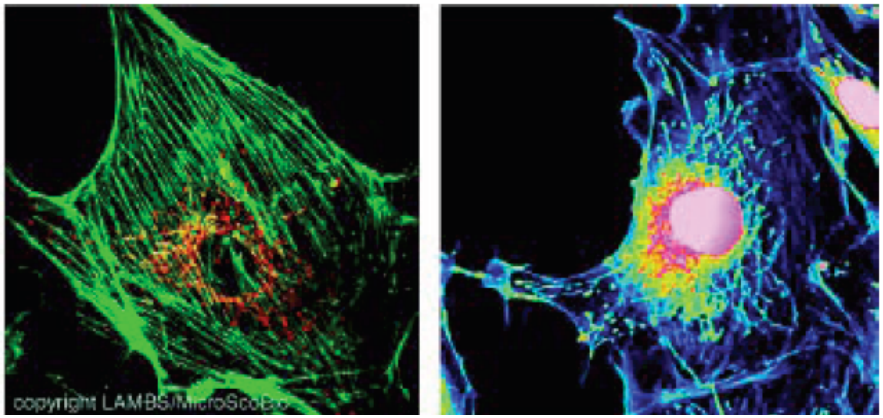
INVESTIGAZIONI FOTONICHE



ALBERTO DIASPRO
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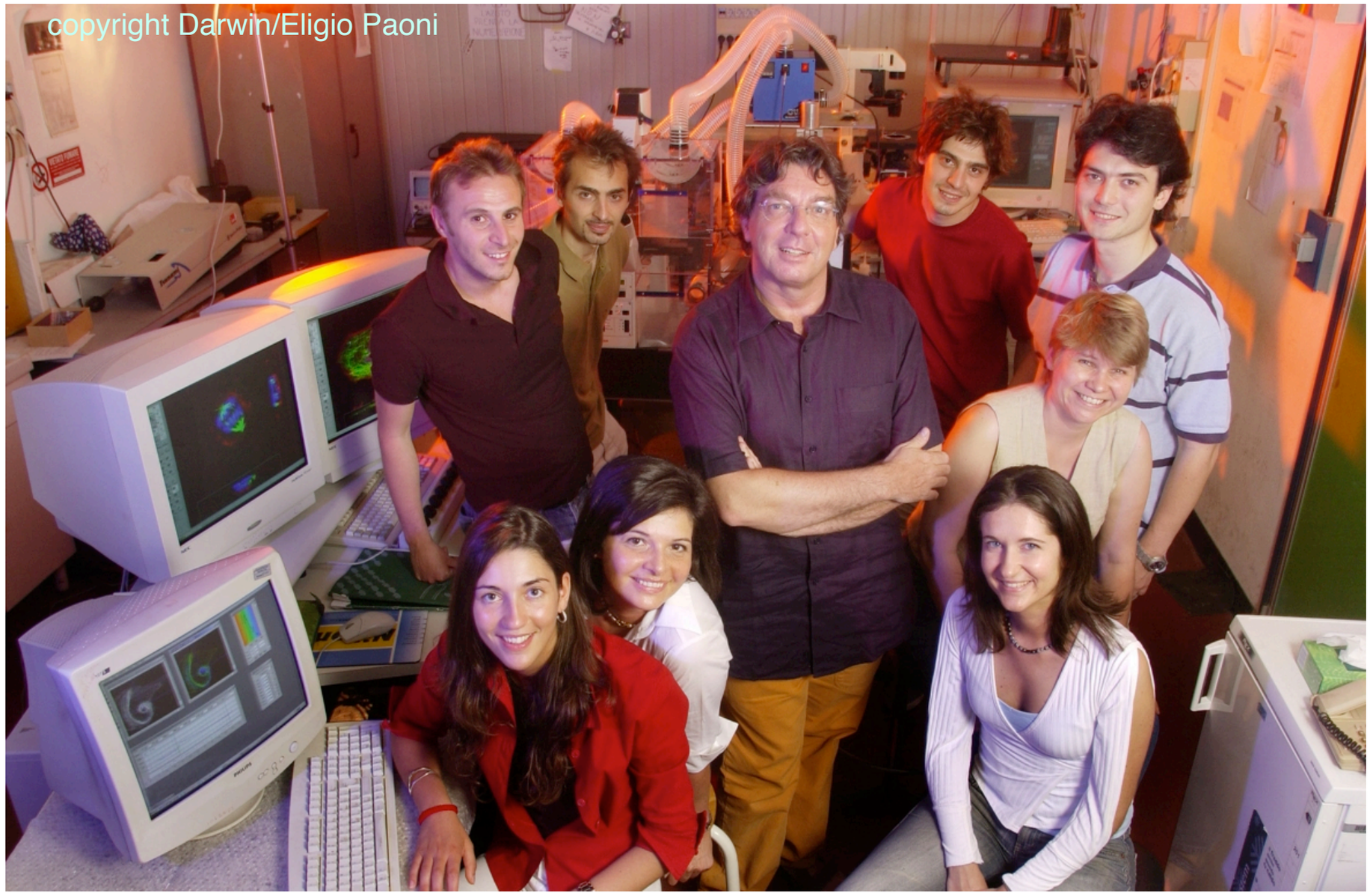
Davide Mazza
Ilaria Testa
Mattia Pesce
Federica Morotti
Francesco Di Fato
Francesca Cella
Emiliano Ronzitti
Cristina Barmo
Johnatan Roberts



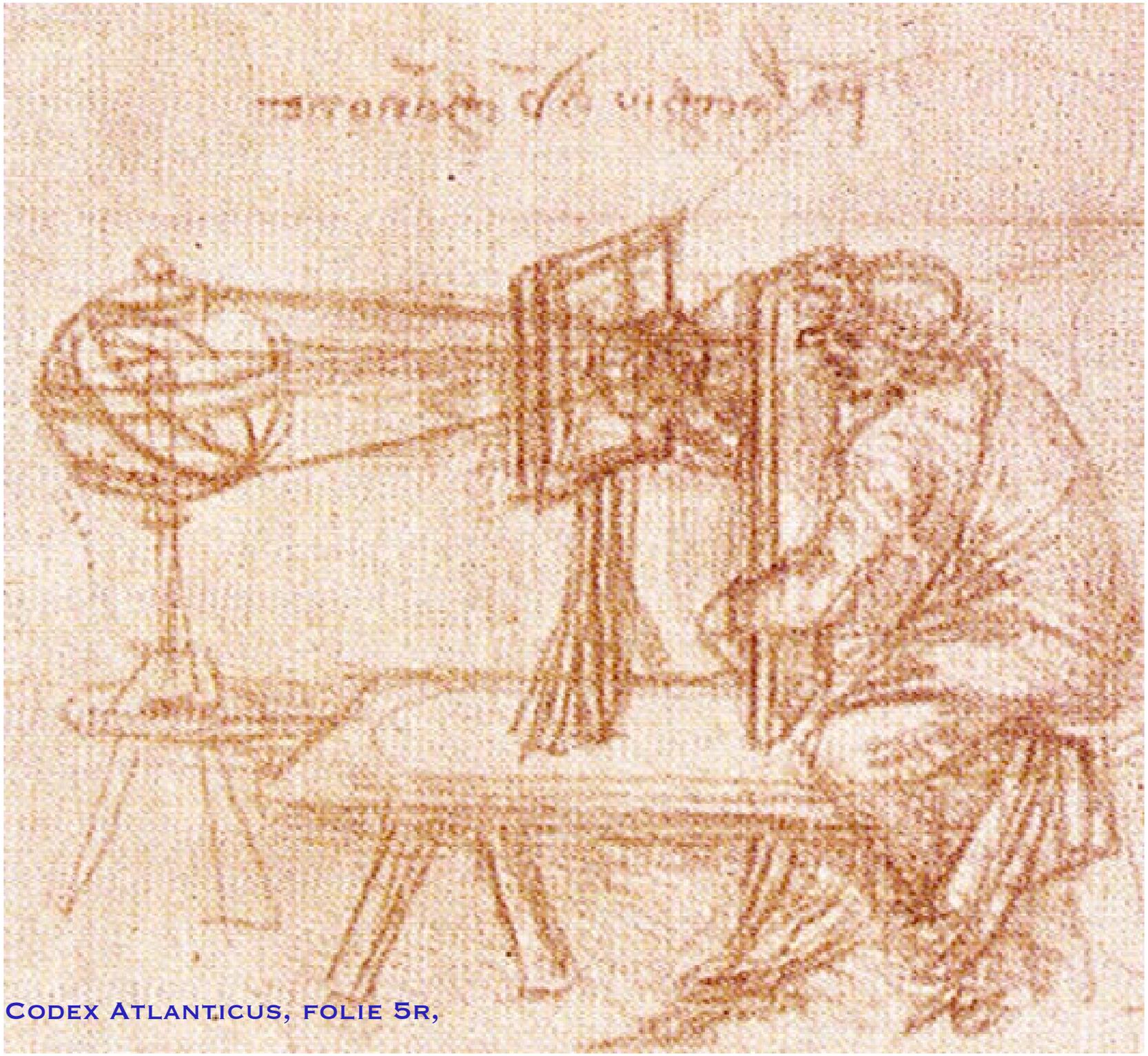
NanoMed Labs
Nanotechnology for bioMedicine

IFOM
ISTITUTO FIRC
DI ONCOLOGIA MOLECOLARE

The IFOM-IEO campus

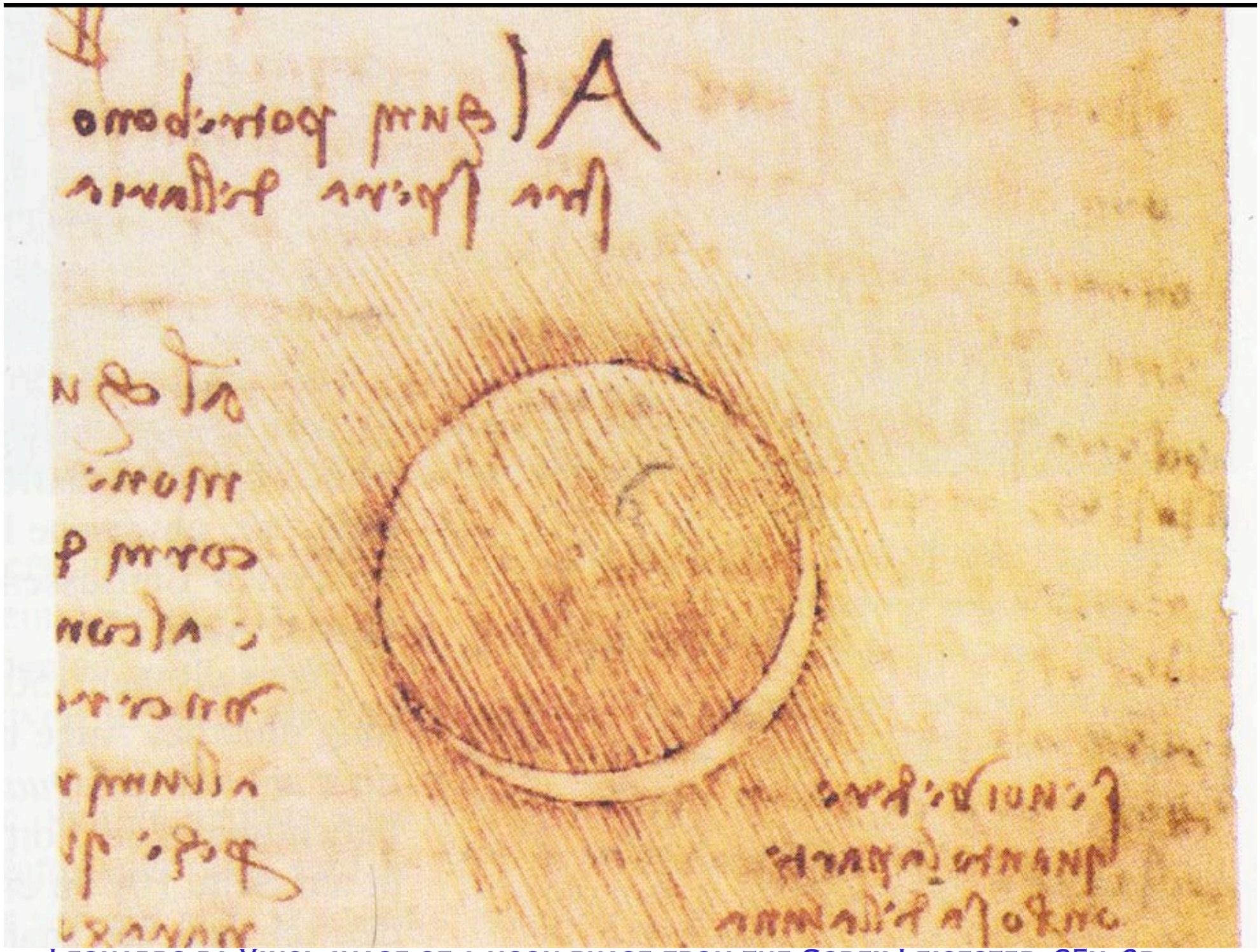
ISTITUTO EUROPEO
DI ONCOLOGIA

OBSERVING EVENTS



Codex Atlanticus, folie 5r,

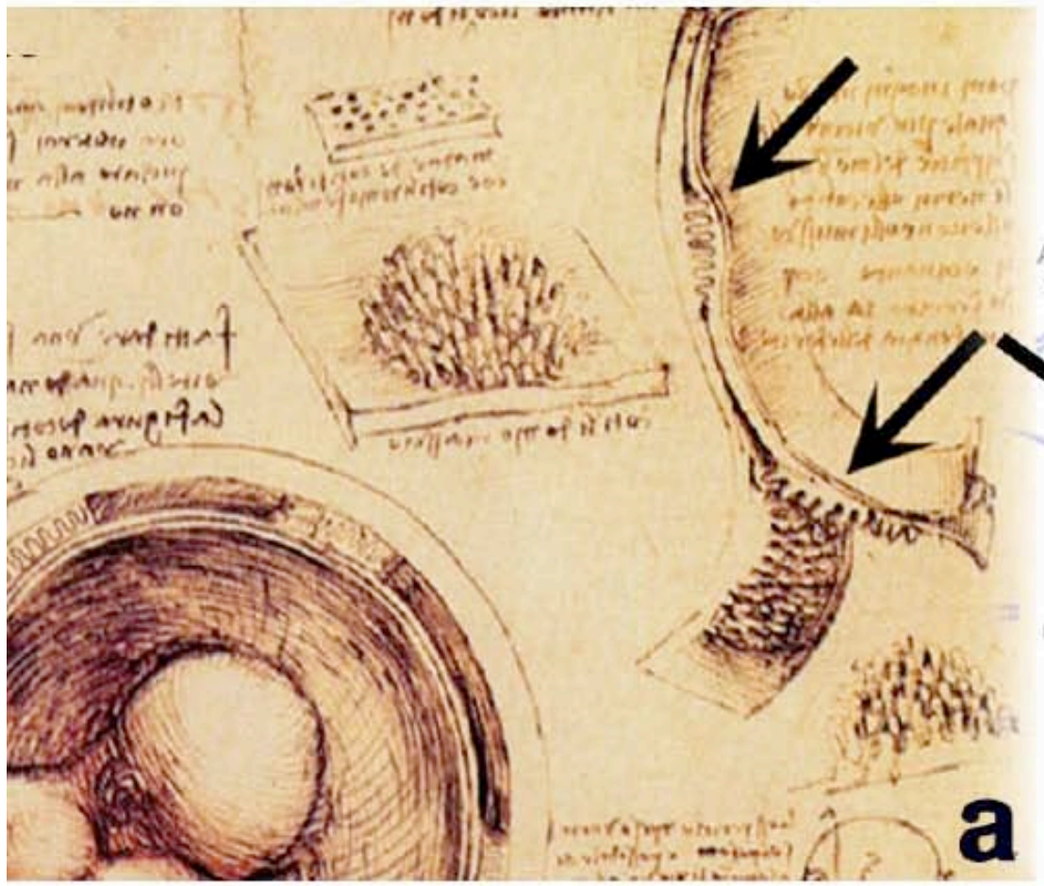
SLIDE CREDIT: Irina Majoul Royal Holloway University of London

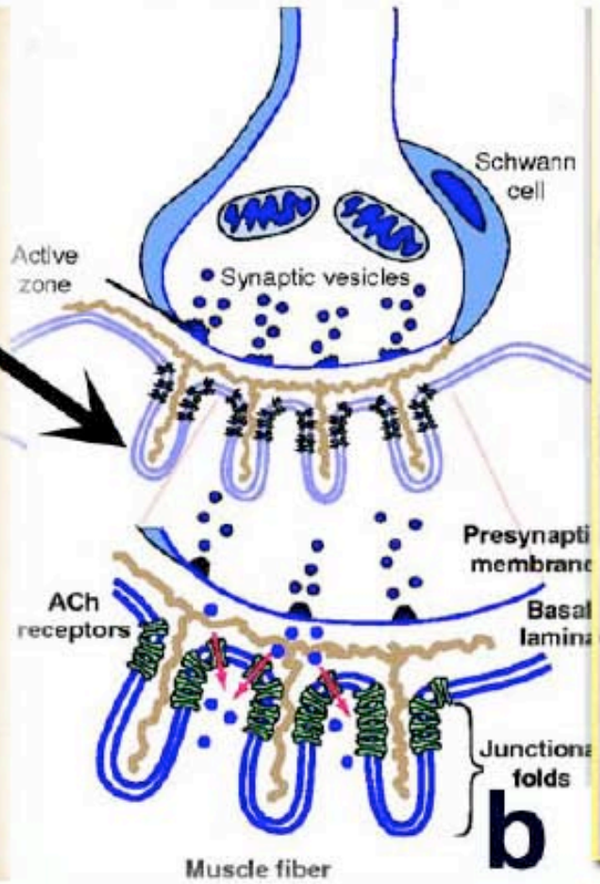


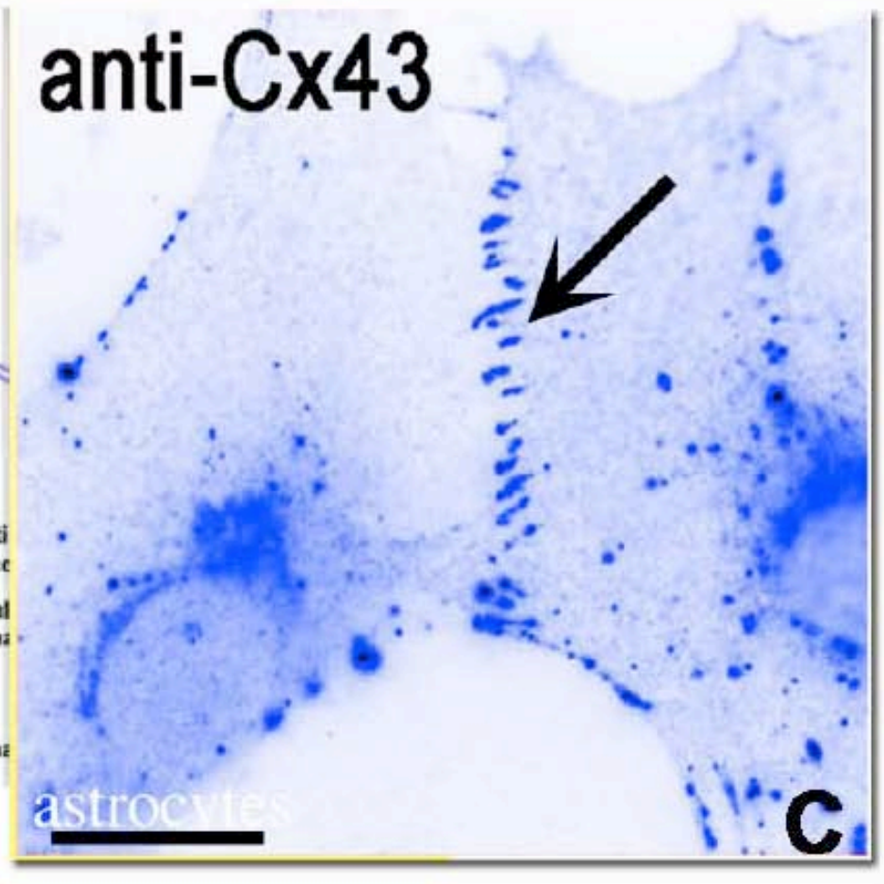
Leonardo da Vinci, image of a moon phase from the Codex Leicester: 35v, 2r

SLIDE CREDIT: Irina Majoul Royal Holloway University of London

OBSERVING EVENTS



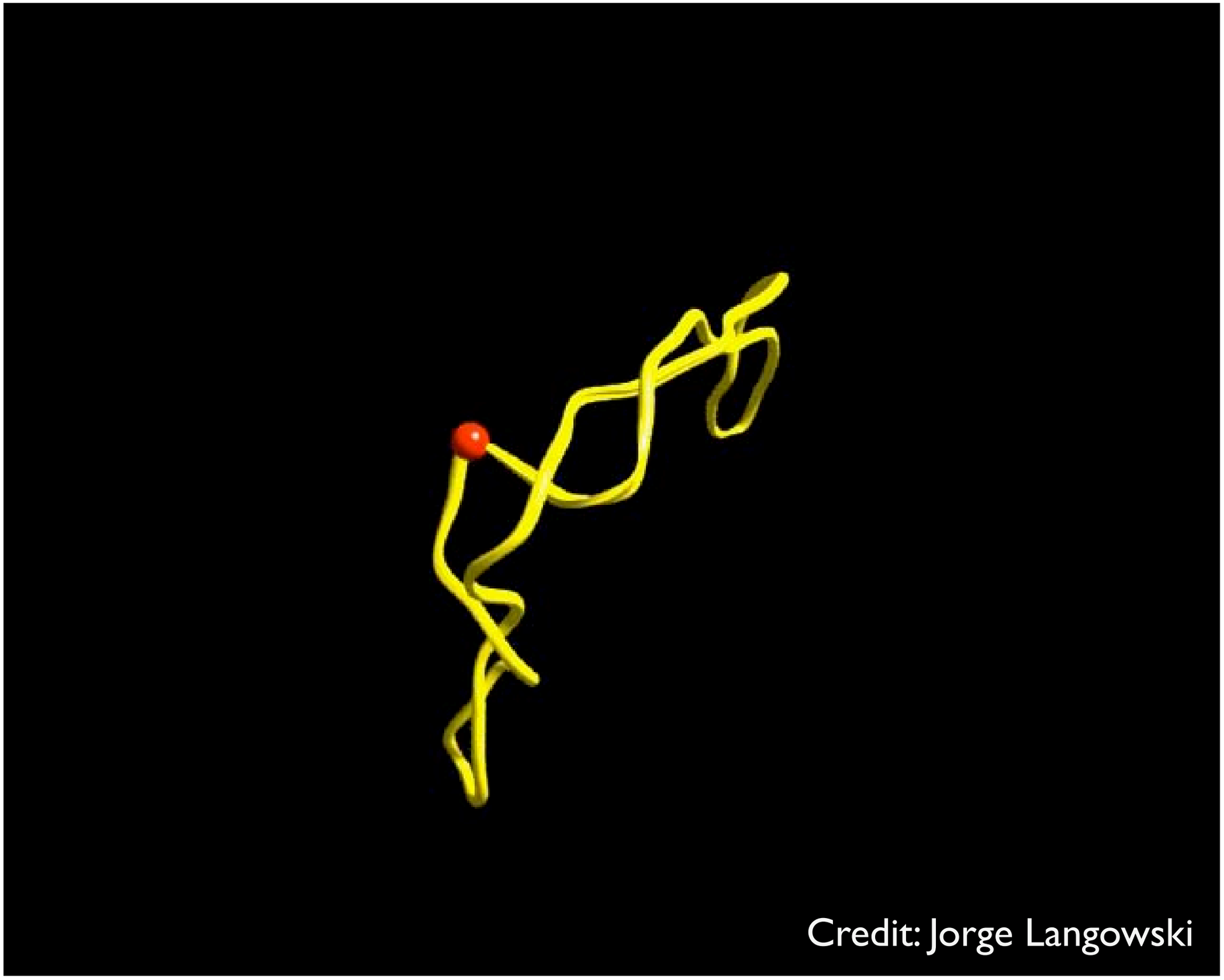




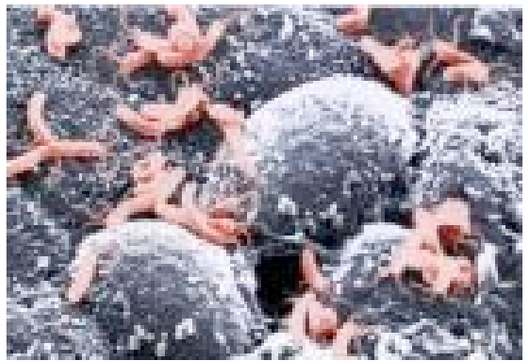
a) – Taken from an anatomical study of Leonardo da Vinci 1508 - 1510 (Manuscript RL, 19102r, Windsor Library, UK). The left panel shows the interface between dissected tissues (wall of a womb) where Leonardo used the tools available for him to analyse how two biological surfaces create an adhesive junctional interface; b) – An insert representing junctional folds of a synaptic terminal that creates integrity of the structure (Cohen-Cory, 2002). c) – Junctional cell-cell interface between two cultured primary astrocytes revealed with antibodies against a gap junction protein, Connexin-43 (Butkevich et al., 2004).

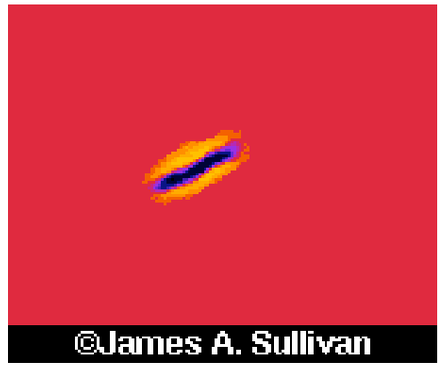
SLIDE CREDIT: Irina Majoul Royal Holloway University of London

OBSERVING EVENTS



OBSERVING EVENTS





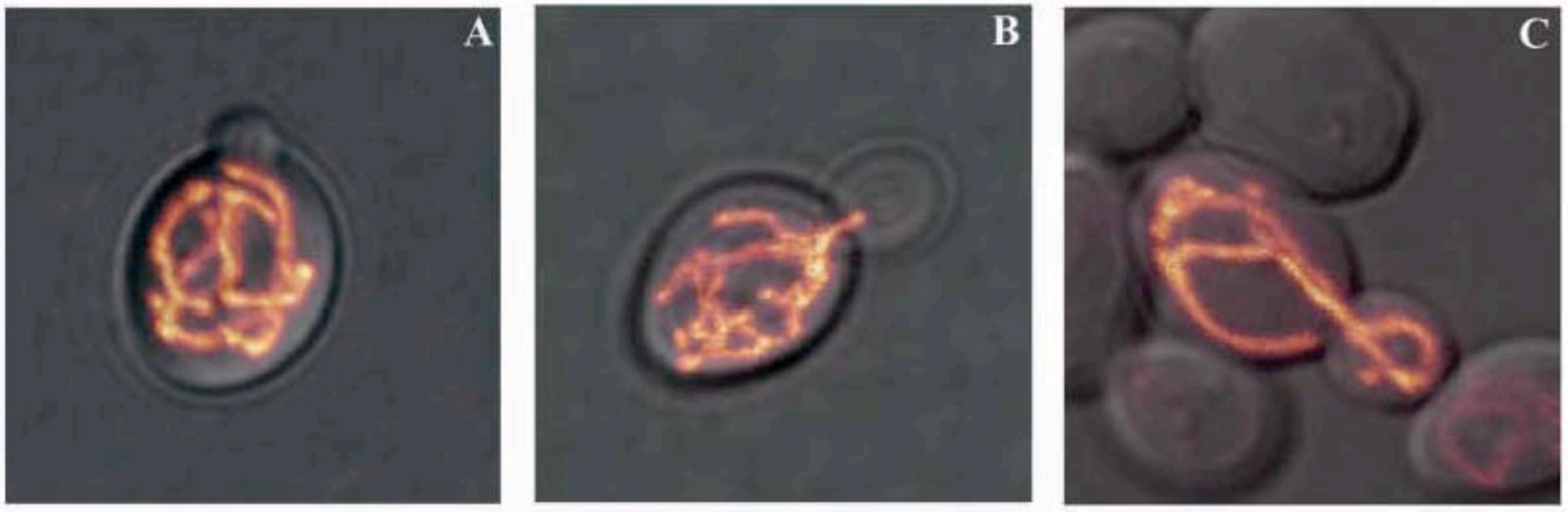
The Nobel Prize in Physiology or Medicine 2005

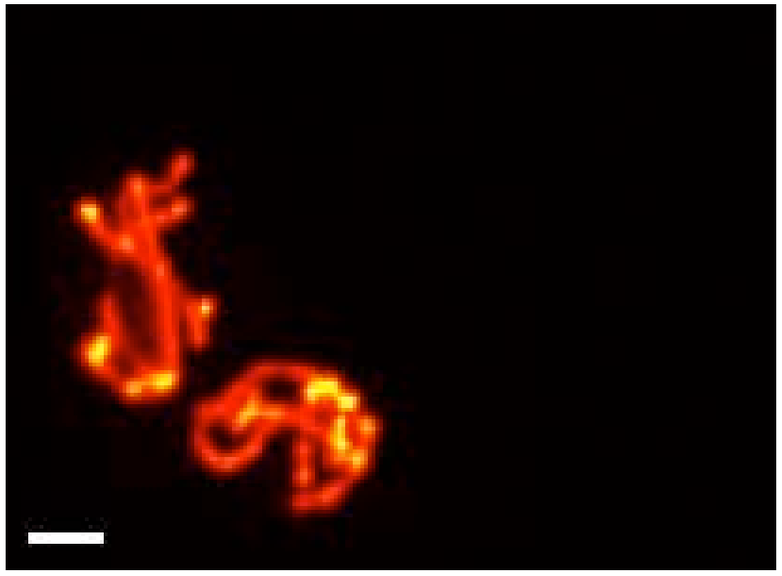
Helicobacters were discovered in Italy in 1892: An episode in the scientific life of an eclectic pathlogist, Giulio Bizzozero

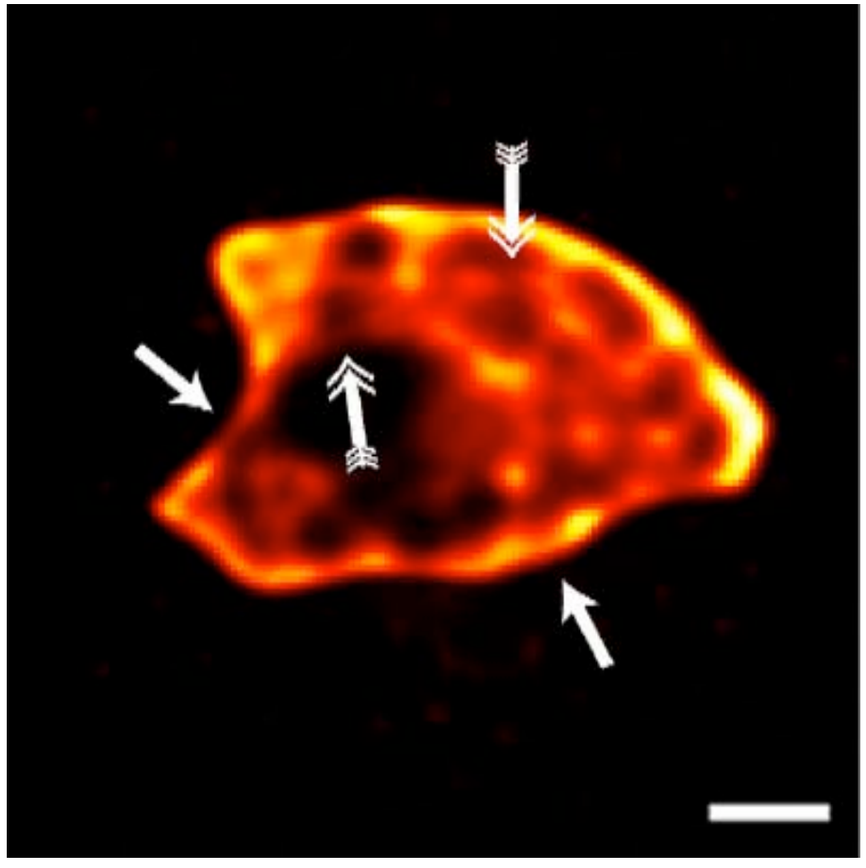
Bizzozero G. Sulle ghiandole tubulari del tube gastroenterico e sui rapporti del loro coll'epitelio de rivestimento della mucosa. Atti R Accad Sci Torino 1892;28:233-51

"H.pylori image courtesy of www.hpylori.com.au"

OBSERVING EVENTS







Credit: Stefan Hell, JCS web site

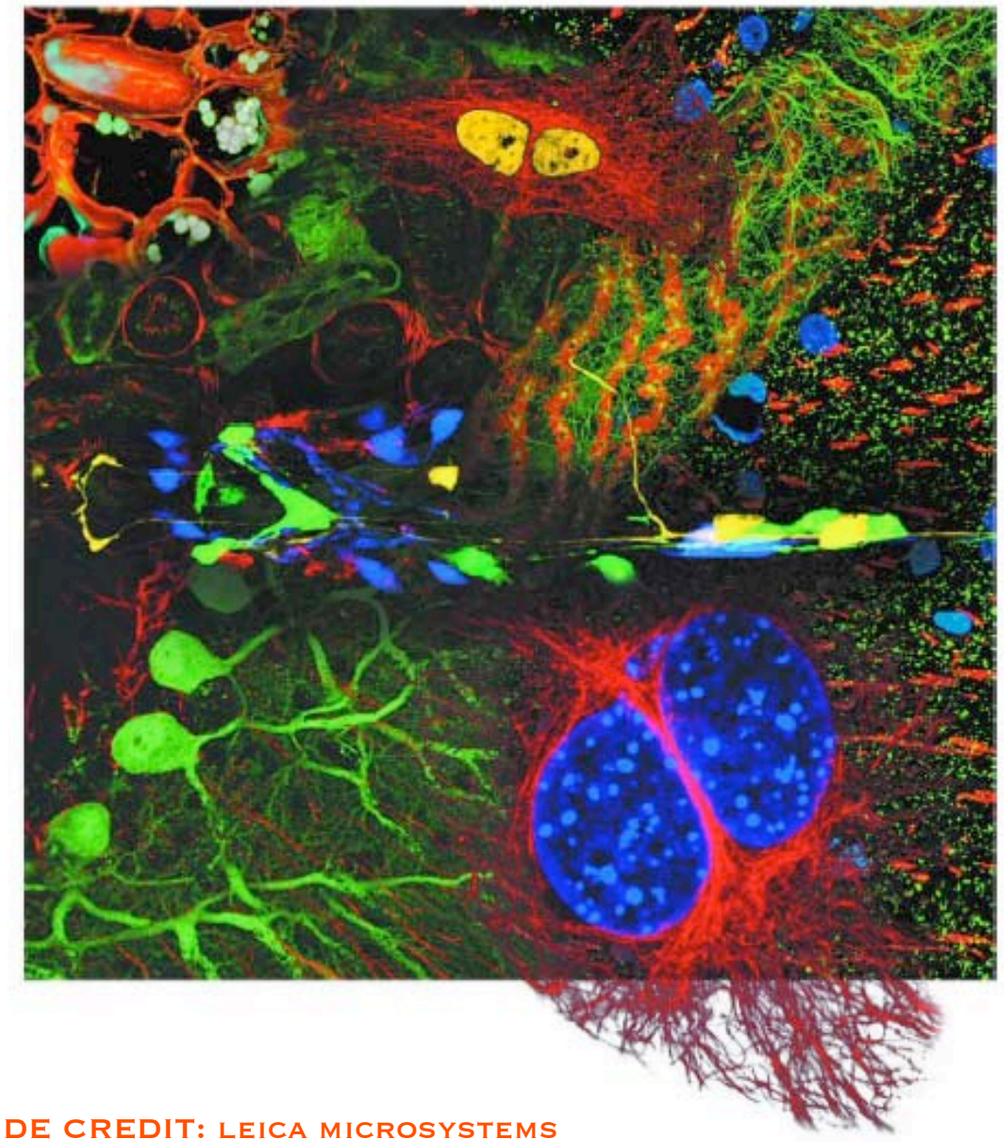
Journal of Cell Science 116 (10), 2005
Spatial and temporal dynamics of budding yeast mitochondria lacking the division component Fis1p

CAPTURING EVENTS

MICROSCOPY

A microscope is an instrument magnifying objects by means of a specific interaction – more commonly by means of lenses – so as to capture details invisible to the naked eye.

(Oxford dictionary, after Colin Sheppard)



SLIDE CREDIT: LEICA MICROSYSTEMS

OPTICAL MICROSCOPY

MICROGRAPHIA:

OR SOME

Physiological Descriptions

OF

MINUTE BODIES

MADE BY

MAGNIFYING GLASSES

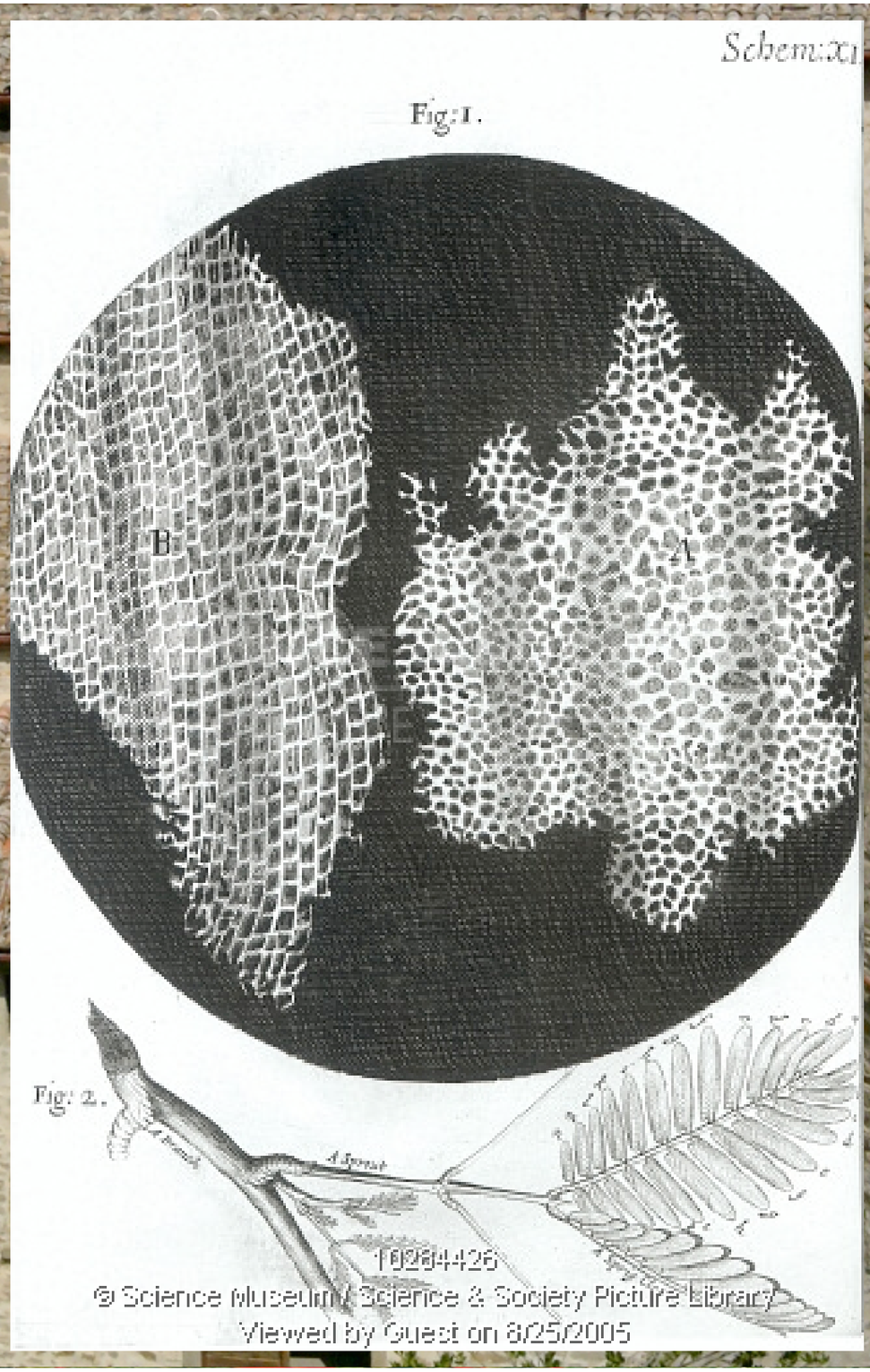
WITH

Observations and Inquiries thereupon.

By *R. HOOKE*, Fellow of the Royal Society.

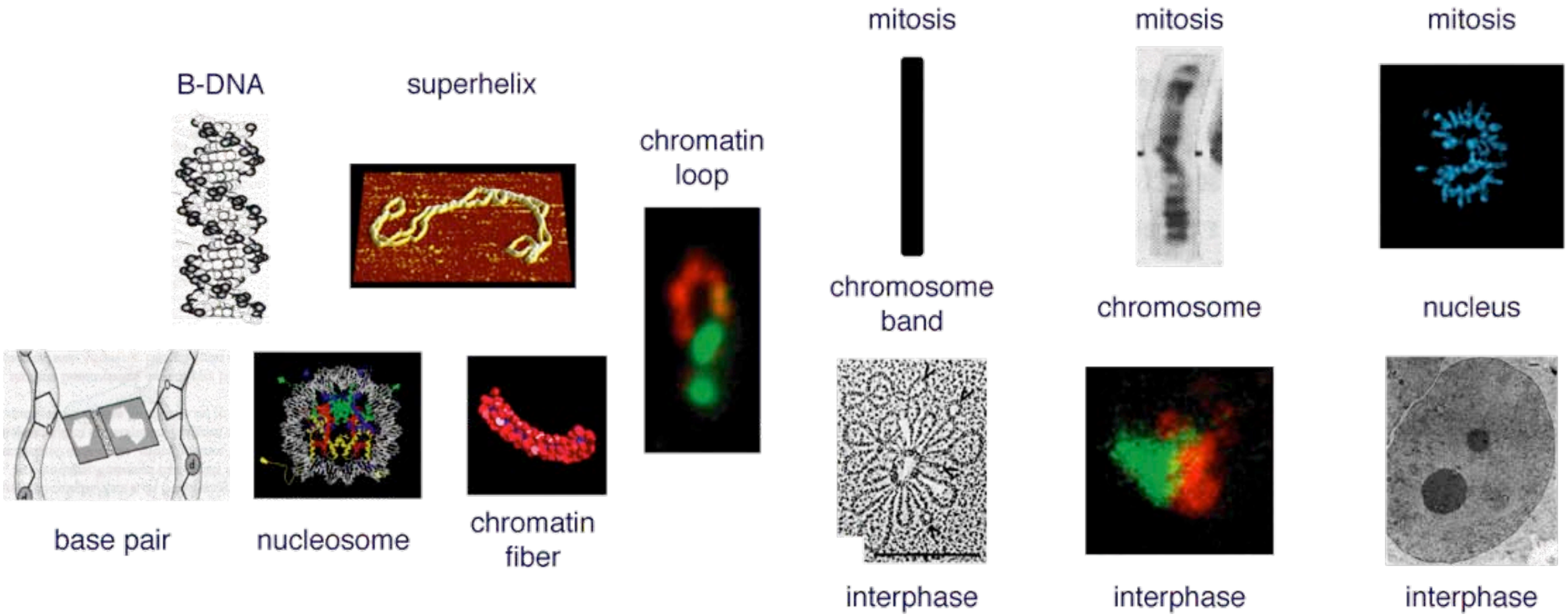
Nanpossis oculo quantum contendere Lynceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.

