

# Bi9393 Analytická cytometrie

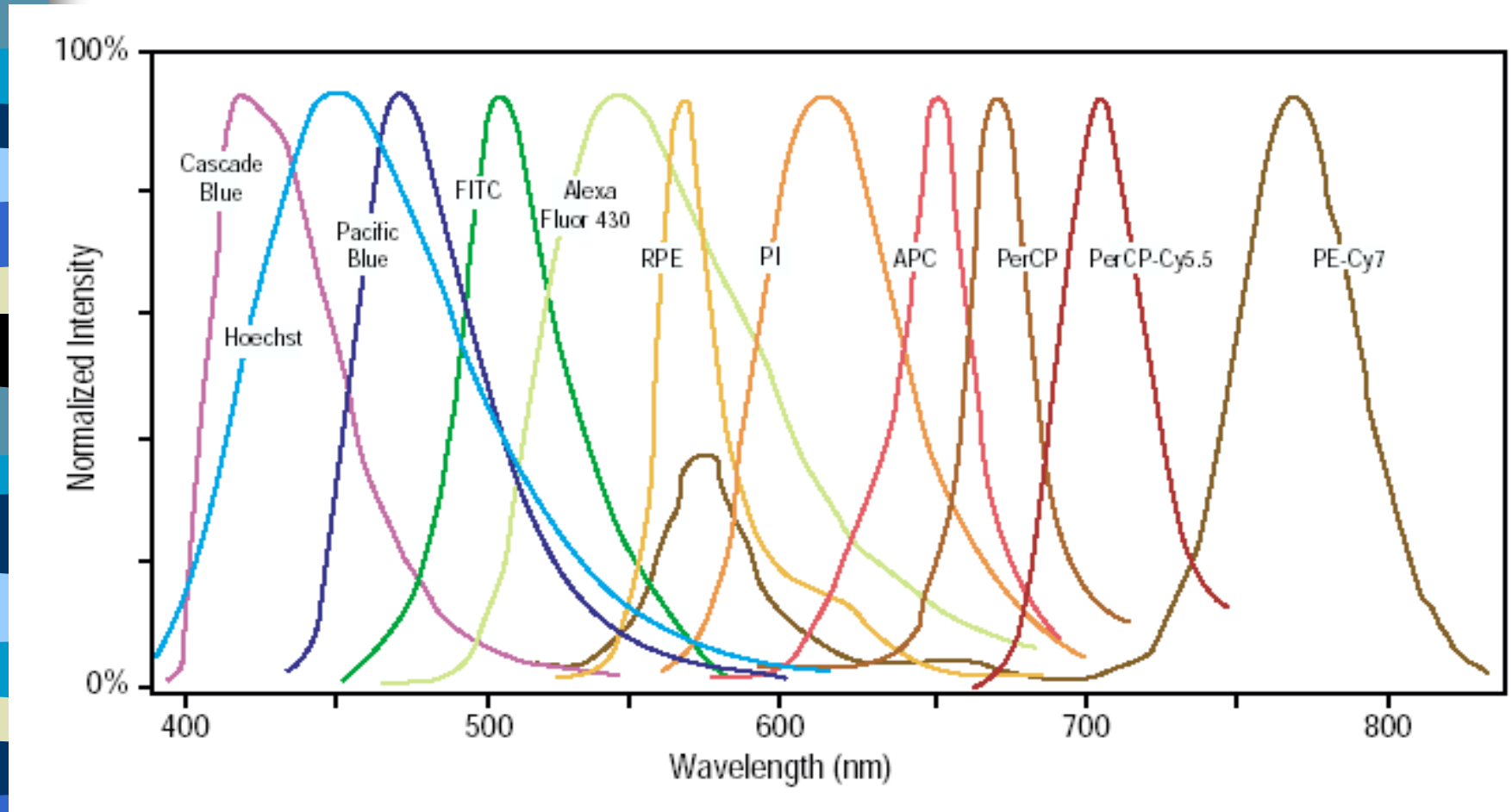


**Karel Souček, Ph.D.**

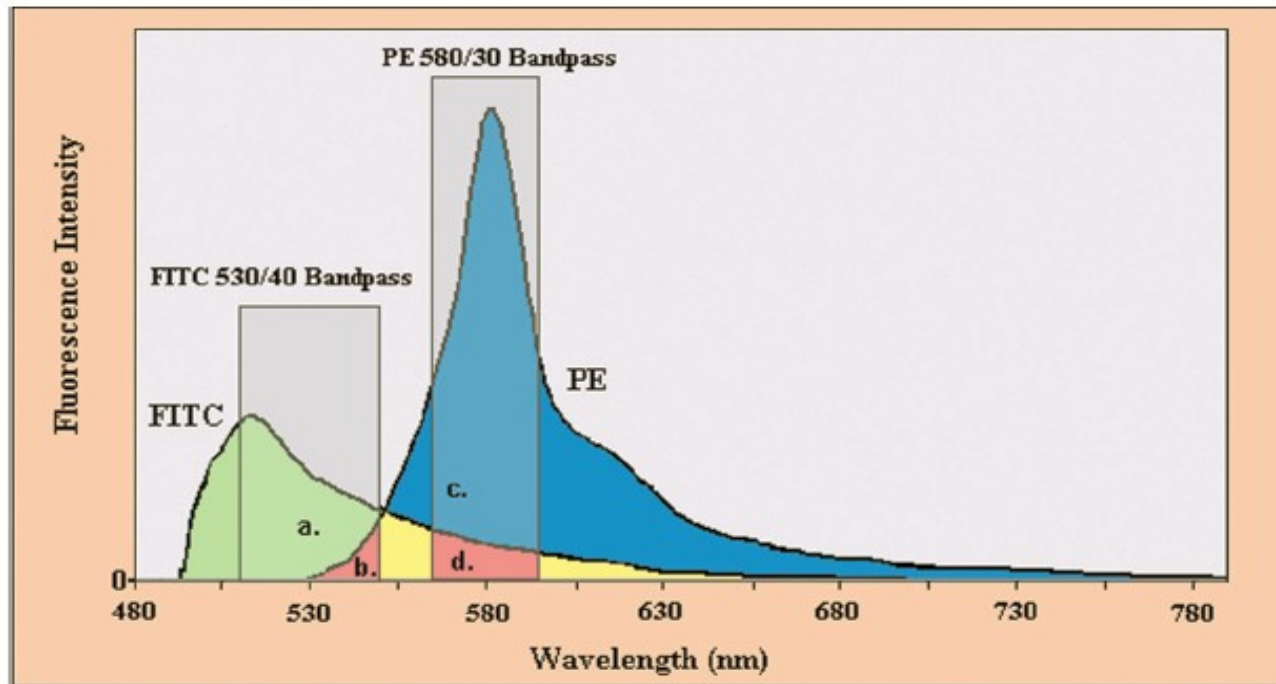
Oddělení cytokinety  
Biofyzikální ústav AVČR, v.v.i.  
Královopolská 135  
612 65 Brno

**e-mail: [ksoucek@ibp.cz](mailto:ksoucek@ibp.cz)**  
tel.: 541 517 166

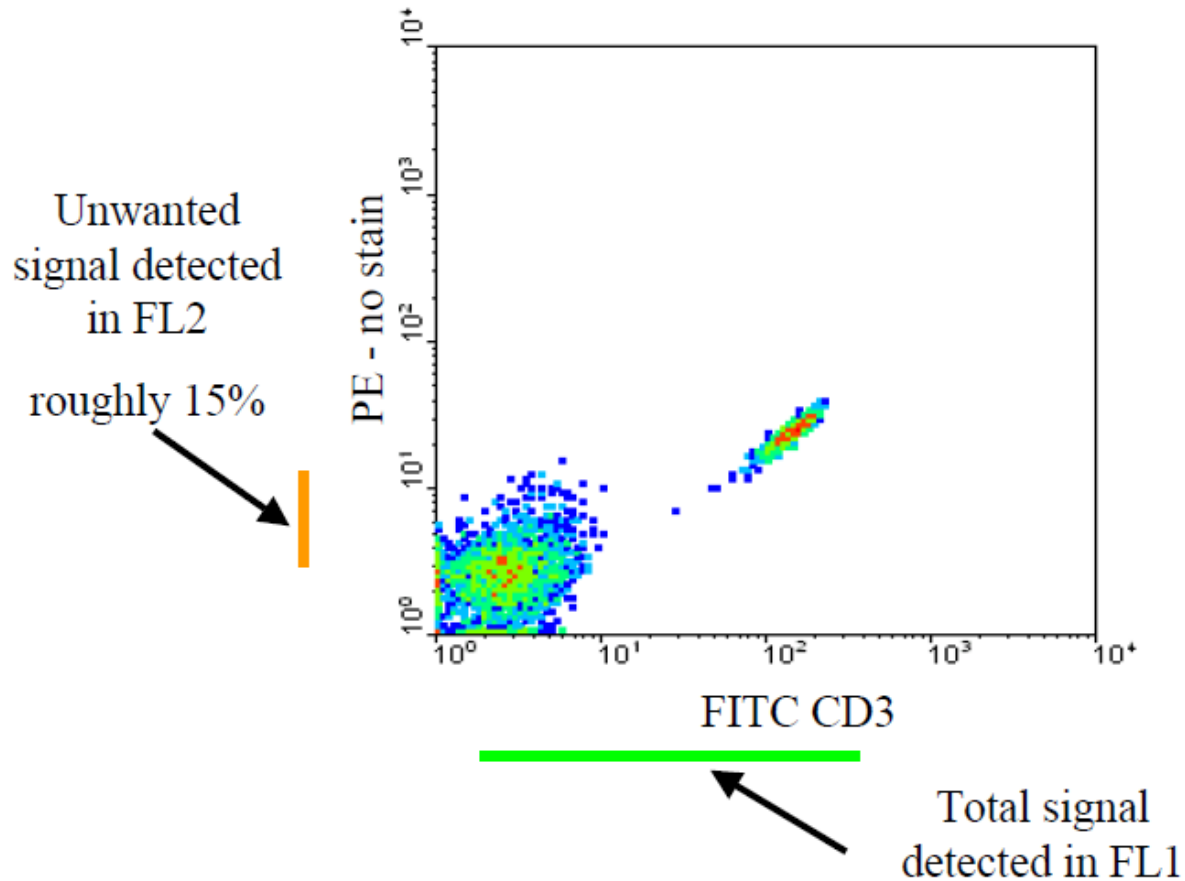
# Emission Spectra–Spectral Overlap



# Co je problém při vícebarevné detekci?

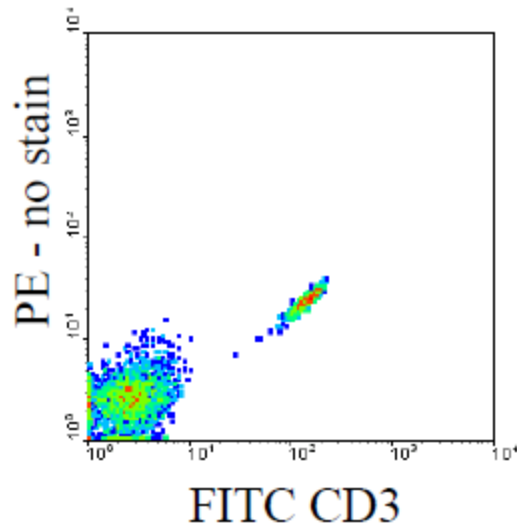


# Uncompensated FITC Single stain Control



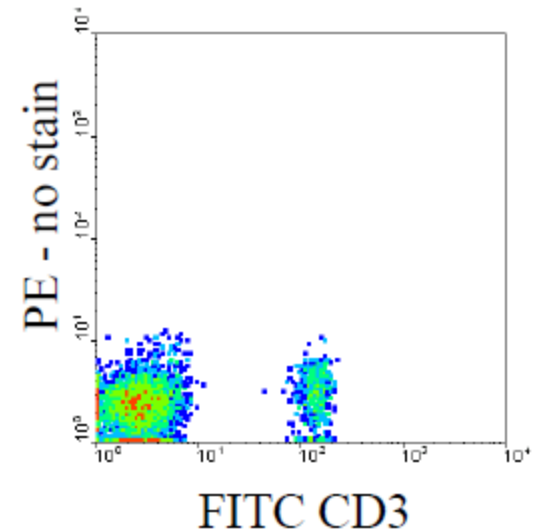
# FITC Single Stain Control

Uncompensated

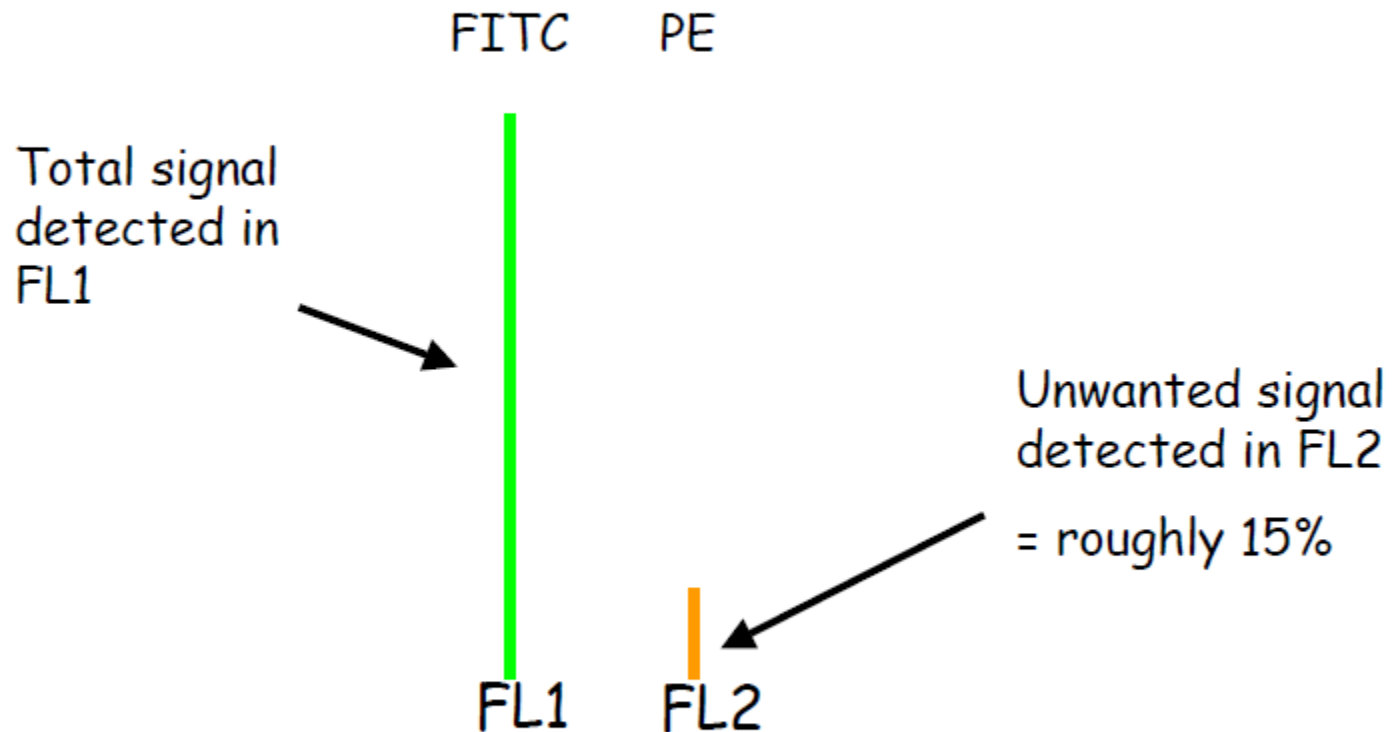


FL2-15%FL1

Compensated



# FITC Single Stain Control



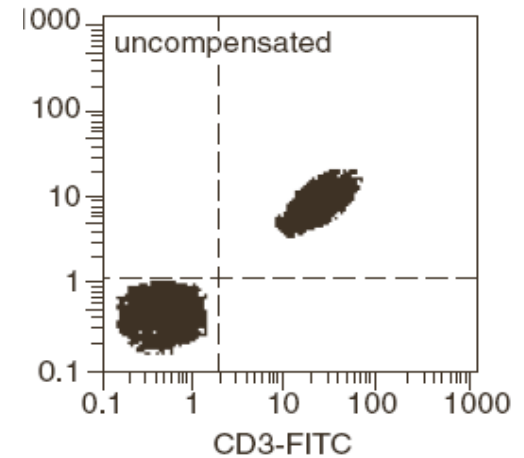
$$\text{True PE} = \text{Total FL2} - 15\% \text{ FL1}$$

# Kompenzace fluorescenčního signálu

#2

FITC positive & negative

PE negative beads



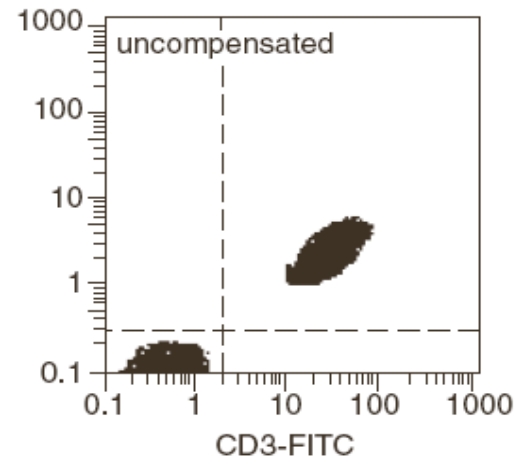
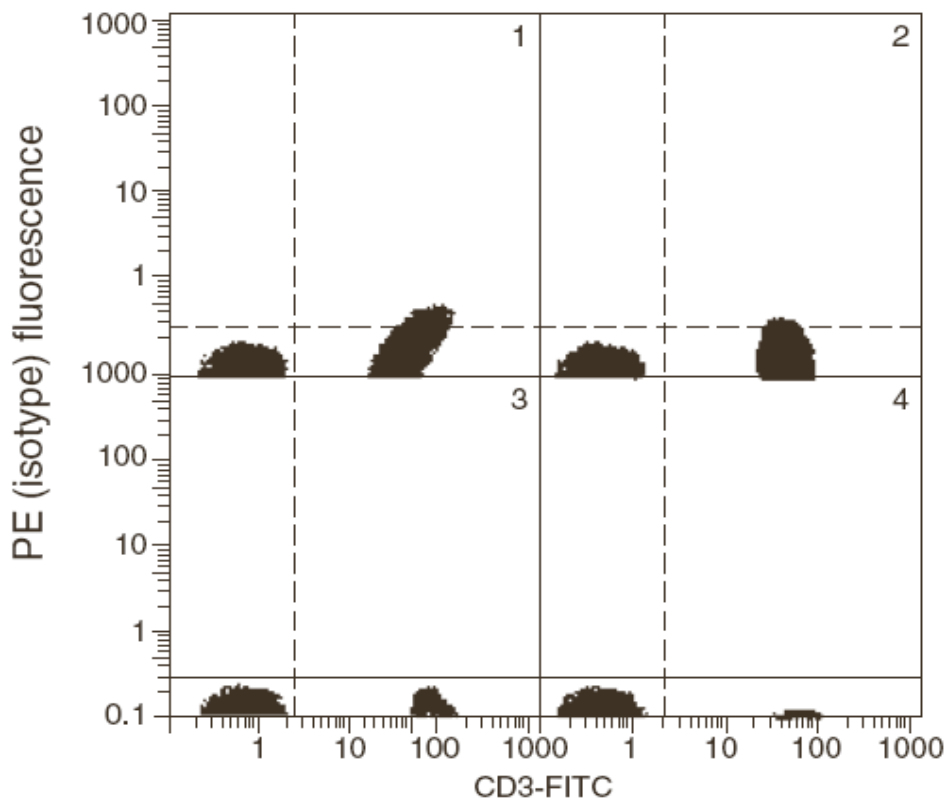
Current Protocols in Cytometry

# Kompenzace fluorescenčního signálu

FITC positive & negative

PE negative beads

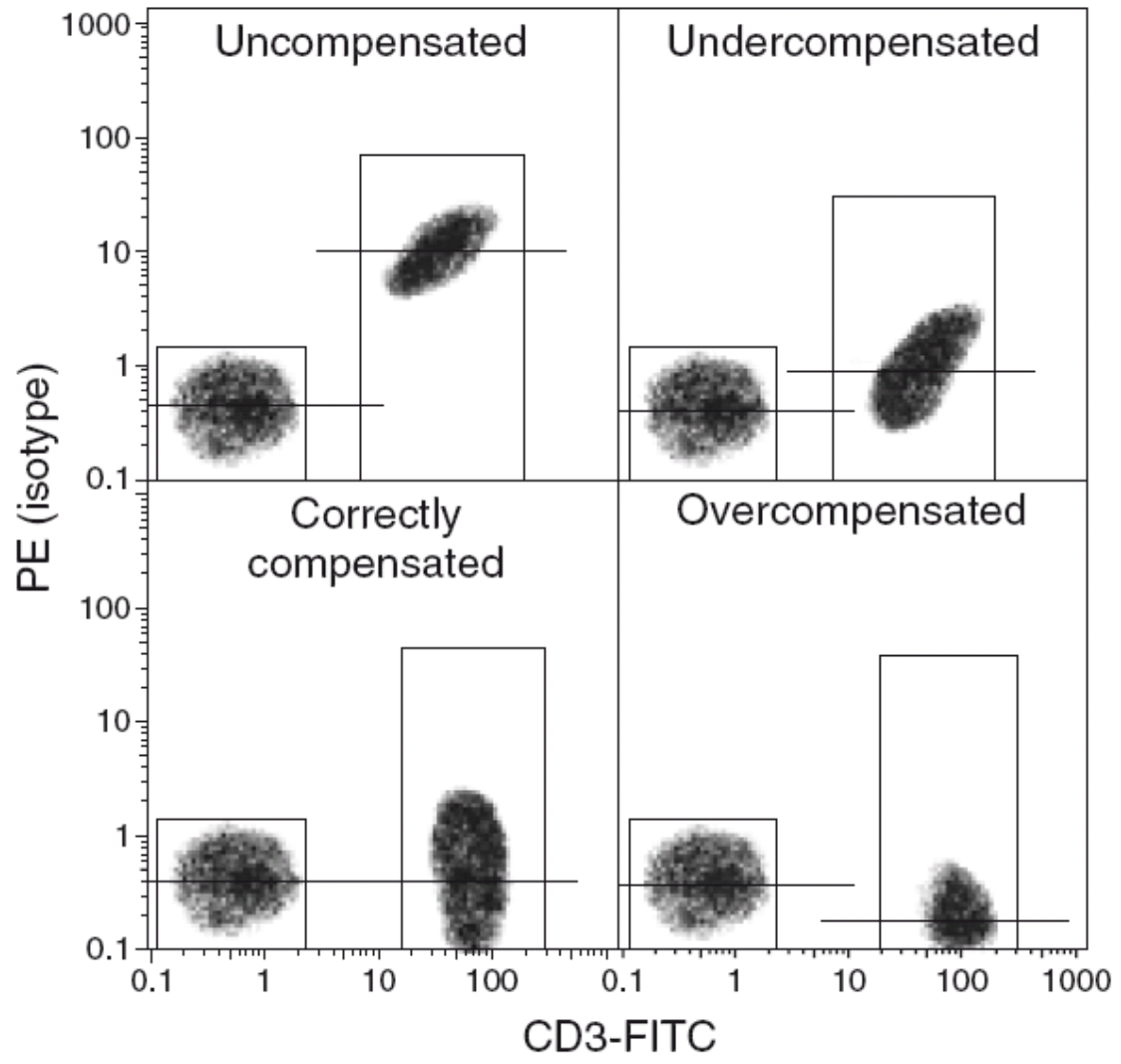
**NONE!**



Current Protocols in Cytometry



# Kompenzace fluorescenčního signálu



# Nastavení kompenzací

- značené mikročástice – pro běžně konjugované fluorochromy



## CaliBRITE Beads

CaliBRITE 3 three-color kit–Catalog No. 340486

CaliBRITE two-color kit–Catalog No. 349502

CaliBRITE PerCP Beads–Catalog No. 340497

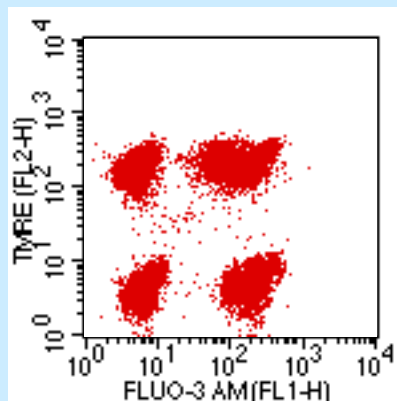
CaliBRITE APC Beads–Catalog No. 340487

CaliBRITE PerCP-Cy5.5 Beads with Bead Dilution Buffer–Catalog No. 345036

For In Vitro Diagnostic Use with FACS brand flow cytometers

Setup	Tube <sup>a</sup>	Unlabeled	FITC	PE	PerCP or PerCP-Cy5.5 <sup>b</sup>	APC
two-color	A	1 drop				
	B	1 drop	1 drop	1 drop		
three-color	A	1 drop				
	B	1 drop	1 drop	1 drop	1 drop	
four-color	A	1 drop				1 drop
	B	1 drop	1 drop	1 drop	1 drop	1 drop

- značené buňky – pro vitální značení



parametr - detektor amp.

FL1 - 544

FL2 - 434

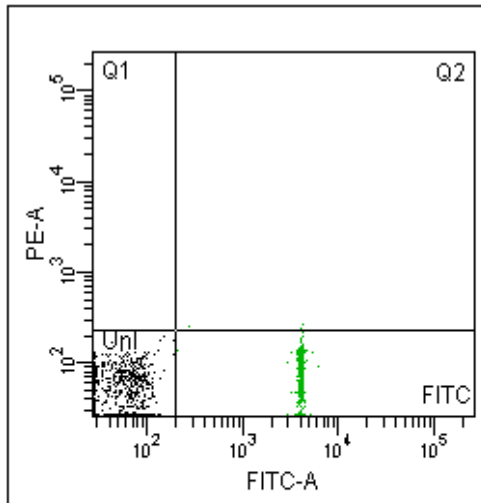
kompenzace

FL1 - 1.1%FL2

FL2 - 17.5%FL1

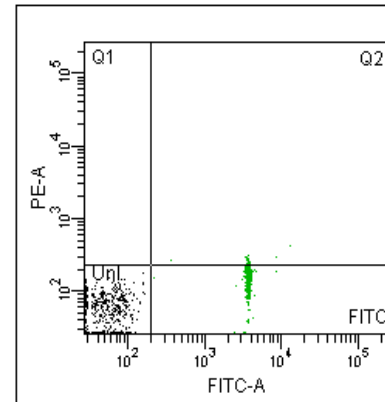
# Effects of Changing PMT Values

Correct Compensation



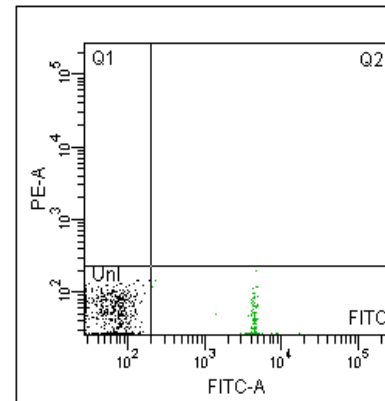
Population	PE-A Mean
Unl	69
FITC	64

FITC Voltage Decreased by 5 V



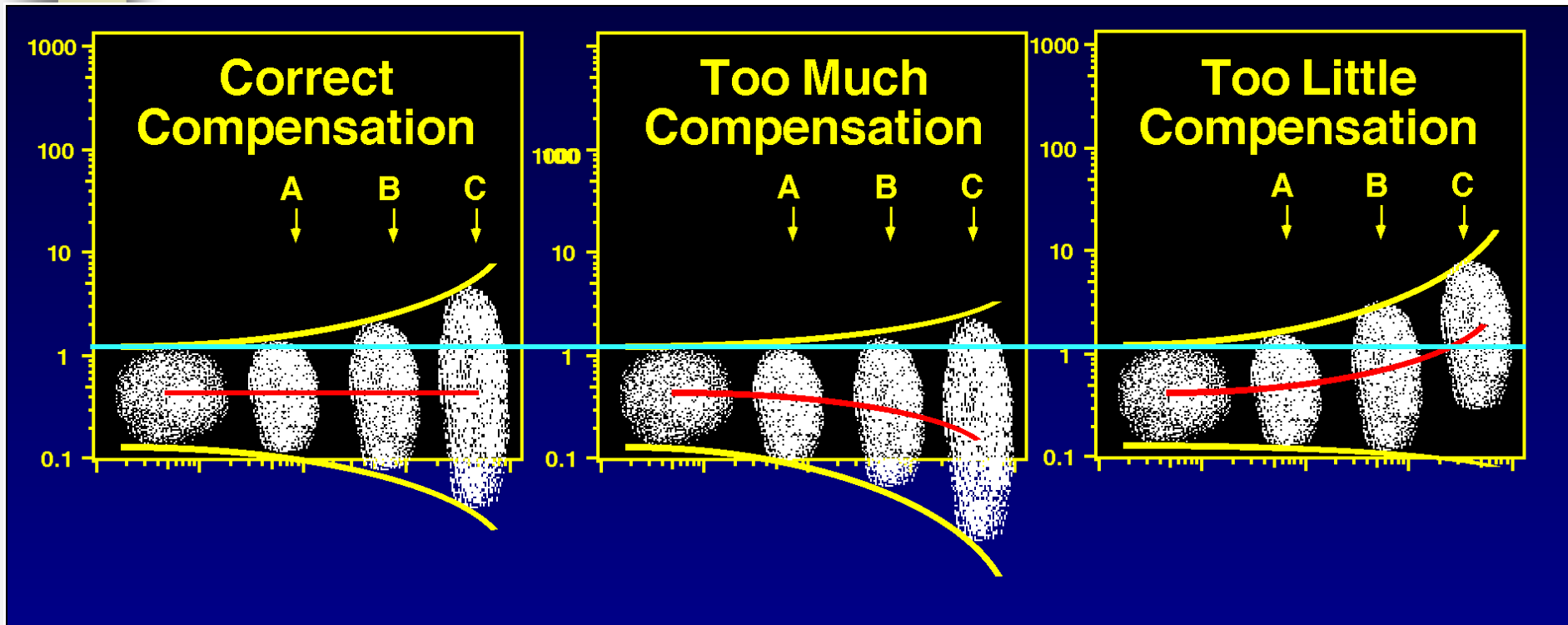
Population	PE-A Mean
Unl	68
FITC	132

FITC Voltage Increased by 5 V



Population	PE-A Mean
Unl	67
FITC	49

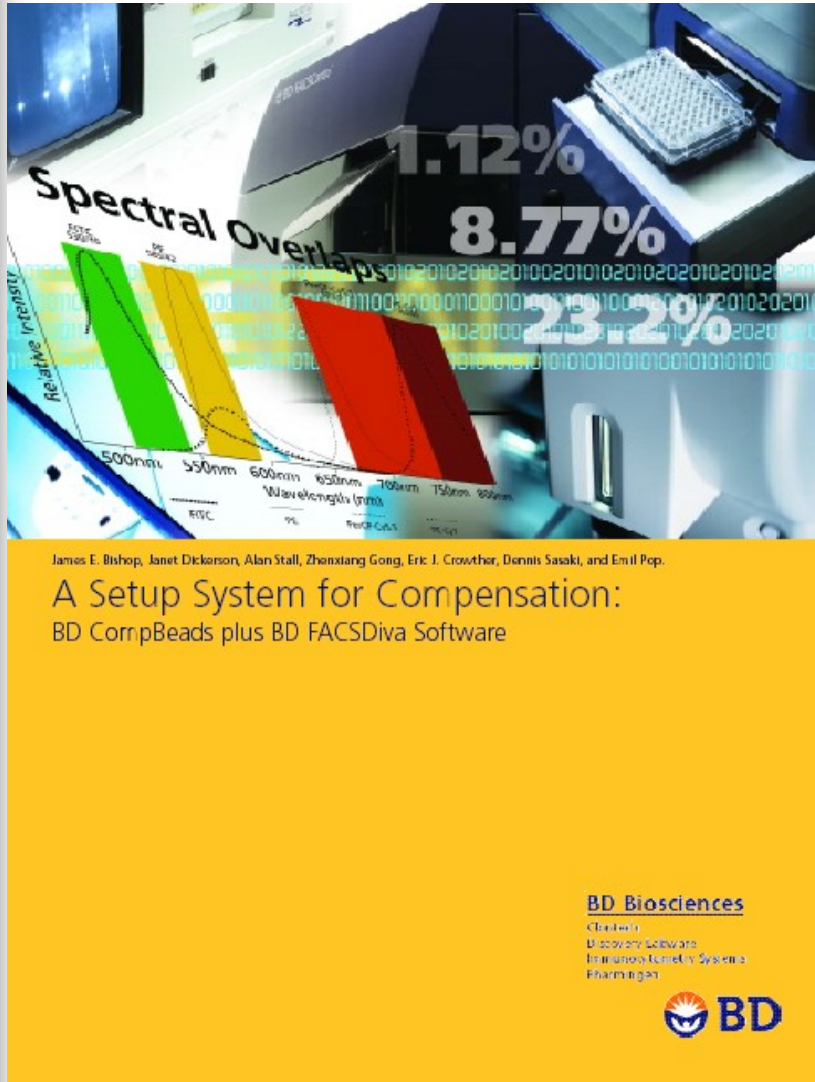
# Which marker for compensation?



Small errors in compensation of a dim control (A) can result in large compensation errors with bright reagents (B & C).

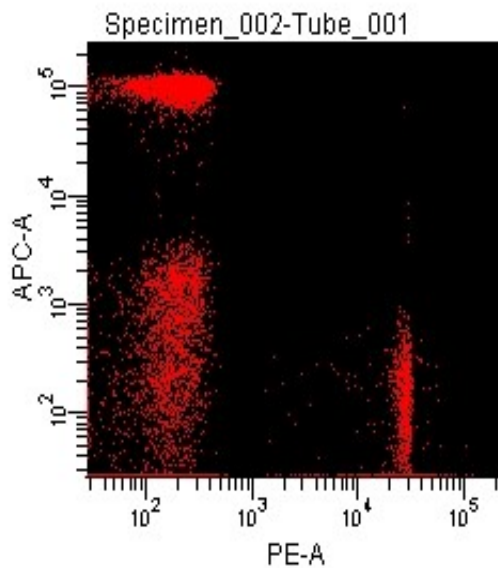
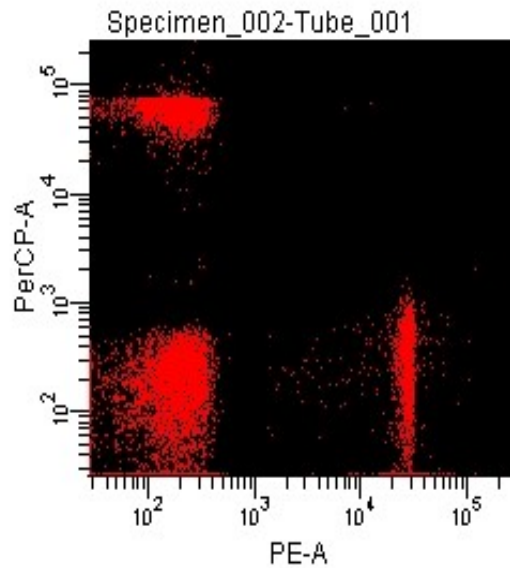
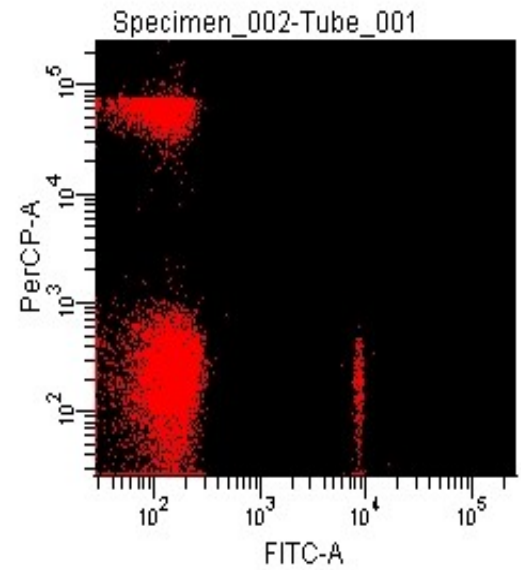
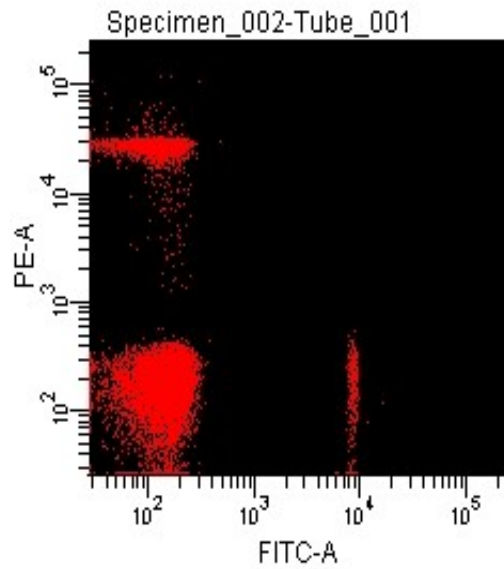
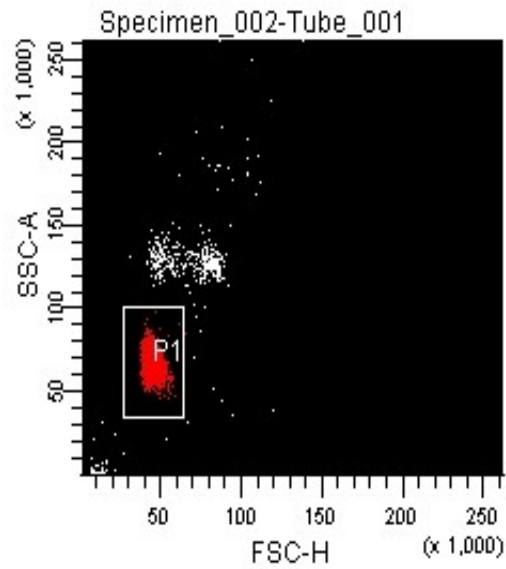
**Use bright markers to setup proper compensation.**

# BD Comp Beads



- Always positive
- Bright staining
- Save sample (HIV patients)
- Use the same antibody for compensation and the real experiment

