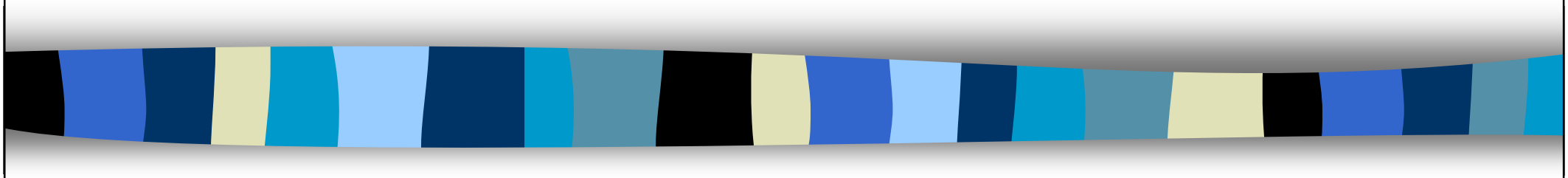
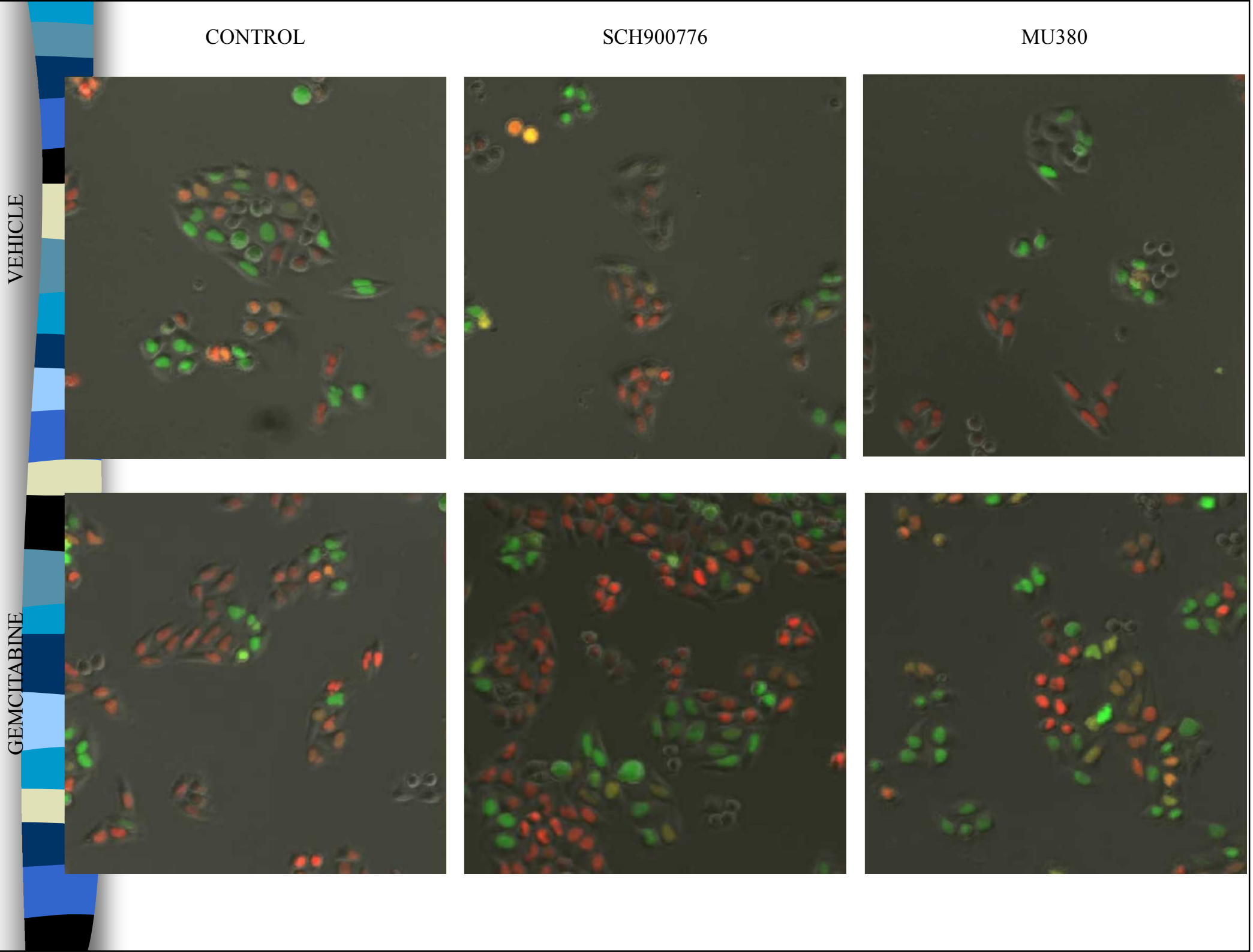
Bi9393 Analytická cytometrie



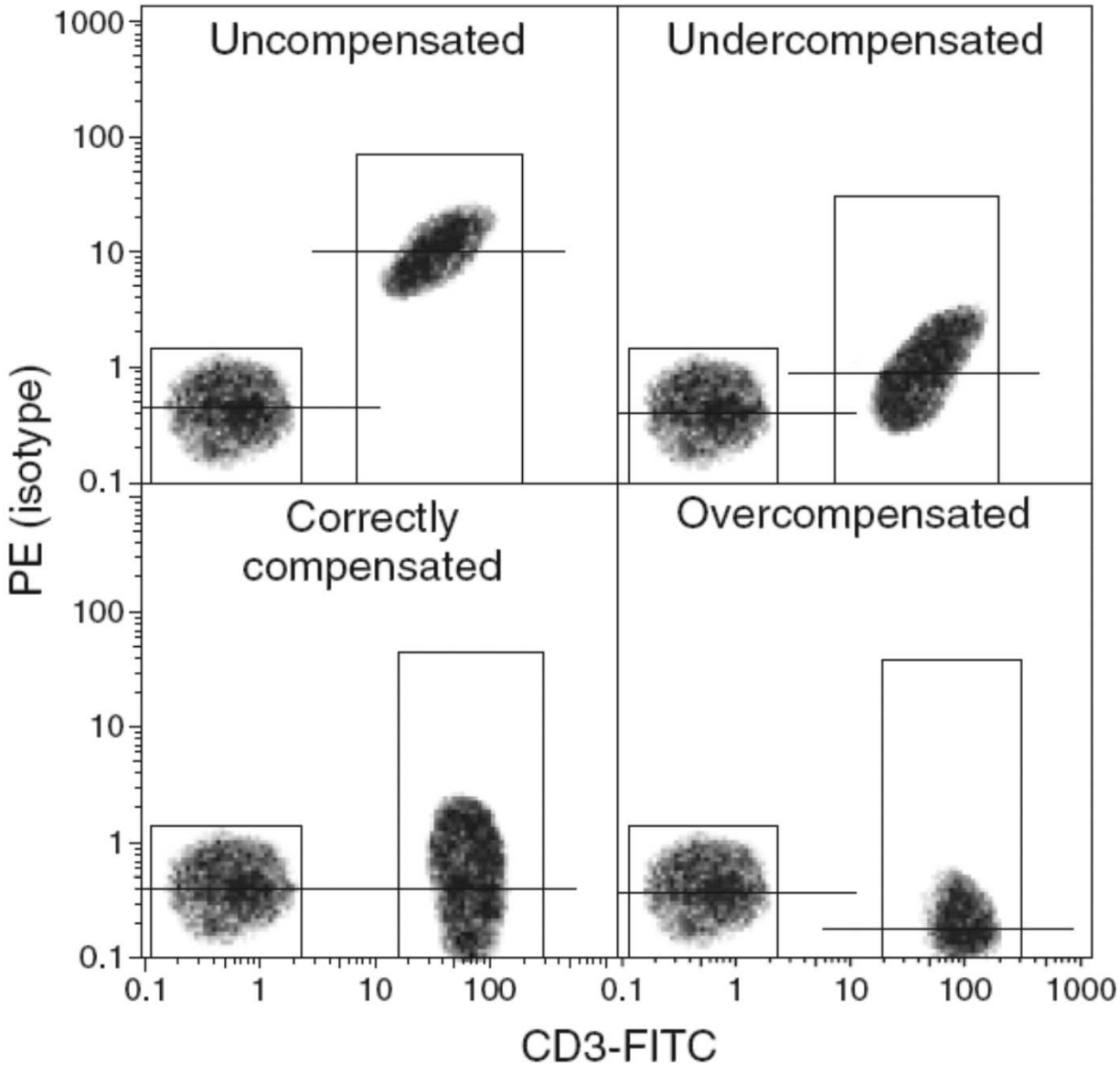
Karel Souček, Ph.D.

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

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


CONTROL
SCH900776
MU380
VEHICLE
GEMCITABINE

Kompenzace fluorescenčního signálu



Factors that Effect Compensation

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

BD FACSAria™ III and Special Order BD FACSAria (typical setup)[b]	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	561	Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	PE-Cy5			
		Infrared	PE-Cy7			
	640	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700			
		Infrared	BD APC-H7	APC-Cy7		
	405	Blue	Brilliant Violet™ 421	BD Horizon™ V450	VPD450	Pacific Blue™
		Green	BD Horizon V500	AmCyan		
		Orange	Brilliant Violet™ 605[a]			
	375[a]	Blue	Hoechst 33342			

BD

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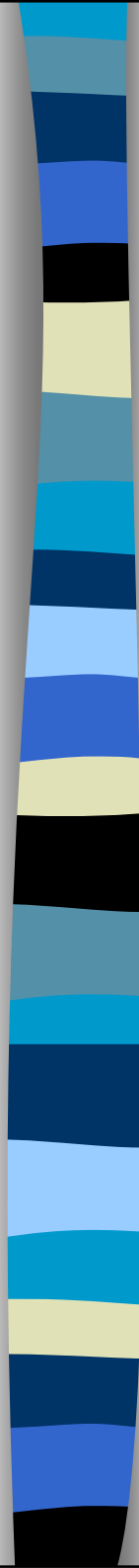
FLUORISH

Streamlining Flow Cytometry

chromocyte



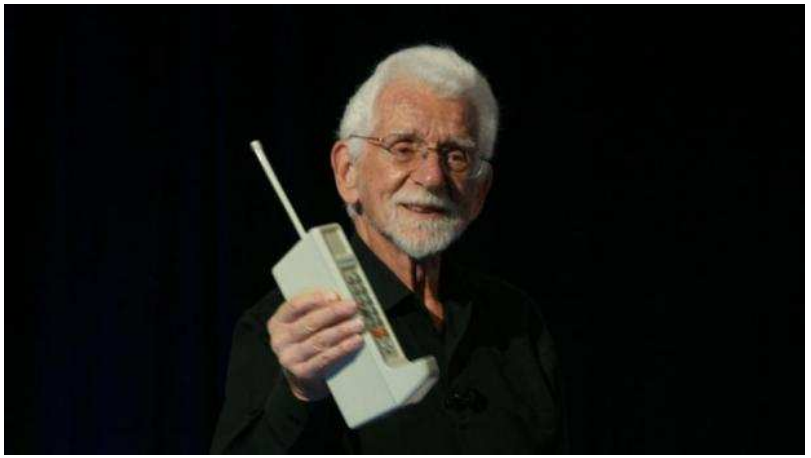
- Řešení?

  #1

Idea (1931)

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

Invention (1973)



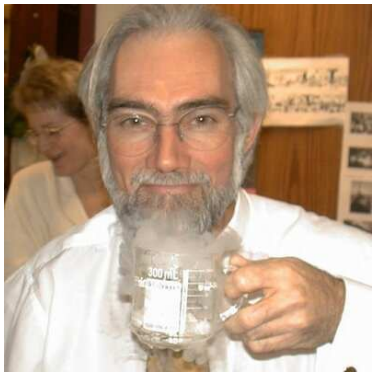
Martin Cooper, Motorola

Innovation (2007)

Steve Jobs, Apple

Spectral flow cytometry

J.P. Robinson, Purdue University



Cytometry Part A • 81A: 35–44, 2012

ORIGINAL ARTICLE

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

MIFlowCyt

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

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

2
DOI: 10.1017/S1431927605510328

Microsc Microanal 11(Suppl 2), 2005

Multispectral Flow Cytometry: Next Generation Tools For Automated Classification

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

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

US007280204B2

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

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

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

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

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

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

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
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	Silcott et al.	356/73
6,947,134	B2 *	9/2005	Chang et al.	356/318
7,057,712	B2 *	6/2006	Beck et al.	356/72

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 315 939	5/1989	

(Continued)

Spectral flow cytometry

SONY

Sony Biotechnology Inc.

US

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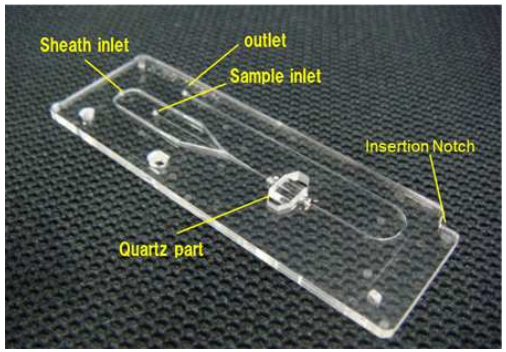
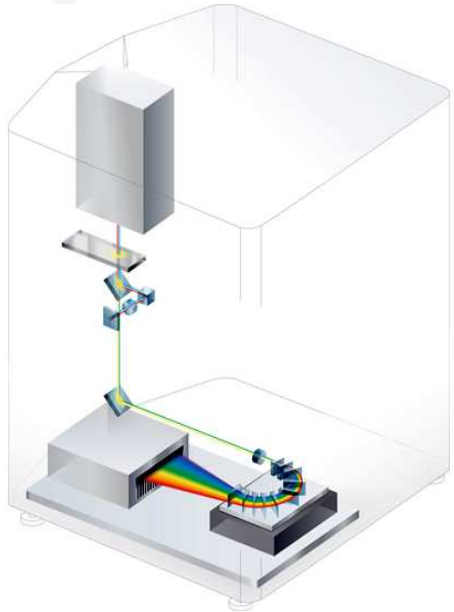
Overview Features Applications Specifications Literature

See Everything

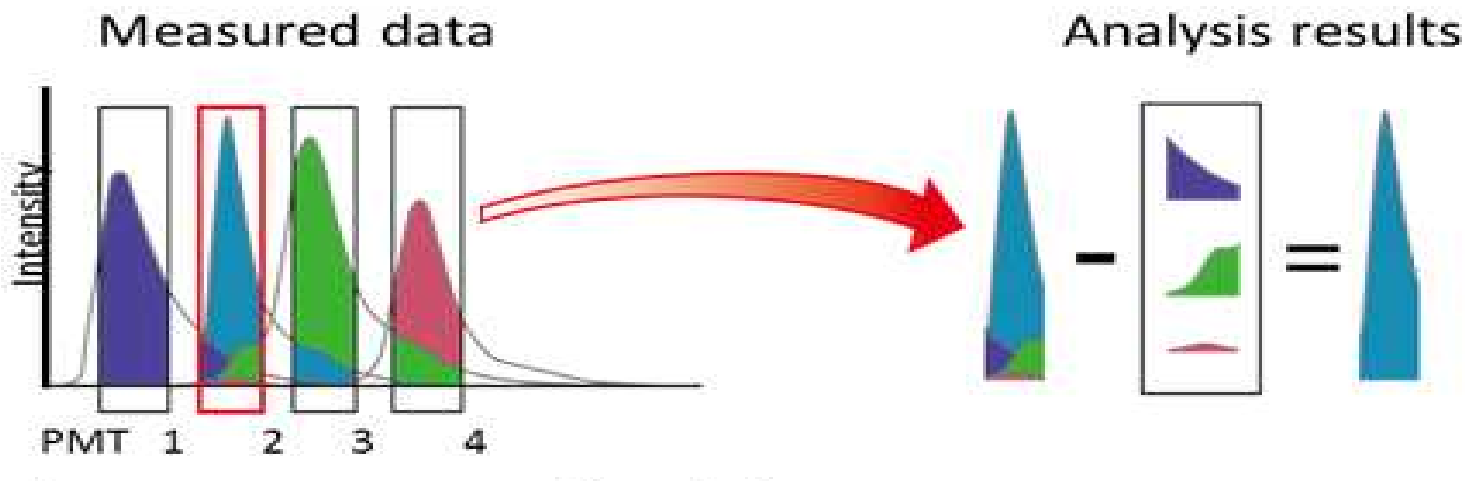
The SP6800 Spectral Analyzer is Sony Biotechnology Inc.'s newest innovative life science system fundamentally expanding the way cell and biomarker analysis can be performed. This system incorporates a unique optical bench, Blu-ray™ disc technology, and advanced algorithms to deliver some of the most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

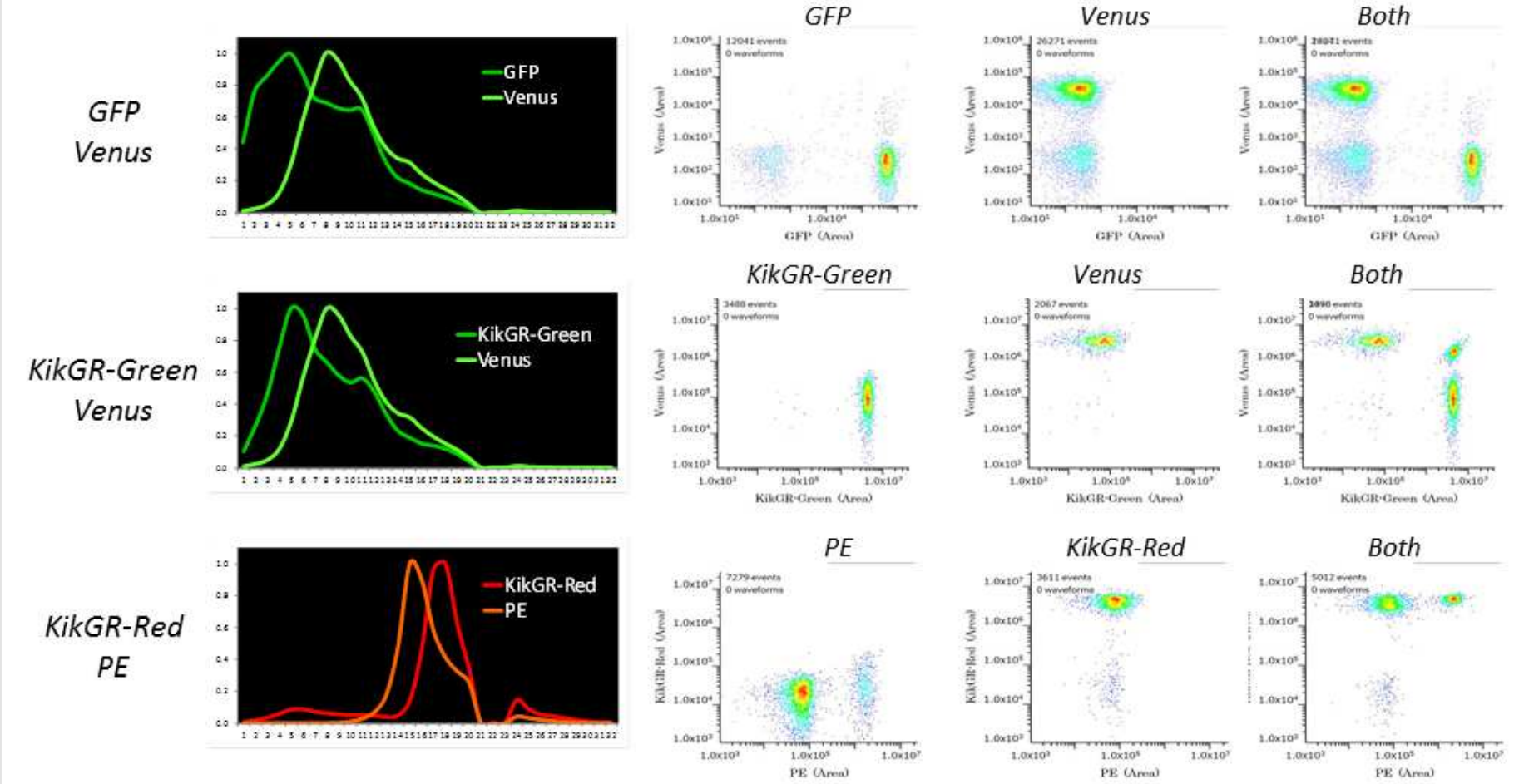
The SP6800 Spectral Analyzer also introduces new Flow Point technology to analyze core stream and sample event location within the flow cell. To improve accuracy of data, this system also provides unique functions to display and analyze cellular autofluorescence and allows the user to easily automatically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





Conventional vs. spectral analysis





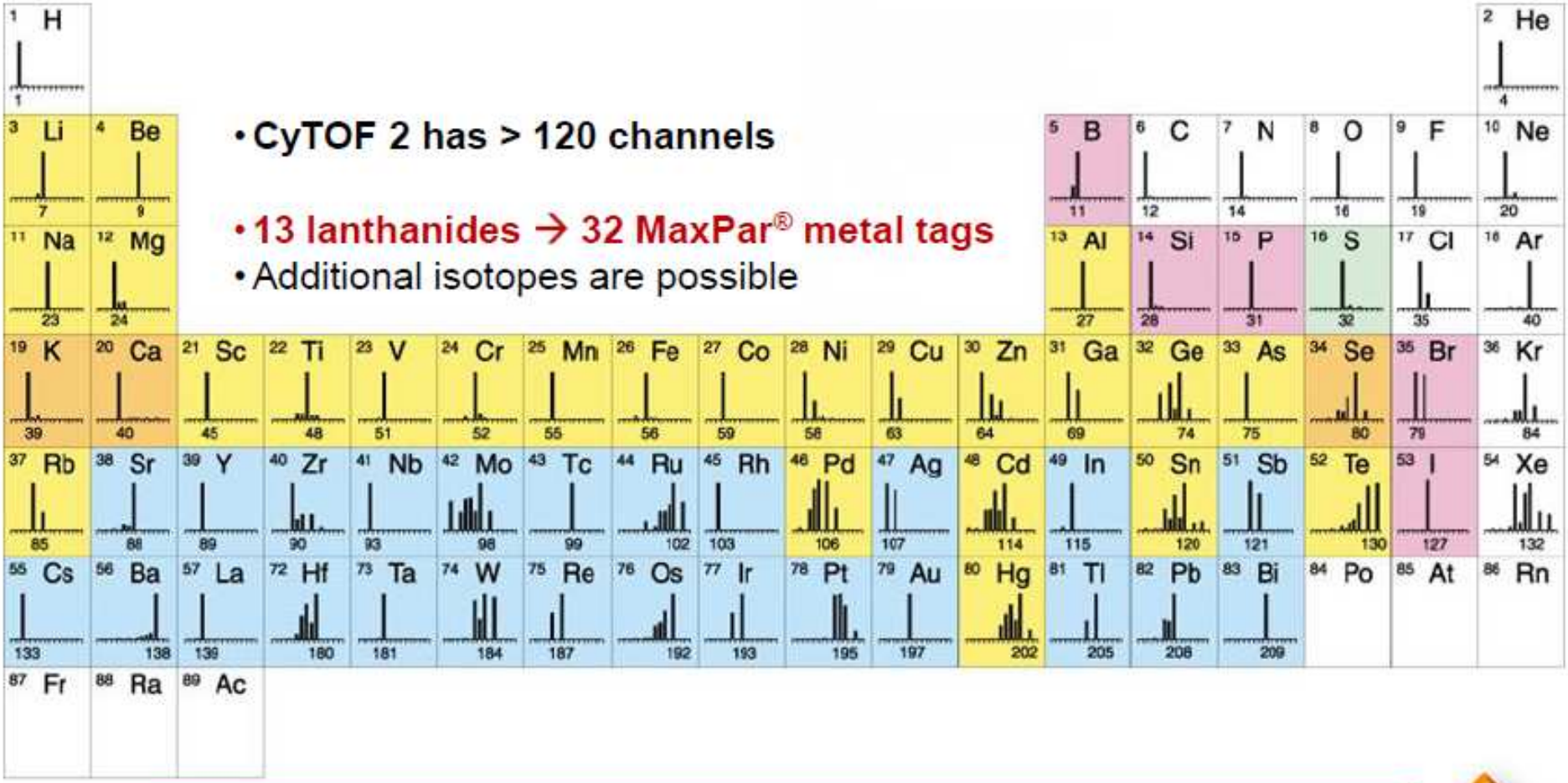
For revealing spatiotemporal regulation of immune cells, fluorescent proteins are very useful, which can be difficult to analyze with traditional flow cytometry technologies. These figures show how easily the SP6800 Spectral Analyzer can separate overlapping spectra of fluorescent proteins and fluorochromes.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

http://www.sonybiotechnology.com/images/sp6800/Fluorescent_Proteins_and_Fluorochromes.jpg

- Řešení?

  #2

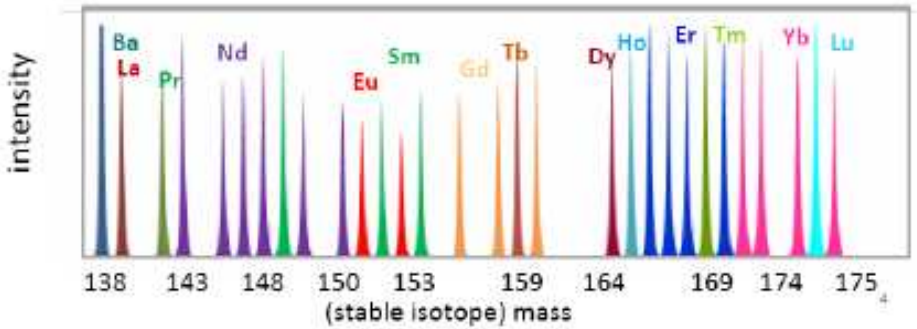
Probing with Isotopes

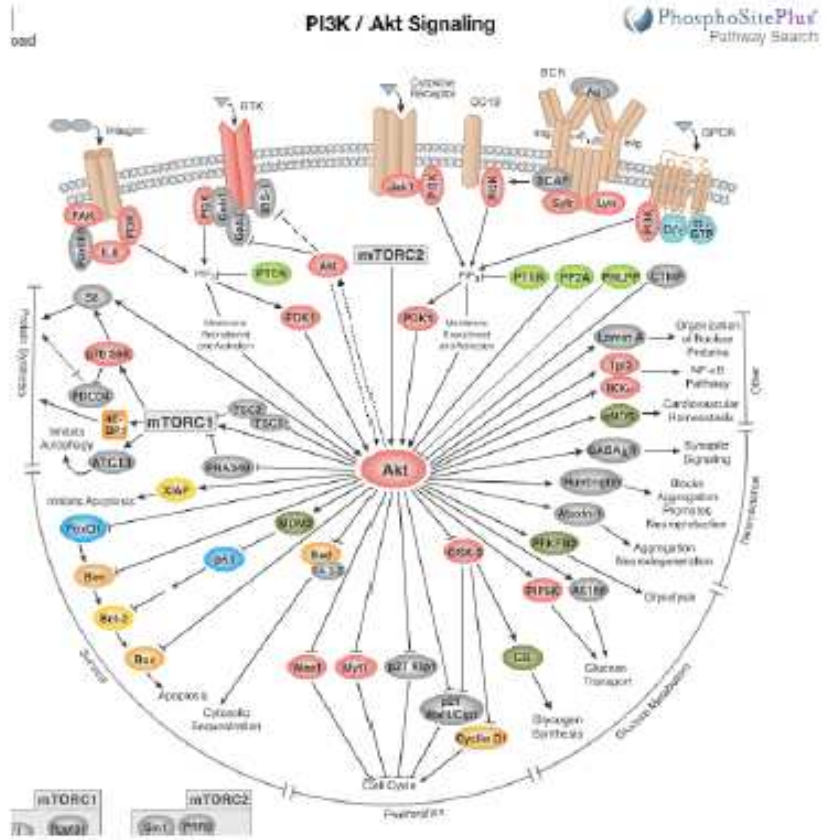


- **CyTOF 2 has > 120 channels**
- **13 lanthanides → 32 MaxPar® metal tags**
- Additional isotopes are possible

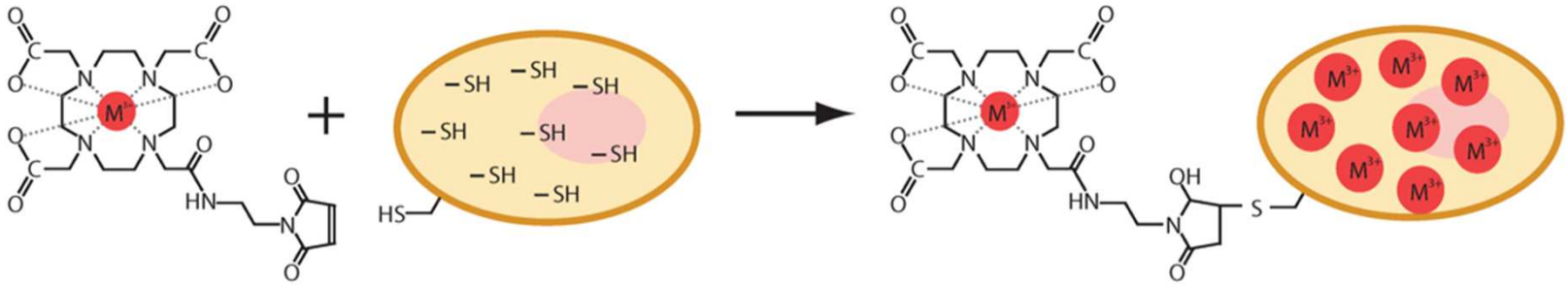
Why Mass Cytometry?

