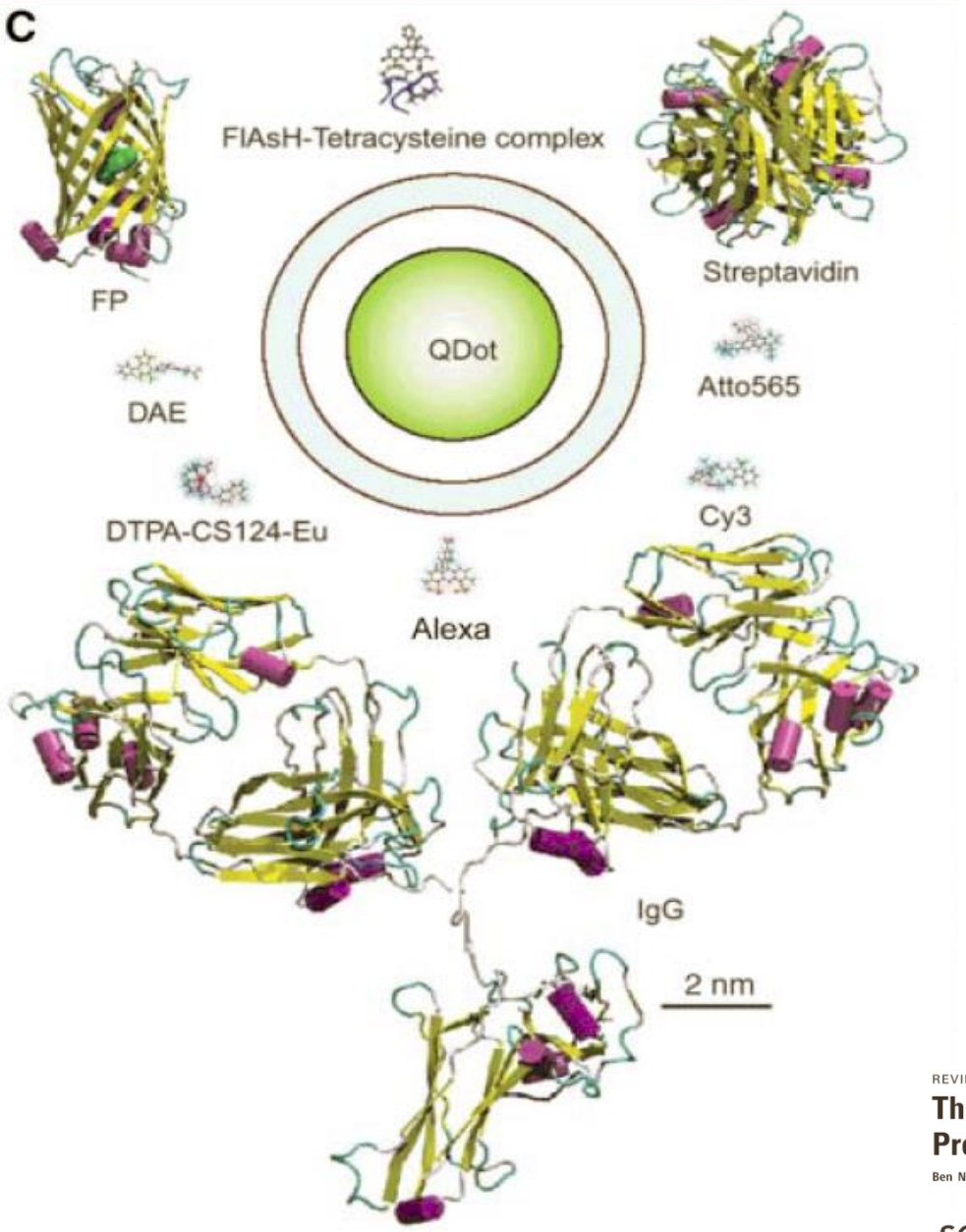
Bi9393 Analytická cytometrie

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

Targeting proteins & fluorophores



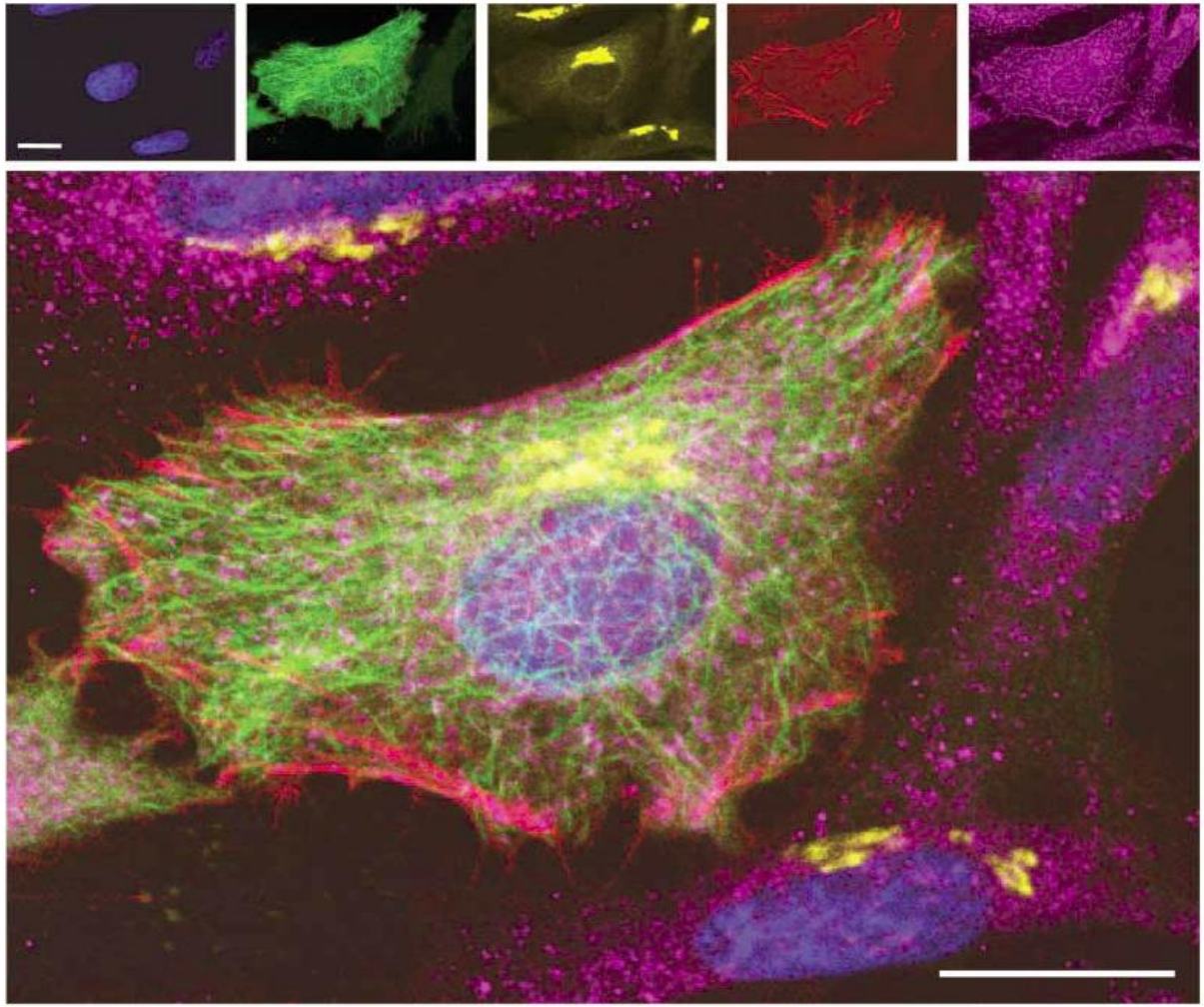
REVIEW

The Fluorescent Toolbox for Assessing Protein Location and Function

Ben N. G. Giepmans,[1,2] Stephen R. Adams,[2] Mark H. Ellisman,[1] Roger Y. Tsien[2,3]*

SCIENCE VOL 312 14 APRIL 2006

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



REVIEW

The Fluorescent Toolbox for Assessing Protein Location and Function

Ben N. G. Giepmans,[1,2] Stephen R. Adams,[2] Mark H. Ellisman,[1] Roger Y. Tsien[2,3]*

SCIENCE VOL 312 14 APRIL 2006

Vitální analýza buněčných funkcí

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

Detekce viability

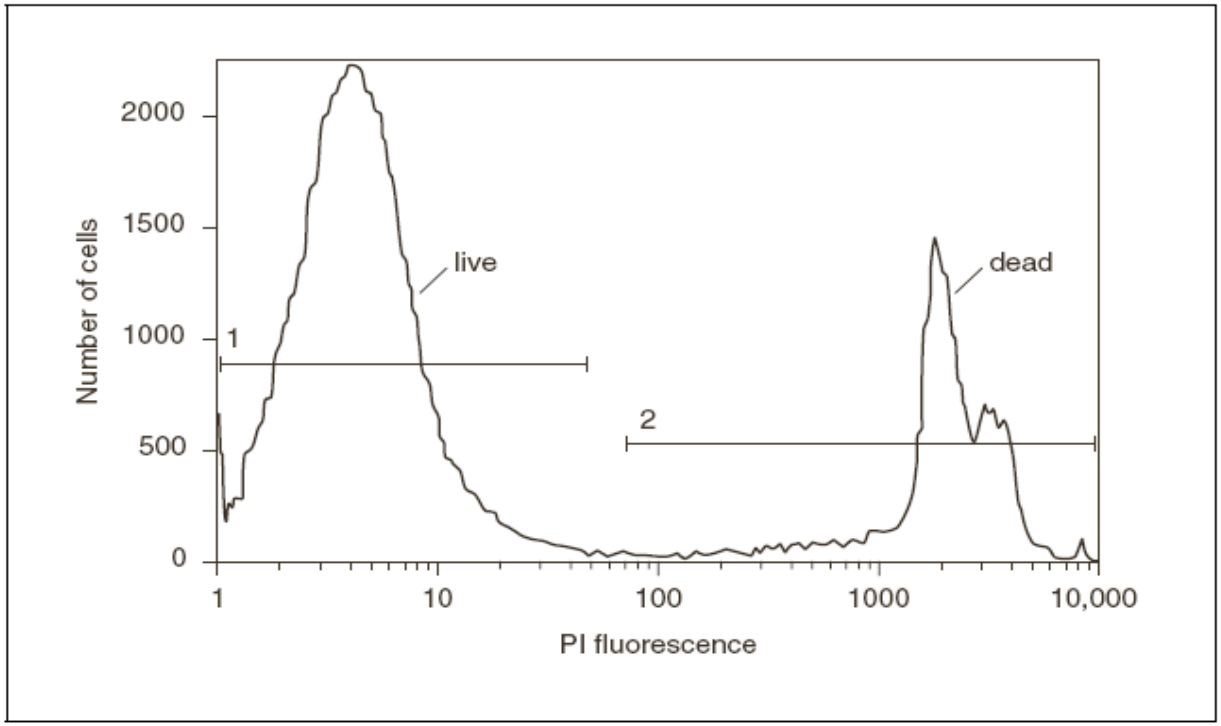
- jedna z nejjednodušších analýz
- funguje na principu:
  - detekce membránové integrity - neprůchodnosti některých fluorescenčních značek cytoplazmatickou membránou živých buněk **– propidium iodide, ethidium bromide, 7-amino actinomycin D**
  - detekce fyziologického stavu buněk – použití fluorescenčních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

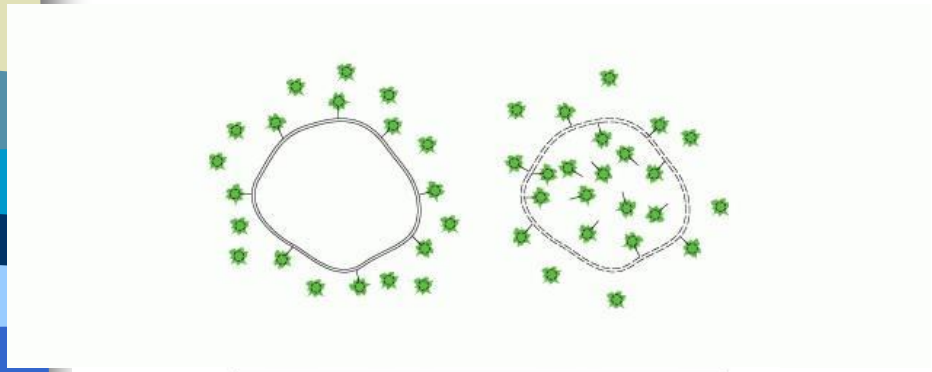
*Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm.

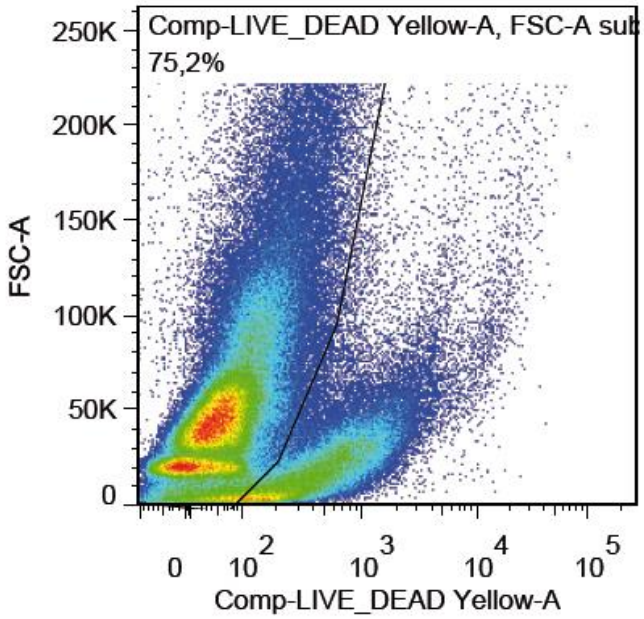
Detekce viability



Current Protocols in Cytometry

VIABILITY using LIVE/DEAD fixable stains





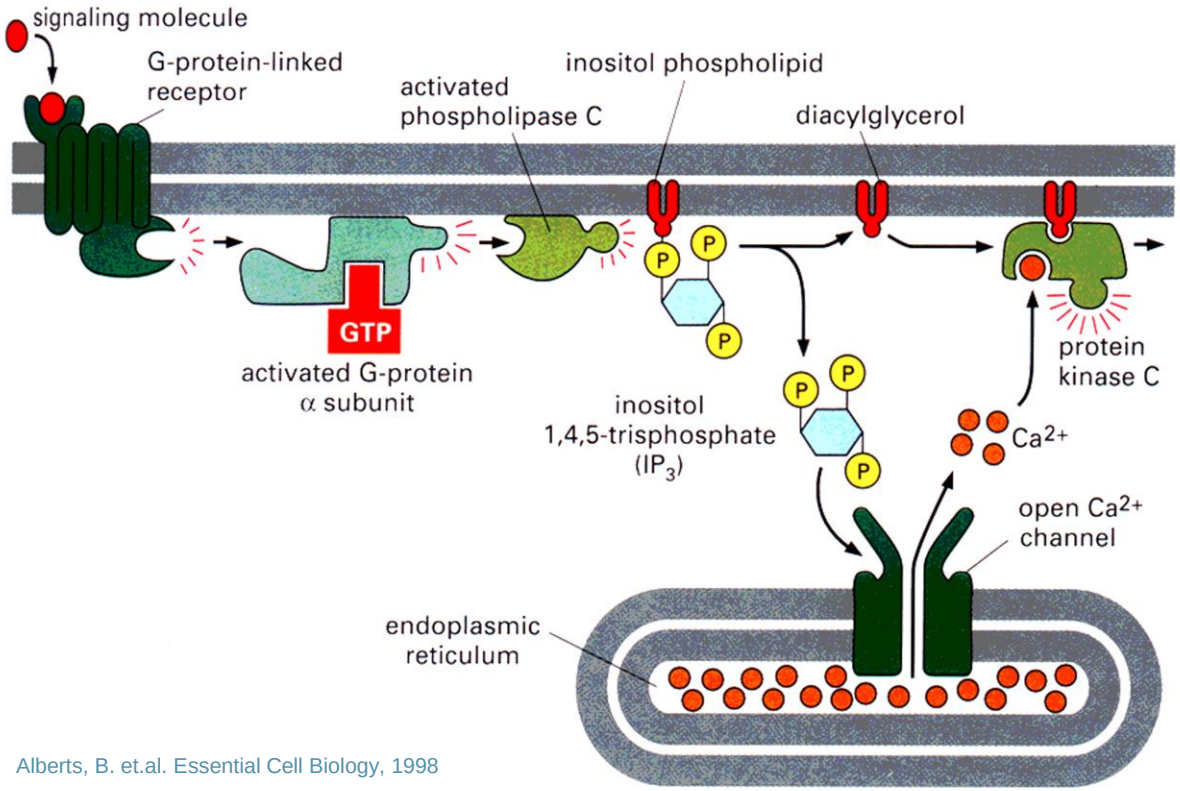
Principle: *reaction of a fluorescent reactive dye with cellular amines, in necrotic cells react with free amines both in the interior and on the cell = intense staining, live cells stained on surface only = dim signal*

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

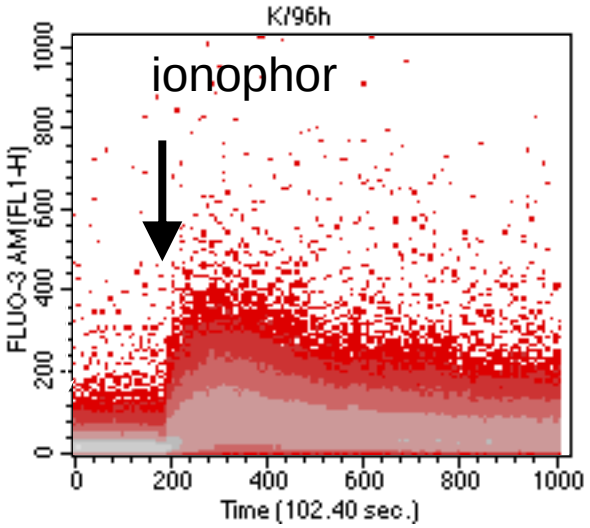
Cons: *live cells have signal, stain only in buffers w/o BSA or serum, Tris or azide*

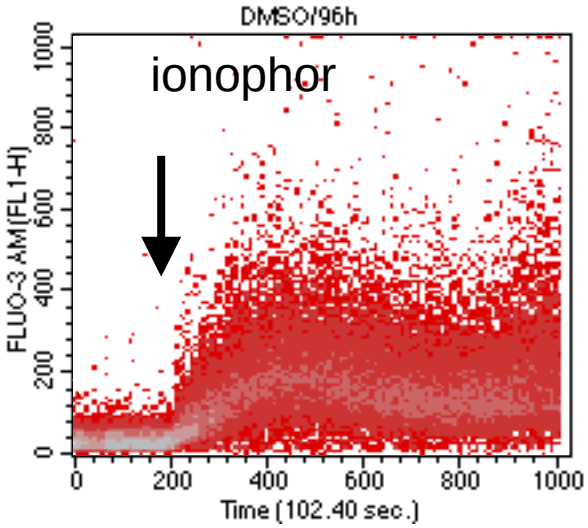
Přenos signálu pomocí Ca^{2+}

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



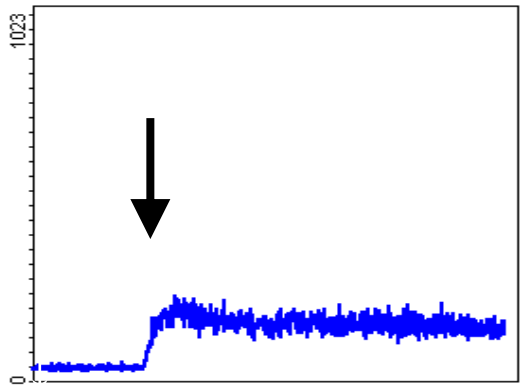
Alberts, B. et.al. Essential Cell Biology, 1998

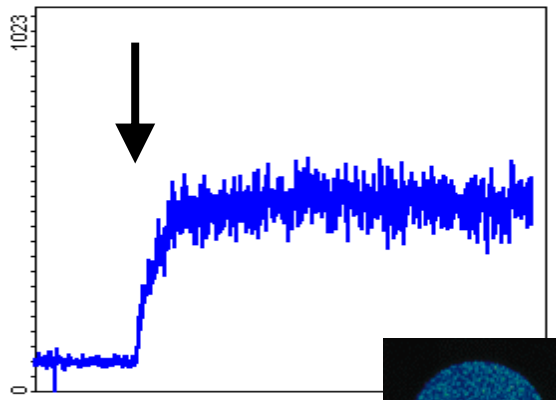


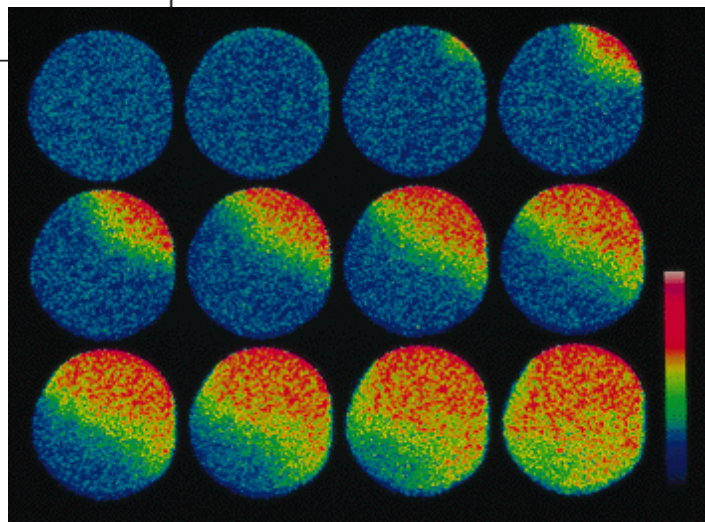


Ca $^{2+}$ influx

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







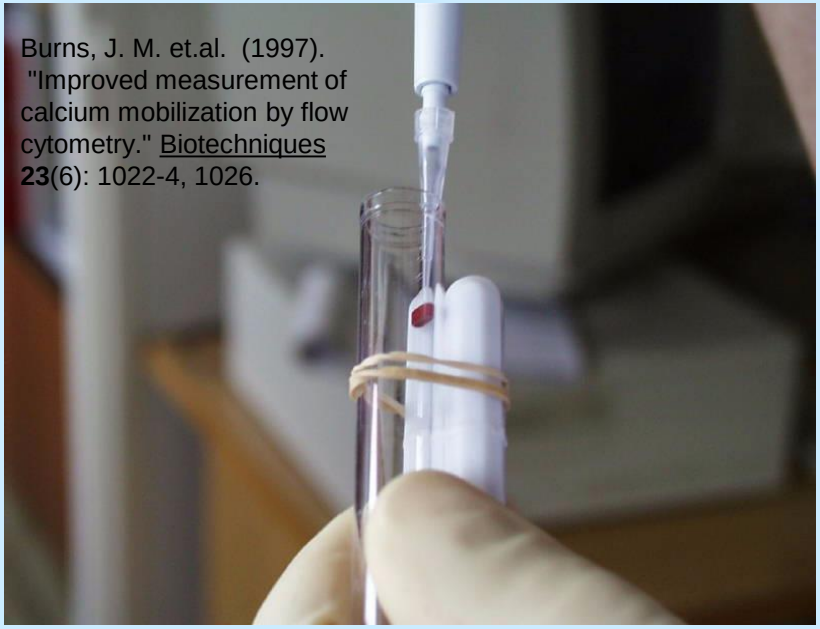
Molecular Probes

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

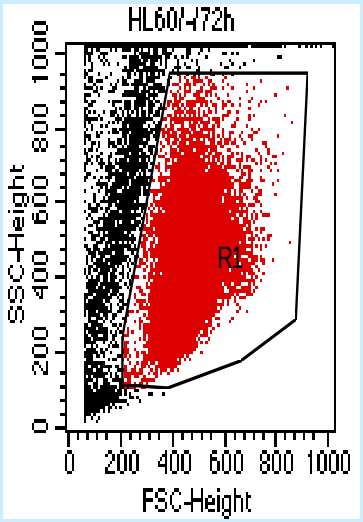

Burns, J. M. et.al. (1997). "Improved measurement of calcium mobilization by flow cytometry." Biotechniques **23**(6): 1022-4, 1026.

