Bi9393 Analytická cytometrie
Lekce 5

Karel Souček, Ph.D.

Oddělení cytokinetiky
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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**.

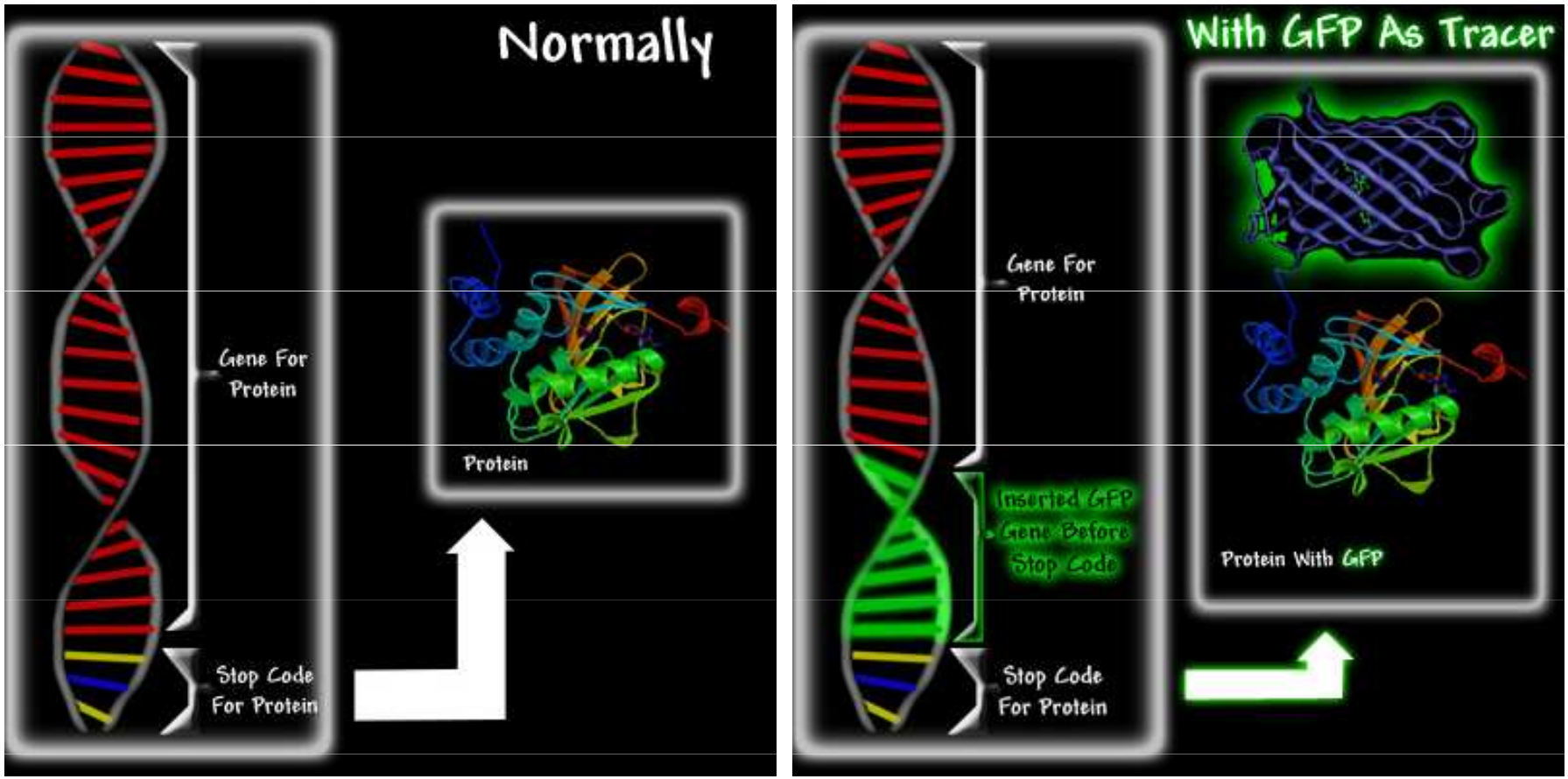
Aequorea victoria "Crystal jelly "

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



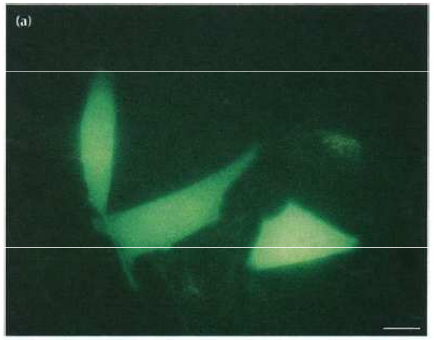
http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP2.htm

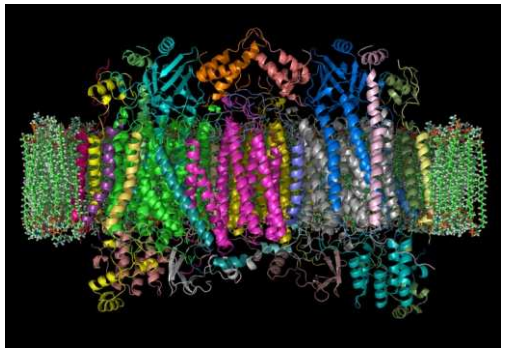
Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells

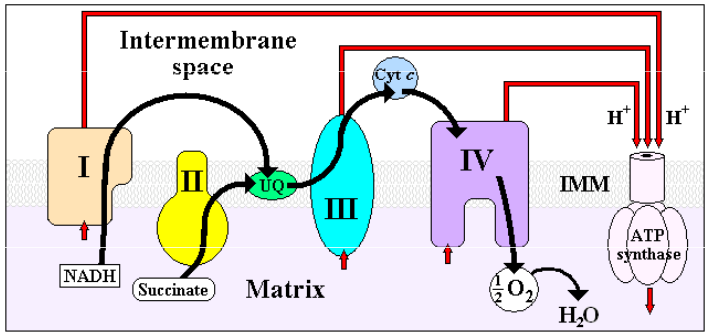
Rosario Rizzuto, Marisa Brini, Paola Pizzo, Marta Murgia and Tullio Pozzan

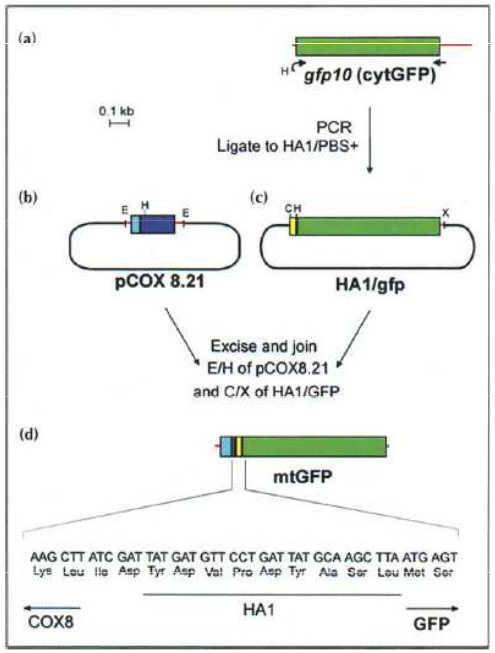
Department of Biomedical Sciences and CNR Center for the Study of Mitochondrial Physiology, University of Padova, Via Trieste 75, 35121 Padova, Italy.

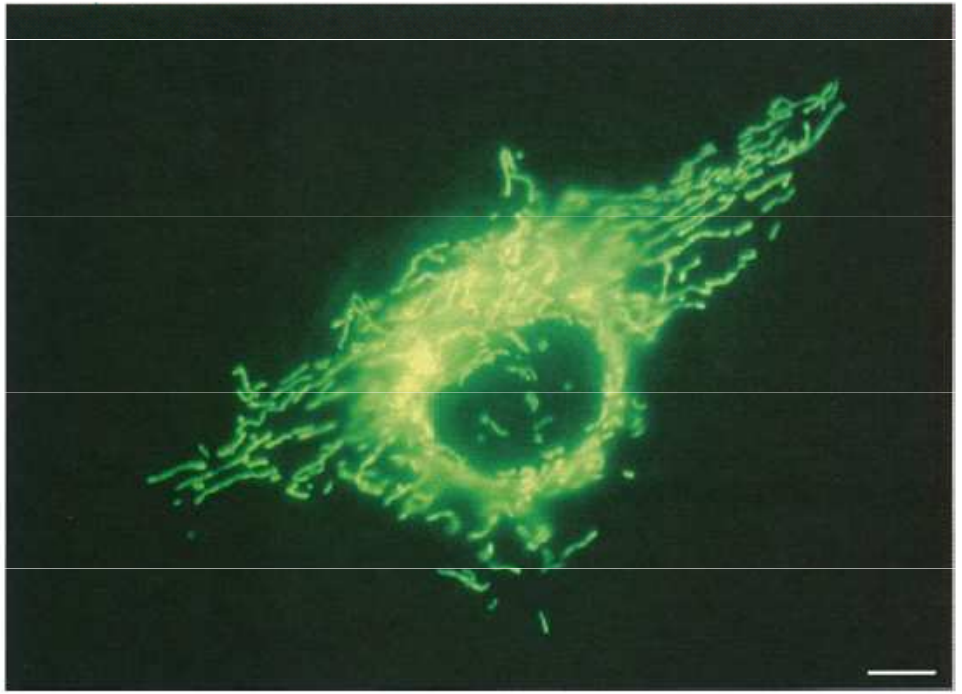
Current Biology 1995, 5:635–642

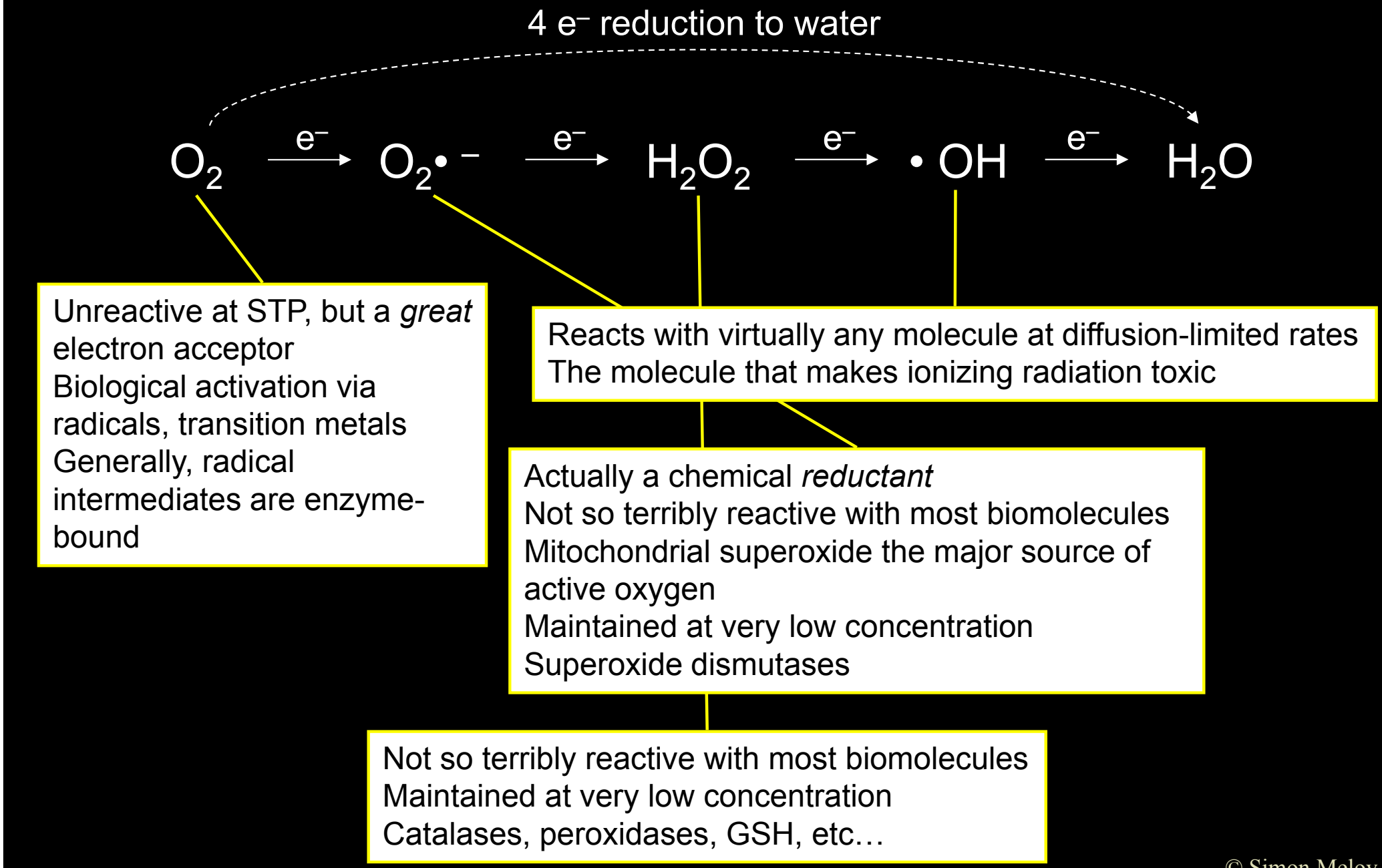






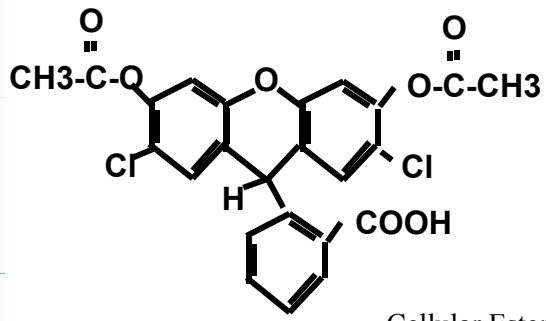





4 e⁻ reduction to water
O_2
e⁻
$O_2\bullet^-$
e⁻
H_2O_2
e⁻
• OH
e⁻
H_2O
Unreactive at STP, but a *great* electron acceptor
Biological activation via radicals, transition metals
Generally, radical intermediates are enzyme-bound
Reacts with virtually any molecule at diffusion-limited rates
The molecule 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…

DCFH-DA → DCFH → DCF

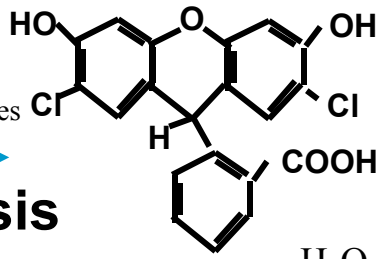
2',7'-dichlorofluorescin diacetate



Cellular Esterases

Hydrolysis

2',7'-dichlorofluorescin

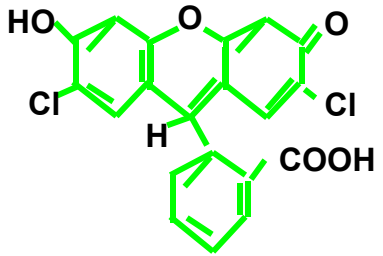


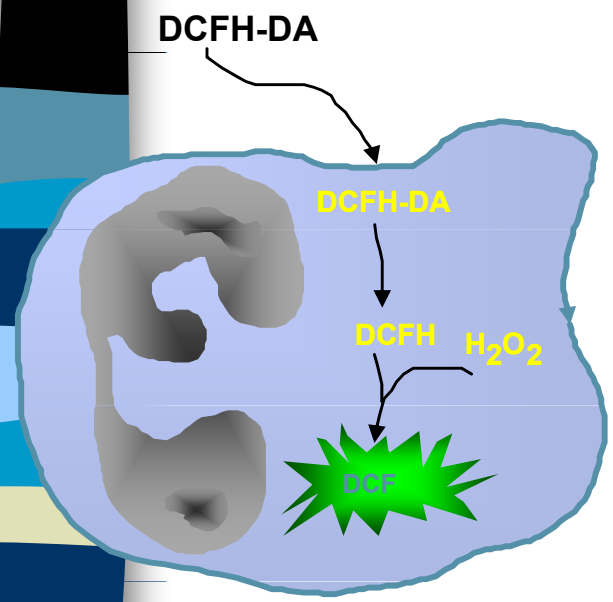
H_2O_2

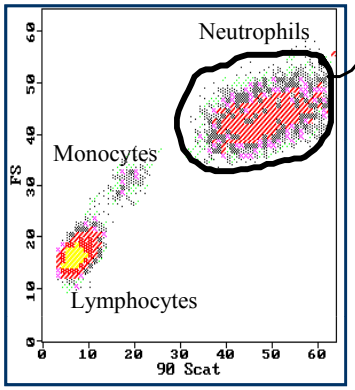
Oxidation

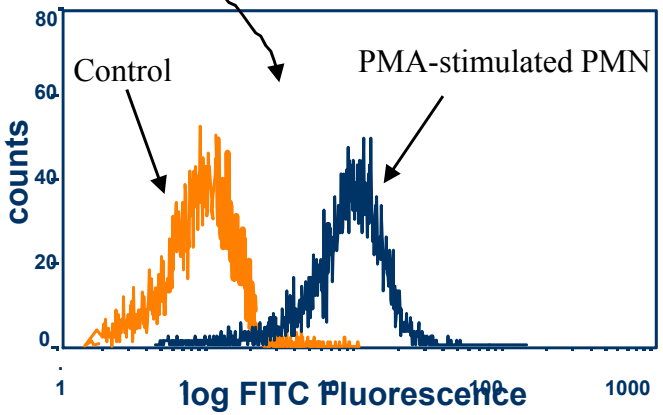
Fluorescent

2',7'-dichlorofluorescein

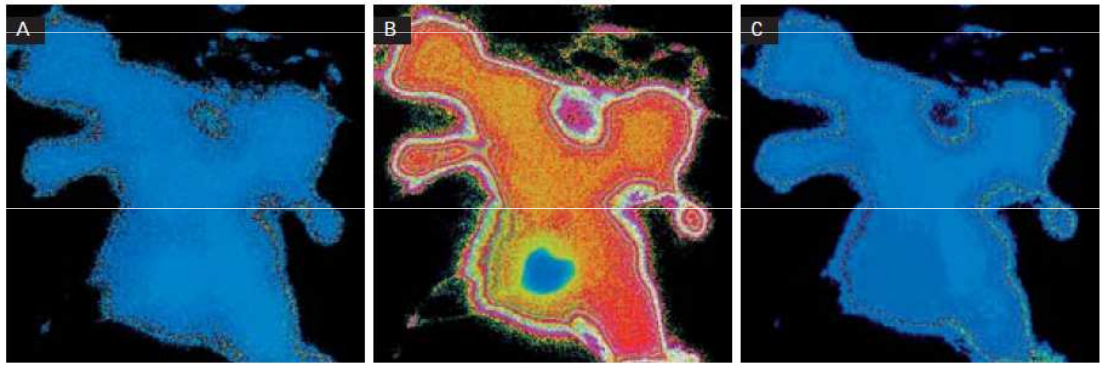






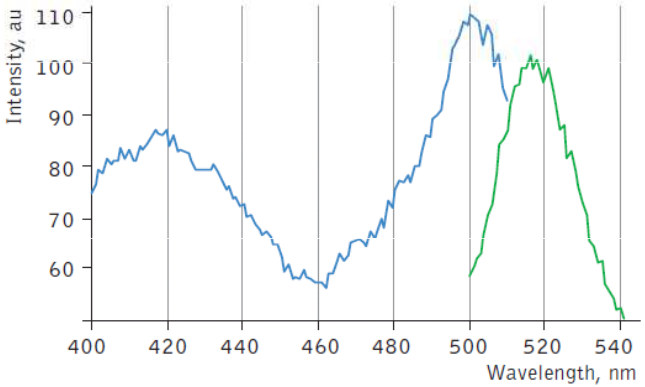


Fluorescent sensors for detection of H_2O_2

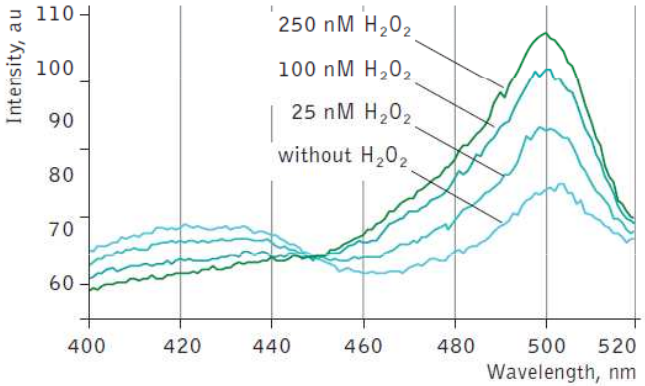


Ratiometric images of the group of HeLa cells before (A), 20 sec after (B), and 600 sec after (C) addition of 180 ul of H_2O_2. Images were pseudocolored using "ratio" lookup table of NIH ImageJ software: blue-green-red-white colors represent lowest-intermediate-high-highest level of H_2O_2.

evrogen



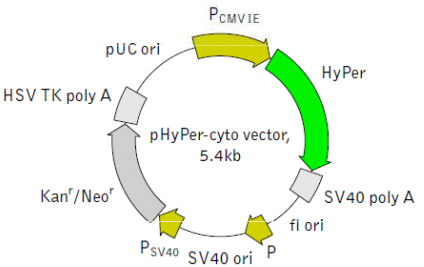
HyPer excitation (blue line) and emission (green line) spectra.

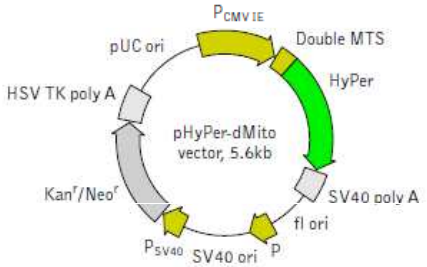


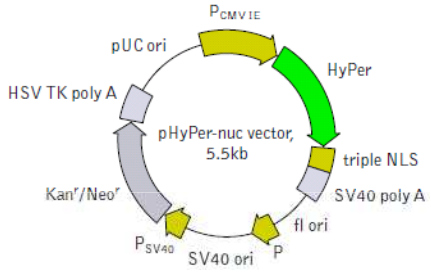
Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition. Emission was measured at 530 nm.

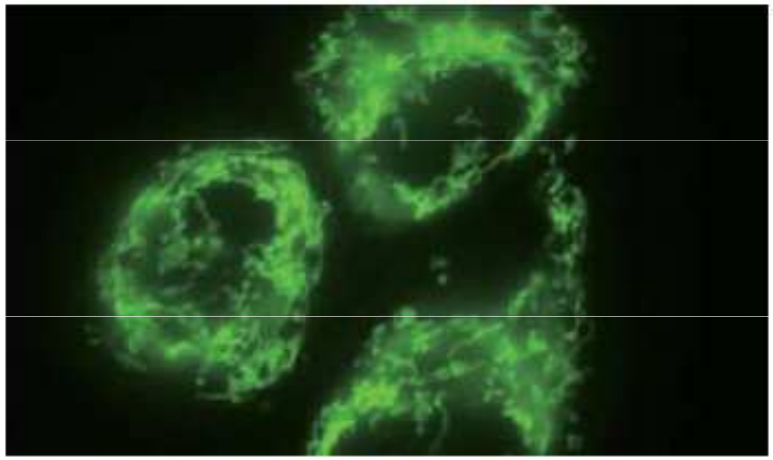
Variants & fusions

- **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]**



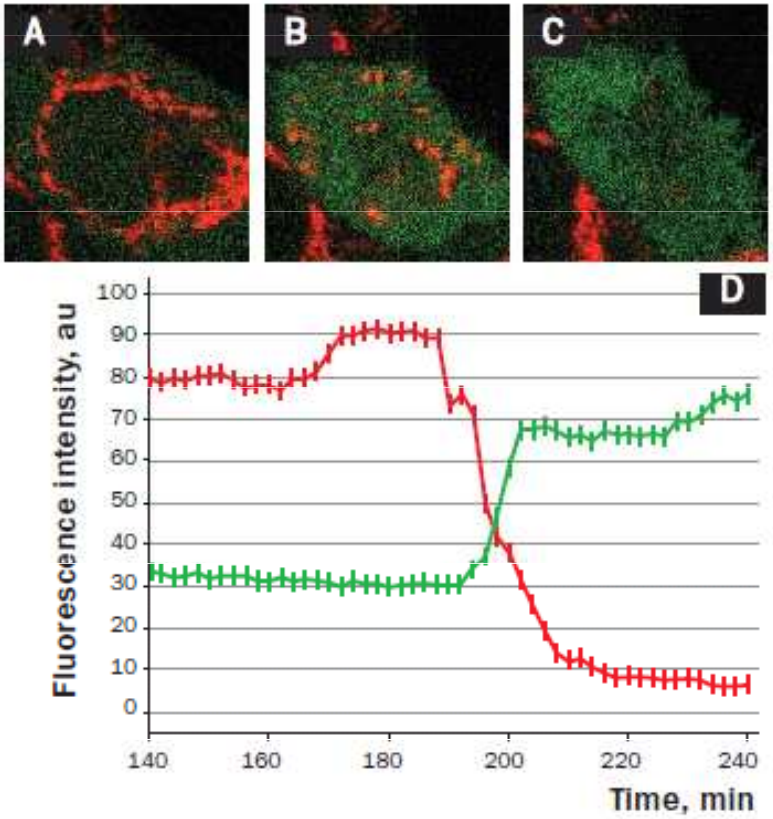






Stably transfected HeLa cells expressing mitochondria-targeted HyPer.

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

„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

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)

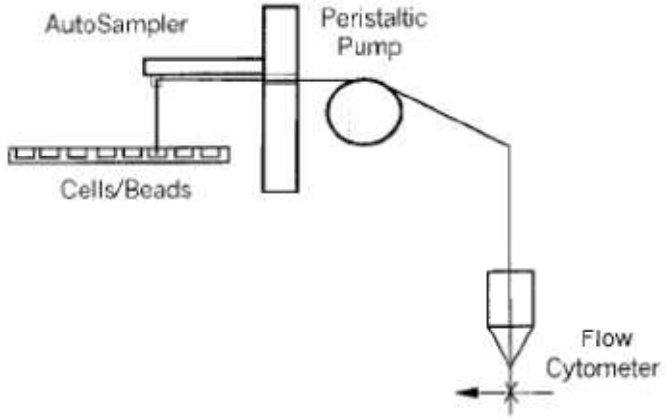
High Throughput Flow Cytometry

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

A



B

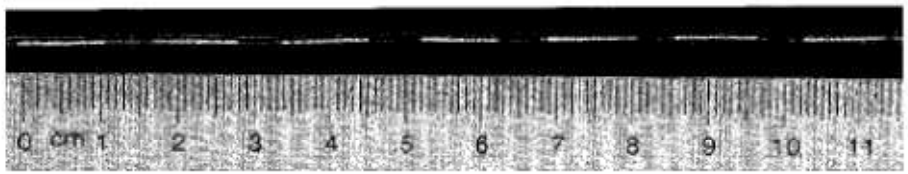
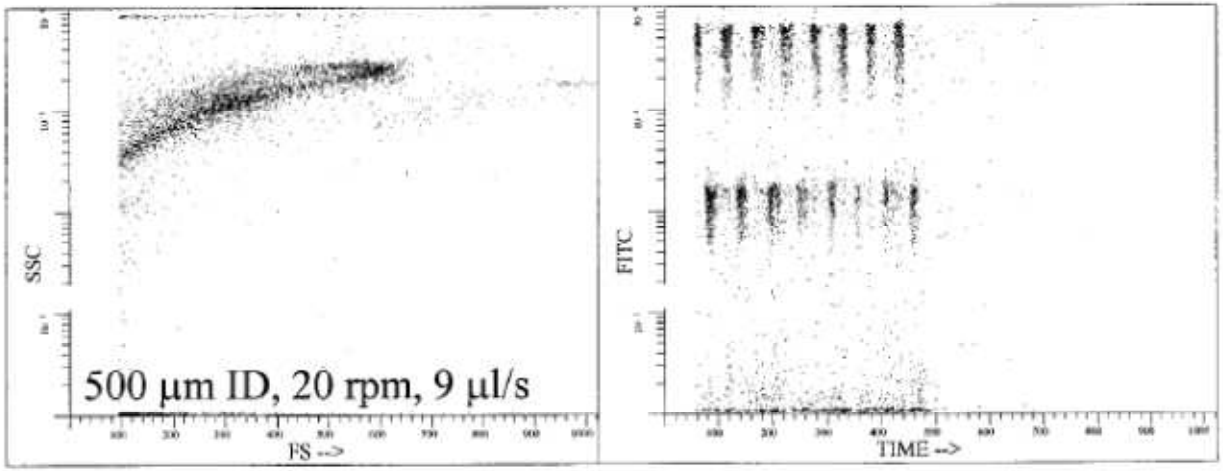
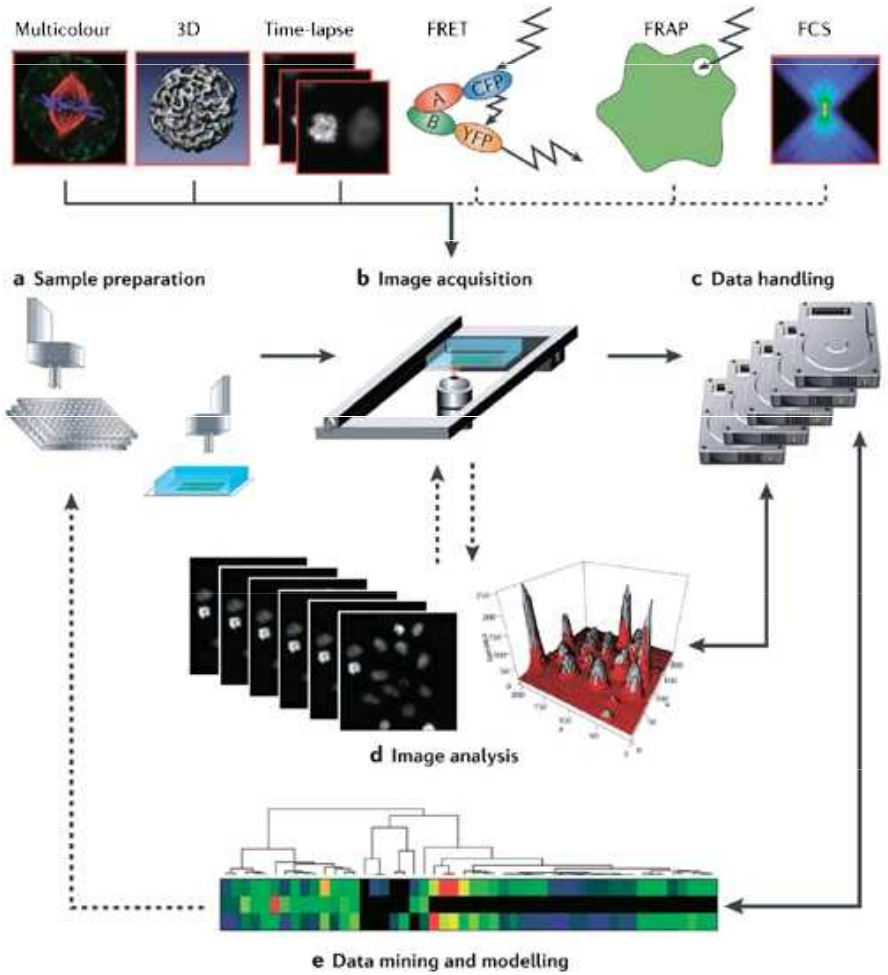


FIG. 1. High throughput flow cytometry. A: Schematic view of the flow cytometer, autosampler, and peristaltic pump. B: Adjacent samples of latex microspheres separated by air in the 0.02-in (254-μm) ID tubing between the peristaltic pump and the flow cytometer.

