

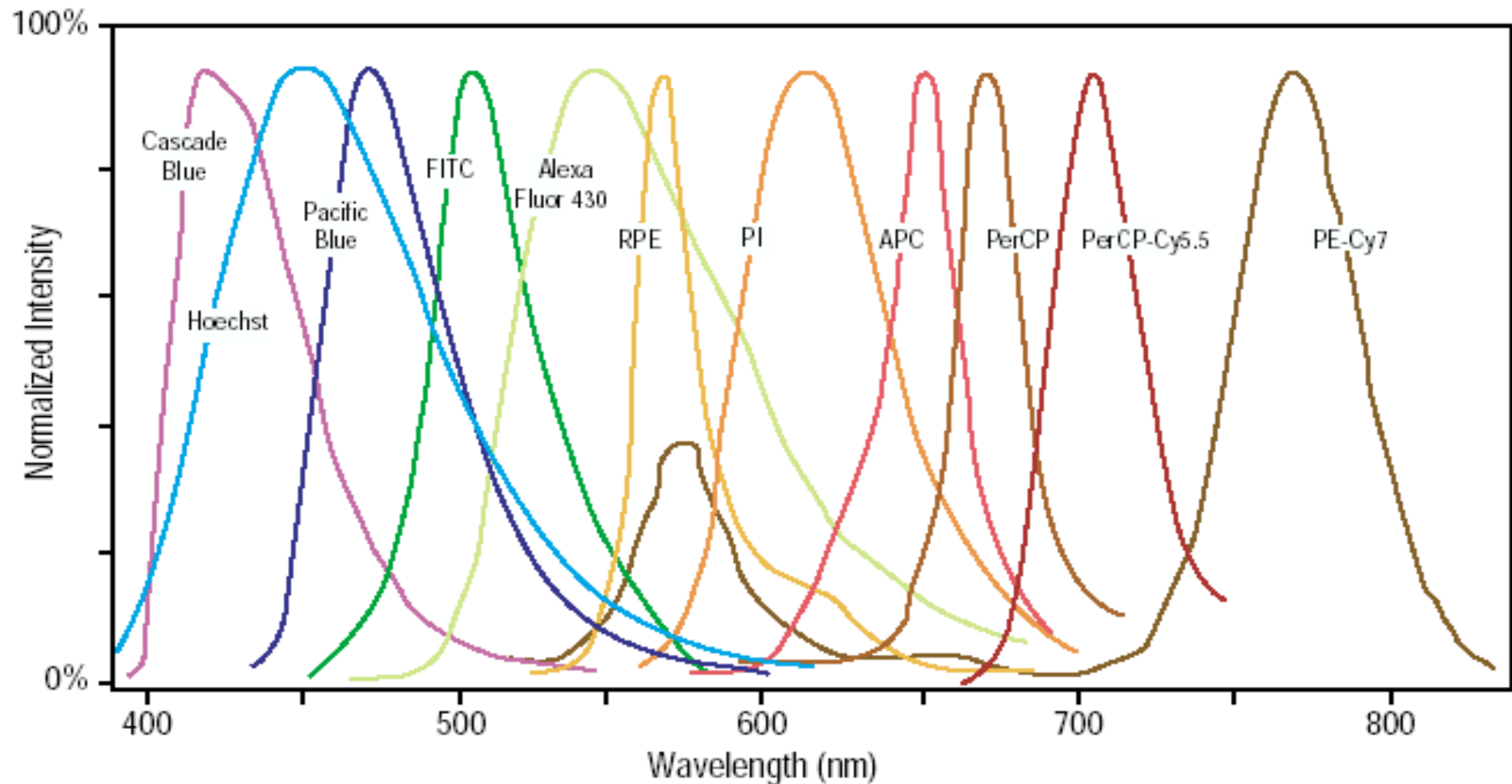
Bi9393 Analytická cytometrie

Karel Souček, Ph.D.

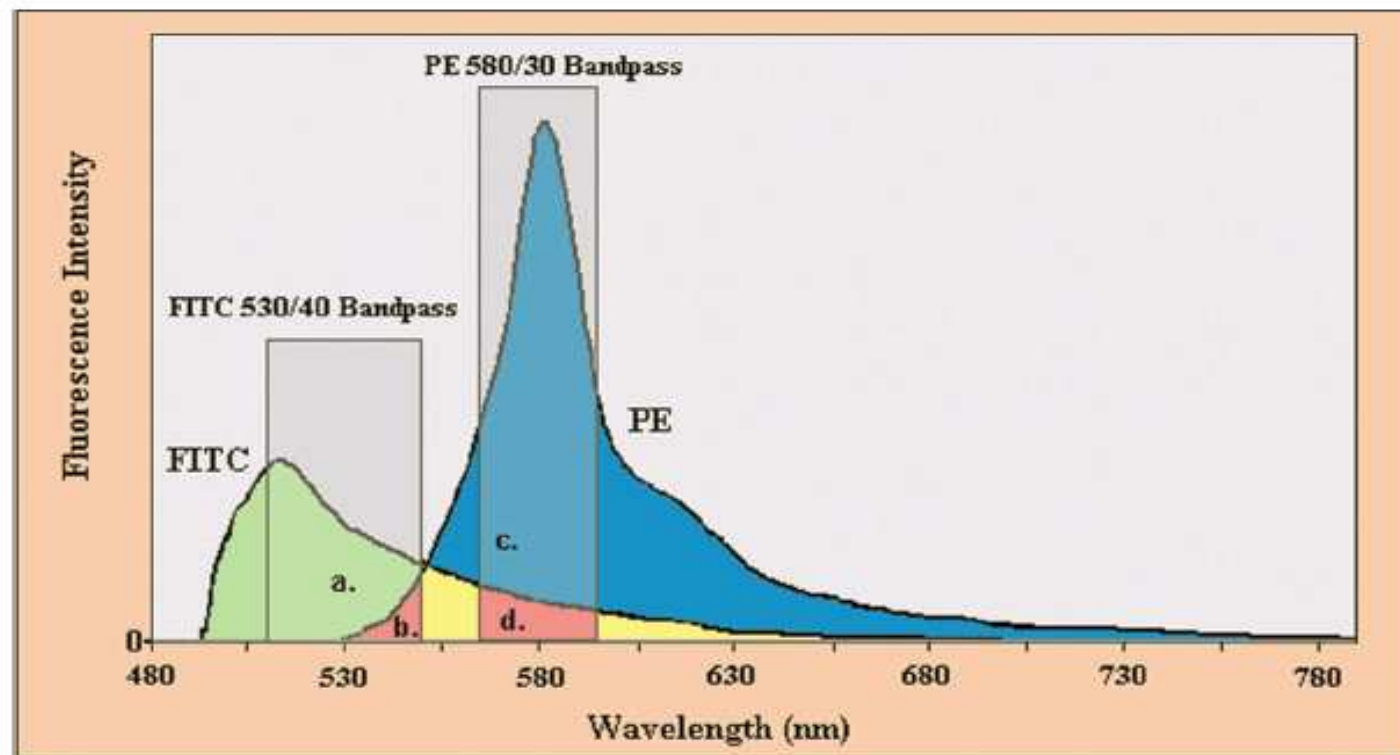
Oddělení cytokinetiky
Biofyzikální ústav AVČR, v.v.i.
Královopolská 135
612 65 Brno

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

Emission Spectra–Spectral Overlap

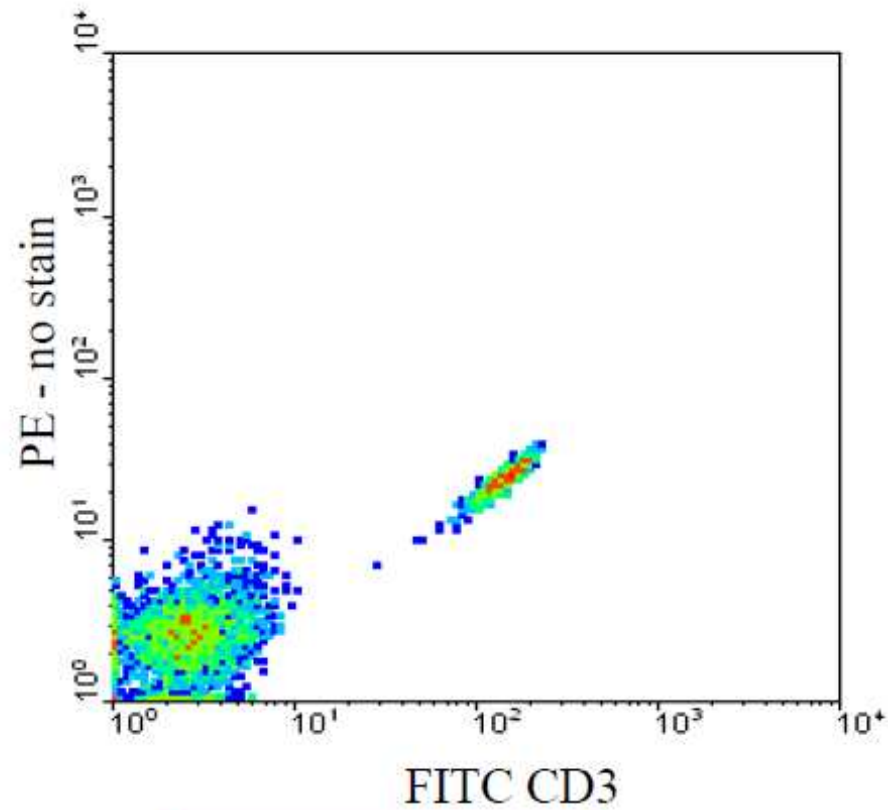


Co je problém při vícebarevné detekci?



Uncompensated FITC Single stain Control

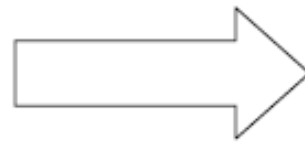
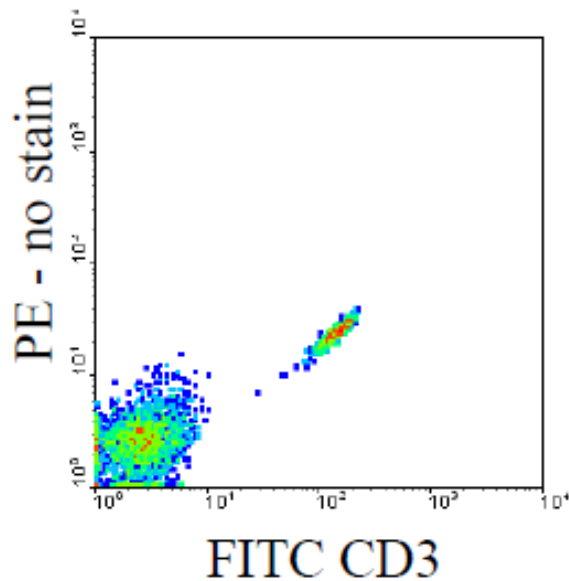
Unwanted
signal detected
in FL2
roughly 15%



Total signal
detected in FL1

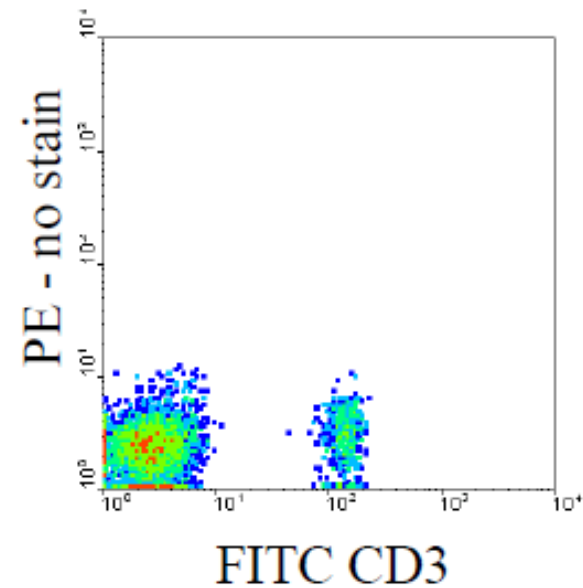
FITC Single Stain Control

Uncompensated

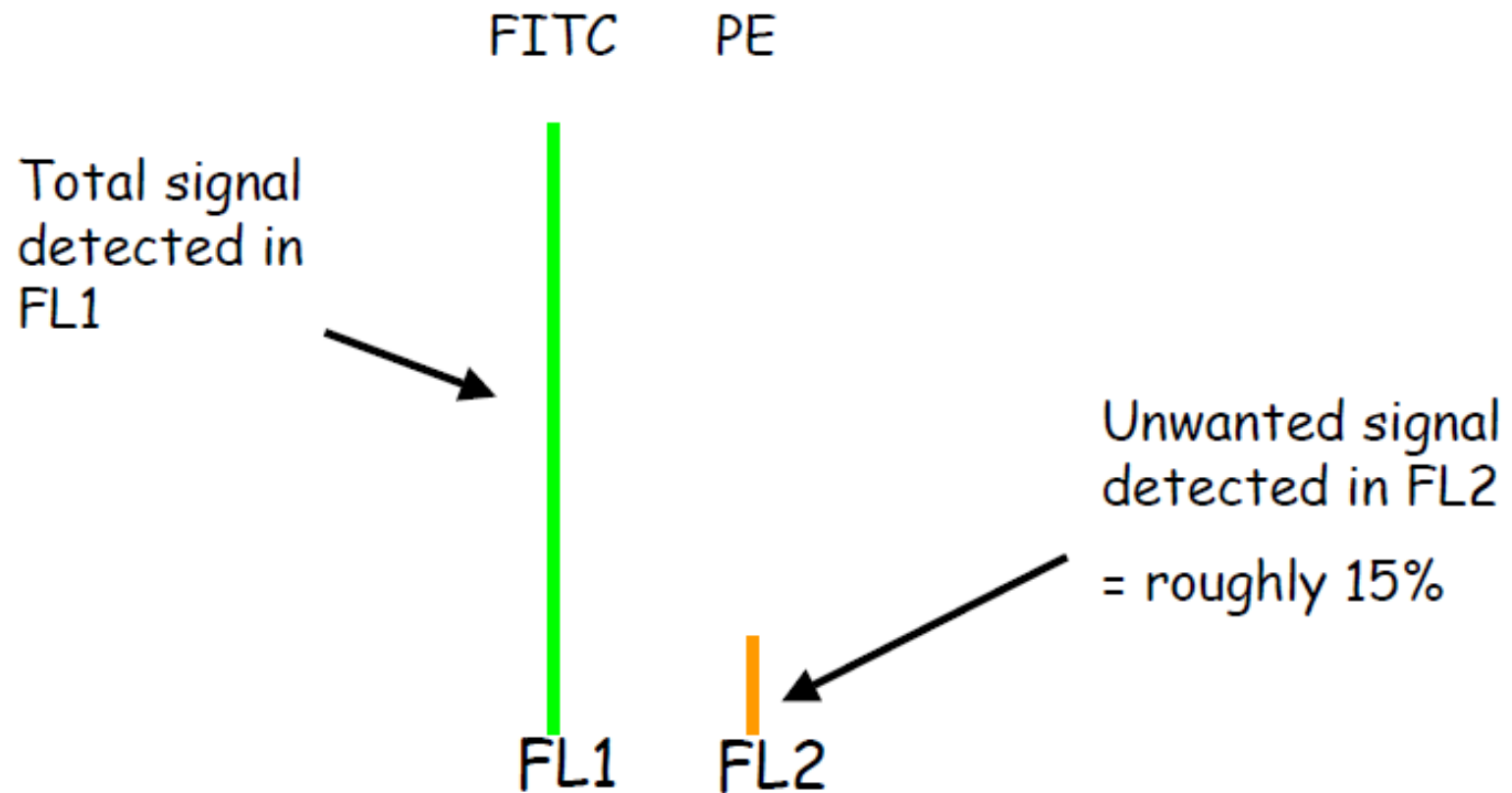


FL2-15%FL1

Compensated

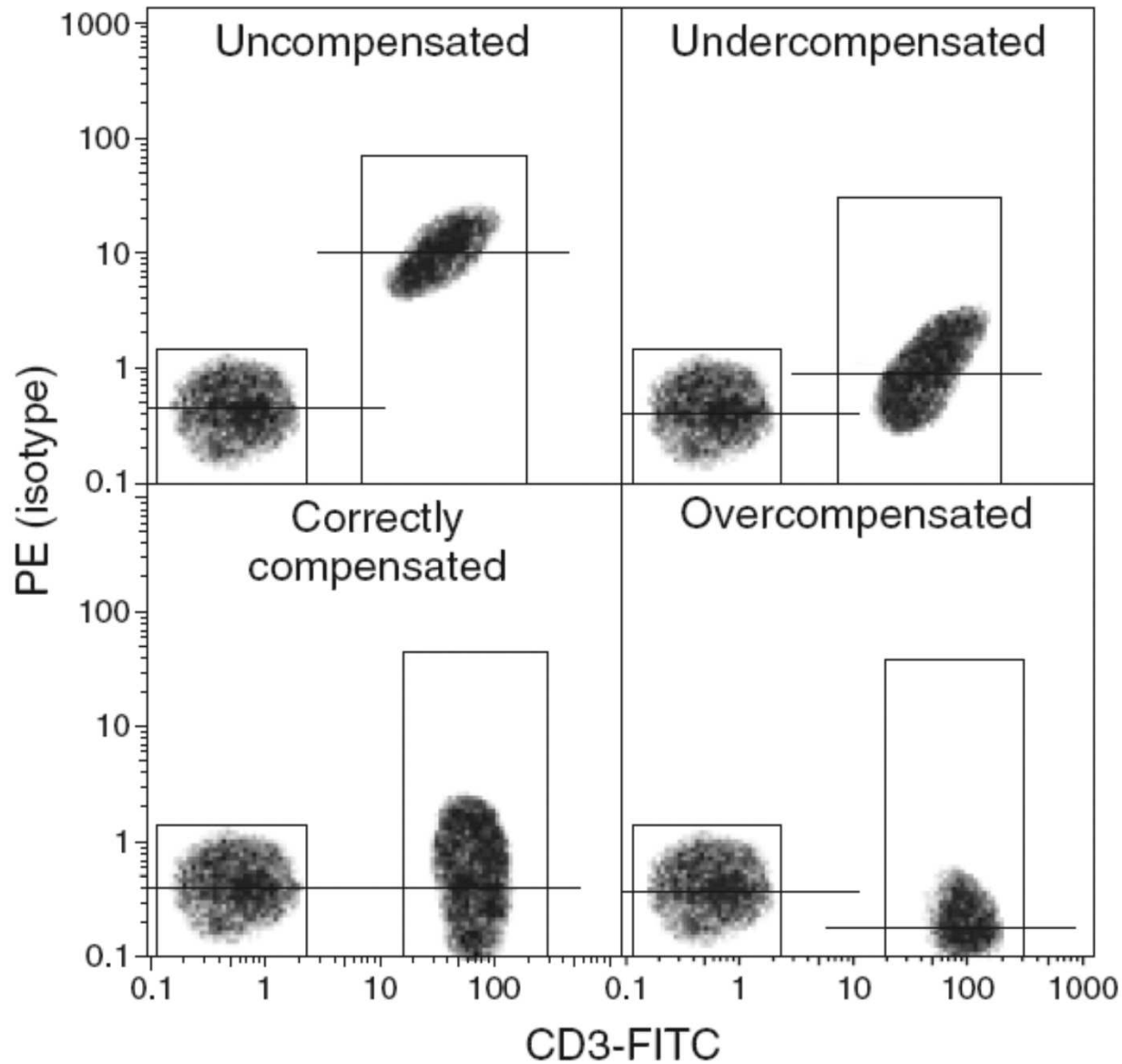


FITC Single Stain Control



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

Kompenzace fluorescenčního signálu





Factors that Effect Compensation

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

Wavelength (nm)	Filter	Excitation	Emission	Fluorochrome	Fluorochrome
488	Green	FTTC	Alexa Fluor® 488		
	Yellow	PE	PI		
	Orange	BD Horizon PE-CF504	PE-Texas Red®		
	Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
561	Infrared	PE-Cy7			
	Yellow	PE	PI		
	Orange	BD Horizon PE-CF504	PE-Texas Red®		
	Red	PE-Cy5			
640	Infrared	PE-Cy7			
	Red	APC	Alexa Fluor® 647		
	Far Red	Alexa Fluor® 700			
405	Infrared	BD APC-H7	APC-Cy7		
	Blue	Brilliant Violet™ 421	BD Horizon™ V450	VPD450	Pacific Blue™
	Green	BD Horizon V500	AmCyan		
375*	Orange	Brilliant Violet™ 605*			
	Blue	Hoehchst 33342			





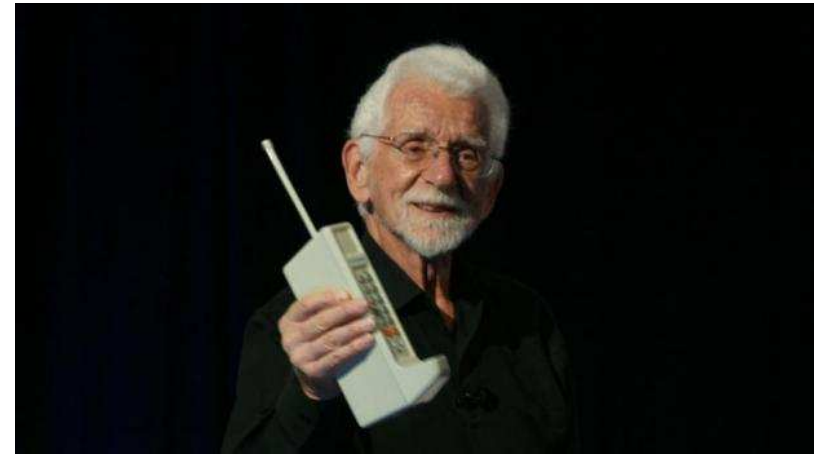
■ Řešení?
#1

Idea (1931)



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

Invention (1973)



Martin Cooper, Motorola

Innovation (2007)



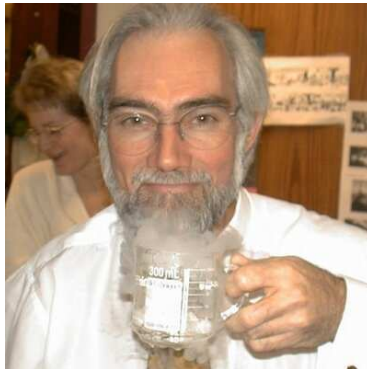
Steve Jobs, Apple

Spectral flow cytometry

J.P. Robinson, Purdue University

Cytometry Part A • 81A: 35–44, 2012

ORIGINAL ARTICLE



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



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

Gérald Grégori,^{1,2} Valery Patsekina,^{1,3} Bartek Rajwa,^{1,3} James Jones,⁴ Kathy Ragheb,^{1,3} Cheryl Holdman,^{1,3} J. Paul Robinson^{1,3,4*}

2
DOI: 10.1017/S1431927605510328

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

Multispectral Flow Cytometry: Next Generation Tools For Automated Classification

J. Paul Robinson^{a,b}, Valery Patsekina^a, Gerald Gregori^a, Bartek Rajwa^{a,b}, and James Jones^{a,b}

^aDepartment of Basic Medical Science, School of Veterinary Medicine, and ^bWeldon 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) **MULTI-SPECTRAL DETECTOR AND ANALYSIS SYSTEM**
5,394,237 A 2/1995 Chang et al. 188/79-51
5,422,712 A 6/1995 Ogino 356/73
5,675,517 A 10/1997 Stokdijk 702/85
(75) Inventors: Joseph Paul Robinson, West Lafayette, IN (US); Bartłomiej Rajwa, West Lafayette, IN (US); Gérald Grégori, Marseille (FR); Valery Patsekina, West Lafayette, IN (US)
5,719,667 A * 2/1998 Miers 356/73
6,249,341 B1 * 6/2001 Basiji et al. 356/73
6,630,307 B2 * 10/2003 Bruchez et al. 435/6
6,885,440 B2 * 4/2005 Silcoit 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)

FOREIGN PATENT DOCUMENTS

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

EP 0 315 939 5/1989

(Continued)

Spectral flow cytometry

SONY Sony Biotechnology Inc.

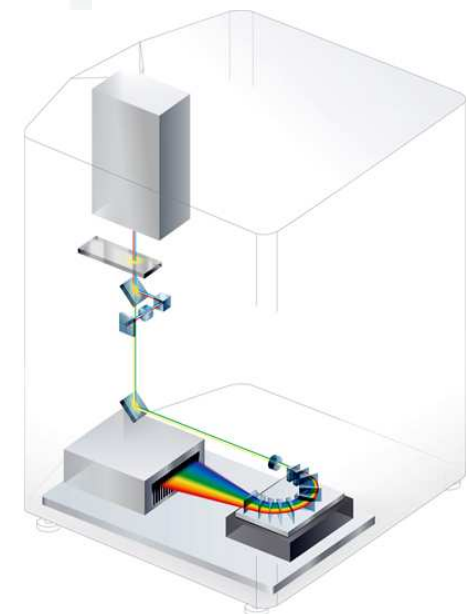
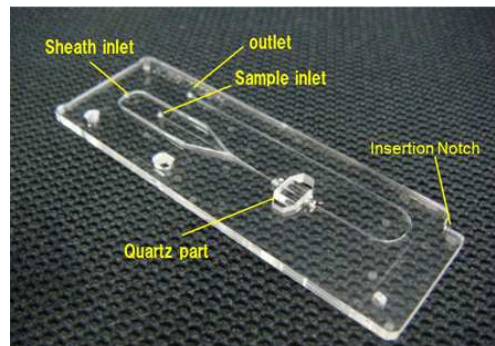
Products ▸ Resources ▸ Support ▸ About Us ▸ Contact Us ▸ Order Info ▸ Site Q

Overview **Features** Applications Specifications Literature

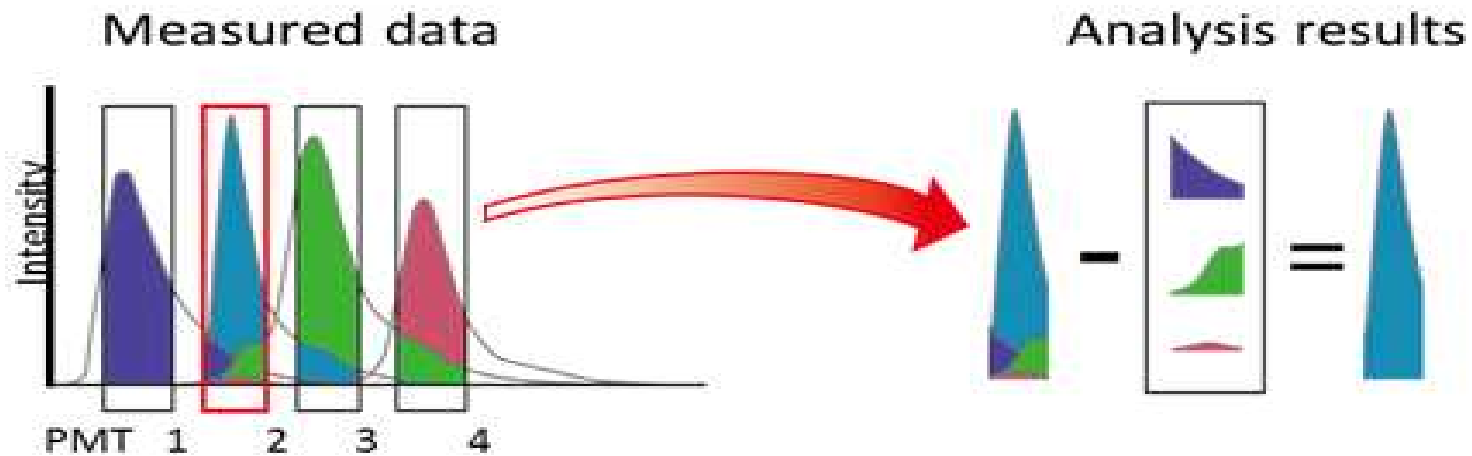
See Everything

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.

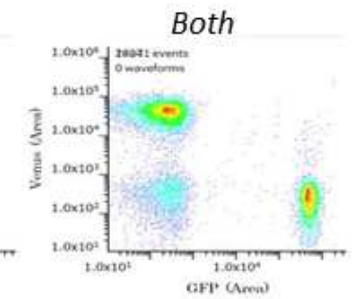
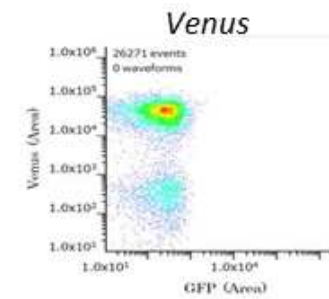
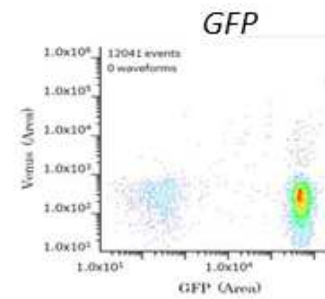
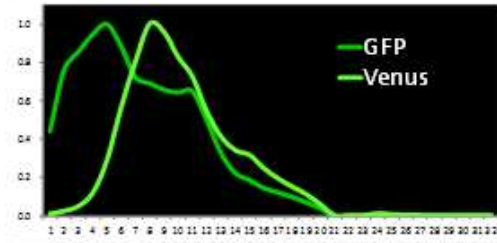
The SP6800 Spectral Analyzer also introduces new Flow Point technology to analyze core stream and sample event location within the flow cell. To improve accuracy of data, this system also provides unique functions to display and analyze cellular autofluorescence and allows the user to easily automatically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



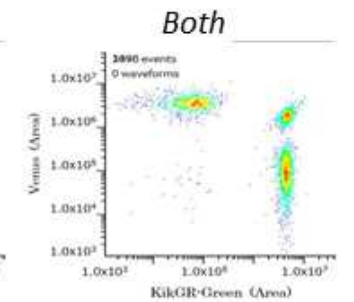
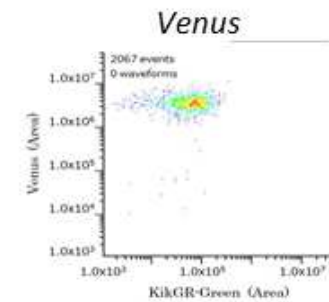
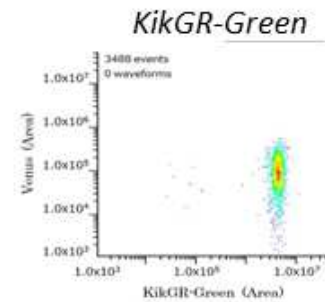
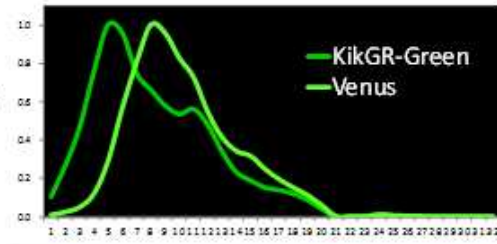
Conventional vs. spectral analysis



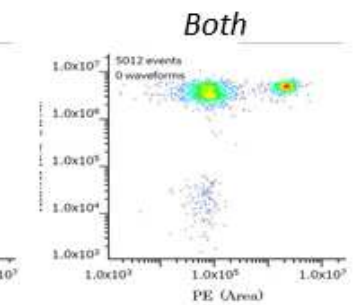
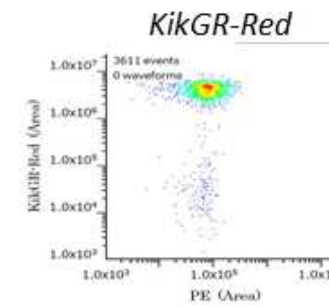
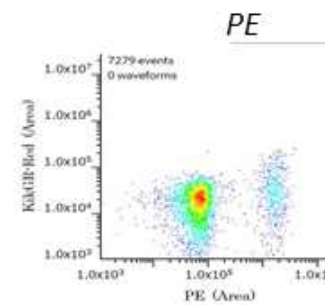
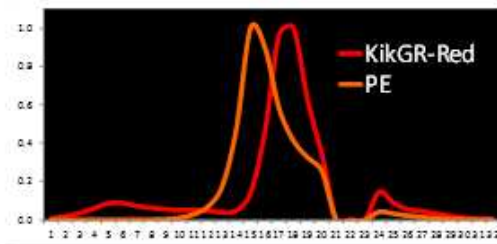
GFP
Venus