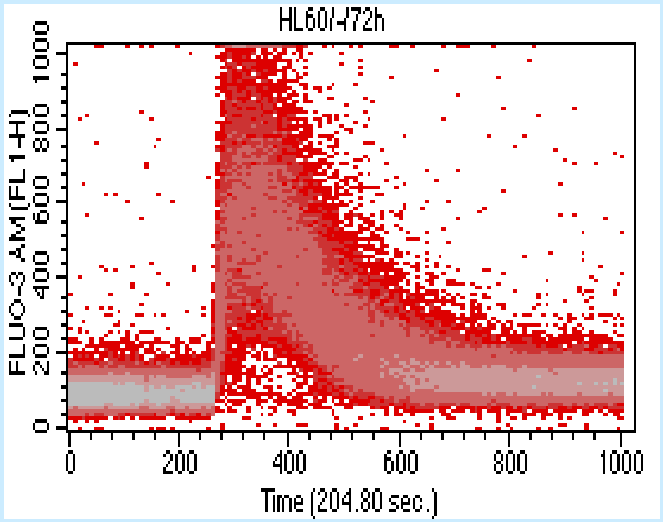

OMNIBIO

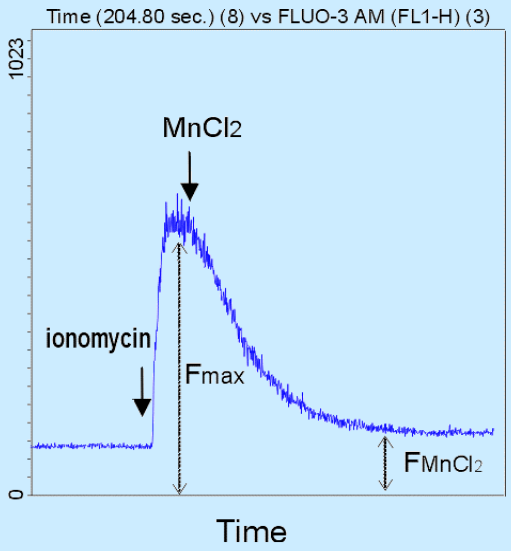
Kalibrace

(pro jednu vlnovou délku)

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





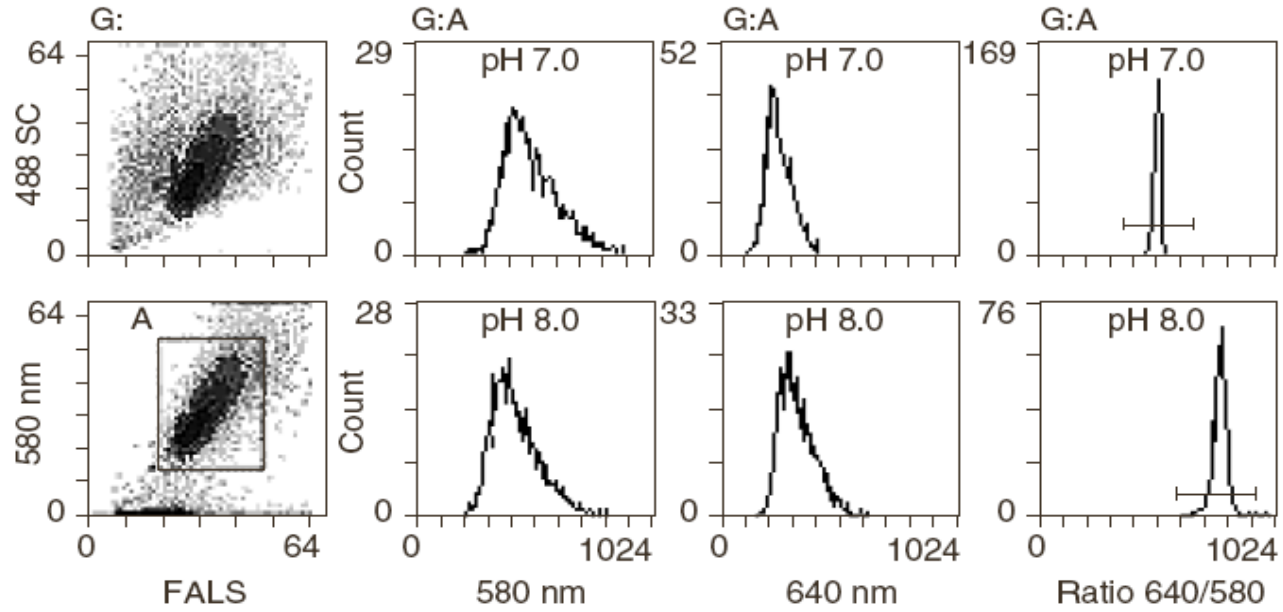


Fluo-3 (Kd ~ 400nM, 22°C; 864 nM, 37°C)

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

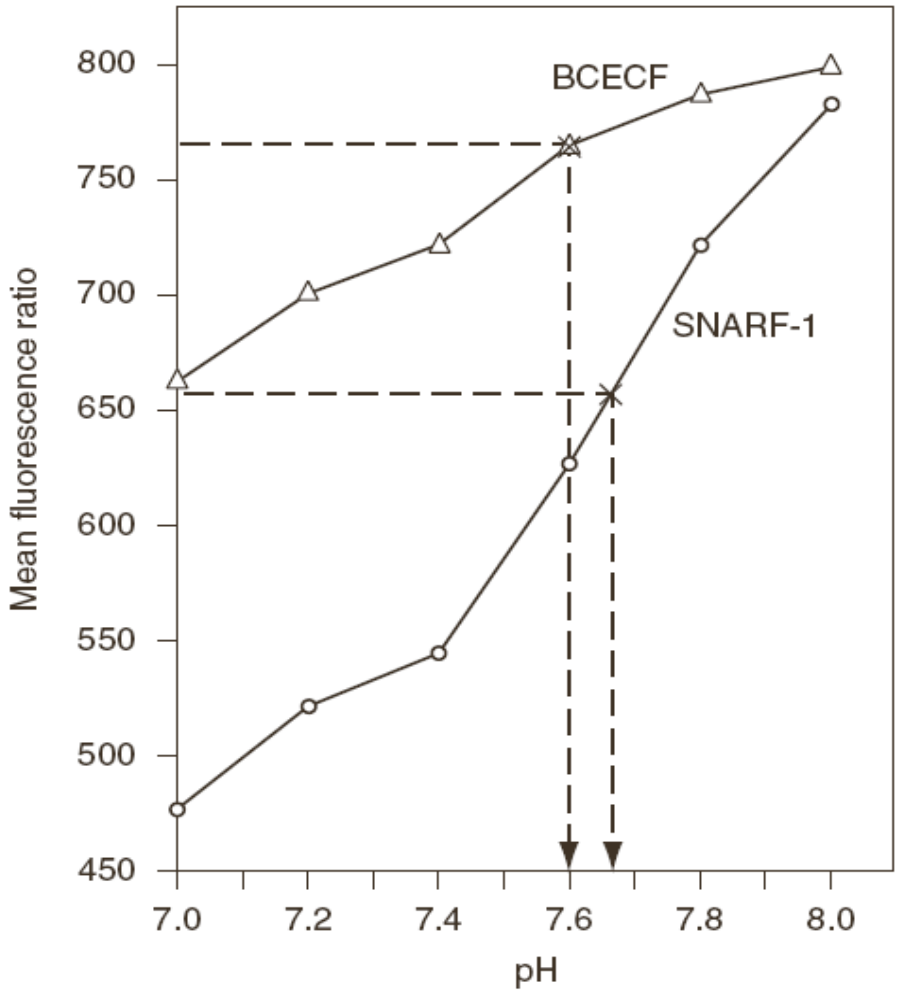
Detekce intracelulárního pH

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



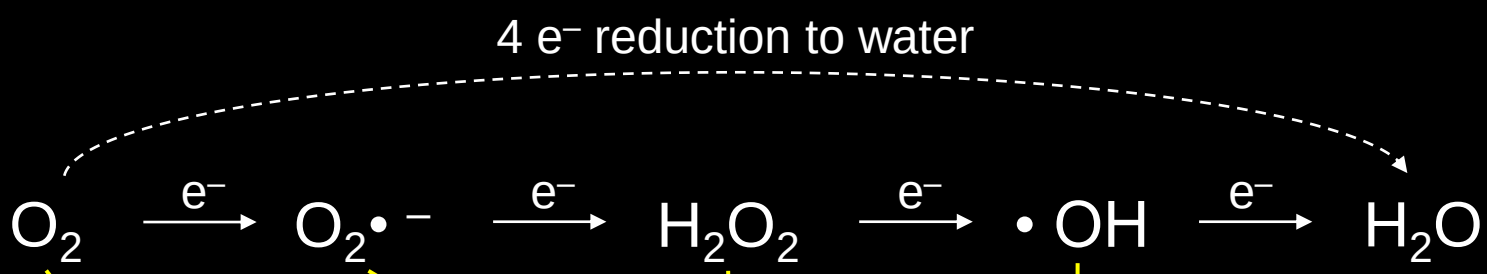
Detekce intracelulárního pH

- 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



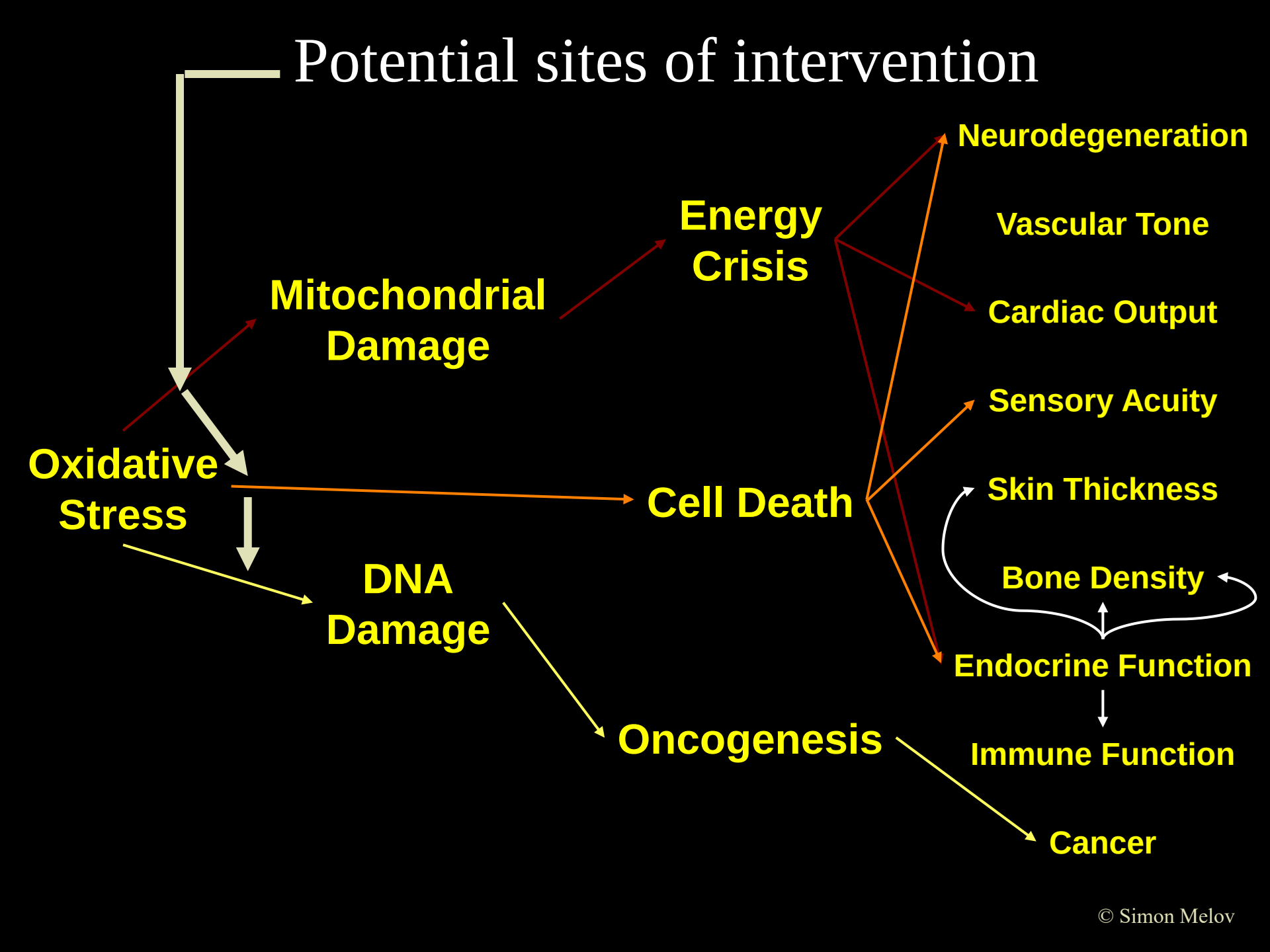
Unreactive at STP, but a *great* electron acceptor
Biological activation via radicals, transition metals
Generally, radical intermediates are enzyme-bound

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

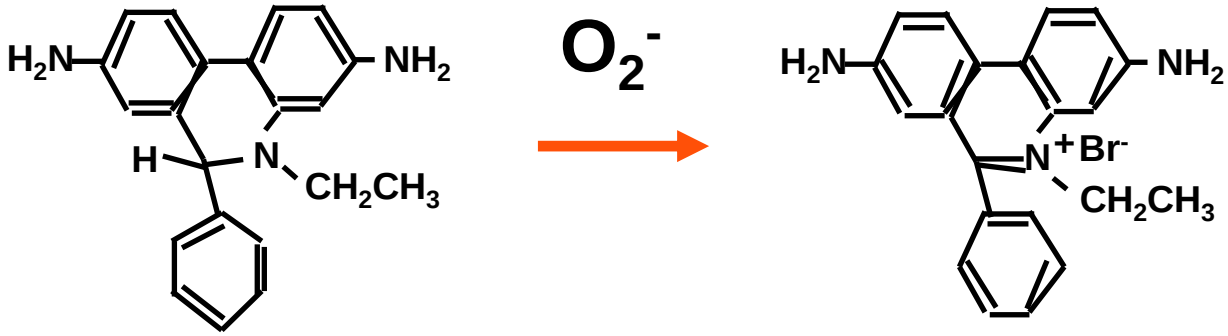
Not so terribly reactive with most biomolecules
Maintained at very low concentration
Catalases, peroxidases, GSH, etc…

Potential sites of intervention

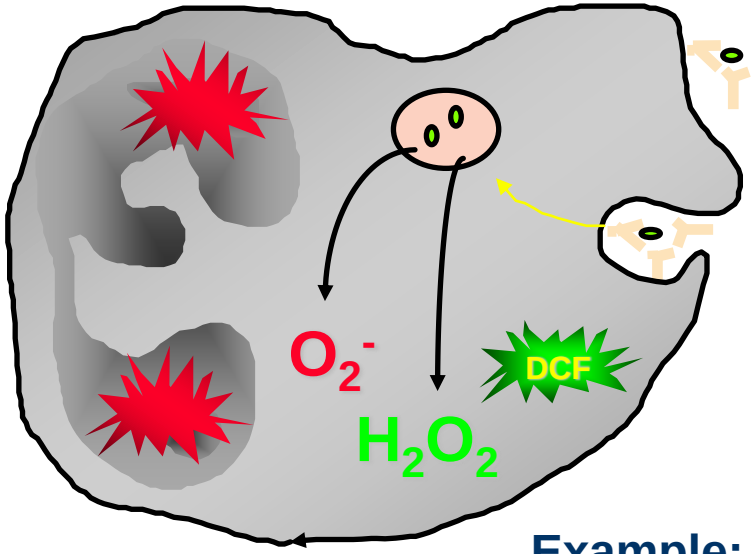


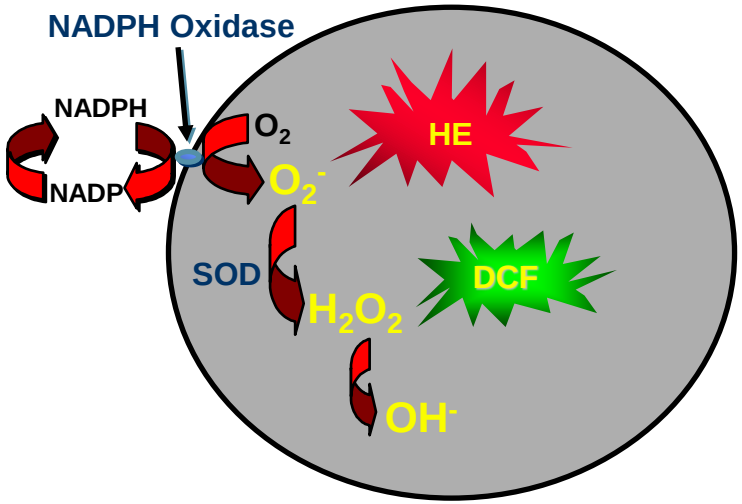
Hydroethidine

HE → **EB**



Phagocytic Vacuole

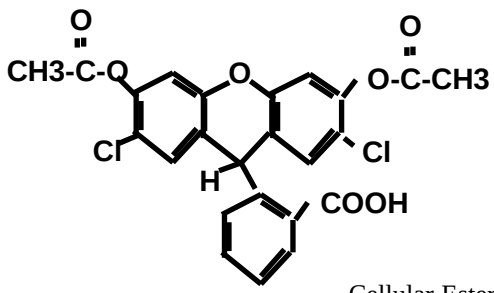




Example: Neutrophil Oxidative Burst

DCFH-DA → DCFH → DCF

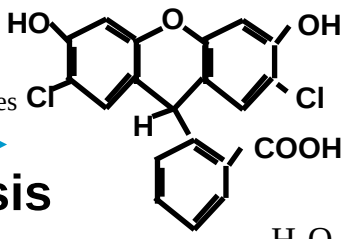
2',7'-dichlorofluorescin diacetate



Cellular Esterases

Hydrolysis

2',7'-dichlorofluorescin

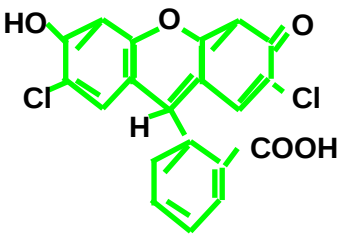


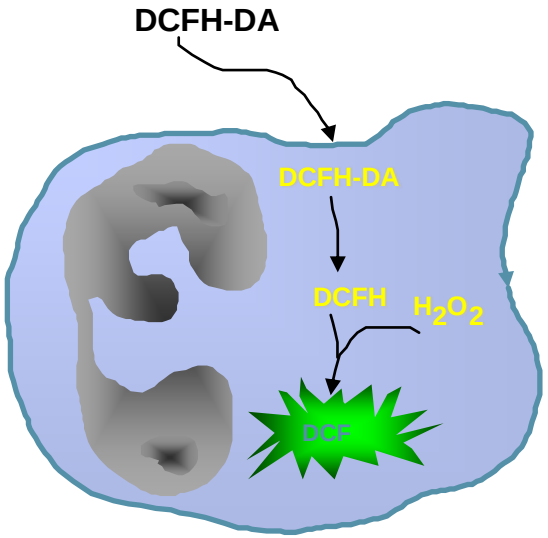
H_2O_2

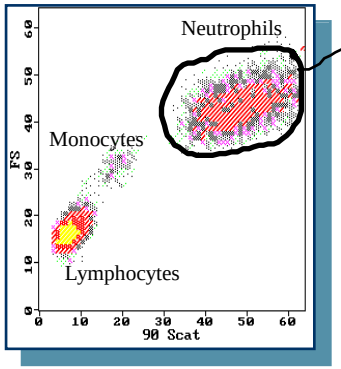
Oxidation

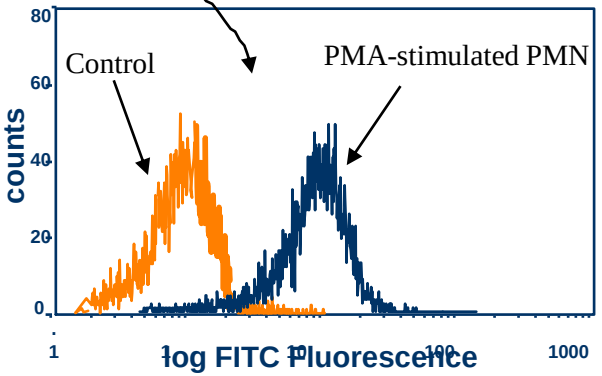
Fluorescent

2',7'-dichlorofluorescein



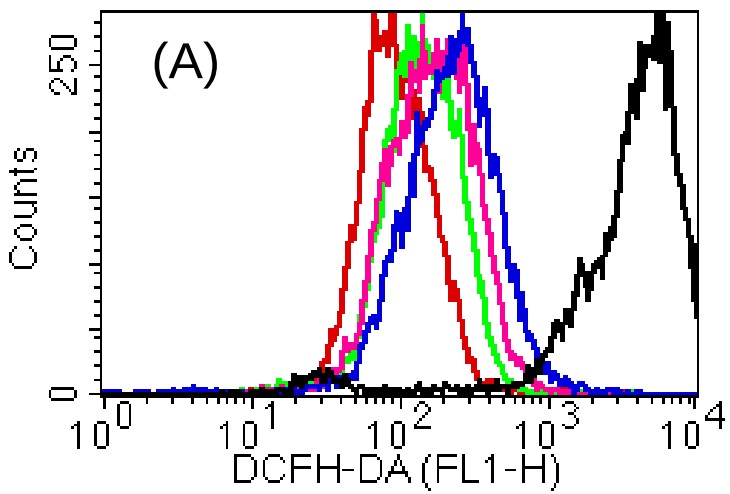


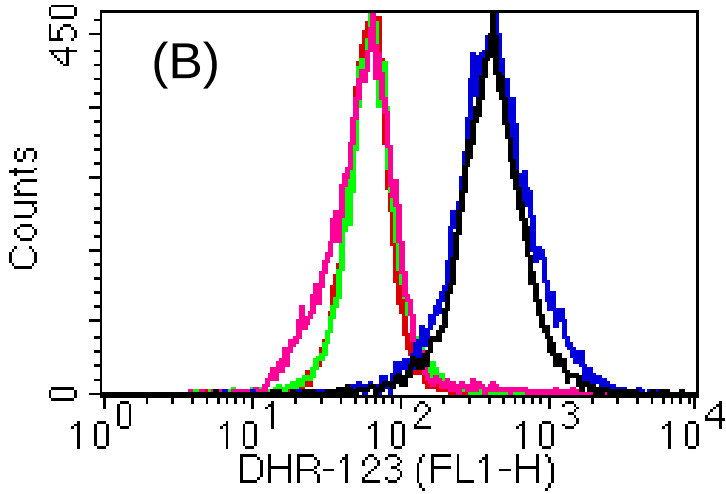


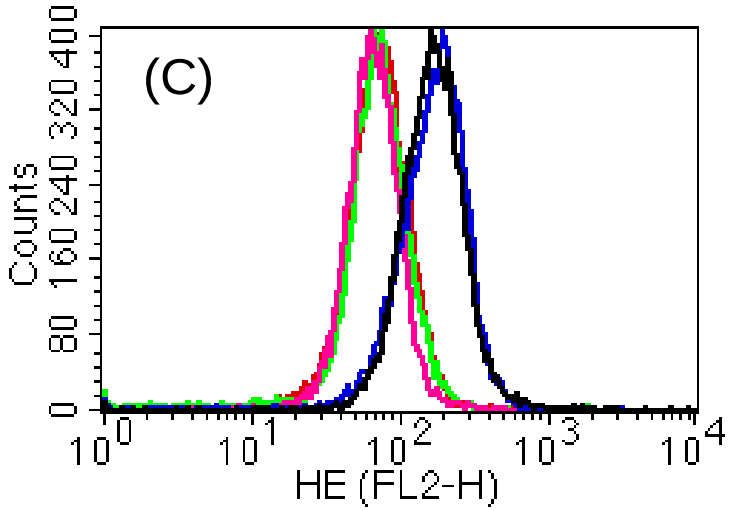


Oxidative Burst

- 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**.