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





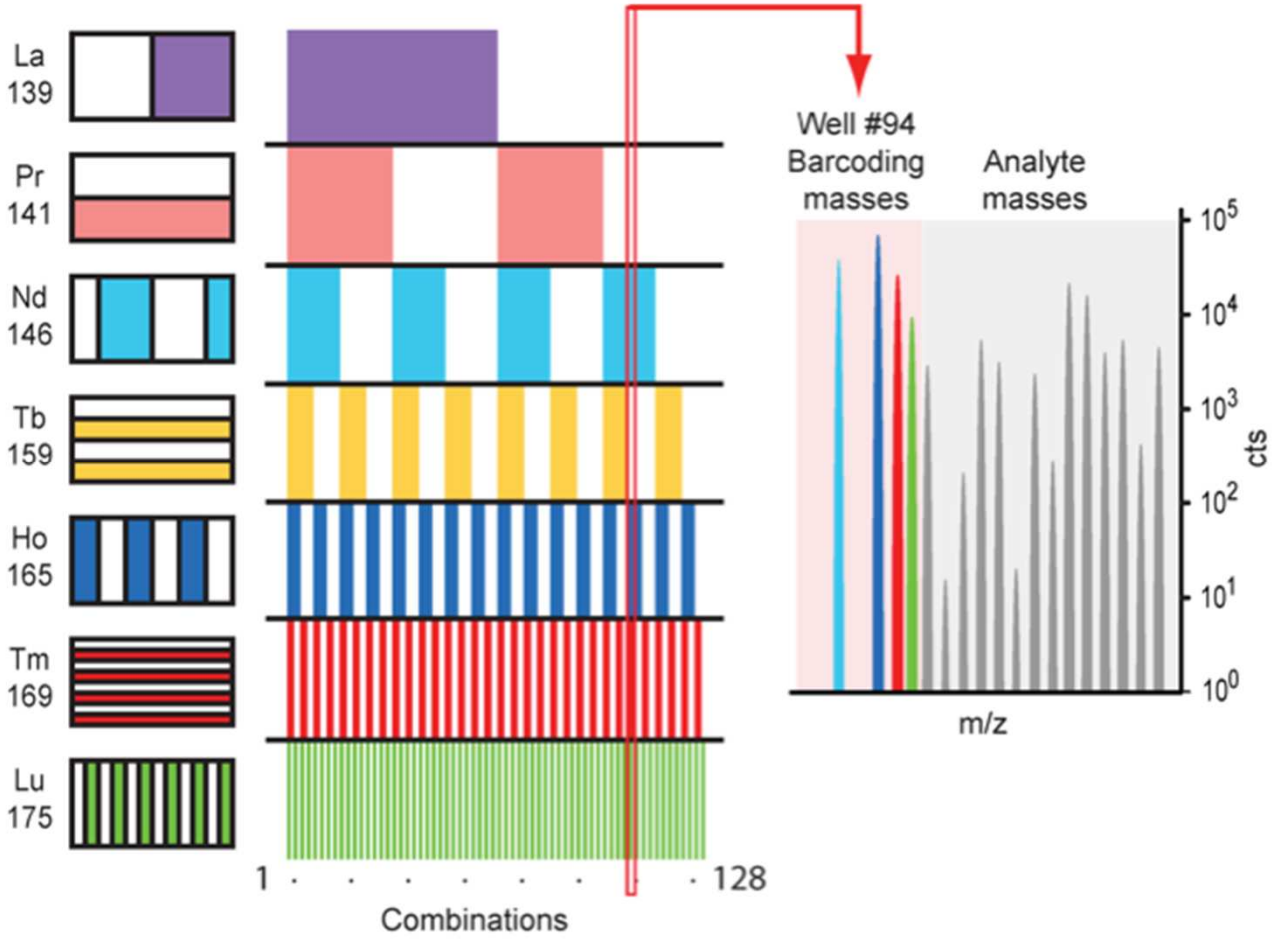
Single Cell Mass Cytometry



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

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

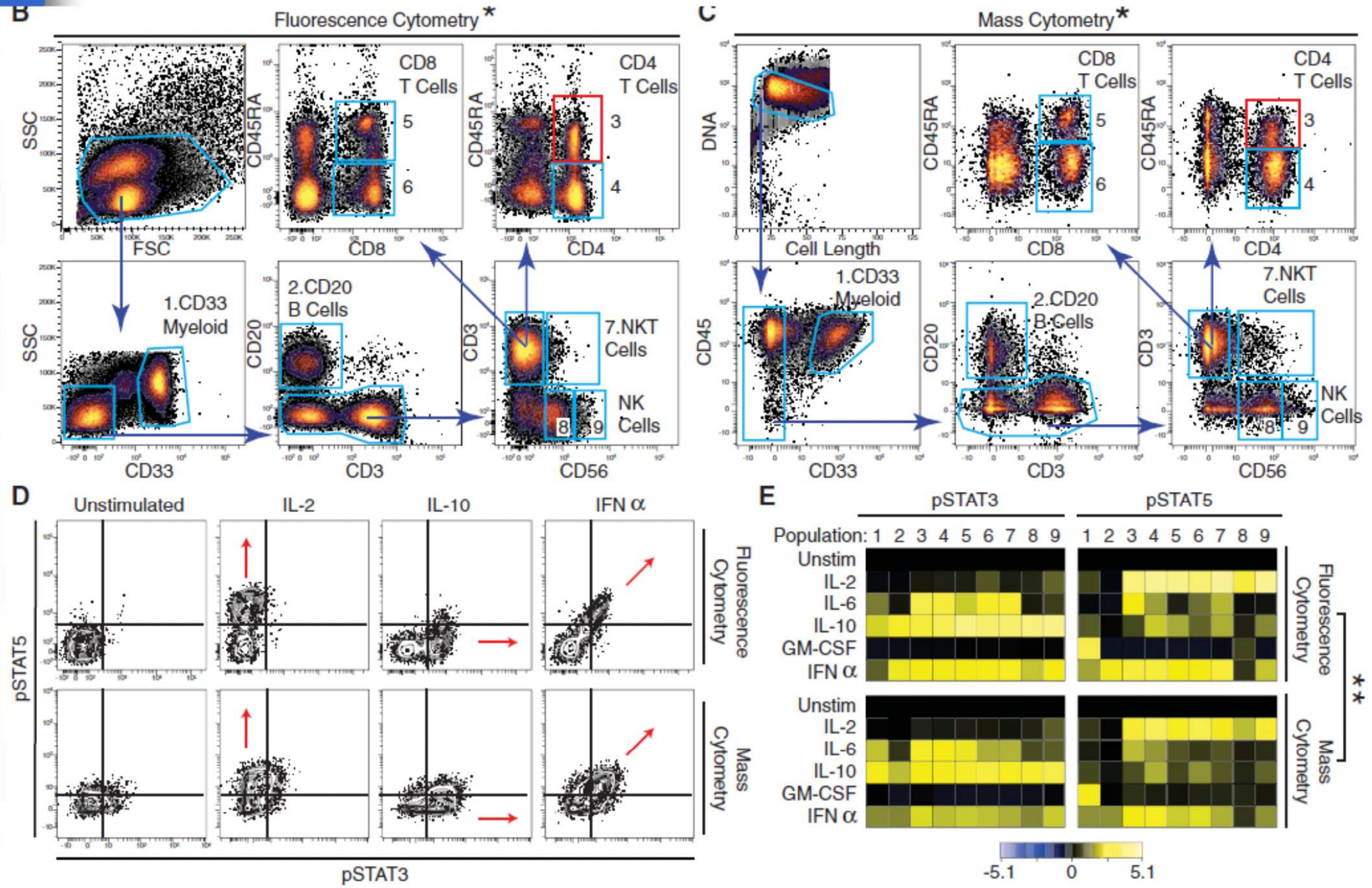
Single Cell Mass Cytometry



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

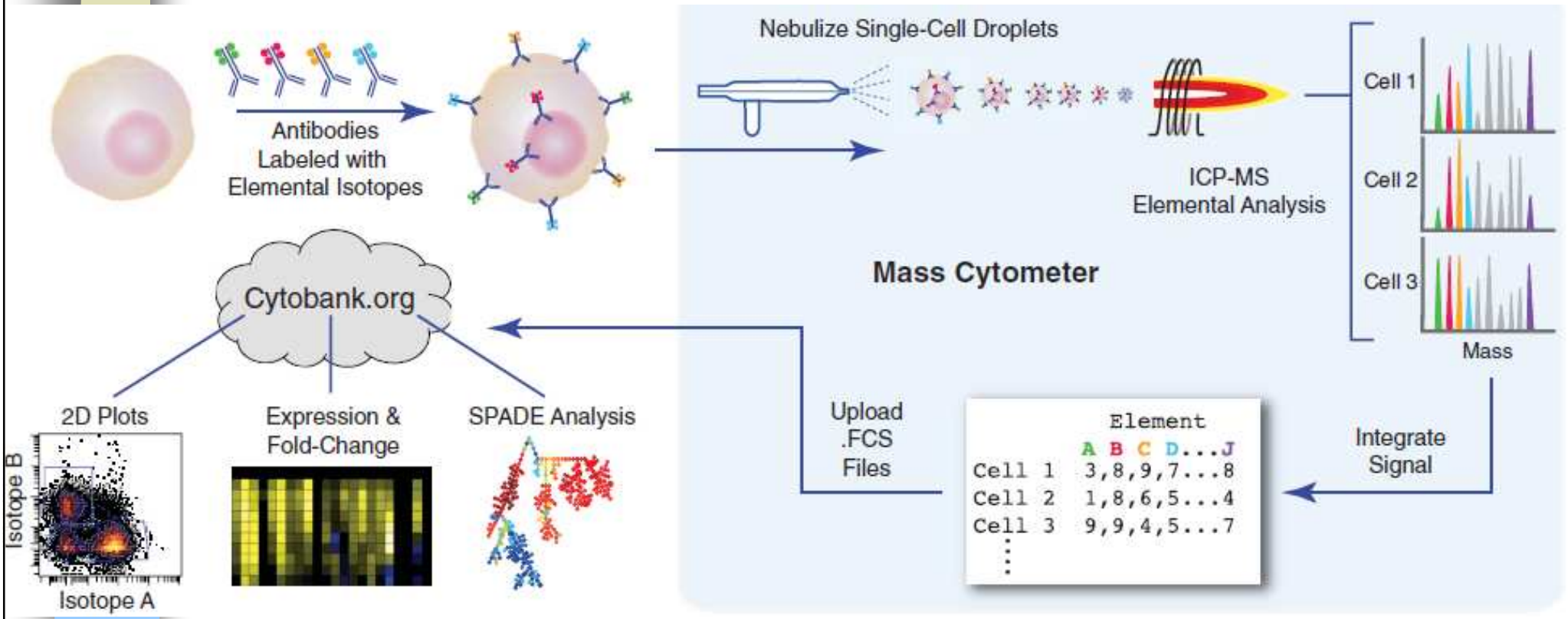
Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum
Sean C. Bendall, *et al.*
Science **332**, 687 (2011);

Single Cell Mass Cytometry



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

Single Cell Mass Cytometry

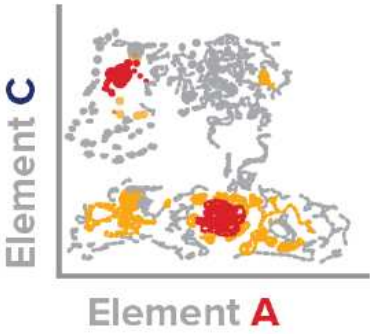


Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum
Sean C. Bendall, *et al.*
Science **332**, 687 (2011);

Mass Cytometry: 50+ Parameters on Millions of Cells

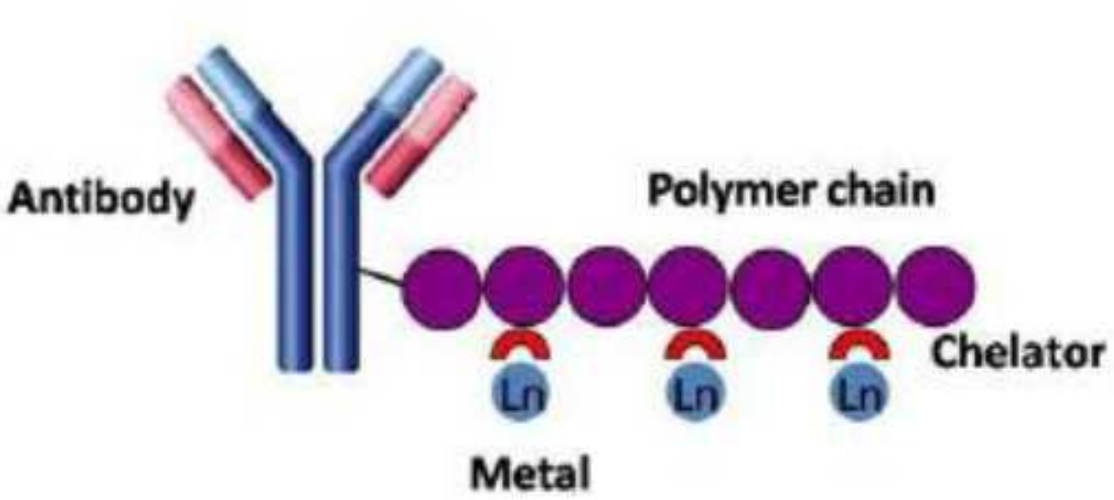
Discovery of new biology
Comprehensive functional profiling

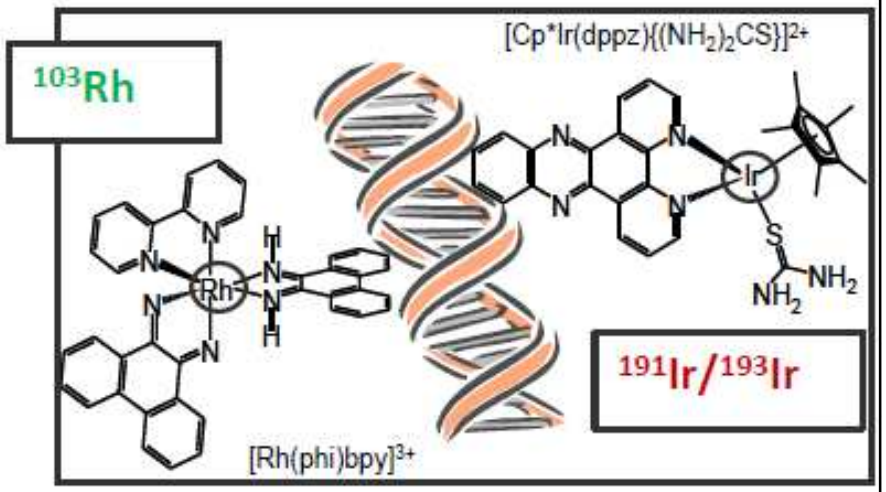
Basic research
Drug discovery
Clinical research



FLUIDIGM

MaxPar® metal-tagged probes

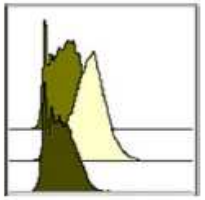




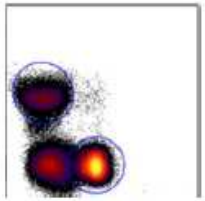
- Lanthanide tags: 32 isotopes from 13 elements
- IgG antibody probes:
  - Pre-conjugated antibodies (220 currently available and growing)
  - MAXPAR® labeling kits (for 32 stable isotopes)
- Nucleic acid-binding metallo-intercalators
  - Identifies single cell events
  - Live/dead indicator

Analyze: Cytobank

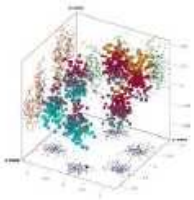
Plot raw data



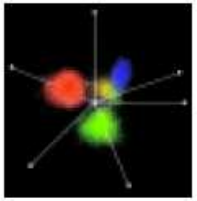
Histogram



Biaxial plot

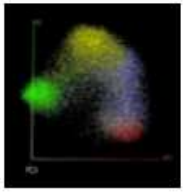


3D plot

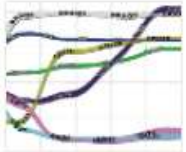


Radar

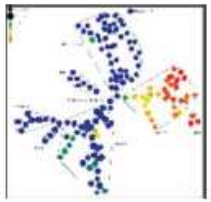
Reduce dimensionality



PCA

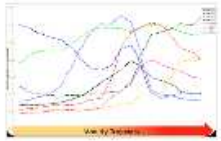


Gemstone



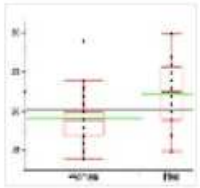
SPADE

viSNE



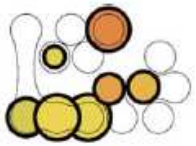
Wanderlust

Summarize statistics



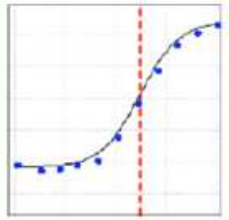
Box plot

Heat map



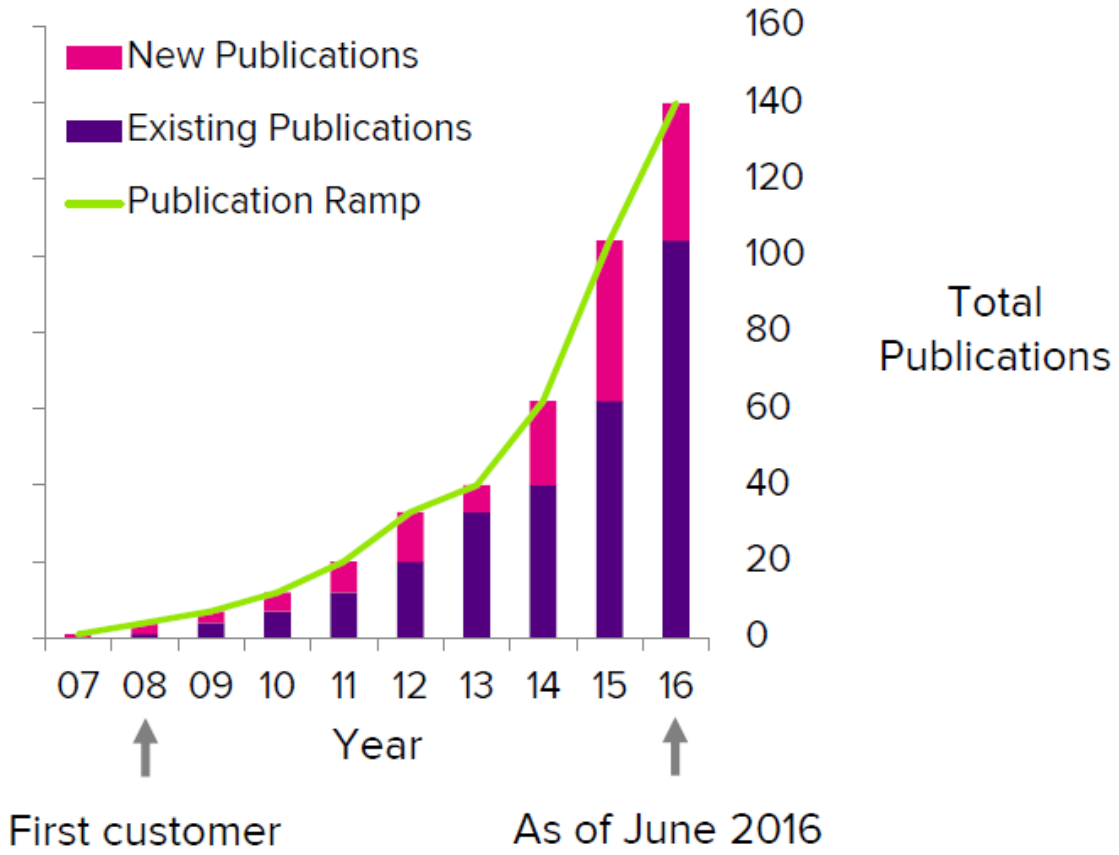
Network

Sunburst



Dose curve

FLUIDIGM


New Publications
Existing Publications
Publication Ramp
160
140
120
100
80
60
40
20
0
Total Publications
07
08
09
10
11
12
13
14
15
16
Year
First customer
As of June 2016

FLUIDIGM

Analyze: Cytobank

Analysis toolkit designed for mass cytometry

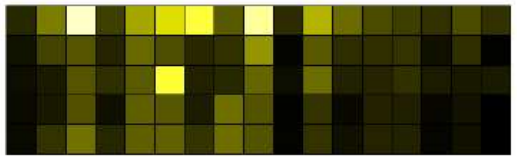
Cloud-based—accessible from anywhere

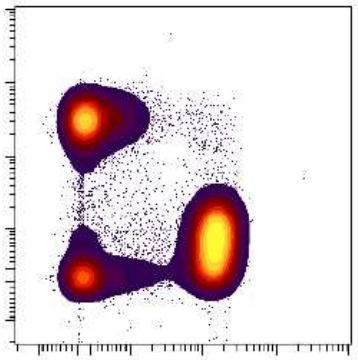
Data storage and backup included

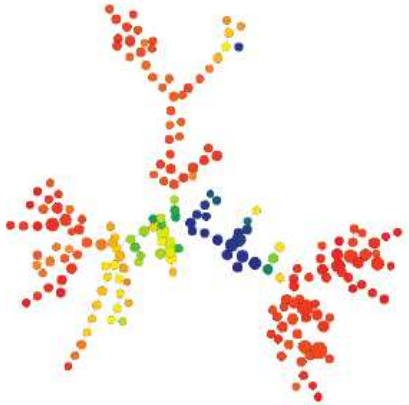
Demo datasets and tutorials

Strong scientific support

fluidigm.cytobank.org



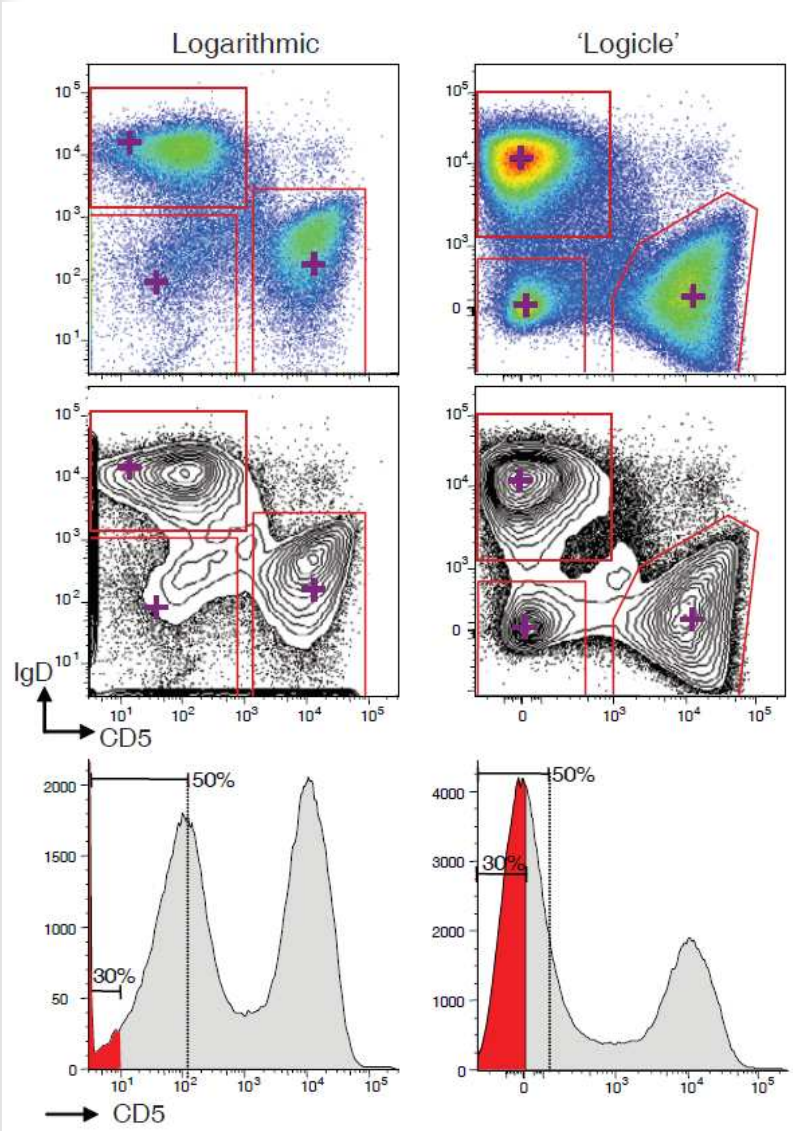


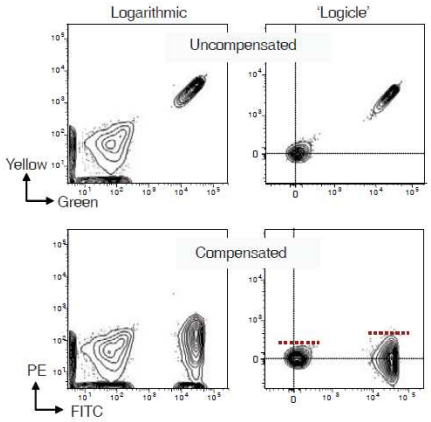


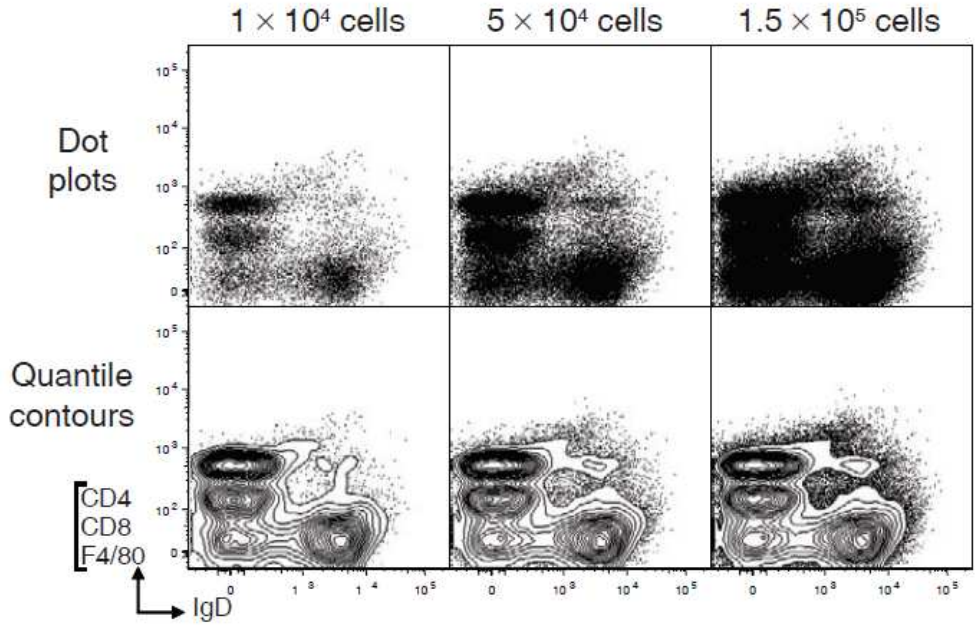
Cytobank

FLUIDIGM

Vizualizace dat a intepretace dat







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

BOX 1 SUGGESTED GUIDELINES FOR FACS DATA PRESENTATION[4]

Instrument: Identify the FACS instrument and the software used to collect, compensateand 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.

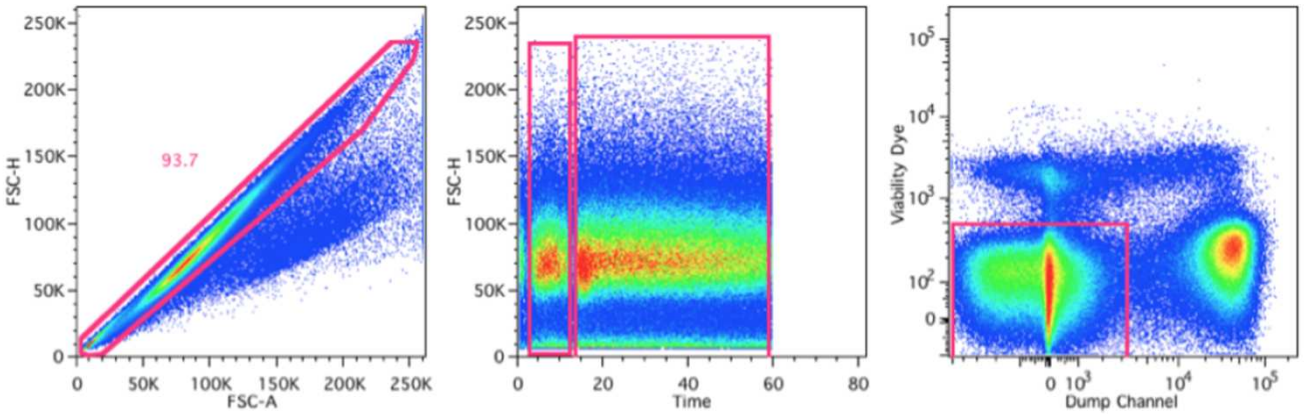
Frequency measurements: Show the frequencies (or percentages) of cells in gates of importance in the study. Compute these values relative to the total number of cells presented in the display on which the values appear. If a different frequency computation is used, define the method that was used and 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.

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***

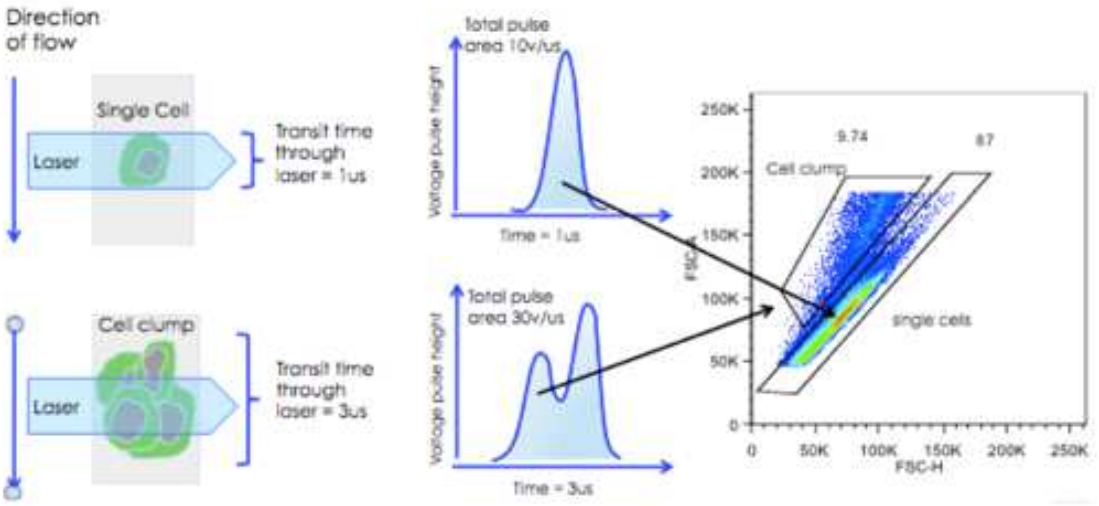
Gating a kontrola kvality

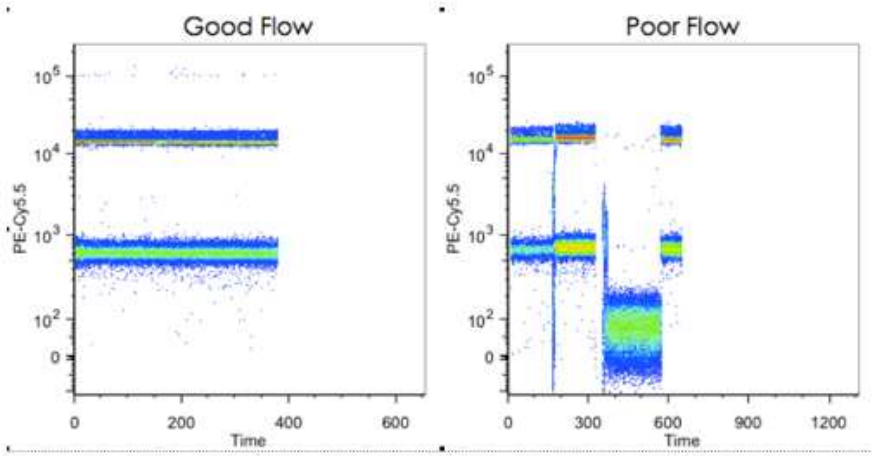
1) Singlets
2) Time
3) viabilita



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

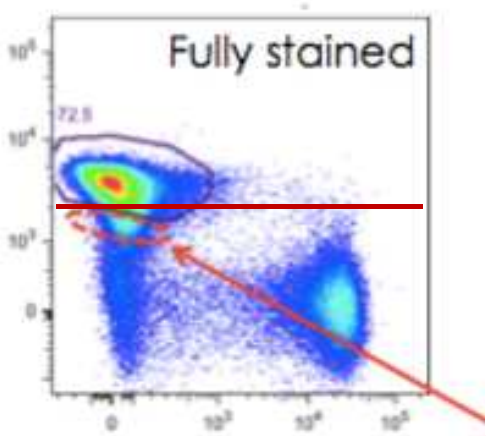
Gating a kontrola kvality



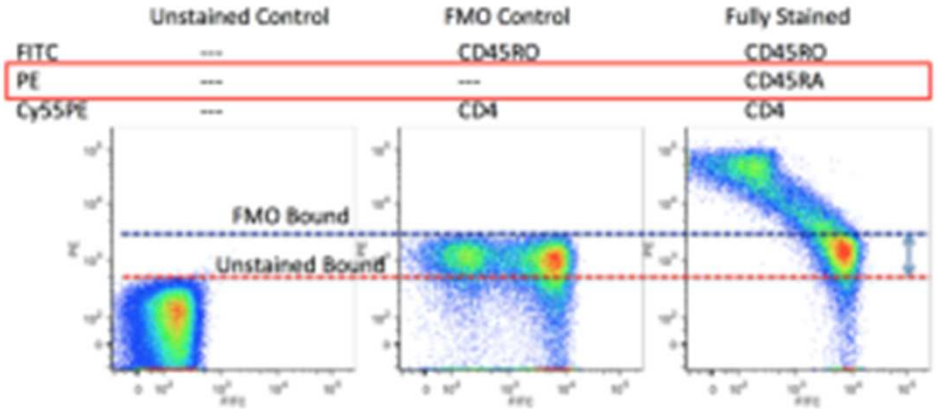


http://expertcytometry.com/6-flow-cytometry-gating-tips-that-most-scientists-forget/

Gating a kontrola nastavení



FMO ~ Fluorescence Mines One

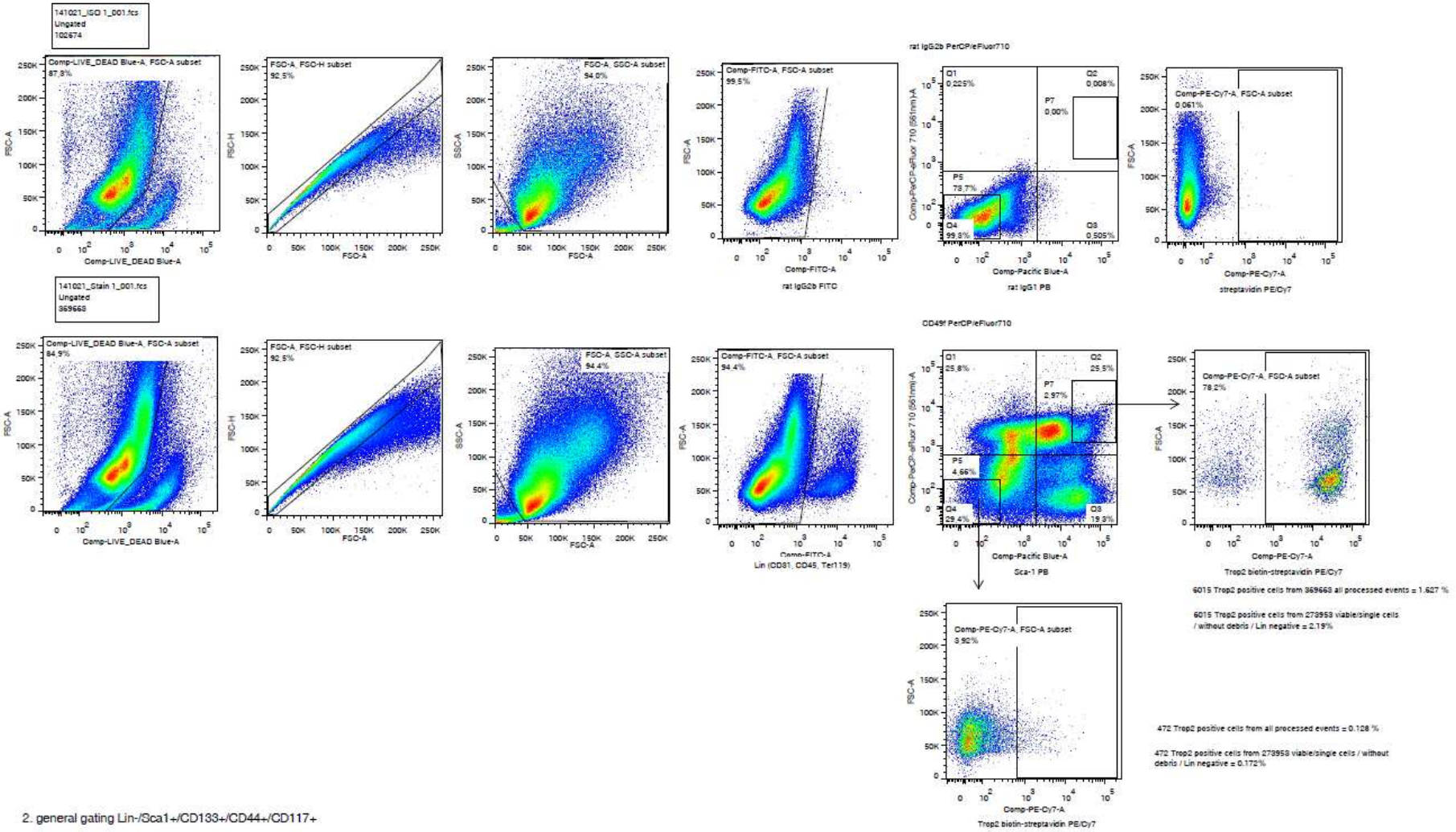


Antigen	FITC	PE	Cy5-PE	APC
CD3 FMO	---	CD4	CD8	CD19
CD4 FMO	CD3	---	CD8	CD19
CD8 FMO	CD3	CD4	---	CD19
CD19 FMO	CD3	CD4	CD8	---

