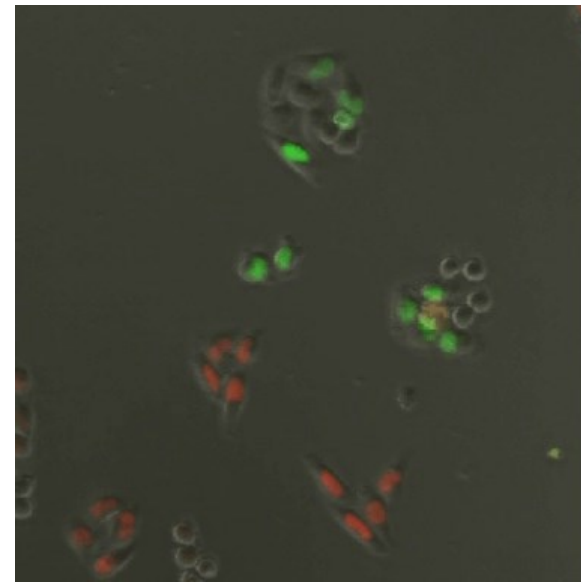
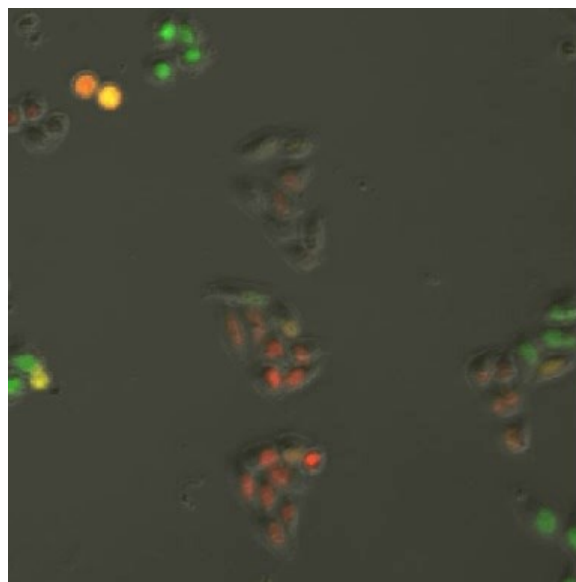
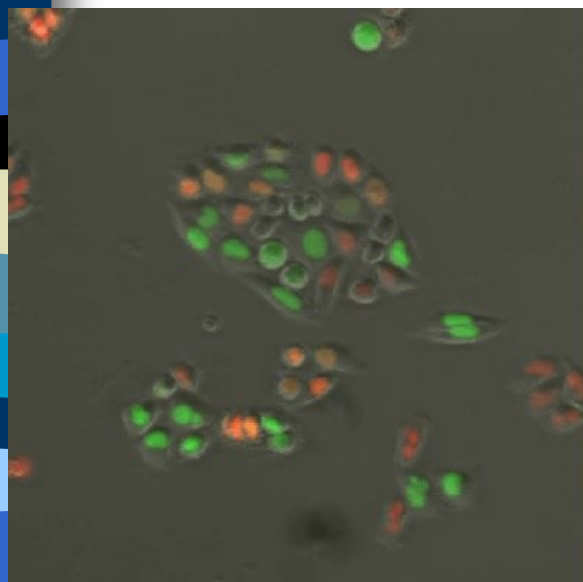


CONTROL

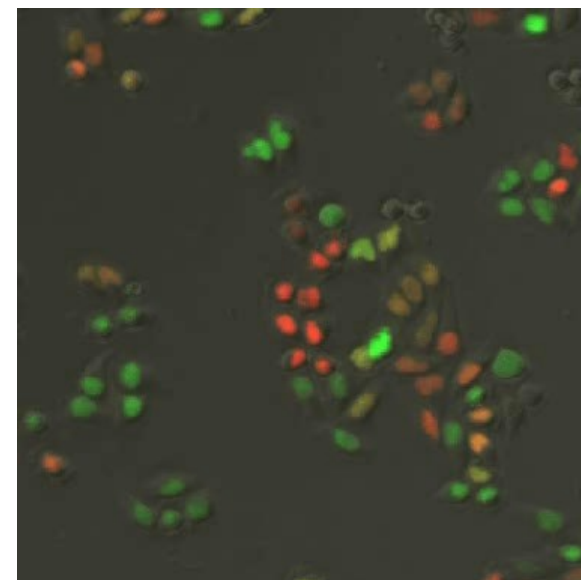
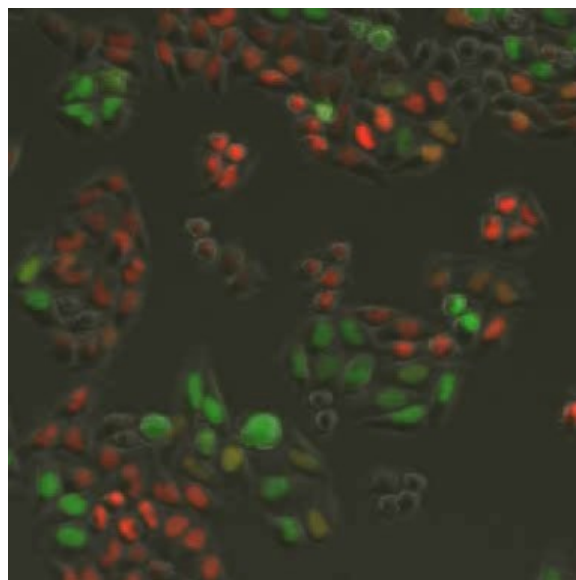
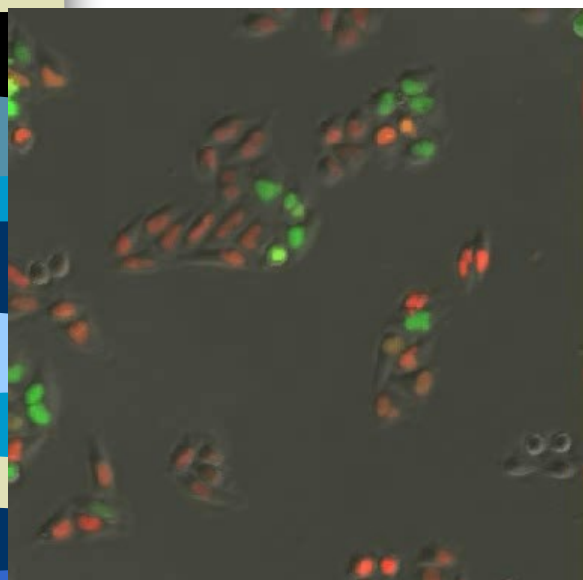
SCH900776

MU380

VEHICLE



GEMCITABINE



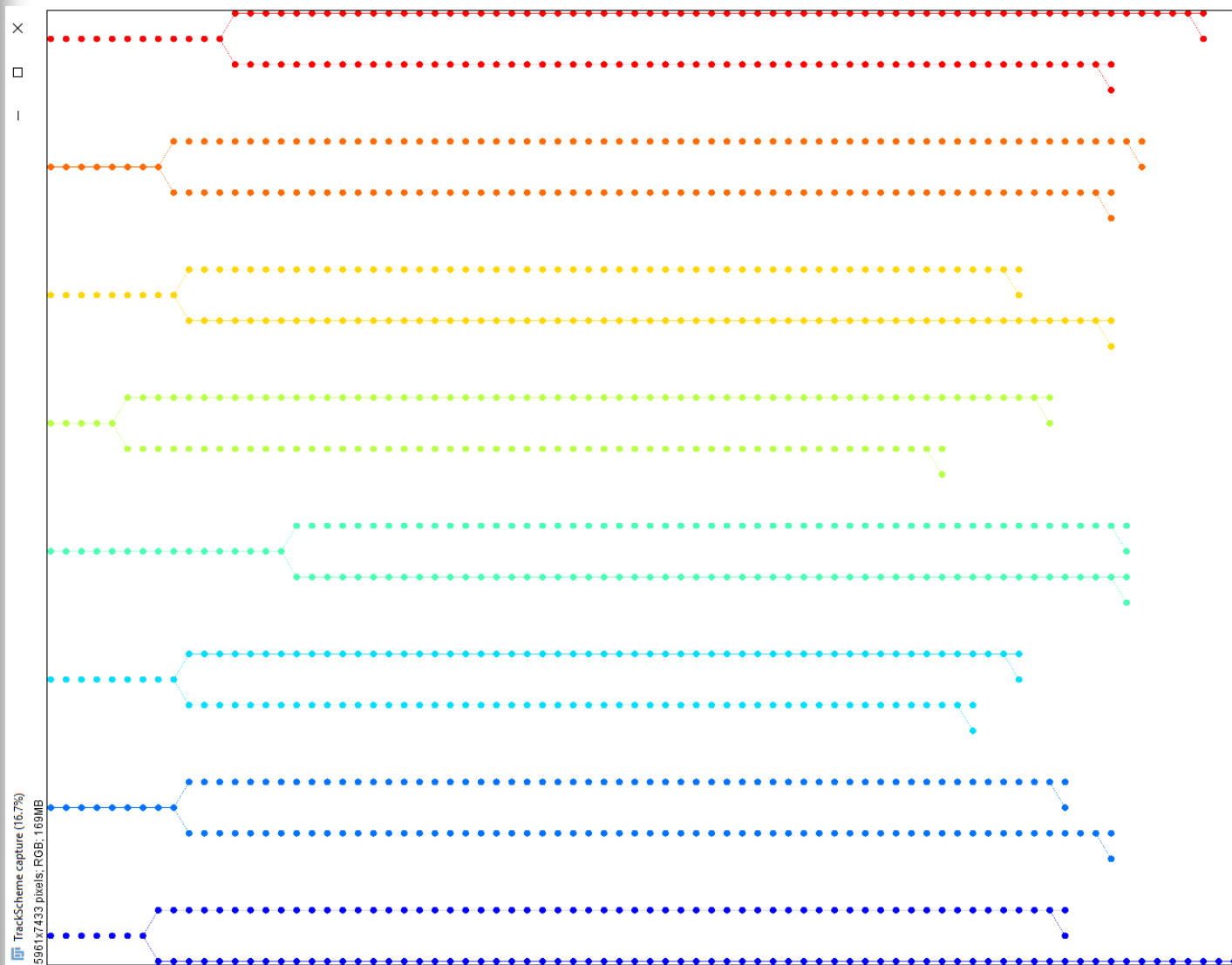


...lot of questions, but how to answer them?

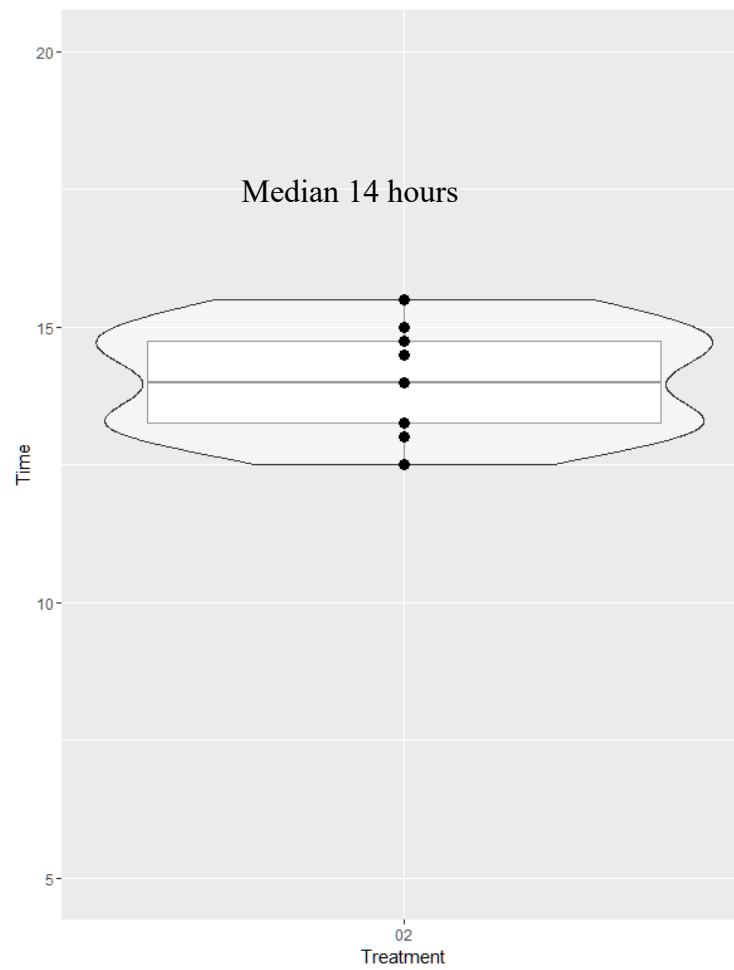
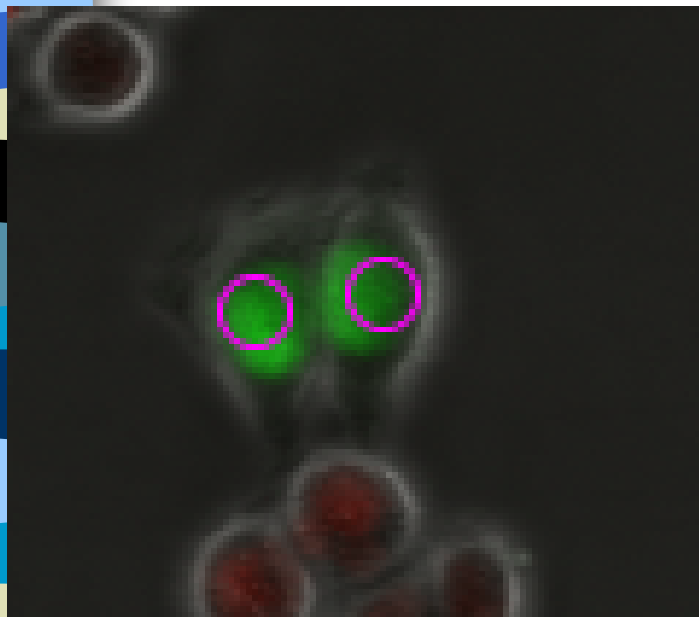
- How many times cells divided?
- What is a length of cell cycle phases?
- Is there a difference in time between first and second division?
- How it is all affected by my drugs?

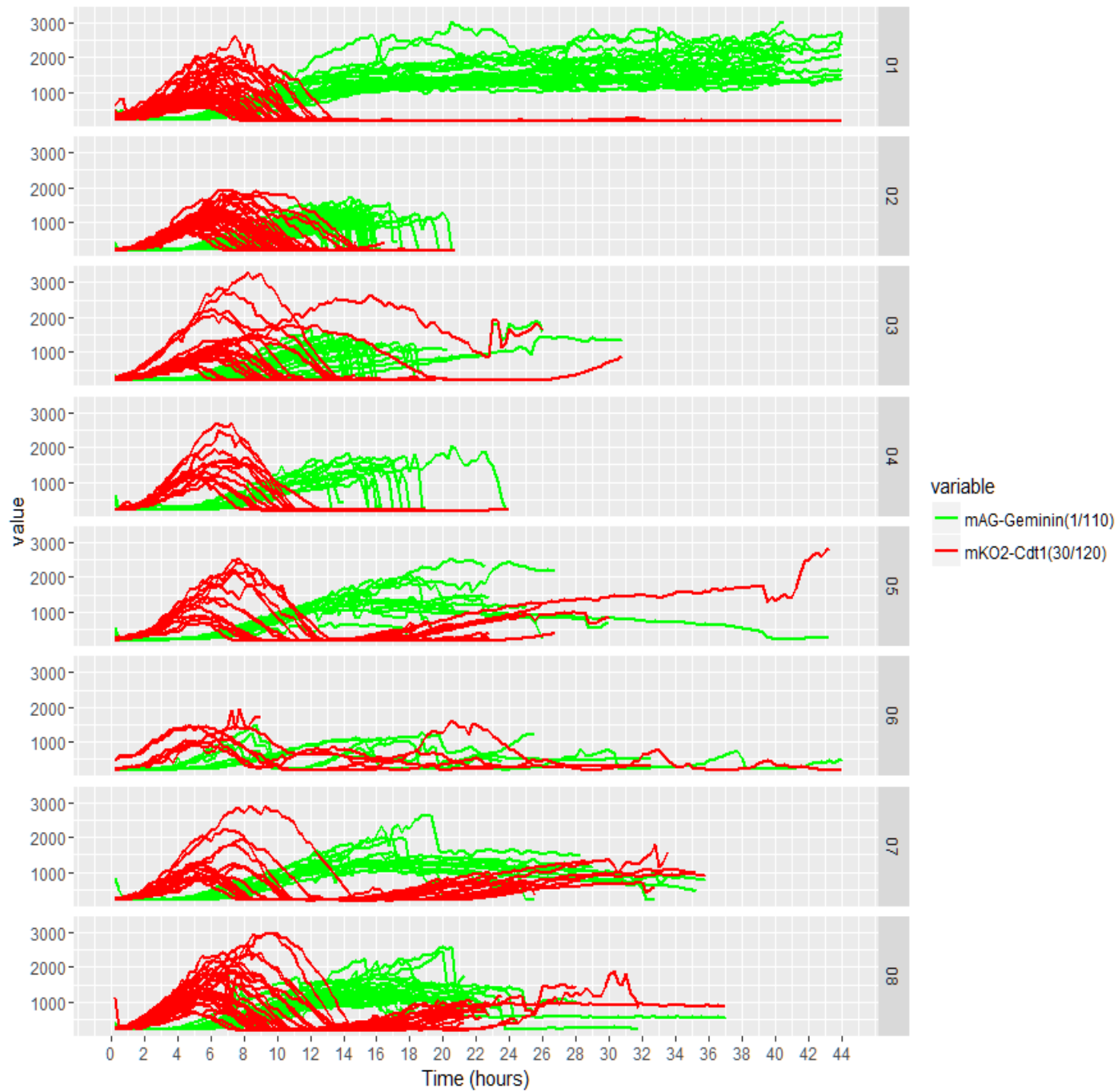
Branches (divisions) analysis

02_02_01_01

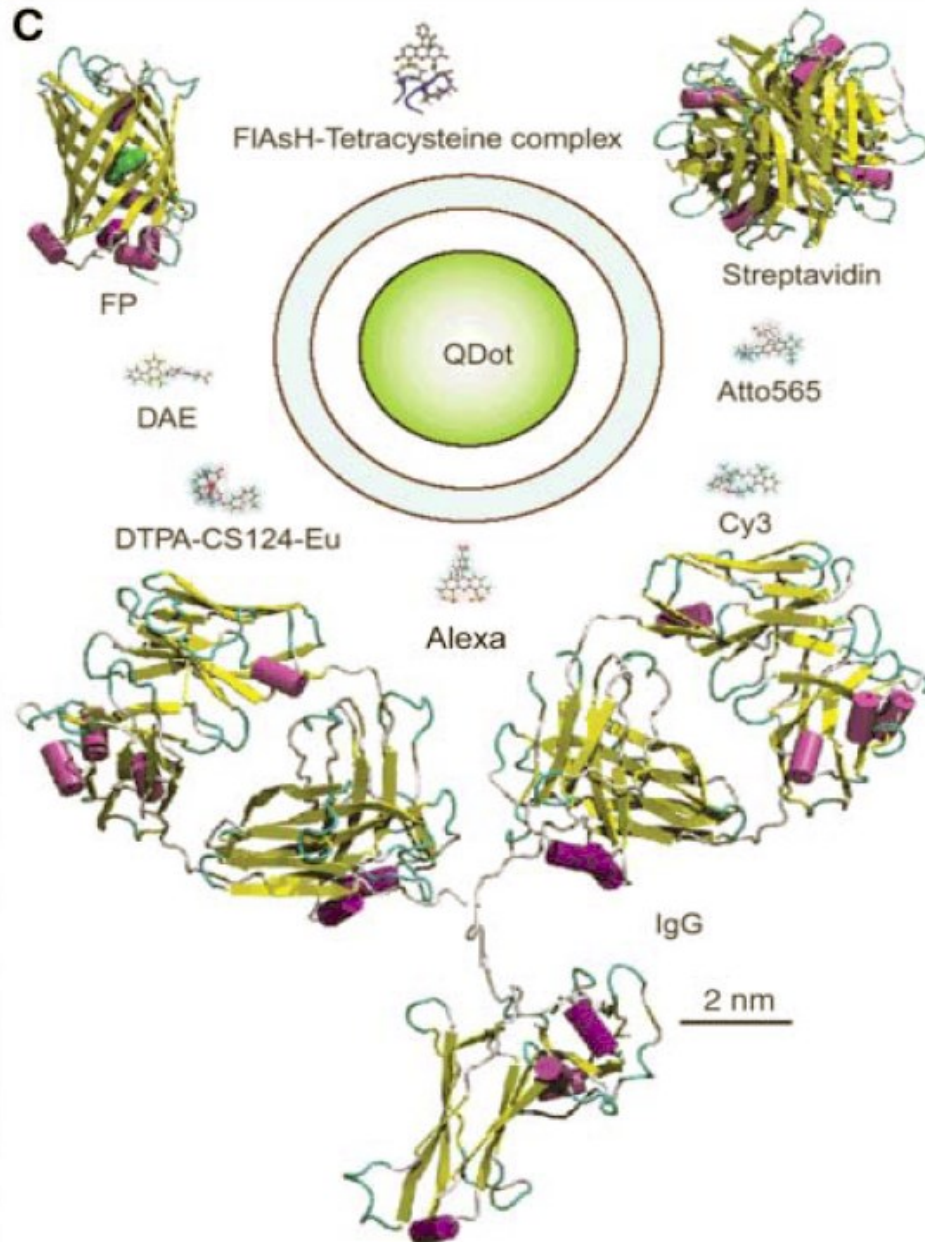


02_02_01_01





Targeting proteins & fluorophores

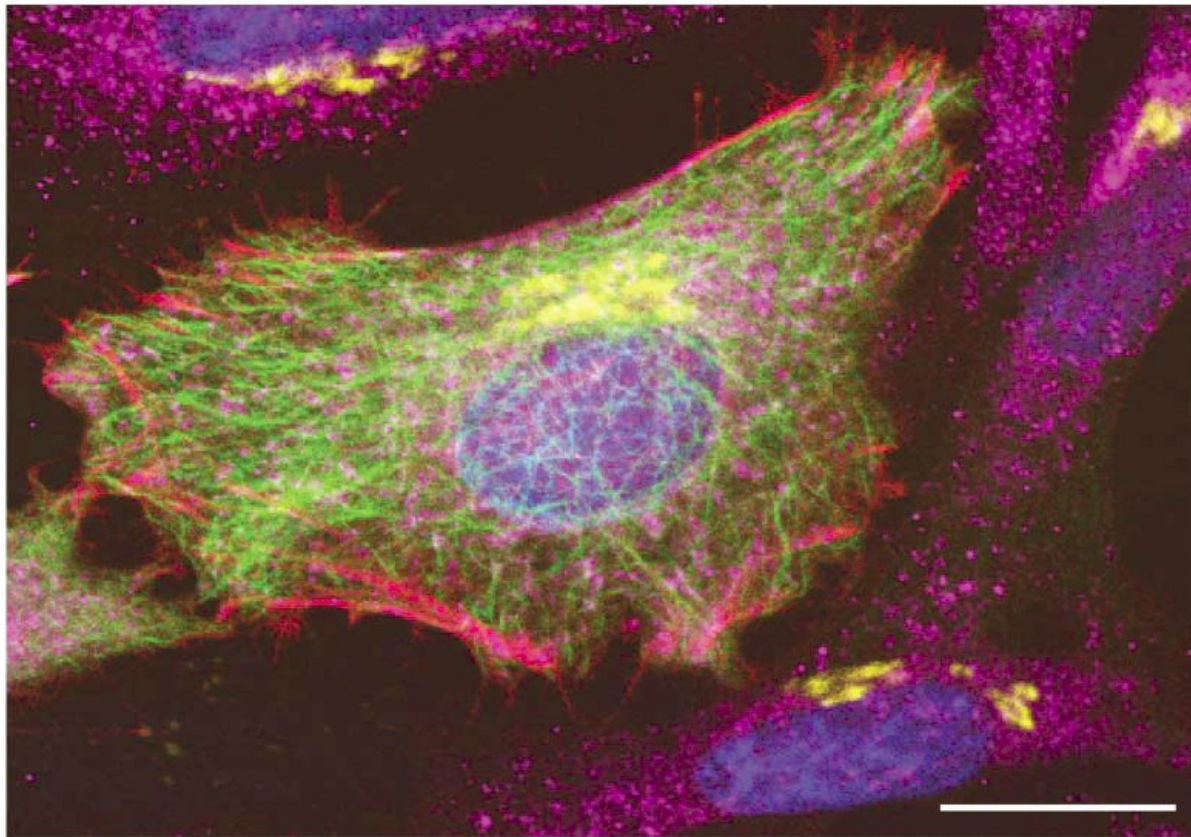
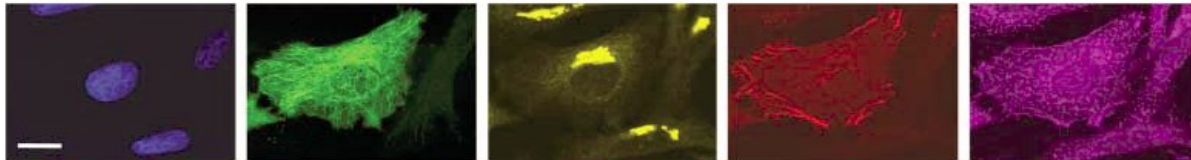


REVIEW

The Fluorescent Toolbox for Assessing Protein Location and Function

Ben N. G. Giepmans,^{1,2} Stephen R. Adams,² Mark H. Ellisman,¹ Roger Y. Tsien^{2,3*}

Emission (nm):	410-490	500-530	555-565	580-620	>660
Fluorophore:	Hoechst	GFP	QD565	ReAsH	Cy5
Targeting:	direct affinity	genetic	immuno	genetic	immuno
Target:	DNA	α -tubulin	giantin	β -actin	Cytochrome c
Structure:	nuclei	microtubules	golgi	stress fibers	mitochondria

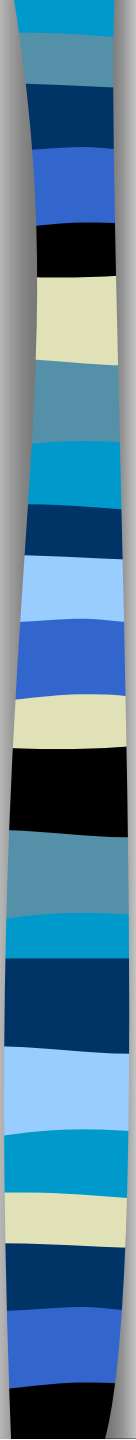


REVIEW

The Fluorescent Toolbox for Assessing Protein Location and Function

Ben N. G. Geipmans,^{1,2} Stephen R. Adams,² Mark H. Ellisman,¹ Roger Y. Tsien^{2,3*}

SCIENCE VOL 312 14 APRIL 2006



Shrnutí přednášky

- Kompenzace
- Kontrola kvality, zásady
- analýza proliferace
- fluorescenční proteiny

Na konci dnešní přednášky byste měli:

1. Jaké jsou základní principy multispektrální a hmotnostní cytometrie
2. vědět jakým způsobem je možné analyzovat buněčný cyklus.
3. umět navrhnout další parametr kombinovatelný s DNA analýzou.
4. znát příklady buněčných funkcí které je možné analyzovat na průtokovém cytometru.
5. vědět co jsou to fluorescenční proteiny a jaké jsou výhody jejich využití v buněčné biologii.
6. co je to click-IT.