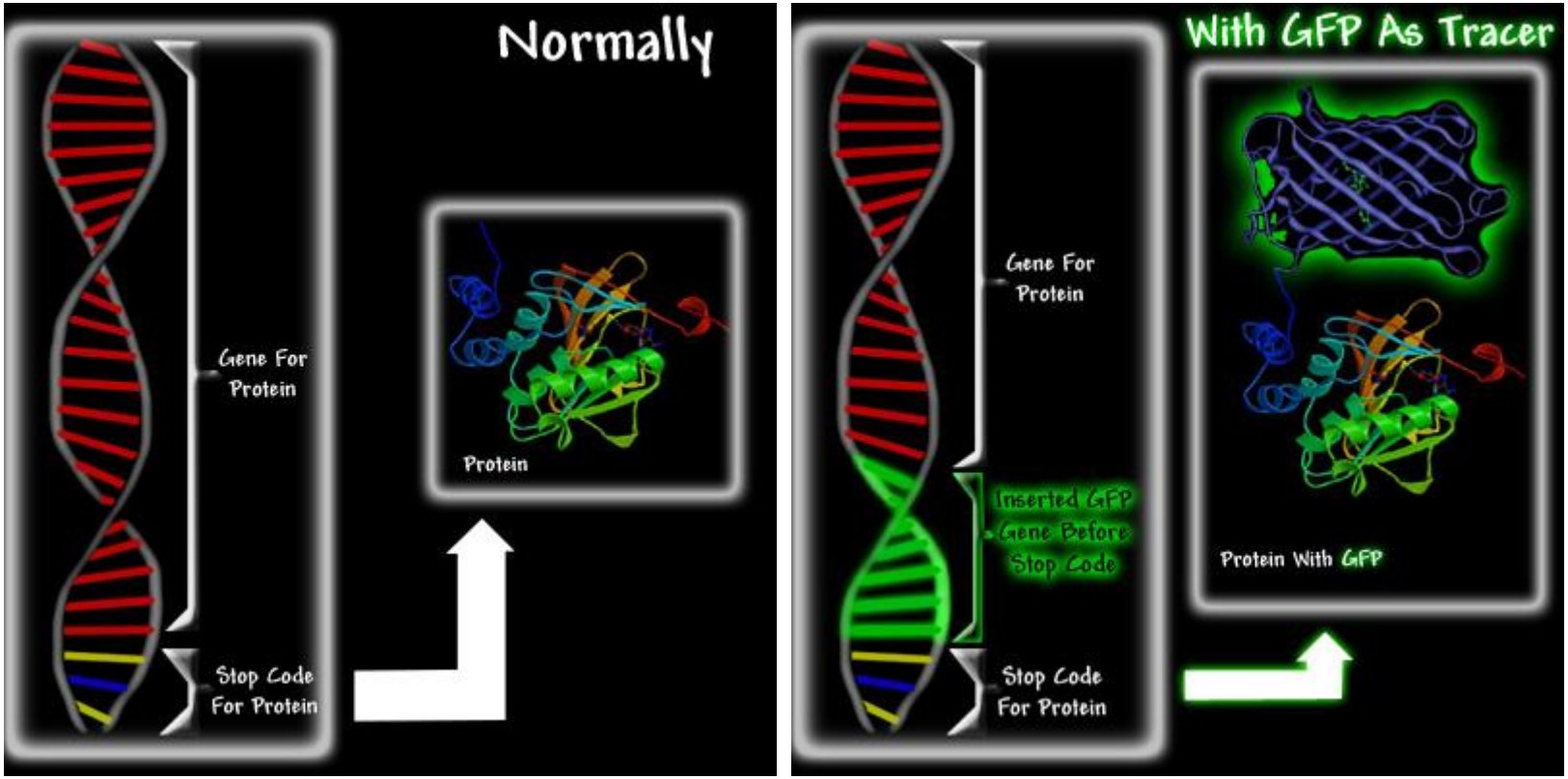
Aequorea victoria "Crystal jelly "

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



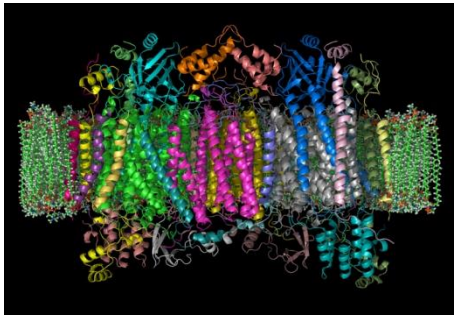
http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm

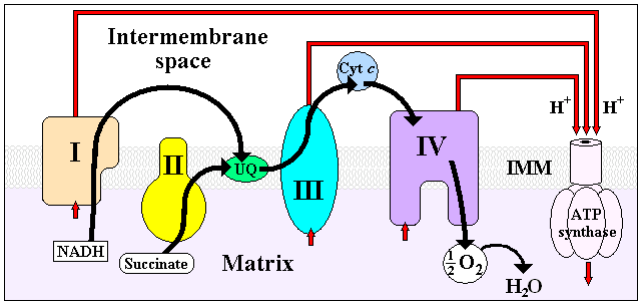
Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells

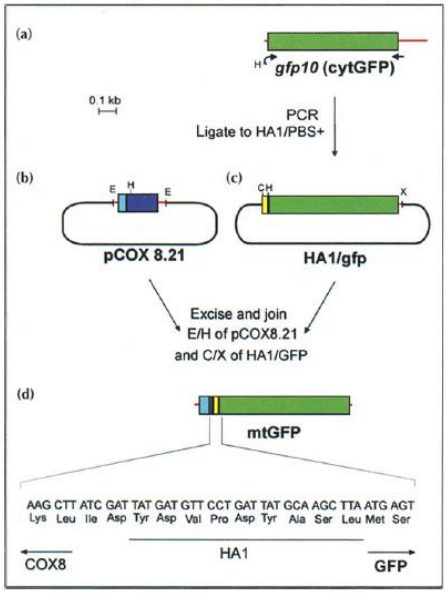
Rosario Rizzuto, Marisa Brini, Paola Pizzo, Marta Murgia and Tullio Pozzan

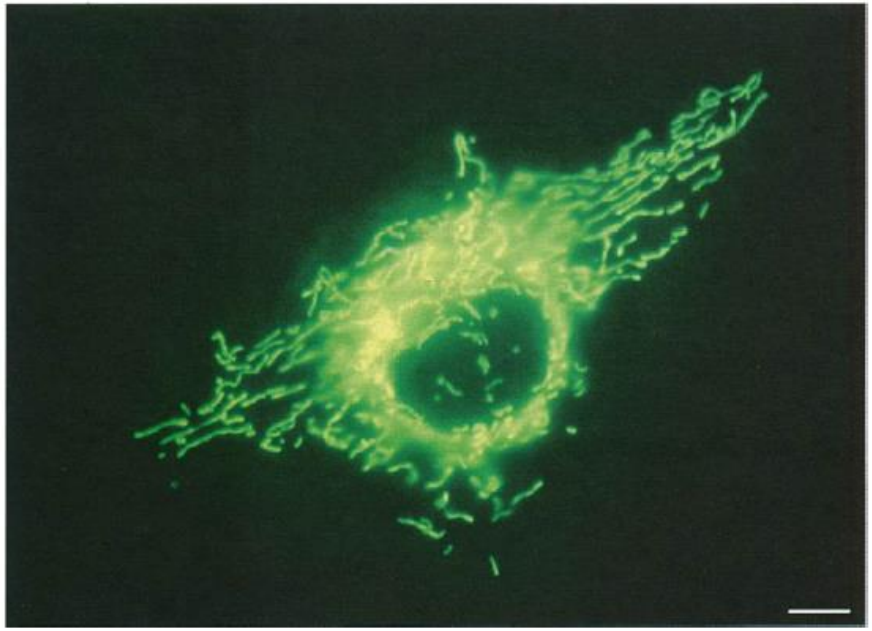
Department of Biomedical Sciences and CNR Center for the Study of Mitochondrial Physiology, University of Padova, Via Trieste 75, 35121 Padova, Italy.

Current Biology 1995, **5**:635–642

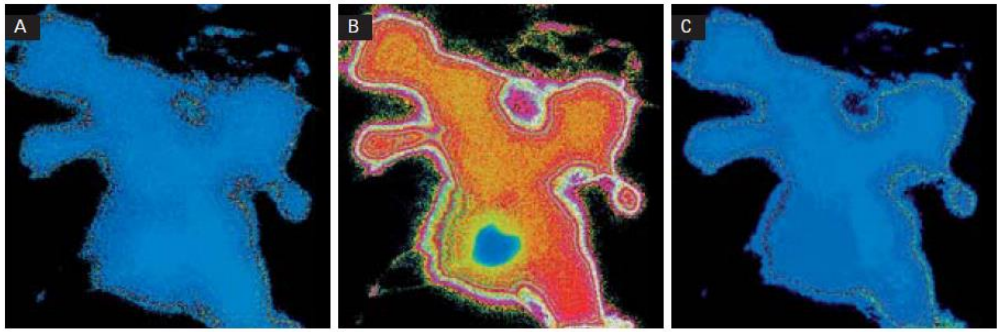






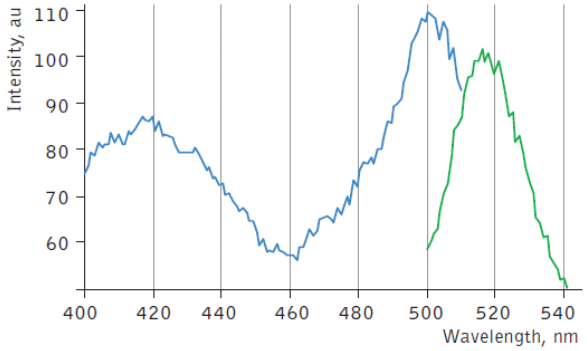


Fluorescent sensors for detection of H_2O_2

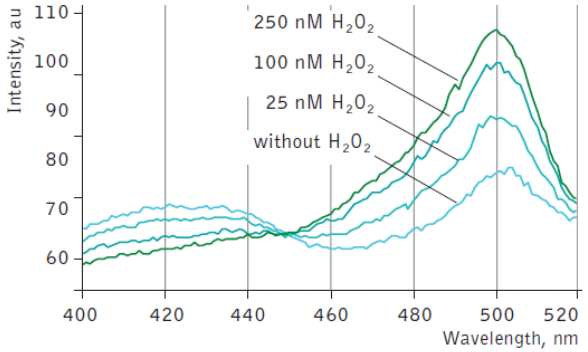


Ratiometric images of the group of HeLa cells before (A), 20 sec after (B), and 600 sec after (C) addition of 180 ul of H_2O_2. Images were pseudocolored using "ratio" lookup table of NIH ImageJ software: blue-green-red-white colors represent lowest-intermediate-high-highest level of H_2O_2.

evrogen



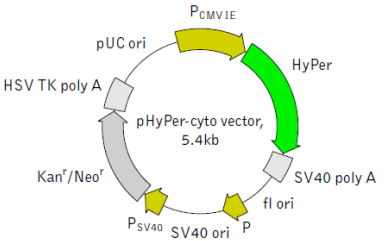
HyPer excitation (blue line) and emission (green line) spectra.

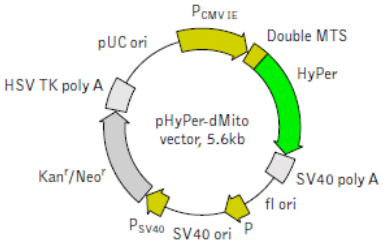


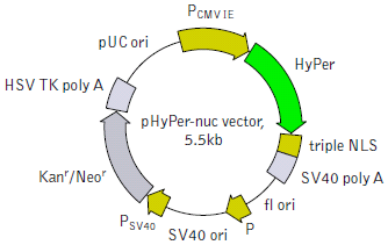
Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition. Emission was measured at 530 nm.

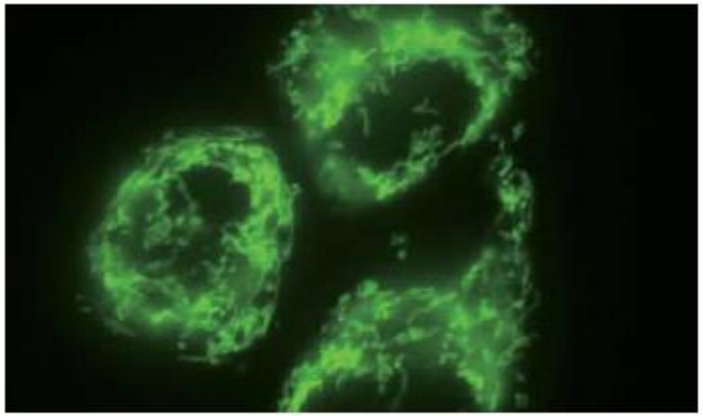
Variants & fusions

- **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]**



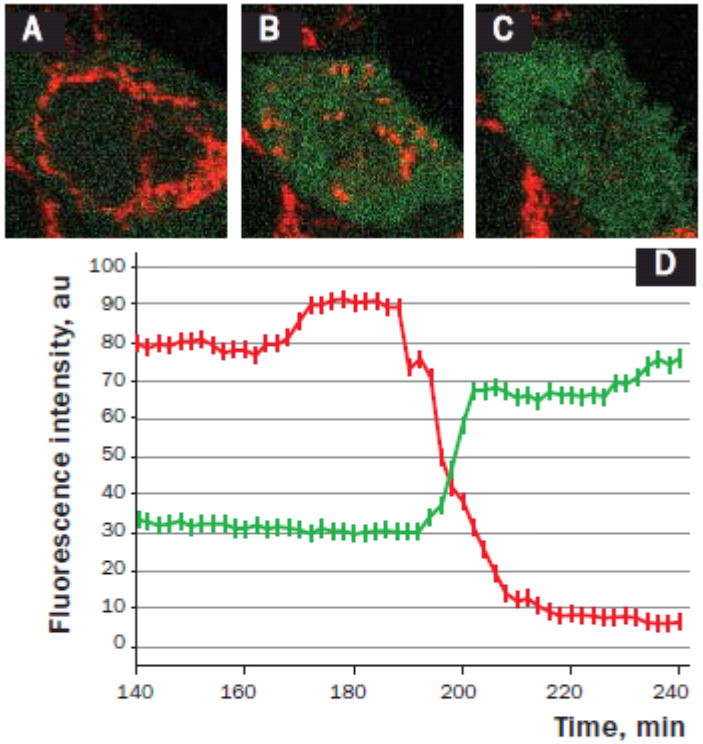






Stably transfected HeLa cells expressing mitochondria-targeted HyPer.

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

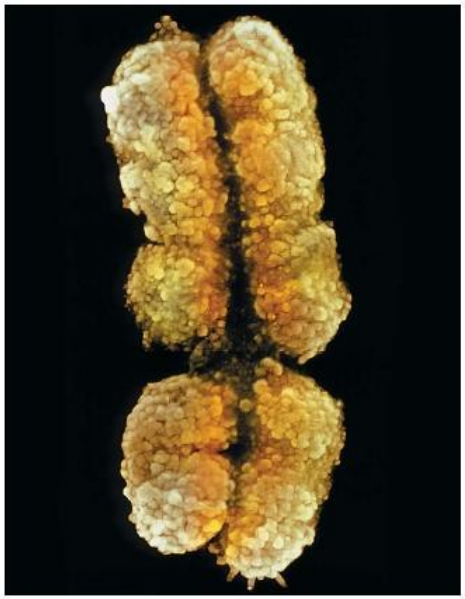
Biomedical Sciences Division, L-452, Lawrence Livermore Laboratory, Livermore, California 94550

Communicated by Donald A. Glaser, December 18, 1978

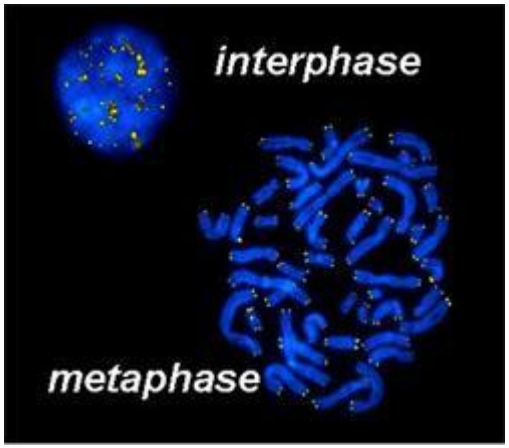
Analýza a sortrování chromozómů

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

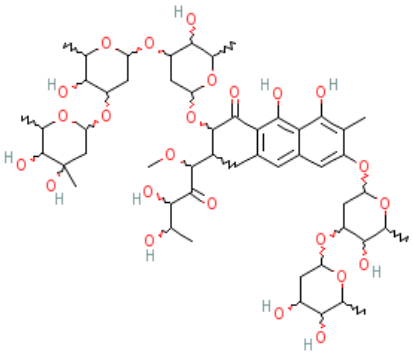
= celková DNA vs. G/C-bohaté oblasti



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

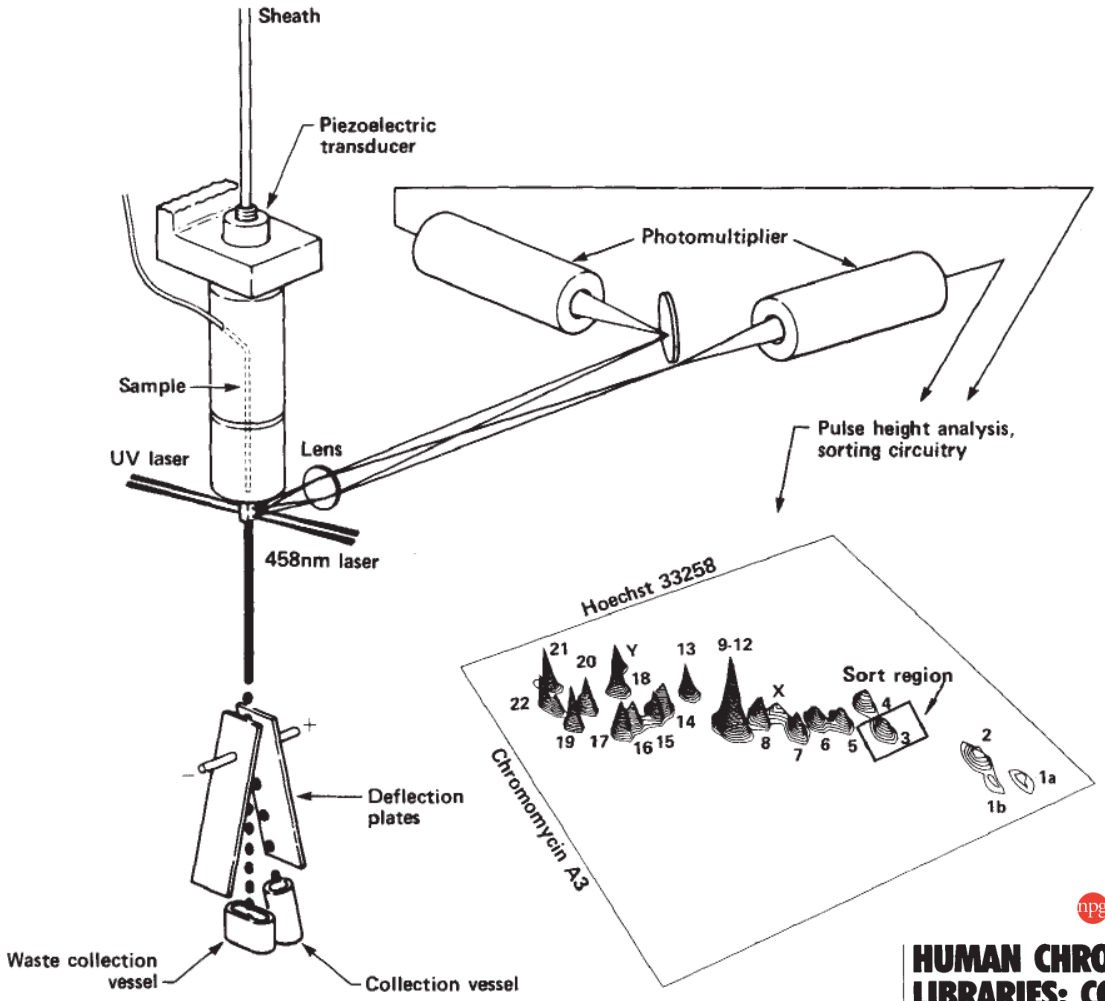


http://www.nccr-oncology.ch/scripts/page9243.html



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*.

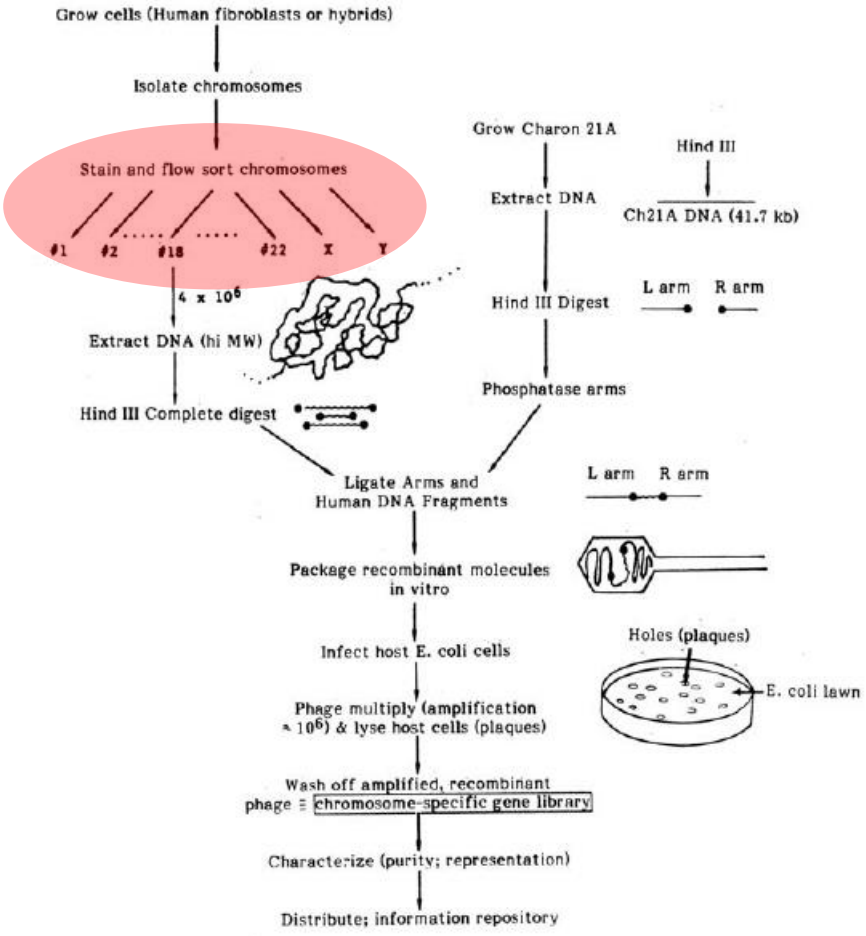
National Laboratory Gene Library Project, * Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, California 94550, † Los Alamos National Laboratory, Life Sciences Division, University of California, Los Alamos, New Mexico 87545,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

HUMAN CHROMOSOME-SPECIFIC DNA LIBRARIES: CONSTRUCTION AND AVAILABILITY

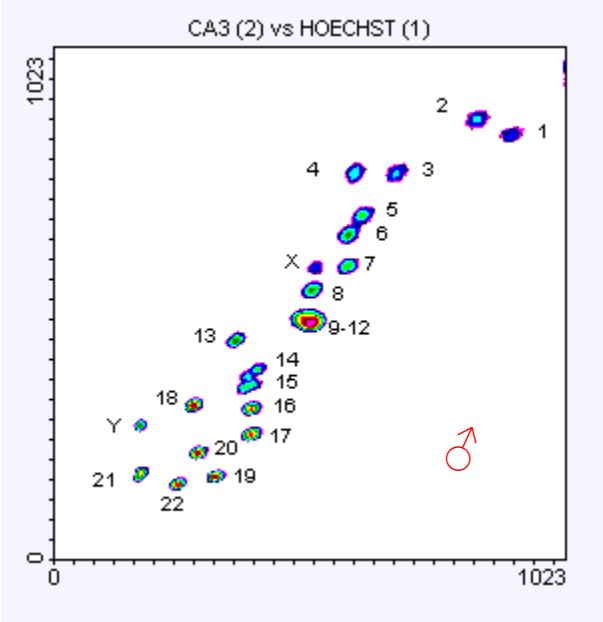
M.A. Van Dilla*□, L.L. Deaven†□, K.L. Albright†, N.A. Allen*, M.R. Aubuchon*, M.F. Bartholdi†, N.C. Brown†, E.W. Campbell†, A.V. Carrano*, L.M. Clark†, L.S. Cram†, B.D. Crawford†, J.C. Fuscoe*, J.W. Gray*, C.E. Hildebrand†, P.J. Jackson†, J.H. Jett†, J.L. Longmire†, C.R. Lozes*, M.L. Luedemann†, J.C.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

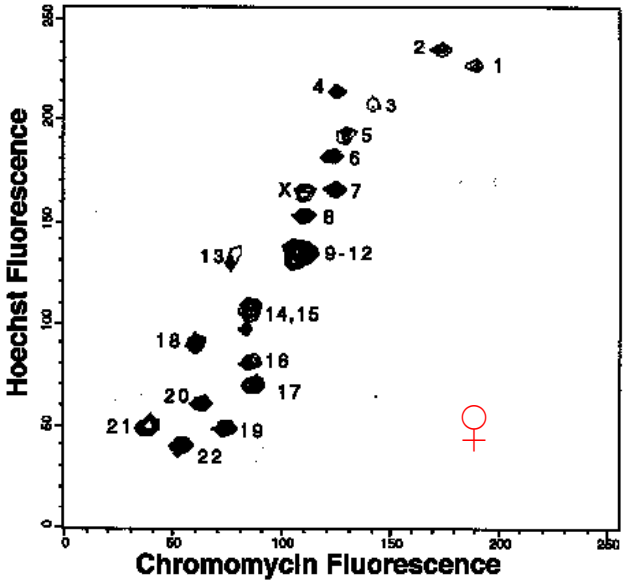
CONSTRUCTION OF A PHASE I CHROMOSOME-SPECIFIC (#18) HUMAN GENE LIBRARY IN CHARON 21A USING HIND III (LLNL)

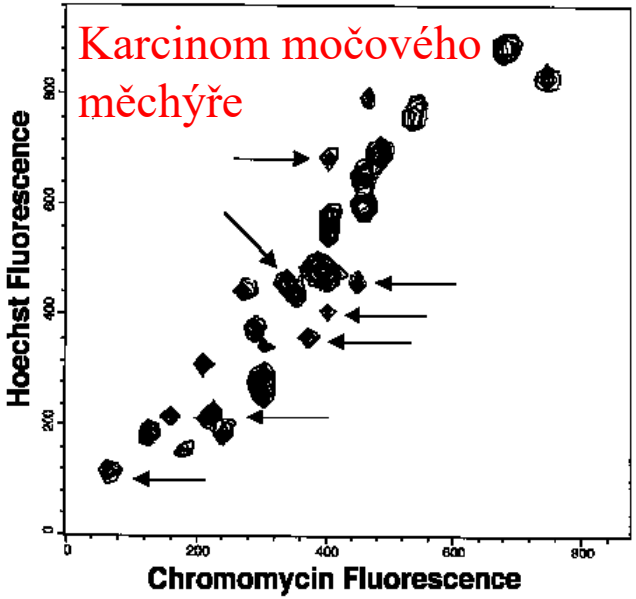


„Flow karyotype“



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





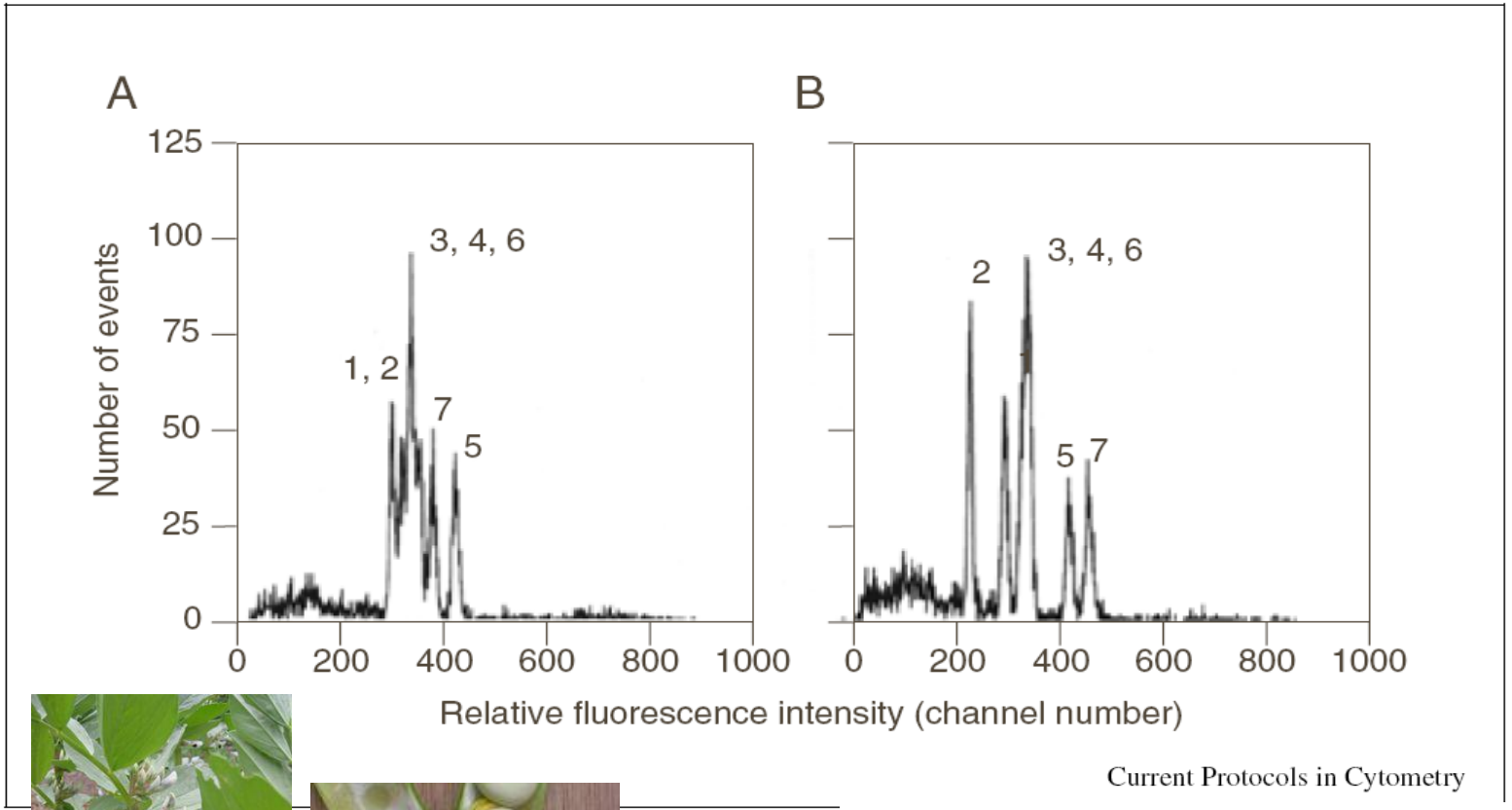
The Preparation of Human Chromosomes for Flow Cytometry