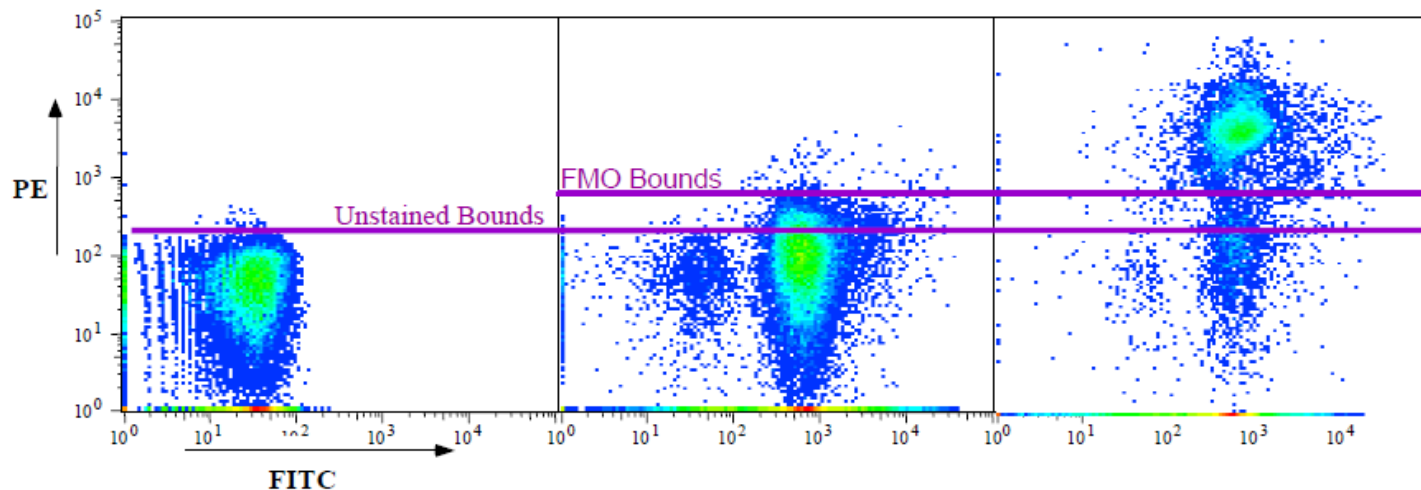
# BD Comp Beads



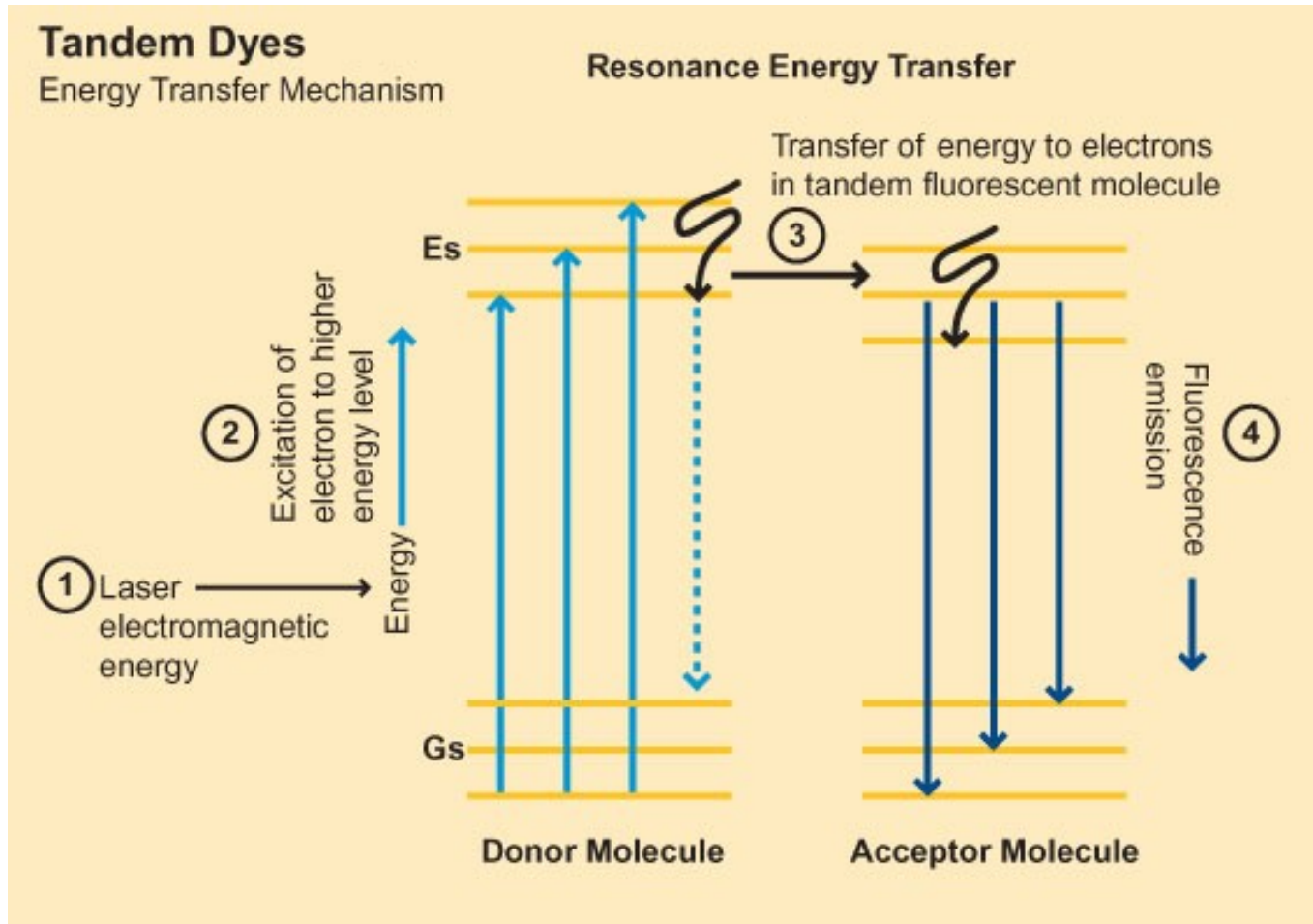
# Fluorescence Minus One

PBMC were stained as shown in a 3-color experiment. Compensation was properly set for all spillovers

	Unstained Control	FMO Control	Fully Stained
FITC	-	CD3	CD3
PE	-	-	CD4
Cy5PE	-	CD8	CD8

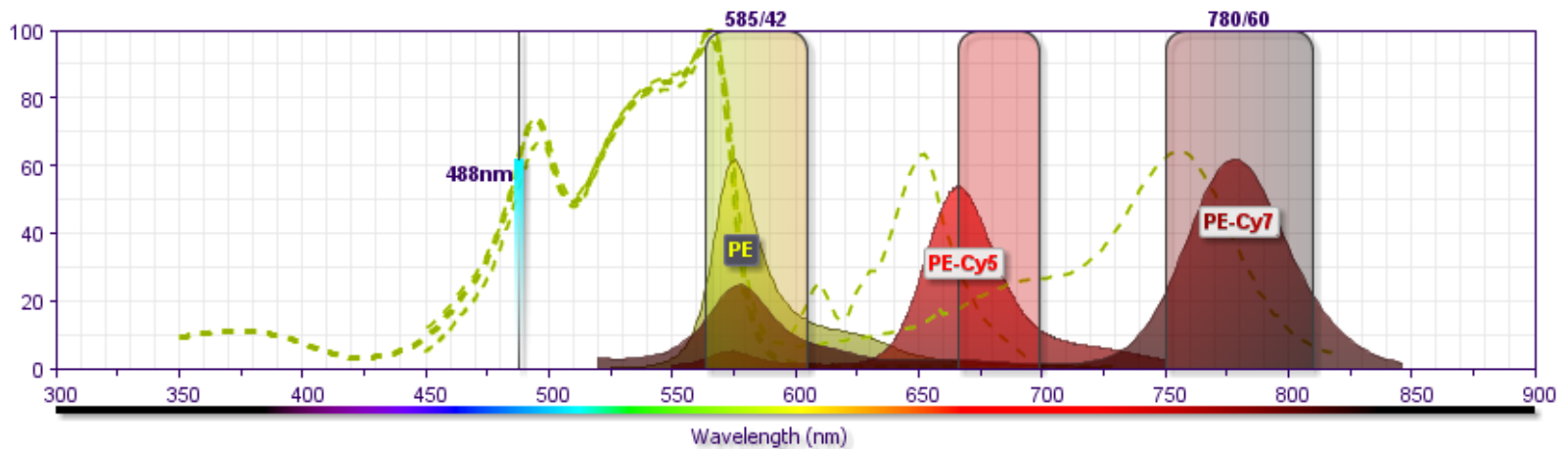
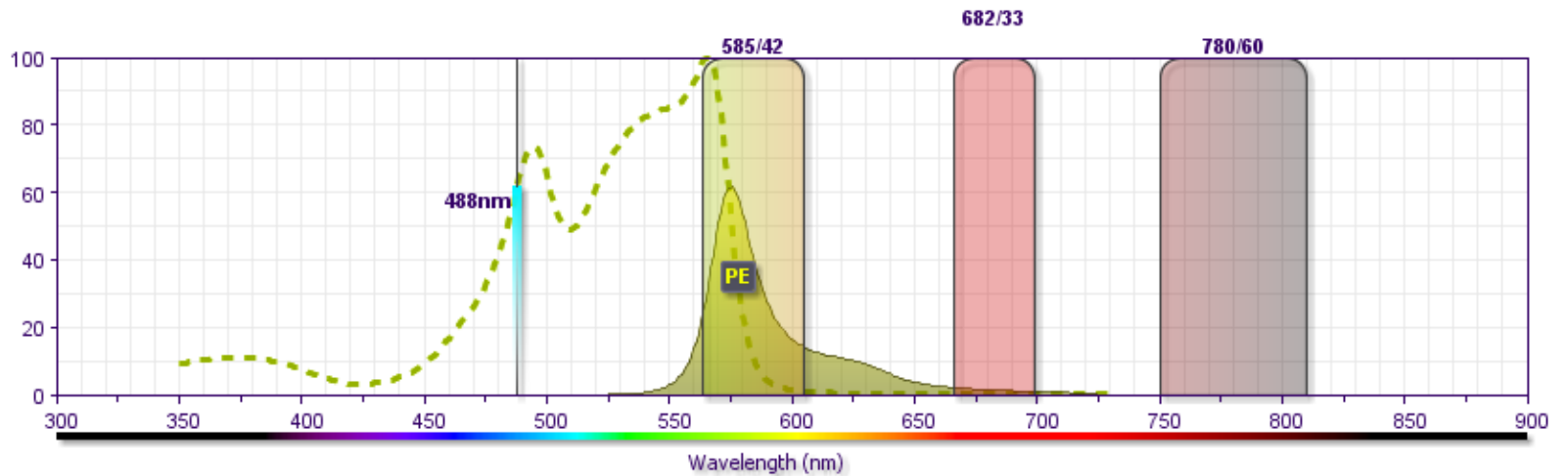


# Tandemové značky

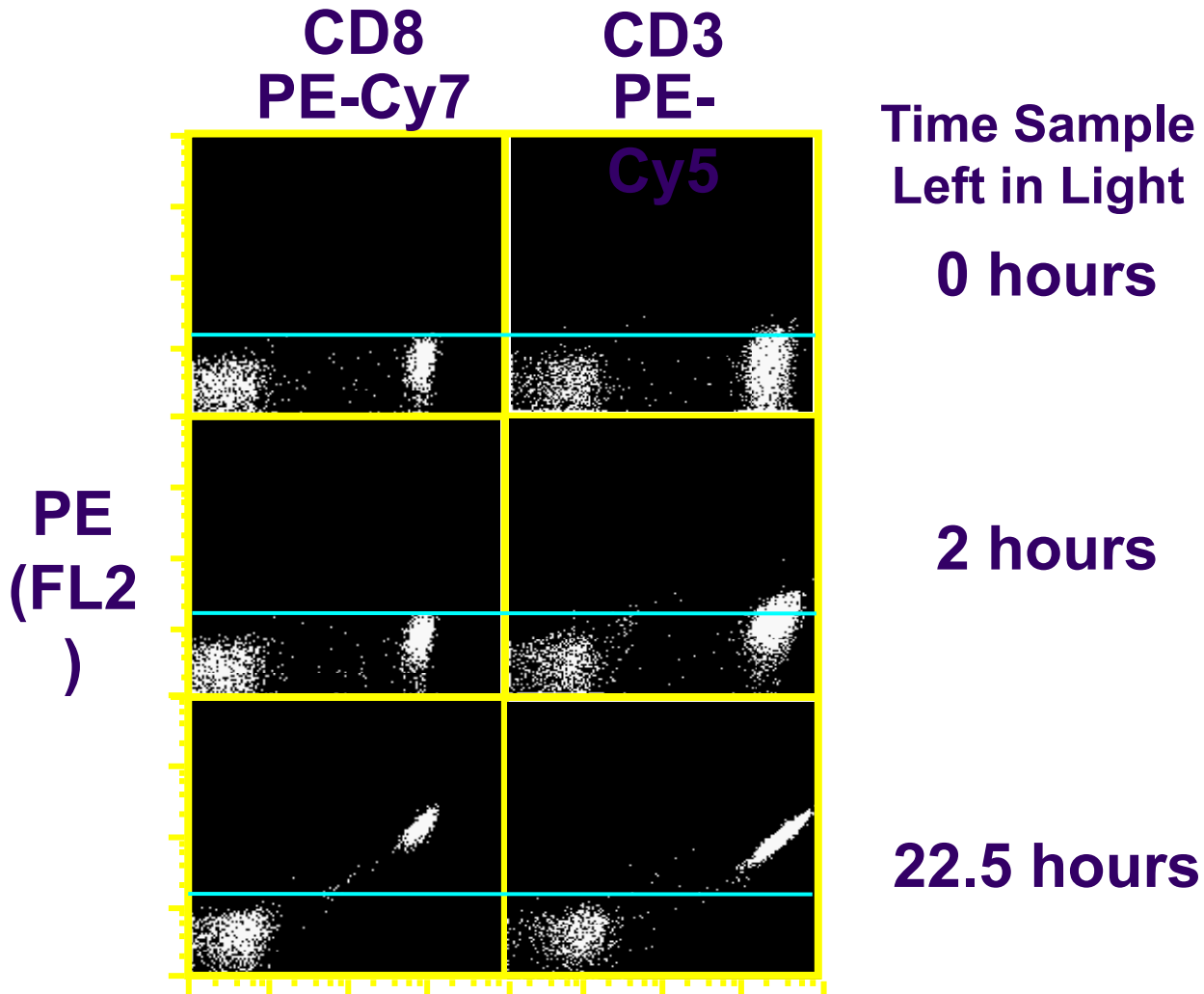




# Tandemové značky - příklad



# Tandems are light sensitive





# Factors that Effect Compensation

- Reagent Lot-to-Lot Variation
- Fluorochrome Stability
- Sample-to-Sample Variation
- Assay Staining Conditions

BD FACSAria™ III and Special Order BD FACSAria (typical setup) <sup>2</sup>						
488	Green	FTC	Alexa Fluor® 488			
	Yellow	PE	PI			
	Orange	BD Horizon PE-CF594	PE-Texas Red®			
	Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5	
	Infrared	PE-Cy7				
561	Yellow	PE	PI			
	Orange	BD Horizon PE-CF594	PE-Texas Red®			
	Red	PE-Cy5				
	Infrared	PE-Cy7				
640	Red	APC	Alexa Fluor® 647			
	Far Red	Alexa Fluor® 700				
	Infrared	BD APC-H7	APC-Cy7			
405	Blue	Brilliant Violet™ 421	BD Horizon™ V450	VPD450	Pacific Blue™	
	Green	BD Horizon V500	AmCyan			
	Orange	Brilliant Violet™ 605*				
375*	Blue	Hoehst 33342				



Enabling Legendary Discovery™





- Řešení?  
#1

## Idea (1931)



"Simplicissimus Karl Arnold Mobile Telephony" by Source (WP:NFCC#4). Licensed under Fair use via Wikipedia

## Invention (1973)



Martin Cooper, Motorola

## Innovation (2007)



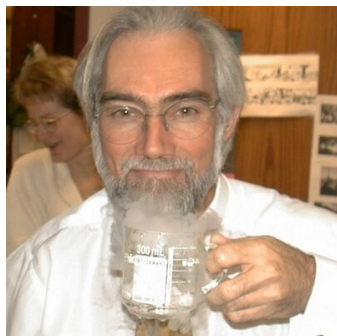
Steve Jobs, Apple

# Spectral flow cytometry

## J.P. Robinson, Purdue University

Cytometry Part A • 81A: 35–44, 2012

ORIGINAL ARTICLE



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



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

Gérald Grégori,<sup>1,2</sup> Valery Patsekina,<sup>1,3</sup> Bartek Rajwa,<sup>1,3</sup> James Jones,<sup>4</sup> Kathy Ragheb,<sup>1,3</sup> Cheryl Holdman,<sup>1,3</sup> J. Paul Robinson<sup>1,3,4\*</sup>

2  
DOI: 10.1017/S1431927605510328

Microsc Microanal 11(Suppl 2), 2005  
Copyright 2005 Microscopy Society of America

#### Multispectral Flow Cytometry: Next Generation Tools For Automated Classification

J. Paul Robinson<sup>a,b</sup>, Valery Patsekina<sup>a</sup>, Gerald Grégori<sup>a</sup>, Bartek Rajwa<sup>a,b</sup>, and James Jones<sup>a,b</sup>

<sup>a</sup>Department of Basic Medical Science, School of Veterinary Medicine, and <sup>b</sup>Weldon Department of Biomedical Engineering, Purdue University, West Lafayette, IN, 47907, USA



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

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

(54) <b>MULTI-SPECTRAL DETECTOR AND ANALYSIS SYSTEM</b>	5,394,237 A	2/1995	Chang et al.	188/79-51
	5,422,712 A	6/1995	Ogano	356/73
	5,675,517 A	10/1997	Stokdijk	702/85
(75) Inventors: <b>Joseph Paul Robinson</b> , West Lafayette, IN (US); <b>Bartłomiej Rajwa</b> , West Lafayette, IN (US); <b>Gérald Grégori</b> , Marseille (FR); <b>Valery Patsekina</b> , West Lafayette, IN (US)	5,719,667 A *	2/1998	Miers	356/73
	6,240,541 B1 *	6/2001	Baraji et al.	356/73
	6,630,307 B2 *	10/2003	Broschek et al.	435/6
	6,885,440 B2 *	4/2005	Silcott et al.	356/73
	6,947,134 B2 *	9/2005	Chang et al.	356/318
	7,057,712 B2 *	6/2006	Beck et al.	356/72

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

(Continued)



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

FOREIGN PATENT DOCUMENTS

EP 0 315 939 5/1989

(Continued)

# Spectral flow cytometry

**SONY** Sony Biotechnology Inc.   US

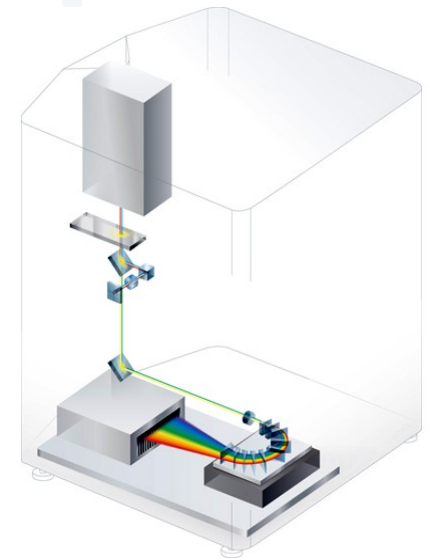
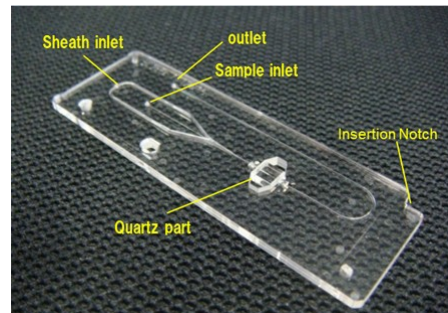
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Overview Features Applications Specifications Literature

## See Everything

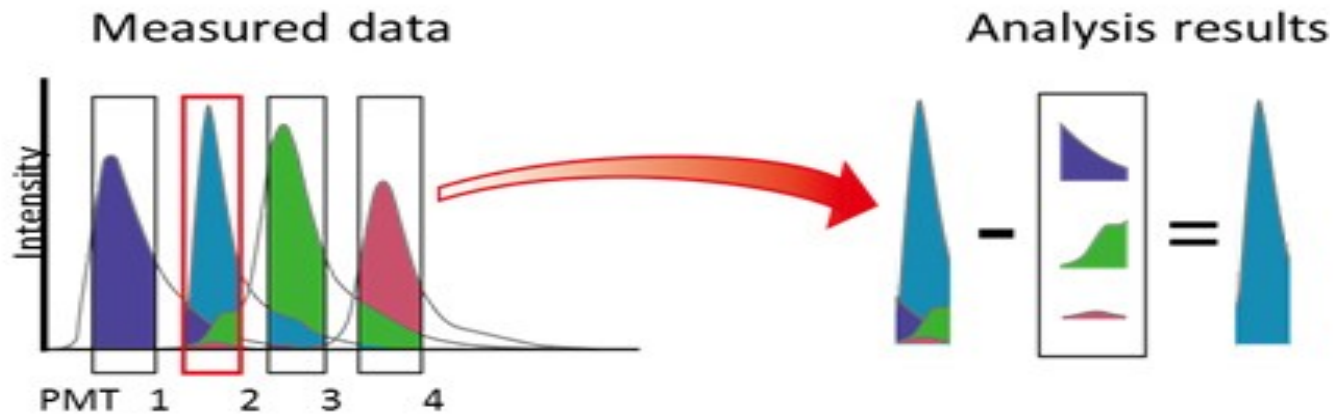
The SP6800 Spectral Analyzer is Sony Biotechnology Inc.'s newest innovative life science system fundamentally expanding the way cell and biomarker analysis can be performed. This system incorporates a unique optical bench, Blu-ray™ disc technology, and advanced algorithms to deliver some of the most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

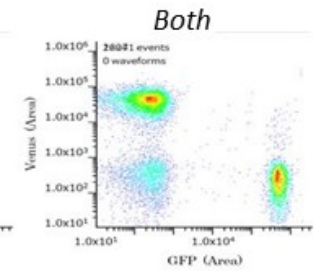
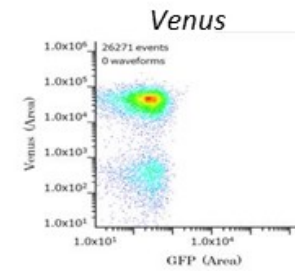
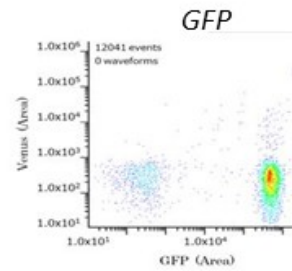
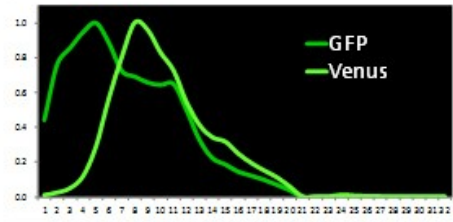




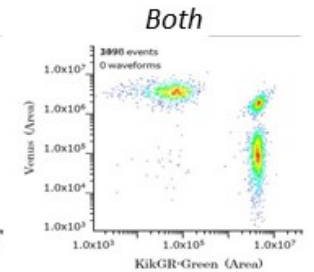
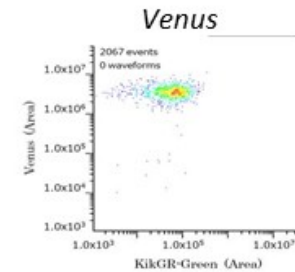
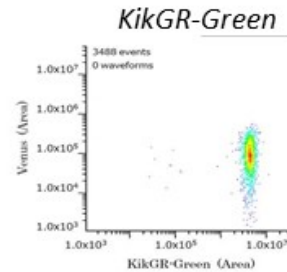
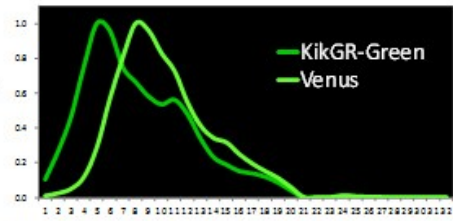
# Conventional vs. spectral analysis



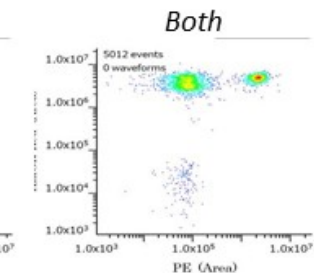
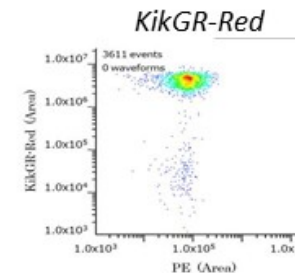
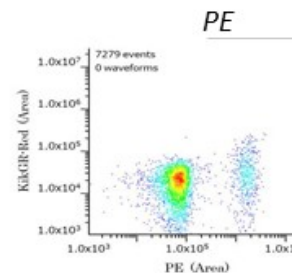
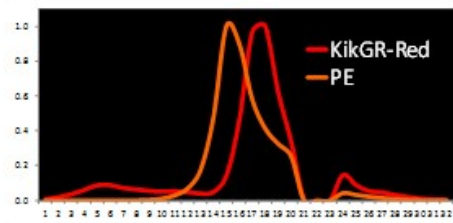
*GFP*  
*Venus*



*KikGR-Green*  
*Venus*



*KikGR-Red*  
*PE*

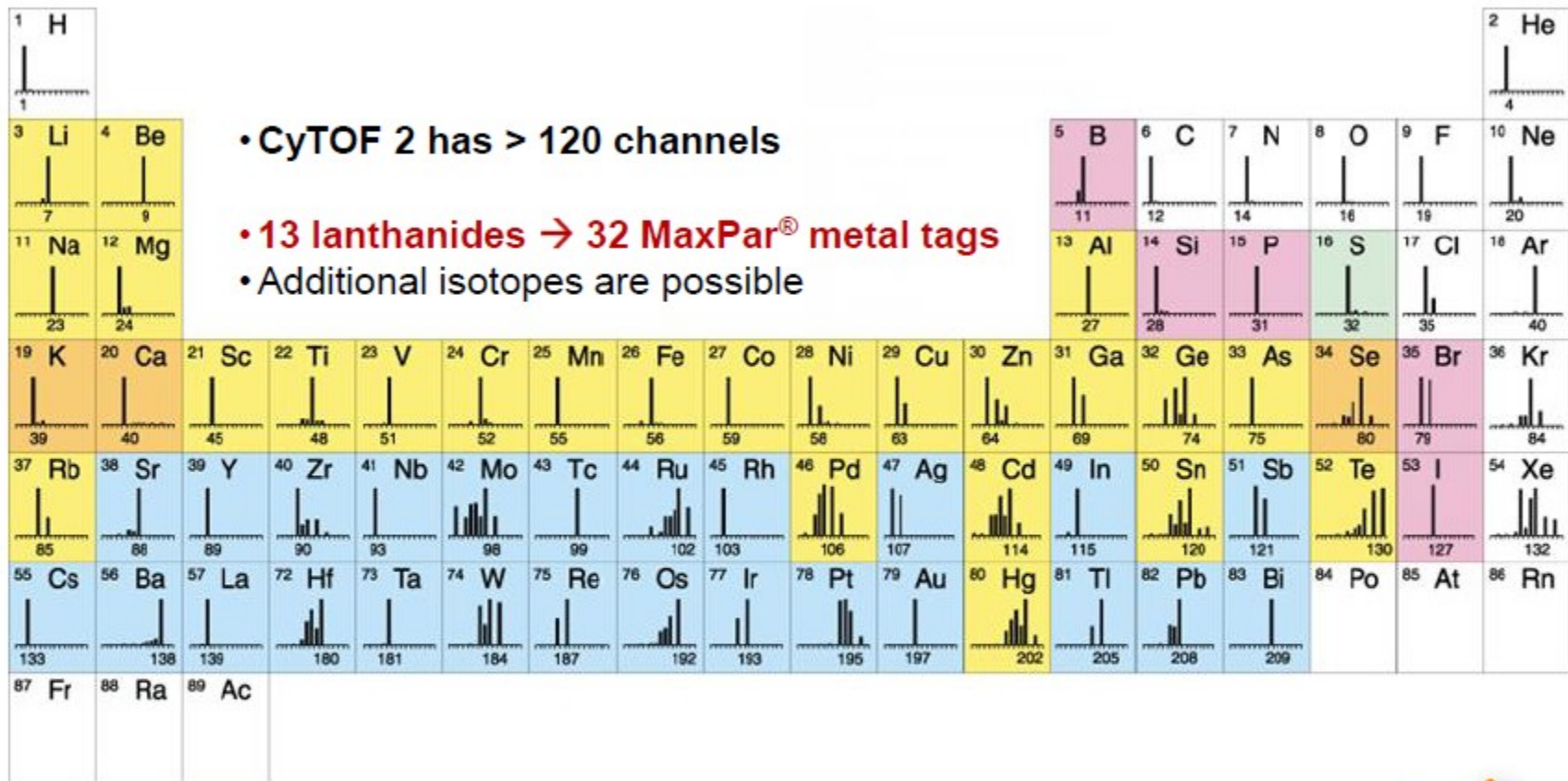


For revealing spatiotemporal regulation of immune cells, fluorescent proteins are very useful, which can be difficult to analyze with traditional flow cytometry technologies. These figures show how easily the SP6800 Spectral Analyzer can separate overlapping spectra of fluorescent proteins and fluorochromes. Data courtesy of M. Tomura of Kyoto University.



- Řešení?  
#2

# Probing with Isotopes



• CyTOF 2 has > 120 channels

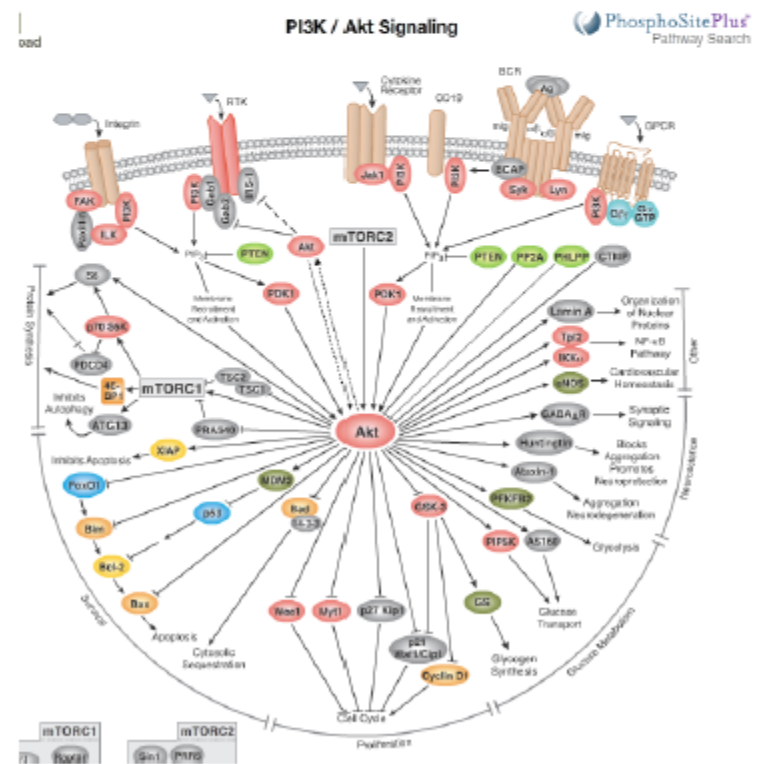
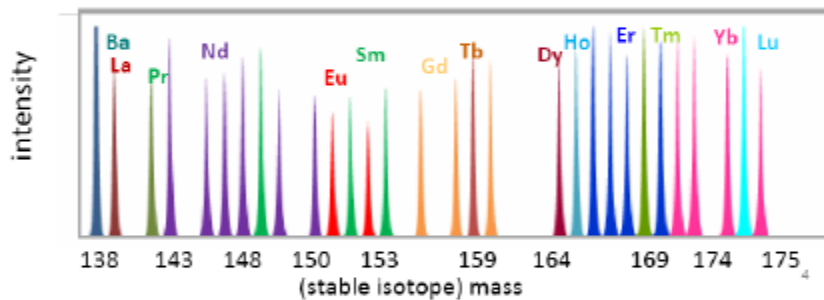
• 13 lanthanides → 32 MaxPar<sup>®</sup> metal tags

• Additional isotopes are possible

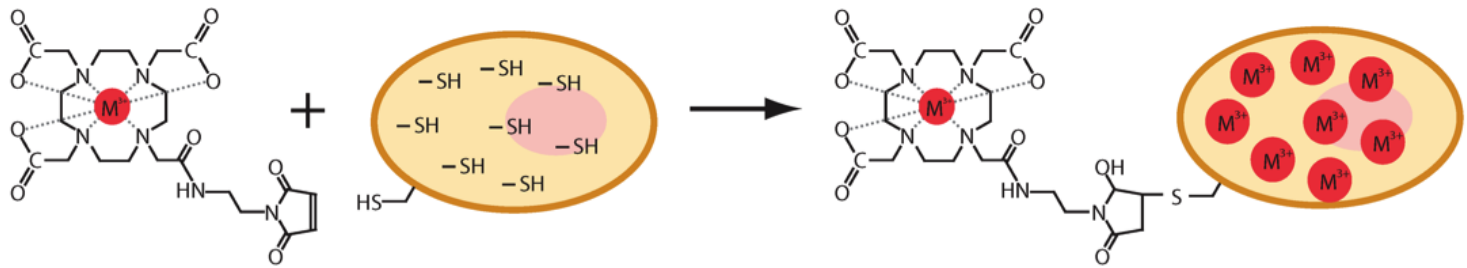


# Why Mass Cytometry?

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



# Single Cell Mass Cytometry



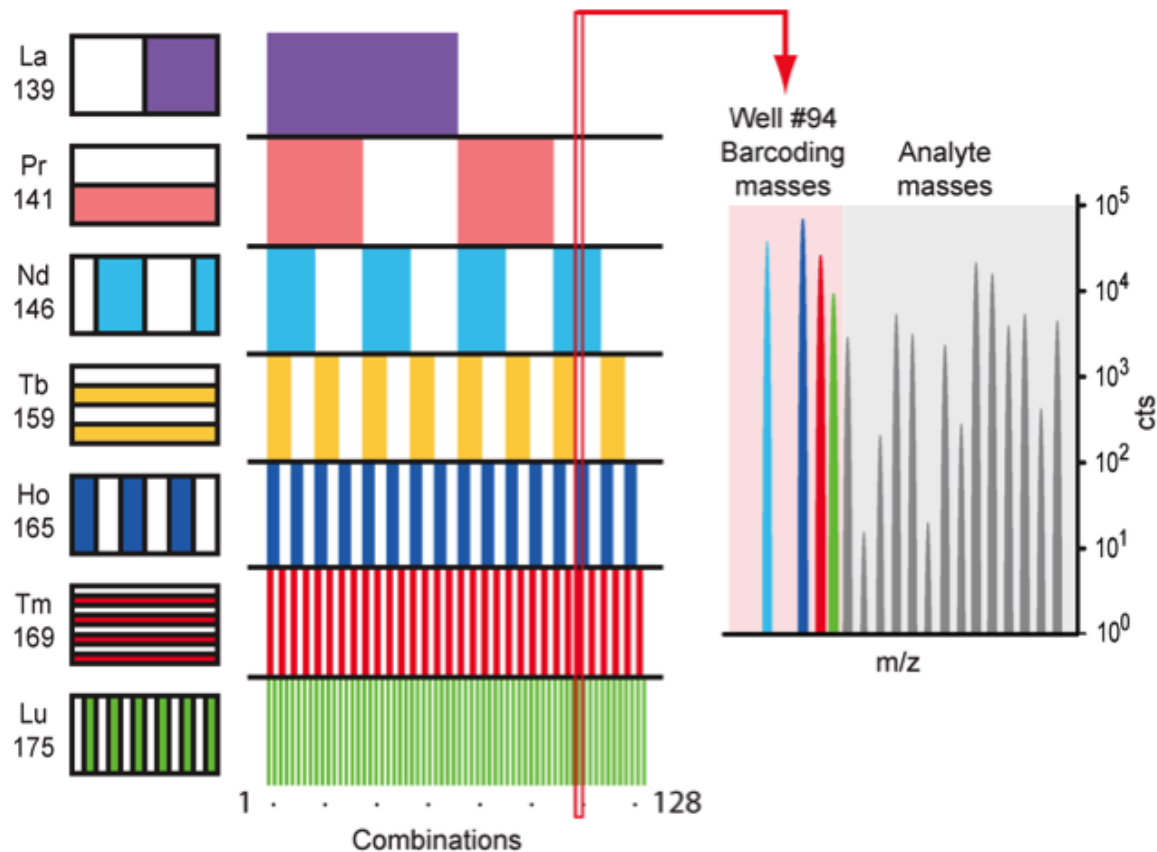
Cells were covalently labeled with a bifunctional compound, maleimido-mono-amide-DOTA (mDOTA). This compound can be loaded with a lanthanide(III) isotope ion, and reacts covalently with cellular thiol groups through the maleimide moiety.

**Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum**

Sean C. Bendall, *et al.*

*Science* **332**, 687 (2011);

# Single Cell Mass Cytometry

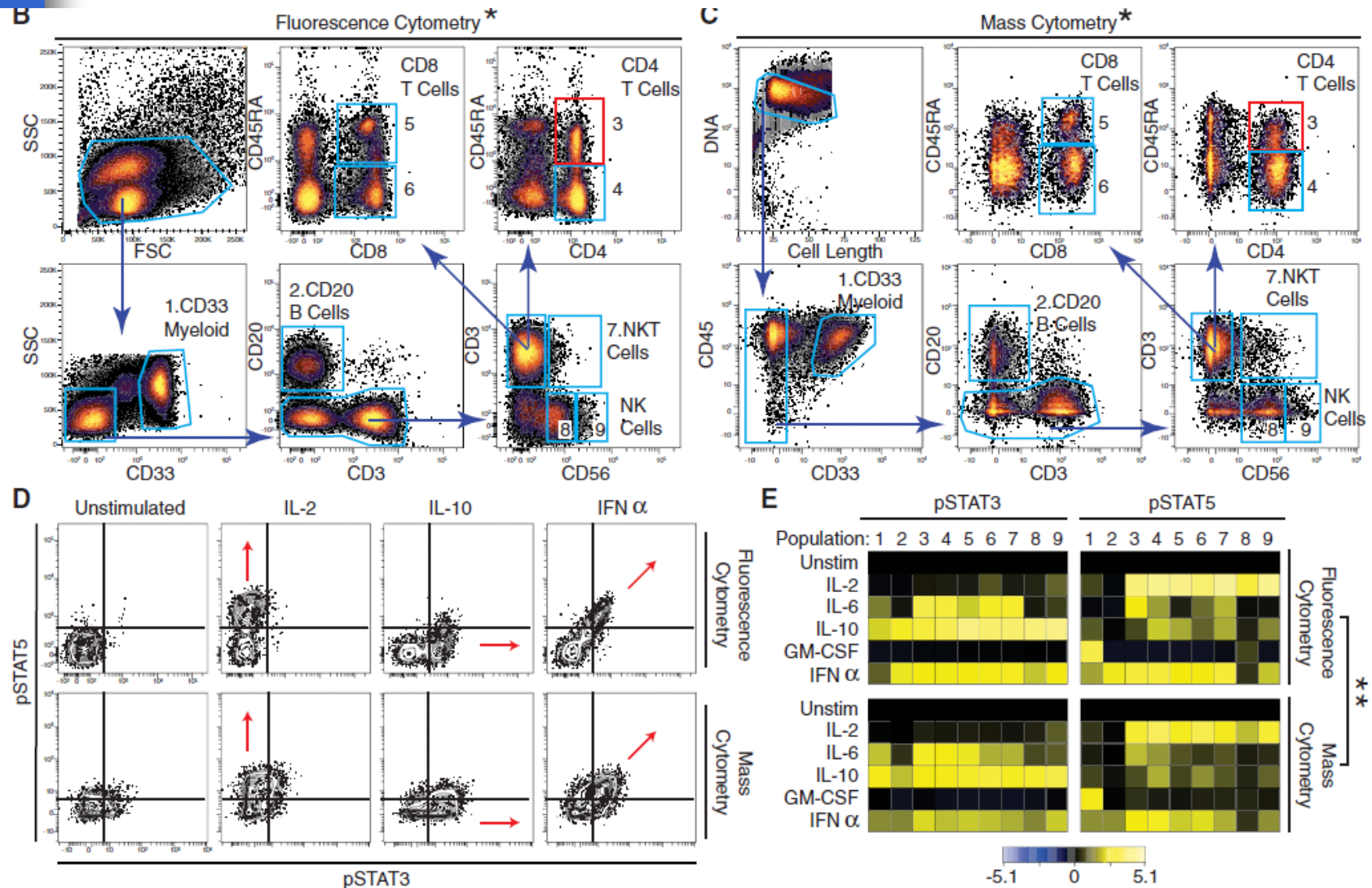


Seven unique lanthanide isotopes were used to generate 128 combinations, enough to barcode each sample in a 96-well plate. The seven lanthanide isotopes, their masses and their locations on the 96-well plate are shown.

**Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum**

Sean C. Bendall, *et al.*  
*Science* **332**, 687 (2011);

# Single Cell Mass Cytometry



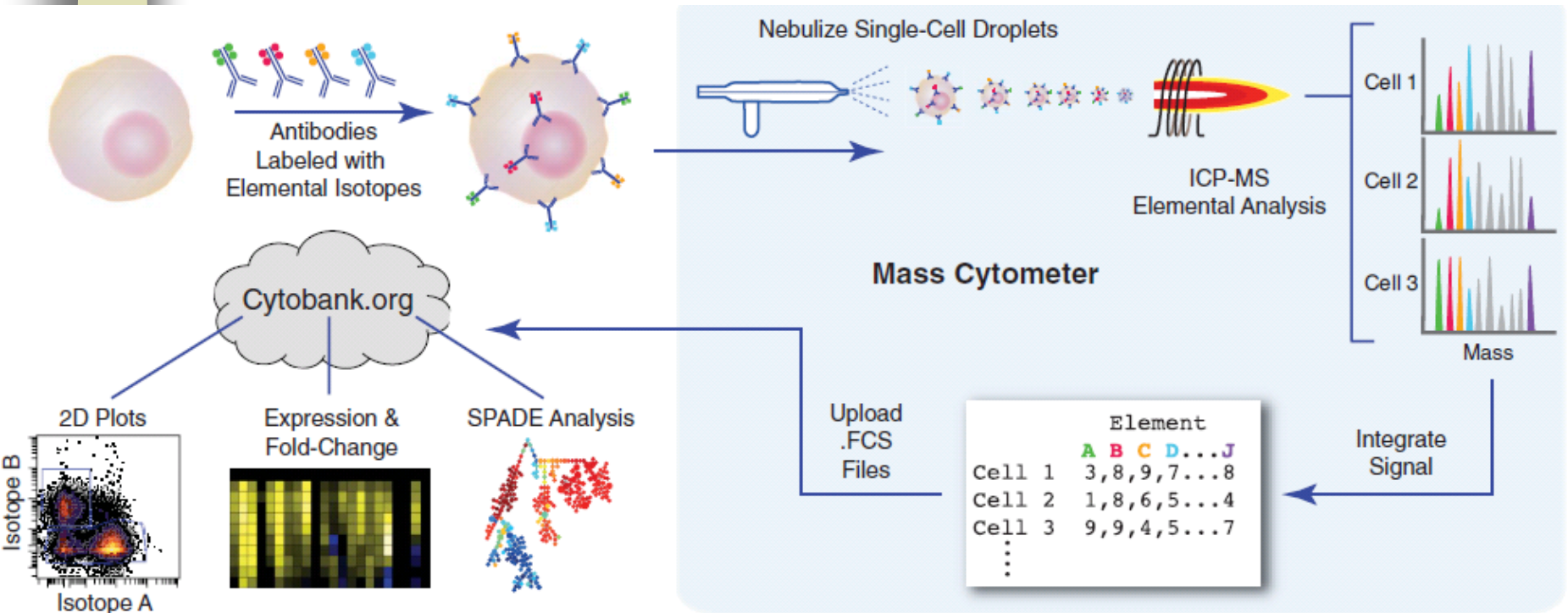
Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum

Sean C. Bendall, *et al.*

*Science* 332, 687 (2011);



# Single Cell Mass Cytometry



**Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum**

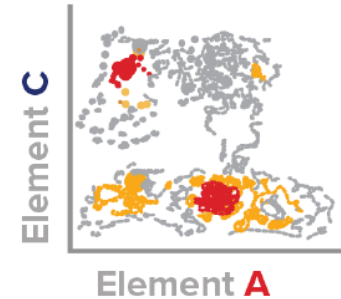
Sean C. Bendall, *et al.*

*Science* **332**, 687 (2011);

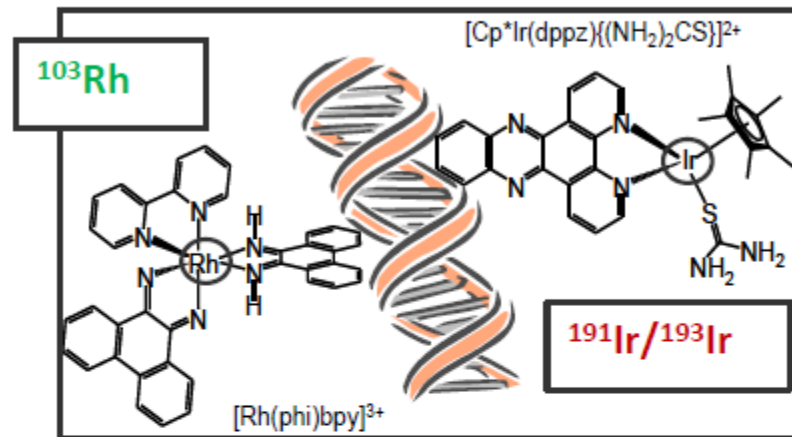
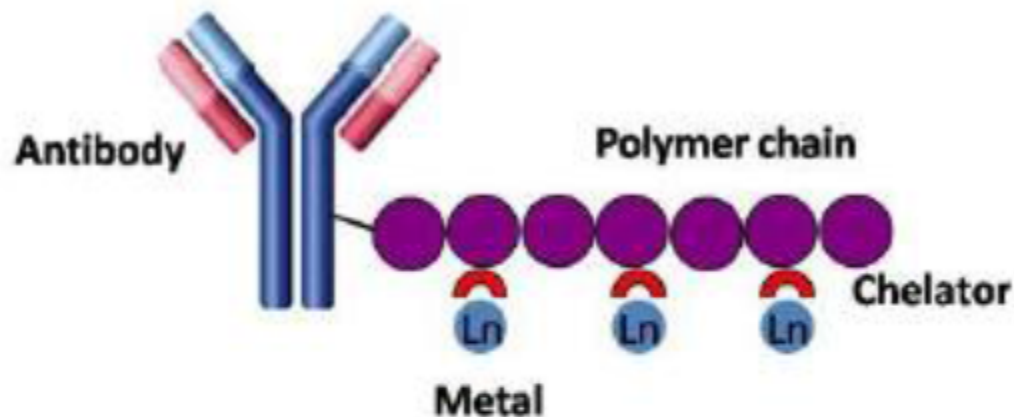
# Mass Cytometry: 50+ Parameters on Millions of Cells

Discovery of new biology  
Comprehensive functional profiling

Basic research  
Drug discovery  
Clinical research



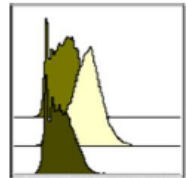
# MaxPar<sup>®</sup> metal-tagged probes



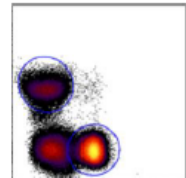
- Lanthanide tags: 32 isotopes from 13 elements
- IgG antibody probes:
  - Pre-conjugated antibodies (220 currently available and growing)
  - MAXPAR<sup>®</sup> labeling kits (for 32 stable isotopes)
- Nucleic acid-binding metallo-intercalators
  - Identifies single cell events
  - Live/dead indicator

# Analyze: Cytobank

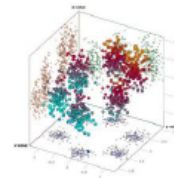
Plot raw data



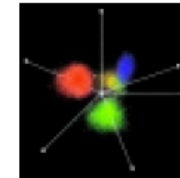
Histogram



Biaxial plot

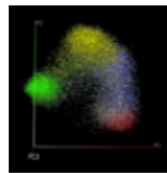


3D plot

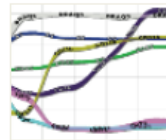


Radar

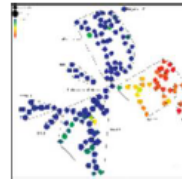
Reduce dimensionality



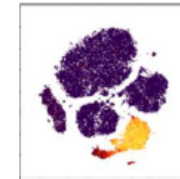
PCA



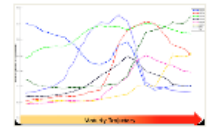
Gemstone



SPADE

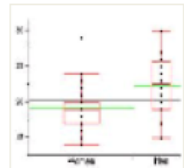


viSNE

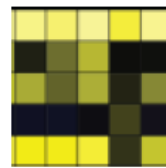


Wanderlust

Summarize statistics



Box plot



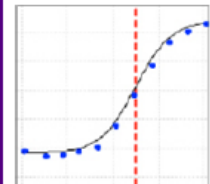
Heat map



Network



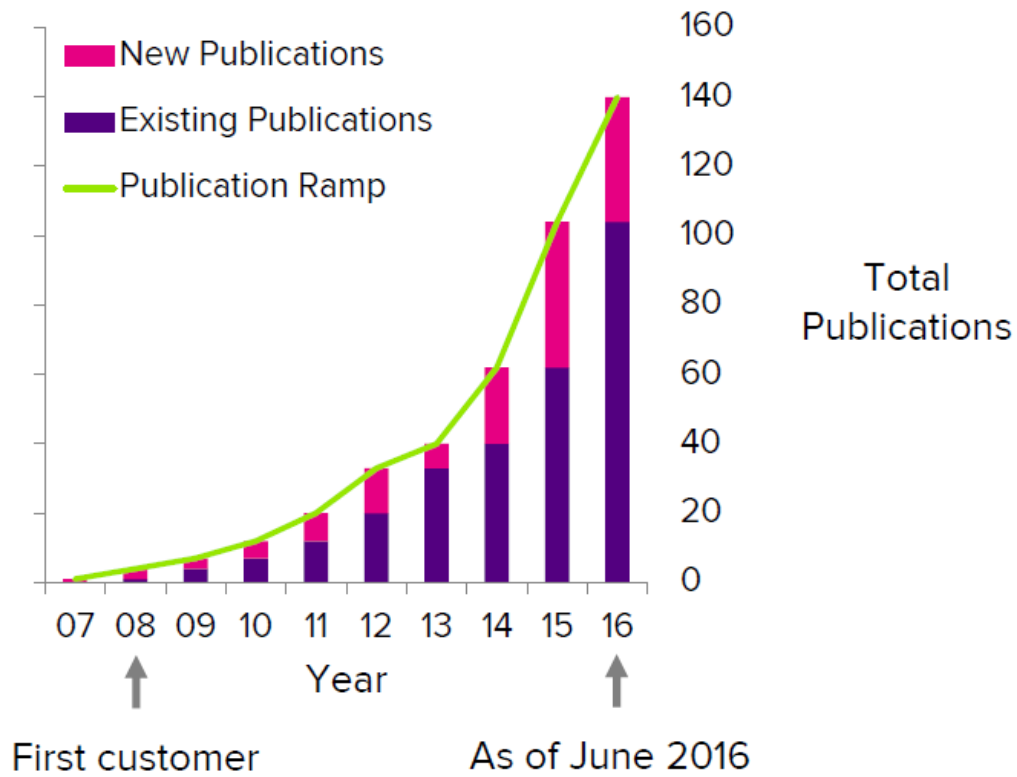
Sunburst



Dose curve



FLUIDIGM®



FLUIDIGM®



# Analyze: Cytobank

Analysis toolkit designed for mass cytometry

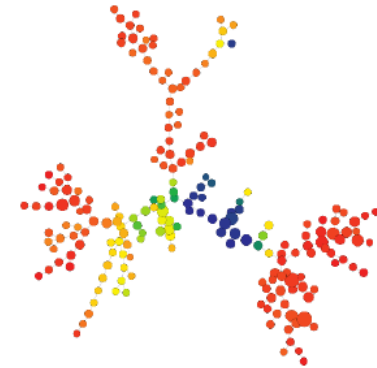
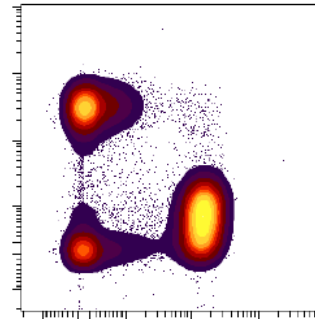
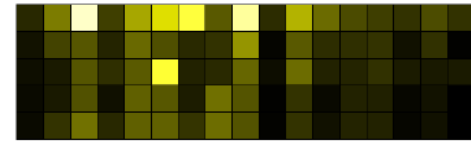
Cloud-based—accessible from anywhere

Data storage and backup included

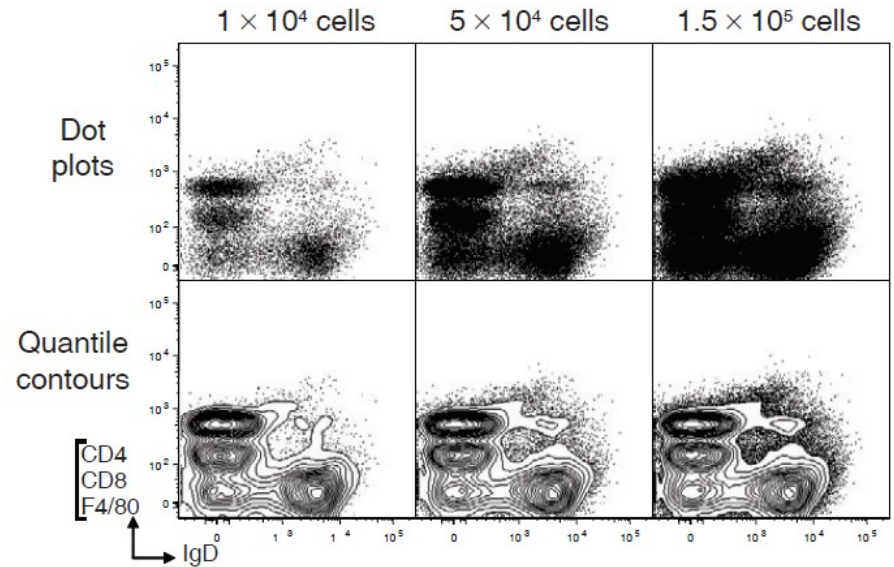
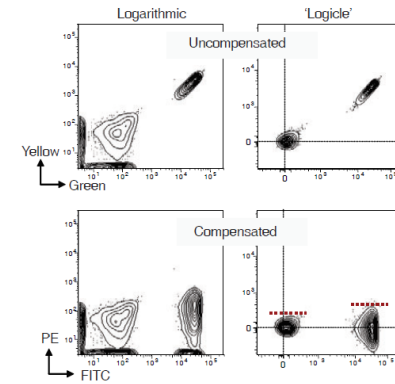
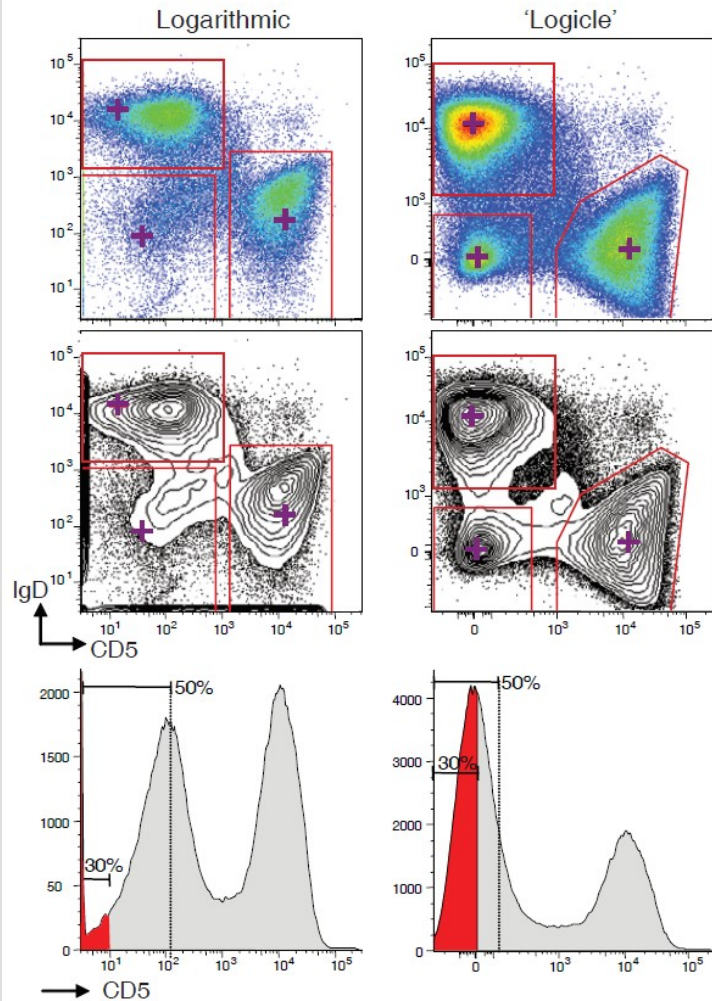
Demo datasets and tutorials

Strong scientific support

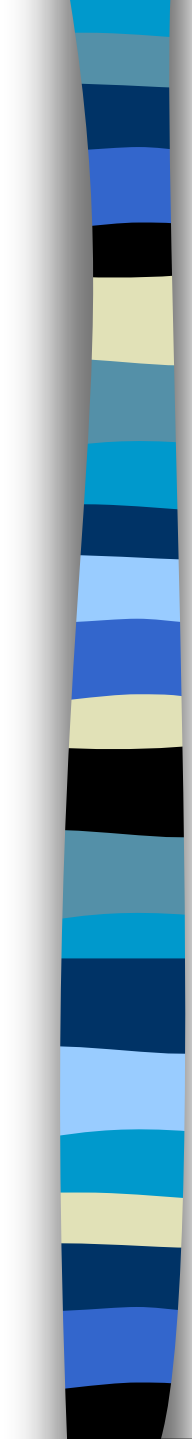
[fluidigm.cytobank.org](http://fluidigm.cytobank.org)



# Vizualizace dat a intepretace dat



Herzenberg LA, Tung J, Moore WA, Herzenberg LA, Parks DR (2006) Interpreting flow cytometry data: a guide for the perplexed. *Nat Immunol* 7: 681-685



## BOX 1 SUGGESTED GUIDELINES FOR FACS DATA PRESENTATION<sup>4</sup>

**Instrument:** Identify the FACS instrument and the software used to collect, compensate and analyze the data. Include model and version number where more than one exists.

**Graphic displays:** Choose smoothing, graph and display options according to the dictates of the study. Be consistent across all displays in an analysis. Indicate the number of cells for which data are displayed and, where applicable, the contour or color density intervals used in the figure.

**Scaling:** Show all parts of the plot axis necessary to indicate the scaling that was used (such as log, linear or 'logicle'). Numerical values for axis 'ticks' can be eliminated except when necessary to clarify the scaling. For univariate (one-dimensional) histograms, the scale for the abscissa (x axis) should be linear and should begin at zero unless otherwise indicated. Numerical axis values should not be included with the zero-based linear axes but should be shown for other axes.

**Gating:** Display the gates used at each step in the gating sequence when gates are set manually (subjective gating). Show data for control samples when these are used to set gates. If necessary, present this information in supplementary figures. When an algorithm is used to set gates, define it explicitly and state that it has been used. Gating is assumed to be subjective unless otherwise stated.

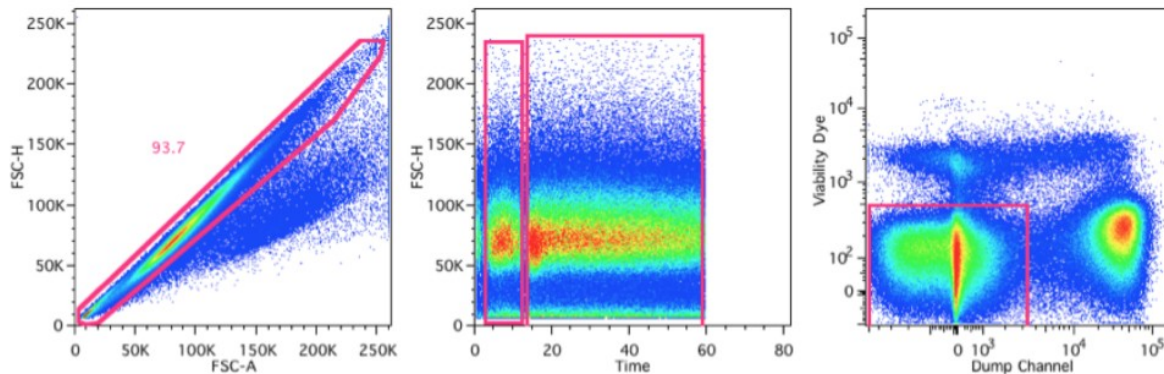
**Frequency measurements:** Show the frequencies (or percentages) of cells in gates of importance in the study. Compute these values relative to the total number of cells presented in the display on which the values appear. If a different frequency computation is used, define the method that was used and where it was applied. The graph itself cannot convey this requisite information.

**Intensity measurements:** Explicitly define the statistic applied (mean, median or a particular percentile). All statistics should be applied to the 'scaled' intensity measurement rather than to 'channel' numbers.



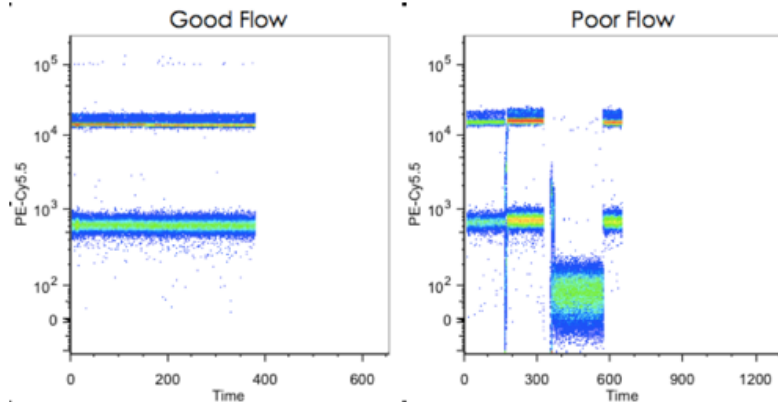
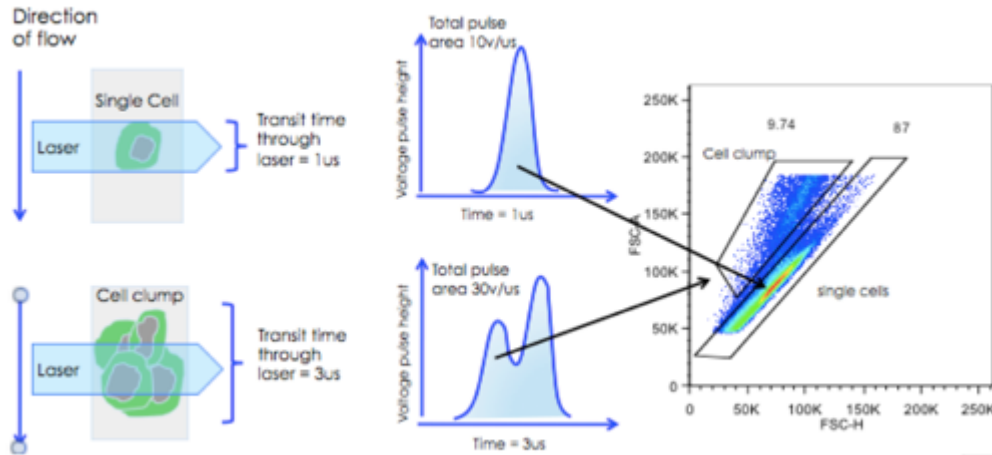
# Gating a kontrola kvality

- 1) Singlets
- 2) Time
- 3) viabilita



<http://expertcytometry.com/3-flow-cytometry-gates-that-will-improve-the-accuracy-of-your-facs-data-analysis/>

# Gating a kontrola kvality



<http://expertcytometry.com/6-flow-cytometry-gating-tips-that-most-scientists-forget/>

# FlowClean Plugin

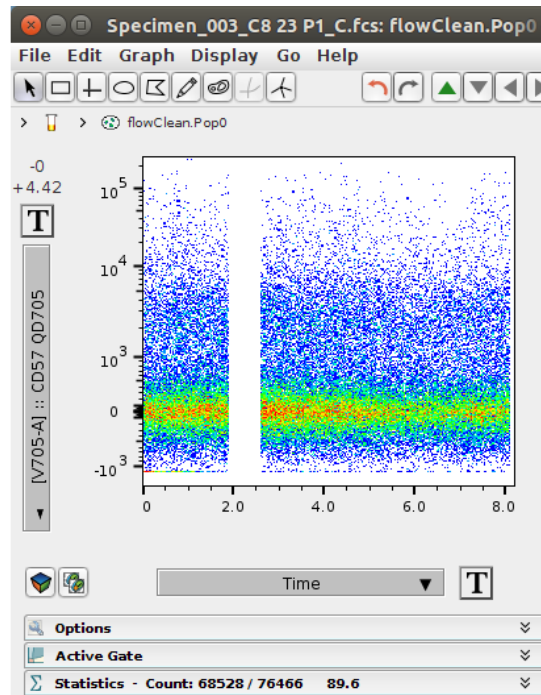
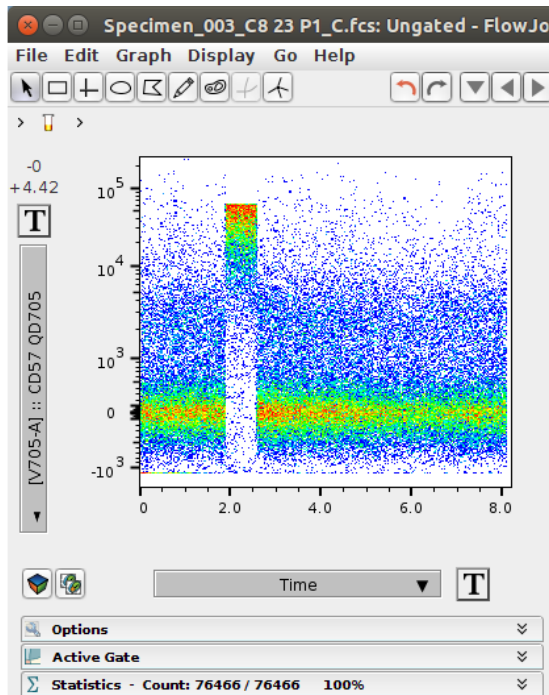


Josef Spidlen

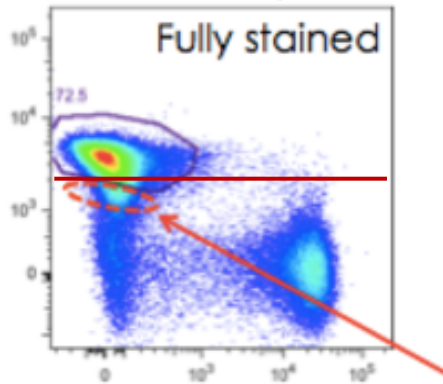
## Clean up your data with the FlowClean plugin

Do you analyze a lot of samples? If so, data quality control may be challenging, especially when a large number of parameters is measured. In particular, fluorescence measurements for a sample over the collection time may not remain stable due to fluctuations in fluid dynamics. As many as 13.7% of publicly available FCS files [have been shown to have this problem](#). But don't worry, we are here to help!

As you may know, our latest release, FlowJo v10.2, contains new and improved architecture for plugins. One of our featured plugins—FlowClean—has been designed to address exactly this issue. It automatically identifies and flags fluorescence anomalies in your FCS files by tracking cell populations in the centered log ratio space. This has been shown to provide a sensitive and consistent method of quality control. Do you want to give it a try?

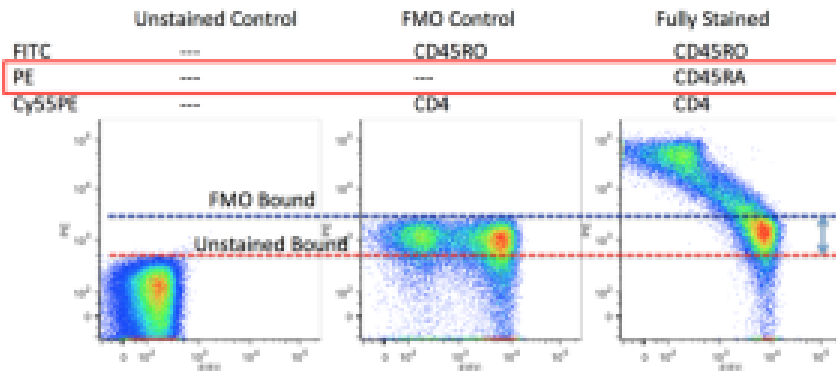


# Gating a kontrola nastavení



Cells in spread of fluorescence

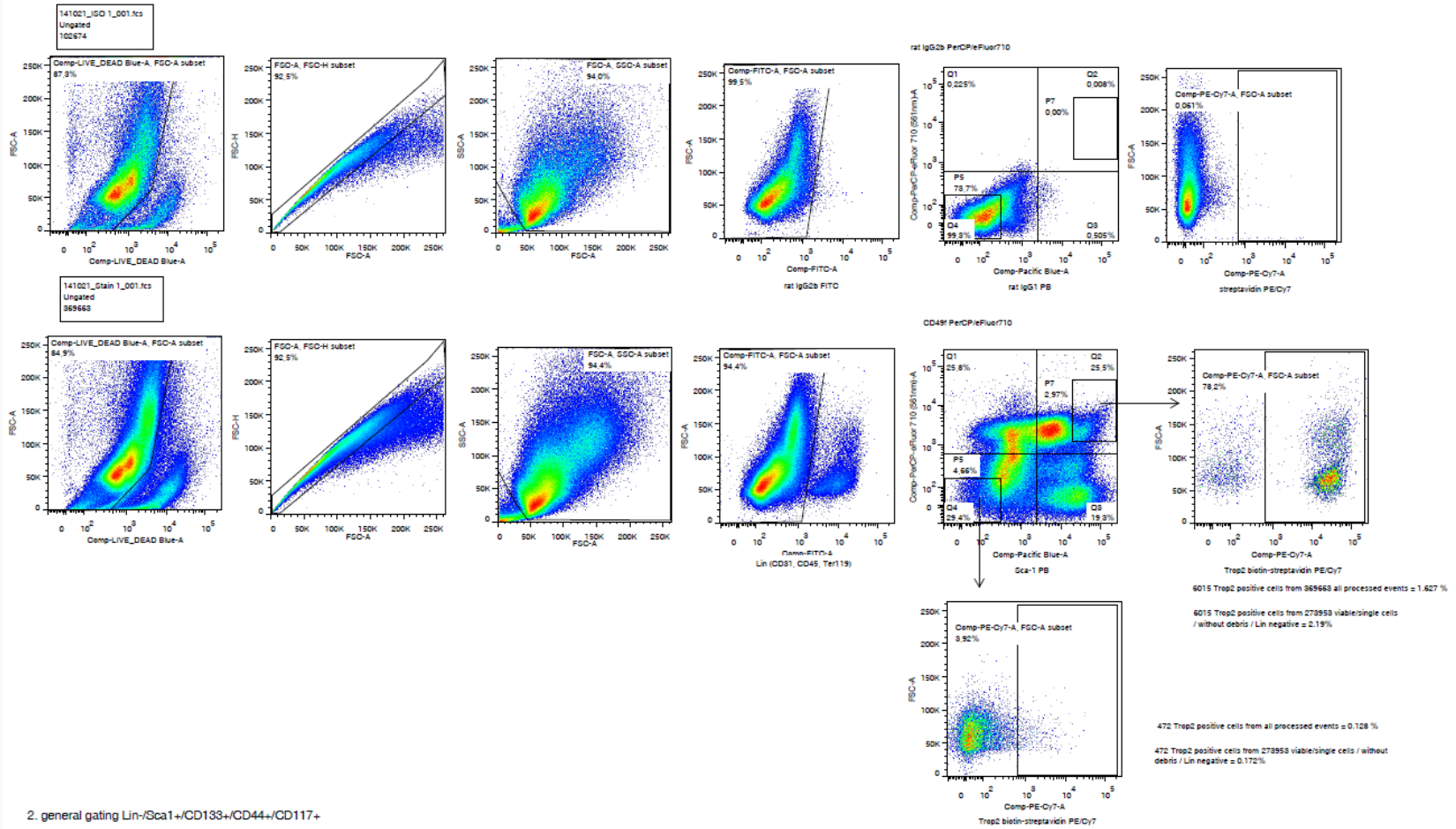
FMO ~ Fluorescence Mines One



Antigen	FITC	PE	Cy5-PE	APC
CD3 FMO	---	CD4	CD8	CD19
CD4 FMO	CD3	---	CD8	CD19
CD8 FMO	CD3	CD4	---	CD19
CD19 FMO	CD3	CD4	CD8	---

# Gating – příklad hodnocení

## 1. general gating Lin-/Sca1+ CD49f+/Trop2+



## 2. general gating Lin-/Sca1+/CD133+/CD44+/CD117-



# 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 Ca<sup>2+</sup>**

stanovení intracelulárních cytokinů

Natural Killer ligace značených buněk

analýza reportérových genů



# Biologické aplikace průtokové cytometrie

- analýza proliferace
- fluorescenční proteiny



# Buněčný cyklus

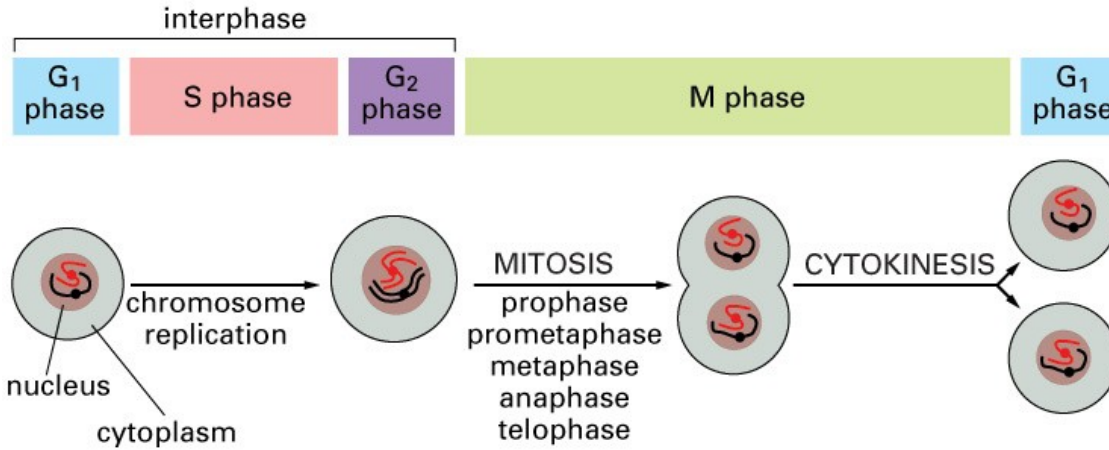


Figure 18-1. Molecular Biology of the Cell, 4th Edition.

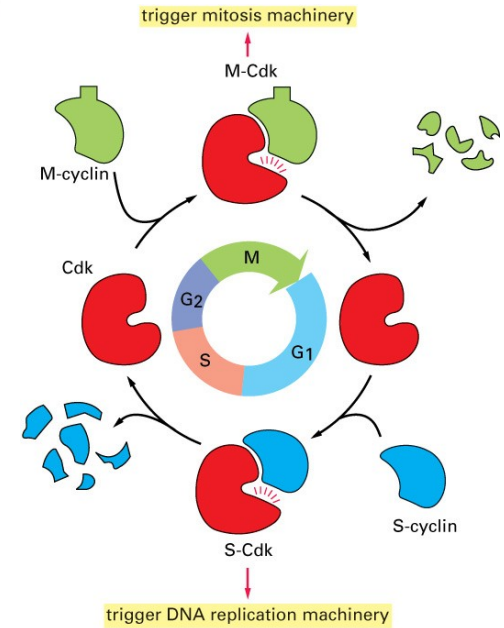
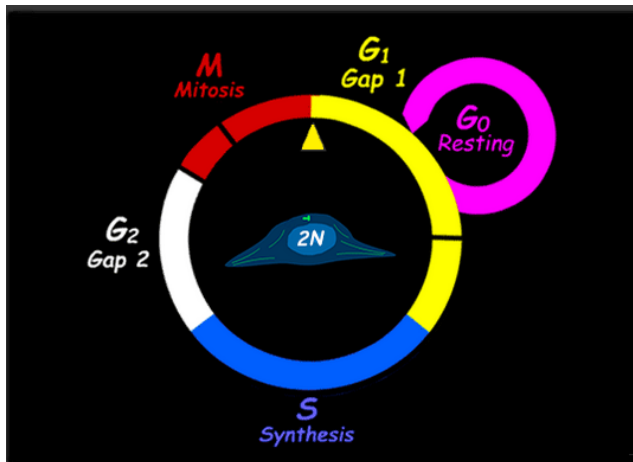


Figure 17-16. Molecular Biology of the Cell, 4th Edition.

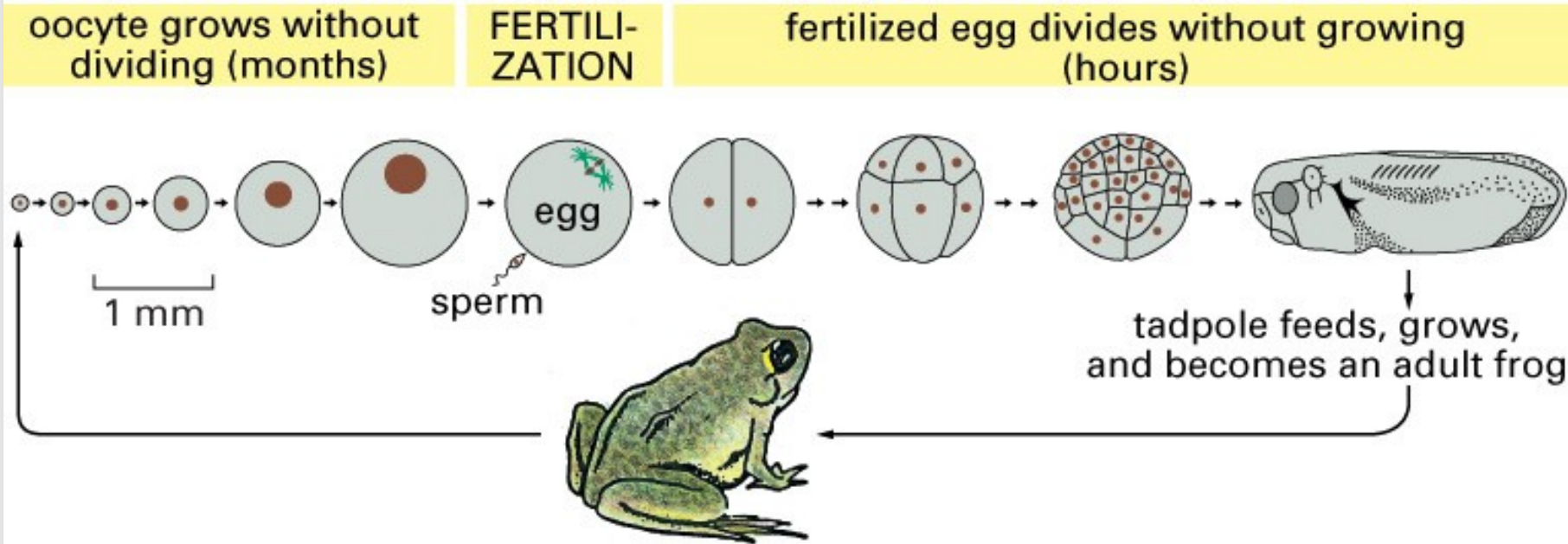
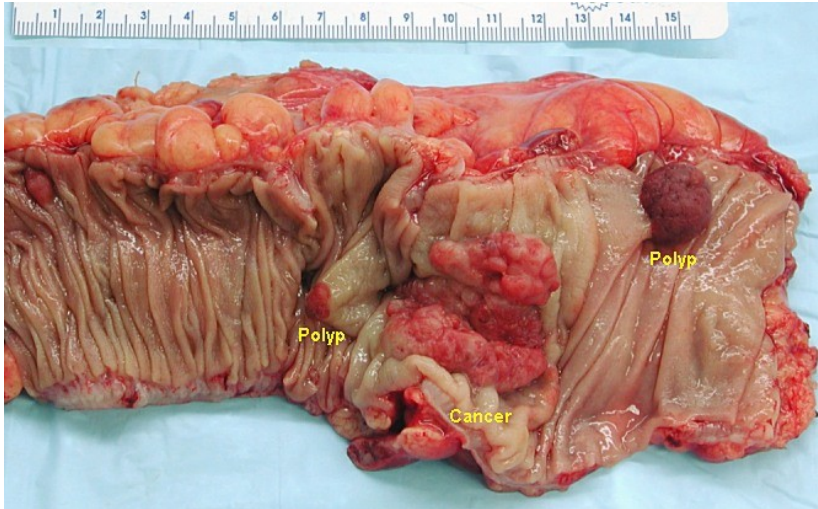


Figure 17-8. Molecular Biology of the Cell, 4th Edition.





