Bogomoletz Institute of Physiology
Kiev
Ukraine

Dr. Pavel Belan

Оптогенетические методы исследования

15.05.2014

l dz·dz

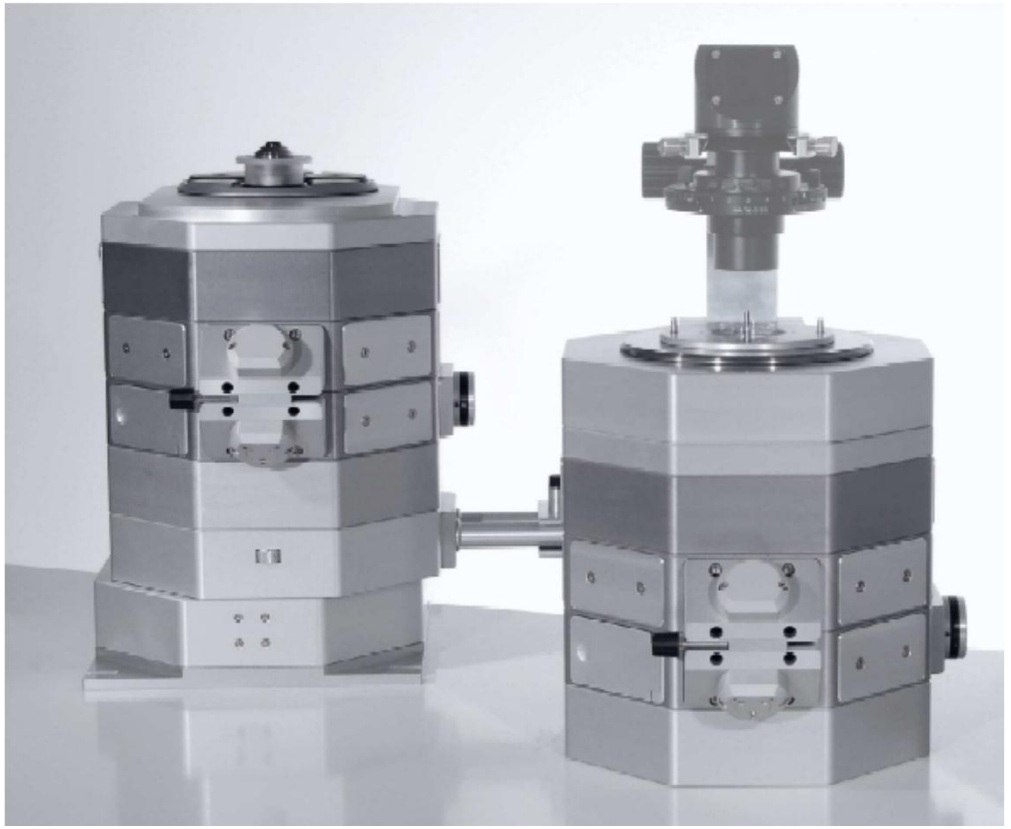
- *Флуоресцентная микроскопия основанная на применении флуоресцентных белков, Green Fluorescent Protein (GFP)*