Vitální analýza buněčných funkcí

- Průtoková cytometrie umožňuje vícebarevnou vitální analýzu buněk
 - intracelulární koncentrace iontů,
 - pH,
 - produkce reaktivních skupin,
 - životnost

Detekce viability

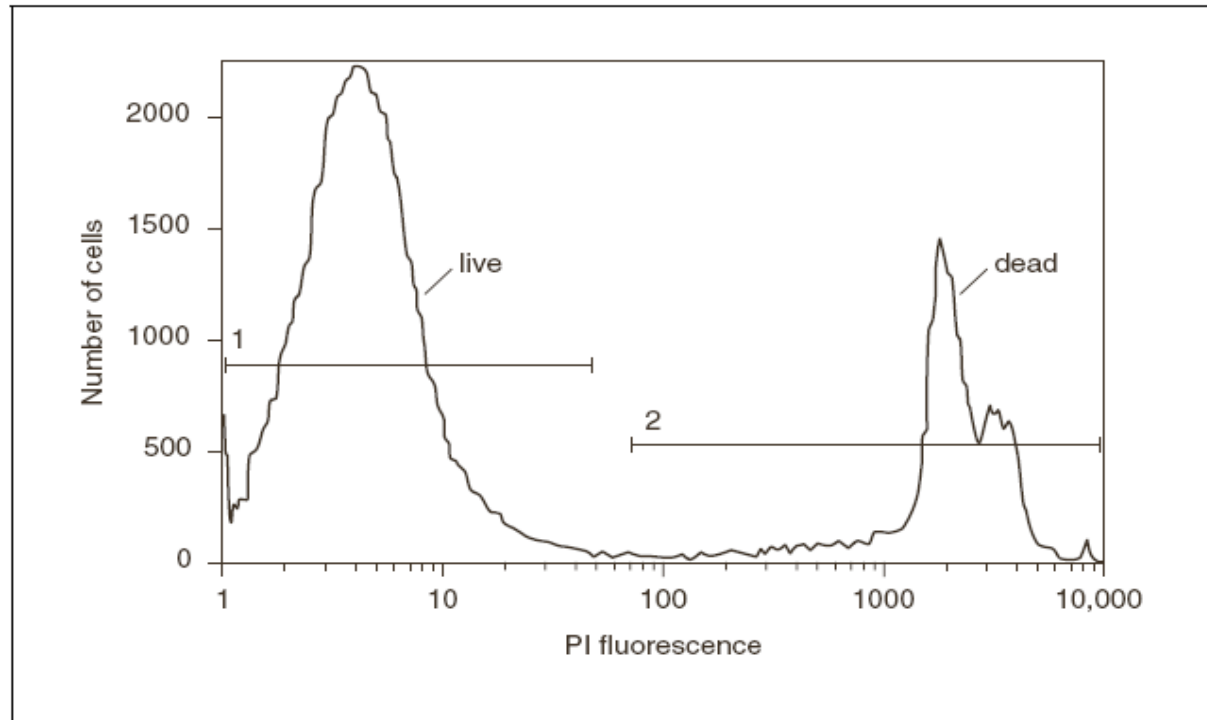
- jedna z nejjednodušších analýz
- funguje na principu:
 - detekce membránové integrity - neprůchodnosti některých fluorescenčních značek cytoplazmatickou membránou živých buněk – **propidium iodide, ethidium bromide, 7-amino actinomycin D**
 - detekce fyziologického stavu buněk – použití fluorescenčních značek barvicích pouze živé buňky - **Rhodamine-123, Calcein-AM**
- **ethidium monoazide** – lze jím obarvit mrtvé buňky a následně fixovat
- Pomocí **LDS-751** (laser dye styryl-751) je možné odlišit mrtvé buňky i po fixaci
- LIVE/DEAD® Fixable Dead Cell Stain Kits



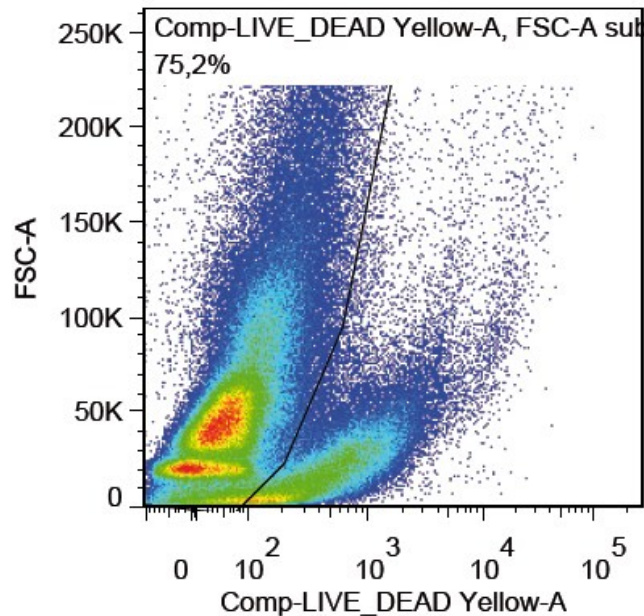
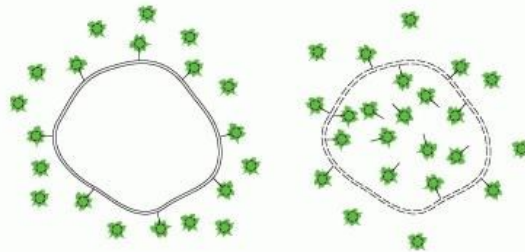
Reactive dye	Excitation source	Ex*	Em*
blue fluorescent reactive dye (L23105)	UV	350	450
violet fluorescent reactive dye (L34955)	405 nm	416	451
aqua fluorescent reactive dye (L34957)	405 nm	367	526
yellow fluorescent reactive dye (L34959)	405 nm	400	575
green fluorescent reactive dye (L23101)	488 nm	495	520
red fluorescent reactive dye (L23102)	488 nm	595	615
far red fluorescent reactive dye (L10120)	633/635 nm	650	665
near-IR fluorescent reactive dye (L10119)	633/635 nm	750	775

*Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm.

Detekce viability



VIABILITY using LIVE/DEAD fixable stains



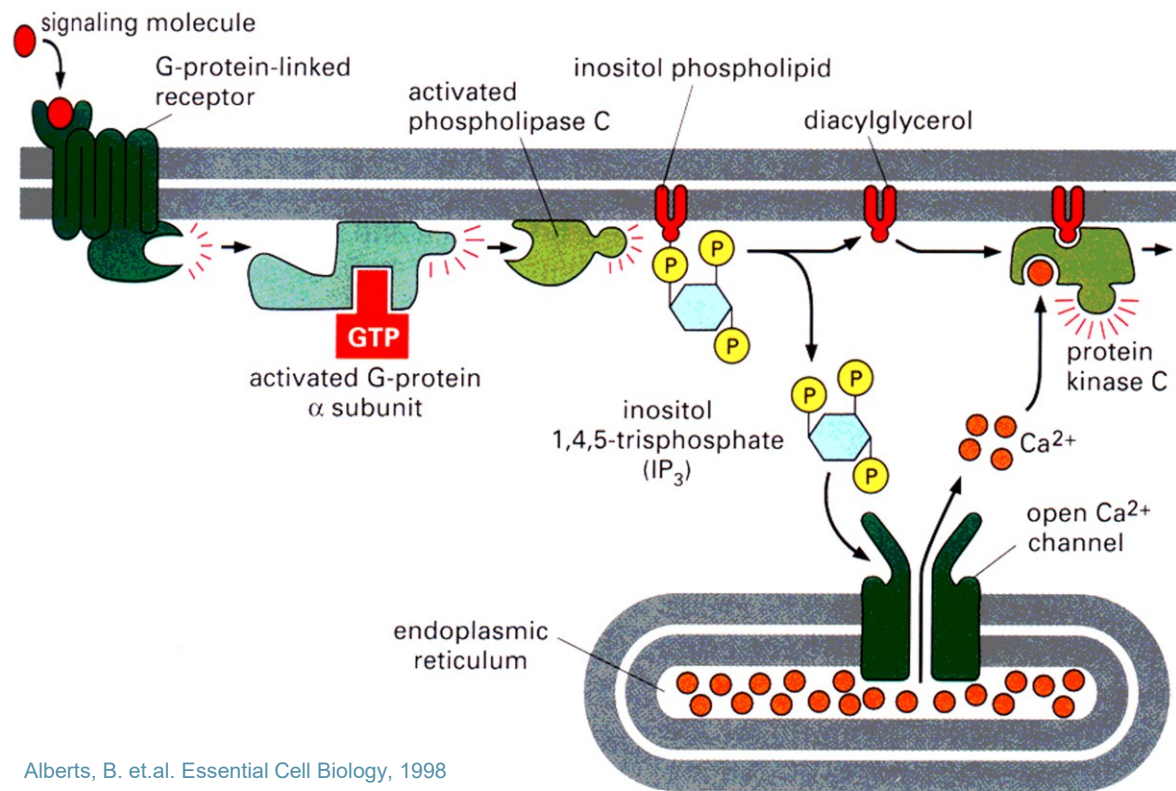
Principle: reaction of a fluorescent reactive dye with cellular amines, in necrotic cells react with free amines both in the interior and on the cell = intense staining, live cells stained on surface only = dim signal

Pros: simple, wide spectrum of dyes, fixable, The ArC™ Amine Reactive Compensation Bead Kit

Cons: live cells have signal, stain only in buffers w/o BSA or serum, Tris or azide

Přenos signálu pomocí Ca^{2+}

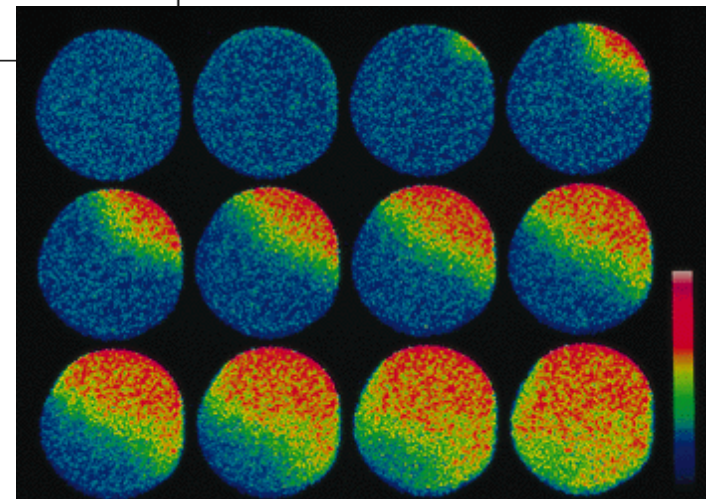
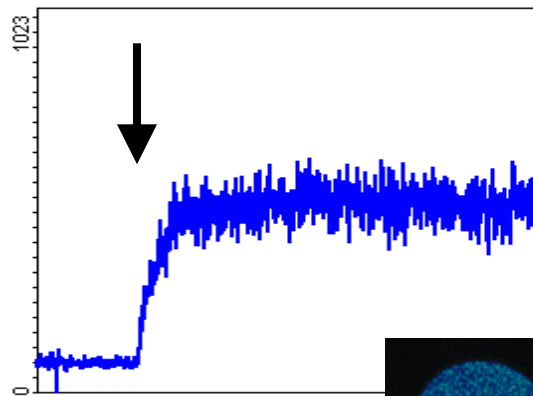
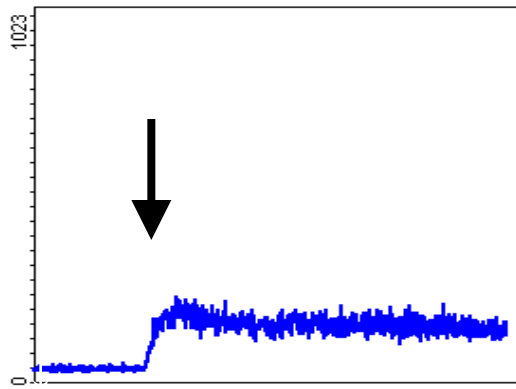
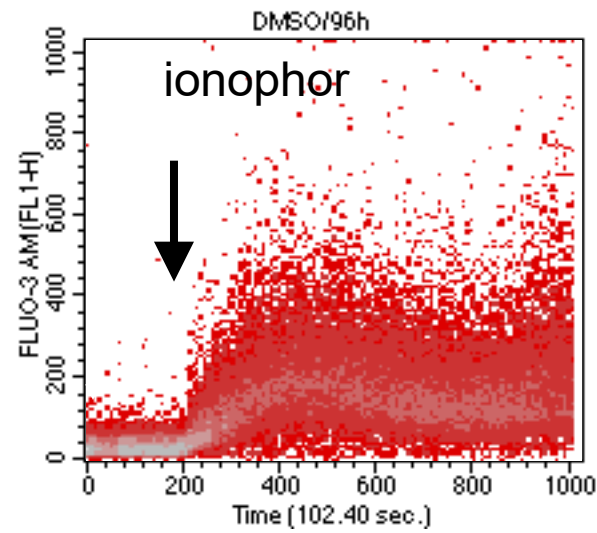
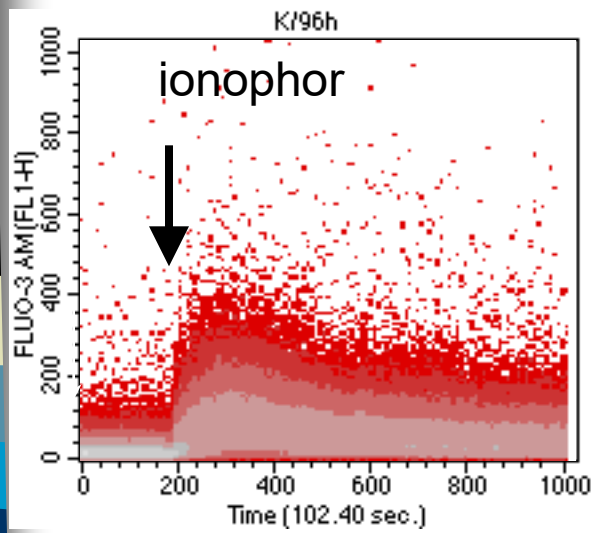
- **Cytosol** (koncentrace - „klidová“ 100 nM vs. 1-10 μM aktivovaná)
- $[\text{Ca}^{2+}]_c$ aktivuje proteinkinázu C
- interaguje s „ Ca^{2+} - binding proteins“



Alberts, B. et al. Essential Cell Biology, 1998

Ca²⁺ influx

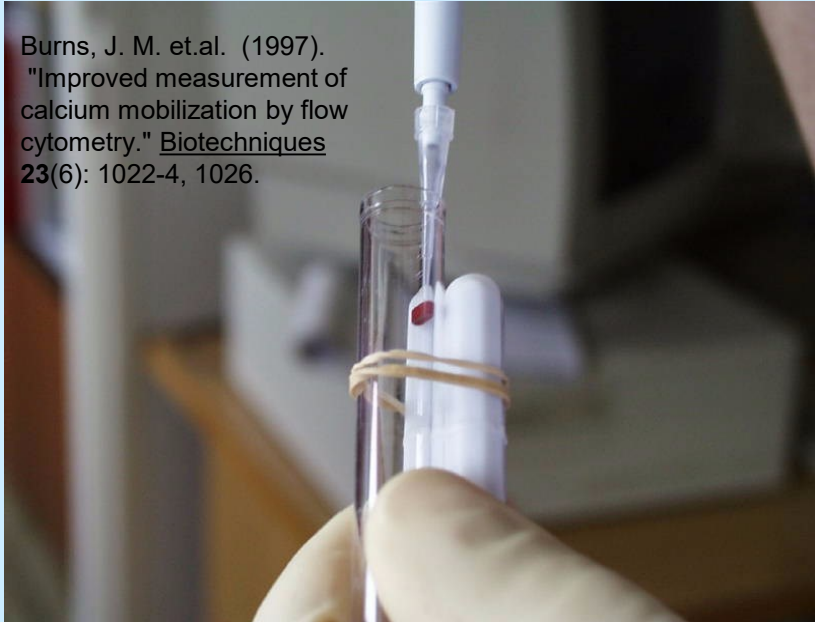
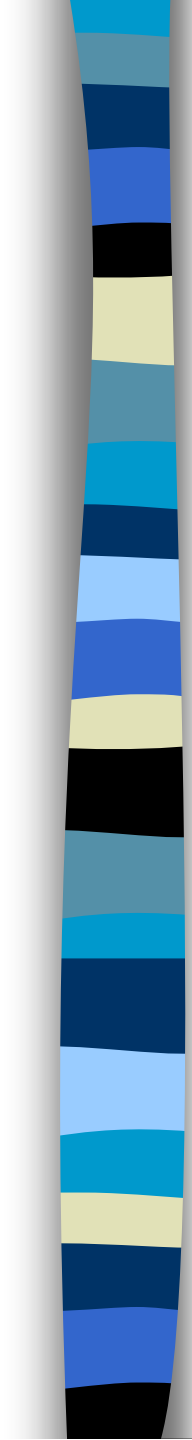
- Fura-2
- Fluo-3
- Indo-1





Zajištění vhodných podmínek pro detekci $[Ca^{2+}]_i$

- standardizace barvení a kalibrace
- temperace vzorku po celou dobu měření
- standardizace způsobu přidávání induktoru
 - zlepšení rozpustnosti AM estery modifikovaných indikátorů (BSA, Pluronic® -127)
 - inhibice aktivního vylučování indikátoru buňkou (Probecid)
 - pro kalibraci vhodné AM estery modifikované chelátory (BAPTA-AM)

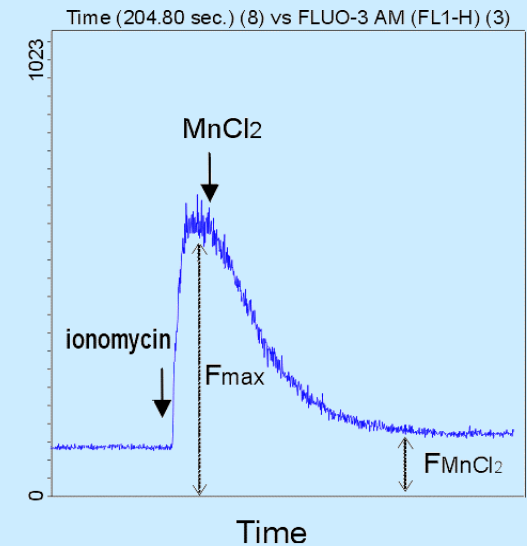
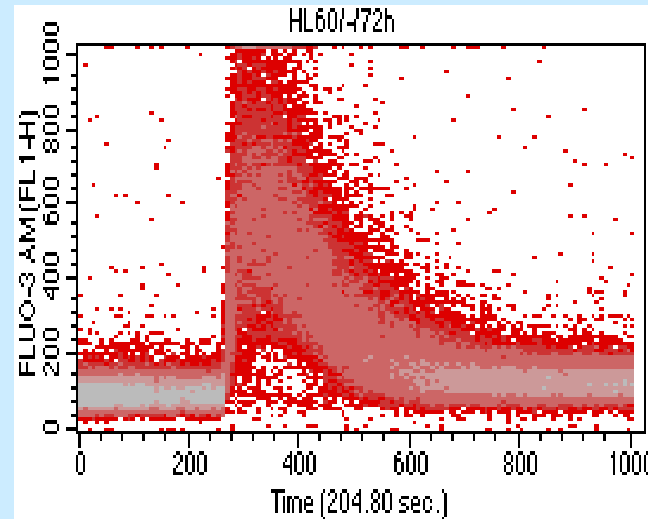
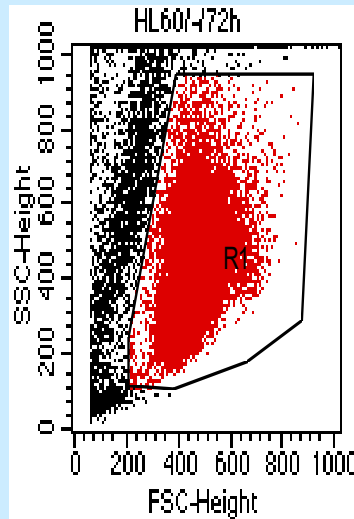




Kalibrace

(pro jednu vlnovou délku)

$$[Ca^{2+}] = K_d \times \frac{(F - F_{\min})}{(F_{\max} - F)}$$

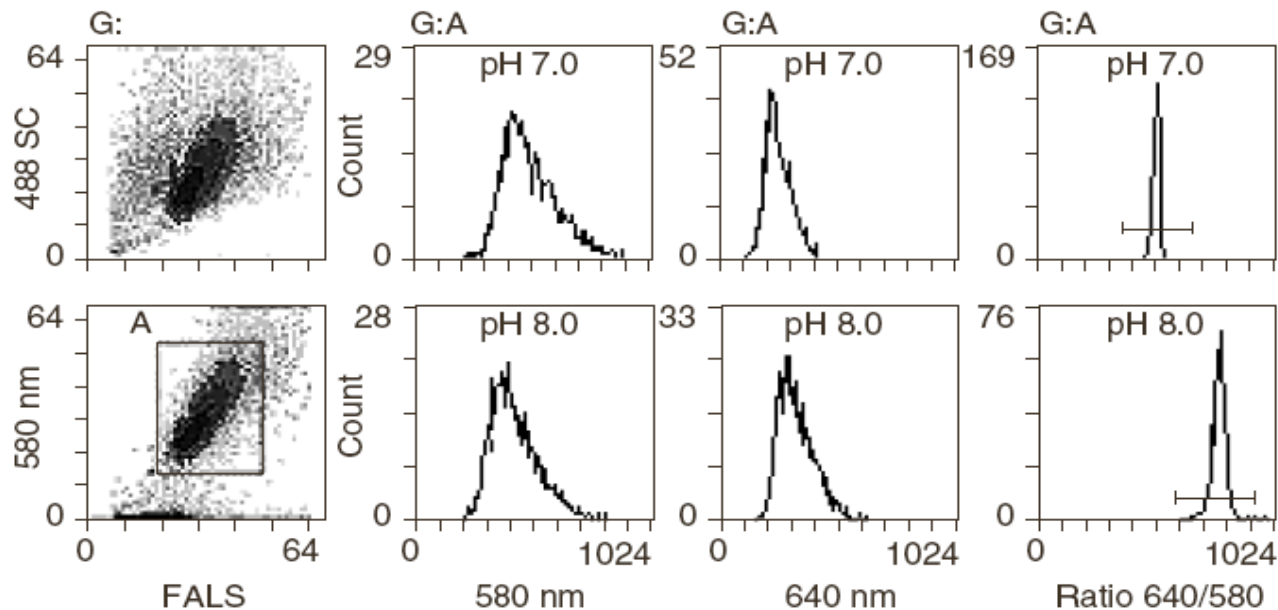


Fluo-3 ($K_d \sim 400\text{nM}$, 22°C ; 864 nM , 37°C)

$$F_{\min} = 1.25 \times F_{\text{MnCl}_2} - 0.25 \times F_{\max}$$

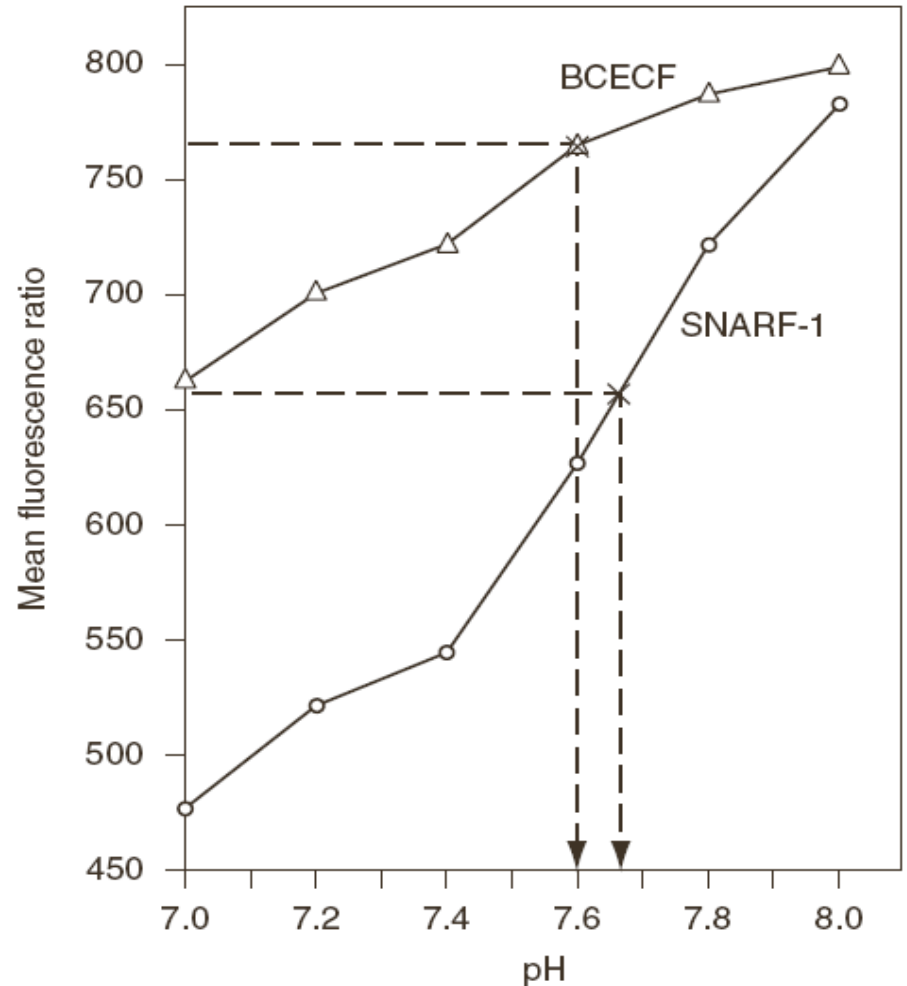
Detekce intracelulárního pH

- Fluorescenční značky měnící intenzitu fluorescence v závislosti na pH
- SNARF-1, BCECF



Detekce intracelulárního pH

- Nutná kalibrace pomocí draslíkových pufrů a ionoforu (nigericin)

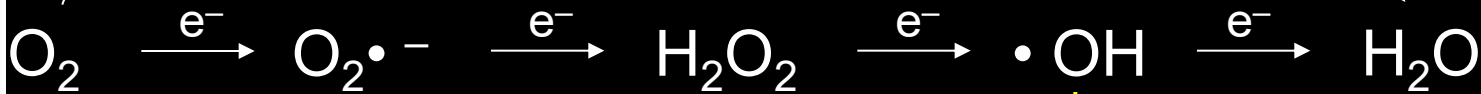




Detekce reaktivních kyslíkových skupin

- **Reaktivní kyslíkové skupiny hrají klíčovou roli v celé řadě biologických procesů**
 - posttranslační modifikace proteinů
 - regulace transkripce
 - regulace struktury chromatinu
 - přenos signálu
 - funkce imunitního systému
 - fyzický a metabolický stres
 - neurodegenerace, stárnutí

4 e⁻ reduction to water



Unreactive at STP, but a *great* electron acceptor
Biological activation via radicals, transition metals
Generally, radical intermediates are enzyme-bound

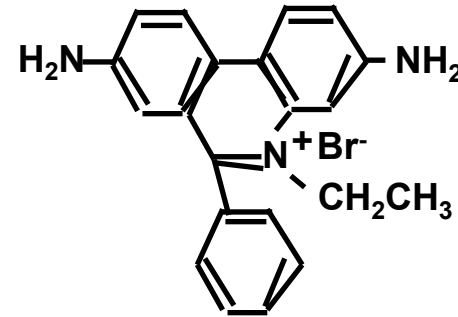
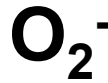
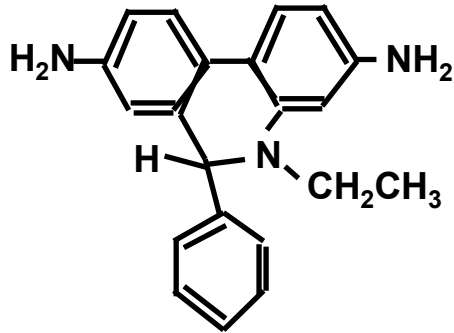
Reacts with virtually any molecule at diffusion-limited rates
The molecule that makes ionizing radiation toxic

Actually a chemical *reductant*
Not so terribly reactive with most biomolecules
Mitochondrial superoxide the major source of active oxygen
Maintained at very low concentration
Superoxide dismutases

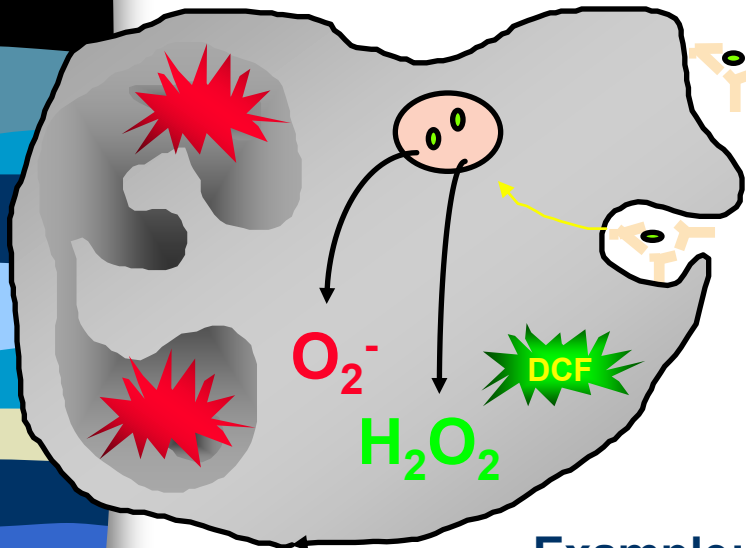
Not so terribly reactive with most biomolecules
Maintained at very low concentration
Catalases, peroxidases, GSH, etc...

