Fundamentals in Biophotonics

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17.02.2025.

Plan for the class

- **WHO?**
- Lectures: Aleksandra Radenovic (aleksandra.radenovic@epfl.ch)
- Exercises:
- Wei Guo (wei.guo@epfl.ch)

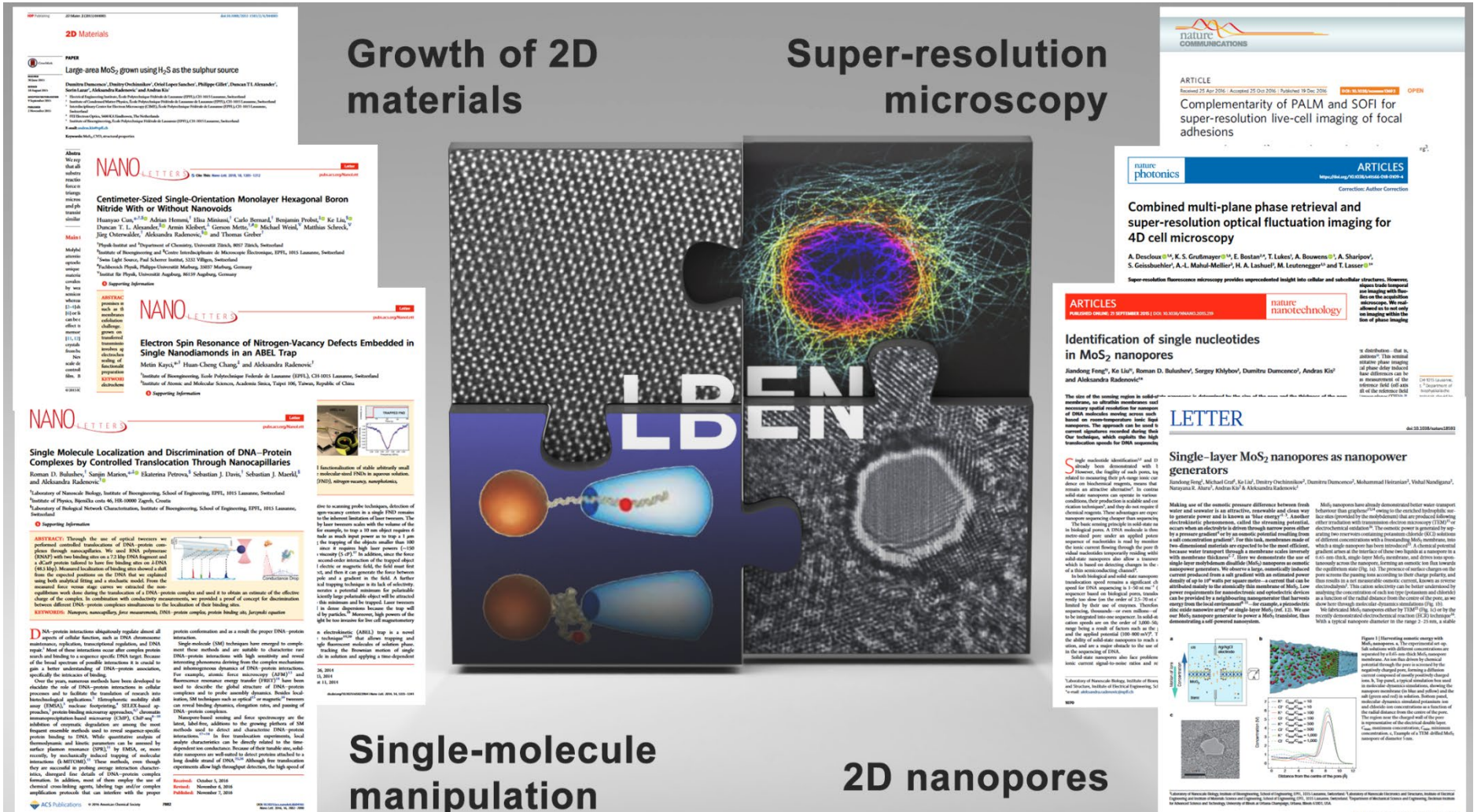
- **WHEN and WHERE?**
- Lectures: Mondays 10.15– 12.00
- Exercises: problem sets that will follow after 2nd week

- Moodle : https://moodle.epfl.ch/course/view.php?id=15044

- **Grade** during the semester **homework** and **flipped classroom**

Prof Radenovic

- Office hours 14-15.00 every Friday –for Prof Radenovic and Wei Guo
- E mail alakesandra.radenovic@epfl.ch
- Lab LBEN



- YOU

Schedule

- https://docs.google.com/spreadsheets/d/1rYpU327ekuptJWSO3Ve7fA2KkFle4t6n/edit?gid=483657210#gid=483657210

Presentation schedule						
Date	1st Student	Assigned Topic	2nd Student	Assigned Topic	3nd Student	Assigned Topic
17.02.2025.	INTRO SESSION					
24.02.2025	Selection of the topics /schedule of the topics /material preparation /No Class					
03.03.2025.	Lecture 1					
10.03.2025.	Lecture 2					
17.03.2025.	Lecture 3					
23.03.2025.	Lecture 5					
31.03.2025.	Lecture 6					
07.04.2025.	Lecture 7					
14.04.2025.	Lecture 8					
21.04.2025	Easter holidays(Vacances) Easter Monday(Jour férié)					
28.04.2025.						
05.05.2025.						
12.05.2025.						
19.05.2025.						
26.05.2025.						

TOPICS

EndoscopicTecnniques_1
OCT
Latice_light
Single cell quantitative measurements_1
Single cell quantitative measurements_2
Optical tweezers 1
Optical tweezers 2
Optogenetics_1
Optogenetics_2
SMLM
FSTORM-blinking
PAINT
DNAnano
SIM
STED

Outline

- Total 13 weeks
- Fundamentals of Light and Matter 2 weeks
  - Introducing basic concepts :
  - Light as photons carries energy
  - Light as waves exhibits *interference* and *diffraction* consequences/applications phase contrast microscopy optical coherence tomography
  - Light propagation in a medium is dependent on its optical characteristics - consequences/applications in spectroscopy and fluorescent microscopy
  - (Born and Wolf)
  - Fundamentals of matter - the concept that electron energies in atoms and molecules have only certain permissible discrete values and conditions called *quantization of energies* (**electronic, vibrational, rotational** and translational)
  - Its (electronic, vibrational) significance to biophotonics / spectroscopy , bioimaging, biosensing , photodynamic therapy , and biomaterials for photonics
  - Qualitative demonstrate the use of Schrödinger equation to obtain energy levels
  - Bonding in organic molecules σ and π
  - π electron delocalization effect in conjugated structures its importance in understanding spectroscopy and fluorescence behavior of fluorophores used in bioimaging and biosensing

Outline

- Fundamentals of Light and Matter interaction 2 weeks
  - Absorption *Beer-Lambert's law* (absorbance, transmittance, optical density)
  - IR absorption
  - Refraction
  - Reflection

  - Polarization
  - Spontaneous emission
  - Simulated emission
  - Raman scattering
  - Introduce various spectroscopic approaches (electronic and vibrational)
  - Introduce spectroscopies that are using optical activity of the chiral media (circular Dichroism CD, Vibraitonal Circular Dichroism
  - (conventional basic elements of conventional spectrometer and FT Spectrometer)

  Light emission intrinsic and extrinsic
  - Fluorescence (fluorescence spectra , lifetime, quantum efficiency , depolarization , introduce concept of dipole emission dipole absorption dipole)
  - Phosphorescence

Outline

- Principles of Laser and Detectors (EMCCD) 1 week
- Lasers (light amplification by stimulated emission radiation) action
  - Principle
  - Terminology
  - Various types of laser /different classification schemes
  - Non linear optical processes with intense laser beam
  - Mechanism of frequency conversion
  - Multiphoton absorption

- Detectors EM CCD done
  - Speed of acquisition
  - Sensitivity of detection (low light)
  - Signal detection efficiency (QE)
  - Size of pixel / frame
  - Dynamic Range

Outline

- Photobiology Interaction of light with cells 1 week
  - Light absorption in the cells

    endogenous (cell constituents)

    exogenous (photosensitizers)
  - Radiative processes

    Autofluorescence (NADH, flavins , aromatic amino acid such as tryptophan tyrosine phenylalanine)

    Protein degradation (photoaddition, photofragmentation , photo-oxidation, photo hydration, cis-trans isomerization, photorearegment)

    Principle of photosensitized oxidation process
  - Nonradiative process

    photochemical, excited state chemistry

    Photodisruption

    Photoablation
  - Light Scattering
  - Elastic (Rayleigh and Mie)
  - Inelastic (Brillouin and Raman)
  - Photosynthesis

