BRAIN AWARENESS WEEK

SETTIMANA DEL CERVELLO 2018

TECNOLOGIE E NEUROSCIENZE

dall'Homo sapiens all'Homo technologicus

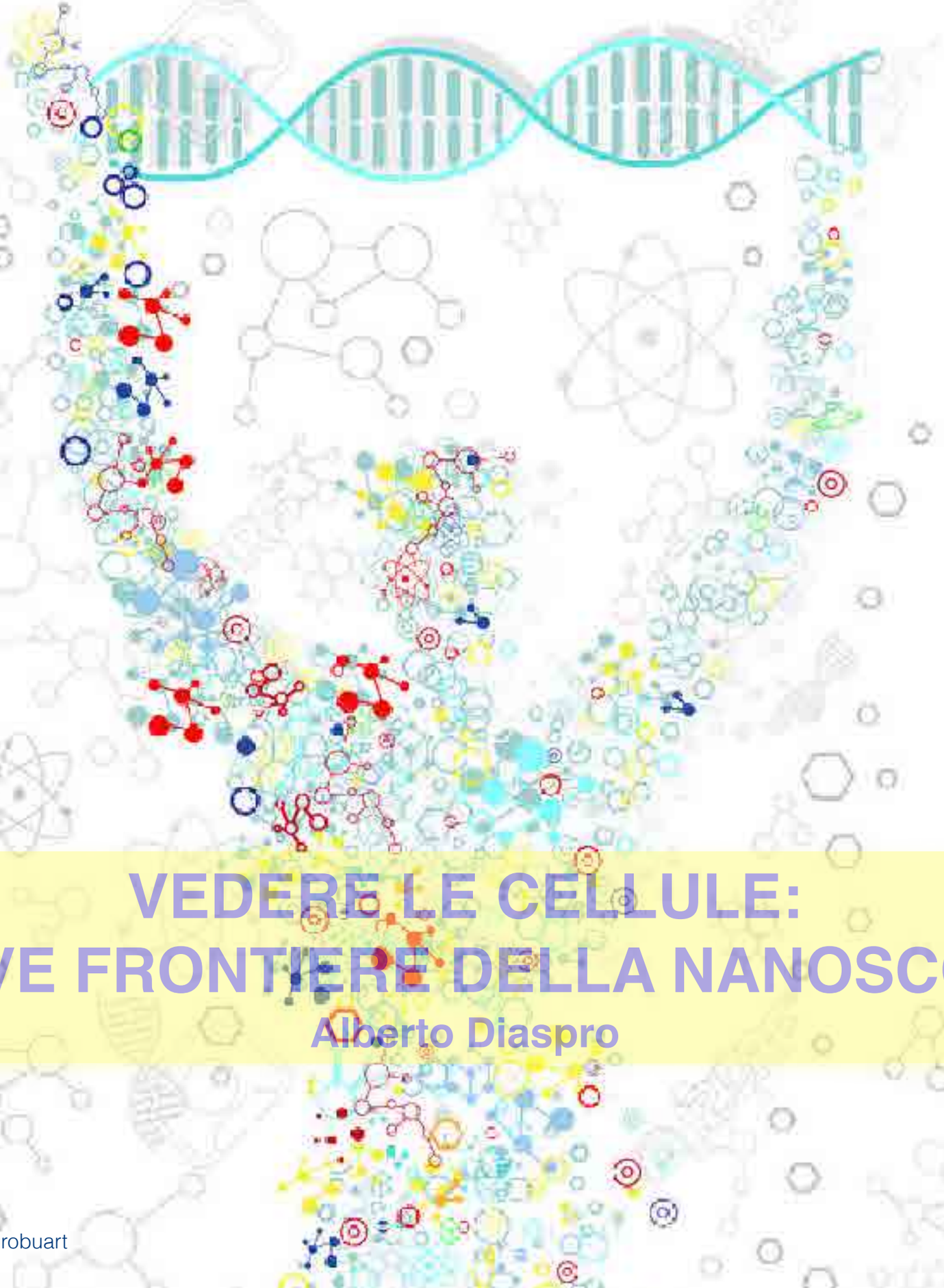
IL CIRCOLO DEI LETTORI

12 › 17 MARZO 2018

CIRCOLO DEI LETTORI

VIA BOGINO 9 - TORINO

IL CIRCOLO DEI LETTORI



VEDERE LE CELLULE: NUOVE FRONTIERE DELLA NANOSCOPIA

Alberto Diaspro

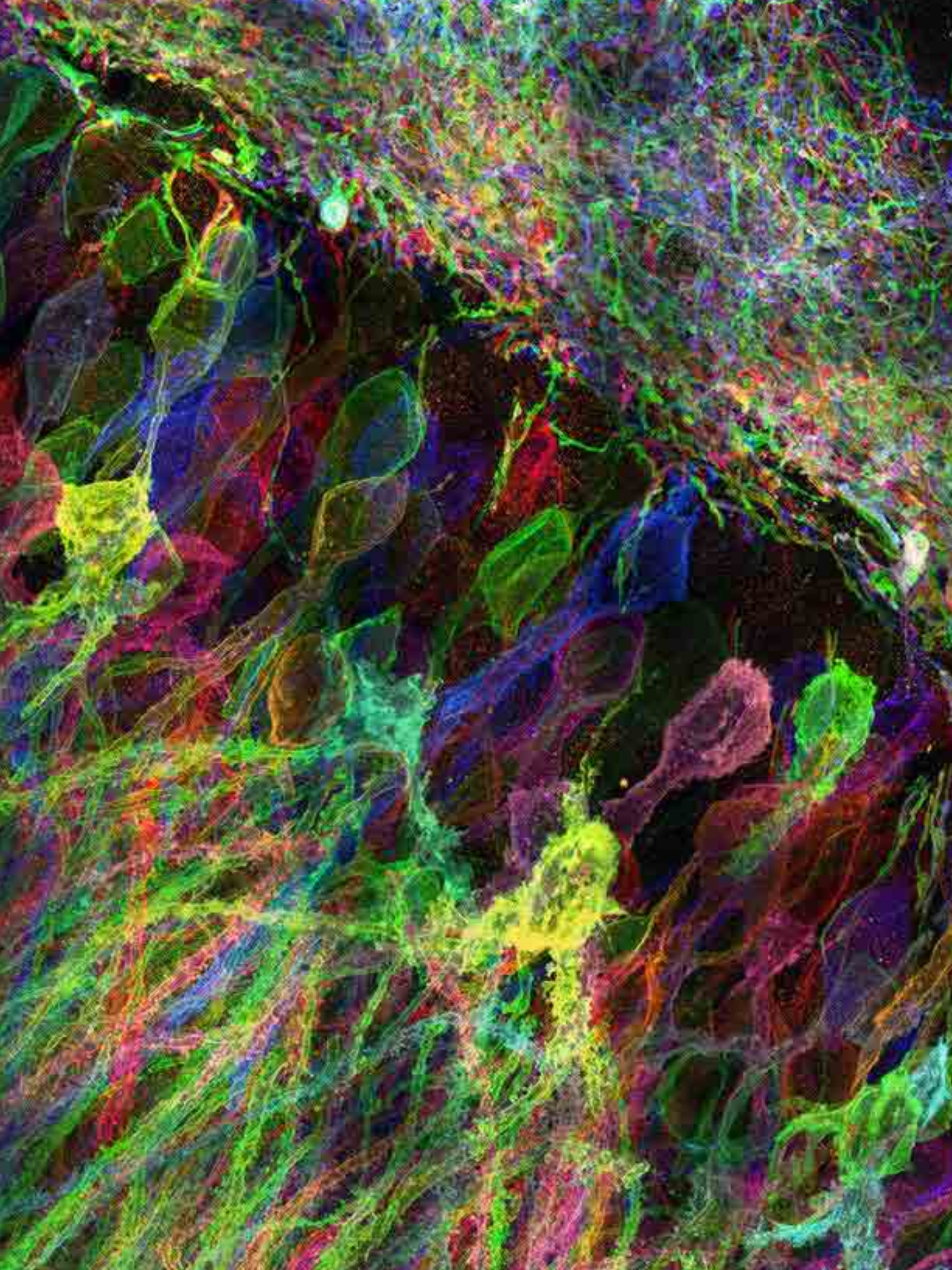
credit:robuart

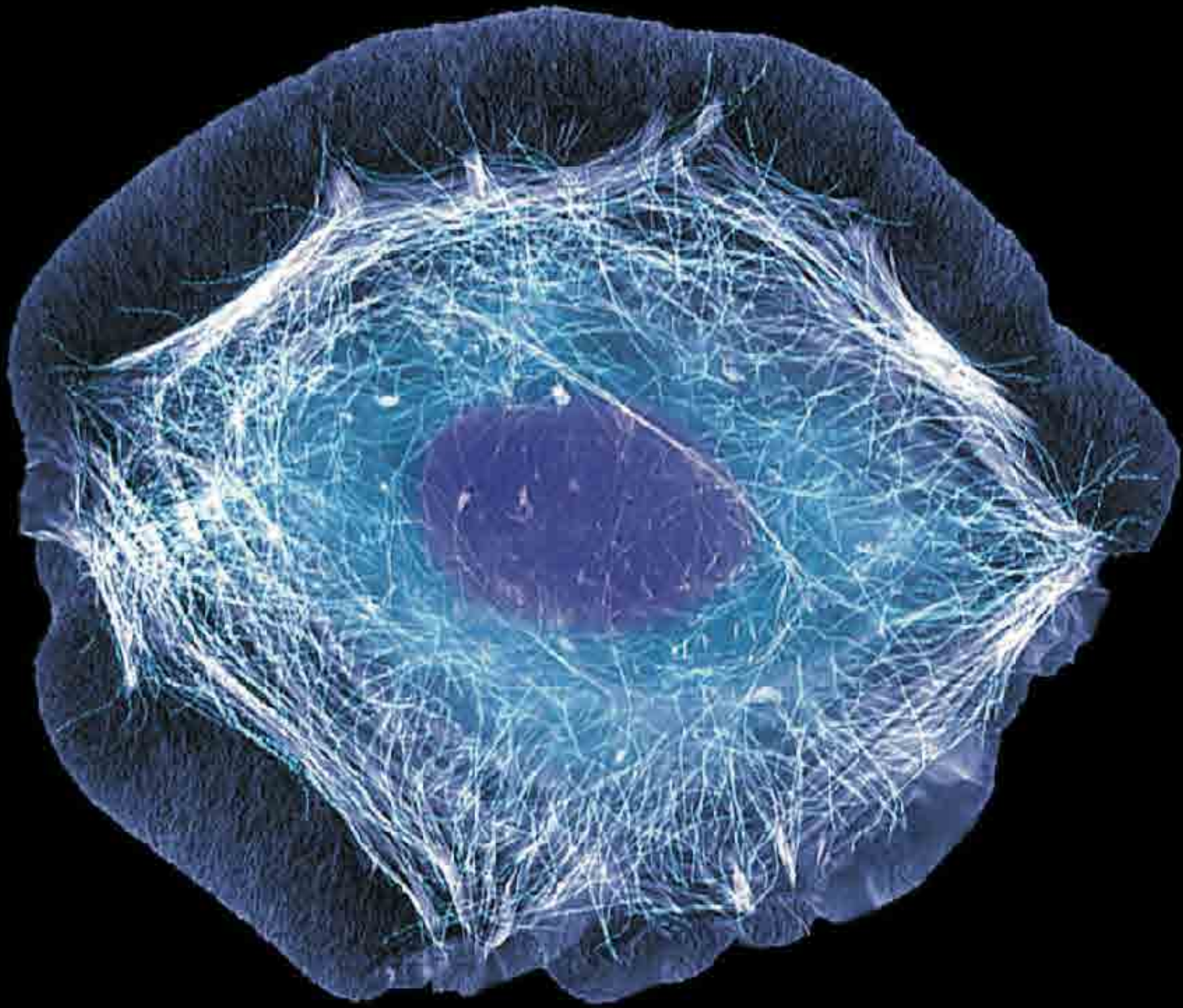
ISTITUTO ITALIANO DI TECNOLOGIA

OPTICAL NANOSCOPY

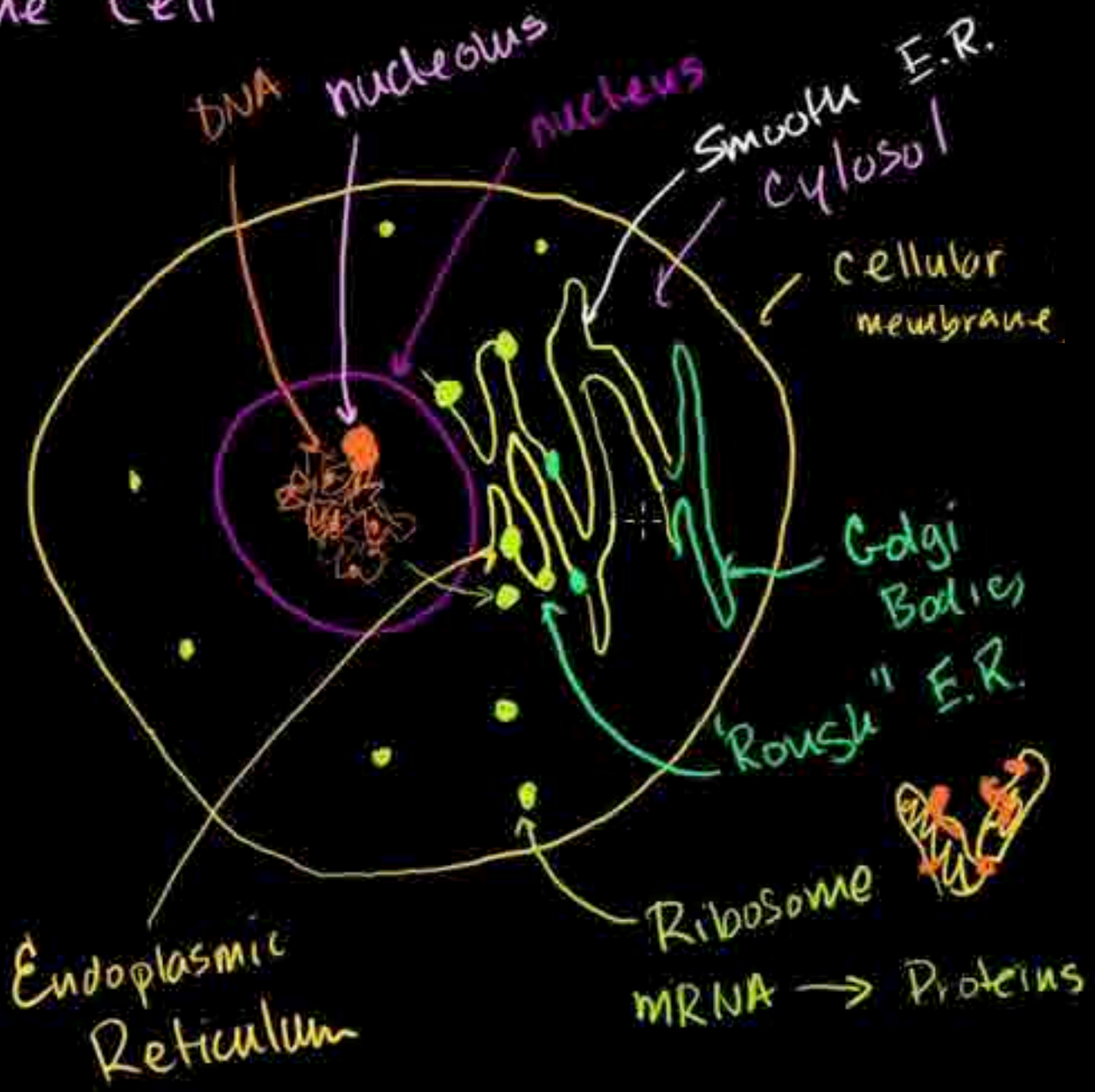
7 miliardi di persone… circa 76 organi
100000 miliardi di cellule

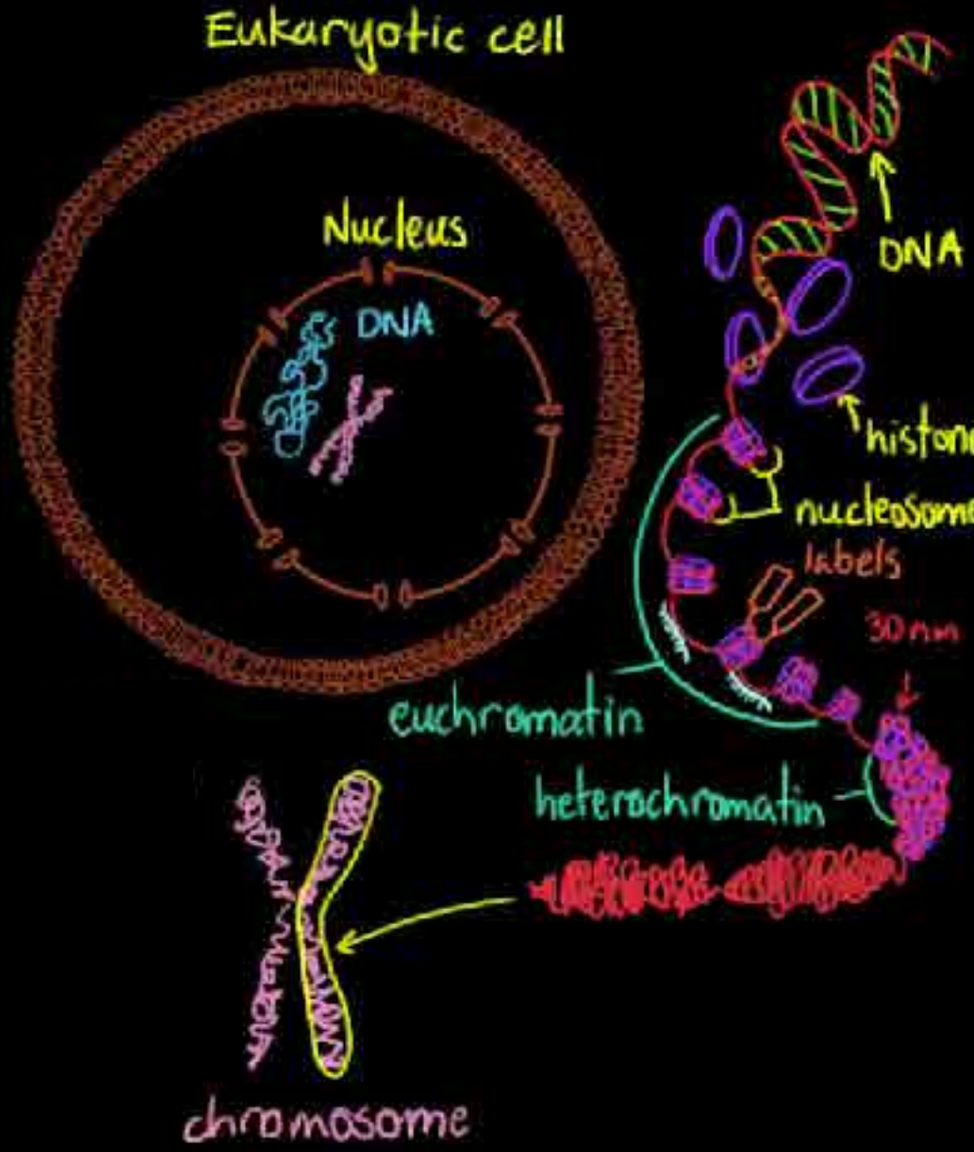

A–Z + 0–9 SOWIE " _ " SIND ZULÄSSIG F. NAMEN PROB/PR





The Cell


DNA
nucleolus
nucleus
Smooth E.R.
cytosol
cellular membrane
Golgi Bodies
"Rough" E.R.
Ribosome
mRNA → Proteins
Endoplasmic Reticulum


Eukaryotic cell
Nucleus
DNA
DNA
histone
nucleosome
labels
30 nm
euchromatin
heterochromatin
chromosome

La Biblioteca Nazionale di Firenze conserva circa 8 milioni di scritti, consultati centinaia di migliaia di volte ogni anno.

“You can observe a lot by watching.”

Yogi Berra

Nascita di Venere, Sandro Botticelli, 1482–1485, Uffizi, Firenze.

LIQUITOPY

Actor's Mask, Paul Klee, 1924.

LIQUITOPY

How to reveal the reality that is behind visible things?

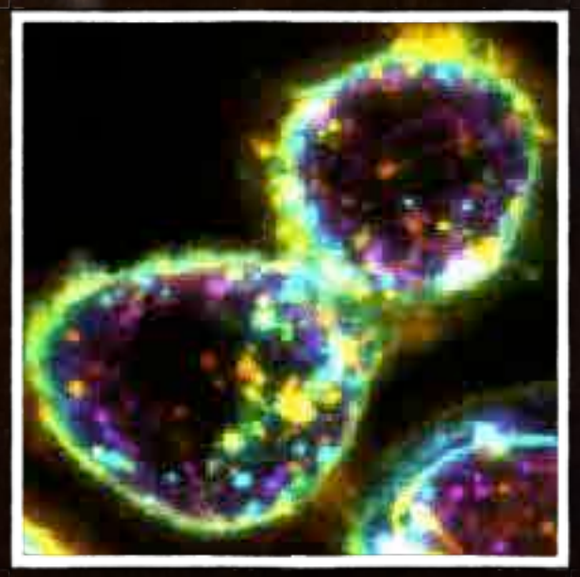
(Adapted after Paul Klee, 1879-1940)

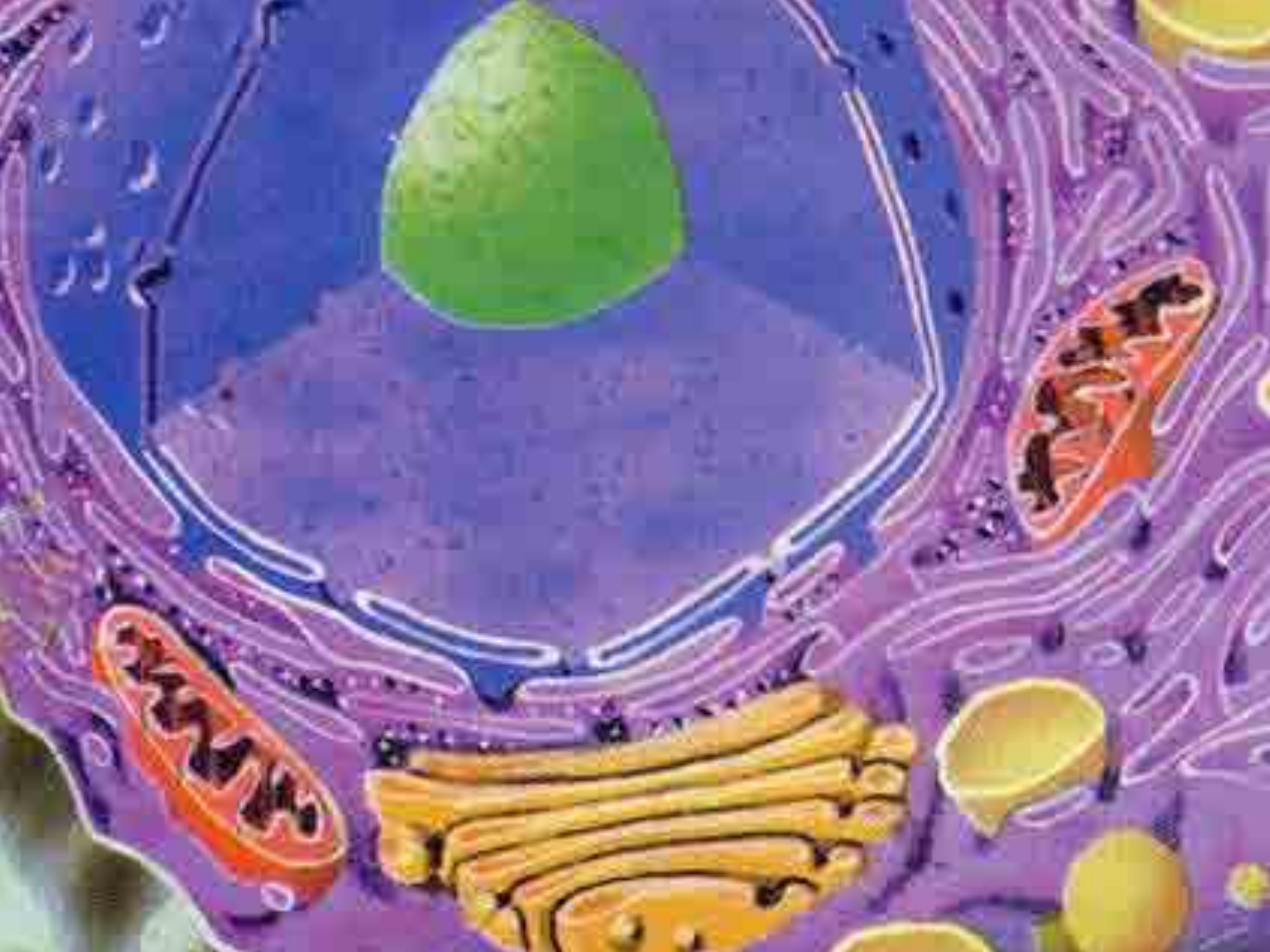
VIAGGIO AL CENTRO DELLA CELLULA

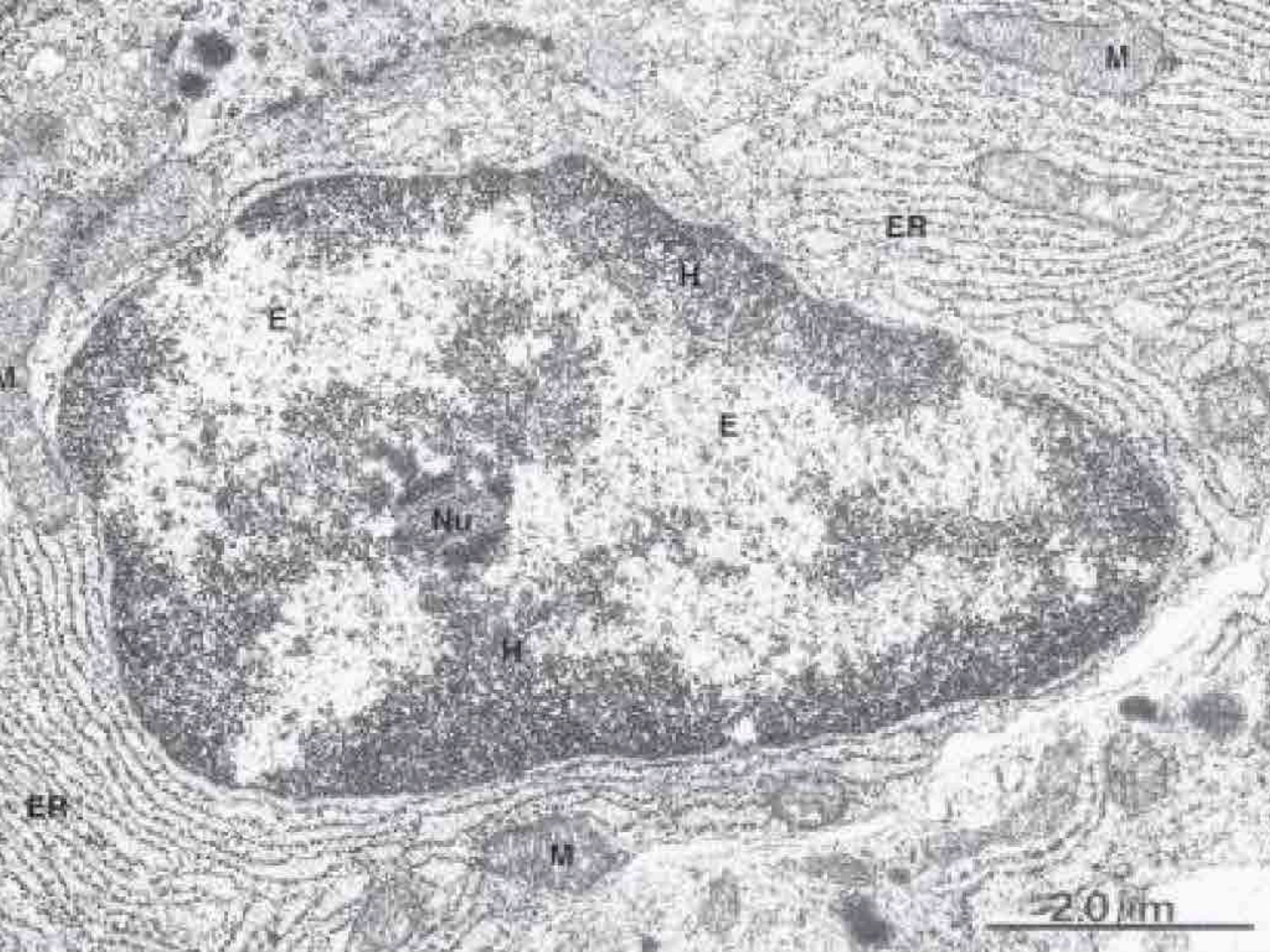
Testo di Federico Taddia –
Foto di Andrea Samaritani
e Pop Microscopy.

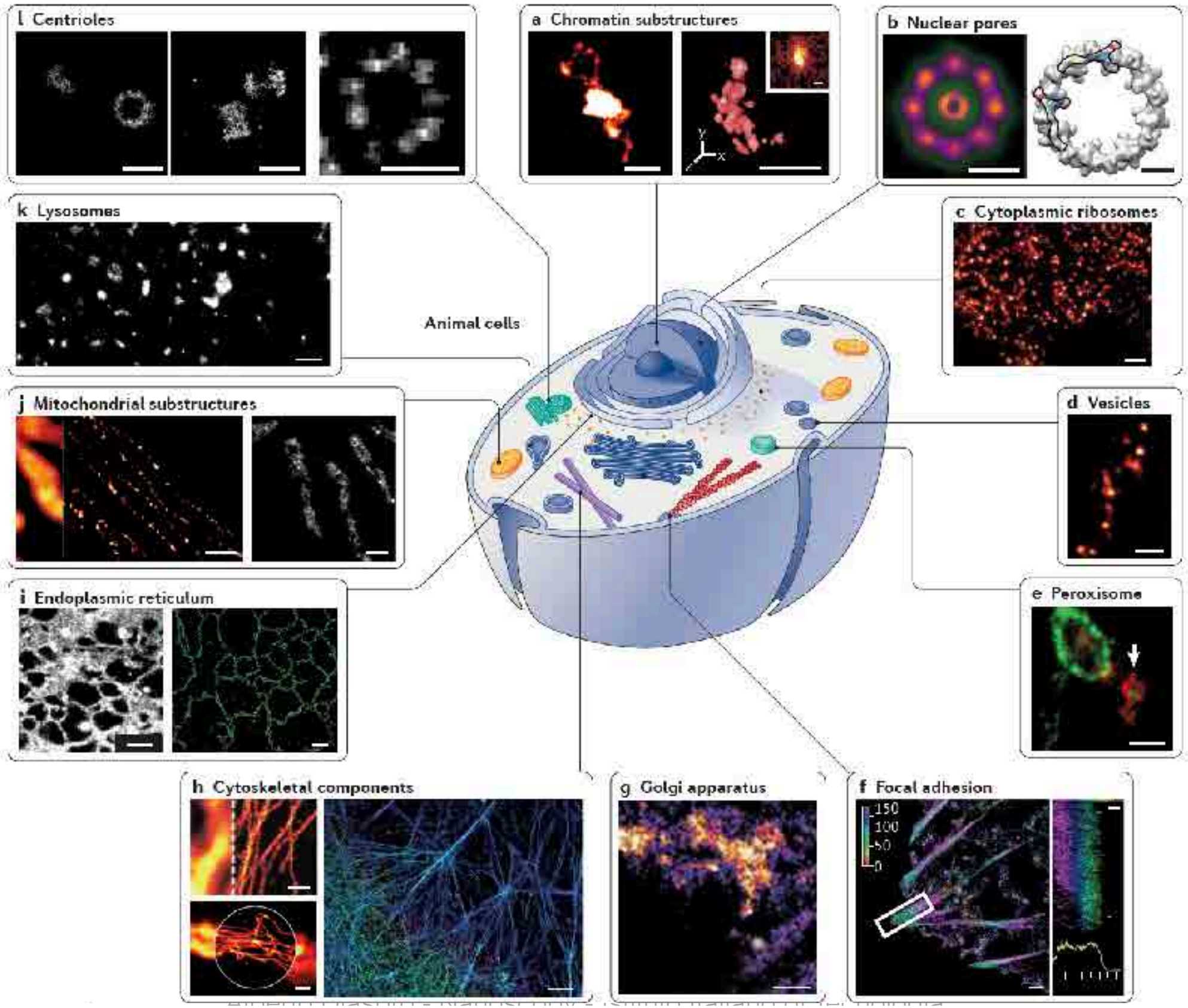
ENTRARE NELLE **CELLULE** CON DEI **FASCI DI LUCE** PER VEDERE QUELLO CHE SUCCEDE. FANTASCIENZA? PER NULLA! ACCADE TUTTI I GIORNI **ALL'ISTITUTO ITALIANO DI TECNOLOGIA DI GENOVA**, DOVE SI TROVANO ALCUNI DEI **MICROSCOPI OTTICI** PIÙ AVANZATI AL MONDO. IL RISULTATO: TANTA RICERCA, UN SACCO DI INNOVAZIONE E **NANOFOTOGRAFIE** CHE SONO VERE OPERE D'ARTE!

Light microscopy turns invisible into visible

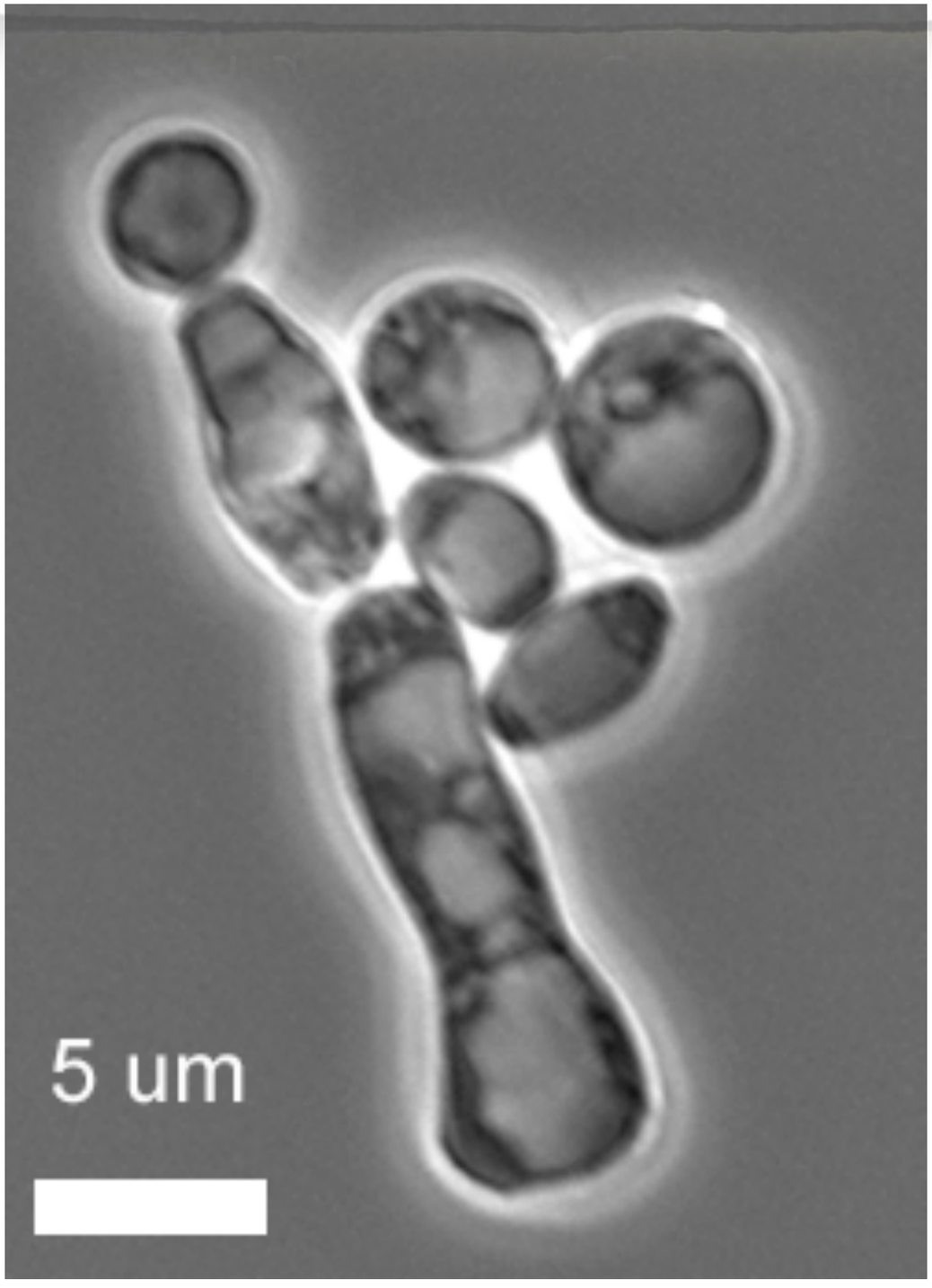





M
ER
H
E
M
E
Nu
H
ER
M
2.0 µm


l Centrioles
a Chromatin substructures
b Nuclear pores
k Lysosomes
Animal cells
c Cytoplasmic ribosomes
j Mitochondrial substructures
d Vesicles
i Endoplasmic reticulum
e Peroxisome
h Cytoskeletal components
g Golgi apparatus
f Focal adhesion
150
100
50
0

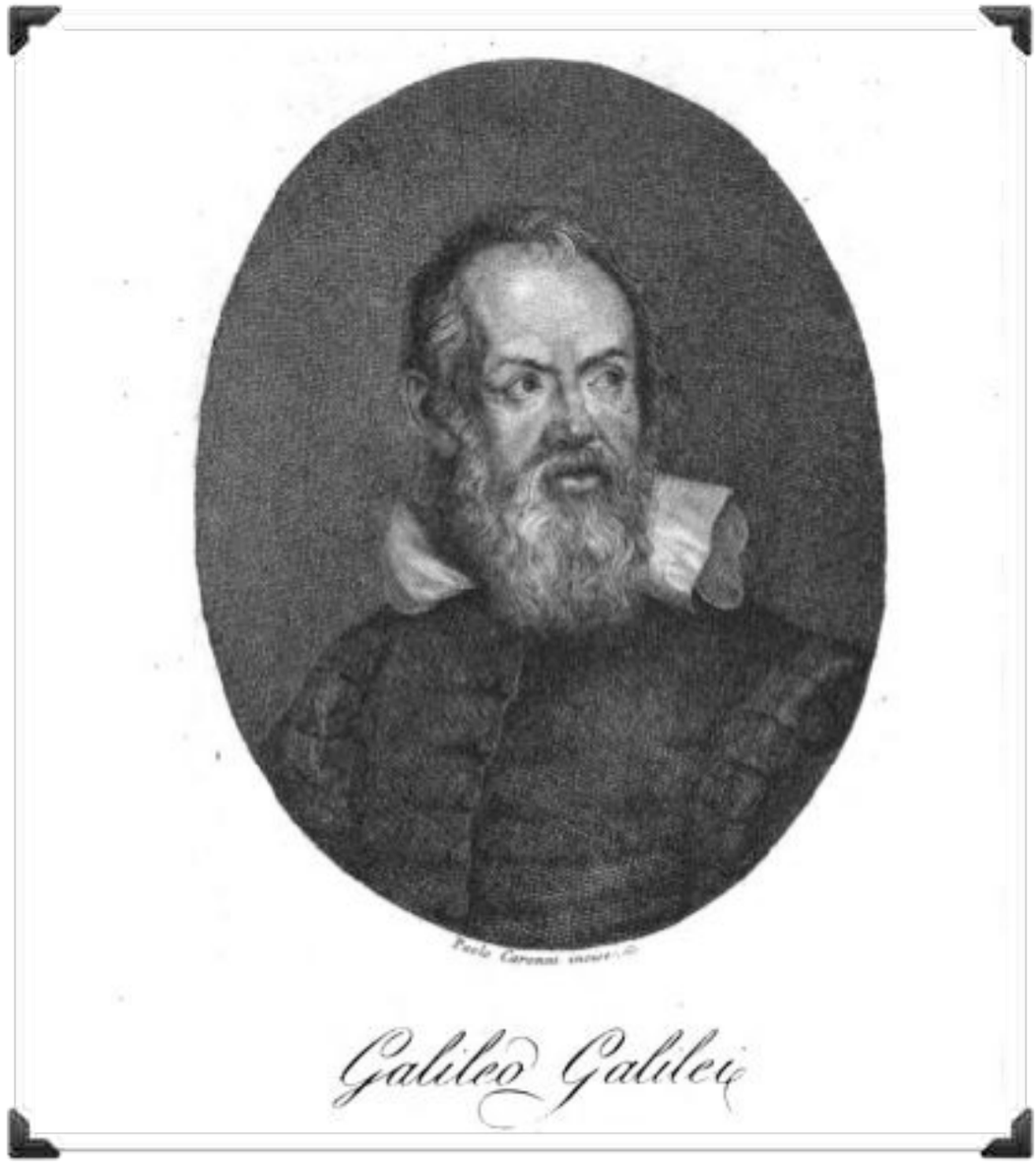
Light microscopy turning invisible into visible



MICROSCOPIUM **EXTRAORDINARIUM** NOMINARE LIBUIT…

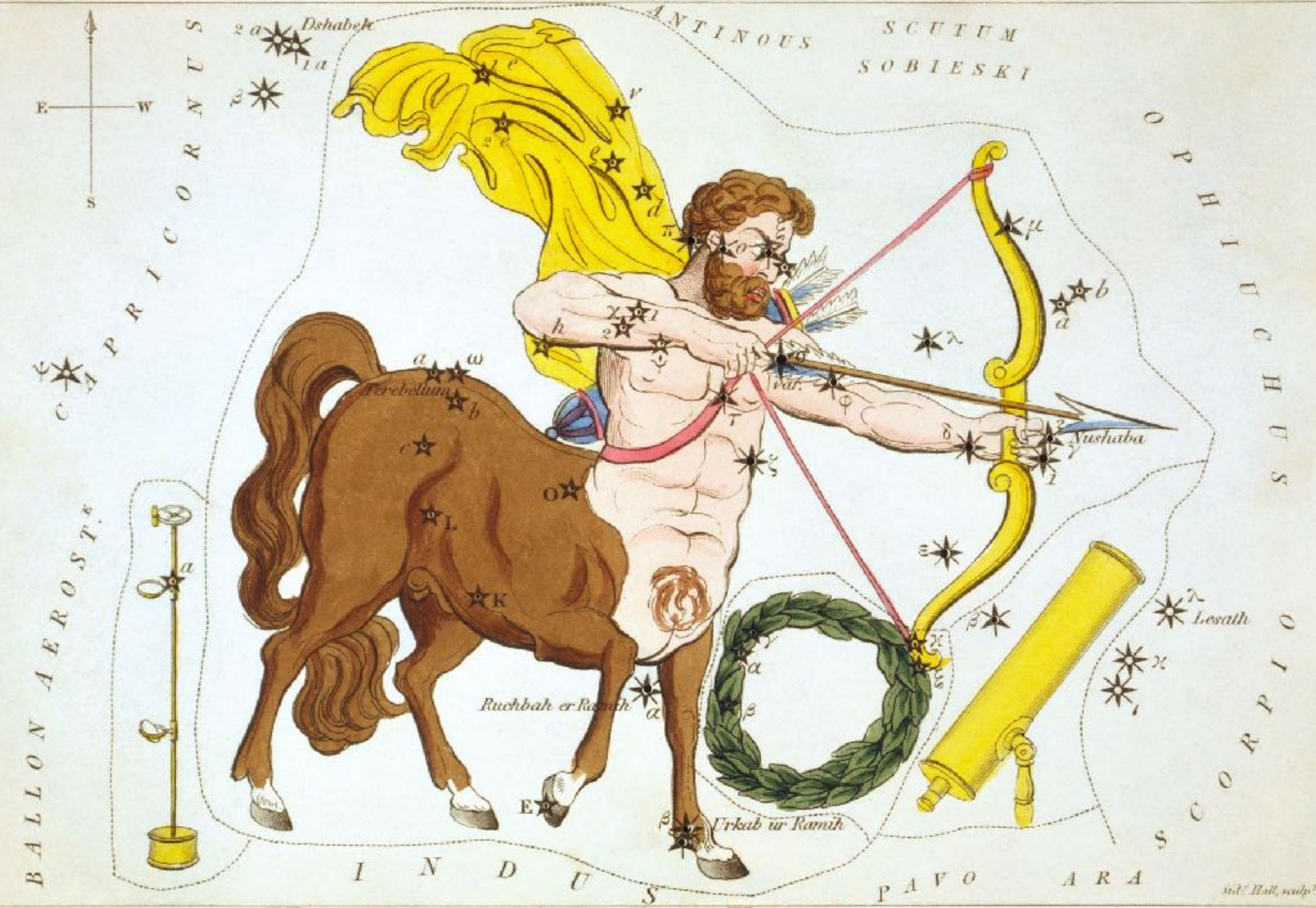
...microscopium nominare libuit...Faber, Lincei, 1625

Since the early years of the Galileo Galilei's "Occhialino" - "*un occhialino per veder da vicino le cose minime*" - brillantly renamed, on April 13th 1625 in a letter written to Federico Cesi, as "Microscopium" by Johannes Faber (1574-1629), it was evident the potential of such an optical tool.

