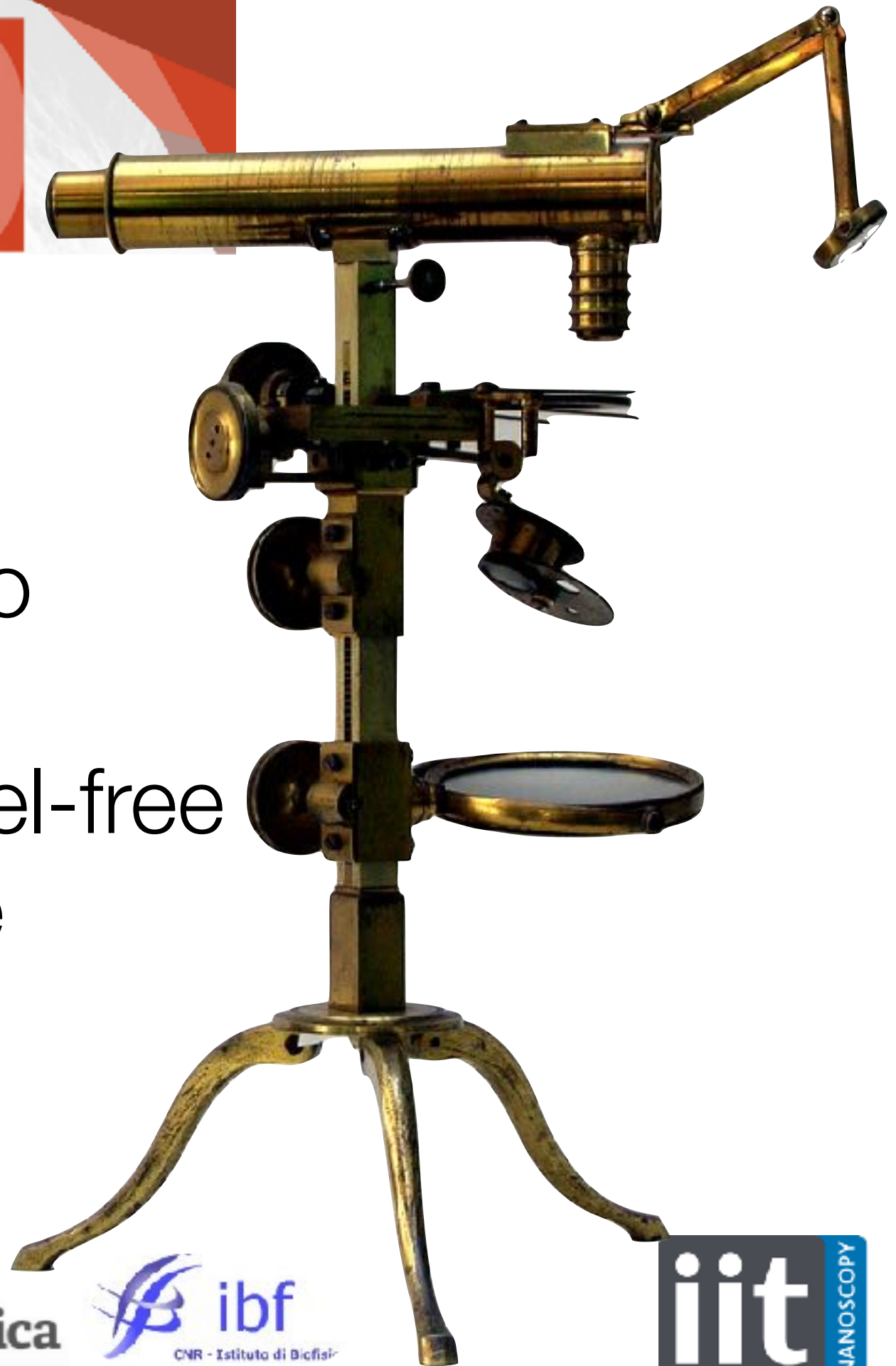


Alberto Diaspro

Il microscopio ottico
intelligente:
dalla fluorescenza al label-free
a super risoluzione
molecolare.



early days...

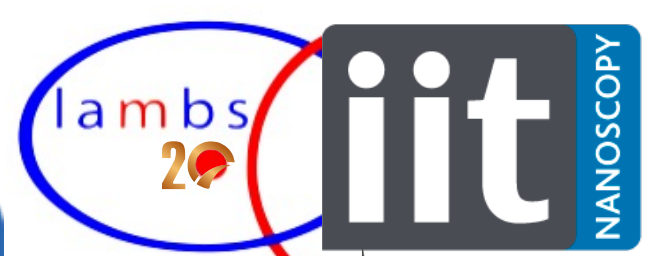


Premio Fermi - Fisica 2024



DiasproLab

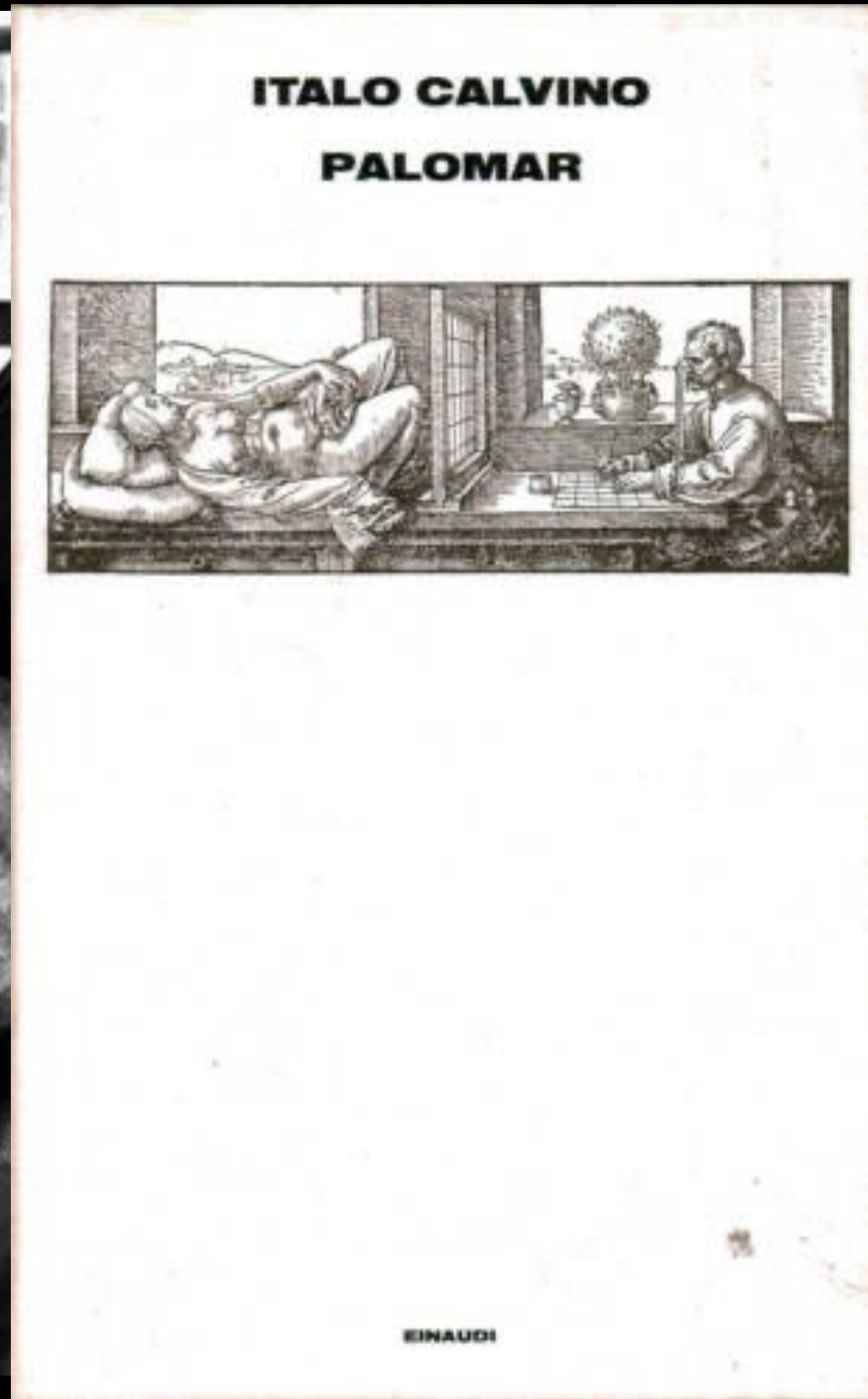
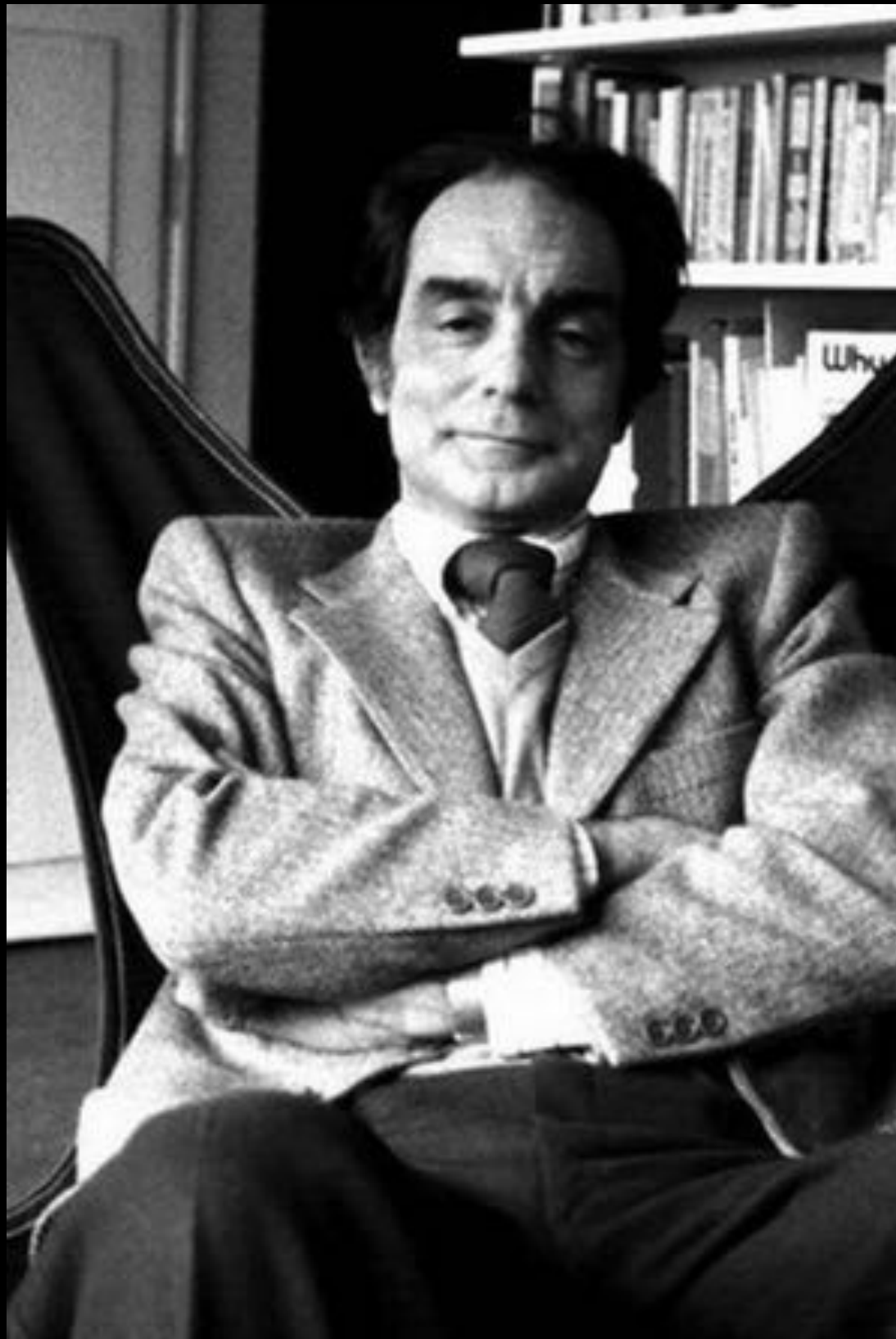
I-ALM node Genoa



DiasproLab retreat - Dinty Snipe Club, Genova - 2023



When there is a terrific starry night, Mr Palomar says:
I have to go watching at the stars

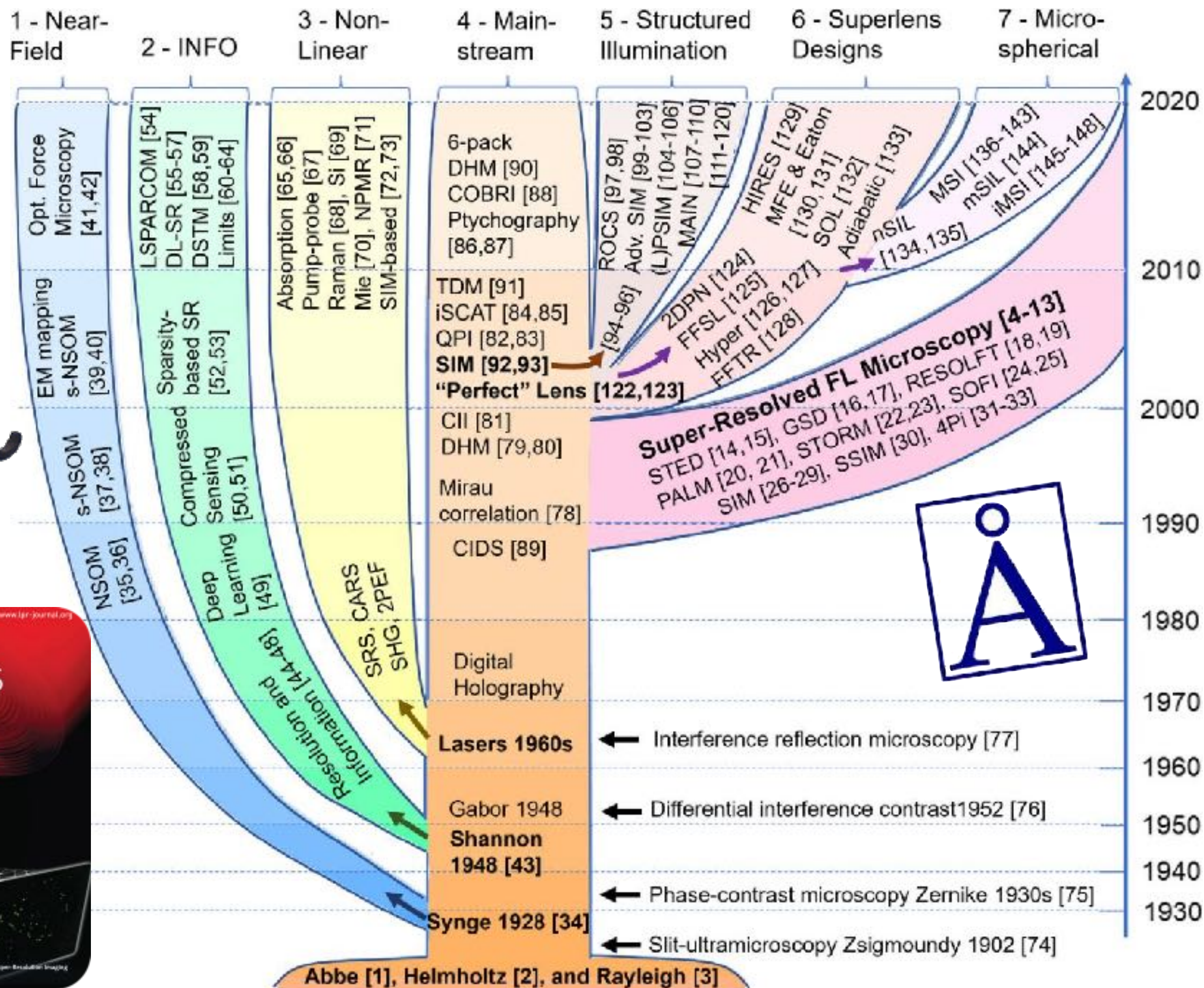


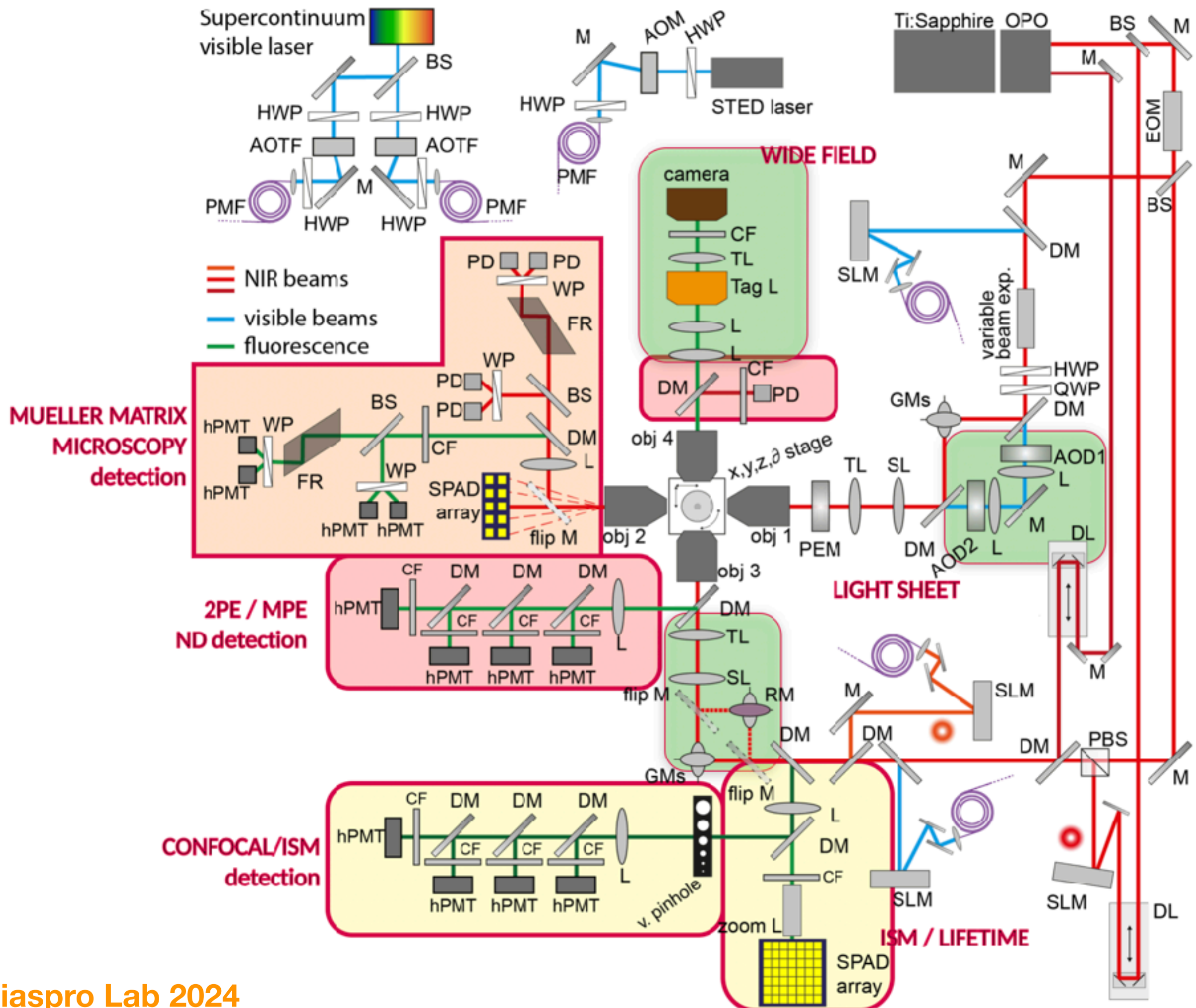
Italo Calvino (Santiago de Las Vegas de La Habana, 15 ottobre 1923 – Siena, 19 settembre 1985)

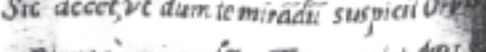




Diaspro Lab, set-up







ADA LOVELACE

FIRST COMPUTER PROGRAMMER



The Analytical Engine

Lovelace's program turned a complex formula into simple calculations that could be encoded on punched cards and fed into Charles Babbage's Analytical Engine, a mechanical computer that he designed but never built. She published it in 1843, a century before the modern computer age.

"I want to put in something about Bernoulli's Number, in one of my Notes, as an example of how an explicit function may be worked out by the engine, without having been worked out by human head and hands first."



$$\frac{x}{e^x - 1} = \frac{1}{1 + \frac{x}{2} + \frac{x^2}{2 \cdot 3} + \frac{x^3}{2 \cdot 3 \cdot 4} + \&c.}$$



A Universal Computer

Lovelace did more than write the first computer program. She was also the first person to realise that a general purpose computer could do anything, given the right data and instructions.

"The Analytical Engine weaves algebraic patterns just as the Jacquard loom weaves flowers and leaves."

"Supposing, for instance, that the fundamental relations of pitched sounds in the science of harmony and of musical composition were susceptible of such expression and adaptations, the engine might compose elaborate and scientific pieces of music of any degree of complexity or extent."



**Augusta Ada King,
Countess of Lovelace**
Born: 10 December 1815
Died: 27 November 1852



Ada Lovelace Day
FindingAda.com



LOVELACE

ADA LOVELACE

FIRST COMPUTER PROGRAMMER



The Analytical Engine

Lovelace's program turned a complex formula into simple calculations that could be encoded on punched cards and fed into Charles Babbage's Analytical Engine, a mechanical computer that he designed but never built. She published it in 1843, a century before the modern computer age.

"I want to put in something about Bernoulli's Number, in one of my Notes, as an example of how an explicit function may be worked out by the engine, without having been worked out by human head and hands first."



$$\frac{x}{e^x - 1} = \frac{1}{1 + \frac{x}{2}}$$



A Universal Calculator

Lovelace did more than write the first computer program. She was also the first person to realise that a general purpose computer could do anything, given the right data and instructions.

"The Analytical Engine weaves algebraic patterns just as the Jacquard loom weaves flowers and leaves."

Lovelace did more than write the first computer program. She was also the first person to realise that a general purpose computer could do anything, given the right data and instructions.

and of musical composition were susceptible of such expression and adaptations, the engine might compose elaborate and scientific pieces of music of any degree of complexity or extent."



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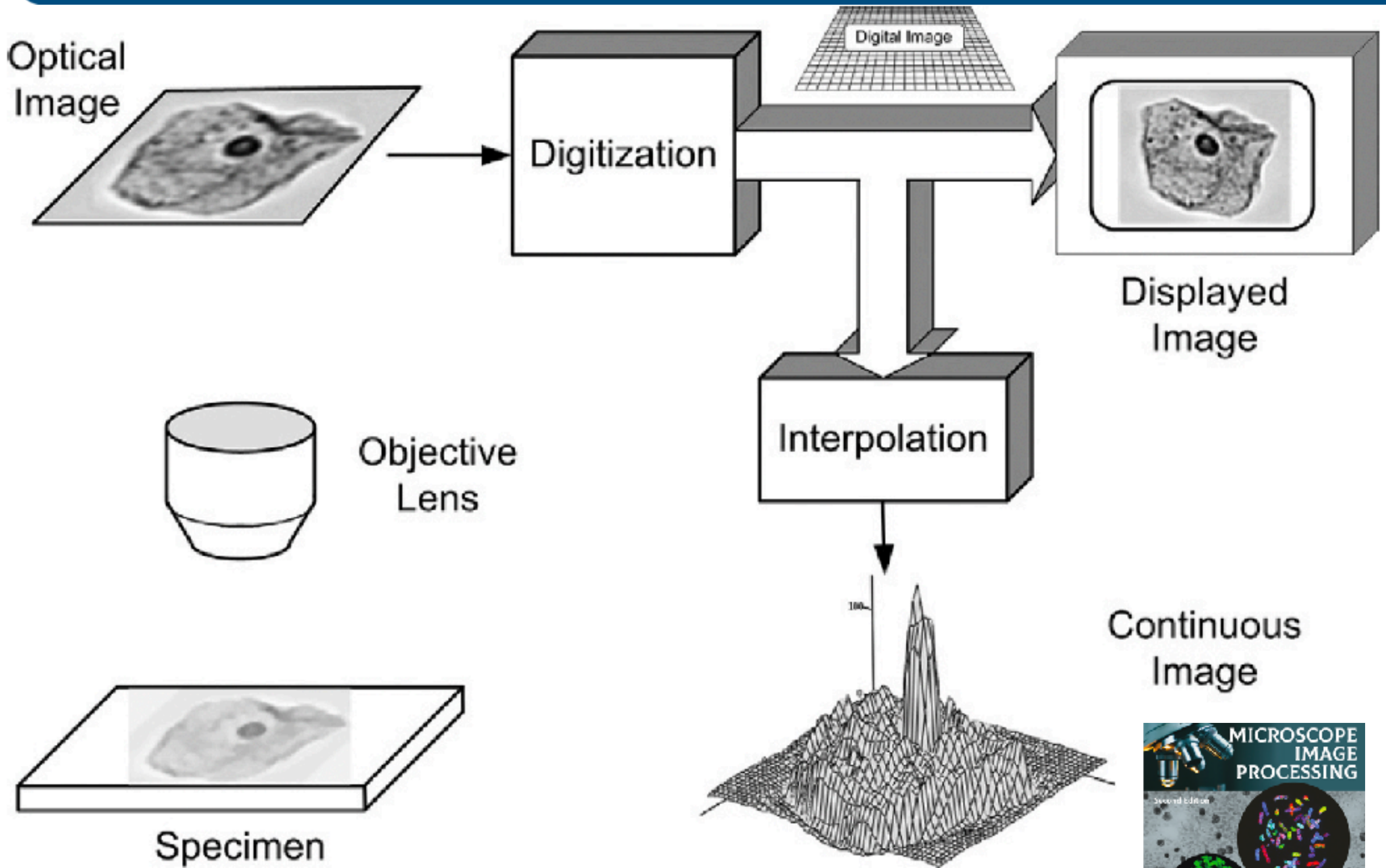


Ada Lovelace Day
FindingAda.com



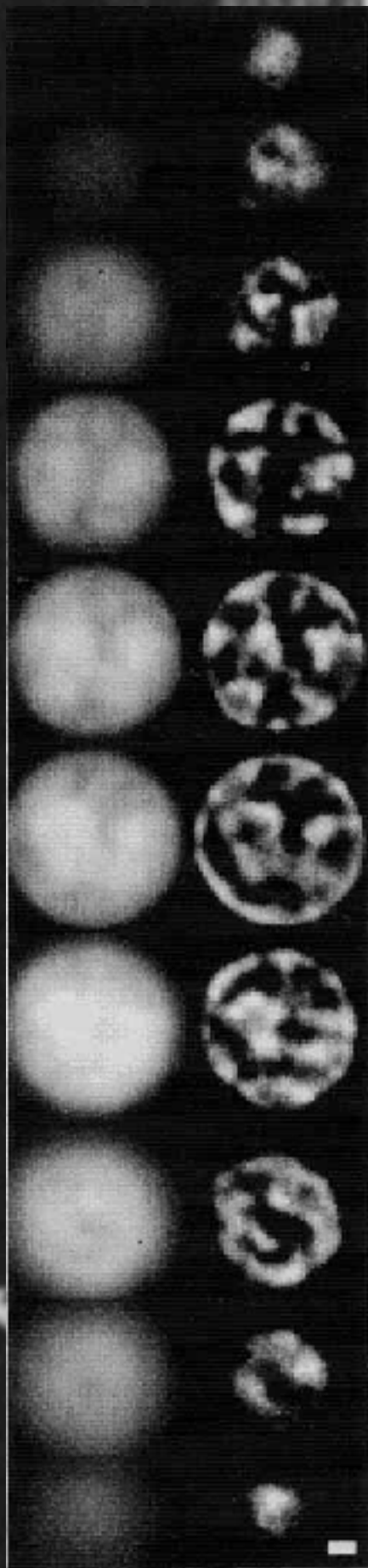
LOVELACE

The four images of digital microscopy. The microscope forms an optical image of the specimen. This is digitized to produce the digital image, which can be displayed and interpolated to form the continuous image. The displayed image allows the original image to be visualized.



Fatima A. Merchant, Alberto Diaspro, Chapter 11th - Three-Dimensional Imaging,
Editor(s): Fatima A. Merchant, Kenneth R. Castleman,
Microscope Image Processing Academic Press, 2023





A.Diaspro et al., Image and Vision Computing, 1990

13 years before the early days...

3D representation of biostructures imaged with an optical microscope

A Diaspro et al

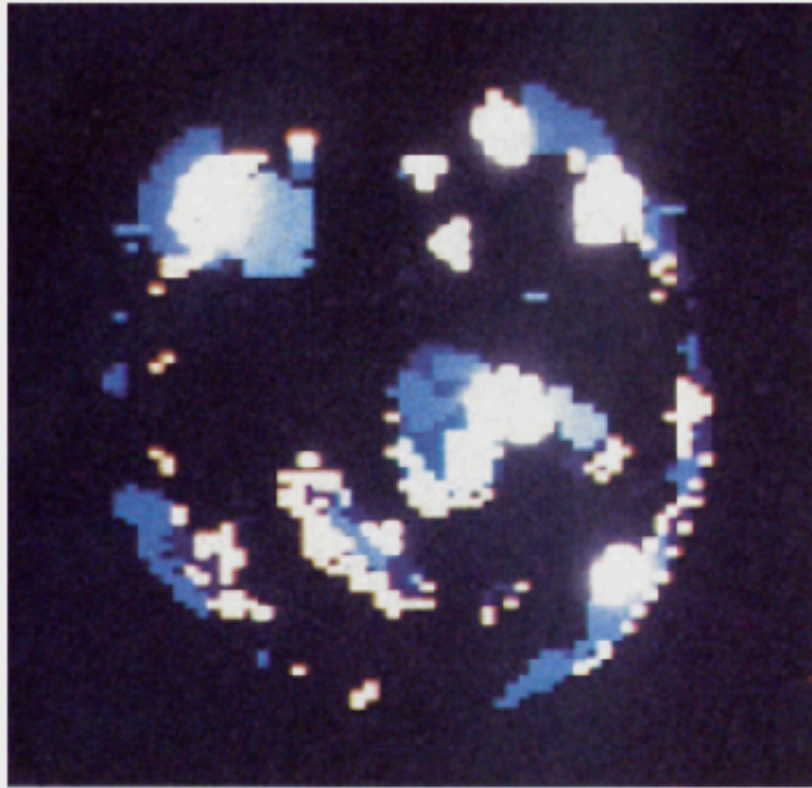


Plate 9. Top view of chromatin layouts. The pixel intensity is directionally proportional to the distance of the observer from the optical section contributing to the overall picture

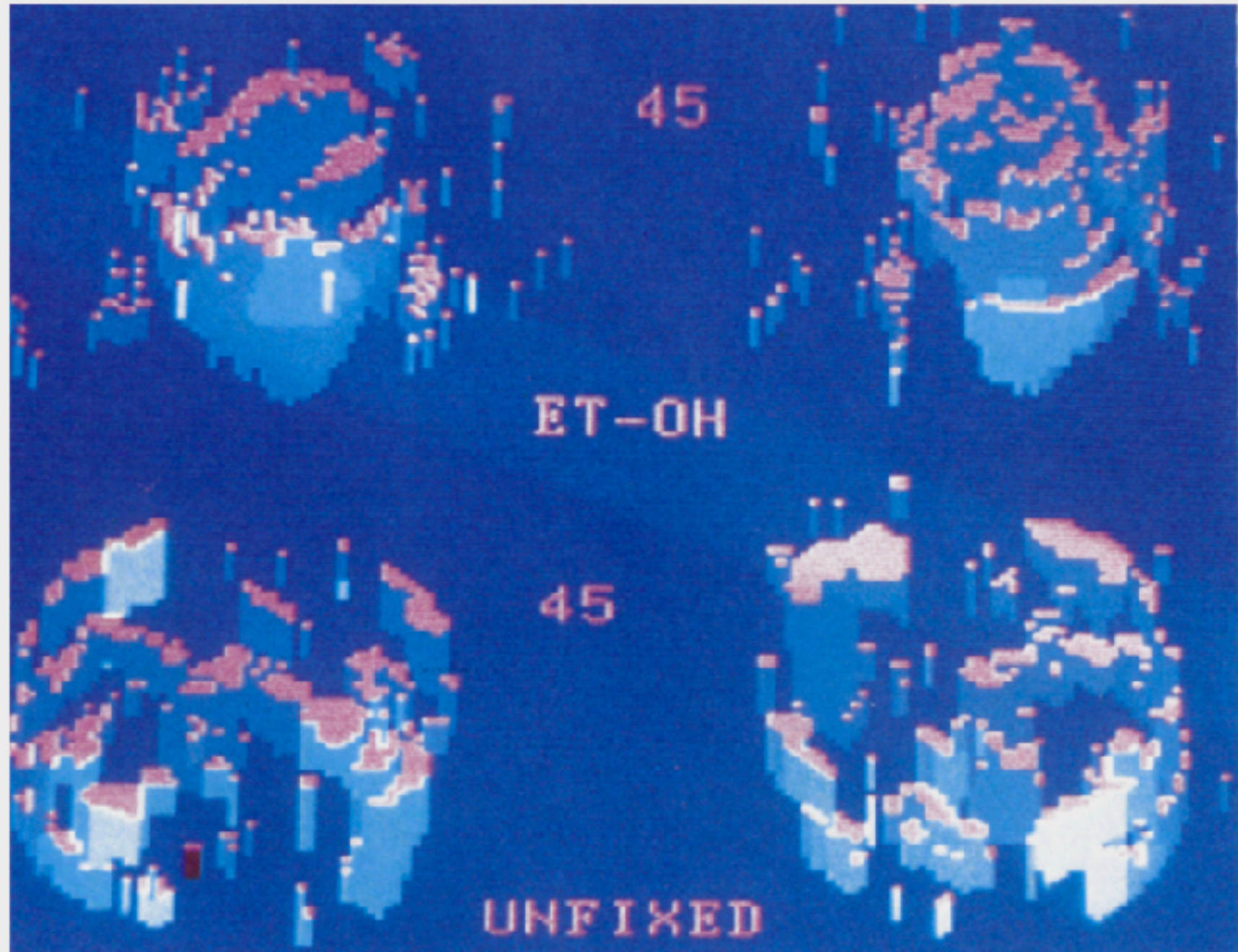


Plate 10. Four 3D representations of differently viewed chromatin layouts (see text)

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

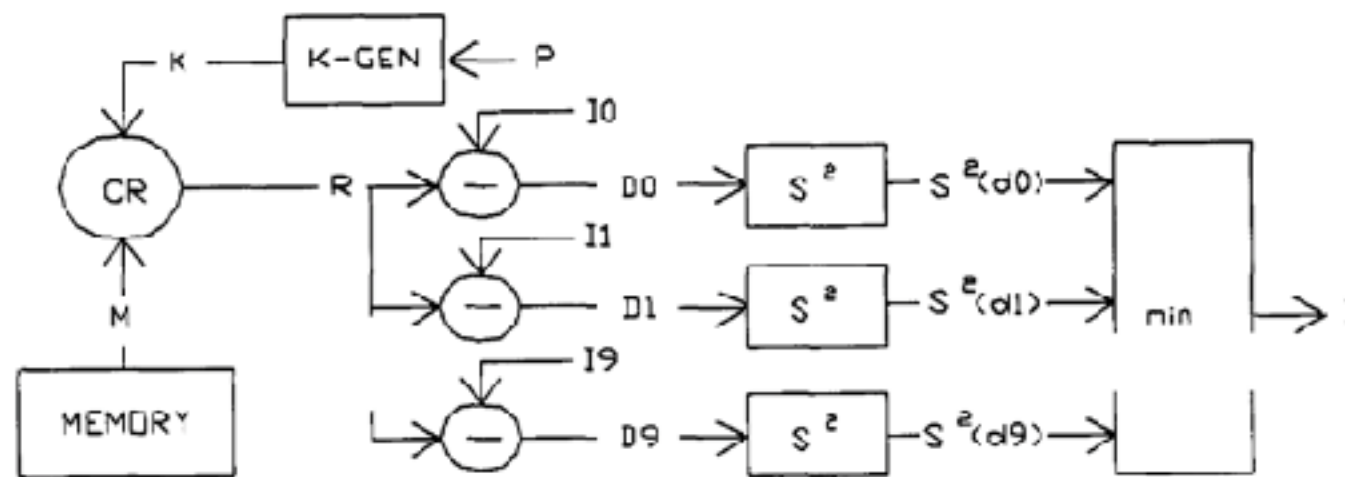
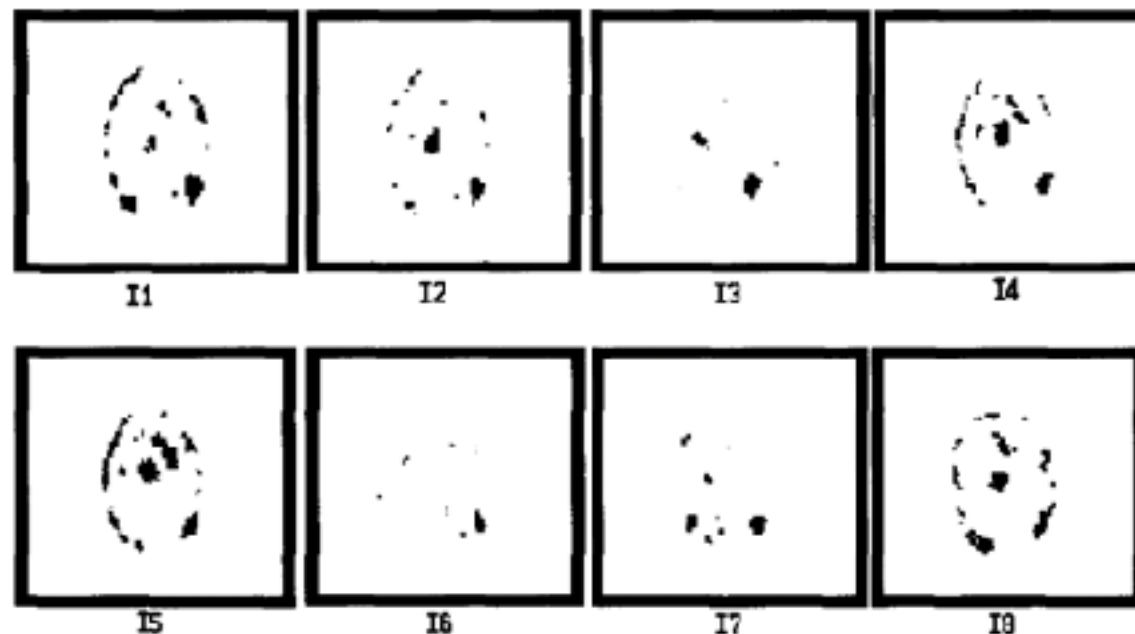


Figure 1. The schema of the associative classifier.



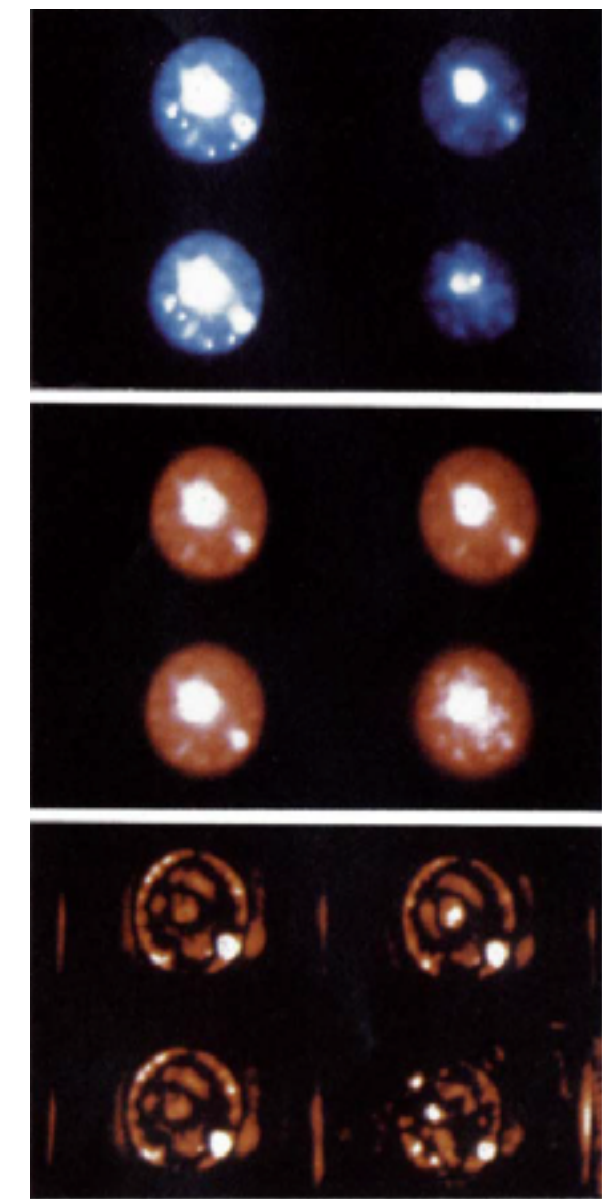
The Nobel Prize in Physics 2024



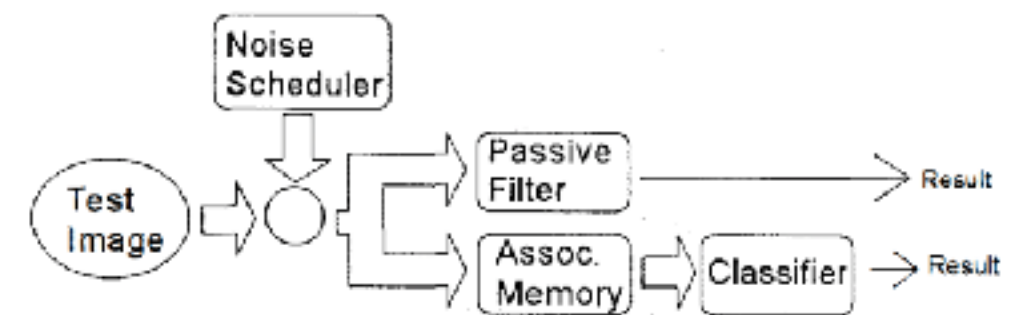
2024 Nobel Prize in Physics
Dutch
John J. Hopfield
1928-2024



2024 Nobel Prize in Physics
Dutch
Geoffrey E. Hillson
1928-2024



A.Diaspro, Image and Vision Computing, 1990

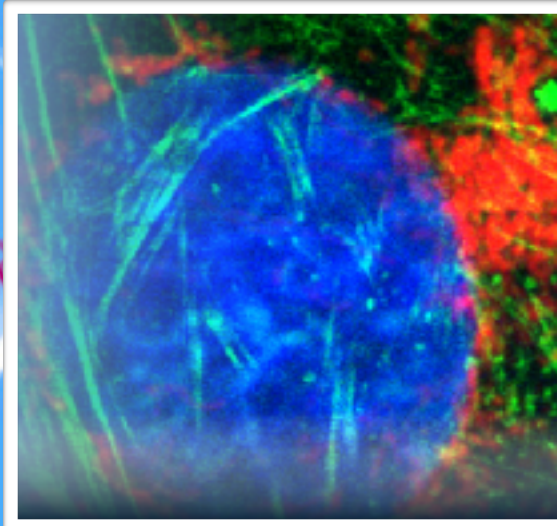


A.Diaspro, G.Parodi, R.Zunino, **Studia Biophysica**, vol.139, no.2, pp.69-16, 1990

13 years before the early days...

8 43 2020

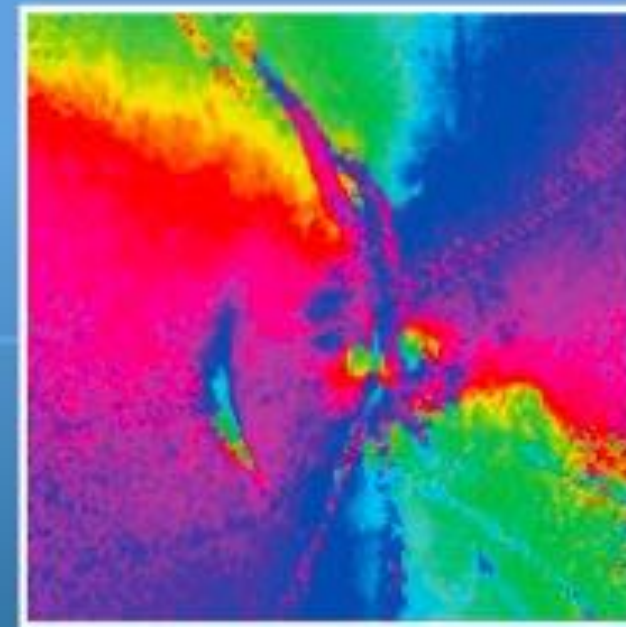
La Rivista del Nuovo Cimento *della Società Italiana di Fisica*



La Rivista del Nuovo Cimento *della Società Italiana di Fisica*

Emerging Mueller matrix microscopy applications
in biophysics and biomedicine

ALBERTO DIASPRO • PAOLO BIANCHINI • FABIO CALLEGARI •
LISA CUNEO • RICCARDO MARONGIU • AYMERIC LE GRATIET •
ALI MOHEBI • M. SCOTTO • COLIN J. R. SHEPPARD



Optical nanoscopy

A. Diaspro, P. Bianchini

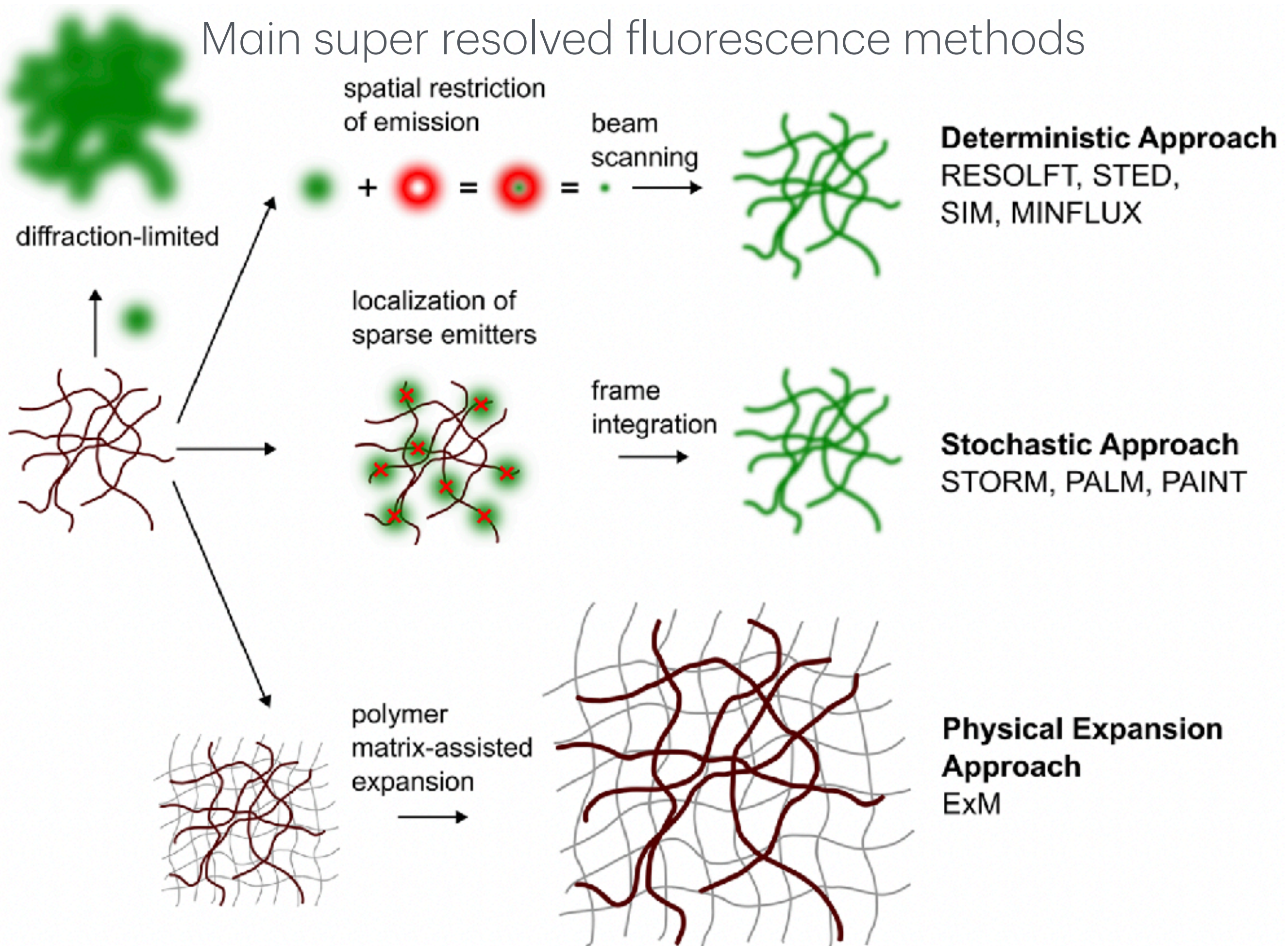


Recognized by the European Physical Society
Associated to the European Physical Journal



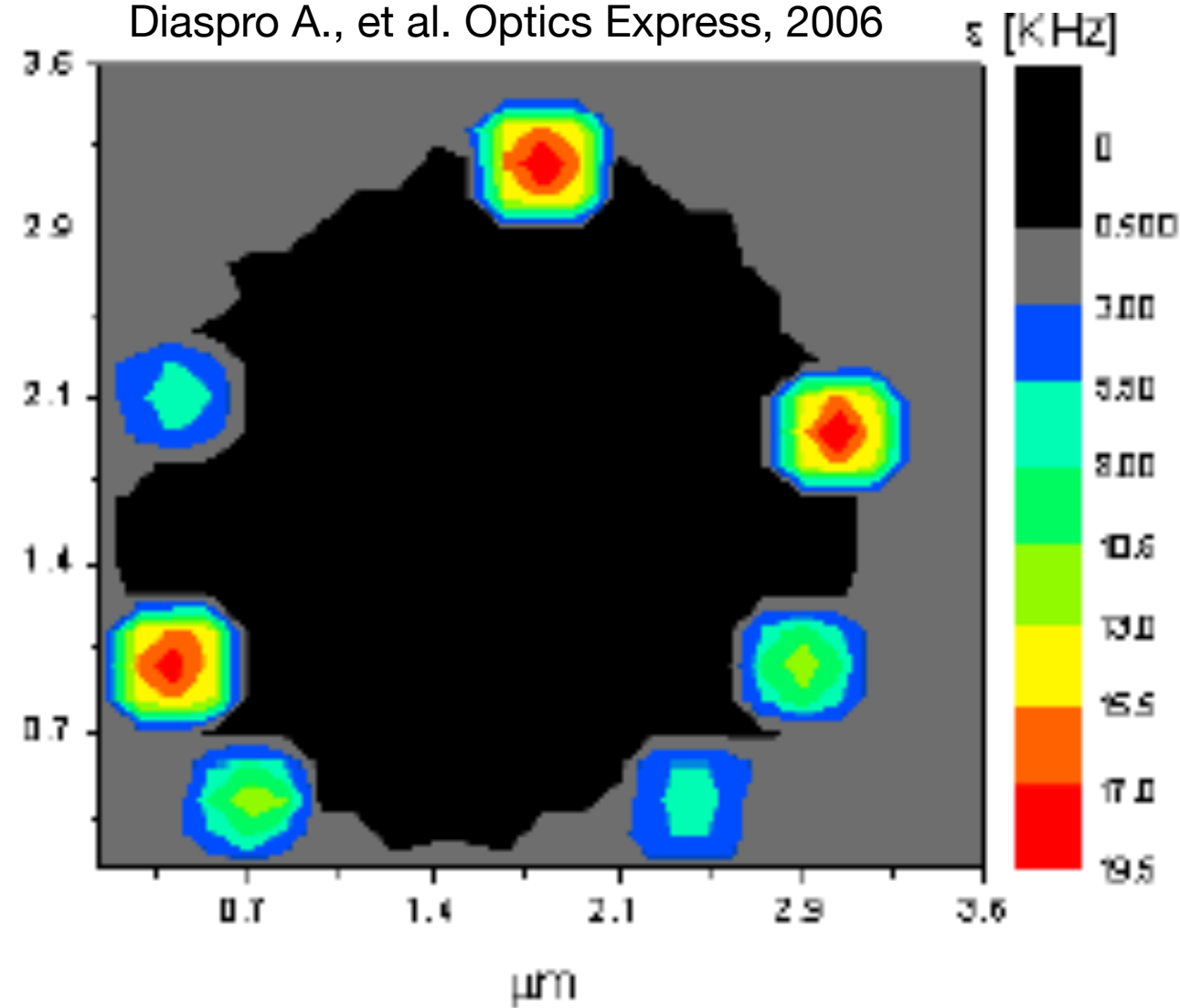
Springer

Main super resolved fluorescence methods



Single Molecule and Super resolved Microscopy

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, 18148 Genoa, Italy; and [§]IFOM, Istituto FIRC di Oncologia Molecolare, 20139 Milan, Italy

INSTITUTE OF PHYSICS PUBLISHING

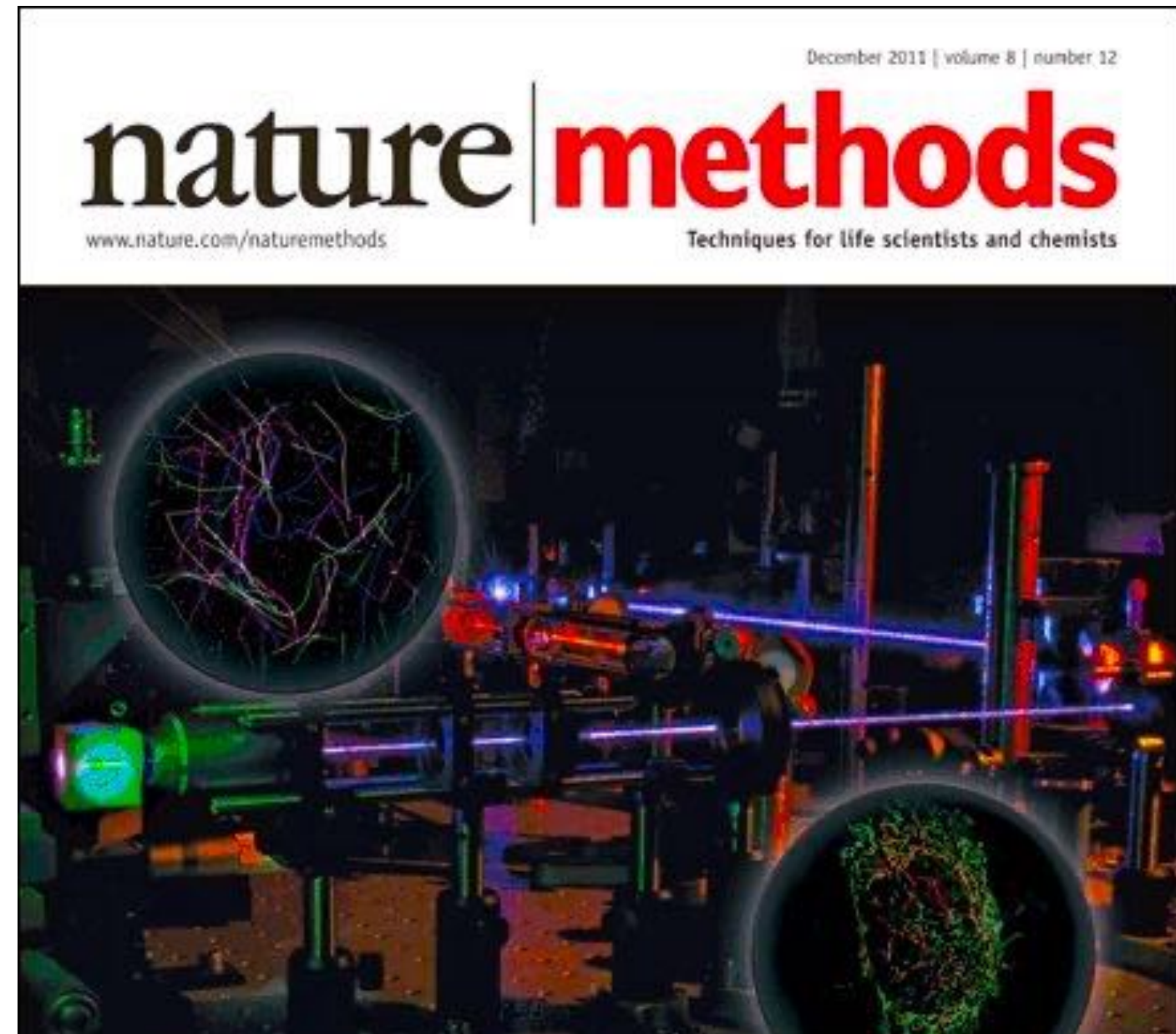
J. Phys. D: Appl. Phys. 36 (2003) 1682–1688

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



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

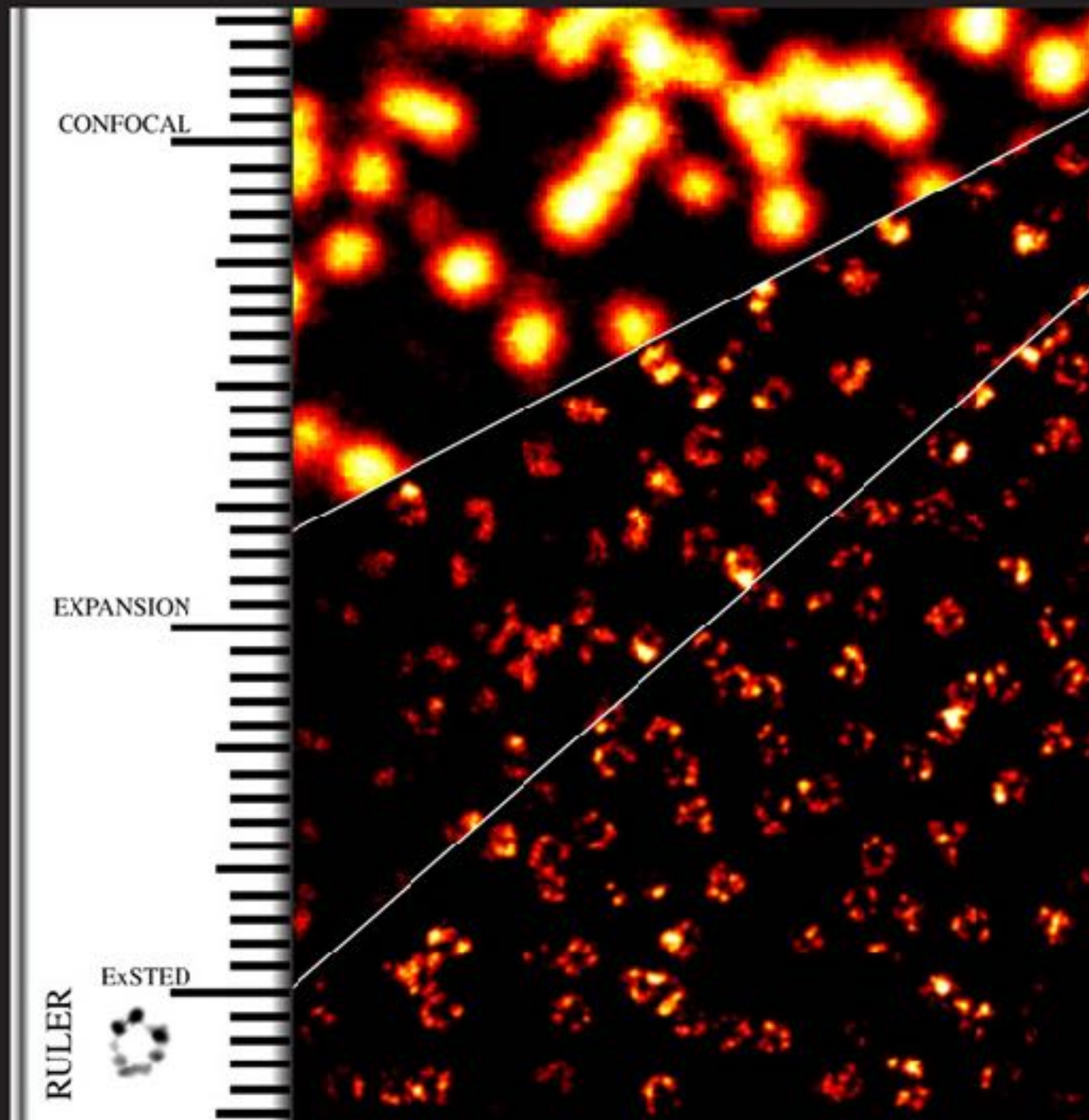
Paolo Bianchini^{*}, Benjamin Harke^{*}, Silvia Galiani^{*,§}, Giuseppe Vicidomini^{*}, and Alberto Diaspro^{*,§,1}



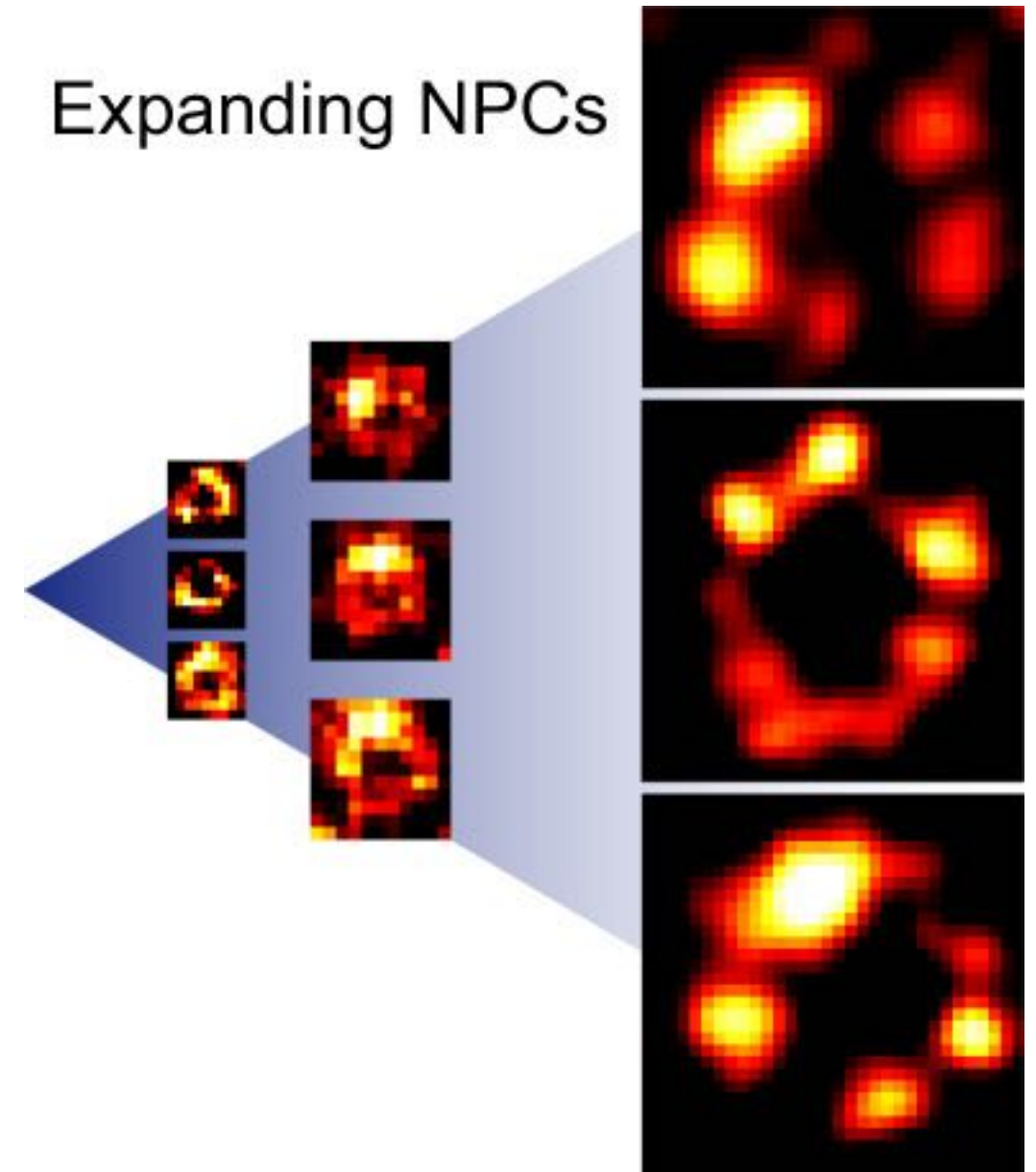
STED super-resolved microscopy

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

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



Expanding NPCs



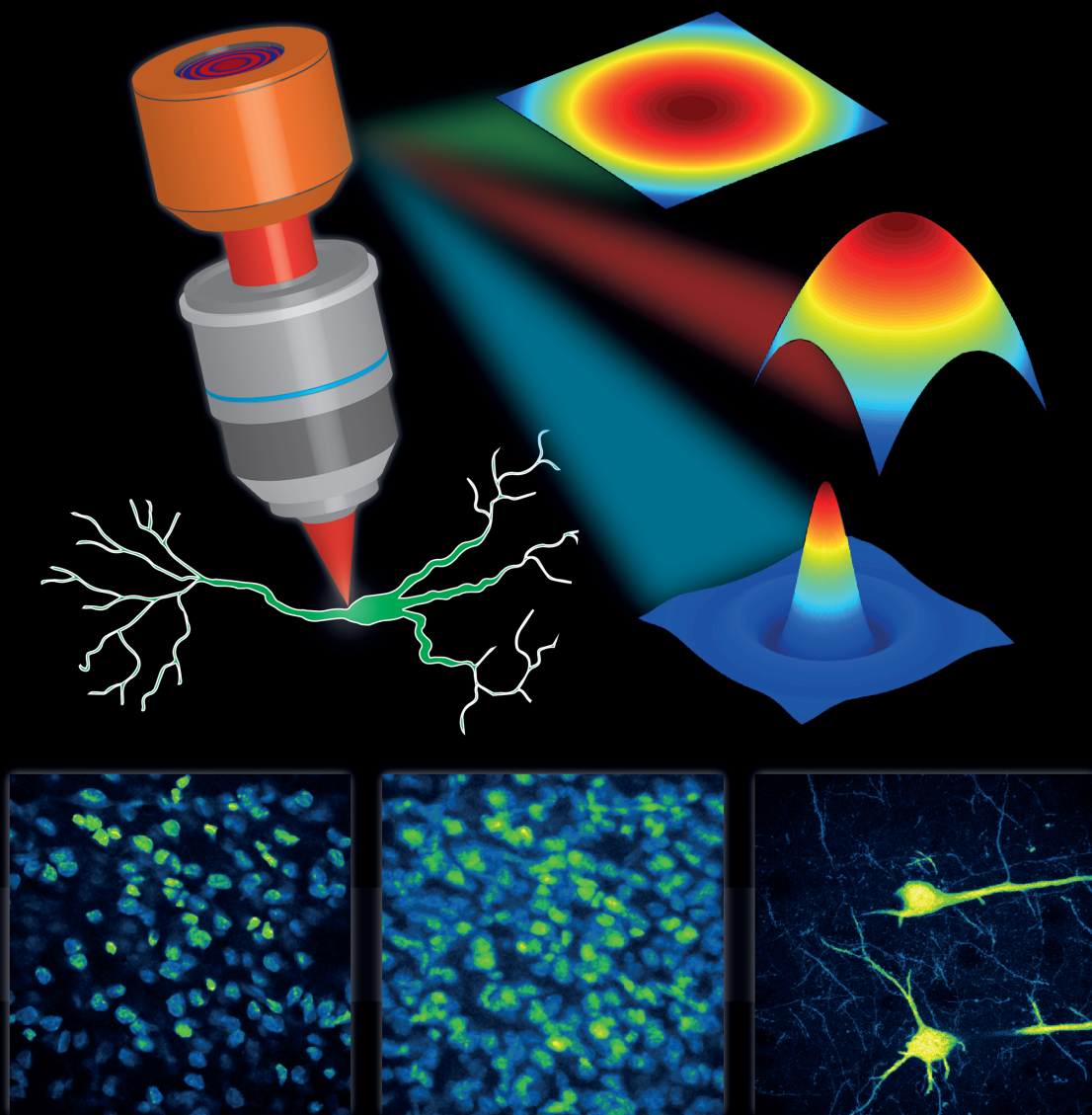
Beam shaping and Label-free Microscopy

Journal of

BIOPHOTONICS

2/18

www.biophotonics-journal.org



Enhanced volumetric imaging in 2-photon microscopy via acoustic lens beam shaping
Simonluca Piazza, Paolo Bianchini, Colin Sheppard, Alberto Diaspro, Martí Duocastella

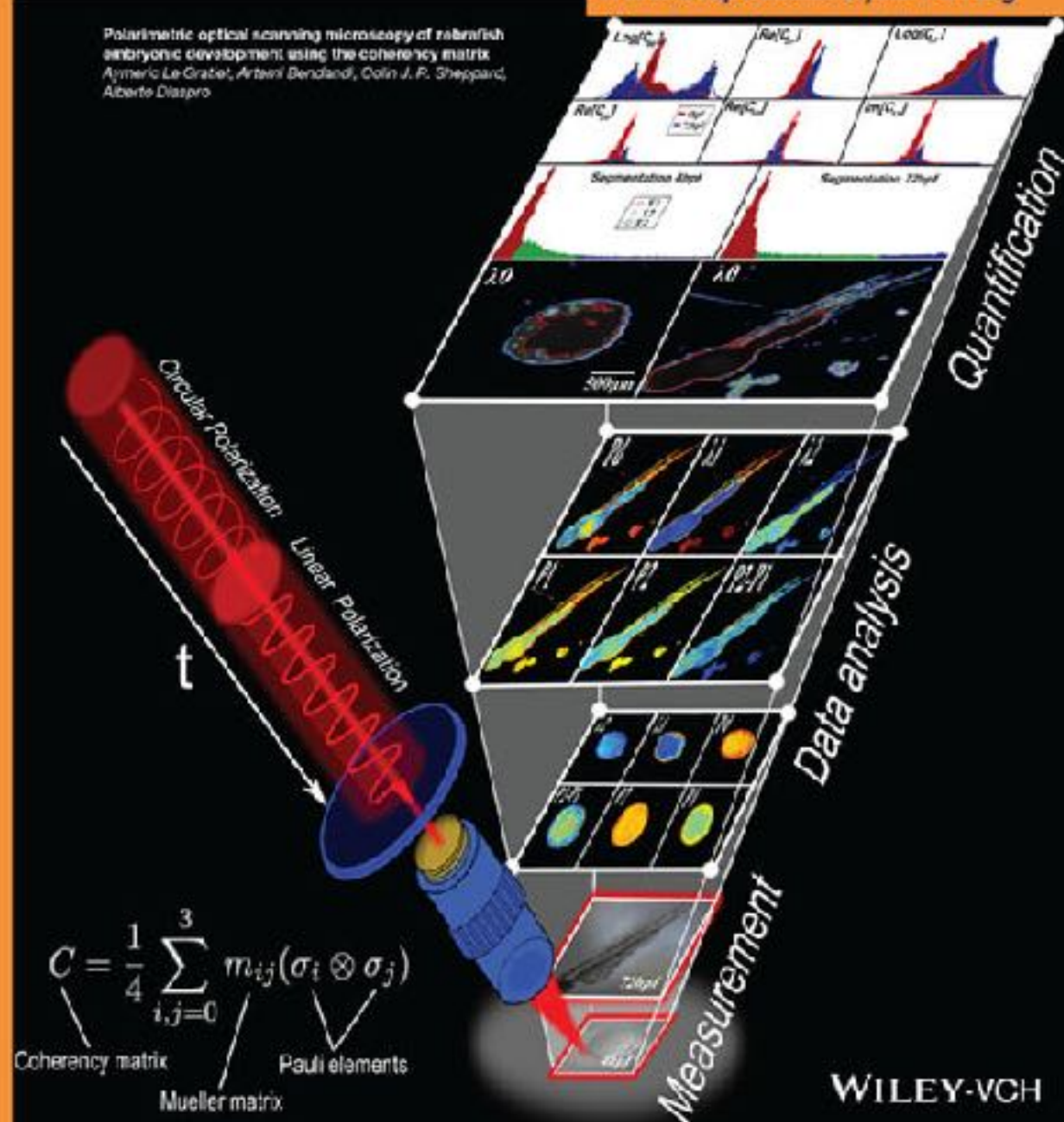
WILEY-VCH

6/2021

JOURNAL OF
BIOPHOTONICS

www.biophotonics-journal.org

Polarimetric optical scanning microscopy of zebrafish embryonic development using the coherency matrix
Aymeric Le Griset, Arseni Bendandi, Colin J. R. Sheppard, Alberto Diaspro



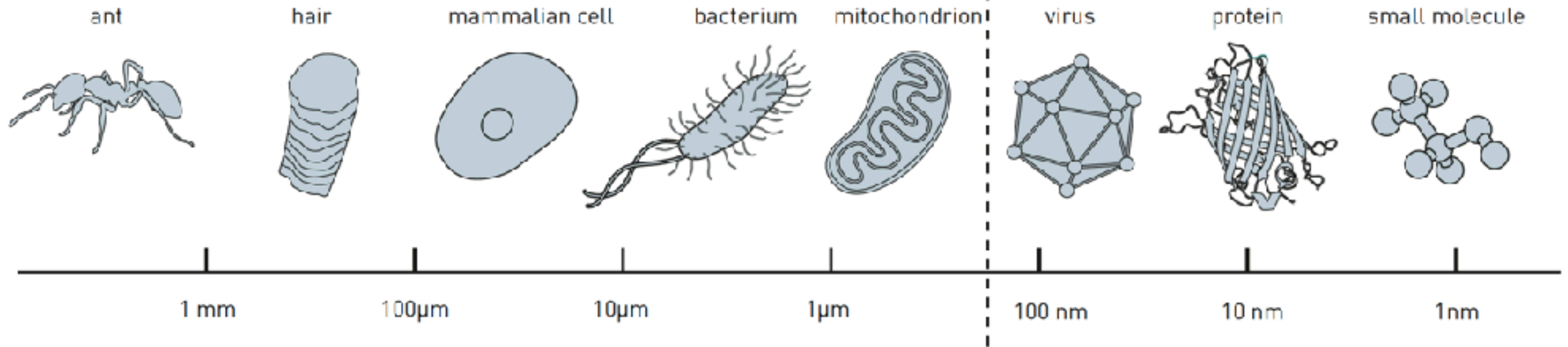
WILEY-VCH

Multi-modal molecular content





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.