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

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

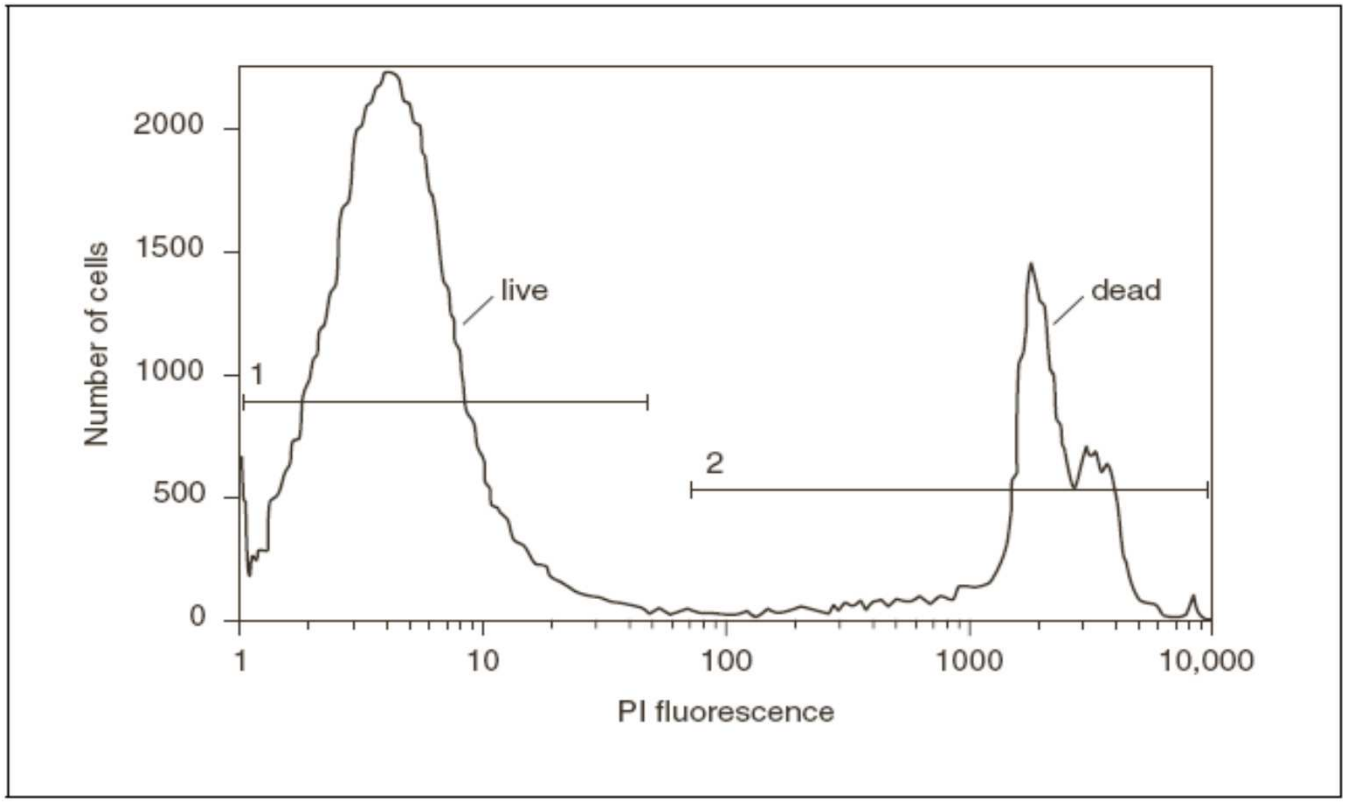
- jedna z nejjednodušších analýz
- funguje na principu:
  - detekce membránové integrity - neprůchodnosti některých fluorescenčních značek cytoplazmatickou membránou živých buněk **– propidium iodide, ethidium bromide, 7-amino actinomycin D**
  - detekce fyziologického stavu buněk – použití fluorescenčních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

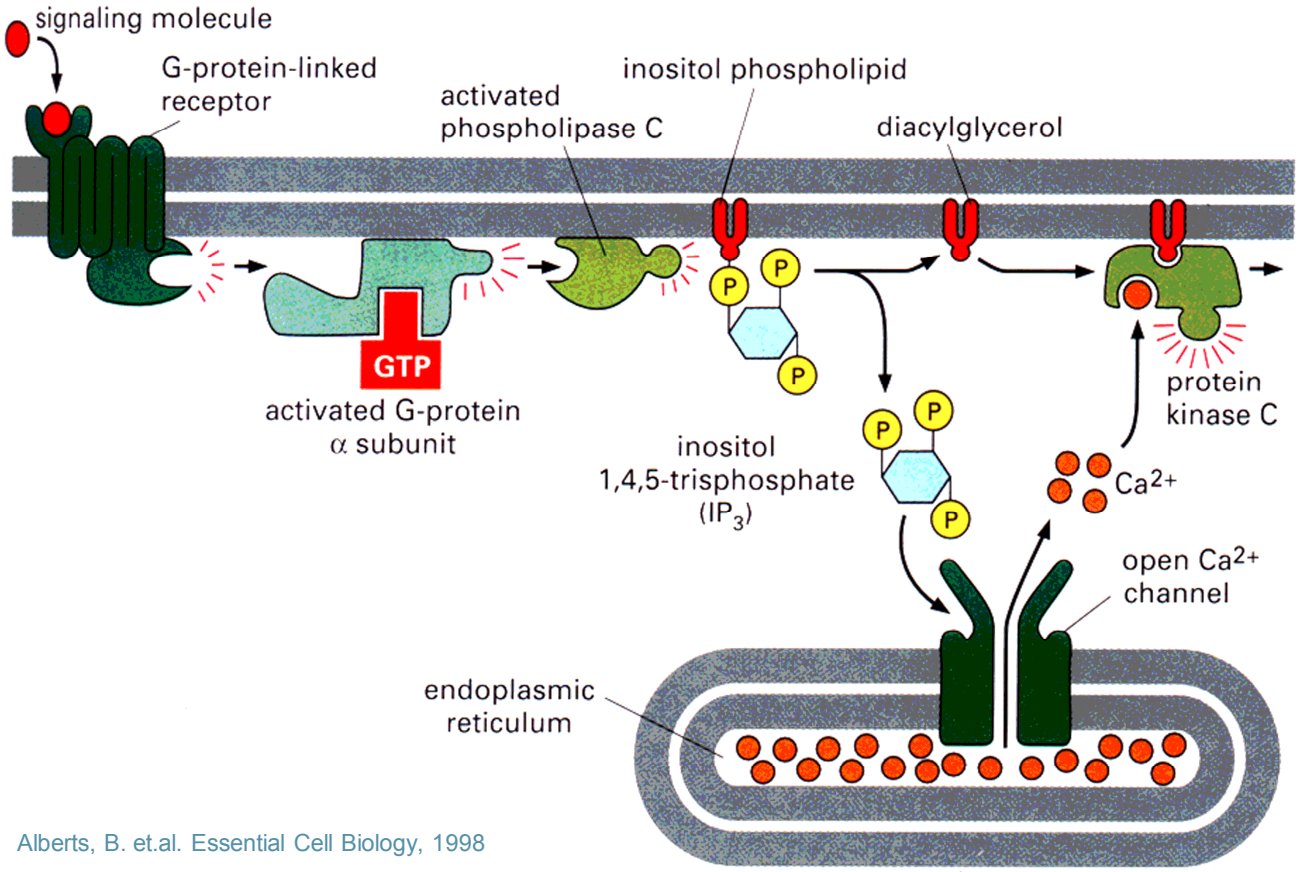
Detekce viability



Current Protocols in Cytometry

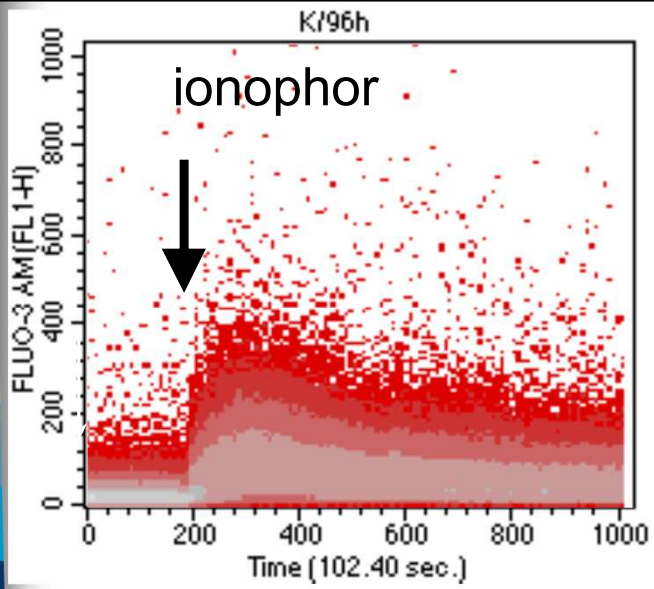
Přenos signálu pomocí Ca^{2+}

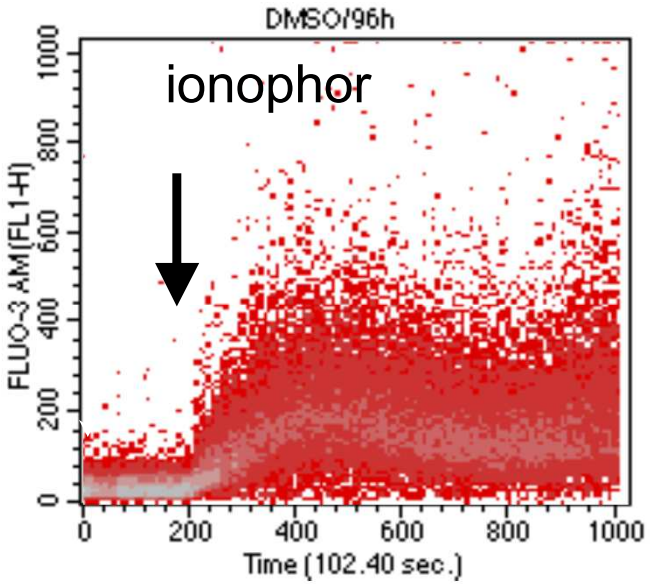
- **Cytosol** (koncentrace - „klidová“ 100 nM vs. 1-10 μM aktivovaná)
- $[Ca^{2+}]_c$ aktivuje proteinkinázu C
- interaguje s „Ca^{2+} - binding proteins“

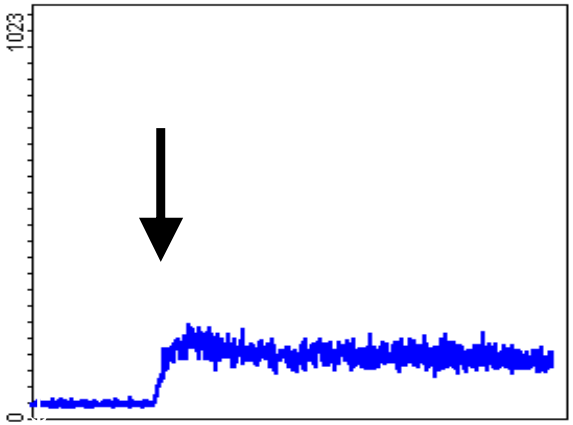


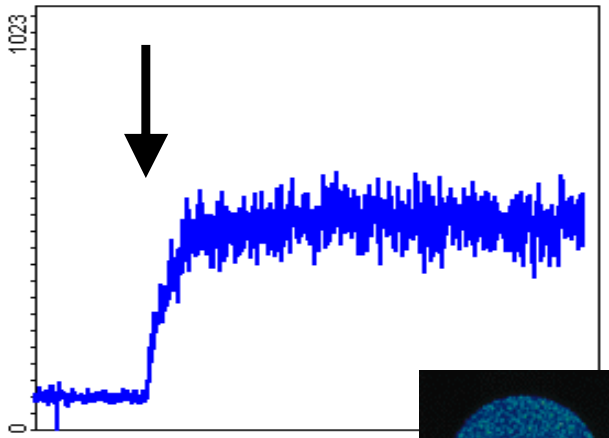
Alberts, B. et.al. Essential Cell Biology, 1998

Ca $^{2+}$ influx

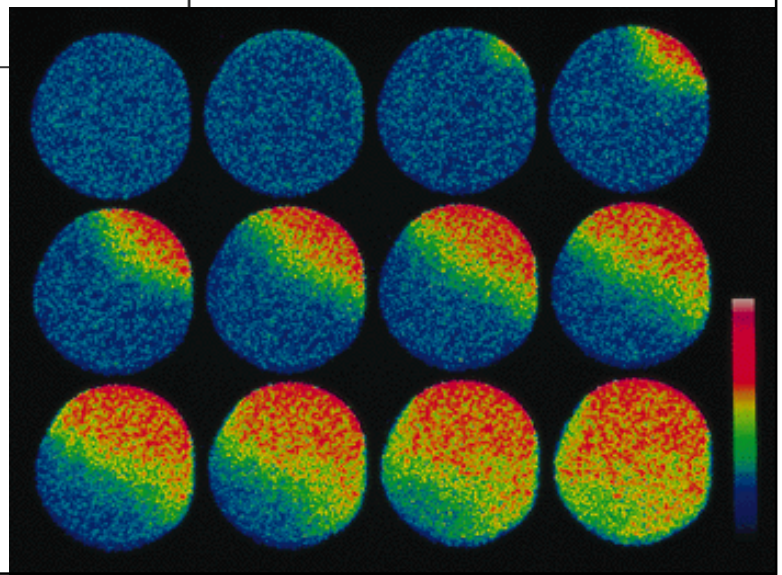








- Fura-2
- Fluo-3
- Indo-1

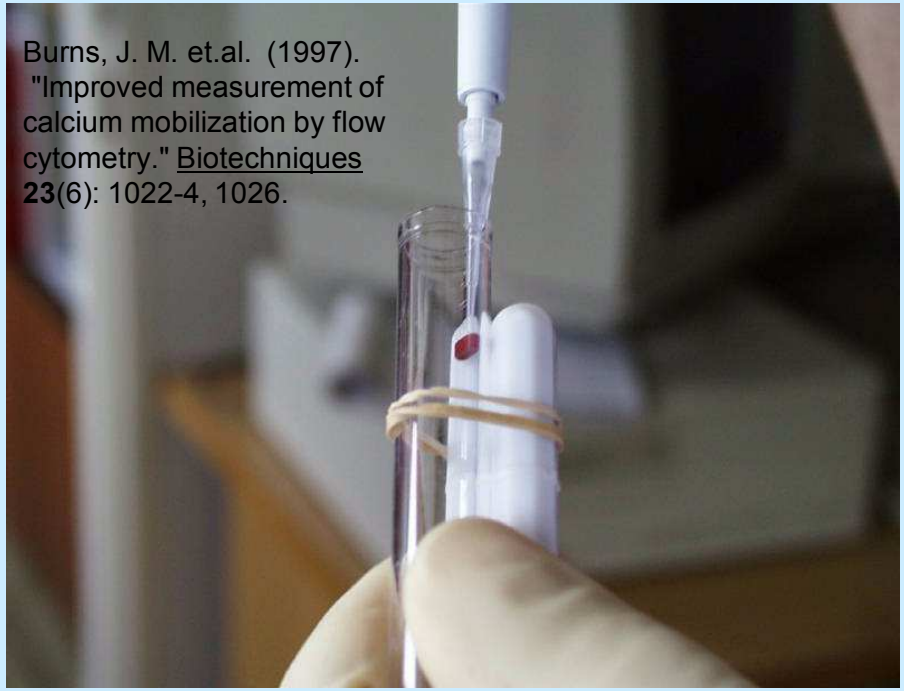


Zajištění vhodných podmínek pro detekci $[Ca^{2+}]_i$

- 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
Cytek
FLOW CYTOMETRY PRODUCTS

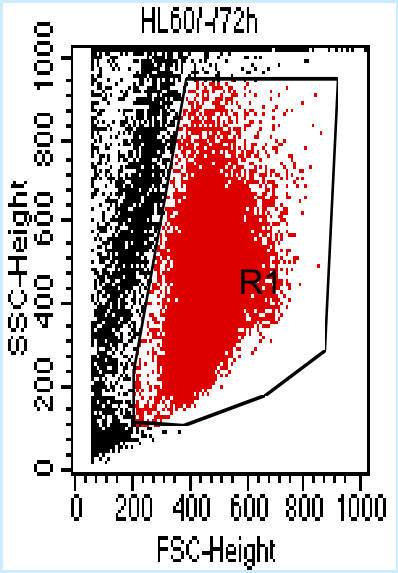

Burns, J. M. et.al. (1997). "Improved measurement of calcium mobilization by flow cytometry." Biotechniques **23**(6): 1022-4, 1026.

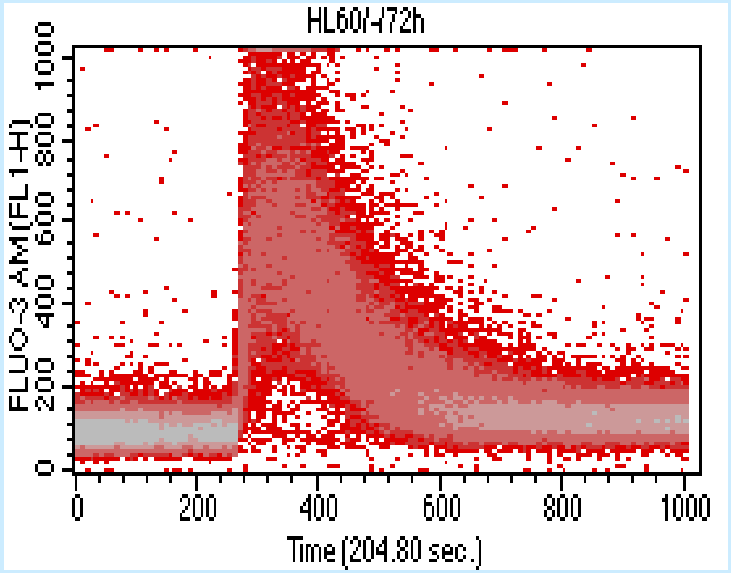

OMNIBIO

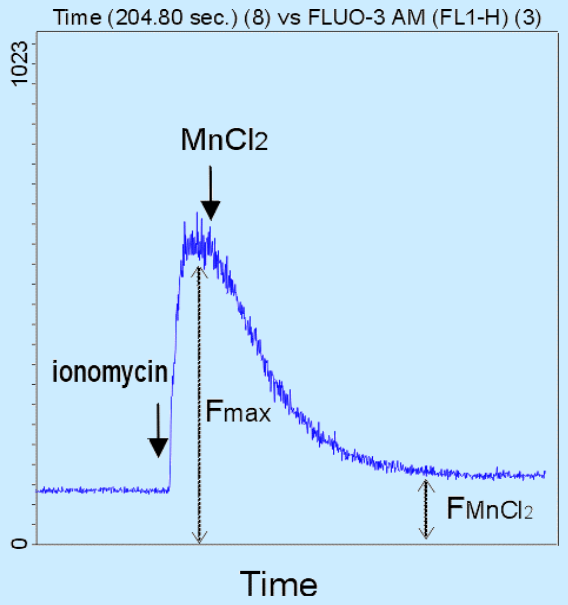
Kalibrace

(pro jednu vlnovou délku)

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





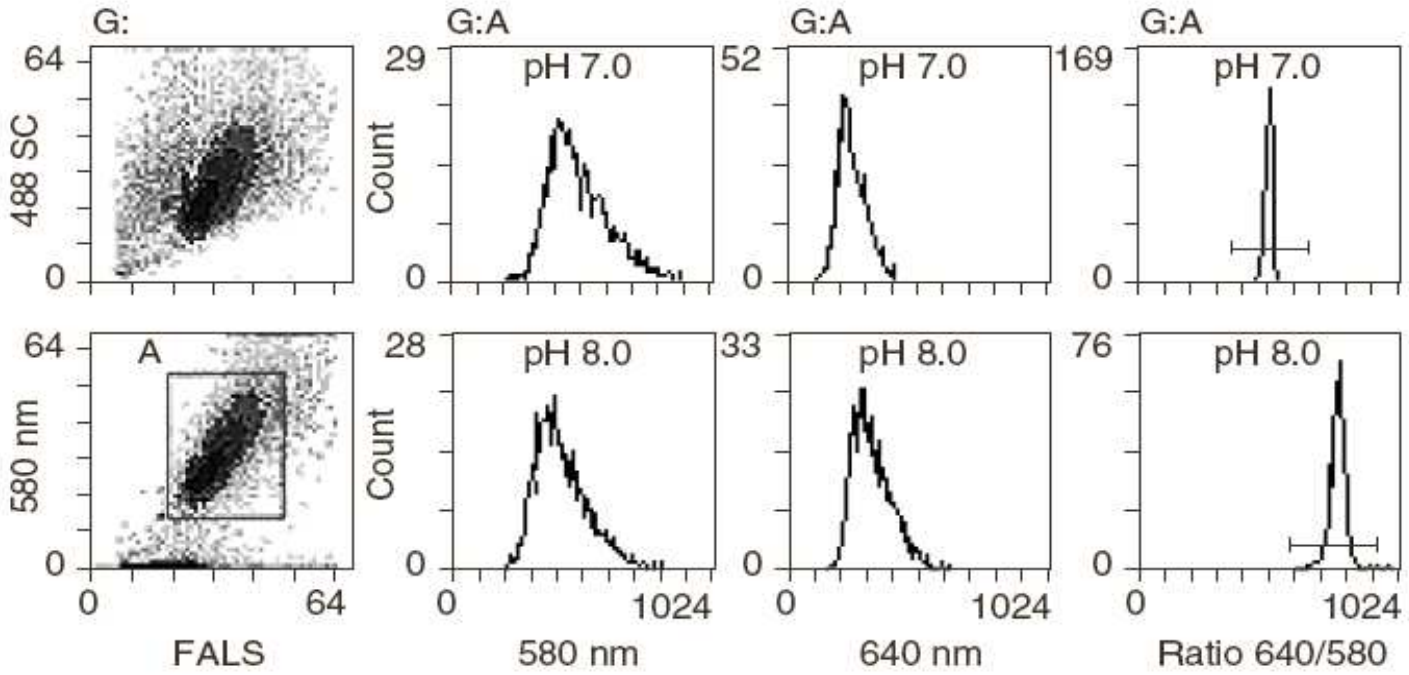


Fluo-3 (Kd ~ 400nM, 22°C; 864 nM, 37°C)

$F_{min} = 1.25 \times F_{MnCl2} - 0.25 \times F_{max}$

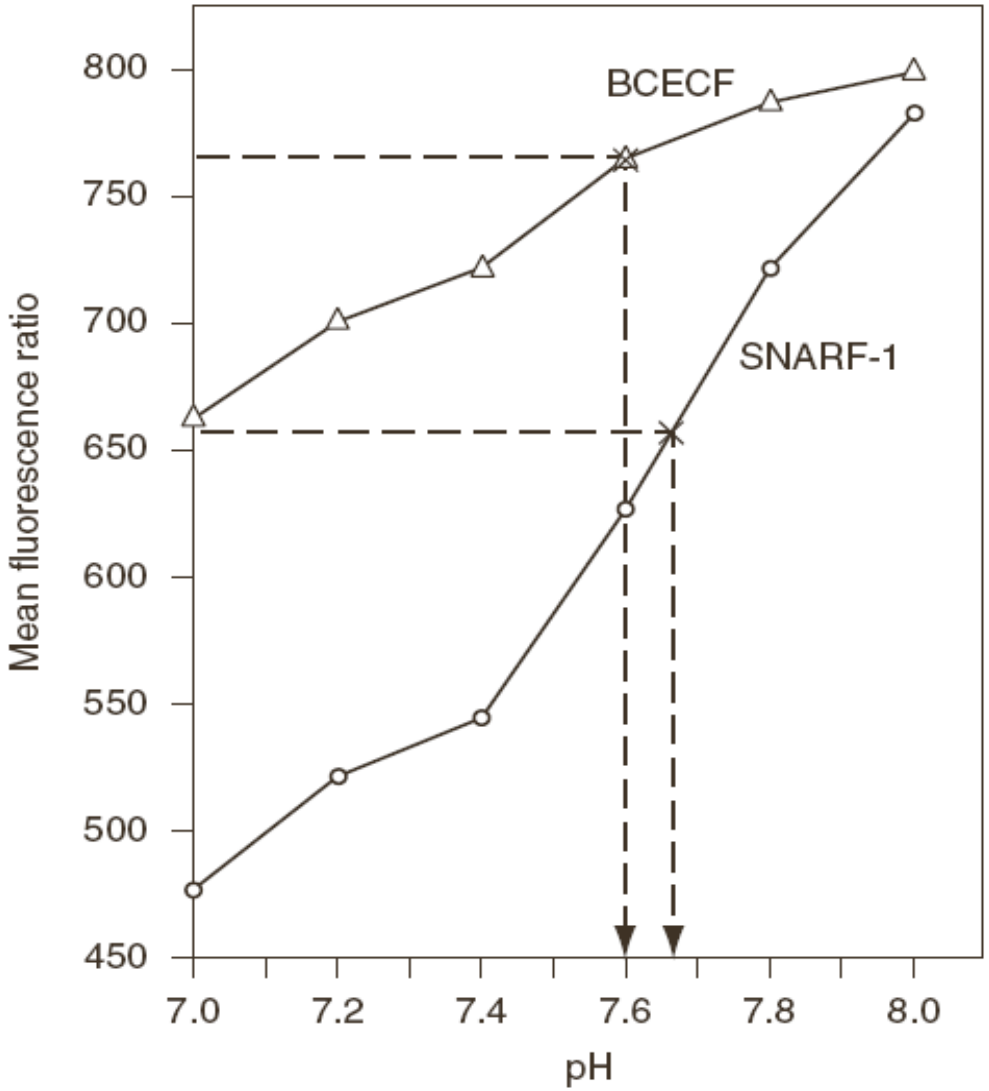
Detekce intracelulárního pH

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



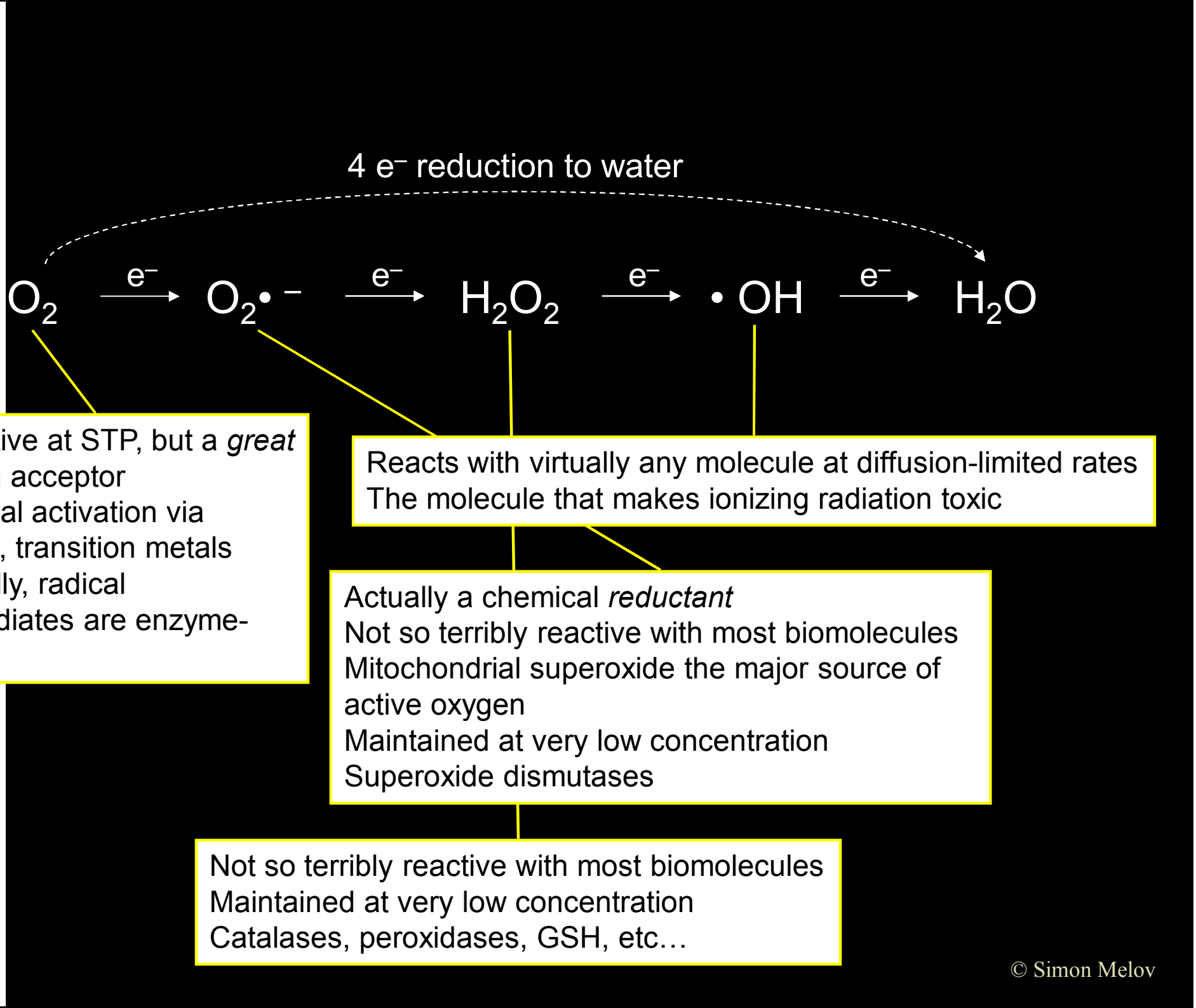
Detekce intracelulárního pH

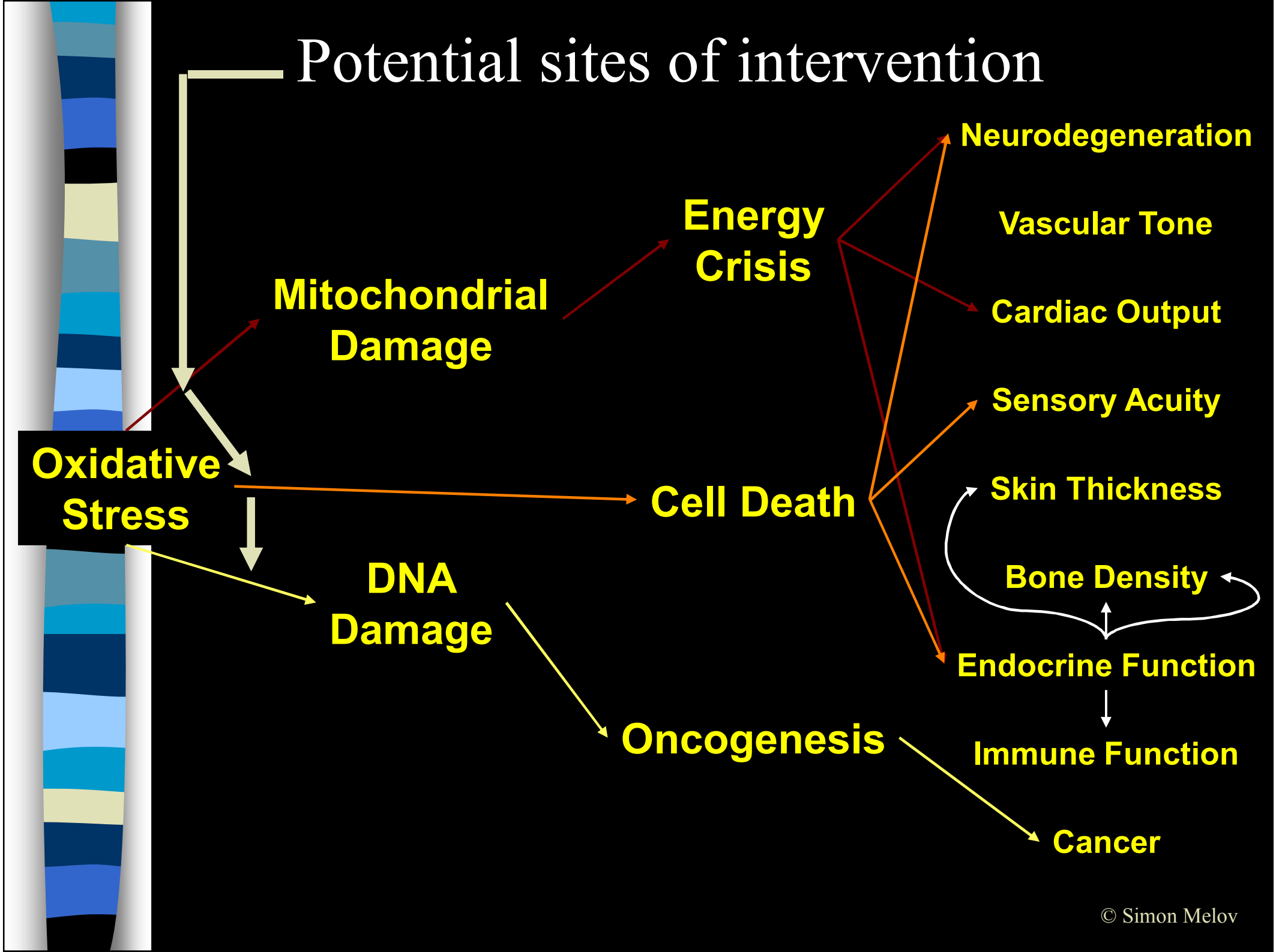
- Nutná kalibrace pomocí draslíkových pufrů a ionoforu (nigericin)



