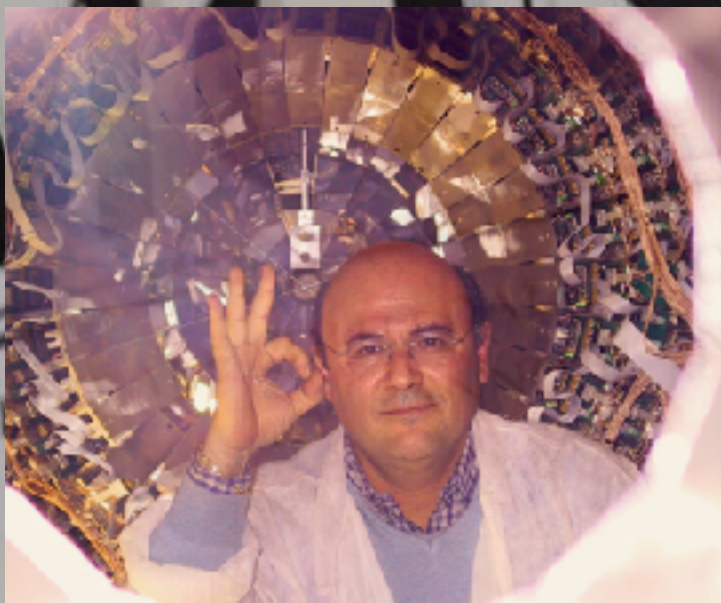


FATA 2025

FAst Timing Applications for Nuclear Physics and Medical Imaging
Catania - Italy, 8th - 10th October 2025

The second edition of FATA will be held in memory of Angelo Pagano, chair of the previous editions

Il LNS è un trasformatore di energia in informazioni scientifiche e non produce nessuna scoria radiattiva.



Esso permette di studiare il microcosmo nucleare osservando le modificazioni che subisce il nucleo quando vengono assegnate certe condizioni sperimentali che cercherò di chiarire in seguito. Ma come si può osservare il nucleo atomico dal momento che esso rimane nascosto all'esperienza comune?



Alberto Diaspro

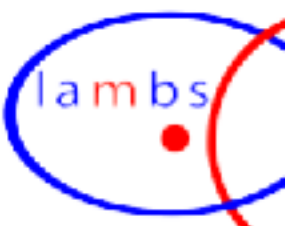
FATA 2025

Fast Timing Applications for Nuclear Physics and Medical Imaging
Catania - Italy, 8th - 10th October 2025

The second edition of FATA will be held in memory of Angelo Fagano, chair of the previous editions



NANOSCOPY



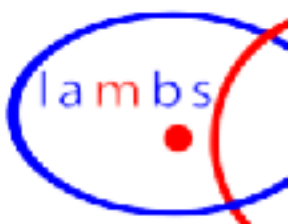


Alberto Diaspro

FATA 2025

Fast Timing Applications for Nuclear Physics and Medical Imaging
Catania - Italy, 8th - 10th October 2025

The second edition of FATA will be held in memory of Angelo Fagano, chair of the previous editions

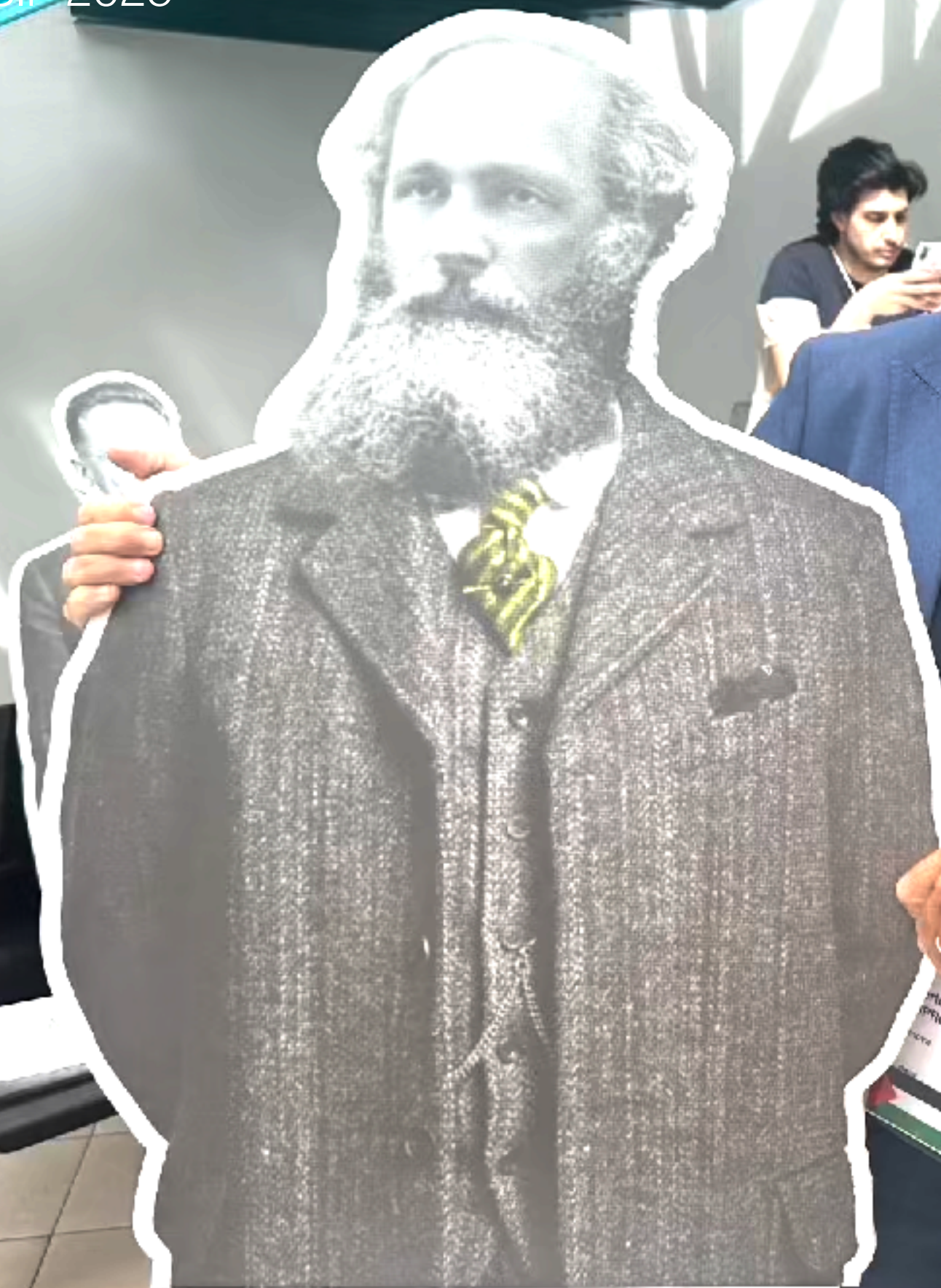




Alberto Diaspro

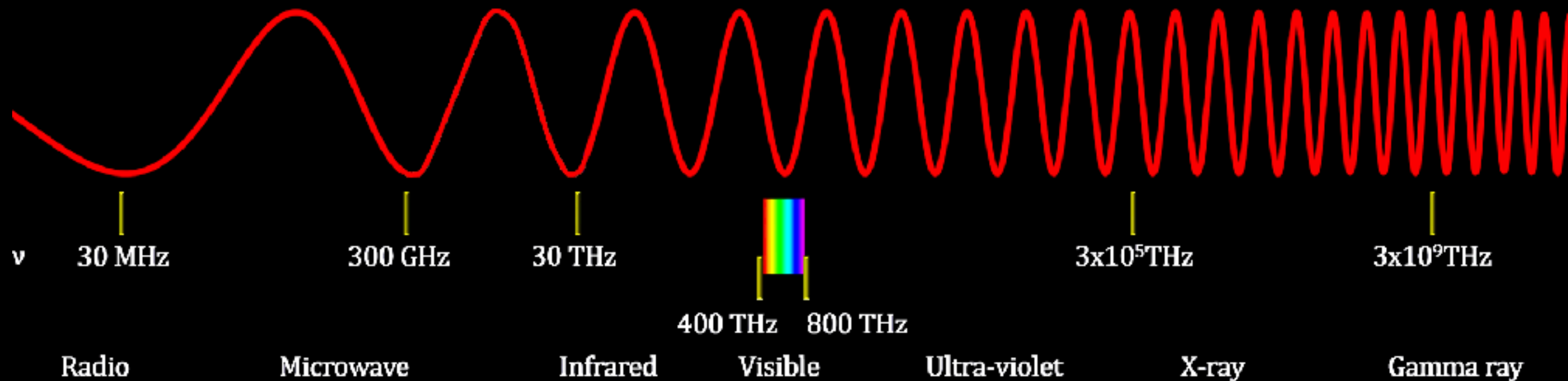






James Clerk Maxwell

Electromagnetic Spectrum



Let

ν = frequency

λ = wavelength

c = speed of light

Then

$$\lambda \nu = c$$

And

$$\lambda = c/\nu$$

For Red Light

$$\nu = 400 \text{ THz}$$

$$\lambda = 3 \times 10^8 \text{ ms}^{-1} / 400 \times 10^{12} \text{ s}^{-1}$$

$$= 750 \text{ nm} = 0.00003 \text{ inches}$$

EARTH AND MOON

— to scale —

Speed of light in real-time
Surface-to-surface in 1.255 seconds

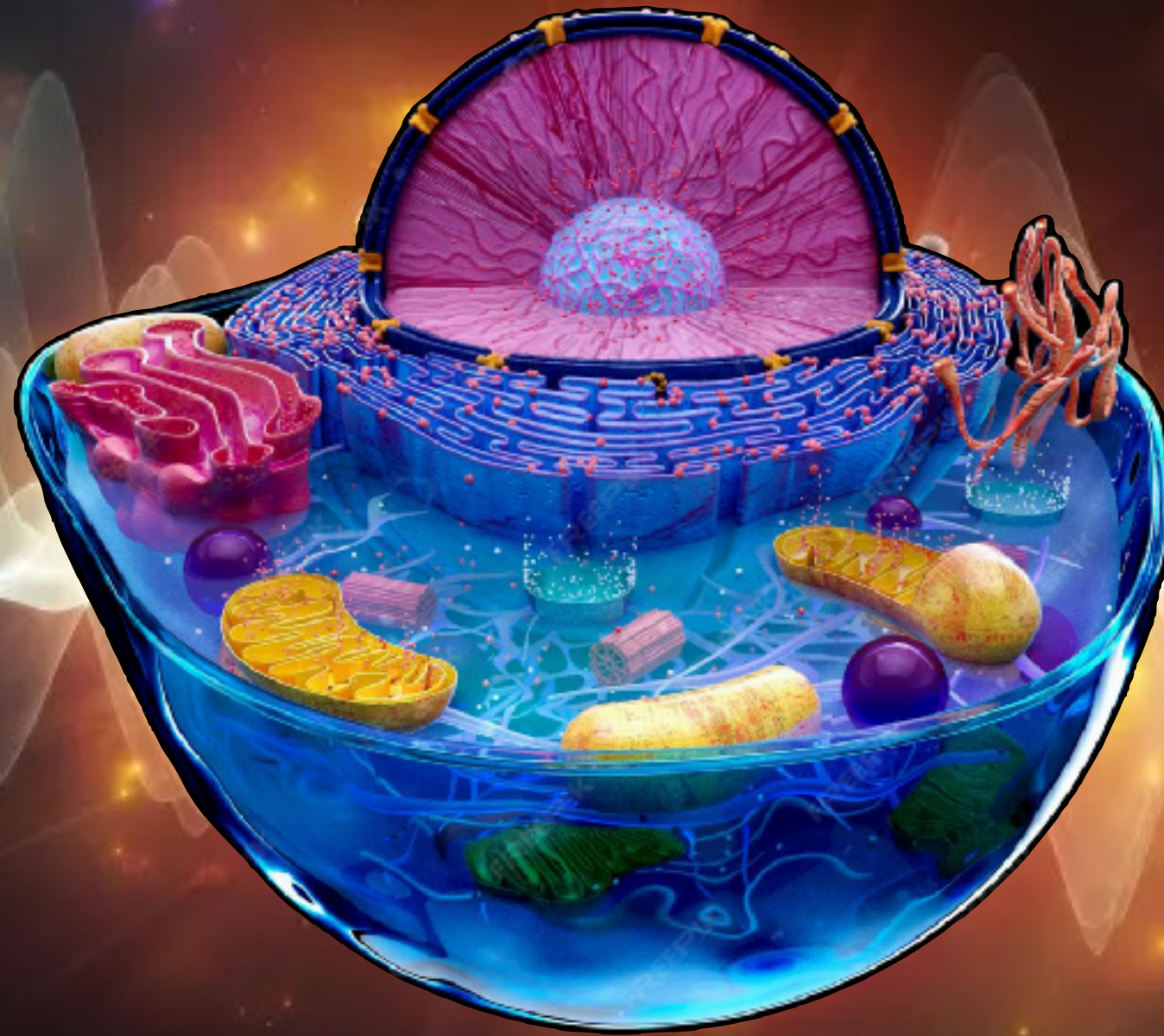


EARTH

MOON

Average distance
384,400 km

James O'Donoghue / NASA imagery — T: @physicsJ IG: jameslikesspace



For a cell with a refractive index, n , between 1.3 and 1.5 in the visible spectrum, light traveling through a thickness of 10 micrometers (10^{-6} meters) would take approximately 0.033 femtoseconds (3.3×10^{-14} seconds) to pass through.

Object

$$t(x, y) = a(x, y)e^{i\phi(x, y)}$$

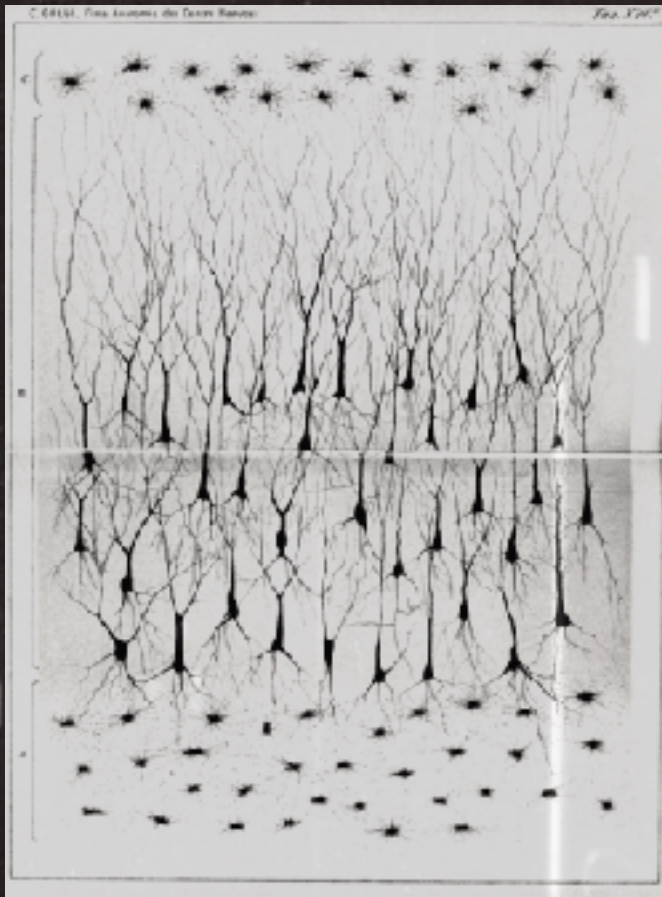
$a(x, y)$ is modulus (amplitude), real

$\phi(x, y)$ is phase, real

Perfect image

$$I(x, y) = \left| a(x, y)e^{i\phi(x, y)} \right|^2 = a^2(x, y)$$

Credit: Colin JR Sheppard



1906

Camillo Golgi (1843 - 1926)



1953



5 μm







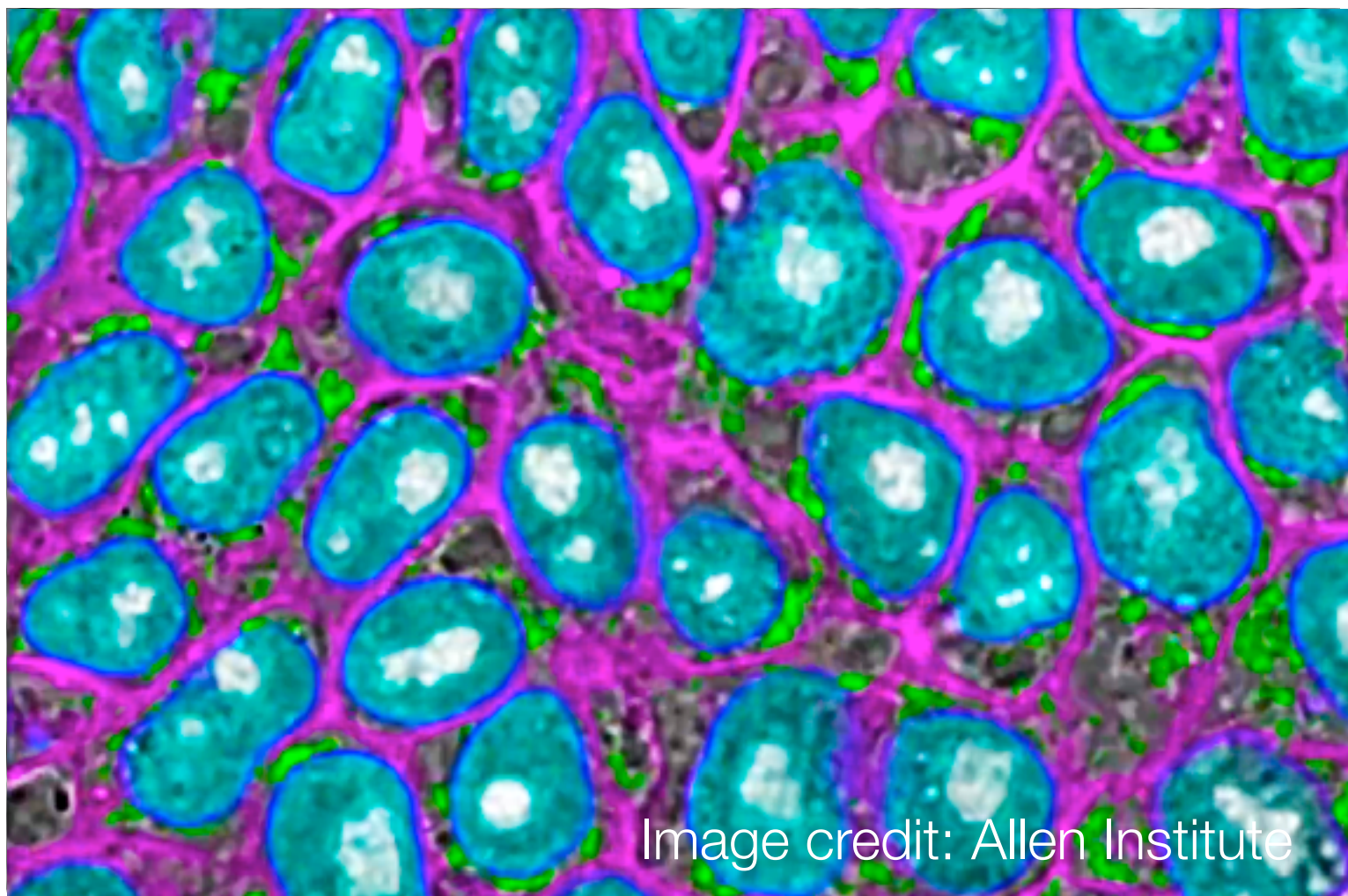


Image credit: Allen Institute

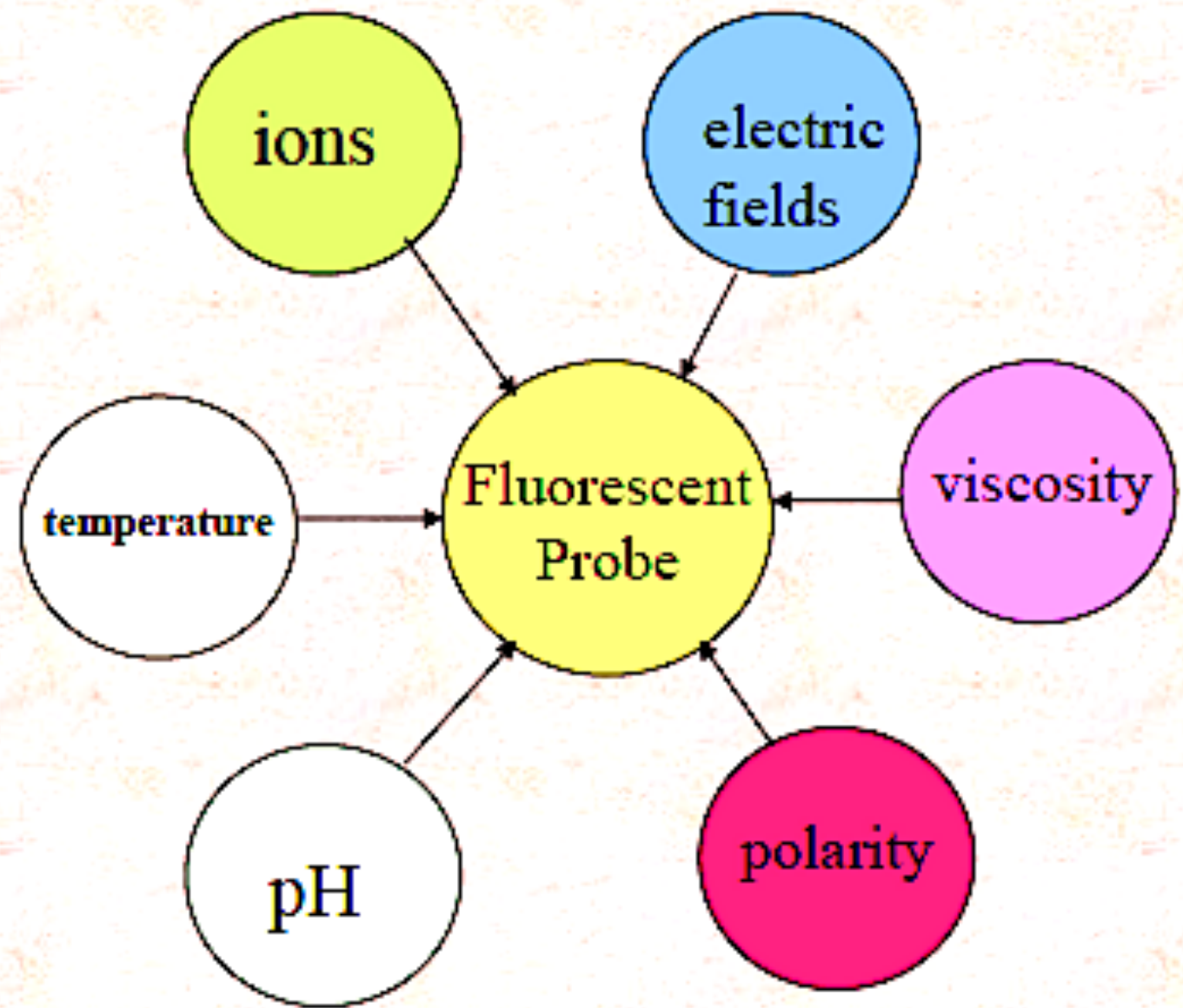
FLUORE -

SCIENCE

Why fluorescence?

- its pretty!
- it provides information on the molecular environment
- it provides information on dynamic processes on the nanosecond timescale

Credit:David Jameson

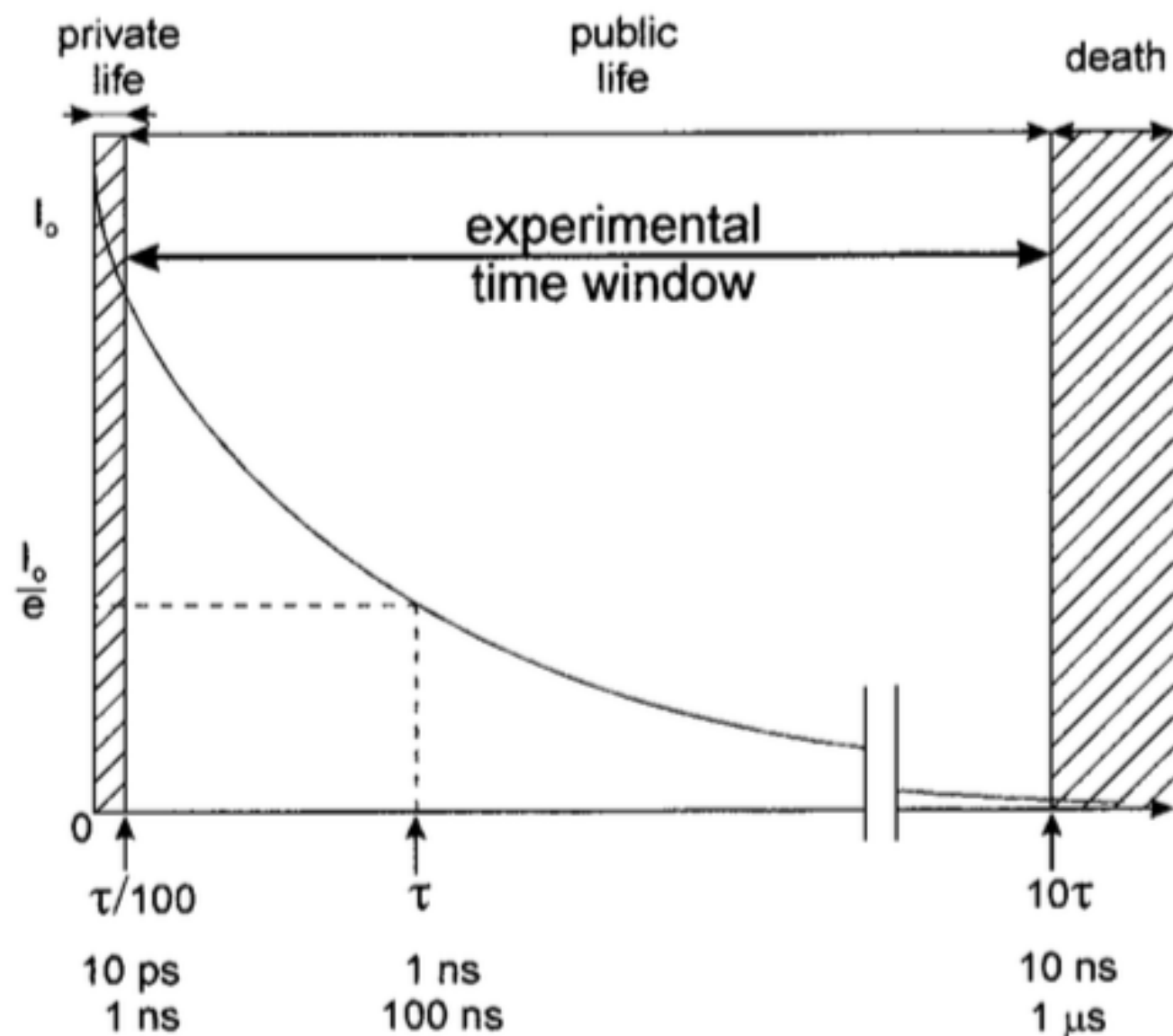


Fluorescence Probes are essentially molecular stopwatches which monitor *dynamic* events which occur during the excited state lifetime – such as movements of proteins or protein domains

FLUORESCENCE DECAY

$$I = I_0 \exp(-t/\tau)$$

lifetime



EXAMPLES OF LIFETIMES

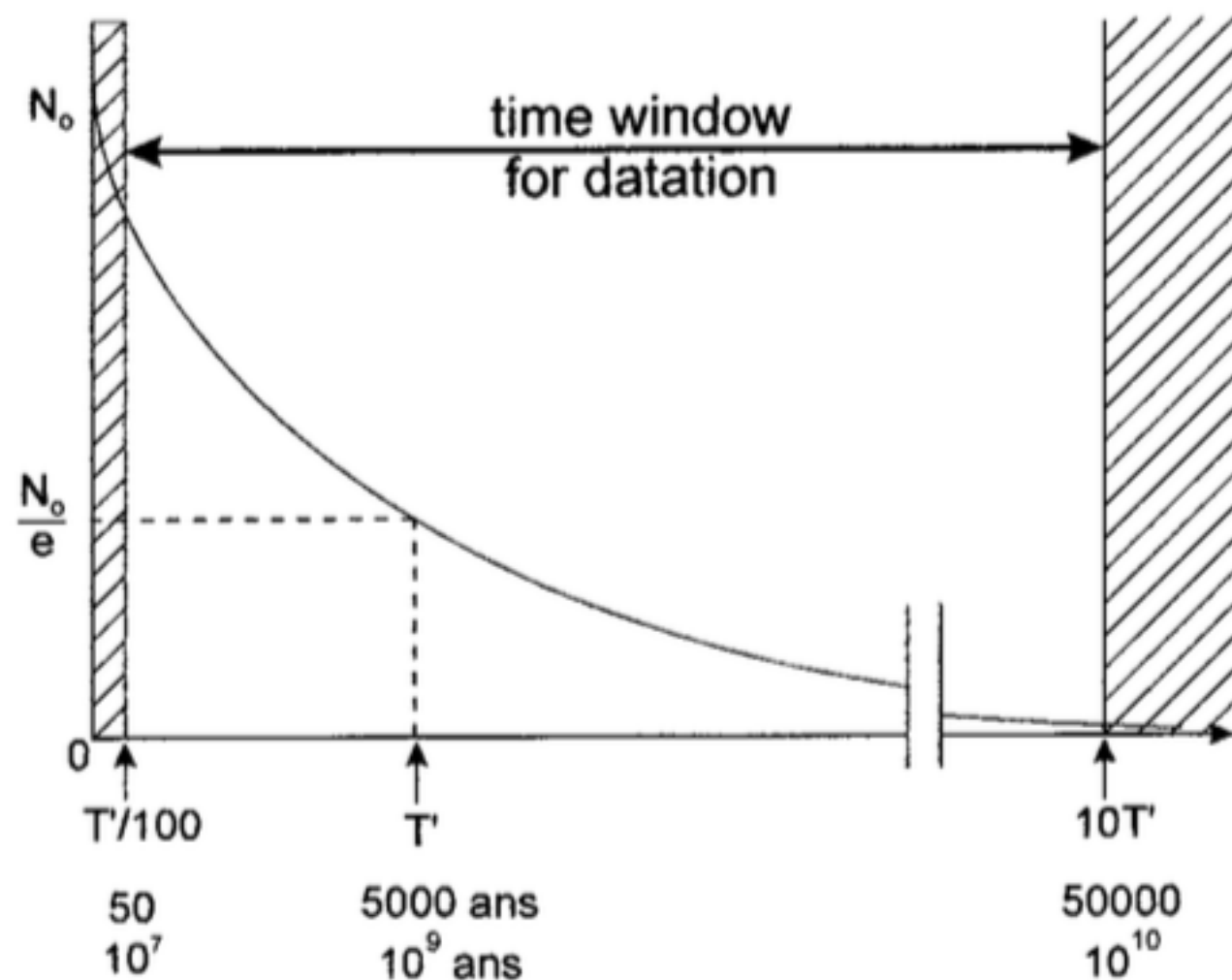
Pyrene/EtOH	410 ns
Anthracene/EtOH	5.1 ns
Erythrosine/H ₂ O	90 ps

$$\tau = \frac{1}{k_f + k_i}$$

RADIOACTIVE DECAY

$$N = N_0 \exp(-t/T')$$

period x1.35



EXAMPLES OF PERIODS

¹⁴ C	5700 years
⁴⁰ K	$1.5 \cdot 10^9$ years



Cy2, exc 491 nm, emis 499 – 505 nm,
NMDA receptors

Lp, exc 474 nm, 509 – 514 nm,
beads in vacuols

Acridine Orange, exc 484 nm,
emis 520 – 532 nm, nucleus

Cy3, exc 551 nm, emis 555 – 575 nm,
Gaba-B-R1 receptors

TexasRed, exc 562 nm, emis 605 – 612 nm,
vacuoles and endosomes

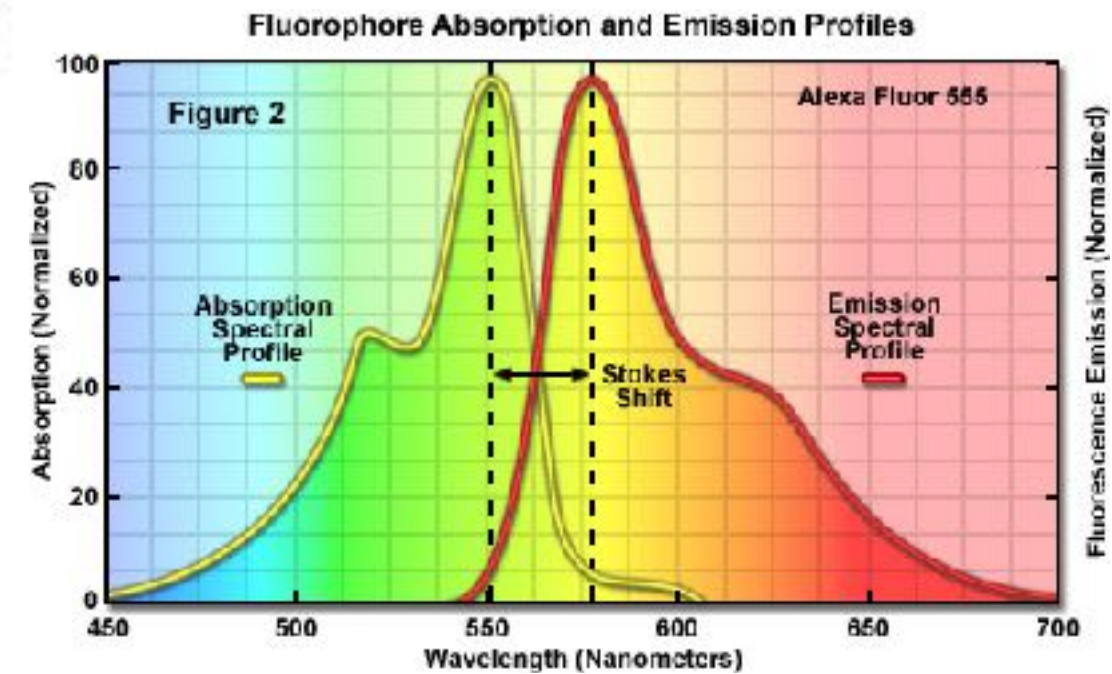
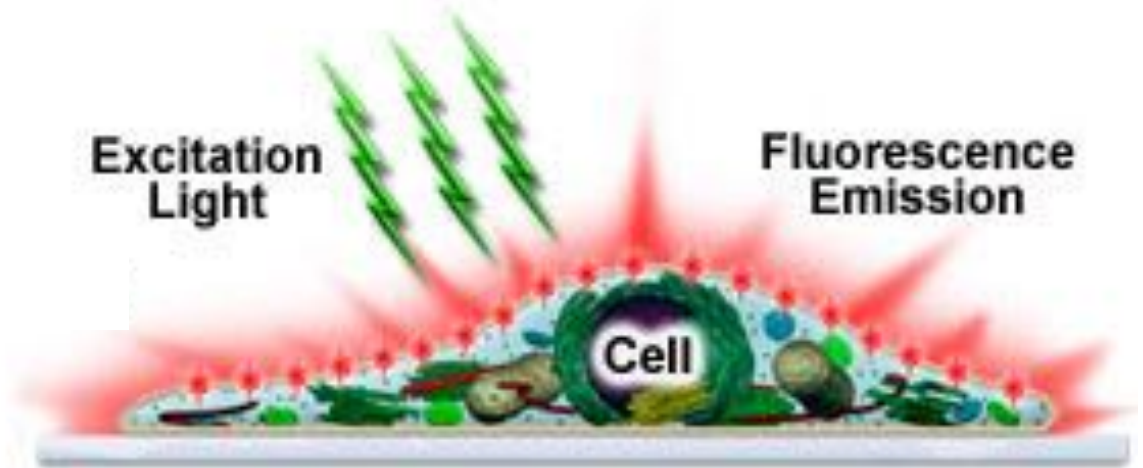
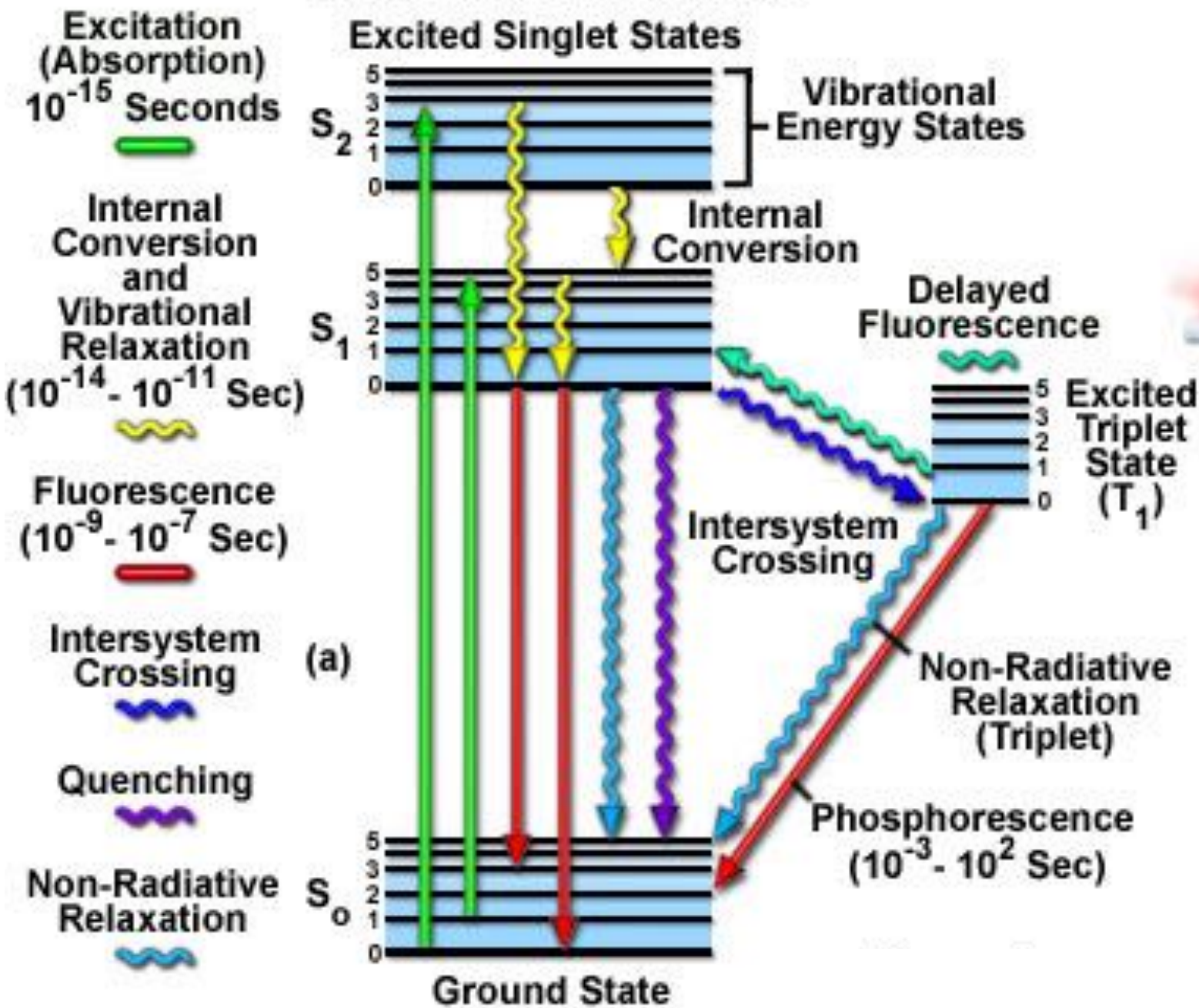
Alexa 594, exc 594, emis 615 – 625 nm,
nuclear and cellular membrane