Object

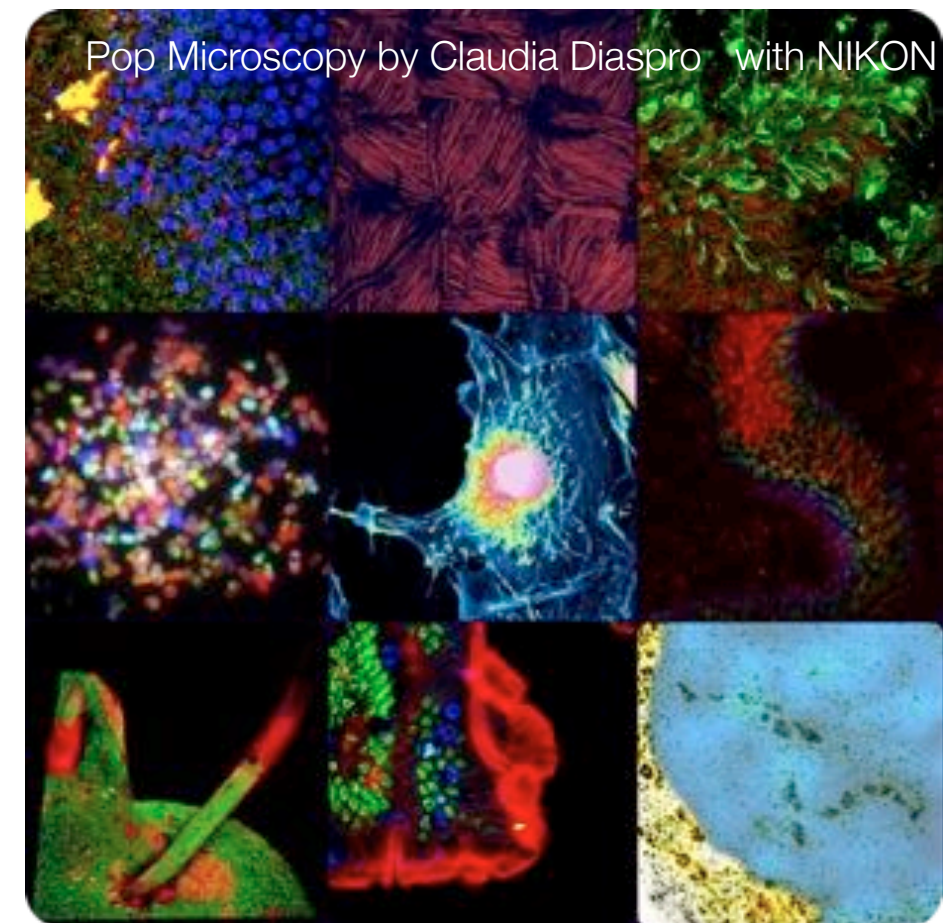
$$t(x, y) = a(x, y)e^{i\phi(x, y)}$$

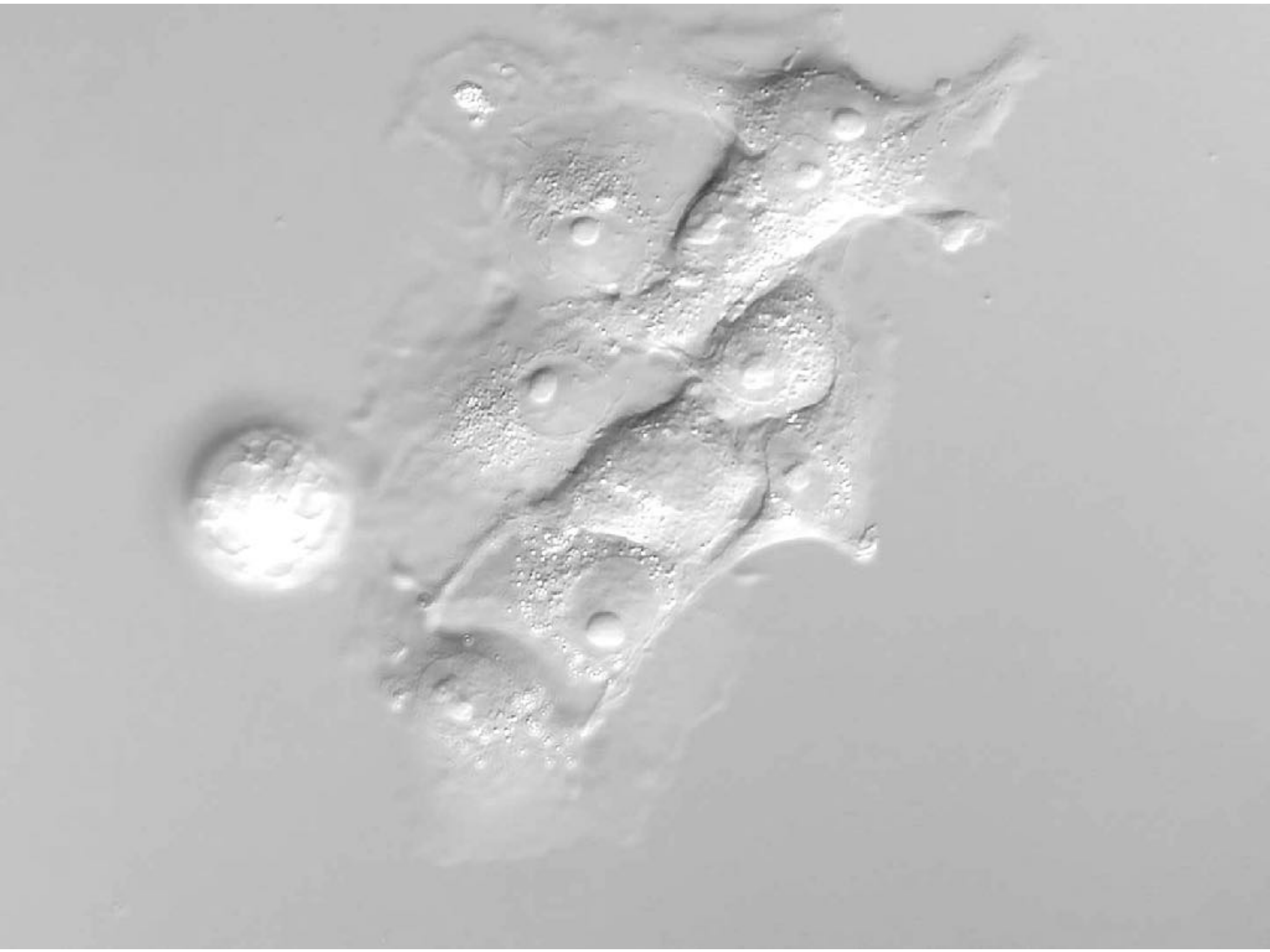
$a(x, y)$ is modulus (amplitude), real

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

Perfect image

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







1906

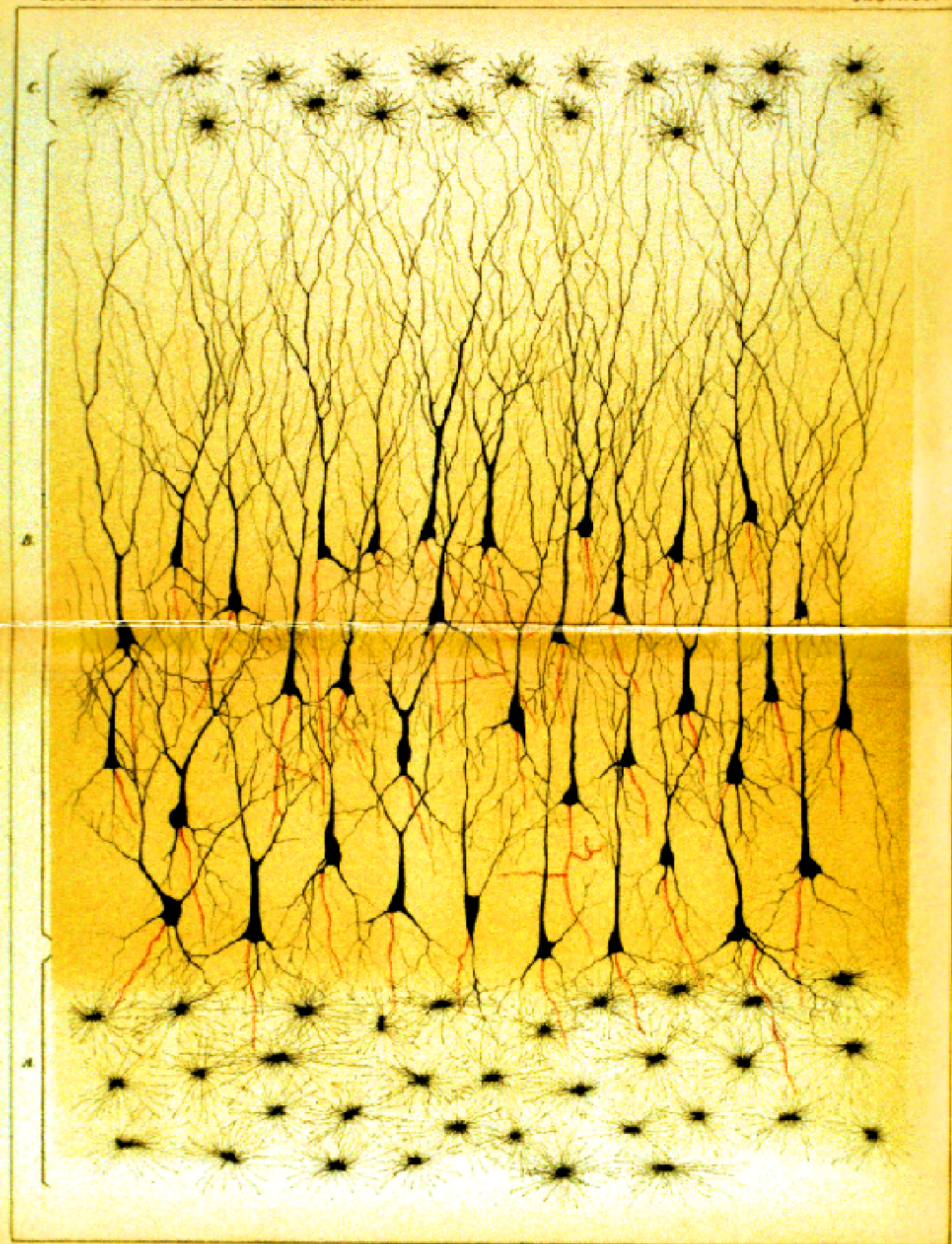


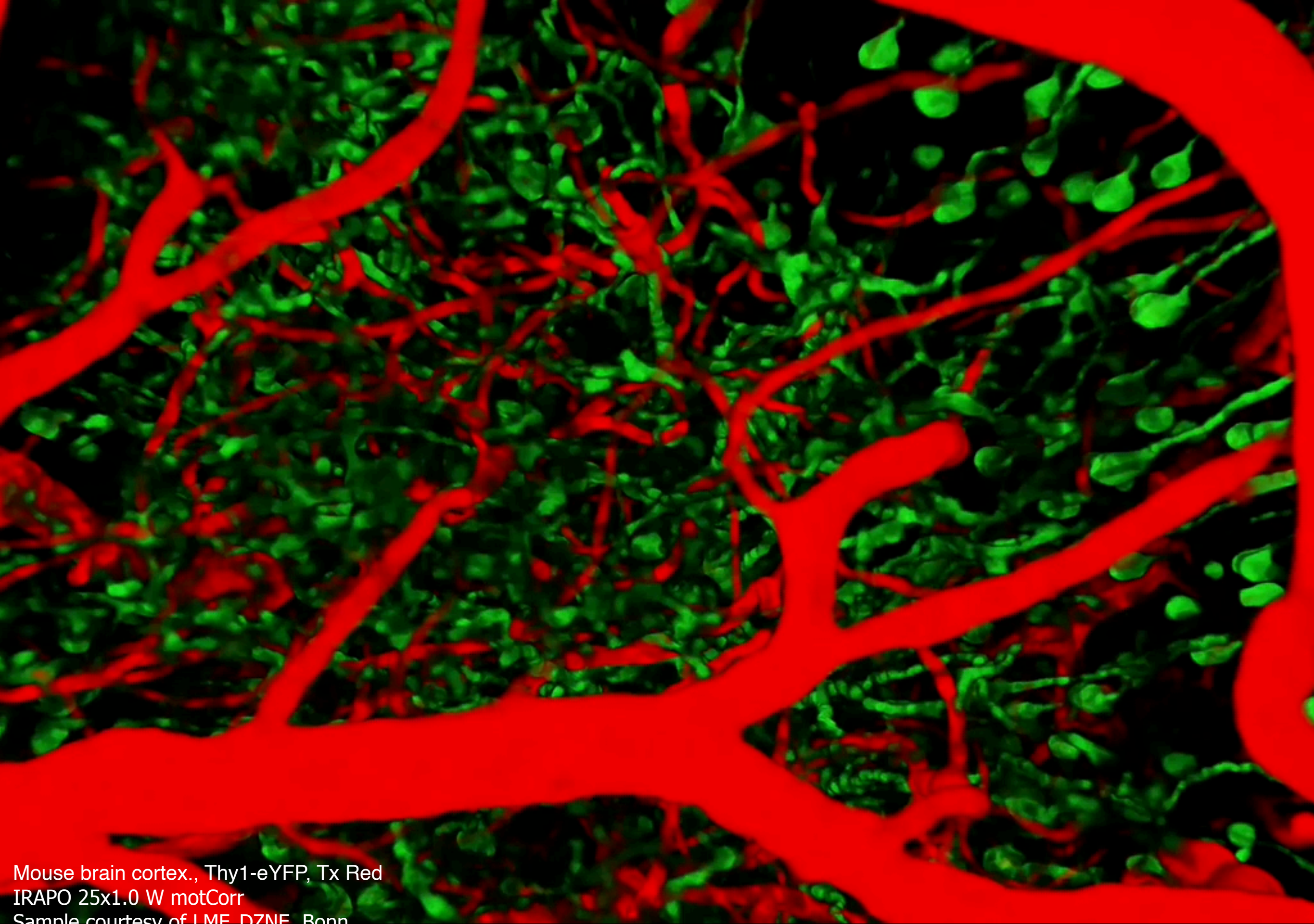


1906

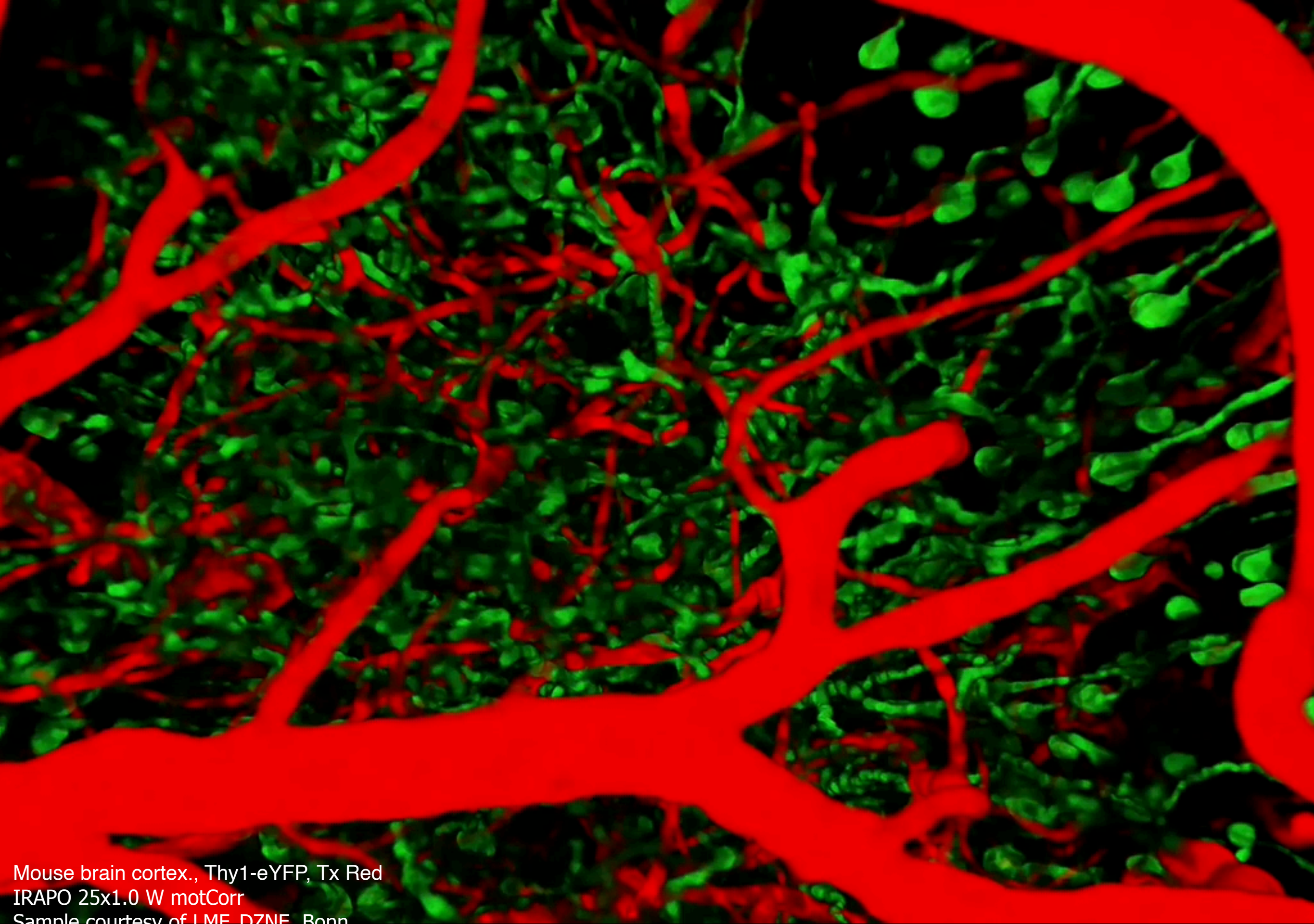


1906

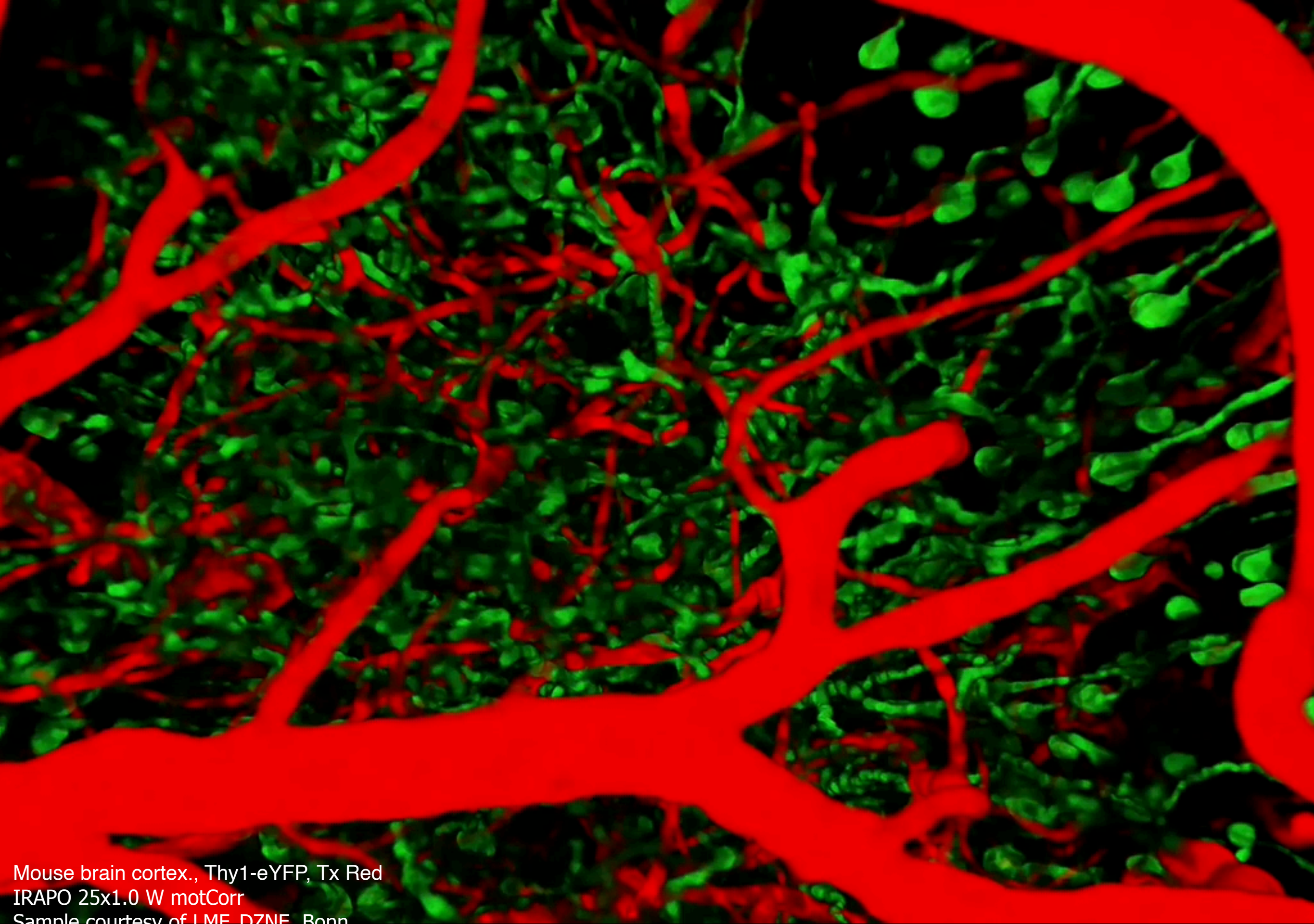




Mouse brain cortex., Thy1-eYFP, Tx Red
IRAPO 25x1.0 W motCorr
Sample courtesy of LMF, DZNE, Bonn



Mouse brain cortex., Thy1-eYFP, Tx Red
IRAPO 25x1.0 W motCorr
Sample courtesy of LMF, DZNE, Bonn



Mouse brain cortex., Thy1-eYFP, Tx Red
IRAPO 25x1.0 W motCorr
Sample courtesy of LMF, DZNE, Bonn



1953

FLUORESCENCE

Springer Series in Fluorescence 17
Series Editor: Martin Heide

David M. Jameson · Editor

Perspectives on Fluorescence

A Tribute to Gregorio Weber

Springer

Pavla Puri Mondal
Alberto Diaspro

Fundamentals of Fluorescence Microscopy

Exploring Life with Light

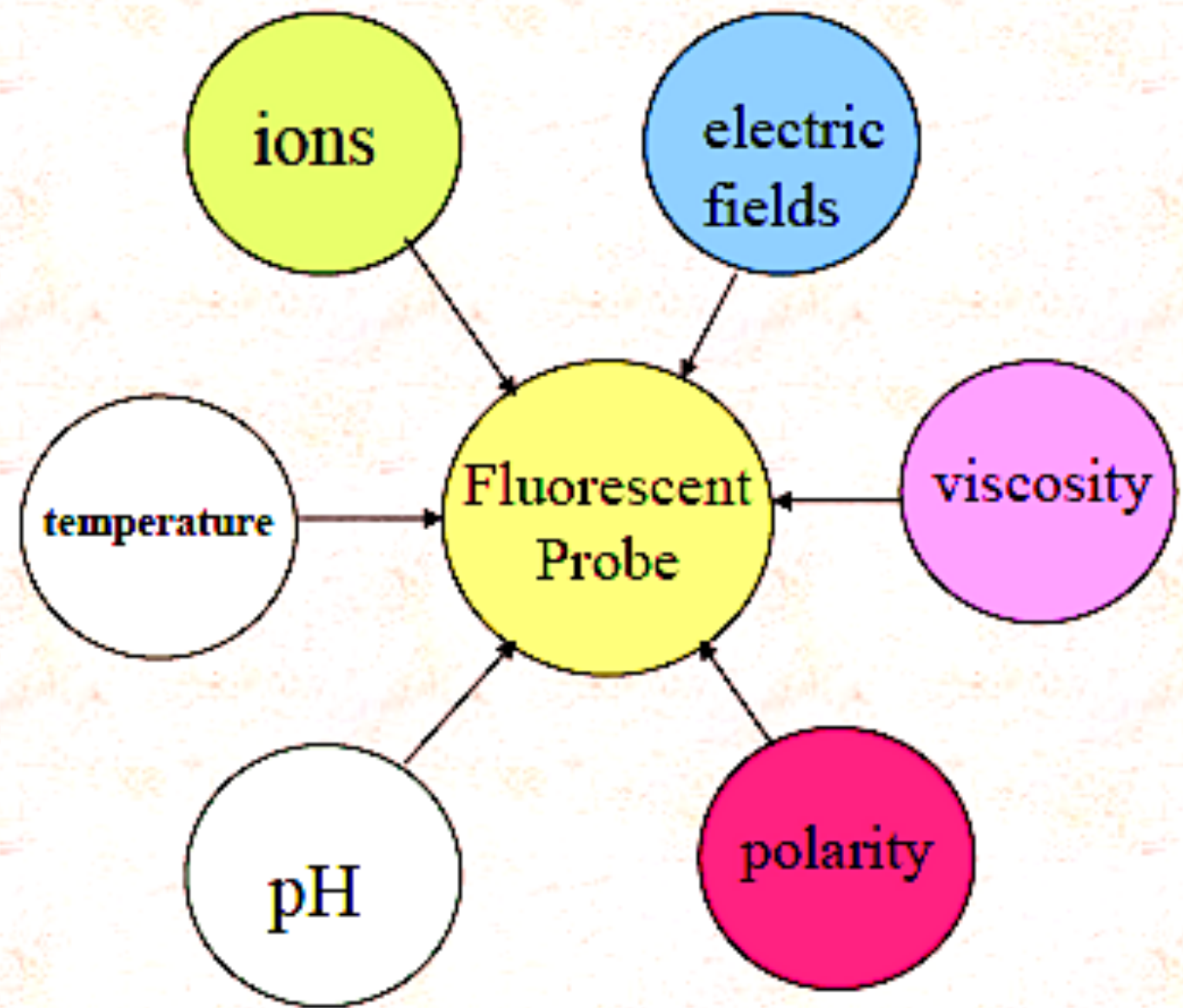


Springer

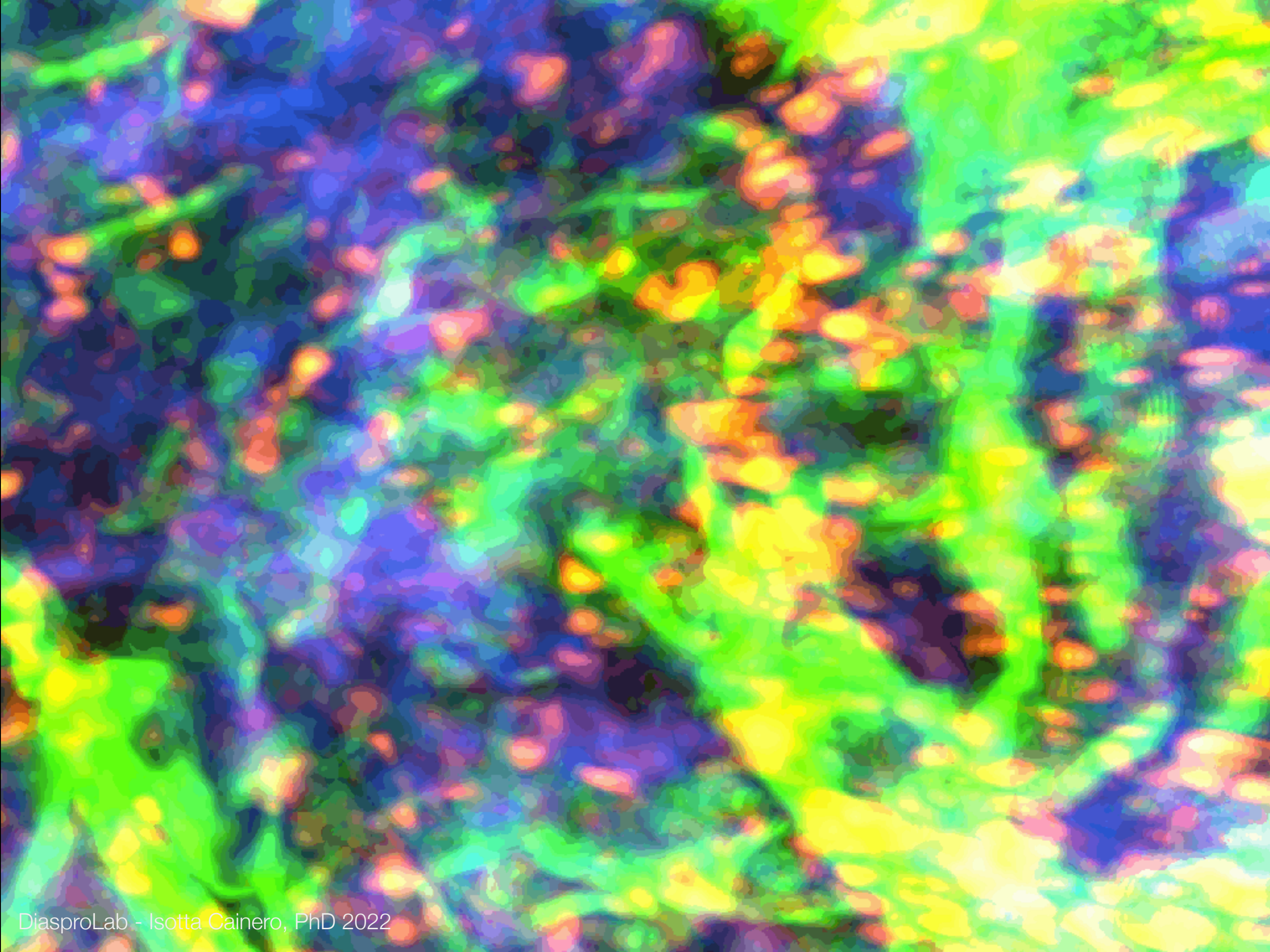
Why fluorescence?

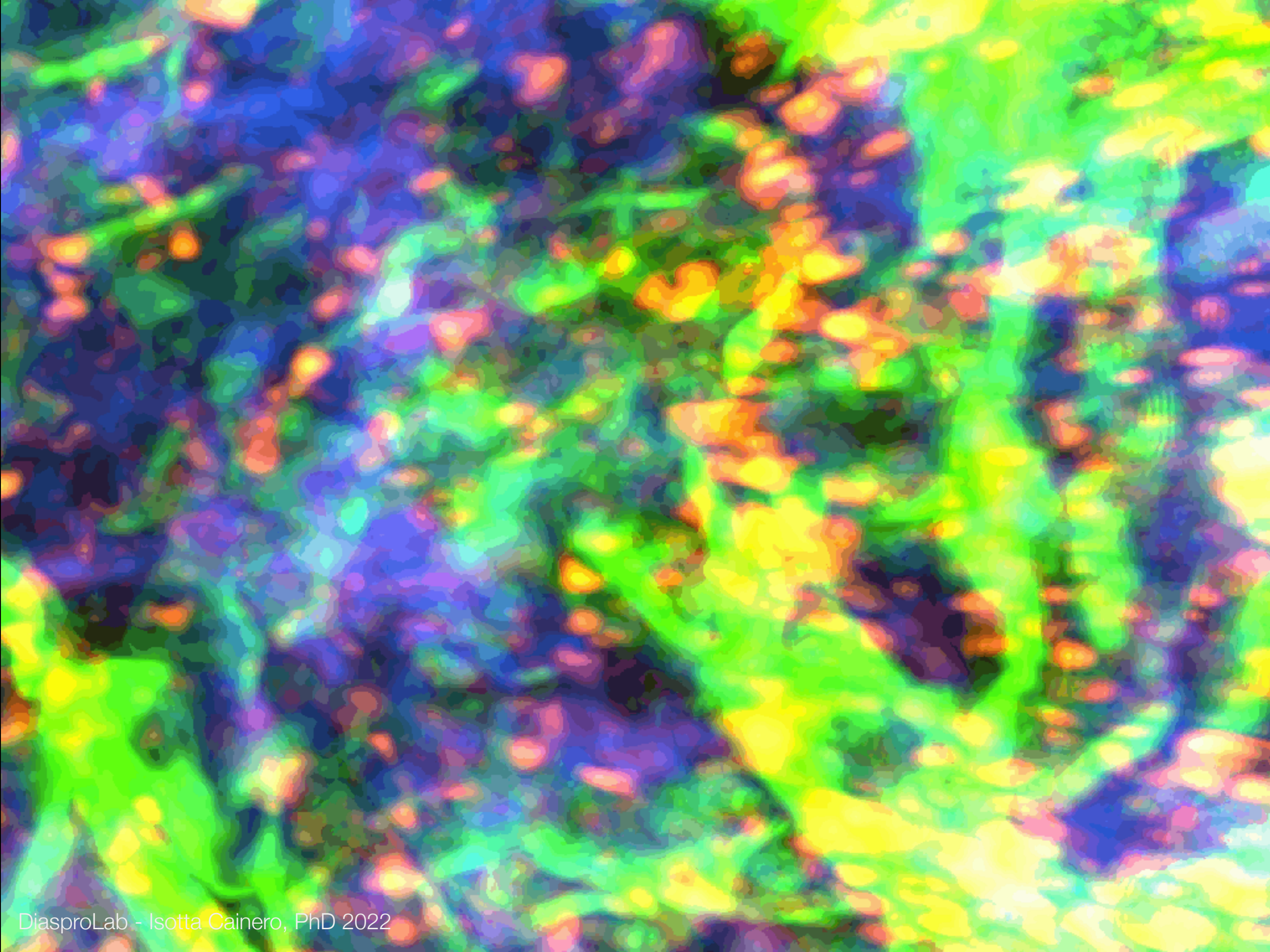
- its pretty!
- it provides information on the molecular environment
- it provides information on dynamic processes on the nanosecond timescale

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





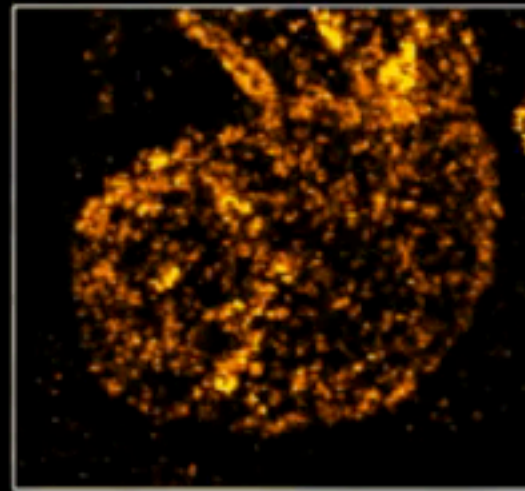
- ▶ 3DSIM acquisitions of 4-color stained samples
(FOV. 15 μm^2)

- ▶ **Nuclear Pore Complex**

- ▶ **Lamin or Progerin eGFP**

- ▶ **DNA Hoechst**

- ▶ **Histone H3K9me2**



[M. Oneto, et al.
Biophysical Journal,
2019]

[I. Cainero, et al.
Sensors, 2021]

[I. Cainero, et al.
Biophysical Reports,
in prep. 2023]

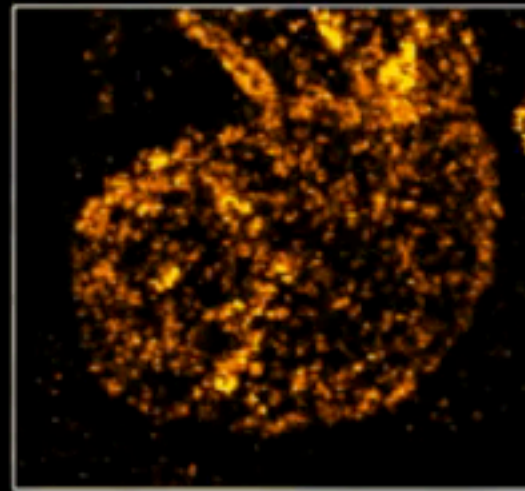
- ▶ 3DSIM acquisitions of 4-color stained samples
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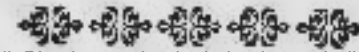
[M. Oneto, et al.
Biophysical Journal,
2019]

[I. Cainero, et al.
Sensors, 2021]

[I. Cainero, et al.
Biophysical Reports,
in prep. 2023]

PROPOSITIO I.

*Lumen propagatur seu diffunditur non solum Directè,
Refractè, ac Reflexè, sed etiam alio quodam
Quarto modo, DIFFRACTE'.*



Francesco Maria Grimaldi, Physico-mathesis de lumine, coloribus et iride (Bononiae, 1665).

4λ

REVIEW

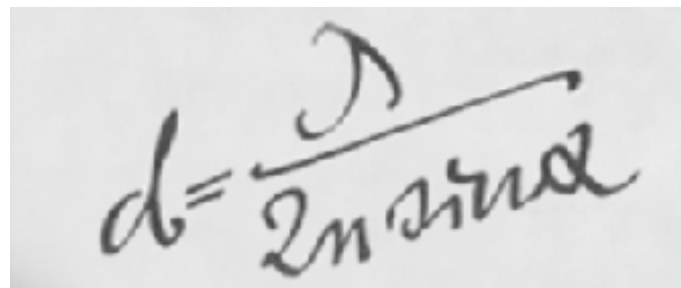
Resolution and super-resolution

Colin J. R. Sheppard 

Department of Nanophysics, Istituto Italiano di Tecnologia, via Morego 30, Genova, 16163, Italy

Correspondence

Colin J. R. Sheppard, Department of Nanophysics, Istituto Italiano di Tecnologia, via Morego 30, Genova 16163, Italy.
Email: colinjrsheppard@gmail.com


$$d = \frac{\lambda}{2n \sin \alpha}$$

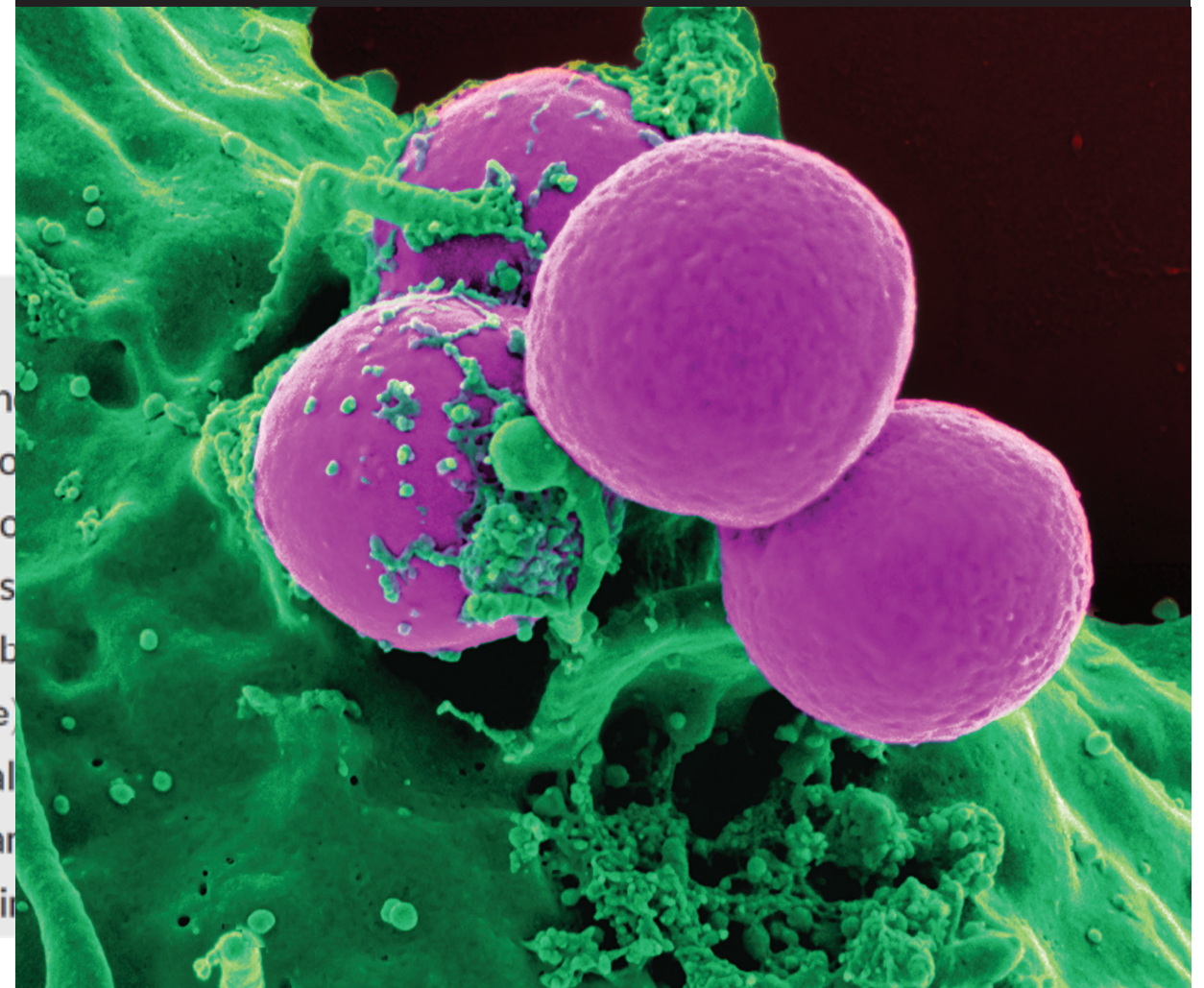
Abstract

Many papers have claimed that resolution can be improved classically, by measurement of the contrast of the image of a point source. However, the resolution, but of detection sensitivity, is limited by the bar of 0.735 that in the Rayleigh criterion, $\lambda/(13.9 \times \text{numerical aperture})$, compares with $\lambda/(\text{numerical aperture})$ for the classical case, and for points, two lines, arrays of lines.

VOLUME 80, ISSUE 4, MAY 2017

Editor-in-Chief
ALBERTO DIASPRO

MICROSCOPY RESEARCH & TECHNIQUE



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WILEY Blackwell

Sheppard CJR. Resolution and super-resolution. Microsc Res Tech. 2017;00:1–9.

REVIEW

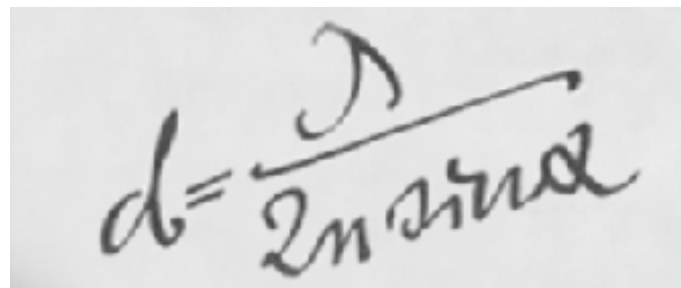
Resolution and super-resolution

Colin J. R. Sheppard 

Department of Nanophysics, Istituto Italiano di Tecnologia, via Morego 30, Genova, 16163, Italy

Correspondence

Colin J. R. Sheppard, Department of Nanophysics, Istituto Italiano di Tecnologia, via Morego 30, Genova 16163, Italy.
Email: colinjrsheppard@gmail.com


$$d = \frac{\lambda}{2n \sin \alpha}$$

Abstract

Many papers have claimed that resolution can be improved classically, by measurement of the contrast of the image of a point source. However, the Rayleigh criterion, which is the bar of 0.735 that in the limit of large numerical aperture compares with $\lambda/(\text{numerical aperture})$, holds for the classical case, and applies to points, two lines, arrays of lines, and so on.

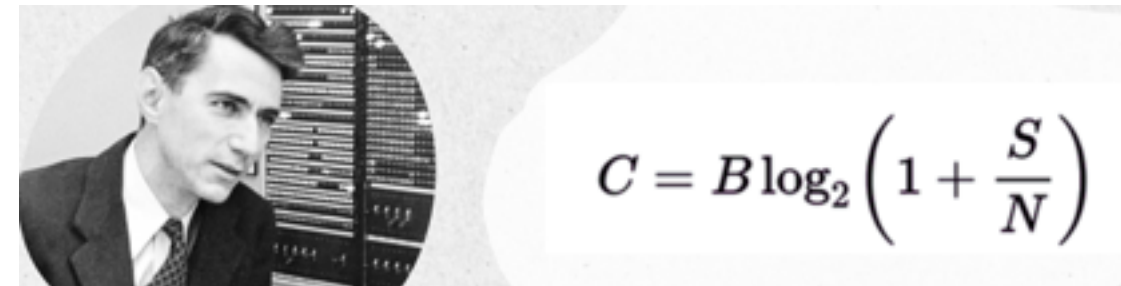


Sheppard CJR. Resolution and super-resolution. Microsc Res Tech. 2017;00:1–9.



G. Toraldo di Francia, Rev. Opt. 28, 597 (1949).

The Information Channel Revolution



JOURNAL OF THE OPTICAL SOCIETY OF AMERICA VOLUME 45, NUMBER 7 JULY, 1955

Resolving Power and Information

G. TORALDO DI FRANCIA
Istituto Nazionale di Ottica, Arcetri-Florence, Italy
(Received January 24, 1955)

The degrees of freedom of an image formed by any real instrument are only a finite number, while those of the object are an infinite number. Several different objects may correspond to the same image. It is shown that in the case of coherent illumination a large class of objects corresponding to a given image can be found very easily. Two-point resolution is impossible unless the observer has *a priori* an infinite amount of information about the object.

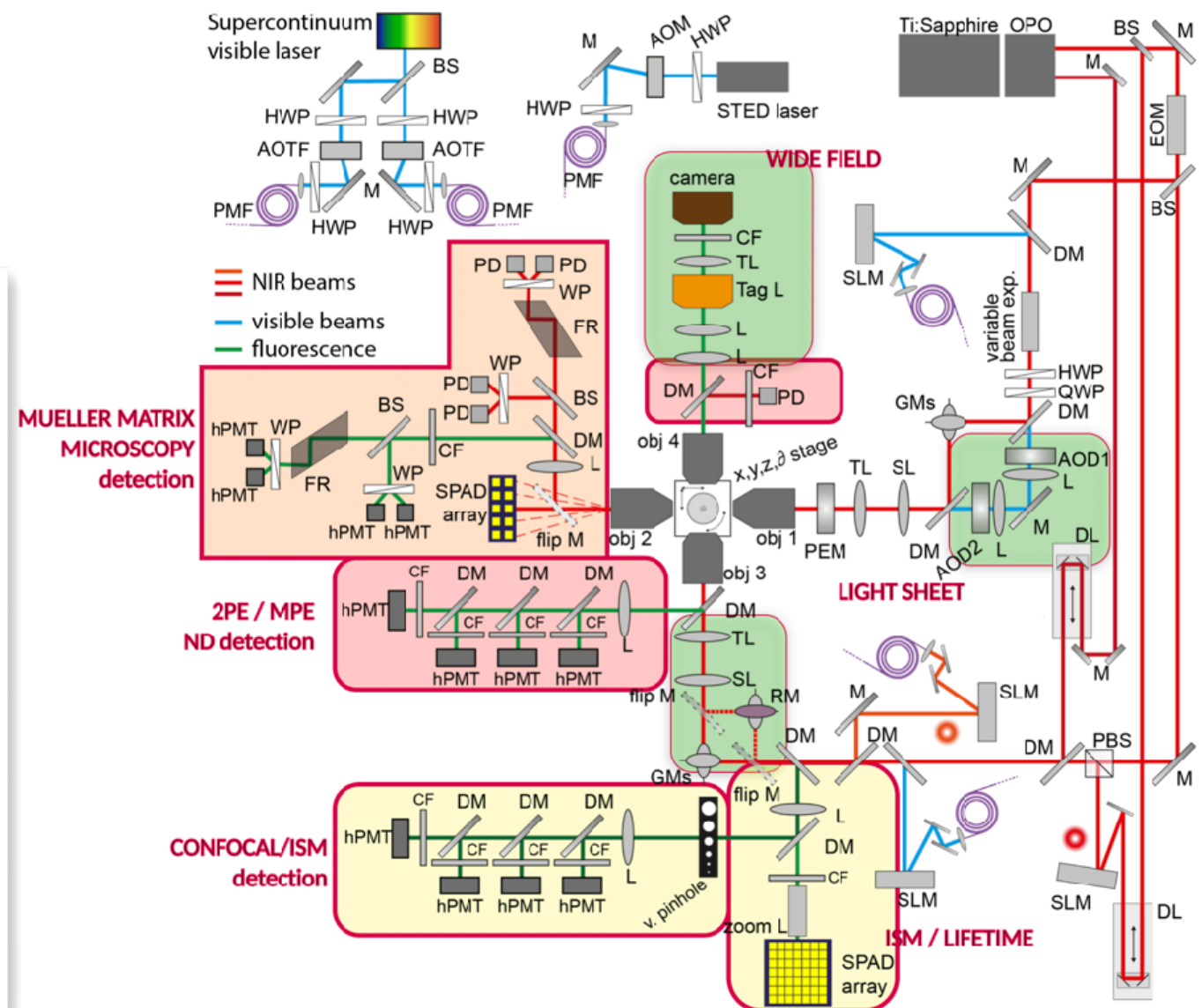
INTRODUCTION

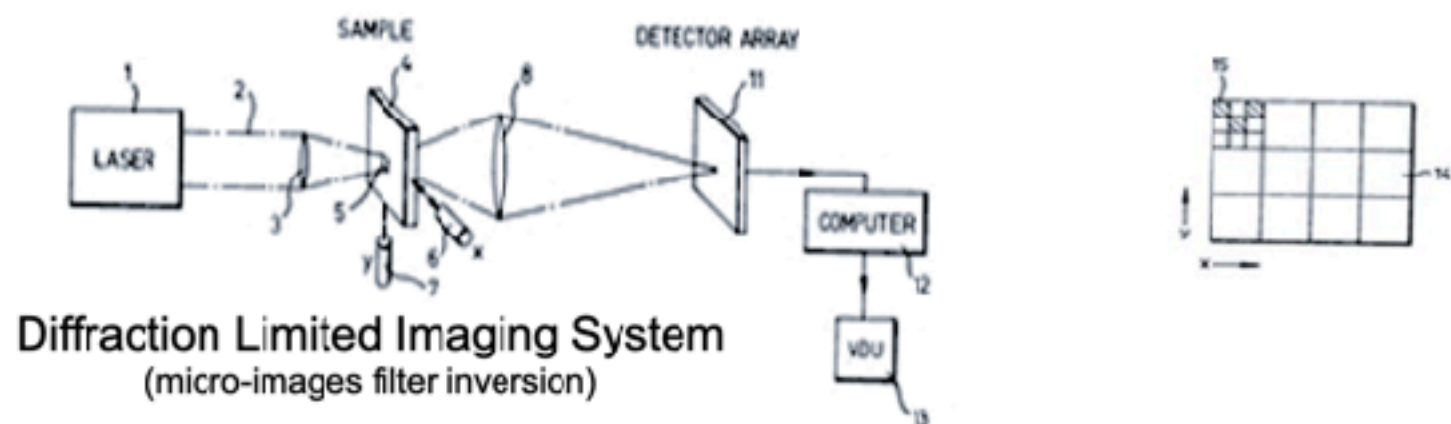
THE theory of resolving power is undergoing a transformation. Many workers agree that its classical formulation cannot be made completely satisfactory.

In the first place it may be remarked that the generally accepted value $1.22\lambda/D$ for the minimum angular separation of two points, which can be seen as distinct with an instrument having a circular pupil of diameter D , cannot be derived solely from the properties of the instrument. Its validity is limited to a given receptor and can be ascertained only by an experiment. However, it has been repeatedly found that even with one and the same receptor, the ability to resolve two points is influenced to a great extent by the light intensity and by other conditions.

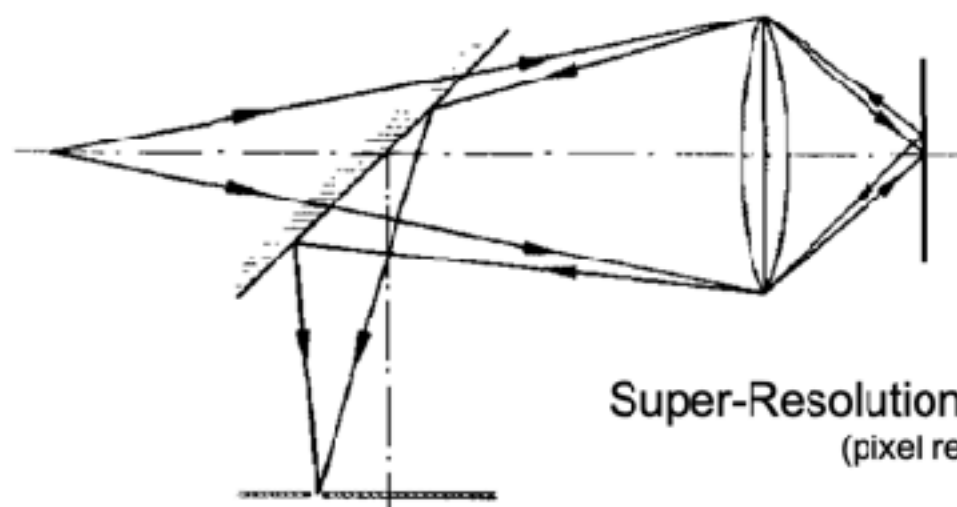
receptors, detecting the difference between the image of a single point and the image of two points located closer and closer to one another. This means that at present there is only a *practical* limit (if any) and not a *theoretical* limit for two-point resolving power.

In the second place, it is well known that the value of the resolving power, when measured experimentally, turns out to depend substantially on the shape of the test object used. Sets of points or of lines, circles, stars, Landolt rings, and many other patterns have been employed as test objects with different values of contrast, and each one gives a different result. After so many investigations about resolving power, one cannot escape the discouraging conclusion that a very common sentence like: "The resolving power of such instrument has such value" has no meaning. Resolving power is not a well-defined classical quantity.





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



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

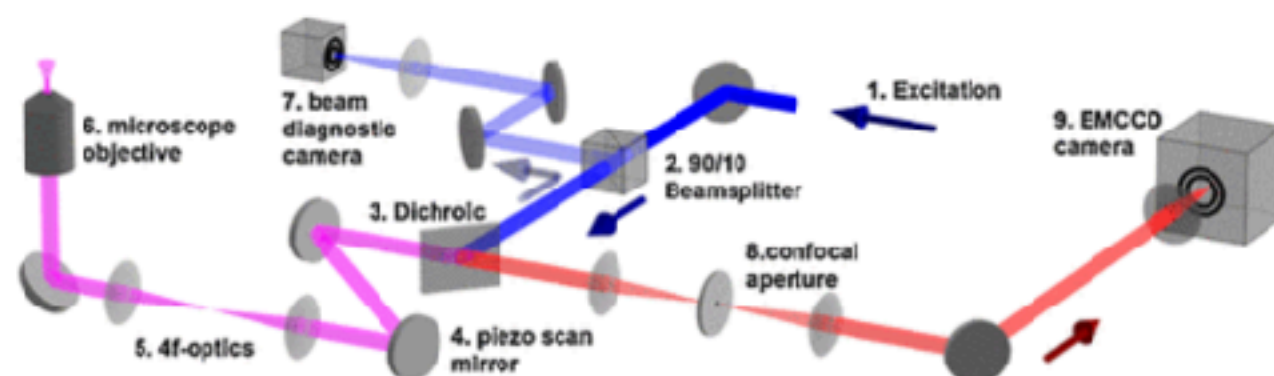


Image Scanning Microscopy (ISM)

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

Research Article

Vol. 34, No. 8 / August 2017 / Journal of the Optical Society of America A

1839

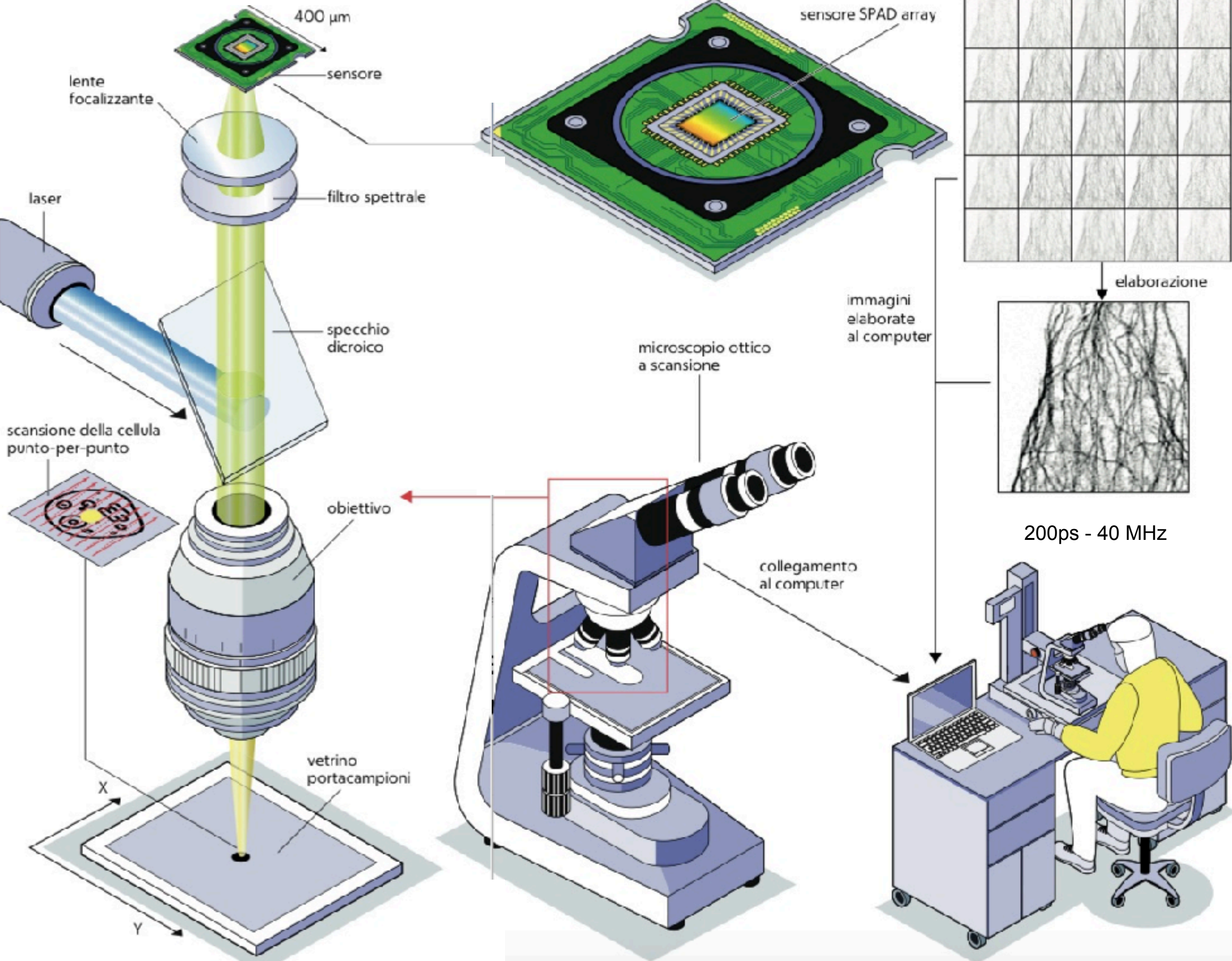
Journal of the
Optical Society
of America