C



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



Analysis

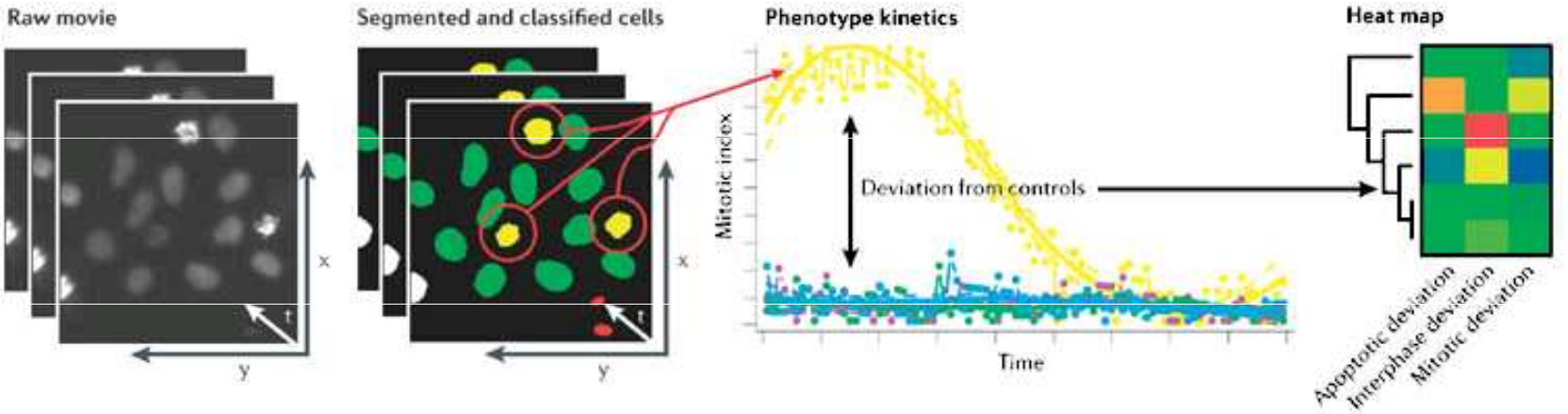


Table 1. Comparison of the Key Attributes of High-Throughput Flow Cytometry and High-Content Microscopy

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

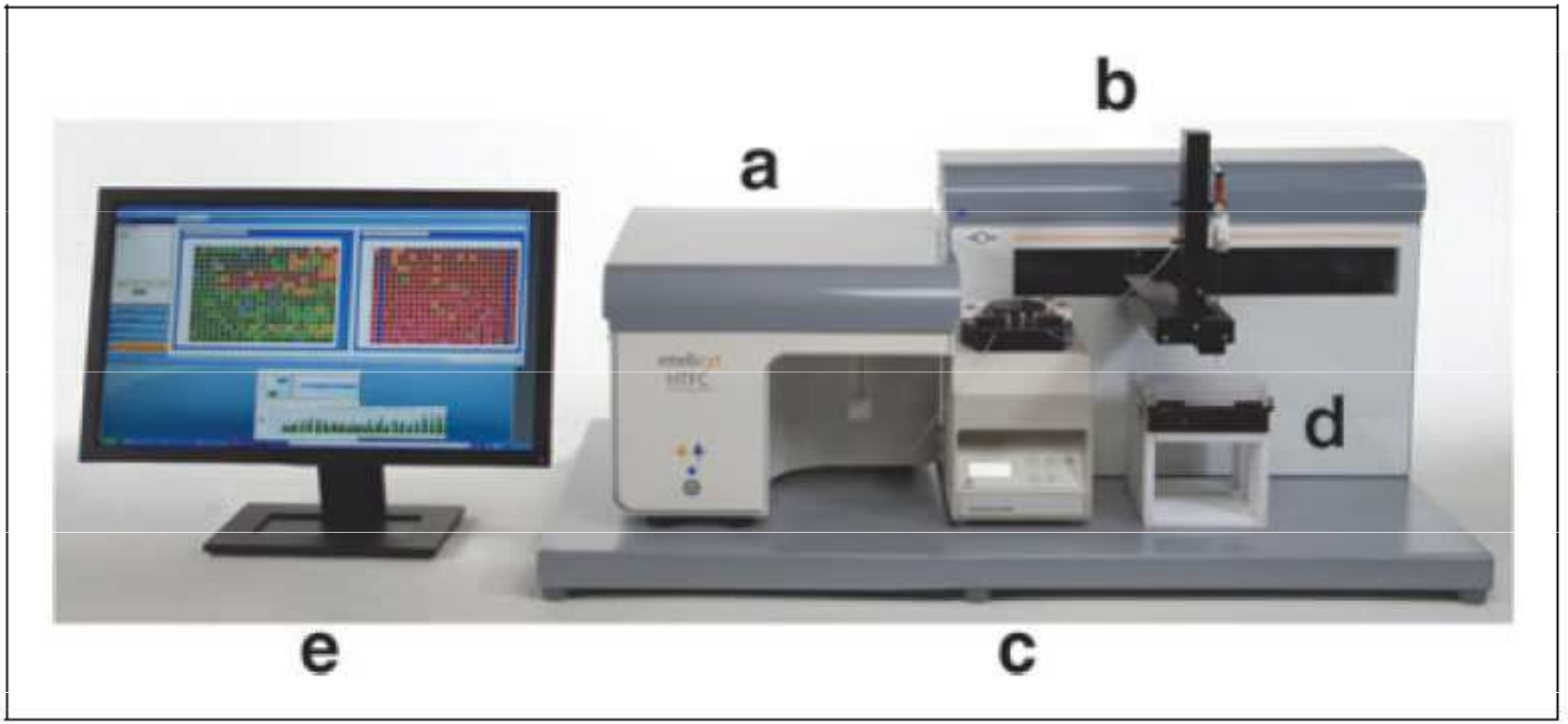


Fig. 1. The HTFC Screening System (IntelliCyt Corporation). **(a)** 2-laser, 4-color flow cytometer; **(b)** an x, y, z autosampler; **(c)** a low pulsation peristaltic pump; **(d)** orbital plate shaker that accommodates 96- and 384-well plates; **(e)** system computer with HyperView installed to set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

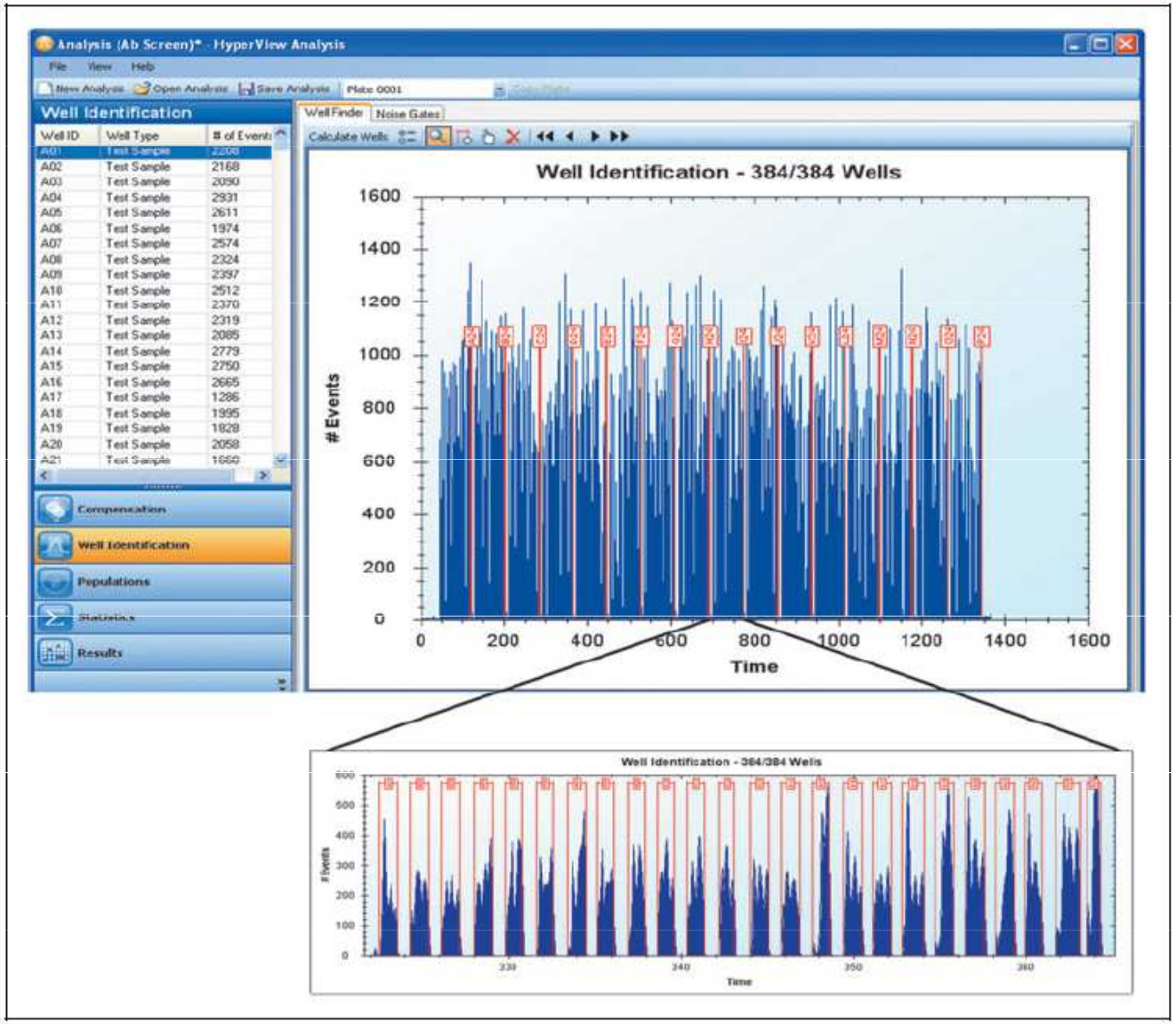
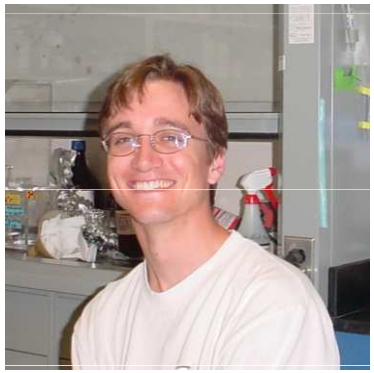
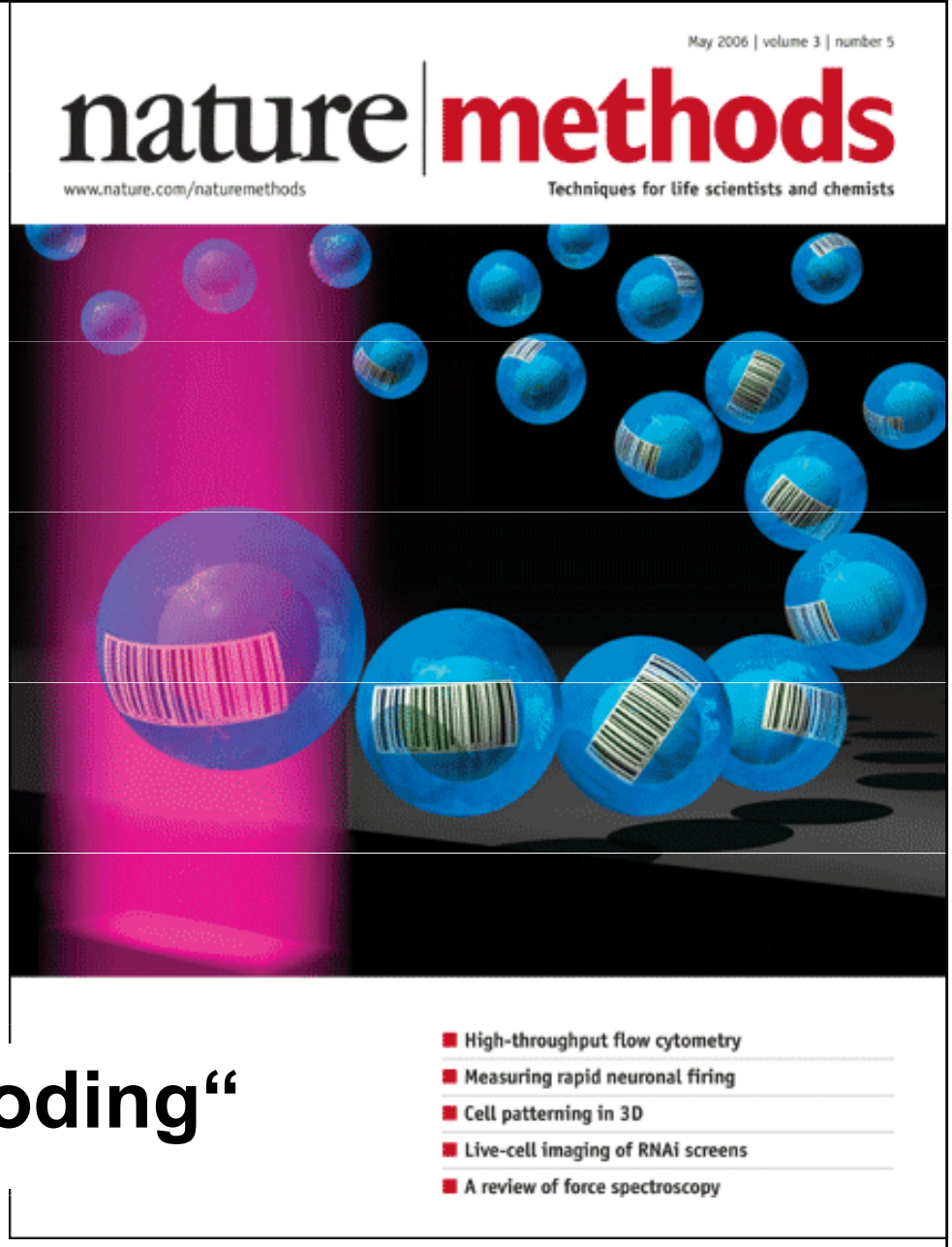


Fig. 4. Screenshot from HyperView showing an example of the Well Identification process. Data from the 384-well plate is collected in to a single flow cytometry standard file, which is shown in the main window. The data are deconvolved by the software algorithm to identify each peak with a well address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



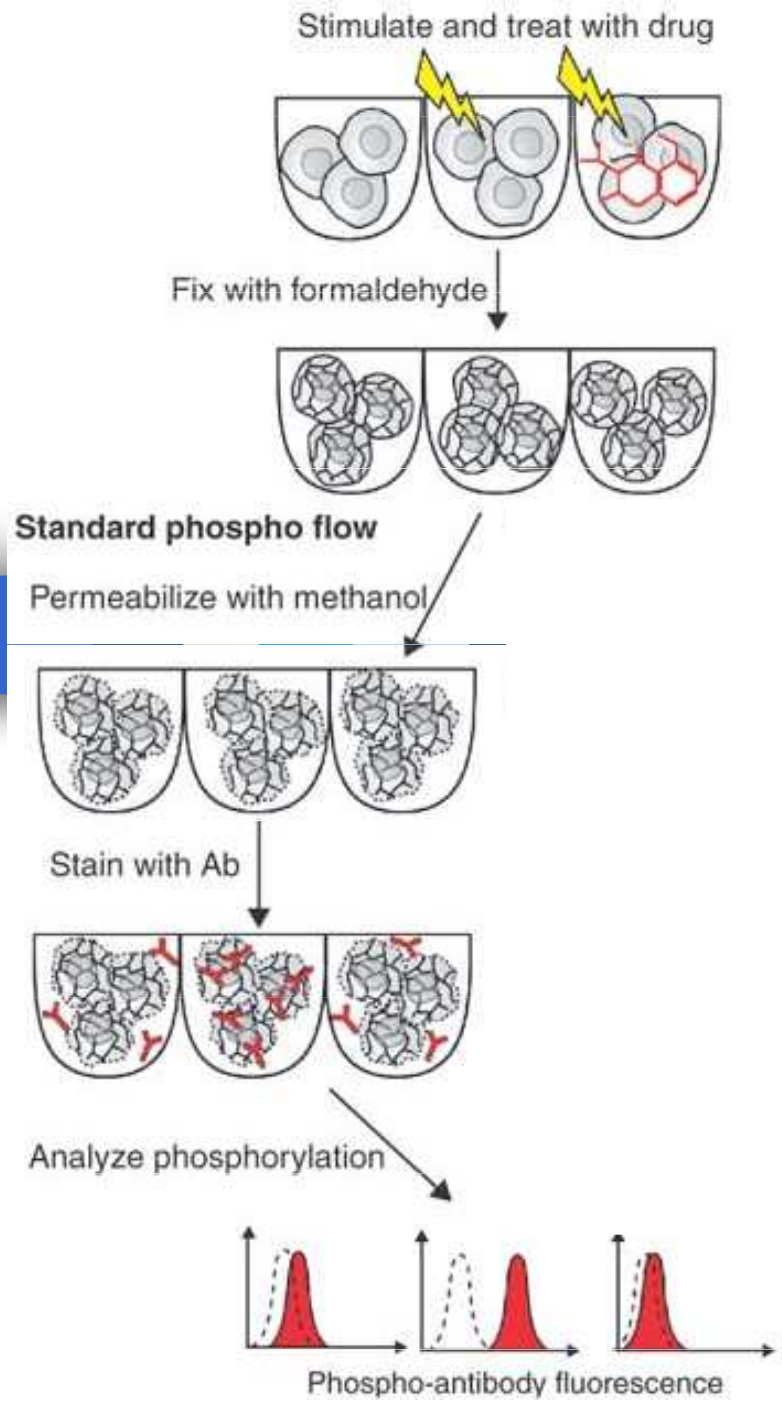
Garry Nolan

Peter Krutzik

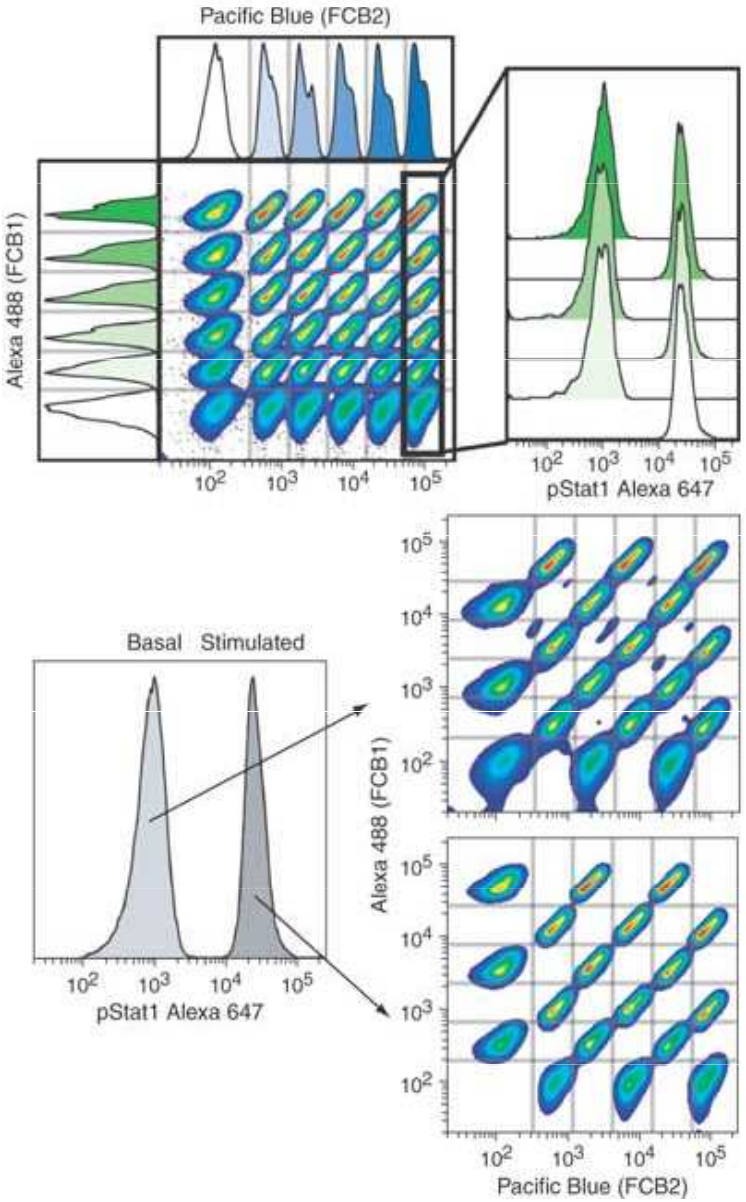


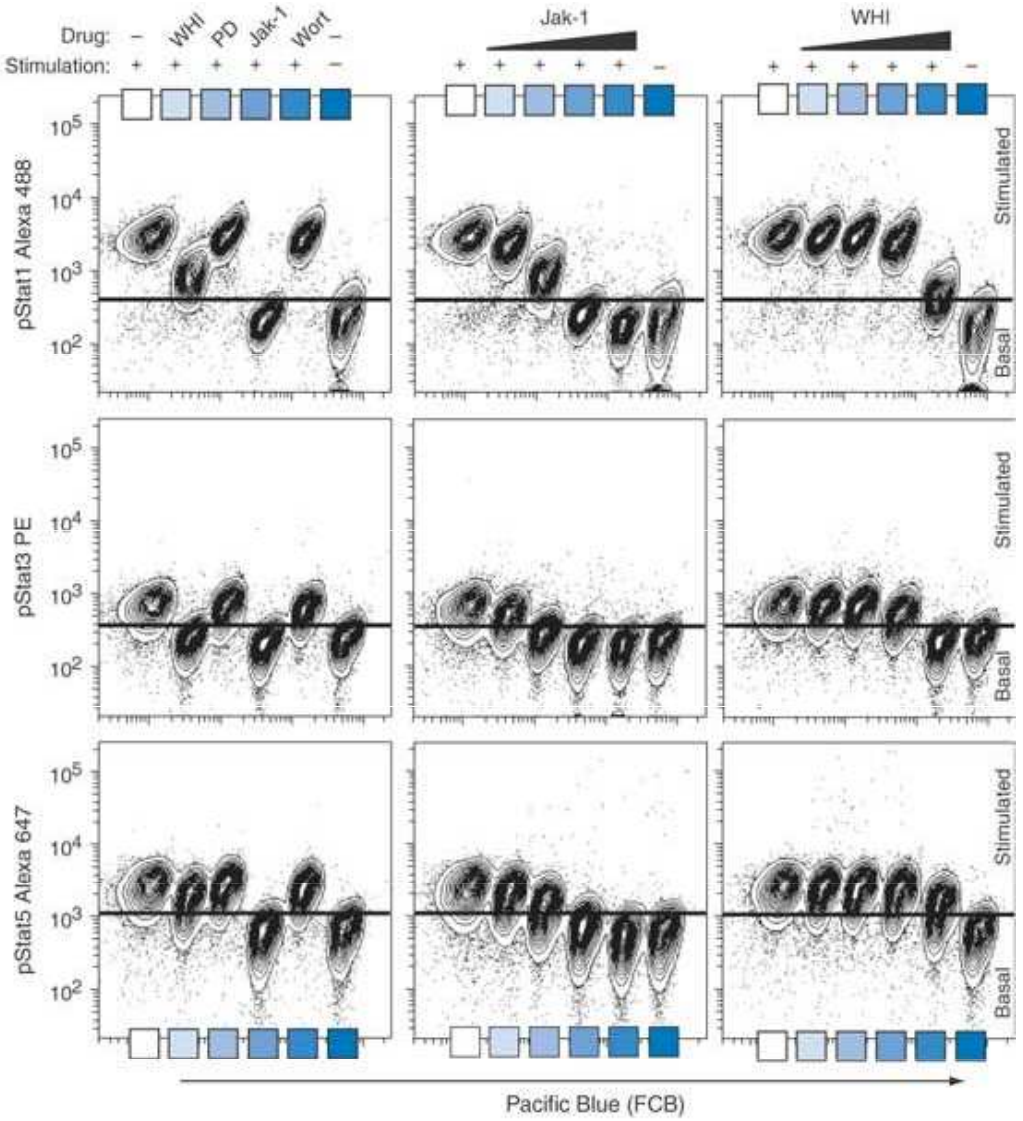
„Fluorescent cell barcoding“

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.

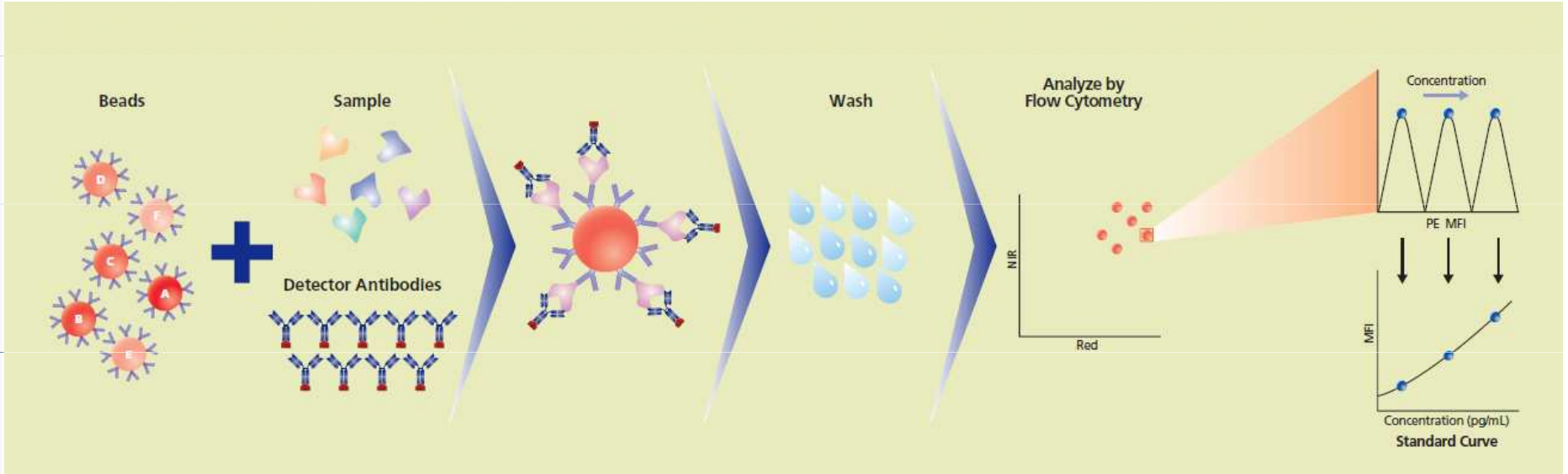




Krutzik PO, Nolan Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling.
Nat Methods. 2006 May;3(5):361-8.

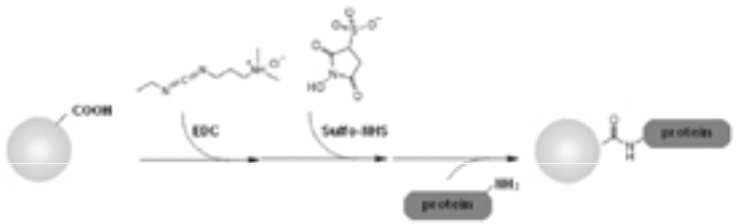
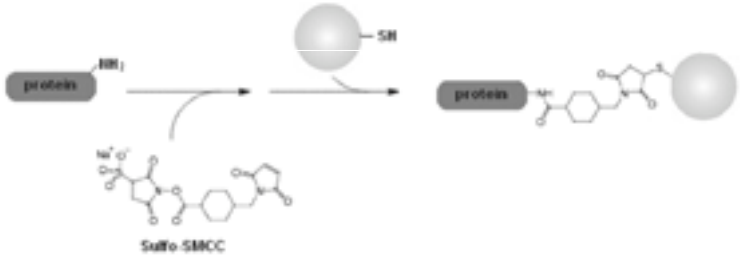
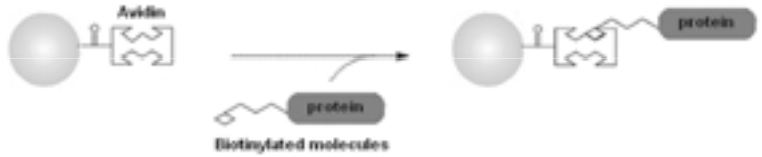
Cytometric bead array (CBA)

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

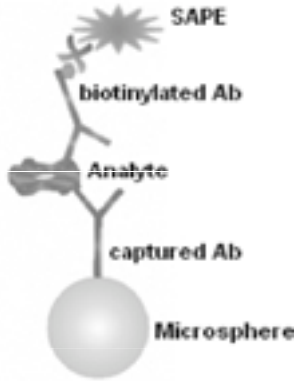


Multiplex microsphere-based flow cytometric platforms for protein analysis and their application in clinical proteomics – from assays to results

A

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

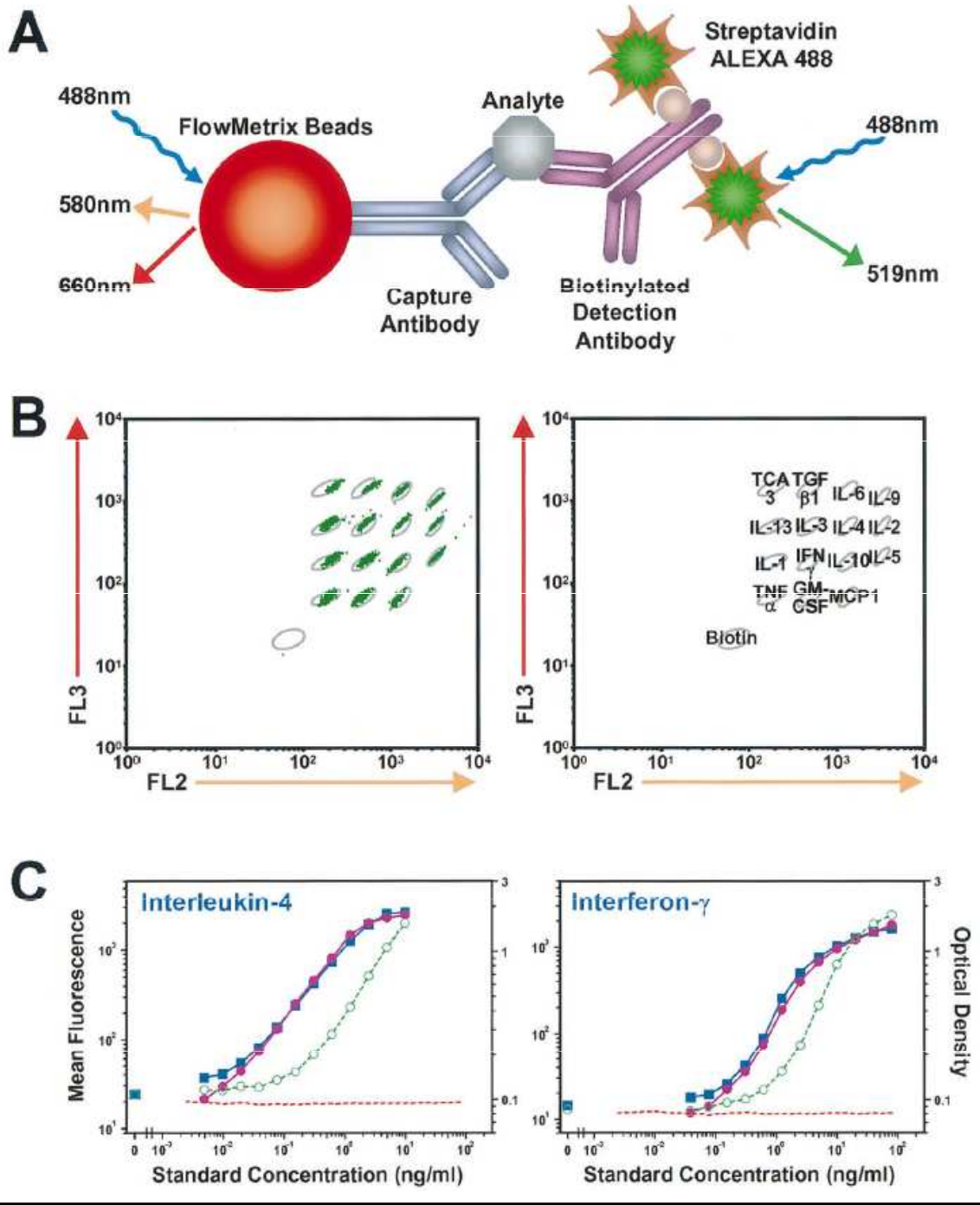
B



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

CBA

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

Biologické aplikace průtokové cytometrie

- Cytogenetika
  - analýza chromozómů
    - karyotyp
    - sortrování
      - chromozómové DNA knihovny
      - FISH značení (chromosome painting)

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

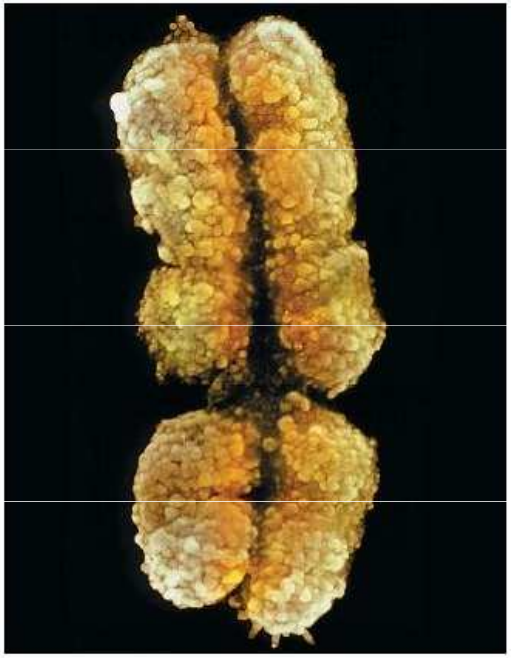
Biomedical Sciences Division, L-452, Lawrence Livermore Laboratory, Livermore, California 94550