Alexa 633, exc 633 nm, emis 655-665 nm,
clathrine vesicles

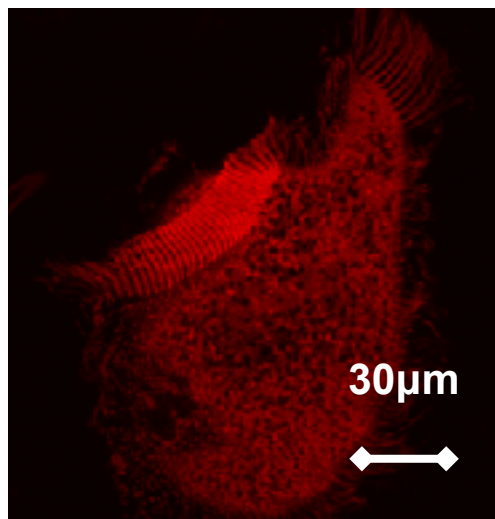
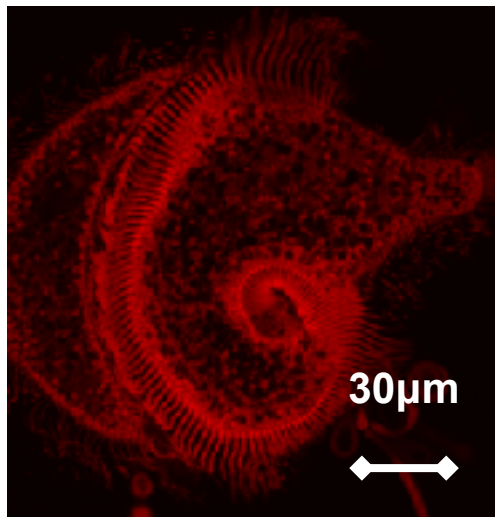
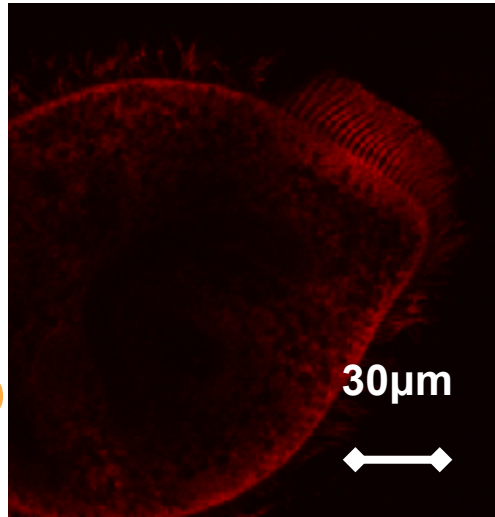
Cy5, exc 640 nm,
emis 661-675 nm, cilia

Fundamental Concepts Underpinning Fluorescence Microscopy

Jablonski Energy Diagram



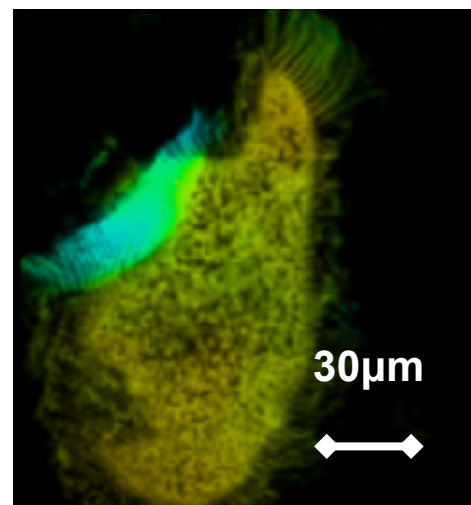
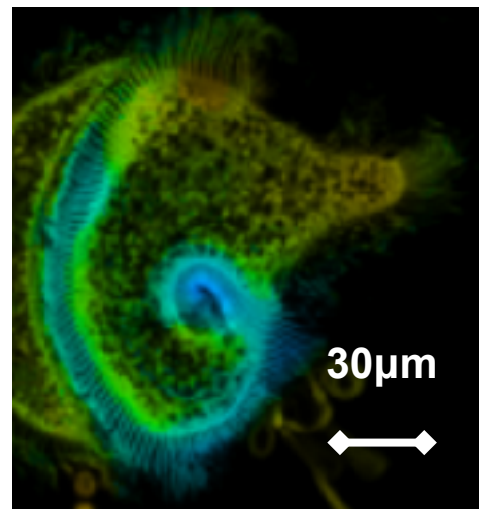
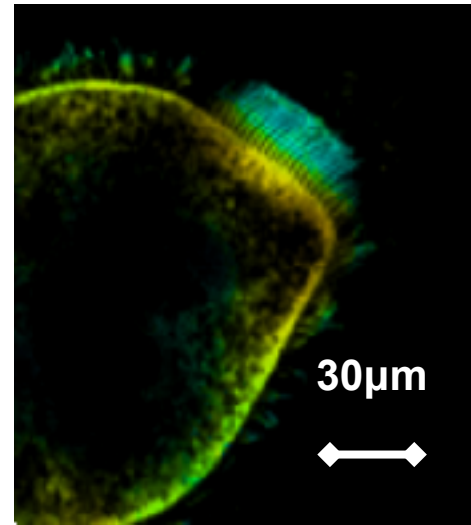
Fluorescence intensity images



Credits: Mattia Pesce

Ciliated Protozoa (Fabrea Salina)

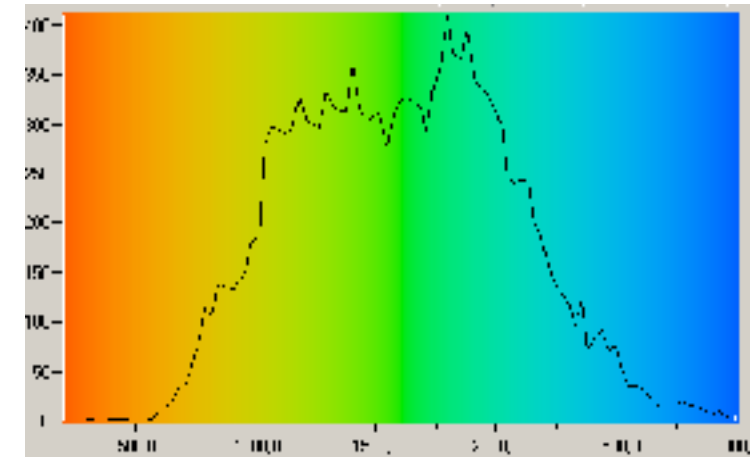
Fluorescence Life time imaging



Credits: Mattia Pesce

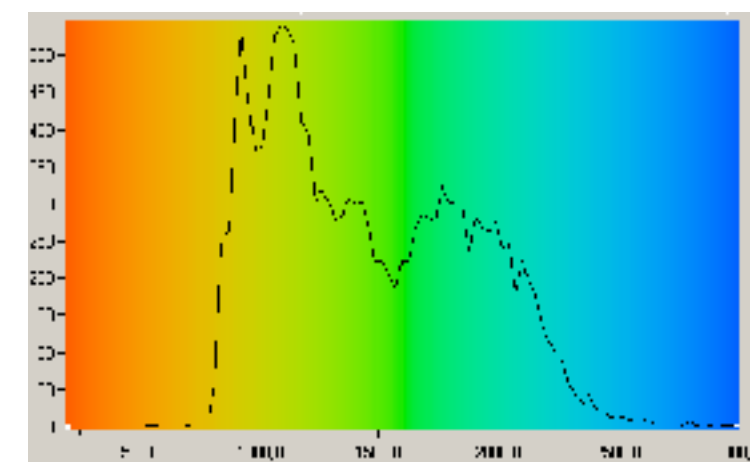
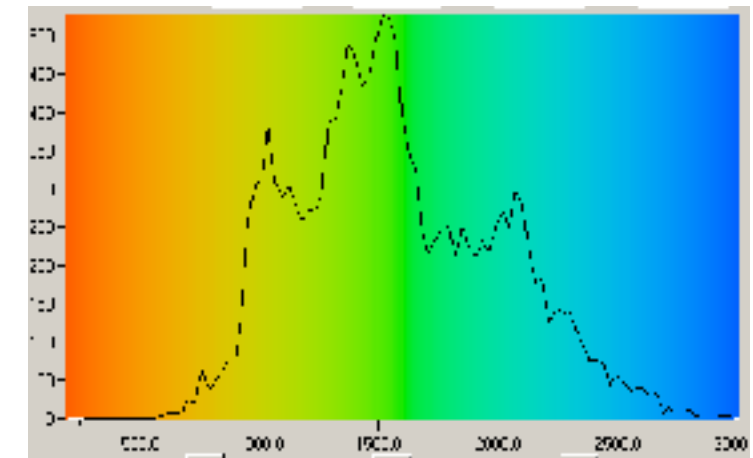
Colombetti G., Checcucci G., Lucia S., Usai C., Ramoino P., Bianchini P., Pesce M., Vicidomini G., Diaspro A. (2007). **Microsc Res Tech.** 70(12), 1028–33

Giuliano Colombetti, IBF CNR, Pisa



200ps

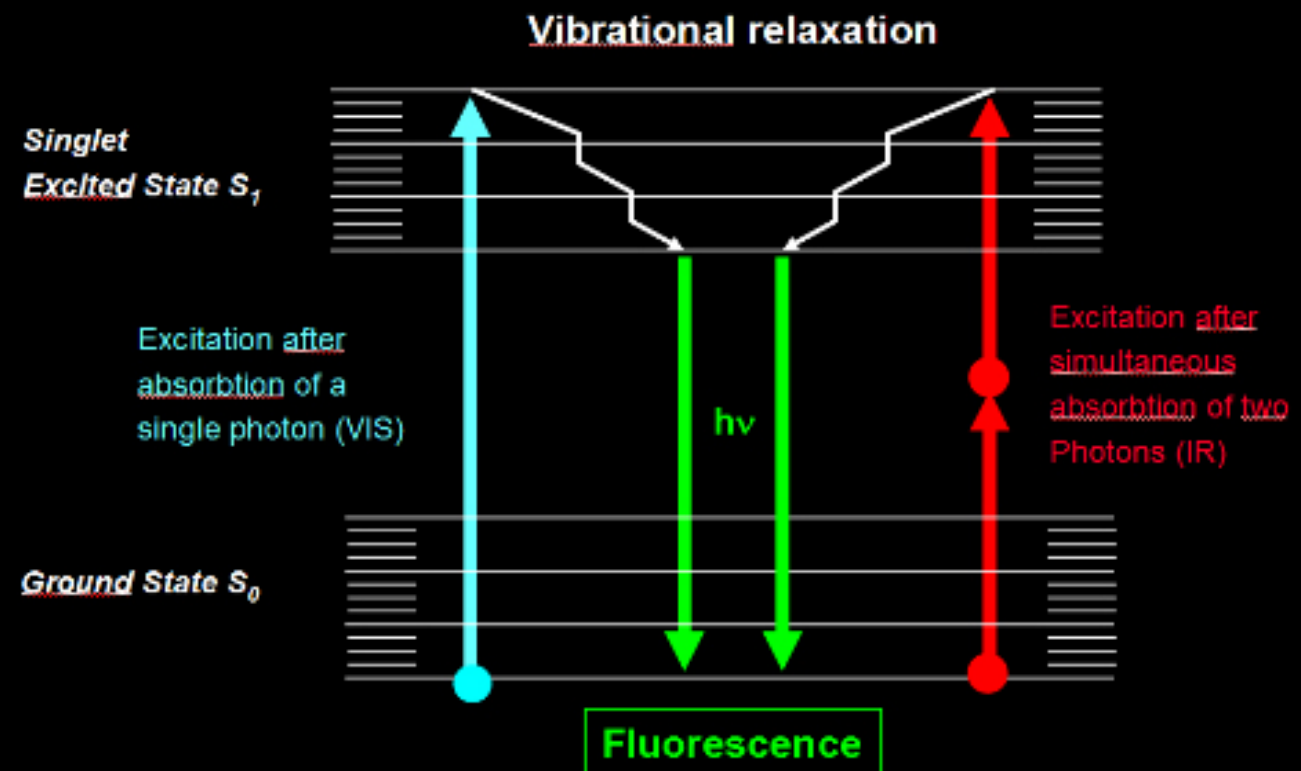
3000ps



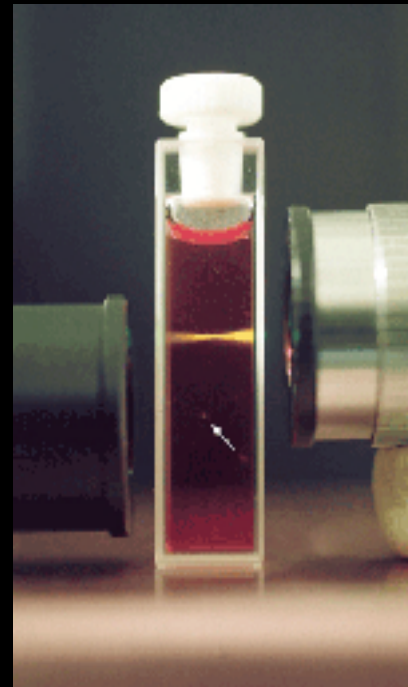
TWO-PHOTON EXCITATION MICROSCOPY



Maria Göppert-Mayer predicted that an atom or a molecule could interact with two photon(s) simultaneously by absorbing them in the very same quantum event (1929).



Sheppard CJR and Kompfner R. Applied Optics .17: 2879–2882 (1978)
Denk, W., Strickler, J.H. & Webb, W.W. Science 248, 73–76 (1990).



$$n_a \approx \delta \cdot \langle P \rangle^2 \cdot f_p^{-1} \cdot \xi \cdot \left(\frac{NA^2}{\eta c \lambda} \right)^2$$

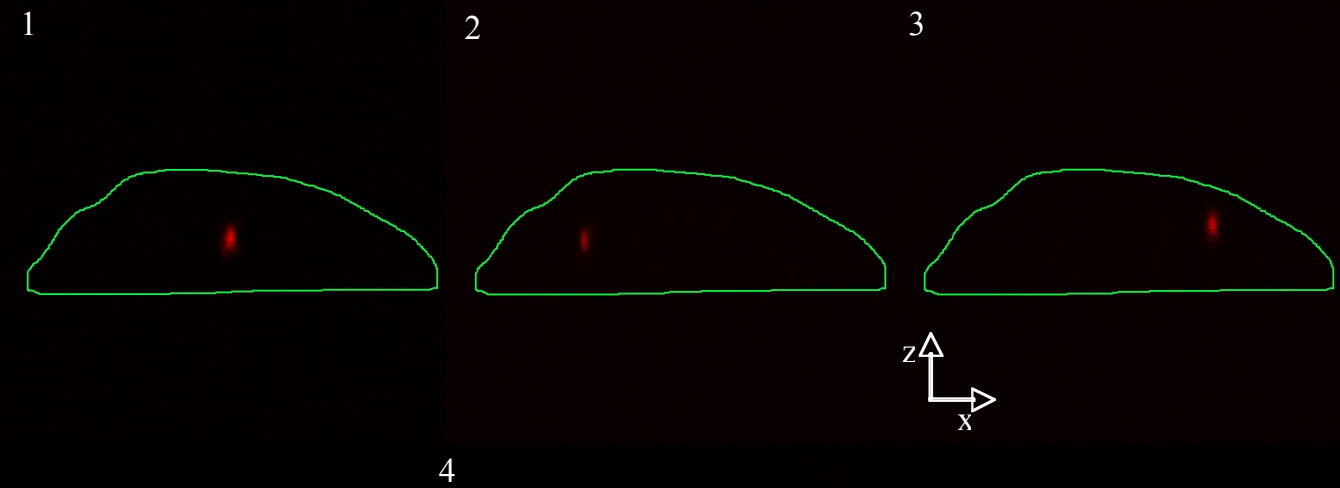
Credit:BRAD AMOS
MRC UK

Diaspro Lab

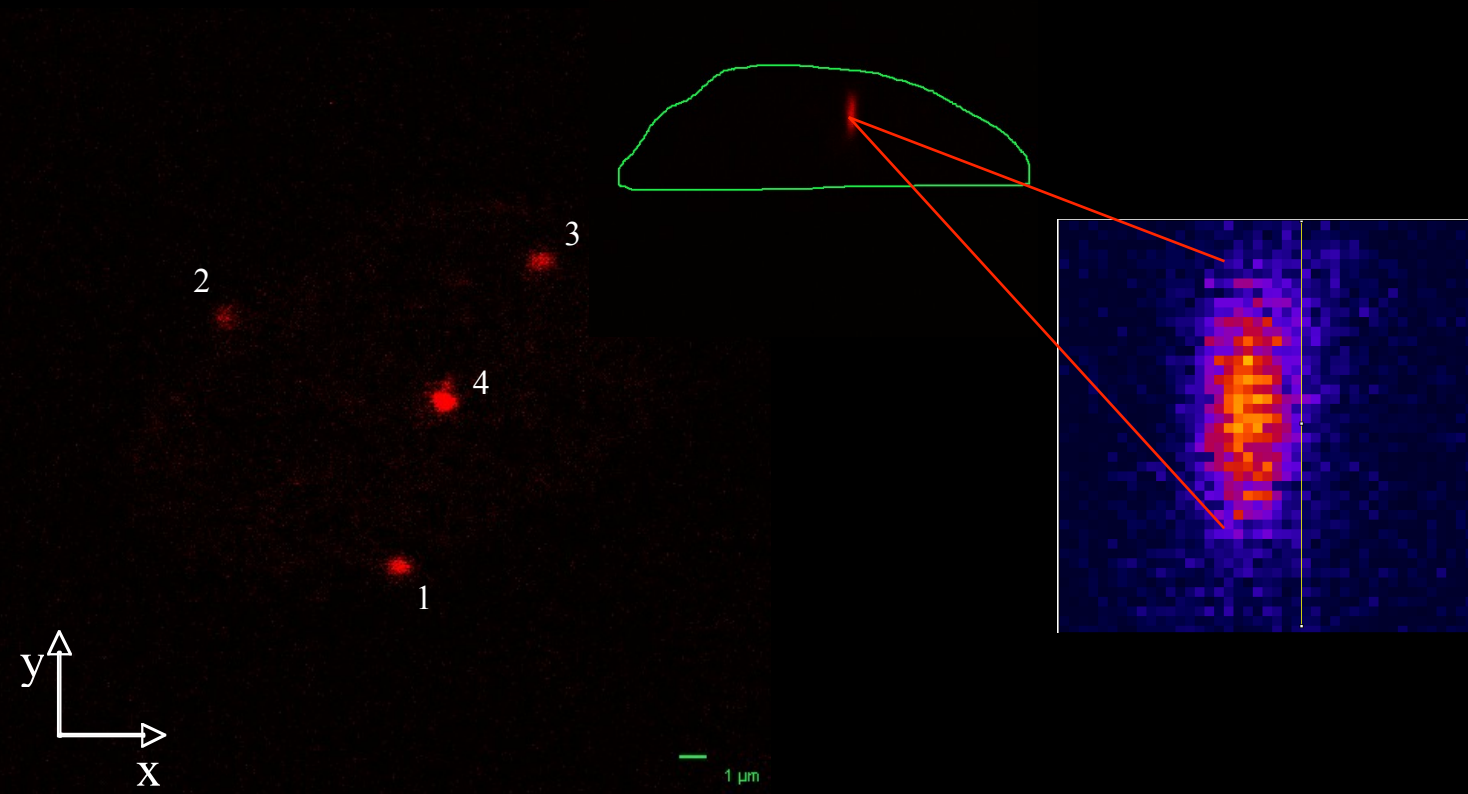
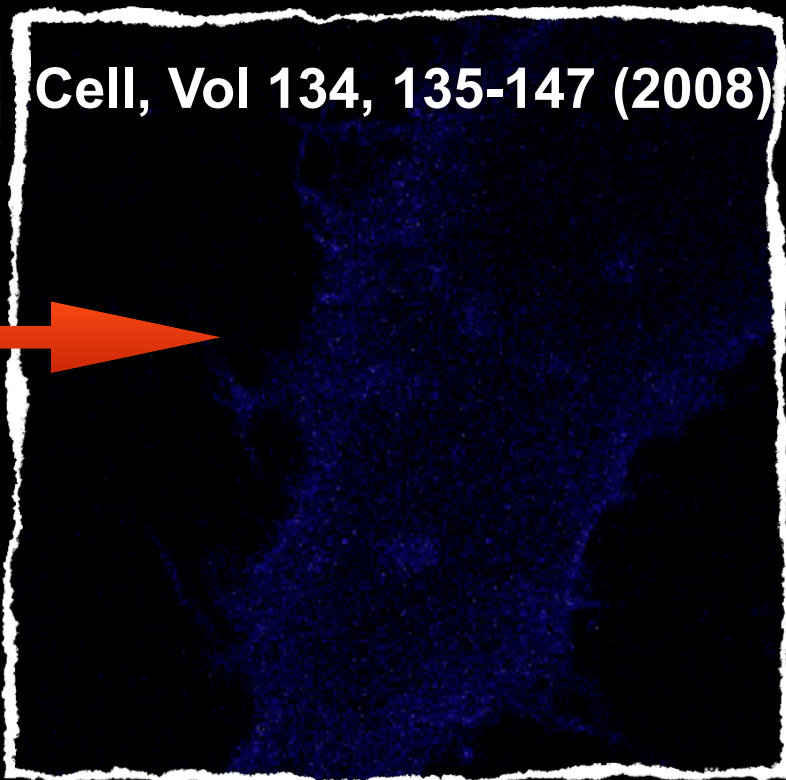


PA-GFP

APL 91, 133902 (2007).



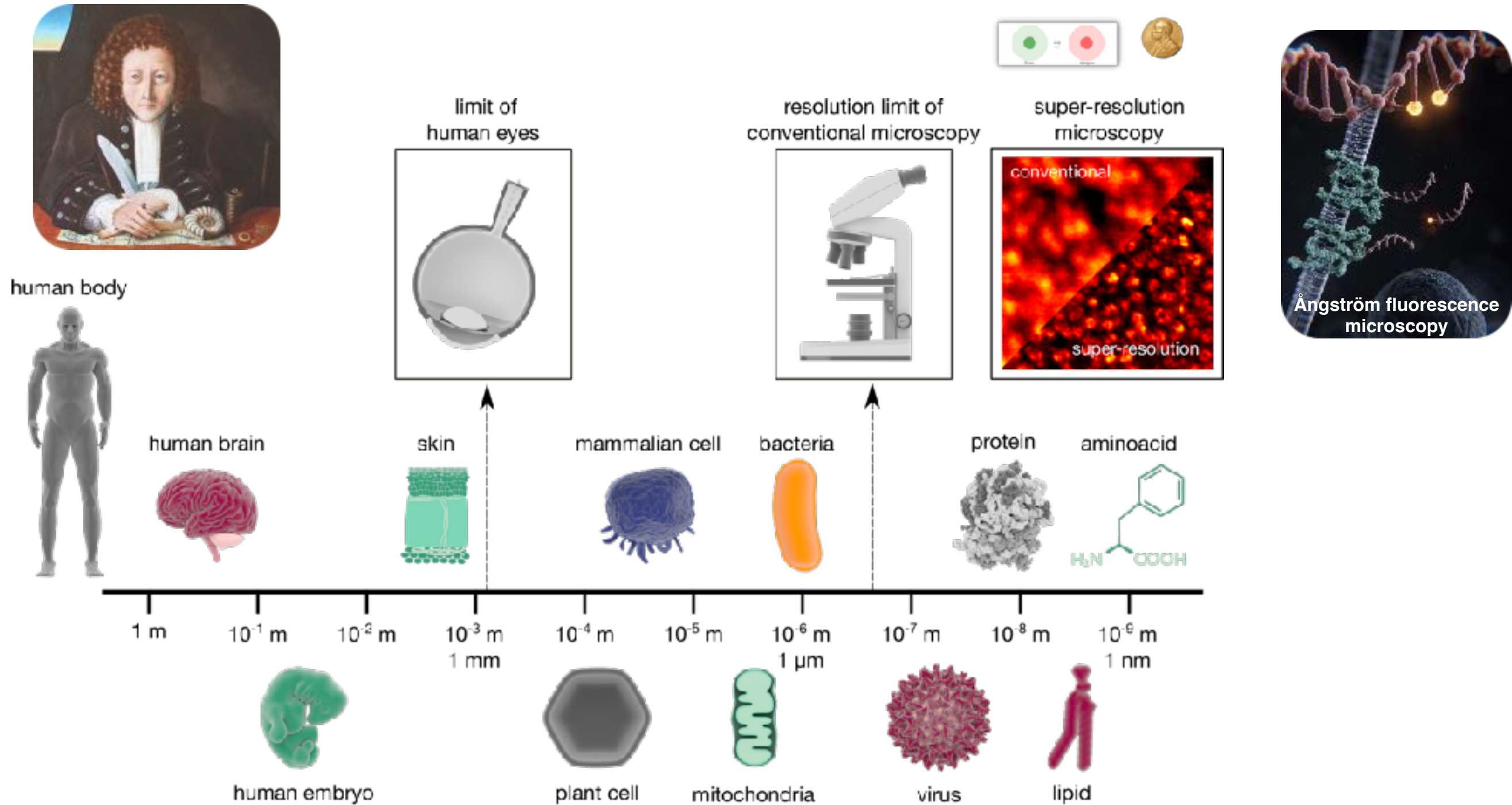
Cell, Vol 134, 135-147 (2008)



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

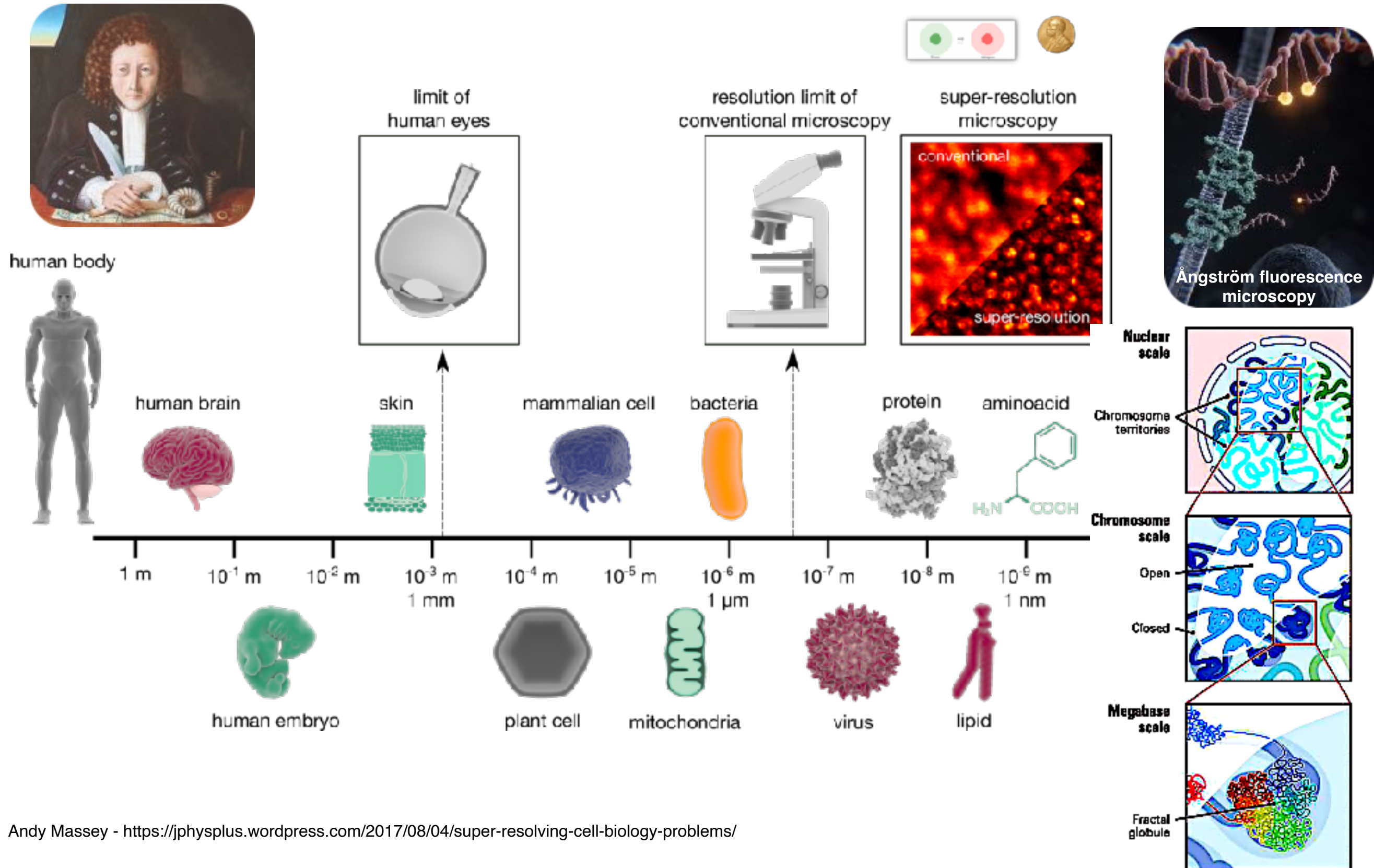
'There is nothing so small, as to escape our enquiry'

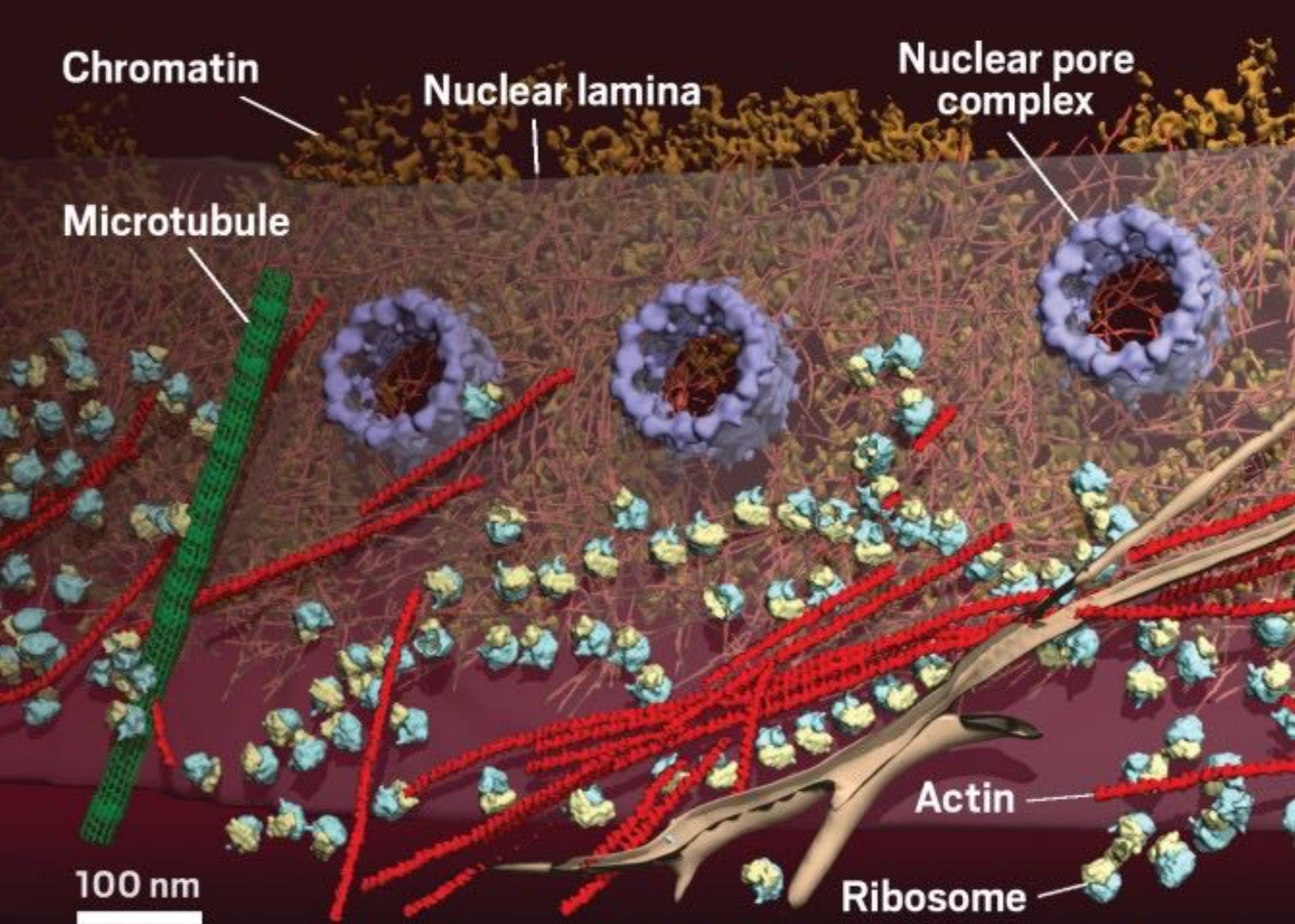
There is a new visible world discovered to the understanding. Robert Hooke, Micrographia 1665



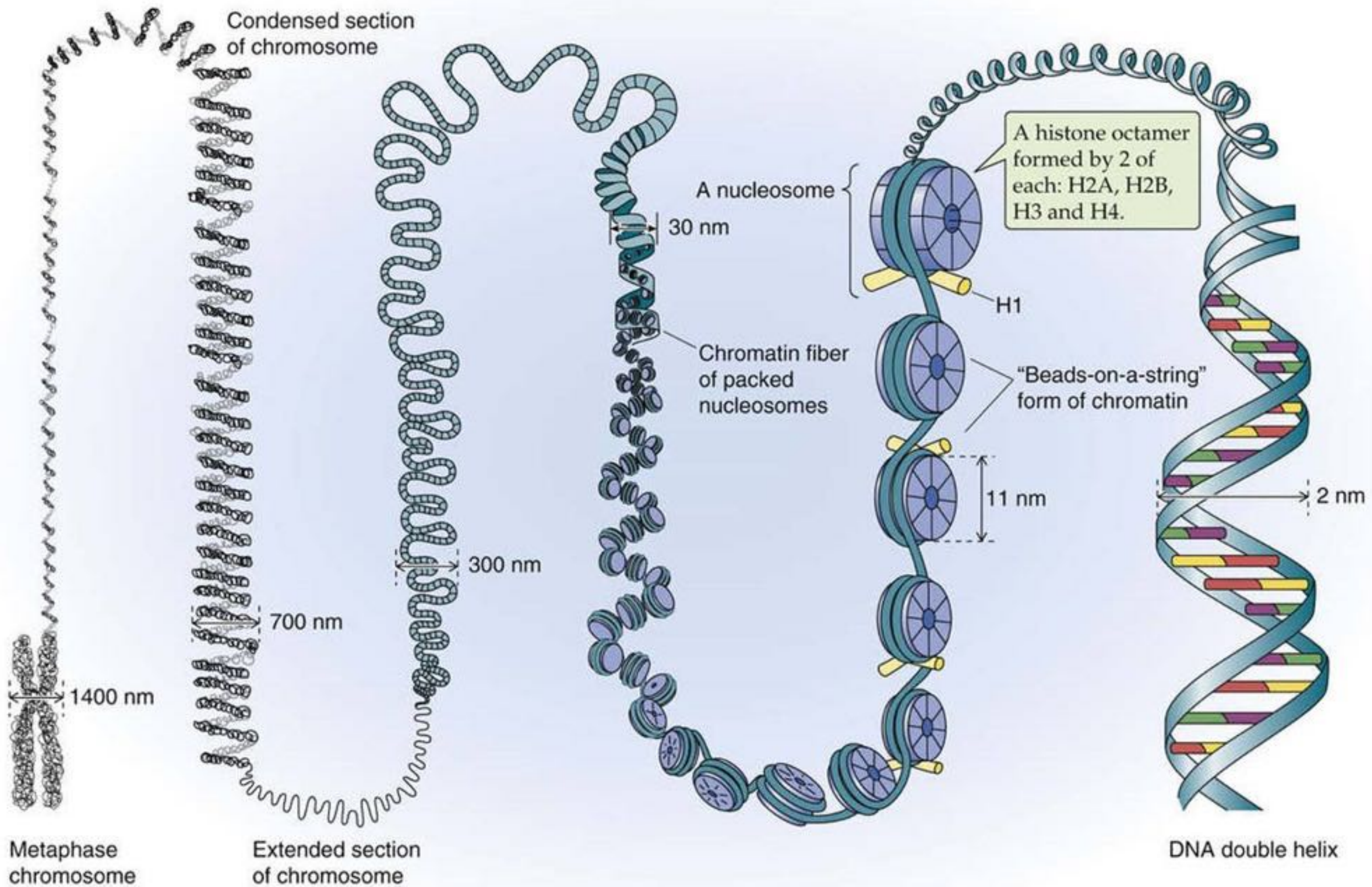
'There is nothing so small, as to escape our enquiry'

There is a new visible world discovered to the understanding. Robert Hooke, Micrographia 1665

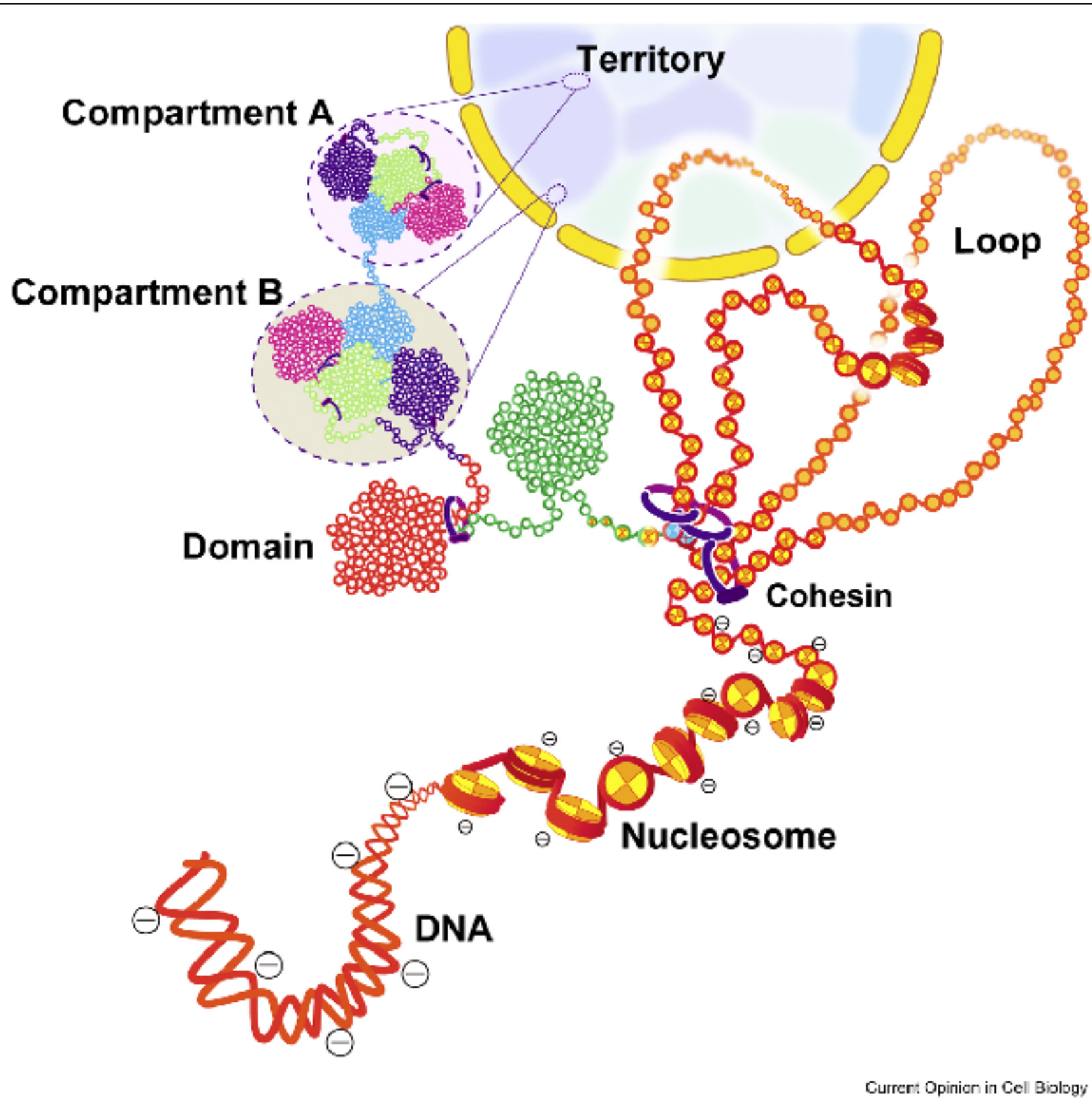




The mission/target: tuning the optical microscope across spatial dimension from micron to nanometers



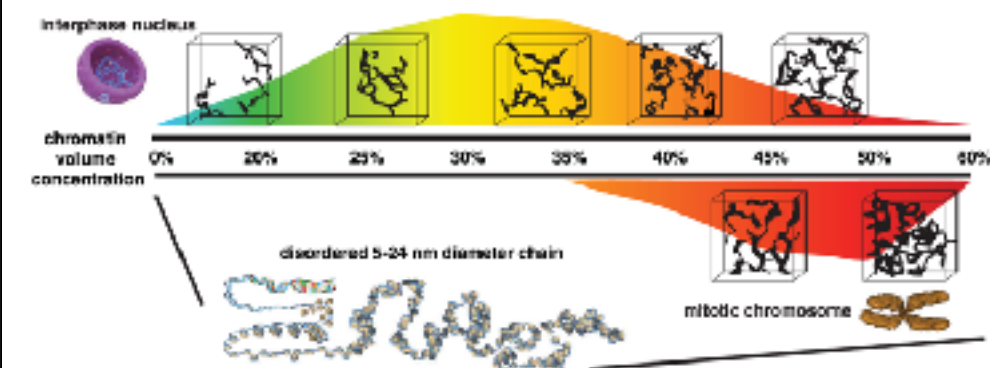
A scheme for hierarchical chromatin organization inside the cell nucleus.