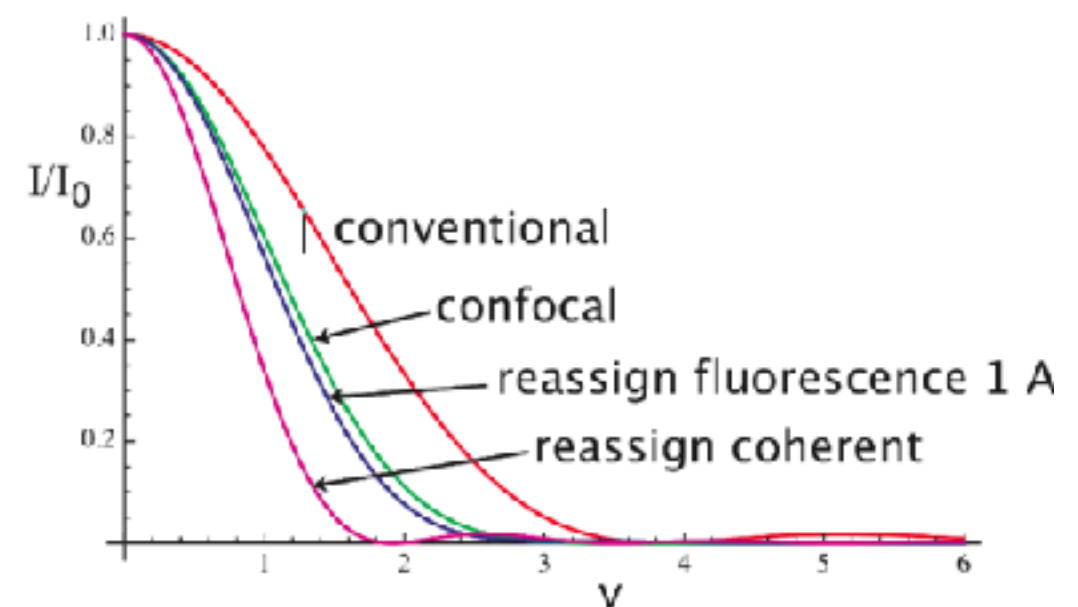
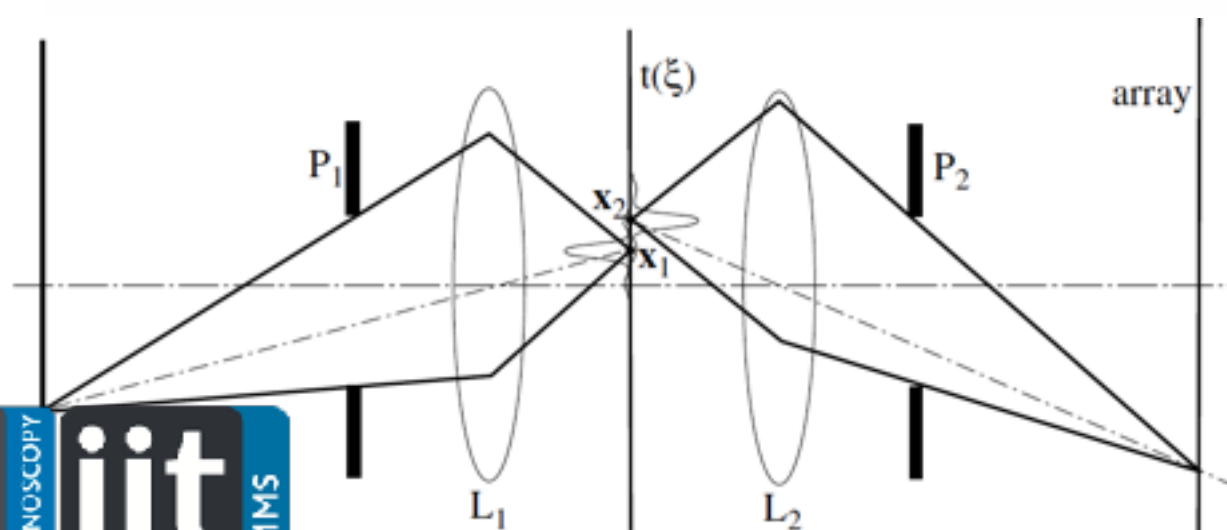
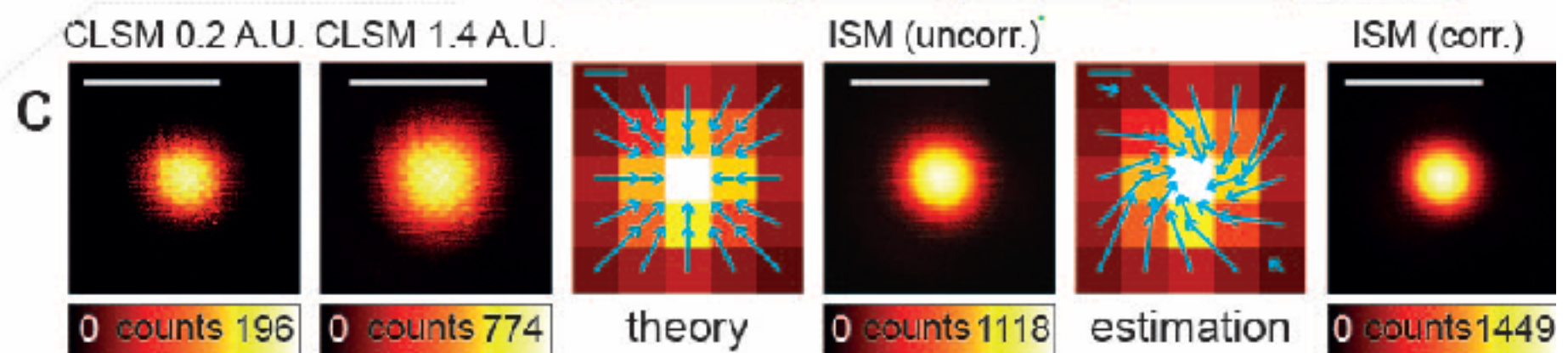
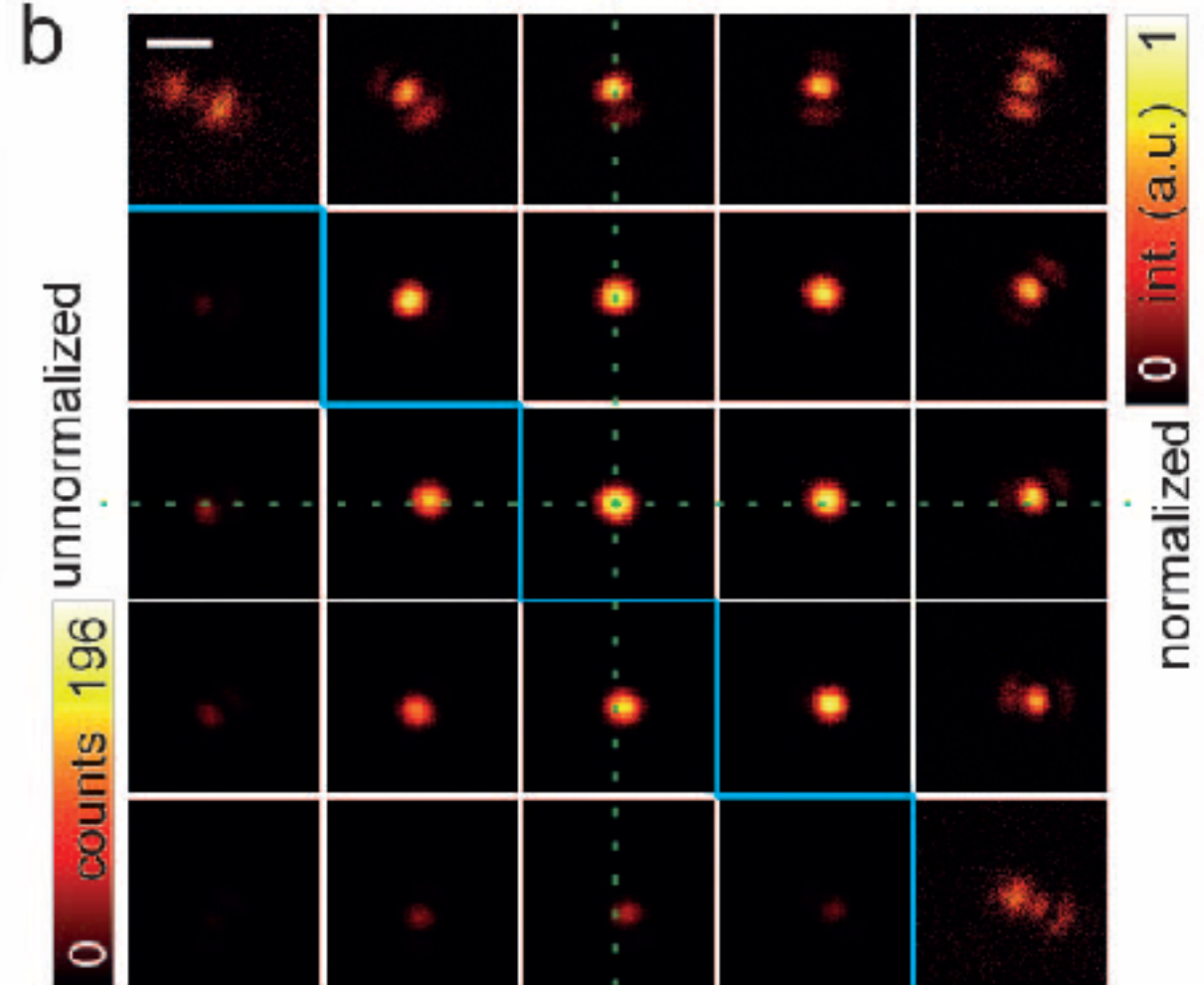
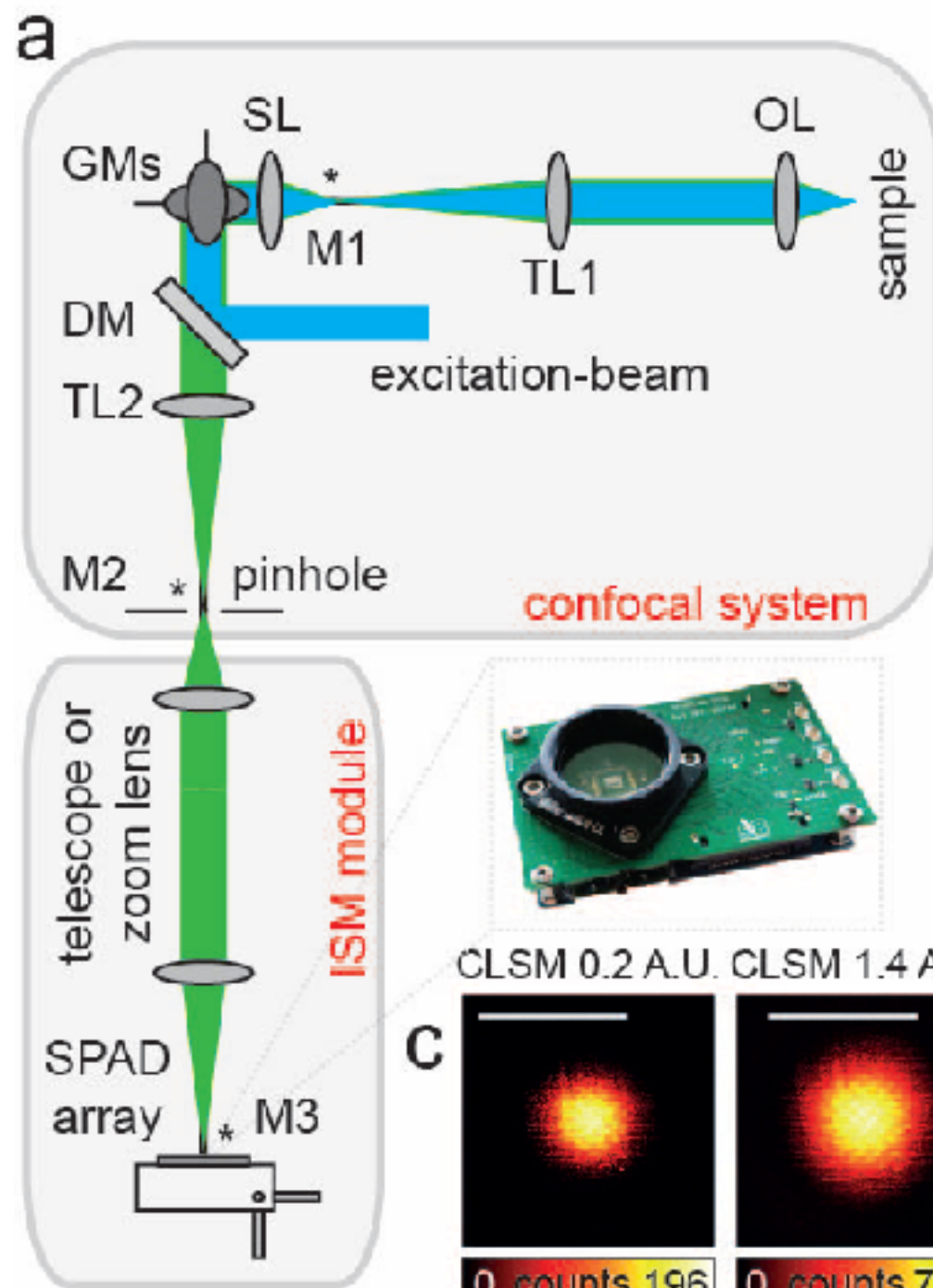
A

OPTICS, IMAGE SCIENCE, AND VISION

Image formation in image scanning microscopy, including the case of two-photon excitation

COLIN J. R. SHEPPARD,^{1,*} MARCO CASTELLO,^{1,2} GIORGIO TORTAROLO,^{1,2} GIUSEPPE VOIGDINI,¹ AND ALBERTO DIASPRO^{1,2,3}

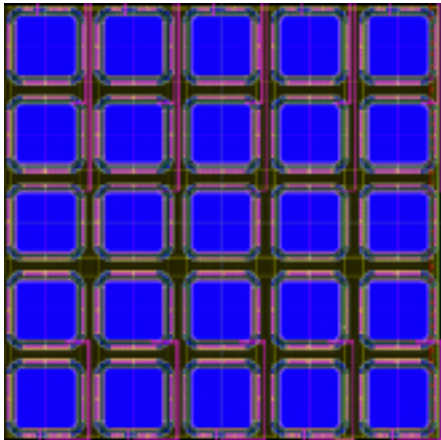
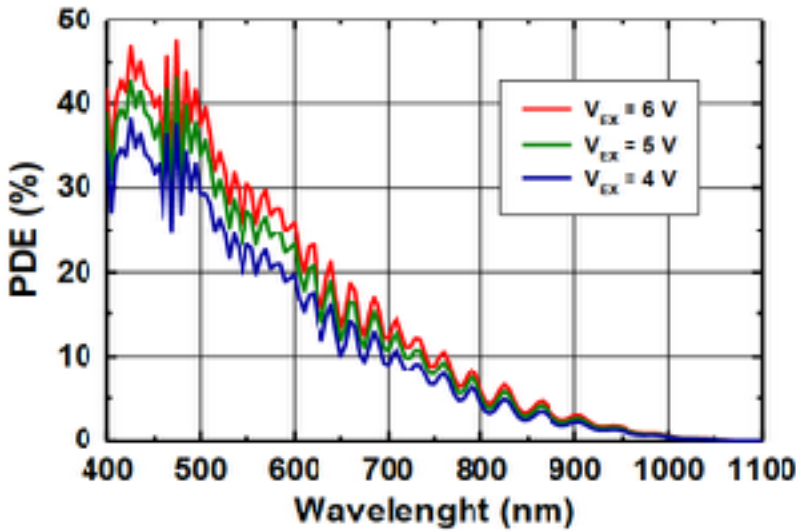




the first detector array specifically designed for microscopy



Time jitter 120/150 ps



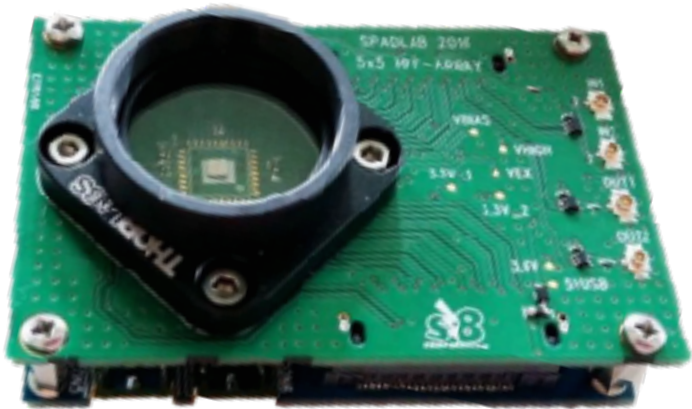
features:



- ◆ 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

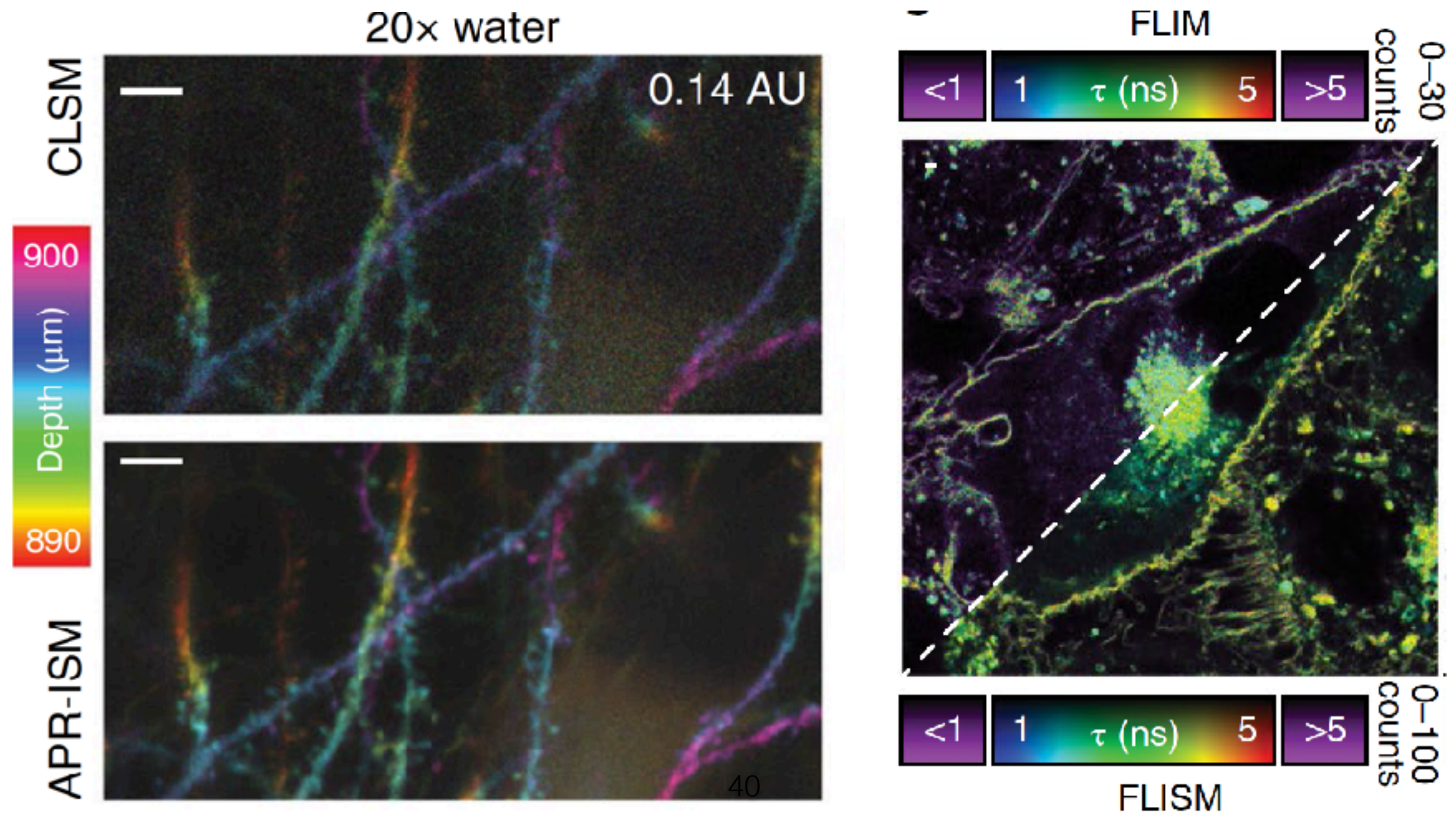
Hold-OFF Time	Afterpulsing probability
50 ns	6,5%
100 ns	2,4%
200 ns	1,4%

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

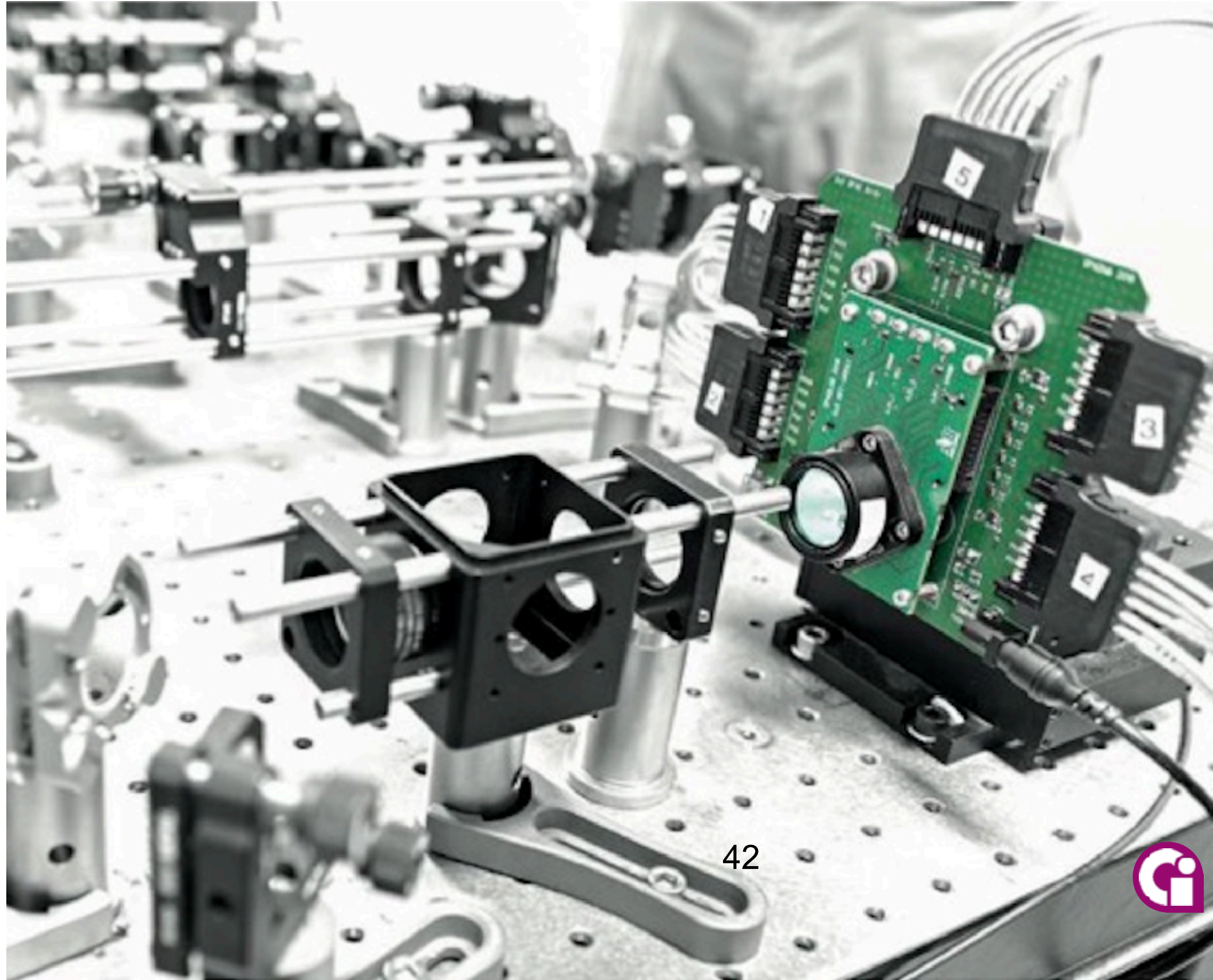


M.Castello et al. (2019)

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







PRISM 

super-resolution
"a better look at your sample"

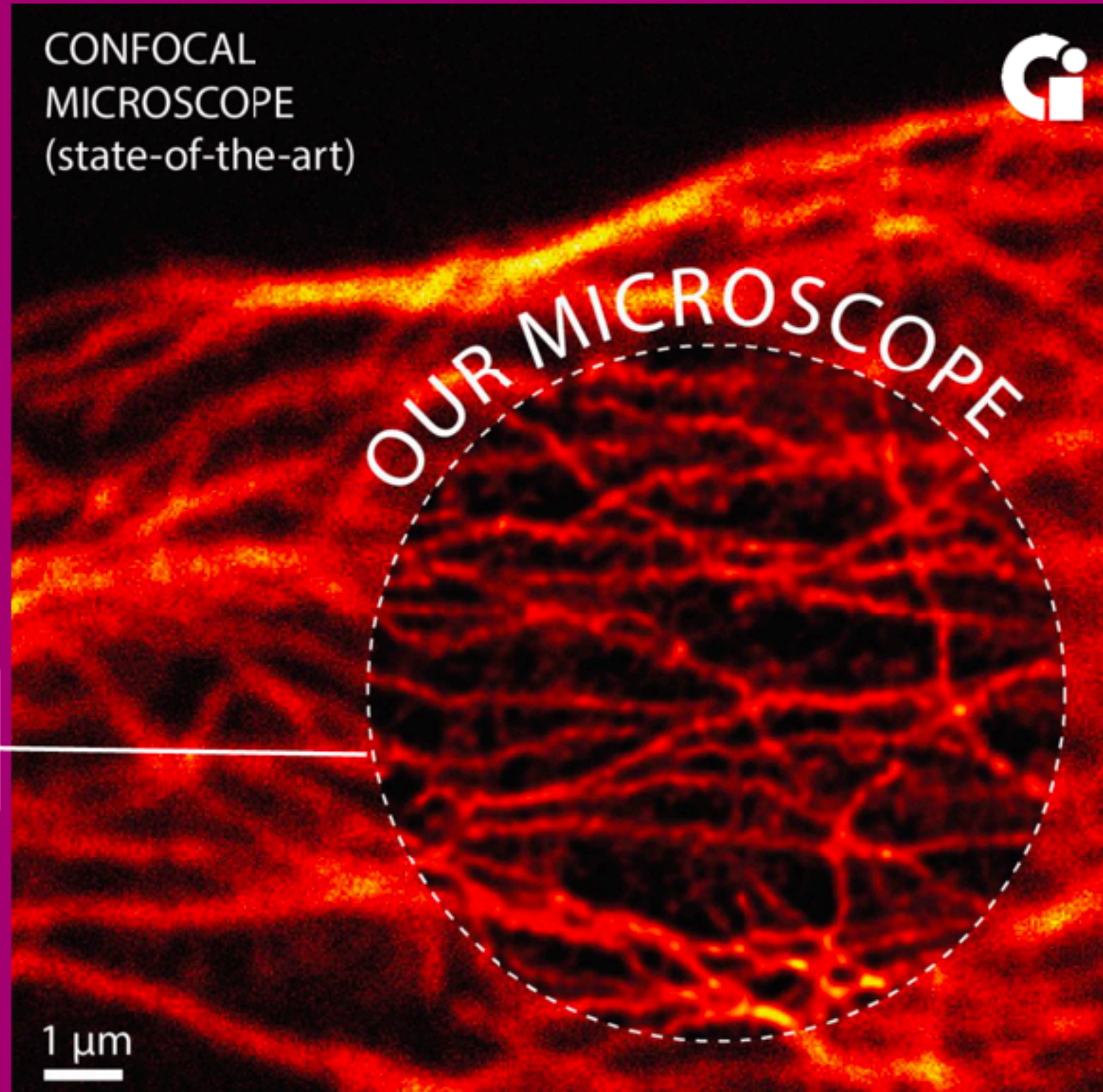
down to
100 nm

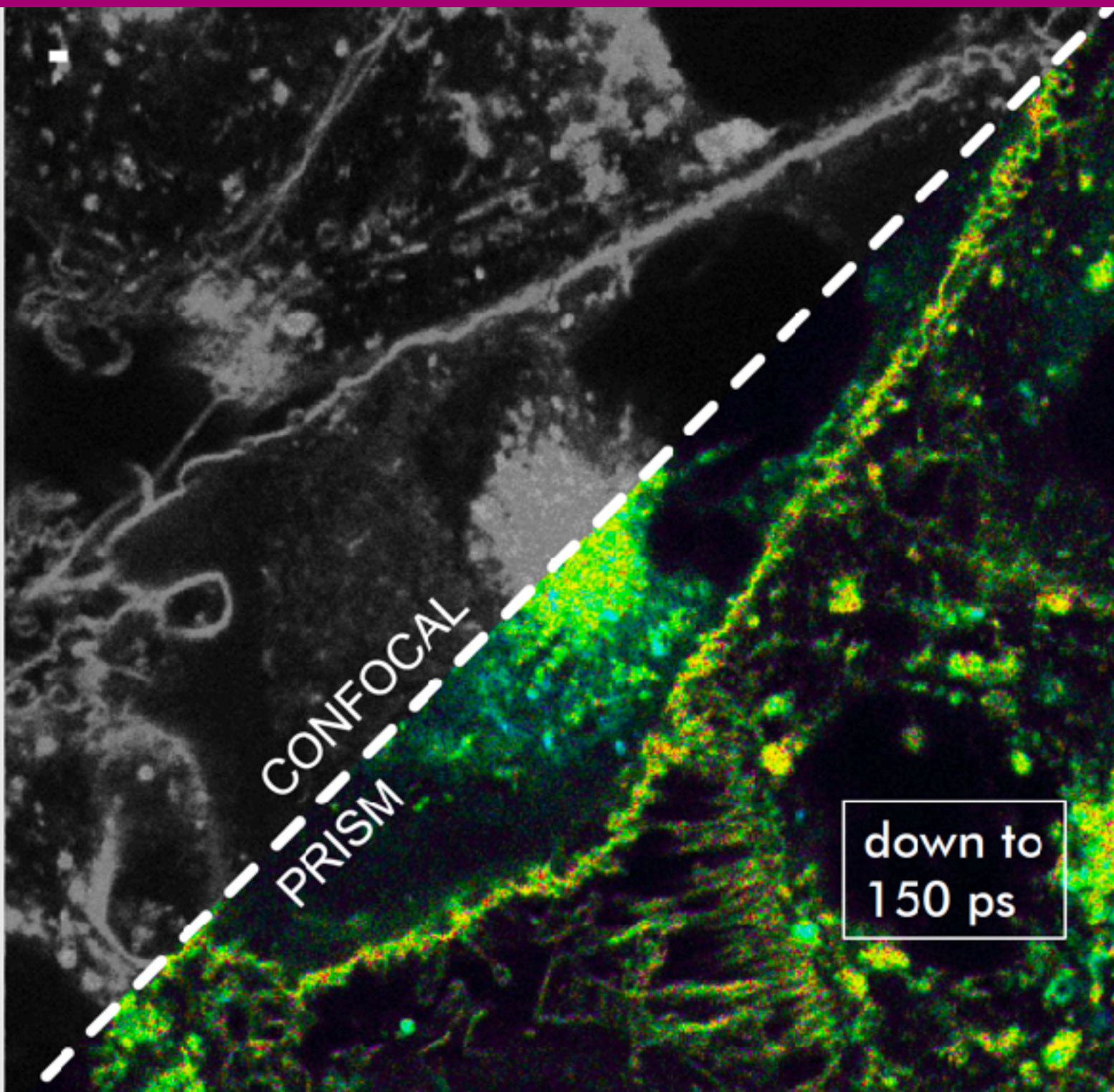
CONFOCAL
MICROSCOPE
(state-of-the-art)



OUR MICROSCOPE

1 μm





PRISM 

Cell metabolism



slow

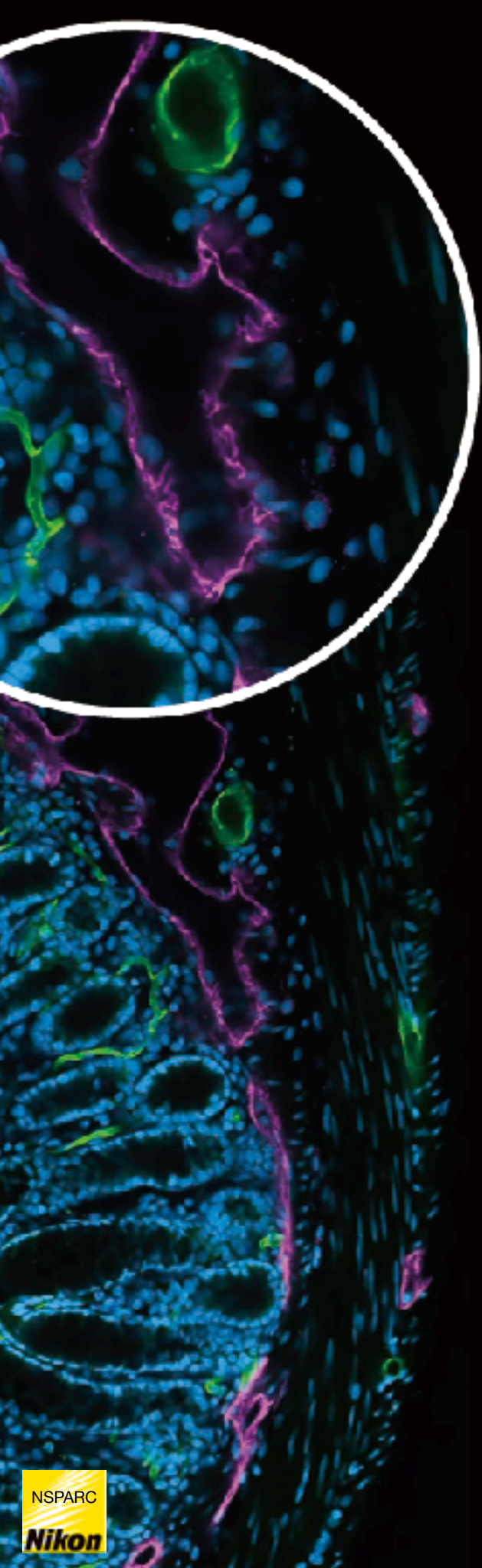


fast

**temporal
super-resolution**
"e.g. non-invasive detection of cancer"

AX AX R with NSPARC

Confocal based Super Resolution Microscope



Shedding New Light On **MICROSCOPY**

製品情報 > 共焦点顕微鏡 >

AX / AX R

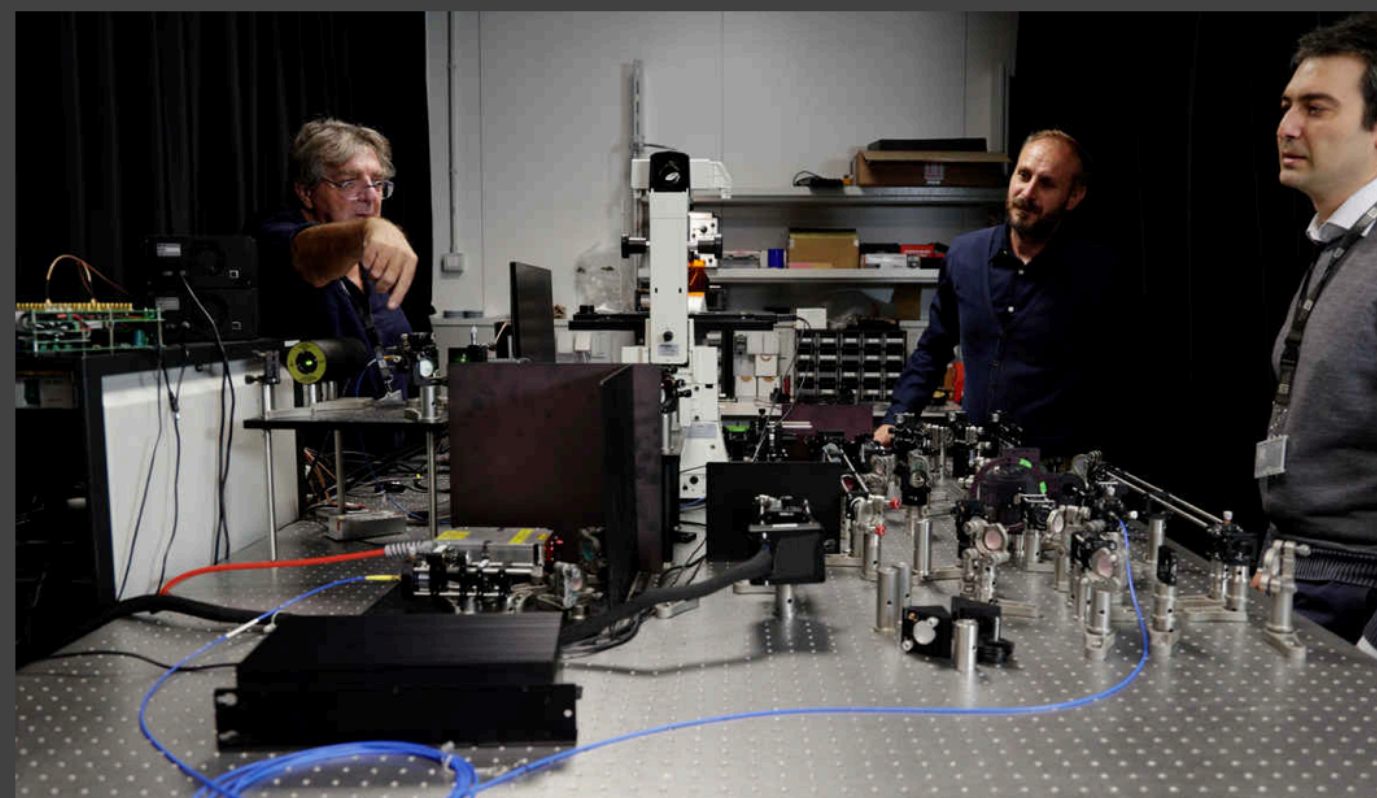
超解像共焦点レーザー顕微鏡システム

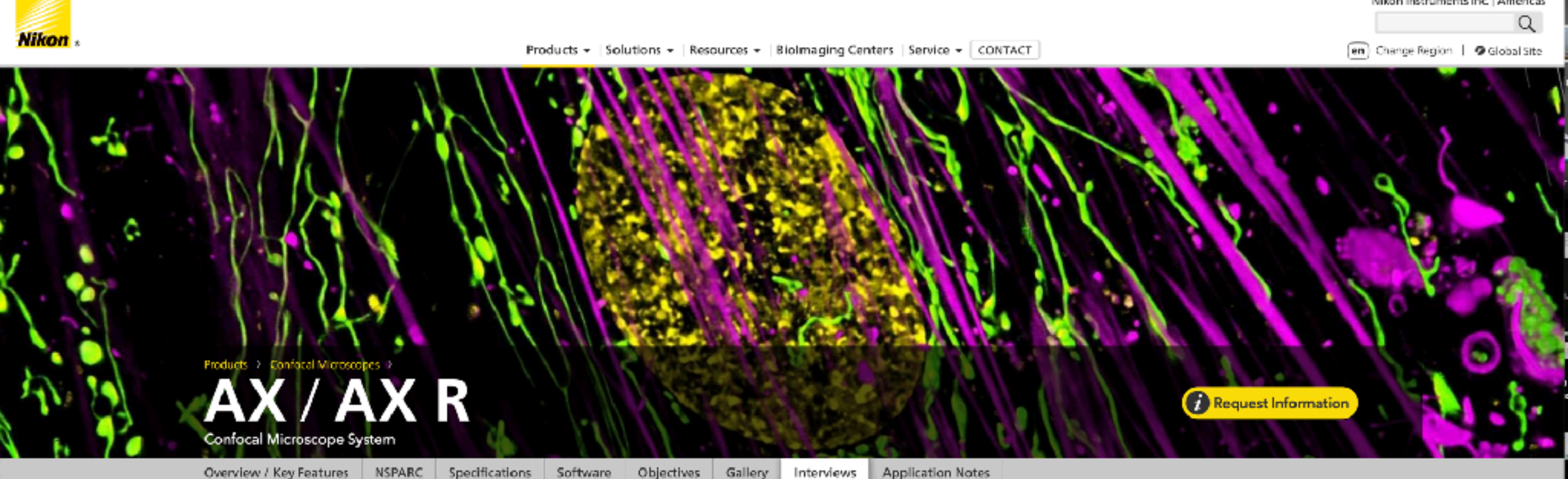
製品に関するお問い合わせ

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。

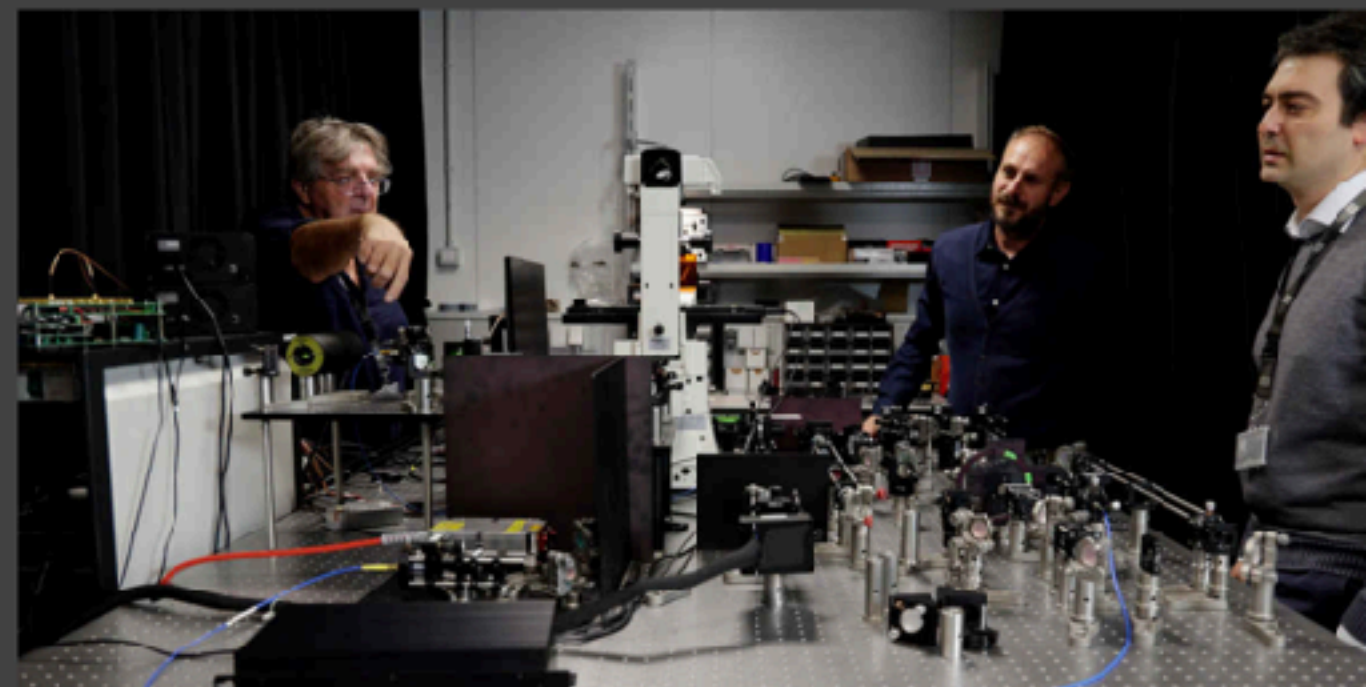




An Inside Look

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.



AX / AX R with NSPARC

Confocal-based Super Resolution Microscope

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

Colin J. R. Sheppard, Marco Castello, Giorgio Tortarolo, Giuseppe Vicidomini, and Alberto Diaspro, "Image formation in image scanning microscopy, including the case of two-photon excitation," J. Opt. Soc. Am. A 34, 1339-1350 (**2017**)

Traditional Detector

NSPARC



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



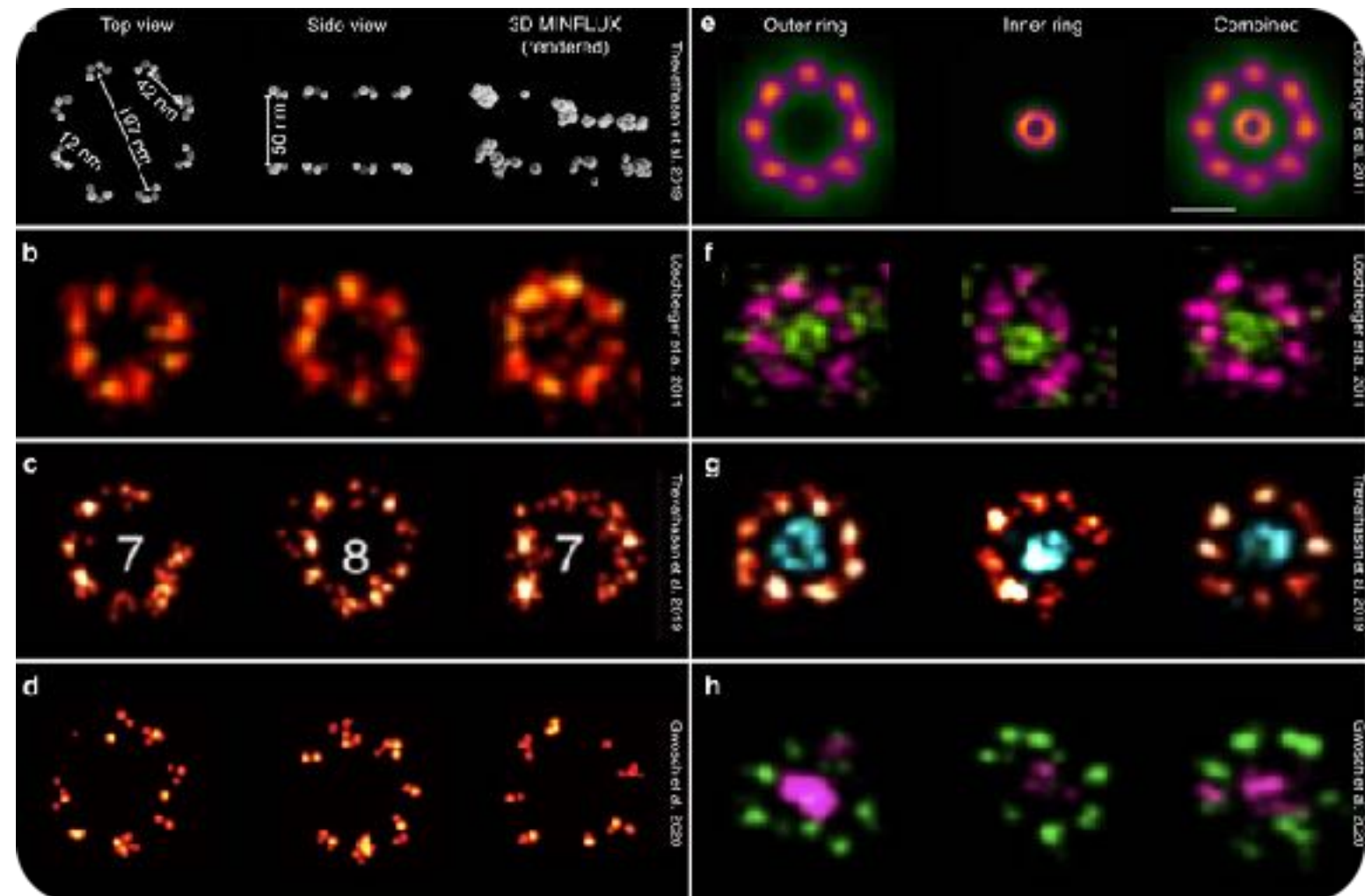
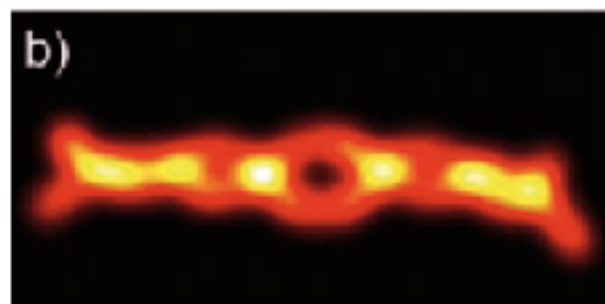
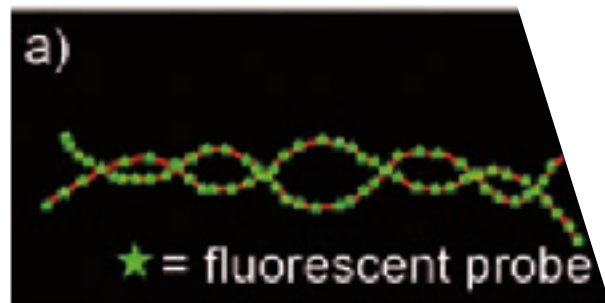
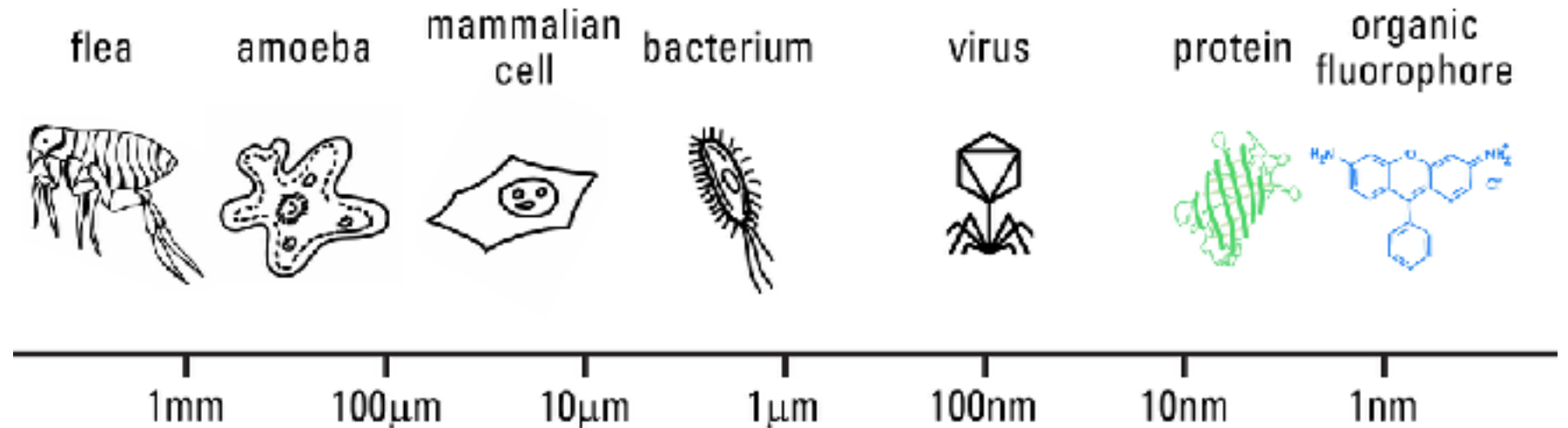
© Nobel Media AB. Photo: A. Mahmoud
Eric Betzig
Prize share 1/3



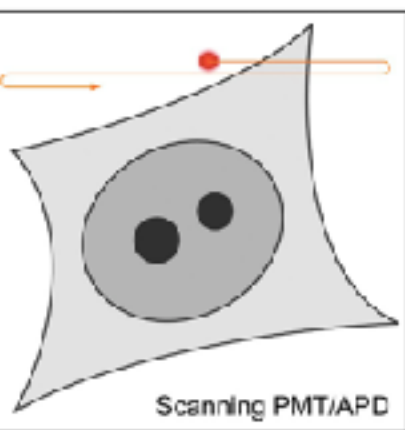
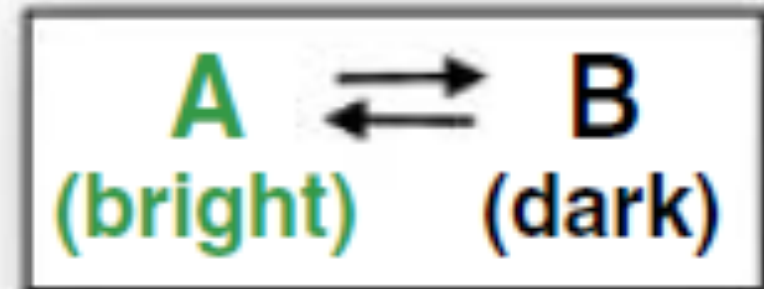
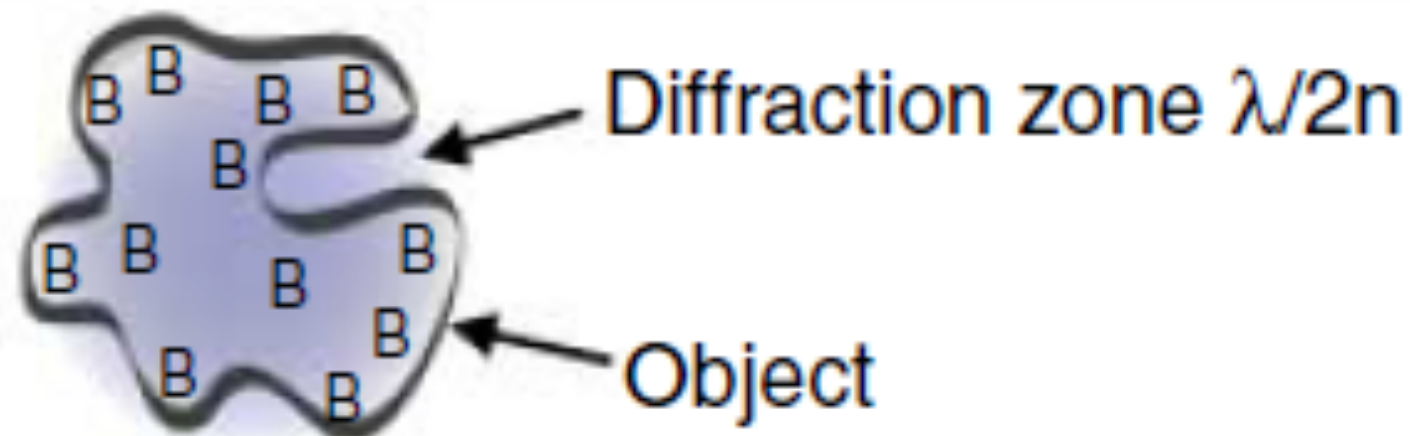
© Nobel Media AB. Photo: A. Mahmoud
Stefan W. Hell
Prize share 1/3



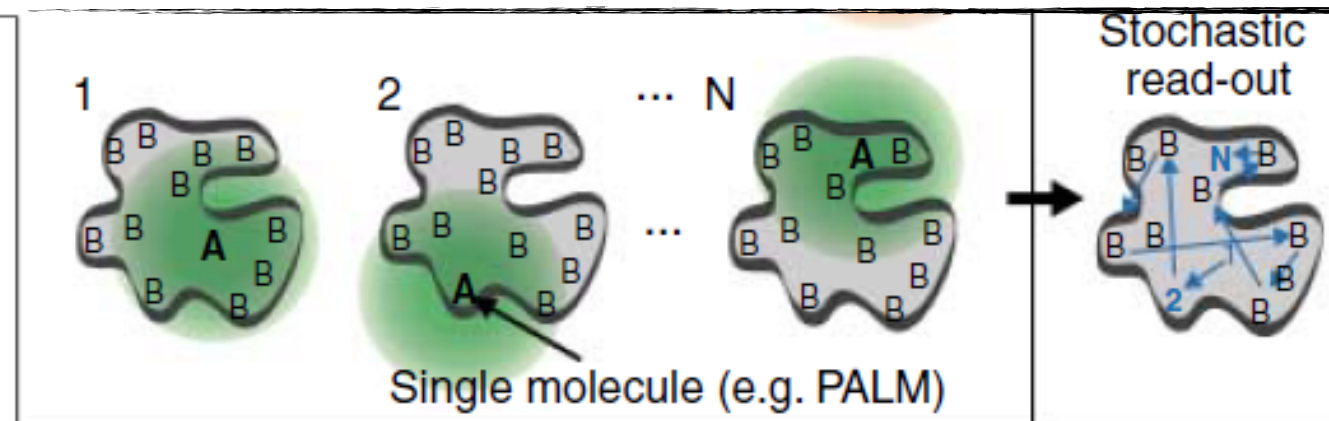
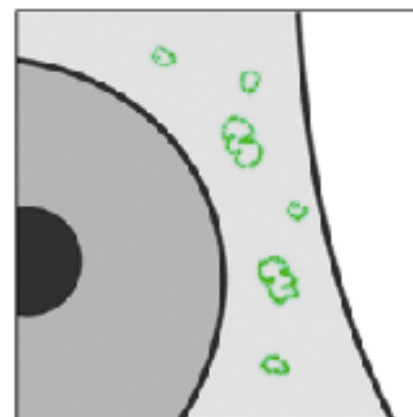
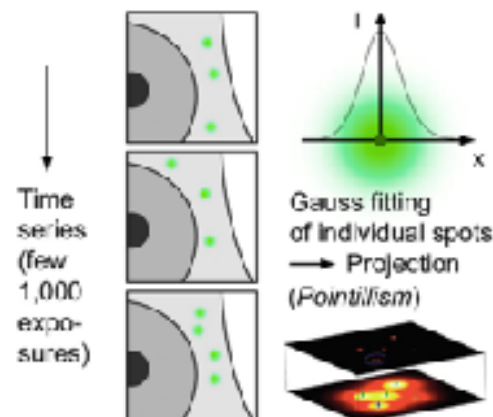
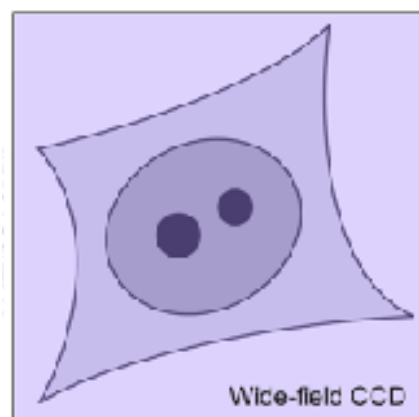
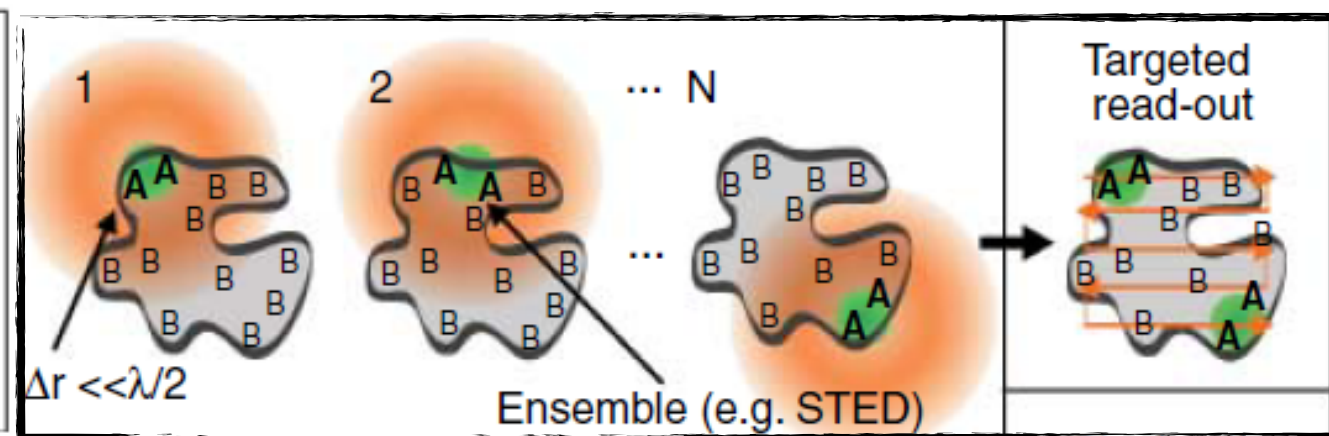
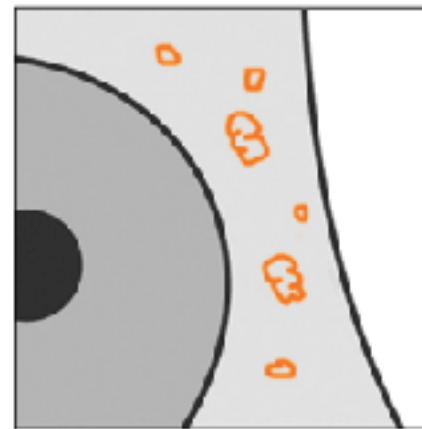
© Nobel Media AB. Photo: A. Mahmoud
William E. Moerner
Prize share 1/3



SUPER RESOLVED FLUORESCENCE MICROSCOPY



Excitation PSF
 +
 STED pulse PSF
 =
 Effective PSF

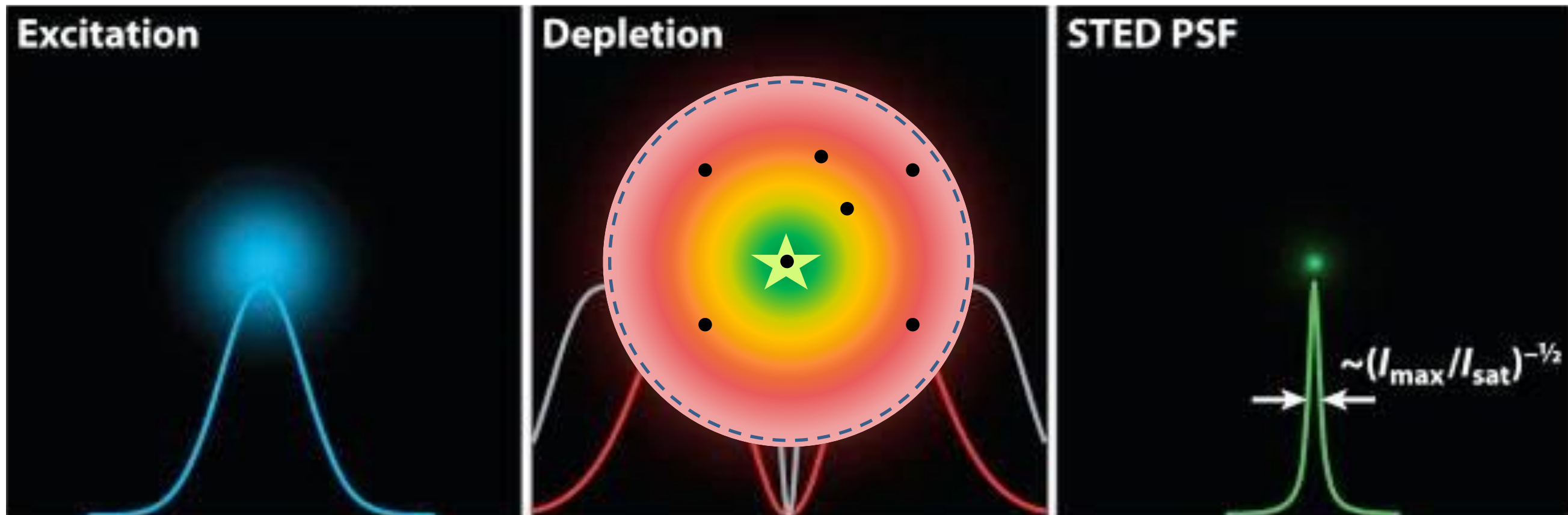




STED super-resolved microscopy

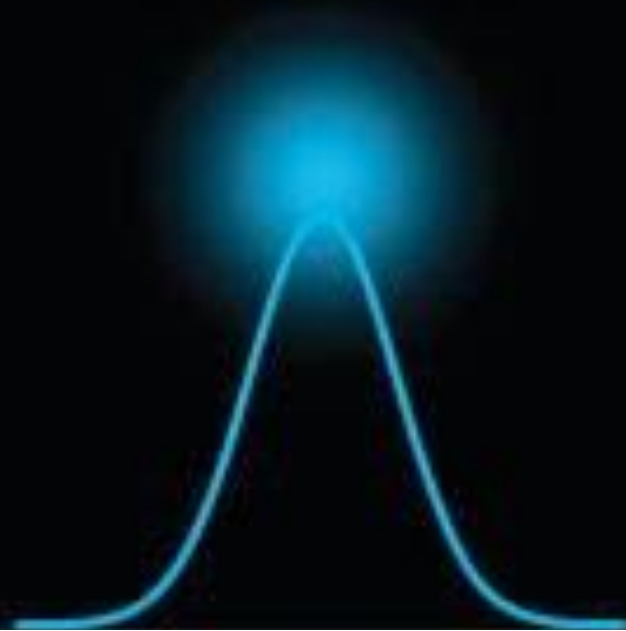
Giuseppe Vicidomini¹ , Paolo Bianchini²  & Alberto Diaspro^{2,3} 

PUBLISHED ONLINE 29 JANUARY 2018; DOI:10.1038/NMETH.4593 **NATURE METHODS** | ADVANCE ONLINE PUBLICATION |

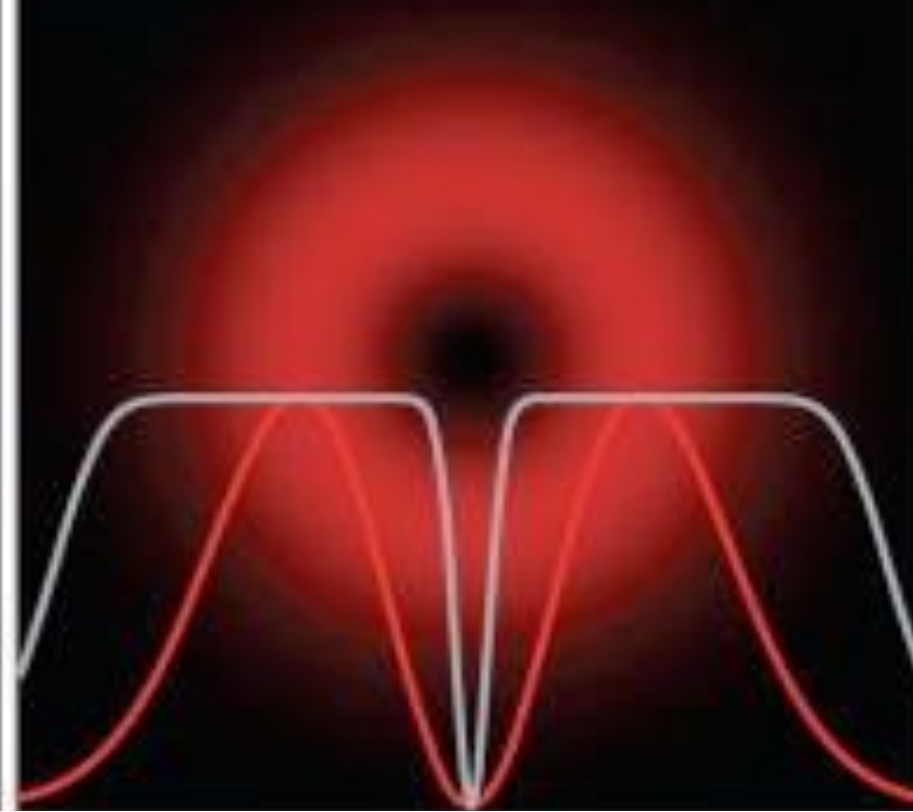


Pump-probe/Spectroscopy

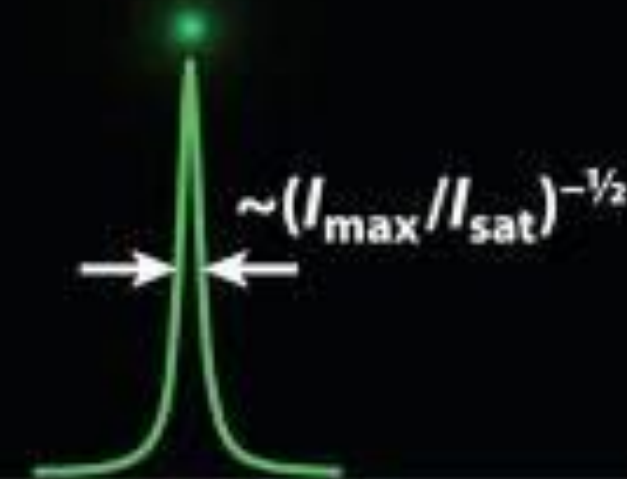
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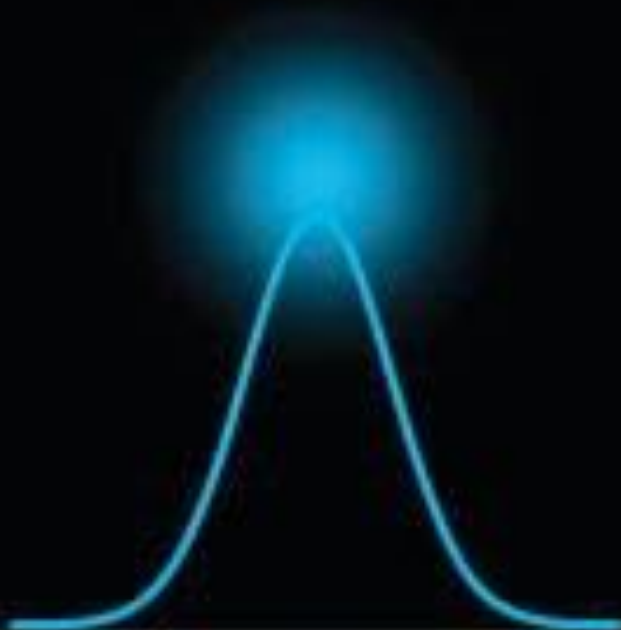
Depletion



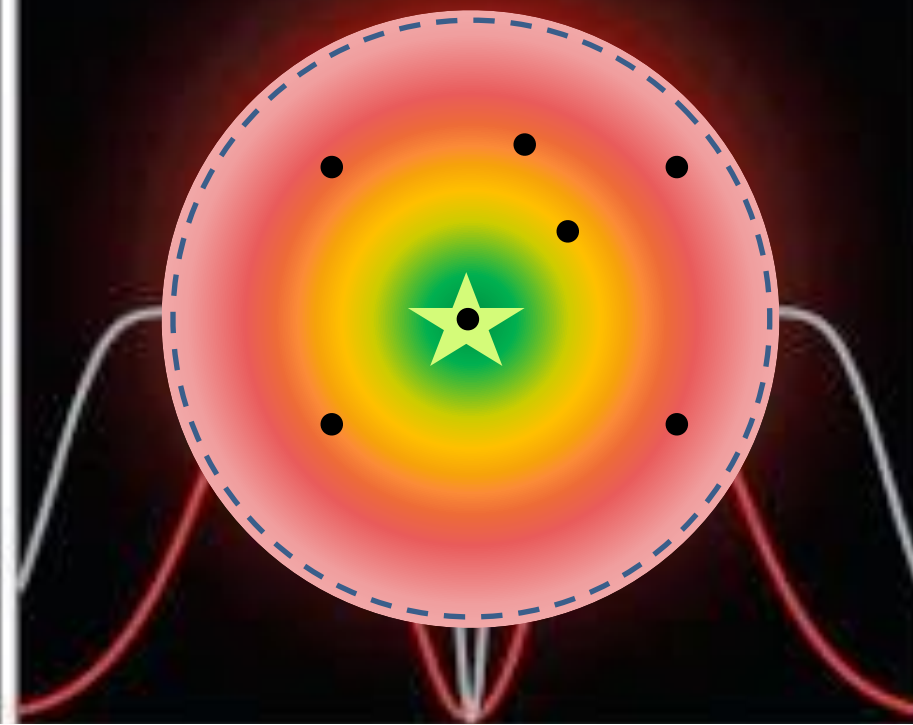
STED PSF



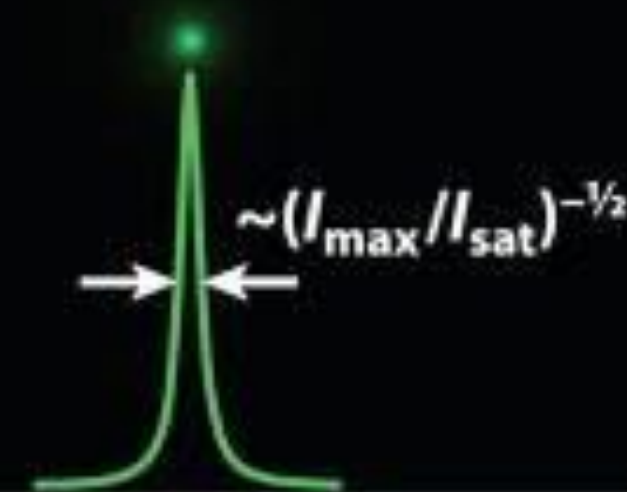
Excitation



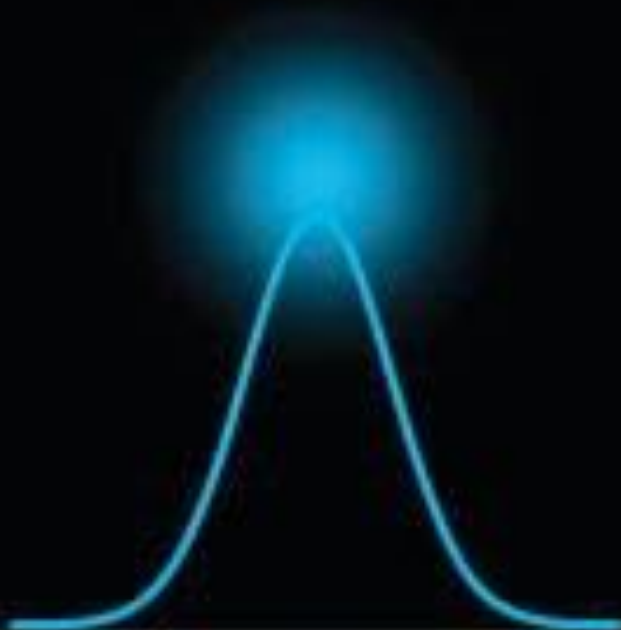
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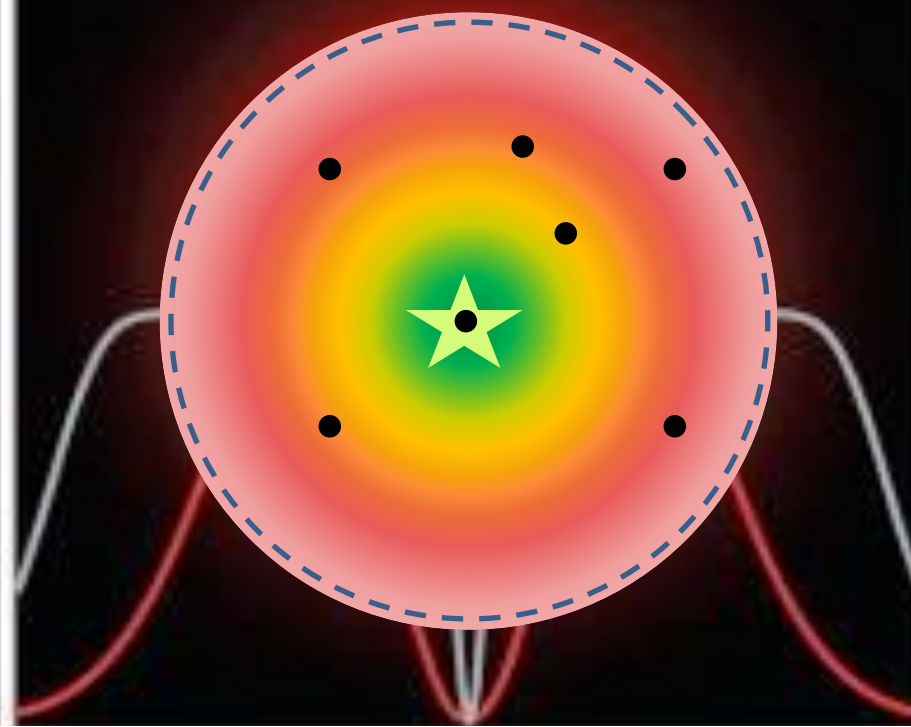
STED PSF