- *Оптогенетика*

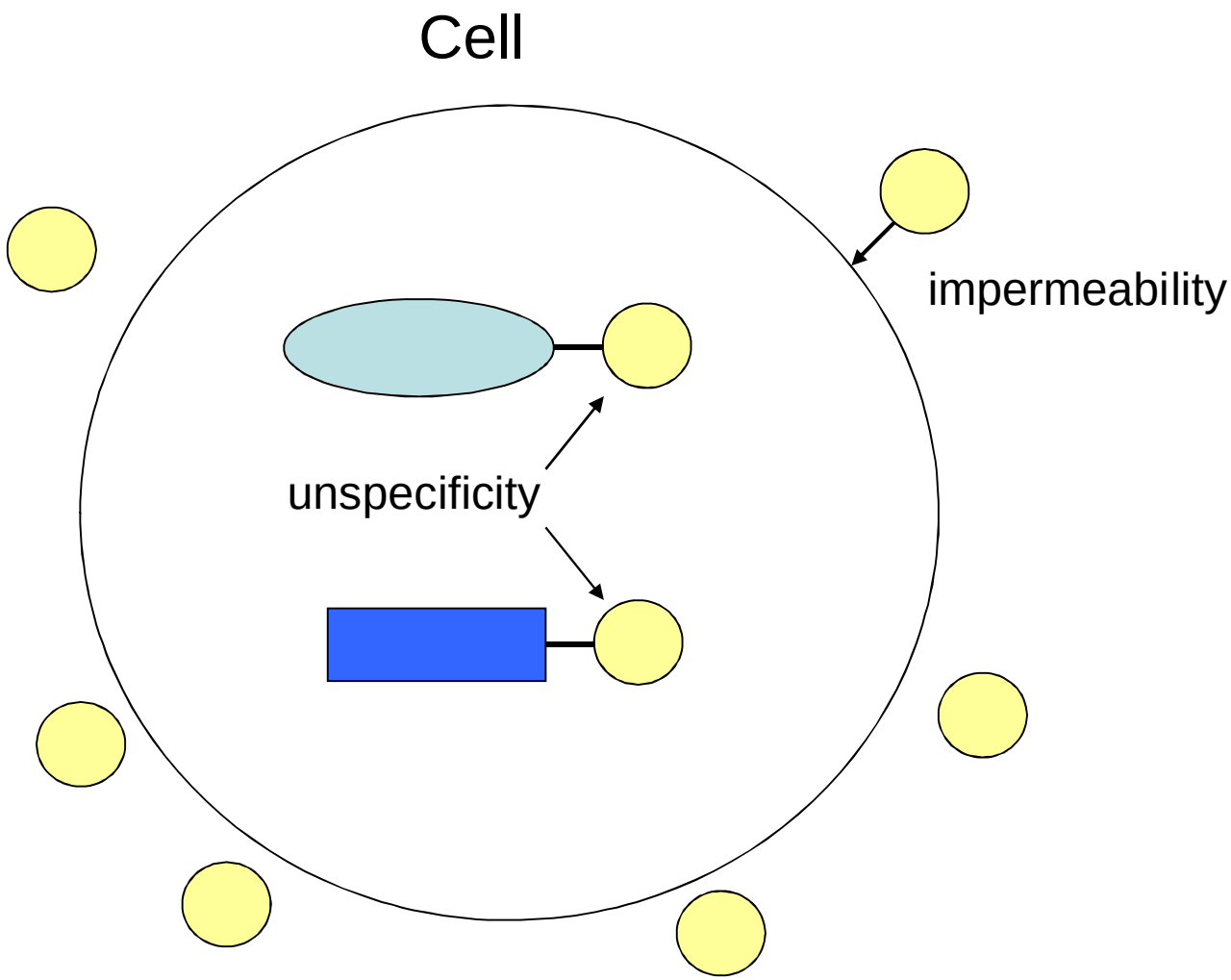
State-of-the-Art microscopes

Compound microscope

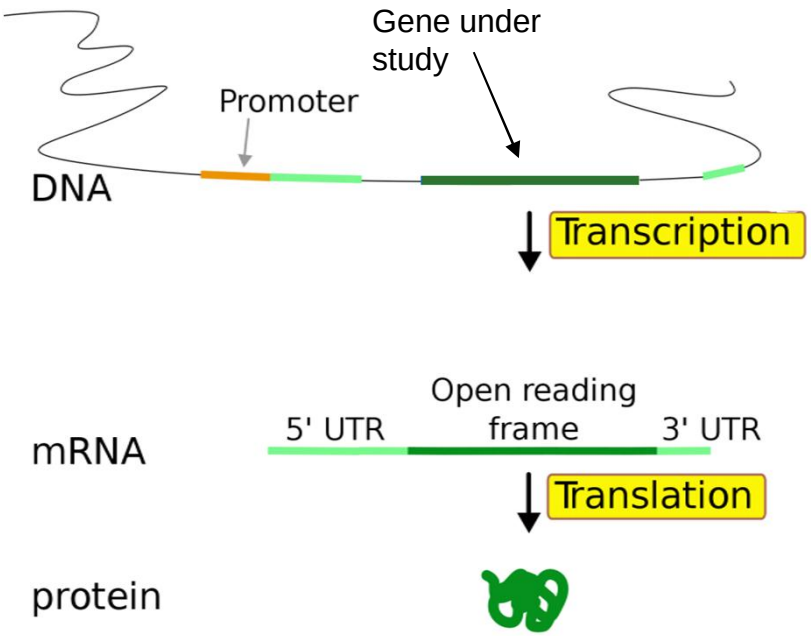
iMIC Digital Microscopes

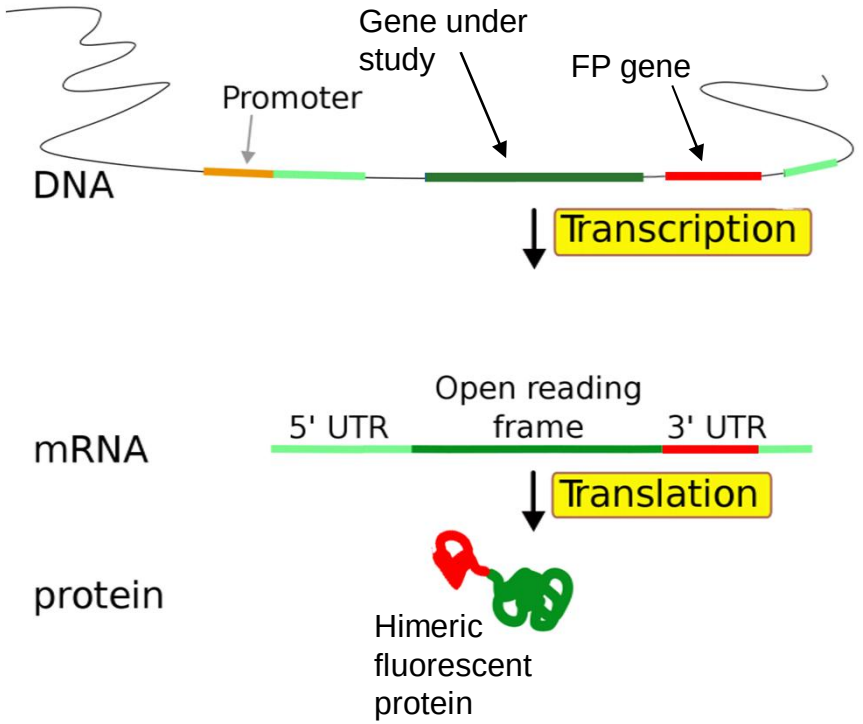


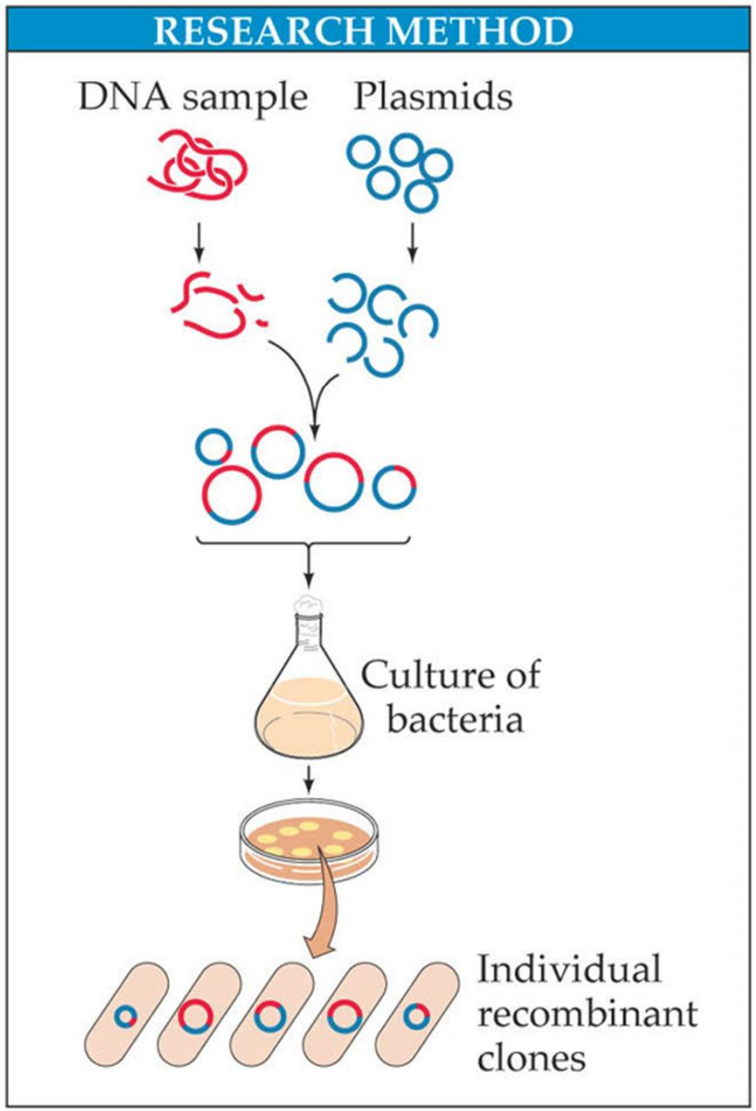
Problems with fluorescent tags



Himeric gene expression

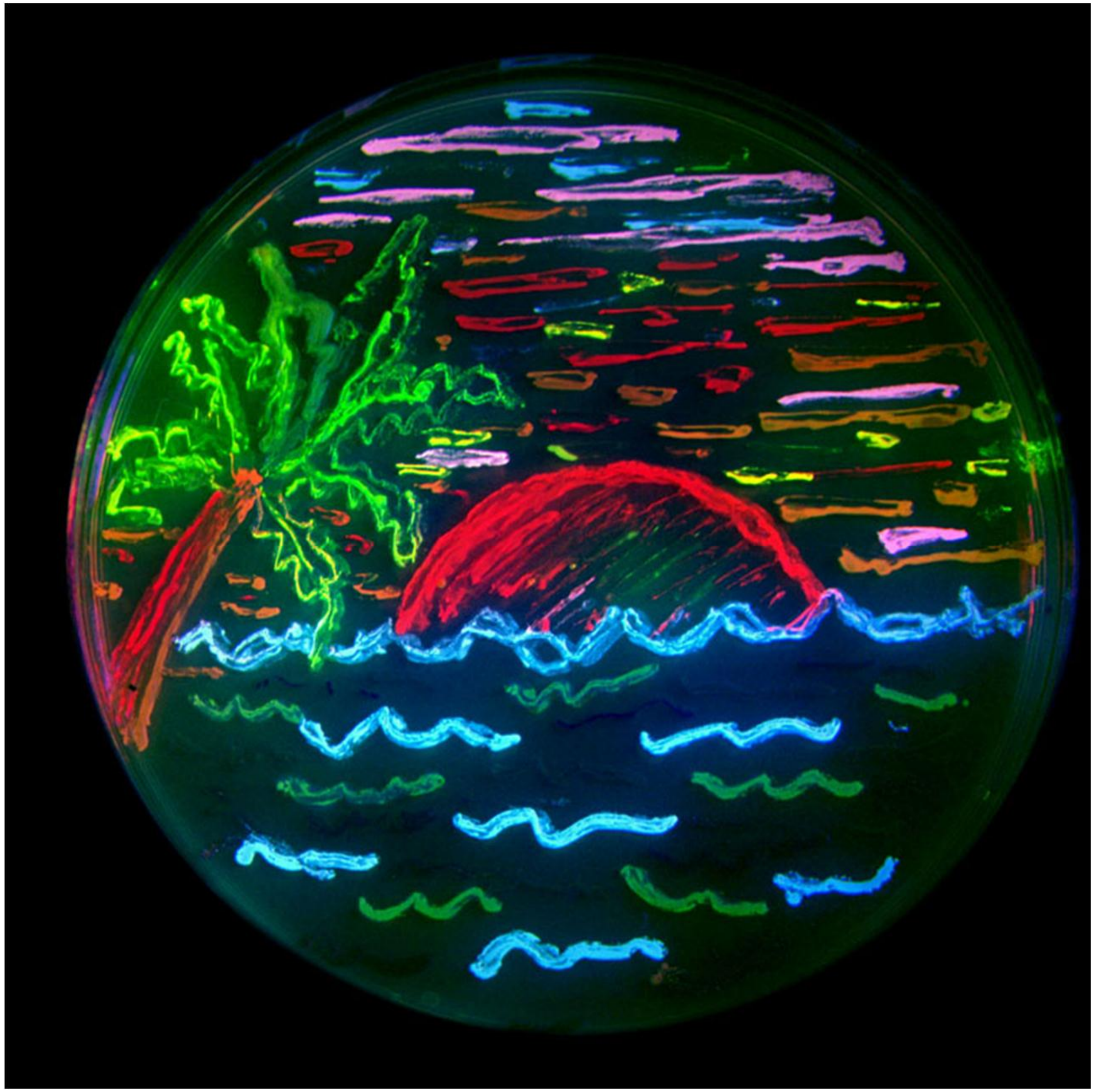




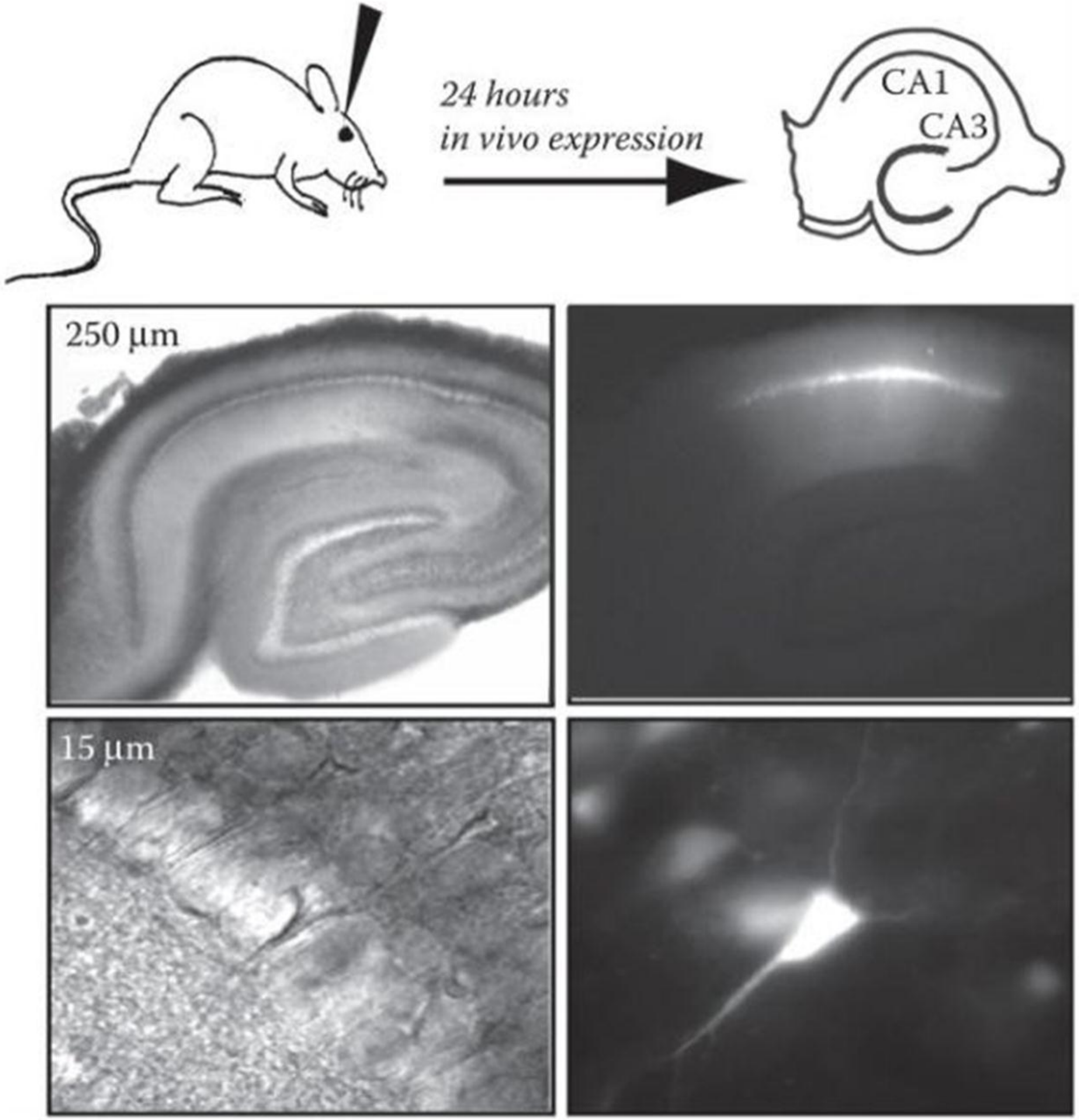


LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, **Figure 16.7 Constructing a Gene Library**

ɿ ʝ dʑʝ dʑ ʔ ʬ dʑtstɕʝ fŋɰʝ dʑsdʑ ʔ ʙʝ dʑsʕ (GFP)