Detekce reaktivních kyslíkových skupin

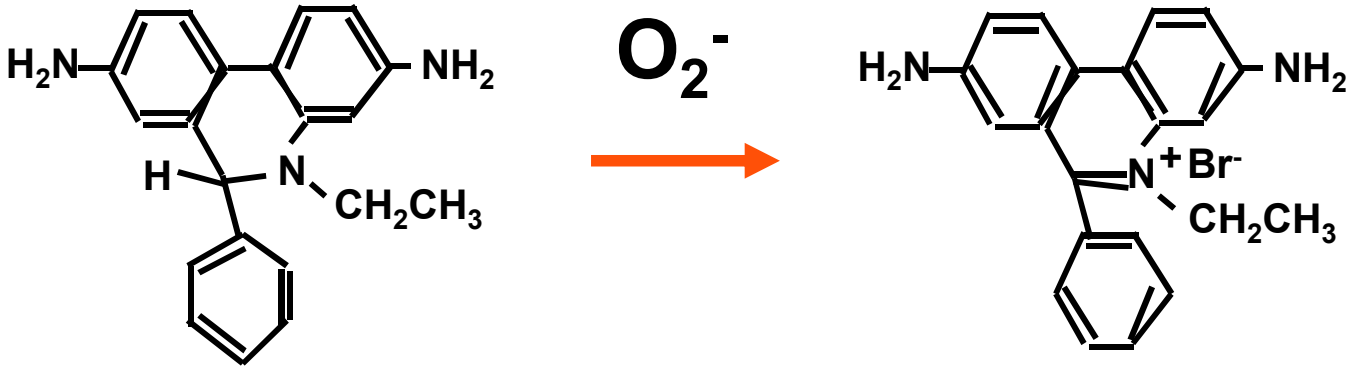
- Reaktivní kyslíkové skupiny hrají klíčovou roli v celé řadě biologických procesů
  - posttranslační modifikace proteinů
  - regulace transkripce
  - regulace struktury chromatinu
  - přenos signálu
  - funkce imunitního systému
  - fyzický a metabolický stres
  - neurodegenerace, stárnutí

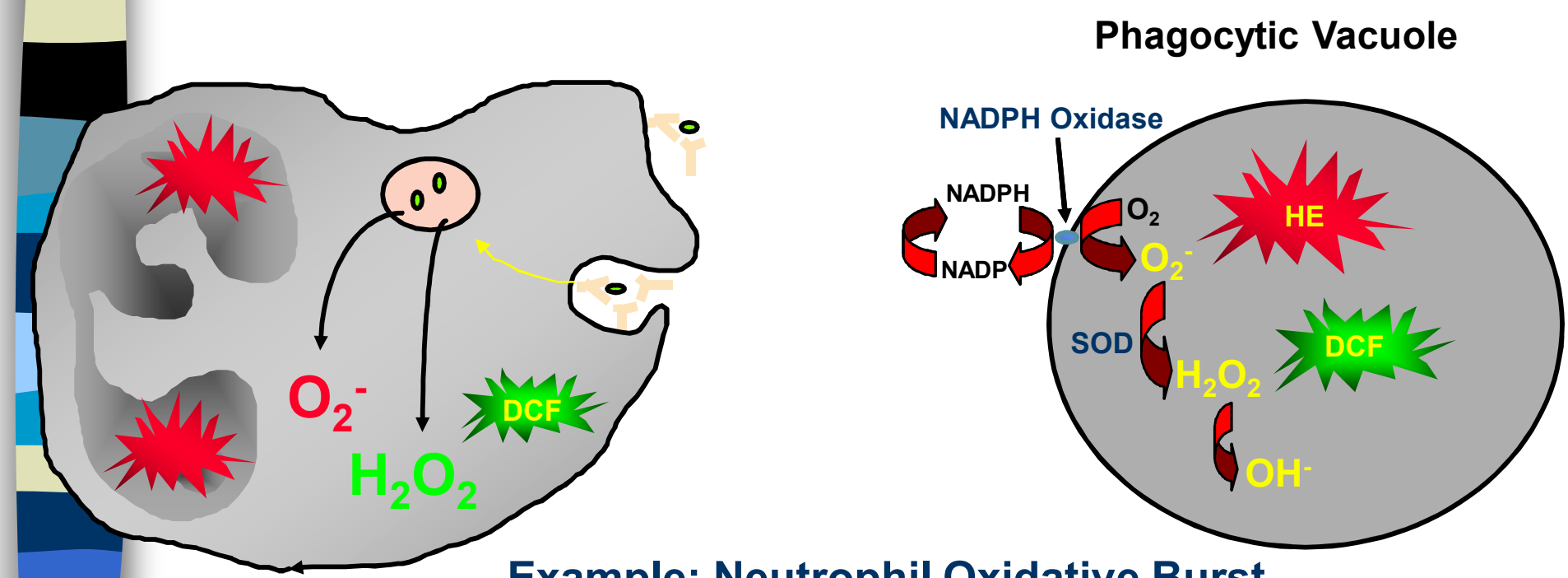

4 e⁻ reduction to water
O_2
e⁻
$O_2\bullet^-$
e⁻
H_2O_2
e⁻
• OH
e⁻
H_2O
Unreactive at STP, but a *great* electron acceptor
Biological activation via radicals, transition metals
Generally, radical intermediates are enzyme-bound
Reacts with virtually any molecule at diffusion-limited rates
The molecule 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
Neurodegeneration
Energy Crisis
Vascular Tone
Mitochondrial Damage
Cardiac Output
Sensory Acuity
Oxidative Stress
Cell Death
Skin Thickness
Bone Density
DNA Damage
Endocrine Function
Oncogenesis
Immune Function
Cancer
© Simon Melov

Hydroethidine

HE → **EB**

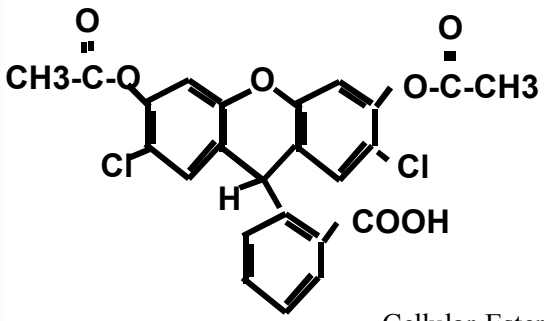




Example: Neutrophil Oxidative Burst

DCFH-DA → DCFH → DCF

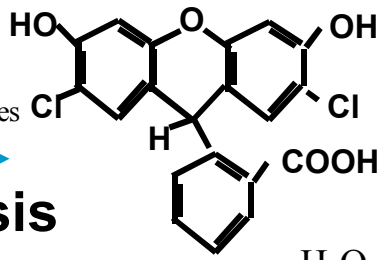
2',7'-dichlorofluorescin diacetate



Cellular Esterases

Hydrolysis

2',7'-dichlorofluorescin

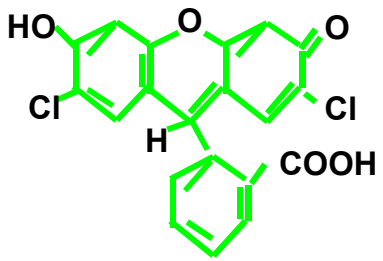


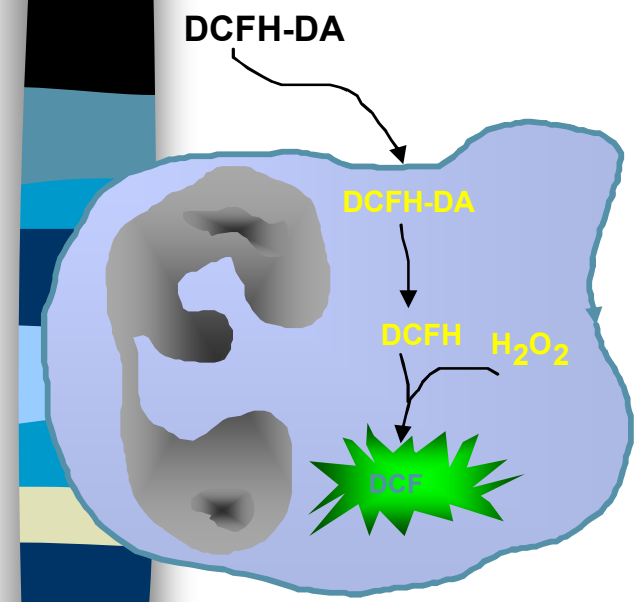
H_2O_2

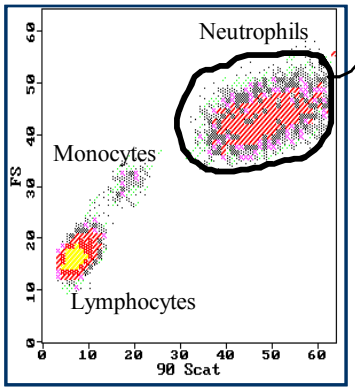
Oxidation

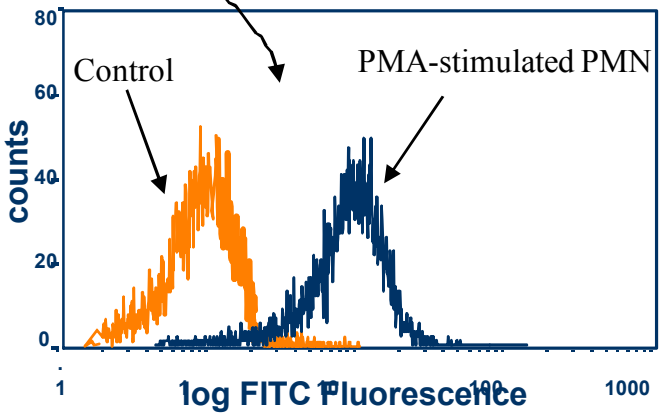
Fluorescent

2',7'-dichlorofluorescein



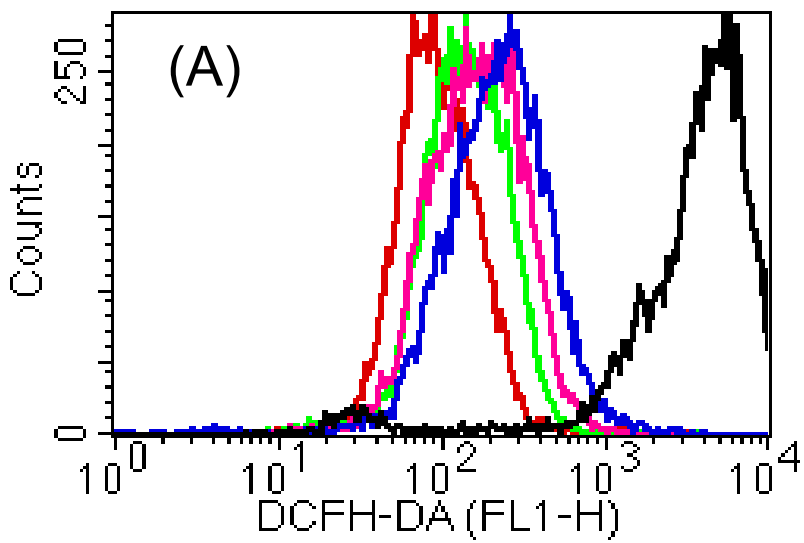


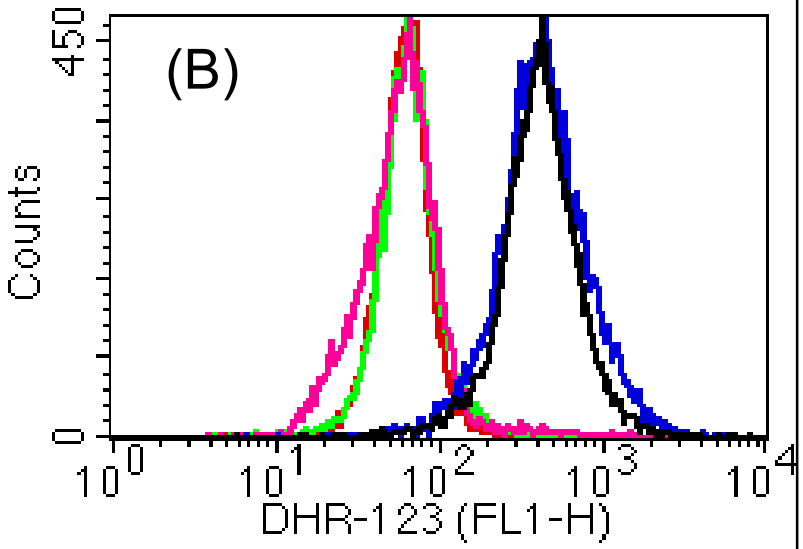


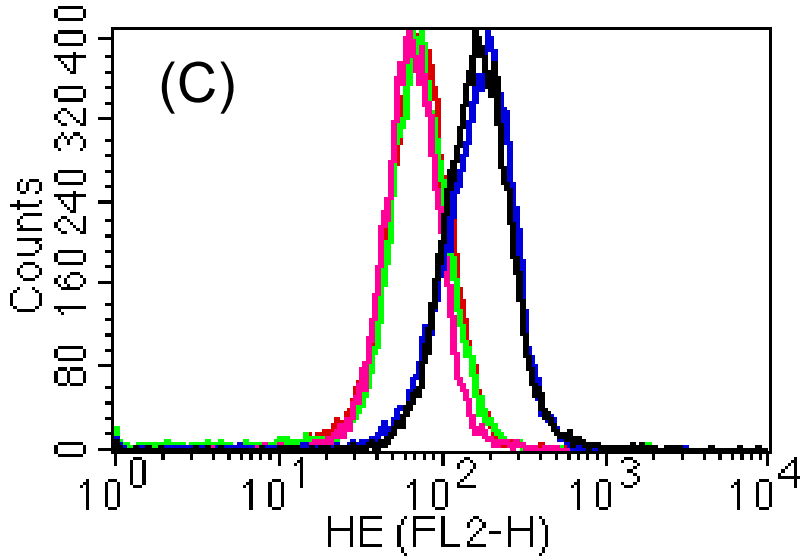


Oxidative Burst

- 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.

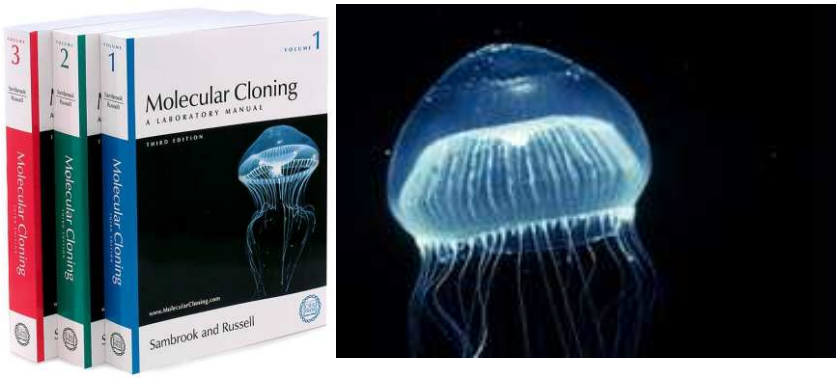
– modré světlo excituje **green fluorescent protein**.

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 "

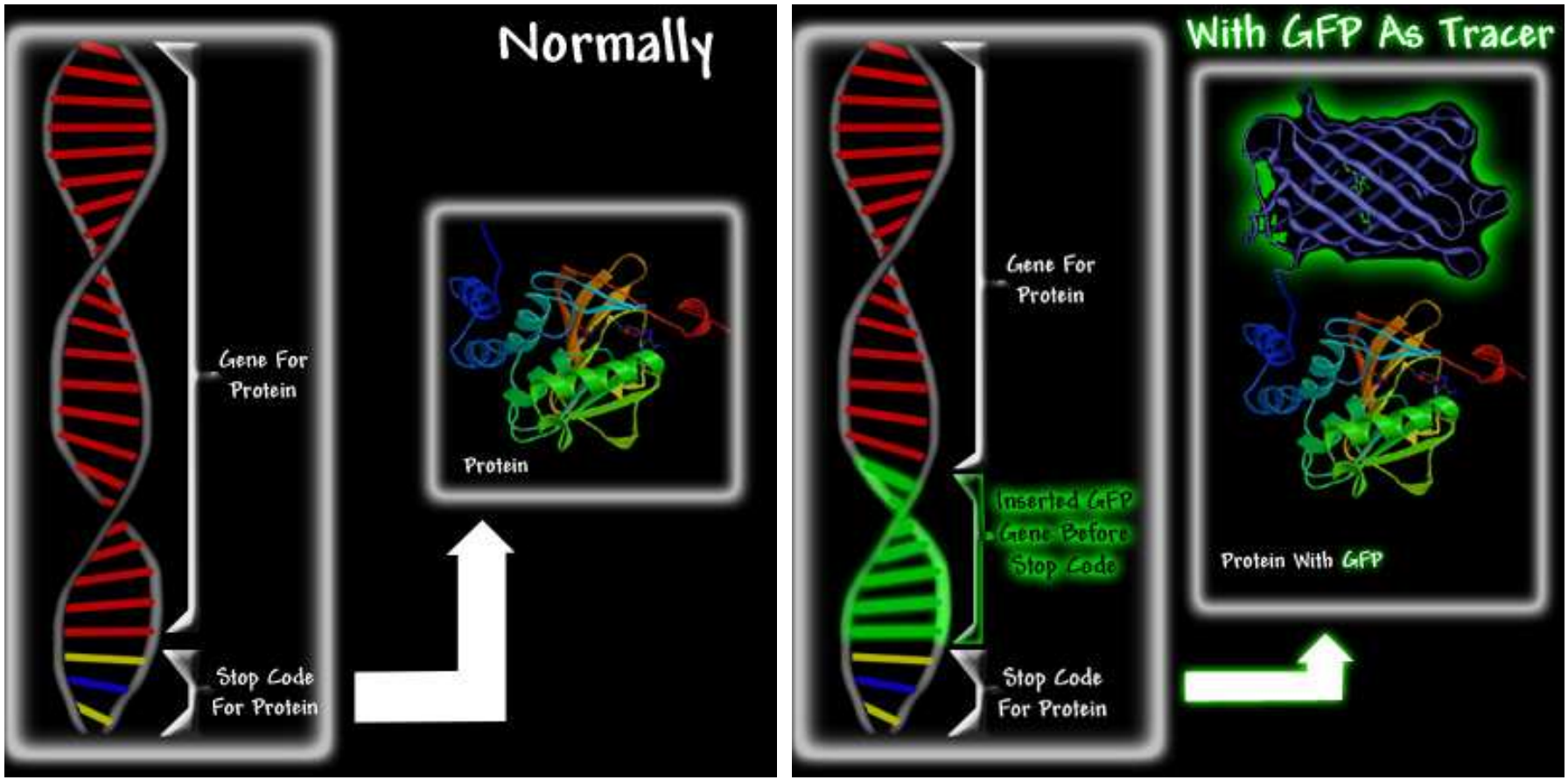


http://www.mbayaq.org/efc/living_species/default.asp?hOri=1&inhab=440

Renilla reniformis "Sea Pansy"

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

Fluorescenční proteiny



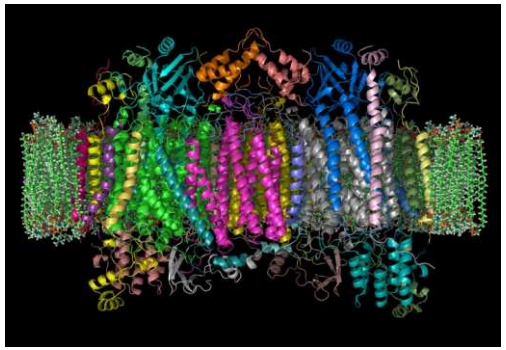
http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm

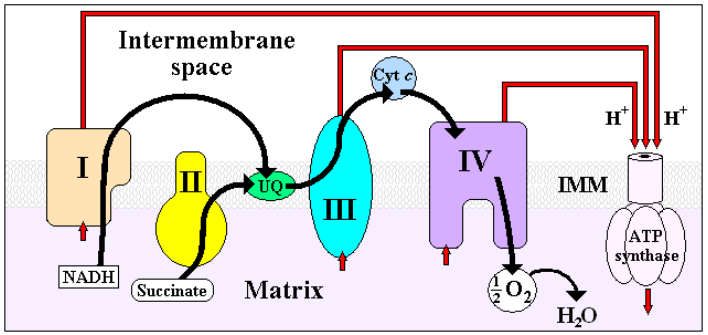
Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells

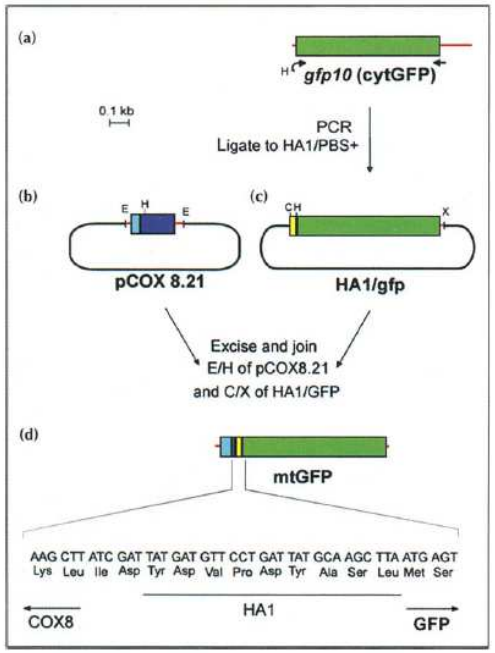
Rosario Rizzuto, Marisa Brini, Paola Pizzo, Marta Murgia and Tullio Pozzan

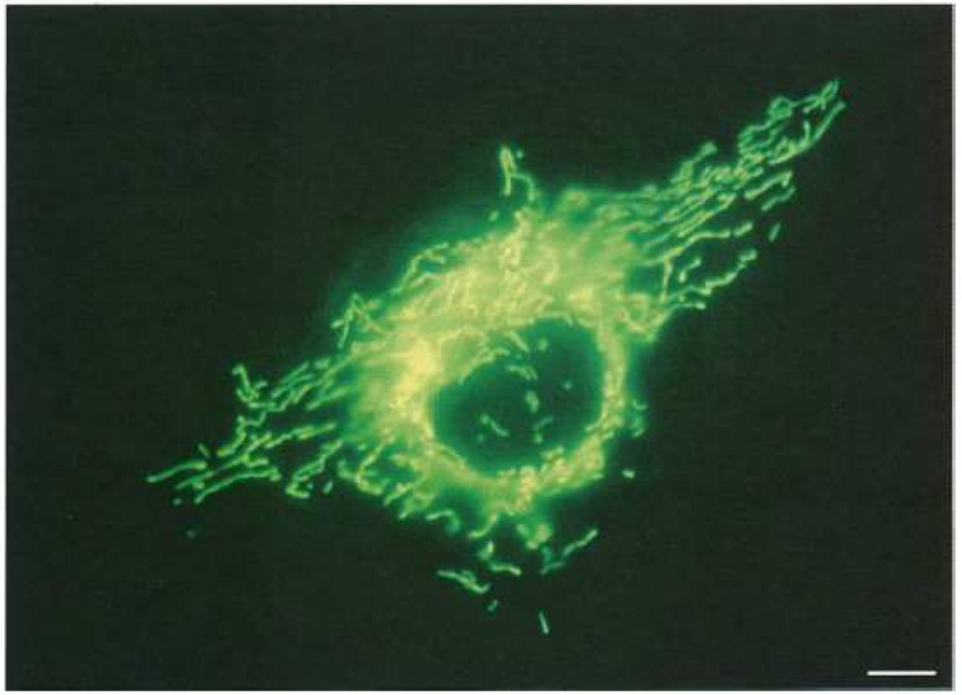
Department of Biomedical Sciences and CNR Center for the Study of Mitochondrial Physiology, University of Padova, Via Trieste 75, 35121 Padova, Italy.

Current Biology 1995, 5:635–642

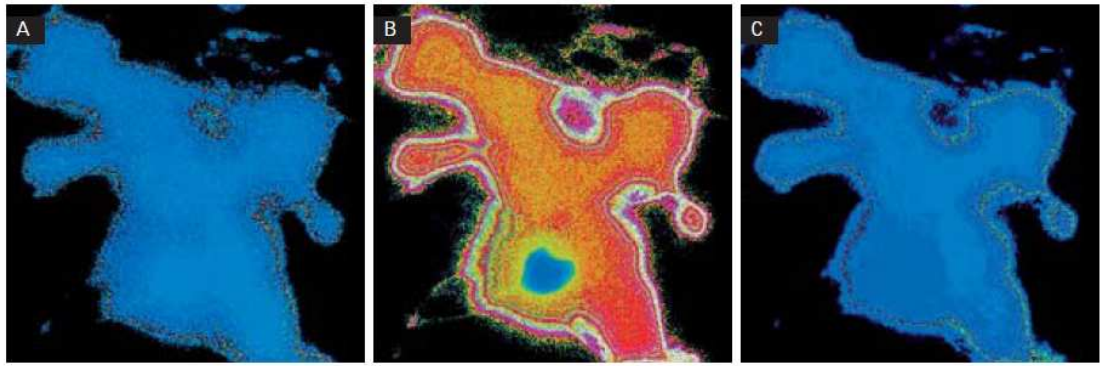






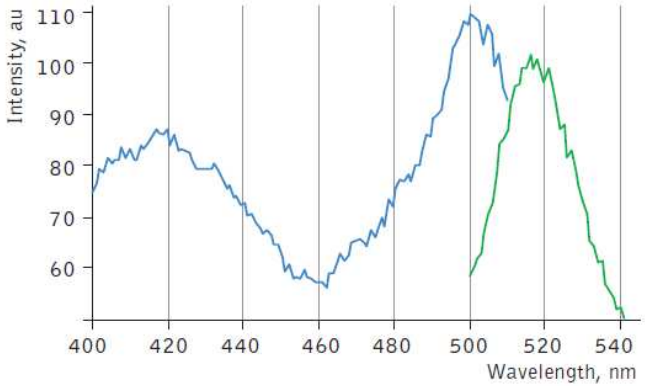


Fluorescent sensors for detection of H_2O_2

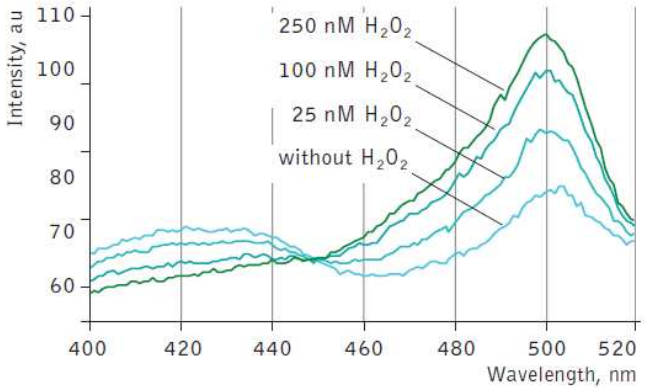


Ratiometric images of the group of HeLa cells before (A), 20 sec after (B), and 600 sec after (C) addition of 180 ul of H_2O_2. Images were pseudocolored using "ratio" lookup table of NIH ImageJ software: blue-green-red-white colors represent lowest-intermediate-high-highest level of H_2O_2.

evrogen



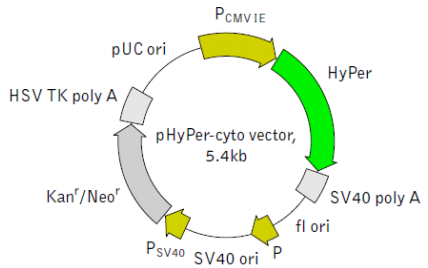
HyPer excitation (blue line) and emission (green line) spectra.

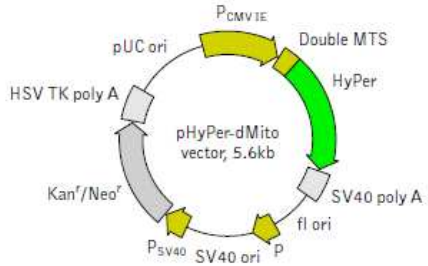


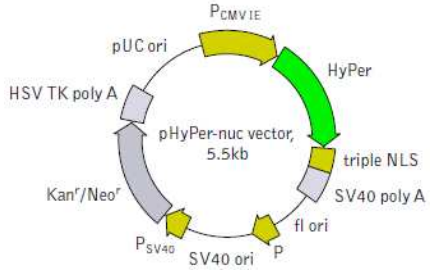
Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition. Emission was measured at 530 nm.

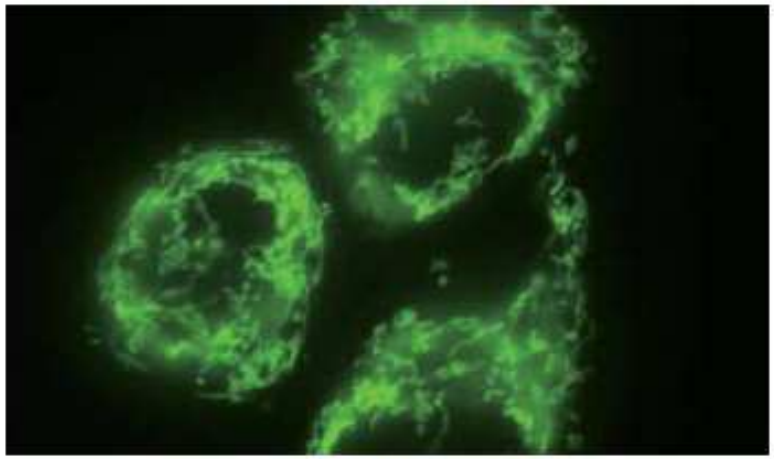
Variants & fusions

- **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]**



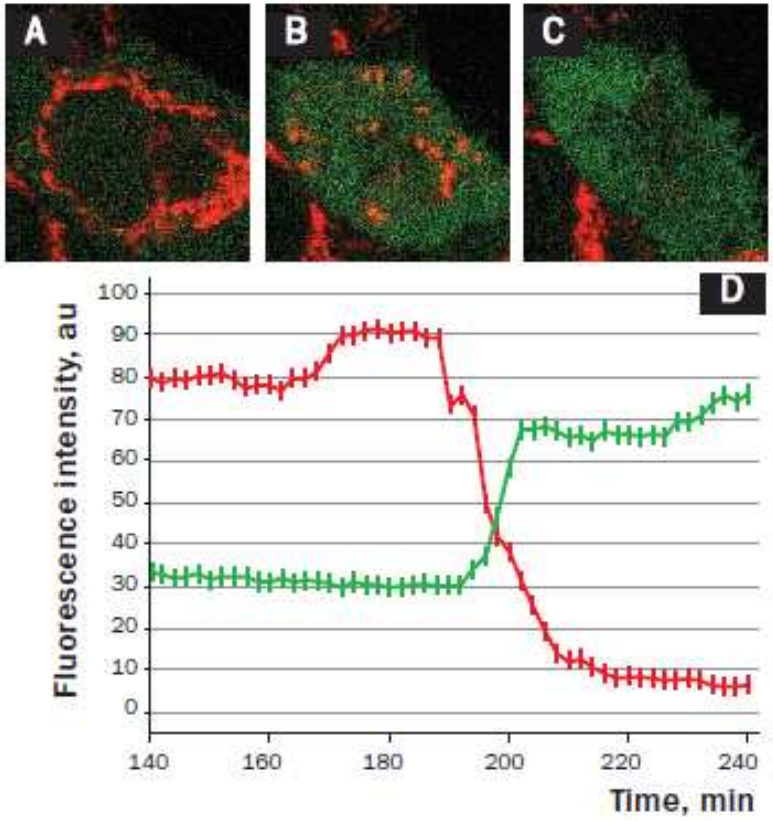






Stably transfected HeLa cells expressing mitochondria-targeted HyPer.