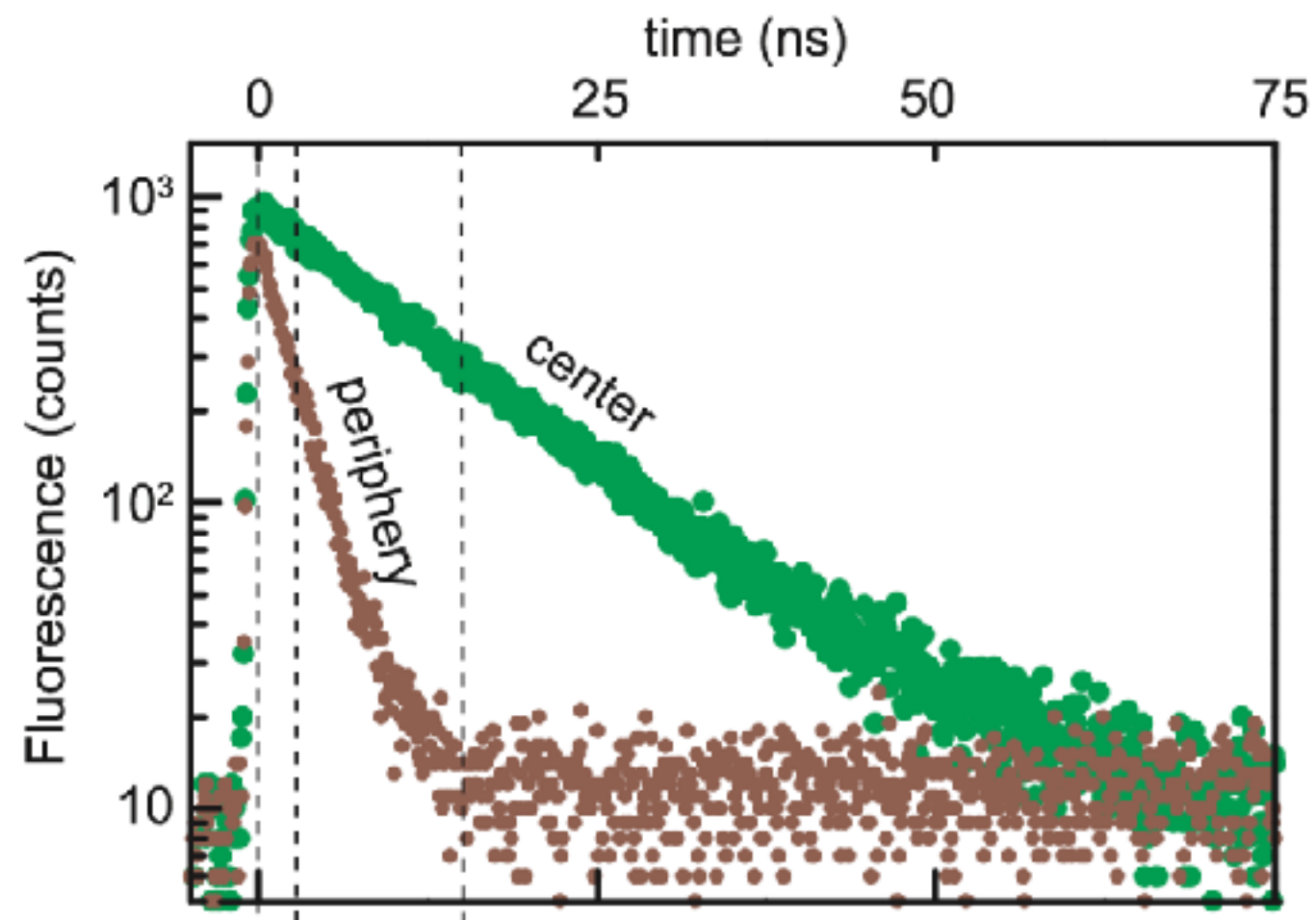
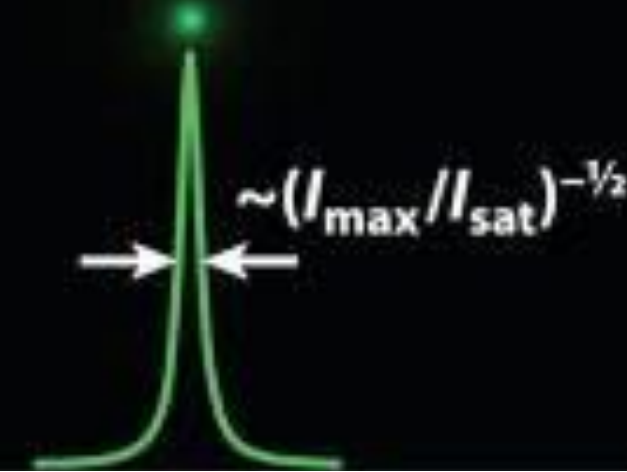
Excitation



Depletion



STED PSF

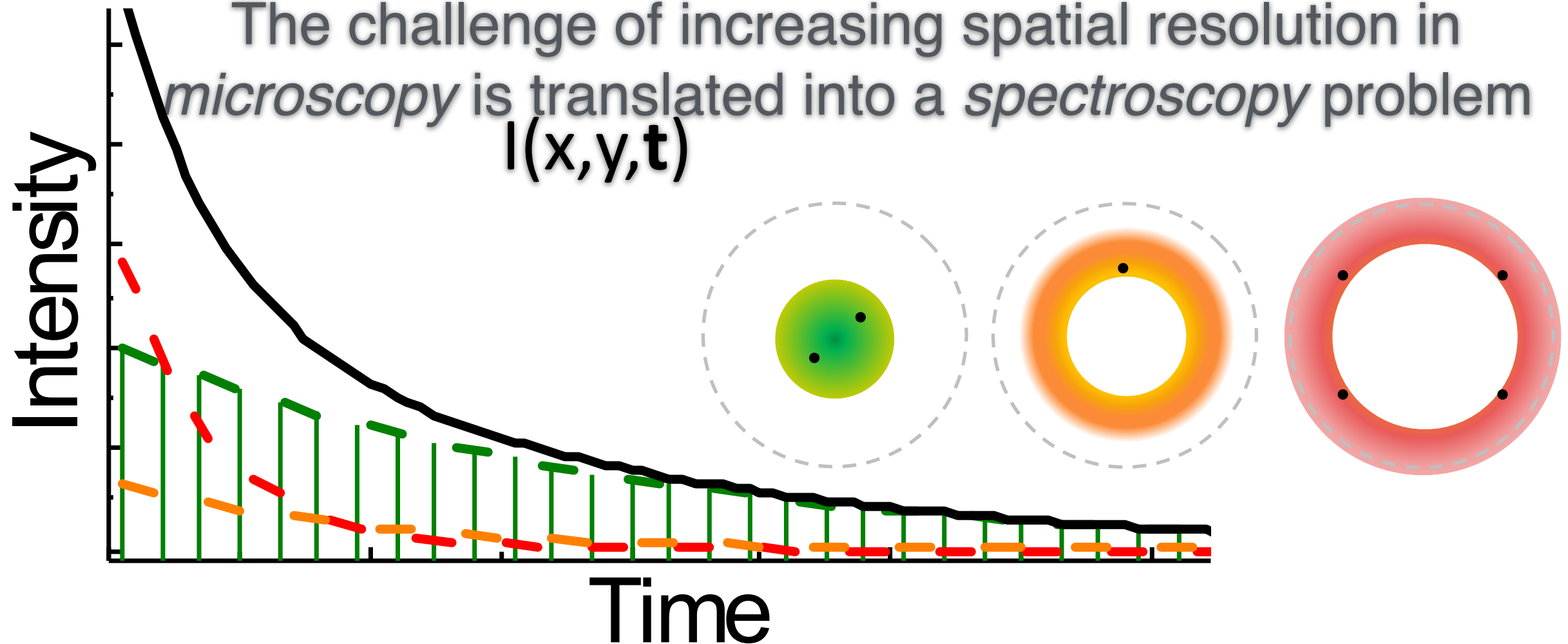


ENCODING TIME - DECODING SPACE

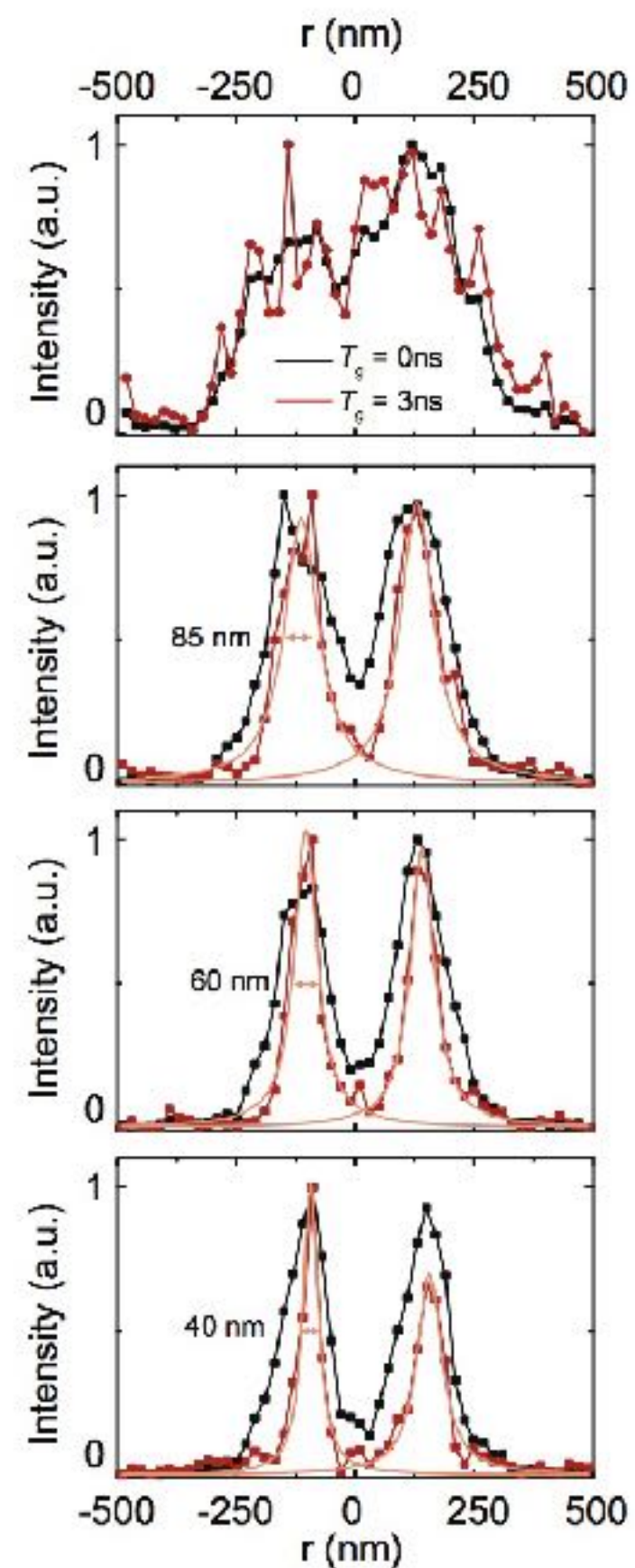
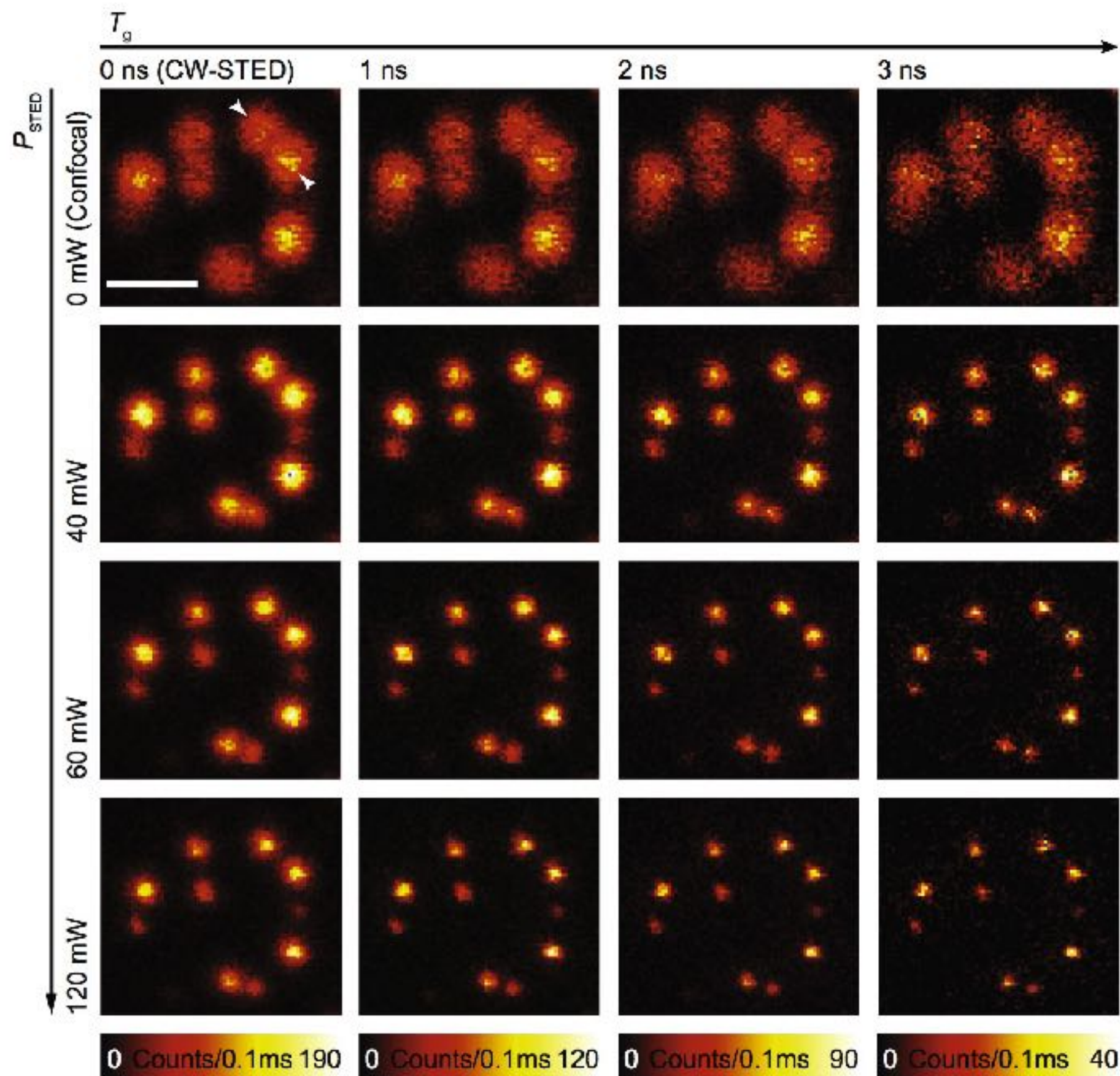
Decoding of hidden spatial information

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

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



For instance, spatial information can be 'encoded' in the temporal dynamics of fluorescence

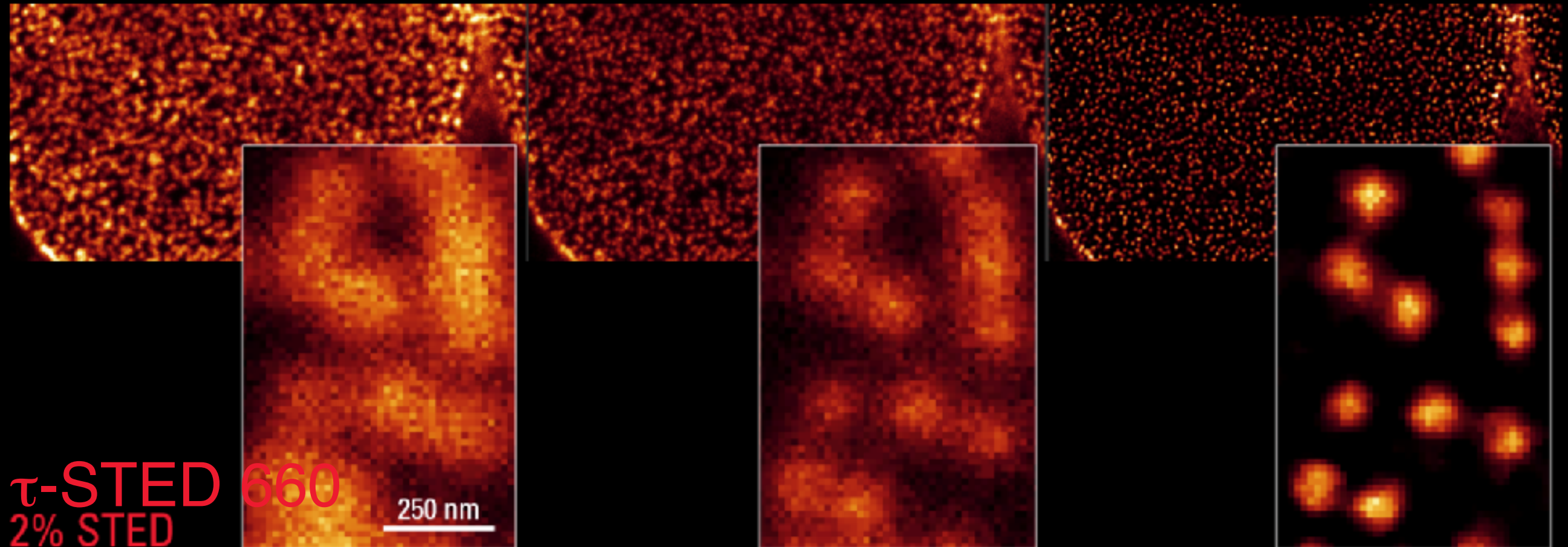


τ -STED

Confocal

Conventional STED

τ -STED



τ -STED 660 at extremely low STED power on COS7 cells immunostained for nuclear pores (NUPs). The primary antibody recognizes several proteins of the nuclear pore basket, so the NUPs are labeled in the central region and as a result they appear as point-like structures.

STED LABS, STED, RIMM, WCCG, U503, 10-2020

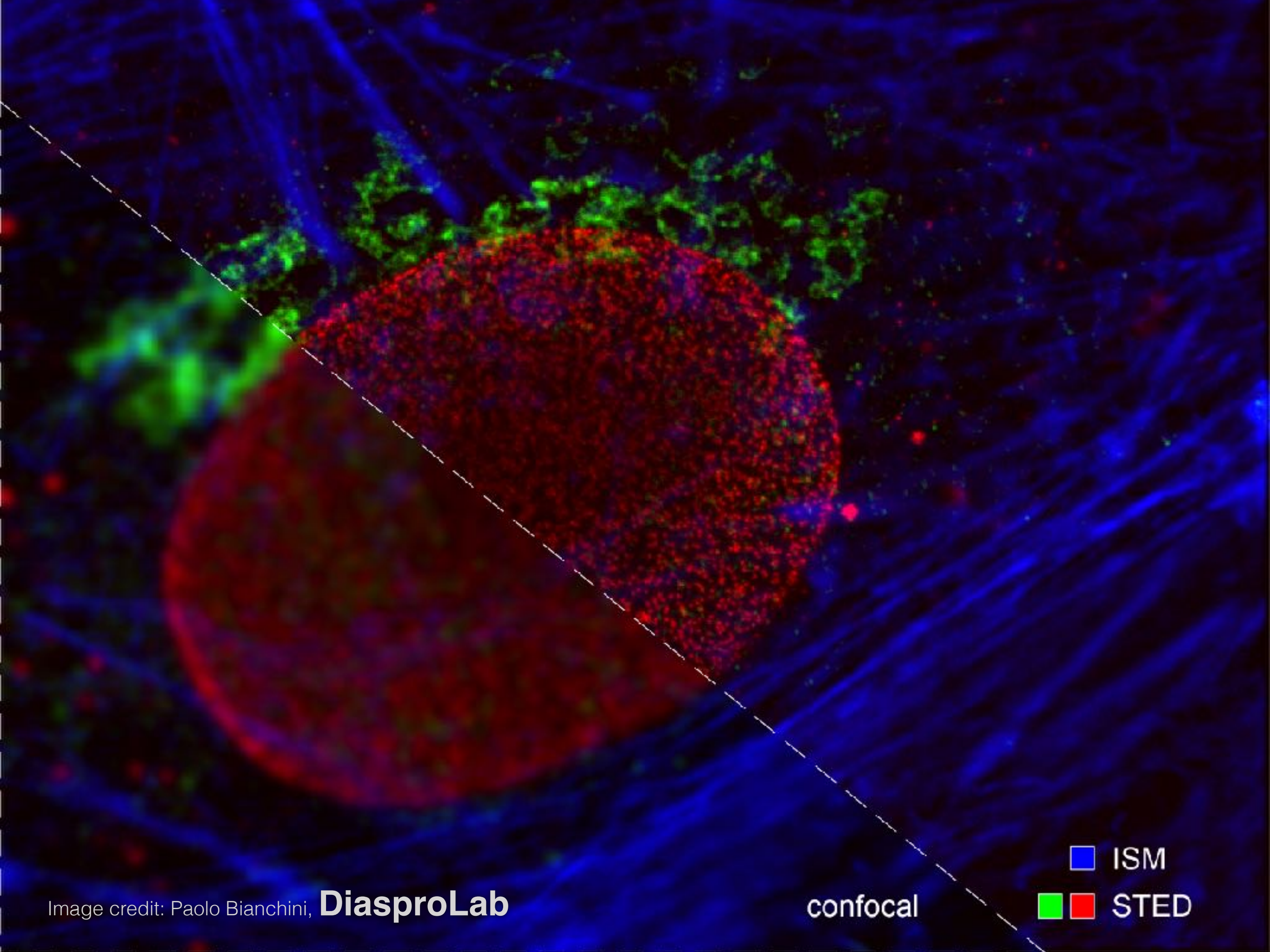


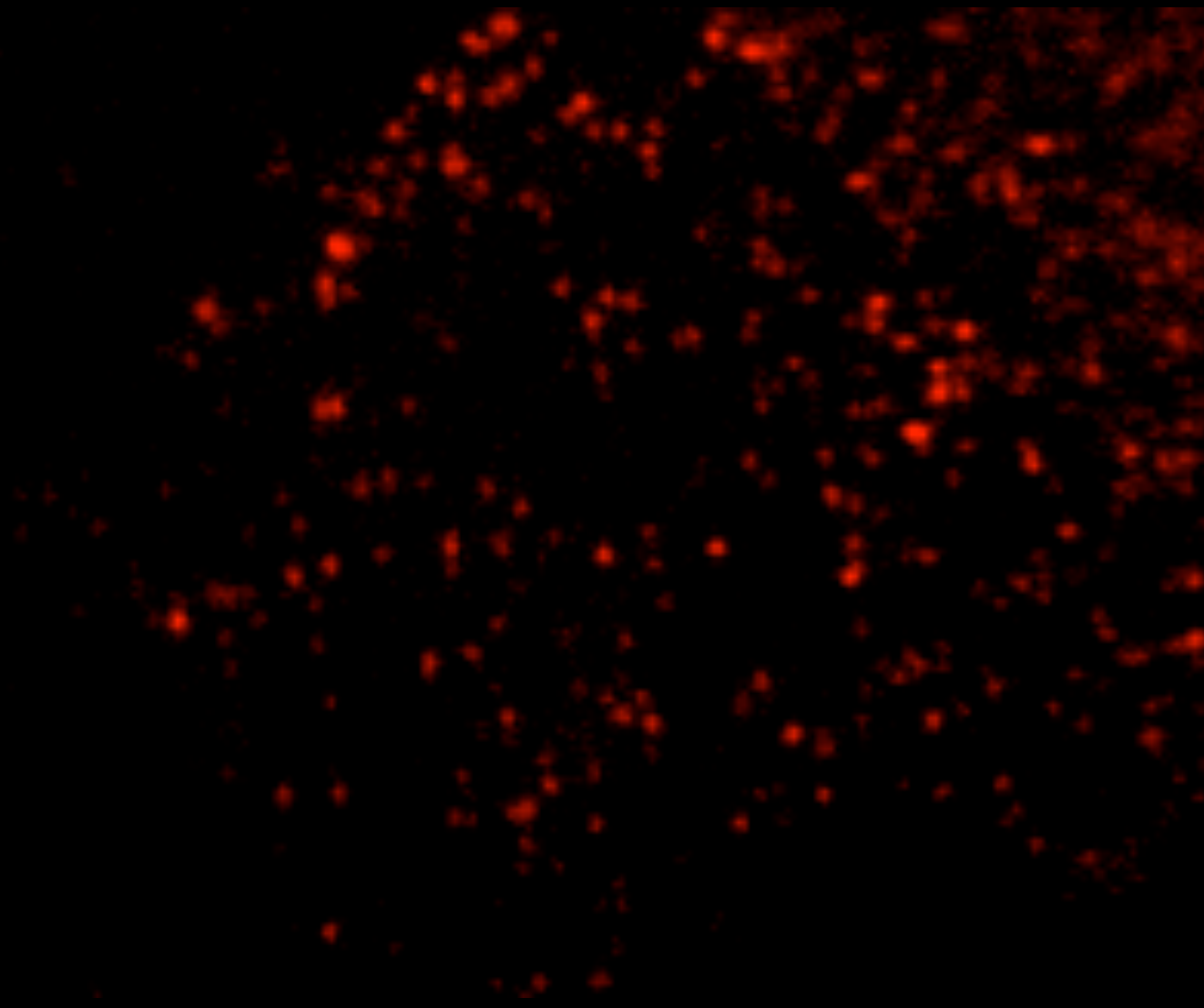
Image credit: Paolo Bianchini, **DiasproLab**

confocal

ISM
STED



Paraffin section of gut biopsy stained for Ki67 (abberior STAR ORANGE), Muc2 (abberior STAR RED), and DAPI.

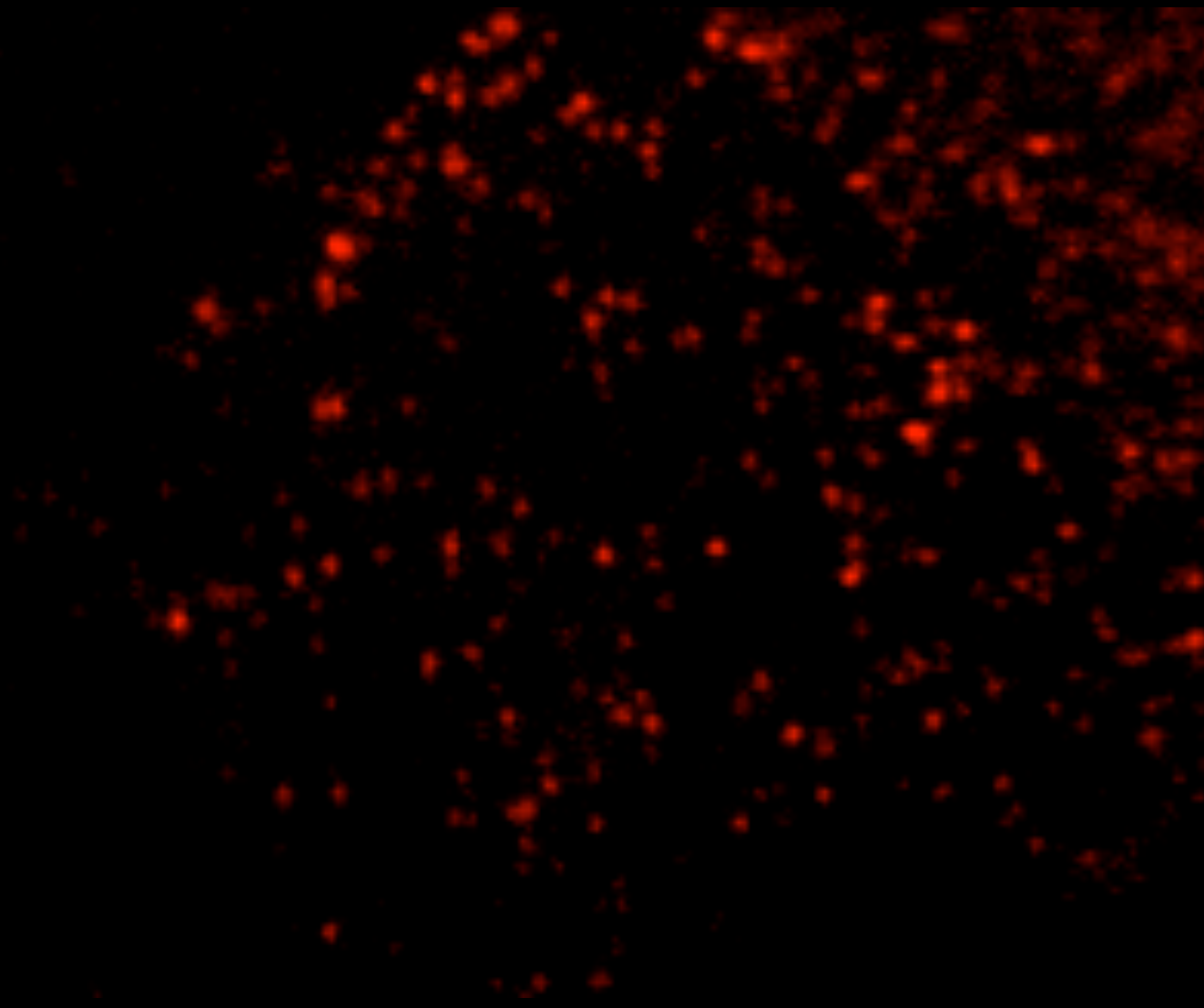


20 ms/frame - alfa-tubulin

Diaspro Lab - Francesca Cella Zanacchi project

localization precision 15 nm





20 ms/frame - alfa-tubulin

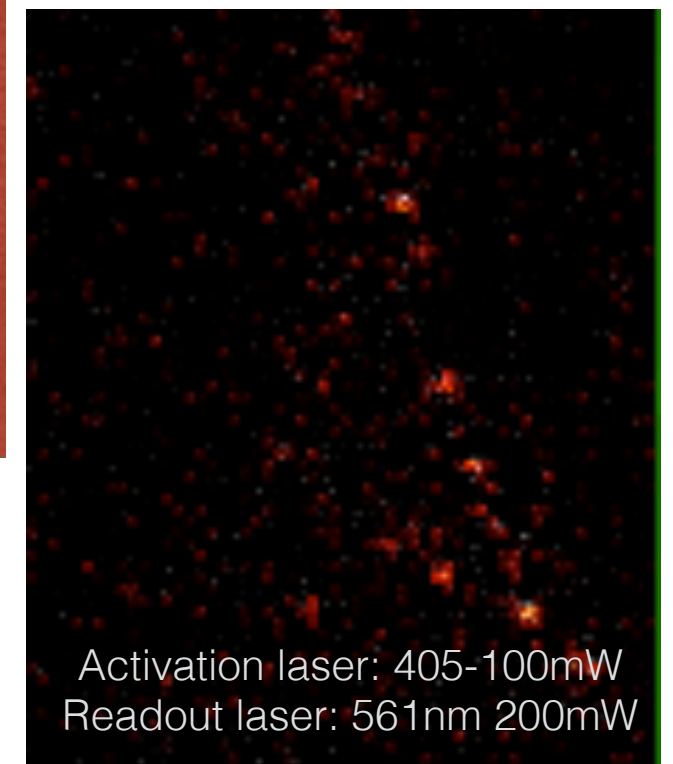
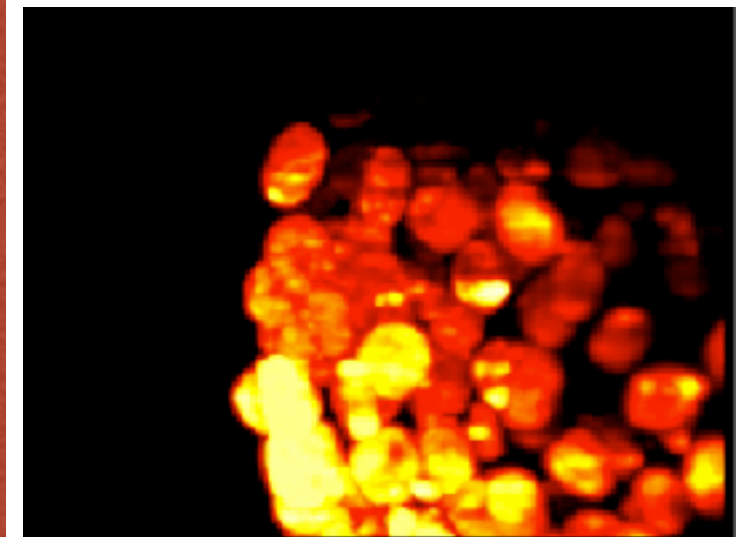
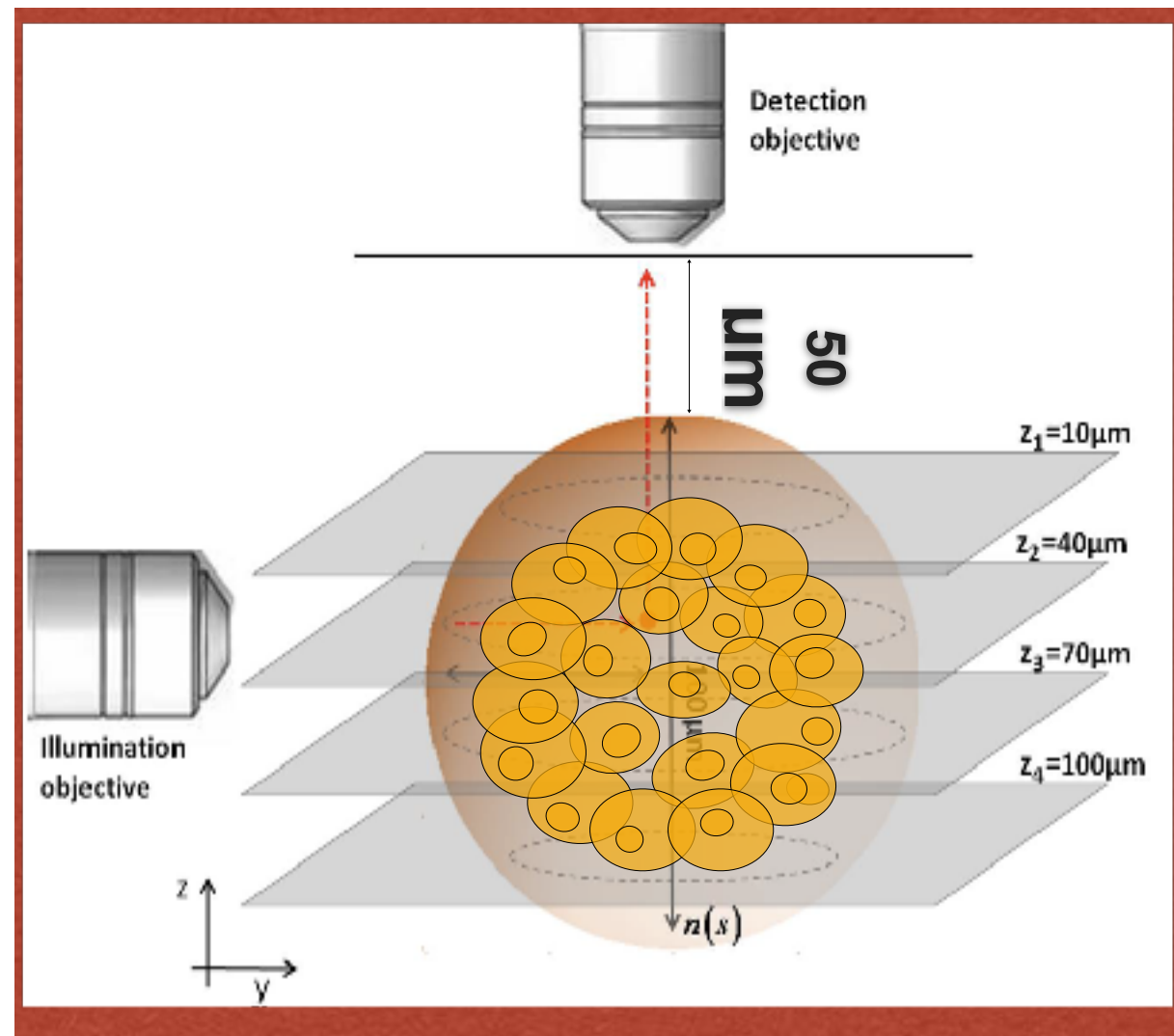
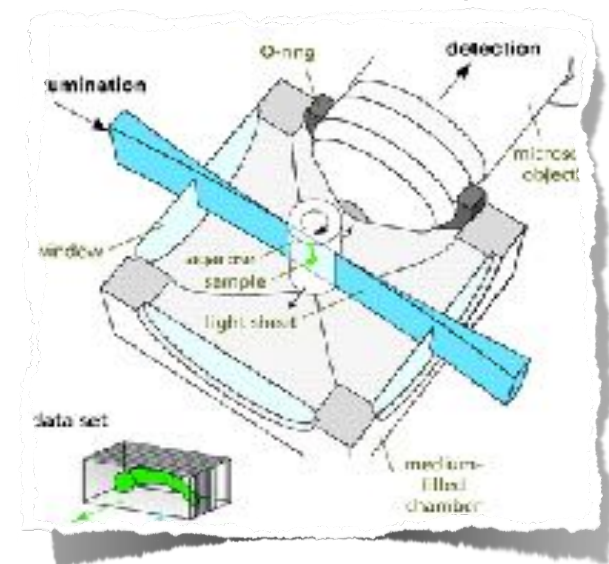
Diaspro Lab - Francesca Cella Zanacchi project

localization precision 15 nm



IML SPIM

(Individual Molecule Localization - Selective Plane Illumination Microscopy)

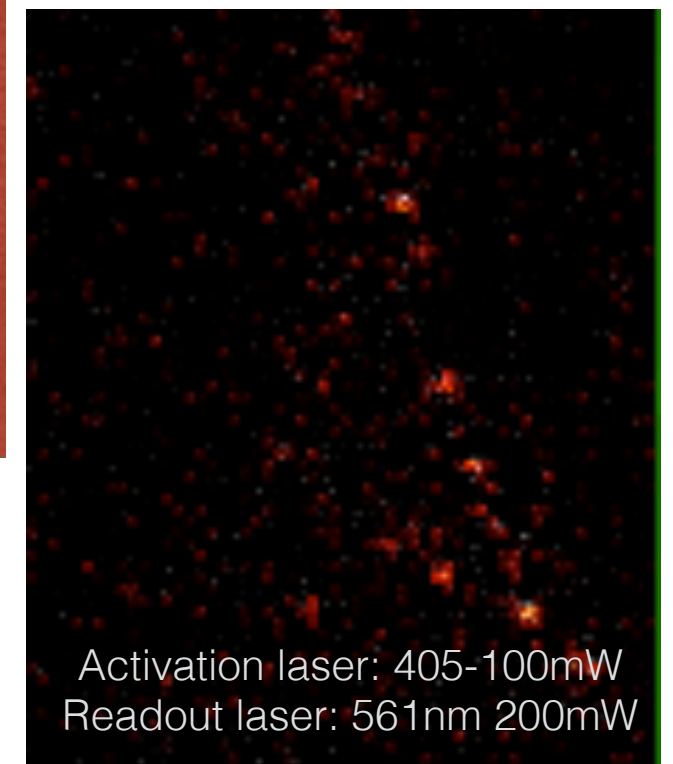
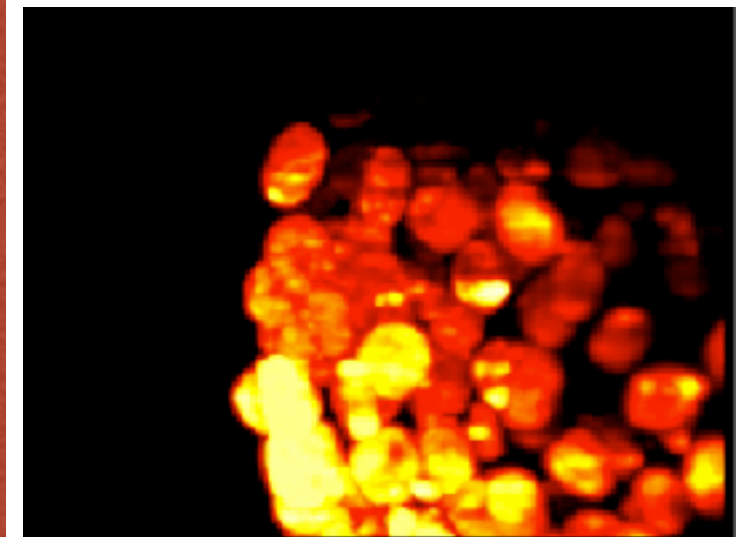
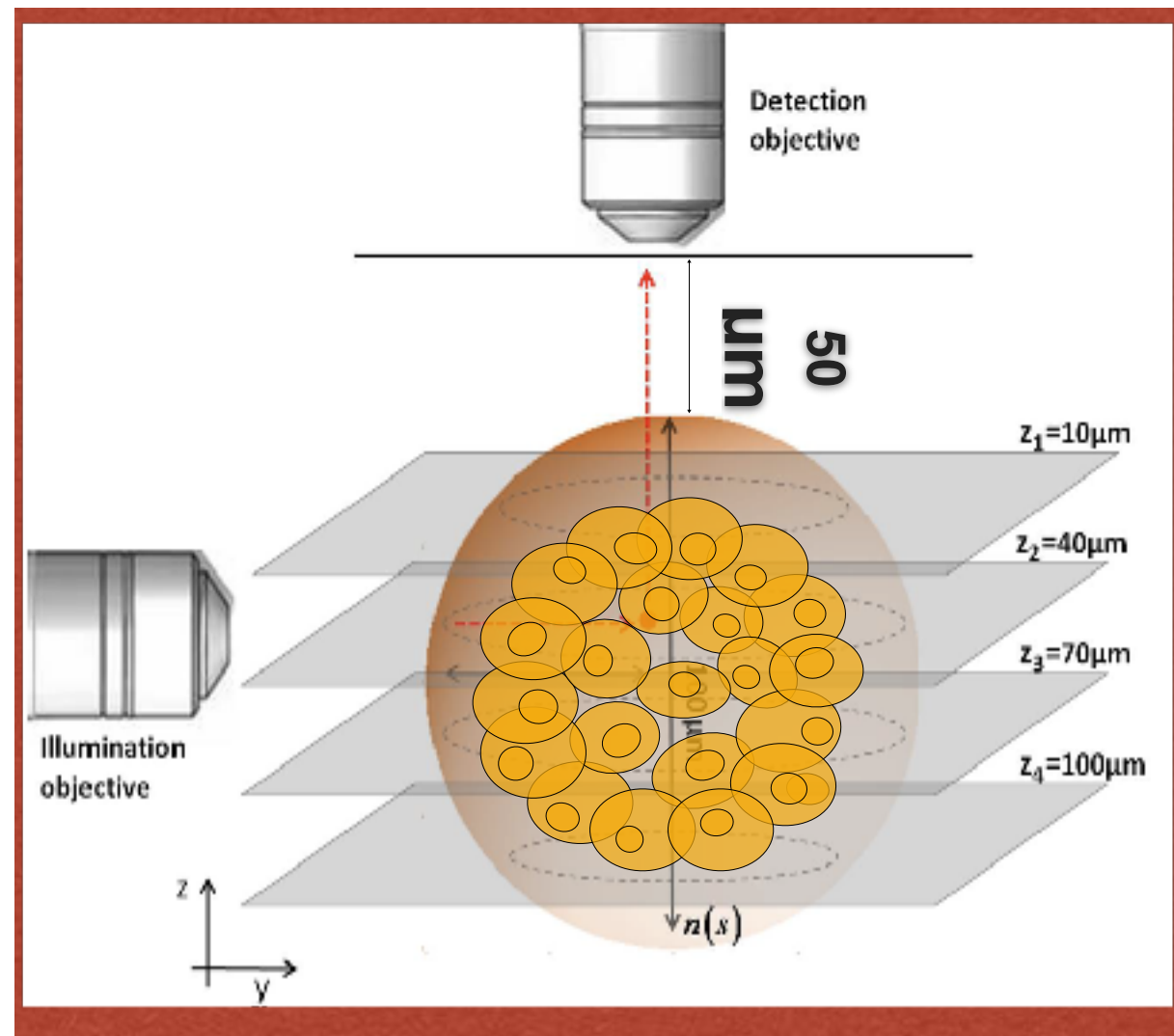
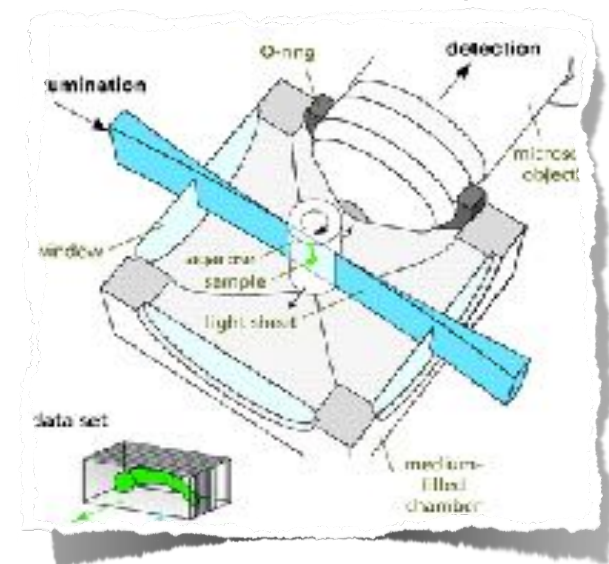


NATURE METHODS | VOL.8 NO.12 | DECEMBER 2011 |

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

IML SPIM

(Individual Molecule Localization - Selective Plane Illumination Microscopy)

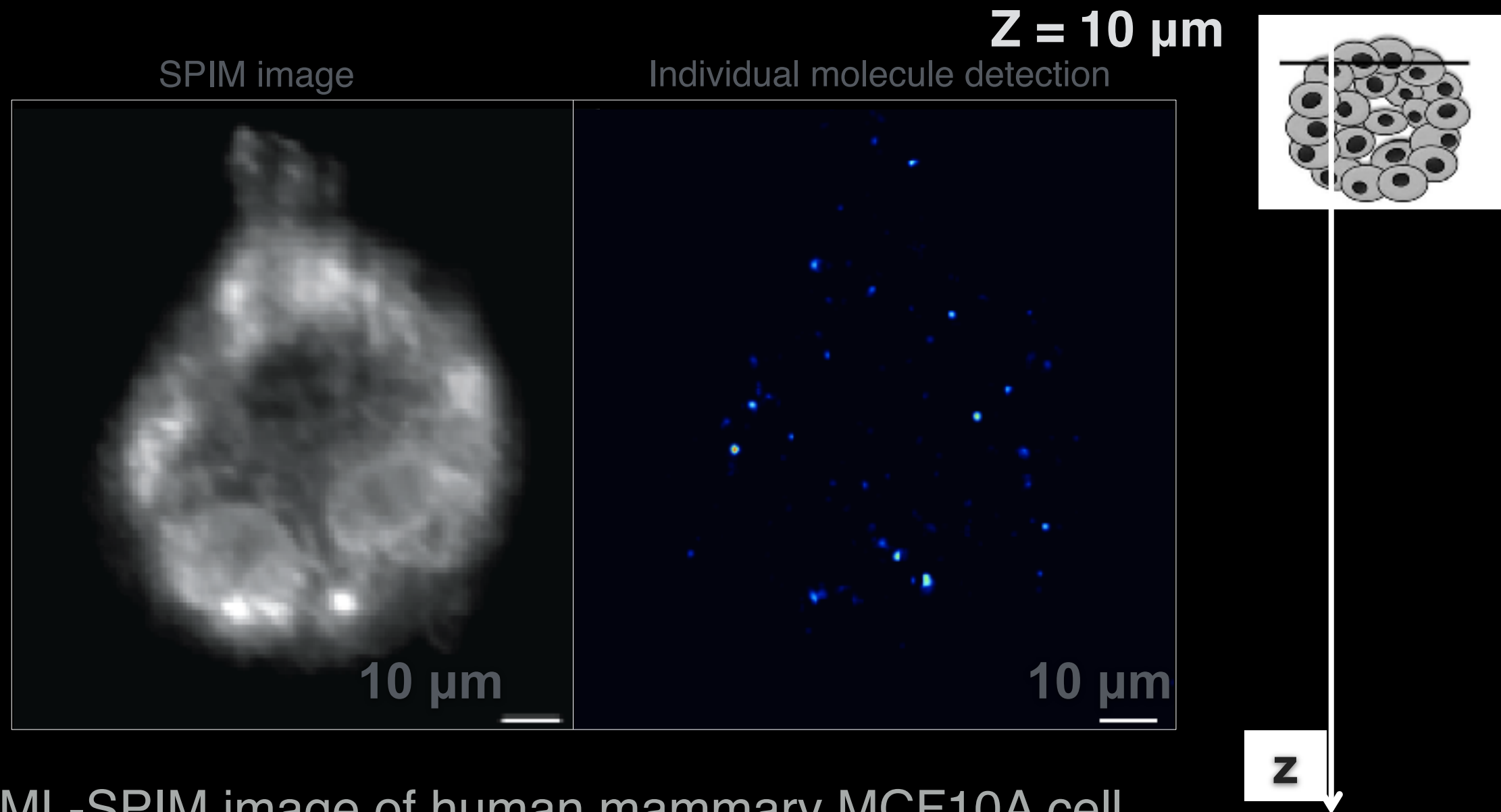


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Nature Methods (2011)

IML-SPIM

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

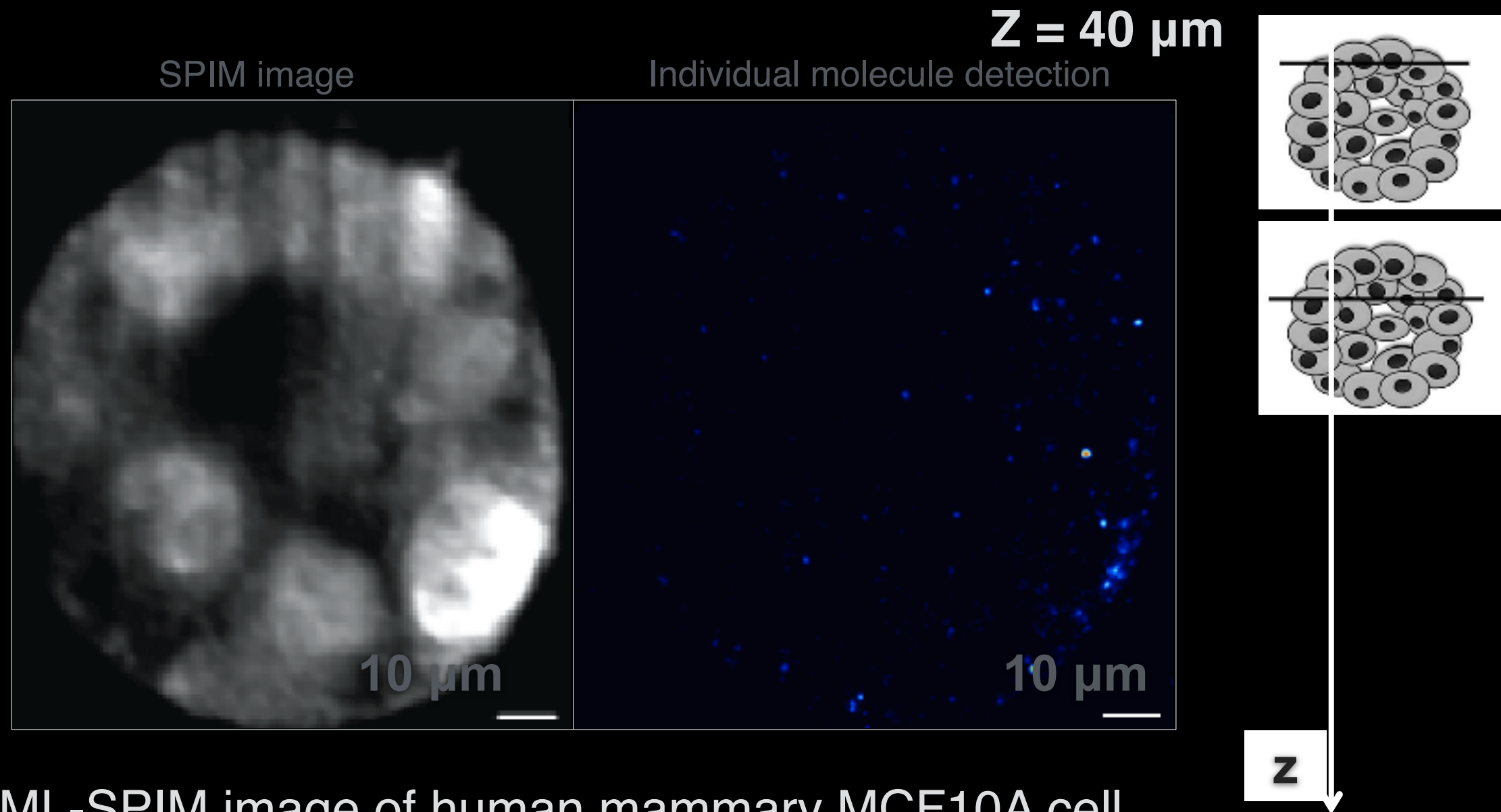


IML-SPIM image of human mammary MCF10A cell spheroids expressing H2B-PAmCherry.

NATURE METHODS | VOL.8 NO.12 | DECEMBER 2011 |

IML-SPIM

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

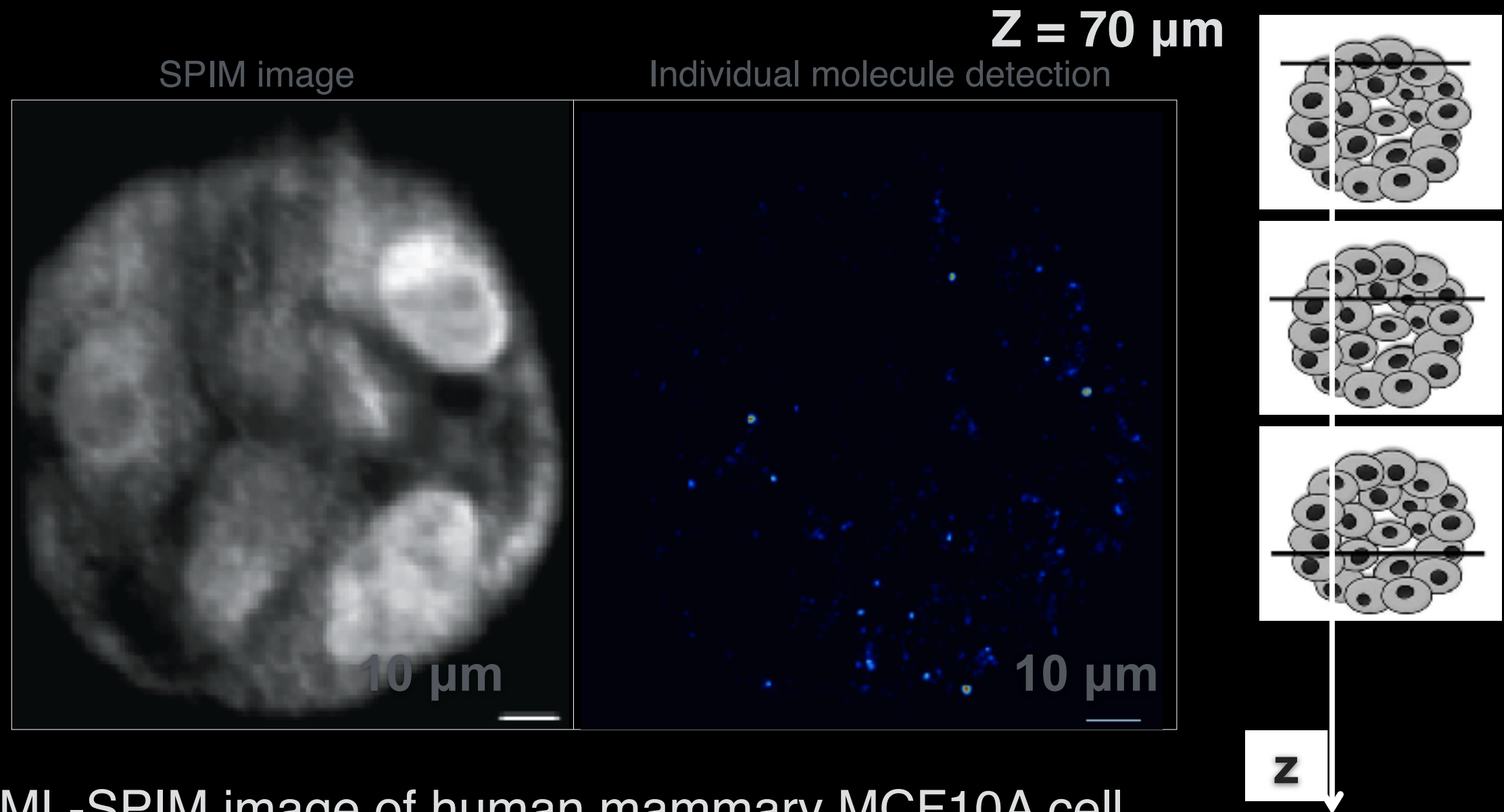


IML-SPIM image of human mammary MCF10A cell spheroids expressing H2B-PAmCherry.

NATURE METHODS | VOL.8 NO.12 | DECEMBER 2011 |

IML-SPIM

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

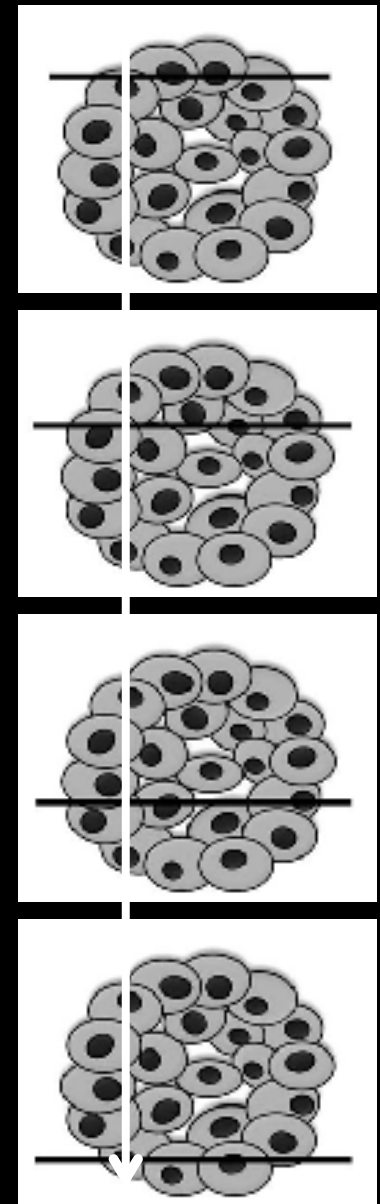
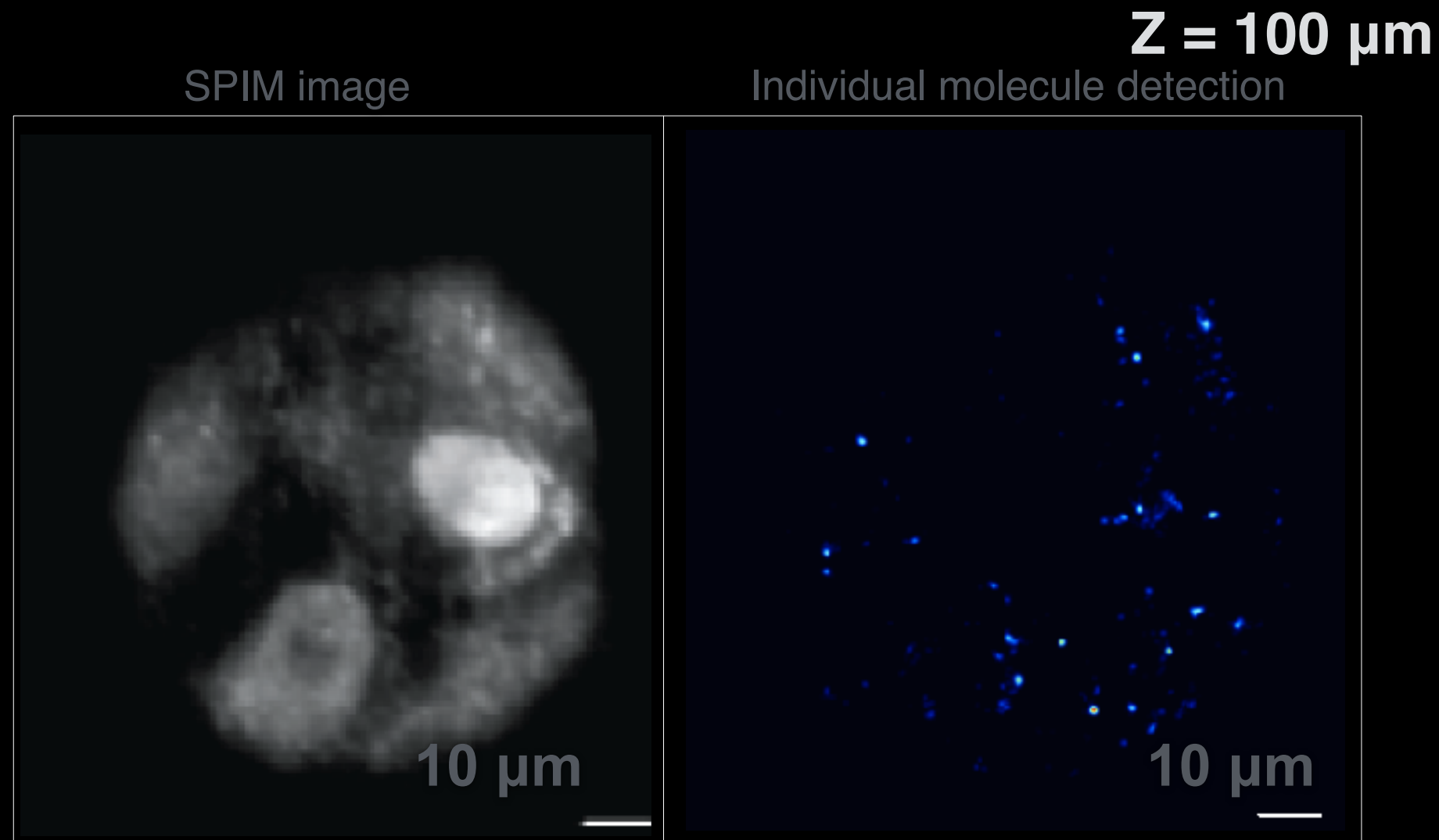


IML-SPIM image of human mammary MCF10A cell spheroids expressing H2B-PAmCherry.

NATURE METHODS | VOL.8 NO.12 | DECEMBER 2011 |

IML-SPIM

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



IML-SPIM image of human mammary MCF10A cell spheroids expressing H2B-PAmCherry.