Outline

- Bioimaging : Principles and Techniques 2 weeks
  - Utilizes an optical contrast such as difference in light transmission , reflection and fluorescence between the region to be imaged and the surrounding region (background)
- Short overview on Optical imaging
  - Short overview on Optical imaging
  - Transmission
  - Fluorescence microscopy
  - Confocal
  - Multiphoton
  - Optical coherence tomography
  - Near field optical microscopy
  - FRET imaging
  - FLIM
  - Nonlinear optical imaging
  - SH Microscopy
  - CARS
  - Super-resolution

Outline

- Bioimaging applications 2 week
- Fluorophores as bioimaging markers
  - Endogenous
  - Exogenous (fluorophores targeting specific biological molecules without prior coupling and fluorophores that need to be conjugated)

    Organ metallic

    Near-IR and IR

    Two-Photon

    Inorganic Nanoparticles (QD etc)

    GFP based labeling falls between endogenous and exogenous

    Organelle targeting fluorophores

    DNA targeting fluorophores -FISH (Fluorescence in situ hybridization)
- Probing the Celular Ionic Environment
  - Ca imaging
  - pH sensing fluorophores
  - Two photon tracking of drug cell interactions
  - Optogenetics
  -

Outline

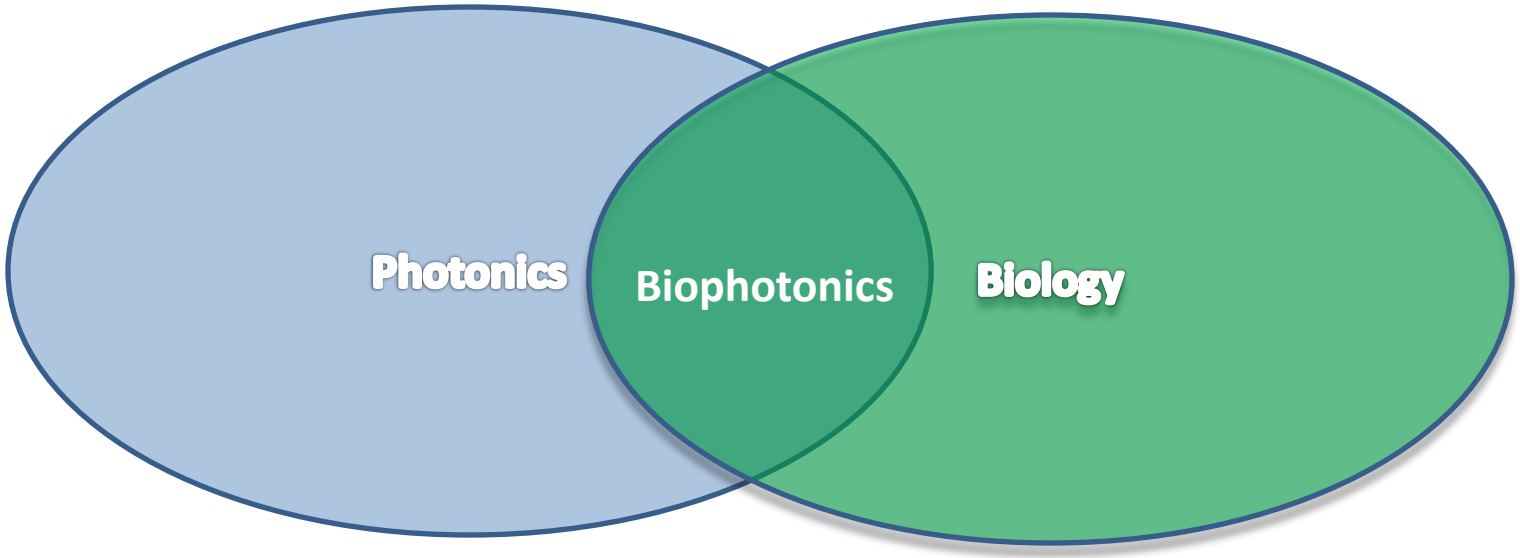
- Tissue imaging
  - Optical coherence tomography (OCT)
  - Two-photon excitation laser scanning microscopy (TPLSM)
- In vivo imaging
  - Corneal imaging
  - Retinal imaging
  - Optical mammography by NIRF
  - Catheter based OCT

Outline

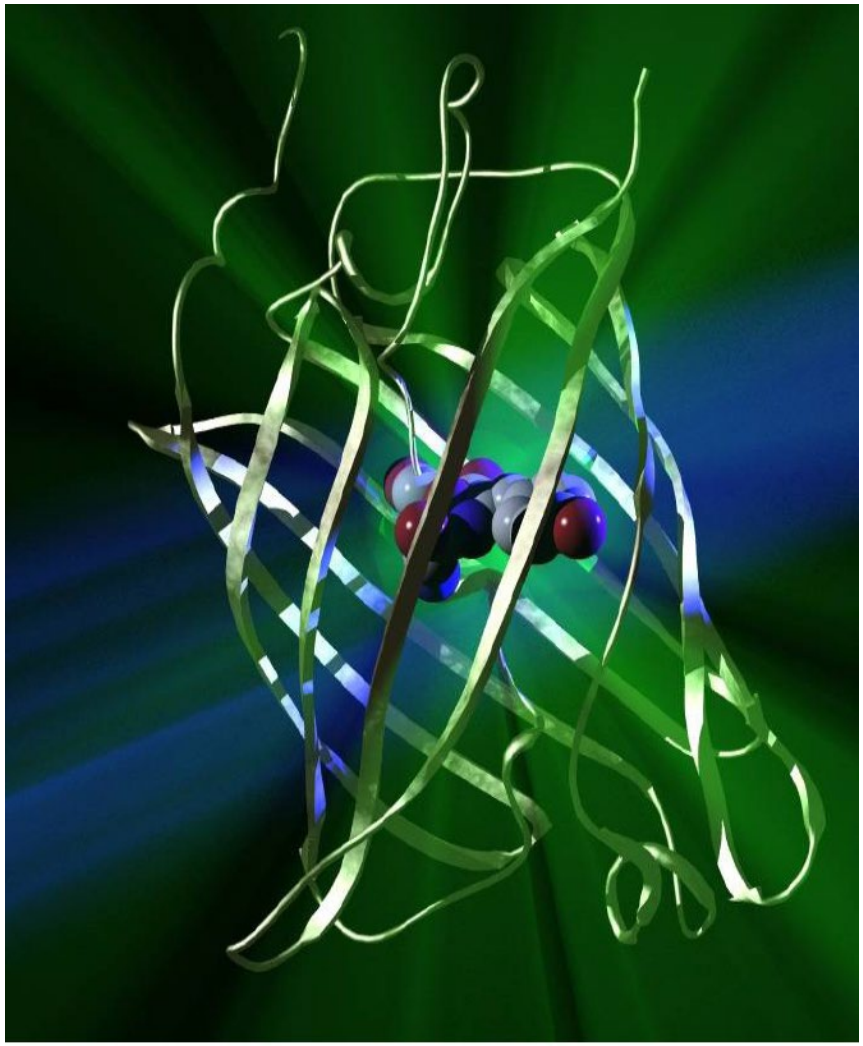
- Optical Biosensors and Microarray 1 week
- Optogenetics 1 week
- Labelfree: Surface Plasmon resonance (SPR) and dielectric waveguide methods, biosensors based on whispering gallery modes in microresonators. 1 week
- Recapitulation and preparation for exam 1 week

Biophotonics

- Biophotonics, the ‘marriage’ between **photonics** and **biology**, is an emerging interdisciplinary frontier that deals with **interactions between light and biological matter.**
- Through the integration of four principle technologies, lasers, photonics, nanotechnology, and biotechnology, biophotonics offers immense hope for the early detection and treatment of diseases and for new modalities of light guided and light activated therapies.