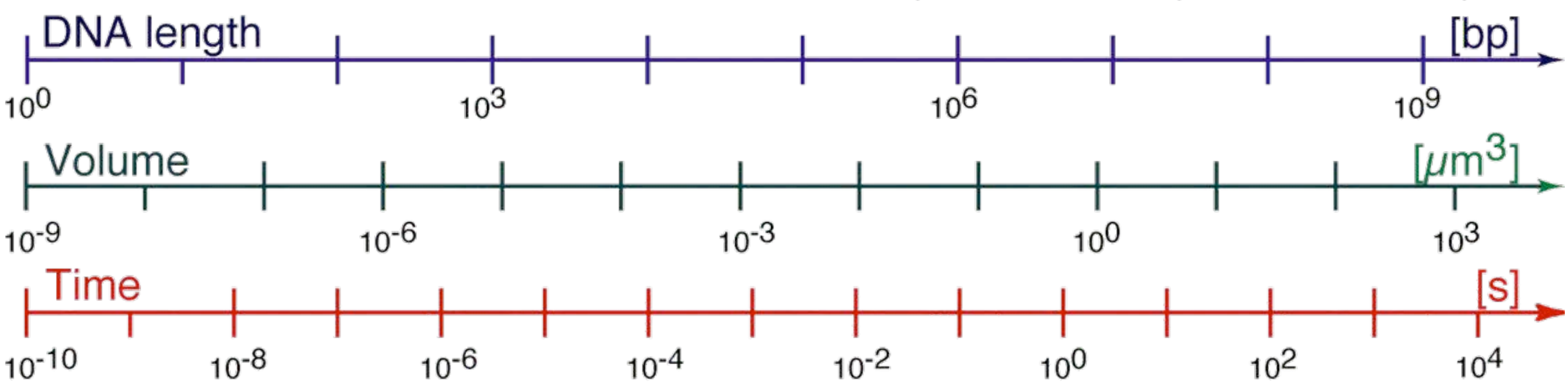
LONDON, Printed by *Jo. Martyn*, and *Ja. Allestry*, Printers to the Royal Society, and are to be sold at their Shop at the *Bell* in S. *Paul's* Church-yard. M DC LX V.



OPTICAL MICROSCOPY

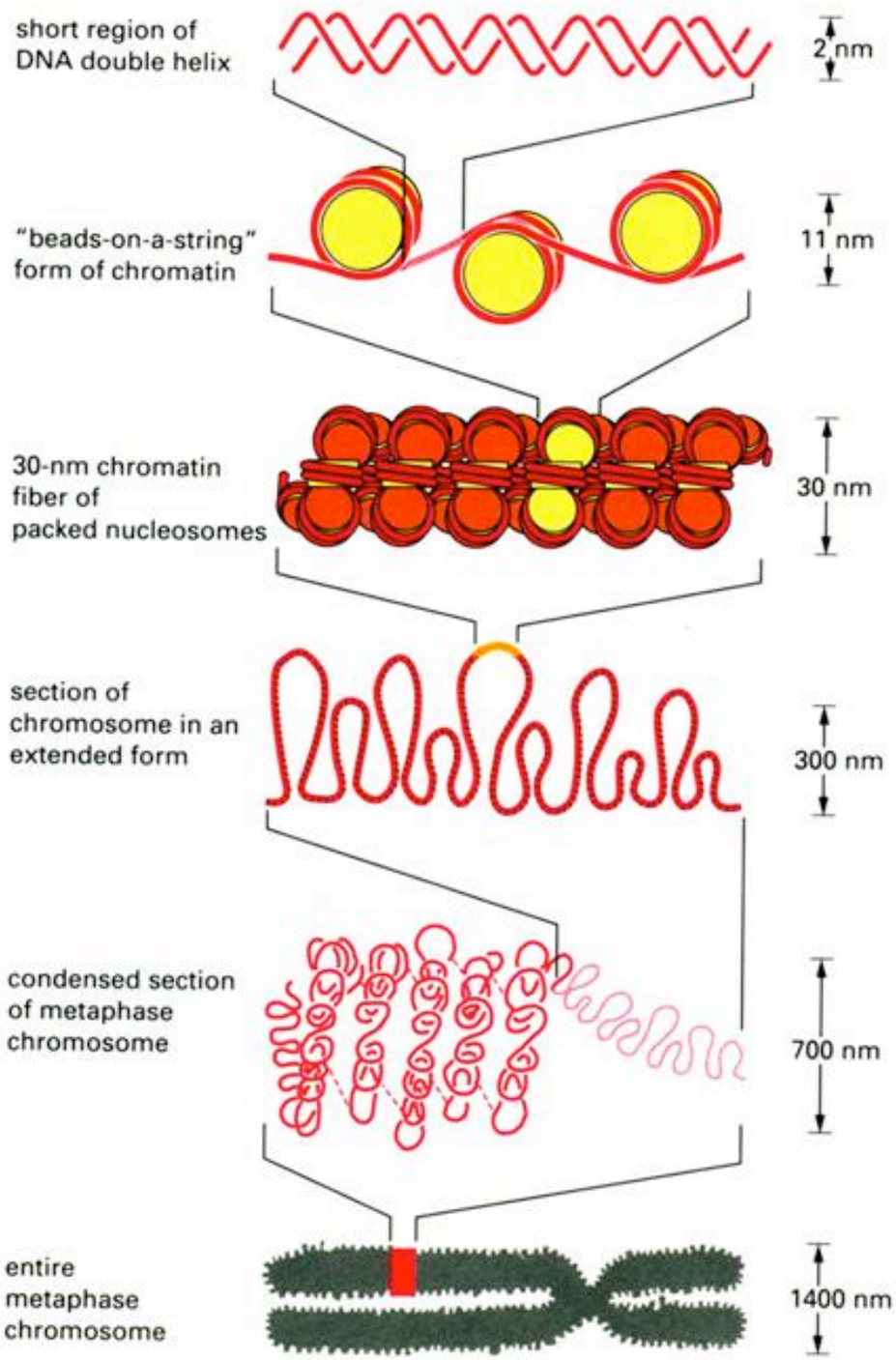
The Biological Scenario

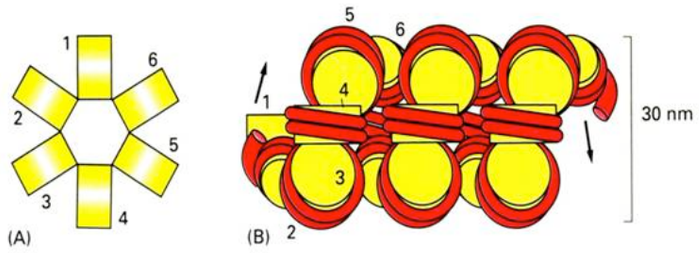


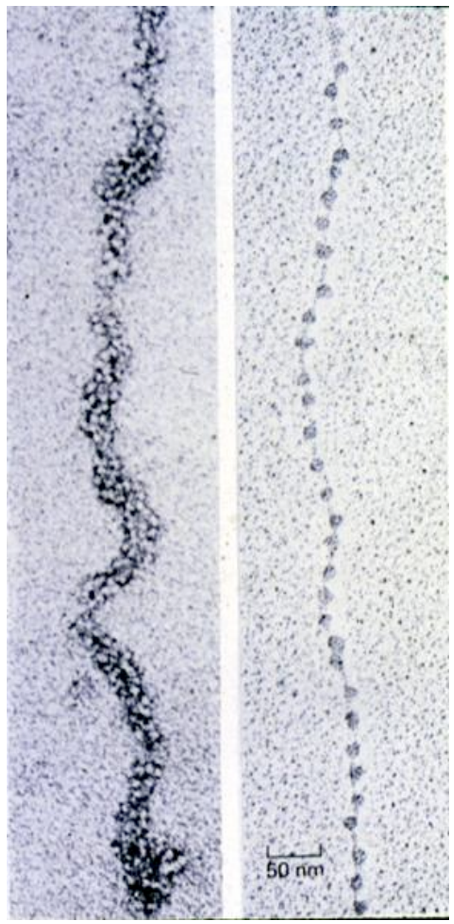


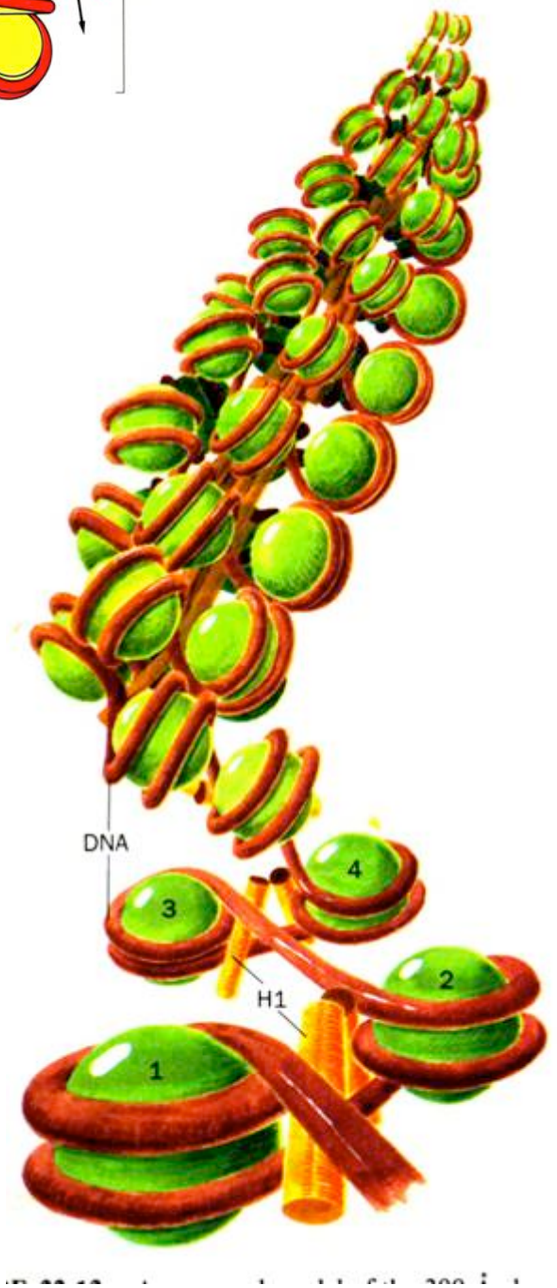
Slide credit: Jörg Langowski, DKFZ, Heidelberg

The Biological Scenario



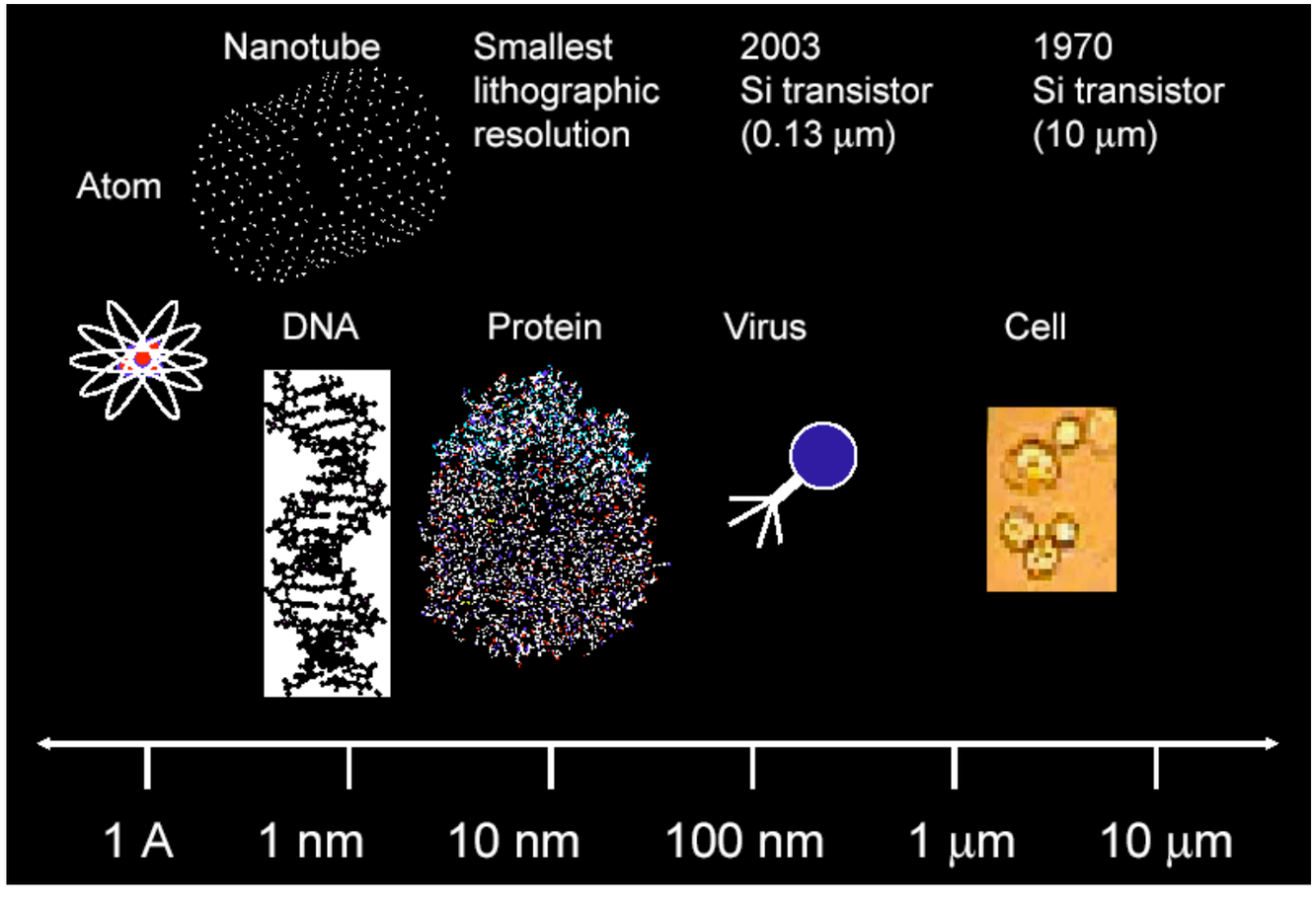






RESOLUTION OBSESSION

HUMAN EYE	100 000 nm
OPTICAL MICROSCOPE	200 nm
ELECTRON MICROSCOPE	0.4 nm 3 nm
SCANNING PROBE MICROSCOPE	0.1-10 nm



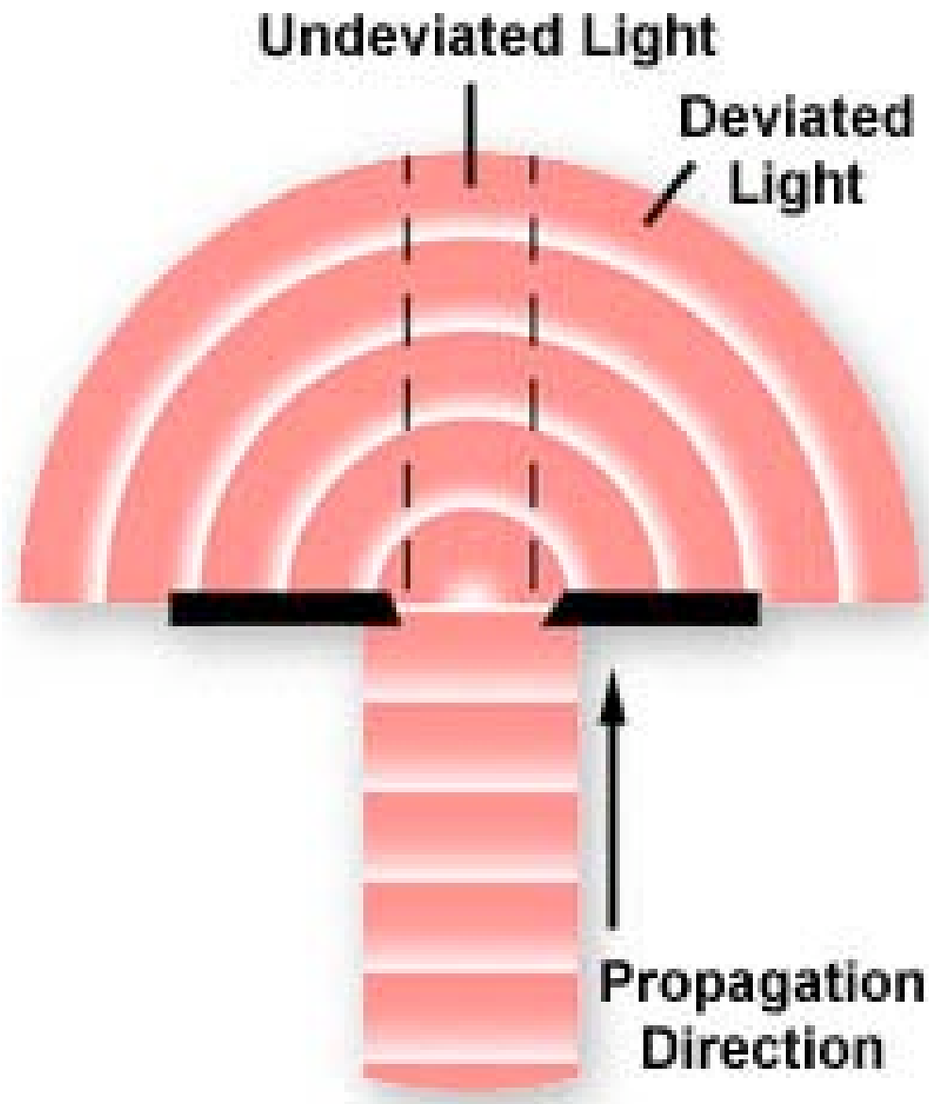
OPTICAL MICROSCOPY IMAGING

Georges Seurat (1859-1891), Una domenica pomeriggio all'isola della Grande Jatte,1883-85

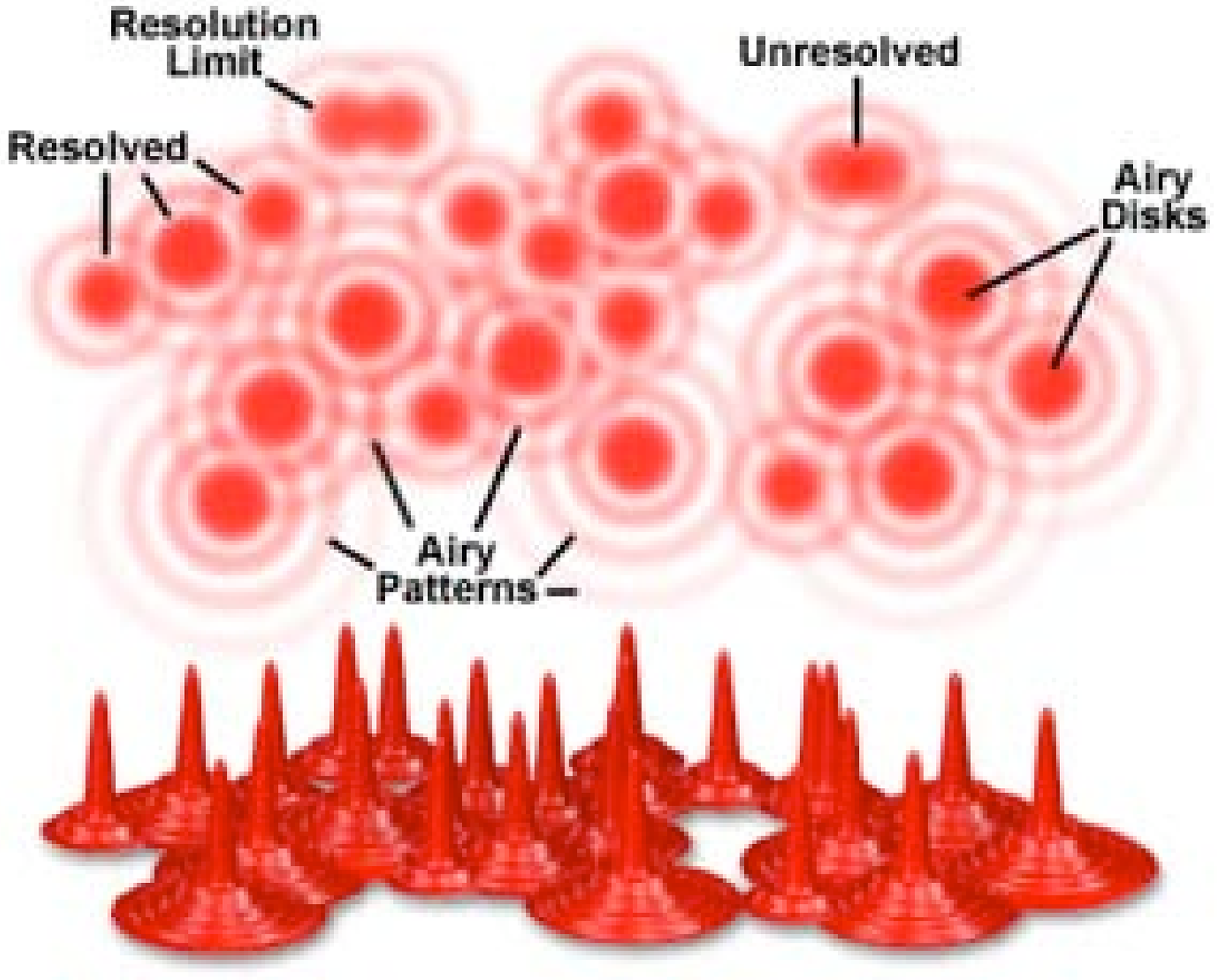
Lens Effect

POINTLIKE OBJECT VS. QUASI POINTLIKE IMAGE

Optical RESOLUTION

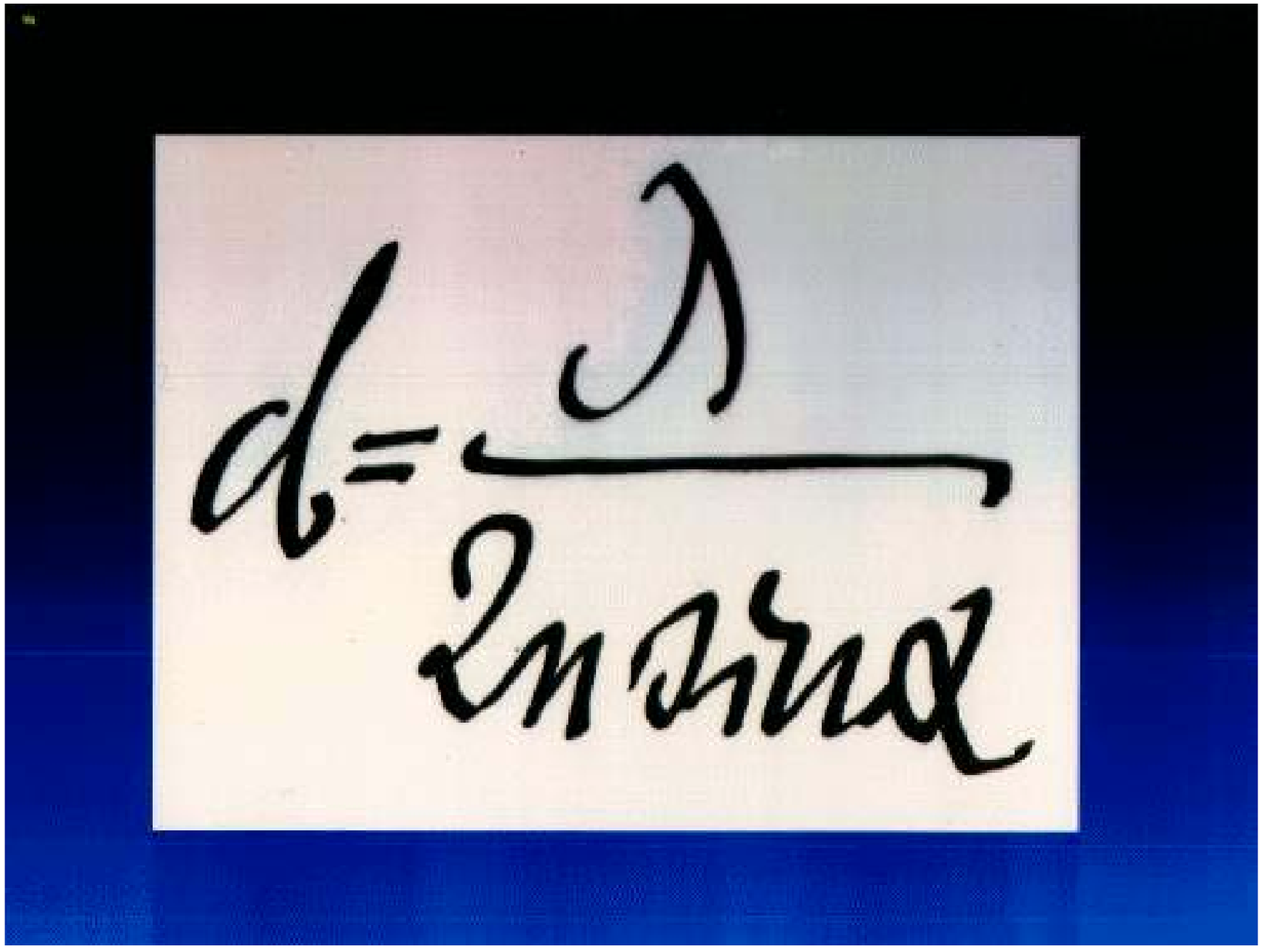


Optical RESOLUTION



Vignette credit: http://www.microscopyu.com/tutorials/

Optical RESOLUTION

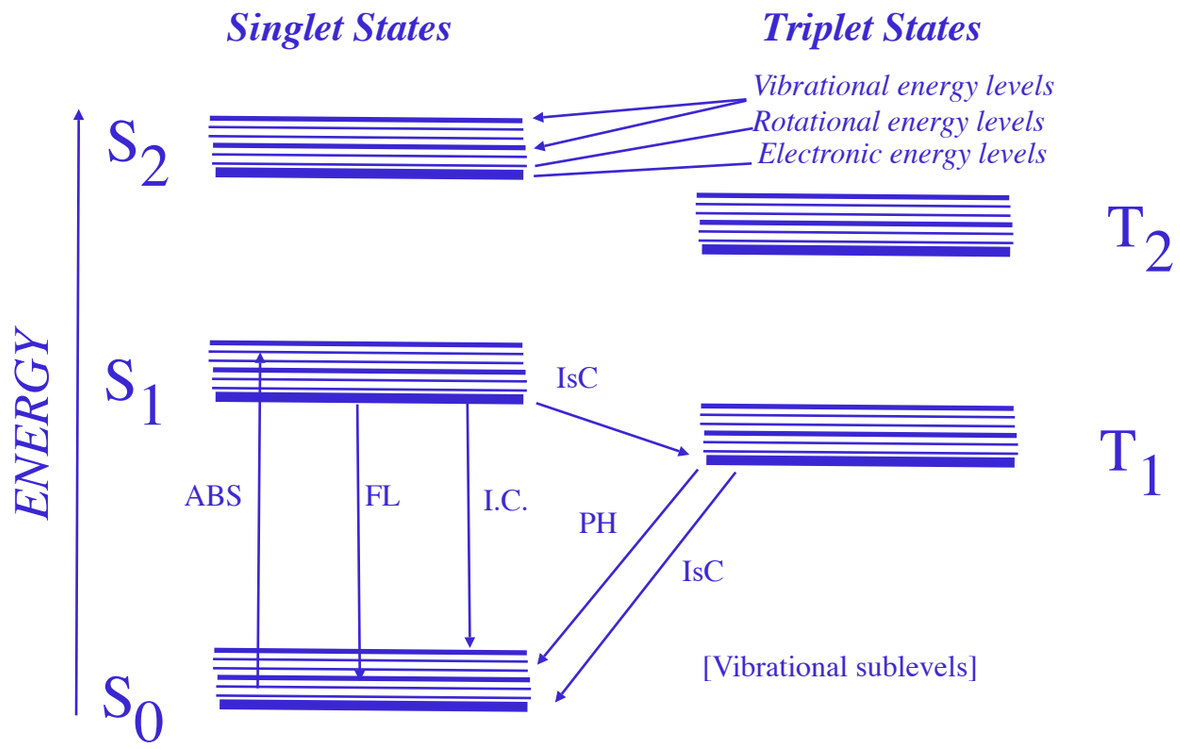


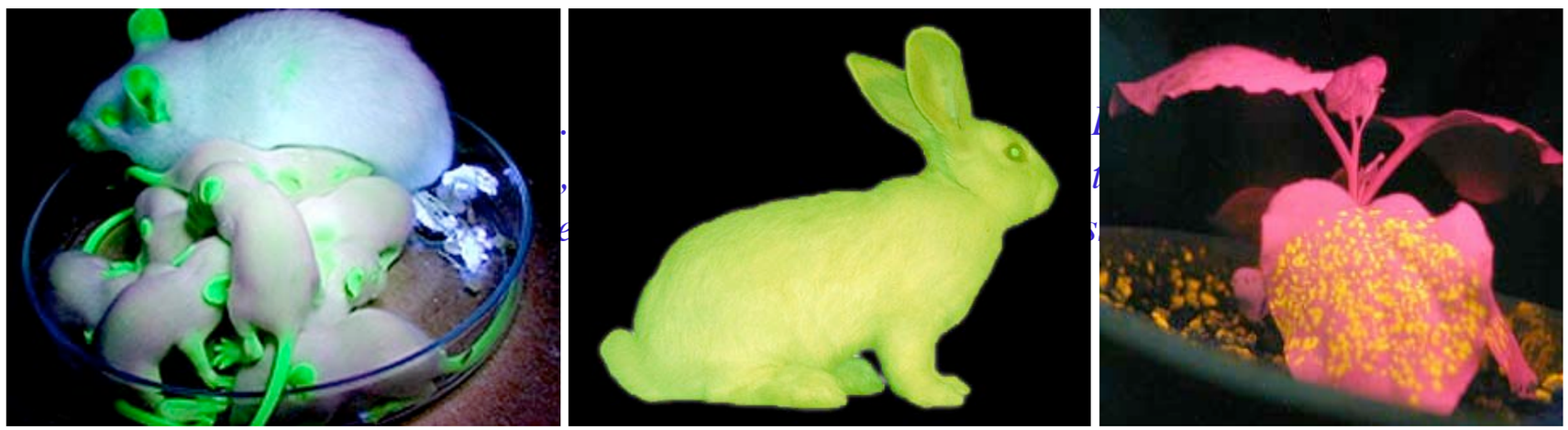
ABBE, E (1873) ARCHIVE F. MIKROSKOP.ANAT. 9, 413-420

Optical Contrast

Giudizio Universale: immagine nel VIS (sinistra) e in fluorescenza indotta UV (destra): l'azzurro di lapislazzuli, utilizzato da Michelangelo, e' riconoscibile dalla presenza di una fluorescenza bianco-verdastra

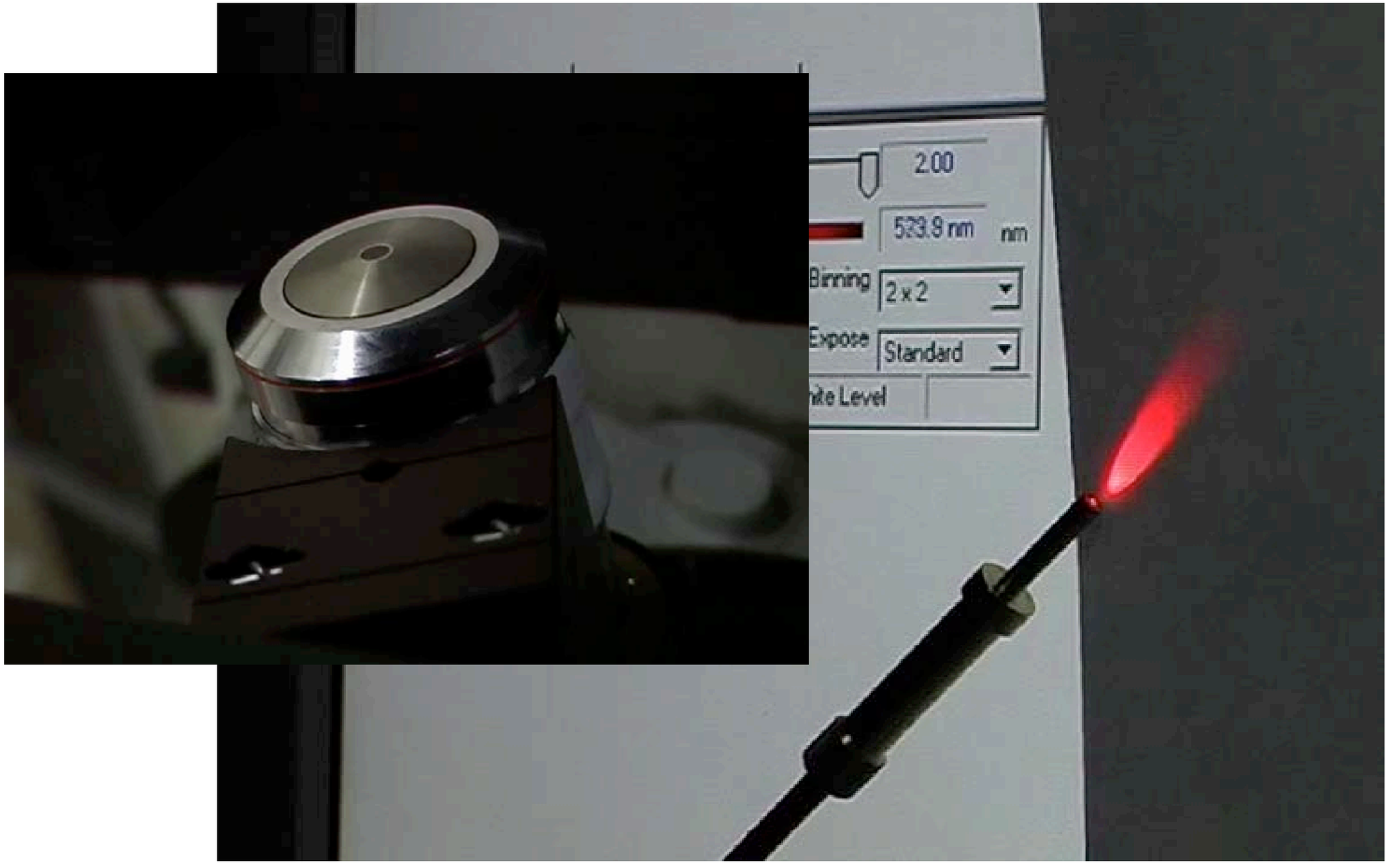
Perrin-Jablonski Diagram





Slide Credit: Paul Robinson, Purdue and Paolo Bianchini, LAMBS

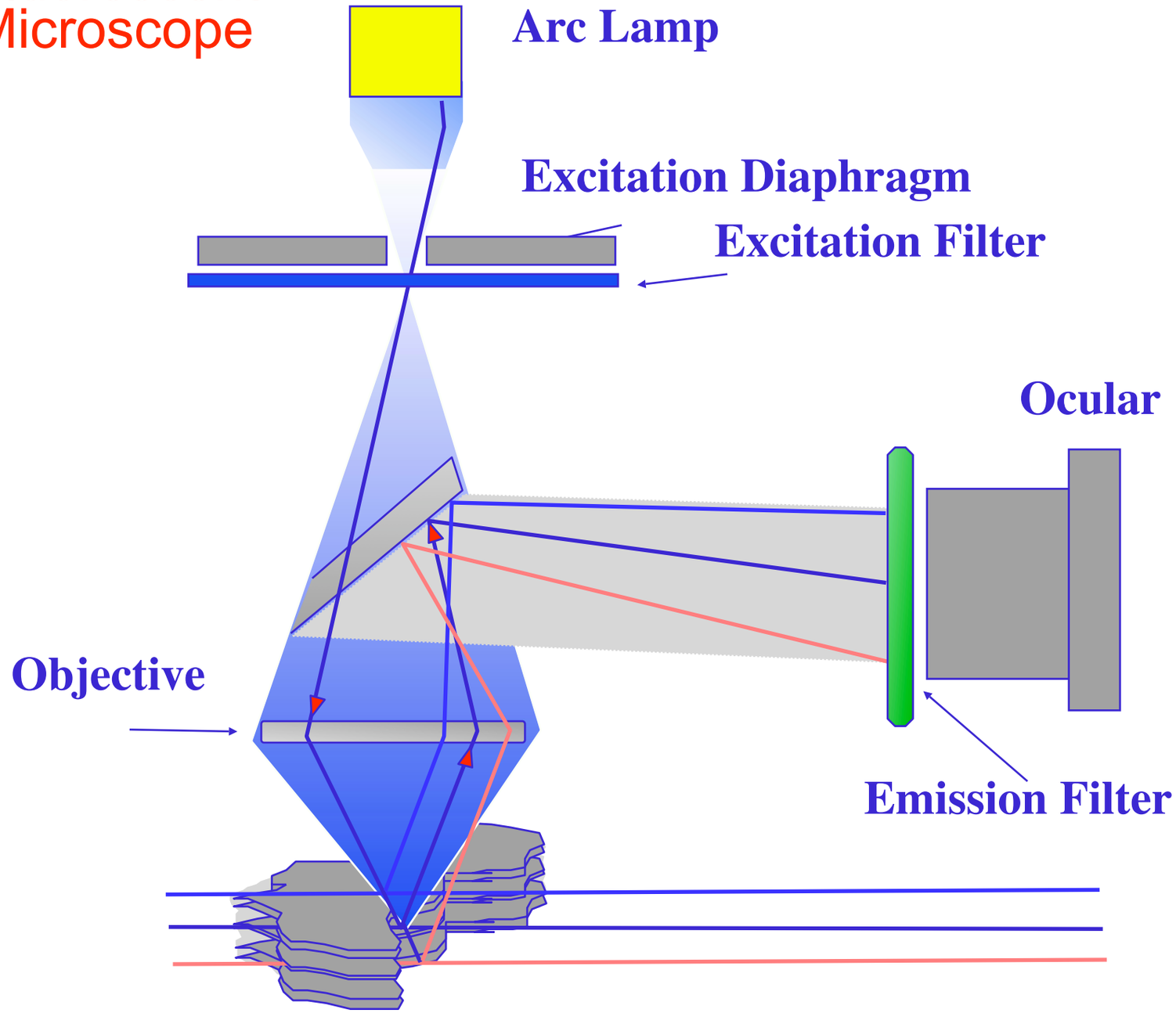
THE OPTICAL MICROSCOPE



Credit: Leica Microsystems, Mannheim, Germany

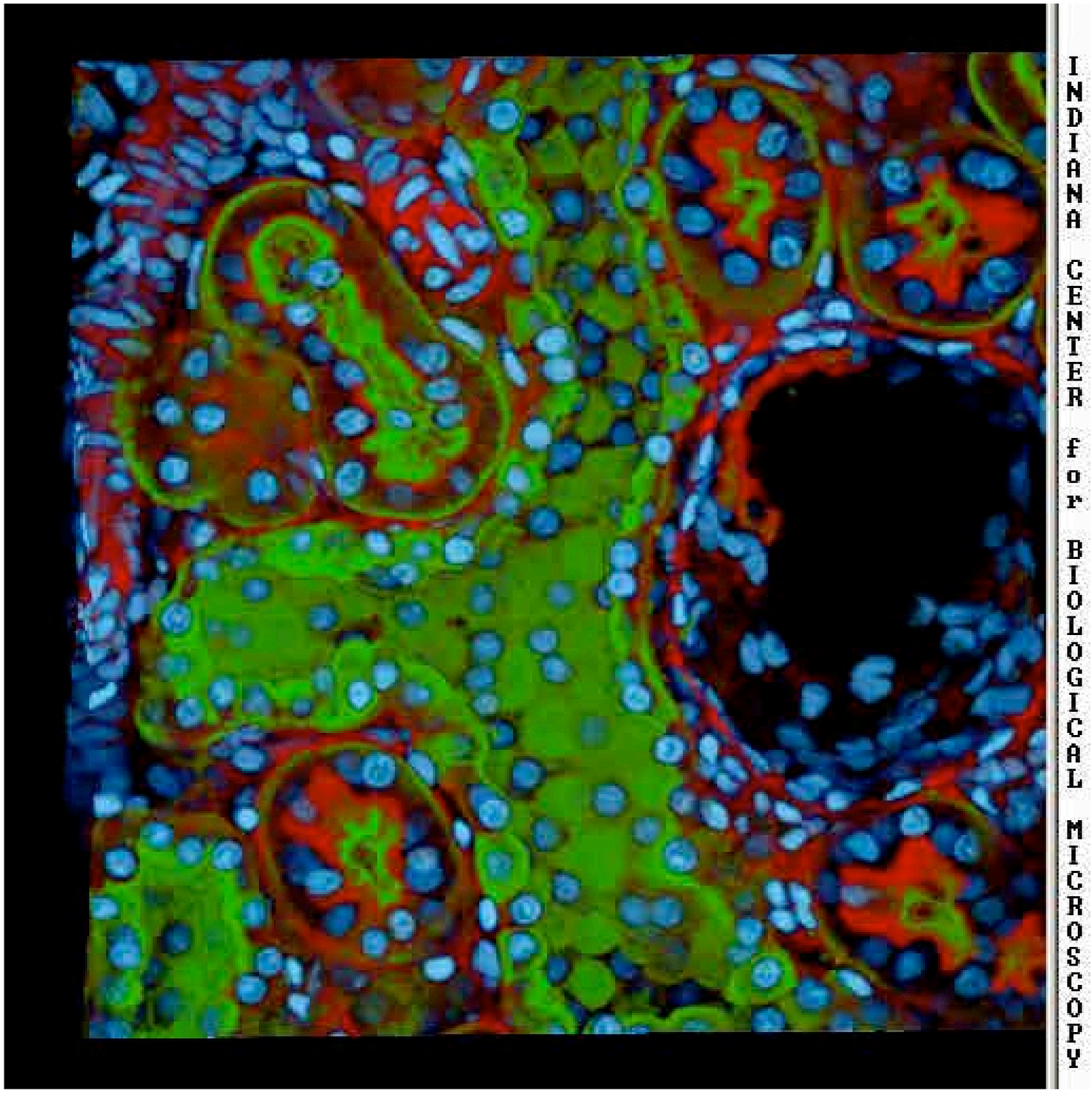
THE OPTICAL MICROSCOPE

Fluorescent Microscope



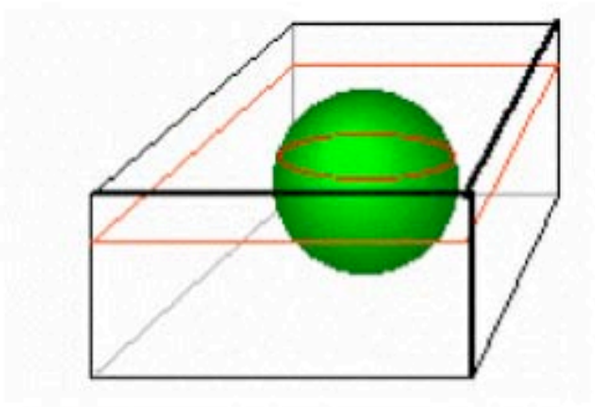
(COURTESY: PAUL ROBINSON, PURDUE UNIVERSITY)