Communicated by Donald A. Glaser, December 18, 1978

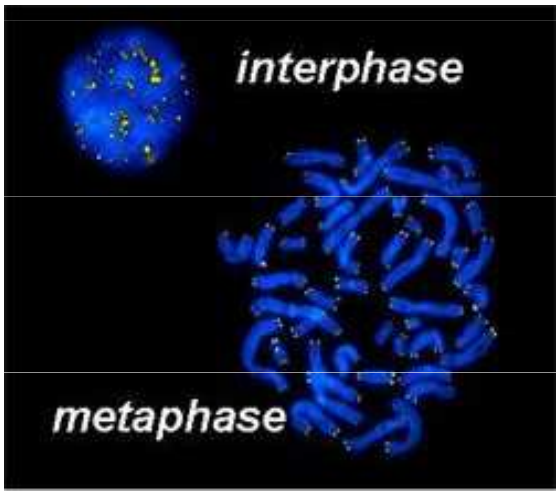
Analýza a sortrování chromozómů

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

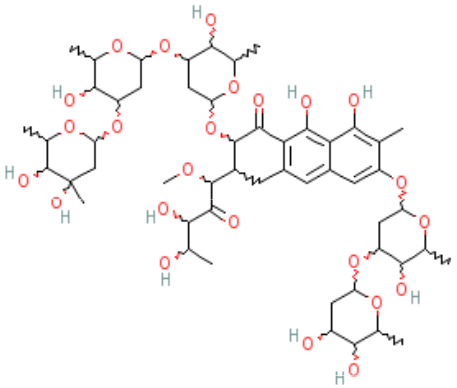
= celková DNA vs. G/C-bohaté oblasti



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

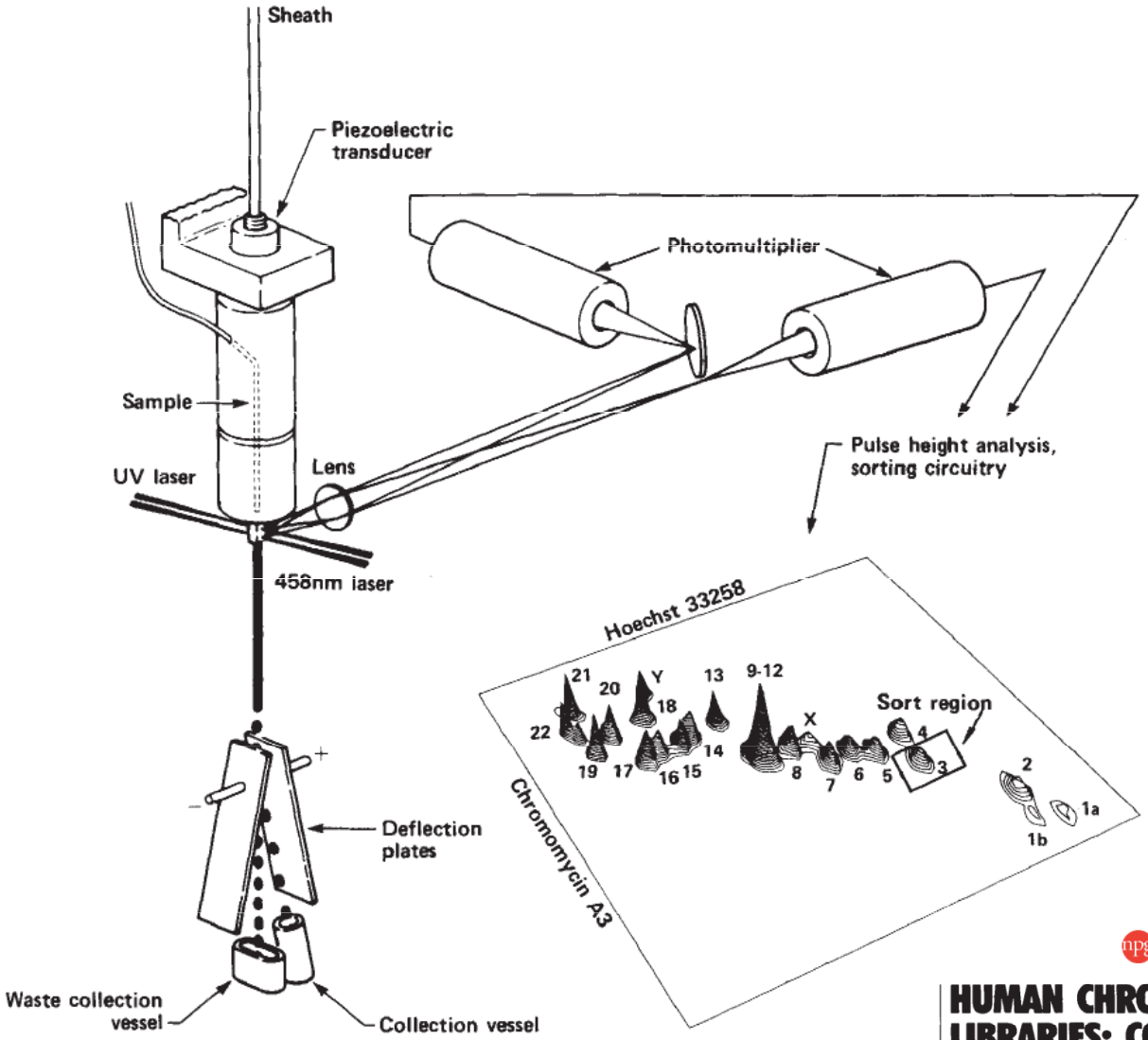


http://www.nccr-oncology.ch/scripts/page9243.html



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

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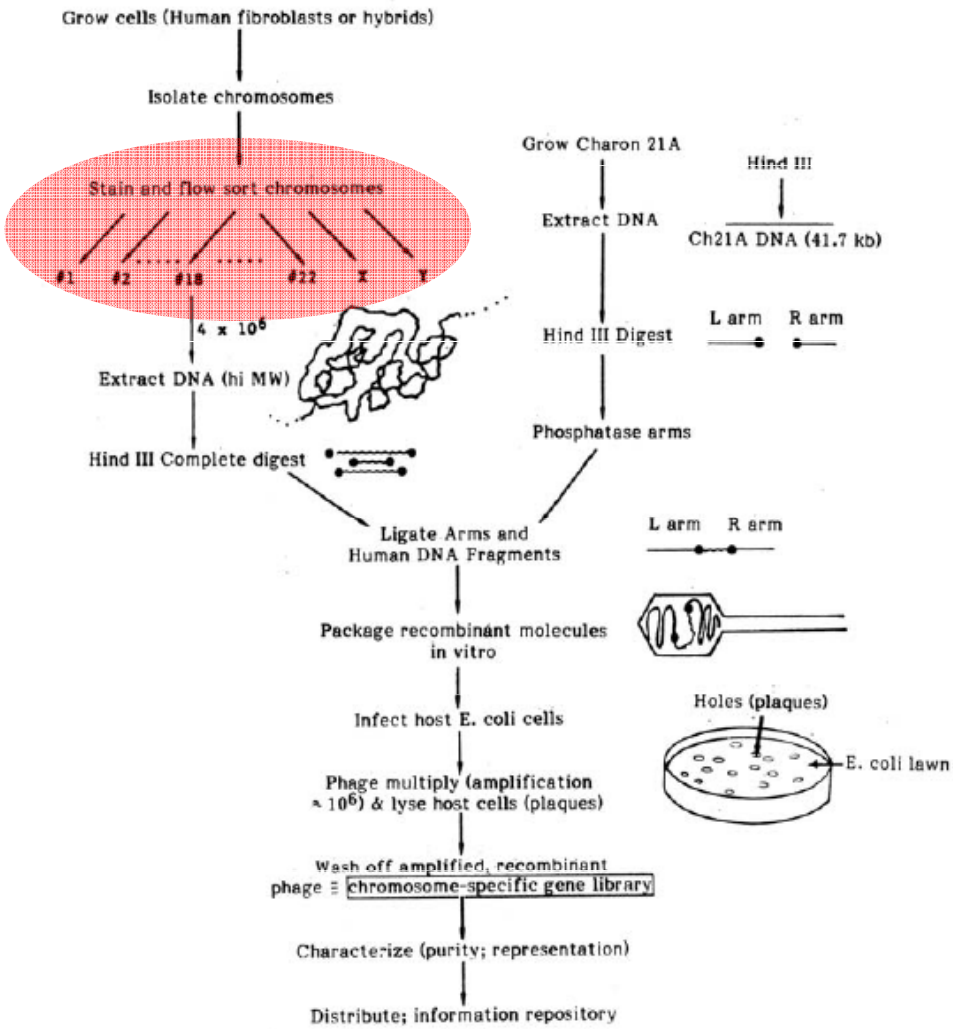
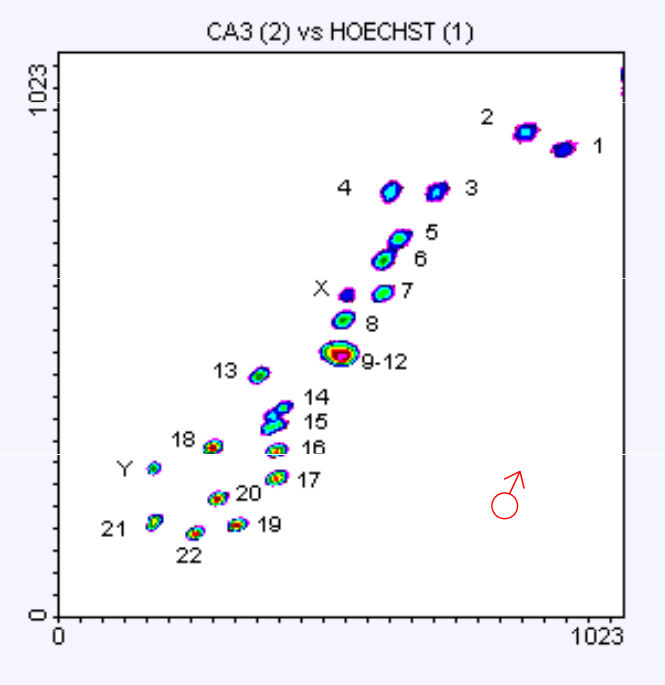
National Laboratory Gene Library Project, * Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, California 94550, † Los Alamos National Laboratory, Life Sciences Division, University of California, Los Alamos, New Mexico 87545,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

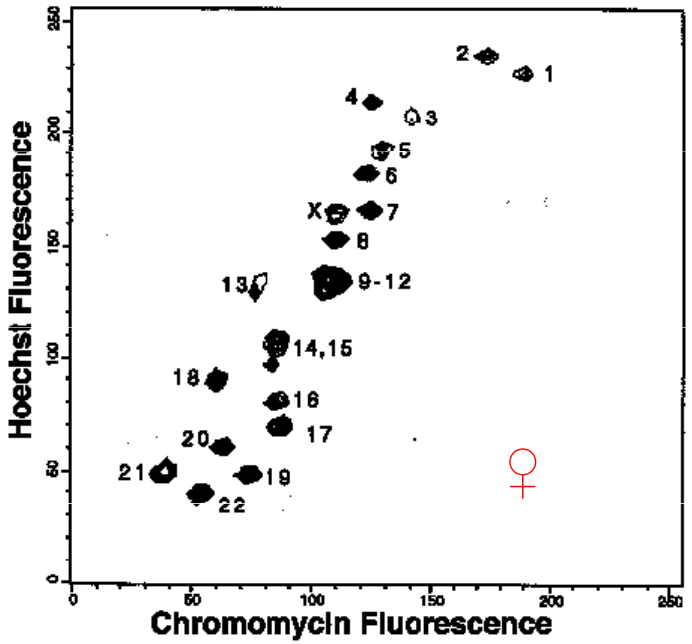
CONSTRUCTION OF A PHASE I CHROMOSOME-SPECIFIC (#18) HUMAN GENE LIBRARY IN CHARON 21A USING HIND III (LLNL)

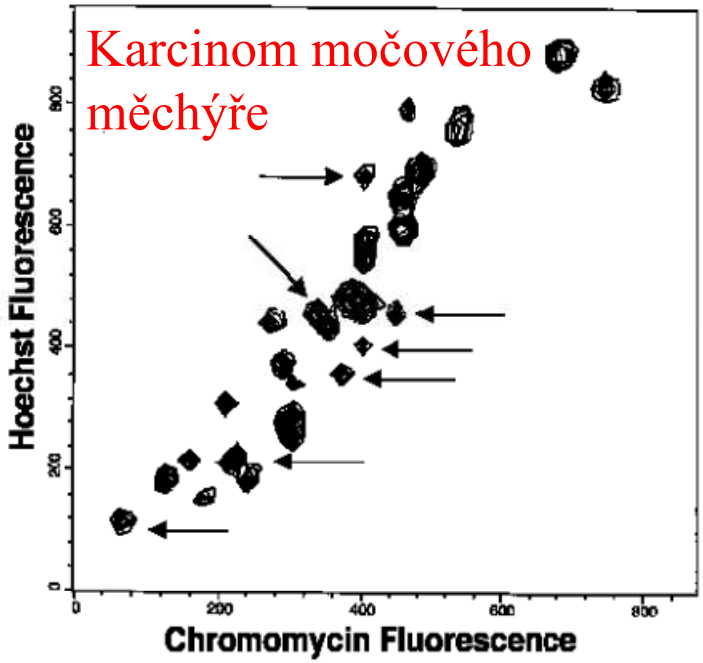


„Flow karyotype“



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





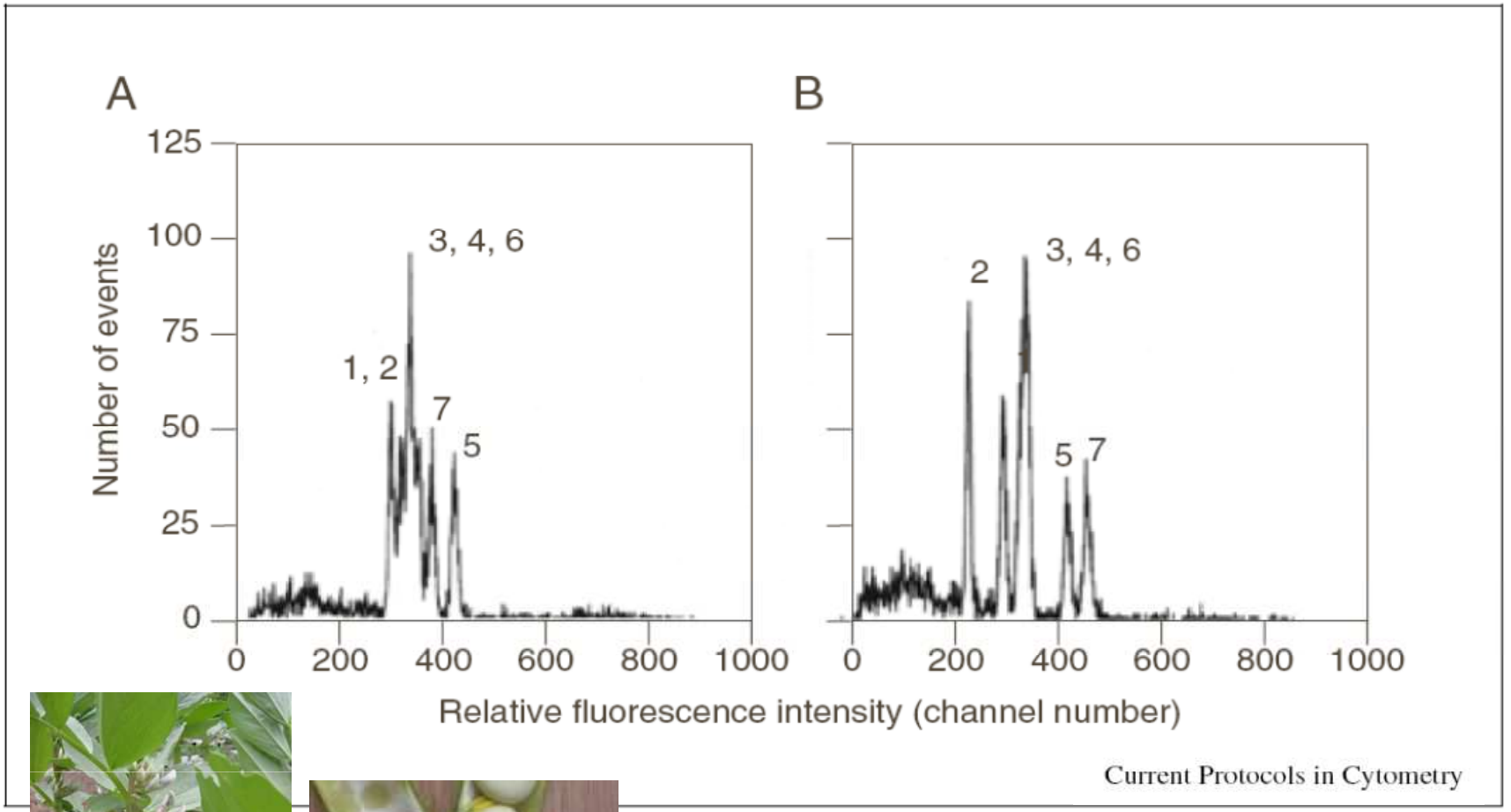
The Preparation of Human Chromosomes for Flow Cytometry

DEREK DAVIES

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

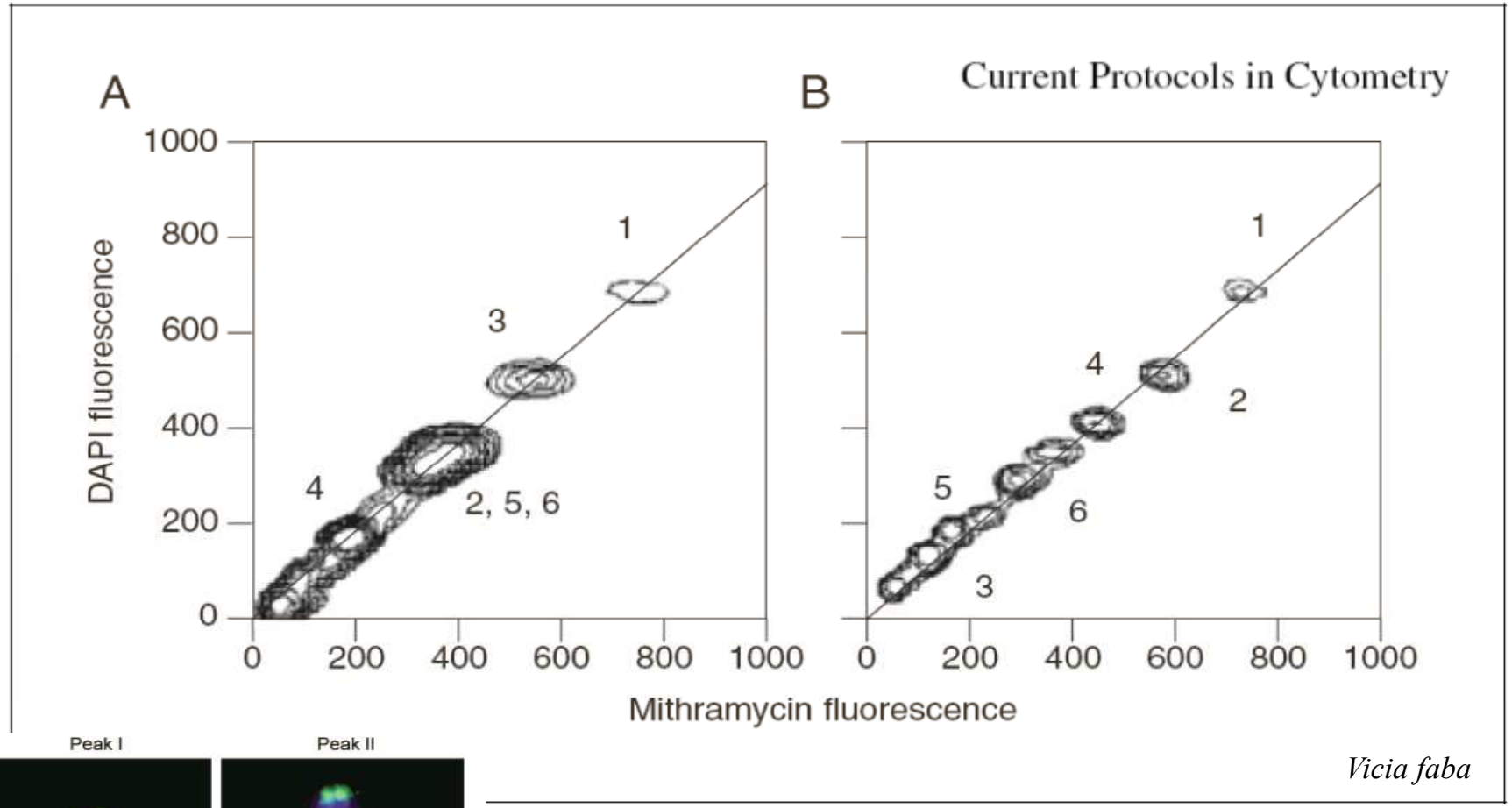
Vol. 33/2 Proceedings RMS *June* 1998

Sortrování chromozómů

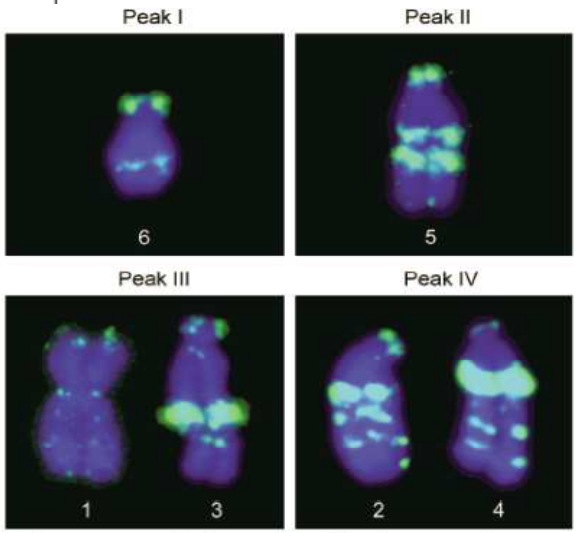


Pisum sativum

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
- bioterorismus

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

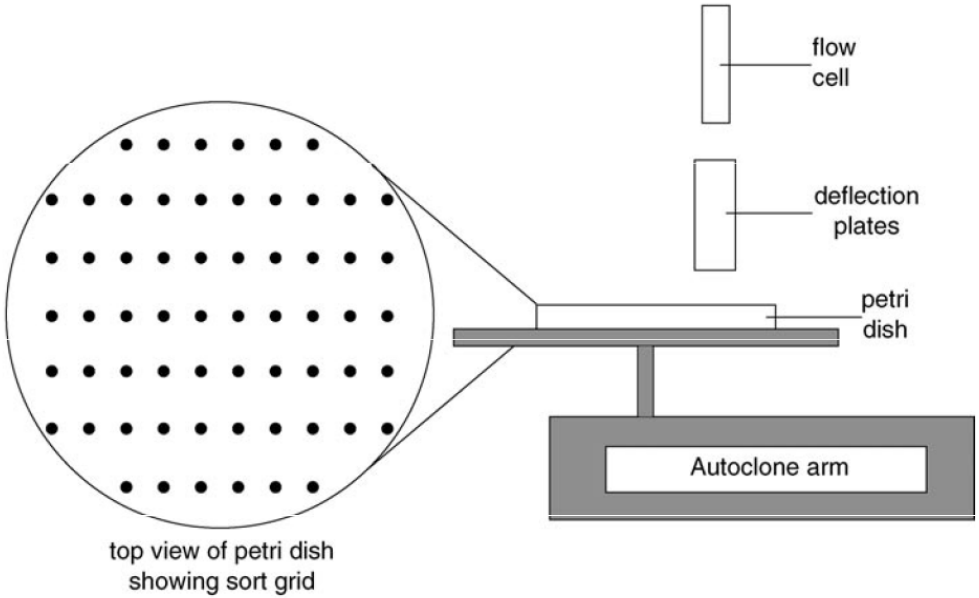
Current Protocols in Cytometry

Aplikace průtokové cytometrie v mikrobiologii

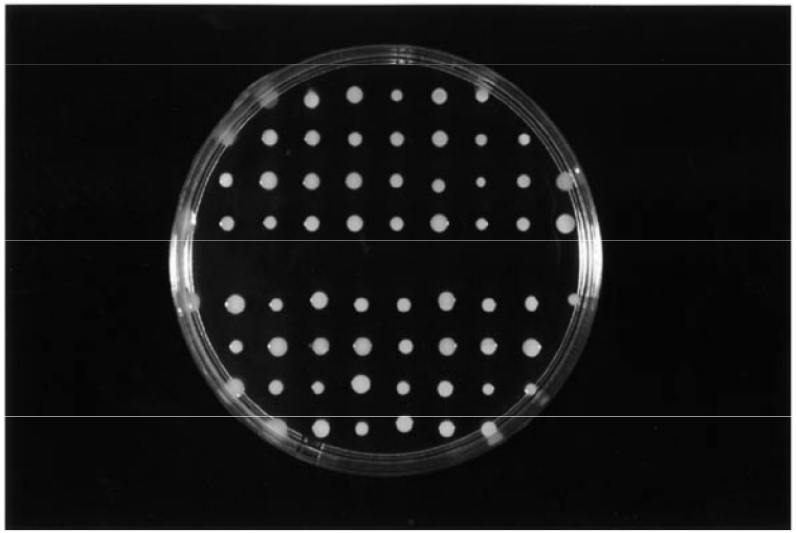
- viabilita
- metabolické funkce
- sortrování
- analýza aerosolů (Fluorescence Aerodynamic Particle Sizer (Flaps))

Aplikace průtokové cytometrie v mikrobiologii

- Sortrování
  - EPICS + Autoclone® modul

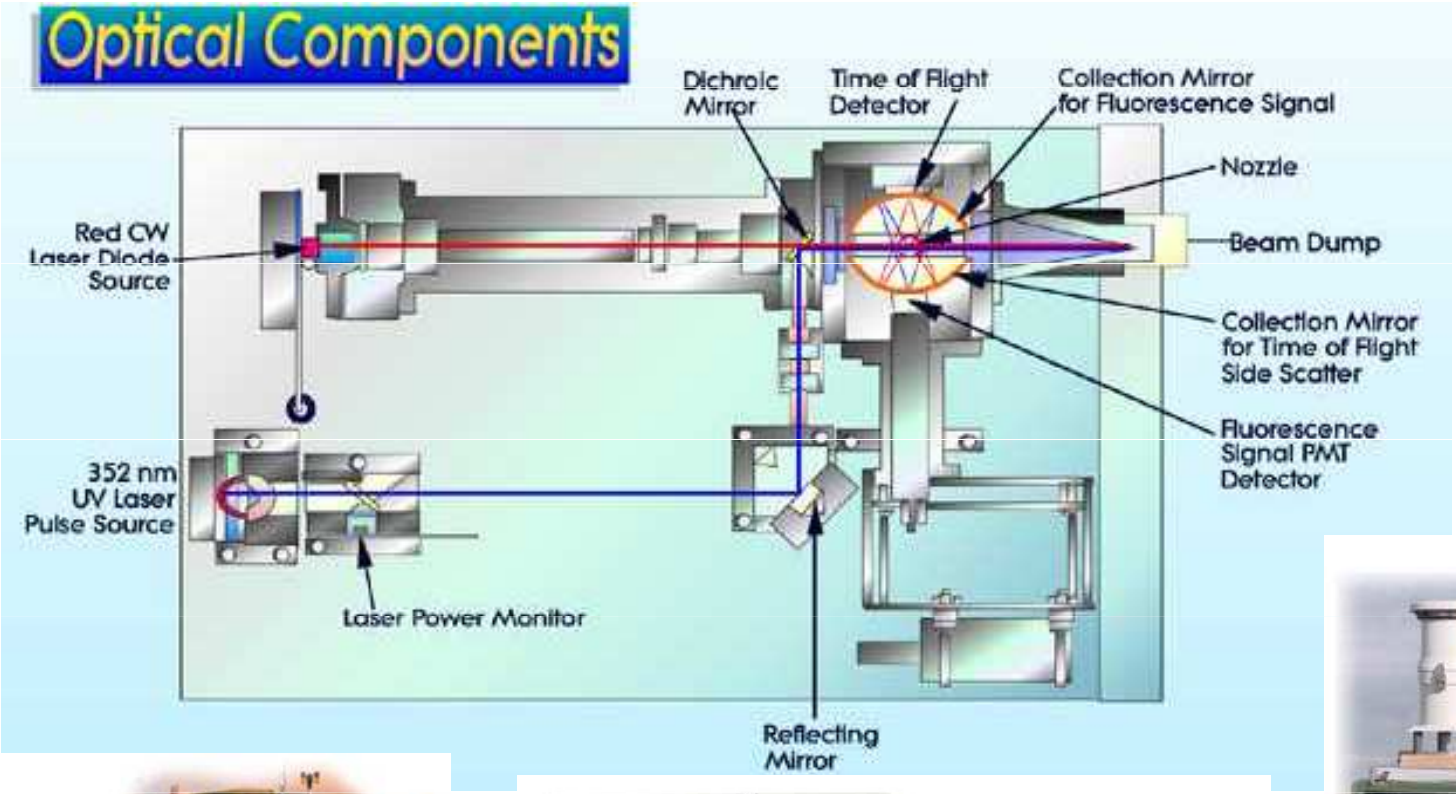


top view of petri dish showing sort grid



Current Protocols in Cytometry

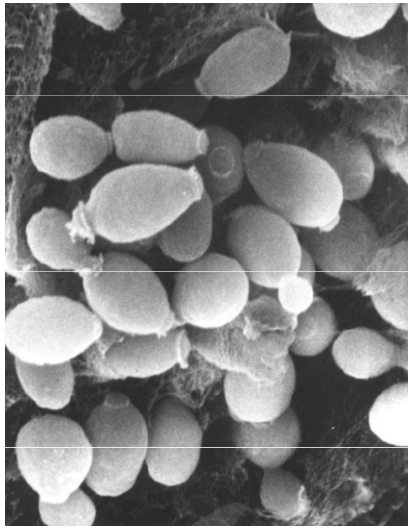
Fluorescence Aerodynamic Particle Sizer (Flaps)



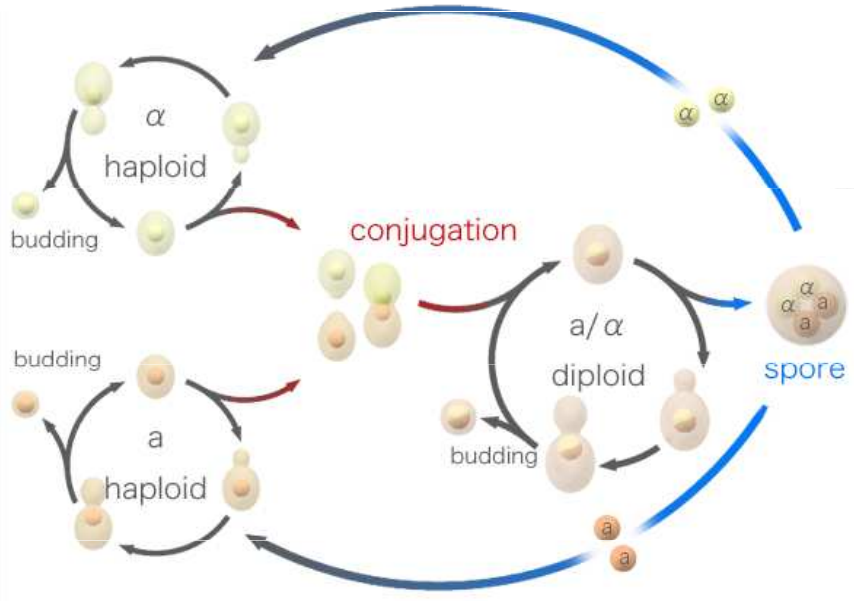
http://www.dres.dnd.ca/ResearchTech/Products/CB_PRODUCTS/RD98001/index_e.html

