

Bi9393 Analytická cytometrie



Karel Souček, Ph.D.

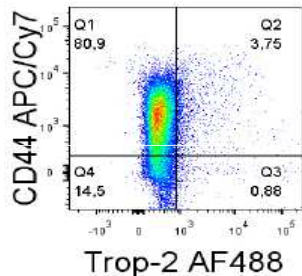
Oddělení cytokinetiky
Biofyzikální ústav AVČR, v.v.i.
Královopolská 135
612 65 Brno

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

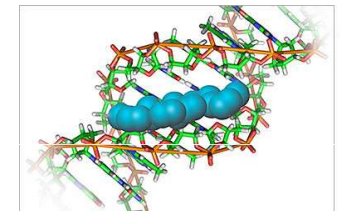
Flow cytometry

most common applications

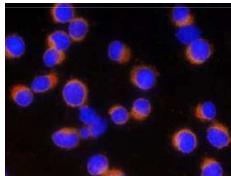
Immunophenotype characterisation of the cells
(CSCs markers, differentiation, ...)



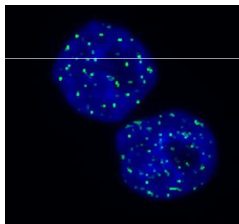
Cell Cycle (DNA content, Cell cycle modulation after treatment)



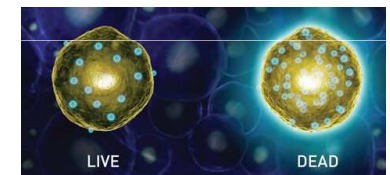
Cell Death analysis
(AnnexinV, Cleaved Caspase3, ...)



DNA damage (γ H2AX,...)



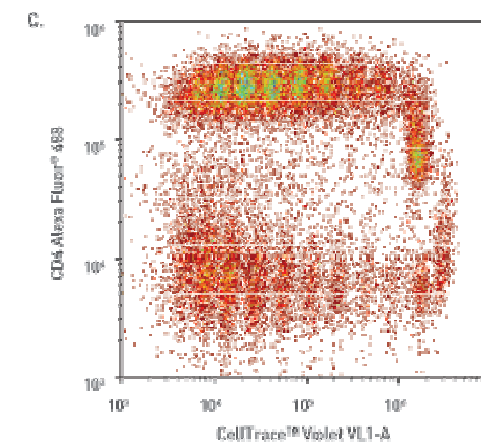
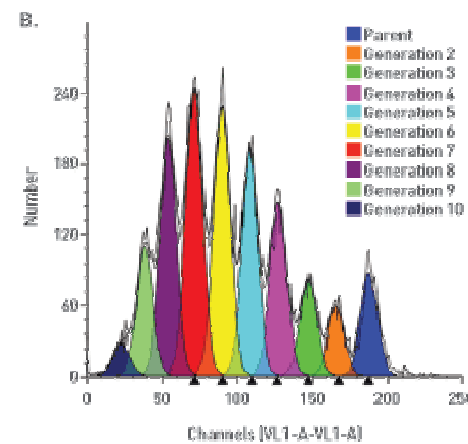
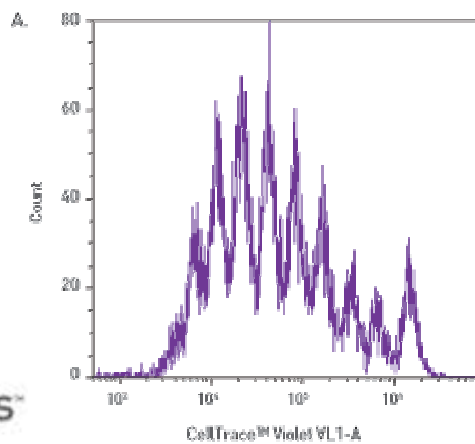
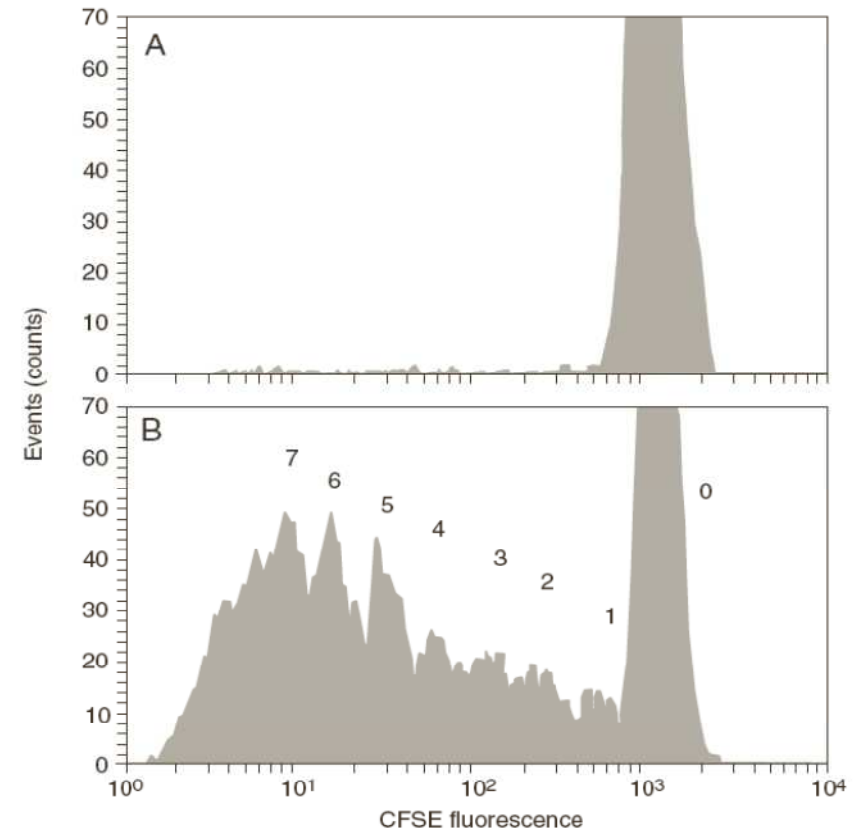
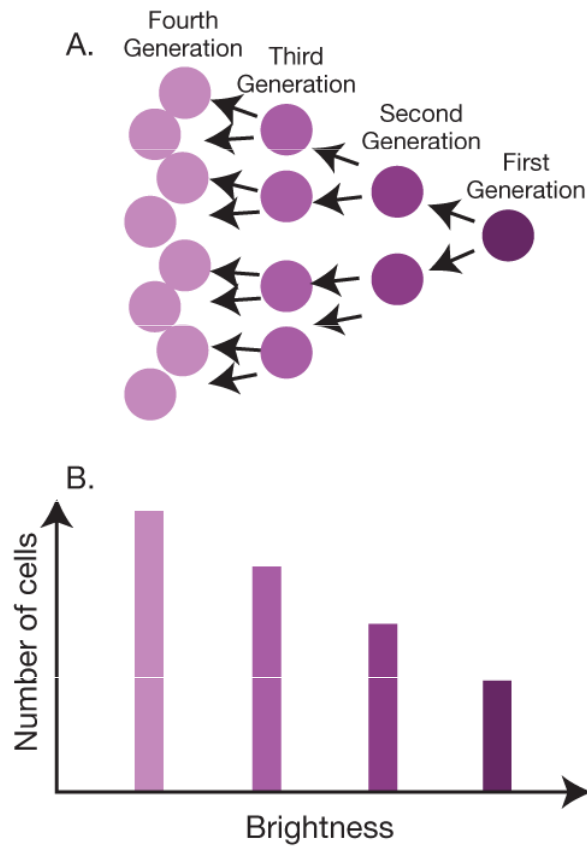
Viability assays (propidium iodid, Calcein AM, ...)

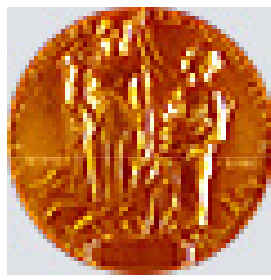


Proliferation (BrdU, EdU, mitosis - pH3)



Detekce počtu buněčného dělení





The Nobel Prize in Chemistry 2008

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



Photo: J. Henriksson/SCANPIX

Osamu Shimomura

🕒 1/3 of the prize

USA

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

b. 1928
(in Kyoto, Japan)



Photo: J. Henriksson/SCANPIX

Martin Chalfie

🕒 1/3 of the prize

USA

Columbia University
New York, NY, USA

b. 1947



Photo: UCSD

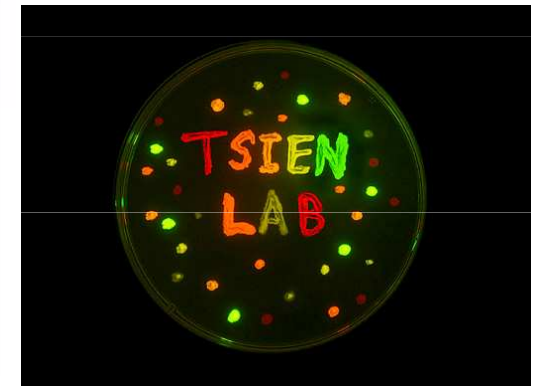
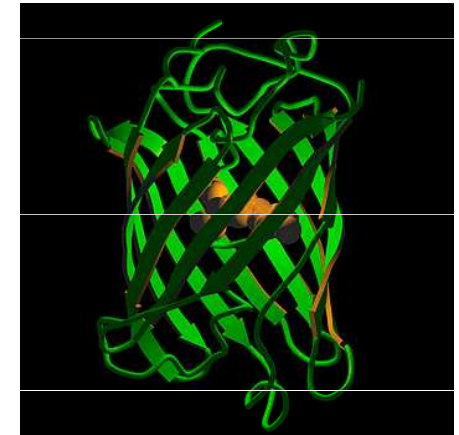
Roger Y. Tsien

🕒 1/3 of the prize

USA

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

b. 1952



Fluorescenční proteiny

■ bioluminescence resonance energy transfer (BRET)

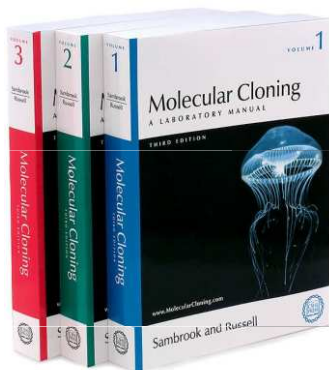
Aequorea victoria - medúza žijící ve vodách na pobřeží Severní Ameriky.

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

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

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

Aequorea victoria “Crystal jelly “



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

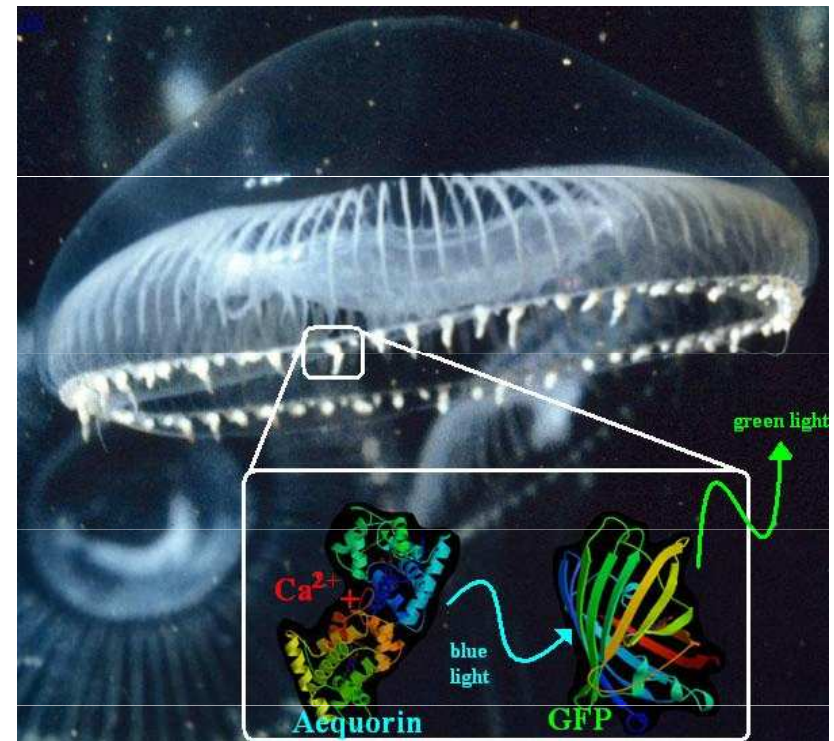
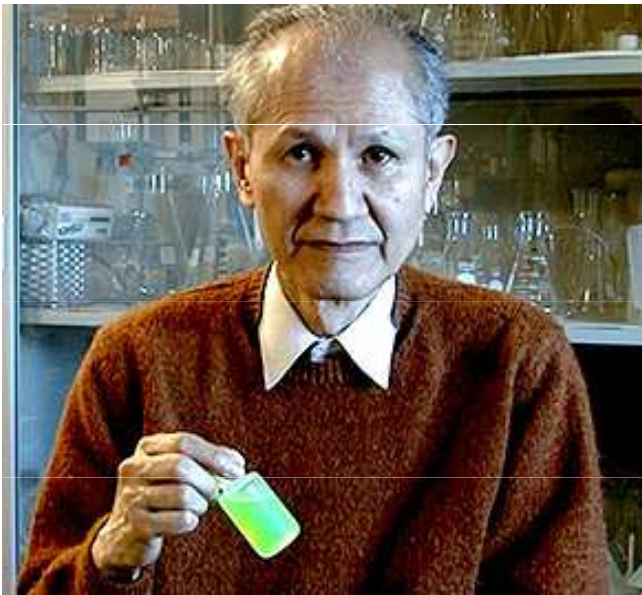
Renilla reniformis "Sea Pansy"



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

Fluorescenční proteiny

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



Fluorescenční proteiny

- Douglas Prasher
- Martin Chalfie

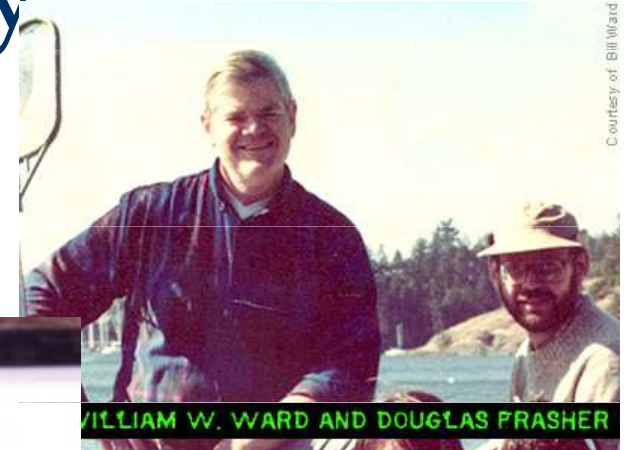
Science. 1994 Feb 11;263(5148):

Green fluorescent protein as a marker for gene expression.

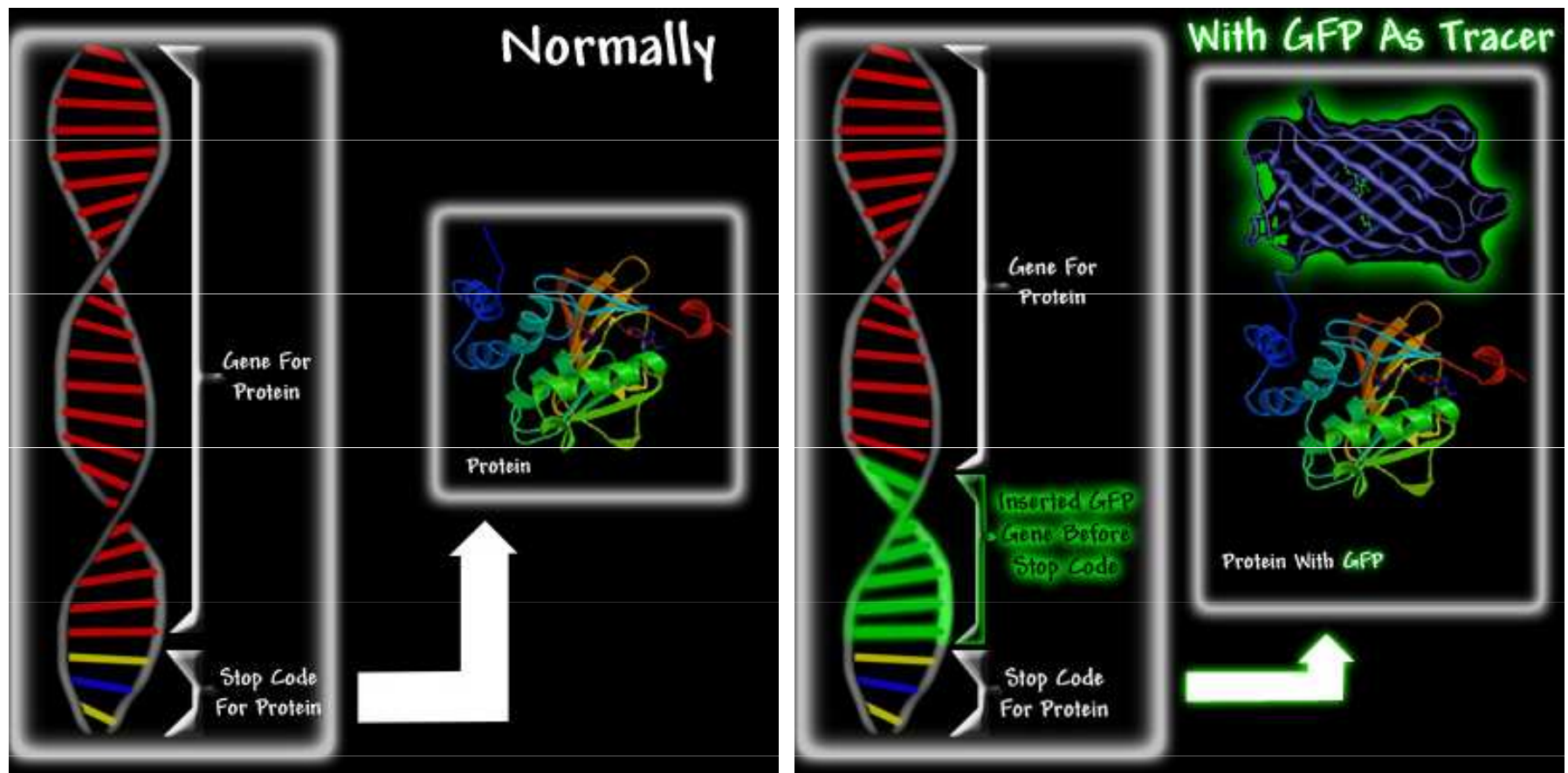
Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC.

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

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

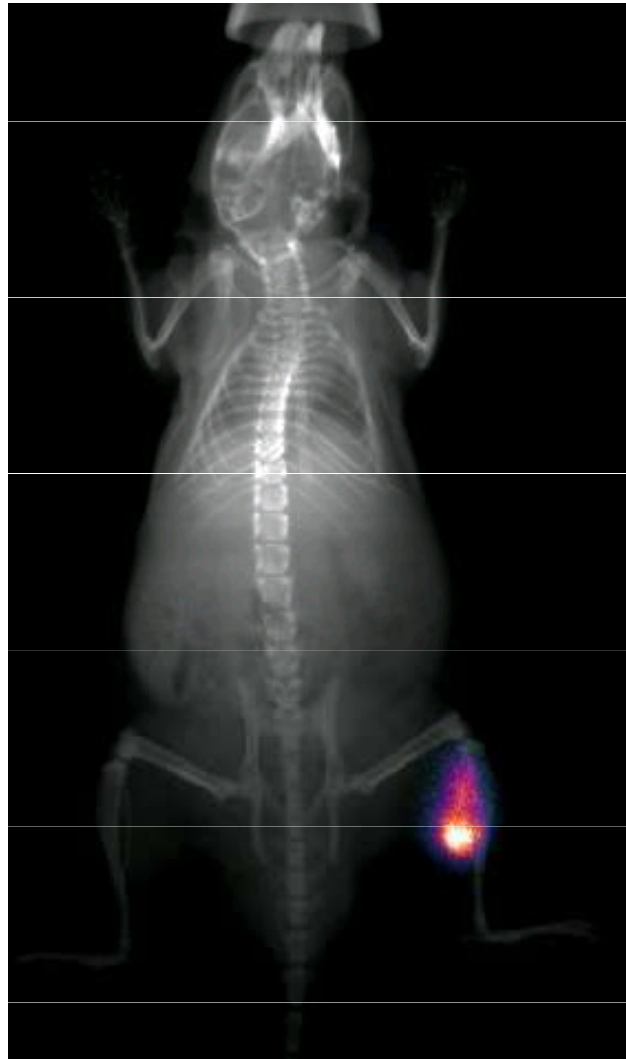


Fluorescenční proteiny

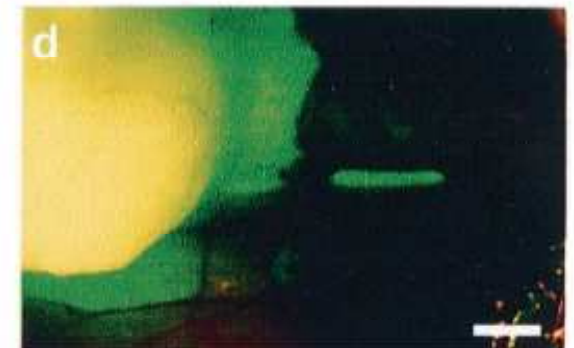
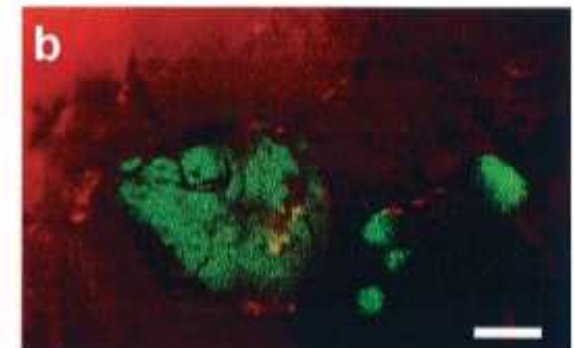
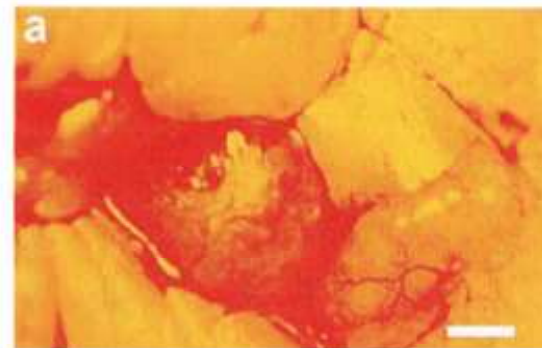
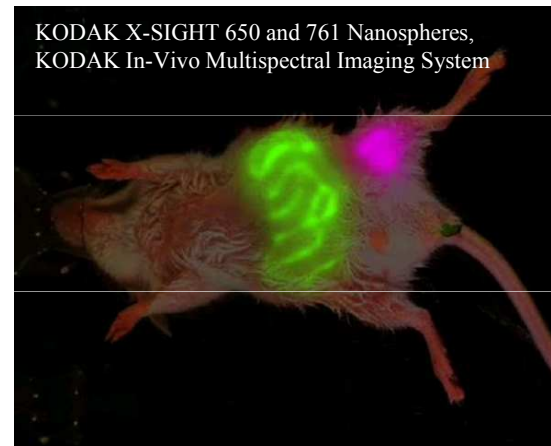


<http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm>

in vivo molekulární vizualizace



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

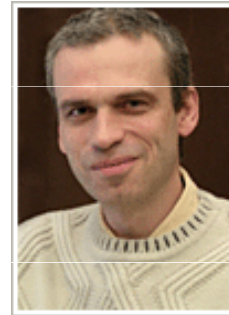


Hasegawa, S., Yang, M., Chishima, T., Miyagi, Y., Shimada, H., Moossa, A. R., and Hoffman, R. M. In vivo tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. *Cancer Gene Ther*, 7: 1336-1340, 2000.

Fluorescenční proteiny

■ Sergey A. Lukyanov

- Objevil „GFP-like“ proteiny u nesvětélkujících korálů



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RESEARCH

Fluorescent proteins from nonbioluminescent Anthozoa species

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

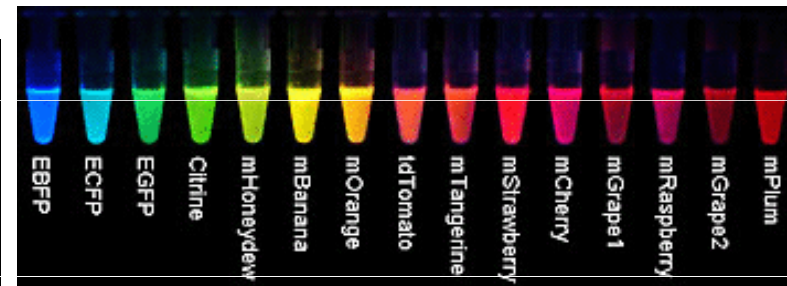
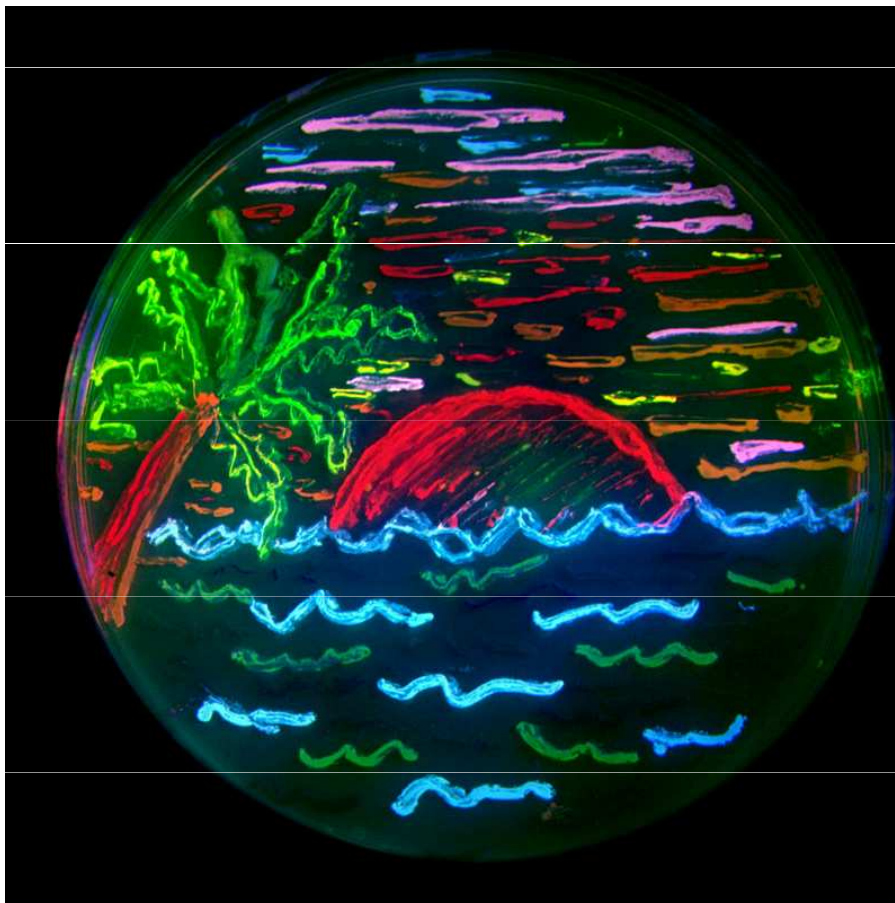
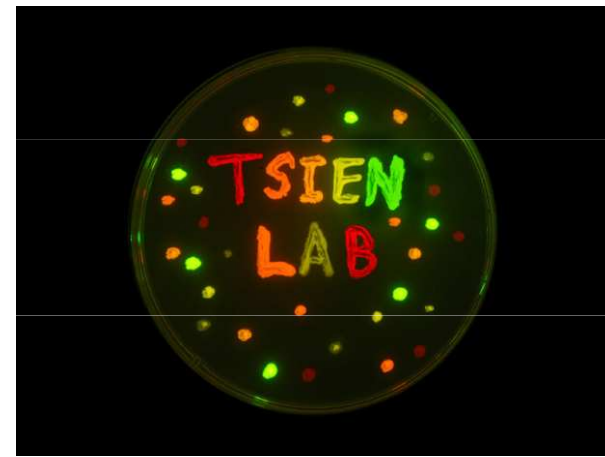
*Institute of Bioorganic Chemistry, Russian Academy of Science, 117871 Moscow, Russia. ¹Institute of Ecology and Evolution, and ²Institute of Biochemistry Russian Academy of Science, 17071 Moscow, Russia. *Corresponding author (e-mail: luk@ibch.siobc.ras.ru).*

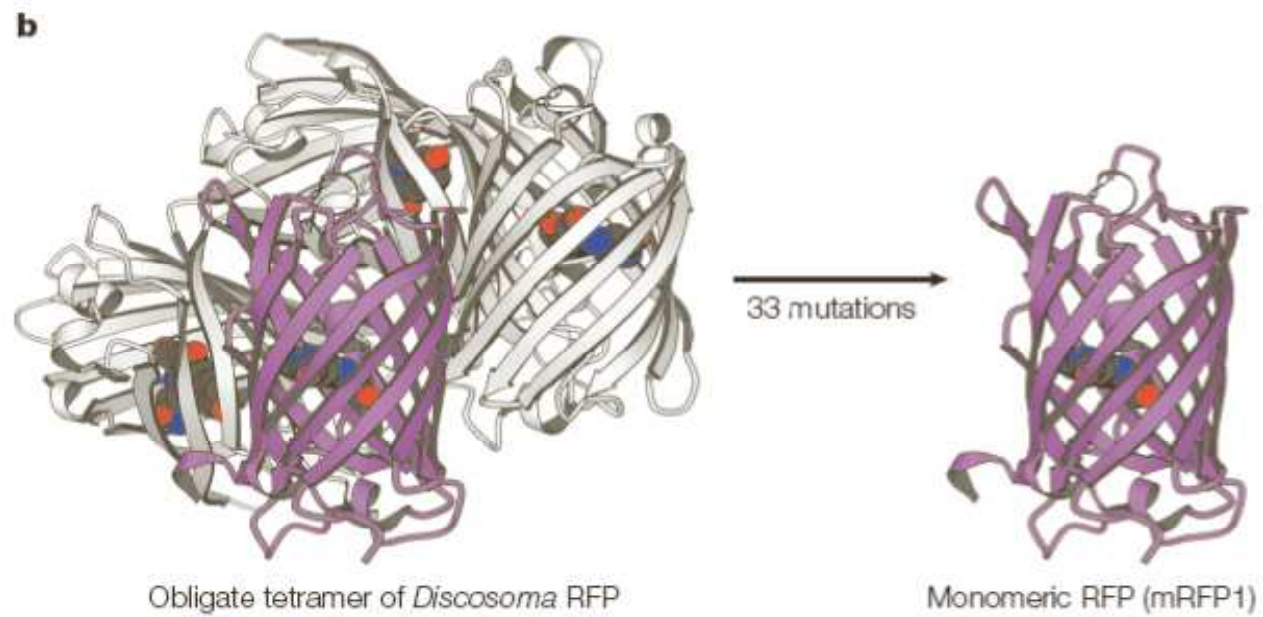
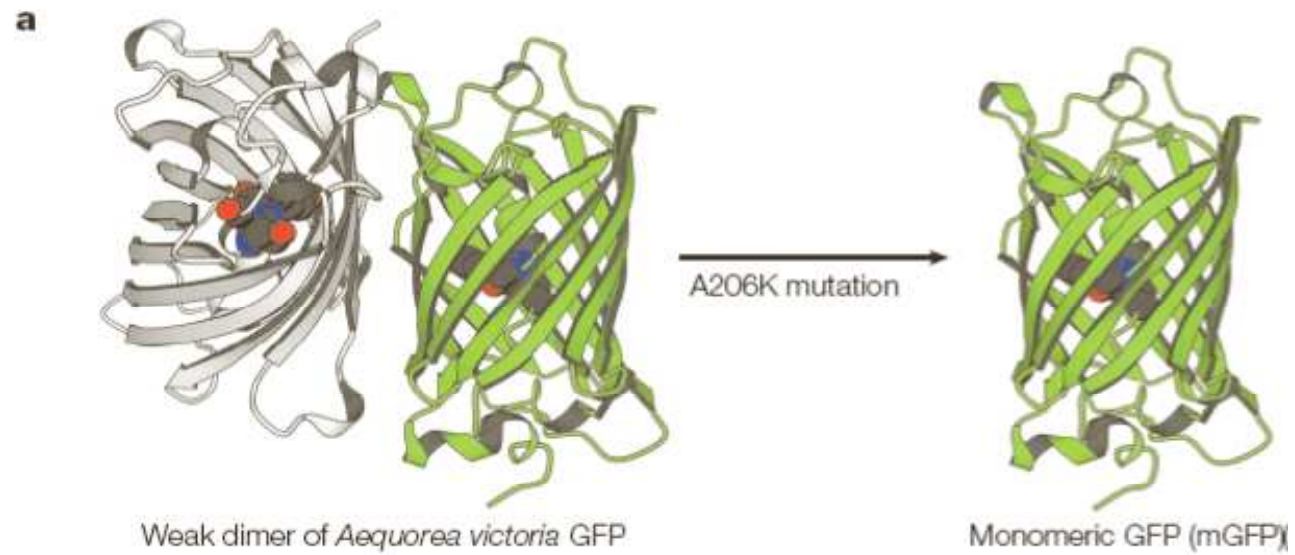
Received 28 May 1999; accepted 18 July 1999

Roger Tsien

- ~ 2002 – mutace FP = barevné spektrum

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





CREATING NEW FLUORESCENT PROBES FOR CELL BIOLOGY

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

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

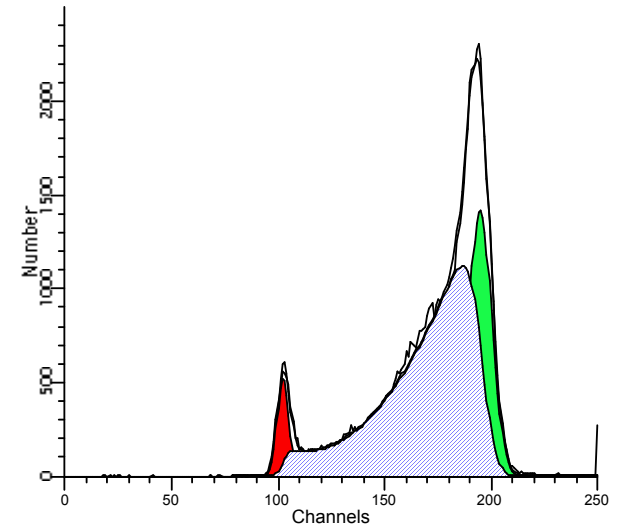
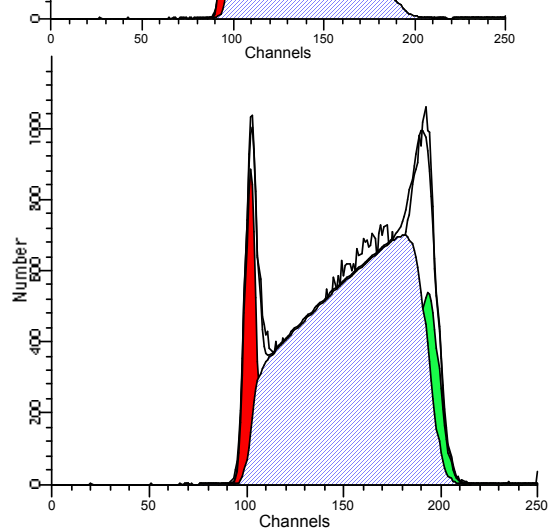
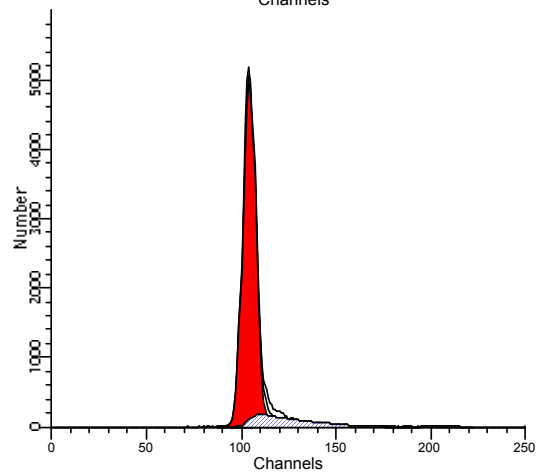
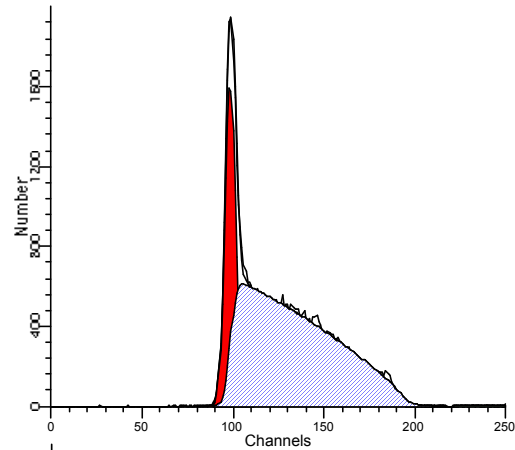
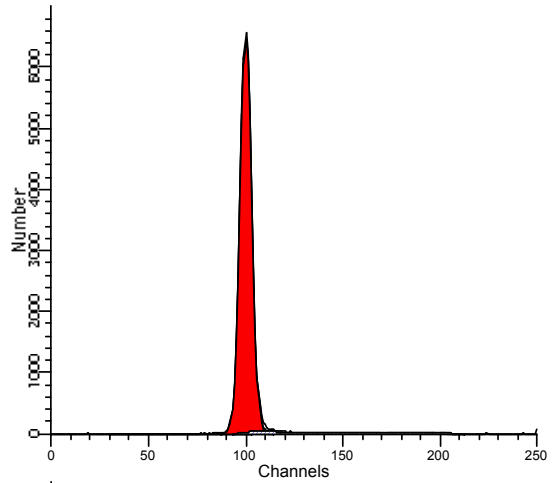
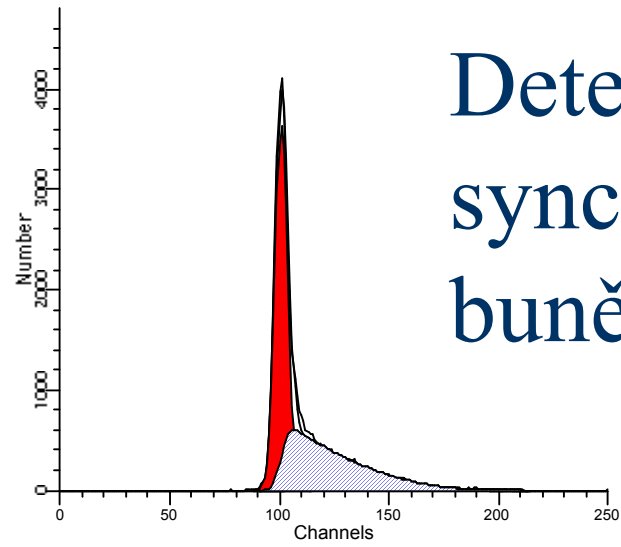
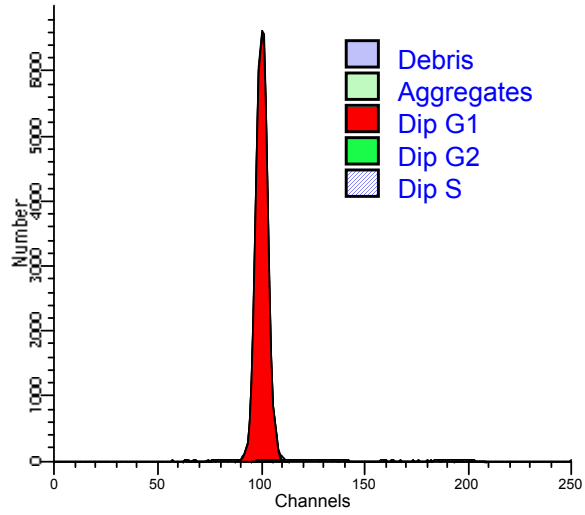
Class	Protein	Source laboratory (references)	Excitation ^c (nm)	Emission ^d (nm)	Brightness ^e	Photostability ^f	pKa	Oligomerization
Far-red	mPlum ^g	Tsien (5)	590	649	4.1	53	<4.5	Monomer
Red	mCherry ^g	Tsien (4)	587	610	16	96	<4.5	Monomer
	tdTomato ^g	Tsien (4)	554	581	95	98	4.7	Tandem dimer
	mStrawberry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