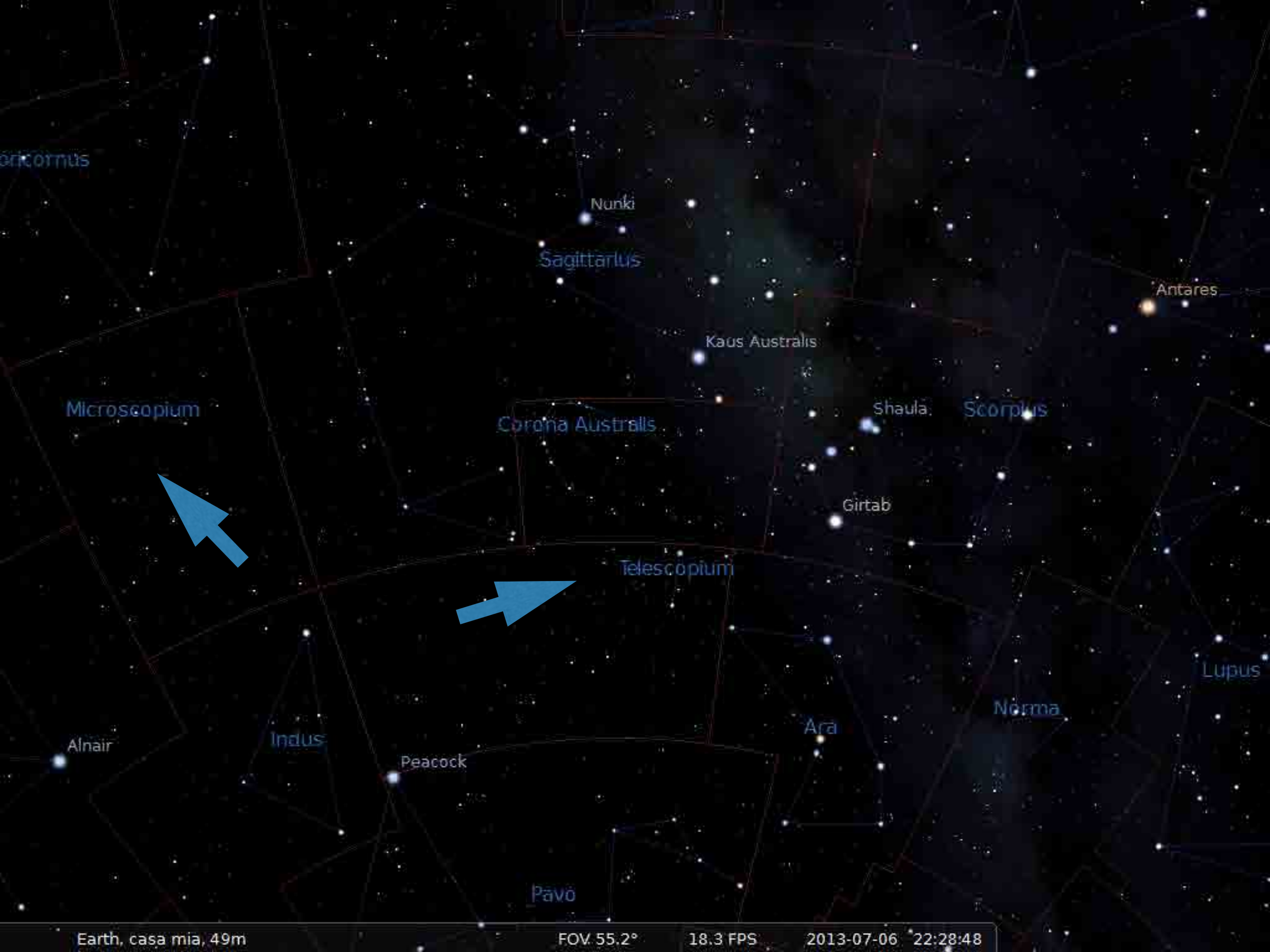


Ottavio Leoni, portrait of Galileo Galilei, 1624

SAGITTARIUS AND CORONA AUSTRALIS.



MICROSCOPIUM. AND TELESCOPIUM.


Nunki
Sagittarius
Antares
Kaus Australis
Microscopium
Corona Australis
Shaula
Scorpius
Girtab
Telescopium
Lupus
Norma
Ara
Alnair
Indus
Peacock
Pavo
Earth, casa mia, 49m
FOV 55.2°
18.3 FPS
2013-07-06
22:28:48

MICROGRAPHIA:

OR SOME

Physiological Descriptions

OF

MINUTE BODIES

MADE BY

MAGNIFYING GLASSES

WITH

OBSERVATIONS and INQUIRIES thereupon.

By *R. HOOKE*, Fellow of the ROYAL SOCIETY.

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

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

Fig: 1.

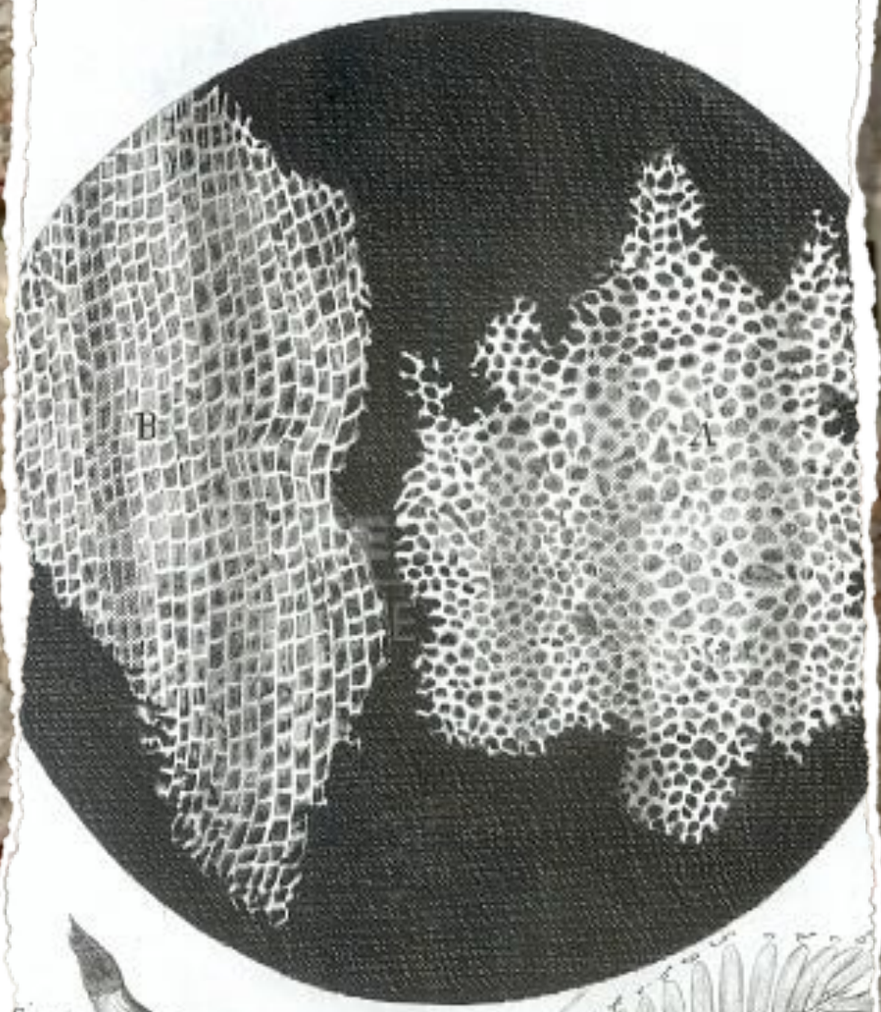


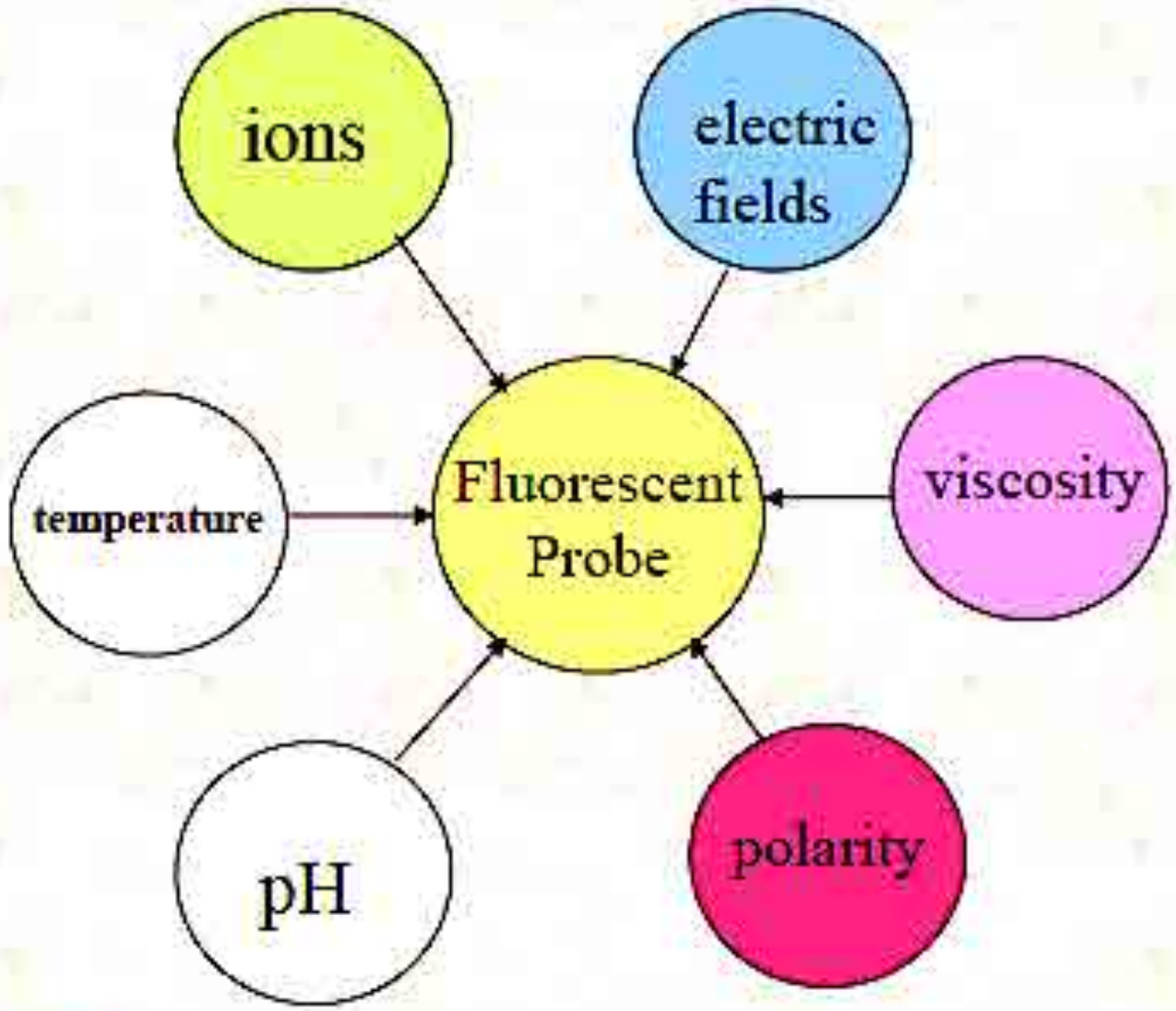
Fig: 2.

Fluorescence Microscopy

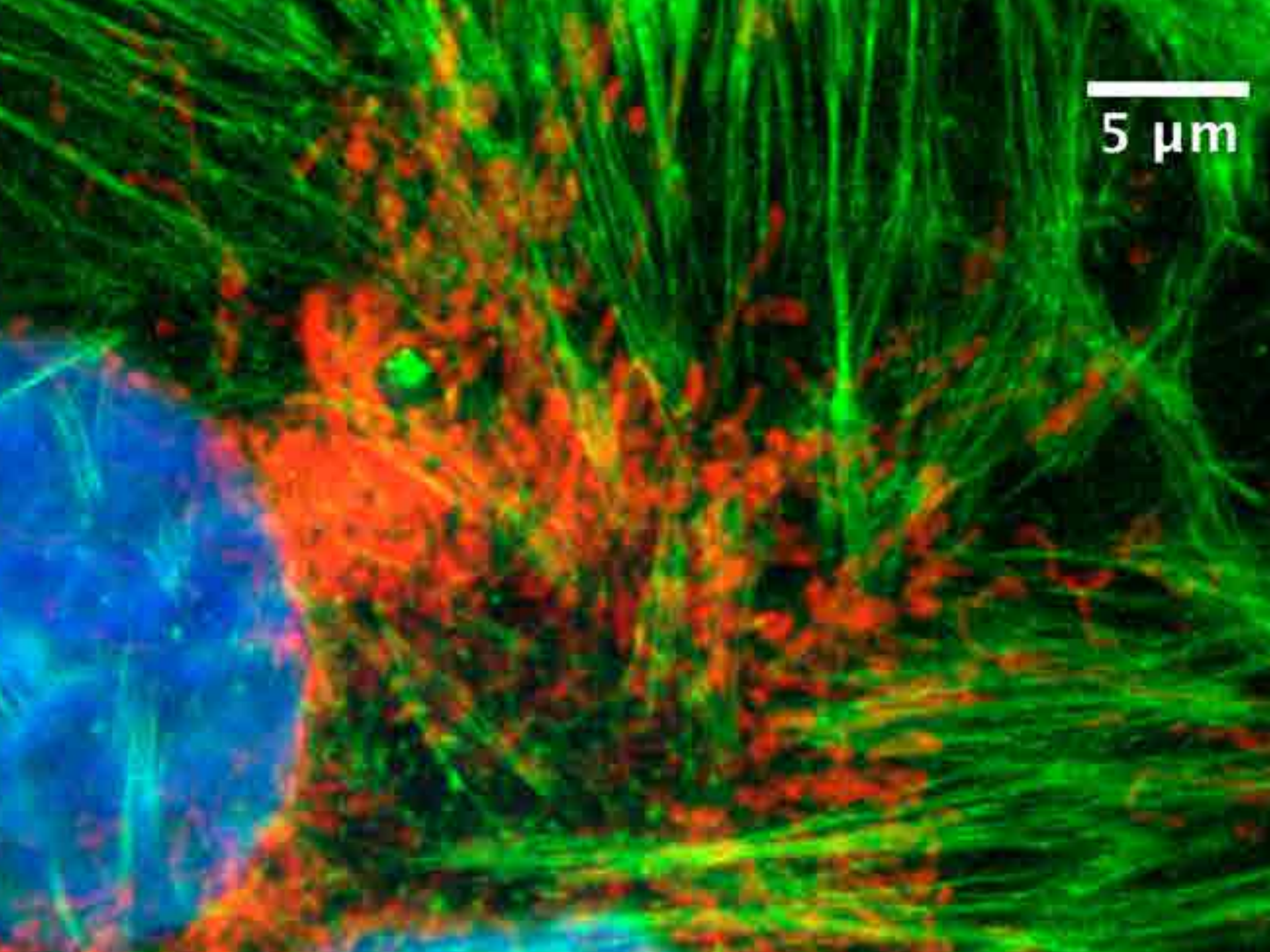
Why fluorescence?

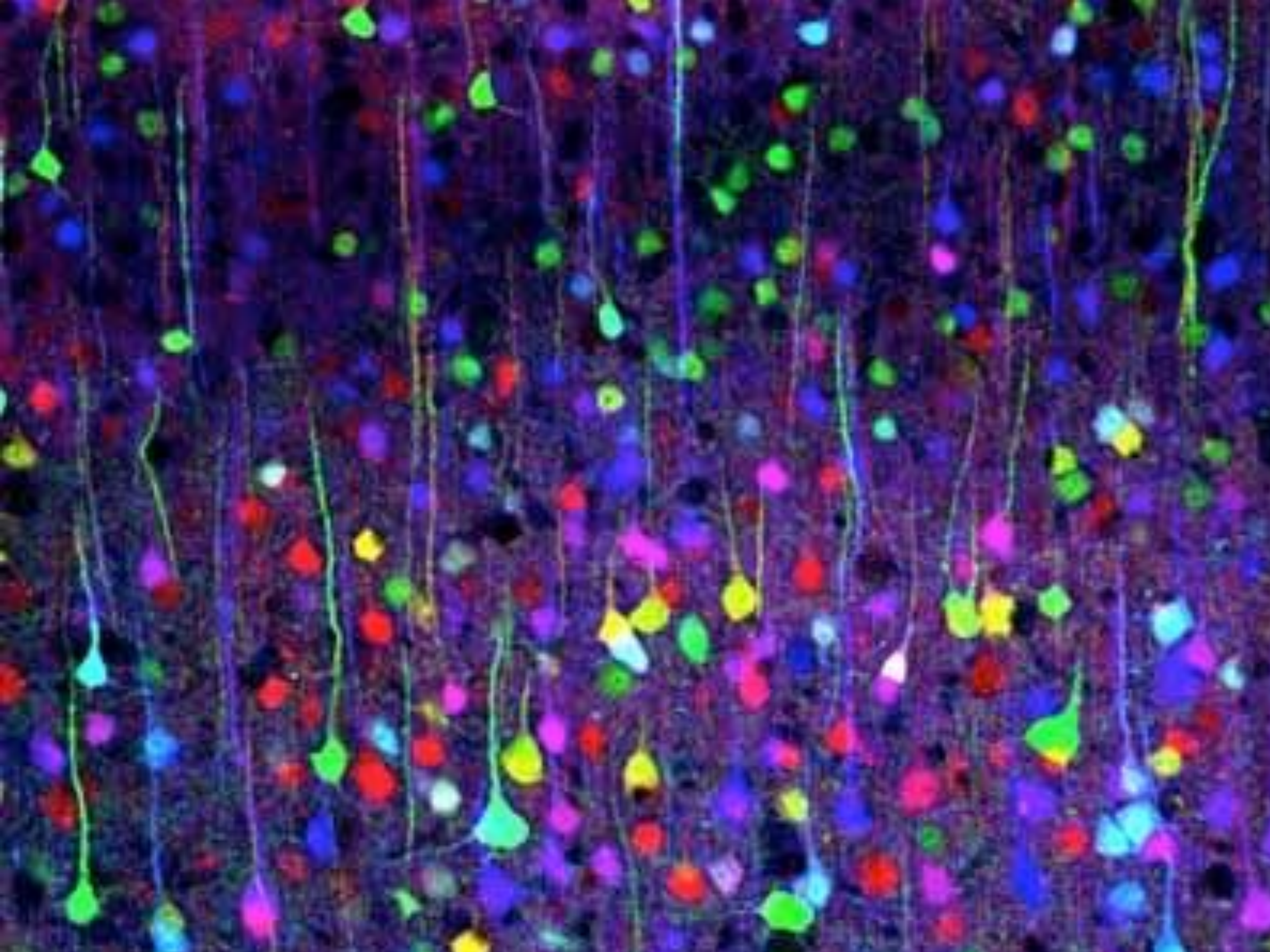
- its pretty!
- it provides information on the molecular environment
- it provides information on dynamic processes on the nanosecond timescale

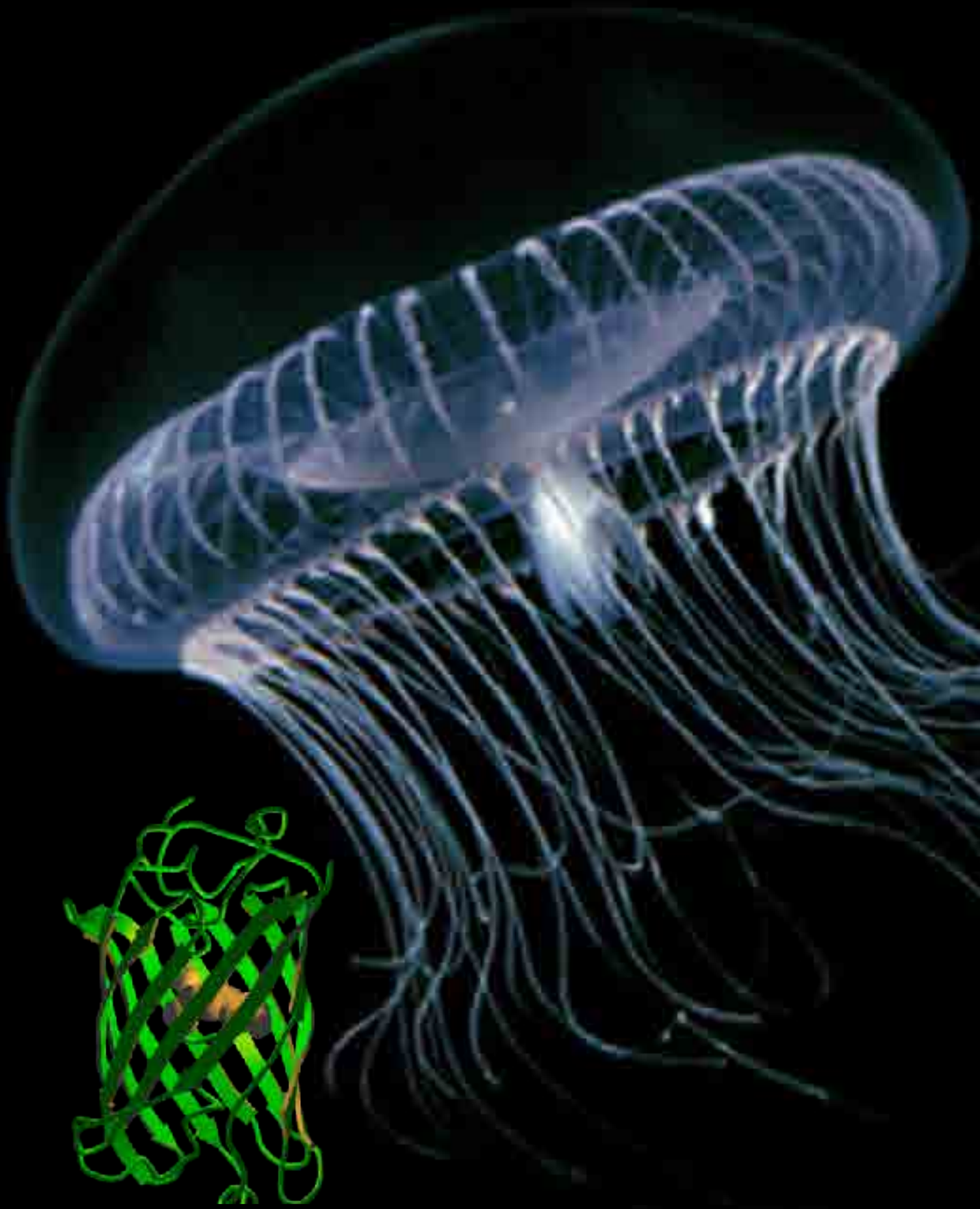
Credit:David Jameson

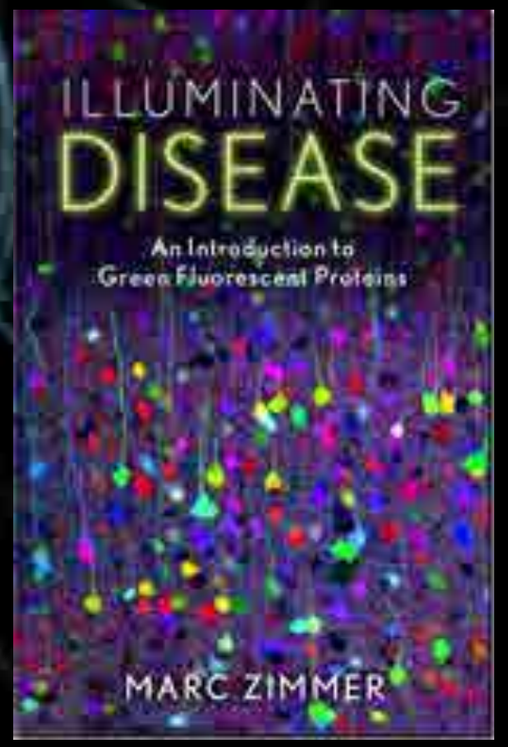


Fluorescence Probes are essentially molecular stopwatches which monitor *dynamic* events which occur during the excited state lifetime – such as movements of proteins or protein domains


5 µm







The Nobel Prize in Chemistry 2008

"for the discovery and development of the green fluorescent protein, GFP"

Photo: J. Henriksson/SCANPIX

Martin Chalfie

1/3 of the prize

USA

Columbia University
New York, NY, USA

b. 1947

Photo: UCSD

Roger Y. Tsien

1/3 of the prize

USA

University of California
San Diego, CA, USA

b. 1952

Photo: J. Henriksson/SCANPIX

Osamu Shimomura

1/3 of the prize

USA

Marine Biological
Laboratory (MBL)
Woods Hole, MA, USA

b. 1928

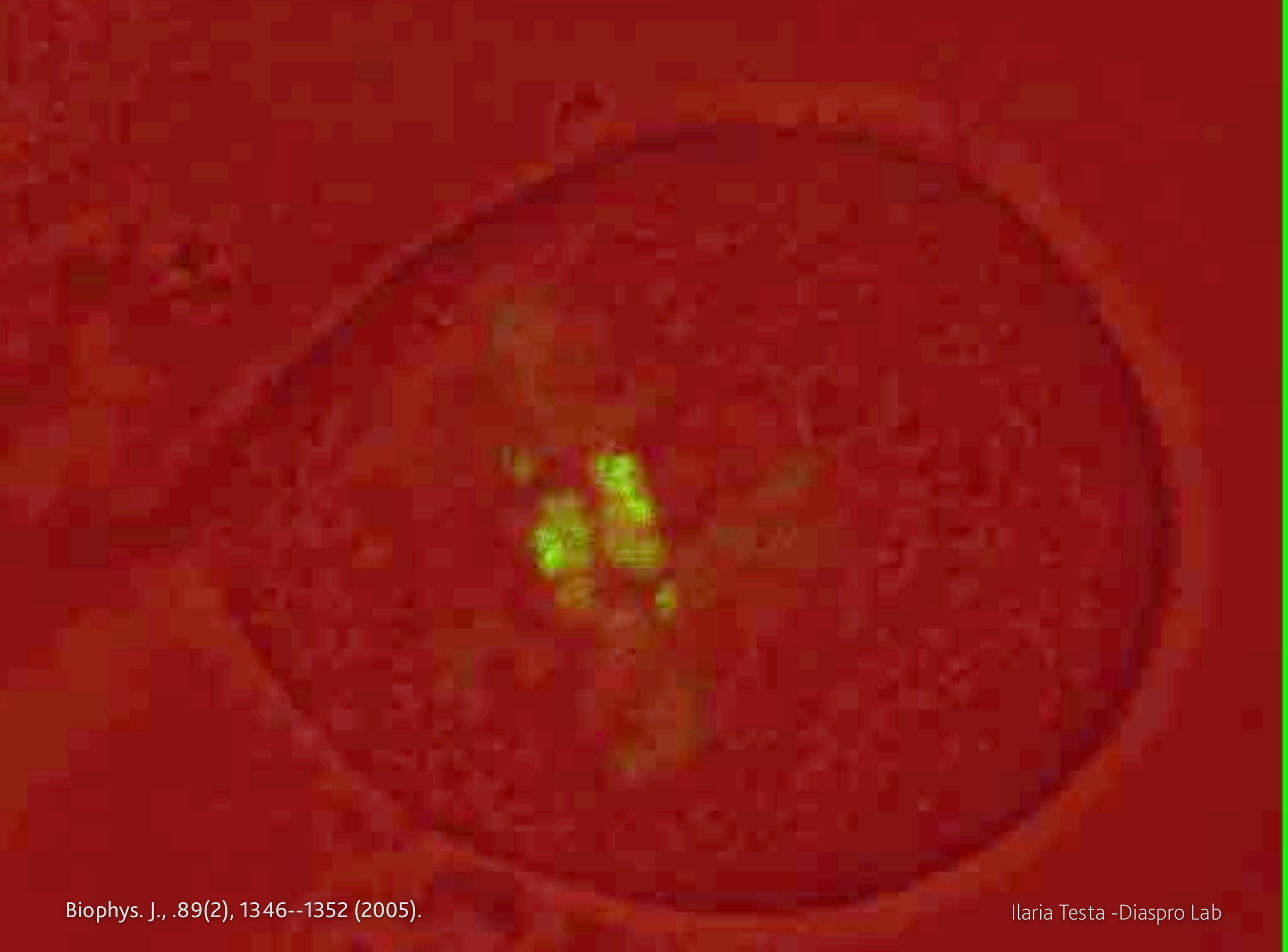
Credit: M Chalfie - Nobel Laureate 2008

C. Elegans PH-GFP H2B-mCherry

4D

Credit: E. Betzig - Nobel Laureate 2008

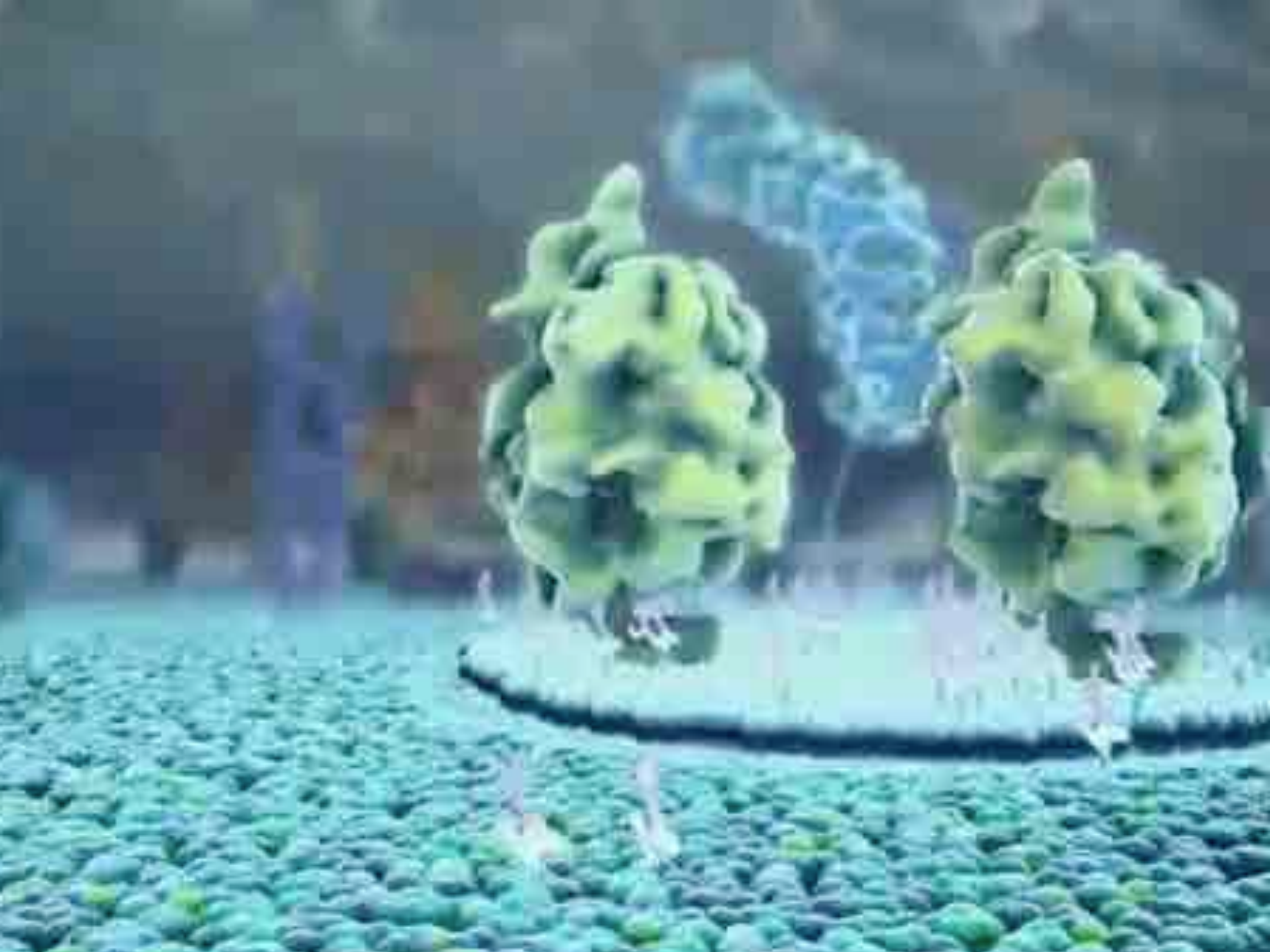
Credit: Eric Betzig - Janelia Farm (2015)

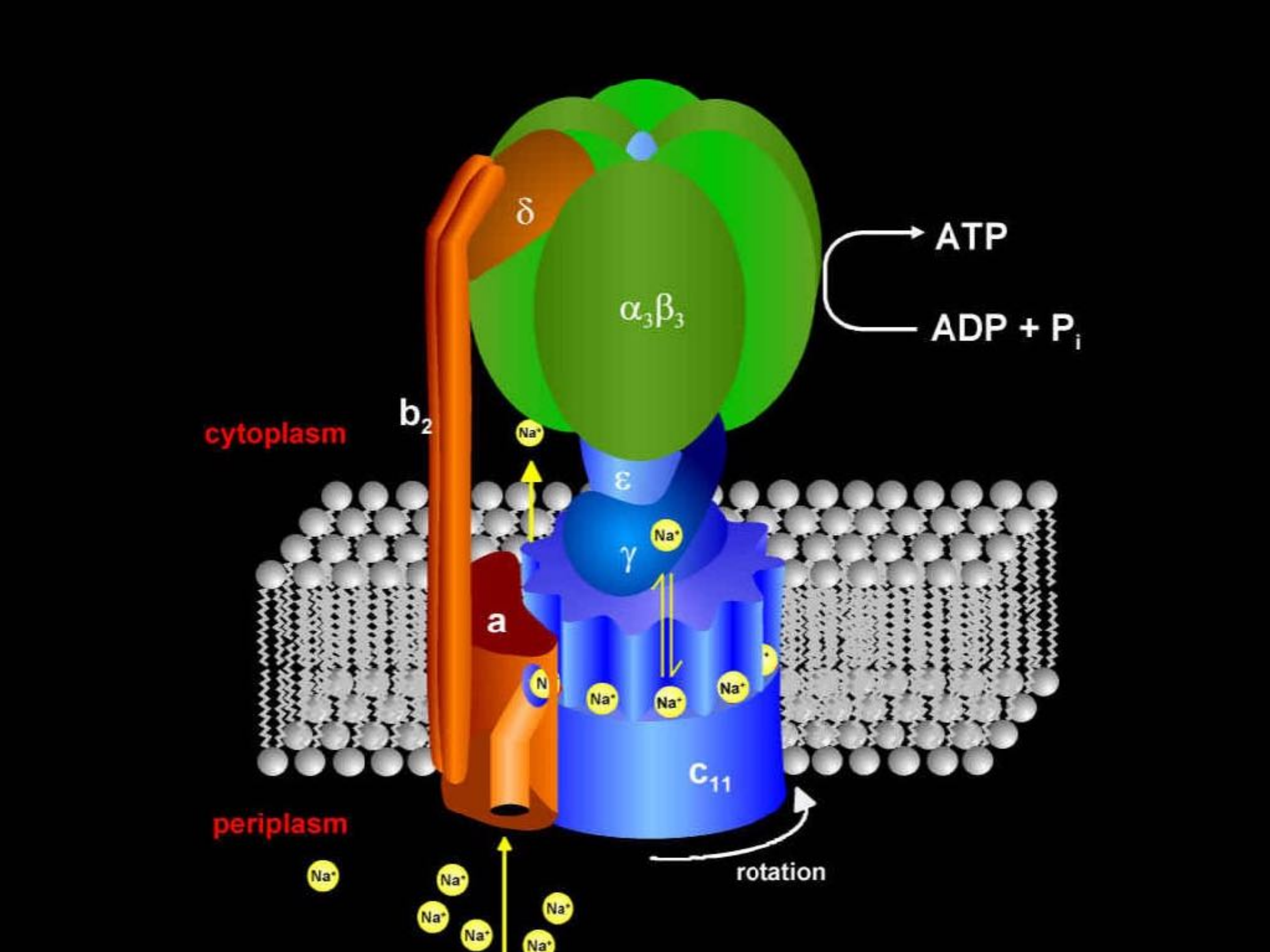


Biophys. J., .89(2), 1346--1352 (2005).