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



Current Protocols in Cytometry

Pisum sativum

PROTOCOL

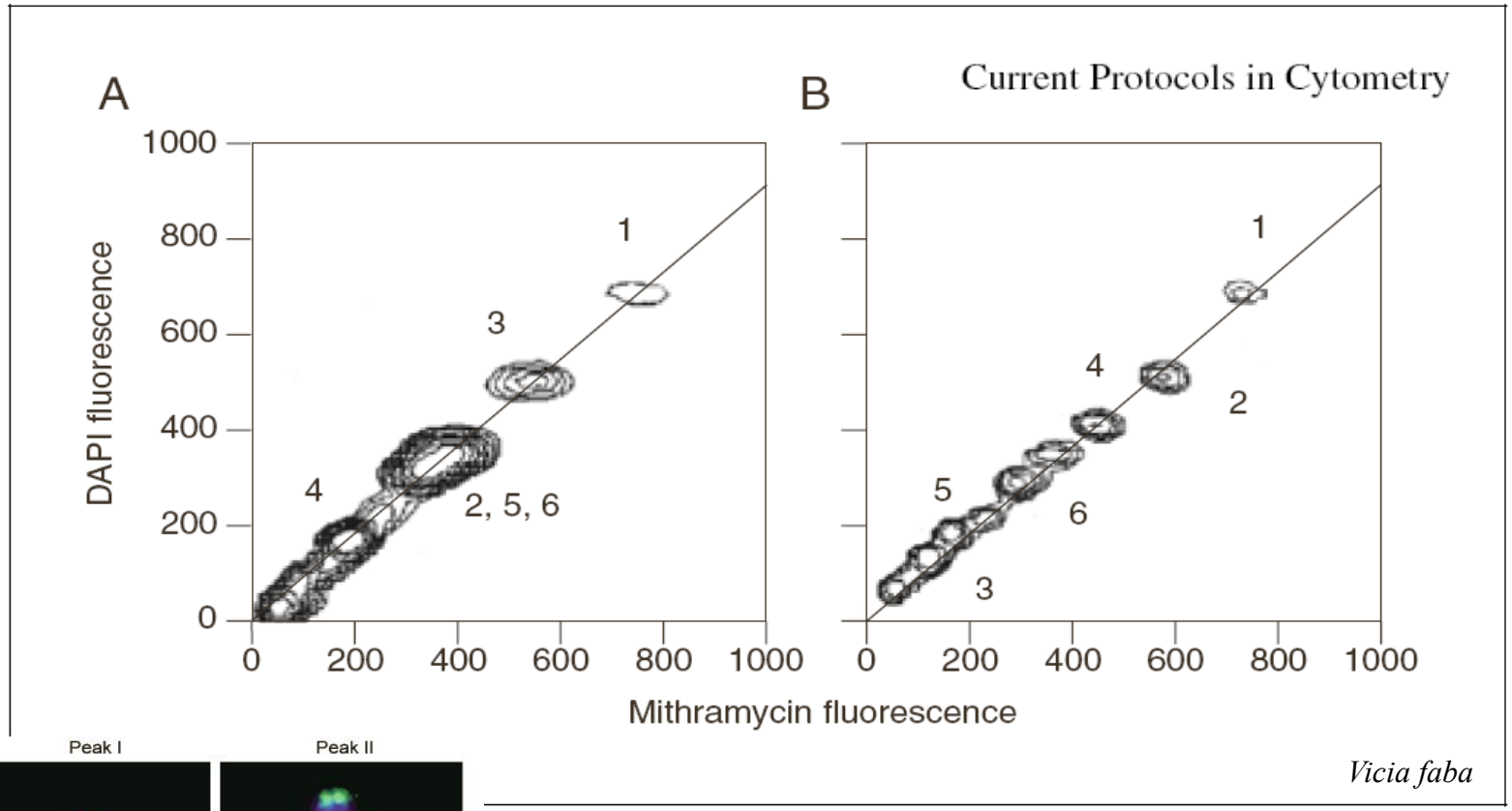
Estimation of nuclear DNA content in plants using flow cytometry

Jaroslav Doležel[1,2], Johann Greilhuber[3] & Jan Suda[4,5]

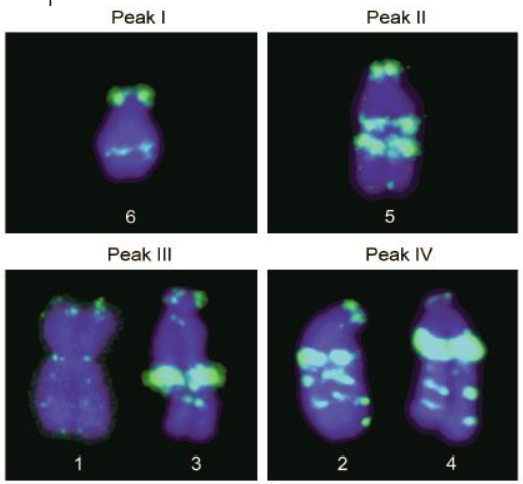
[1]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovská 6, CZ-77200 Olomouc, Czech Republic. [2]Department of Cell Biology and Genetics, Palacký University, Šlechtitelů 11, CZ-78371 Olomouc, Czech Republic. [3]Department of Systematic and Evolutionary Botany, Faculty of Life Sciences, University of Vienna, Rennweg 14, A-1030 Vienna, Austria. [4]Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-12801 Prague, Czech Republic. [5]Institute 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

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]*

Cytogenetic and Genome Research

Cytogenet Genome Res 2010;129:211–223
DOI: 10.1159/000313072

Published online: May 26, 2010

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 Šafář[1], Jan Bartoš[1], Jaroslav Janda[1], Arnaud Bellec[2], Marie Kubaláková[1,3], Miroslav Valárik[1], Stéphanie Pateyron[2], Jitka Weiserová[1], Radka Tušková[1], Jarmila Čihalíková[1,3], Jan Vrána[1], Hana Šimková[1], Patricia Faivre-Rampant[2], Pierre Sourdille[4], Michel Caboche[2], Michel Bernard[4], Jaroslav Doležel[1,3] and Boulos Chalhoub[2,*]

[1]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovska 6, CZ-77200 Olomouc, Czech Republic,
[2]Laboratory of Genome organization, Unité de Recherches en Génomique Végétale (INRA-URGV), 2 rue Gaston Crémieux, CP 5708, F-91057 Évry Cedex, France,
[3]Department of Cell Biology and Genetics, Palacký University, Šlechtitelů 11, Olomouc, Czech Republic, and
[4]Génétique Moléculaire des Céréales, UMR INRA-UBP, Domaine de Crouelle, 234 Avenue du Brézet, F-63039 Clermont-Ferrand Cedex 2, France

Received 1 February 2004; revised 5 May 2004; accepted 11 May 2004.
*For correspondence (fax 33 1 60874549; e-mail chalhoub@evry.inra.fr).

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,

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,

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

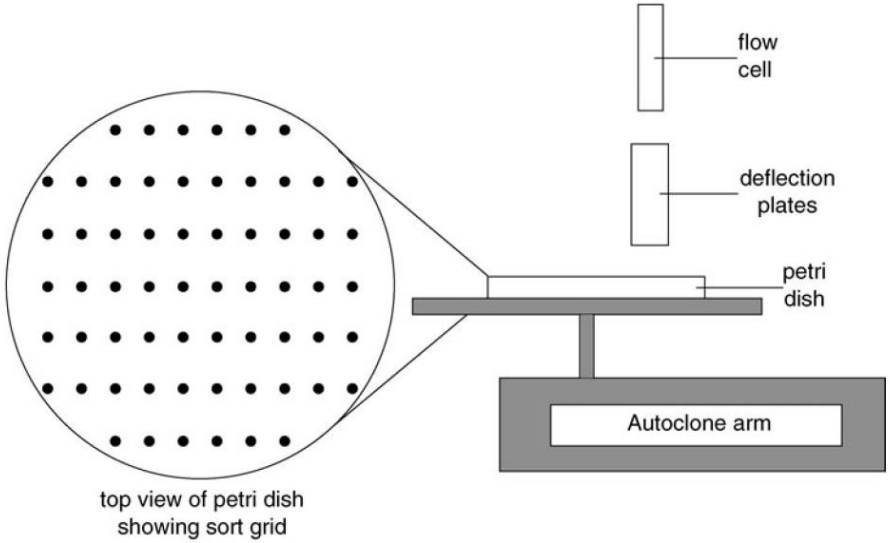
Current Protocols in Cytometry

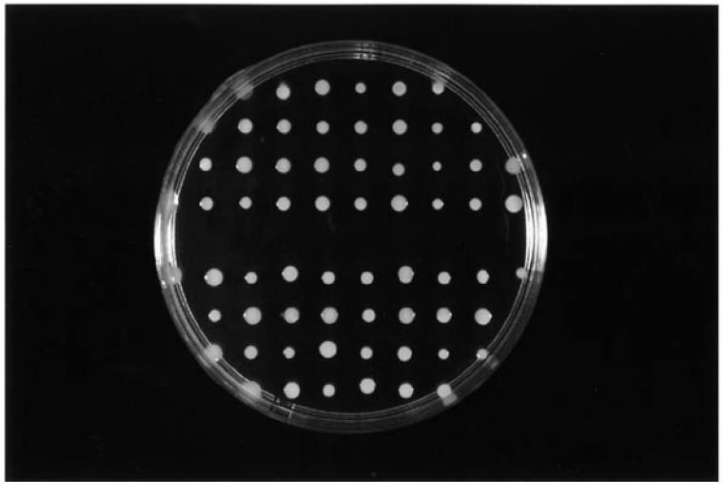
Aplikace průtokové cytometrie v mikrobiologii

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

Aplikace průtokové cytometrie v mikrobiologii

- Sortrování
  - EPICS + Autoclone® modul





Current Protocols in Cytometry

BactoSense™

Automated flow cytometer for online monitoring of bacteria in water

Fast Results available within 20 minutes, analyses up to 48 samples in 24h

Safe No handling of chemicals, all components are in a sealed and recyclable cartridge

Accurate Flow cytometry technology allows precise detection of more than 99.9% of microbial cells

Secure 24/7 monitoring: set a threshold value to get an alarm in time to act accordingly

Reliable Self-check routines, factory calibration and low maintenance

bNovate TECHNOLOGIES

For your PROCESS

In the FIELD

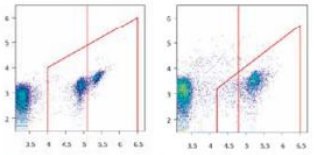
In the LAB

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

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

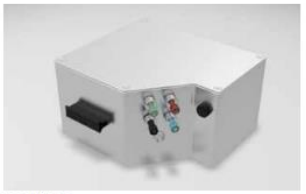
Instrument	Factory calibrated
Display	Touchscreen
Data storage	32 GB
Protection level enclosure	IP 65
Dimensions (WxDxH)	350 x 240 x 373 mm
Weight	14.5 kg
Power supply	100 - 240 VAC, 50/60 Hz, 1.4 A, IP 67
Power consumption	20 W
Ambient temperature	5..30°C
Relative humidity	10 - 90% RH
Cartridge	Hermetically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

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



Dotplots showing TCC and ICC

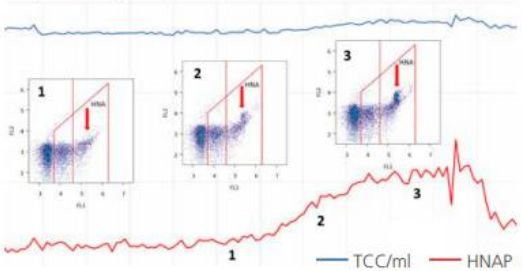
Online sampler / manual sampler



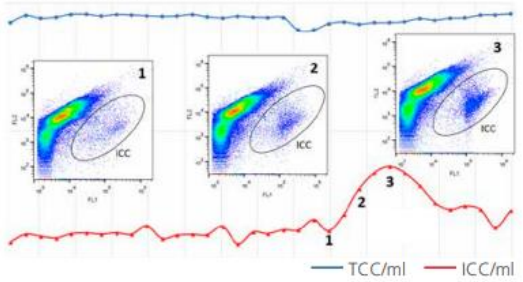
Cartridge

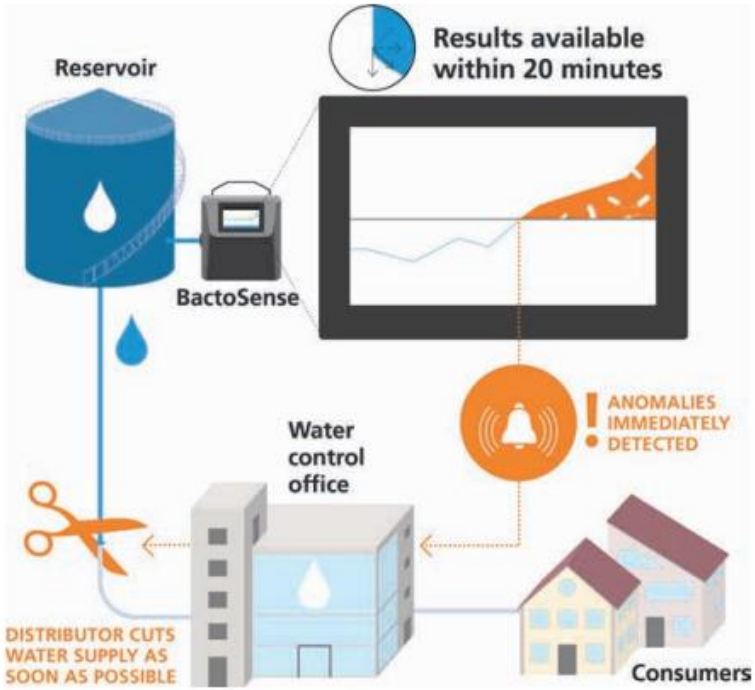
IO box

Graph showing TCC and HNAP measurements over one week



Graph showing TCC and ICC measurements every 3 hours

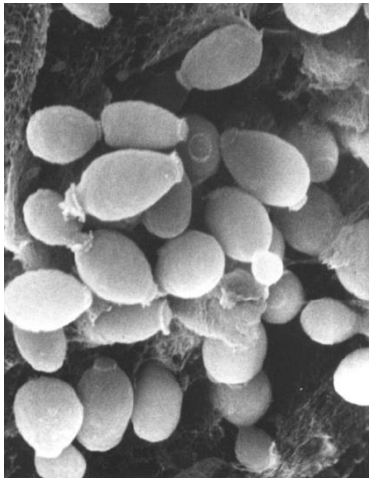




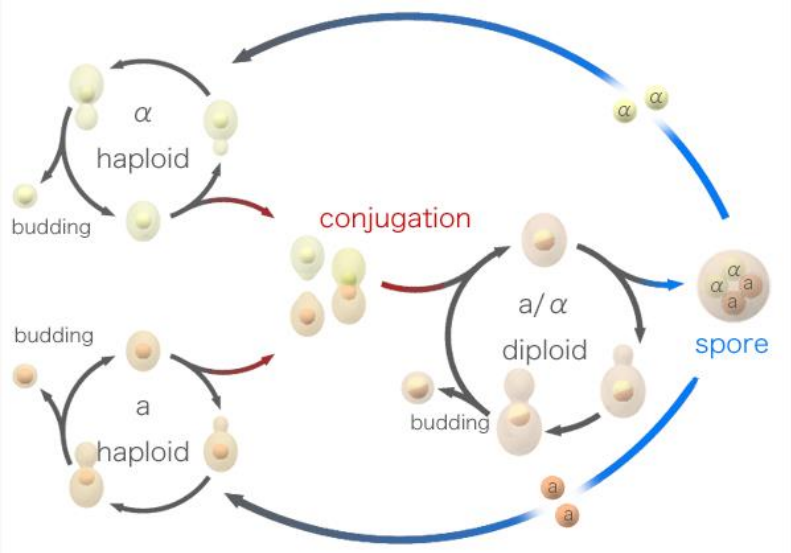
312020/02EN

Průtoková cytometrie kvasinek

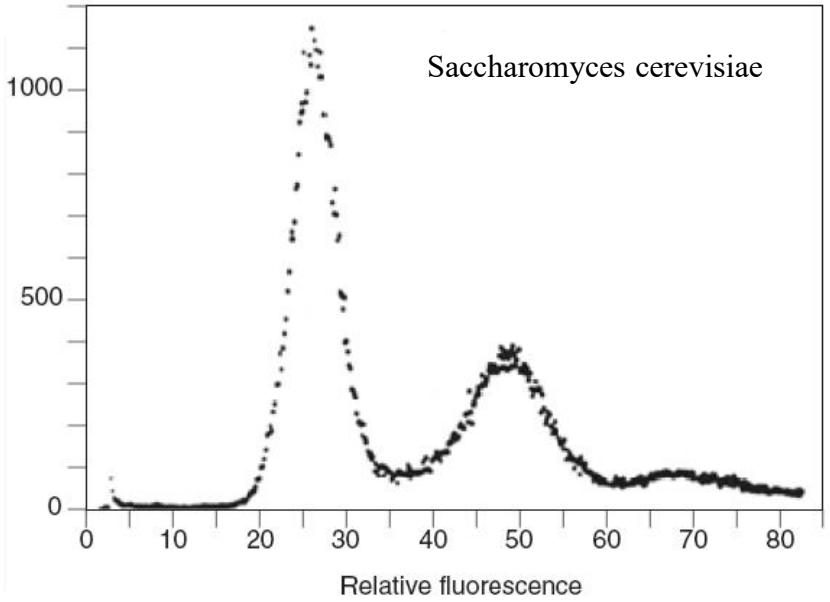
- **buněčné dělení**
- viabilita
- membránový potenciál
- respirace
- produkce H_2O_2
- citlivost k antibiotikům
- separace



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

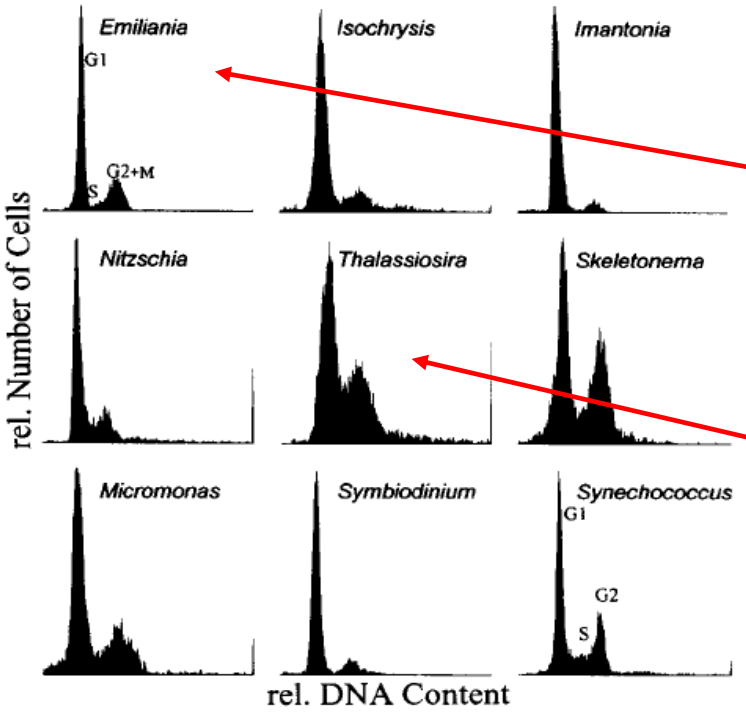
Vol. 185: 301–307, 1999 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published August 20

NOTE

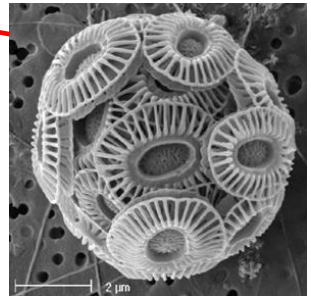
Cytometric measurement of the DNA cell cycle in the presence of chlorophyll autofluorescence in marine eukaryotic phytoplankton by the blue-light excited dye YOYO-1

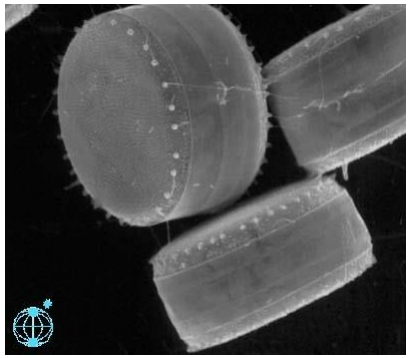
Frank J. Jochem[1,*], Doris Meyerdierks[2]

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



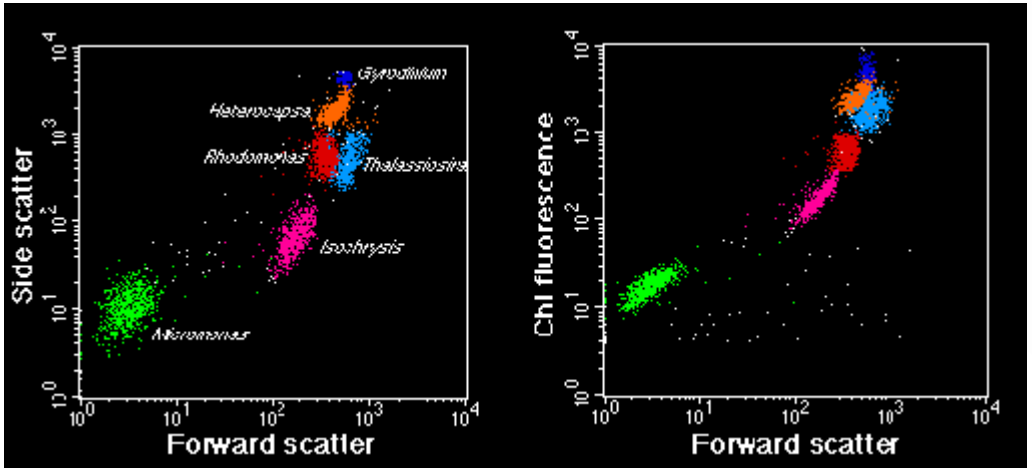
http://www.soes.soton.ac.uk/staff/tt/

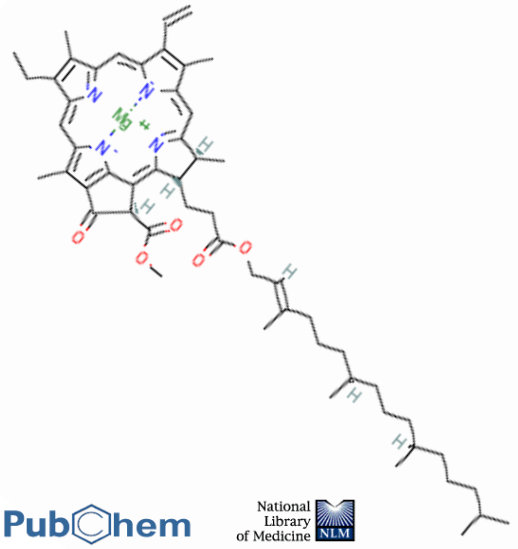


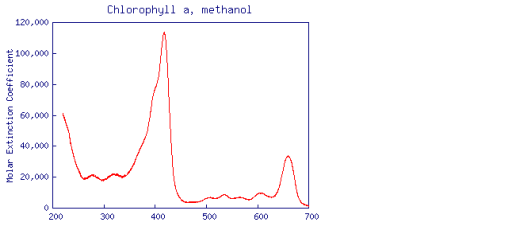


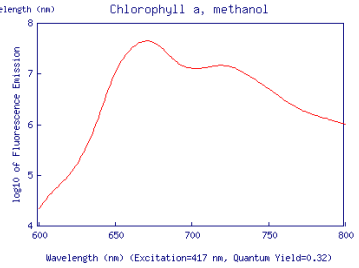
http://planktonnet.awi.de/portal.php?pagetitle=assetfactsheet&asset_id=15127