Průtoková cytometrie kvasinek

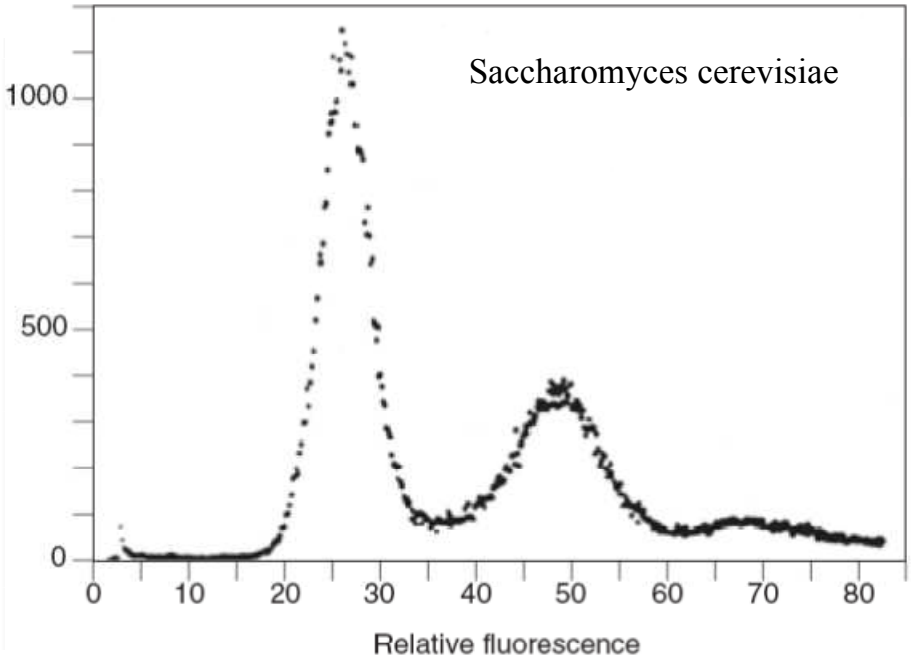
- **buněčné dělení**
- viabilita
- membránový potenciál
- respirace
- produkce H_2O_2
- citlivost k antibiotikům
- separace



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

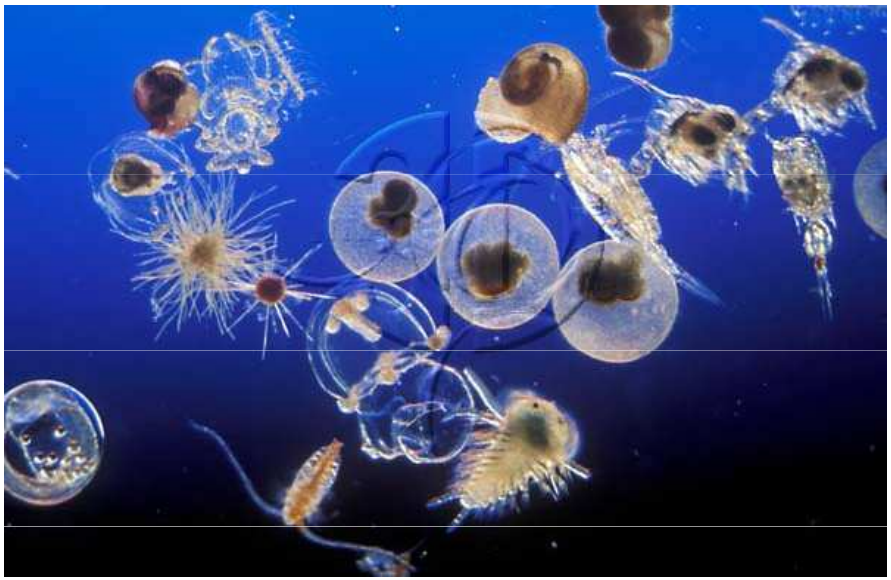
Yeast Cell Cycle During Fermentation and Beer Quality

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

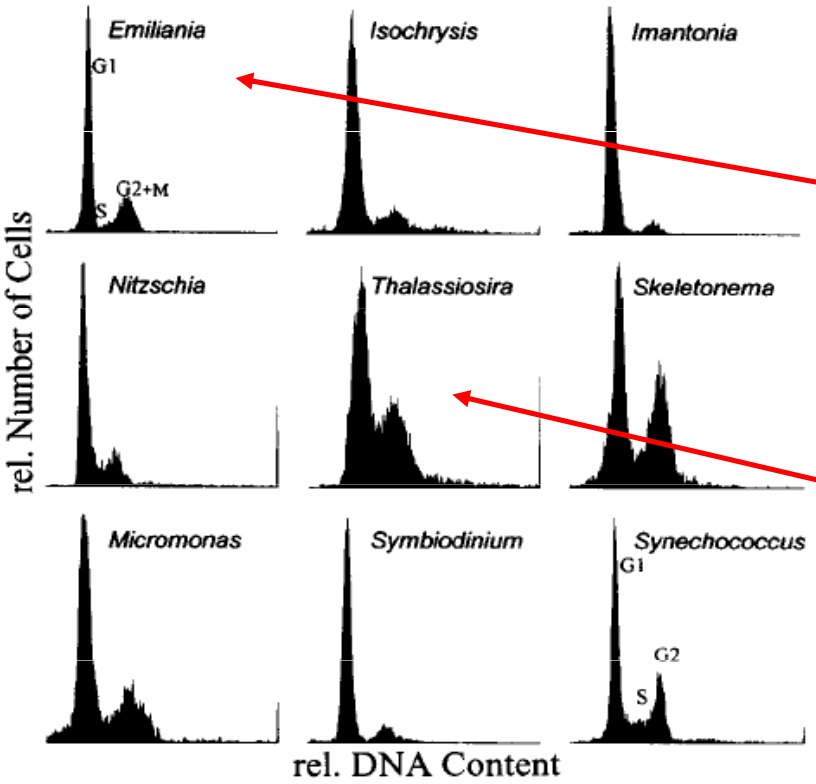
Vol. 185: 301–307, 1999 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published August 20

NOTE

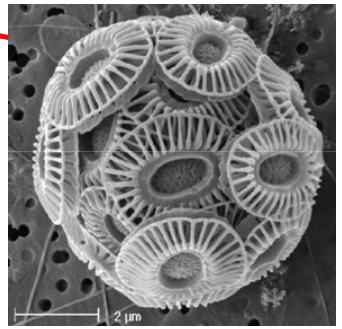
Cytometric measurement of the DNA cell cycle in the presence of chlorophyll autofluorescence in marine eukaryotic phytoplankton by the blue-light excited dye YOYO-1

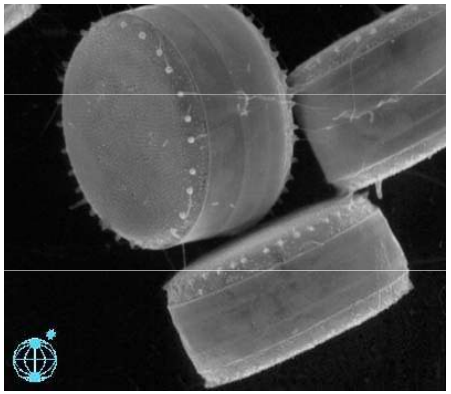
Frank J. Jochem[1,*], Doris Meyerdierks[2]

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



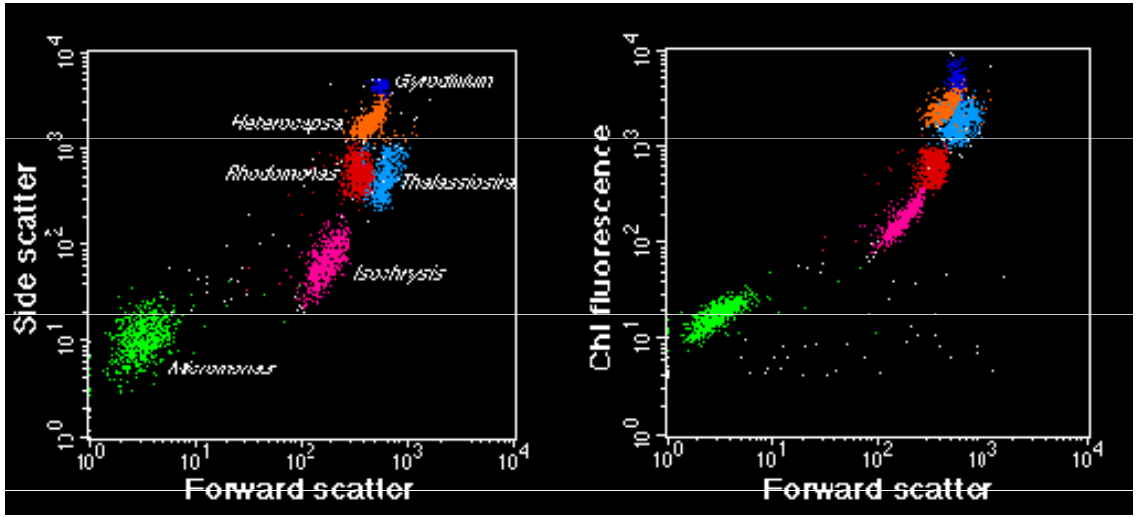
http://www.soes.soton.ac.uk/staff/tt/

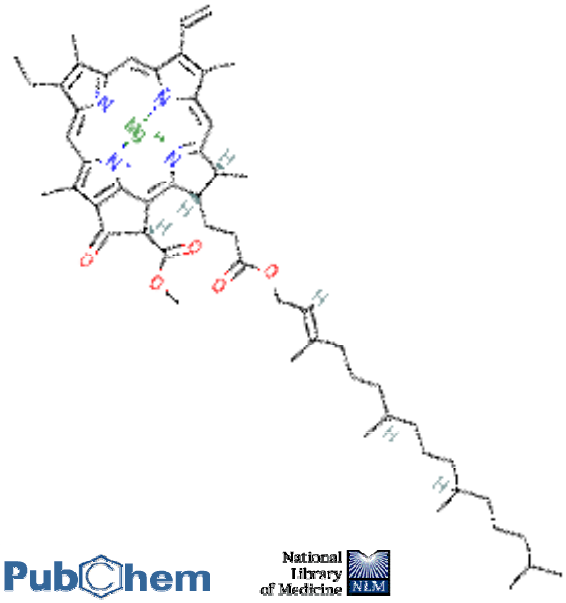




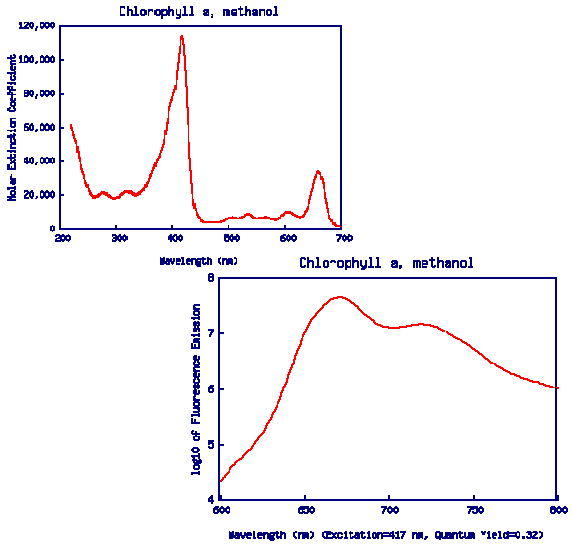
http://planktonnet.awi.de/portal.php?pagetitle=assetfactsheet&asset_id=15127

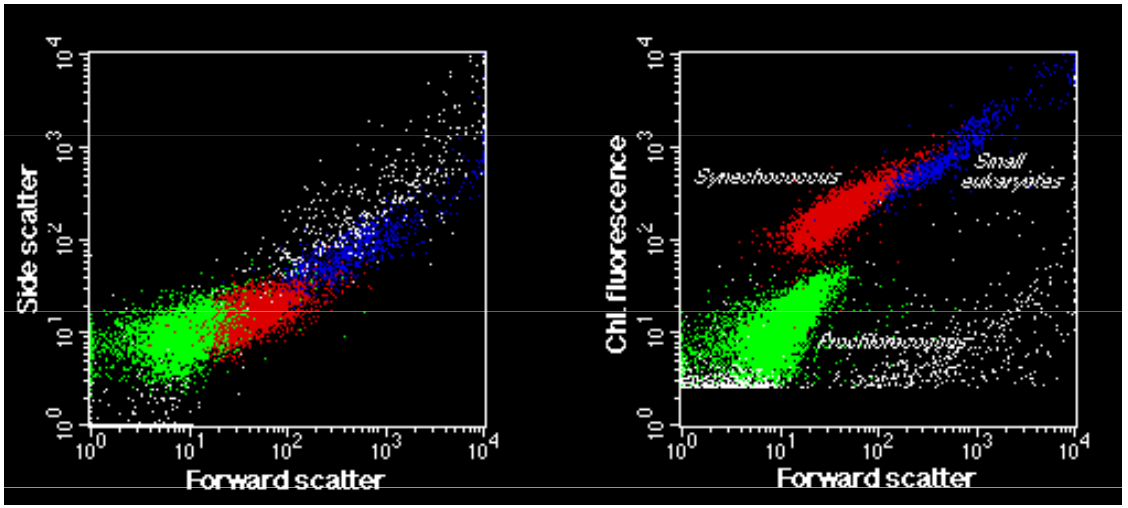
Průtoková cytometrie v hydrobiologii





PubChem National Library of Medicine NLM





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

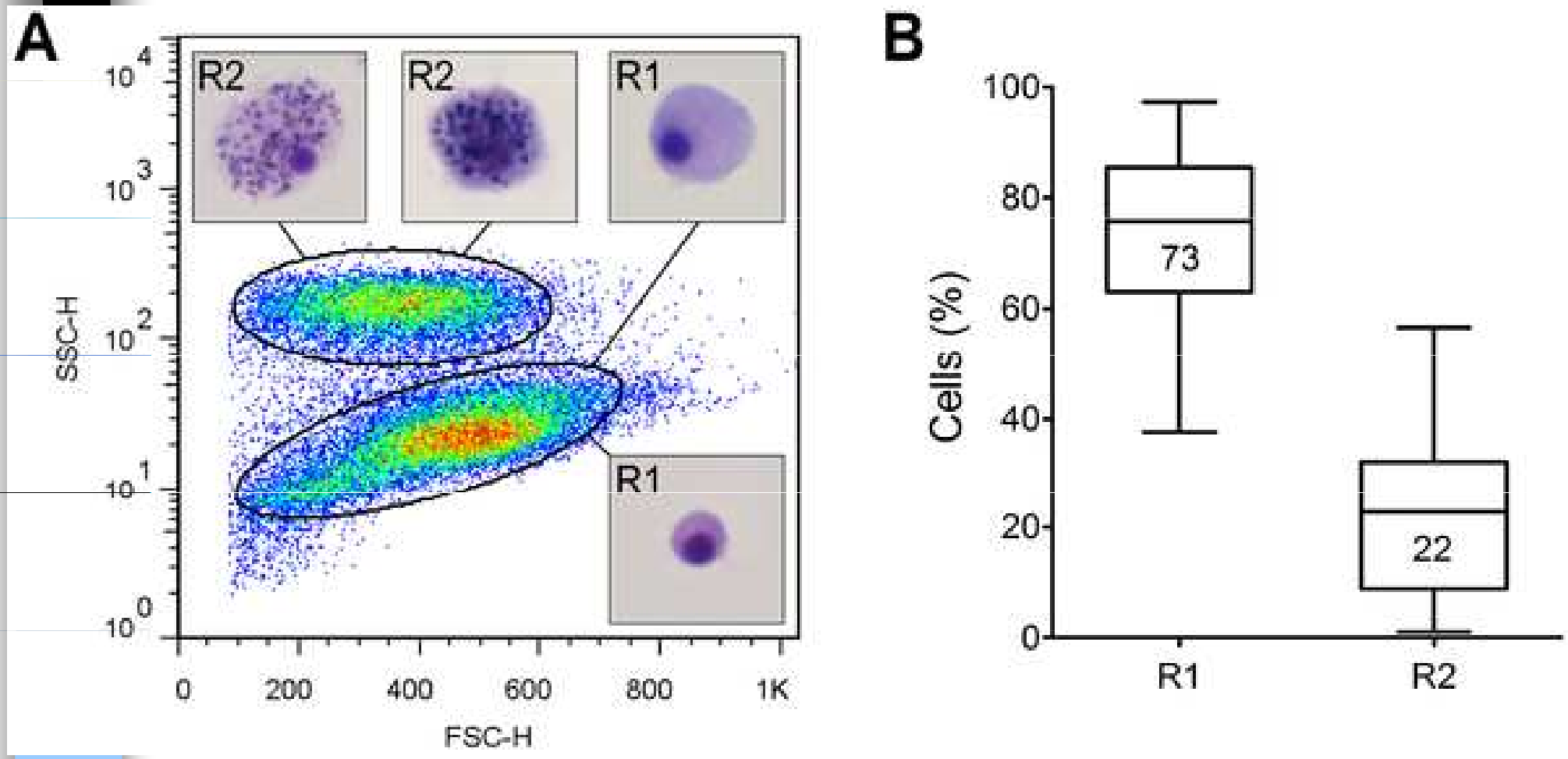
Flow cytometry as a tool for analysing invertebrate cells

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*

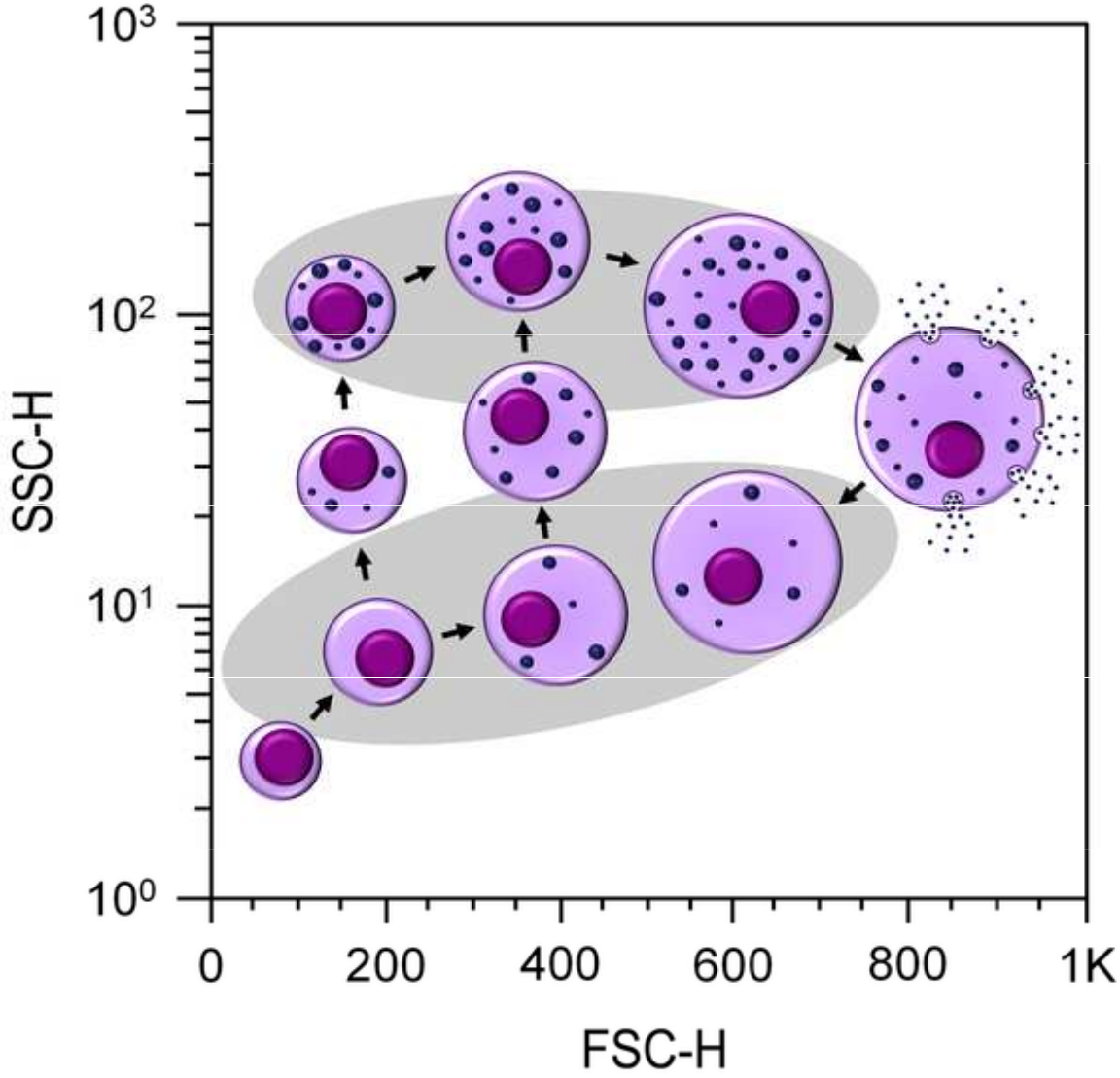
http://www.icms.qmul.ac.uk/flowcytometry/uses/insects/index.html

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

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 vizualizace - limitace

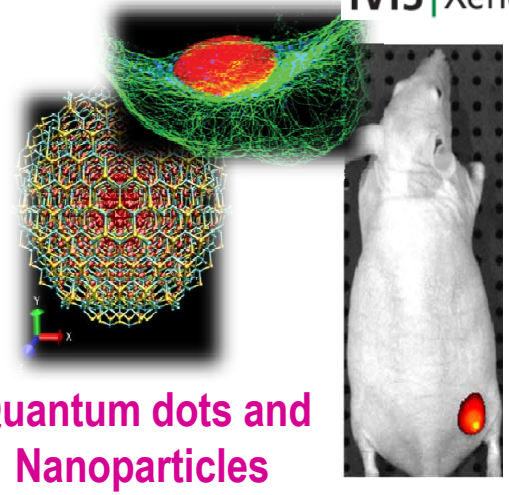
- Tloušťka tkáně

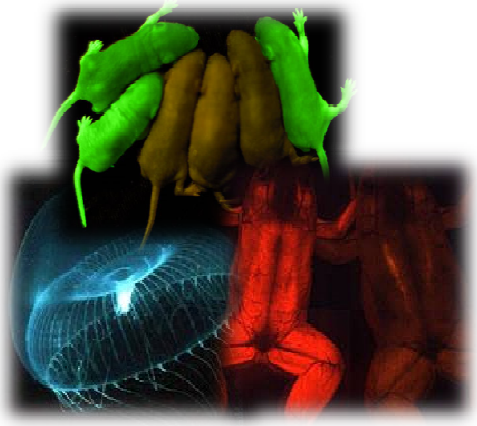
Imaging Basics – Reporter Molecules

Luciferase

Fluorescent dyes

IVIS | XenoLight DiR





Fluorescent Proteins

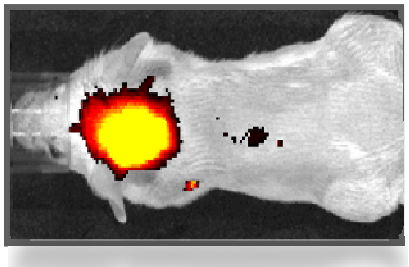
Quantum dots and Nanoparticles

+ ATP and O_2 – Live cells

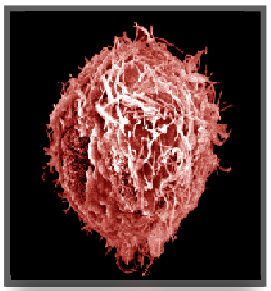
+ D-luciferin substrate

+ excitation light source

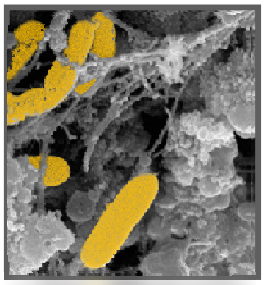
Transfection



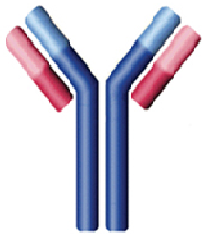
Genetic Marker



Label Cells



Label Bacteria



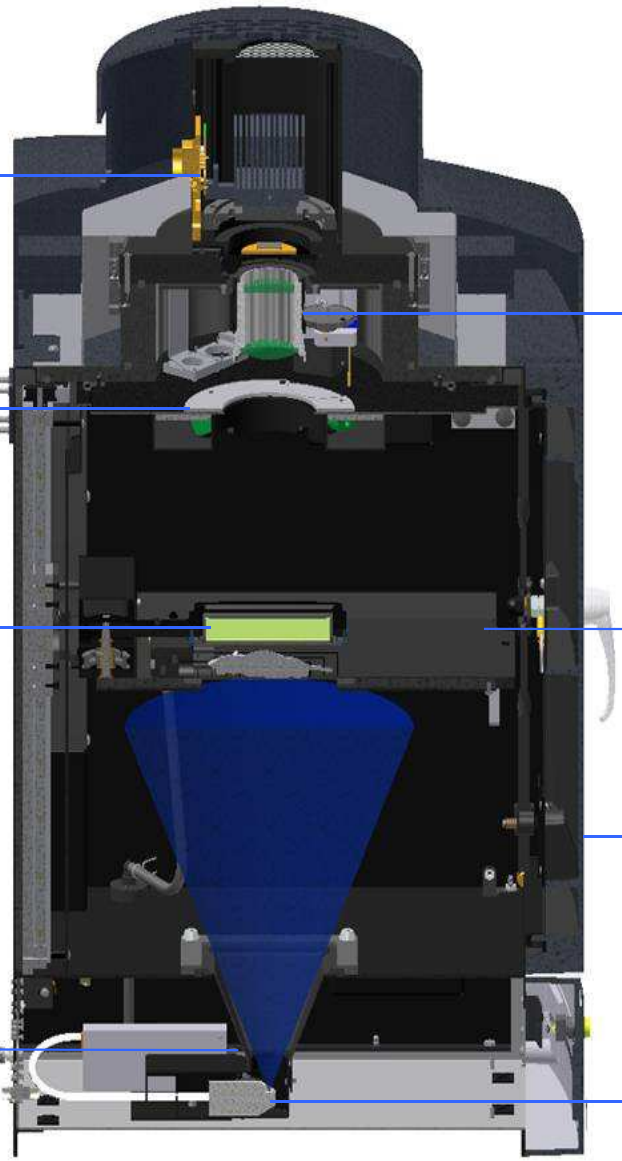
Label Proteins

Biology

IVIS® Lumina XR – Hardware

- Customized for *in-vivo* imaging
- High sensitivity from 300-900 nm
- Large dynamic range

The IVIS® Lumina XR Imaging Chamber



CCD Camera
Cooled -90C

f/1 Lens & Aperture

Interchangeable
Emission Filter
Wheel

Cesium Iodide
(CsI(Tl)) scintillator

Heated
Sample Stage

Light-tight
and shielded
imaging
chamber

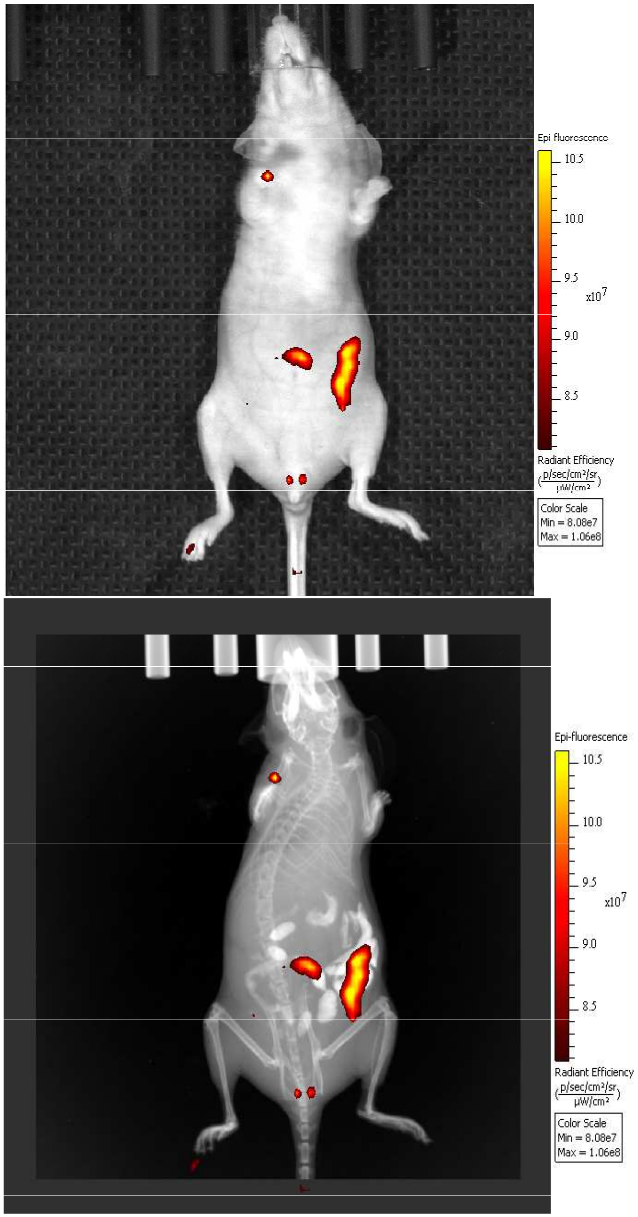
Automated
aluminum filter

X-Ray Source
(40kVp,100mA)
Tungsten Anode

The Value of X-Ray + Optical Imaging

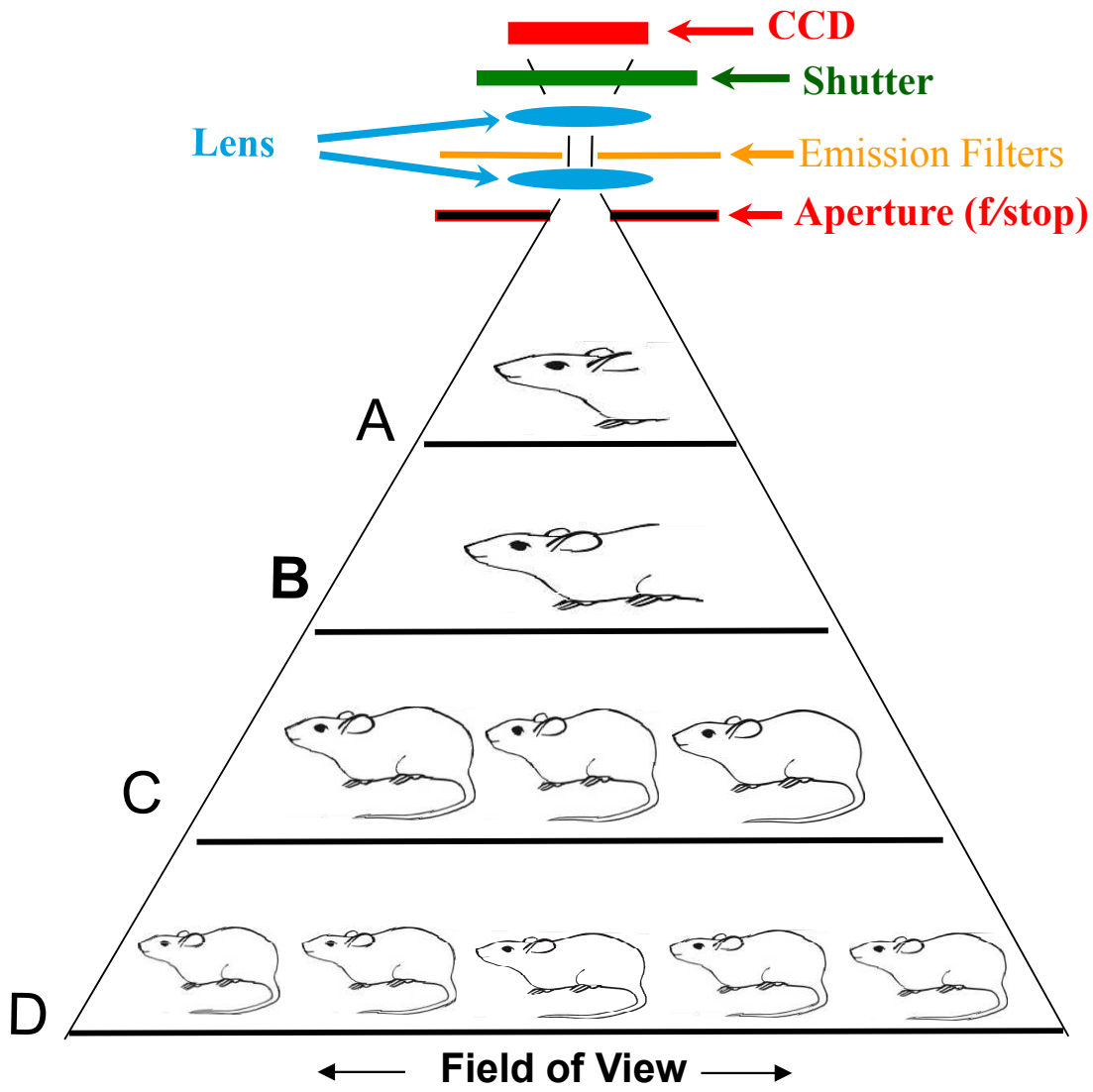
X-Ray Provides a Fixed Anatomical Reference

- The Question: Where is the source origin relative to the surface signal?
- The Problem: Tissue attenuation/ scattering makes 2D optical signals difficult to locate at a defined location.
- The Solution: A co-registered X-ray image provides a fixed anatomical reference, defining skeletal structure and soft tissue organs and enabling better localization of the optical signal.