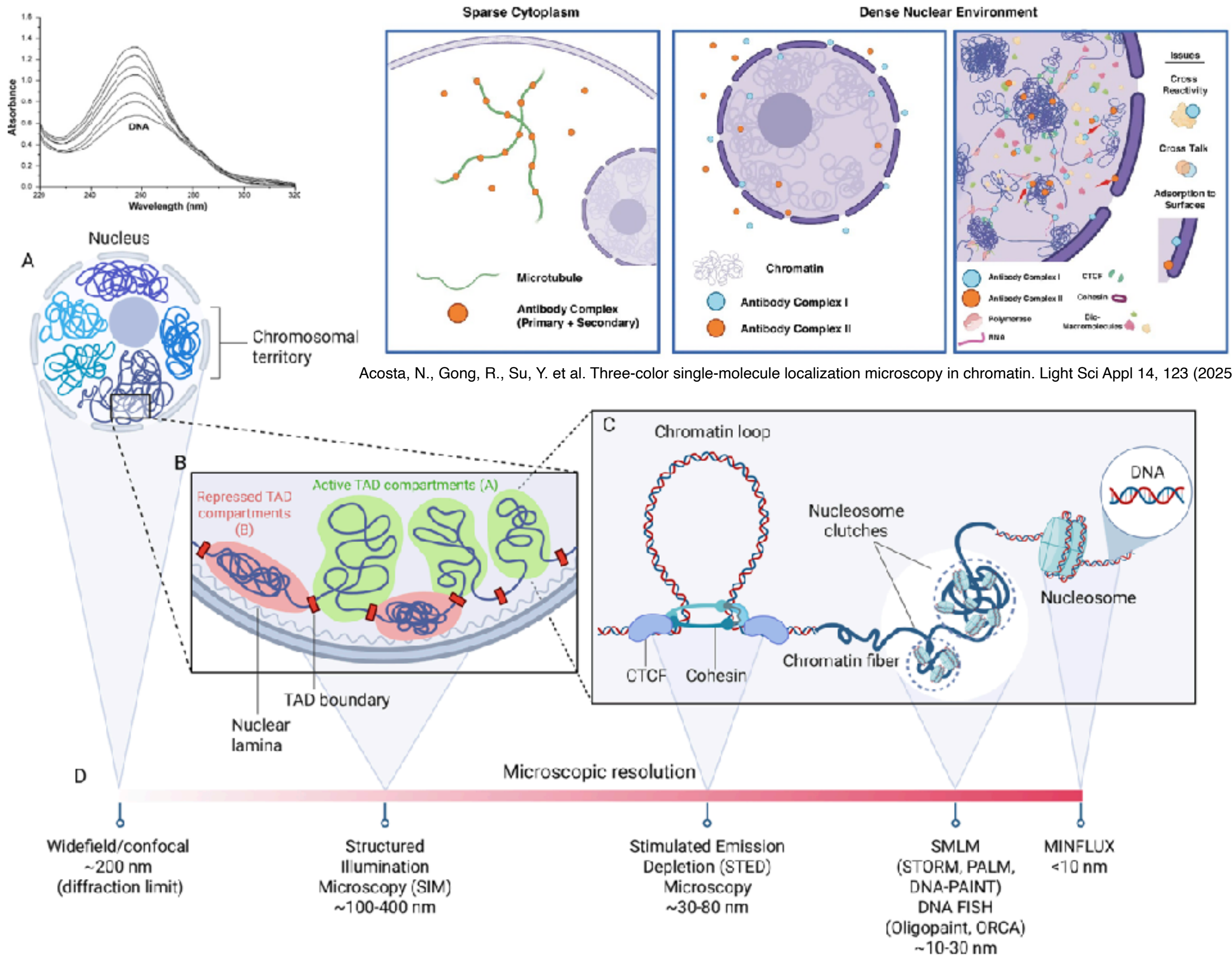


Kazuhiro Maeshima, Sachiko Tamura, Jeffrey C. Hansen, Yuji Itoh,
Fluid-like chromatin: Toward understanding the real chromatin organization present in the cell,
Current Opinion in Cell Biology, 2020.

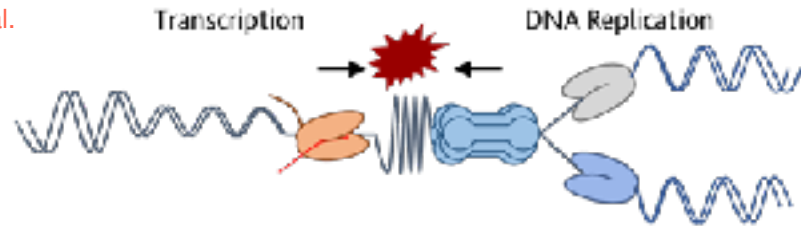


Ou, H.D., S. Phan, T.J. Deerinck, A. Thor, M.H. Ellisman, and C.C. O'Shea. ChromEMT: Visualizing 3D chromatin structure and compaction ninterphase and mitotic cells. Science, 2017



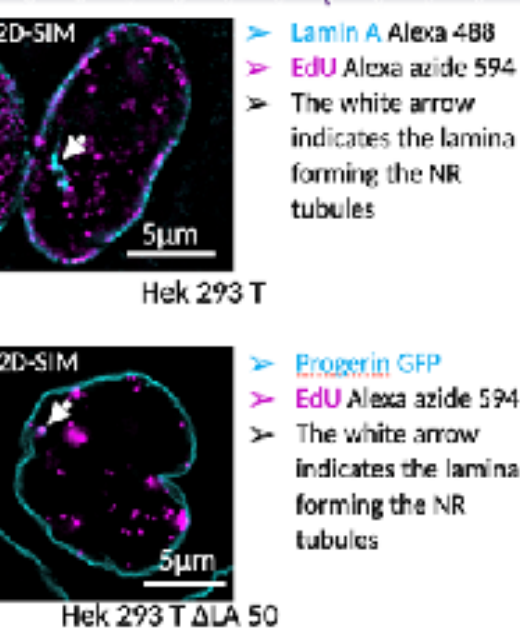
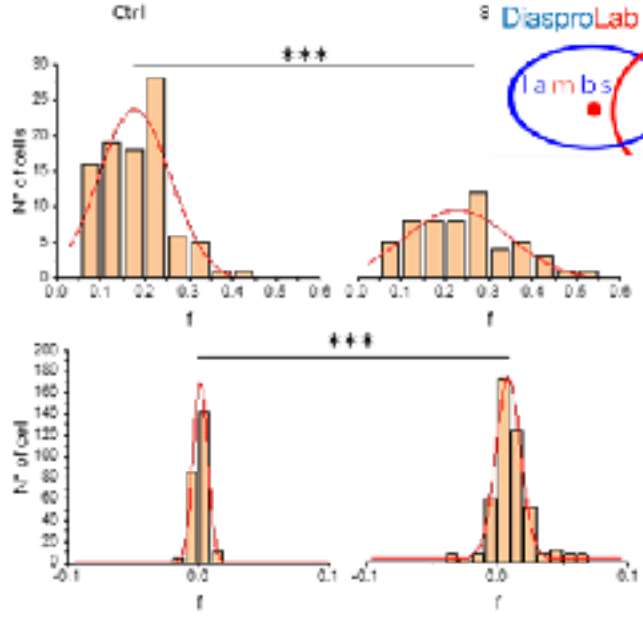
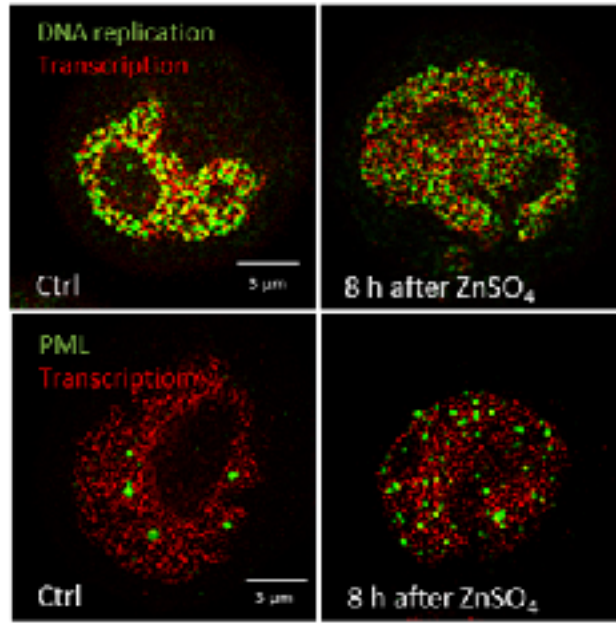
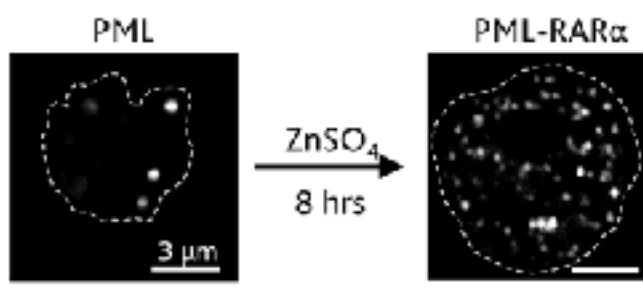
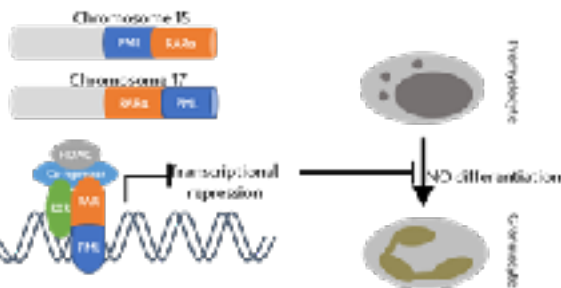


Acosta, N., Gong, R., Su, Y. et al. Three-color single-molecule localization microscopy in chromatin. *Light Sci Appl* 14, 123 (2025)

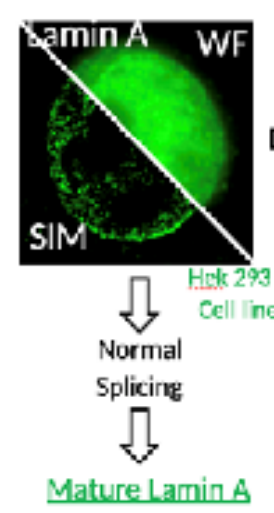


Oncogene model: U937-PR9 engineered cell line carrying the inducible fusion protein PML-RAR α

Acute promyelocytic leukemia

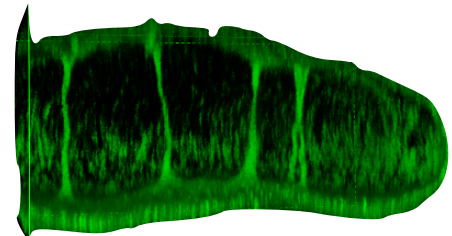


Normal LMNA gene



Physiological Phenotype

Healthy kid



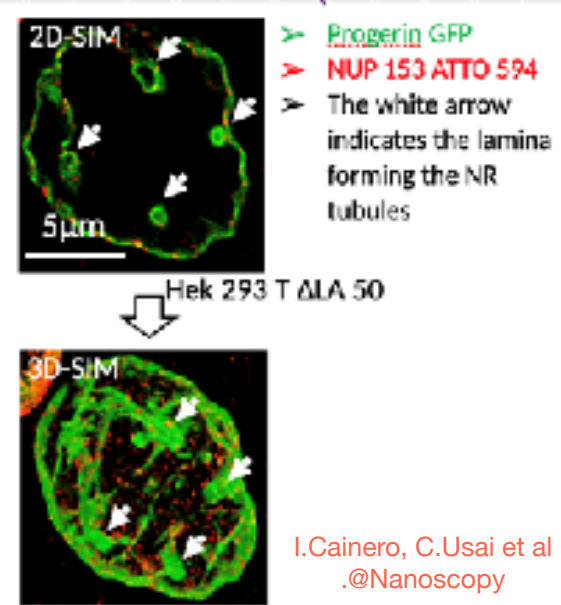
1 mutation Causes the production of 1 mutated protein

Mutant LMNA gene HGPS patient



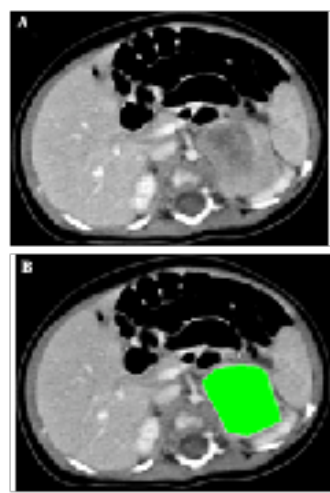
Pathological Phenotype

HGPS disease

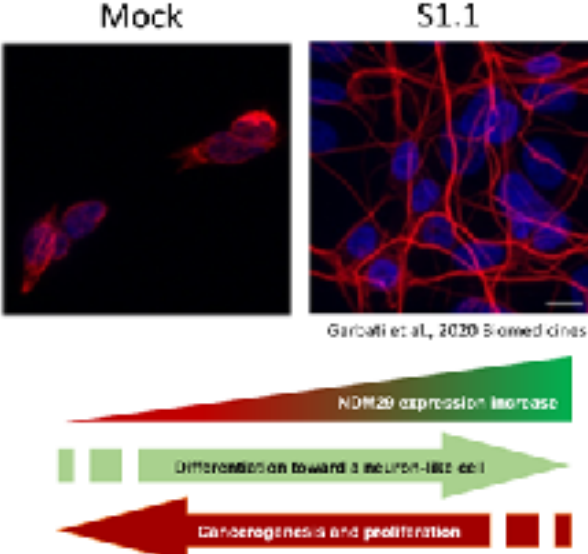


I.Cainero, C.Usai et al.
@Nanoscopy

NEUROBLASTOMA

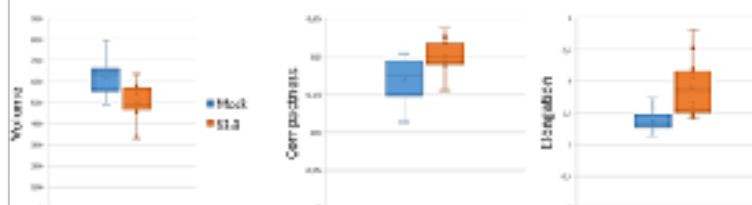
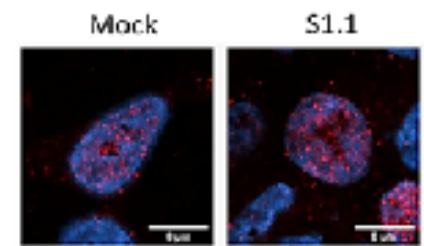


In vitro model of neuroblastoma

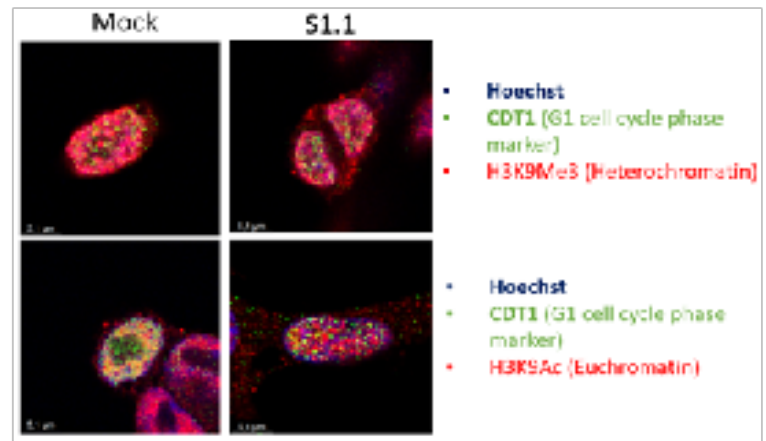


Confocal approach

→ Goal: Evaluation of the nuclear morphology of malignant (Mock) and neuron-like cells (S1.1)



Stimulated emission depletion (STED) microscopy



→ Goal: Analysis of the different spatial arrangements of domains rich in acetylated and methylated H3K9 and, therefore, in chromatin compaction



Diaspro Lab, set-up



Luciano Berio (Imperia, 24 ottobre 1925 – Roma, 27 maggio 2003)

NAXOS

Luciano BERIO

Sequenzas I-XIV

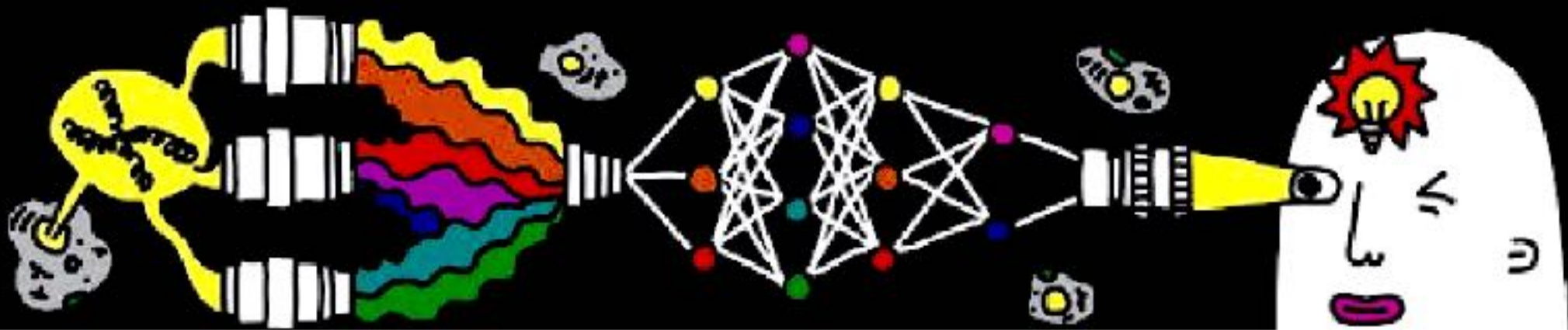
for Solo Instruments

Flute • Harp
Soprano • Piano
Trombone • Viola
Oboe • Violin
Clarinet • Trumpet
Guitar • Bassoon
Accordion • Cello
Saxophones
(1925-2003)



Luciano Berio (Imperia, 24 ottobre 1925 – Roma, 27 maggio 2003)





MOMIX

Multimodal Optical Microscopy Image Correlation Sensing



Super Resolution

"...for realistic, quantitative descriptions at nano-scale resolution of the dynamics of the complex, multidimensional molecular biological processes that define the phenotypes of all life forms."

Måns Ehrenberg Nobel Committee for Chemistry - Scientific Background on the Nobel Prize in Chemistry 2014

The Nobel Prize in Chemistry 2014



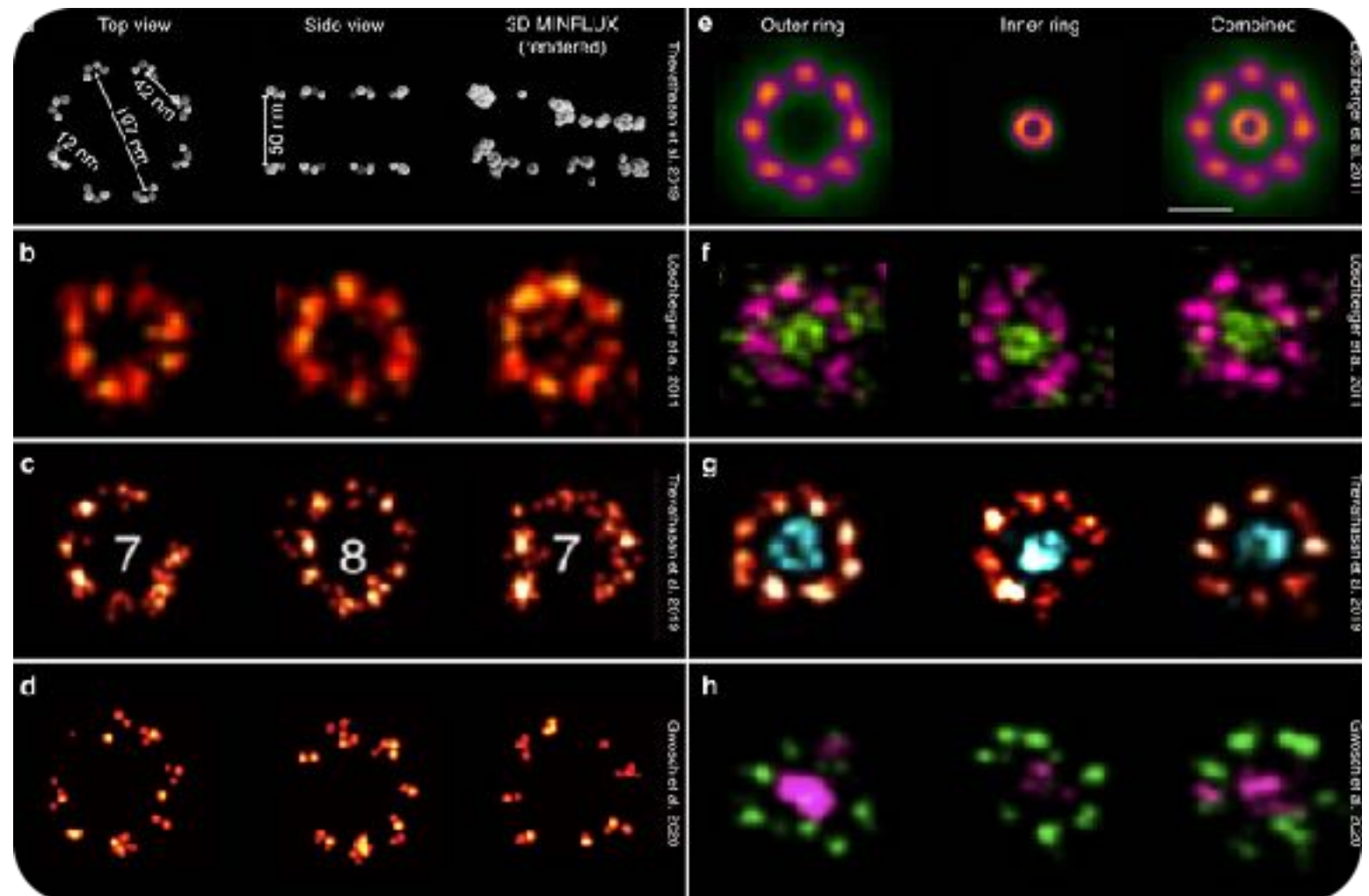
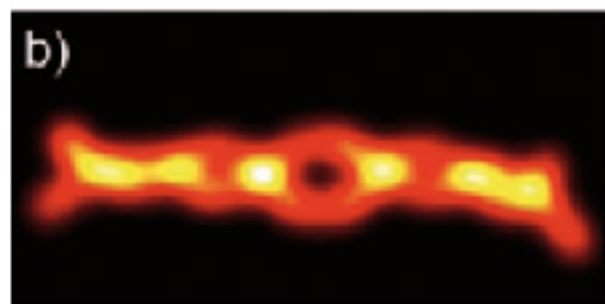
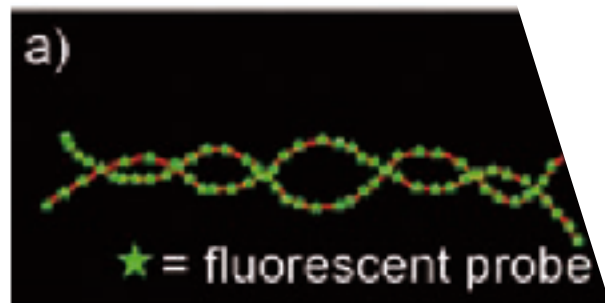
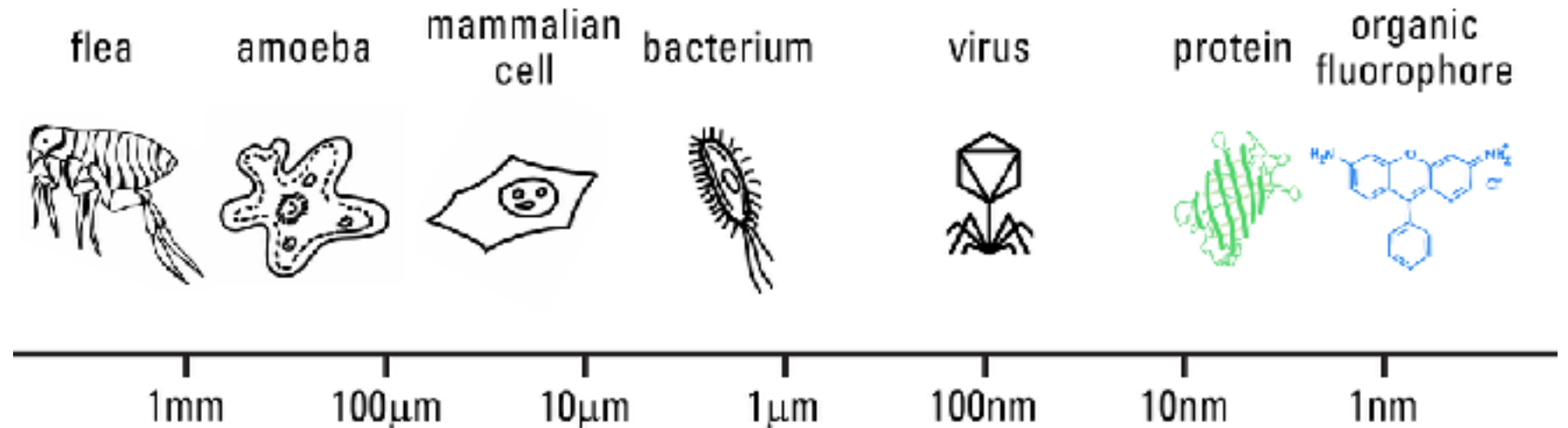
© Nobel Media AB. Photo: A. Mahmoud
Eric Betzig
Prize share 1/3



© Nobel Media AB. Photo: A. Mahmoud
Stefan W. Hell
Prize share 1/3

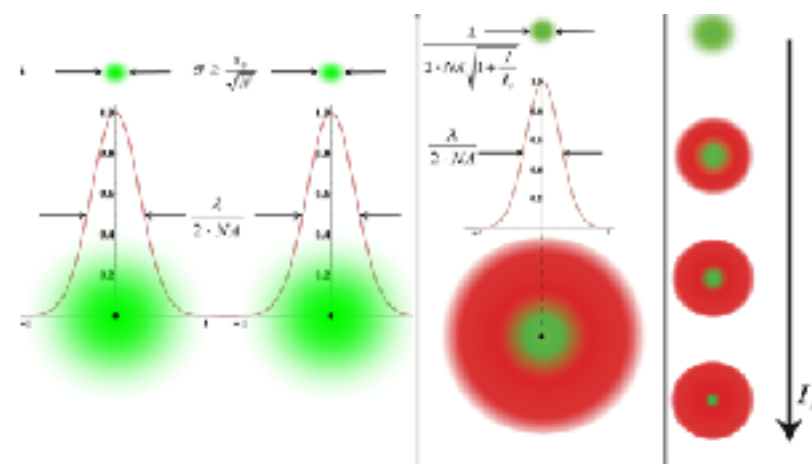
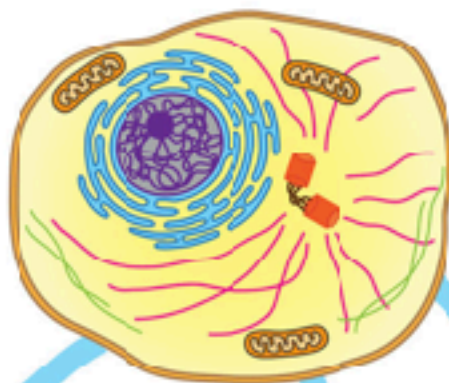


© Nobel Media AB. Photo: A. Mahmoud
William E. Moerner
Prize share 1/3





2014

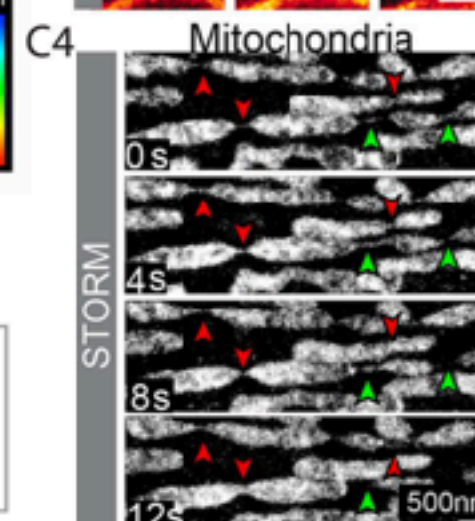
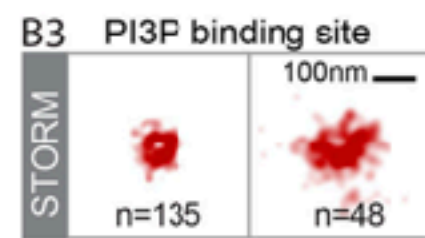
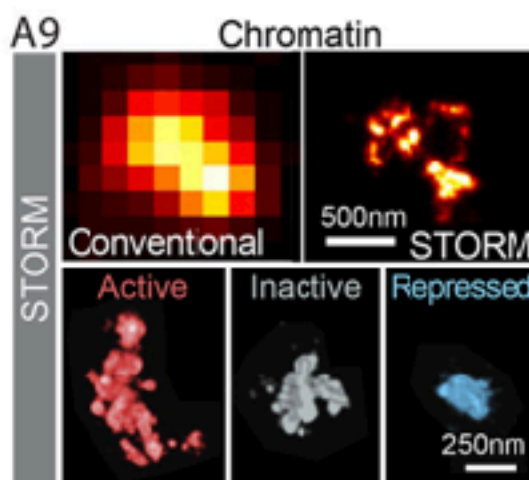
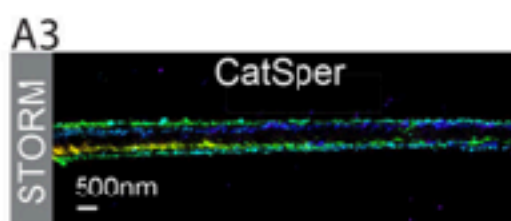
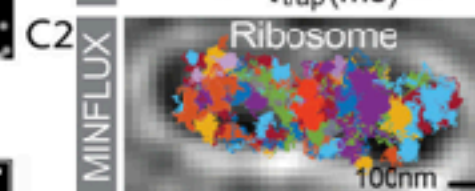
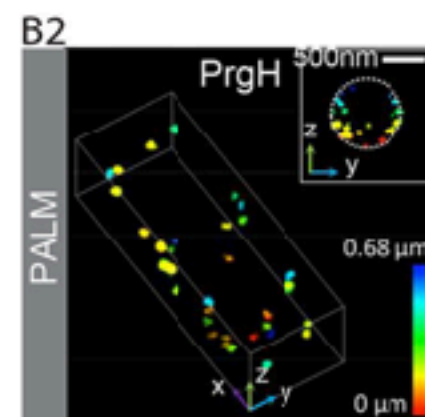
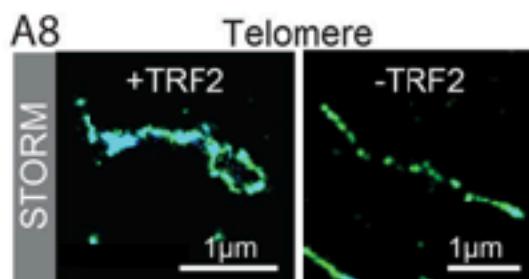
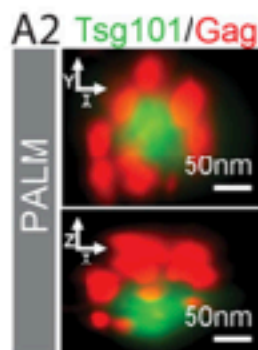
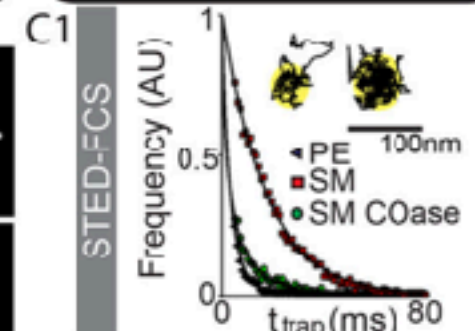
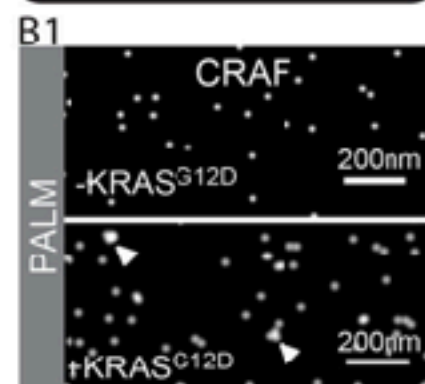
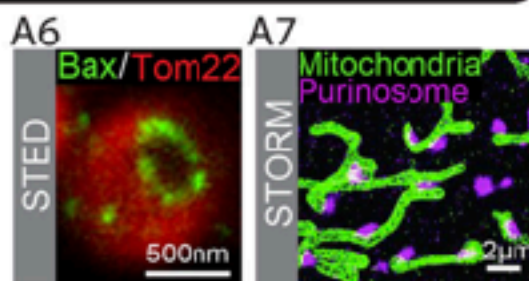
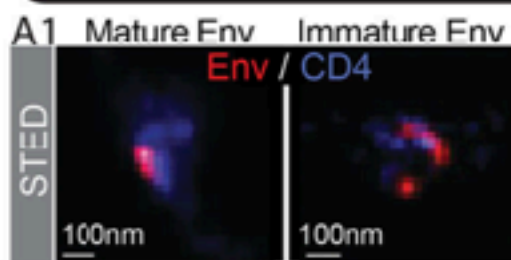