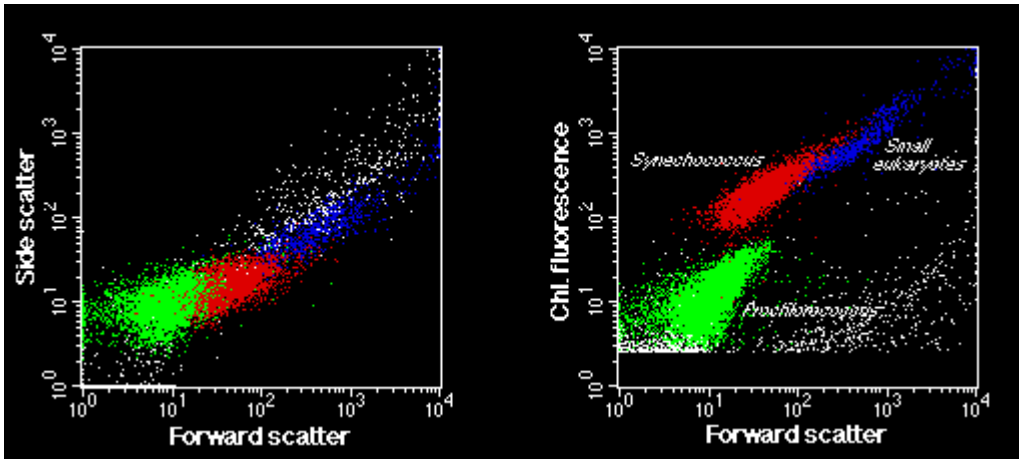
Průtoková cytometrie v hydrobiologii











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

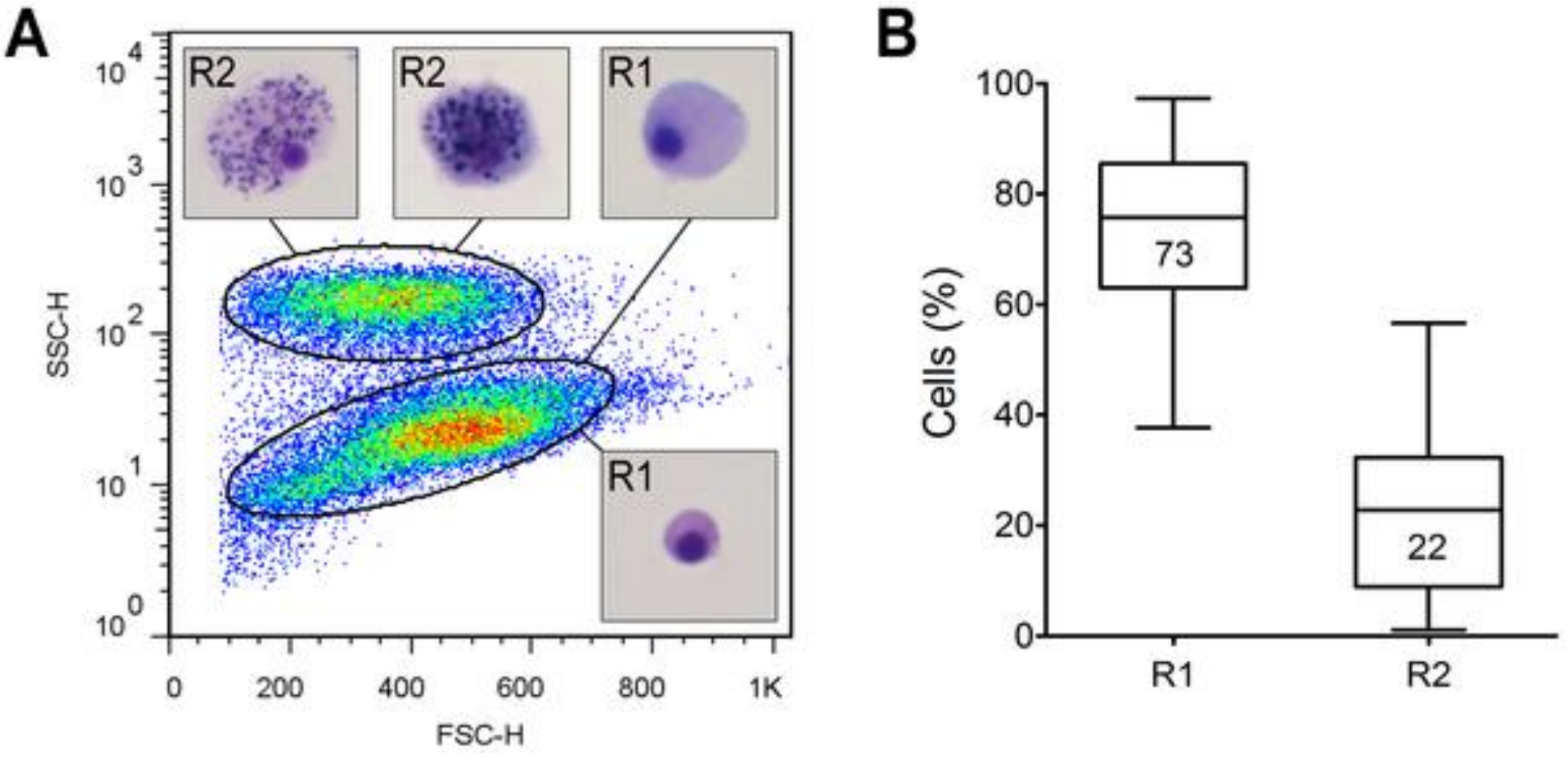
Flow cytometry as a tool for analysing invertebrate cells

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*

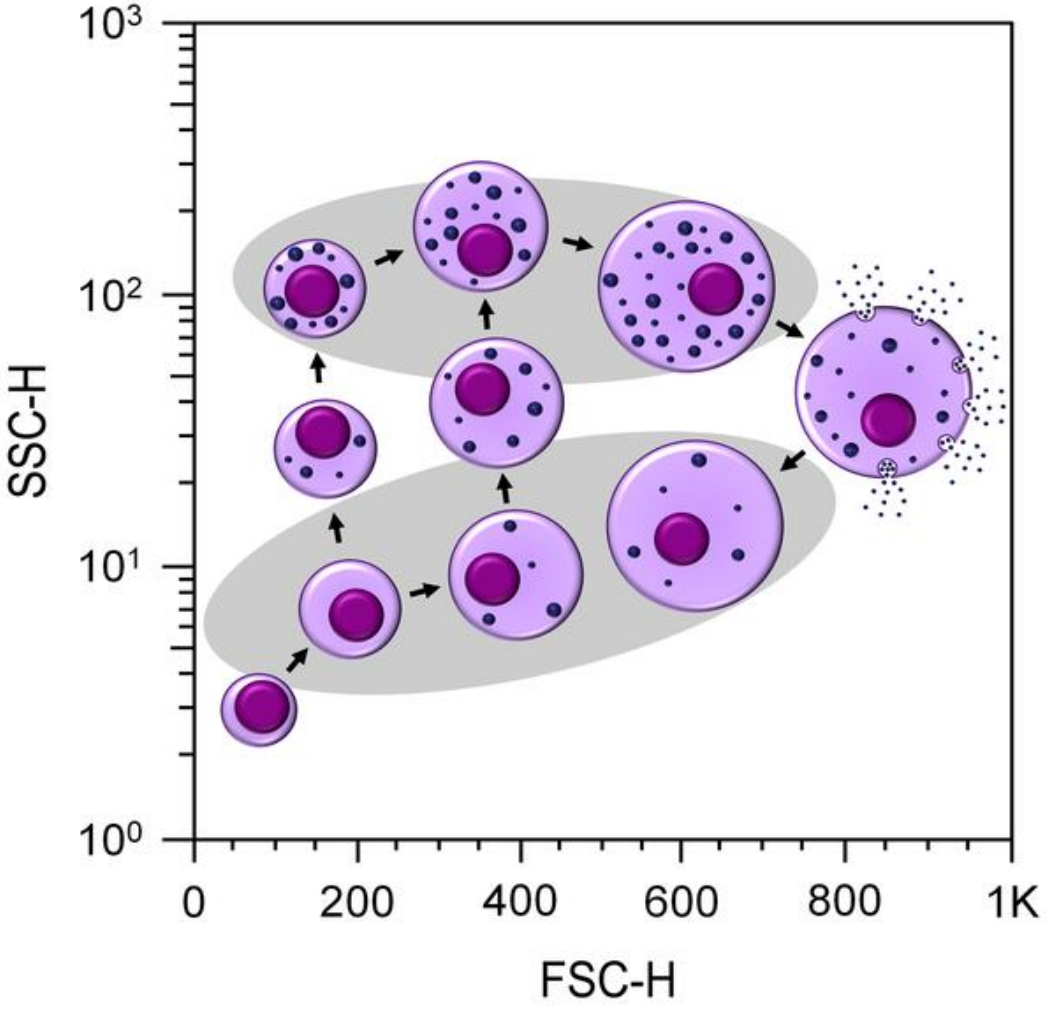
http://www.icms.qmul.ac.uk/flowcytometry/uses/insects/index.html

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

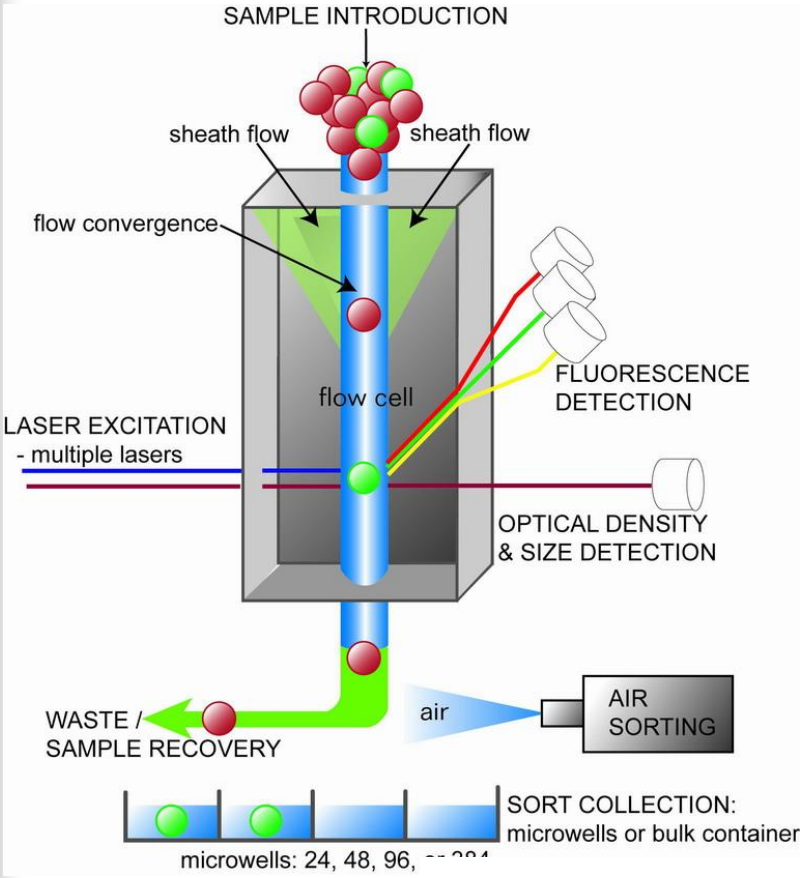


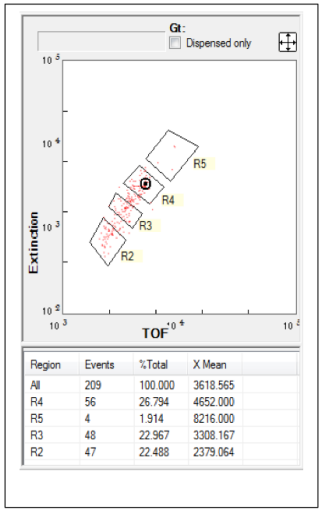
Rebelo MdF, Figueiredo EdS, Mariante RM, Nóbrega A, et al. (2013) New Insights from the Oyster Crassostrea rhizophorae on Bivalve Circulating Hemocytes. PLoS ONE 8(2): e57384. doi:10.1371/journal.pone.0057384
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0057384

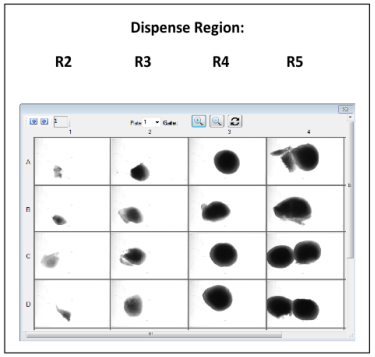
Figure 6. Proposed model for hemocyte maturation, as seen by flow cytometry.



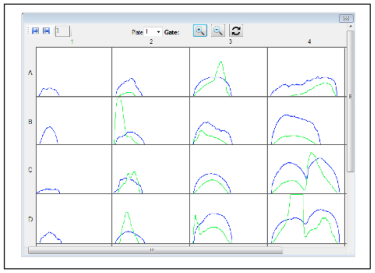
Rebelo MdF, Figueiredo EdS, Mariante RM, Nóbrega A, et al. (2013) New Insights from the Oyster Crassostrea rhizophorae on Bivalve Circulating Hemocytes. PLoS ONE 8(2): e57384. doi:10.1371/journal.pone.0057384
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0057384



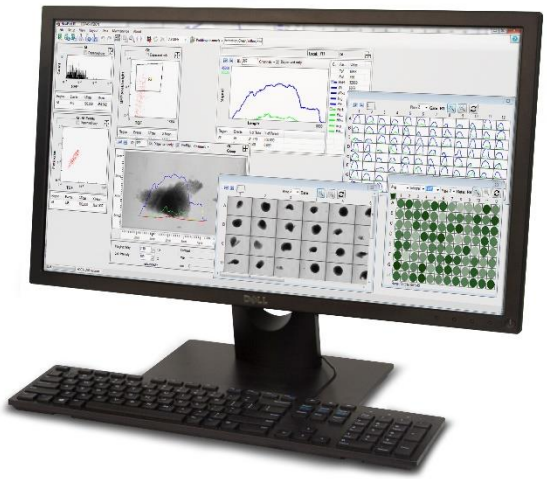




Dispensed Object **Images:**

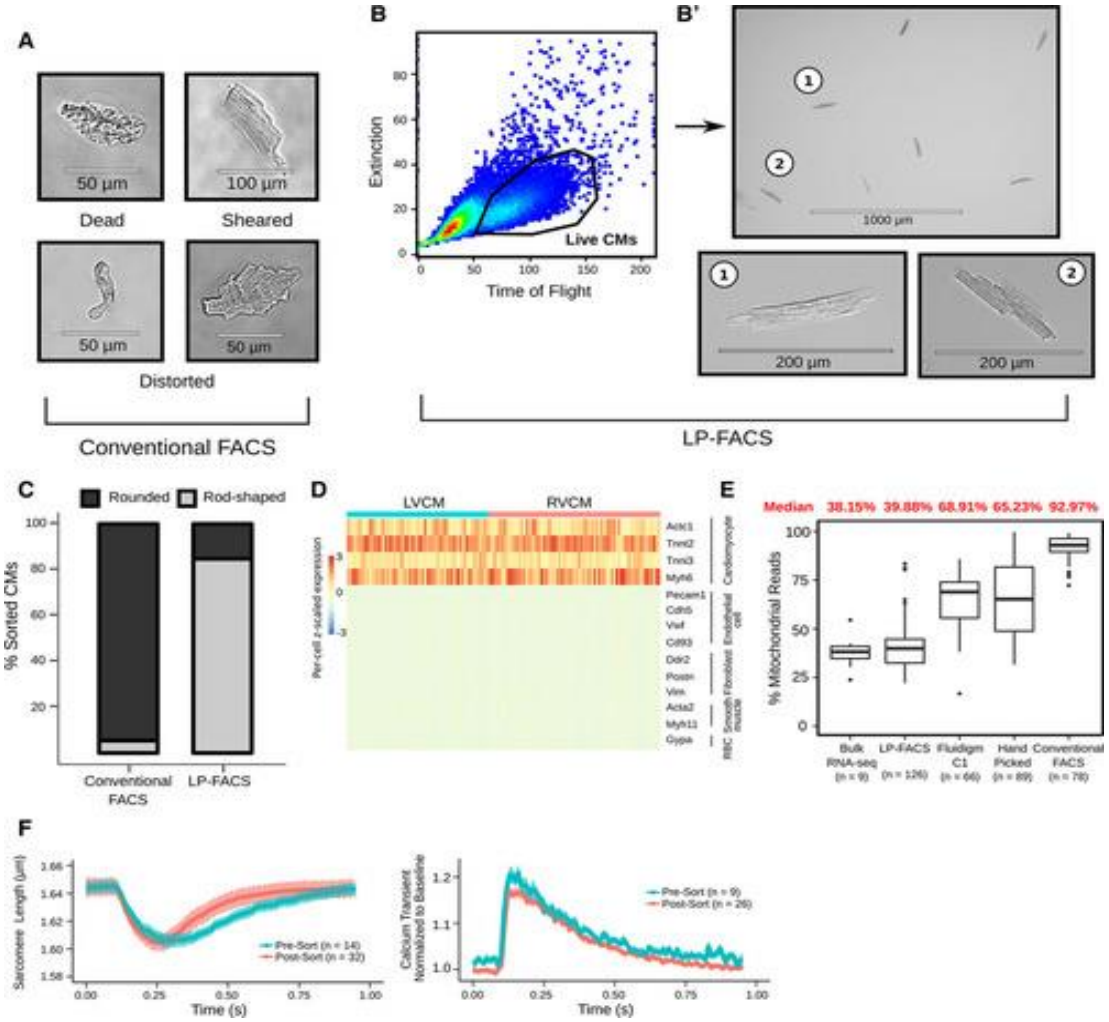


Corresponding Object **Profiles:**



https://www.unionbio.com/copas/

UNION BIOMETRICA



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

„High Throughput Flow Cytometry“

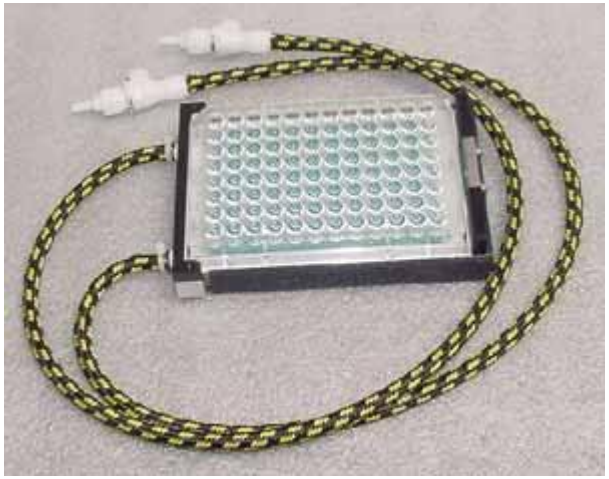
- automatizace + robotizace = urychlení a efektivita sběru dat (měření desítky vzorků za hodinu s minimálním zásahem operátora)
- využití principu vícebarevné analýzy

Automatizované systémy měření vzorků

Automatický karusel (autosampler)

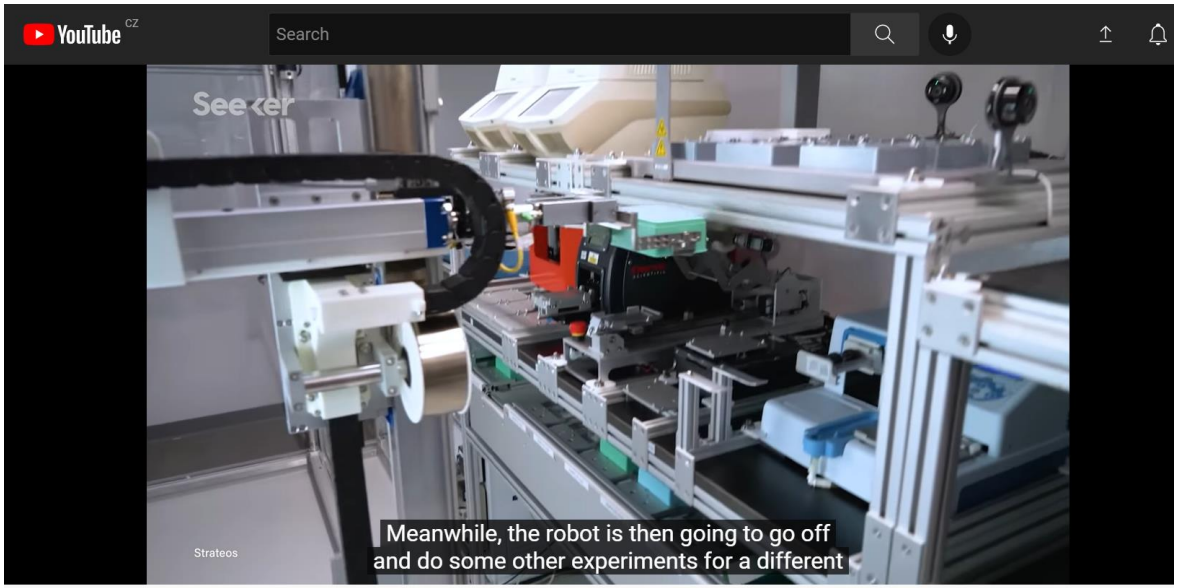
Adaptér pro nasávání vzorků z mikrotitrační desky

Automatizovaný „microsampler“ systém



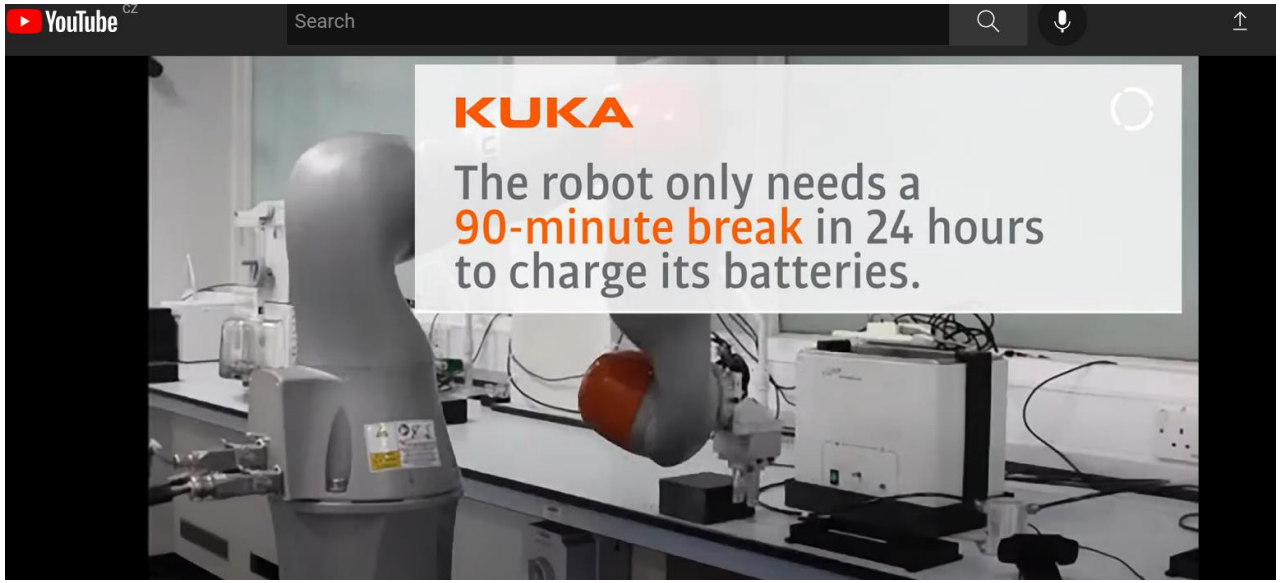
Cytek FLOW CYTOMETRY PRODUCTS

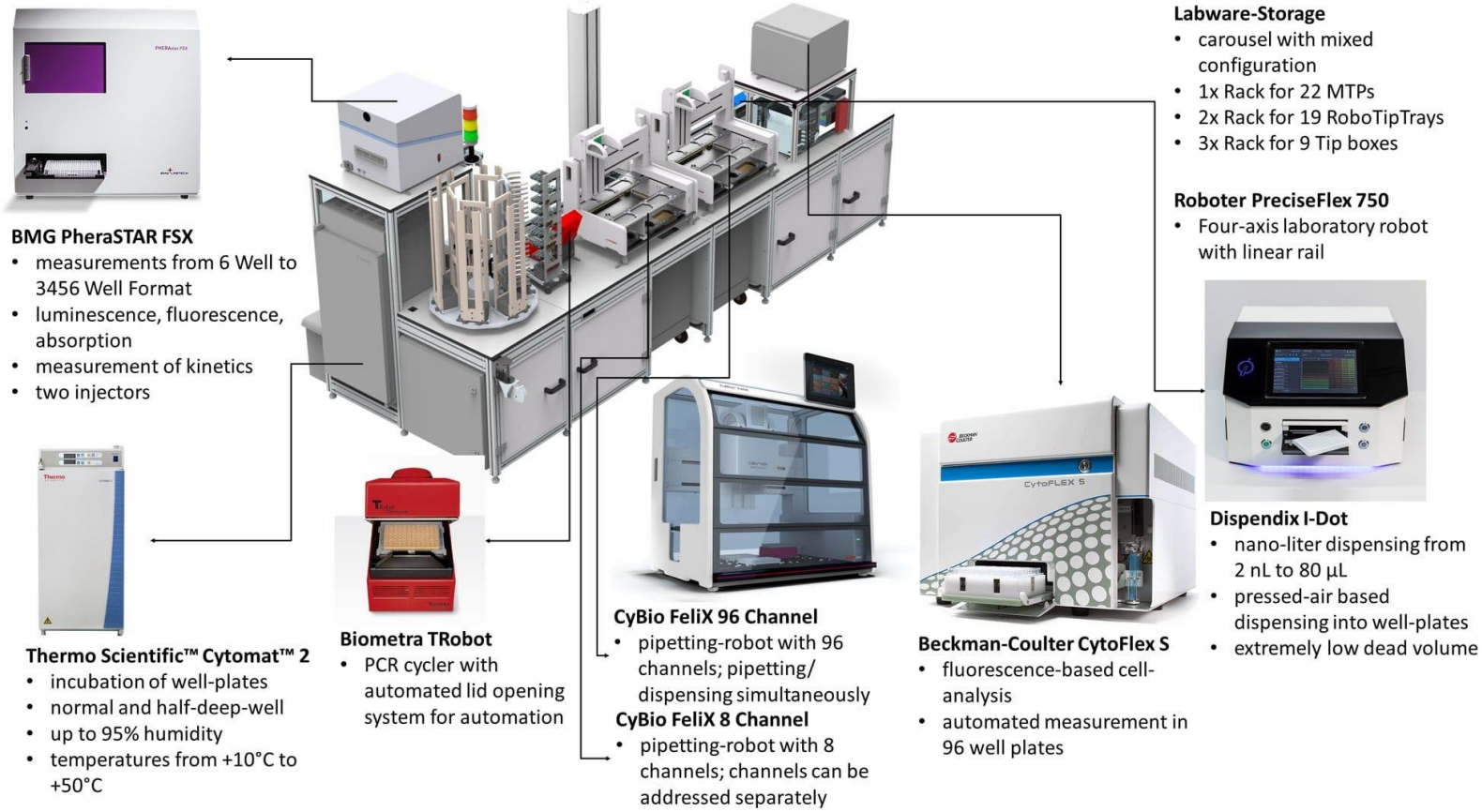
BD



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

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





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

Thermo Scientific™ Cytomat™ 2
- incubation of well-plates
- normal and half-deep-well
- up to 95% humidity
- temperatures from +10°C to +50°C