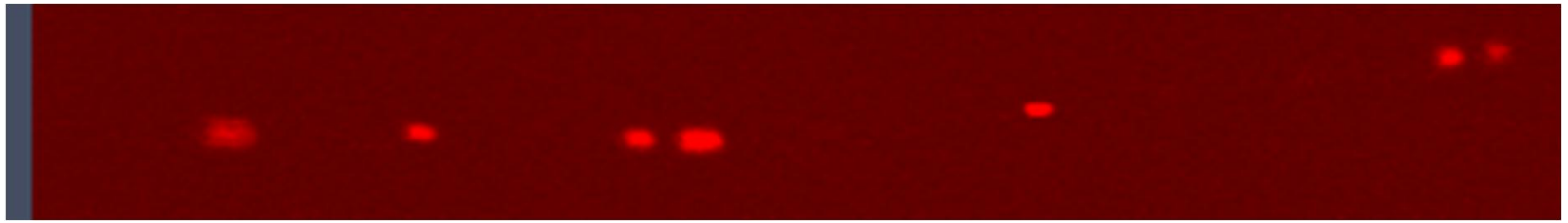


http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP-1.htm


24 hours
in vivo expression
CA1
CA3
250 µm
15 µm

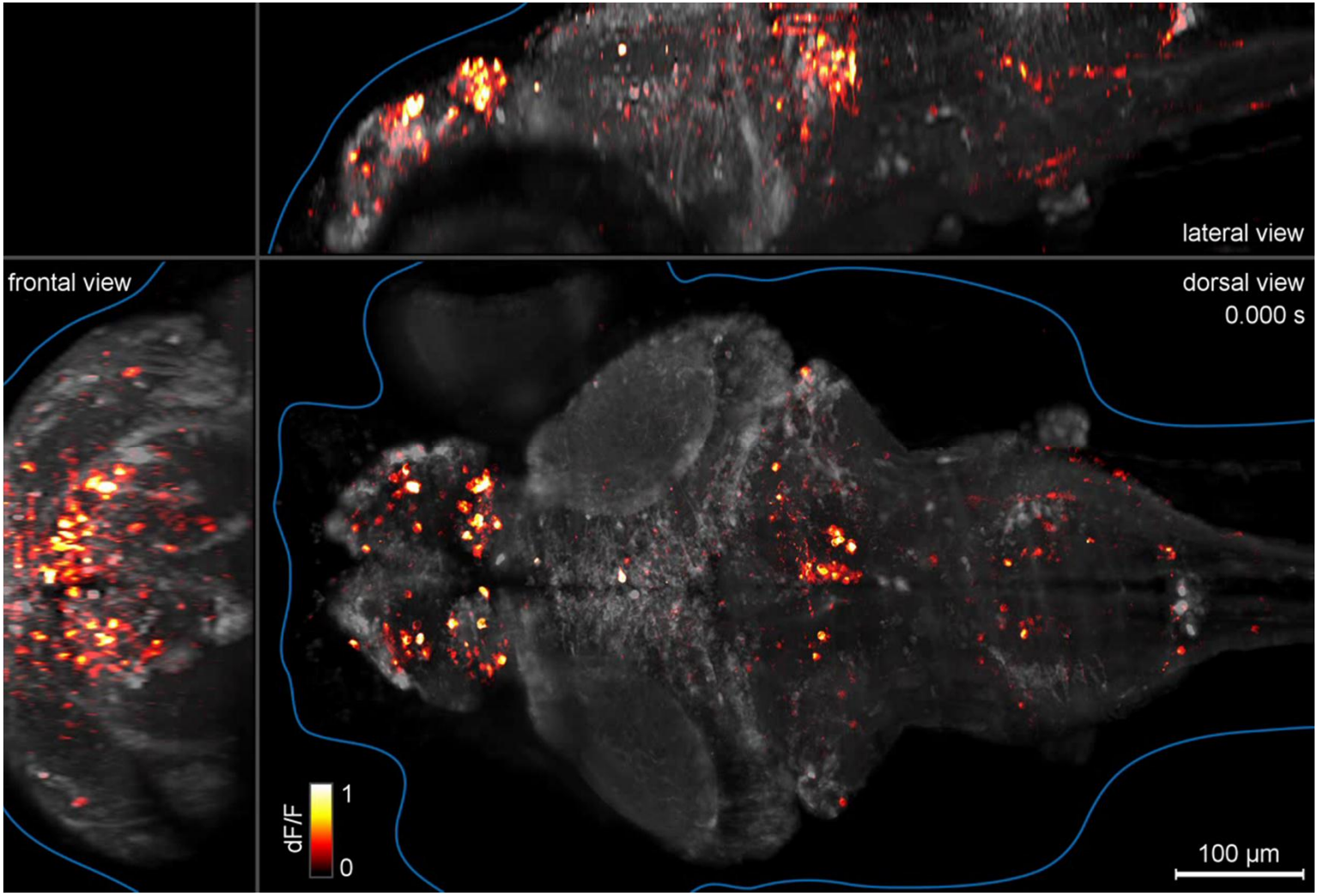
ɬ ɑʃtɷʘɔdʒɑʃʜdʑ ʝ dʑʝʔtɷtsdʑ «tɷʘʜlzʞ dʑ ⊓» ʕtɷʳ fŋ

Mitochondrial dynamics in axons of hippocampal pyramidal neurons



J Biol Chem. 2011 Mar 30. [Epub ahead of print]

Whole-brain imaging of neuronal activity



Nature Method (2013) doi:10.1038/nmeth.2434

Å ʌ ʣʣʦʦʨʝ fŋɥʝ ʣʑʦʣʑ ʝ ʙʝ ʣɮɖʃ ɘʤʒʝ fŋʃsʝ fŋ
ʨʘʟ ʣʑʦʦʙ ʨʘʟ ʣʑ ʤɖʃ fŋʧʦfŋʦʙ ʘʤɖʃ ʜʦfŋsʘɘ ʢɖʃ
ɖʃ ɕʝ ʣʑsɘ ɘʣʑlʦʨ ʢʣʑ lʦʦʢ ʷ ɘʣʑʷʁ lsfŋʷ
ʤʦʰ ʣʑ ʤɖʃ ɖʃʣʑŋʃsʨʣzʤʒʝ ʣʑʦʤʒ ɖʃfŋʃfŋʣʑ ʜʦɘ ʘʣʑʃʡ
ɘ ʦʙ ʣʘfŋʃsɖʃ ʢʣʑ lʦʦɥʣʑʡ ʙɖʃʦʣʦɢɖʃɖʃ,
ʧʦʟ ɘʦʣʷʁ ʰ ɖʃʤɖʃ ɖʃfŋʃfŋʣʑ ʜʦɘ ʘls ʜɖʃʣʑʘʤɖʃʢlz
ʙʝ ʣɮʦɘ ɖʃ ʢʣʑ lʦʦɥʣʑ [illegible] ʦʨɢʘʣʑ ʣzɘ ʞ ɖʃɘʳ [illegible]
ʢʣʑ lʦʦɥʣʑ [illegible] fŋɖʃfŋʃsʝ ʤʘ[illegible].

Å ɿ ʦ ʤʦʞ ʥʦ ʣʠ ʥʝ ʪʦʣ ʢʦ ʠɲɲʥʝ ʜ ʦɘʘʪʮ

ʢʥʝ ʪʦɻʥ ʝ ʧ ʨʦɻʝ ɲɲ , ʥʦ ʠ ʣʧ ʨʘɘ ʣʷ ʪʮ

ʠʤʠ?

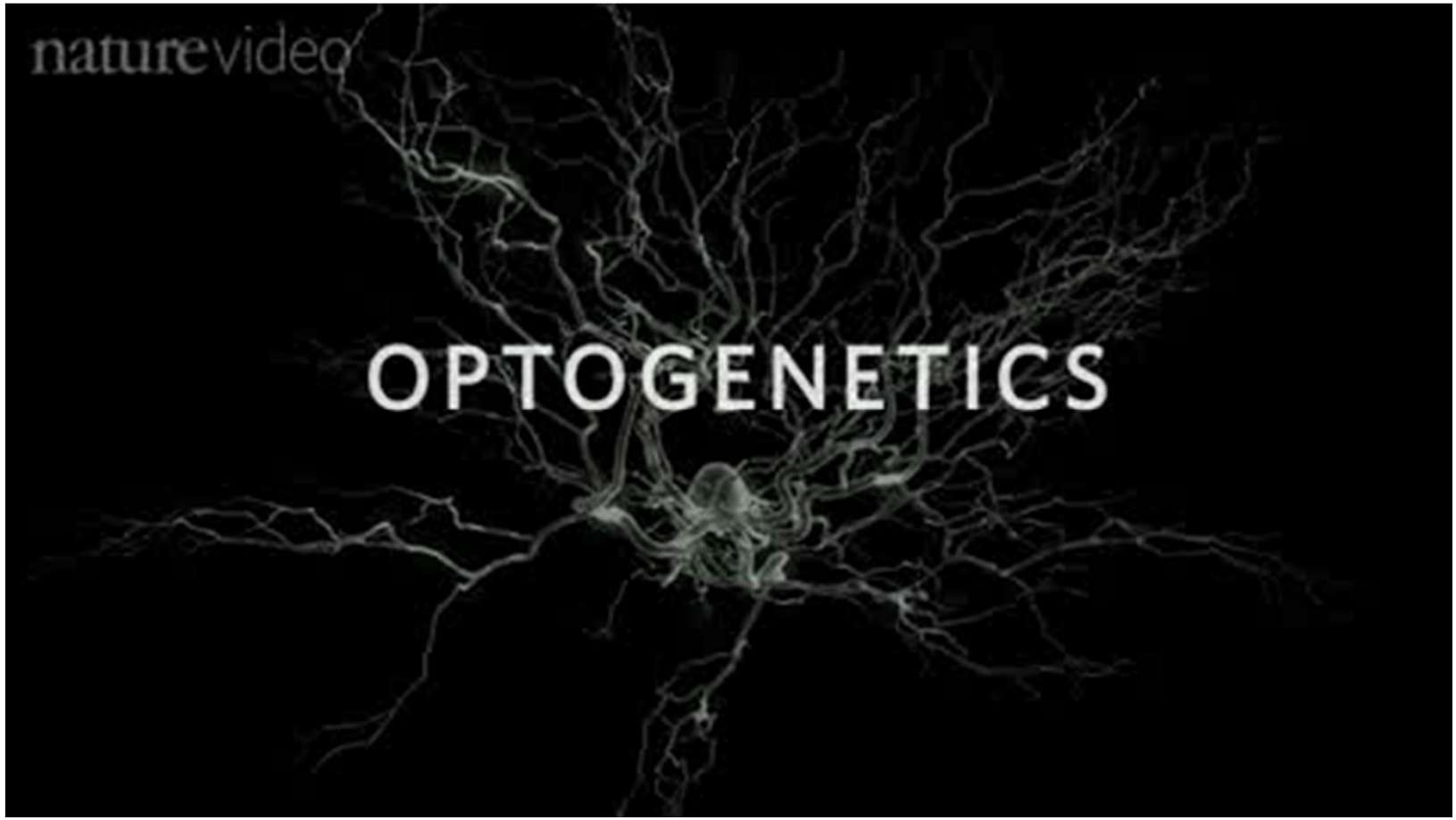
ʃ tʃlstsgj dʒjlsɑʃʕʘ(Optogenetics)

ʃ tʃlstsgj dʒjlsɑʃʕʘɻ lsts ʕtsdʒɜɑʃdʑʊɑʃʷ ɑgj dʒjlsɑʃʊjfɲʕɑʃ ɑʃ tsʃlsɑʃʊjfɲʕɑʃ dʒjlstsʜtsɔ fɲ
ʊjdzʁ ʕtsdʑstɑtsdzɑʃtɑtsɔʘlsʏ frʃʃ ʊɑʃʷ ɑʃʊjfɲʕɑʃ frʃtsʁʳ lsɑʃʷ ɔ tsʃtɑjʜjdʒjdʑdzʑ ɲ
ʕdʒjlsʕʘɲ ʞ ɑʃɔʳ ɲ lsʕʘdʒjʔ fɲdʒʃɑdzɑʃfɲjʕtzdʑʜdzʑ dʒɔtɑjdʒjdʑdzʑ dʒtɑʘʟtɑjʰ ʃjdʑdʒʃjdʒ

Optogenetics is the combination of genetic and optical methods to control specific events in targeted cells of living tissue, even within freely moving mammals and other animals, with the temporal precision (millisecond-timescale) needed to keep pace with functioning intact biological systems.