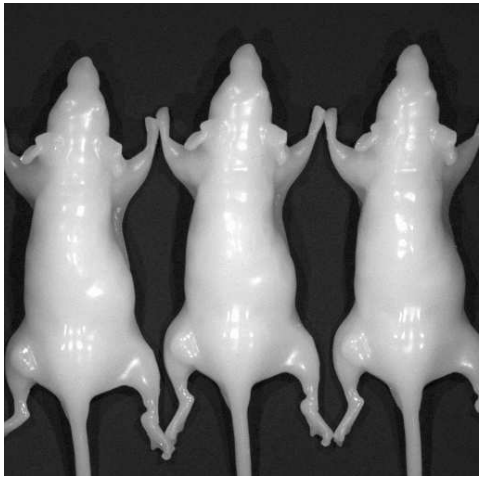
Camera and Lens Settings are Analogous to Photography

- Field of View (FOV) is dependent on the distance from the lens to the sample
- Light collected is proportional to how long the shutter is open (exposure time)
- Aperture (*f*/stop) controls the amount of light collected
- Digital pixel binning is possible on the CCD – alters sensitivity/resolution

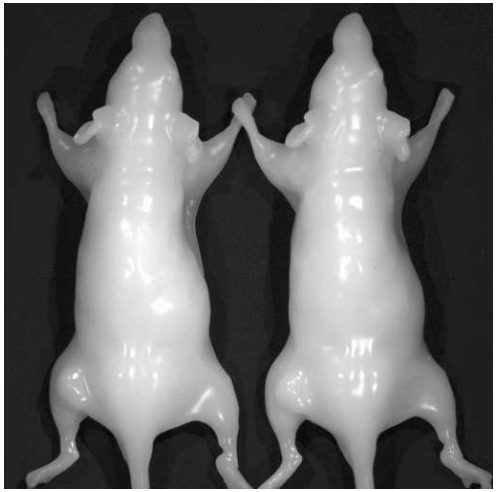


Field of View

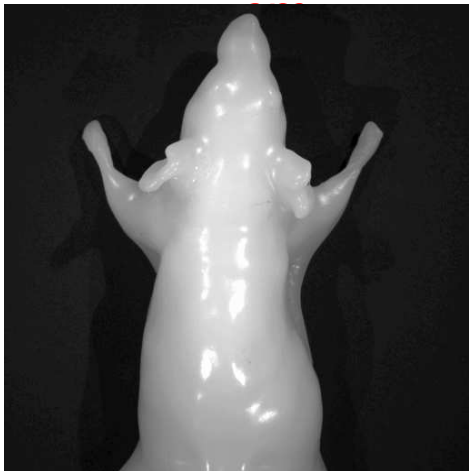
FOV D = 12.5 x 12.5 cm



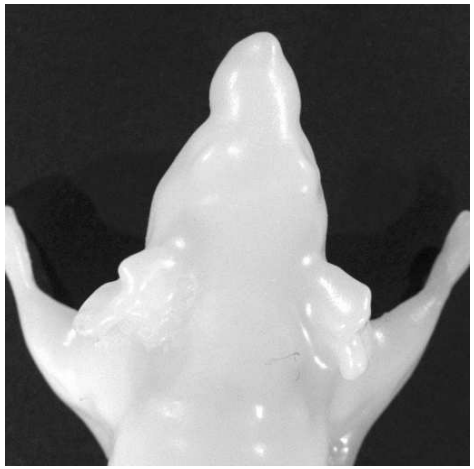
FOV C = 10 x 10 cm



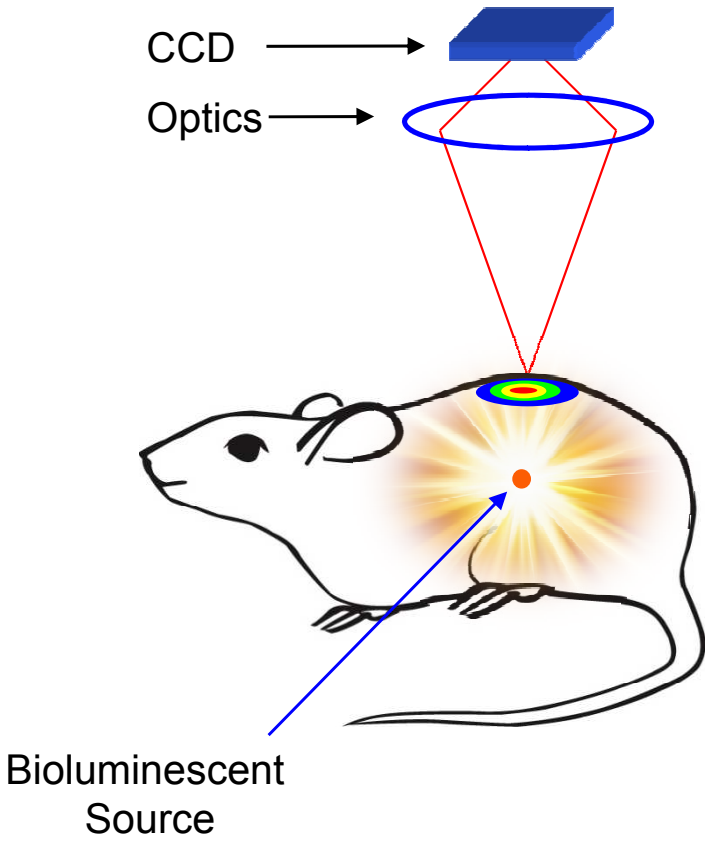
FOV B = 7.5 x 7.5



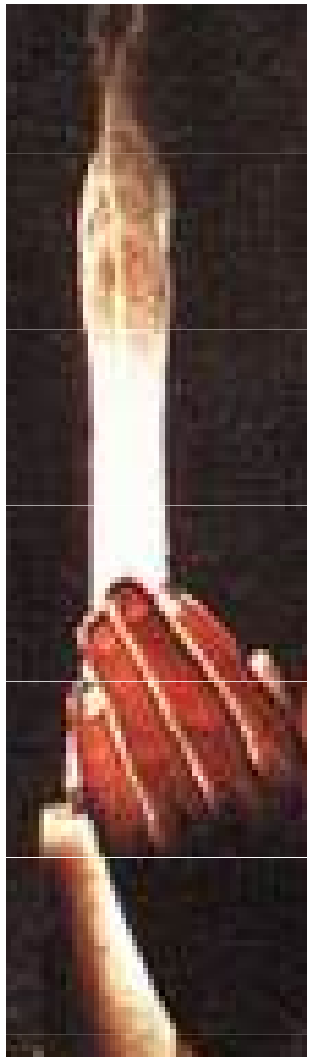
FOV A = 5 x 5 cm

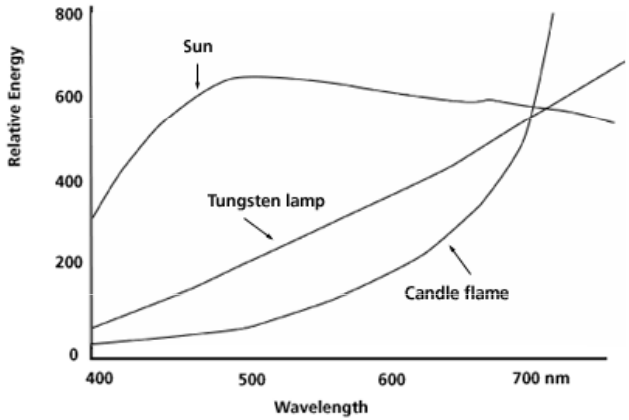


Photons Diffuse Through Tissue – Surface Light Pattern is Recorded



- Light traveling through tissue scatters many times creating a “fuzzy” image at the surface of the animal
- The IVIS® views the diffuse image on the camera-facing (top) surface of the subject
- Not all light from the source will make it to the camera – light absorption will occur as signal exits the animal

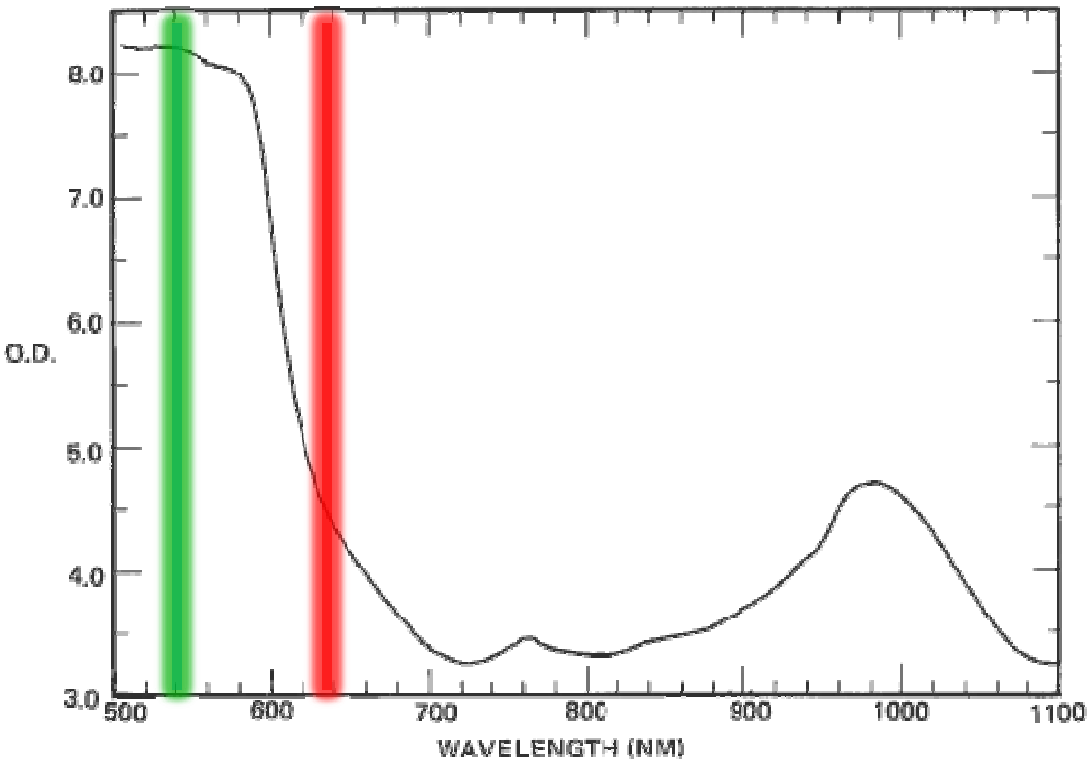


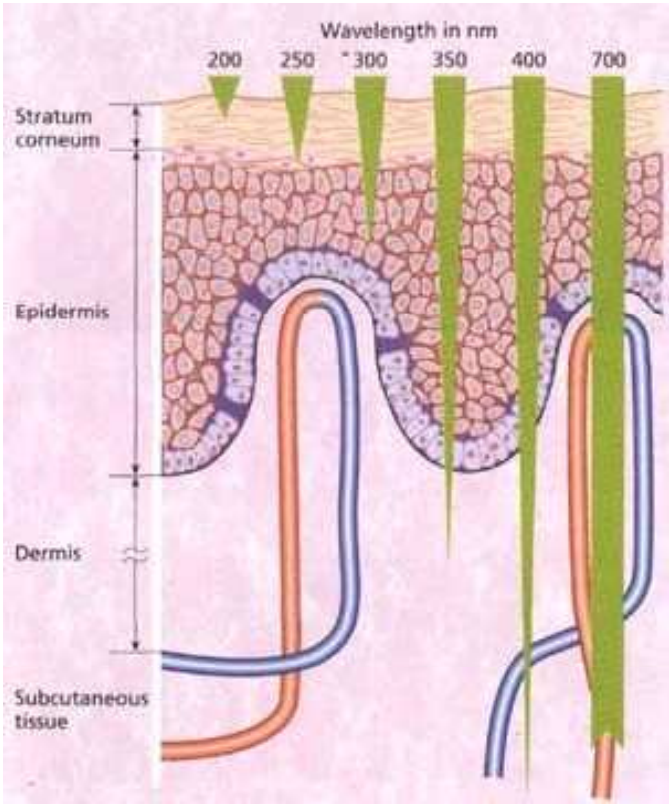


Christ with St. Joseph in the Carpenter's Shop
Georges De La Tour, ~ 1640 (Musee du Louvre, Paris).

http://osa.magnet.fsu.edu/teachersparents/articles/sourcesoflight.html

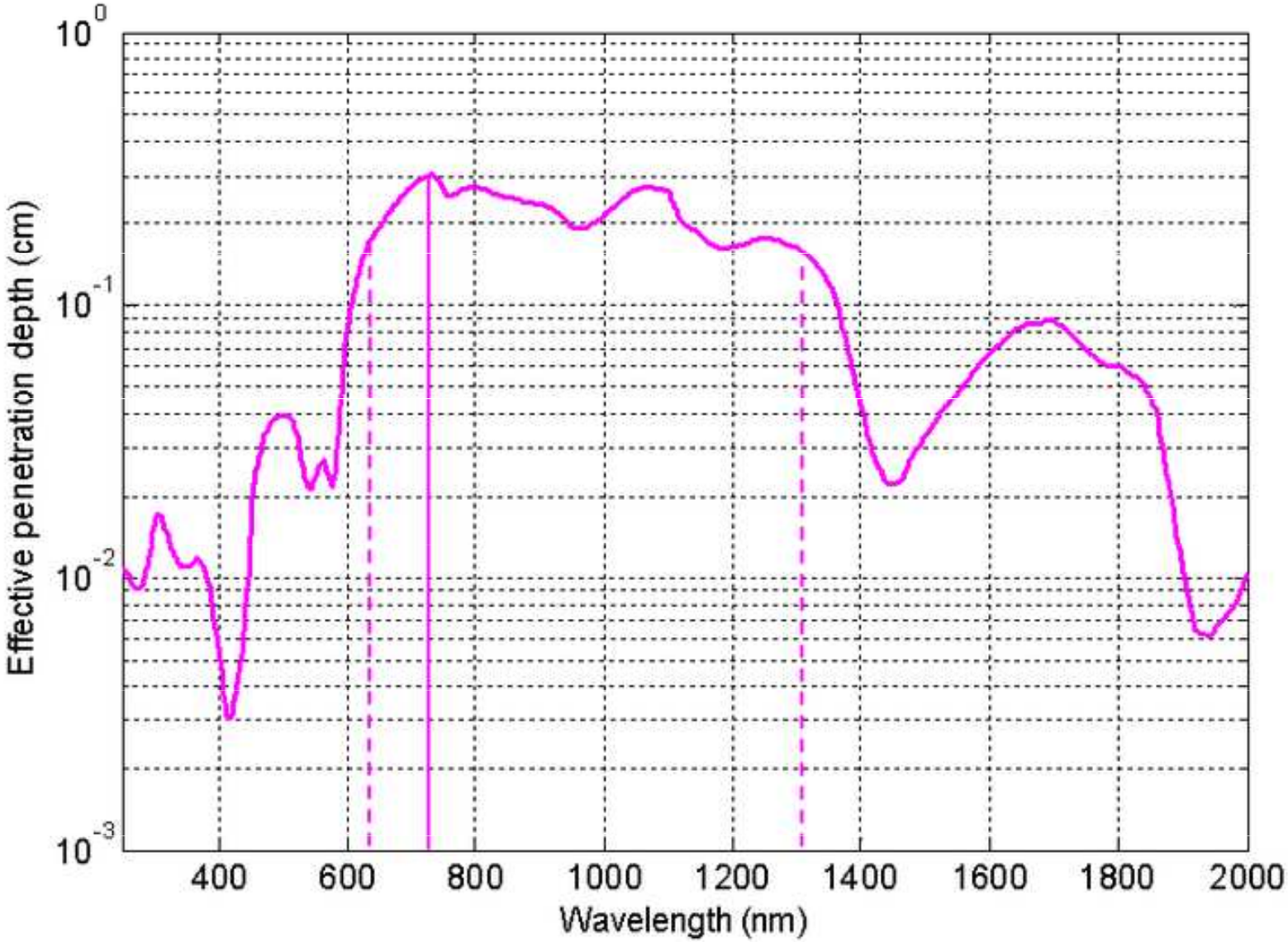
Průchod světla tkání





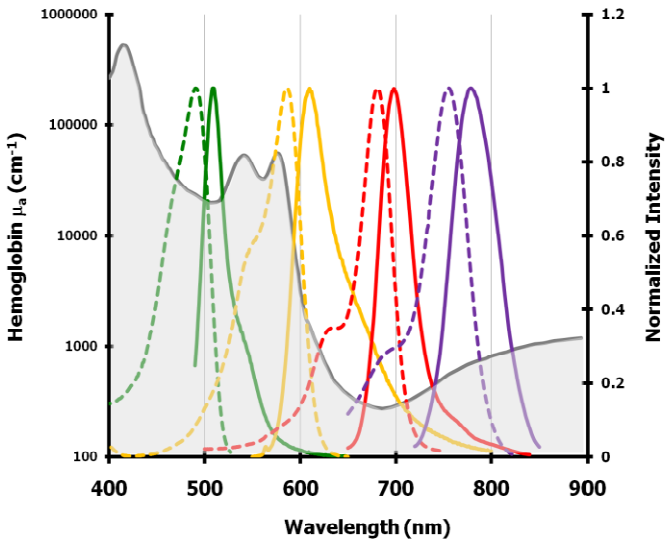
The absorption spectrum of a human hand.
The spectrum was recorded with a very sensitive spectrophotometer with the hand in close juxtaposition with the photocathode (unpublished data of Karl H. Norris, from The Science of Photobiology (KC Smith, ed., Plenum Press, 1977; p. 400).

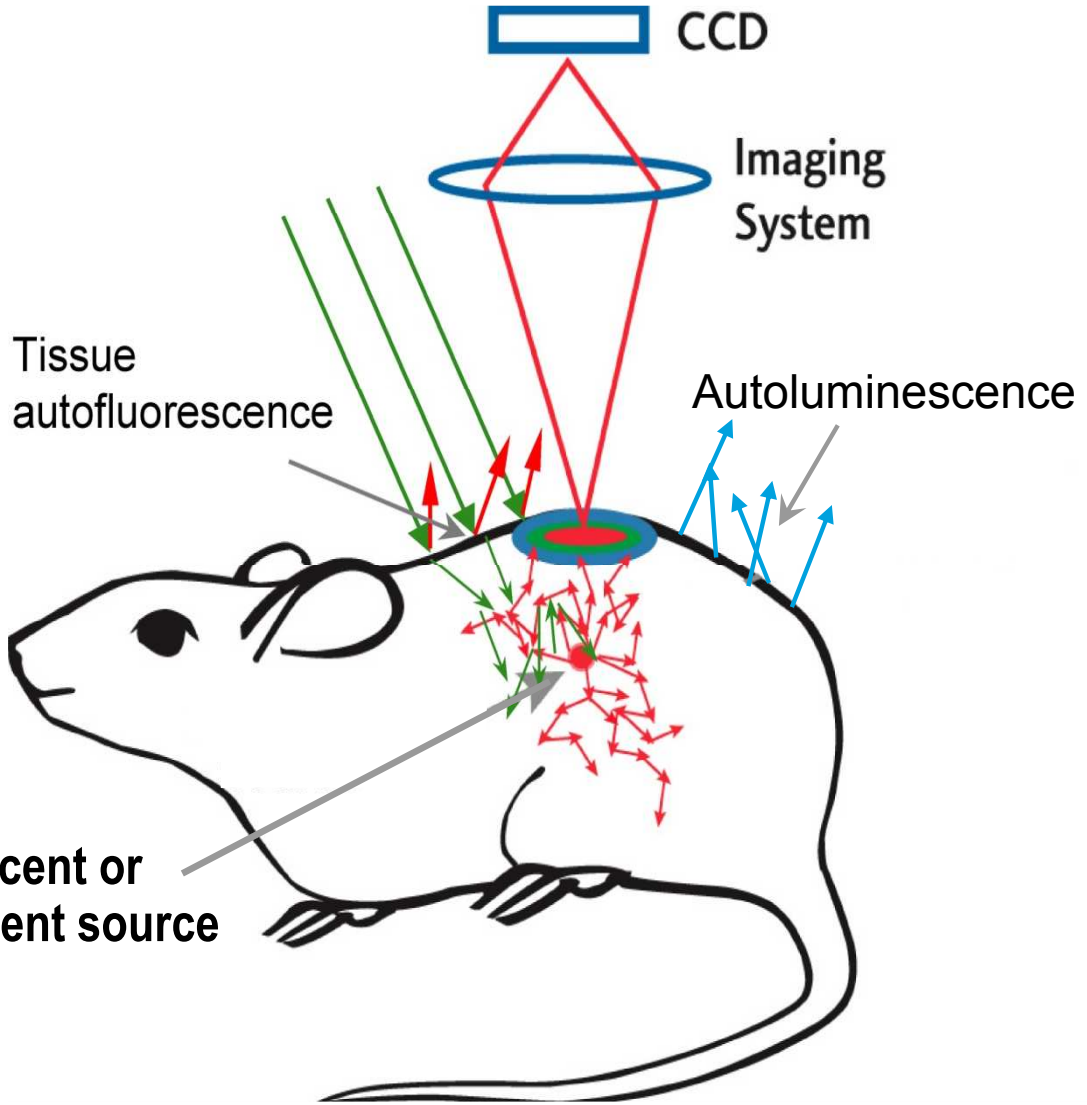
Effective penetration depth in breast tissue



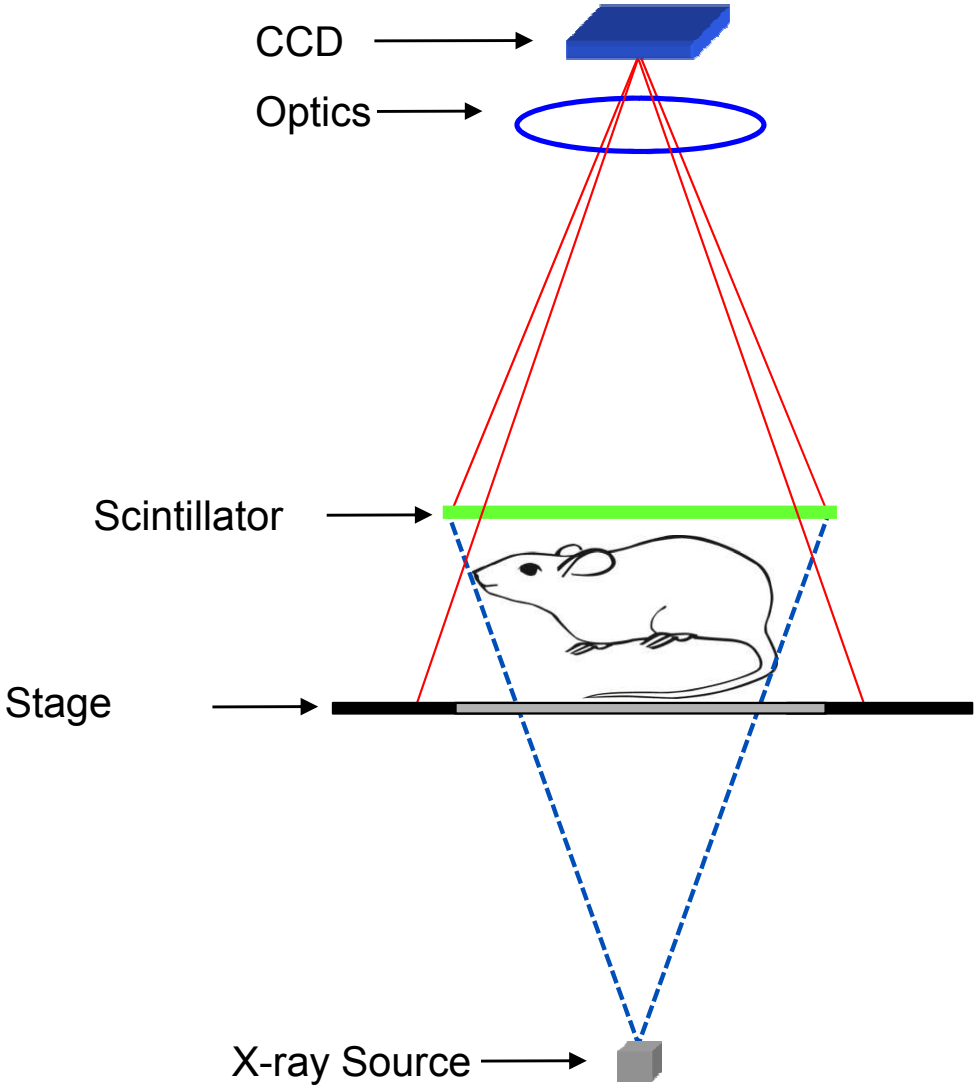
Challenge of *in vivo* Optical Imaging

- Photons are absorbed and scattered in tissue
- Surface signal depends on source depth
- Tissue is both autoluminescent and autofluorescent
- Autofluorescence levels are much higher that autoluminescence





How an X-Ray Image is Acquired



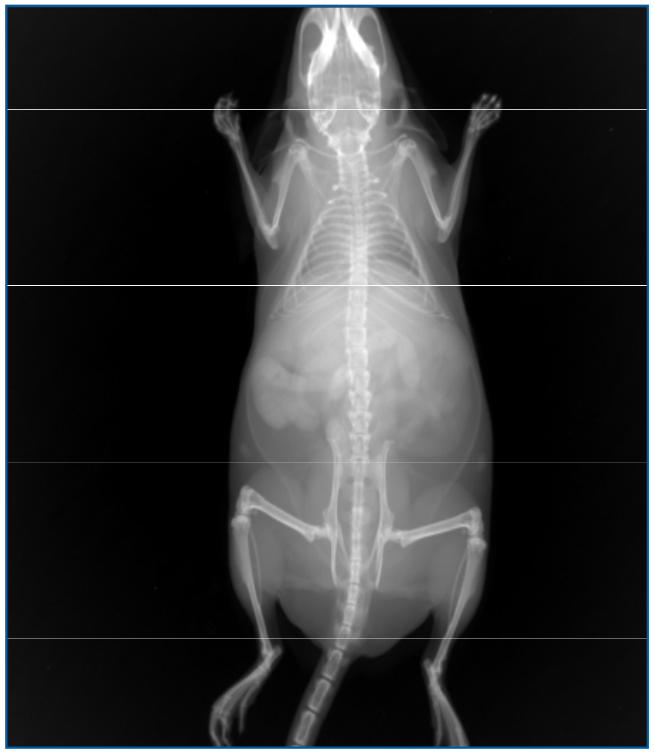
- X-rays will be attenuated in tissue differently resulting in an image on the scintillator
- The CCD views the scintillator resulting in a planar X-ray image
- X-ray and Optical images have different path lengths. To correct this geometrical difference, the X-ray image is registered to the optical image

Close Up of Scintillator in Position

Field of View (X-ray)

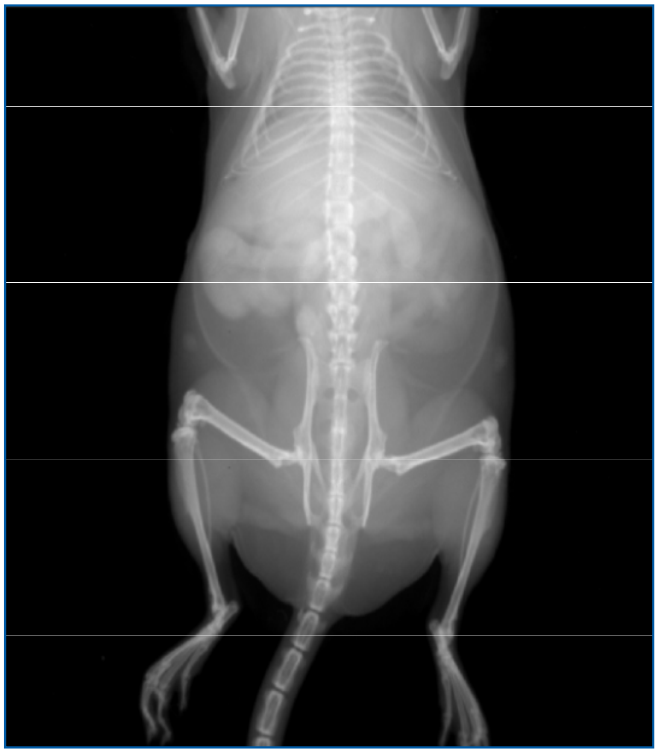
FOV C

10 x 10 cm



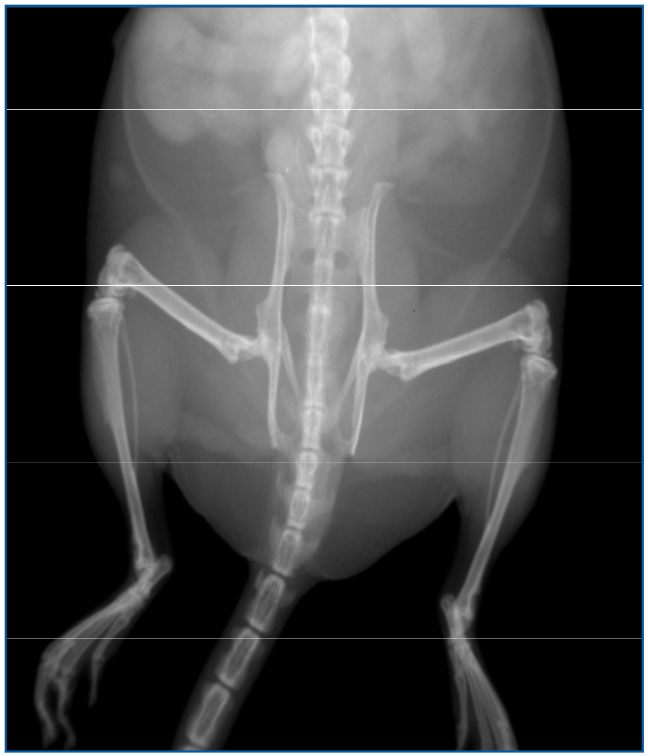
FOV B

7.5 x 7.5 cm

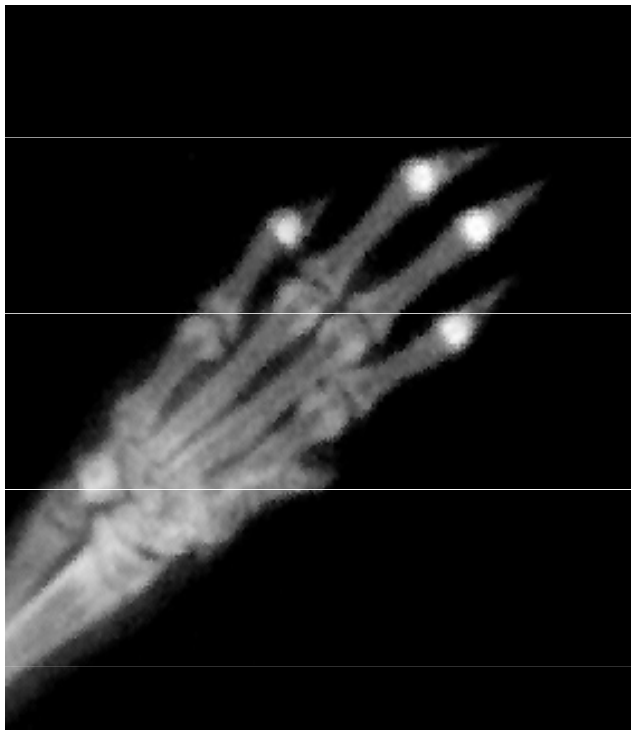


FOV A

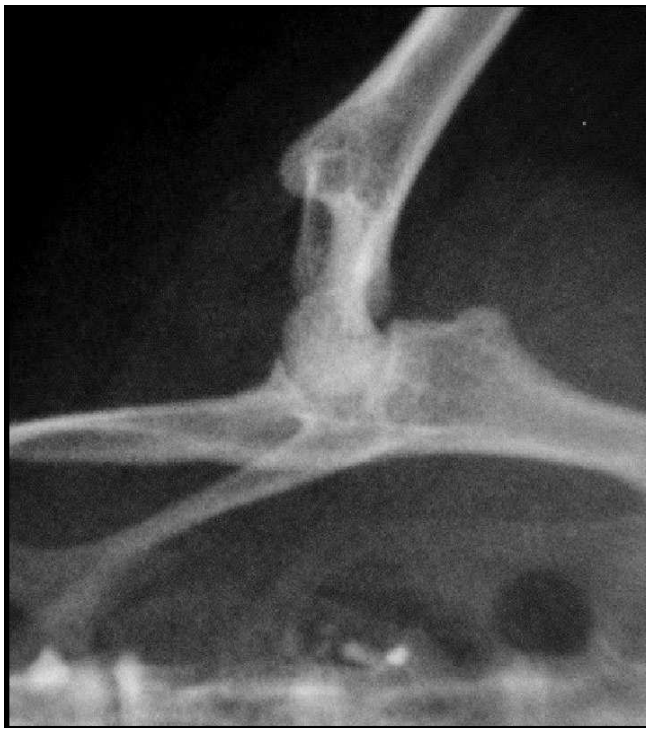
5 x 5 cm



Optional Z-FOV (2.5)