Biometra TRobot
- PCR cycler with automated lid opening system for automation

CyBio FeliX 96 Channel
- pipetting-robot with 96 channels; pipetting/ dispensing simultaneously

CyBio FeliX 8 Channel
- pipetting-robot with 8 channels; channels can be addressed separately

Beckman-Coulter CytoFlex S
- fluorescence-based cell-analysis
- automated measurement in 96 well plates

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

Roboter PreciseFlex 750
- Four-axis laboratory robot with linear rail

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

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

Incorporating Automation into a Flow Cytometry Workflow for Antibody Discovery

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

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

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*]

[1]Department of Pathology and Cancer Research Facility, University of New Mexico Health Sciences Center, Albuquerque, New Mexico
[2]Department of Mechanical Engineering, University of New Mexico College of Engineering, Albuquerque, New Mexico
[3]Department 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

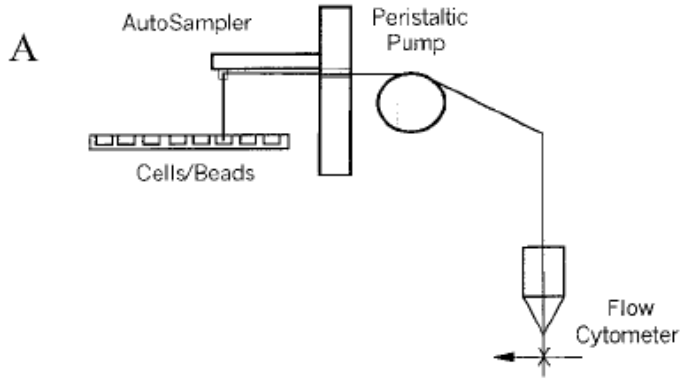
Cytometry 44:83–90 (2001)

High Throughput Flow Cytometry

Frederick W. Kuckuck,[1] Bruce S. Edwards,[1,2*] and Larry A. Sklar[1,2*]

[1]Cytometry, Cancer Research and Treatment Center, University of New Mexico Health Sciences Center, Albuquerque, New Mexico
[2]Department of Pathology, University of New Mexico Health Sciences Center, Albuquerque, New Mexico

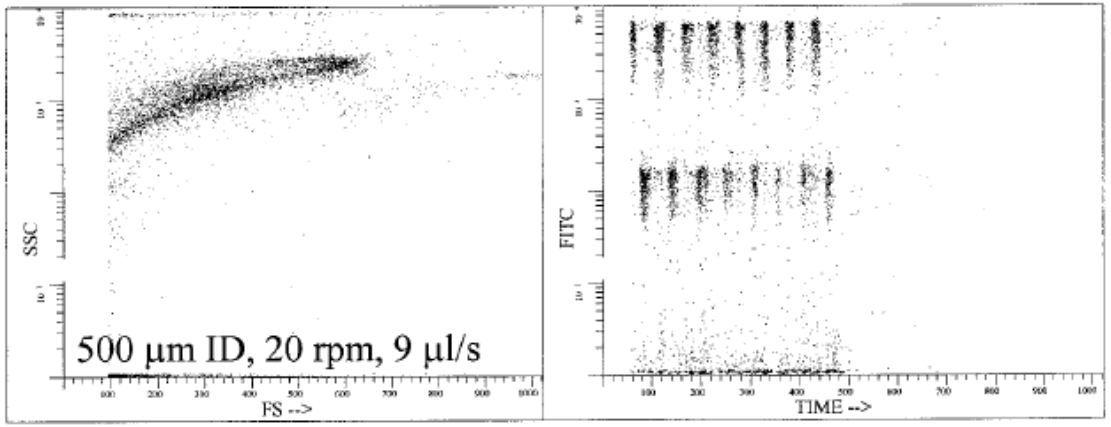
Received 18 September 2000; Revision Received 4 January 2001; Accepted 13 January 2001



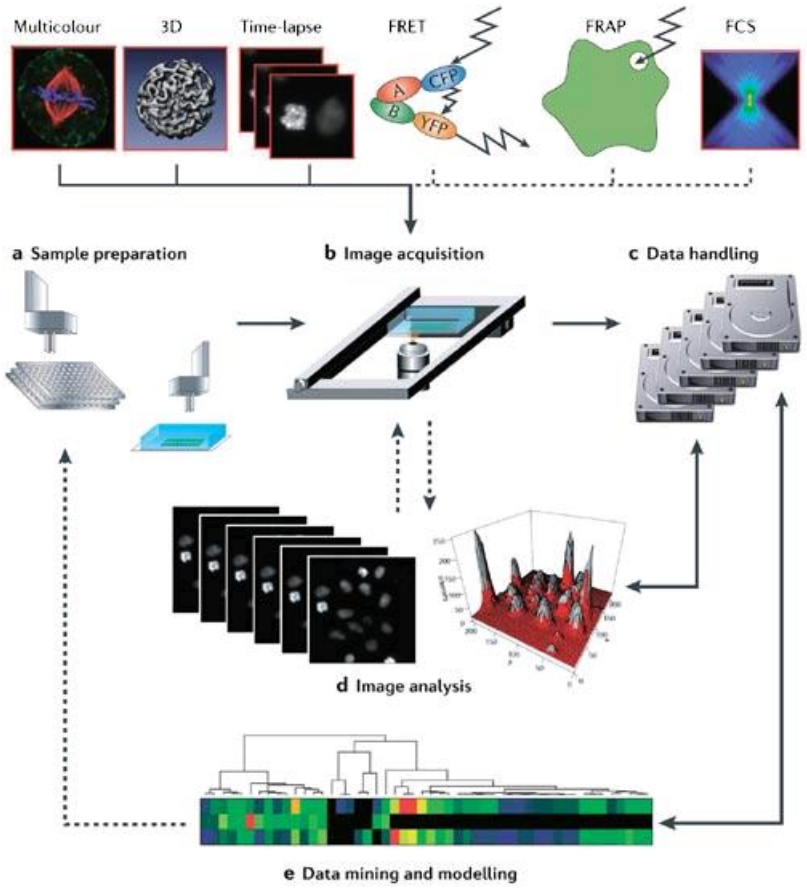
B

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

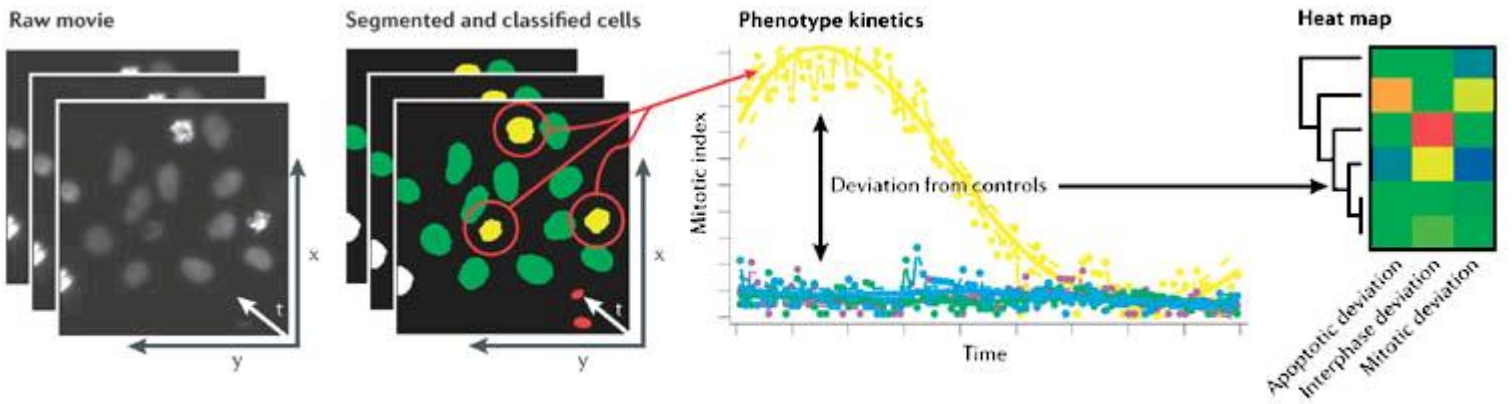


Table 1. Comparison of the Key Attributes of High-Throughput Flow Cytometry and High-Content Microscopy		
Key Attributes	**HT Flow Cytometry**	**High Content Microscopy**
Cell types	Optimal for suspension cells; adherent cells need to be detached before sampling.	Optimal for adherent cells; suspension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

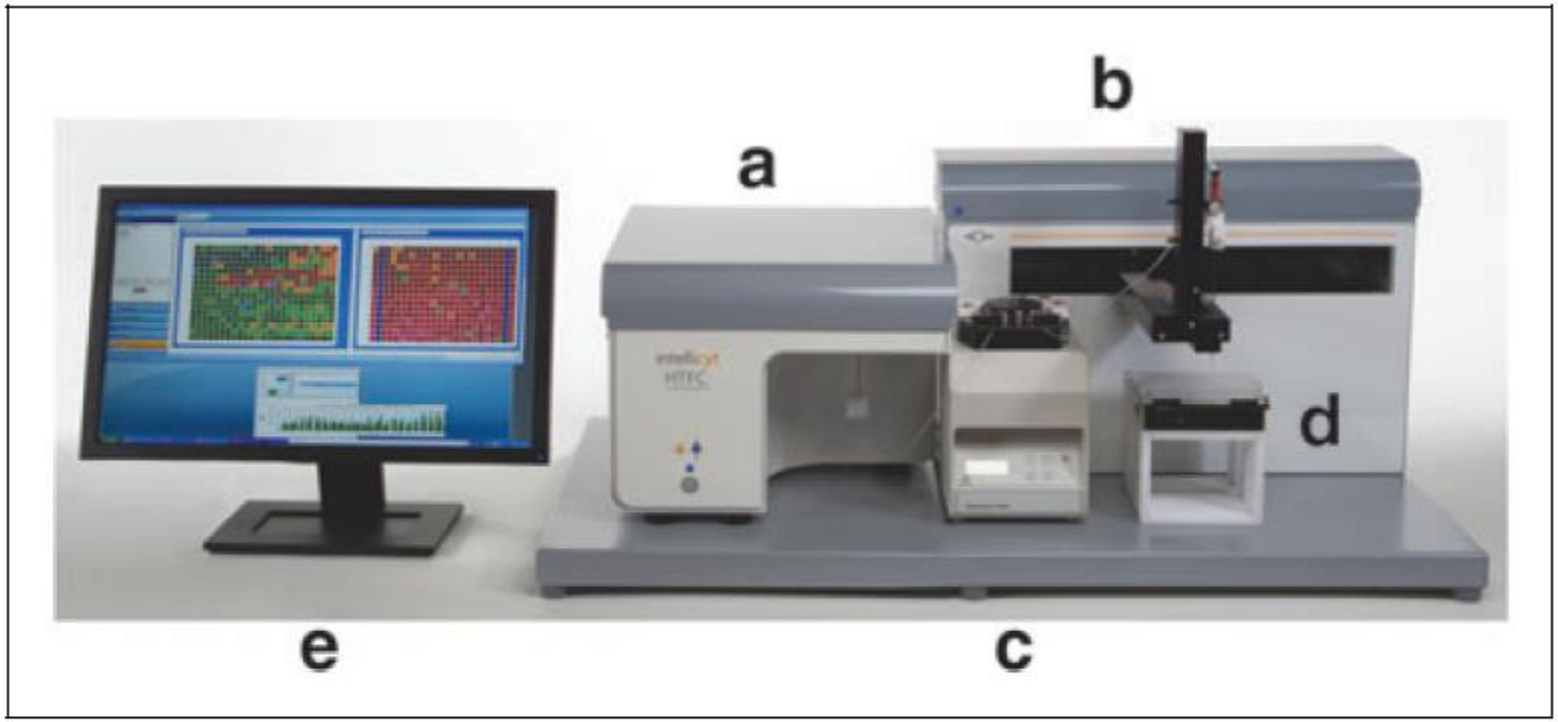
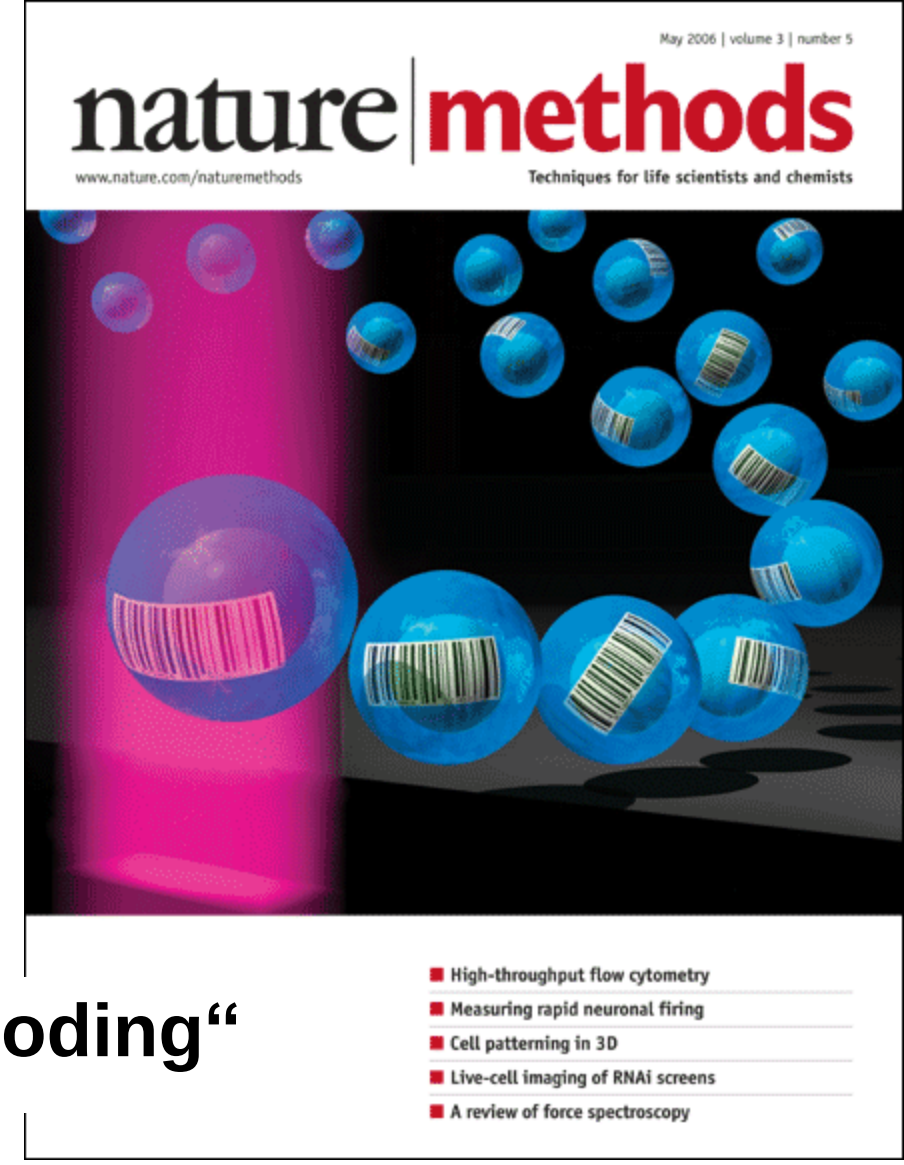


Fig. 1. The HTFC Screening System (IntelliCyt Corporation). **(a)** 2-laser, 4-color flow cytometer; **(b)** an x, y, z autosampler; **(c)** a low pulsation peristaltic pump; **(d)** orbital plate shaker that accommodates 96- and 384-well plates; **(e)** system computer with HyperView installed to set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