# 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™

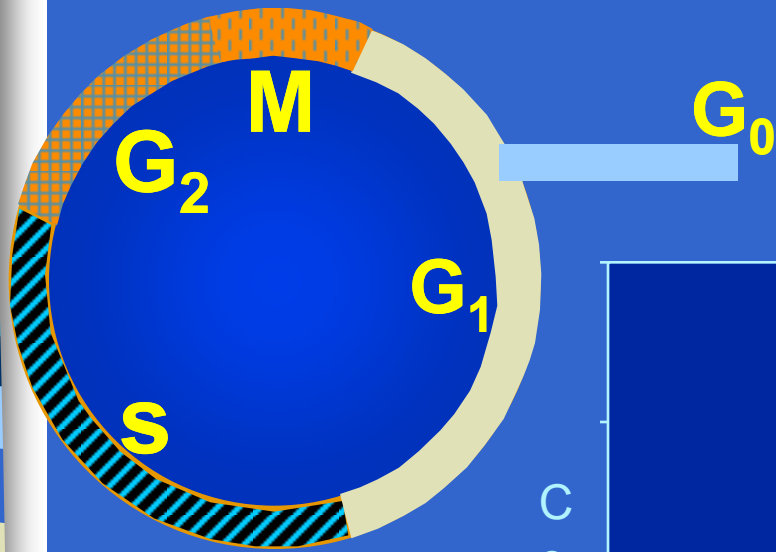
- DRAQ5

- Quaternary benzo[c]phenanthridine alkaloids (QBAs)

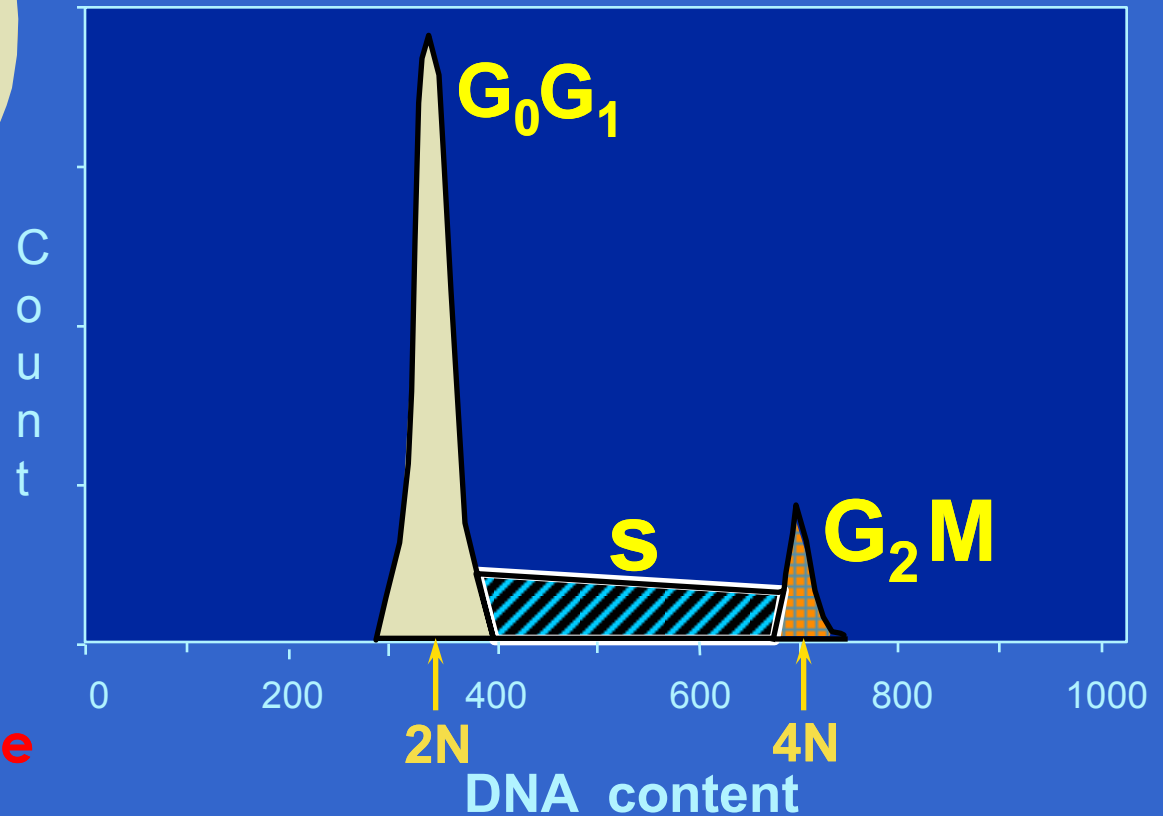
I. Slaninova, J. Slanina and E. Taborska, "Quaternary benzo[c]phenanthridine alkaloids--novel cell permeant and red fluorescing DNA probes," *Cytometry A*, vol. 71, no. 9, pp. 700-708, 2007.

- mohou být používány pro značení viabilních buněk.

# Normal Cell Cycle



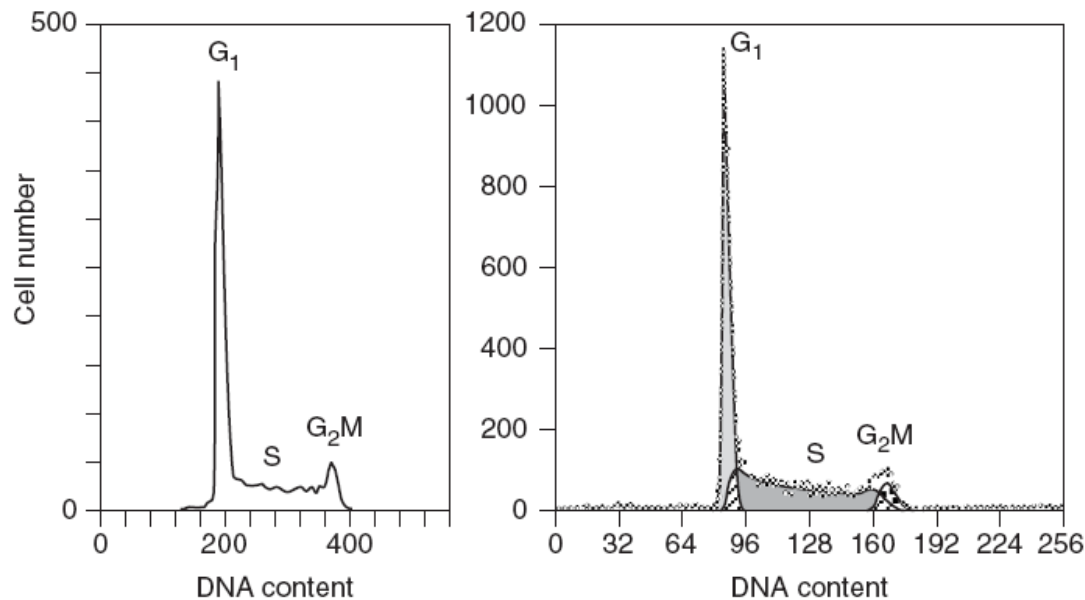
## DNA Analysis



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

# Analýza histogramu buněčného cyklu

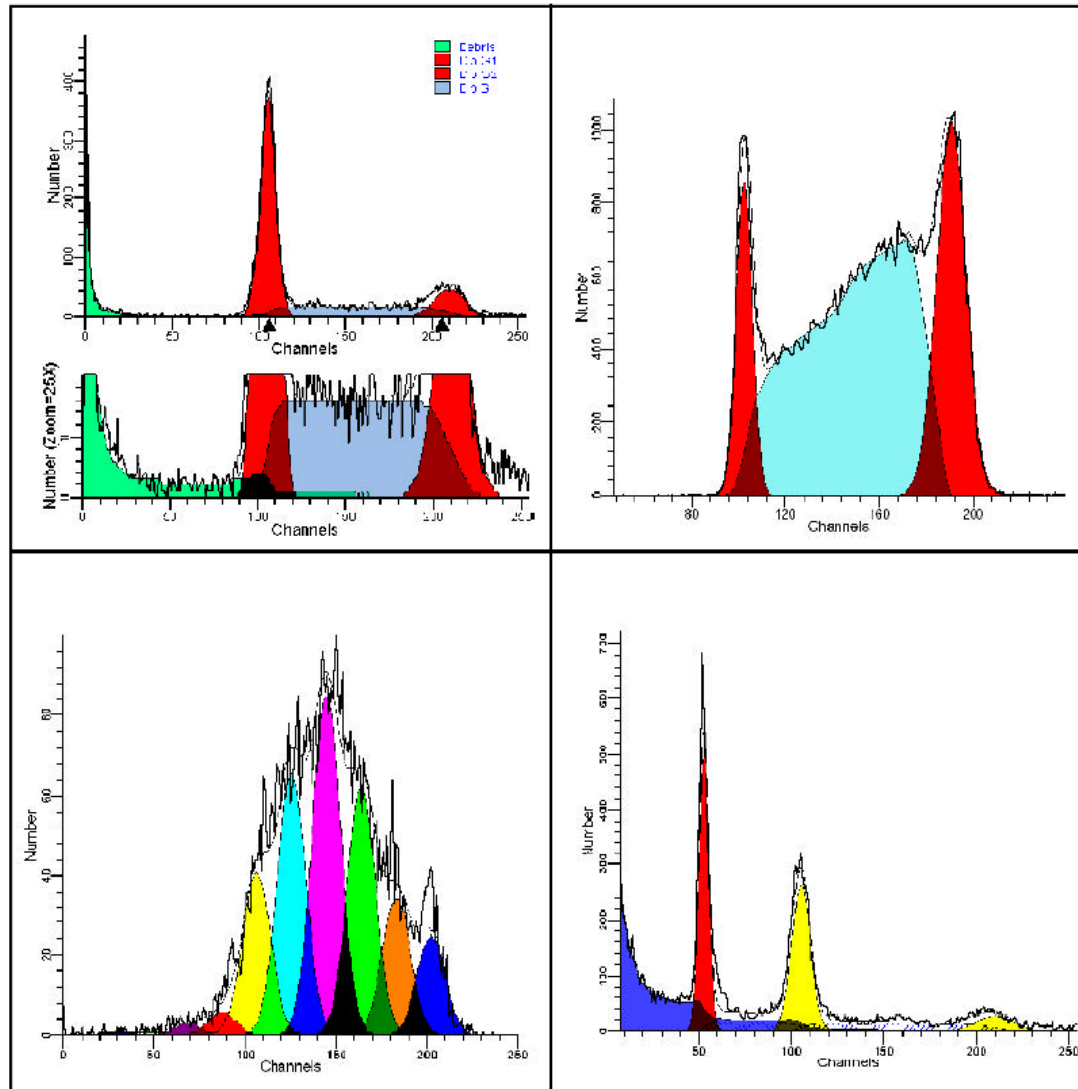
- **nepoužívá** se běžná analýza pomocí úseček (regionů) v histogramu
- **je nutné** používat speciální software pro modelování analýzu distribuce jednotlivých fází



# ModFit LT™



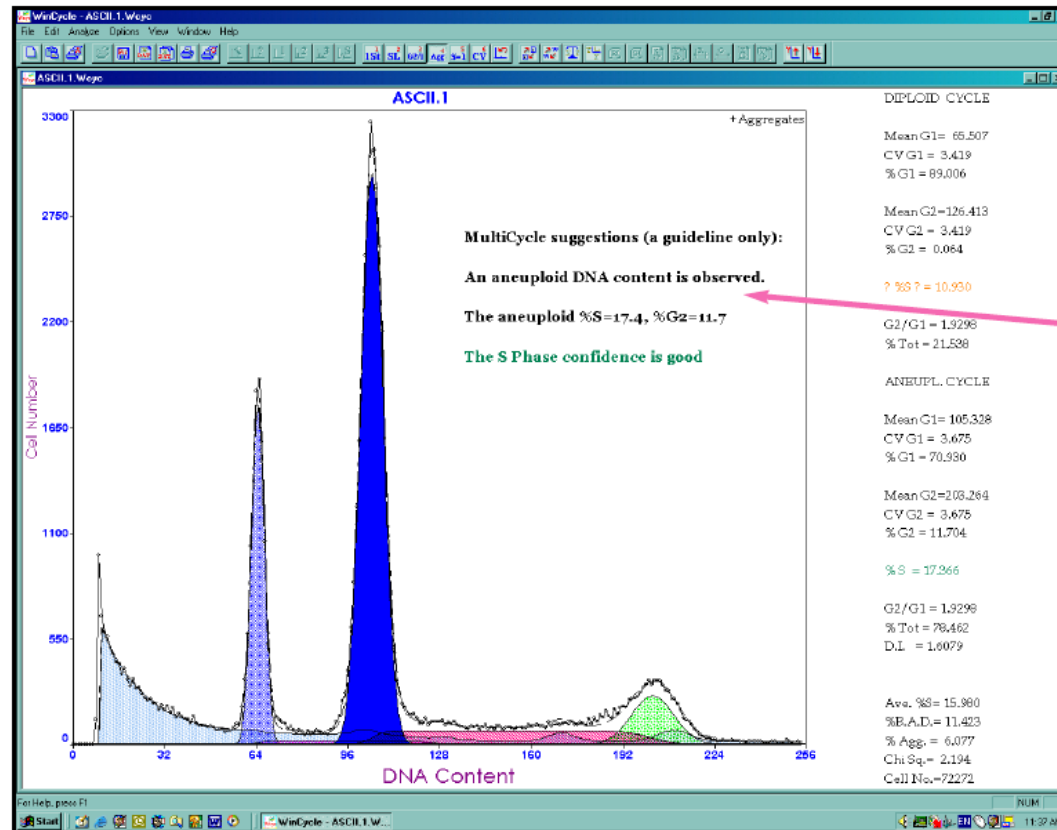
*An impressive new version of the industry standard.*



# MultiCycle for Windows

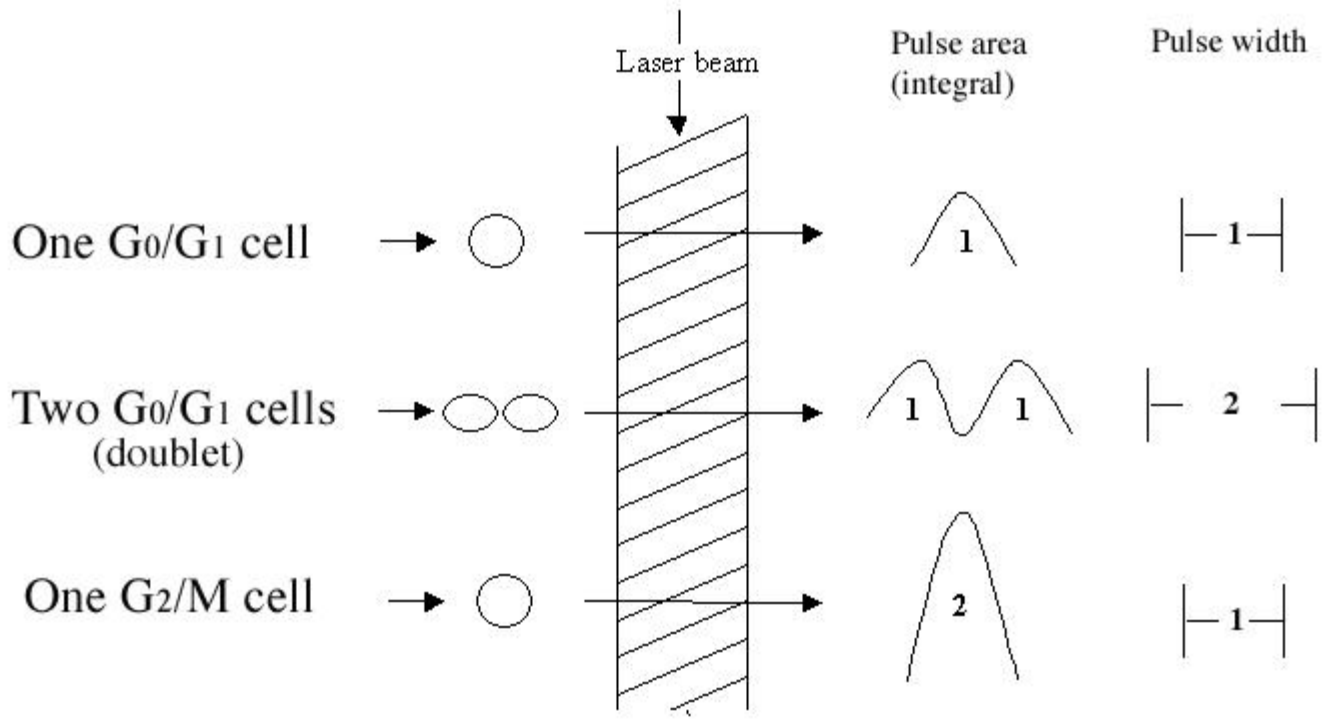
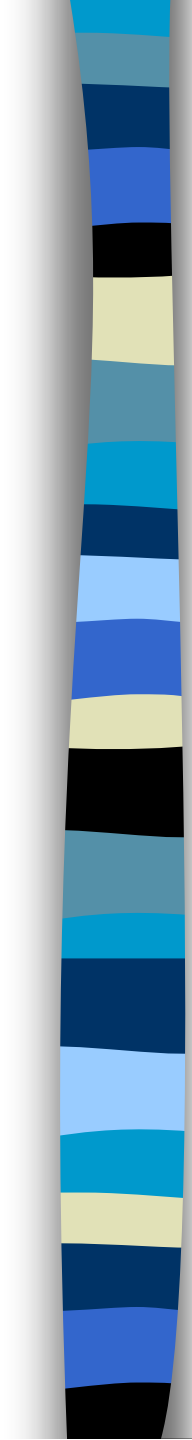
## *Advanced* DNA Cell Cycle Analysis Program

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

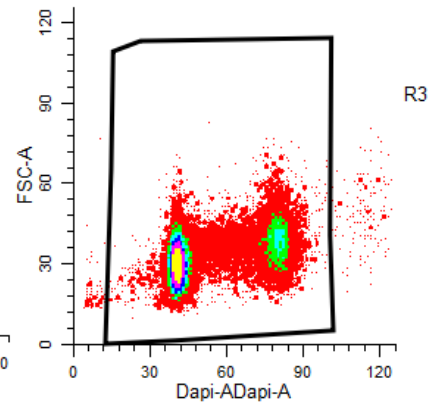
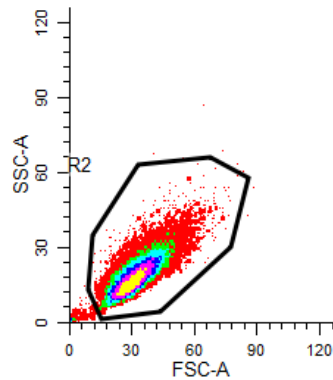
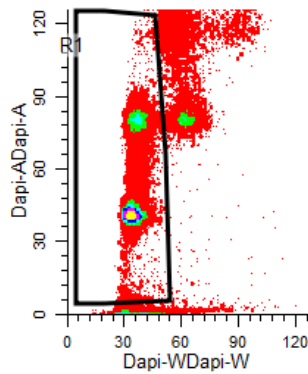
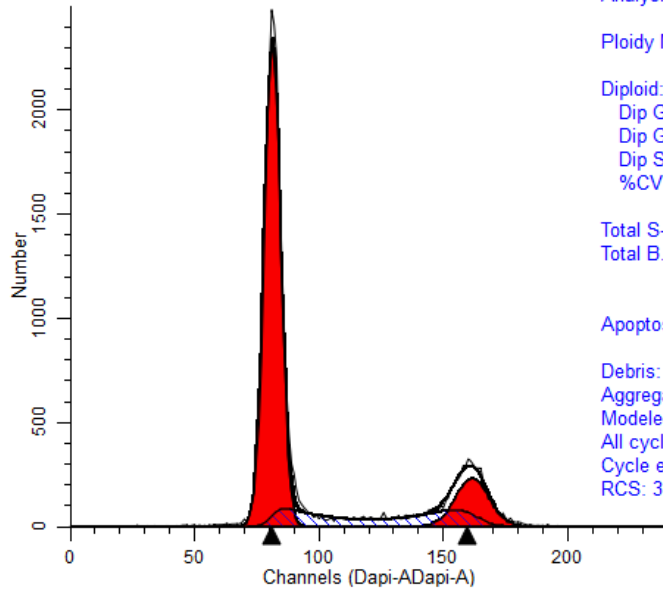
Ploidy Mode: First cycle is diploid

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

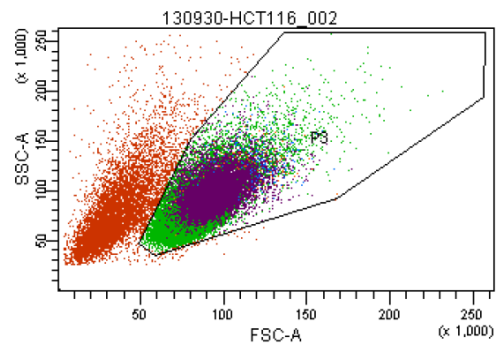
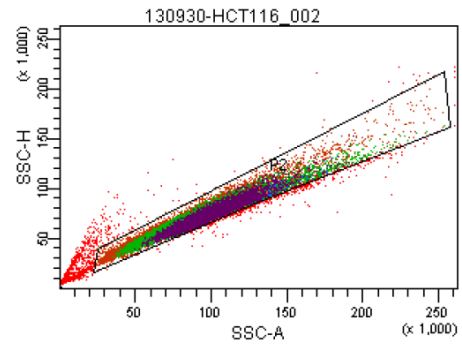
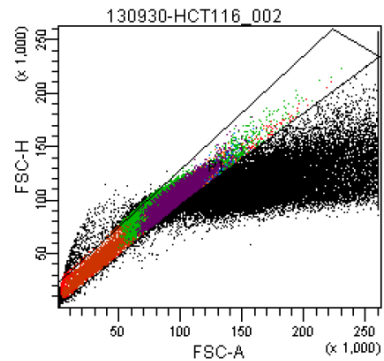
Total S-Phase: 17.76 %  
Total B.A.D.: 0.00 % no debris no aggs

Apoptosis: % Mean:

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



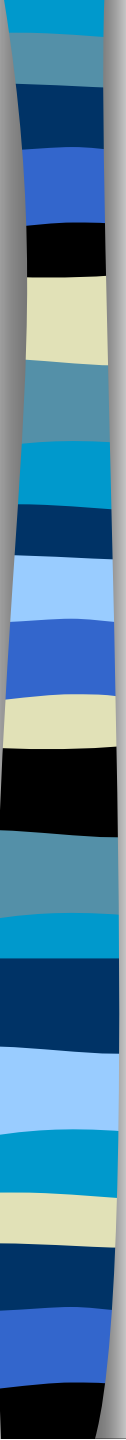
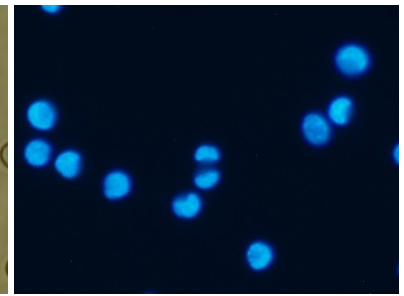
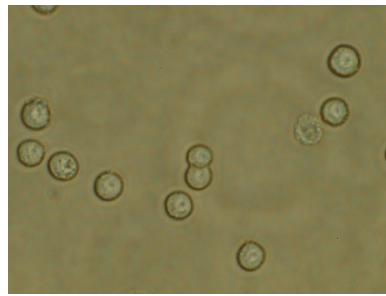
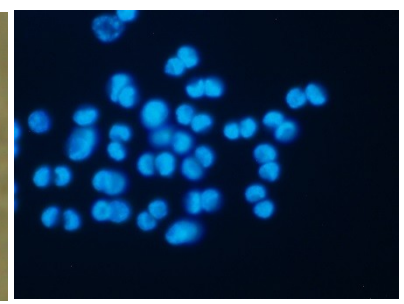
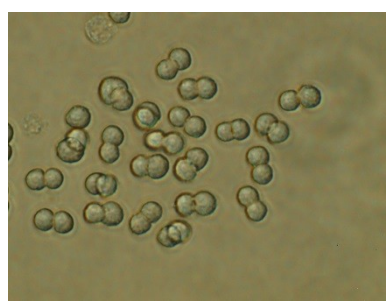
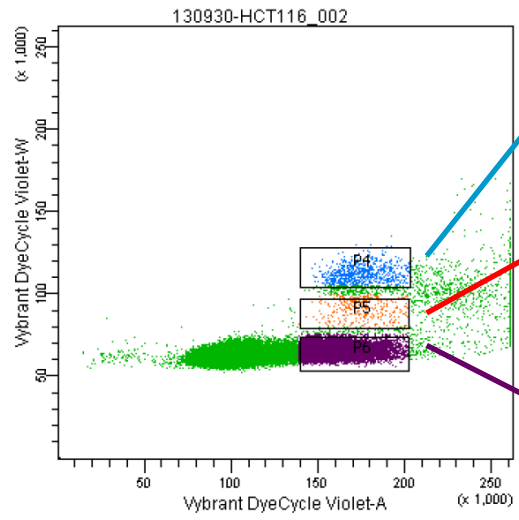
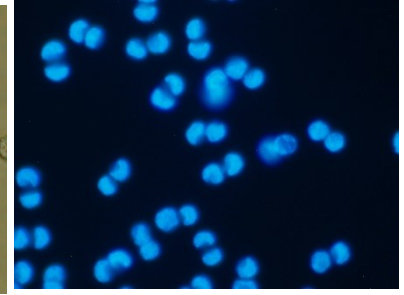
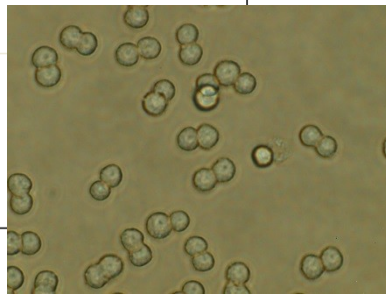
# VybrantDCV\_CellCycleSorting



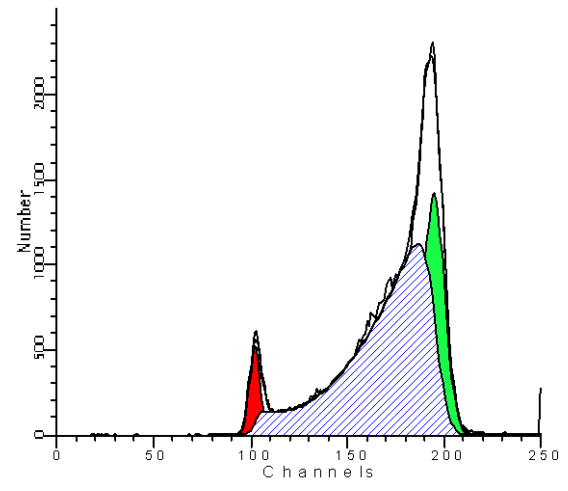
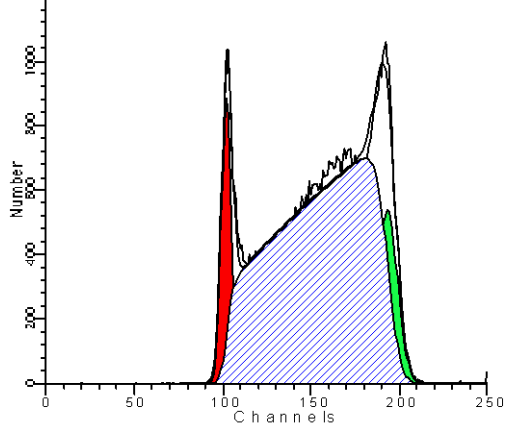
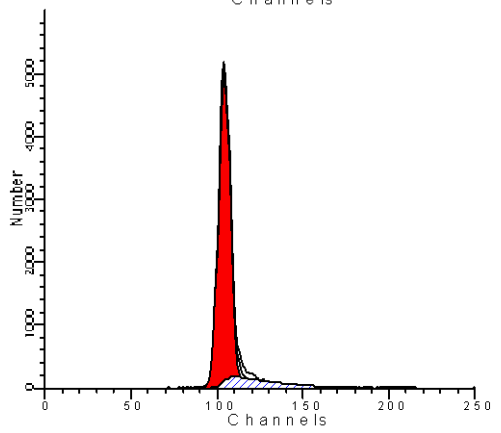
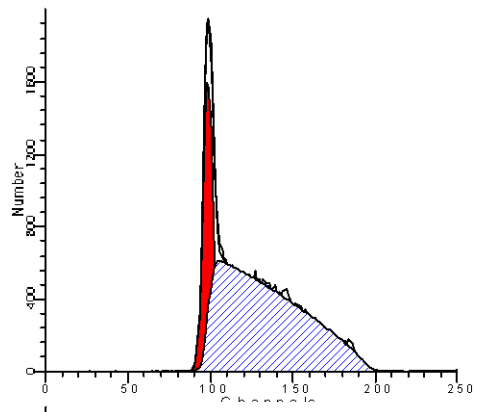
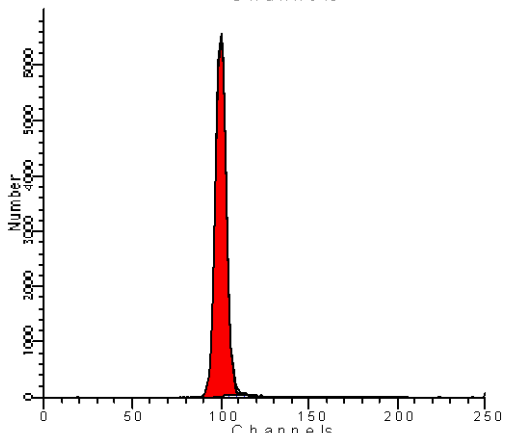
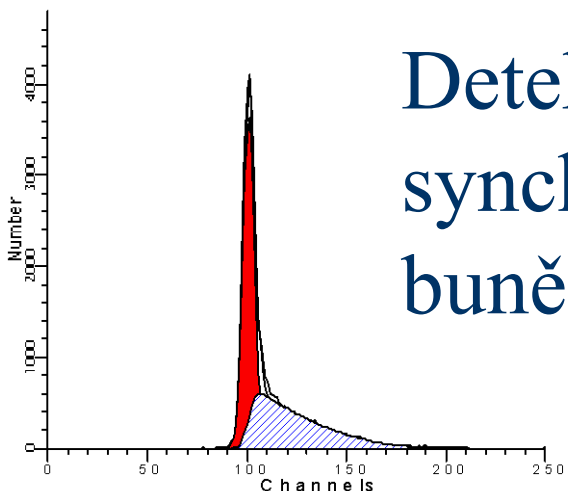
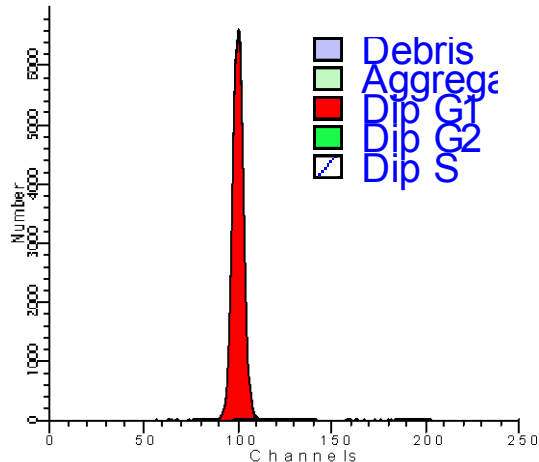
Tube: HCT116\_002

Population

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

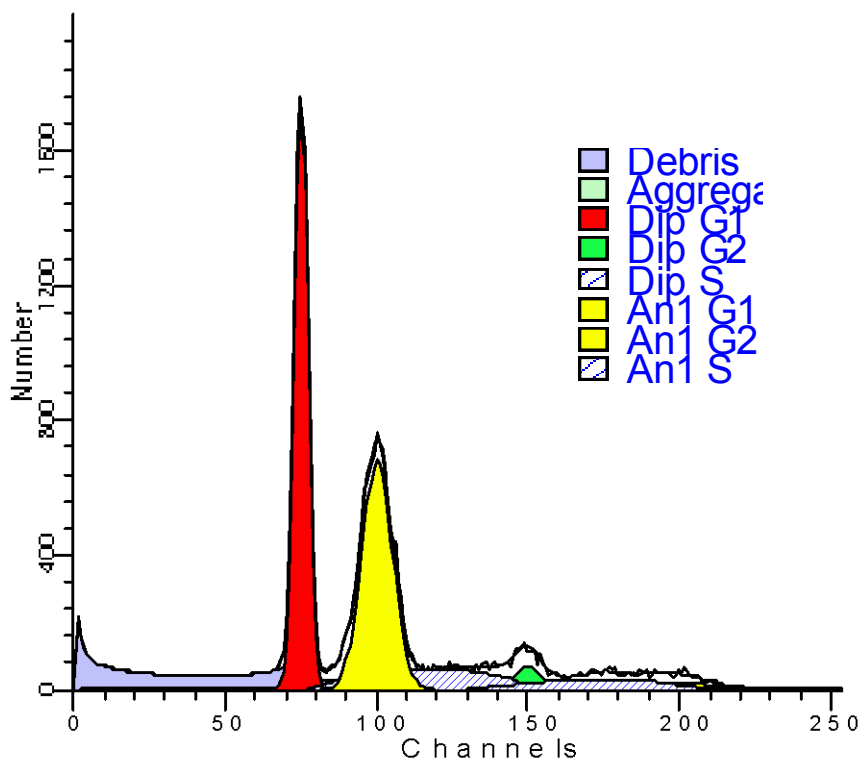


# Detekce buněk v synchronizovaném buněčném cyklu



# 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*

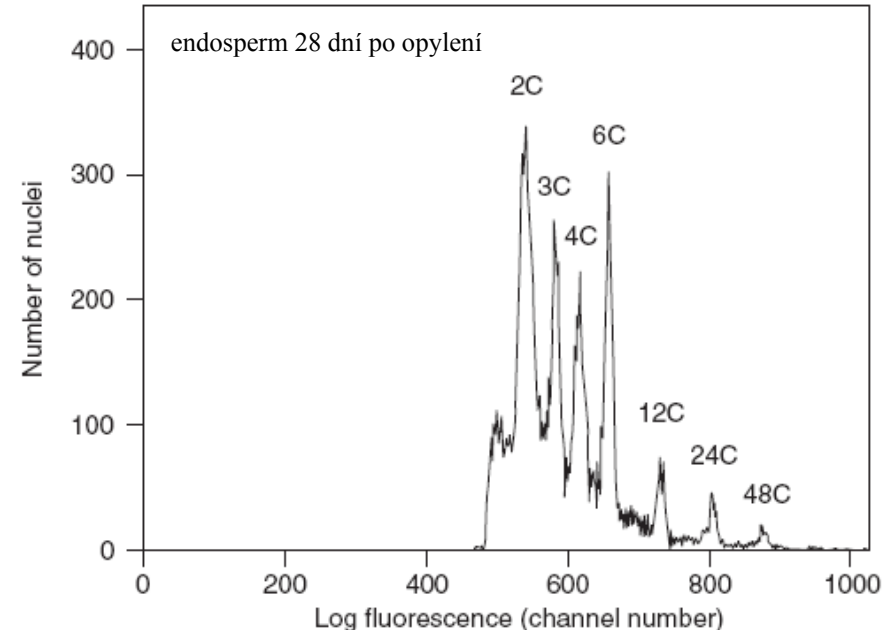
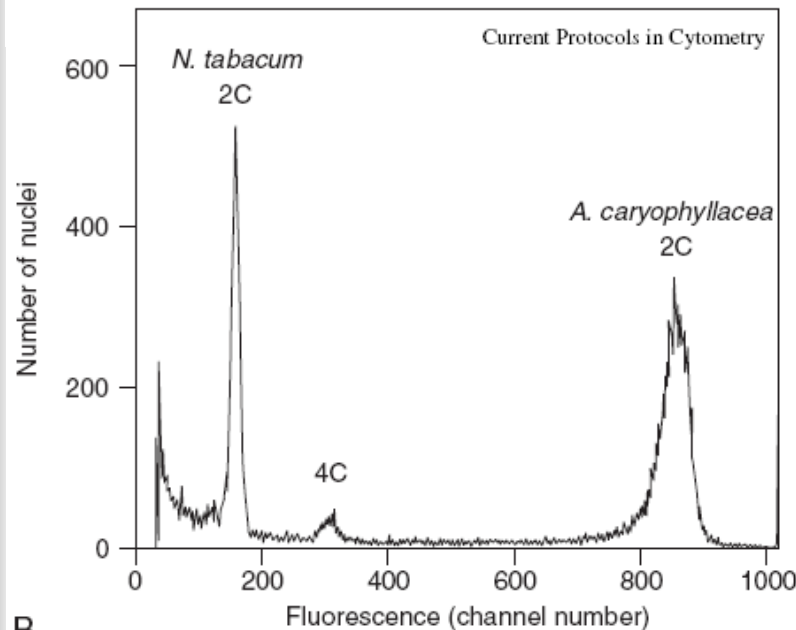