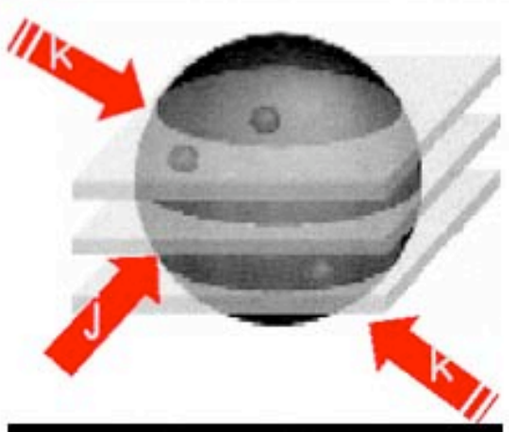
3D Optical Microscopy

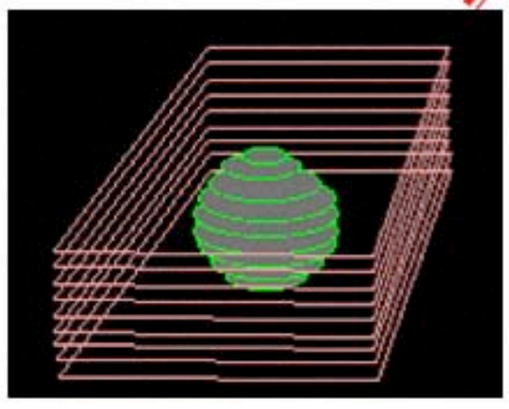


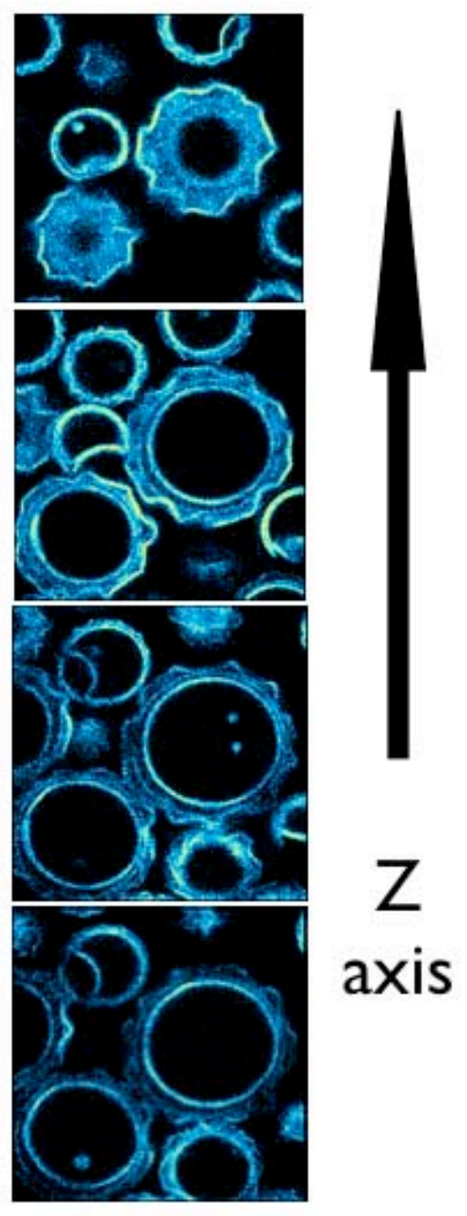
3D Optical Microscopy

OPTICAL SECTIONING





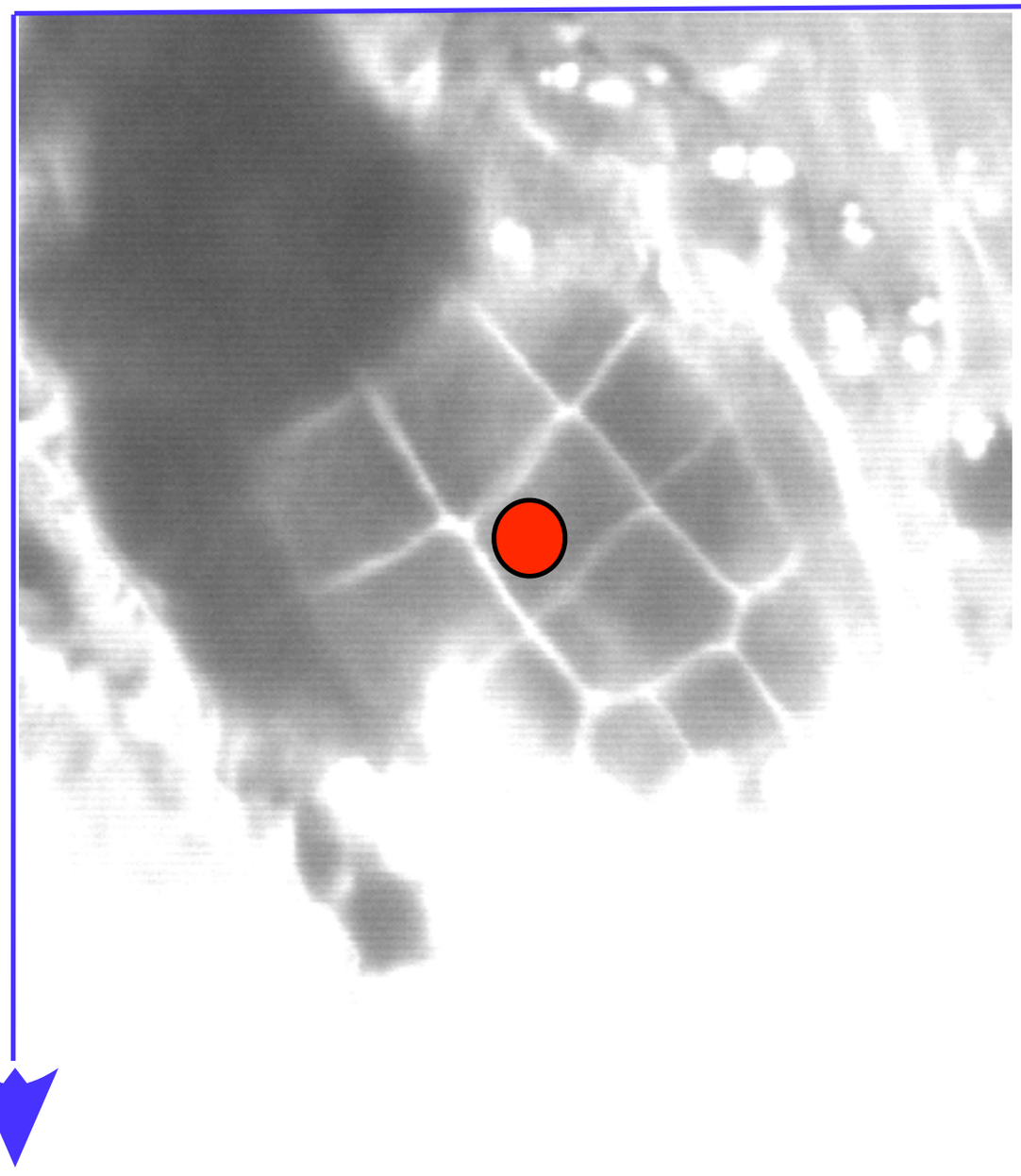


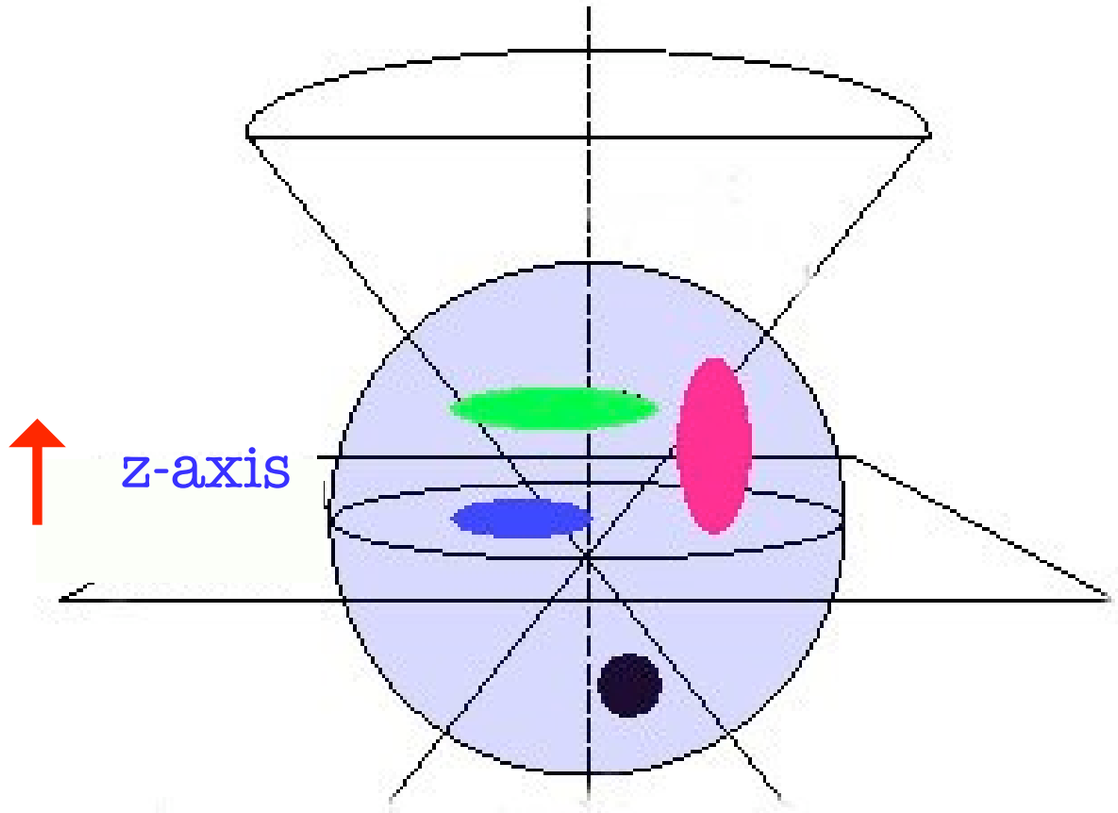


B. Bianco, A. Diaspro, Cell Biophysics, 15 (3), pp.189-200, 1989.
A. Diaspro, et al., Image Vision and Computing, 8 (2), pp.130-141, 1990.

a.diaspro, g.chirico, m.collini (2004) quart. rev. .biophys.,vol.38, nr.2, pp.1-72 (2006).

NAVIGATING INTO CELLS AND TISSUES

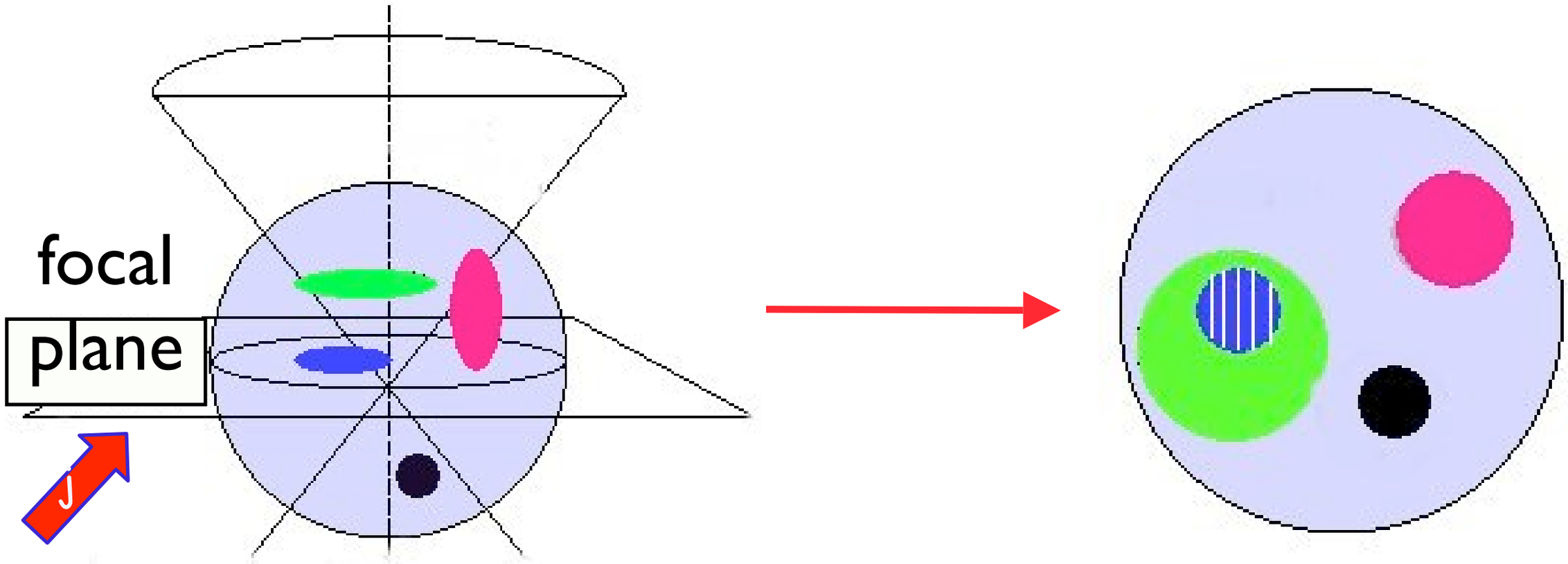




Vignette credit:Giuseppe Vicidomini, LAMBS-MicroScoBio, Genova

3D Optical Microscopy

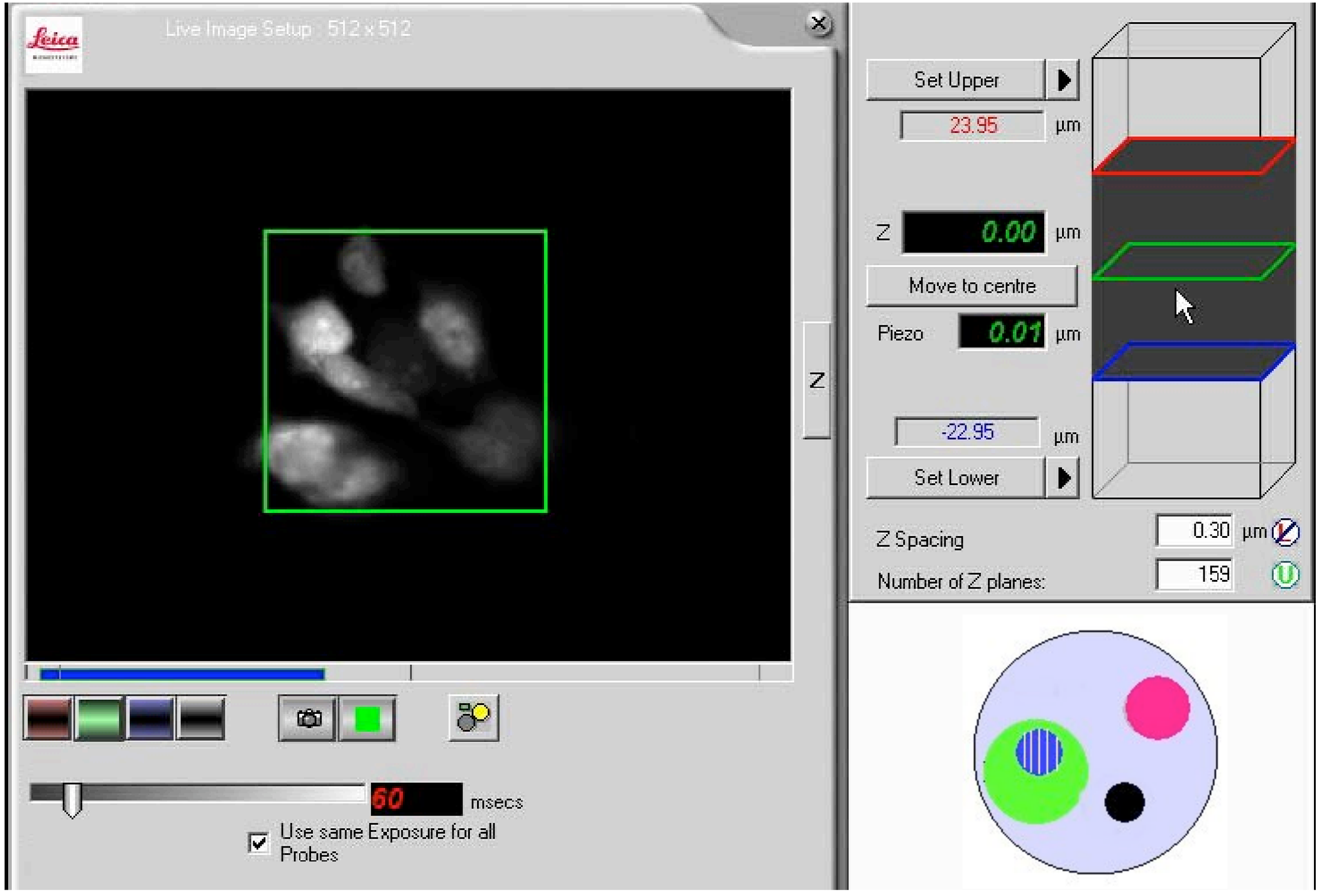
OPTICAL SECTIONING



B. Bianco, A. Diaspro, Cell Biophysics, 15 (3), pp.189-200, 1989.
A. Diaspro, et al., Image Vision and Computing, 8 (2), pp.130-141, 1990.

Slide credit:Giuseppe Vicidomini, LAMBS-MicroScoBio, Genova

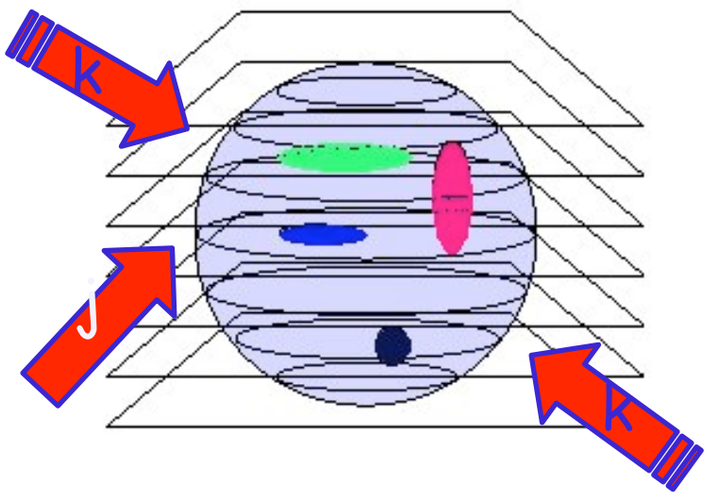
OPTICAL SECTIONING



OPTICAL SECTIONING

Within the optical sectioning scheme the situation is that the observed image O at a plane j is produced by the true fluorescence distribution at plane j, distorted by the microscope through S, plus contributions from adjacent k planes and noise N.

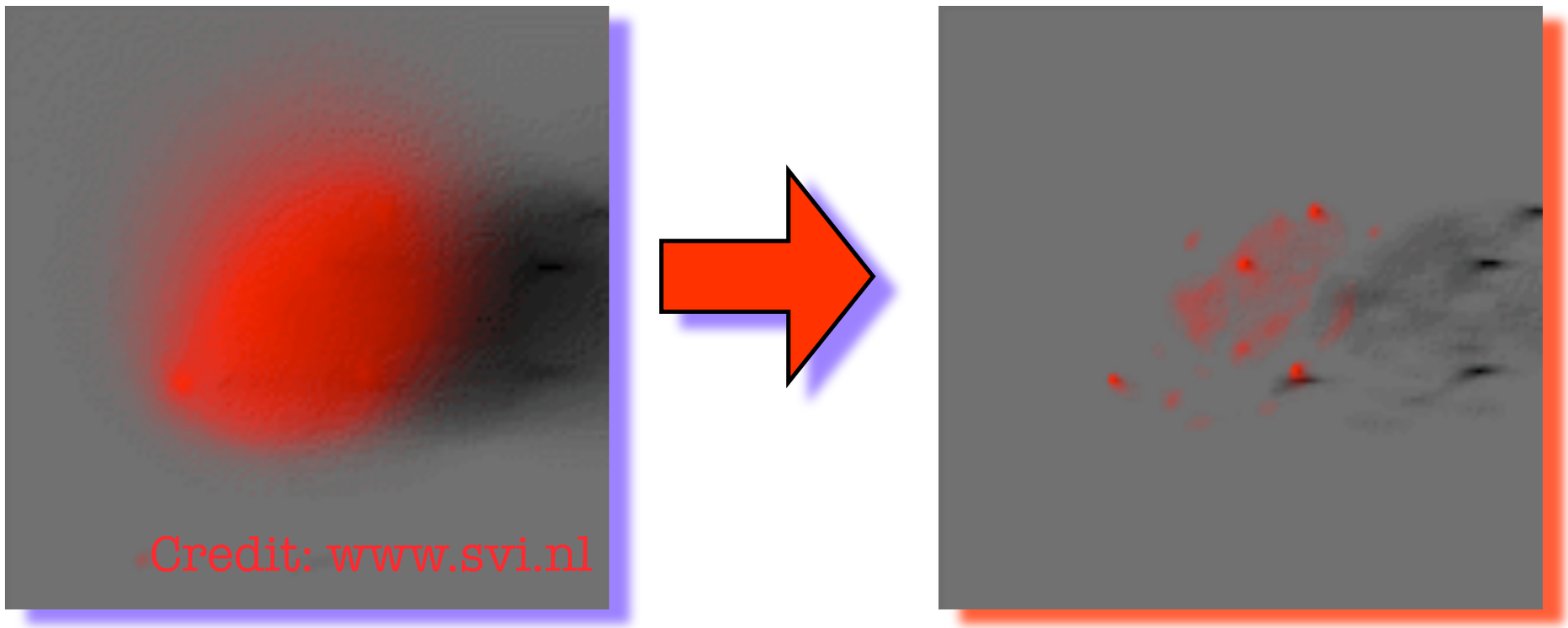
$$O_j = I_j S_j + \Sigma_{k \neq j} I_k S_k + N$$



3D Optical Microscopy

Solving the equation set...

$$O_j = I_j S_j + \Sigma_{k \neq j} I_k S_k + N$$



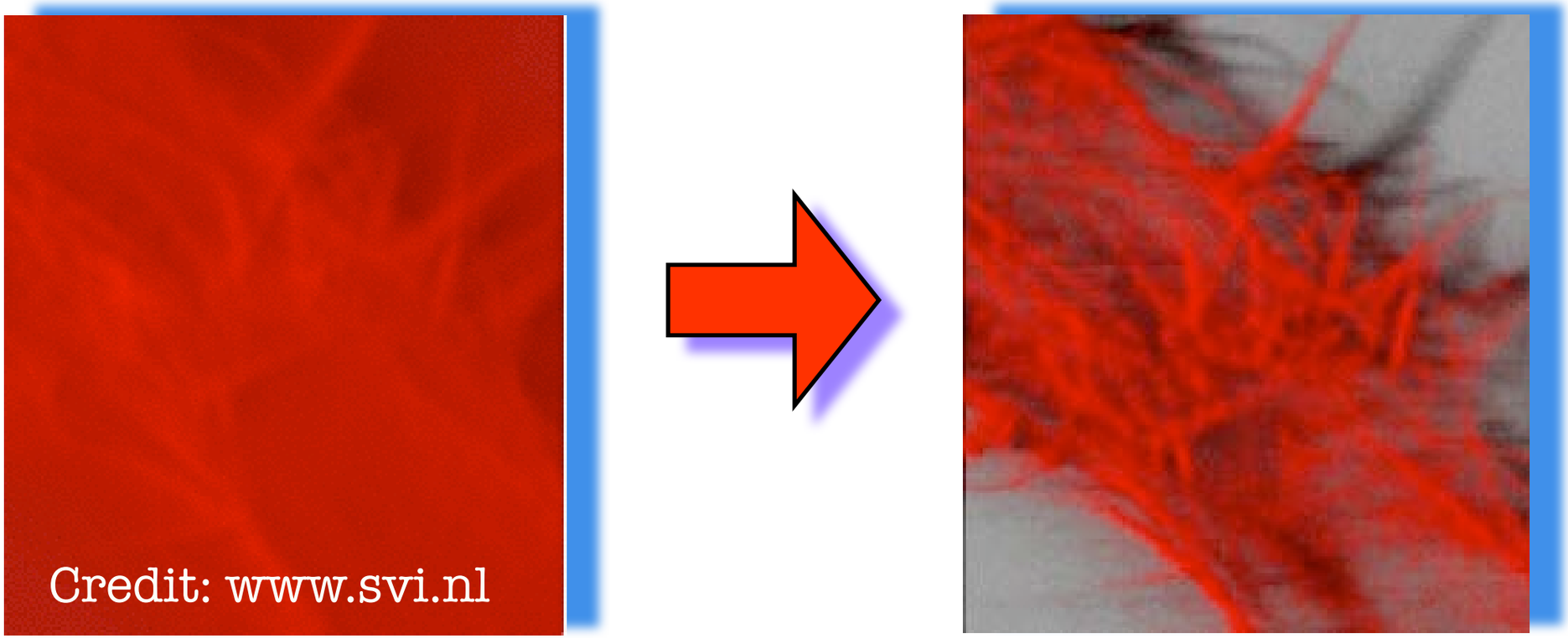
$$h(x,y,z) * f(x,y,z) + n(x,y,z) + b(x,y,z) = g(x,y,z)$$

www.powermicroscope.com

BONETTO P., BOCCACCI P., SCARITO M., DAVOLIO M., EPIFANI M., VICIDOMINI G., TACCHETTI C., RAMOINO P., USAI C., DIASPRO A. (2004) MICROSCOPY RESEARCH AND TECHNIQUE. vol. 64, pp. 196-203.

Solving the equation set...

$$O_j = I_j S_j + \Sigma_{k \neq j} I_k S_k + N$$



$$h(x,y,z) * f(x,y,z) + n(x,y,z) + b(x,y,z) = g(x,y,z)$$

www.powermicroscope.com

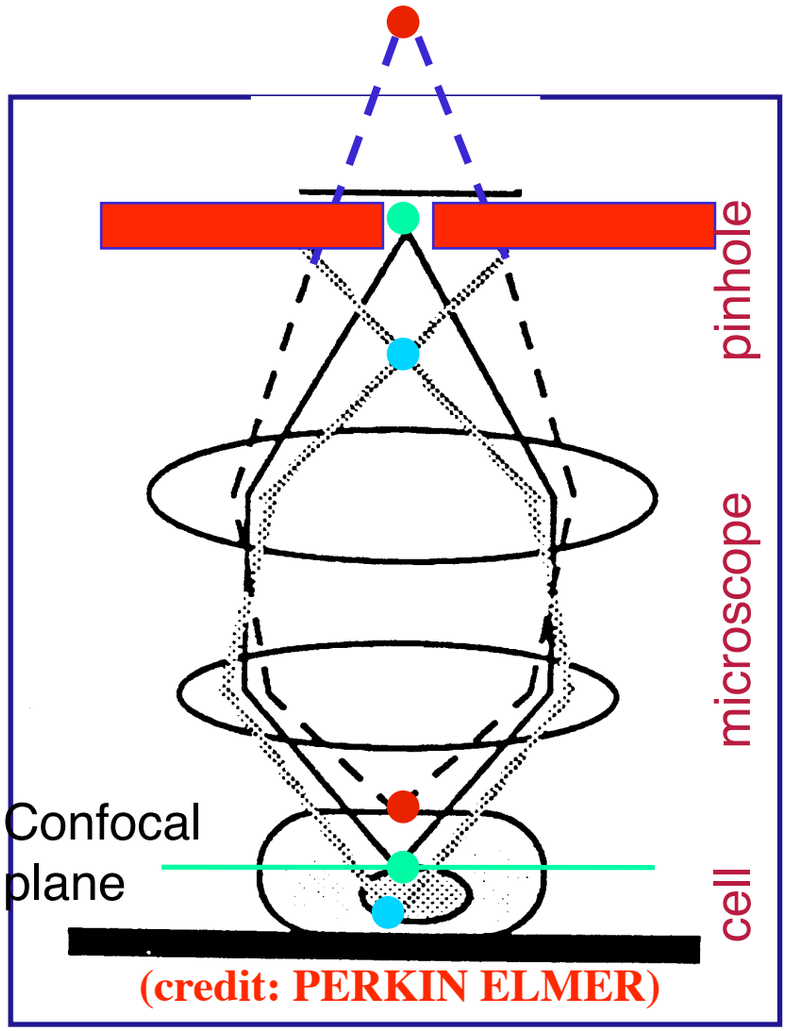
BONETTO P., BOCCACCI P., SCARITO M., DAVOLIO M., EPIFANI M., VICIDOMINI G., TACCHETTI C., RAMOINO P., USAI C., DIASPRO A. (2004) MICROSCOPY RESEARCH AND TECHNIQUE. vol. 64, pp. 196-203.

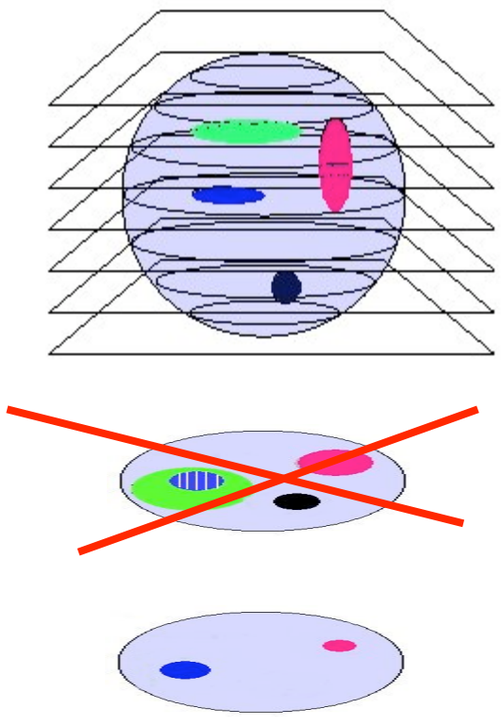
Solving the equation set...by means of a confocal set-up

$\sum I_k S_k \rightarrow 0$

$S_j \rightarrow 1$

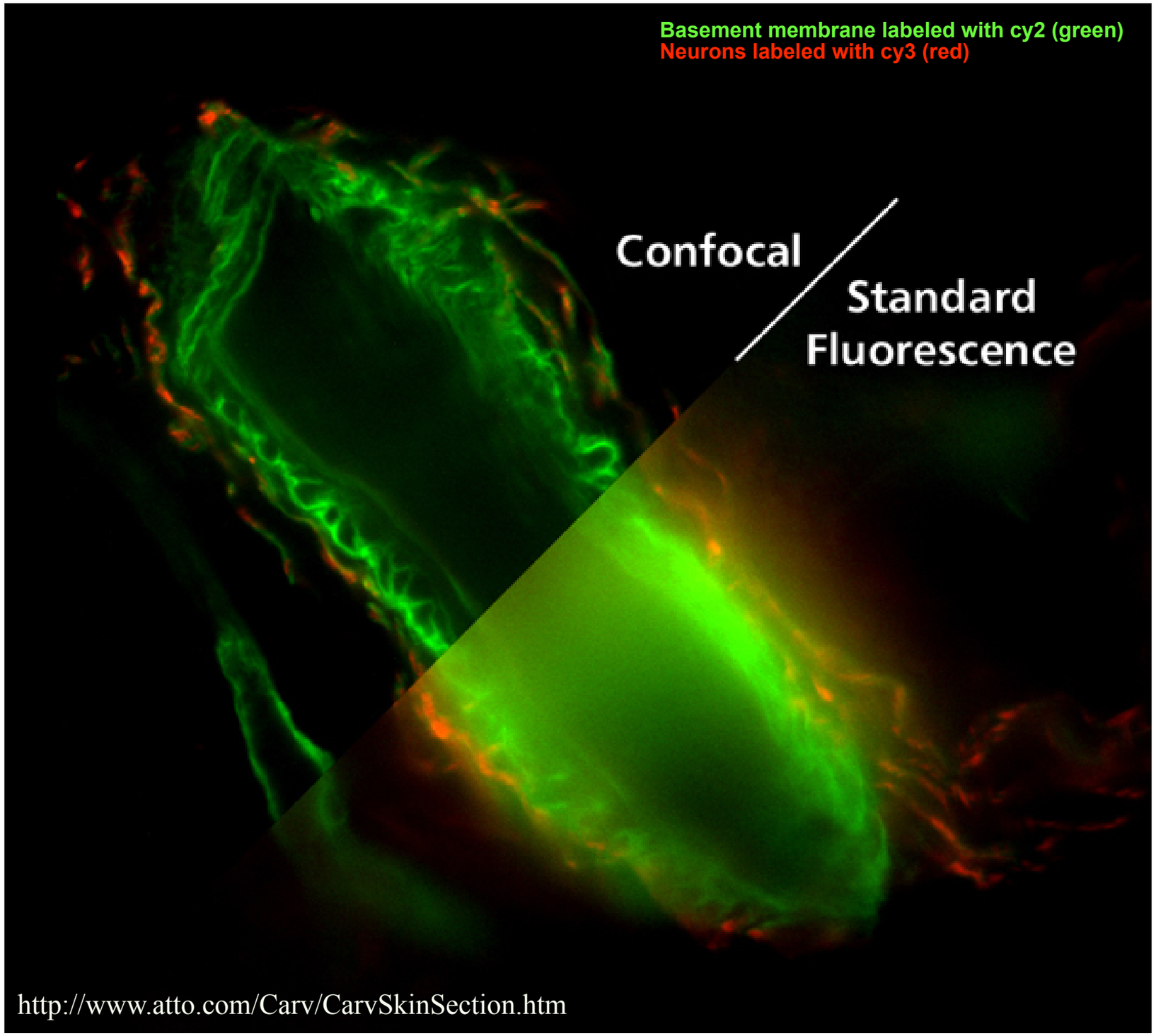
$O_j \rightarrow I_j$





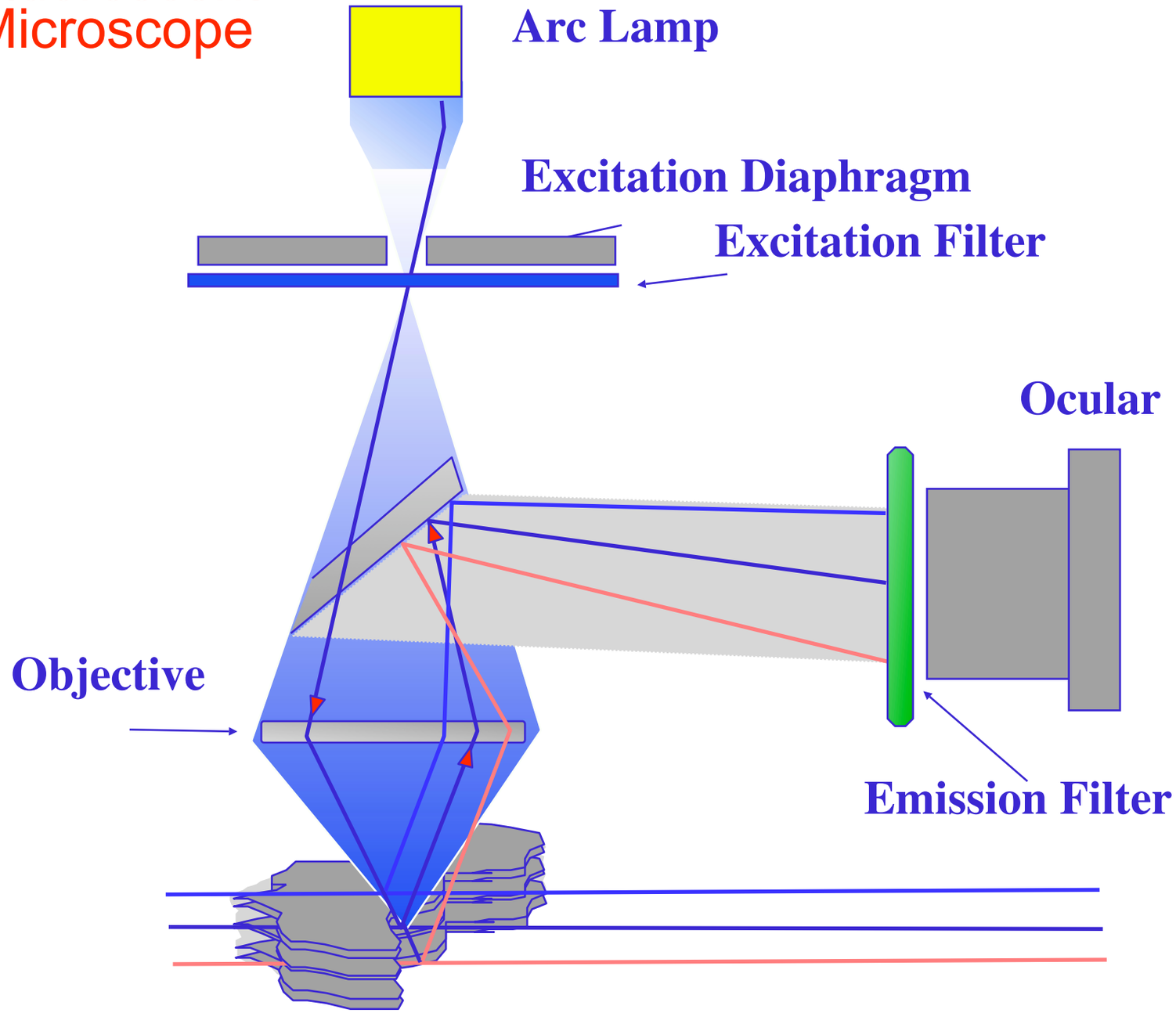
$O_j = I_j S_j + \sum_{k \neq j} I_k S_k + N$

Confocal Microscopy



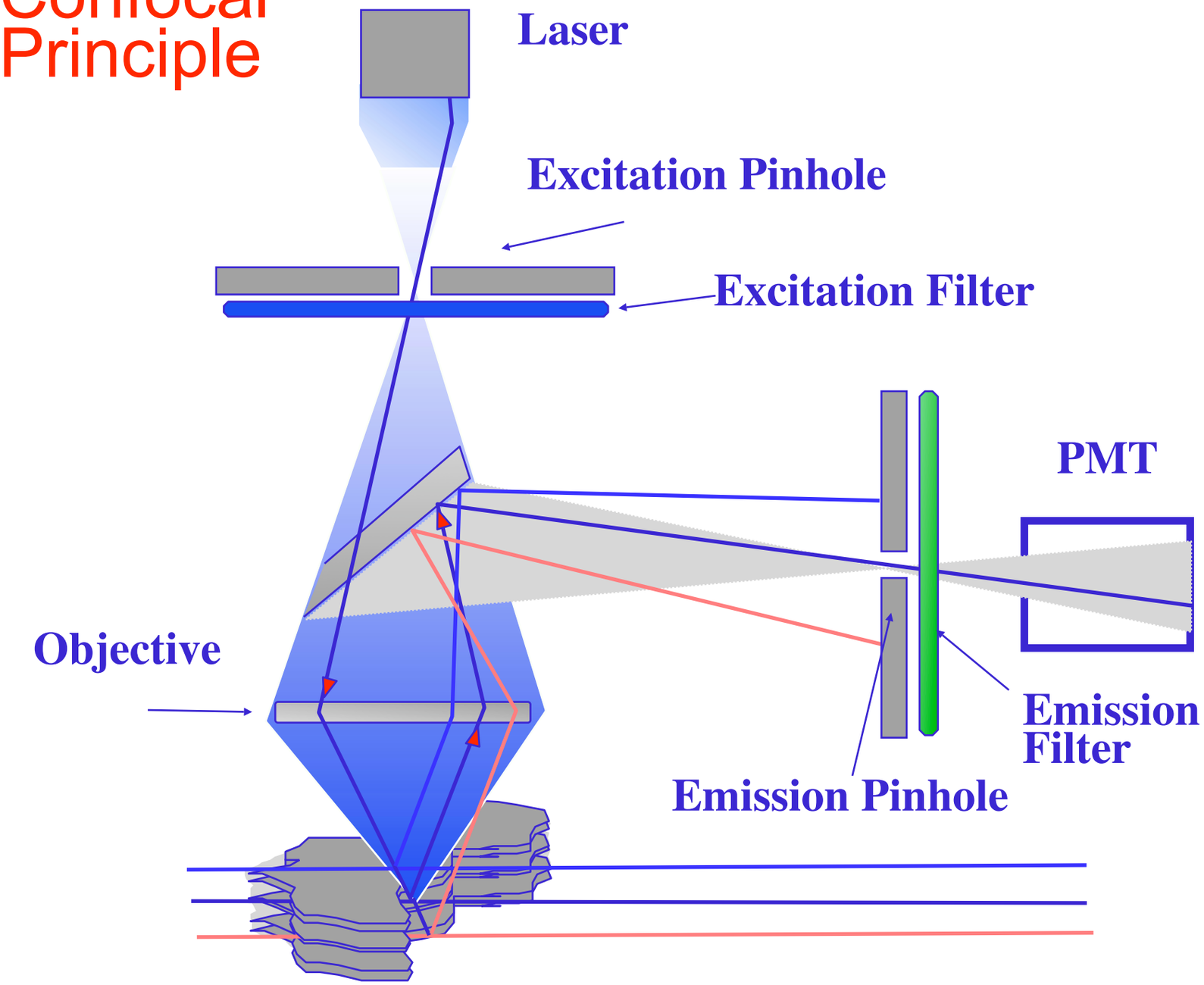
THE OPTICAL MICROSCOPE

Fluorescent Microscope



(COURTESY: PAUL ROBINSON, PURDUE UNIVERSITY)

Confocal Principle



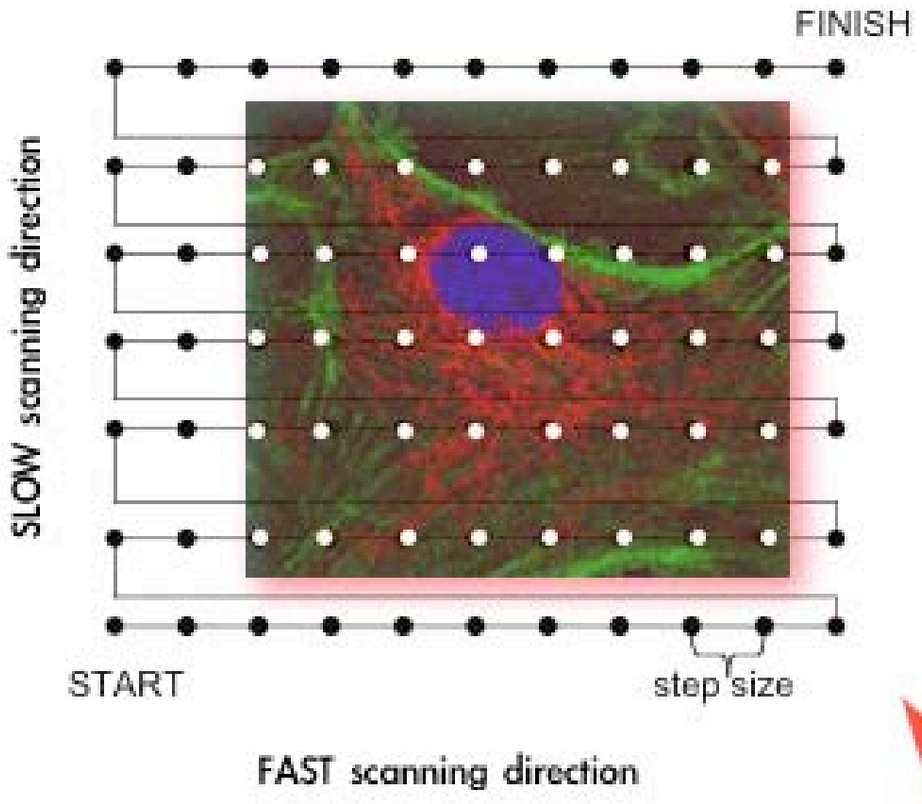
(COURTESY: PAUL ROBINSON, PURDUE UNIVERSITY)

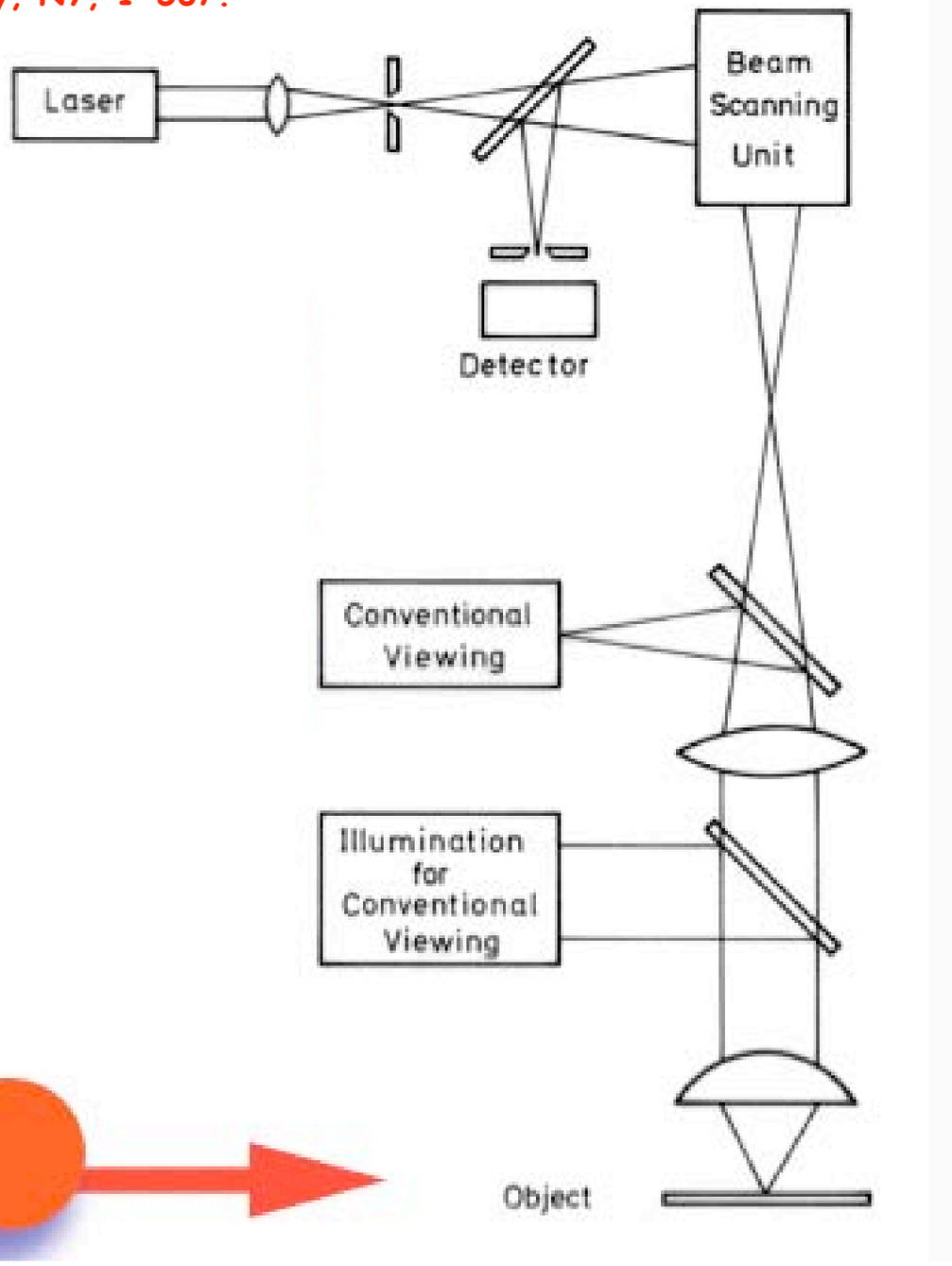
THE BILL...