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.

evrogen

Analýza a sortrování 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

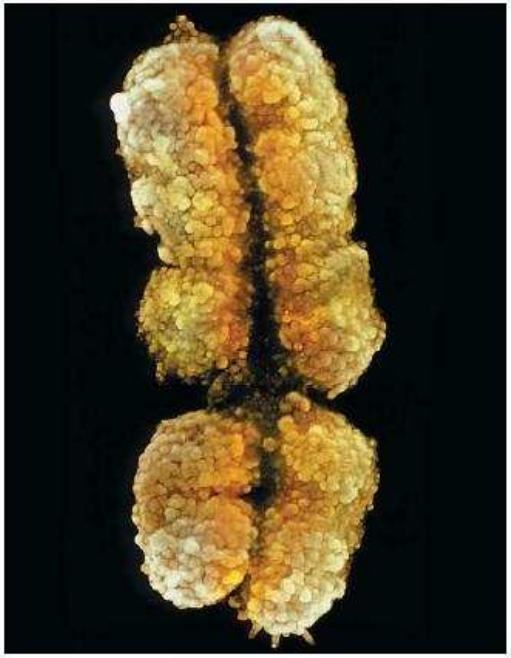
Biomedical Sciences Division, L-452, Lawrence Livermore Laboratory, Livermore, California 94550

Communicated by Donald A. Glaser, December 18, 1978

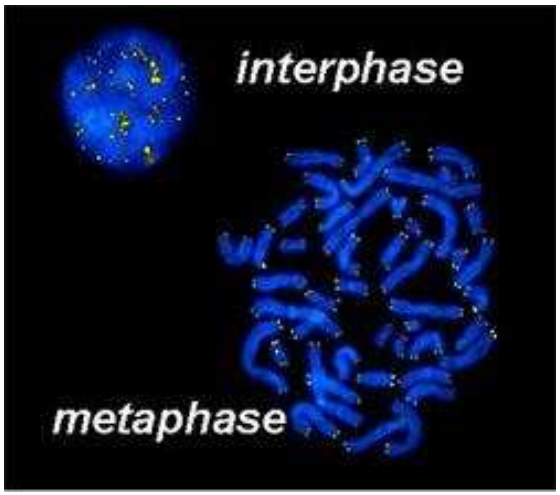
Analýza a sortrování chromozómů

- synchronizace buněk – zisk metafázních chromozómů (colcemid, hydroxyurea)
- izolace chromozómů
- značení DAPI nebo **Hoechst** vs. **chromomycin A3** (CA3) nebo mithramycin

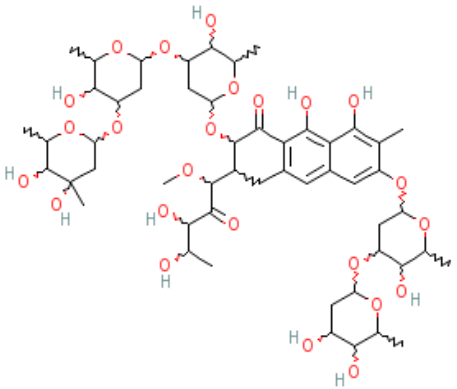
= celková DNA vs. G/C-bohaté oblasti



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

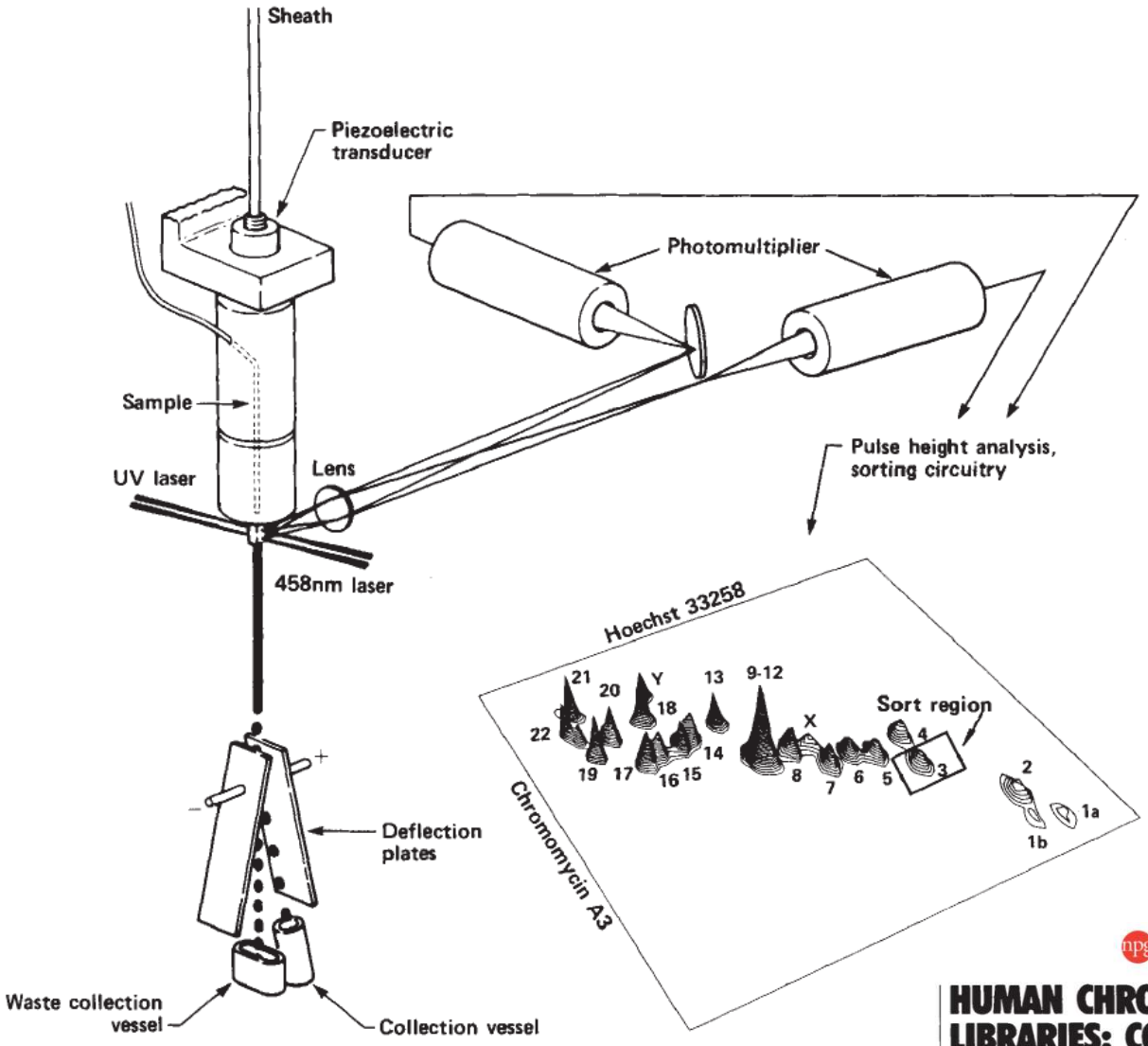


http://www.nccr-oncology.ch/scripts/page9243.html



PubChem National Library of Medicine NLM

Analýza a sortrování chromozómů



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*.

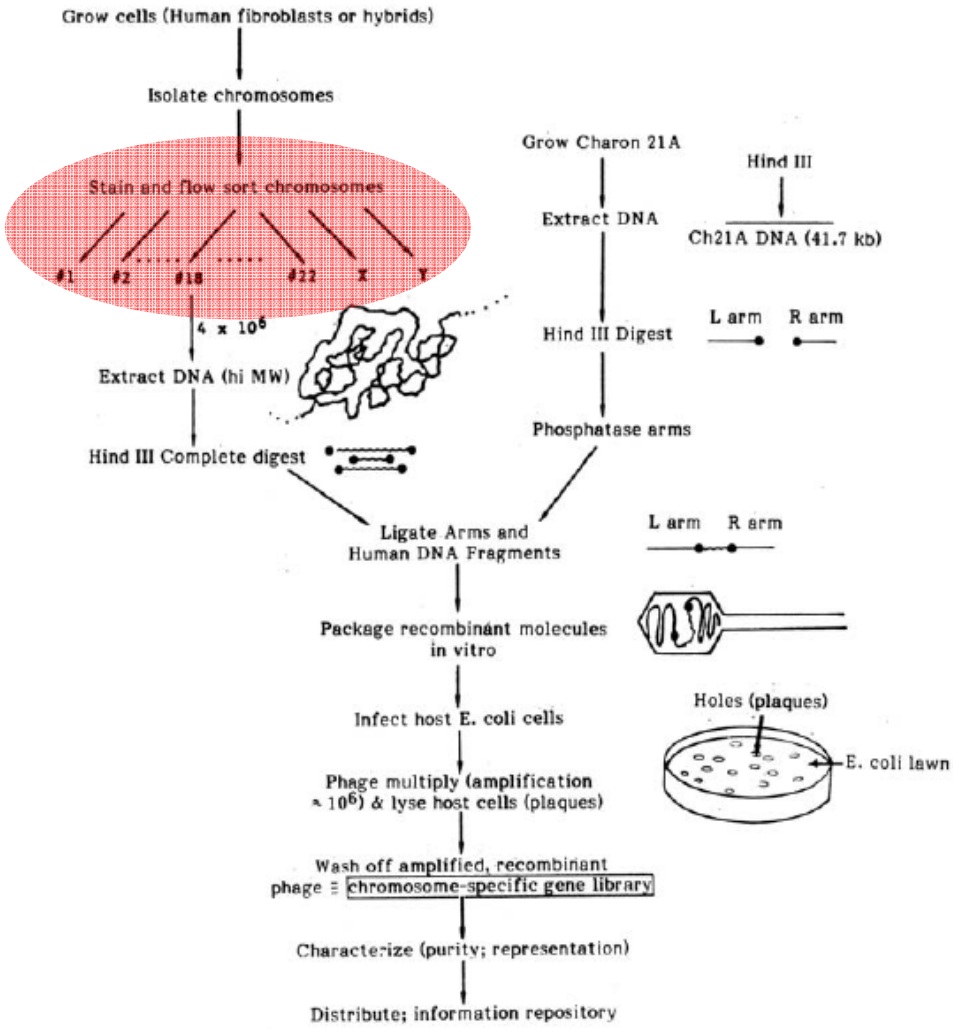
National Laboratory Gene Library Project, * Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, California 94550, † Los Alamos National Laboratory, Life Sciences Division, University of California, Los Alamos, New Mexico 87545,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

HUMAN CHROMOSOME-SPECIFIC DNA LIBRARIES: CONSTRUCTION AND AVAILABILITY

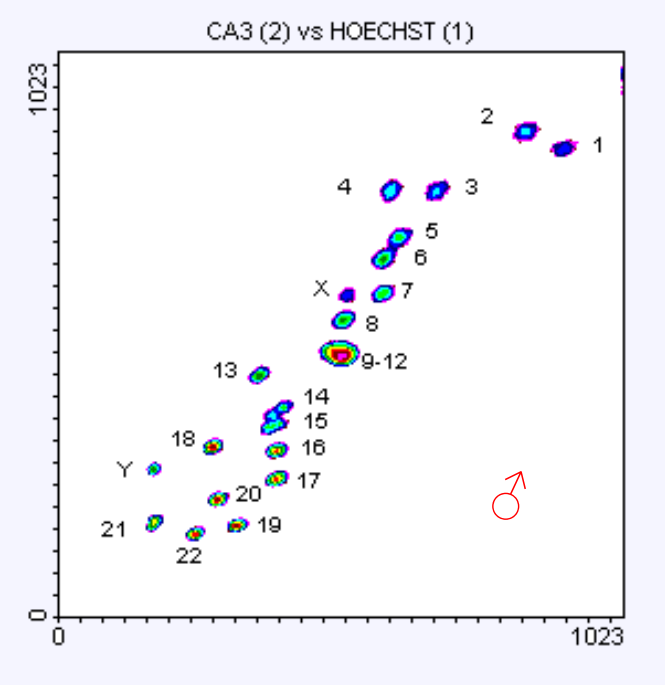
M.A. Van Dilla*□, L.L. Deaven†□, K.L. Albright†, N.A.Allen*, M.R. Aubuchon*, M.F.Bartholdi†, N.C.Brown†, E.W.Campbell†, A.V.Carrano*, L.M.Clark†, L.S.Cram†, B.D.Crawford†, J.C.Fuscoe*, J.W.Gray*, C.E.Hildebrand†, P.J.Jackson†, J.H.Jett†, J.L.Longmire†, C.R.Lozes*, M.L.Luedemann†, J.C.Martin†, J.S.McNinch*, 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.

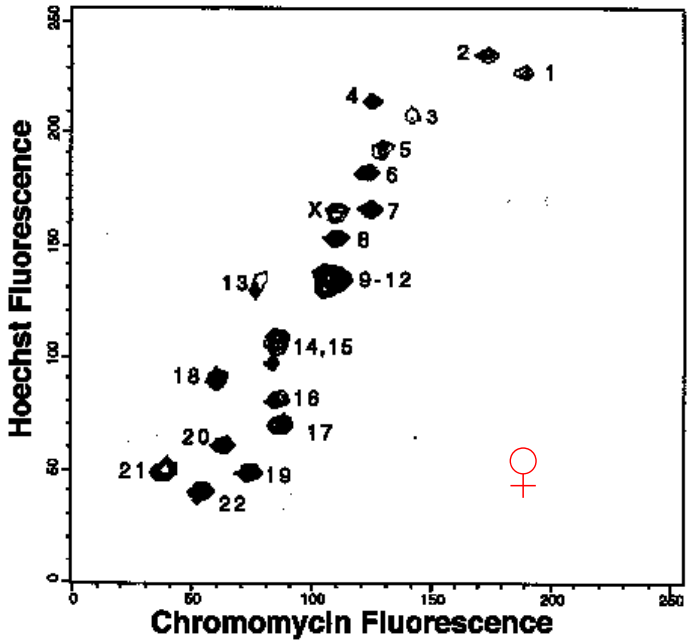
CONSTRUCTION OF A PHASE I CHROMOSOME-SPECIFIC (#18) HUMAN GENE LIBRARY IN CHARON 21A USING HIND III (LLNL)

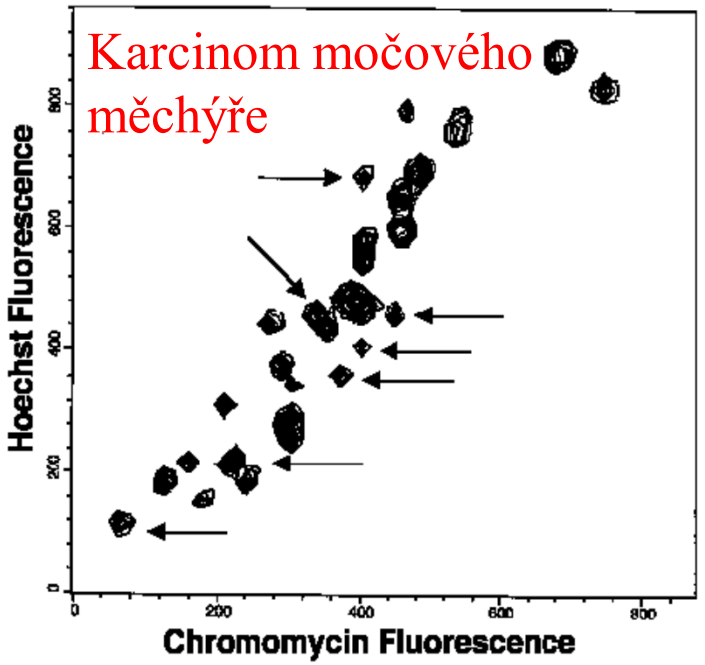


„Flow karyotype“



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





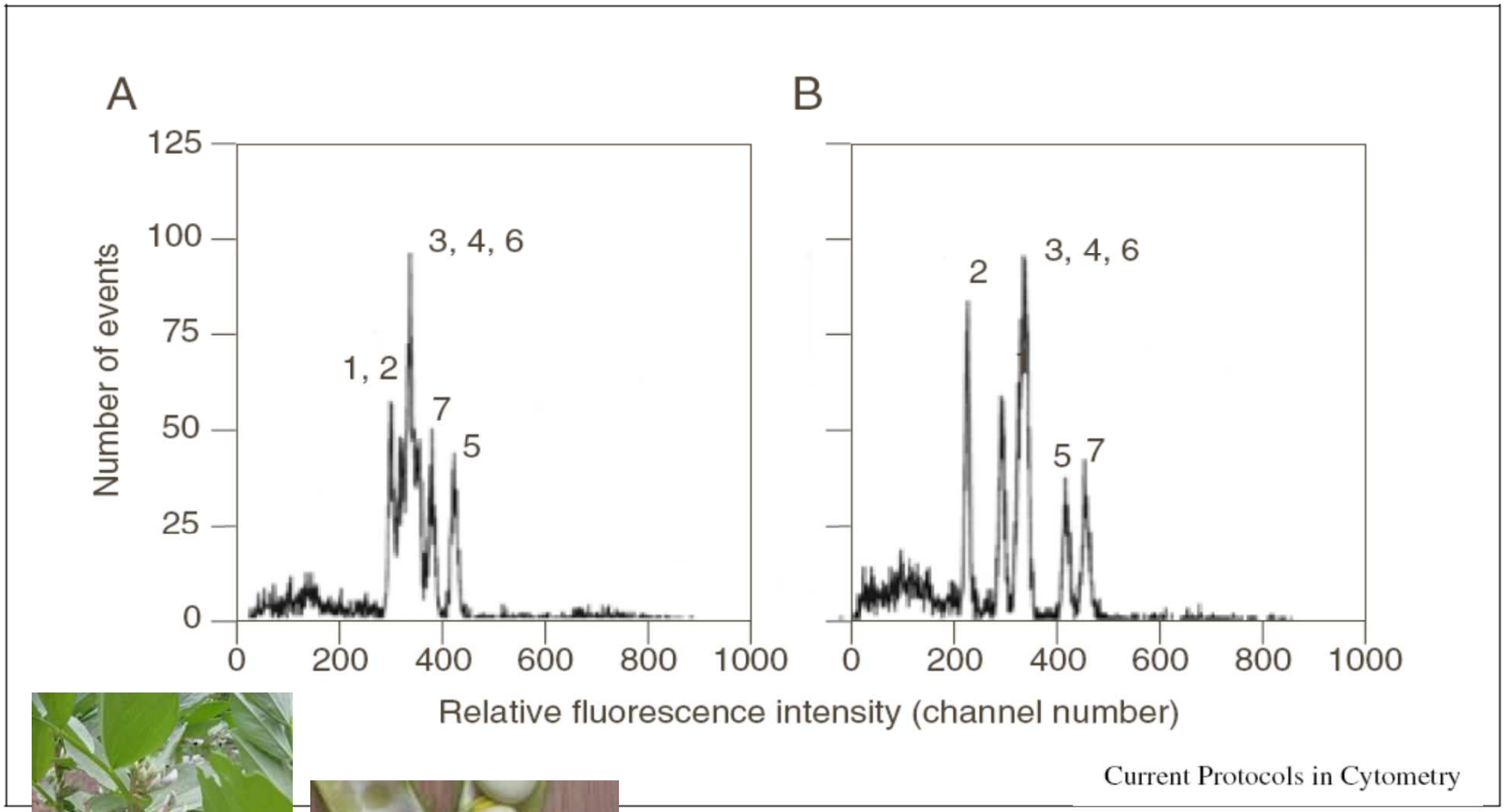
The Preparation of Human Chromosomes for Flow Cytometry

DEREK DAVIES

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

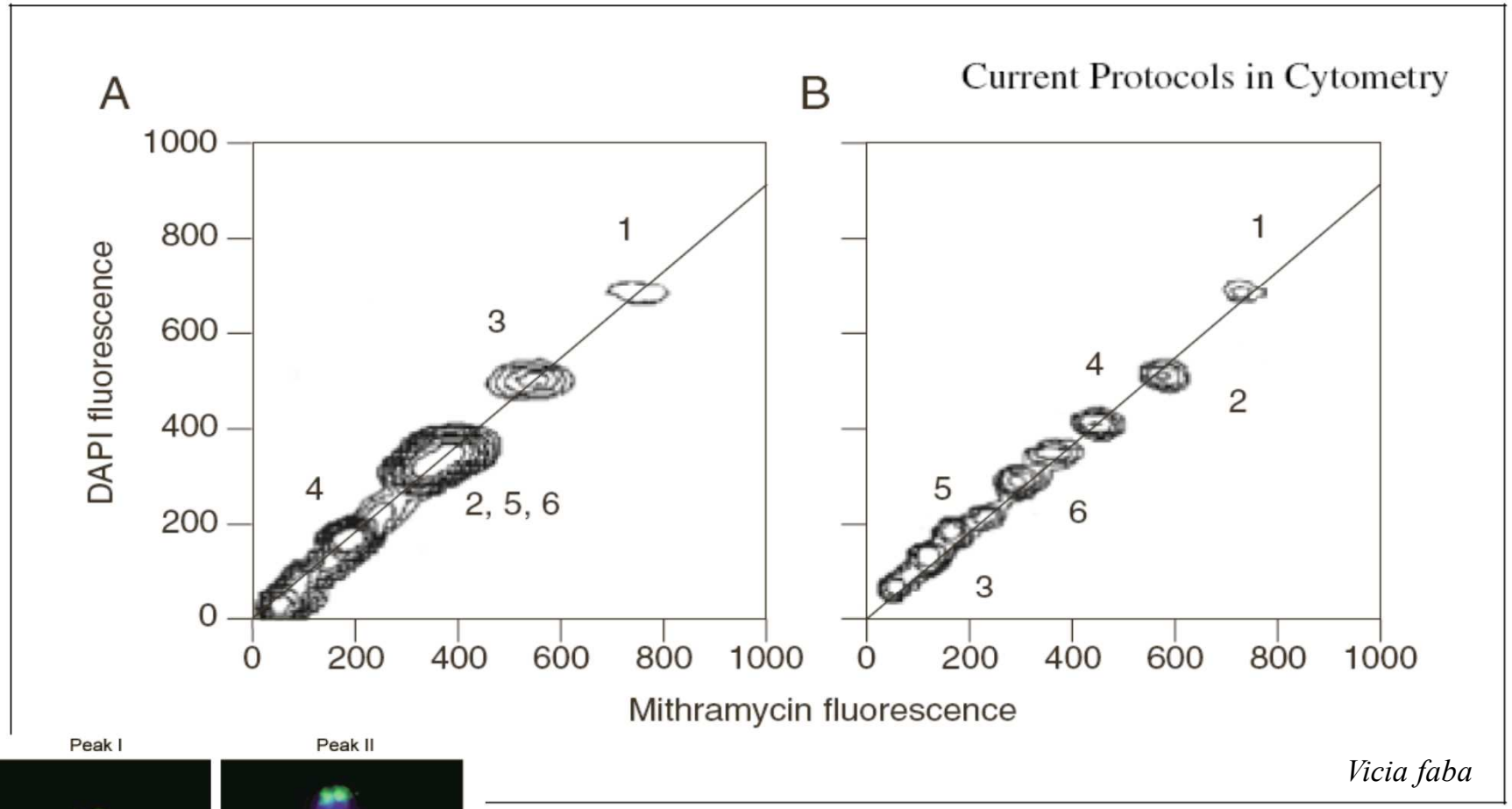
Vol. 33/2 Proceedings RMS *June* 1998

Sortrování chromozómů

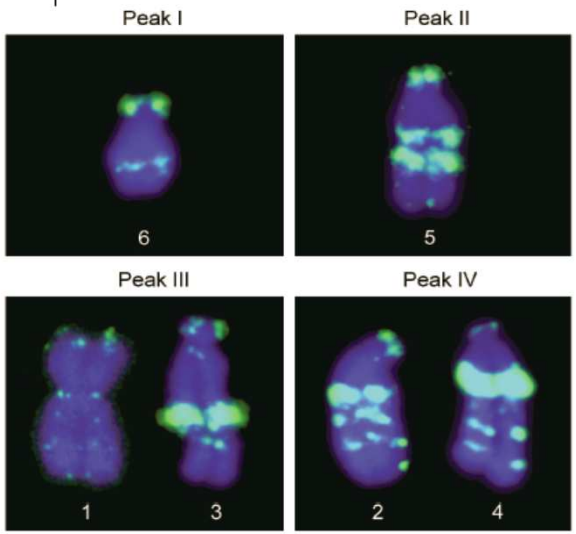


Pisum sativum

Sortrování chromozómů



Vicia faba



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

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

P. KOVÁŘOVÁ[1], A. NAVRÁTILOVÁ[2], J. MACAS[2] and J. DOLEŽEL[1,3]*

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

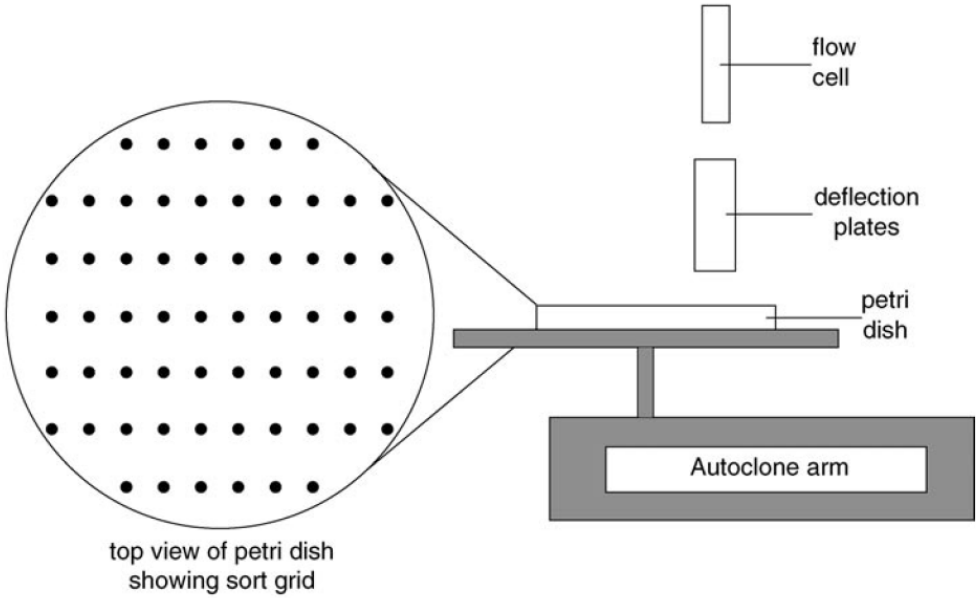
Current Protocols in Cytometry

Aplikace průtokové cytometrie v mikrobiologii

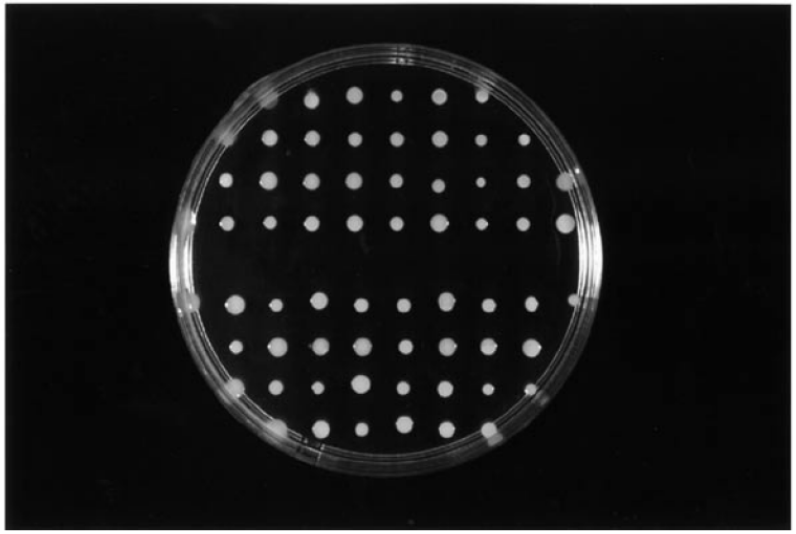
- viabilita
- metabolické funkce
- sortrování
- analýza aerosolů (Fluorescence Aerodynamic Particle Sizer (Flaps))

Aplikace průtokové cytometrie v mikrobiologii

- Sortrování
  - EPICS + Autoclone® modul



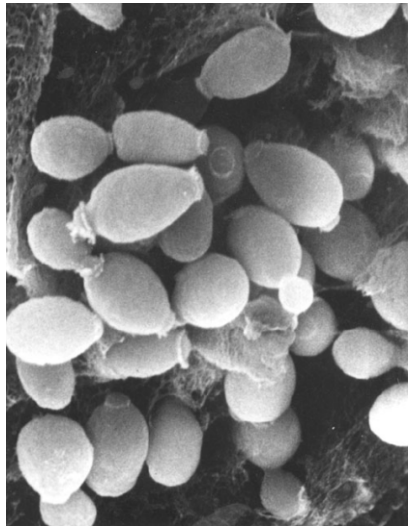
top view of petri dish showing sort grid



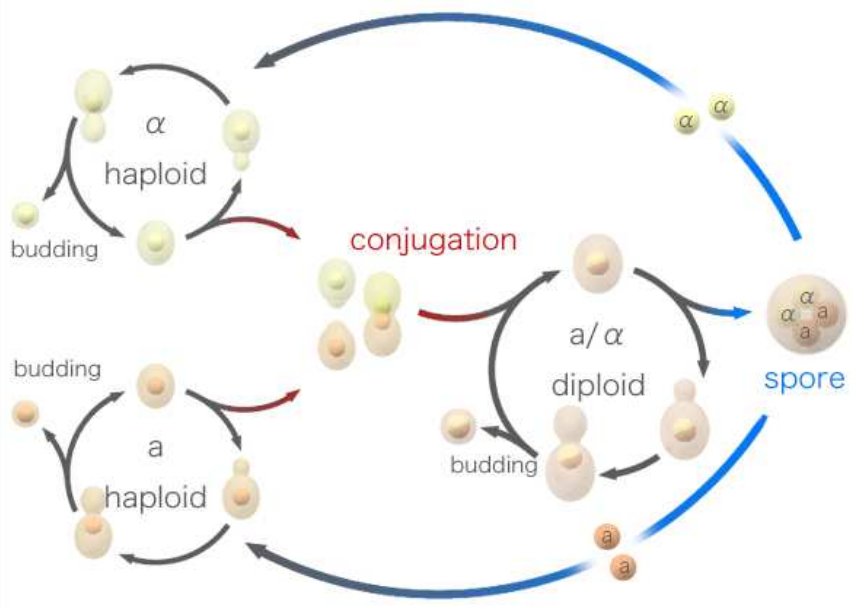
Current Protocols in Cytometry

Průtoková cytometrie kvasinek

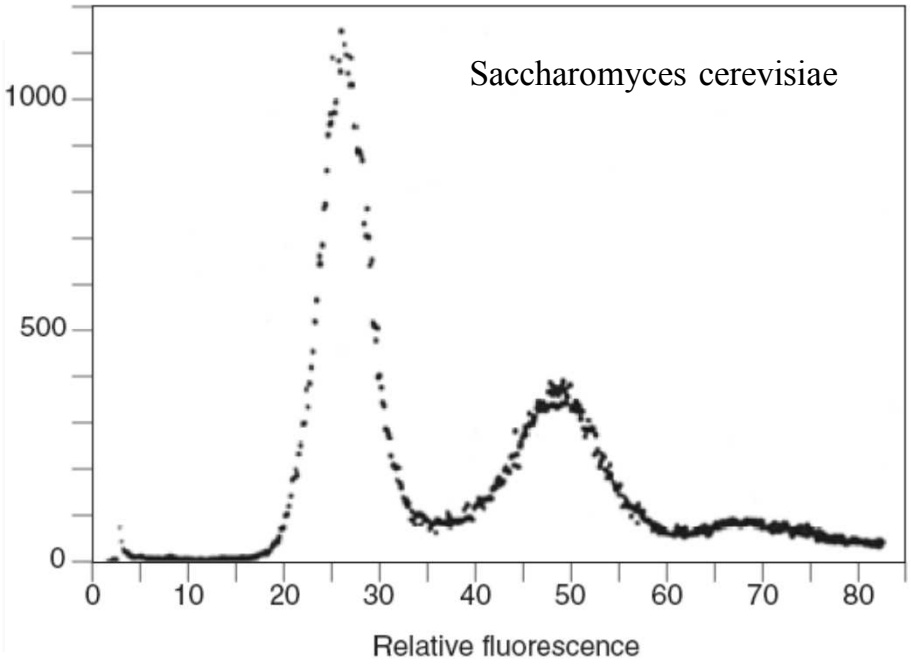
- **buněčné dělení**
- viabilita
- membránový potenciál
- respirace
- produkce H_2O_2
- citlivost k antibiotikům
- separace



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



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



Current Protocols in Cytometry

Průtoková cytometrie kvasinek

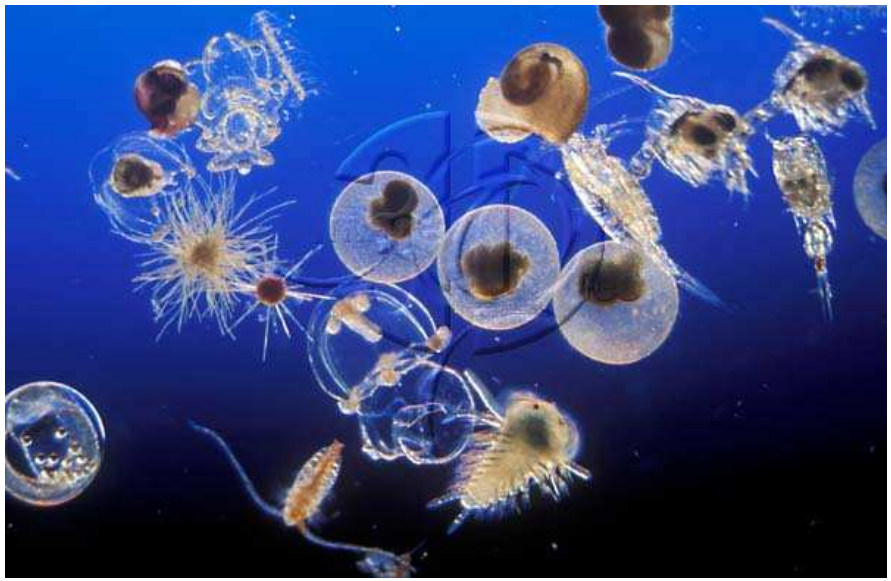
Yeast Cell Cycle During Fermentation and Beer Quality

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 nano-fytoplanktonu (< 20 μM)
- analýza metabolických funkcí planktonu
- studium pigmentace (analýza chlorofylu a fykoeritrinu)



Průtoková cytometrie v hydrobiologii

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

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

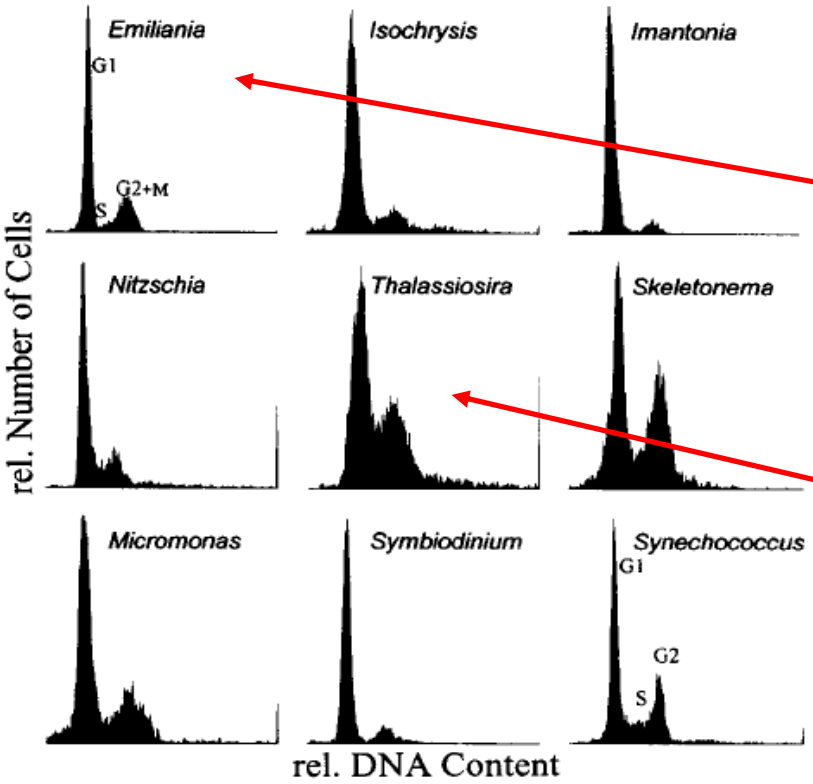
Vol. 185: 301–307, 1999 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published August 20