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

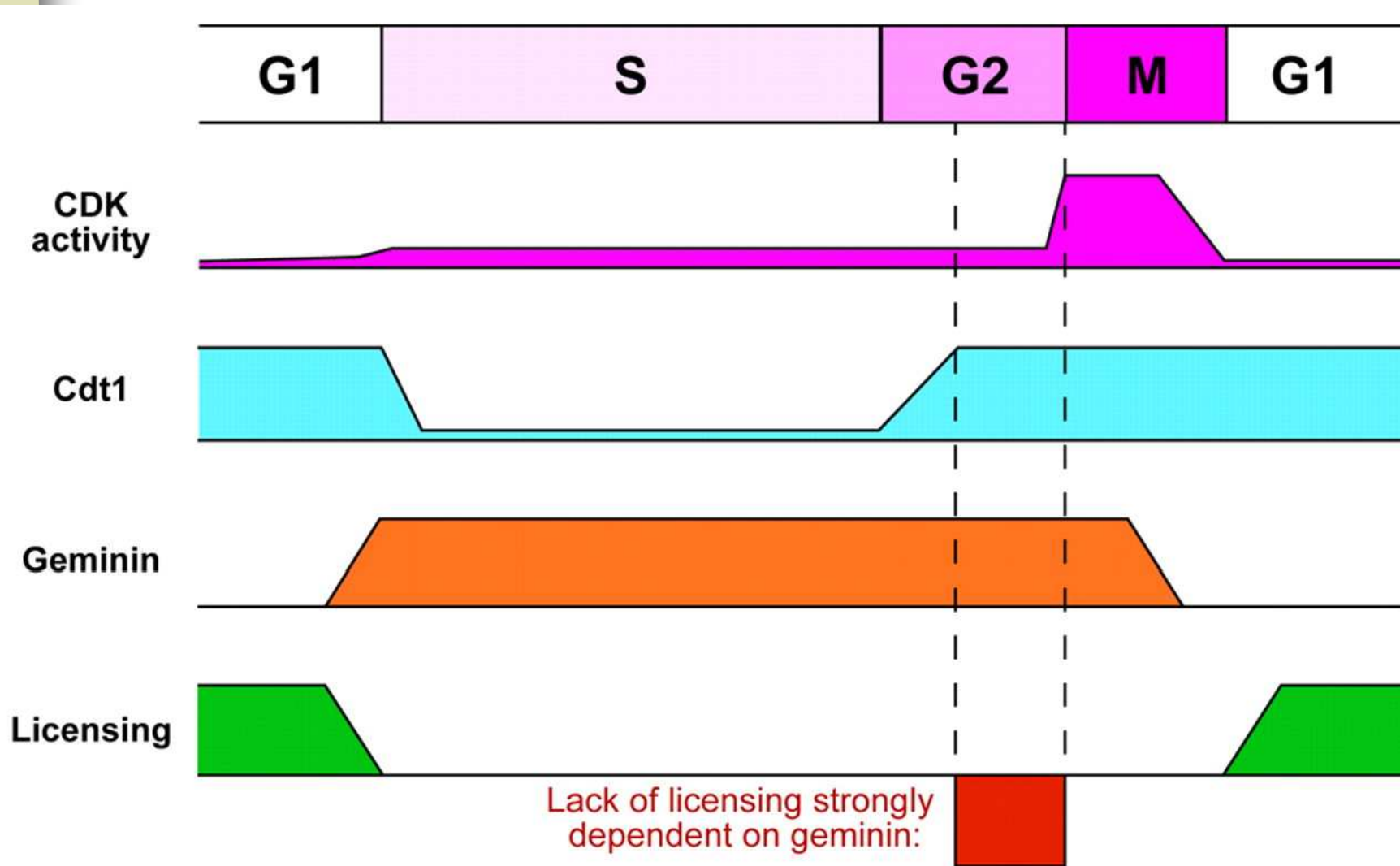
A guide to choosing fluorescent proteins

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

Detekce buněk v synchronizovaném buněčném cyklu

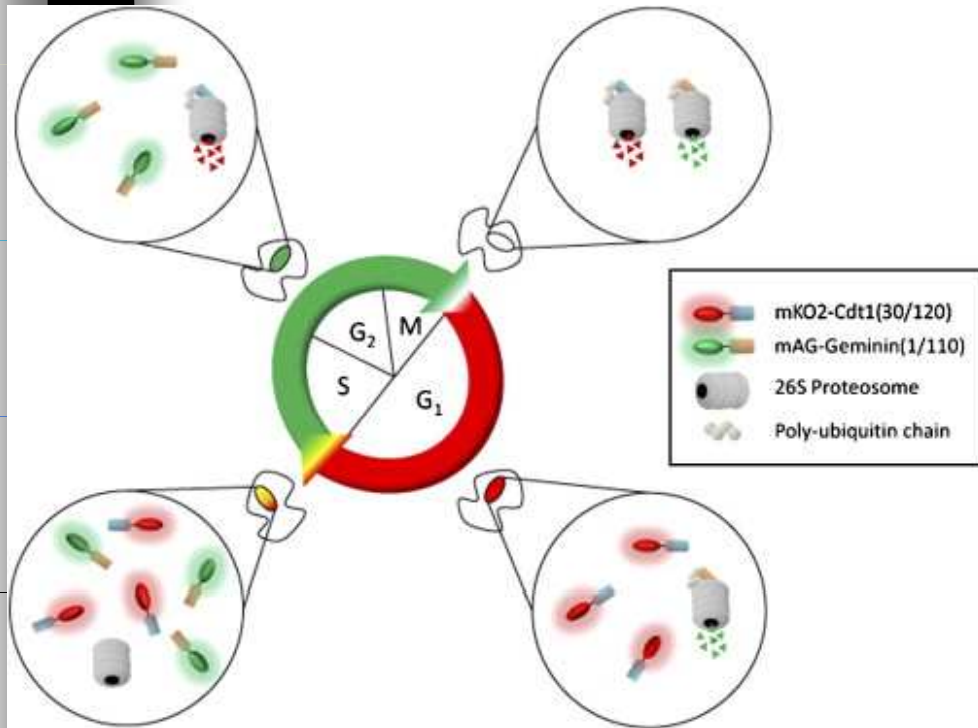


Licensing control by Cdt1 and geminin



Fucci

(fluorescent ubiquitination-based cell cycle indicator) cells



Ubiquitin E3 ligase complexes

G₁ - APC^{Cdh1}

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

S, G₂, M- SCF^{Skp2}

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

Fucci sensors - 1st generation, coral FP

monomeric Kusabira orange 2 – hCdt1 (30/120)

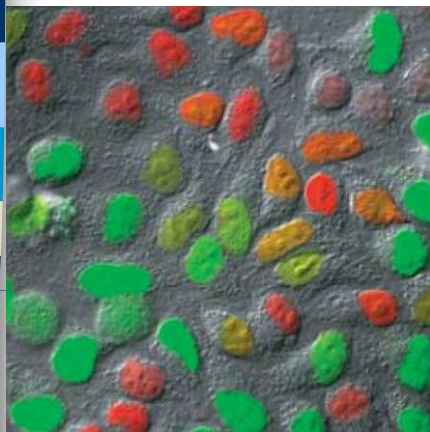
Monomeric Azami-Green – hGeminin (1/110)

Fucci sensors – 2nd generation, *Aequorea* FP

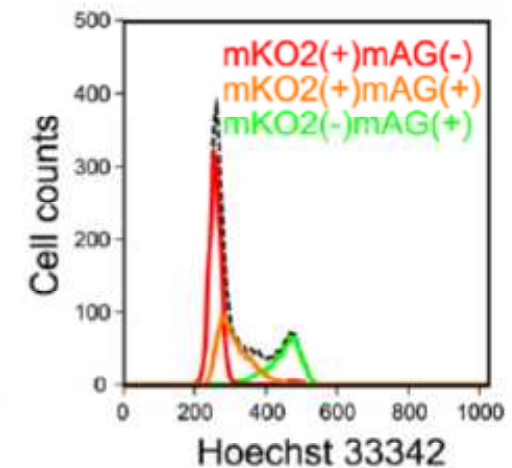
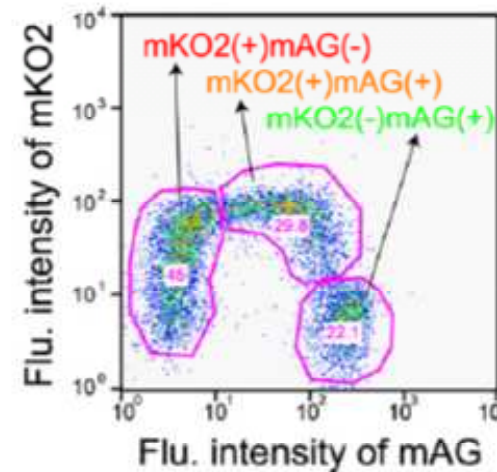
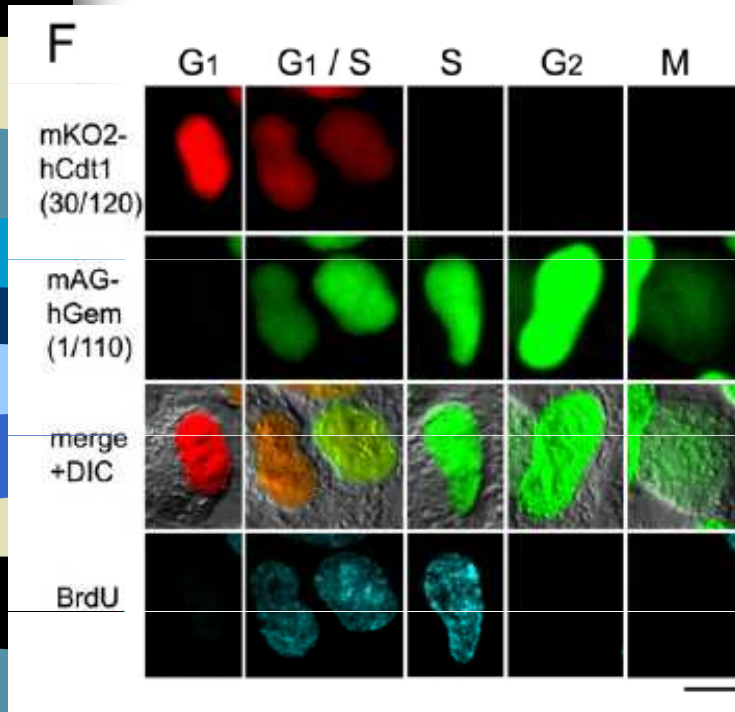
red monomeric fluorescent protein - mCherry -
hCdt1 (30/120)

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

Chemistry & Biology 15, February 2008 ©2008 Elsevier Ltd



Fucci



Resource

Cell

Visualizing Spatiotemporal Dynamics of Multicellular Cell-Cycle Progression

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

¹Laboratory for Cell Function and Dynamics

²Laboratory for Cell Culture Development

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

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

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

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

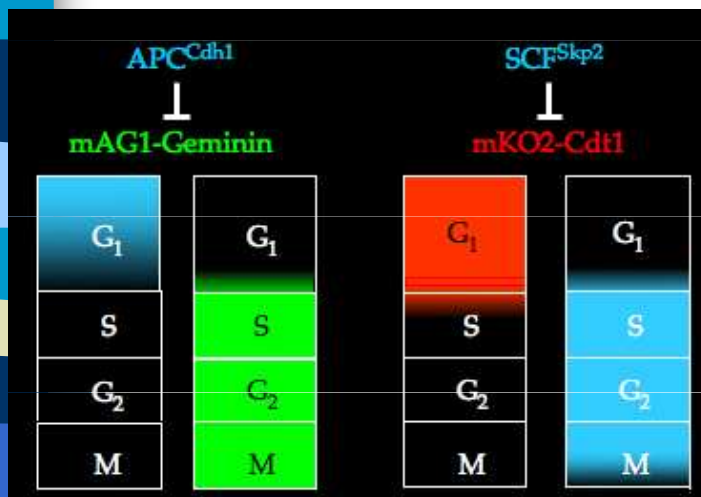
⁷Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Syowa-ku, Nagoya, Aichi 466-8550, Japan

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

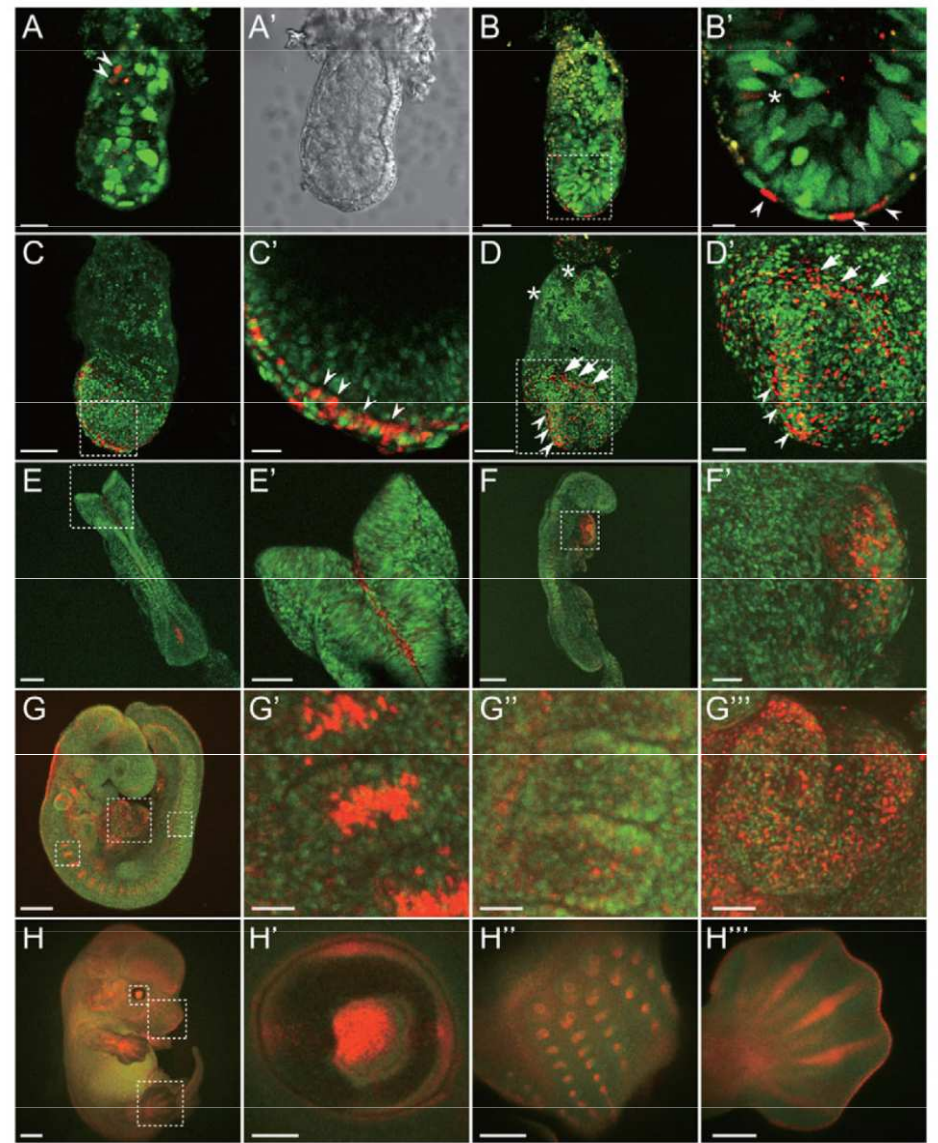
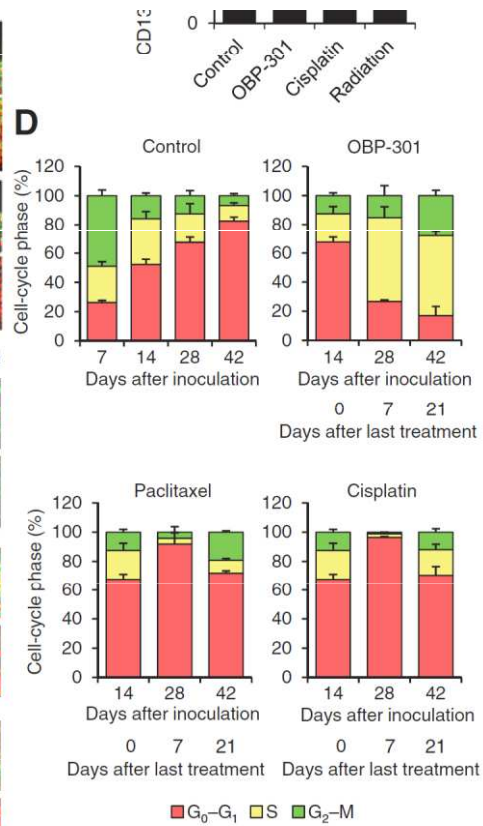
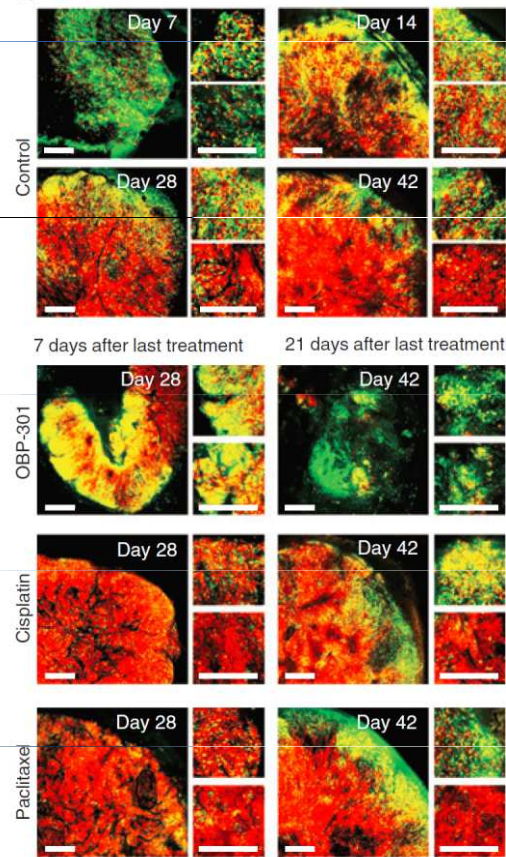
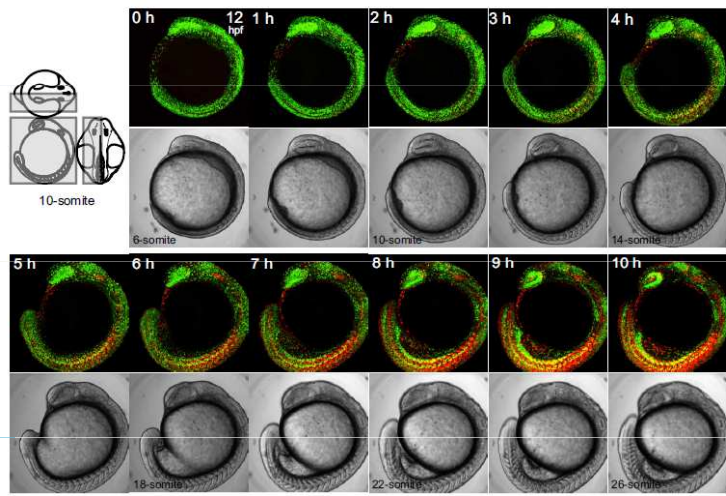
⁹Genome Dynamics Project, Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan

*Correspondence: matsushi@brain.riken.jp

DOI 10.1016/j.cell.2007.12.033



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

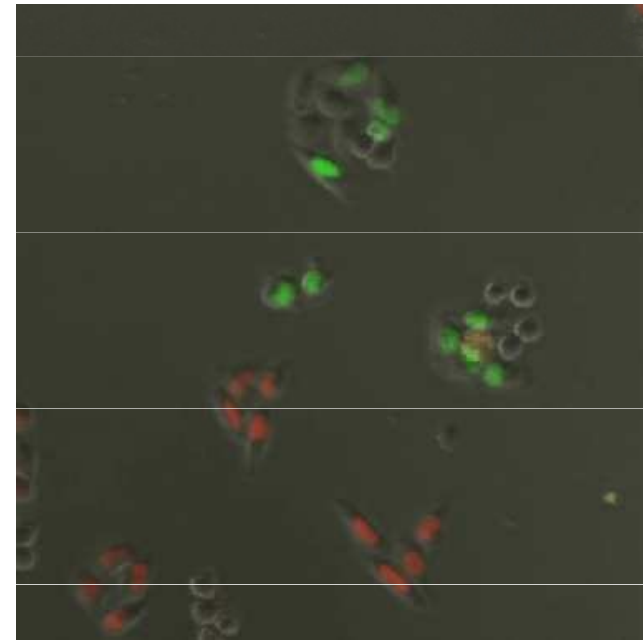
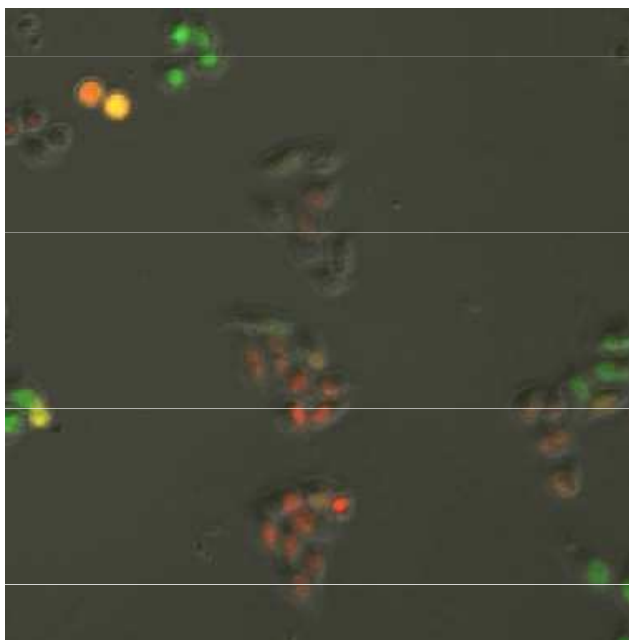
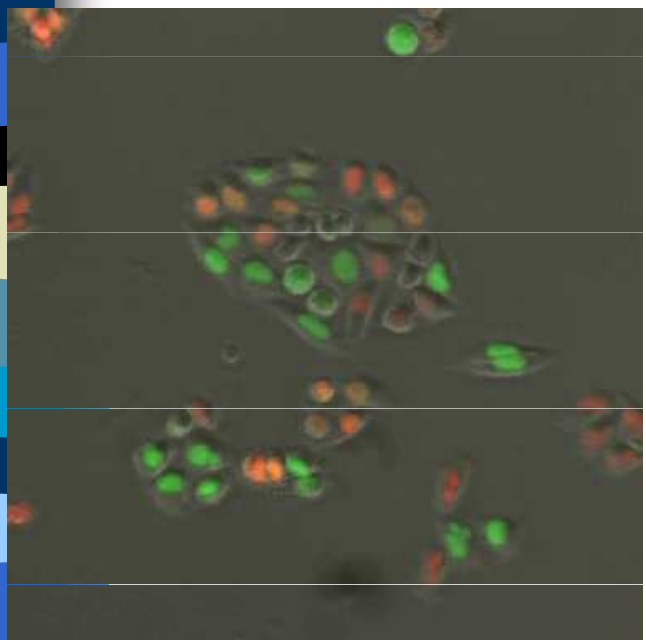


CONTROL

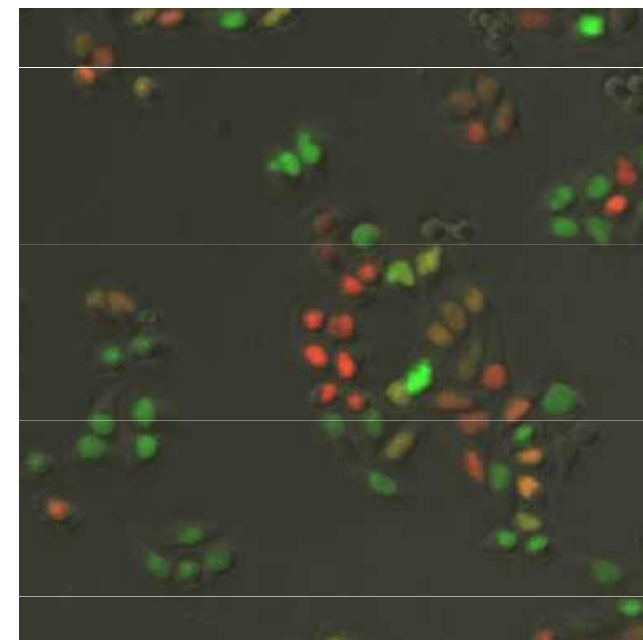
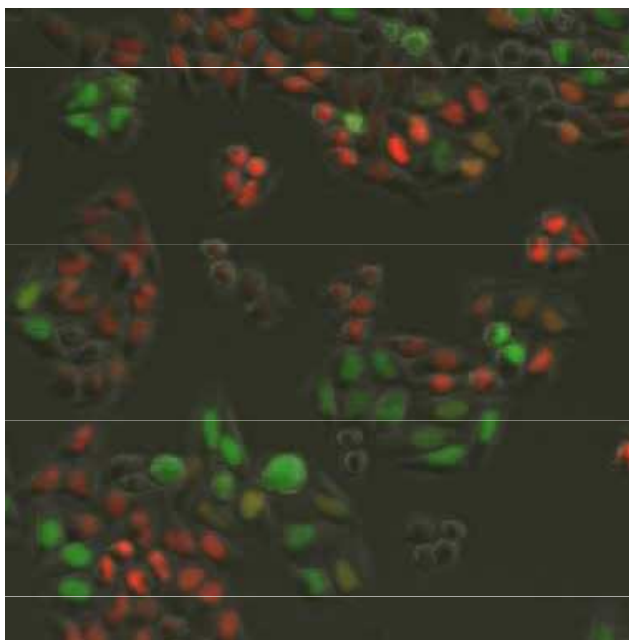
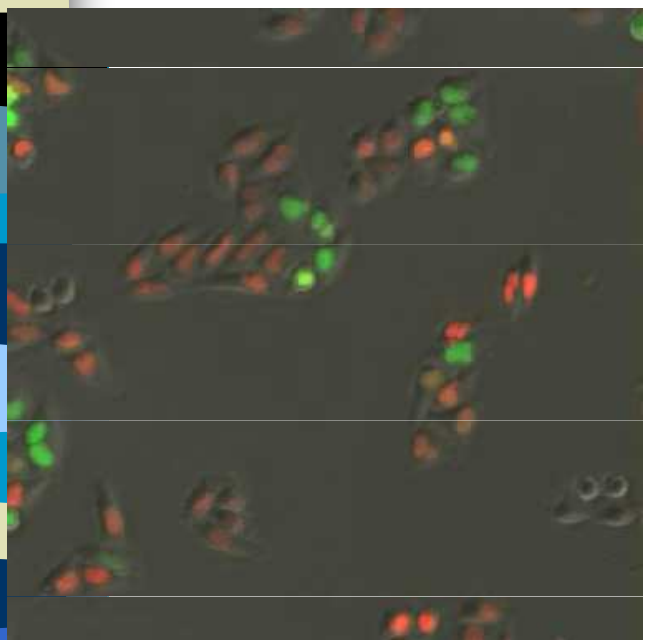
SCH900776

MU380

VEHICLE



GEMCITABINE



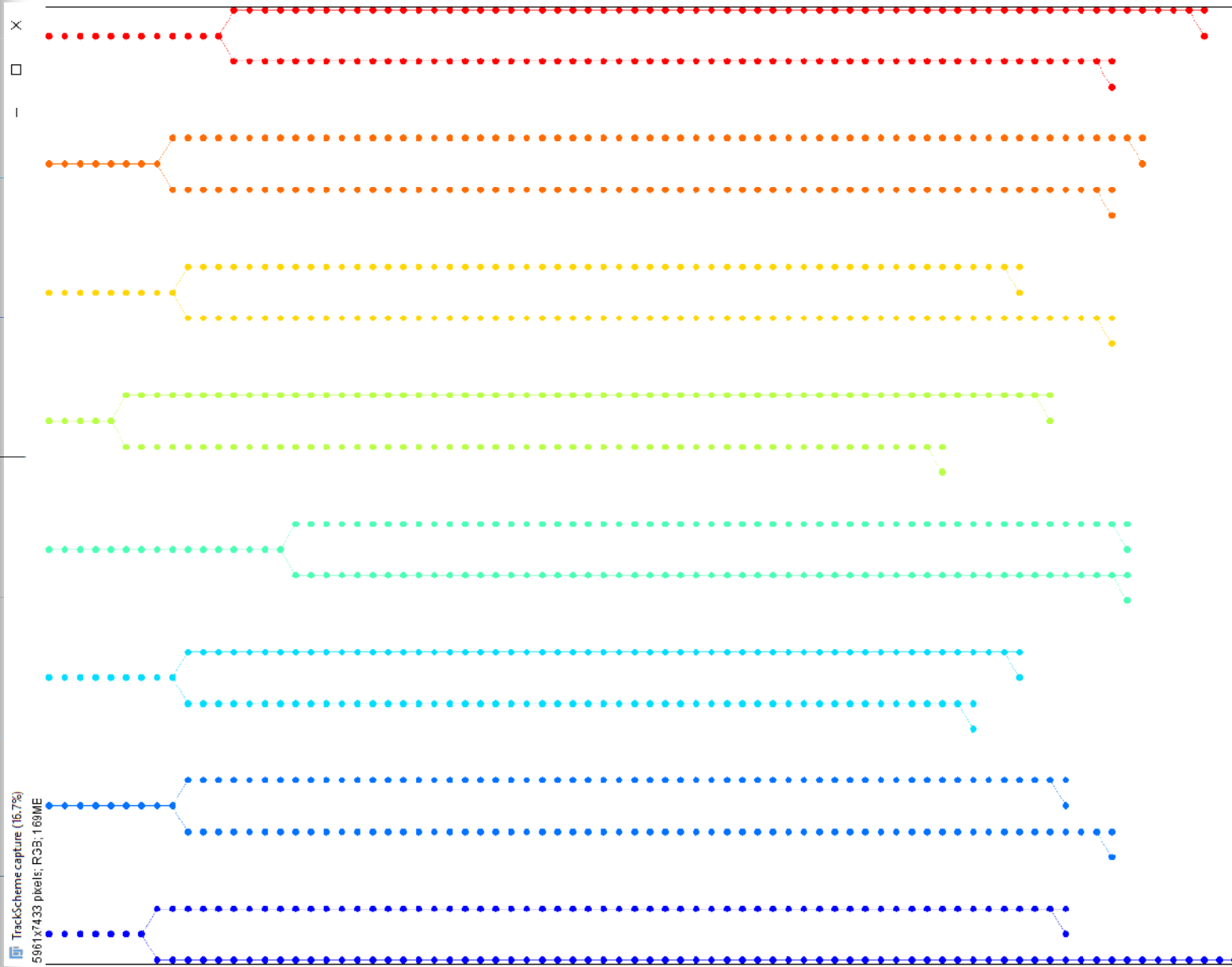


...lot of questions, but how to answer them?

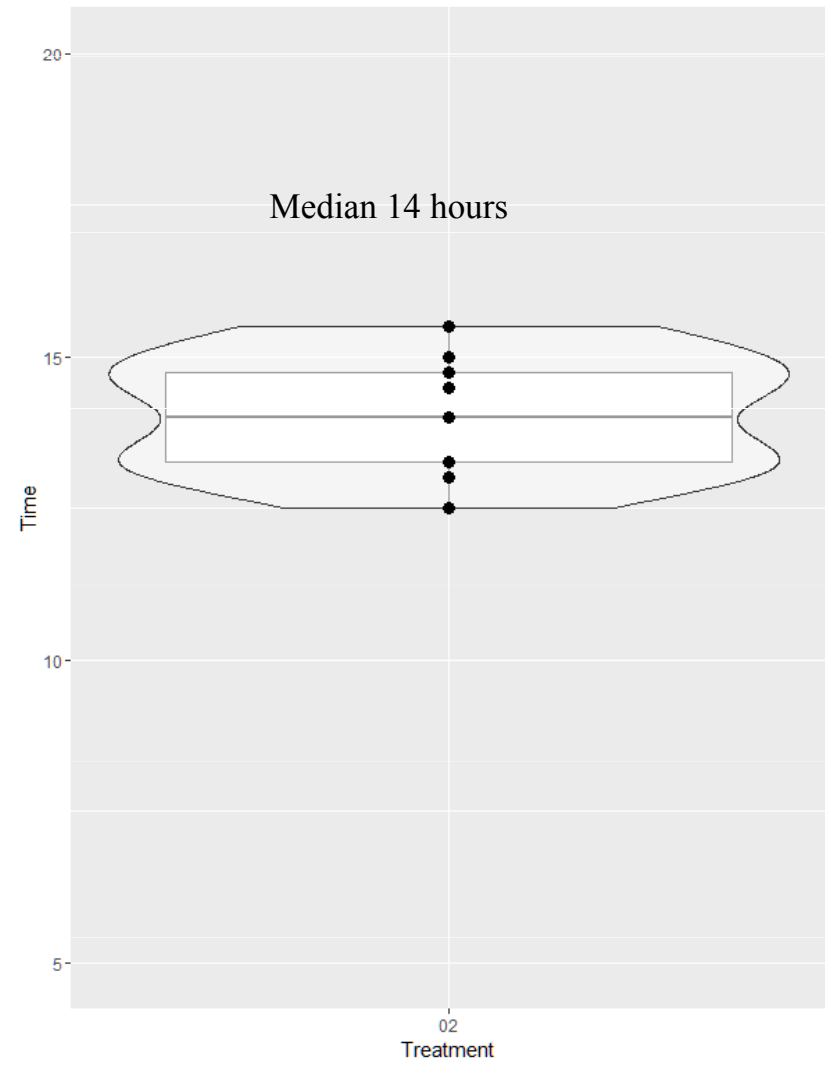
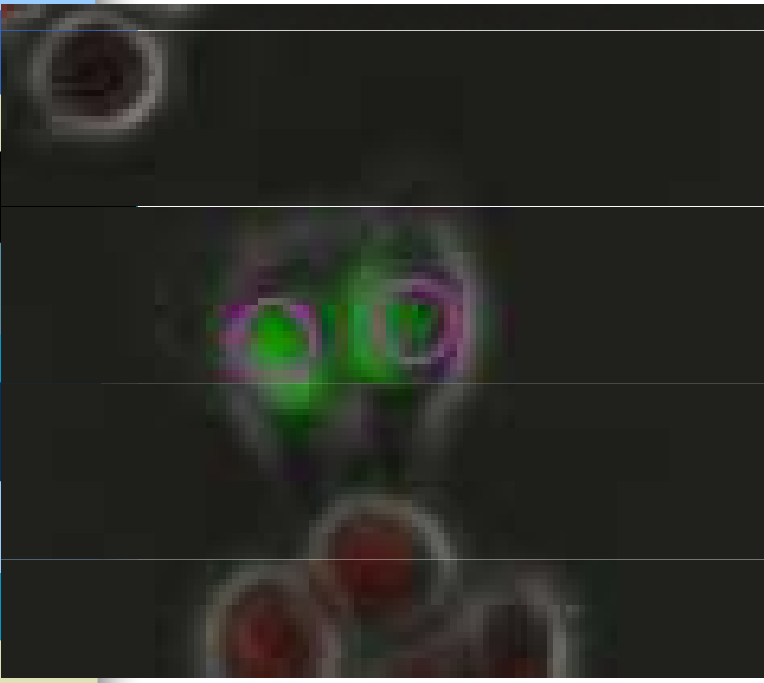
- How many times cells divided?
- What is a length of cell cycle phases?
- Is there a difference in time between first and second division?
- How it is all affected by my drugs?