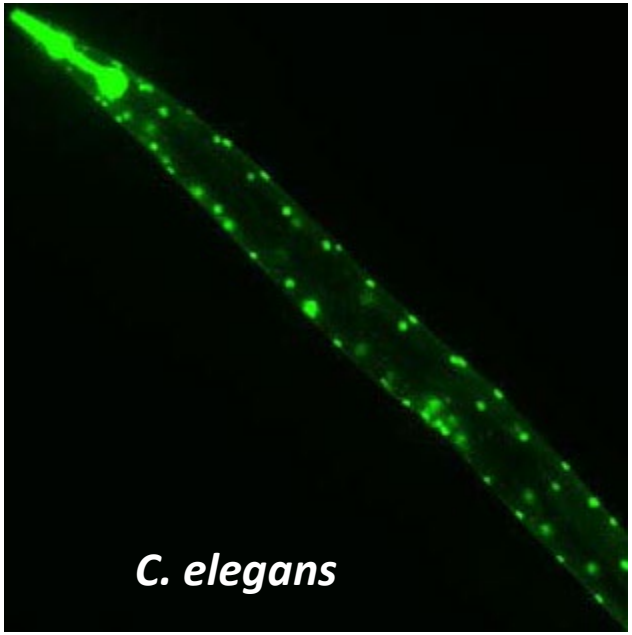
3D-Structure of GFP

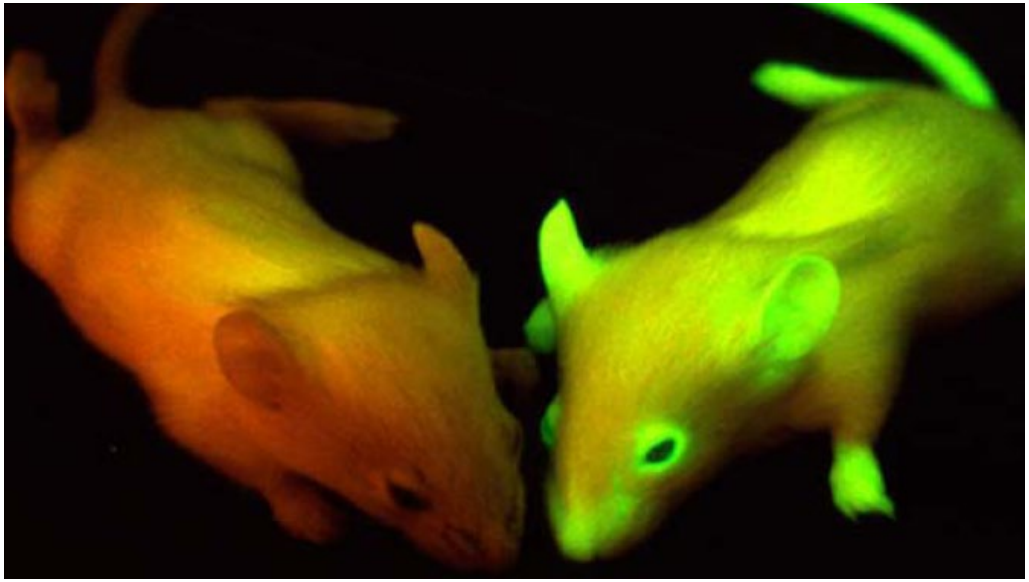


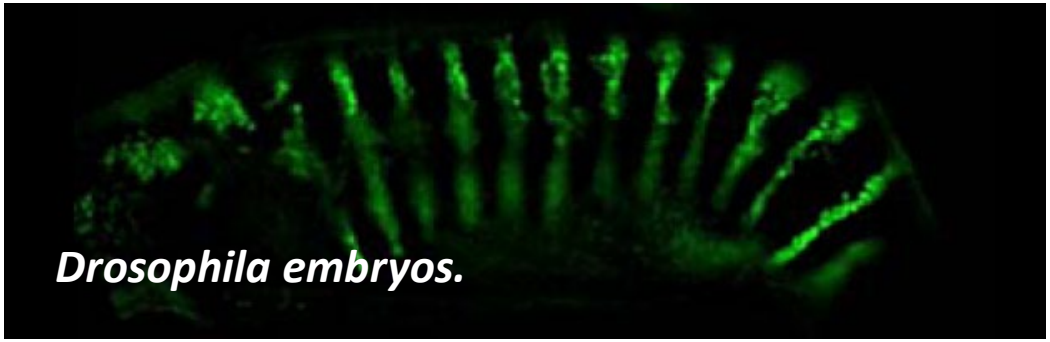
- “Paint in a can”
- Composed of 238 amino acids (27KDa).
- Each monomer composed of a central helix surrounded by an eleven stranded cylinder of anti-parallel beta-sheets (shields fluorophore from solvent)
- Cylinder has a diameter of about 30 Å and is about 40 Å long
- Fluorophore is located on the central helix
- Deprotonated phenolate of Tyr66 is cause of fluorescence

What organisms have been transformed?

bacteria, fungi, Dictyostelium, C. elegans, plants, Drosophila, mammals

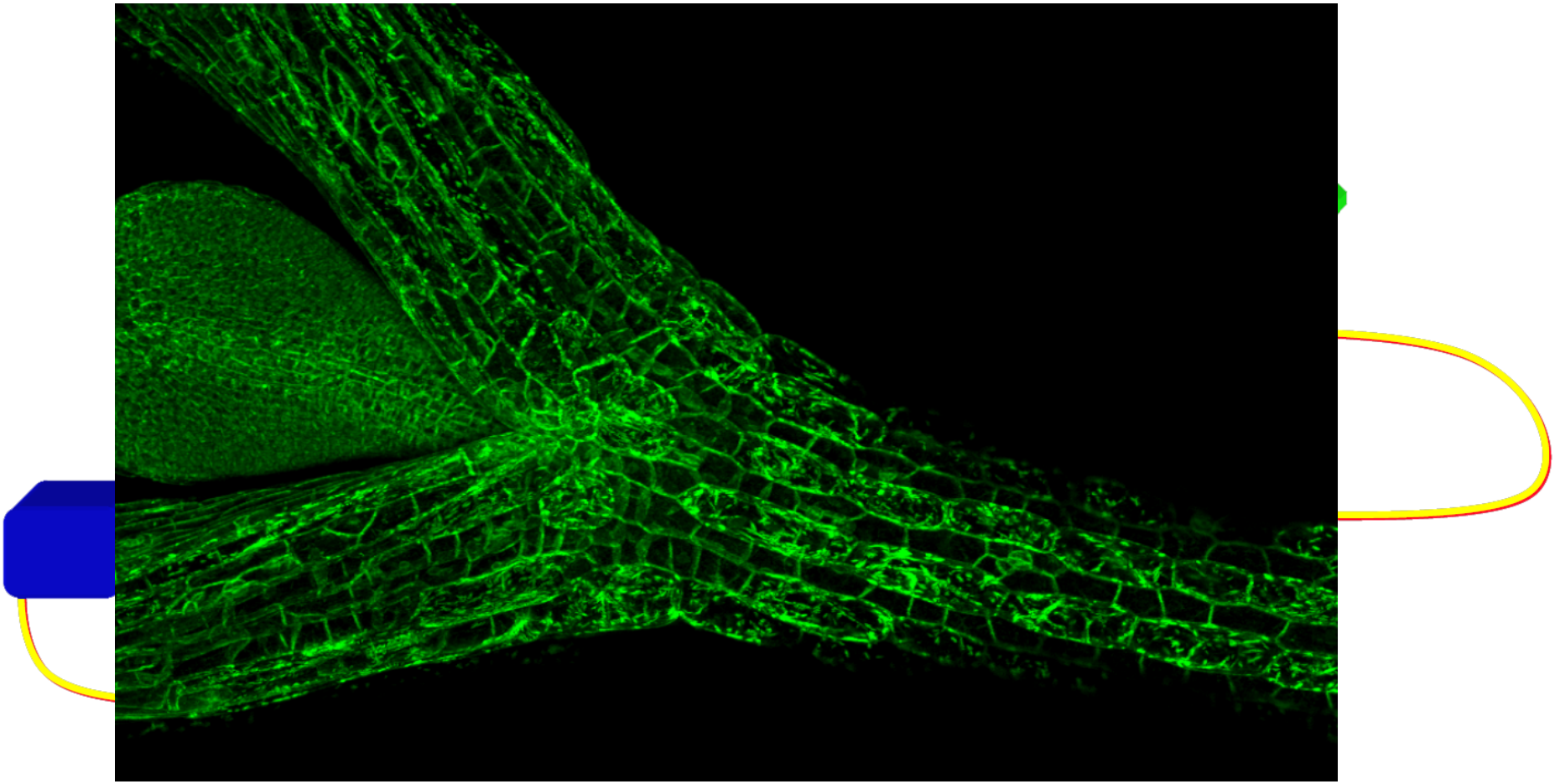






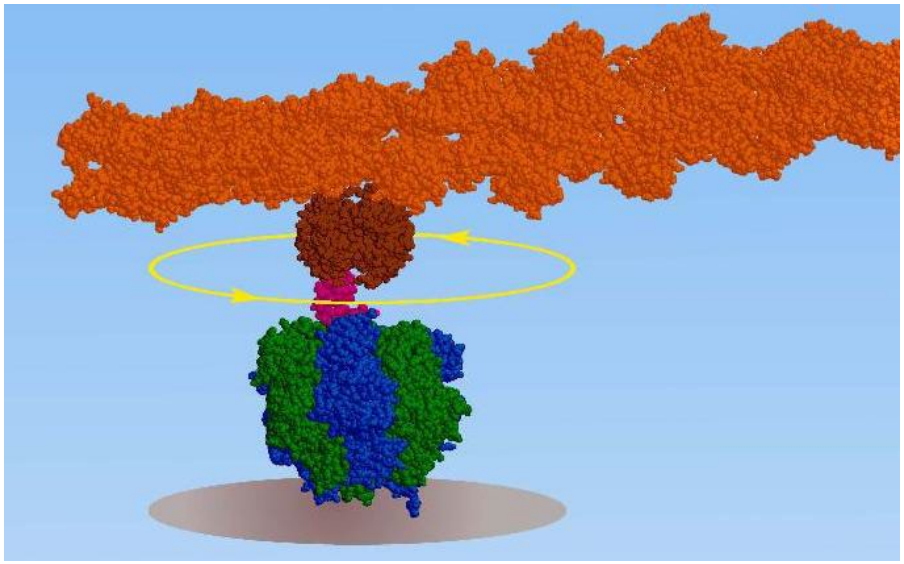
How do we use fluorescent proteins?