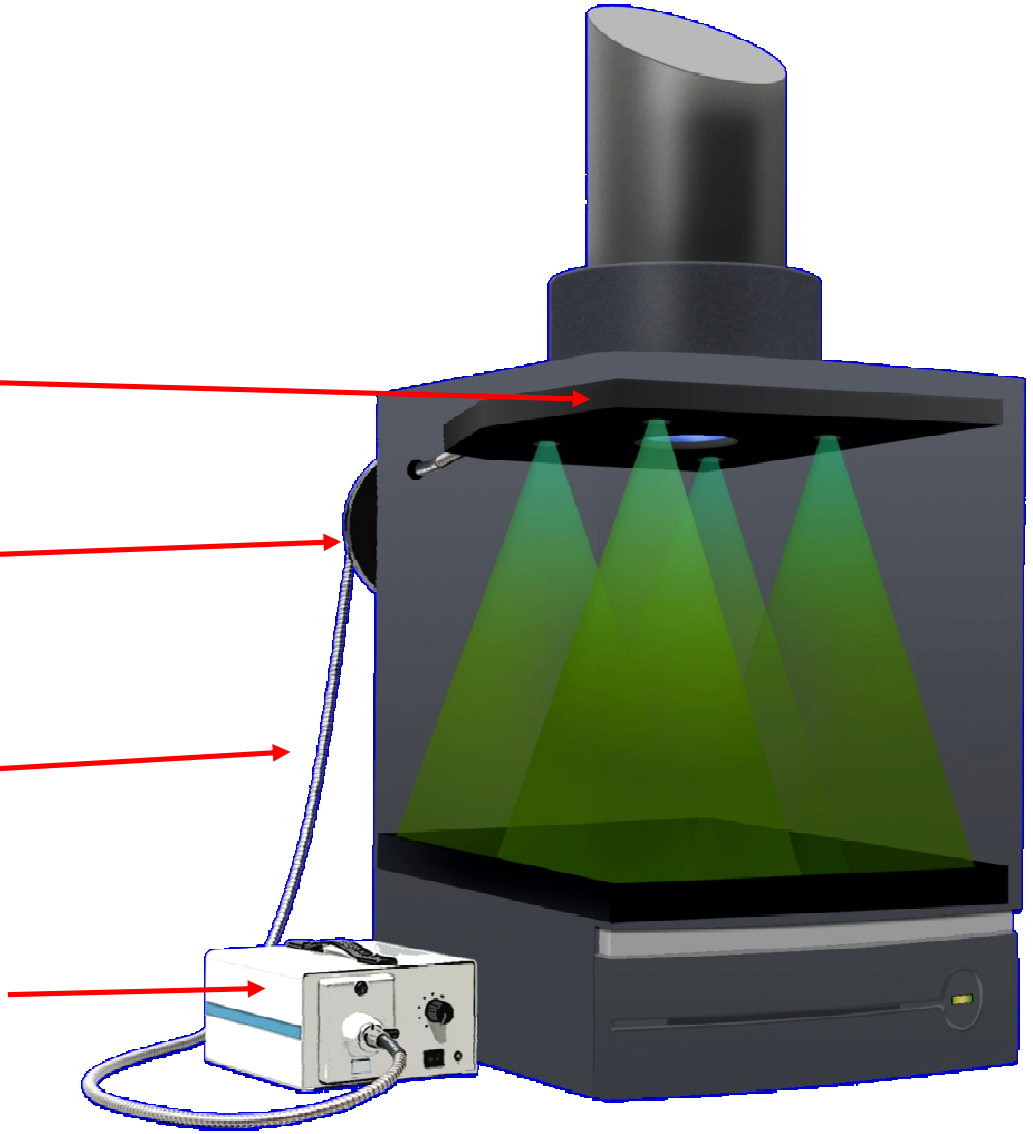
Magnified Mouse Paw



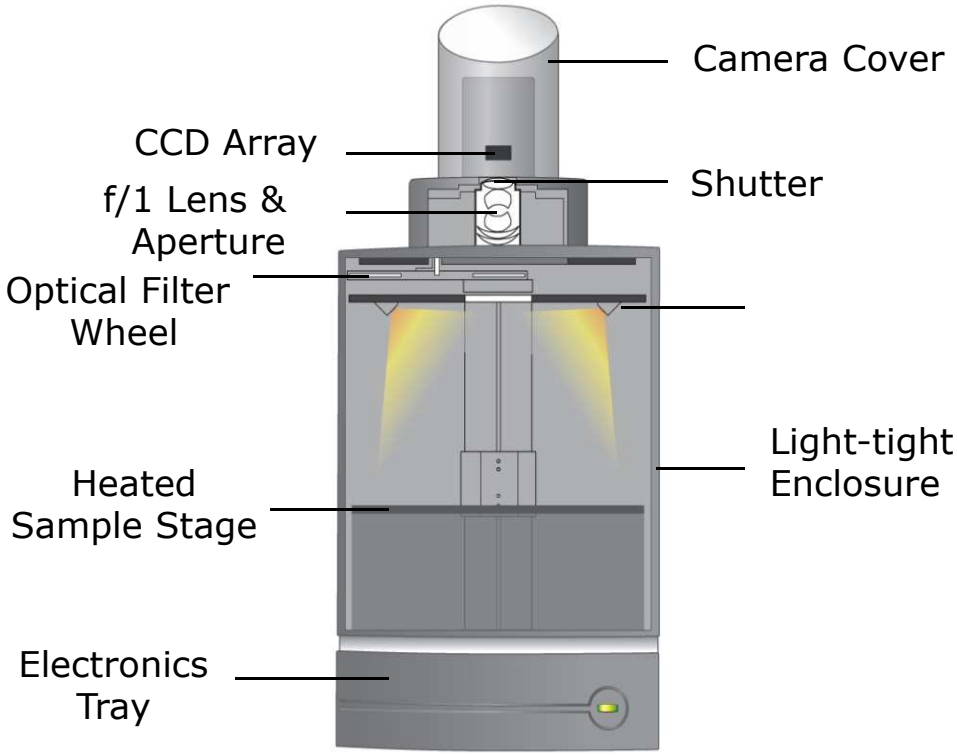
Magnified Hip Ball and Socket

IVIS® Lumina Fluorescence Components

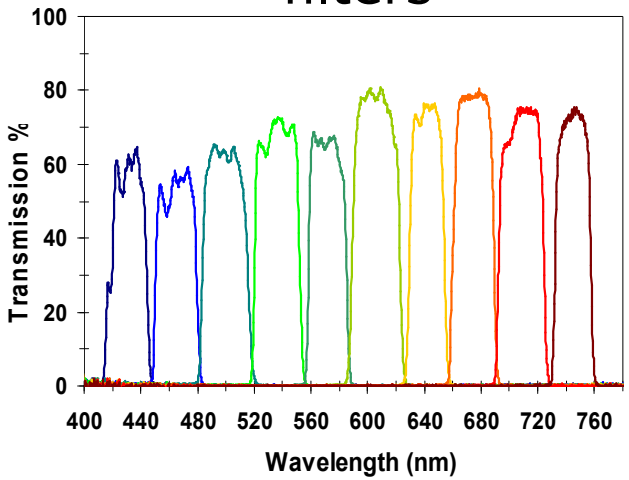
- Fully computer controlled
- User interchangeable eight position Emission filter wheel
- Twelve position Excitation filter wheel
- Low Auto Fluorescence optics and fiber optics
- 150 Watt Tungsten/Halogen lamp with computer controlled intensity



IVIS® Lumina XR



10 excitation filters

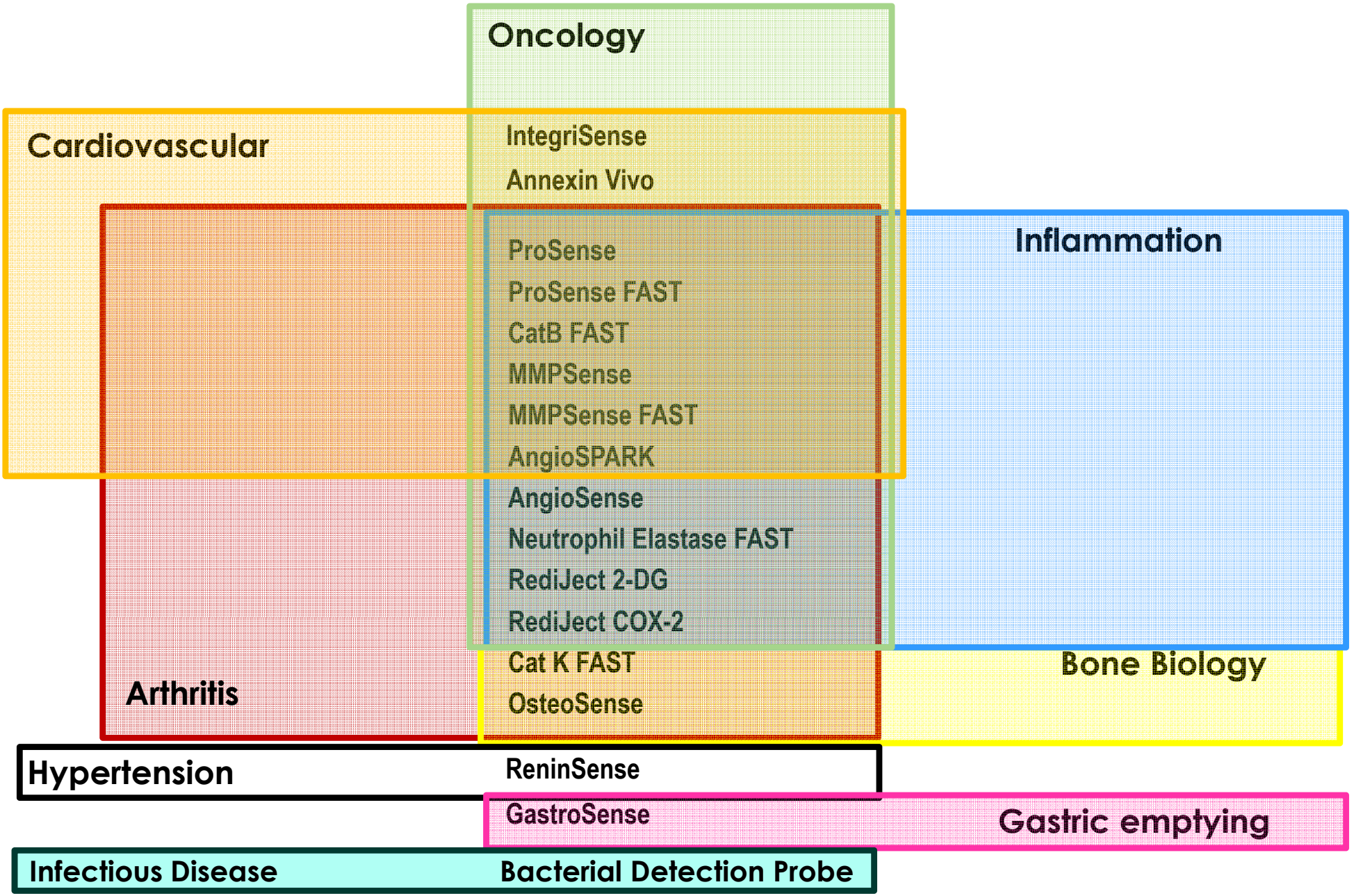


Fluorophores	Standard High Resolution Excitation Filter Set (Built-In)**	Emission Filter Options
GFP, YFP and PKH26	430, 465, 500, 535, 570, 605, 640, 675, 710, 745	***500 Series (Low Range)** 500, 520, 540, 560, 580, 600 and 620 nm
DsRed and Tomato		***600 Series (Mid Range)** 580, 600, 620, 640, 660, 680 and 700 nm
Cy5.5, XenoLight 680, Katushka and Cherry FP		***Mid-High Range** 640,660,680,700,720,740 and 760nm
Indocyanine Green and XenoFluor 750, 770		***700 Series (High Range)** 720, 740, 760, 780, 800, 820, and 840 nm
Multiple Fluorophores Spanning 500-900 nm Broad Imaging Solution		**Standard Emission Filter Set** 515-575, 575-650, 695-770, 810-875 nm

*20 nm bandpass emission filter

**35 nm bandpass excitation filters

In Vivo Fluorescent Agent Applications

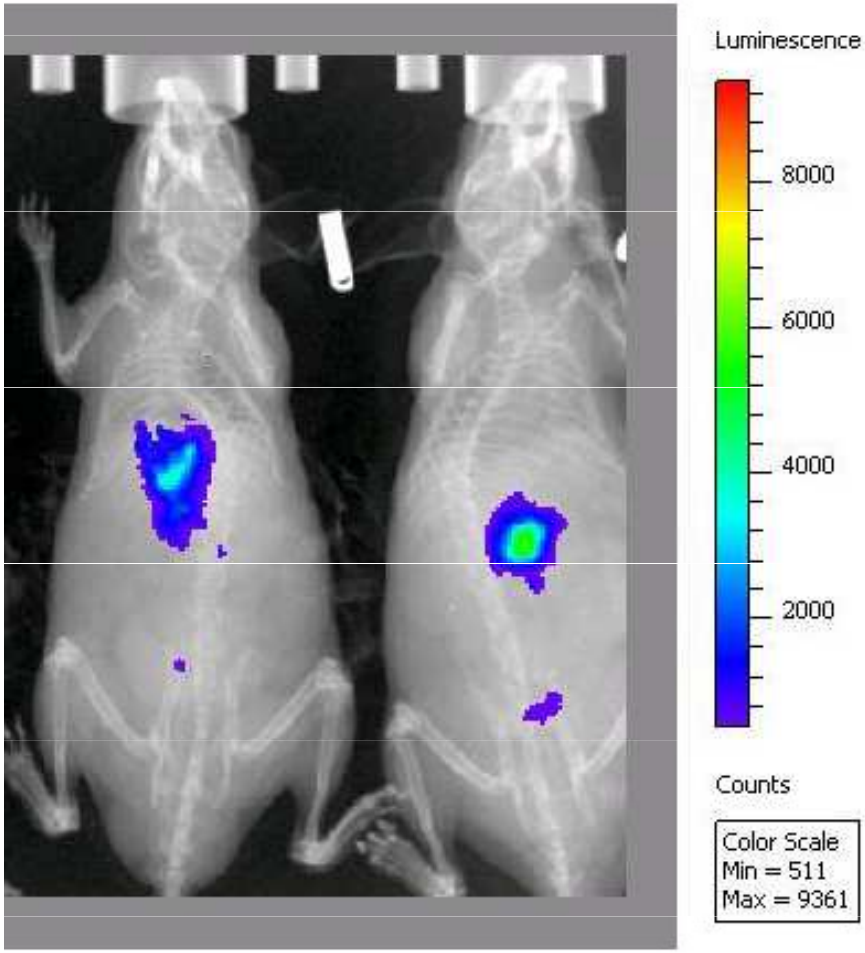


- Agents support multiple *in vivo* research areas

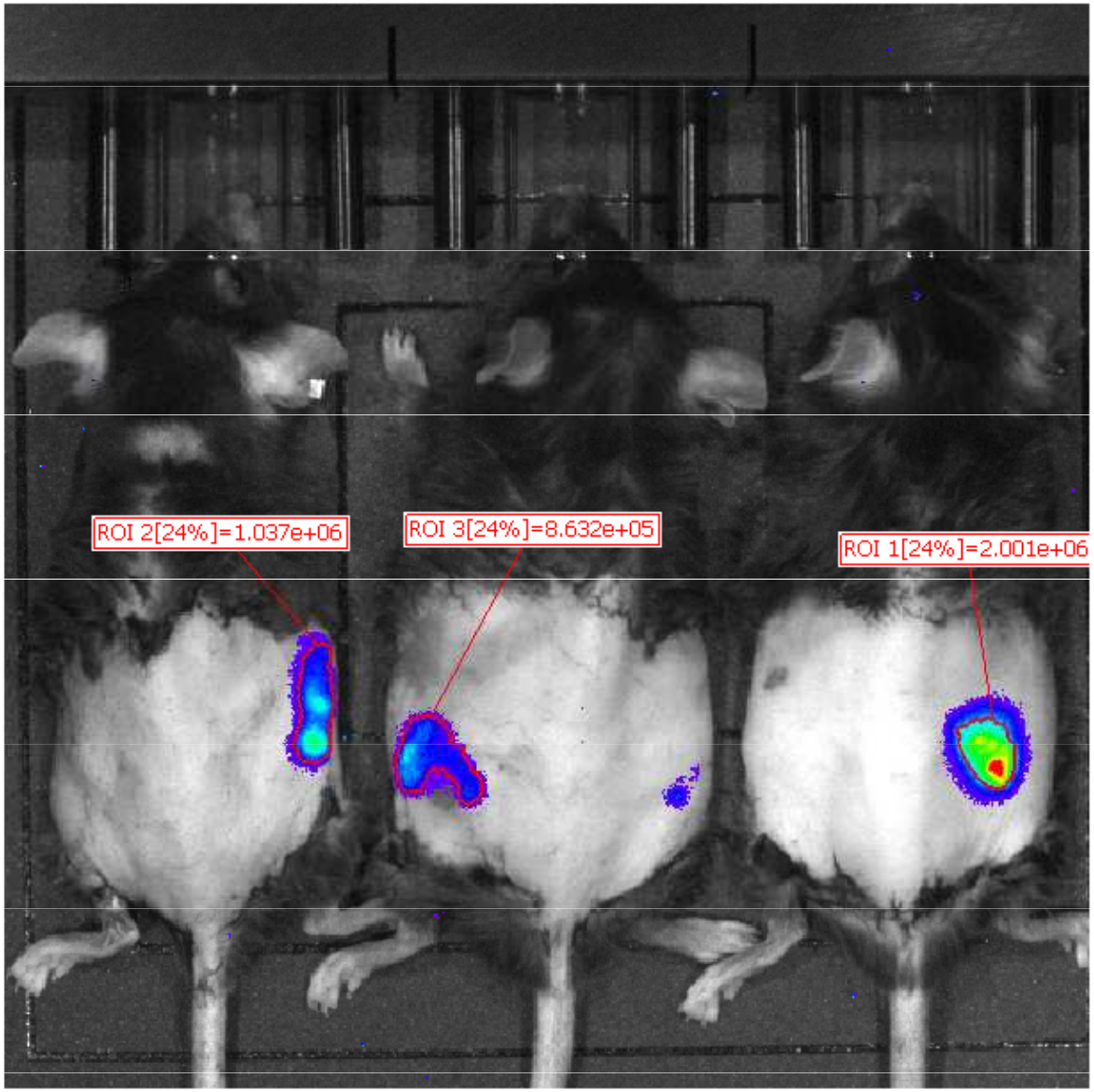
- tools for *in vivo* molecular imaging
  - **Prostate, Breast, Melanoma, and Colon Carcinoma Models**
    - for syngeneic immunocompetent mice strains C57Bl/6 or BALB/c
    - stable transfected with lentiviral *luc* vector
      - CT26 luc, GFP - mouse colon cancer *in process*
      - 4T1 luc - mouse breast cancer *done, tested in vivo*
      - B16 F10 luc, GFP – mouse melanoma *done, tested in vivo*
      - TRAMP-C1 GFP, luc – mouse prostate cancer *in process*

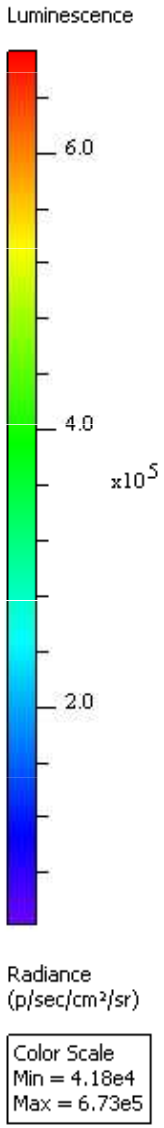
Future plan: transfect cells with pLKO.1-CMV-fLuc-IRES-mCherry

4T1 luc - s.c. injection



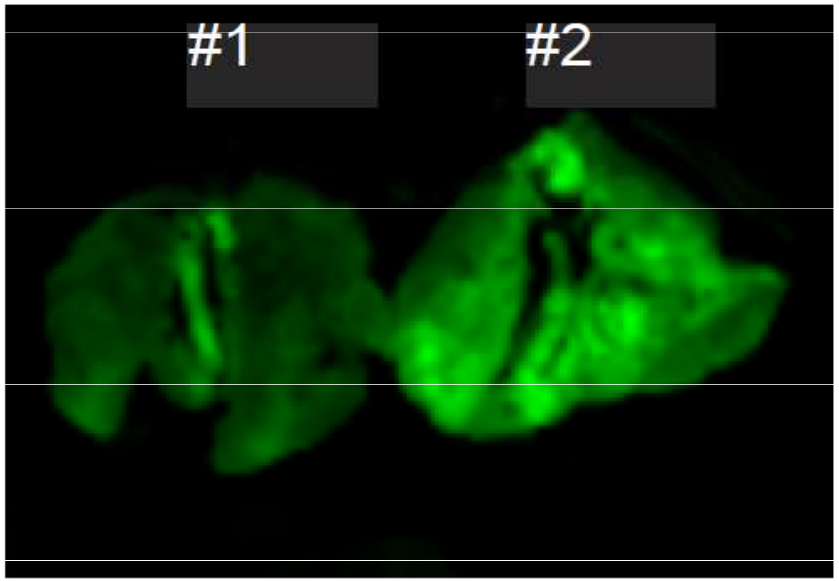
B16 F10 luc - s.c. injection

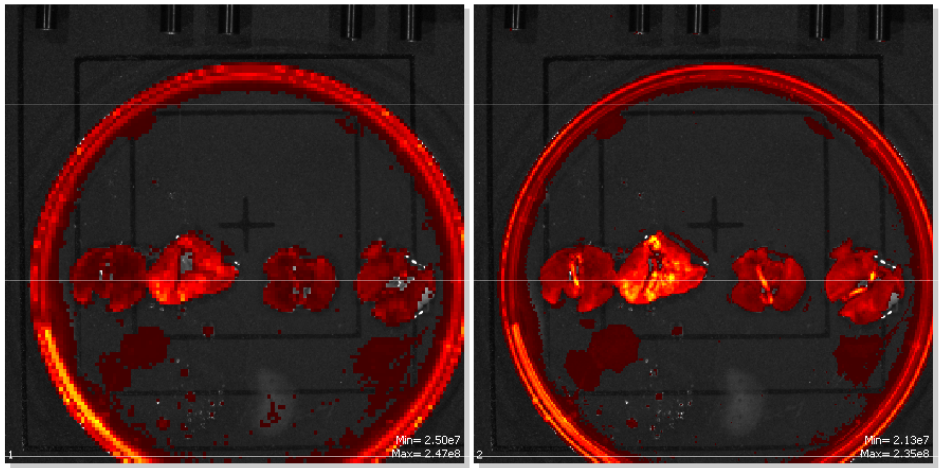




GFP *ex-vivo* imaging: example

Lungs

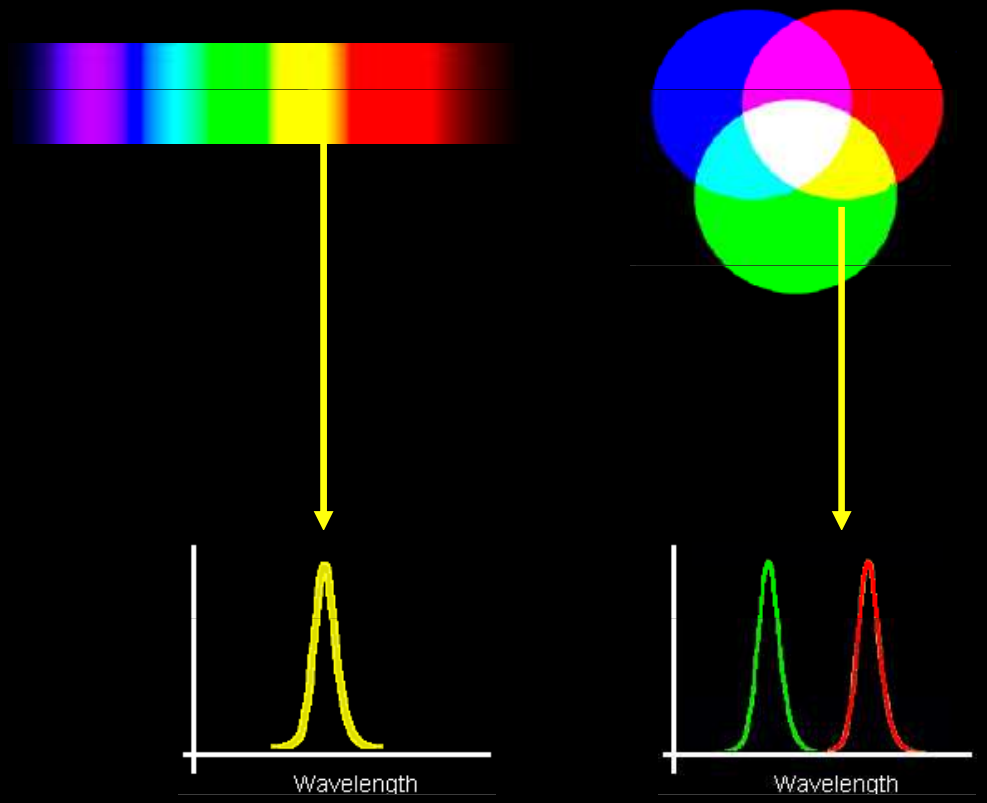




#1 female C57Bl/6, intact
#2 female C57Bl/6, B16F10 GFP
injected i.v. 1x10^6

harvest after 7 days

Color is a “pigment” of our imagination



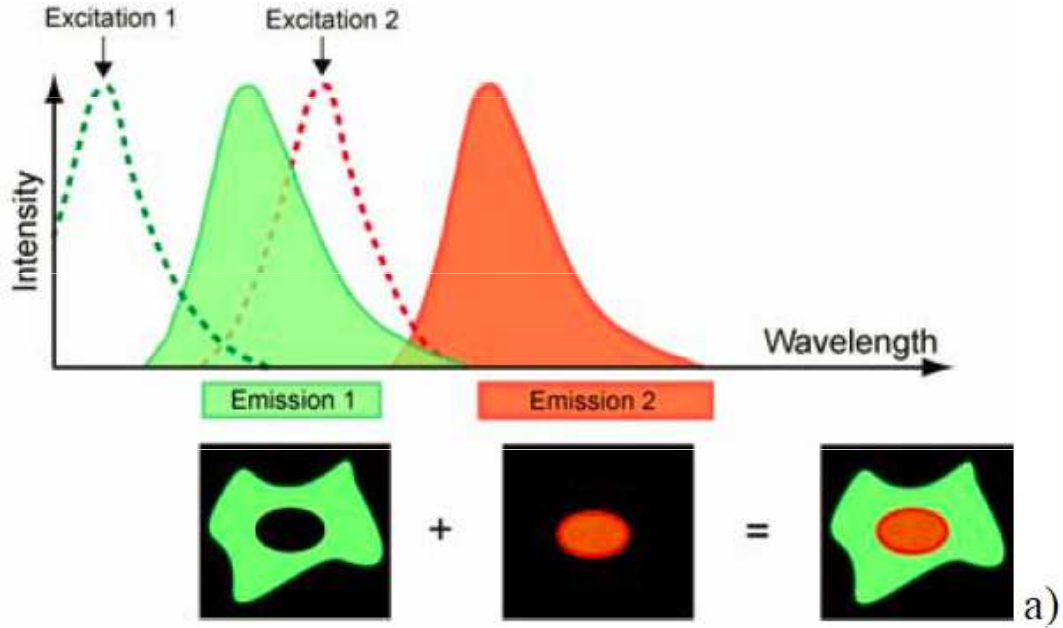
Light has no color

“Color” resides in the eye of the observer

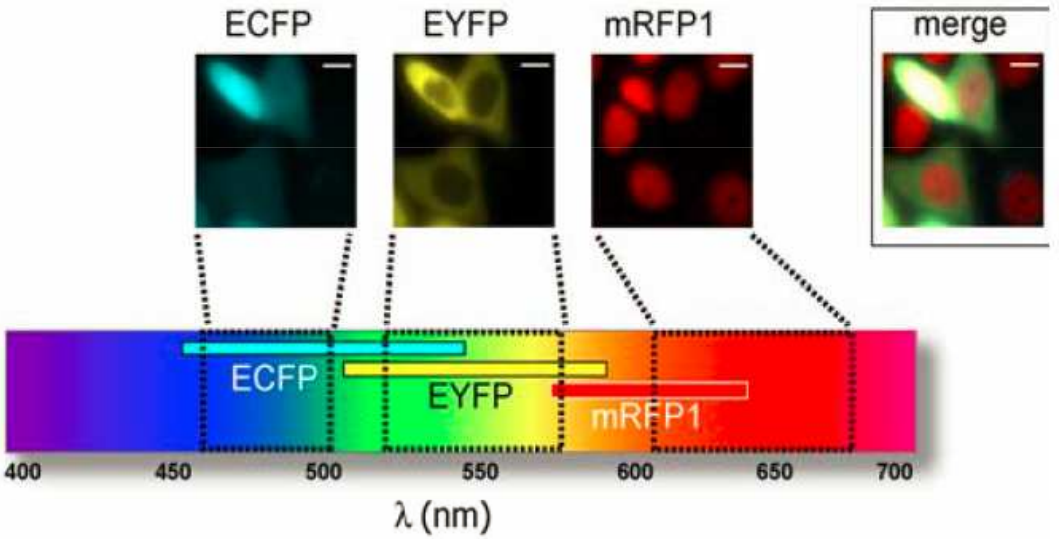
These two yellows are *visually* indistinguishable

BUT multispectral imaging can be used to resolve their spectral differences

Fluorescence unmixing in cell biological research



a)



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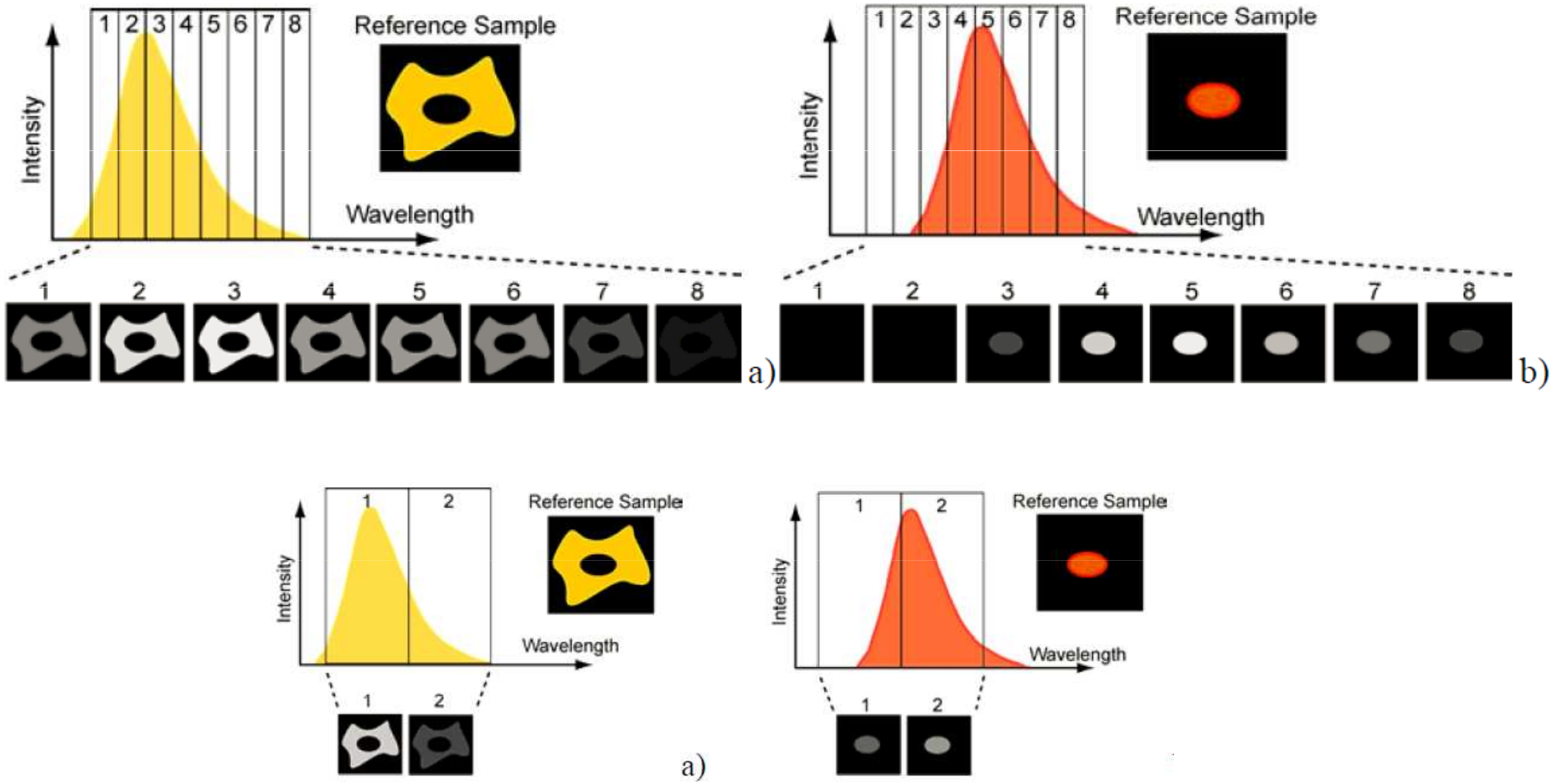
Spectral unmixing: basic principle