(GOYA, Blindman's buff; credit:BRUNO SAMORI')

POINT BY POINT IMAGING

A.Diaspro (ed.), (2001) "Confocal and Two-photon Microscopy. Wiley, NY, 1-567.

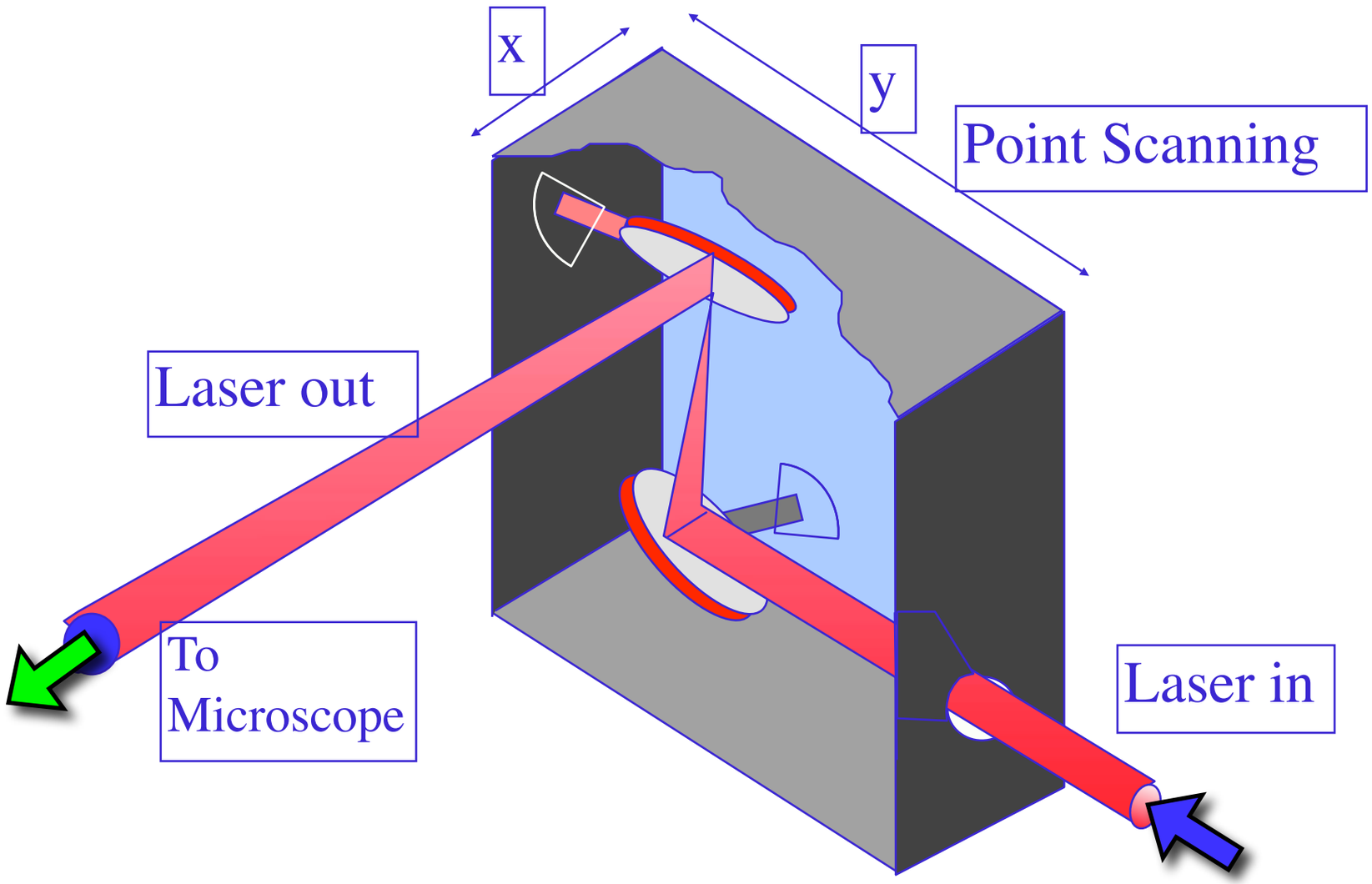


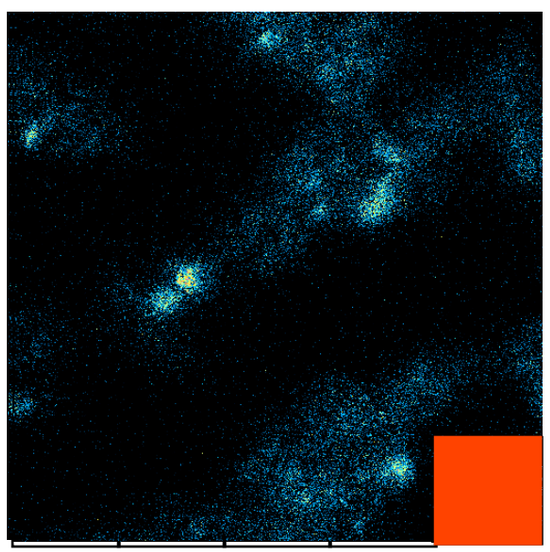


POINT BY POINT IMAGING

LIKE A FLORIAN'S COFFE IN VENICE

2 ms/line



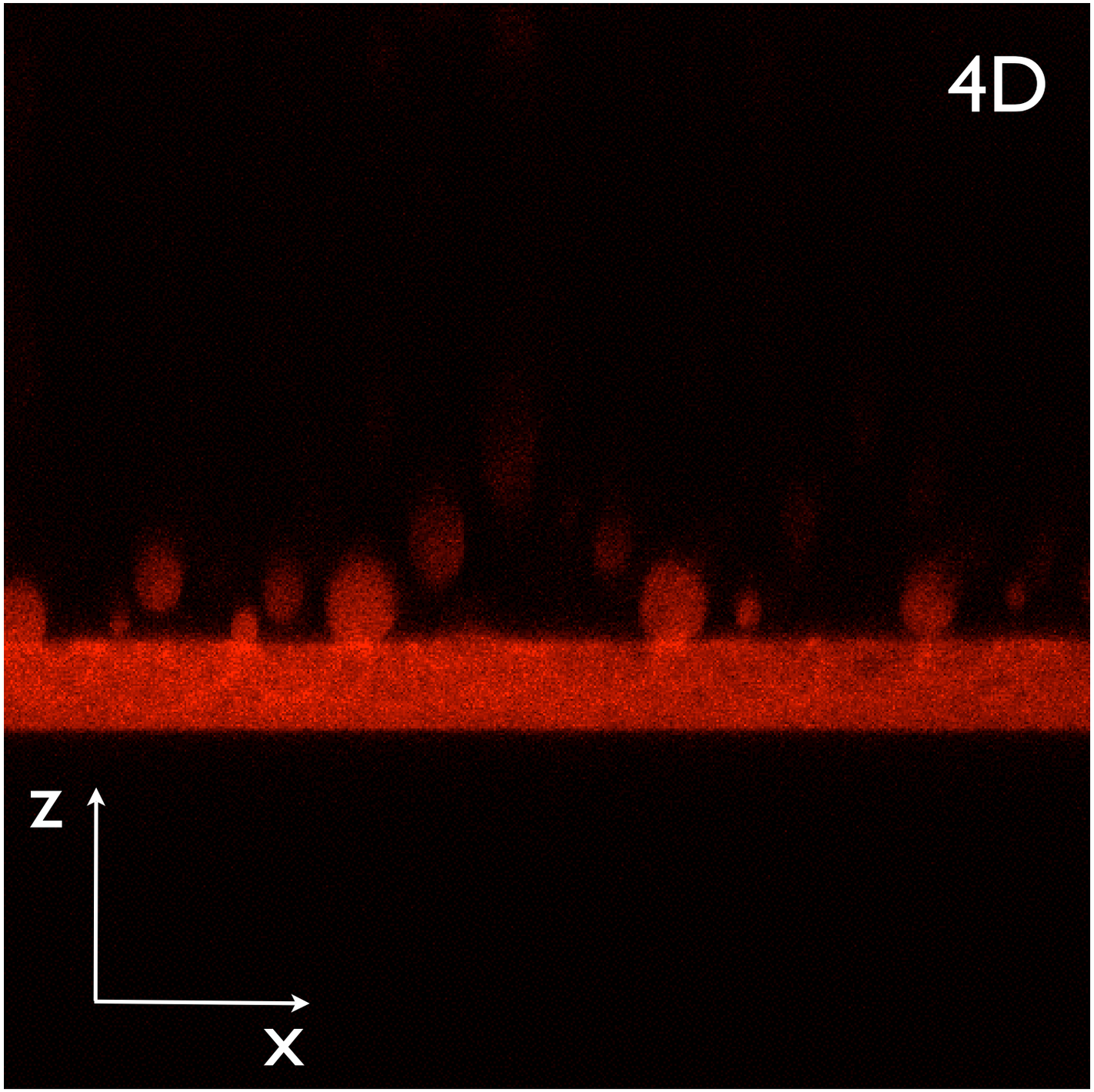


Slide credit: Fabio Cannone

POINT BY POINT IMAGING

LIKE A FLORIAN'S COFFE IN VENICE

2 ms/line

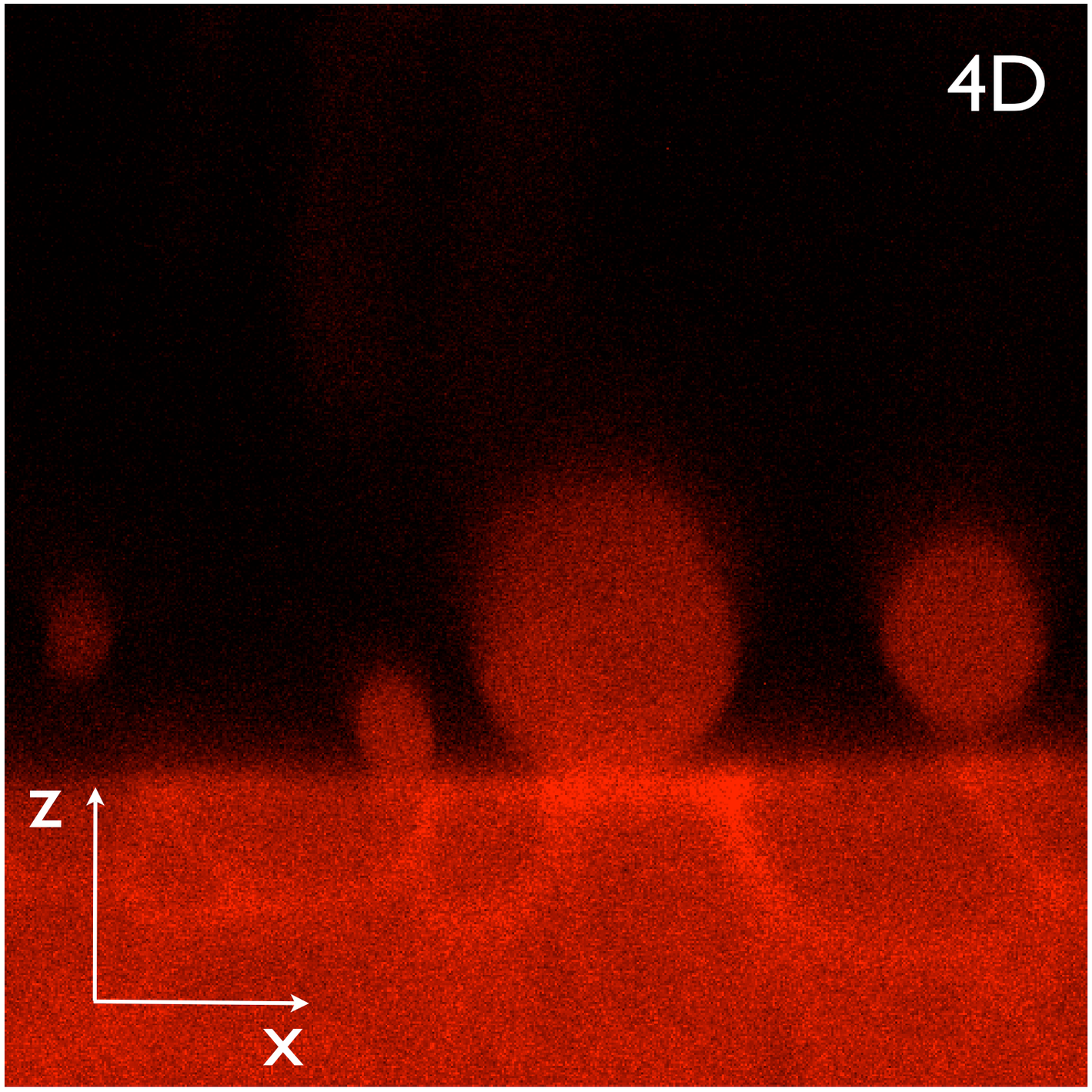


Slide credit:Davide Mazza, LAMBS-MicroScoBio, Genova

POINT BY POINT IMAGING

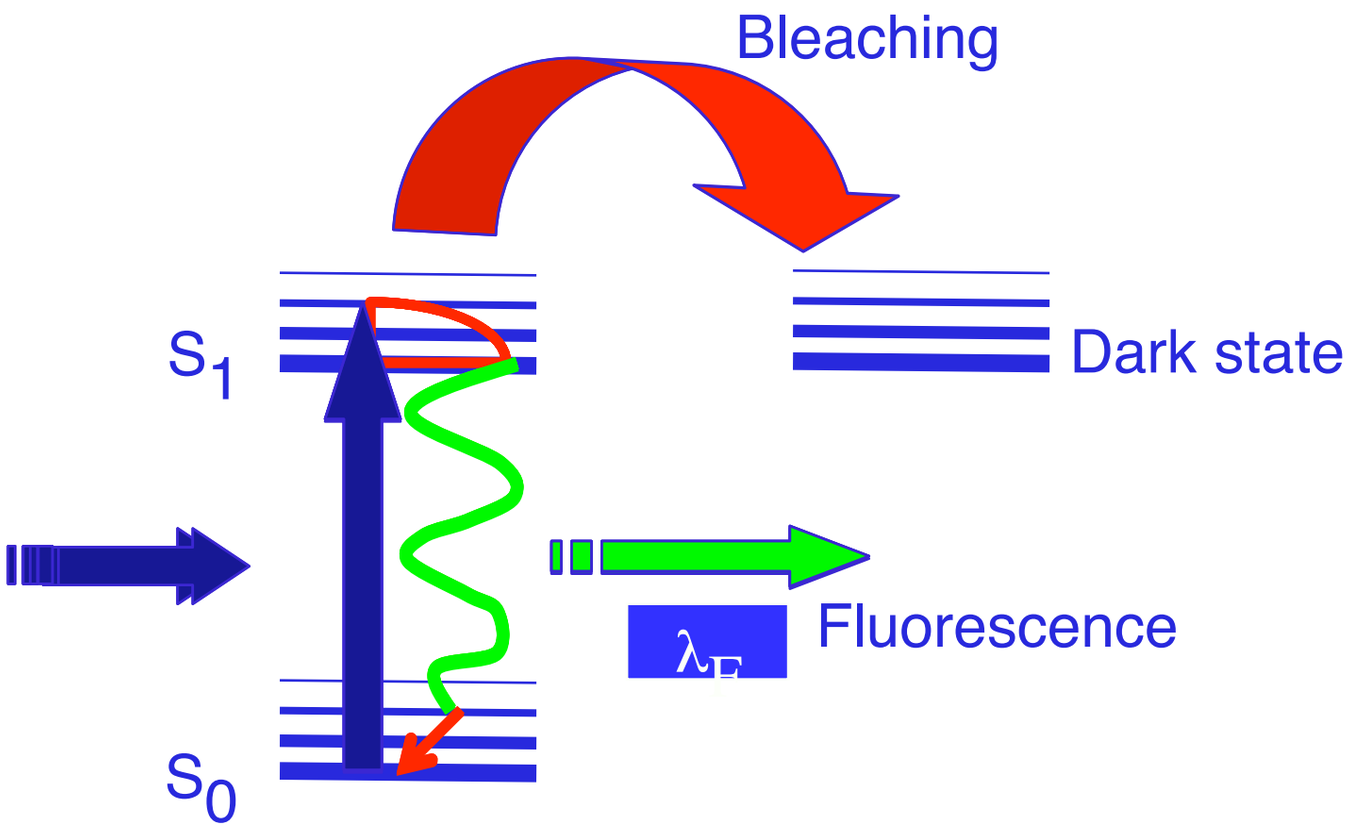
LIKE A FLORIAN'S COFFE IN VENICE

2 ms/line



Giuseppe Pellizza da Volpedo (1868-1907)
Panni al sole (1894), olio su tela, 87x131 cm, Domodossola, collezione privata

PHOTOBLEACHING PROBLEM



- Photochemical lifetime: fluorescein will undergo 30-40,000 emissions before bleaching.
- At low excitation intensities, pb occurs but at lower rate.
- Bleaching is often photodynamic--involves light and oxygen.

Diaspro A. et al., Photobleaching, in Handbook of Confocal Microscopy (J.Pawley ed.), Plenum, NEW edition, 2006

Slide credit: Giberto Chirico, Enrico Gratton

PHOTOBLEACHING PROBLEM

Diaspro A. et al., Photobleaching, in Handbook of Confocal Microscopy (J.Pawley ed.), Plenum, NEW edition, 2006

Image credit:David Piston, Vanderbilt University

TWO-PHOTON EXCITATION MICROSCOPY

A.Diaspro (ed.), (2001) "Confocal and Two-photon Microscopy. Wiley, NY, 1-567.

TWO-PHOTON EXCITATION MICROSCOPY

A.Diaspro (ed.), (2001) "Confocal and Two-photon Microscopy. Wiley, NY, 1-567.

Giuseppe Pellizza da Volpedo (1868–1907)

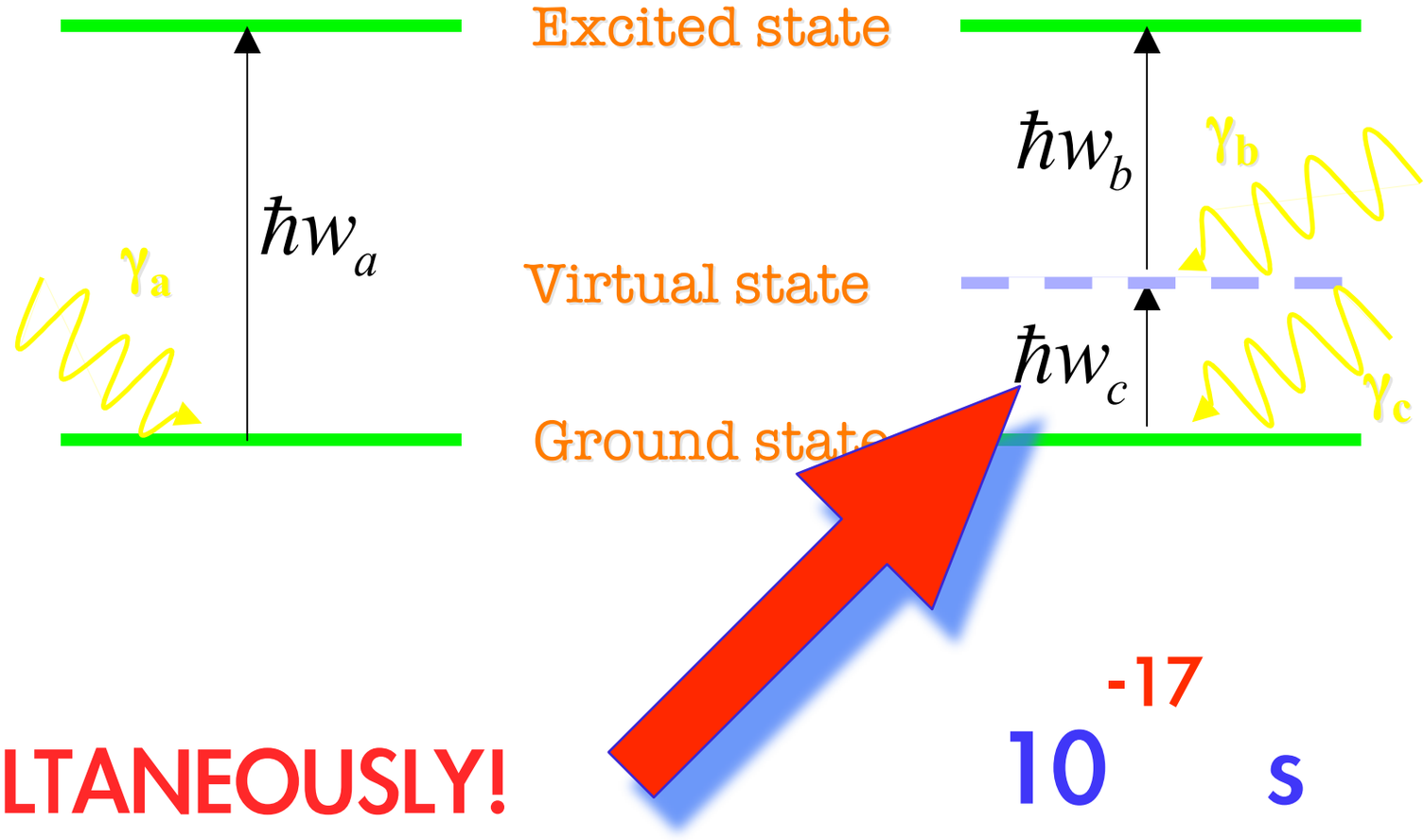
Il quarto stato (1901), olio su tela, 293x545 cm, Milano, Civica Galleria d'Arte Moderna

TWO-PHOTON EXCITATION MICROSCOPY

(Credit: CLAUDIA DIASPRO from JOVANOTTI'S POP CONCERT)

TWO-PHOTON EXCITATION MICROSCOPY

(credit: EMILIO SEGRE' archive)



SIMULTANEOUSLY!

10^{-17} s

Maria Göppert-Mayer predicted that an atom or a molecule could interact with two photons simultaneously by absorbing them in the very same quantum event.

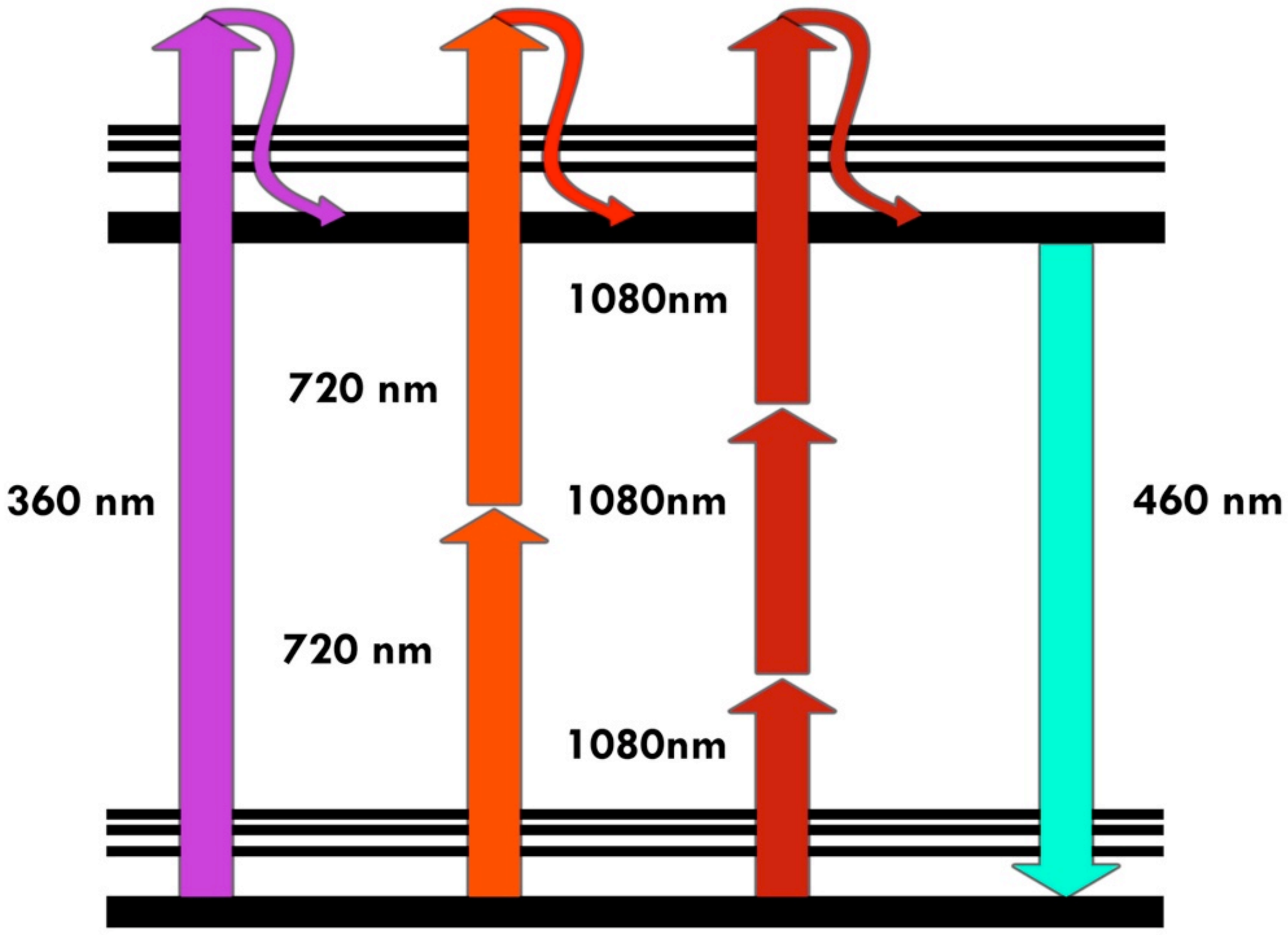
TWO-PHOTON EXCITATION MICROSCOPY

$E_a = E_b + E_c$

$$\lambda_a = \left(\frac{1}{\lambda_b} + \frac{1}{\lambda_c} \right)^{-1}$$

Movie Credit: Elio Cocchia Fabrizio Papalia, Paolo Sapuppo - LEICA Microsystems

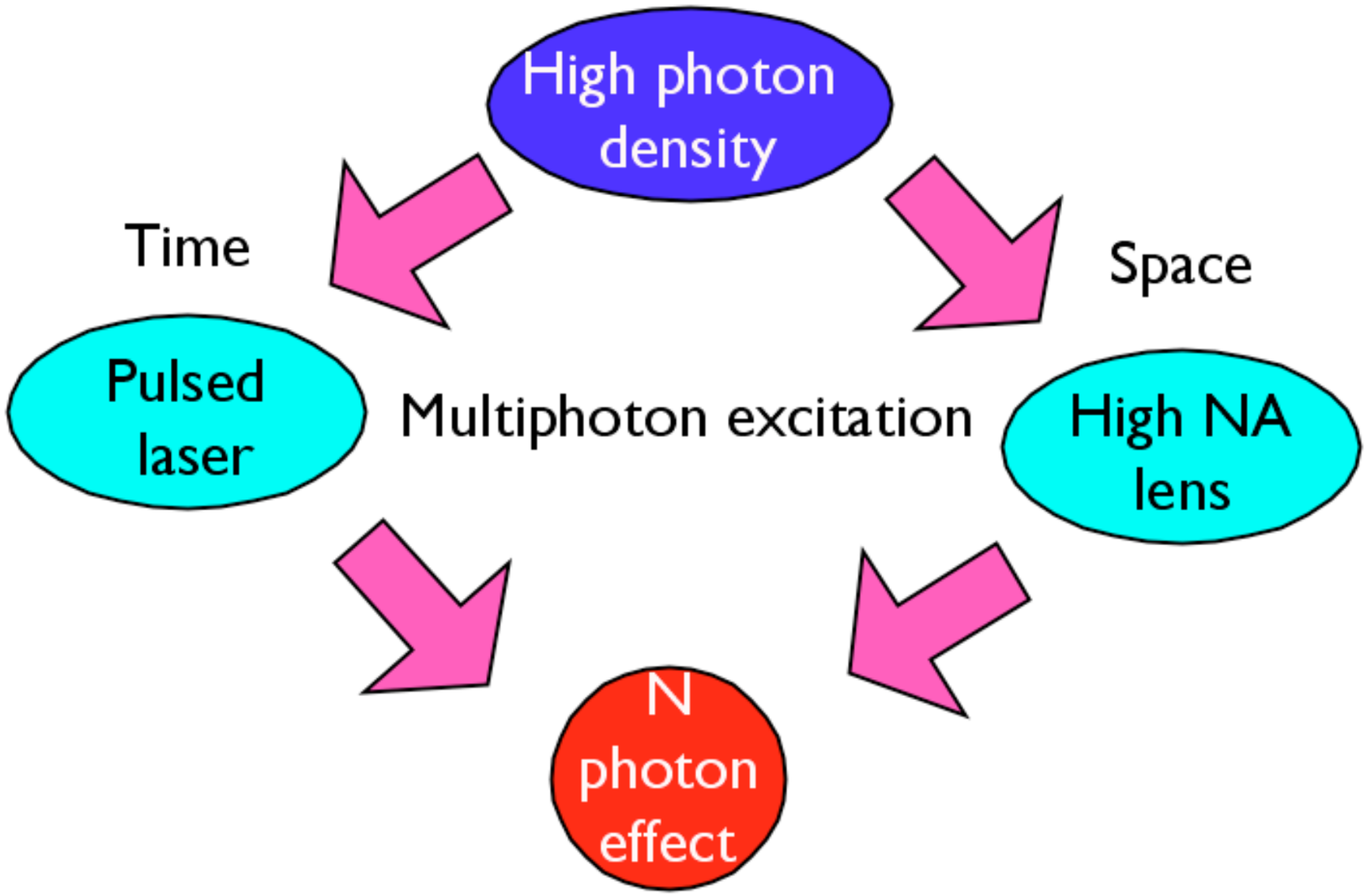
TWO-PHOTON EXCITATION MICROSCOPY



IN BRIGHT SUNLIGHT, A MOLECULE OF RHODAMINE B, AN EXCELLENT 1- OR 2- PHOTON ABSORBER, ABSORBS A PHOTON THROUGH A 1-PHOTON PROCESS ABOUT ONCE A SECOND, A PHOTON PAIR BY 2-PHOTON ABSORPTION EVERY 10 MILLION YEARS...

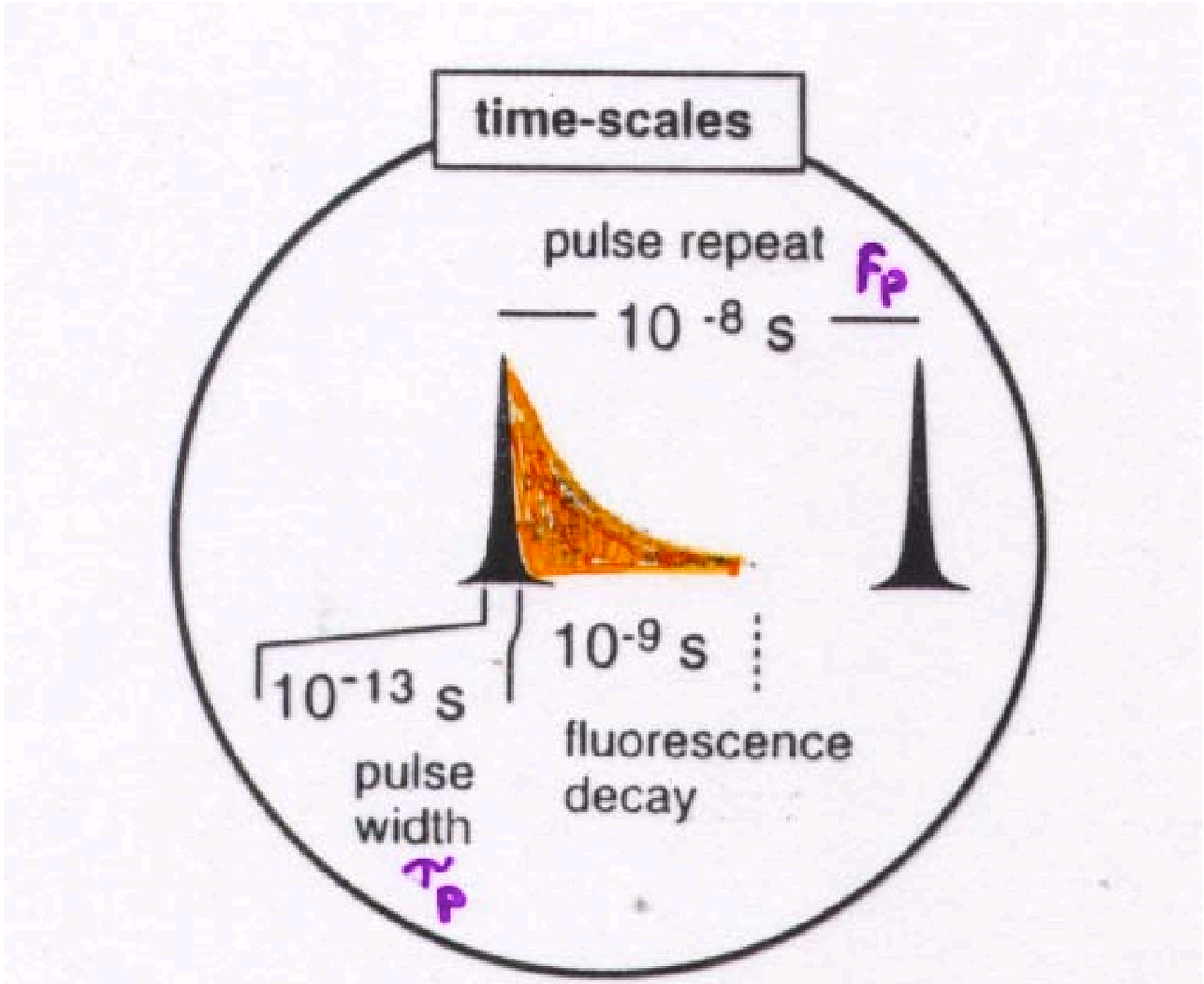
Denk and Svoboda (1997) Neuron, 18:351

TWO-PHOTON EXCITATION MICROSCOPY

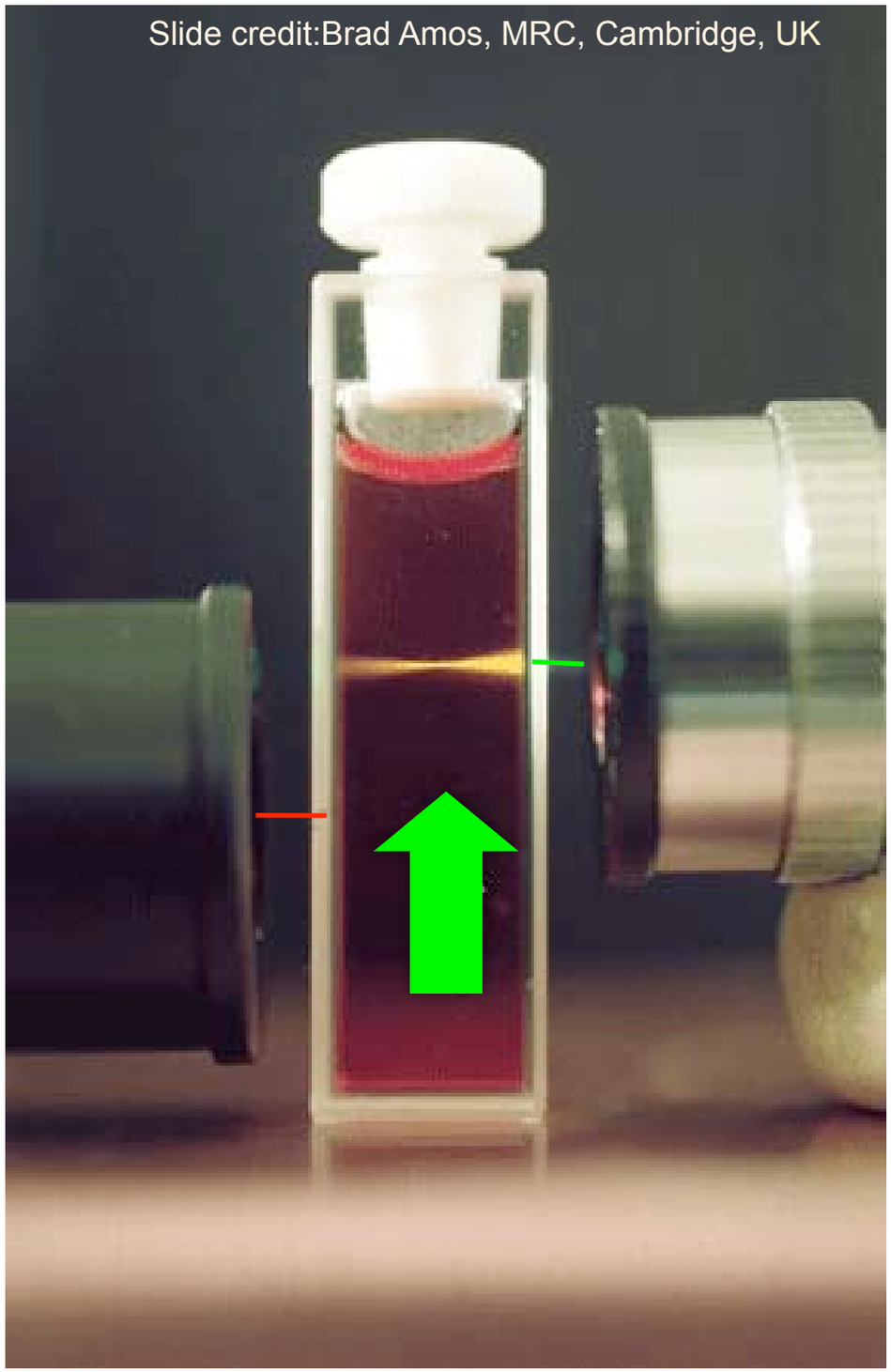


A.DIASPRO, G.CHIRICO, M.COLLINI (2004) QUART. REV. .BIOPHYS.,VOL.38, NR.2, PP.1-72 (2006).