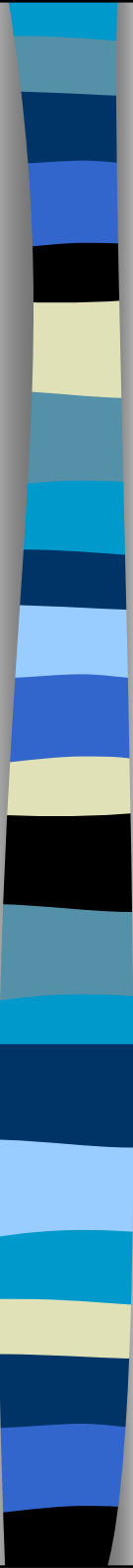
KikGR-Green
Venus



KikGR-Red
PE

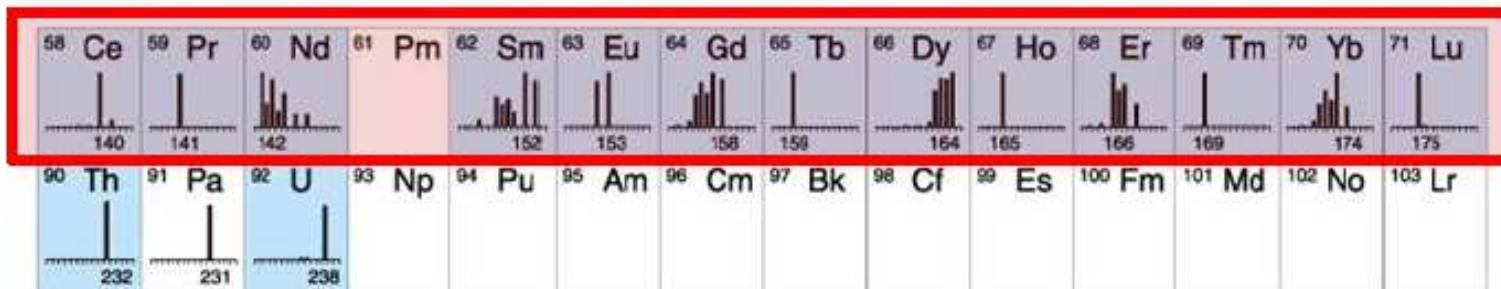
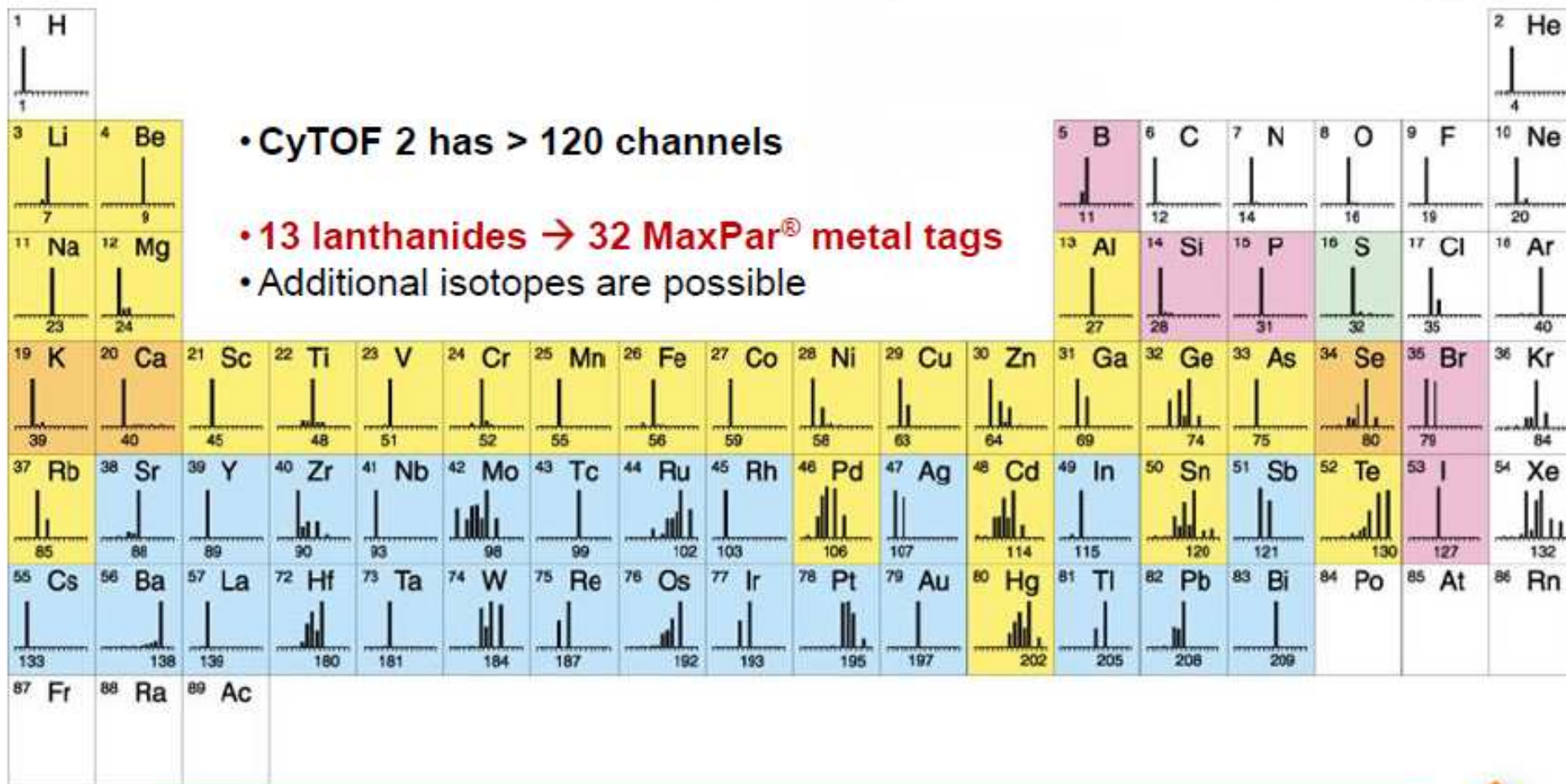


For revealing spatiotemporal regulation of immune cells, fluorescent proteins are very useful, which can be difficult to analyze with traditional flow cytometry technologies. These figures show how easily the SP6800 Spectral Analyzer can separate overlapping spectra of fluorescent proteins and fluorochromes.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

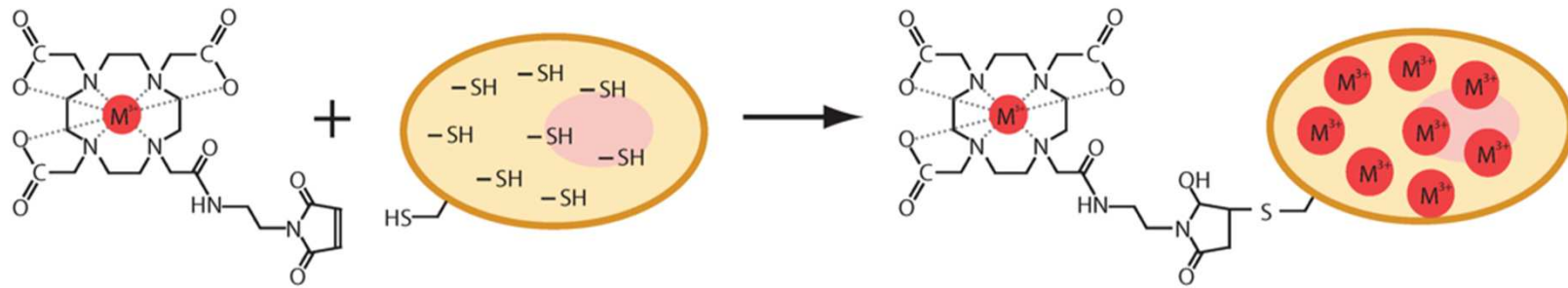


■ Řešení?
#2

Probing with Isotopes



Single Cell Mass Cytometry



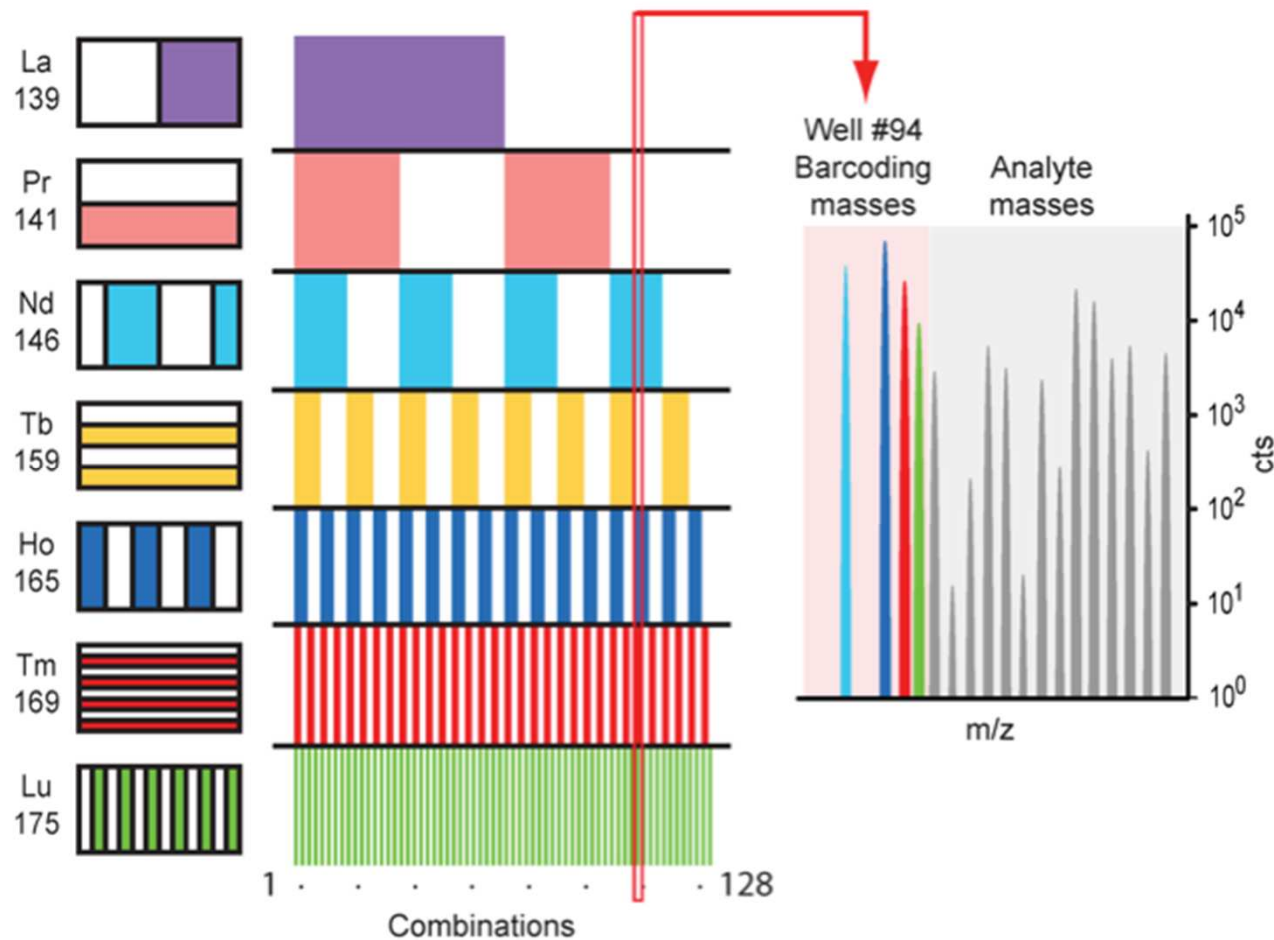
Cells were covalently labeled with a bifunctional compound, maleimido-mono-amide-DOTA (mDOTA). This compound can be loaded with a lanthanide(III) isotope ion, and reacts covalently with cellular thiol groups through the maleimide moiety.

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



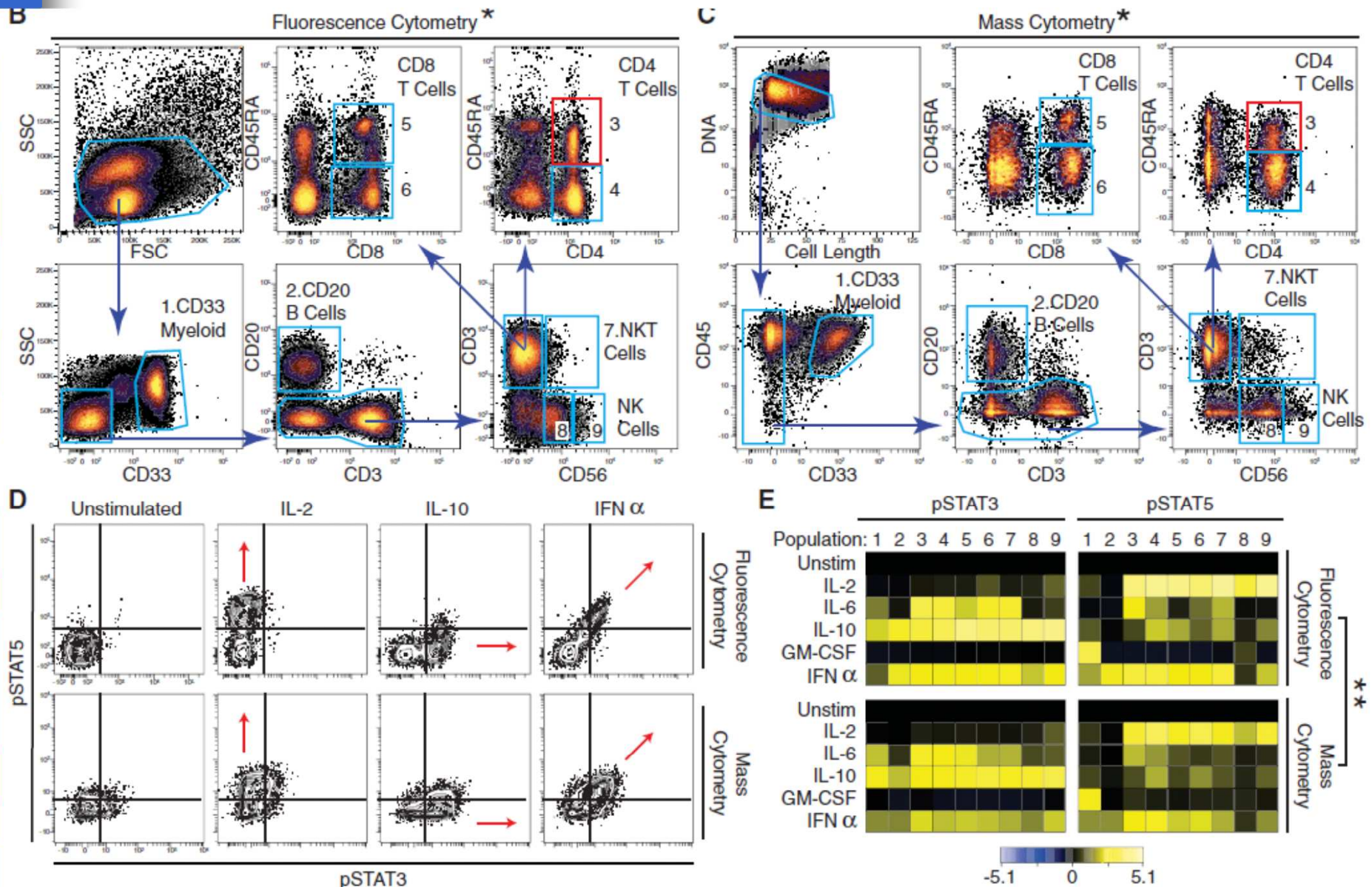
Seven unique lanthanide isotopes were used to generate 128 combinations, enough to barcode each sample in a 96-well plate. The seven lanthanide isotopes, their masses and their locations on the 96-well plate are shown.

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

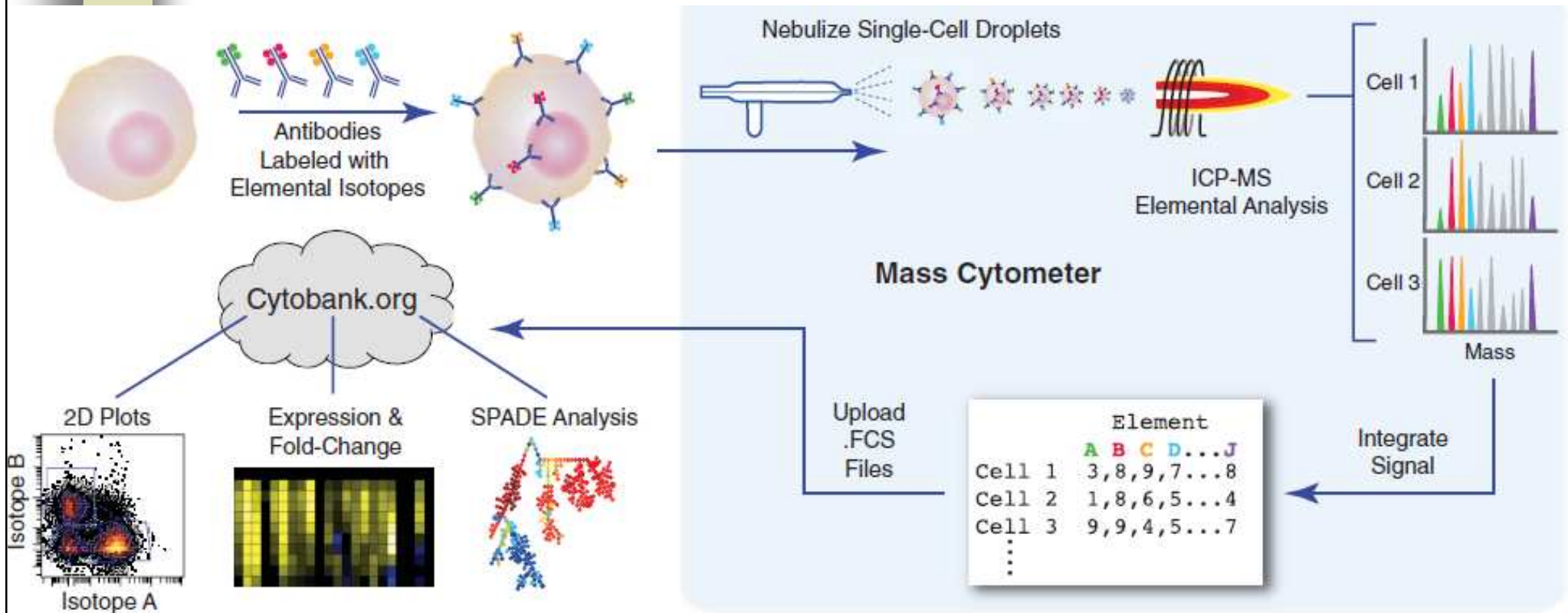


Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum

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

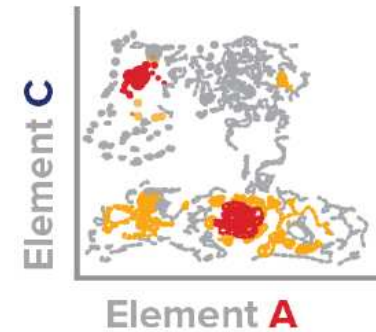
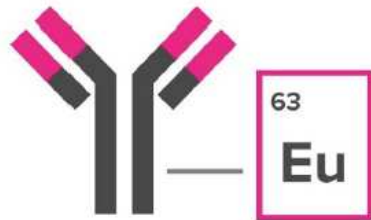
Sean C. Bendall, *et al.*

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

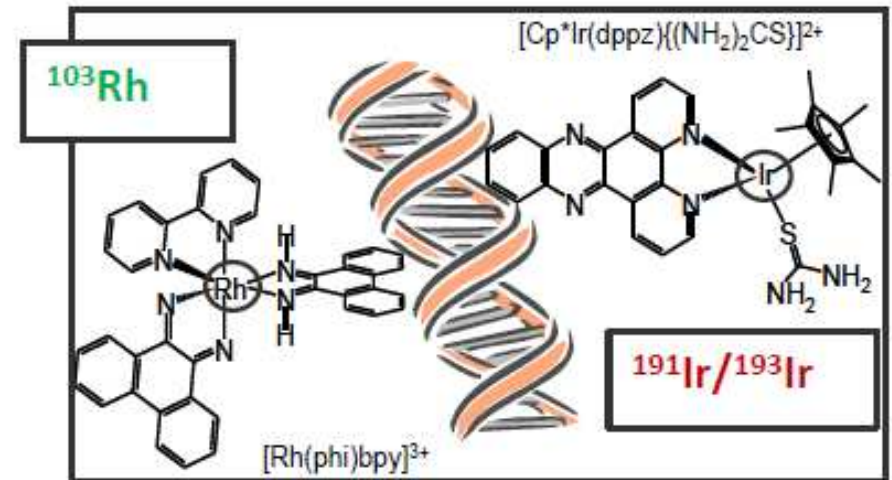
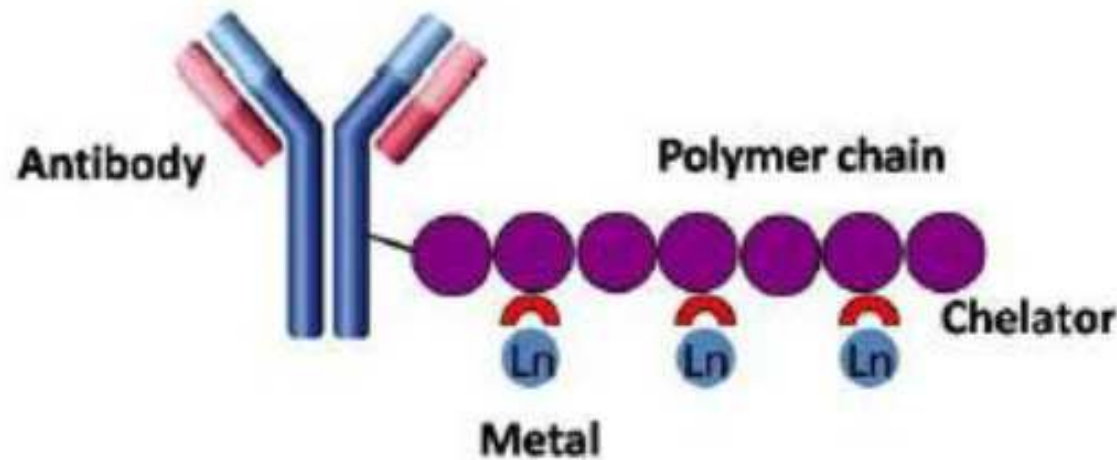
Mass Cytometry: 50+ Parameters on Millions of Cells

Discovery of new biology
Comprehensive functional profiling

Basic research
Drug discovery
Clinical research



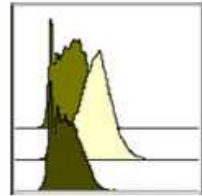
MaxPar[®] metal-tagged probes



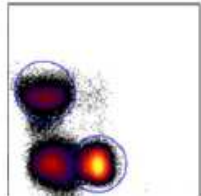
- Lanthanide tags: 32 isotopes from 13 elements
- IgG antibody probes:
 - Pre-conjugated antibodies (220 currently available and growing)
 - MAXPAR[®] labeling kits (for 32 stable isotopes)
- Nucleic acid-binding metallo-intercalators
 - Identifies single cell events
 - Live/dead indicator

Analyze: Cytobank

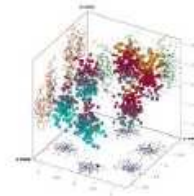
Plot raw data



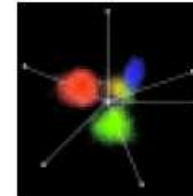
Histogram



Biaxial plot

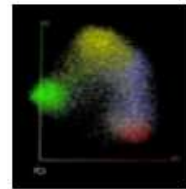


3D plot

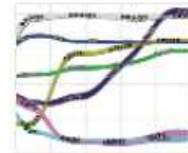


Radar

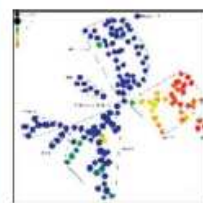
Reduce dimensionality



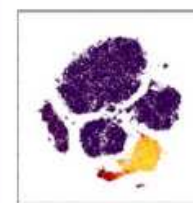
PCA



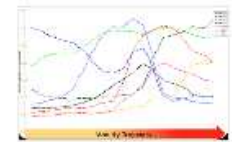
Gemstone



SPADE

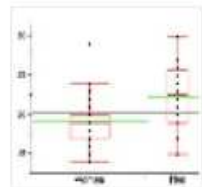


viSNE

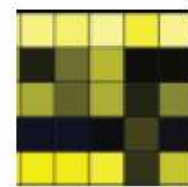


Wanderlust

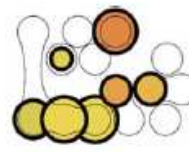
Summarize statistics



Box plot



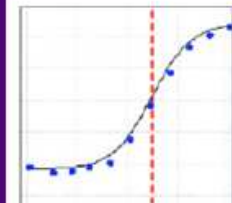
Heat map



Network



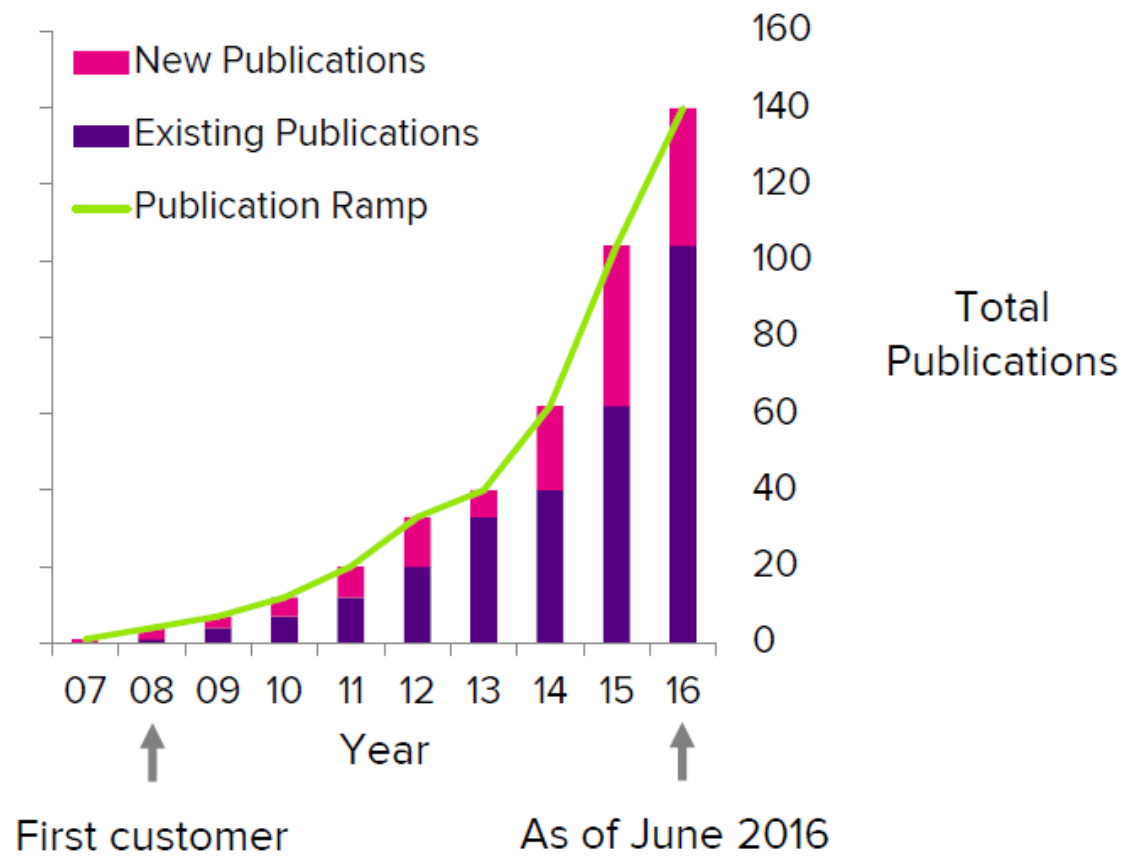
Sunburst



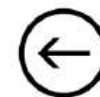
Dose curve



FLUIDIGM®



FLUIDIGM®



Analyze: Cytobank

Analysis toolkit designed for mass cytometry

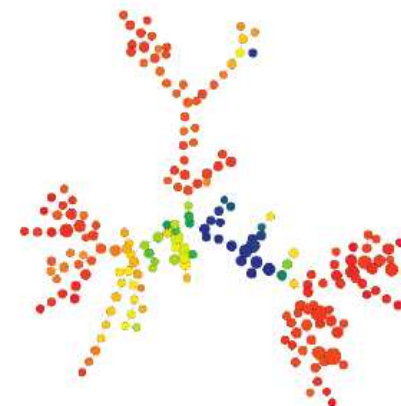
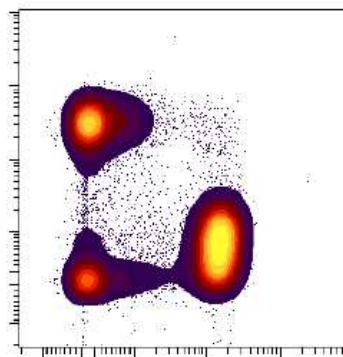
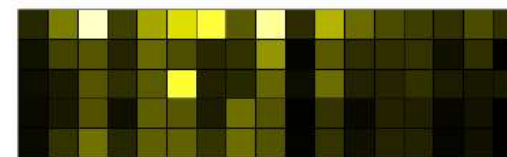
Cloud-based—accessible from anywhere

Data storage and backup included

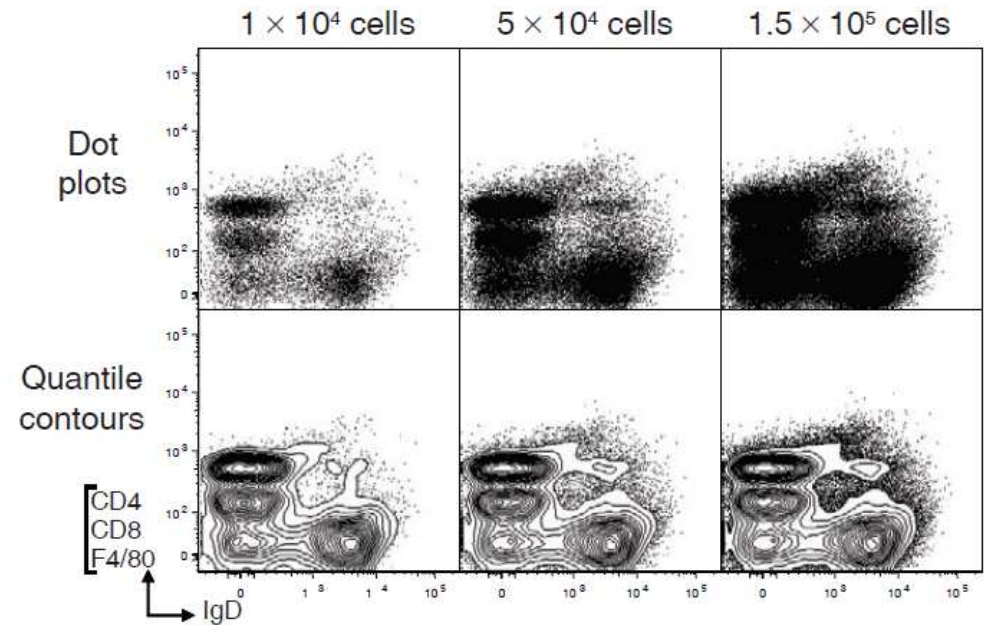
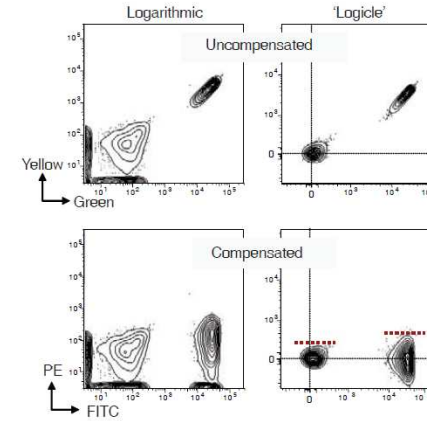
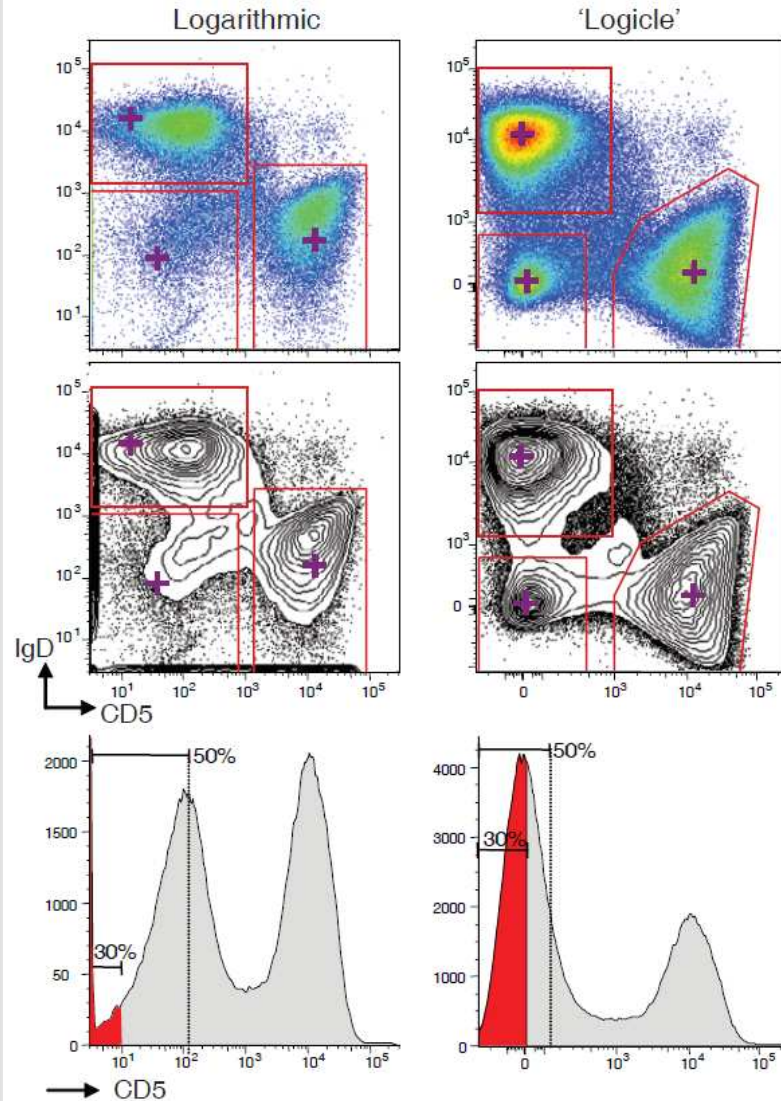
Demo datasets and tutorials

Strong scientific support

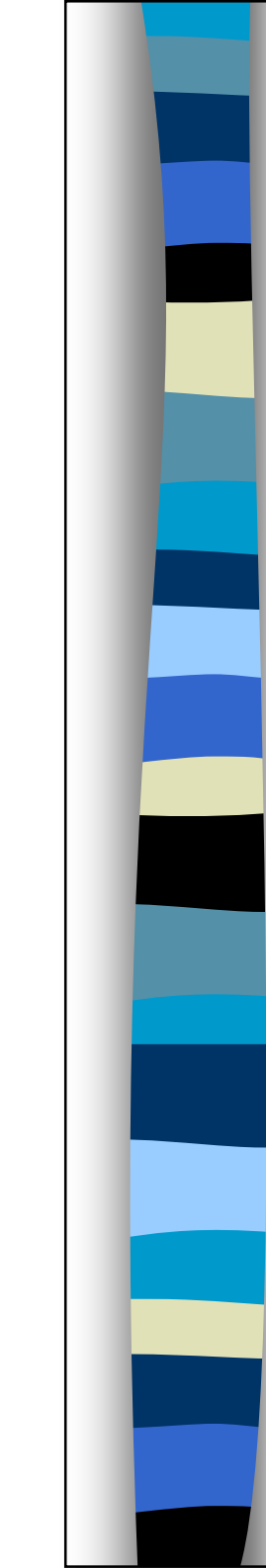
fluidigm.cytobank.org



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 PRESENTATION⁴

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 (y 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.