*Alstroemeria caryophyllacea*

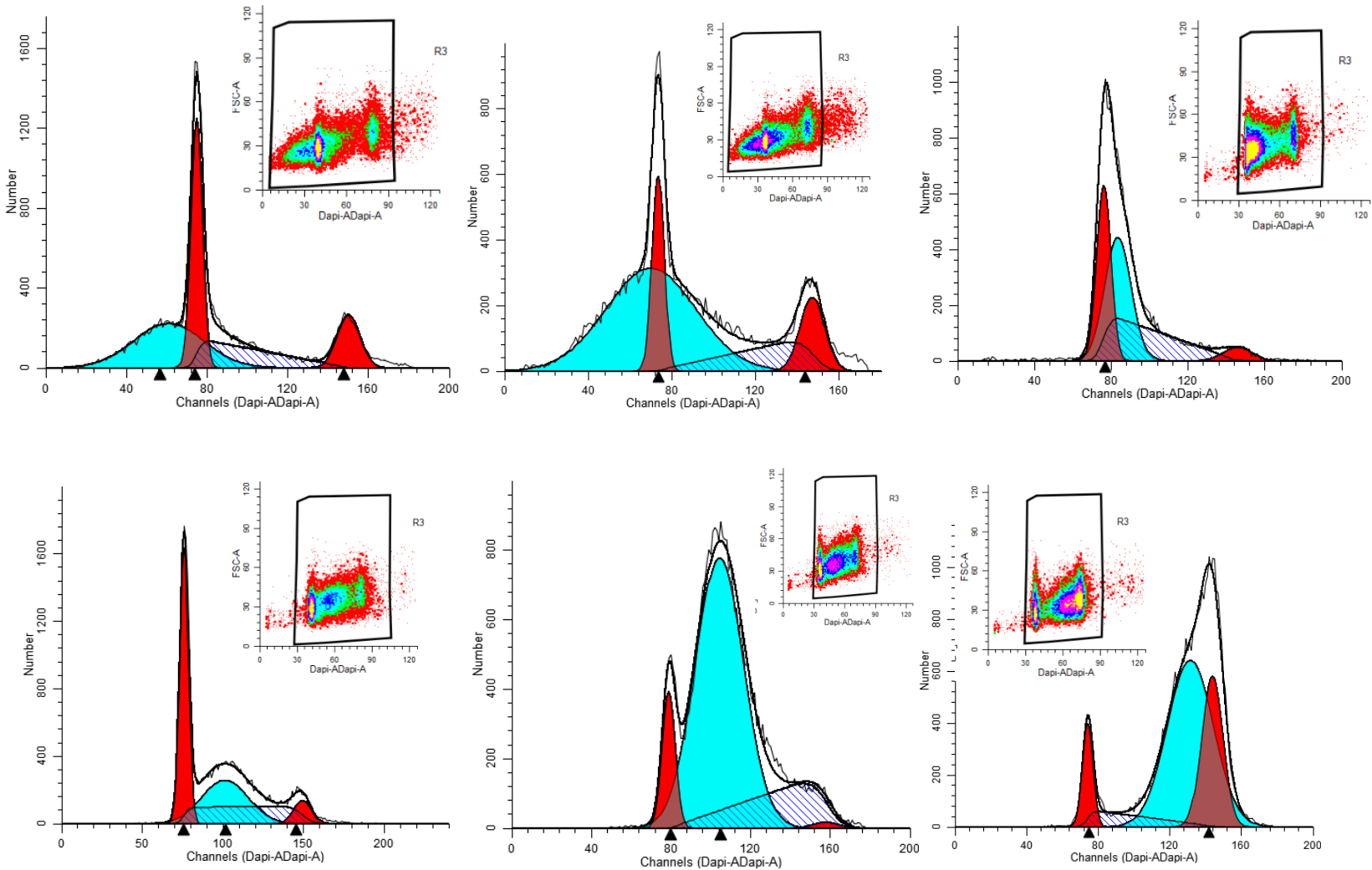


*Zea mays*

CyFlow® Ploidy Analyser



# Cell cycle analysis- limitations



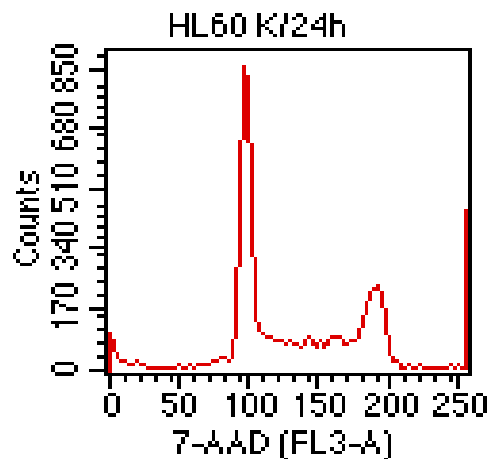
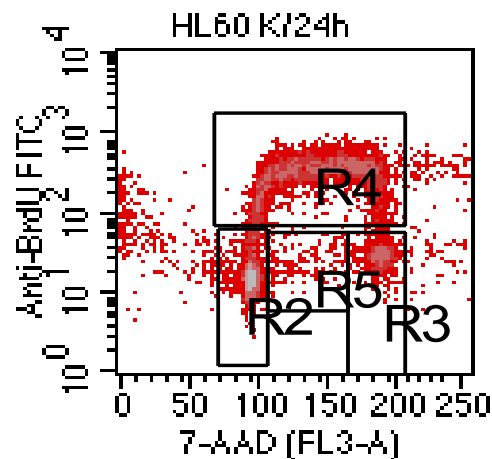
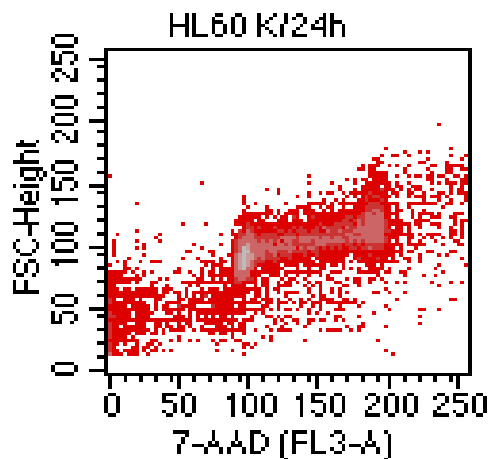


# Analýza inkorporace BrdU

- 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



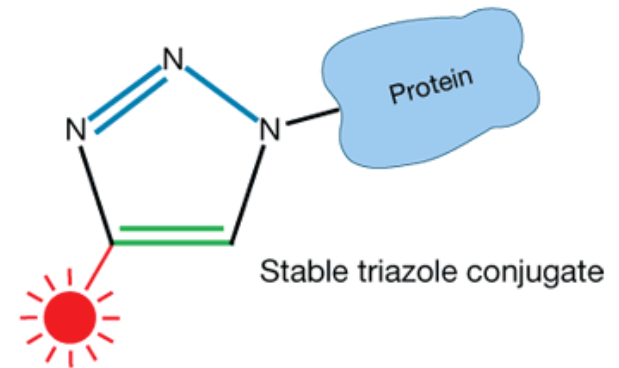
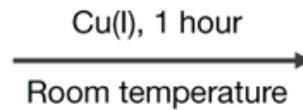
File: HL60 K/24

Regi	% Ga
R1	100.1
R2	35.4
R3	10.2
R4	47.8
R5	1.3

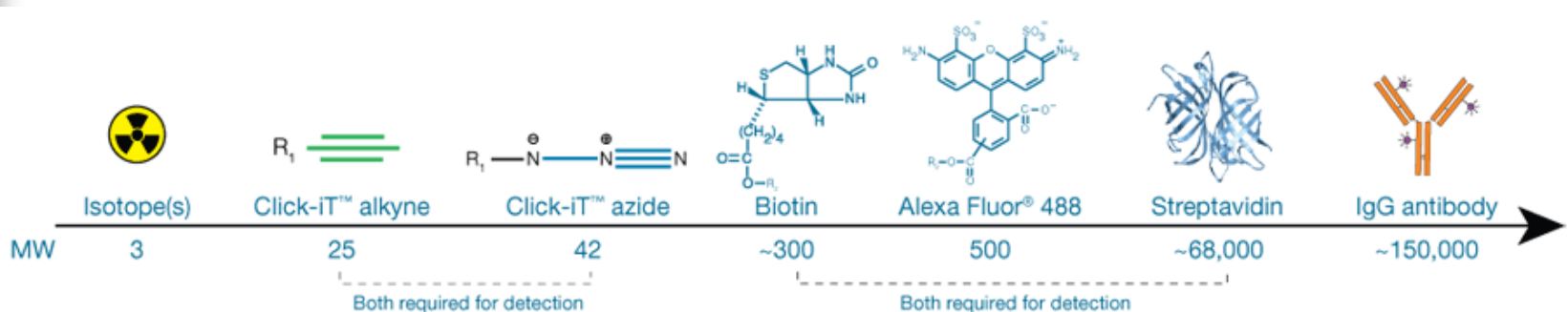
# Click azide/alkyne reaction



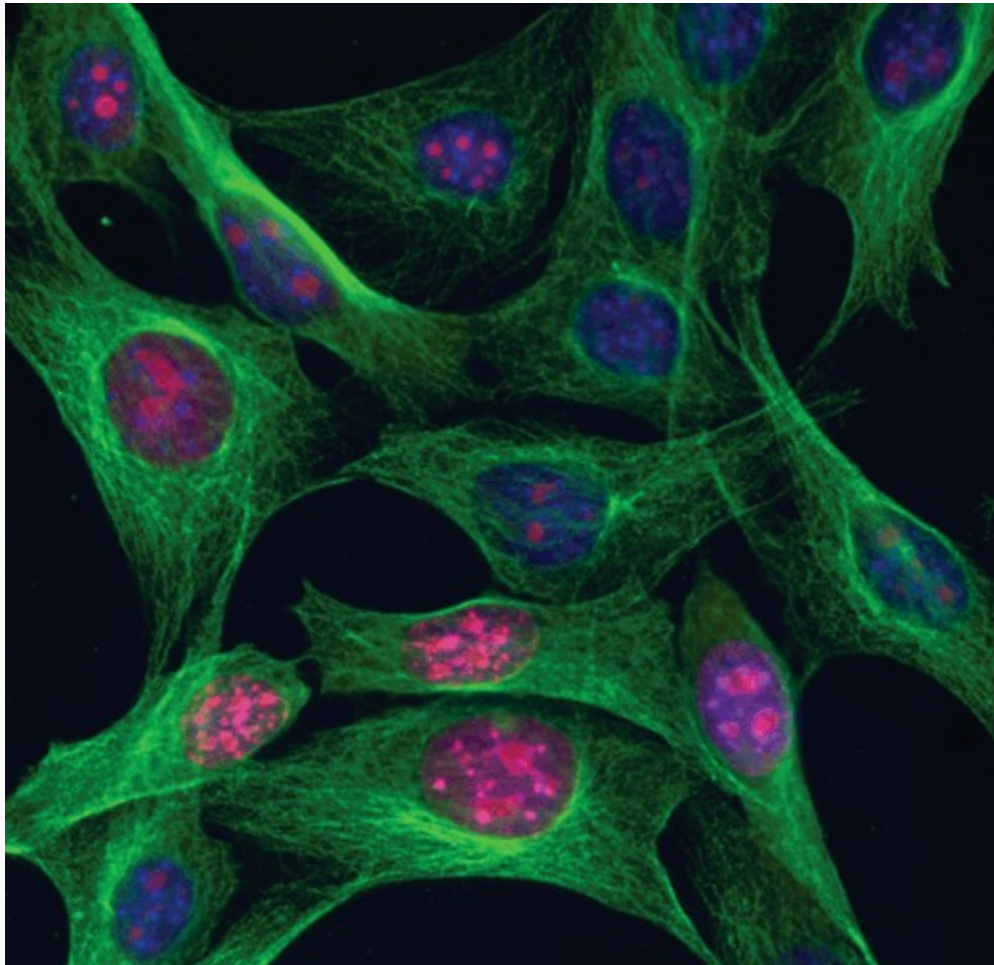
Metabolically or enzymatically  
azide-modified protein



TAMRA, Dapoxyl®, or biotin alkyne



# Aplikace Click-IT (Invitrogen)



## **Multiplex imaging with Click-iT® RNA assays.**

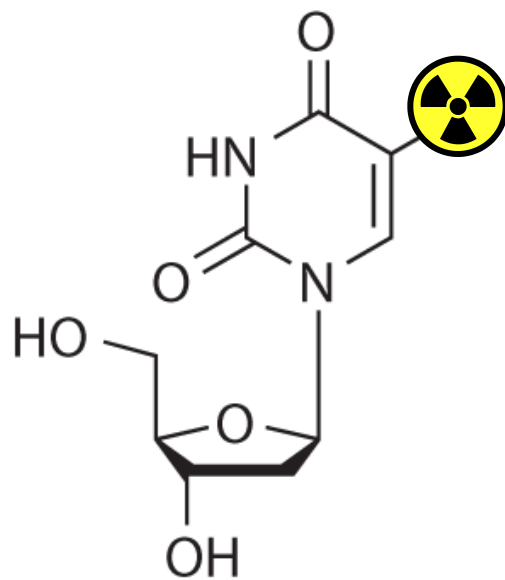
NIH3T3 cells were incubated with 1 mM EU, formaldehyde-fixed, and permeabilized with Triton® X-100. EU incorporated into newly synthesized RNA (red) in some cells was detected using the Click-iT® RNA Alexa Fluor® 594 Imaging Kit. Tubulin (green) was detected with anti-tubulin mouse IgG9 and visualized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



# Aplikace Click-IT (Invitrogen)

analýza syntézy DNA  
(proliferace)

# $^3\text{H}$ -thymidine



Tritiated ( $^3\text{H}$ ) thymidine

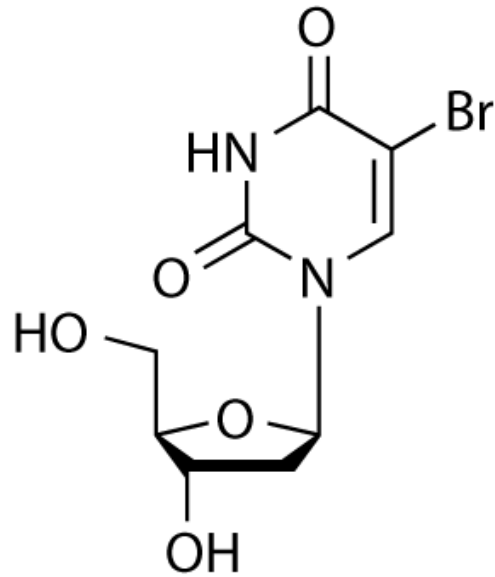


## **$^3\text{H}$ -thymidine**

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



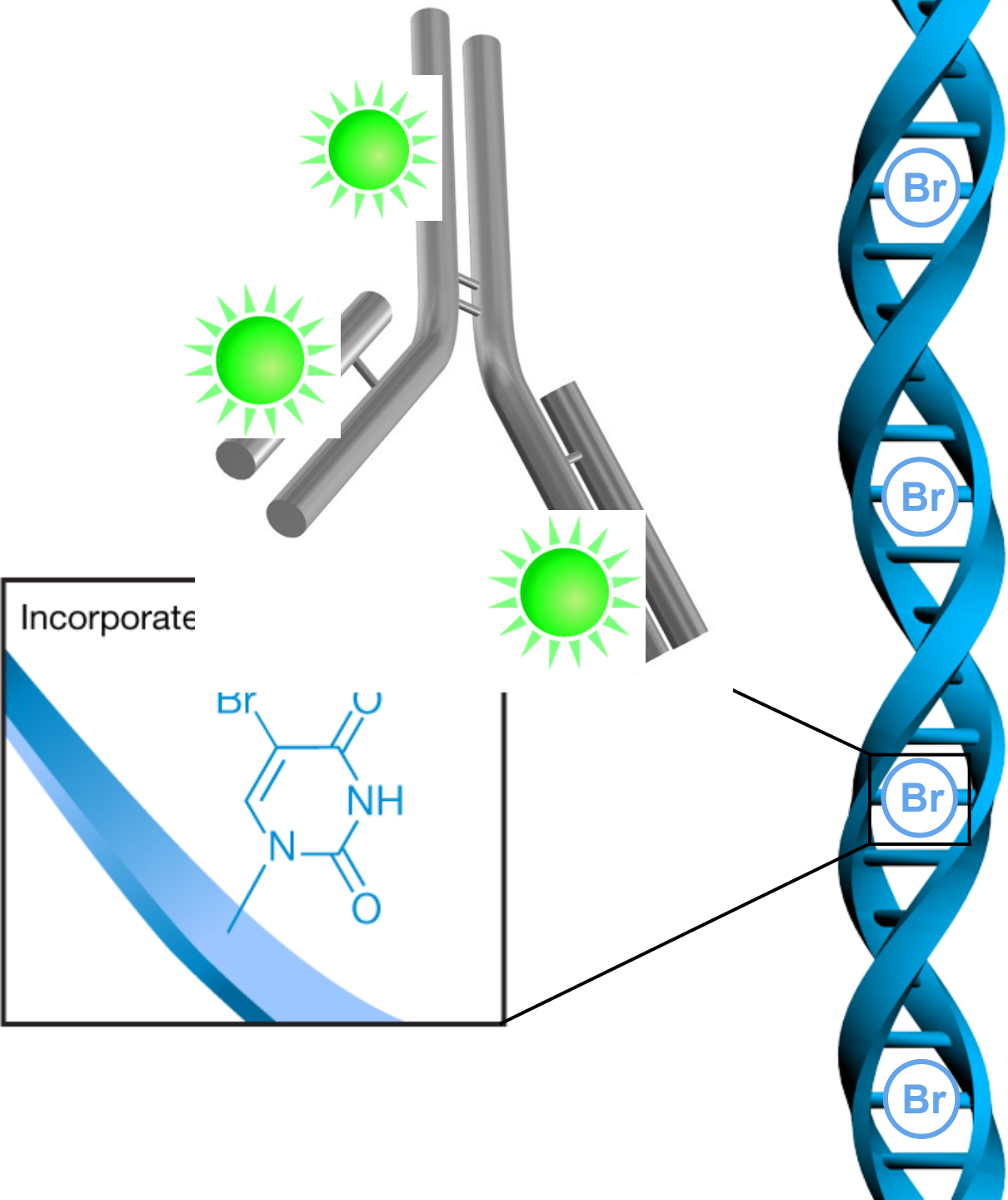
**BrdU**



**BrdU (5-bromo-2'-deoxyuridine)**



**BrdU**



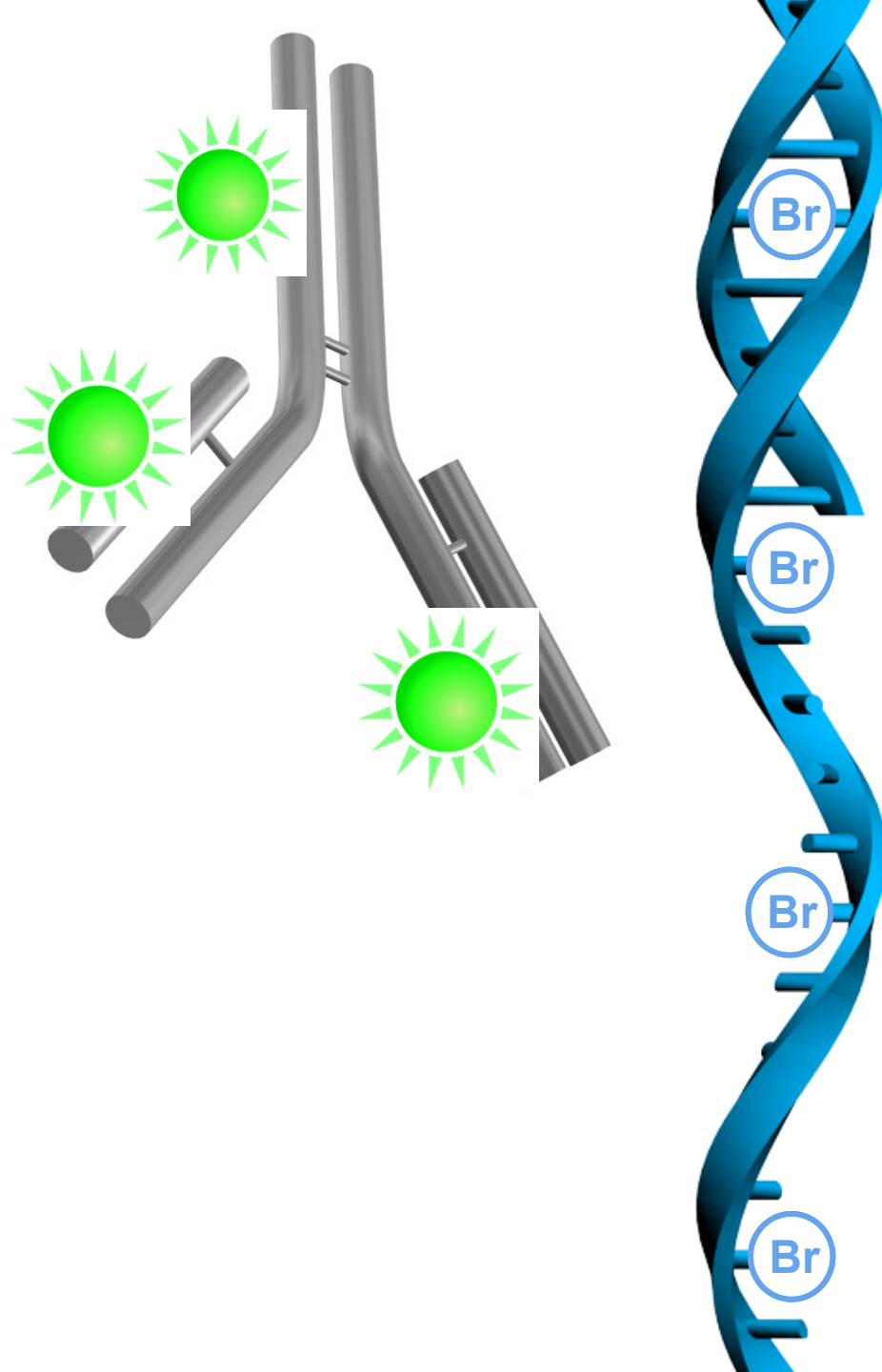


**BrdU**

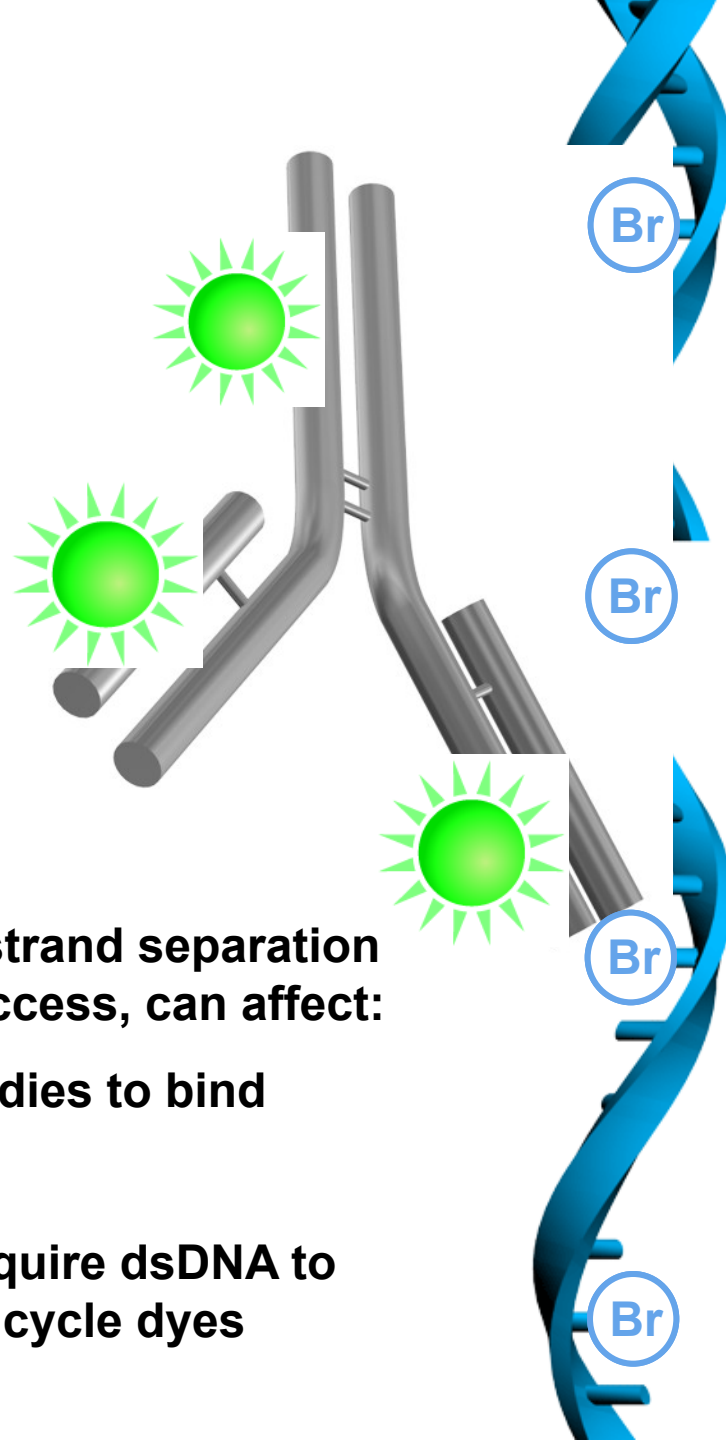
*Acid or DNase*



**BrdU**

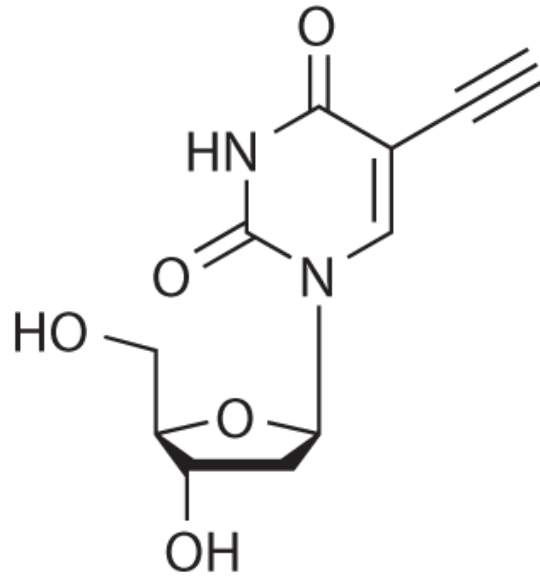


# BrdU



- **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**

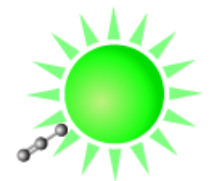
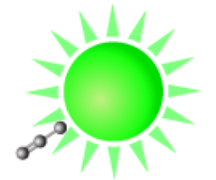
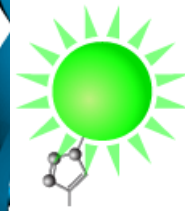
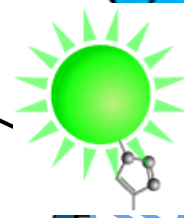
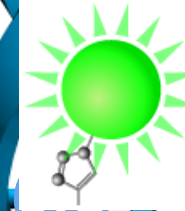
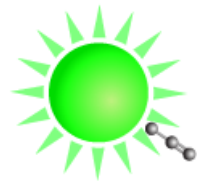
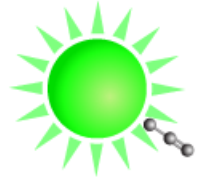
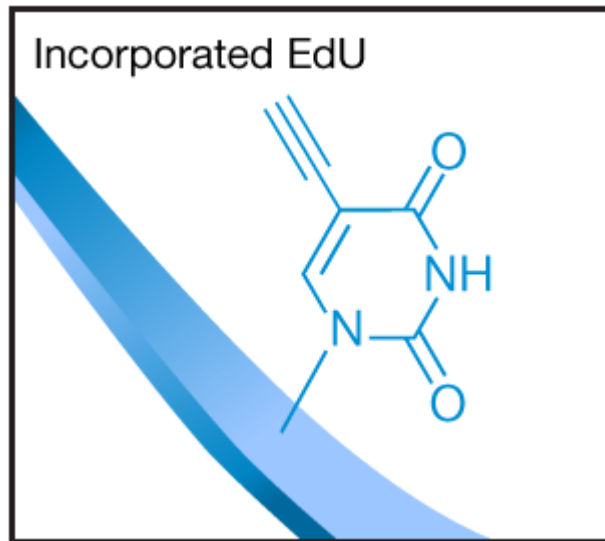
# Click-iT™ EdU



**EdU (5-ethynyl-2'-deoxyuridine)**

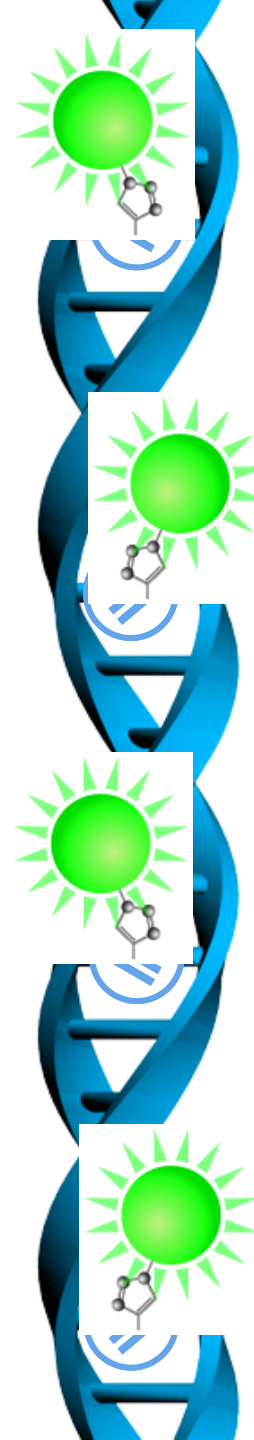


# Click-iT™ EdU



## Click-iT™ Edu

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



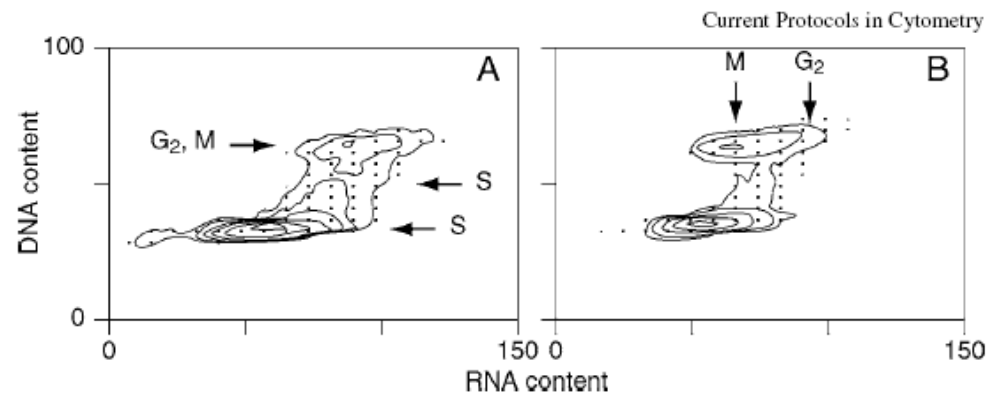
# Analýza DNA a RNA

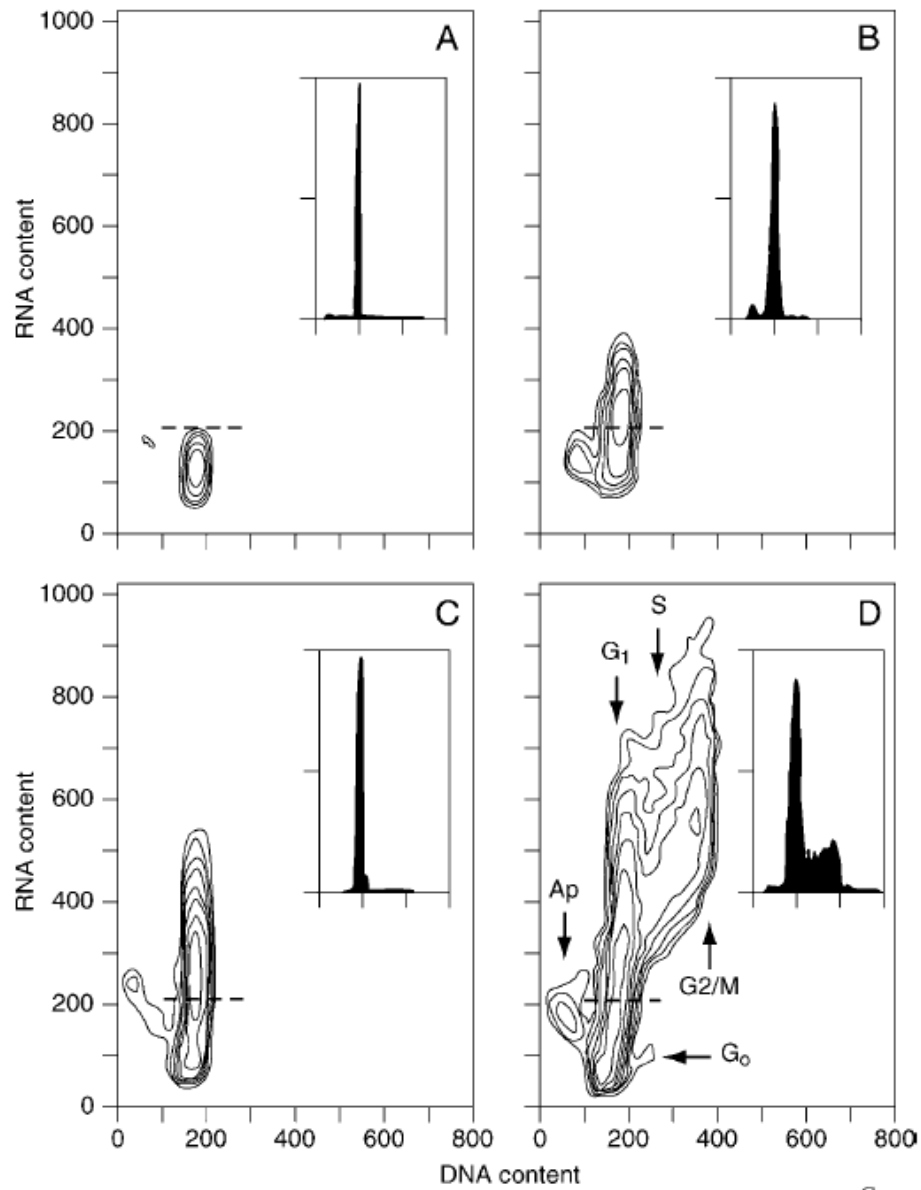
## Pyronin Y vs. Hoechst 33342

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

## ■ Acridine orange

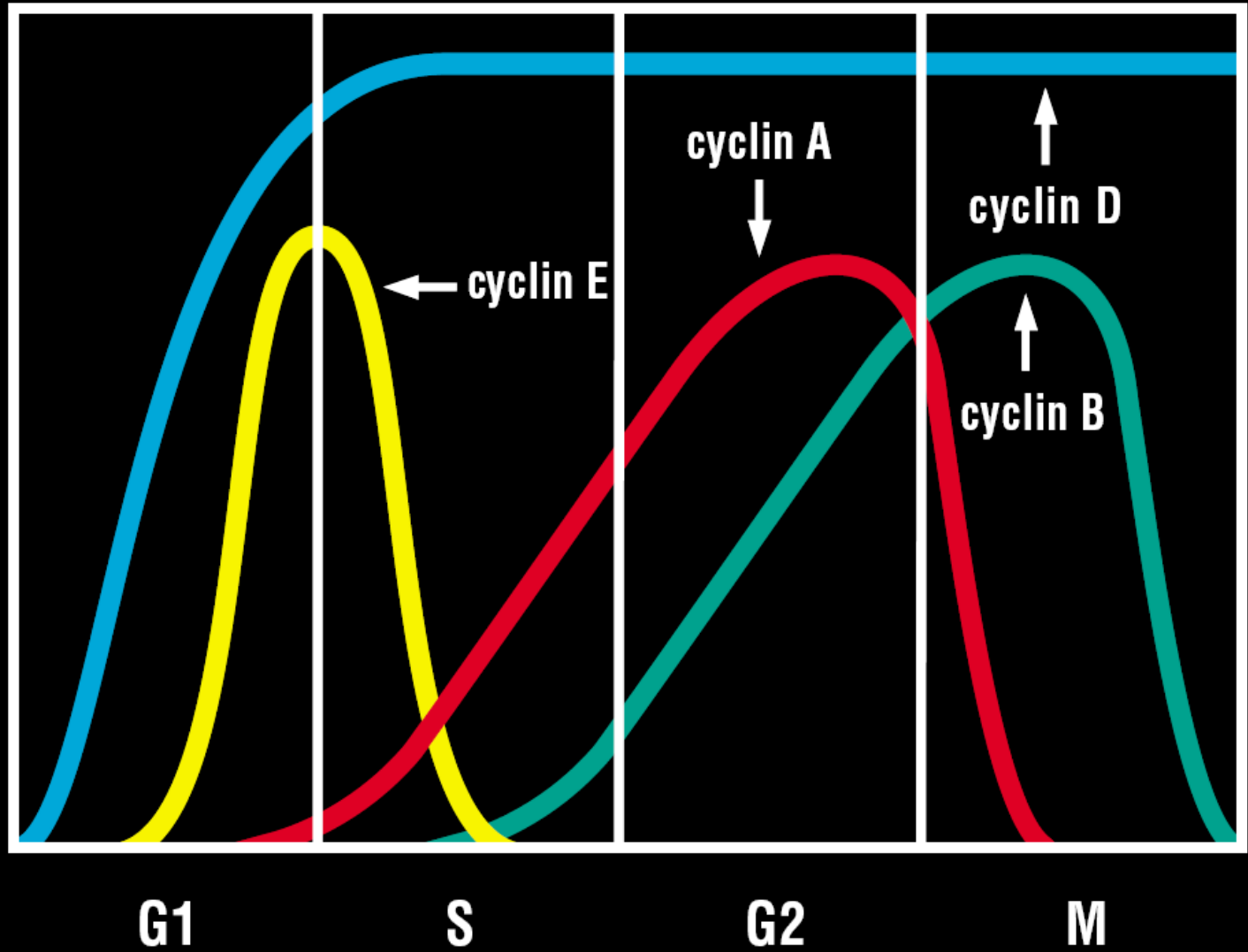
- při interakci s RNA emituje červené světlo a při interakci s DNA zelené



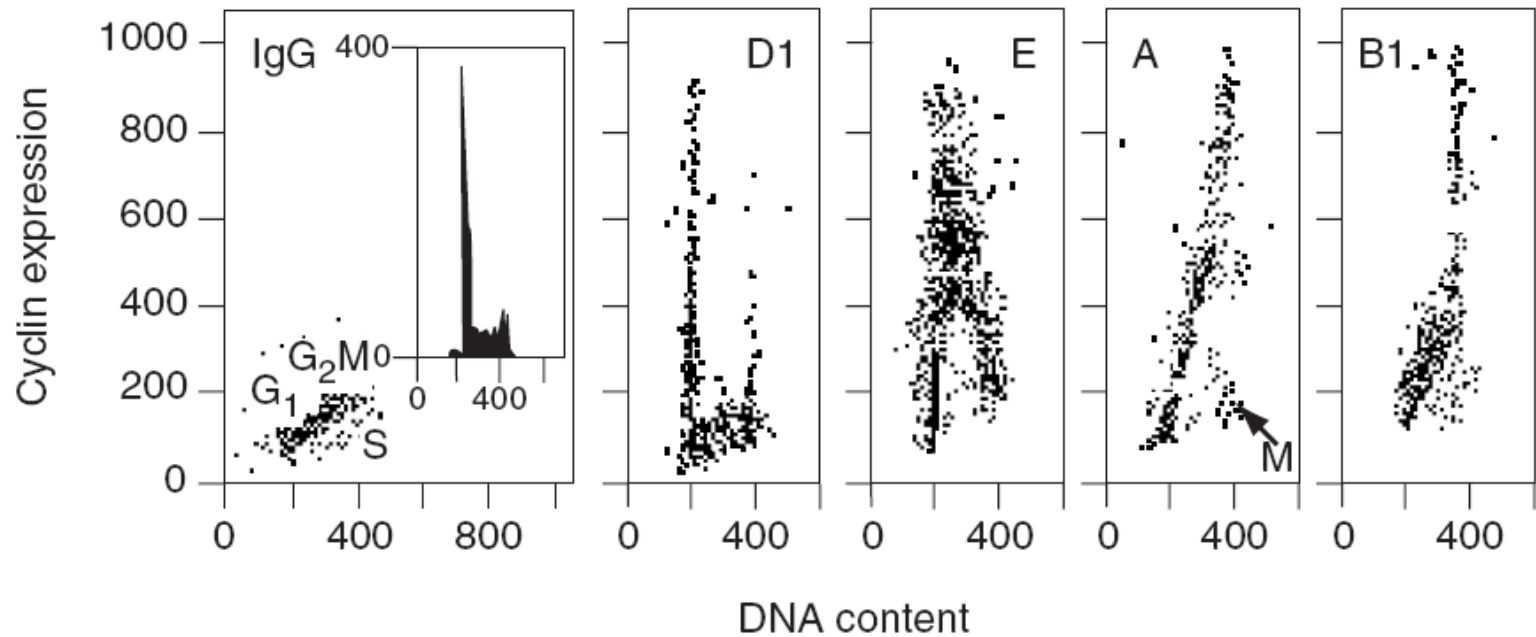




## Cyclin Expression: Periodicity

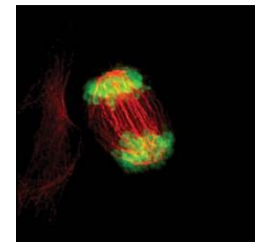
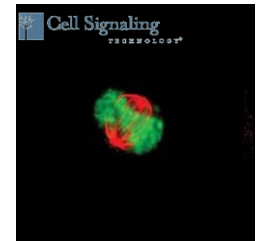
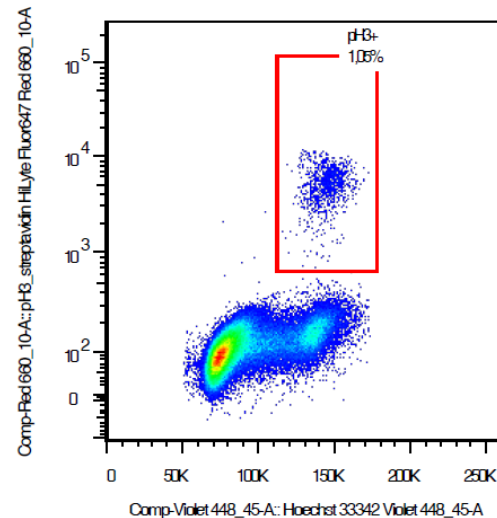
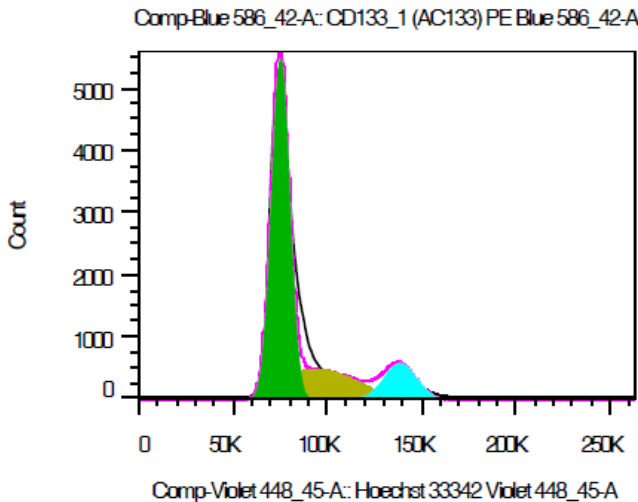


# Detekce intracelulárních proteinů v kombinaci s detekcí DNA



# Detekce mitotických buněk

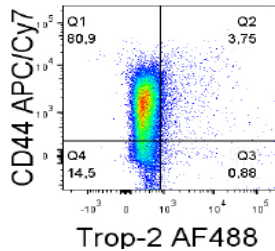
- Histone H3 je specificky fosforylován během mitózy (Ser10, Ser28, Thr11)
- dvojité značení DNA vs. H3-P identifikuje populaci buněk v M-fázi



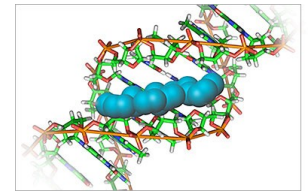
# Flow cytometry

## most common applications

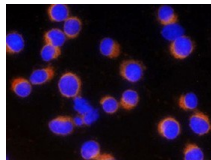
Immunophenotype characterisation of the cells  
(CSCs markers, differentiation, ...)