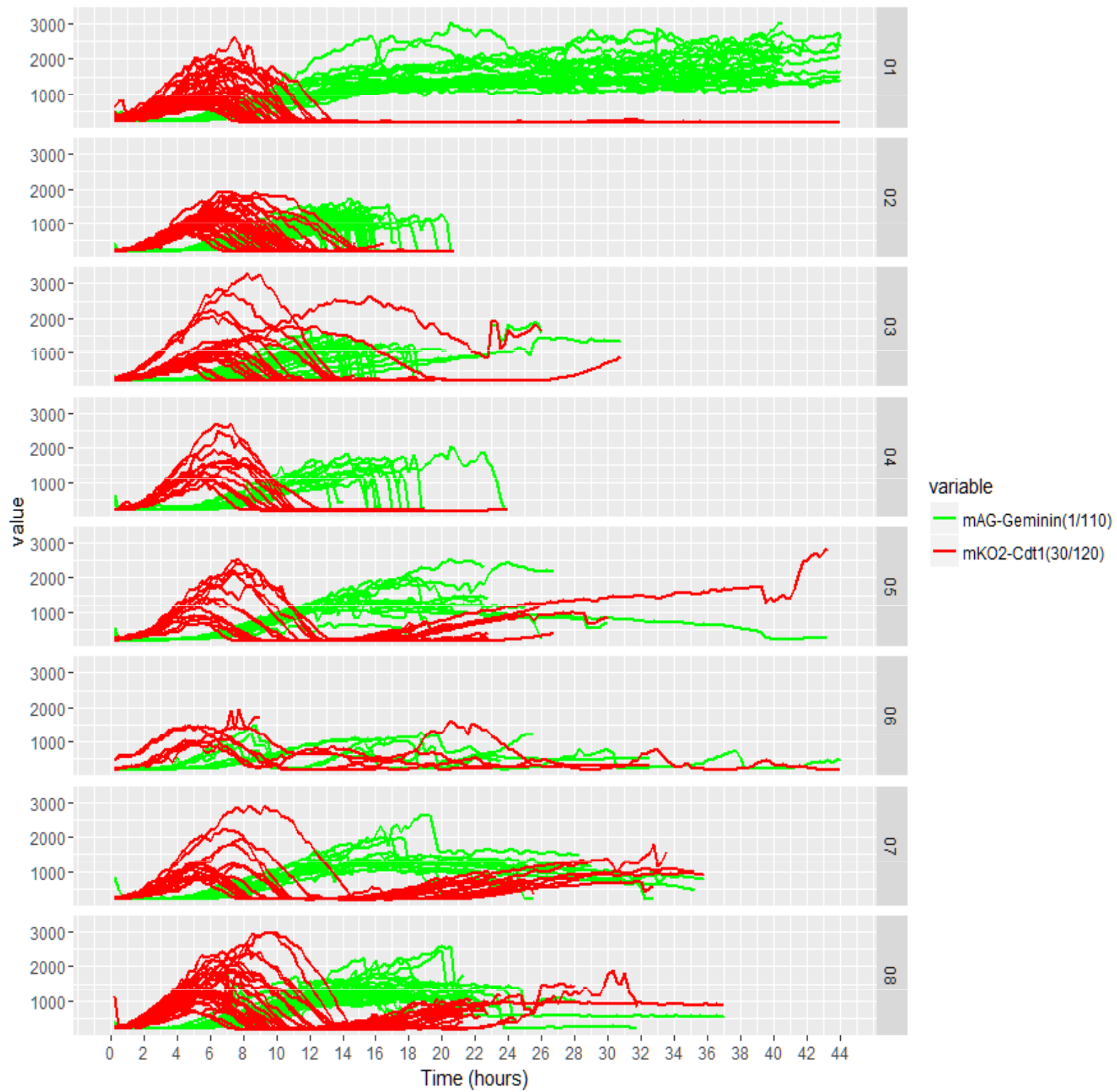
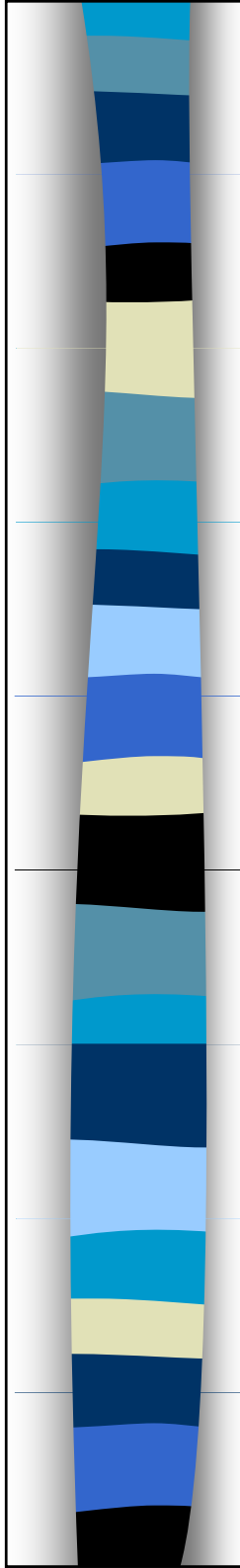
Branches (dvisions) analysis

02_02_01_01

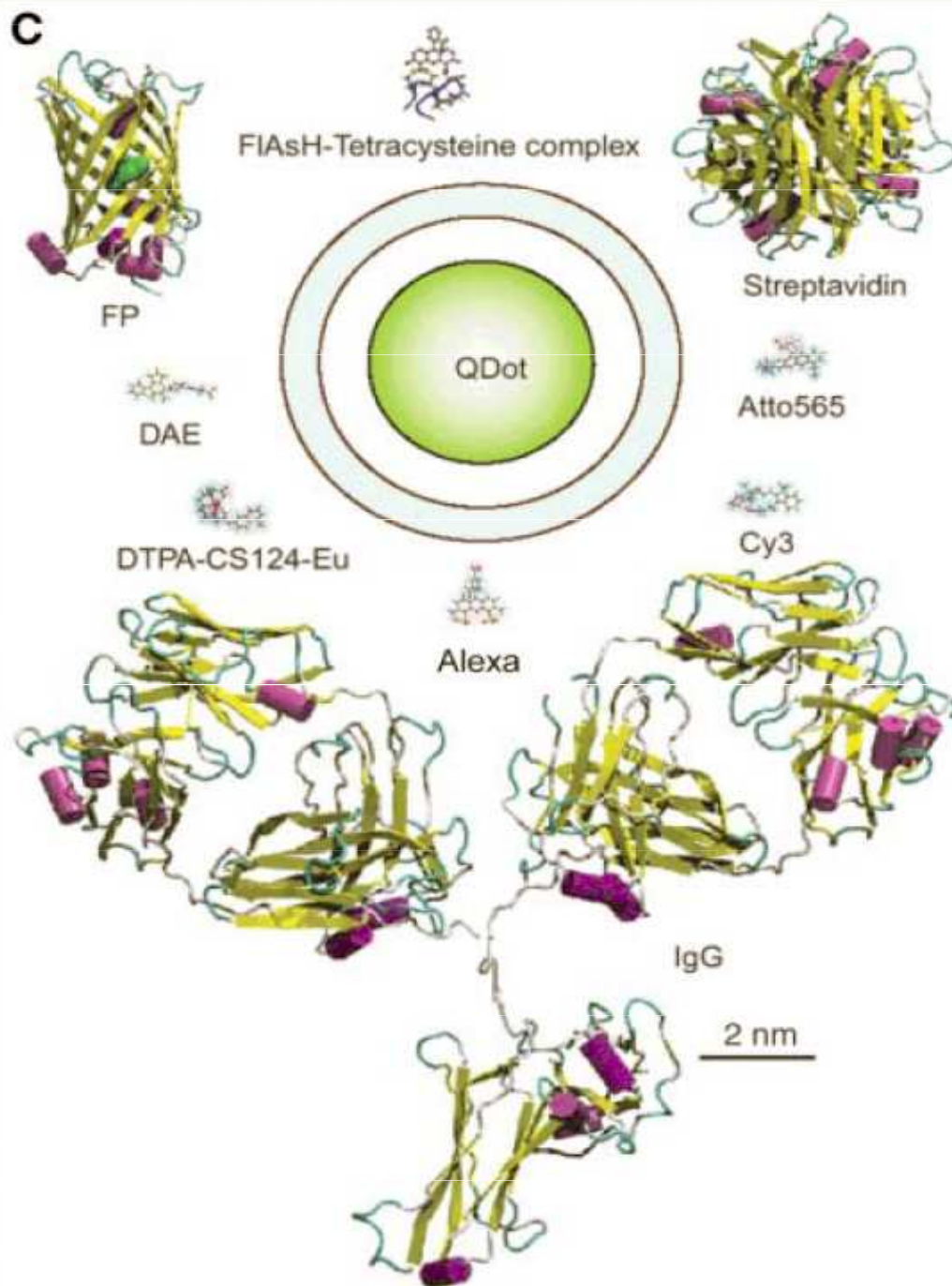


02_02_01_01





Targeting proteins & fluorophores



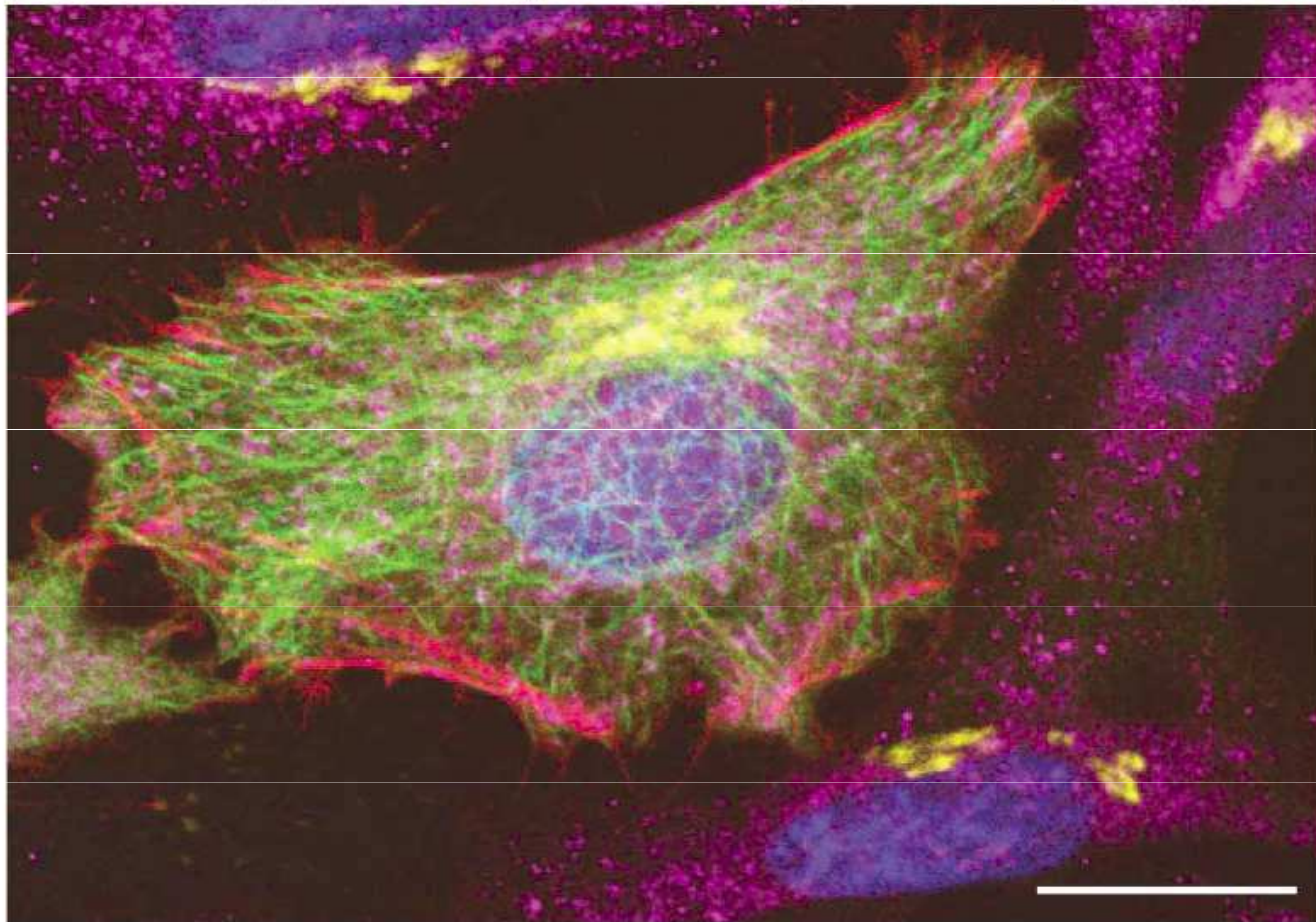
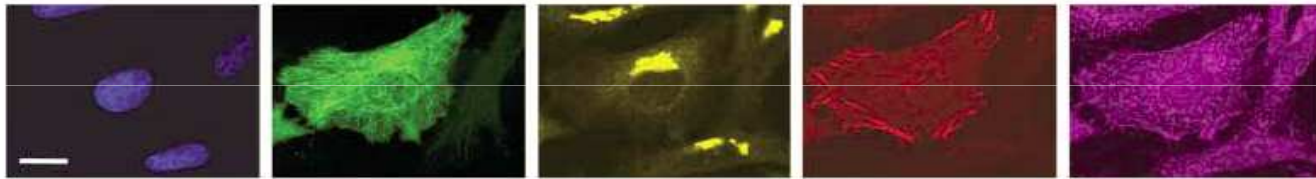
REVIEW

The Fluorescent Toolbox for Assessing Protein Location and Function

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

SCIENCE VOL 312 14 APRIL 2006

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



REVIEW

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SCIENCE VOL 312 14 APRIL 2006

Vitální analýza buněčných funkcí

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

Detekce viability

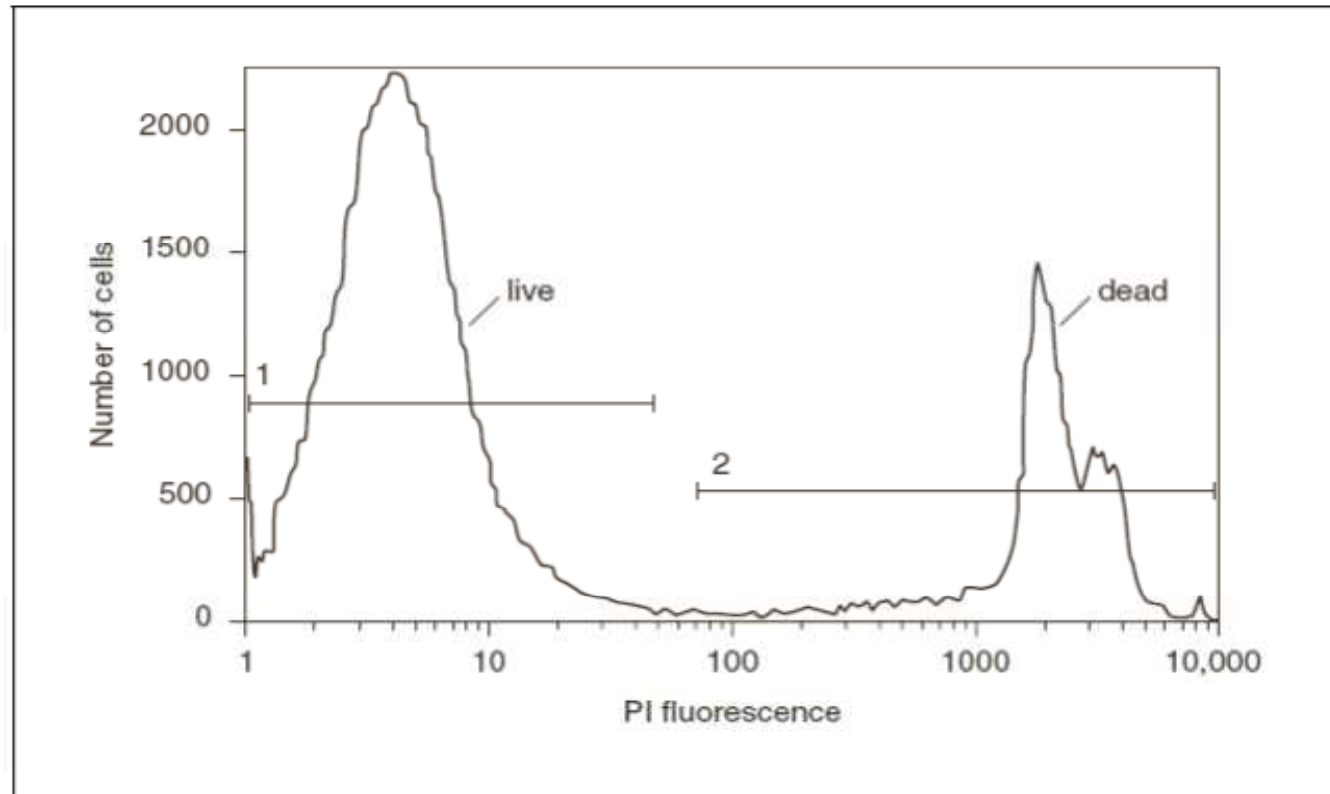
- jedna z nejjednodušších analýz
- funguje na principu:
 - detekce membránové integrity - neprůchodnosti některých fluorescenčních značek cytoplazmatickou membránou živých buněk – **propidium iodide, ethidium bromide, 7-amino actinomycin D**
 - detekce fyziologického stavu buněk – použití fluorescenčních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

 invitrogen™

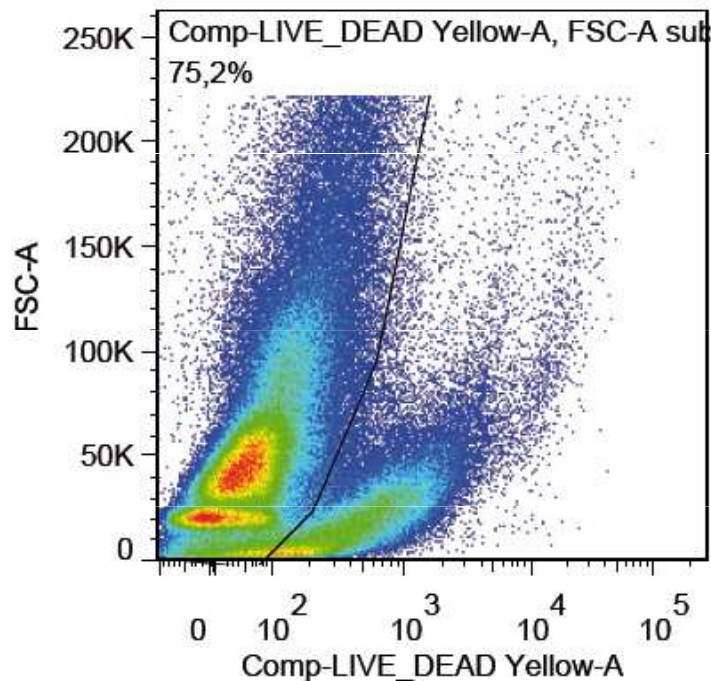
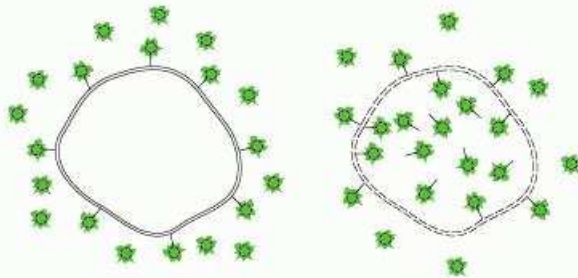
Reactive dye	Excitation source	Ex*	Em*
blue fluorescent reactive dye (L23105)	UV	350	450
violet fluorescent reactive dye (L34955)	405 nm	416	451
aqua fluorescent reactive dye (L34957)	405 nm	367	526
yellow fluorescent reactive dye (L34959)	405 nm	400	575
green fluorescent reactive dye (L23101)	488 nm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

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

Detekce viability



VIABILITY using LIVE/DEAD fixable stains



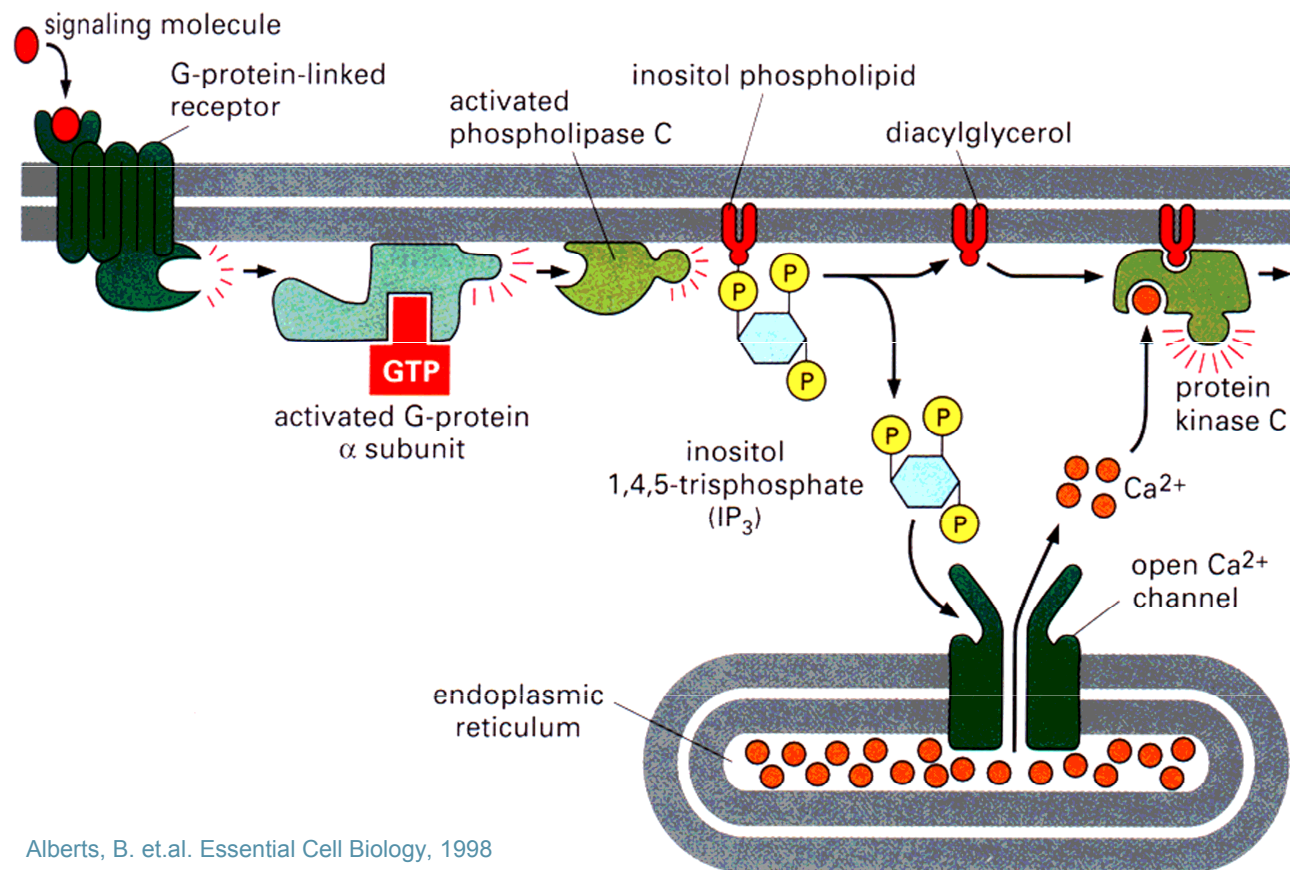
Principle: reaction of a fluorescent reactive dye with cellular amines, in necrotic cells react with free amines both in the interior and on the cell = intense staining, live cells stained on surface only = dim signal

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

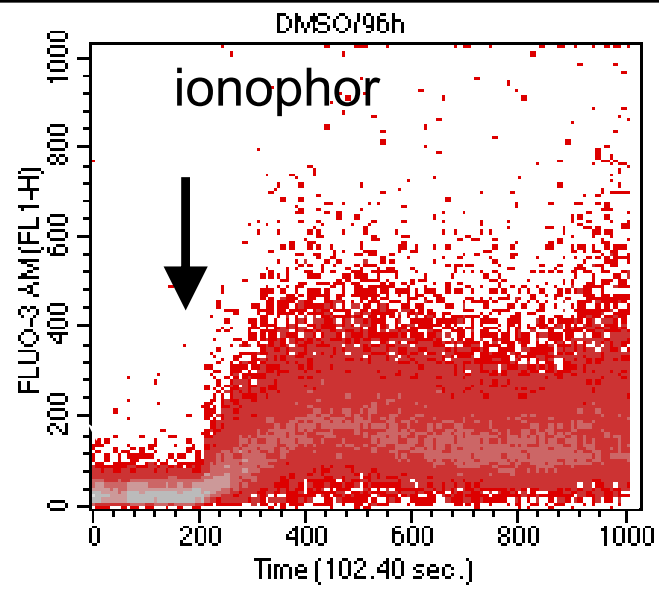
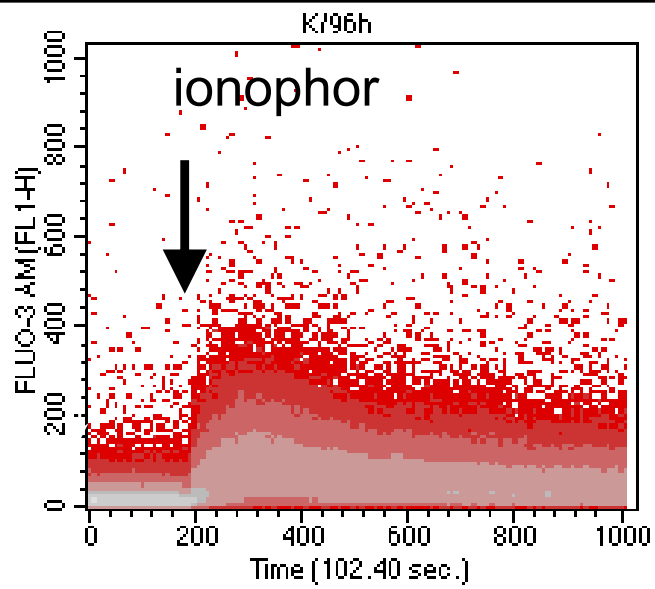
Cons: live cells have signal, stain only in buffers w/o BSA or serum, Tris or azide

Přenos signálu pomocí Ca^{2+}

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

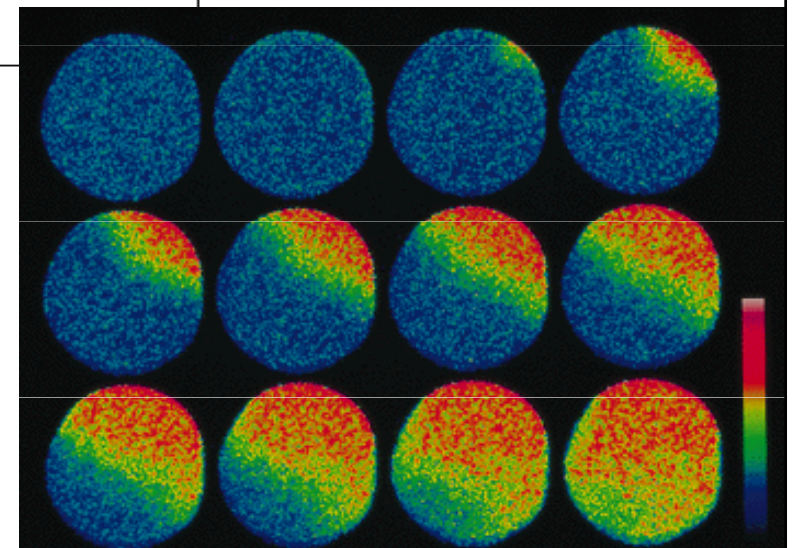
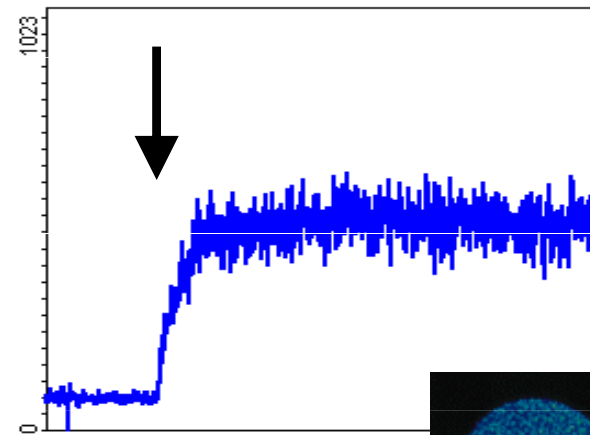
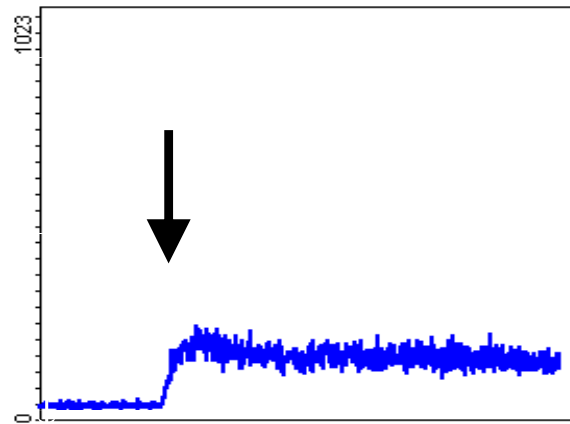


Alberts, B. et.al. Essential Cell Biology, 1998



Ca²⁺ influx

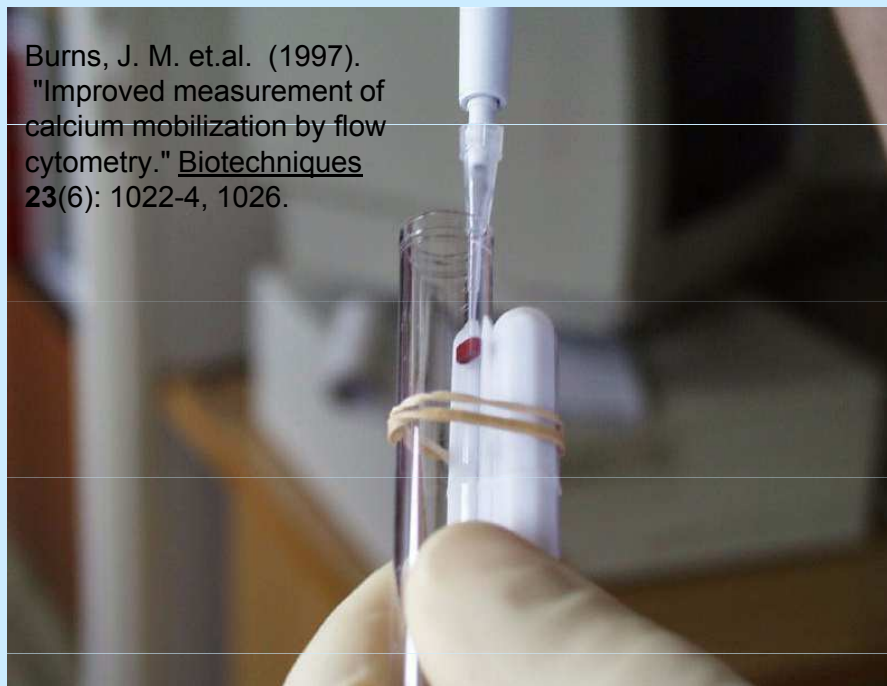
- Fura-2
- Fluo-3
- Indo-1



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

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

<http://www.cytekdev.com>

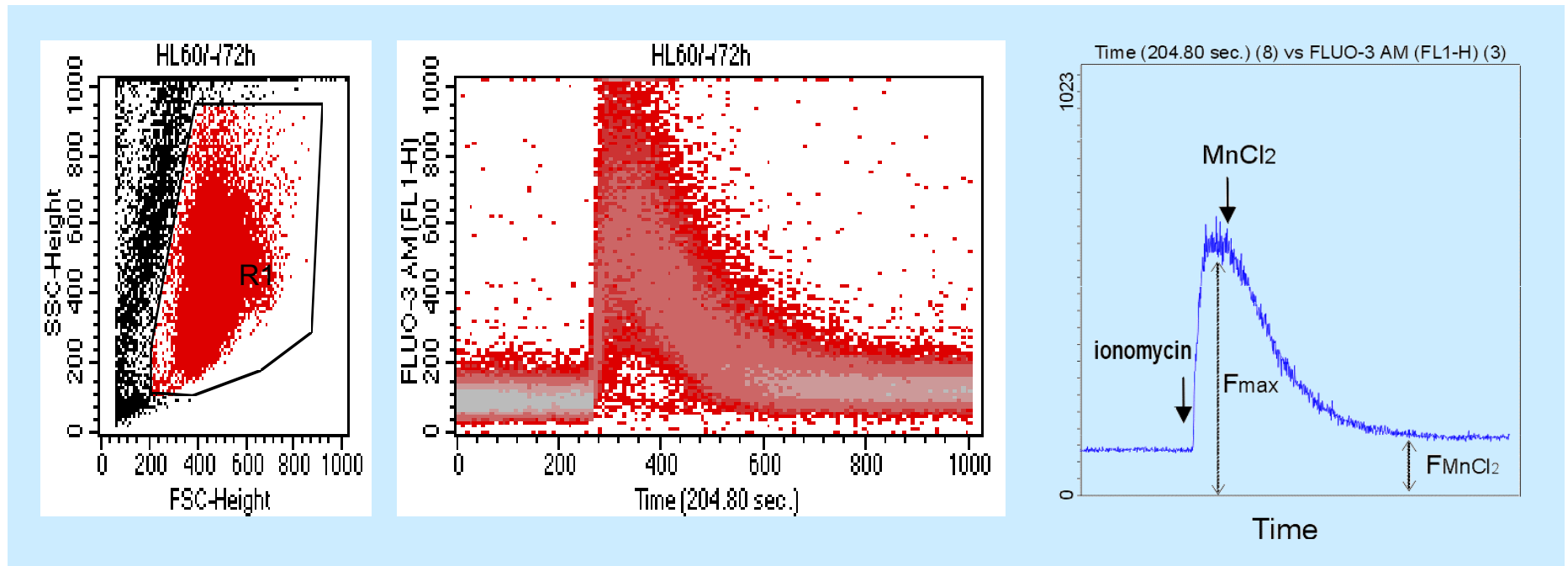


Burns, J. M. et.al. (1997).
"Improved measurement of calcium mobilization by flow cytometry." *Biotechniques* 23(6): 1022-4, 1026.