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Spectral unmixing: results

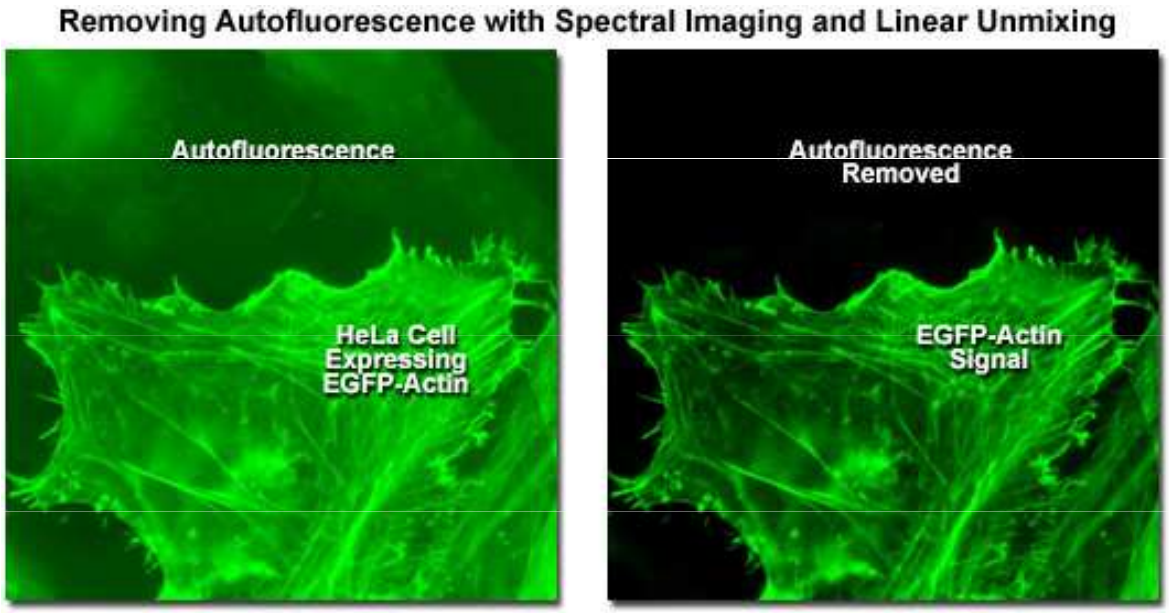
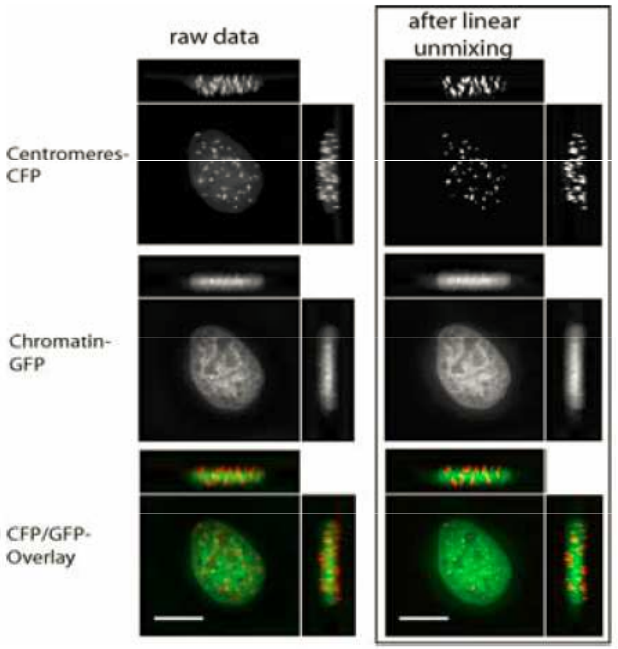


Figure 8



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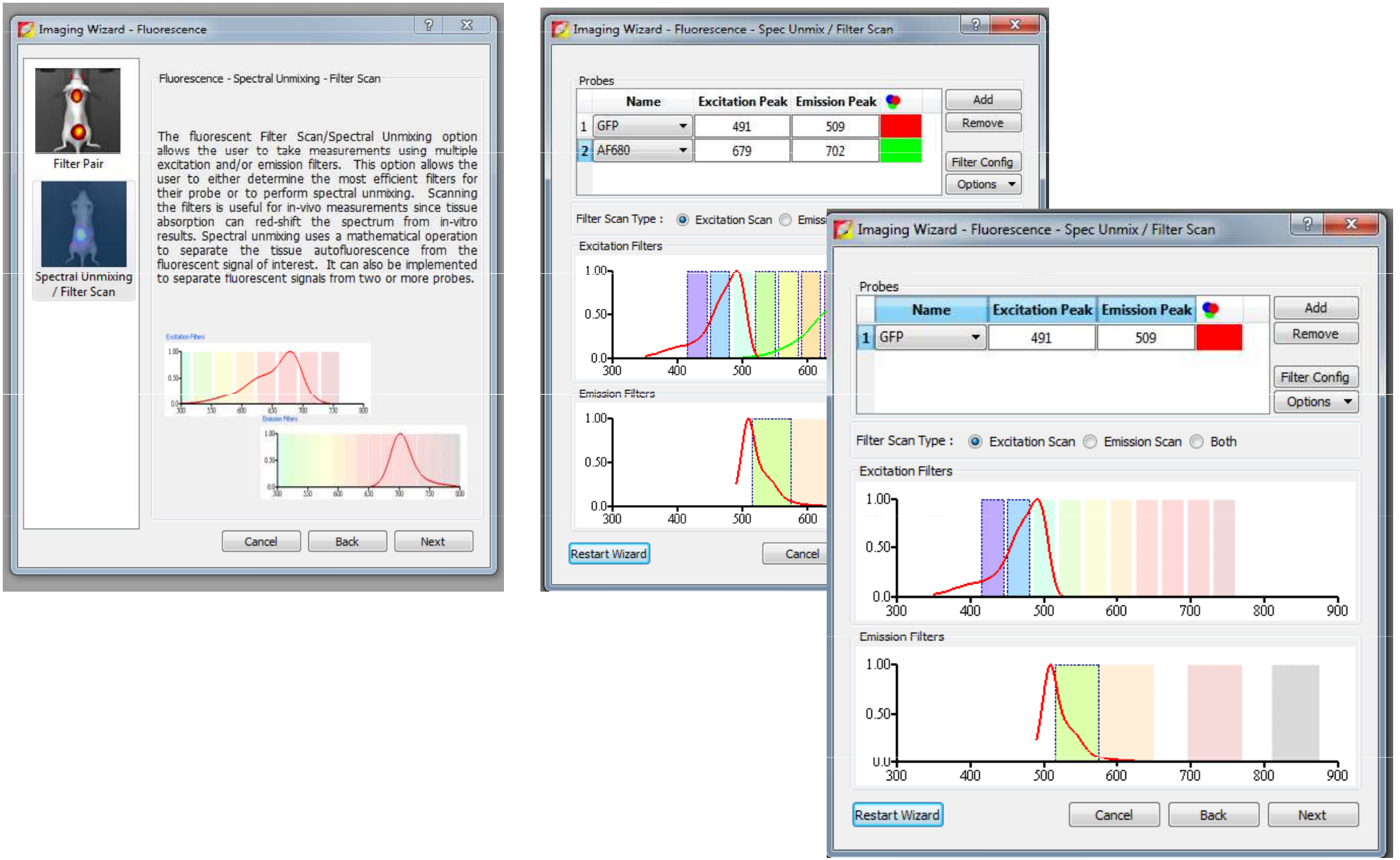
IntegriSense 680 Fluorescent Imaging Agent

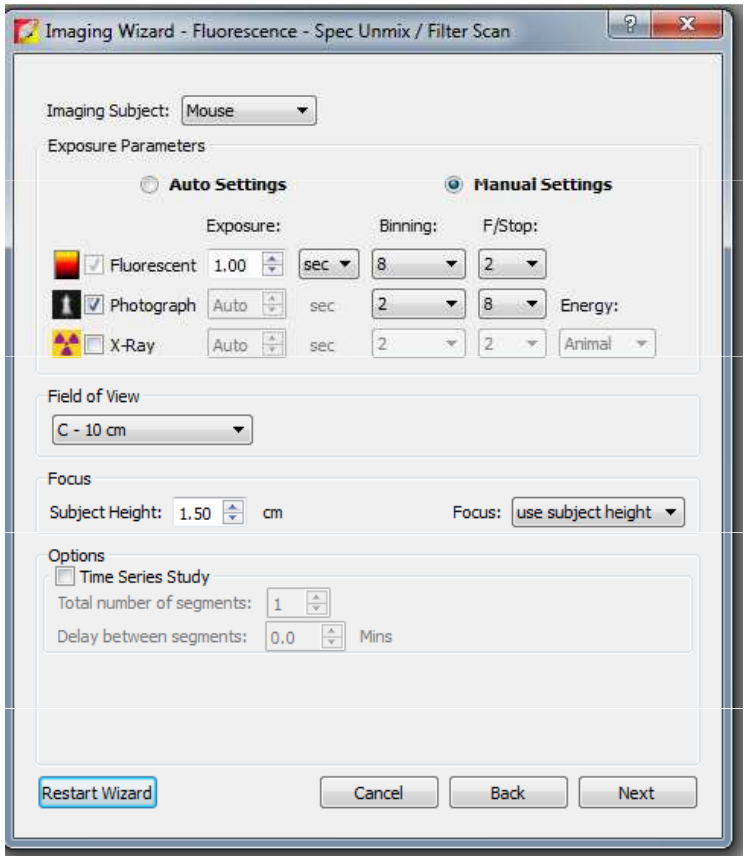
IntegriSense™ 680 fluorescent imaging agent is used for in vivo detection of integrin $\alpha_v\beta_3$ using a low molecular weight peptidomimetic antagonist coupled to a red fluorochrome. IntegriSense 680, an integrin-targeted molecular imaging agent allows the non-invasive imaging of disease status and progression.

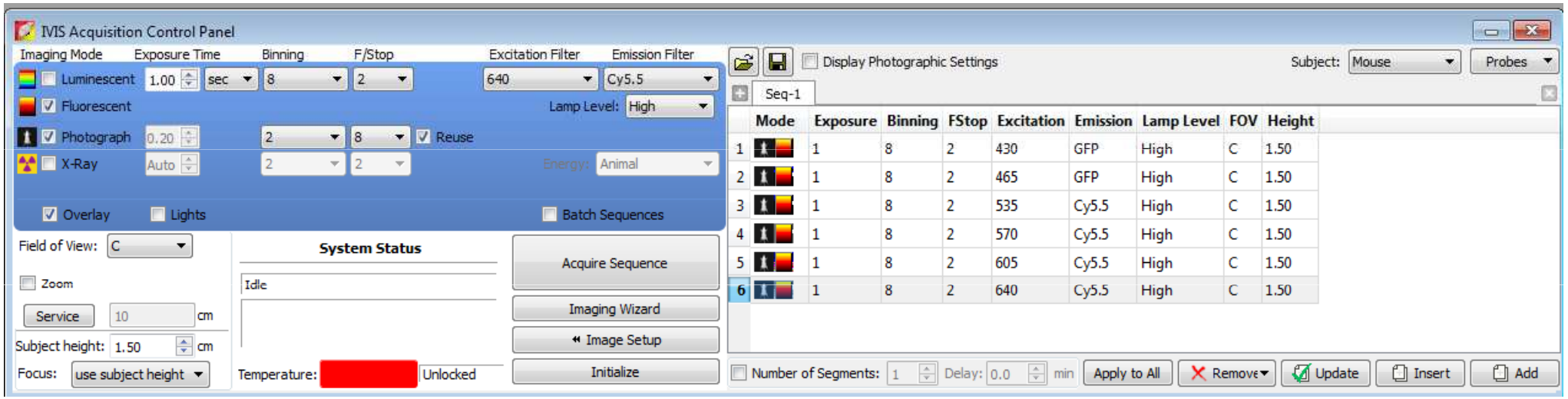
PerkinElmer
For the Better

Excitation unmixing: Setup

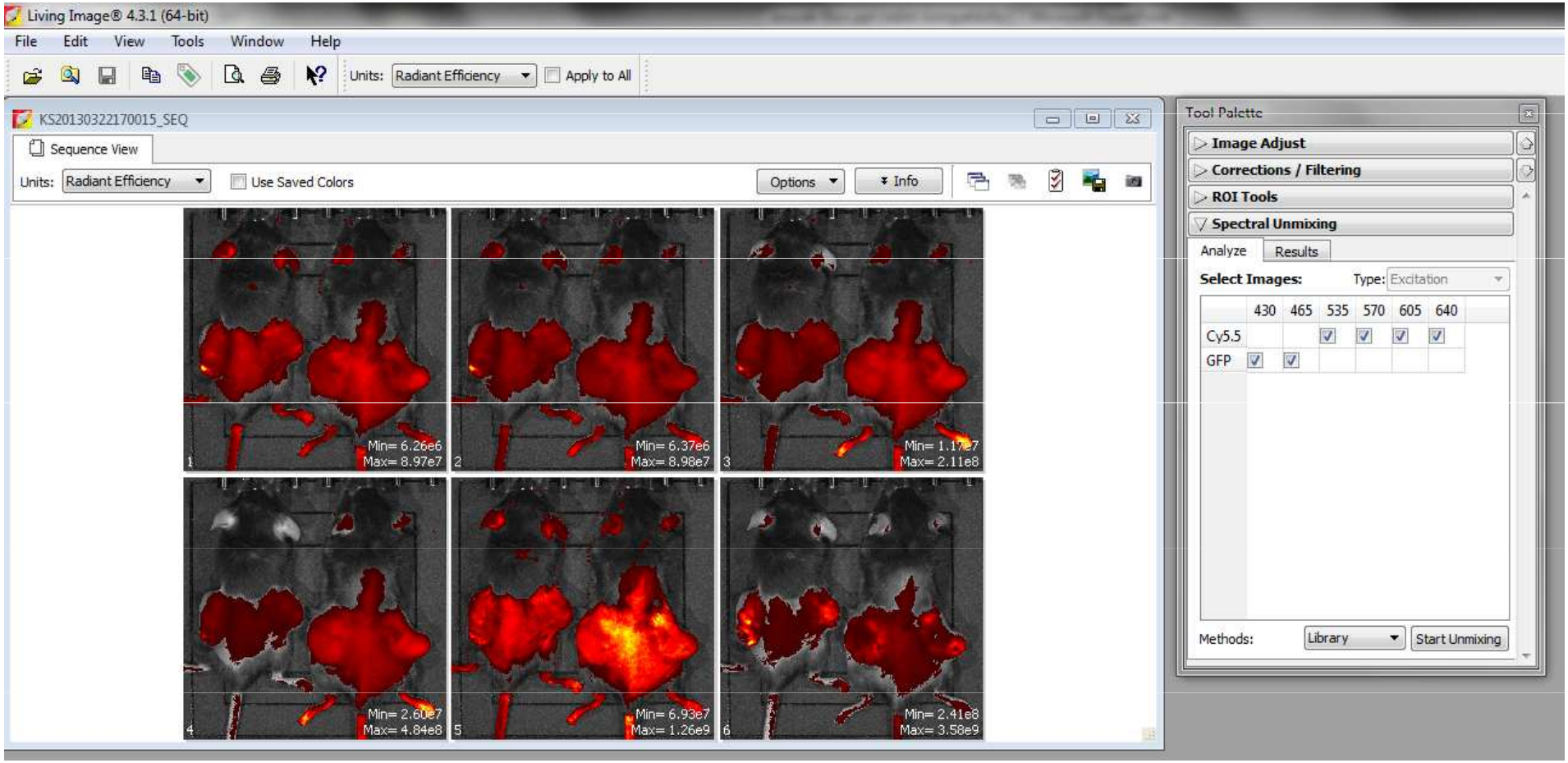


Excitation unmixing: Setup

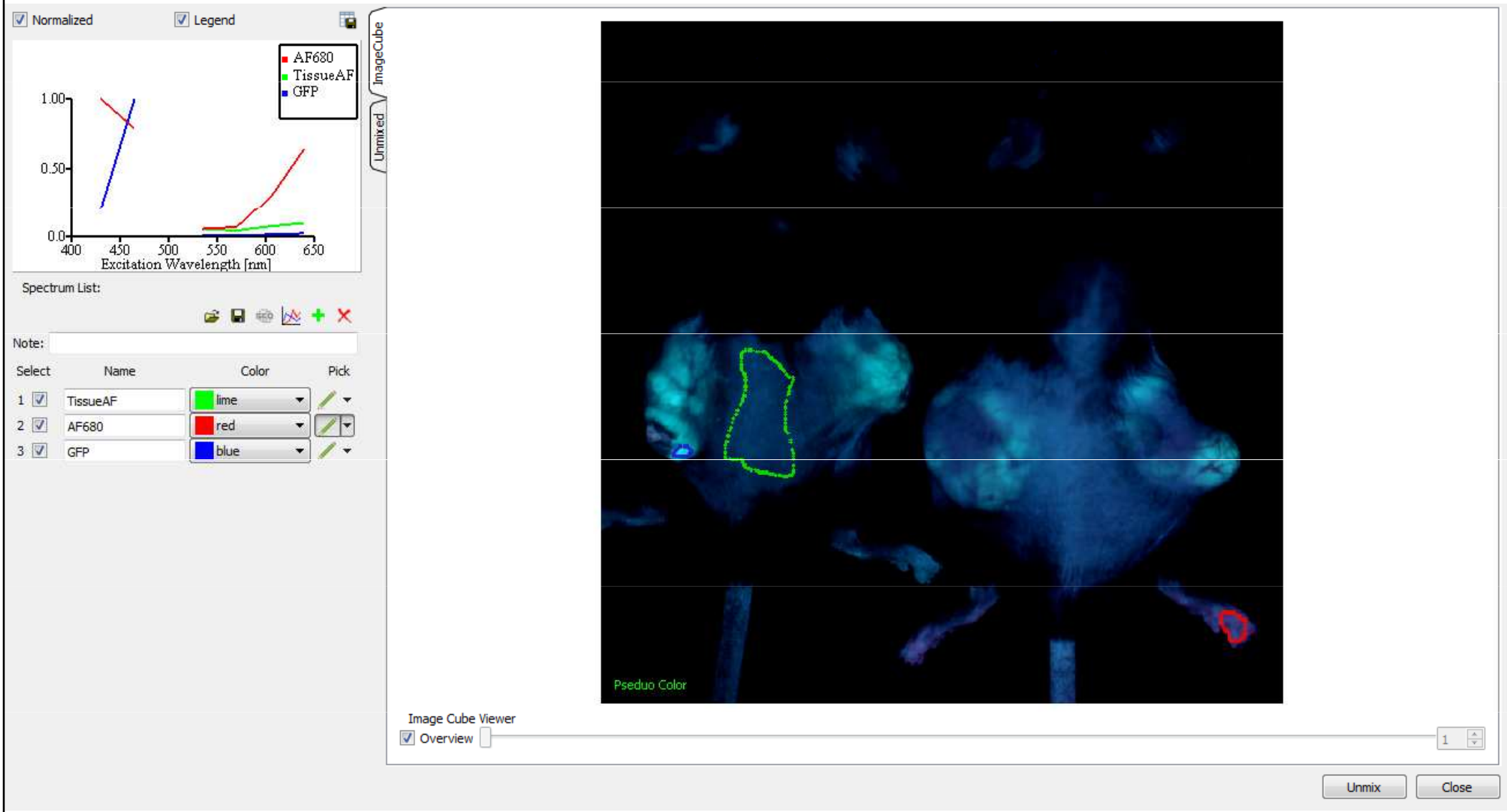




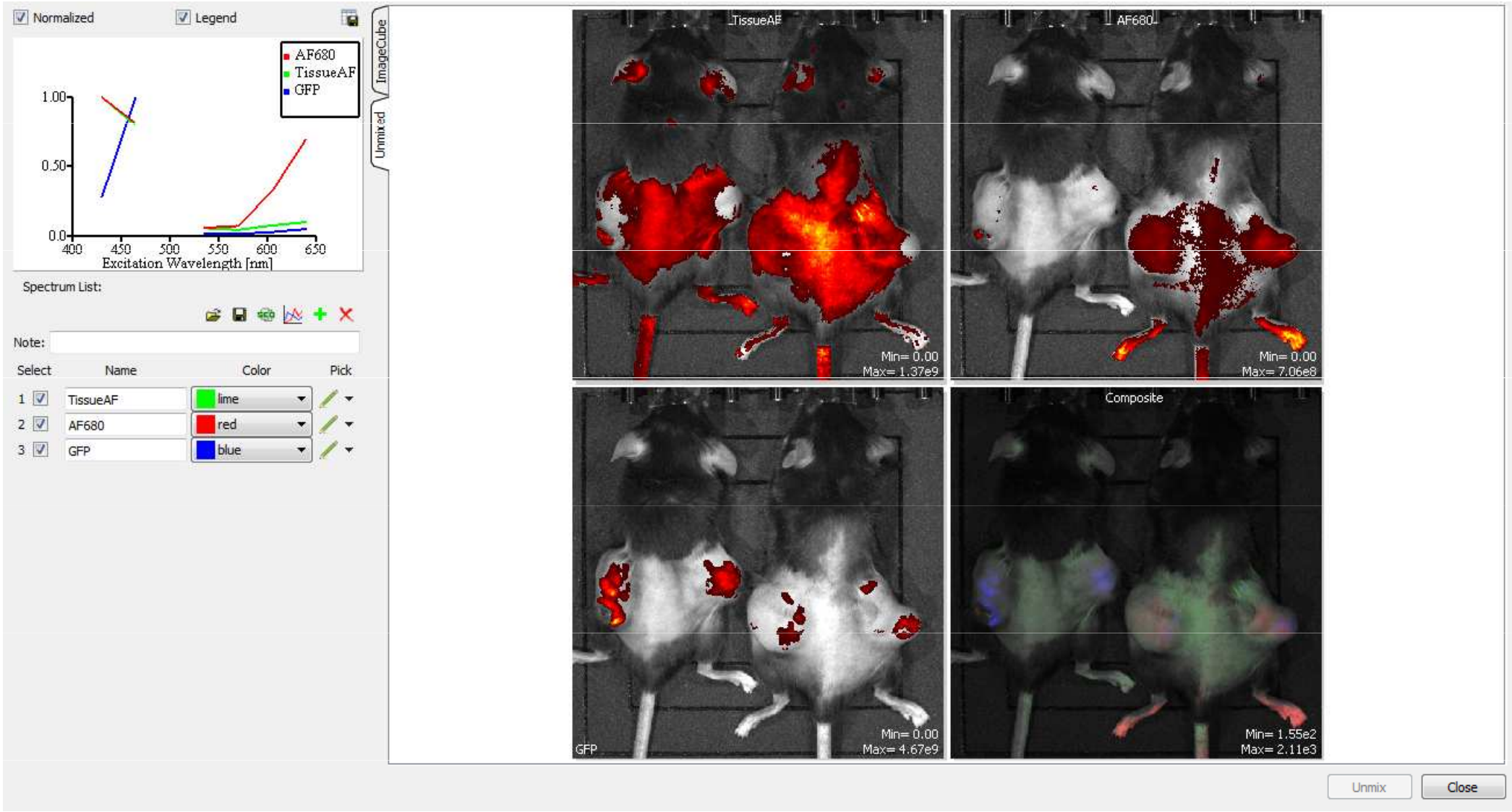
Excitation unmixing: Raw data - Autoflurescence, GFP, AF680



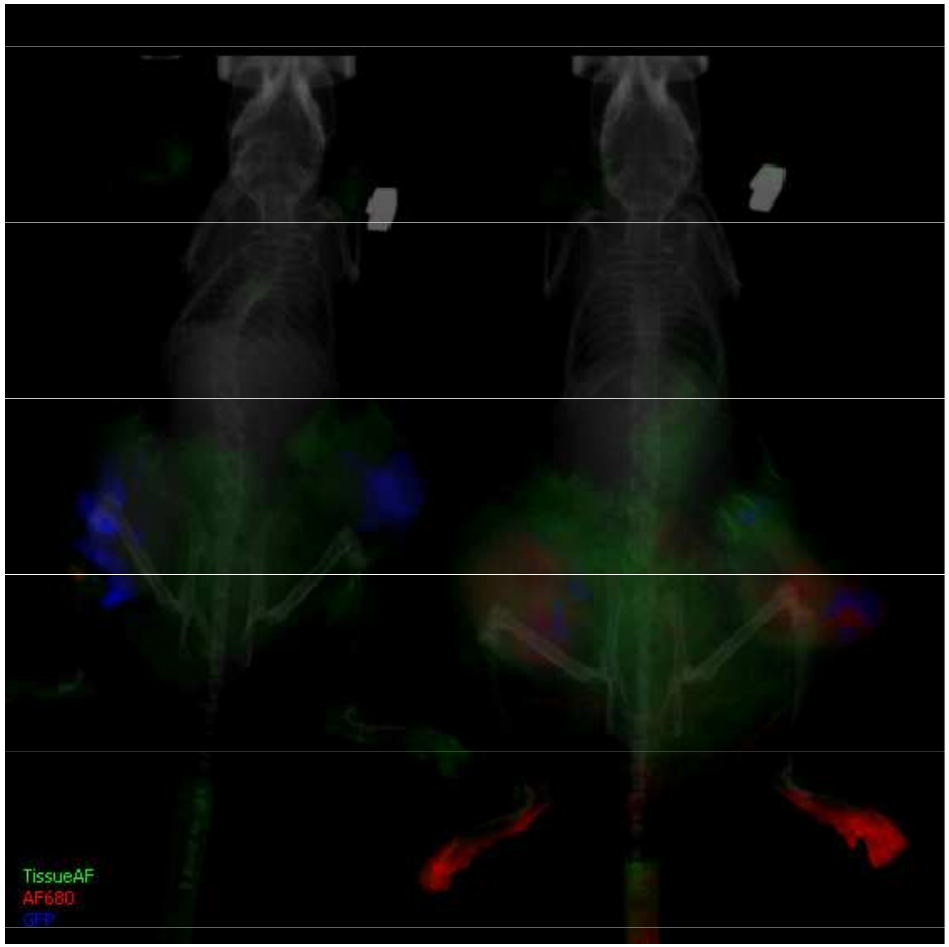
Excitation unmixing: Autoflurescence, GFP, AF680

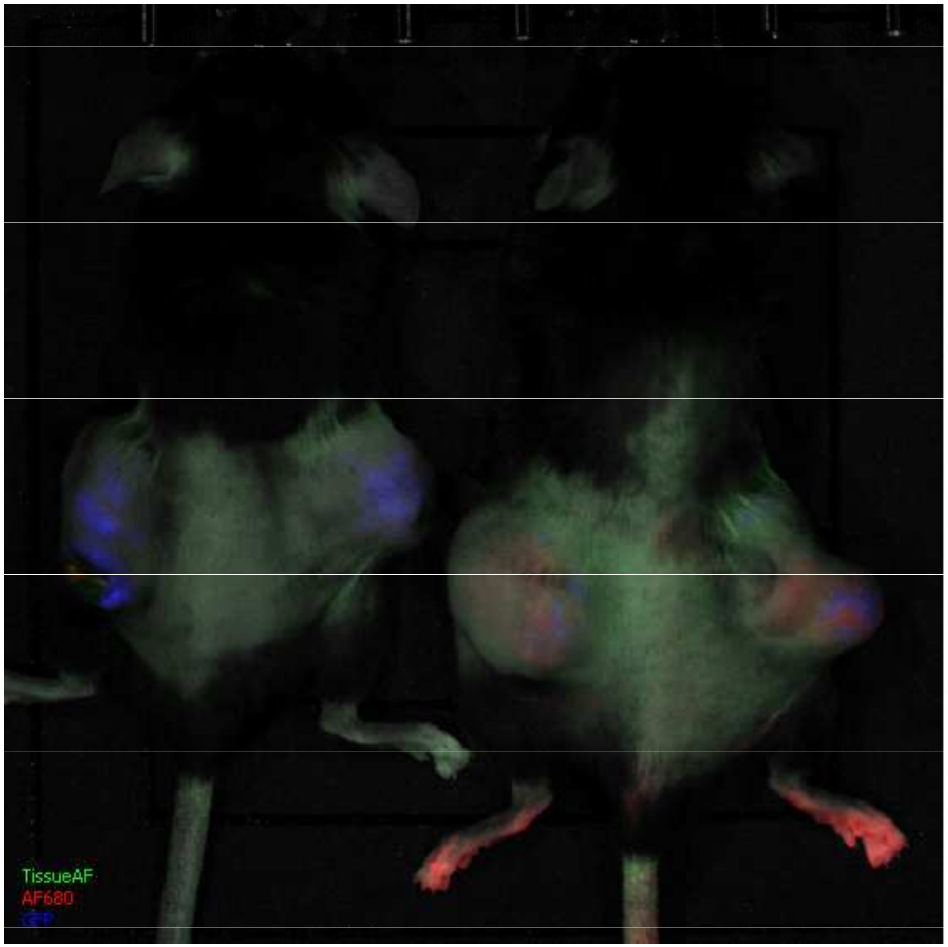


Excitation unmixing: Autoflurescence, GFP, AF680

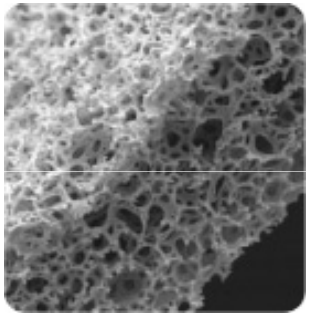


Excitation unmixing: Autoflurescence, GFP, AF680



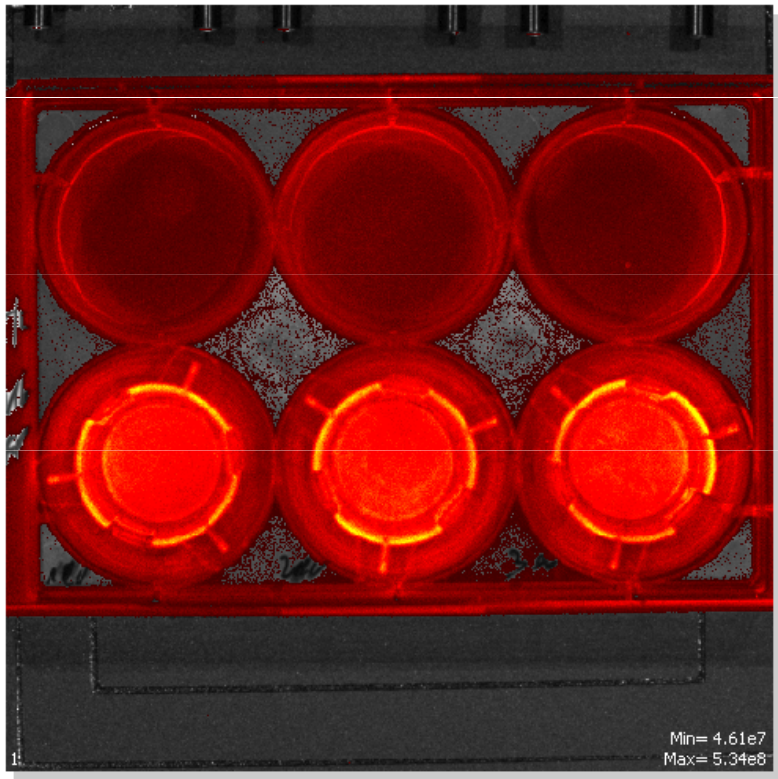


In vitro 3D imaging using Lumina XR: autofluorescence & GFP

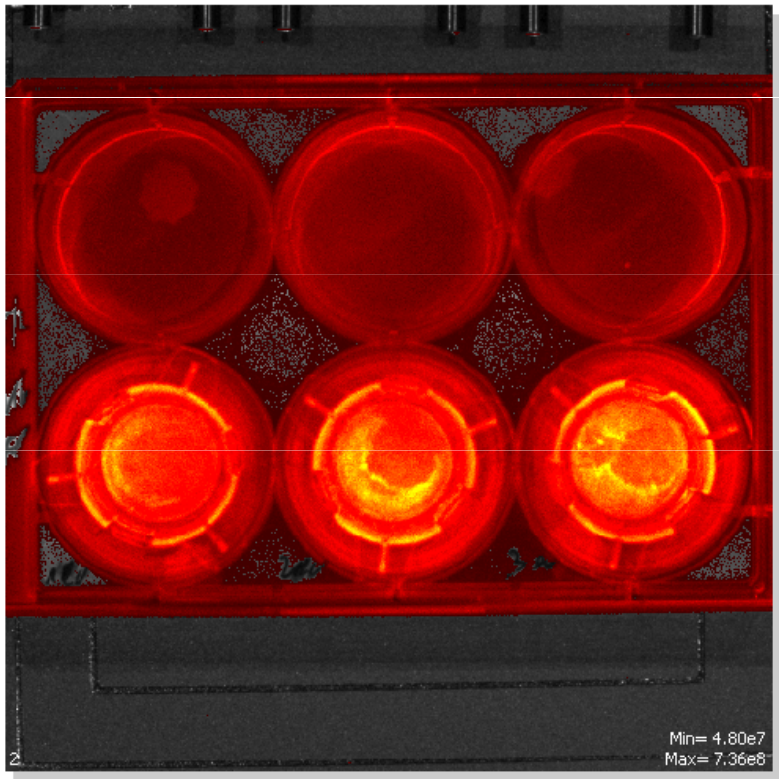


- B16 F10 GFP cells
- seeded 100 000, 200 000, 300 000 cells per insert (**bottom row, 3D**) and the same per well (**top row, 2D**)
- imaging using IVIS Lumina XR
- excitation from the top, plate without the lid
- data analysis using spectral unmixing