Super-resolution imaging

Spatial organization / Molecular interaction

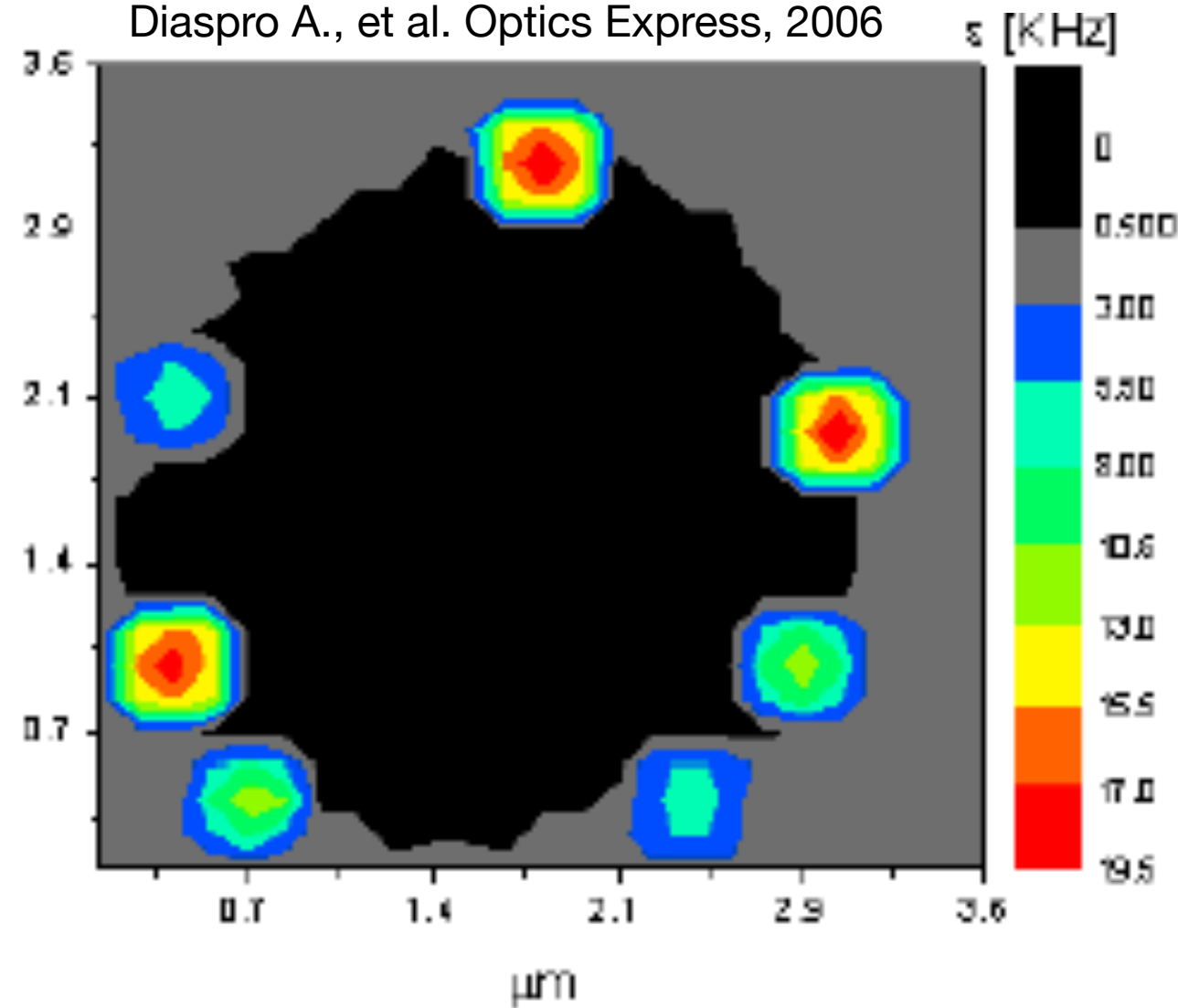
Stoichiometry / Molecular counting

Temporal dynamics



Single-molecule - Super resolution

Diaspro A., et al. Optics Express, 2006



1345

Biophysical Journal Volume 89 August 2005 1345-1352

Two-Photon Activation and Excitation Properties of PA-GFP in the 720–920-nm Region

Marc Schneider,* Sara Barozzi,[†] Ilaria Testa,^{*,‡} Mario Faretta,[‡] and Alberto Diaspro^{*,§}

^{*}INFM Genoa, Department of Physics, University of Genoa, I-10146 Genoa, Italy; [†]European Institute of Oncology, Department of Experimental Oncology, 20141 Milan, Italy; [‡]LAMBS-MicroScBio Research Center, University of Genoa, 15146 Genoa, Italy; and [§]IFOM, Istituto FIRC di Oncologia Molecolare, 20139 Milan, Italy

INSTITUTE OF PHYSICS PUBLISHING

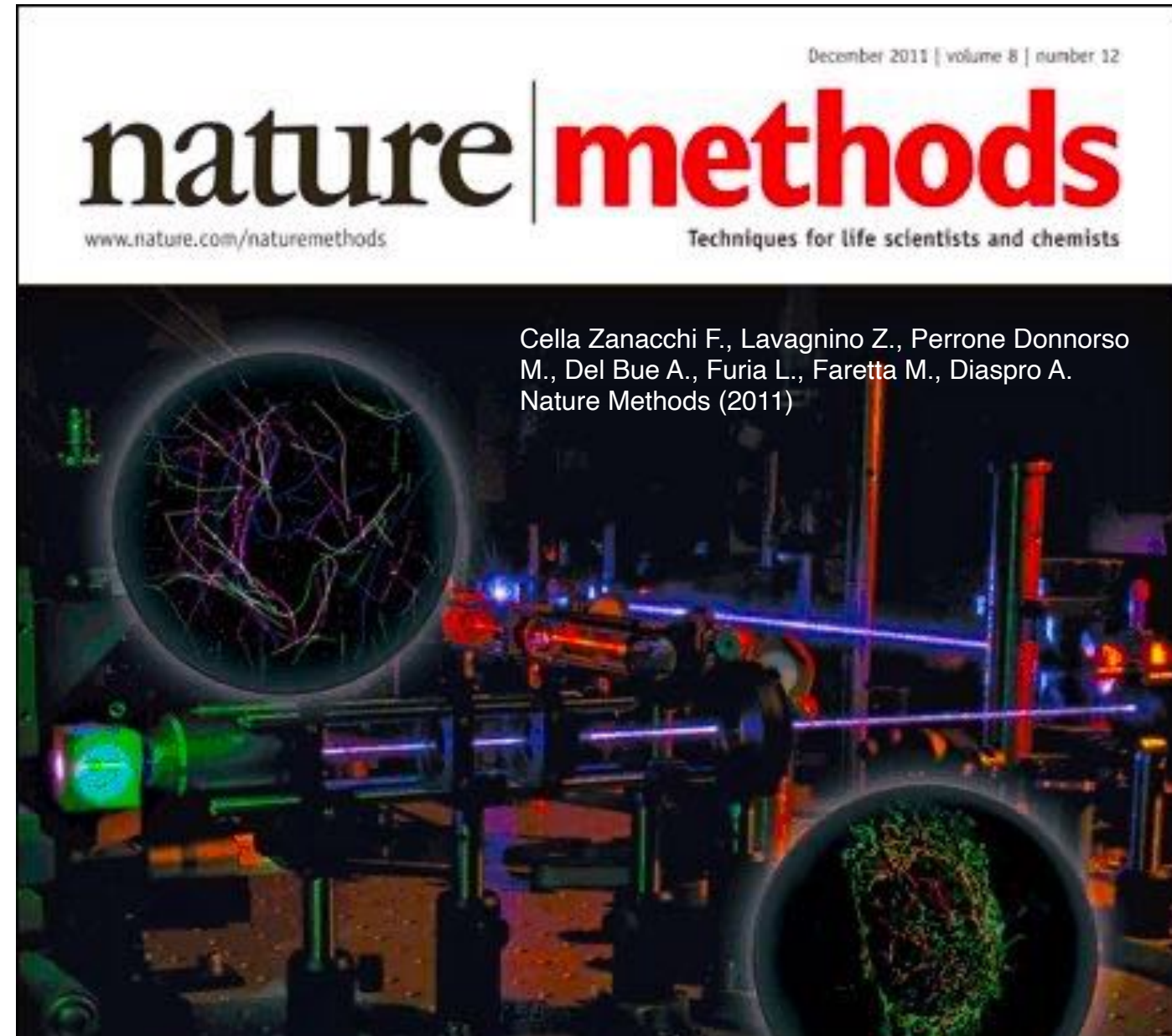
J. Phys. D: Appl. Phys. 36 (2003) 1682-1688

JOURNAL OF PHYSICS D: APPLIED PHYSICS

PII: S0022-3727(03)55973-3

Single molecule photodynamics by means of one- and two-photon approach

Giuseppe Chirico^{1,3}, Fabio Cannone¹ and Alberto Diaspro²



Cella Zanacchi F., Lavagnino Z., Perrone Donnorso M., Del Bue A., Furia L., Faretta M., Diaspro A.
Nature Methods (2011)

Single-wavelength two-photon excitation–stimulated emission depletion (SW2PE-STED) superresolution imaging

Paolo Bianchini¹, Benjamin Harke², Silvia Galiani^{*,h}, Giuseppe Vicidomini¹, and Alberto Diaspro^{*,h,1}



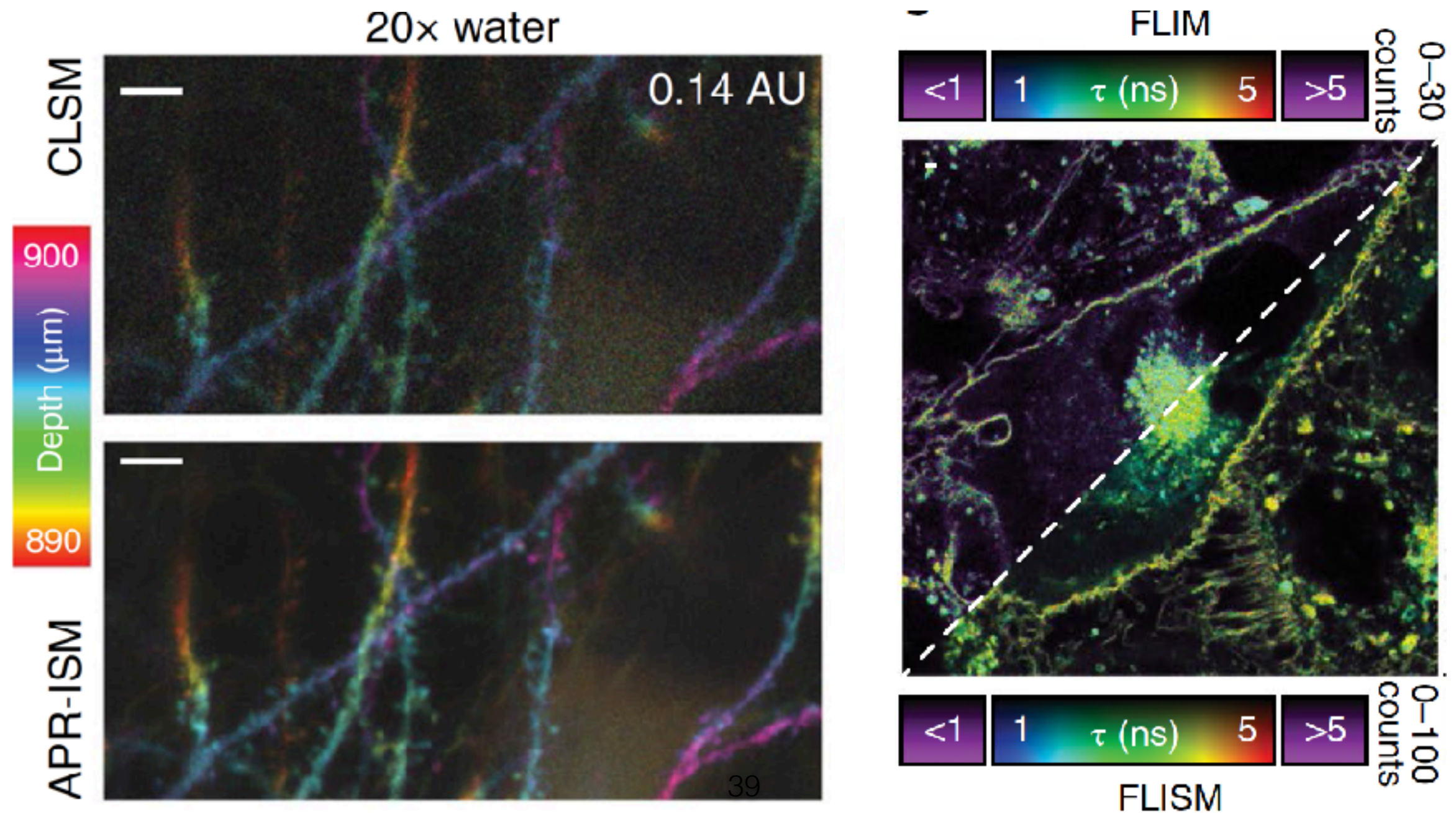
STED super-resolved microscopy

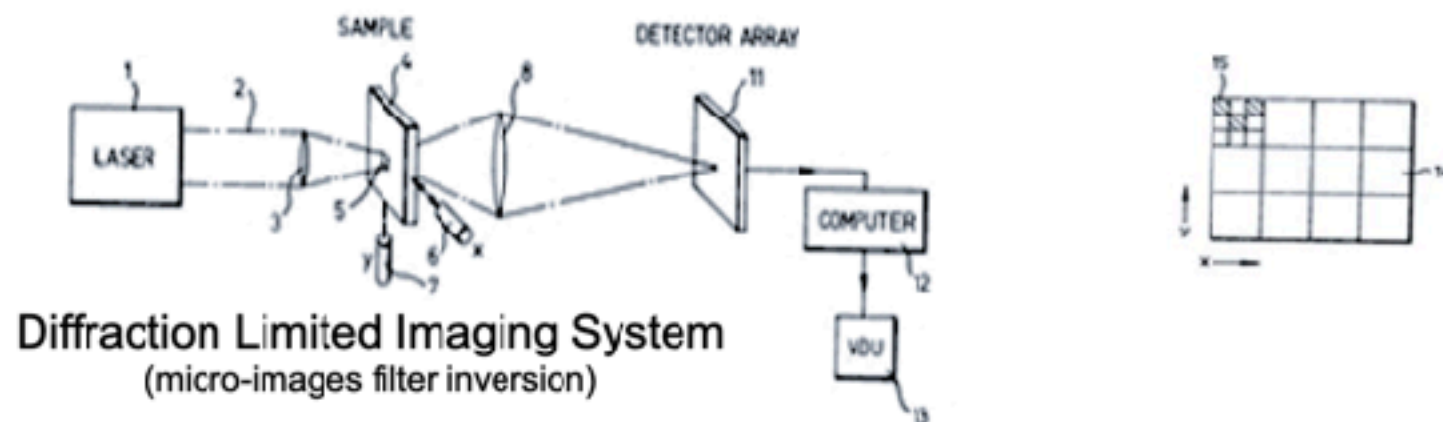
Giuseppe Vicidomini¹, Paolo Bianchini² & Alberto Diaspro^{2,3}

PUBLISHED ONLINE 29 JANUARY 2012; DOI:10.1038/NMETH.14503 NATURE METHODS | ADVANCE ONLINE PUBLICATION |

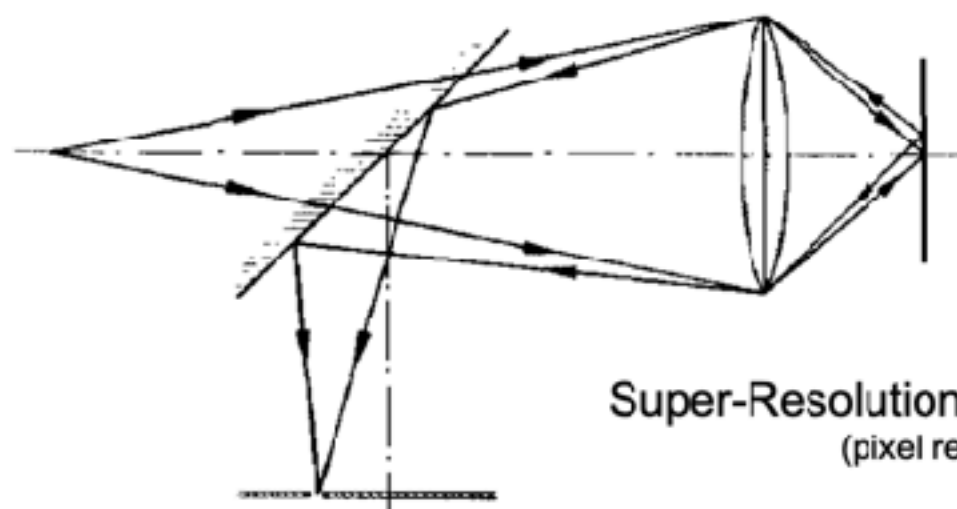
M.Castello et al. (2019)

A robust and versatile platform for image scanning microscopy enabling super-resolution FLIM





Bertero M., Pike E.R., US4549204 A, (1981)



Sheppard C.J.R., Optik, 80(2):53-54 (1988)

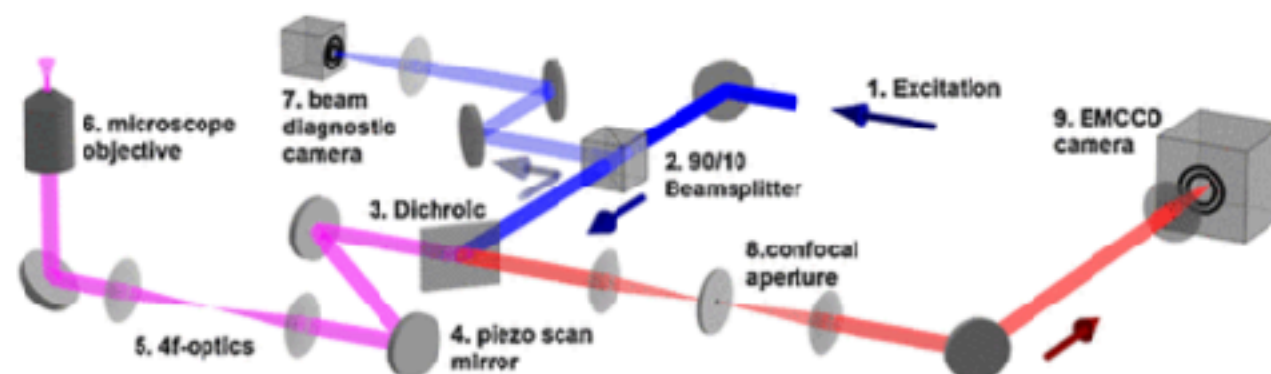
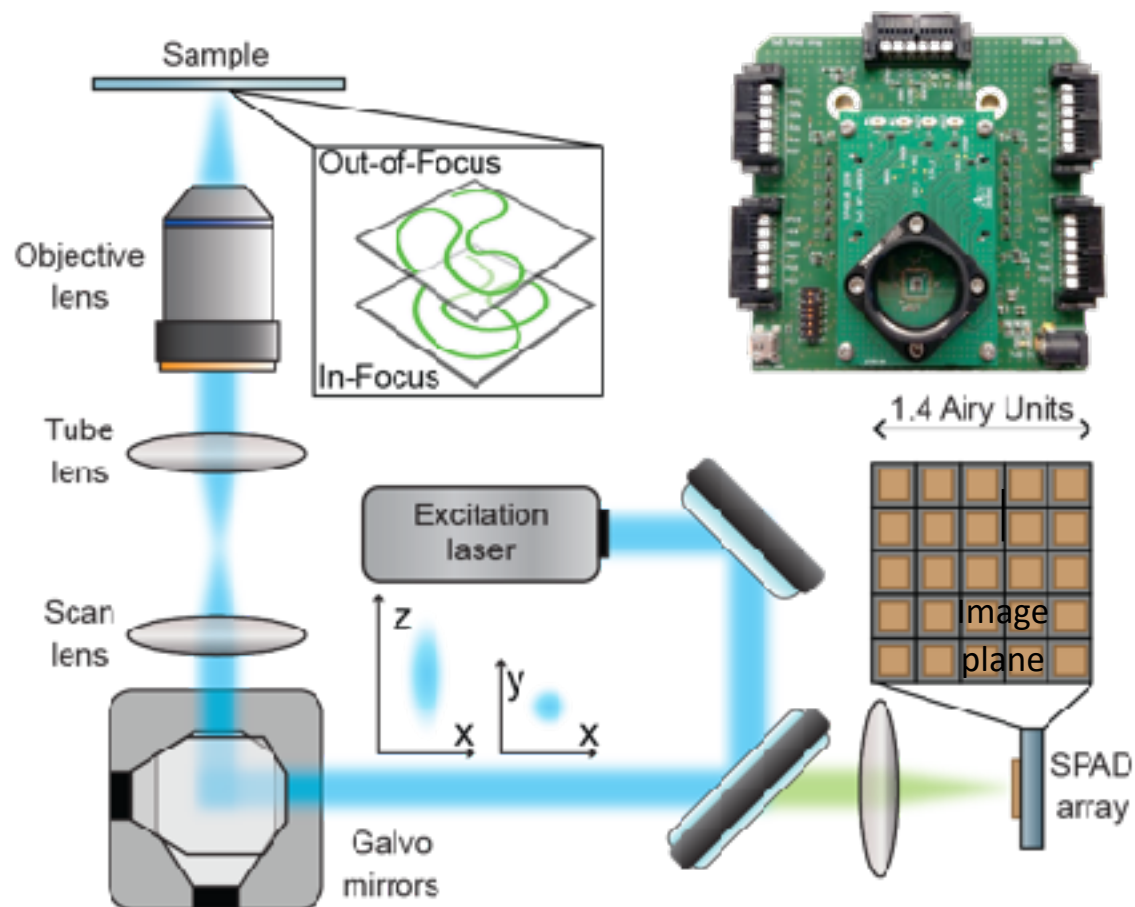
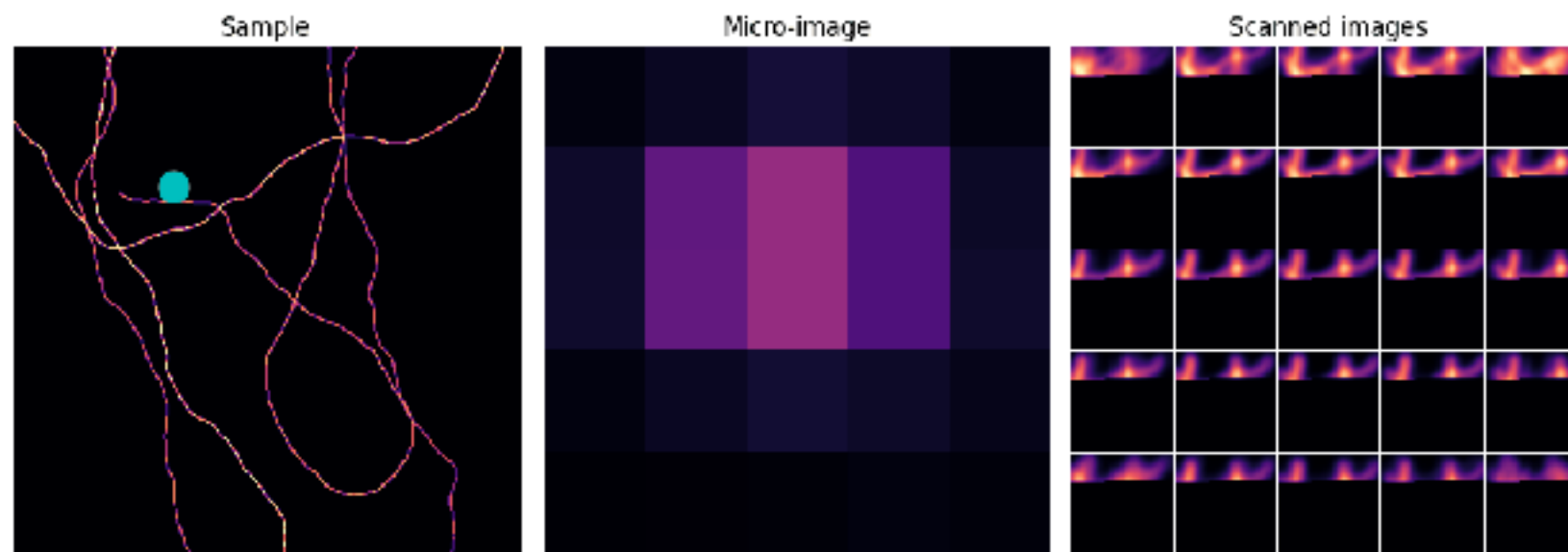


Image Scanning Microscopy (ISM)

Muller C.B., Enderlein J., Phys. Rev. Lett., 104:108101 (2010)



The resolution/SNR trade-off is overcome with a single photon avalanche diode (SPAD) array detector.

Detector arrays transformed CLSM into ISM: a practical **super-resolution** technique

- Every pixel (0.28 AU) acts as a small pinhole
→ **high resolution**
- A large detector ensures a good collection efficiency → **high SNR**

- Marco Castello, Colin J. R. Sheppard, Alberto Diaspro, and Giuseppe Vicidomini, "Image scanning microscopy with a quadrant detector," Opt. Lett. 40, 5355-5358 (2015)

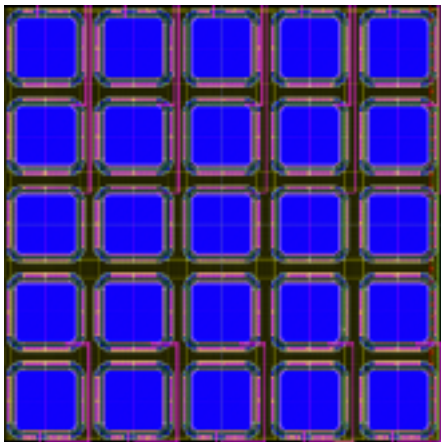
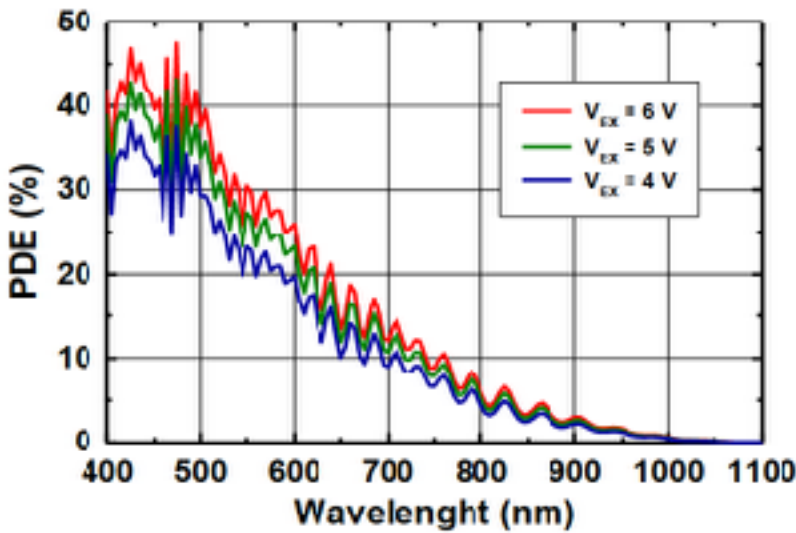
- Sheppard CJR, Castello M, Tortarolo G, Zunino A, Slenders E, Bianchini P, Vicidomini G, Diaspro A. Background Rejection in Two-Photon Fluorescence Image Scanning Microscopy. Photonics. 2023;10(5):601.

- Tortarolo G, Zunino A, Fersini F, Castello M, Piazza S, Sheppard CJR, Bianchini P, Diaspro A, Koho S, Vicidomini G. Focus image scanning microscopy for sharp and gentle super-resolved microscopy. Nat Commun. 2022;13(1):7723.

the first detector array specifically designed for microscopy



Time jitter 120/150 ps



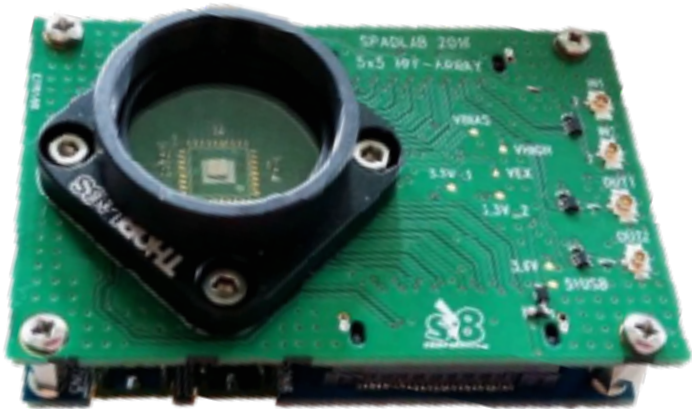
features:



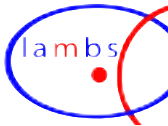
- ◆ 5 x 5 matrix
- ◆ 75 μm pitch
- ◆ each detector: 50 μm x 50 μm active area
- ◆ fill factor = $\frac{50^2}{75^2} \approx 44\%$ (future improvement: micro lenses array)
- ◆ 25 TTL-like signals, up to 20 MHz
- ◆ PDE up to 45%
- ◆ dark count <100 cps @25°C

Hold-OFF Time	Afterpulsing probability
50 ns	6,5%
100 ns	2,4%
200 ns	1,4%

SPADs	Crosstalk probability
First neighbors - orthogonal	< 1%
First neighbors - diagonal	< 0,2%



Marco Castello, Colin J. R. Sheppard, Alberto Diaspro, and Giuseppe Vicidomini, "Image scanning microscopy with a quadrant detector," Opt. Lett. 40, 5355-5358 (2015)
Mauro Buttafava, et al. SPAD-based asynchronous-readout array detectors for image scanning microscopy. Optica, 2020



製品情報 > 共焦点顕微鏡 >

AX / AX R

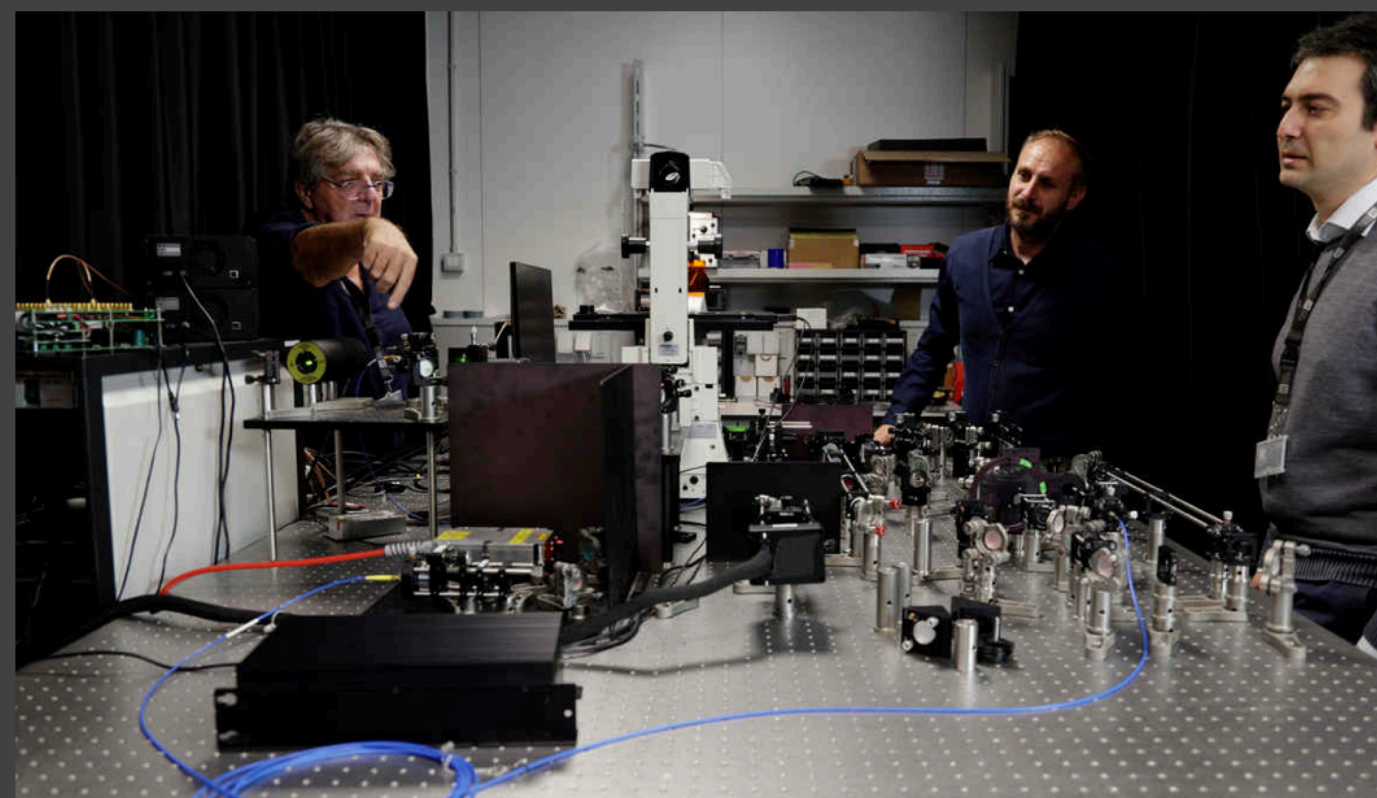
超解像共焦点レーザー顕微鏡システム

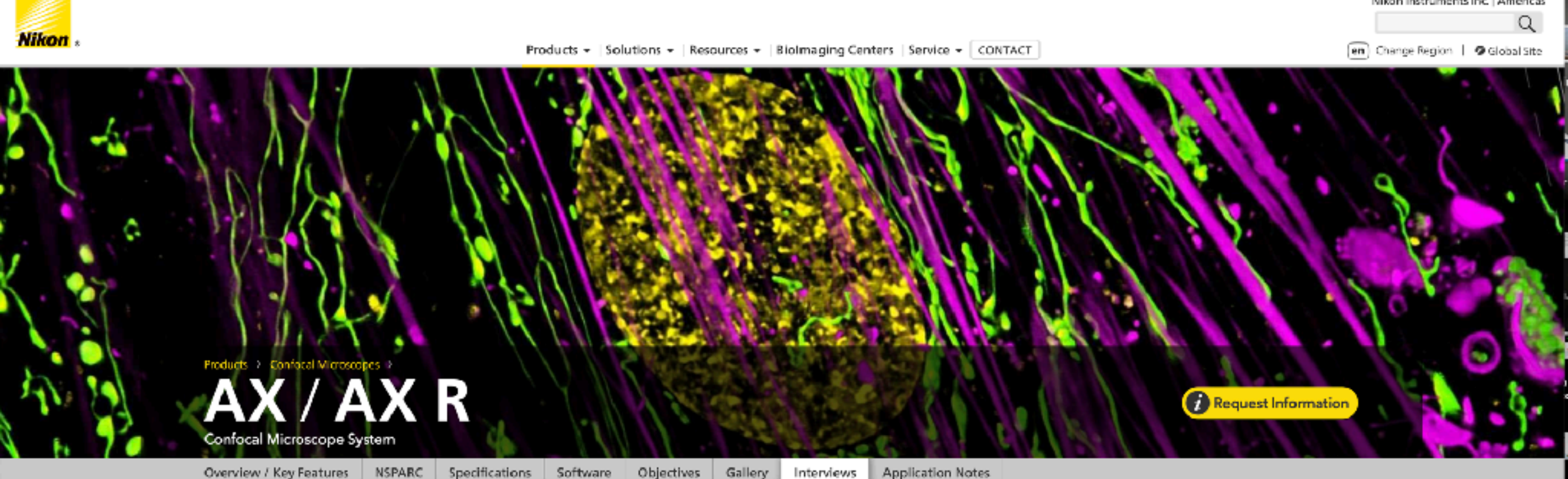
製品に関するお問い合わせ

概要・特長・機能 NSPARC 仕様 ソフトウェア 対物レンズ 作例画像集 インタビュー アプリケーションノート

開発・企画インタビュー 超解像共焦点レーザー顕微鏡システムAX/AX R with NSPARC。 その開発はイタリア技術研究所から

NSPARCの源流は、IIT（イタリア技術研究所）にあります。IIT内にある、ニコンイメージングセンター（NIC@IIT）、分子顕微鏡法および分光法ラボ（MMS）で研究・開発に取り組まれた、アルベルト・ディアスプロ教授、パオロ・ビアンキーニ博士、ジュゼッペ・ヴィチドミーニ博士にお話を伺いました。

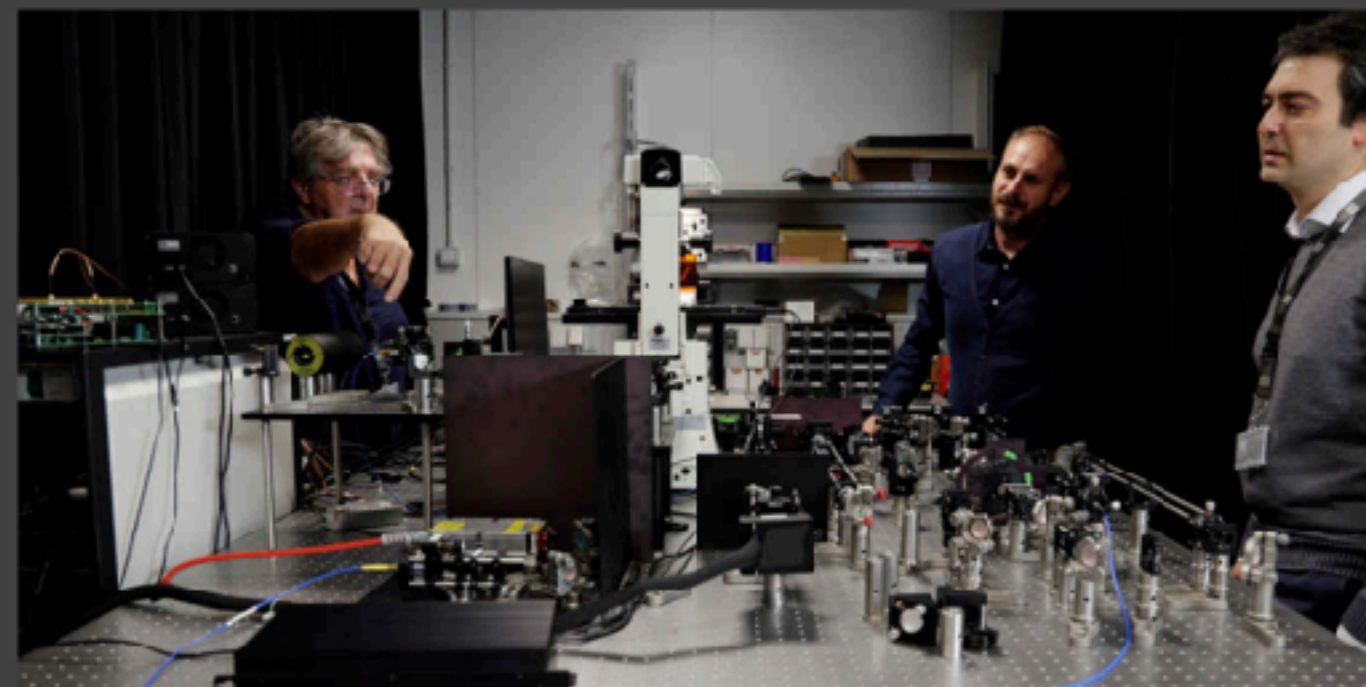




An Inside Look

The confocal-based super-resolution microscope AX/AX R with NSPARC. This development started at the Istituto Italiano di Tecnologia.