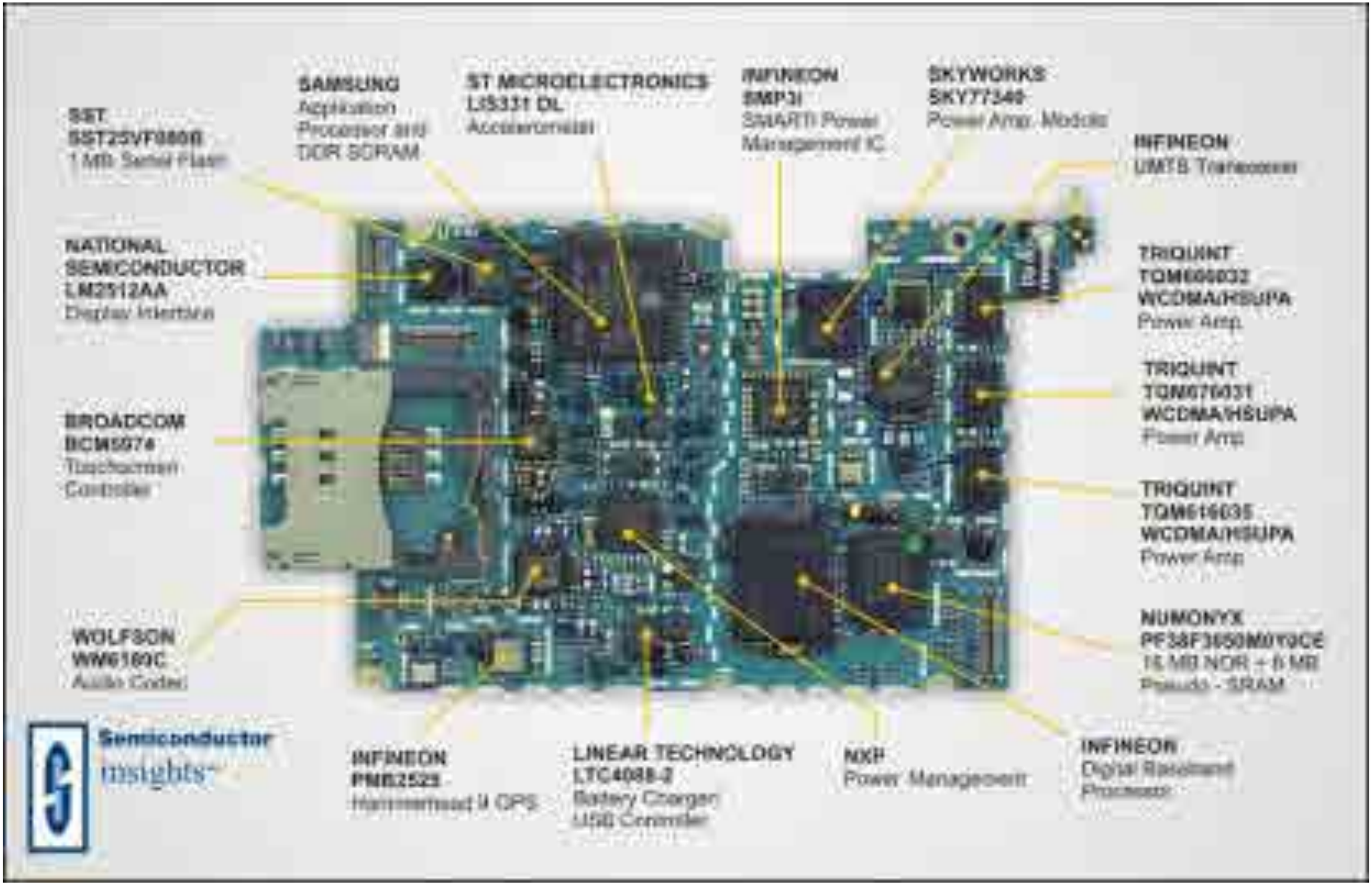
Ilaria Testa -Diaspro Lab

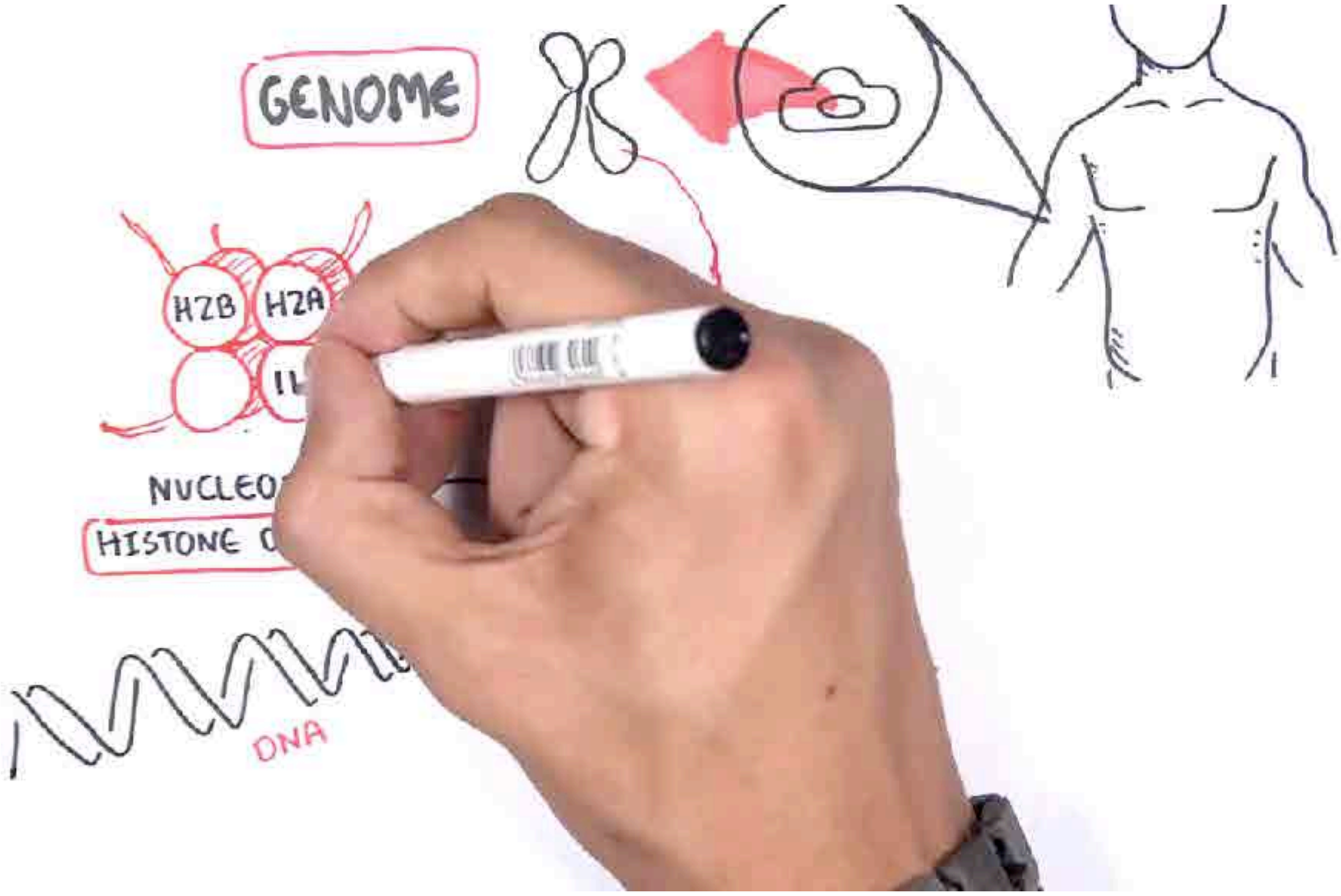
Xiaowei Zhuang, Harvard

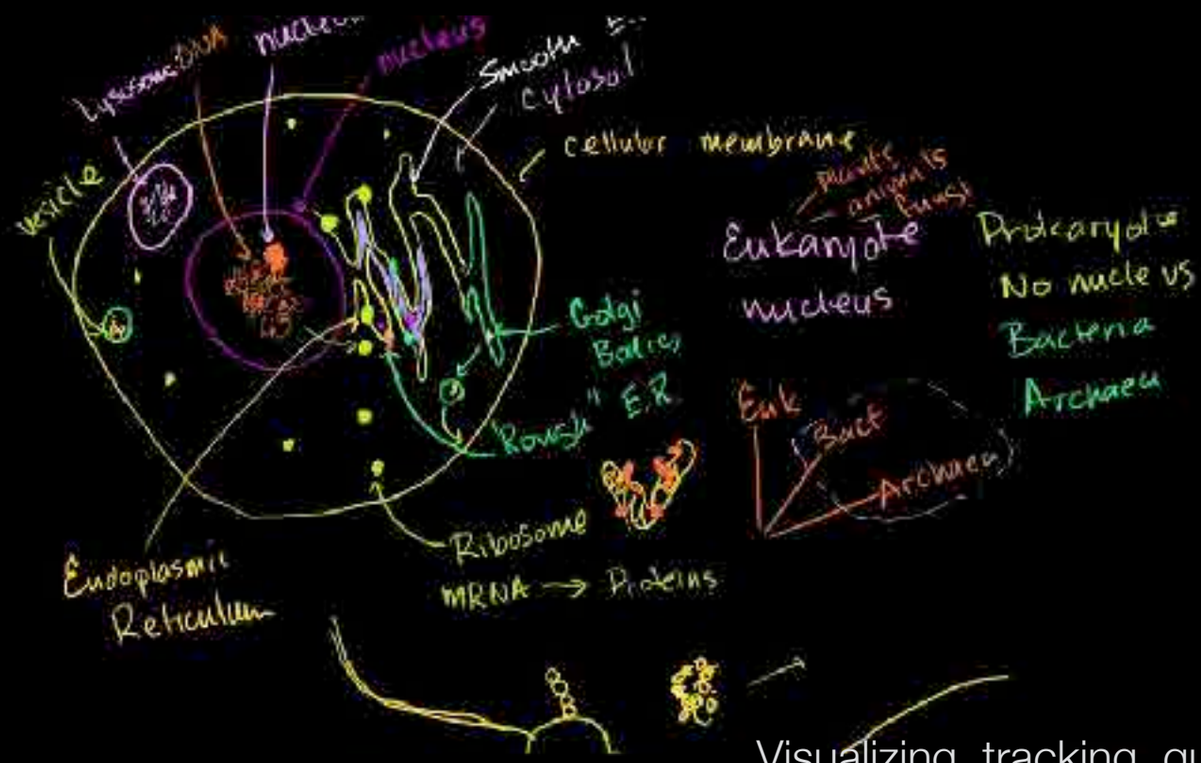



δ
$\alpha_3\beta_3$
ATP
ADP + P_i
b_2
cytoplasm
Na+
ε
γ
a
c_{11}
periplasm
rotation

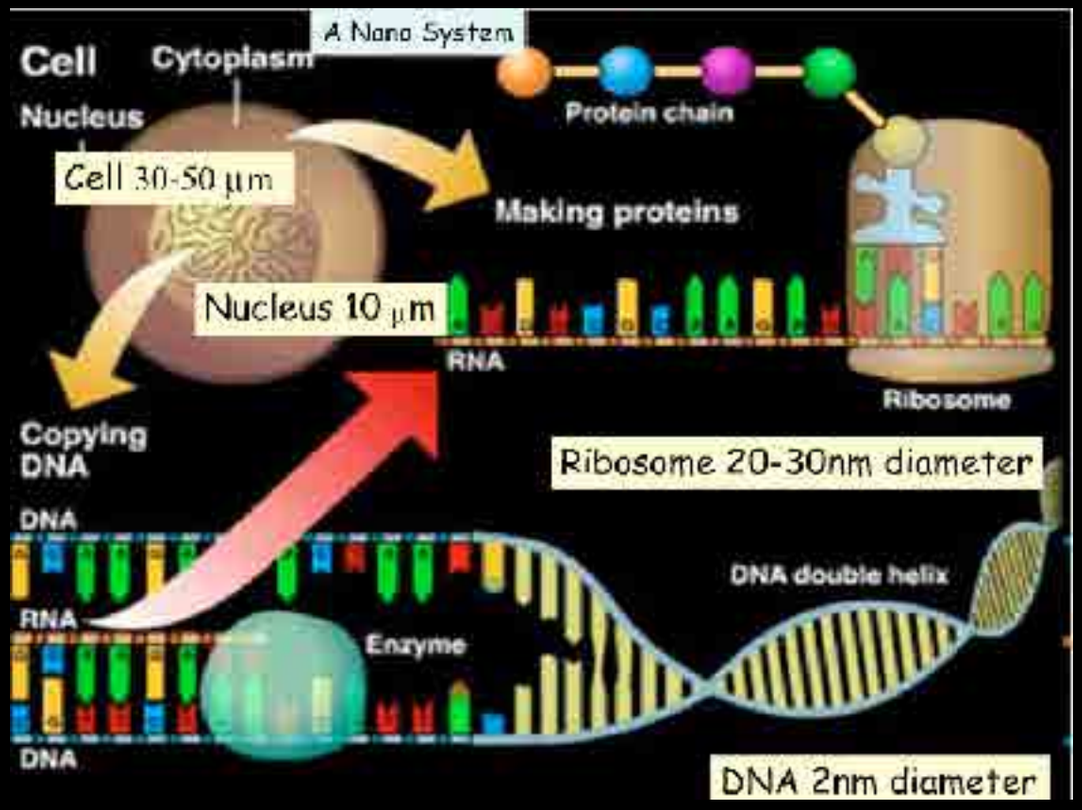
Chromatin


SST
SST25VF080B
1 Mb Serial Flash
SAMSUNG
Application
Processor and
DDR SDRAM
ST MICROELECTRONICS
LIS331 DL
Accelerometer
INFINEON
SMP3i
SMART Power
Management IC
SKYWORKS
SKY77340
Power Amp. Module
INFINEON
UMTS Transceiver
NATIONAL
SEMICONDUCTOR
LM2512AA
Display Interface
TRIQUINT
TQM666032
WCDMA/HSUPA
Power Amp.
TRIQUINT
TQM676031
WCDMA/HSUPA
Power Amp.
BROADCOM
BCM5974
Touchscreen
Controller
TRIQUINT
TQM616035
WCDMA/HSUPA
Power Amp.
WOLFSON
WM6180C
Audio Codec
NUMONYX
PF38F3650M0Y0CE
16 MB NOR + 8 MB
Pseudo - SRAM
Semiconductor
insights
INFINEON
PMB2523
Hammerhead II GPS
LINEAR TECHNOLOGY
LTC4088-2
Battery Charger
USB Controller
NXP
Power Management
INFINEON
Digital Baseband
Processor

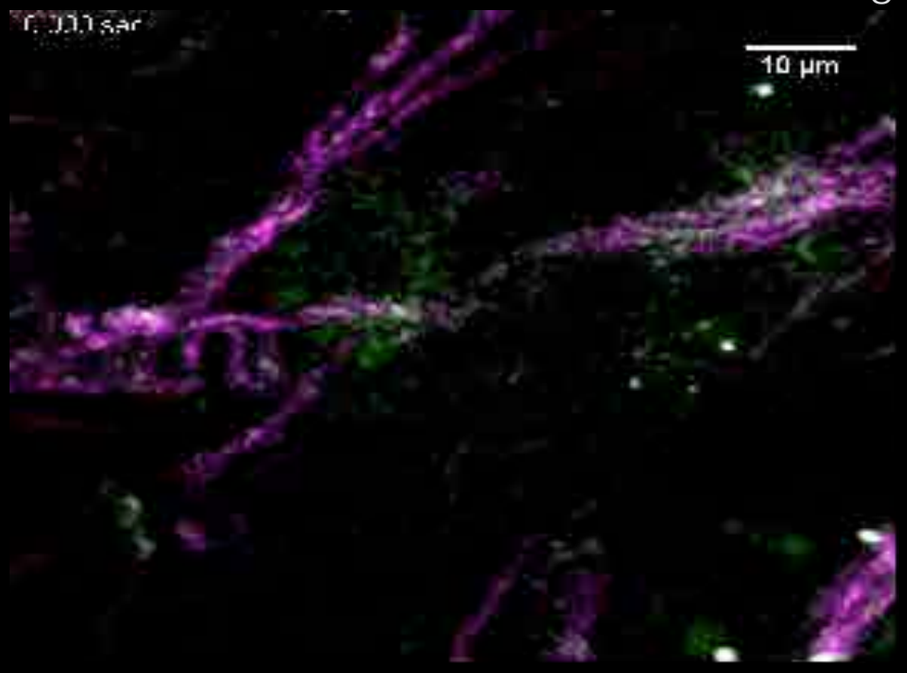

GENOME
H2B
H2A
DNA

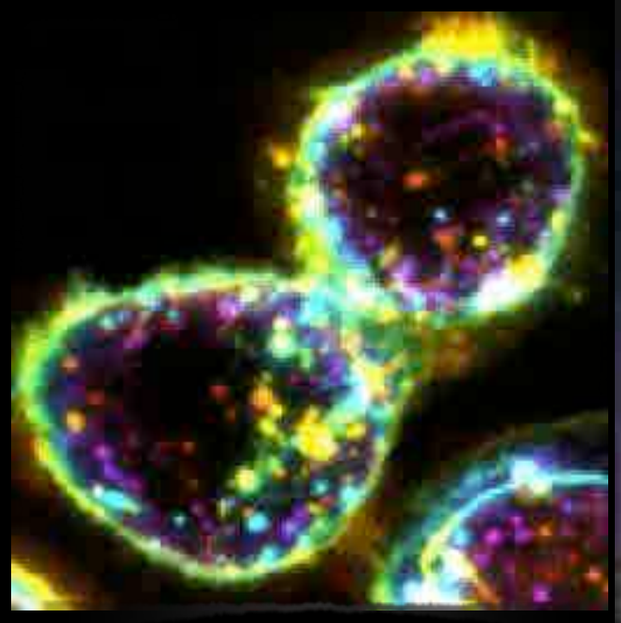


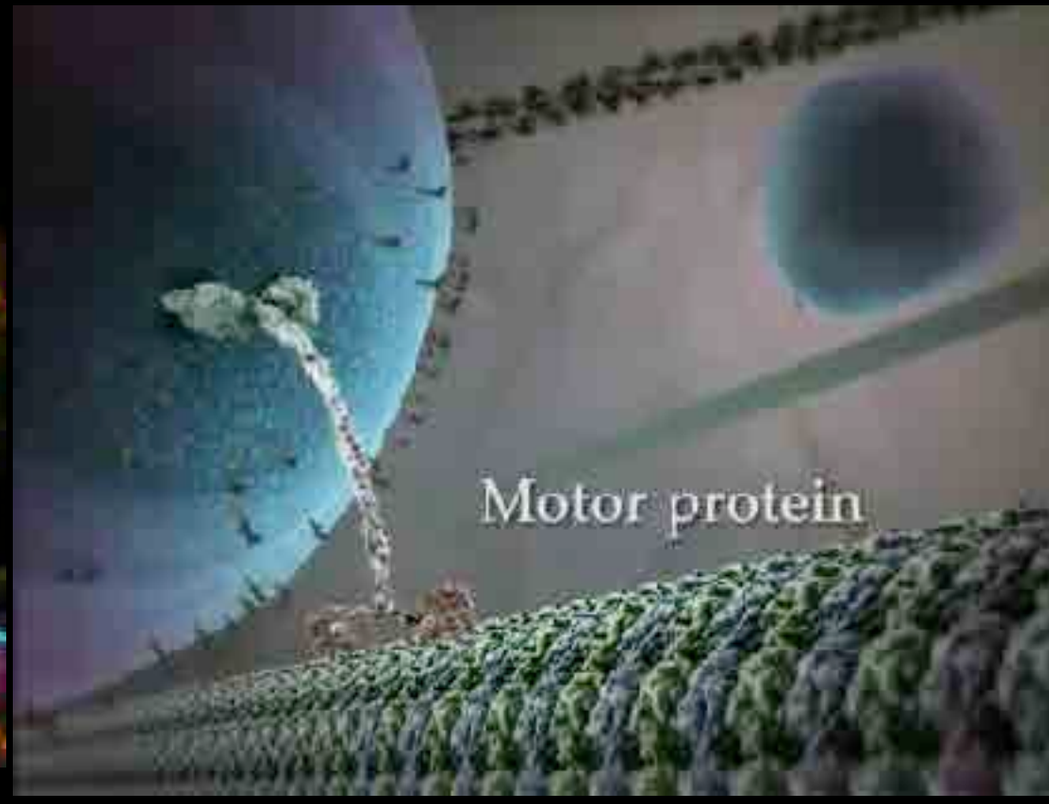
Visualizing, tracking, quantifying events in biological systems…

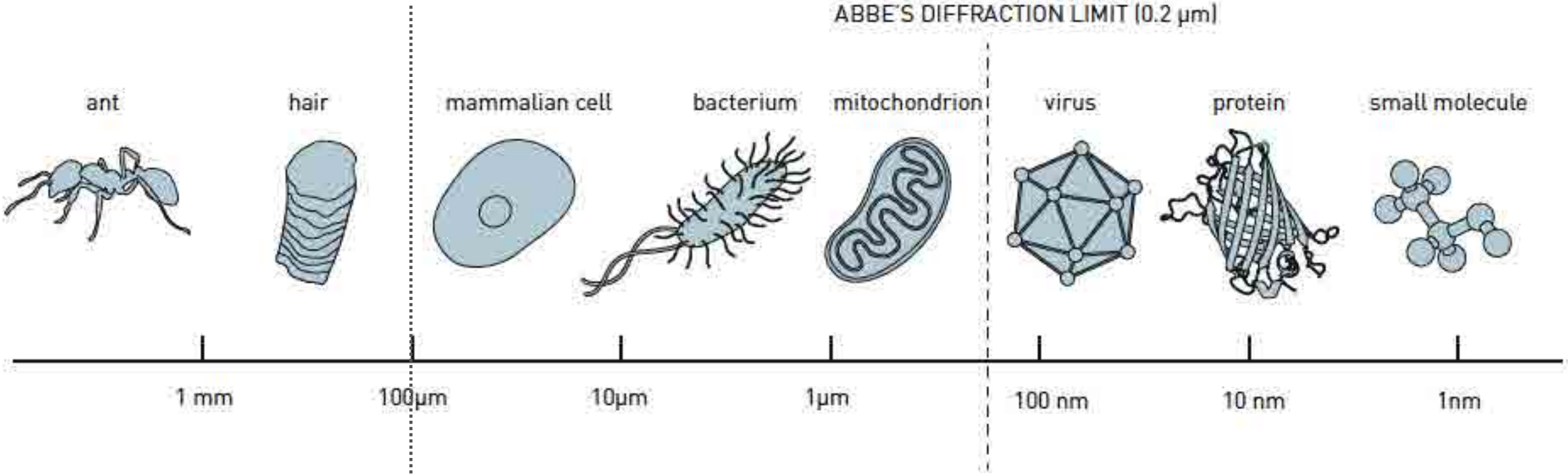


Cell aggregates, small organisms, organs, tissues...



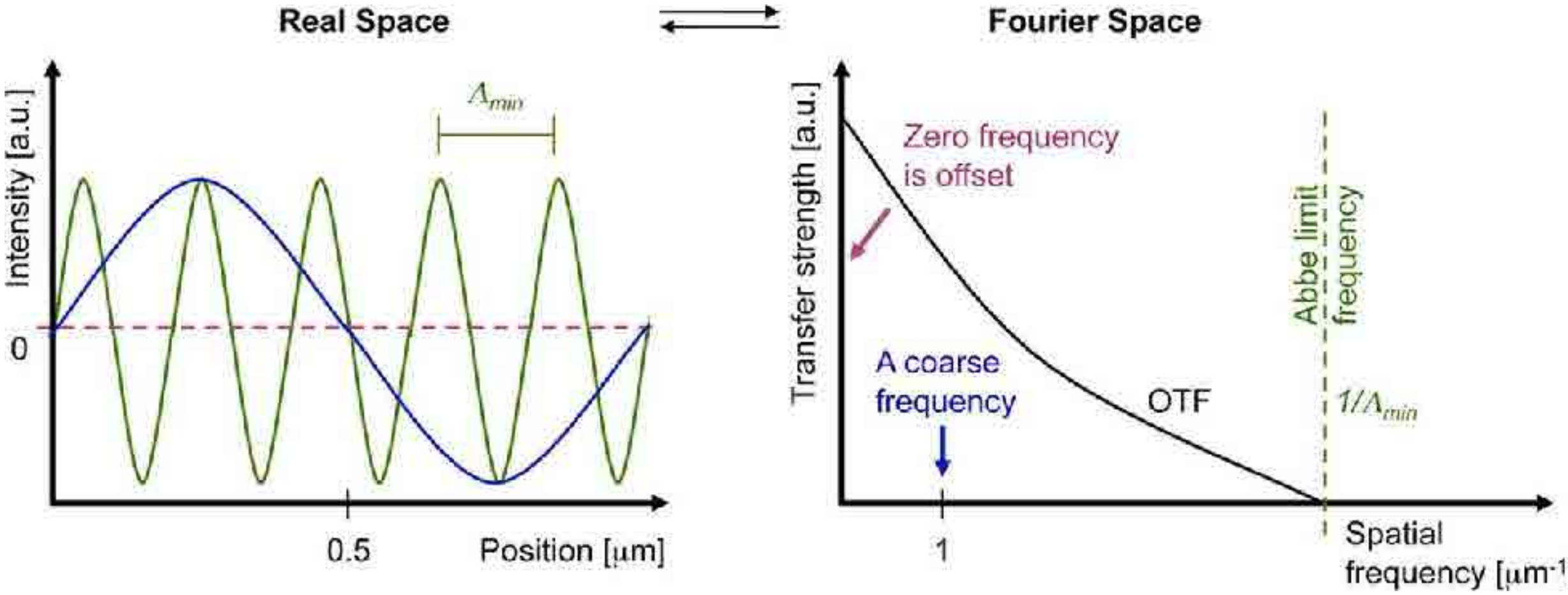


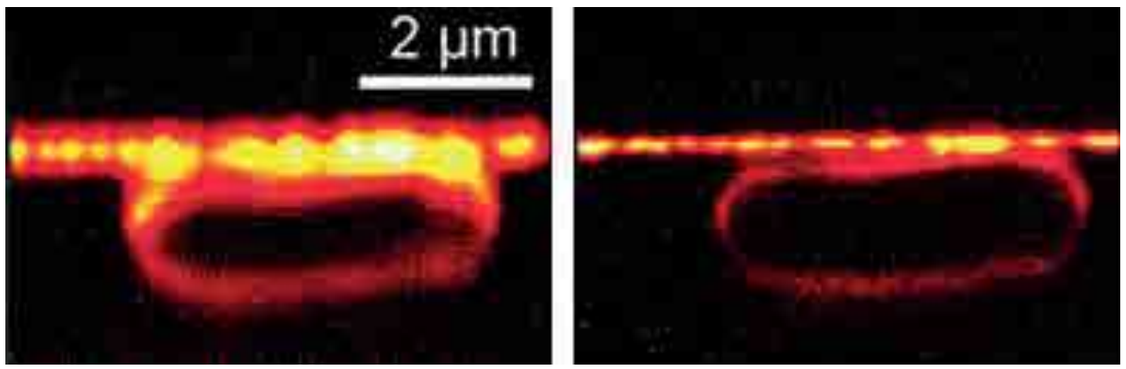




THE NOBEL PRIZE IN CHEMISTRY 2014 - THE ROYAL SWEDISH ACADEMY OF SCIENCES - HTTP://KVA.SE

Resolution





Schermelleh et al., 2010, JCB vol. 190 no. 2 165-175.

$$(\delta x_{min}, \delta y_{min}) = \Delta_{min} = \frac{\lambda}{2n\sin\alpha}$$

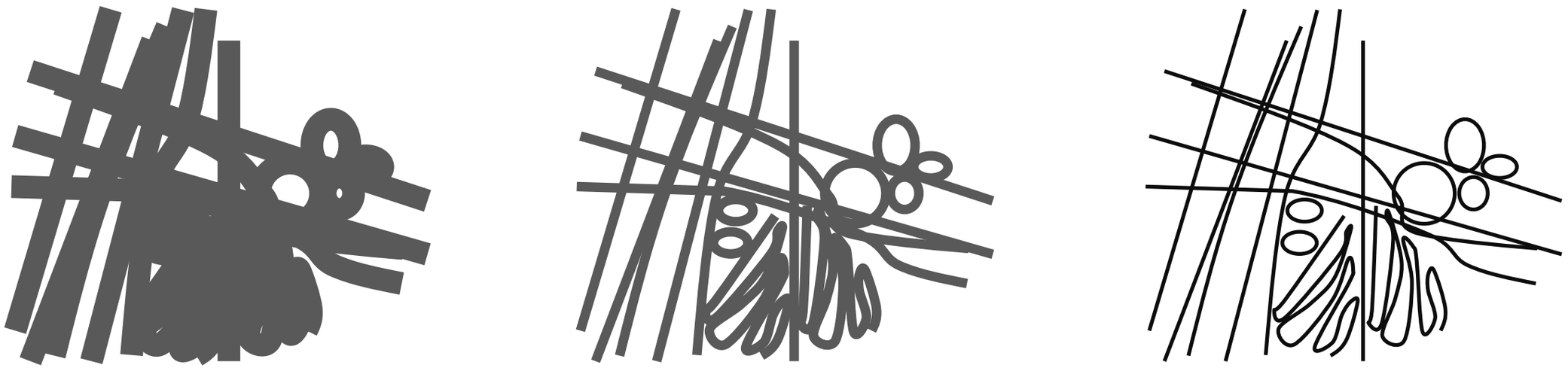
$$\delta z_{min} \approx \frac{\lambda}{2n(\sin\alpha)^2}$$

Klar et al, 2000, Proc. Natl. Acad. Sci. USA 97: 8206–8210.

Like a tuneable Radio station

In optical microscopy we can get access to ‘nanoscale’ details
by increasing the optical resolution of the microscope

500 nm



Increasing spatial resolution

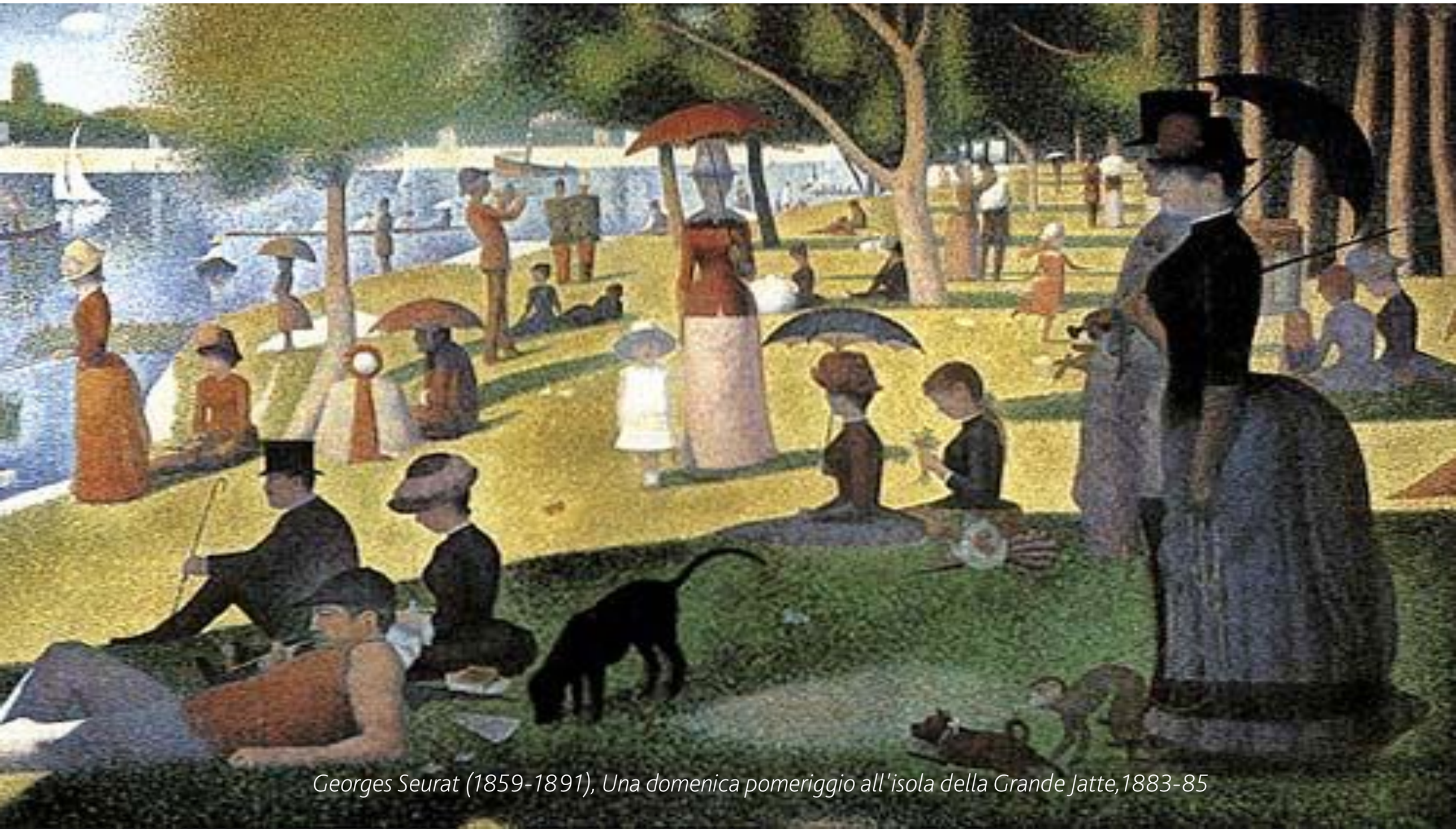
Point Spread Function (PSF)

250 nm

100 nm

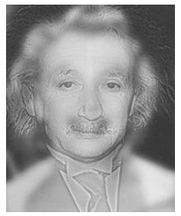
25 nm

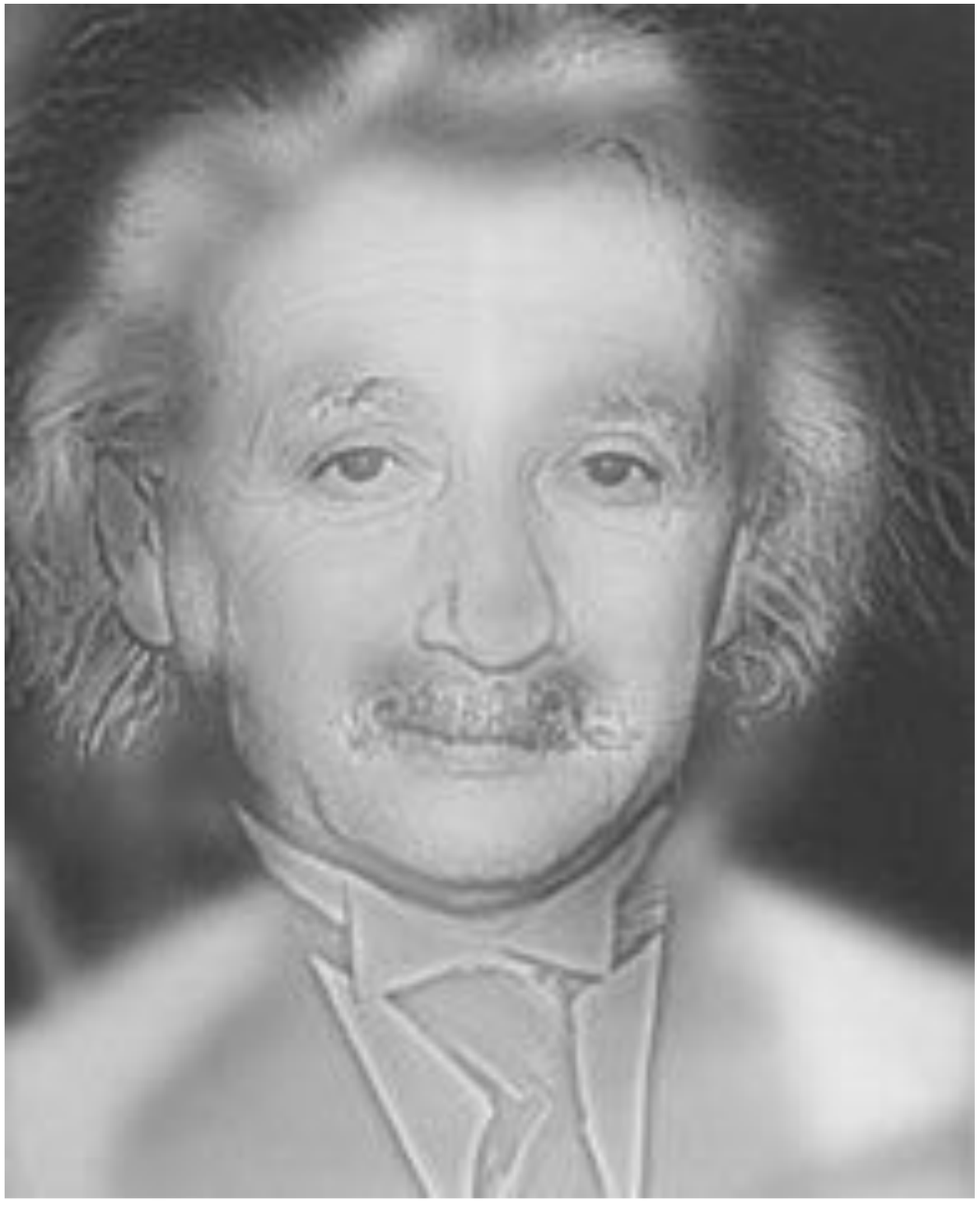
slide credit: Luca Lanzano’, IIT

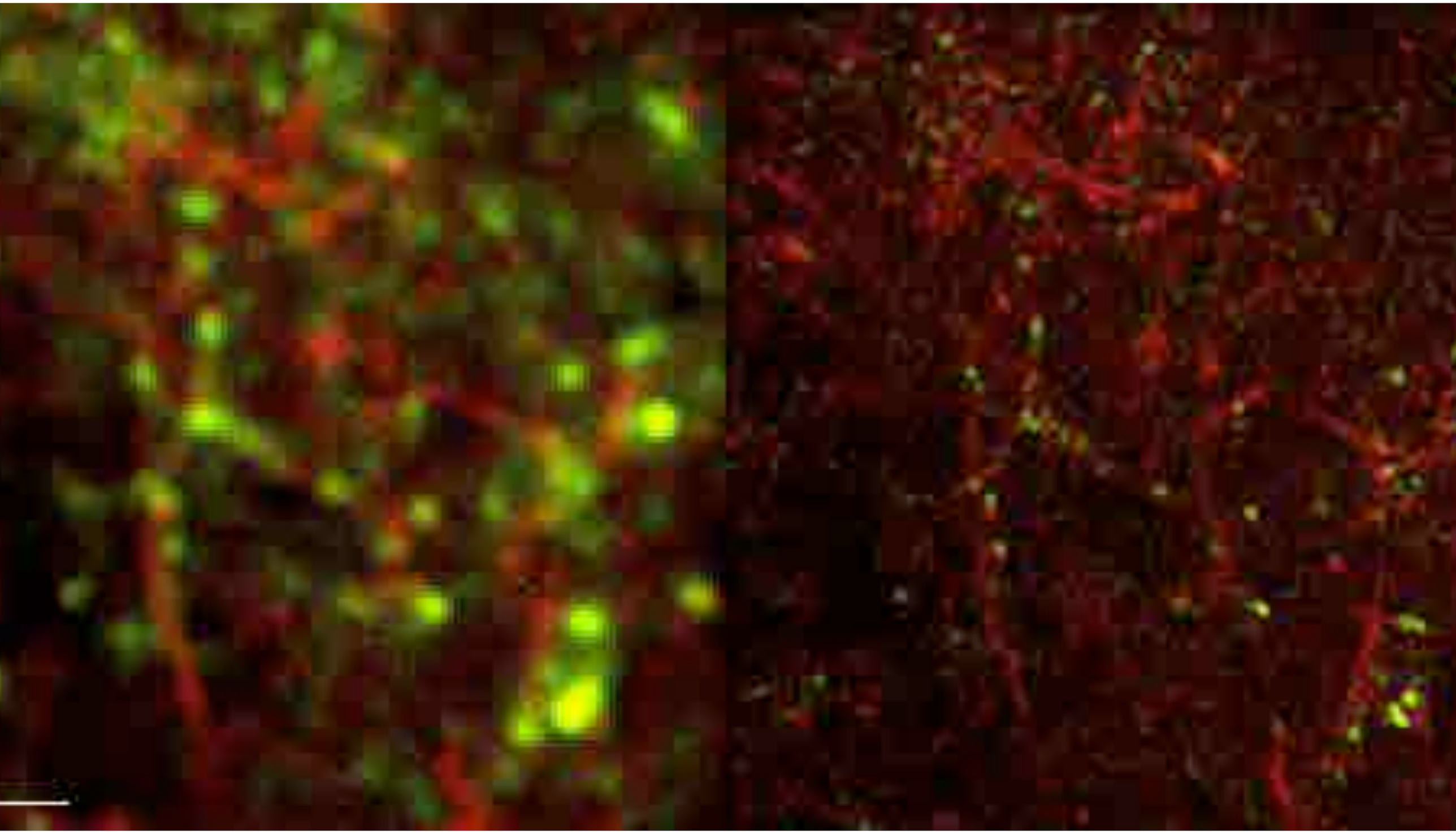


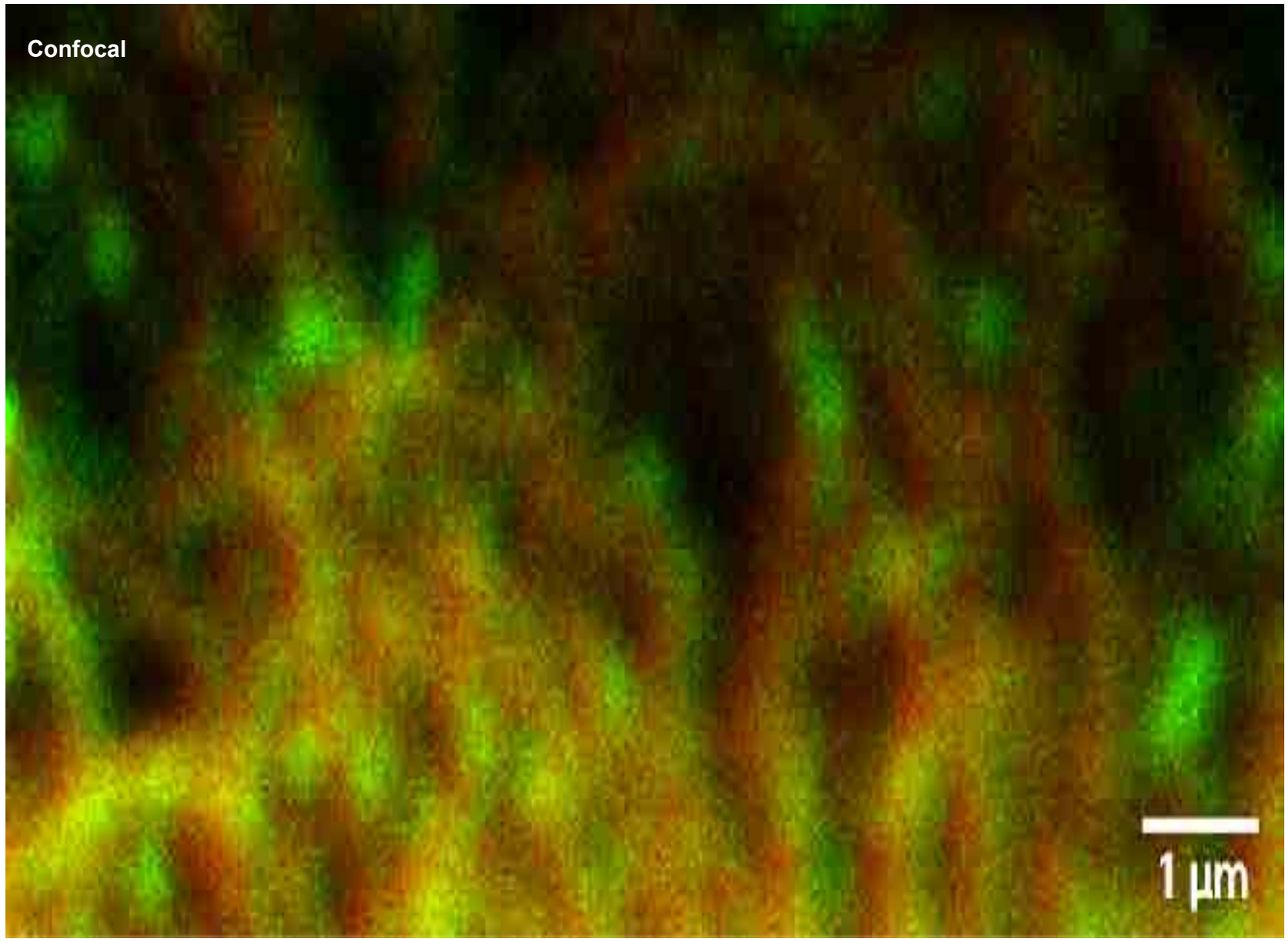
Georges Seurat (1859-1891), Una domenica pomeriggio all'isola della Grande Jatte,1883-85

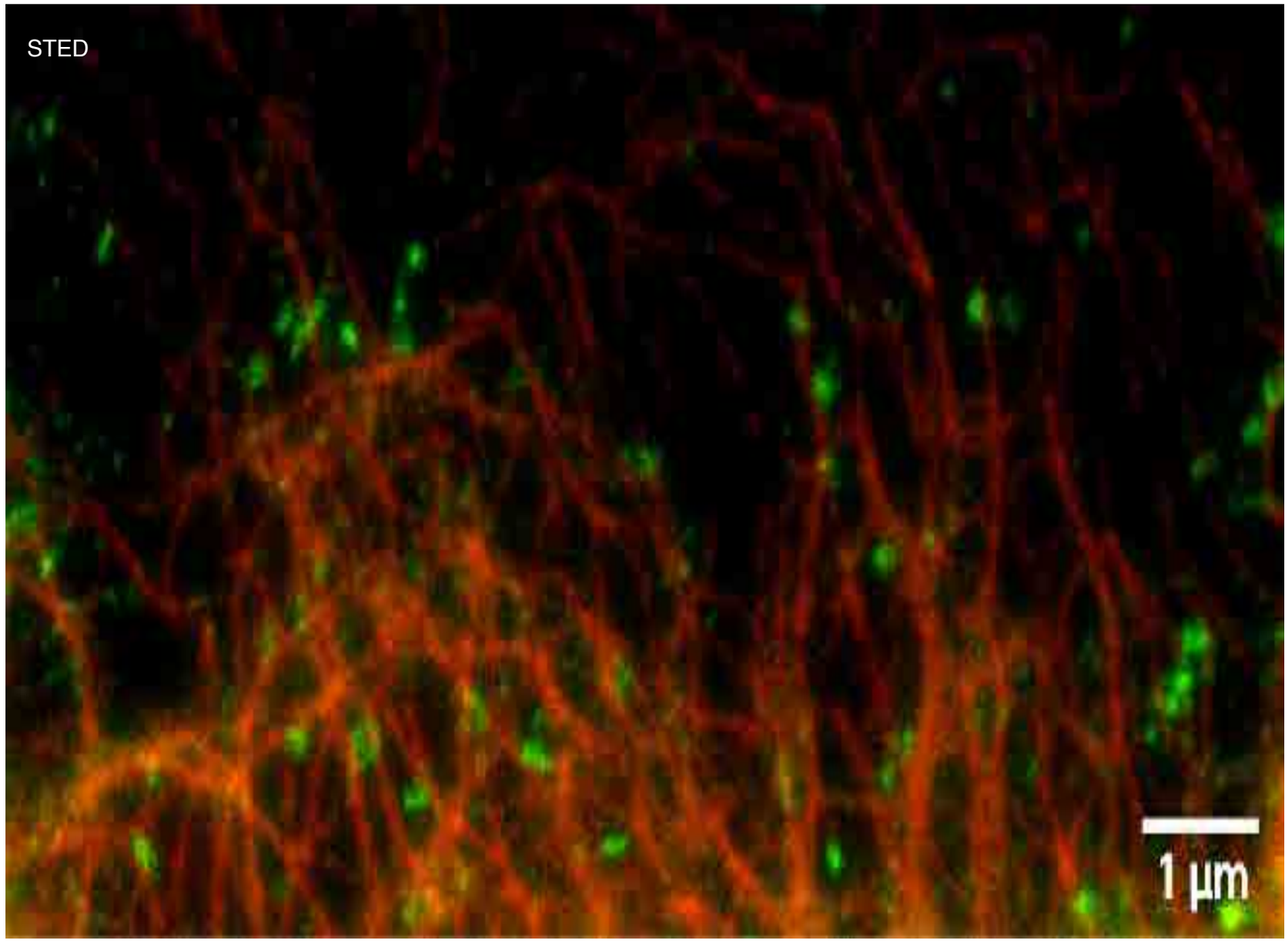
Jean-Etienne Liotard, La bella cioccolataia, 1745








Confocal
1 µm


STED
1 µm

Super Resolution

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 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.