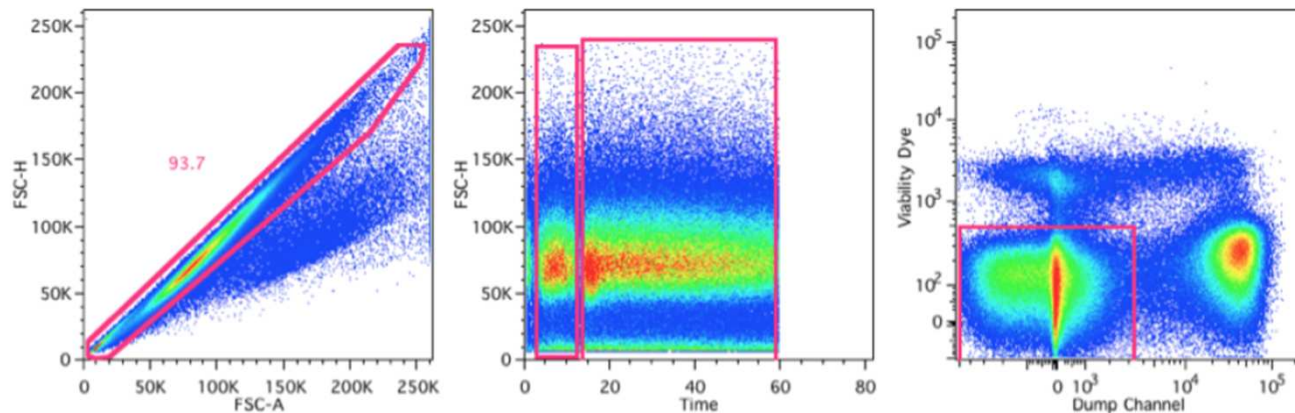
Gating: Display the gates used at each step in the gating sequence when gates are set manually (subjective gating). Show data for control samples when these are used to set gates. If necessary, present this information in supplementary figures. When an algorithm is used to set gates, define it explicitly 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.

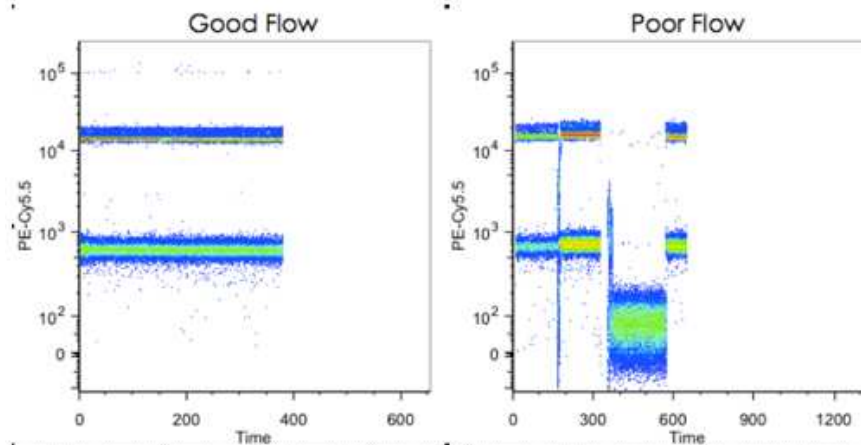
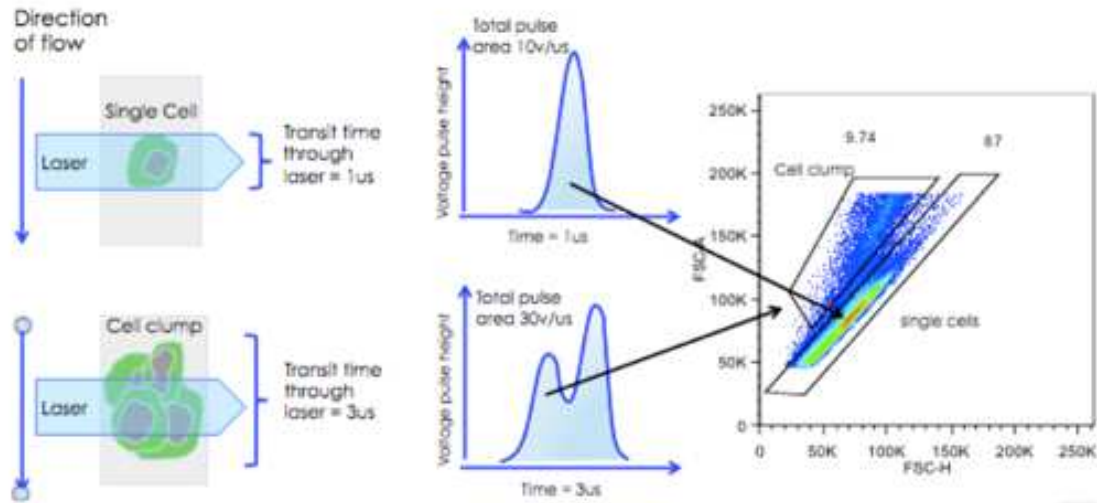
Gating a kontrola kvality

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

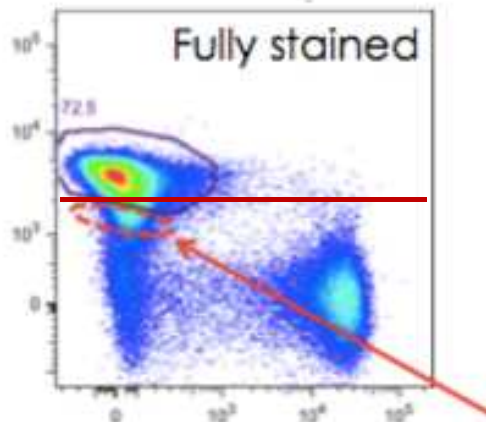


<http://expertcytometry.com/3-flow-cytometry-gates-that-will-improve-the-accuracy-of-your-facs-data-analysis/>

Gating a kontrola kvality

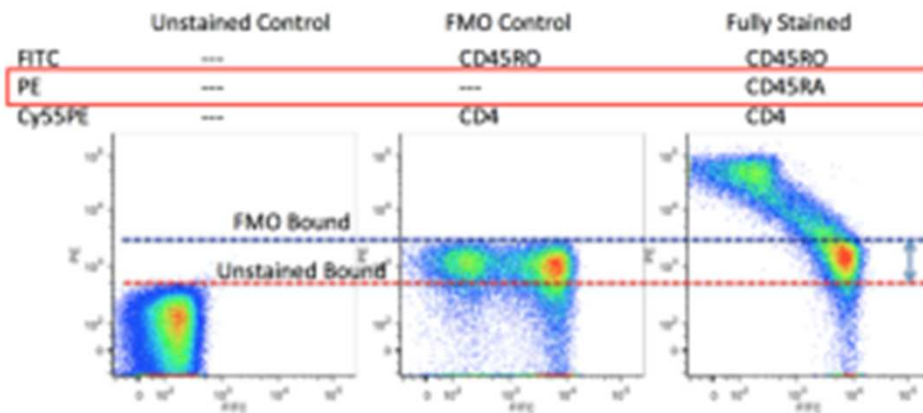


Gating a kontrola nastavení



Cells in spread of fluorescence

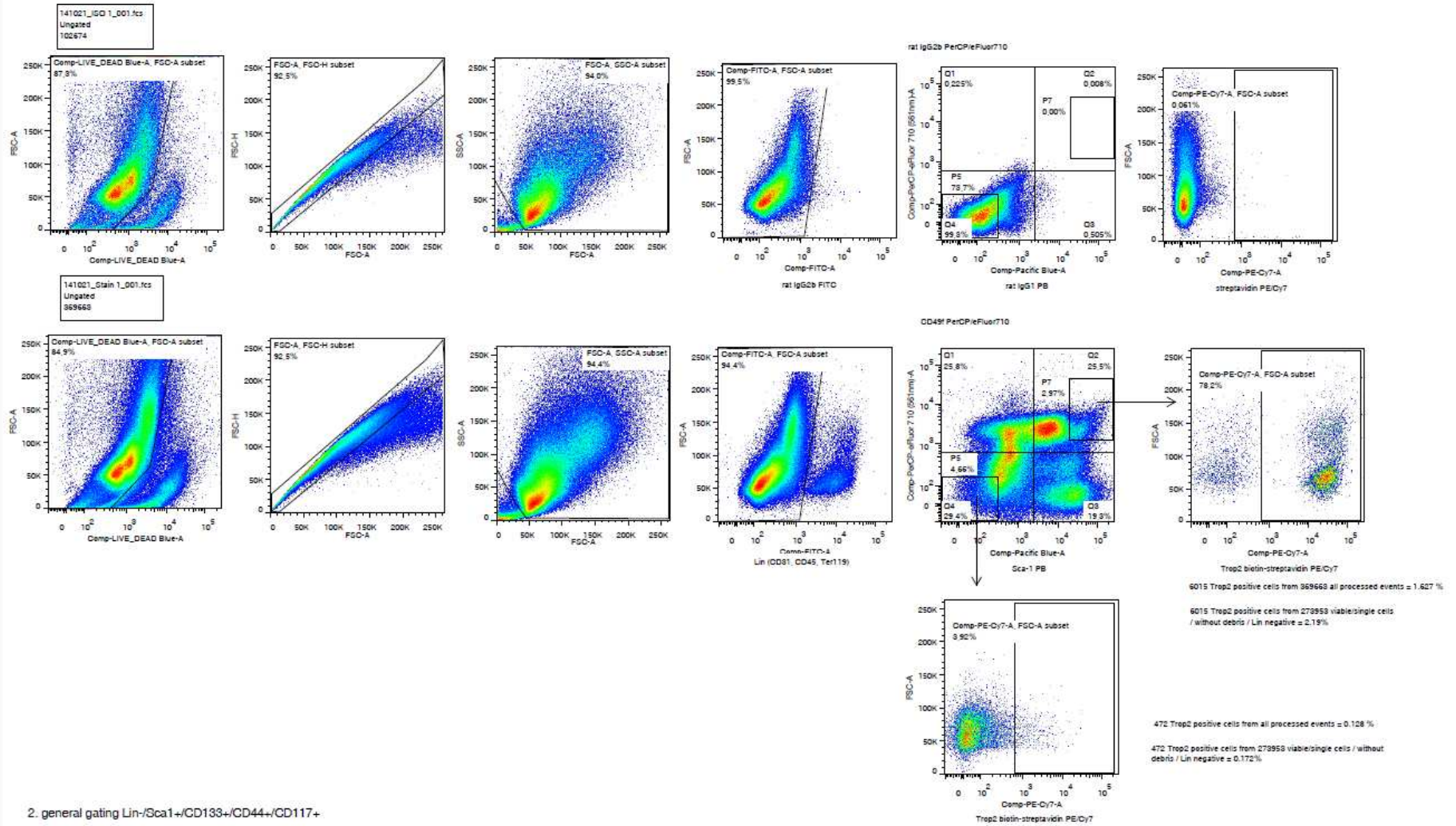
FMO ~ Fluorescence Mines One



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+



Vitální analýza buněčných funkcí

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

Detekce viability

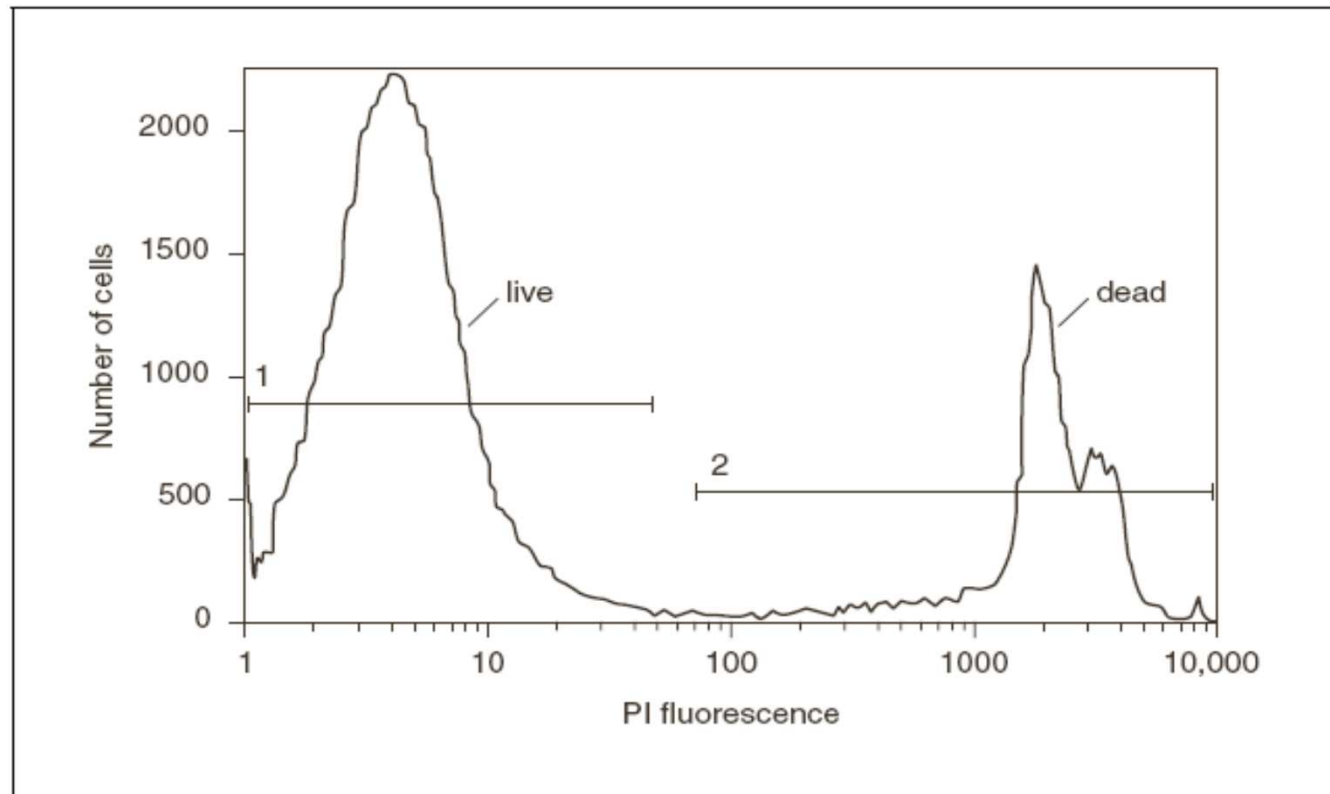
- jedna z nejjednodušších analýz
- funguje na principu:
 - detekce membránové integrity - neprůchodnosti některých fluorescenčních značek cytoplazmatickou membránou živých buněk – **propidium iodide, ethidium bromide, 7-amino actinomycin D**
 - detekce fyziologického stavu buněk – použití fluorescenčních 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

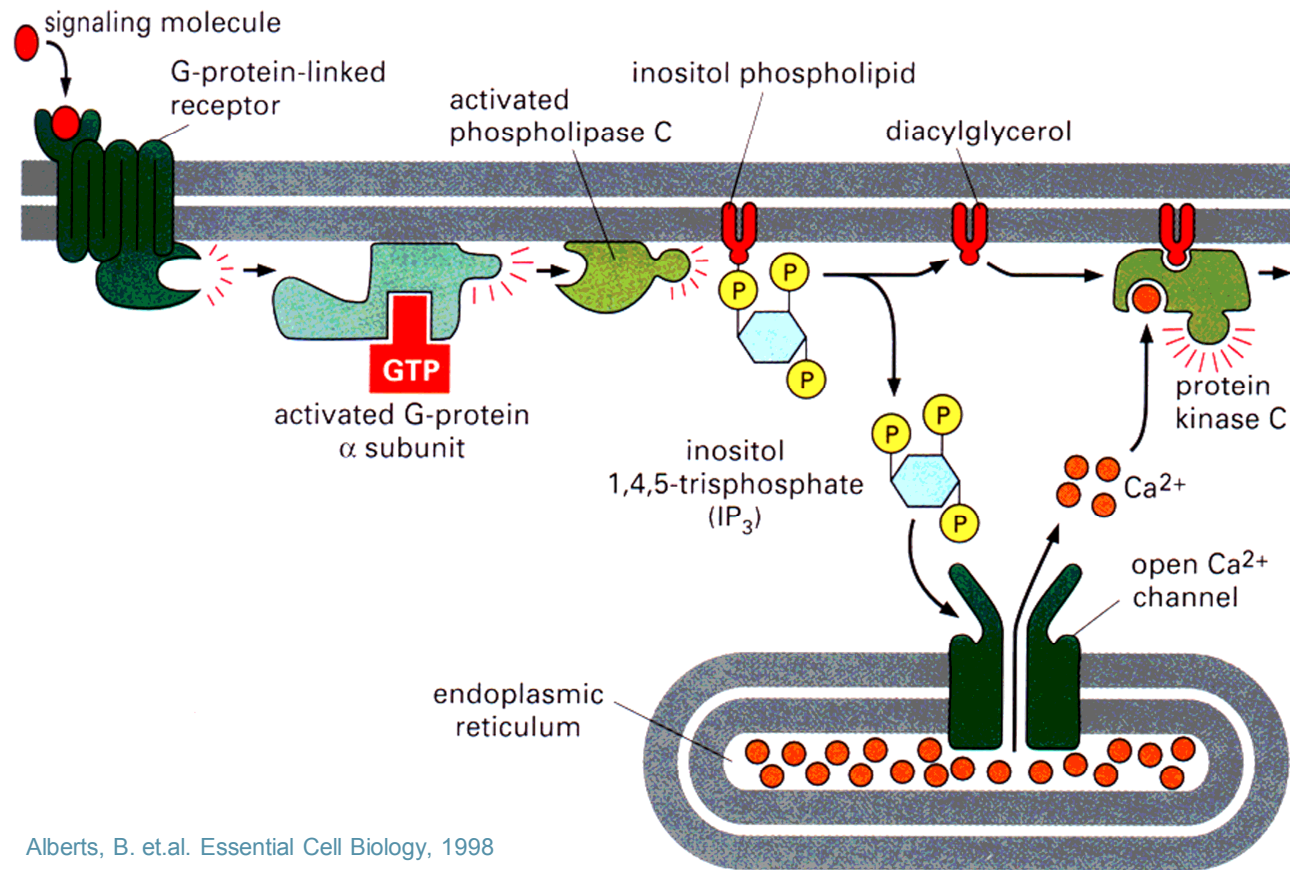
*Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm.

Detekce viability



Přenos signálu pomocí Ca^{2+}

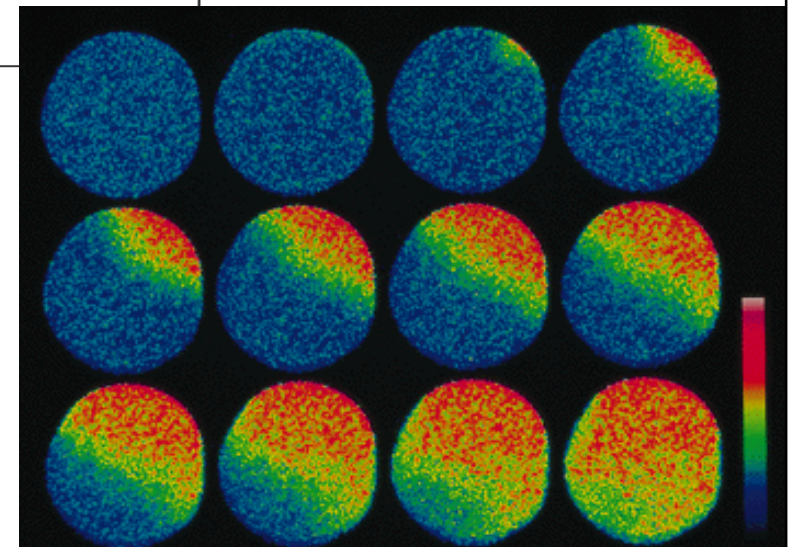
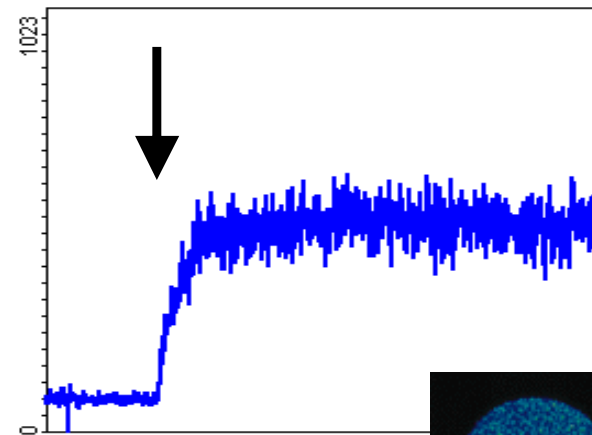
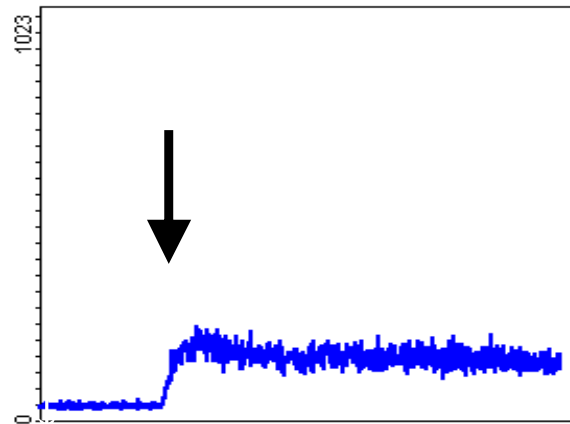
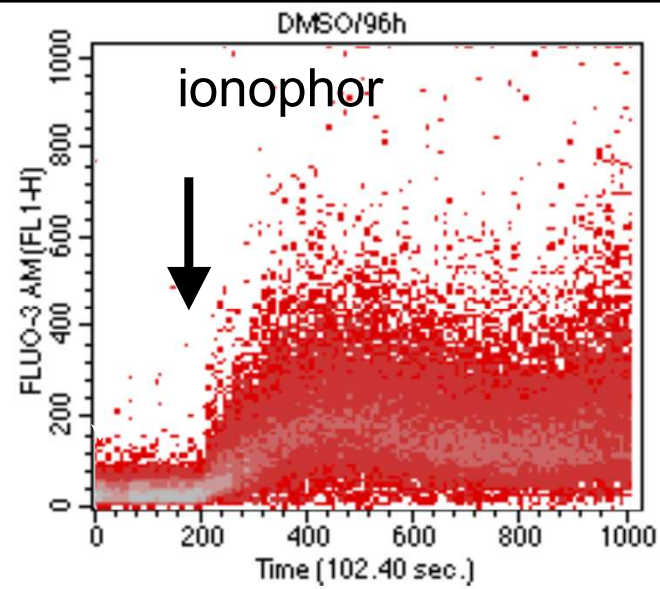
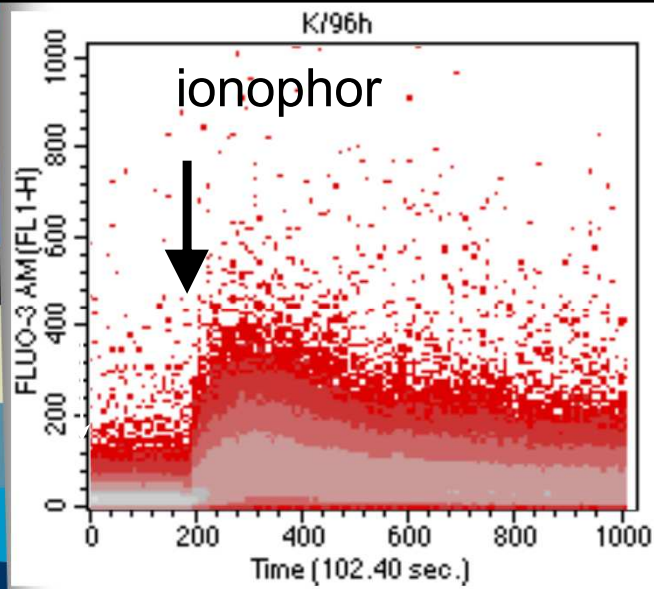
- **Cytosol** (koncentrace - „klidová“ 100 nM vs. 1-10 μM aktivovaná)
- $[\text{Ca}^{2+}]_c$ aktivuje proteinkinázu C
- interaguje s „ Ca^{2+} - binding proteins“



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

Ca²⁺ influx

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

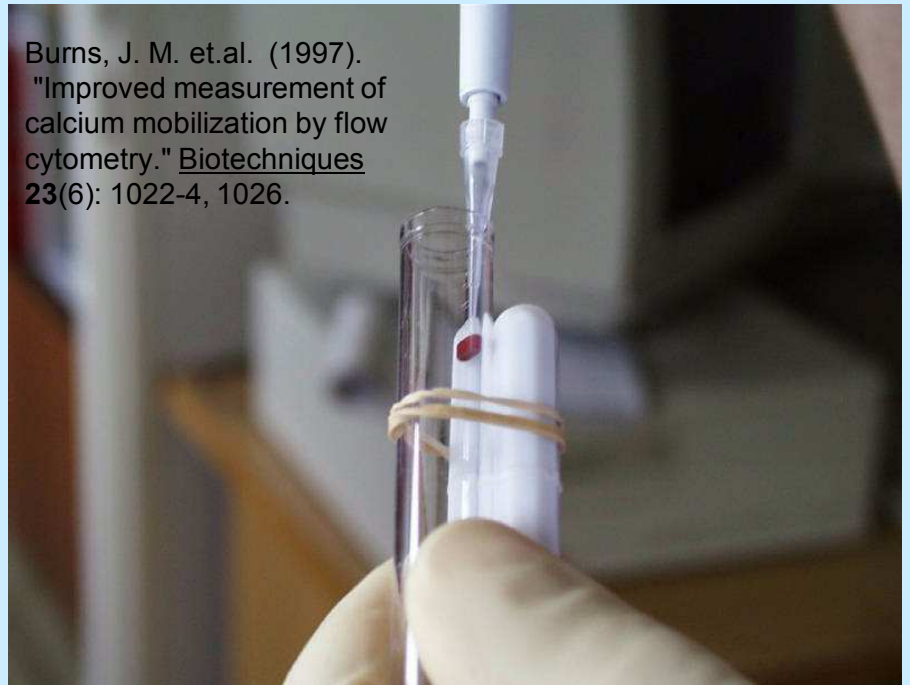




Zajištění vhodných podmínek pro detekci $[Ca^{2+}]_i$

- 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>



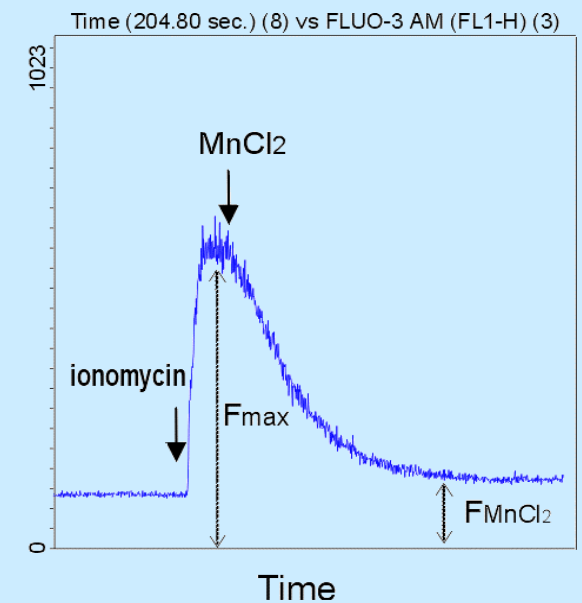
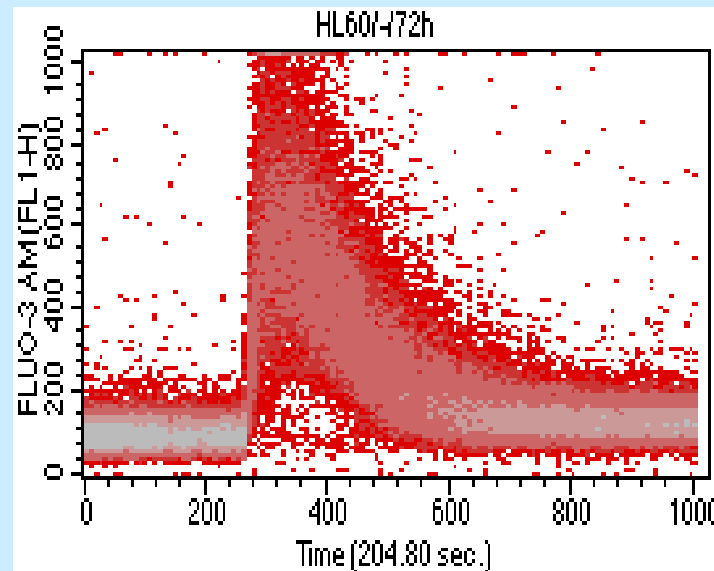
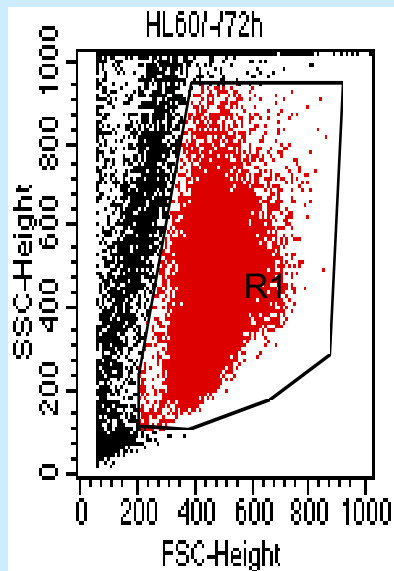
Burns, J. M. et.al. (1997).
"Improved measurement of calcium mobilization by flow cytometry." Biotechniques 23(6): 1022-4, 1026.