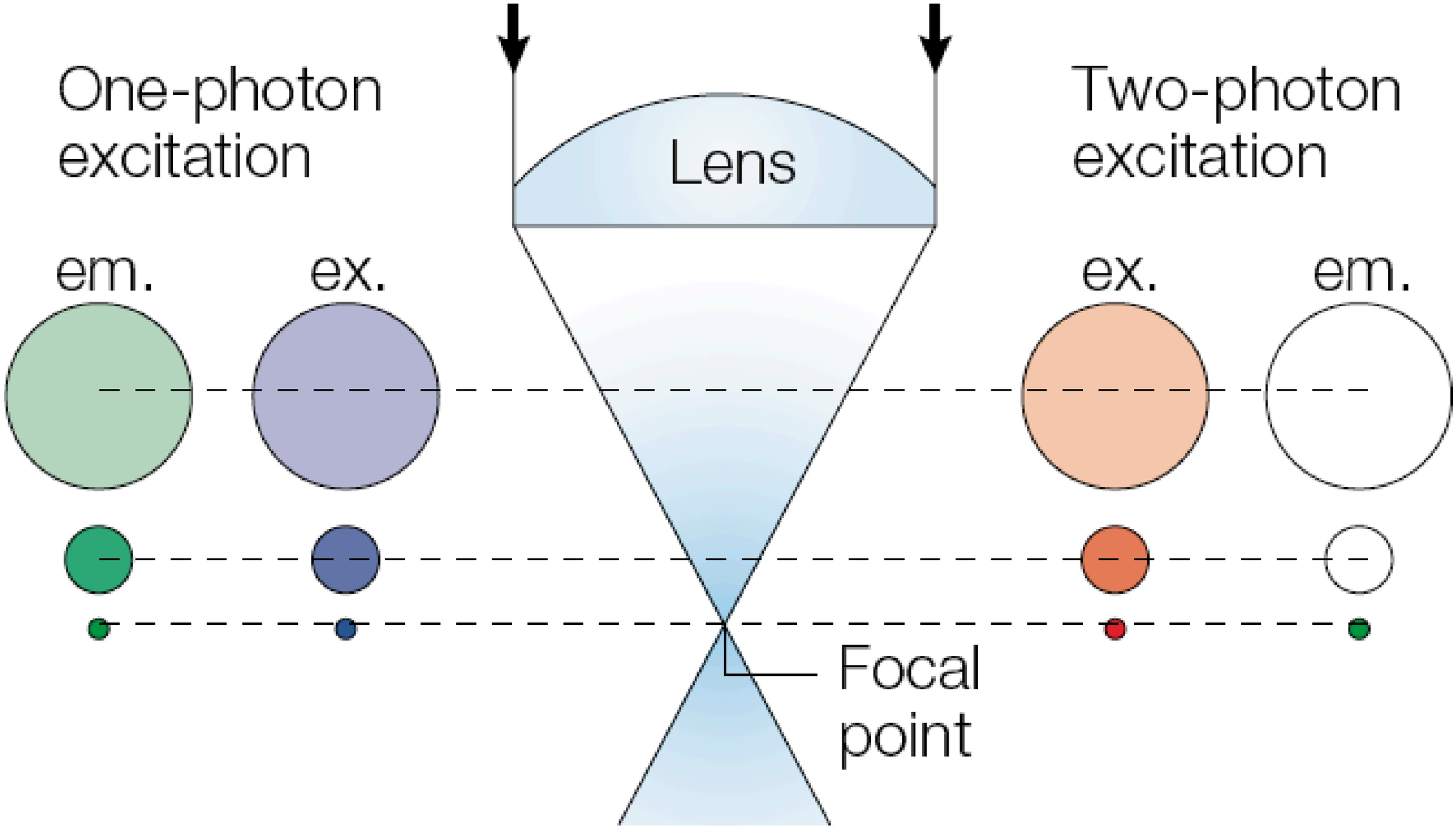
CORE ELEMENTS ARE LASER SOURCES



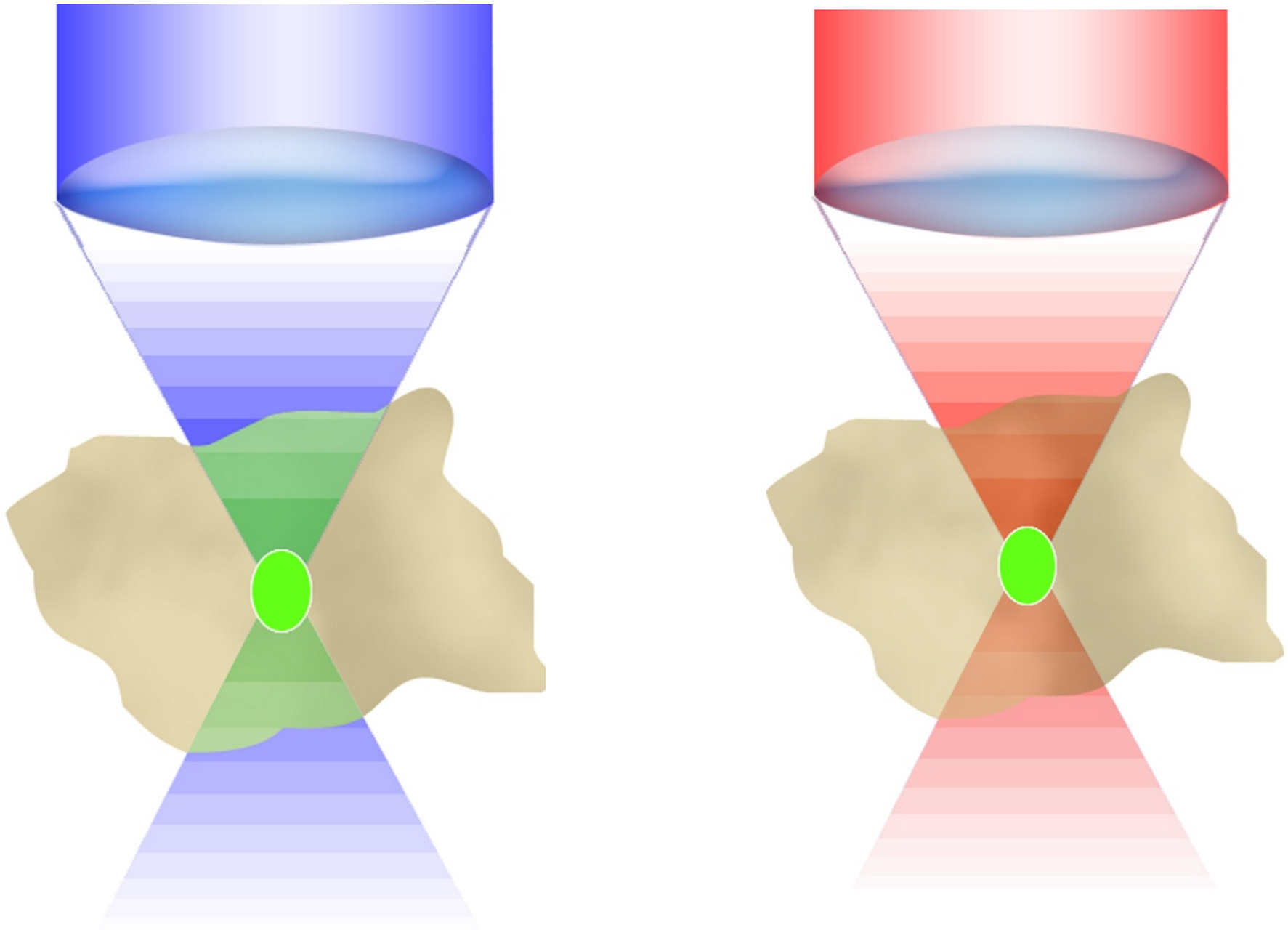
Pav= D*Ppk; D=t*F


Slide credit:Brad Amos, MRC, Cambridge, UK

TWO-PHOTON EXCITATION MICROSCOPY

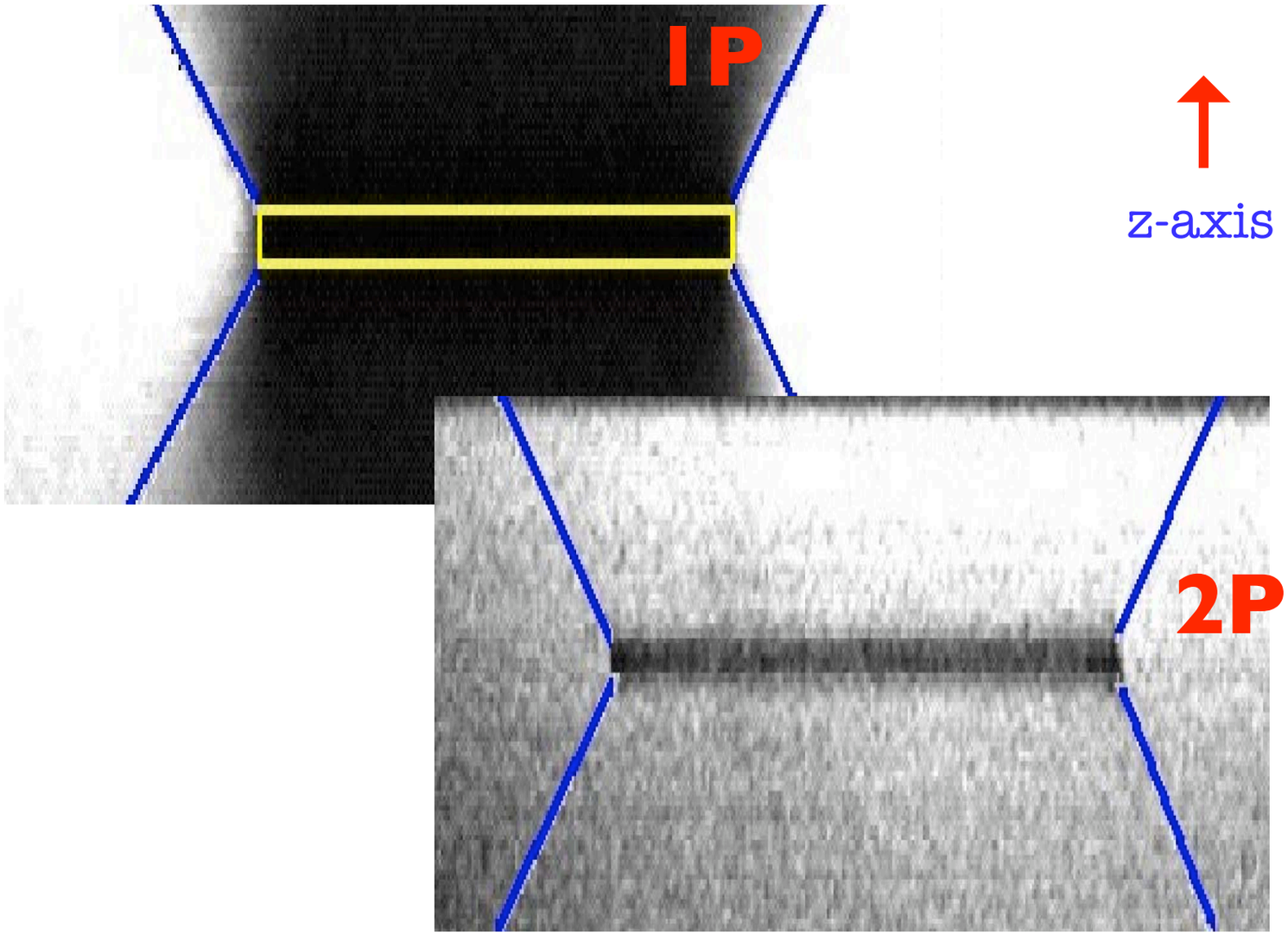


M.D.Cahalan et al. (2002) Nat.Rev.Immunol., 2: 872



Slide credit:Paolo Bianchini, LAMBS-MicroScoBio, Genova

TWO-PHOTON EXCITATION MICROSCOPY



Slide credit:David Piston, Vanderbilt University

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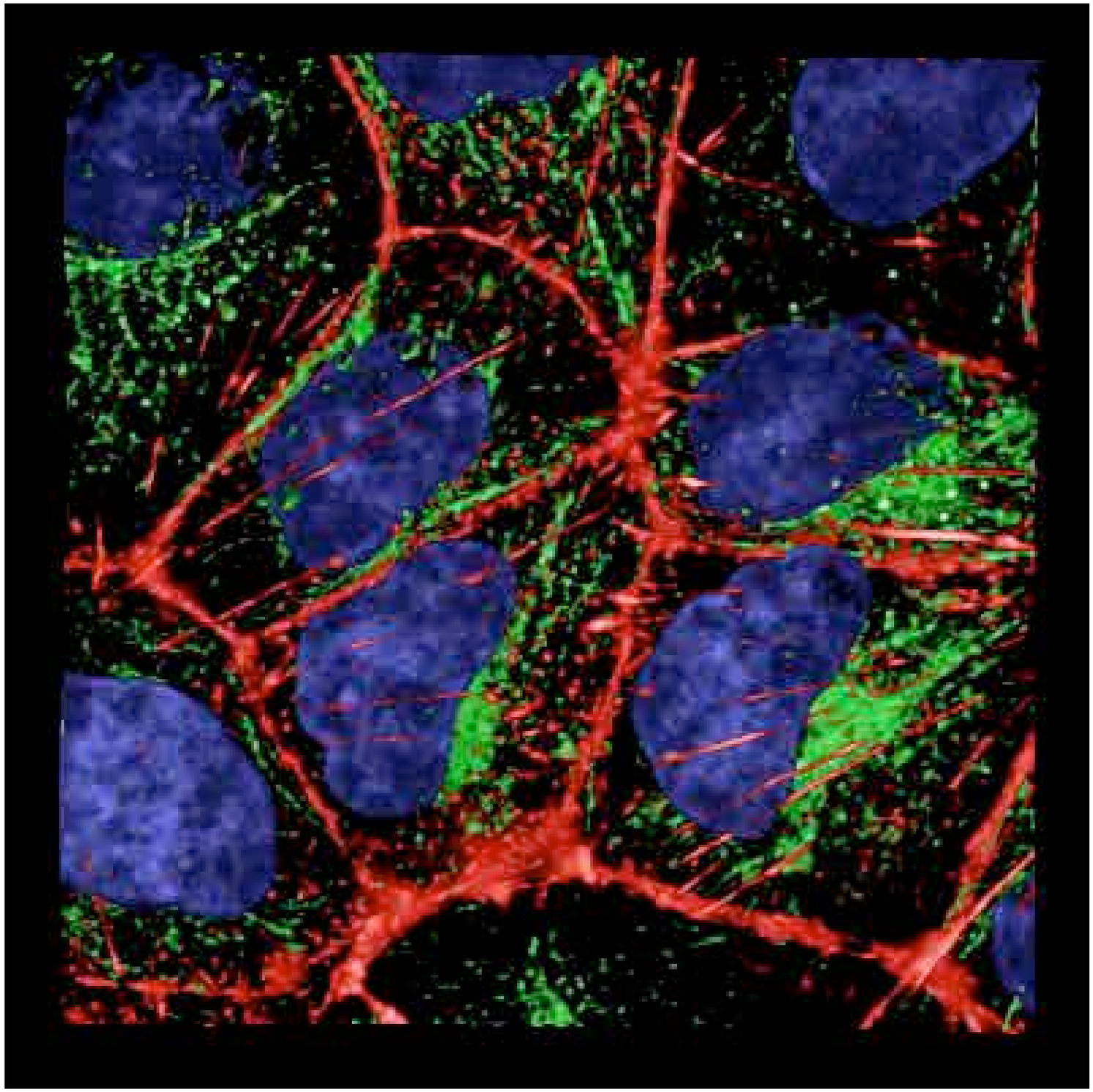
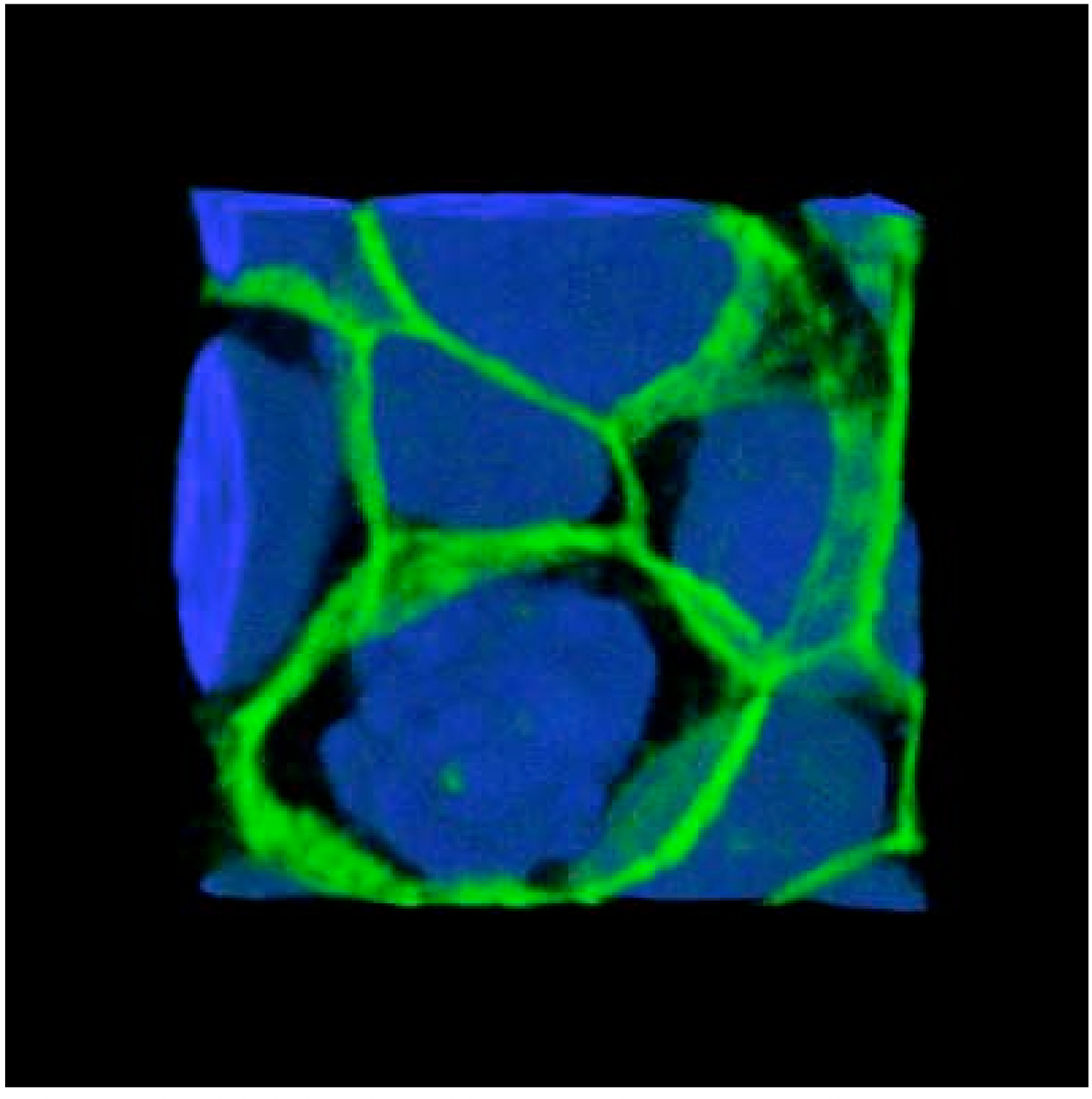


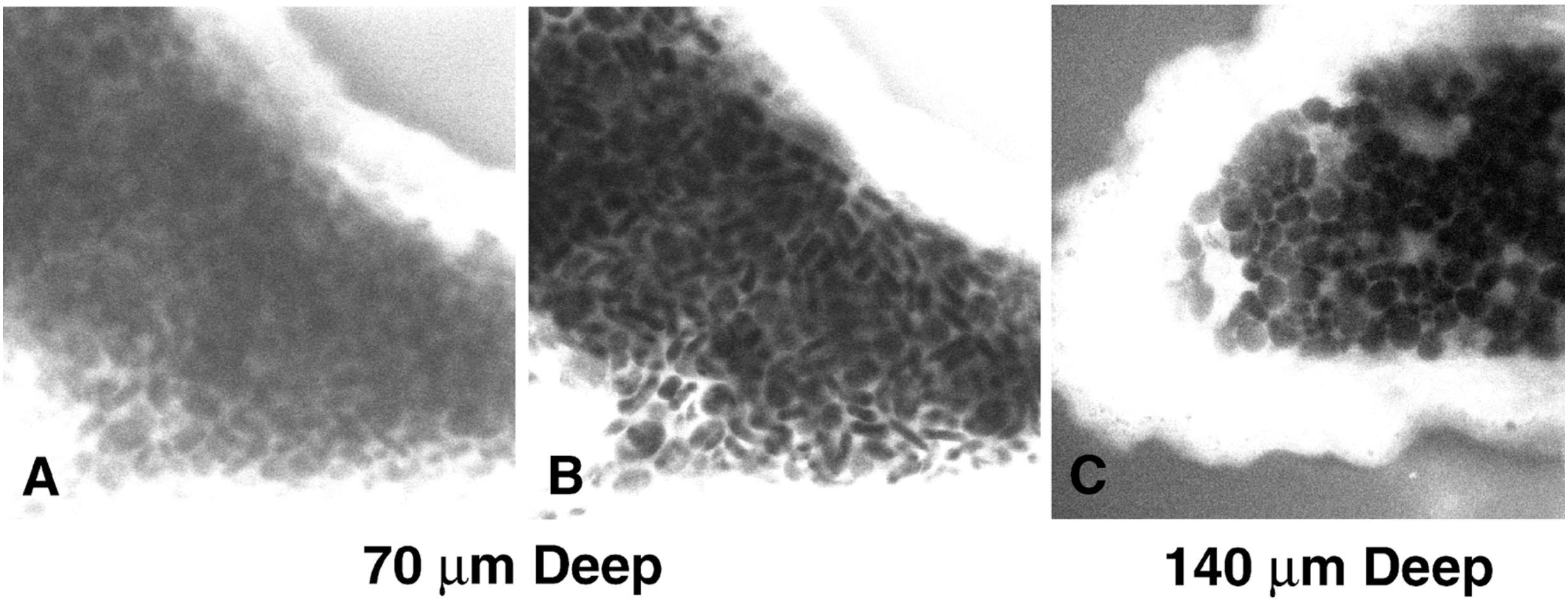
Tissue cultured PTR cells, TexasRed-Phalloidin, FITC-a tubulin, and DAPI. (Drs. Wang and Dunn, ICBM)

TWO-PHOTON EXCITATION MICROSCOPY



Two photon volume of dapi labeled nuclei and fluorescein labeled anti-cadherin. (Dr. Dunn, ICBM)

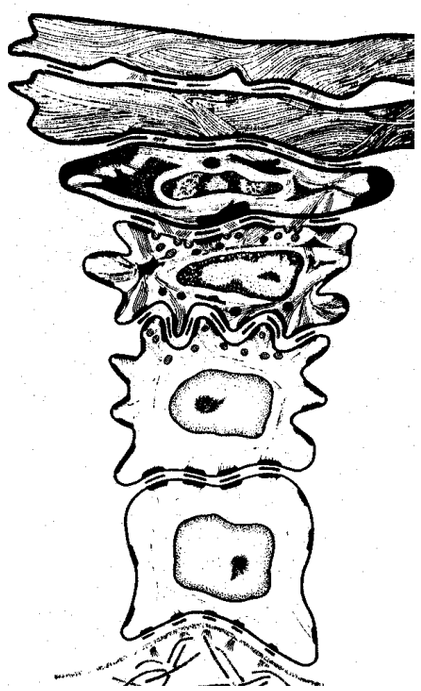
Penetration depth



Images of a Shark Choroid Plexus Stained with Fluorescein

Piston DW (2005), PLoS Biol 3(6): e207

Penetration depth



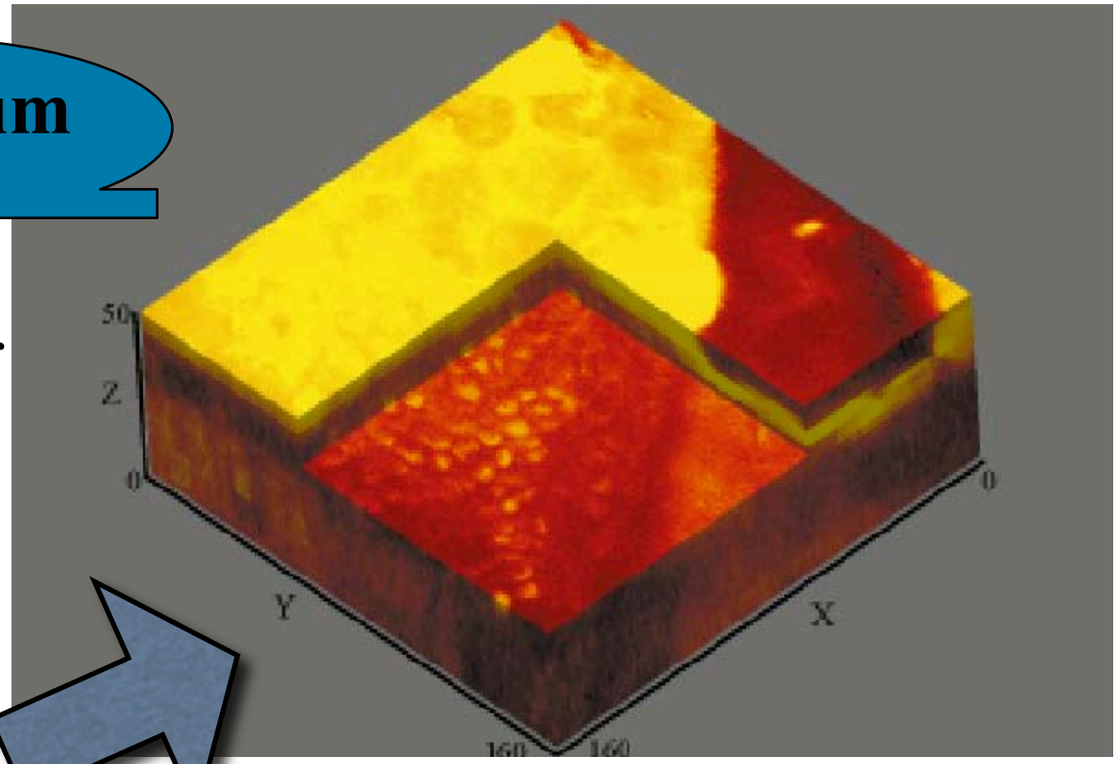
Stratum

Corneum

Granular

Spinous

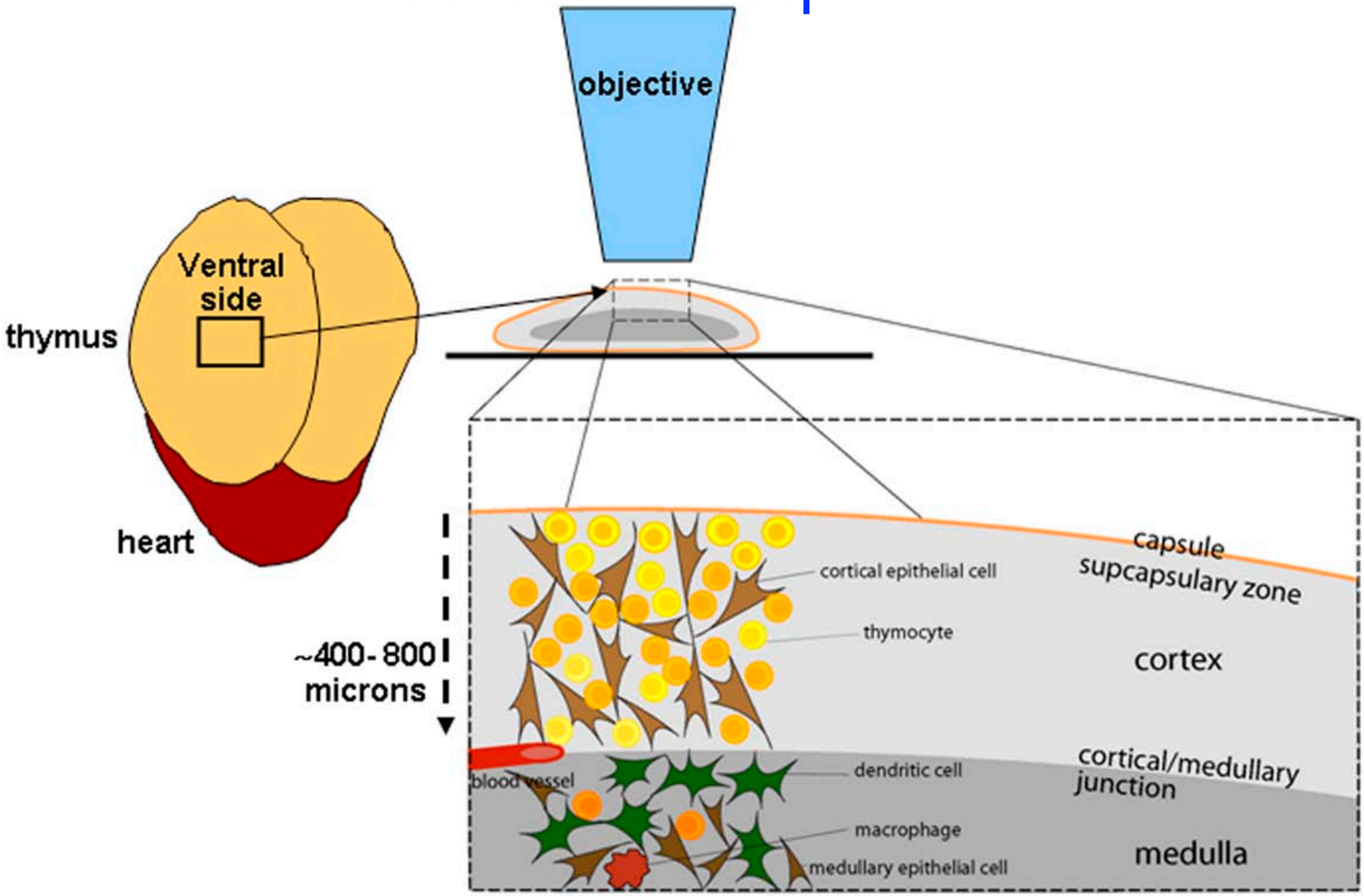
Basal



Three-dimensional reconstructed two-photon images of in vivo human skin. The strata corneum and the basal layers are clearly visible.

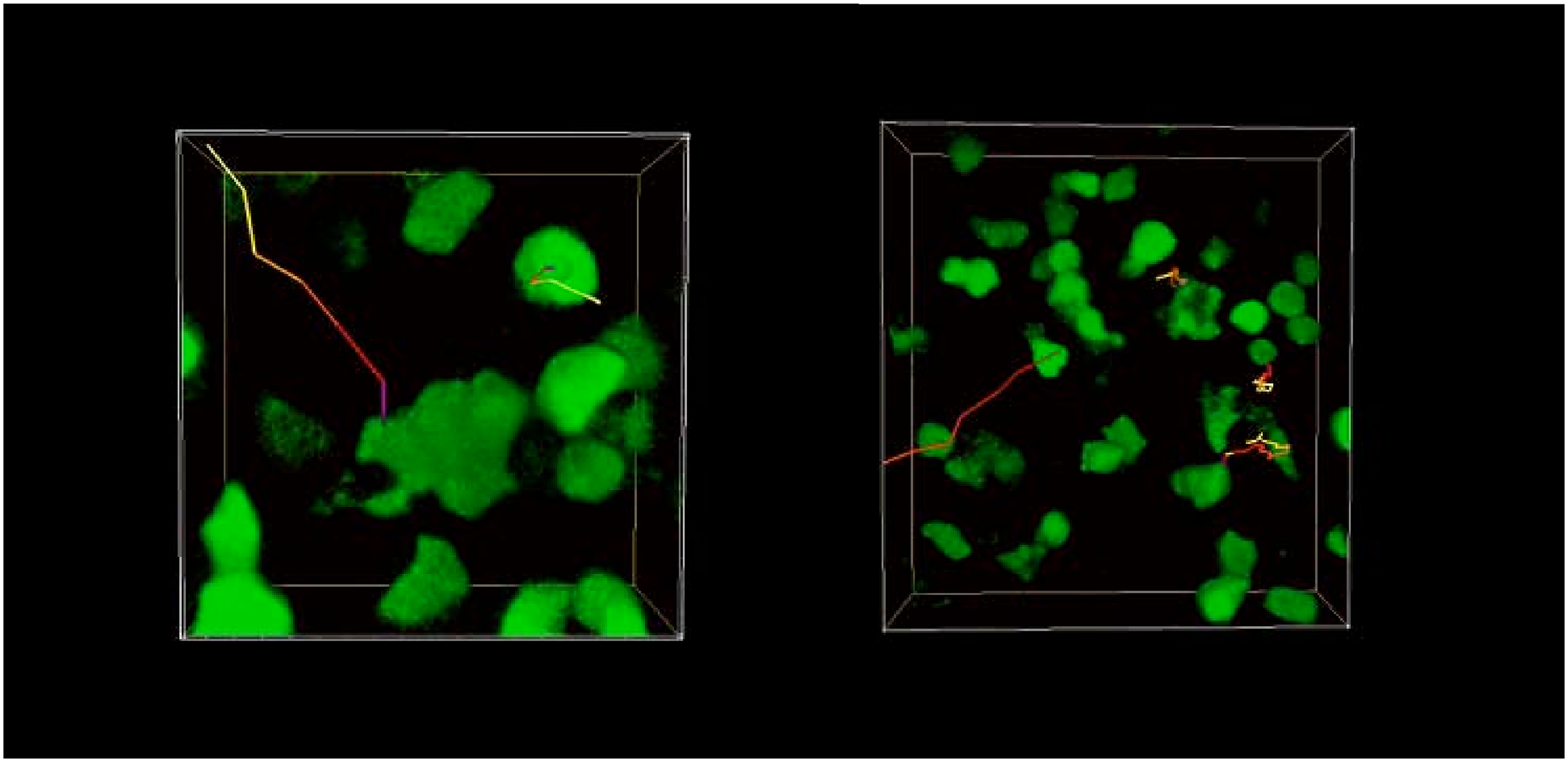
(Image credit: PETER SO, BARRY MASTERS)

Penetration depth



Real-time visualization of thymocytes within intact thymic lobes using two-photon microscopy (2005) PLoS Biol 3(6): e205

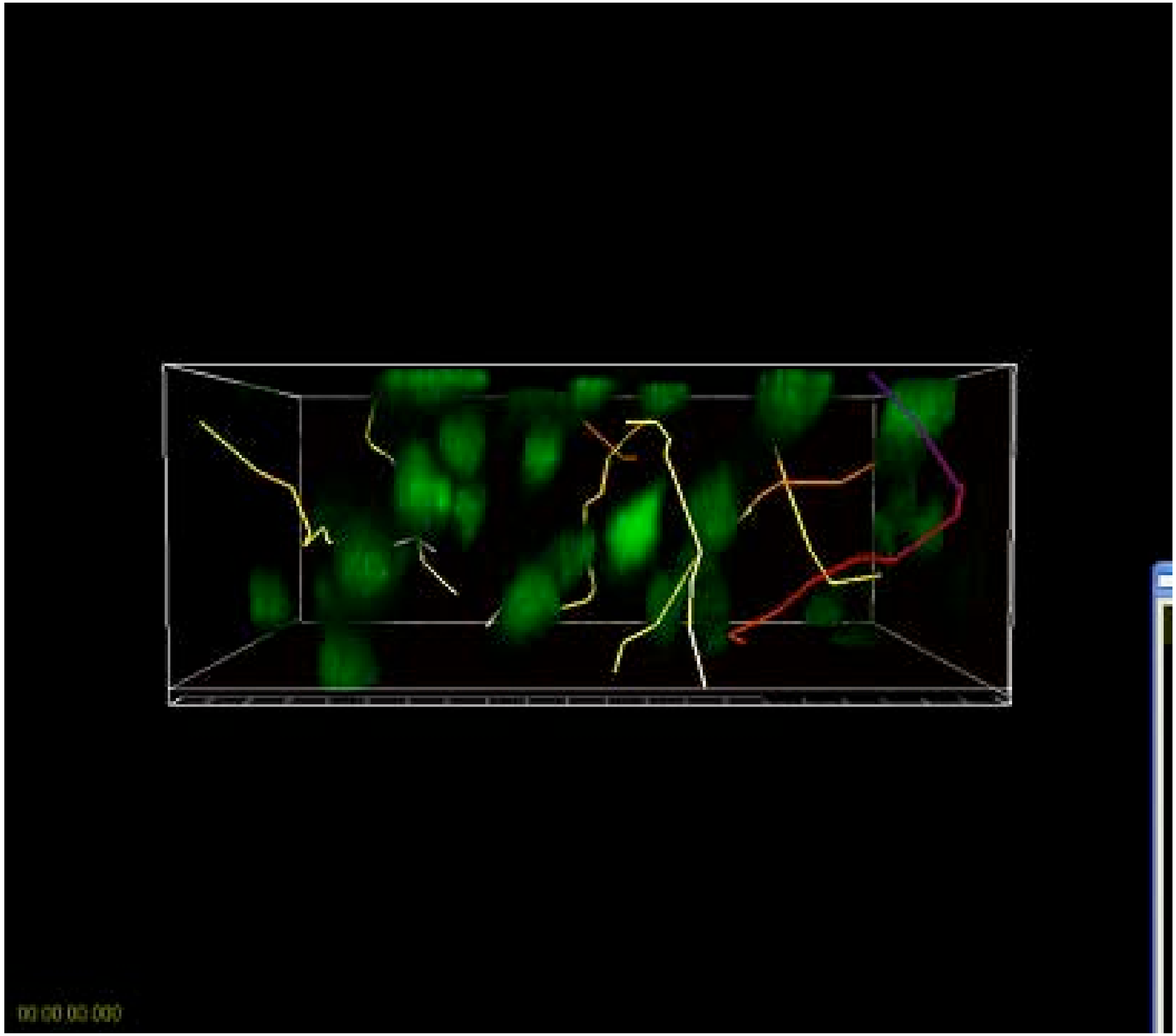
TWO-PHOTON EXCITATION MICROSCOPY



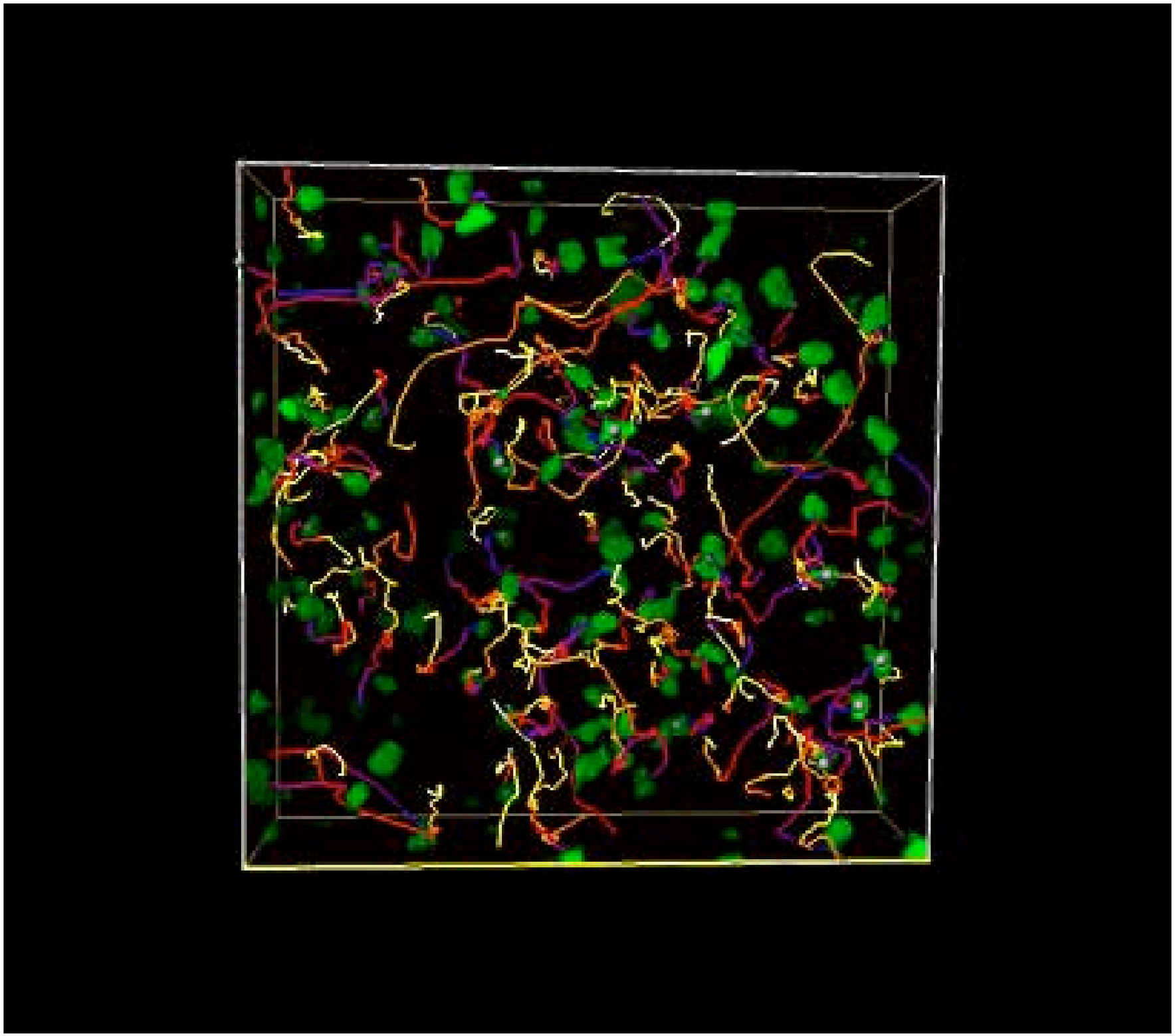
Two-photon microscopy reveals the three-dimensional dynamics of B cell (red) and T cell (green) conjugate within lymph tissue in real time

Citation: (2005) Tracking the Details of an Immune Cell Rendezvous in 3-D. PLoS Biol 3(6): e206

TWO-PHOTON EXCITATION MICROSCOPY

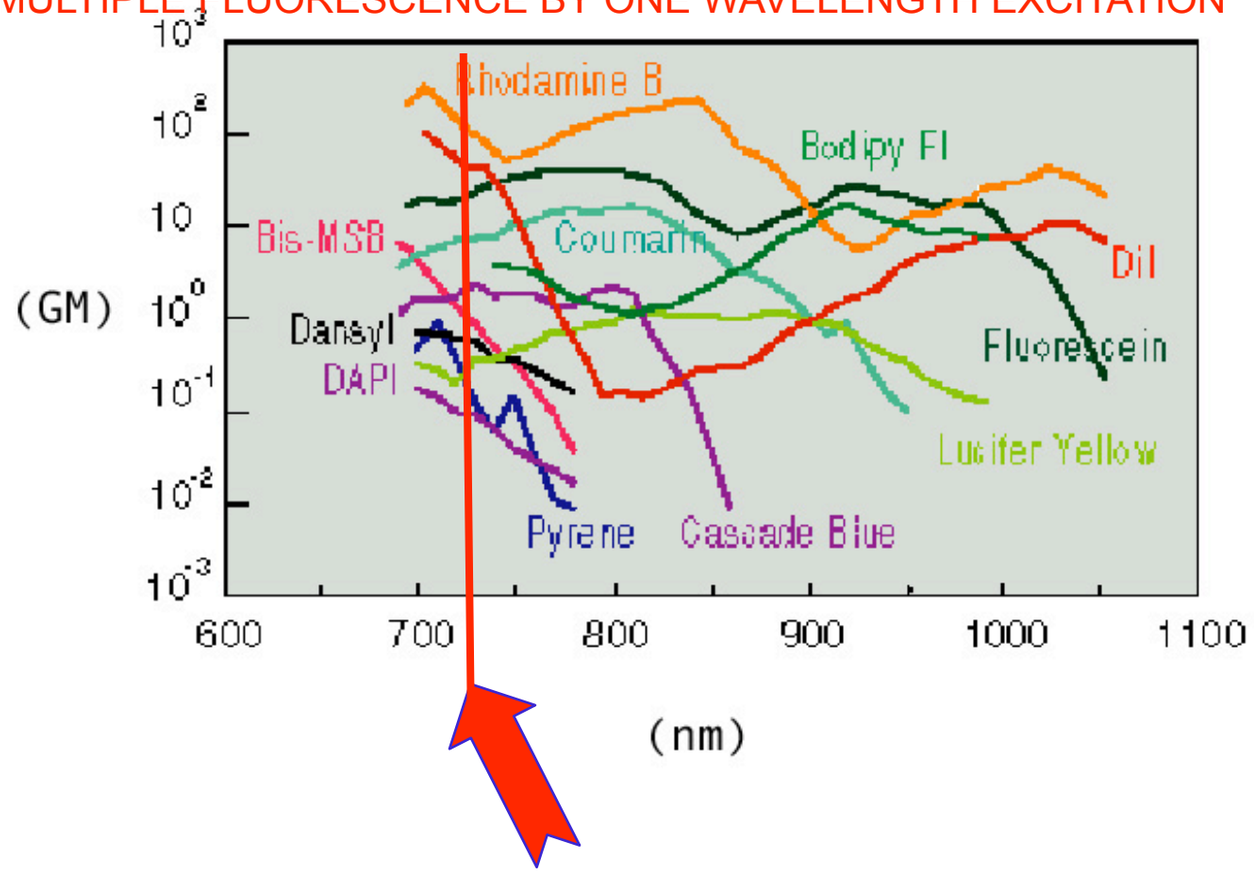


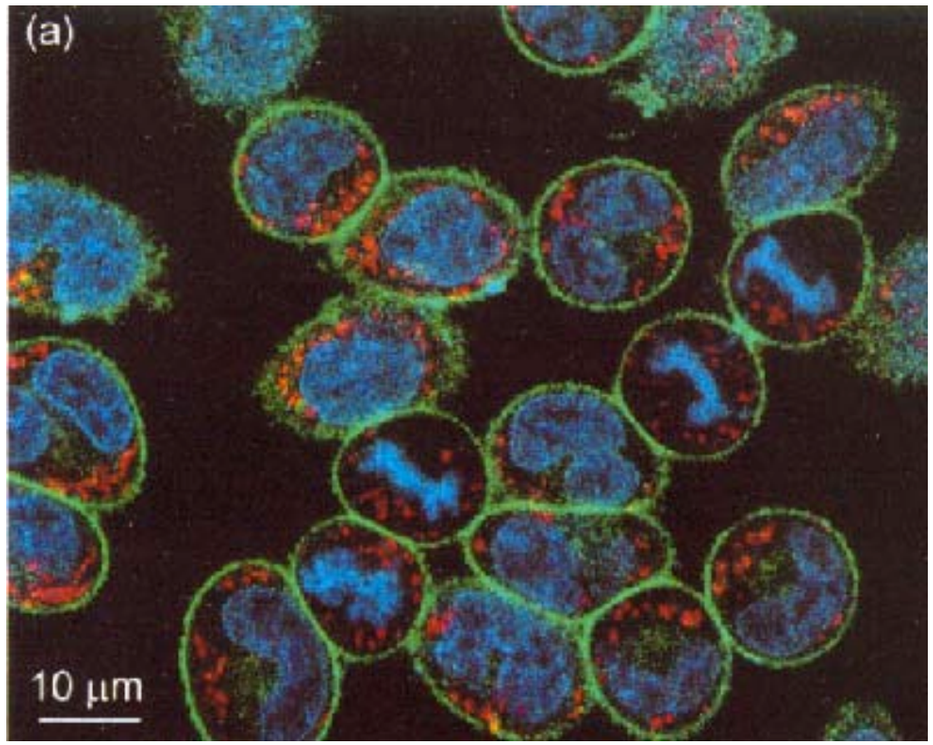
TWO-PHOTON EXCITATION MICROSCOPY



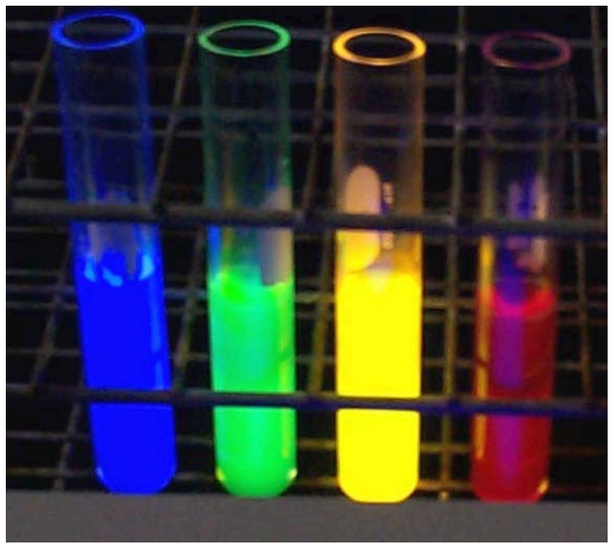
TWO-PHOTON EXCITATION MICROSCOPY

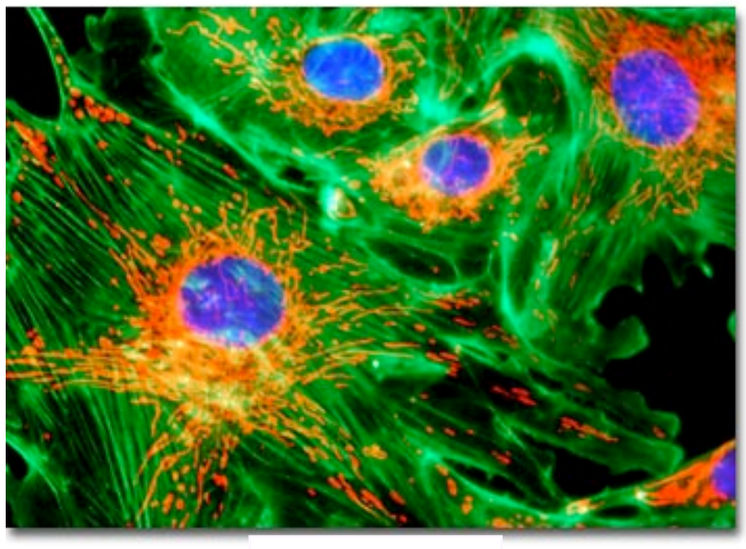
MULTIPLE FLUORESCENCE BY ONE WAVELENGTH EXCITATION

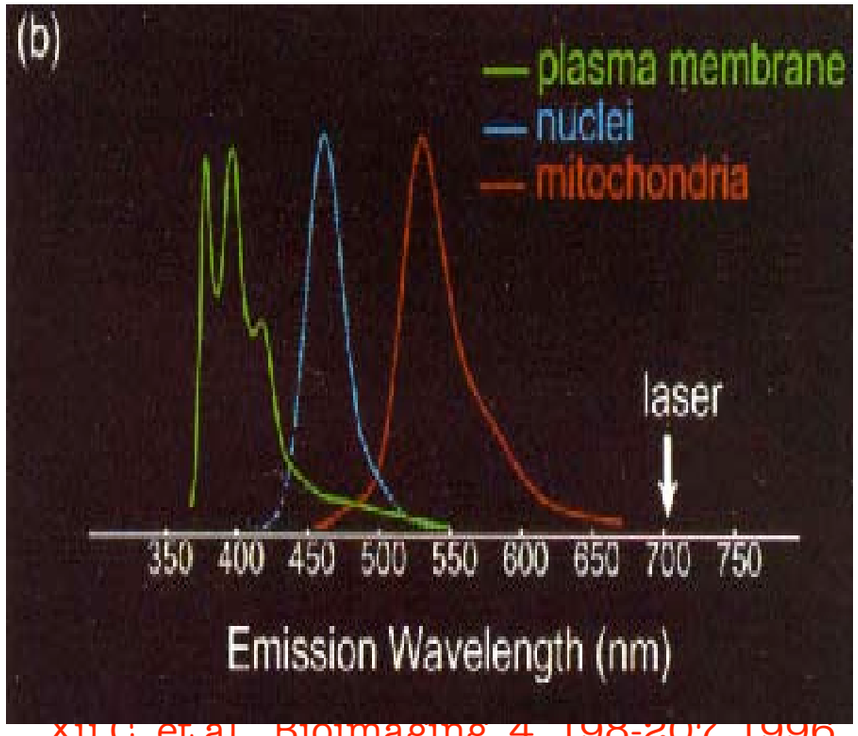




One dice...two dices...to get 5!