- Structural studies Genetic modification of an organism for fluorescent protein expression. Promotor can be constitutive or tissue-specific.

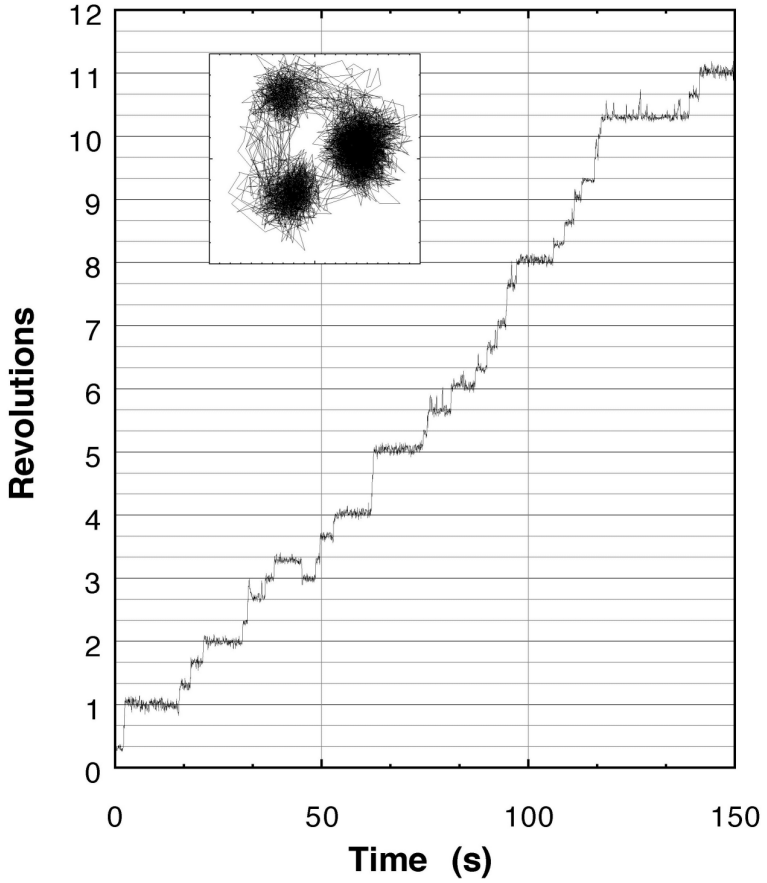


Rotary Molecular motor : The F1- ATP synthetas

- The F1-ATPase: a rotationary motor



Rotation by 120 steps

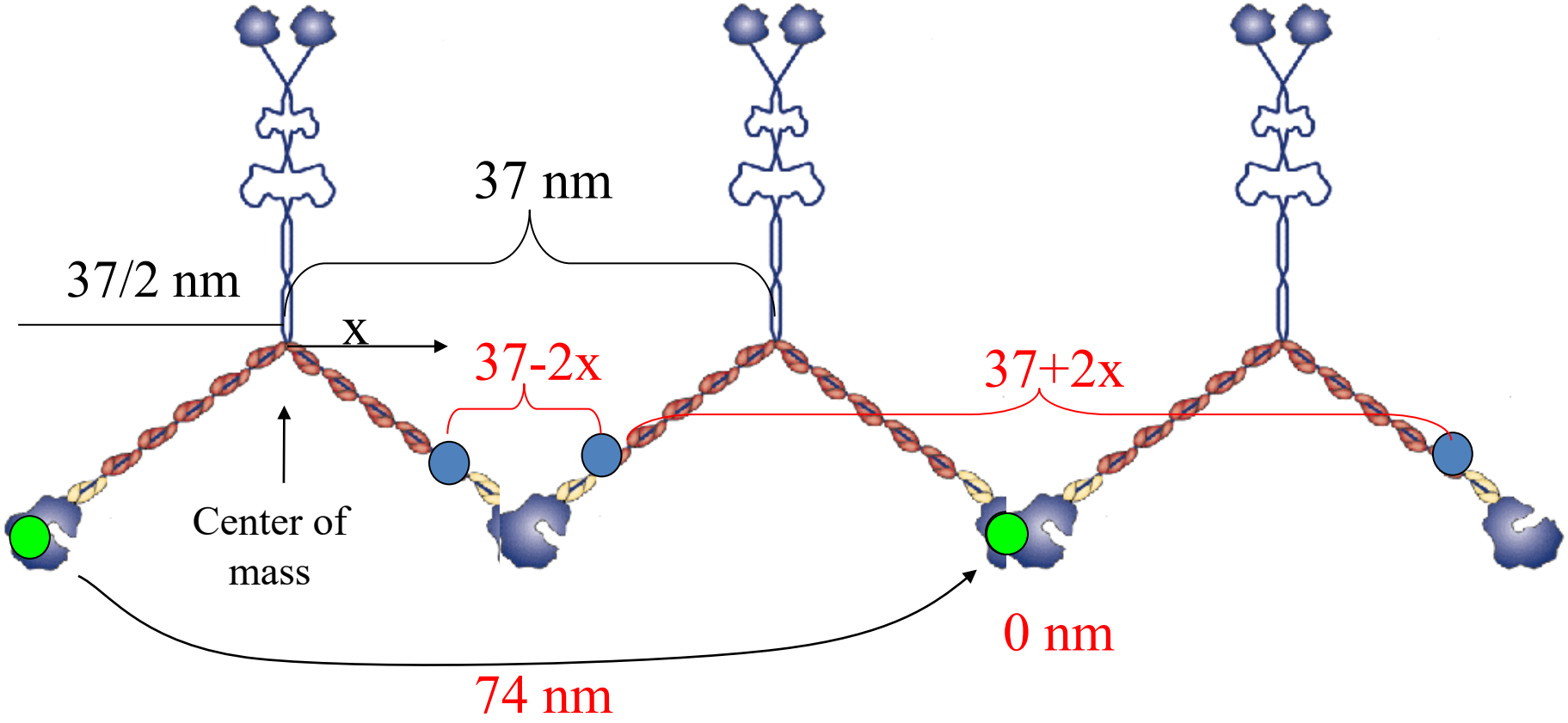


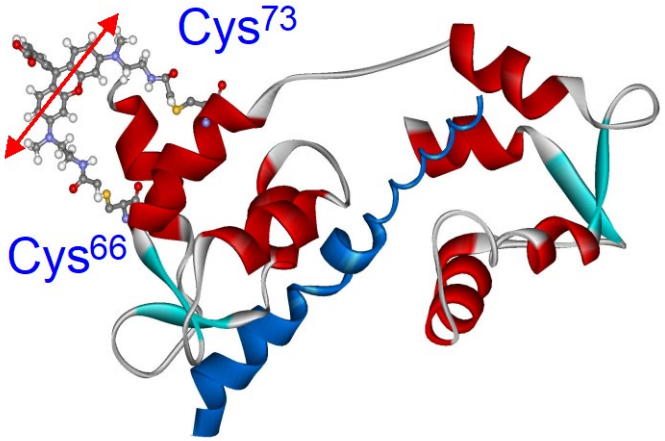
The ATP motor/generator is coupled to a proton flux [H+]

The F0 and F1 subunits are connected by a shaft γ

The ATP hydrolysis/synthesis occurs on the β subunits

Using in Vitro FIONA to analyze Myosin V movement





Expected step size

Hand-over-hand:

Head = 2 x 37 nm= 74, 0, 74 nm

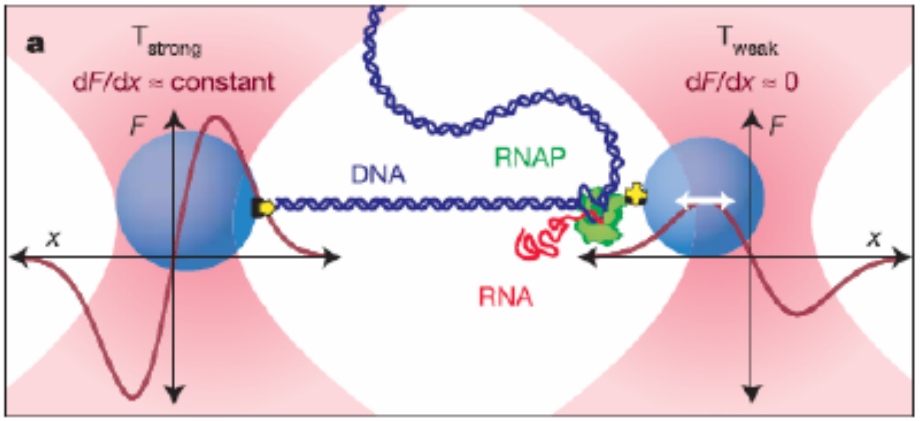
CaM-Dye: 37-2x, 37+2x, …

Inchworm: always S_{cm} = 37 nm

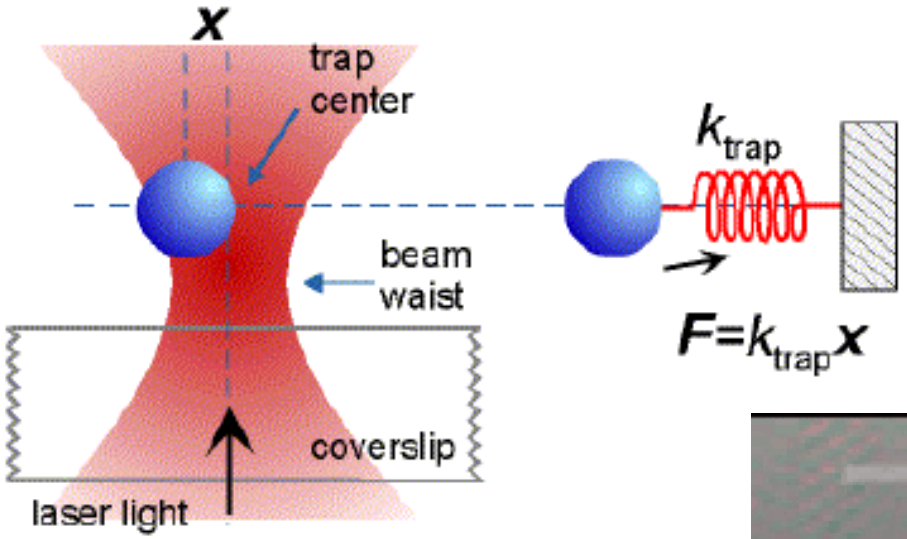
Optical tweezers

(aka. Optical traps, laser tweezers, photonic force microscope, etc.)

- Trapping and applications
- Principles
- Design
  - Layout
  - Trapping laser
  - Objective
- Position control
  - Stage motion
  - Mirrors / AODs / Holograms
- Position detection
- Calibration
  - Position calibration
  - Force calibration
- Examples



What are Optical Tweezers(OT) ?



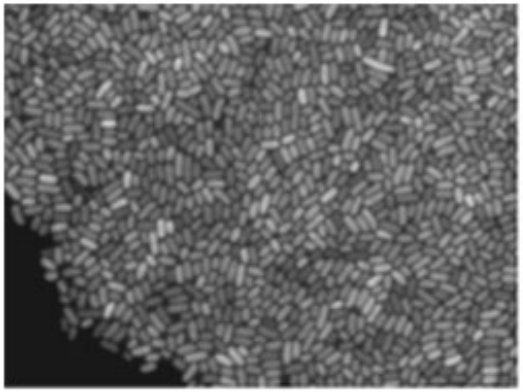
- Optical Tweezers = Focused Laser beam

- OT works by transfer of momentum

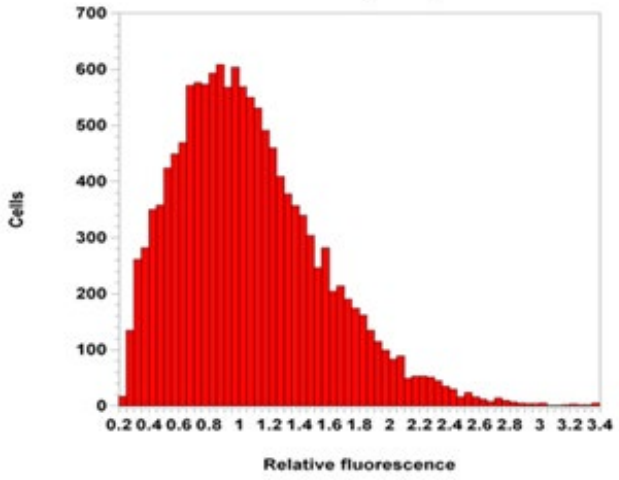
- Particles with higher n than surrounding medium are trapped in an approximately harmonic potential

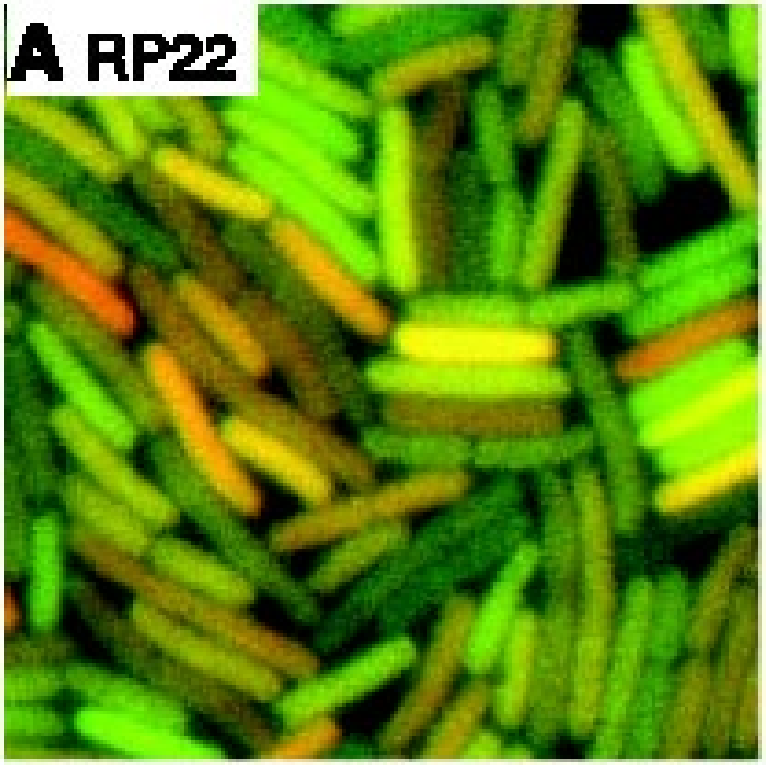
k ≈ 0,001– 0,1 pN/nm

Biophotonics tool in quantitative biology



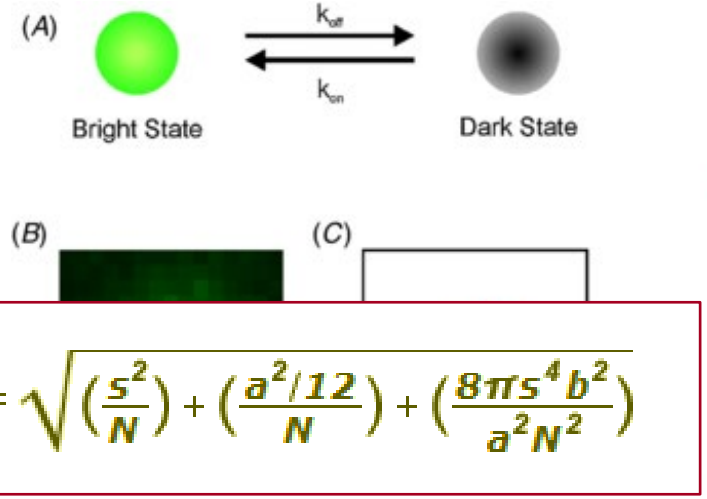
(a) Cells expressing YFP under rpoH, aggregate-sensitive chromosomal promoter