http://en.wikipedia.org/wiki/Optogenetics

Photosensitive bacteria are founders of optogenetics



Nature web site

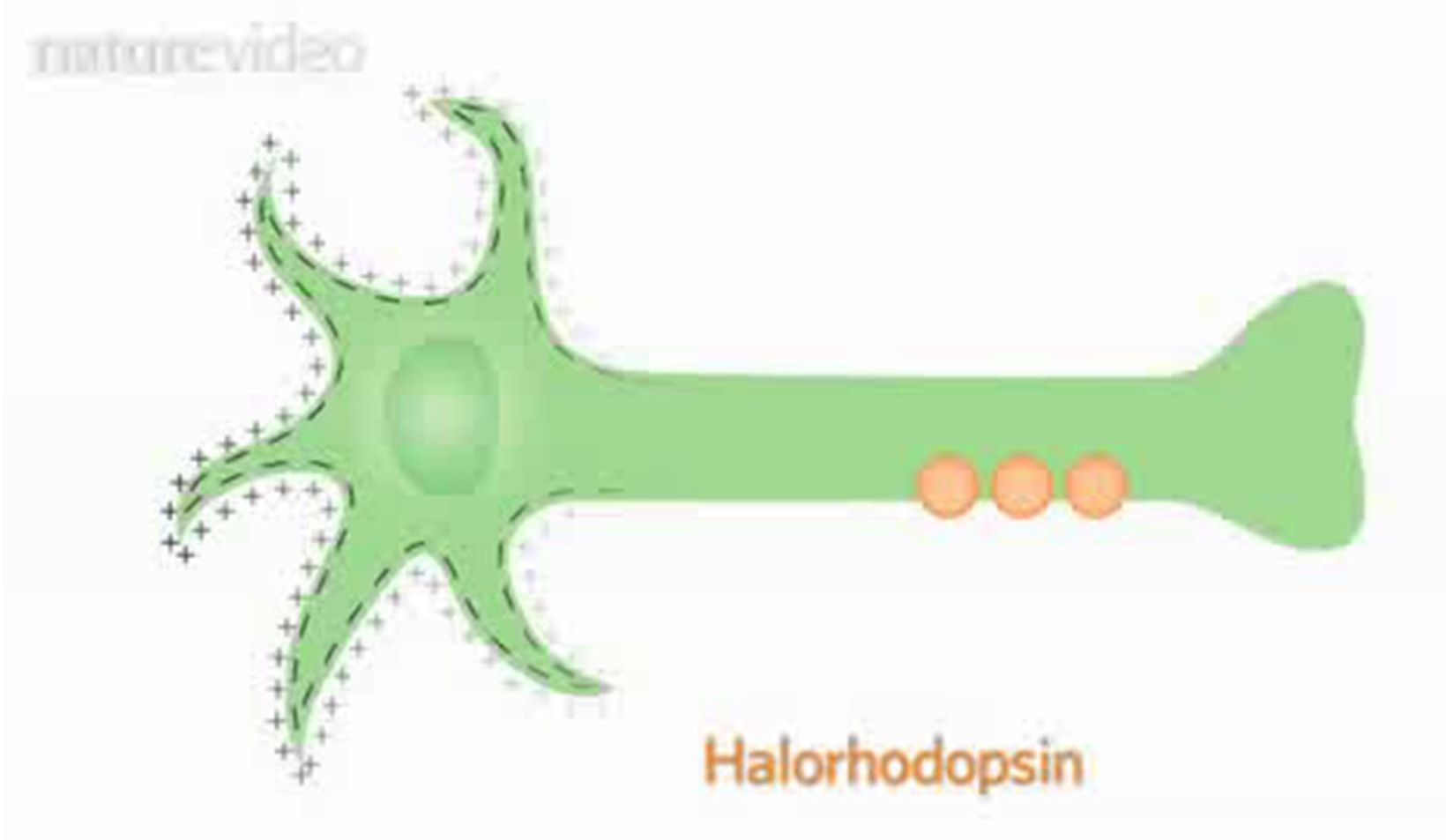
Channelrhodopsin (ChR2) an electronic photoswitch exciting neurons