Ex. 430/Em. 515-575

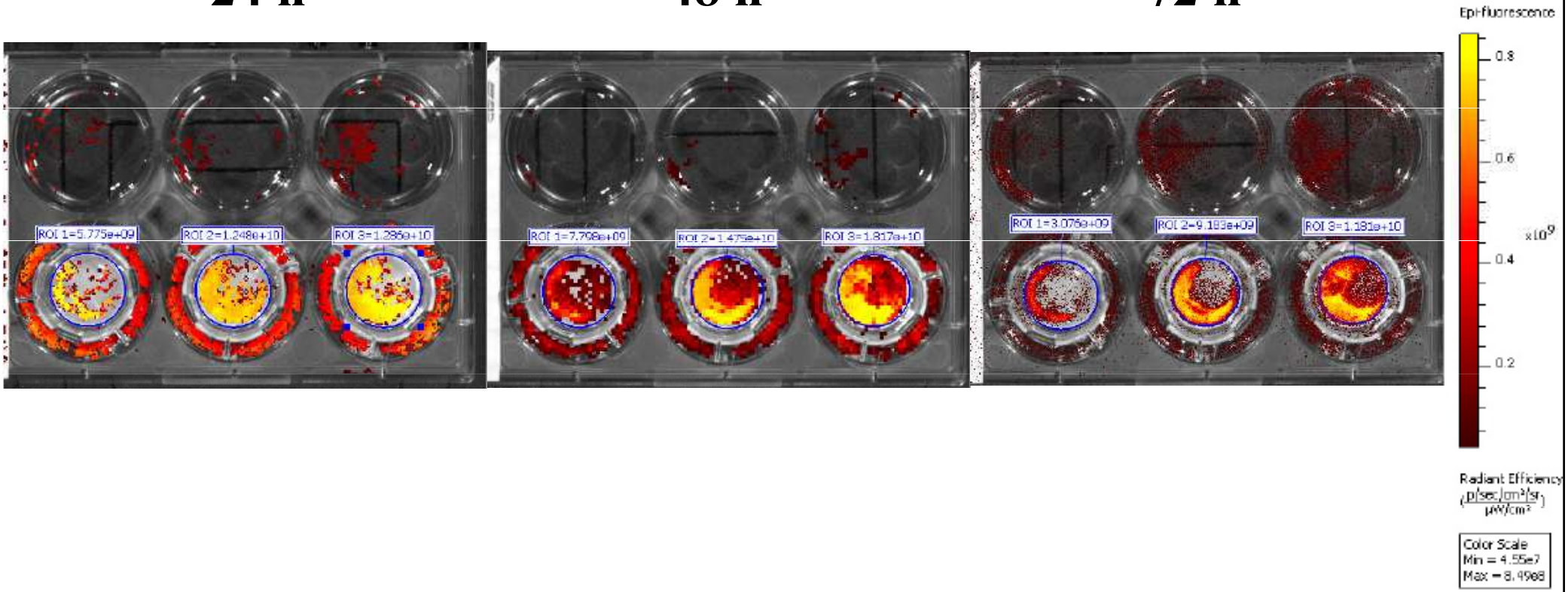


Ex. 465/Em. 515-575



In vitro 3D imaging using Lumina XR: autofluorescence & GFP

24 h **48 h** **72 h**



IVIS Lumina XR souhrn

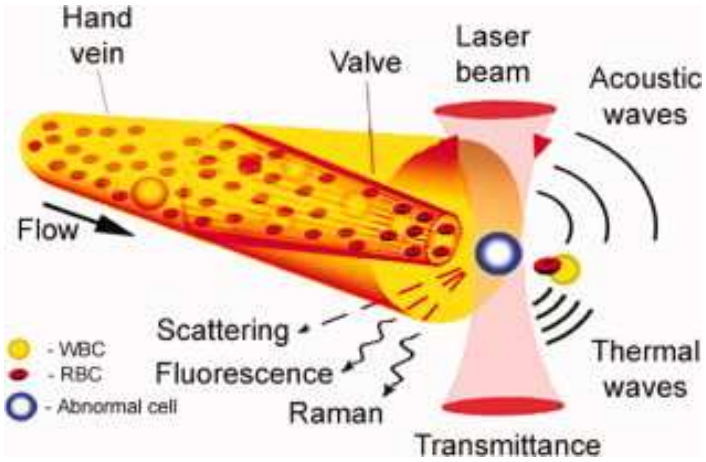
Výhody:

- možnost kombinace detekce luminescence, fluorescence, X-ray
- intuitivní ovládání
- option Z-FOV 2.5 x 2.5 cm
- spektrální unmixing

Limitace:

- omezený počet emisních filtrů
- aktuální konfigurace FOV max. 12.5 x 12.5 cm (tendr option 24 x 23 cm)
- zatím jen syngenní *in vivo* modely
- X-ray pouze pro malé hlodavce a FOV max. 10 x 10 cm
- 3D object - > 2D image

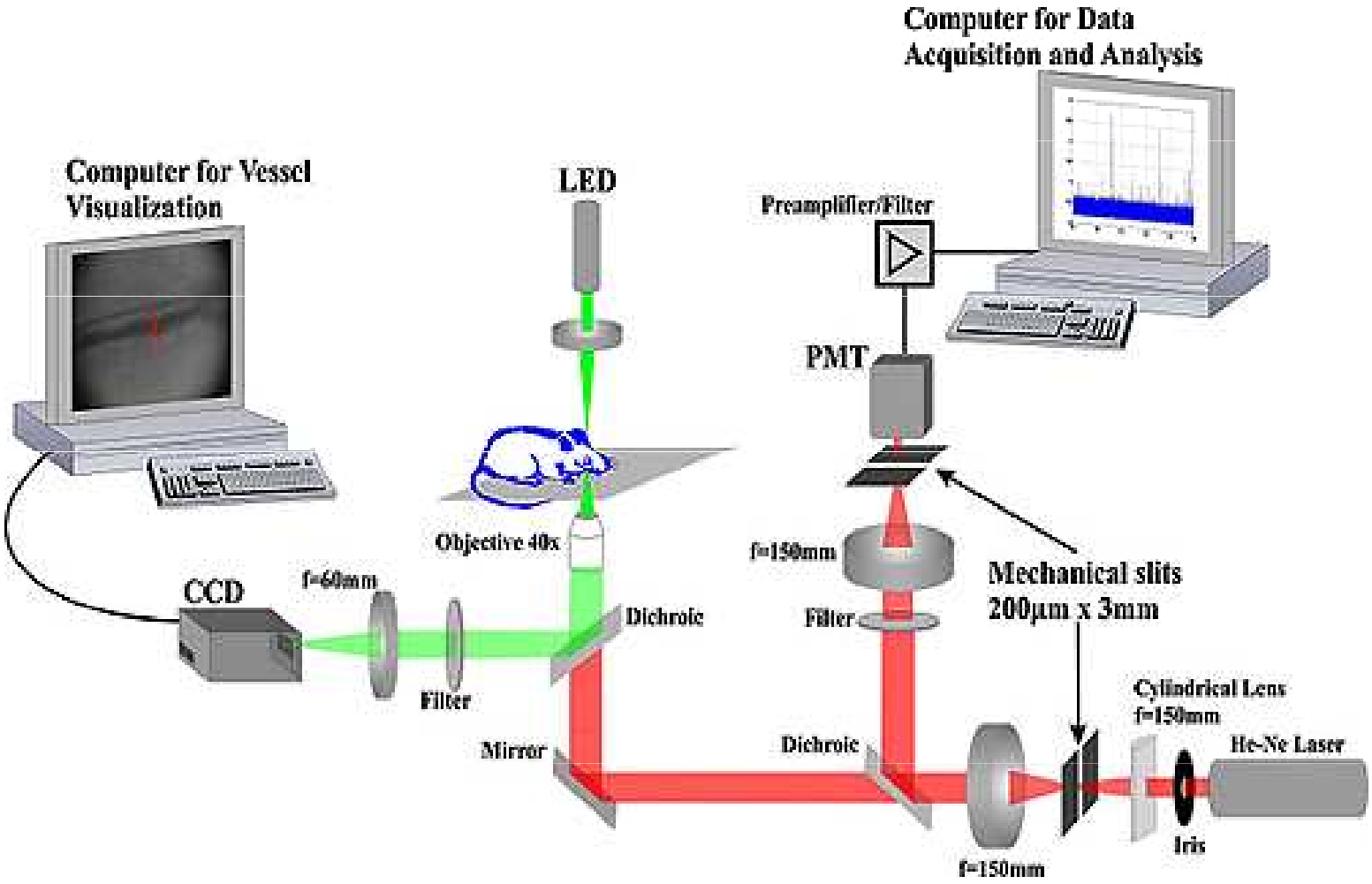
in vivo flow cytometry – základní principy



- 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

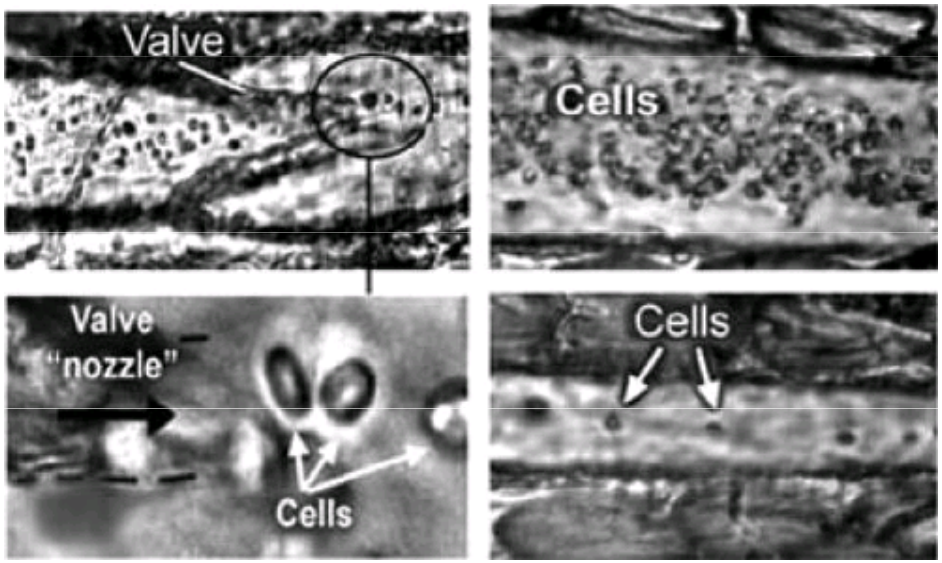
in vivo flow cytometry



Cytometry part A 79A: 737-745, 2011

in vivo flow cytometry – bez značení

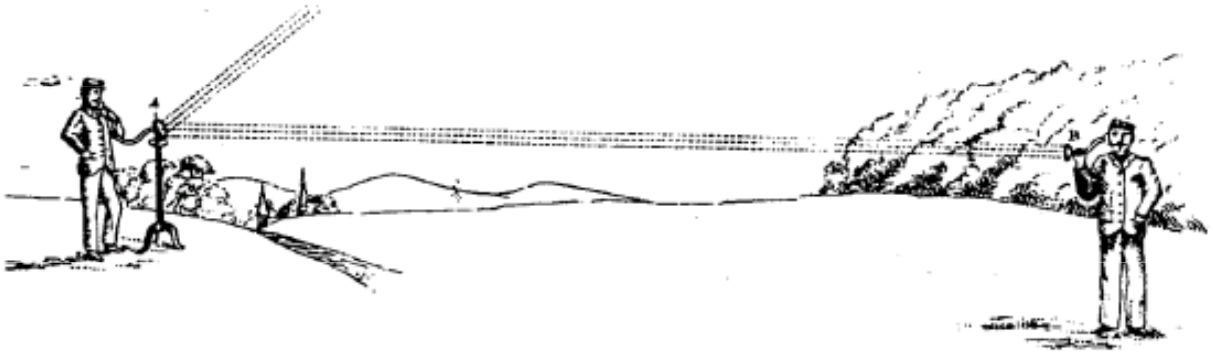
- Nahrávka videa pomocí vysokorychlostní CCD nebo CMOS kamery s vysokým rozlišením v režimu propustnosti nebo odrazu.
- Příklad: high-speed transmittance digital microscopy (TDM)
- Limity: hloubka tkáně.
- TDM může sloužit k navedení zdrojů záření pro další analýzu do určené oblasti.



Cytometry part A 79A: 737-745, 2011

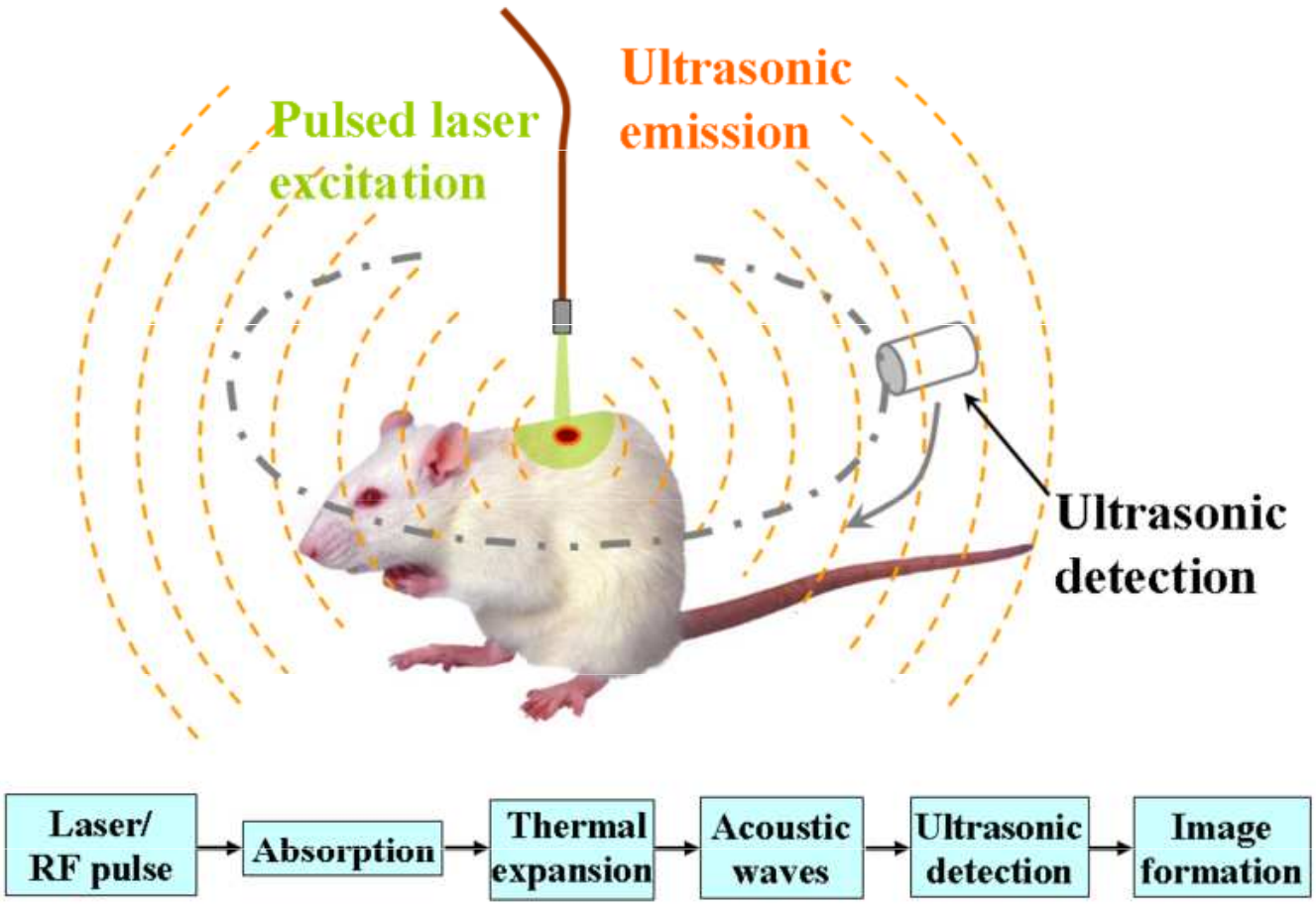
photoacoustic and photothermal imaging

- 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[†,*]

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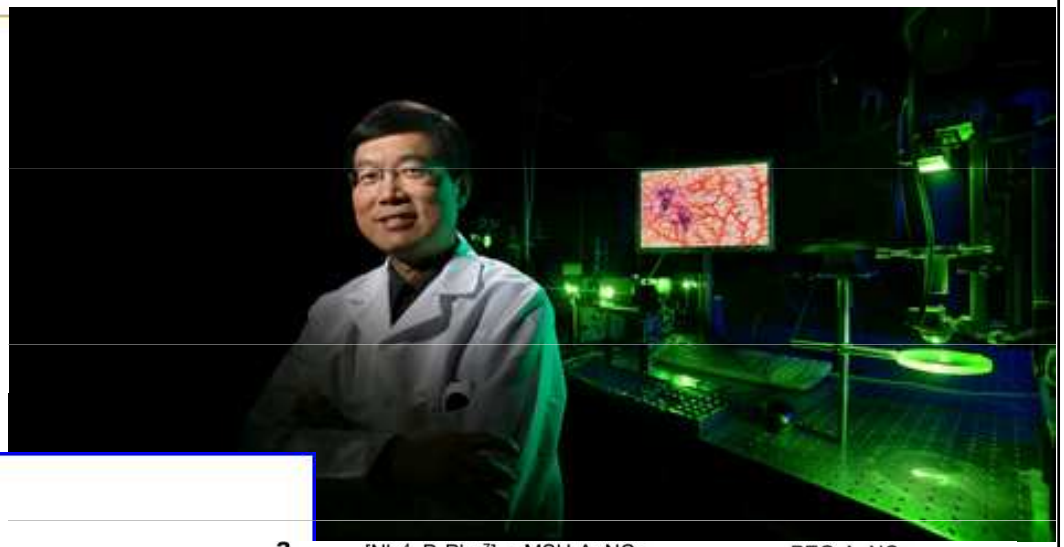
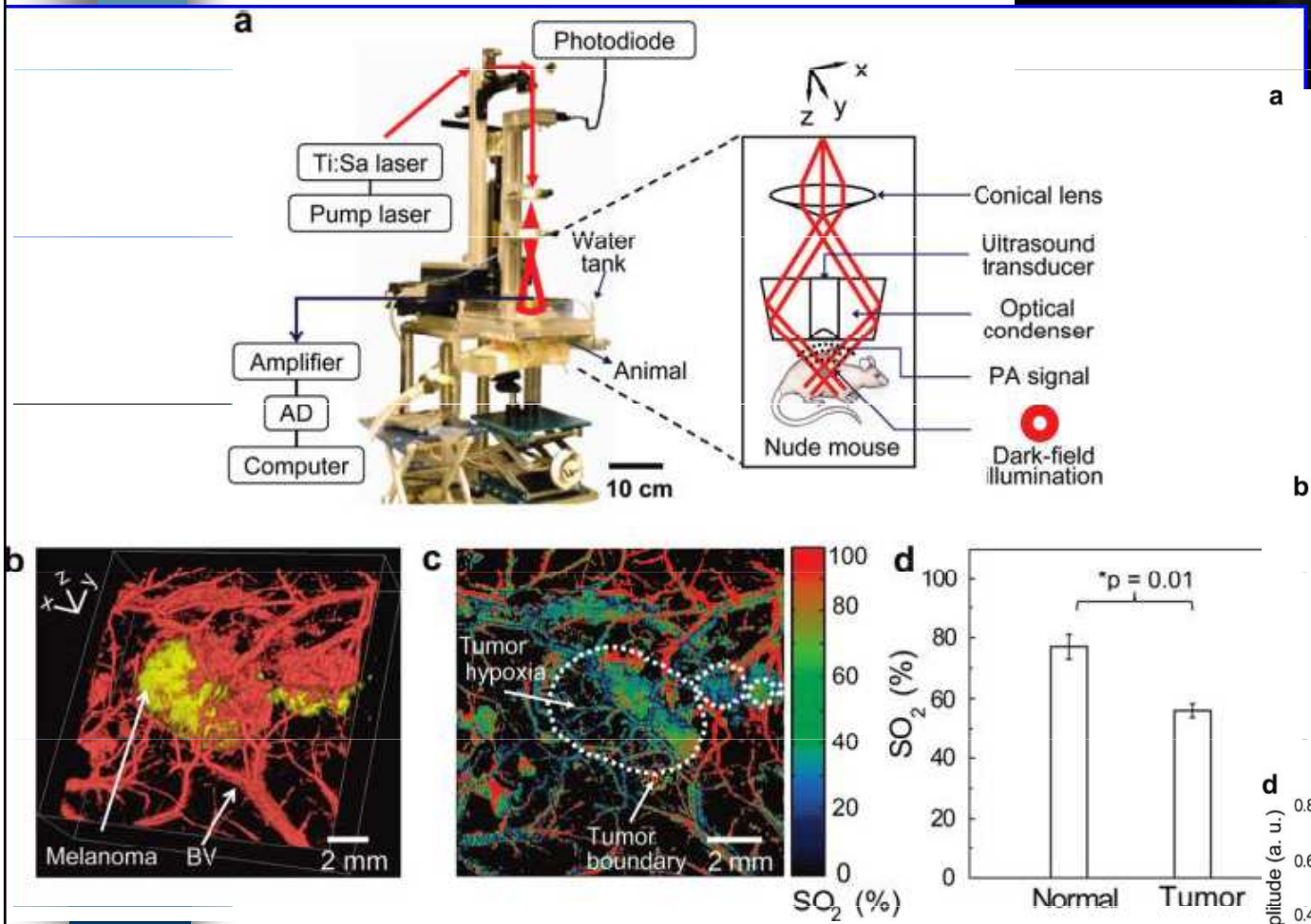
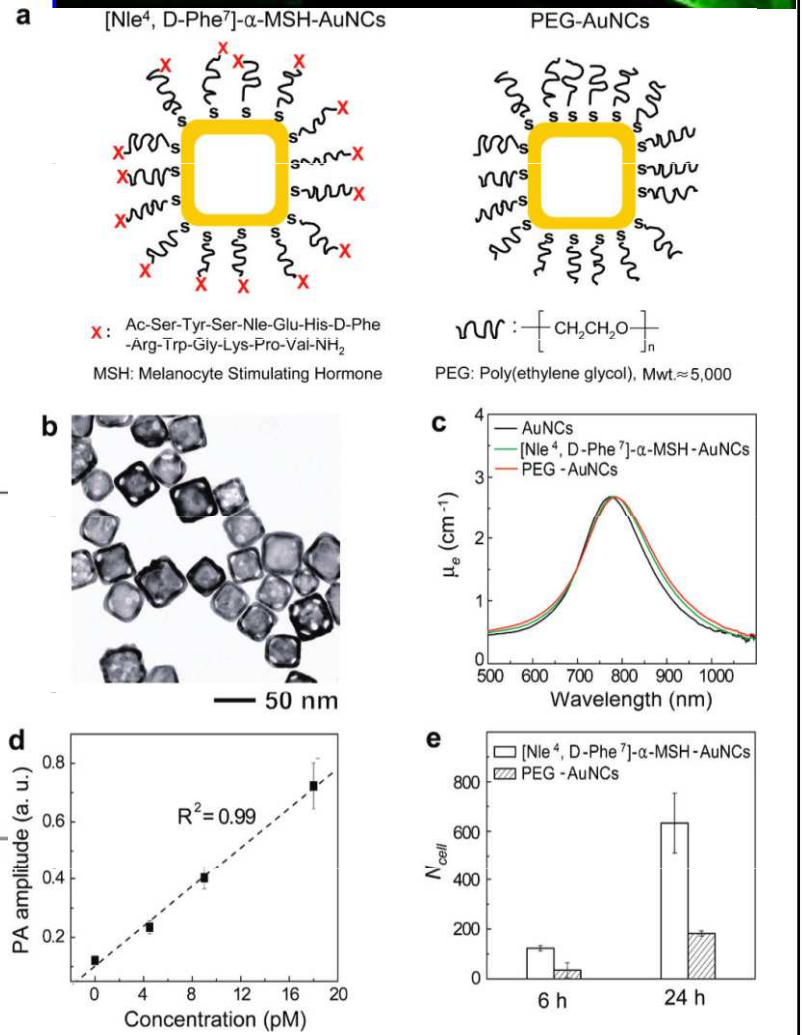


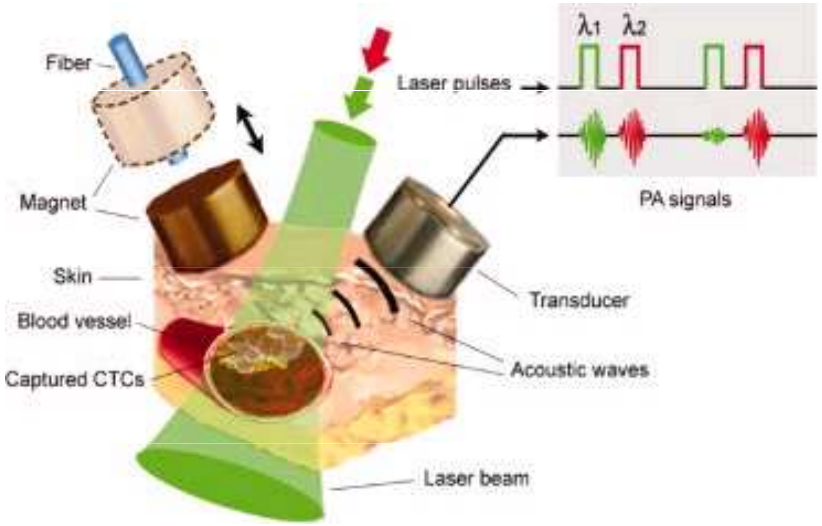


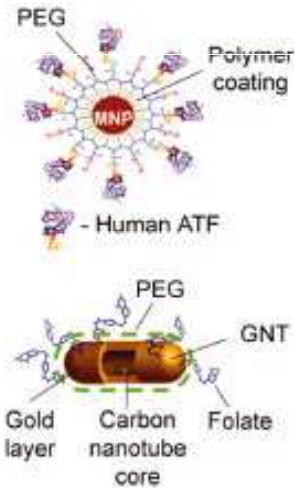


ACS Nano **2010** *4* (8), 4559-4564

In vivo flow cytometrie – detekce specifických signálů

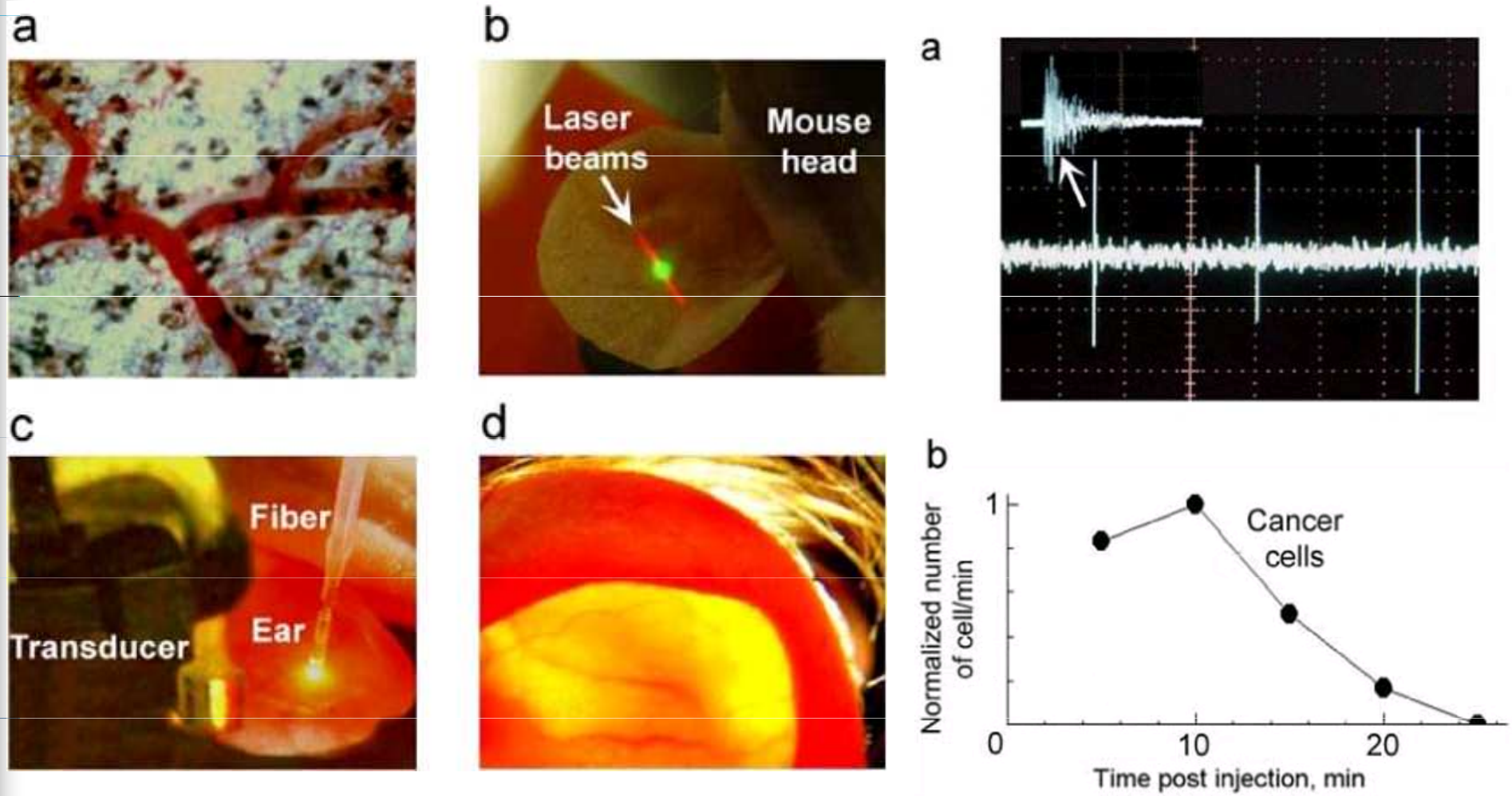
- Detekce fotoakustických a fototermálních jevů





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
- *in vivo* zabrazovací metody

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* zobrazovaní a *in vivo* průtokové cytometrie.