Kalibrace

(pro jednu vlnovou délku)

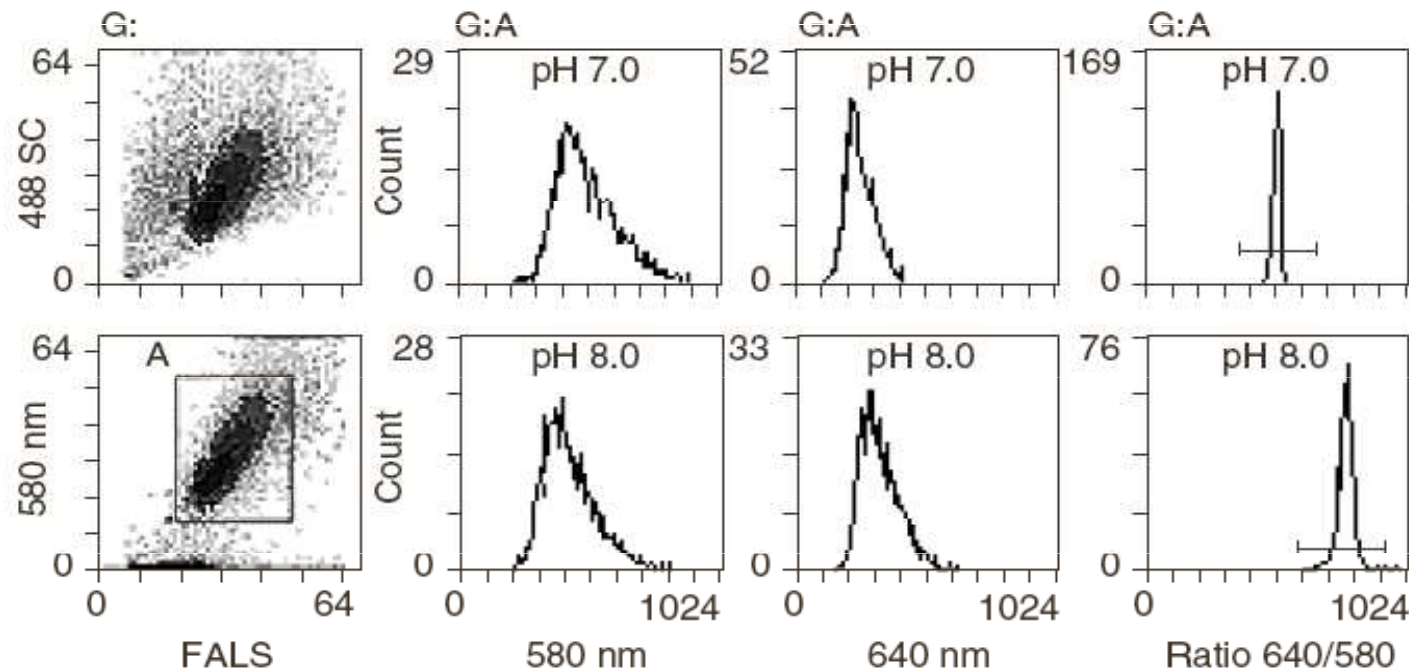


Fluo-3 ($K_d \sim 400\text{nM}$, 22°C ; 864 nM , 37°C)

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

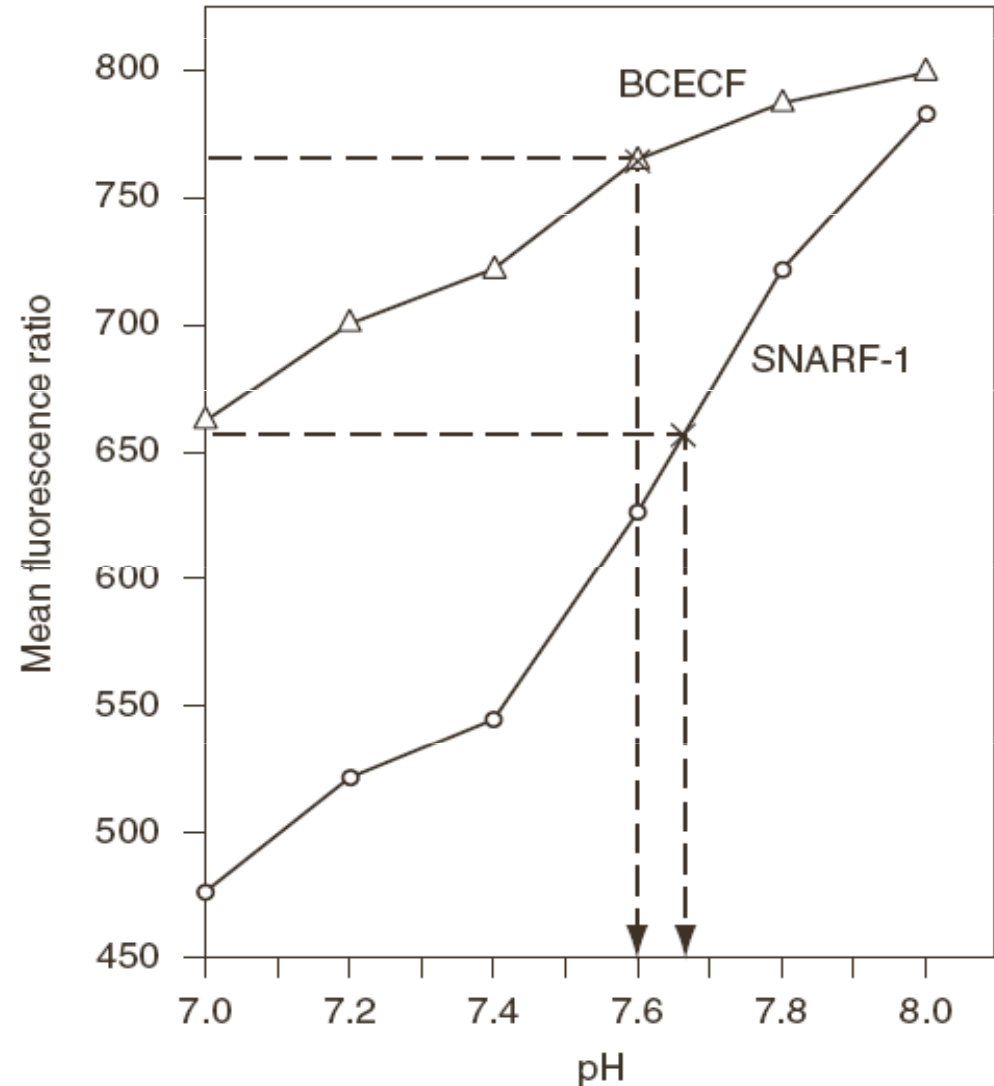
Detekce intracelulárního pH

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



Detekce intracelulárního pH

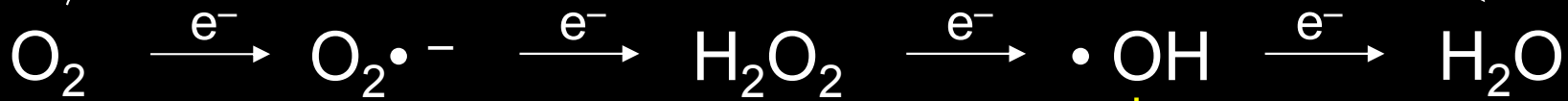
- Nutná kalibrace pomocí draslíkových pufrů a ionoforu (nigericin)



Detekce reaktivních kyslíkových skupin

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

4 e⁻ reduction to water



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

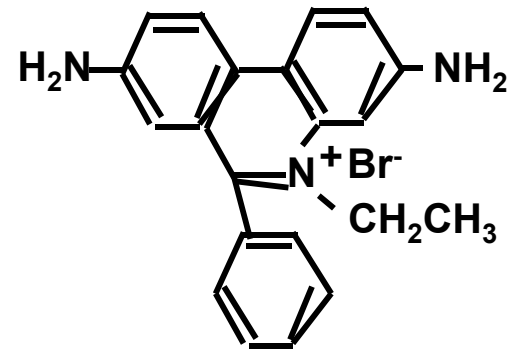
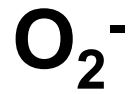
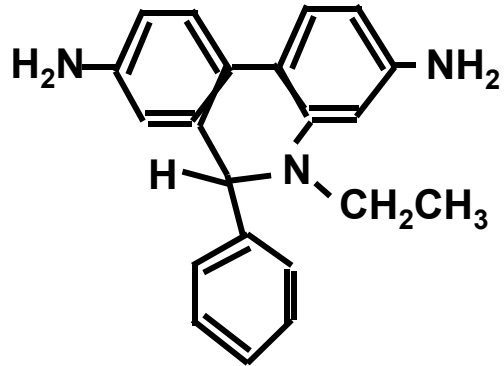
Reacts with virtually any molecule at diffusion-limited rates
The molecule that makes ionizing radiation toxic

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

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

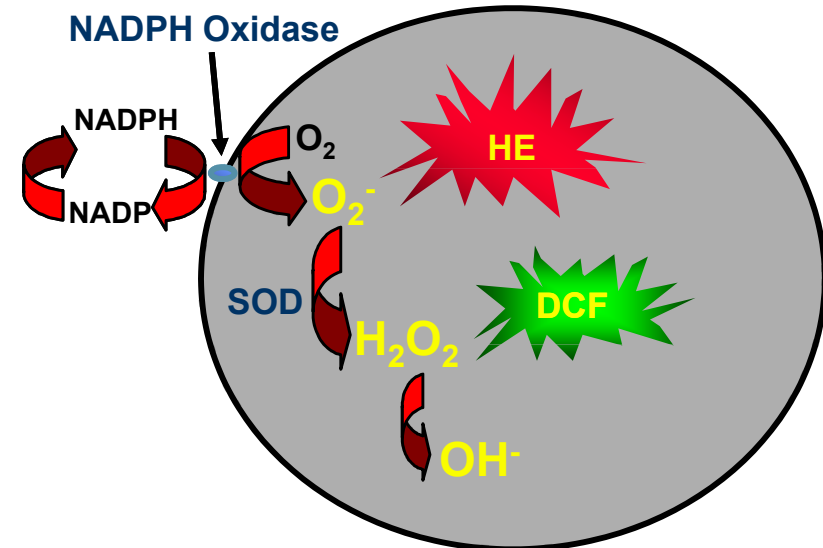
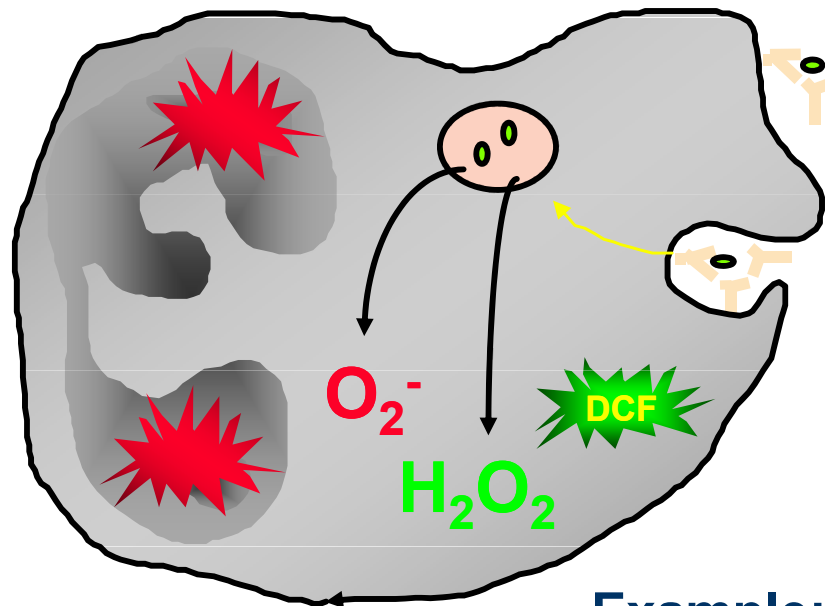
Hydroethidine

HE

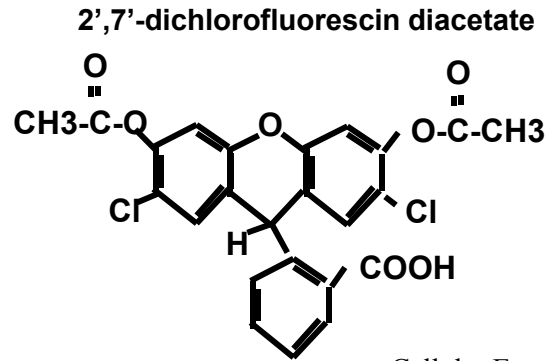


EB

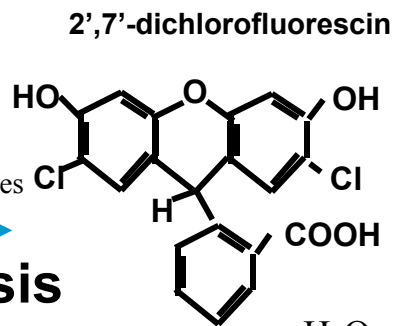
Phagocytic Vacuole



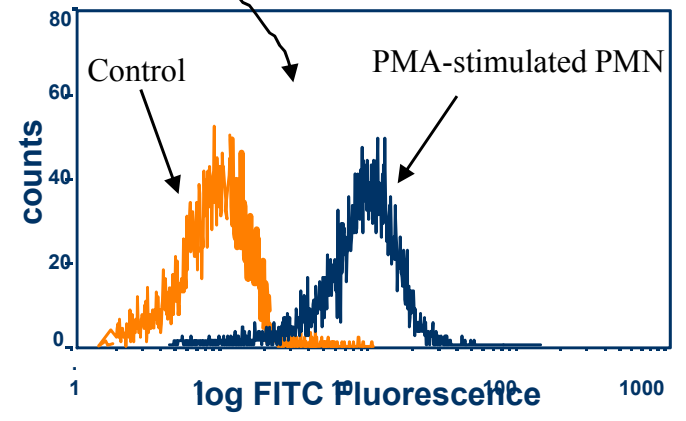
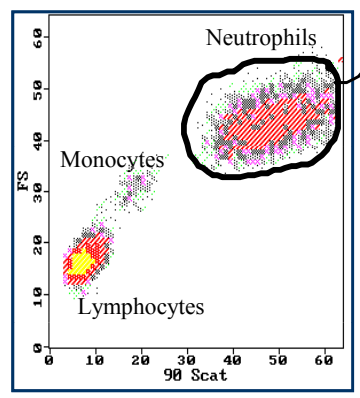
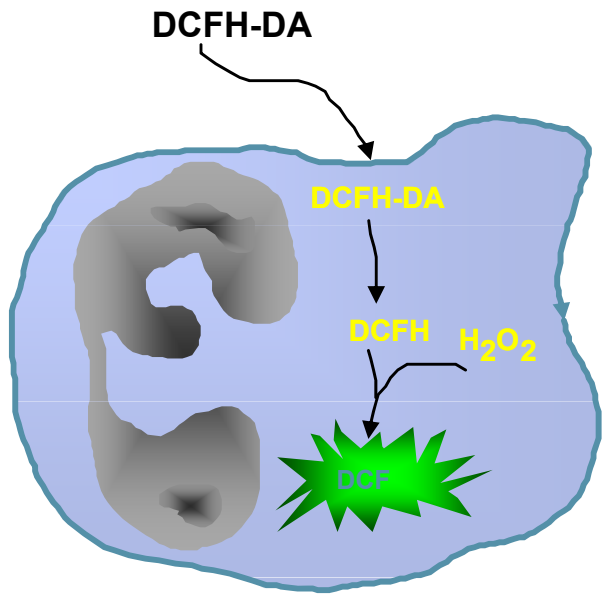
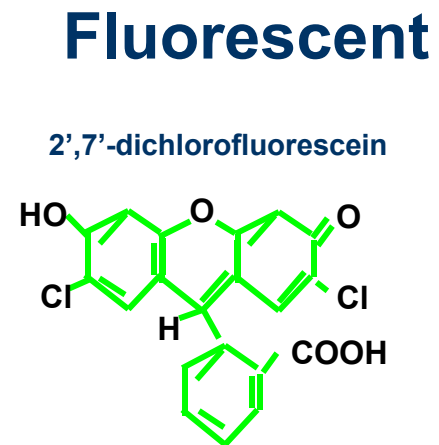
Example: Neutrophil Oxidative Burst



Cellular Esterases
 \longrightarrow
Hydrolysis

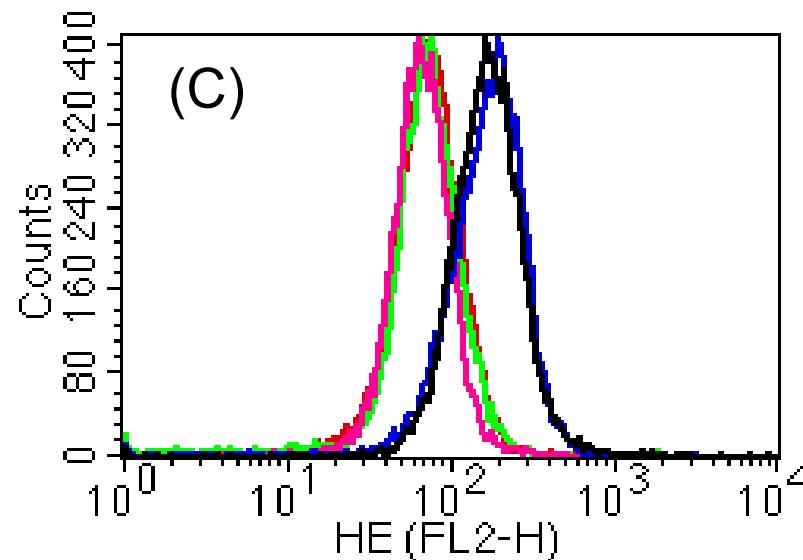
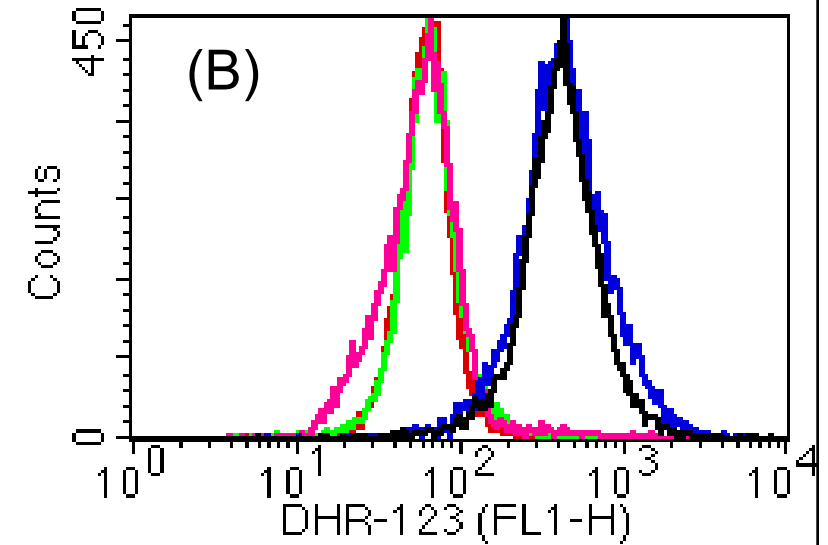
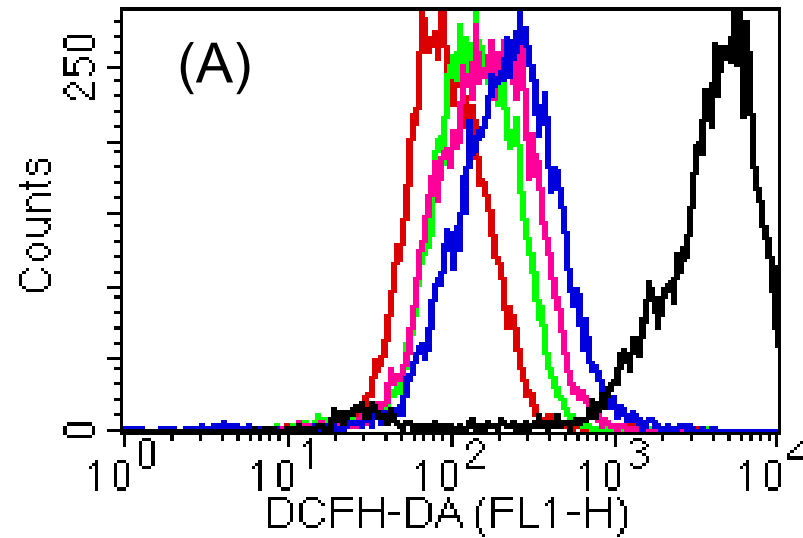


H_2O_2
 \longrightarrow
Oxidation



Oxidative Burst

- DCFH-DA
- DHR-123
- HE



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

Fluorescenční proteiny

■ bioluminescence resonance energy transfer (BRET)

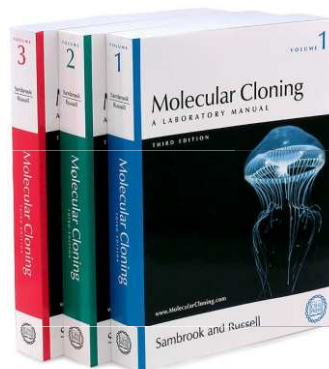
Aequorea victoria - medúza žijící ve vodách na pobřeží Severní Ameriky.

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

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

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

Aequorea victoria “Crystal jelly “



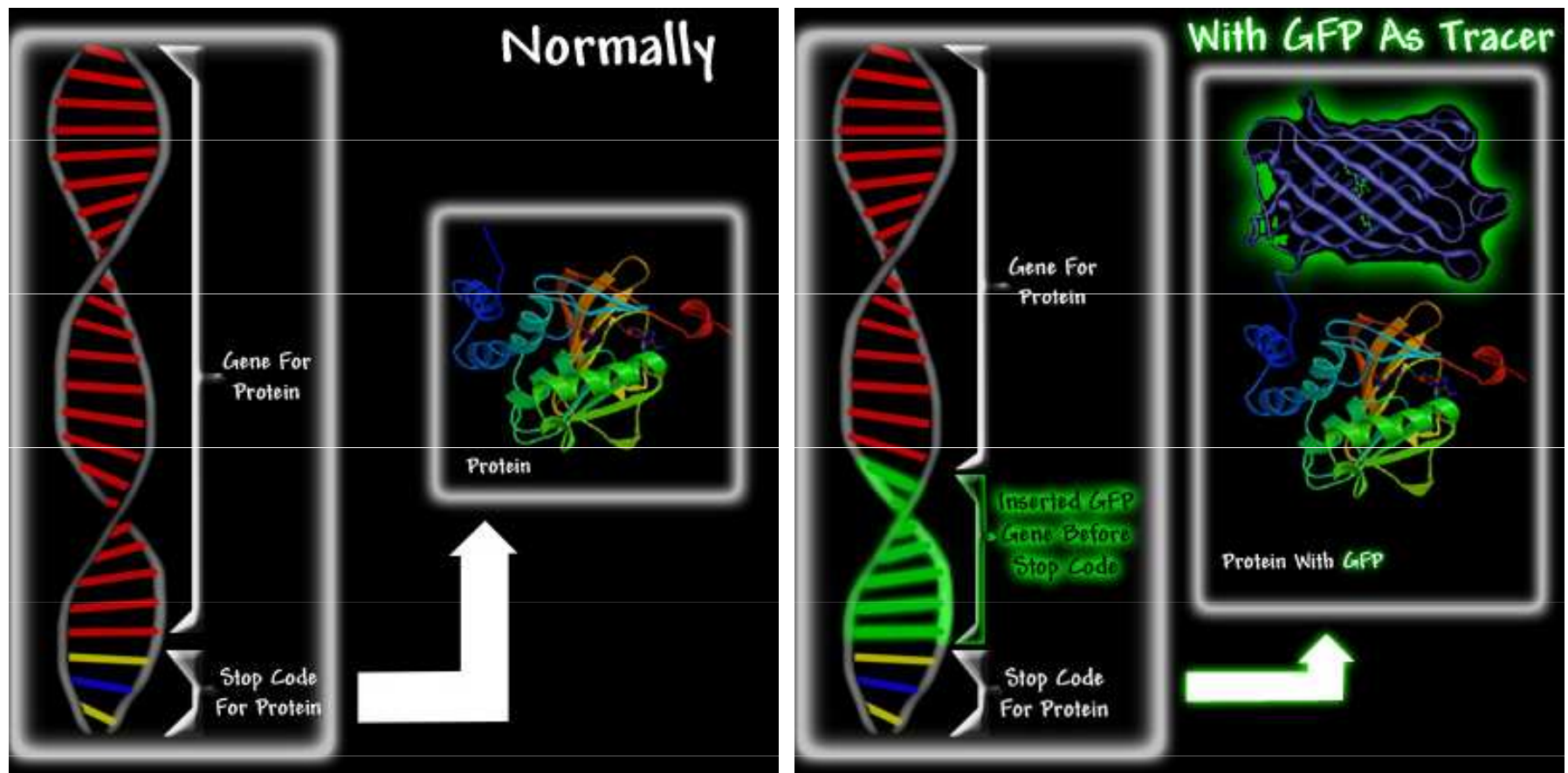
http://www.mbayaq.org/efc/living_species/default.asp?hOri=1&inhab=440

Renilla reniformis "Sea Pansy"



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

Fluorescenční proteiny



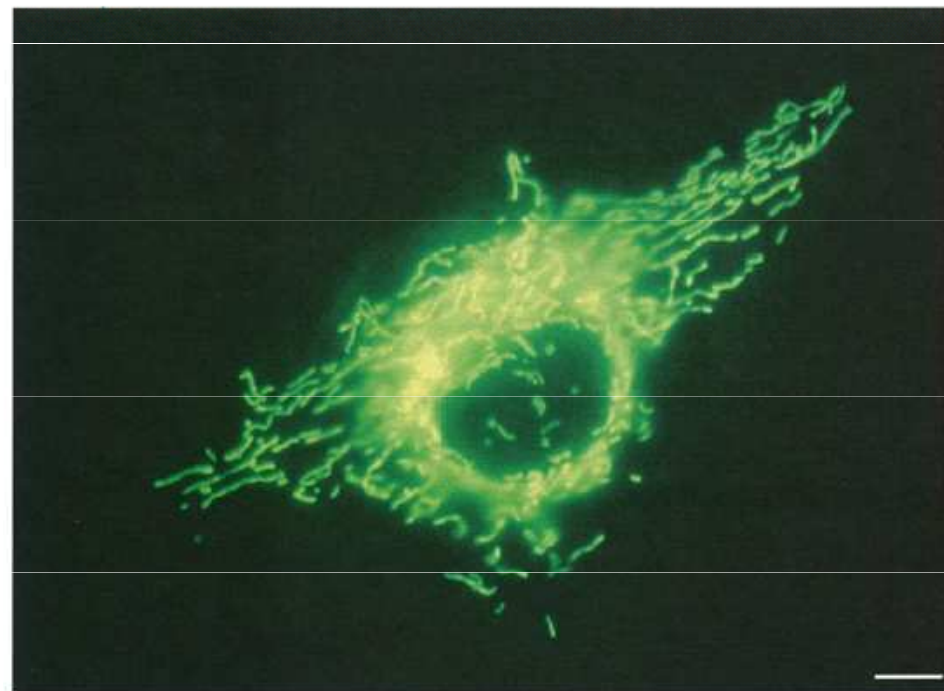
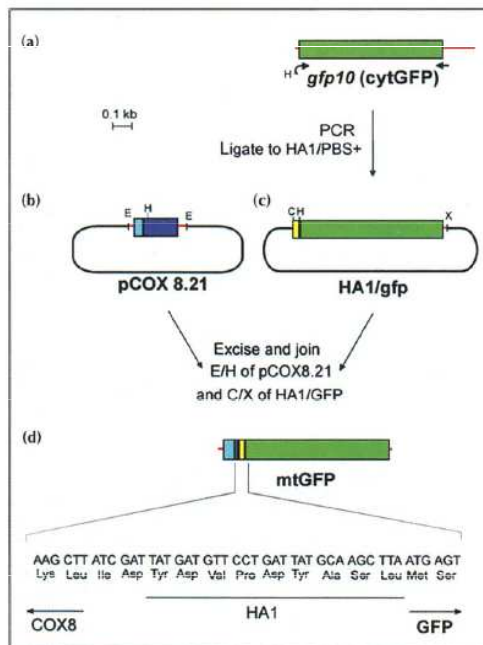
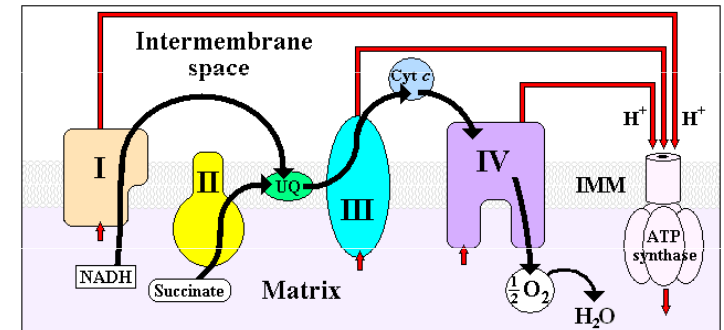
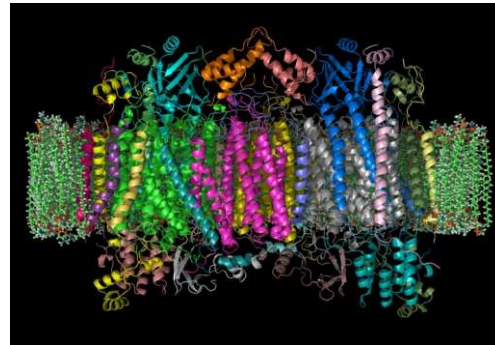
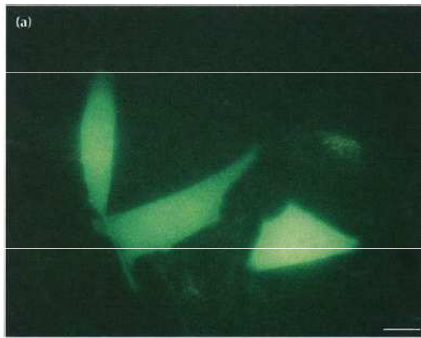
<http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm>

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

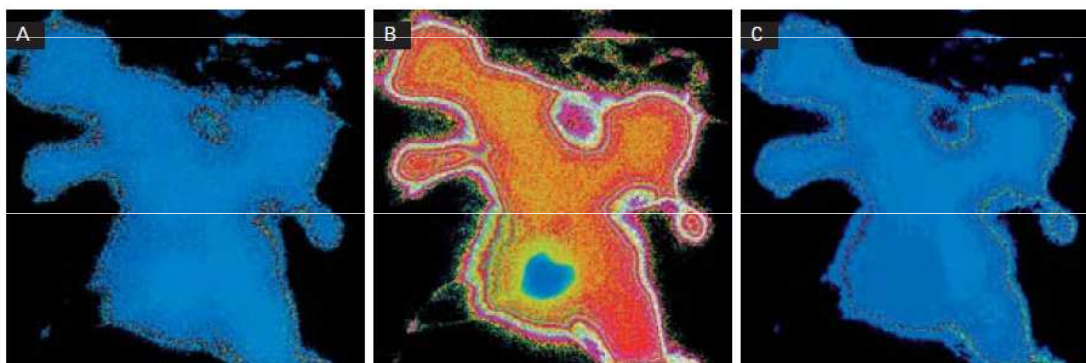
Rosario Rizzuto, Marisa Brini, Paola Pizzo,
Marta Murgia and Tullio Pozzan

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

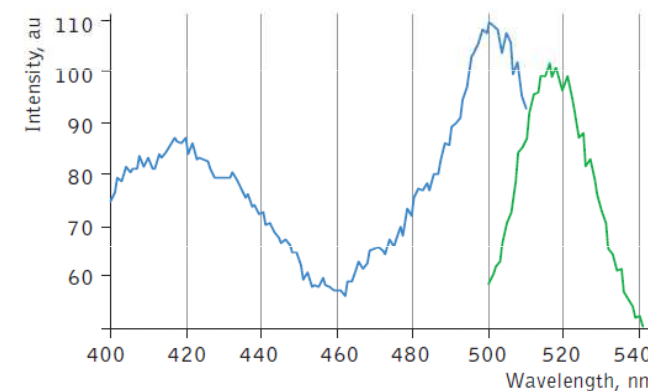
Current Biology 1995, 5:635–642



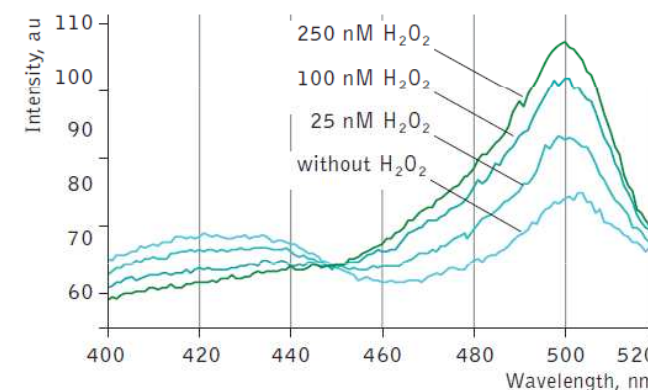
Fluorescent sensors for detection of H_2O_2



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



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



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

Variants & fusions

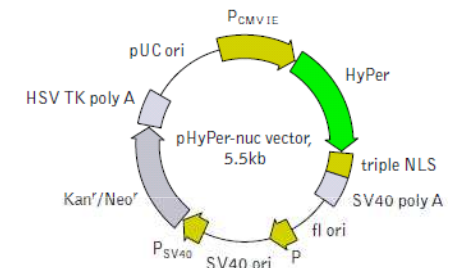
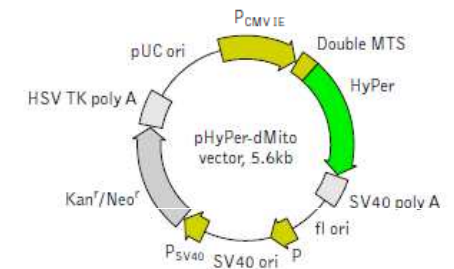
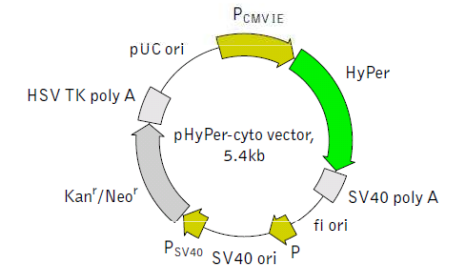
- **pHyPer-cyto vector**

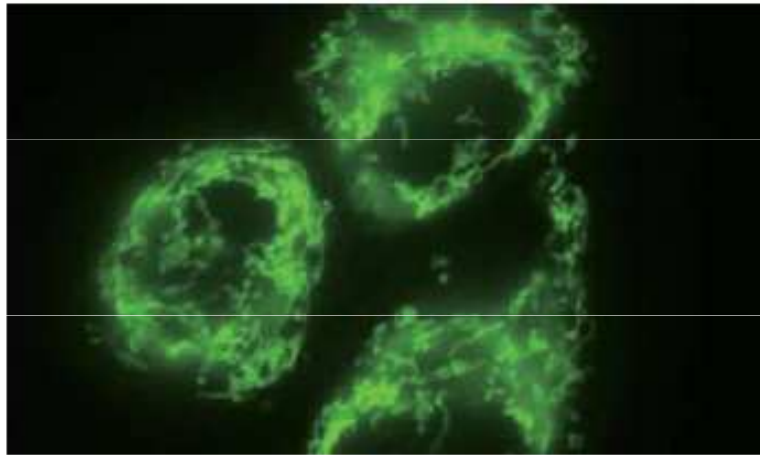
- **pHyPer-dMito vector**

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

- **pHyPer-nuc vector**

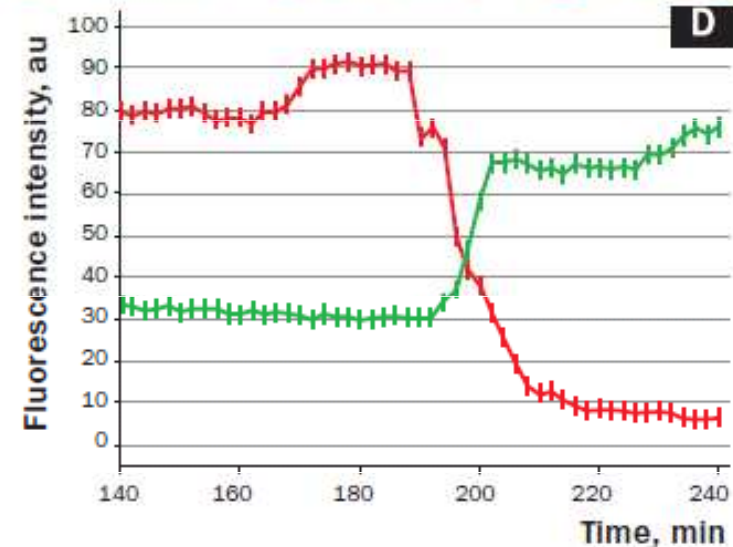
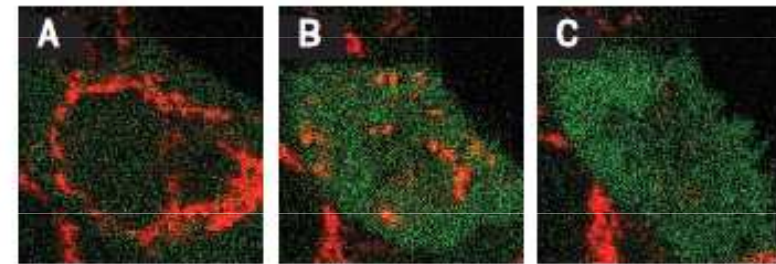
- Three copies of the nuclear localization signal (NLS) fused to the HyPer C-terminus provide for efficient translocation of HyPer to the nuclei of mammalian cells [Fischer-Fantuzzi and Vesco, 1988]





Stably transfected HeLa cells expressing mitochondria-targeted HyPer.

Image from Dr. Christian Petzelt (Marinpharm).



Dynamics of intracellular H_2O_2 production in a HeLa cell undergoing Apo2L/TRAIL-induced apoptosis.