Nature web site

Examples of how Channelrhodopsins can control cells and animal behavior

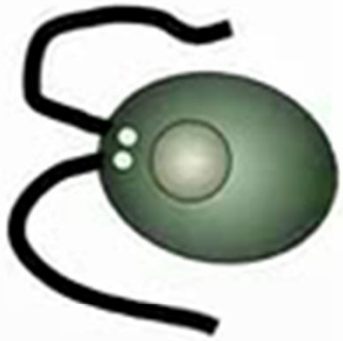
Nature web site

Halorhodopsin (NpHR) - an electronic photoswitch inhibiting neurons



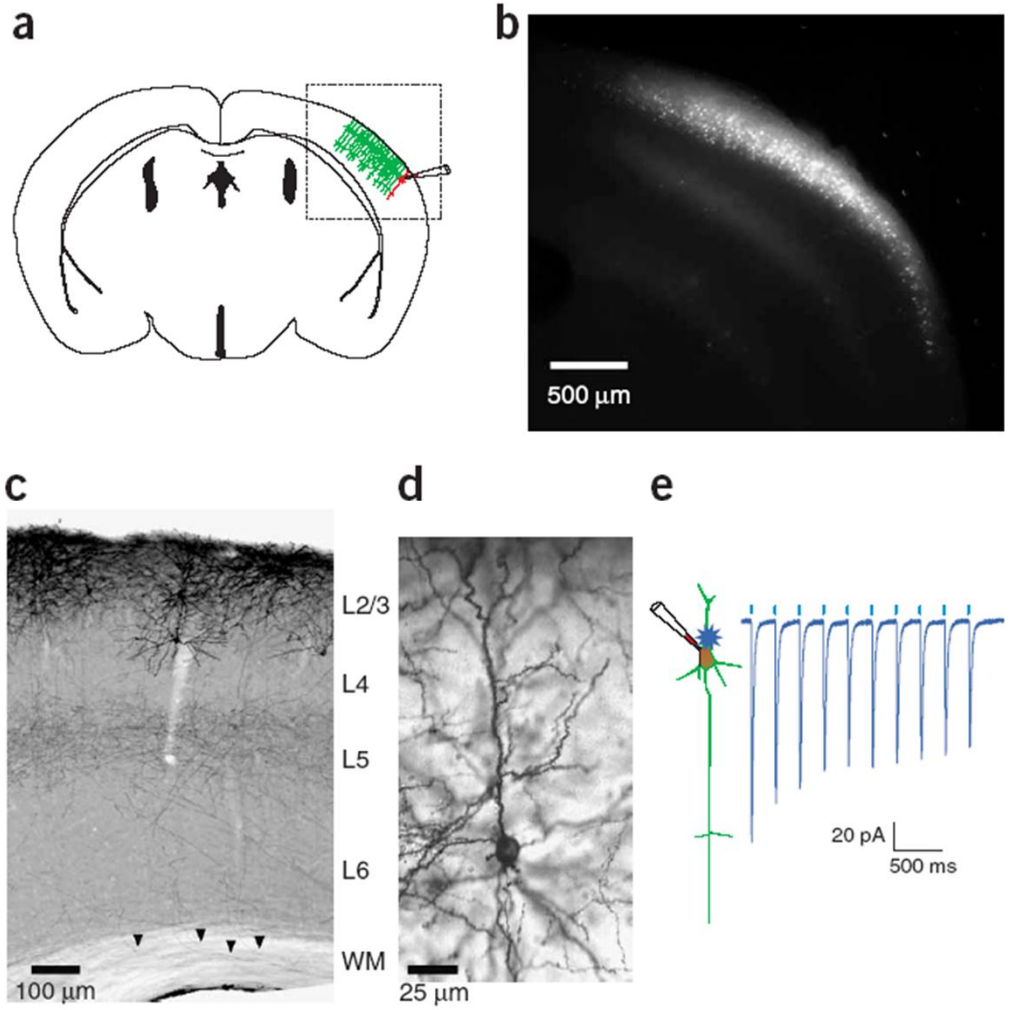
Nature web site

Molecular-genetic approach to indroduce optogenetic constructs



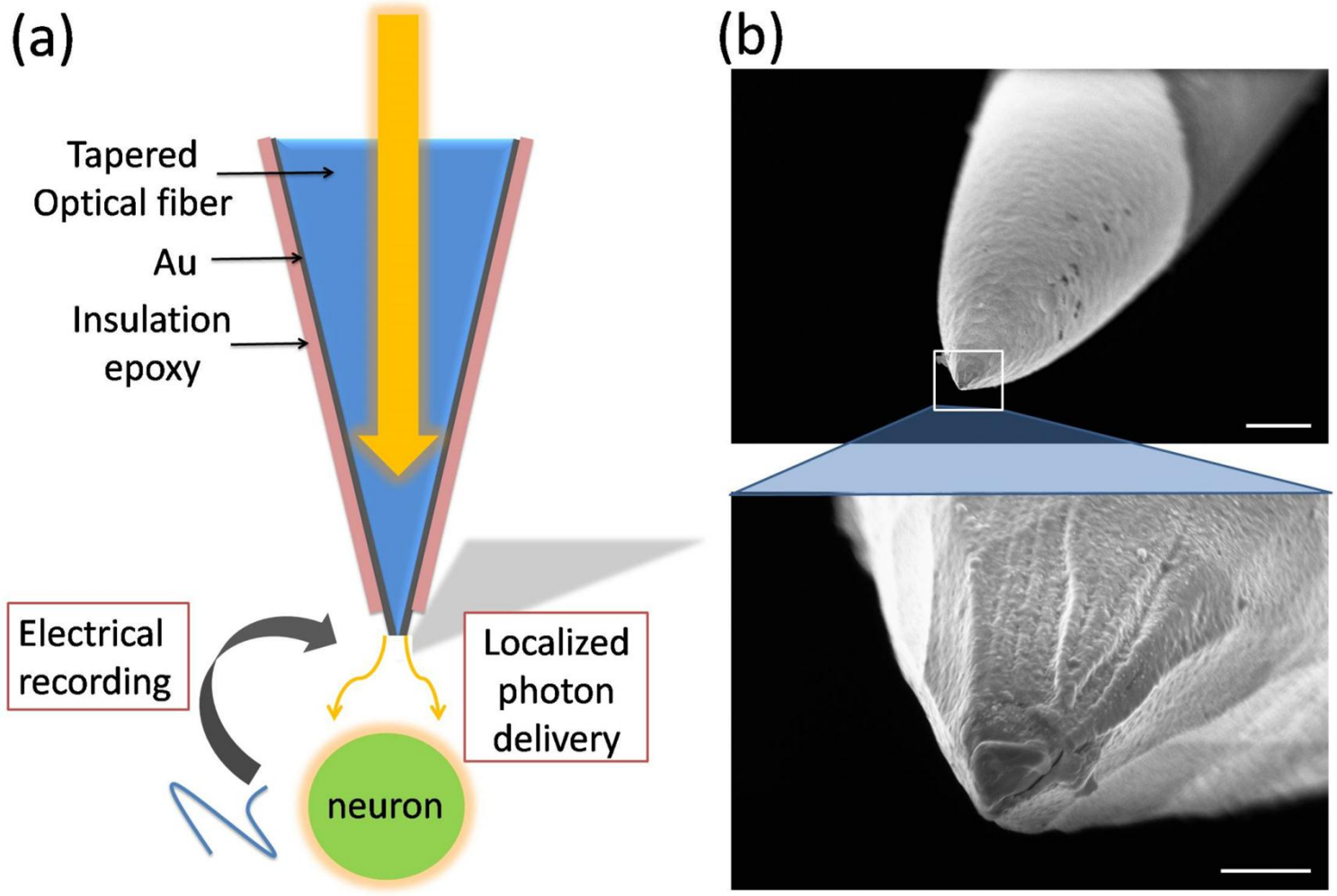
Nature web site

Photostimulation of ChR2-positive neocortical L2/3 pyramidal neurons



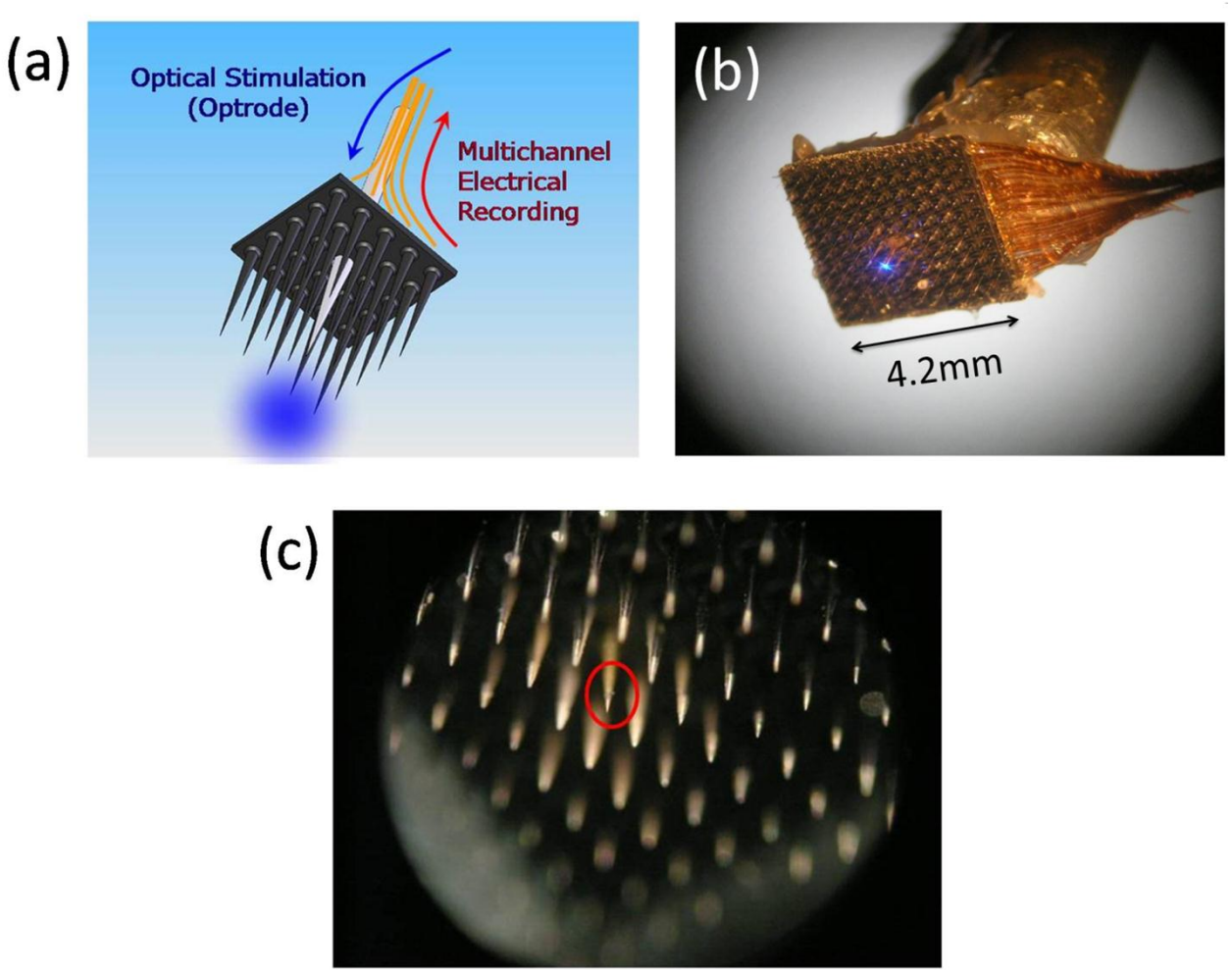
NATURE NEUROSCIENCE 10:663 2007.