Kalibrace

(pro jednu vlnovou délku)

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

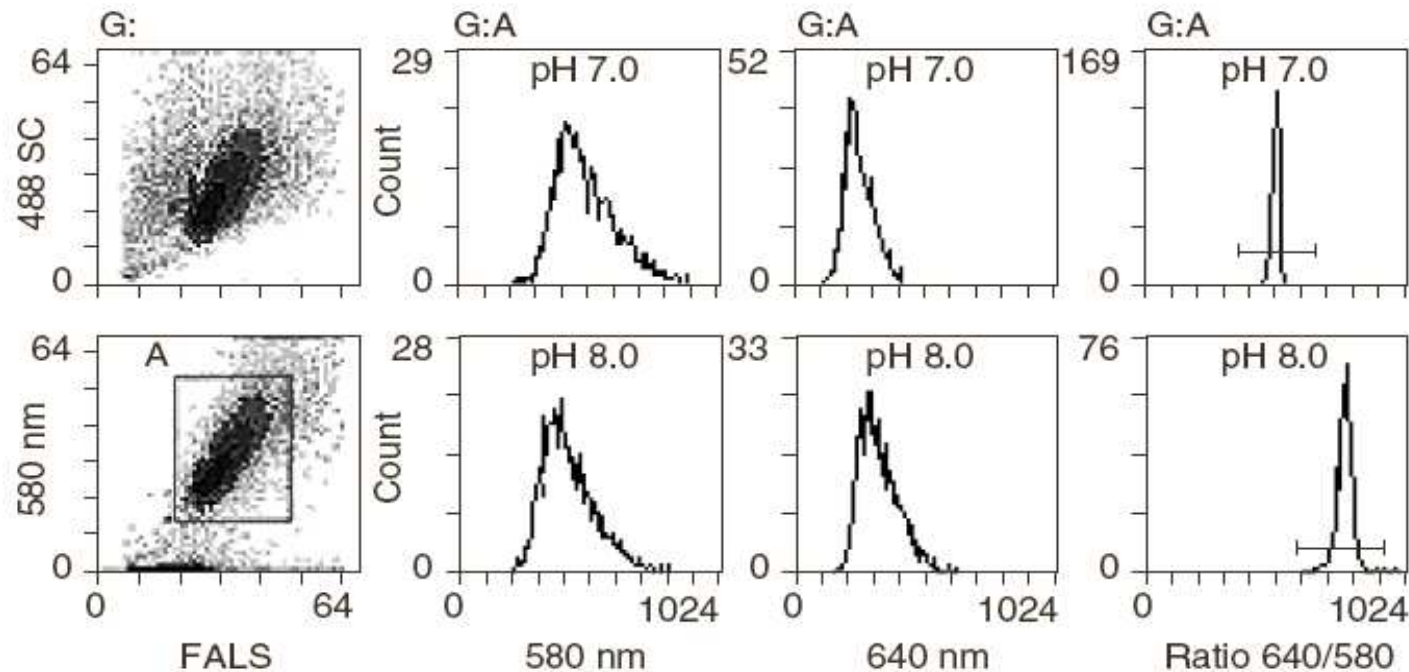


Fluo-3 (K_d ~ 400nM, 22°C; 864 nM, 37°C)

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

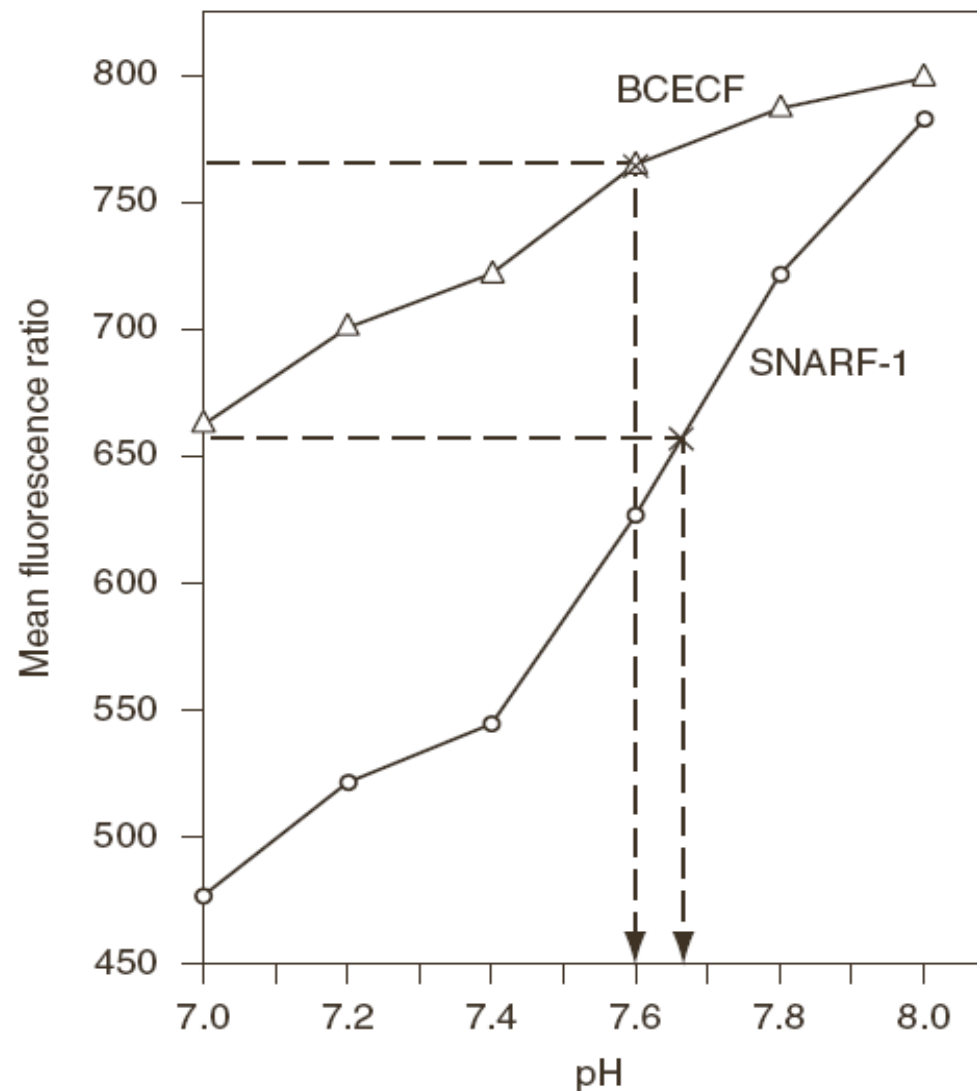
Detekce intracelulárního pH

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



Detekce intracelulárního pH

- Nutná kalibrace pomocí draslíkových pufrů a ionoforu (nigericin)

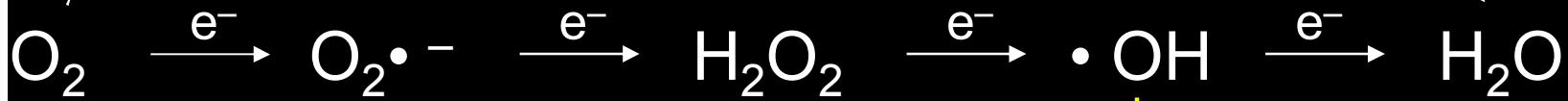




Detekce reaktivních kyslíkových skupin

- **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



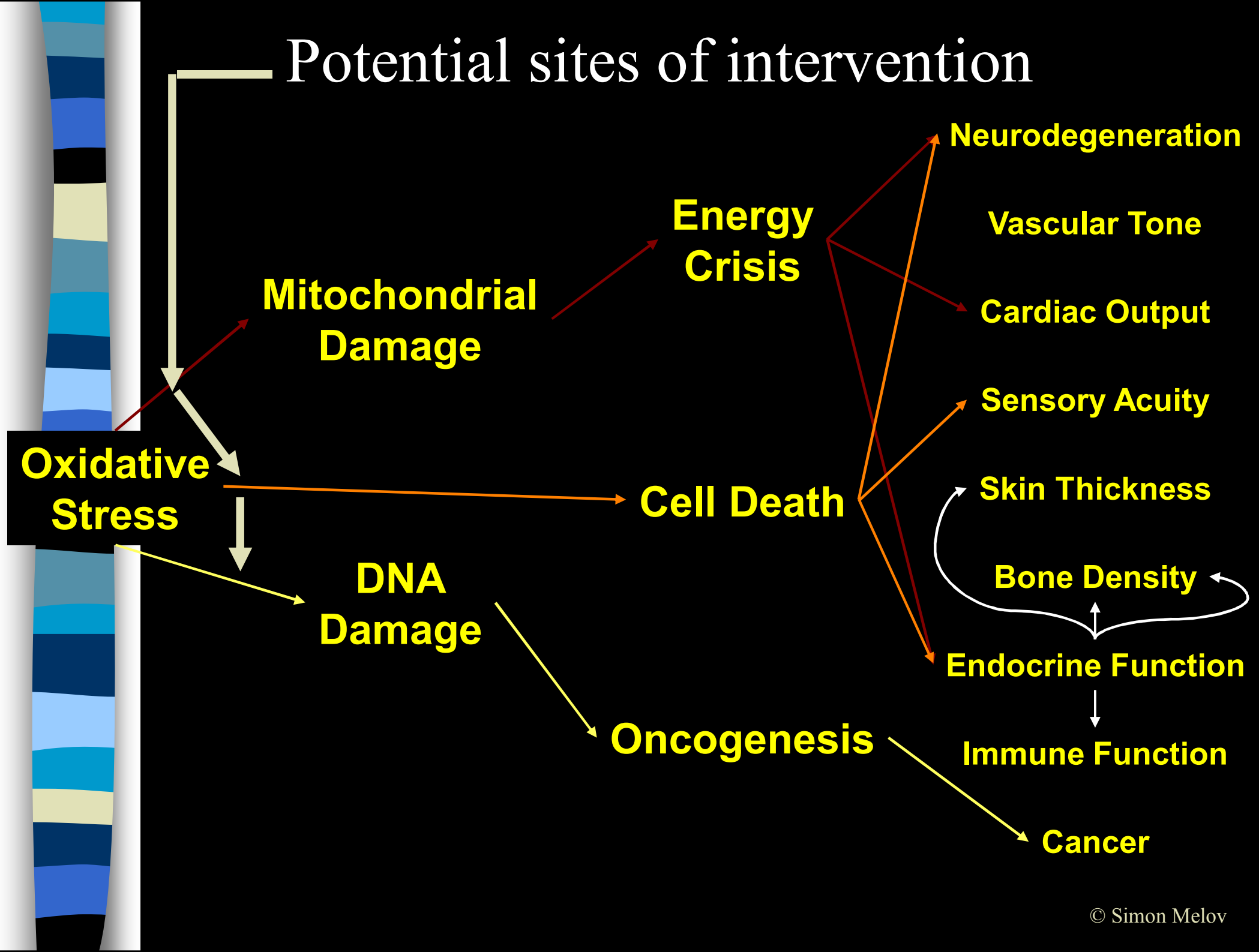
Unreactive at STP, but a *great* electron acceptor
Biological activation via radicals, transition metals
Generally, radical intermediates are enzyme-bound

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

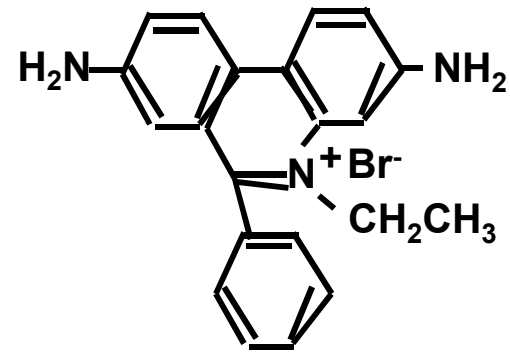
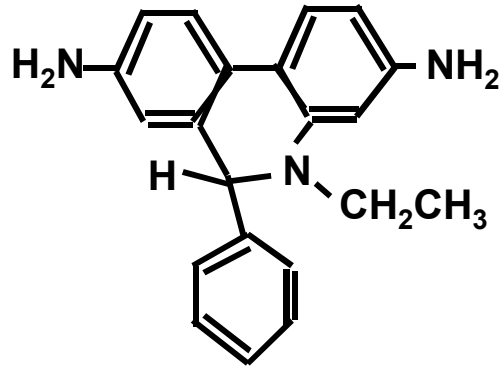
Not so terribly reactive with most biomolecules
Maintained at very low concentration
Catalases, peroxidases, GSH, etc...

Potential sites of intervention



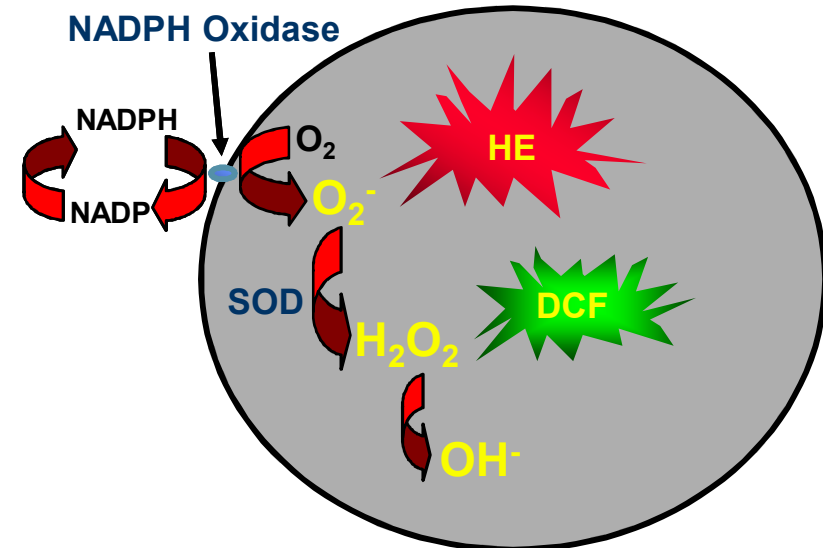
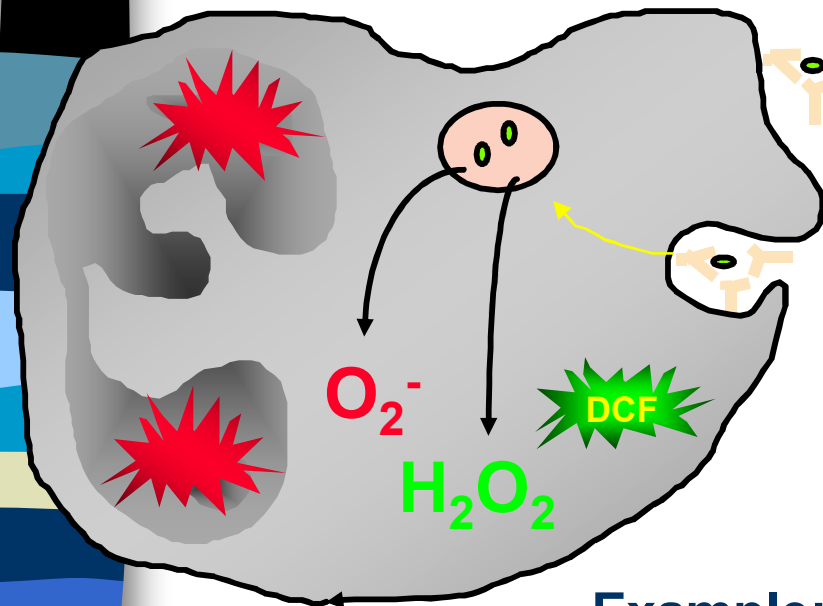
Hydroethidine

HE

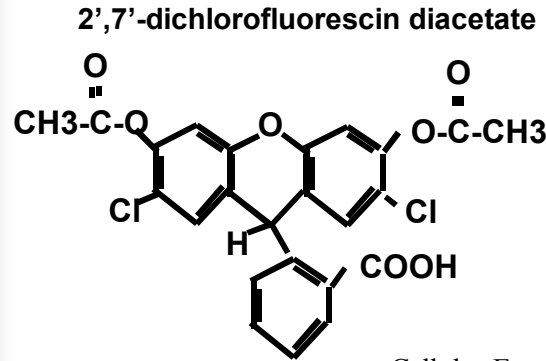


EB

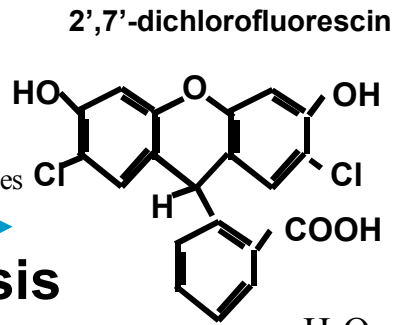
Phagocytic Vacuole



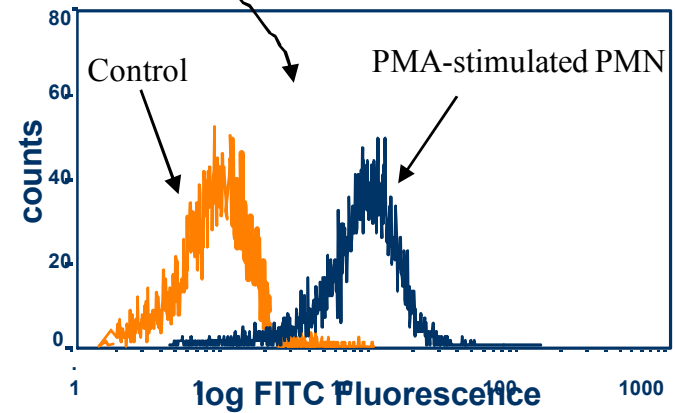
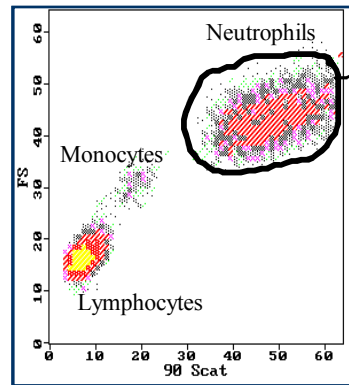
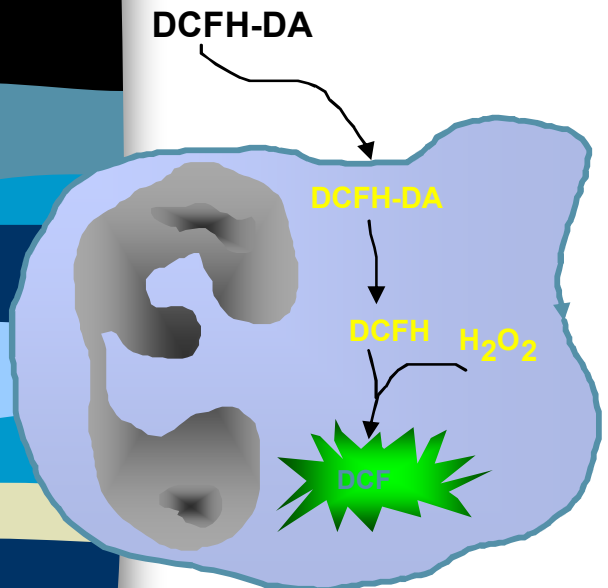
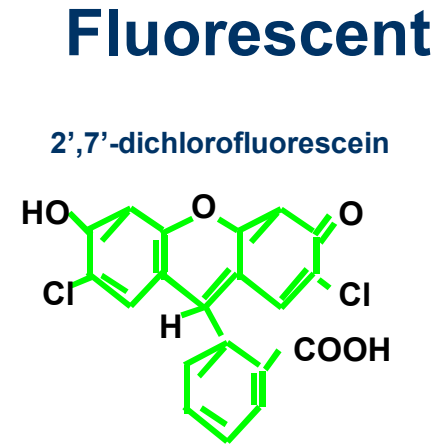
Example: Neutrophil Oxidative Burst



Cellular Esterases
 $\xrightarrow{\text{blue arrow}}$
Hydrolysis

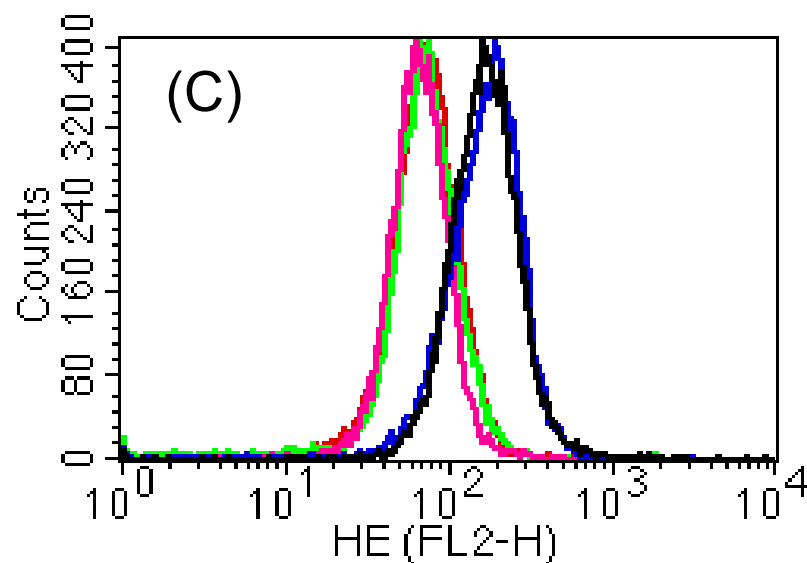
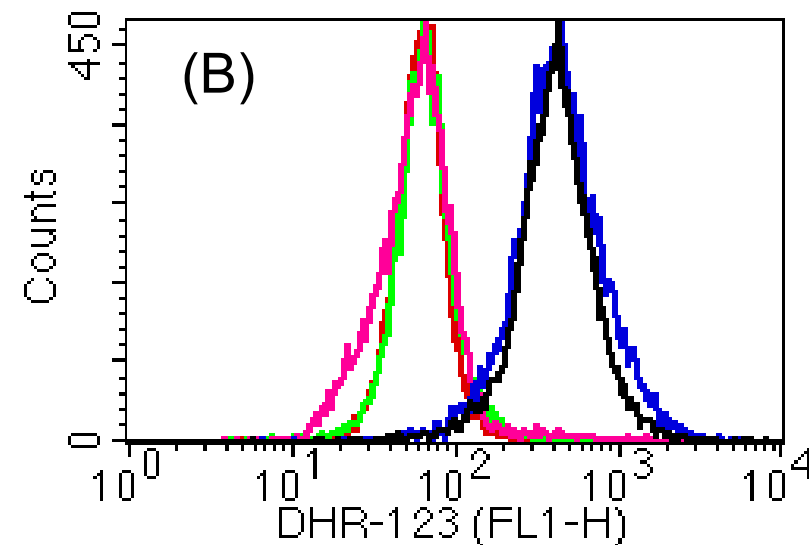
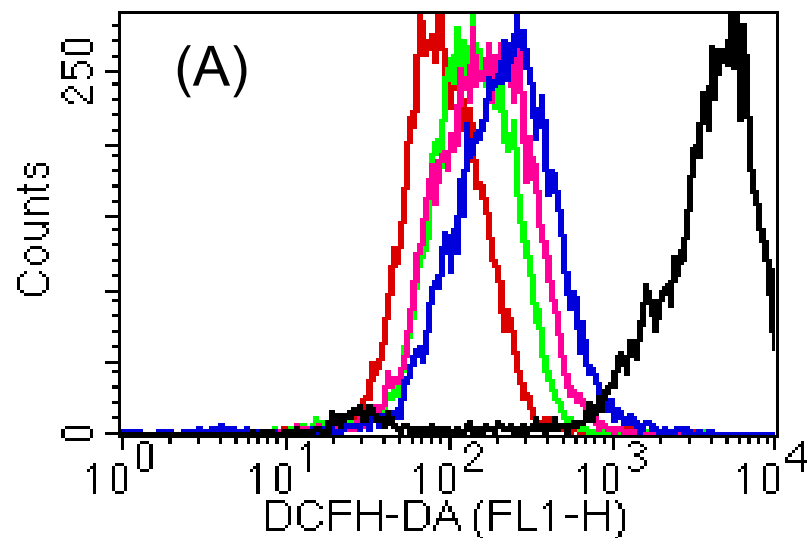


$\xrightarrow{\text{orange arrow}}$
Oxidation



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)

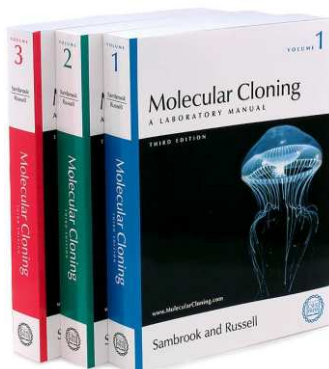
Aequorea victoria - medúza žijící ve vodách na pobřeží Severní Ameriky.

- 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 “



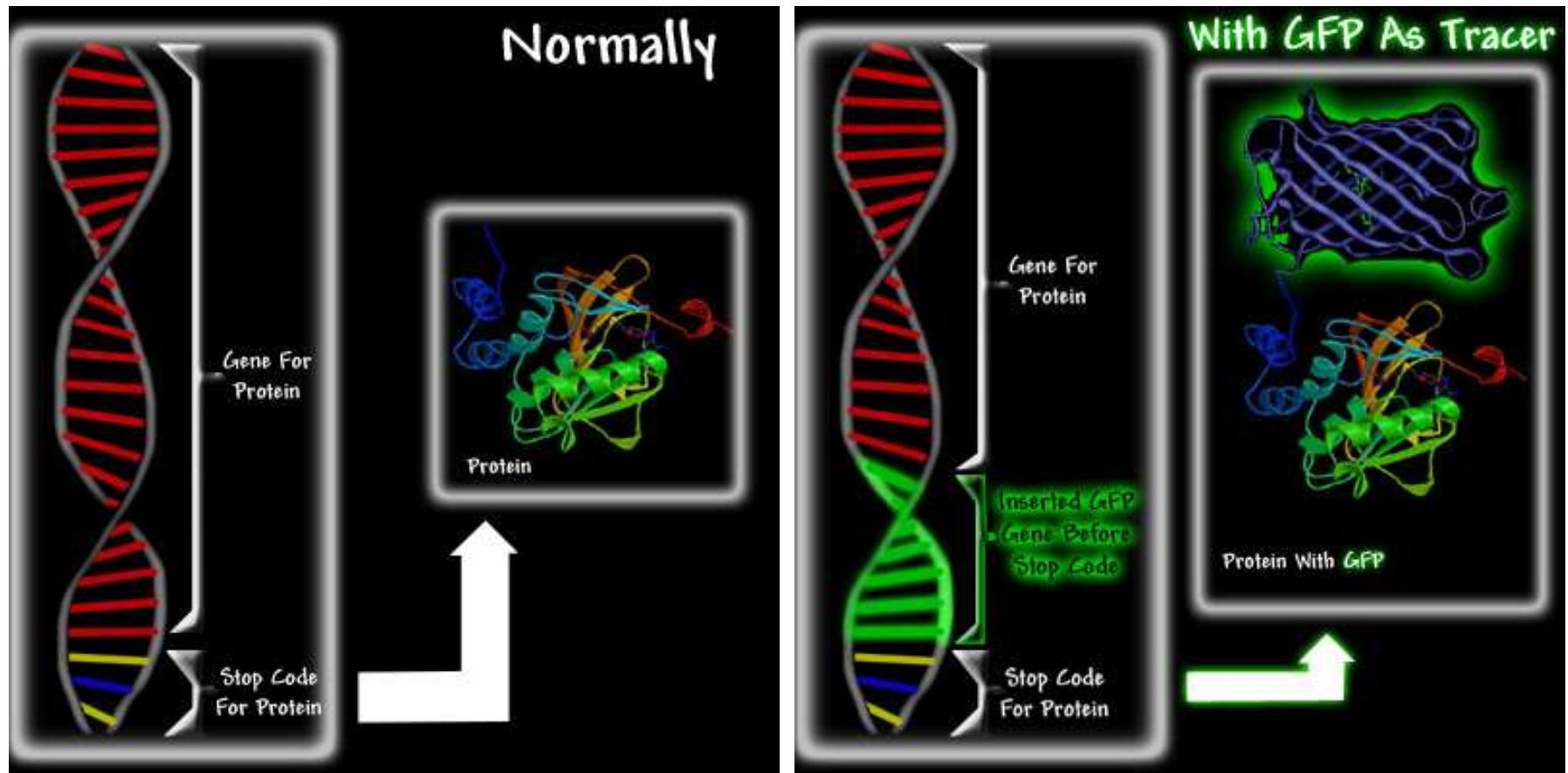
http://www.mbayaq.org/efc/living_species/default.asp?hOri=1&inhab=440

Renilla reniformis "Sea Pansy"



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

Fluorescenční proteiny



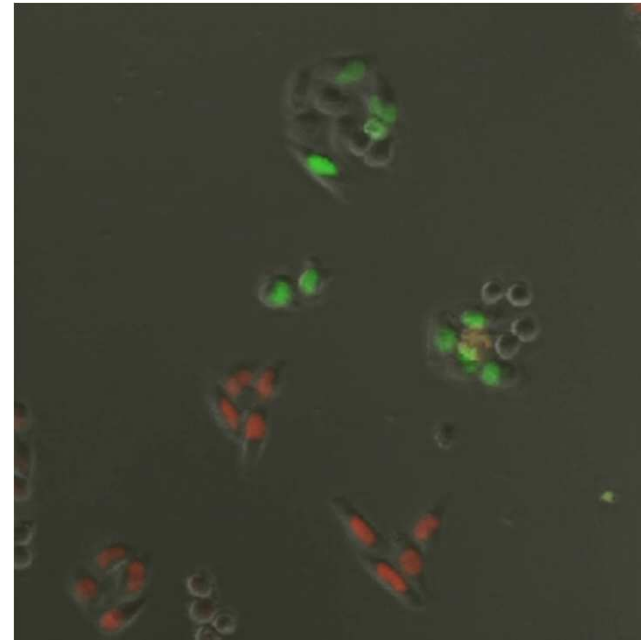
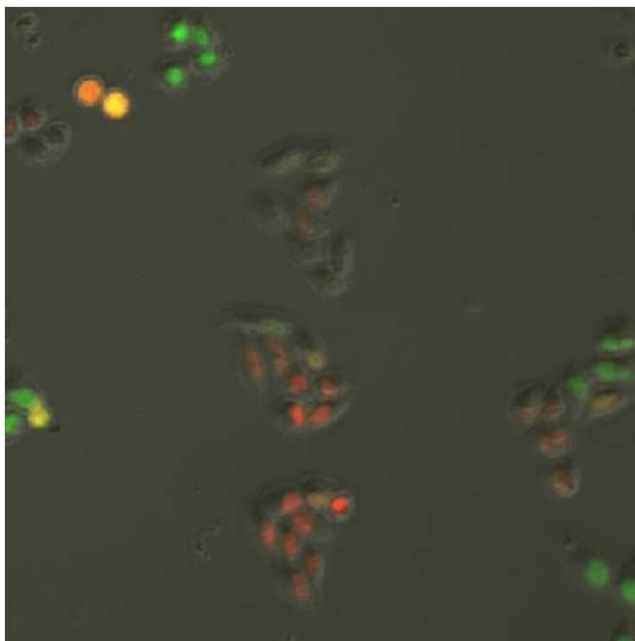
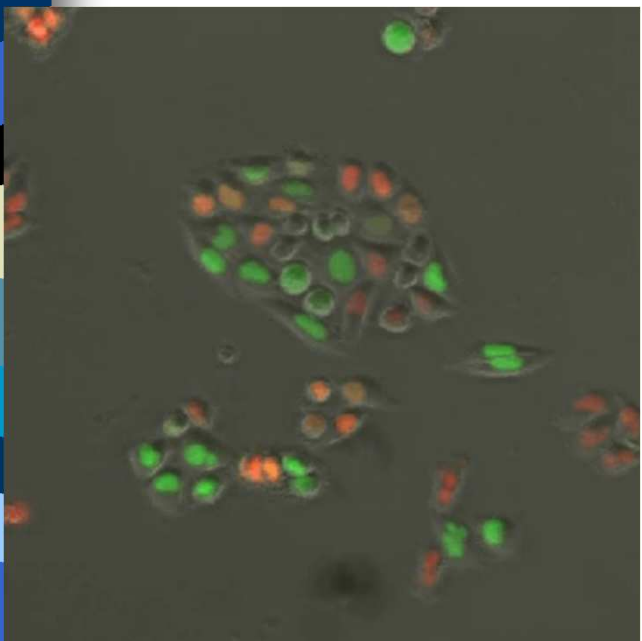
<http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm>

CONTROL

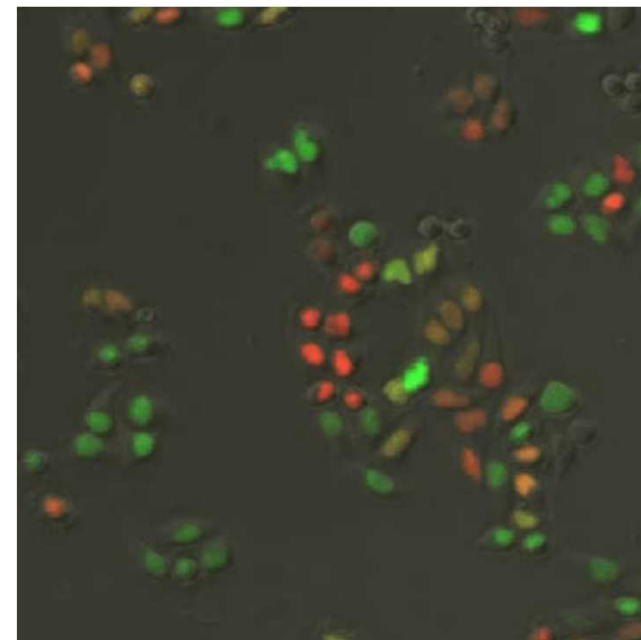
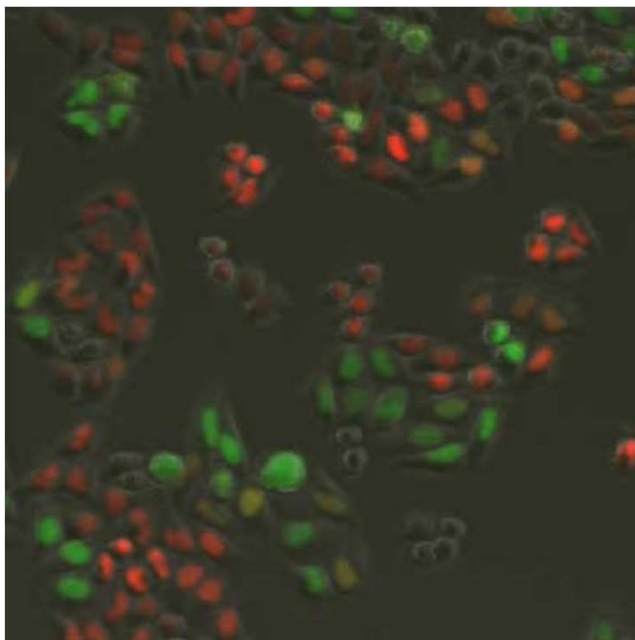
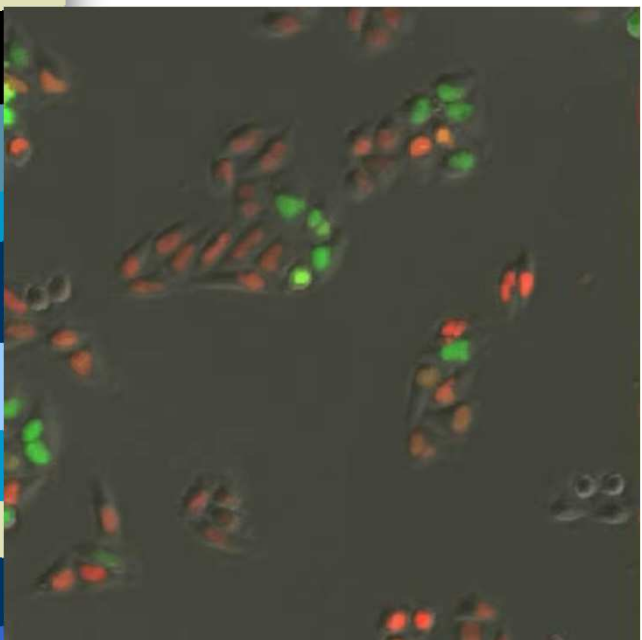
SCH900776

MU380

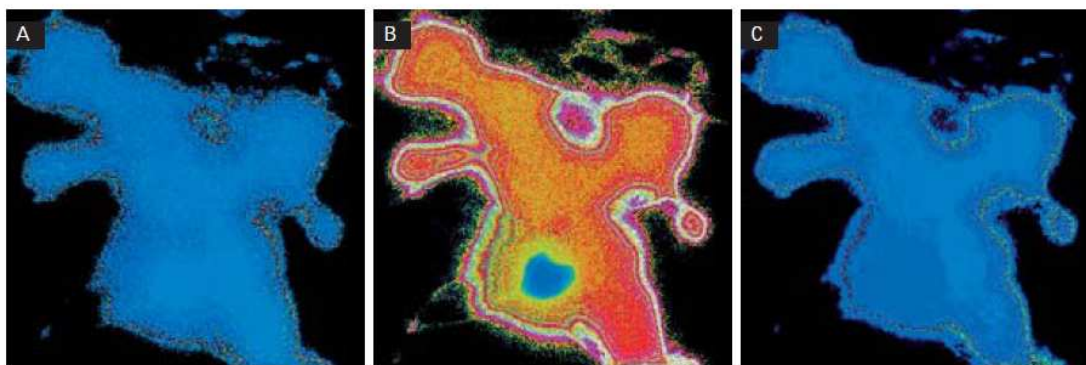
VEHICLE



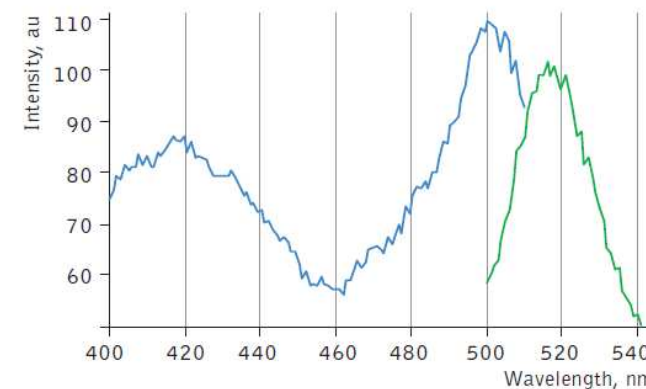
GEMCITABINE



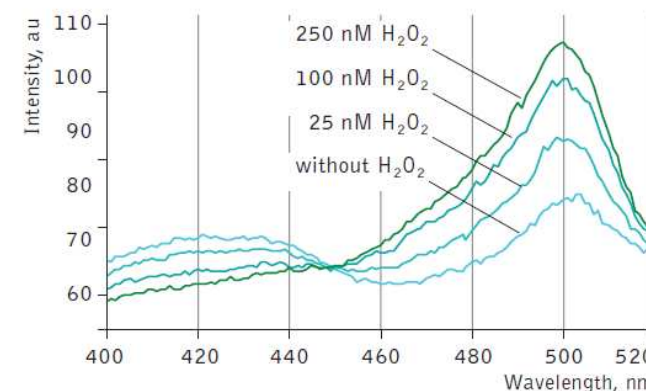
Fluorescent sensors for detection of H₂O₂



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 H₂O₂. Images were pseudocolored using "ratio" lookup table of NIH ImageJ software: blue-green-red-white colors represent lowest-intermediate-high-highest level of H₂O₂.