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

Analýza a sortování chromozómů

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

Measurement and purification of human chromosomes by flow cytometry and sorting

(isolated chromosomes/DNA cytophotometry/flow microfluorometer)

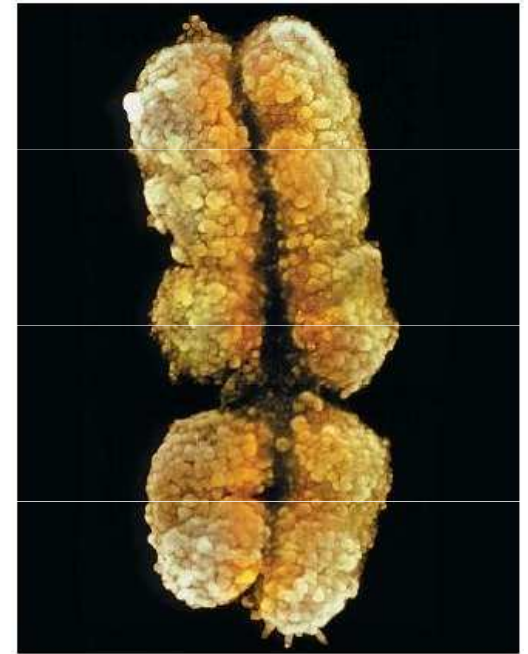
A. V. CARRANO, J. W. GRAY, R. G. LANGLOIS, K. J. BURKHART-SCHULTZ, AND M. A. VAN DILLA

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

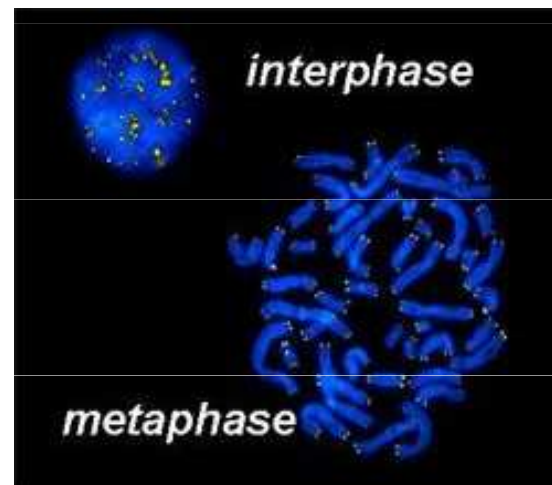
Communicated by Donald A. Glaser, December 18, 1978

Analýza a sortování chromozómů

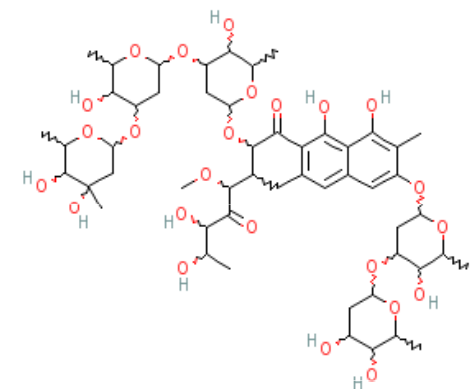
- synchronizace buněk – získání metafázních chromozómů (colcemid, hydroxyurea)
 - izolace chromozómů
 - značení DAPI nebo **Hoechst** vs. **chromomycin A3** (CA3) nebo mithramycin
- = celková DNA vs. G/C-bohaté oblasti



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



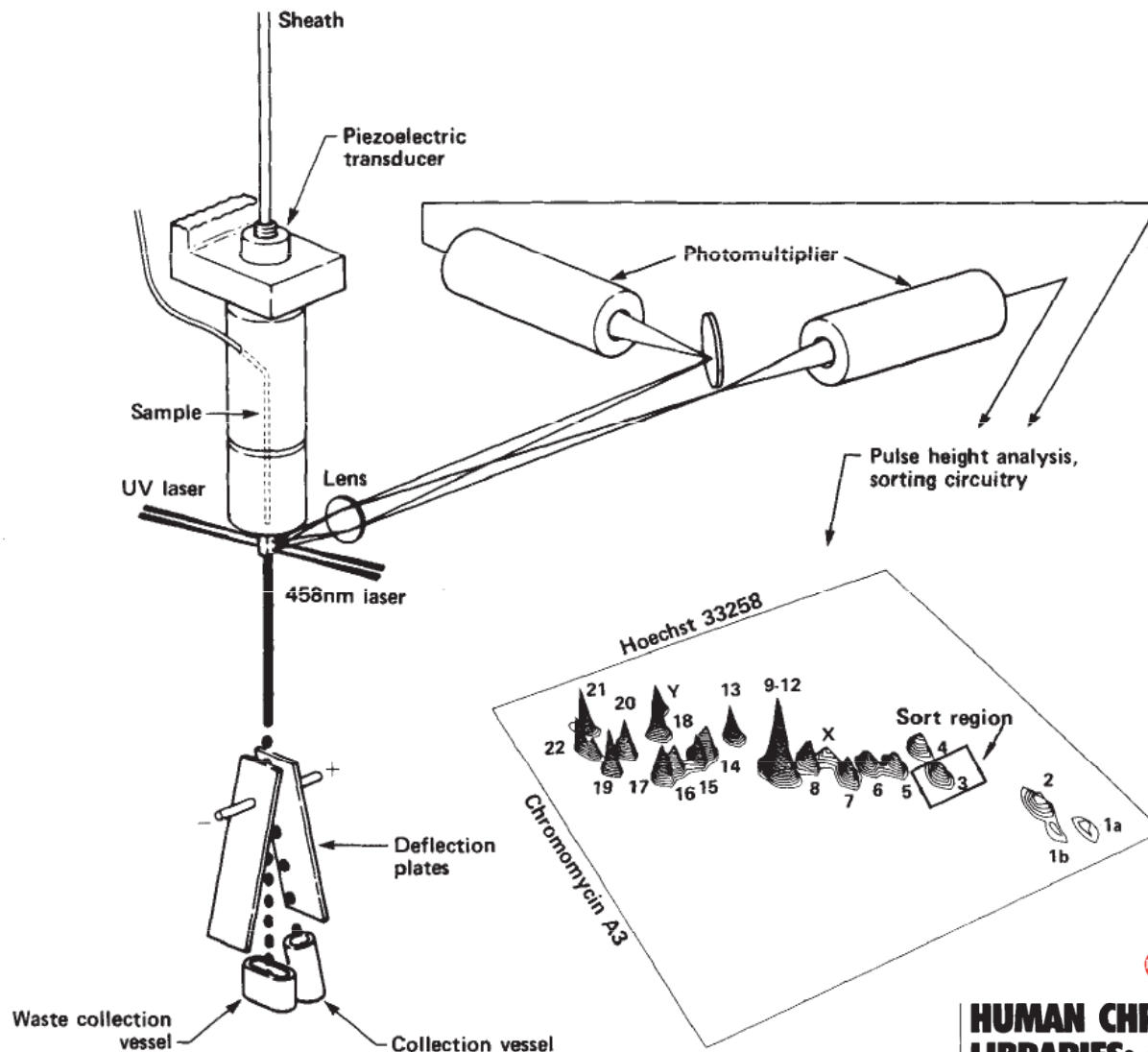
<http://www.nccr-oncology.ch/scripts/page9243.html>



PubChem

National
Library
of Medicine
NLM

Analýza a sortování chromozómů



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HUMAN CHROMOSOME-SPECIFIC DNA LIBRARIES: CONSTRUCTION AND AVAILABILITY

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

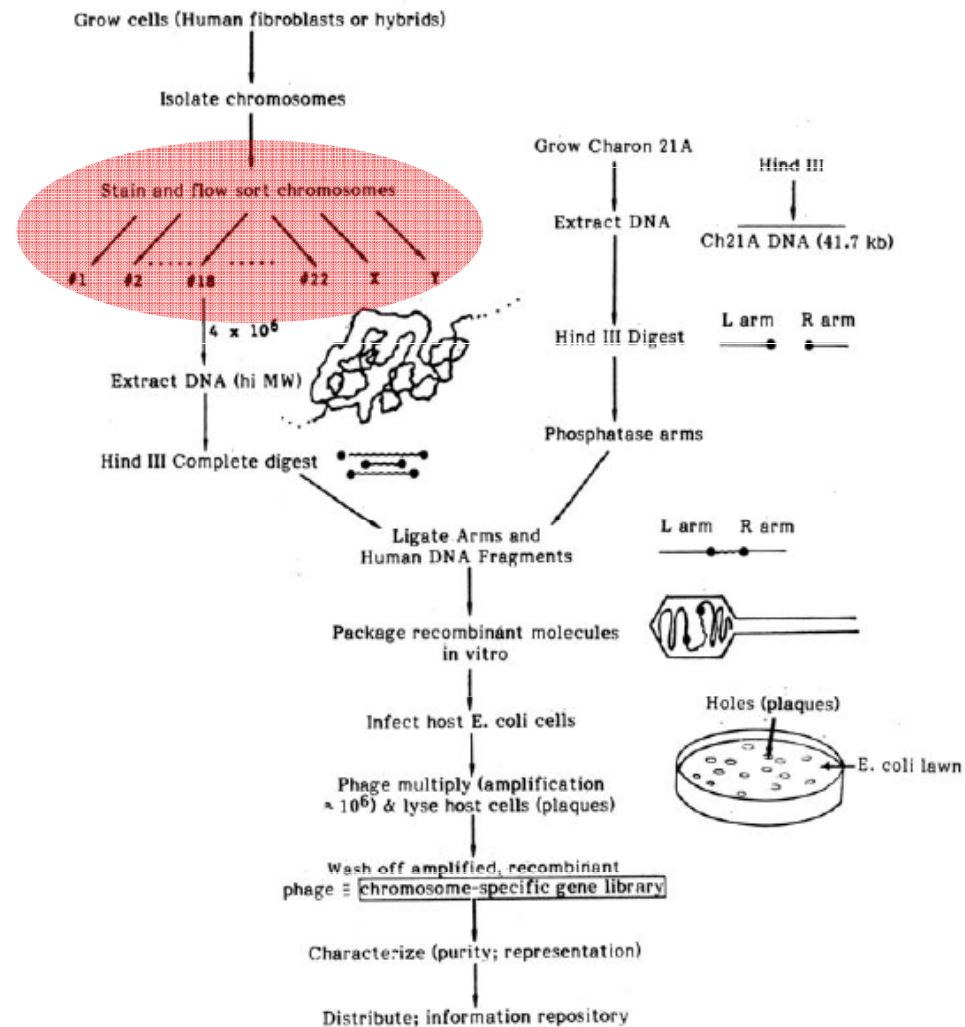
National Laboratory Gene Library Project. ^{*} Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, California 94550. [†] Los Alamos National Laboratory, Life Sciences Division, University of California, Los Alamos, New Mexico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

HUMAN CHROMOSOME-SPECIFIC DNA LIBRARIES: CONSTRUCTION AND AVAILABILITY

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

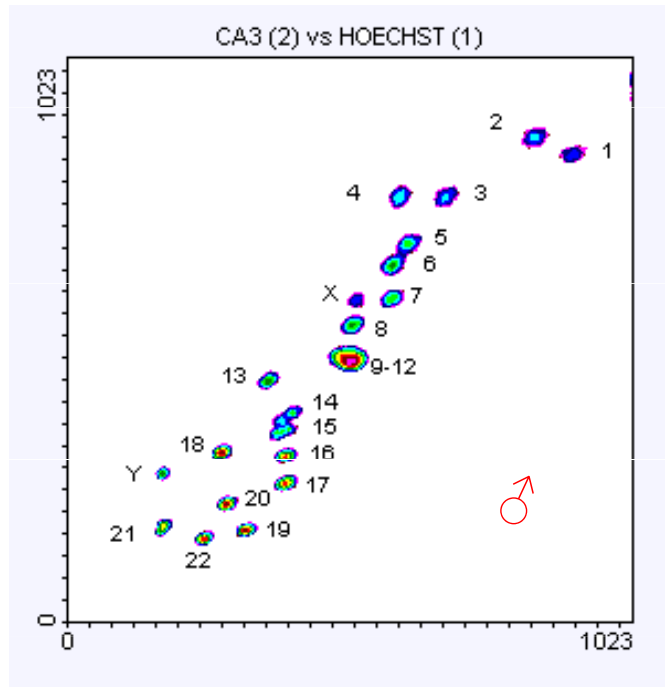
National Laboratory Gene Library Project. ^{*} Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, California 94550. [†] Los Alamos National Laboratory, Life Sciences Division, University of California, Los Alamos, New Mexico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

CONSTRUCTION OF A PHASE I CHROMOSOME-SPECIFIC (#18) HUMAN GENE LIBRARY IN CHARON 21A USING HIND III (LLNL)



e!

„Flow karyotype“



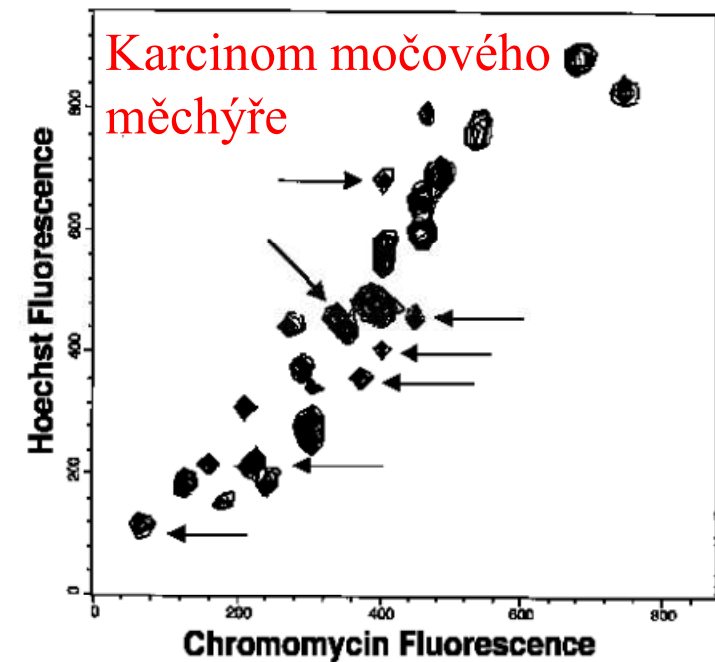
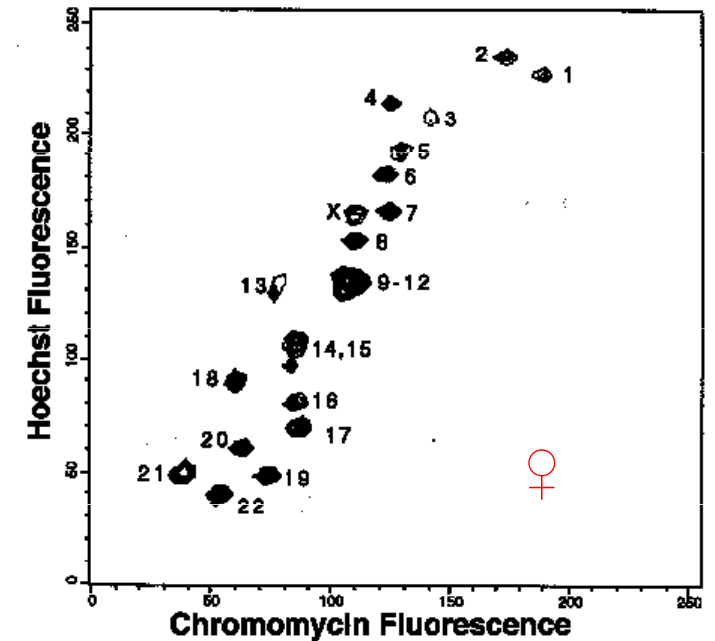
<http://www.sanger.ac.uk/HGP/Cytogenetics/>

The Preparation of Human Chromosomes for Flow Cytometry

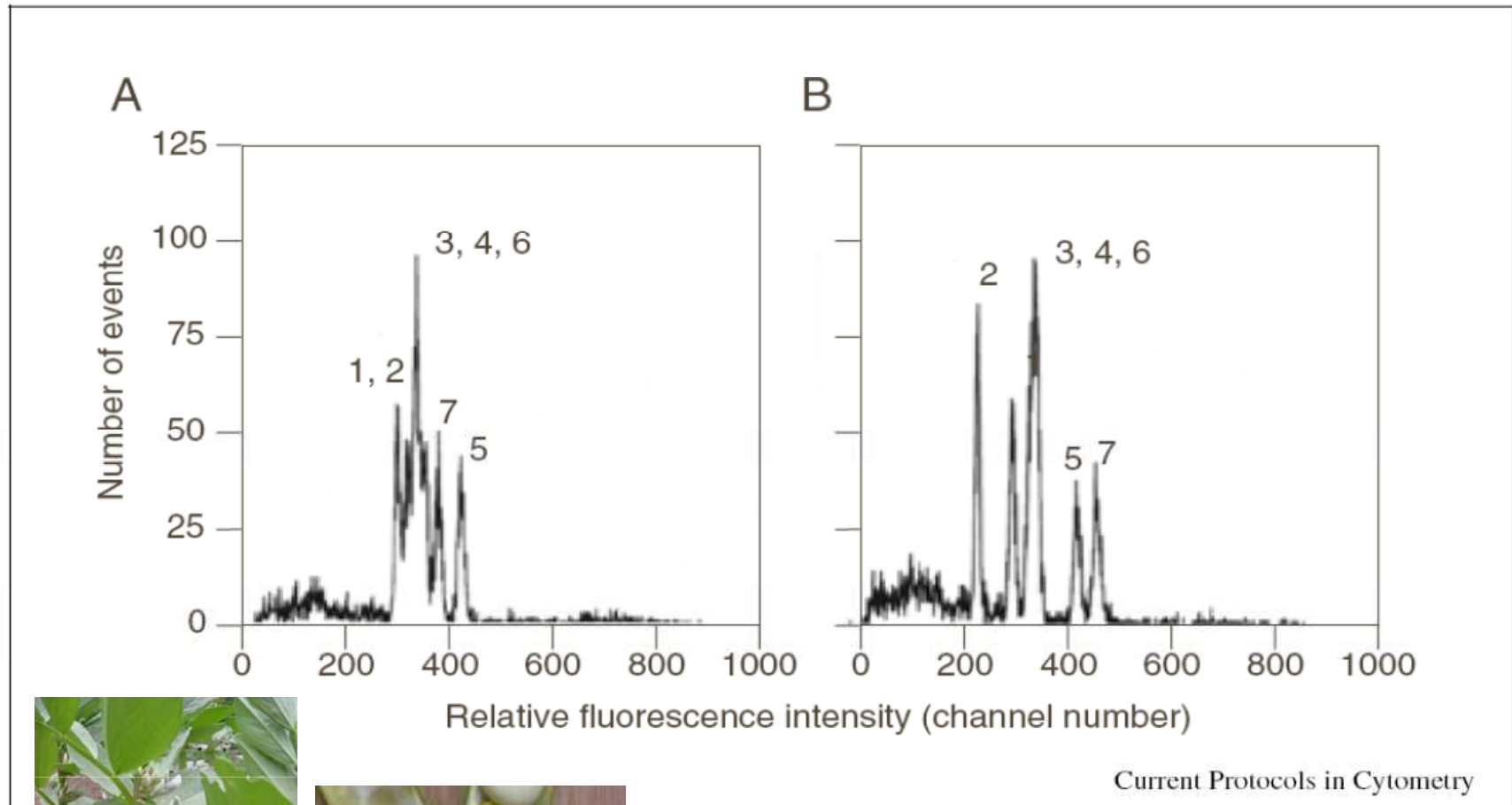
DEREK DAVIES

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

Vol. 33/2 Proceedings RMS June 1998



Sortování chromozómů



Pisum sativum

Current Protocols in Cytometry

PROTOCOL

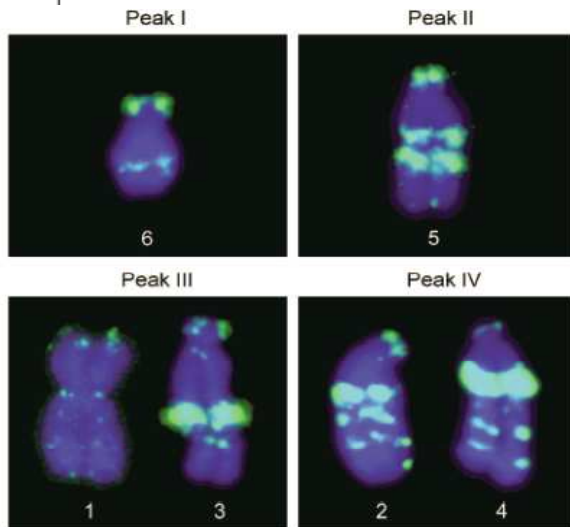
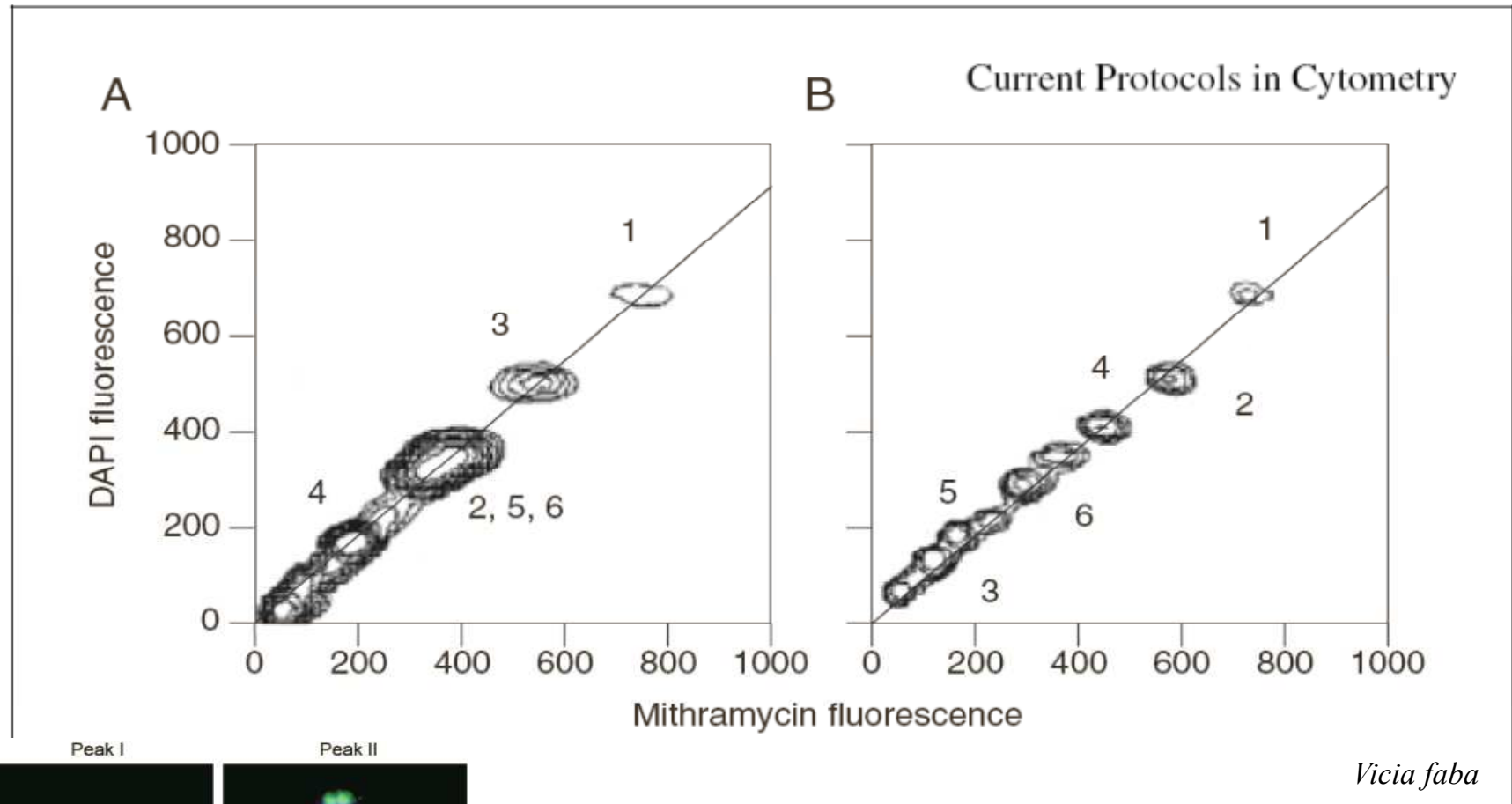
Estimation of nuclear DNA content in plants using flow cytometry

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

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

Published online 6 September 2007; doi:10.1038/nprot.2007.310

Sortování chromozómů



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

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

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



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

J. Šafář H. Šimková M. Kubaláková J. Číhalíková P. Suchánková J. Bartoš
J. Doležel

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

The Plant Journal (2004) 39, 960–968

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

TECHNICAL ADVANCE

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

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

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

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

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

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

Science

July 17, AUGUST 3, 2018
sciencemag.org



ROAD MAP FOR WHEAT

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

RESEARCH

RESEARCH ARTICLE

WHEAT GENOME

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

International Wheat Genome Sequencing Consortium (IWGSC)*

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

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

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

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

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

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

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

Aplikace průtokové cytometrie v mikrobiologii

- ekologie
- potravinářství

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

Aplikace průtokové cytometrie v mikrobiologii

Relative Size Ratios for Bacteria, Yeast, and Eukaryotes

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

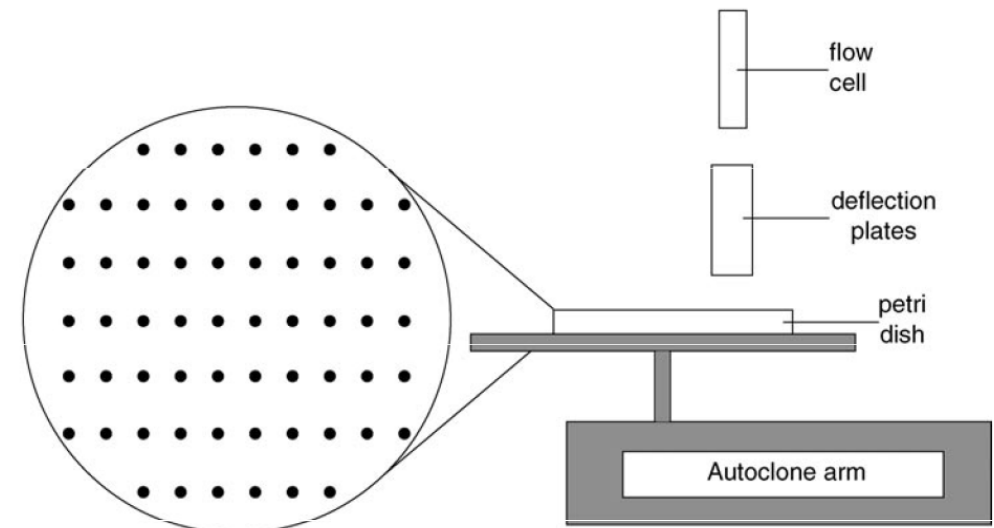
Current Protocols in Cytometry

Aplikace průtokové cytometrie v mikrobiologii

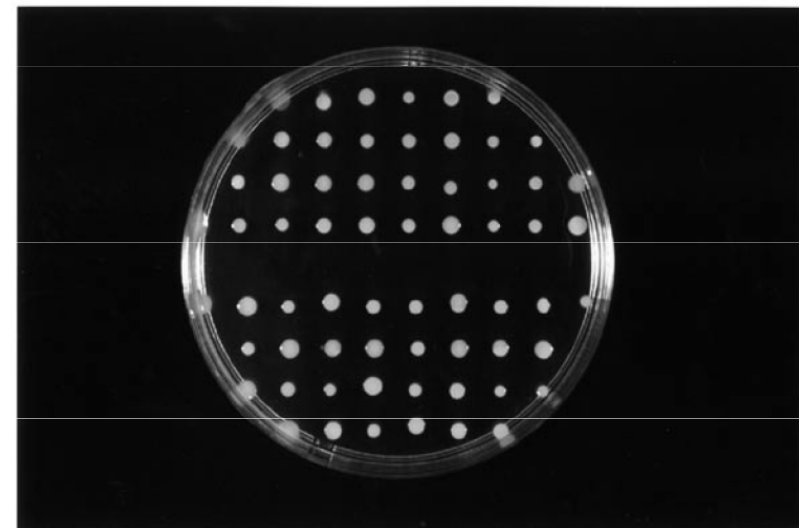
- viabilita
- metabolické funkce
- sortování
- analýza aerosolů (Fluorescence Aerodynamic Particle Sizer (Flaps))

Aplikace průtokové cytometrie v mikrobiologii

- Sortování
 - EPICS + Autoclone® modul



top view of petri dish showing sort grid



BactoSense™

Automated flow cytometer for online monitoring of bacteria in water



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



For your PROCESS



In the FIELD



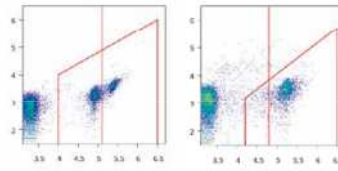
In the LAB

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

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

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

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



Dotplots showing TCC and ICC



Online sampler / manual sampler

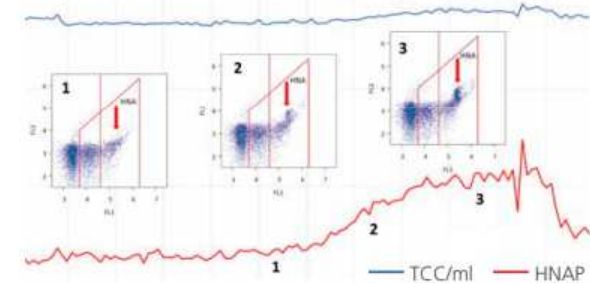


Cartridge

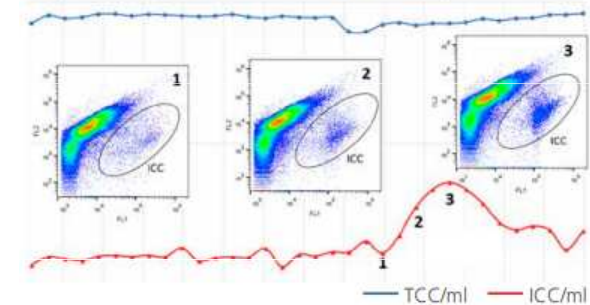


IO box

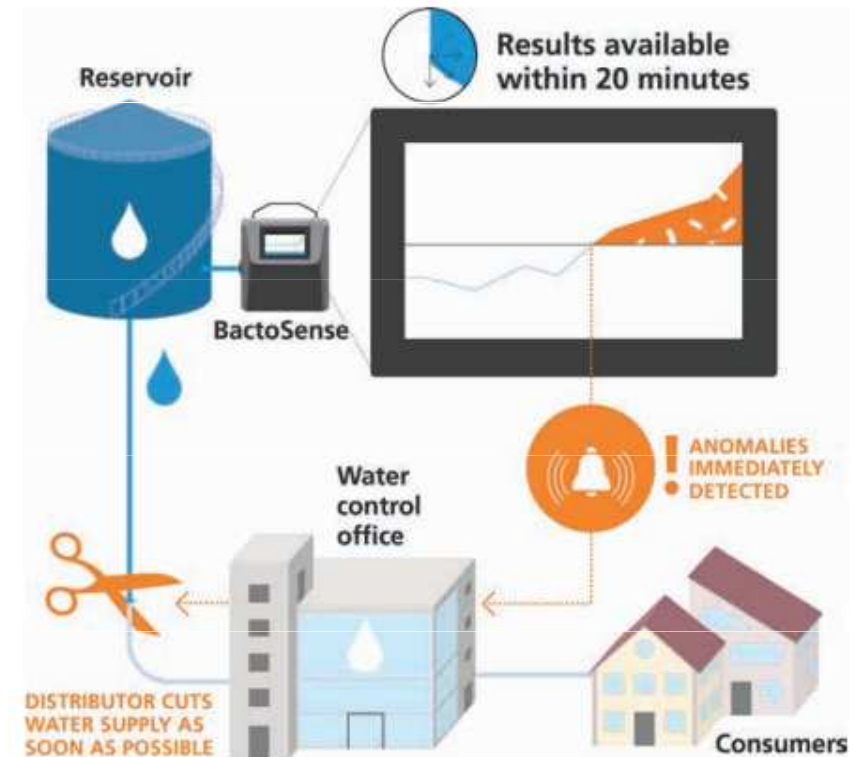
Graph showing TCC and HNAP measurements over one week



Graph showing TCC and ICC measurements every 3 hours

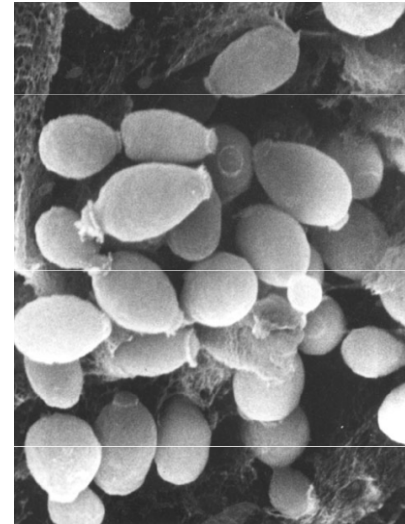


3120200ZEN

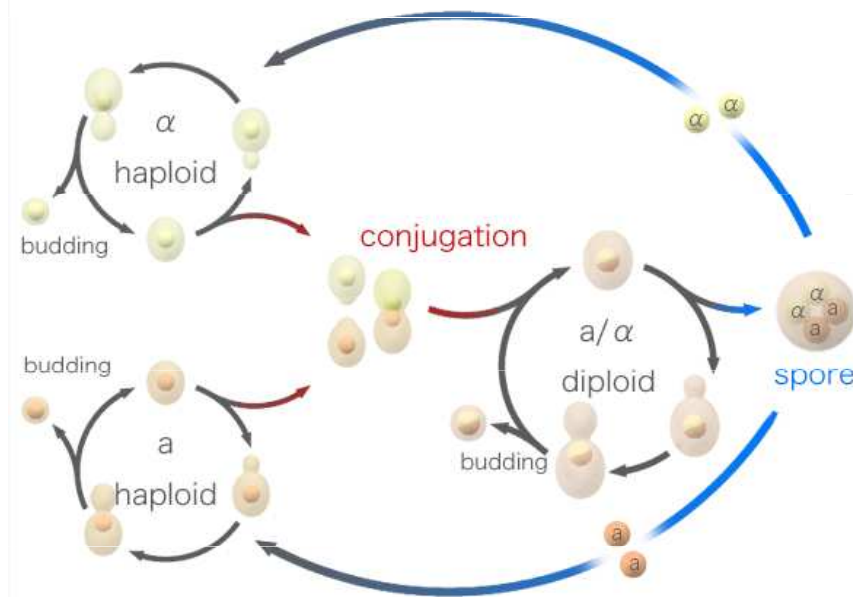


Průtoková cytometrie kvasinek

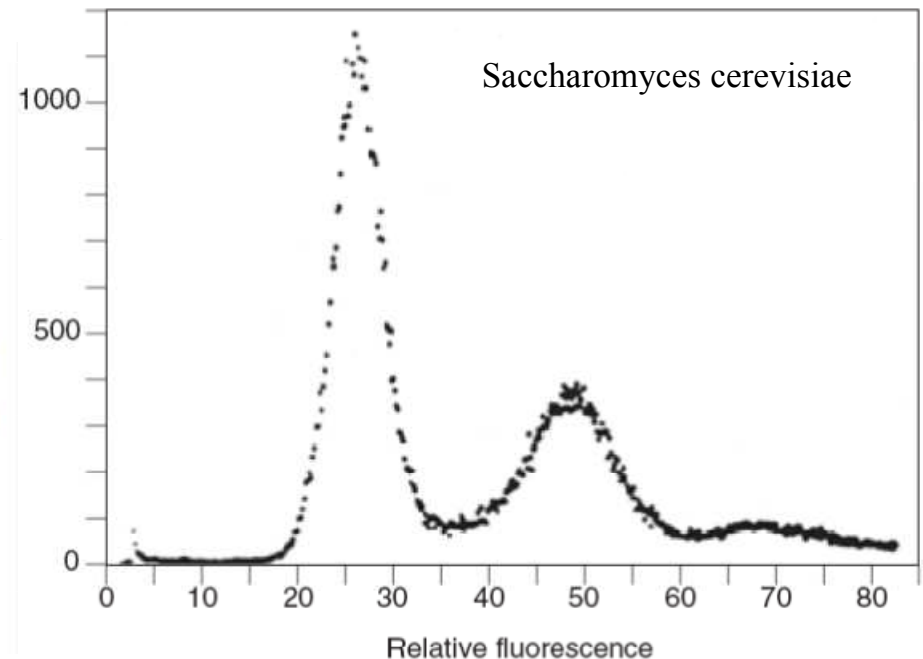
- buněčné dělení
- viabilita
- membránový potenciál
- respirace
- produkce H_2O_2
- citlivost k antibiotikům
- separace



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



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



Průtoková cytometrie kvasinek

Yeast Cell Cycle During Fermentation and Beer Quality

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

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



Průtoková cytometrie v hydrobiologii

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



Průtoková cytometrie v hydrobiologii

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Cytometry 44:236–246 (2001)

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

W.K.W. Li^{*} and P.M. Dickie

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

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

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Cytometry 10:659–669 (1989)