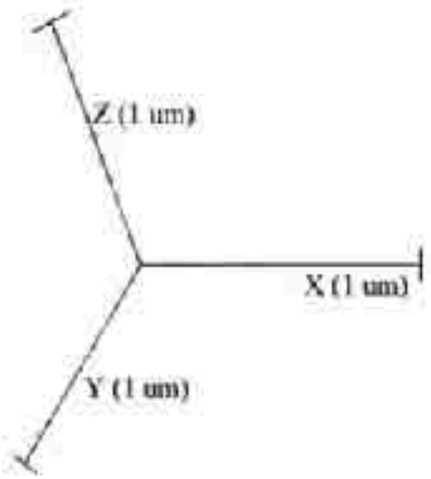
In the first place it may be remarked that the generally accepted value $1.22\lambda/D$ for the minimum angular separation of two points, which can be seen as distinct with an instrument having a circular pupil of diameter D, cannot be derived solely from the properties of the instrument. Its validity 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.

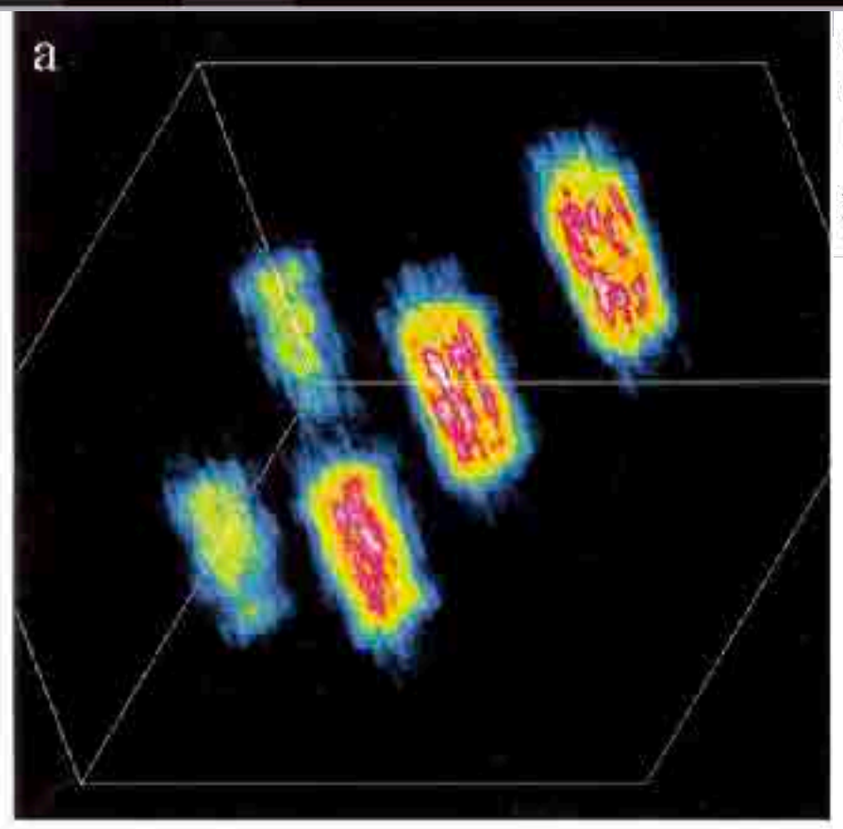
receptors, detecting the difference between the image of a single point and the image of two points located closer and closer to one another. This means that at present there is only a *practical* limit (if any) and not a *theoretical* limit for two-point resolving power.

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 tool. 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 physical quantity

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

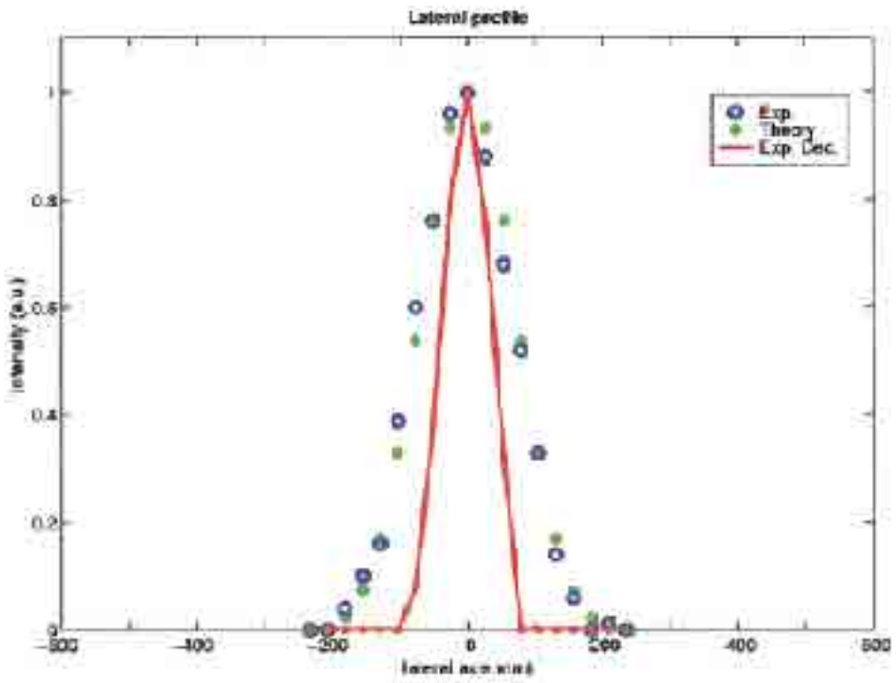
MICROSCOPY
RESEARCH & TECHNIQUE

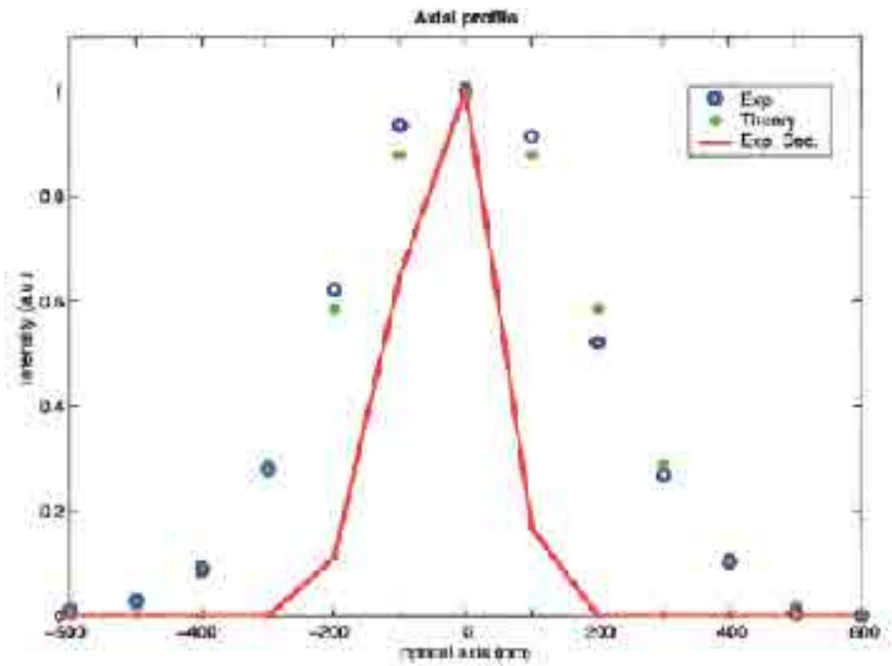


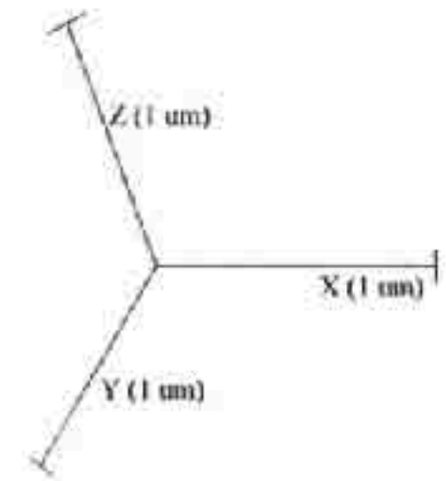


Single-Pinhole Confocal Imaging of Sub-Resolution Sparse Objects Using Experimental Point Spread Function and Image Restoration

A. DIASPRO,* S. ANNUNZIATA, AND M. ROBELLO
INFM, Biophysical Section, Genoa Research Unit and Department of Physics, University of Genoa, Via Dodecaneso 33, 16146 Genova, Italy







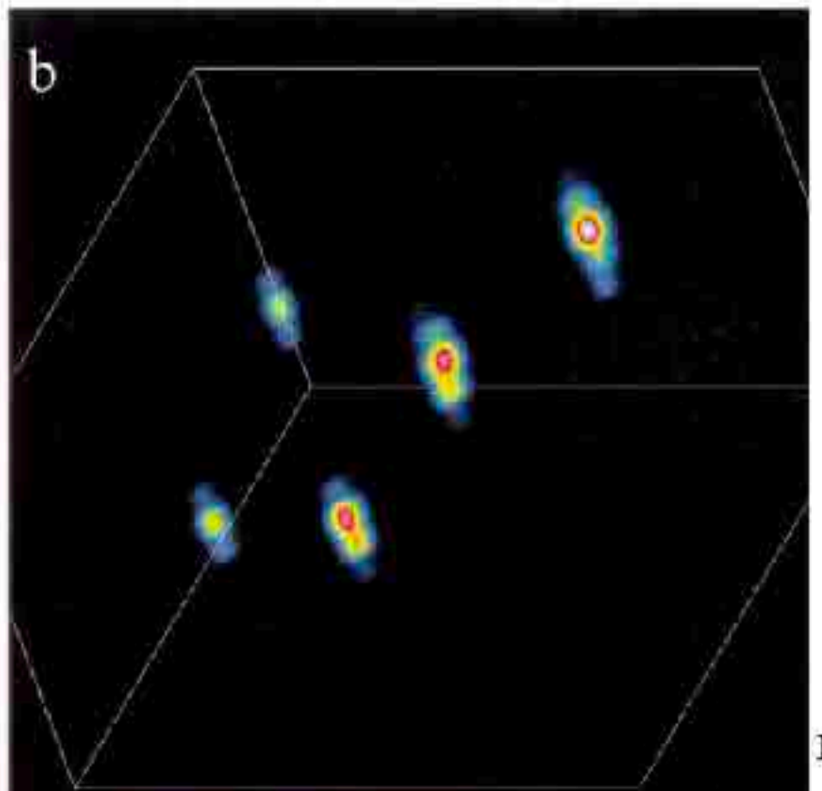
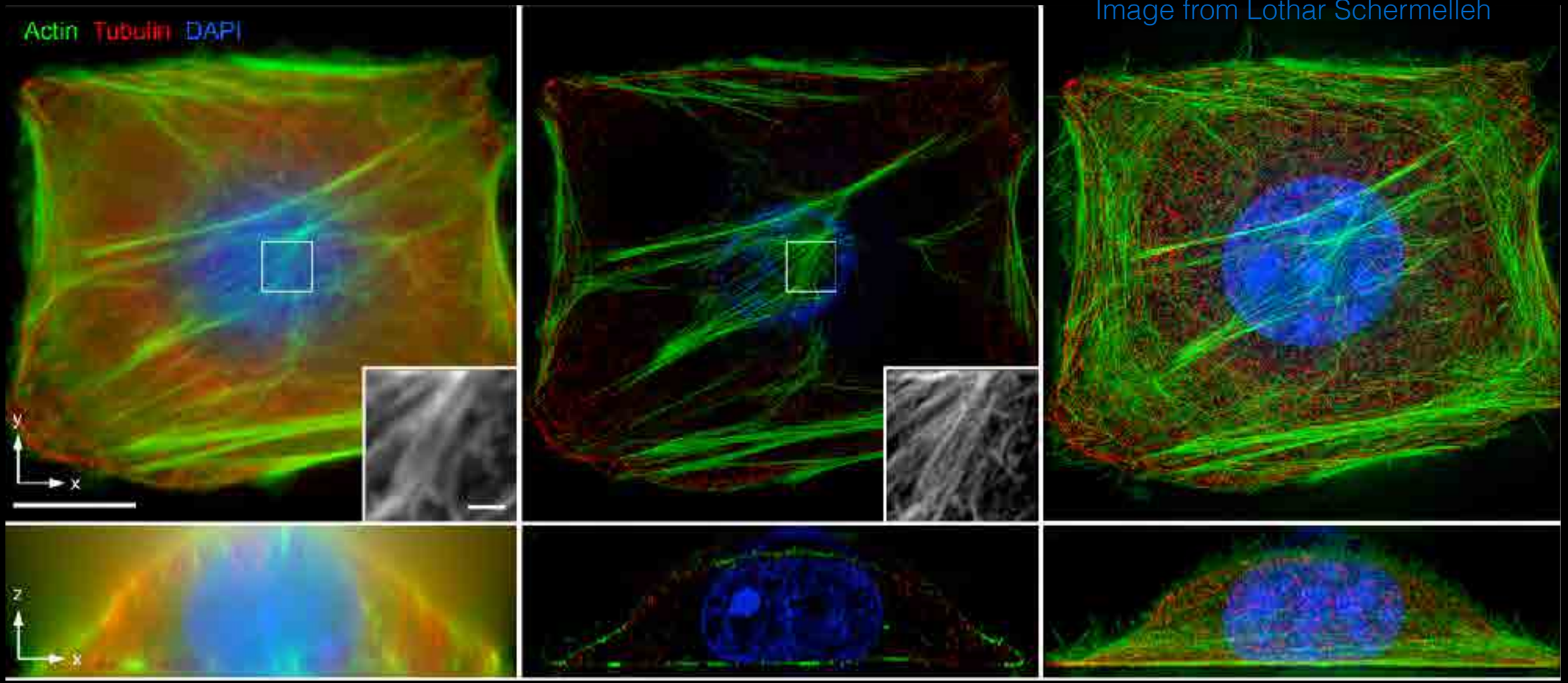


Image from Lothar Schermelleh



wide field

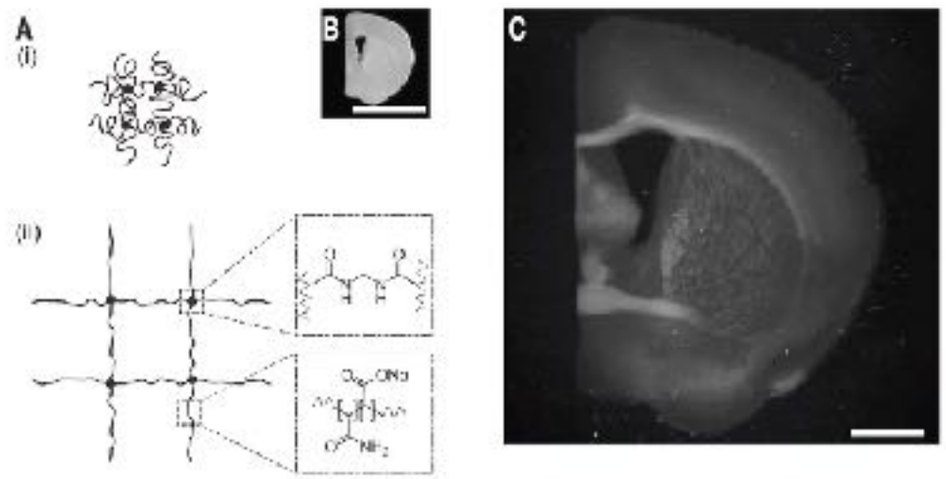
3D SIM

M. G. L. Gustafsson, et al. Biophys. J. 2008, 94, 4957 – 4970.

Expansion Microscopy

Irene's question:
Can we install polymer chains of
a swellable material?.

…throughout cells, winding their way around biomolecules, so that we can pull the biomolecules apart?



Chen*, Tillberg*, Boyden (2015)
Science 347(6221):543-548.

Expansion Microscopy

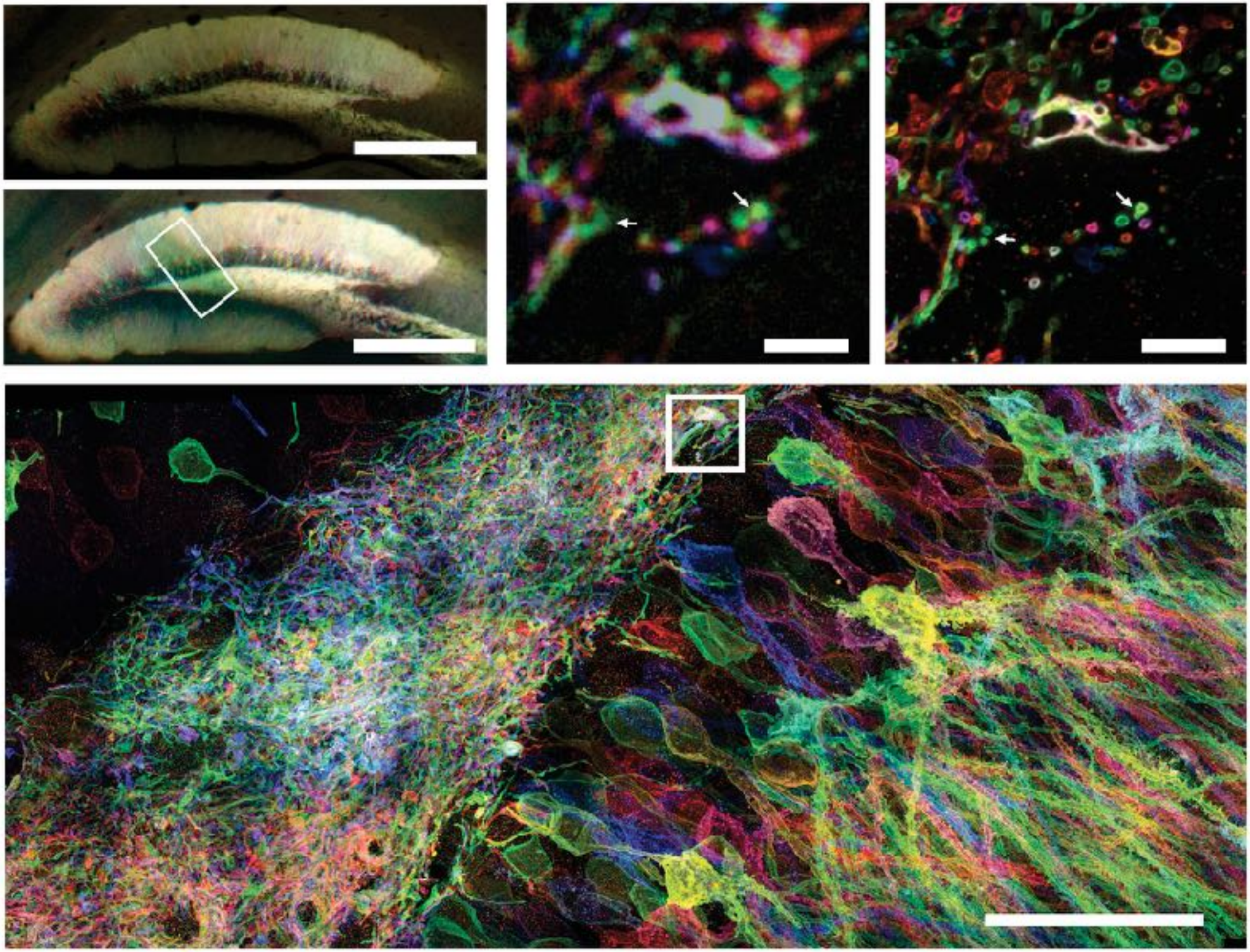
Brainbow

Confocal, pre-expansion

Confocal, post-expansion

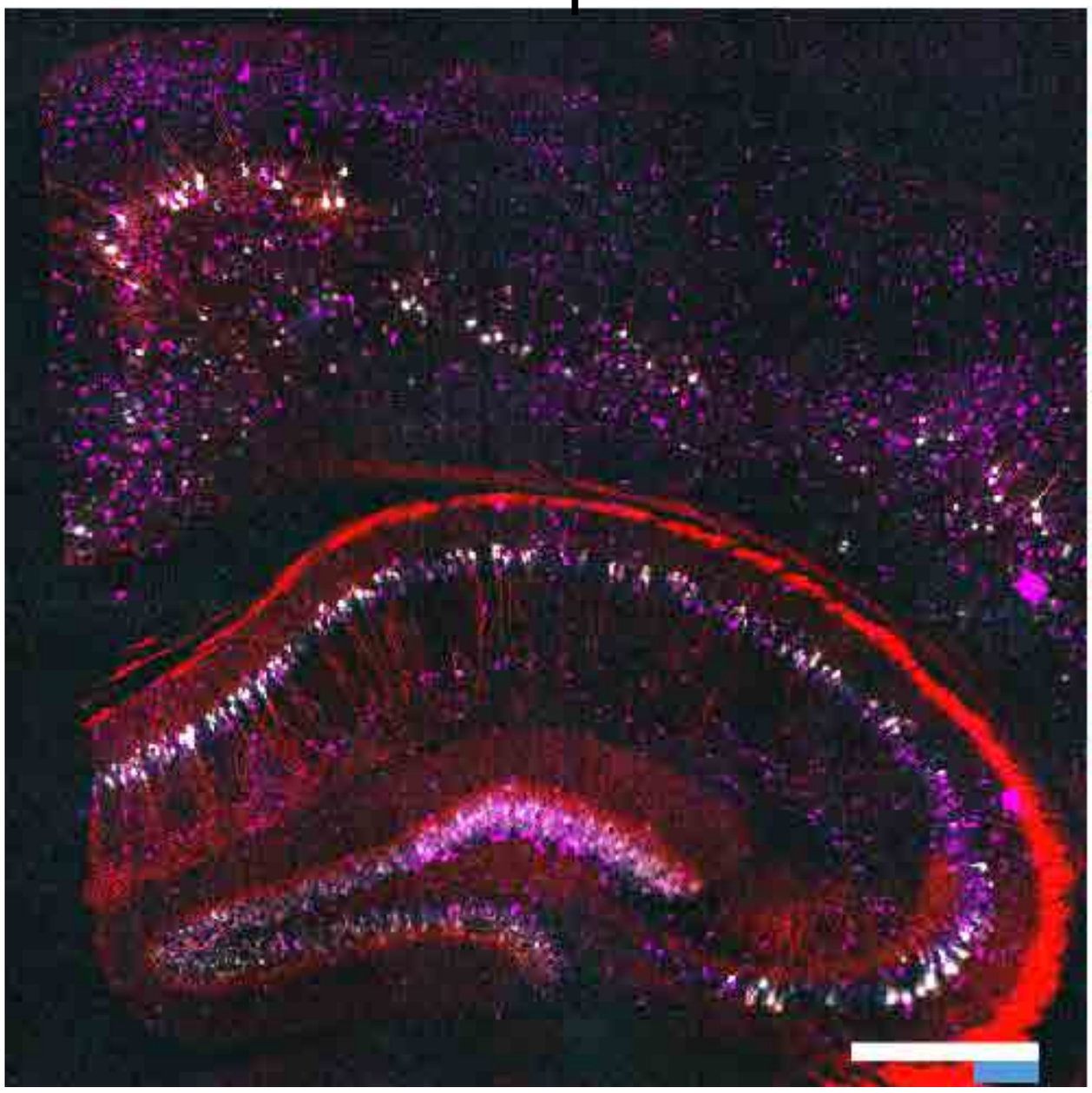
Widefield, pre-expansion

Widefield, post-expansion

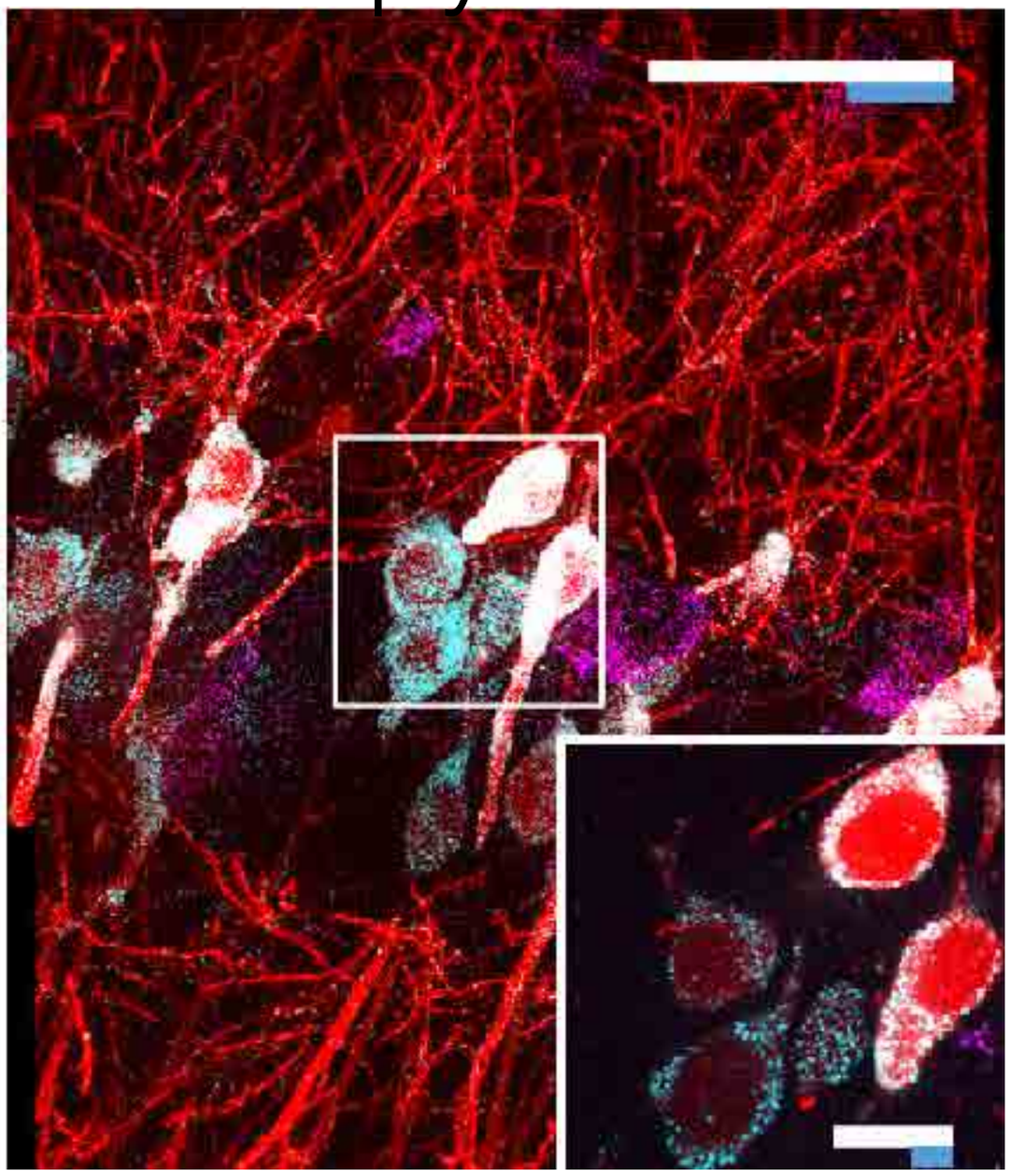


Tillberg*, Chen*, Piatkevich, Zhao, Yu et al. (2016) Nature Biotechnology 34:987–992.

Expansion Microscopy



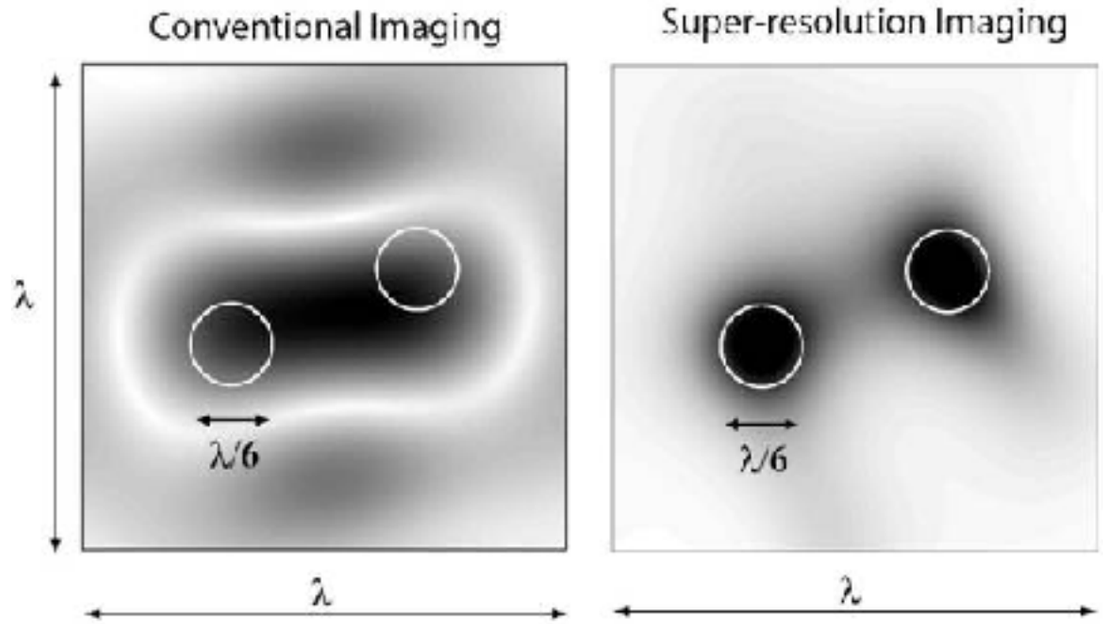
bar:500 µm (expansion factor 2.9×)



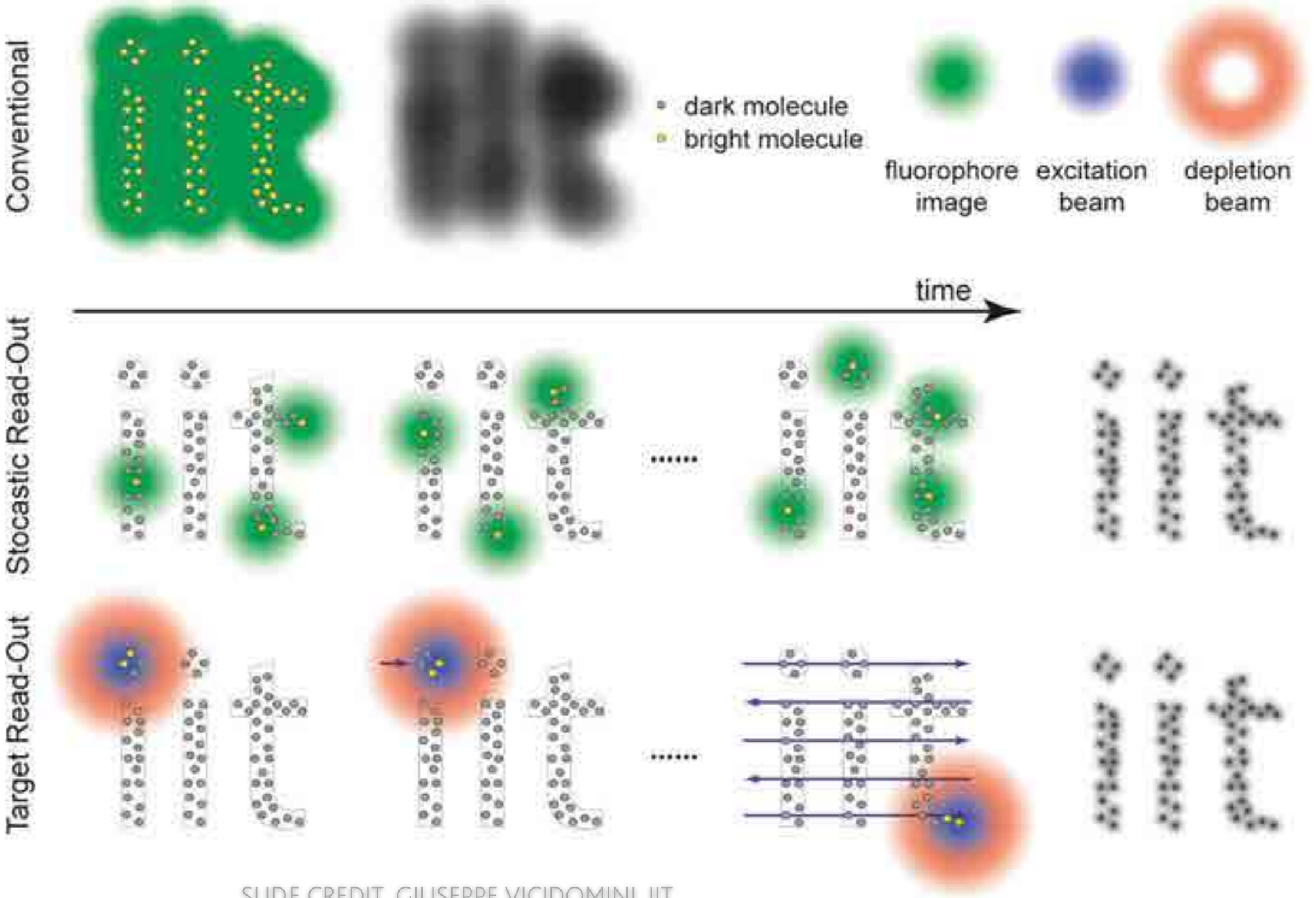
bar:50 µm (2.9×), inset 10 µm

Chen, F., Wassie, A.T., Cote, A.J., Sinha, A., Alon, S., Asano, S., Daugharthy, E.R., Chang, J.-B., Marblestone, A., Church, G.M., Raj, A., Boyden, E.S. (2016) Nanosca)le Imaging of RNA with Expansion Microscopy,
Nature Methods