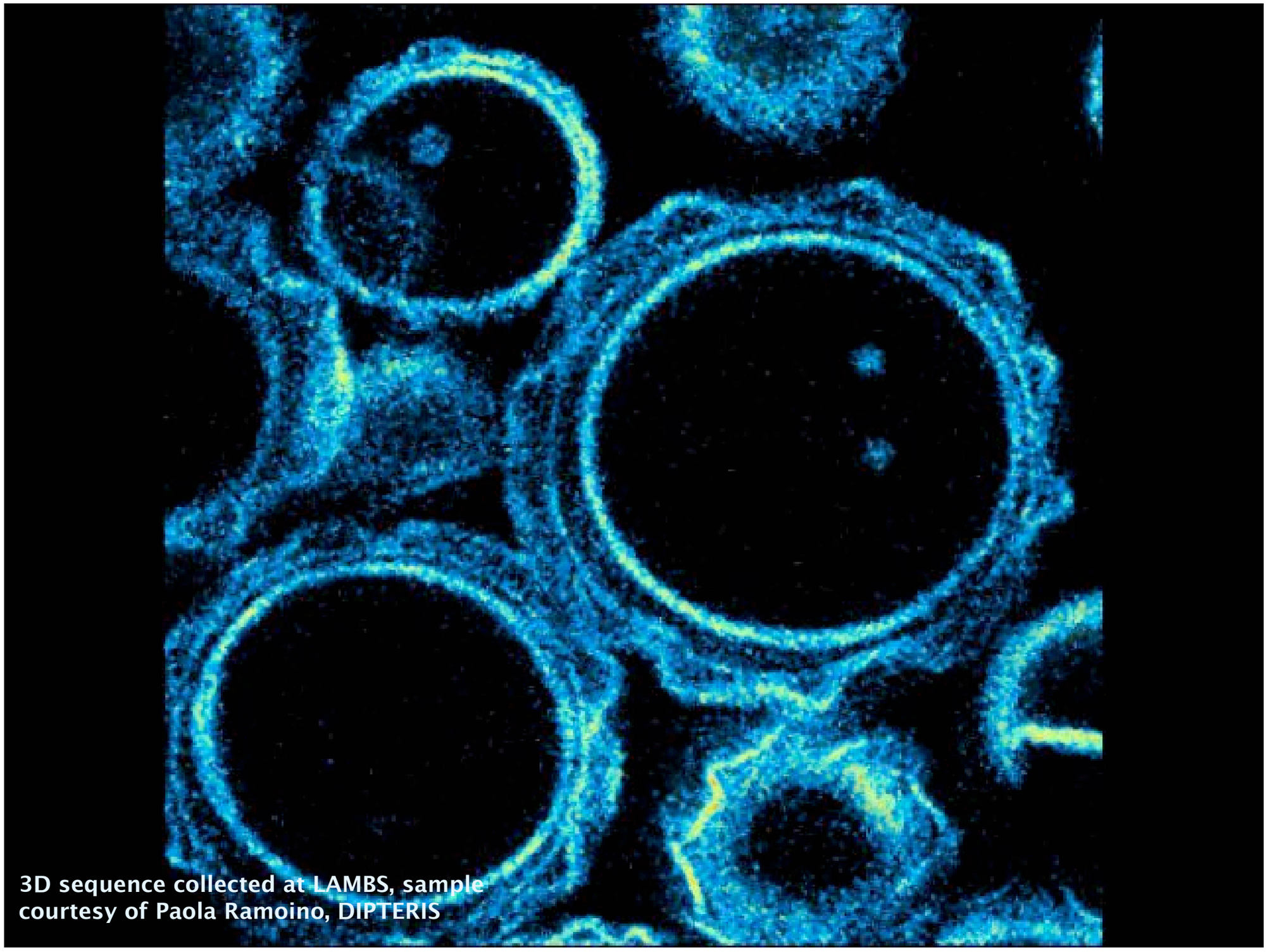






Xu C. et al., Biomaging, 4, 198-207, 1996

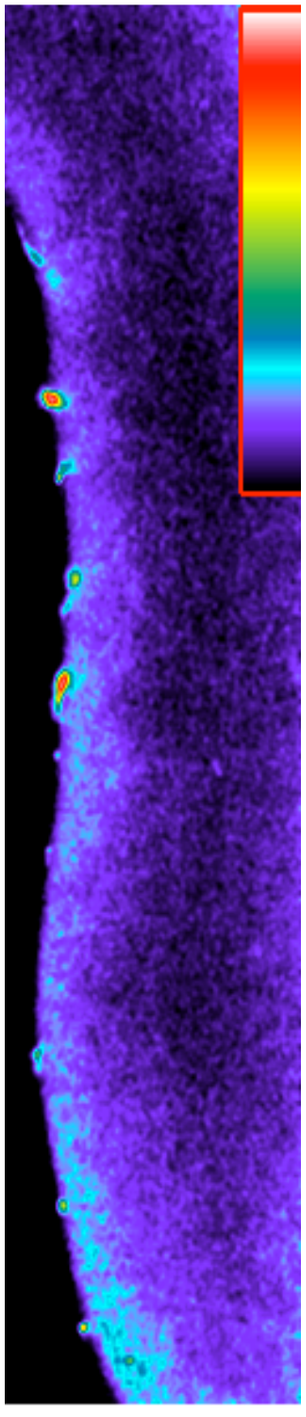
TWO-PHOTON EXCITATION MICROSCOPY



3D sequence collected at LAMBS, sample courtesy of Paola Ramoino, DIPTERIS

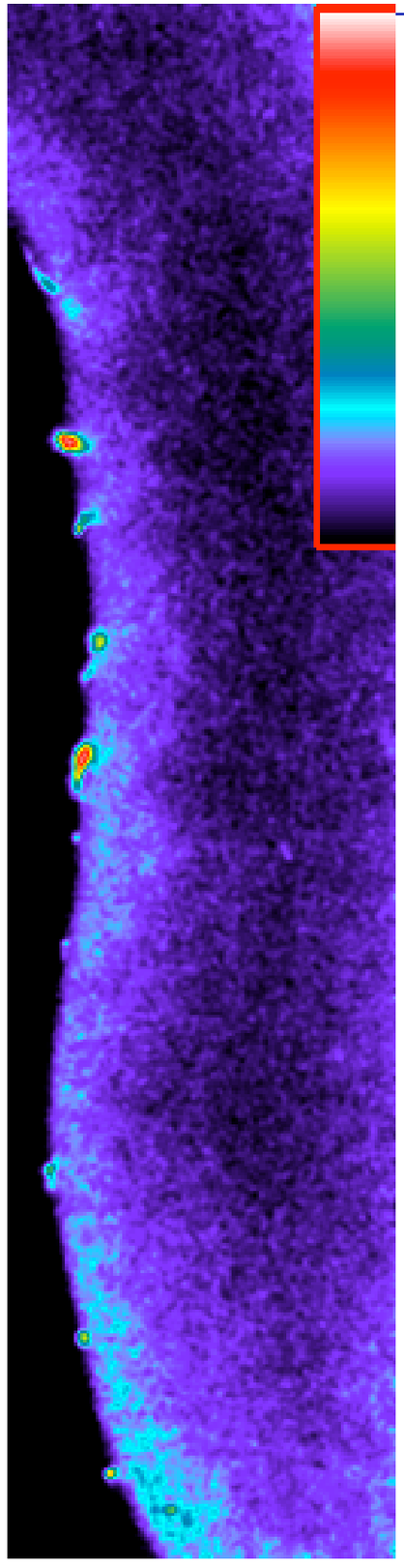
A.DIASPRO (2005) TRENDS IN BIOTECHNOLOGY, IN PRESS

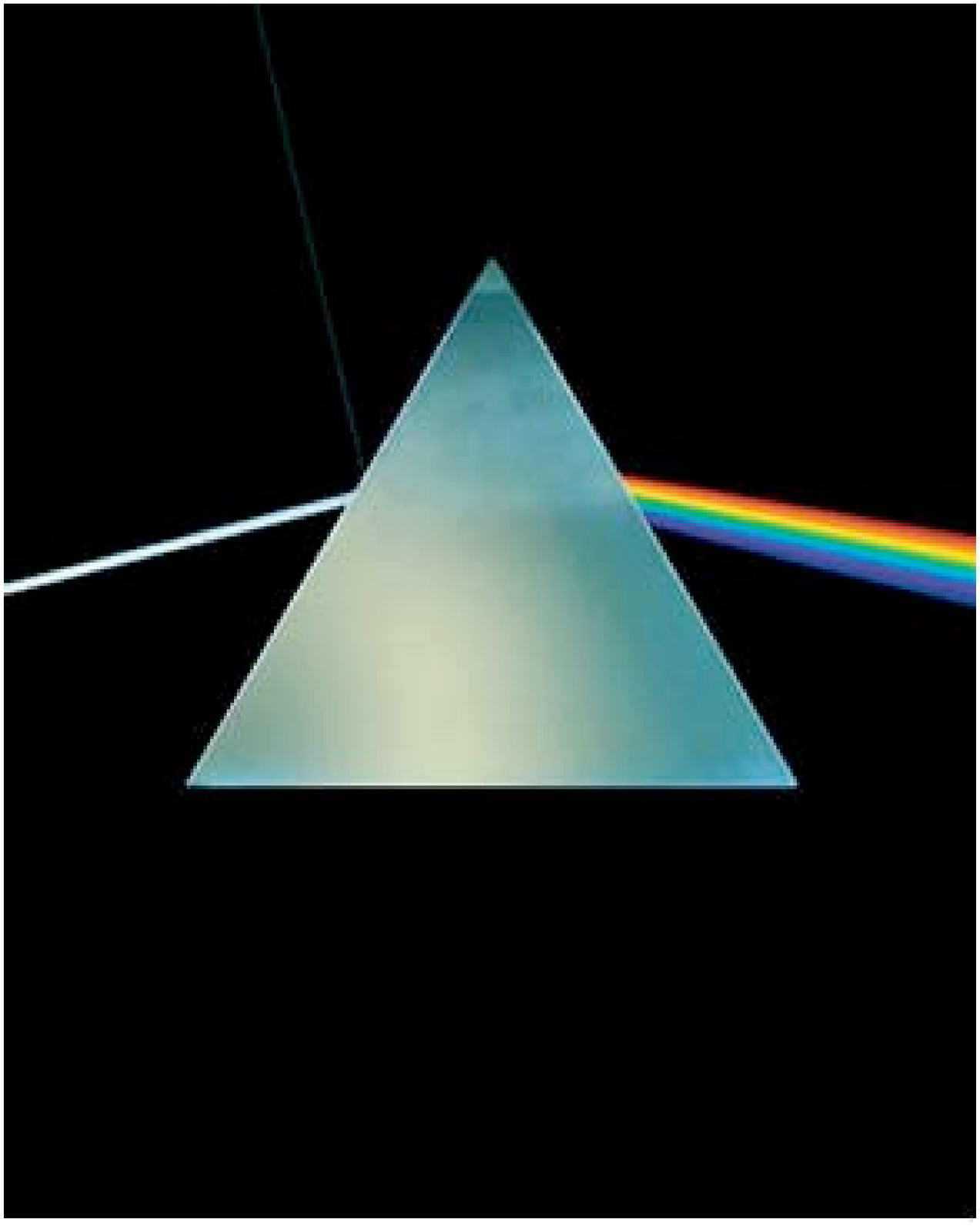
Spectral INFORMATION



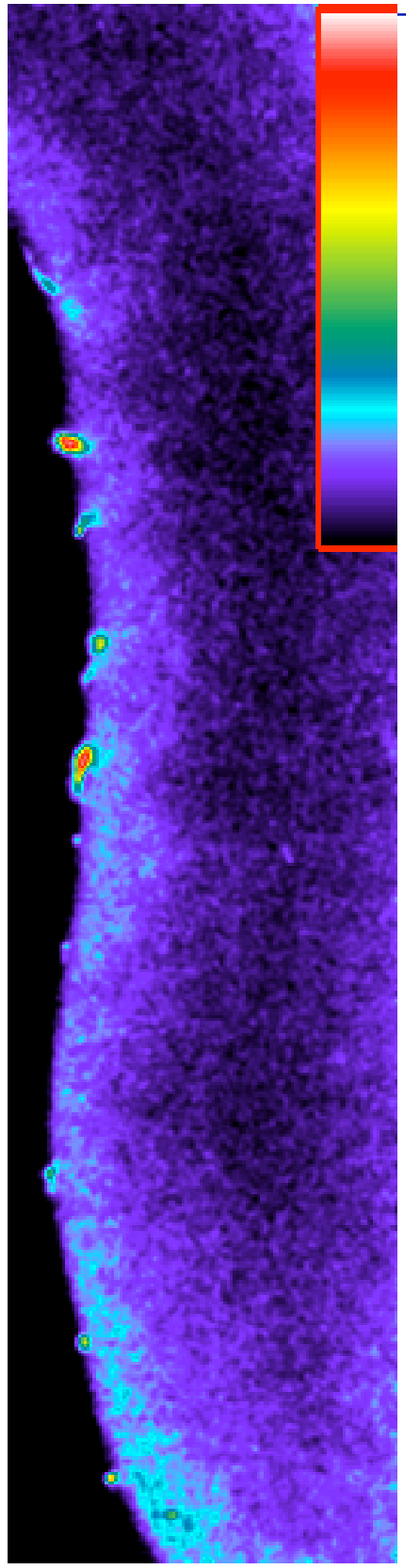
TCS SP5 resonant scanner 50 Hz framerate. Simulataneous record of CFP, GFP, YFP, DsRed, DiD and transmitted light.
Credits: LEICA microsystems and Dr. Harald Hutter, Max Planck Institute (MPI) for Medical Research, Heidelberg, Germany

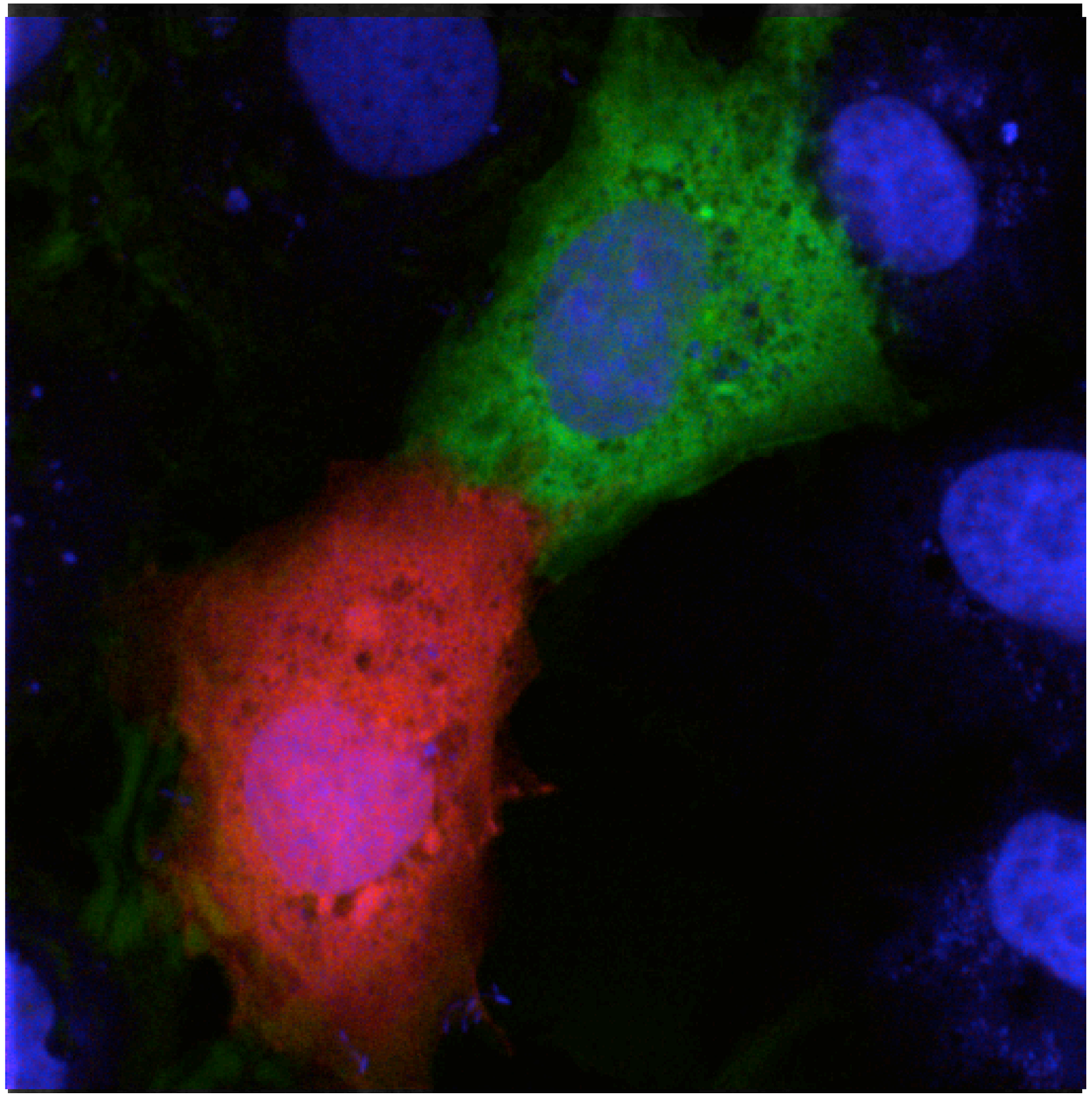
Spectral separation problem



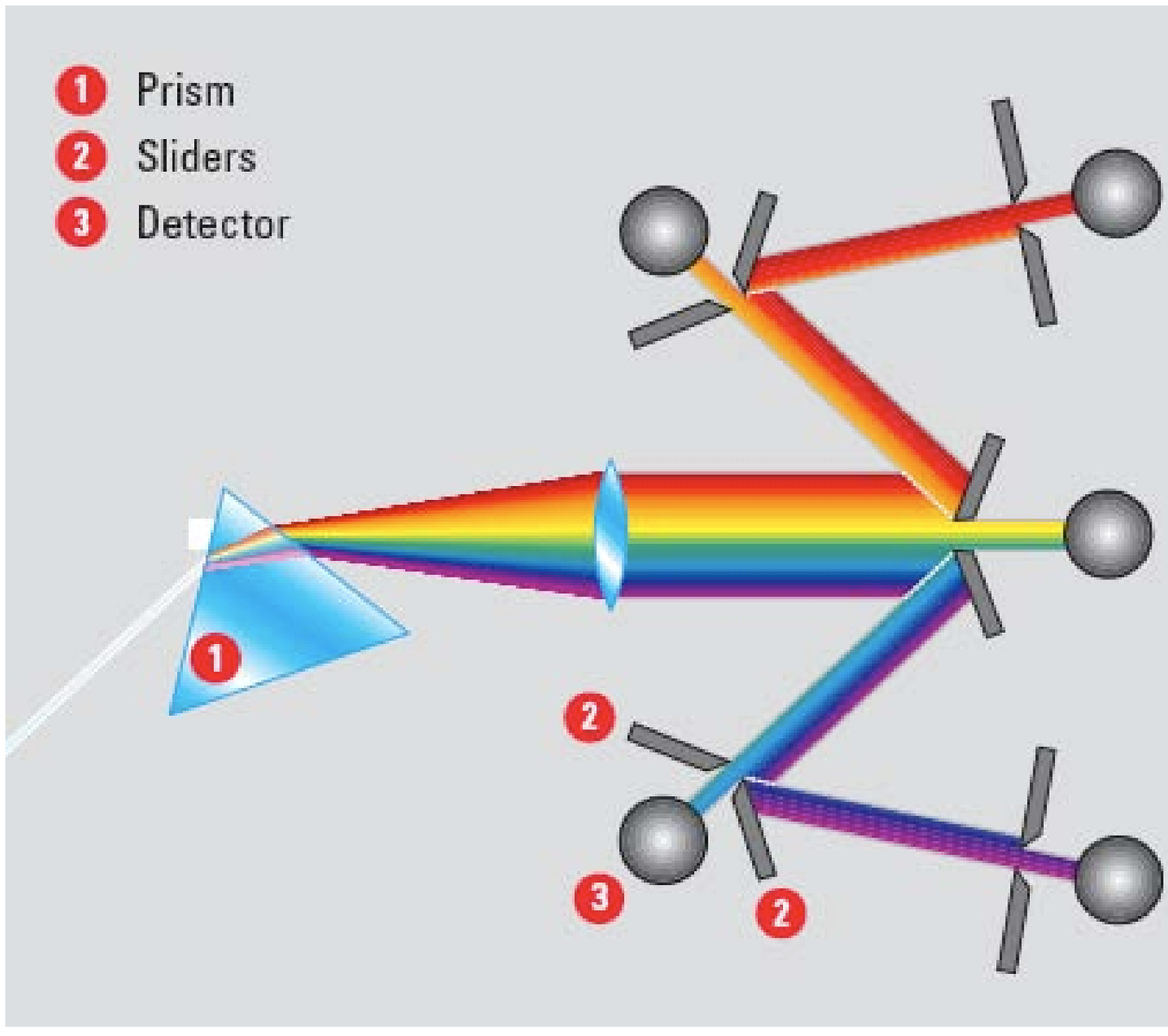


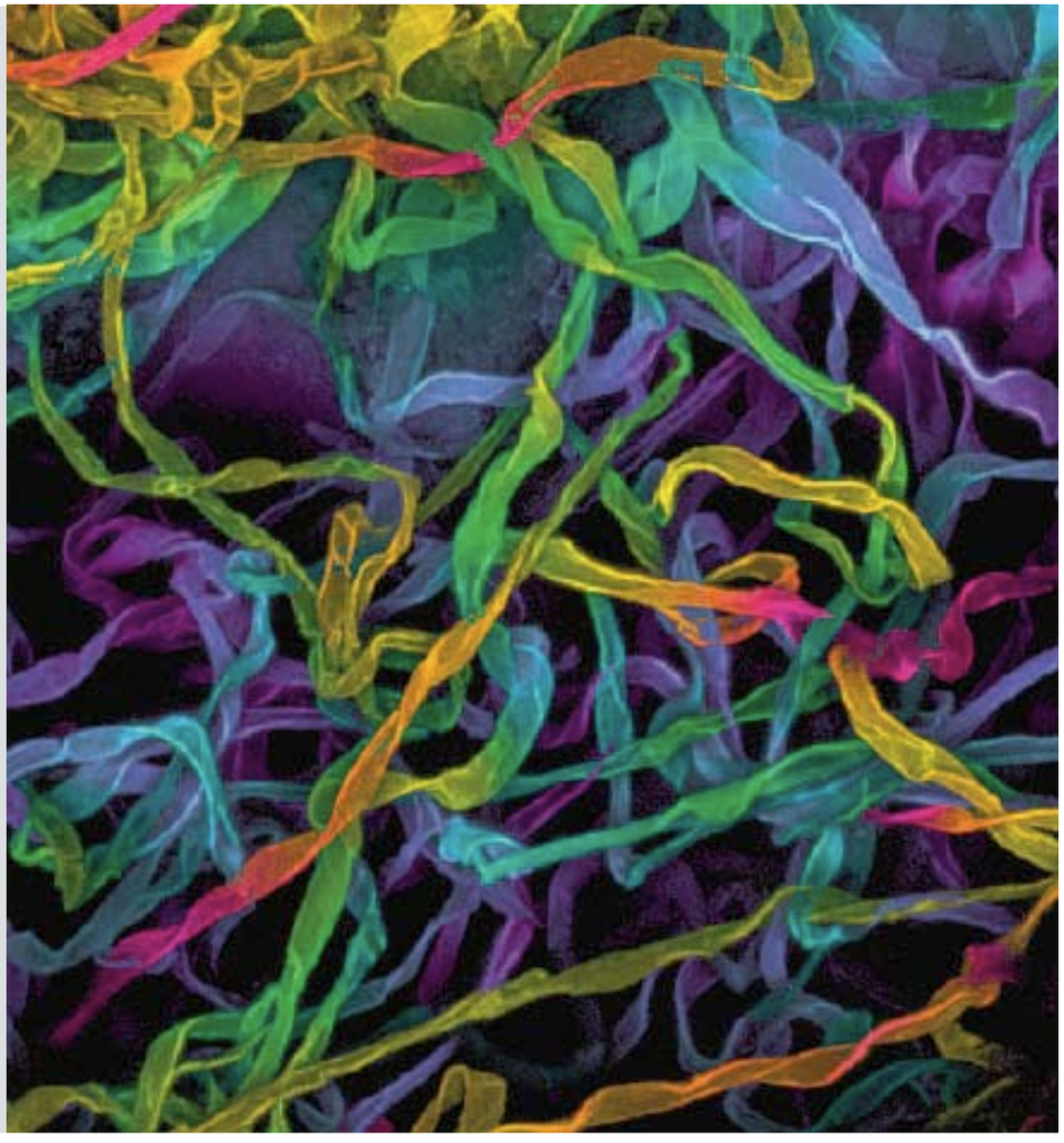
Spectral separation problem





Spectral separation problem

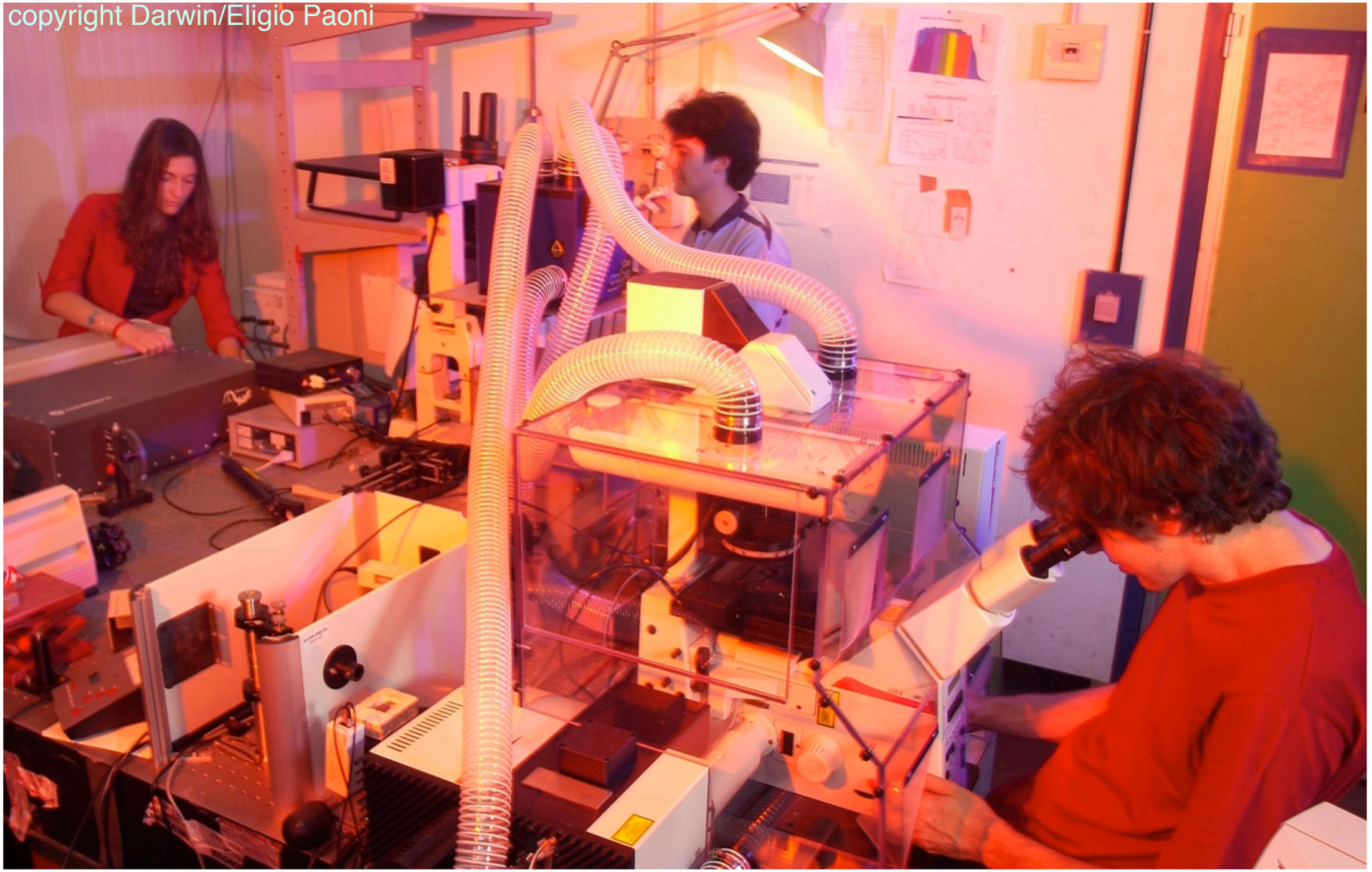




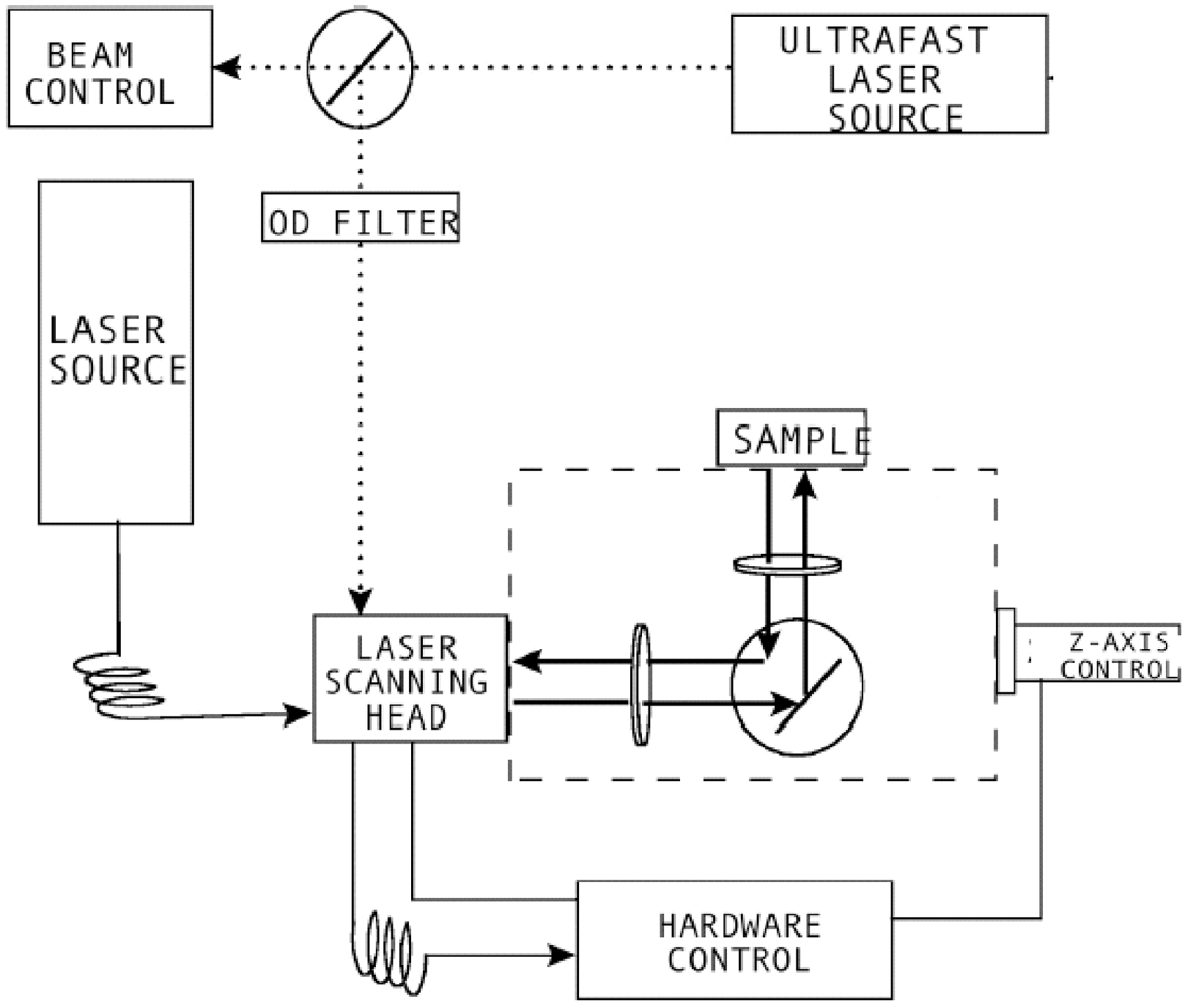
TCS SP5 resonant scanner 71 Hz framerate.
Beating cilia in reflected light and DiI-Fluorescence together with transmitted light.