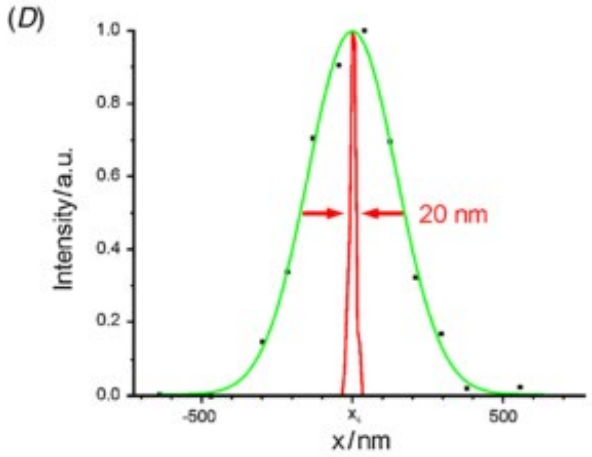


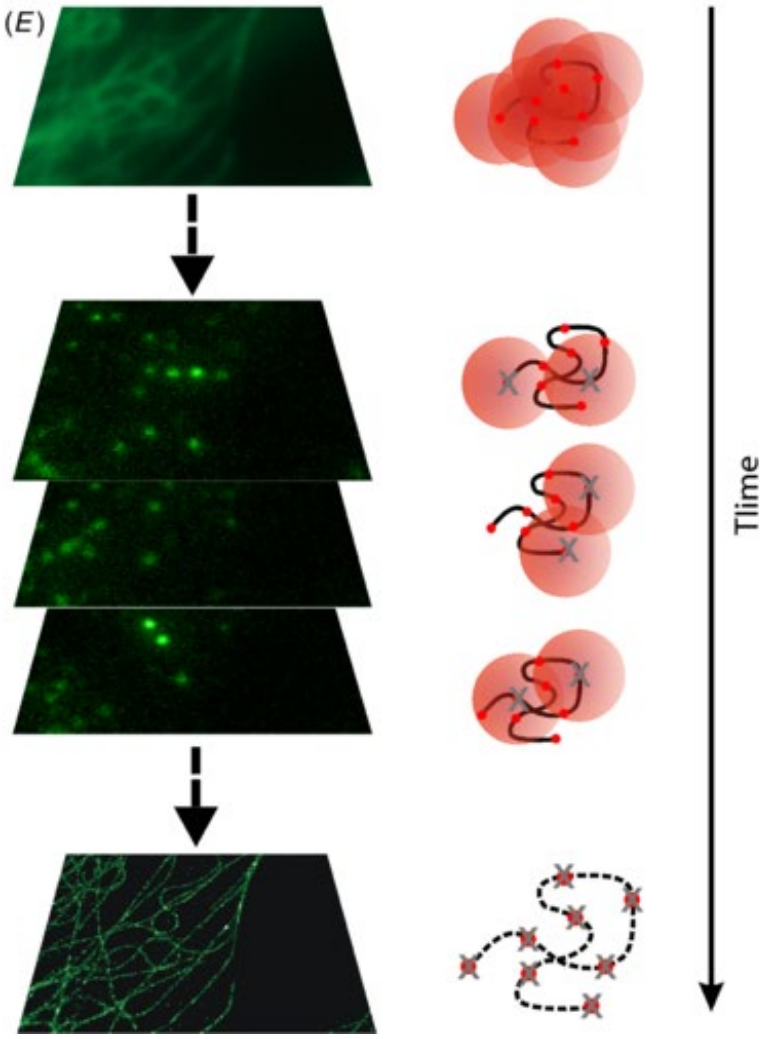
Localization microscopy PALM, STORM, fPALM, SMACAM

- Spatial localization accuracy below 10 and Nyquist-Shannon limited resolution of approximately 20 nm



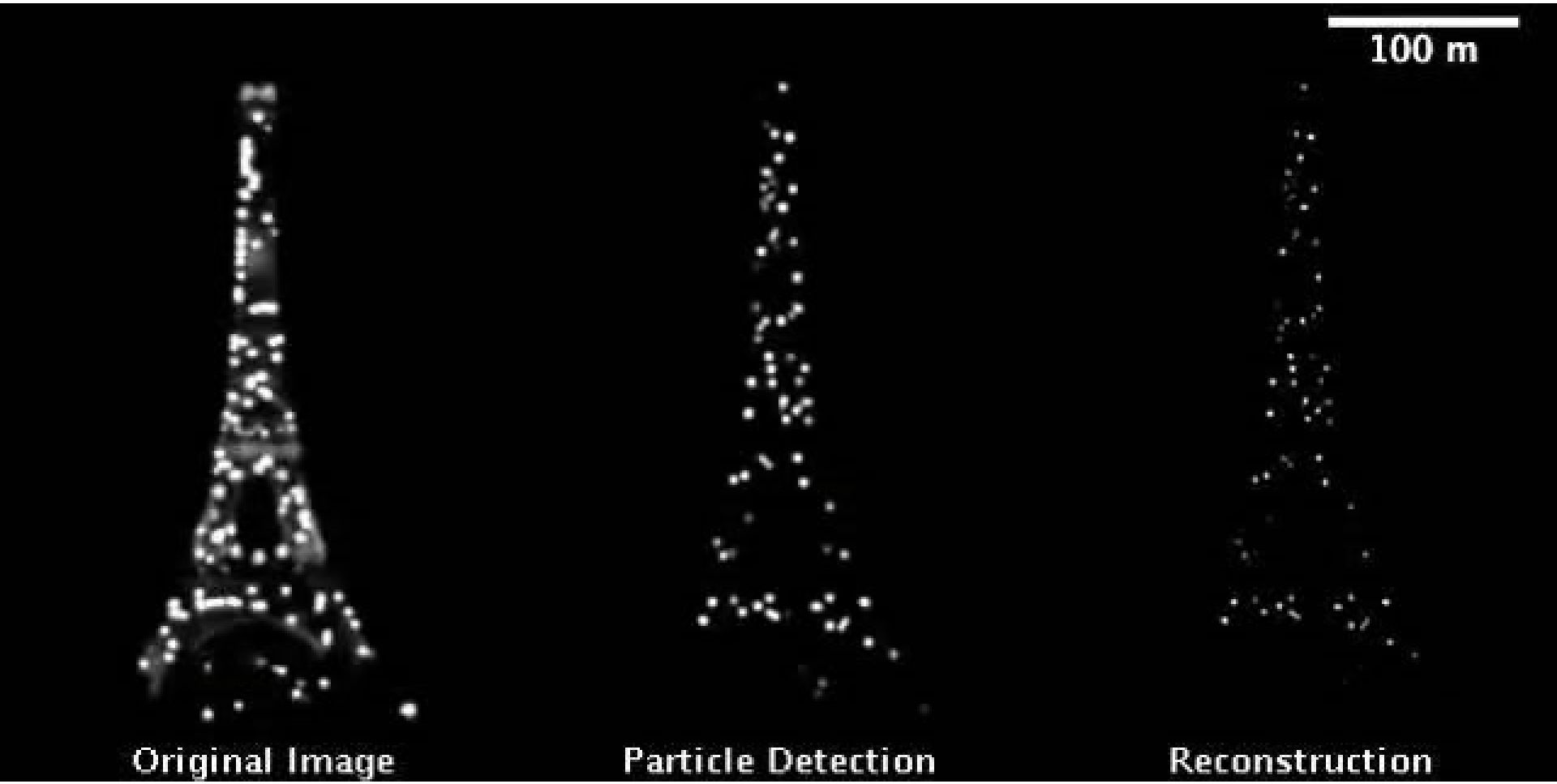
$$\sigma = \sqrt{\left(\frac{s^2}{N}\right) + \left(\frac{a^2/12}{N}\right) + \left(\frac{8\pi s^4 b^2}{a^2 N^2}\right)}$$