Hydroethidine

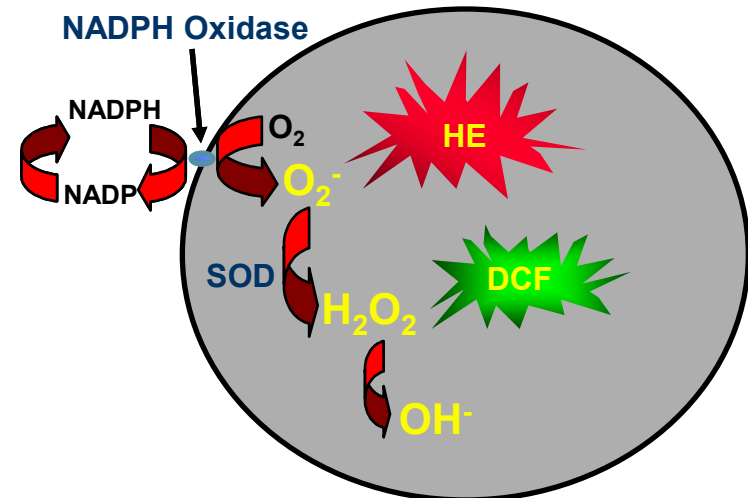
HE



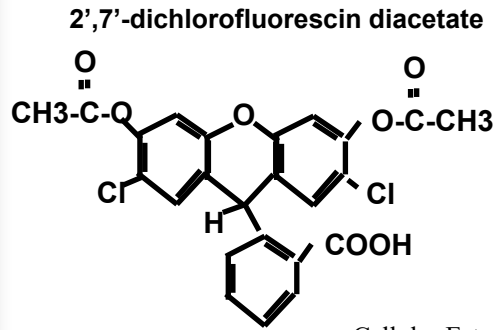
EB



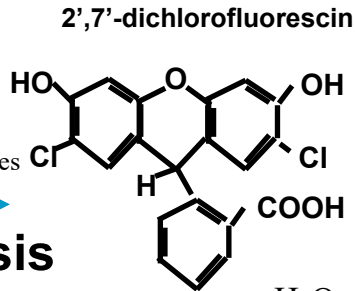
Phagocytic Vacuole



Example: Neutrophil Oxidative Burst

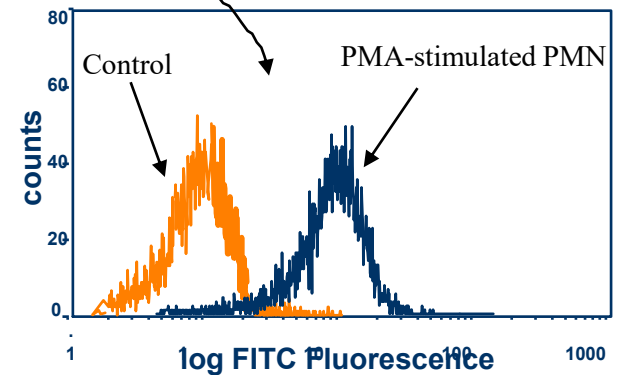
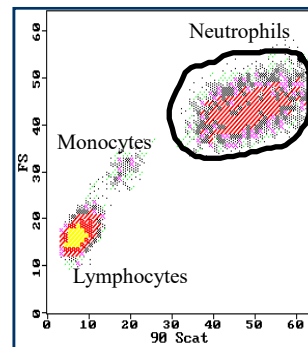
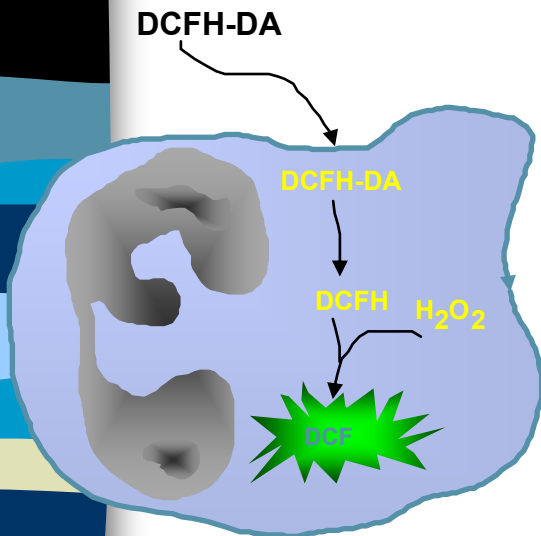
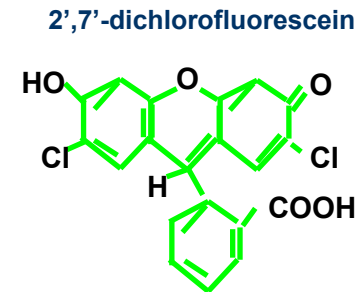


Cellular Esterases
 \longrightarrow
Hydrolysis



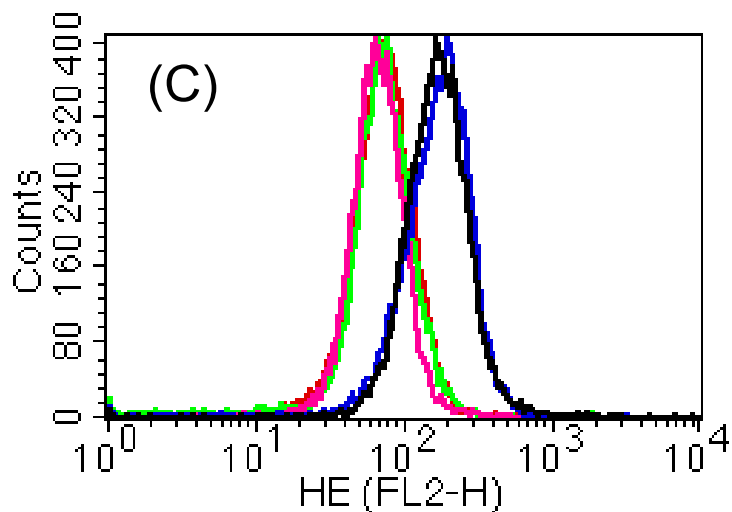
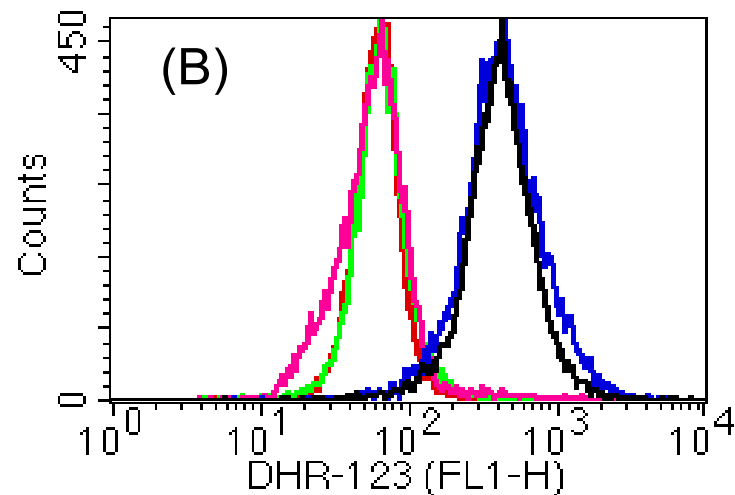
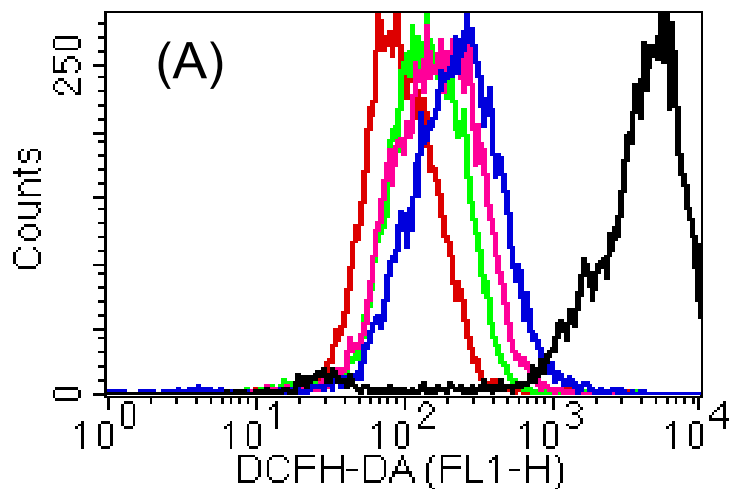
H_2O_2
 \longrightarrow
Oxidation

Fluorescent



Oxidative Burst

- DCFH-DA
- DHR-123
- HE



Key	Name
—	K/72h+PMA
—	ATRA/72h+PMA
—	DMSO/72h+PMA
—	NaBT/72h+PMA
—	vit. D3/72h+PMA

Fluorescenční proteiny

■ bioluminescence resonance energy transfer (BRET)

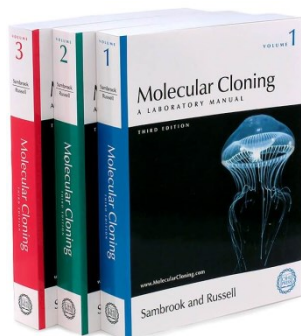
Aequorea victoria - medúza žijící ve vodách na pobřeží Severní Ameriky.

- je schopna modře světélkovat (bioluminescence). Ca^{2+} interaguje s fotoproteinem aequorinem.
- modré světlo excituje **green fluorescent protein**.

Renilla reniformis – korál žijící ve vodách na severním pobřeží Floridy.

- luminescence vzniká degradací coelenterazinu za katalytického působení luciferázy.
- modré světlo excituje **green fluorescent protein**.

Aequorea victoria “Crystal jelly “



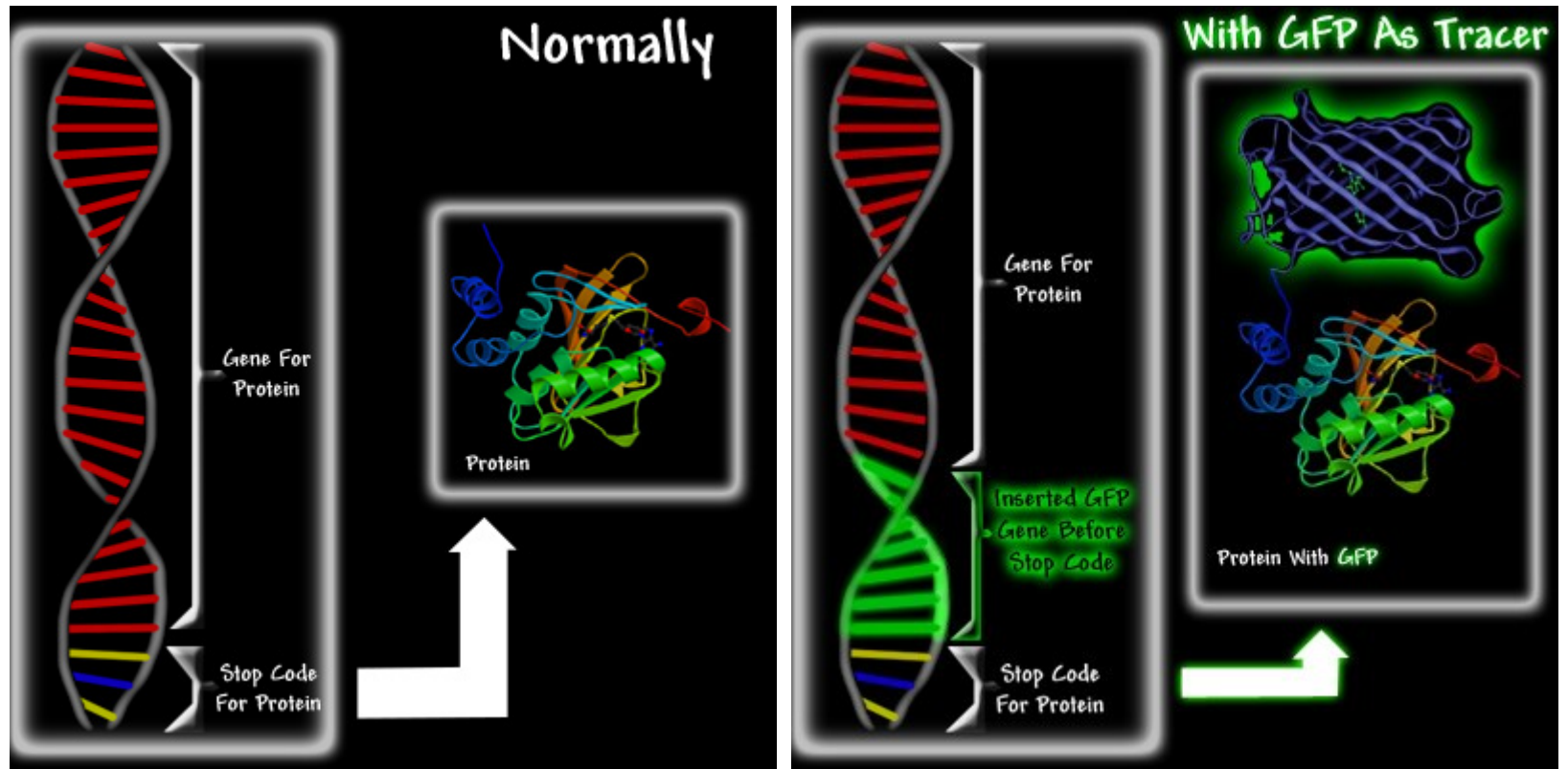
http://www.mbayaq.org/efc/living_species/default.asp?hOri=1&inhab=440

Renilla reniformis "Sea Pansy"



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

Fluorescenční proteiny



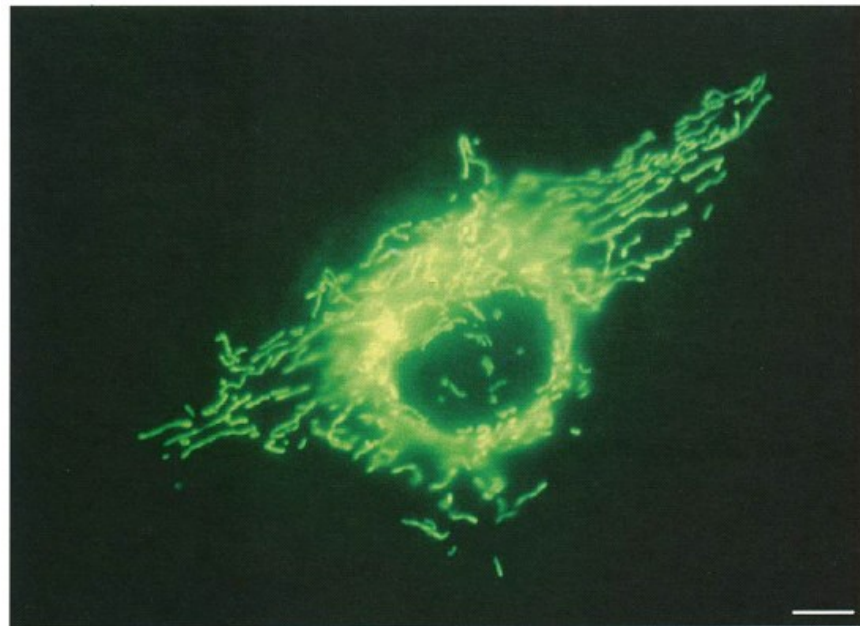
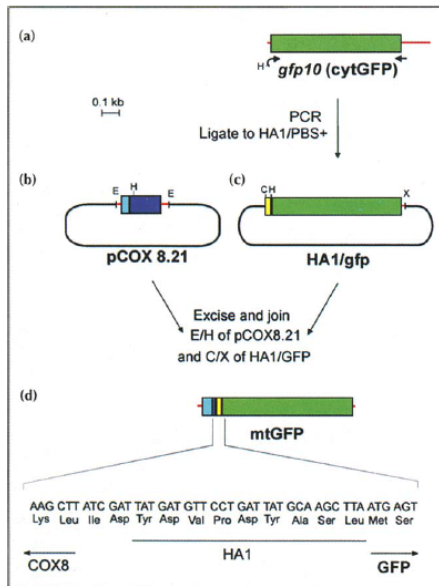
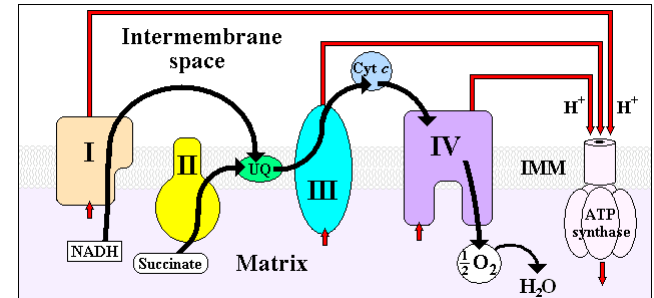
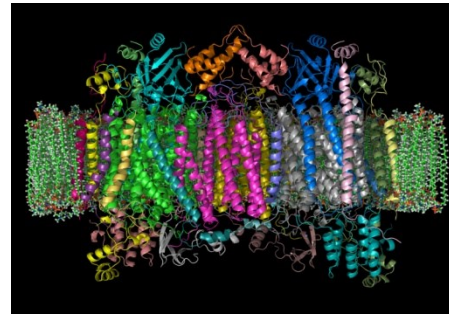
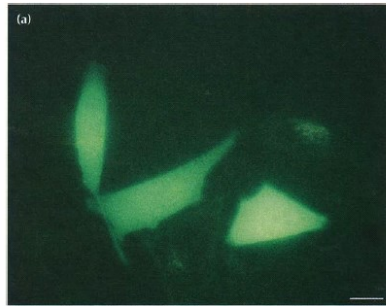
<http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm>

Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells

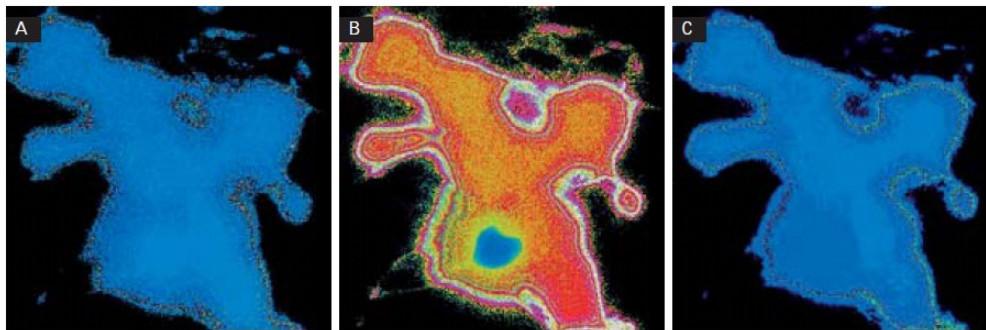
Rosario Rizzuto, Marisa Brini, Paola Pizzo,
Marta Murgia and Tullio Pozzan

Department of Biomedical Sciences and CNR Center for the Study of Mitochondrial Physiology, University of Padova, Via Trieste 75, 35121 Padova, Italy.

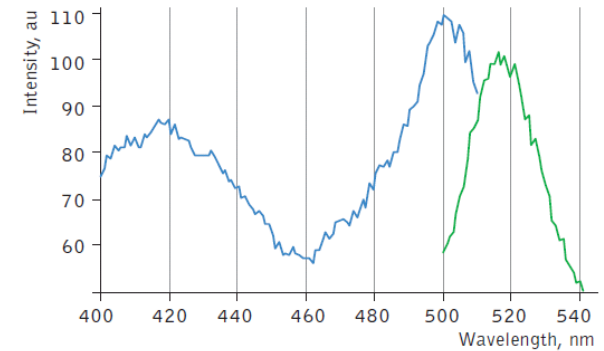
Current Biology 1995, 5:635-642



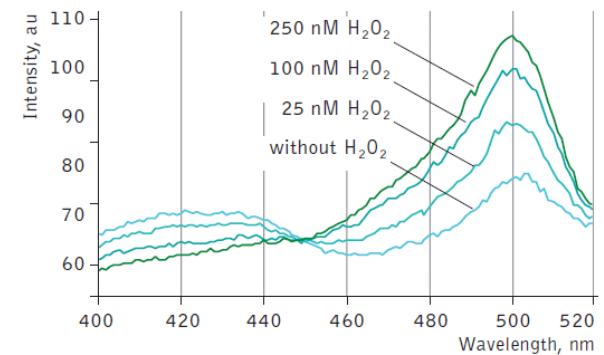
Fluorescent sensors for detection of H_2O_2



Ratiometric images of the group of HeLa cells before (A), 20 sec after (B), and 600 sec after (C) addition of 180 μ l of H_2O_2 . Images were pseudocolored using "ratio" lookup table of NIH ImageJ software: blue-green-red-white colors represent lowest-intermediate-high-highest level of H_2O_2 .



HyPer excitation (blue line) and emission (green line) spectra.



Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition. Emission was measured at 530 nm.

Variants & fusions

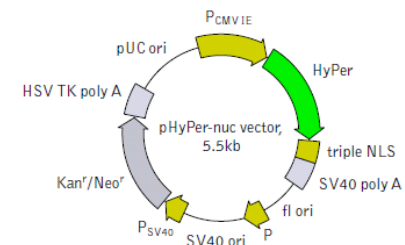
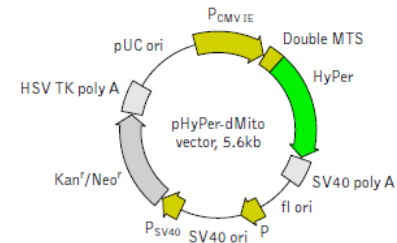
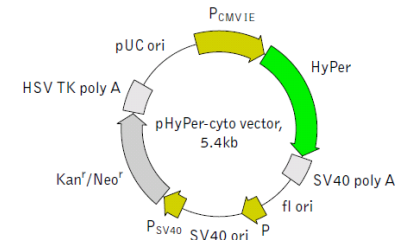
■ pHyPer-cyto vector

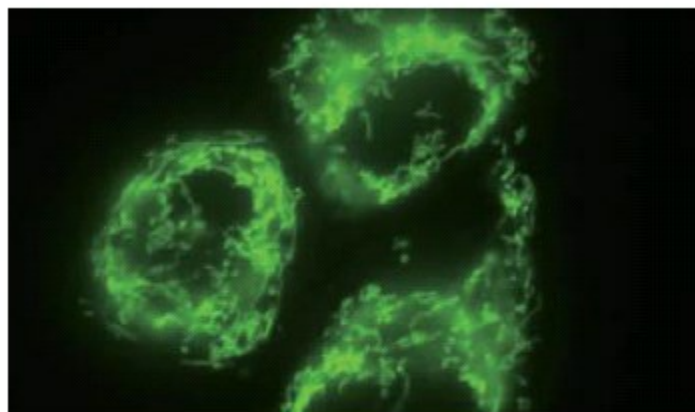
■ pHyPer-dMito vector

- Duplicated mitochondrial targeting sequence (MTS) is fused to the HyPer N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase [Rizzuto et al., 1989; Rizzuto et al., 1995].

■ pHyPer-nuc vector

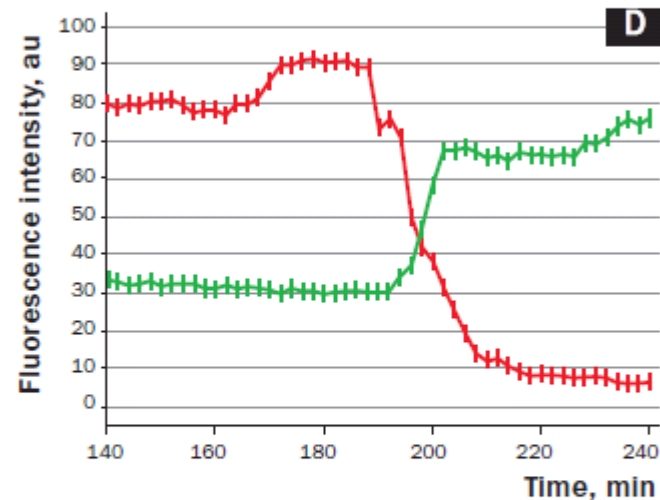
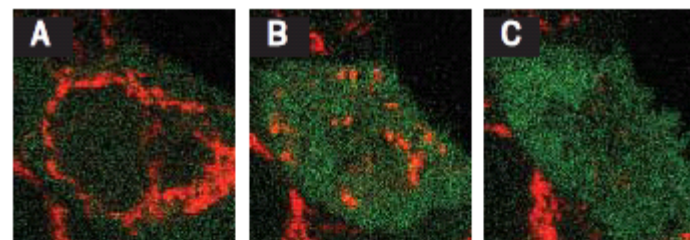
- Three copies of the nuclear localization signal (NLS) fused to the HyPer C-terminus provide for efficient translocation of HyPer to the nuclei of mammalian cells [Fischer-Fantuzzi and Vesco, 1988]





Stably transfected HeLa cells expressing mitochondria-targeted HyPer.

Image from Dr. Christian Petzelt (Marinpharm).



Dynamics of intracellular H₂O₂ production in a HeLa cell undergoing Apo2L/TRAIL-induced apoptosis.

A-C — confocal images of HeLa cells expressing cytosolic HyPer in 176 min (A), 200 min (B) and 240 min (C) after Apo2L/TRAIL addition; D — Intensities of HyPer (green) and TMRM (red) fluorescence in the cell.



Analýza a sortování chromozómů

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 3, pp. 1382-1384, March 1979
Genetics

Measurement and purification of human chromosomes by flow cytometry and sorting

(isolated chromosomes/DNA cytophotometry/flow microfluorometer)

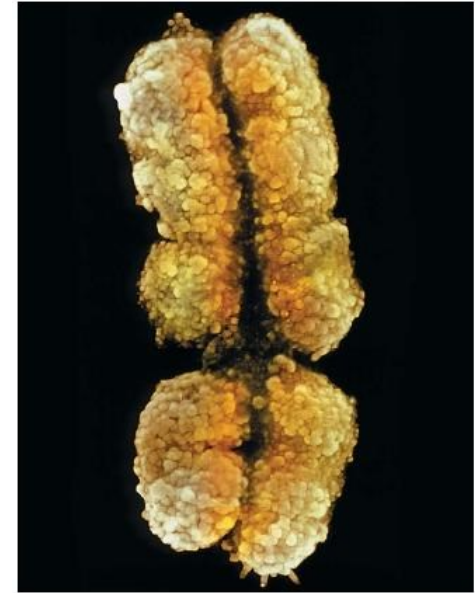
A. V. CARRANO, J. W. GRAY, R. G. LANGLOIS, K. J. BURKHART-SCHULTZ, AND M. A. VAN DILLA

Biomedical Sciences Division, L-452, Lawrence Livermore Laboratory, Livermore, California 94550

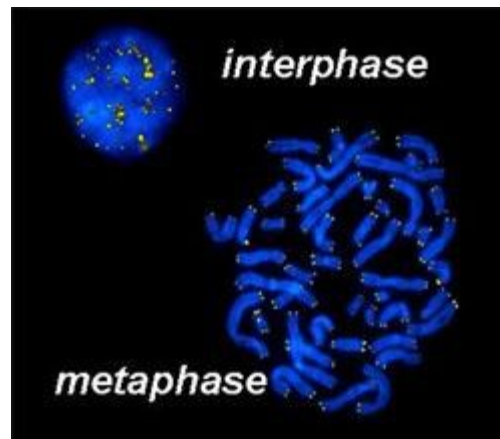
Communicated by Donald A. Glaser, December 18, 1978

Analýza a sortování chromozómů

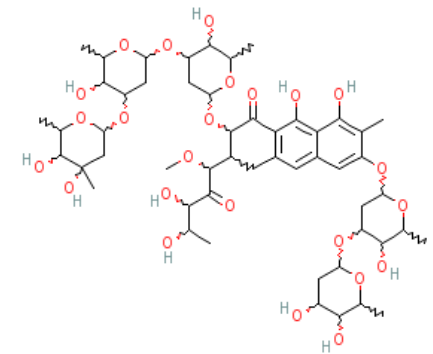
- synchronizace buněk – získání metafázních chromozómů (colcemid, hydroxyurea)
 - izolace chromozómů
 - značení DAPI nebo **Hoechst** vs. **chromomycin A3** (CA3) nebo mithramycin
- = celková DNA vs. G/C-bohaté oblasti



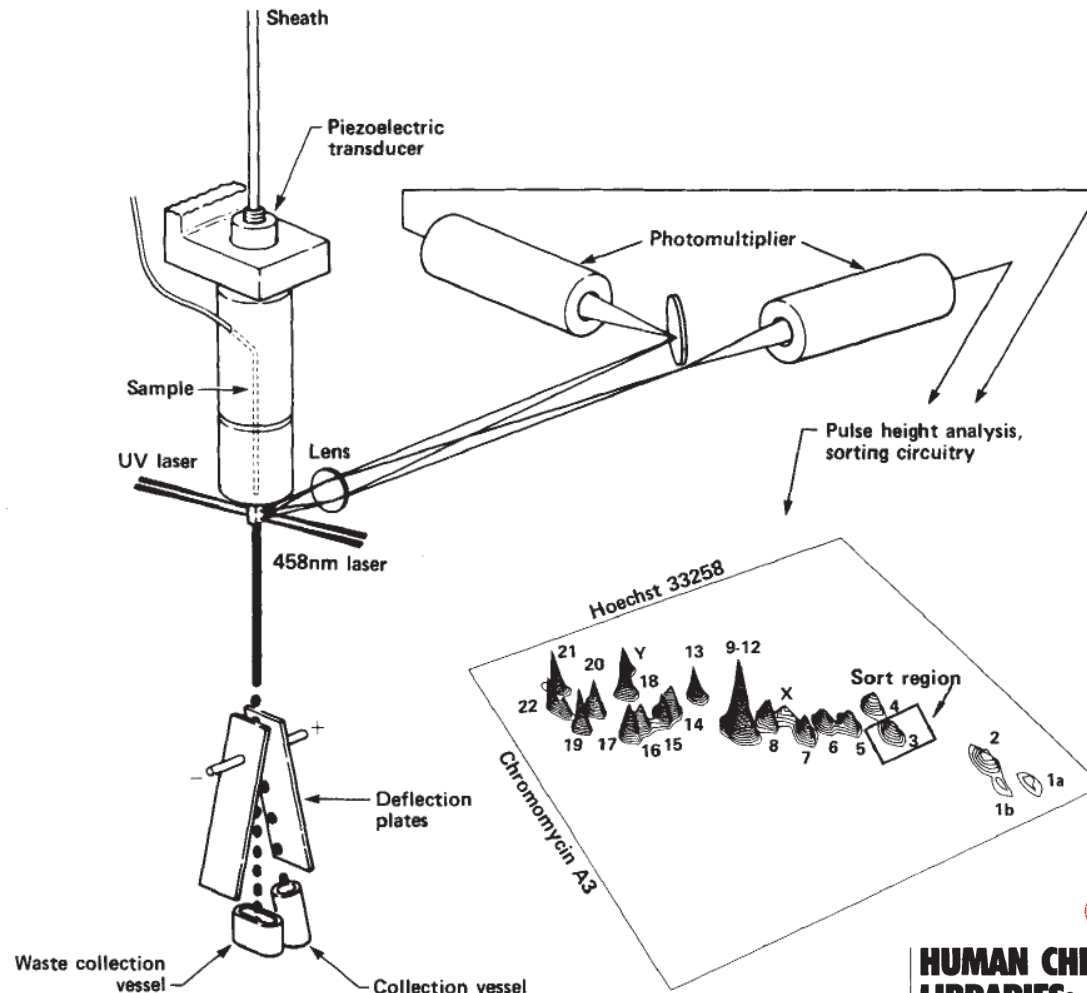
<http://www.scienceclarified.com/Ca-Ch/Chromosome.html>



<http://www.nccr-oncology.ch/scripts/page9243.html>



Analýza a sortování chromozómů



 © 1986 Nature Publishing Group <http://www.nature.com/naturebiotechnology>

HUMAN CHROMOSOME-SPECIFIC DNA LIBRARIES: CONSTRUCTION AND AVAILABILITY

M.A. Van Dilla[□], L.L. Deaven[□], K.L. Albright[†], N.A. Allen^{*}, M.R. Aubuchon^{*}, M.F. Bartholdi[†], N.C. Brown[†], E.W. Campbell[†], A.V. Carrano^{*}, L.M. Clark[†], L.S. Cram[†], B.D. Crawford[†], J.C. Fusco^{*}, J.W. Gray^{*}, C.E. Hildebrand[†], P.J. Jackson[†], J.H. Jett[†], J.L. Longmire[†], C.R. Lozes^{*}, M.L. Luedemann[†], J.C. Martin[†], J.S. McNinch^{*}, L.J. Meincke[†], M.L. Mendelsohn^{*}, J. Meyne[†], R.K. Moyzis[†], A.C. Munk[†], J. Perlman^{*}, D.C. Peters^{*}, A.J. Silva^{*}, and B.J. Trask^{*}.