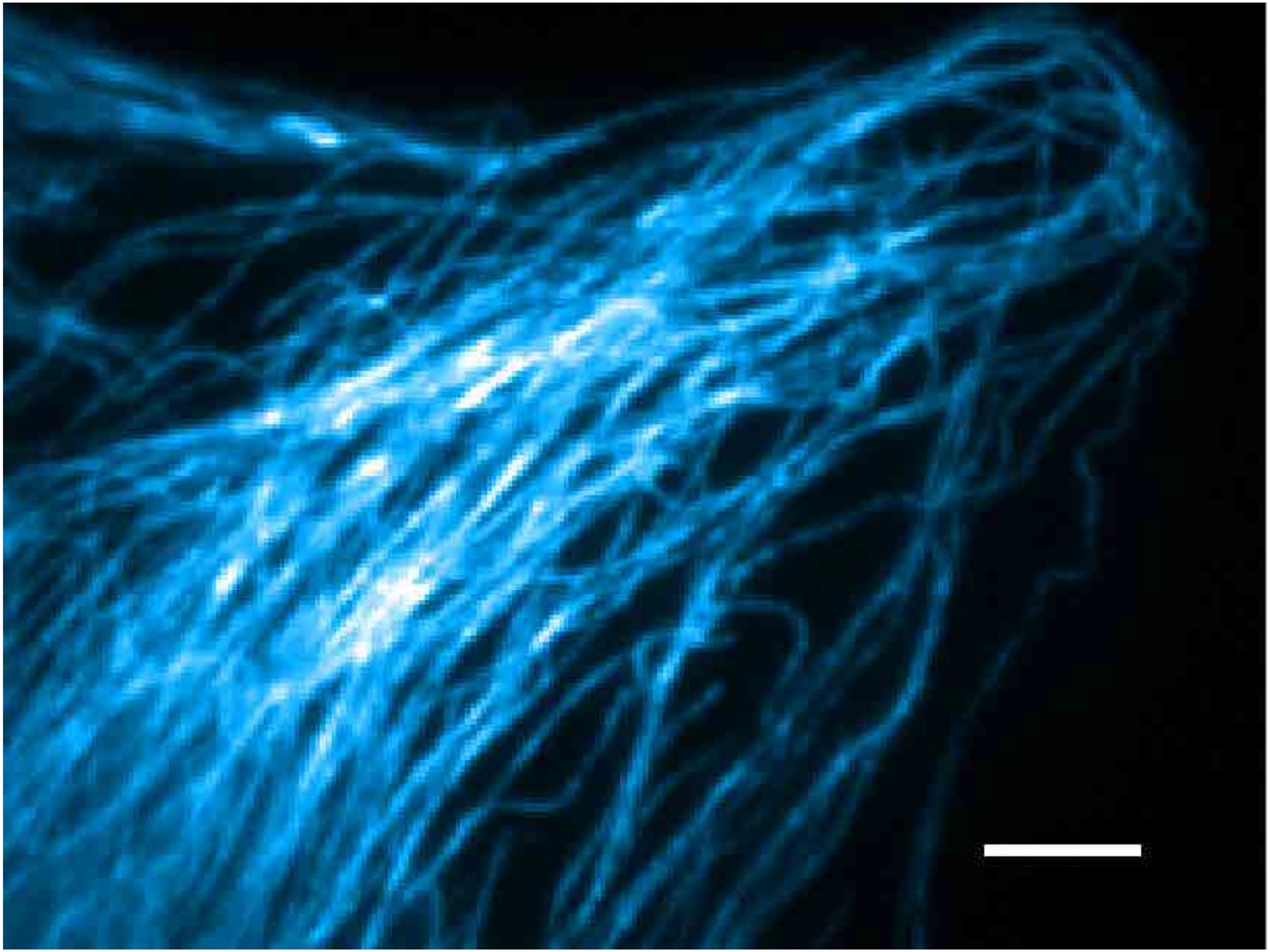
Super Resolution

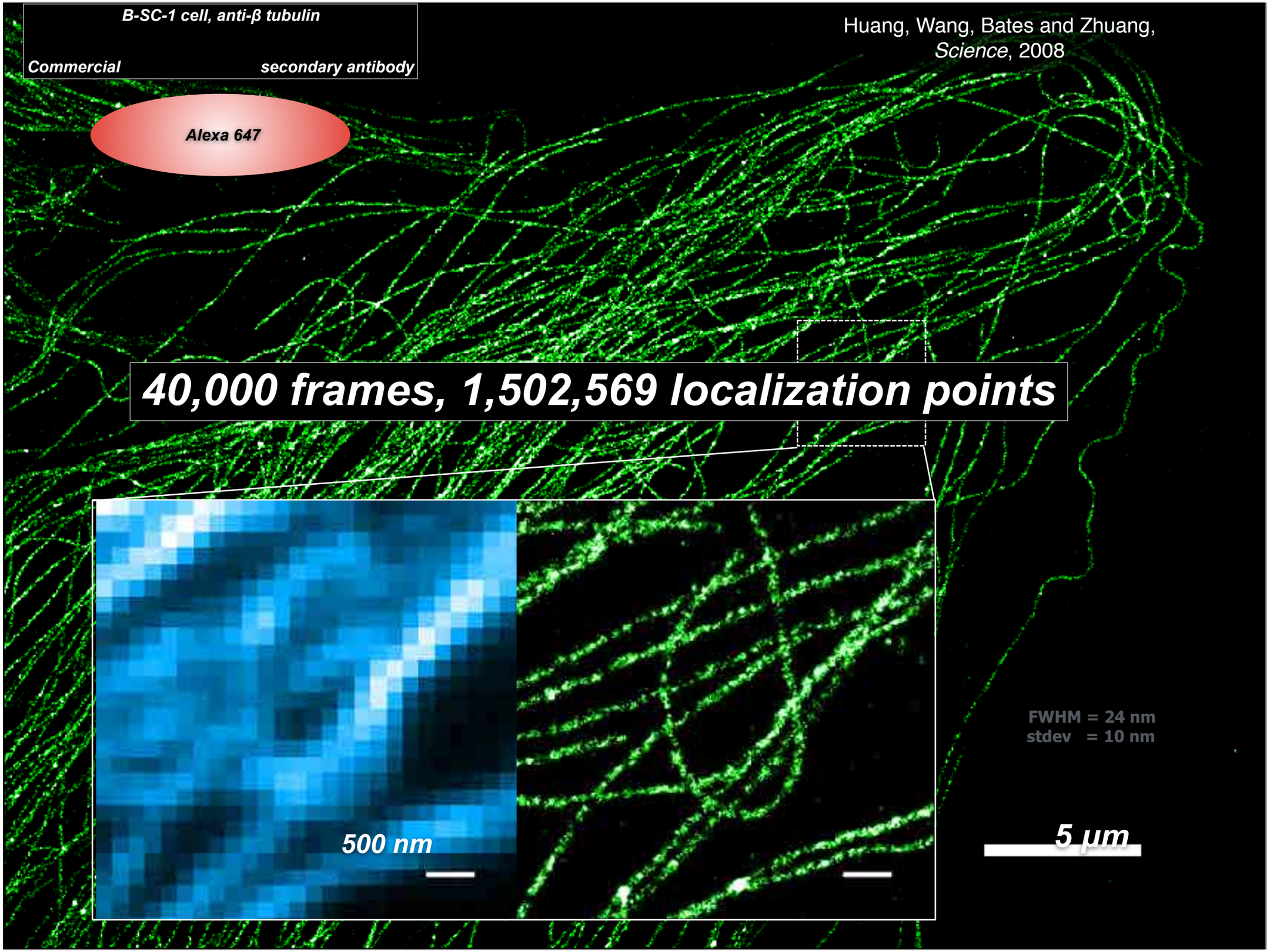


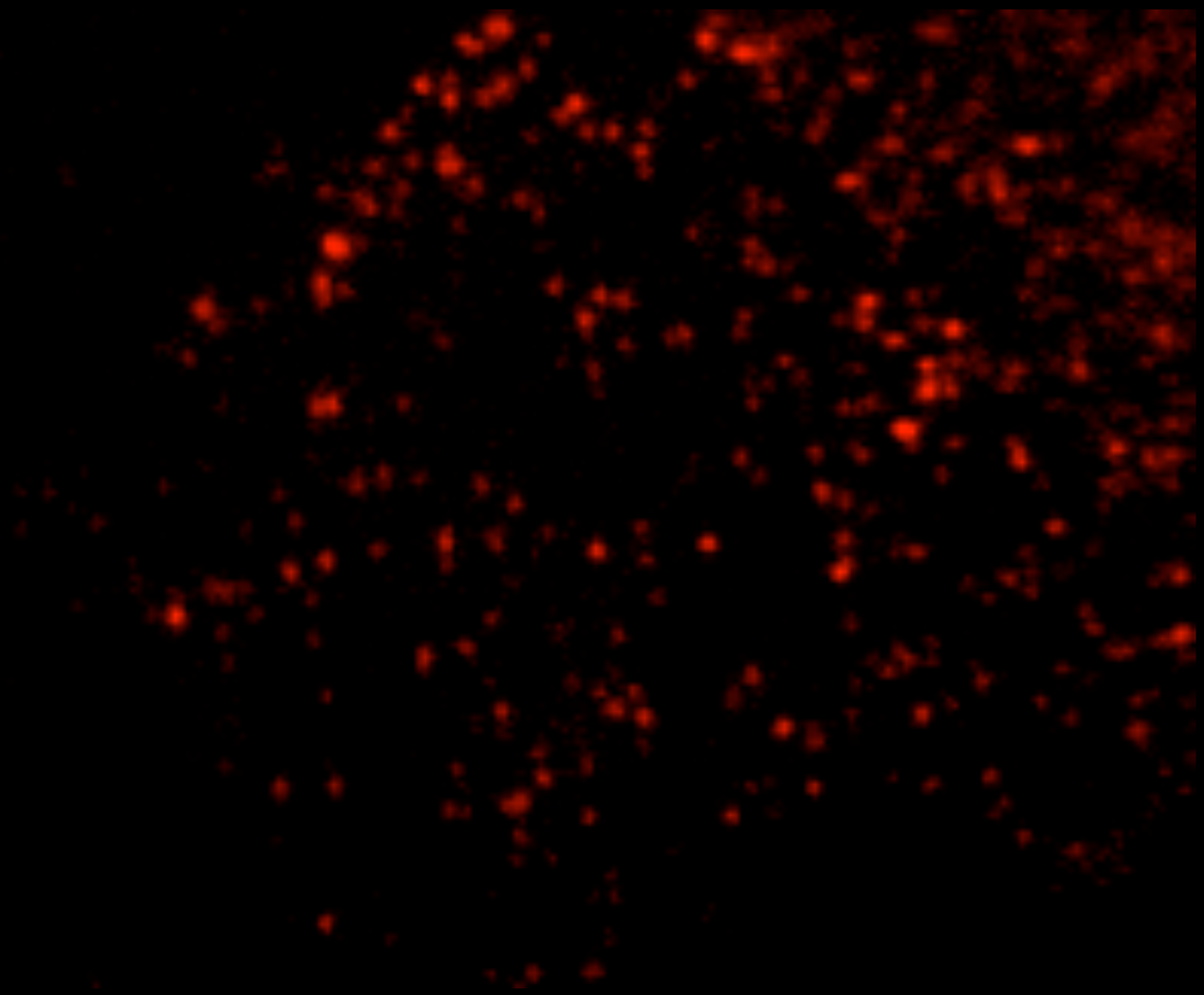
Super resolved Fluorescence Microscopy



SLIDE CREDIT, GIUSEPPE VICIDOMINI, IIT




B-SC-1 cell, anti-β tubulin
Commercial
secondary antibody
Alexa 647
Huang, Wang, Bates and Zhuang,
Science, 2008
40,000 frames, 1,502,569 localization points
500 nm
FWHM = 24 nm
stdev = 10 nm
5 μm



Diaspro Lab - Francesca Cella Zanacccchi project

20 ms/frame - alfa-tubulin

localization precision 15 nm

ACS Photonics

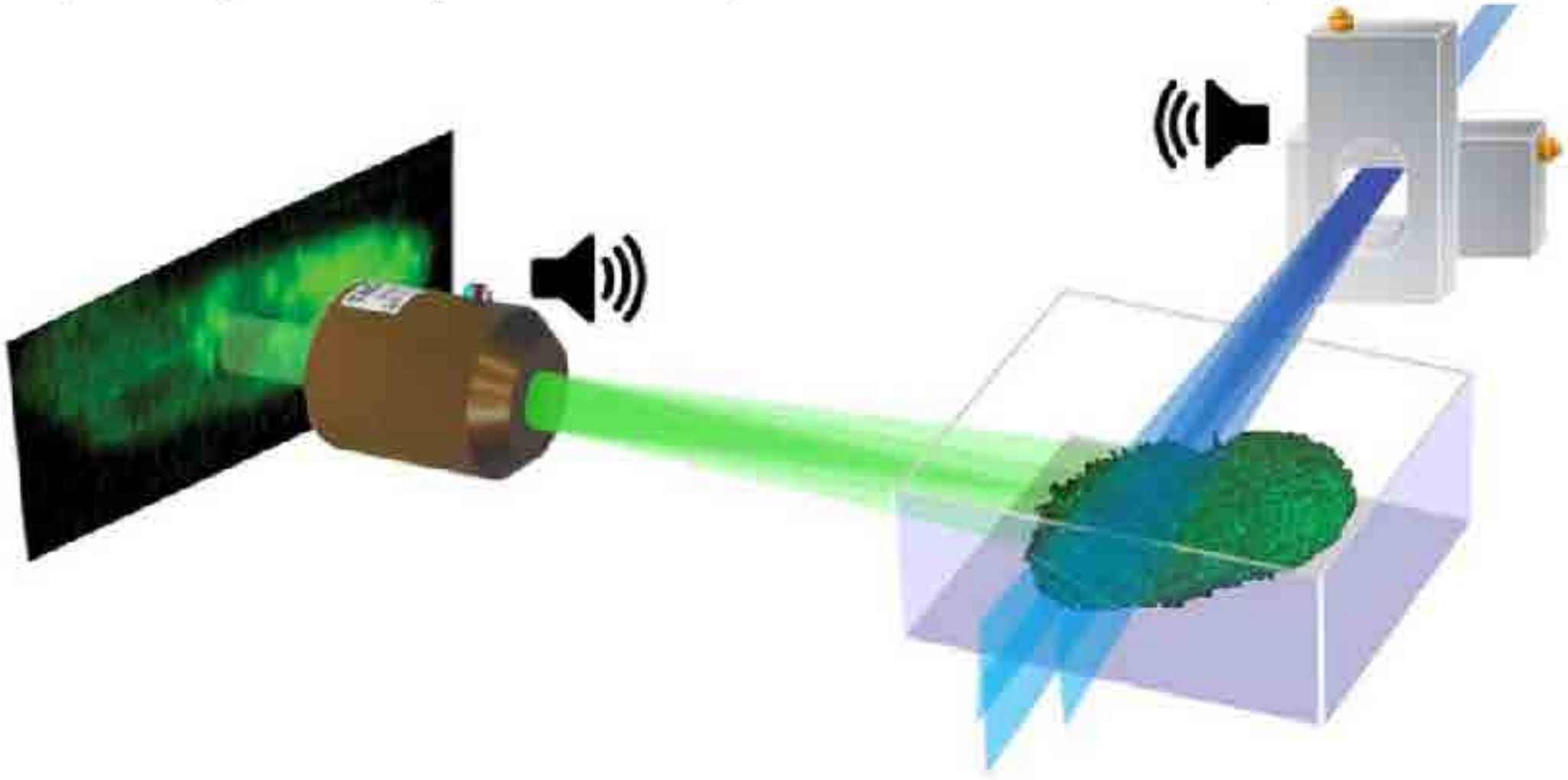
Subscriber access provided by IST ITALIANO DI TECH IIT

Article

Fast inertia-free volumetric light-sheet microscope

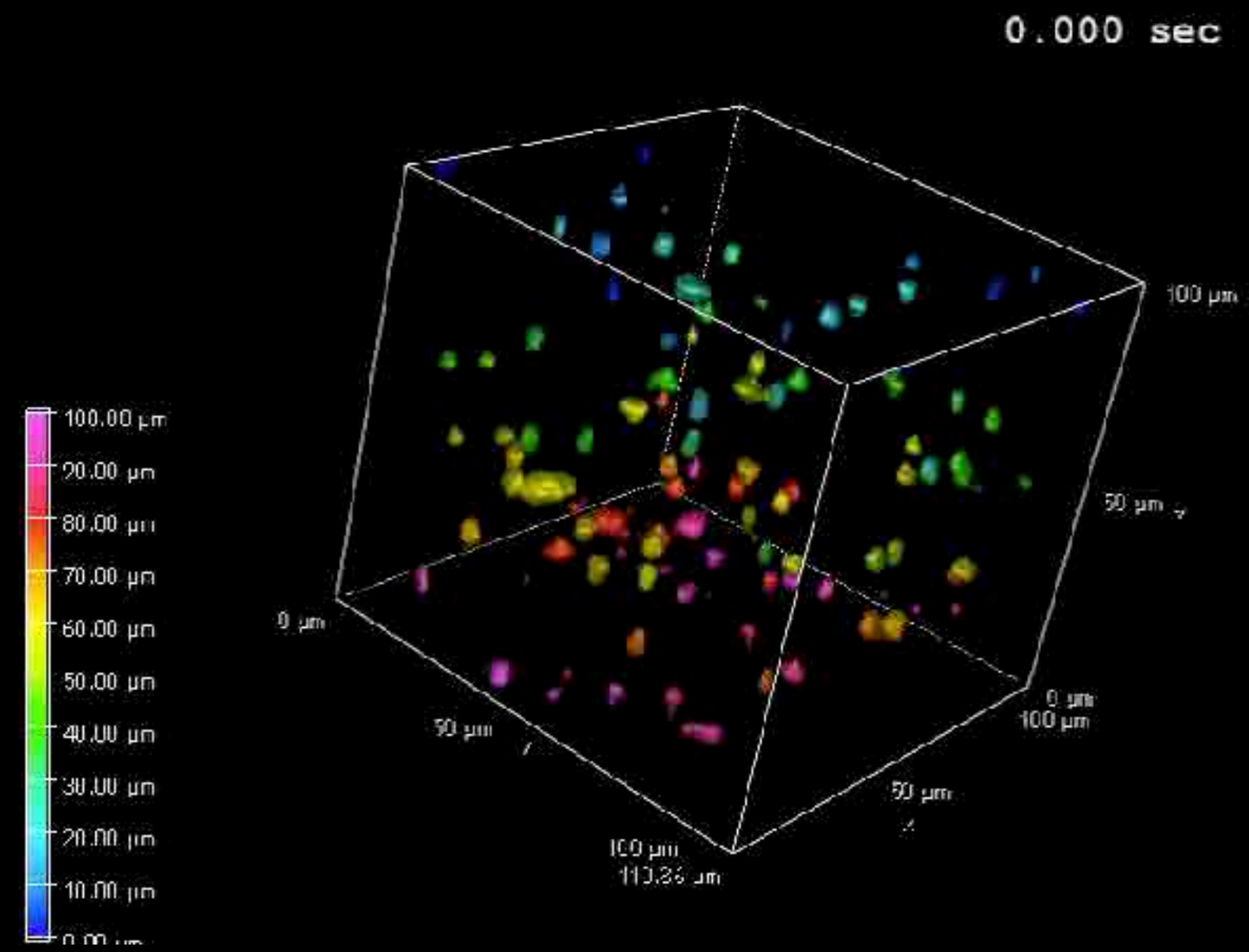
Marti Duocastella, Giuseppe Sancataldo, Peter Saggau, Paola Ramoino, Paolo Bianchini, and Alberto Diaspro

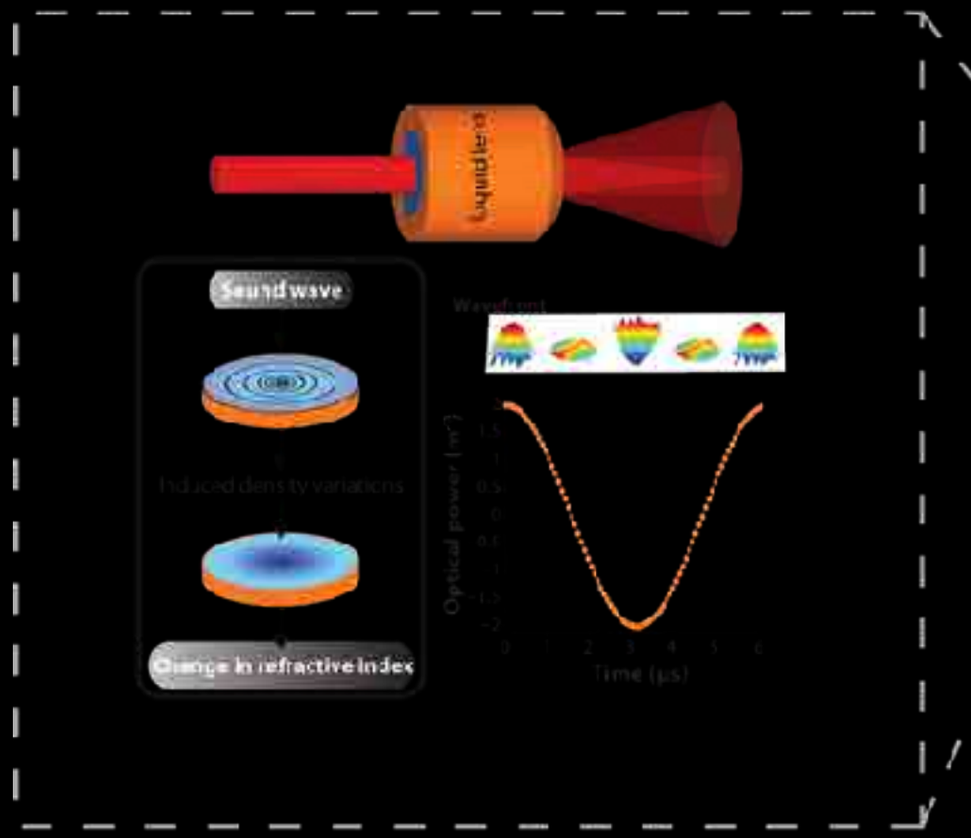
ACS Photonics, **Just Accepted Manuscript** • DOI: 10.1021/acsphotonics.7b00382 • Publication Date (Web): 22 Jun 2017



200 Volumes per Second
10000 Frames per Second
600 · 10^6 Voxels per Second

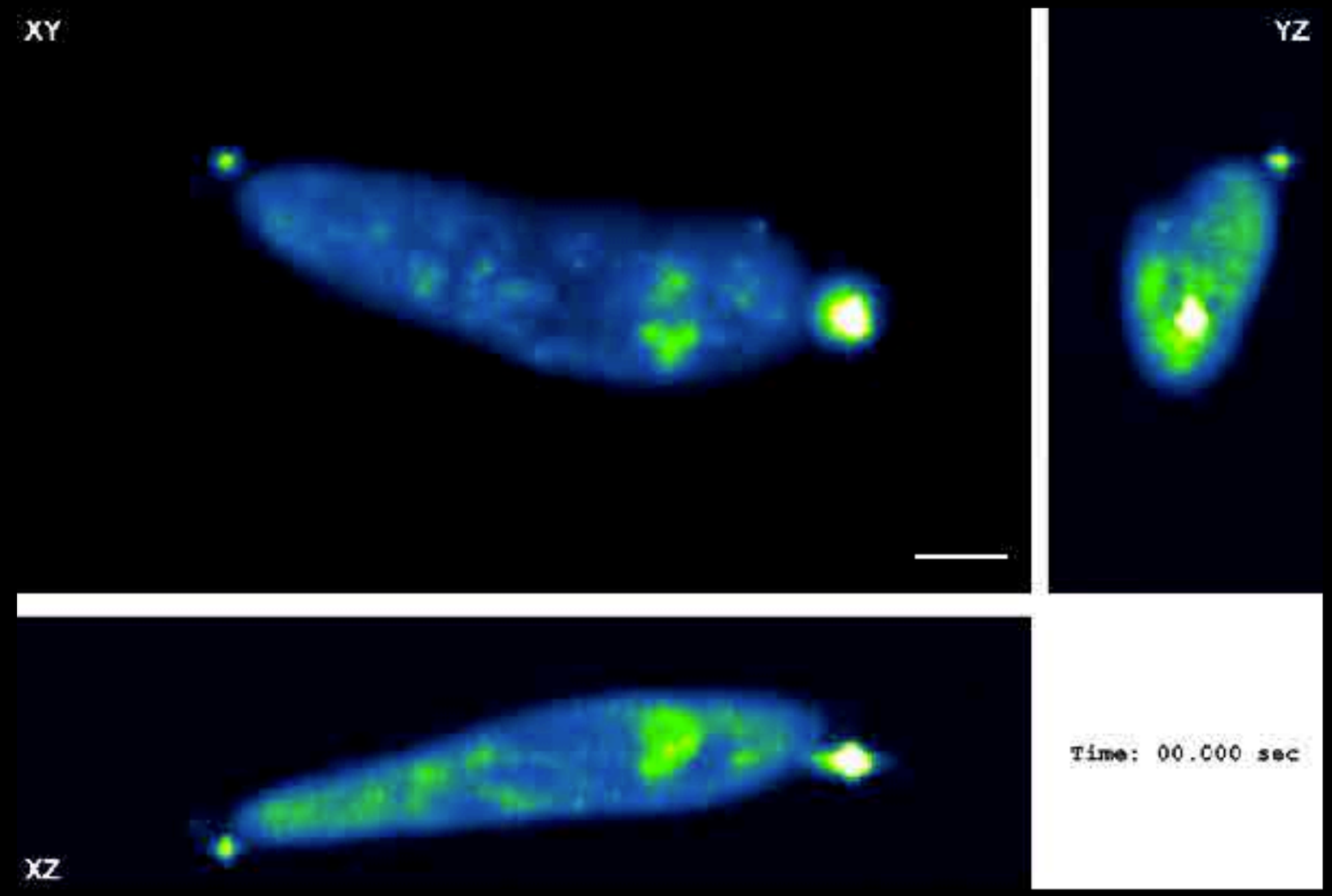
SPIM - liquid lens

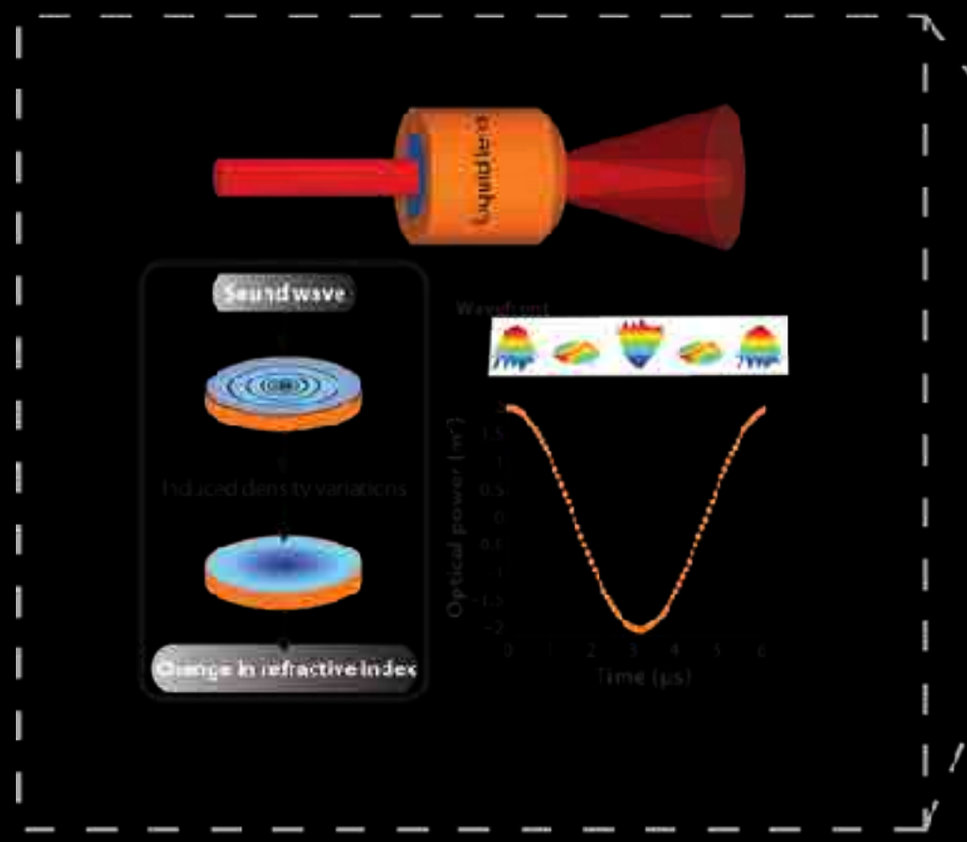




Credits: Giuseppe Sancataldo, Paolo Bianchini, Marti Duocastella - Diaspro Lab, IIT - 2016

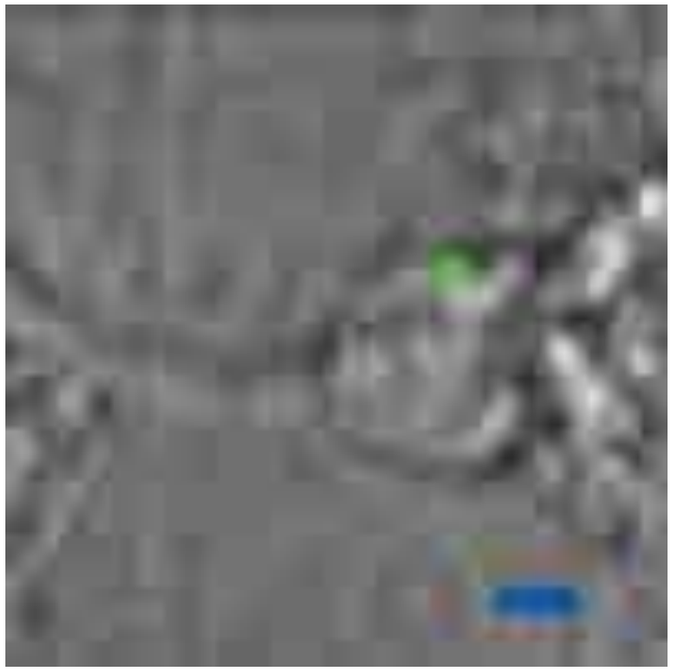
Diaspro Lab, ACS NANO 2017

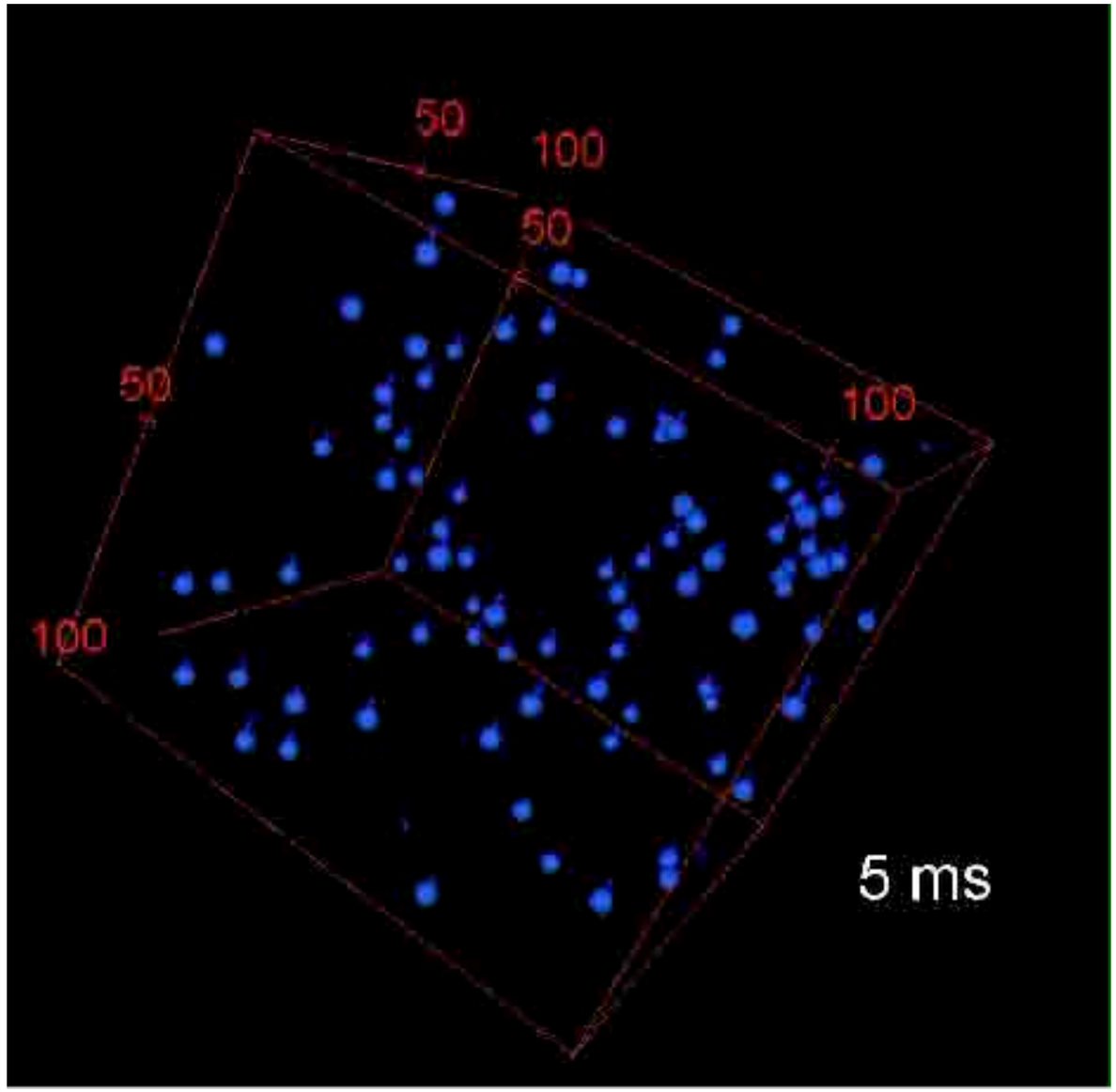


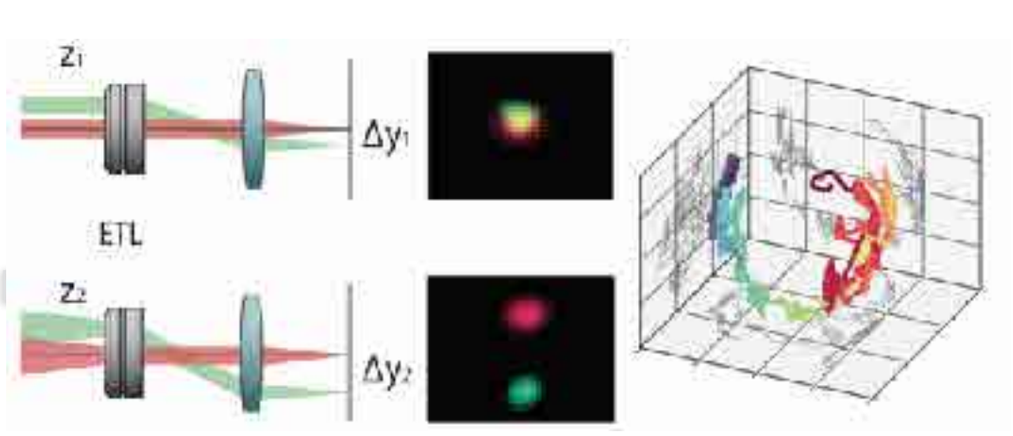


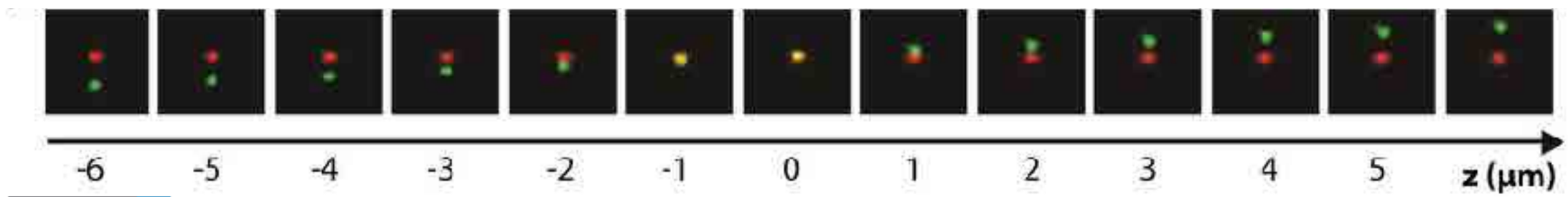
- Volume Size 138×138×60 μm^3 (XYZ)
- 11 Volumes/Second
- 333 Frame/Second
- 3 ms/frame
- 30 planes/Volume
- Nile Red Fluorescence Signal
- Andor NEO 5.5 camera

Credits: Giuseppe Sancataldo, Paolo Bianchini, Marti Duocastella - Diaspro Lab, IIT - 2016







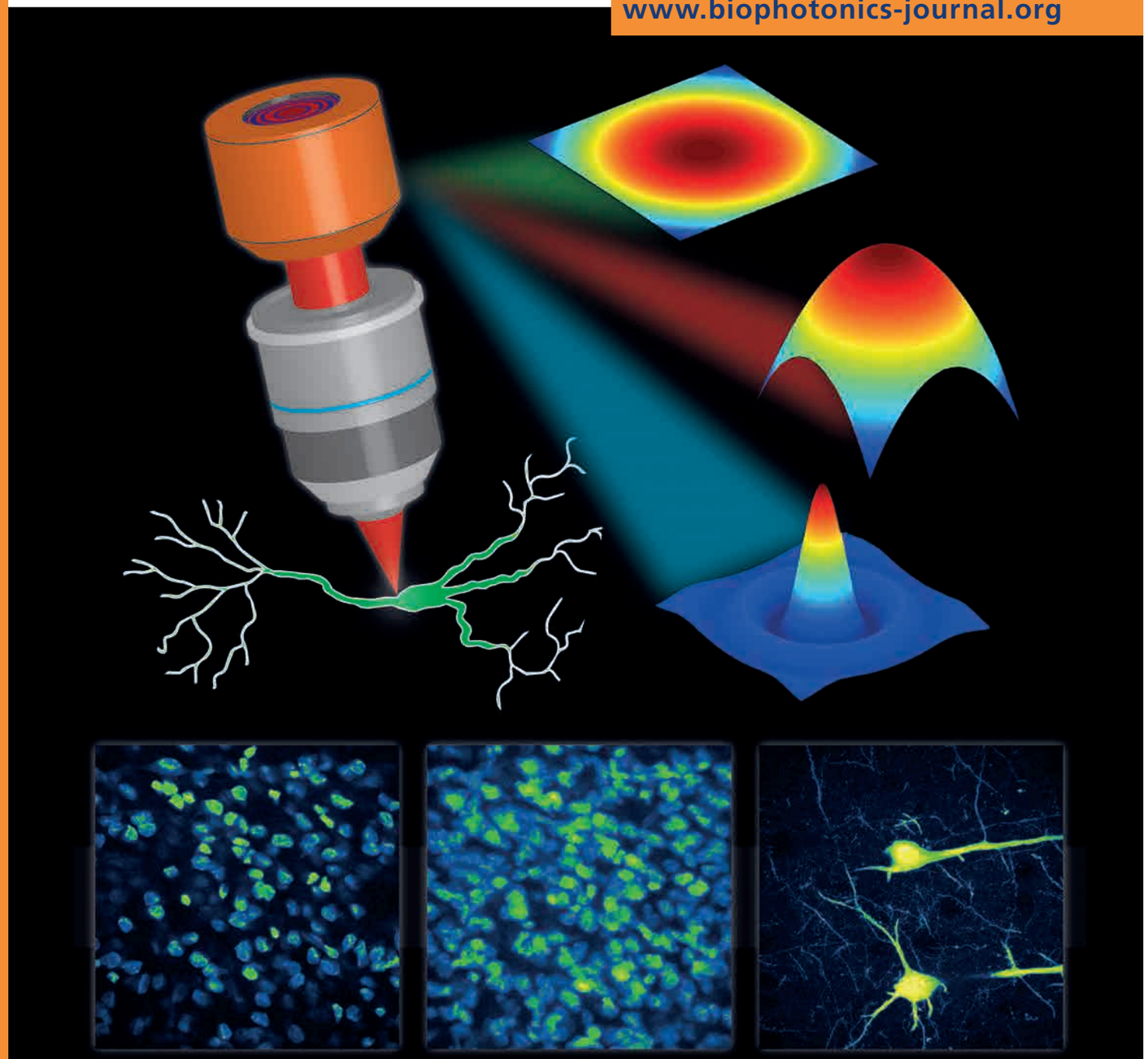


Optica 4, 367-373 (2017) - Diaspro lab

Slide Credit: Giuseppe Sancatldo,

Alberto Diaspro

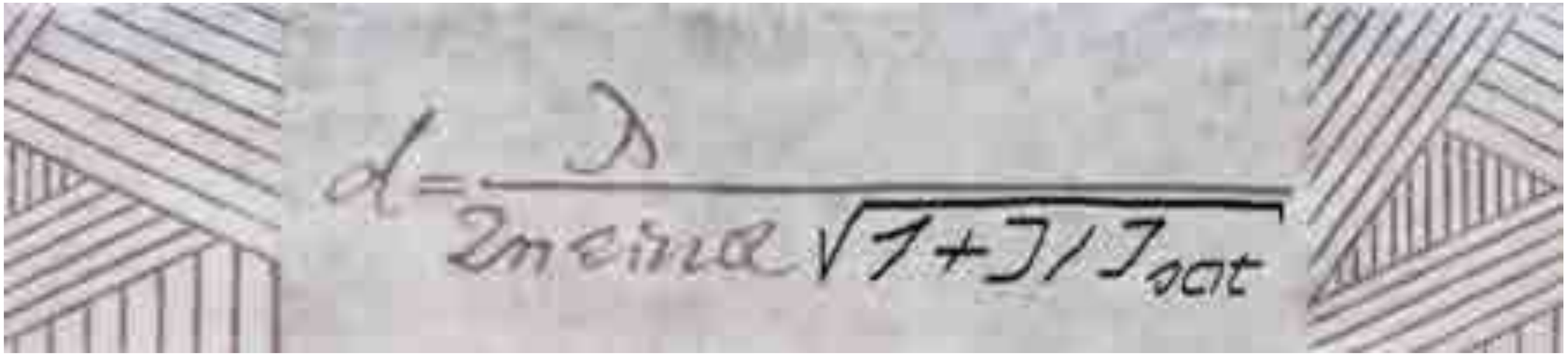
Journal of
BIOPHOTONICS
2/18
www.biophotonics-journal.org

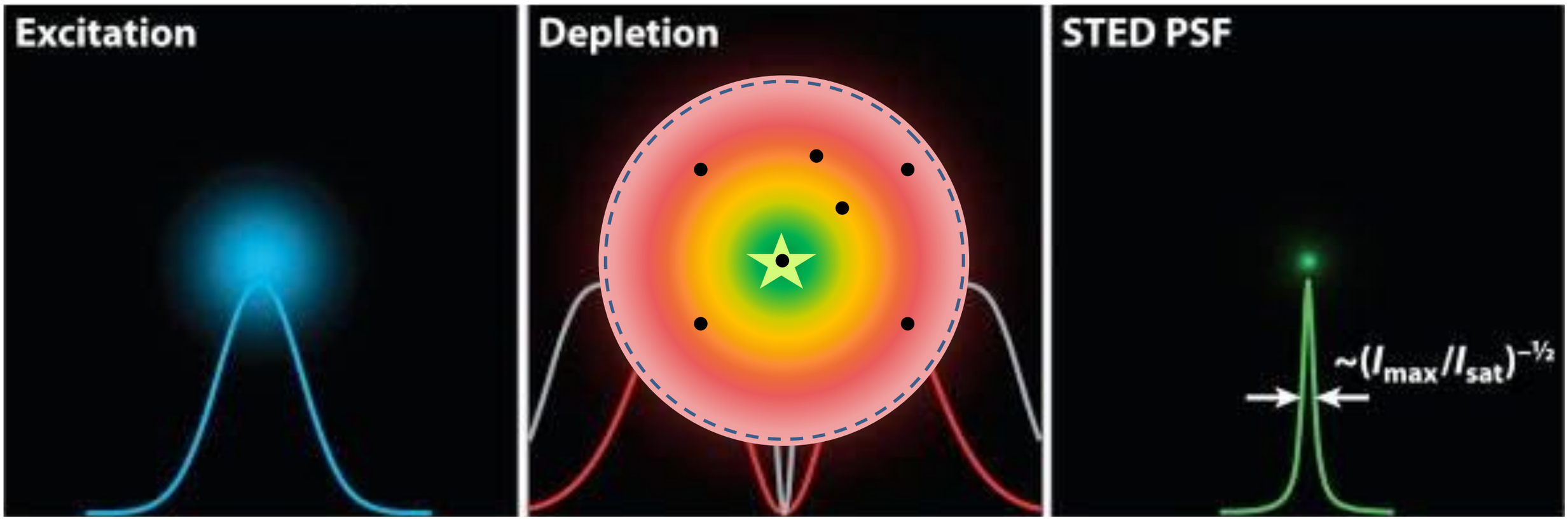


Enhanced volumetric imaging in 2-photon microscopy via acoustic lens beam shaping
Simonluca Piazza, Paolo Bianchini, Colin Sheppard, Alberto Diaspro, Martí Duocastella

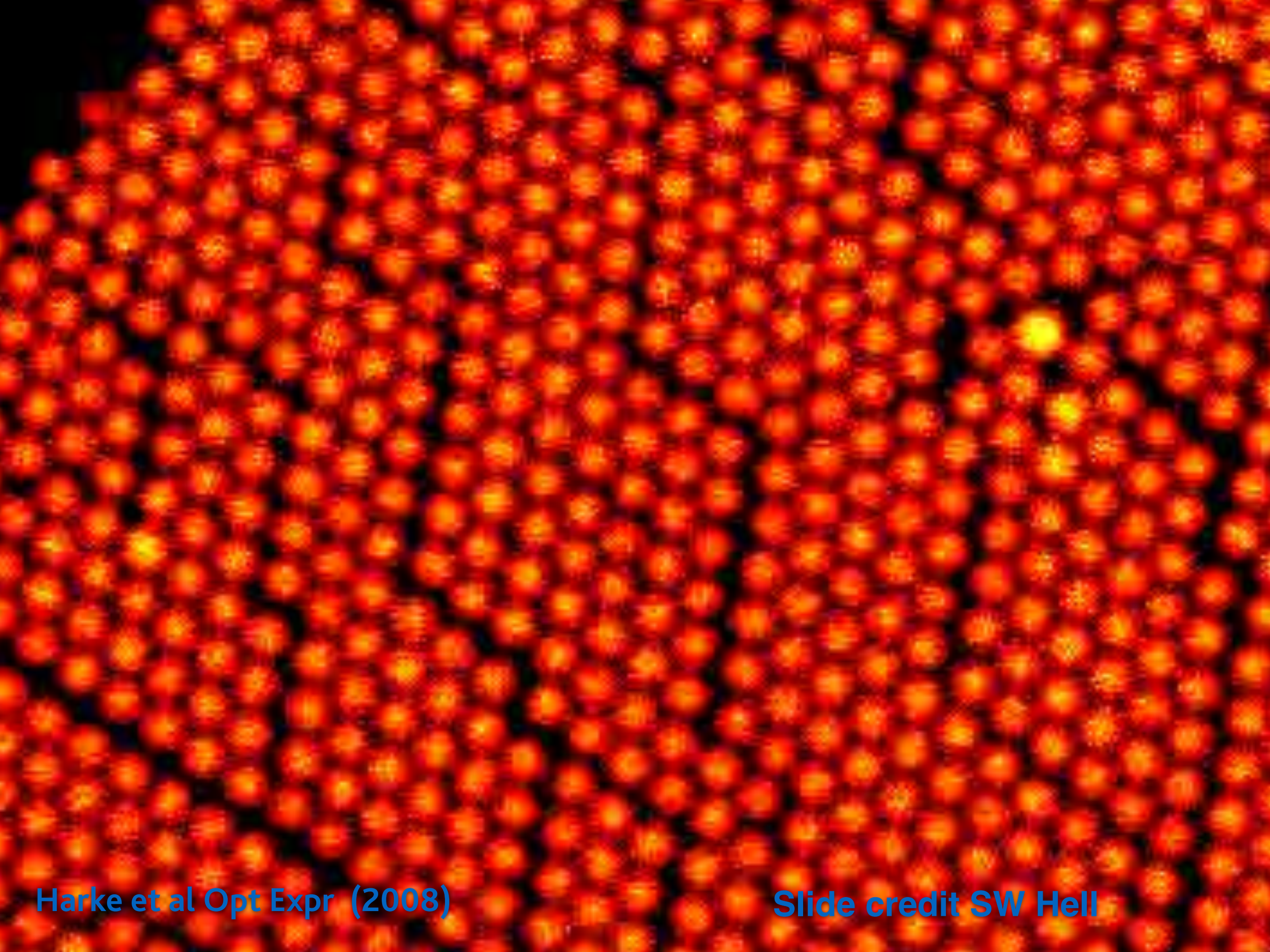
WILEY-VCH

PELLIZZA DA VOLPEDO - PANNI AL SOLE
(1894), olio su tela, 87x131 cm, Domodossola, collezione privata