NOTE

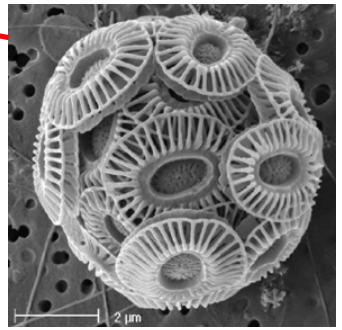
Cytometric measurement of the DNA cell cycle in the presence of chlorophyll autofluorescence in marine eukaryotic phytoplankton by the blue-light excited dye YOYO-1

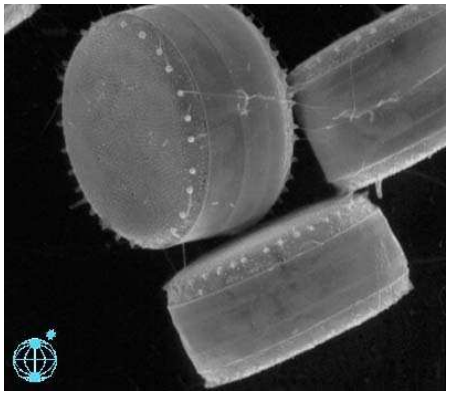
Frank J. Jochem[1,*], Doris Meyerdierks[2]

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



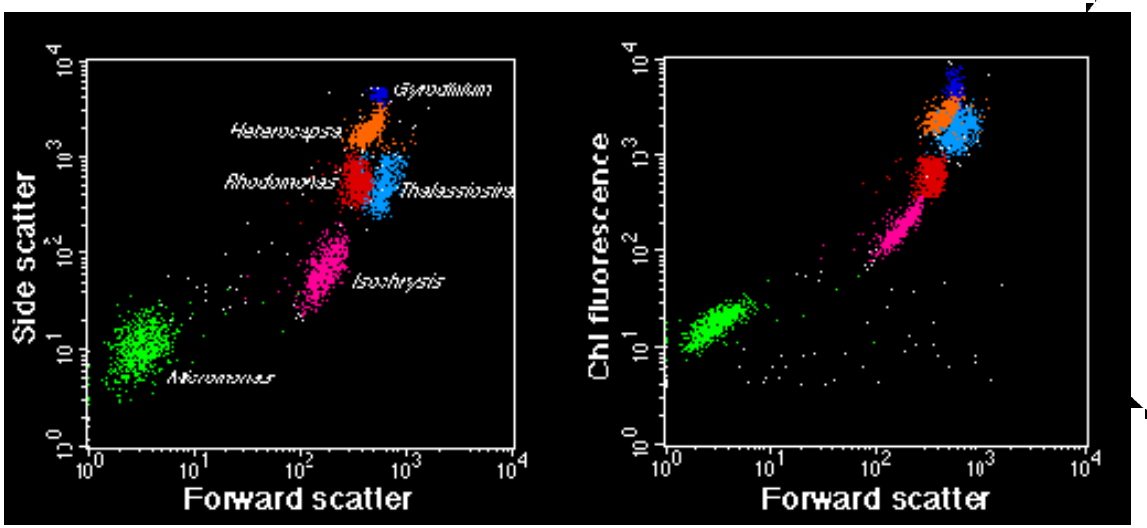
http://www.soes.soton.ac.uk/staff/tt/

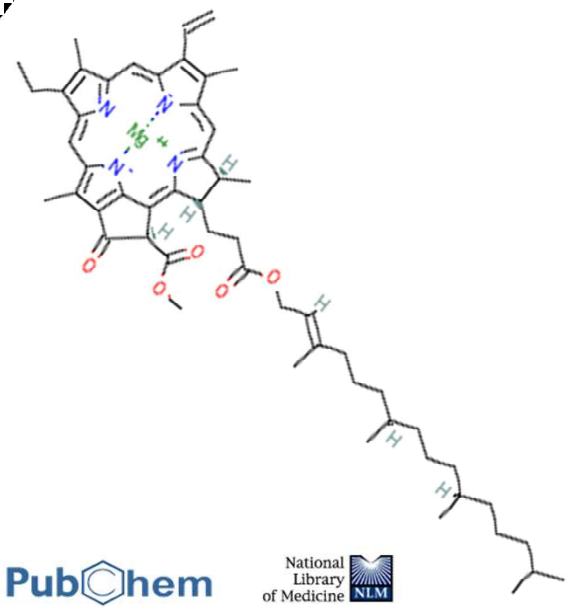


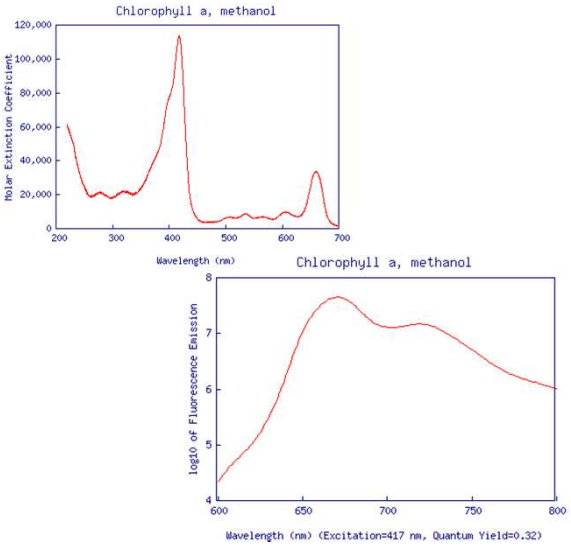


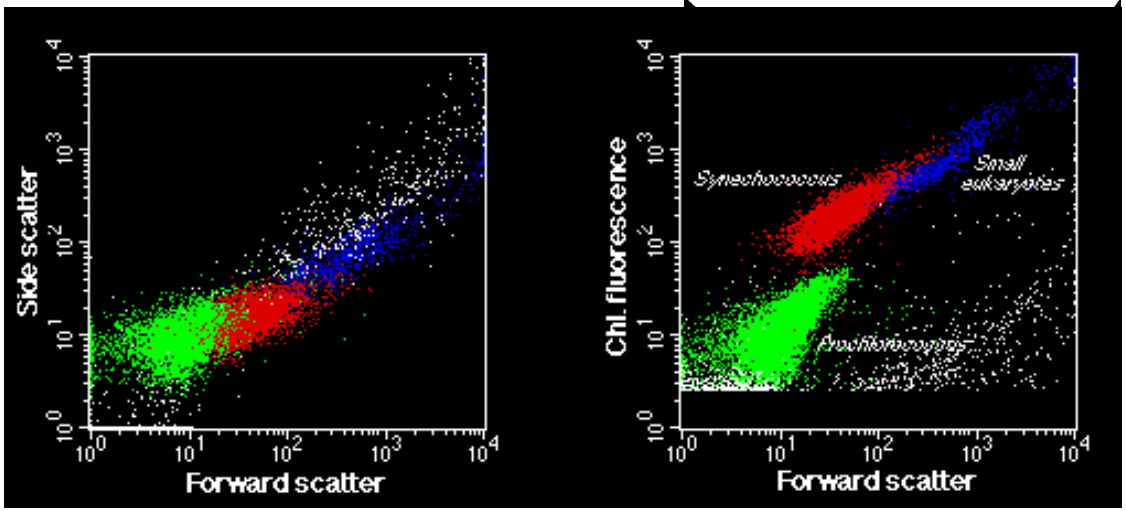
http://planktonnet.awi.de/portal.php?pagetitle=assetfactsheet&asset_id=15127

Průtoková cytometrie v hydrobiologii









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

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

Available online at www.sciencedirect.com

ScienceDirect

Journal of Environmental Sciences 2012, 24(9) 1709–1716

JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

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

Quan Zhou[1,2], Wei Chen[1], Huiyong Zhang[3], Liang Peng[1], Liming Liu[1], Zhiguo Han[3], Neng Wan[4], Lin Li[1], 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

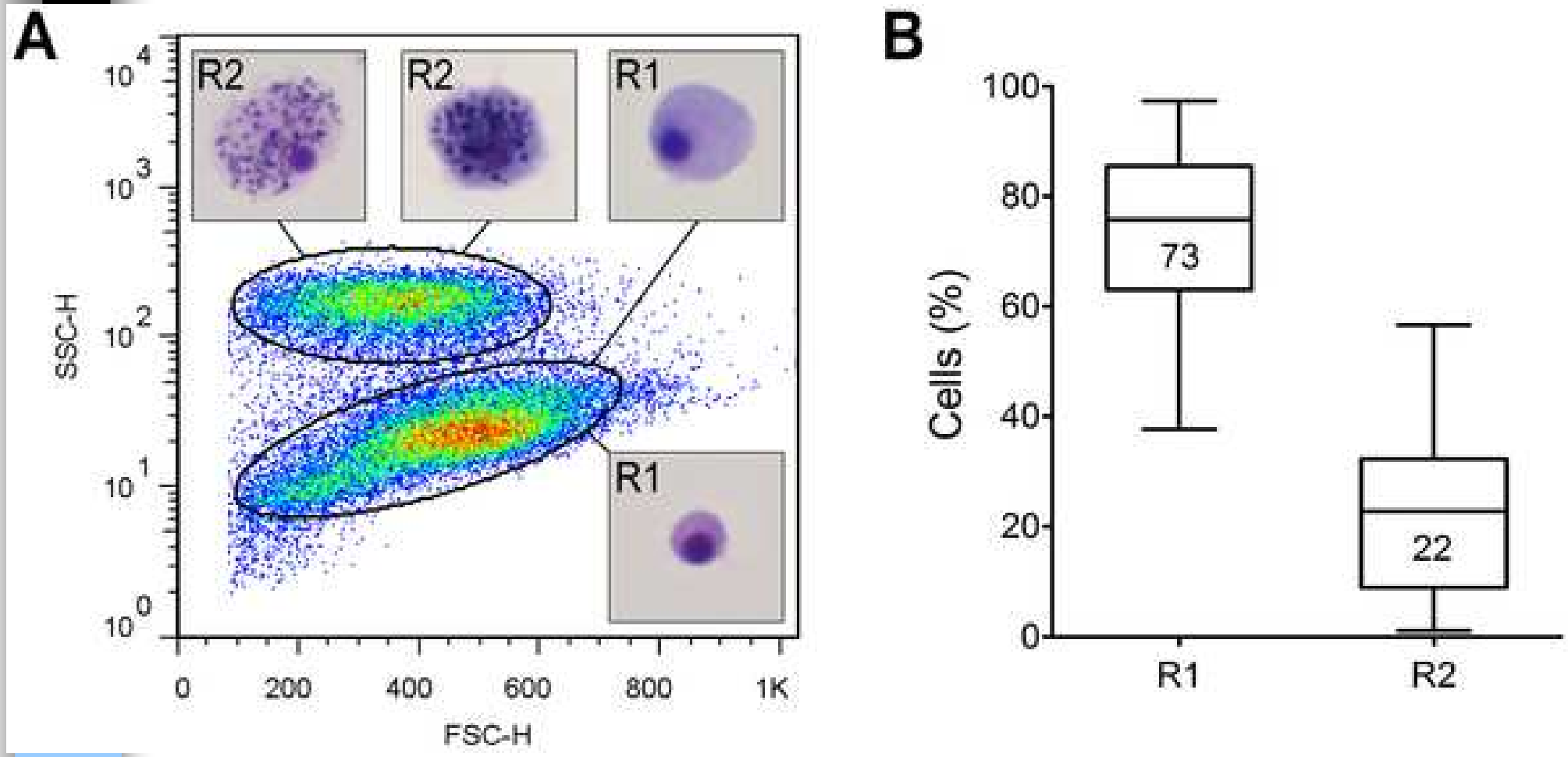
Flow cytometry as a tool for analysing invertebrate cells

A Cossarizza[1], M Pinti[1], L Troiano[1], EL Cooper[2]

[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*

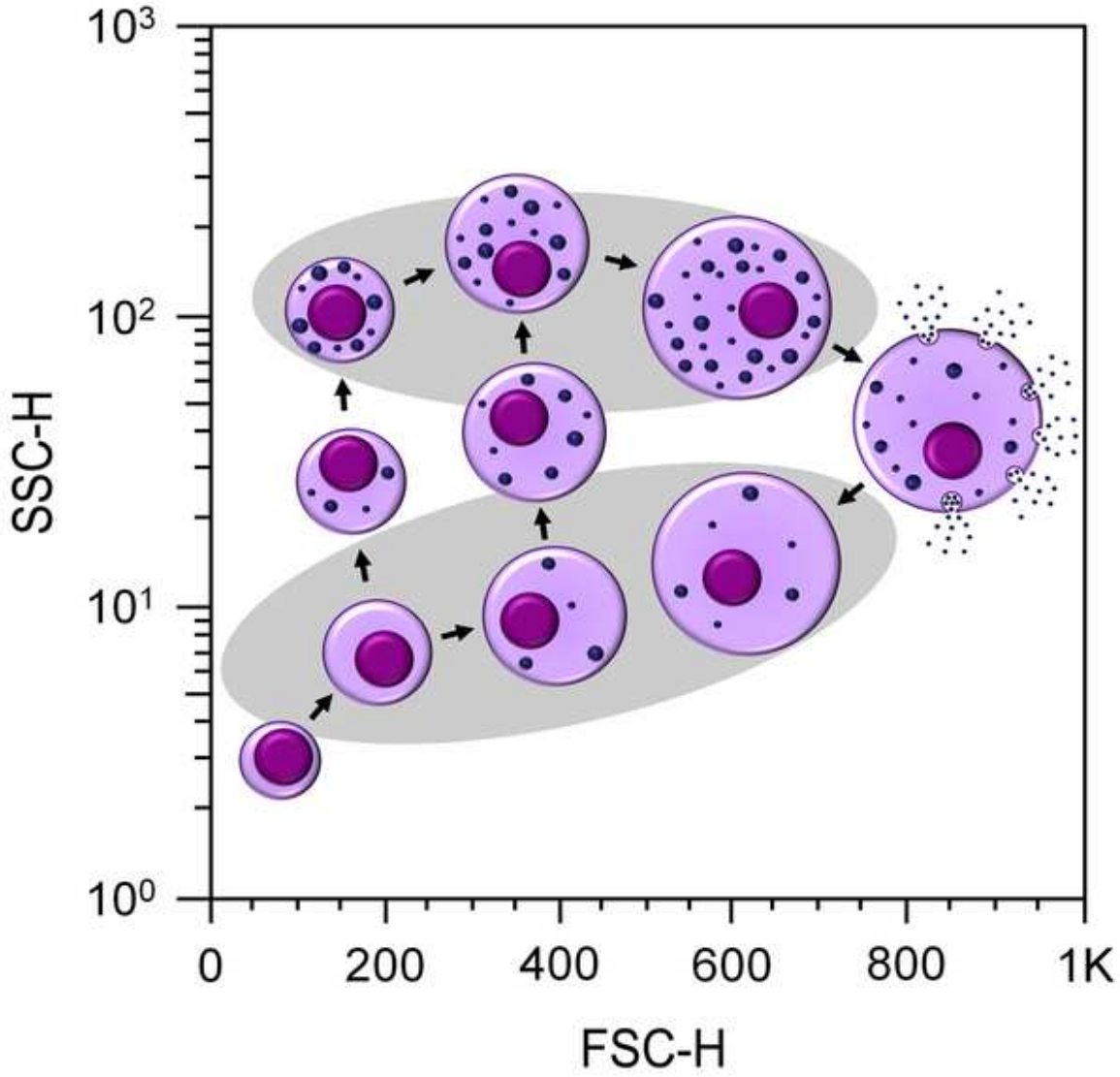
http://www.icms.qmul.ac.uk/flowcytometry/uses/insects/index.html

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



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

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

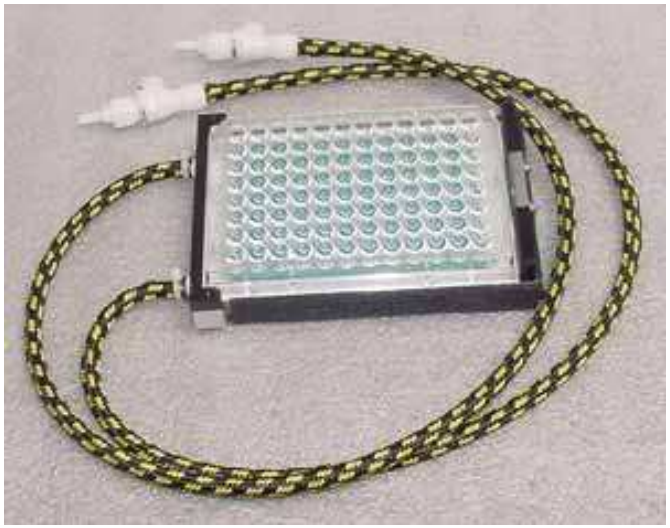
Automatizované systémy měření vzorků

Automatický karusel (autosampler)

Adaptér pro nasávání vzorků z mikrotitrační desky

BD

Automatizovaný „microsampler“ systém



Cytek FLOW CYTOMETRY PRODUCTS

BD

Cytometry 47:183–191 (2002)
DOI 10.1002/cyto.10067

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

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High Throughput Flow Cytometry

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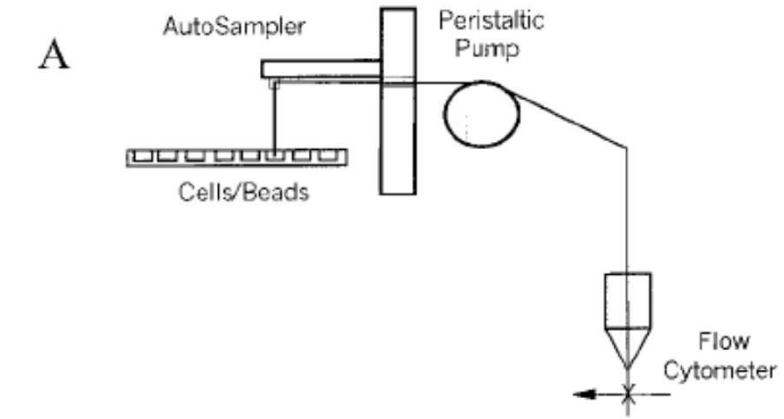
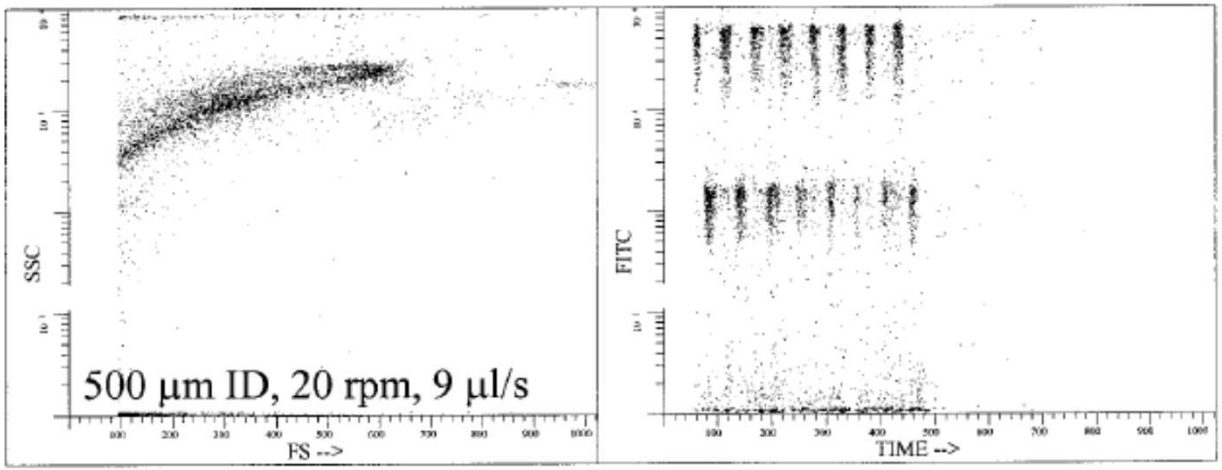
Received 18 September 2000; Revision Received 4 January 2001; Accepted 13 January 2001



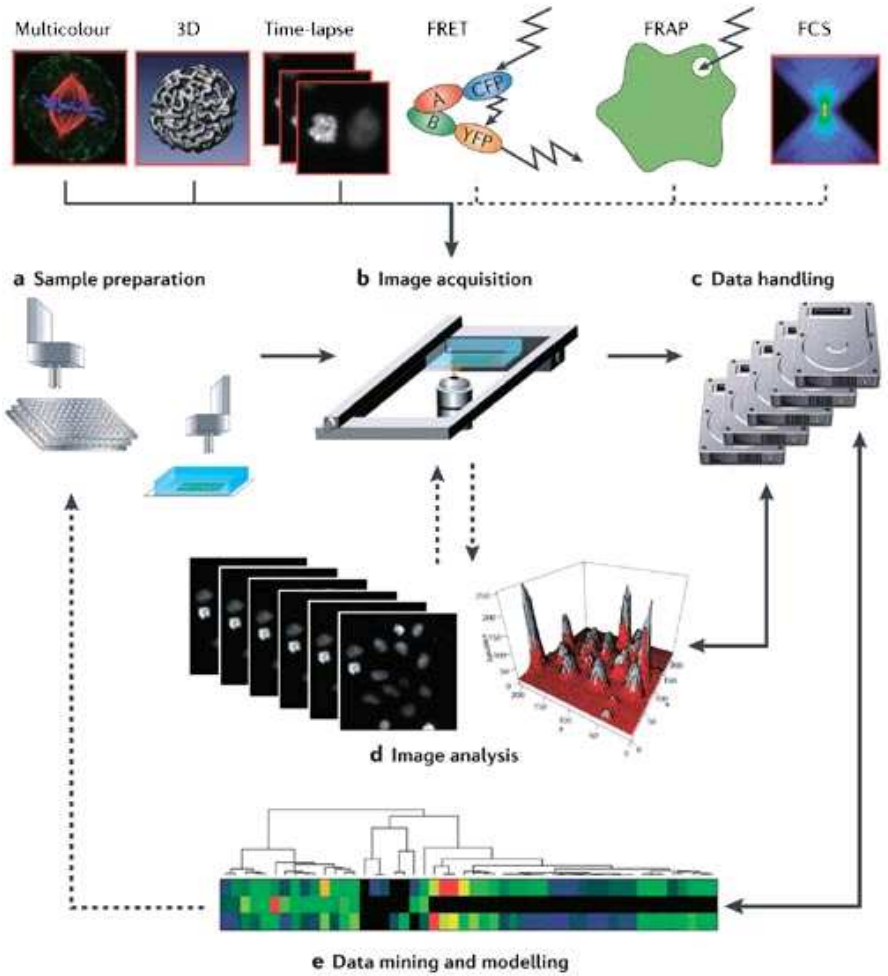
B

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-μm) ID tubing between the peristaltic pump and the flow cytometer.

C



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



Analysis

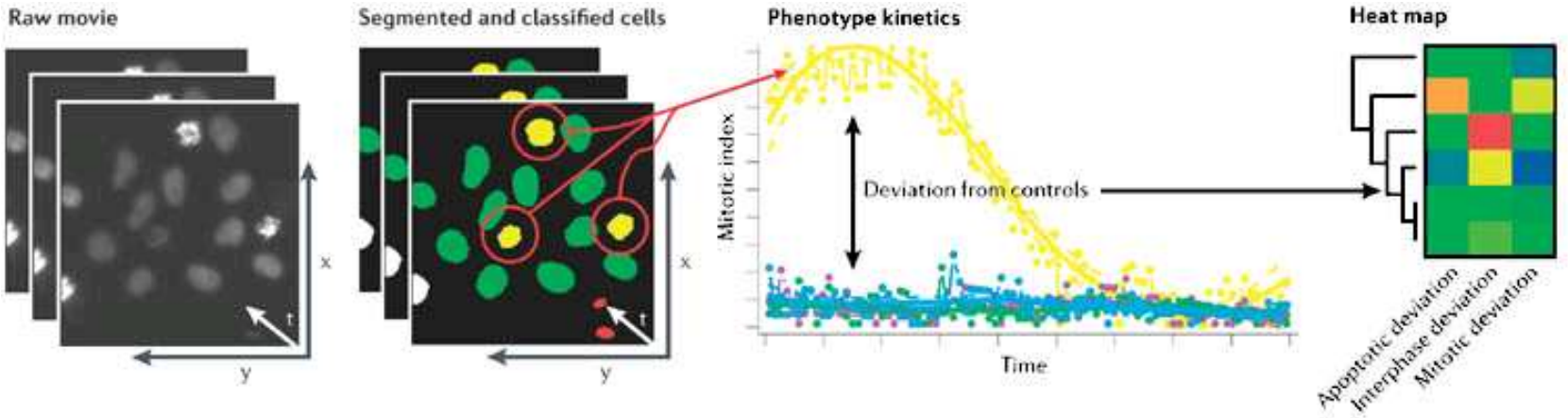


Table 1. Comparison of the Key Attributes of High-Throughput Flow Cytometry and High-Content Microscopy		
Key Attributes	**HT Flow Cytometry**	**High Content Microscopy**
Cell types	Optimal for suspension cells; adherent cells need to be detached before sampling.	Optimal for adherent cells; suspension 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

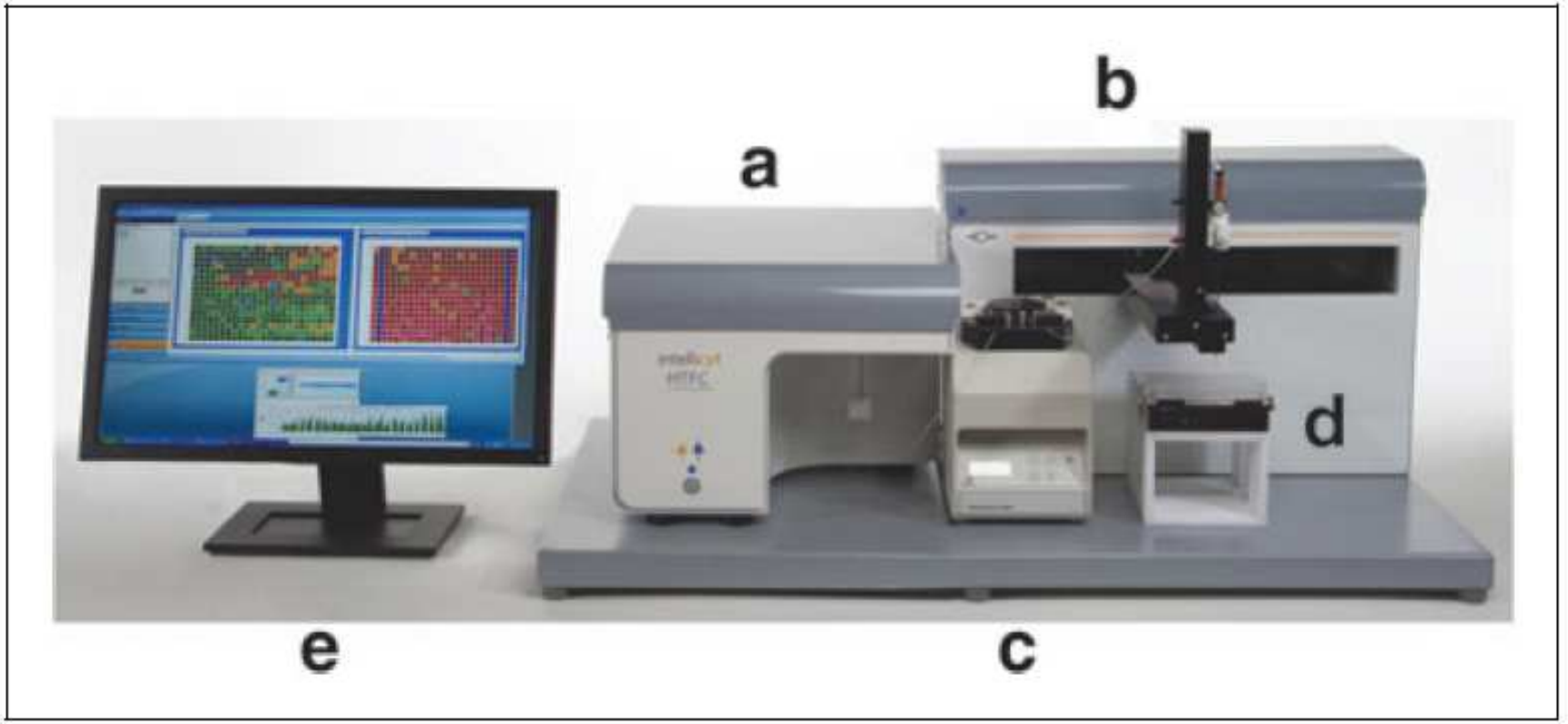


Fig. 1. The HTFC Screening System (IntelliCyt Corporation). **(a)** 2-laser, 4-color flow cytometer; **(b)** an x, y, z autosampler; **(c)** a low pulsation peristaltic pump; **(d)** orbital plate shaker that accommodates 96- and 384-well plates; **(e)** system computer with HyperView installed to set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

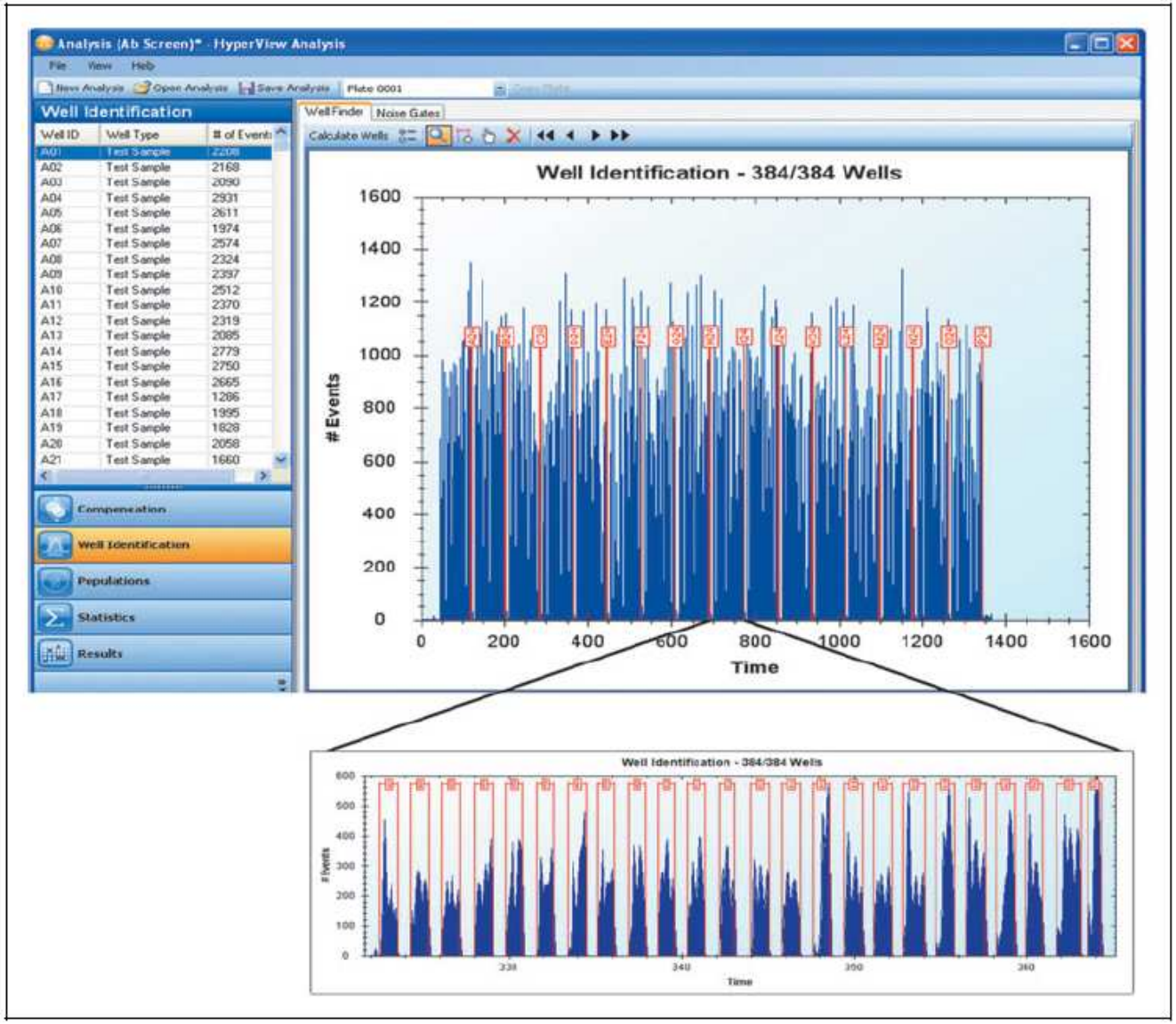
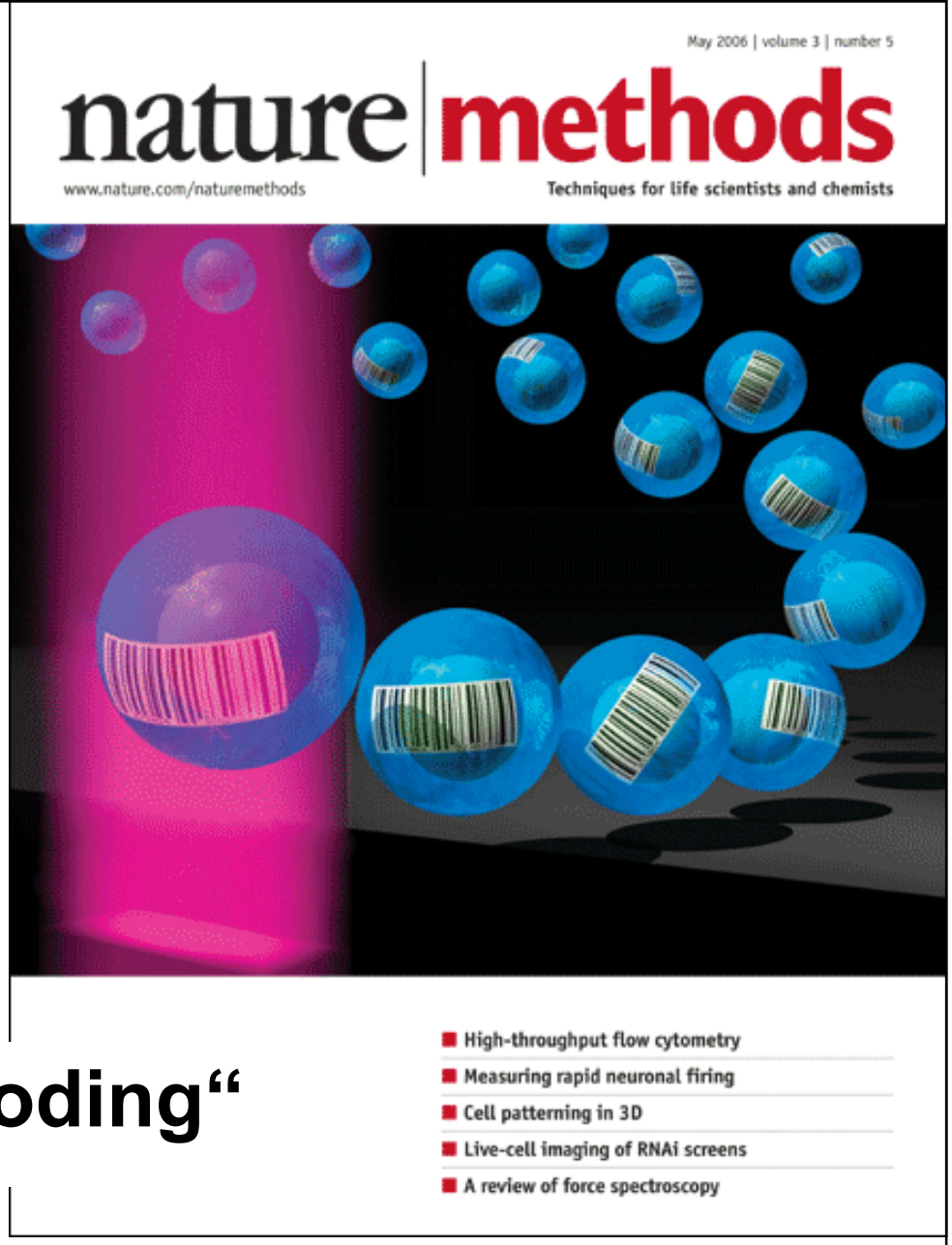