National Laboratory Gene Library Project. ^{*} Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, California 94550. [†] Los Alamos National Laboratory, Life Sciences Division, University of California, Los Alamos, New Mexico 87545. [□] To whom correspondence should be directed.

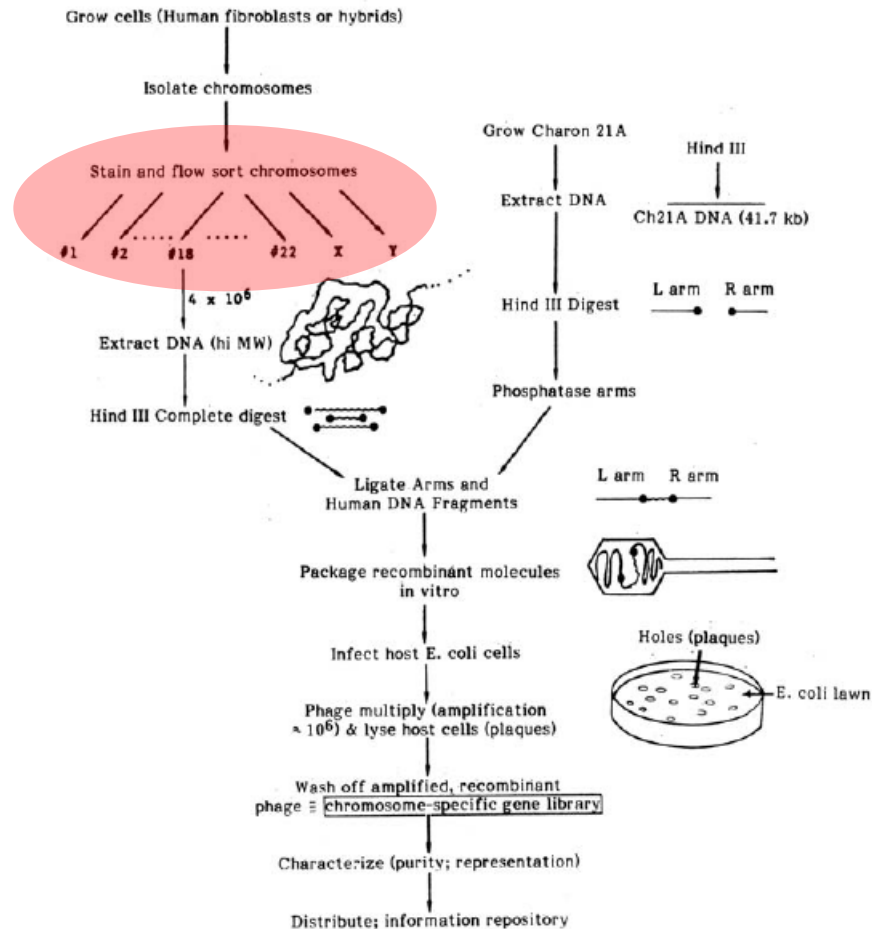
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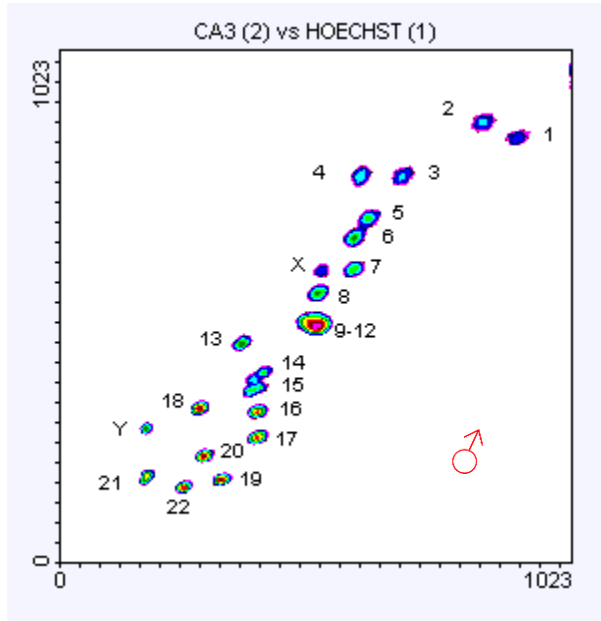
CONSTRUCTION OF A PHASE I CHROMOSOME-SPECIFIC (#18)

HUMAN GENE LIBRARY IN CHARON 21A USING HIND III (LLNL)



e!

„Flow karyotype“



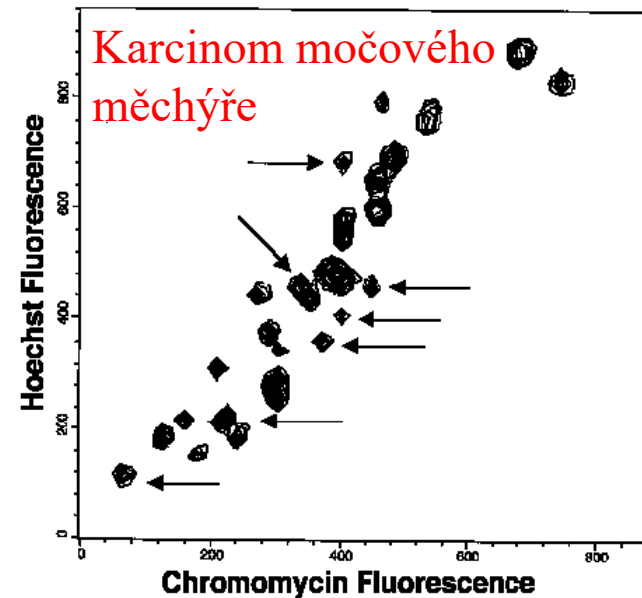
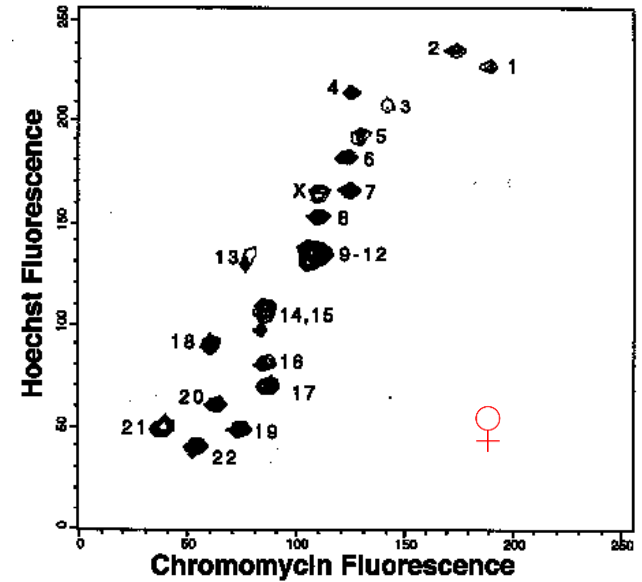
<http://www.sanger.ac.uk/HGP/Cytogenetics/>

The Preparation of Human Chromosomes for Flow Cytometry

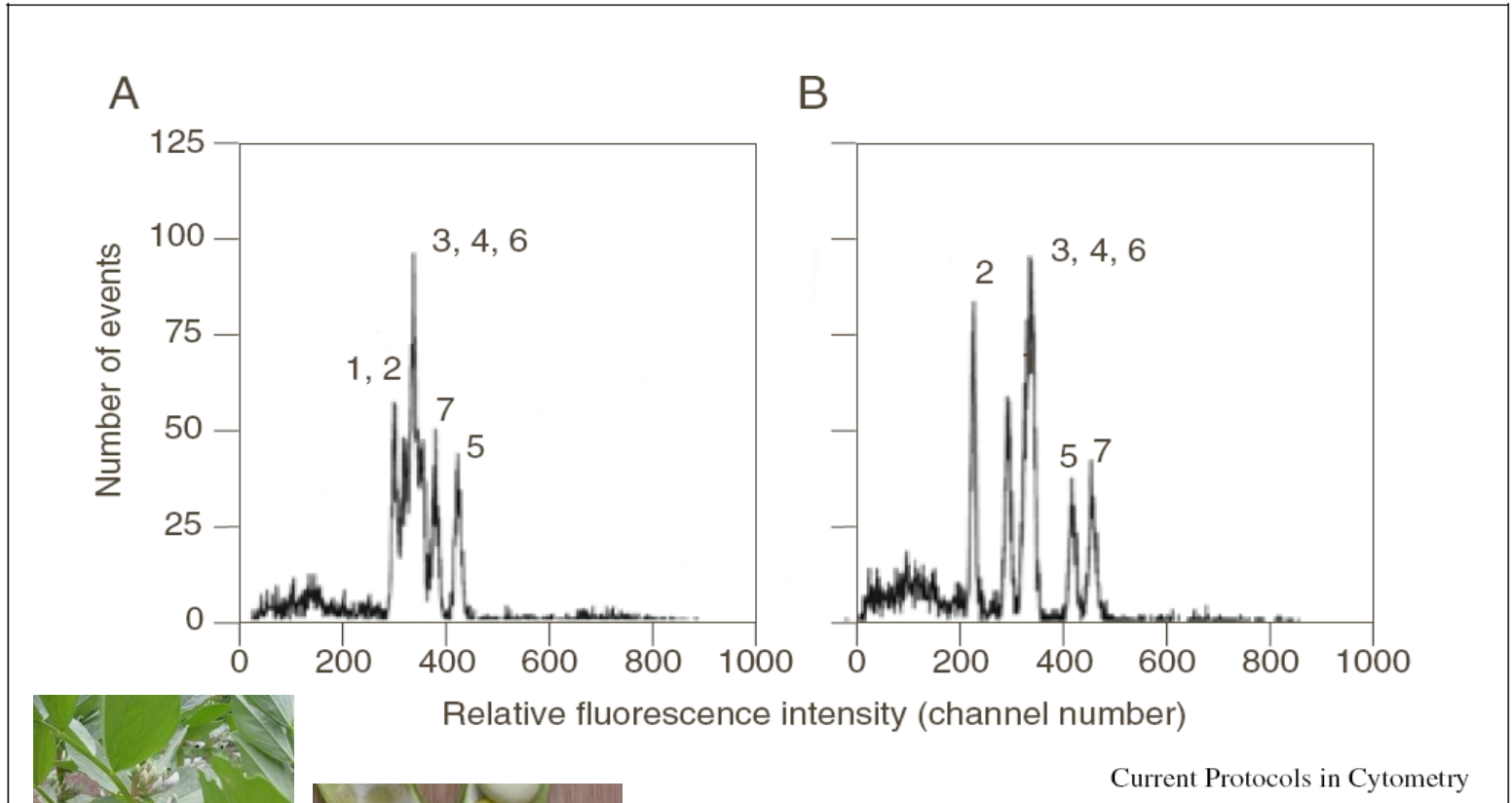
DEREK DAVIES

FACS Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX

Vol. 33/2 Proceedings RMS June 1998



Sortování chromozómů



Pisum sativum

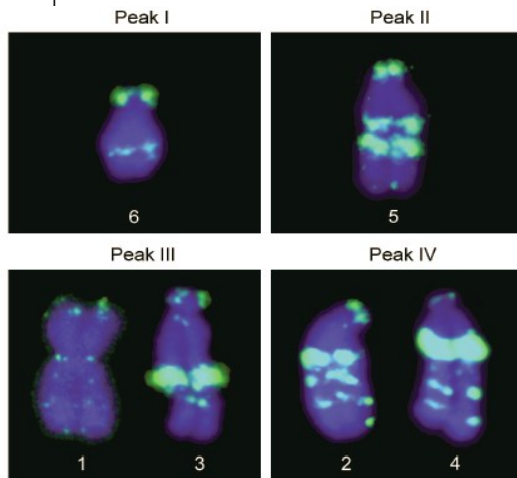
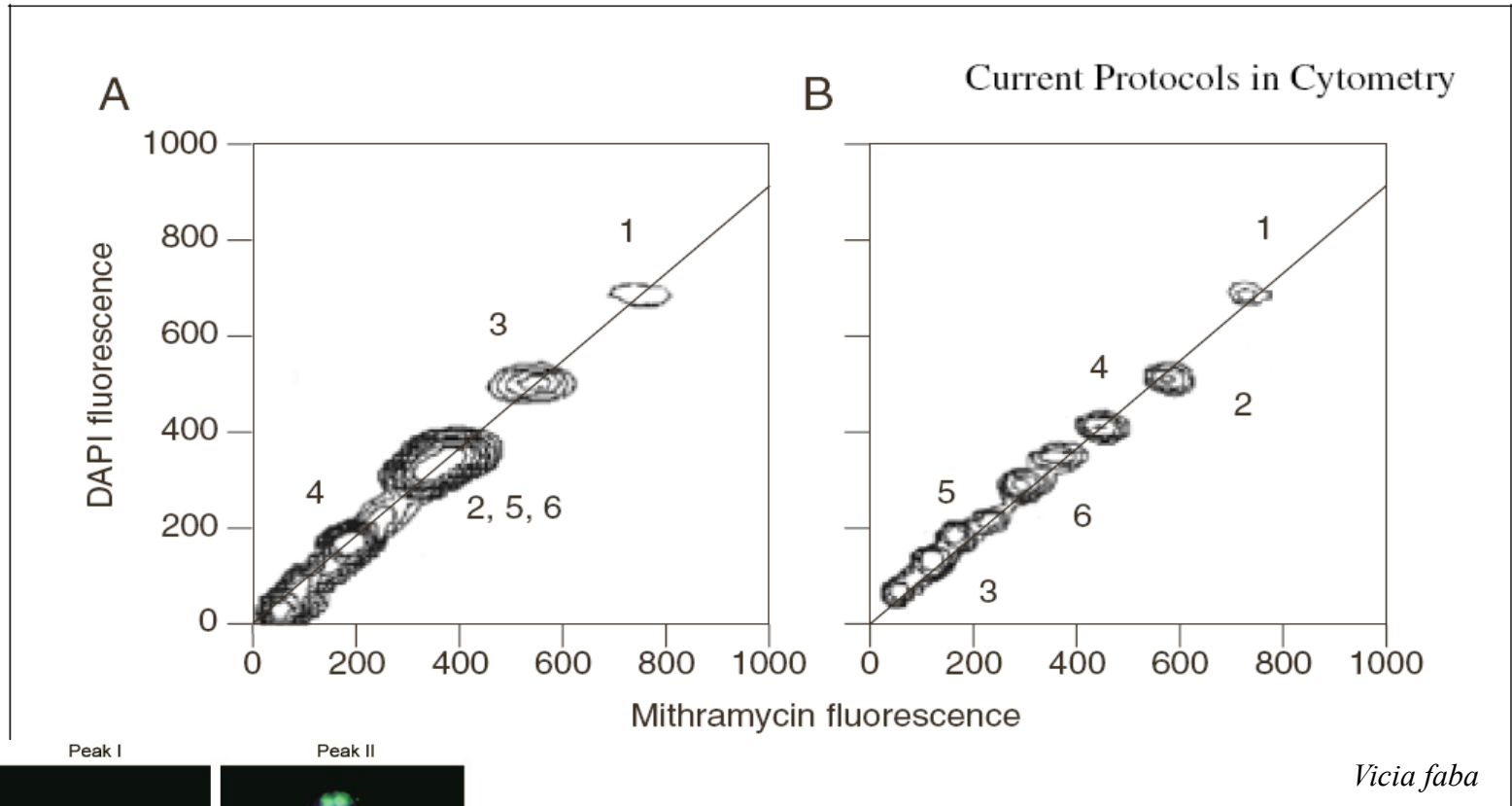
PROTOCOL

Estimation of nuclear DNA content in plants using flow cytometry

Jaroslav Doležel^{1,2}, Johann Greilhuber³ & Jan Suda^{4,5}

¹Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovská 6, CZ-77200 Olomouc, Czech Republic; ²Department of Cell Biology and Genetics, Palacký University, Šlechtitelů 11, CZ-78371 Olomouc, Czech Republic; ³Department of Systematic and Evolutionary Botany, Faculty of Life Sciences, University of Vienna, Rennweg 14, A-1030 Vienna, Austria; ⁴Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-12801 Prague, Czech Republic; ⁵Institute of Botany, Academy of Sciences of the Czech Republic, Příhonice 1, CZ-25243, Czech Republic. Correspondence should be addressed to J.D. (dolez@ueb.cas.cz).

Sortování chromozómů



BIOLOGIA PLANTARUM 51 (1): 43-48, 2007

Chromosome analysis and sorting in *Vicia sativa* using flow cytometry

P. KOVÁŘOVÁ¹, A. NAVRÁTILOVÁ², J. MACAS² and J. DOLEŽEL^{1,3*}



Development of Chromosome-Specific BAC Resources for Genomics of Bread Wheat

J. Šafář¹ H. Šimková¹ M. Kubaláková¹ J. Číhalíková¹ P. Suchánková¹ J. Bartoš¹
J. Doležel¹

Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Olomouc, Czech Republic

The Plant Journal (2004) **39**, 960–968

doi: 10.1111/j.1365-313X.2004.02179.x

TECHNICAL ADVANCE

Dissecting large and complex genomes: flow sorting and BAC cloning of individual chromosomes from bread wheat

Jan Šafář¹, Jan Bartoš¹, Jaroslav Janda¹, Arnaud Bellec², Marie Kubaláková^{1,3}, Miroslav Valárik¹, Stéphanie Pateyron², Jitka Weiserová¹, Radka Tušková¹, Jarmila Číhalíková^{1,3}, Jan Vrána¹, Hana Šimková¹, Patricia Faivre-Rampant², Pierre Sourdille⁴, Michel Caboche², Michel Bernard⁴, Jaroslav Doležel^{1,3} and Boulos Chalhou^{2,*}

¹Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovska 6, CZ-77200 Olomouc, Czech Republic,

²Laboratory of Genome organization, Unité de Recherches en Génomique Végétale (INRA-URGV), 2 rue Gaston Crémieux, CP 5708, F-91057 Évry Cedex, France,

³Department of Cell Biology and Genetics, Palacký University, Šlechtitelů 11, Olomouc, Czech Republic, and

⁴Génétique Moléculaire des Céréales, UMR INRA-UBP, Domaine de Crouelle, 234 Avenue du Brézet, F-63039 Clermont-Ferrand Cedex 2, France

Received 1 February 2004; revised 5 May 2004; accepted 11 May 2004.

*For correspondence (fax 33 1 60874549; e-mail chalhou@evry.inra.fr).



ROAD MAP FOR
WHEAT

Ordered sequence will
speed research pp. 635, 661, & 662

RESEARCH

RESEARCH ARTICLE

WHEAT GENOME

Shifting the limits in wheat research and breeding using a fully annotated reference genome

International Wheat Genome Sequencing Consortium (IWGSC)*

An annotated reference sequence representing the hexaploid bread wheat genome in 21 pseudomolecules has been analyzed to identify the distribution and genomic context of coding and noncoding elements across the A, B, and D subgenomes. With an estimated coverage of 94% of the genome and containing 107,891 high-confidence gene models, this assembly enabled the discovery of tissue- and developmental stage-related coexpression networks by providing a transcriptome atlas representing major stages of wheat development. Dynamics of complex gene families involved in environmental adaptation and end-use quality were revealed at subgenome resolution and contextualized to known agronomic single-gene or quantitative trait loci. This community resource establishes the foundation for accelerating wheat research and application through improved understanding of wheat biology and genomics-assisted breeding.

Wheat (*Triticum aestivum* L.), the most widely cultivated crop on Earth, contributes about a fifth of the total calories consumed by humans and provides more protein than any other food source (1, 2). Breeders strive to develop improved varieties by fine-tuning genetically complex yield and end-use quality parameters while maintaining yield stability and regional adaptation to specific biotic and abiotic stresses (3). These efforts are limited, however, by insufficient knowledge and understanding of the molecular basis of key

the wheat genome through gene loss, gain, and duplication (6). The lack of global sequence contiguity and incomplete coverage (only 10 Gb were assembled), however, did not provide the wider regulatory genomic context of genes. Subsequent whole-genome assemblies improved contiguity (7–9) but lacked full annotation and did not resolve the intergenic space or present the genome in the correct physical order.

Here we report an ordered and annotated assembly (IWGSC RefSeq v1.0) of the 21 chromosomes of the allohexaploid wheat cultivar CS.

St
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ir
tv

Final assembly chromosome (pac)-based sequence assemblies. Finally, IWGSC RefSeq v1.0 was assessed with independent data derived from coding and noncoding sequences, revealing that 99 and 98% of the previously known coding exons (6) and transposable element (TE)-derived (ISBP) markers (table S9), respectively, were present in the assembly. The approximate 1-Gb size difference between IWGSC RefSeq v1.0 and the new genome size estimates of 15.4 to 15.8 Gb (14) can be accounted for by collapsed or unassembled sequences of highly repeated clusters, such as ribosomal RNA coding regions and telomeric sequences.

A key feature distinguishing the IWGSC RefSeq v1.0 from previous draft wheat assemblies (6–9) is the long-range organization, with 90% of the genome represented in superscaffolds larger than 4.1 Mb and with each chromosome represented.

Downloaded from <http://science.sciencemag.org/> on 7



Aplikace průtokové cytometrie v mikrobiologii

- ekologie
- potravinářství

<http://www.cyto.purdue.edu/flowcyt/research/micrflow/>

Aplikace průtokové cytometrie v mikrobiologii

Relative Size Ratios for Bacteria, Yeast, and Eukaryotes

Measurement	Bacteria	Yeast	Eukaryote
Diameter	0.5-5	3-5	10-30
Surface area	3-12	30-75	300-3000
Volume	0.3-3	20-125	500-1500
Dry cell mass	1	10	300-3000

Current Protocols in Cytometry

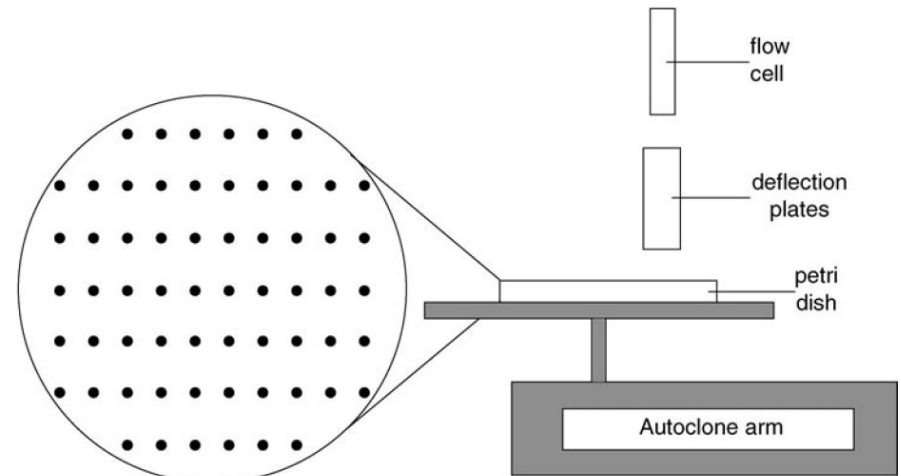


Aplikace průtokové cytometrie v mikrobiologii

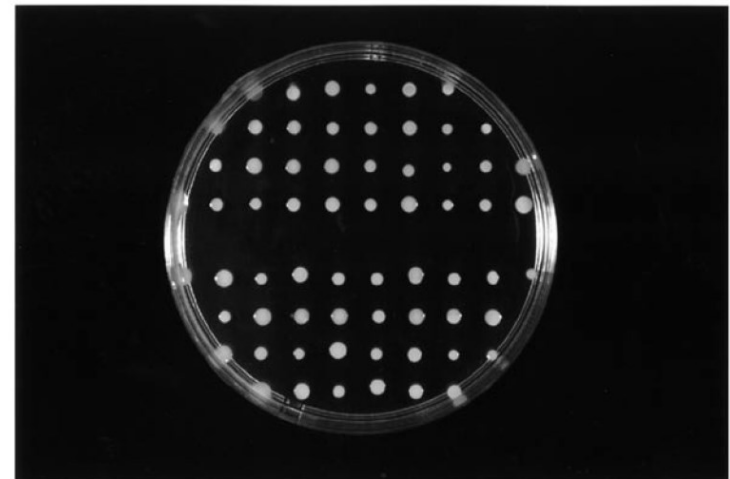
- viabilita
- metabolické funkce
- sortování
- analýza aerosolů (Fluorescence Aerodynamic Particle Sizer (Flaps))

Aplikace průtokové cytometrie v mikrobiologii

- Sortování
 - EPICS + Autoclone® modul



top view of petri dish showing sort grid



BactoSense™

Automated flow cytometer for online monitoring of bacteria in water



- Fast** Results available within 20 minutes, analyses up to 48 samples in 24h
- Safe** No handling of chemicals, all components are in a sealed and recyclable cartridge
- Accurate** Flow cytometry technology allows precise detection of more than 99.9% of microbial cells
- Secure** 24/7 monitoring: set a threshold value to get an alarm in time to act accordingly
- Reliable** Self-check routines, factory calibration and low maintenance



For your PROCESS



In the FIELD



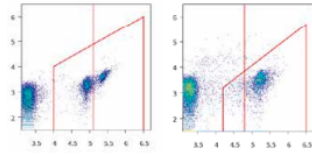
In the LAB

Measuring principle	Flow cytometry
Light source	Laser diode 488nm
Optical detection	Fluorescence: 535/43 (FL1), 715 LP (FL2), Side scatter 488/10 (SSC)
Lower size detection limit	0.1 µm
Measuring range	1'000 - 2 Million cells/ml
Detection limit	100 - 5 Million cells/ml
Accuracy	< 5 % relative
Automatic measuring interval	Minimum 30 minutes, maximum 6 hours
Microbial parameters	TCC/ml, ICC/ml, LNA/ml, HNA/ml, HNAP(%)

Sampling	Online or manual
Sample volume	260 µl sampled, 90 µl for analysis
flow rate (online)	200 - 400 ml/min
chlorine concentration	max. 3 mg/l
turbidity	1 - 10 FTU
pH-value	5 - 12
temperature range	5..40°C
conductivity	0 - 100'000 µs/cm à 20°C

Instrument	Factory calibrated
Display	Touchscreen
Data storage	32 GB
Protection level enclosure	IP 65
Dimensions (WxDxH)	350 x 240 x 373 mm
Weight	14.5 kg
Power supply	100 - 240 VAC, 50/60 Hz, 1.4 A, IP 67
Power consumption	20 W
Ambient temperature	5..30°C
Relative humidity	10 - 90% RH
Cartridge	Hermetically sealed enclosure for reagents, cleaning liquids and waste
Cartridge capacity	Max. 1'000 measurements, 9 months validity

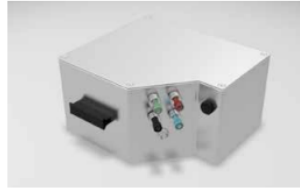
Interface	Digital and analogue
Inputs	4 x digital, freely configurable
Outputs analogue	2 x 0/4 .. 20 mA, galvanically isolated
Outputs digital	4 x digital, freely configurable
Digital interfaces	Sealed USB, Ethernet connections, Modbus