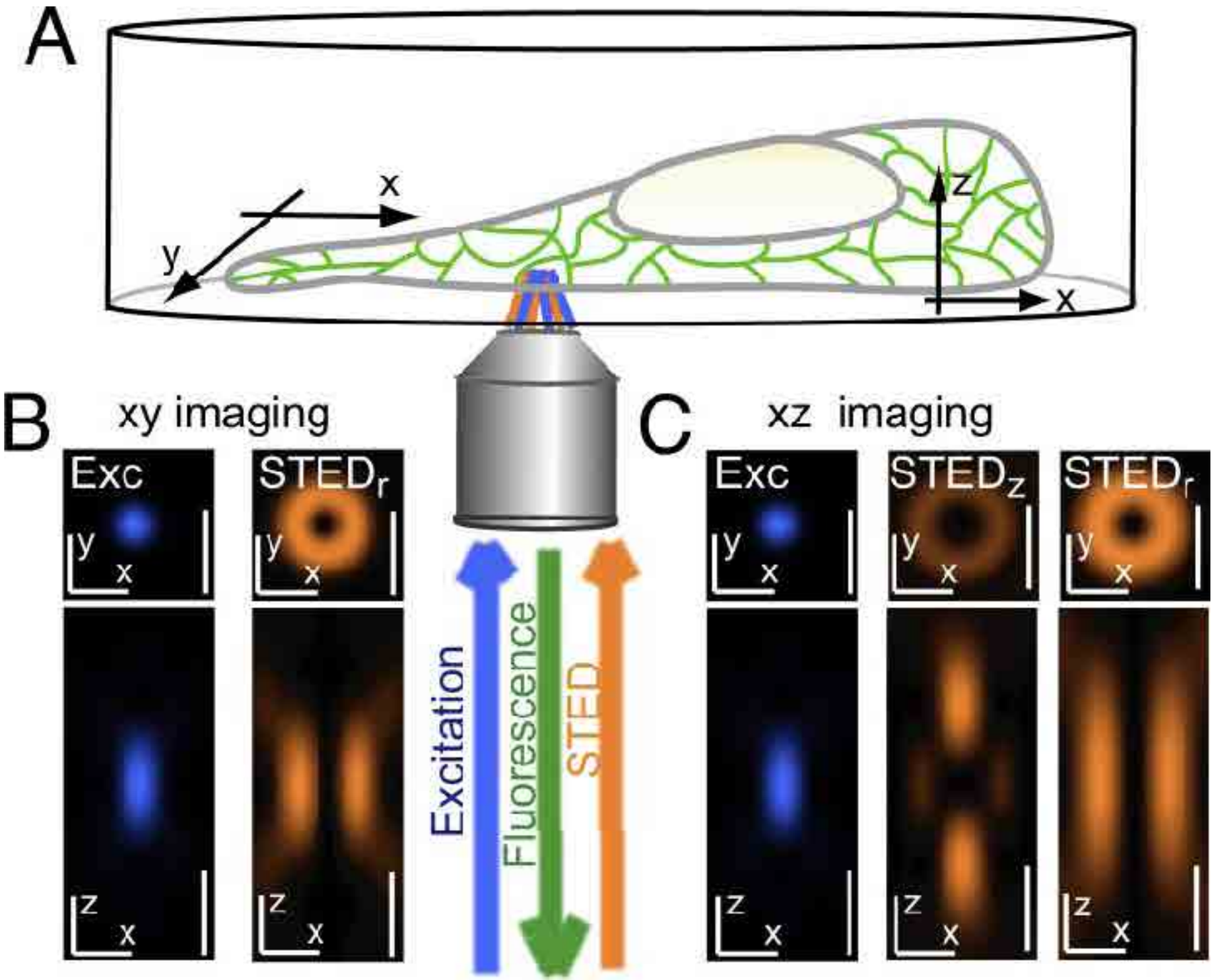
Pump-probe/Spectroscopy



Harke et al Opt Expr (2008)

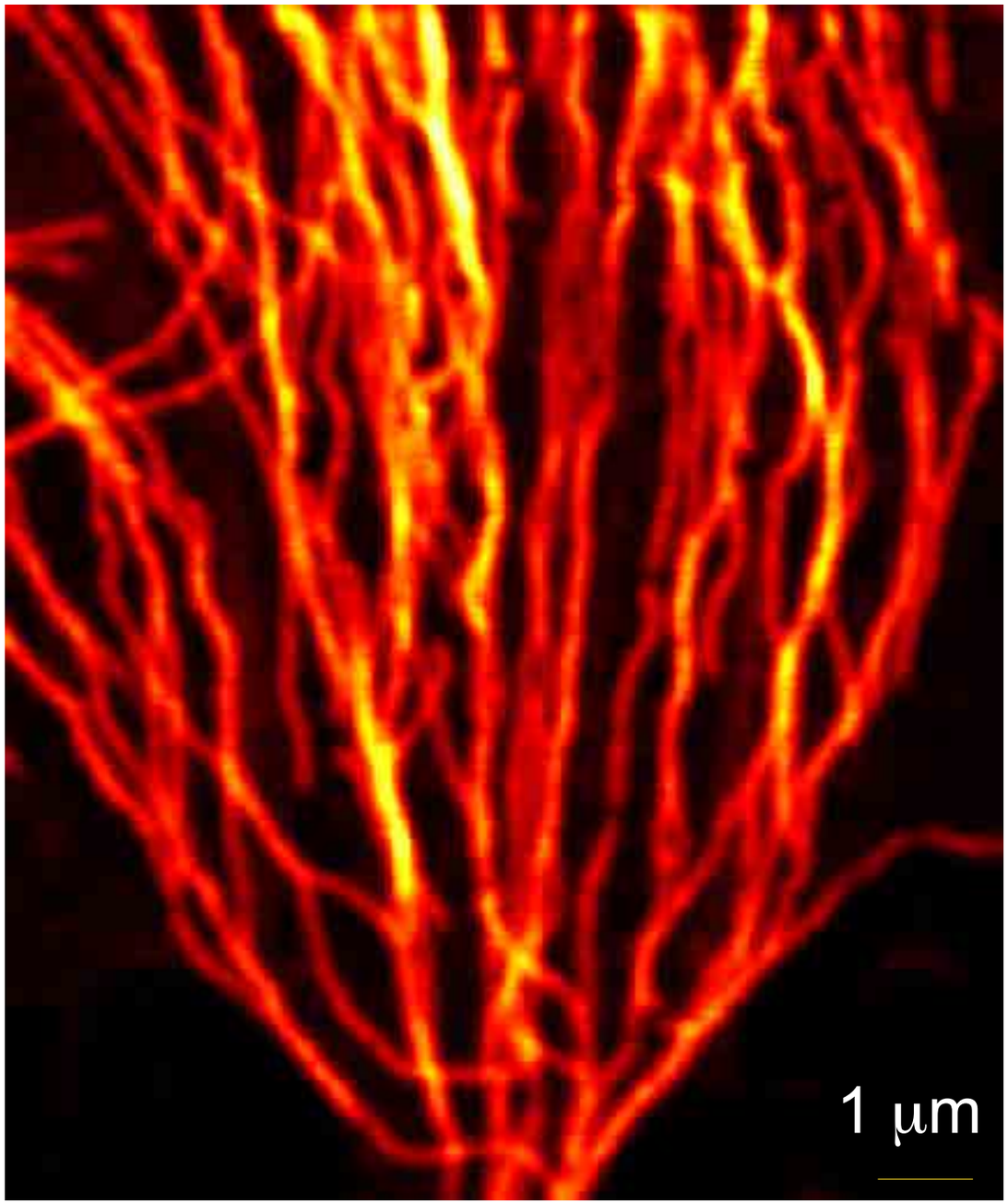
Slide credit SW Hell

STED

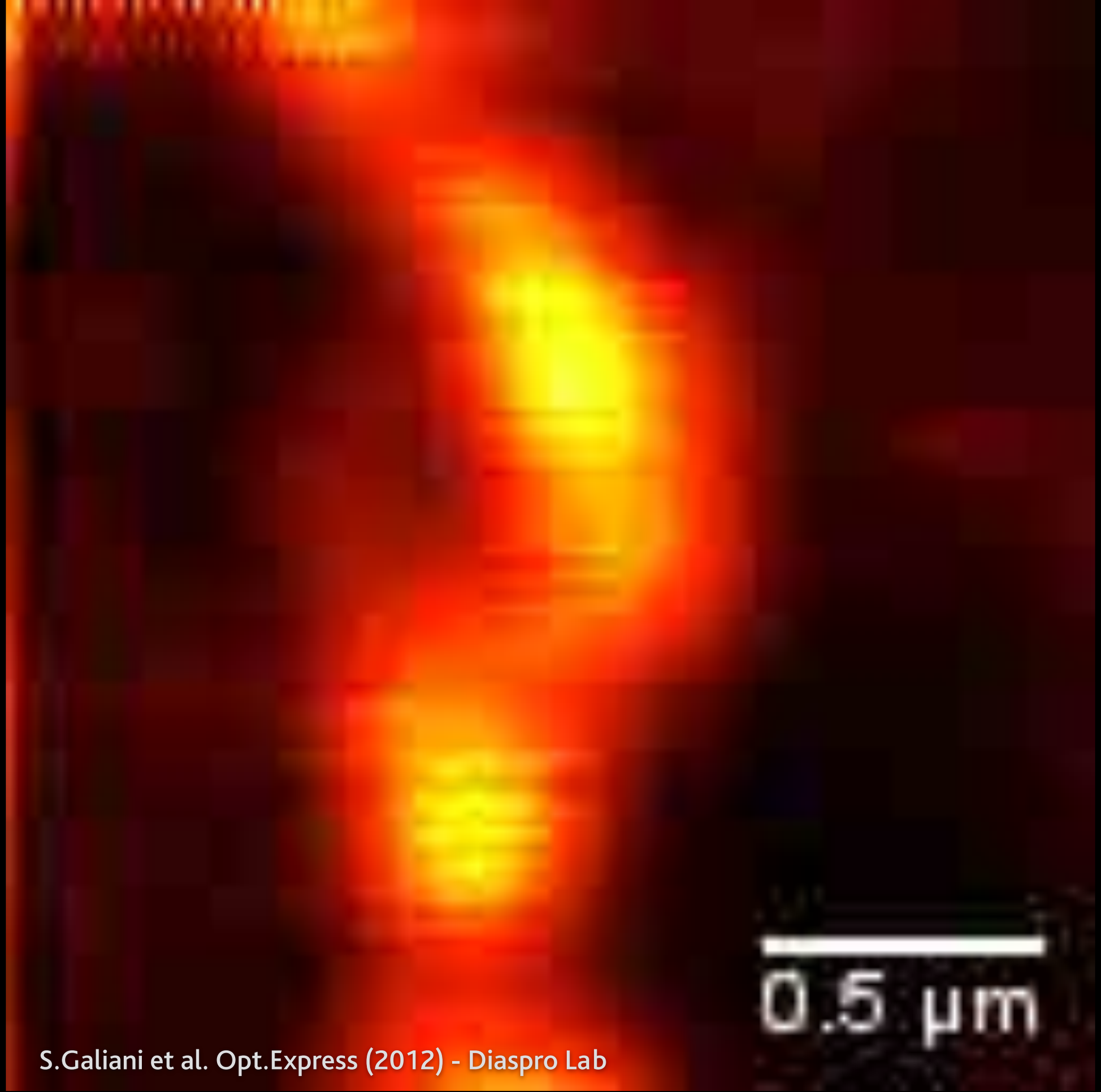

A
x
y
z
x
B
xy imaging
Exc
STED$_r$
y
x
z
x
C
xz imaging
Exc
STED$_z$
STED$_r$
Excitation
Fluorescence
STED

STED

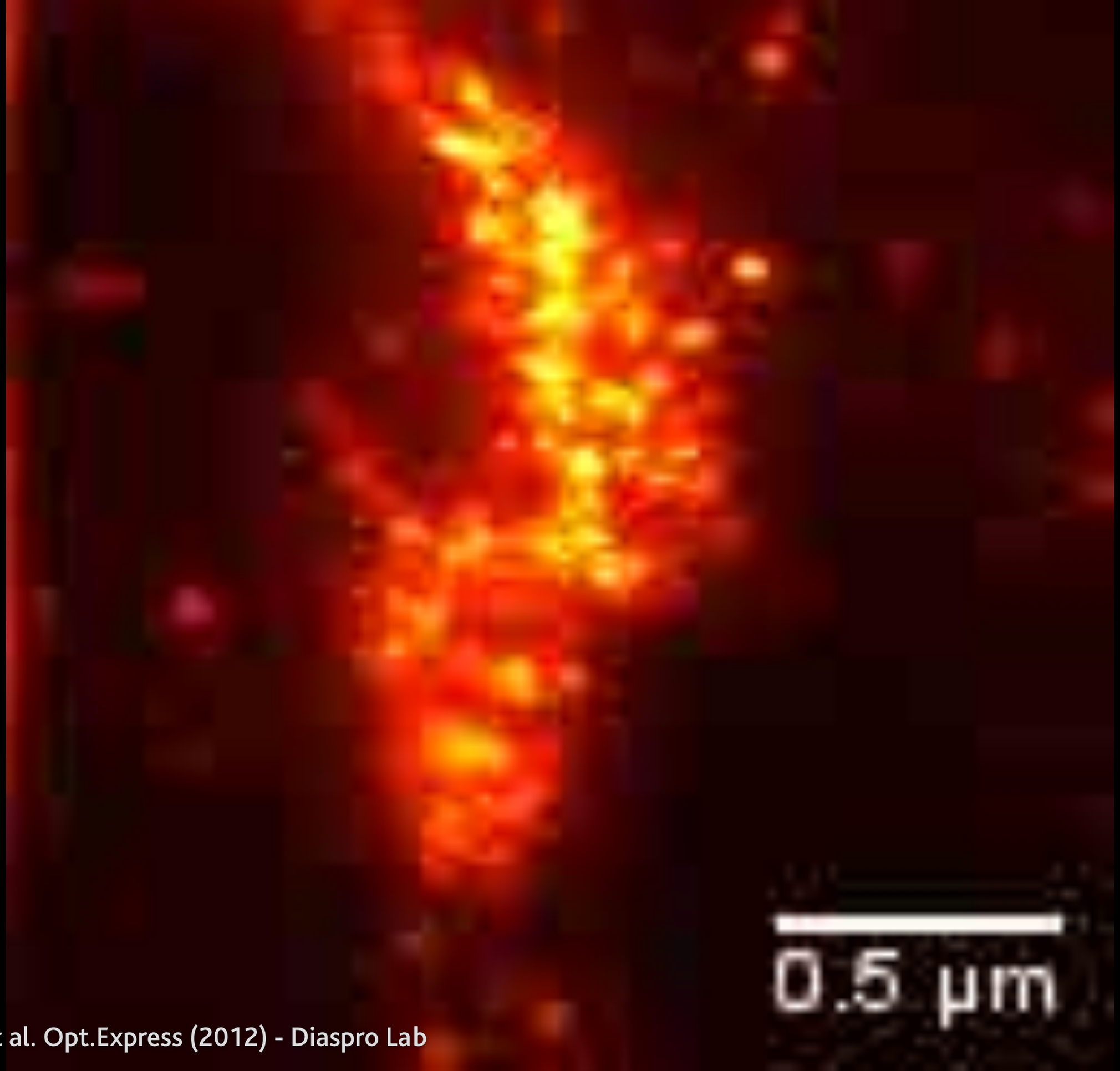
Tubulin fibers in PtK2 cells



Slide credit B. Harke

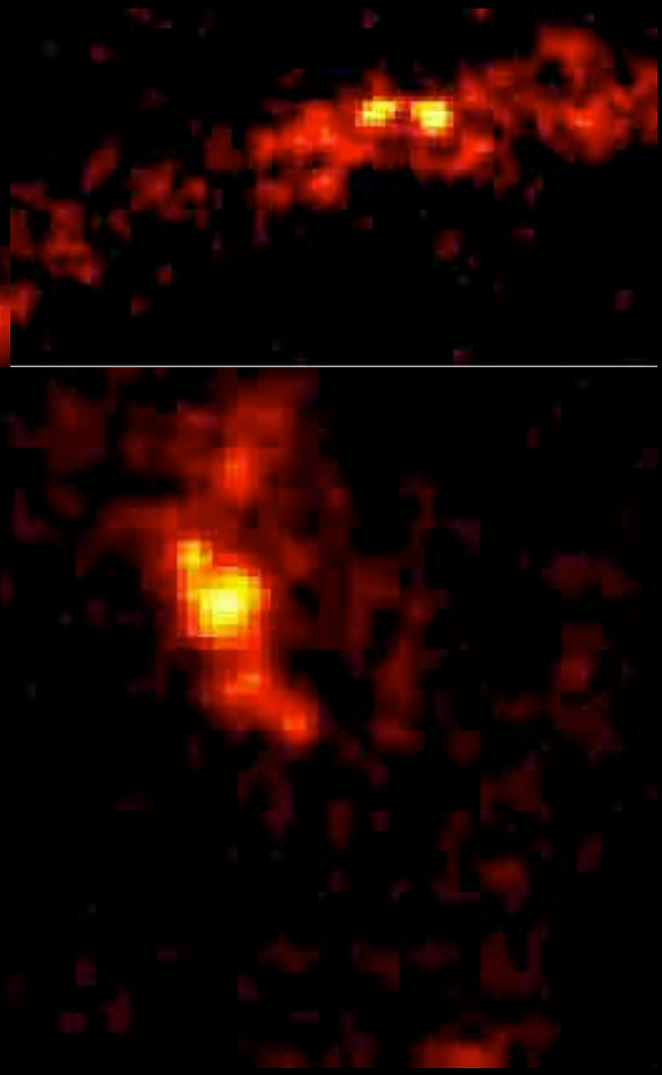


S.Galiani et al. Opt.Express (2012) - Diaspro Lab

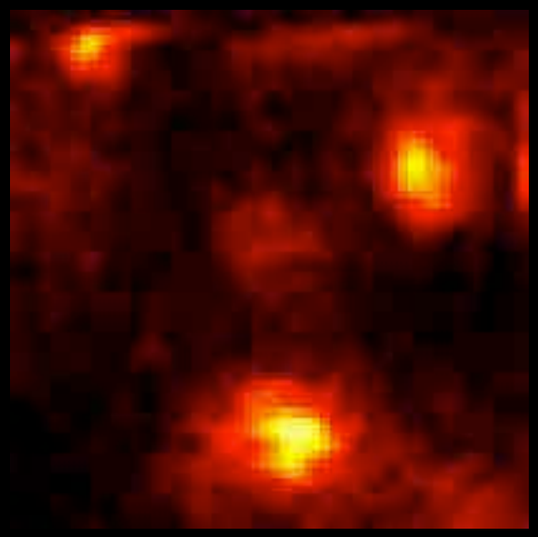


S.Galiani et al. Opt.Express (2012) - Diaspro Lab

sinapsi in vitro



biglie fluorescenti
di 40 nm



1µm

vescicole presinaptiche

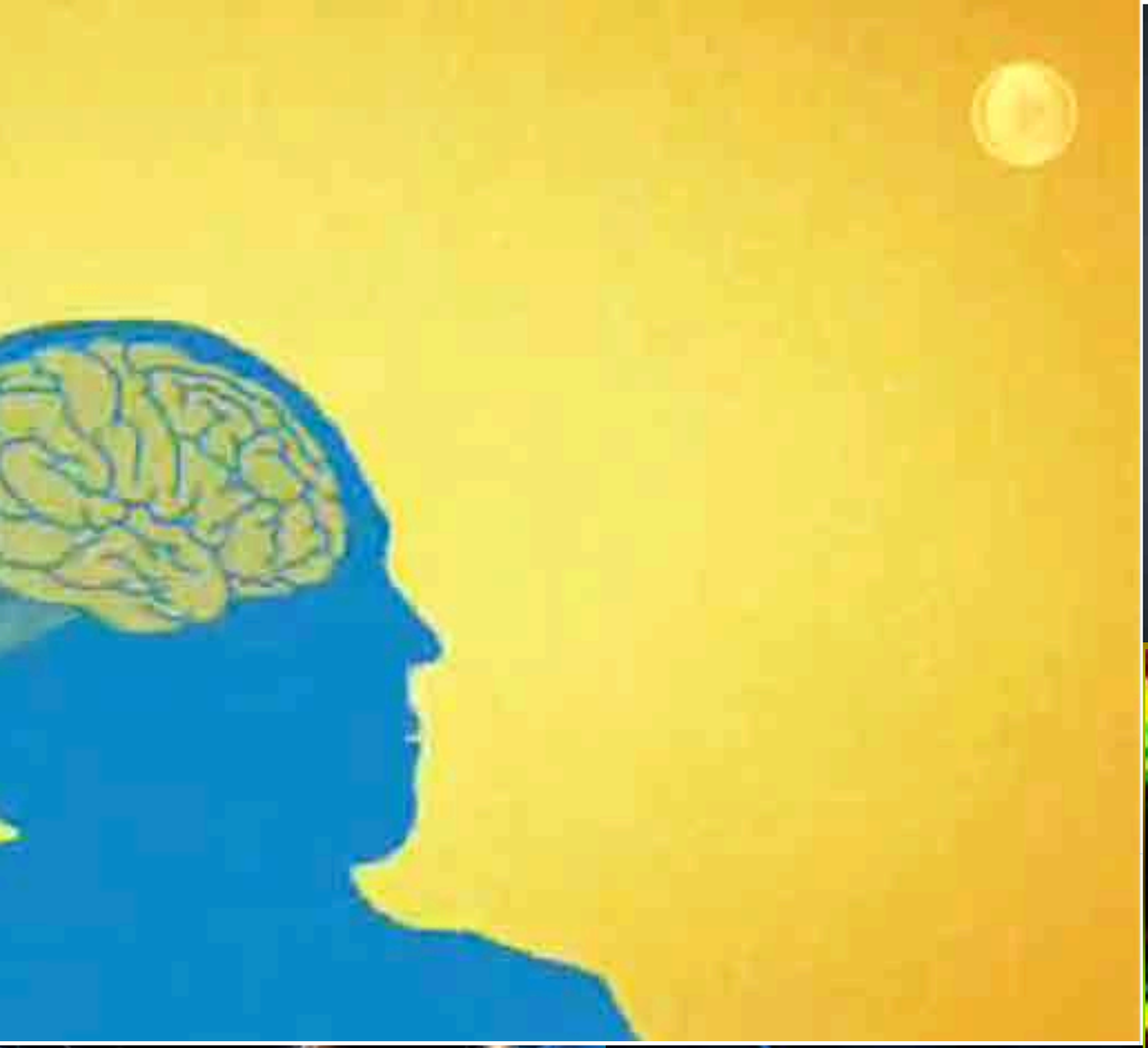
S.Galiani, P.Bianchini - Diaspro Lab

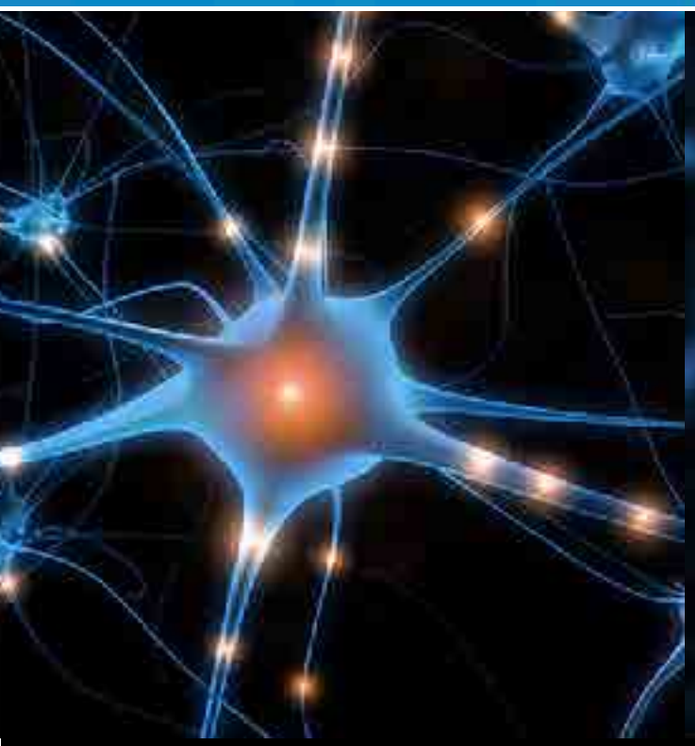
NIKON SUPER RESOLUTION SYSTEM

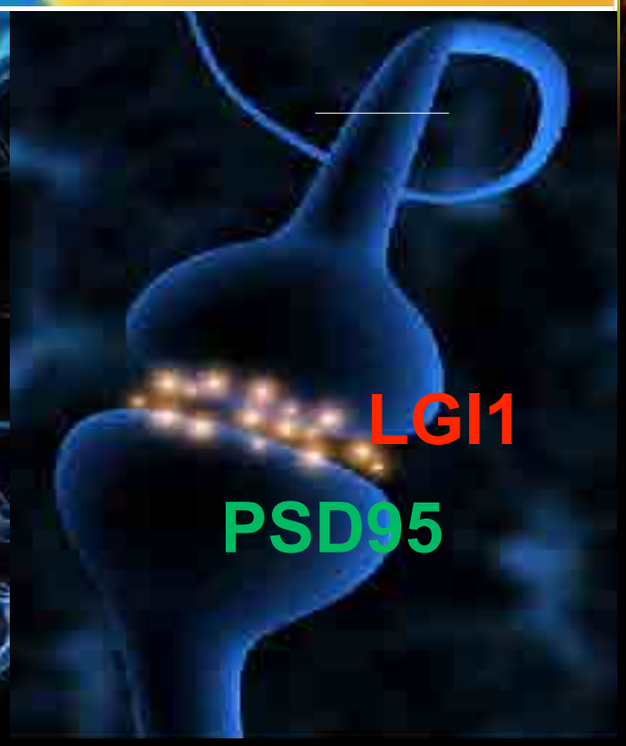
N-STORM Super Resolution System on TiE inverted Microscope and Andor camera

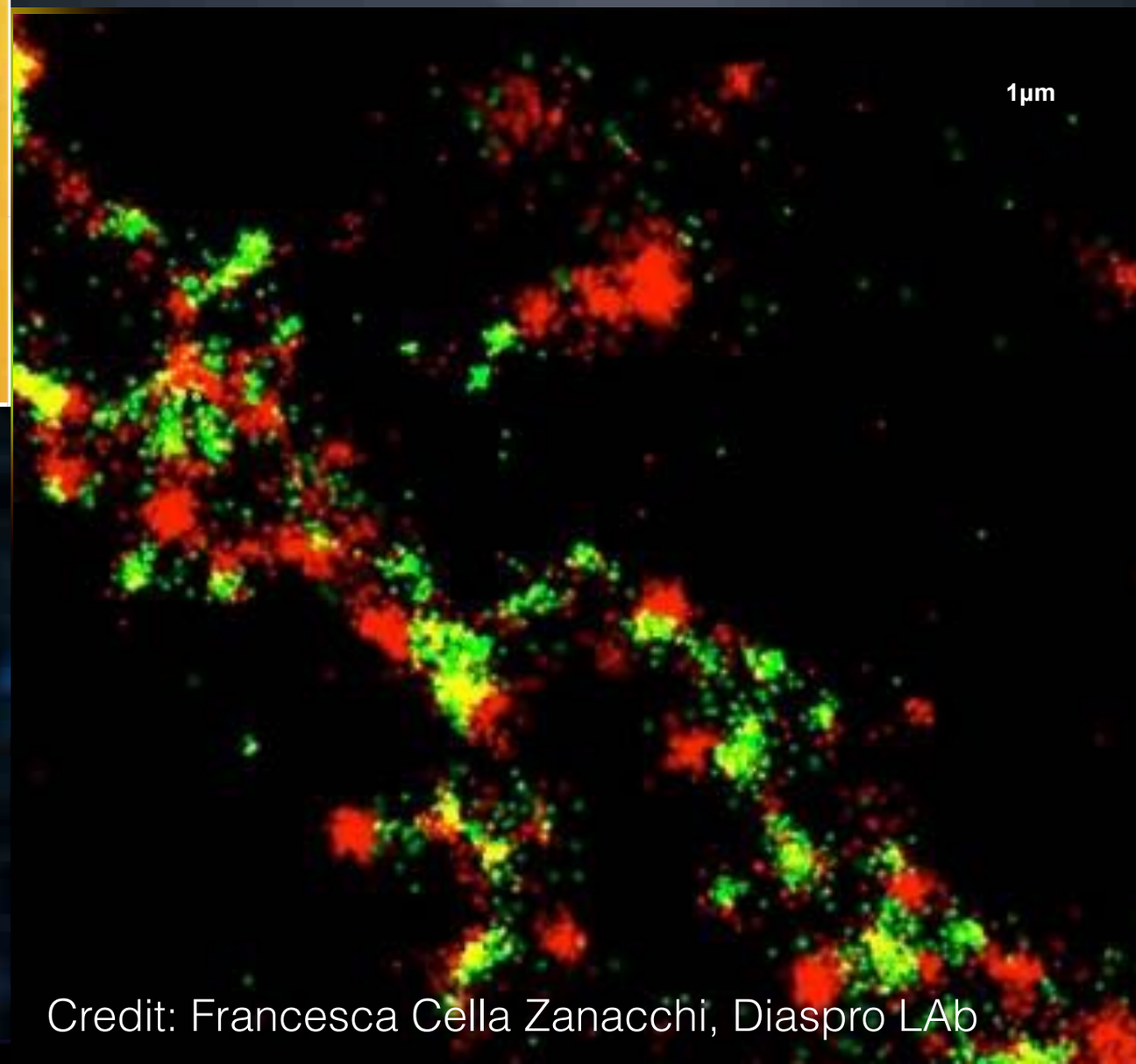
blips

magnify the world





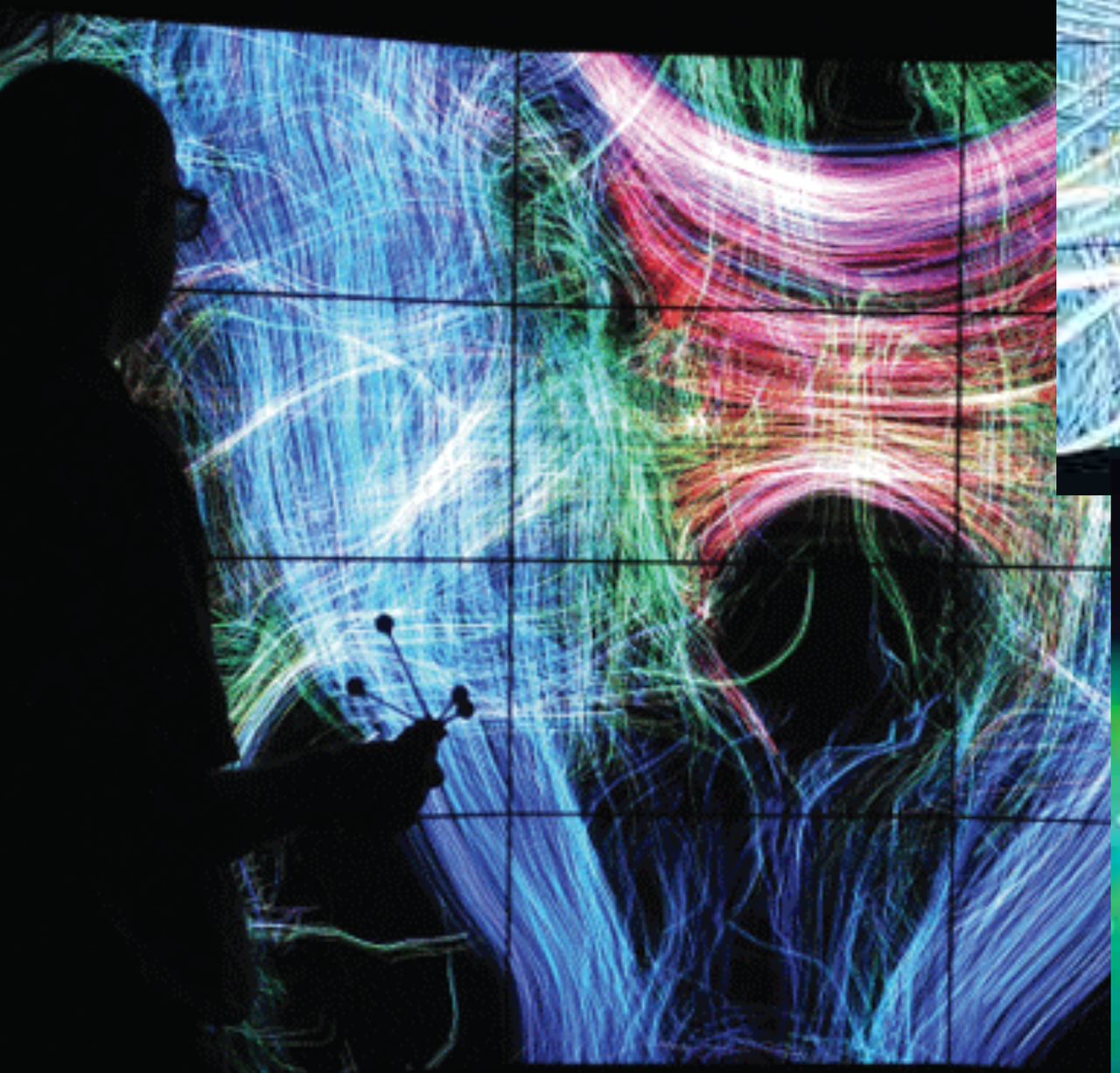




Credit: Francesca Cella Zanacchi, Diaspro LAb

nature medicine

VOLUME 20 NUMBER 3 MARC
www.nature.com/nature



Virtual reality labs for biomedicine

Targeting p38 to rejuvenate muscle stem cells

Role of Fas ligand in T-cell surveillance of tumors

Future Microscopy

Liquid Tunable Microscopy

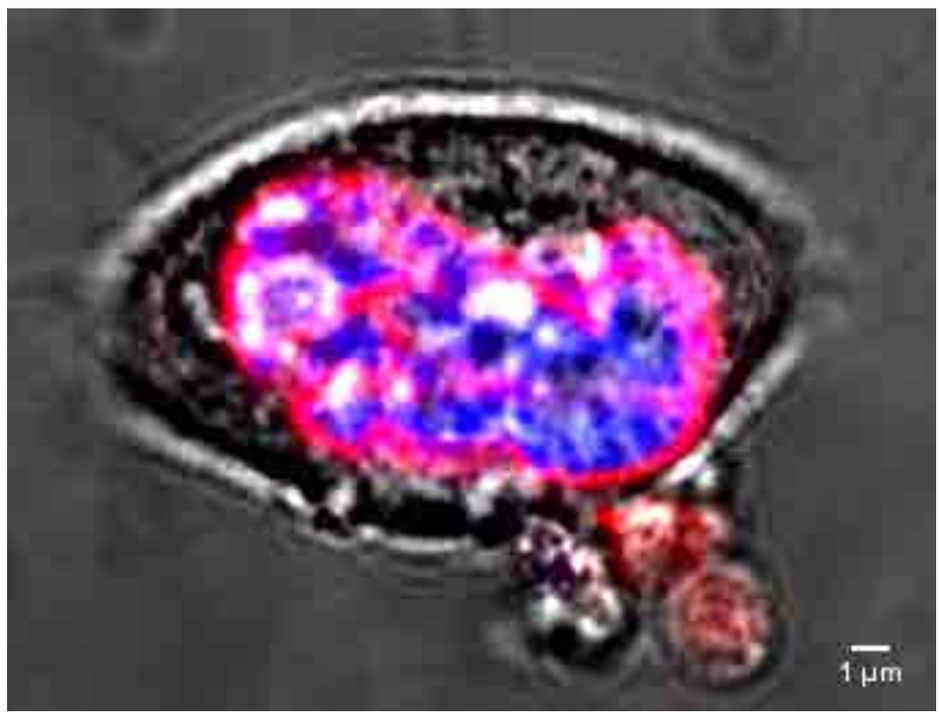
LIQUITOPY ®

Label free

Mueller Matrix, CIDS, Phase

Fluorescence dynamics

Exploring the environment at the nanoscale



Credit Luca Pesce,Diaspro Lab 2017

LIQUITOPY ®

Converging Approach

CORRELATIVE EM/SPM

FRET

FCS

FRAP

FLIM

Anisotropy

Probes Approach
Photo-Physical Properties

Optics Approach
Optics/Light Properties

clearing

EXPANSION

Non-Linear

Saturation

Interference Techniques

stochastic read out

targeted read-out

2PE

SHG MPE

Computational

LABEL FREE MUELLER

Confocal ISM

SPIM

STORM GSDIM

PALM

IML-SPIM

STED

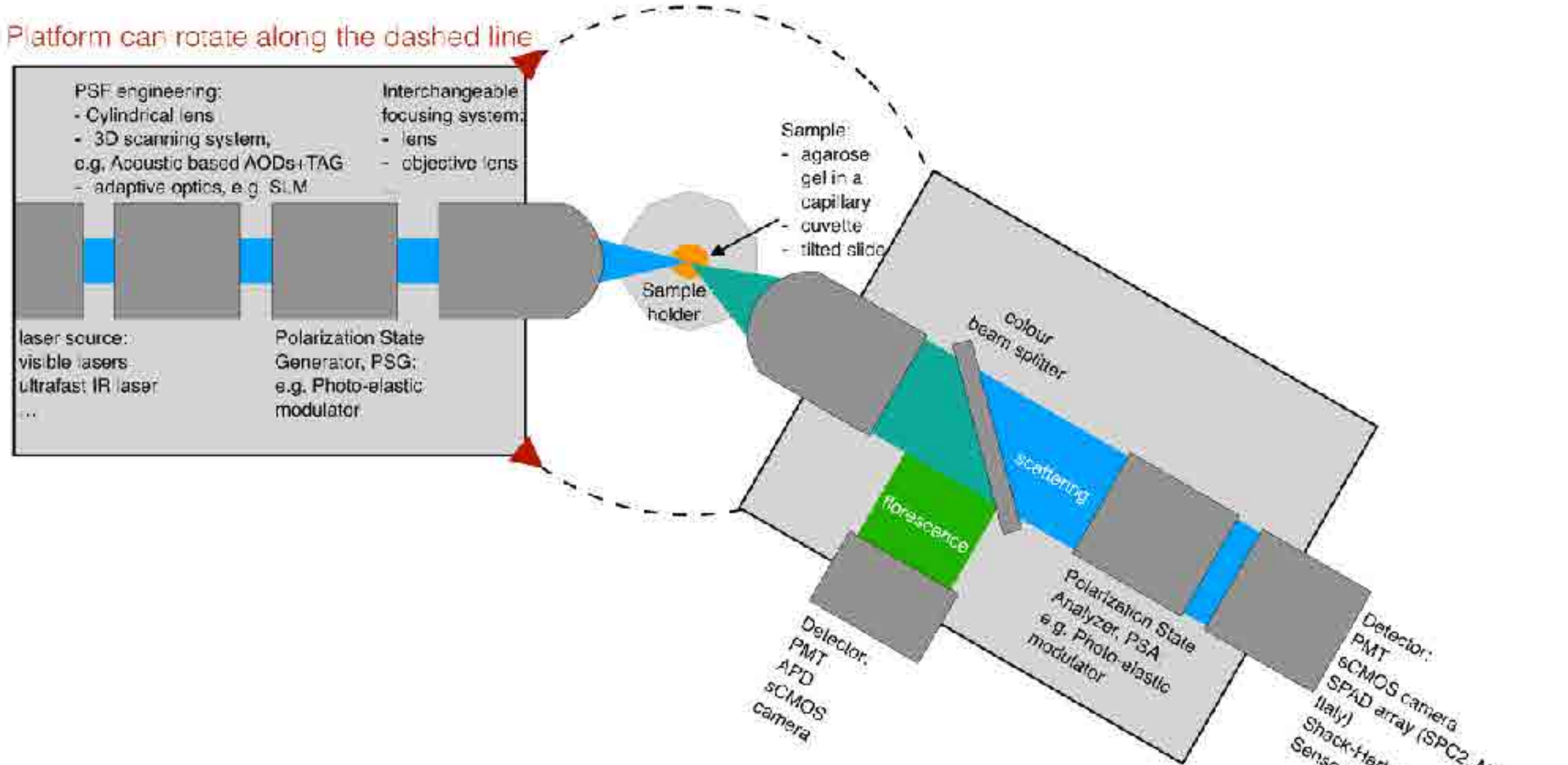
SPLIT-STED

MPE-STED

SW 2PE-STED

FCS-STED

credit: Paolo Bianchini, LAMBS IIT



credit: Paolo Bianchini, Diaspro Lab, LAMBS IIT

LIQUID

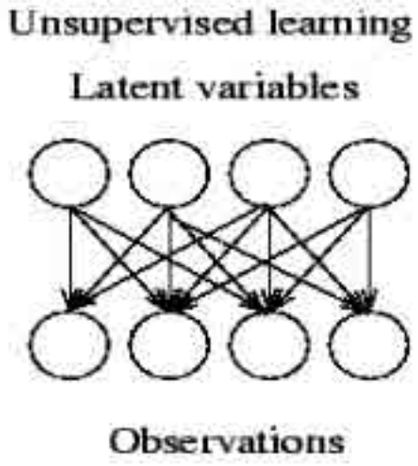
SOLID

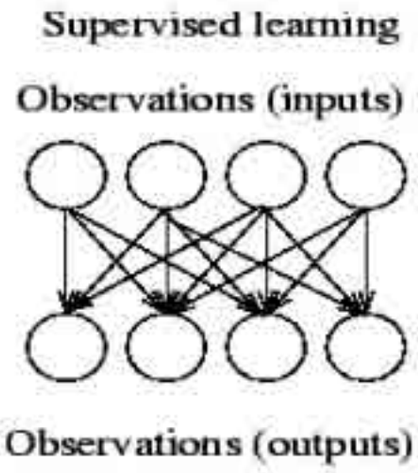
Paul Klee, The Castle and the sun, 1928

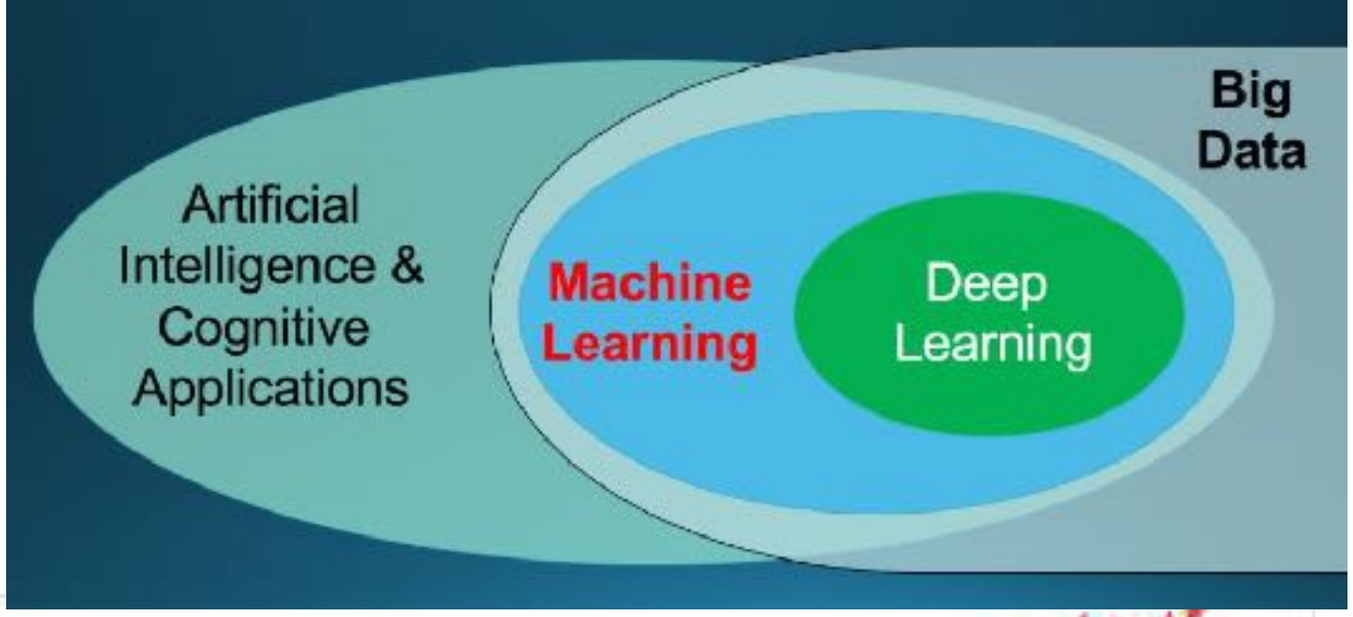
Sandro Botticelli, Nascita di Venere (1482-1485)