NATURE METHODS | VOL.8 NO.12 | DECEMBER 2011 |

LENDL 6 6 3 3 3
CHANG 4 4 6 6 4



CONVEGNO DELLA SERA
ONE more thing
di Massimo Sestini



1989

CHANG - LENDL

LENDL 6 6 3 3 3
CHANG 4 4 6 6 4



CONVEGNO DELLA SERA
ONE more thing
di Massimo 36



1989

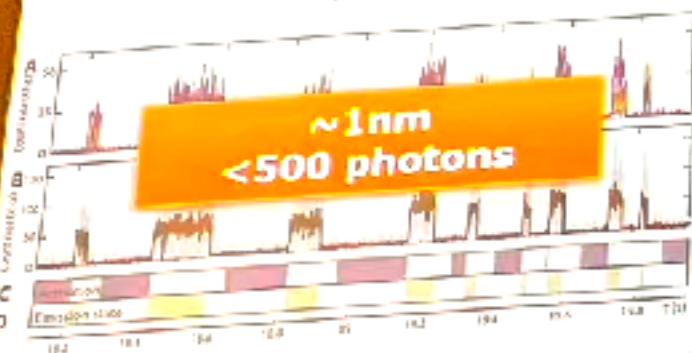
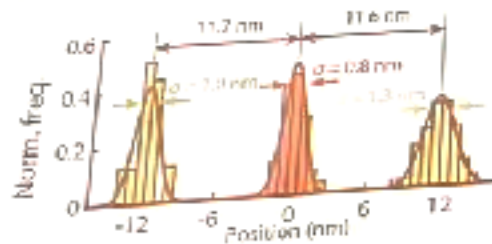
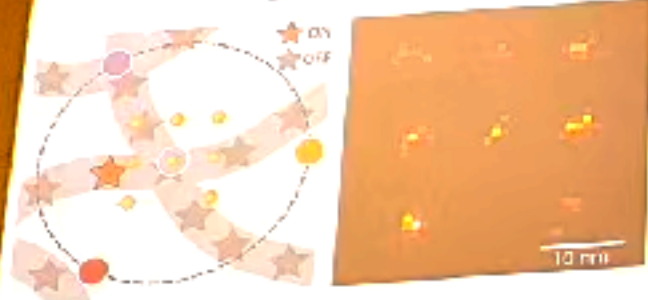
CHANG - LENDL

MINIFLUX APPLICATIONS

TRACKING ~nm RANGE

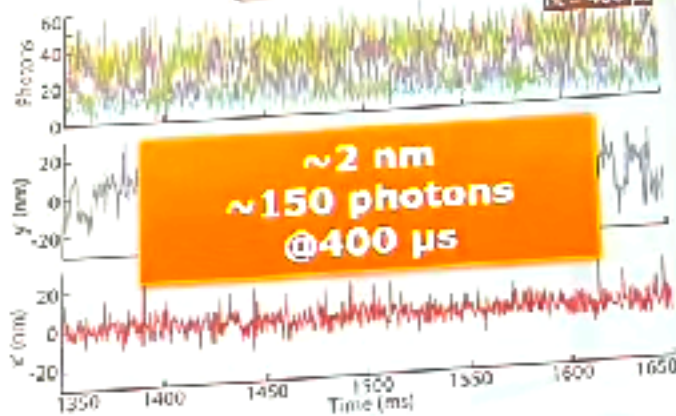
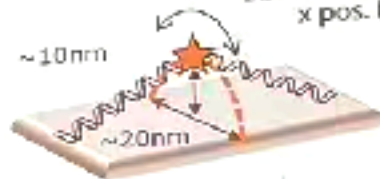
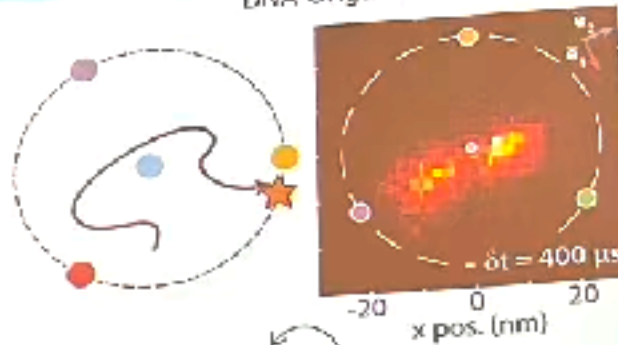
NANOSCOPY

DNA Origami



**~1nm
<500 photons**

DNA Origami



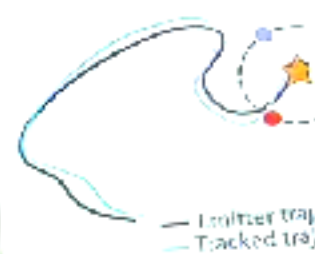
**~2 nm
~150 photons
@400 μs**

TRACKING ~nm RANGE

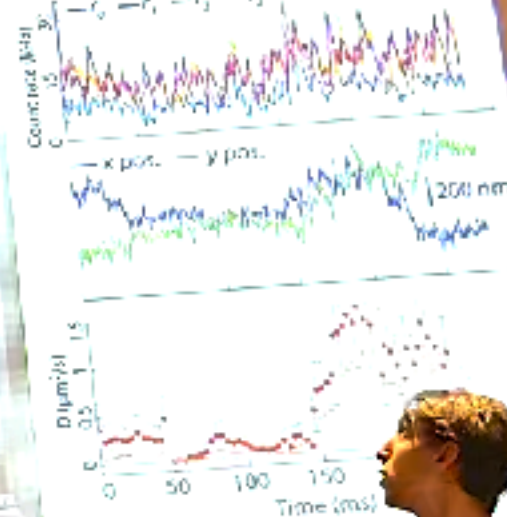
Ribosomal subunit proteins



Johan Elf
Uppsala University



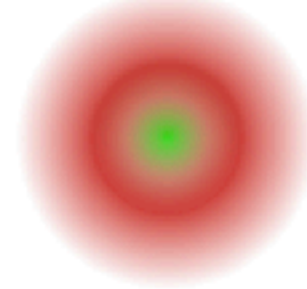
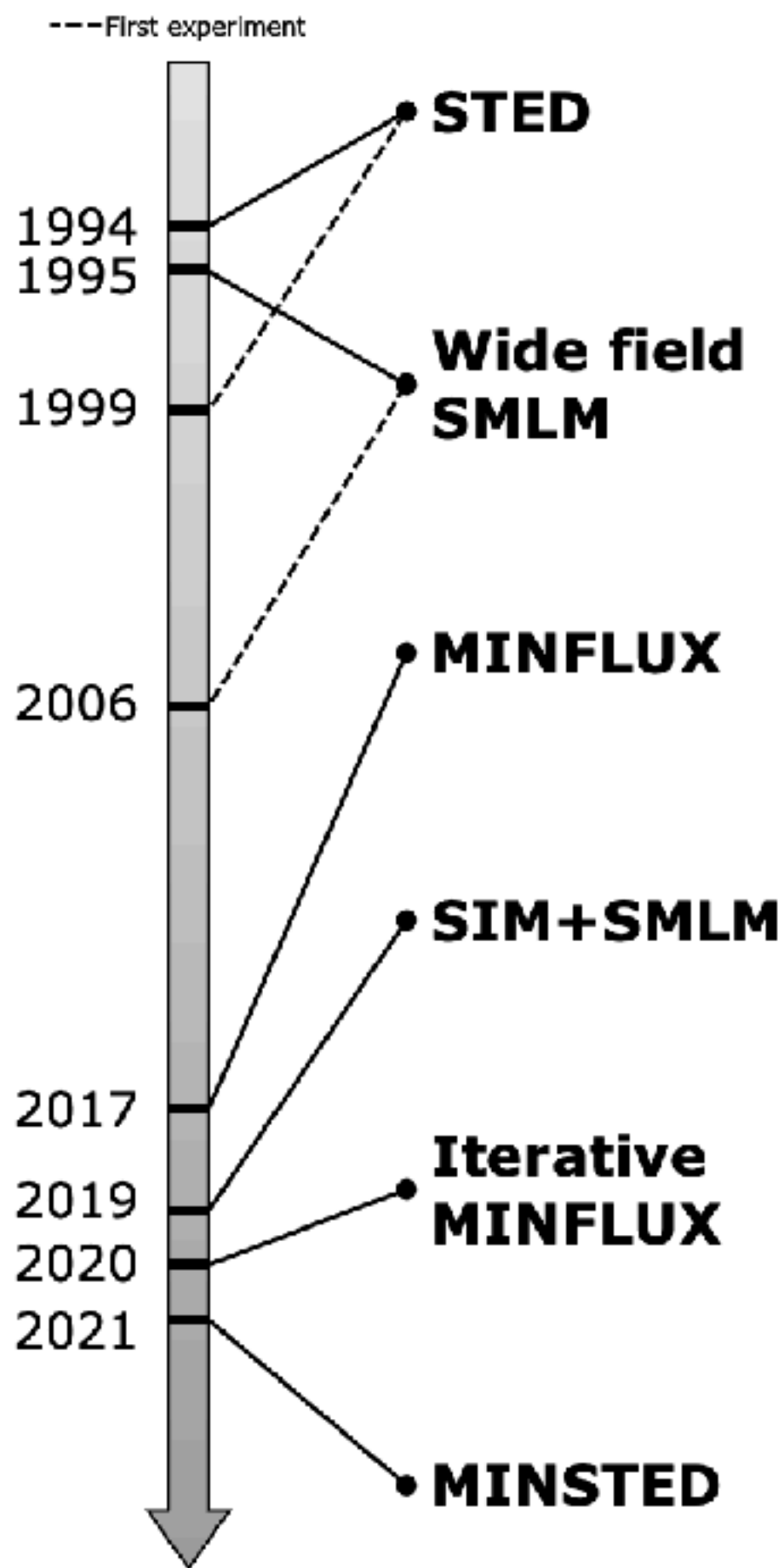
Localizations	8-15	100k	747
Time resolution	10-50 ms	125 μs	
Tracking error	20-60 nm	50 nm	



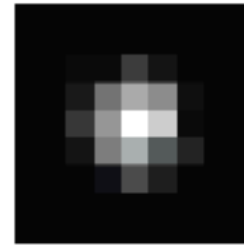
@Balzarotti

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

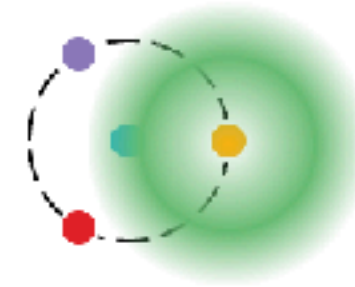
Y. Elf, et. al. *PNAS*, 115, 6117-6122 (2018)



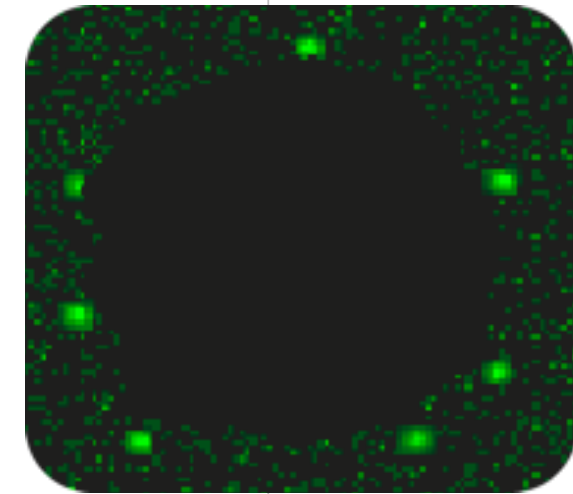
$$\sigma_{STED} \propto \frac{\lambda}{NA} \frac{1}{\sqrt{1 + I/I_s}}$$



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



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



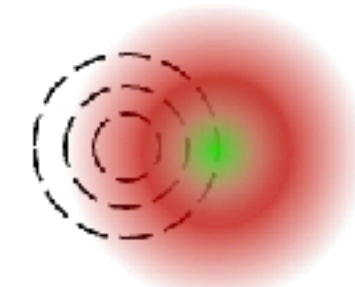
Diaspro A., et al. (2006) Optics Express



$$\sigma_{SIM} \propto \frac{\lambda}{NA} \frac{1}{\sqrt{N}} \frac{1}{2.4}$$

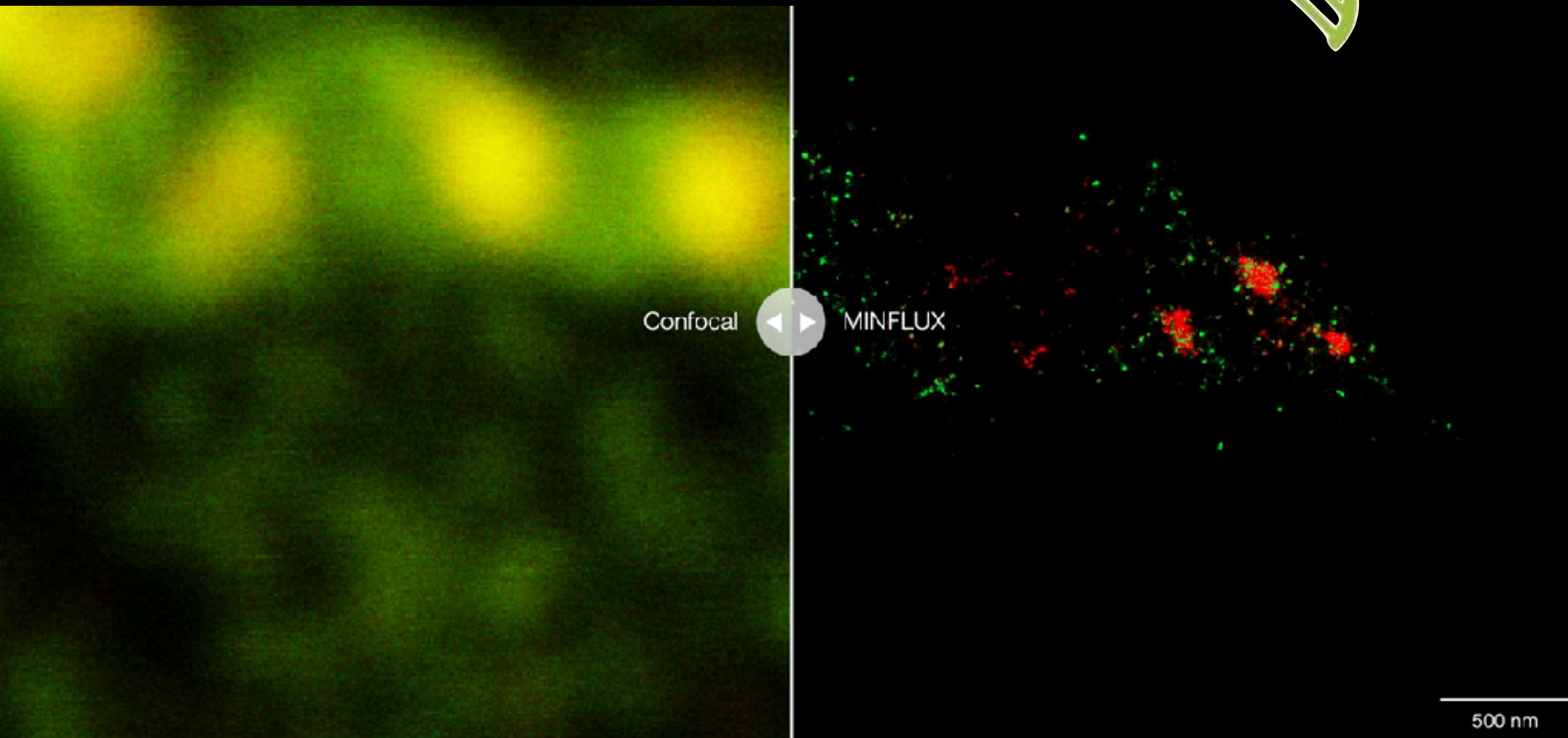
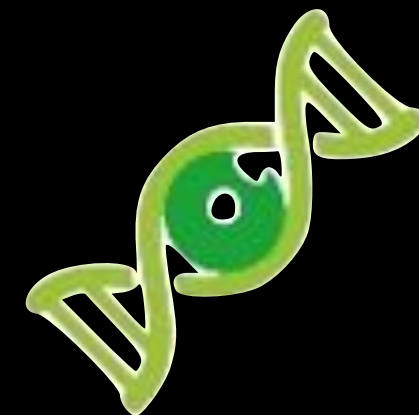


$$\sigma_{iMF} \propto \frac{L}{N^{K/2}}$$



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

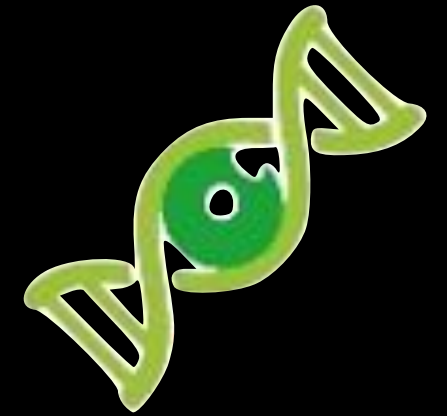




Localization precision:
better than 2 nm

Localization rate:
10kHz

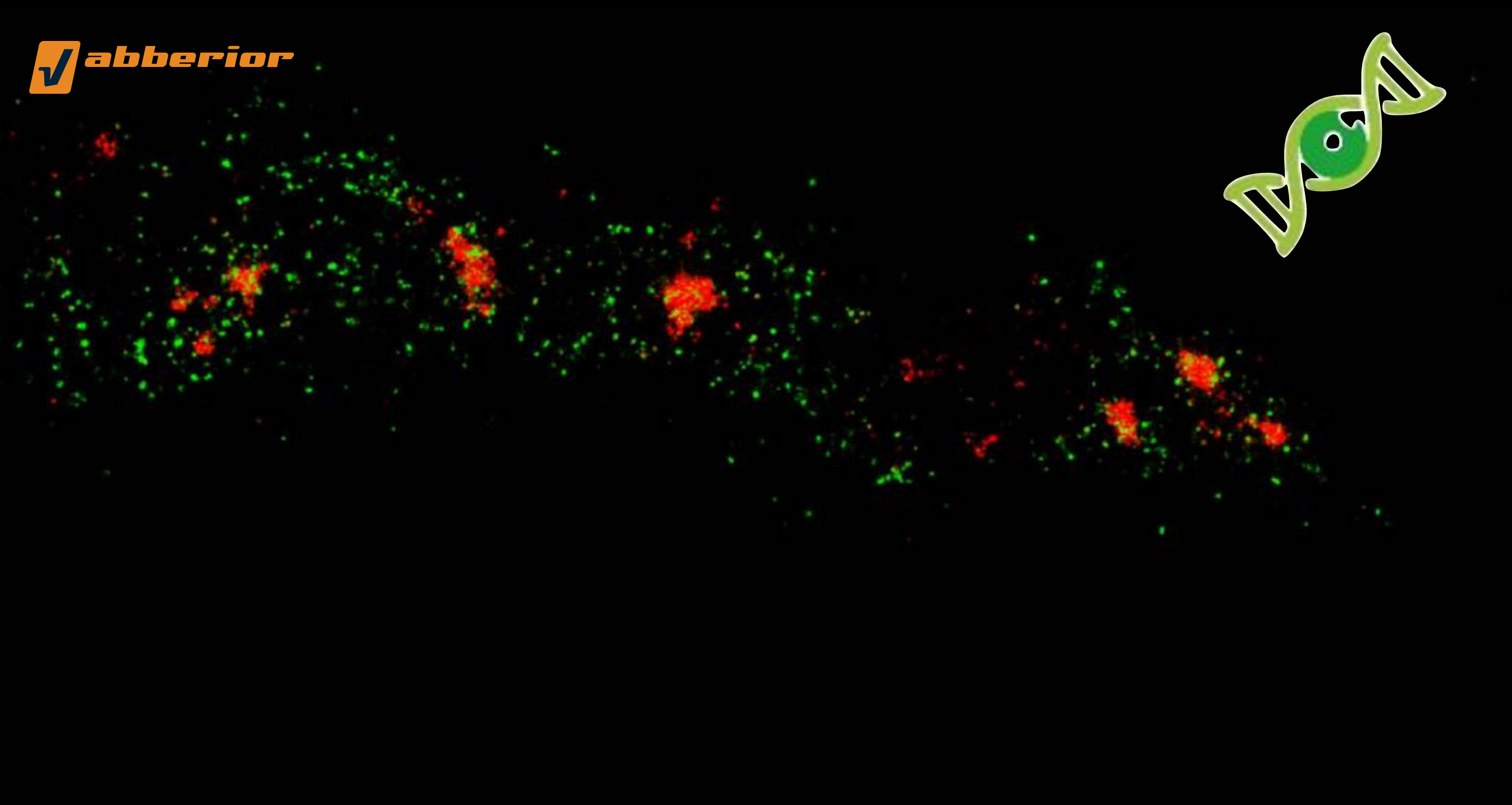
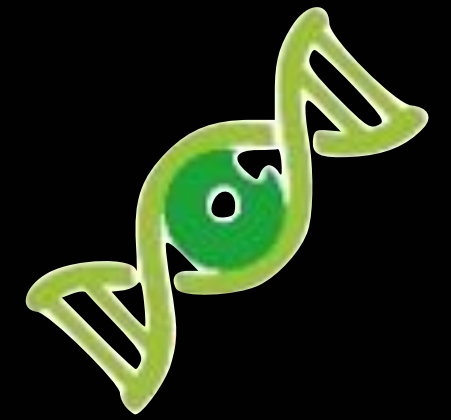
Typical track length:
> 28000 @ 20 nm precision



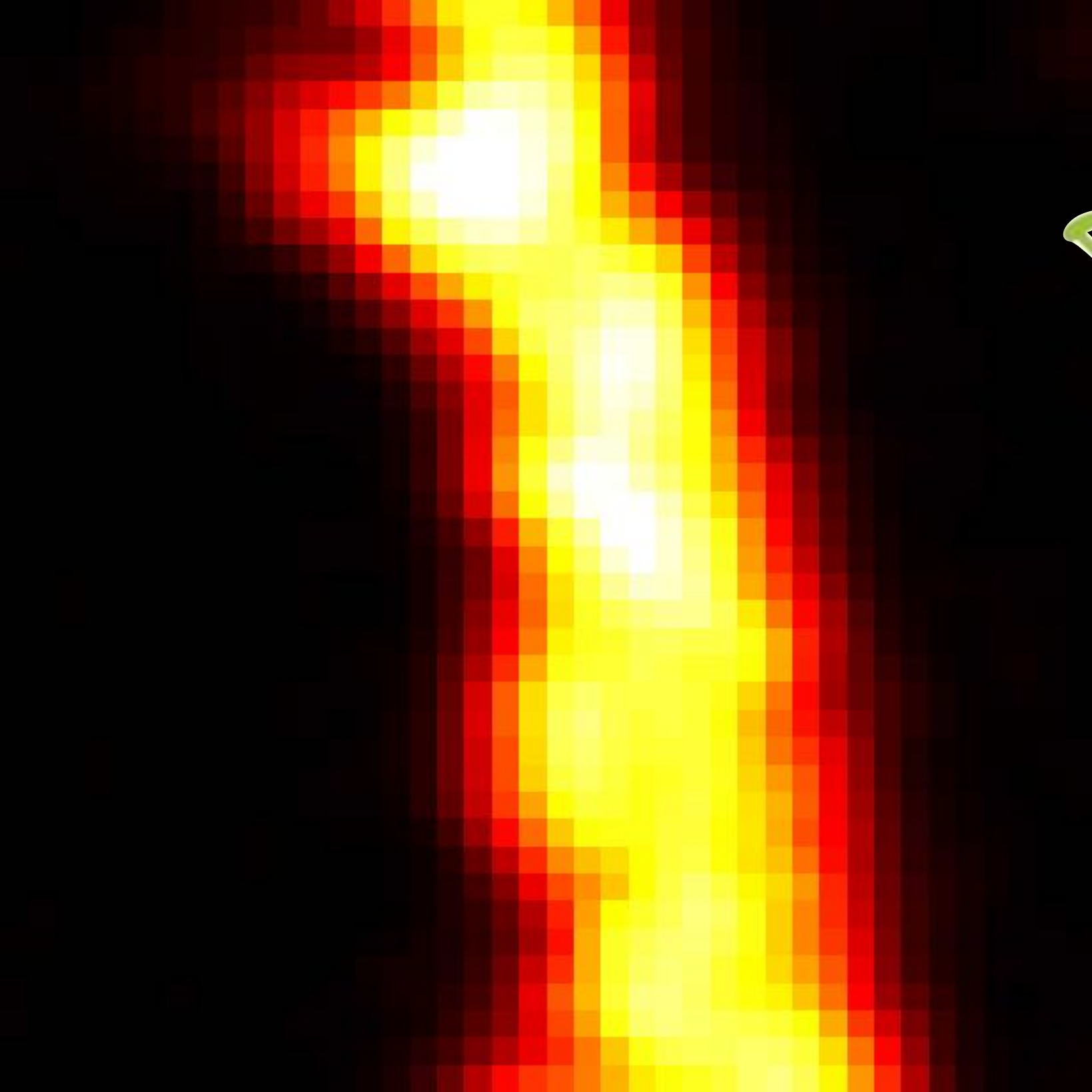
Two-color *MINFLUX* on mitochondria samples. The mitochondrial proteins TOM20 (green) and mtDNA (red) were labeled in mammalian cells with indirect immunofluorescence using secondary antibodies coupled to sCy5 and CF680. Two-color confocal (A) and *MINFLUX* (B) was performed using a ratiometric detection strategy. Please note that the labeling density of both structures is highly dissimilar. For TOM20 single proteins are labeled in the mitochondrial membrane, whereas numerous binding sites are decorated in the mtDNA. *MINFLUX* enables the visualization and separation of both structures.



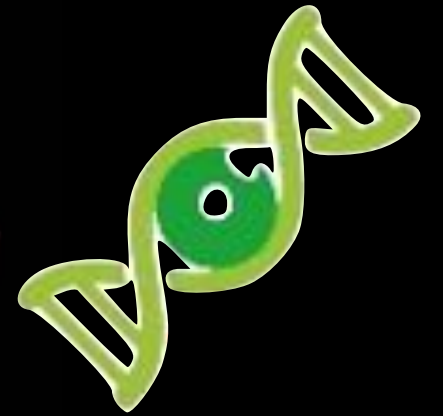
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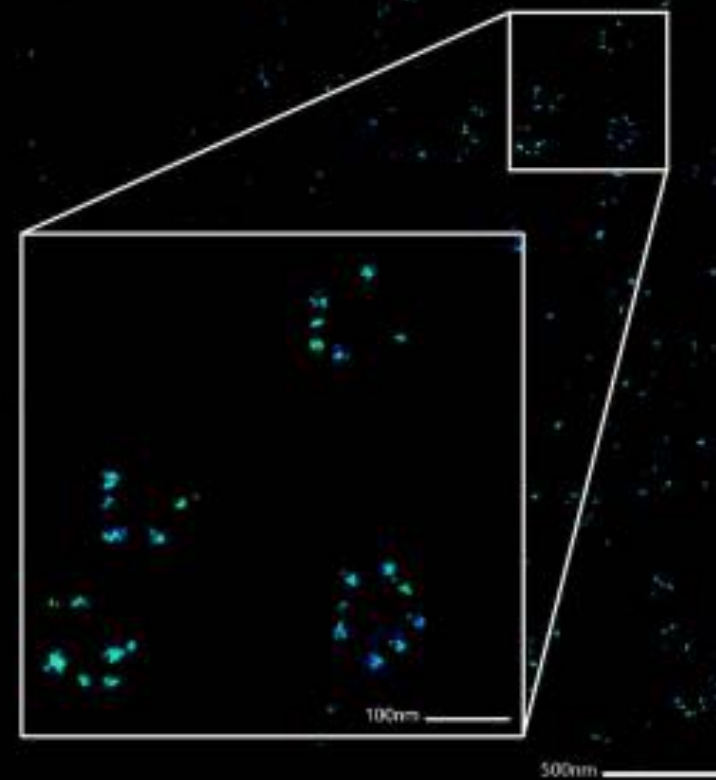
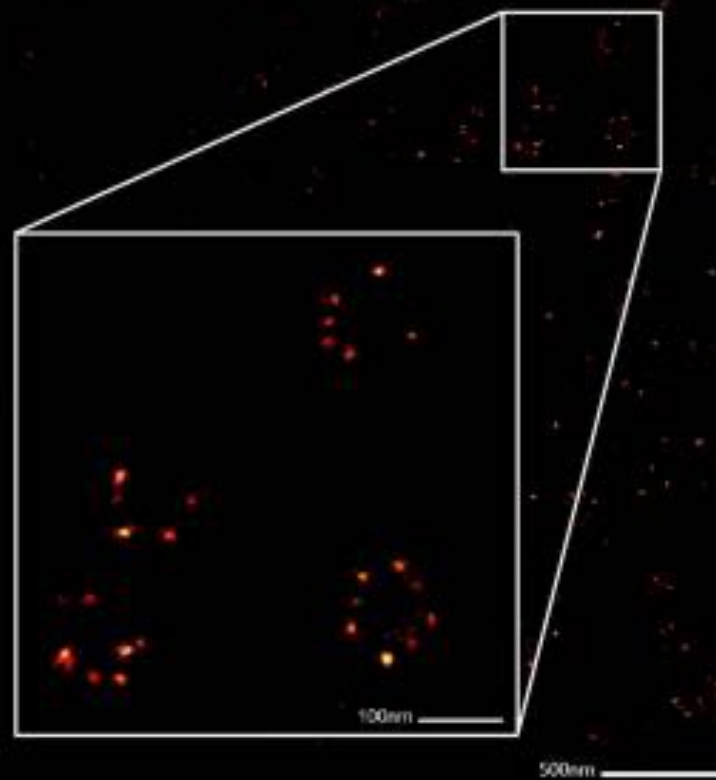
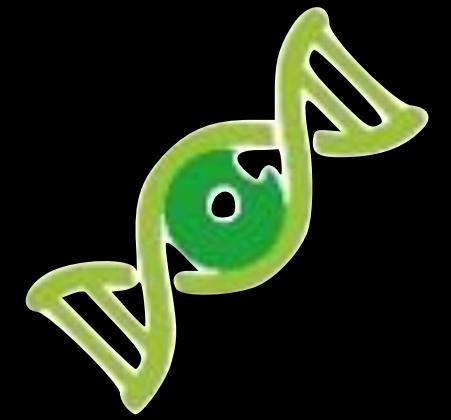
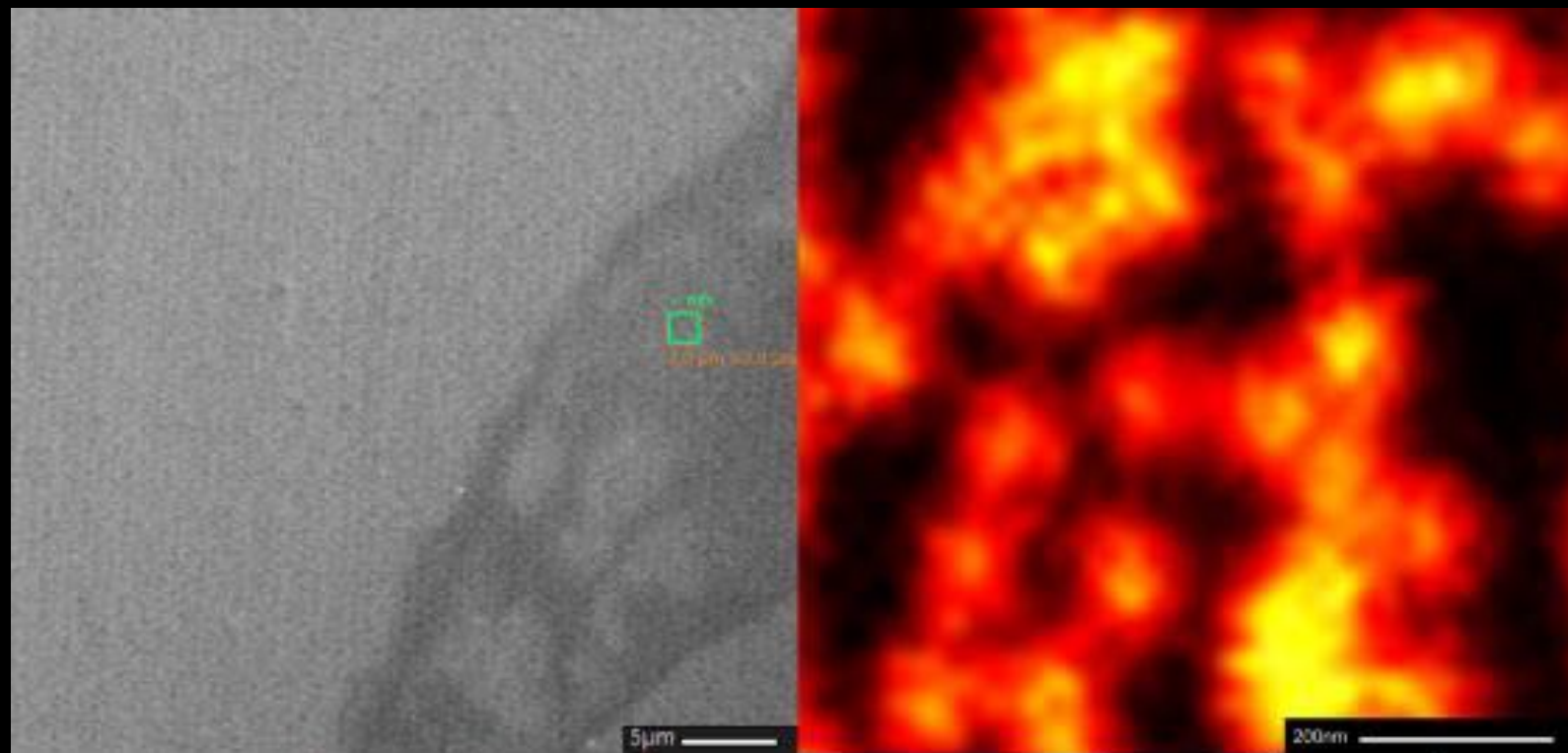
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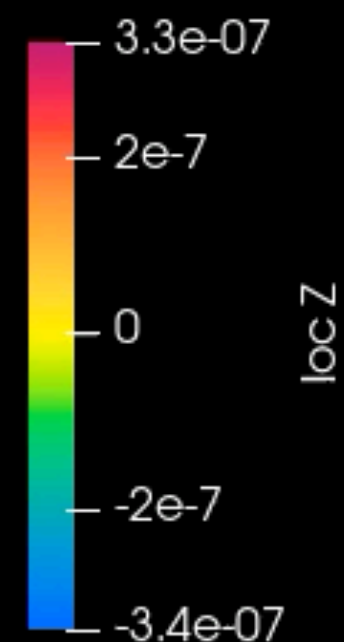
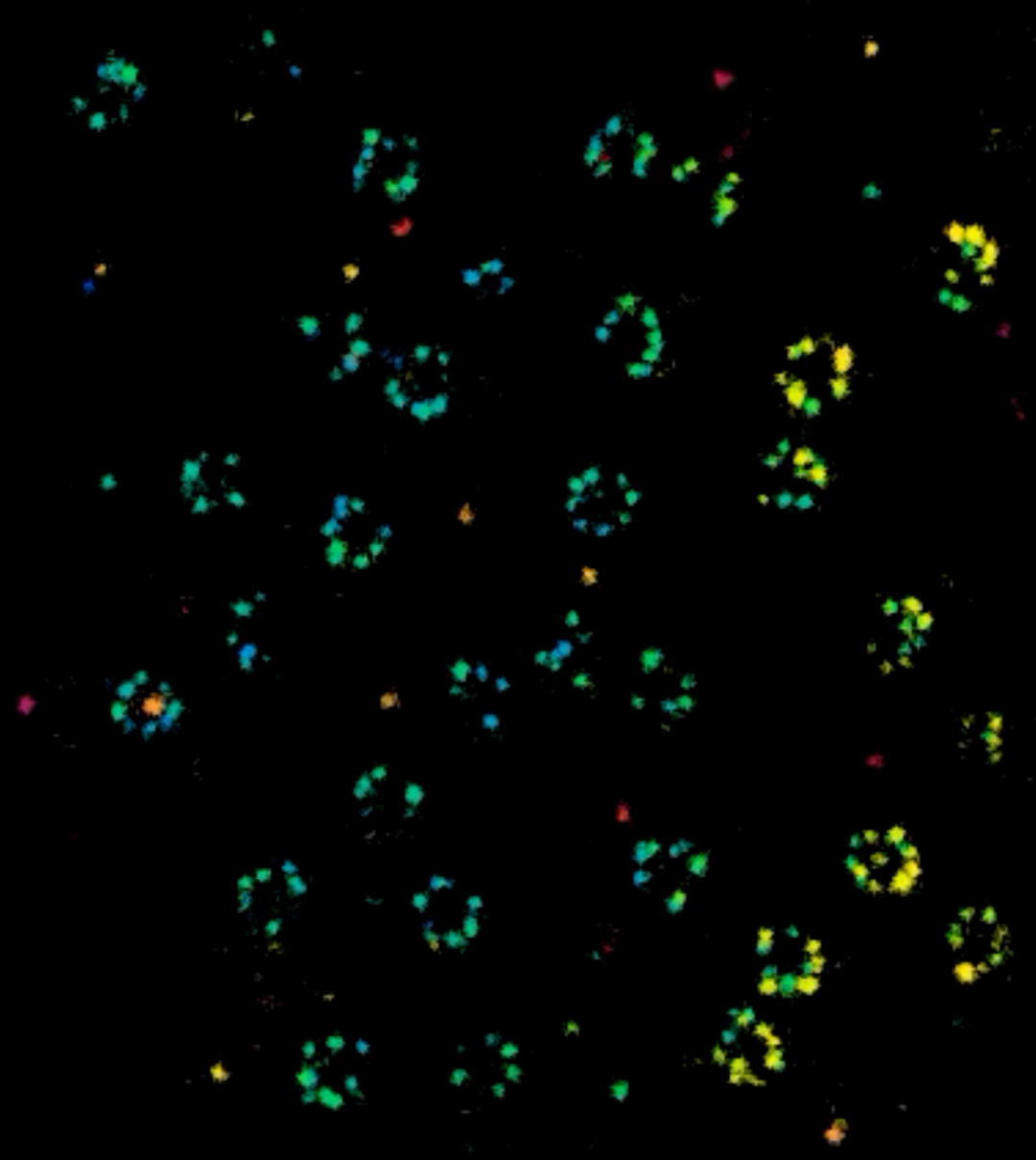
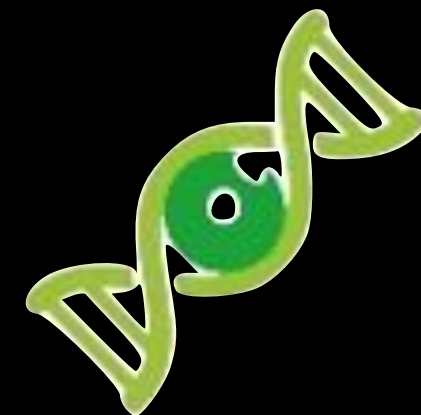


MINFLUX image of axonal β II spectrin in primary hippocampal neurons with < 2 nm resolution. Note the periodic arrangement of spectrin along the axon, and the absence of any details in the confocal counterpart image.

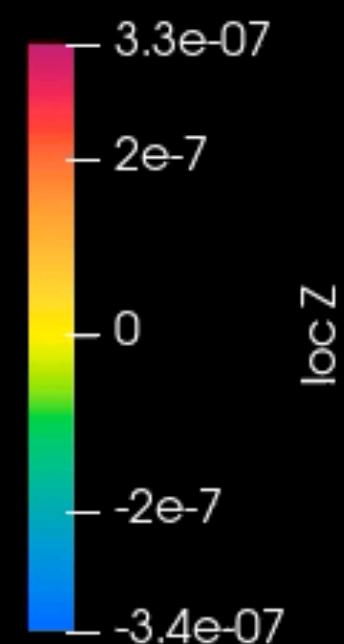
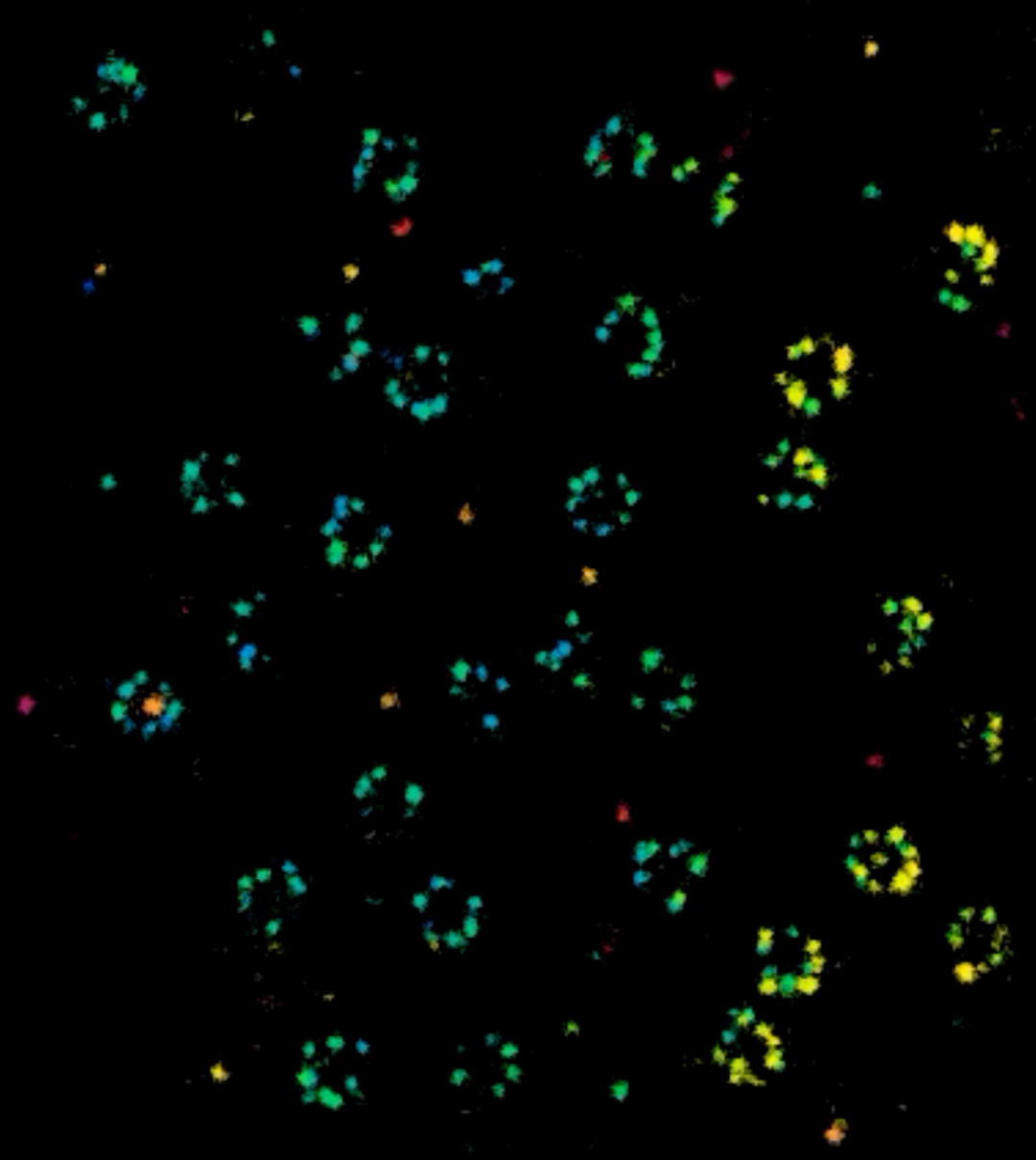
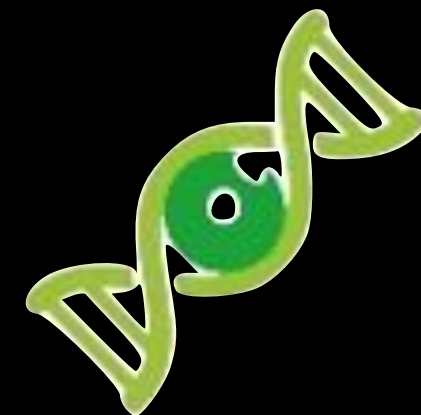


MINFLUX image of axonal β II spectrin in primary hippocampal neurons with < 2 nm resolution. Note the periodic arrangement of spectrin along the axon, and the absence of any details in the confocal counterpart image.



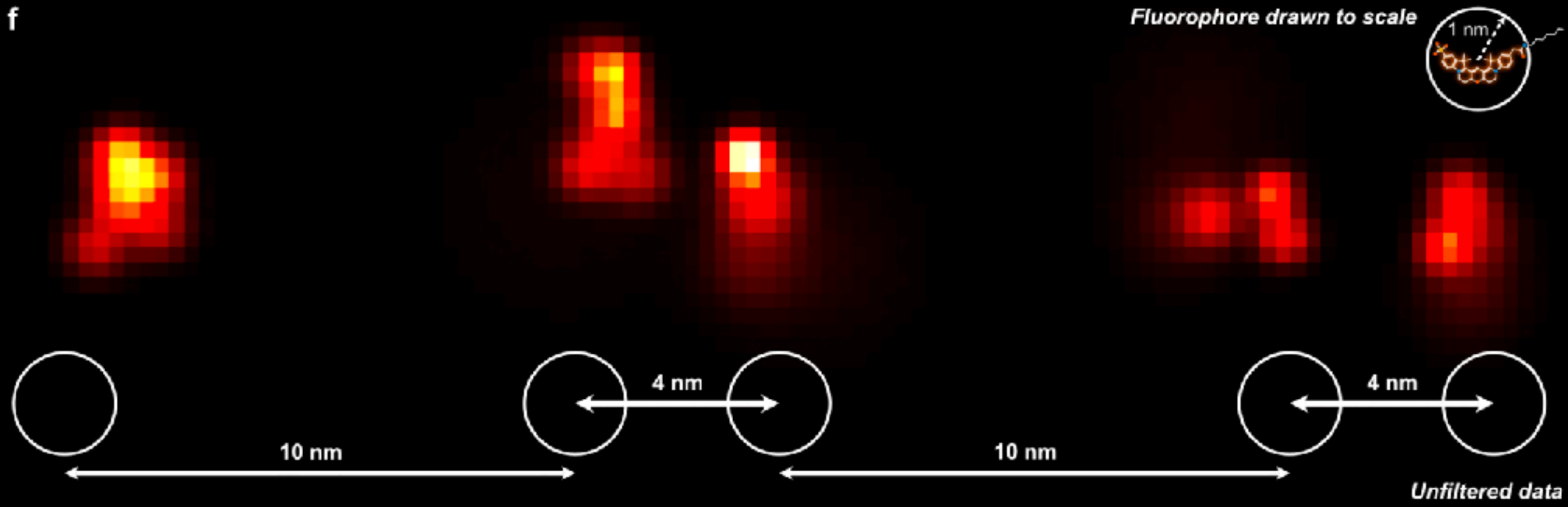


3D of nuclear pores, z-position coded.
Image by Clara Guerth & Virginia Bazzurro,
Heidelberg, EMBL course 21-26 October 2024



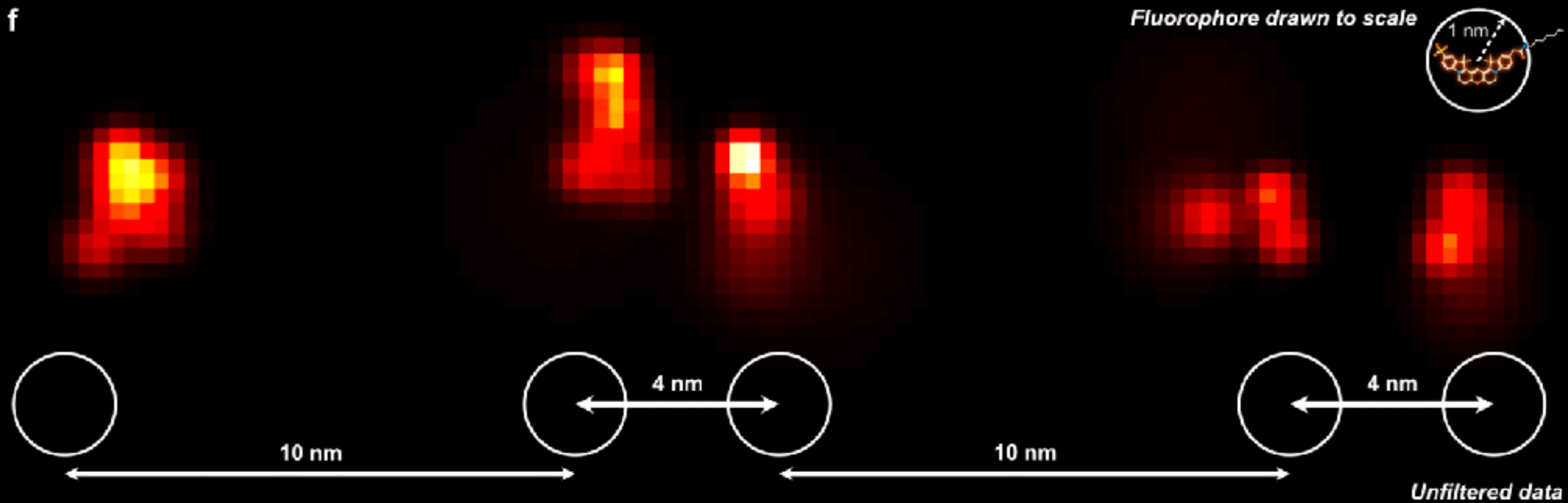
3D of nuclear pores, z-position coded.
Image by Clara Guerth & Virginia Bazzurro,
Heidelberg, EMBL course 21-26 October 2024

f





f



MINSTED nanoscopy enters the Ångström localization range

Received: 25 March 2062

Michael Weber^{1,2}, Henrik von der Esche^{1,2}, Marcel Leuninger¹,
Philip Dunkel², Steinhilber Bombardieri^{1,2}, Teakker A. Khan¹,
Jon Koller-Findeken¹, Willem C. Gerdes² and Stefan W. Hell^{1,2}✉

Accepted: 2015 September 16; Accepted Manuscript: 2015 September 16

Published online: 7 November 2021

Ångström-resolution fluorescence microscopy

<http://dx.doi.org/10.1038/941686-023-05225-0>

Received: 21 July 2022

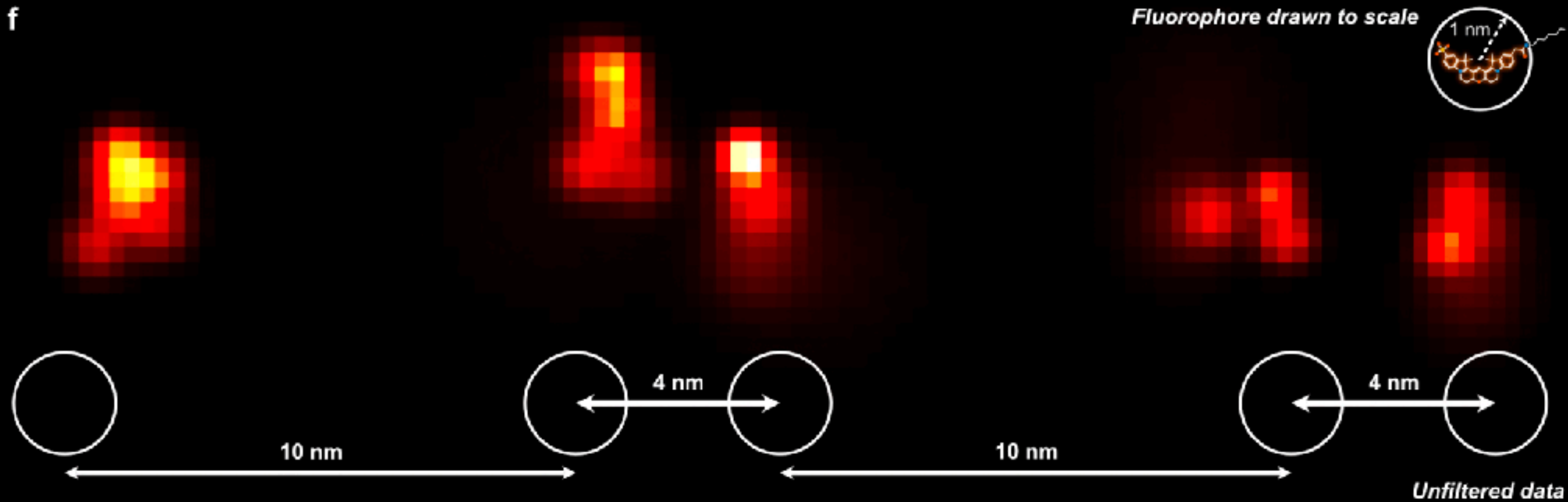
Accepted: 7 March 2023

Published online 24 May 2023

Suzanne C. M. Reinhardt^{1,2}, Luciano A. Masullo¹, Isabelle Baudraux^{1,2}, Philipp H. Stoen^{2,3},
Rafal Krowczyński^{1,4}, Alexandra S. Eklund^{1,5}, Sebastian Strauss^{1,2}, Edward M. Unterwiesing^{1,2},
Thomas Schlichthaele^{1,2}, Maximilian T. Strauss^{1,2}, Christian Klein^{3,4} & Ralf Jungmann^{1,2,6}

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

f



No limits, Jonathan? he thought, and he smiled. His race to learn had begun.

Richard Bach
Jonathan Livingston Seagull
Scribner, New York, 1970

<https://doi.org/10.1038/s41586-023-06226-9>

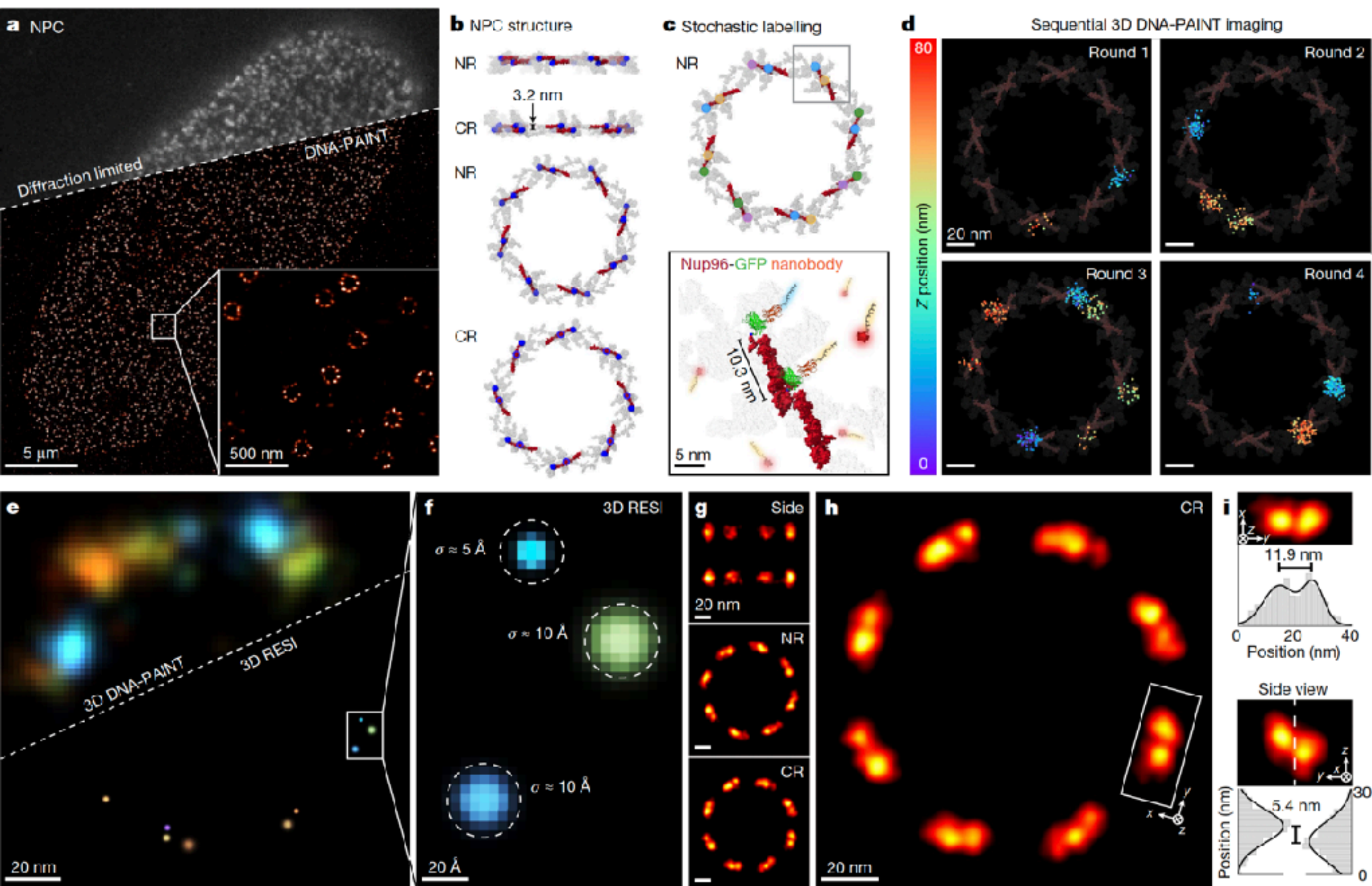
Received: 21 July 2022

Accepted: 7 March 2023

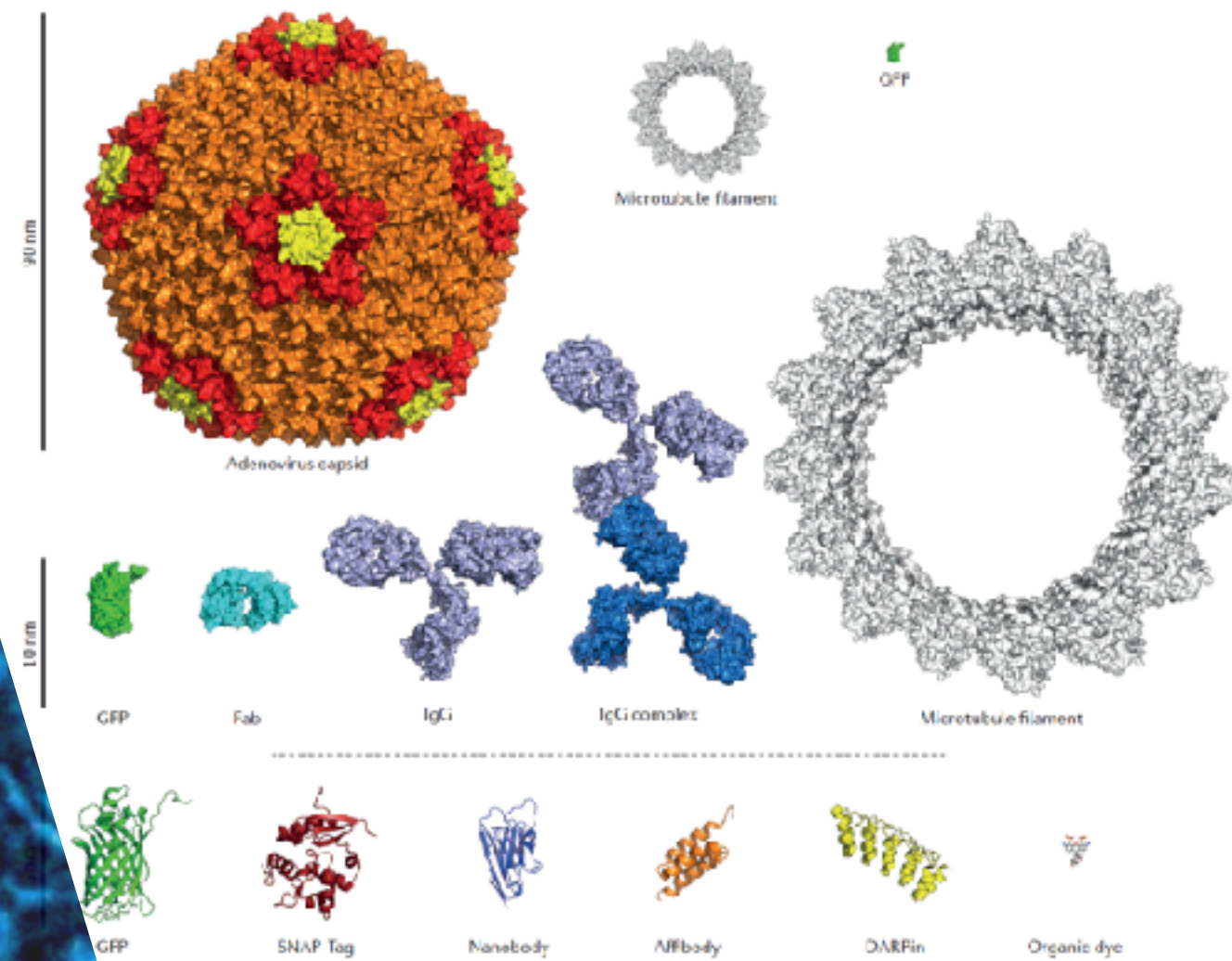
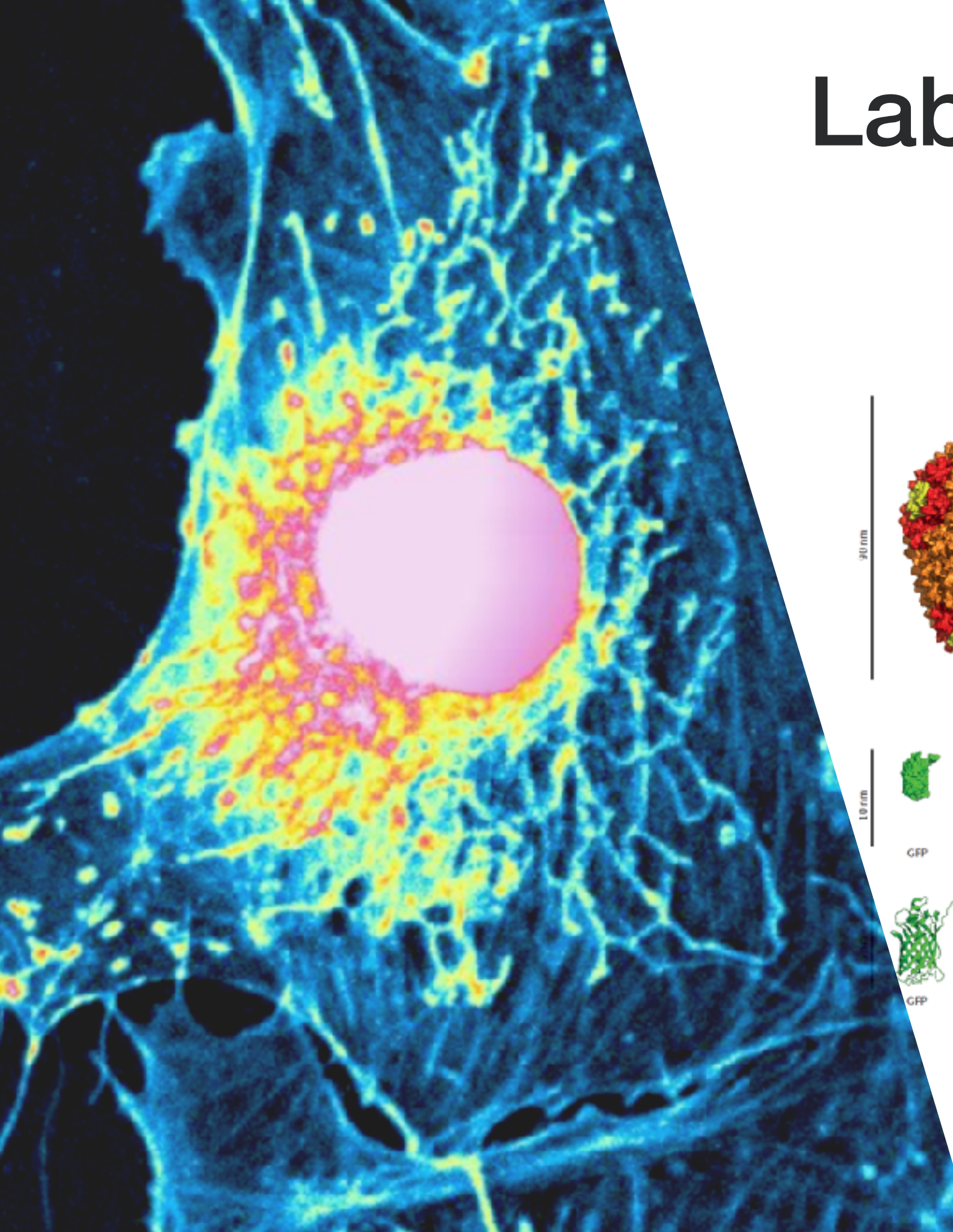
Published online: 21 May 2023

Susanne C. M. Reinhardt^{1,2,3,4}, Luciano A. Morille^{1,2}, Isabelle Baudron^{1,2,3}, Philippe R. Spon^{1,2}, Ralf Kowalewski^{1,2}, Alexandre S. Filant^{1,2}, Sebastian Straus^{1,2}, Edward M. Unterwiesing^{1,2}, Thomas Schlichthaefer^{1,2}, Maximilian T. Straus^{1,2}, Christian Klein^{1,2} & Ralf Jungmann^{1,2,3,4}

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



Label-free Trend

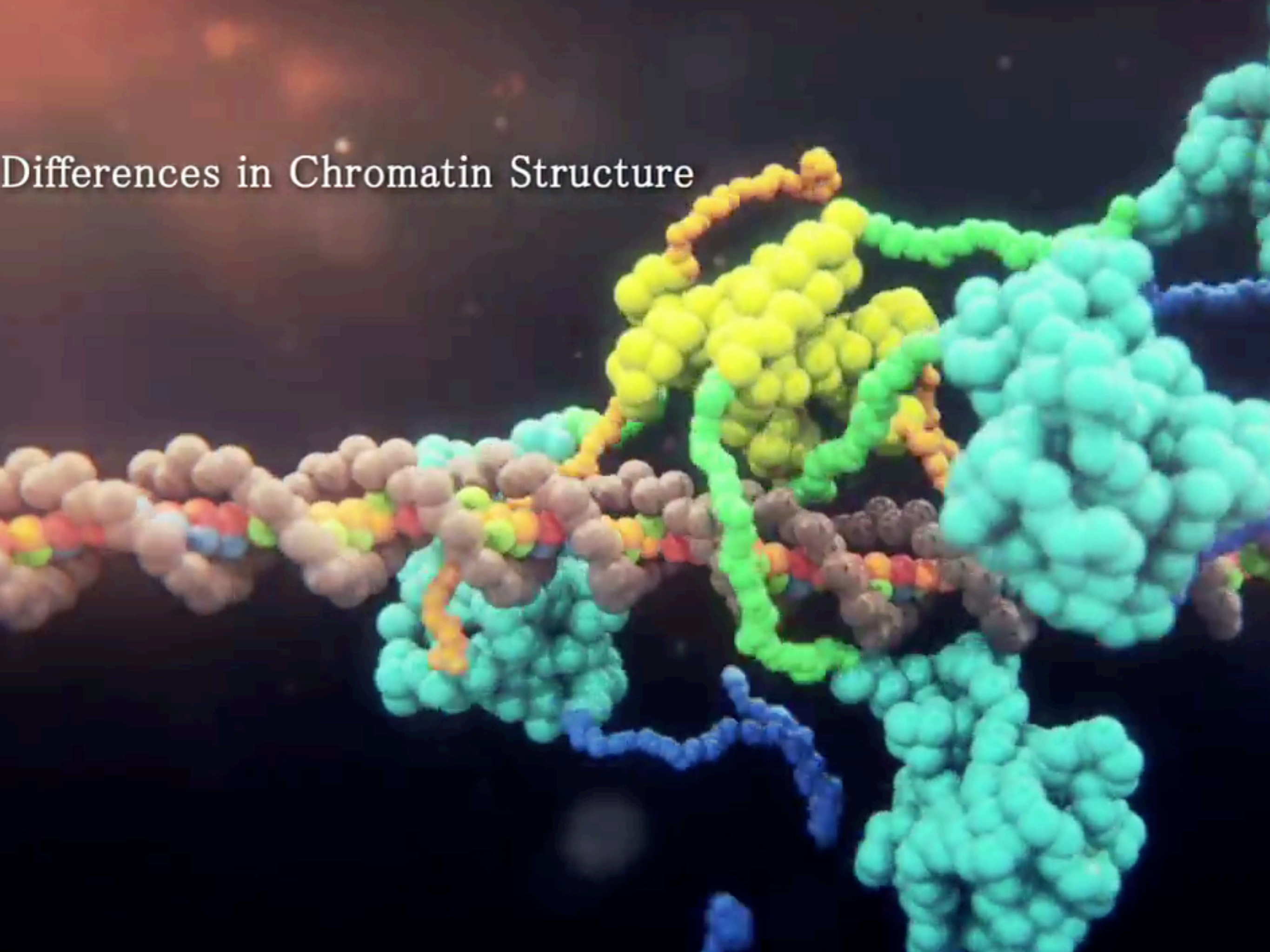




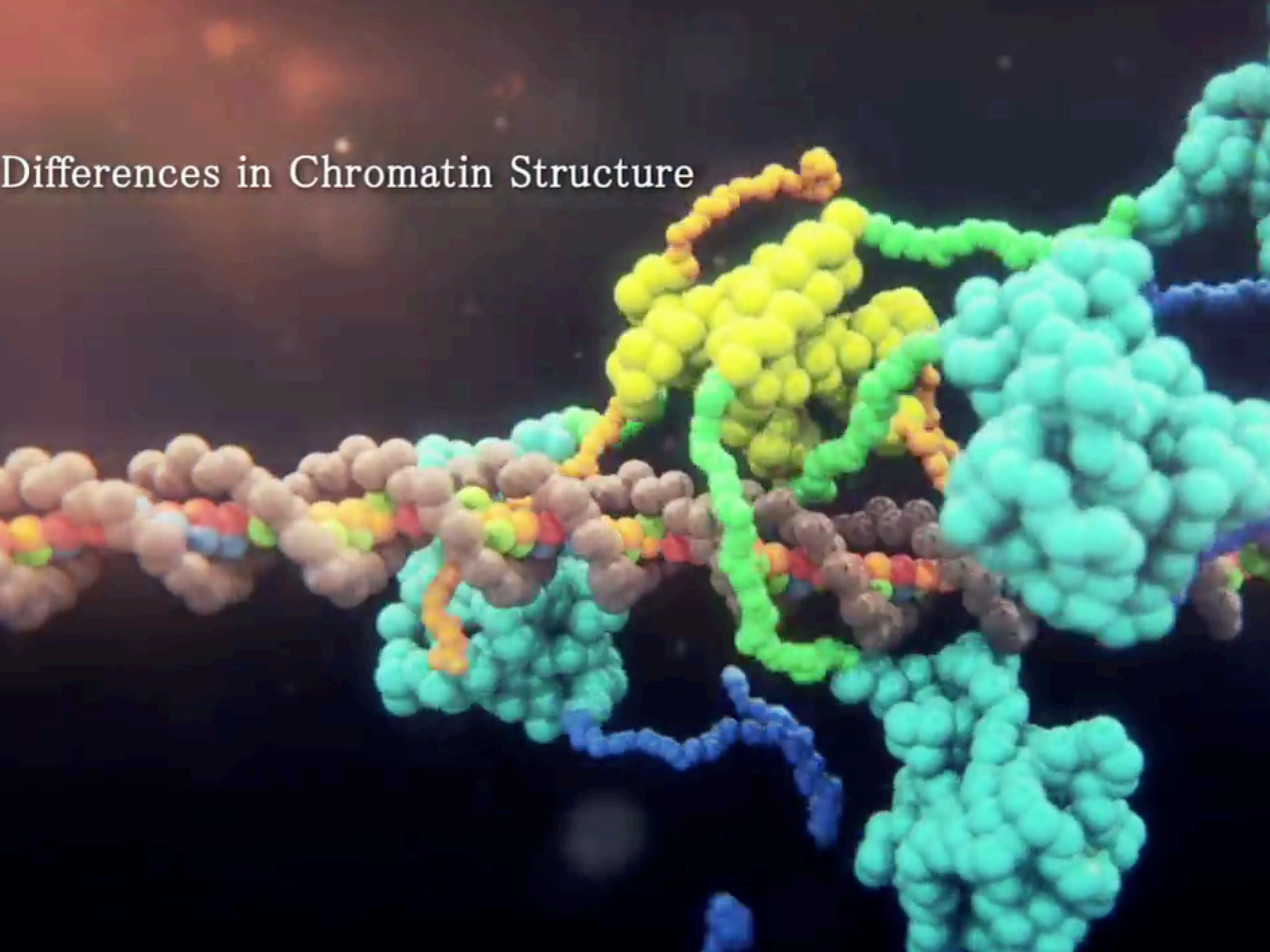


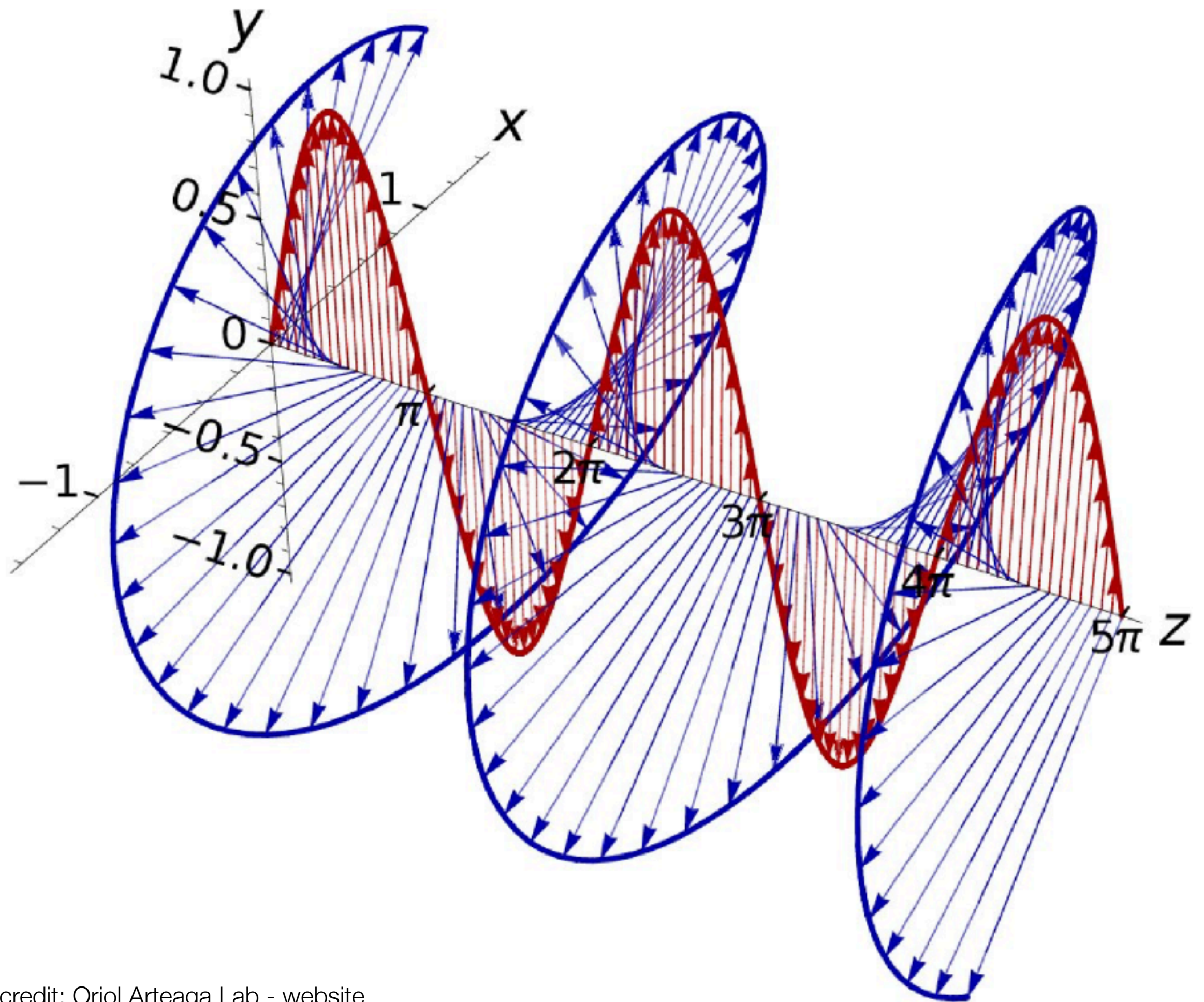


Differences in Chromatin Structure

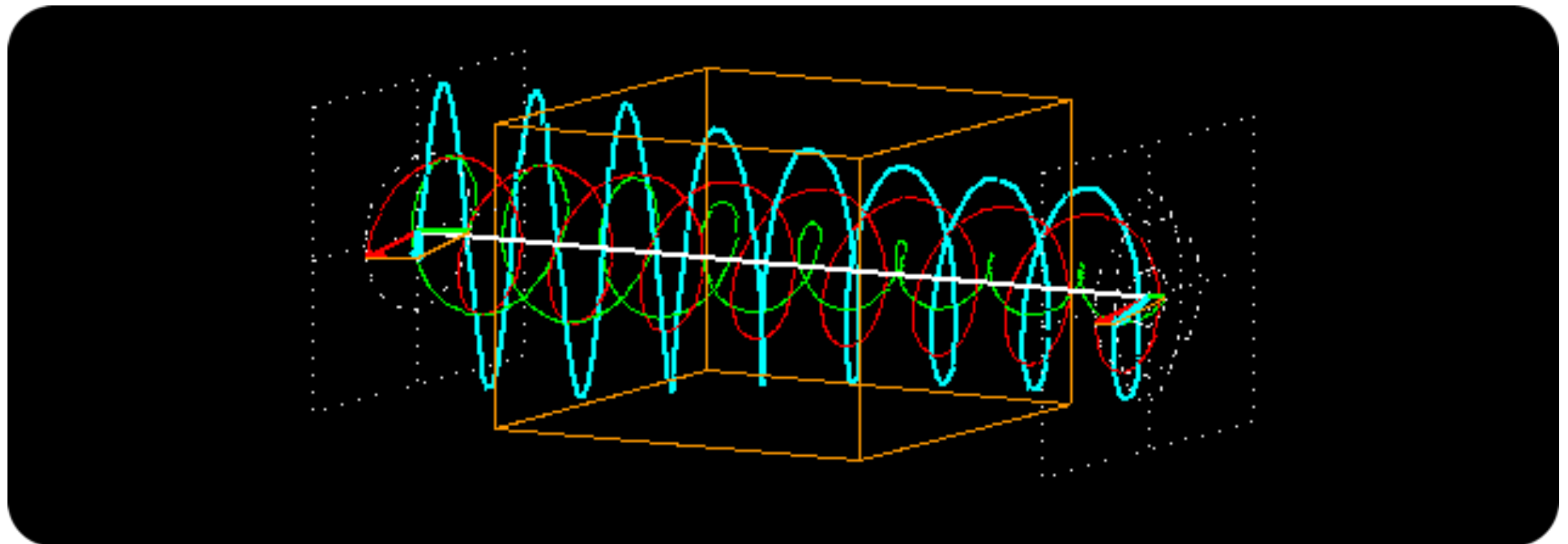
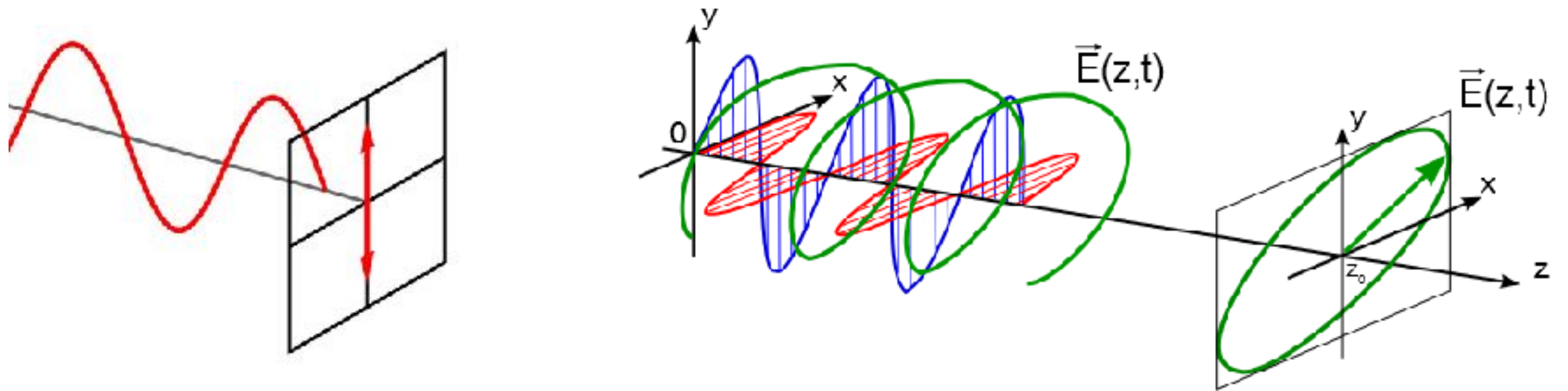