Dotplots showing TCC and ICC



Online sampler / manual sampler

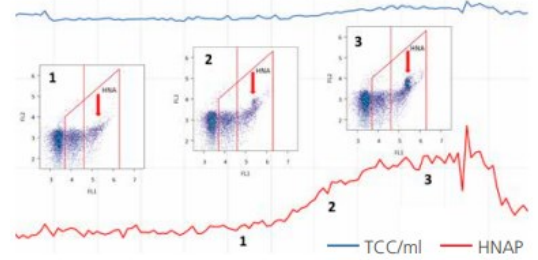


Cartridge

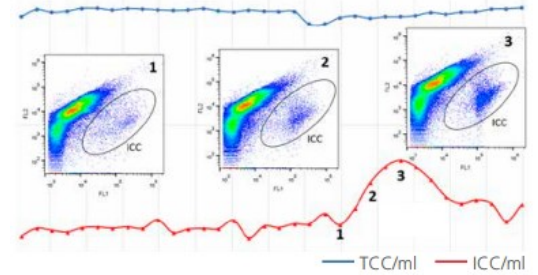


IO box

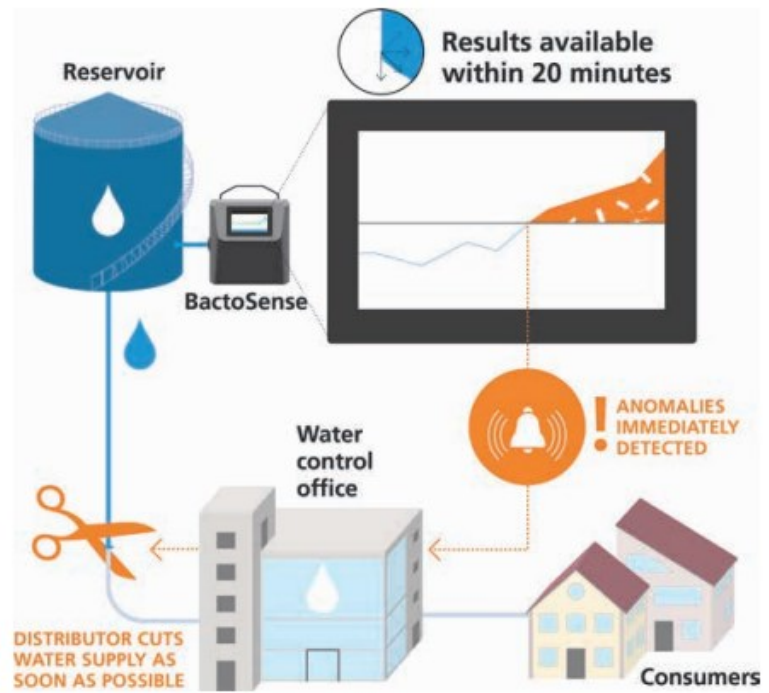
Graph showing TCC and HNAP measurements over one week



Graph showing TCC and ICC measurements every 3 hours

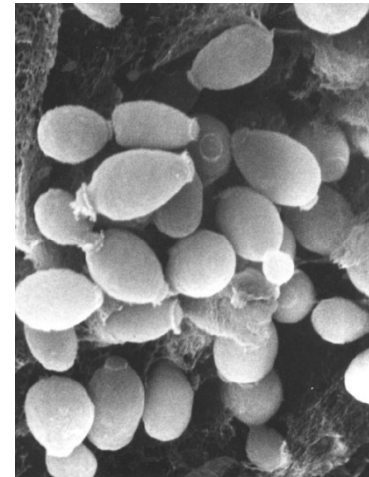


312.02.002.EN

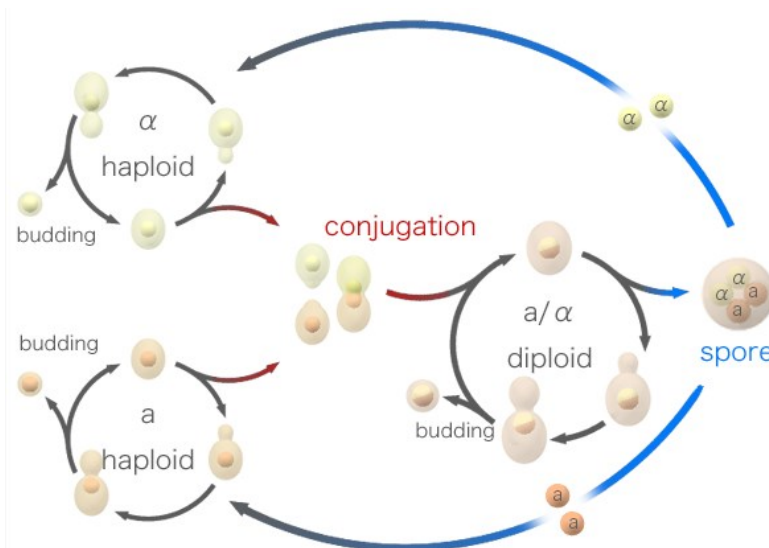


Průtoková cytometrie kvasinek

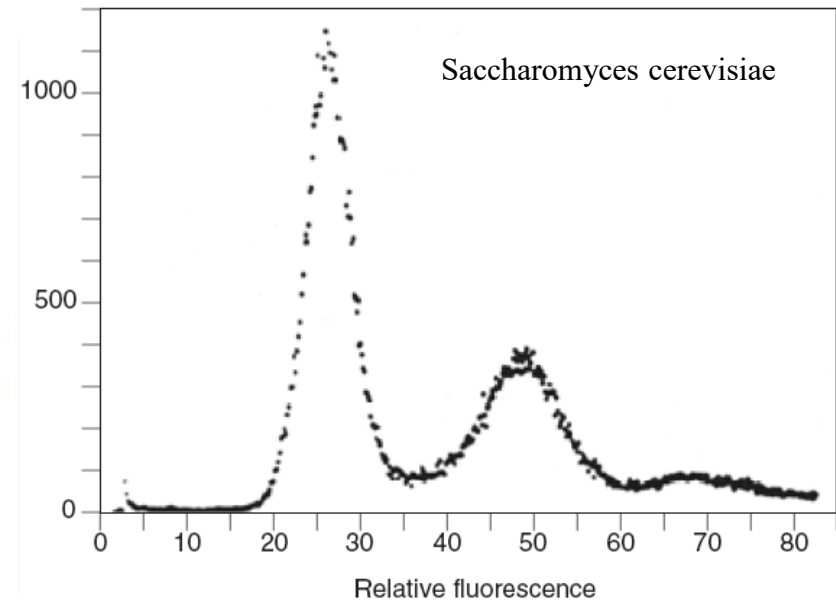
- buněčné dělení
- viabilita
- membránový potenciál
- respirace
- produkce H_2O_2
- citlivost k antibiotikům
- separace



http://www.sbs.utexas.edu/mycology/sza_images_SEM.htm



http://en.wikipedia.org/wiki/Image:Budding_yeast_Lifecycle.png



Průtoková cytometrie kvasinek

Yeast Cell Cycle During Fermentation and Beer Quality

Masahito Muro,¹ Kenichiro Izumi, Takeo Imai, Yutaka Ogawa, and Motoo Ohkochi, *Research Laboratories for Brewing, Kirin Brewery Co., Ltd., 1-17-1, Namamugi, Tsurumi-ku, Yokohama, 230-8628 Japan*

J. Am. Soc. Brew. Chem. 64(3):151-154, 2006



Průtoková cytometrie v hydrobiologii

- studium pico- a nanofytoplanktonu ($< 20 \mu\text{M}$)
- analýza metabolických funkcí planktonu
- studium pigmentace (analýza chlorofylu a fykoeritrinu)





Průtoková cytometrie v hydrobiologii

© 2001 Wiley-Liss, Inc.

Cytometry 44:236–246 (2001)

Monitoring Phytoplankton, Bacterioplankton, and Virioplankton in a Coastal Inlet (Bedford Basin) by Flow Cytometry

W.K.W. Li* and P.M. Dickie

Biological Oceanography Section, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

Received 4 October 2000; Revision Received 2 May 2001; Accepted 2 May 2001

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Cytometry 10:659–669 (1989)

Using Phytoplankton and Flow Cytometry to Analyze Grazing by Marine Organisms

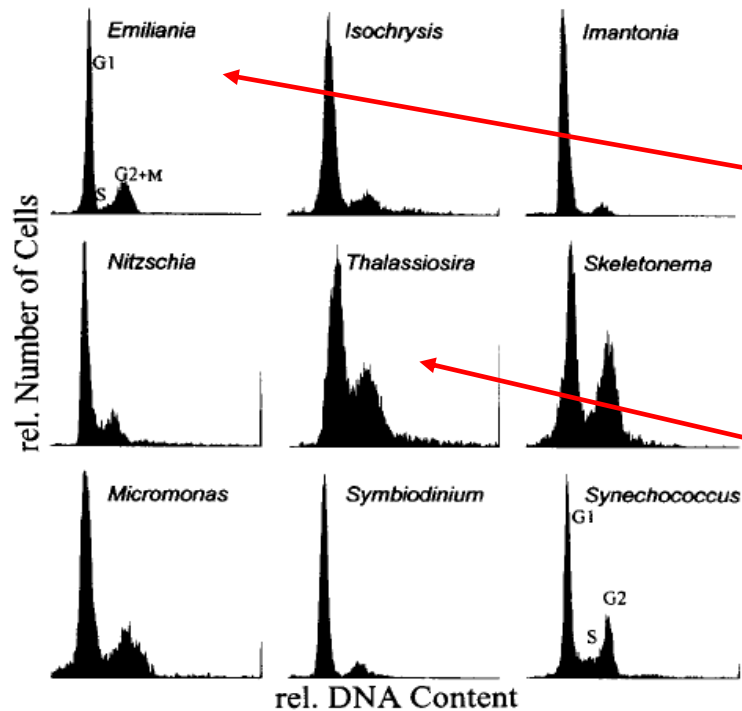
Terry L. Cucci, Sandra E. Shumway, Wendy S. Brown, and Carter R. Newell

Department of Marine Resources (S.E.S.) and Bigelow Laboratory for Ocean Sciences (T.L.C., S.E.S.), West Boothbay Harbor, Maine 04575; Chemistry Department, Bowdoin College (W.S.B.), Brunswick, Maine 04011; Great Eastern Mussel Farms (C.R.N.), Tenants Harbor, Maine 04857

Received for publication November 2, 1988; accepted April 17, 1989

Průtoková cytometrie v hydrobiologii

■ analýza DNA



Vol. 185: 301–307, 1999

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published August 20

NOTE

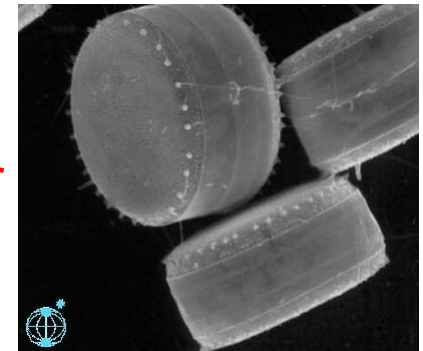
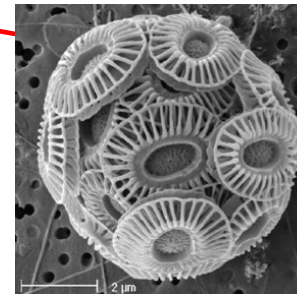
Cytometric measurement of the DNA cell cycle in the presence of chlorophyll autofluorescence in marine eukaryotic phytoplankton by the blue-light excited dye YOYO-1

Frank J. Jochem^{1,*}, Doris Meyerdierks²

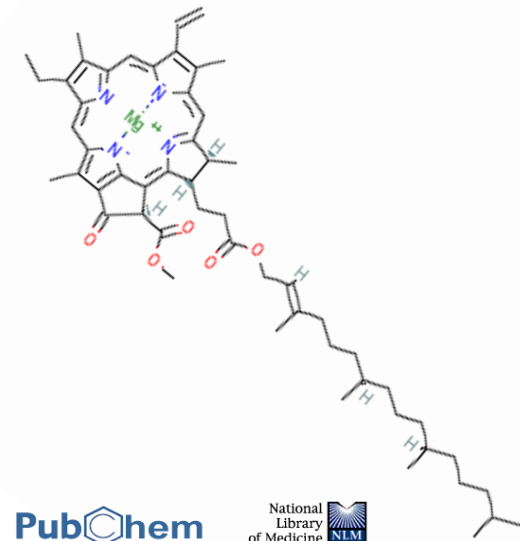
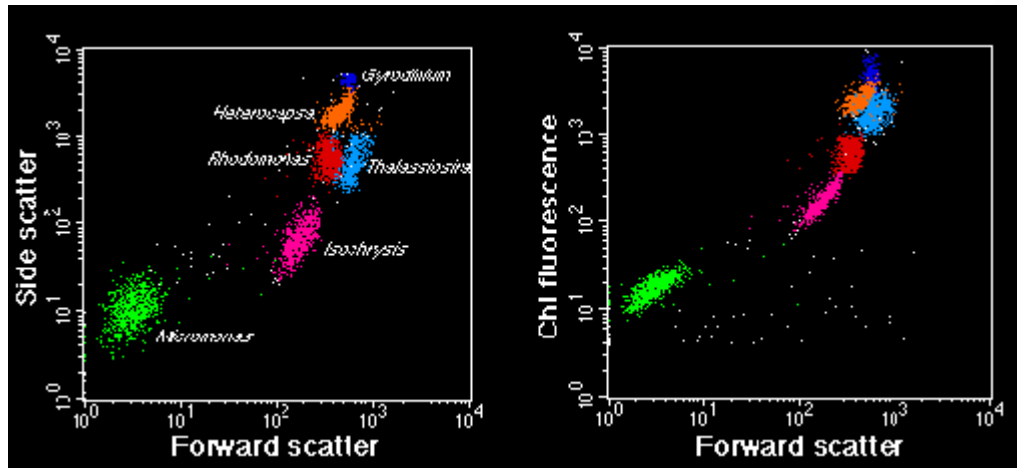
¹Institut für Meereskunde, Düsterbrookweg 20, D-24105 Kiel, Germany

²Universität Bremen, FB II Meeresbotanik, Postfach 330440, D-28334 Bremen, Germany

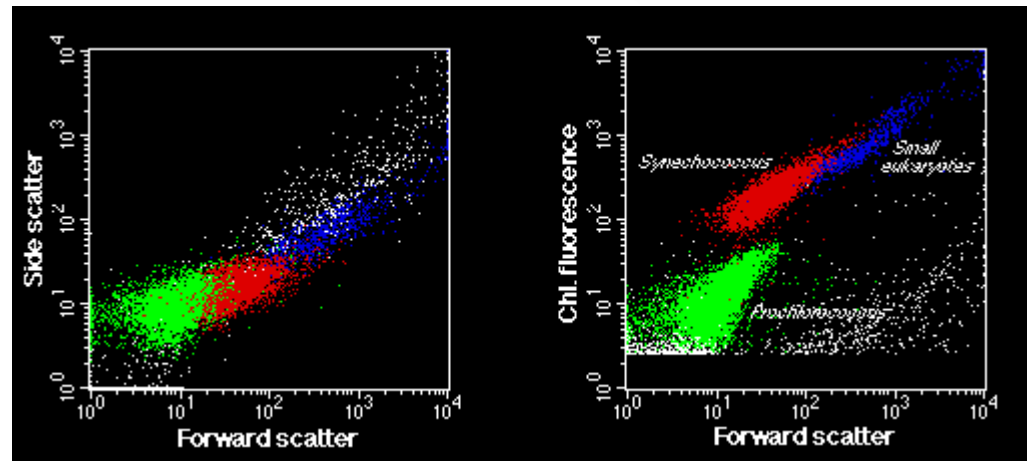
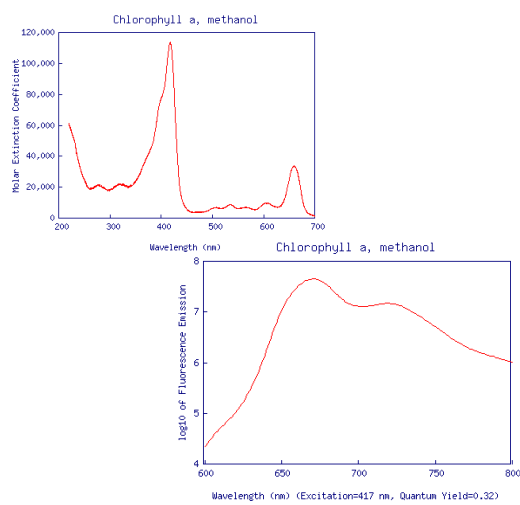
<http://www.soes.soton.ac.uk/staff/tt/>



Průtoková cytometrie v hydrobiologii



PubChem National Library of Medicine NLM



[http://omlc.ogi.edu/spectra/PhotochemCAD/html/chlorophyll-a\(MeOH\).html](http://omlc.ogi.edu/spectra/PhotochemCAD/html/chlorophyll-a(MeOH).html)

<http://www.cyto.purdue.edu/flowcyt/research/micrflow/sieracki/sierack2.htm>



A flow cytometer based protocol for quantitative analysis of bloom-forming cyanobacteria (*Microcystis*) in lake sediments

Quan Zhou^{1,2}, Wei Chen¹, Huiyong Zhang³, Liang Peng¹, Liming Liu¹, Zhiguo Han³,
Neng Wan⁴, Lin Li¹, Lirong Song^{1,*}

1. State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China.

E-mail: quanzhou1985@yahoo.com.cn

2. Graduate School of Chinese Academy of Sciences, Beijing 100039, China

3. Zealquest Laboratory for Ecological Research, Zealquest Scientific Technology Co., Ltd., Shanghai 200333, China

4. Changshu Institute of Technology, Changshu 215500, China

Received 06 November 2011; revised 20 March 2012; accepted 28 April 2012

Flow cytometry assessment of bacterioplankton in tropical marine environments

L. Andrade^a, A.M. Gonzalez^a, F.V. Araujo^{a,b}, R. Paranhos^{a,*}

^aDepartment of Marine Biology, Institute of Biology, University of Brazil, Prédio do CCS, bloco A, sala A1-071-Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ 21944-970, Brazil

^bFaculty of Teacher Formation, University of the State of Rio de Janeiro-UERJ, Brazil

Průtoková cytometrie bezobratlých

- lze aplikovat běžné metodické přístupy a fluorescenční značky
- Příklady aplikací:
 - buněčný cyklus
 - cytotoxicita
 - apoptóza





Invertebrate Survival Journal

ISJ 2: 32-40, 2005

ISSN 1824-307X

Review

Flow cytometry as a tool for analysing invertebrate cells

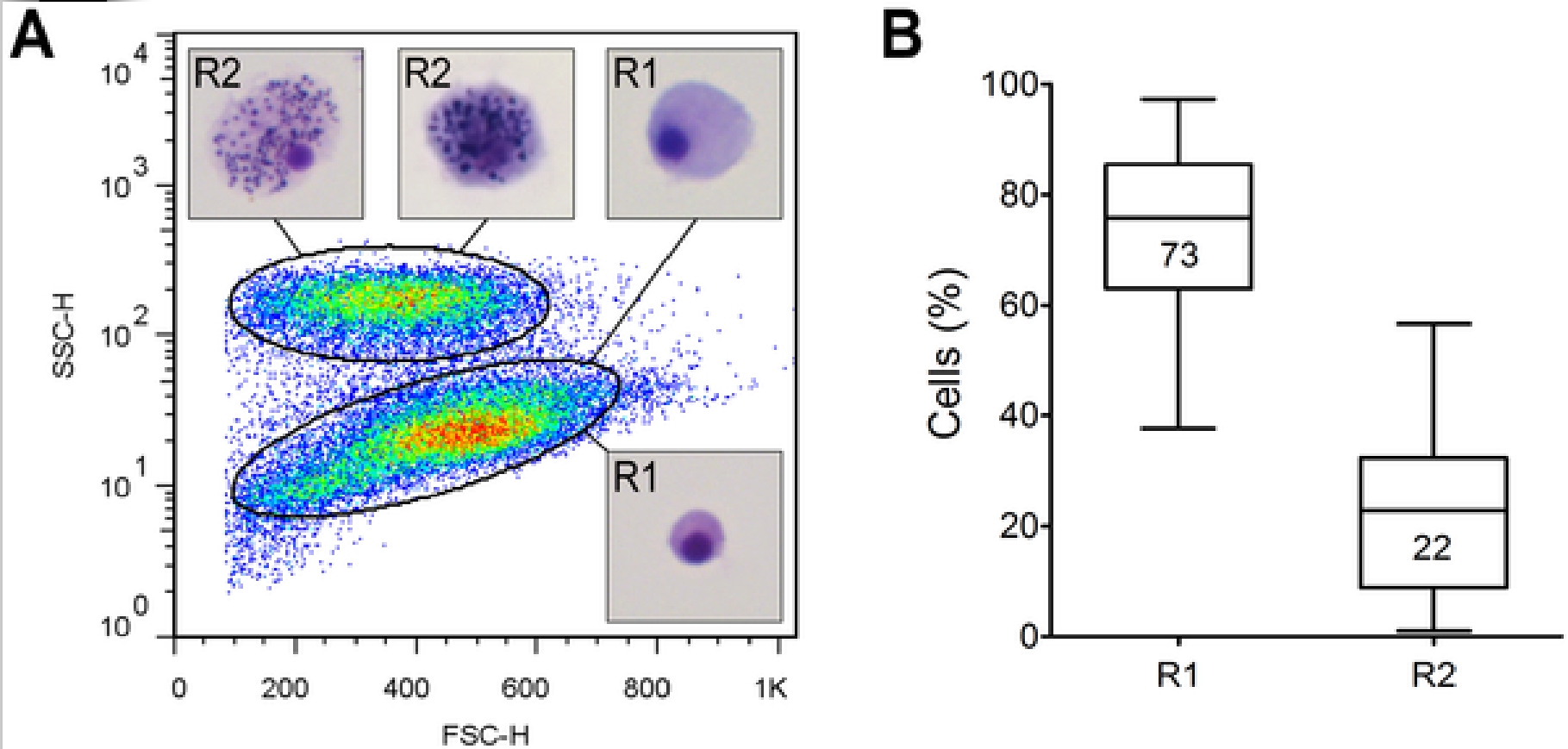
A Cossarizza¹, M Pinti¹, L Troiano¹, EL Cooper²

¹ *Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy*

² *Department of Neurobiology, UCLA School of Medicine, Los Angeles, CA, USA*

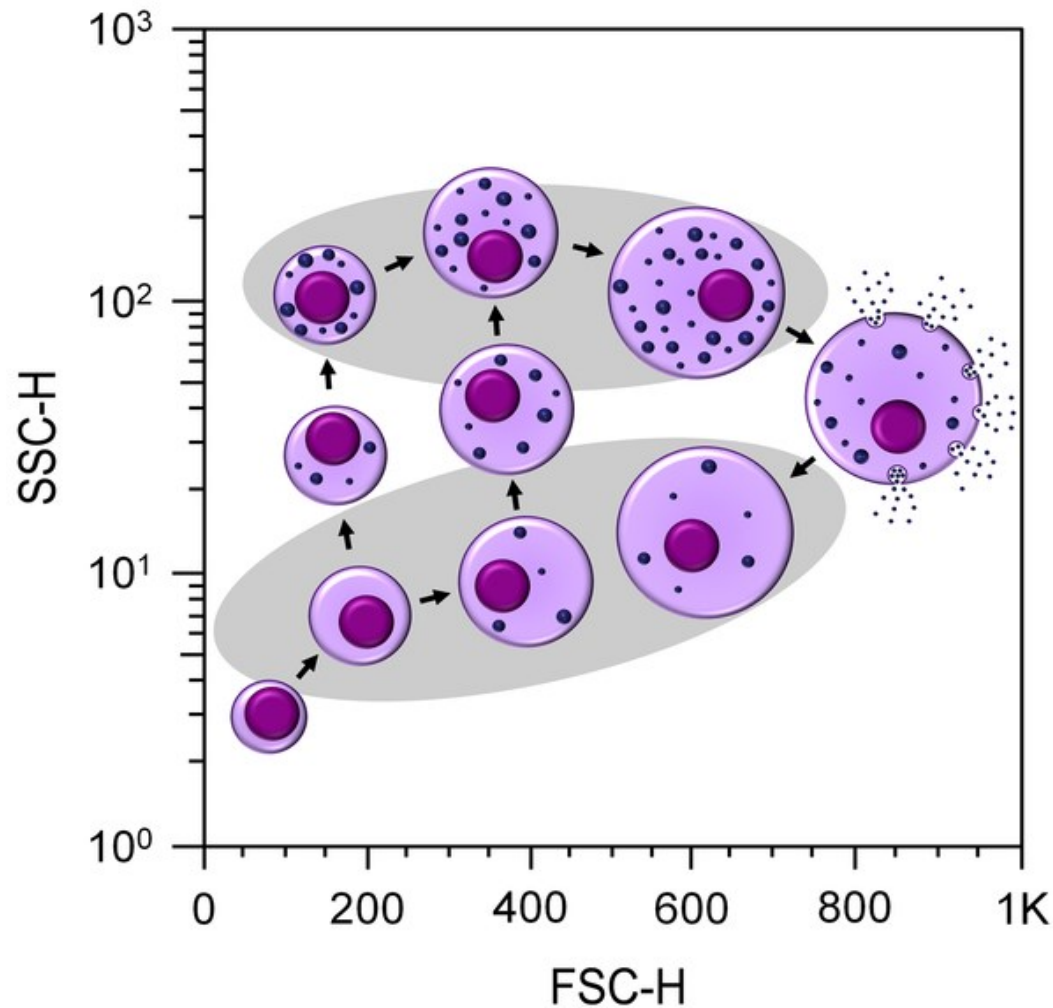
<http://www.icms.qmul.ac.uk/flowcytometry/uses/insects/index.html>

Figure 5. Representative flow-cytometry scatter plot of hemocytes from 25 oysters.