Using Phytoplankton and Flow Cytometry to Analyze Grazing by Marine Organisms

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

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

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

Průtoková cytometrie v hydrobiologii

■ analýza DNA

Vol. 185: 301–307, 1999

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

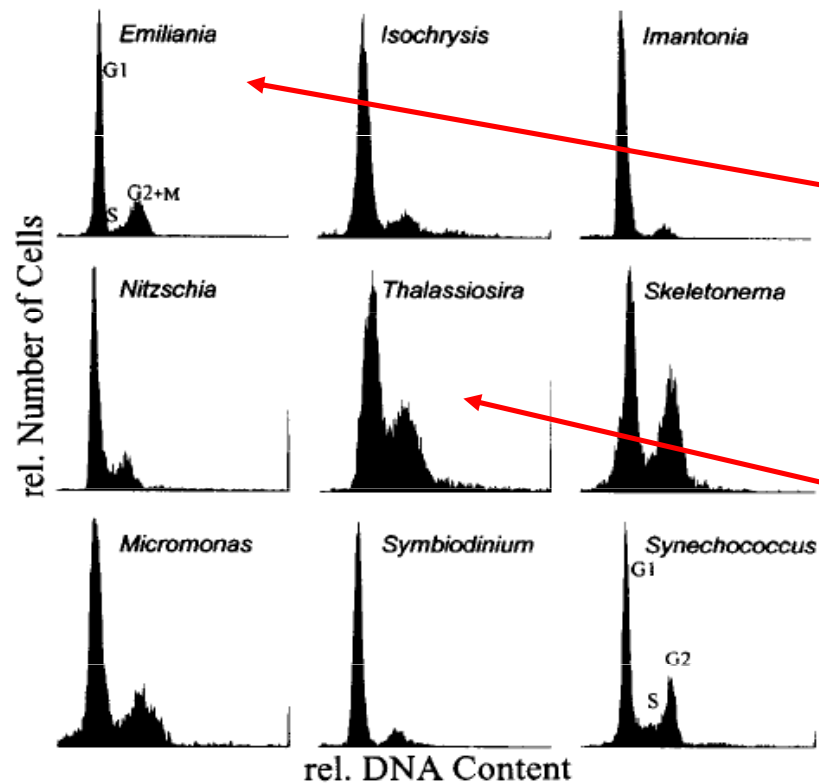
Published August 20

NOTE

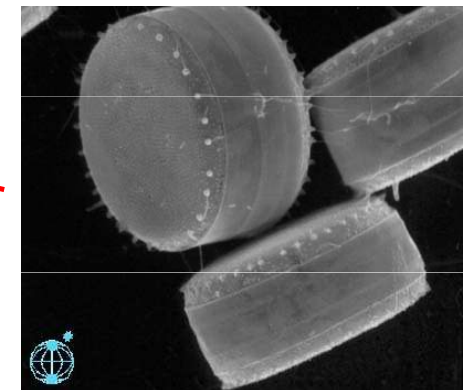
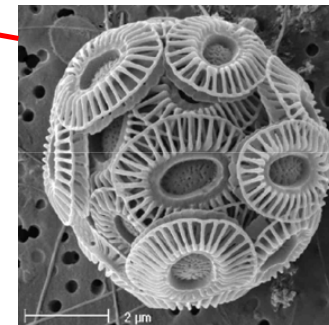
Cytometric measurement of the DNA cell cycle in the presence of chlorophyll autofluorescence in marine eukaryotic phytoplankton by the blue-light excited dye YOYO-1

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

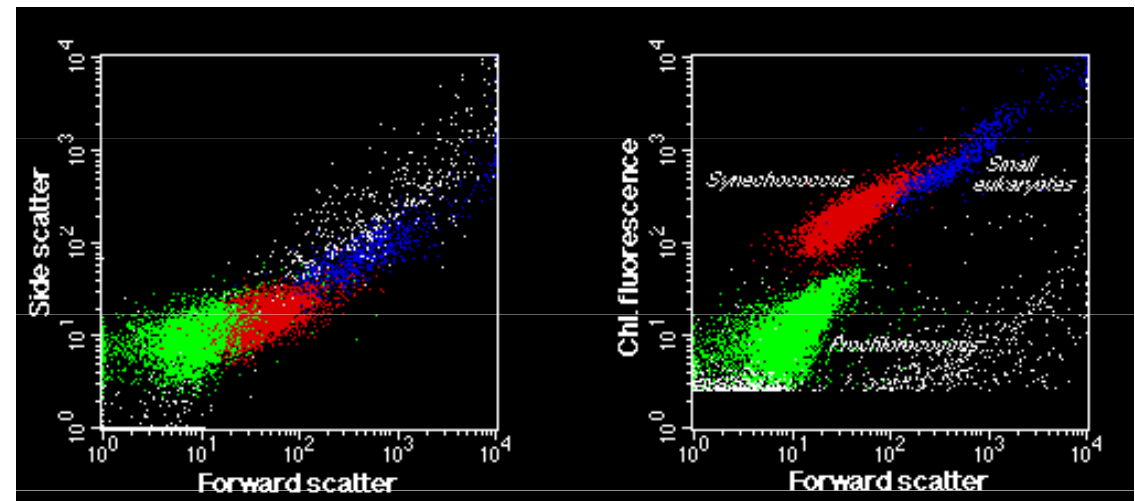
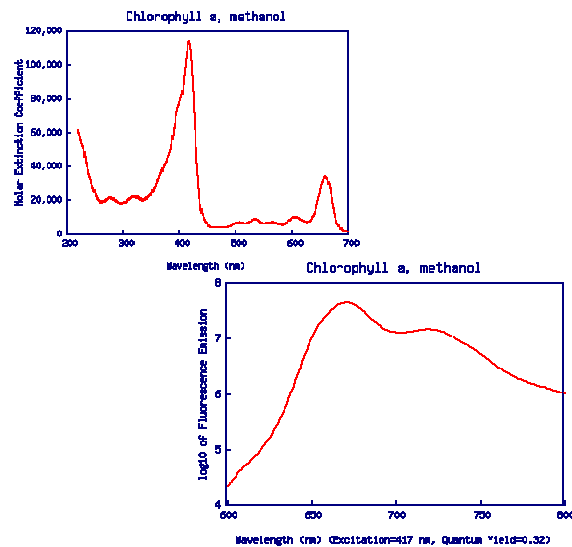
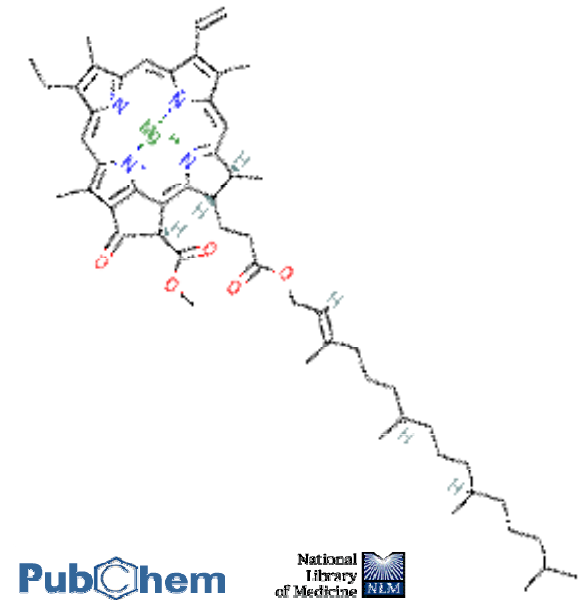
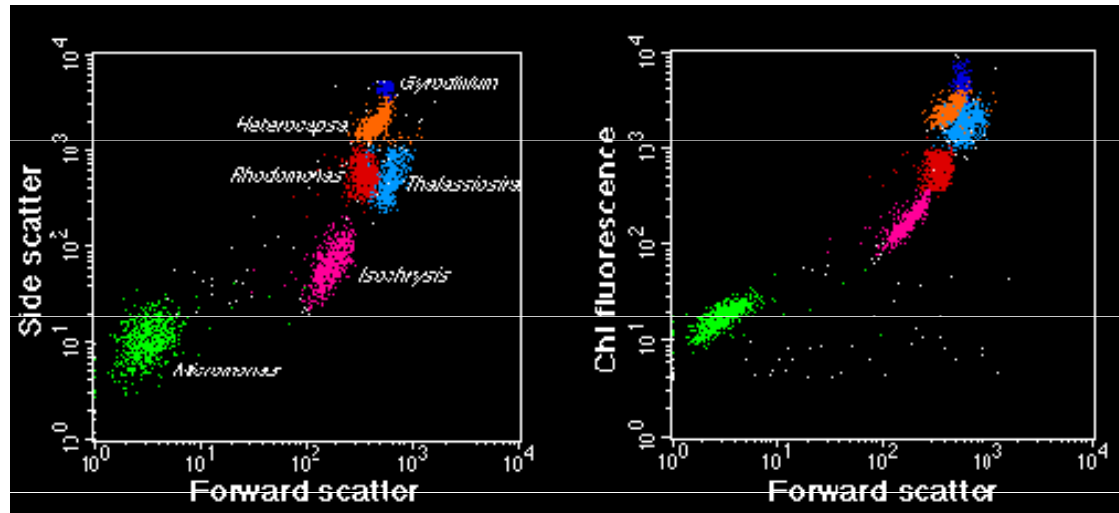
¹Institut für Meereskunde, Düsternbrooker Weg 20, D-24105 Kiel, Germany
²Universität Bremen, FB II Meeresbotanik, Postfach 330440, D-28334 Bremen, Germany



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



Průtoková cytometrie v hydrobiologii





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

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

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

E-mail: quanzhou1985@yahoo.com.cn

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

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

4. Changshu Institute of Technology, Changshu 215500, China

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

Flow cytometry assessment of bacterioplankton in tropical marine environments

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

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

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

Průtoková cytometrie bezobratlých

- lze aplikovat běžné metodické přístupy a fluorescenční značky

- Příklady aplikací:

- buněčný cyklus
- cytotoxicita
- apoptóza



Invertebrate Survival Journal

ISJ 2: 32-40, 2005

ISSN 1824-307X

Review

Flow cytometry as a tool for analysing invertebrate cells

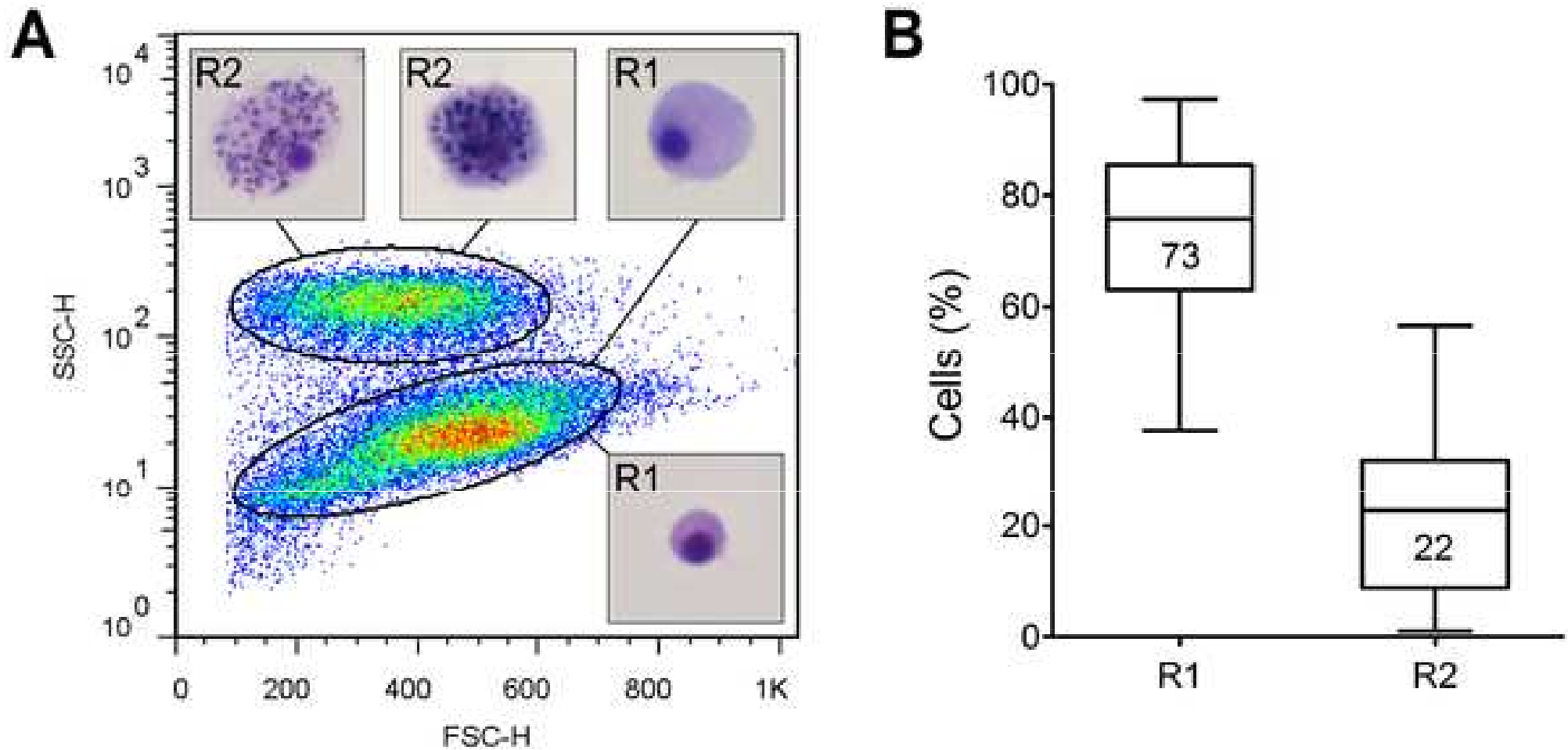
A Cossarizza¹, M Pinti¹, L Troiano¹, EL Cooper²

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

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

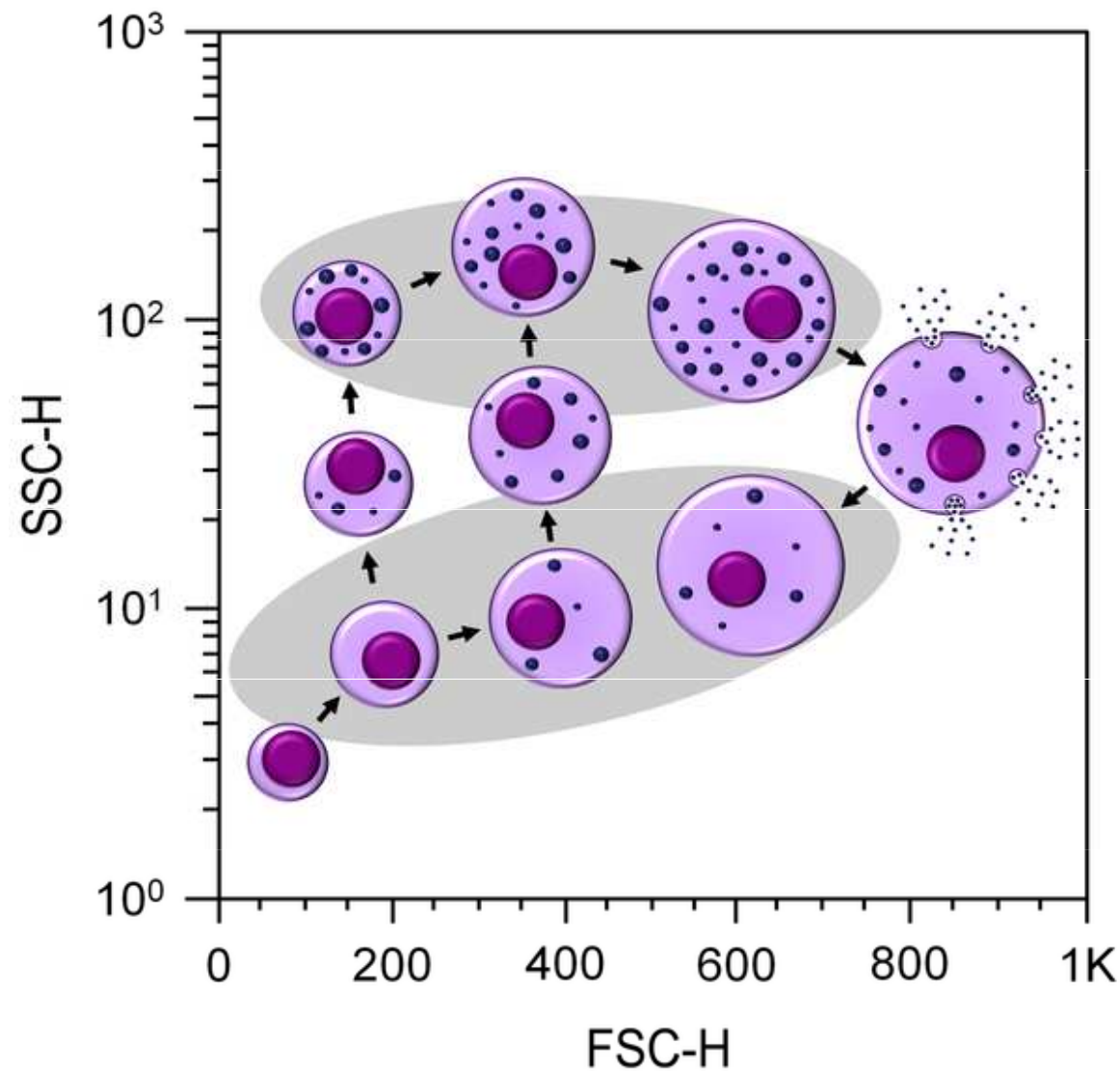
<http://www.icms.qmul.ac.uk/flowcytometry/uses/insects/index.html>

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



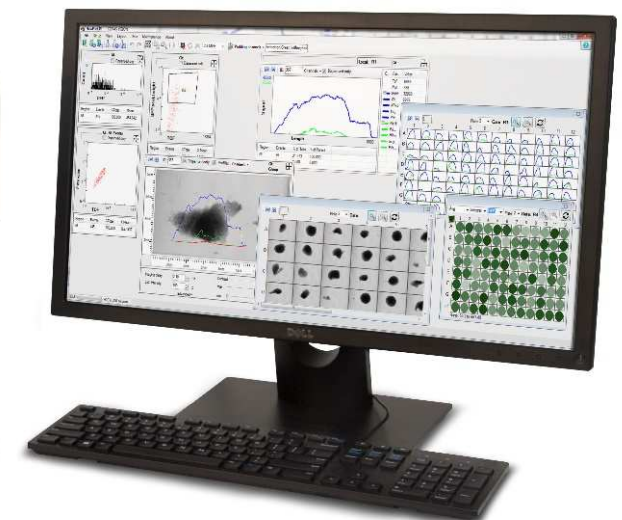
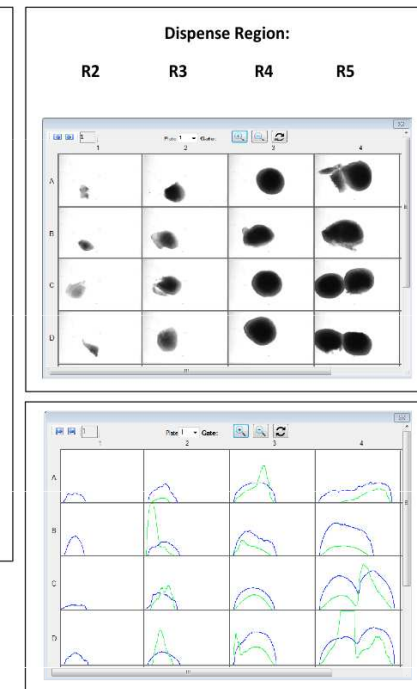
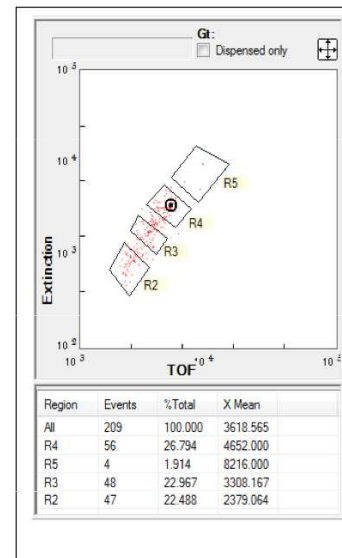
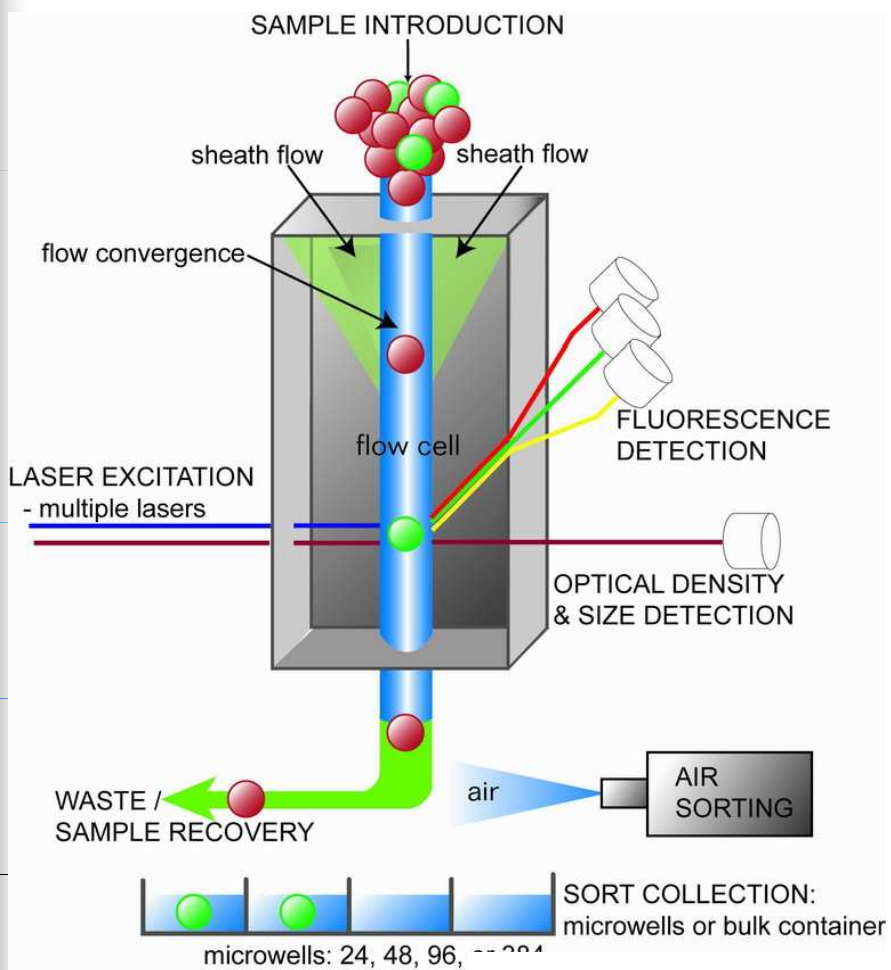
Rebello MdF, Figueiredo EdS, Mariante RM, Nóbrega A, et al. (2013) New Insights from the Oyster *Crassostrea rhizophorae* on Bivalve Circulating Hemocytes. PLoS ONE 8(2): e57384. doi:10.1371/journal.pone.0057384
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0057384>

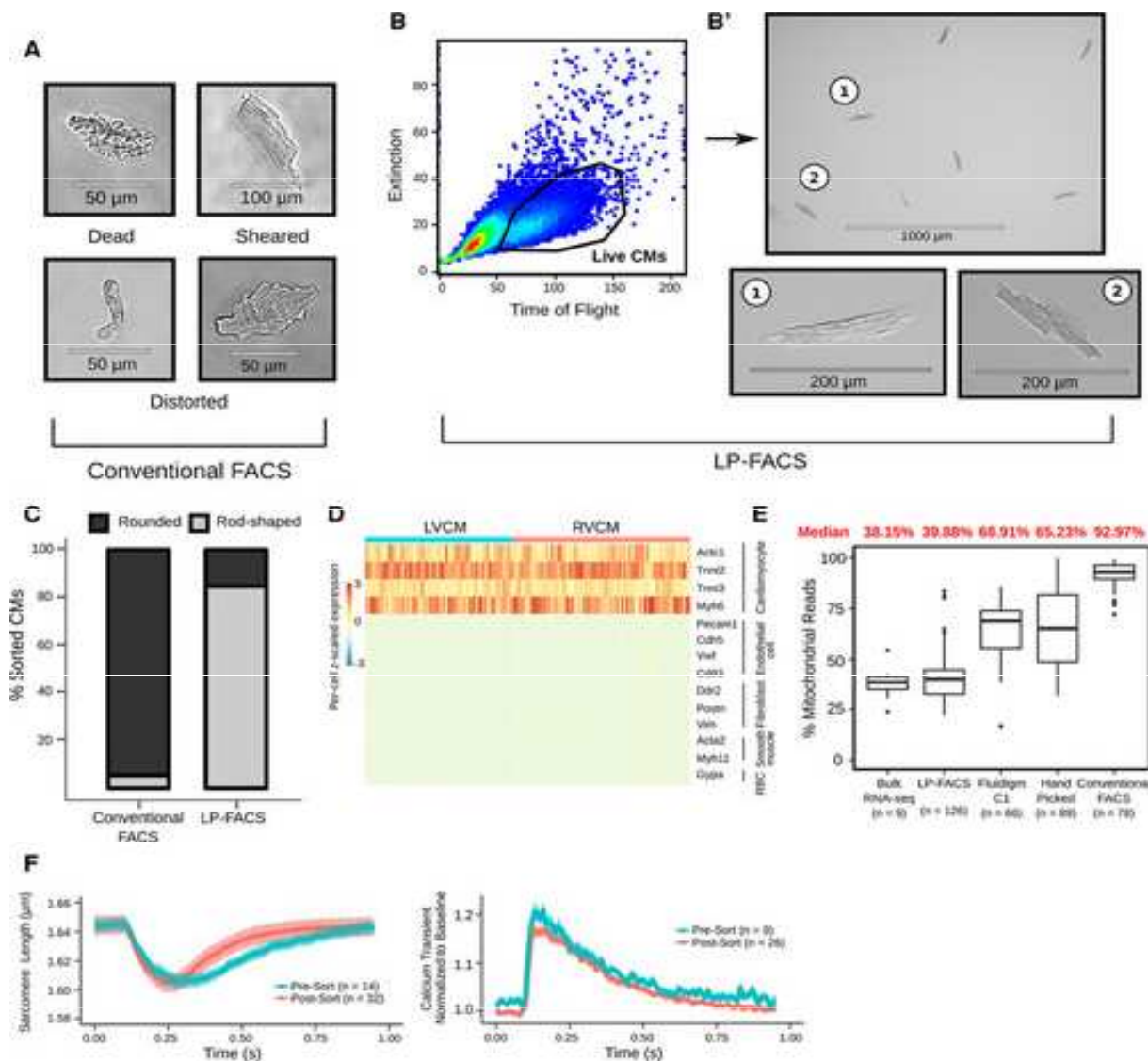
Figure 6. Proposed model for hemocyte maturation, as seen by flow cytometry.



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

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0057384>





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

„High Throughput Flow Cytometry“

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

Automatizované systémy měření vzorků



Automatický karusel (autosampler)

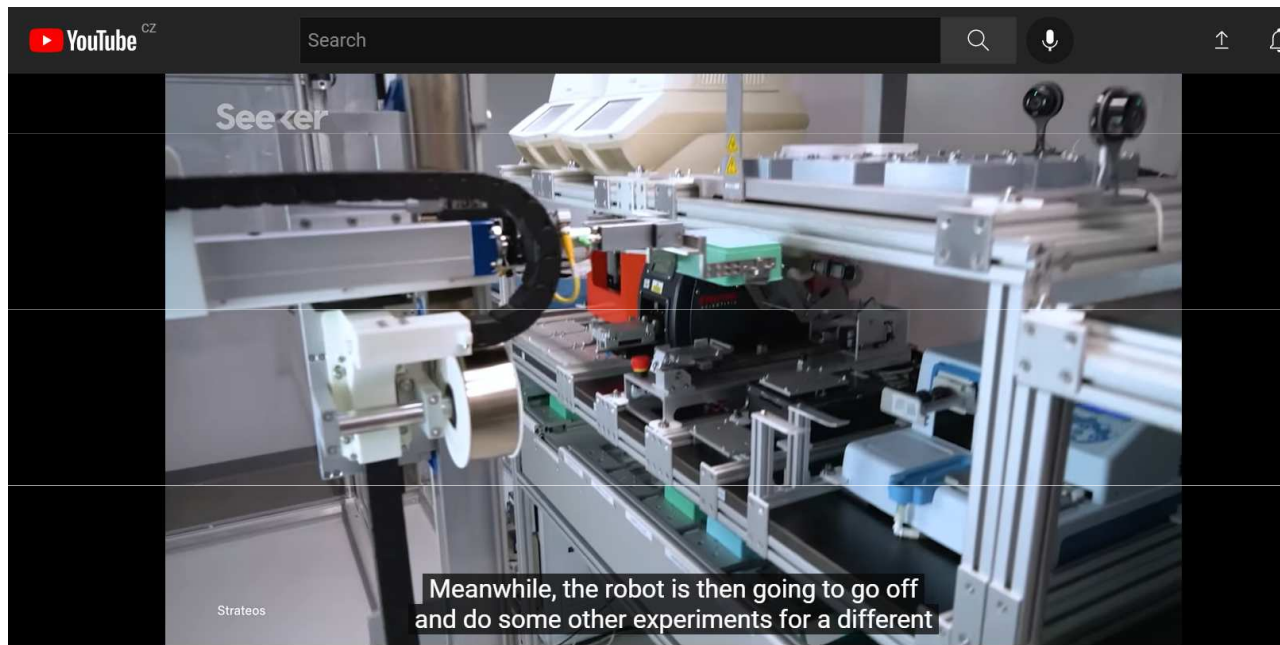


Adaptér pro nasávání vzorků z mikrotitrační desky



Automatizovaný „microsampler“ systém





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

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





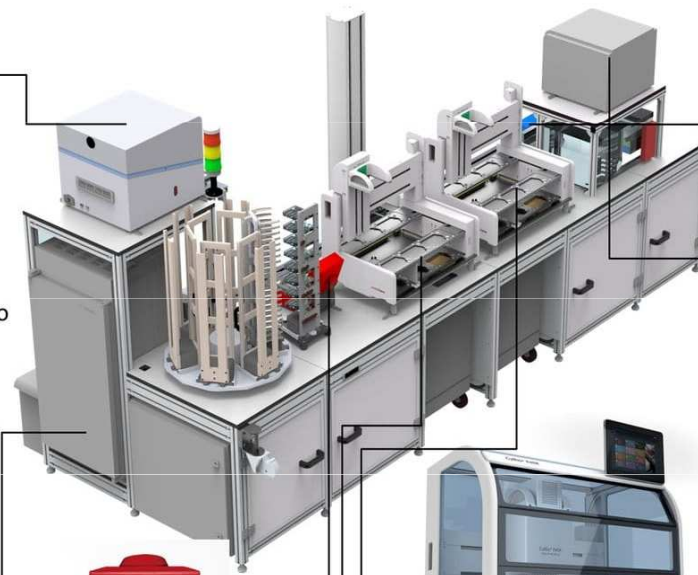
BMG PheraSTAR FSX

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



Thermo Scientific™ Cytomat™ 2

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



Biometra TRobot

- PCR cycler with automated lid opening system for automation

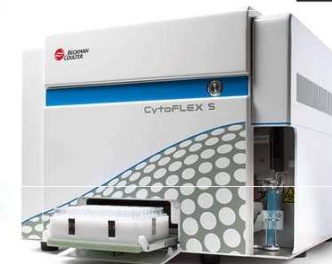


CyBio Felix 96 Channel

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

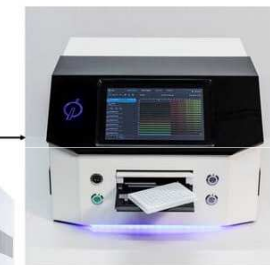
CyBio Felix 8 Channel

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



Beckman-Coulter CytoFlex S

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



Labware-Storage

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

Roboter PreciseFlex 750

- Four-axis laboratory robot with linear rail

Dispindex I-Dot

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

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

Incorporating Automation into a Flow Cytometry Workflow for Antibody Discovery

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

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

W. Coyt Jackson,¹ F. Kuckuck,¹ B.S. Edwards,¹ A. Mammoli,² C.M. Gallegos,² G.P. Lopez,³
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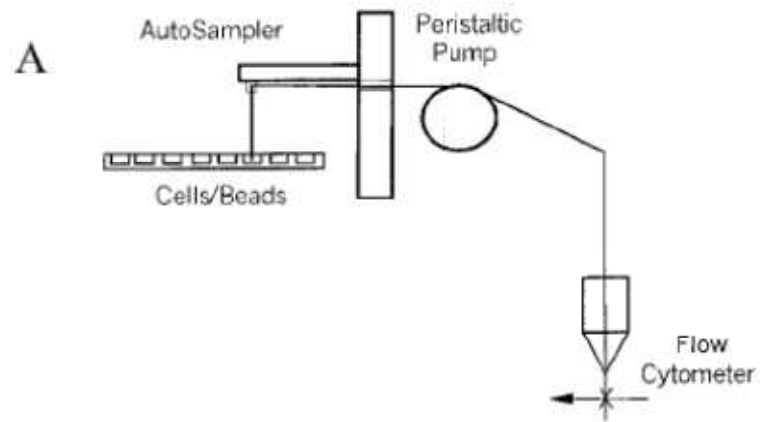
High Throughput Flow Cytometry

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



B

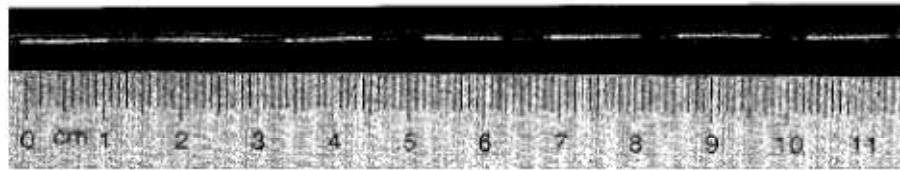
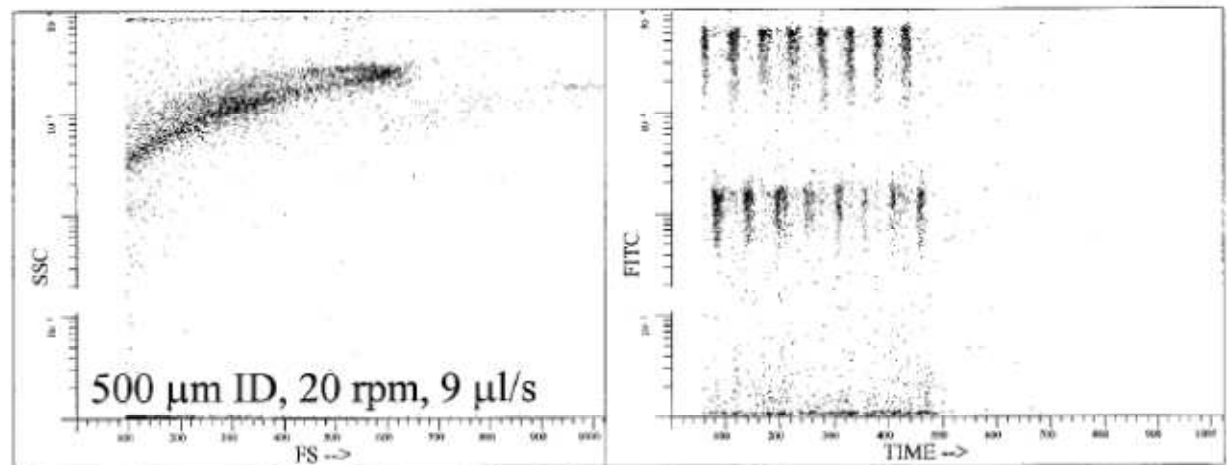
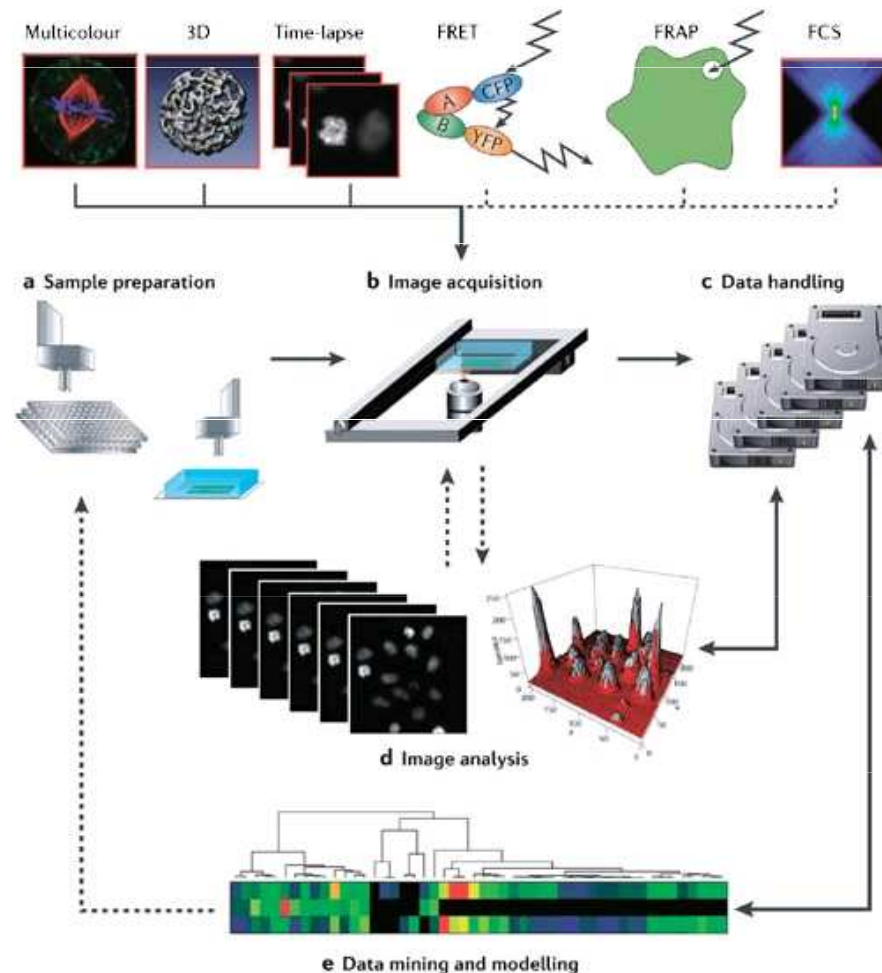


FIG. 1. High throughput flow cytometry. **A:** Schematic view of the flow cytometer, autosampler, and peristaltic pump. **B:** Adjacent samples of latex microspheres separated by air in the 0.02-in (254- μ m) ID tubing between the peristaltic pump and the flow cytometer.