Differences in Chromatin Structure

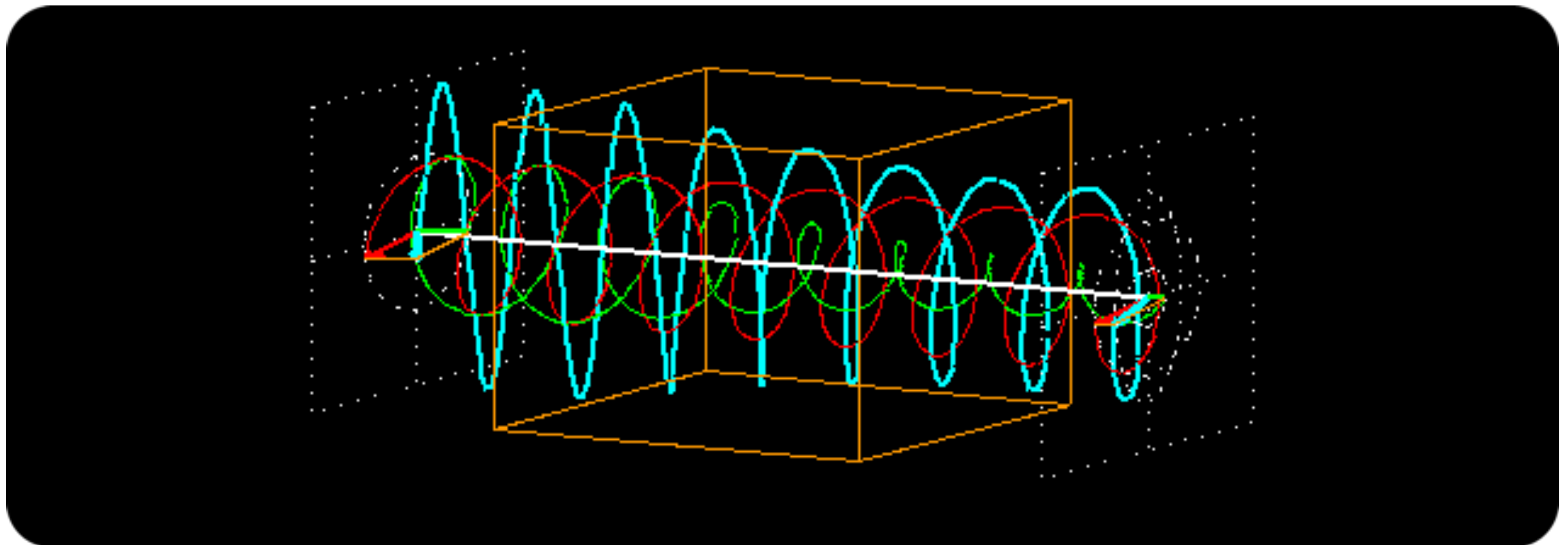
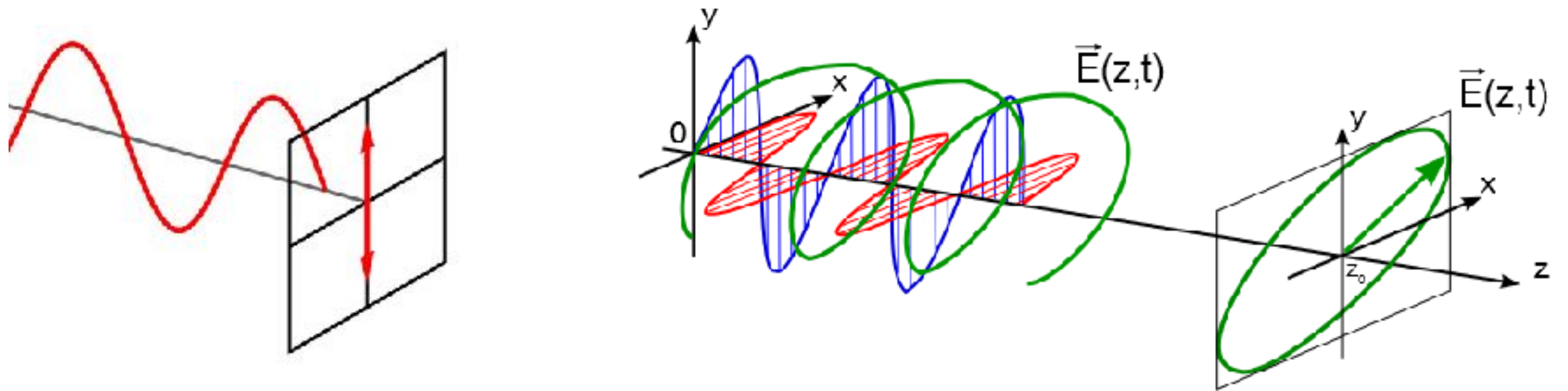




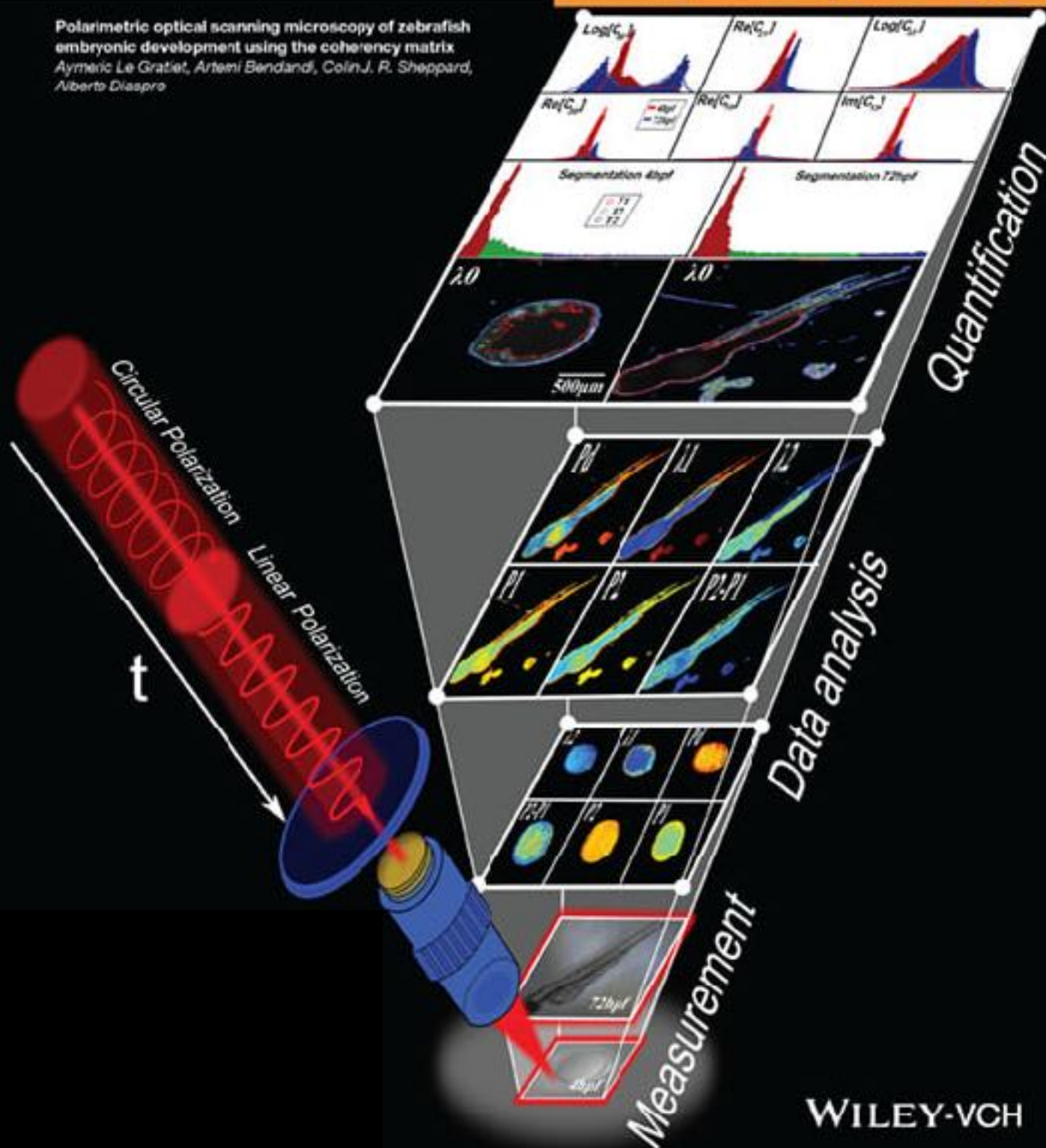
Polarized Light



Polarized Light



Polarimetric optical scanning microscopy of zebrafish embryonic development using the coherency matrix
Aymeric Le Gratiet, Artemi Bendant, Colin J. R. Sheppard, Alberto Diaspro



George Gabriel Stokes
(1819-1903)

Stokes Vector

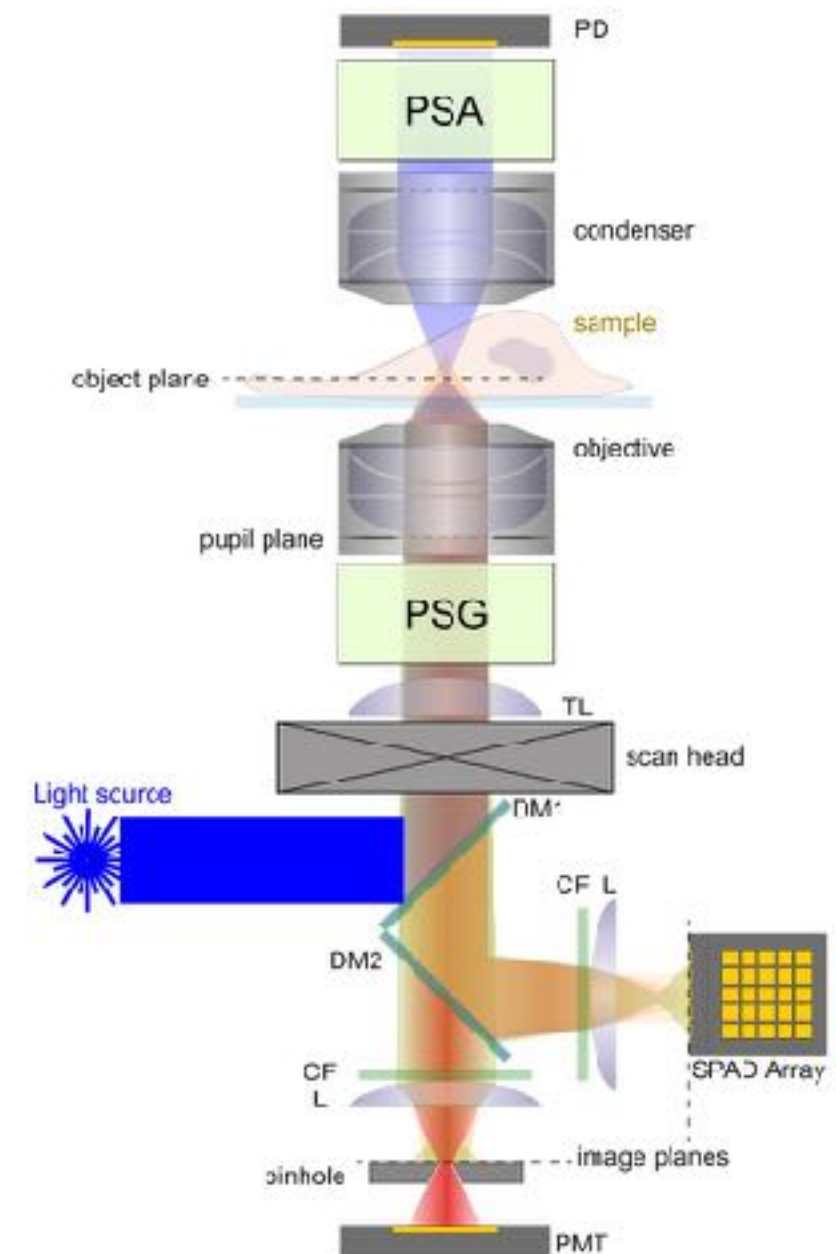
$$\vec{S} = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix} = \begin{bmatrix} I_0 \\ I_x - I_y \\ I_{-45^\circ} - I_{+45^\circ} \\ I_R - I_L \end{bmatrix}$$



Hans Mueller
(1900-1965)

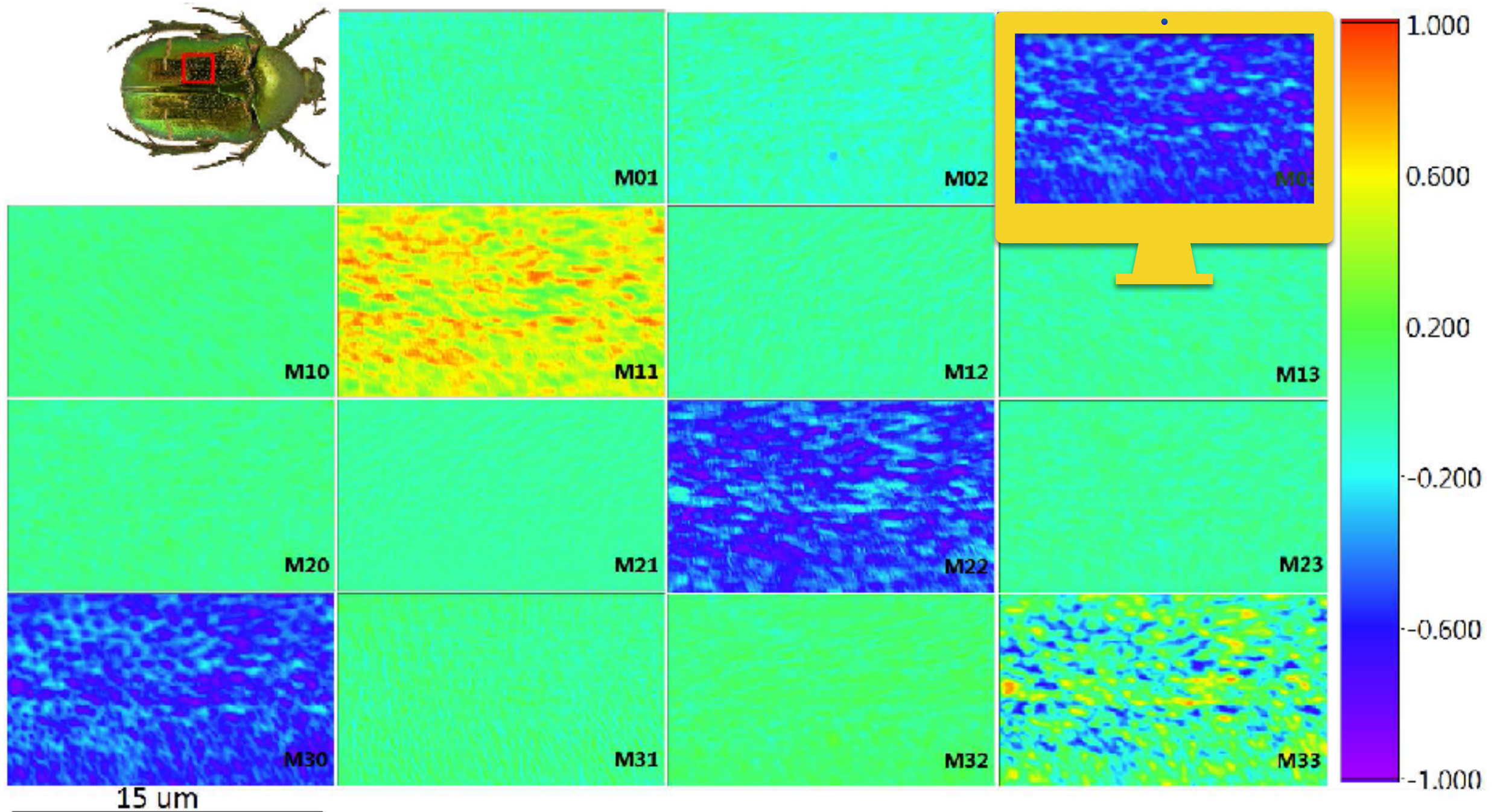
$$\vec{S}_i \xrightarrow{[M]} \vec{S}_s$$

$$\vec{S}_s = [M] \cdot \vec{S}_i$$

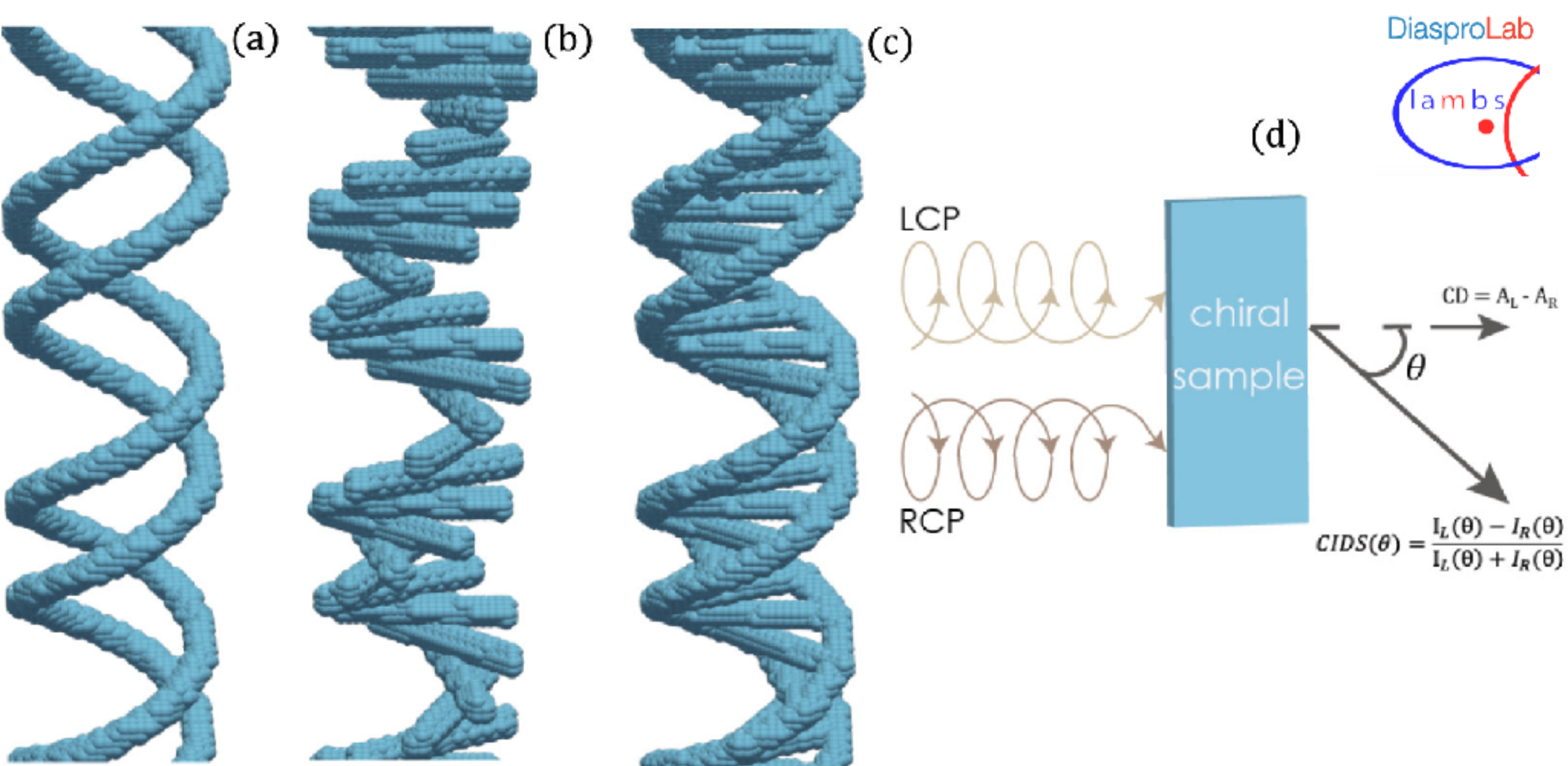


Cetonia Aurata beetle, structural chirality

O.Arteaga O. Lab
Department of Applied Physics and Optics
University of Barcelona



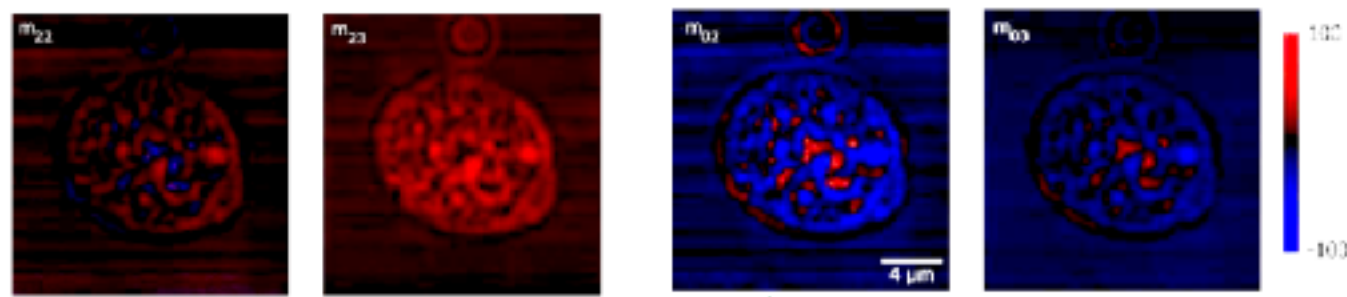
Arteaga, Oriol & Kuntman, Ertan. (2014). Beyond polarization microscopy: Mueller matrix microscopy with frequency demodulation. Proceedings of SPIE - The International Society for Optical Engineering. 9099



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.

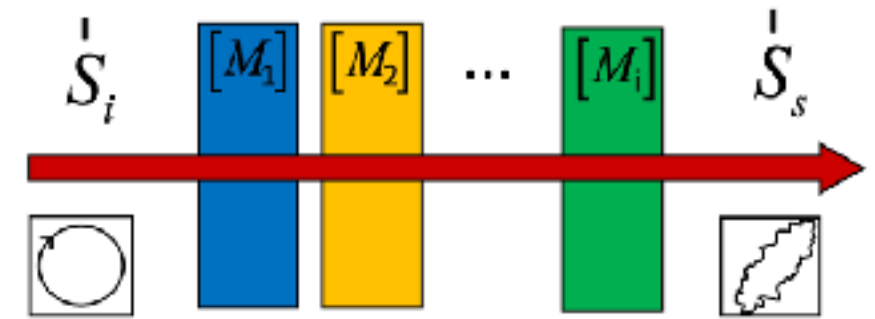




m_{22} = Depolarization
 m_{23} = Linear Birefringence
 m_{02} = Linear Dichroism
 m_{03} = Circular Dichroism

THE SIXTEEN ENTRIES OF MUELLER MATRIX EXPRESSED IN TERMS OF THE MEASURABLE INTENSITIES

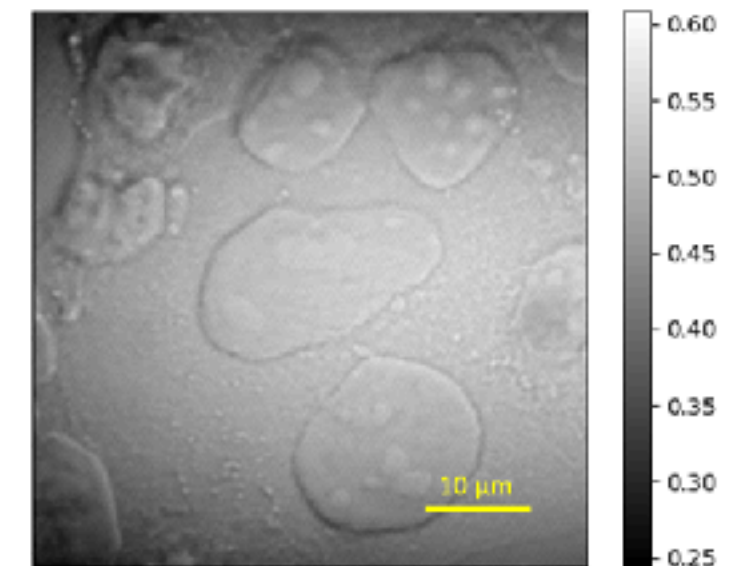
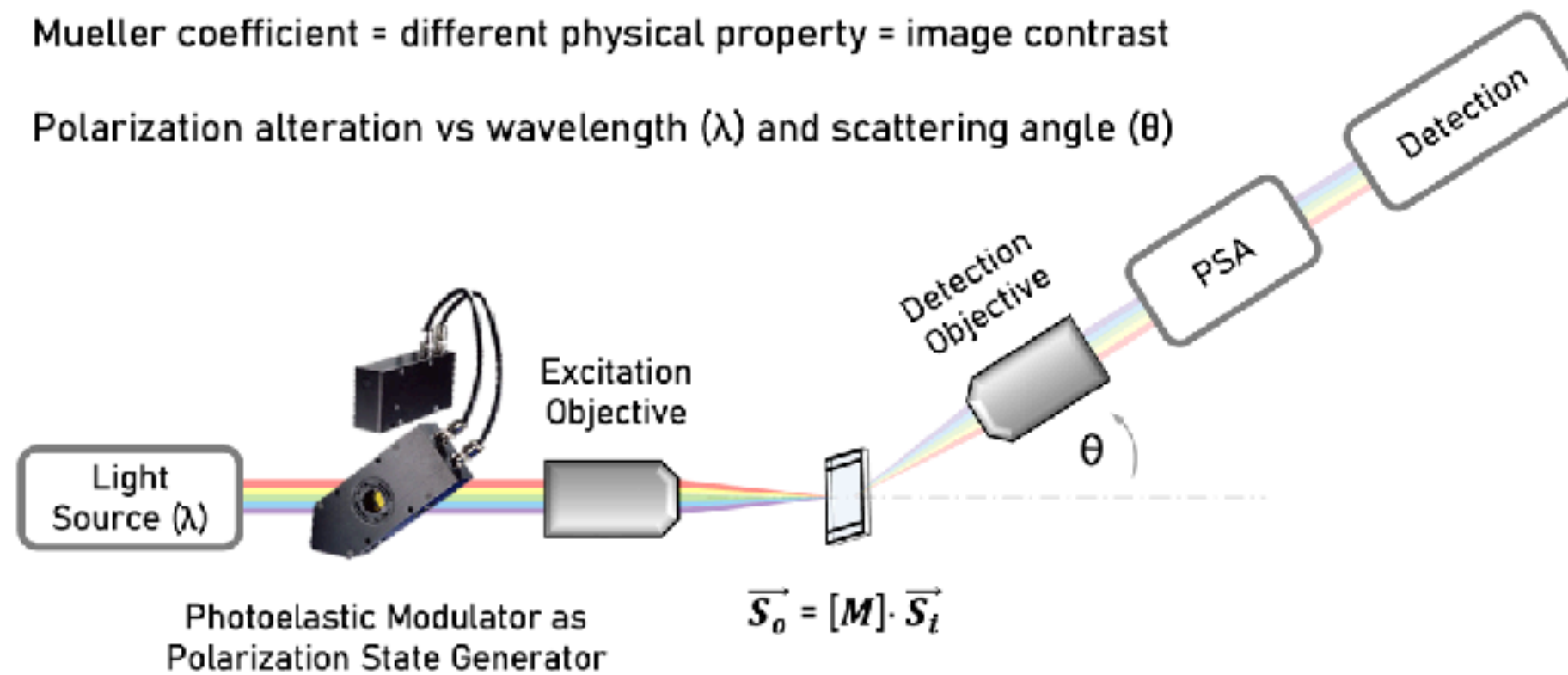
$M_{11} = \frac{I}{I_0}$	$M_{12} = \frac{I_H - I_V}{I_H + I_V}$	$M_{13} = \frac{I_{45} - I_{-45}}{I_{45} + I_{-45}}$	$M_{14} = \frac{I_R - I_L}{I_R + I_L}$
$M_{21} = \frac{I_H^H - I_H^V}{I_H^H + I_H^V}$	$M_{22} = \frac{I_H^H - I_H^V - (I_V^H - I_V^V)}{I_H^H + I_H^V + I_V^H + I_V^V}$	$M_{23} = \frac{I_{45}^H - I_{45}^V - (I_{-45}^H - I_{-45}^V)}{I_{45}^H + I_{45}^V + I_{-45}^H + I_{-45}^V}$	$M_{24} = \frac{I_R^H - I_R^V - (I_L^H - I_L^V)}{I_R^H + I_R^V + I_L^H + I_L^V}$
$M_{31} = \frac{I^{45} - I^{-45}}{I^{45} + I^{-45}}$	$M_{32} = \frac{I_H^{45} - I_H^{-45} - (I_V^{45} - I_V^{-45})}{I_H^{45} + I_H^{-45} + I_V^{45} + I_V^{-45}}$	$M_{33} = \frac{I_{45}^{45} - I_{45}^{-45} - (I_{-45}^{45} - I_{-45}^{-45})}{I_{45}^{45} + I_{45}^{-45} + I_{-45}^{45} + I_{-45}^{-45}}$	$M_{34} = \frac{I_R^{45} - I_R^{-45} - (I_L^{45} - I_L^{-45})}{I_R^{45} + I_R^{-45} + I_L^{45} + I_L^{-45}}$
$M_{41} = \frac{I_R^H - I_L^H}{I_R^H + I_L^H}$	$M_{42} = \frac{I_H^R - I_H^L - (I_V^R - I_V^L)}{I_H^R + I_H^L + I_V^R + I_V^L}$	$M_{43} = \frac{I_{45}^R - I_{45}^L - (I_{-45}^R - I_{-45}^L)}{I_{45}^R + I_{45}^L + I_{-45}^R + I_{-45}^L}$	$M_{44} = \frac{I_R^R - I_R^L - (I_L^R - I_L^L)}{I_R^R + I_R^L + I_L^R + I_L^L}$

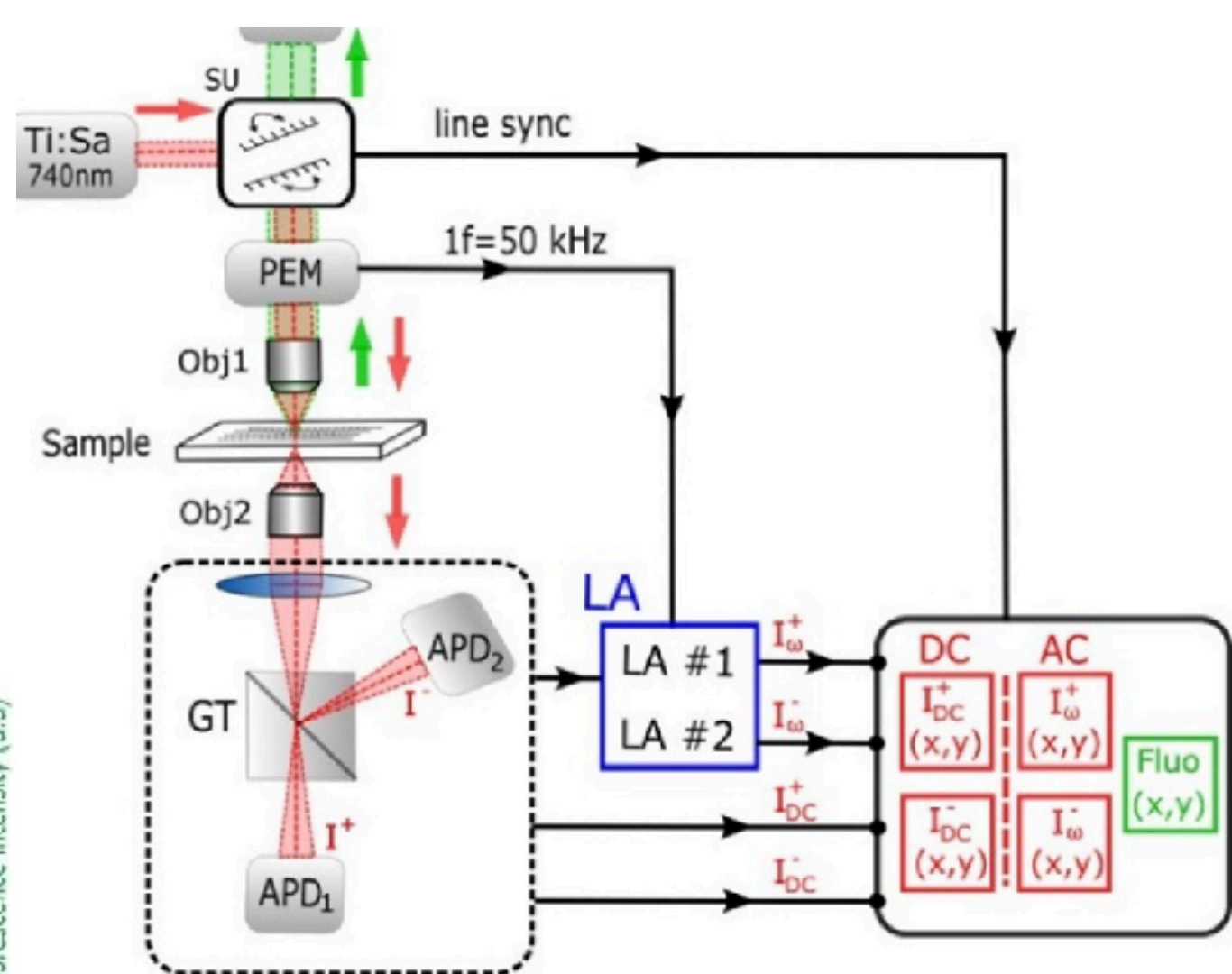
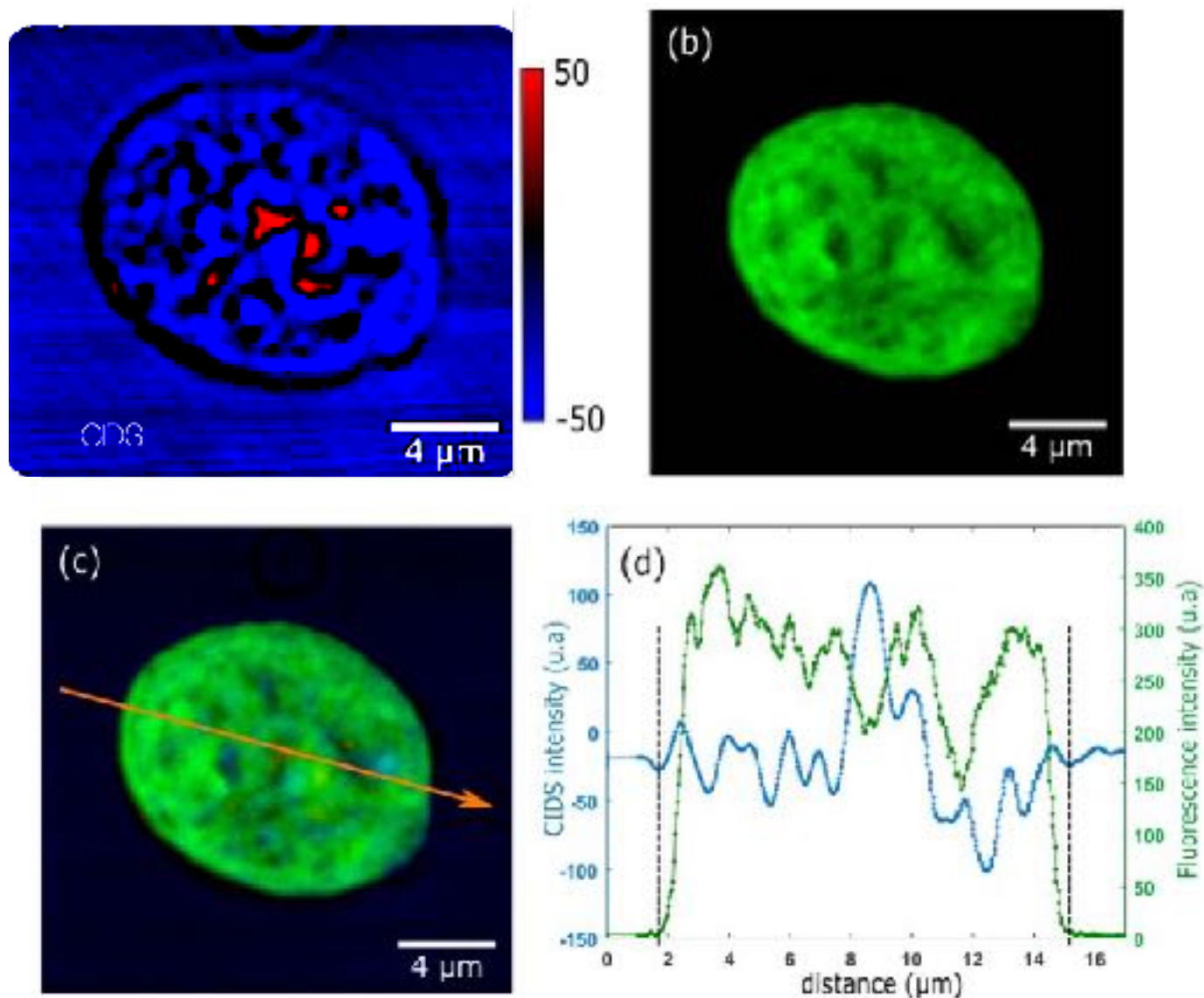


H. Mueller, "The foundation of optics", JOSA 38 (1941)

$$[M] = \begin{bmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{bmatrix}$$

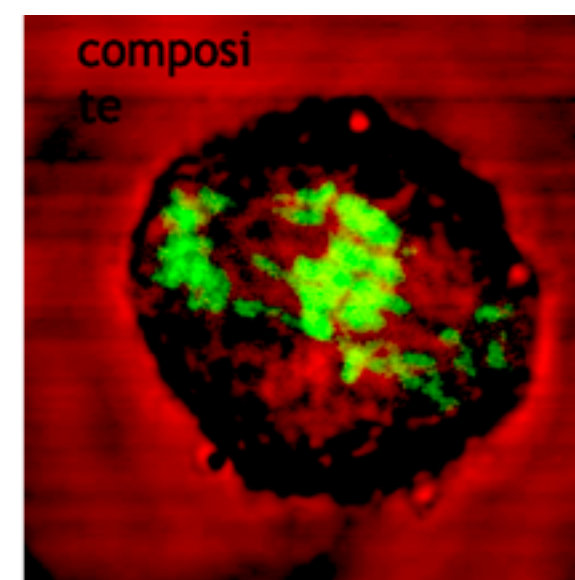
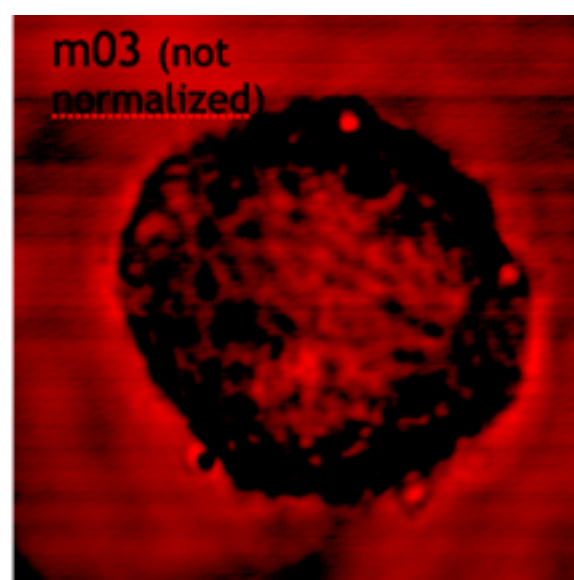
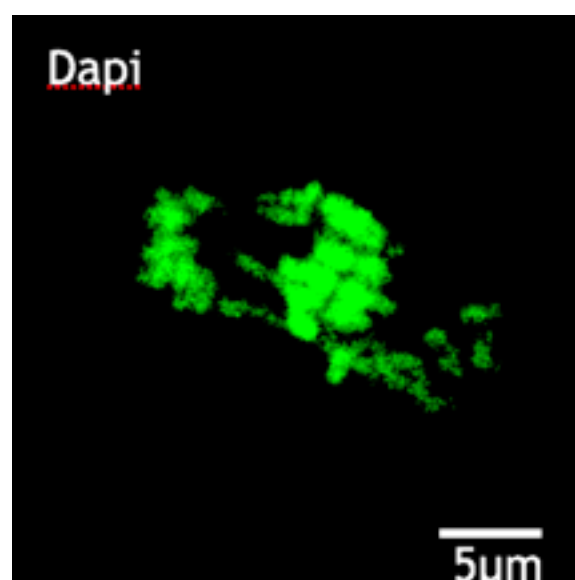
$$\vec{S} = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix} = \begin{bmatrix} I_0 \\ I_H - I_V \\ I_D - I_A \\ I_R - I_L \end{bmatrix} \begin{matrix} \leftrightarrow \\ \otimes \\ \otimes \end{matrix}$$

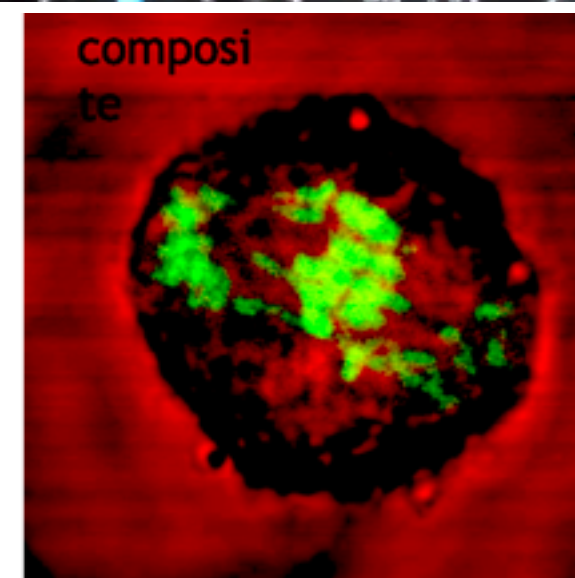
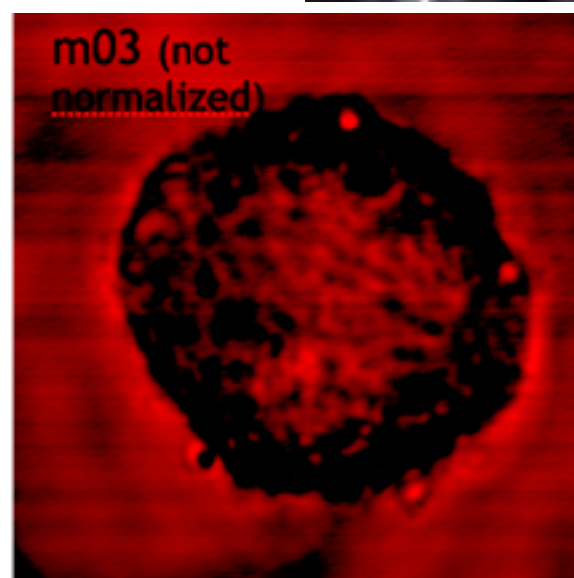
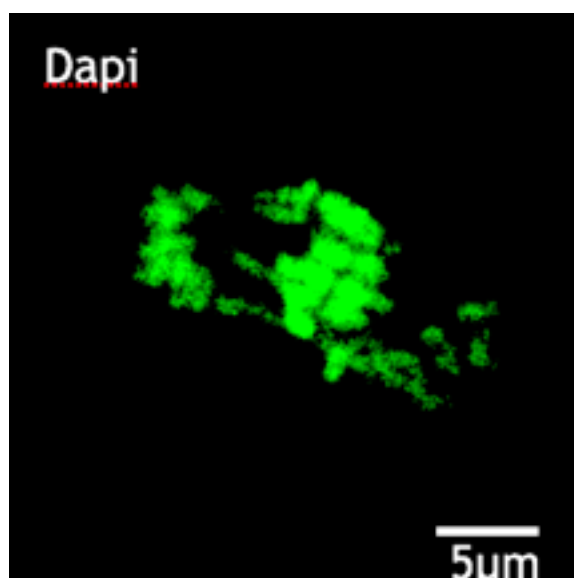
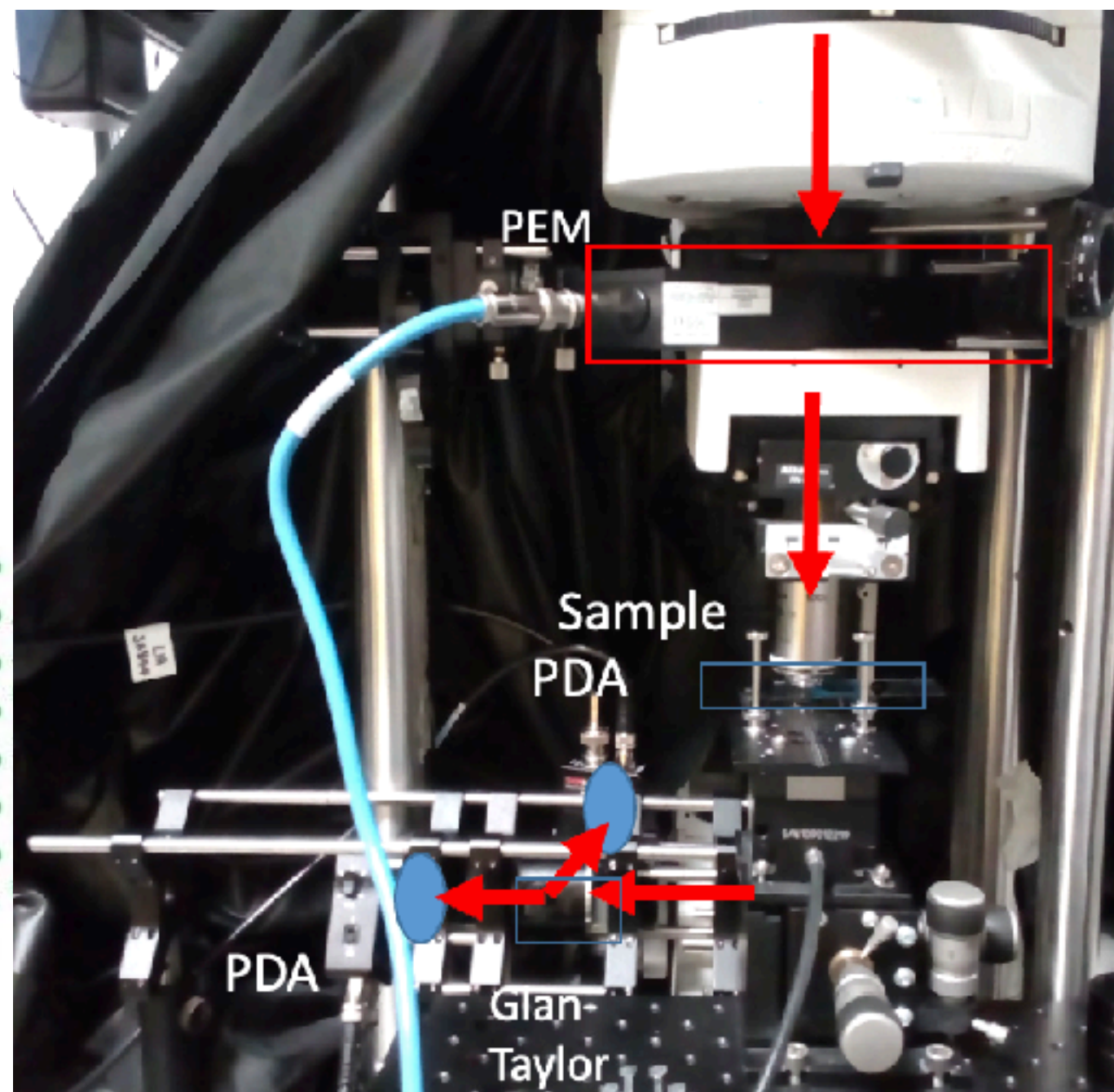
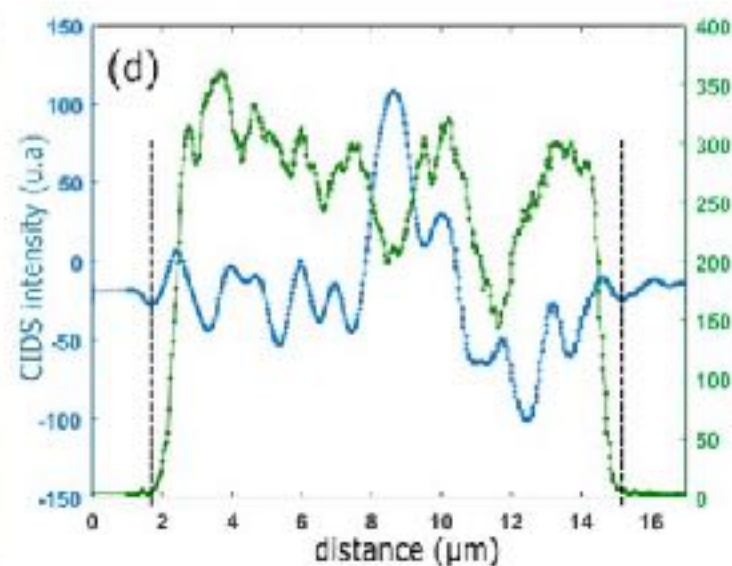
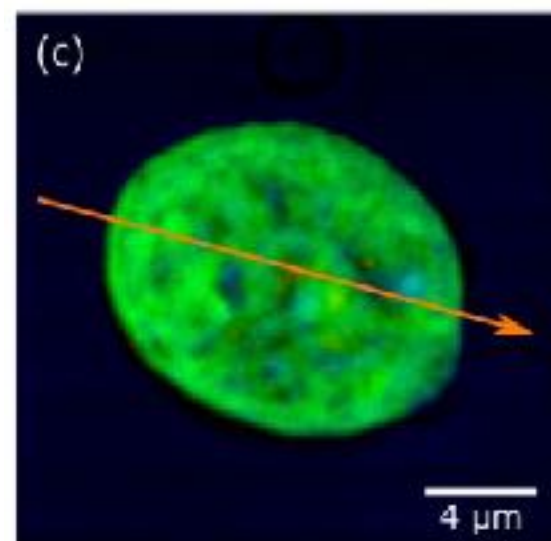
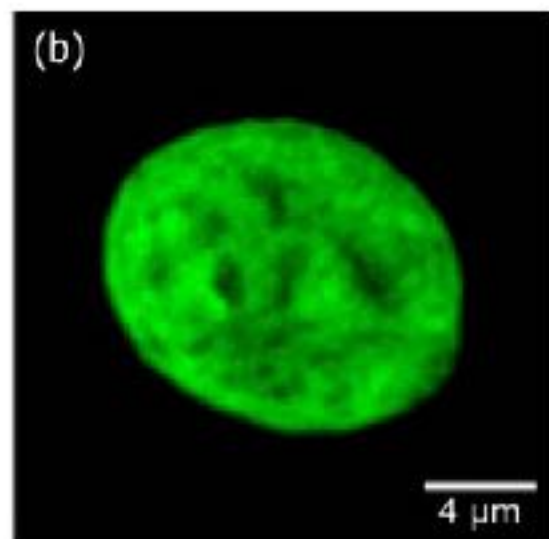
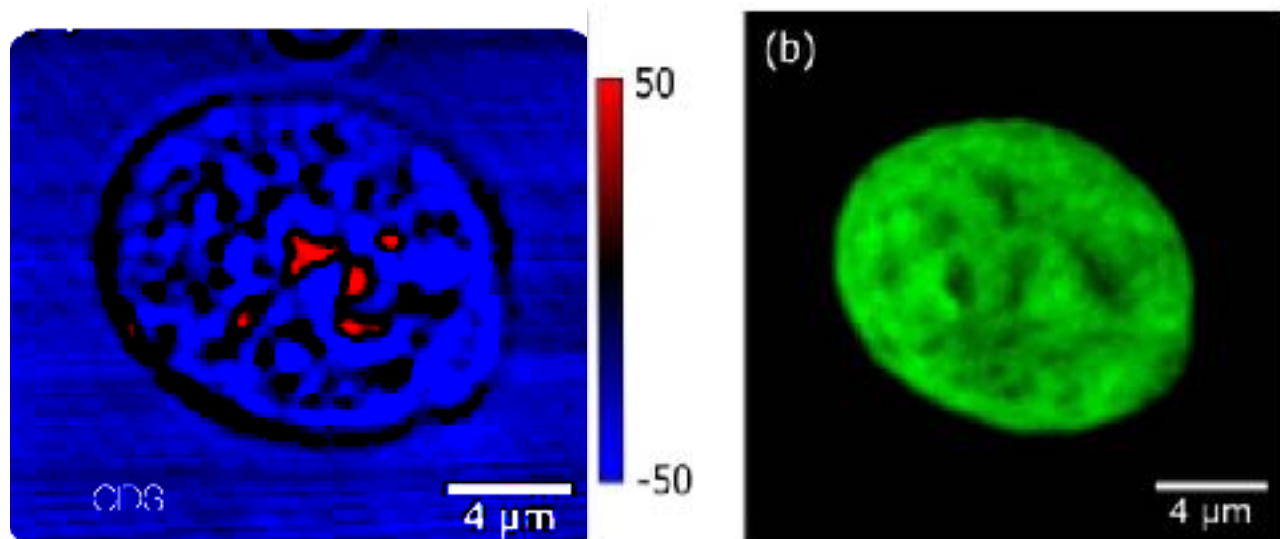


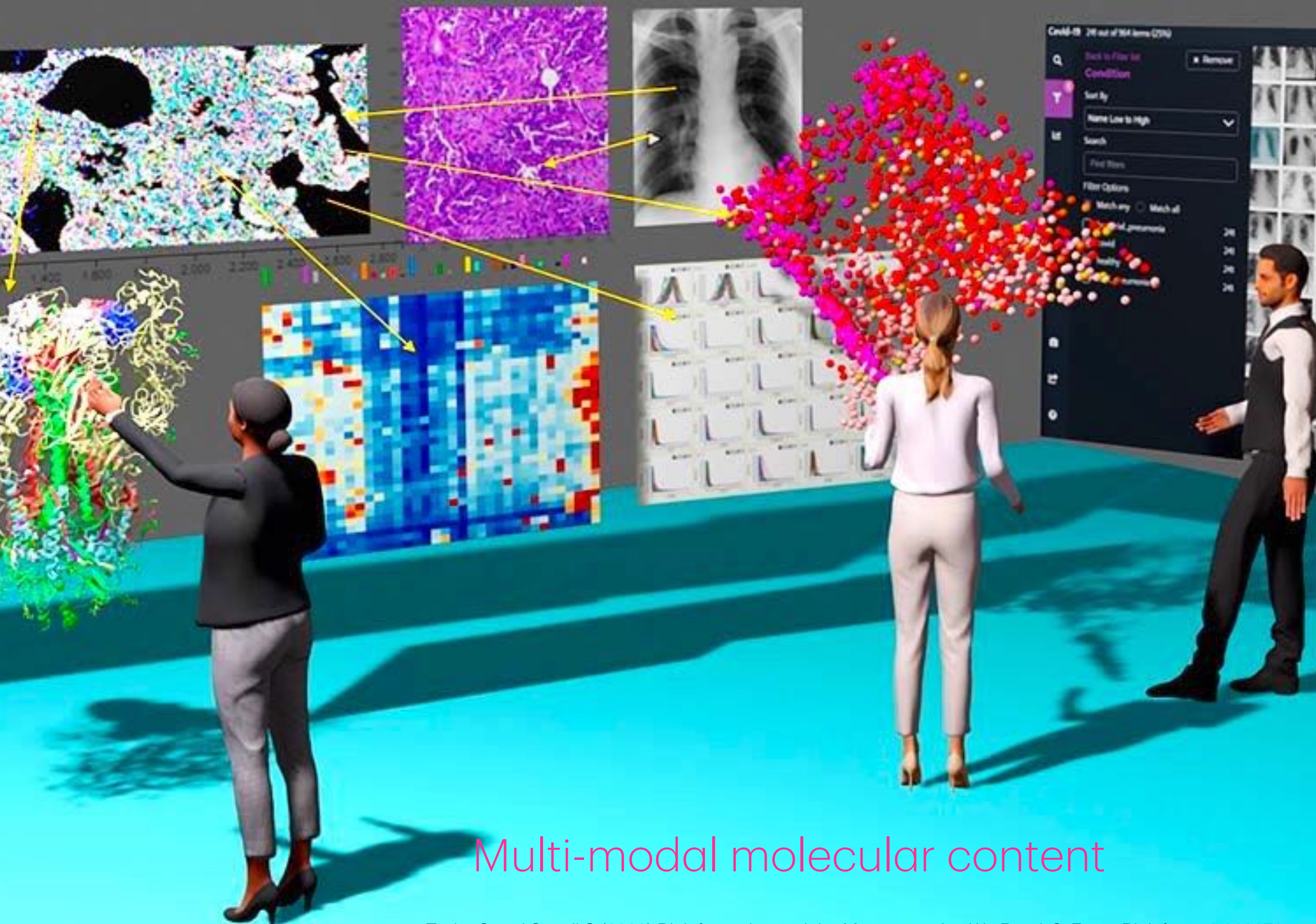


Signal: I_{ω} ($\omega = 50 \text{ kHz}$)

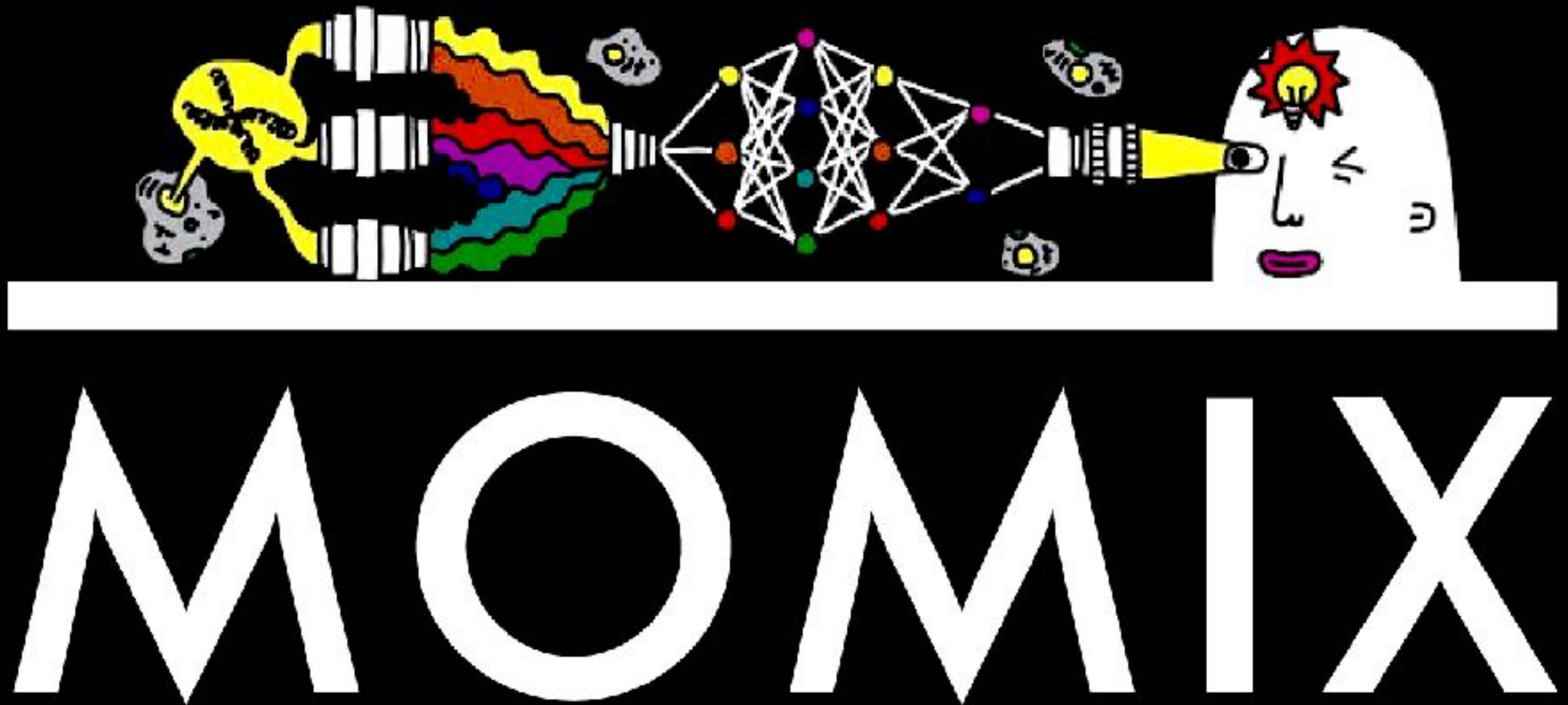
Lock-in demodulates I_{ω} pixel by pixel







Multimodal Optical Microscopy Image Correlation Sensing



How to deal with all these data?

The large amount of data coming from the different modalities of MOMIX need to be structured.

"*Less is more*": we need to reduce the complexity of the data.

The goal is to merge data coming from different mechanisms of contrast.

The ambition is to catch the chance to see things differently and to get an original insight under a new point of view.



AI: the first 60 years in one slide

1943

Arturo Rosenblyeth, Norbert Wiener and Julian Bigelow coin the term "cybernetics". Wiener's popular book by that name published in 1948.

.....

1948

John von Neumann (quoted by E.T. Jaynes) in response to a comment at a lecture that it was impossible for a machine to think: "You insist that there is something a machine cannot do. If you will tell me precisely what it is that a machine cannot do, then I can always make a machine which will do just that". Von Neumann was presumably alluding to the Church-Turing thesis which states that any effective procedure can be simulated by a (generalized) computer.

....

1950

Alan Turing proposes the Turing Test as a measure of machine intelligence.

1950

Claude Shannon published a detailed analysis of chess playing as search.

1955

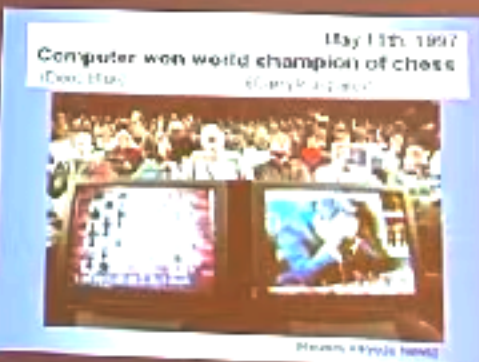
The first Dartmouth College summer AI conference is organized by John McCarthy, Marvin Minsky, Nathan Rochester of IBM and Claude Shannon.

Late 1990s

Web crawlers and other AI-based information extraction programs become essential in widespread use of the World Wide Web.