Courtesy of Kristin Tessmar und Detlev Arendt
European Molecular Biology Laboratory (EMBL)
Heidelberg, Germany

TWO-PHOTON EXCITATION MICROSCOPY

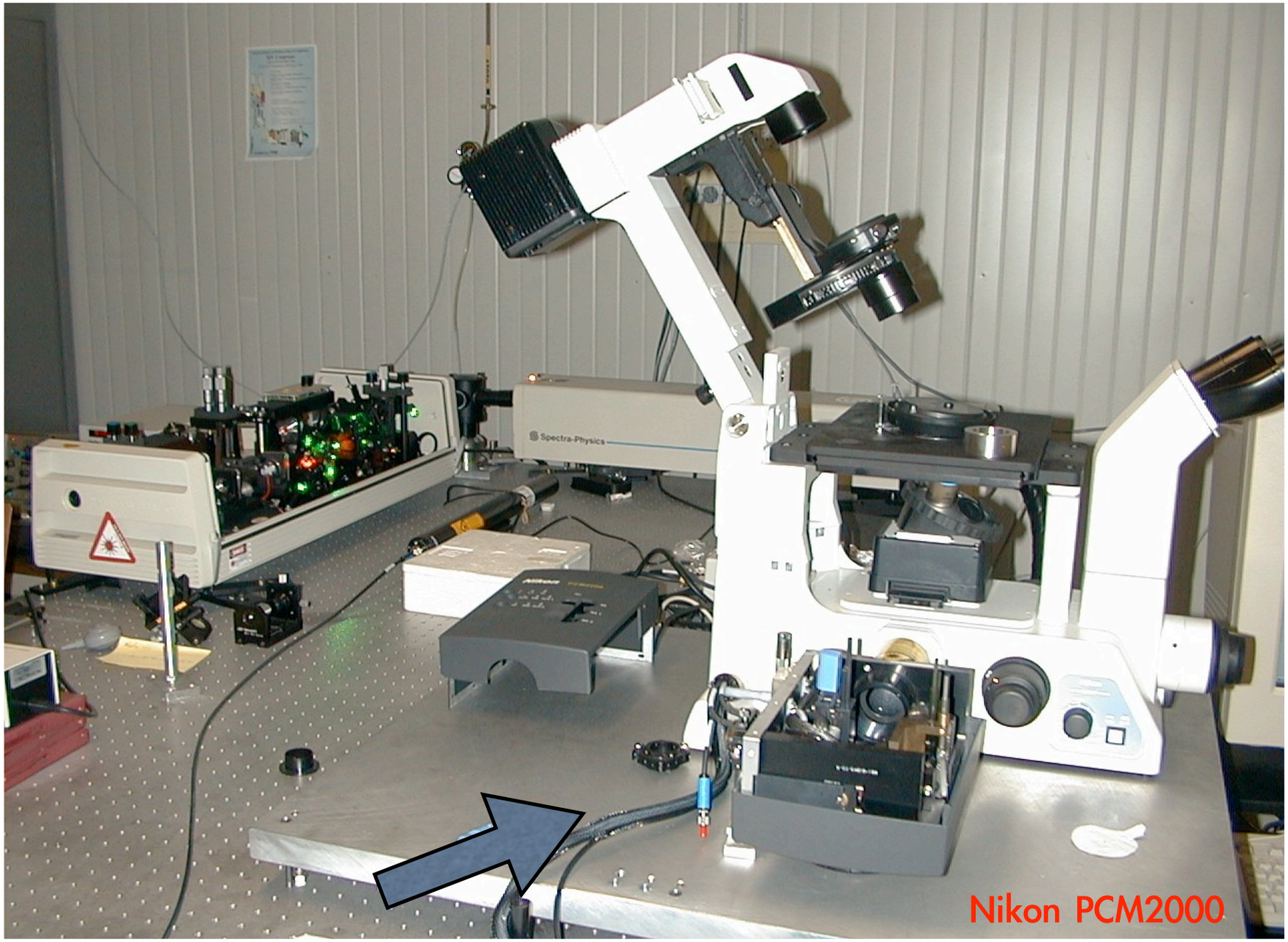

copyright Darwin/Eligio Paoni

TWO-PHOTON EXCITATION MICROSCOPY



DIASPRO ET AL., MICROSC.RES.TECH., 47(3), 196-205, 1999

TWO-PHOTON EXCITATION MICROSCOPY



DIASPRO ET AL., MICROSC.RES.TECH., 47(3), 196-205, 1999

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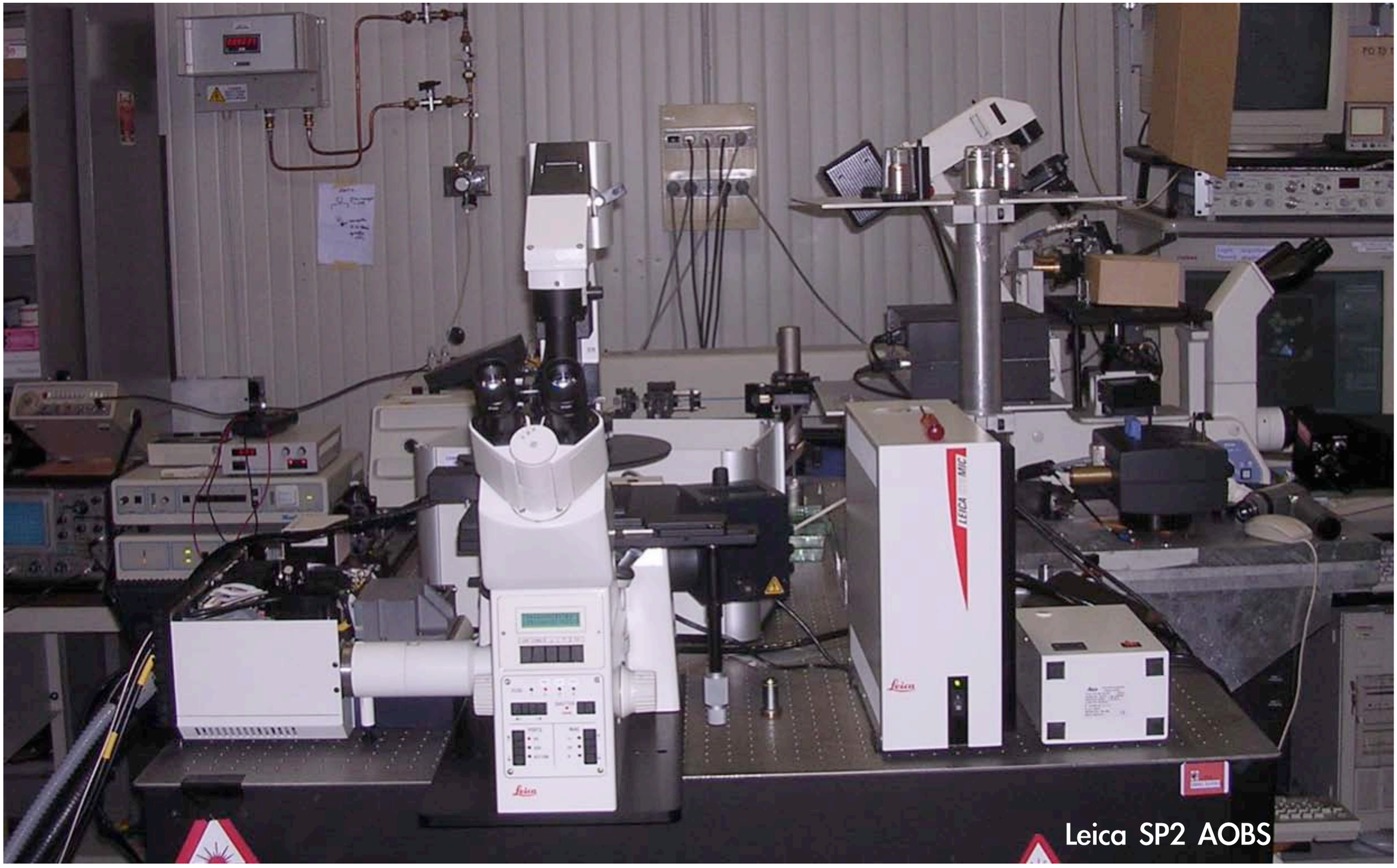
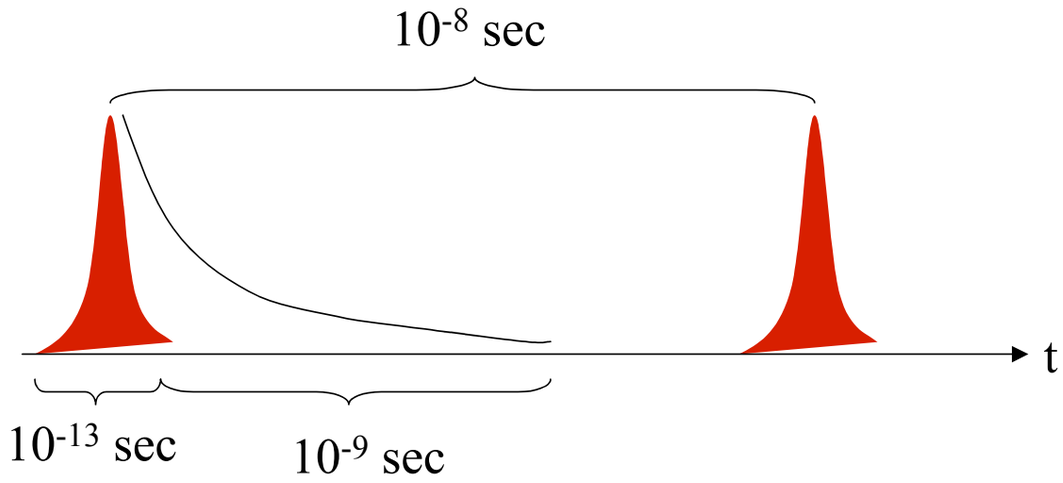
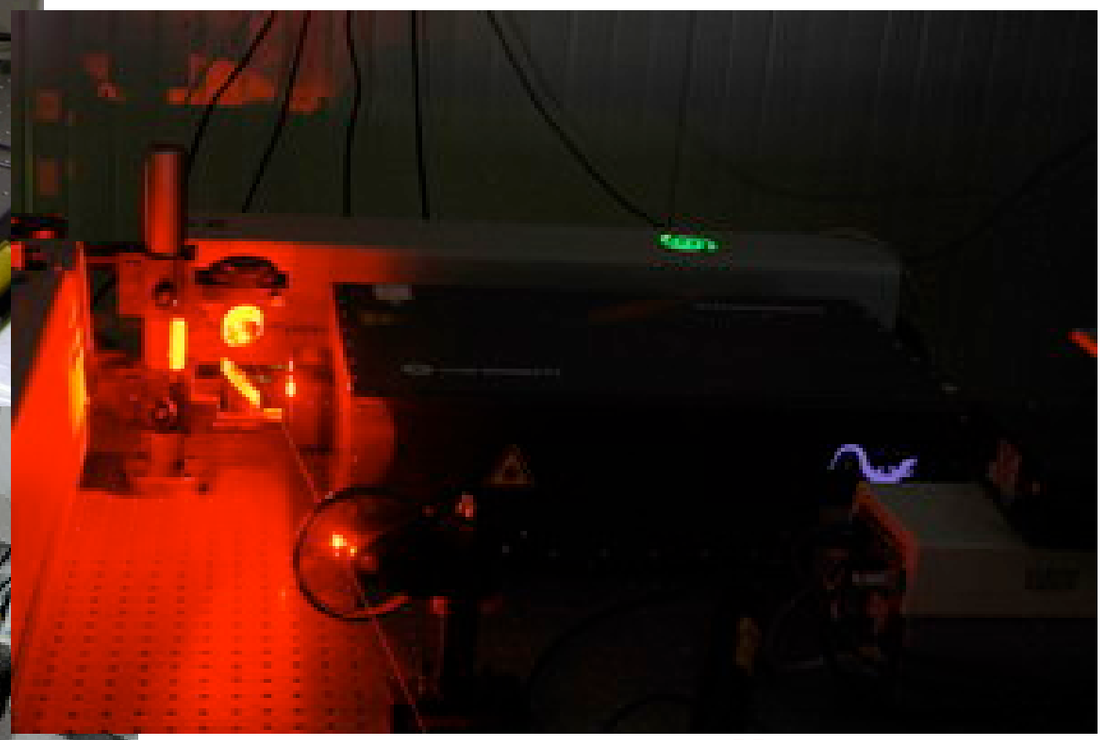


A.DIASPRO, G.CHIRICO, M.COLLINI (2004) QUART. REV. .BIOPHYS.,VOL.38, NR.2, PP.1-72 (2006).

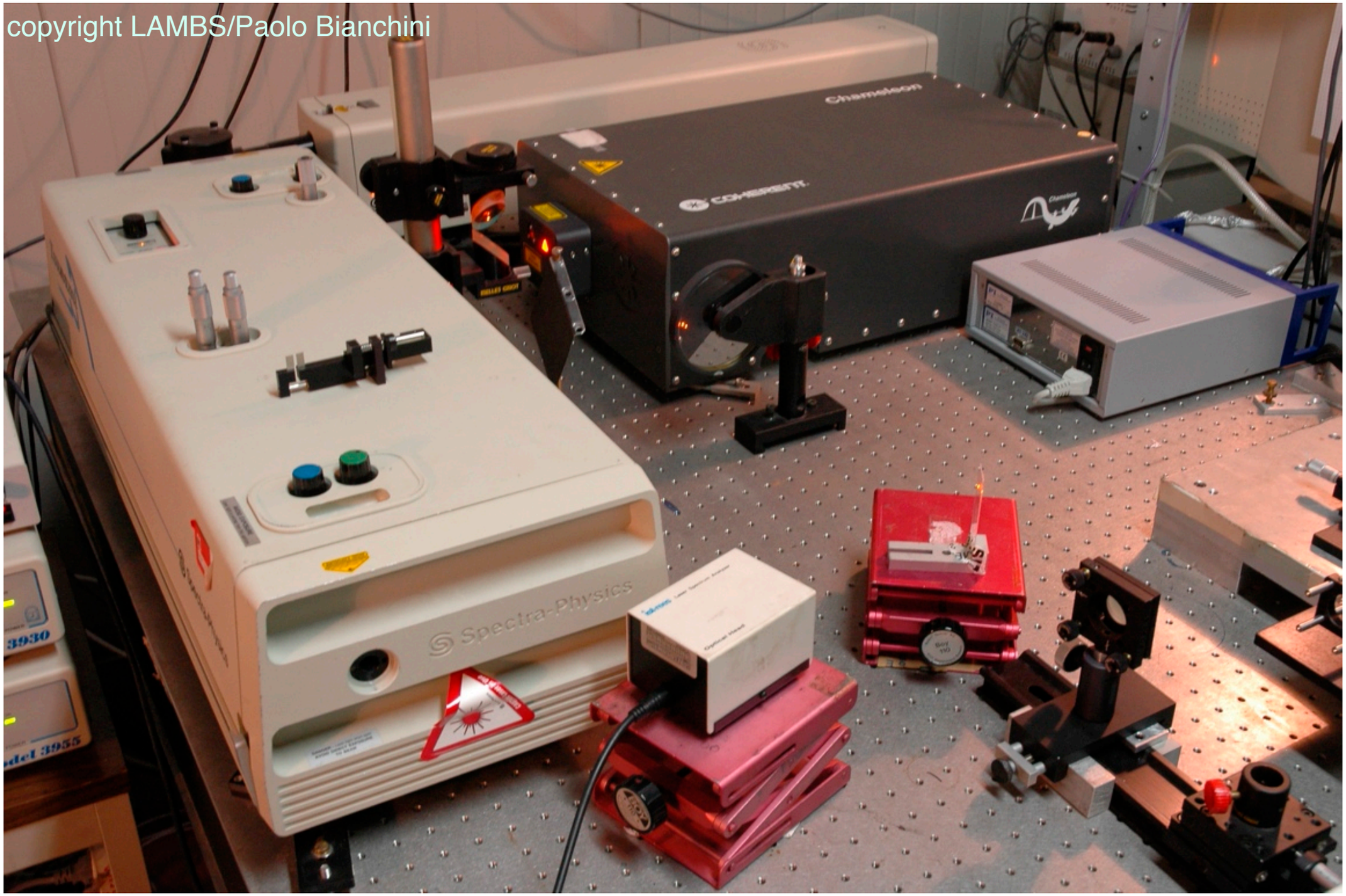
TWO-PHOTON EXCITATION MICROSCOPY





Tsunami-Millennia (680-830/780-930), Chameleon XR (720-980)

TWO-PHOTON EXCITATION MICROSCOPY



A.DIASPRO, G.CHIRICO, M.COLLINI (2004) QUART. REV. .BIOPHYS.,VOL.38, NR.2, PP.1-72 (2006).

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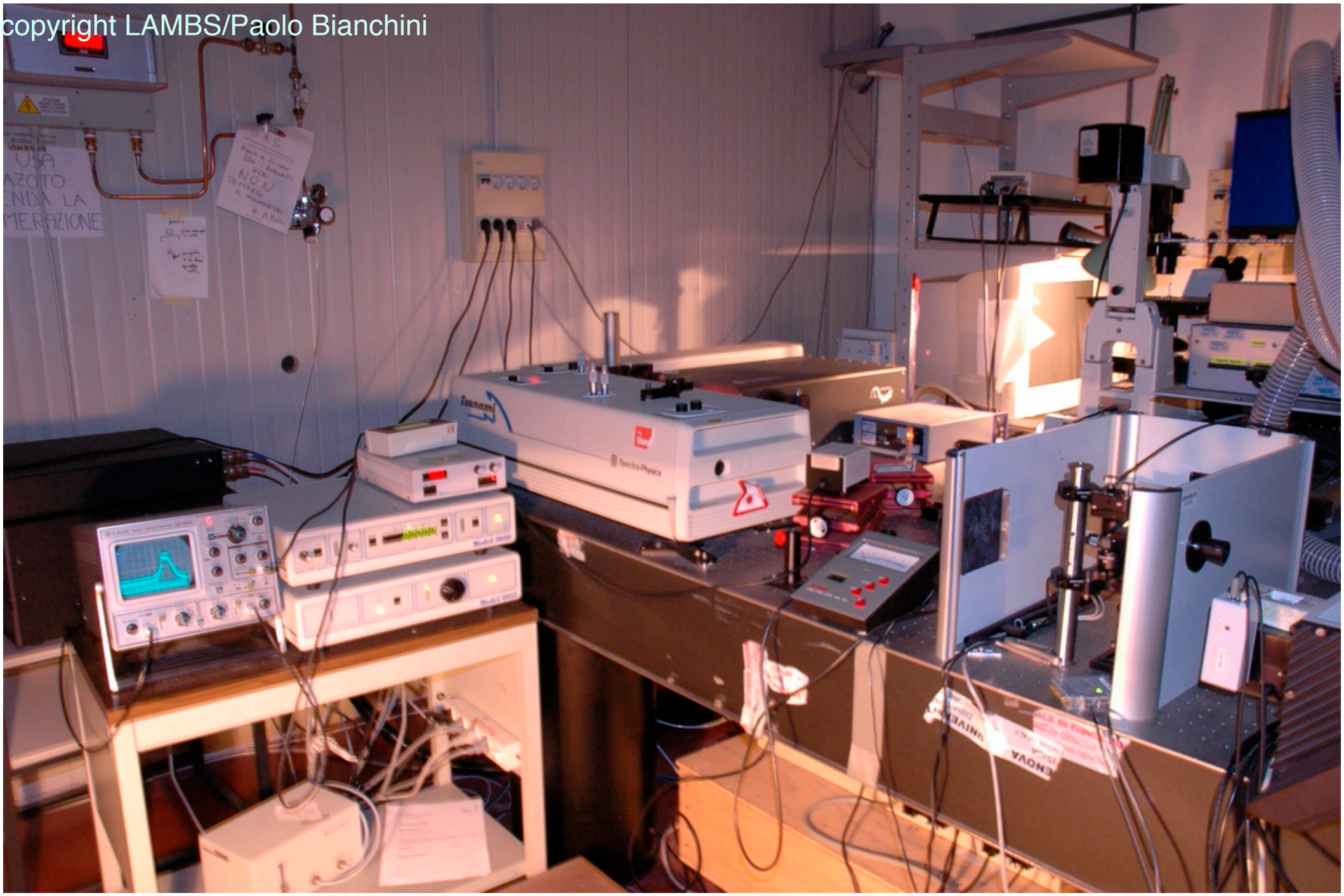
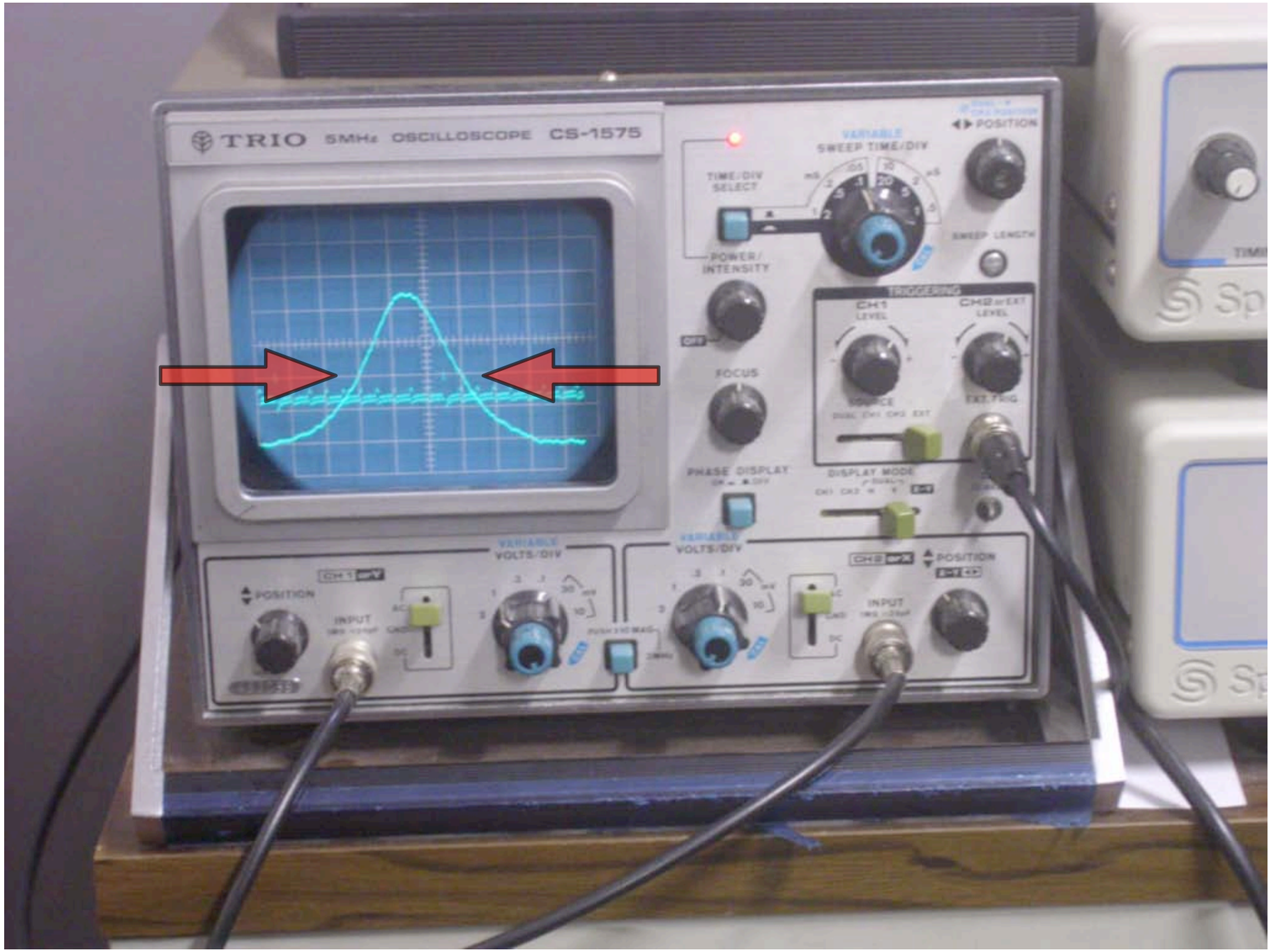
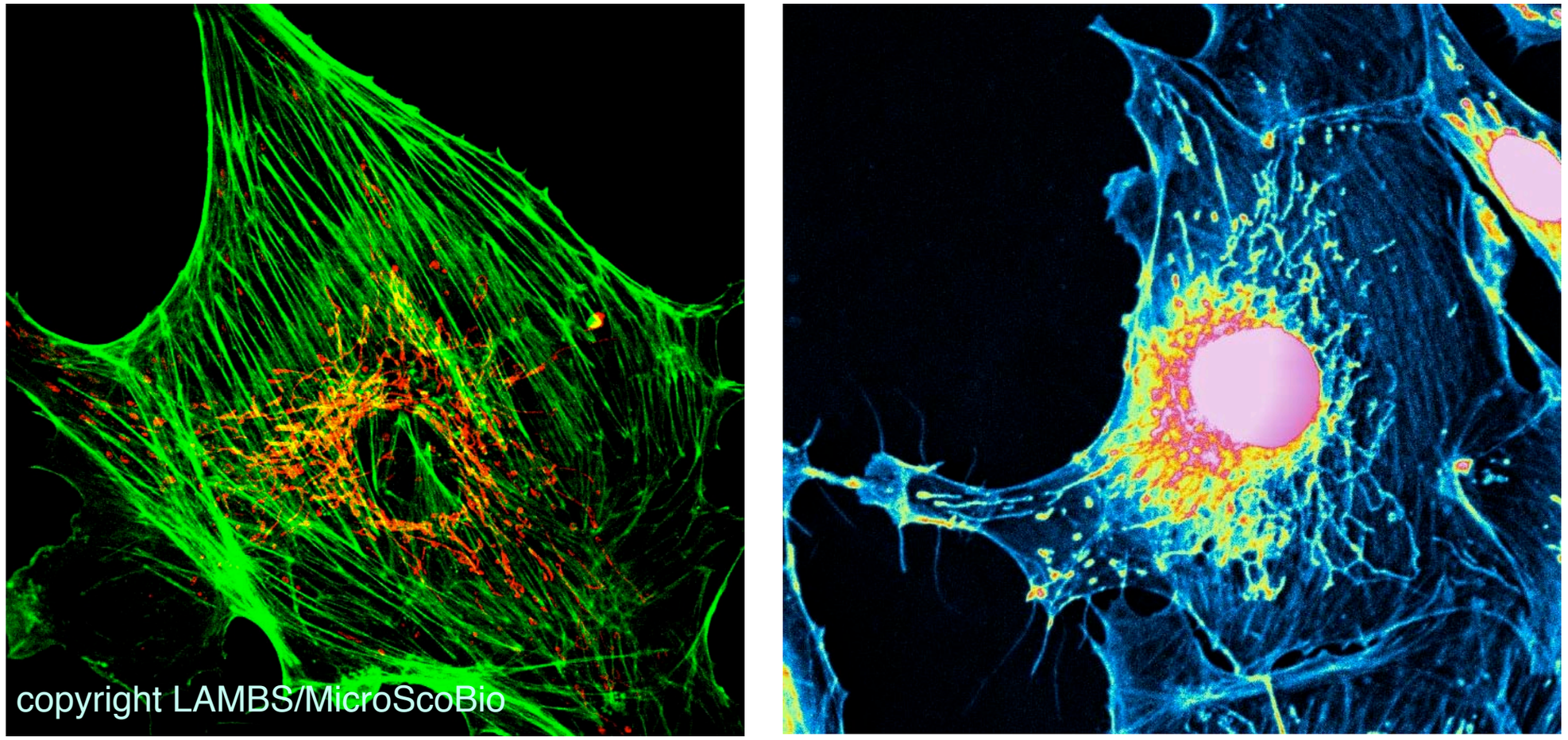


TWO-PHOTON EXCITATION MICROSCOPY



Slide credit:Mario Arace, 1978

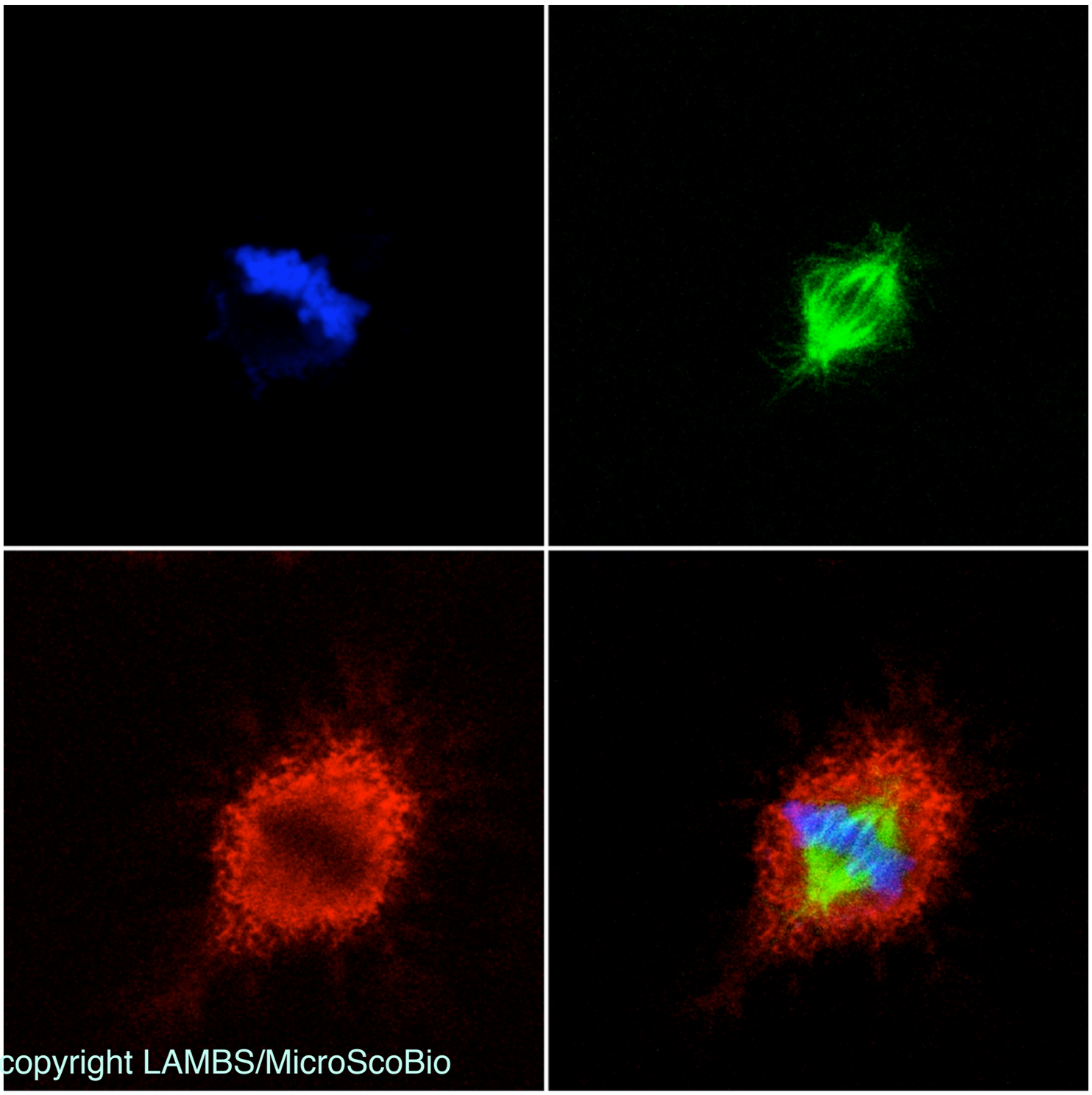
Confocal (left) and TPE (right) multiple fluorescence.



Cells are labelled with DAPI (DNA), mitotracker red (mithocondria) and bodipy (actin); F-14780 Molecular Probes slide.

Voce "Cellula", Supplemento Piccola Treccani

TWO-PHOTON EXCITATION MICROSCOPY



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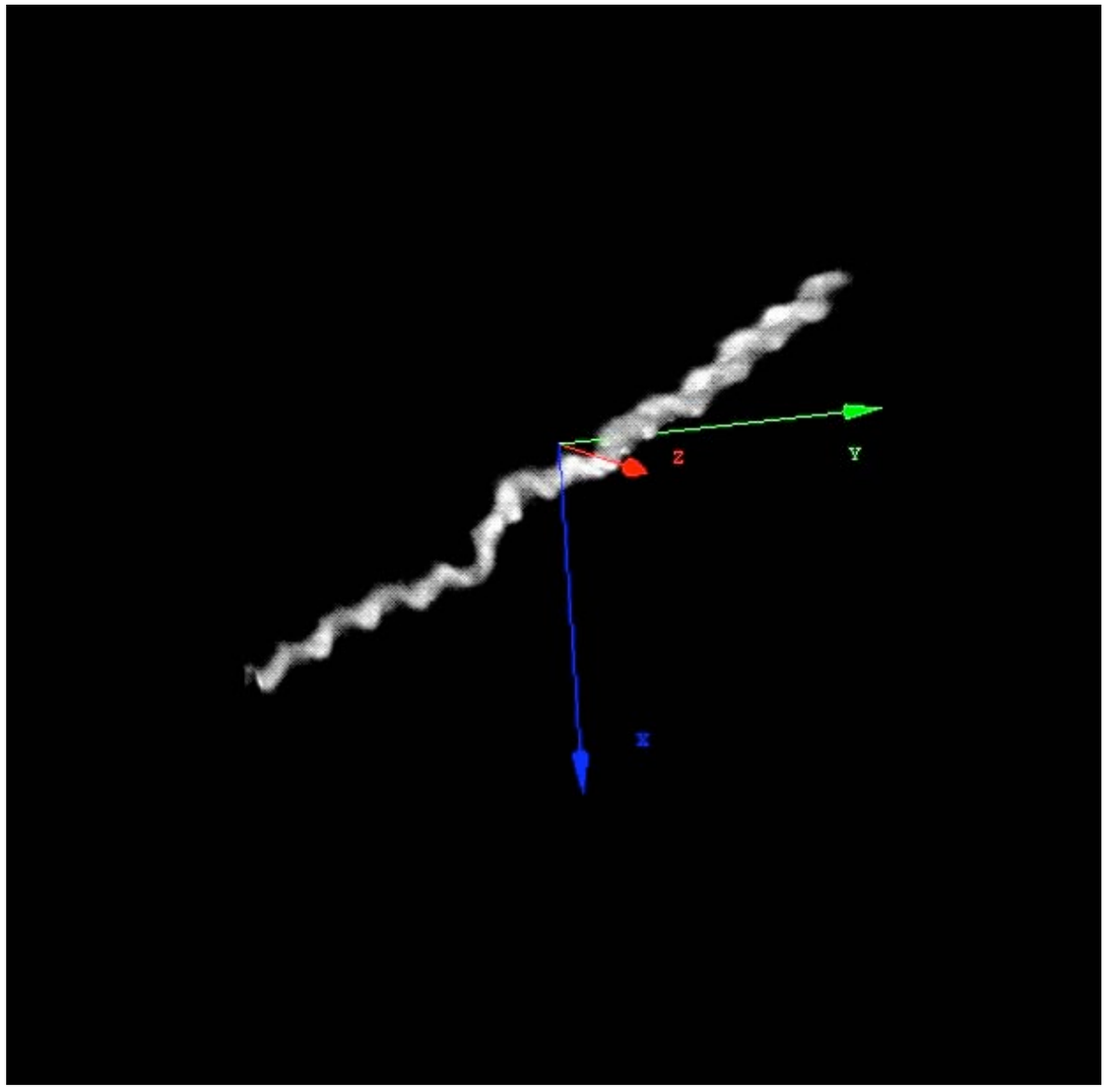
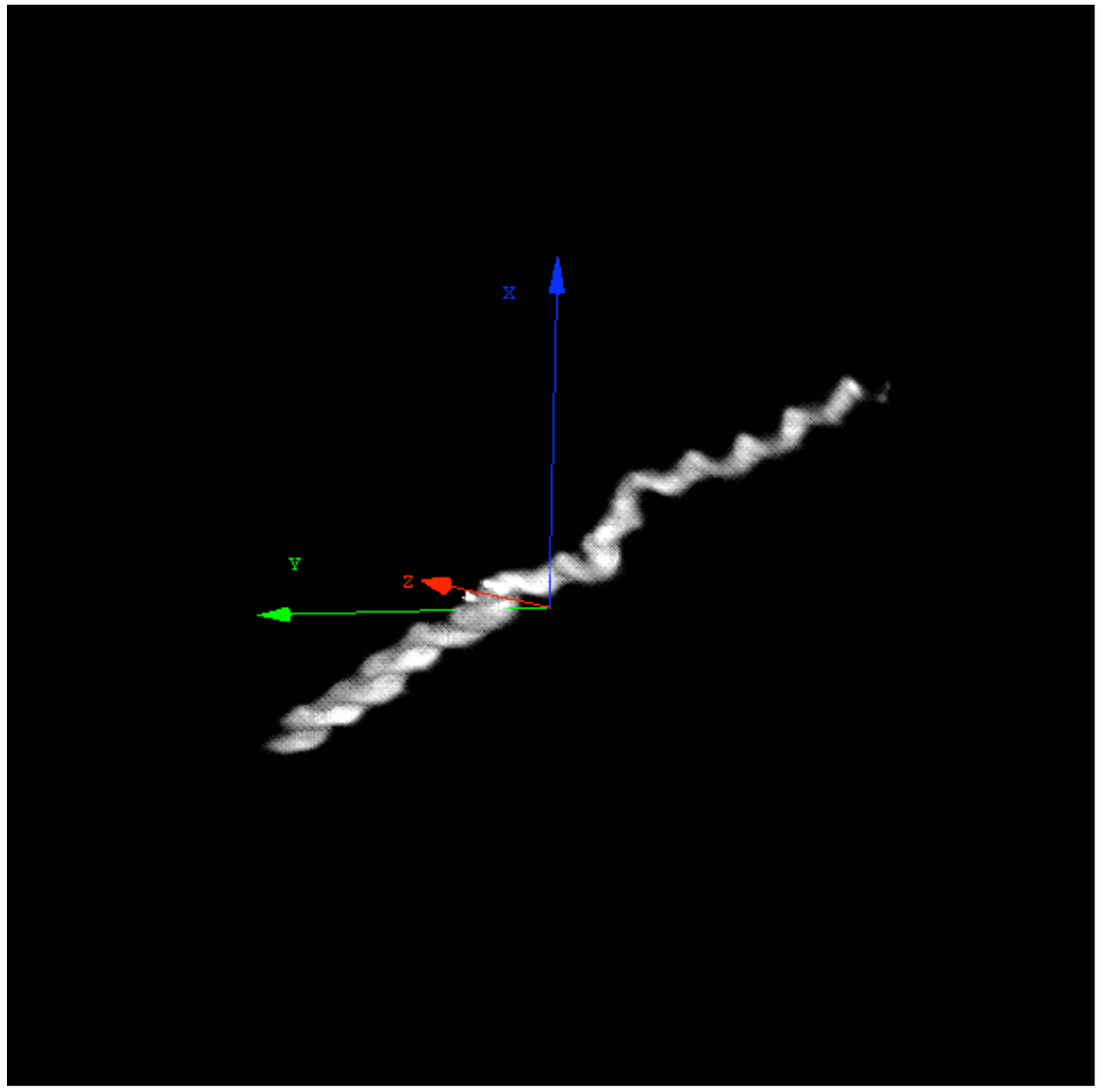
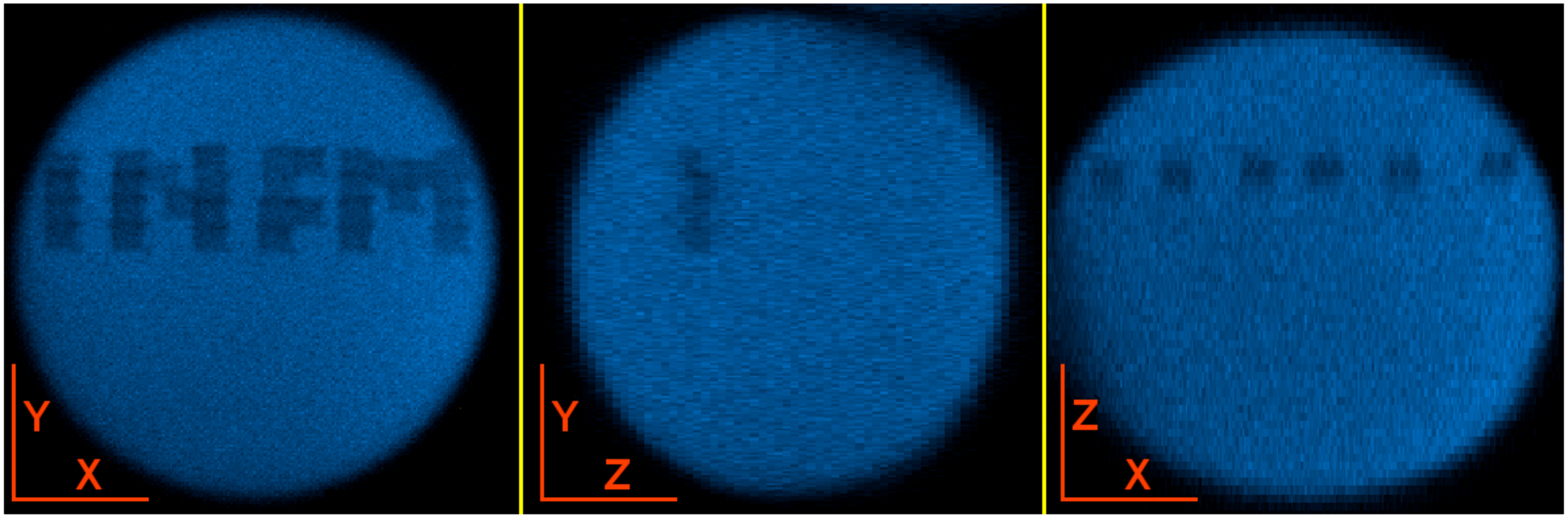




Eledone cirrhosa (octopus) sperm heads

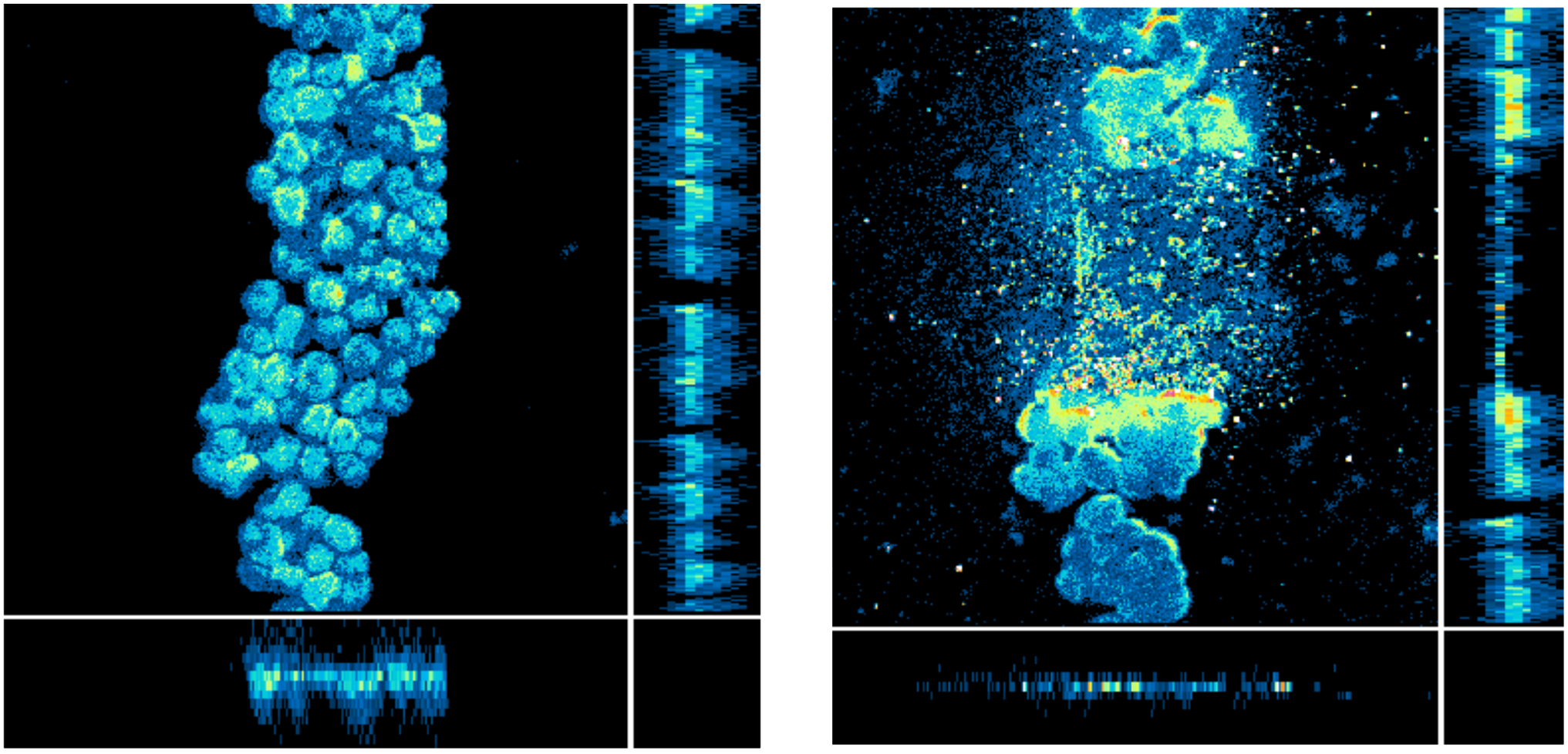
DIASPRO ET AL., MICROSC.RES.TECH., 47(3), 196-205, 1999

Two-photon micropatterning on fluorescent microspheres (22 μm) using photobleaching



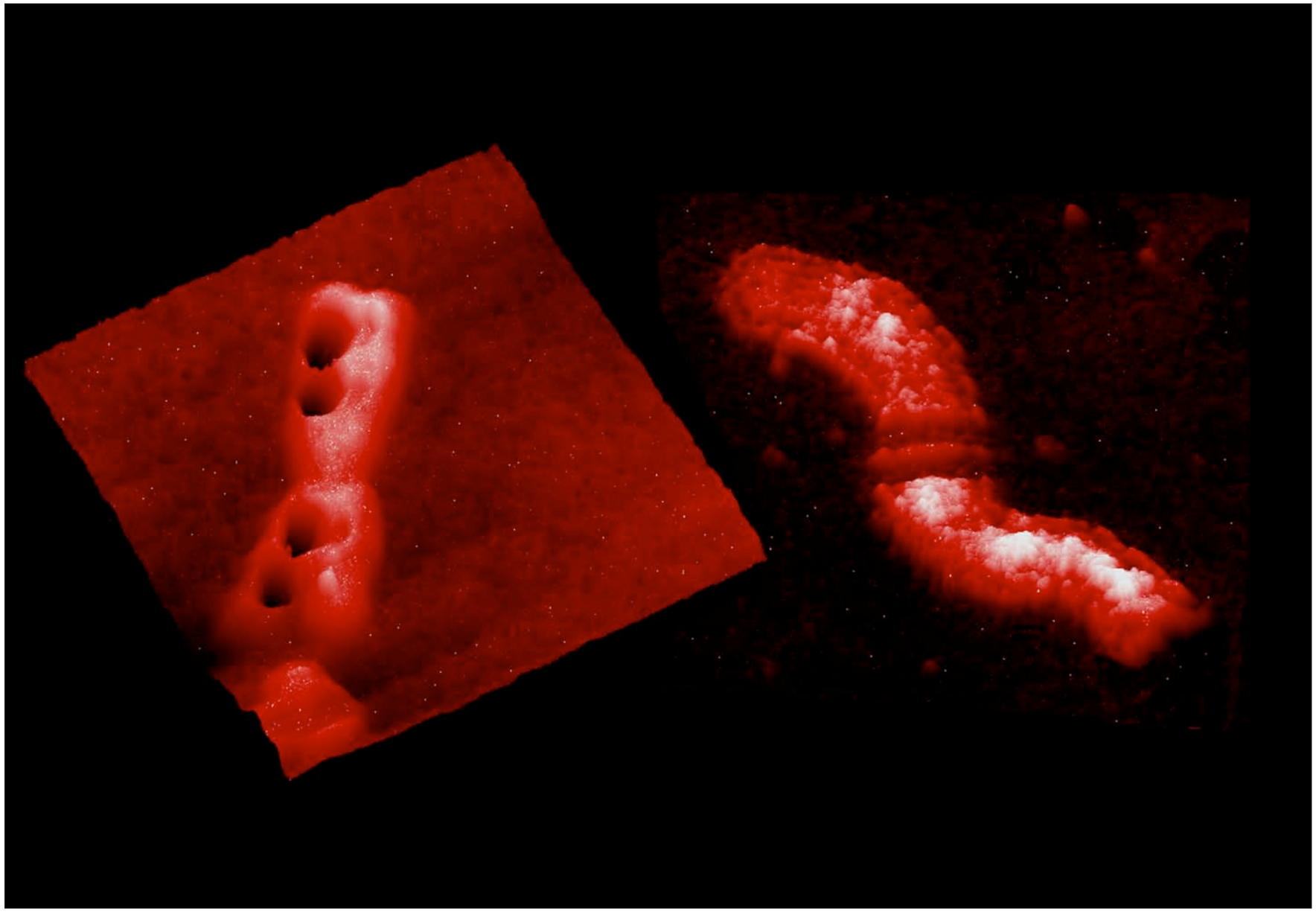
DIASPRO ET AL., MICROSC.RES.TECH., 47(3), 196-205, 1999