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



Changes in the excitation spectrum of isolated HyPer in response to H₂O₂ addition. Emission was measured at 530 nm.

Variants & fusions

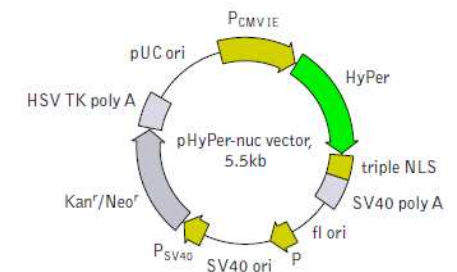
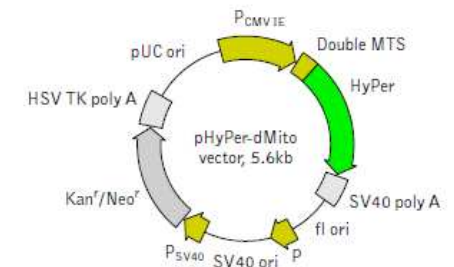
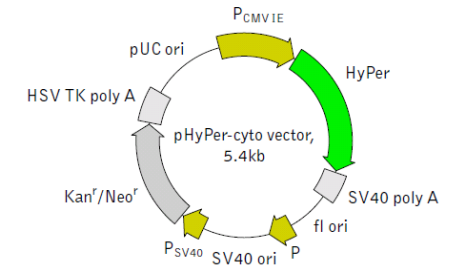
■ pHyPer-cyto vector

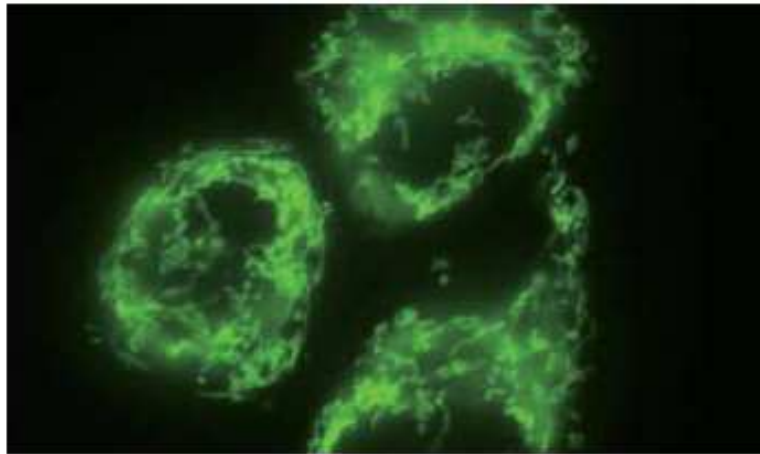
■ pHyPer-dMito vector

- 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

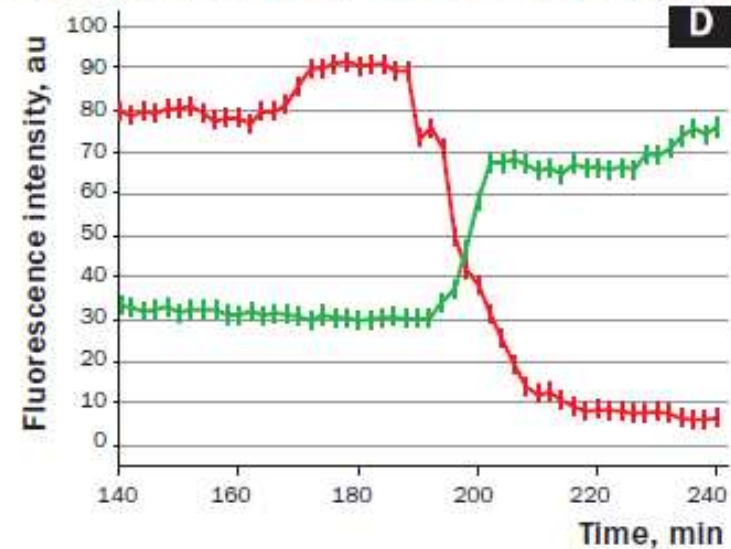
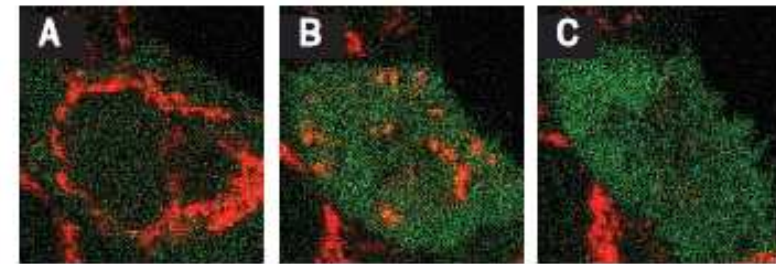
- Three copies of the nuclear localization signal (NLS) fused to the HyPer C-terminus provide for efficient translocation of HyPer to the nuclei of mammalian cells [Fischer-Fantuzzi and Vesco, 1988]





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.



evrogen



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)

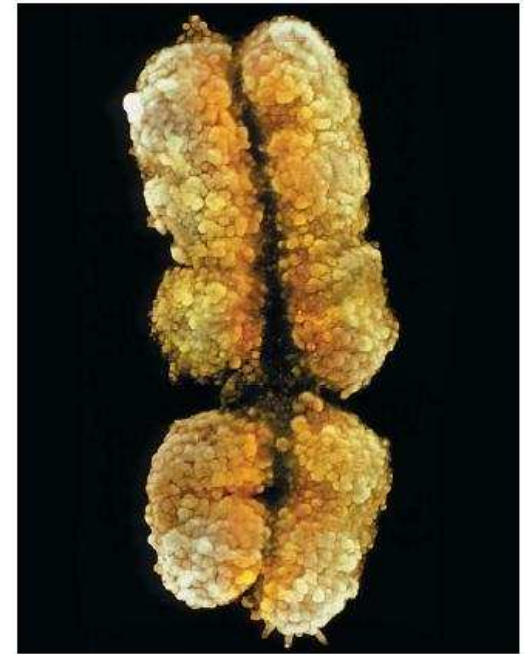
A. V. CARRANO, J. W. GRAY, R. G. LANGLOIS, K. J. BURKHART-SCHULTZ, AND M. A. VAN DILLA

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

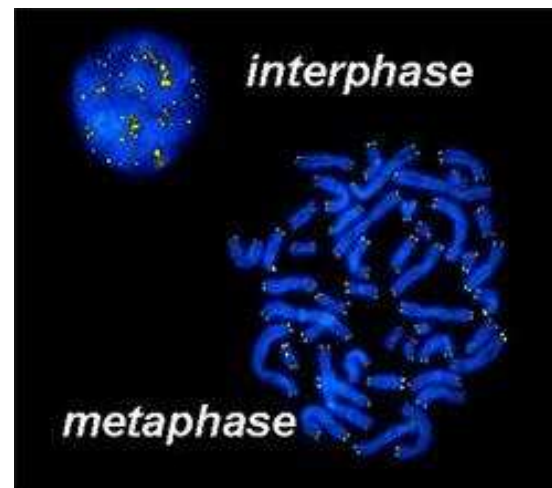
Communicated by Donald A. Glaser, December 18, 1978

Analýza a sortování chromozómů

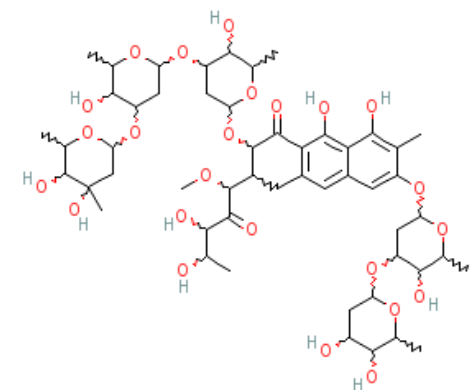
- synchronizace buněk – získání metafázních chromozómů (colcemid, hydroxyurea)
 - izolace chromozómů
 - značení DAPI nebo **Hoechst** vs. **chromomycin A3** (CA3) nebo mithramycin
- = celková DNA vs. G/C-bohaté oblasti



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



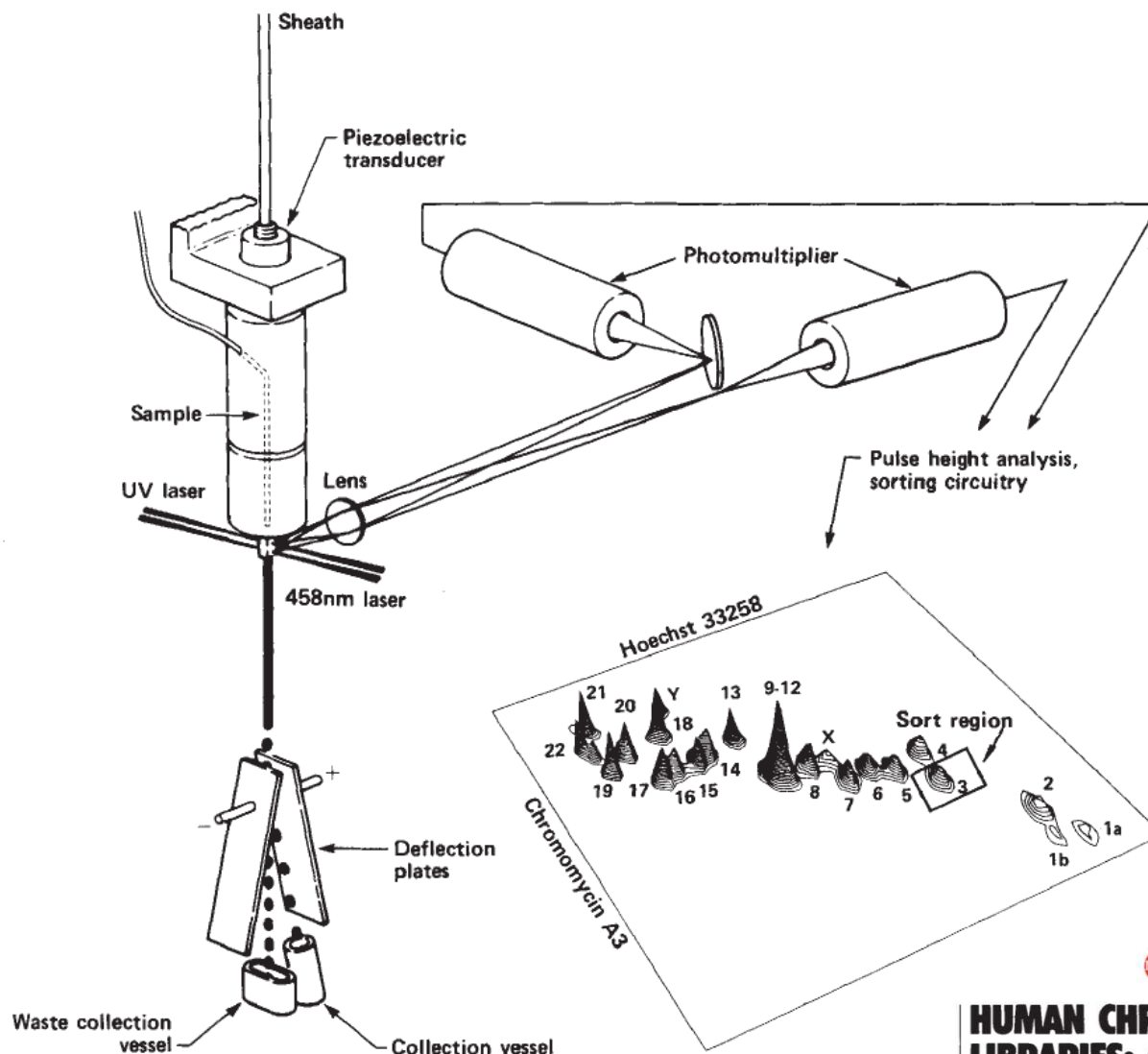
<http://www.nccr-oncology.ch/scripts/page9243.html>



PubChem

National Library of Medicine NLM

Analýza a sortování chromozómů



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

HUMAN CHROMOSOME-SPECIFIC DNA LIBRARIES: CONSTRUCTION AND AVAILABILITY

M.A. Van Dilla[□], L.L. Deaven[□], K.L. Albright[†], N.A. Allen^{*}, M.R. Aubuchon^{*}, M.F. Bartholdi[†], N.C. Brown[†], E.W. Campbell[†], A.V. Carrano^{*}, L.M. Clark[†], L.S. Cram[†], B.D. Crawford[†], J.C. 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^{*}.

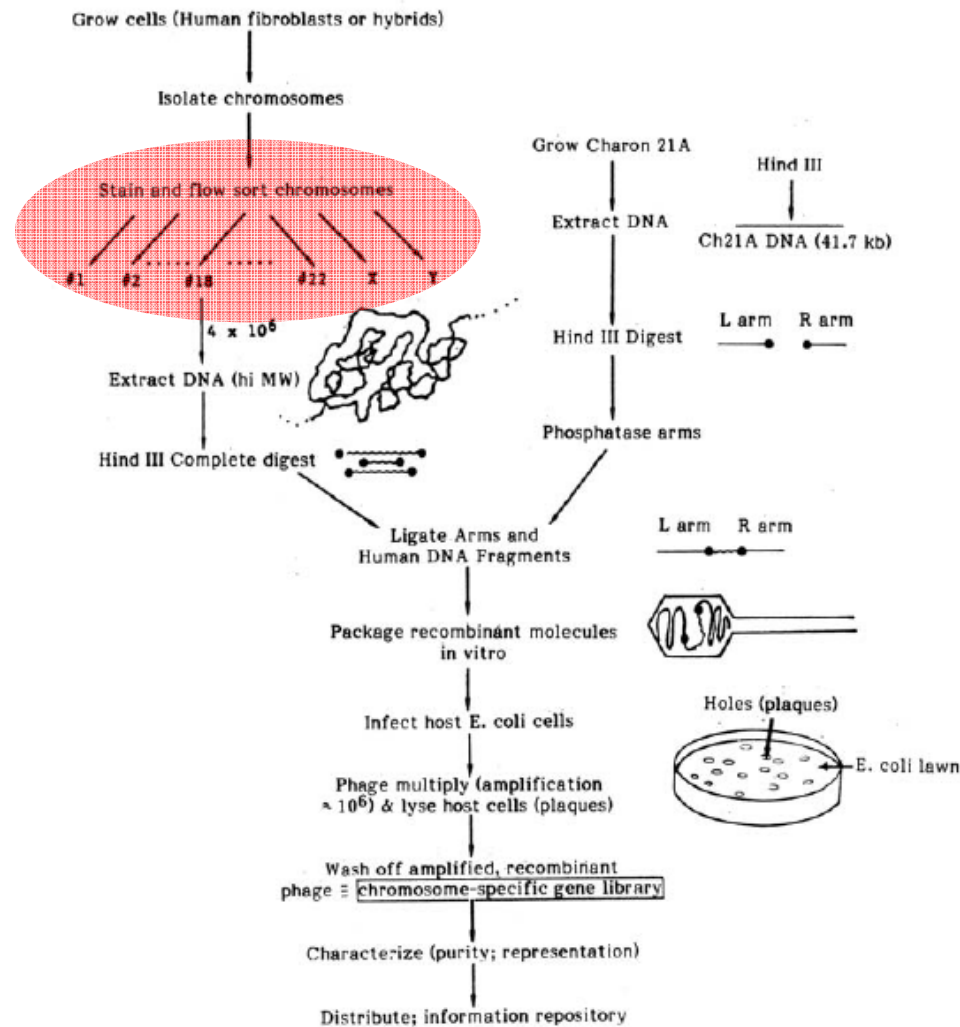
National Laboratory Gene Library Project. ^{*} Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, California 94550. [†] Los Alamos National Laboratory, Life Sciences Division, University of California, Los Alamos, New Mexico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

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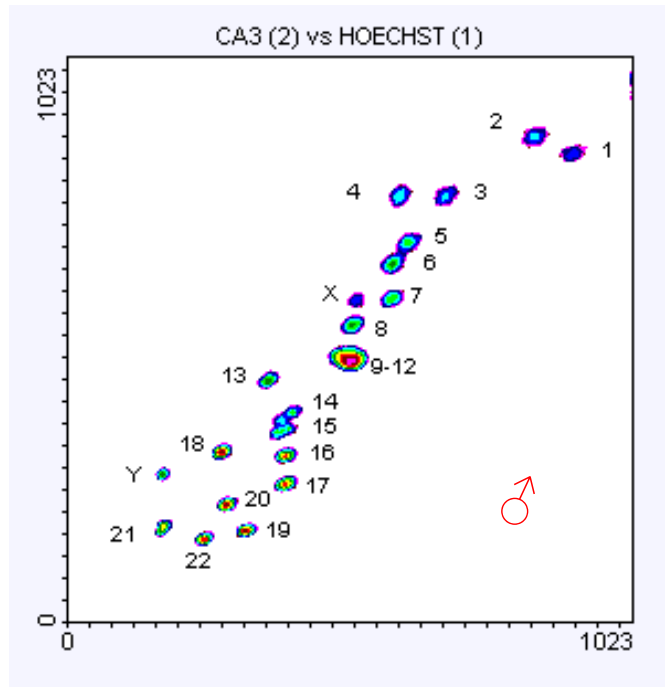
National Laboratory Gene Library Project. ^{*} Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, California 94550. [†] Los Alamos National Laboratory, Life Sciences Division, University of California, Los Alamos, New Mexico 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“



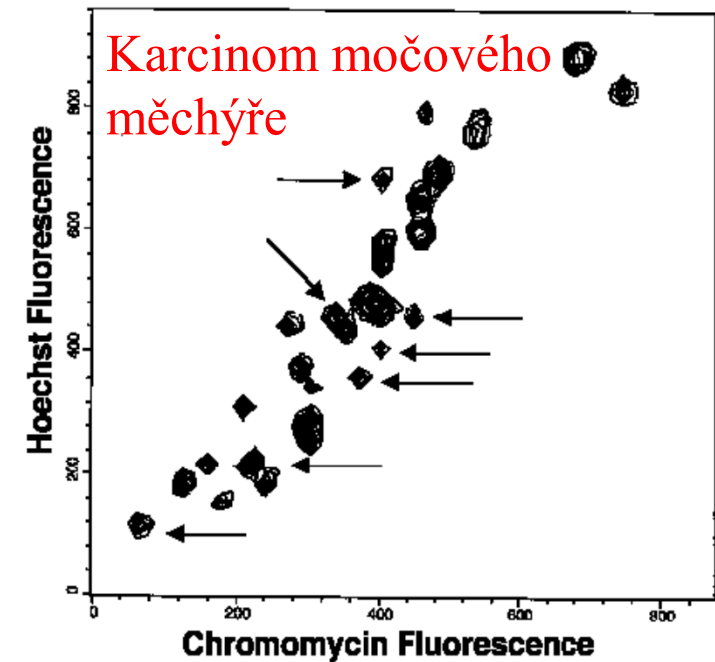
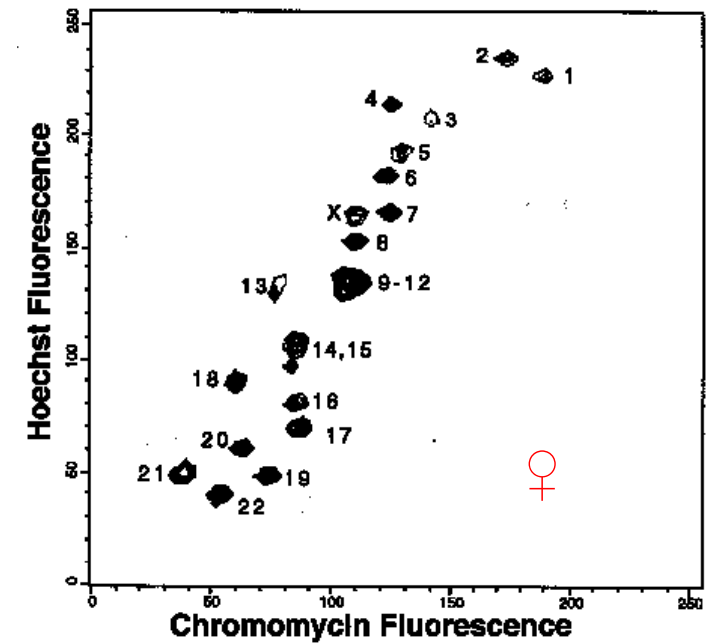
<http://www.sanger.ac.uk/HGP/Cytogenetics/>

The Preparation of Human Chromosomes for Flow Cytometry

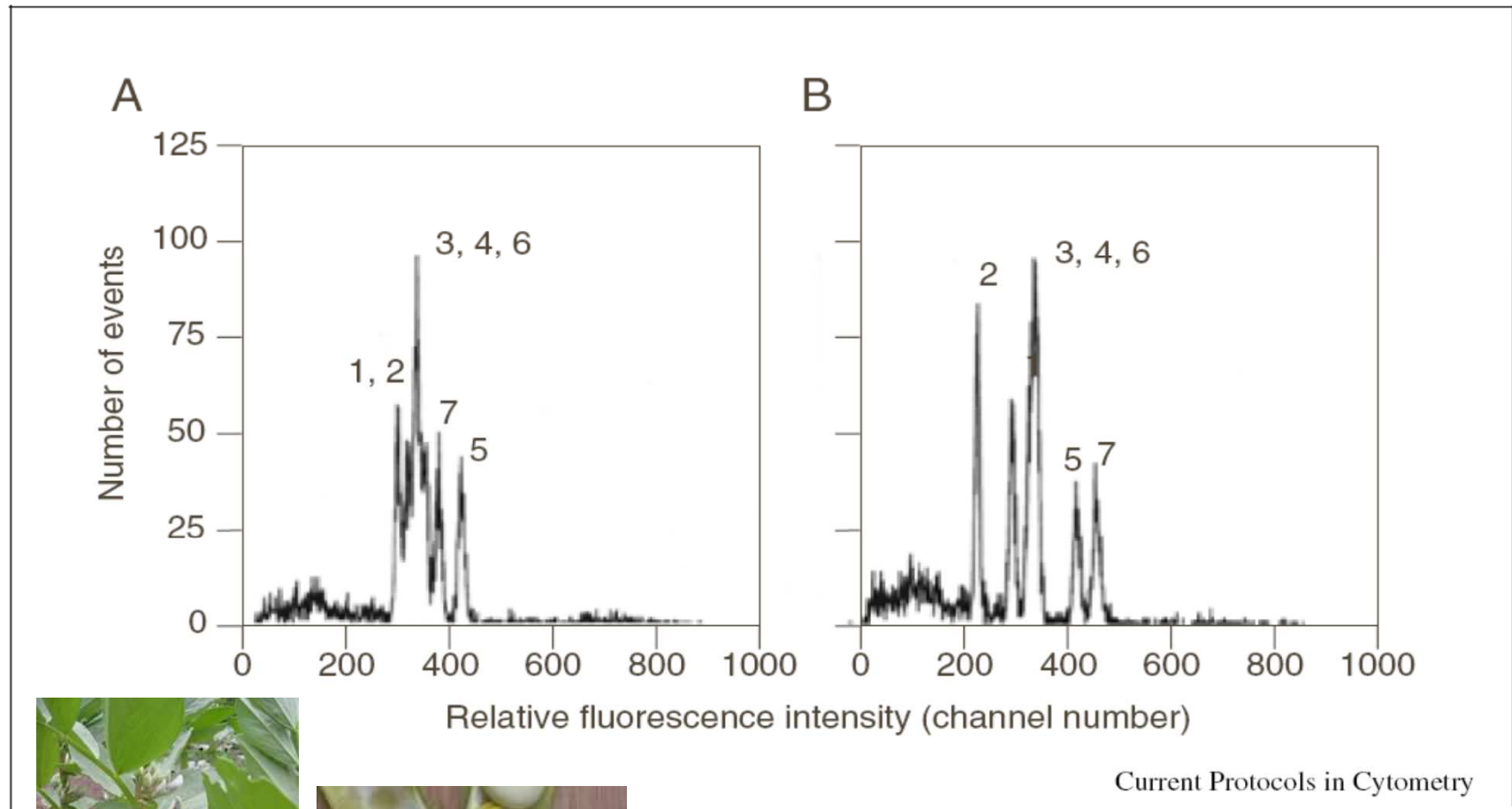
DEREK DAVIES

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

PROTOCOL

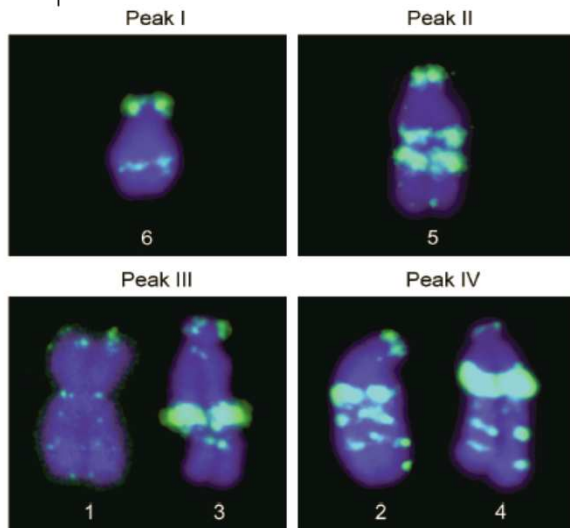
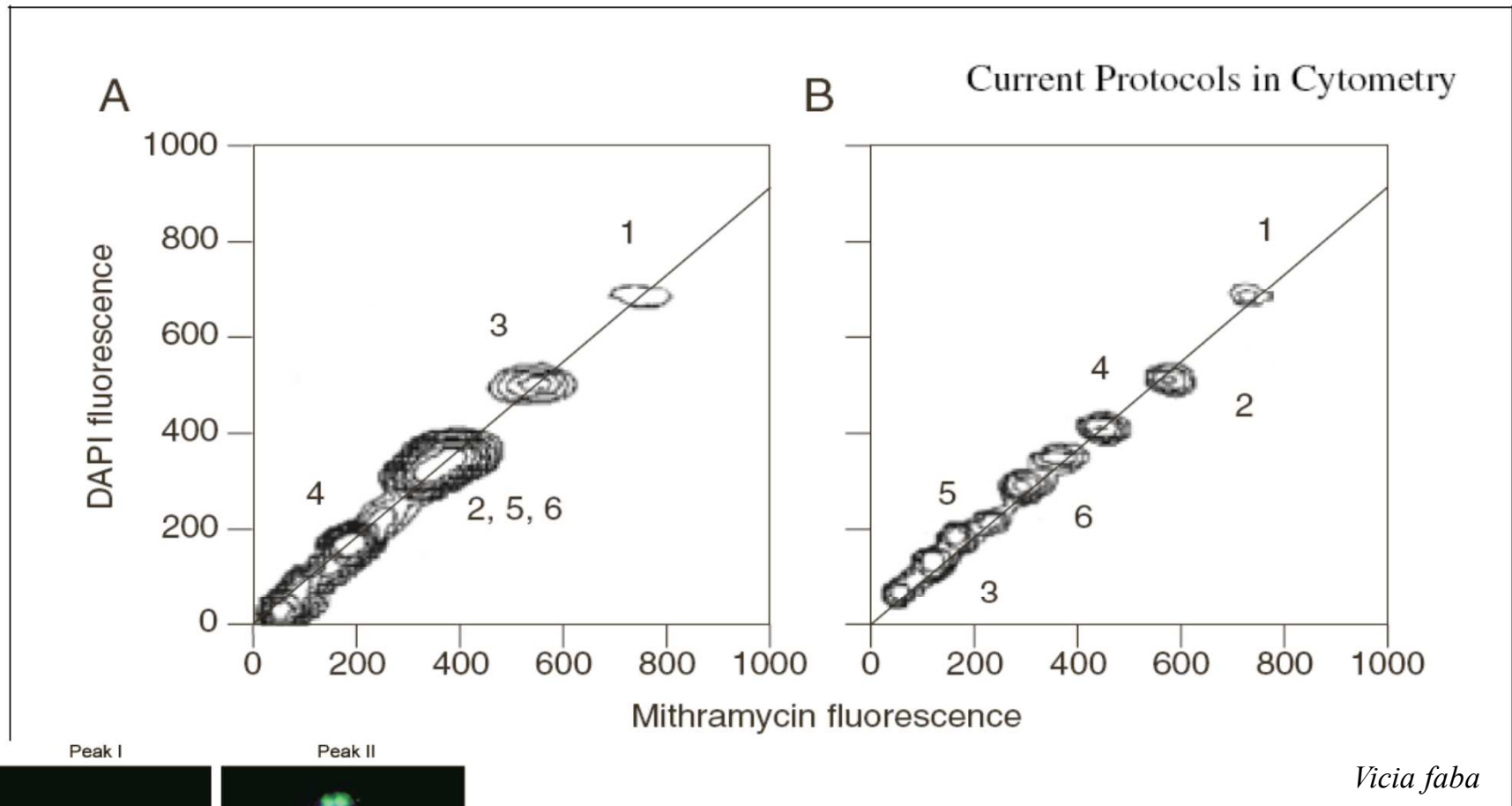
Estimation of nuclear DNA content in plants using flow cytometry

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-78571 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. (dolezel@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

\$15
17 AUGUST 2018
science.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,

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

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

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



Aplikace průtokové cytometrie v mikrobiologii

- ekologie
- potravinářství

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

Aplikace průtokové cytometrie v mikrobiologii

Relative Size Ratios for Bacteria, Yeast, and Eukaryotes

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

Current Protocols in Cytometry

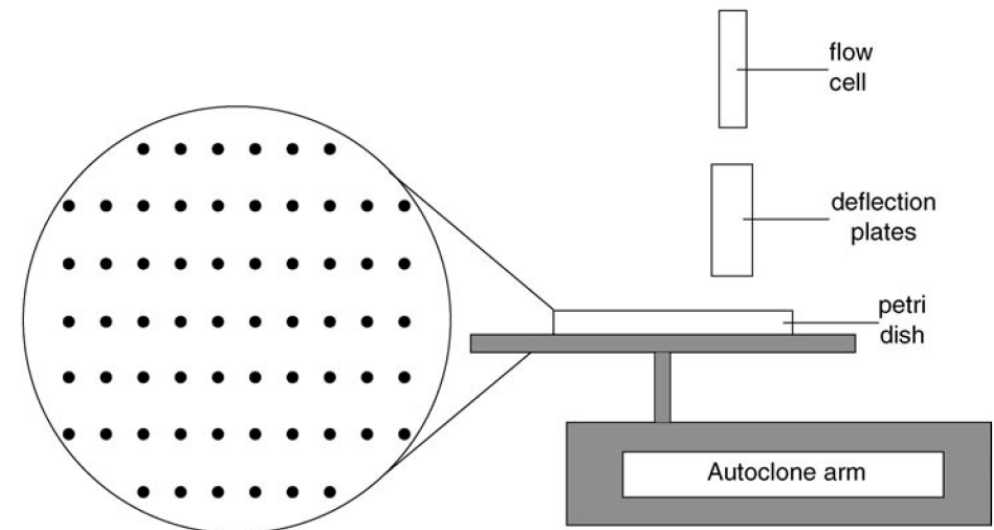


Aplikace průtokové cytometrie v mikrobiologii

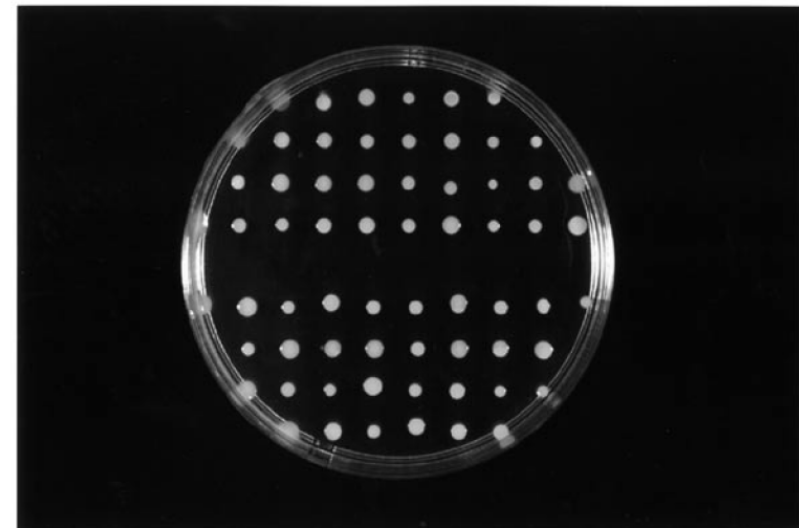
- viabilita
- metabolické funkce
- sortování
- analýza aerosolů (Fluorescence Aerodynamic Particle Sizer (Flaps))