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



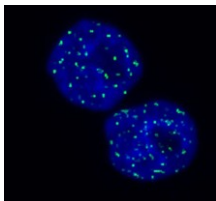
Cell Death analysis  
(AnnexinV, Cleaved Caspase3, ...)



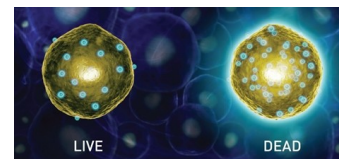
Proliferation (BrdU, EdU, mitosis - pH3)



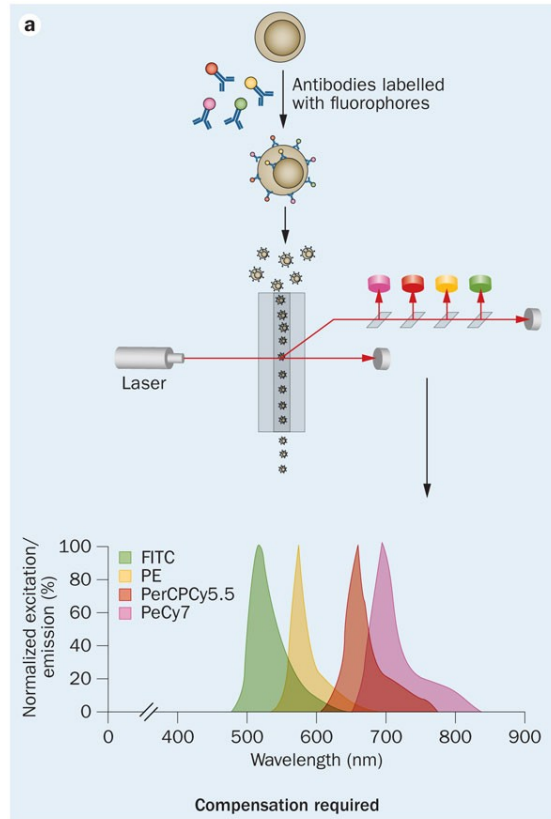
DNA damage ( $\gamma$ H2AX,...)



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



# IMMUNOPHENOTYPING



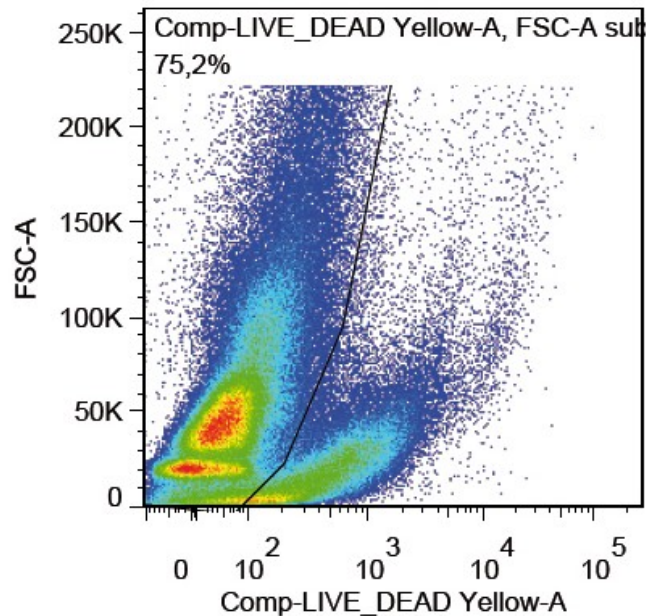
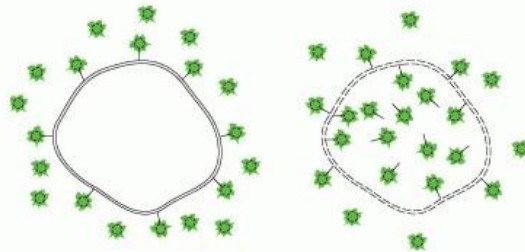
Ermann, J. *et al.* (2015) Immune cell profiling to guide therapeutic decisions in rheumatic diseases  
*Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2015.71

**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

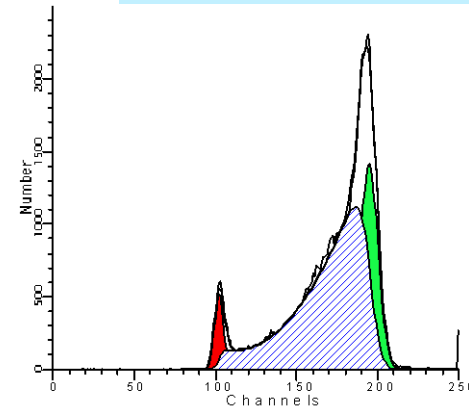
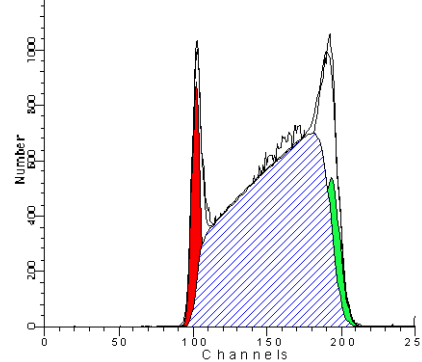
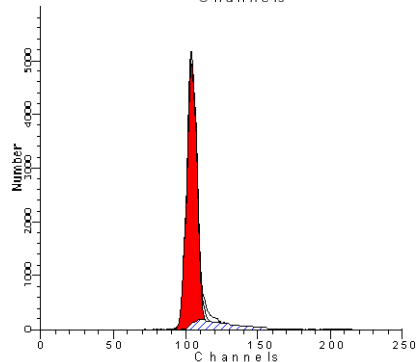
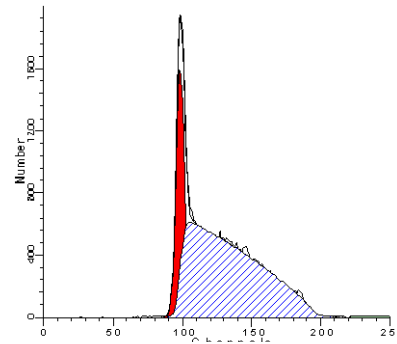
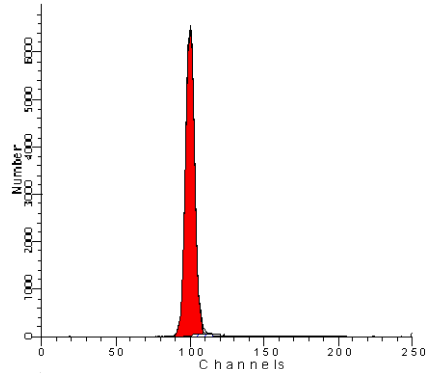
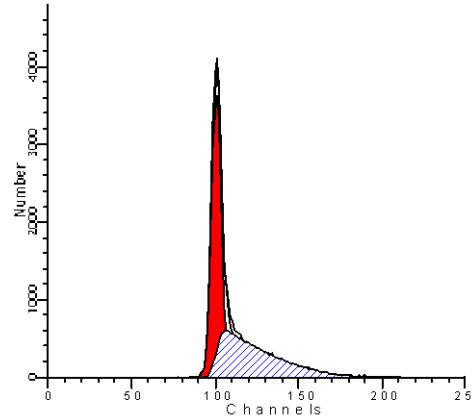
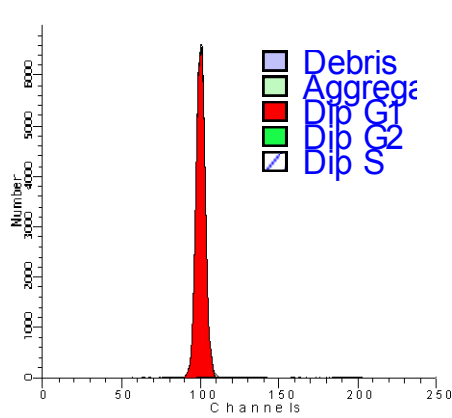


**Principle:** reaction of a fluorescent reactive dye with cellular amines, in necrotic cells react with free amines both in the interior and on the cell = intense staining, live cells stained on surface only = dim signal

**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

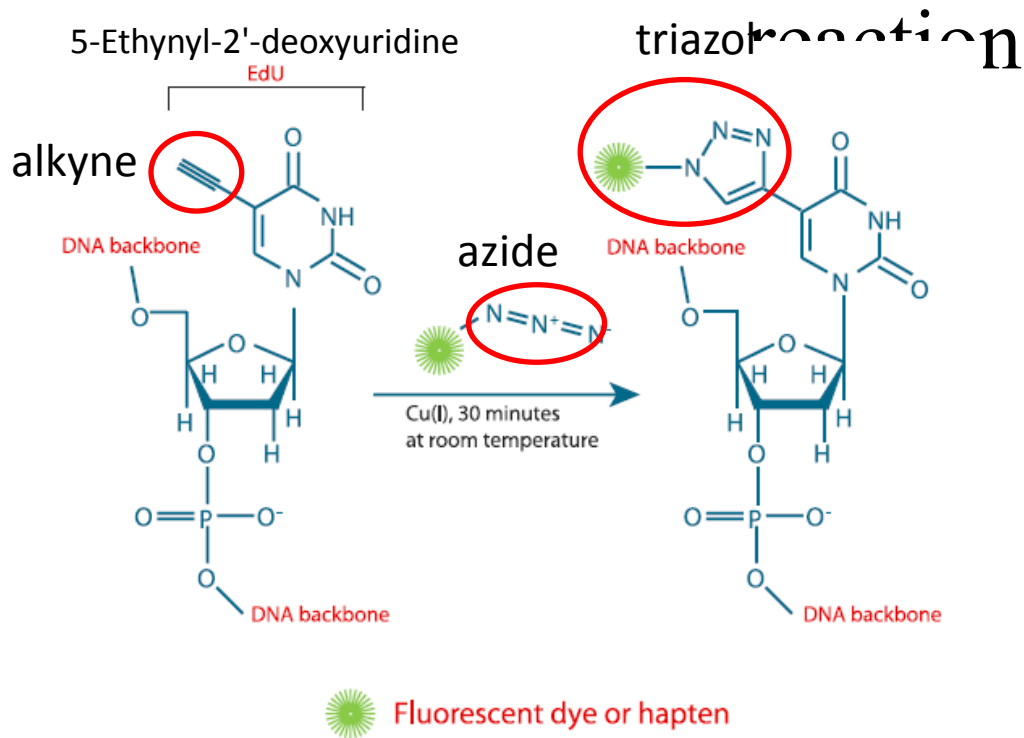


**Principle:** DNA content measurement by fluorescent nucleic-acid-binding dyes

**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

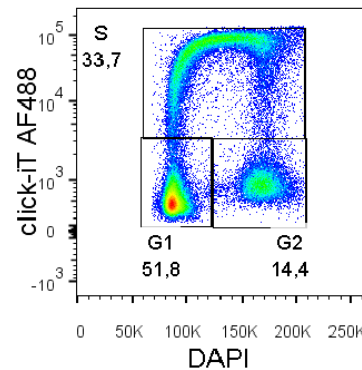
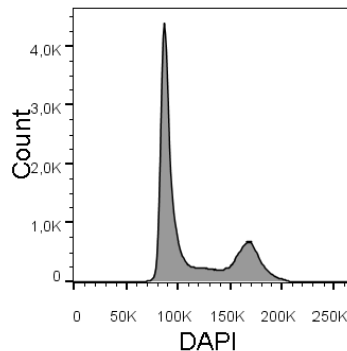
# DNA SYNTHESIS using click azide/alkyne



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

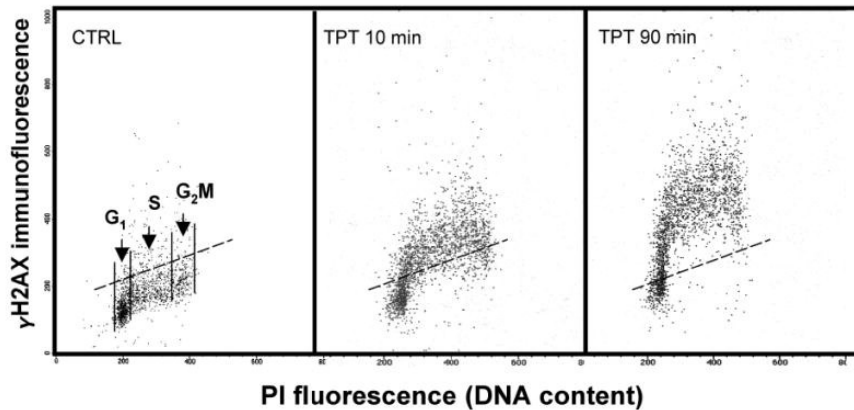
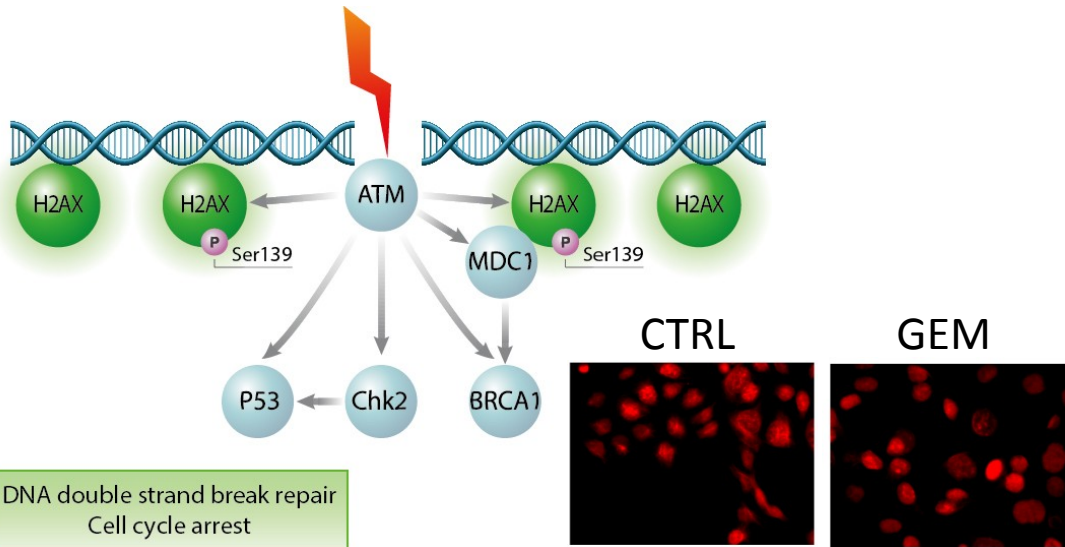
**Pros:** no DNA denaturation required, simplified protocol, small molecule detection, multiplex compatible

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





# DNA DAMAGE using $\gamma$ H2A.X



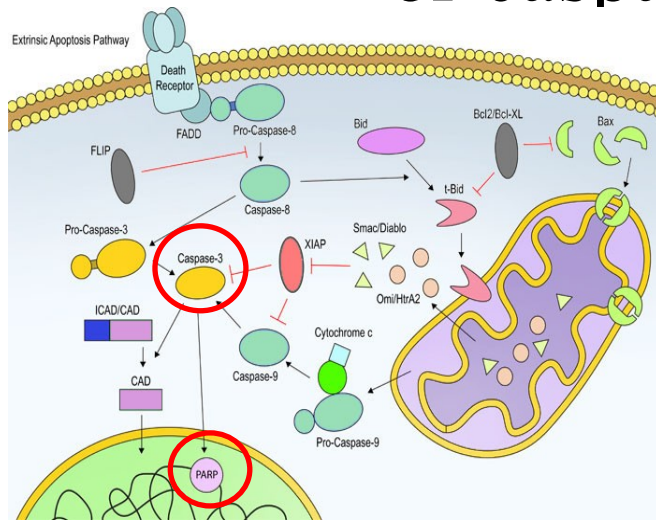
Huang X, Darzynkiewicz Z: **Cytometric Assessment of Histone H2AX Phosphorylation.** In *DNA Repair Protocols: Mammalian Systems.* Edited by Henderson DS. Totowa, NJ: Humana Press; 2006: 73-80

**Principle:** Phosphorylation of the Ser-139 residue of the histone variant H2A.X, forming  $\gamma$ 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

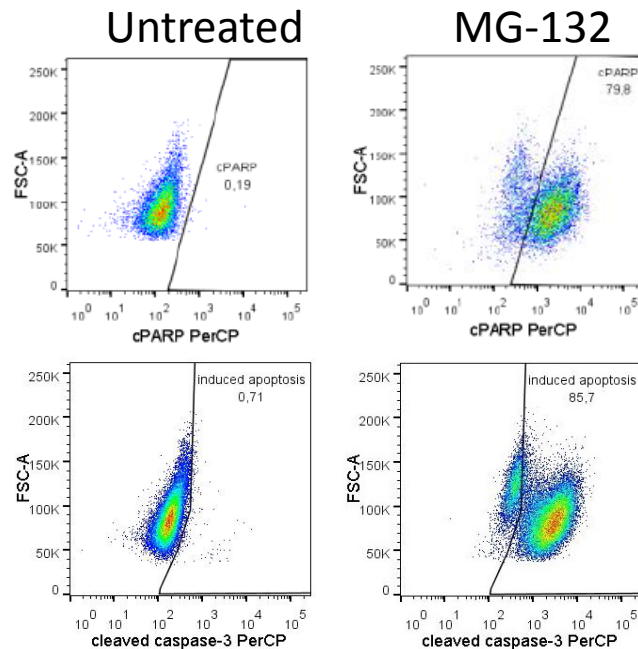
# APOPTOSIS detected via PARP cleavage or caspase-3 activation



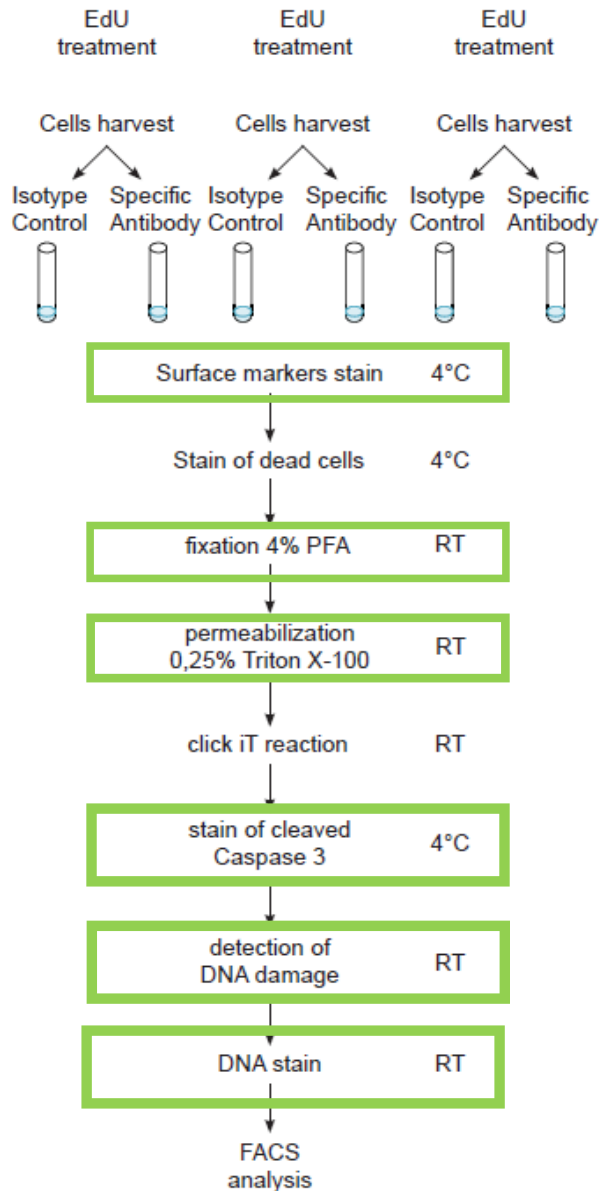
**Principle:** Cleaved Caspase-3 (Asp175) Antibody detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3. Cleaved PARP (Asp214) detects endogenous levels of the large fragment (89 kDa) PARP1 protein produced by caspase cleavage.

**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



# Workflow



## Possible issues Need of optimization

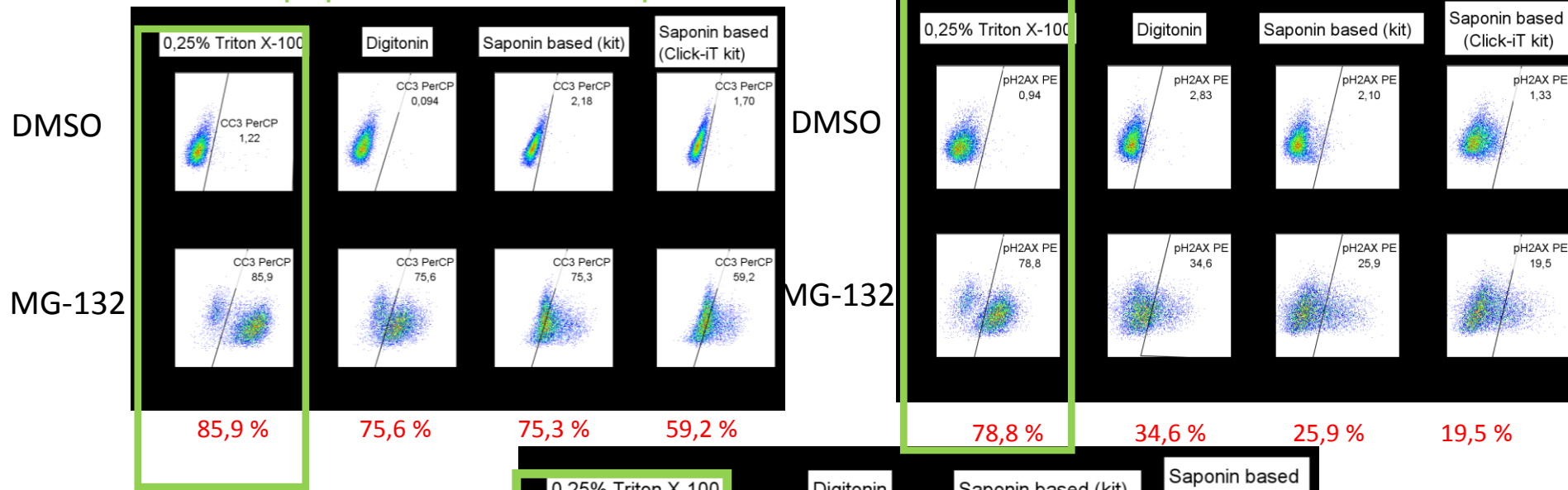
- Incompatibility of Fluorochrome with Click-iT reaction
- Permeabilization
- Over cross-linked
- Insufficient/too high concentration
- Sufficient permeability
- Antibody/ marker selection
- Sufficient permeability
- Antibody specificity
- Compatibility with other fluorochromes

# Permeabilization

Goal: Sufficient for intracellular markers, gentle for surface markers

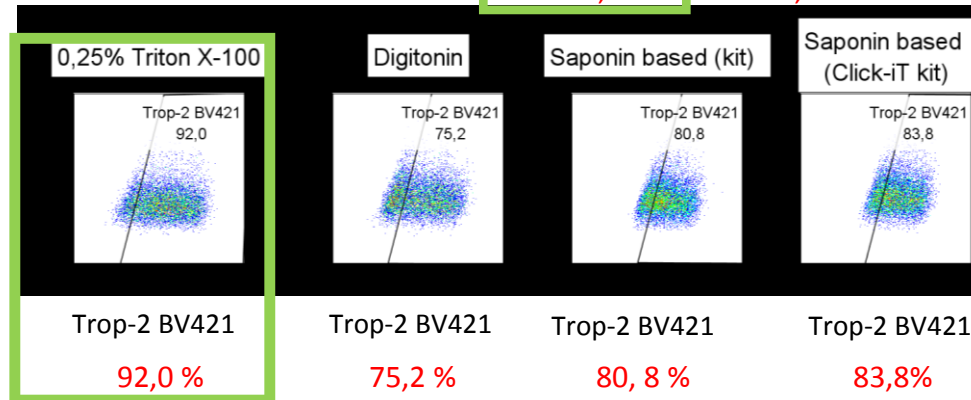
Apoptosis - Cleaved Caspase 3

DNA damage –  $\gamma$ H2AX



Surface marker – Trop-2

DMSO

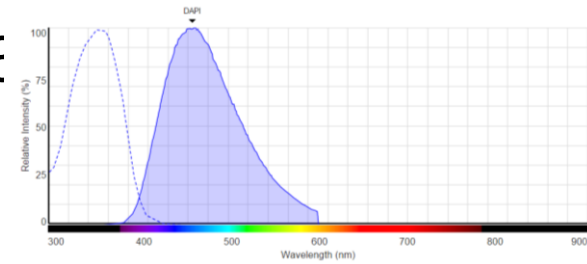


The best solution: 0,25% Triton x-100

# DNA stain

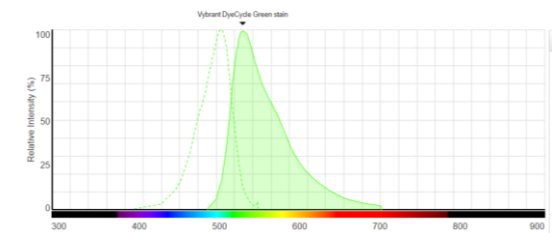
■ Violet laser

DAPI, Hoechst 33342  
FxCycle Violet, ...



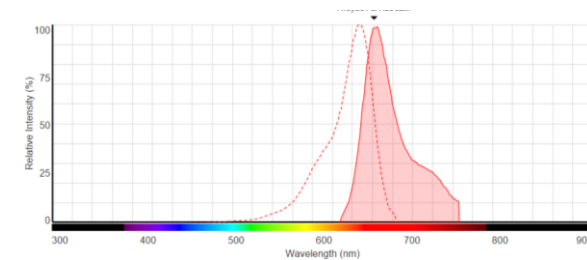
■ Blue laser

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  $\gamma$ 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) .

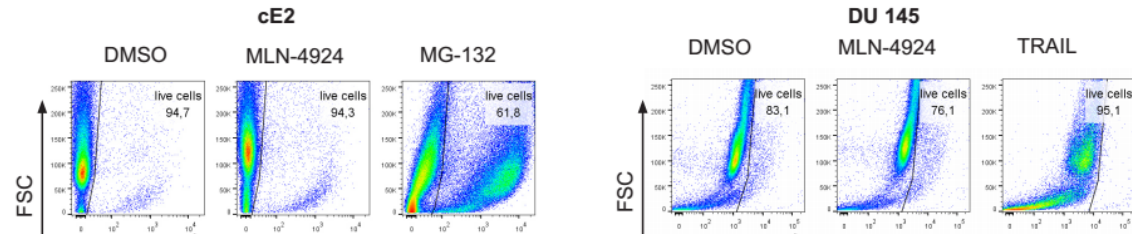
In the process of protocol optimization, FMO controls were measured and revealed DNA dye spillover.

# Example of final set-up

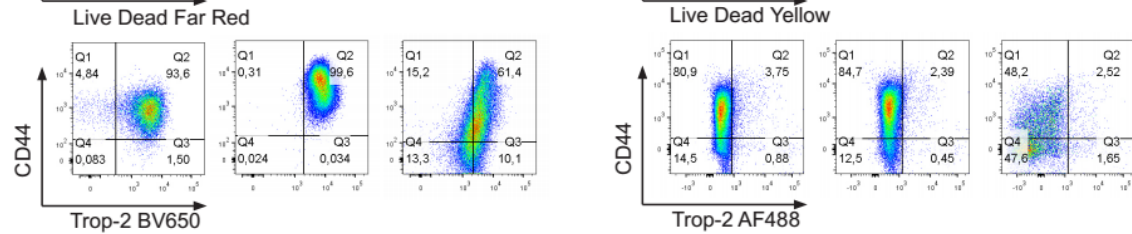
Parametr	Marker	Fluorochrome
Cell Surface Marker	CD44	APC/Cy7
Cell Surface Marker	Trop-2	AF488
Viability	LIVE/DEAD kit	Yellow
DNA synthesis	Click-iT EdU	AF647
Cell Cycle	DNA content	PO-PRO-1
DNA damage	$\gamma$ H2AX	PE
Apoptosis	Cleaved Caspase 3	AF494

# Flow Cytometric Multiparametric Assay was established

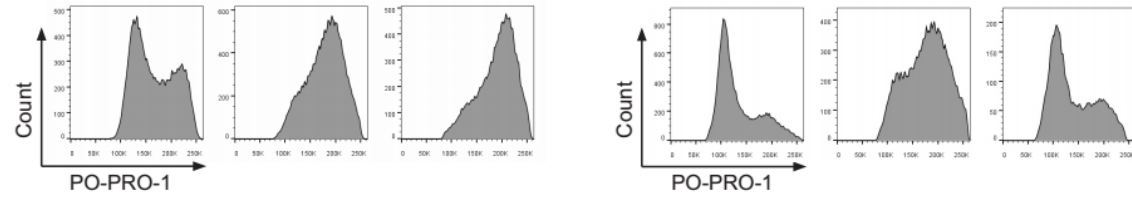
Viability



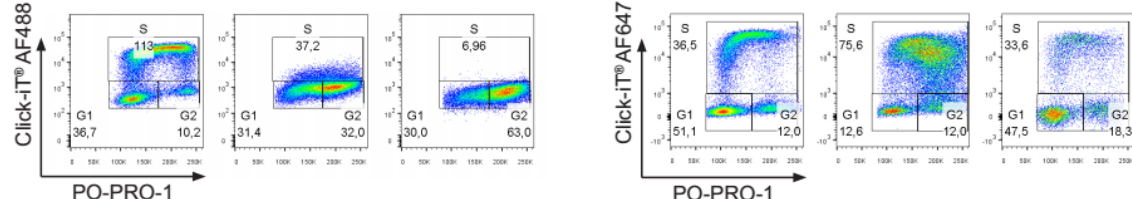
Surface Markers



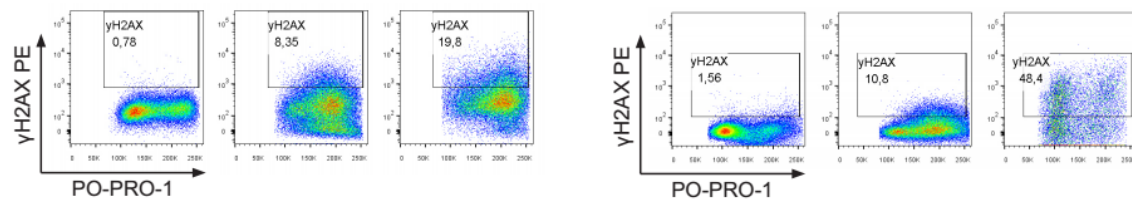
Cell Cycle



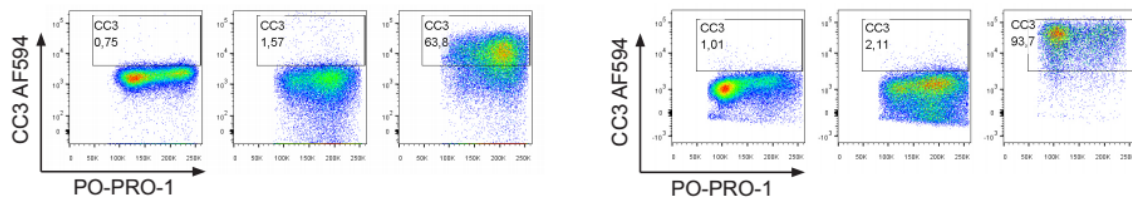
DNA synthesis



DNA damage

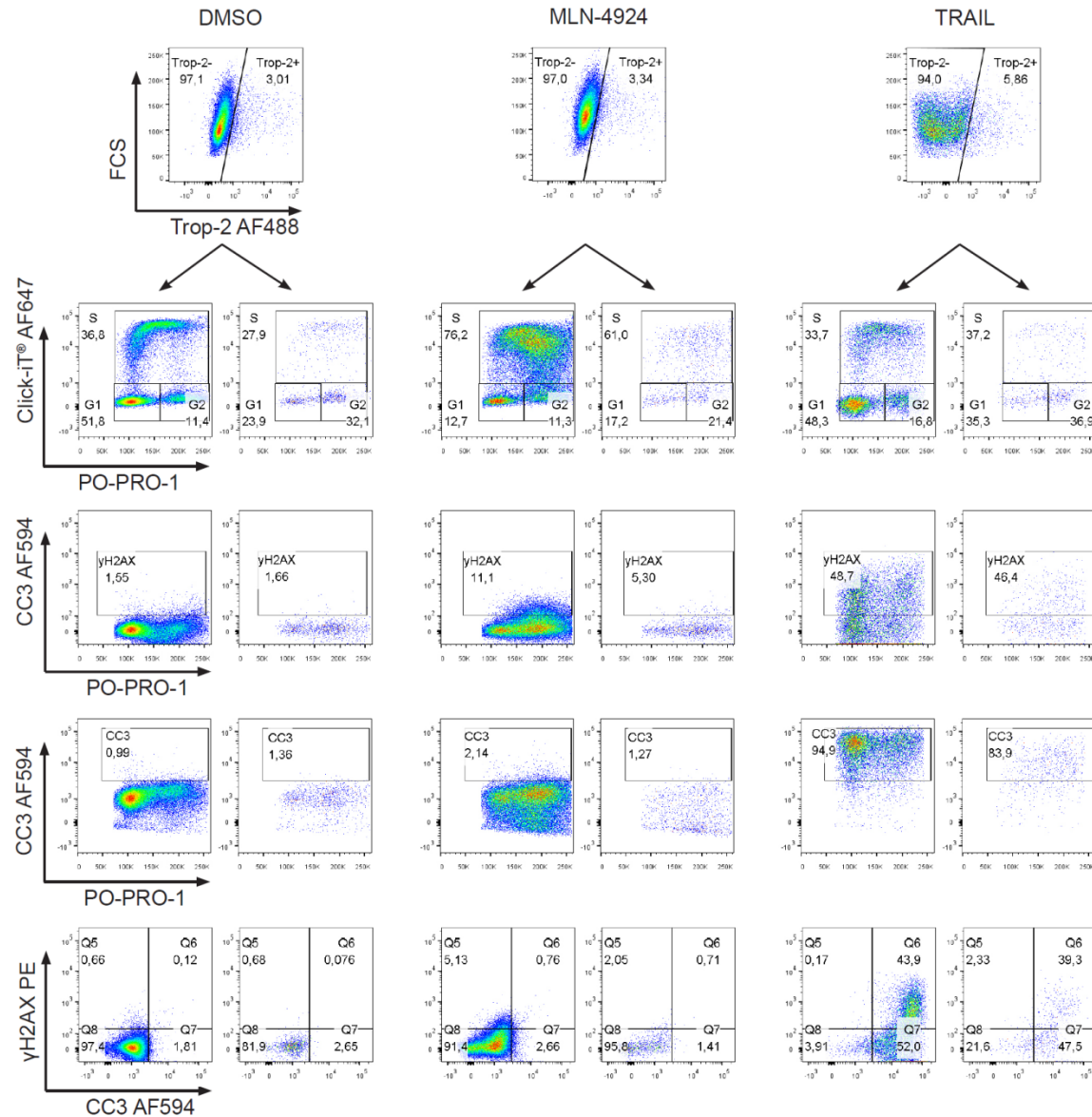


Apoptosis

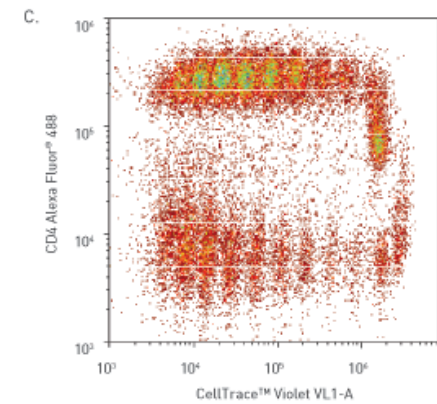
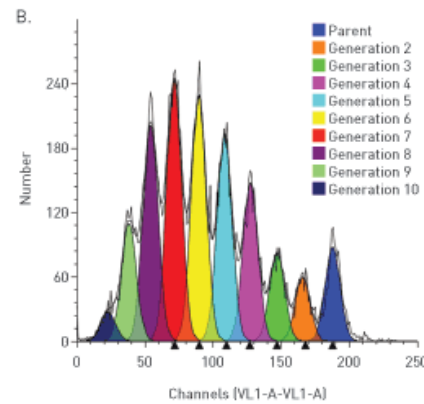
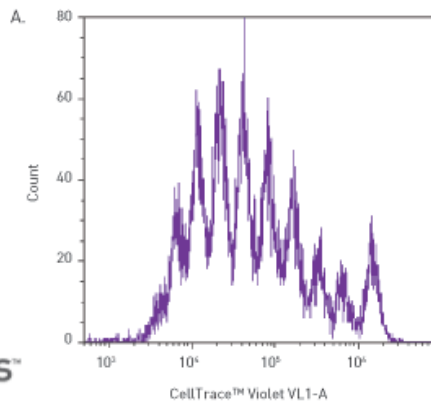
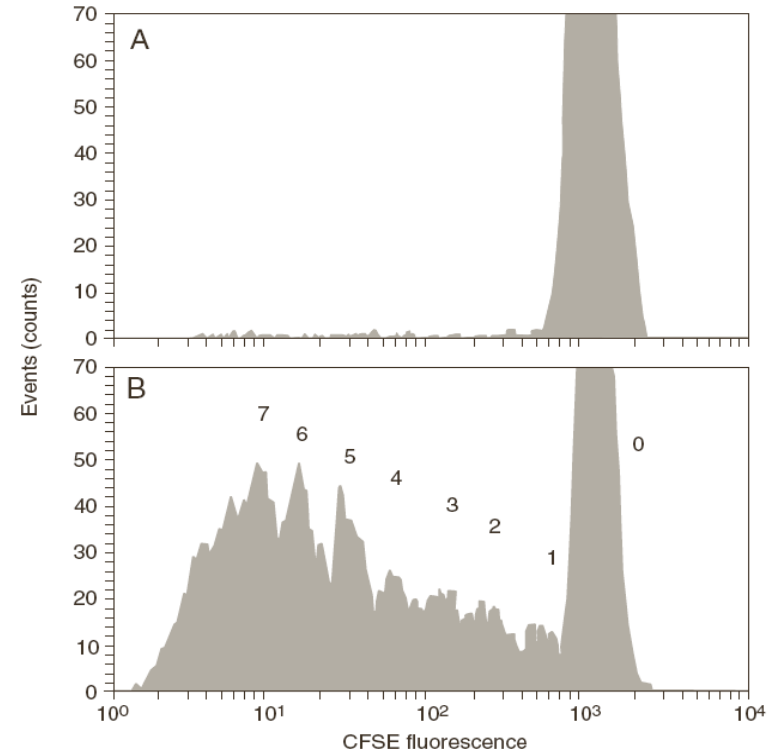
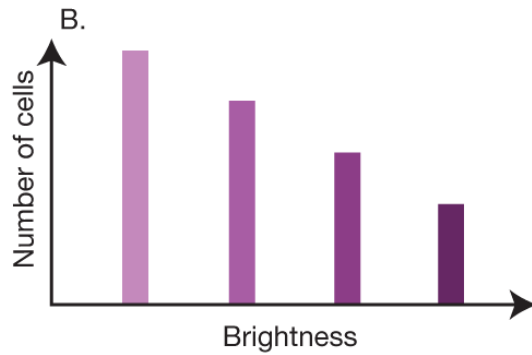
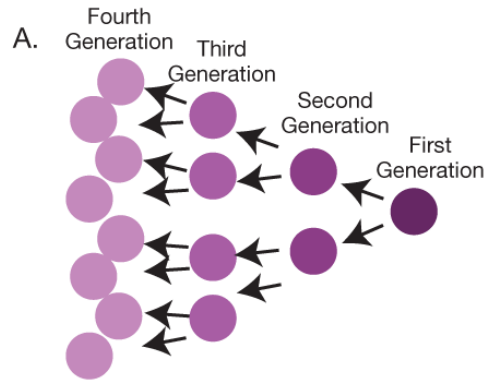