Fig. 4. Screenshot from HyperView showing an example of the Well Identification process. Data from the 384-well plate is collected in to a single flow cytometry standard file, which is shown in the main window. The data are deconvolved by the software algorithm to identify each peak with a well address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

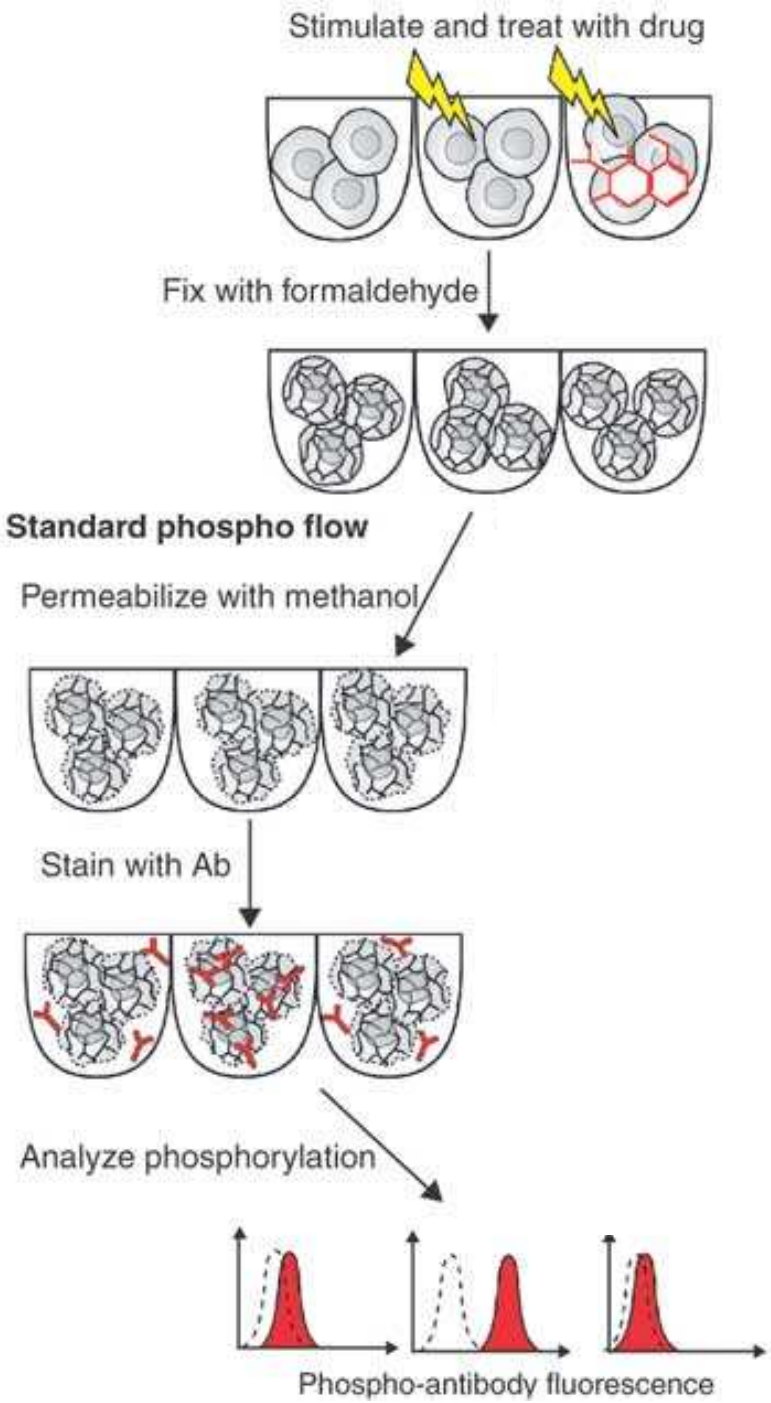
Garry Nolan

Peter Krutzik

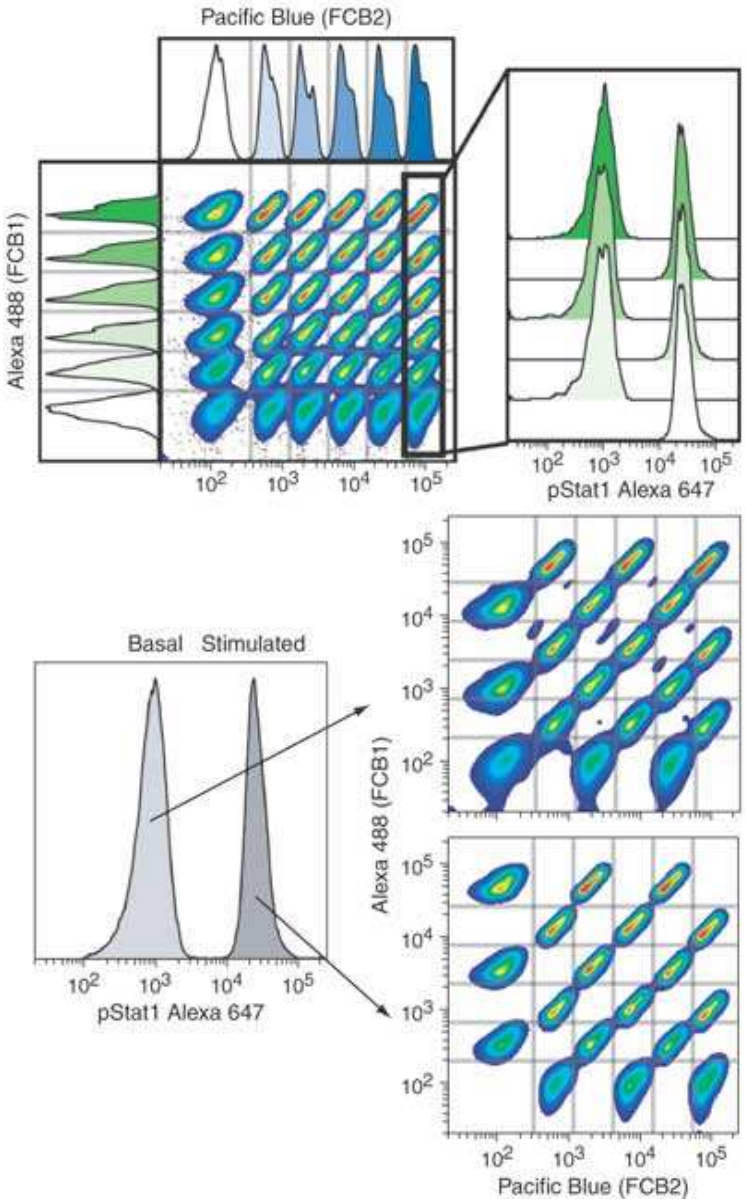


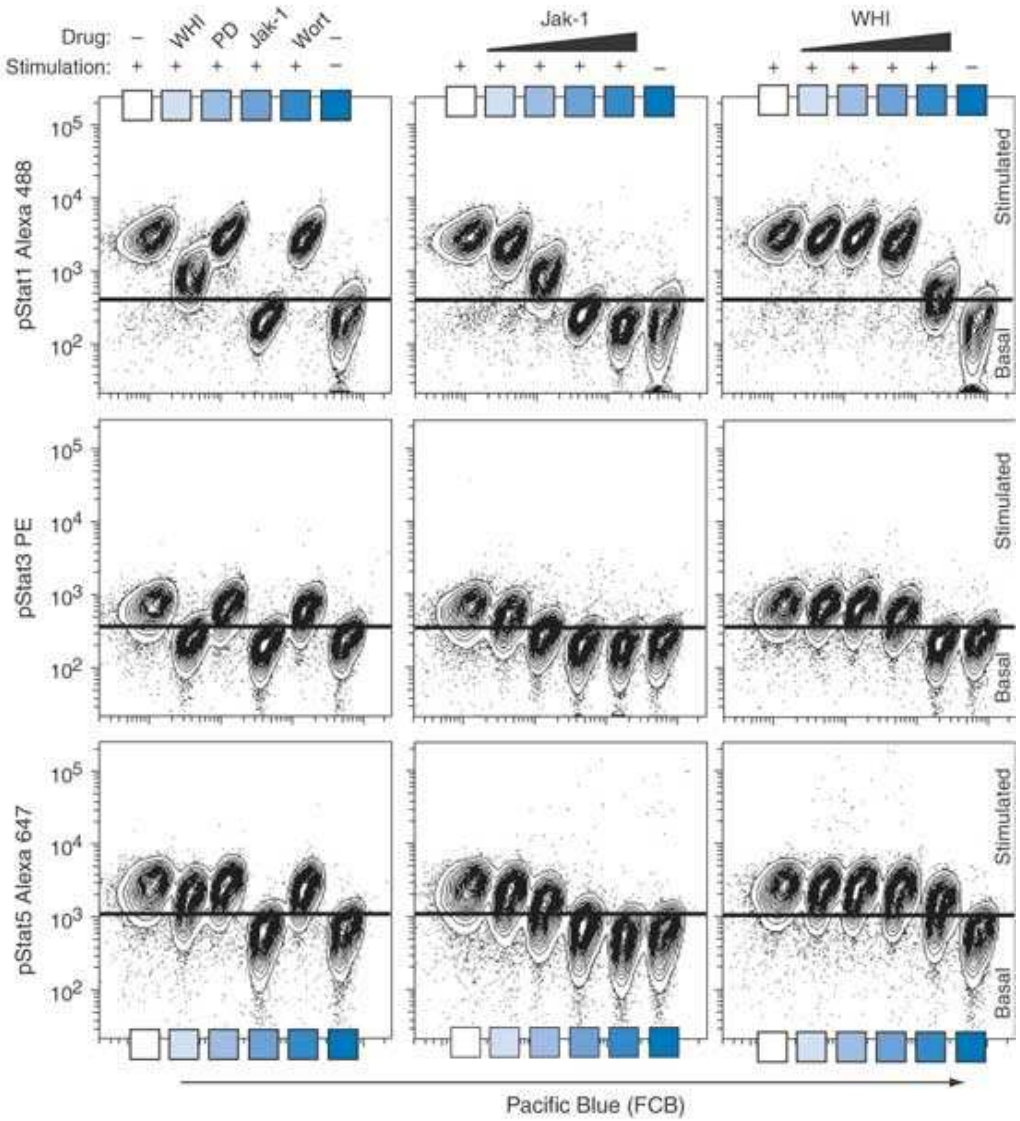
„Fluorescent cell barcoding“

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



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





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

Get the best out of your model

FACS-based surface screen:

- validated antibodies in 96w plates
- several comercially available possibilities, we have gone for…

- LEGENDScreen HUMAN

332 PE conjugated antibodies + ISOs

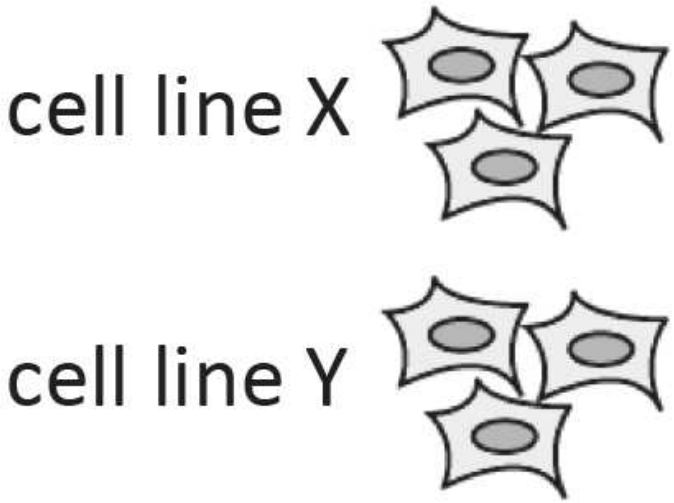
- LEGENDScreen MOUSE

252 PE conjugated antibodies + ISOs

- there are XY vials in LN
- price of kit ≈ 1000 € (27k Kc)

How to get the best of it all?

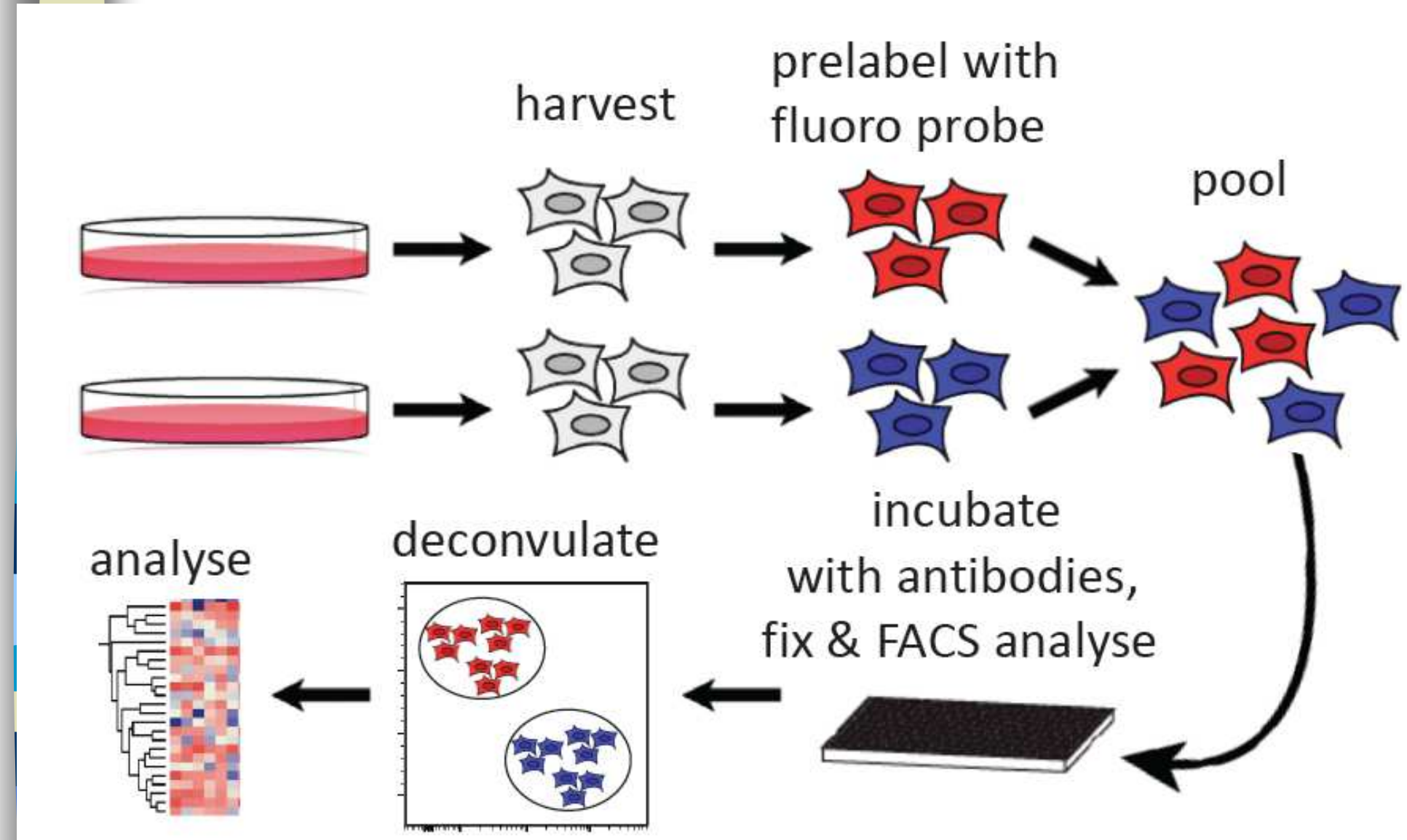
Fluorescent barcode



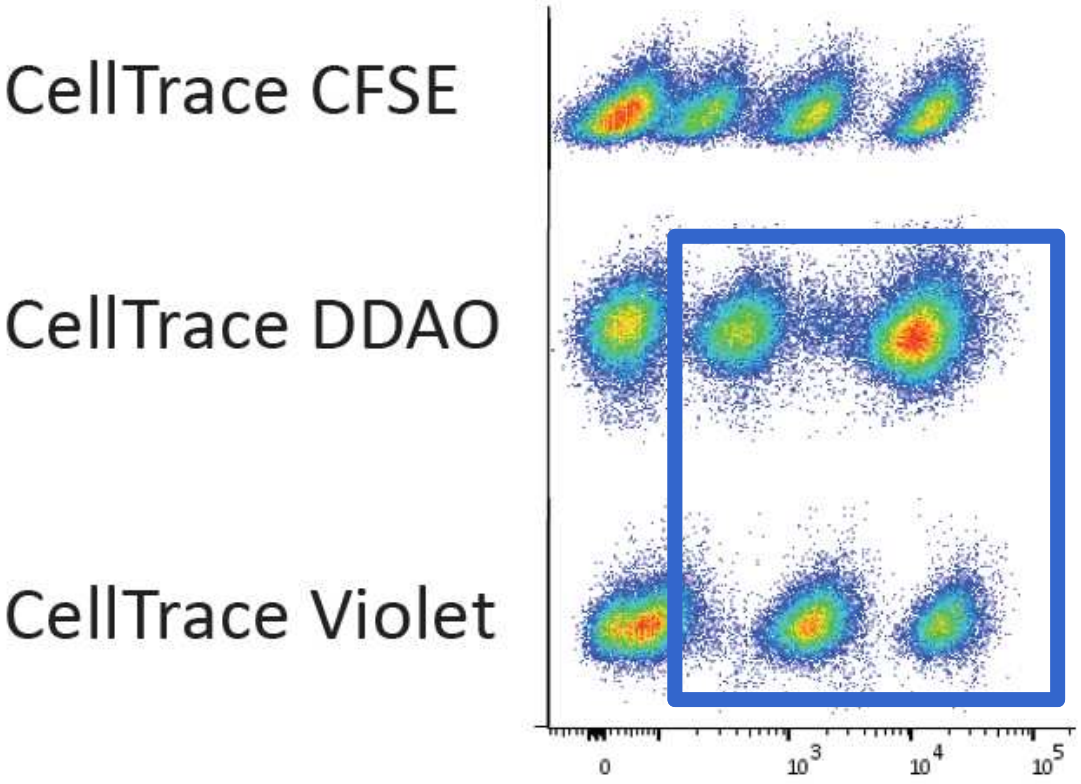
possibility 1 – fluorescent proteins
- which, how many, isn't it too laborous w/out lentiviral TXF?

possibility 2 – amino-reactive probes, lipophilic dyes…
- how to choose the right one?

Final workflow



The optimal concentation issue



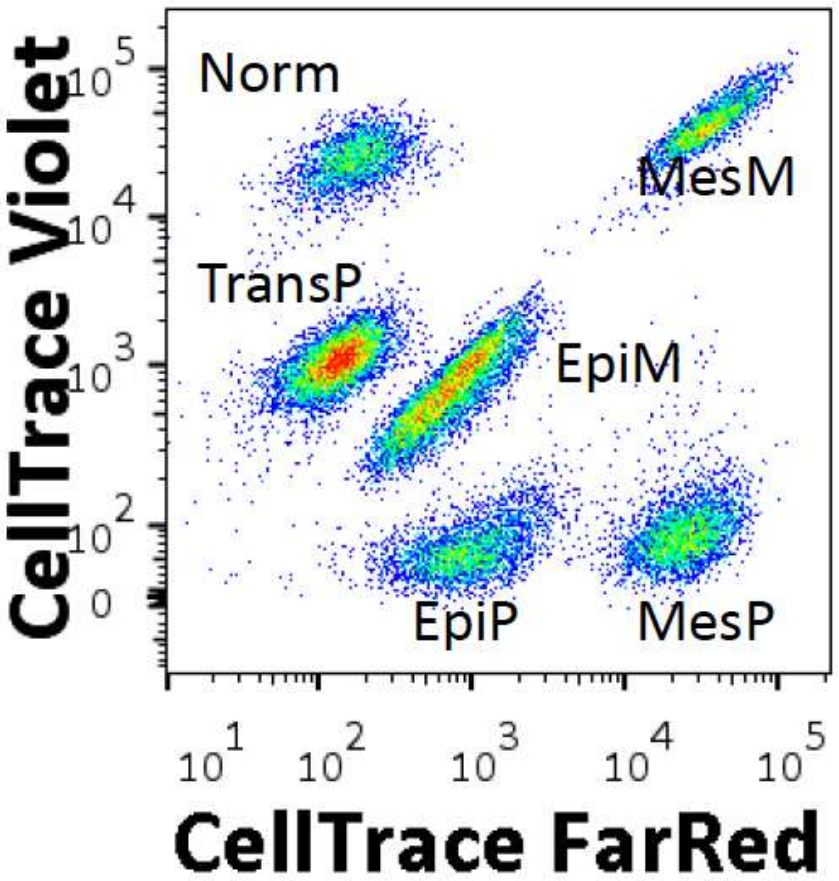
HOW TO TEST IT:

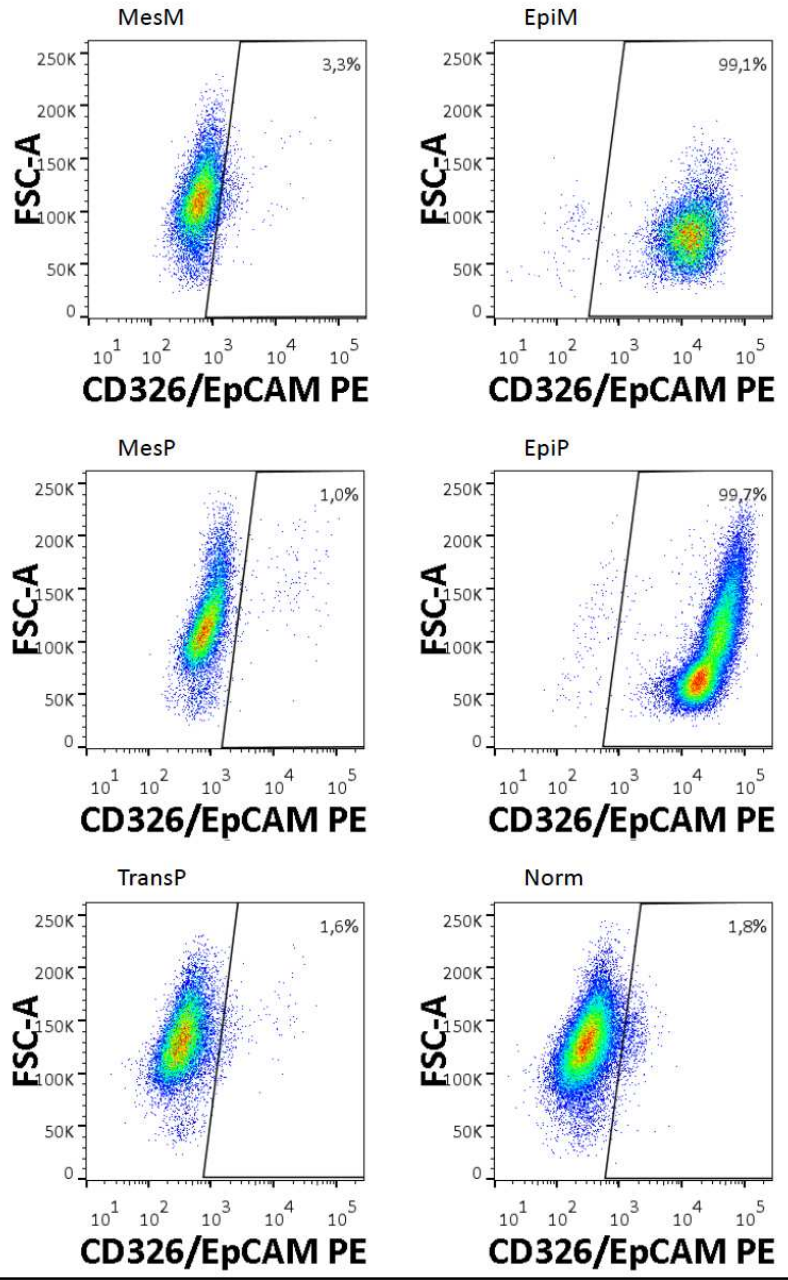
10x serial dilution

REQUIREMENTS:

- optimal resolution
- compatibility w/ PE

Sample results I





EpCAM
- marker of epithelial cells
- commonly lost during EMT

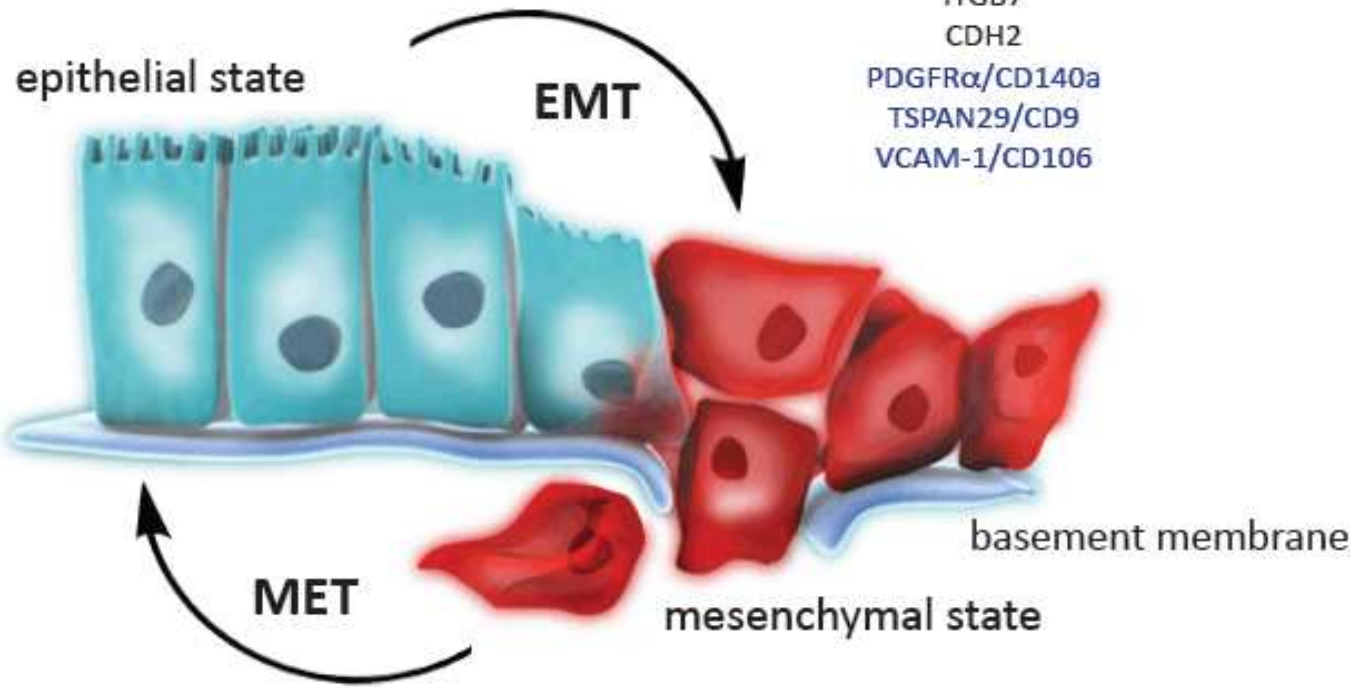
Sample result

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
$CD24^{-}CD44^{high}$

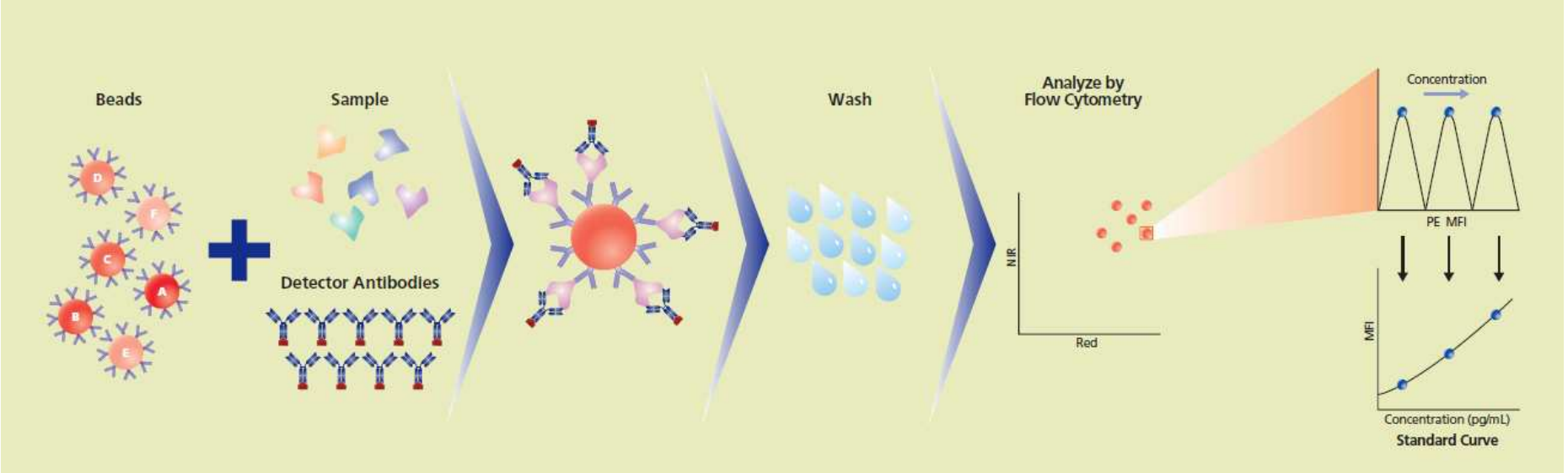
ITGA5/CD49e
ITGA7
ITGB5
ITGB7
CDH2
PDGFRα/CD140a
TSPAN29/CD9
VCAM-1/CD106



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

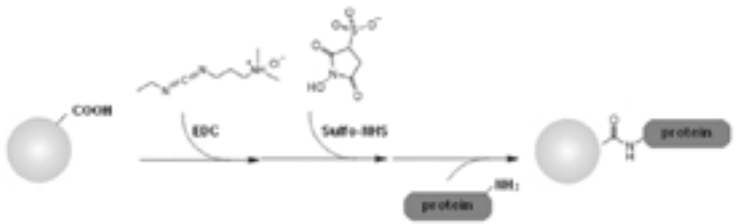
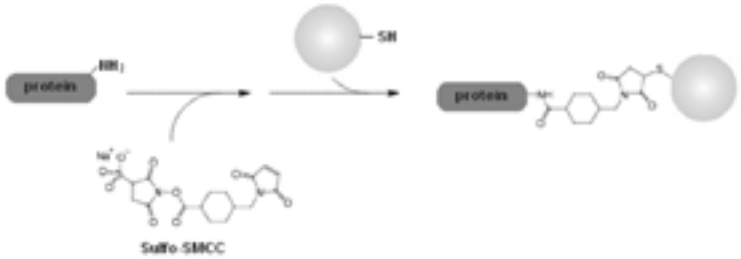
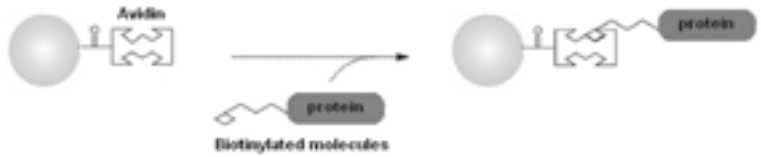
Cytometric bead array (CBA)