Aplikace průtokové cytometrie v mikrobiologii

- Sortování
 - EPICS + Autoclone® modul

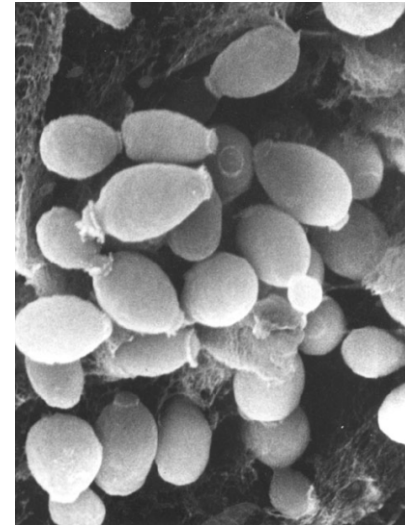


top view of petri dish showing sort grid

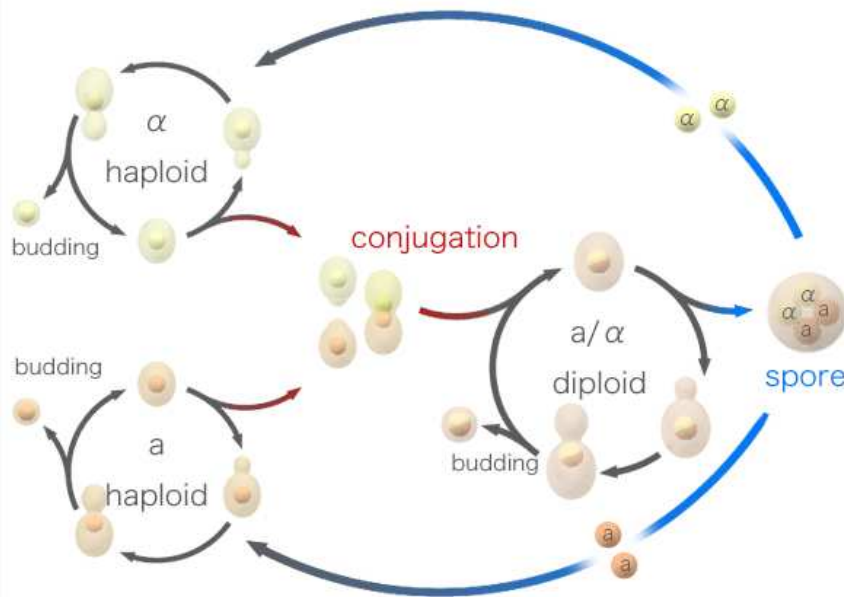


Průtoková cytometrie kvasinek

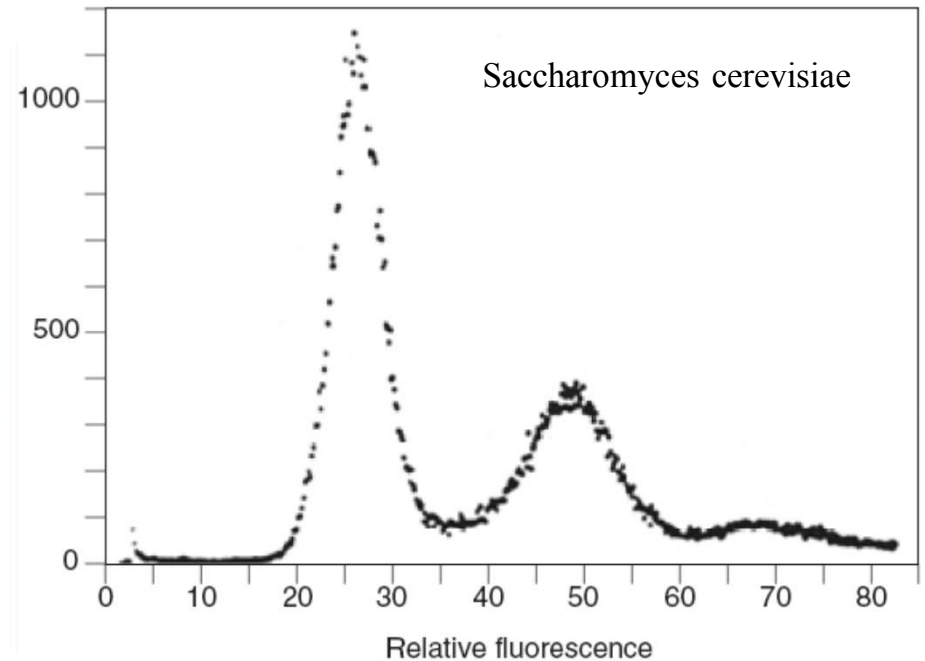
- buněčné dělení
- viabilita
- membránový potenciál
- respirace
- produkce H_2O_2
- citlivost k antibiotikům
- separace



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

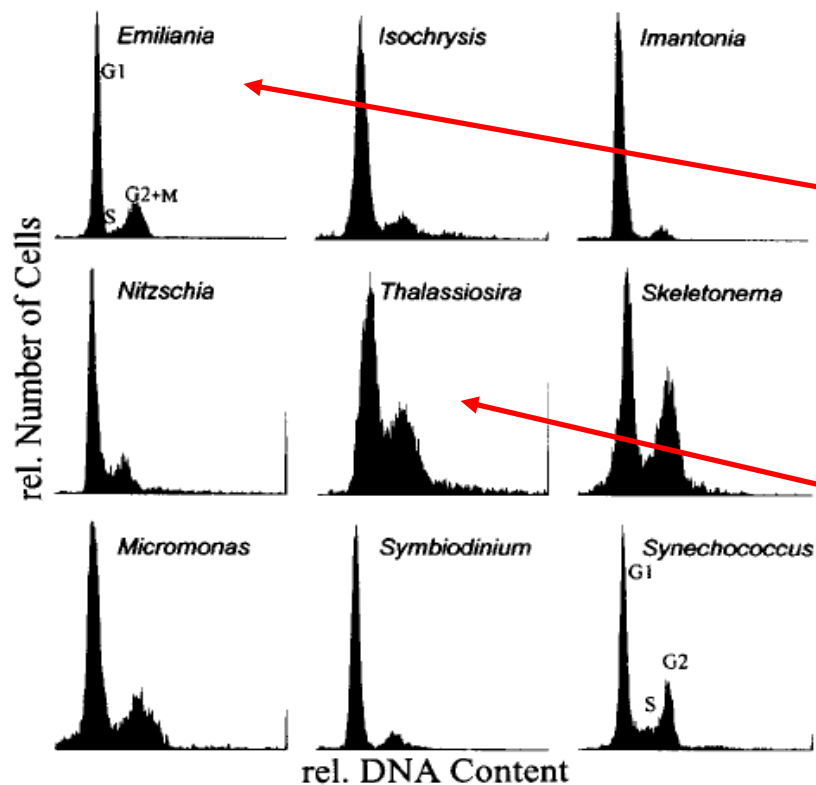
Published August 20

NOTE

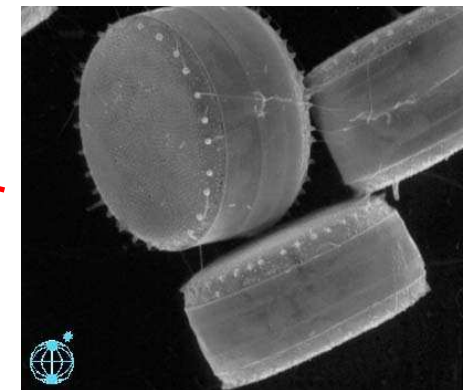
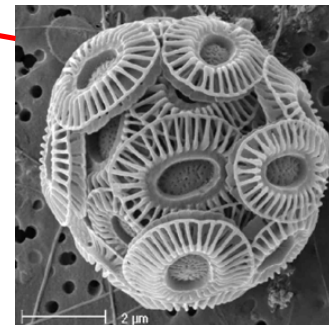
Cytometric measurement of the DNA cell cycle in the presence of chlorophyll autofluorescence in marine eukaryotic phytoplankton by the blue-light excited dye YOYO-1

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

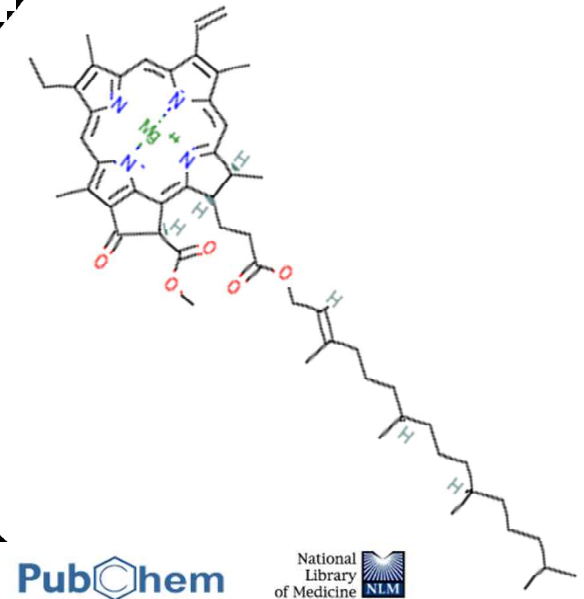
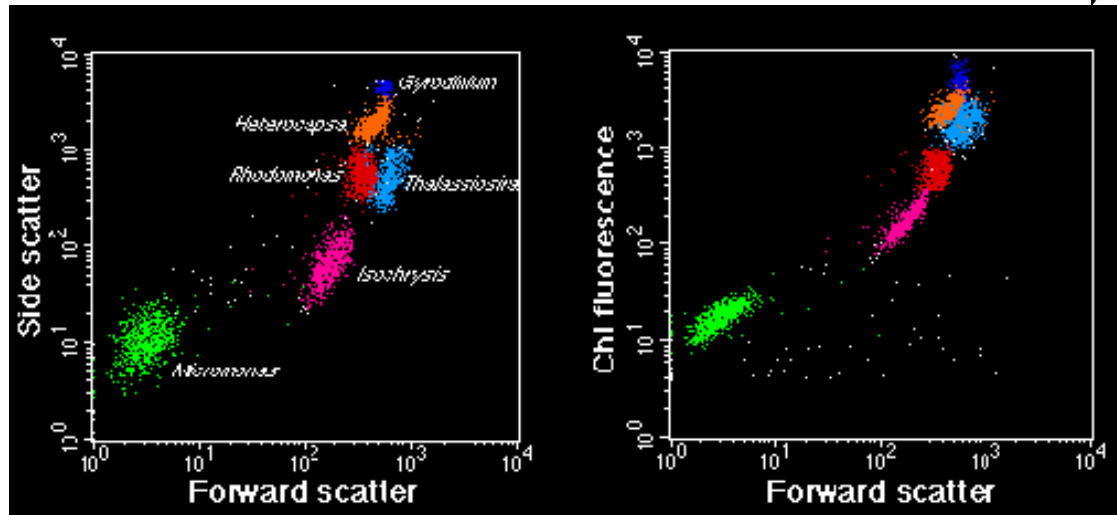
¹Institut für Meereskunde, Düsternbrooker Weg 20, D-24105 Kiel, Germany
²Universität Bremen, FB II Meeresbotanik, Postfach 330440, D-28334 Bremen, Germany



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

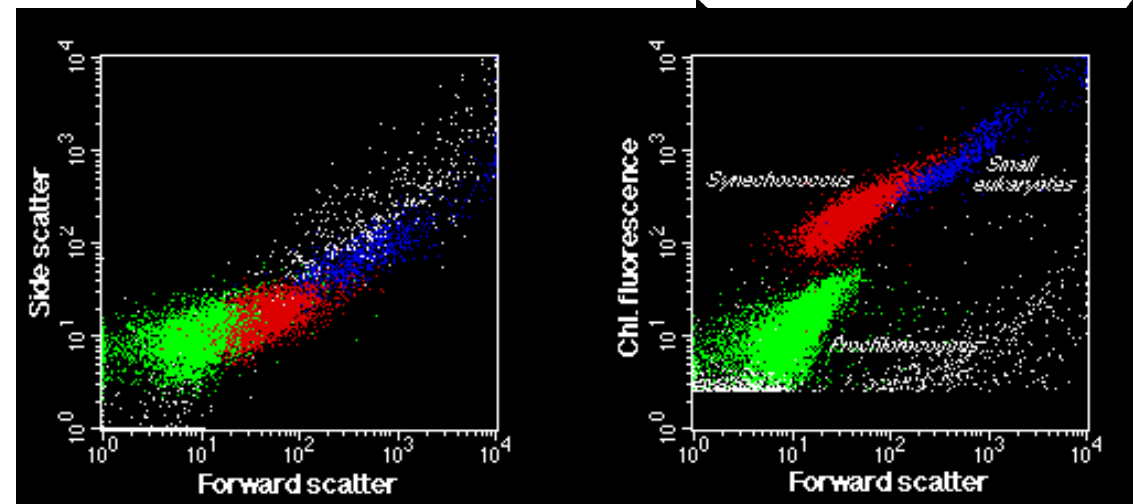
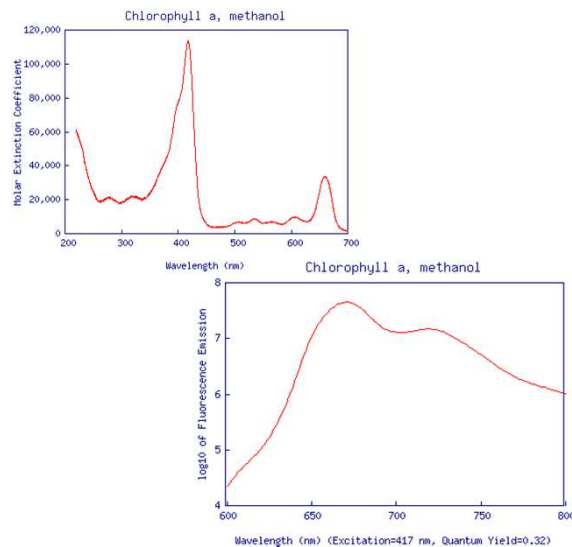


Průtoková cytometrie v hydrobiologii



PubChem

National Library of Medicine NLM





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

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

Flow cytometry assessment of bacterioplankton in tropical marine environments

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^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

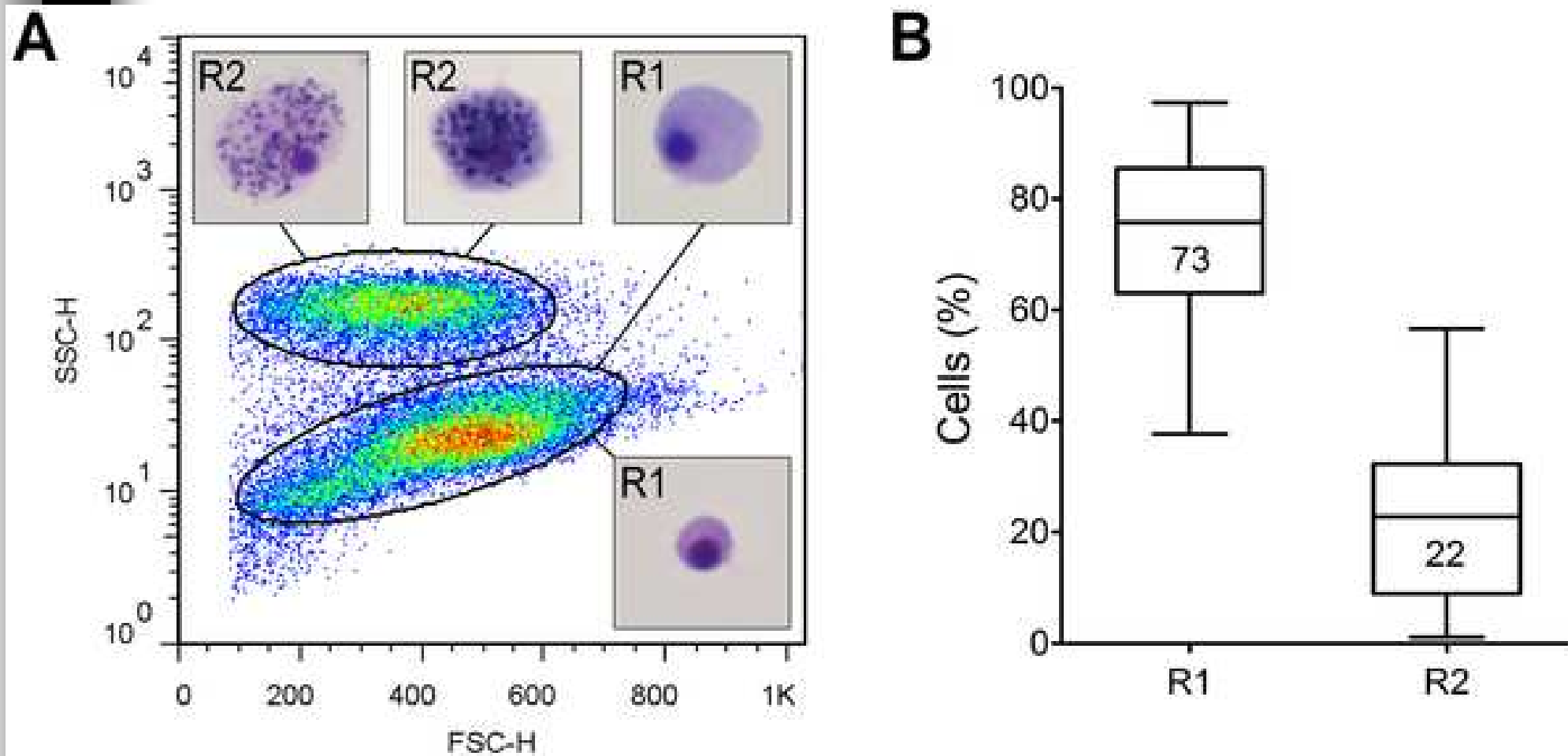
A Cossarizza¹, M Pinti¹, L Troiano¹, EL Cooper²

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*

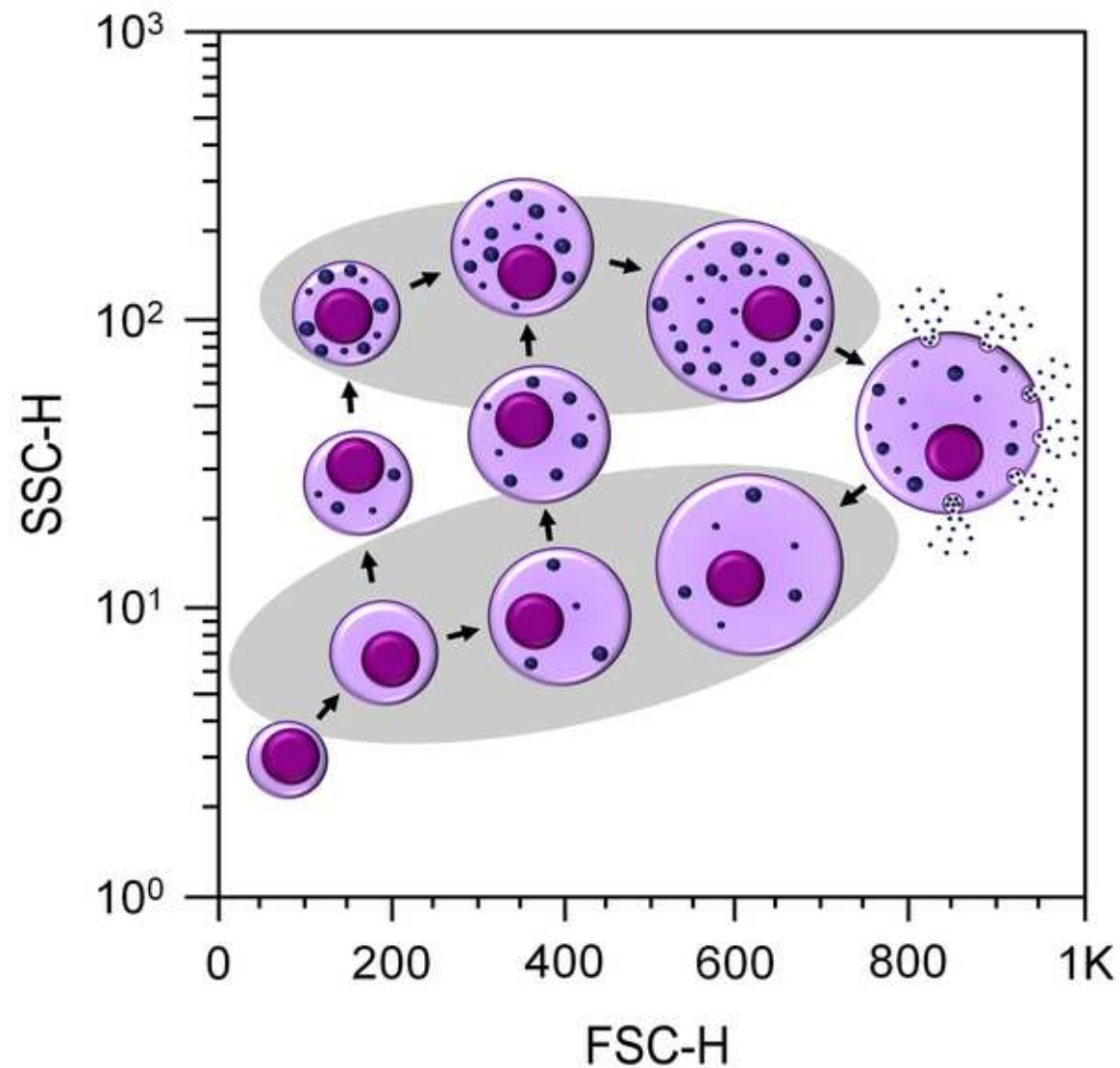
<http://www.icms.qmul.ac.uk/flowcytometry/uses/insects/index.html>

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>



„High Throughput Flow Cytometry“

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

Automatizované systémy měření vzorků



Automatický karusel (autosampler)



Adaptér pro nasávání vzorků z mikrotitrační desky



Automatizovaný „microsampler“ systém



Cyttek FLOW CYTOMETRY PRODUCTS



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

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

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

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

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

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

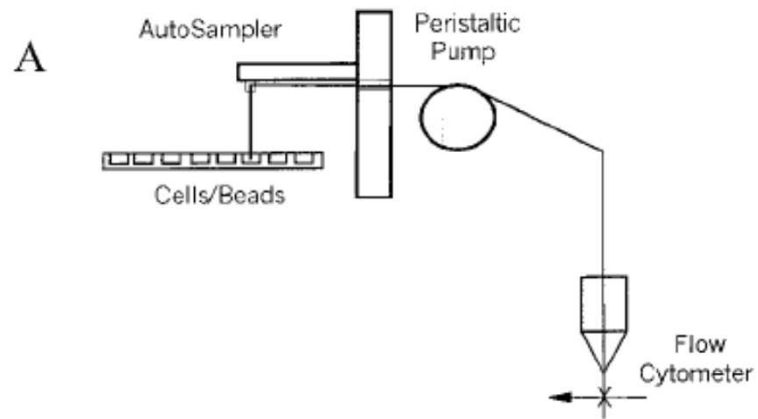
High Throughput Flow Cytometry

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

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

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

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

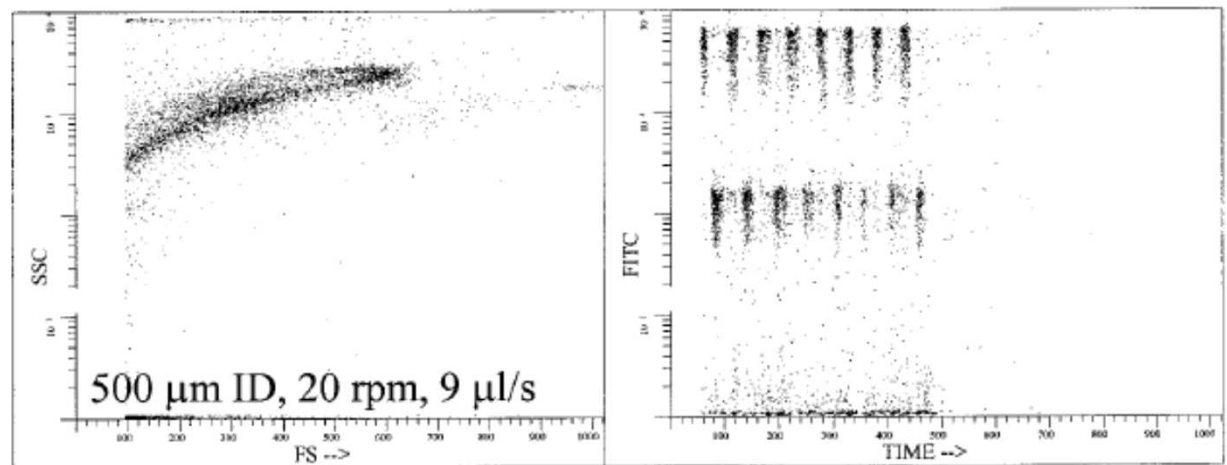


B

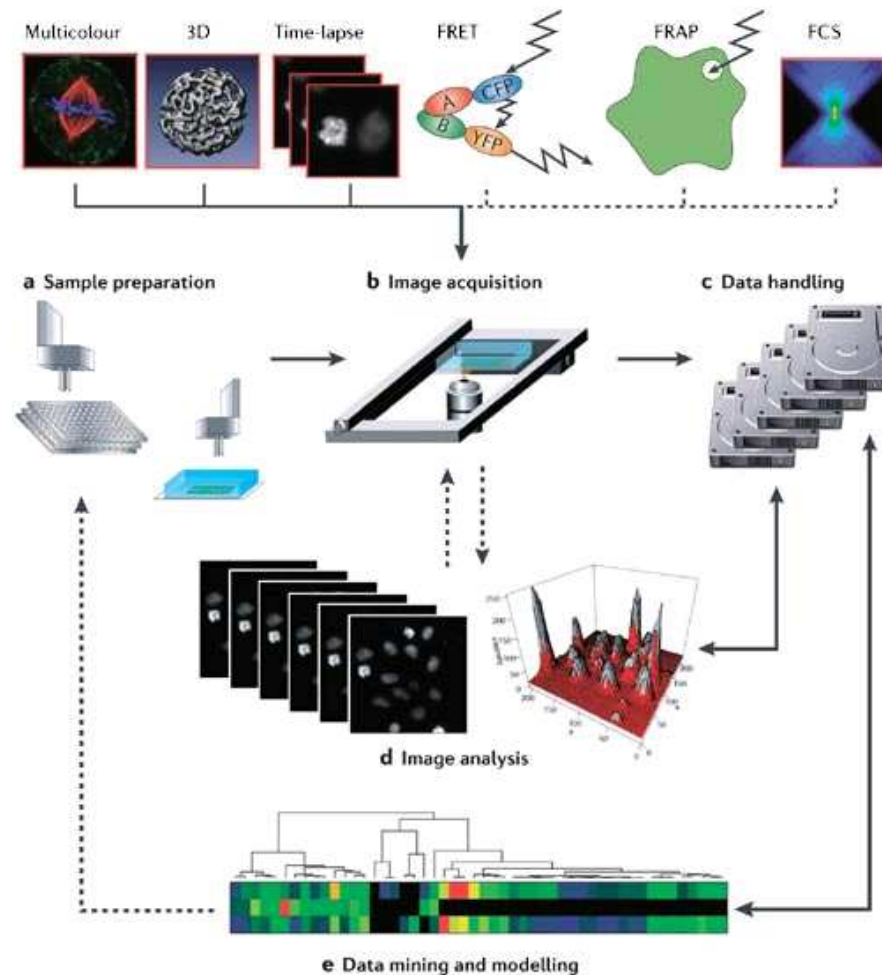


FIG. 1. High throughput flow cytometry. **A**: Schematic view of the flow cytometer, autosampler, and peristaltic pump. **B**: Adjacent samples of latex microspheres separated by air in the 0.02-in (254- μm) ID tubing between the peristaltic pump and the flow cytometer.

C



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



Analysis

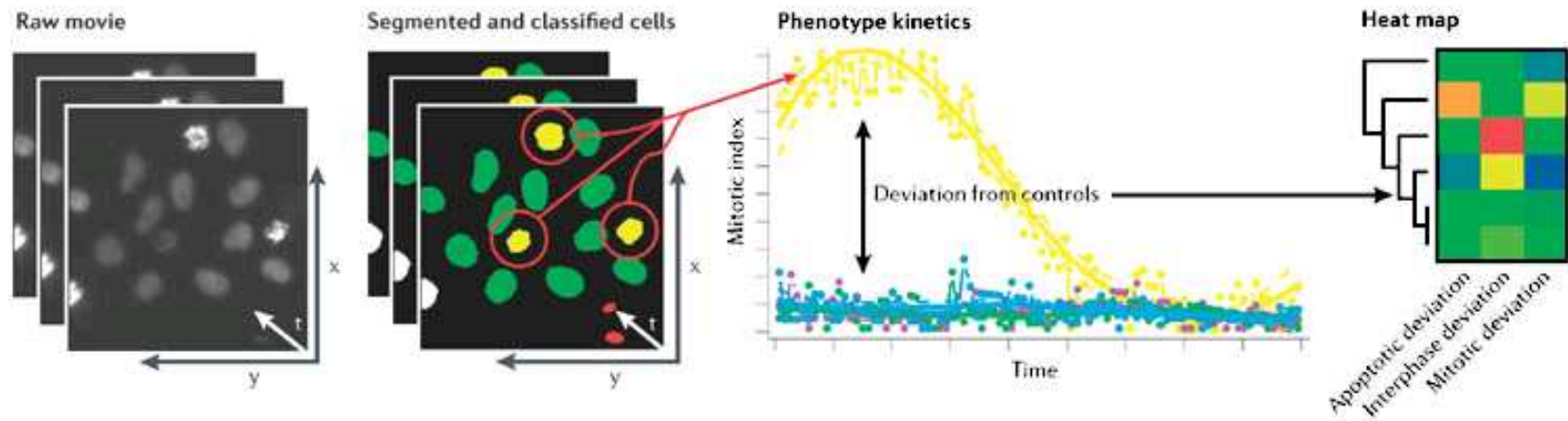


Table 1. Comparison of the Key Attributes of High-Throughput Flow Cytometry and High-Content Microscopy

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

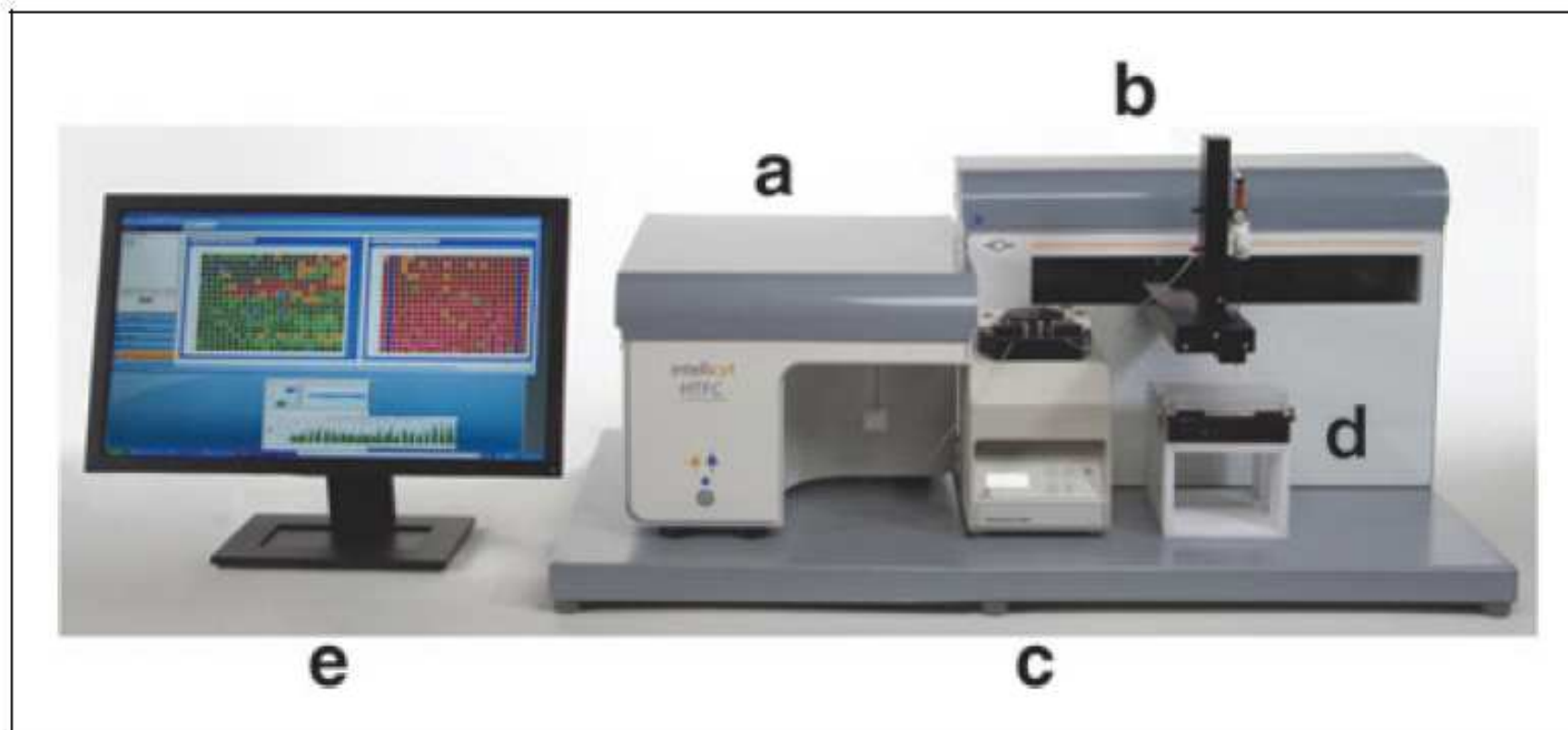


Fig. 1. The HTFC Screening System (IntelliCyt Corporation). **(a)** 2-laser, 4-color flow cytometer; **(b)** an x, y, z autosampler; **(c)** a low pulsation peristaltic pump; **(d)** orbital plate shaker that accommodates 96- and 384-well plates; **(e)** system computer with HyperView installed to set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