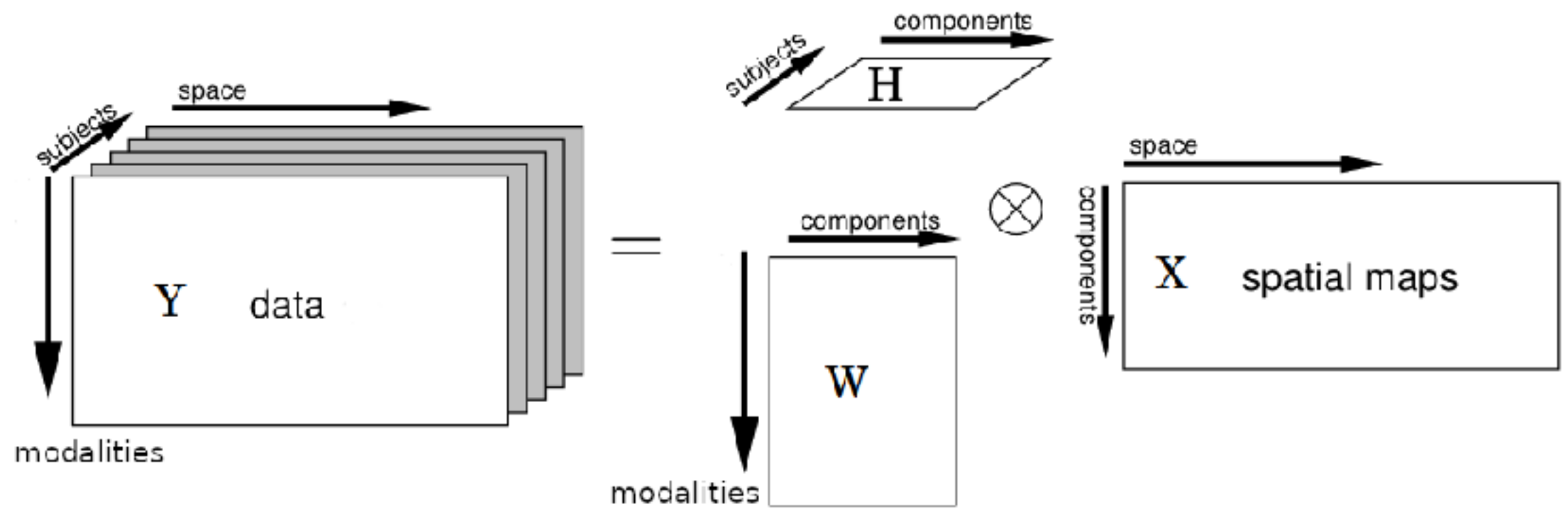
1997

The Deep Blue chess machine (IBM) beats the world chess champion, Garry Kasparov.



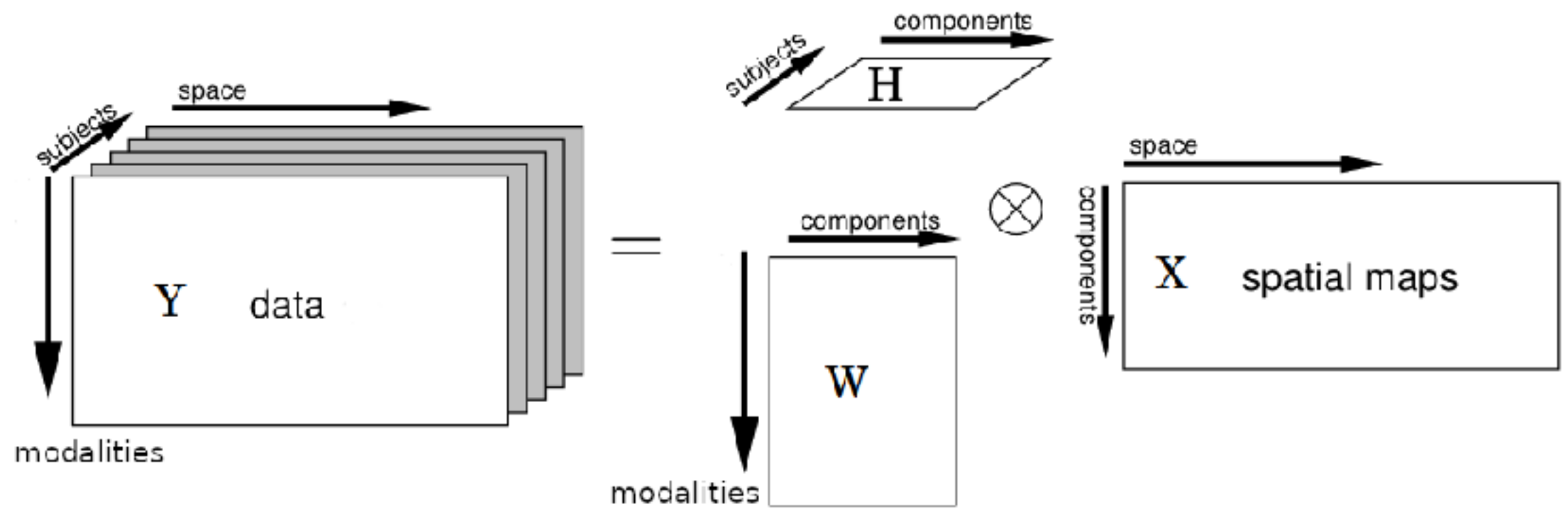
UniGe | MaLGo





The model data set Y expressed as the tensor product of one spatial map ($n=1, \dots, \text{voxel}$), one subject-course ($r=1, \dots, R$) and one modality-course ($t=1, \dots, T$)

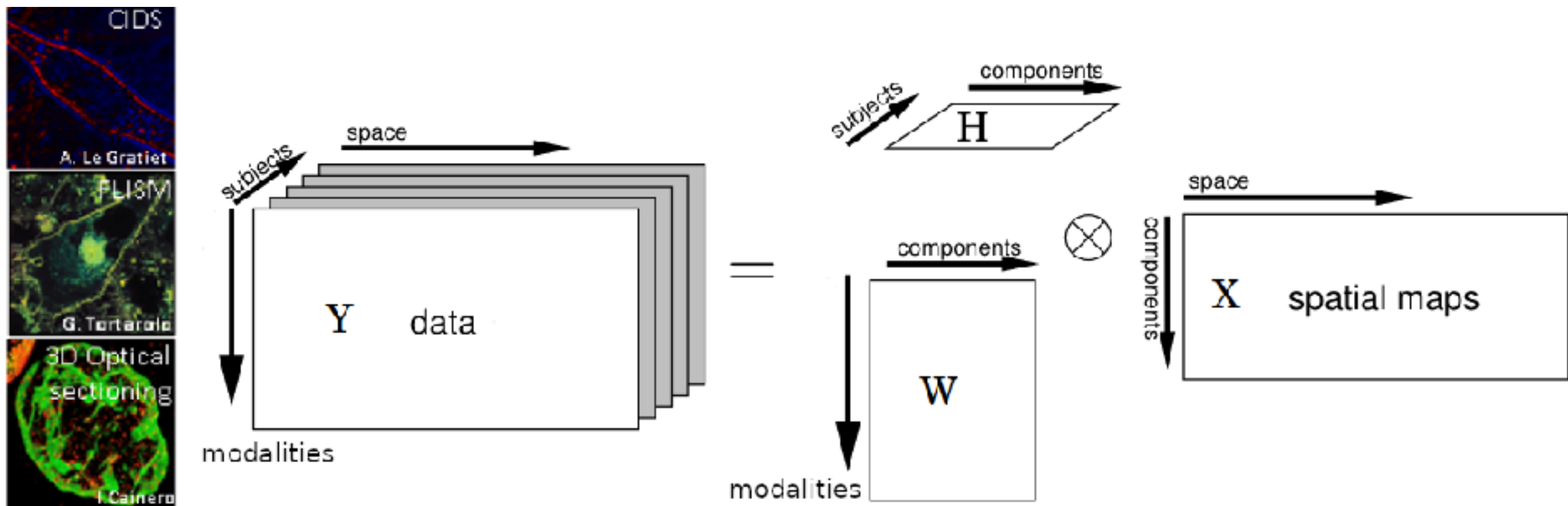
$$Y_{n,t,r} = \sum_{i=1}^L X_{n,i} W_{t,i} H_{i,r} + \varepsilon_{n,t,r} \quad (\text{A1})$$



The model data set Y expressed as the tensor product of one spatial map ($n=1, \dots, \text{voxel}$), one subject-course ($r=1, \dots, R$) and one modality-course ($t=1, \dots, T$)

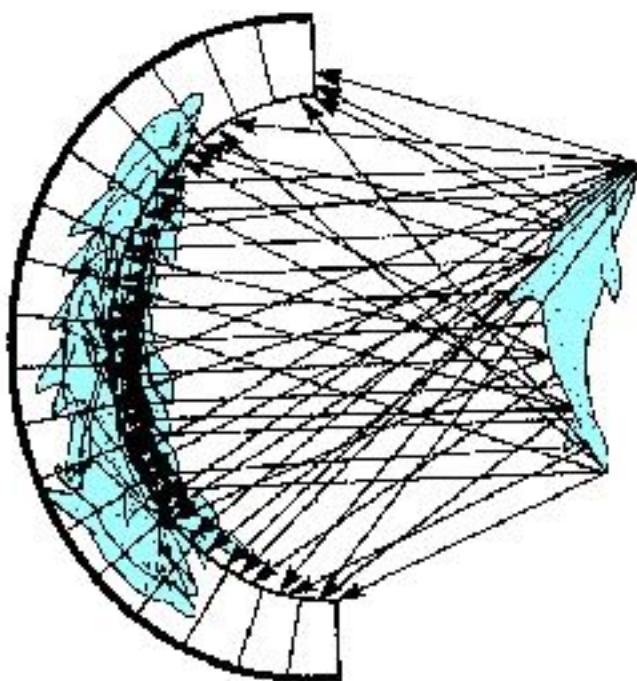
$$Y_{n,t,r} = \sum_{i=1}^L X_{n,i} W_{t,i} H_{i,r} + \varepsilon_{n,t,r} \quad (\text{A1})$$

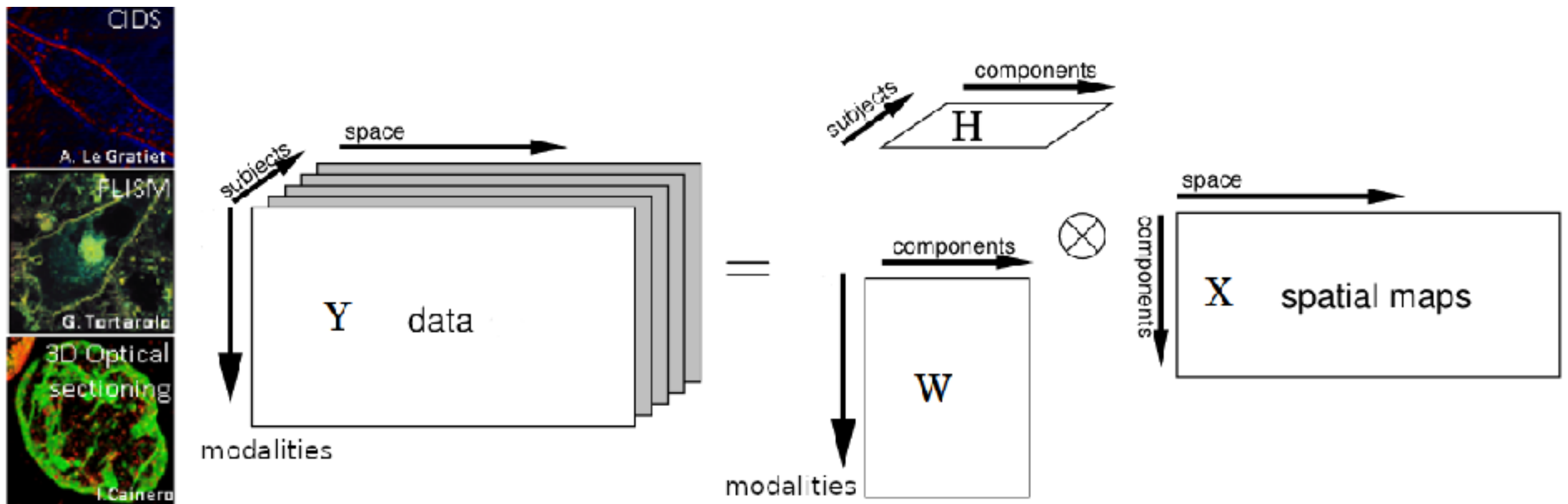




The model data set Y expressed as the tensor product of one spatial map ($n=1, \dots, \text{voxel}$), one subject-course ($r=1, \dots, R$) and one modality-course ($t=1, \dots, T$)

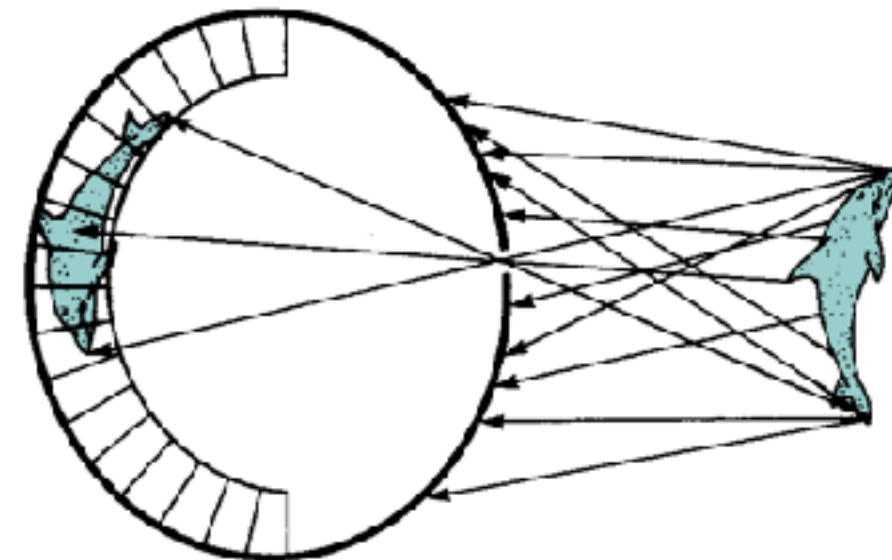
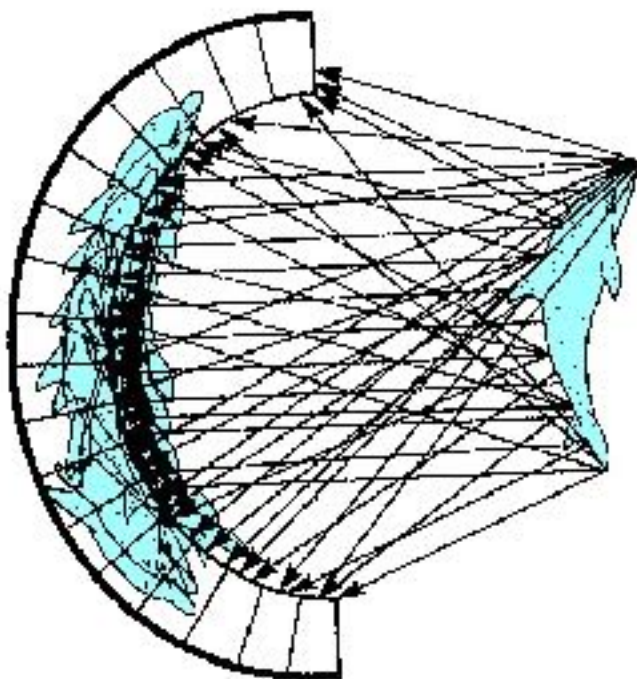
$$Y_{n,t,r} = \sum_{i=1}^L X_{n,i} W_{t,i} H_{i,r} + \varepsilon_{n,t,r} \quad (A1)$$





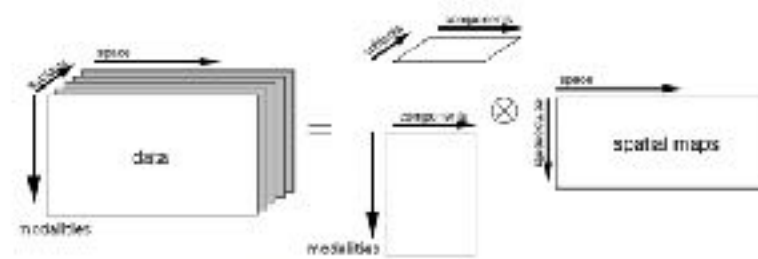
The model data set Y expressed as the tensor product of one spatial map ($n=1, \dots, \text{voxel}$), one subject-course ($r=1, \dots, R$) and one modality-course ($t=1, \dots, T$)

$$Y_{n,t,r} = \sum_{i=1}^L X_{n,i} W_{t,i} H_{i,r} + \varepsilon_{n,t,r} \quad (\text{A1})$$



Multimodal

Data driven cluster
which encode
morphological/functiona
measurements

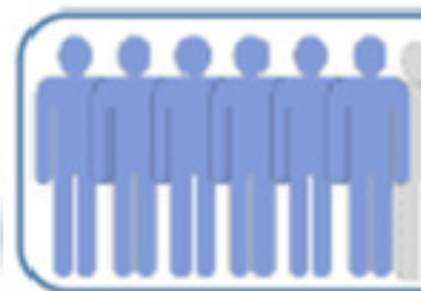


$$\mathbf{X}_{n,t,r}^{(k)} = \sum_{i=1}^L \mathbf{S}_{n,i}^{(k)} \mathbf{W}_{t,i}^{(k)} \mathbf{H}_{i,r} + \mathbf{N}_{n,t,r}^{(k)}$$

where for each modality group $k : 1 \dots K$:

- ▶ $\mathbf{S}_{n,i}$ are the *spatial maps* for all modalities;
- ▶ $\mathbf{W}_{t,i}$ are the *modality weightings* for component i in modality t (tells in which modality to look);
- ▶ $\mathbf{H}_{i,r}$ are the *weights* for component i in subject r (forms a link between the different modalities);
- ▶ $\mathbf{N}_{n,t,r}$ is the *noise*.

Healthy



Unhealthy



Clustering

Table of measurements.

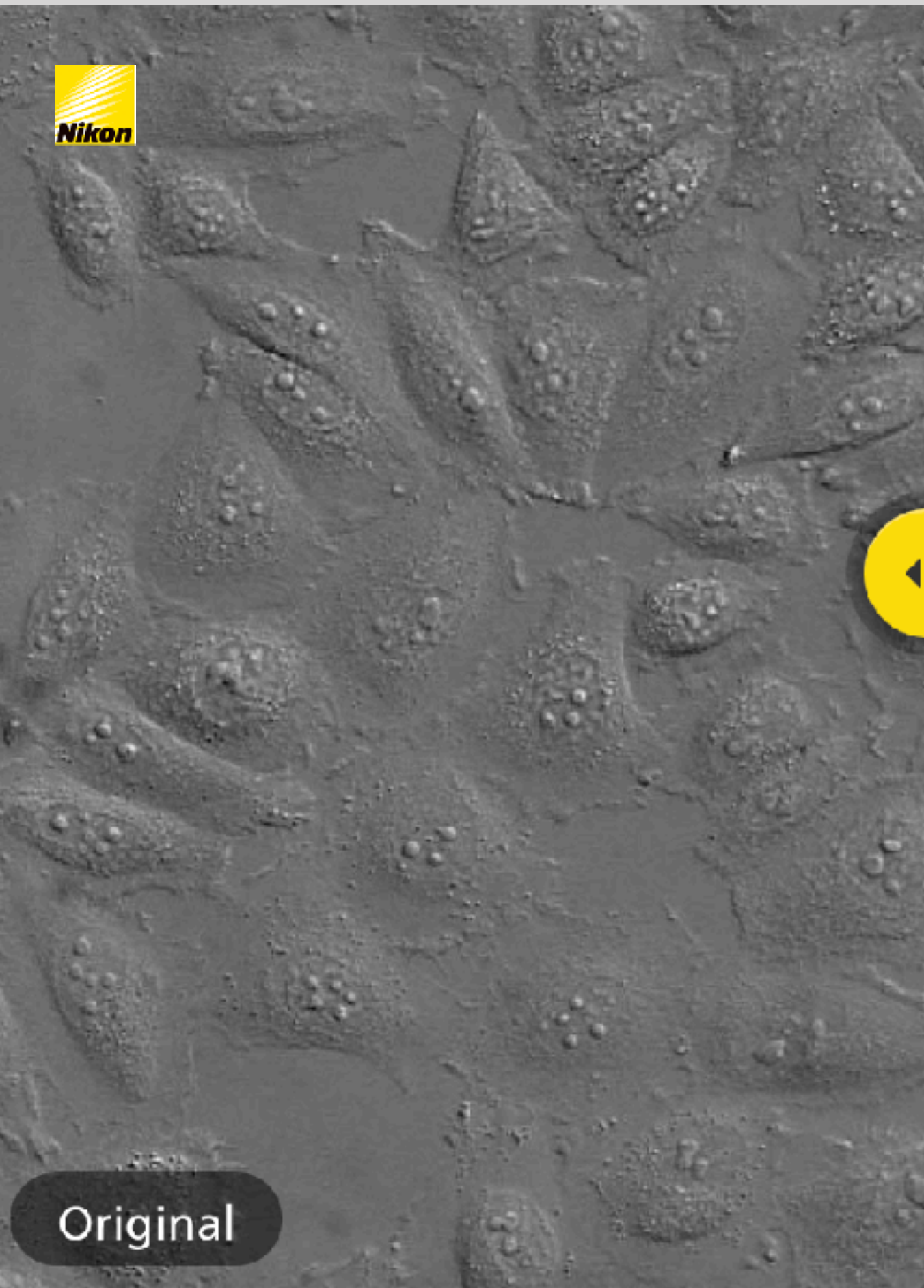
Series	Volume [μm^3]	Surface [μm^2]	Comp.	IntDen [A.U.]	Mean	StdDev	Ch. Comp [%]
1	491.037	555.945	0.156	174.799	654.748	245.297	0.382
2	795.796	718.160	0.193	207.240	478.985	261.747	0.186
3	635.421	646.754	0.169	198.359	574.171	336.499	0.307
4	639.721	640.639	0.176	175.071	503.354	283.191	0.263

Map to clusters

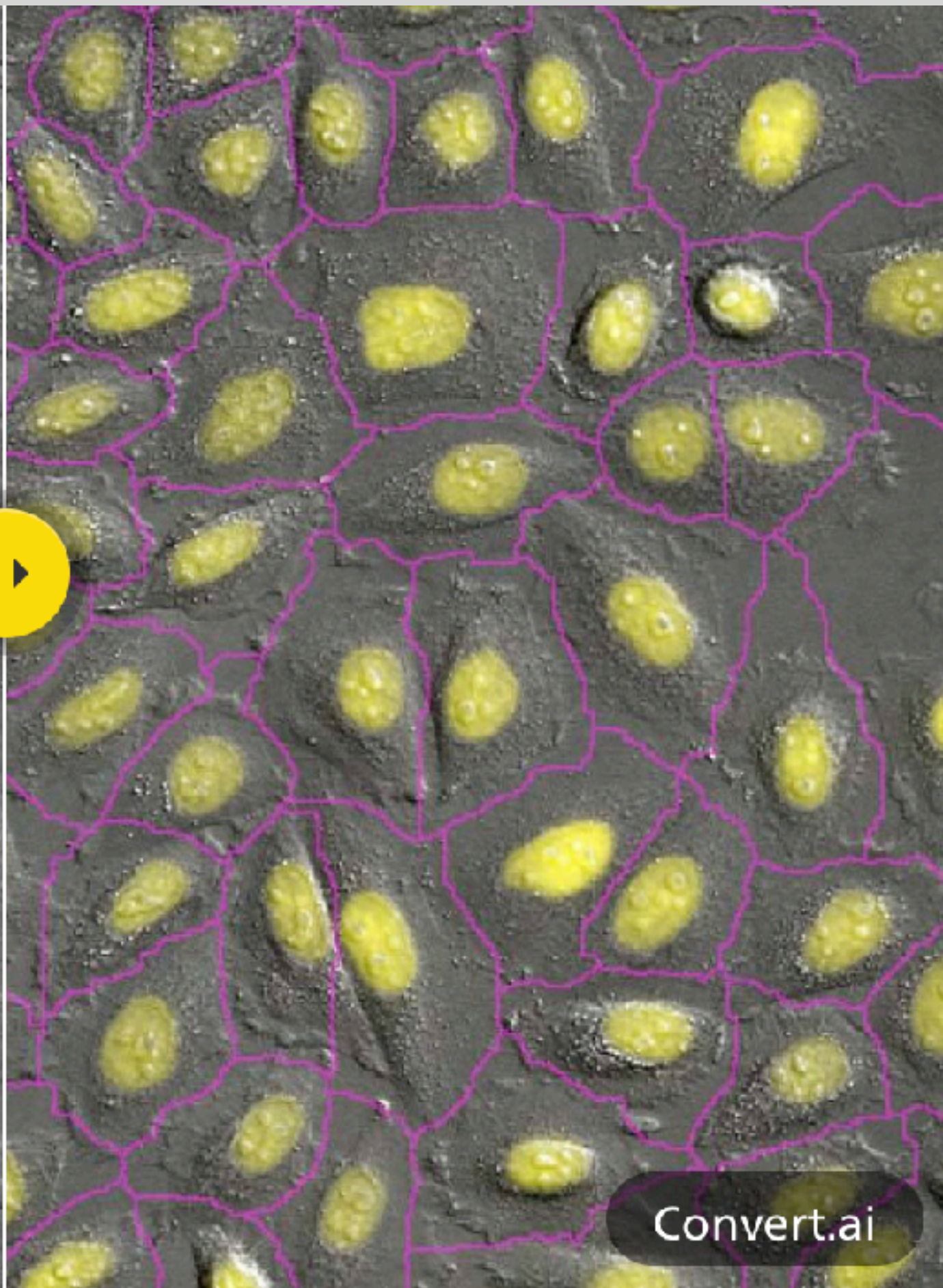


The artificial microscope

A Microscope in the Computer Machine



Original



Convert.ai

The background features several abstract, irregular shapes in vibrant colors including red, blue, yellow, and orange, scattered across the dark field. The text 'LABEL-FREE MICROSCOPY' is centered horizontally and partially overlaid by these shapes.

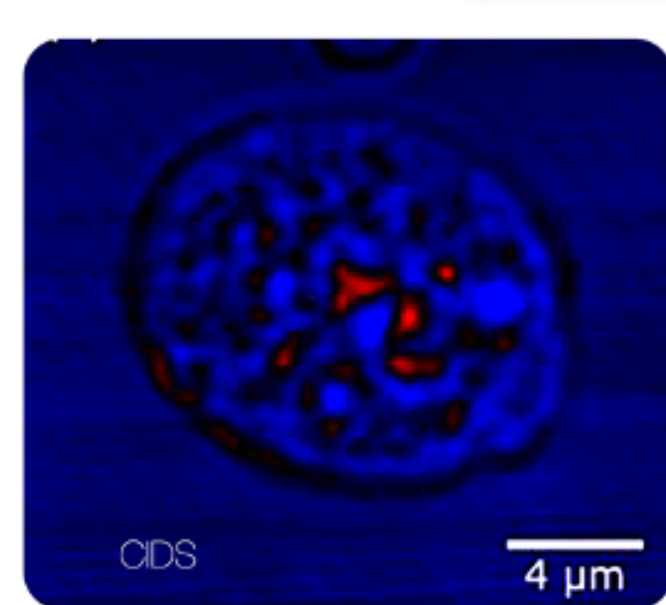
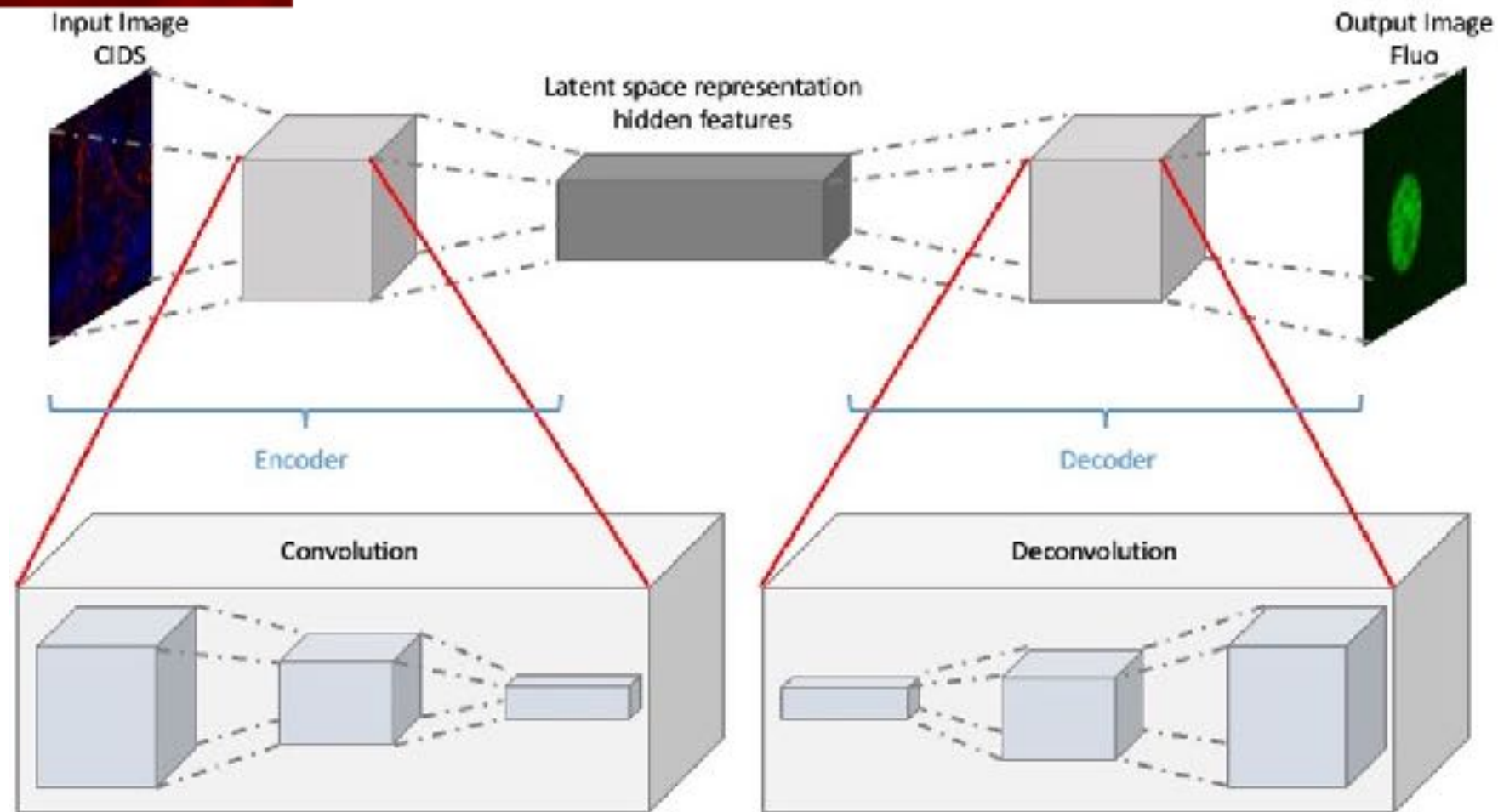
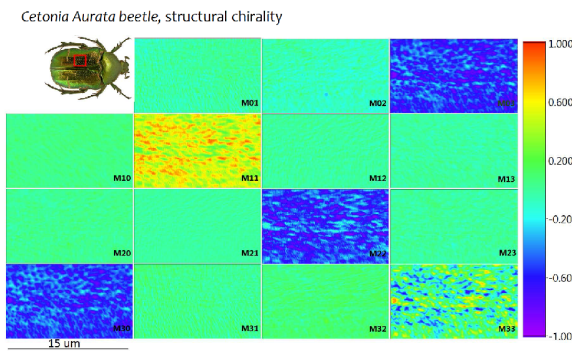
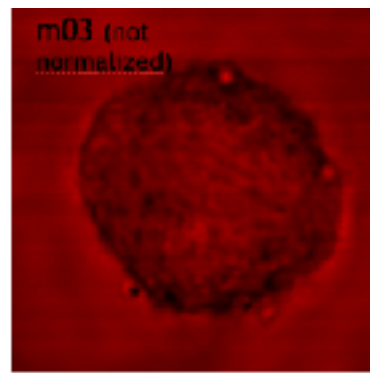
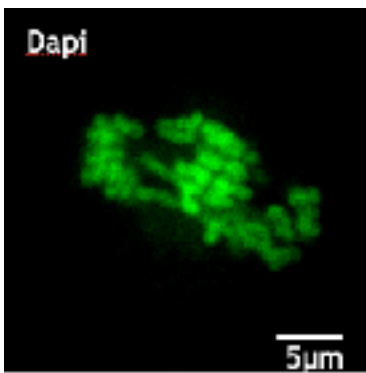
LABEL-FREE MICROSCOPY

Movie credit: Allen Institute

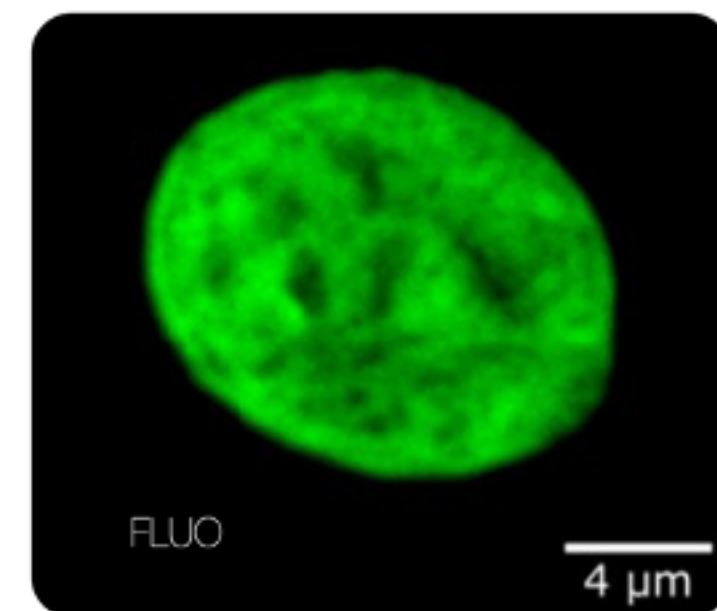
The background of the title features several abstract, irregular shapes in vibrant colors including red, blue, yellow, and orange. These shapes are layered behind the text, creating a sense of depth and movement. The overall aesthetic is modern and scientific.

LABEL-FREE MICROSCOPY

Movie credit: Allen Institute



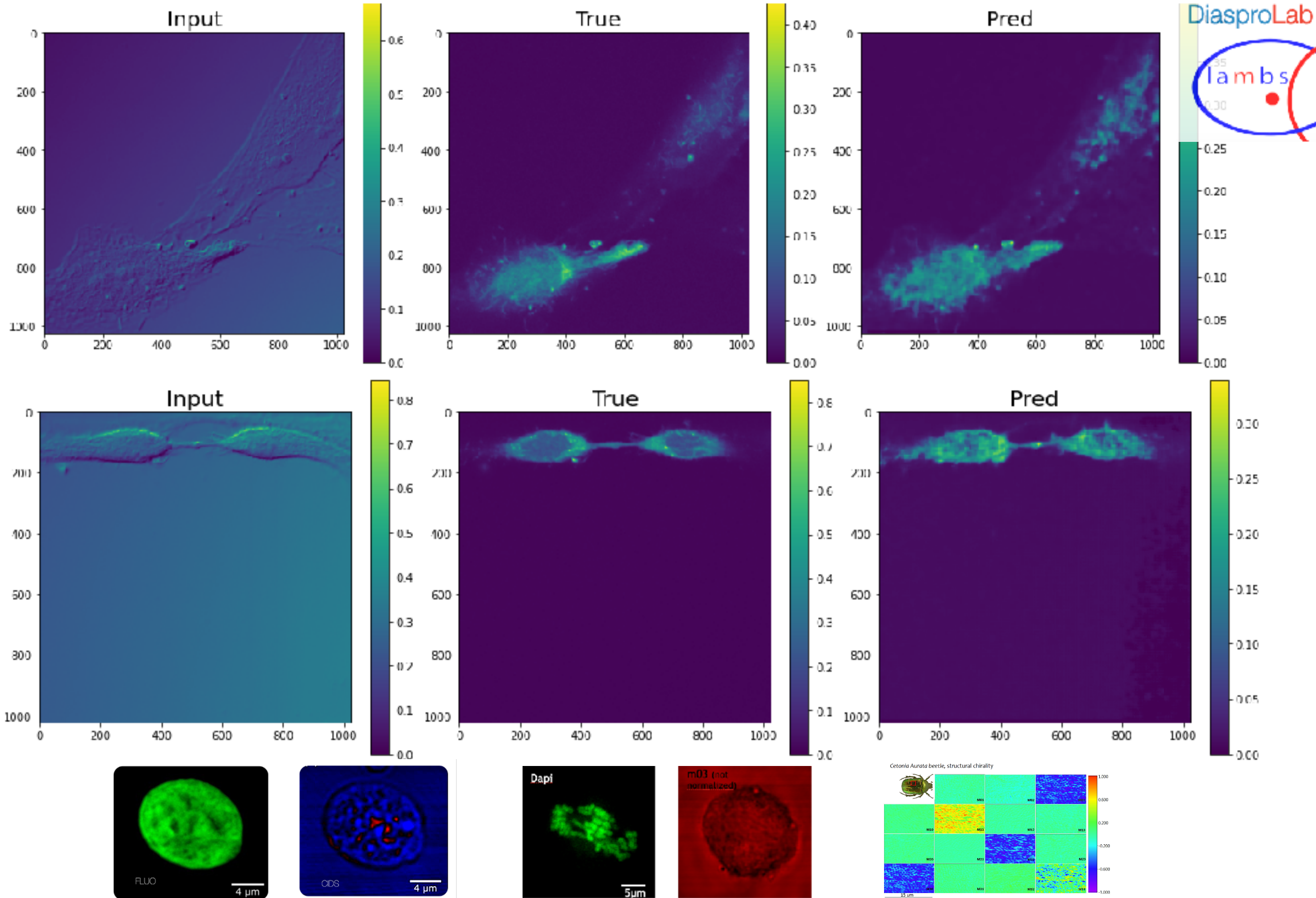
$$[M] = \begin{bmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{bmatrix} \quad \text{CIDS} = \langle (I_L - I_R) \rangle / \langle (I_L + I_R) \rangle$$

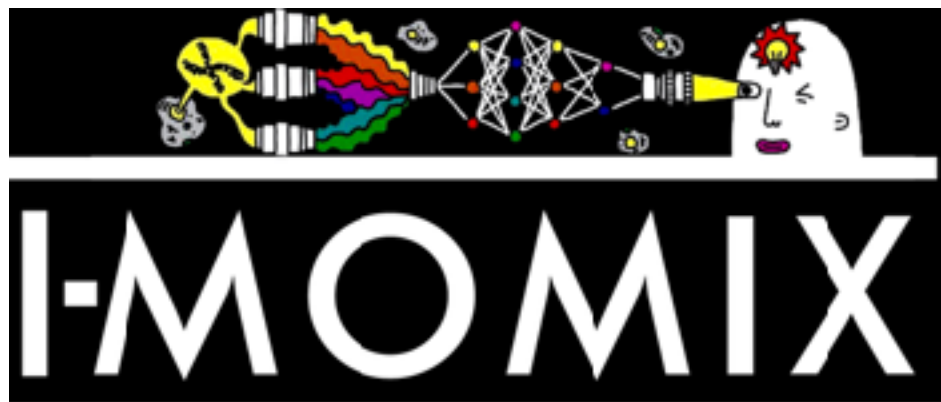




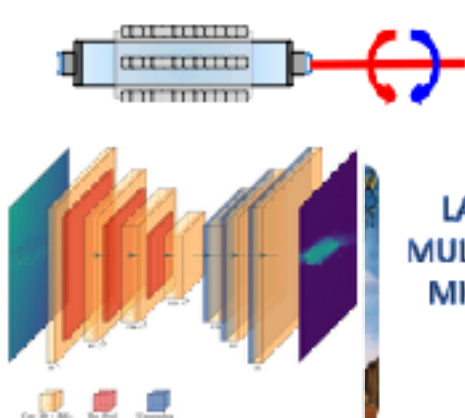
Upsampling





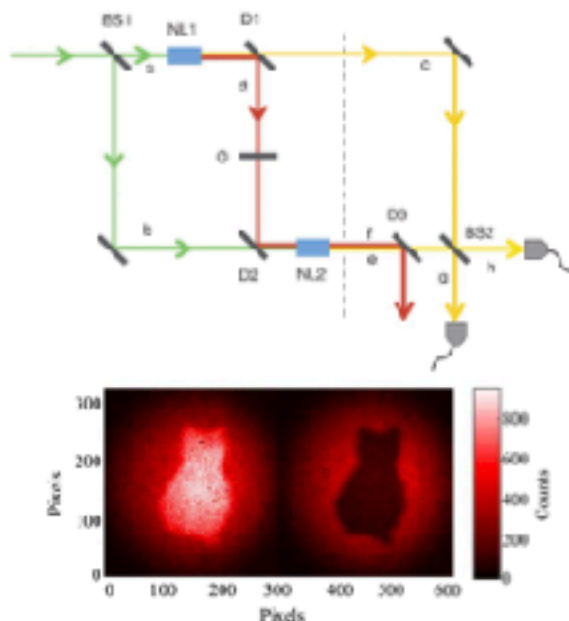


Zeeman effect laser



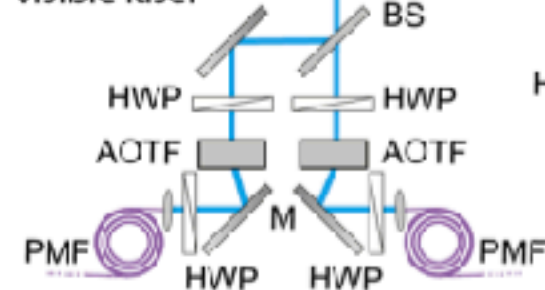
**LABEL-FREE
MULLER MATRIX
MICROSCOPY**

**QUANTUM OPTICAL
MICROSCOPE MODULE**

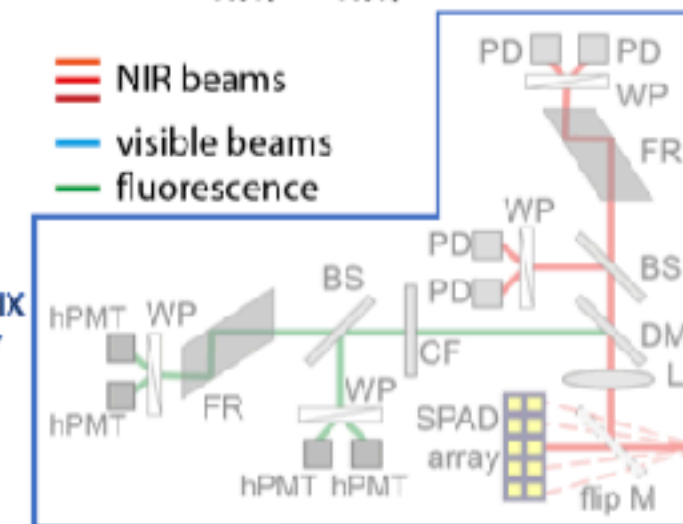


Lemos, G., Quantum imaging with undetected photons.
Nature 512, 409–412 (2014).

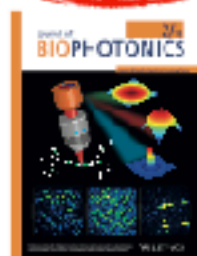
Supercontinuum
visible laser



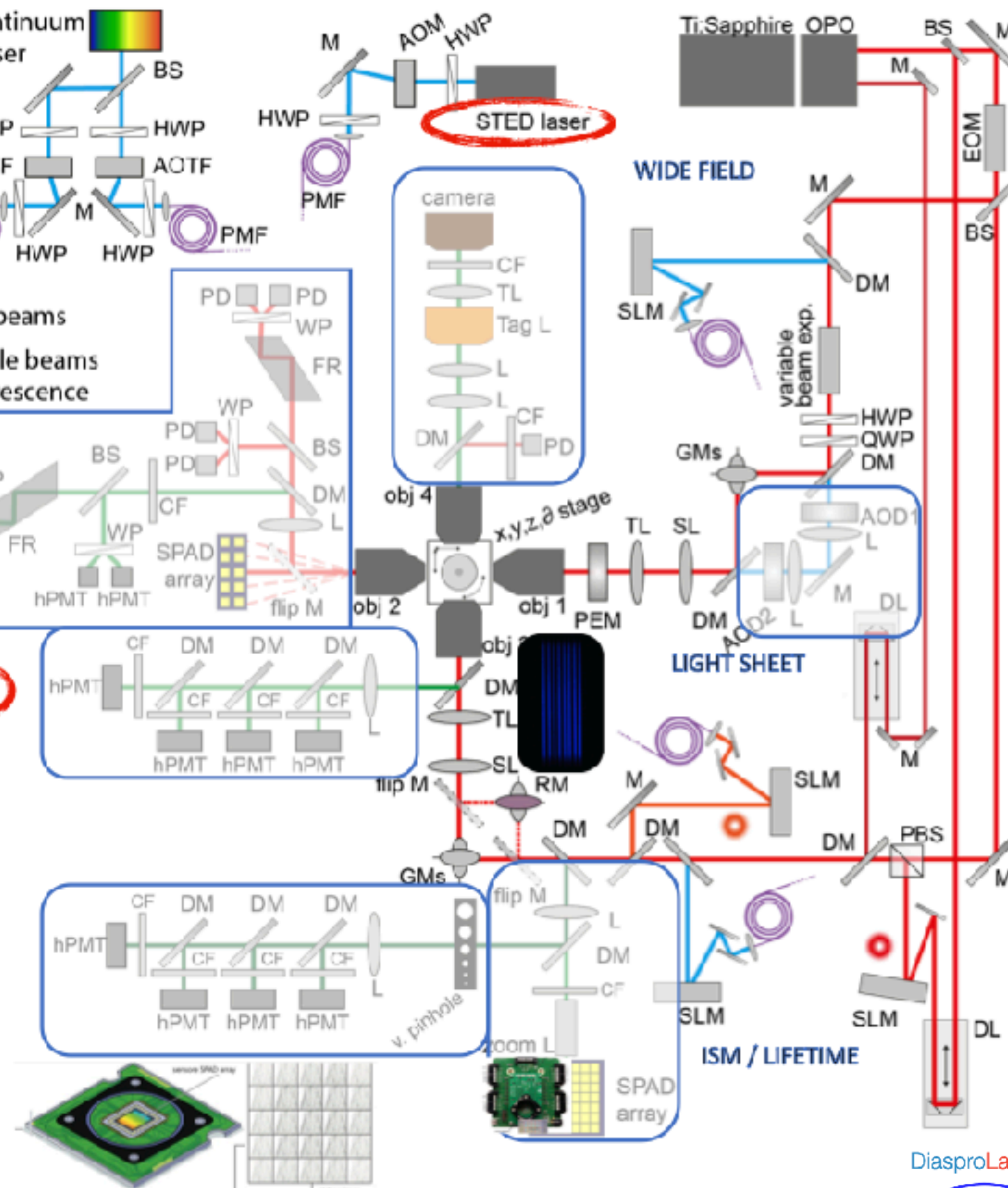
- NIR beams
- visible beams
- fluorescence



2PE / MPE



CONFOCAL

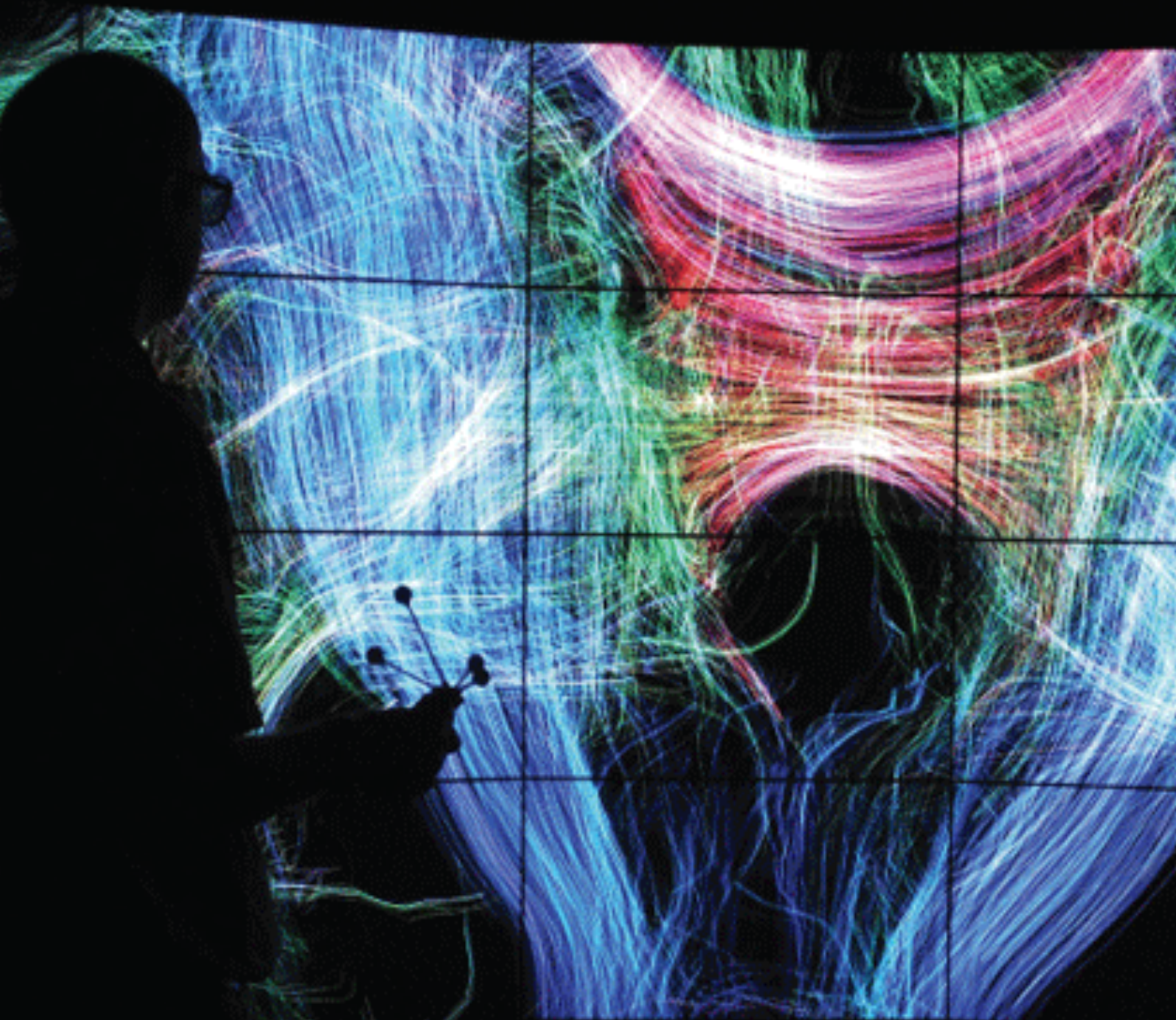


DiasproLab



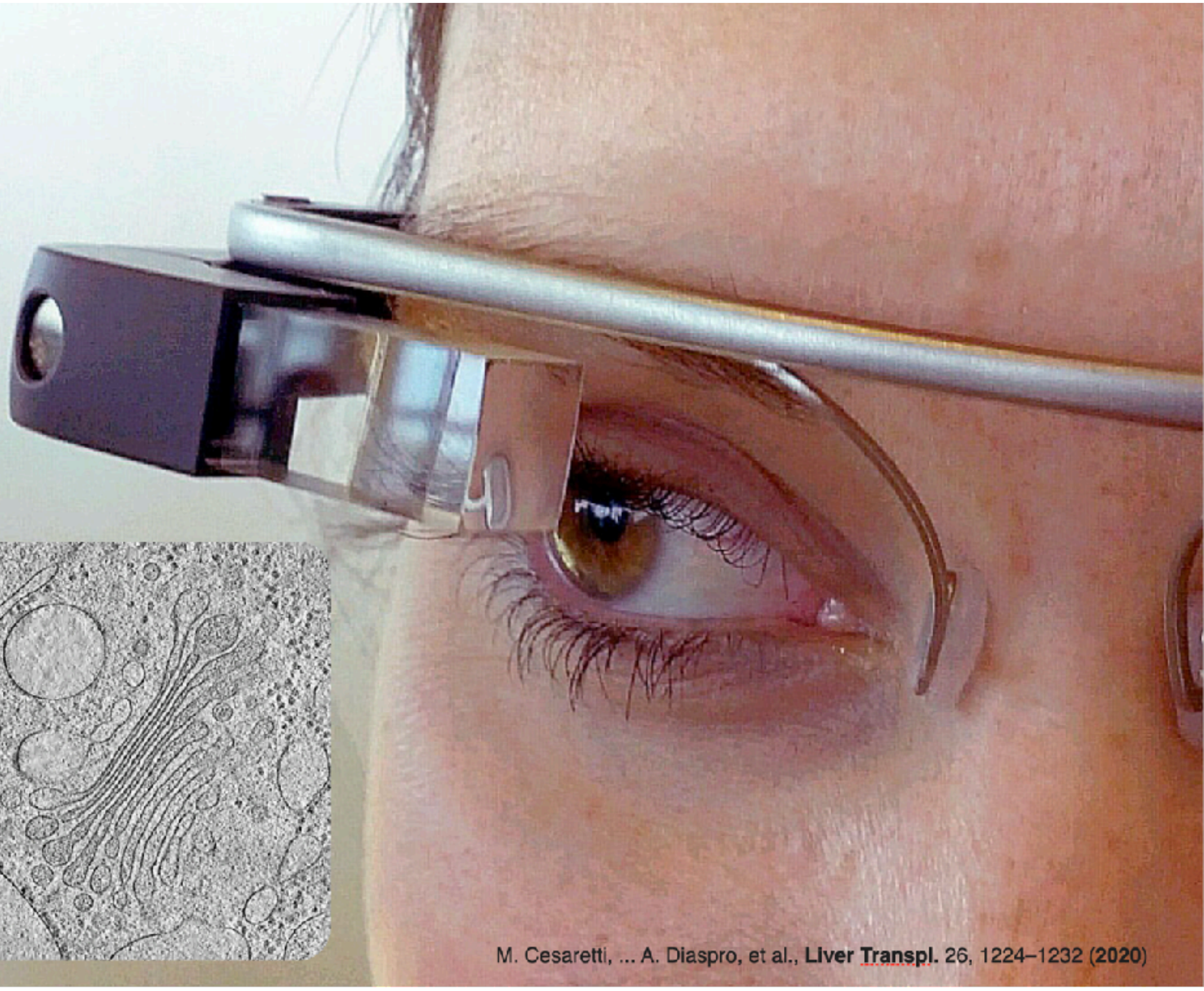
nature medicine

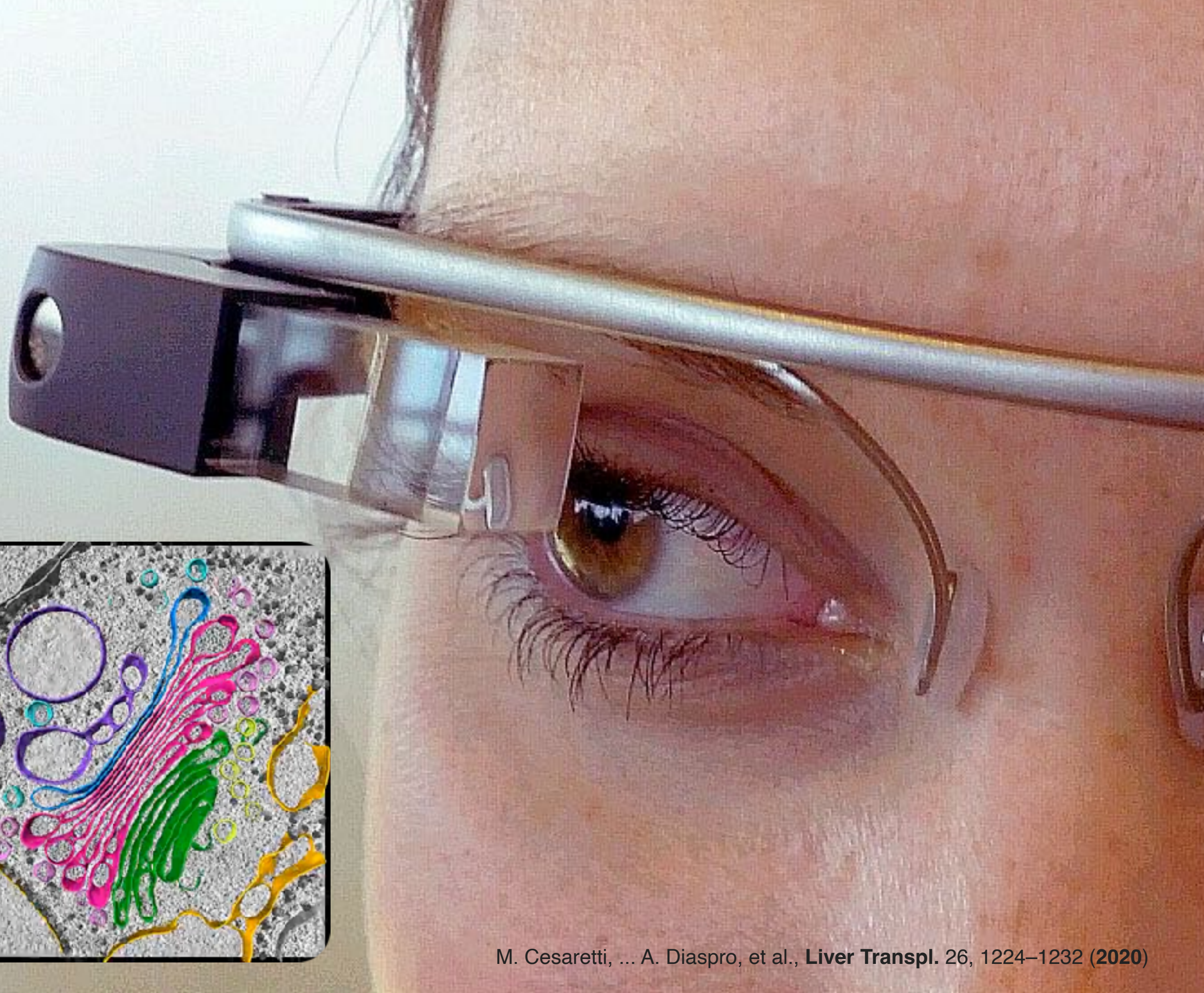
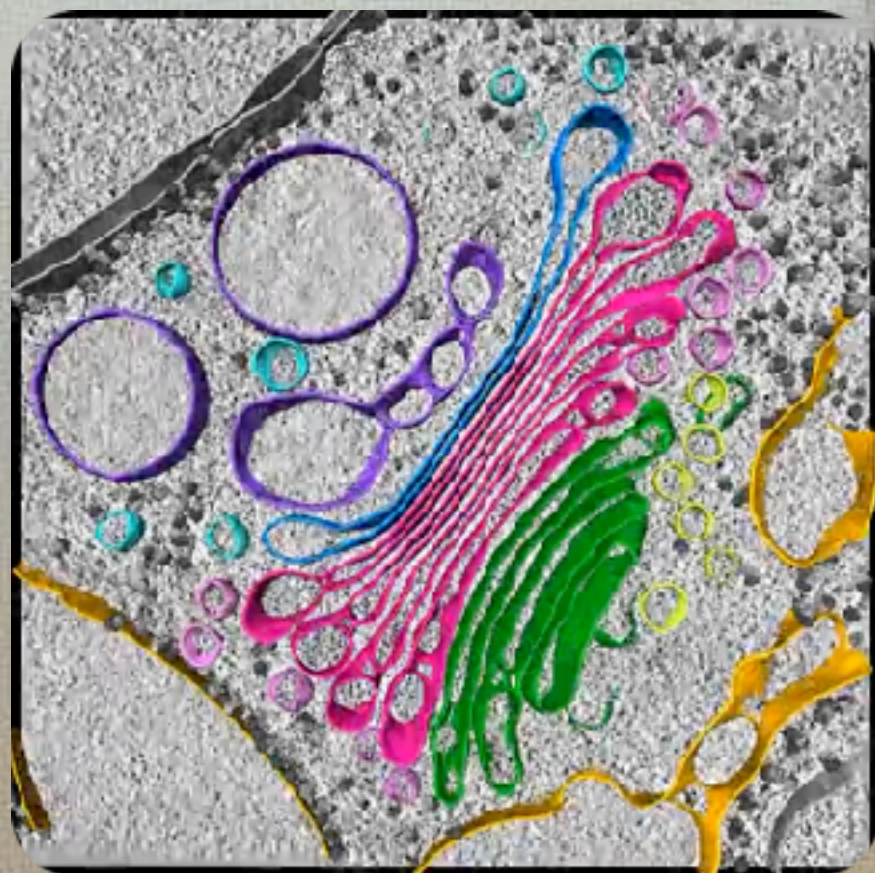
VOLUME 20 NUMBER 3 MARCH 2014
www.nature.com/naturemedicine

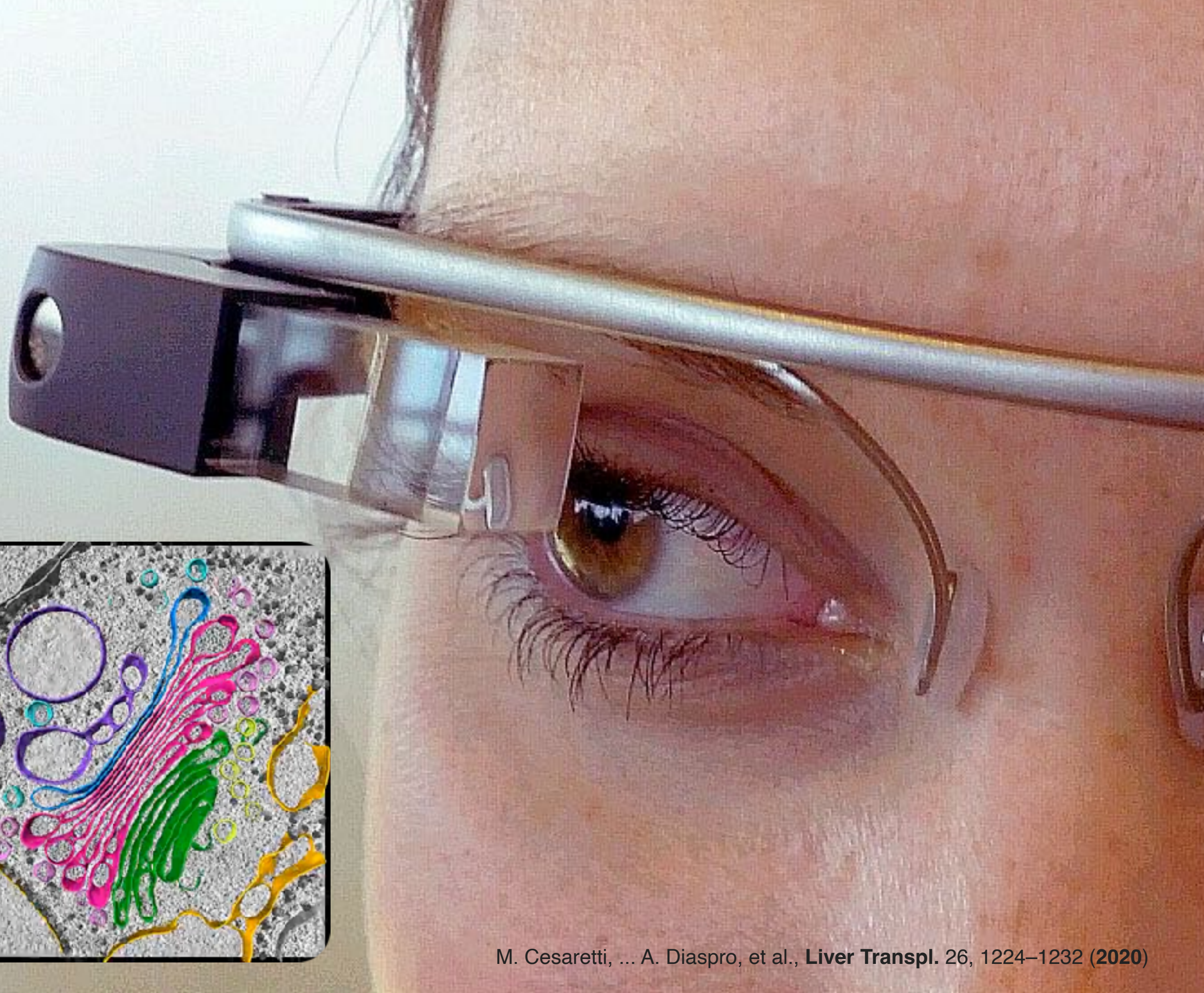
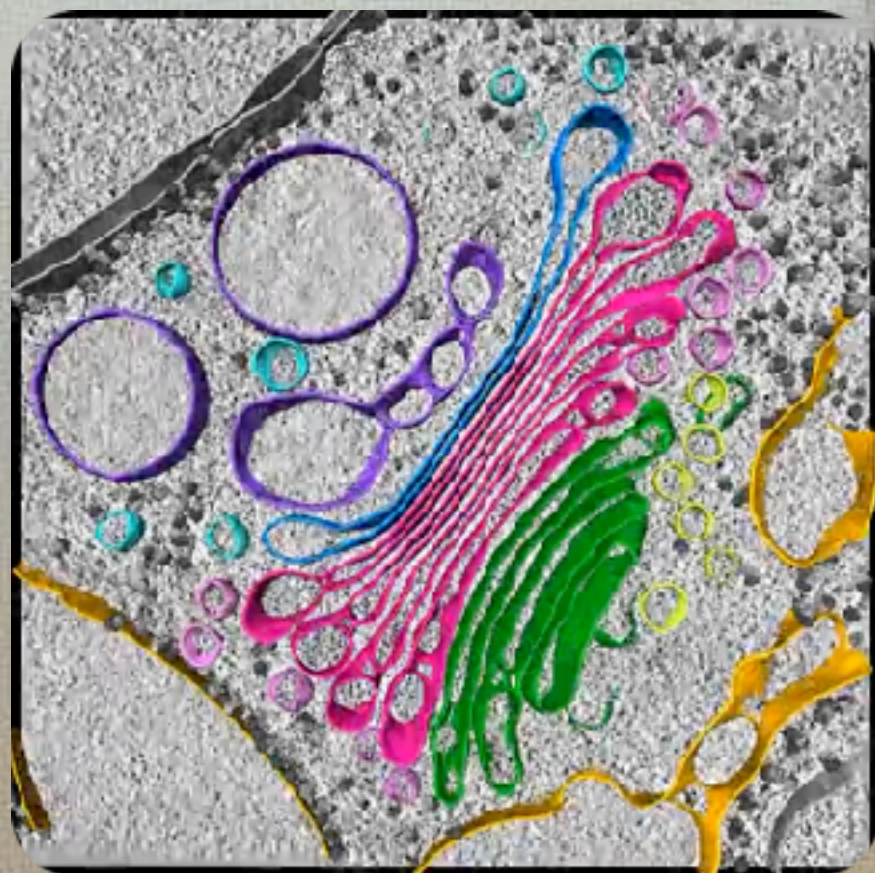


Virtual reality labs for biomedicine









Butch Cassidy & Sundance Kid



Butch Cassidy & Sundance Kid







Giuliana Cuneaz, Quantum quirks, 2023



Giuliana Cuneaz, Quantum quirks, 2023



La meccanica quantistica è la teoria meglio verificata
e di maggior successo nella storia della scienza



The Nobel Prize in Physics 2022



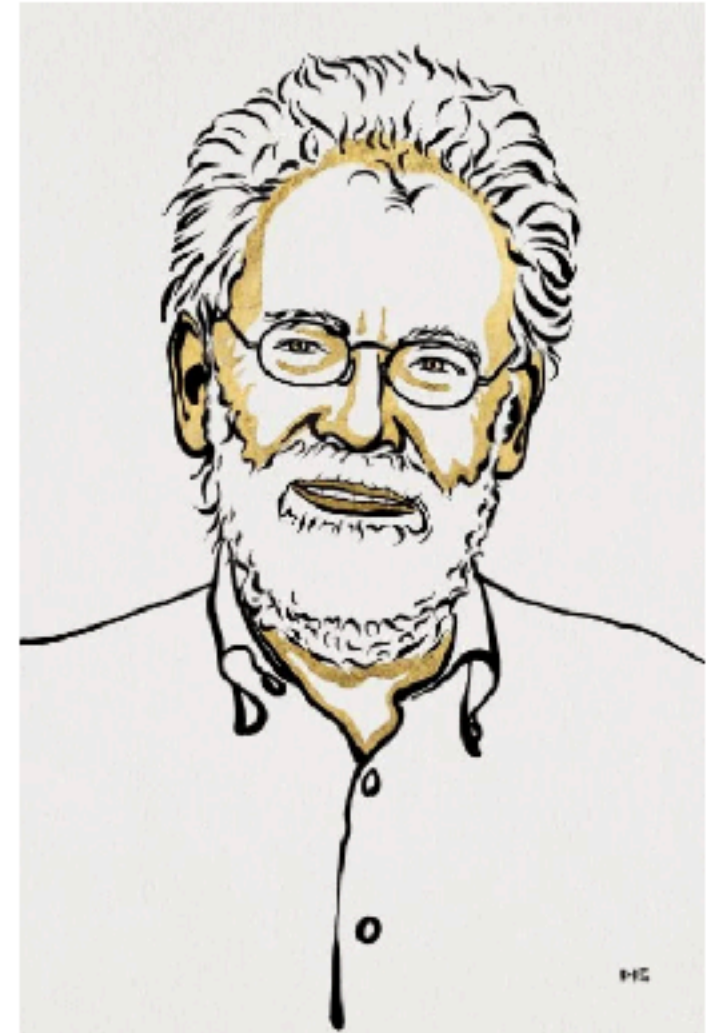
III. Niklas Elmehed © Nobel Prize Outreach