The origin of NSPARC is in the IIT (Italian Institute of Technology). We interviewed Professor Alberto Diaspro, Dr. Paolo Bianchini, and Dr. Giuseppe Vicidomini, who worked on research and development at the Nikon Imaging Center (NIC@IIT) and the Molecular Microscopy and Spectroscopy (MMS) Lab in the IIT.



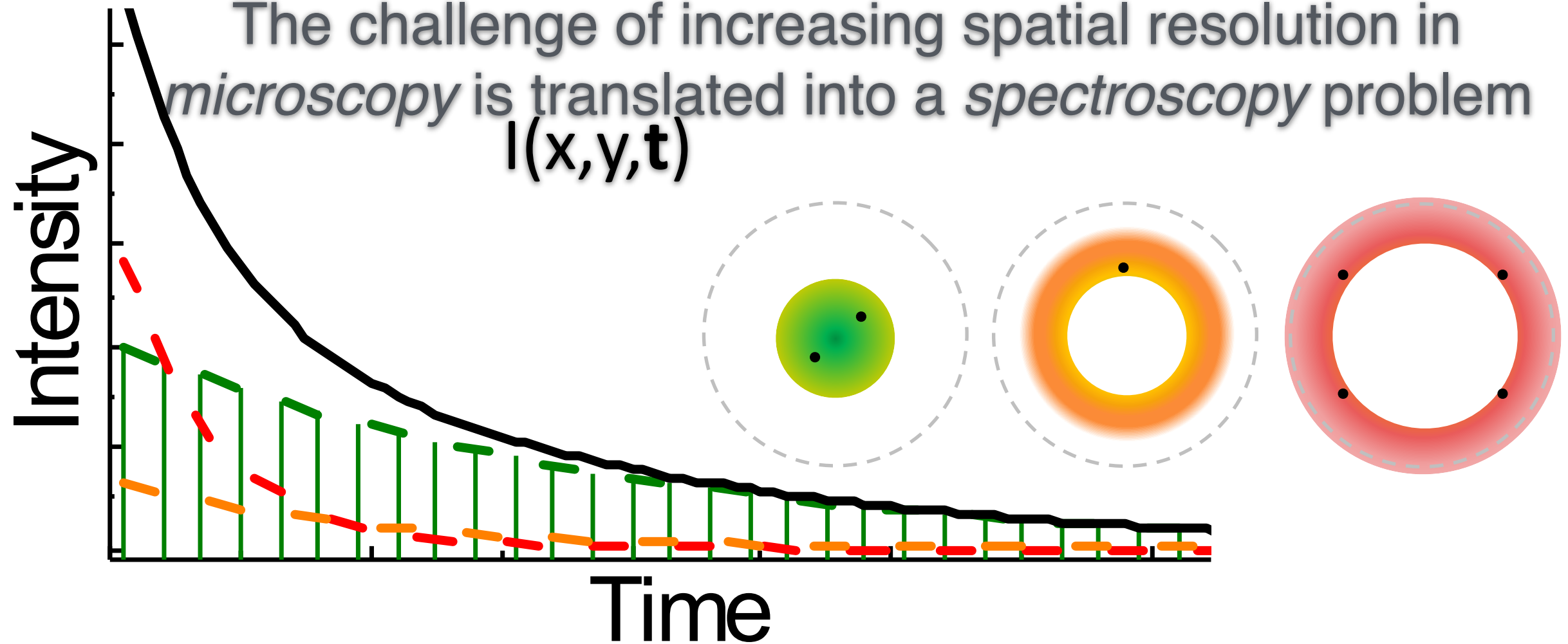
ENCODING TIME - DECODING SPACE

L.Lanzanò, I. Coto Hernandez, M.Castello, E.Gratton, A.Diaspro, G.Vicidomini, **Nature Comm.(2015)** -

Decoding of hidden spatial information

The challenge of increasing spatial resolution in *microscopy* is translated into a *spectroscopy* problem

$$I(x,y,t)$$

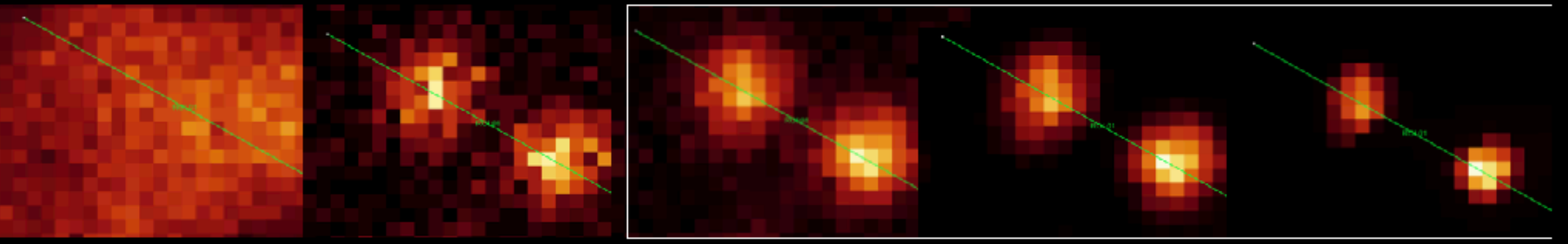


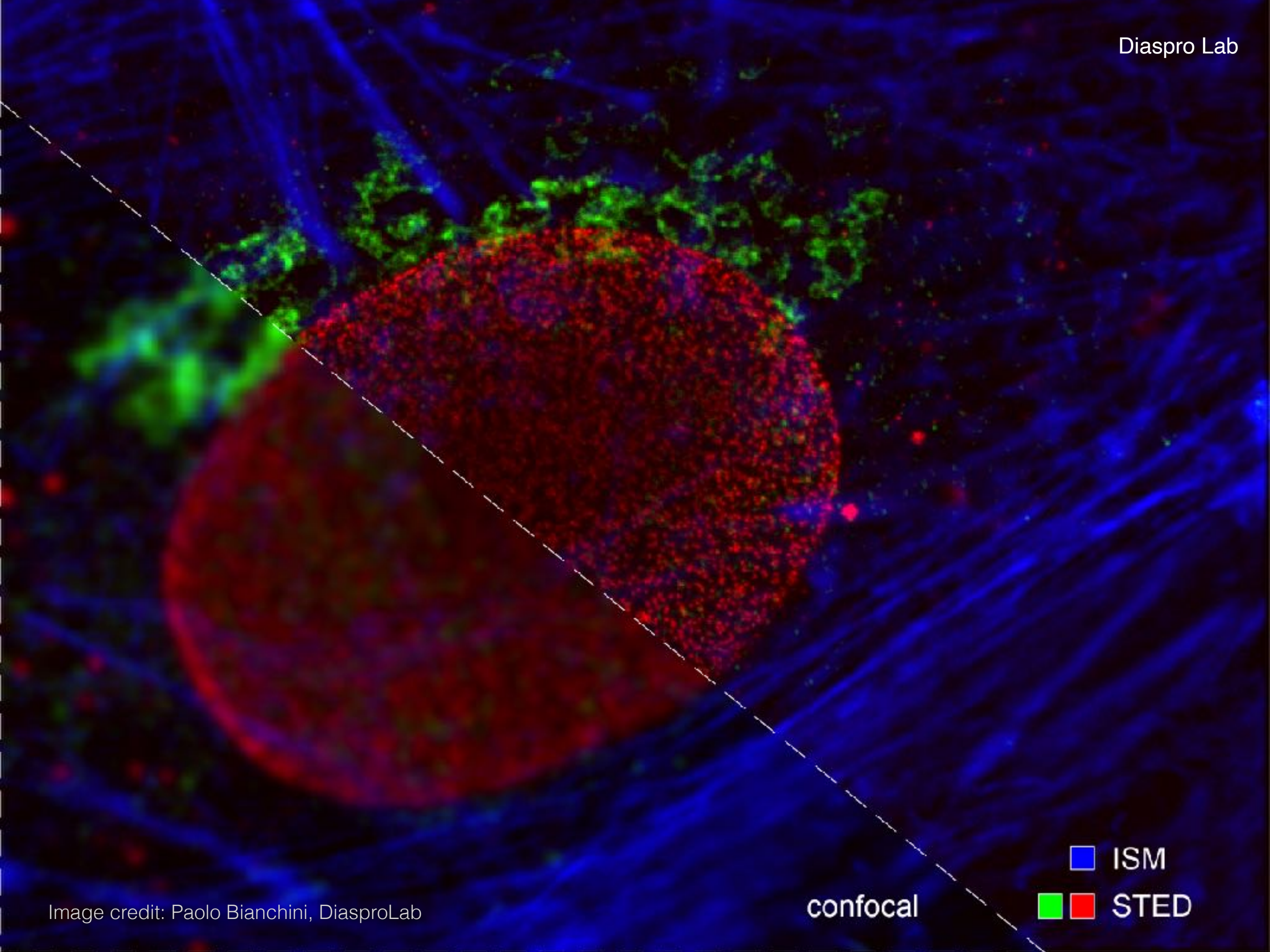
Confocal

without τ -STED

τ -STED

τ -tunable resolution

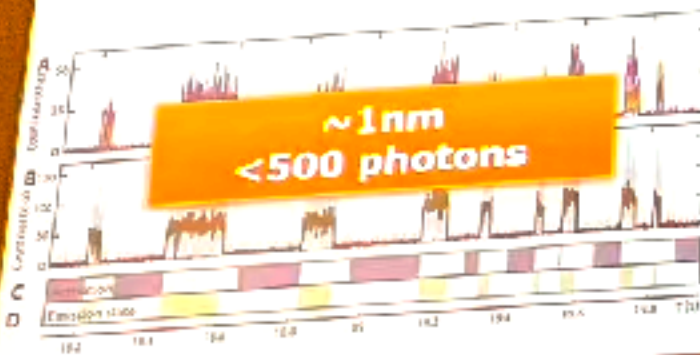
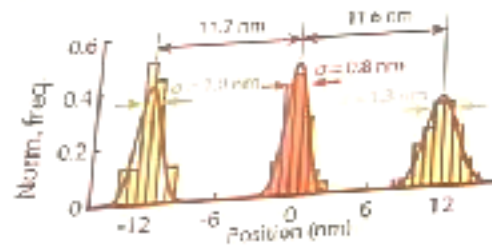
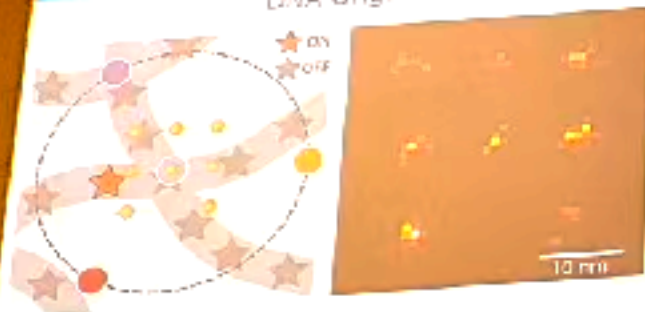




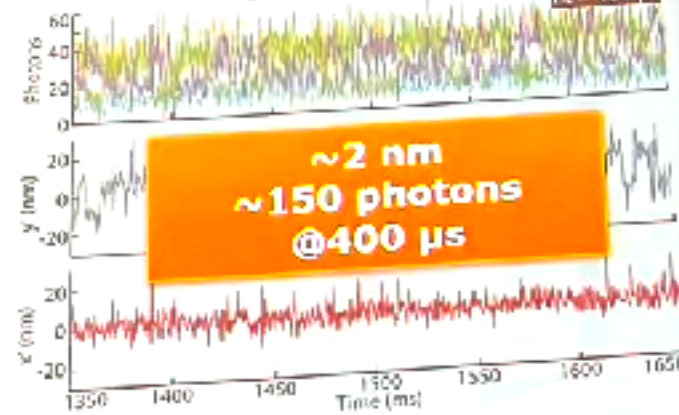
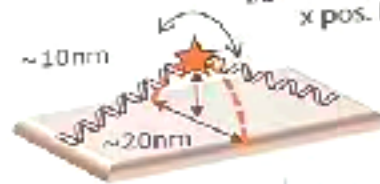
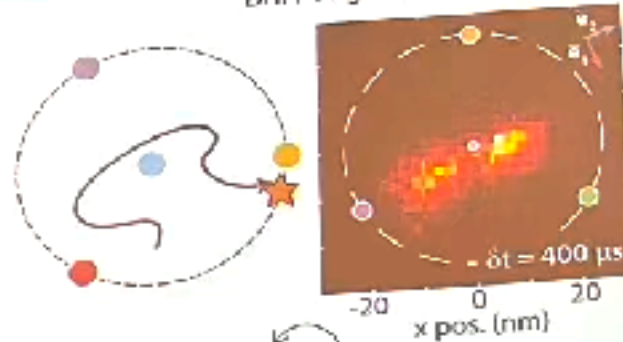
MINIFLUX APPLICATIONS TRACKING ~nm RANGE

NANOSCOPY

DNA Origami



DNA Origami

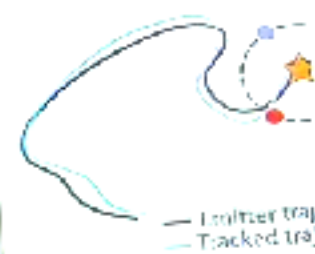


TRACKING ~nm RANGE

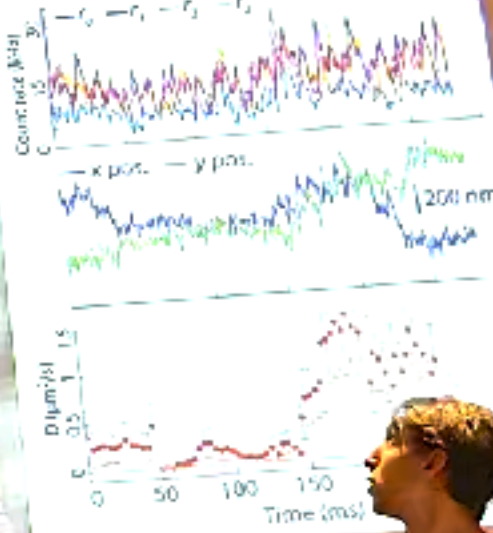
Ribosomal subunit proteins



Johan Elf
Uppsala University



Localizations	0-15	100K	747
Time resolution	10-50 ms	1.25 μs	
Tracking error	20-60 nm	50 nm	



@Balzarotti

Y. Elf, et. al. PNAS, 115, 6117-6122 (2018)

F. Balzarotti, et. al. Science, 355(6325), 606-612 (2017)

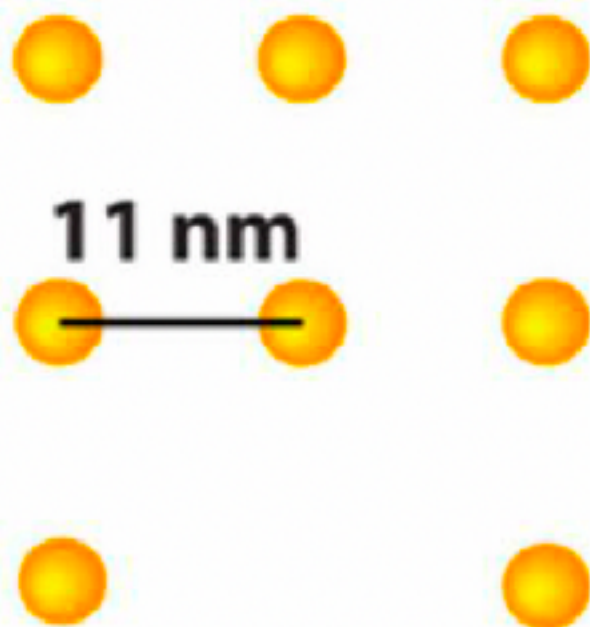
F. Balzarotti, Y. Eilers, K. C. Gwosch, A. H. Gynnå, V. Westphal, F. D. Stefani, J. Elf, S. W. Hell,
Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. Science (2017).

MINFLUX Nanoscopy: A “Brilliant” Technique Promising Major Breakthrough

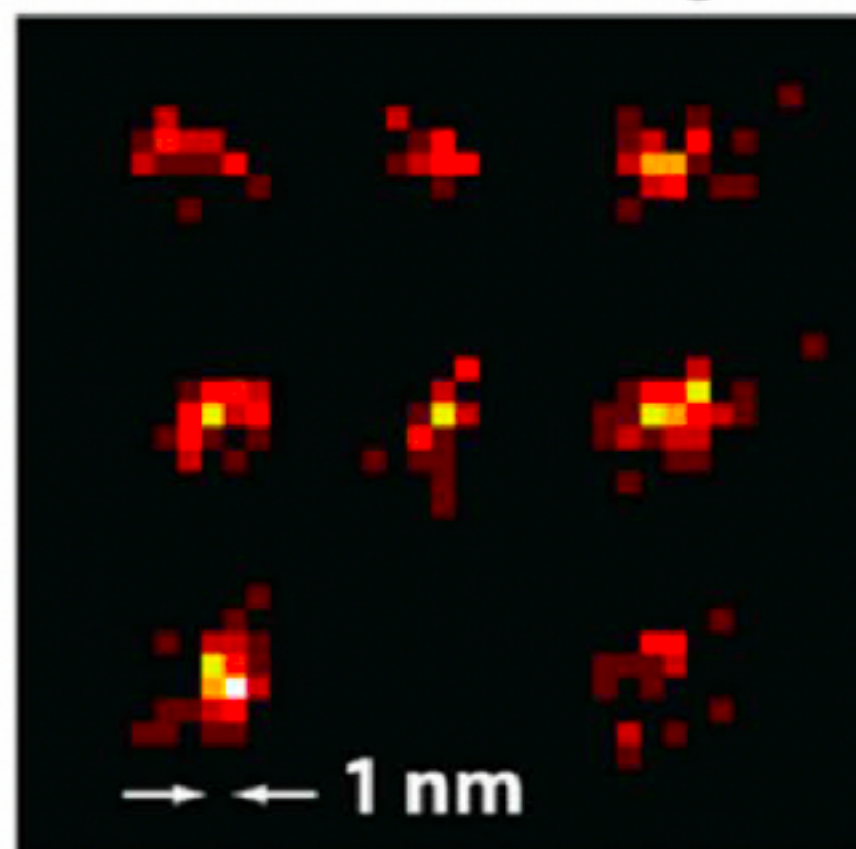
Marco Salerno , Virginia Bazzurro, Elena Angeli, Paolo Bianchini, Mohammadmehdi Roushenas, Kimiya Pakravanan, Alberto Diaspro 

First published: 21 January 2025 | <https://doi.org/10.1002/jemt.24765>

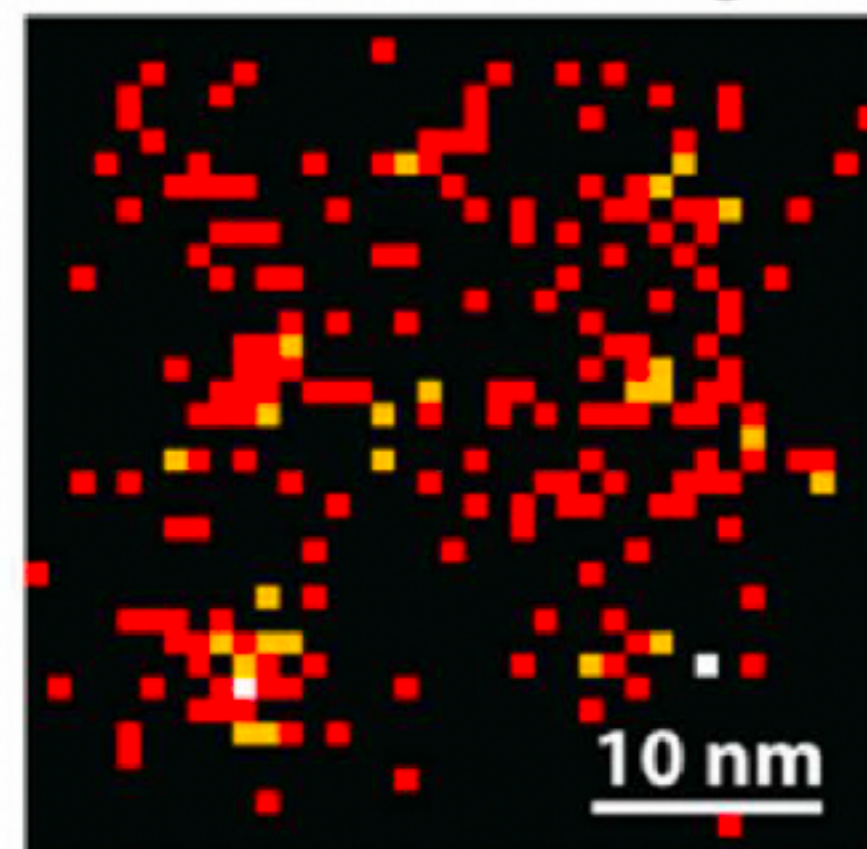
Object
fluorescent molecules

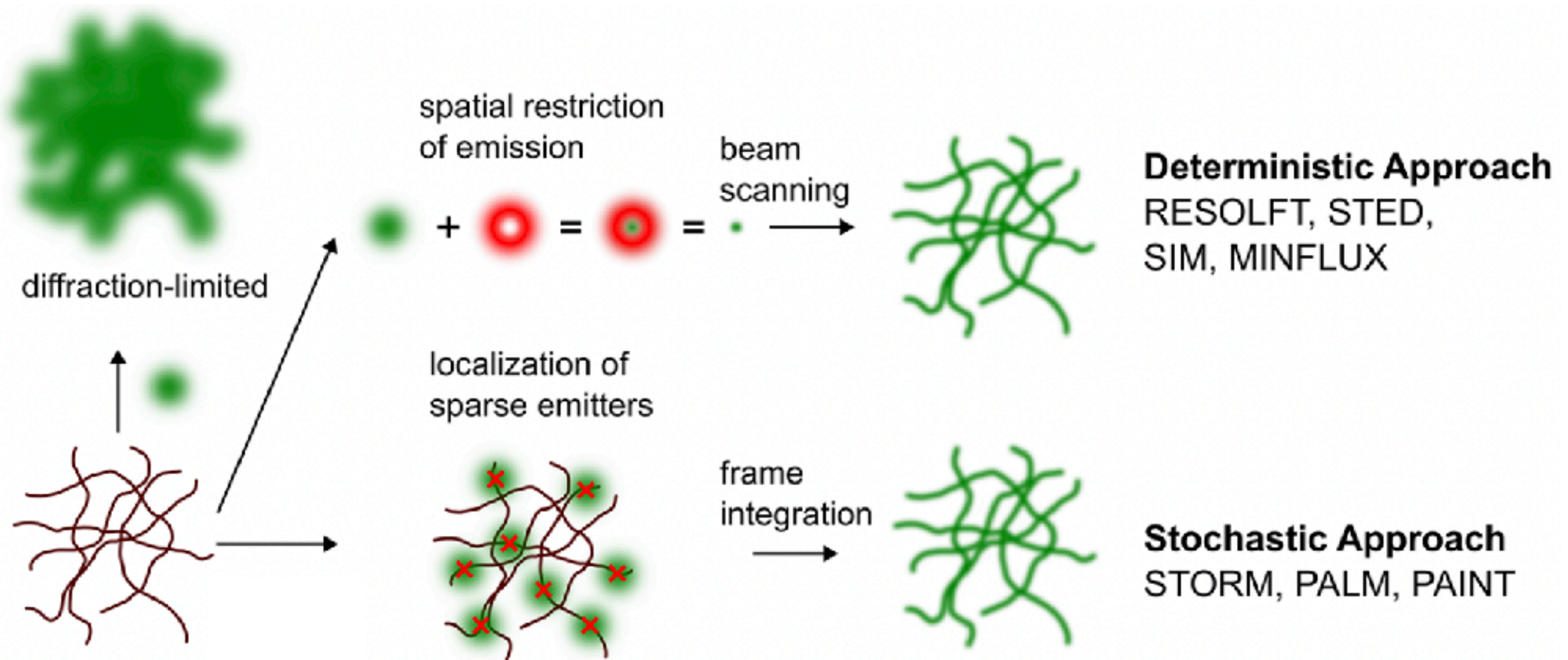


MINFLUX image

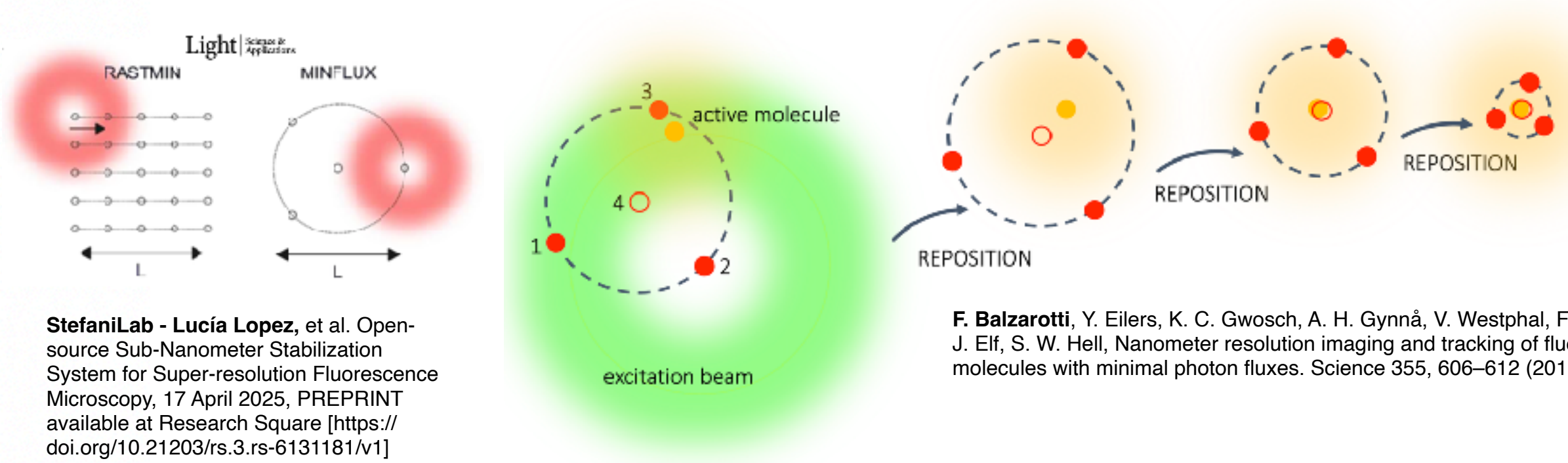


PALM/STORM image





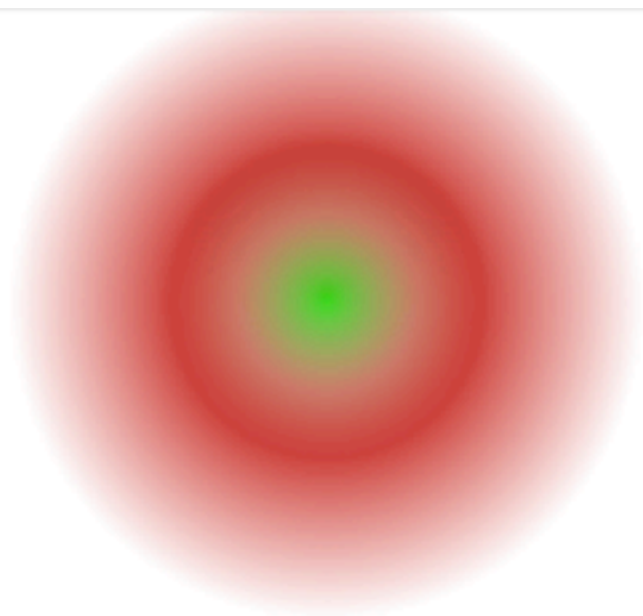
D. Palounek, et al. ACS Photonics 2024, 11, 3907–3921



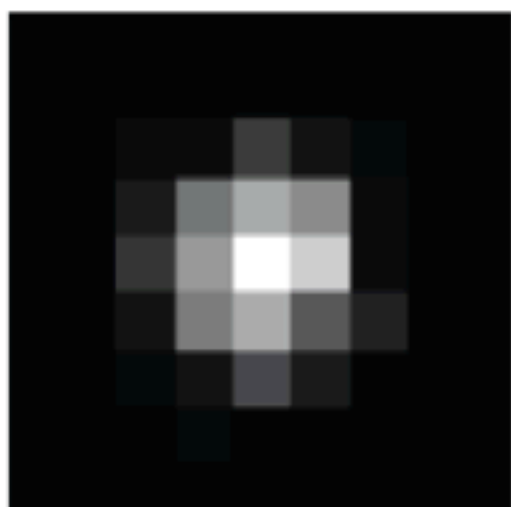
F. Balzarotti, Y. Eilers, K. C. Gwosch, A. H. Gynnå, V. Westphal, F. D. Stefani, J. Elf, S. W. Hell, Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. Science 355, 606–612 (2017).

A.Diaspro, P.Bianchini (2020) Optical Nanoscopy, Riv. Nuovo Cim.

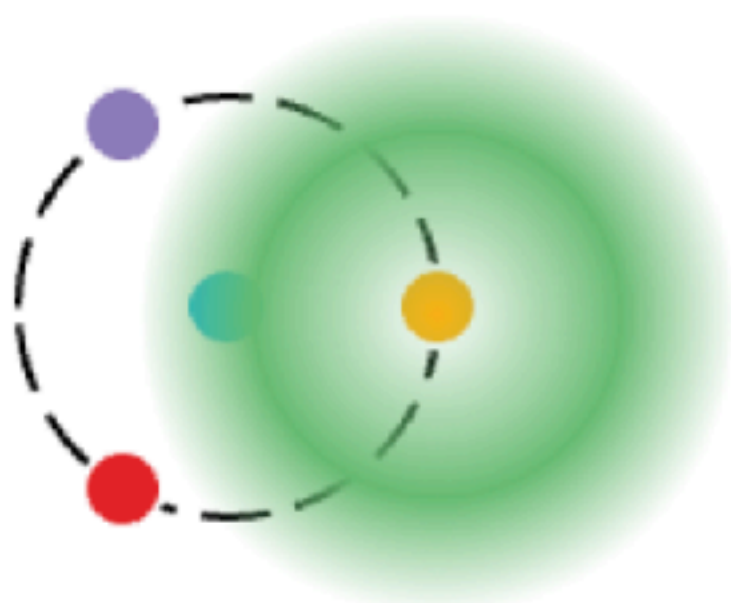
M. Salerno, et al. A. MINFLUX Nanoscopy: A “Brilliant” Technique Promising Major Breakthrough. Microsc Res Tech. (2025),



$$\sigma_{STED} \propto \frac{\lambda}{NA} \frac{1}{\sqrt{1 + I/I_s}}$$



$$\sigma_{CAM} \propto \frac{\lambda}{NA} \frac{1}{\sqrt{N}}$$



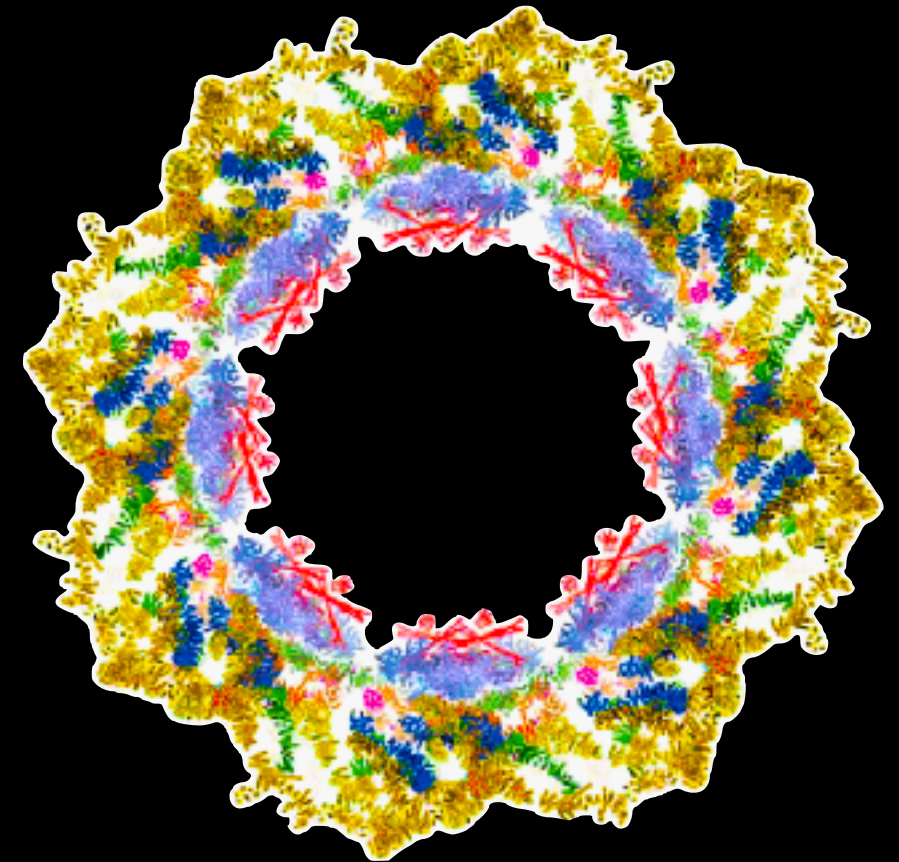
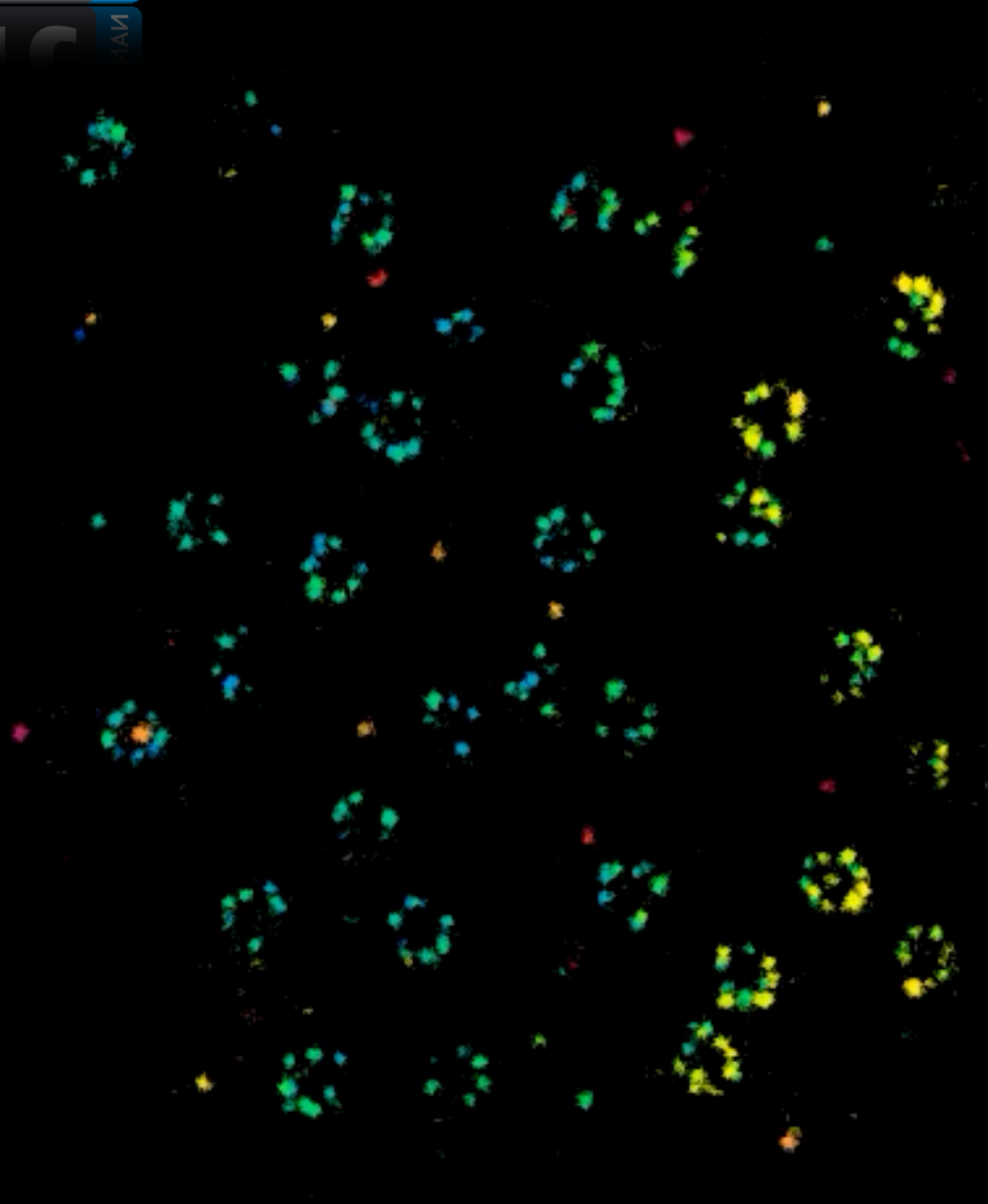
$$\sigma_{MF} \propto \frac{L}{\sqrt{N}}$$



3D of nuclear pores, z-position coded.