Liquid Tunable Microscopy

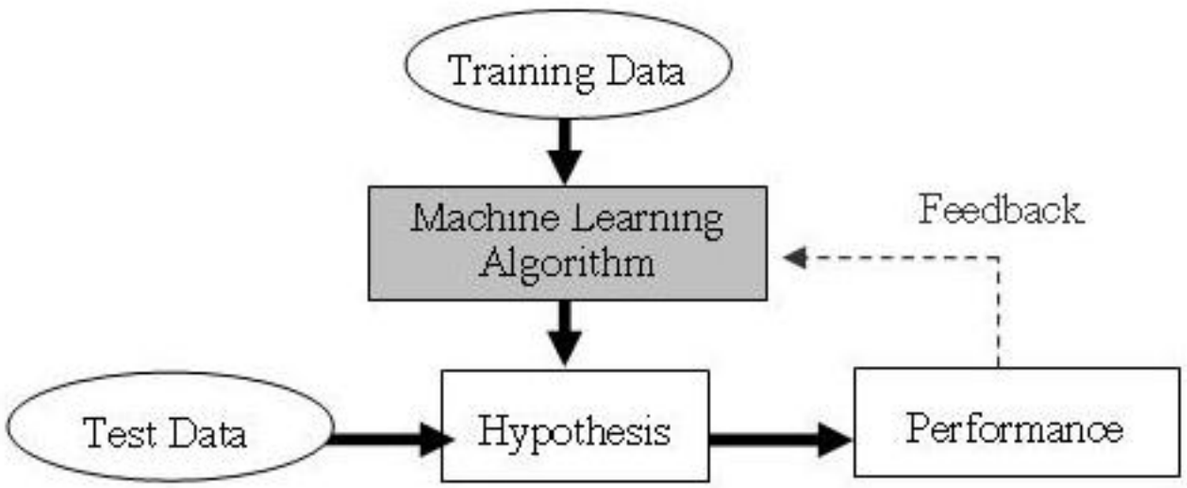
LIQUITOPY

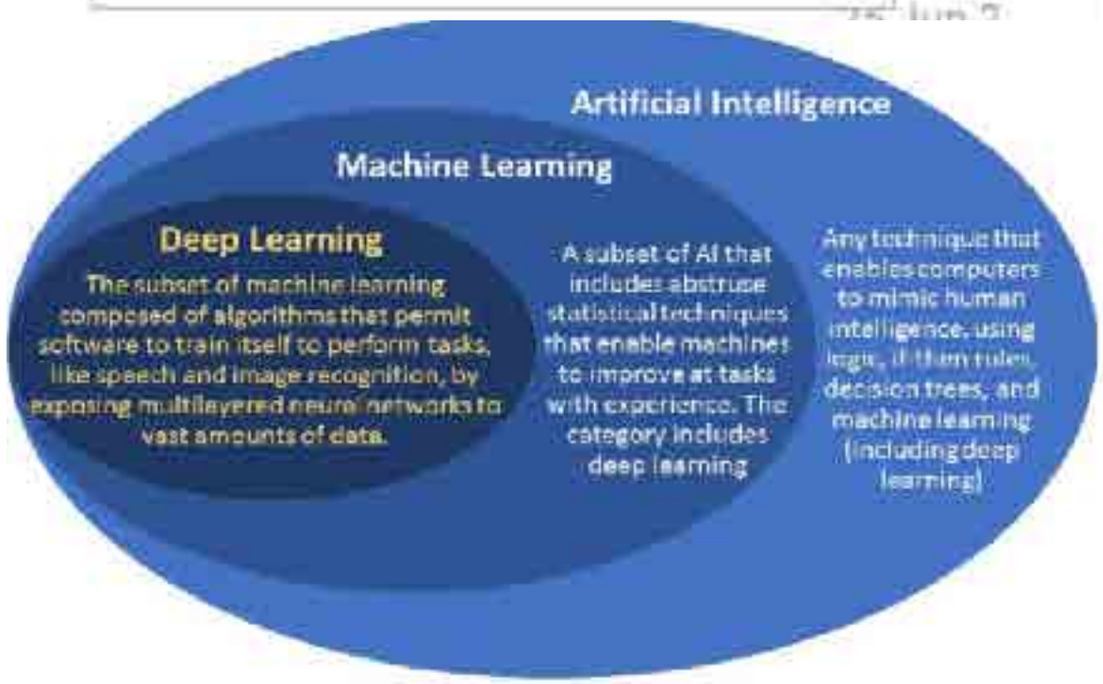

Unsupervised learning
Latent variables
Observations

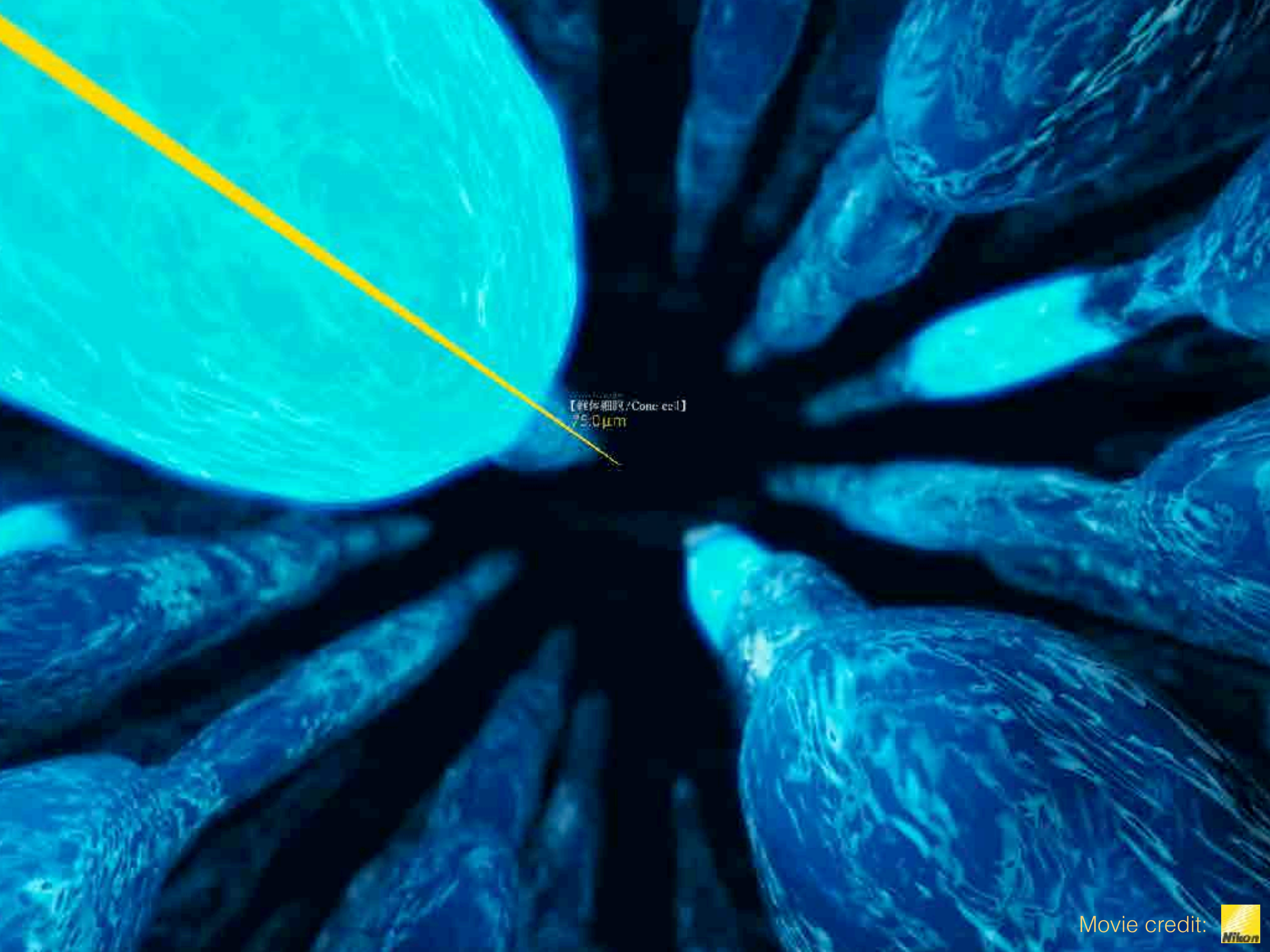

Supervised learning
Observations (inputs)
Observations (outputs)


Big Data
Artificial Intelligence & Cognitive Applications
Machine Learning
Deep Learning


100
75
50
25
26 Aug 20...
25 Jan 2015
20 Aug - 26 Aug 2017
deep learning 46
Machine Learning 100
Values for this point have partial (incomplete) data available


Training Data
Machine Learning Algorithm
Feedback
Test Data
Hypothesis
Performance


Artificial Intelligence
Machine Learning
Deep Learning
The subset of machine learning composed of algorithms that permit software to train itself to perform tasks, like speech and image recognition, by exposing multilayered neural networks to vast amounts of data.
A subset of AI that includes abstruse statistical techniques that enable machines to improve at tasks with experience. The category includes deep learning
Any technique that enables computers to mimic human intelligence, using logic, if-then rules, decision trees, and machine learning (including deep learning)



Movie credit:

LIQUITOPY could provide the opportunity to see things differently and to change our point of view, abandoning the obsession of representing the “real world” when forming an image (AD, 2017).

- **Hugo Pratt, Celtiche**

Liquid Tunable Microscopy
LIQUITOPY

Paolo Bianchini
Francesca Cella Zanacchi
Aymeric Le Gratiet
Colin JR Sheppard
Melody Dibona
Amira El Merhie
Michela Cosentino
Simone Pelicci
Riccardo Marongiu
Luca Lanzanò
Nicholas Anthony
Silvia Dante
Salvatore Surdo
Simonluca Piazza
Luca Pesce
Barbara Salis
Silvia Scalisi
Artemi Bendandi
Martì Duocastella
Claudio Canale
Takahiro Deguchi
Michele Oneto
Lorenzo Scipioni
Giulia Zanini
Marco Cozzolino
Isotta Cainero
Agnieszka Pierzyńska-Mach

Genoa Instruments

Nanoscopy group and Molecular Microscopy and Spectroscopy group retreat in Sestri Levante (Genova, Italy) 2017

"La mejor forma de decir, es hacer" José Marti, Cuba.

POP MICROSCOPY

Collezione di immagini al microscopio di sistemi viventi ideata da Alberto Diaspro e curata da Claudia Diaspro con il supporto tecnico scientifico di Paolo Bianchini, realizzata al NIC@IIT, Nikon Imaging Center presso l'Istituto Italiano di Tecnologia. Si ringraziano per il supporto Camera di Commercio di Genova e Nikon Instruments Italia. Novembre 2015.

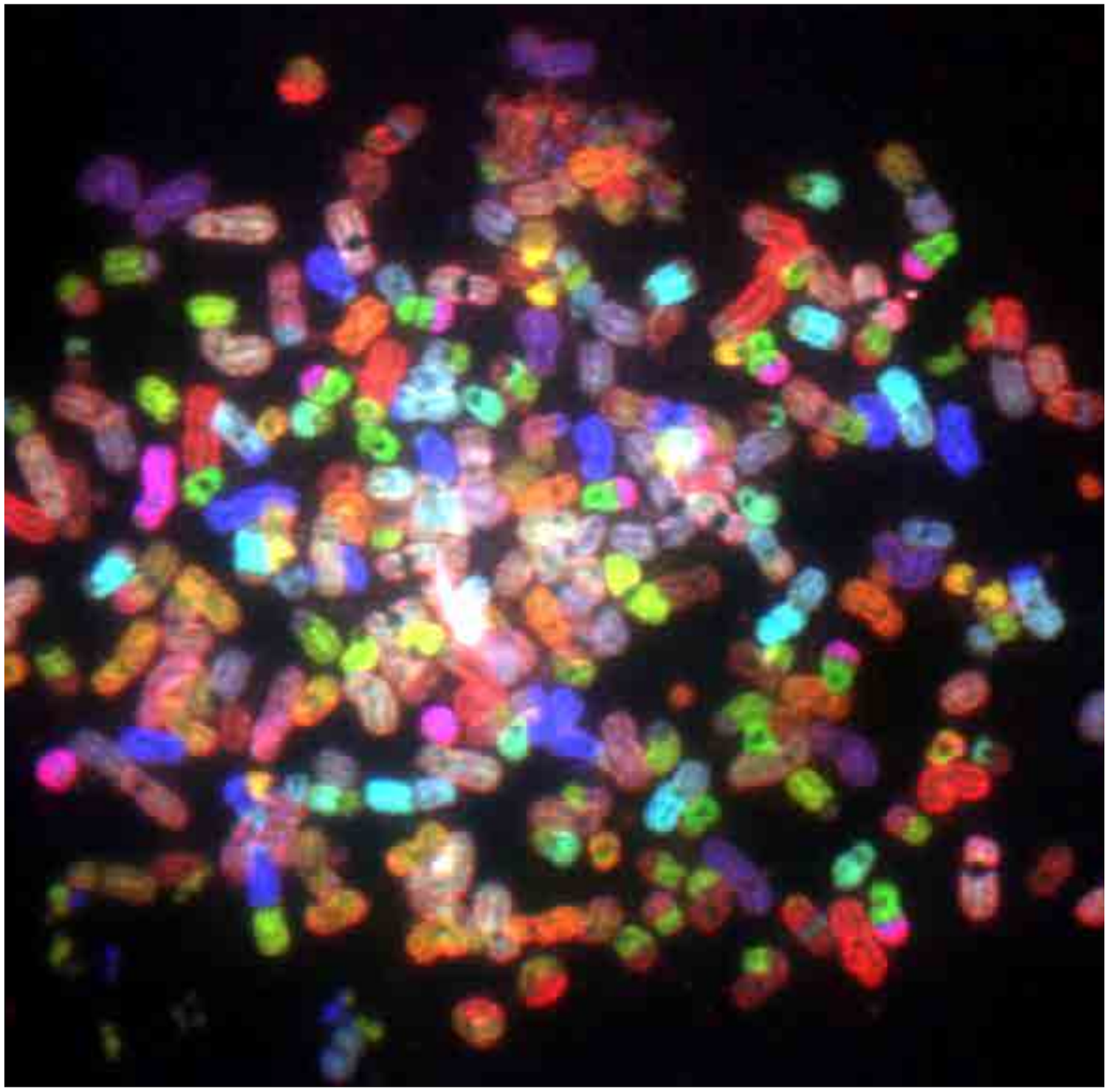
Nikon

NIKON IMAGING CENTRE @ iit ISTITUTO ITALIANO DI TECNOLOGIA

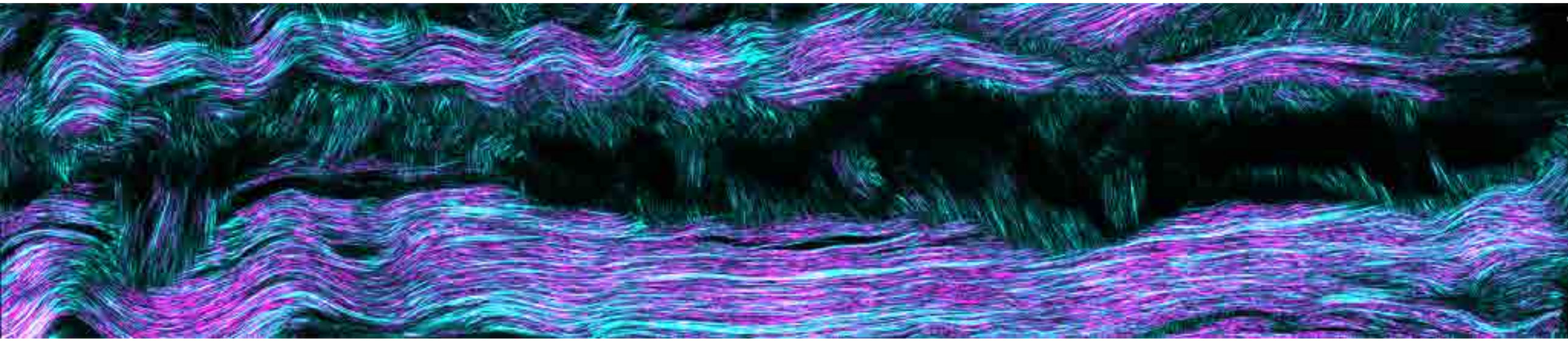
Camera di Commercio Genova

trattamento immagini Lab PHOTO SERVICE

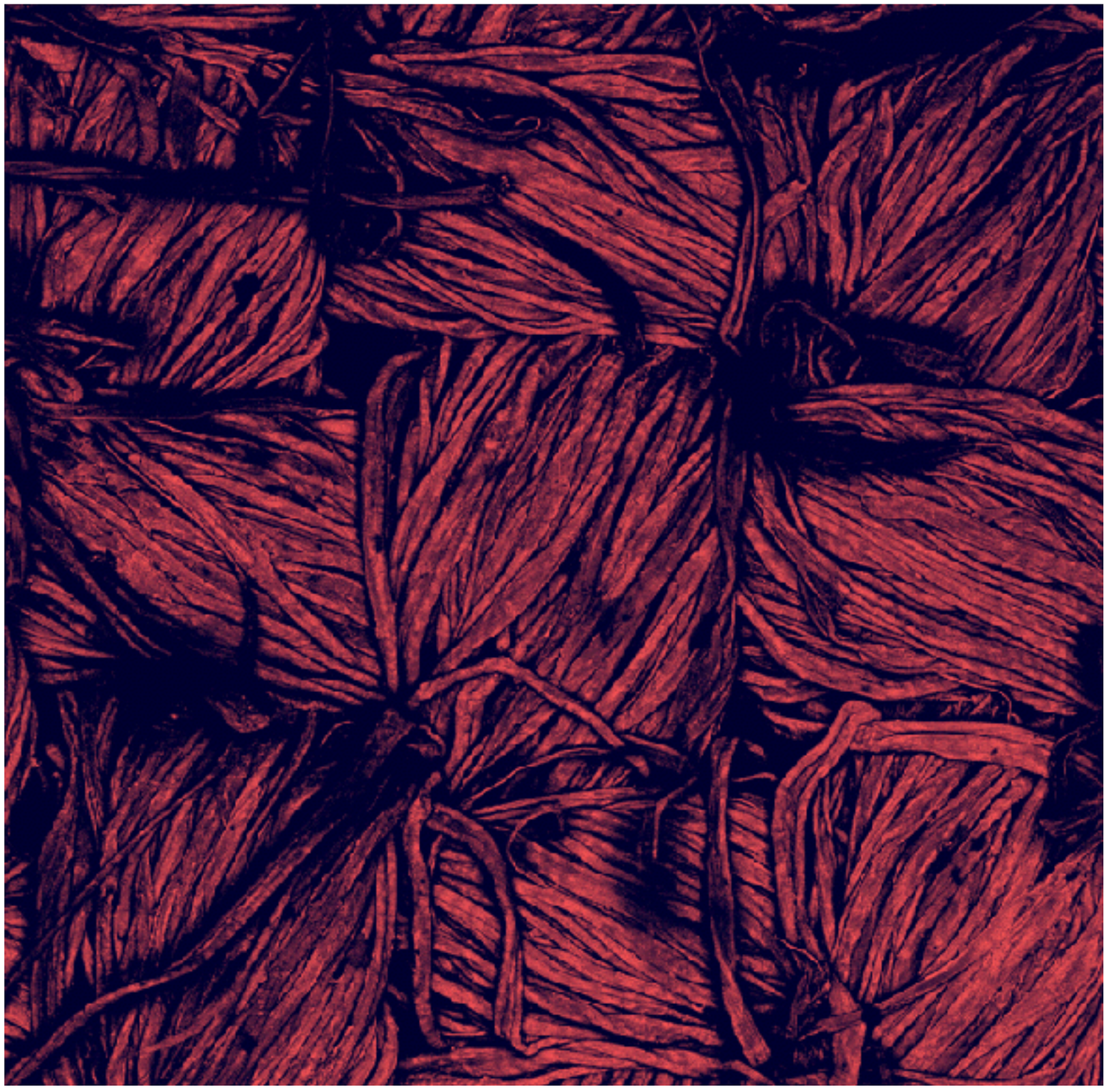
THE SCIENTIST



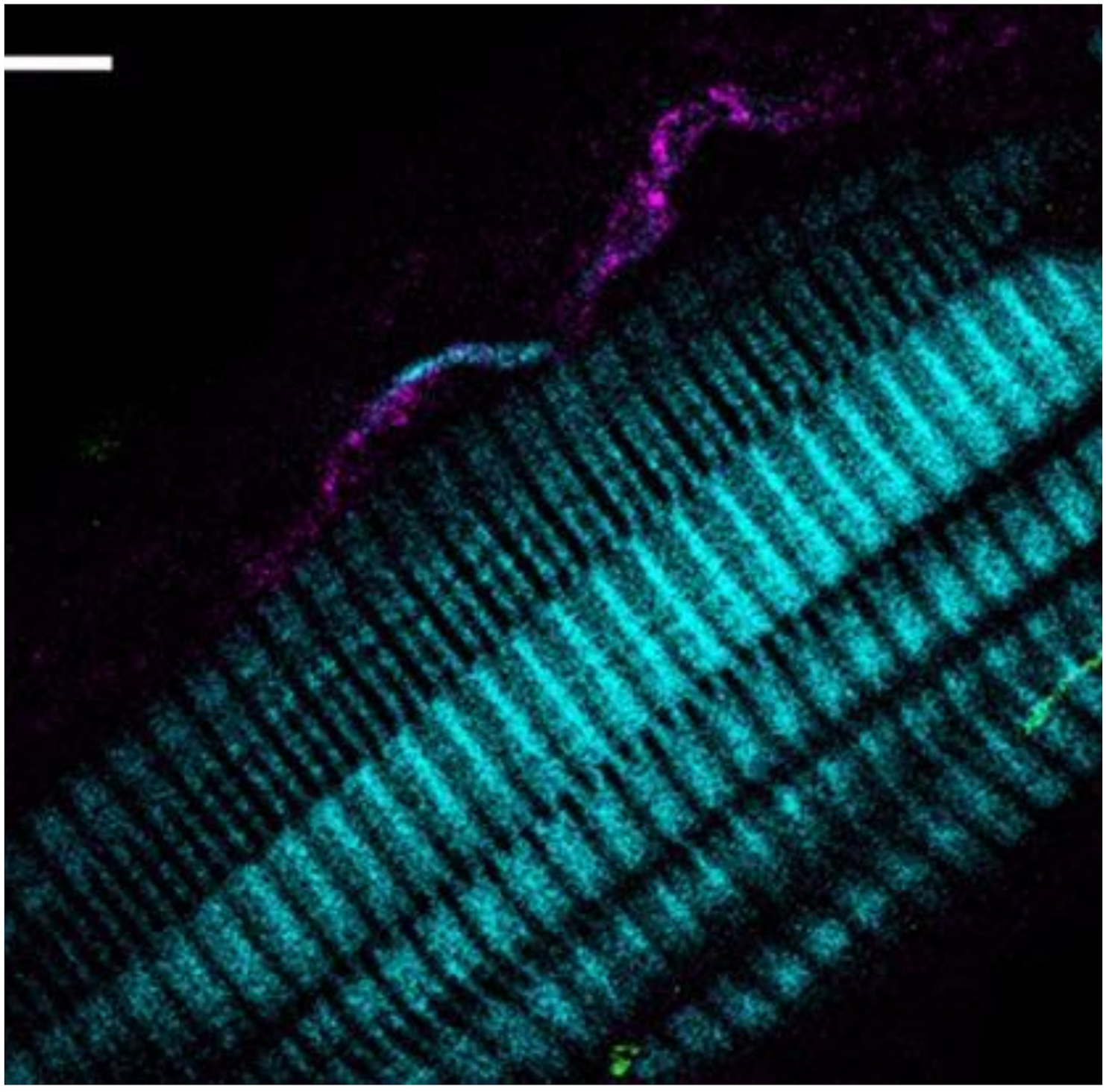
OFELIA



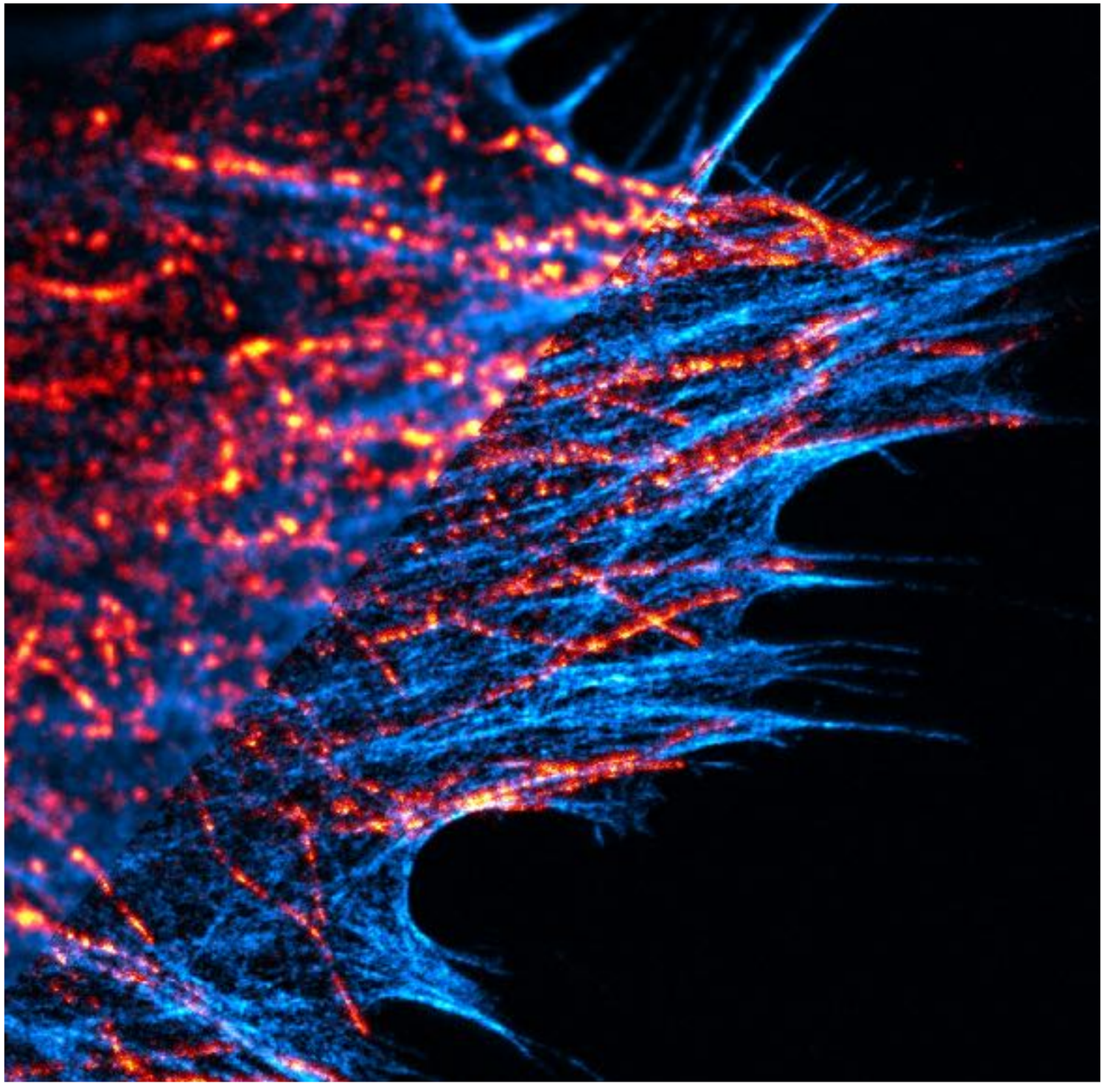
WOOL



MERMAID

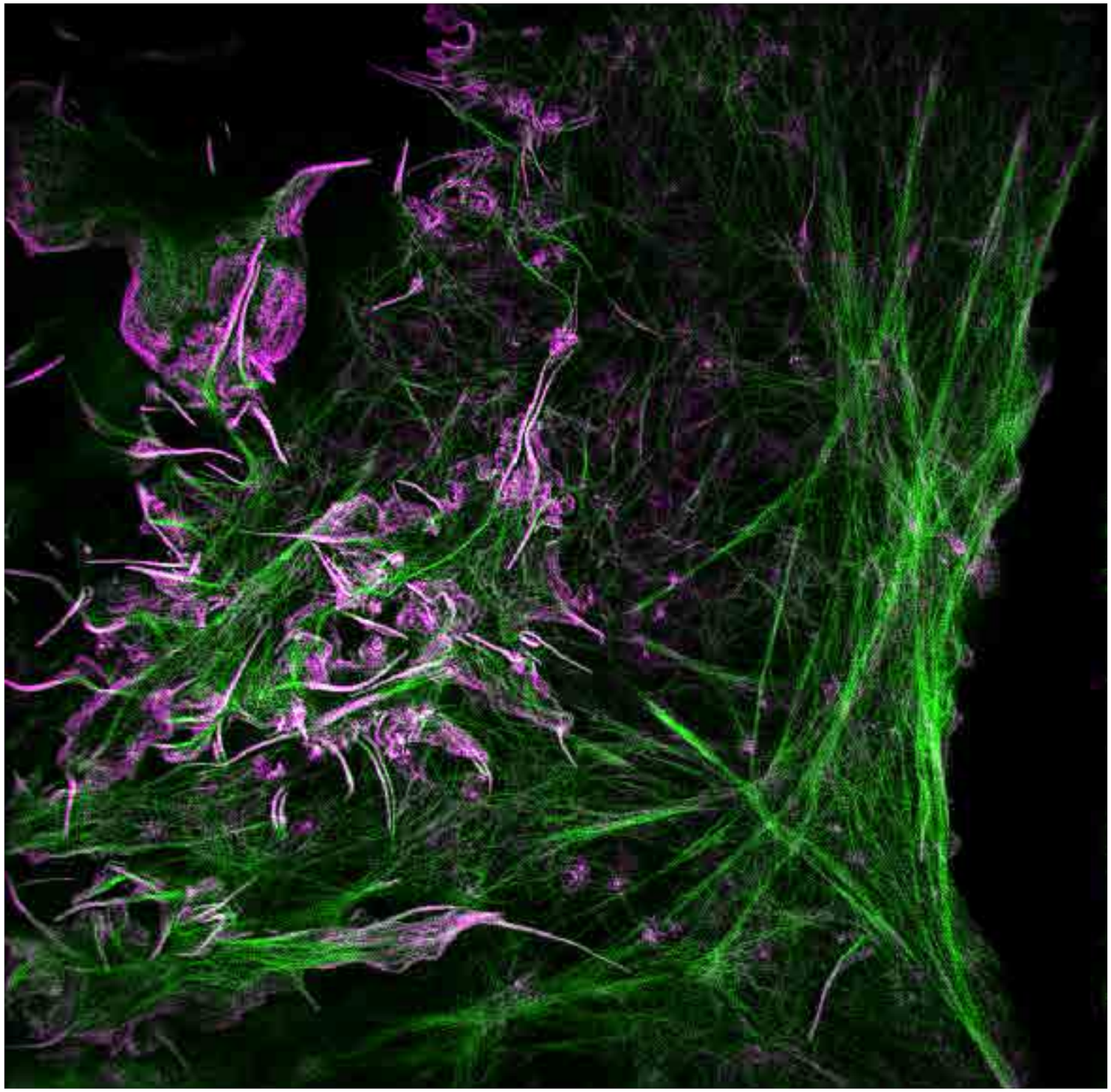


LEAF

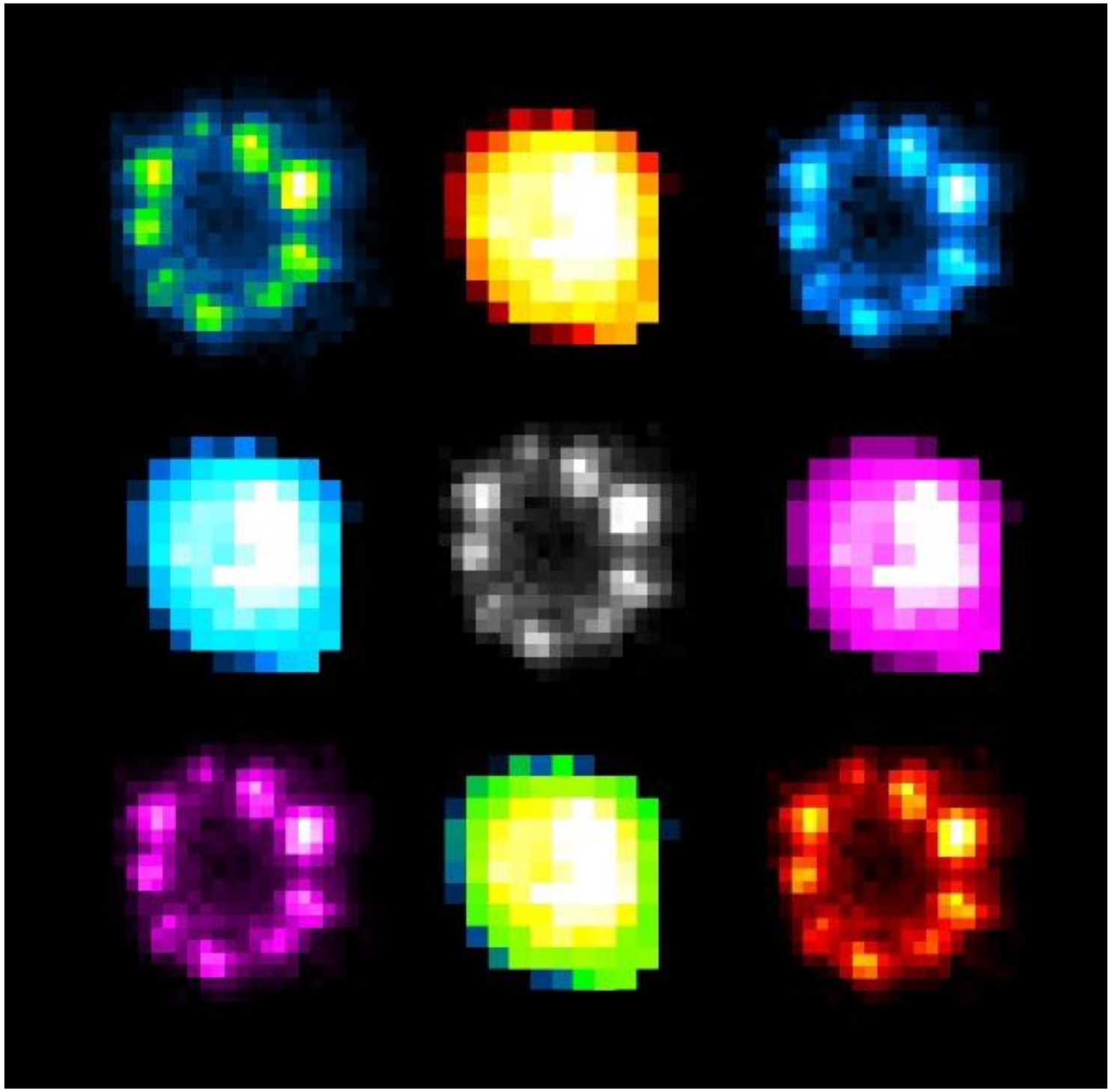


GLICINE

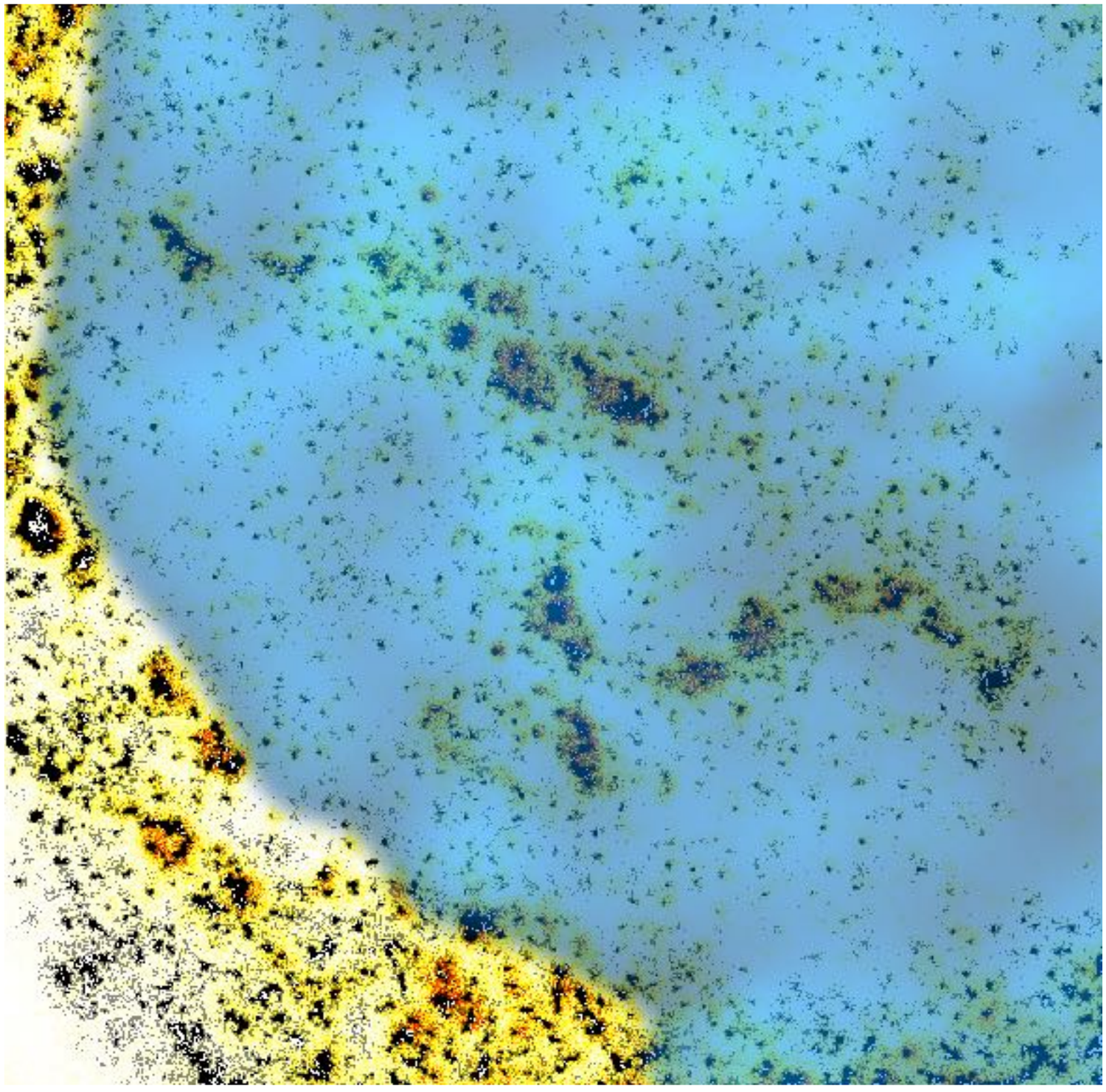
(WISTERIA)