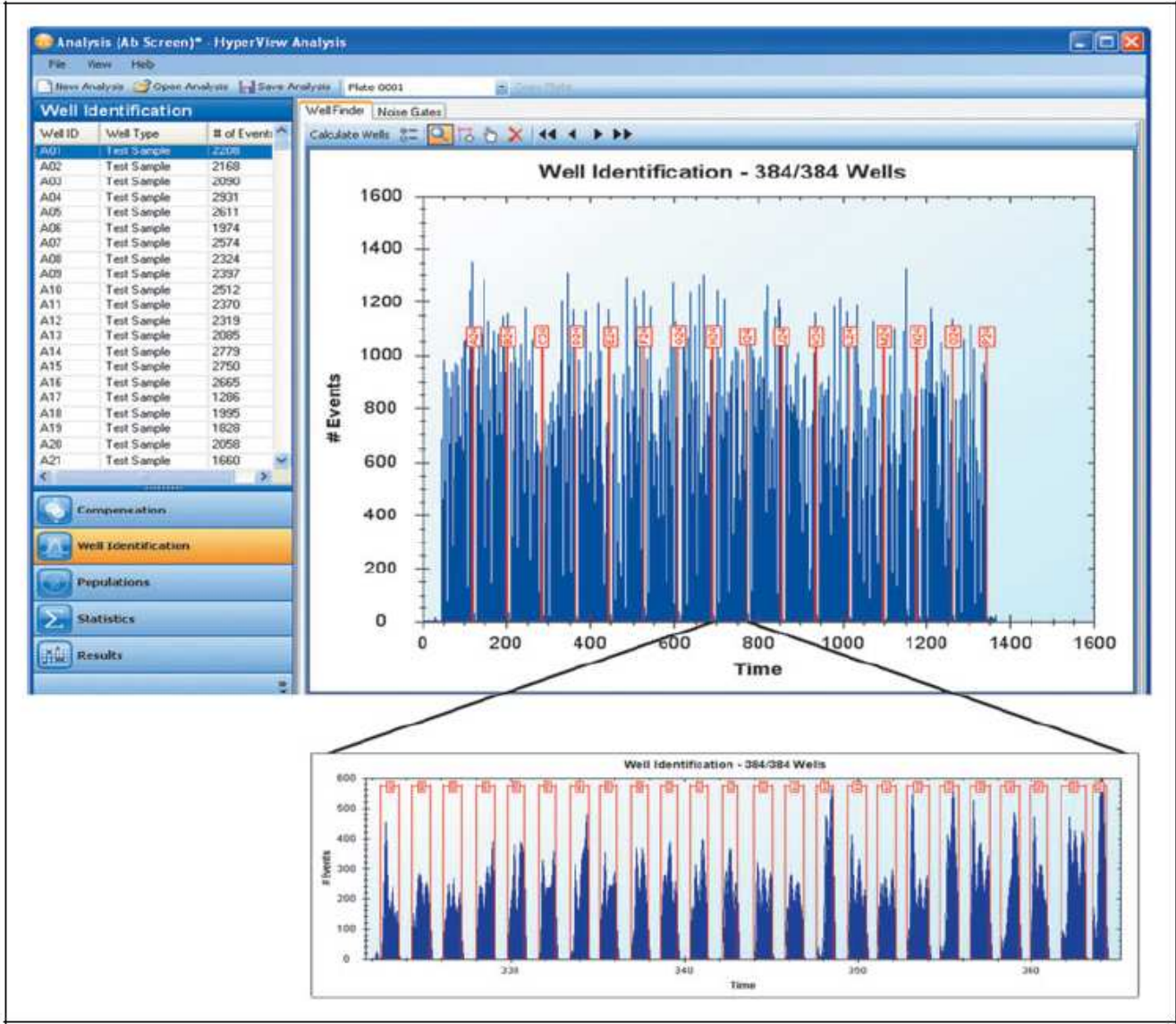


Fig. 4. Screenshot from HyperView showing an example of the Well Identification process. Data from the 384-well plate is collected in to a single flow cytometry standard file, which is shown in the main window. The data are deconvolved by the software algorithm to identify each peak with a well address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

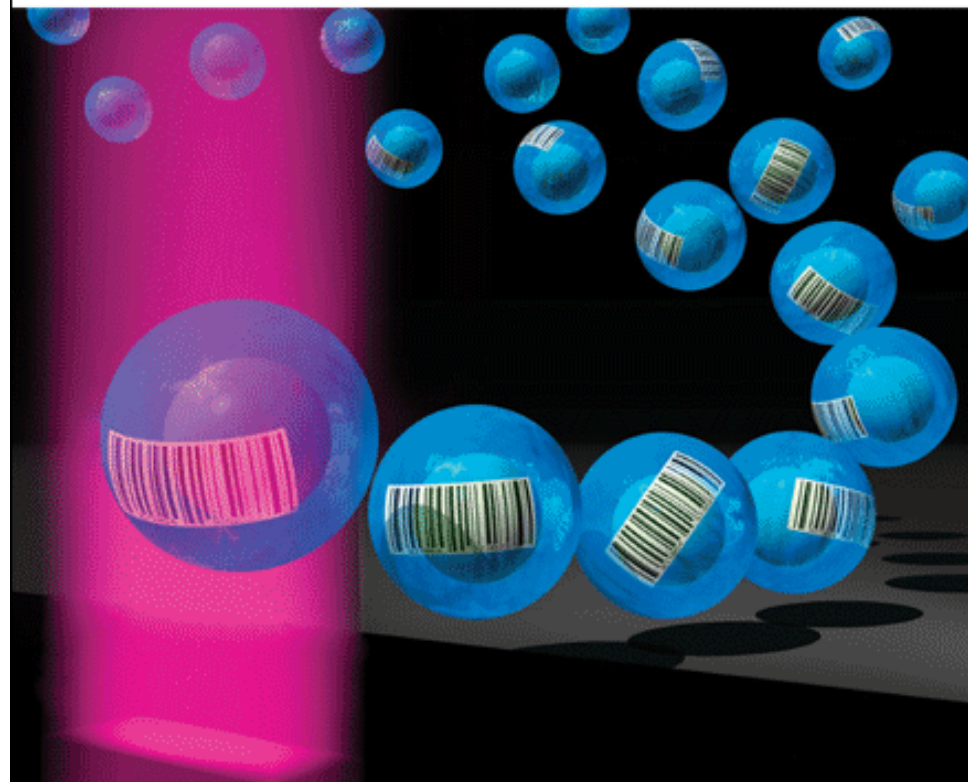


Garry Nolan

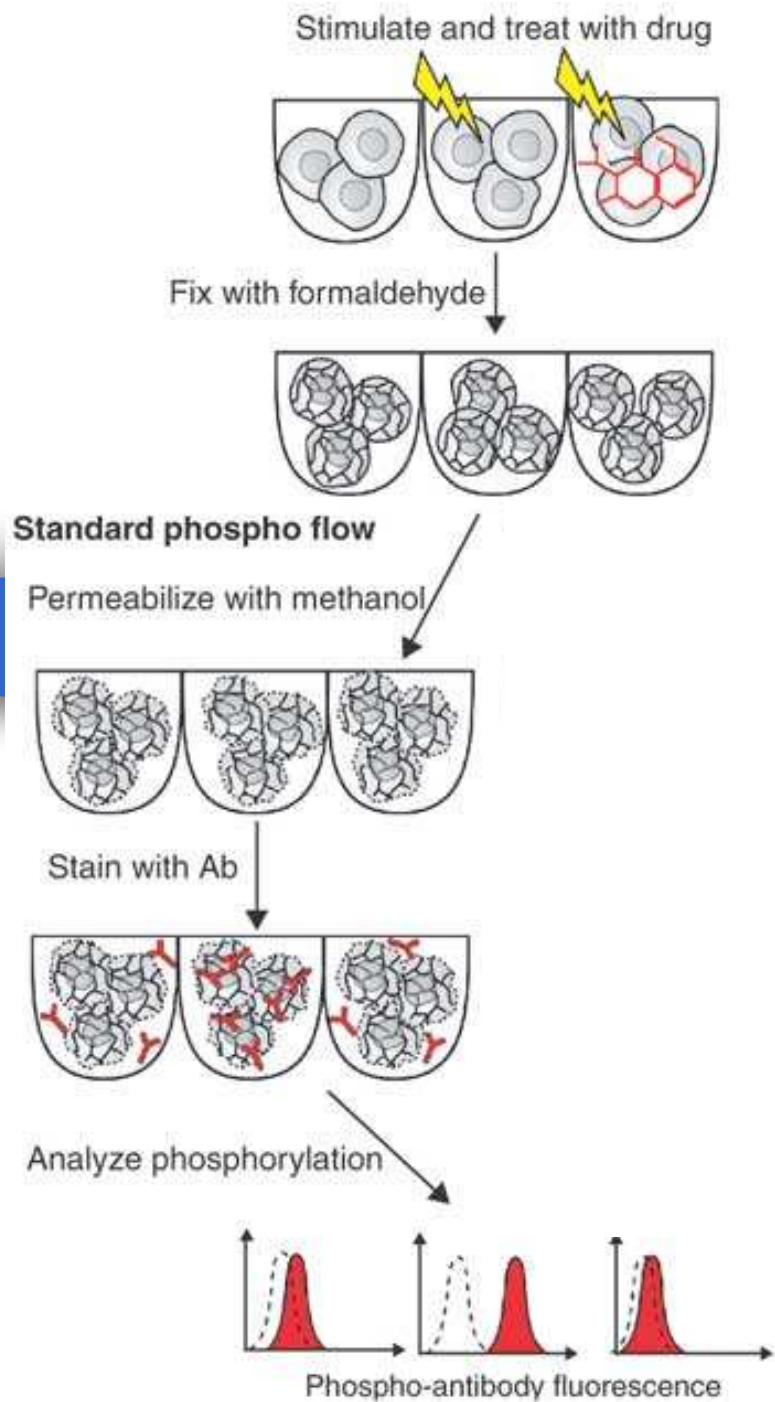


Peter Krutzik

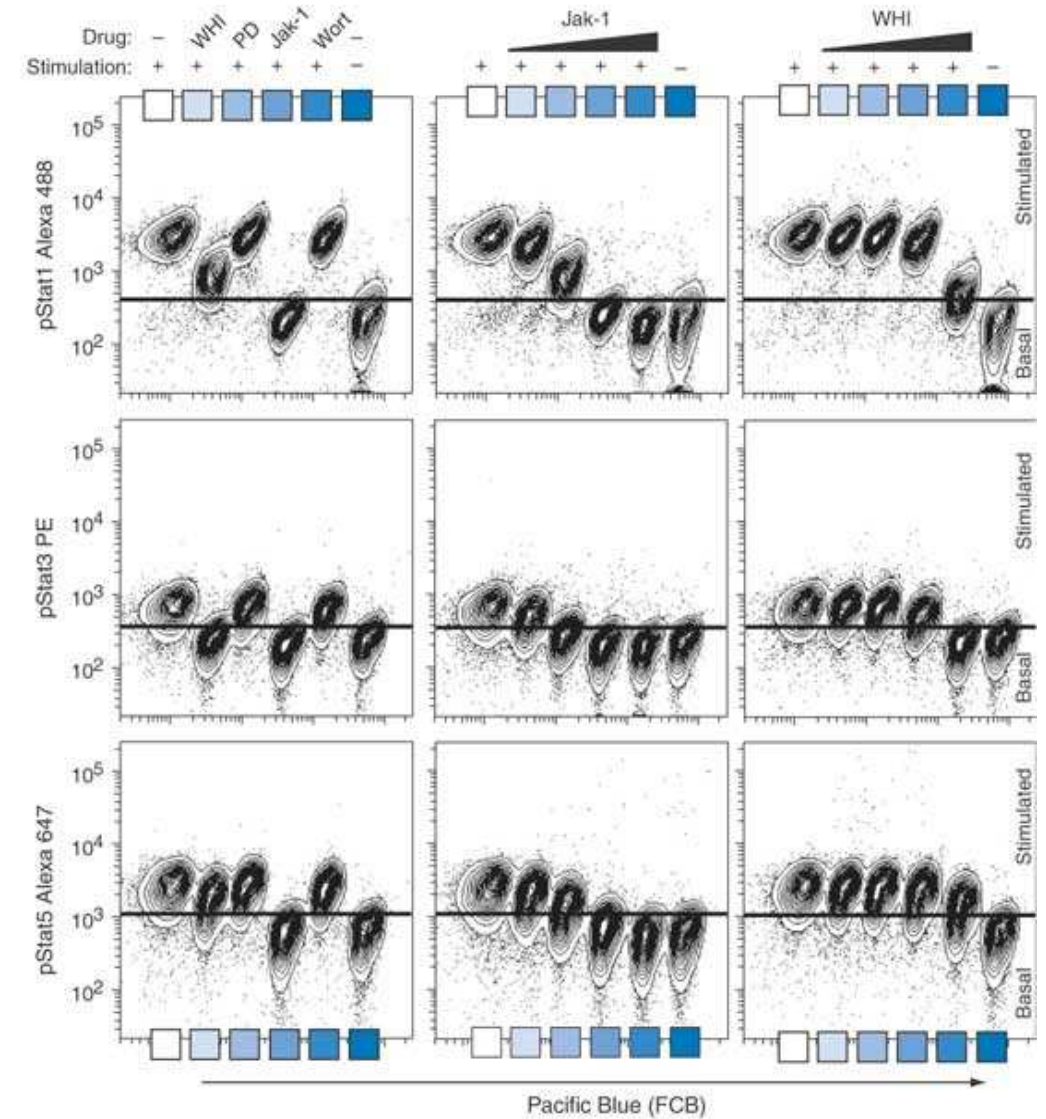
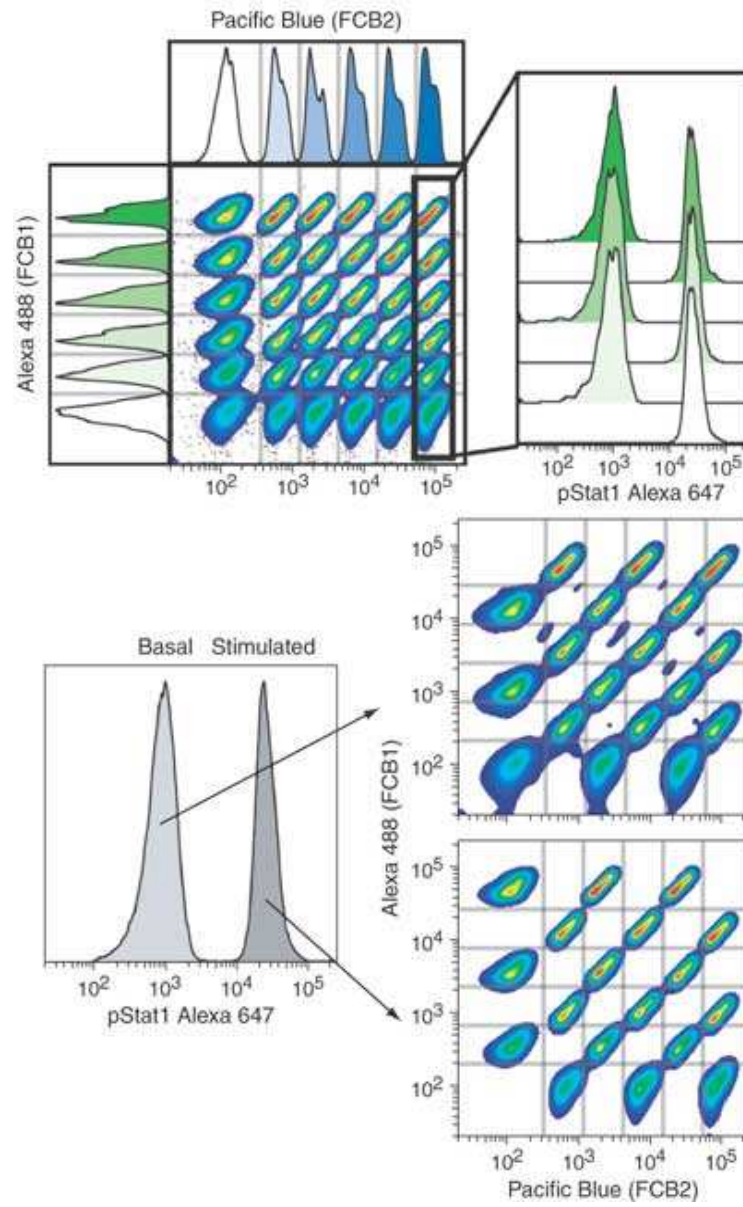
„Fluorescent cell barcoding“



- 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

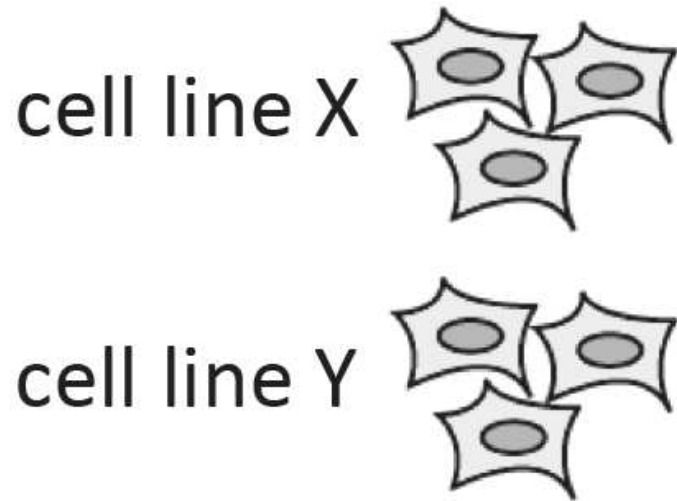
- there are XY vials in LN

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

How to get the best of it all?



Fluorescent barcode



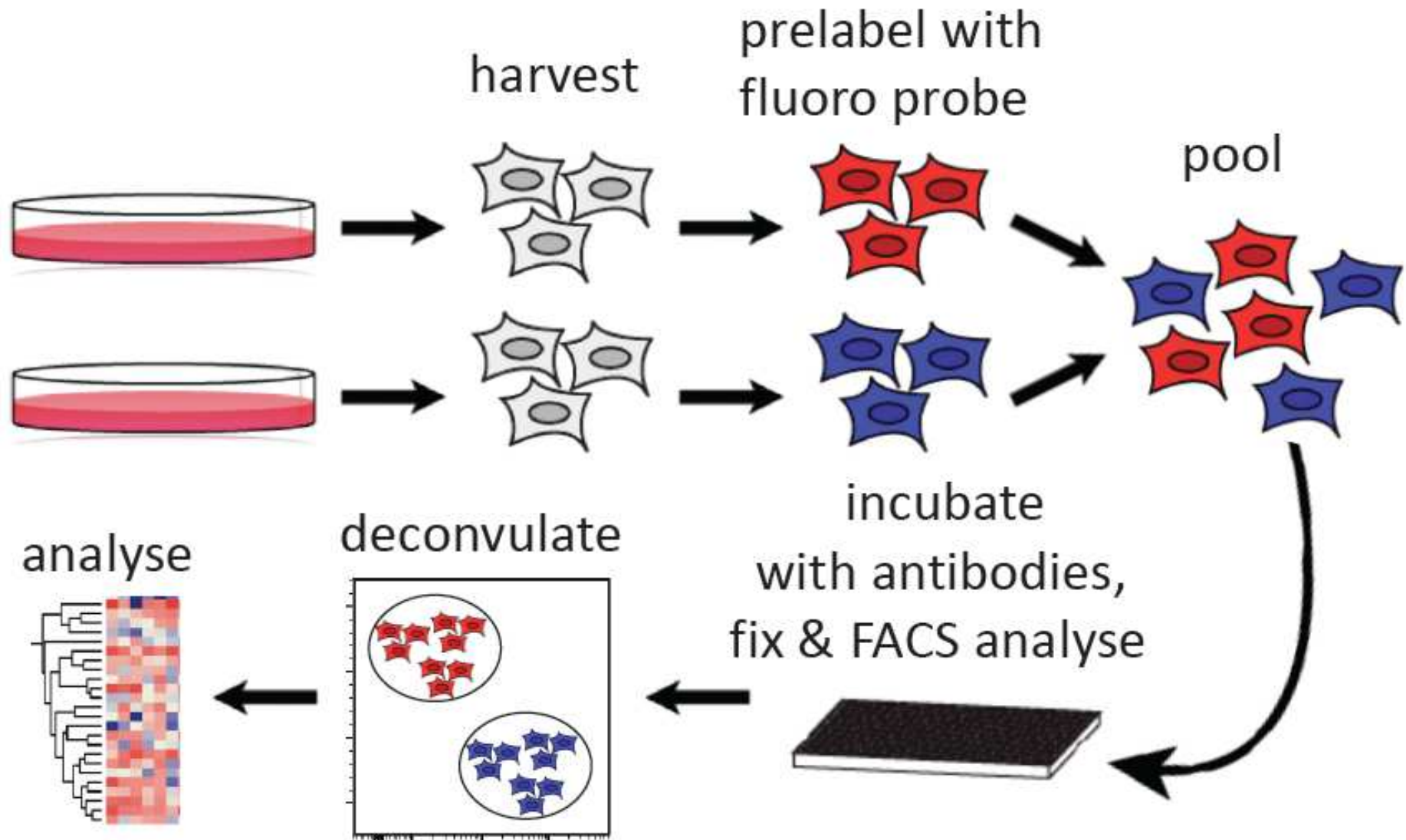
possibility 1 – fluorescent proteins

- which, how many, isn't it too laborous w/out lentiviral TXF?

possibility 2 – amino-reactive probes, lipophilic dyes...

- how to choose the right one?

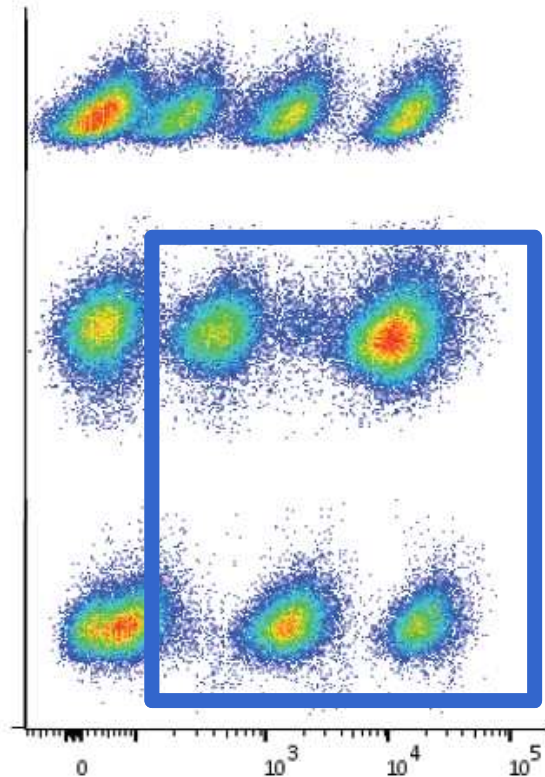
Final workflow



The optimal concentration issue

HOW TO TEST IT:
10x serial dilution

CellTrace CFSE



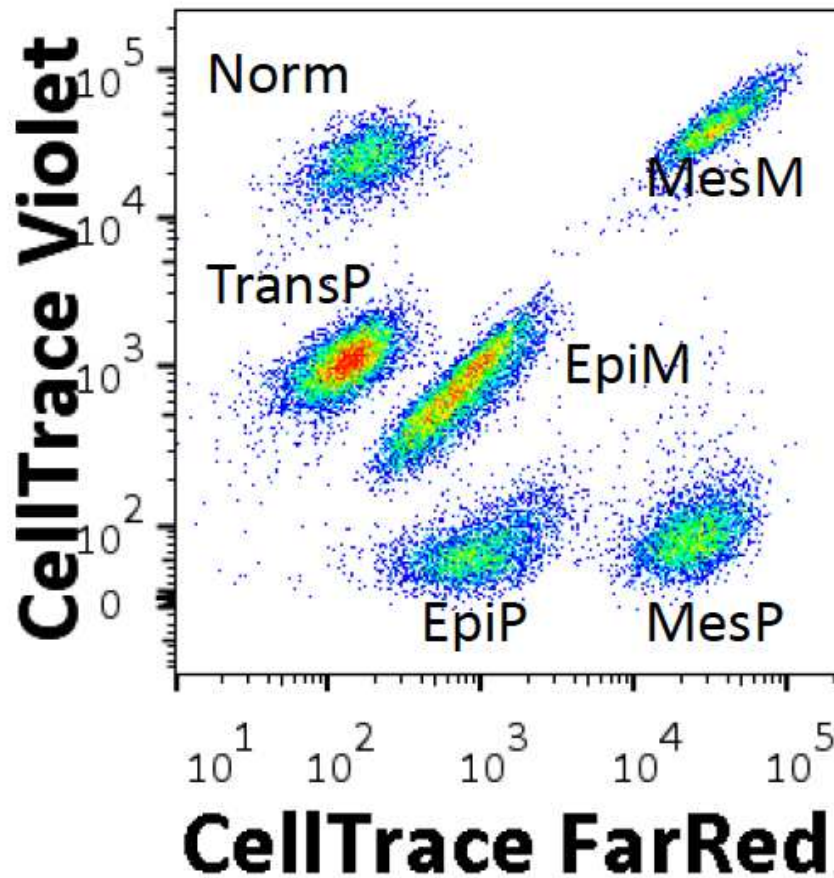
CellTrace DDAO

CellTrace Violet

REQUIREMENTS:

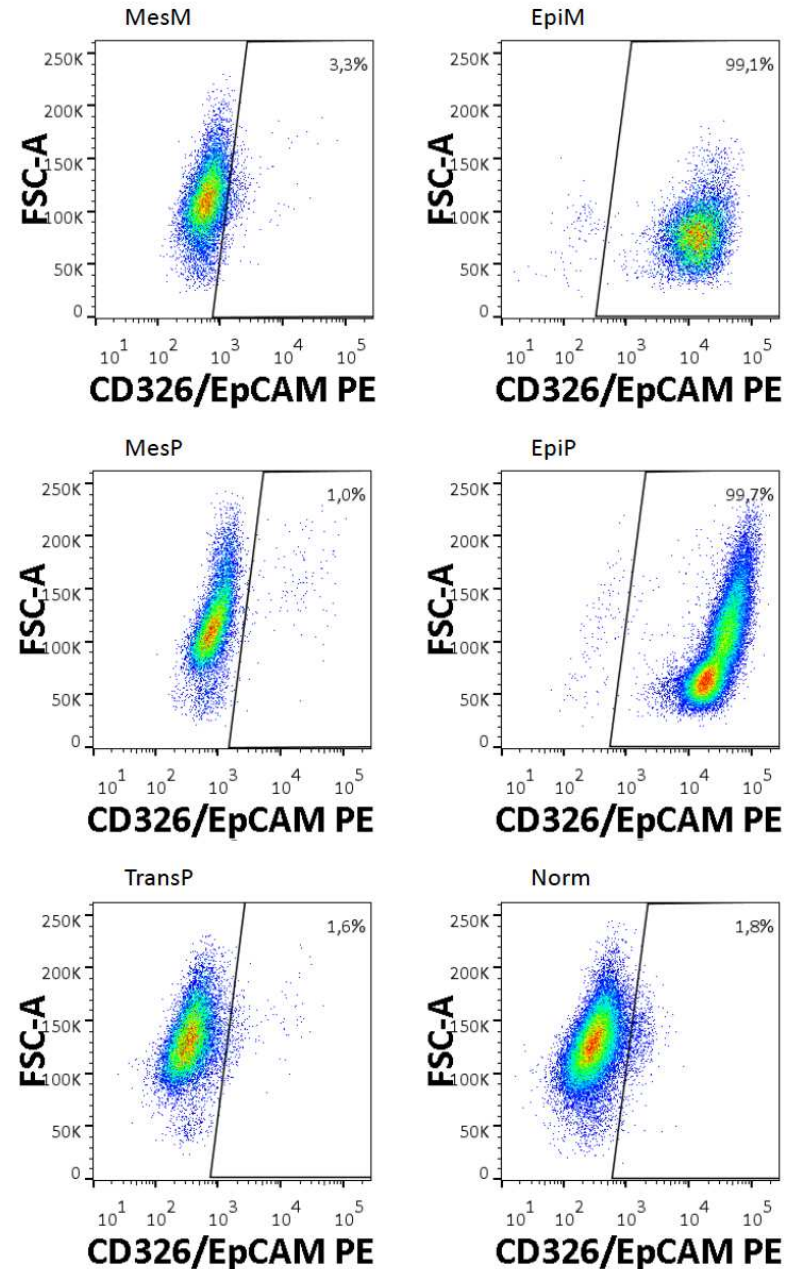
- optimal resolution
- compatibility w/ PE

Sample results I



EpCAM

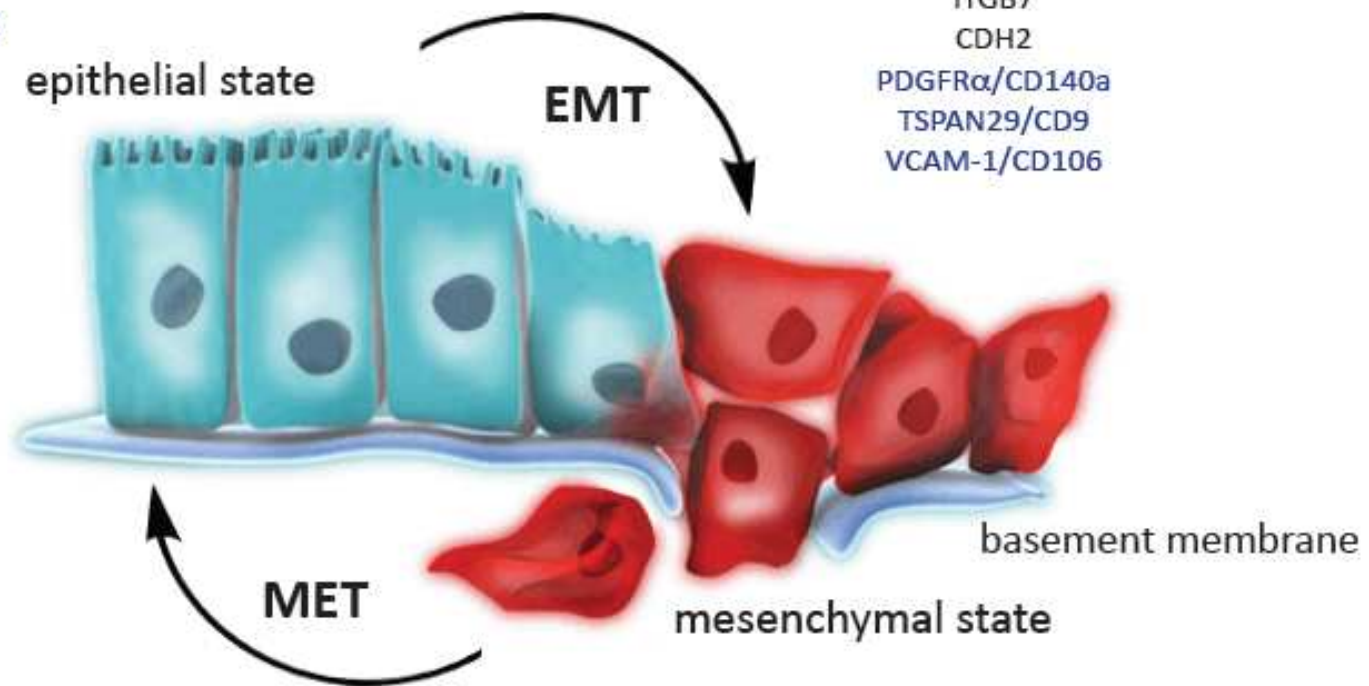
- marker of epithelial cells
- commonly lost during EMT



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



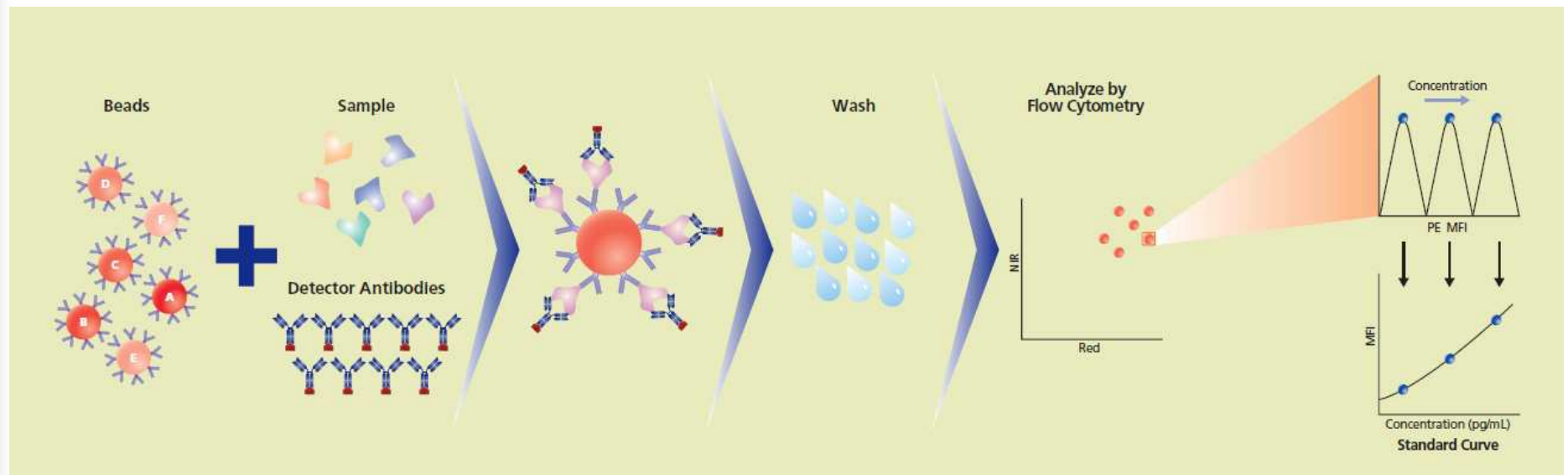
BCSC
CD24⁻CD44^{high}

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

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

Cytometric bead array (CBA)