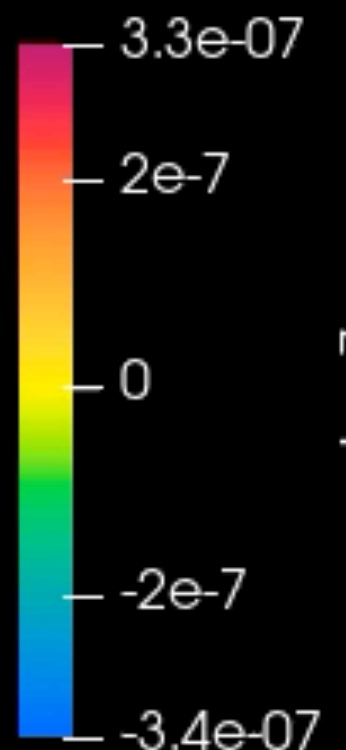
Image by Clara Guerth & Virginia Bazzurro, Heidelberg, EMBL course

Diaspro Lab



Overall Diameter: Around 120 nm

Thickness: 50-70 nm across the nuclear envelope



Salerno, M., Bazzurro, V., Angeli, E., Bianchini, P., Roushenas, M., Pakravanan, K. and Diaspro, A.
MINFLUX Nanoscopy: A “Brilliant” Technique Promising Major Breakthrough. **Microsc Res Tech.** (2025),



MINSTED nanoscopy enters the Ångström localization range

Received: 23 March 2022

Accepted: 20 September 2022

Published online: 1 November 2022

Michael Weber^{1,2}, Henrik von der Linde^{1,2}, Marcel Leutenegger¹, Philip Dunkel³, Shashankar Sambandan^{1,2}, Tanveer A. Khan¹, Jan Koller-Findelken¹, Willem C. Gerdes⁴ and Stefan W. Hell^{1,2,5}

Ångström-resolution fluorescence microscopy

<https://doi.org/10.1038/s41586-023-06026-0>

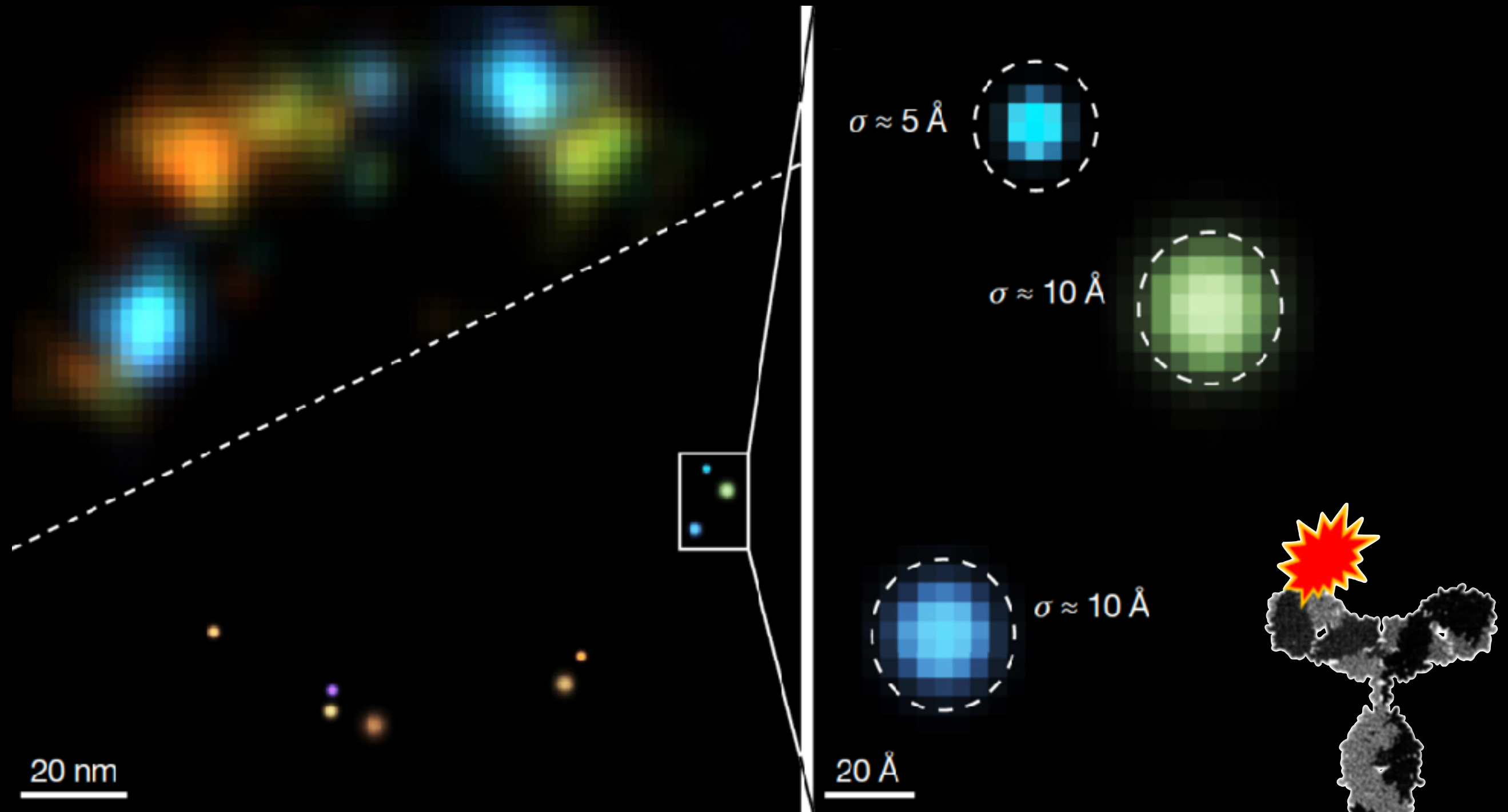
Received: 21 July 2022

Accepted: 7 March 2023

Published online: 21 May 2023

Susanne C. M. Reinhardt^{1,2}, Luciano A. Morillo^{1,2}, Isabelle Baudry^{1,2}, Philipp R. Sören^{1,2}, Ralf Kowalewski^{1,2}, Alexandra S. Eklund^{1,2}, Sebastian Strauss^{1,2}, Edward M. Unterwiesing^{1,2}, Thomas Schlichthaefer^{1,2}, Maximilian T. Strauss^{1,2}, Christian Klein^{1,2} & Ralf Jungmann^{1,2,5}

Fluorescence microscopy, with its molecular specificity, is one of the major

Vignette credit: Alexandra Luca
DiasproLab

IMAGES OBTAINED

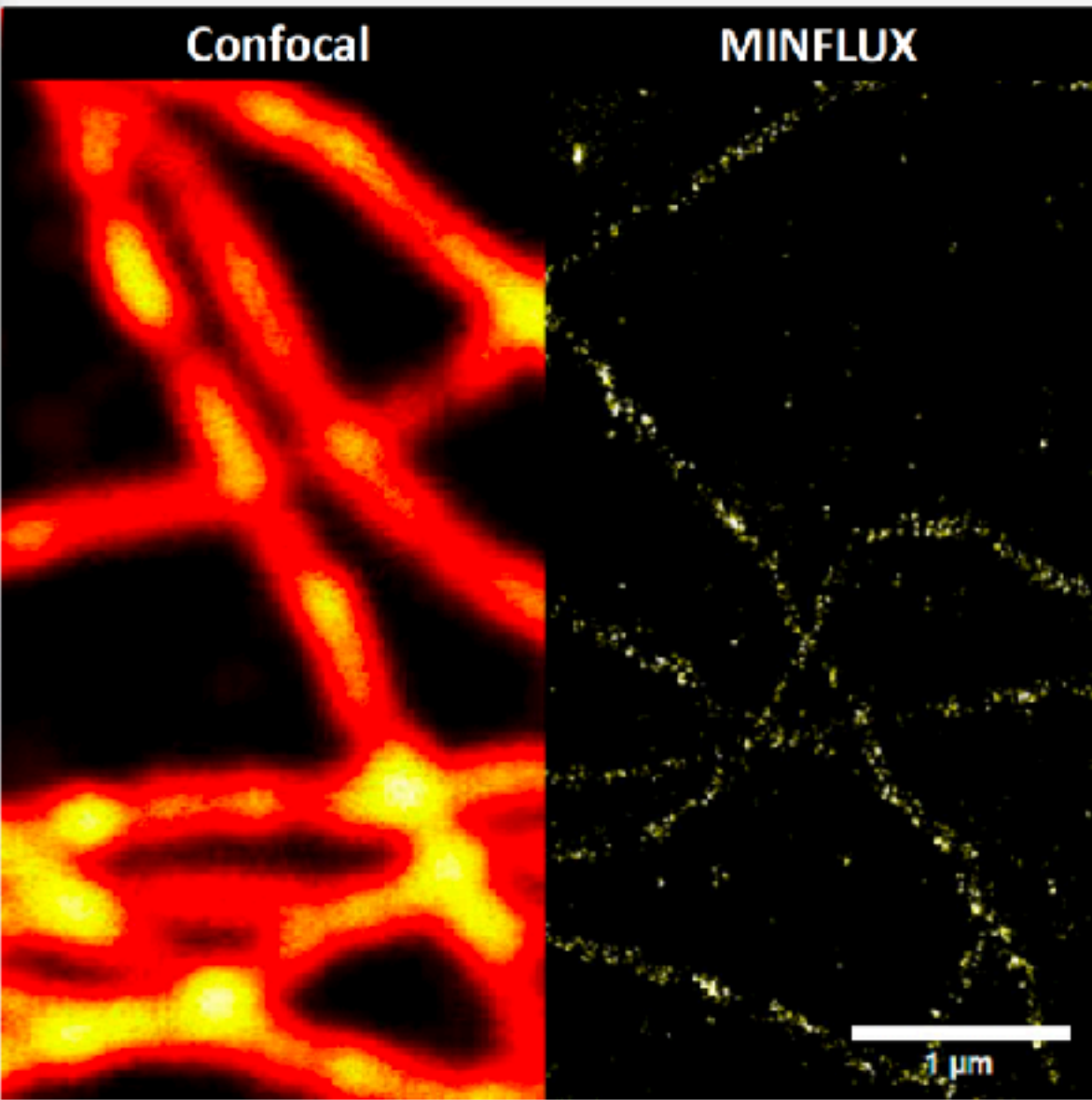


Fig 4: Indirect immunostaining of α -tubulin in HeLa cells using Alexa647. Confocal (left) and MINFLUX output (right)

ESRGAN NEURAL NETWORK

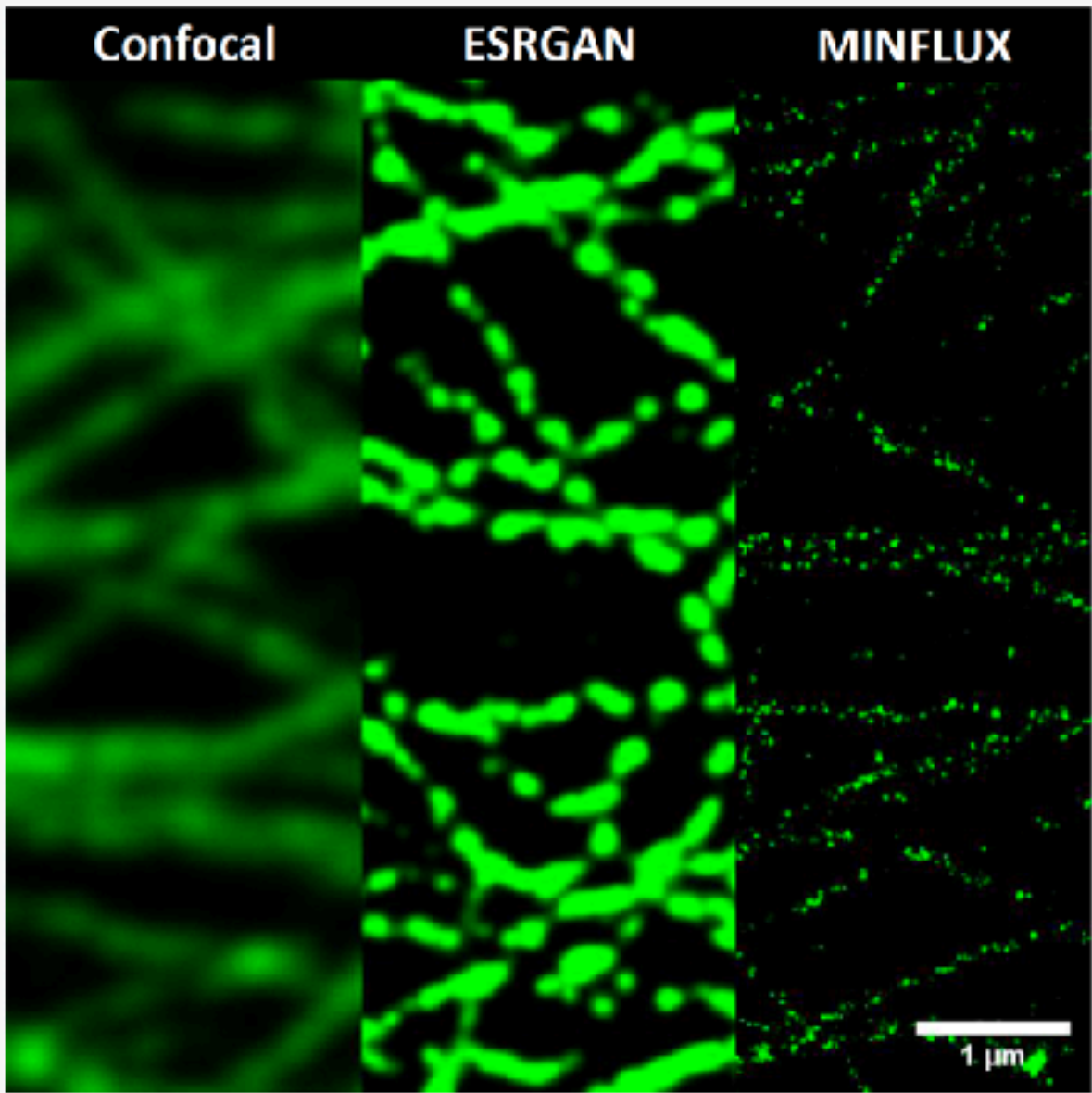
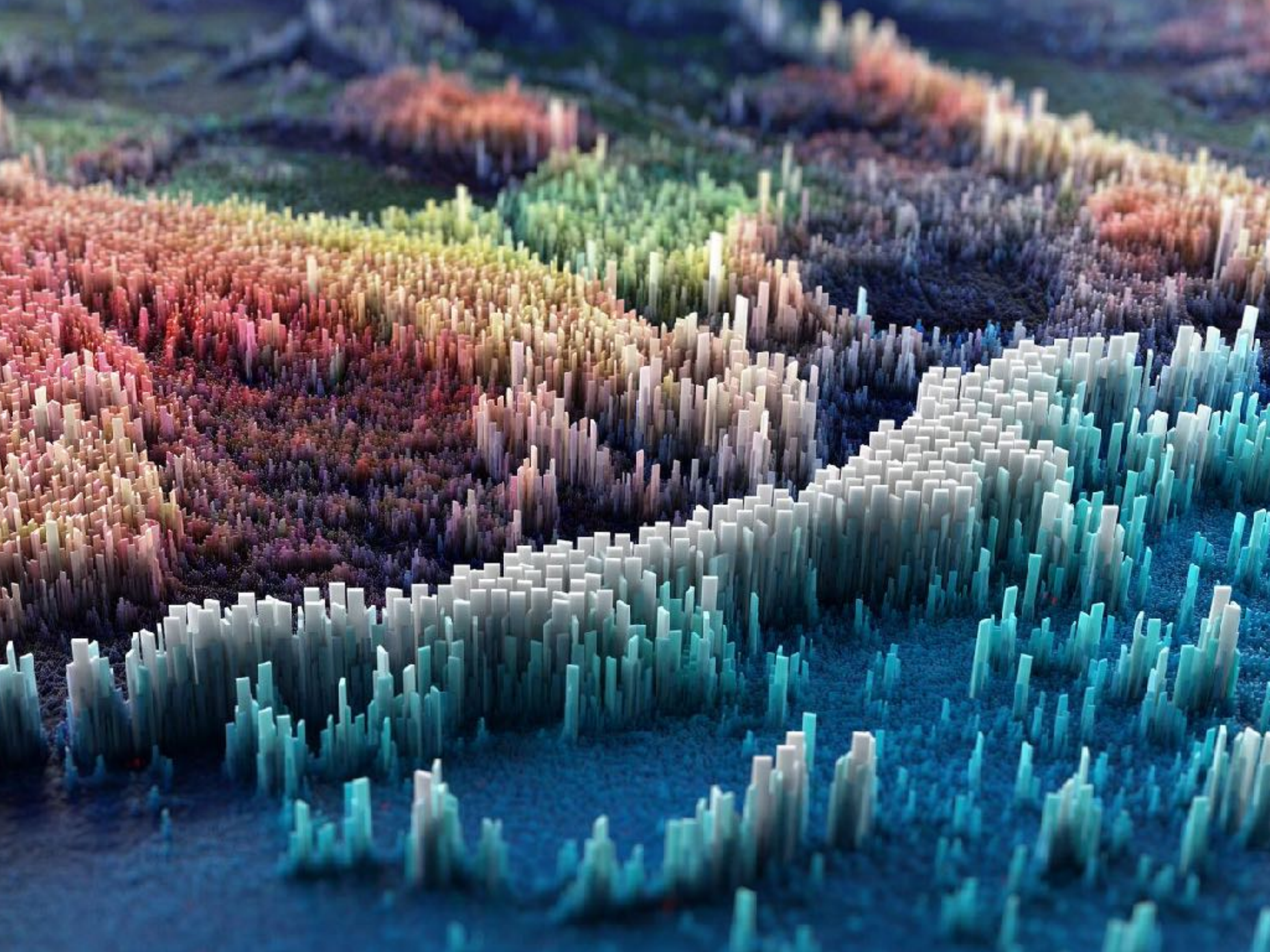
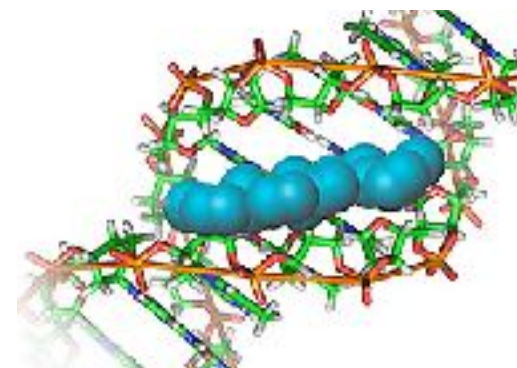
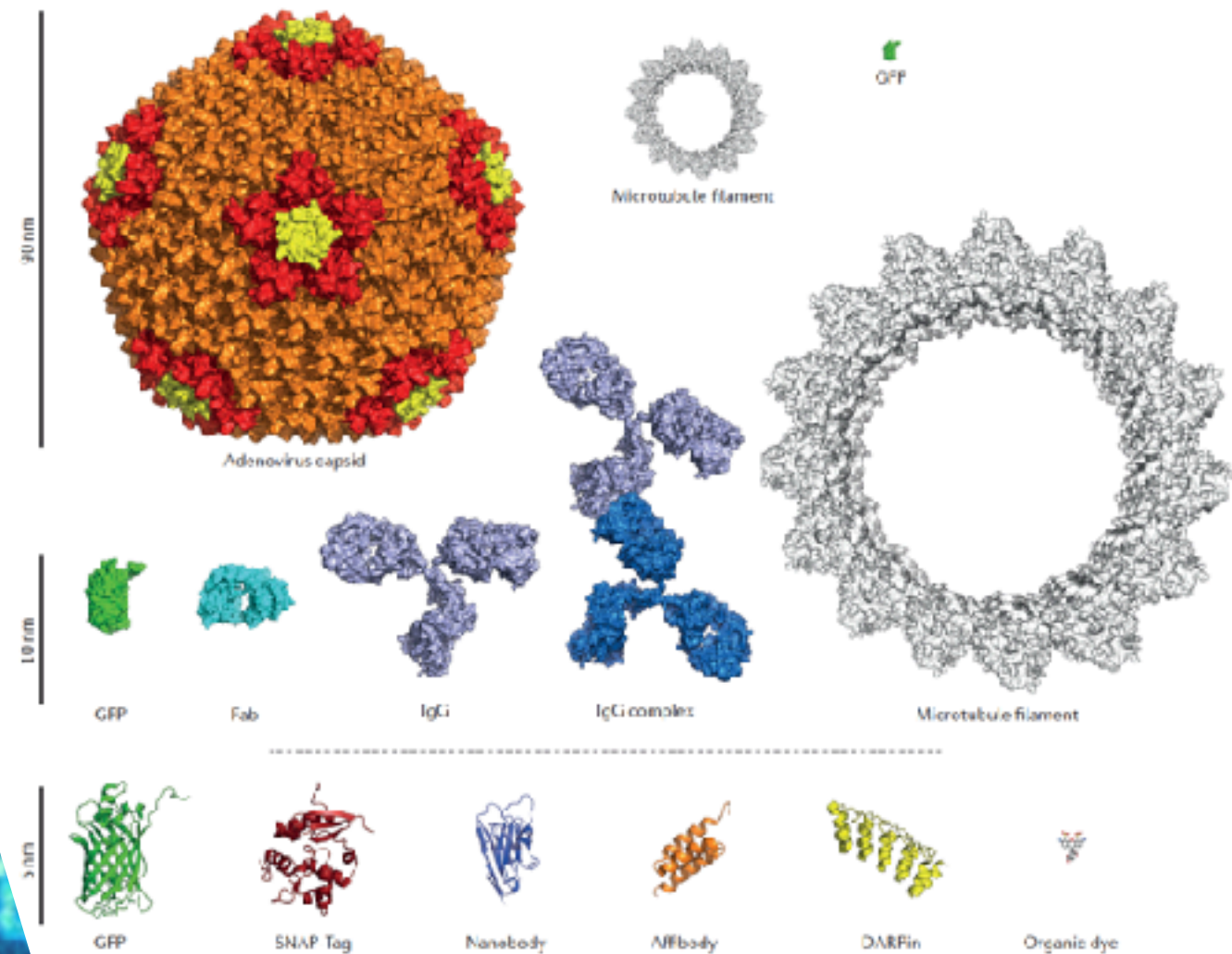
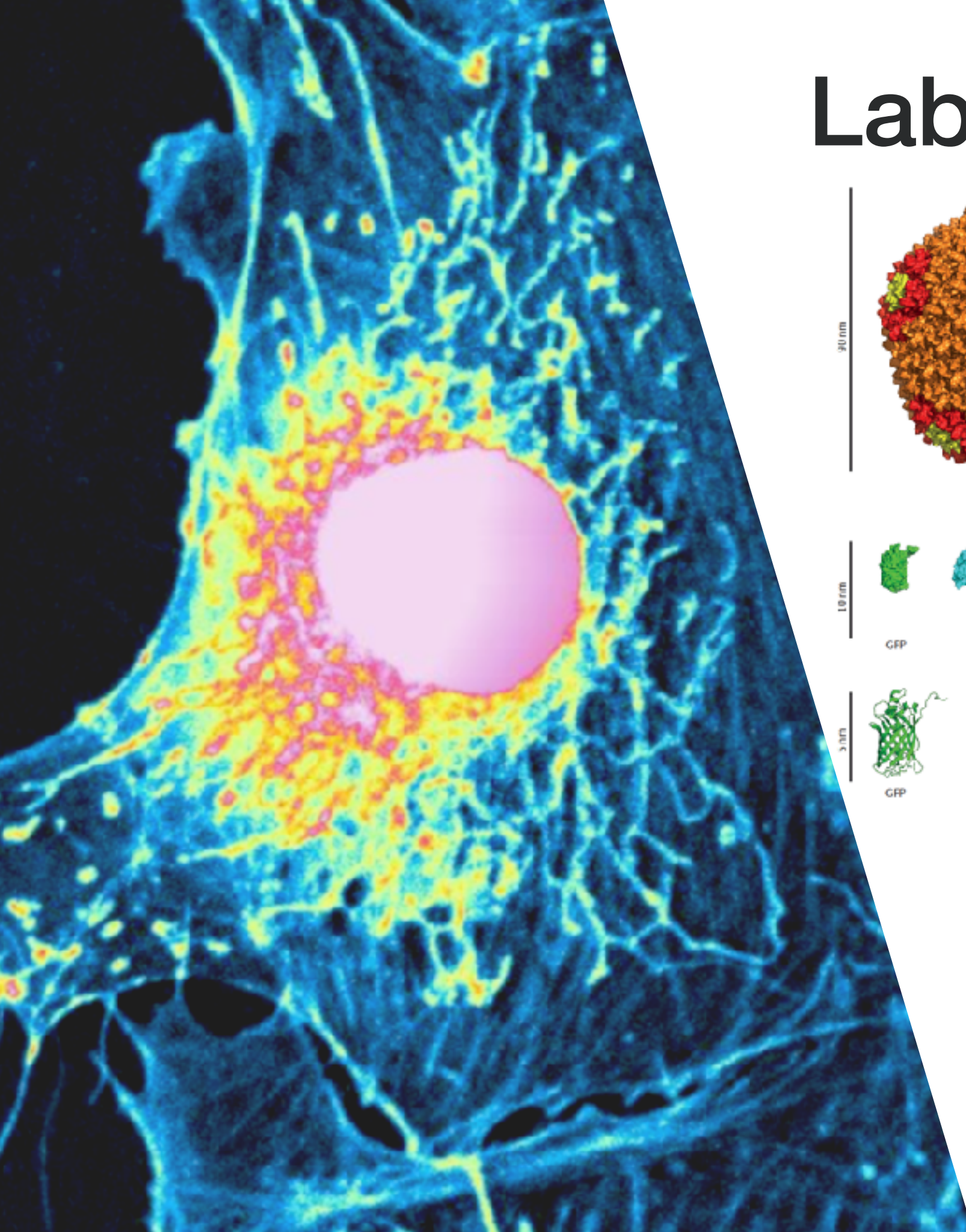


Fig 5: α -tubulin in HeLa cells. Confocal image (left), reconstruction with ESRGAN neural network at STORM resolution (center) and MINFLUX output (right).

ESRGAN (Enhanced Super-Resolution Generative Adversarial Network)



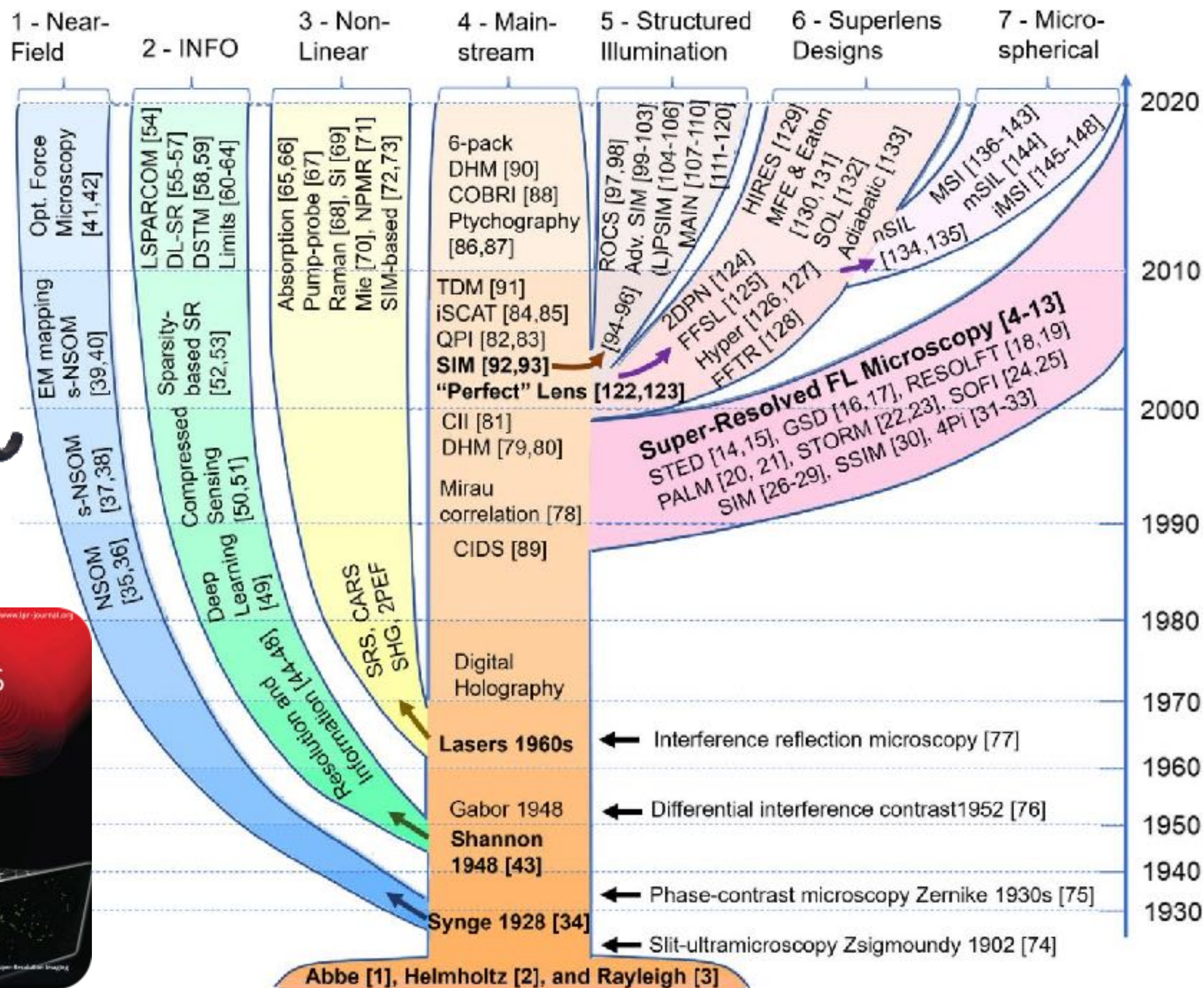
Label-free Trend



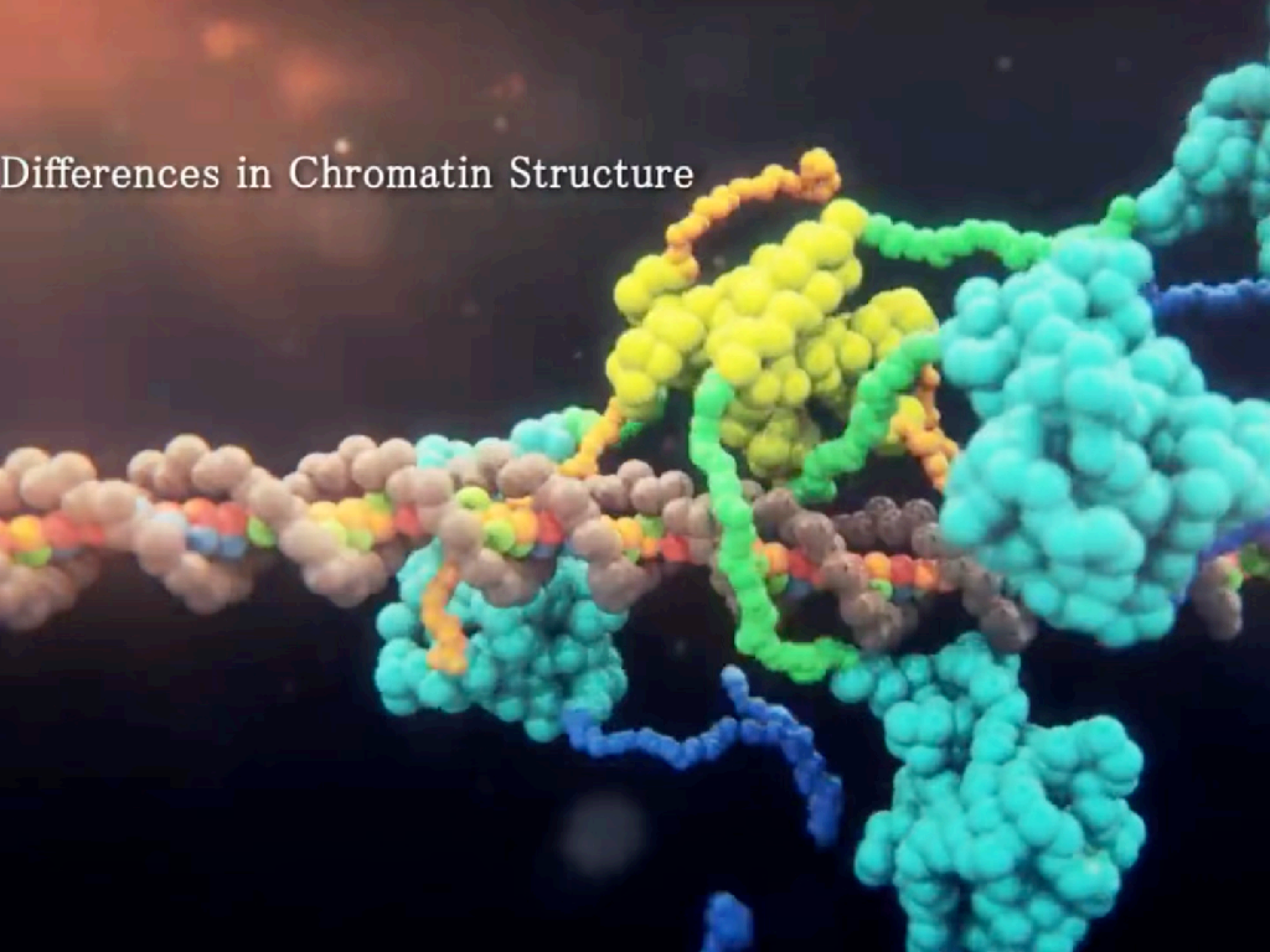
DAPI- minor groove of A/T-rich dsDNA sequences,



Vignette credit: Alexandra Luca
DiasproLab



Differences in Chromatin Structure



Case Study: Eledone Cirrhosa

Sperm heads of the octopus *Eledone Cirrhosa* are left-handed helices

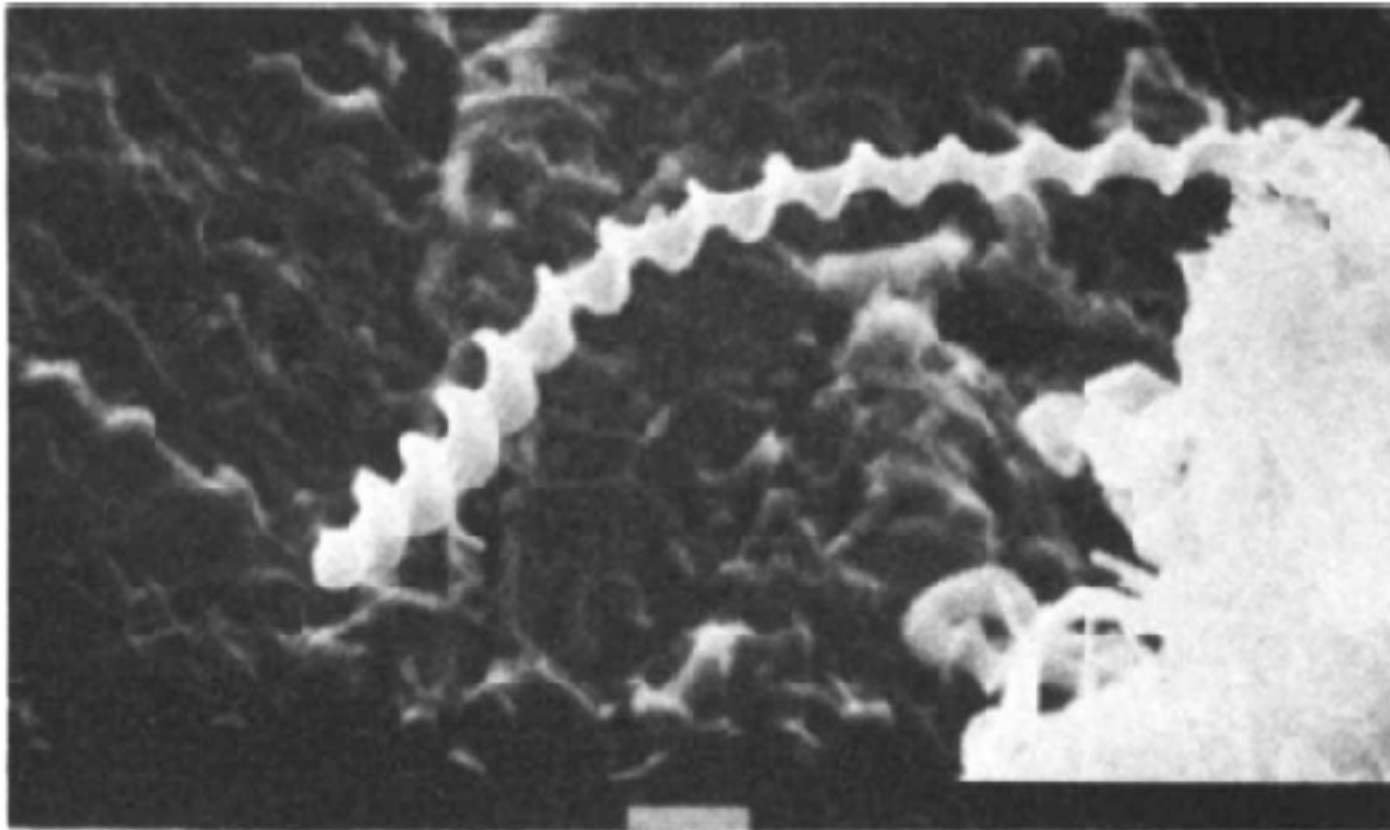


Fig. 1 Scanning electron micrograph of a freeze-dried sperm head from the octopus *Eledone cirrhosa*. The sperm head is a left-handed helix with a pitch of about $0.65 \mu\text{m}$. Scale bar, $1 \mu\text{m}$.