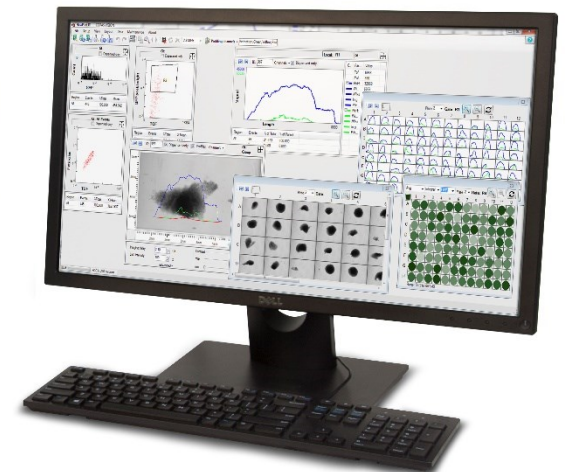
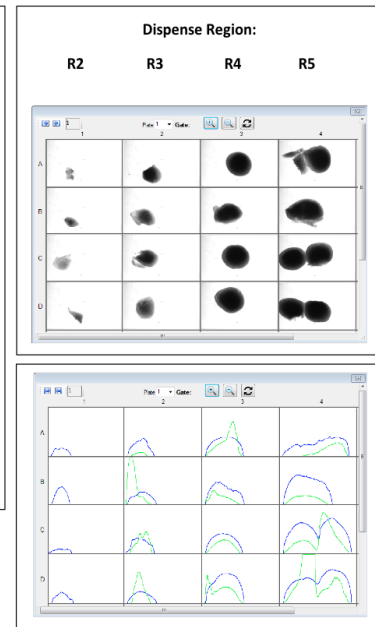
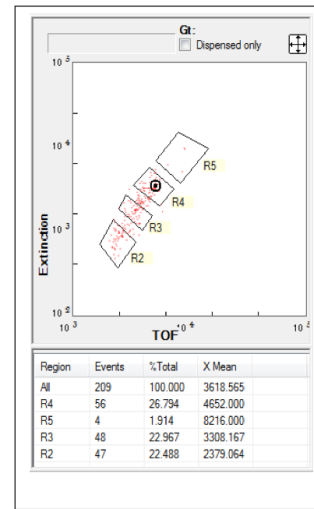
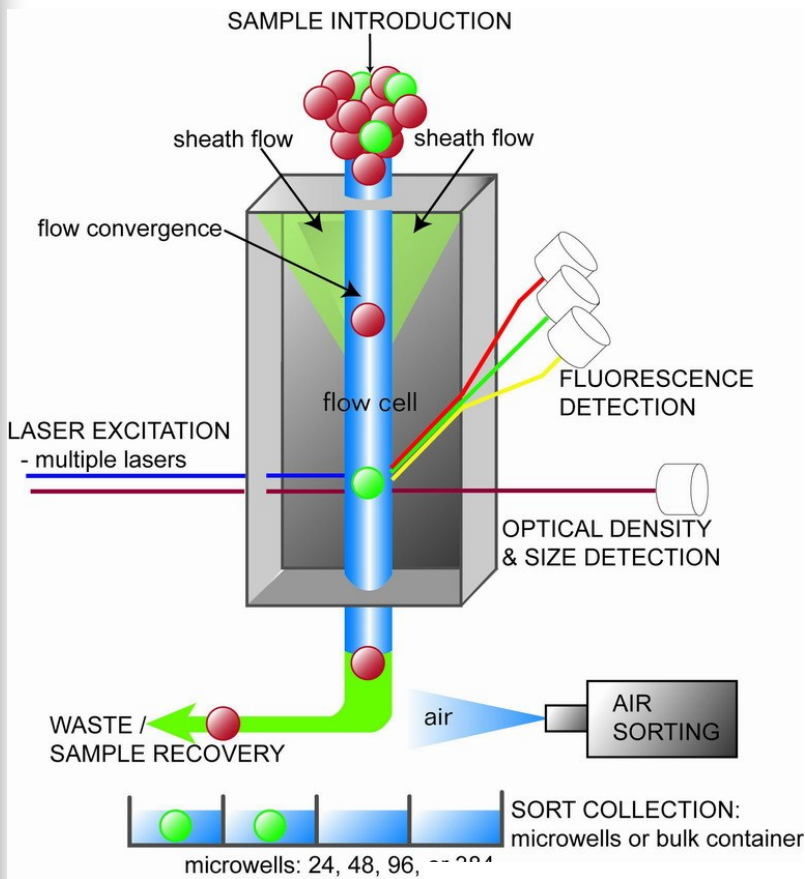


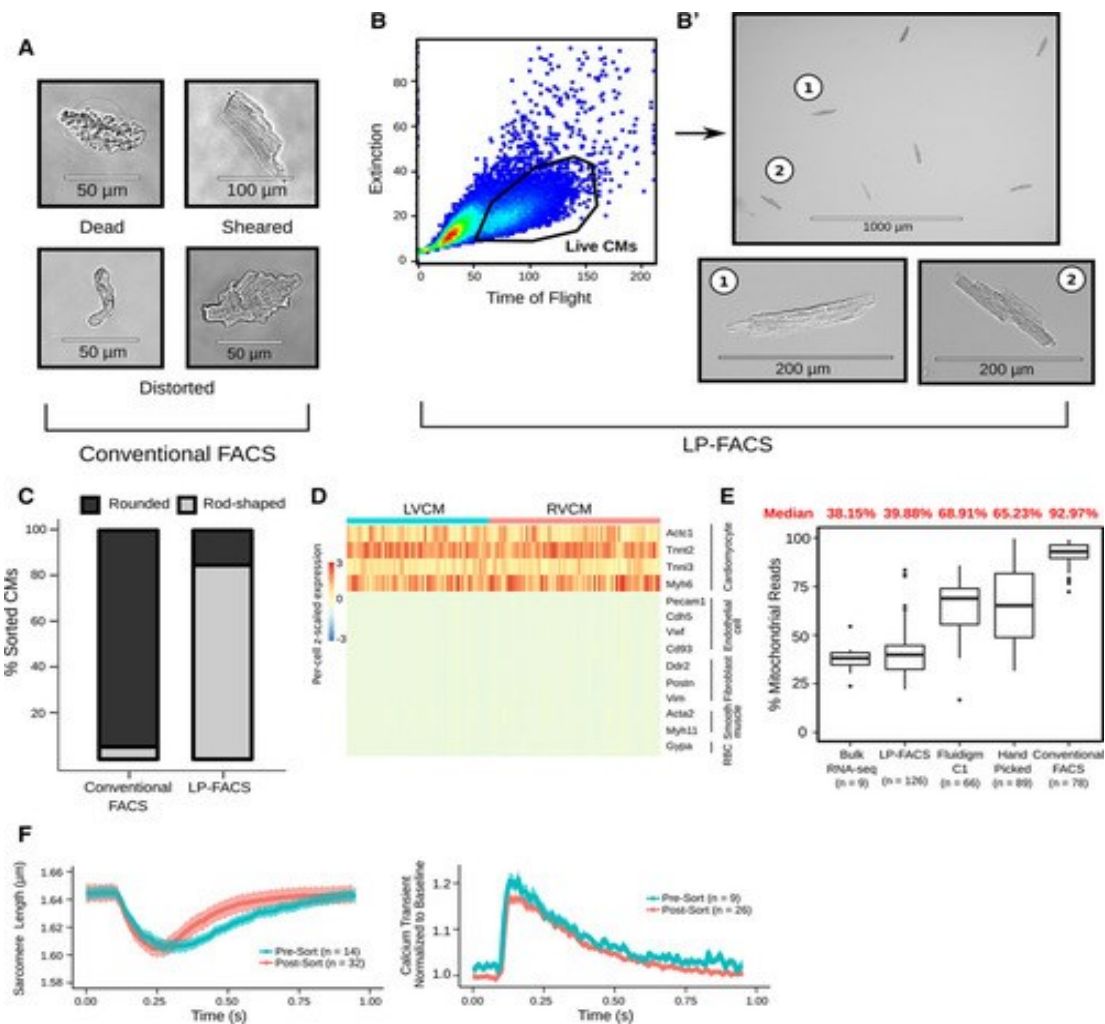
Rebello MdF, Figueiredo EdS, Mariante RM, Nóbrega A, et al. (2013) New Insights from the Oyster *Crassostrea rhizophorae* on Bivalve Circulating Hemocytes. PLoS ONE 8(2): e57384. doi:10.1371/journal.pone.0057384
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0057384>

Figure 6. Proposed model for hemocyte maturation, as seen by flow cytometry.



Rebelo MdF, Figueiredo EdS, Mariante RM, Nóbrega A, et al. (2013) New Insights from the Oyster *Crassostrea rhizophorae* on Bivalve Circulating Hemocytes. PLoS ONE 8(2): e57384. doi:10.1371/journal.pone.0057384
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0057384>





Suraj Kannan. Circulation Research. Large Particle Fluorescence-Activated Cell Sorting Enables High-Quality Single-Cell RNA Sequencing and Functional Analysis of Adult Cardiomyocytes, Volume: 125, Issue: 5, Pages: 567-569, DOI: (10.1161/CIRCRESAHA.119.315493)



„High Throughput Flow Cytometry“

- automatizace + robotizace = urychlení a efektivita sběru dat (měření desítky vzorků za hodinu s minimálním zásahem operátora)
- využití principu vícebarevné analýzy

Automatizované systémy měření vzorků



Automatický karusel (autosampler)



Adaptér pro nasávání vzorků z mikrotitrační desky

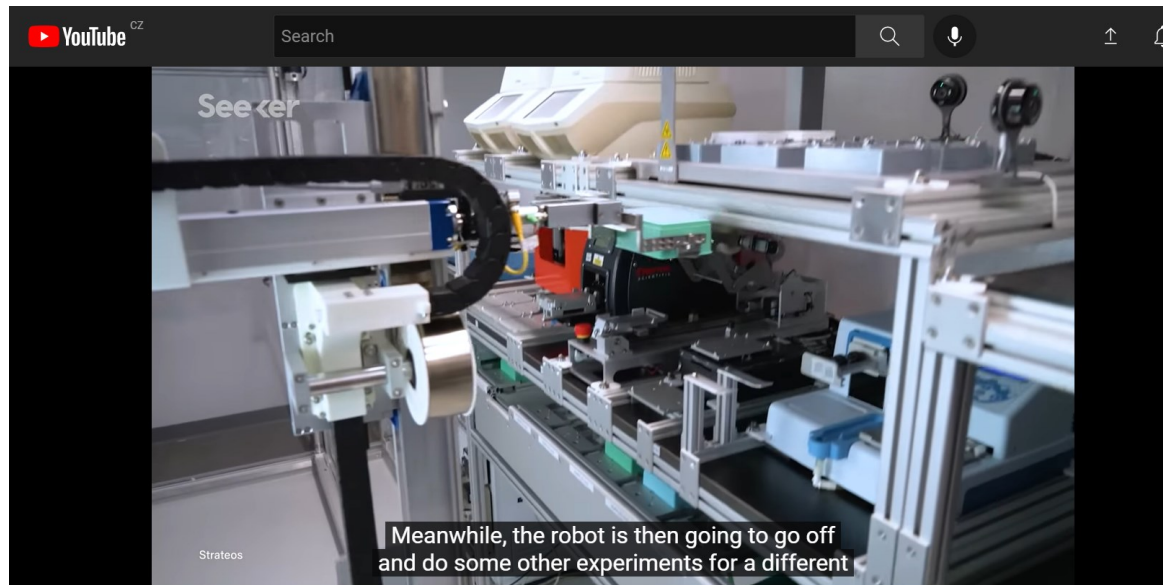


Automatizovaný „microsampler“ systém



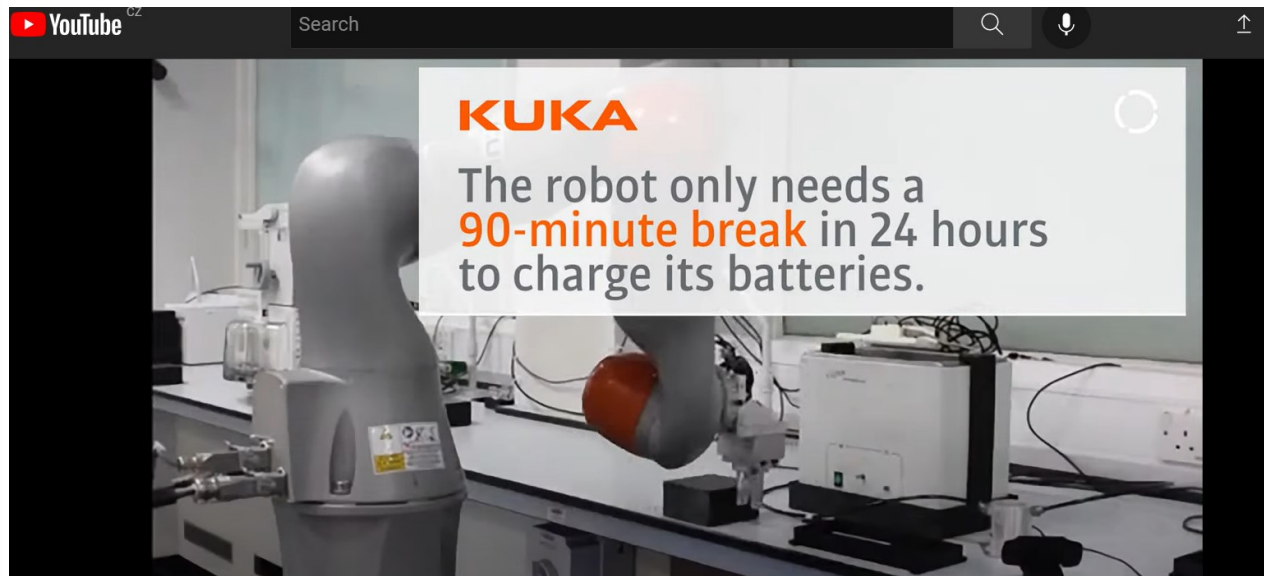
Cyttek FLOW CYTOMETRY PRODUCTS





<https://www.youtube.com/watch?v=L1UgdoP2aeg>

<https://www.youtube.com/watch?v=7MFmbtIb8xA>





BMG PheraSTAR FSX

- measurements from 6 Well to 3456 Well Format
- luminescence, fluorescence, absorption
- measurement of kinetics
- two injectors



Thermo Scientific™ Cytomat™ 2

- incubation of well-plates
- normal and half-deep-well
- up to 95% humidity
- temperatures from +10°C to +50°C



Biometra TRobot

- PCR cycler with automated lid opening system for automation

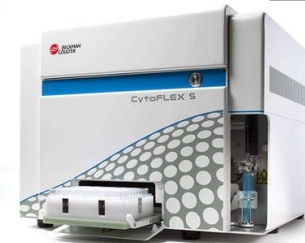


CyBio Felix 96 Channel

- pipetting-robot with 96 channels; pipetting/dispensing simultaneously

CyBio Felix 8 Channel

- pipetting-robot with 8 channels; channels can be addressed separately



Beckman-Coulter CytoFlex S

- fluorescence-based cell-analysis
- automated measurement in 96 well plates



Dispendix I-Dot

- nano-liter dispensing from 2 nL to 80 µL
- pressed-air based dispensing into well-plates
- extremely low dead volume

Labware-Storage

- carousel with mixed configuration
- 1x Rack for 22 MTPs
- 2x Rack for 19 RoboTipTrays
- 3x Rack for 9 Tip boxes

Roboter PreciseFlex 750

- Four-axis laboratory robot with linear rail

<https://www.synbiobeta.com/read/one-lab-in-germany-is-using-robots-to-advance-computer-aided-synthetic-biology>

Incorporating Automation into a Flow Cytometry Workflow for Antibody Discovery

<https://www.youtube.com/watch?v=ERDtmYddNkQ>

Mixing Small Volumes for Continuous High-Throughput Flow Cytometry: Performance of a Mixing Y and Peristaltic Sample Delivery

W. Coyt Jackson,¹ F. Kuckuck,¹ B.S. Edwards,¹ A. Mammoli,² C.M. Gallegos,² G.P. Lopez,³ T. Buranda,¹ and L.A. Sklar^{1*}

¹Department of Pathology and Cancer Research Facility, University of New Mexico Health Sciences Center, Albuquerque, New Mexico

²Department of Mechanical Engineering, University of New Mexico College of Engineering, Albuquerque, New Mexico

³Department of Chemical and Nuclear Engineering, University of New Mexico College of Engineering, Albuquerque, New Mexico

Received 26 July 2001; Revision received 13 December 2001; Accepted 18 December 2001

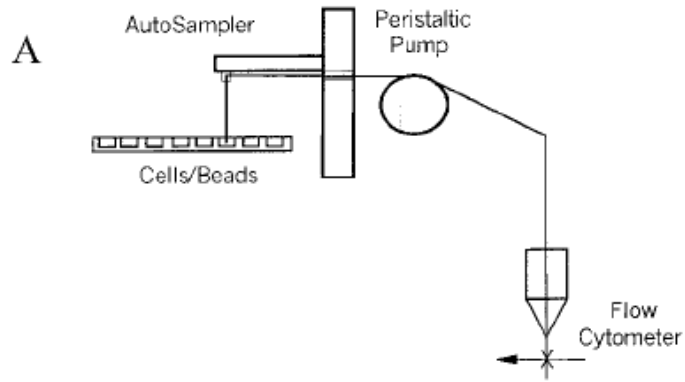
High Throughput Flow Cytometry

Frederick W. Kuckuck,¹ Bruce S. Edwards,^{1,2*} and Larry A. Sklar^{1,2*}

¹Cytometry, Cancer Research and Treatment Center, University of New Mexico Health Sciences Center, Albuquerque, New Mexico

²Department of Pathology, University of New Mexico Health Sciences Center, Albuquerque, New Mexico

Received 18 September 2000; Revision Received 4 January 2001; Accepted 13 January 2001

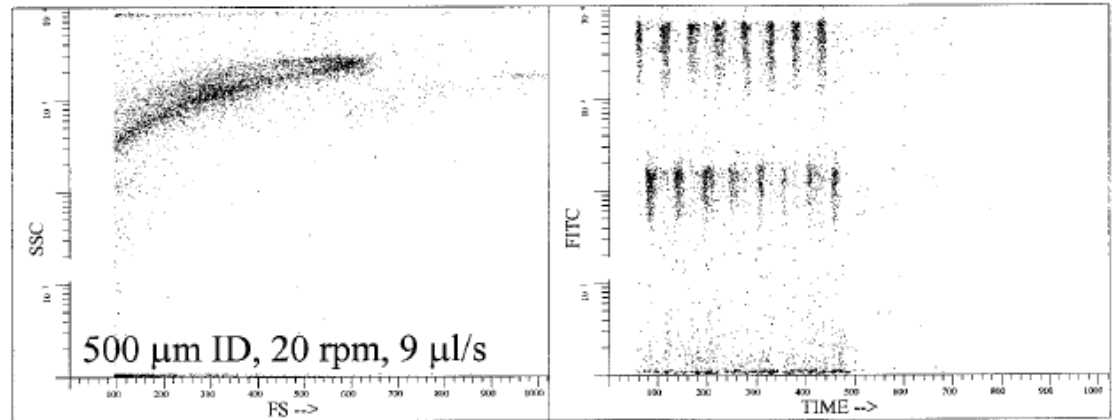


B

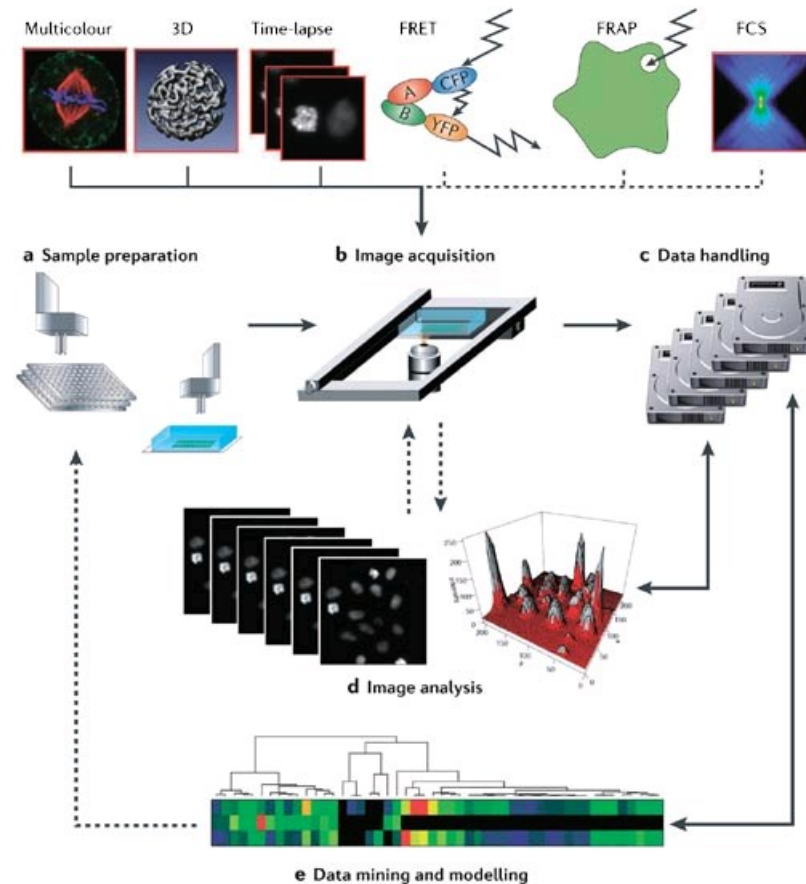


FIG. 1. High throughput flow cytometry. **A:** Schematic view of the flow cytometer, autosampler, and peristaltic pump. **B:** Adjacent samples of latex microspheres separated by air in the 0.02-in (254- μ m) ID tubing between the peristaltic pump and the flow cytometer.

C



The steps in a high-throughput fluorescence-microscopy experiment.



Analysis

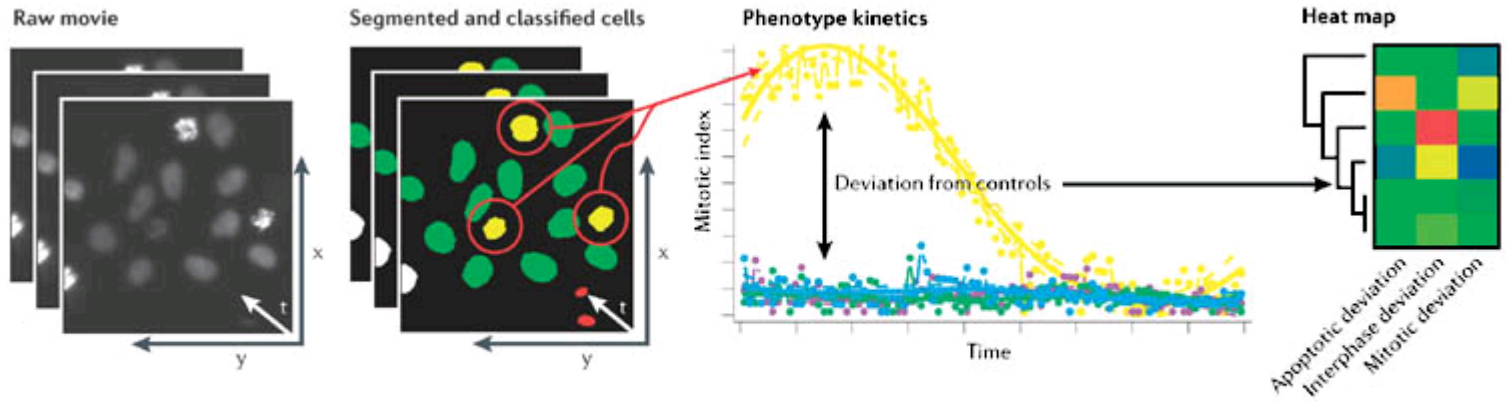


Table 1. Comparison of the Key Attributes of High-Throughput Flow Cytometry and High-Content Microscopy

Key Attributes	HT Flow Cytometry	High Content Microscopy
Cell types	Optimal for suspension cells; adherent cells need to be detached before sampling.	Optimal for adherent cells; suspension cells need to be immobilized before analysis.
Plate requirements	Standard multiwell round-, v-, or flat-bottom plates can be used.	Optically clear plastic or glass bottom plates; uniform flat bottom required.
Bead assays	Optimal technique for performing multiplex bead-based assays	Limited use—beads must be localized to bottom of well.
Label-free measurements	Forward scatter (size) and side scatter (granularity) measurements are standard.	Brightfield microscopy is offered on some instruments.
Cell throughput	Tens of thousands of cells per second	Tens to hundreds of cells per second
Typical 96-well plate read time	<5 min; independent of the number of fluorescent parameters	5–60 min; dependent on the number of fluorescent parameters
Dynamic range	High dynamic range; very faint to very bright signals can be detected in the same sample.	Lower dynamic range
Spatial measurements	No	Yes
Typical data file size	1 to 100 MB per plate	100 to 1,000 MB per plate

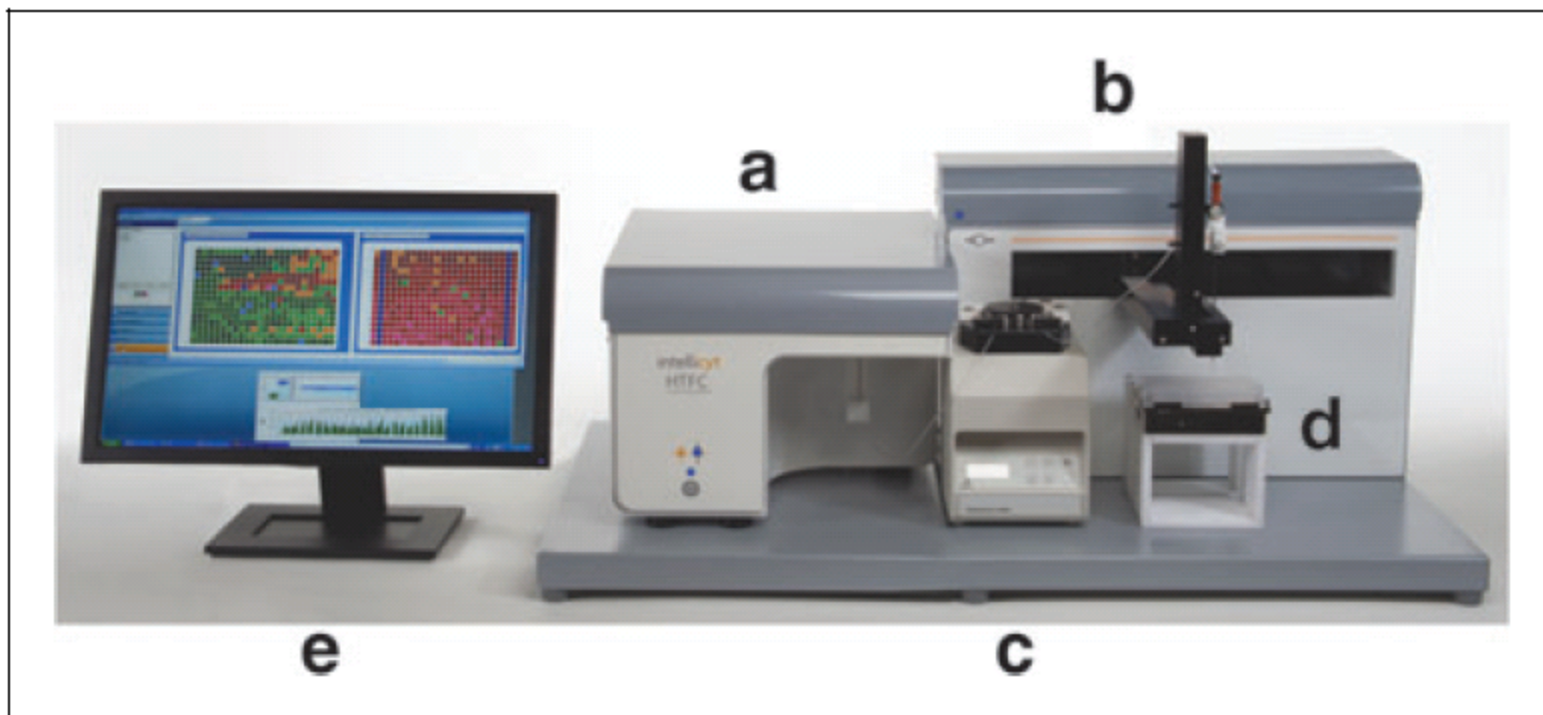


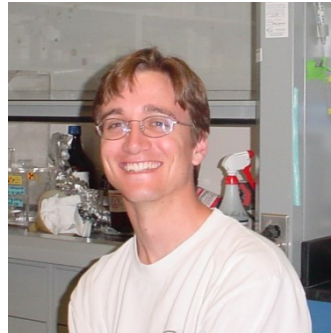
Fig. 1. The HTFC Screening System (IntelliCyt Corporation). **(a)** 2-laser, 4-color flow cytometer; **(b)** an x, y, z autosampler; **(c)** a low pulsation peristaltic pump; **(d)** orbital plate shaker that accommodates 96- and 384-well plates; **(e)** system computer with HyperView installed to set up experiments and process plate data.



Fig. 4. Screenshot from HyperView showing an example of the Well Identification process. Data from the 384-well plate is collected in to a single flow cytometry standard file, which is shown in the main window. The data are deconvolved by the software algorithm to identify each peak with a well address on the plate. One row is expanded to show temporally spaced individual peaks.