Schematics and SEM images of optrode



http://nurmikko.engin.brown.edu/?q=node/33

Optrode array assembly

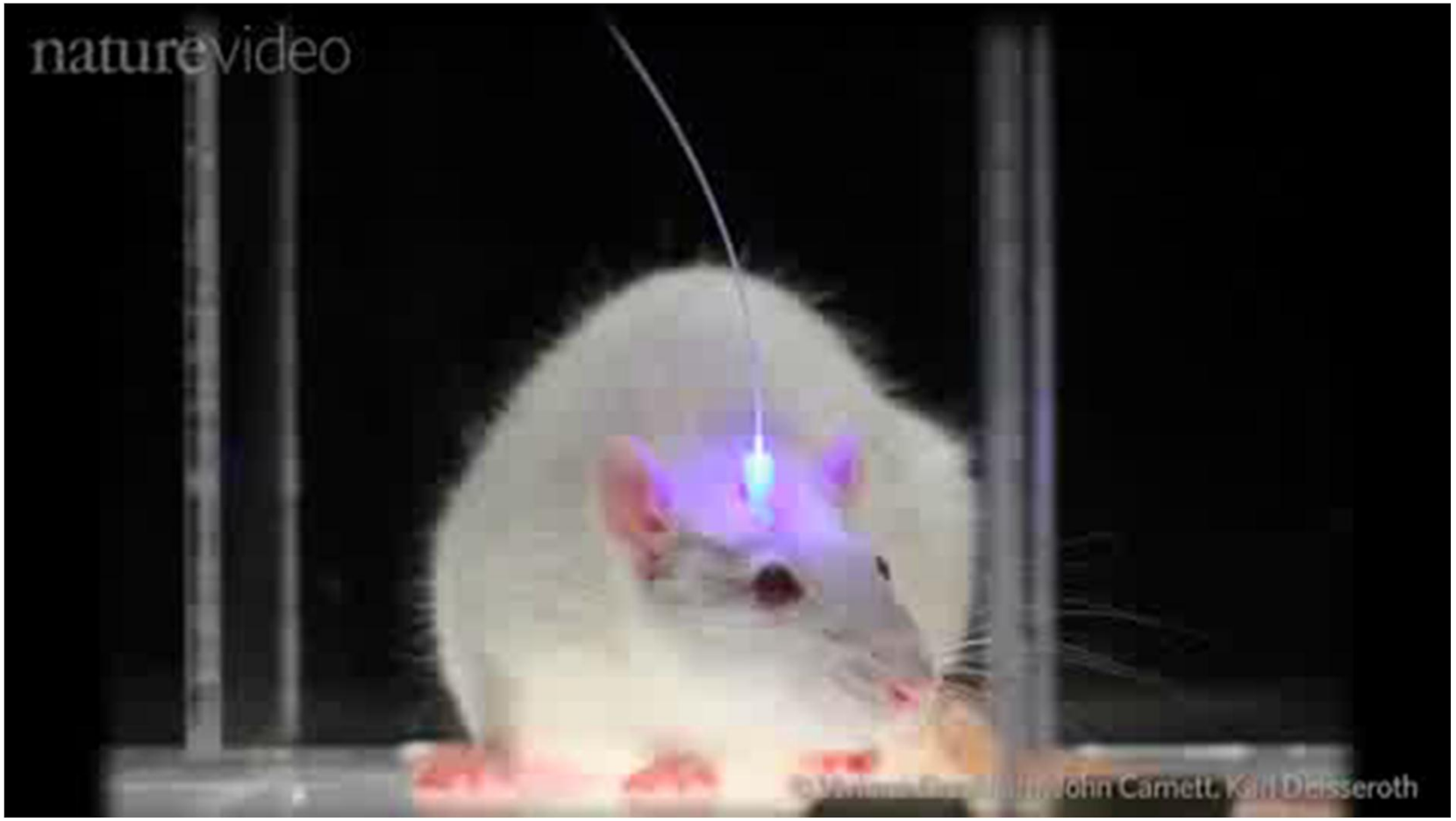


http://nurmikko.engin.brown.edu/?q=node/33

ChR2 mouse

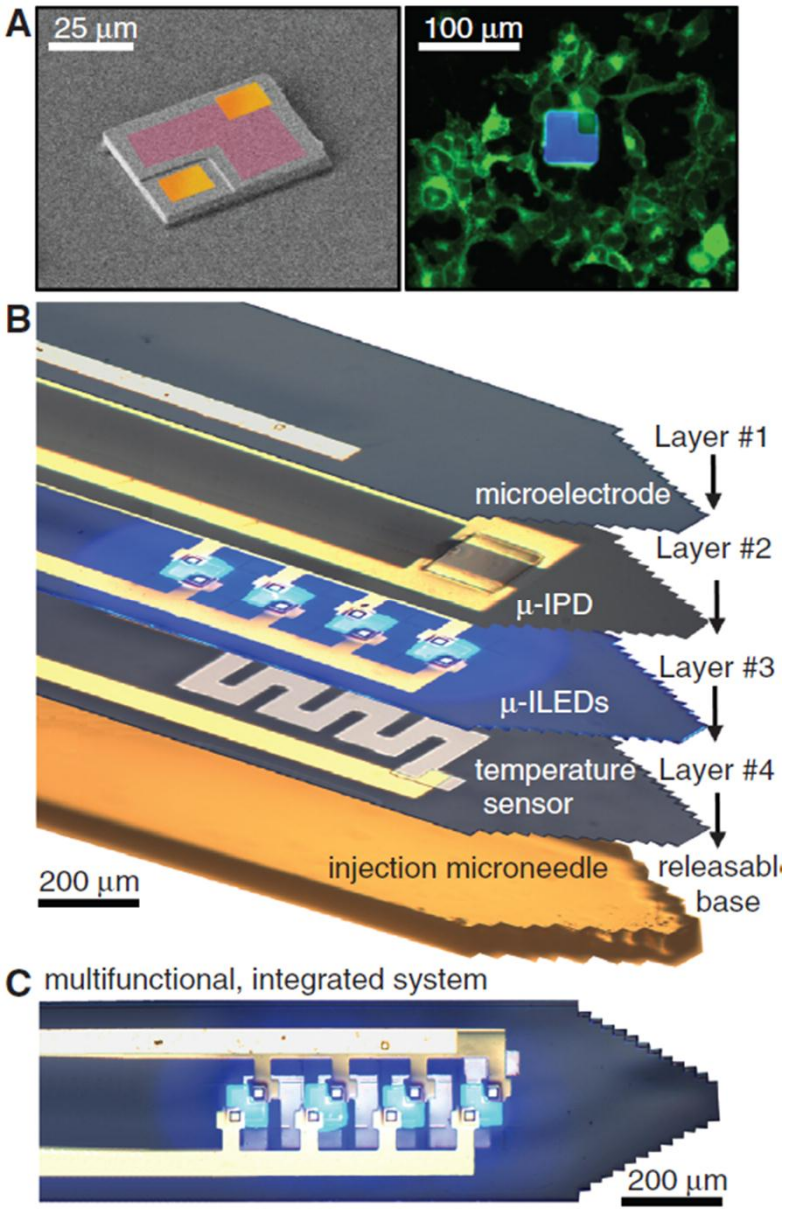
Nature web site

Parkinson disease treatment by optogenetic approaches



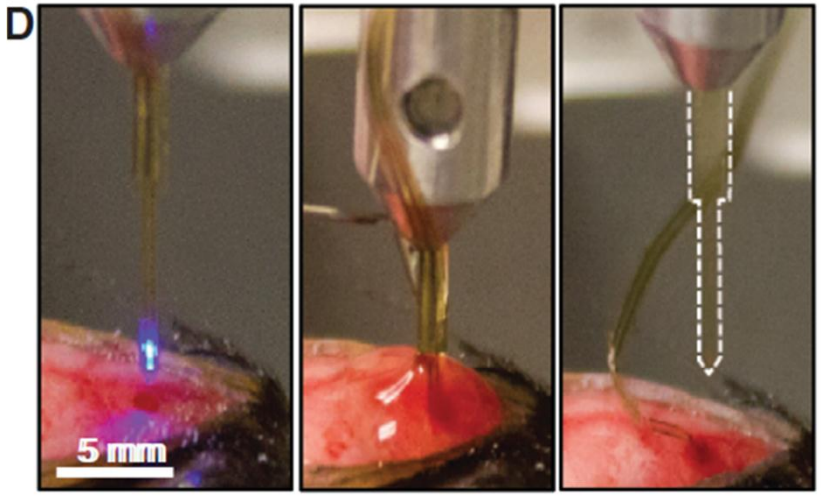
Nature web site

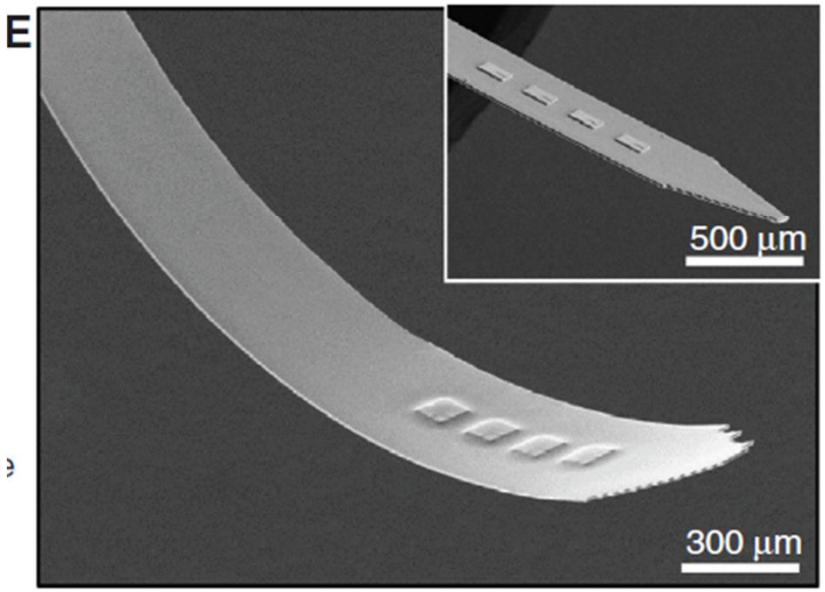
Injectable, cellular-scale semiconductor devices

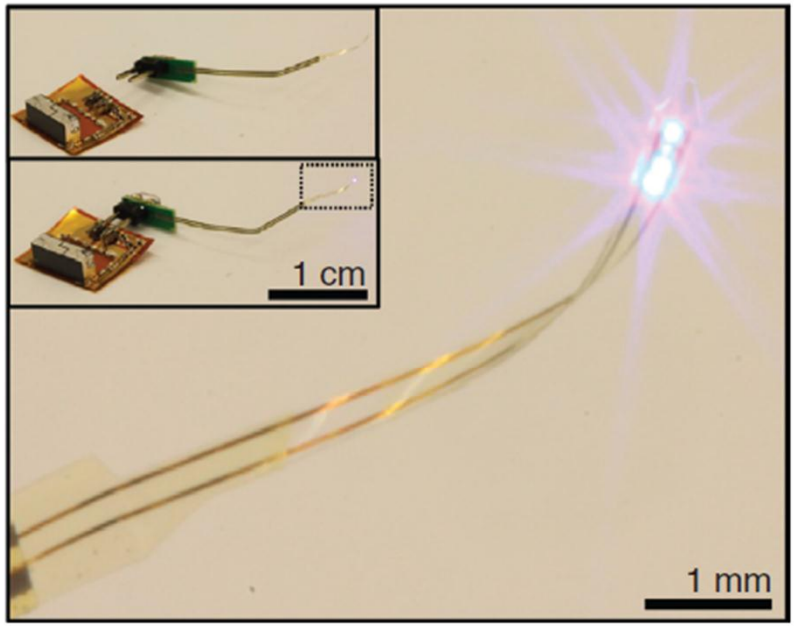


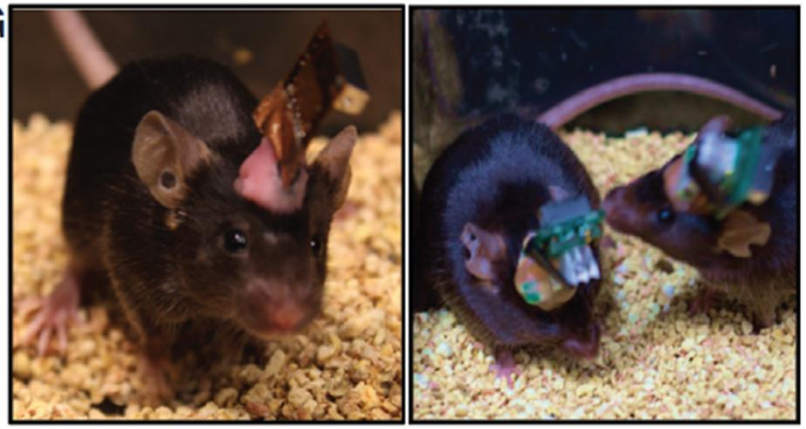
Science **340**, 211 (2013); DOI: 10.1126/science.1232437

Injectable, cellular-scale semiconductor devices



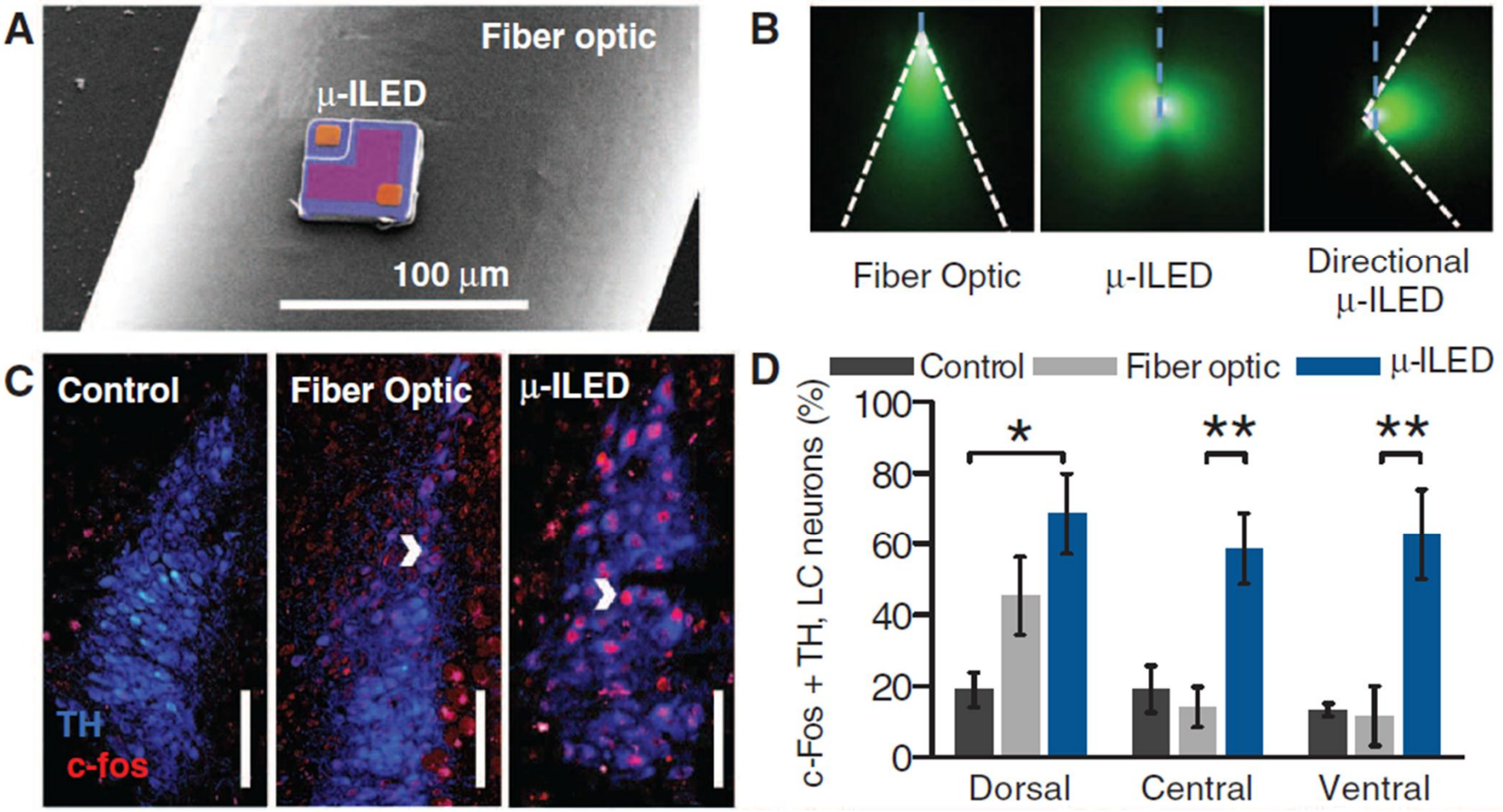






Science **340**, 211 (2013); DOI: 10.1126/science.1232437

μ-ILED devices improve spatial targeting

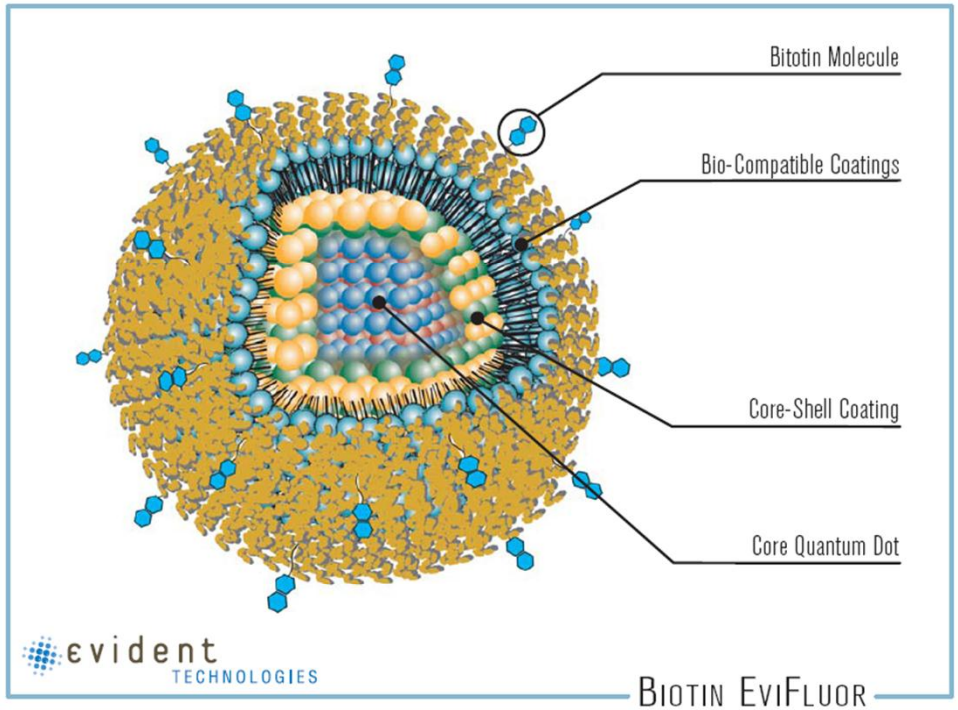


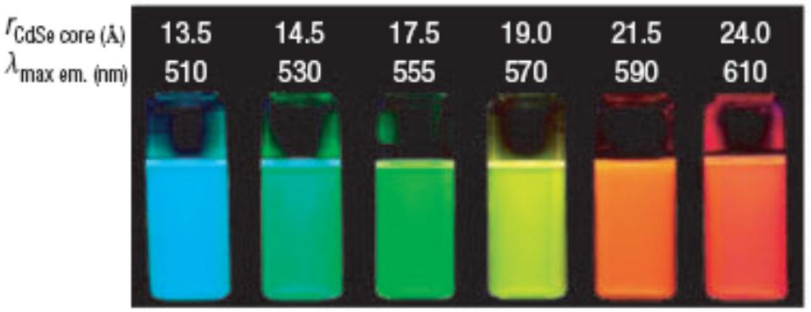
Science **340**, 211 (2013); DOI: 10.1126/science.1232437