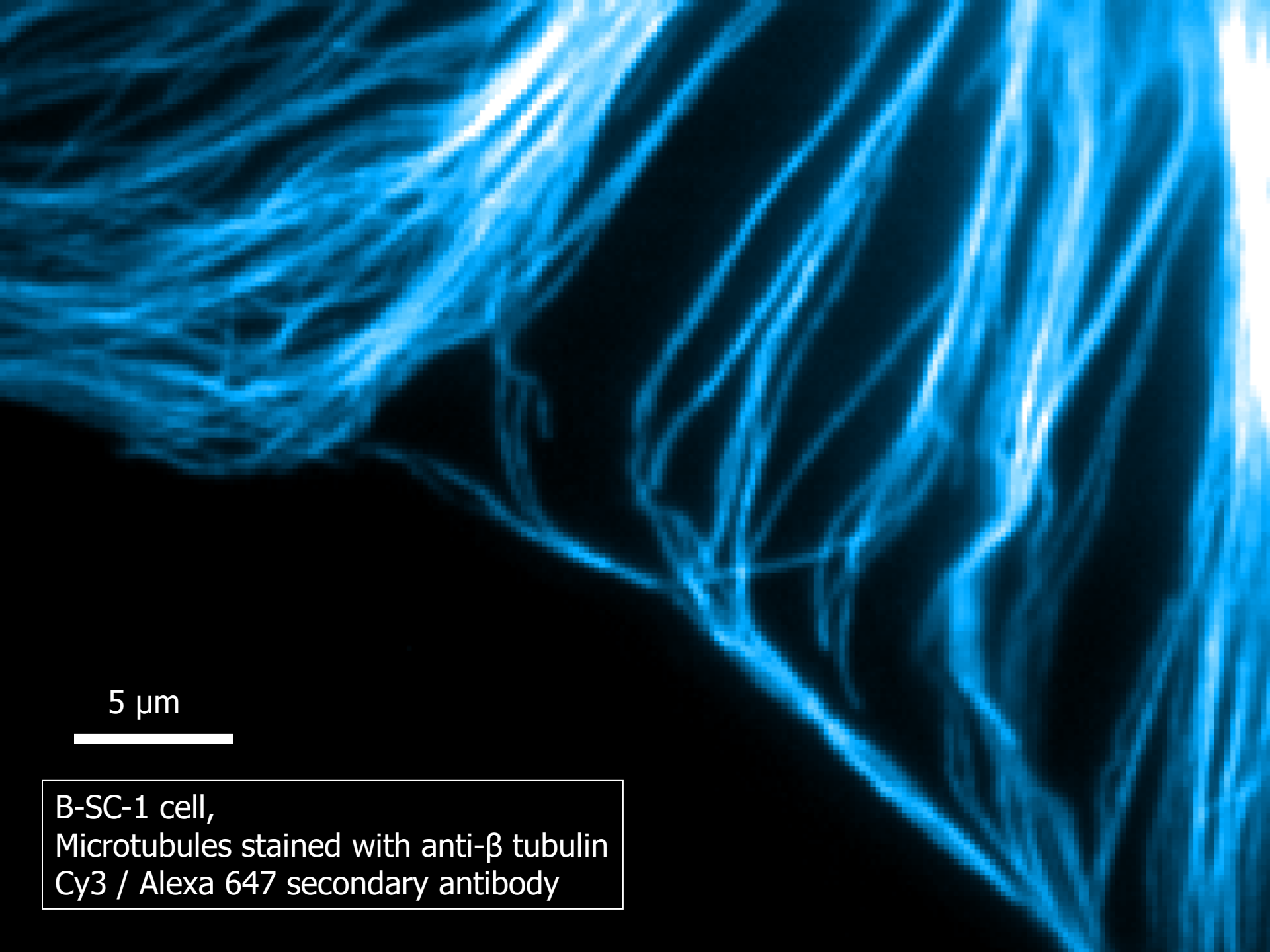


J. Hohlbein et al. Phys. Biol. **7** (2010) 031001 (22pp)

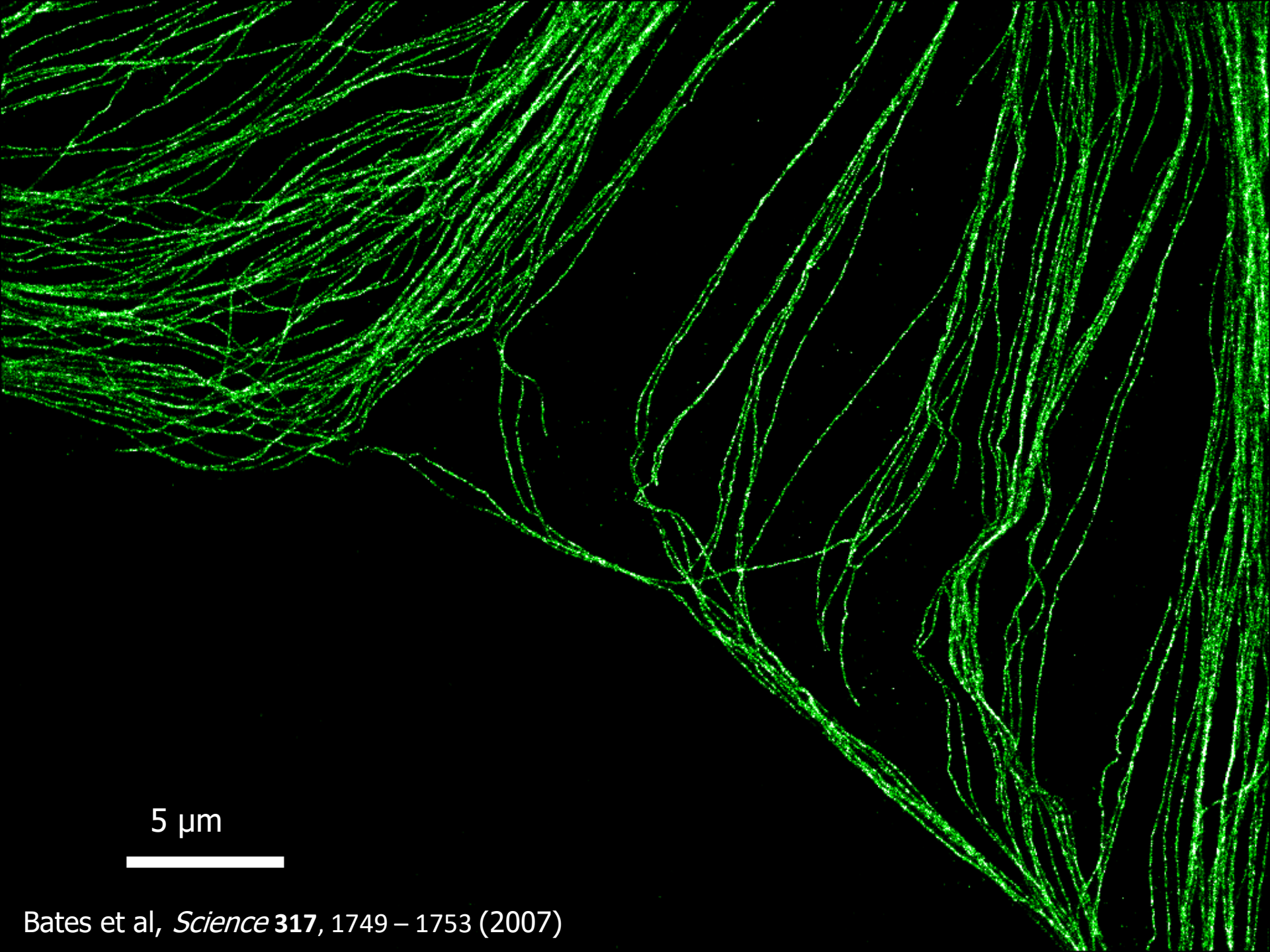
Single molecule localization microscopy



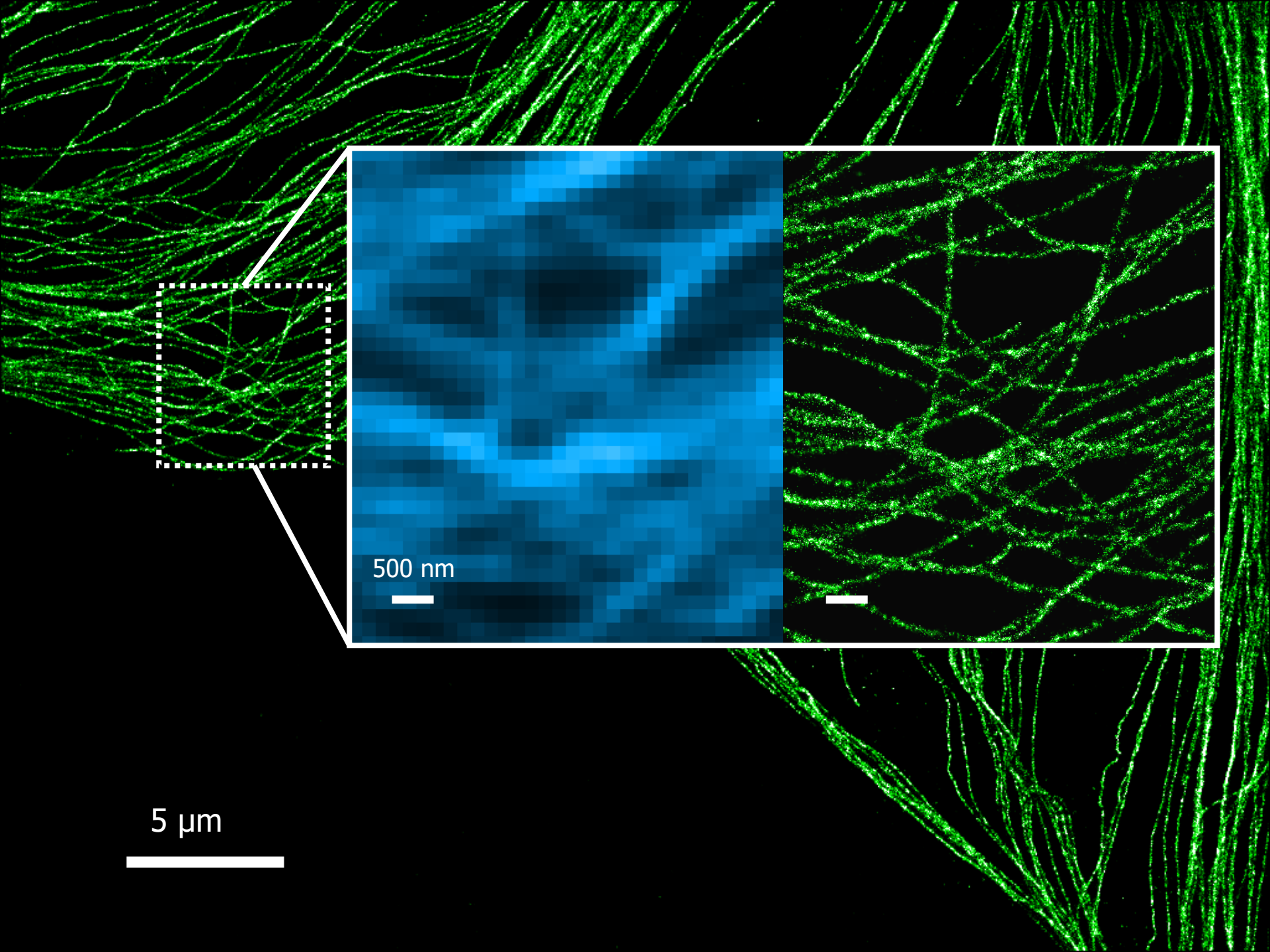
Gaussian spot detection example through QuickPALM - an ultra-fast algorithm for SMLM applied to the PSFs of a common digital camera filming the ***Eiffel Tower stochastic blinking***.