- 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	

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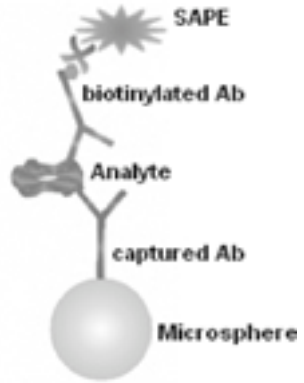
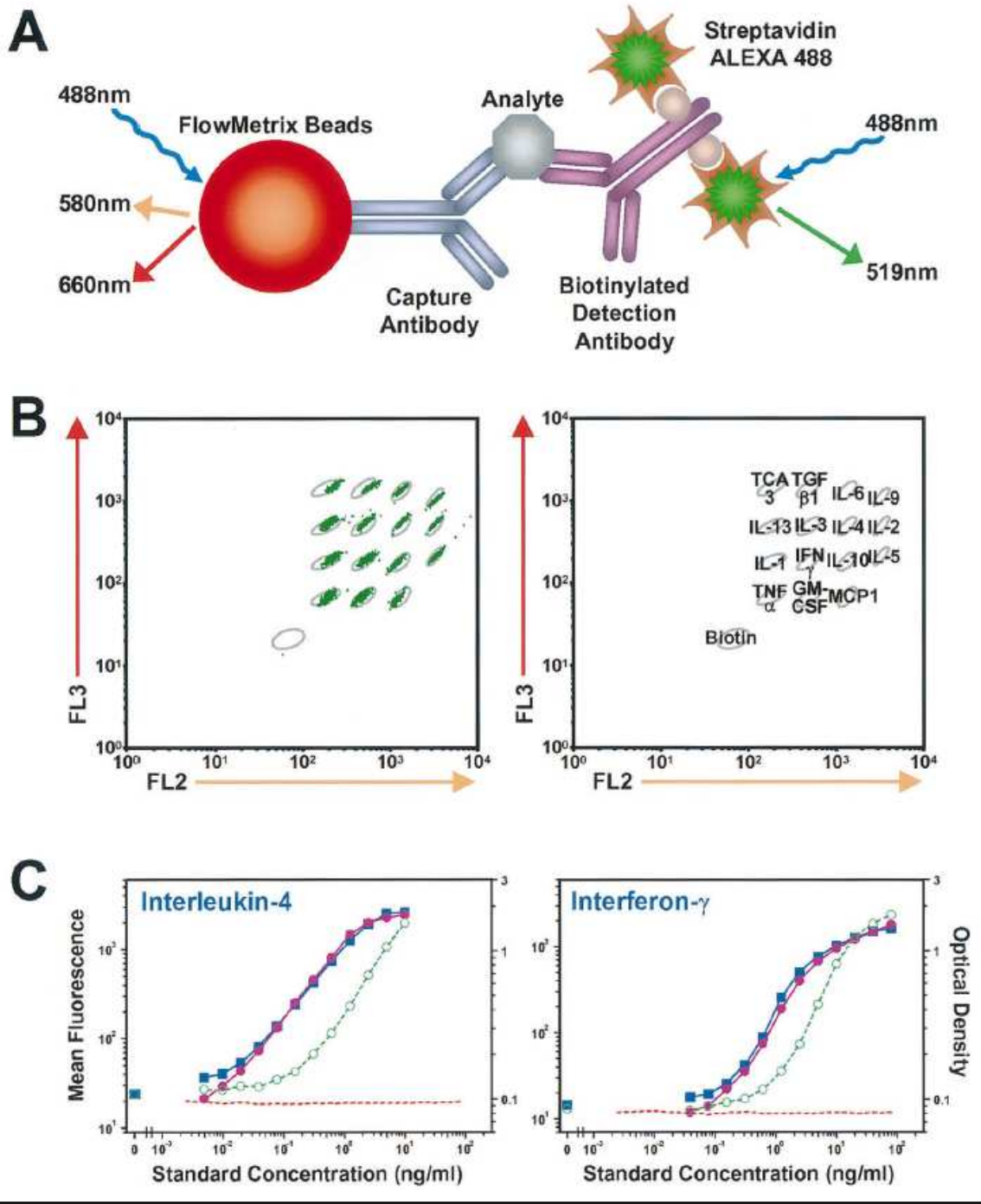


ELECTROPHORESIS
Volume 30, Issue 23, pages 4008-4019, 3 DEC 2009 DOI: 10.1002/elps.200900211
http://onlinelibrary.wiley.com/doi/10.1002/elps.200900211/full#fig1

CBA

D.A.A. Vignali / Journal of Immunological Methods 243 (2000) 243–255



CBA

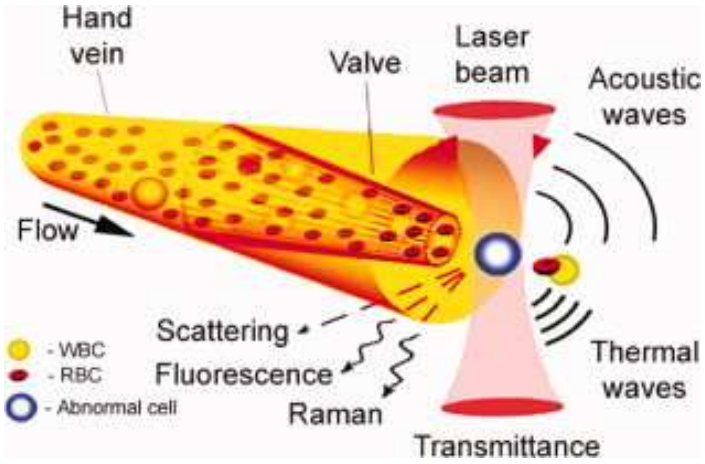
- multiplexing capabilities
- speed
- incorporation of multiple assay formats
- rapid assay development and reasonable cost
- automation

ex vivo flow cytometrie - limitace

- Ovlivnění některých vlastností buněk (morfologie, exprese znaků);
- neumožnuje 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.

Cytometry part A 79A: 737-745, 2011

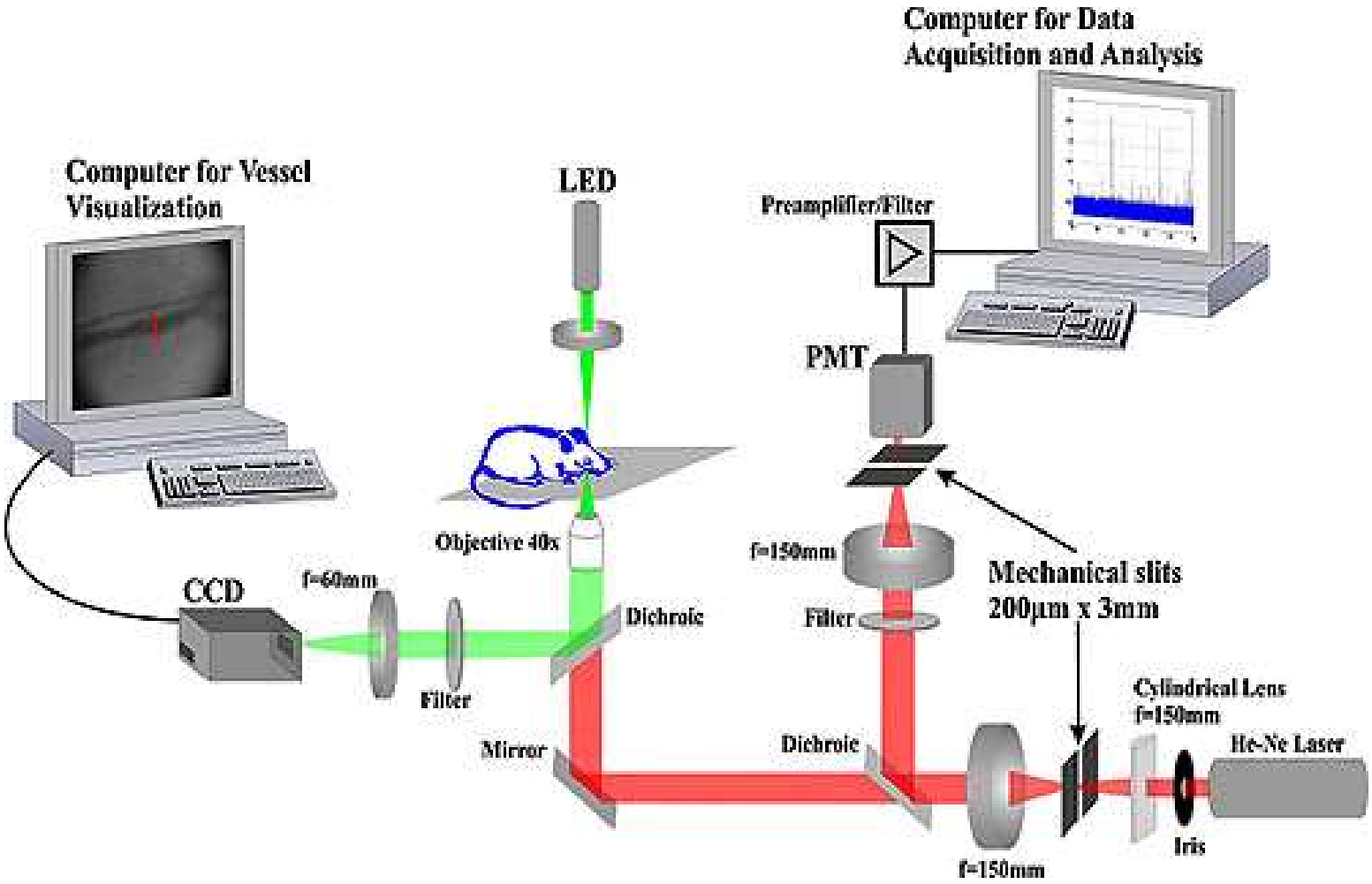
in vivo flow cytometry – základní principy



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

Cytometry part A 79A: 737-745, 2011

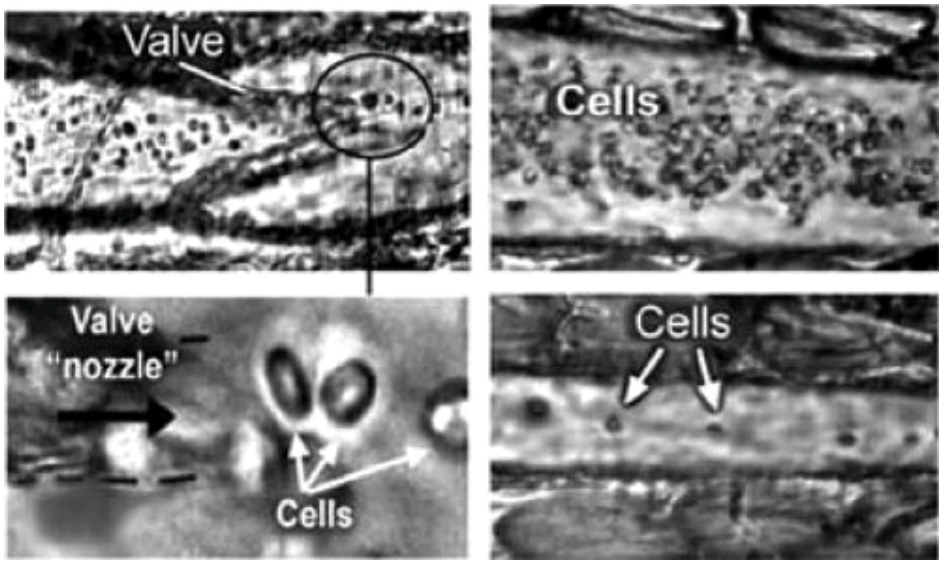
in vivo flow cytometry



Cytometry part A 79A: 737-745, 2011

in vivo flow cytometry – bez značení

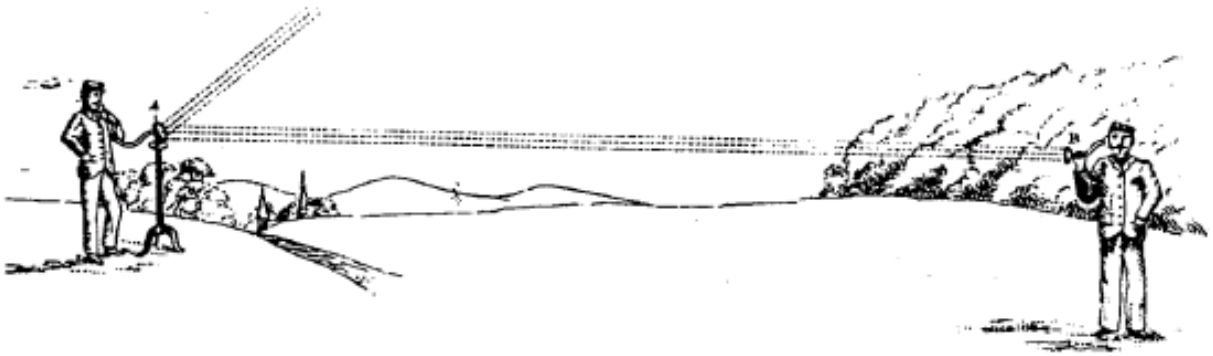
- Nahrávka videa pomocí vysokorychlostní CCD nebo CMOS kamery s vysokým rozlišením v režimu propustnosti nebo odrazu.
- Příklad: high-speed transmittance digital microscopy (TDM)
- Limity: hloubka tkáně.
- TDM může sloužit k navedení zdrojů záření pro další analýzu do určené oblasti.



Cytometry part A 79A: 737-745, 2011

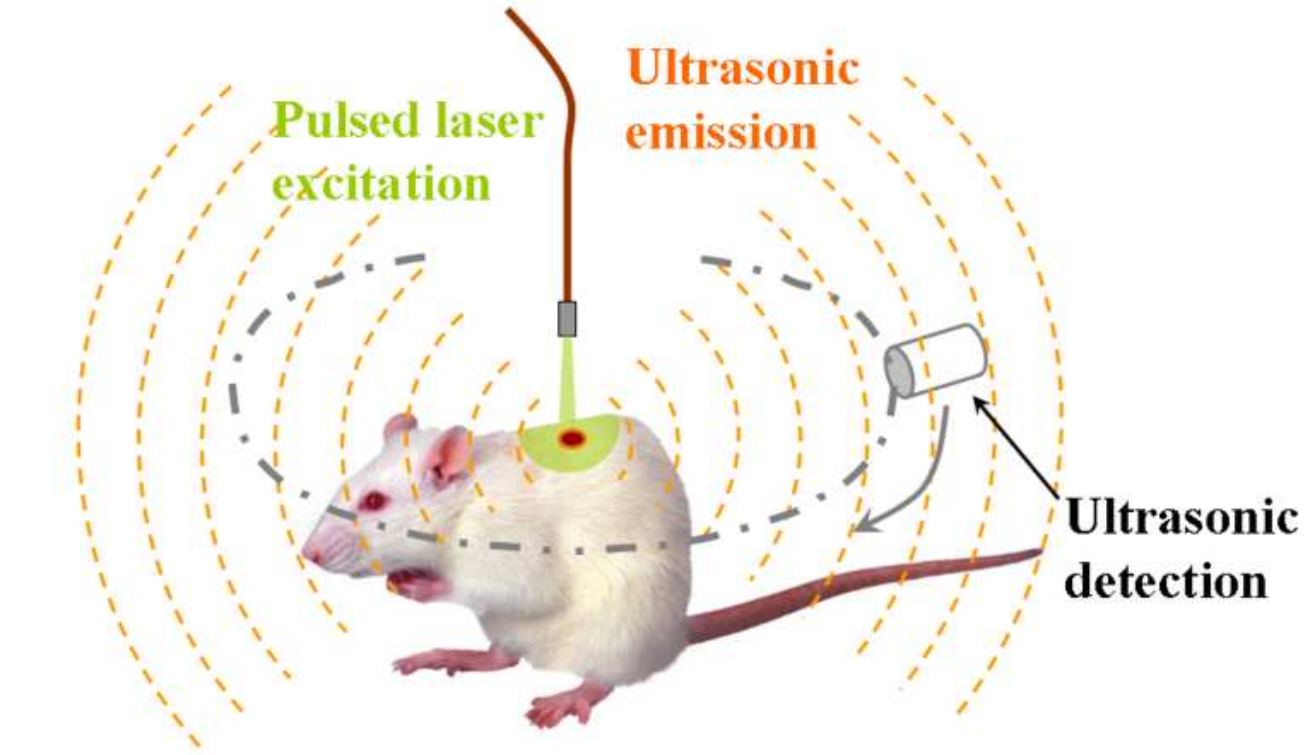
photoacoustic and photothermal imaging

- 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

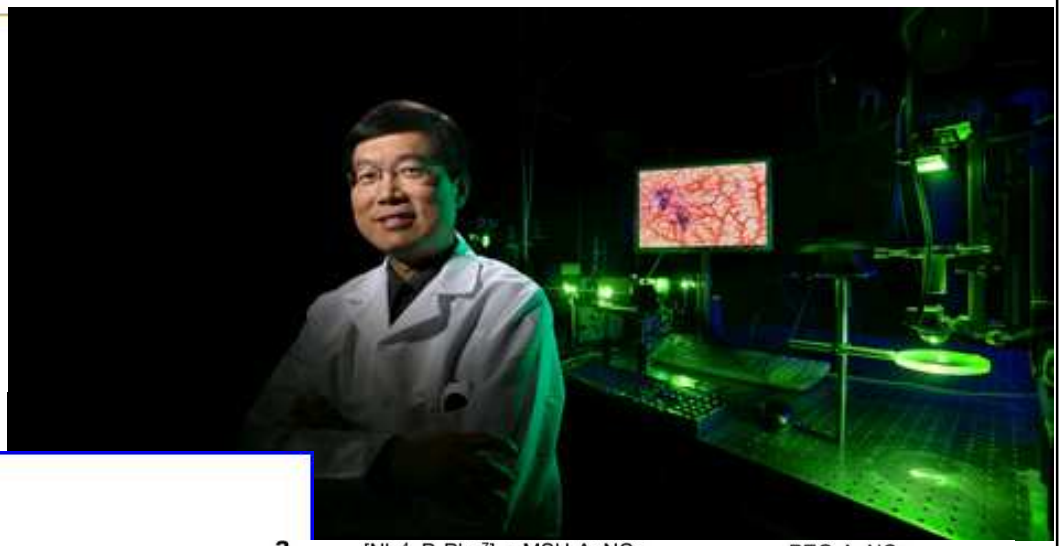


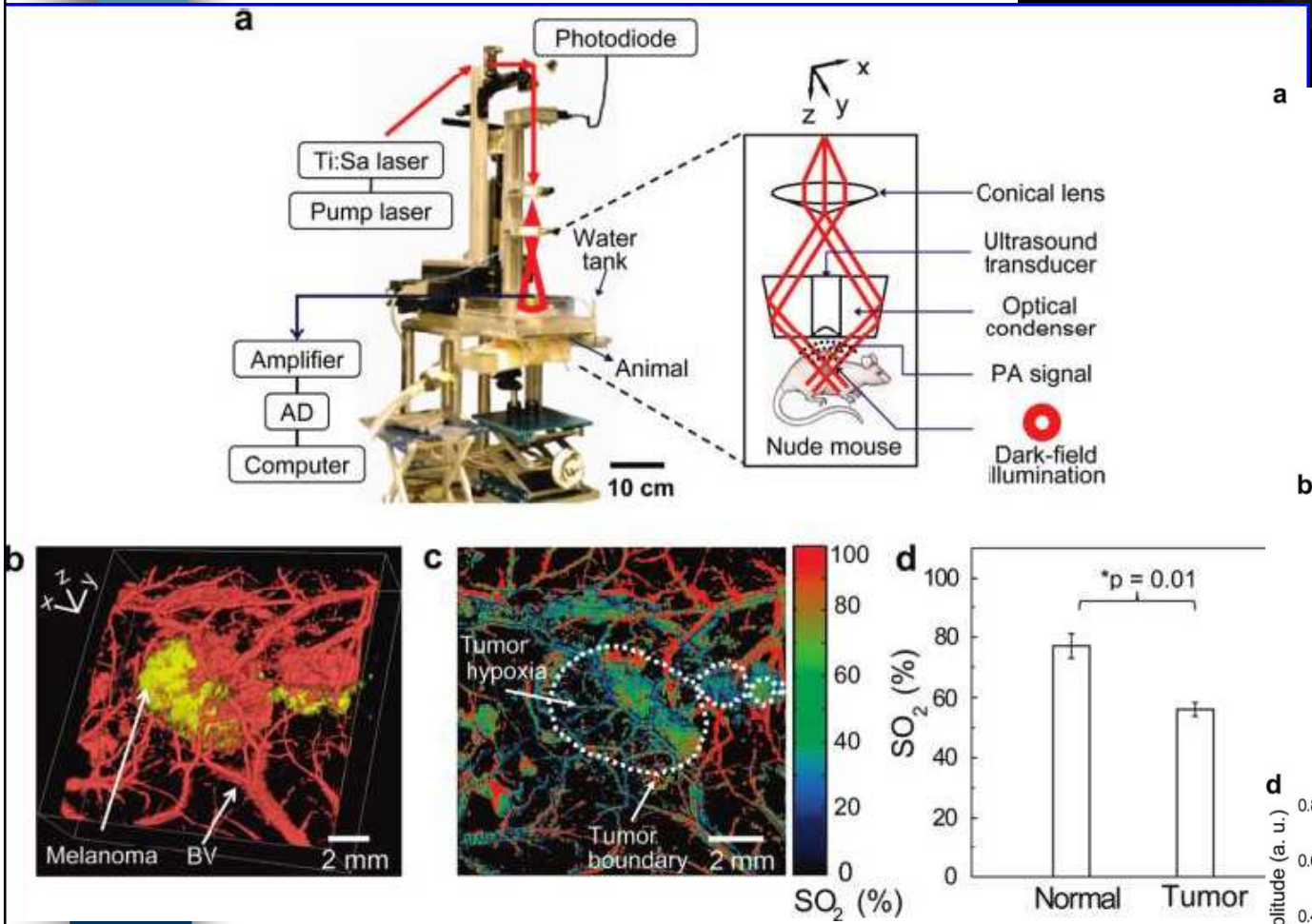
Cytometry part A 79A: 737-745, 2011

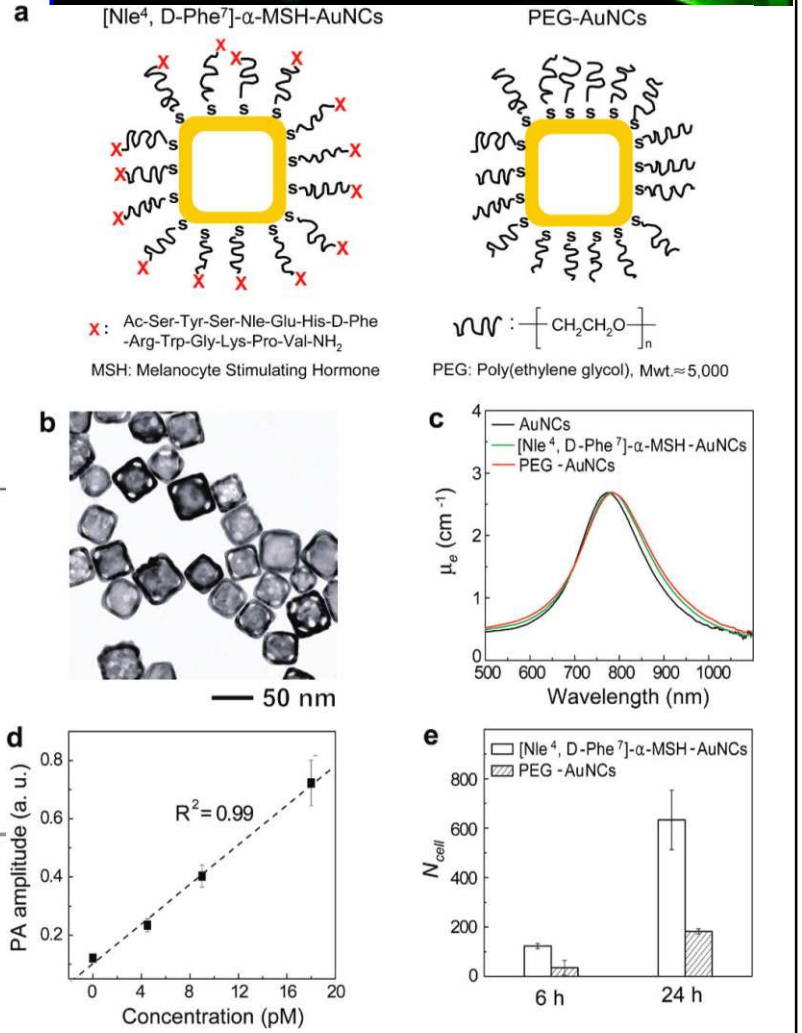
In Vivo Molecular Photoacoustic Tomography of Melanomas Targeted by Bioconjugated Gold Nanocages

Chulhong Kim,[†,§] Eun Chul Cho,[†,§] Jingyi Chen,[†] Kwang Hyun Song,[†] Leslie Au,[†] Christopher Favazza,[†] Qiang Zhang,[†] Claire M. Cobley,[†] Feng Gao,[†] Younan Xia,[†,*] and Lihong V. Wang[†,*]

[†]Department of Biomedical Engineering, Washington University in St. Louis, Campus box 1097, One Brookings Drive, St. Louis, Missouri 63130 and [‡]Division 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.



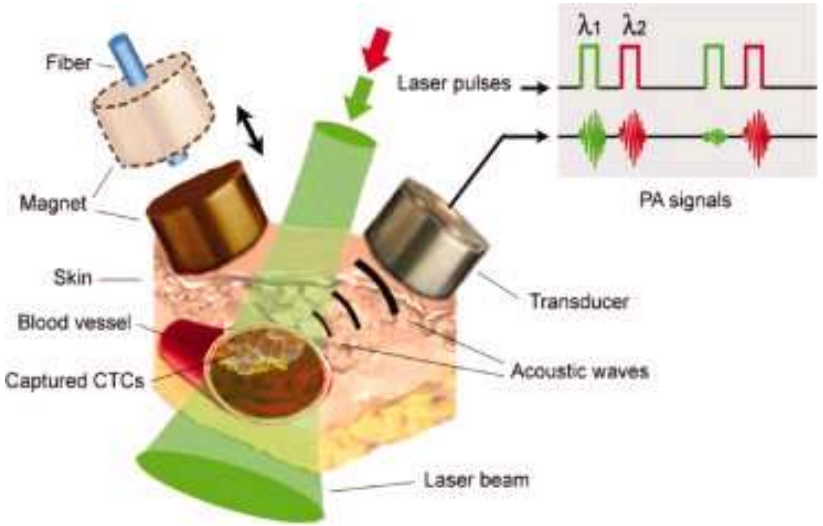


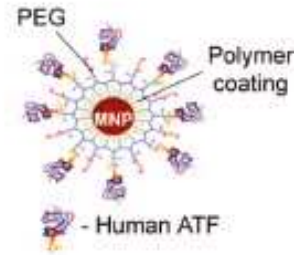


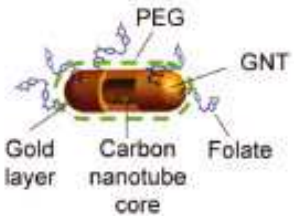
ACS Nano **2010** *4* (8), 4559-4564

In vivo flow cytometrie – detekce specifických signálů

- Detekce fotoakustických a fototermálních jevů

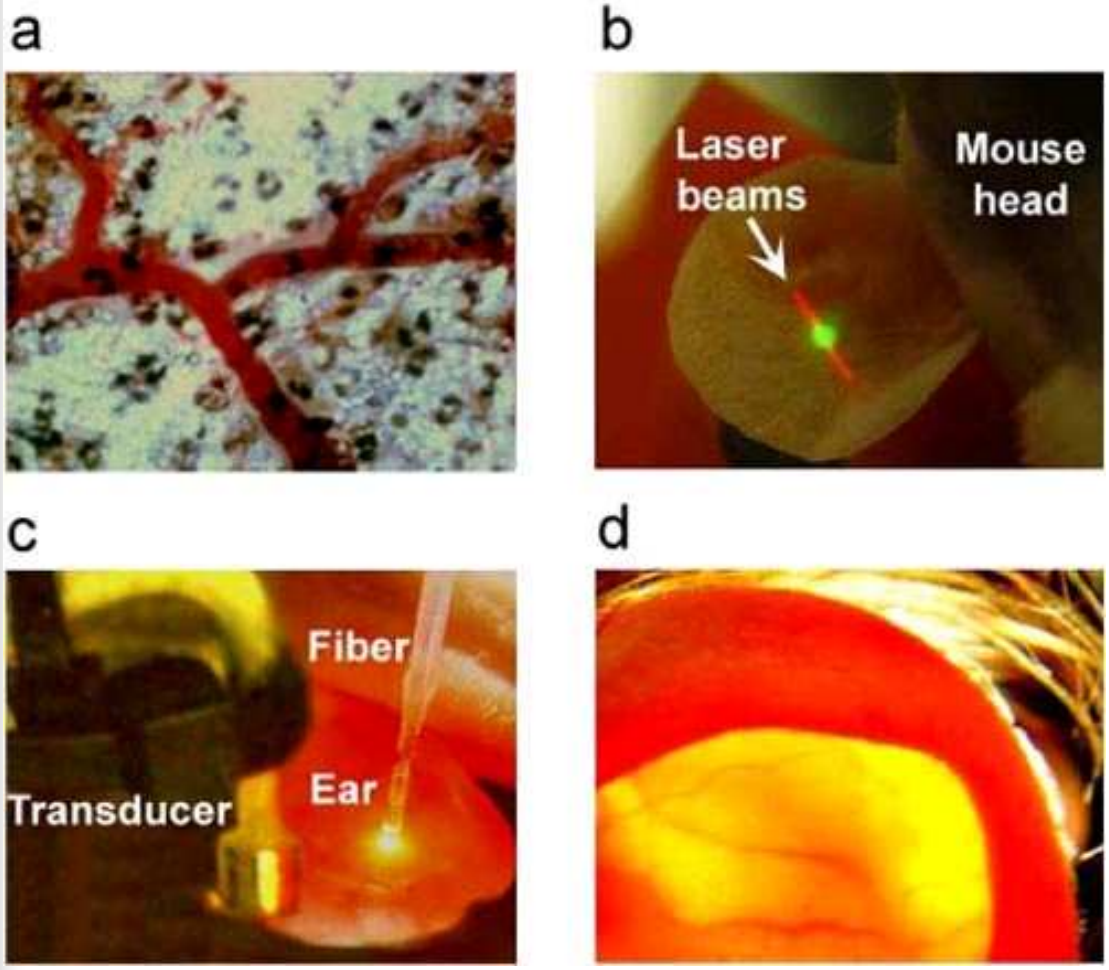


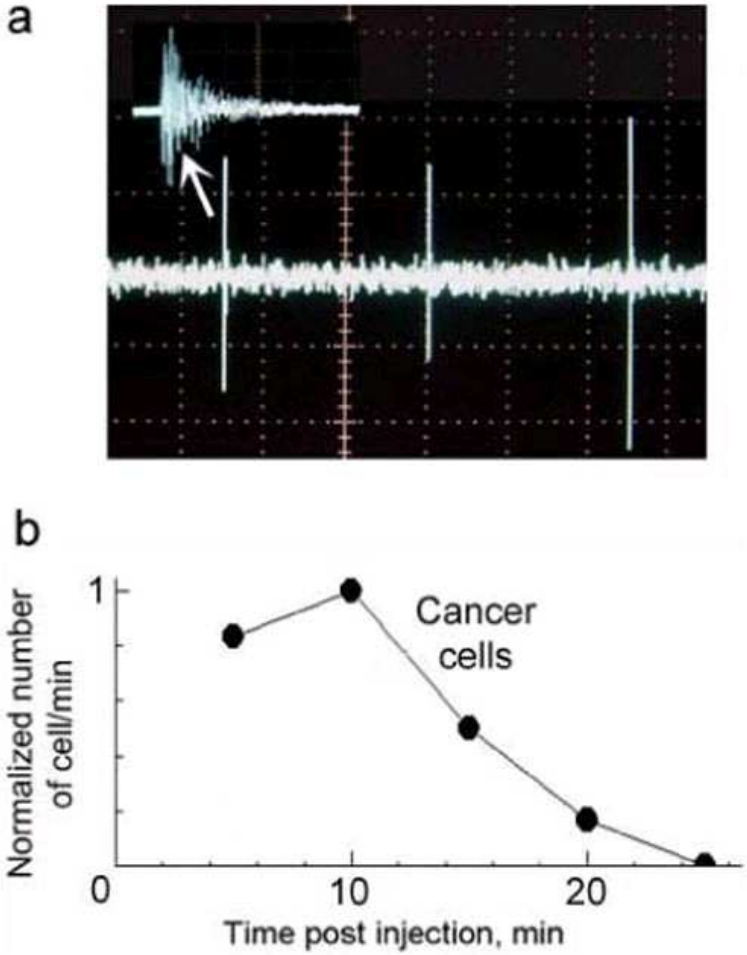




Cytometry part A 79A: 737-745, 2011

in vivo flow cytometry - aplikace





Shrnutí přednášky

- „High-throughput“ průtoková cytometrie …
- … a uplatnění vícebarevné detekce a beads array
- sortrování 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 sortrování 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.