Alain Aspect



III. Niklas Elmehed © Nobel Prize Outreach

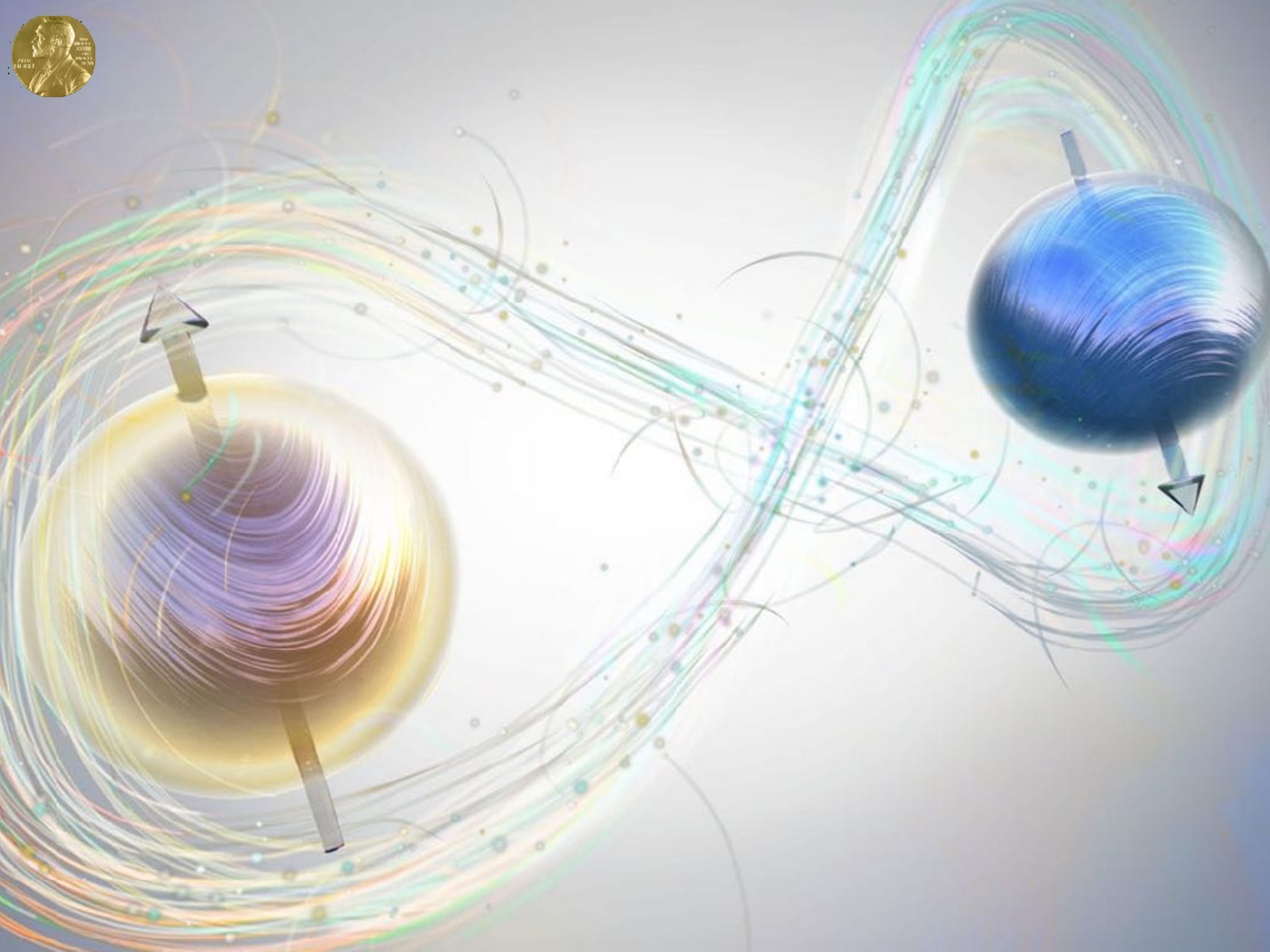
John F. Clauser

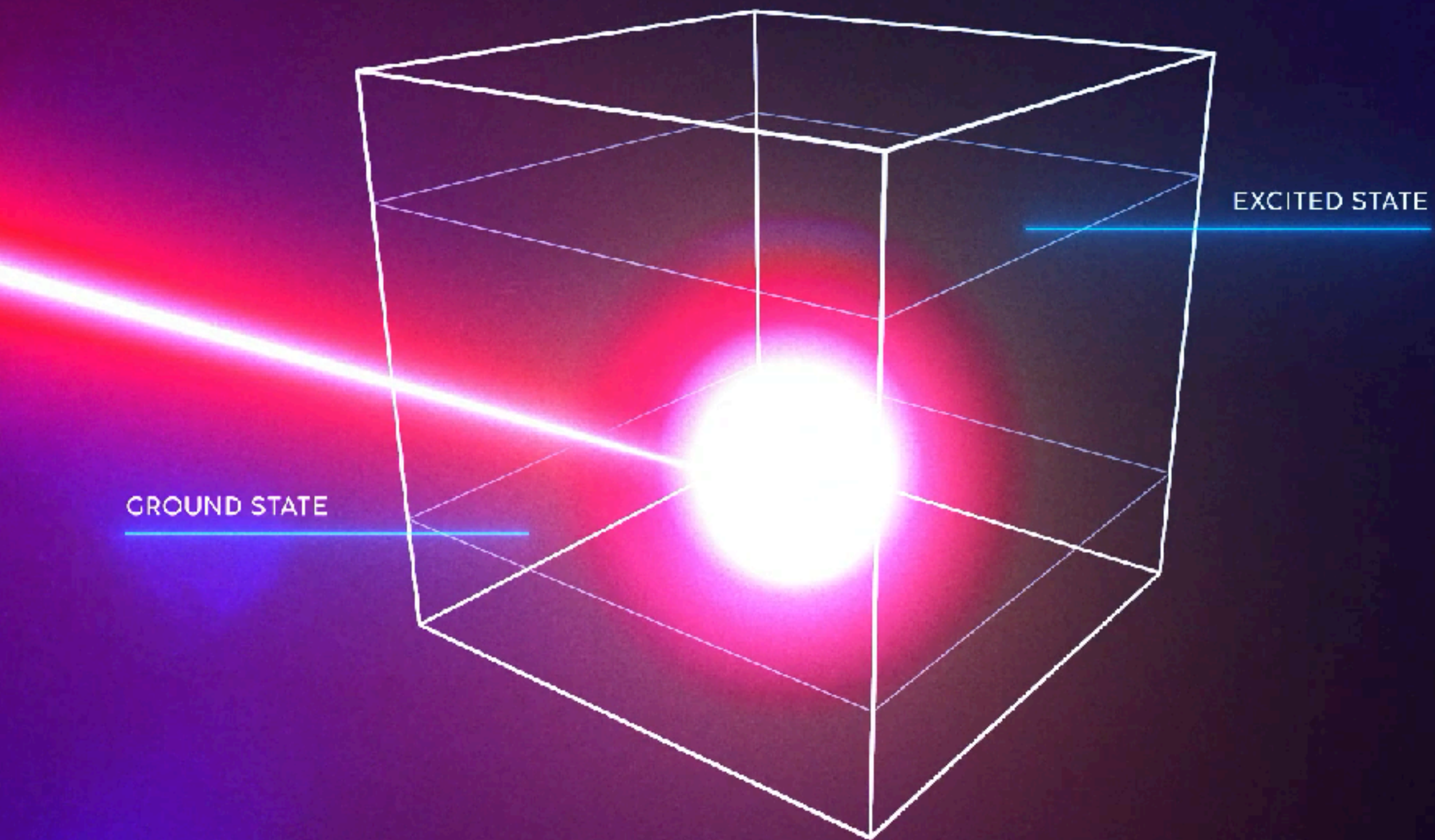


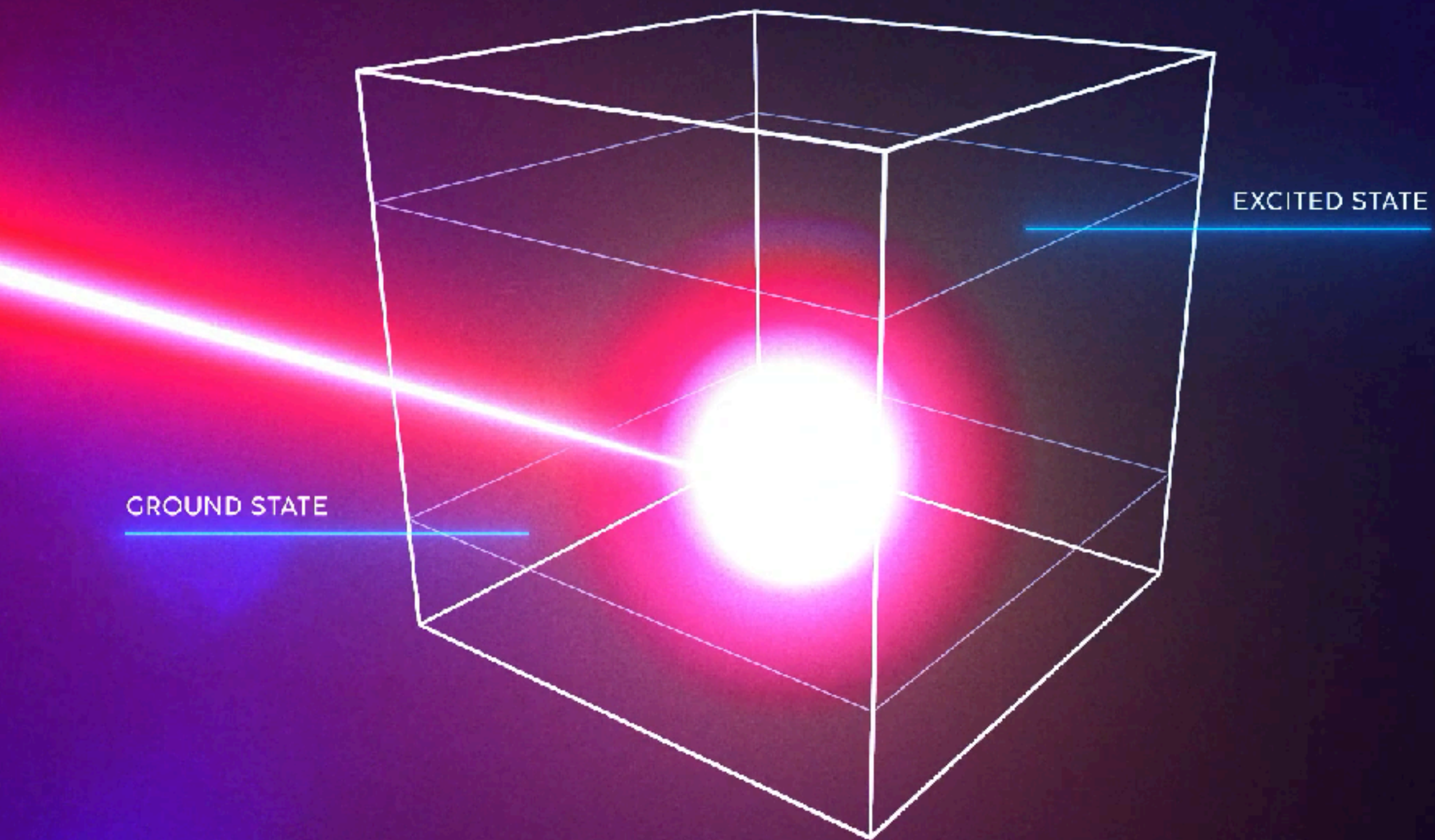
III. Niklas Elmehed © Nobel Prize Outreach

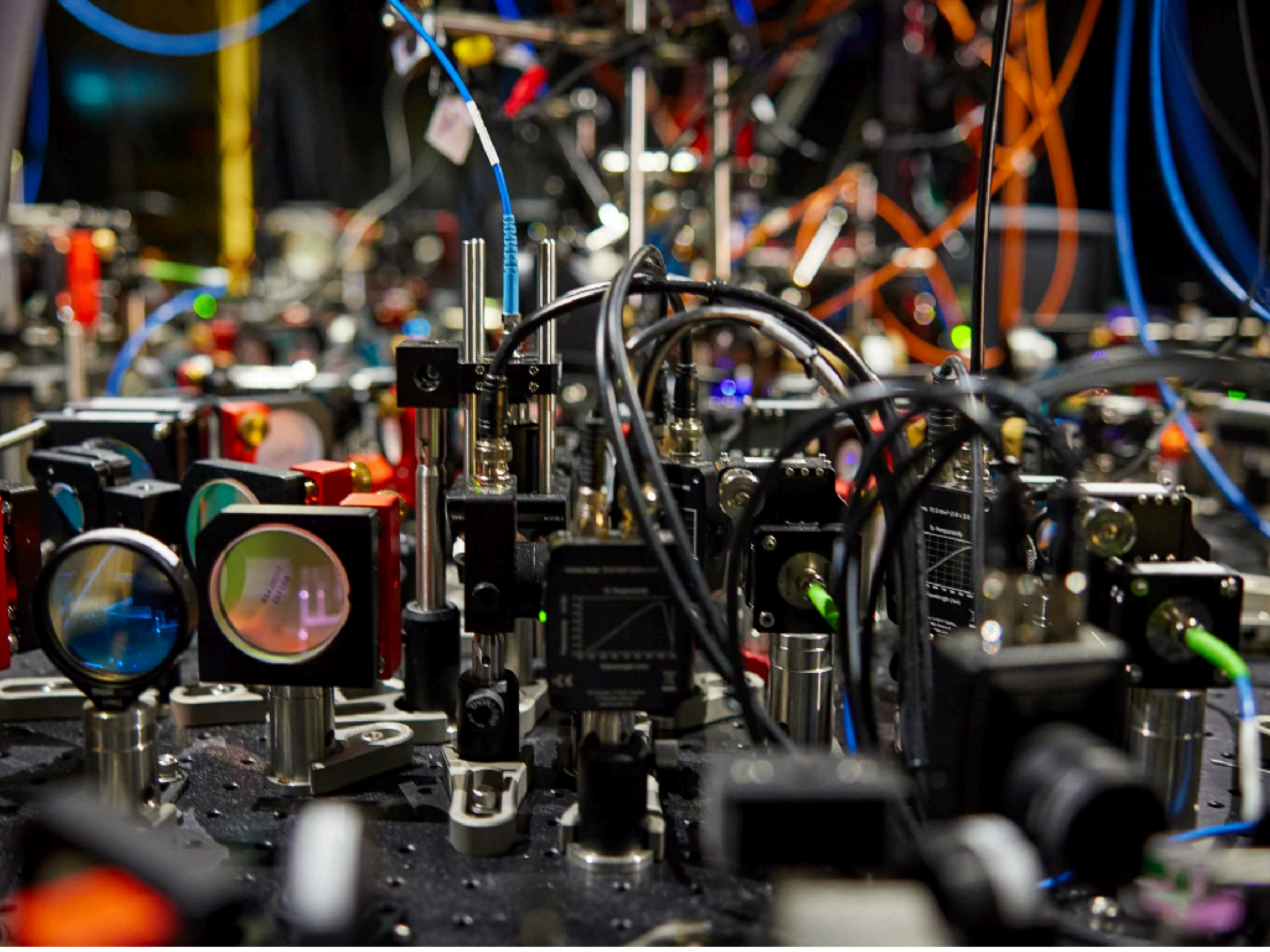
Anton Zeilinger

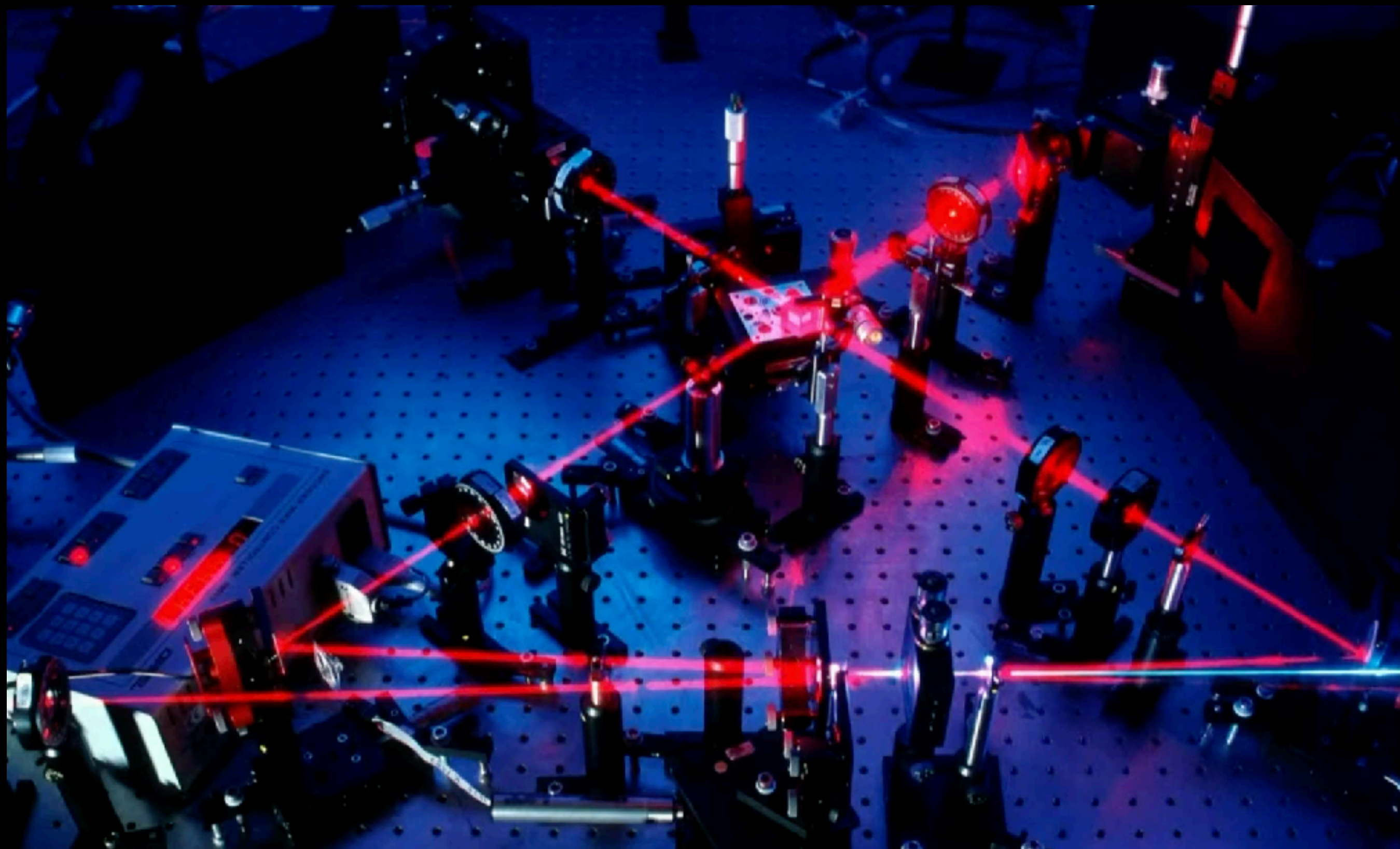
The Nobel Prize in Physics 2022 was awarded jointly to Alain Aspect, John F. Clauser and Anton Zeilinger "for experiments with entangled photons, establishing the violation of Bell inequalities and pioneering quantum information science"

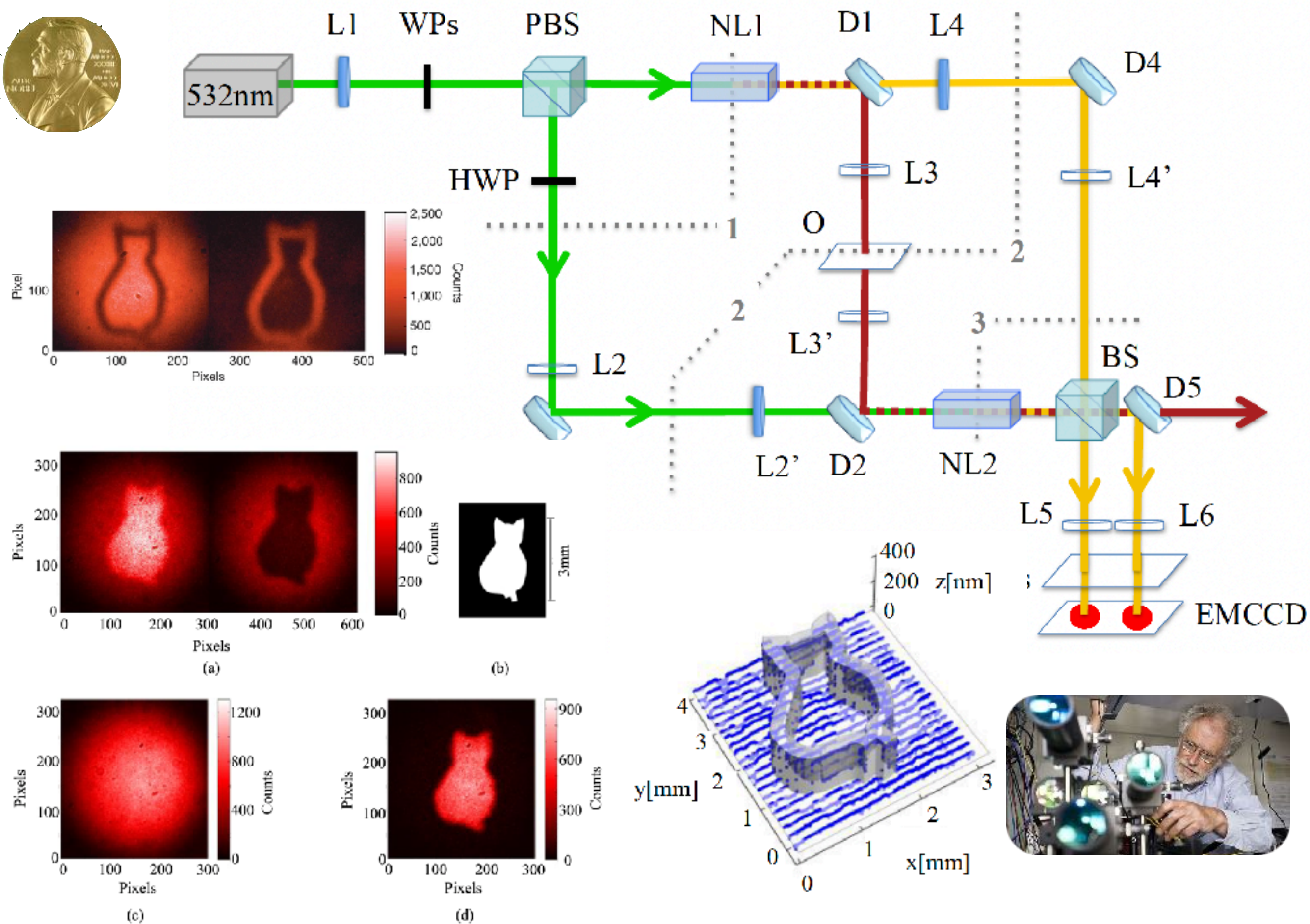


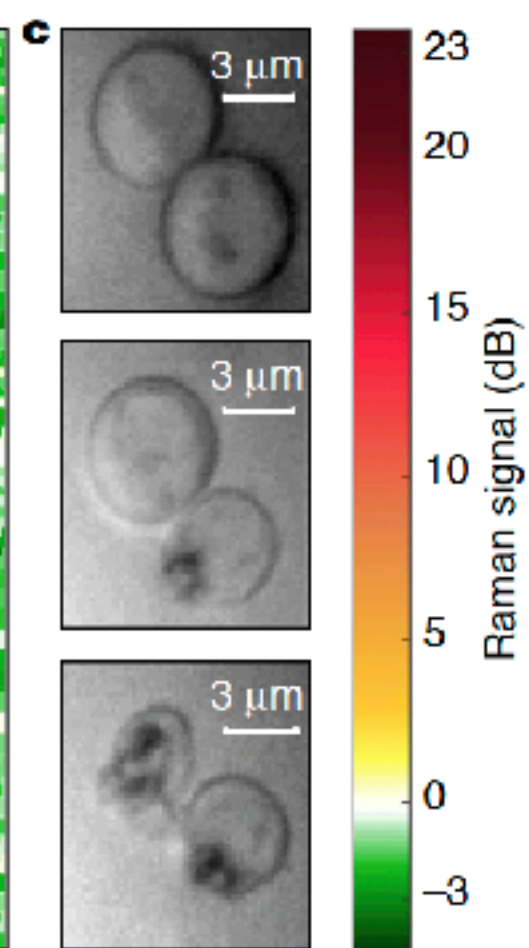
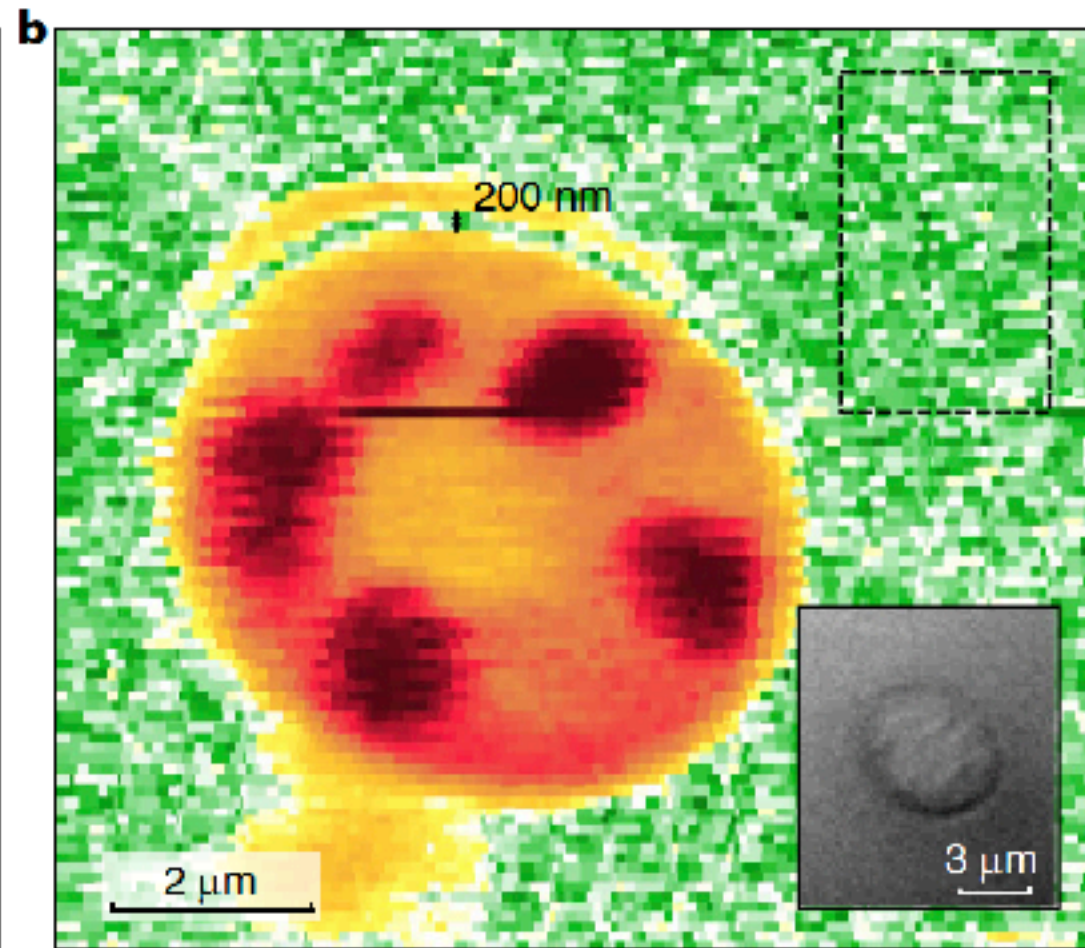
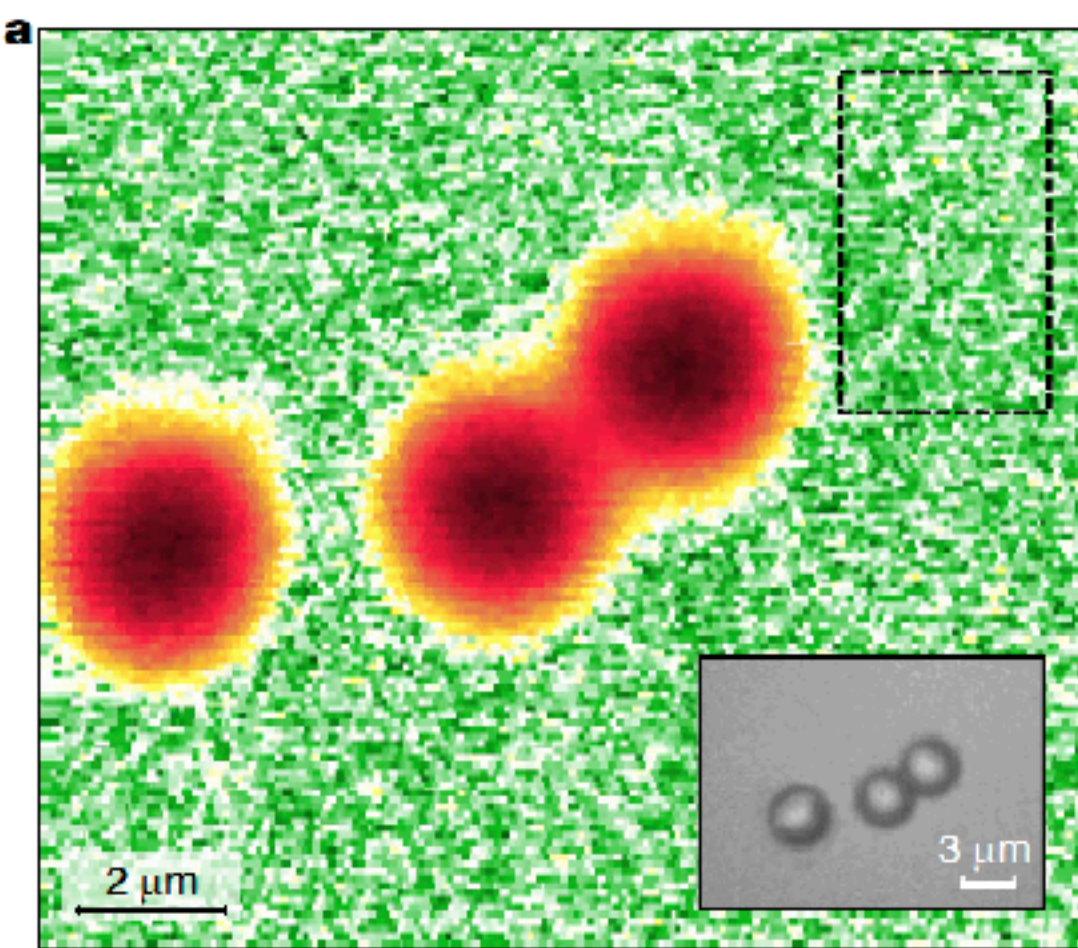
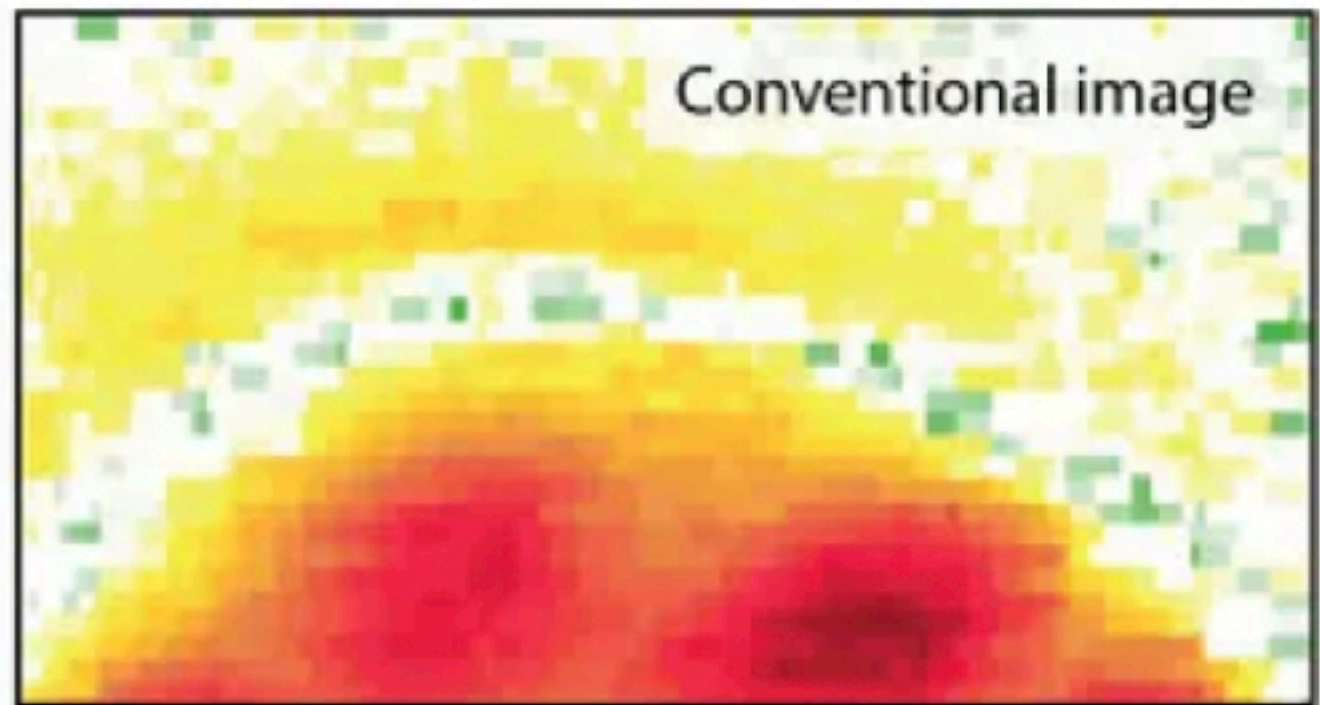
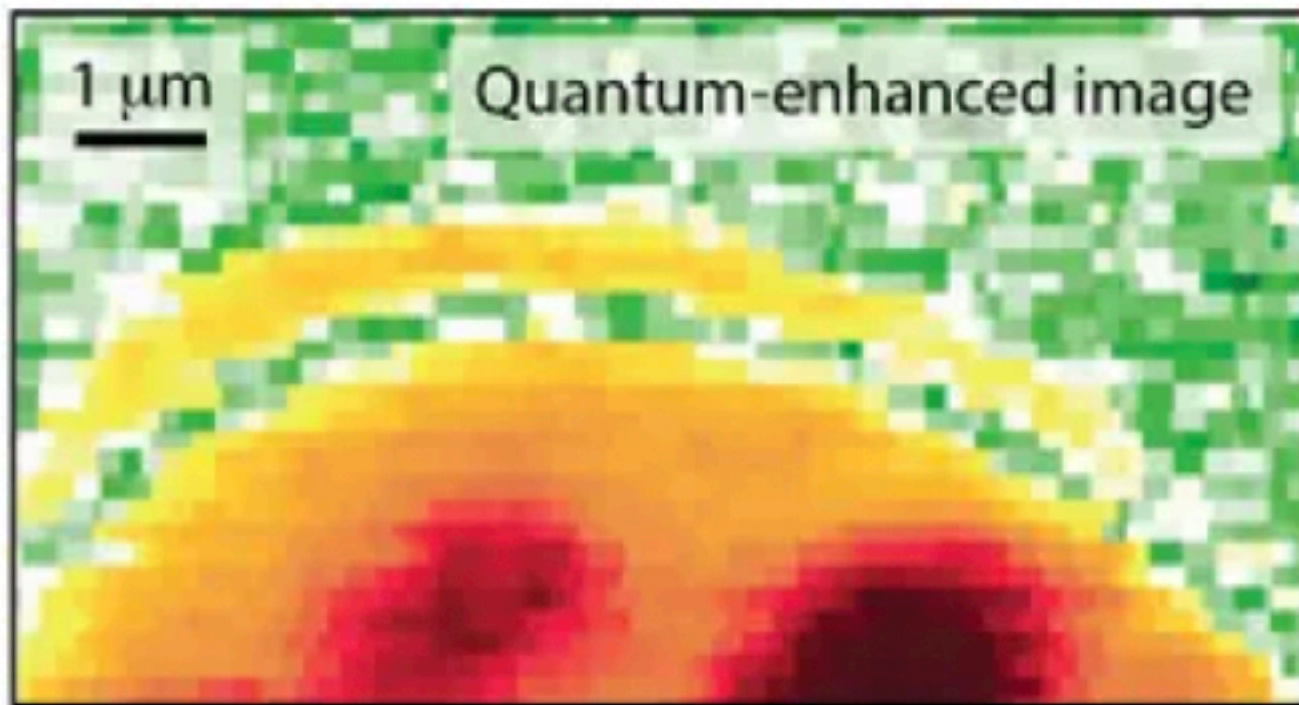




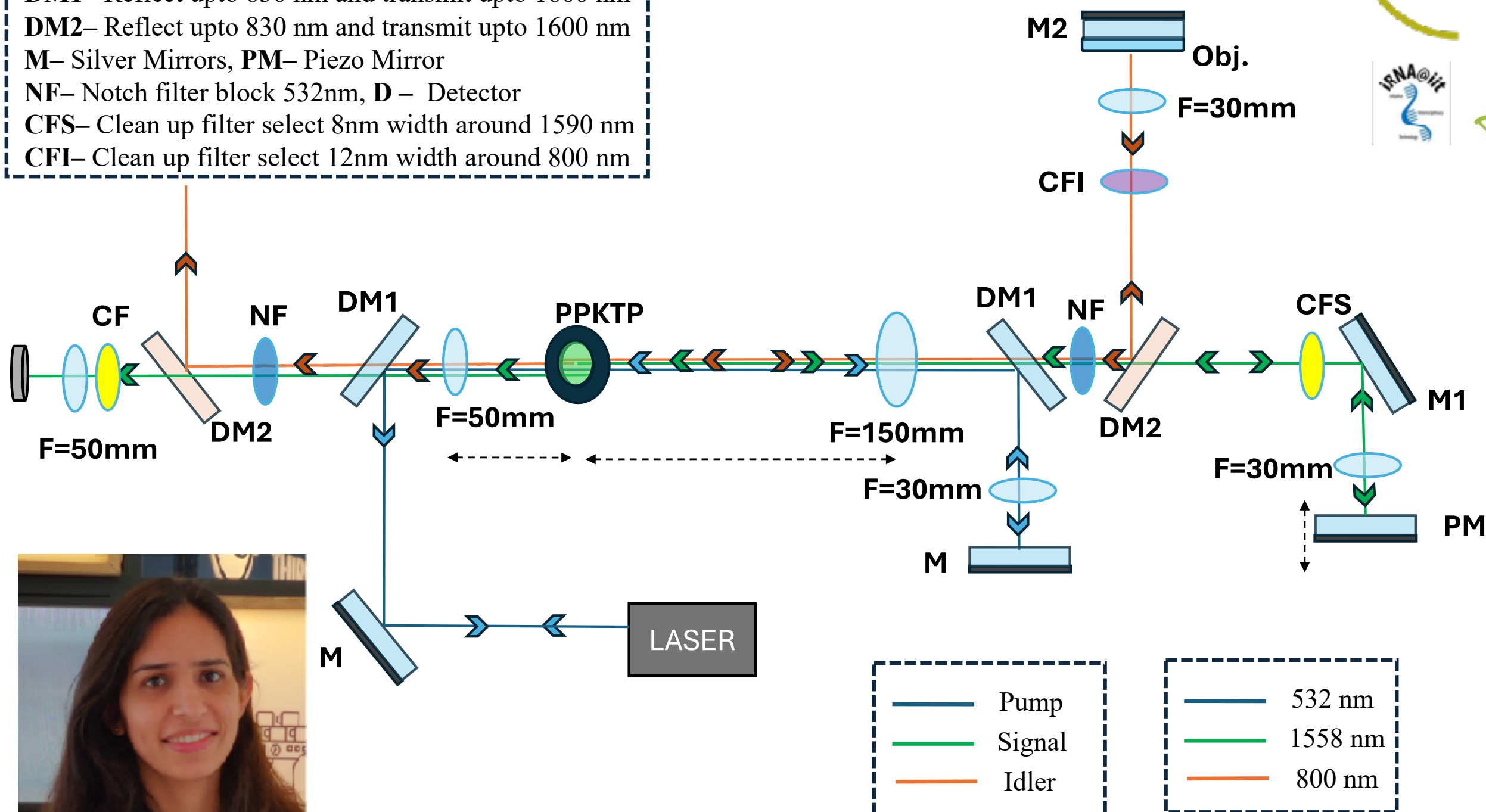






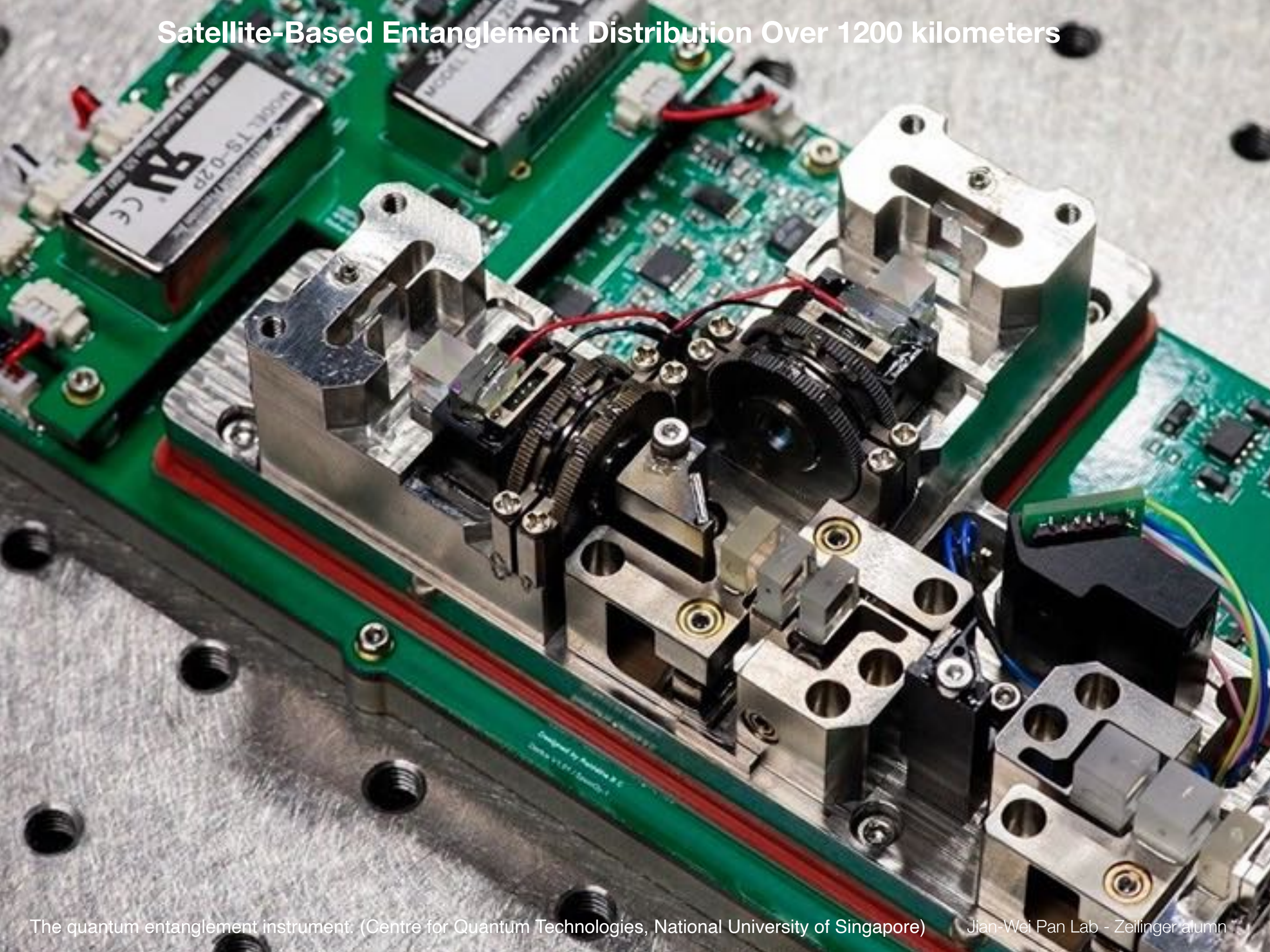


DM1– Reflect upto 650 nm and transmit upto 1600 nm
DM2– Reflect upto 830 nm and transmit upto 1600 nm
M– Silver Mirrors, **PM**– Piezo Mirror
NF– Notch filter block 532nm, **D** – Detector
CFS– Clean up filter select 8nm width around 1590 nm
CFI– Clean up filter select 12nm width around 800 nm

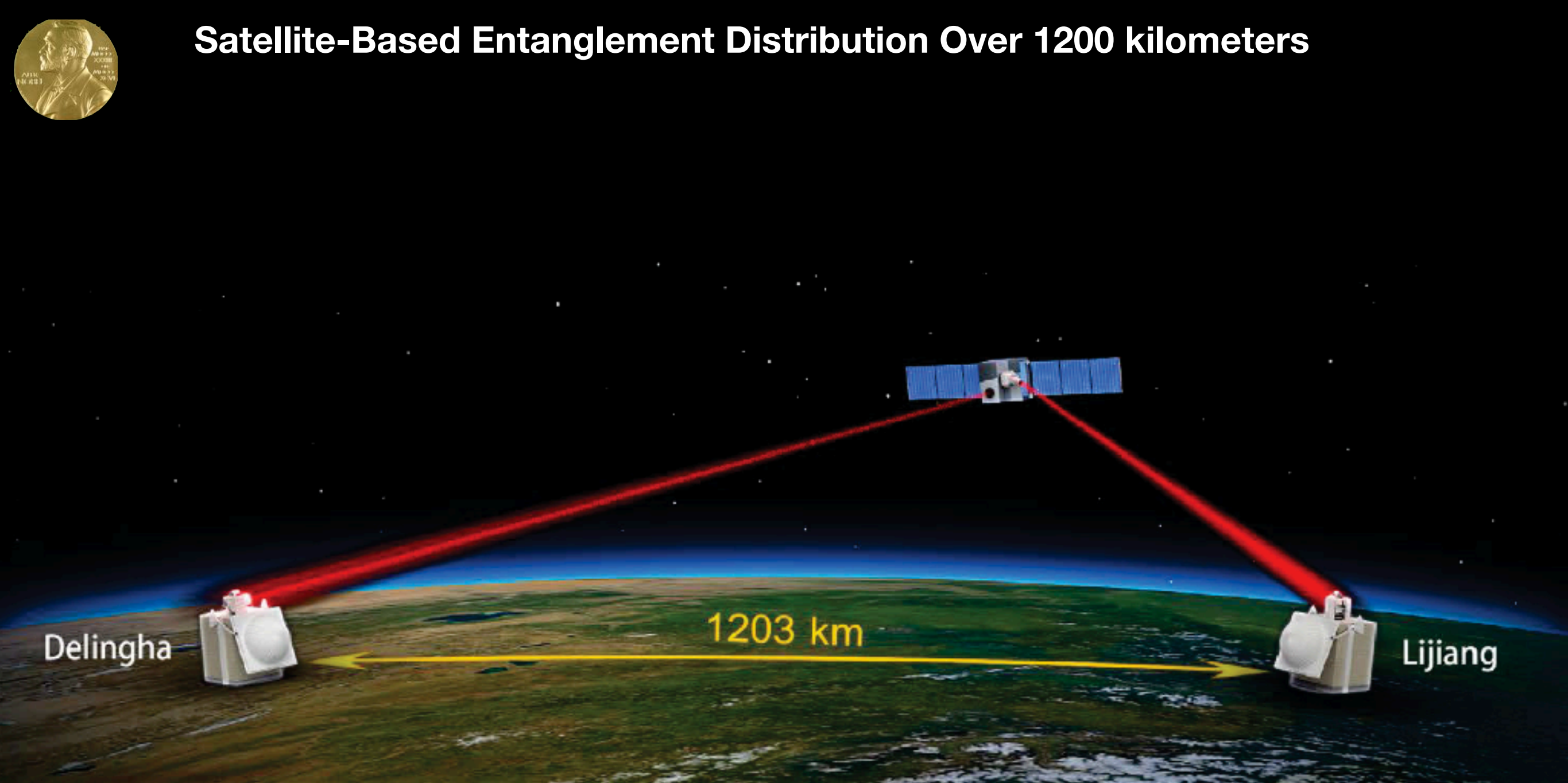


Gaytri Arya

Satellite-Based Entanglement Distribution Over 1200 kilometers

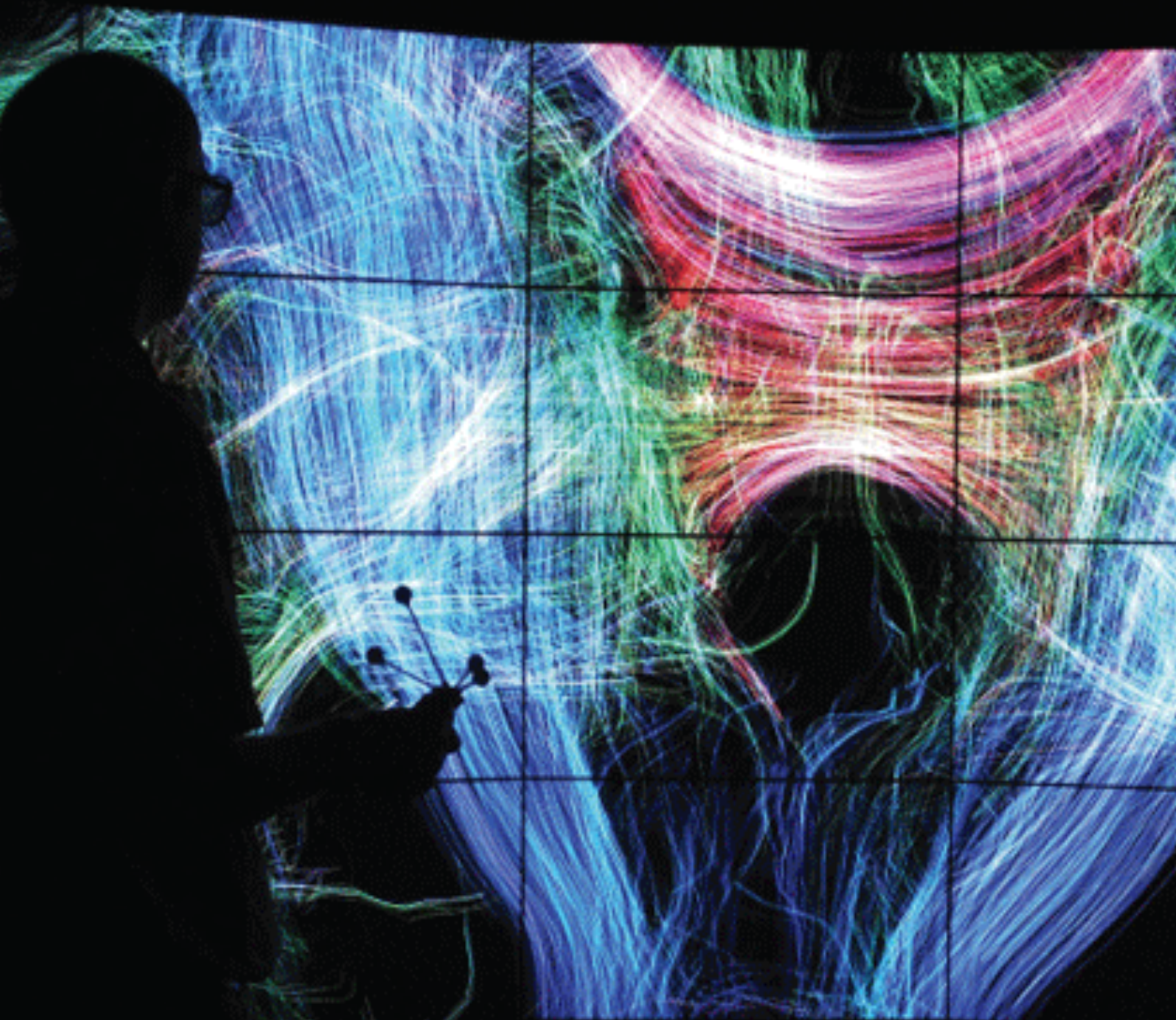


Satellite-Based Entanglement Distribution Over 1200 kilometers



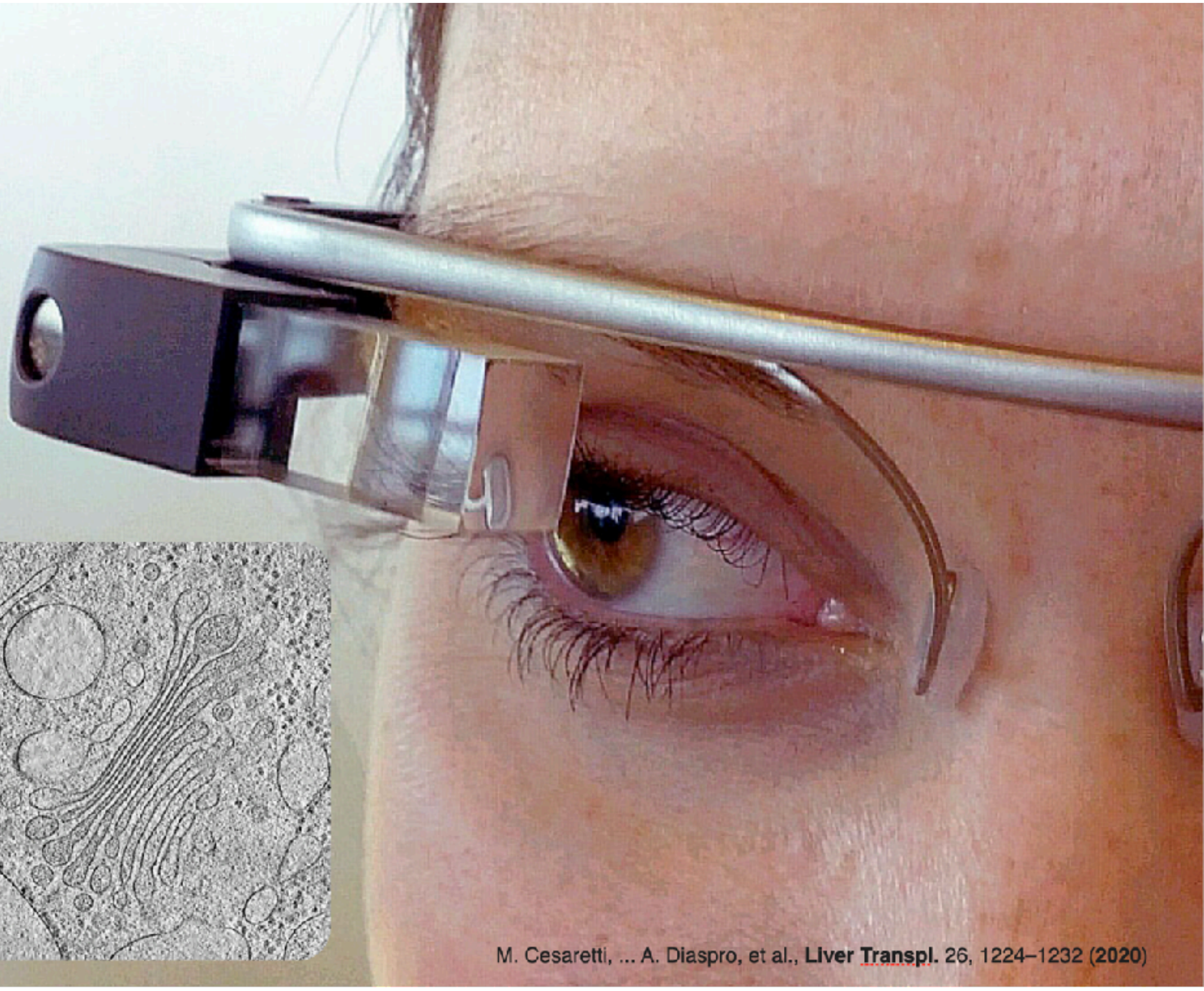
nature medicine

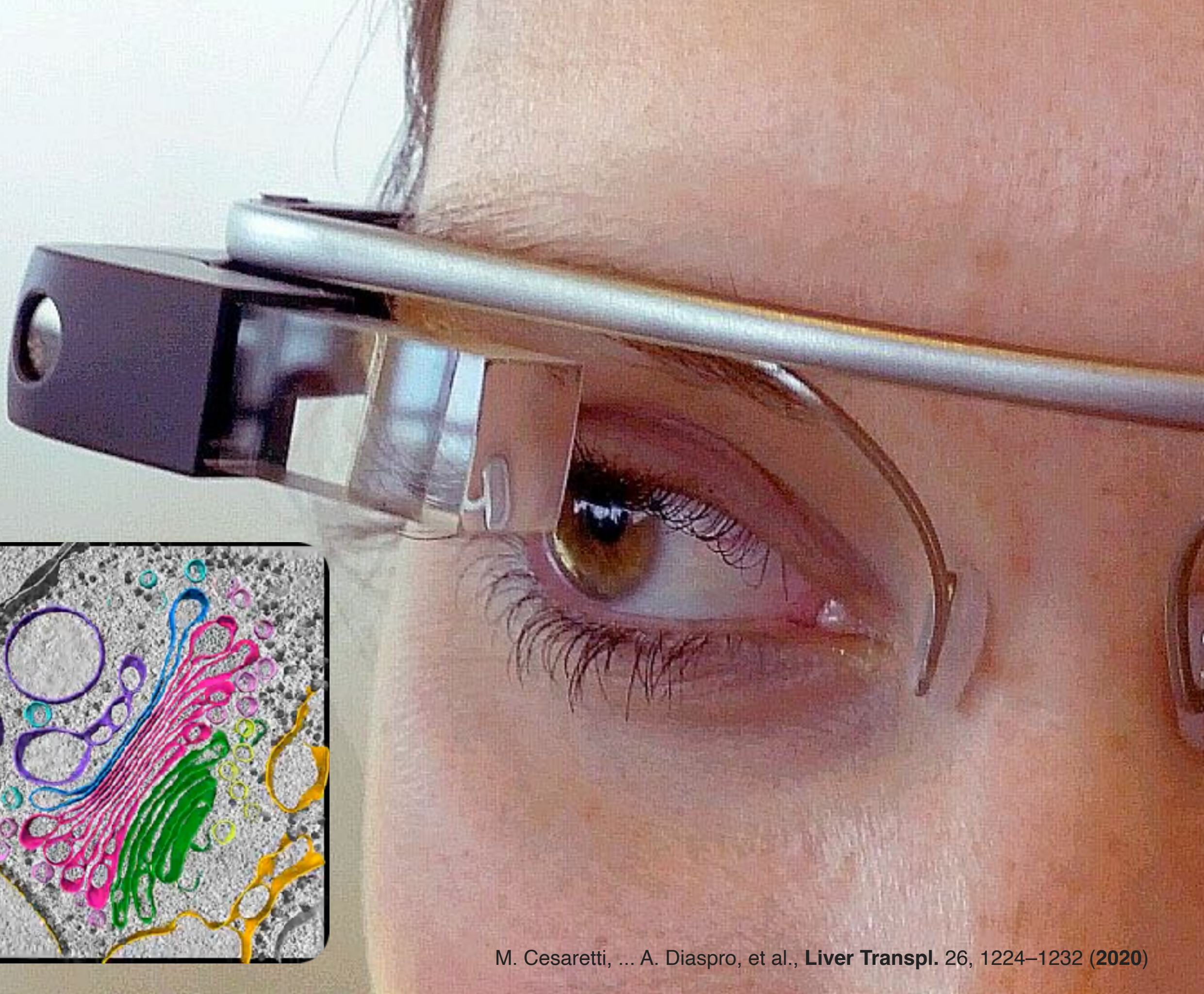
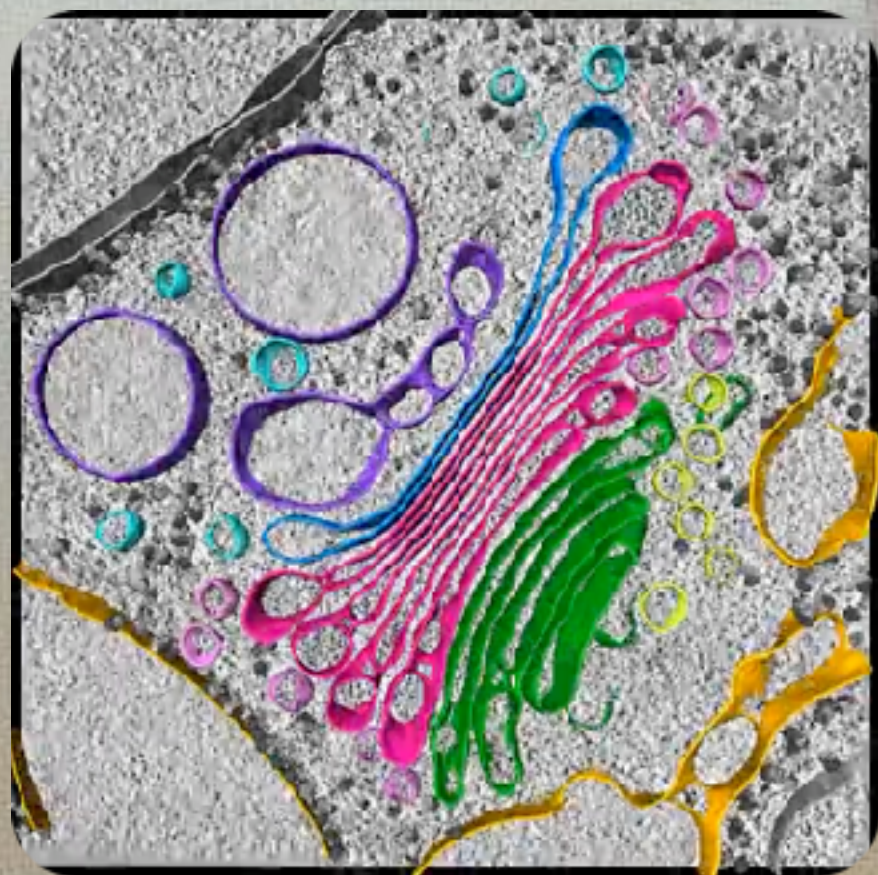
VOLUME 20 NUMBER 3 MARCH 2014
www.nature.com/naturemedicine

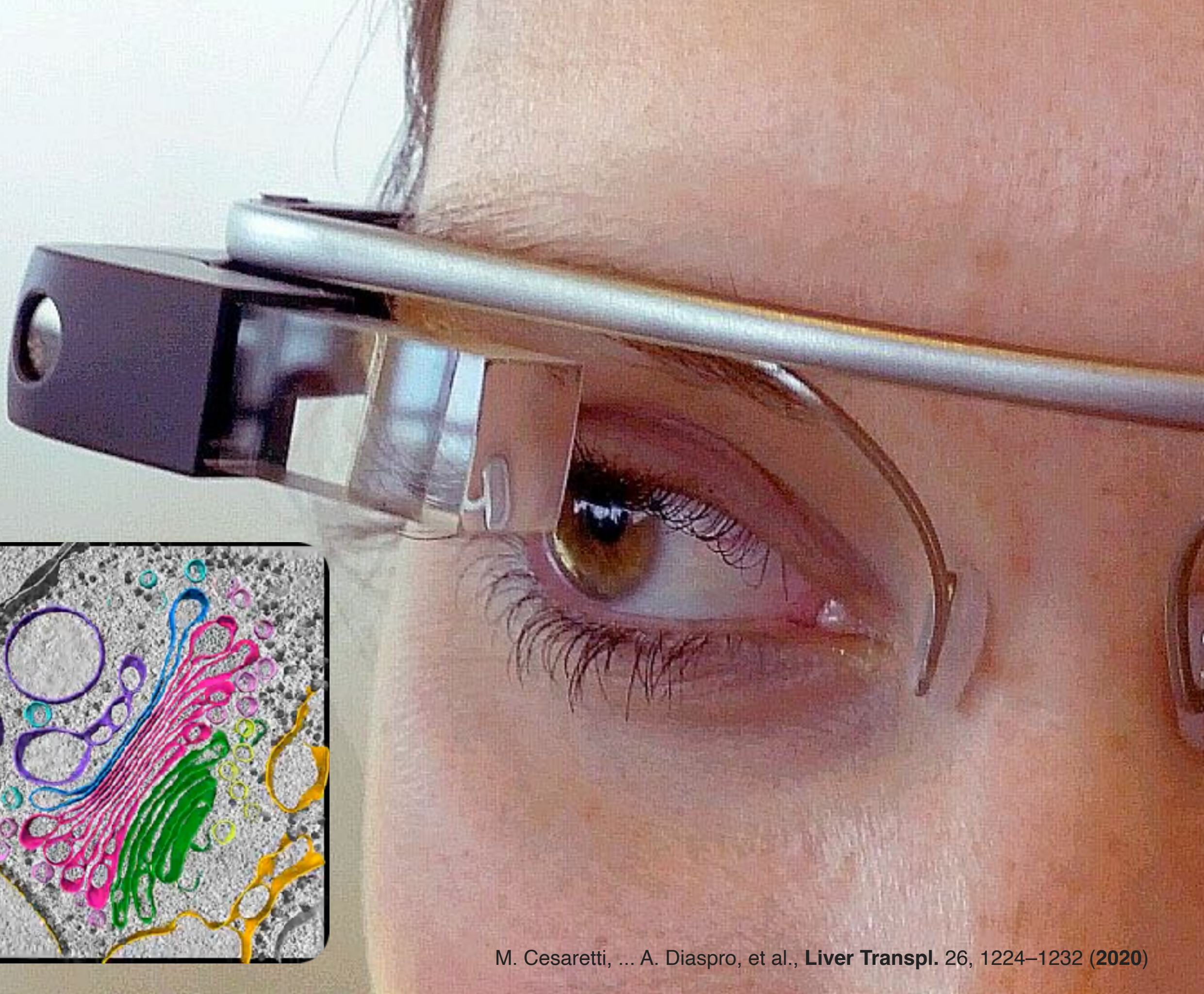
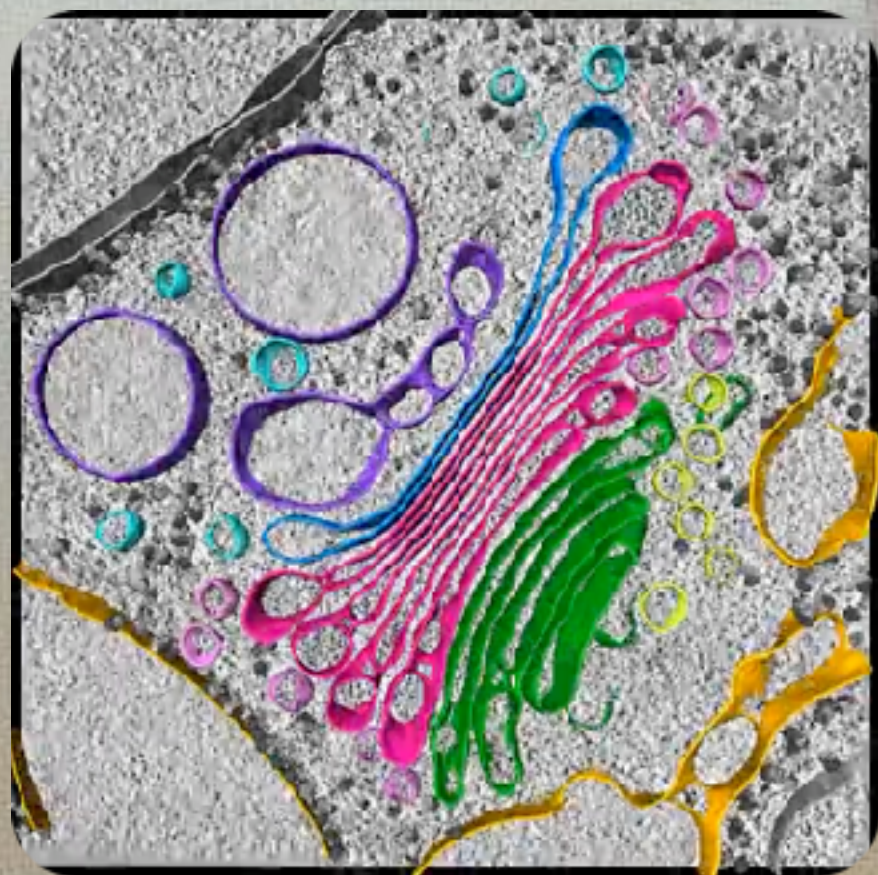


Virtual reality labs for biomedicine













Thanks for the Dance

(Leonard Cohen, 2019)