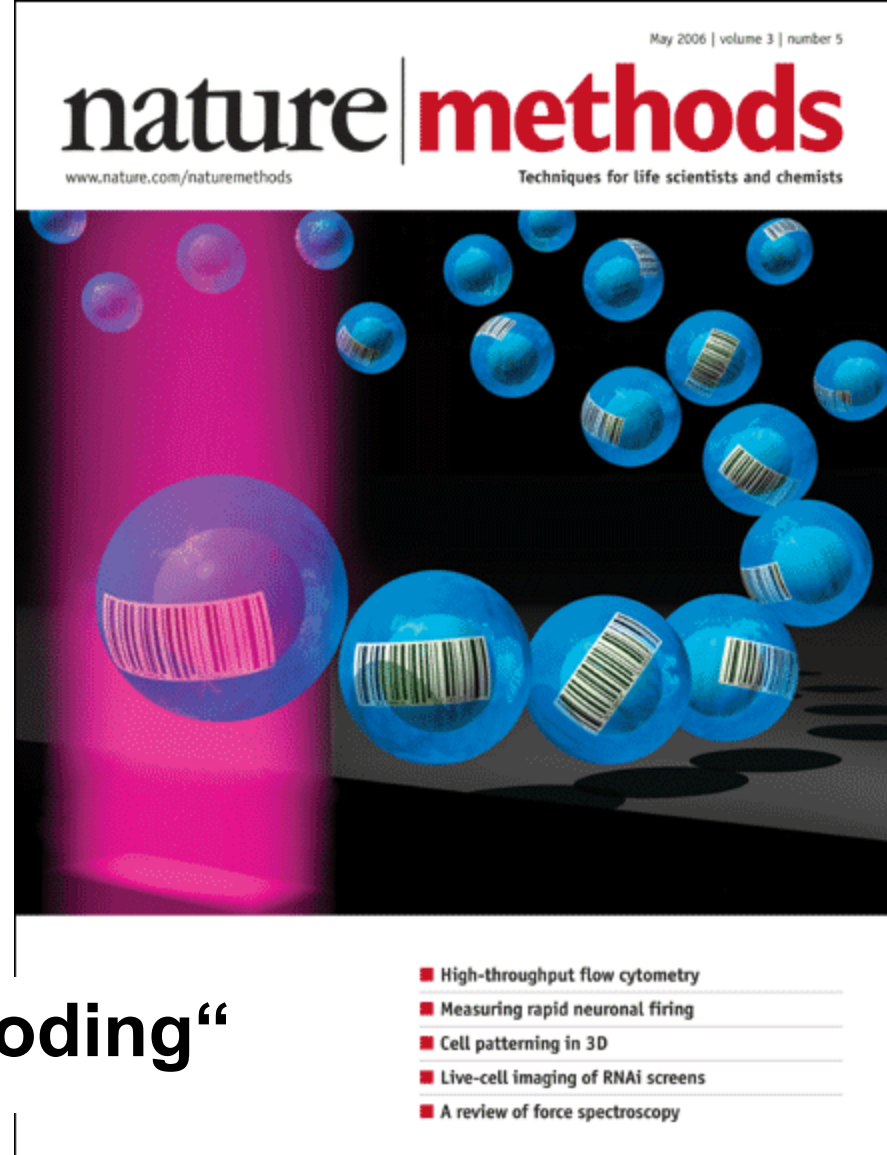


Garry Nolan

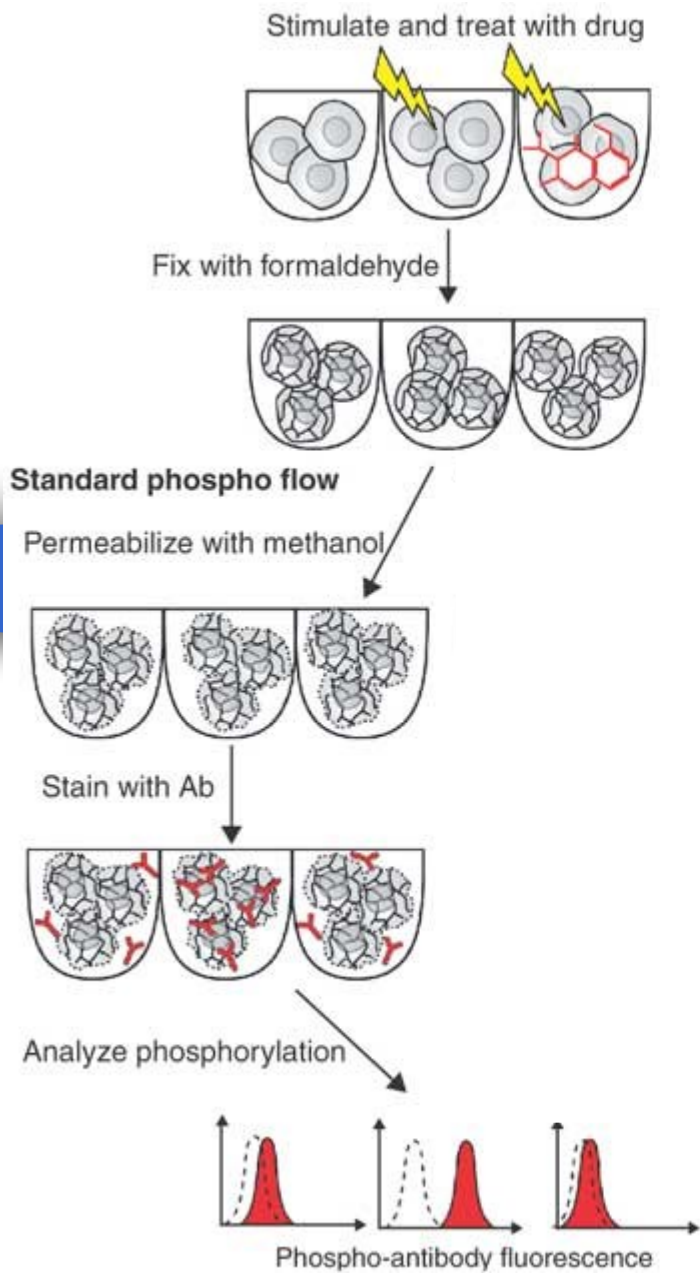


Peter Krutzik

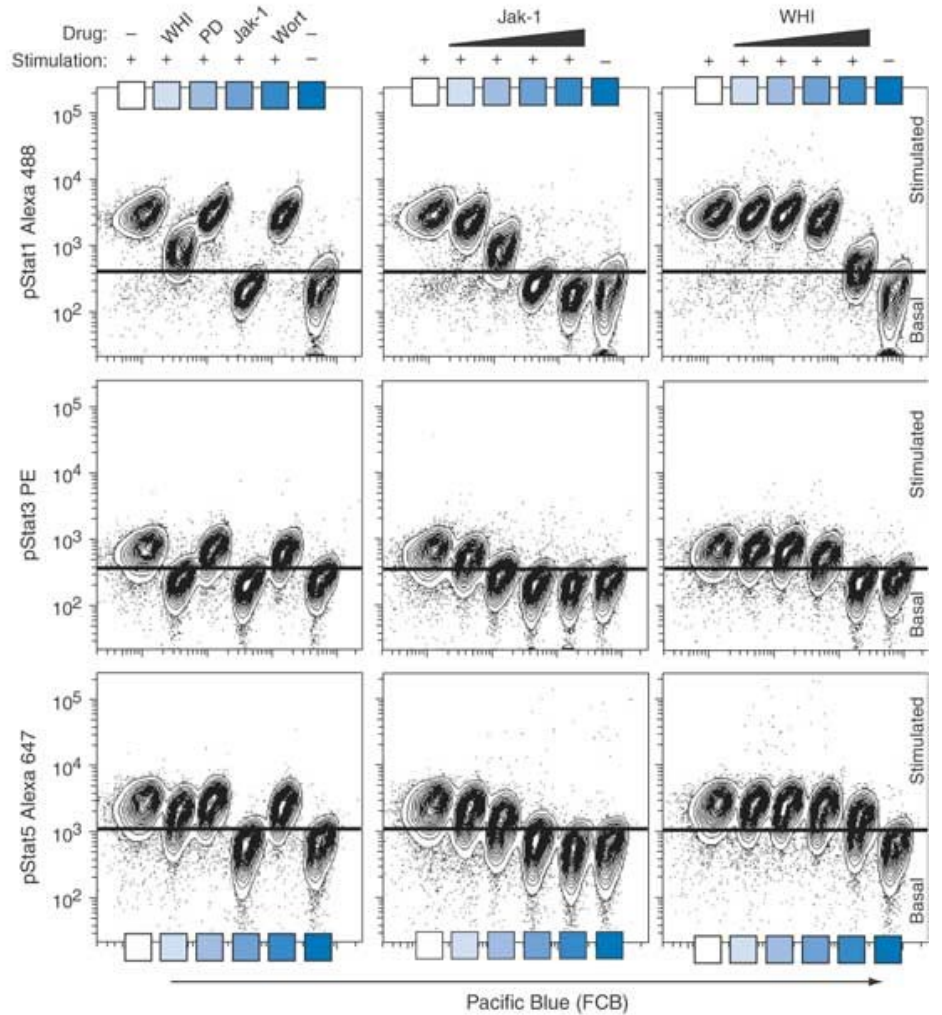
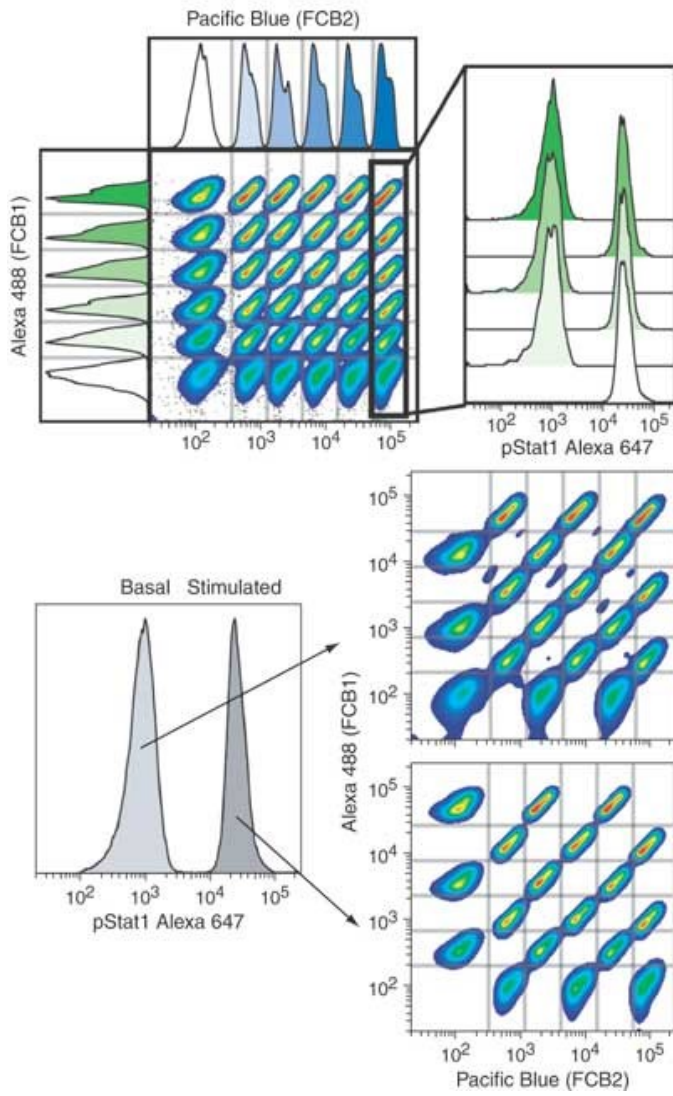
„Fluorescent cell barcoding“



<http://www.stanford.edu/group/nolan/>



[Krutzik PO, Nolan](#) Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling. Nat Methods. 2006 May;3(5):361-8.



[Krutzik PO, Nolan](#) Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling. Nat Methods. 2006 May;3(5):361-8.

Get the best out of your model

FACS-based surface screen:

- validated antibodies in 96w plates
- several commercially available possibilities, we have gone for...

- LEGENDScreen HUMAN

332 PE conjugated antibodies + ISOs

- LEGENDScreen MOUSE

252 PE conjugated antibodies + ISOs

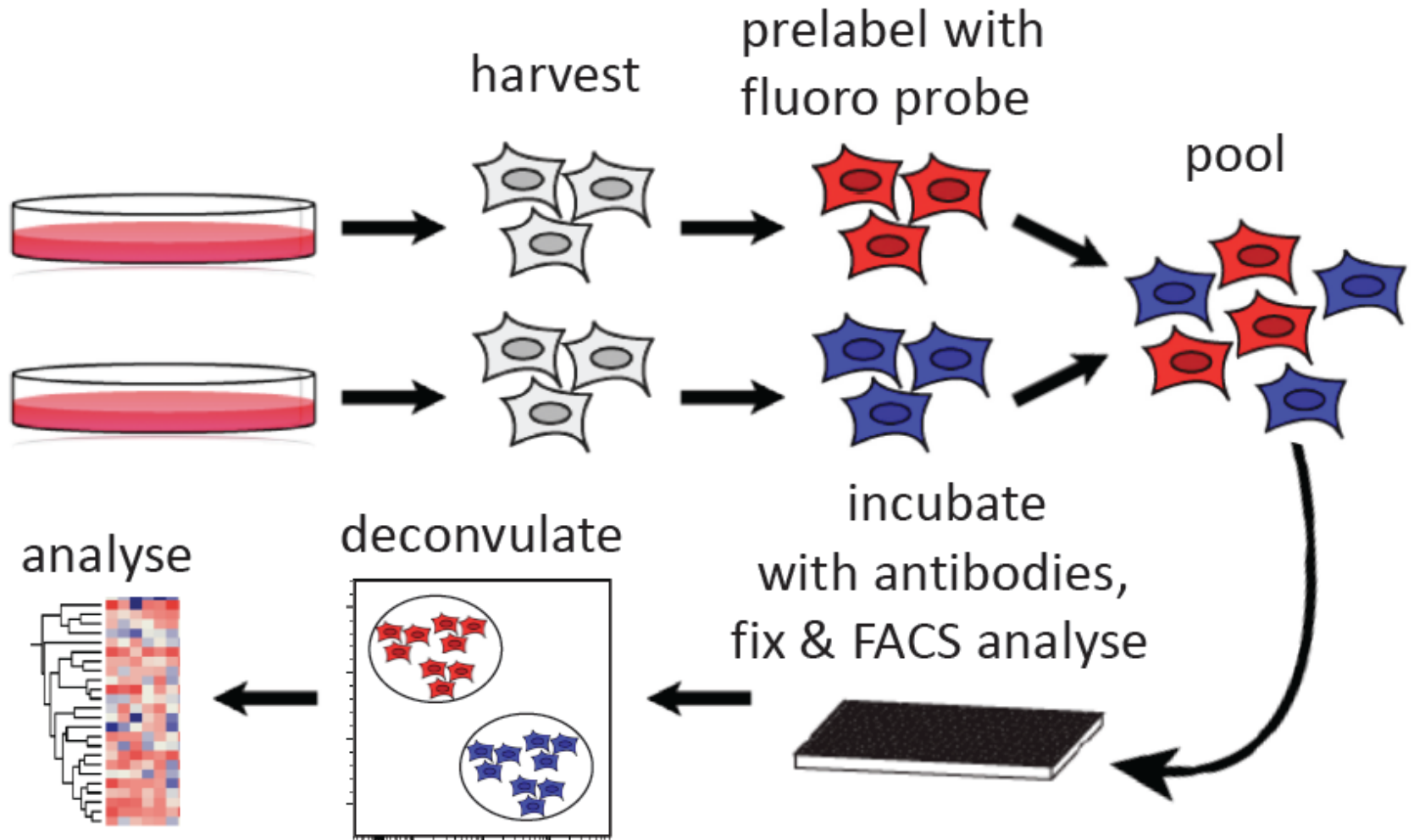
- there are XY vials in LN

- price of kit \approx 1000 € (27k Kc)

How to get the best of it all?



Final workflow



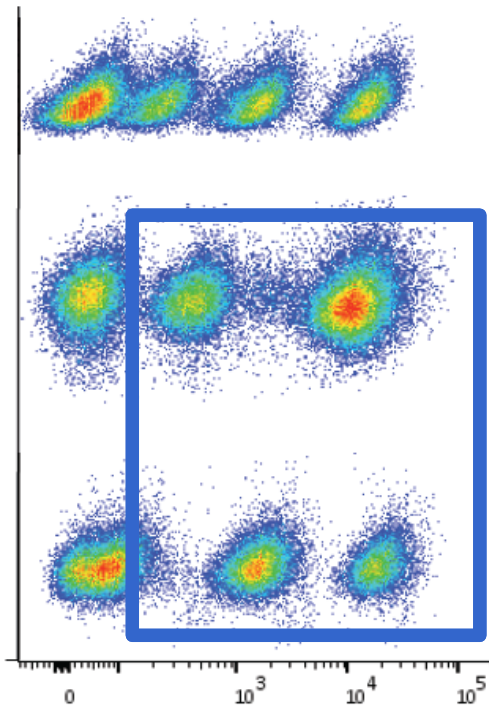
The optimal concentration issue

HOW TO TEST IT:
10x serial dilution

REQUIREMENTS:

- optimal resolution
- compatibility w/ PE

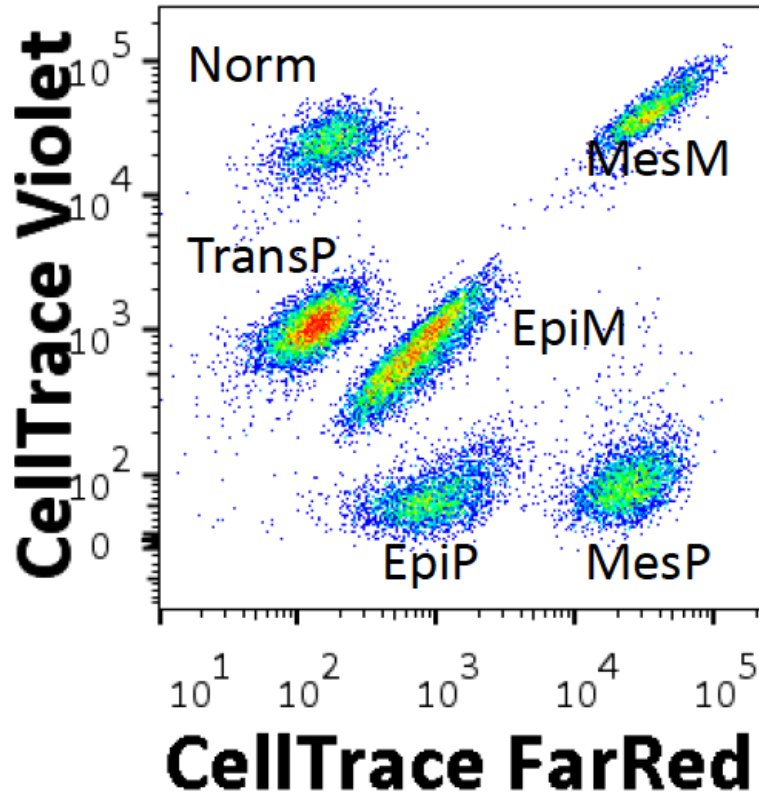
CellTrace CFSE



CellTrace DDAO

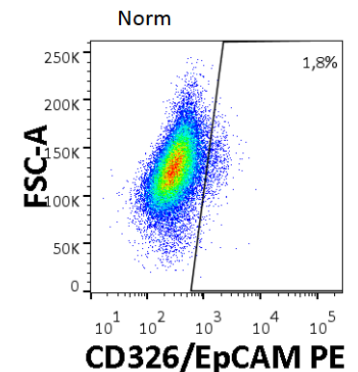
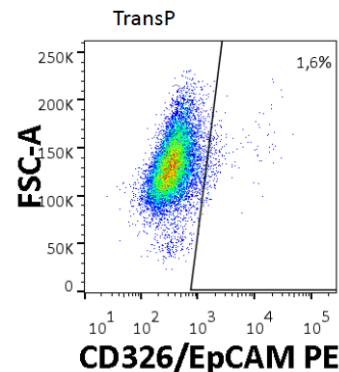
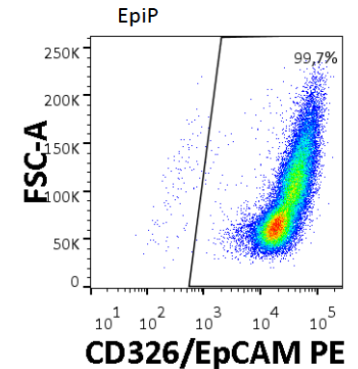
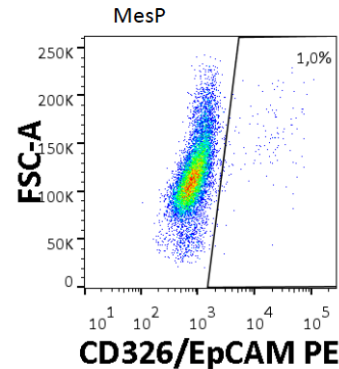
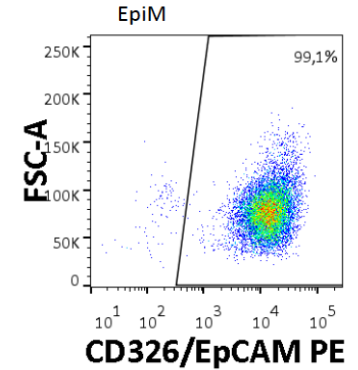
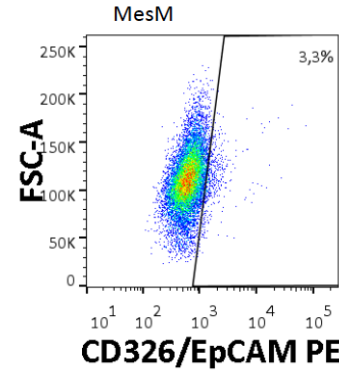
CellTrace Violet

Sample results



EpCAM

- marker of epithelial cells
- commonly lost during EMT



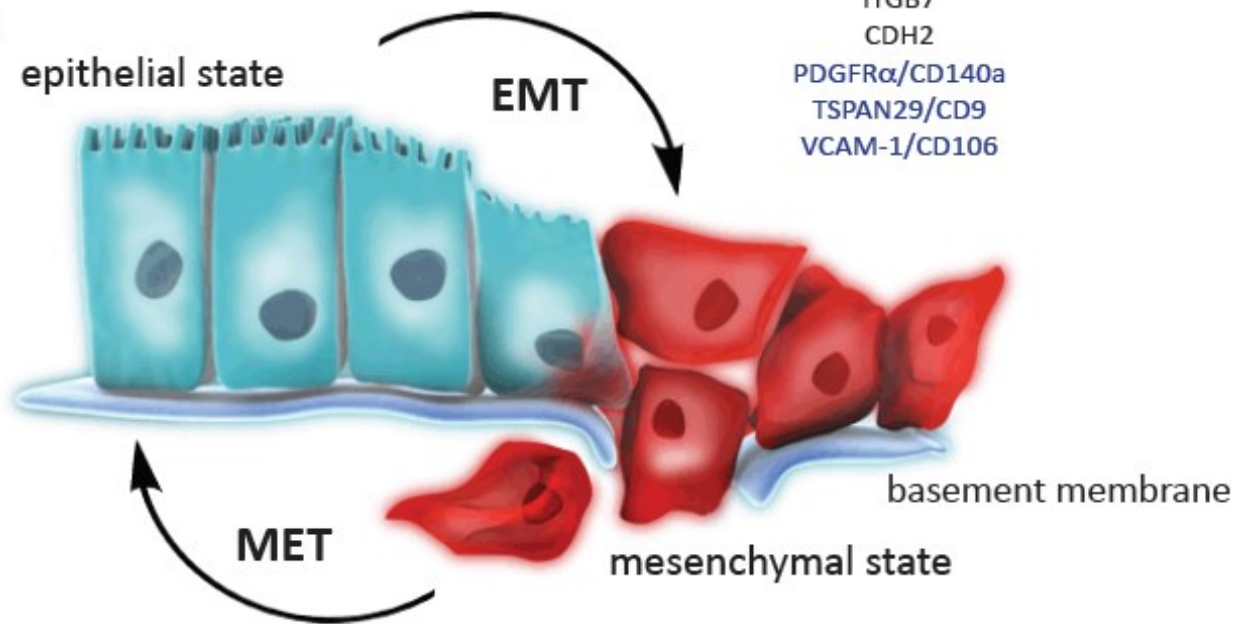
Sample result

non-BCSC
CD24⁺CD44^{low}

5'-nucleotidase/CD73
 basigin/CD147
 BP-3/CD157
 CD38
 CDH1
 CEACAM-1/CD66a
 EpCAM/CD326
 IAP/CD47
 ITGA1/CD49a
 ITGA2/CD49b
 ITGA3/CD49c
 ITGA6/CD49f
 ITGA8
 ITGAV/CD51
 ITGB1/CD29
 ITGB2/CD18
 ITGB3/CD61
 L1CAM
 L-selectin/CD62L
 MCAM/CD146
 TSPAN28/CD30
 TSPAN30/CD63

BCSC
CD24⁻CD44^{high}

ITGA5/CD49e
 ITGA7
 ITGB5
 ITGB7
 CDH2
 PDGFR α /CD140a
 TSPAN29/CD9
 VCAM-1/CD106



dark blue = LEGENDScreen
 black = microarray GSE13259 or qRT-PCR



Chapter 9

High-Throughput, Parallel Flow Cytometry Screening of Hundreds of Cell Surface Antigens Using Fluorescent Barcoding

Stanislav Drápela, Radek Fedr, Ondřej Vacek, Ján Remšík, and Karel Souček

Abstract

Multicolor flow cytometry allows for analysis of tens of cellular parameters in millions of cells at a single-cell resolution within minutes. The lack of technologies that would facilitate feasible and relatively cheap profiling of such a number of cells with an antibody-based approach led us to the development of a high-throughput cytometry-based platform for surface profiling. We coupled the fluorescent cell barcoding with preexisting, commercially available screening tools to analyze cell surface fingerprint at a large scale. This powerful approach will help to identify novel biomarkers and druggable targets and facilitate the discovery of new concepts in immunology, oncology, and developmental biology.

Key words Multicolor flow cytometry, Fluorescent cell barcoding, Cell surface phenotyping, High-throughput screening

Methods in
Molecular Biology 2543

Springer Protocols

Hugo Barcenilla · David Diaz *Editors*

Apoptosis and Cancer

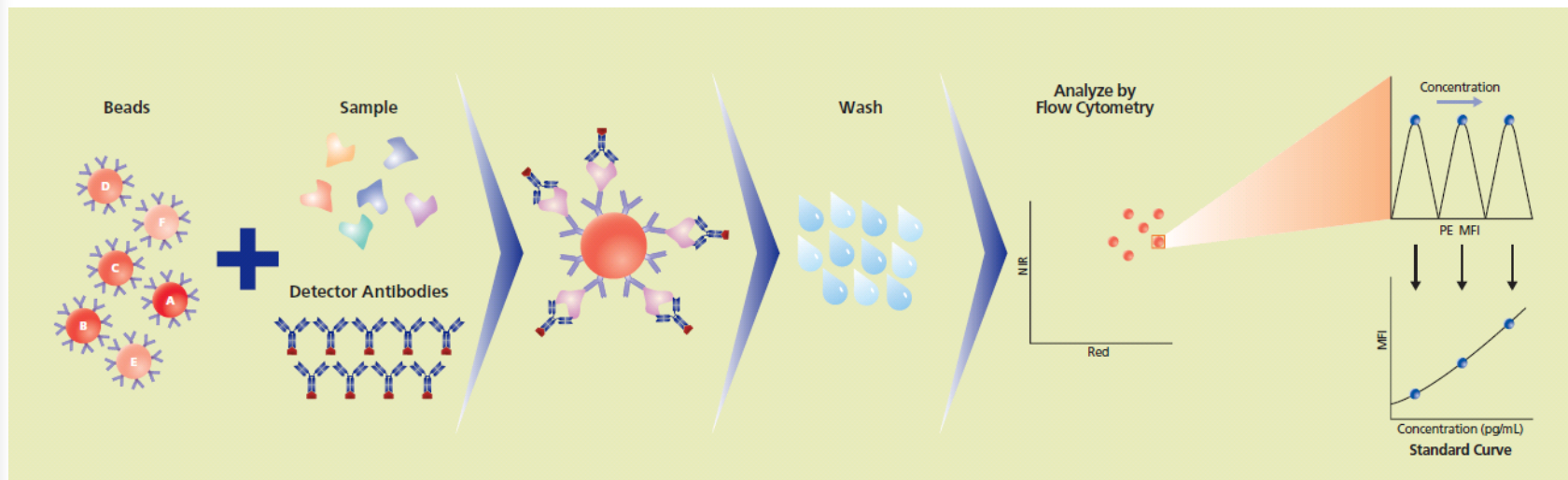
Methods and Protocols



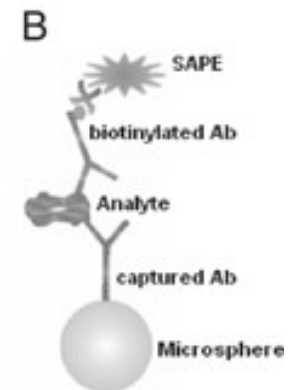
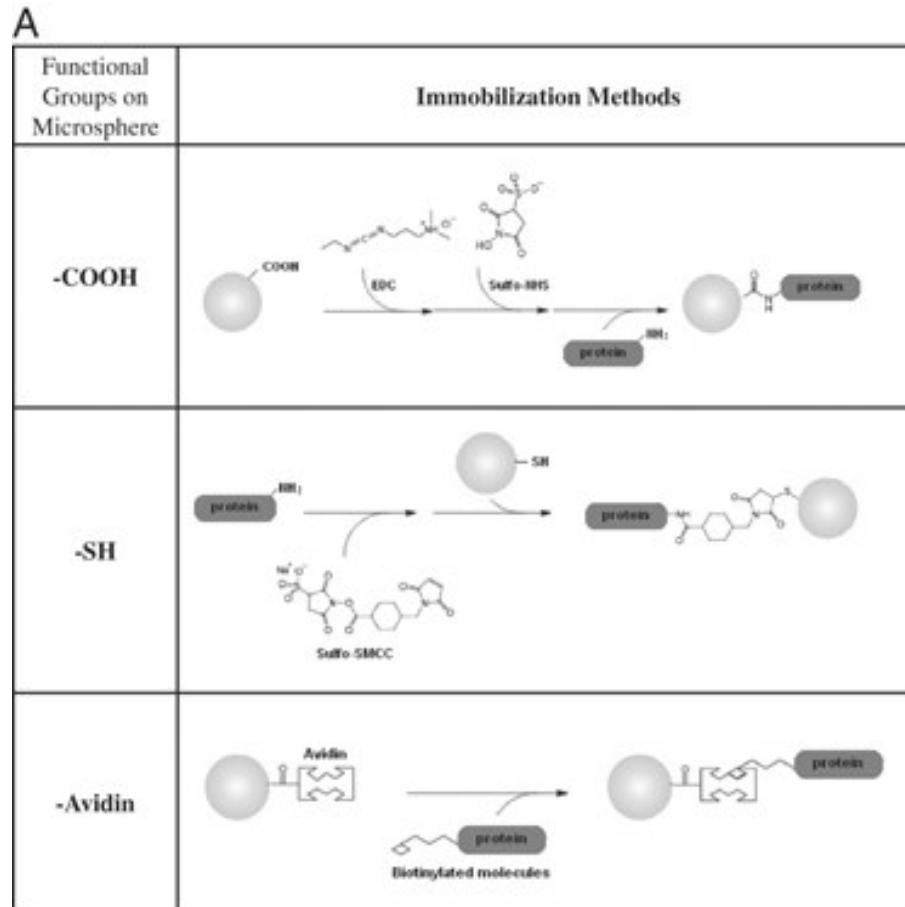
 Humana Press

Cytometric bead array (CBA)

- Multiplexed Bead-Based Immunoassays
- flow cytometry application that allows users to quantify multiple proteins simultaneously



Multiplex microsphere-based flow cytometric platforms for protein analysis and their application in clinical proteomics – from assays to results