C



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



Analysis

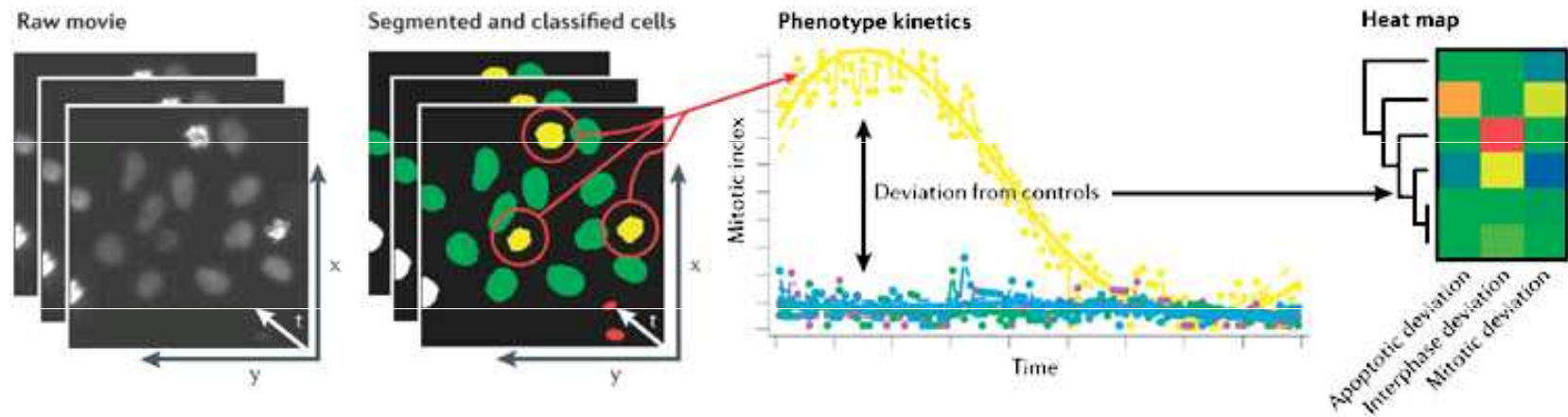


Table 1. Comparison of the Key Attributes of High-Throughput Flow Cytometry and High-Content Microscopy

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

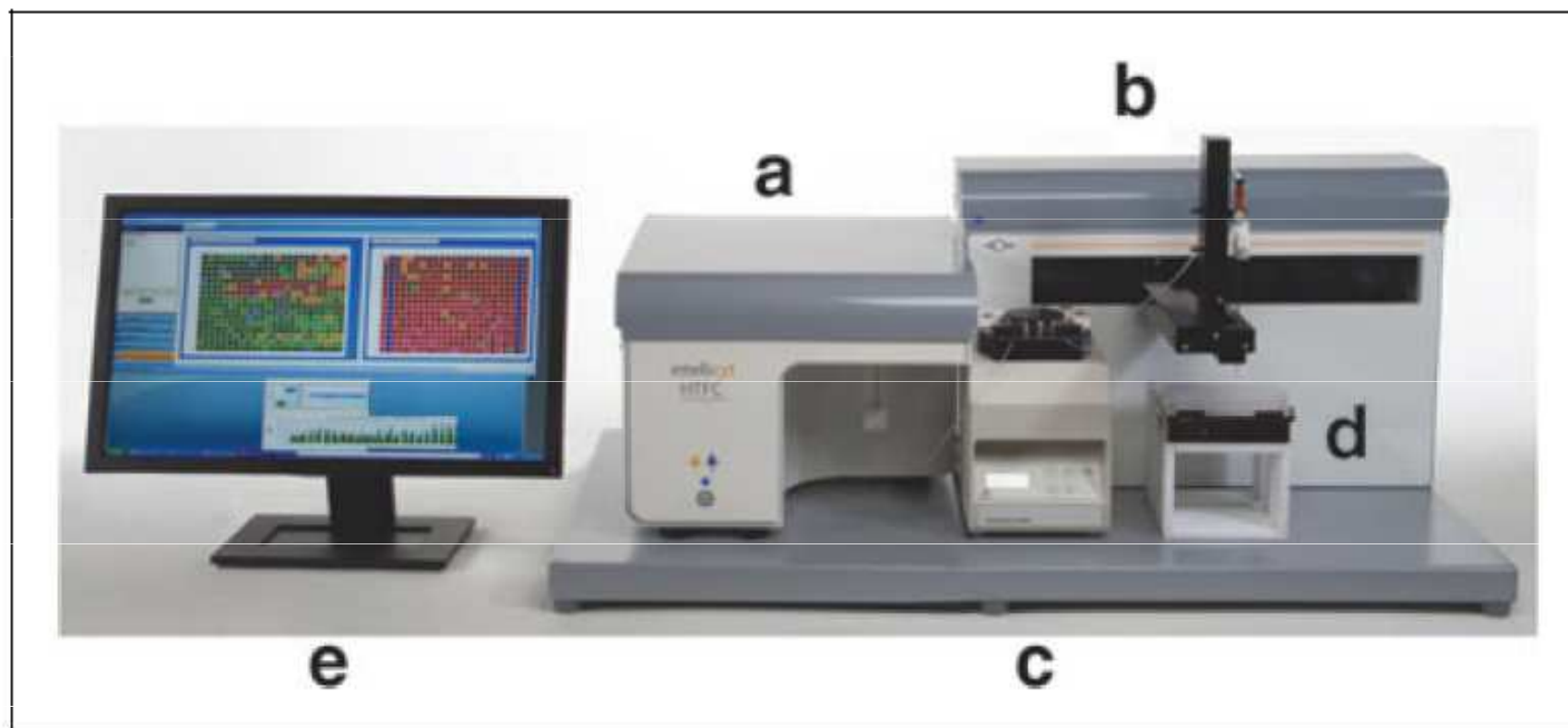


Fig. 1. The HTFC Screening System (IntelliCyt Corporation). **(a)** 2-laser, 4-color flow cytometer; **(b)** an x, y, z autosampler; **(c)** a low pulsation peristaltic pump; **(d)** orbital plate shaker that accommodates 96- and 384-well plates; **(e)** system computer with HyperView installed to set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

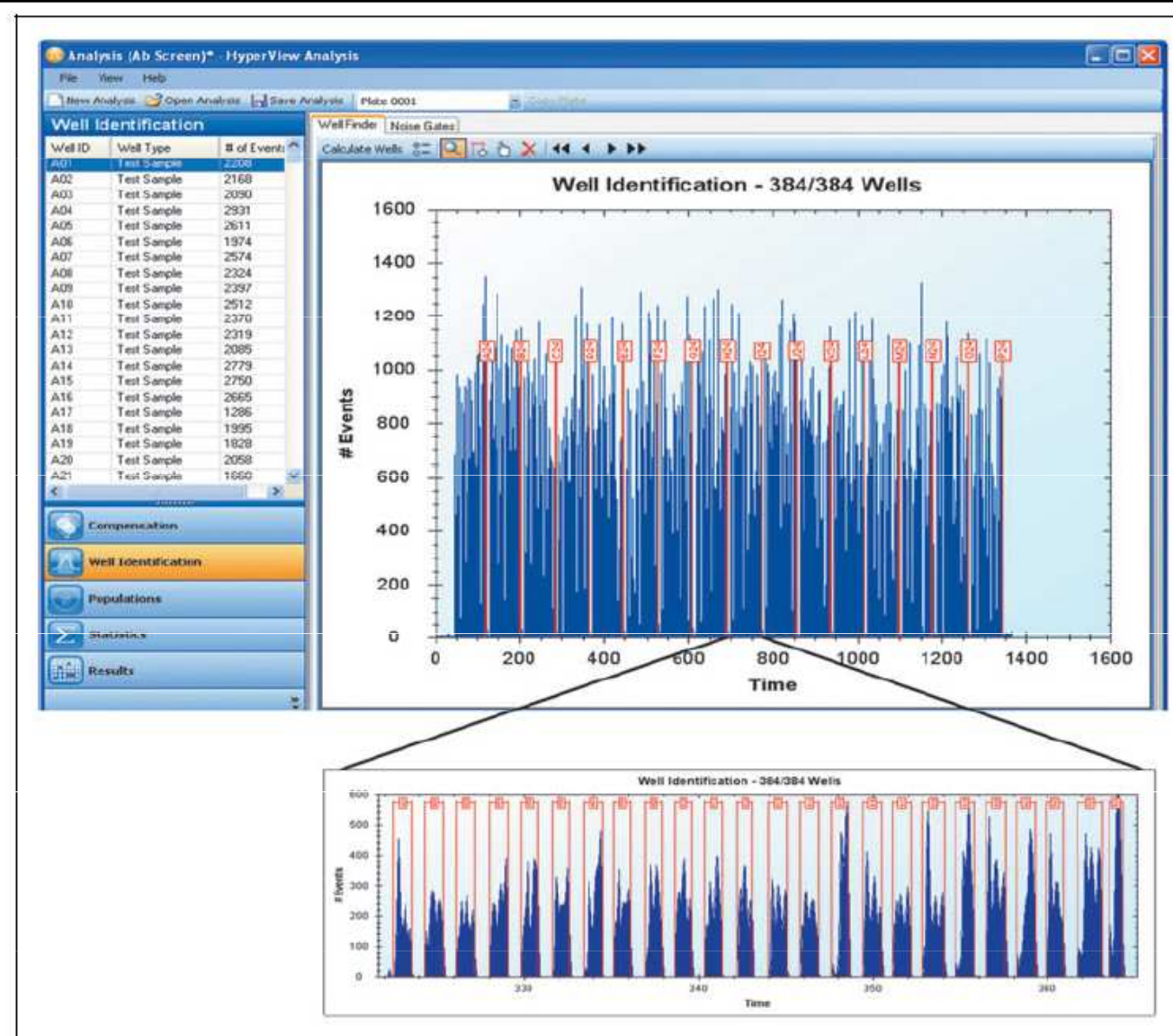
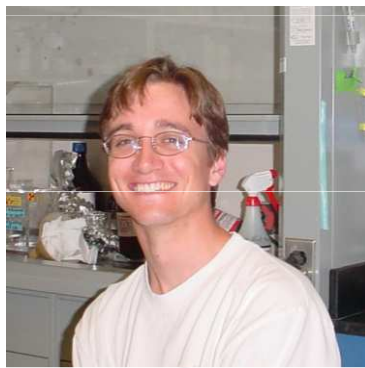


Fig. 4. Screenshot from HyperView showing an example of the Well Identification process. Data from the 384-well plate is collected in to a single flow cytometry standard file, which is shown in the main window. The data are deconvolved by the software algorithm to identify each peak with a well address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

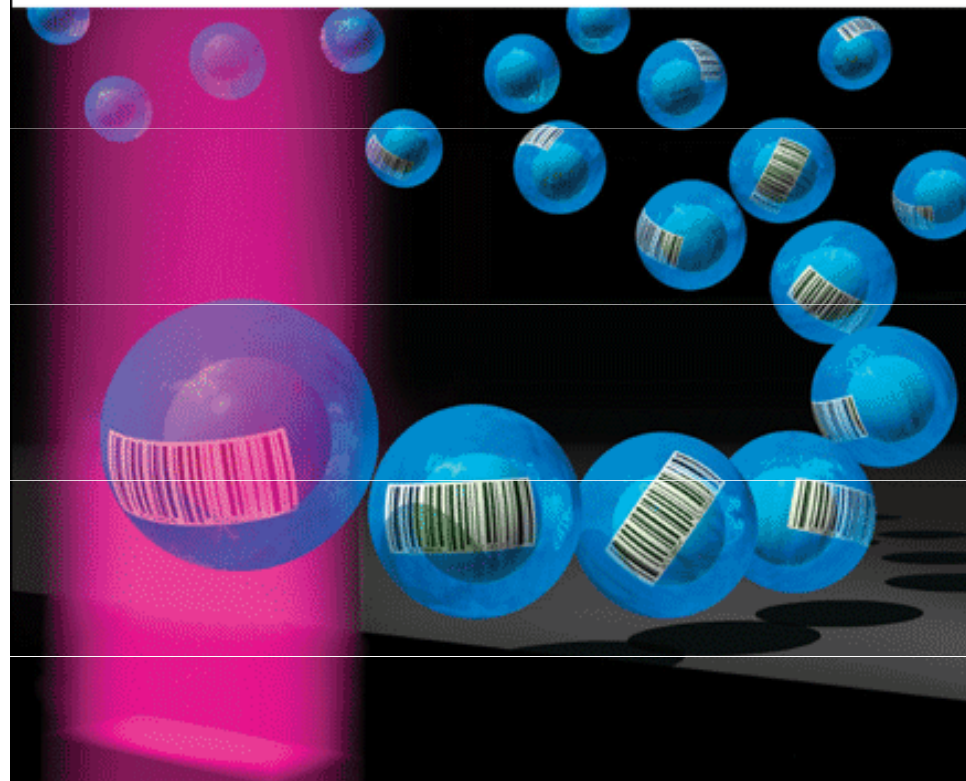


Garry Nolan

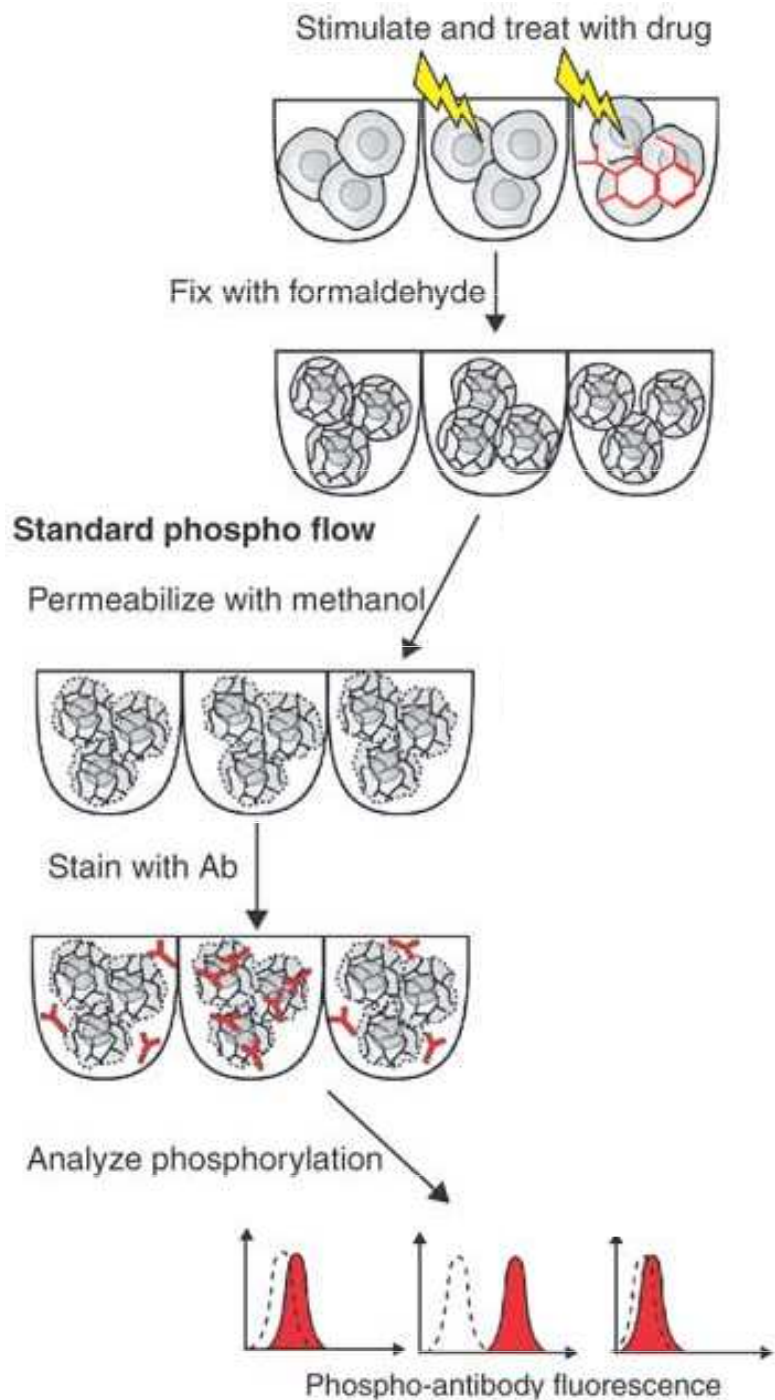


Peter Krutzik

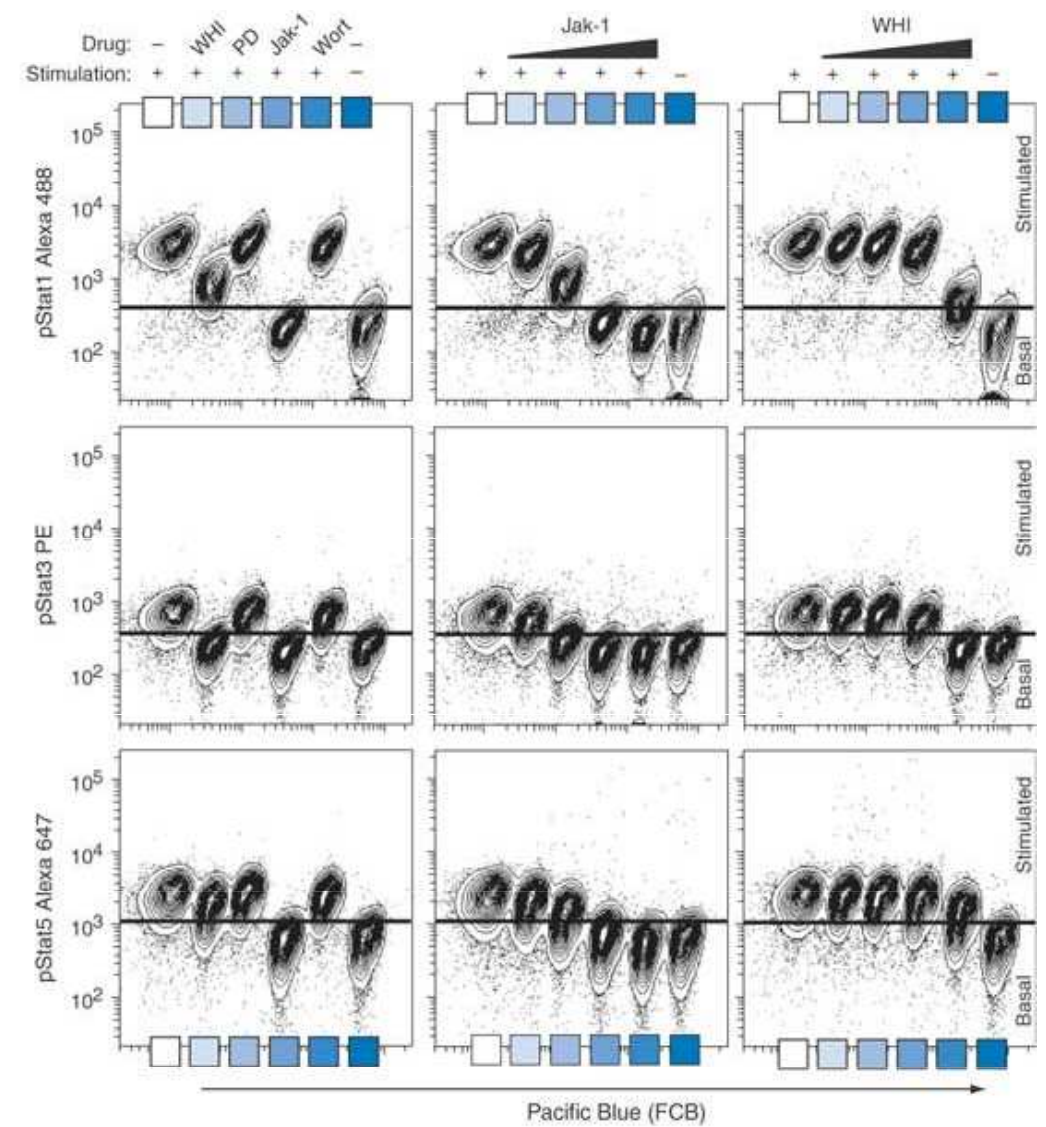
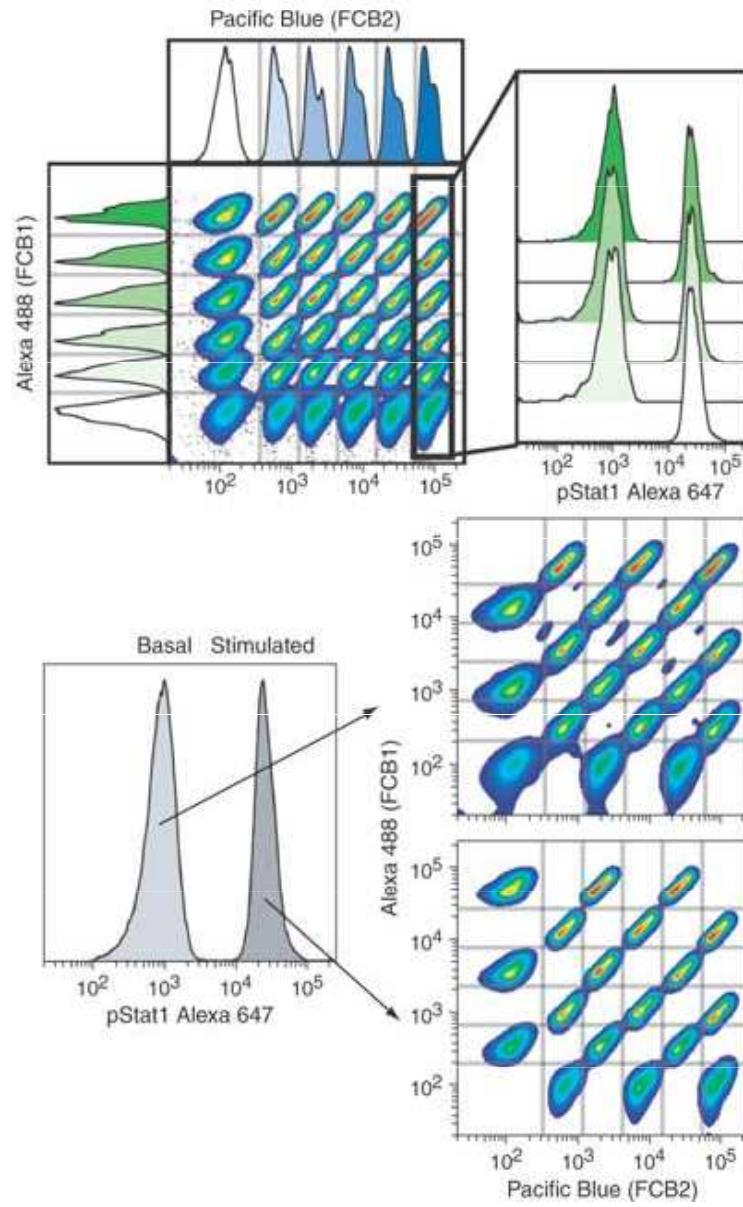
„Fluorescent cell barcoding“



- High-throughput flow cytometry
- Measuring rapid neuronal firing
- Cell patterning in 3D
- Live-cell imaging of RNAi screens
- A review of force spectroscopy

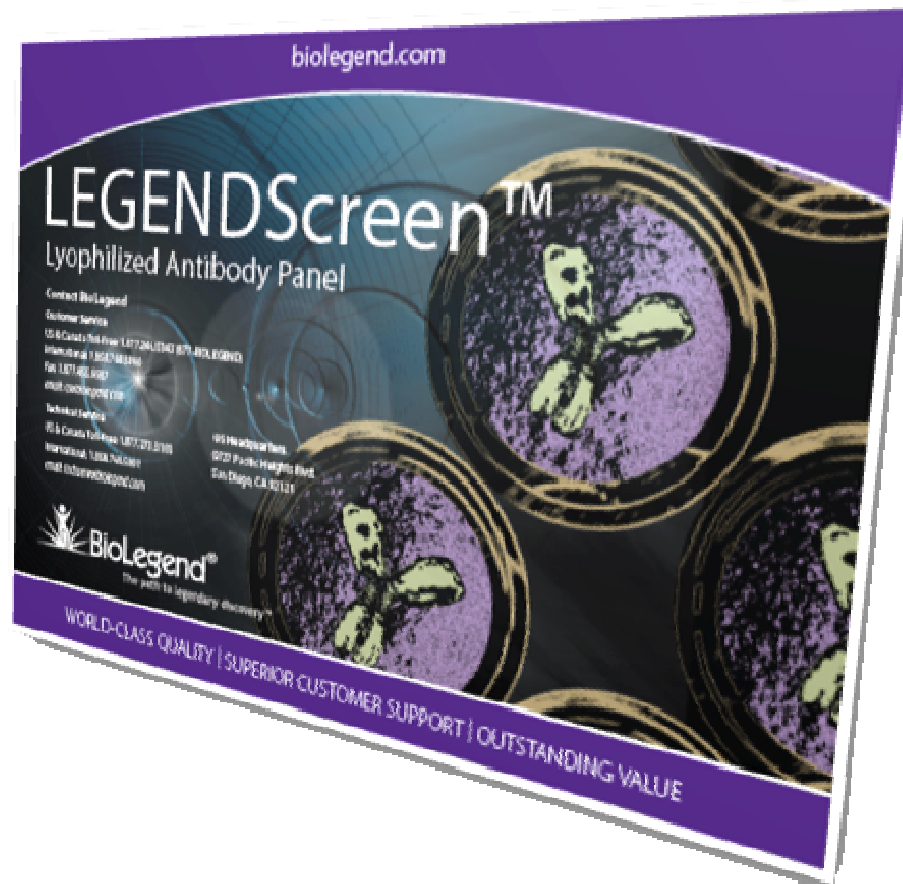


[Krutzik PO, Nolan](#) Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling. Nat Methods. 2006 May;3(5):361-8.



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

Get the best out of your model



FACS-based surface screen:

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

- LEGENDScreen HUMAN

332 PE conjugated antibodies + ISOs

- LEGENDScreen MOUSE

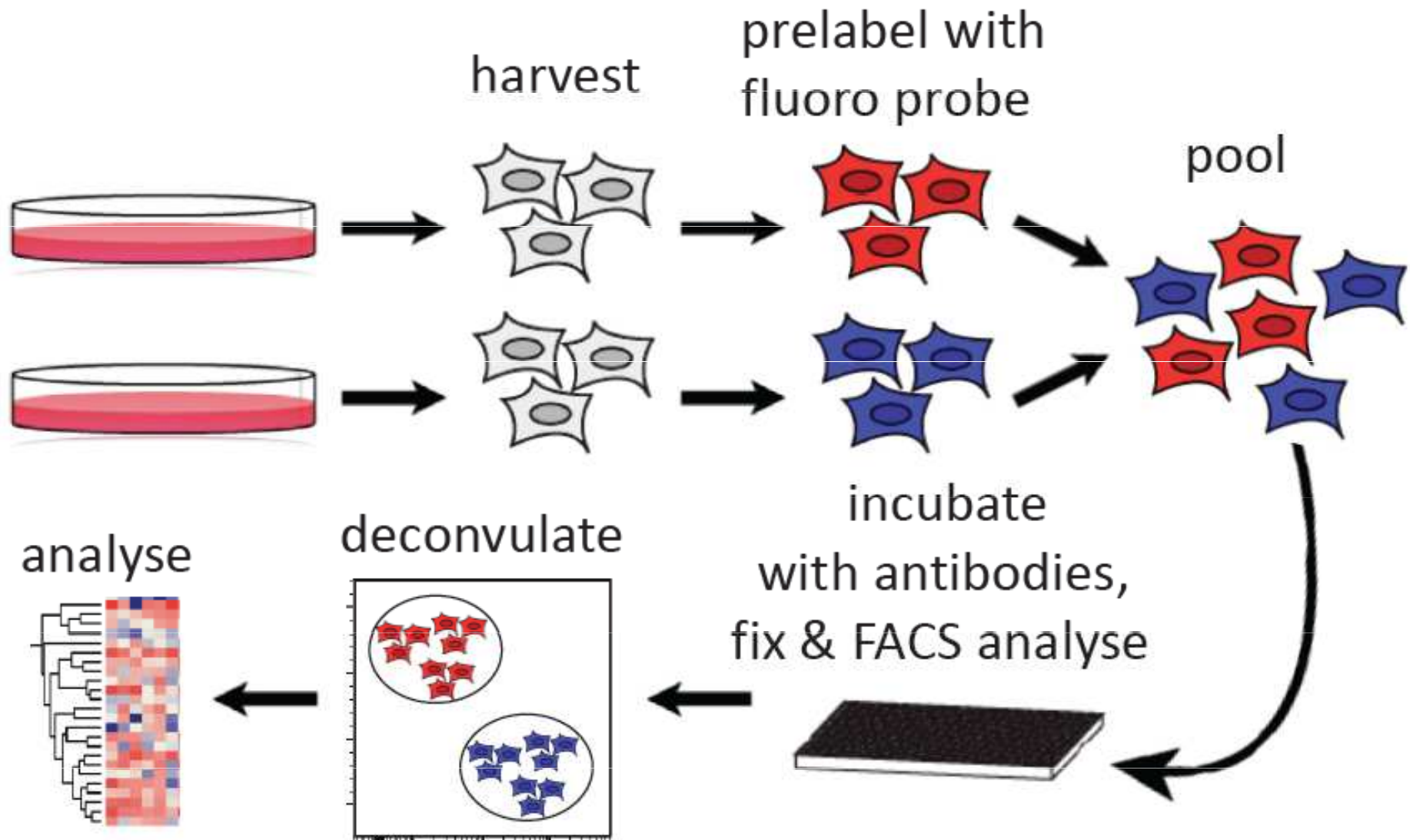
252 PE conjugated antibodies + ISOs

- there are XY vials in LN

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

How to get the best of it all?

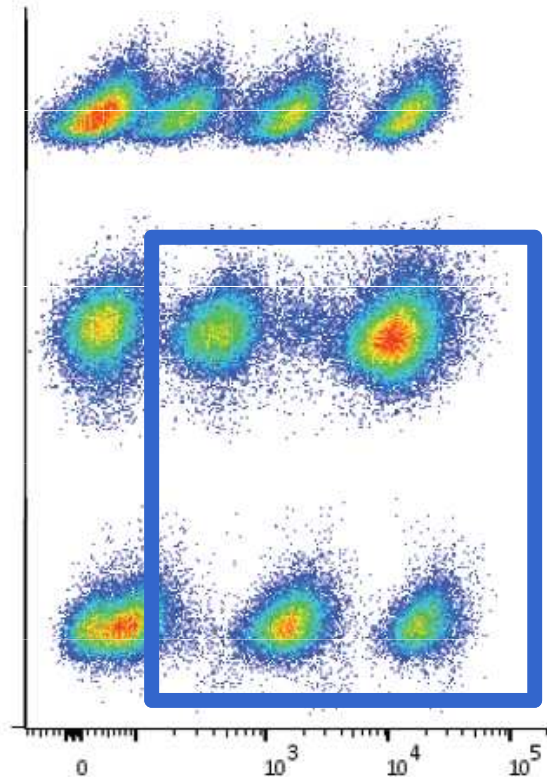
Final workflow



The optimal concentration issue

HOW TO TEST IT:
10x serial dilution

CellTrace CFSE



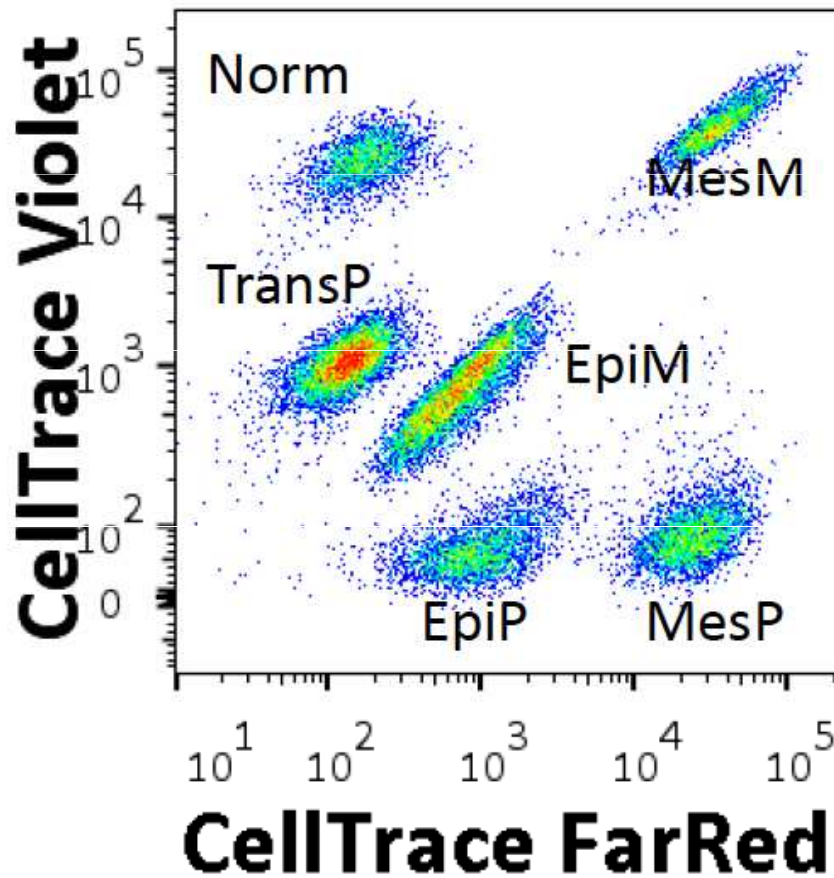
CellTrace DDAO

CellTrace Violet

REQUIREMENTS:

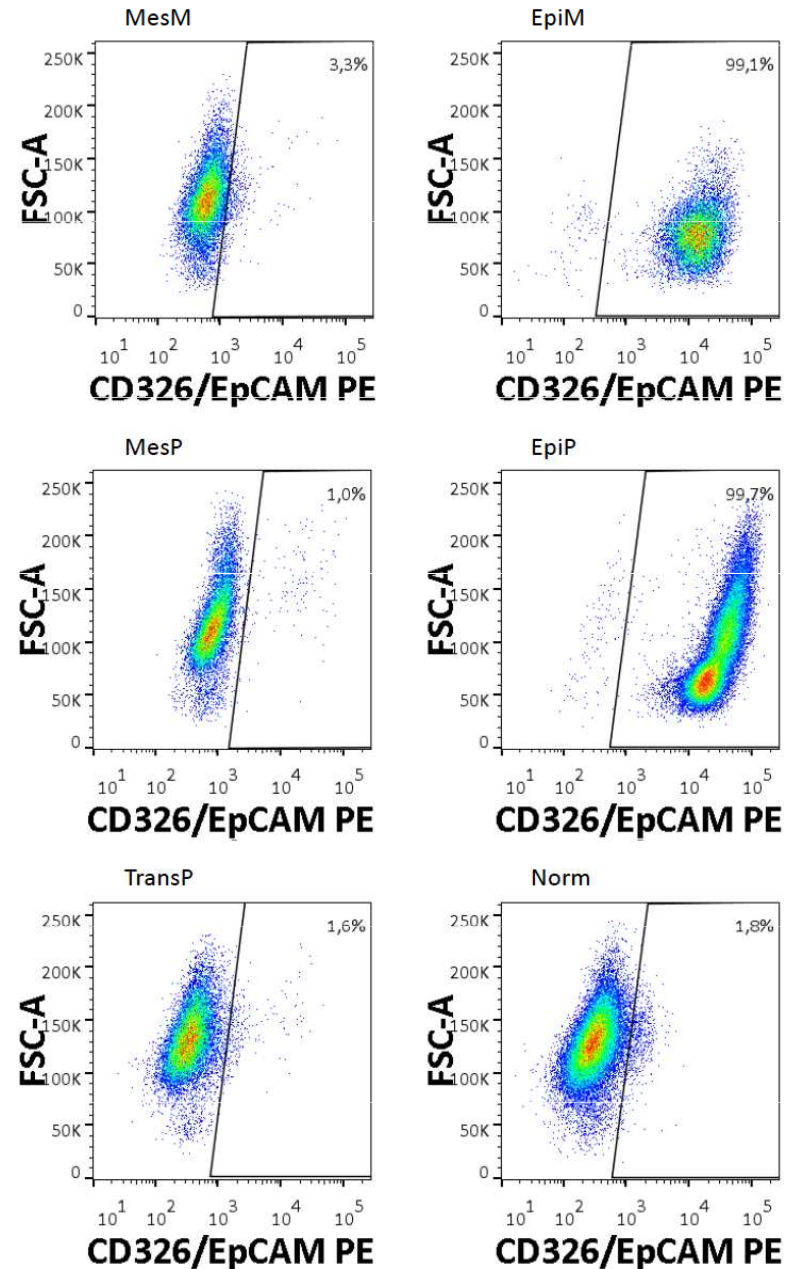
- optimal resolution
- compatibility w/ PE