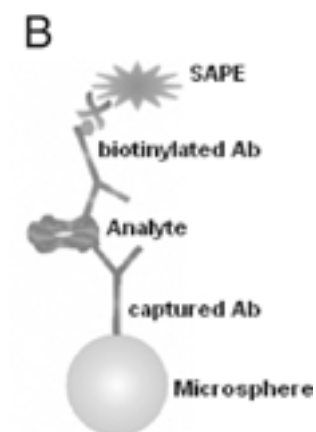
- Multiplexed Bead-Based Immunoassays
- flow cytometry application that allows users to quantify multiple proteins simultaneously



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	

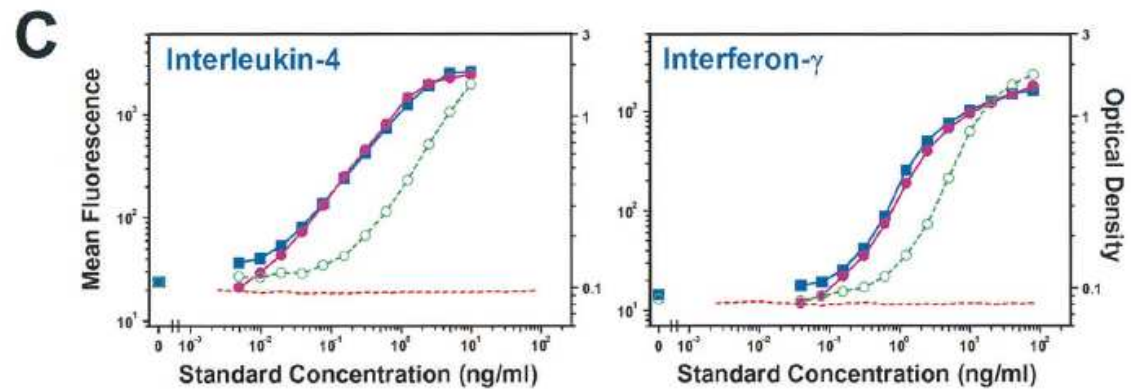
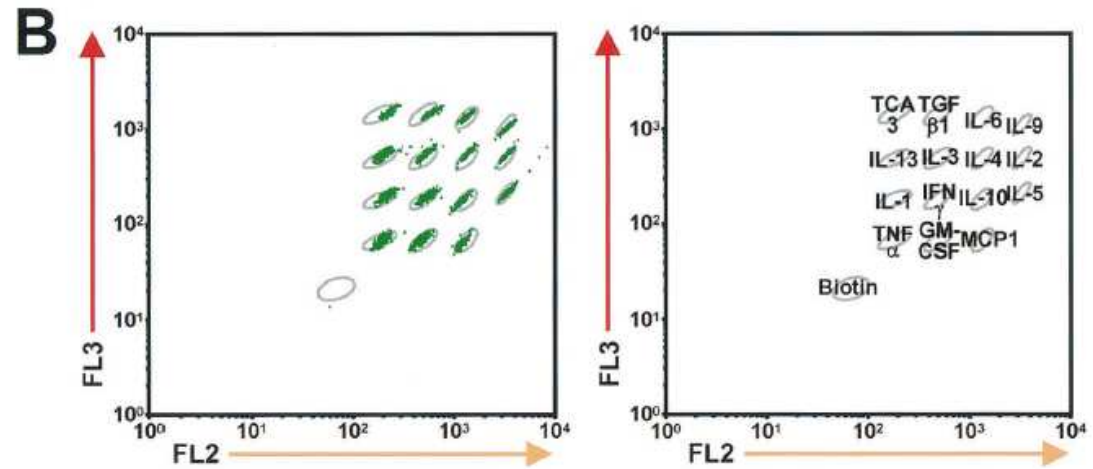
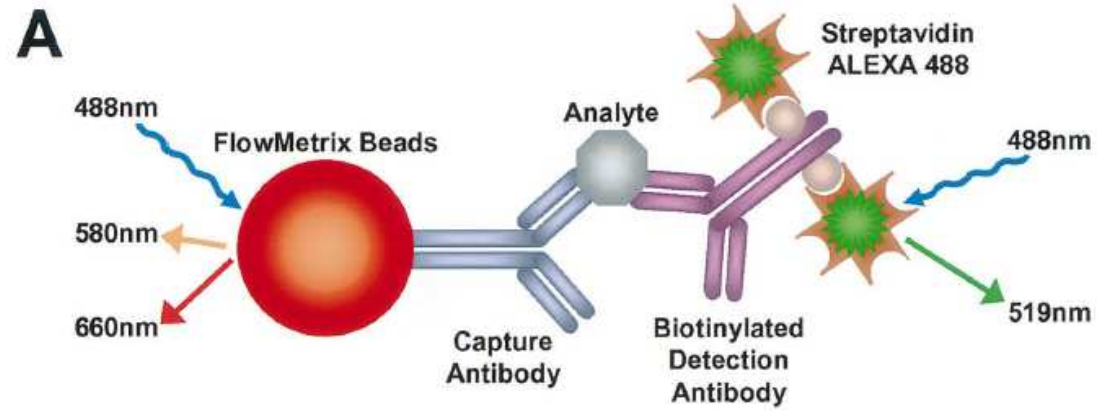


ELECTROPHORESIS

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

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

CBA





CBA

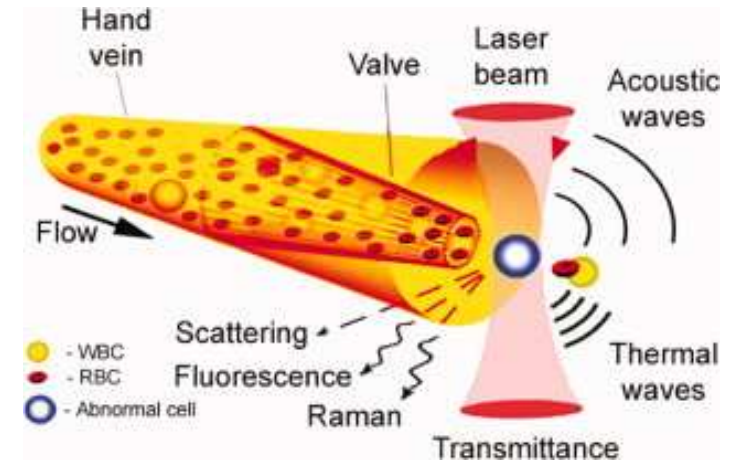
- multiplexing capabilities
- speed
- incorporation of multiple assay formats
- rapid assay development and reasonable cost
- automation



ex vivo flow cytometrie - limitace

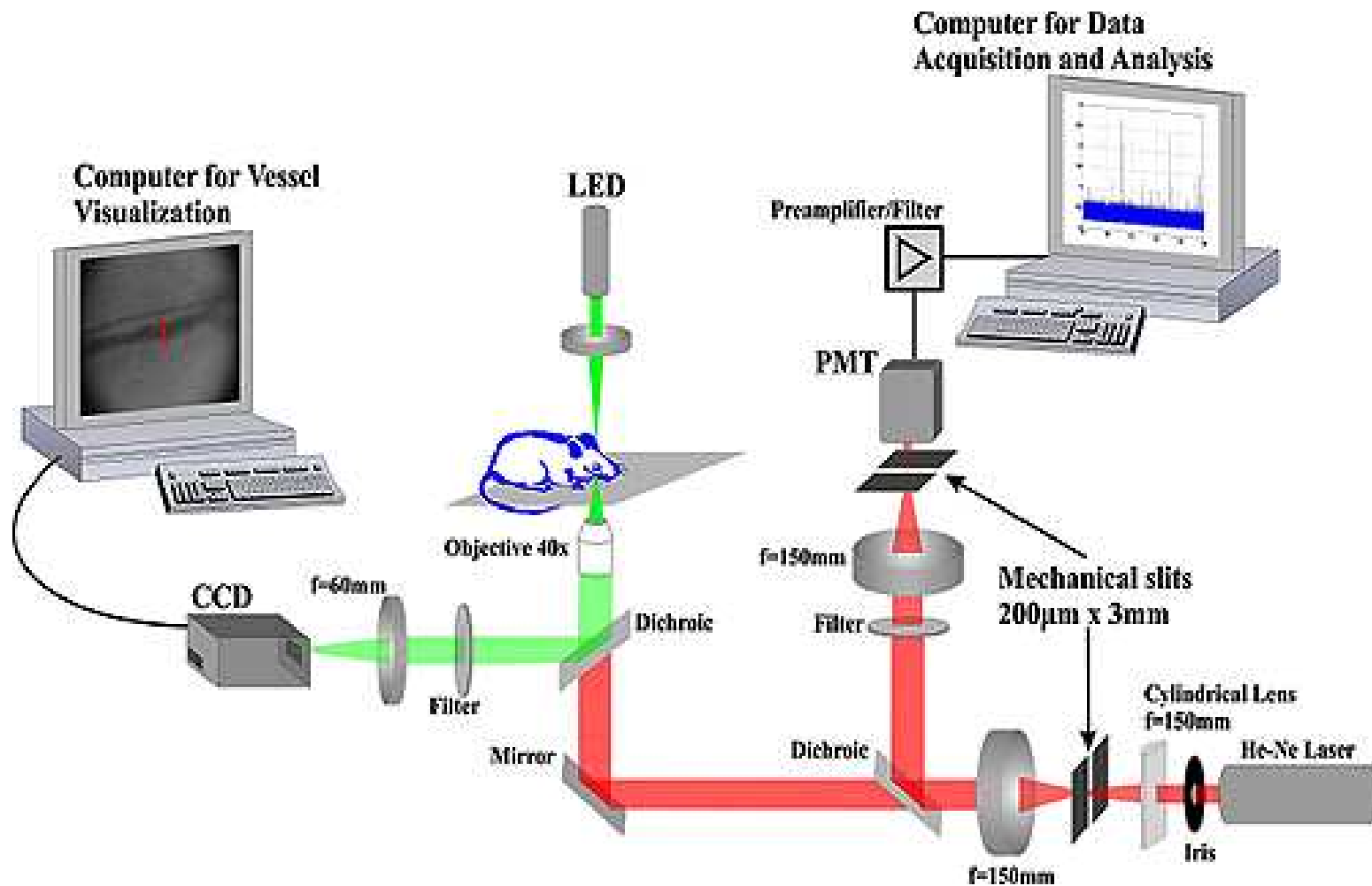
- Ovlivnění některých vlastností buněk (morfologie, exprese znaků);
- neumožňuje dlouhodobější studie buněčného metabolismu a buněčných interakcí (komunikace, adheze) v přirozeném tkáňovém mikroprostředí;
- další:
 - nízká citlivost pro detekci vzácných buněčných subpopulací (1-10 buněk/ml ~ 5000 – 50000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

in vivo flow cytometry – základní principy



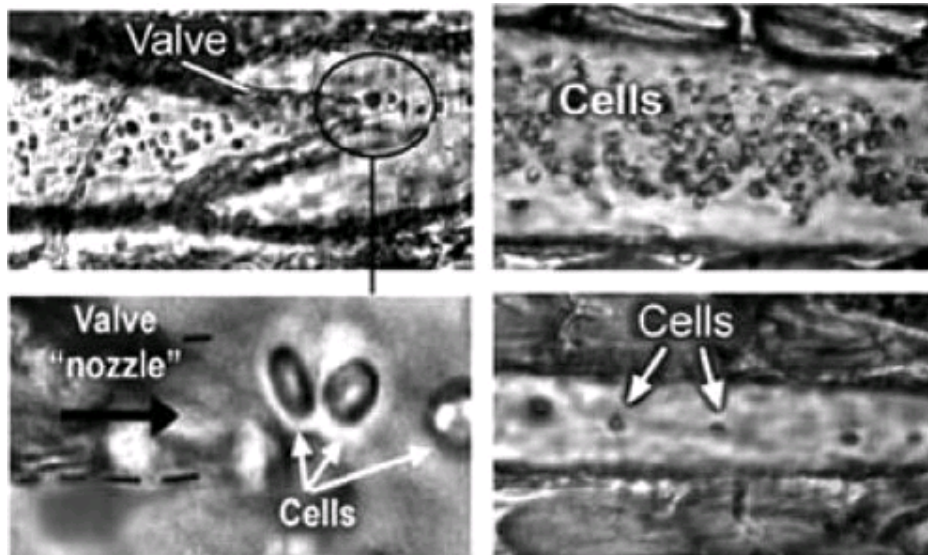
- Zobrazení buněk přímo v krevním nebo lymfatickém řečišti.
- Vizualizace pomocí CCD nebo CMOS kamery po ozáření konvenční mikroskopickou lampou nebo lasery.
- Detekce absorpce, fluorescence, Ramanova spektra, fototermálních nebo fotoakustických signálů.

in vivo flow cytometry



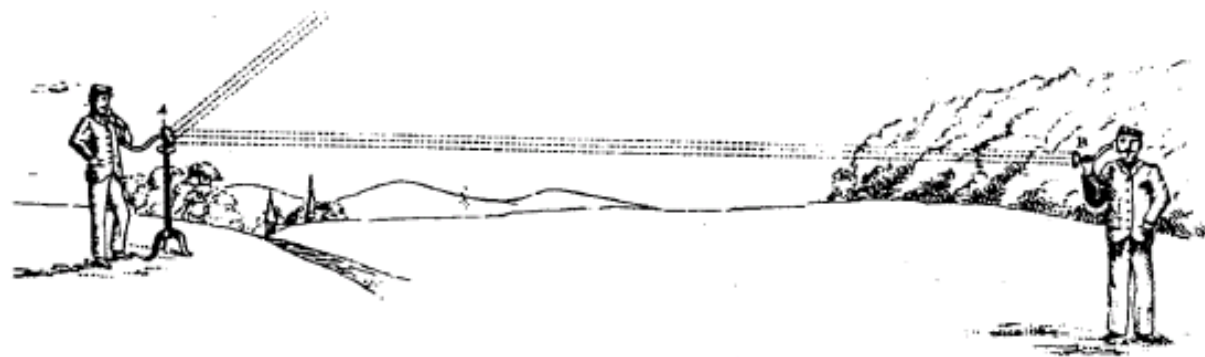
in vivo flow cytometry – bez značení

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



photoacoustic and photothermal imaging

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



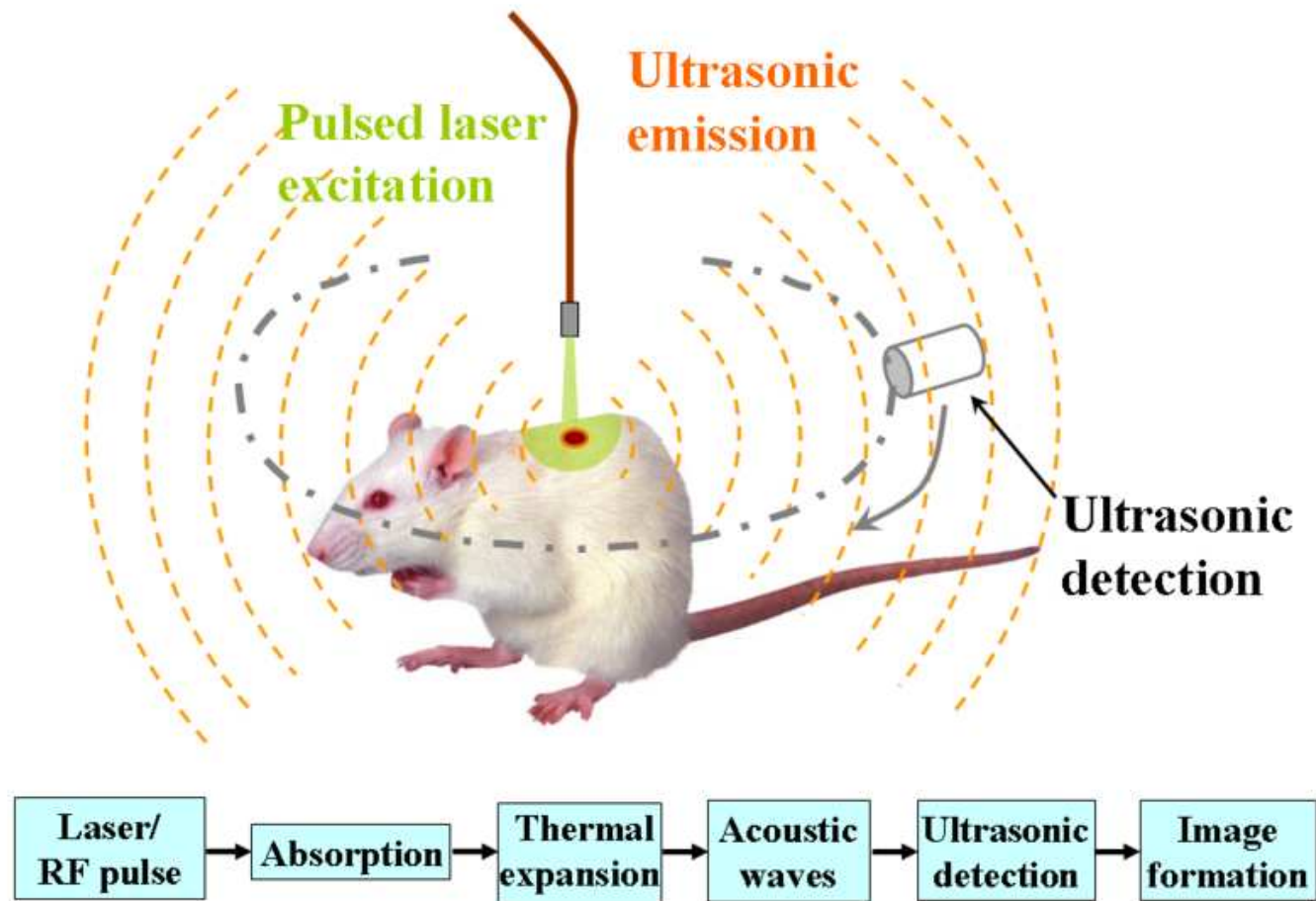
The Photoacoustic Effect

Benjamin T. Spike

Physics 325

April 21, 2006

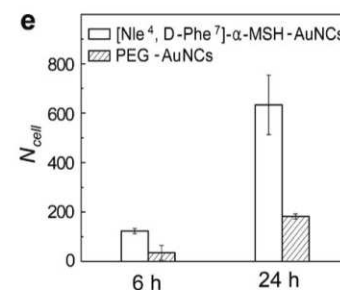
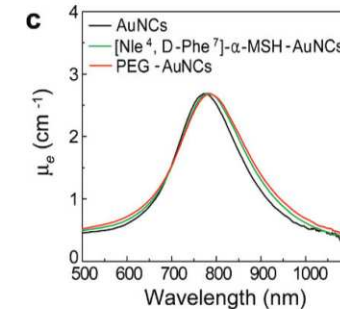
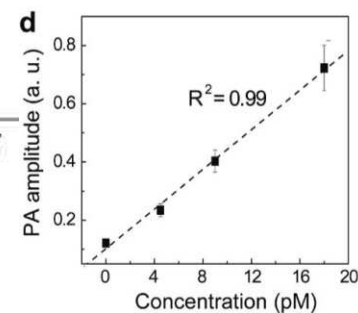
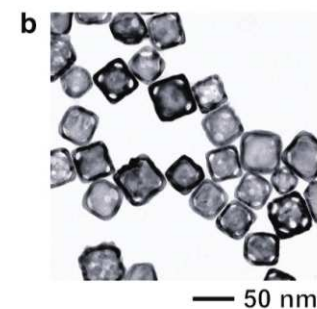
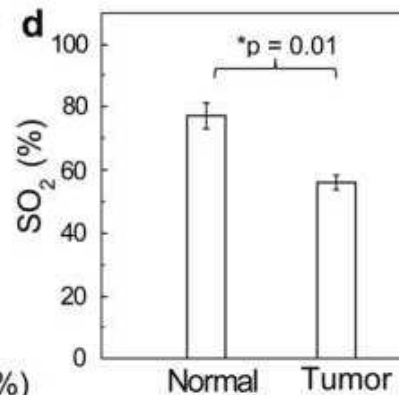
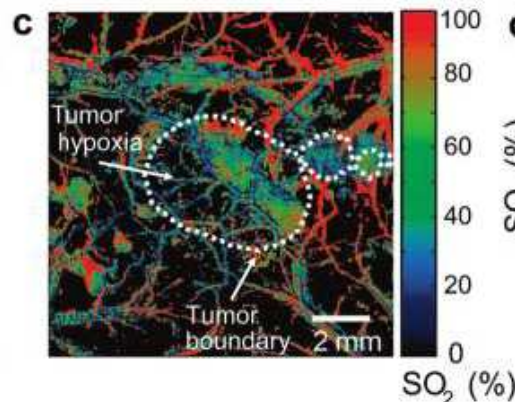
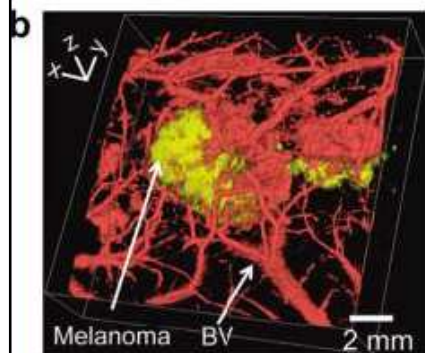
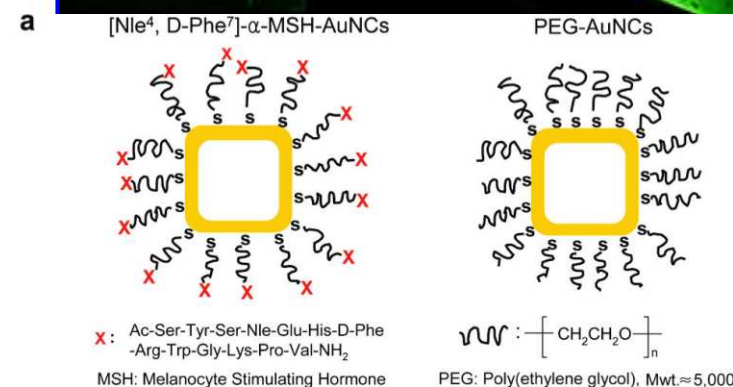
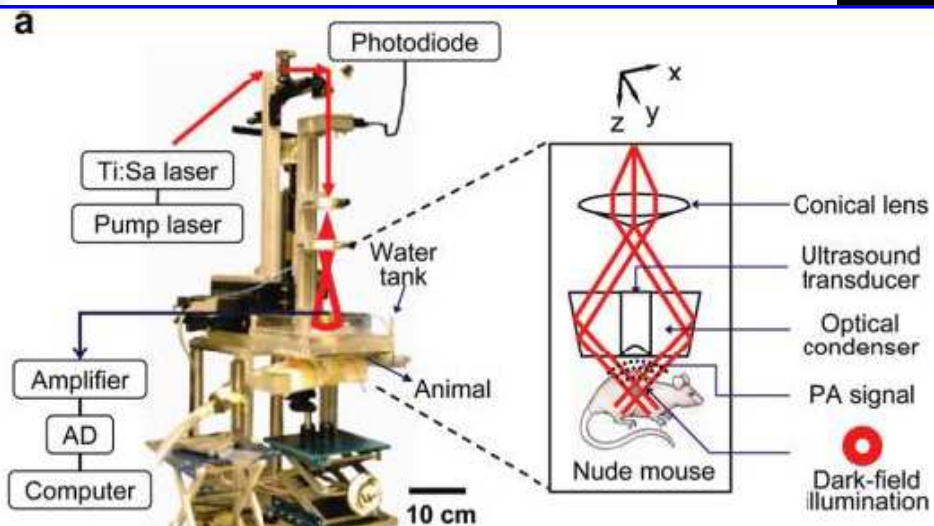
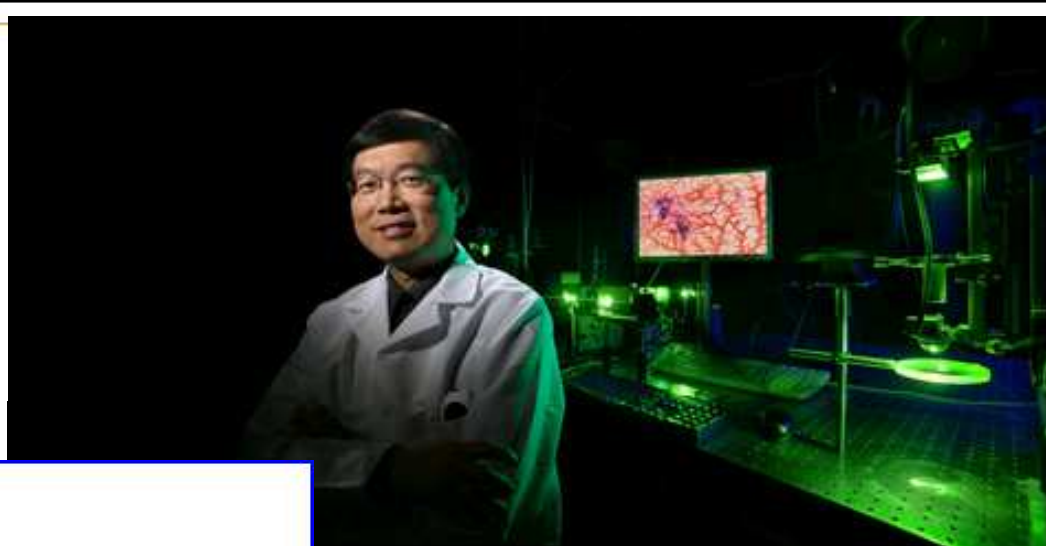
Schematic illustration of photoacoustic imaging



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

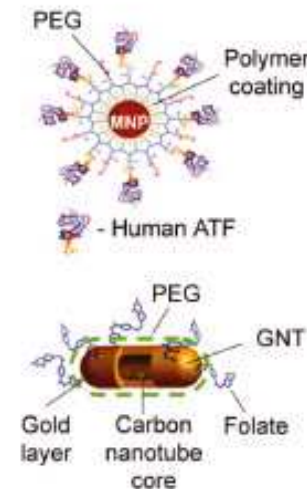
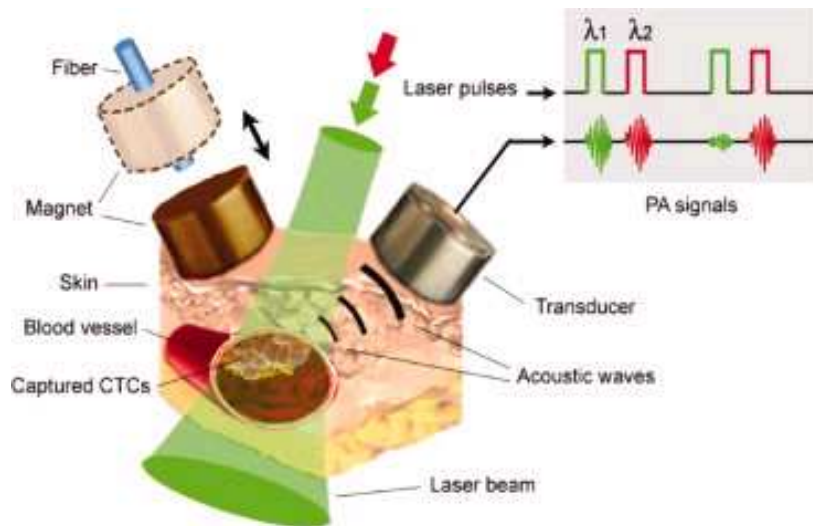
Chulhong Kim,^{1,5} Eun Chul Cho,^{1,5} Jingyi Chen,¹ Kwang Hyun Song,¹ Leslie Au,⁶ Christopher Favazza,¹ Qiang Zhang,⁶ Claire M. Cobley,¹ Feng Gao,⁶ Younan Xia,^{1,*} and Lihong V. Wang^{1,*}

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

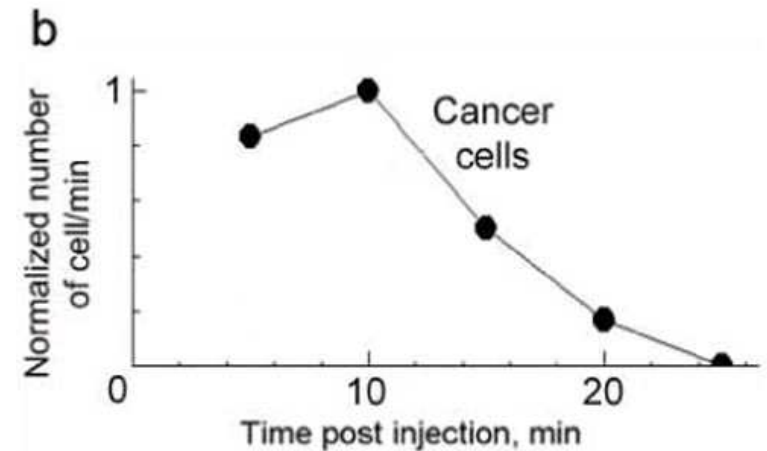
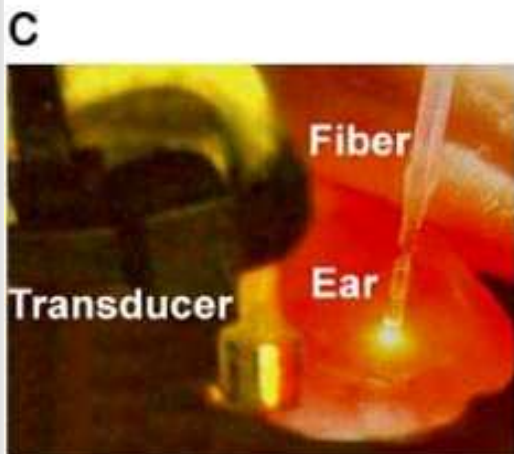
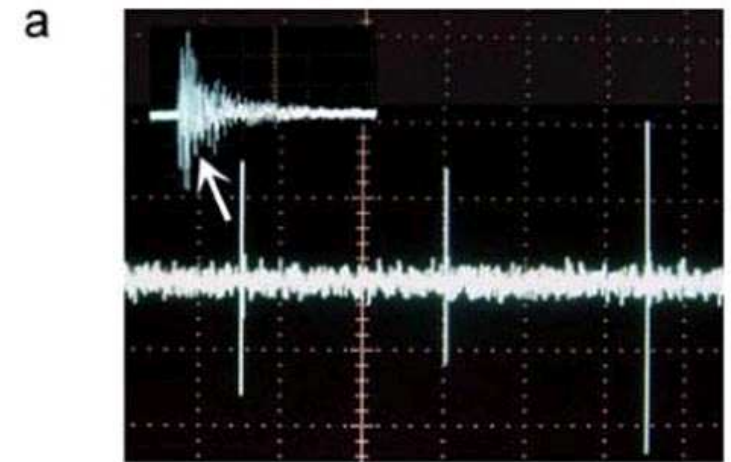
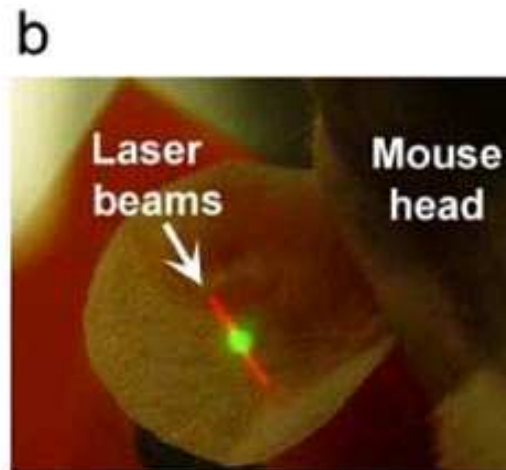
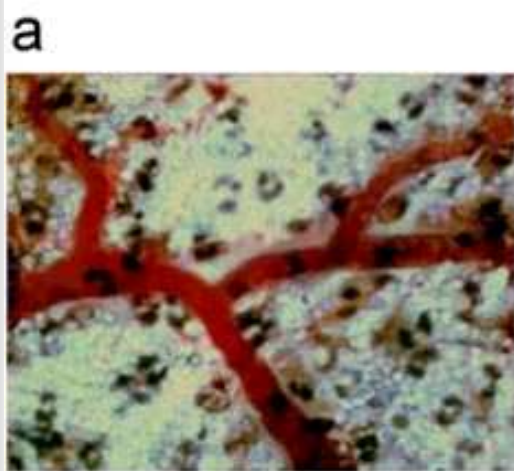


In vivo flow cytometrie – detekce specifických signálů

- Detekce fotoakustických a fototermálních jevů



in vivo flow cytometry - aplikace



Shrnutí přednášky

- „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 co je to „high-throughput“, průtoká cytometrie ...a jak se v ní může uplatnit princip vícebarevného značení.
2. znát základní principy měření a sortování chromozómů pomocí průtokového cytometru;
3. mít představu o možných aplikacích průtokové cytometrie v mikrobiologii, hydrobiologii a studiu bezobratlých;
4. rozumět limitům a principům *in vivo* průtokové cytometrie.