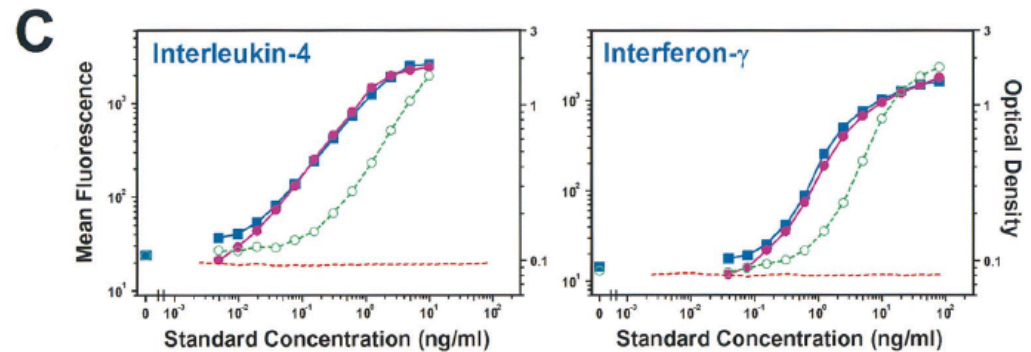
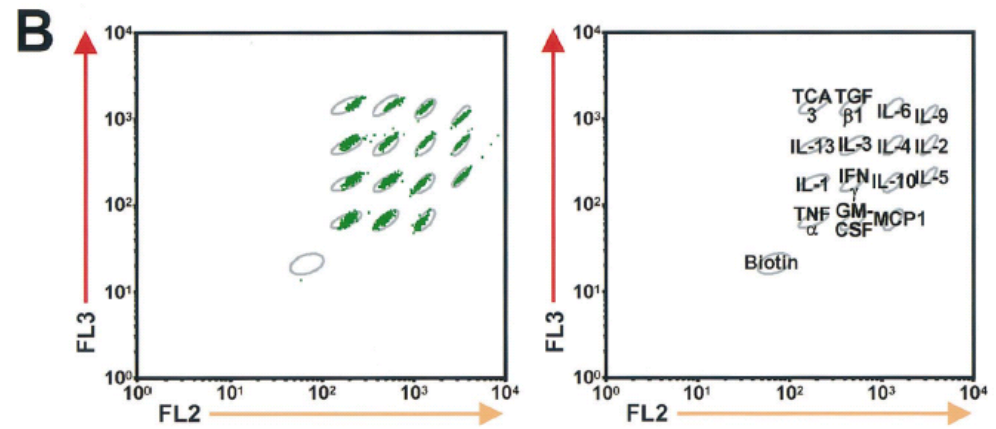
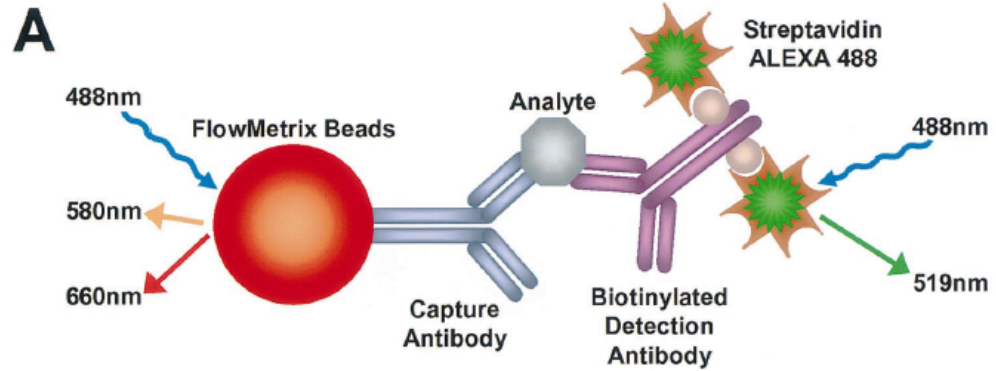


ELECTROPHORESIS

Volume 30, Issue 23, pages 4008-4019, 3 DEC 2009 DOI: 10.1002/elps.200900211

<http://onlinelibrary.wiley.com/doi/10.1002/elps.200900211/full#fig1>

CBA





CBA

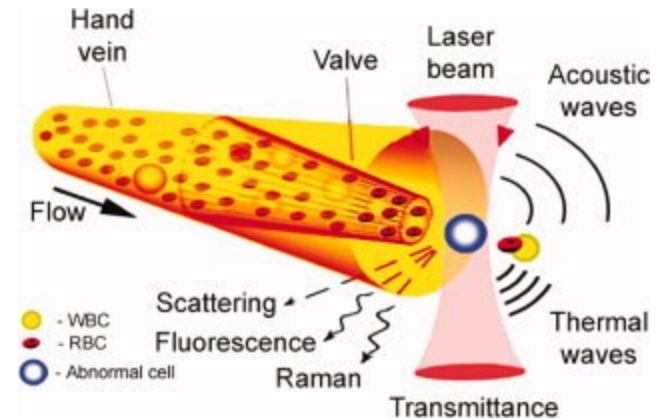
- multiplexing capabilities
- speed
- incorporation of multiple assay formats
- rapid assay development and reasonable cost
- automation



ex vivo flow cytometrie - limitace

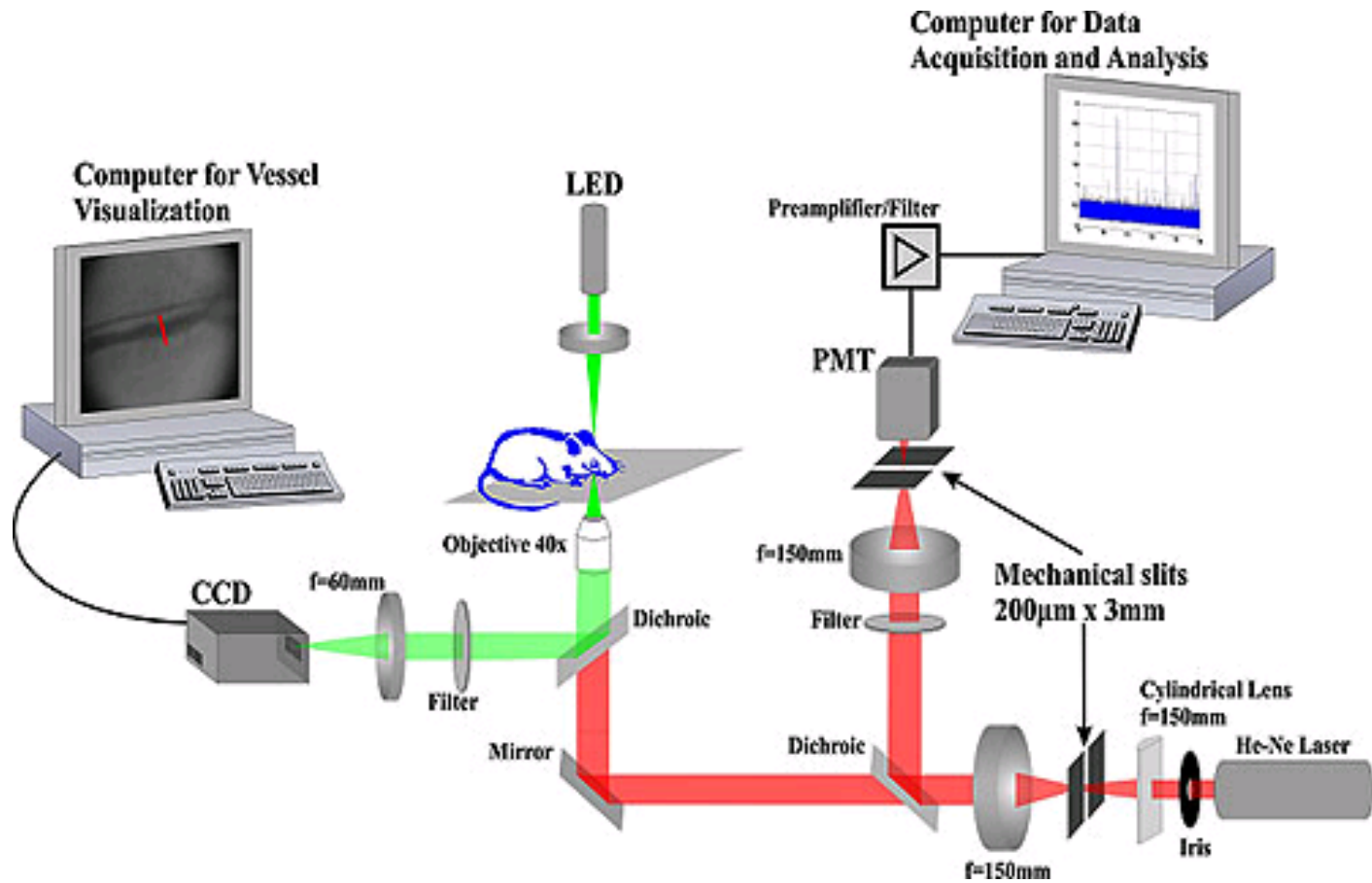
- Ovlivnění některých vlastností buněk (morfologie, exprese znaků);
- neumožňuje dlouhodobější studie buněčného metabolismu a buněčných interakcí (komunikace, adheze) v přirozeném tkáňovém mikroprostředí;
- další:
 - nízká citlivost pro detekci vzácných buněčných subpopulací (1-10 buněk/ml ~ 5000 – 50000 buněk v 5 litrech krve dospělého člověka);
 - časově náročná příprava vzorku (hodiny, dny);
 - diskontinuita odebíraných vzorků.

in vivo flow cytometry – základní principy



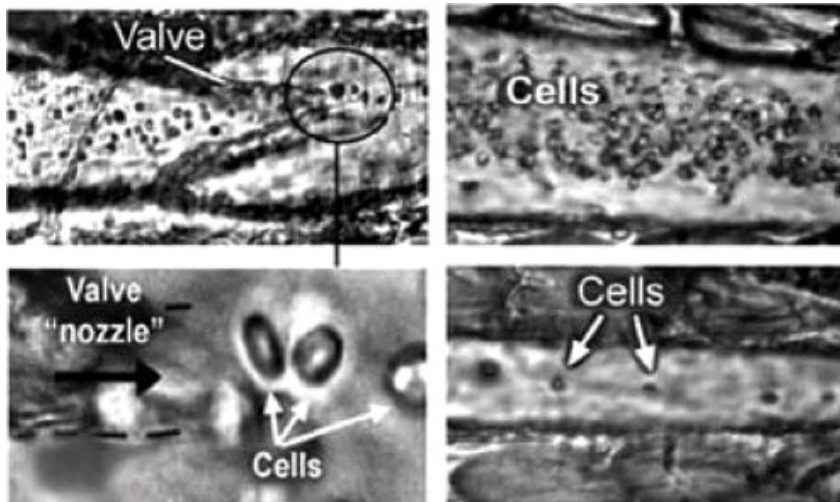
- Zobrazení buněk přímo v krevním nebo lymfatickém řečišti.
- Vizualizace pomocí CCD nebo CMOS kamery po ozáření konvenční mikroskopickou lampou nebo lasery.
- Detekce absorpce, fluorescence, Ramanova spektra, fototermálních nebo fotoakustických signálů.

in vivo flow cytometry



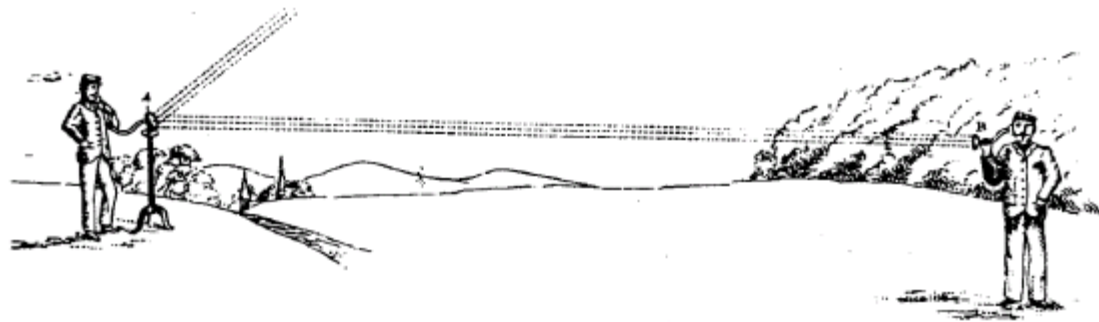
in vivo flow cytometry – bez značení

- Nahrávka videa pomocí vysokorychlostní CCD nebo CMOS kamery s vysokým rozlišením v režimu propustnosti nebo odrazu.
- Příklad: high-speed transmittance digital microscopy (TDM)
- Limity: hloubka tkáně.
- TDM může sloužit k navedení zdrojů záření pro další analýzu do určené oblasti.



photoacoustic and photothermal imaging

- The photoacoustic effect was first discovered by Alexander Graham Bell in his search for a means of wireless communication.¹ Bell succeeded in transmitting sound with an invention he called the “photophone,” which carried a vocal signal with a beam of sunlight that was reflected by a vocally modulated mirror. The sound could be recovered with an ordinary telephone receiver connected to a **selenium cell** illuminated by the light. Bell published the results in a presentation to the American Association for the Advancement of Science in **1880**.



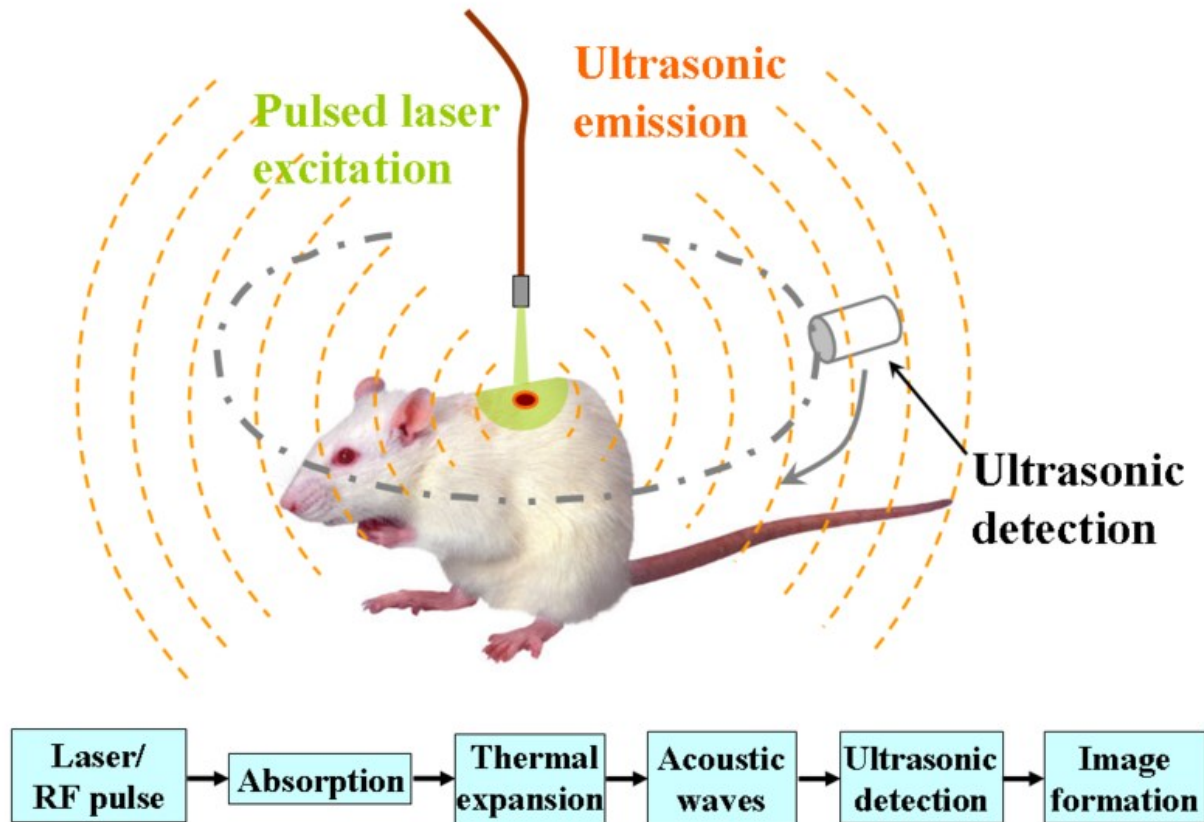
The Photoacoustic Effect

Benjamin T. Spike

Physics 325

April 21, 2006

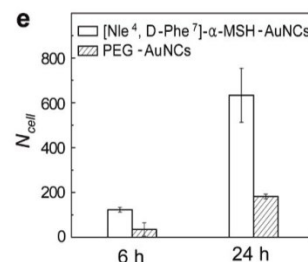
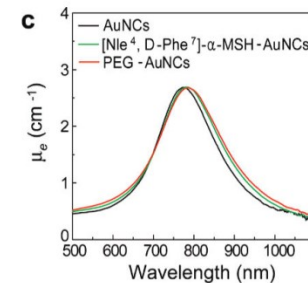
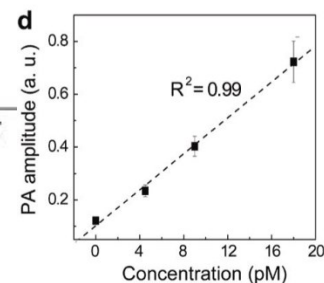
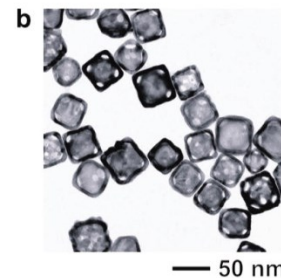
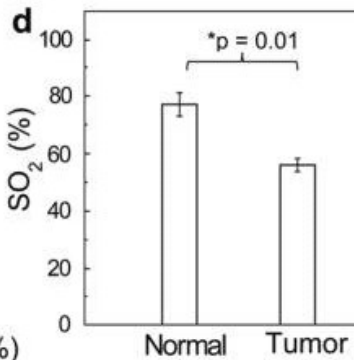
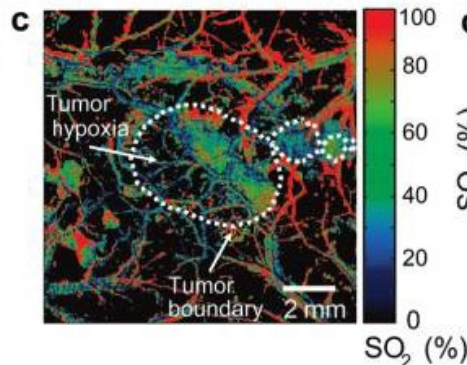
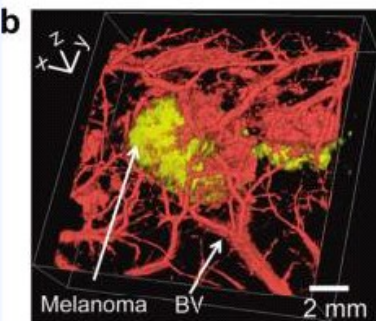
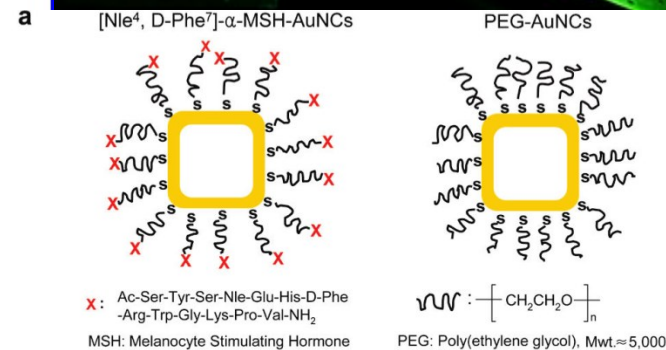
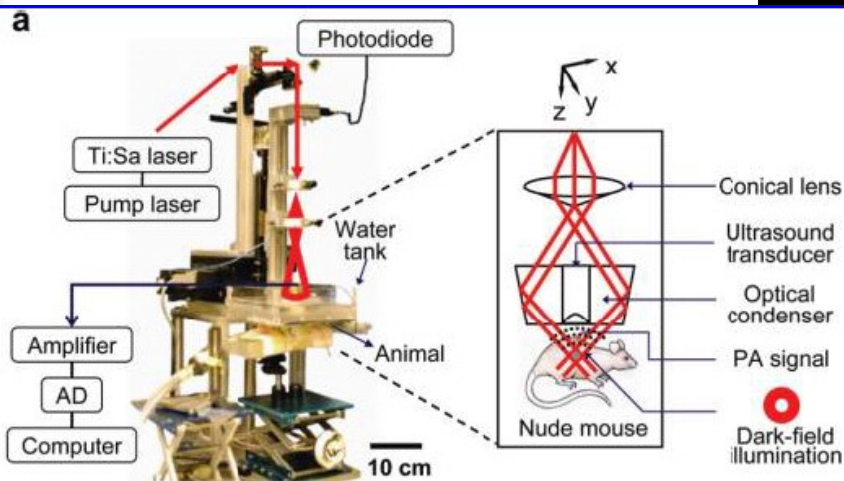
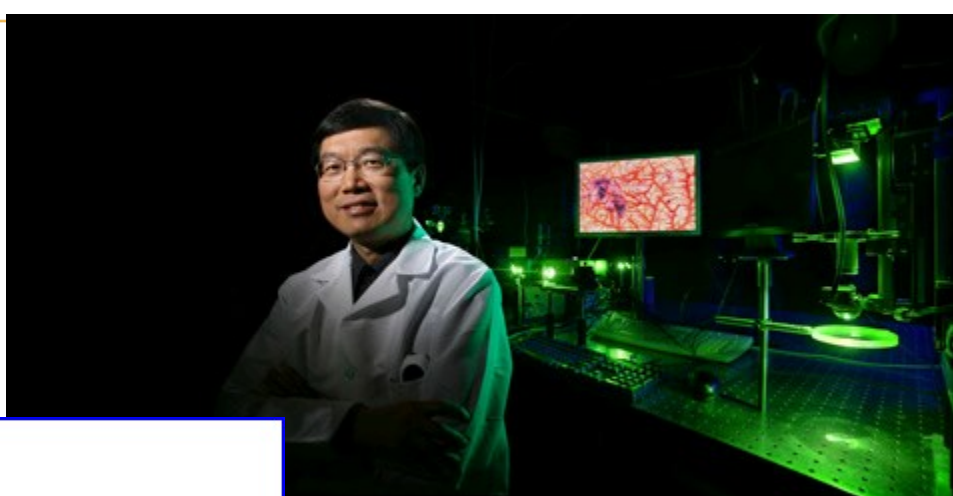
Schematic illustration of photoacoustic imaging



In Vivo Molecular Photoacoustic Tomography of Melanomas Targeted by Bioconjugated Gold Nanocages

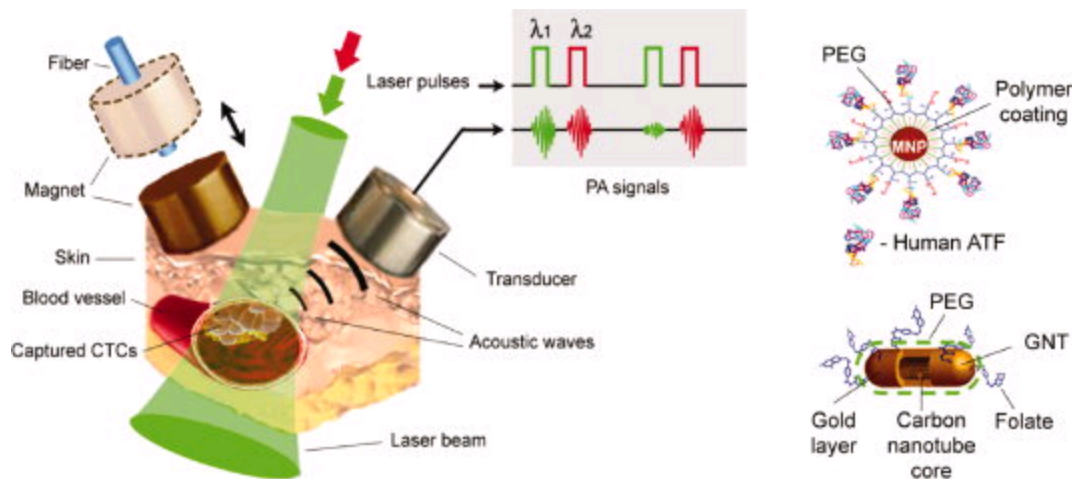
Chulhong Kim,^{1,5} Eun Chul Cho,^{1,5} Jingyi Chen,¹ Kwang Hyun Song,¹ Leslie Au,¹ Christopher Favazza,¹ Qiang Zhang,¹ Claire M. Cobley,¹ Feng Gao,¹ Younan Xia,^{1,*} and Lihong V. Wang^{1,*}

¹Department of Biomedical Engineering, Washington University in St. Louis, Campus box 1097, One Brookings Drive, St. Louis, Missouri 63130 and ²Division of Biostatistics, Washington University School of Medicine, Campus box 8067, 660 South Euclid Avenue, St. Louis, Missouri 63110. *These authors contributed equally to this work.

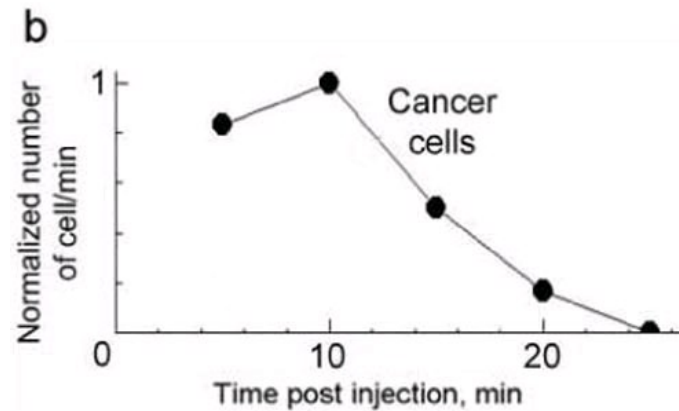
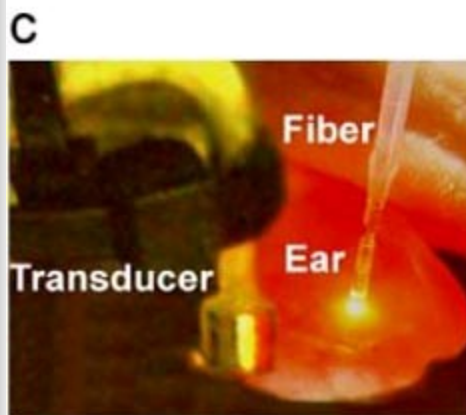
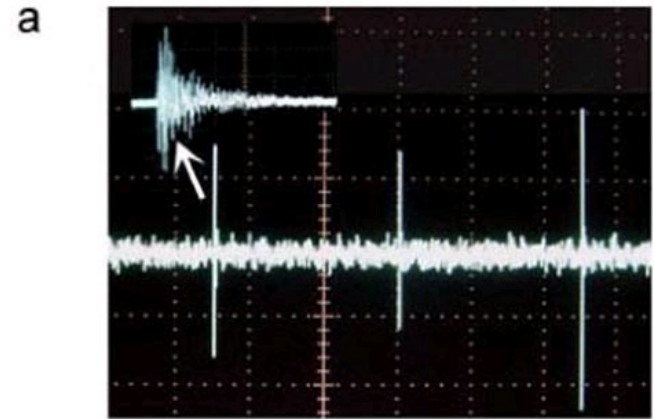
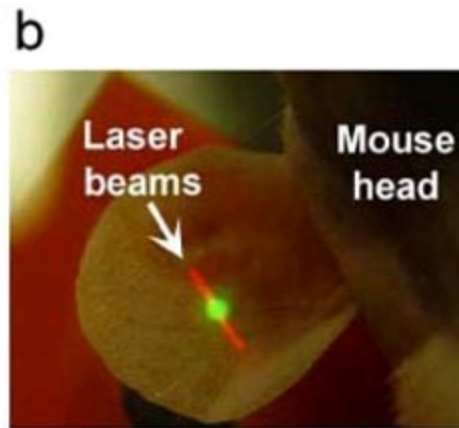
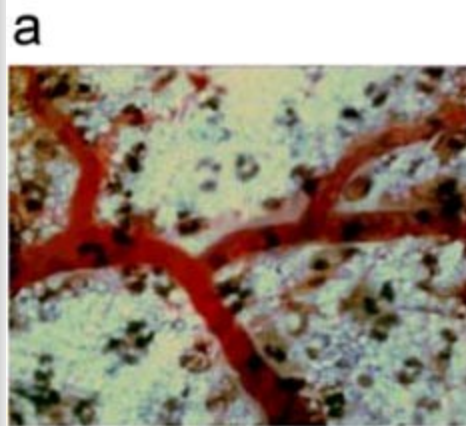


In vivo flow cytometrie – detekce specifických signálů

- Detekce fotoakustických a fototermálních jevů



in vivo flow cytometry - aplikace



Shrnutí přednášky

- Příklady funkčních analýz
- „High-throughput“ průtoková cytometrie ...
- ... a uplatnění vícebarevné detekce a beads array
- sortování chromozómů
- aplikace v mikrobiologii, hydrobiologii a studiu bezobratlých
- *in vivo* průtoková cytometrie

Na konci dnešní přednášky byste měli:

1. vědět jakým způsobem je možné analyzovat buněčný cyklus.
2. umět navrhnout další parametr kombinovatelný s DNA analýzou.
3. znát příklady buněčných funkcí které je možné analyzovat na průtokovém cytometru.
4. vědět co jsou to fluorescenční proteiny a jaké jsou výhody jejich využití v buněčné biologii.
5. vědět co je to „high-throughput“, průtoká cytometrie ... a jak se v ní může uplatnit princip vícebarevného značení.
6. znát základní principy měření a sortování chromozómů pomocí průtokového cytometru;
7. mít představu o možných aplikacích průtokové cytometrie v mikrobiologii, hydrobiologii a studiu bezobratlých;
8. rozumět limitům a principům *in vivo* průtokové cytometrie.