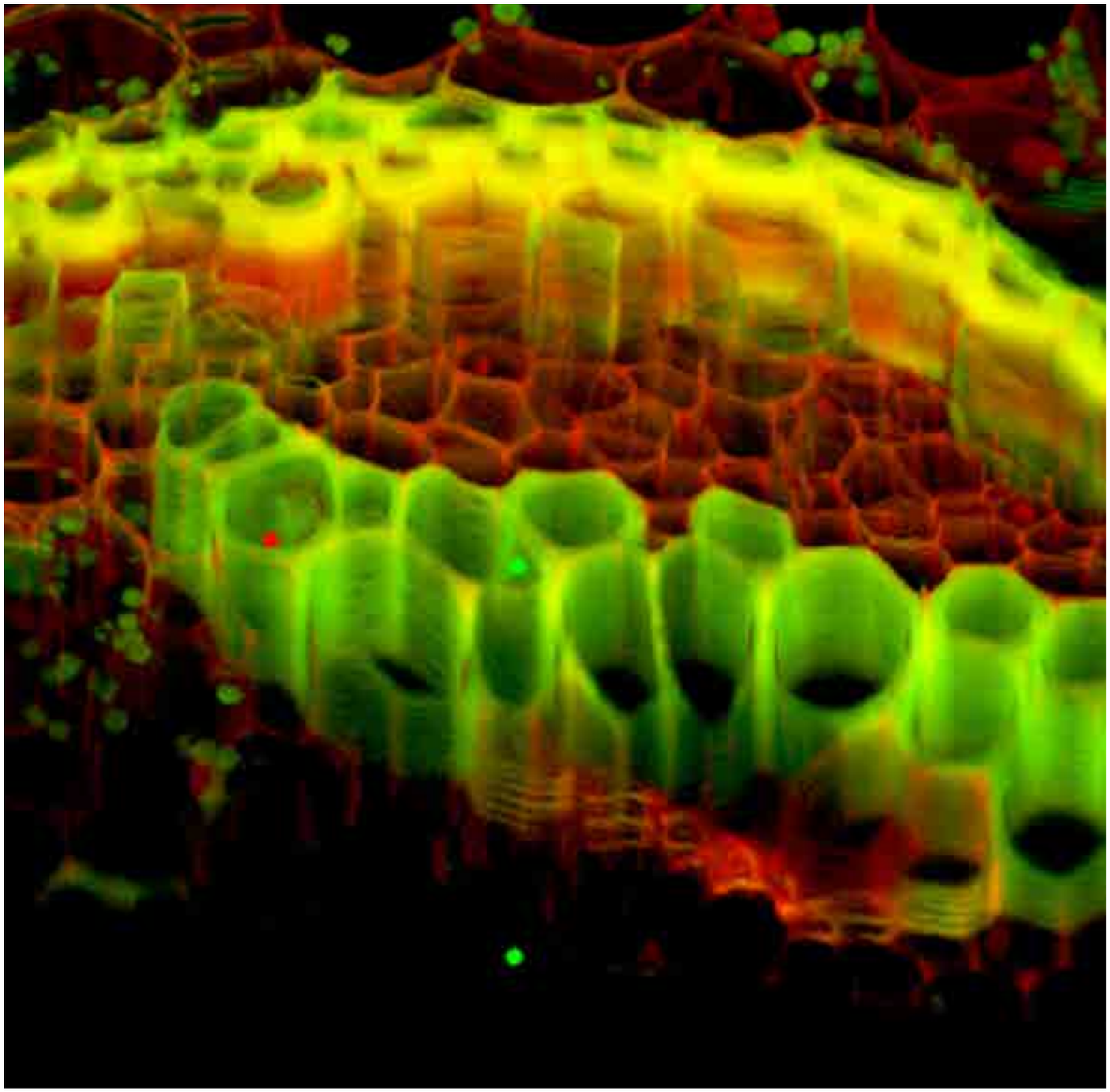
PACMAN



ANTIGUA

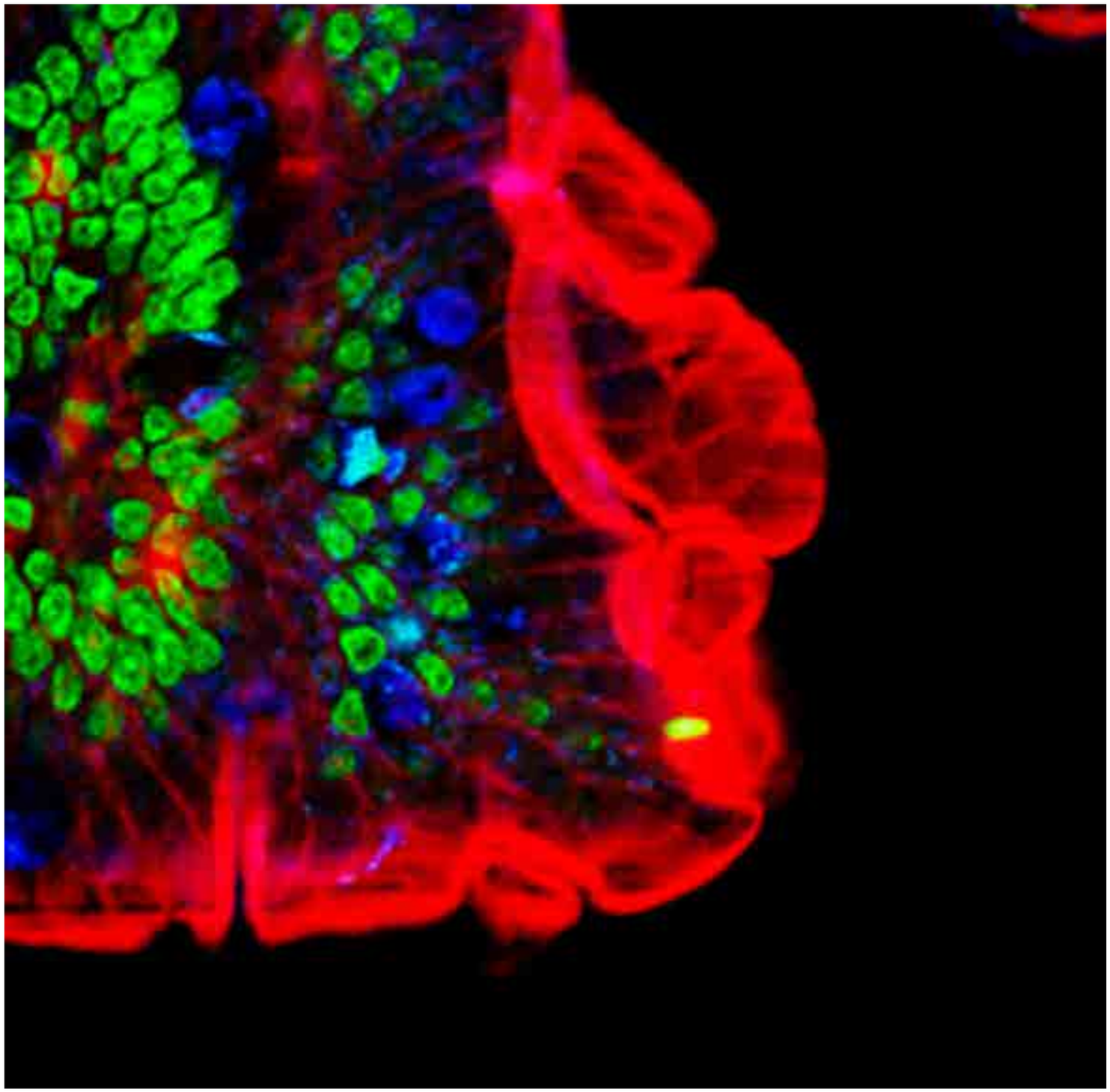


EQUATORE

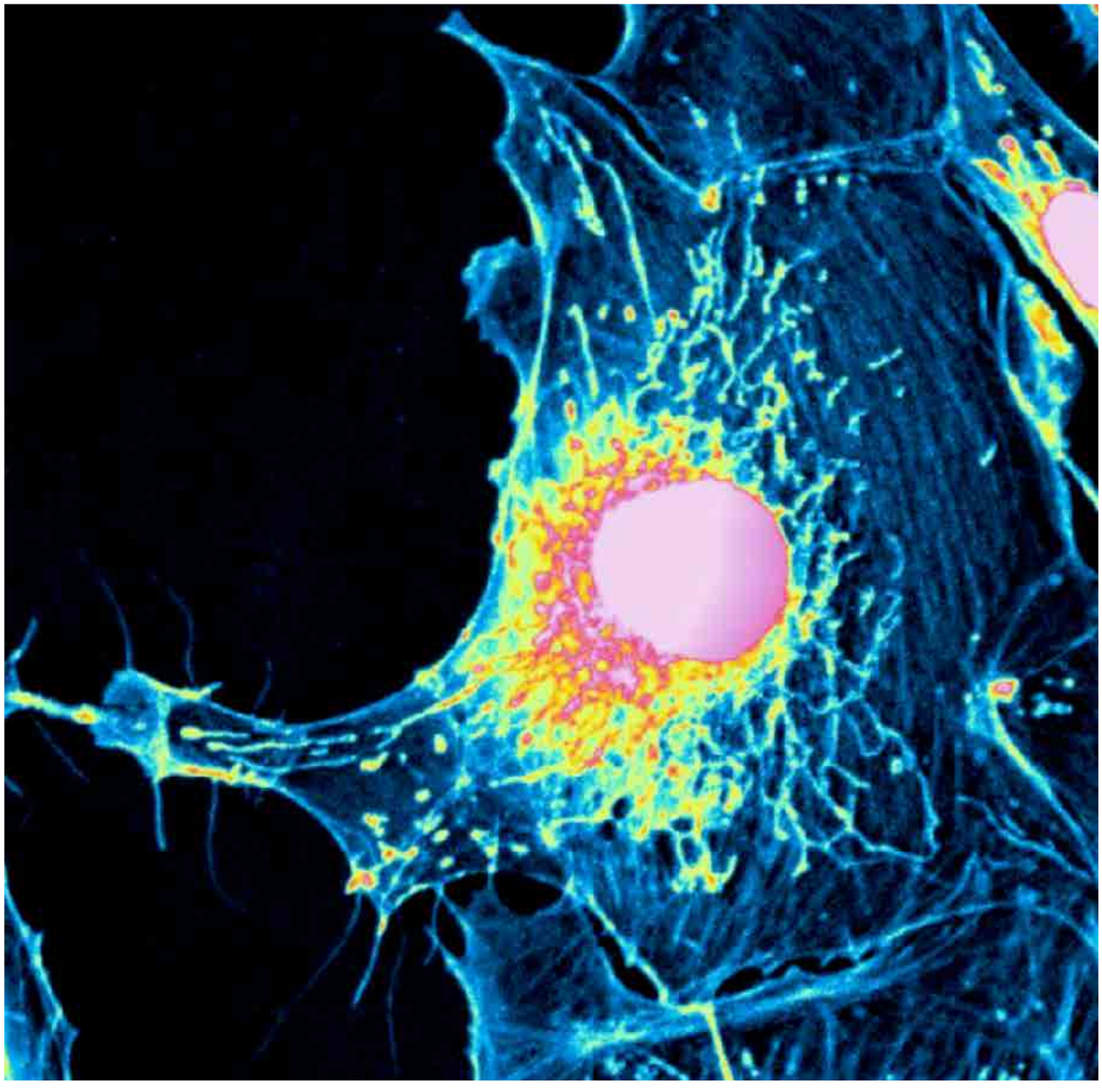


SNAKE

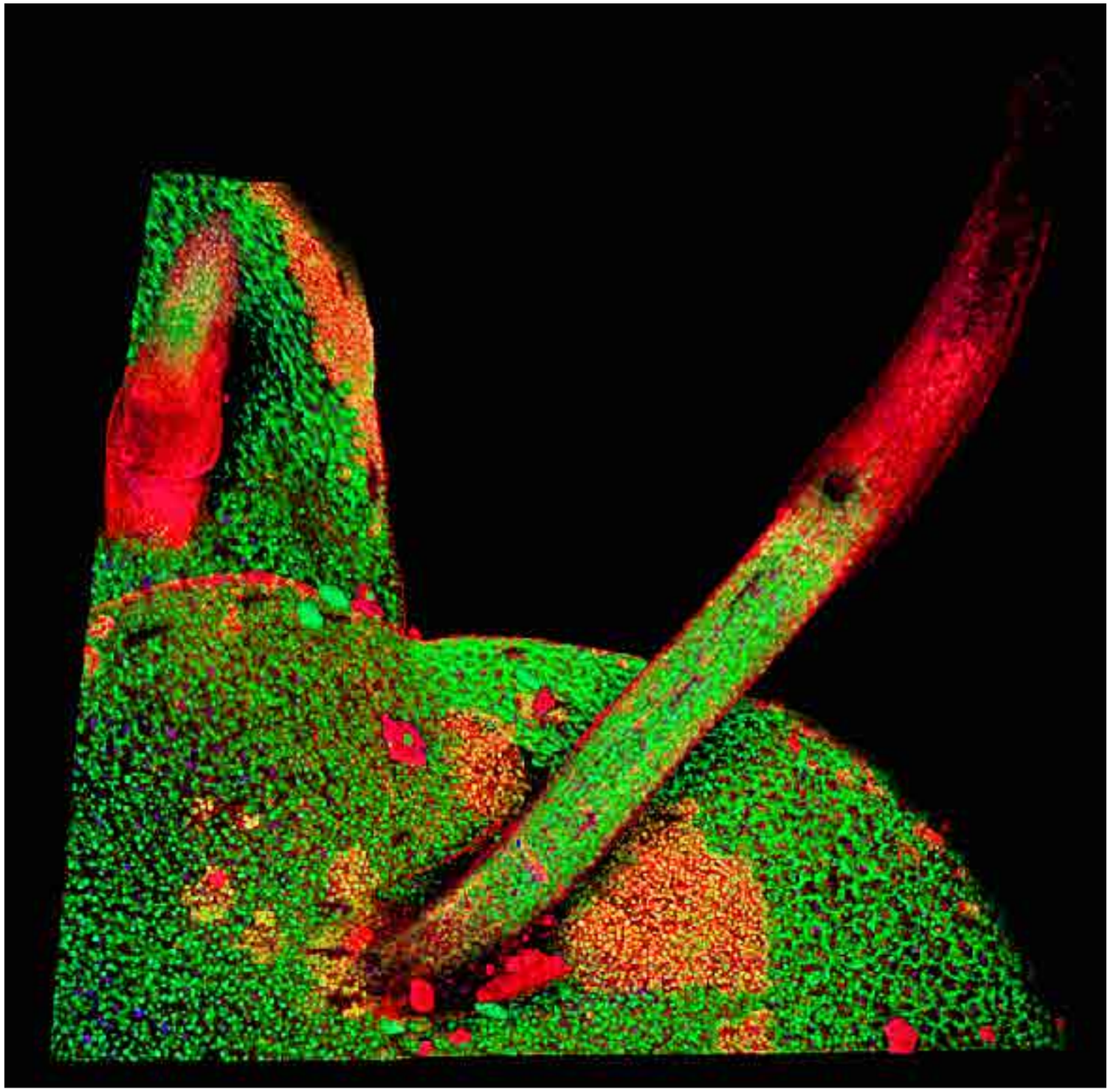
MAGNOLIA



LOST

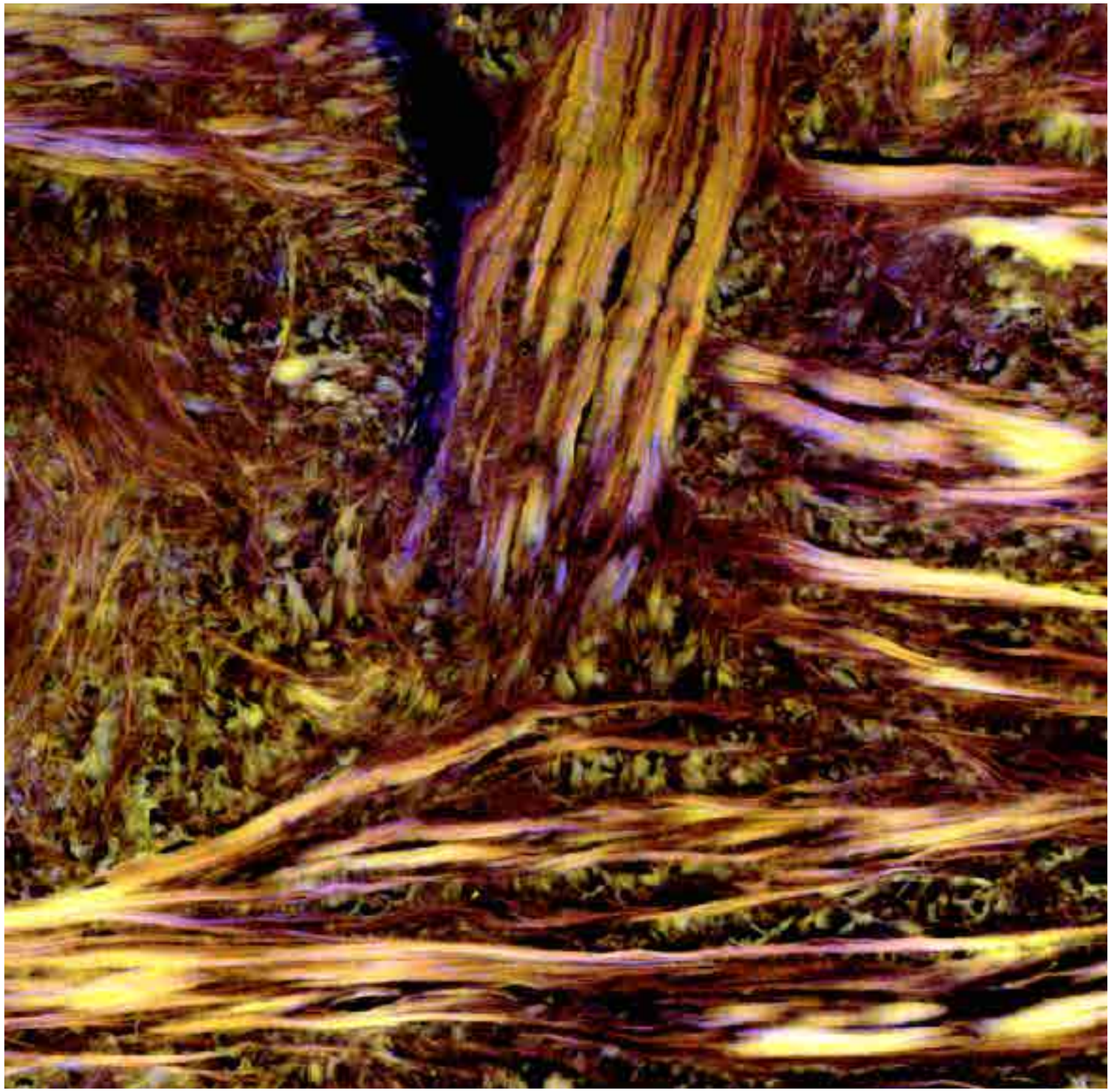


FROG



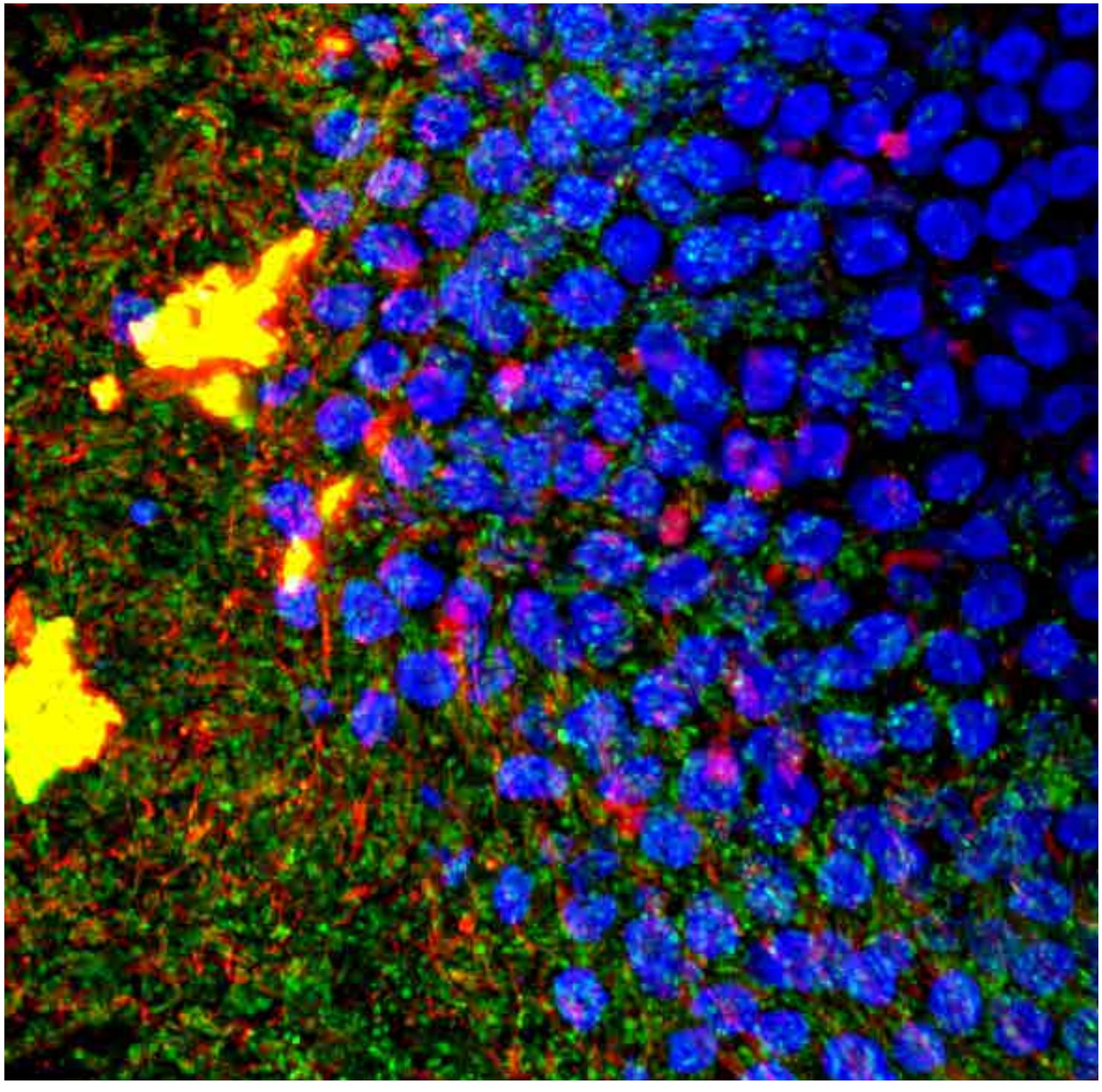
RADICI

(ROOTS)
TRIBUTE TO FRANCESCO GUCCINI

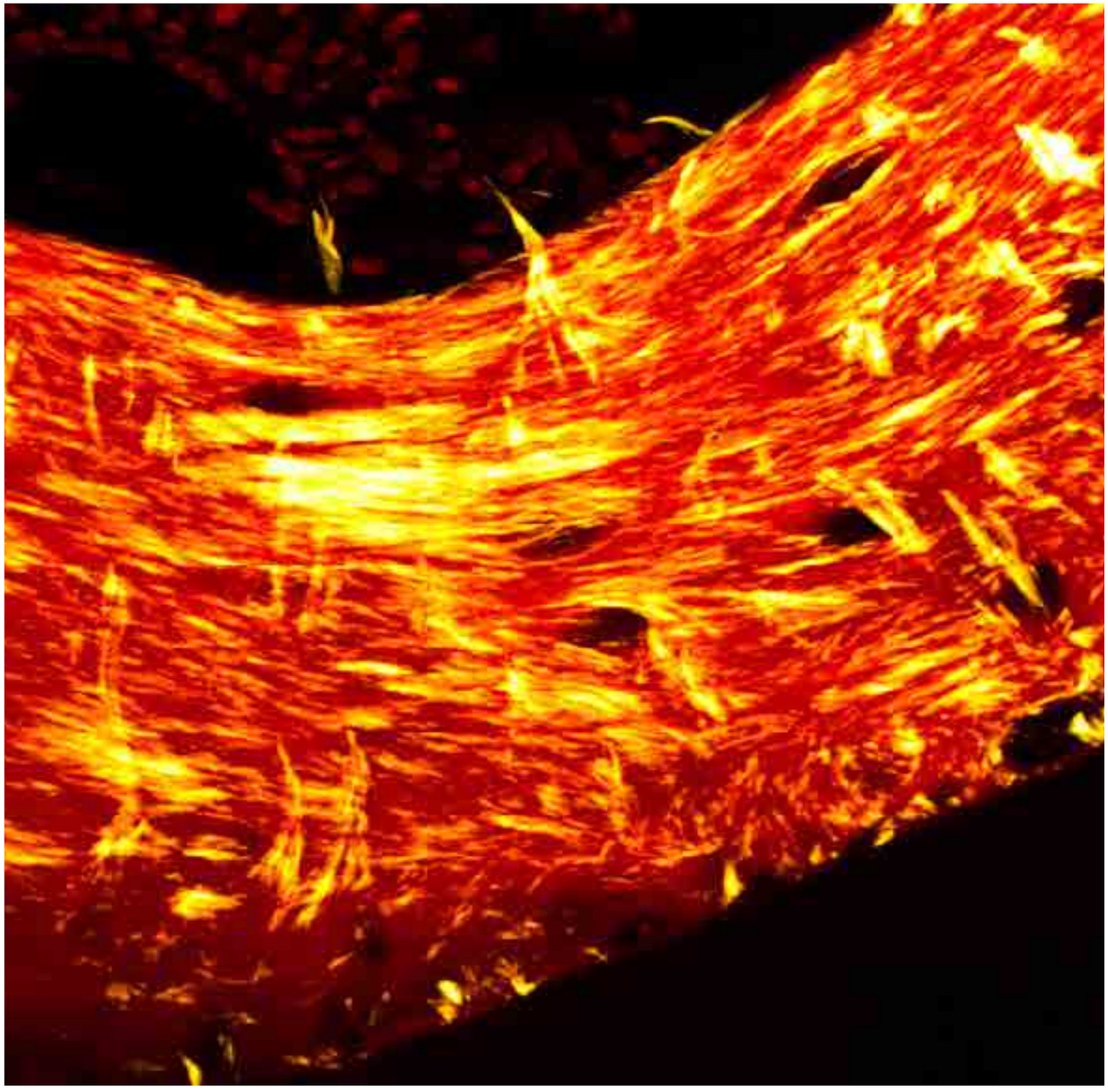


LA ILA

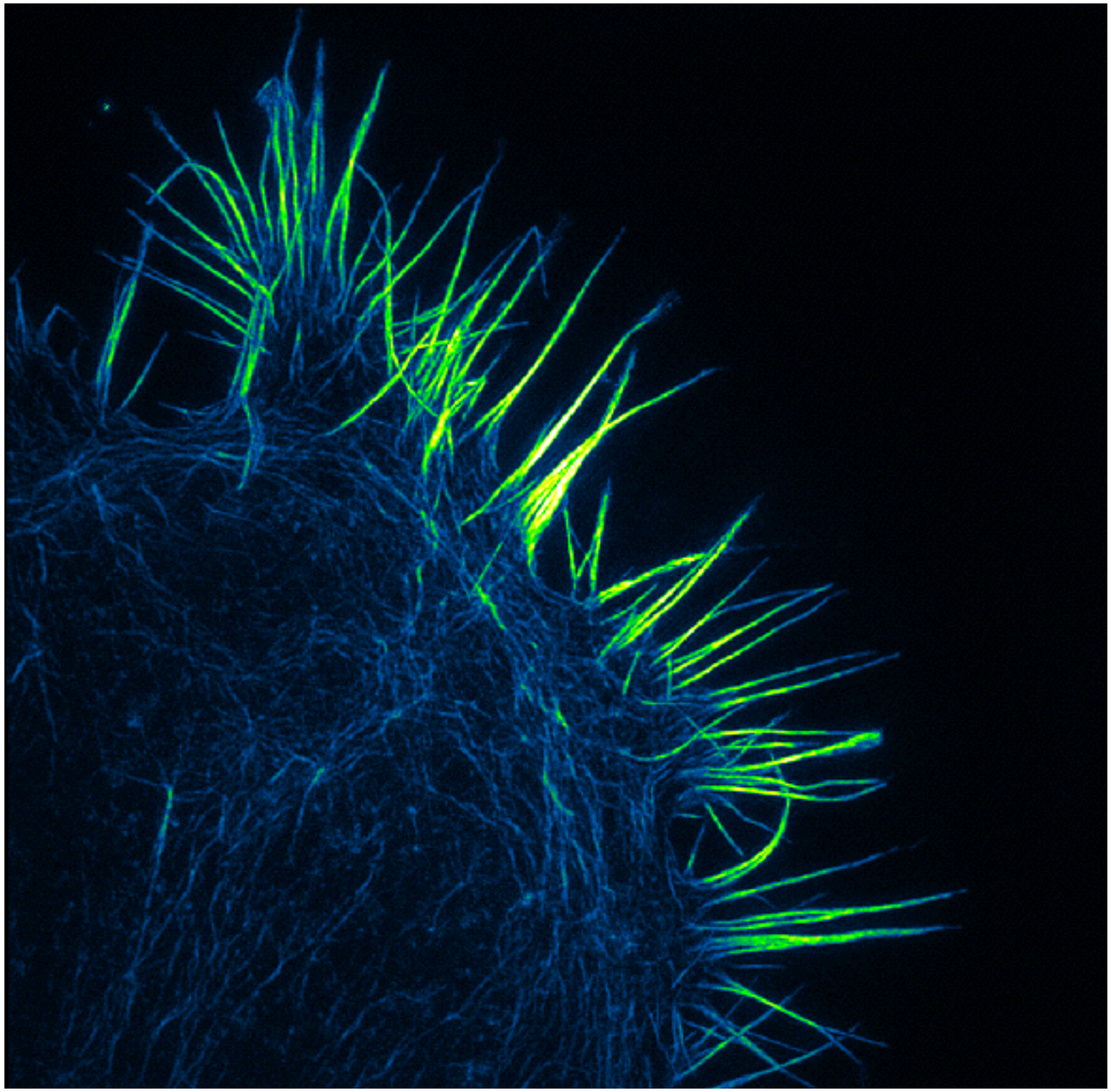
TRIBUTE TO VAL BADIA



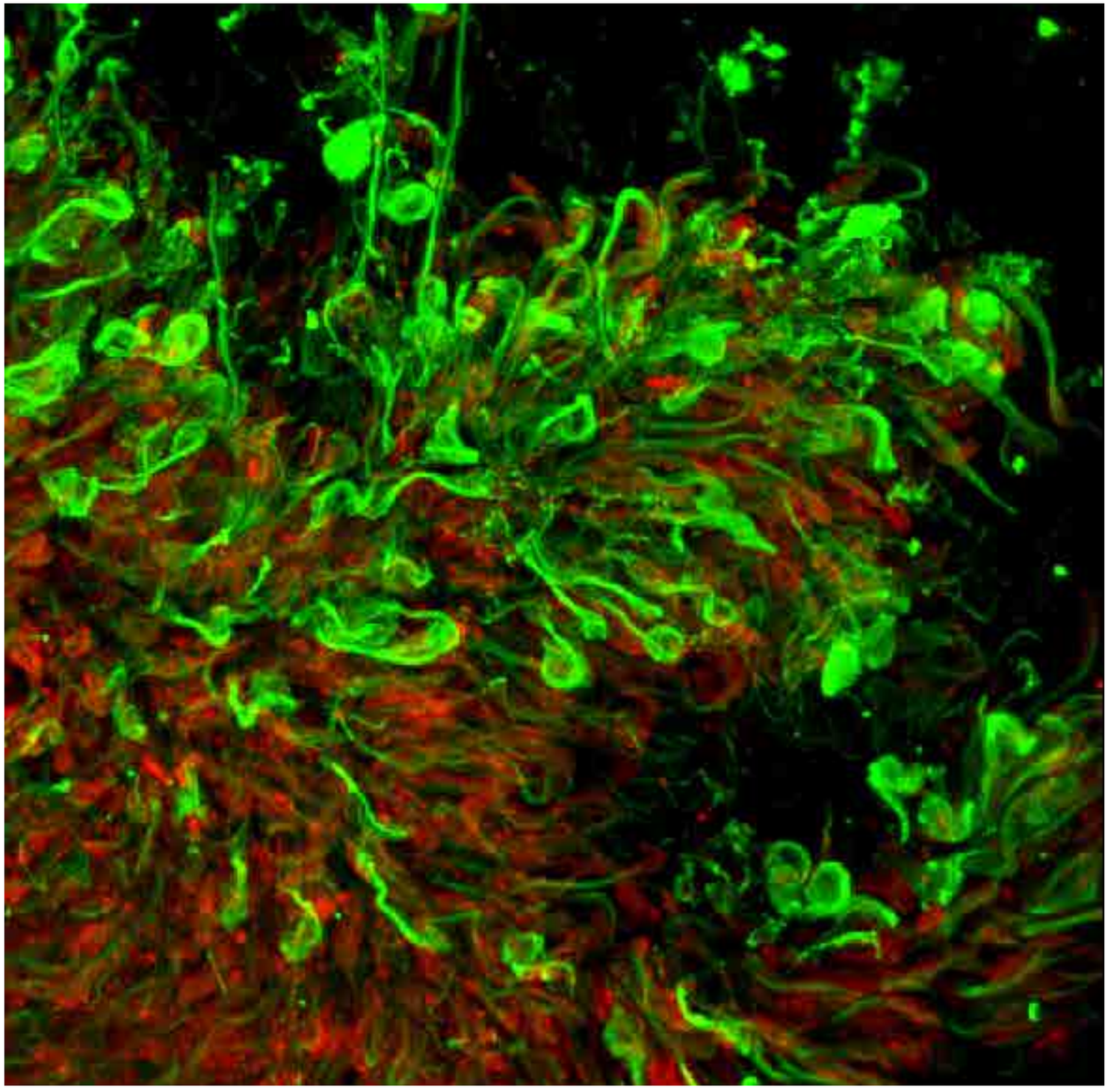
AXEL



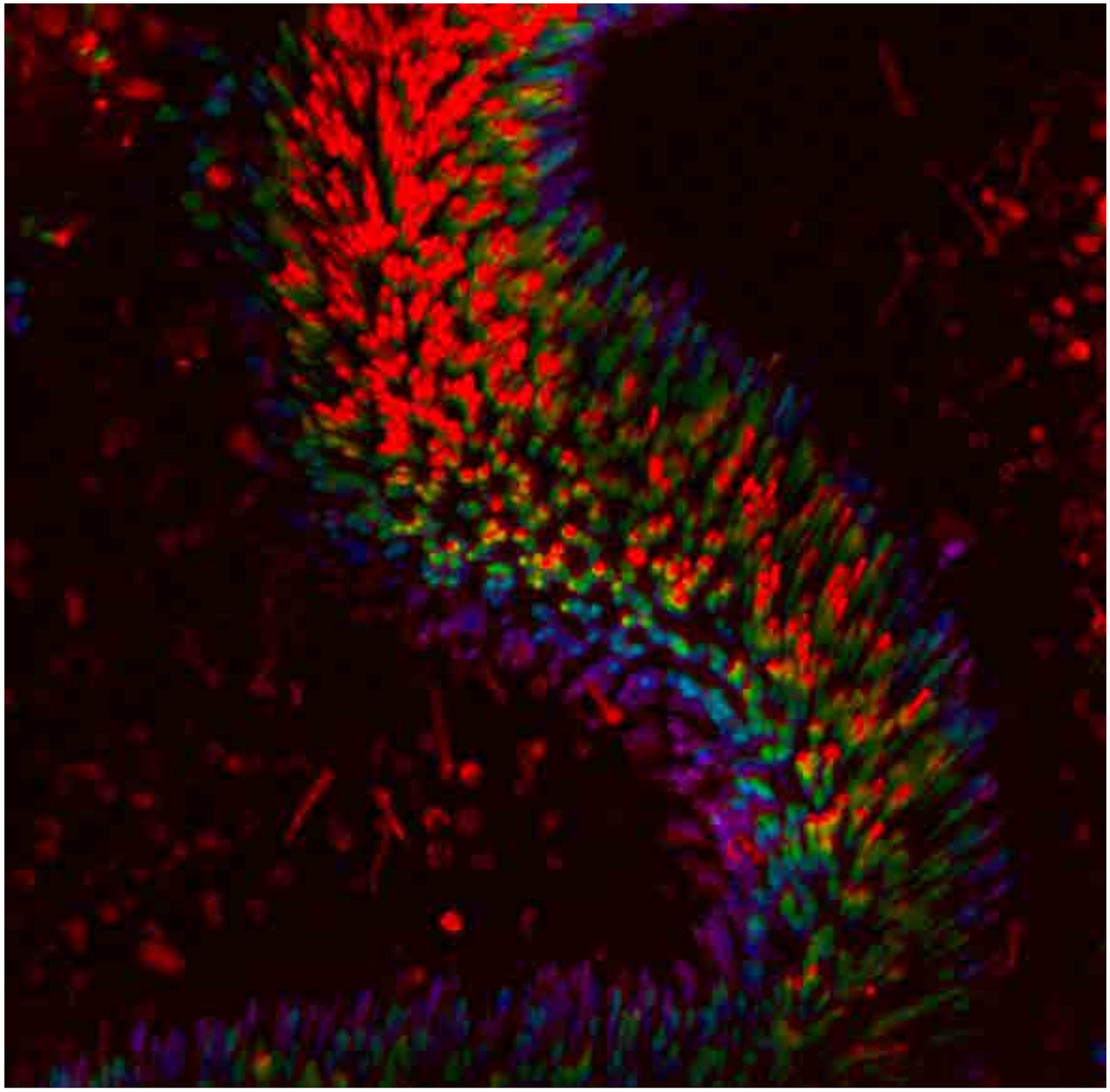
GREEN SLEEVES



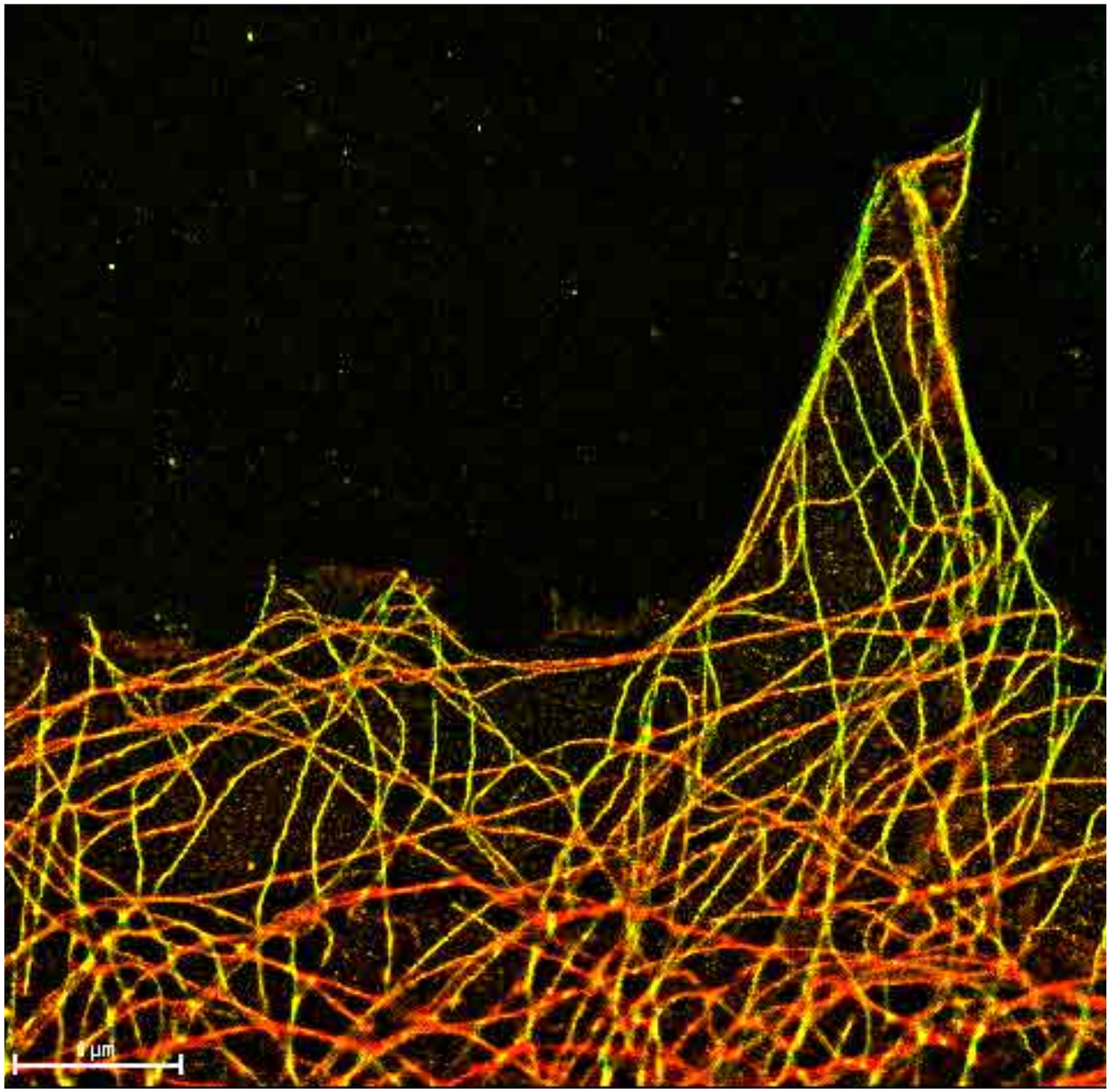
IRELAND



ARIEL



LUNA PARK



POP MICROSCOPY

Collezione di immagini al microscopio di sistemi viventi ideata da Alberto Diaspro e curata da Claudia Diaspro con il supporto tecnico scientifico di Paolo Bianchini, realizzata al NIC@IIT, Nikon Imaging Center presso l'Istituto Italiano di Tecnologia. Si ringraziano per il supporto Camera di Commercio di Genova e Nikon Instruments Italia. Novembre 2015.

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