Selective 3D damage on *Saccharomyces cerevisiae* cell layers



DIASPRO A., EJH, 1999

From Karsten Konig Lab



Journal of Microscopy, Vol. 200, Pt 2, November 2000, pp. 83–104.
Received 20 December 1999; accepted 16 June 2000

From Karsten Konig Lab

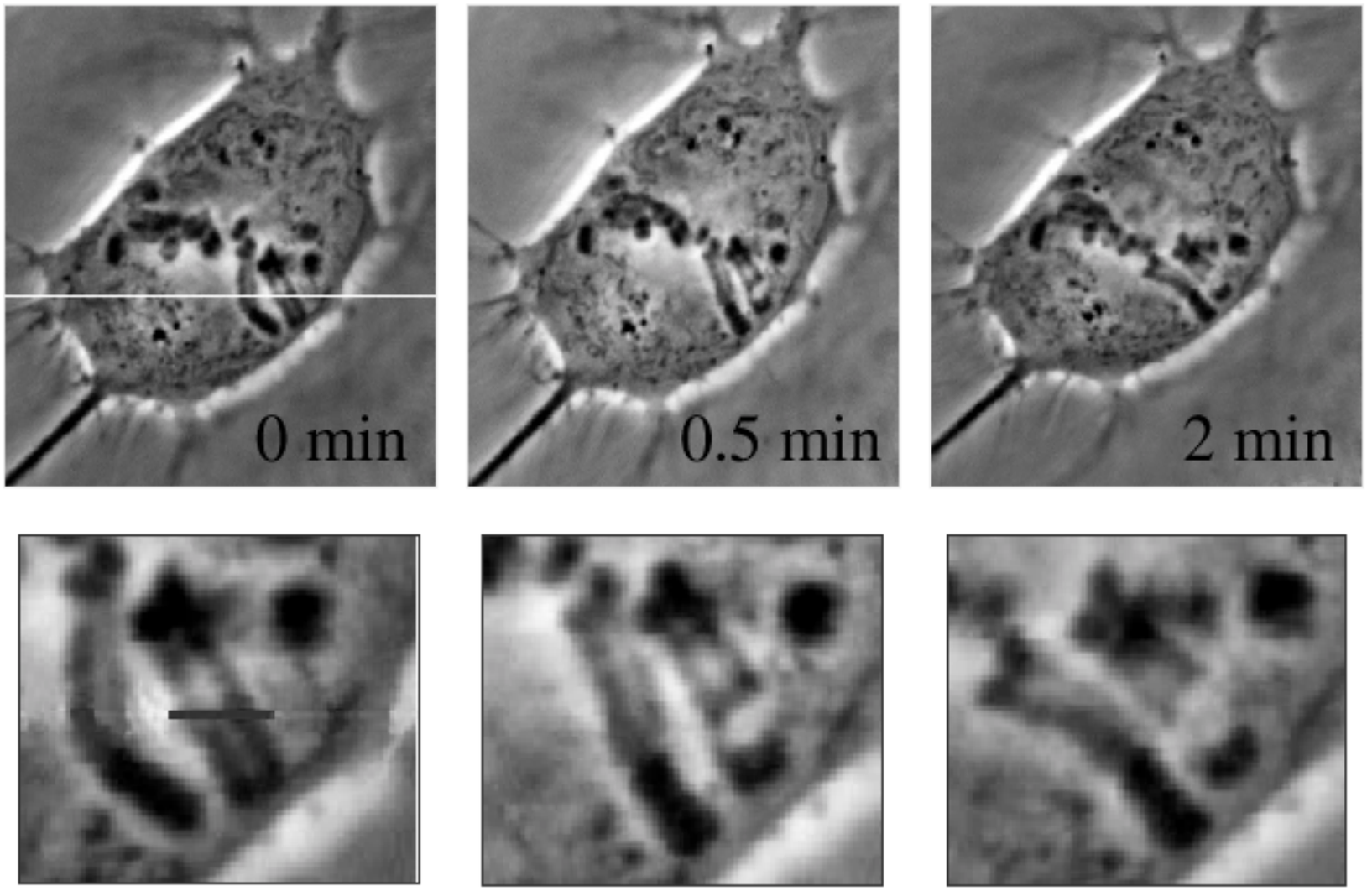


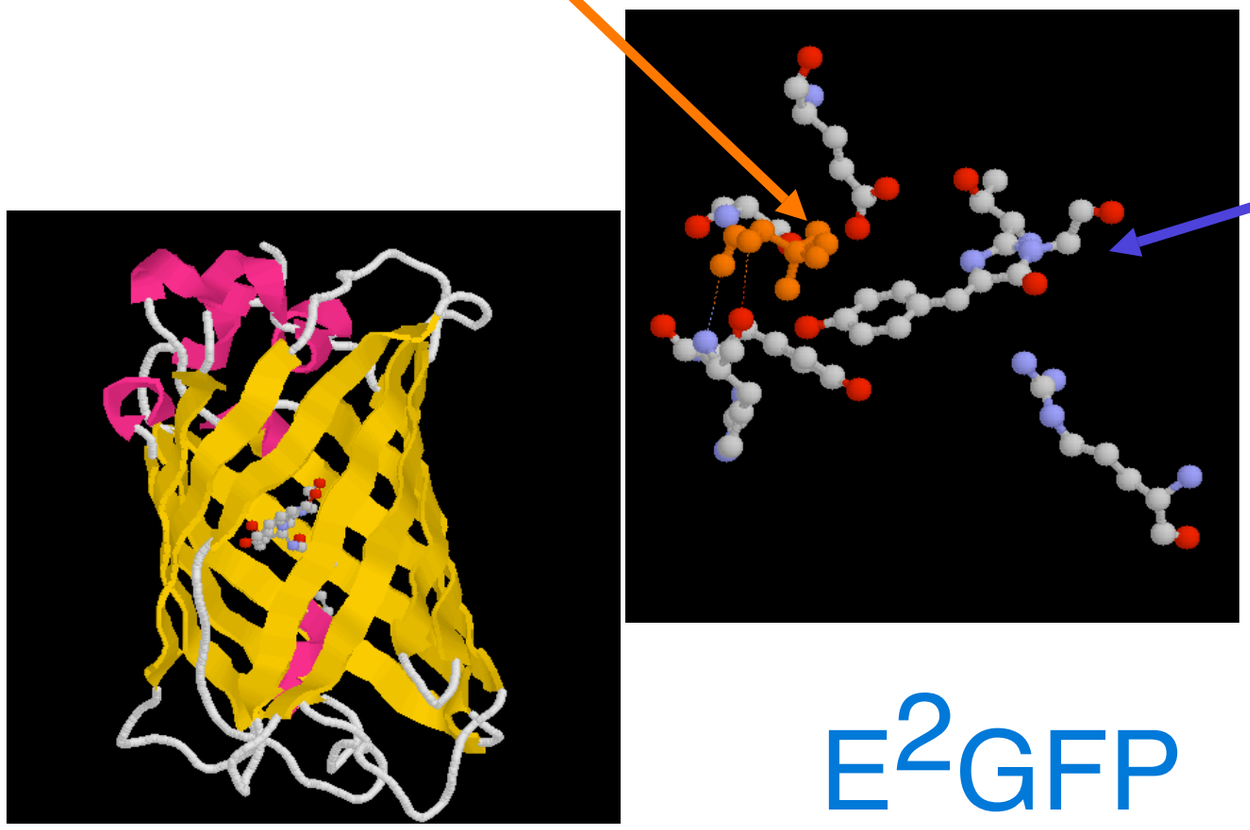
Fig. 18. Chromosome dissection within living cells using the femtosecond NIR laser microscope. The cell remained alive.

Journal of Microscopy, Vol. 200, Pt 2, November 2000, pp. 83–104.
Received 20 December 1999; accepted 16 June 2000

DIASPRO ET AL., MICROSC.RES.TECH., 47(3), 196-205, 1999

SINGEL FLUORESCENT PROTEINS

Threonine$_{203}$ → Tyrosine$_{203}$



Chromophore (65)

Fusion of
Ser 65, Tyr 66, Gly 67

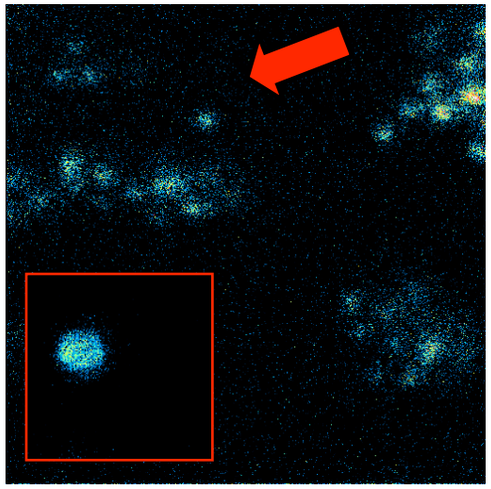
E^{2}GFP

Cinelli RA, Ferrari A, Pellegrini V, Tyagi M, Giacca M, Beltram F.,
Photochem Photobiol. 2000 Jun;71(6):771-6.

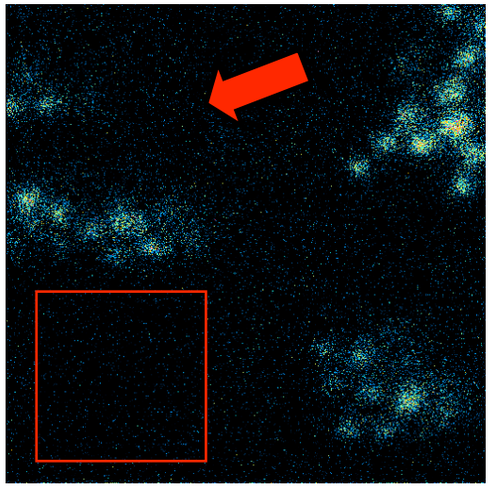
Cinelli RA, Tozzini V, Pellegrini V, Beltram F, Cerullo G, Zavelani-Rossi M, De Silvestri S, Tyagi M, Giacca M.,
Phys Rev Lett. 2001 Apr 9;86(15):3439-42.

Phys Rev E Stat Nonlin Soft Matter Phys. 2004 Sep;70(3 Pt 1):030901

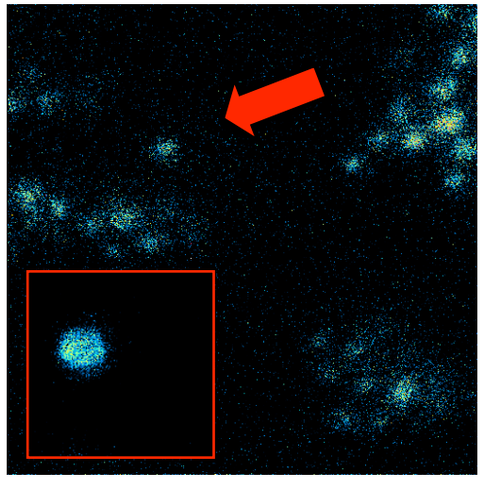
SINGEL FLUORESCENT PROTEINS



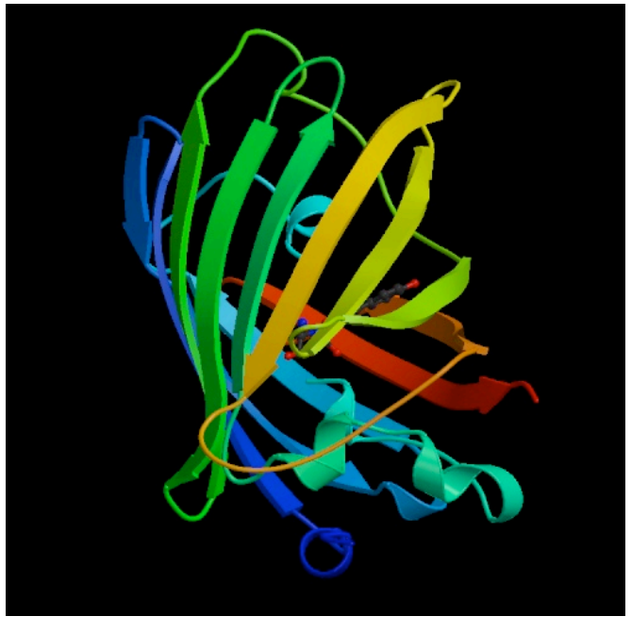
TPE @ 785 nm



TPE @ 720 nm

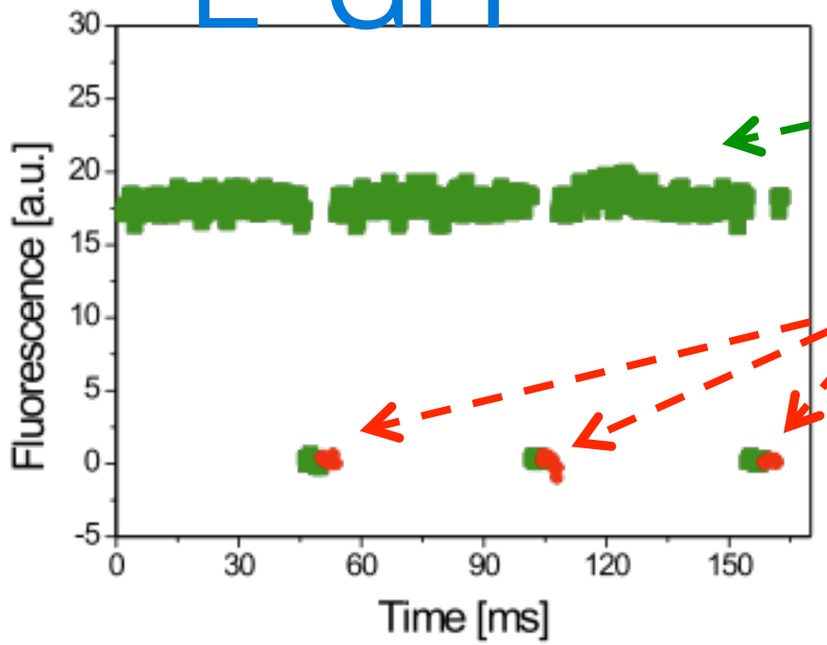


TPE @ 785 nm



E^2GFP in silica GEL, maximum excitation within 700-800 nm range @ 785; after imaging photobleaching @ 785; photocycling @ 720 nm - no photorecovery @ 710/730 -; final imaging @ 785.

E^2GFP



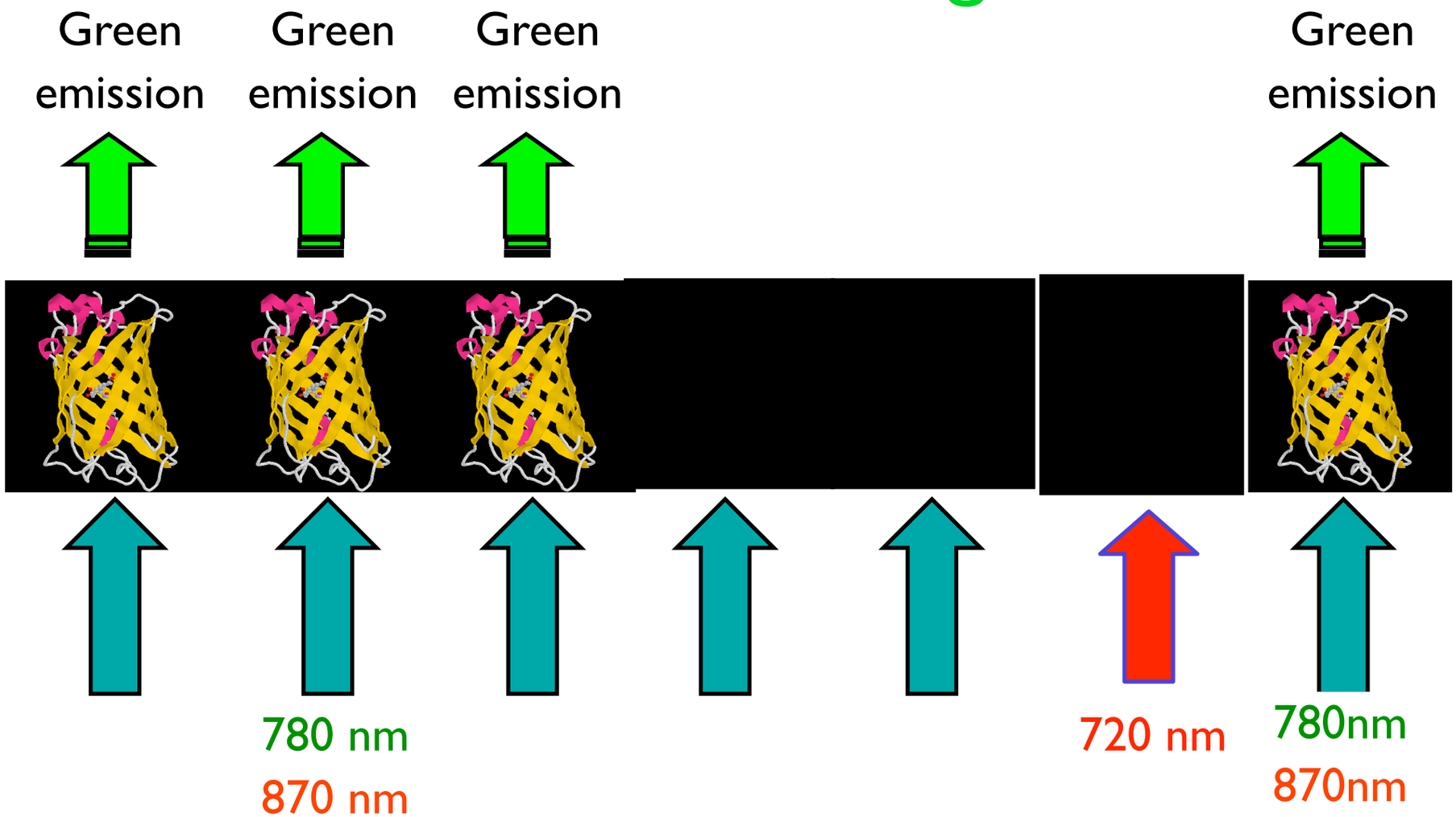
Illumination @ 780nm

Illumination @ 720nm

Phys Rev E Stat Nonlin Soft Matter Phys. 2004 Sep;70(3 Pt 1):030901

E^2GFP

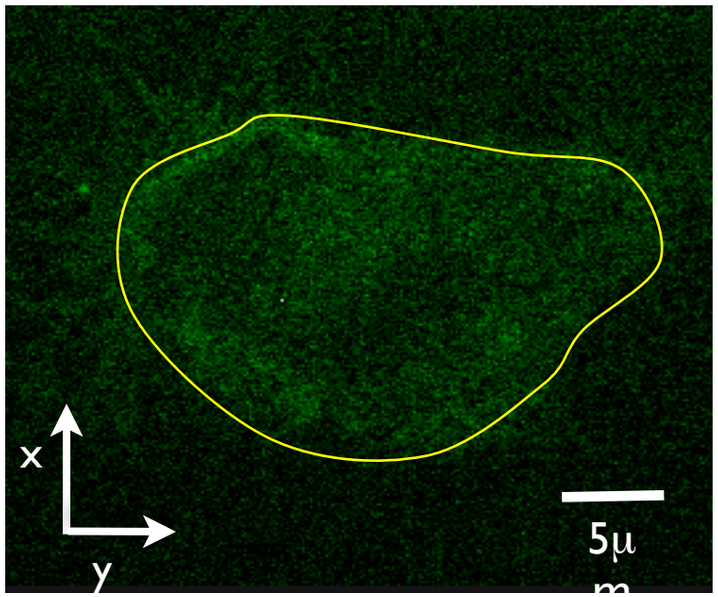
Two Photon Switching Protein



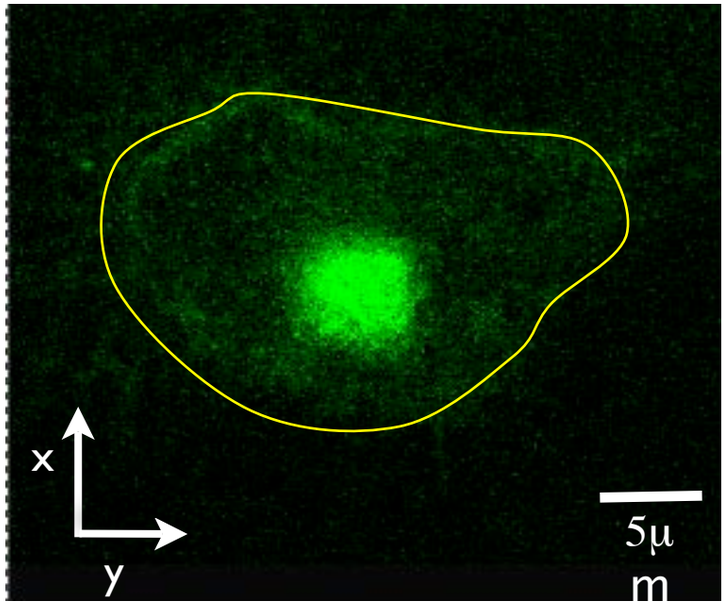
Phys Rev E Stat Nonlin Soft Matter Phys. 2004 Sep;70(3 Pt 1):030901

GFP-PHOTOACTIVATION

Switching on a subset of the expressed protein



PHOTOACTIVATION



PRE ACTIVATION

$\langle P_{EXCITATION} \rangle$= 0,04 mW, $\lambda_{EXCITATION}$= 488nm
Pixel time= 4,9 μs, 512*512 pixels

POST ACTIVATION

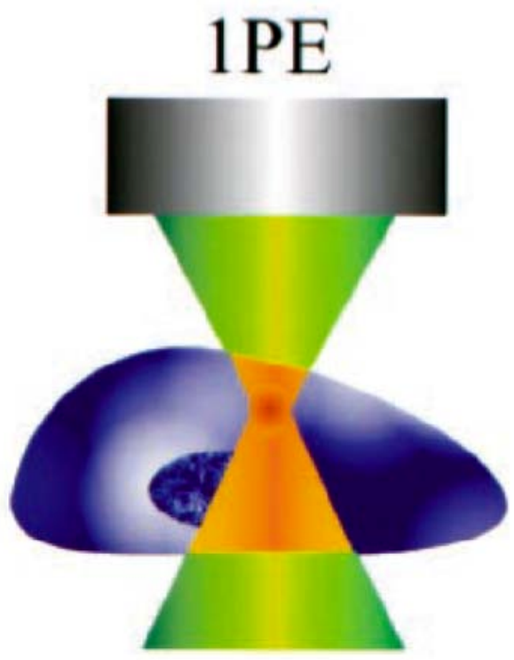
$\langle P_{EXCITATION} \rangle$= 0,04 mW, $\lambda_{EXCITATION}$= 488nm
Pixel time= 4,9 μs, 512*512 pixels

Photoactivation allows you to create a population of labeled proteins in a desired volume inside the cell

I.Testa, S.Barozzi, M.Faretta, A.Diaspro (2005) - LAMBS-IFOM Research Note, 7.

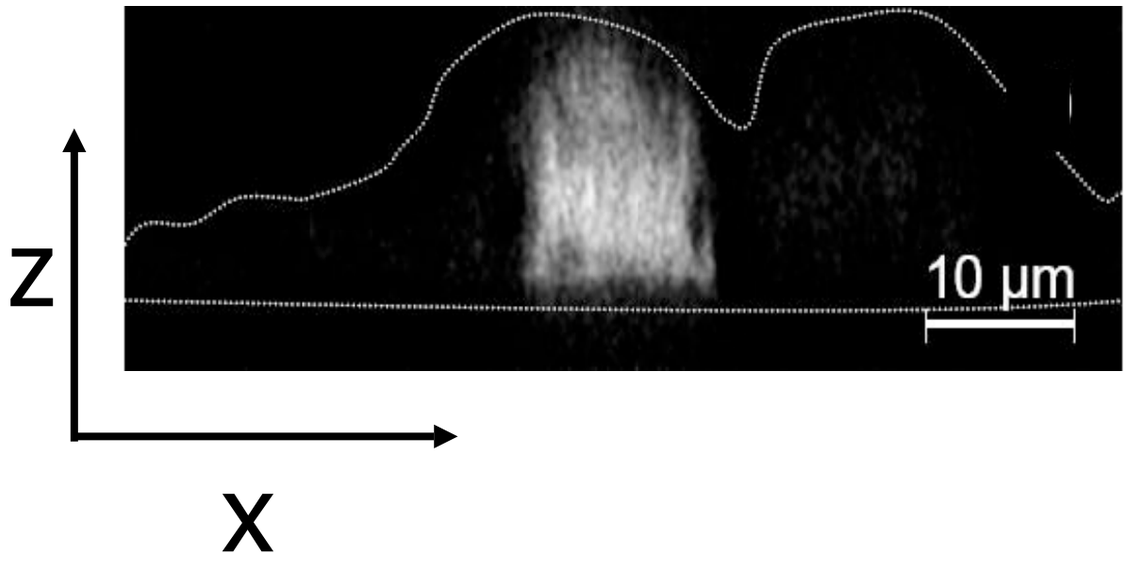
GFP-PHOTOACTIVATION

FLUORESCENCE MICROSCOPY



SINGLE-PHOTON ACTIVATION

UV-Vis
405-413 nm

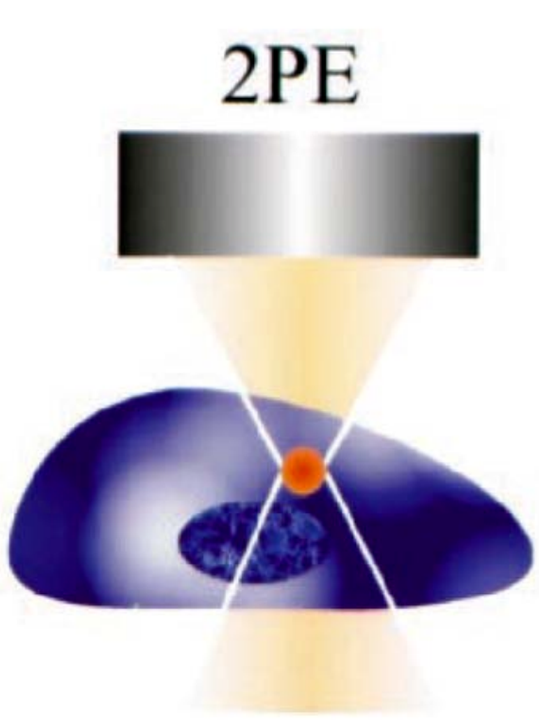


SCHNEIDER M., BAROZZI S., TESTA I., FARETTA M., DIASPRO A. (2005) BIOPHYS. J., VOL.89(2)

GFP-PHOTOACTIVATION

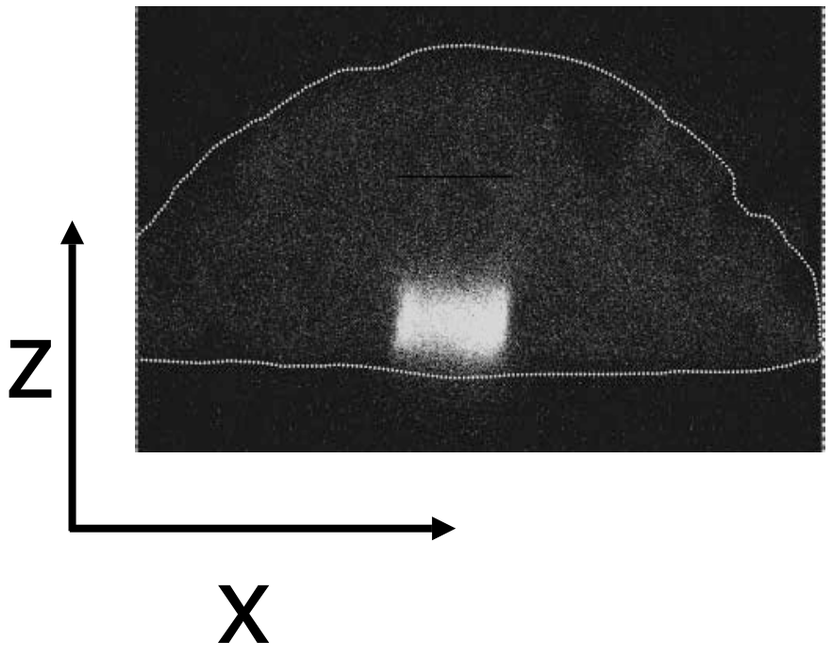
TWO-PHOTON EXCITATION MICROSCOPY

IR-NIR



Denk, W., Strickler, J.H. & Webb, W.W. *Science* **248**, 73–76 (1990).

TWO-PHOTON ACTIVATION

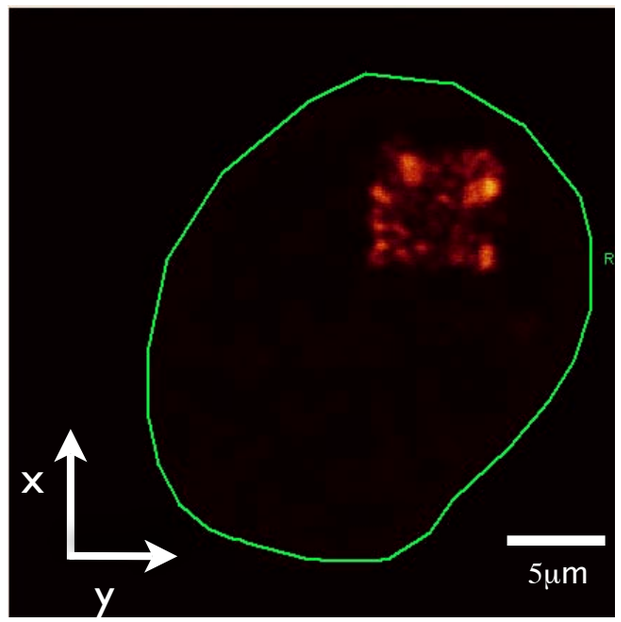


SCHNEIDER M., BAROZZI S., TESTA I., FARETTA M., DIASPRO A. (2005) BIOPHYS. J., VOL.89(2)

TWO PHOTON ACTIVATION IN LIVING CELLS

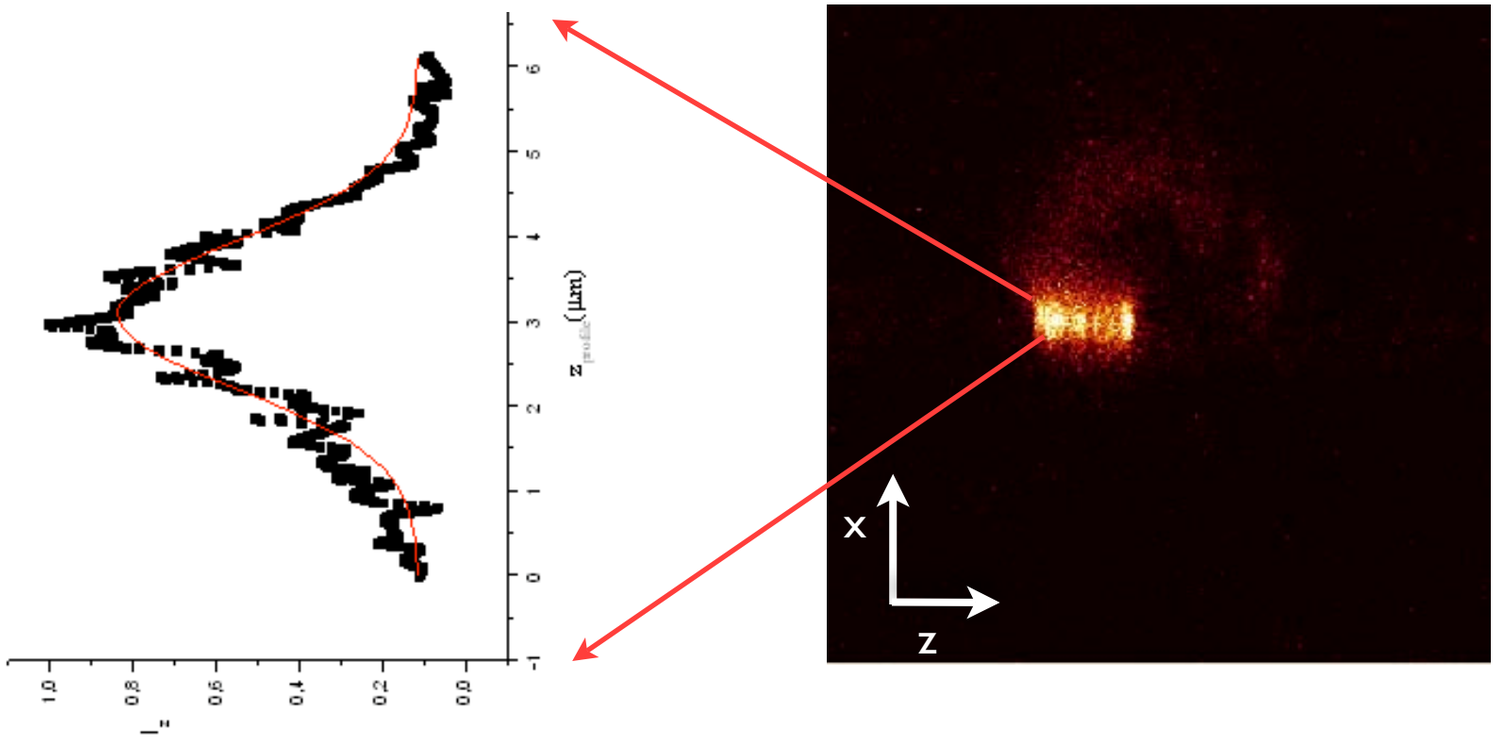
PA-GFP + H2B
He-La cell

$\langle P_{ACTIVATION} \rangle$= 10mW
$\lambda_{ACTIVATION}$= 720nm
Pixel time= 300μs

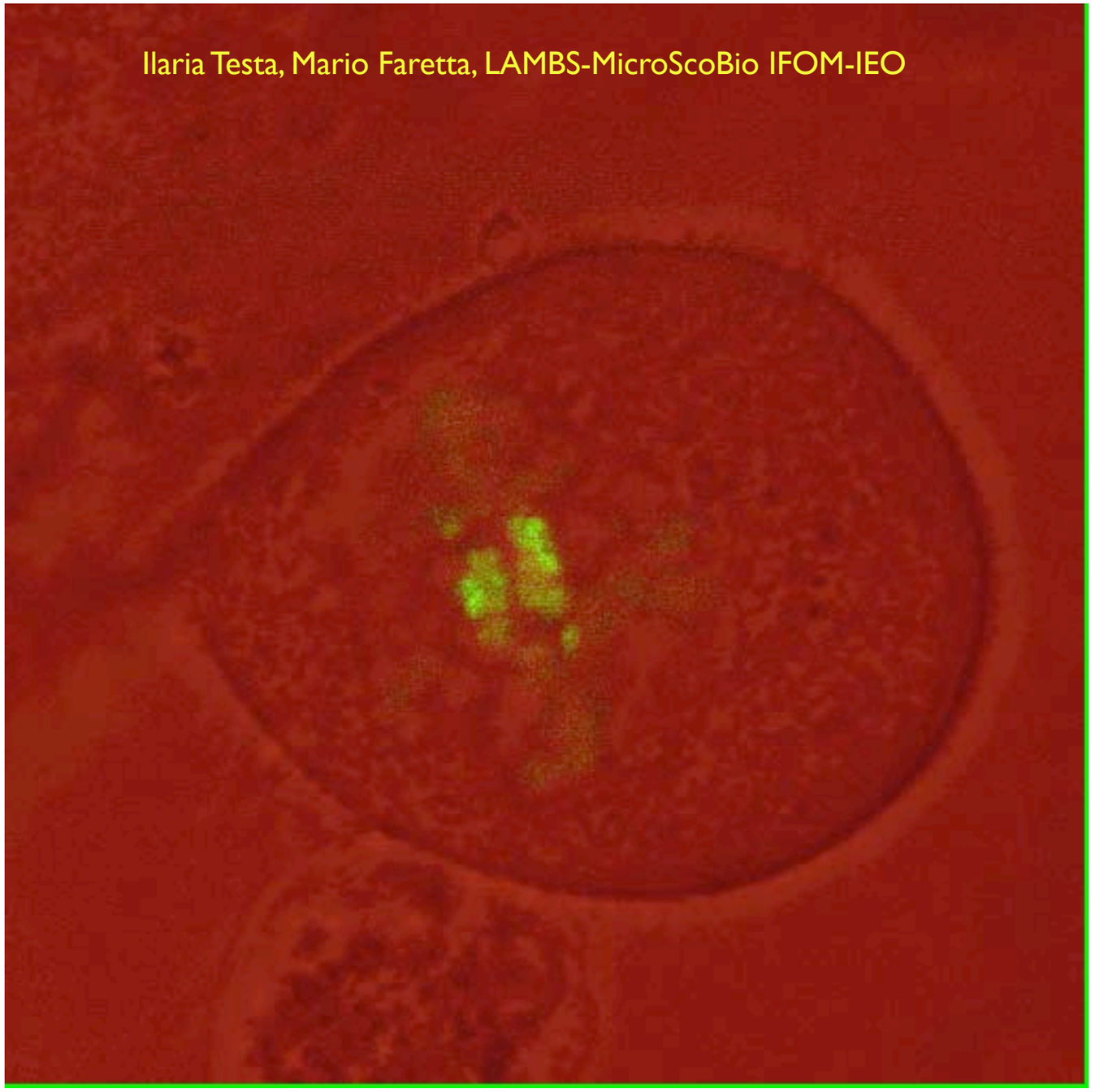


$\langle P_{EXCITATION} \rangle$= 0,04 mW, $\lambda_{EXCITATION}$= 488nm
Pixel time= 4,9 μs, 512*512 pixels

LOCALIZED
INTERACTION

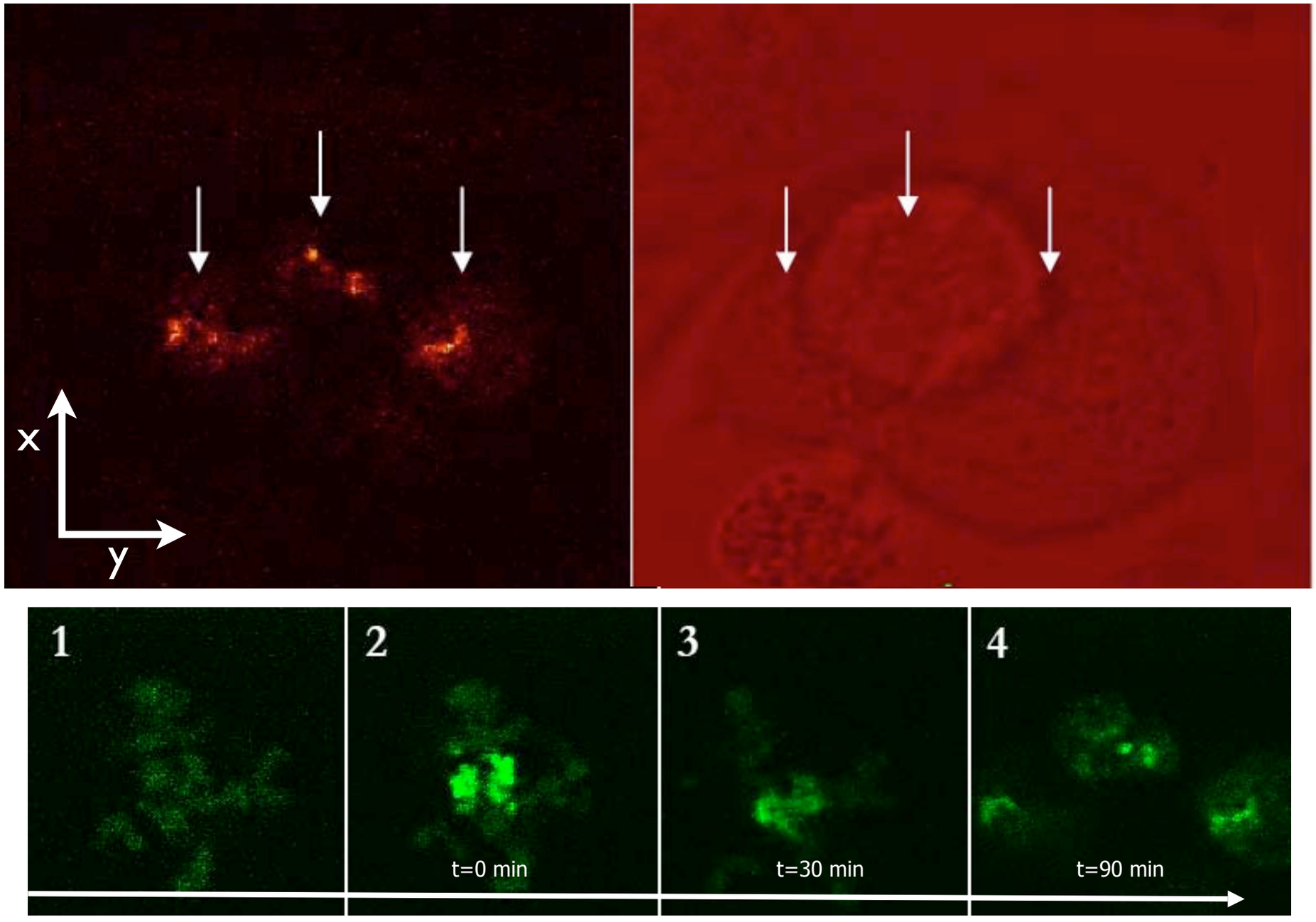


Two photon activation in living cells



I. Testa, S. Barozzi, M. Faretta, A. Diaspro (2005) - LAMBS-IFOM Research Note, 7.
PA-GFP, LEICA SP2 AOBS spectral system, Chameleon-XR Coherent tunable Ti-Sapphire laser

Two photon activation in living cells



I.Testa, S.Barozzi, M.Faretta, A.Diaspro (2005) - LAMBS-IFOM Research Note, 7.
PA-GFP, LEICA SP2 AOBS spectral system, Chameleon-XR Coherent tunable Ti-Sapphire laser

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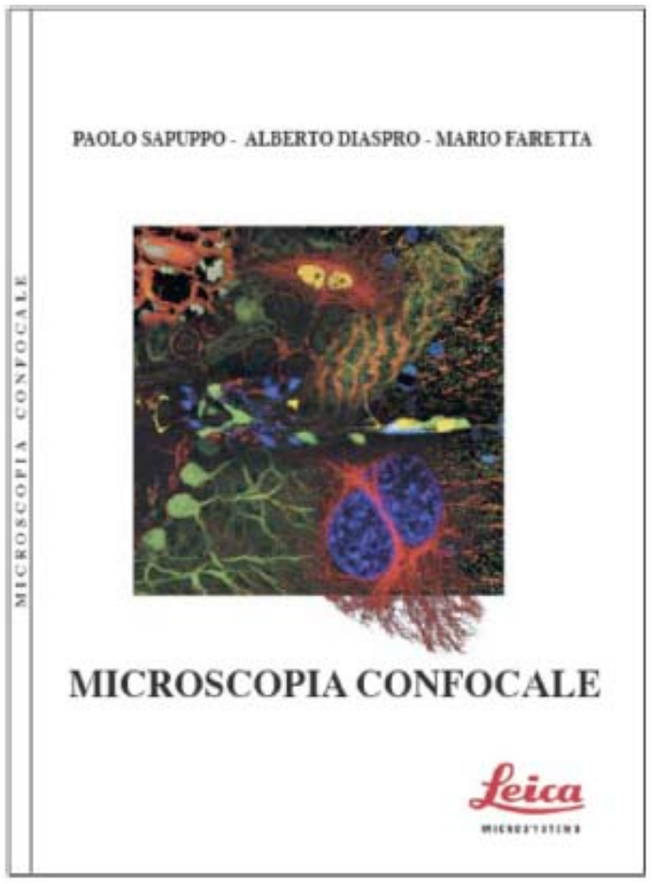

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