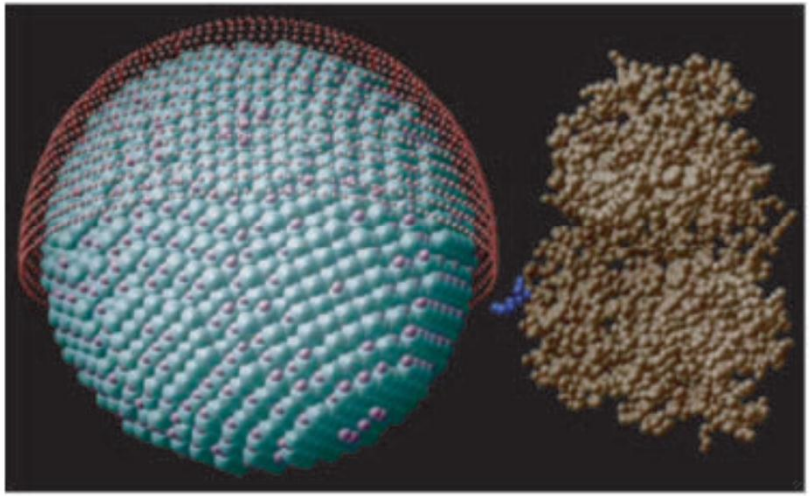
Conclusions

- **Optogenetics** is a new experimental methodology based upon the combination of genetic and optical methods to control specific mainly electrical events in targeted cells of living tissue.
- **Optogenetics** allows to almost instantaneously switch on and off certain neuronal groups in order to study their functions and relationships with other cells of animal body.
- **Optogenetics** also allows to correct pathological changes of signaling processes being a prerequisite for treatment of many neurodegenerative disorders.

ʄ ʦʞ ʝ ʤʣɖ ʤ , ɖfŋʄʦʣ ʟ ʫʷ
ʦʧʪɖʅɥʝ fŋʄɖʝ ʤʝ ʪʦʜʳ ɖfŋfŋʥʝ ʜʦɘʘʪ
ʦʪʜʝ ʣ ʣ ʝ ʙ ɖʦʤʦʥ ʢʫʣ ?



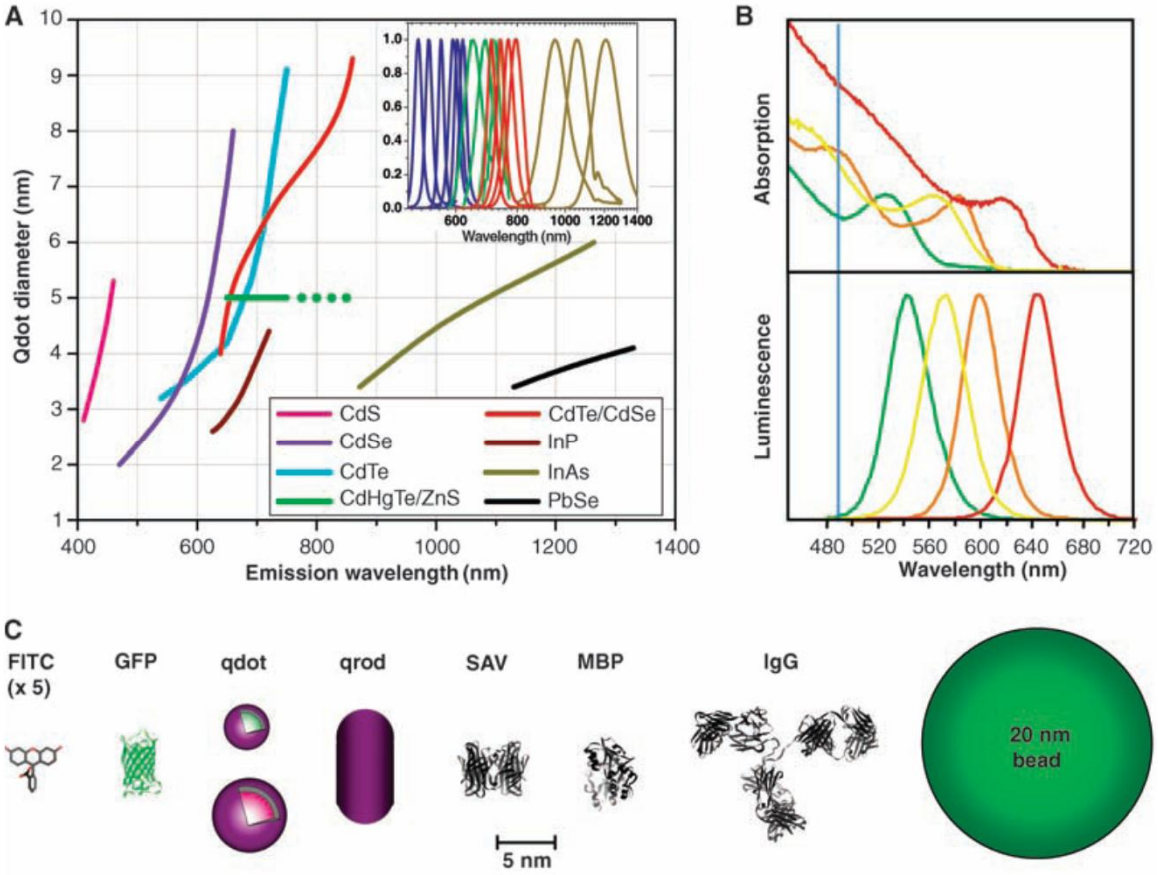




Nature Materials, 4:435, 2005

QDot as compared to MBP, a midsize protein (*M*r ~ 44 kDa) with dimensions of 30x40x65 Å

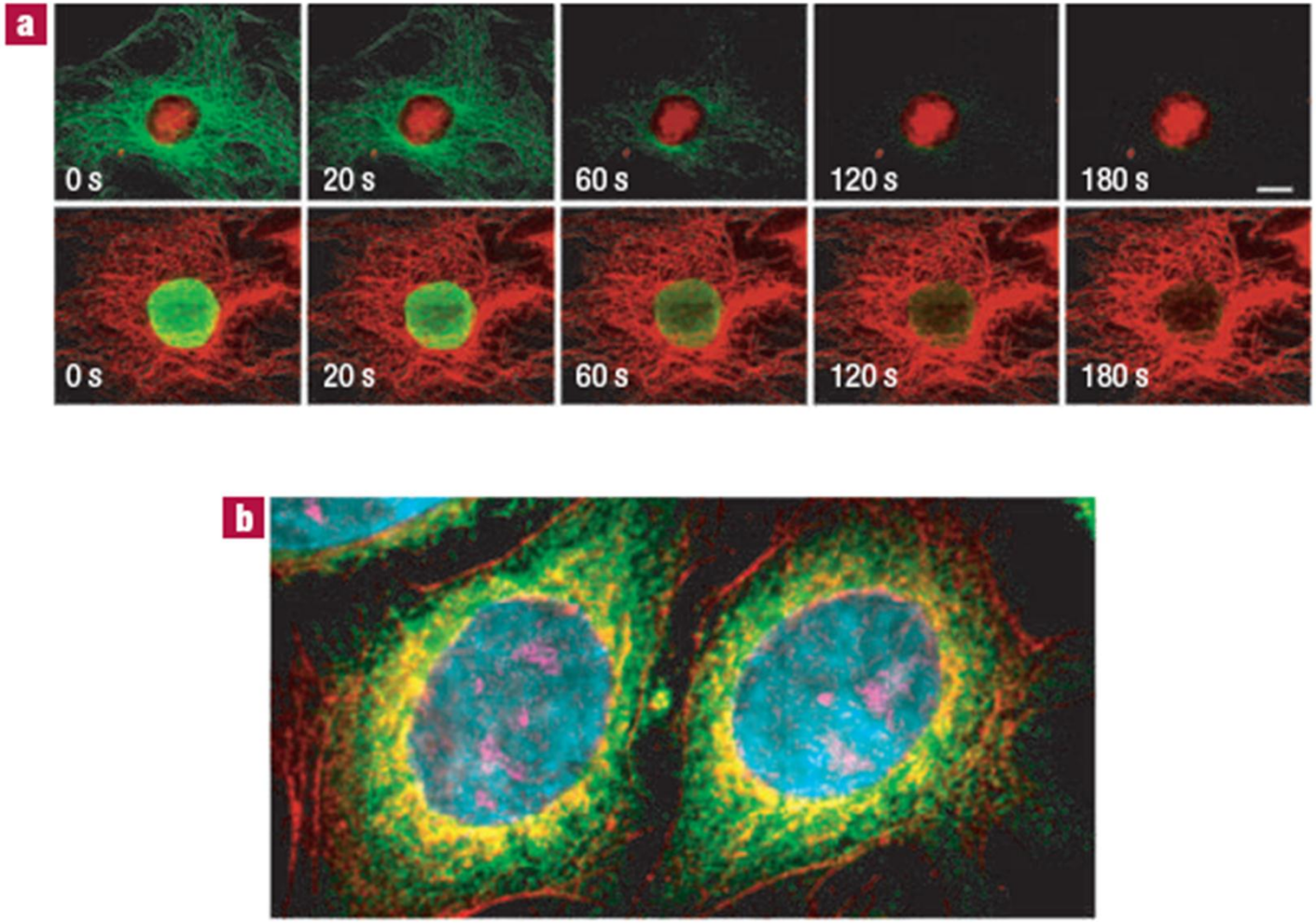
Evident technology web site



(A) Emission maxima and sizes of quantum dots of different composition. Quantum dots can be synthesized from various types of semiconductor materials (II-VI: CdS, CdSe, CdTeI; III-V: InP, InAsI; IV-VI: PbSeI) characterized by different bulk band gap energies. The curves represent experimental data from the literature on the dependence of peak emission wavelength on qdot diameter. The range of emission wavelength is 400 to 1350 nm, with size varying from 2 to 9.5 nm (organic passivation/solubilization layer not included). All spectra are typically around 30 to 50 nm (full width at half maximum). Inset: Representative emission spectra for some materials. (B) Absorption (upper curves) and emission (lower curves) spectra of four CdSe/ZnS qdot samples. The blue vertical line indicates the 488-nm line of an argon-ion laser, which can be used to efficiently excite all four types of qdots simultaneously. (C) Size comparison of qdots and comparable objects. FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; qdot, green (4 nm, top) and red (6.5 nm, bottom) CdSe/ZnS qdot; qrod, rod-shaped qdot; streptavidin (SAV), maltose binding protein (MBP); and immunoglobulin G (IgG).