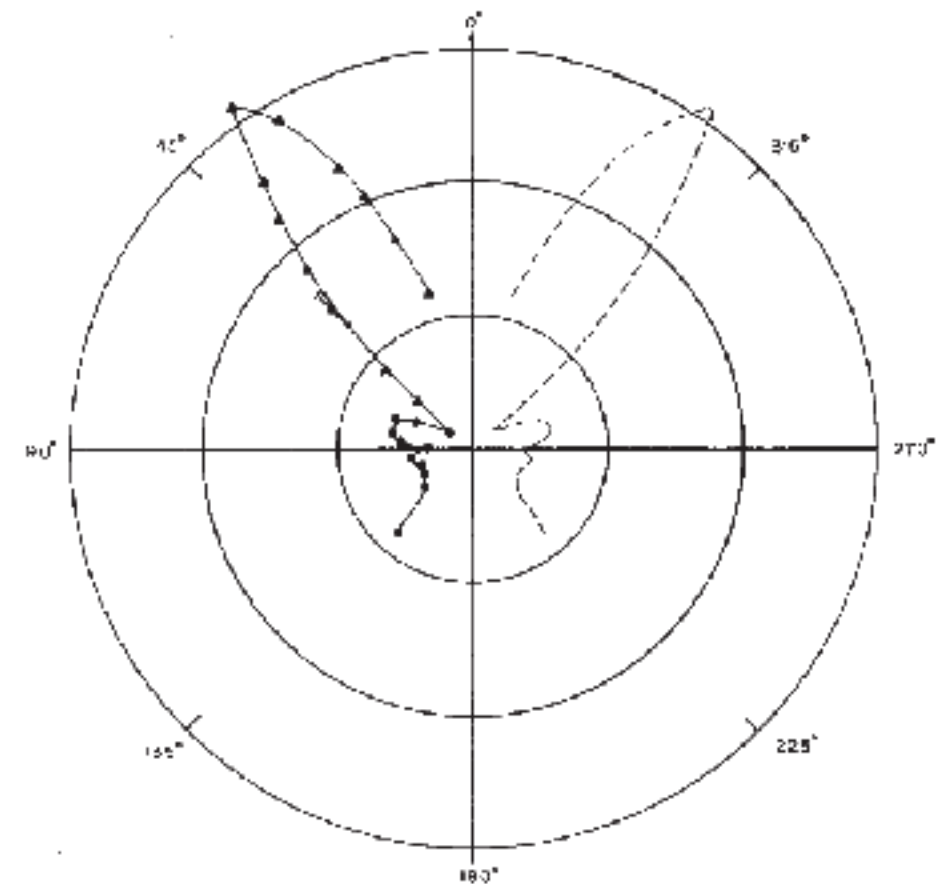
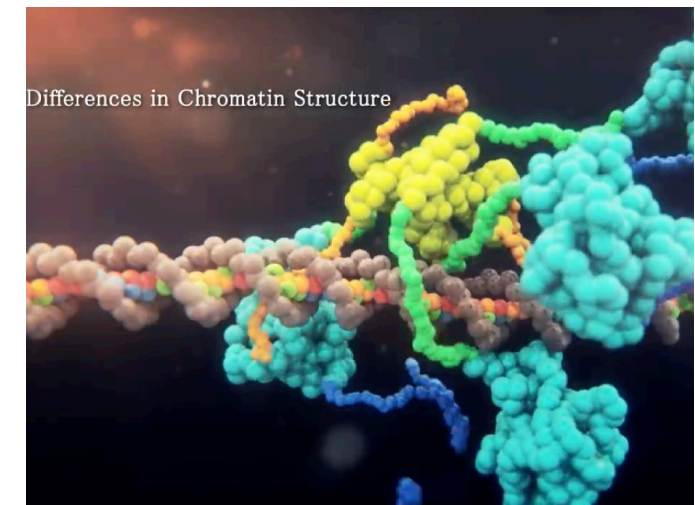
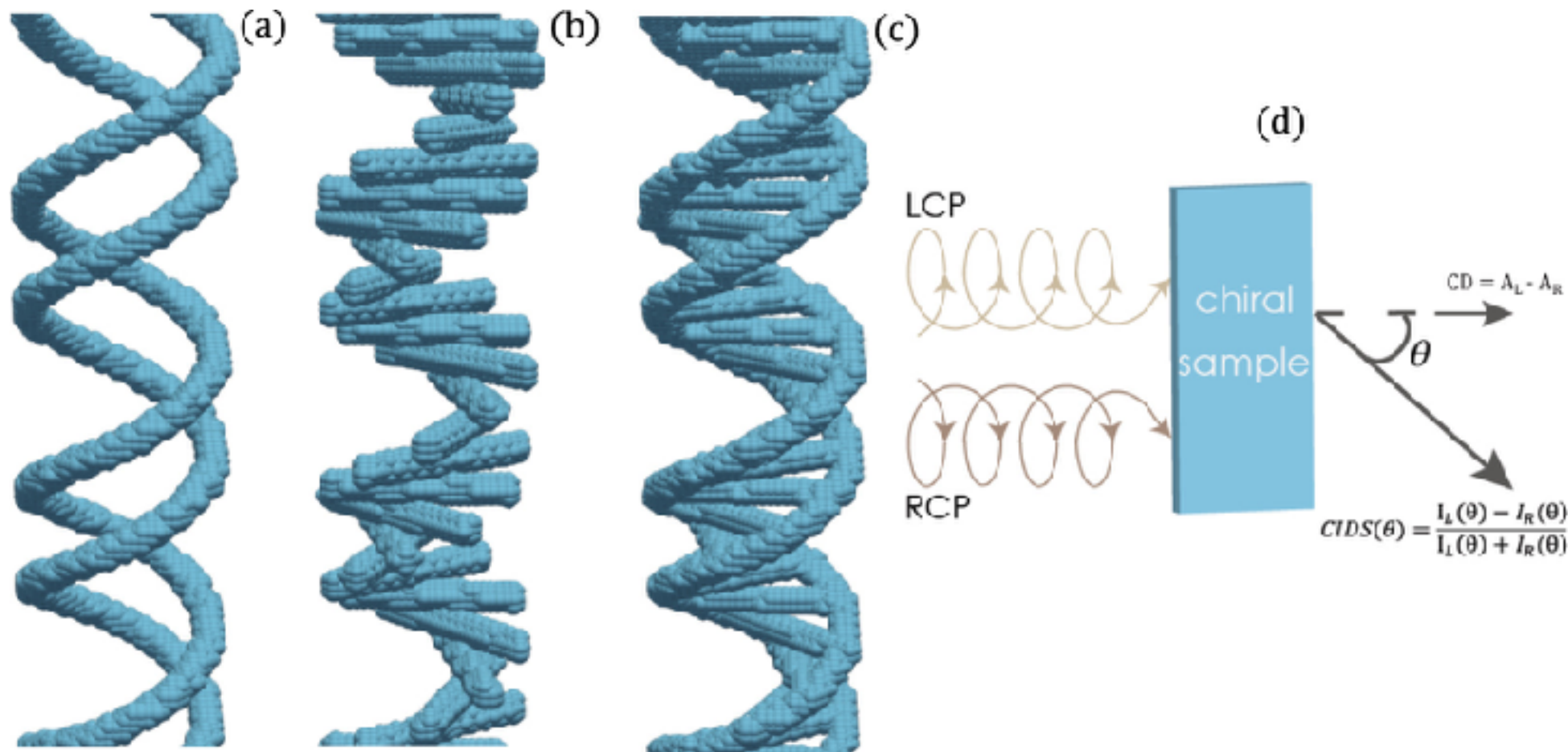


Fig. 3 Plot of CIDS $[(I_L - I_R)/(I_L + I_R)]$ versus scattering angle for *E. cirrhosa* sperm heads at a concentration of 0.25 mg ml^{-1} in the buffer of Fig. 2; the arrow illustrates the direction of incident beam (wavelength = 442 nm). $\blacktriangle$, Positive values; $\bullet$, negative values; ----, measured data; —, constructed by symmetry. The plot has a maximum positive value of 1.4×10^{-2} at about 35° .

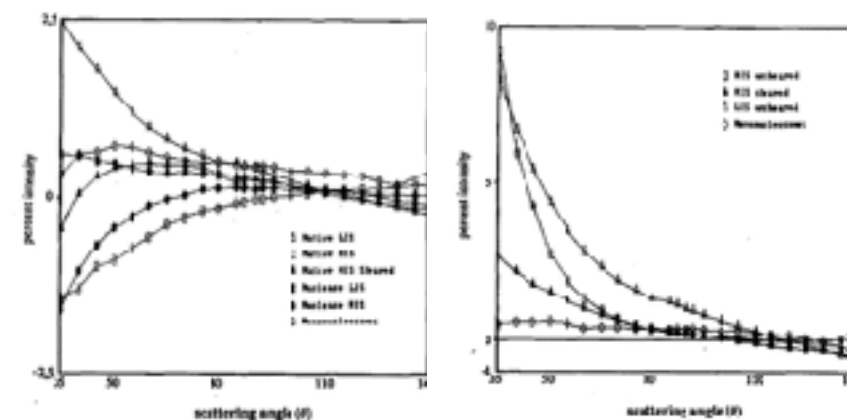
Maestre, Marcos F., et al. "Differential scattering of circularly polarized light by the helical sperm head from the octopus *Eledone cirrhosa*." Nature 298.5876 (1982): 773-774.

Light vs. chromatin interactions modelled by dipole approximation method implemented in ADDA code by discretizing the scattering object of an arbitrary shape into an array of polarizable point dipoles. (M.A. Yurkin, A.G. Hoekstra, 2011, J. Quant. Spectrosc. Radiat. Transfer)



Ashraf M.W., Diaspro A. (2022) **On the structural organization of macromolecules using chiral sensitive differential scattering of circularly polarized light.** Optics Communications. 522: 128639.

Diaspro, A. Circular intensity differential scattering and chromatin-DNA structure. **Cell Biophysics** 10, 45–60 (**1987**).



Mueller Matrix microscopy

Mueller matrix microscopy exploits polarization

Stokes vector describes polarization of light:

$$\vec{S} = \begin{bmatrix} S_1 \\ S_2 \\ S_3 \\ S_4 \end{bmatrix} = \begin{bmatrix} I_0 \\ I_{0^\circ} - I_{90^\circ} \\ I_{45^\circ} - I_{-45^\circ} \\ I_R - I_L \end{bmatrix}$$

$\updownarrow$
+

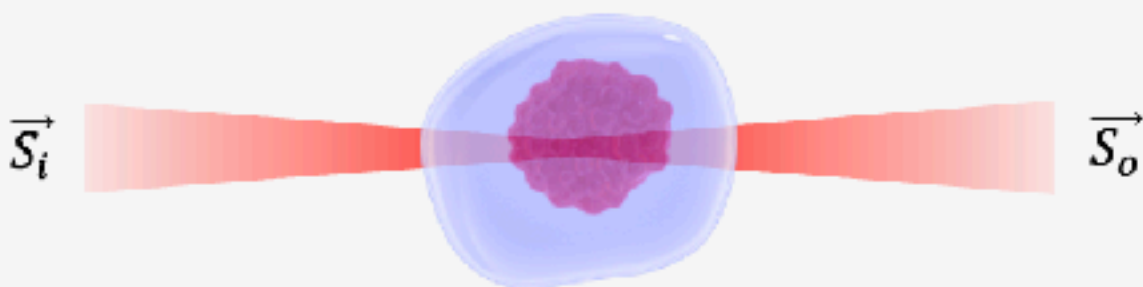
$\times$
-

$\circ$
-

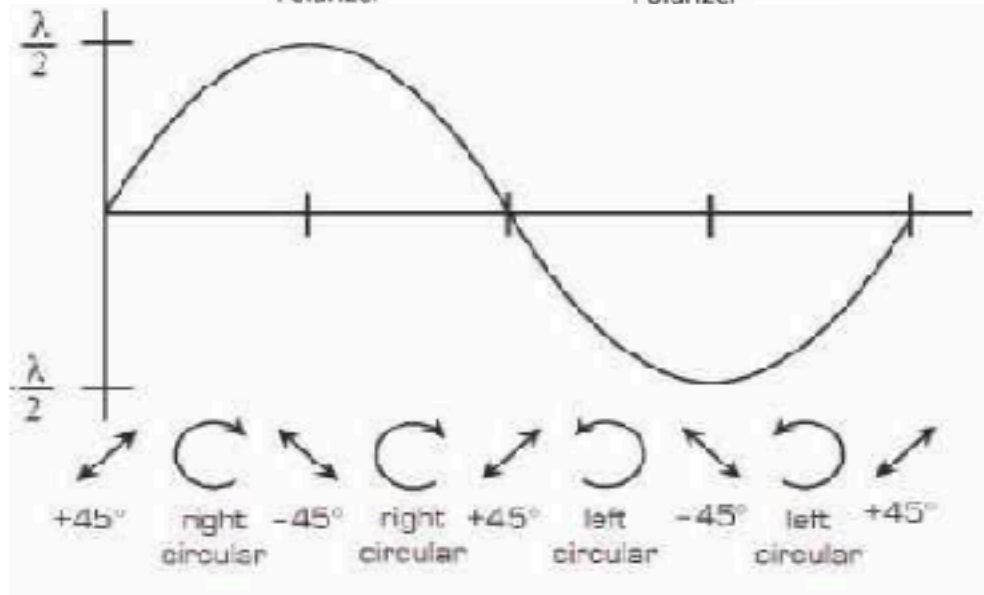
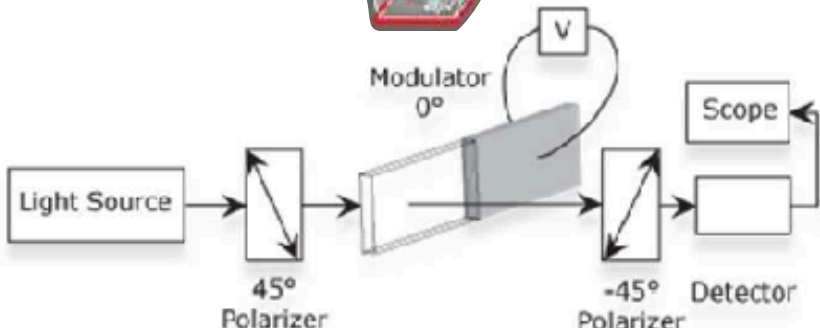
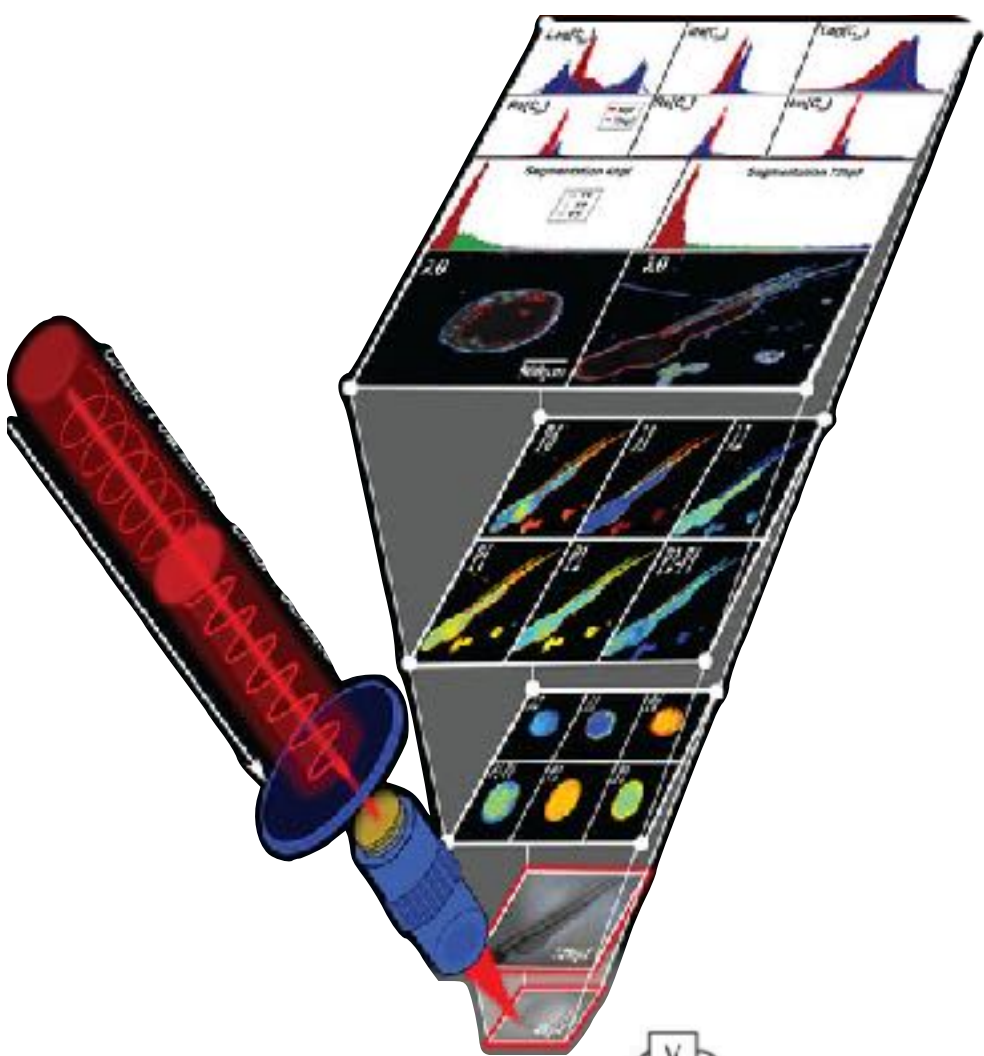
Mueller matrix describes sample anisotropies:

$$[M] = \begin{bmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{bmatrix}, \quad \vec{S}_o = [M] \vec{S}_i$$

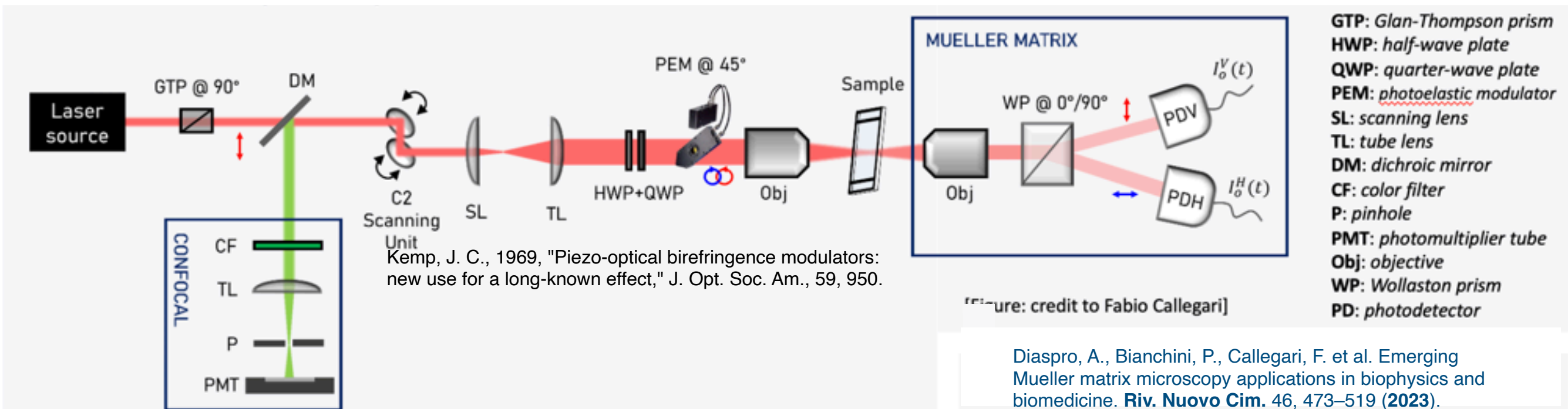
Basic idea:



Generate $\vec{S}_i$
Analyse $\vec{S}_o$ $\rightarrow$ Calculate $[M]$



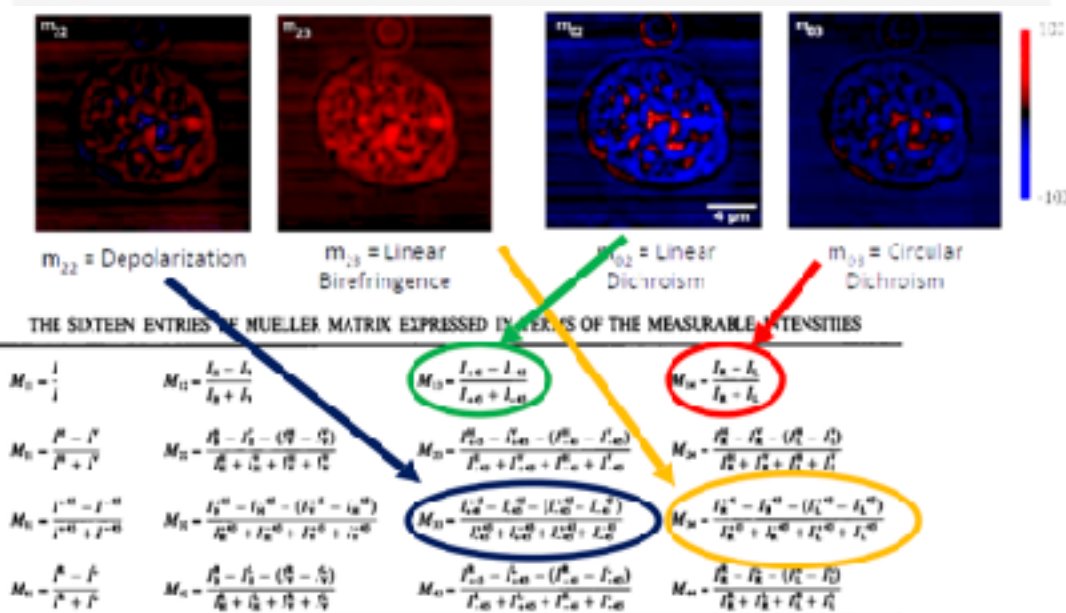
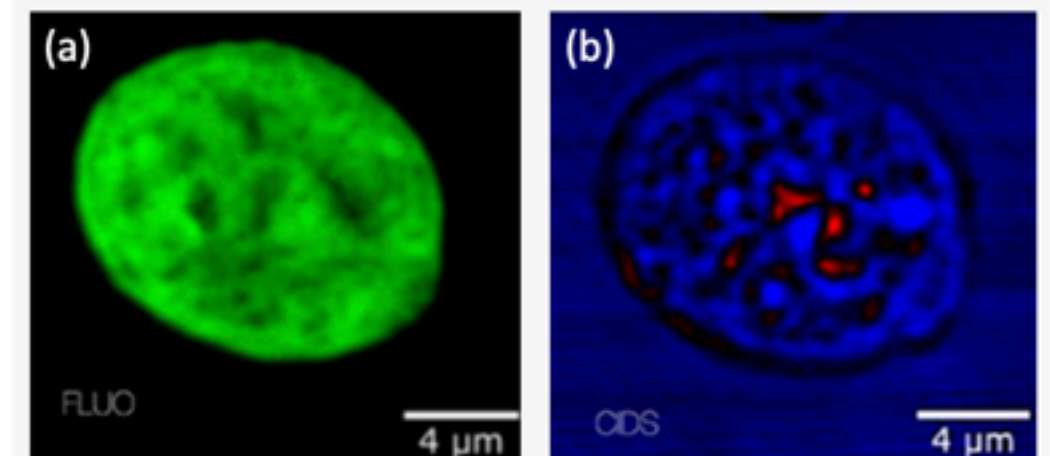
Diaspro, A., Bianchini, P., Callegari, F. et al. Emerging Mueller matrix microscopy applications in biophysics and biomedicine. **Riv. Nuovo Cim.** 46, 473–519 (2023).



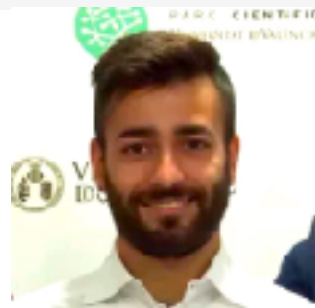
- After PEM: alternate left and right circularly polarized light
- After detection and demodulation, information on 6 elements: $m_{11}, m_{12}, m_{14}; m_{21}, m_{22}, m_{24}$
- Circular intensity differential scattering (CIDS) provides information on chirality:

$$CIDS = m_{14}/m_{11}$$
- Simultaneous acquisition of Mueller matrix and confocal images

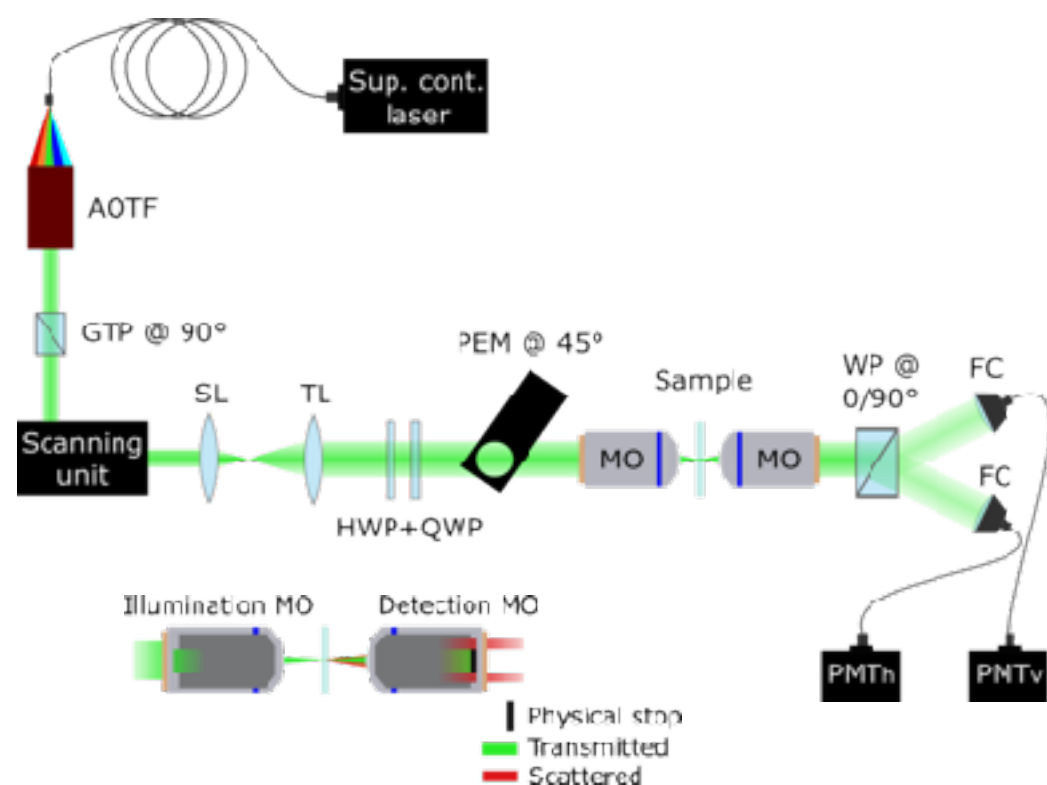
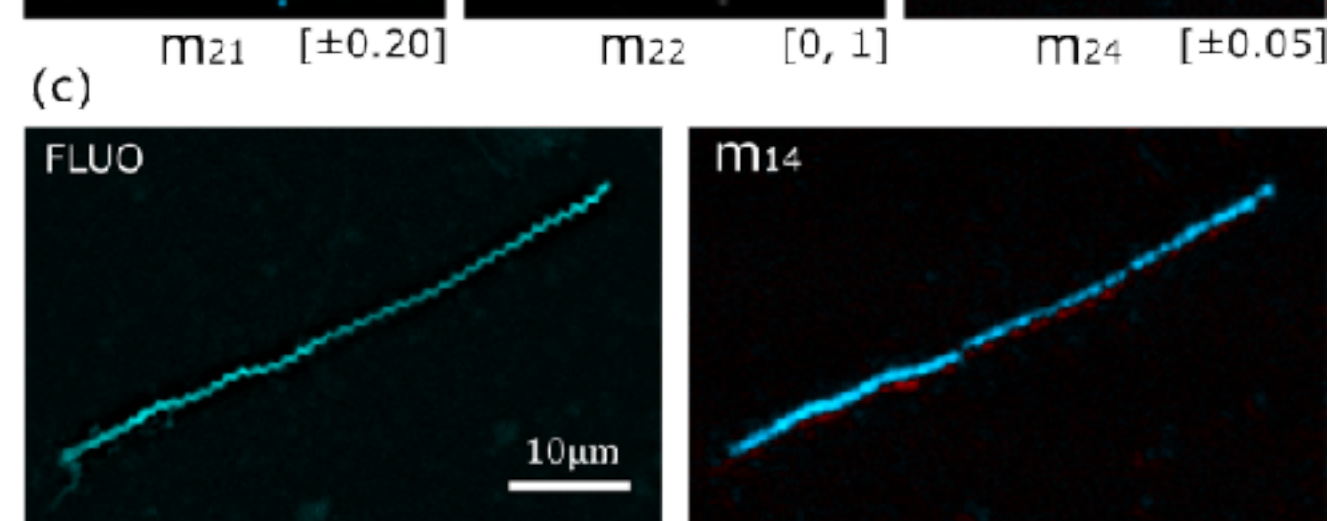
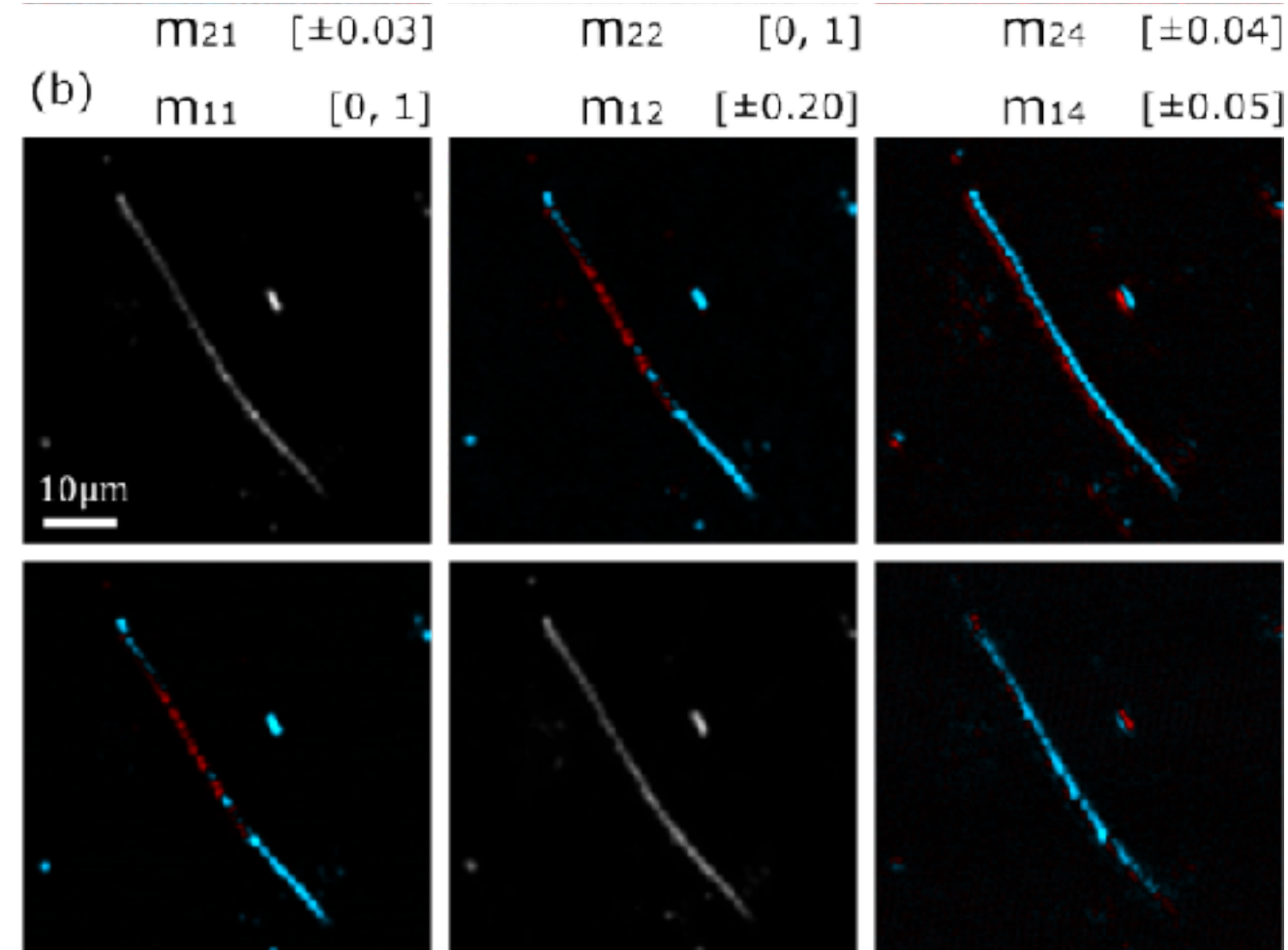
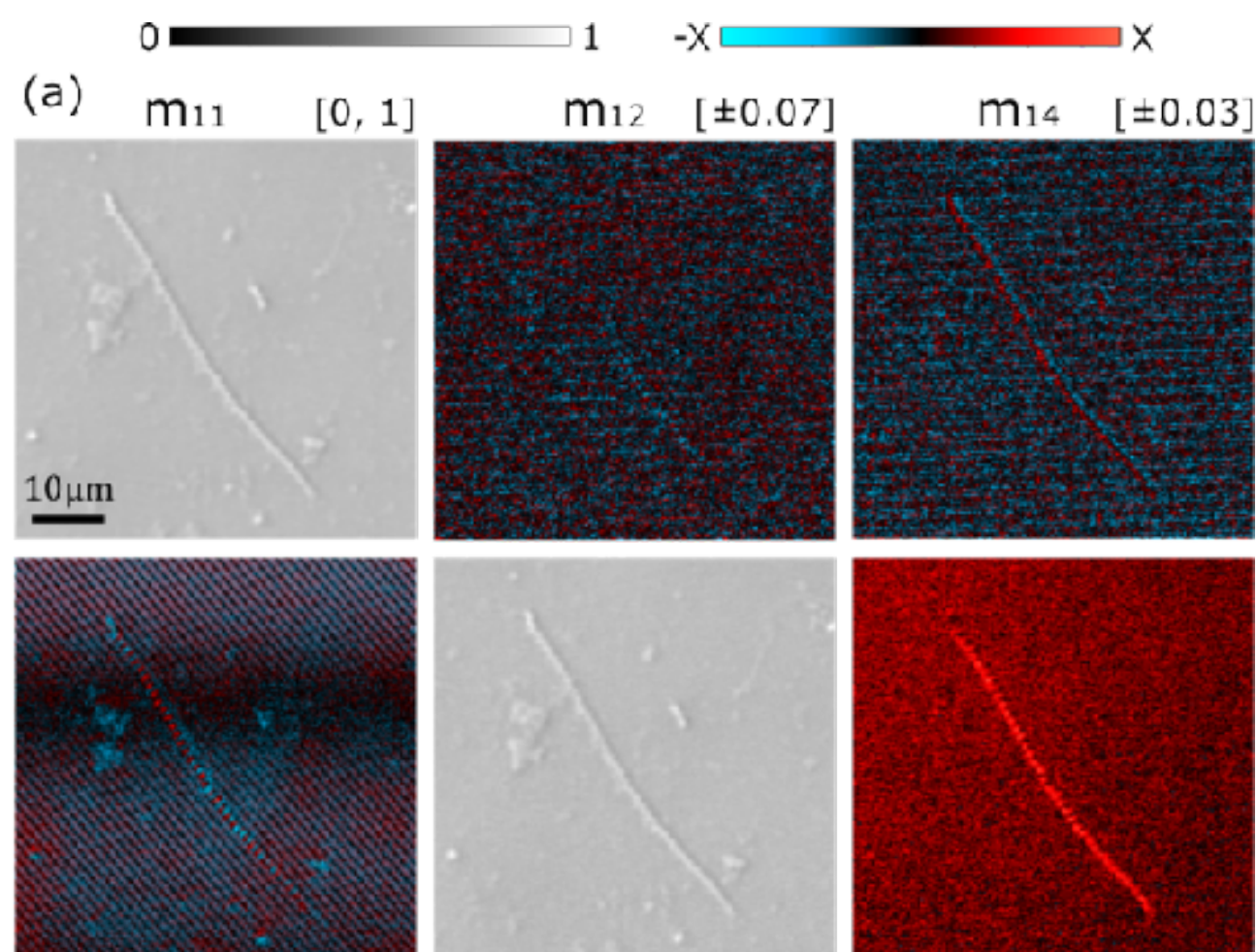
Diaspro, A., Bianchini, P., Callegari, F. et al. Emerging Mueller matrix microscopy applications in biophysics and biomedicine. *Riv. Nuovo Cim.* 46, 473–519 (2023).