# Examination of small subpopulation (Trop-2<sup>+</sup>) in response to experimental treatment



# Detekce počtu buněčného dělení





# The Nobel Prize in Chemistry 2008

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

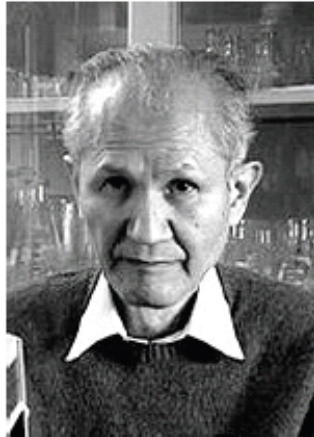


Photo: J. Henriksson/SCANPIX

**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

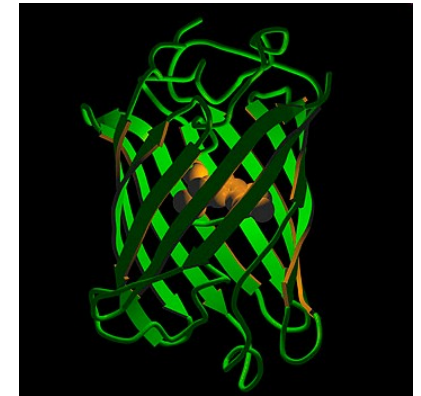
**Roger Y. Tsien**

🕒 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)

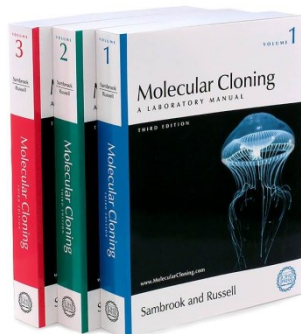
***Aequorea victoria*** - medúza žijící ve vodách na pobřeží Severní Ameriky.

- je schopna modře světélkovat (bioluminescence).  $\text{Ca}^{2+}$  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](http://www.mbayaq.org/efc/living_species/default.asp?hOri=1&inhab=440)

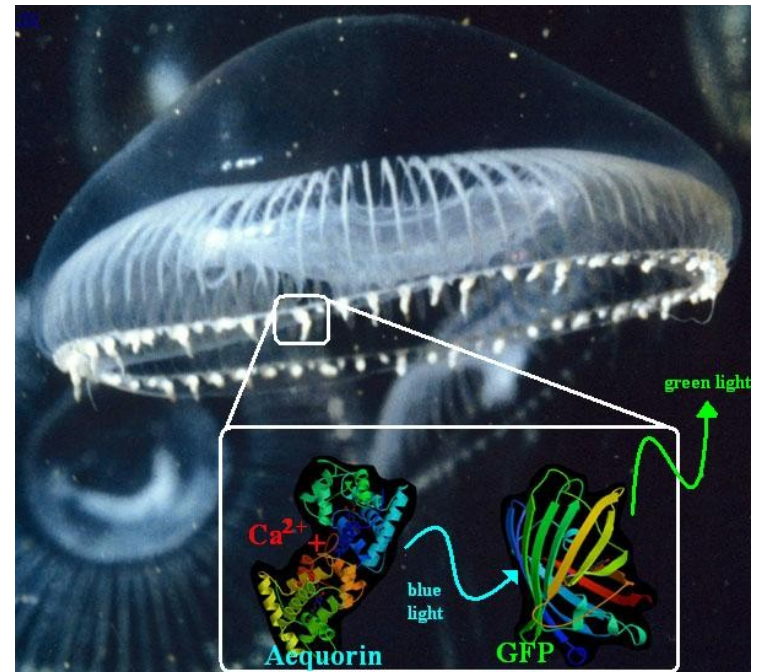
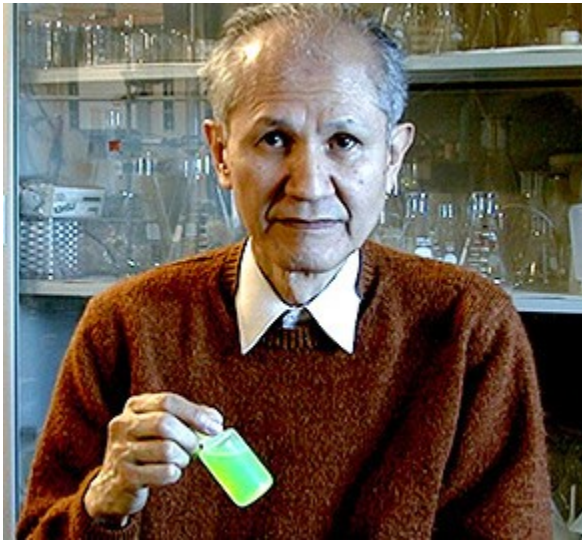
*Renilla reniformis* "Sea Pansy"



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

# Fluorescenční proteiny

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



# Fluorescenční proteiny

## ■ Douglas Prasher

## ■ Martin Chalfie

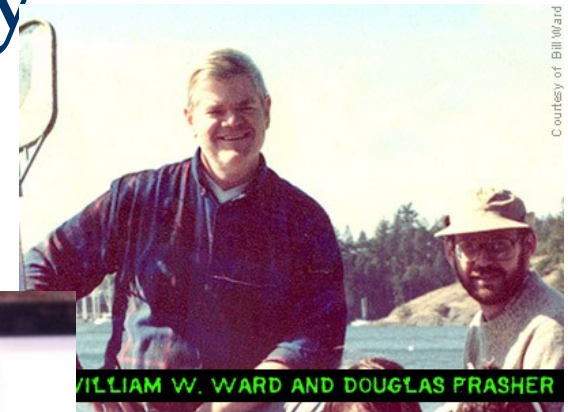
Science. 1994 Feb 11;263(5148):

**Green fluorescent protein as a marker for gene expression.**

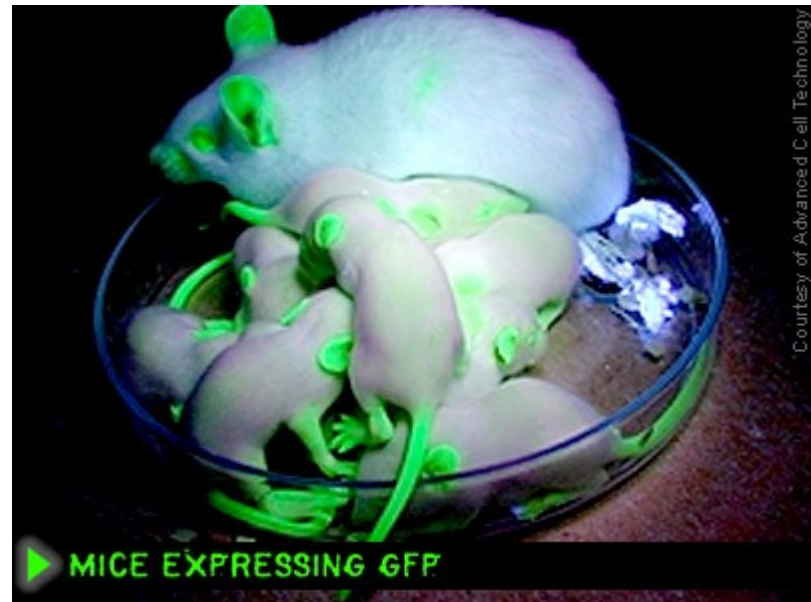
Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC.

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.

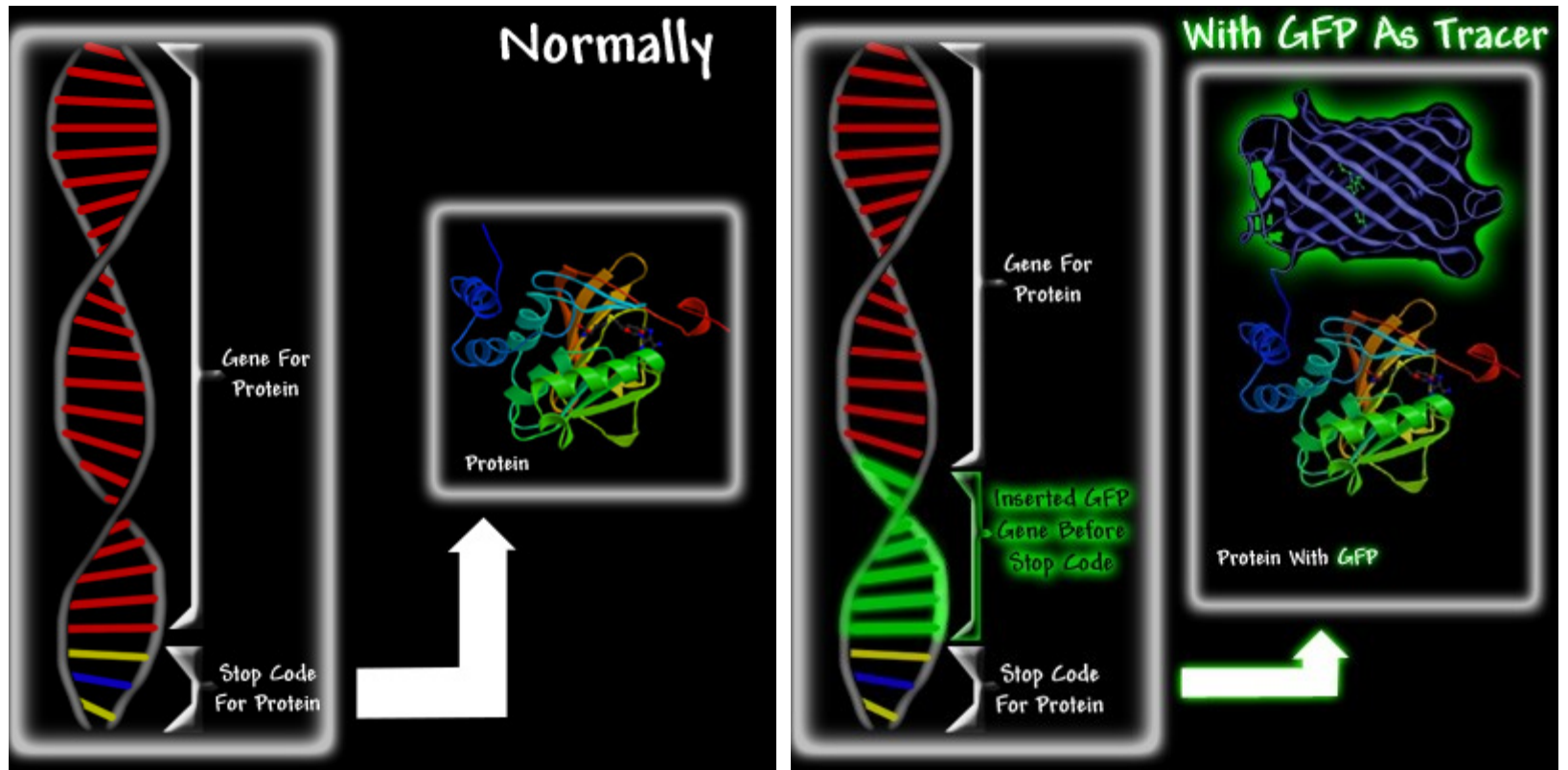


Courtesy of Bill Ward



Courtesy of Advanced Cell Technology

# Fluorescenční proteiny

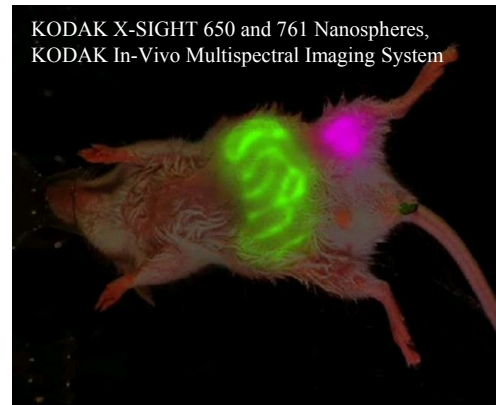


<http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm>

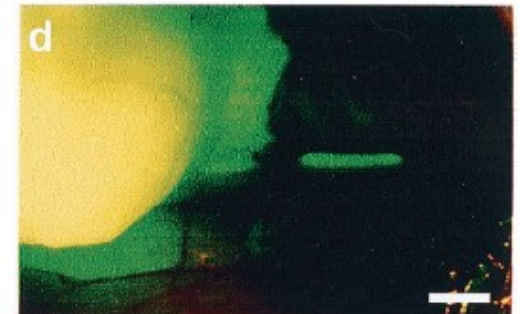
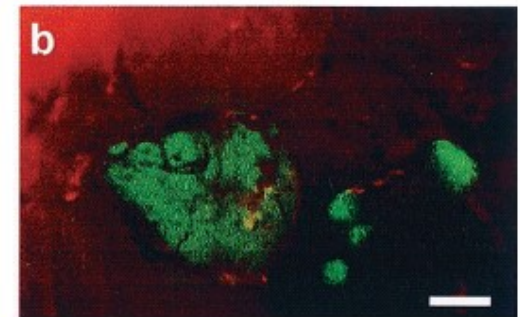
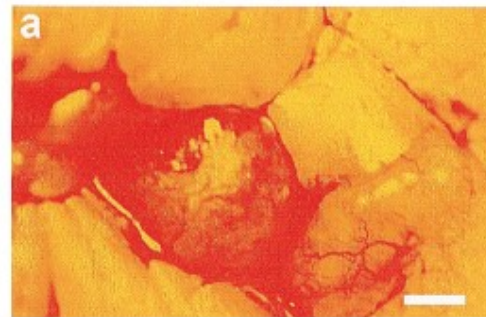
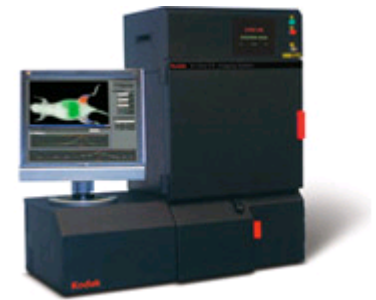
# *in vivo* molekulární vizualizace



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



KODAK X-SIGHT 650 and 761 Nanospheres,  
KODAK In-Vivo Multispectral Imaging System



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. Labas<sup>1</sup>, Aleksandr P. Savitsky<sup>2</sup>, Andrey G. Zaraisky, Mikhail L. Markelov, and Sergey A. Lukyanov\*

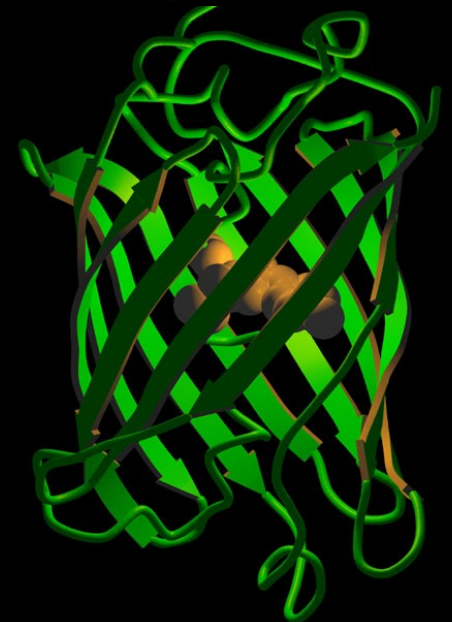
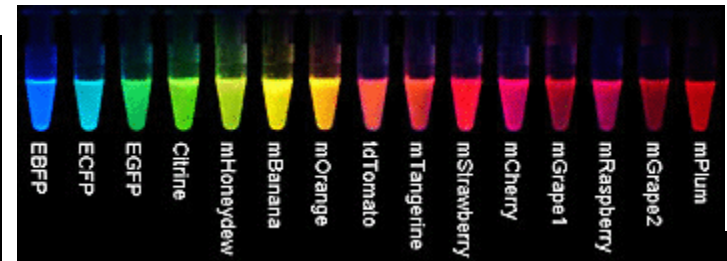
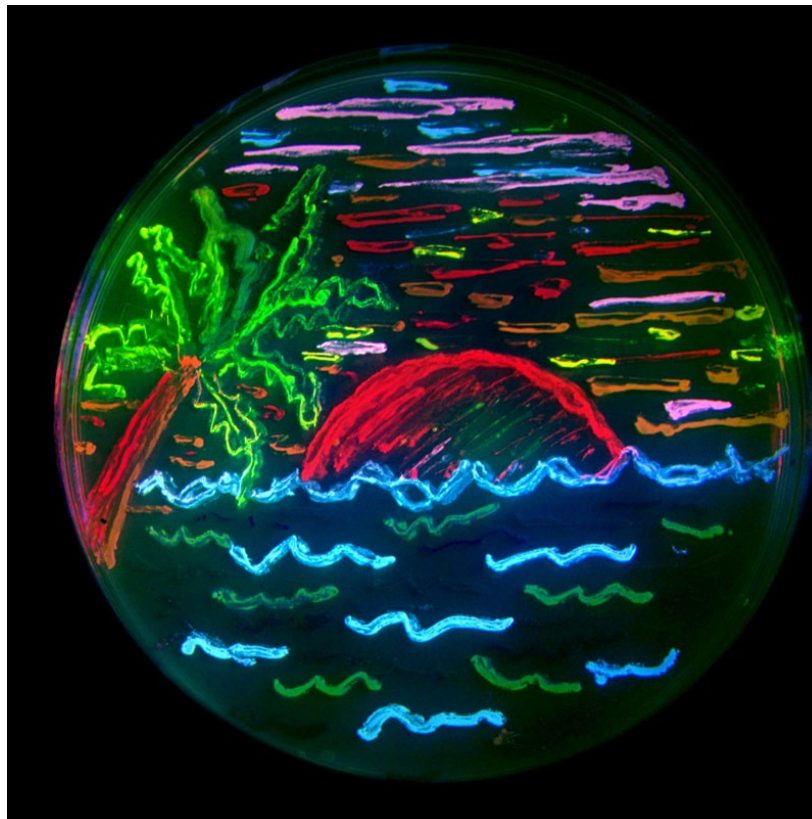
*Institute of Bioorganic Chemistry, Russian Academy of Science, 117871 Moscow, Russia. <sup>1</sup>Institute of Ecology and Evolution, and <sup>2</sup>Institute of Biochemistry Russian Academy of Science, 17071 Moscow, Russia. \*Corresponding author (e-mail: luk@ibch.siobc.ras.ru).*

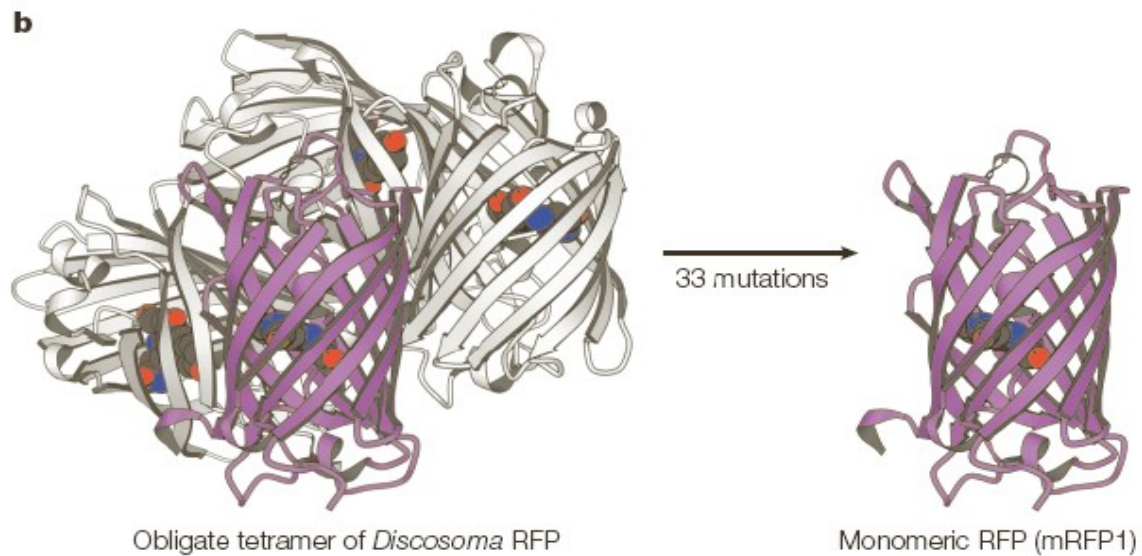
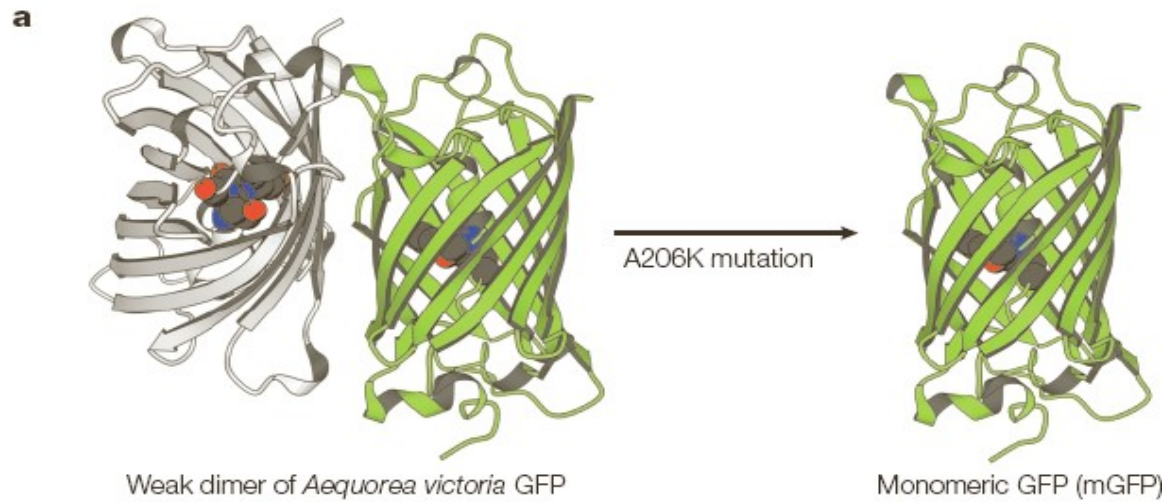
Received 28 May 1999; accepted 18 July 1999

# 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\*<sup>†</sup> and Roger Y. Tsien\*<sup>‡</sup>

**Table 1** | Properties of the best FP variants<sup>a,b</sup>

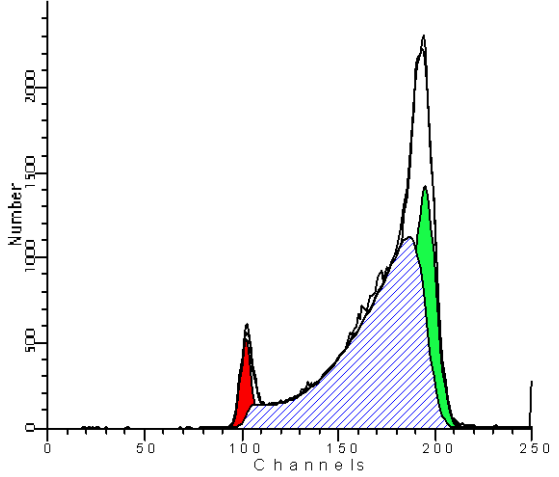
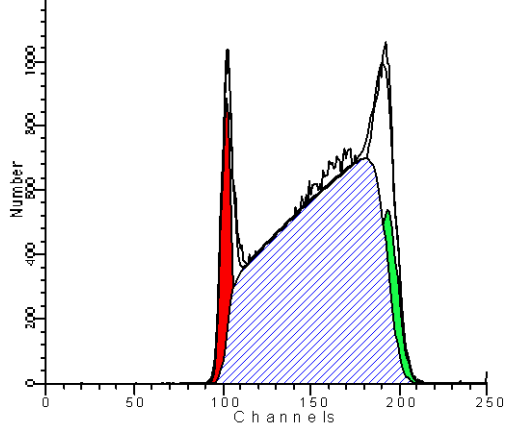
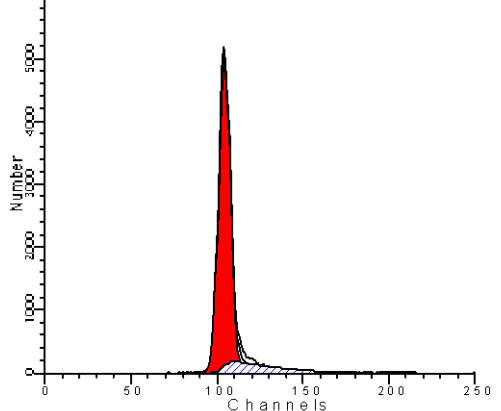
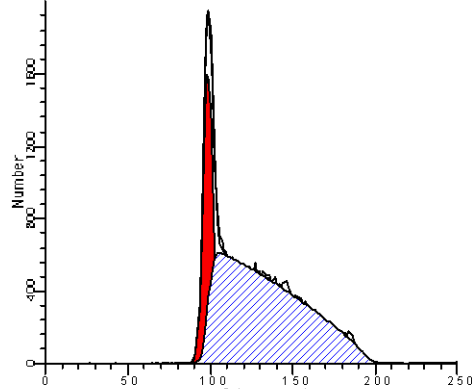
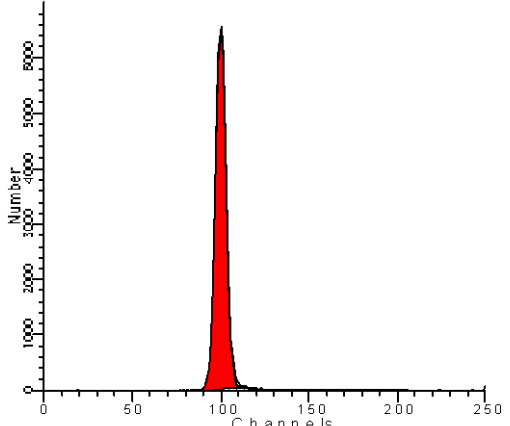
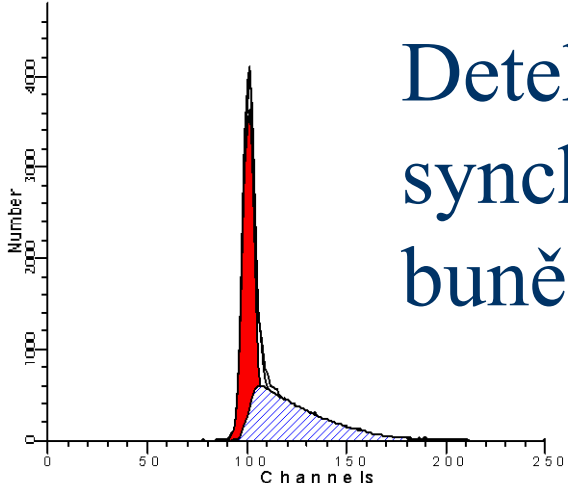
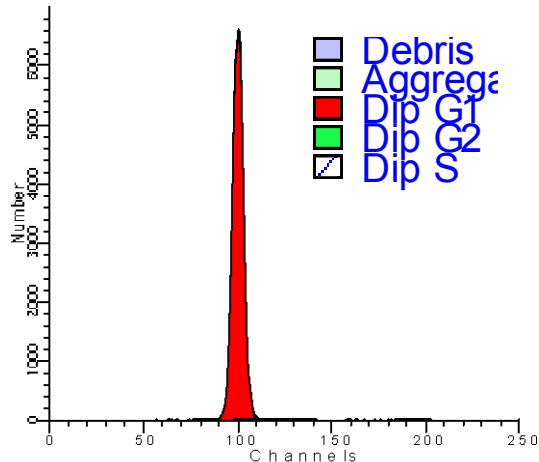
Class	Protein	Source laboratory (references)	Excitation <sup>c</sup> (nm)	Emission <sup>d</sup> (nm)	Brightness <sup>e</sup>	Photostability <sup>f</sup>	pKa	Oligomerization
Far-red	mPlum <sup>g</sup>	Tsien (5)	590	649	4.1	53	<4.5	Monomer
Red	mCherry <sup>g</sup>	Tsien (4)	587	610	16	96	<4.5	Monomer
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

<sup>a</sup>An expanded version of this table, including a list of other commercially available FPs, is available as **Supplementary Table 1**. <sup>b</sup>The mutations of all common AFPs relative to the wild-type protein are available in **Supplementary Table 3**. <sup>c</sup>Major excitation peak. <sup>d</sup>Major emission peak. <sup>e</sup>Product 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)<sup>-1</sup> (for comparison, free fluorescein at pH 7.4 has a brightness of about 69 (mM • cm)<sup>-1</sup>). <sup>f</sup>Time for bleaching from an initial emission rate of 1,000 photons/s down to 500 photons/s (t<sub>1/2</sub>; for comparison, fluorescein at pH 8.4 has t<sub>1/2</sub> of 5.2 s); data are not indicative of photostability under focused laser illumination. <sup>g</sup>Brightest in spectral class. <sup>h</sup>Not recommended (dim with poor folding at 37 °C). <sup>i</sup>Citrine YFP with A206K mutation; spectroscopic properties equivalent to Citrine. <sup>j</sup>Can be made monomeric with A206K mutation. <sup>k</sup>Emerald 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. <sup>l</sup>Formerly sold by Clontech, no longer commercially available. <sup>m</sup>mCFP with A206K mutation; spectroscopic properties equivalent to ECFP.

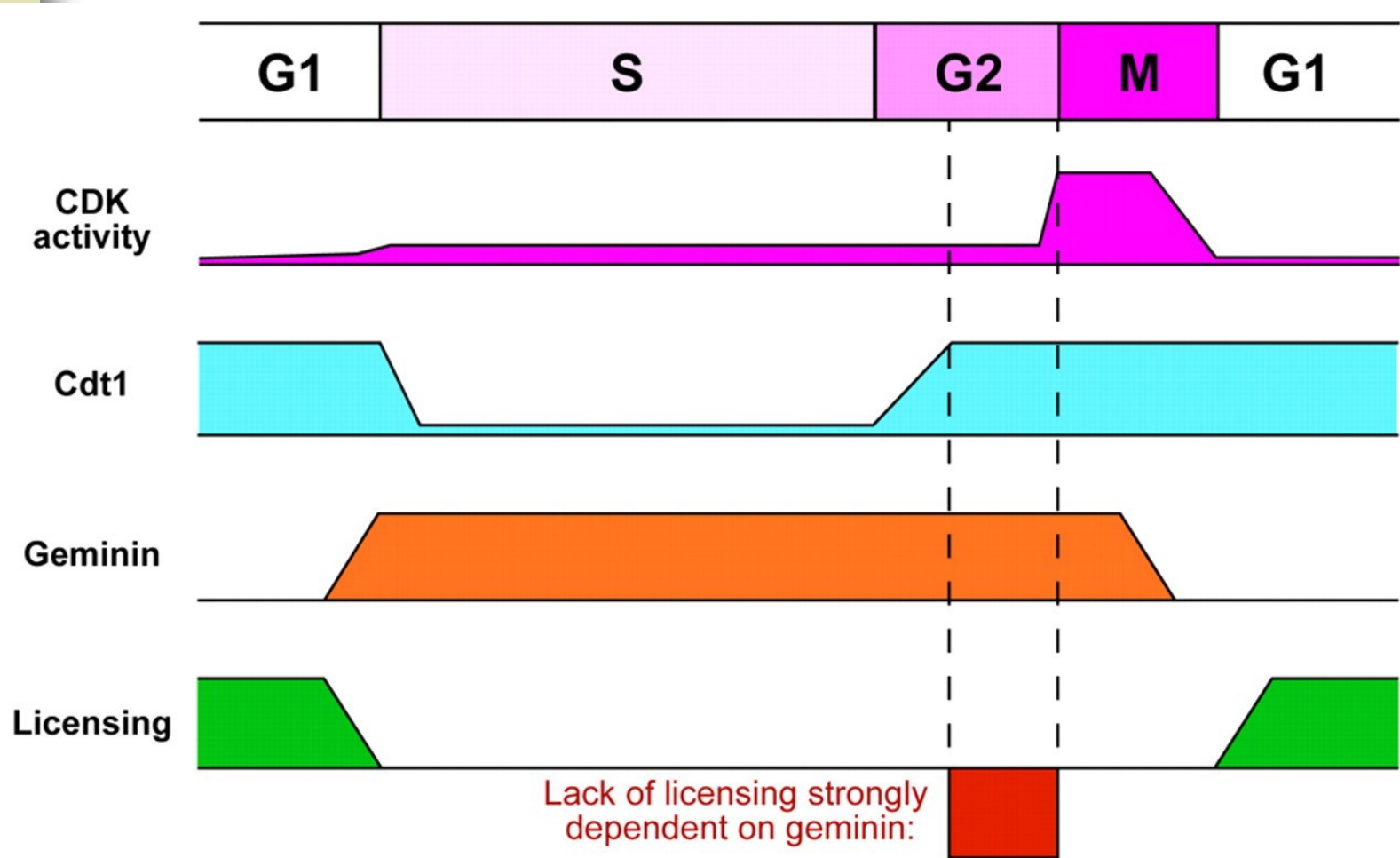
## A guide to choosing fluorescent proteins

Nathan C Shaner<sup>1,2</sup>, Paul A Steinbach<sup>1,3</sup> & Roger Y Tsien<sup>1,3,4</sup>

# Detekce buněk v synchronizovaném buněčném cyklu

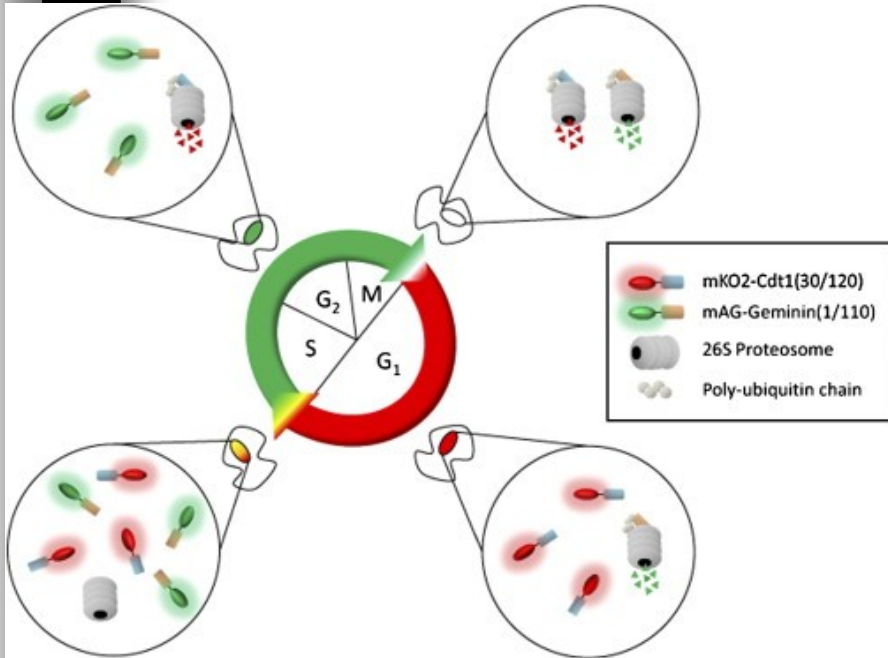


# Licensing control by Cdt1 and geminin

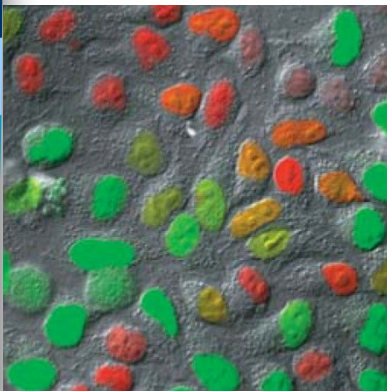


# Fucci

(fluorescent ubiquitination-based cell cycle indicator) cells



Chemistry & Biology 15, February 2008 ©2008 Elsevier Ltd



## Ubiquitin E3 ligase complexes

G<sub>1</sub> - APC<sup>Cdh1</sup>

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

S, G<sub>2</sub>, M- SCF<sup>Skp2</sup>

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

Fucci sensors - 1st generation, coral FP

monomeric Kusabira orange 2 – hCdt1 (30/120)