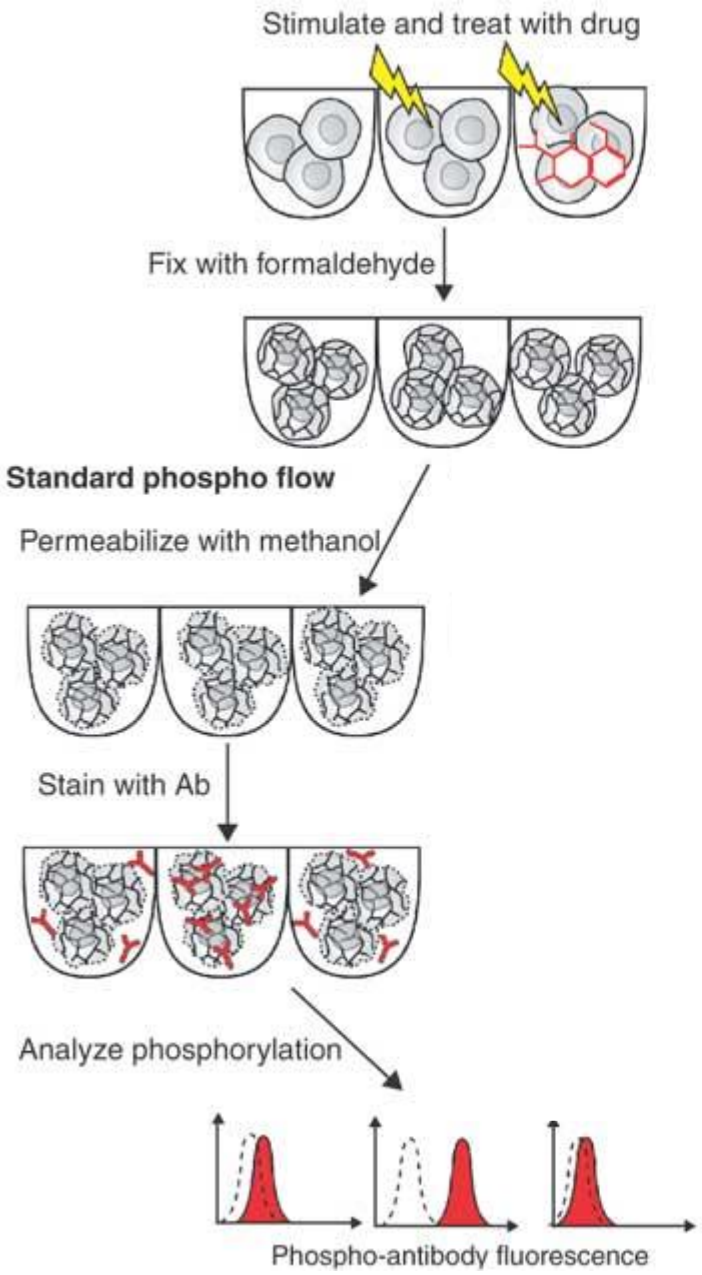
Garry Nolan

Peter Krutzik

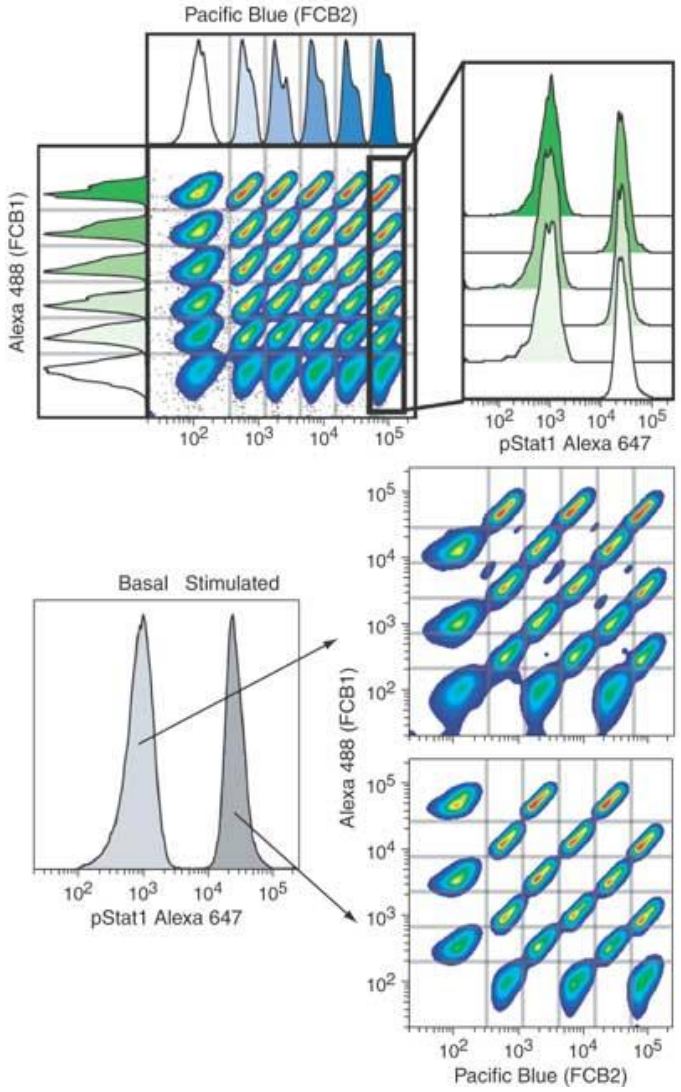


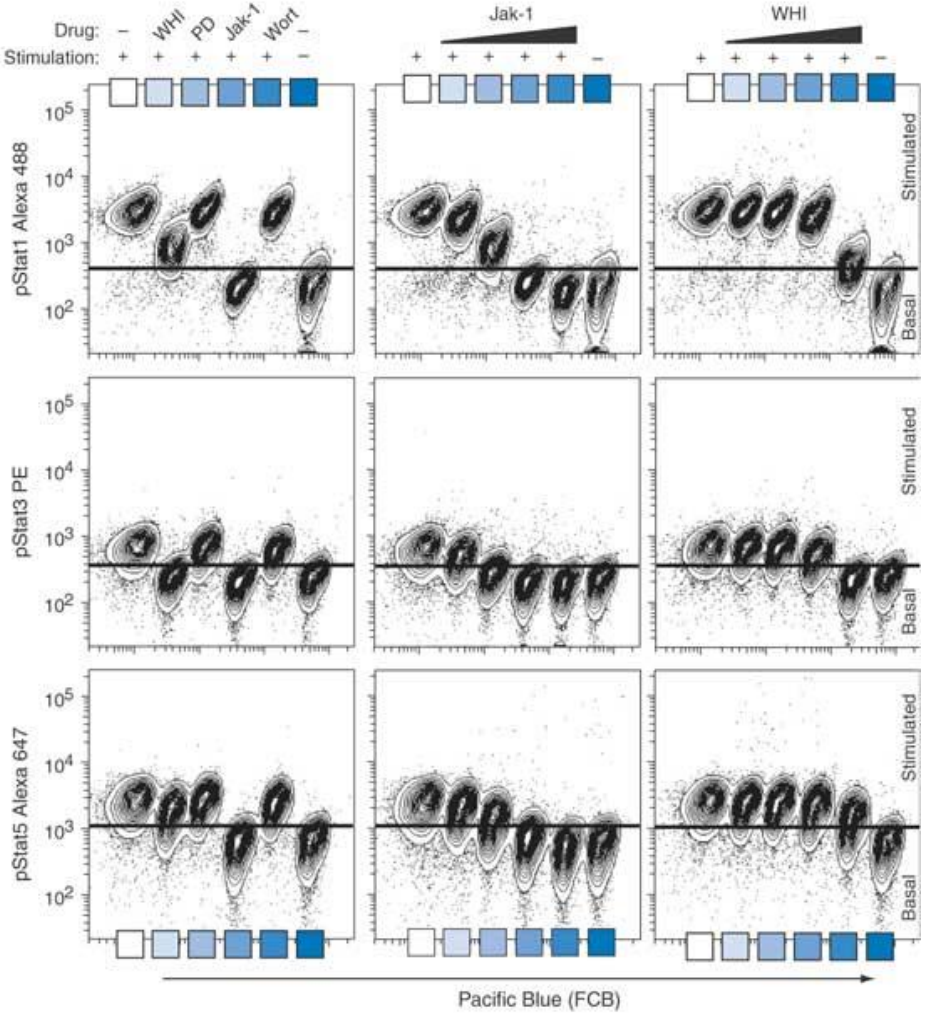
„Fluorescent cell barcoding“

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

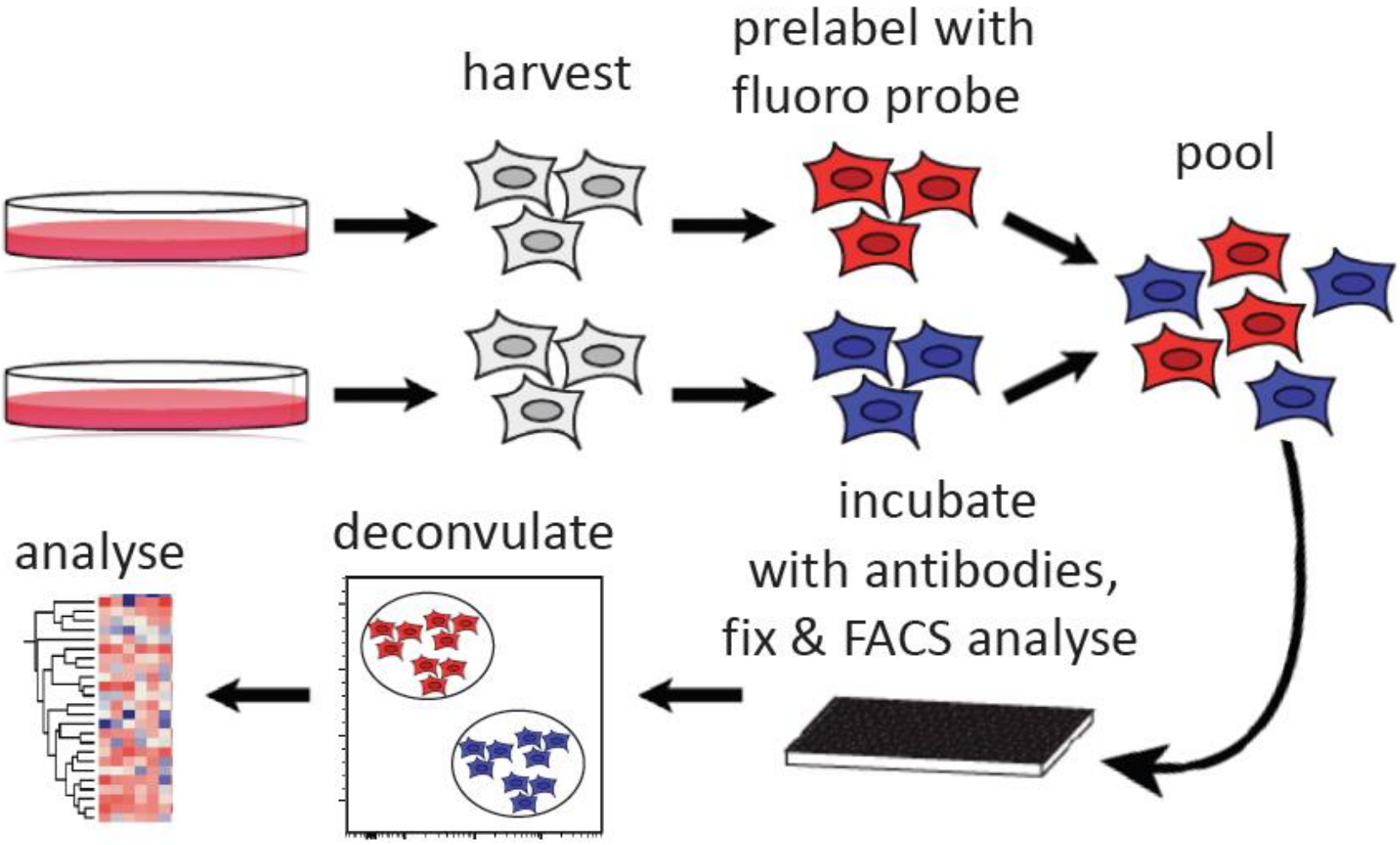
- LEGENDScreen MOUSE

252 PE conjugated antibodies + ISOs

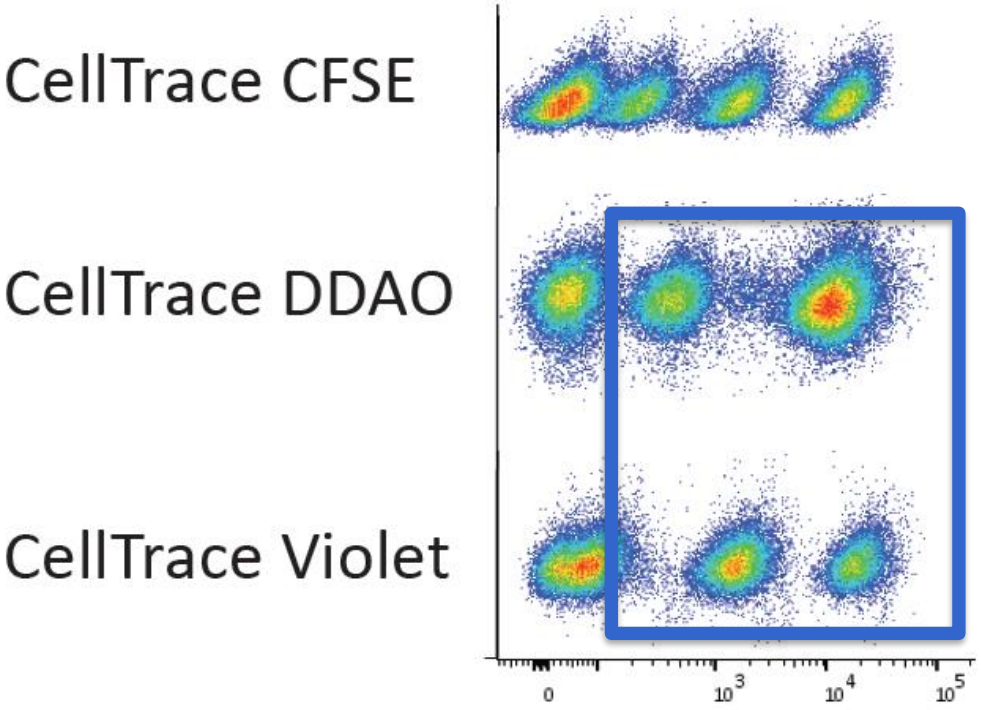
- there are XY vials in LN
- price of kit ≈ 1000 € (27k Kc)

How to get the best of it all?

Final workflow



The optimal concentation issue



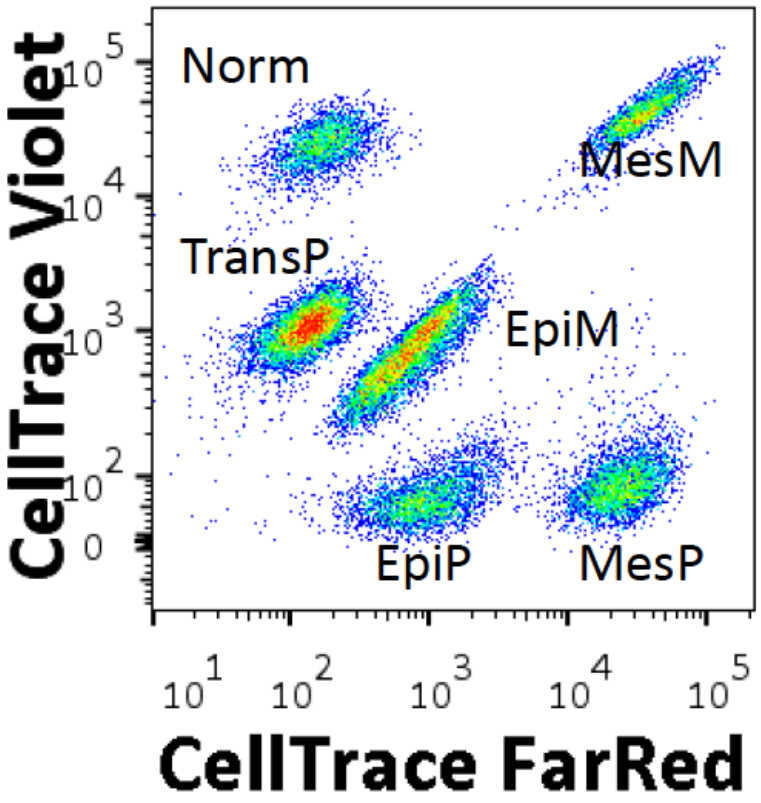
HOW TO TEST IT:

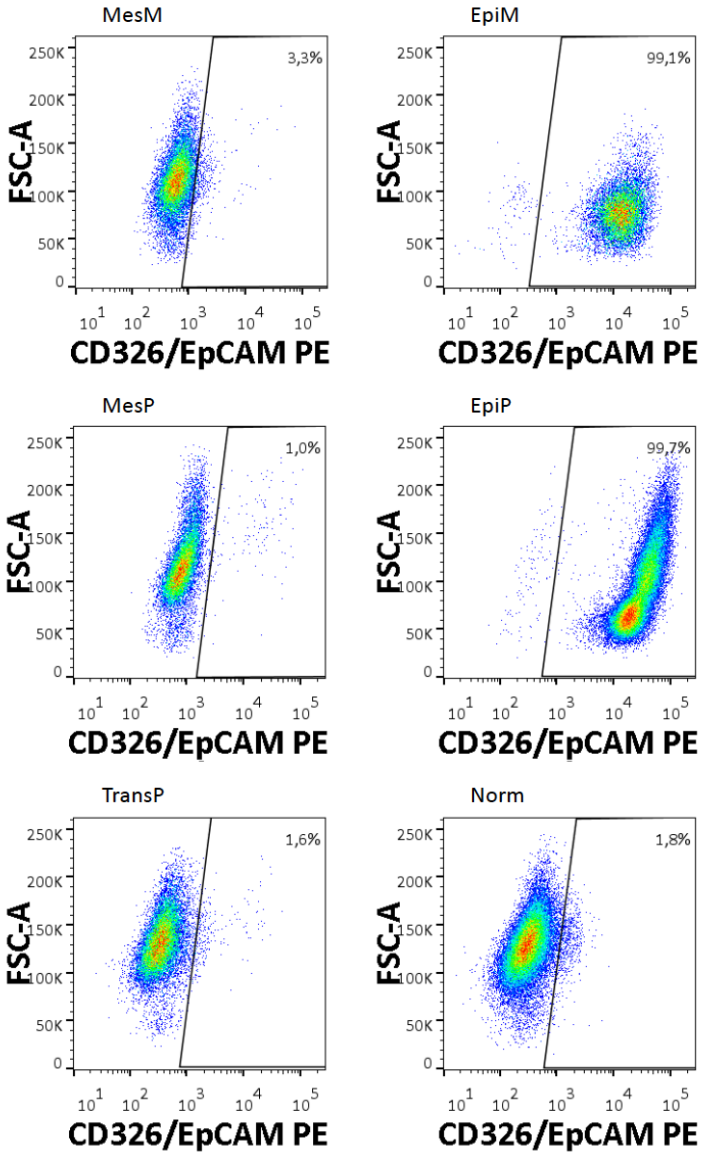
10x serial dilution

REQUIREMENTS:

- optimal resolution
- compatibility w/ PE

Sample results





EpCAM
- marker of epithelial cells
- commonly lost during EMT

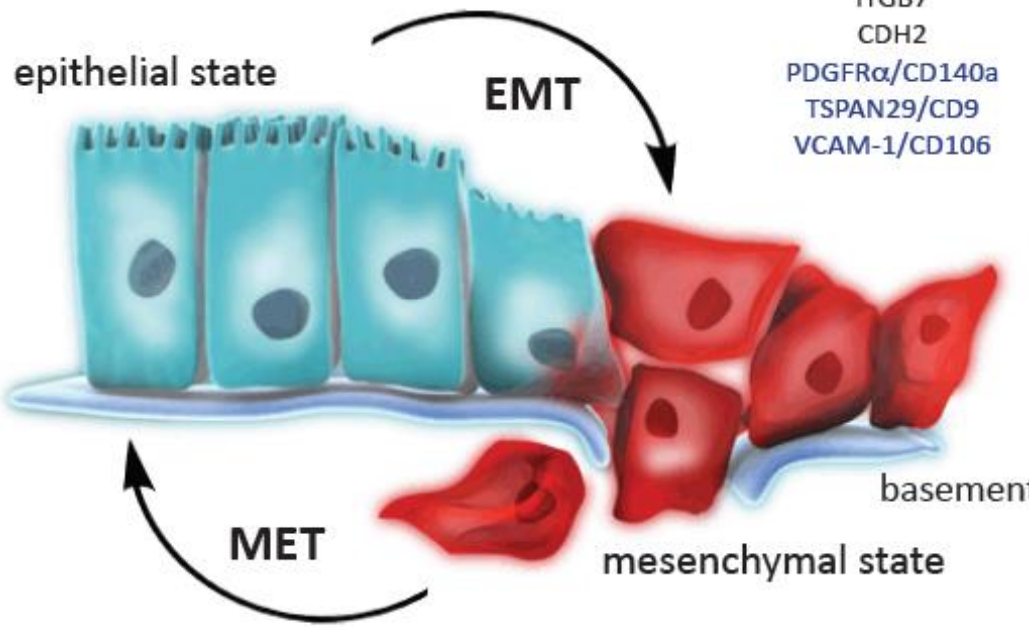
Sample result

non-BCSC
$CD24^{+}CD44^{low}$

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

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

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



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

Chapter 9

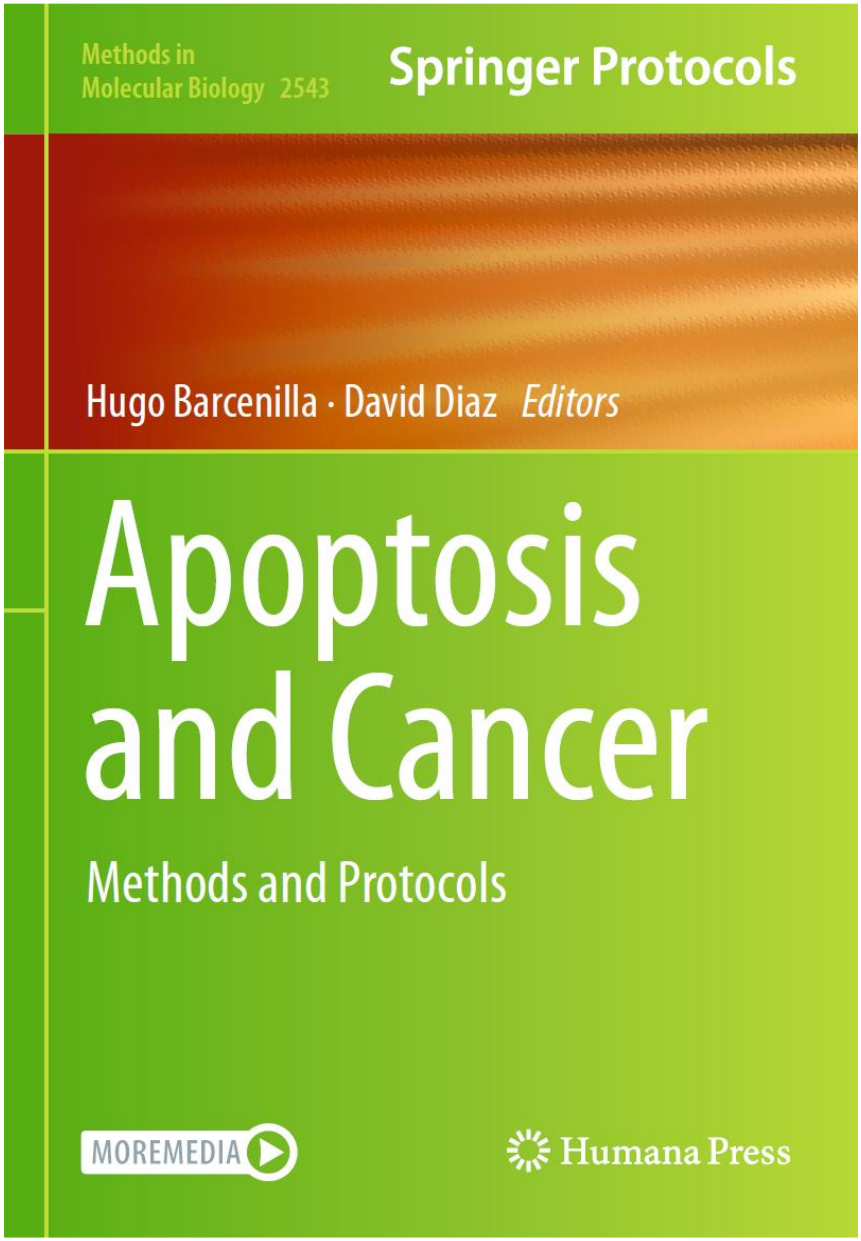
High-Throughput, Parallel Flow Cytometry Screening of Hundreds of Cell Surface Antigens Using Fluorescent Barcoding

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

Abstract

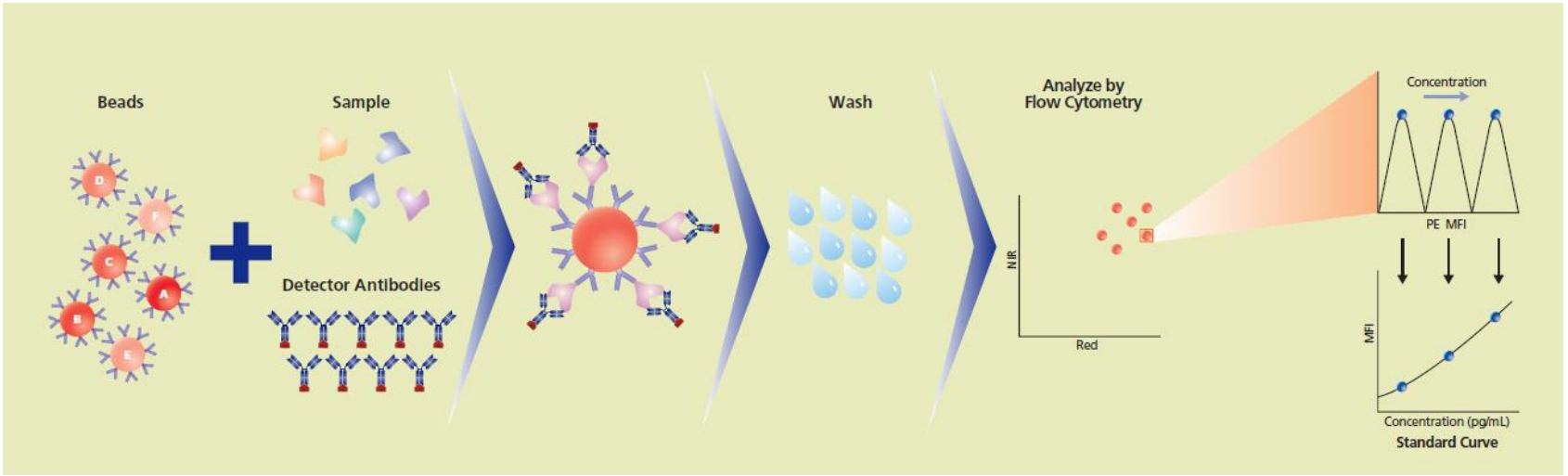
Multicolor flow cytometry allows for analysis of tens of cellular parameters in millions of cells at a single-cell resolution within minutes. The lack of technologies that would facilitate feasible and relatively cheap profiling of such a number of cells with an antibody-based approach led us to the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

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

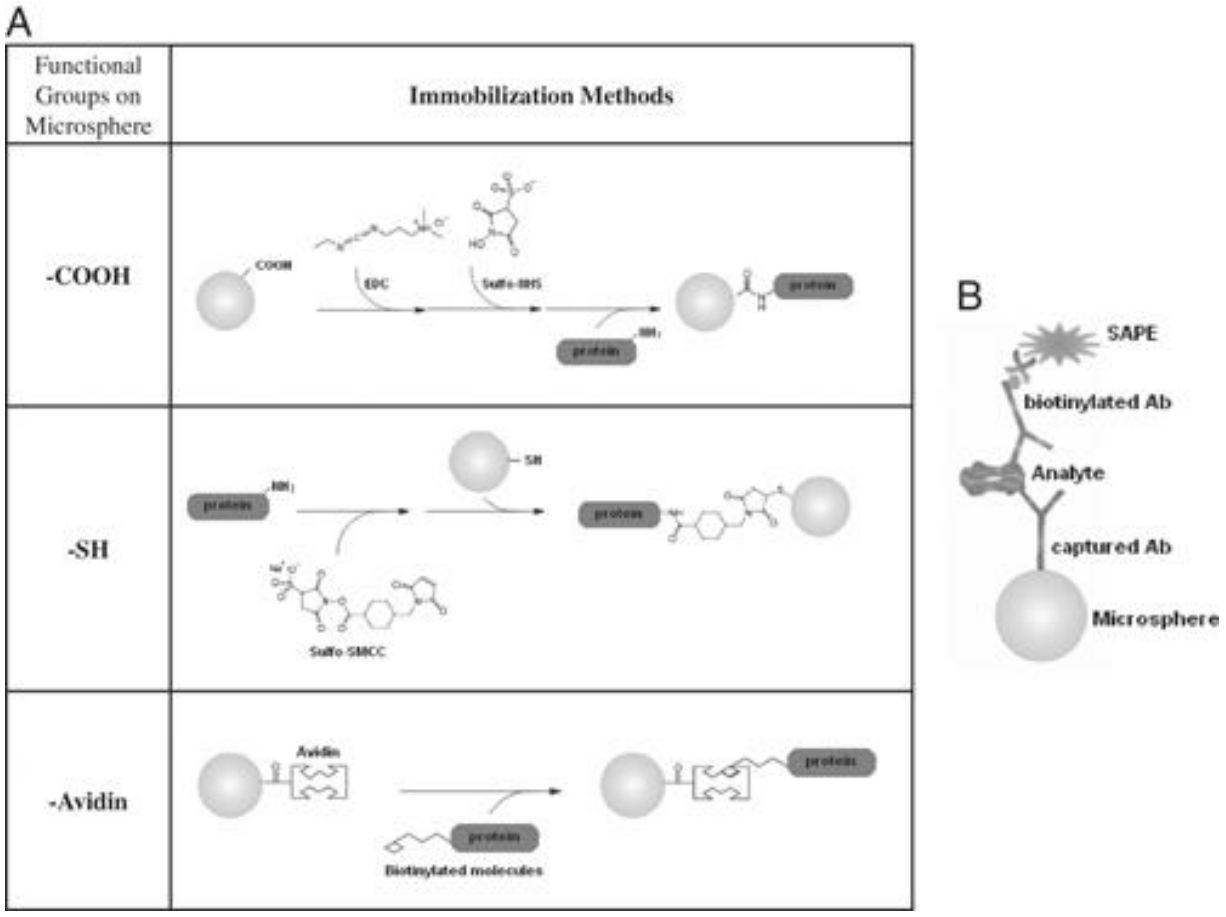


Cytometric bead array (CBA)

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

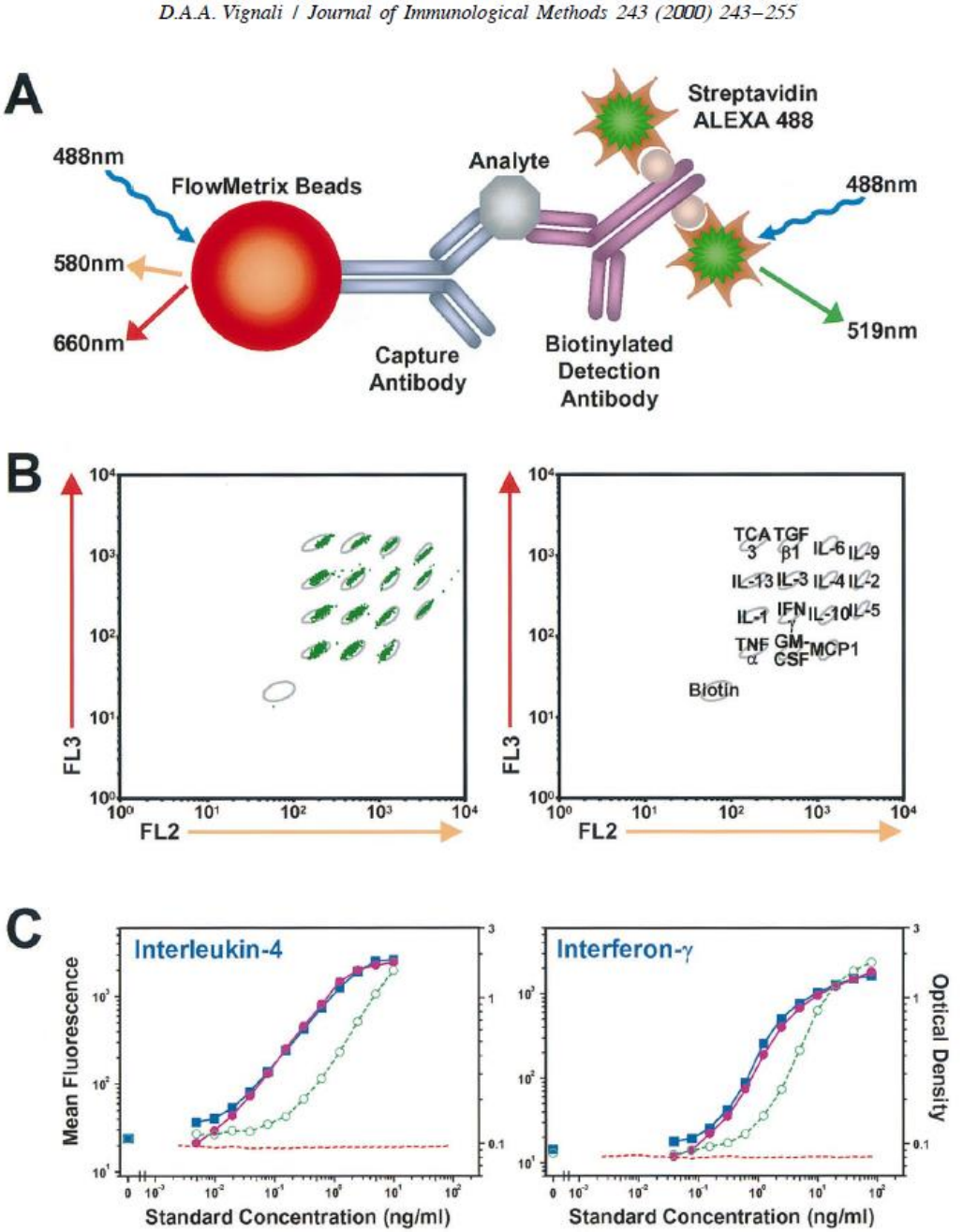


Multiplex microsphere-based flow cytometric platforms for protein analysis and their application in clinical proteomics – from assays to results