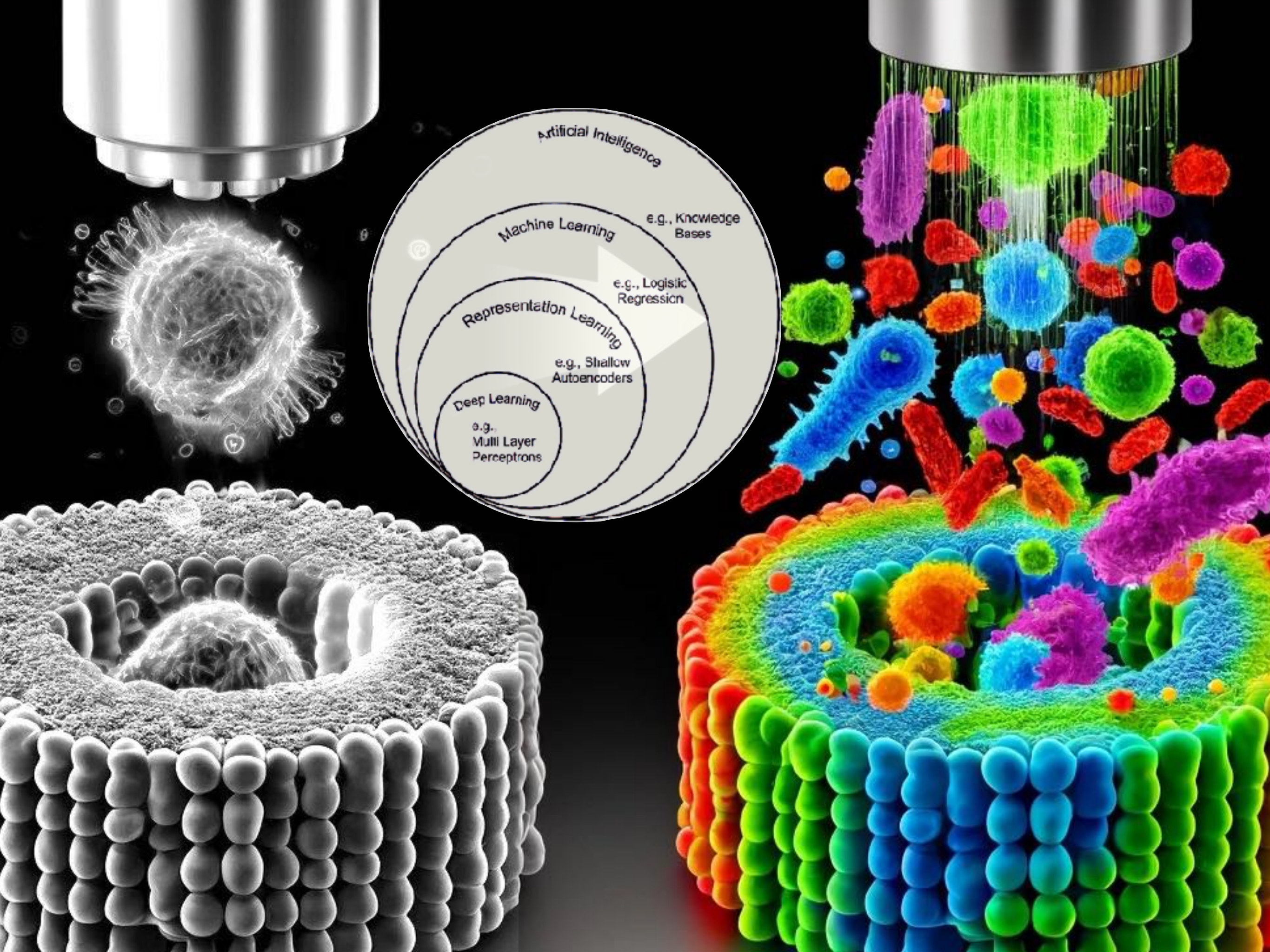
Paolo Bianchini



Nicolò Incardona



Incardona, Nicolo; Bianchini, Paolo; Diaspro, Alberto (2025). Darkfield Mueller matrix microscope allows the observation of single cells with high contrast. **Optica Open**. Preprint. <https://doi.org/10.1364/opticaopen.29715233.v2>



Artificial Intelligence

e.g., Knowledge Bases

Machine Learning

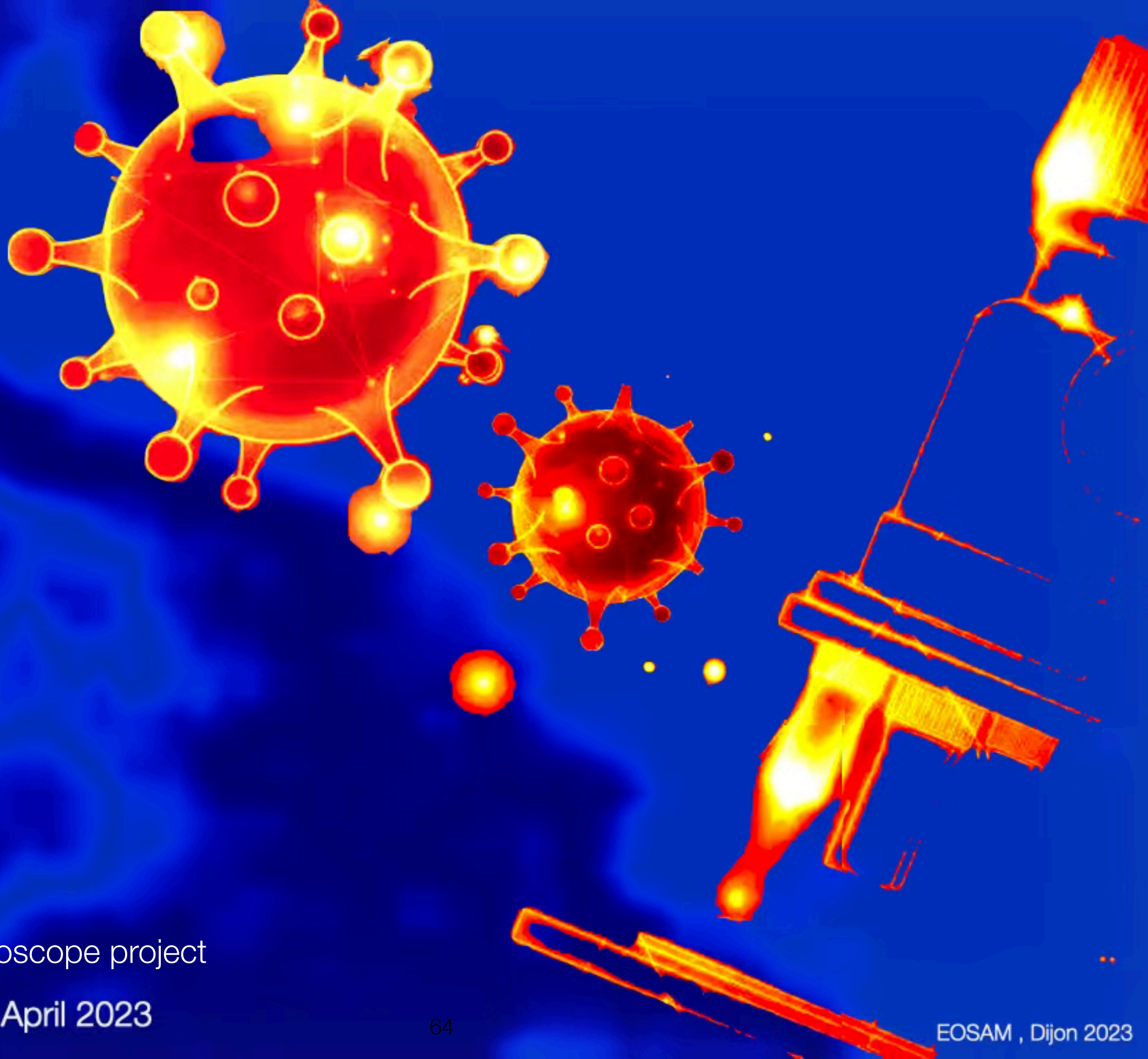
e.g., Logistic Regression

Representation Learning

e.g., Shallow Autoencoders

Deep Learning

e.g., Multi Layer Perceptrons



The artificial microscope project

Alberto Diaspro, April 2023



The Nobel Prize in Physics 2024



Ill. Niklas Elmehed © Nobel Prize Outreach

John J. Hopfield

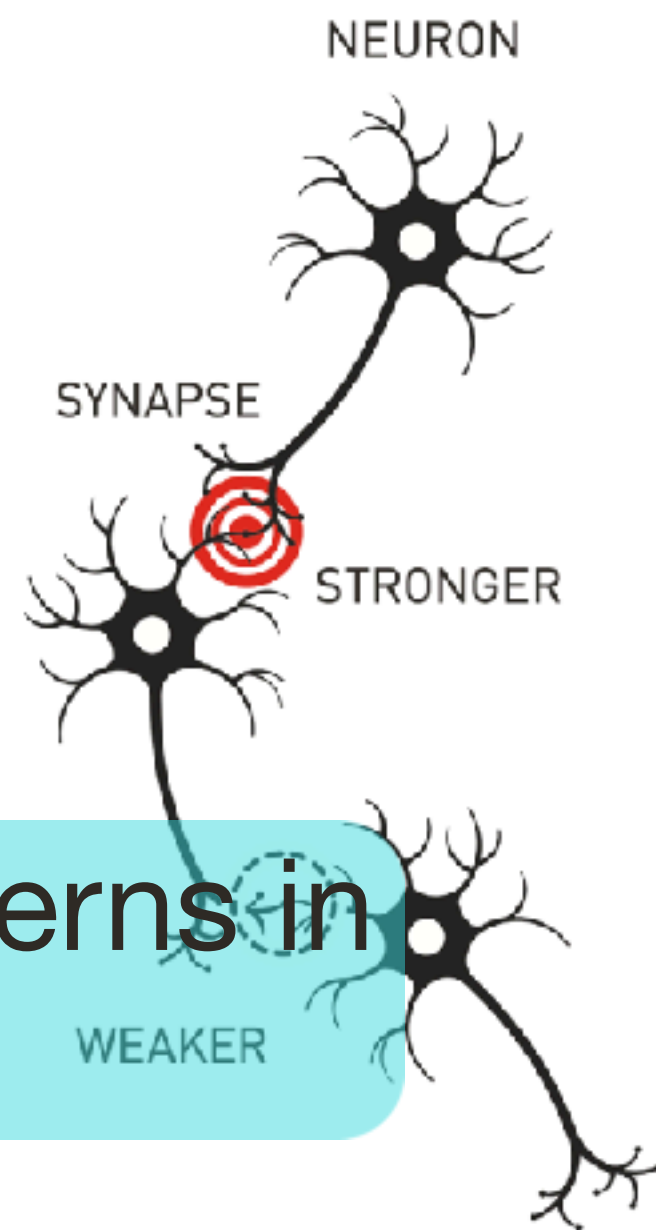
Prize share: 1/2



Ill. Niklas Elmehed © Nobel Prize Outreach

Geoffrey E. Hinton

Prize share: 1/2



They used physics to find patterns in information

A performance analysis of an associative system for image classification

Alberto Diaspro

Institute of Biophysics, University of Genoa, Genoa, Italy

Giancarlo Parodi, Rodolfo Zunino

DIBE, Department of Biophysical and Electronic Engineering, University of Genoa, Genoa, Italy

Received 27 October 1992

Volume 14, Number 11

PATTERN RECOGNITION LETTERS

November 1993

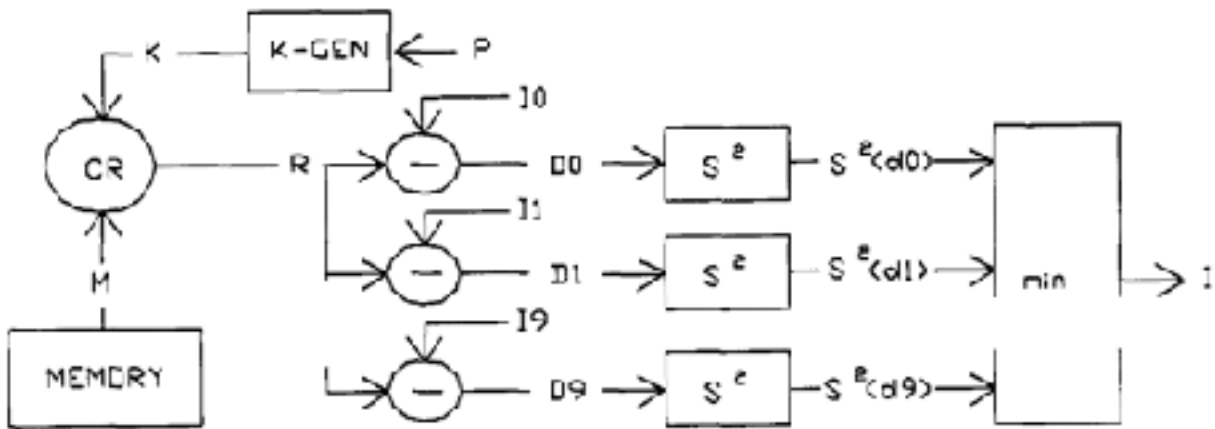


Figure 1. The schema of the associative classifier.

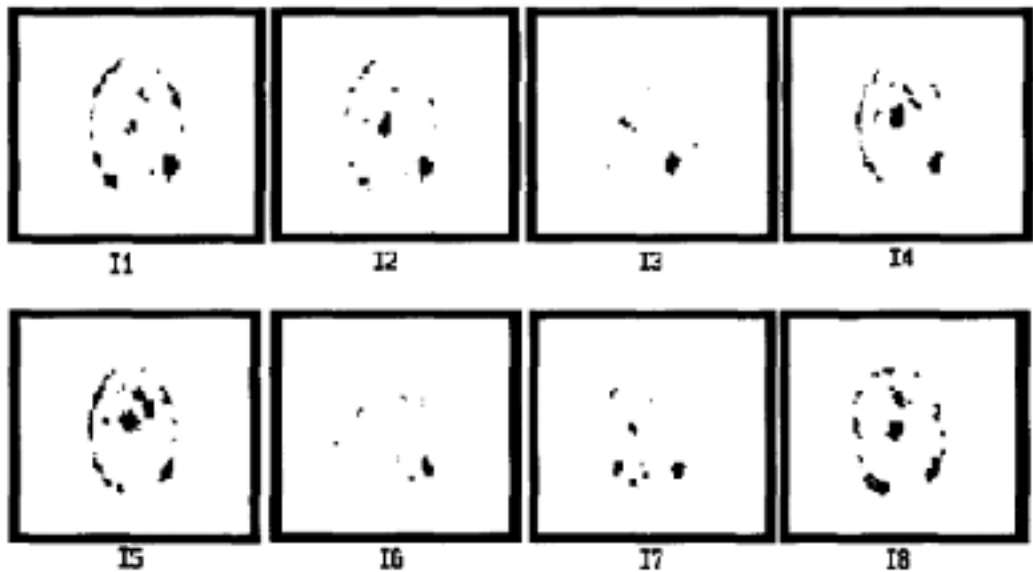


Figure 2. The set of images used for damage-insensitivity tests.

The Nobel Prize in Physics 2024



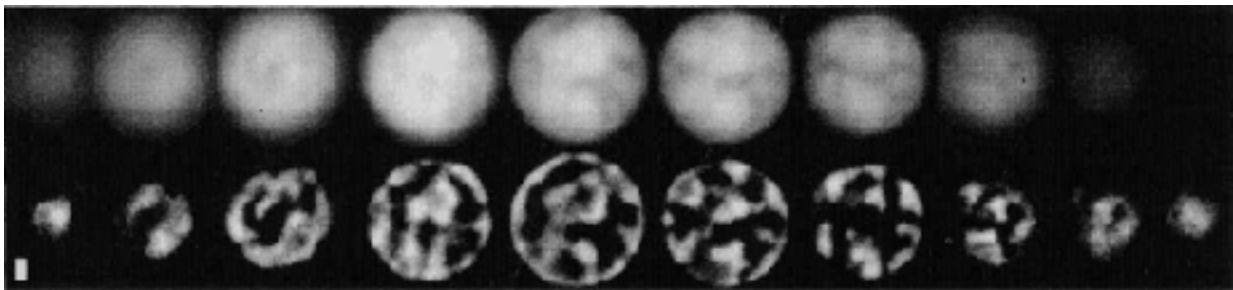
III. Niklas Elmehed © Nobel Prize Outreach
John J. Hopfield
Prize share: 1/2



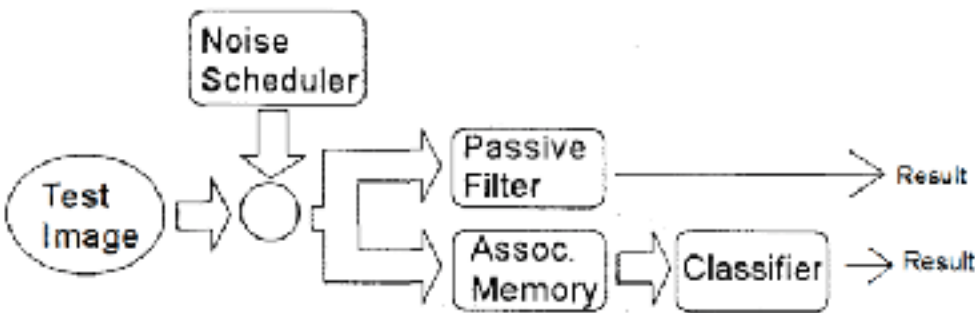
III. Niklas Elmehed © Nobel Prize Outreach
Geoffrey E. Hinton
Prize share: 1/2



J.J. Hopfield, Proc. Natl. Acad. Sci. USA 79, 2554 (1982).



A.Diaspro, Image and Vision Computing, 1990



A.Diaspro, G.Parodi, R.Zunino, **Studia Biophysica**, vol.139, no.2, pp.69-16, 1990

Paired Image-To-Image Translation

- **Image-To-Image Translation:**

- Transformation from One Image Representation to Another
 - Grayscale to RGB | Sketch to Realistic Photo

- **Paired Data:**

- The Image in Domain A has the Corresponding Image in Domain B

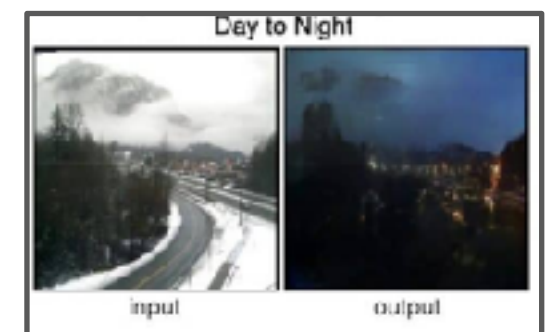
- **Training GANs** involves Two Components,

Trained **Together**:

- a. Training the **Generator**
- b. Training the **Discriminator**

- The **Paired** Images:

- a. **Label Free** & **Fluorescence**

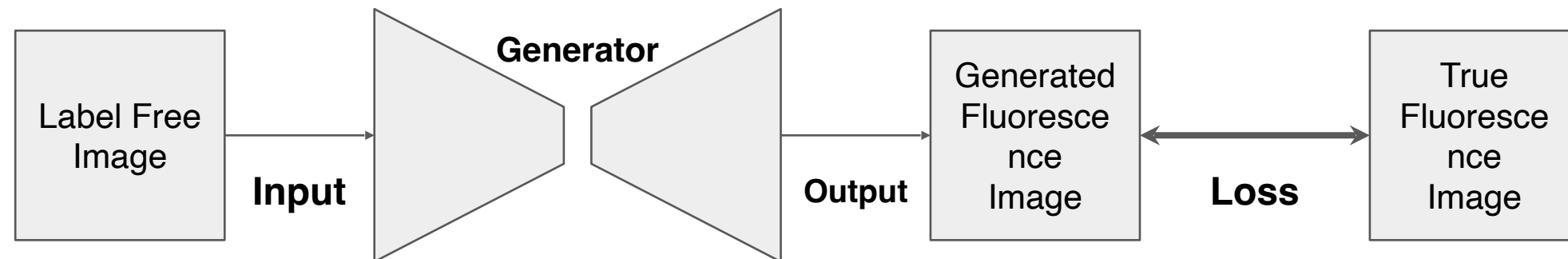


Larbi Toujier

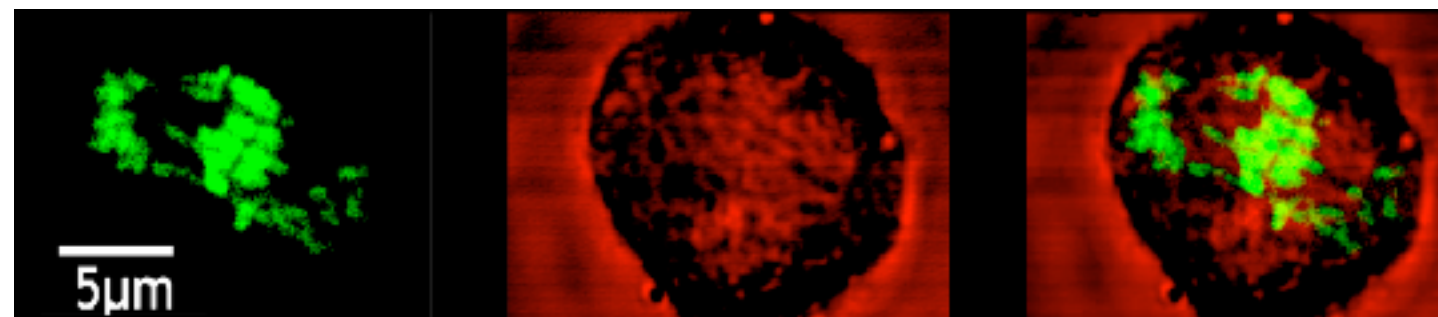
Generative Adversarial Networks (GANs)

Training the Generator

Generative



- **Input:** **Label Free** Image
- **Generator:** Takes **Label Free** and Learns to **Generate Fluorescence**
- **Output:** **Generated Fluorescence** Image, corresponding to the **Input Label Free** Image
- **Loss Function:** **Pixel-Level** Evaluation, **Generated** vs **True Fluorescence** Image

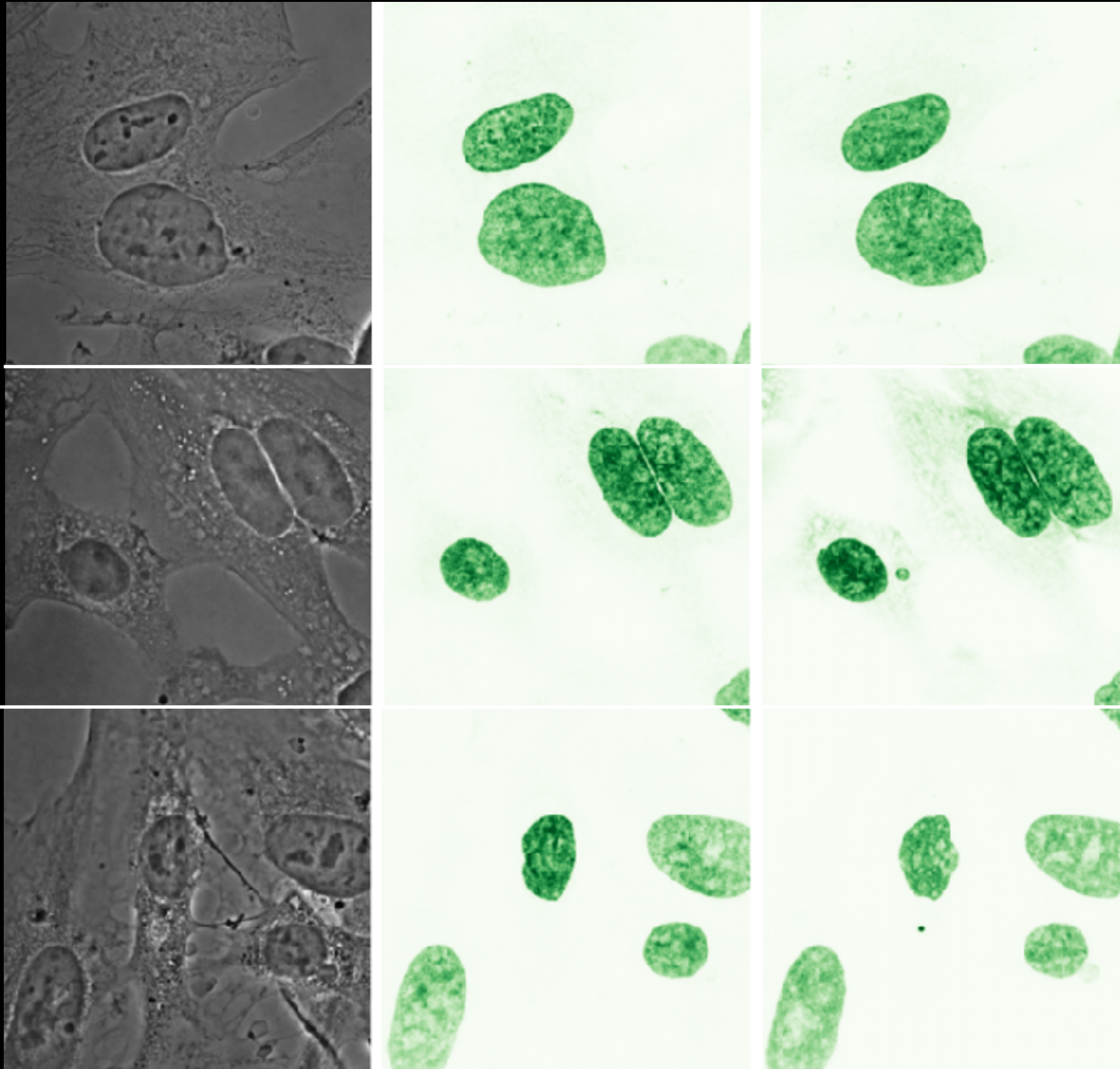


Preliminary results

Label free

Generated

True



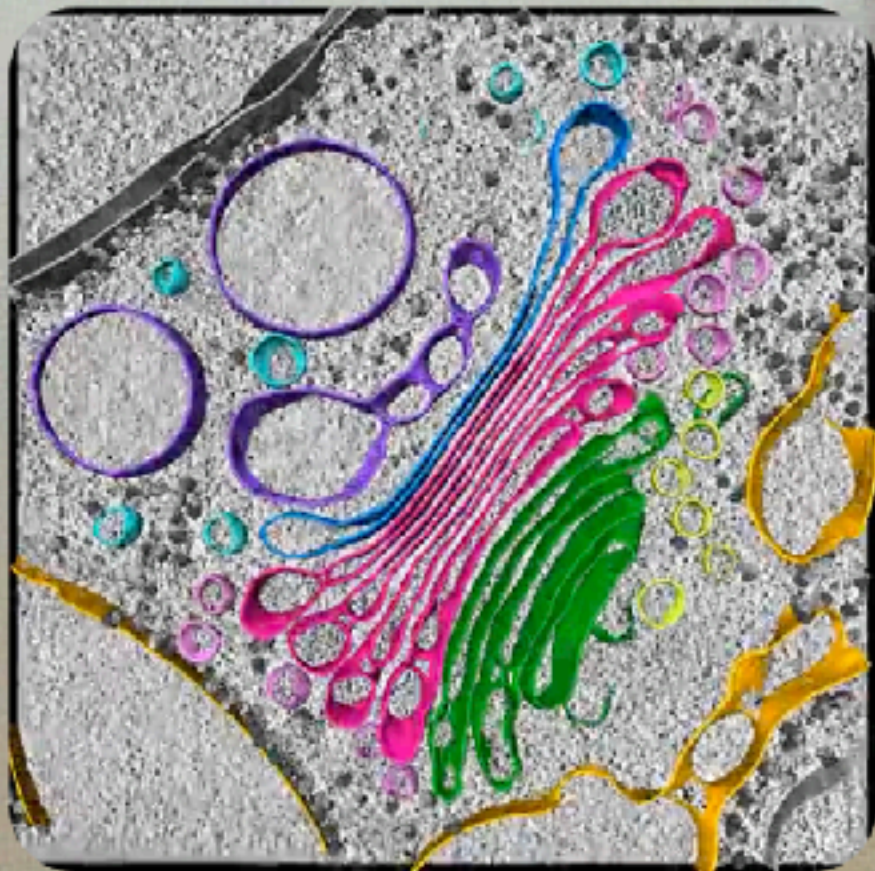
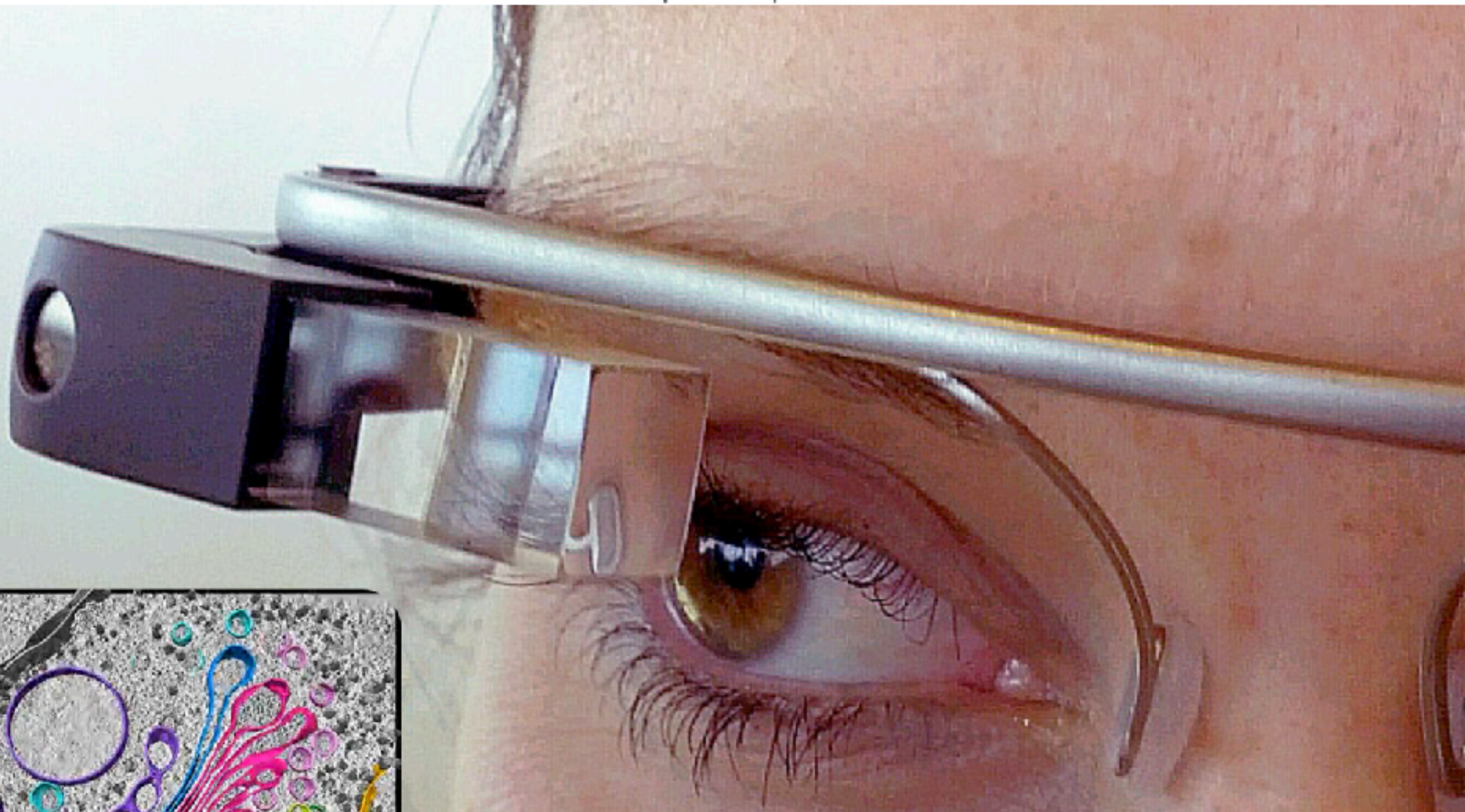
Larbi Toujier

In future perspective...



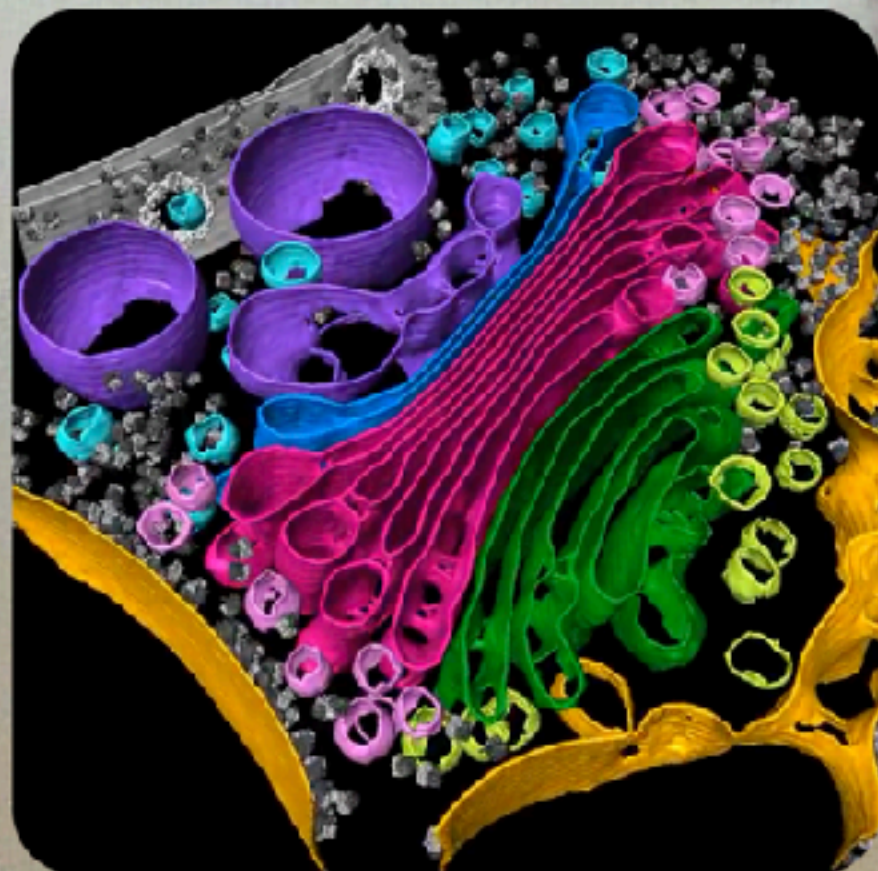
M. Cesaretti, ... A. Diaspro, et al., **Liver Transpl.** 26, 1224–1232 (2020)

In future perspective...



M. Cesaretti, ... A. Diaspro, et al., Liver Transpl. 26, 1224–1232 (2020)

In future perspective...



M. Cesaretti, ... A. Diaspro, et al., **Liver Transpl.** 26, 1224–1232 (2020)

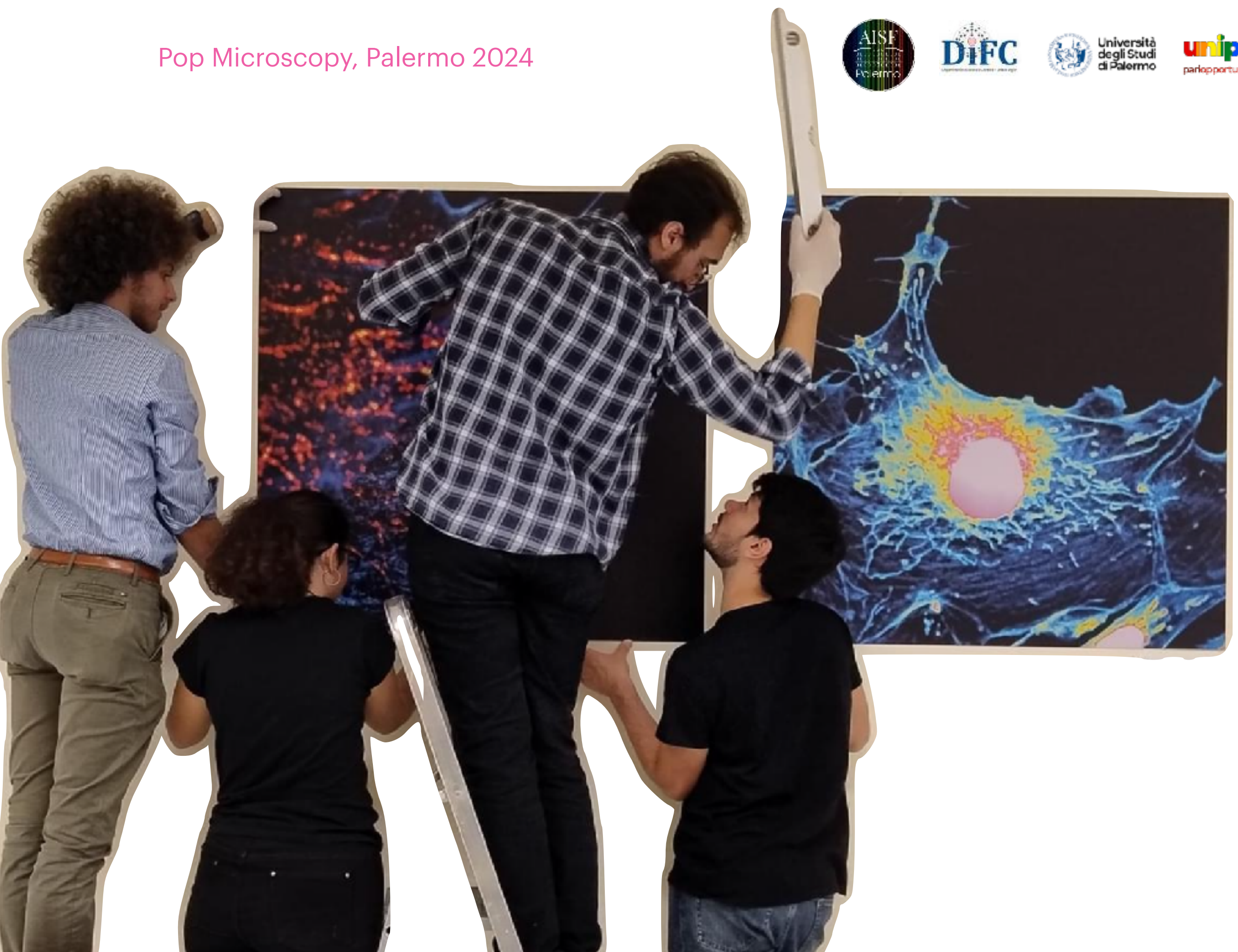


Pop Microscopy, Palermo 2024

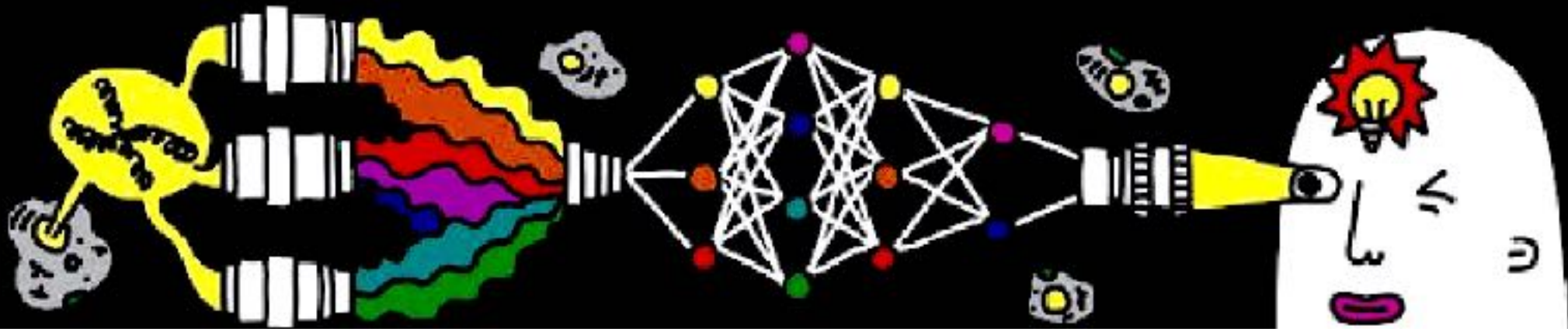


Università
degli Studi
di Palermo

unipa
parlo opportunità



Thank you
for your attention.



MOMIX

Multimodal Optical Microscopy Image Correlation Sensing



GENOA
ICBP 2026
June 28 – July 3

<https://www.icbp2026.org/>



GENOA
ICBP 2026
June 28 – July 3



ICBP2026 GENOA - ITALY

**12TH INTERNATIONAL
CONFERENCE ON
BIOLOGICAL PHYSICS**

JUNE 28 – JULY 3 2026

Magazzini del cotone, Genoa, Italy