Sample results



EpCAM

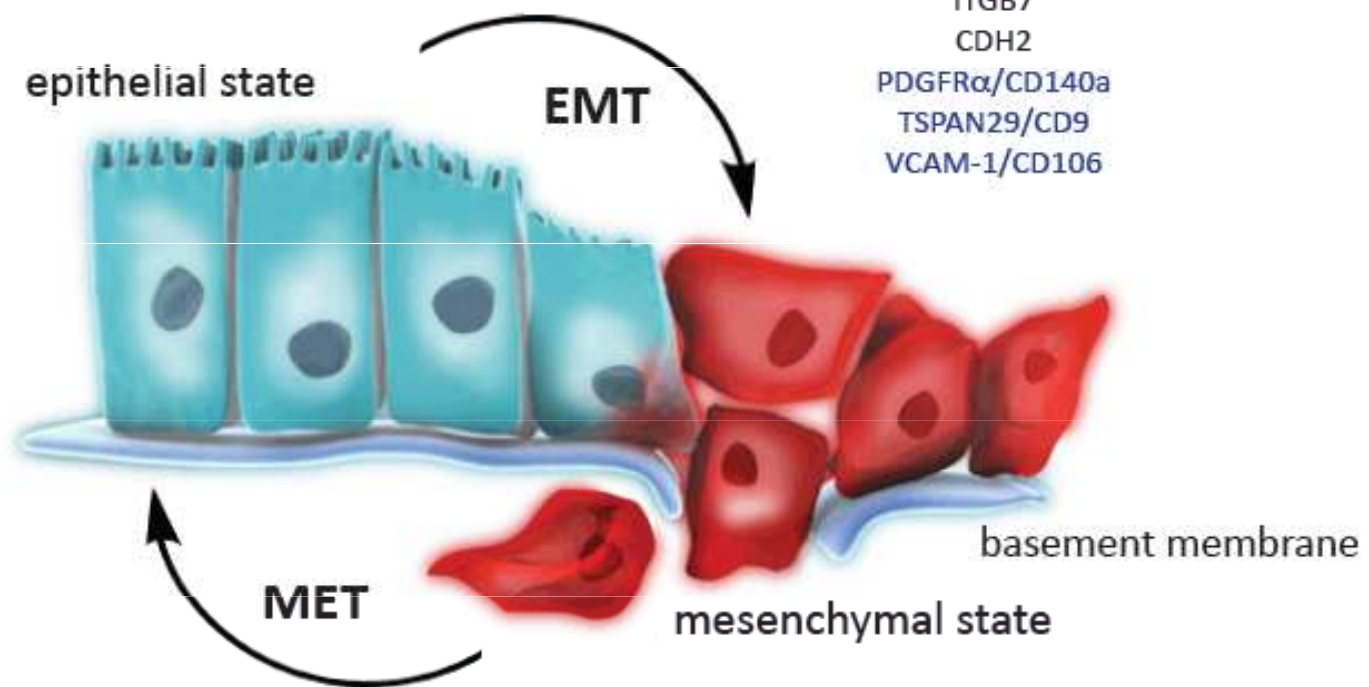
- marker of epithelial cells
- commonly lost during EMT



Sample result

non-BCSC
CD24⁺CD44^{low}

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



BCSC
CD24⁻CD44^{high}

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

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



Chapter 9

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

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

Abstract

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

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

Methods in
Molecular Biology 2543

Springer Protocols

Hugo Barcenilla · David Diaz *Editors*

Apoptosis and Cancer

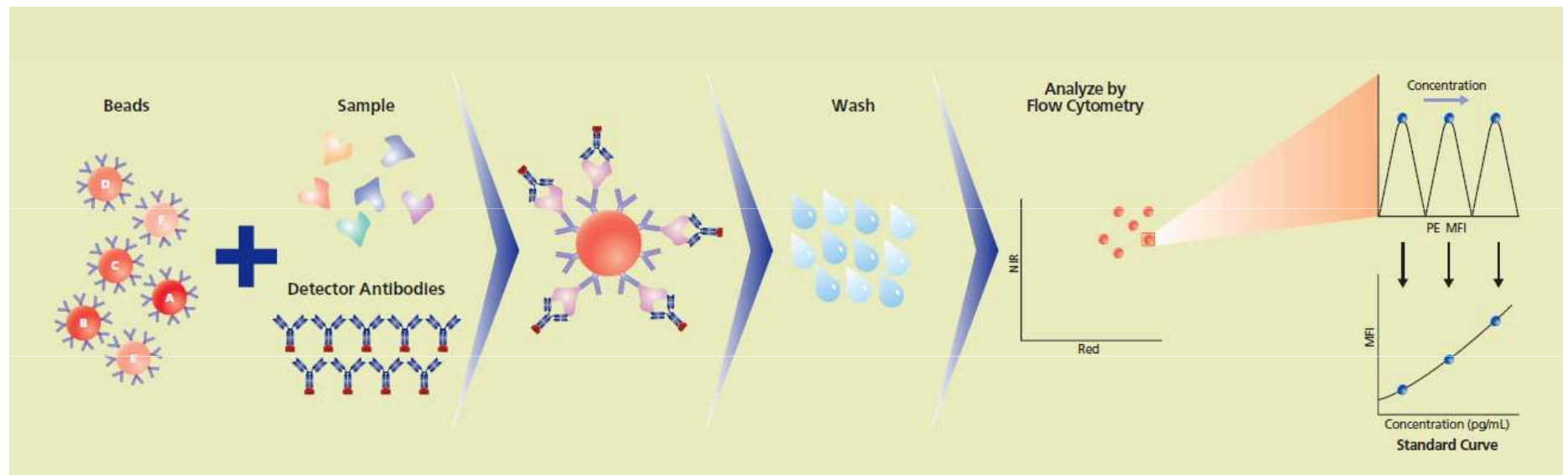
Methods and Protocols



 Humana Press

Cytometric bead array (CBA)

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

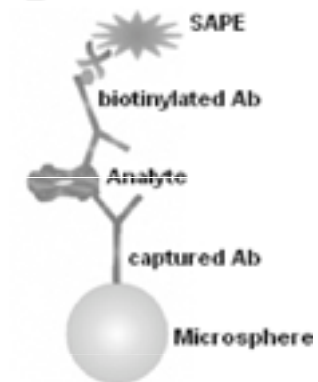


Multiplex microsphere-based flow cytometric platforms for protein analysis and their application in clinical proteomics – from assays to results

A

Functional Groups on Microsphere	Immobilization Methods
-COOH	
-SH	
-Avidin	

B

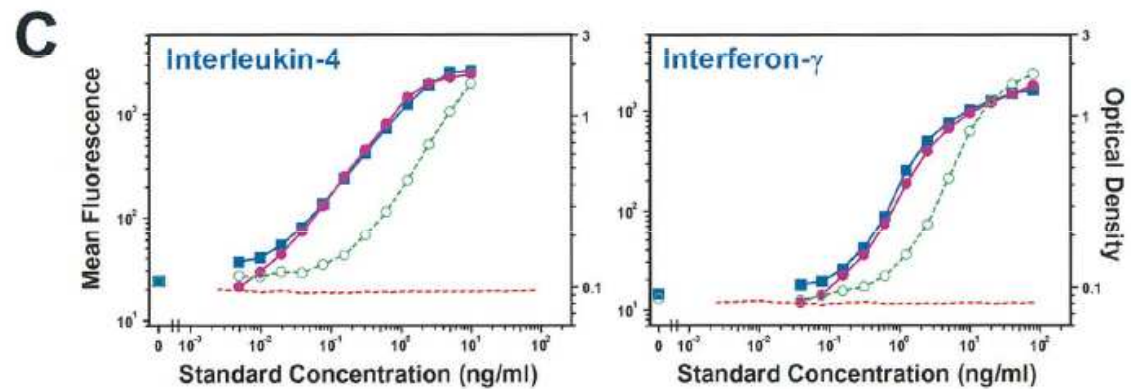
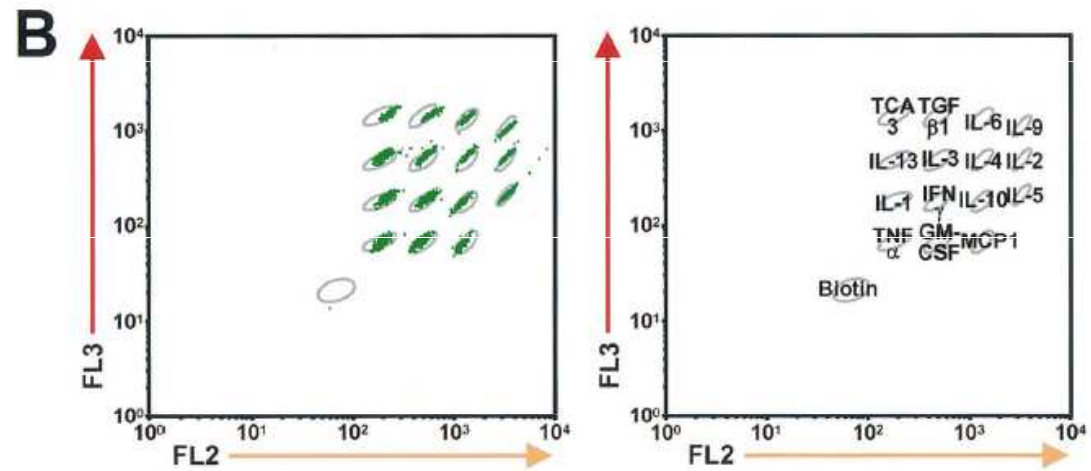
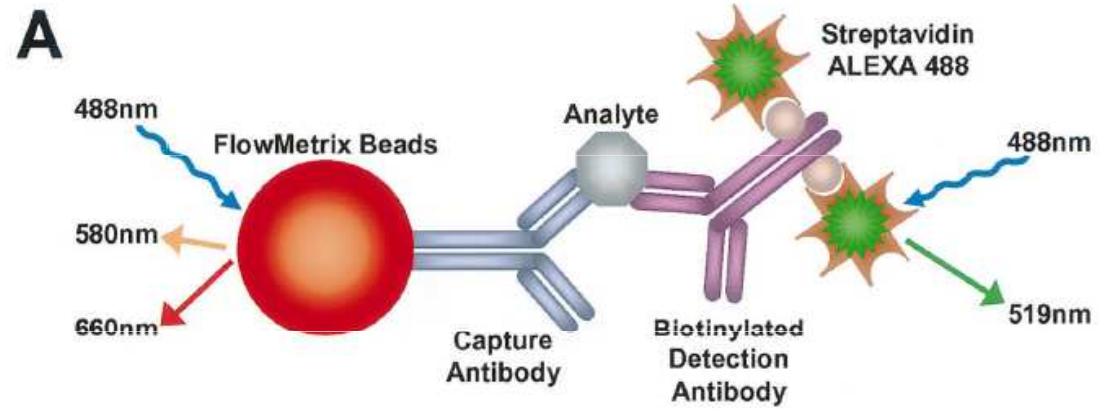


ELECTROPHORESIS

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

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

CBA



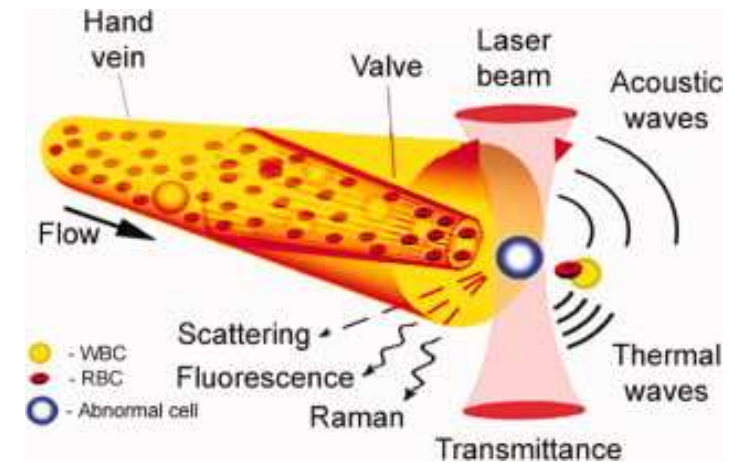
CBA

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

ex vivo flow cytometrie - limitace

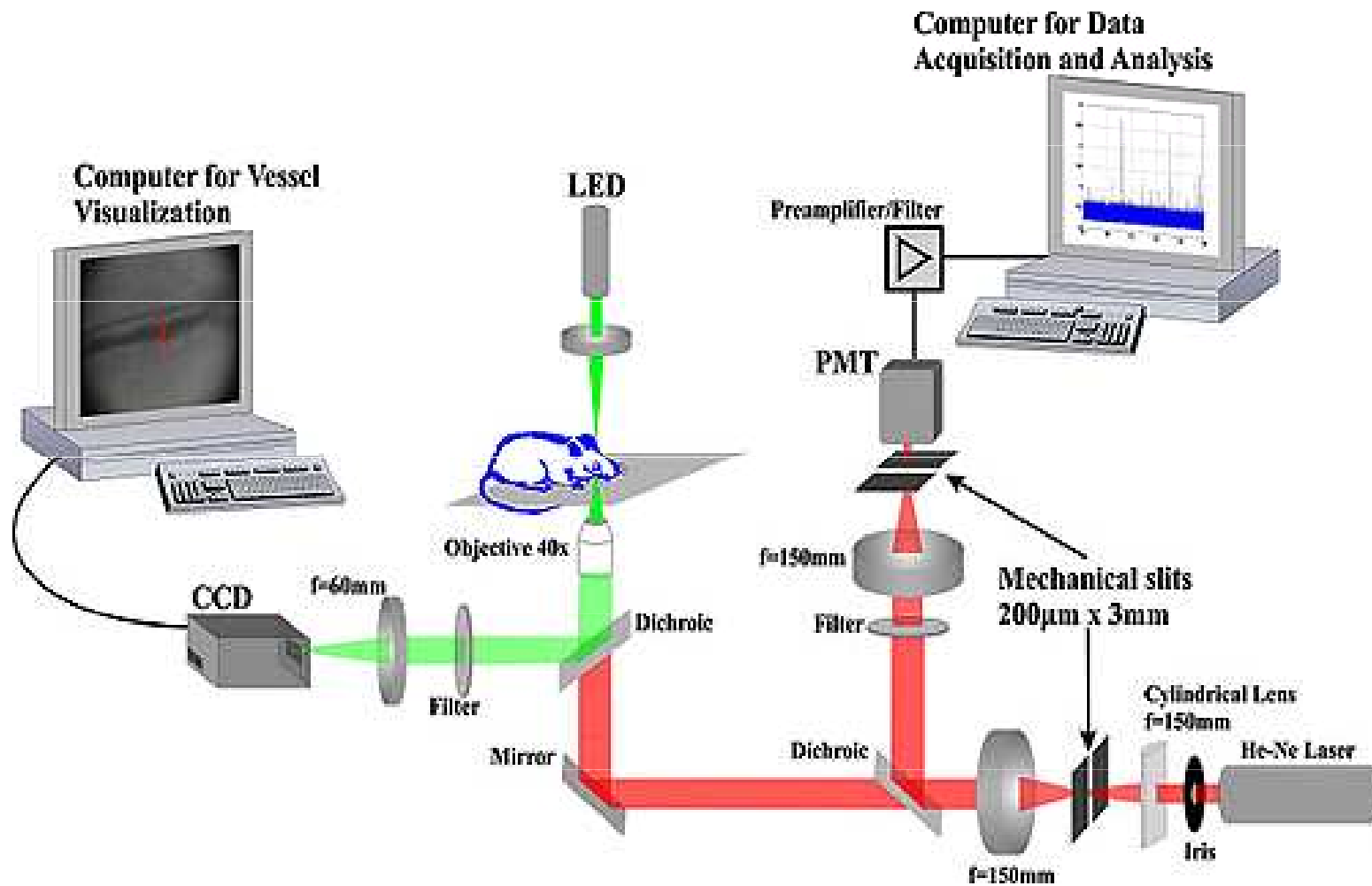
- Ovlivnění některých vlastností buněk (morfologie, exprese znaků);
- neumožňuje dlouhodobější studie buněčného metabolismu a buněčných interakcí (komunikace, adheze) v přirozeném tkáňovém mikroprostředí;
- další:
 - nízká citlivost pro detekci vzácných buněčných subpopulací (1-10 buněk/ml ~ 5000 – 50000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

in vivo flow cytometry – základní principy



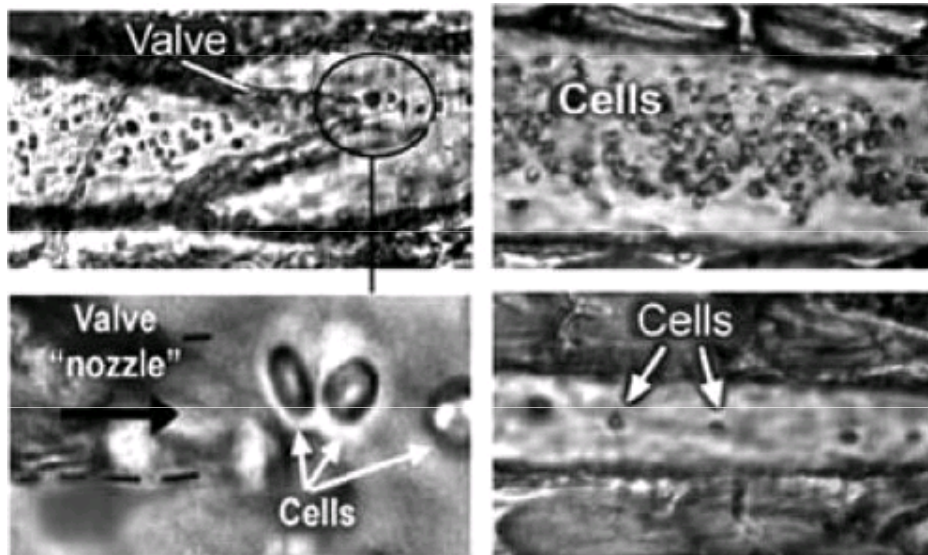
- Zobrazení buněk přímo v krevním nebo lymfatickém řečišti.
- Vizualizace pomocí CCD nebo CMOS kamery po ozáření konvenční mikroskopickou lampou nebo lasery.
- Detekce absorpce, fluorescence, Ramanova spektra, fototermálních nebo fotoakustických signálů.

in vivo flow cytometry



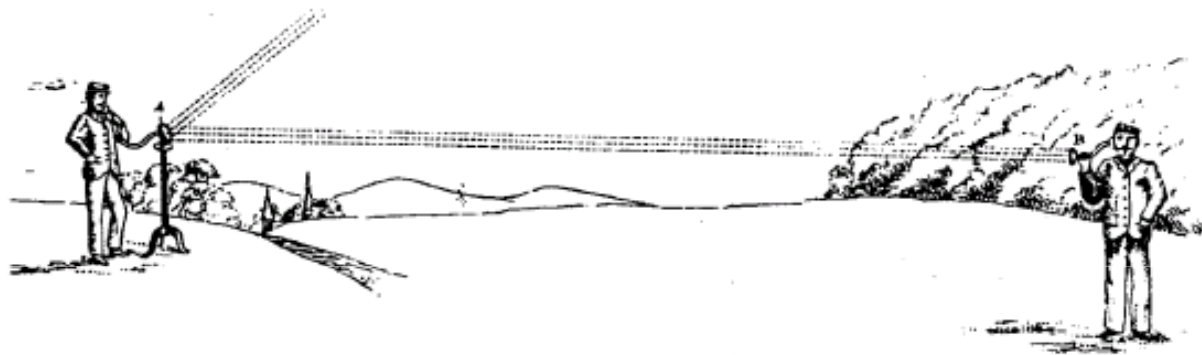
in vivo flow cytometry – bez značení

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



photoacoustic and photothermal imaging

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



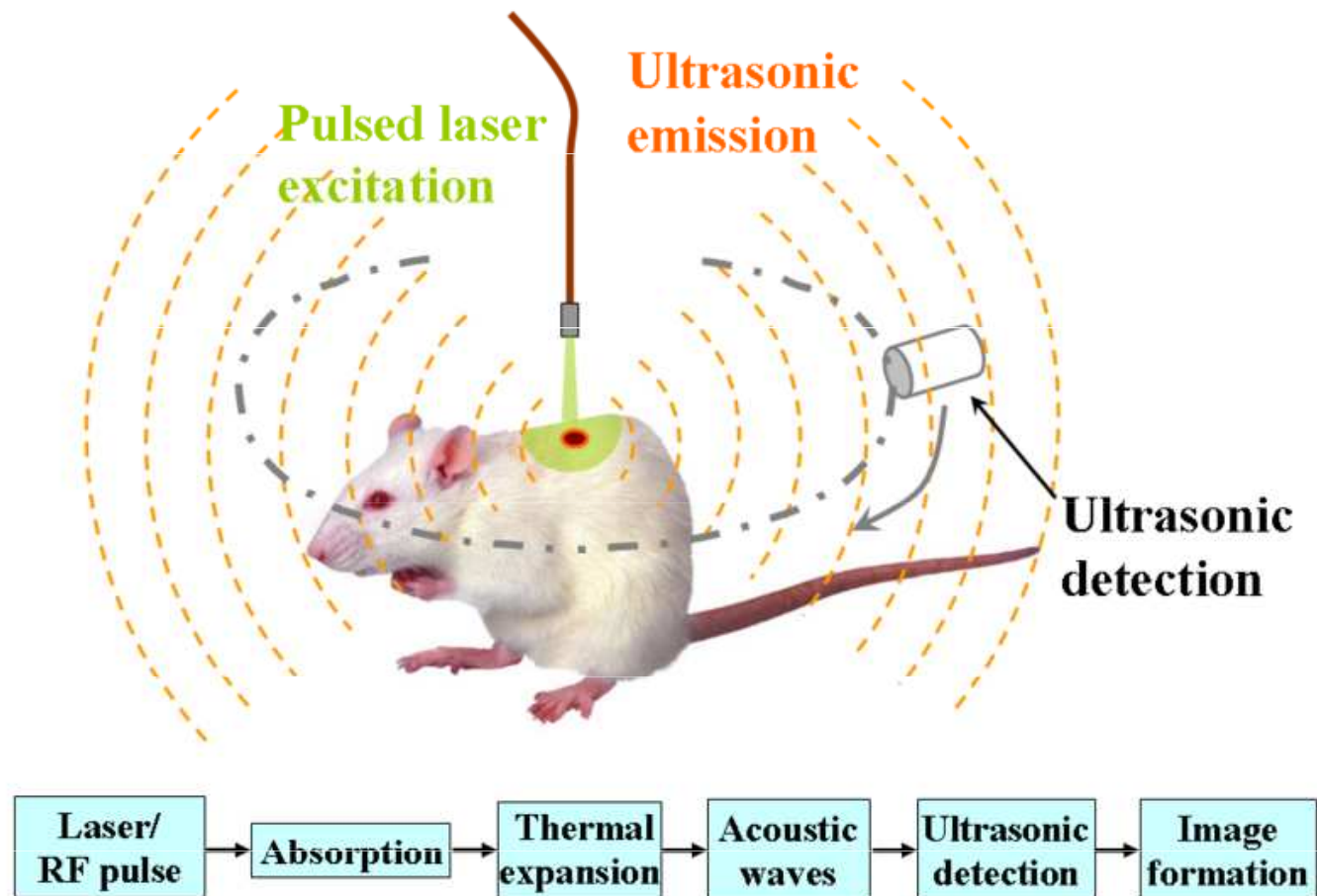
The Photoacoustic Effect

Benjamin T. Spike

Physics 325

April 21, 2006

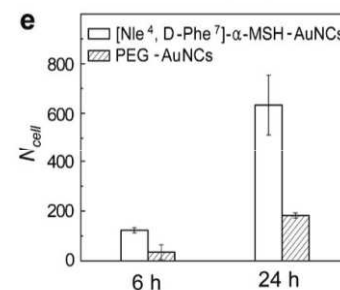
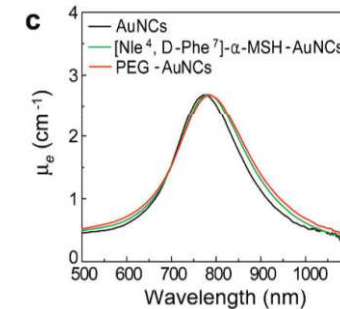
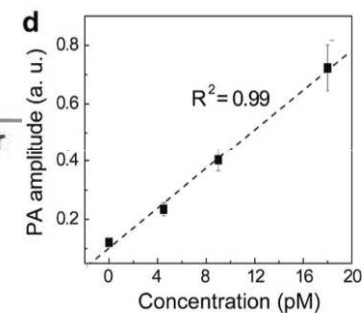
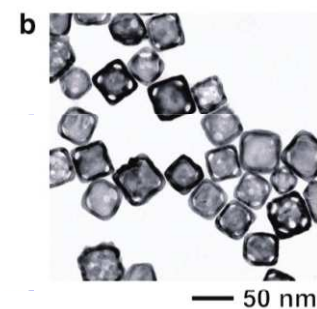
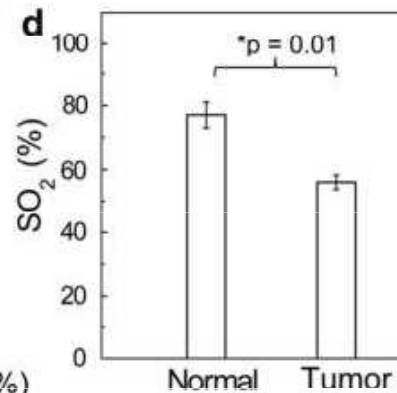
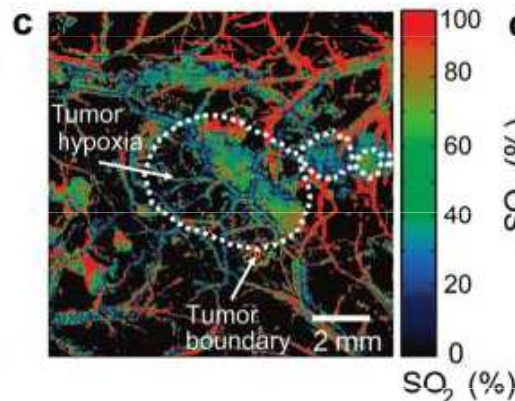
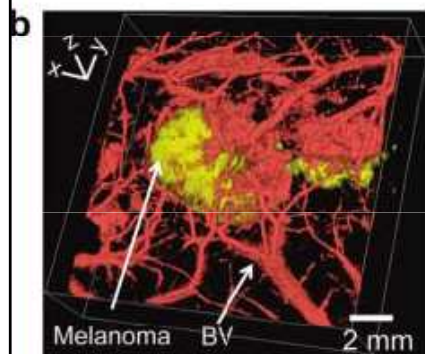
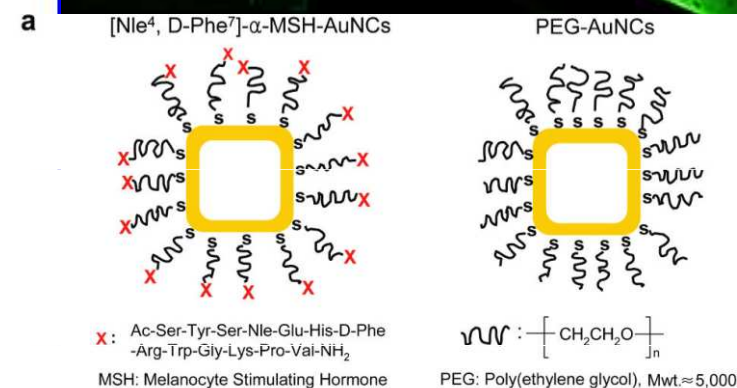
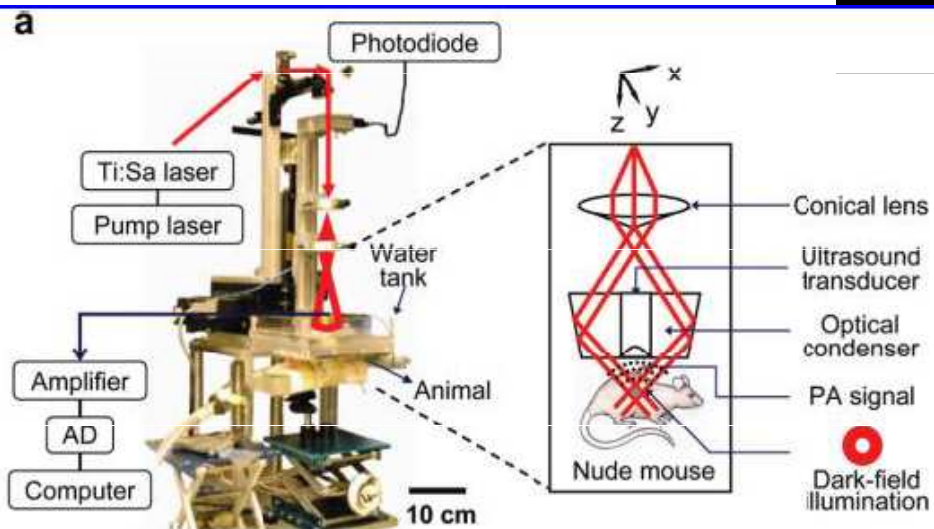
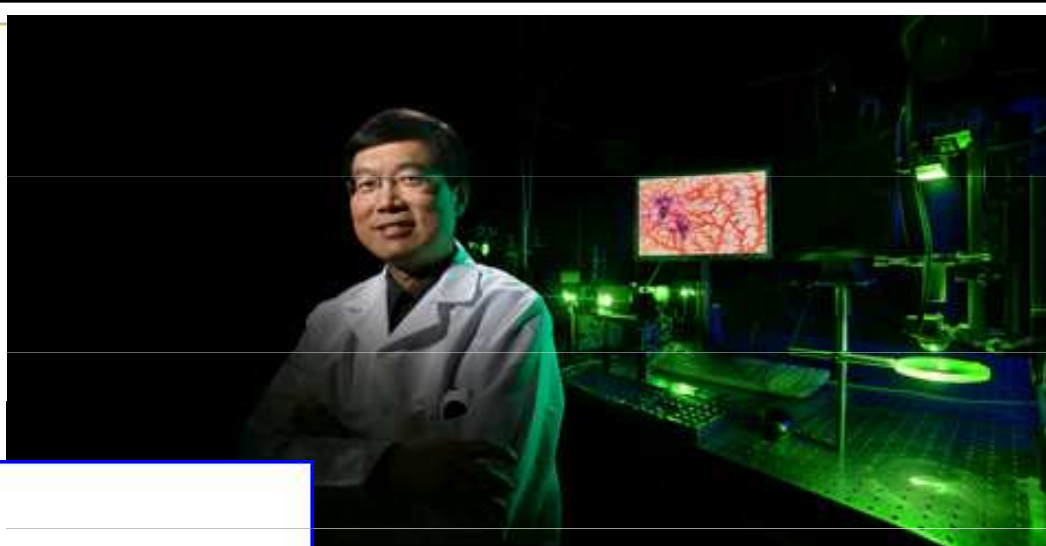
Schematic illustration of photoacoustic imaging



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

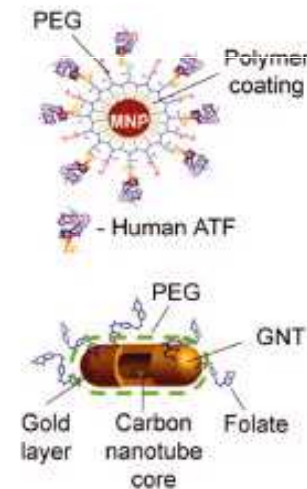
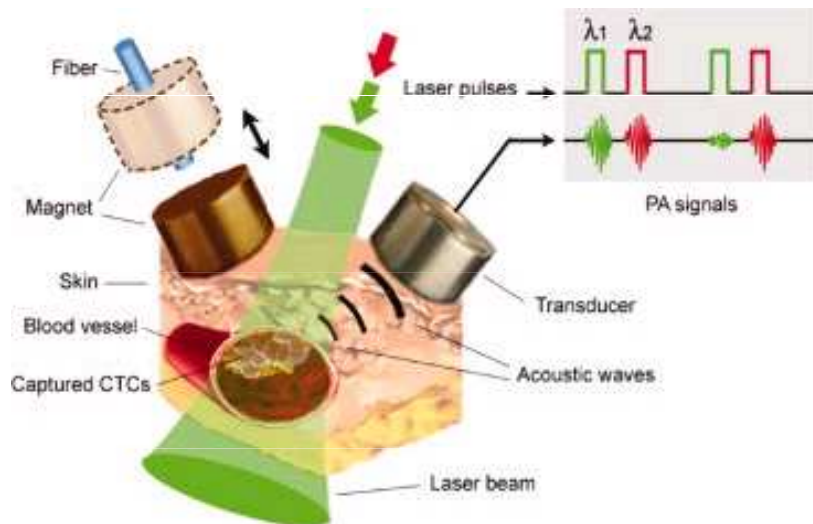
Chulhong Kim,^{1,5} Eun Chul Cho,^{1,5} Jingyi Chen,² Kwang Hyun Song,² Leslie Au,² Christopher Favazza,² Qiang Zhang,² Claire M. Cobley,¹ Feng Gao,⁴ Younan Xia,^{2,*} and Lihong V. Wang^{1,*}

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

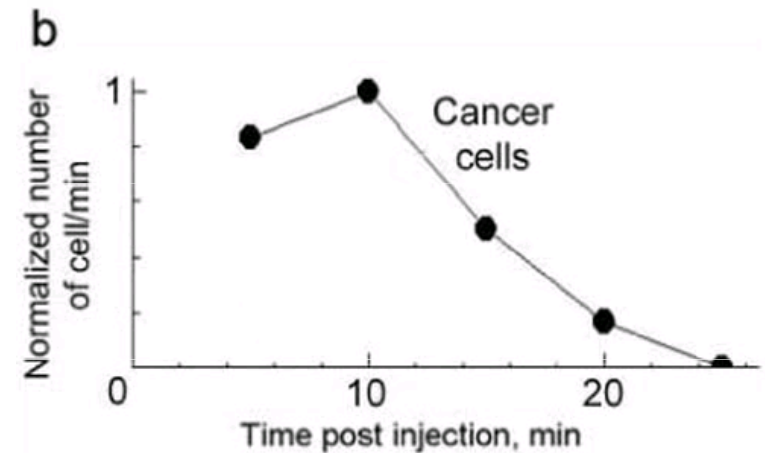
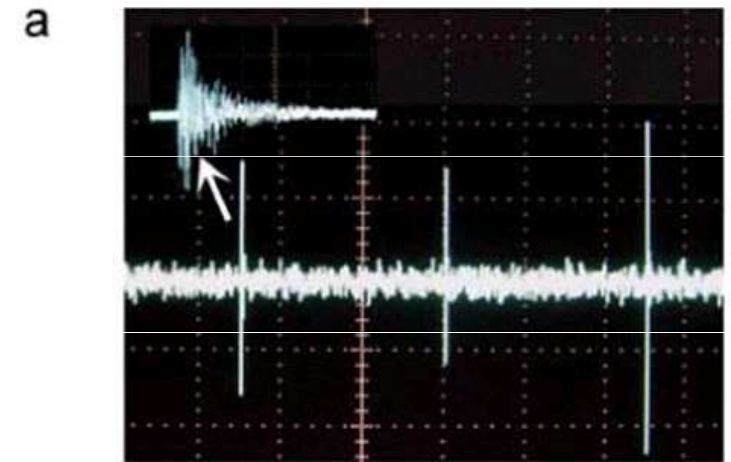
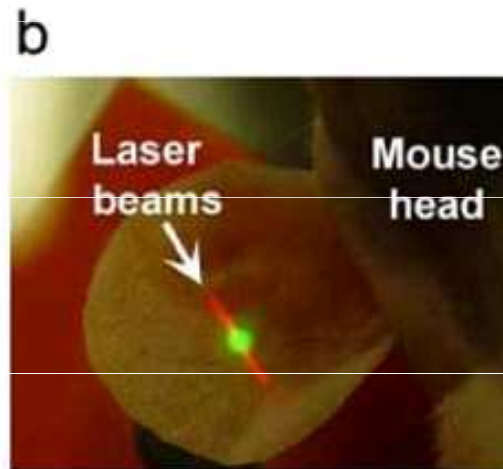
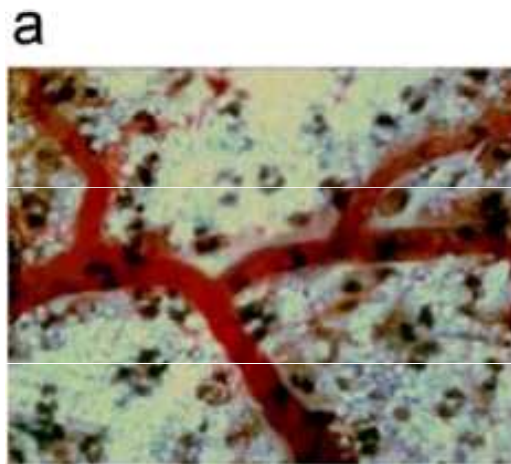


In vivo flow cytometrie – detekce specifických signálů

- Detekce fotoakustických a fototermálních jevů



in vivo flow cytometry - aplikace



Shrnutí přednášky

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

Na konci dnešní přednášky byste měli:

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