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

CBA


D.A.A. Vignali / Journal of Immunological Methods 243 (2000) 243–255
A
Streptavidin
ALEXA 488
488nm
FlowMetrix Beads
Analyte
488nm
580nm
660nm
519nm
Capture
Antibody
Biotinylated
Detection
Antibody
B
FL3
FL2
TCA 3
TGF β1
IL-6
IL-9
IL-13
IL-3
IL-4
IL-2
IL-1
IFN γ
IL-10
IL-5
TNF α
GM-CSF
MCP1
Biotin
C
Interleukin-4
Interferon-γ
Mean Fluorescence
Optical Density
Standard Concentration (ng/ml)
Standard Concentration (ng/ml)

CBA

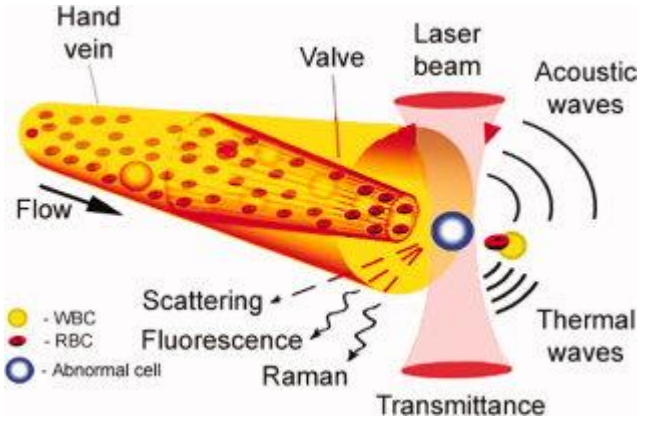
- multiplexing capabilities
- speed
- incorporation of multiple assay formats
- rapid assay development and reasonable cost
- automation

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

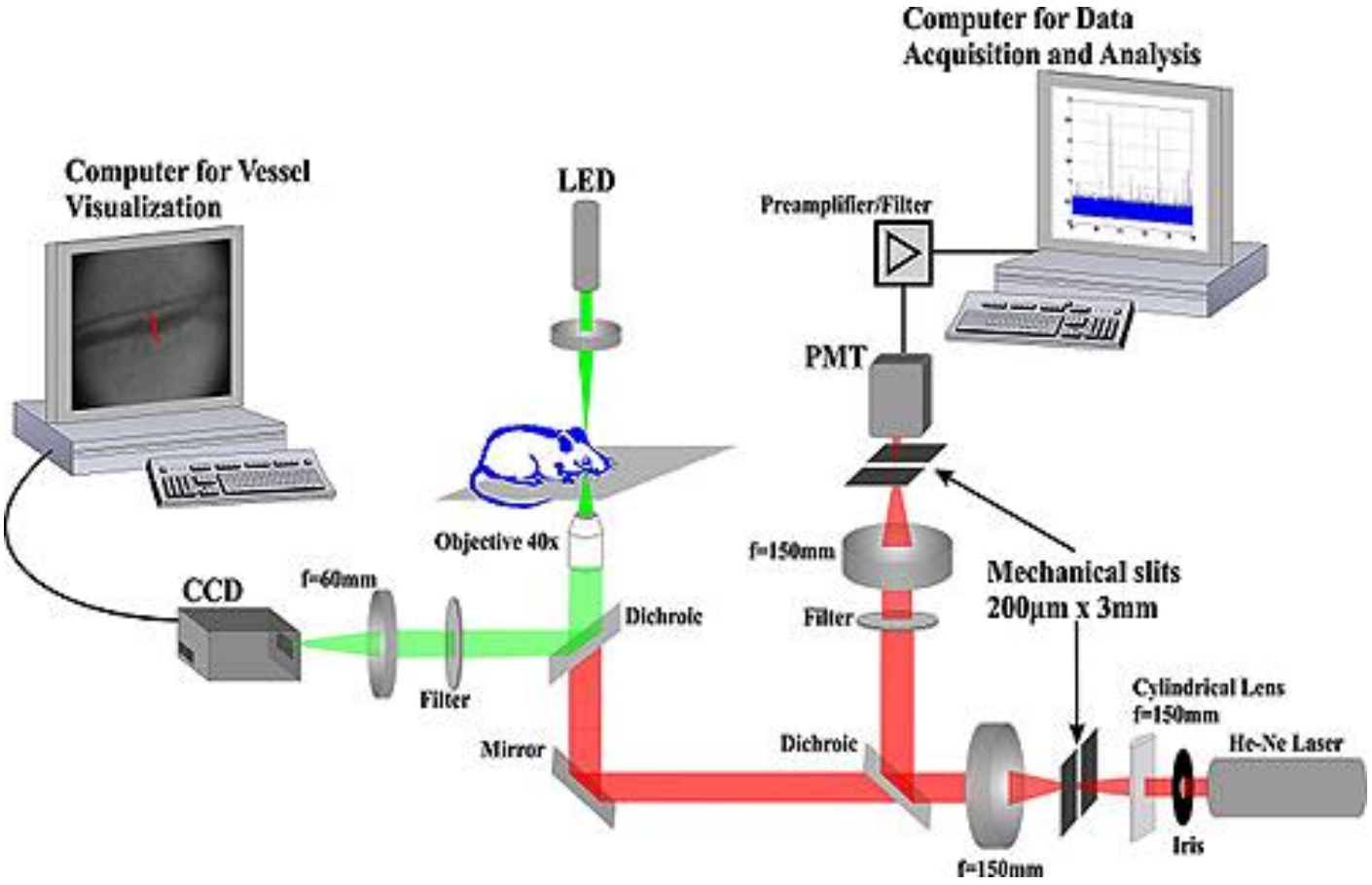
in vivo flow cytometry – základní principy



- 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

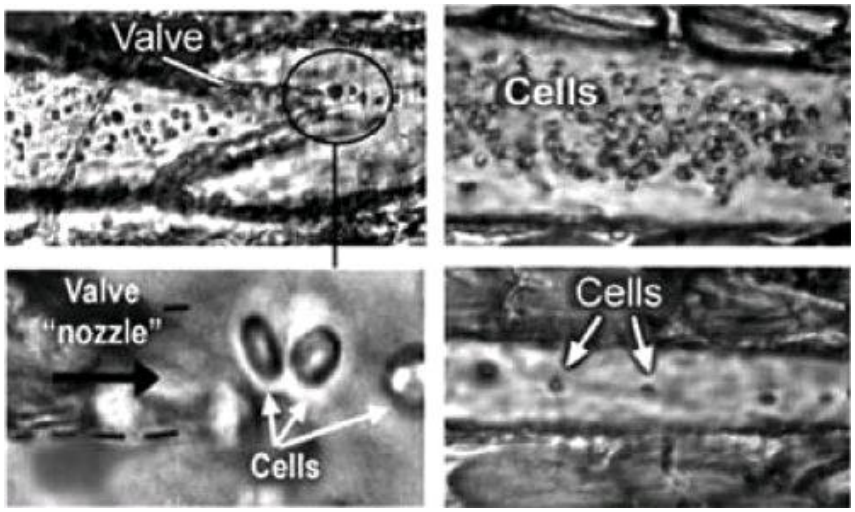
in vivo flow cytometry



Cytometry part A 79A: 737-745, 2011

in vivo flow cytometry – bez značení

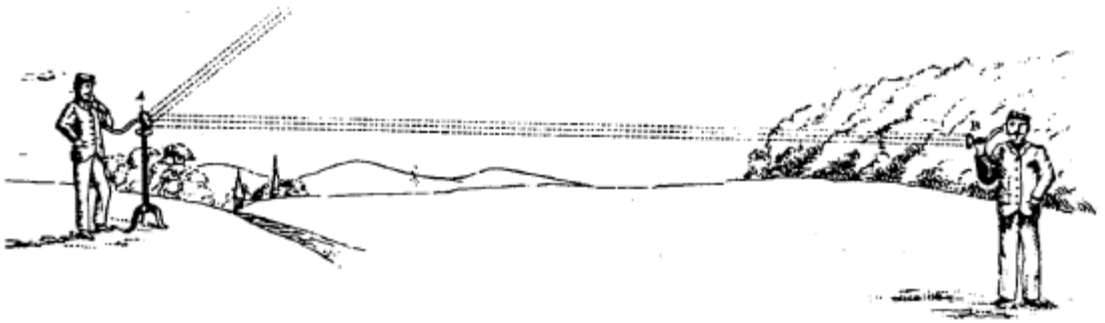
- Nahrávka videa pomocí vysokorychlostní CCD nebo CMOS kamery s vysokým rozlišením v režimu propustnosti nebo odrazu.
- Příklad: high-speed transmittance digital microscopy (TDM)
- Limity: hloubka tkáně.
- TDM může sloužit k navedení zdrojů záření pro další analýzu do určené oblasti.



Cytometry part A 79A: 737-745, 2011

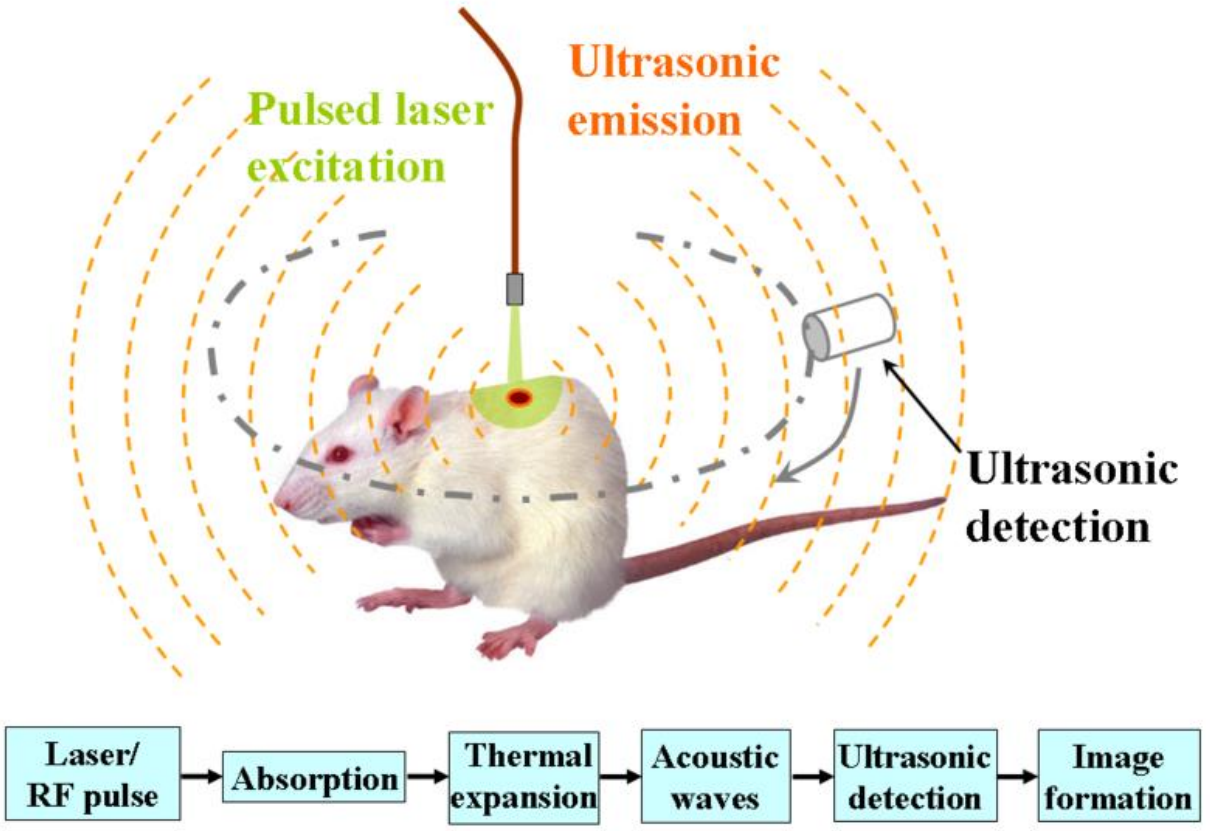
photoacoustic and photothermal imaging

- 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

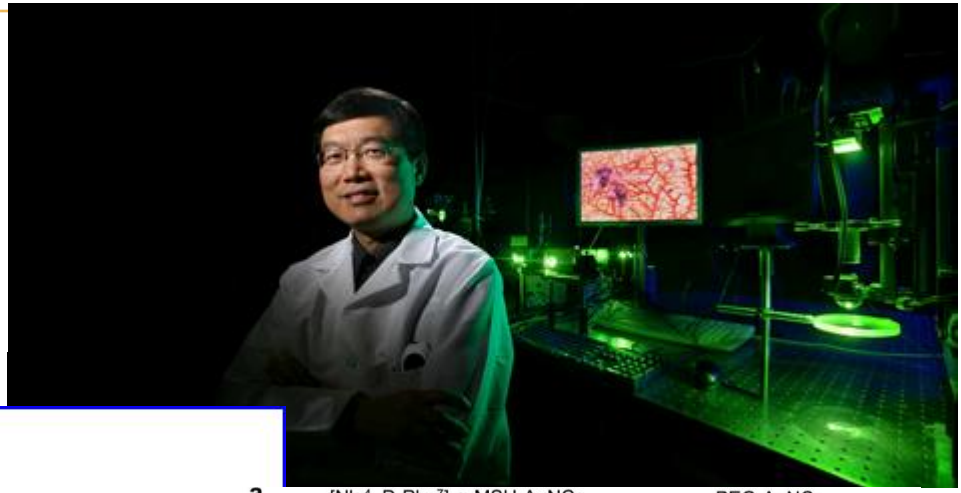


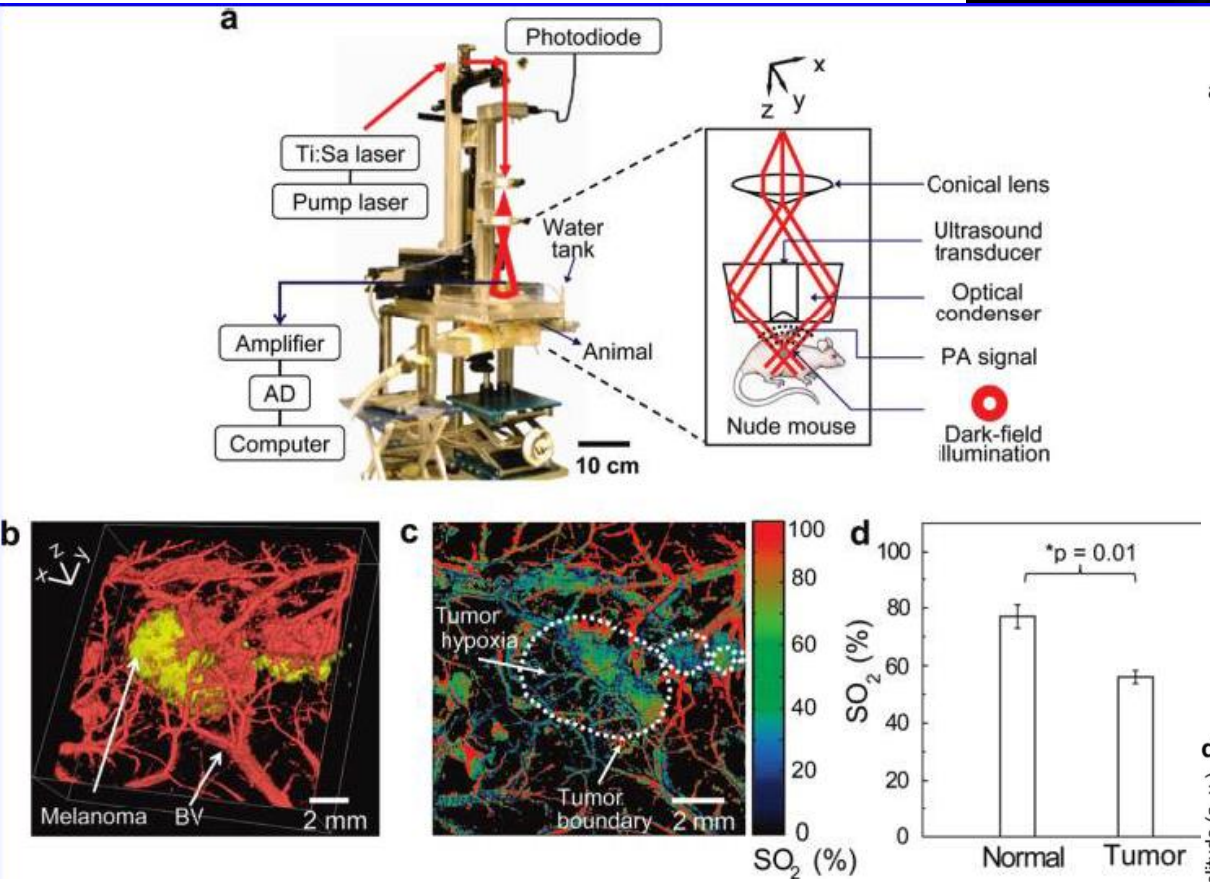
Cytometry part A 79A: 737-745, 2011

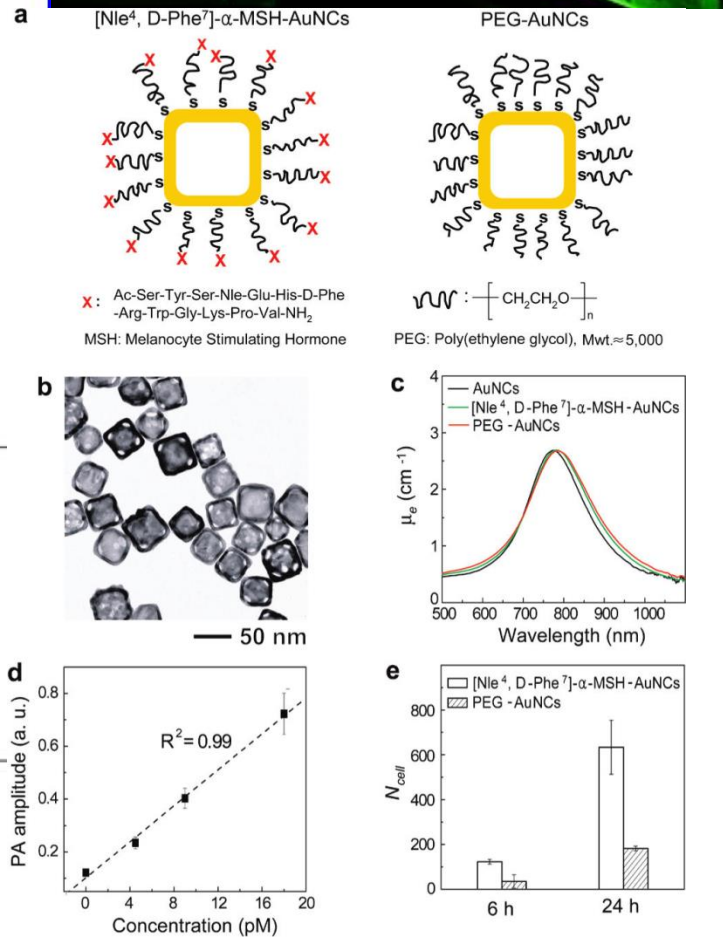
In Vivo Molecular Photoacoustic Tomography of Melanomas Targeted by Bioconjugated Gold Nanocages

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.



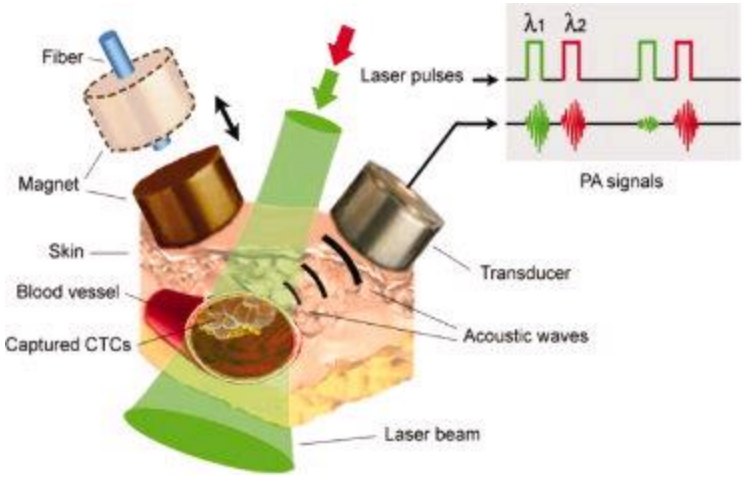


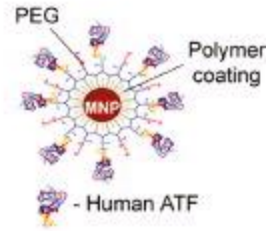


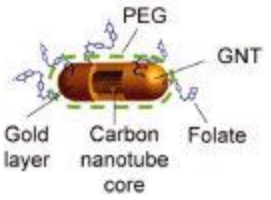
ACS Nano **2010** *4* (8), 4559-4564

In vivo flow cytometrie – detekce specifických signálů

- Detekce fotoakustických a fototermálních jevů

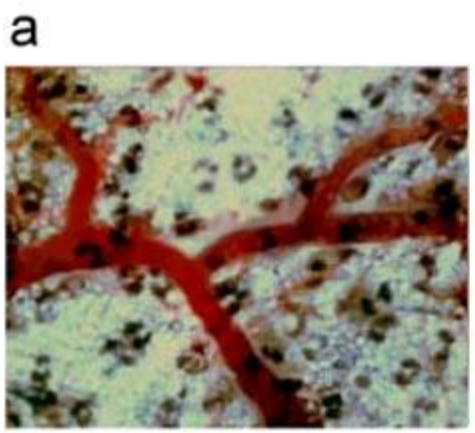


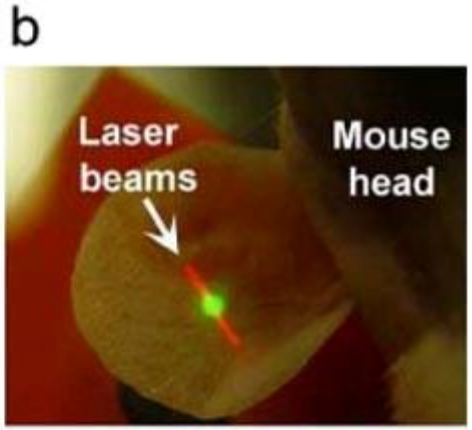


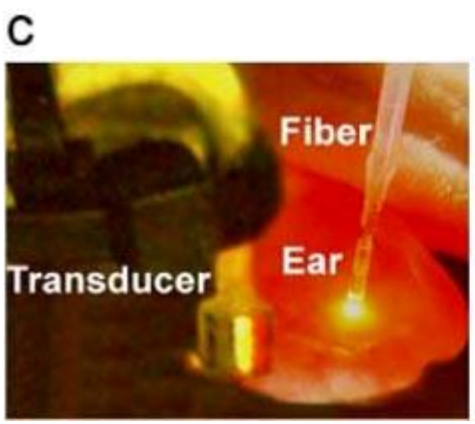


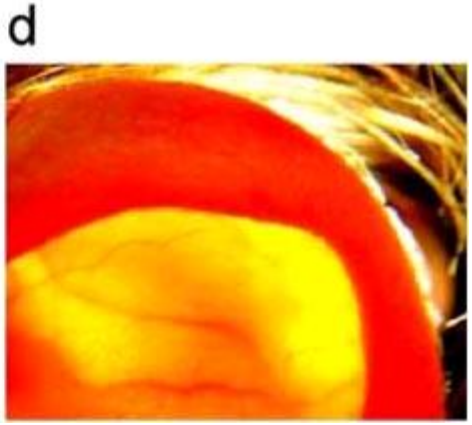
Cytometry part A 79A: 737-745, 2011

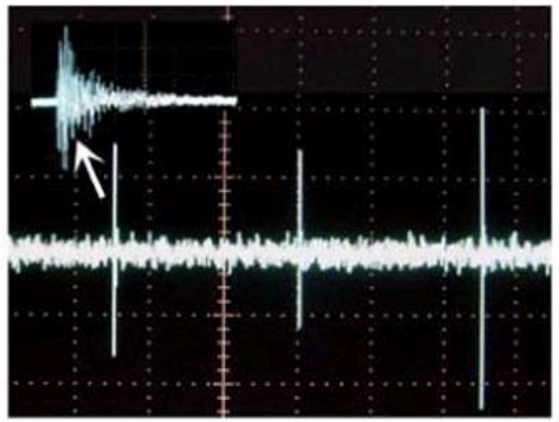
in vivo flow cytometry - aplikace

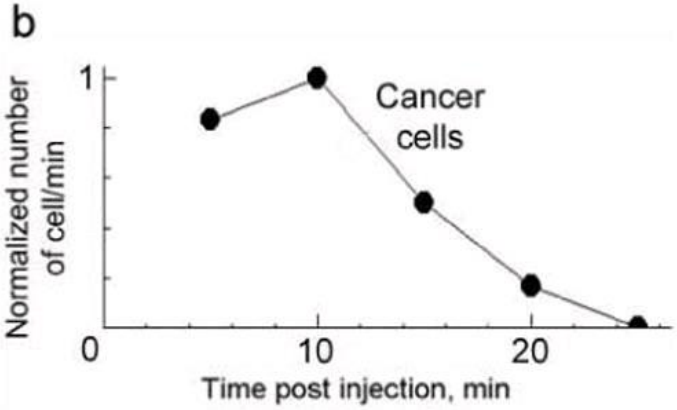












Shrnutí přednášky

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