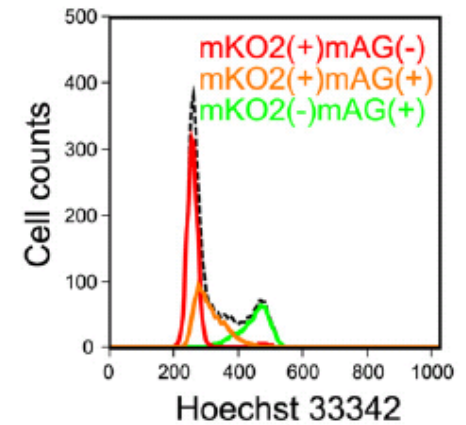
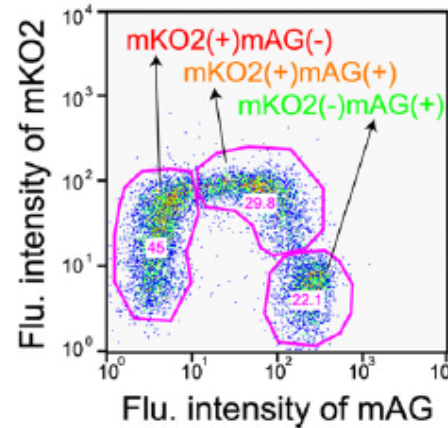
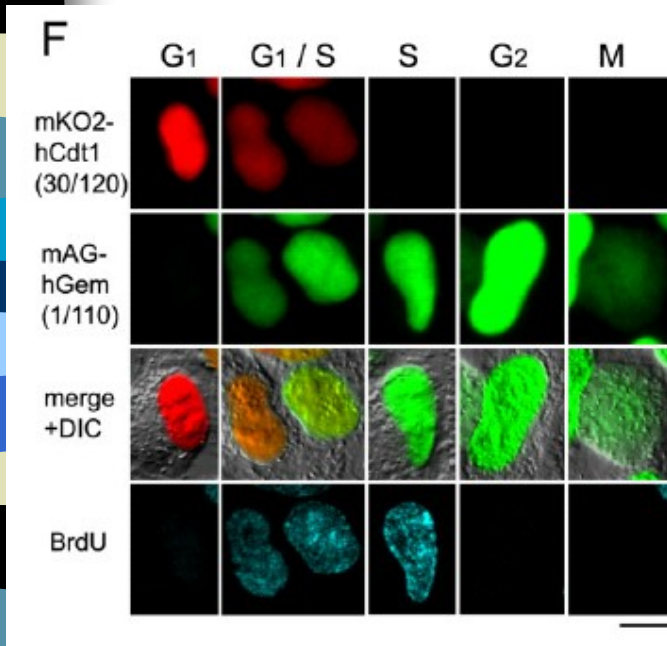
Monomeric Azami-Green – hGeminin (1/110)

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,<sup>1,3</sup> Hiroshi Kurokawa,<sup>1,4</sup> Toshifumi Morimura,<sup>2</sup> Aki Hanyu,<sup>5</sup> Hiroshi Hama,<sup>1</sup> Hatsuki Osawa,<sup>1</sup> Saori Kashiwagi,<sup>2</sup> Kiyoko Fukami,<sup>4</sup> Takaki Miyata,<sup>6</sup> Hiroyuki Miyoshi,<sup>7</sup> Takeshi Imamura,<sup>5</sup> Masaharu Ogawa,<sup>2</sup> Hisao Masai,<sup>8</sup> and Atsushi Miyawaki<sup>1,3,\*</sup>

<sup>1</sup>Laboratory for Cell Function and Dynamics

<sup>2</sup>Laboratory for Cell Culture Development

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

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

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

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

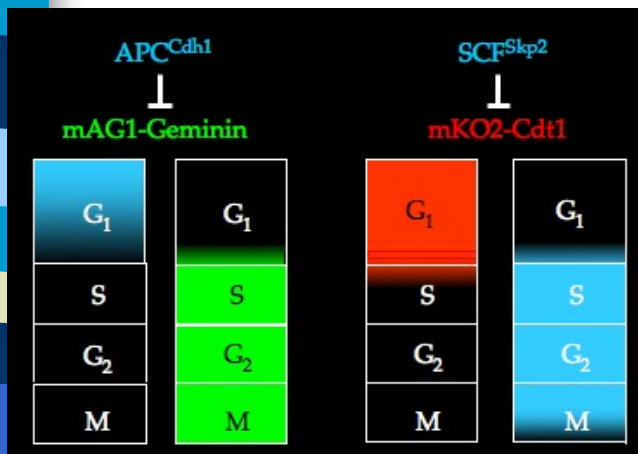
<sup>7</sup>Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Syowa-ku, Nagoya, Aichi 466-8550, Japan

<sup>8</sup>Subteam 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

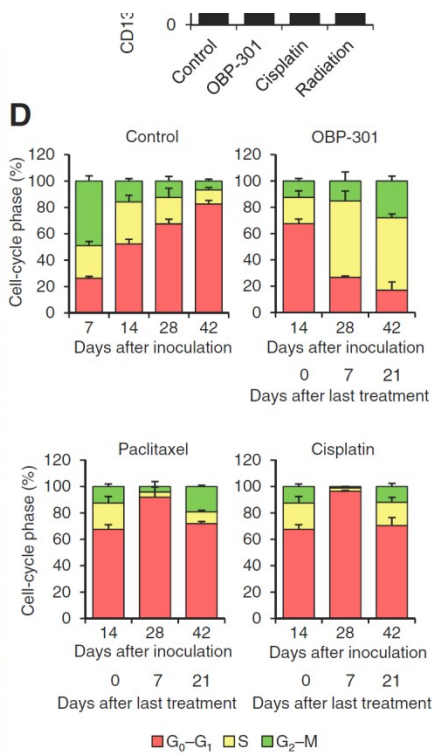
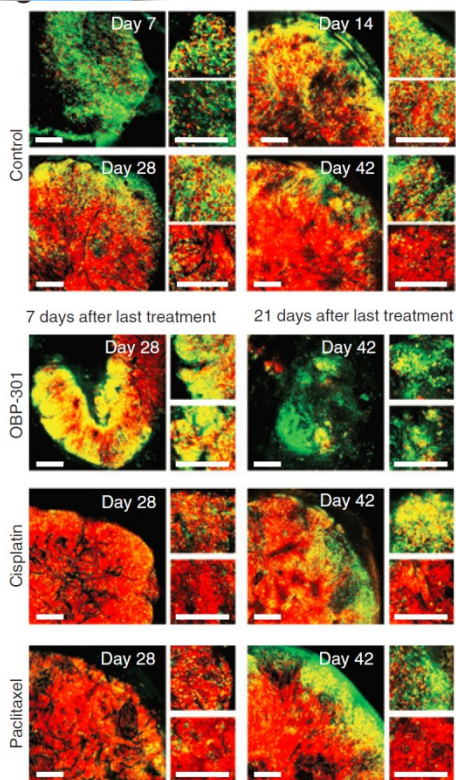
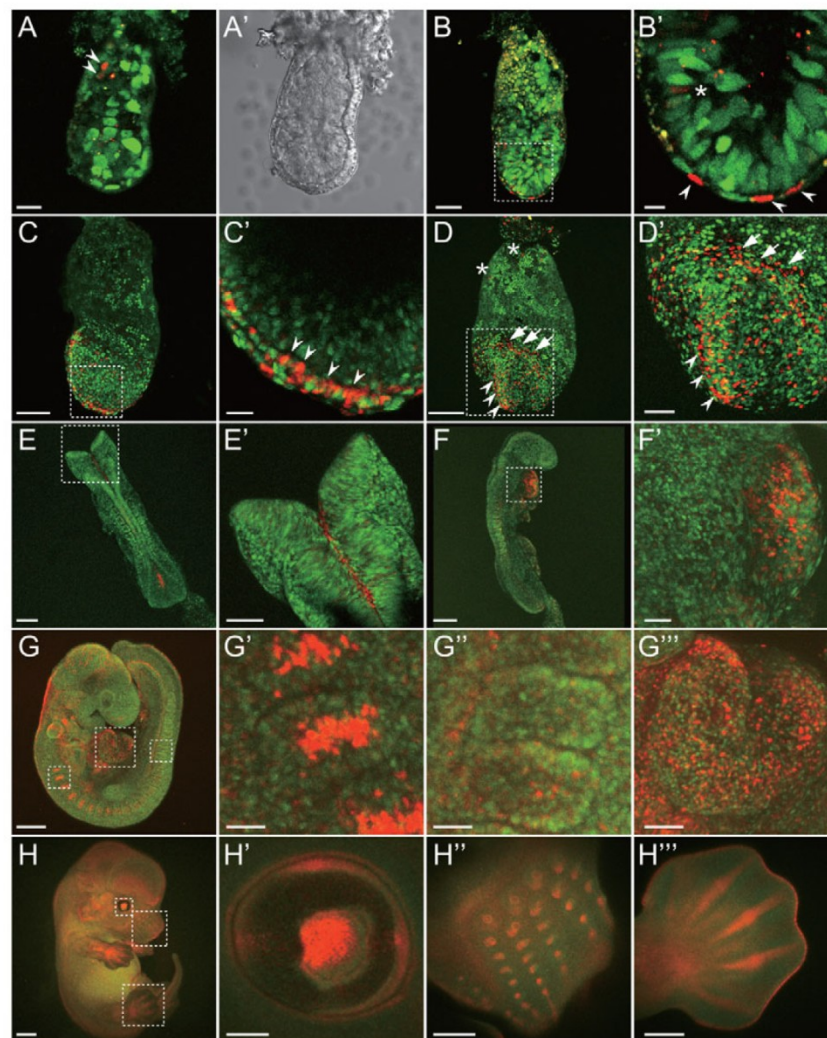
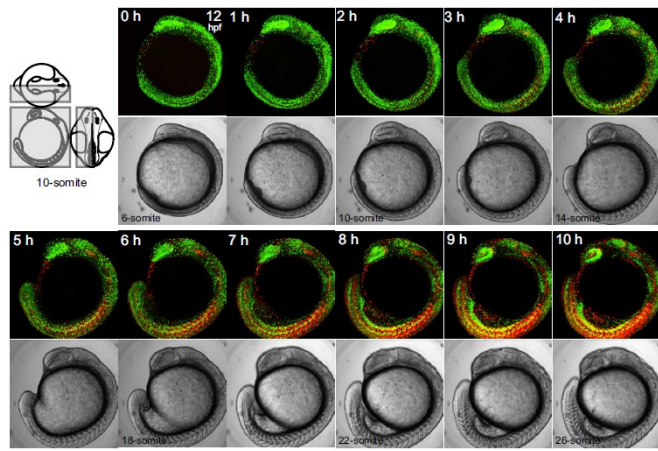
\*Correspondence: matsushi@brain.riken.jp

DOI 10.1016/j.cell.2007.12.033



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



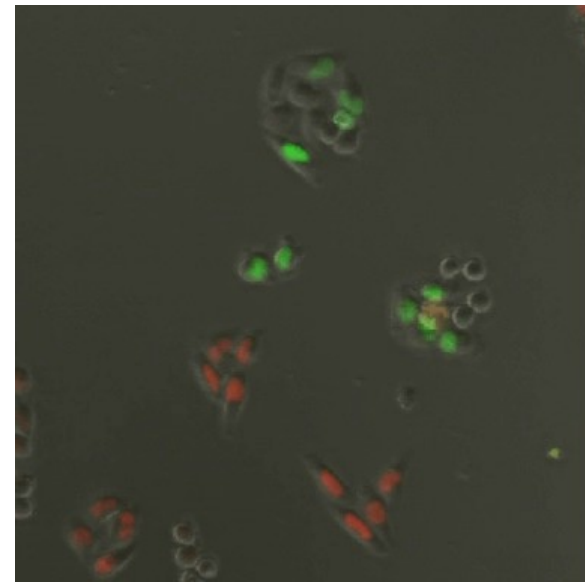
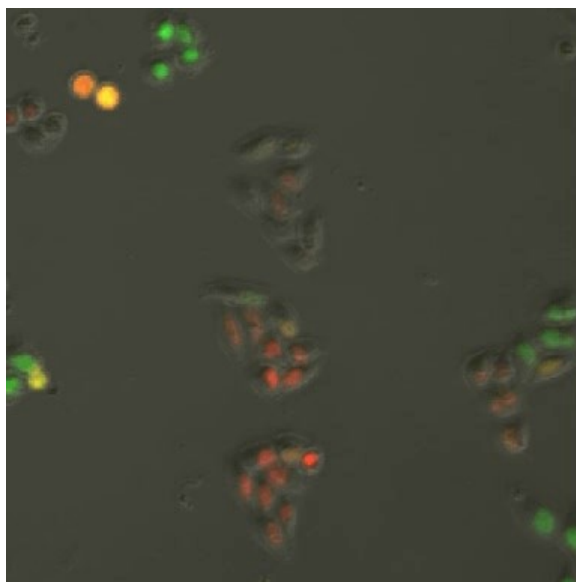
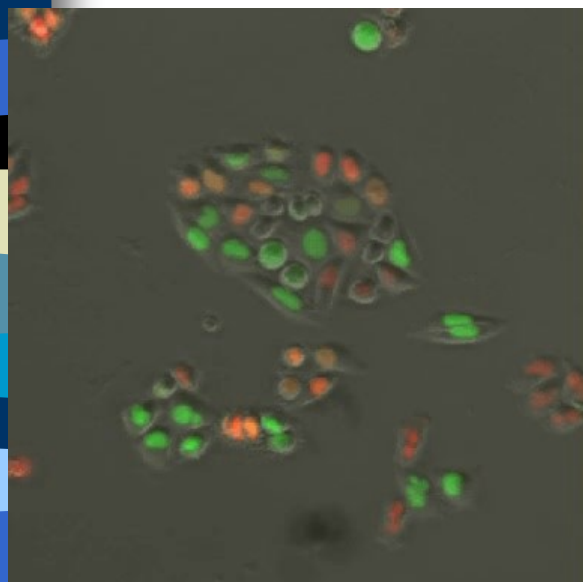


CONTROL

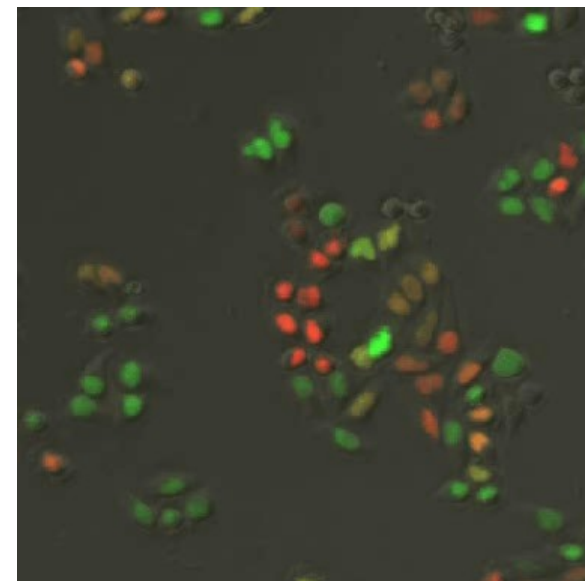
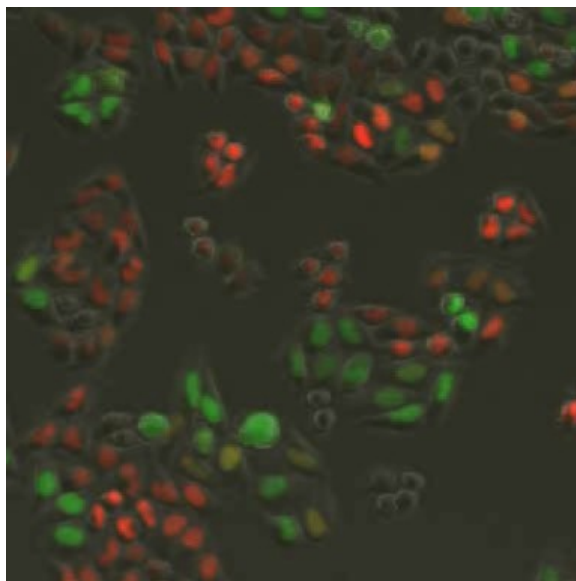
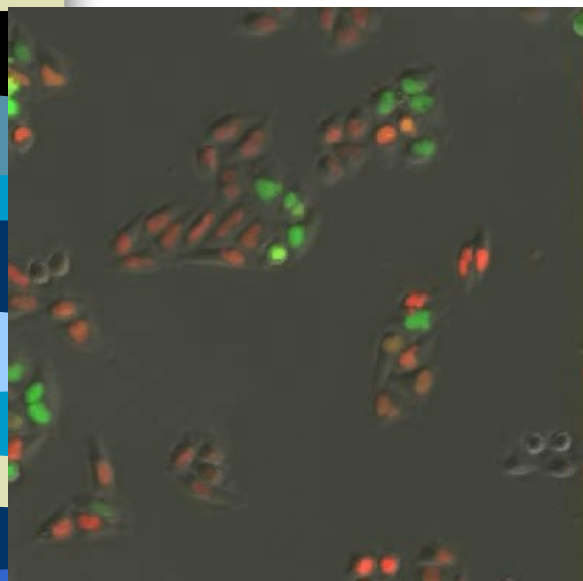
SCH900776

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?

# SOLUTION (Milan\_TrackMate\_(Fiji))



+



## TrackMate\_ (Fiji)

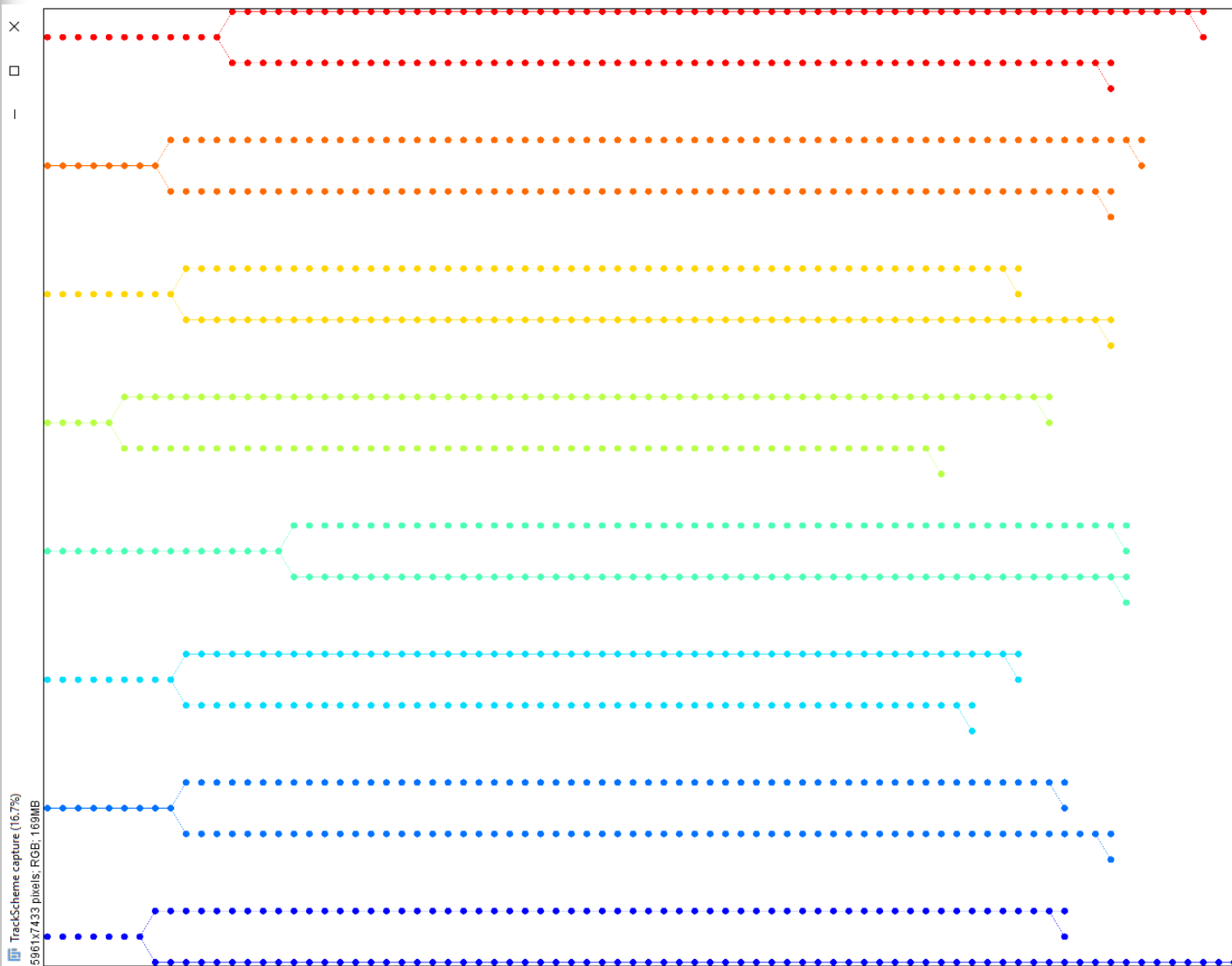
<b>Author</b>	Nick Perry, Jean-Yves Tinevez, Johannes Schindelin
<b>Maintainer</b>	Jean-Yves Tinevez
<b>Source</b>	on GitHub
<b>Initial release</b>	10/05/2012
<b>Development status</b>	v3.4.2, stable
<b>Category</b>	Segmentation, Tracking



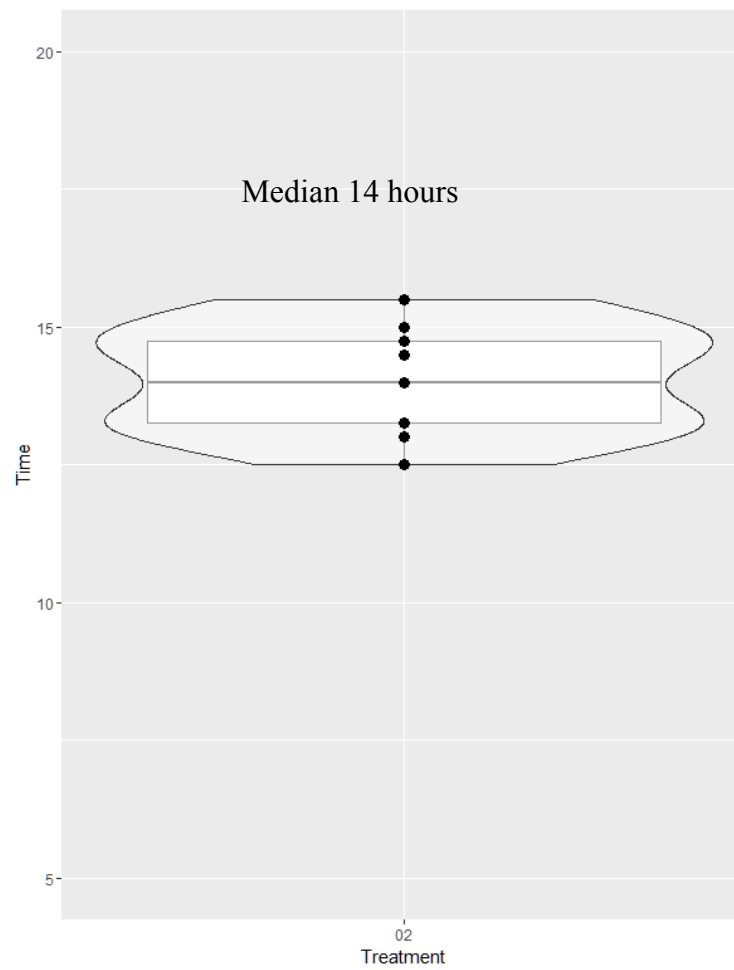
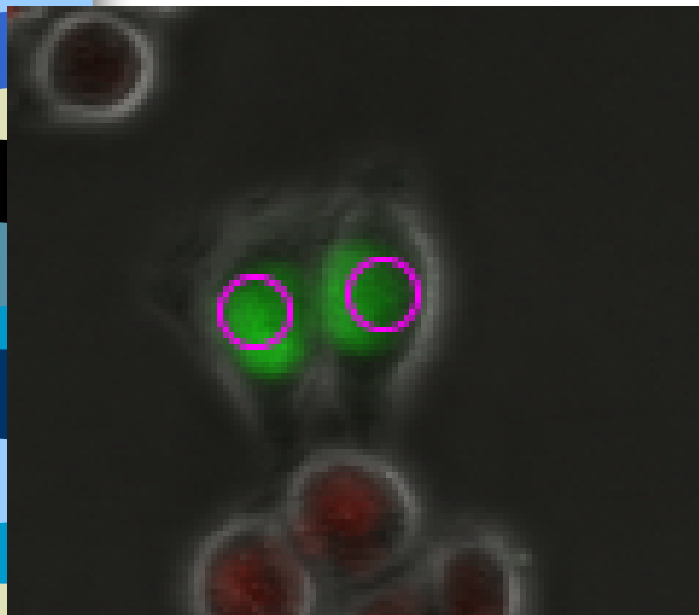
**CELLIM**  
Cellular Imaging Core Facility

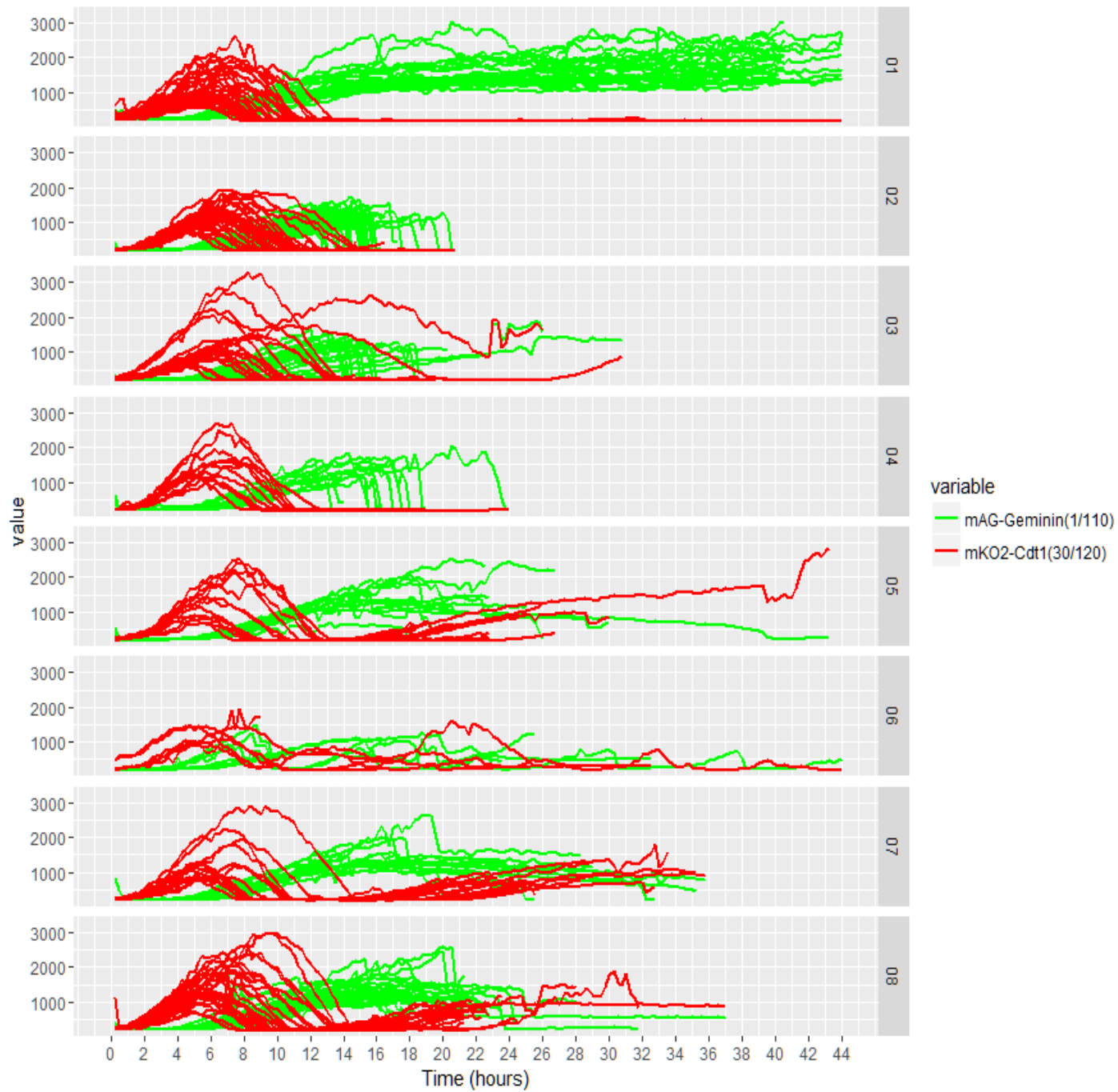
# Branches (divisions) analysis

02\_02\_01\_01

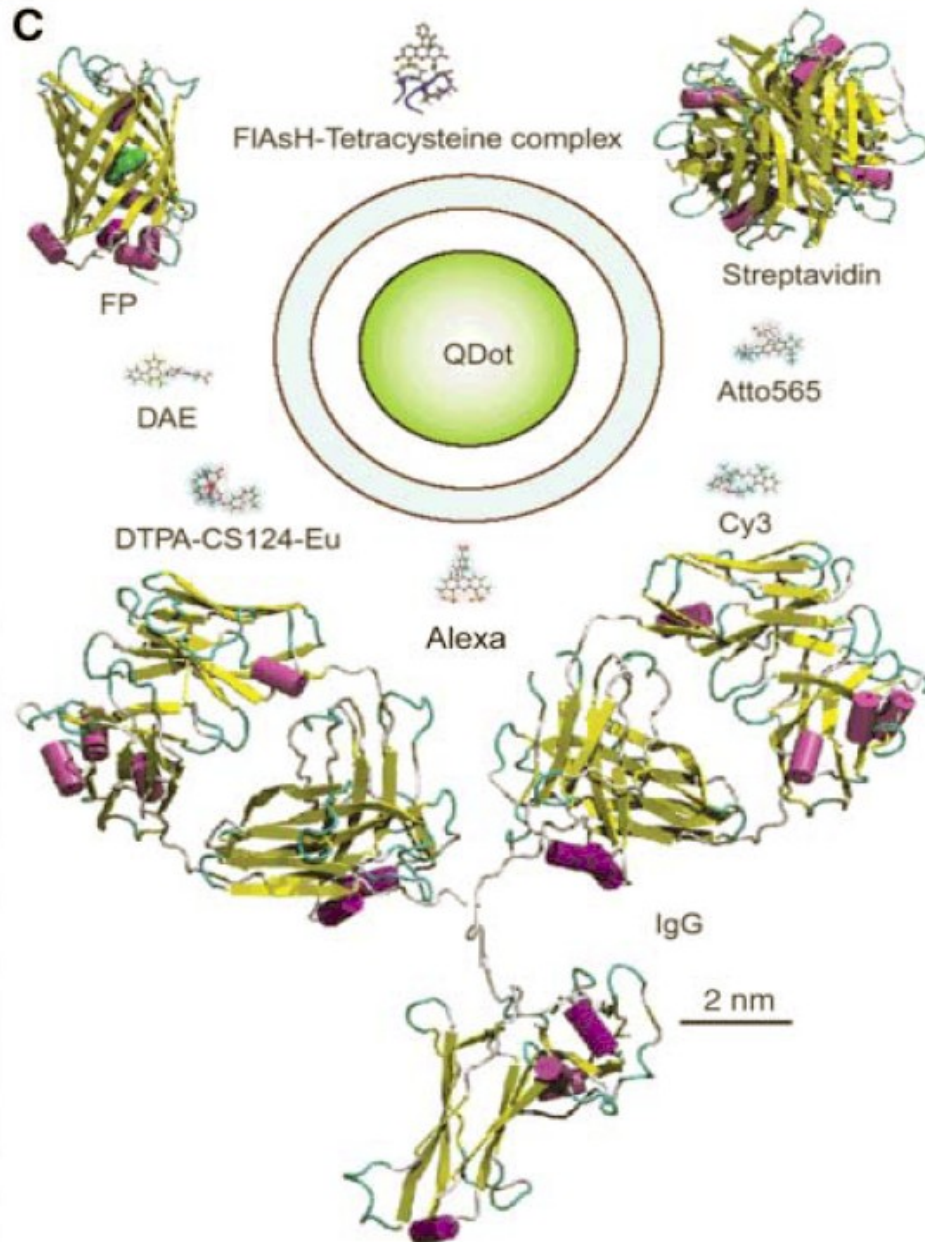


02\_02\_01\_01





# Targeting proteins & fluorophores



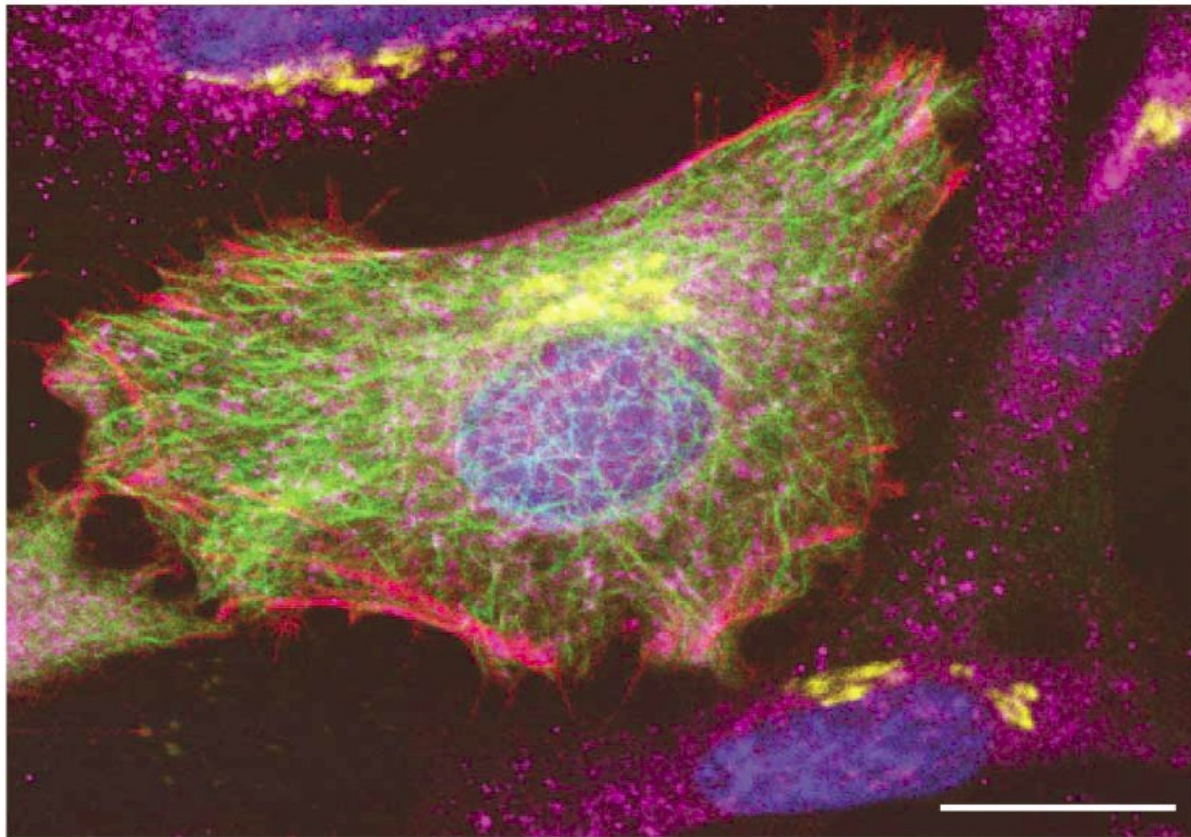
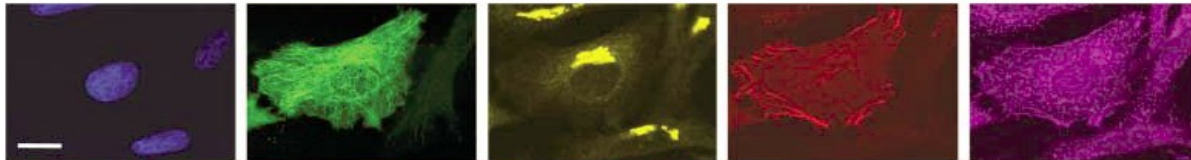
REVIEW

## The Fluorescent Toolbox for Assessing Protein Location and Function

Ben N. G. Giepmans,<sup>1,2</sup> Stephen R. Adams,<sup>2</sup> Mark H. Ellisman,<sup>1</sup> Roger Y. Tsien<sup>2,3\*</sup>



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

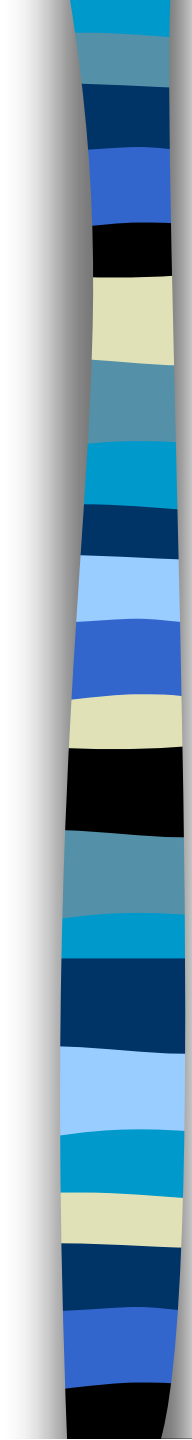


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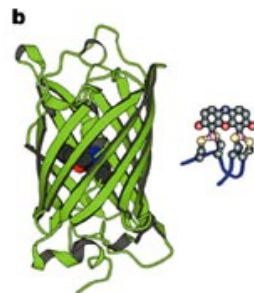
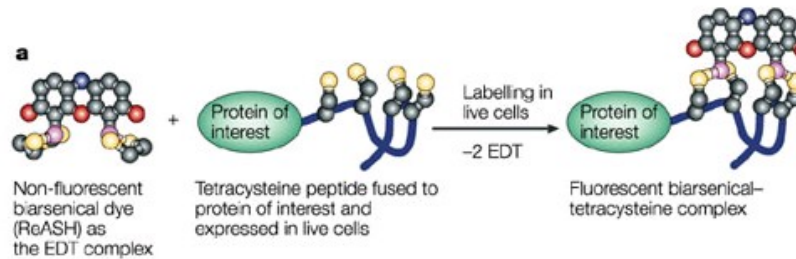




# biarsenical–tetracysteine system

- Nefluorescenční, membránově permeabilní biarsénová značka vytváří kovalentní fluorescenční komplex s jakýmkoliv intracelulárním proteinem obsahujícím krátký tetracysteinový motiv (CCPGCC)

# biarsenical–tetracysteine system



**c**

Biarsenical dye	CHOxAsH	FIAsh	ReAsH
Tetracysteine-complex excitation maximum (nm)	380	508	593
Tetracysteine-complex emission maximum (nm)	430	528	608

# 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.