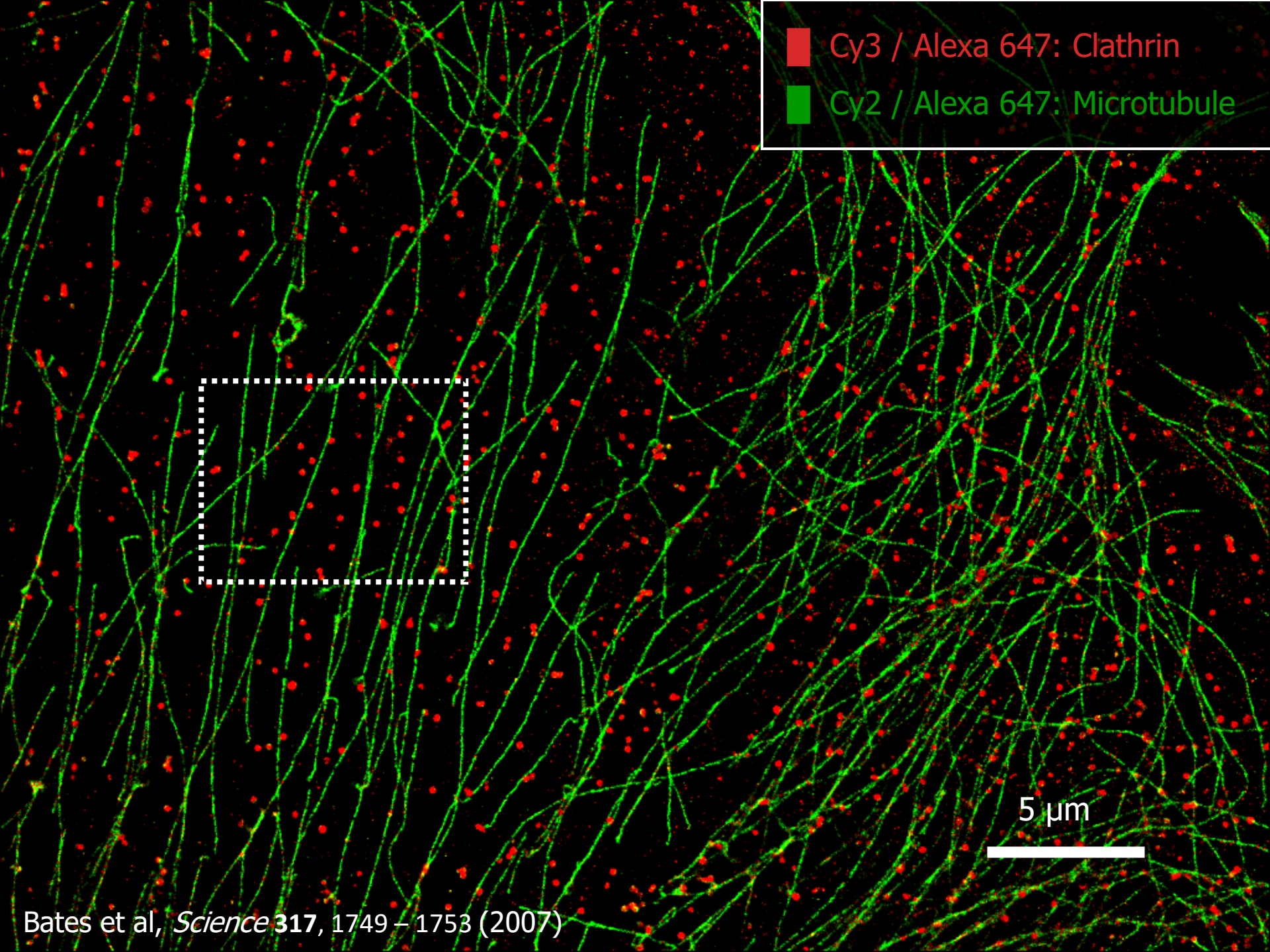


B-SC-1 cell,
Microtubules stained with anti-β tubulin
Cy3 / Alexa 647 secondary antibody



Bates et al, *Science* **317**, 1749 – 1753 (2007)


500 nm
5 µm



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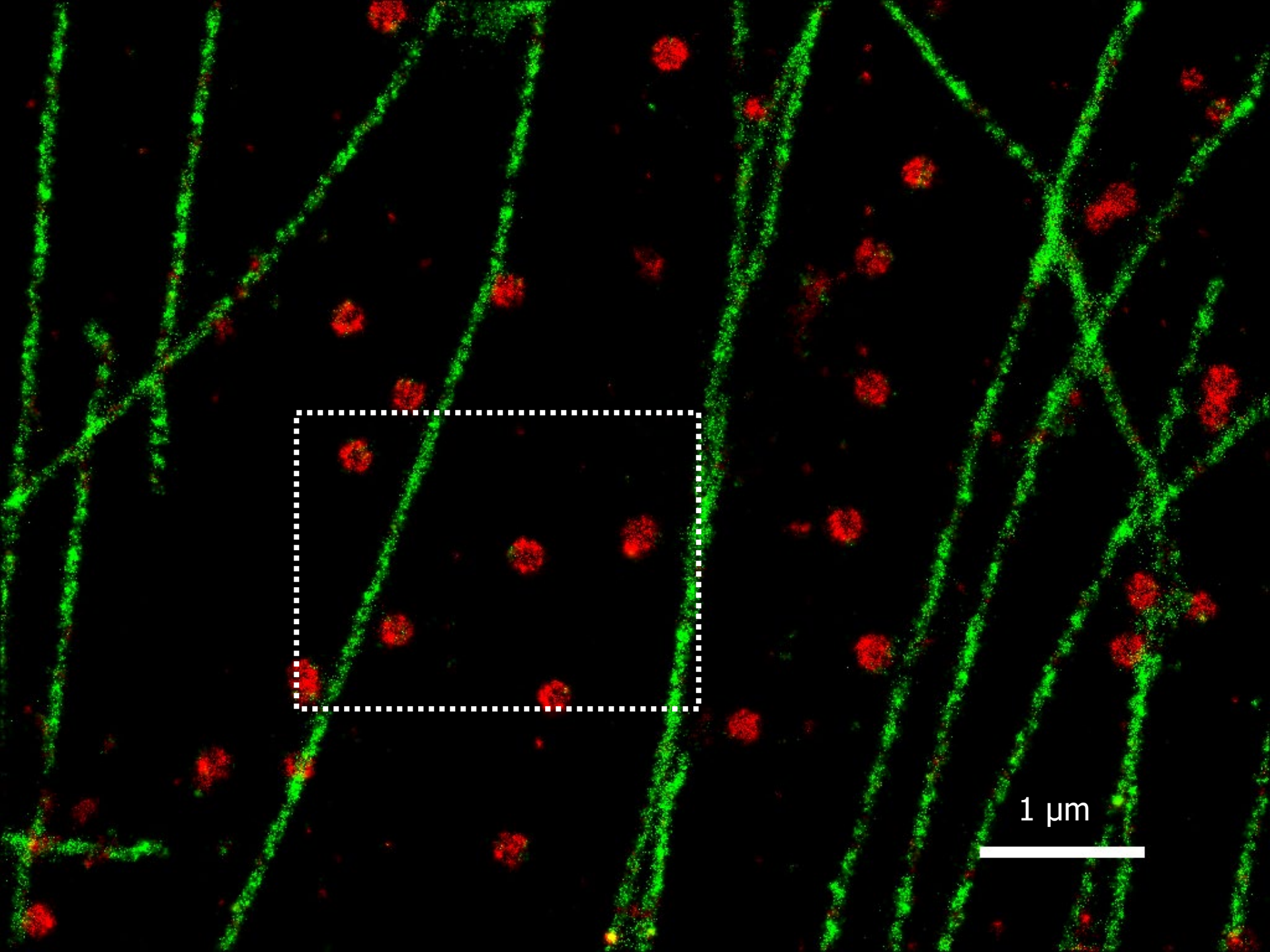
1 µm

The Royal Swedish Academy of Sciences has decided to award the

2014 NOBEL PRIZE IN CHEMISTRY

to:

Eric Betzig, Stefan W. Hell and William E. Moerner

"for the development of super-resolved fluorescence microscopy"

200 nm

Optogenetics