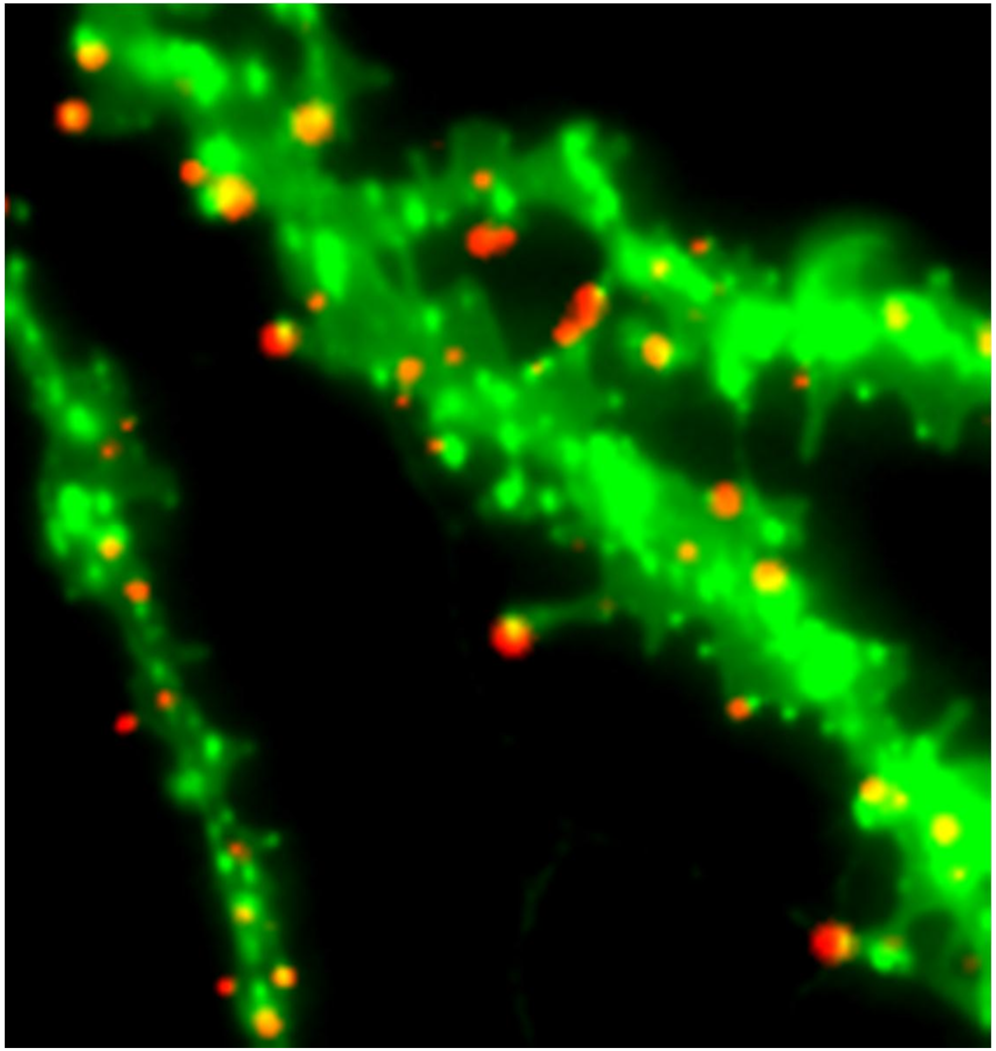
Quantum Dot Corp.'s Web site.

QD resistance to photobleaching and multicolour labelling



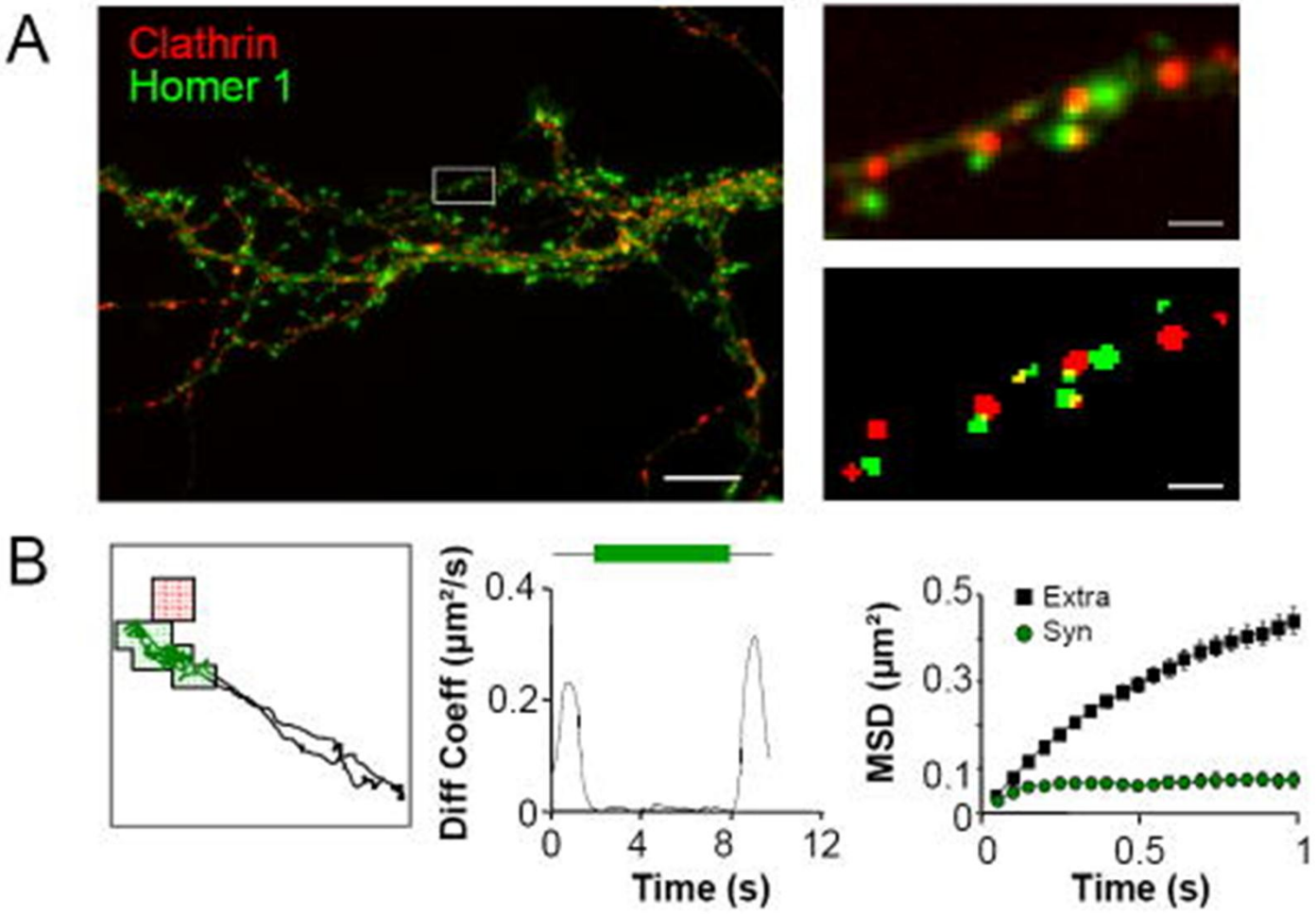
a, Top row: Nuclear antigens were labelled with QD 630ï streptavidin (red), and microtubules were labelled with AlexaFluor 488 (green) simultaneously in a 3T3 cell. Bottom row: Microtubules were labelled with QD 630ï streptavidin (red), and nuclear antigens were stained green with Alexa 488. Continuous exposure times in seconds are indicated (Reprinted by permission of the Nature Publishing Group)34. Note the QD resistance to photobleaching under continuous illumination. **b**, Pseudocoloured image depicting five-colour QD staining of fi xed human epithelial cells. Cyan corresponds to 655-nm Qdots labelling the nucleus, magenta 605-Qdots labelling Ki-67 protein, orange 525-Qdots labelling mitochondria, green 565-Qdots labelling microtubules and red 705-Qdots labelling actin filaments. Nature Materials, 4:435, 2005.

ɼ qʷ ʷ ɮʟqʷ tsʜqʃdʑsuɕdʑ ⁻ AMPA tɕj ɥj lstɑtsɘ dʑʘ tʃtsɘ ȷ tɕ̄dʑsɦʝsɕʃ
ʜ ȷ dʑɪtɑʃlsdʑsɢts ʜ ȷ tɕj ɘʘ dʑʔtɑtsdʑʘ



Neuron web site

AMPA receptors are reversibly stabilized at synapses

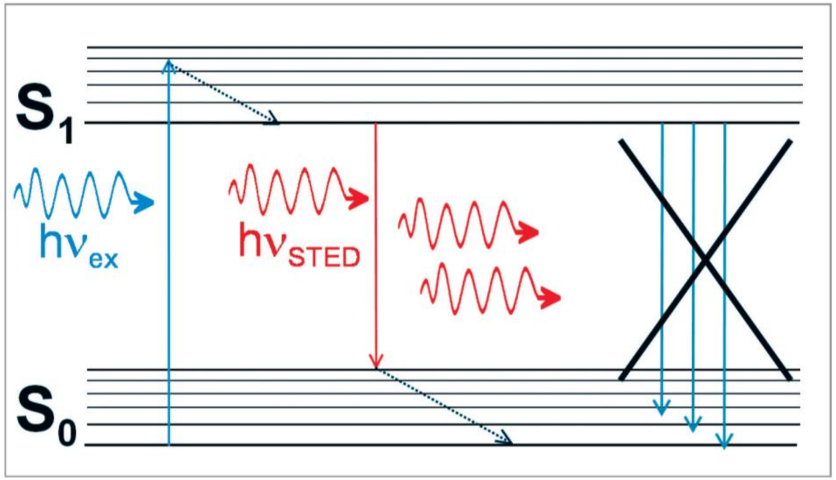


Neuron, Volume 63, Issue 1, 92-105

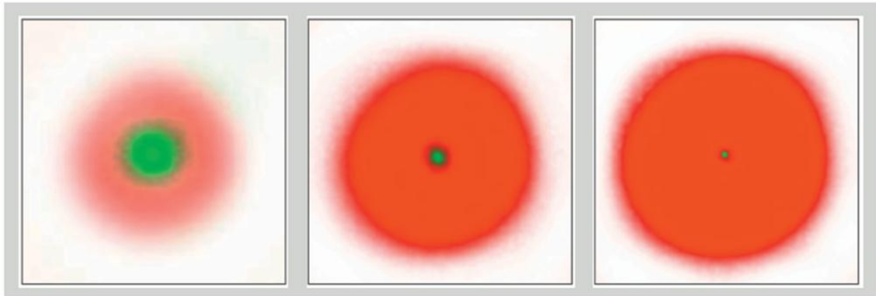
ʆ tʃ lsɷʃɰʝ fŋʗɷʃʝ ʤʝ lstsʜʳ ɷʃfŋʃŋʥʝ ʜtsɘʘʥʃʔ ɷʃʤʝ ʶ ls tsɕtɕʘʥʃɰʝ ʥʃʷ ɘ tɕʘʟ tɕʝʰ ʝ ʥʃɷʃ ʥʘ lztɕtsɘʥʝ 0,5 ʤɷʃʗtɕtsʤʝ lstɕʘ.

ʆ tsʞʝ ʤ ʣɷʃ ʤ , ɷʃfŋʧtsʣ ʟlzʷ tstʃ lsɷʃɰʝ fŋʗɷʃʝ ʤʝ lstsʜʳ ɷʃfŋʃŋʥʝ ʜtsɘʘlsˈ ʗʥʝ lstsɰʥ ʝ ɷʃ ʤtsʥʝ ʗlzʣʷ tɕʣ ʝ tʃ tɕtsɰʝ fŋʃŋ ɘ ʥʘʥsʤʝ lstɕtsɘ tsʤ tɕʘʟ ʤʝ tɕʥsʤ ʜɷʃʘtʃʘʟ tsʥʝ?

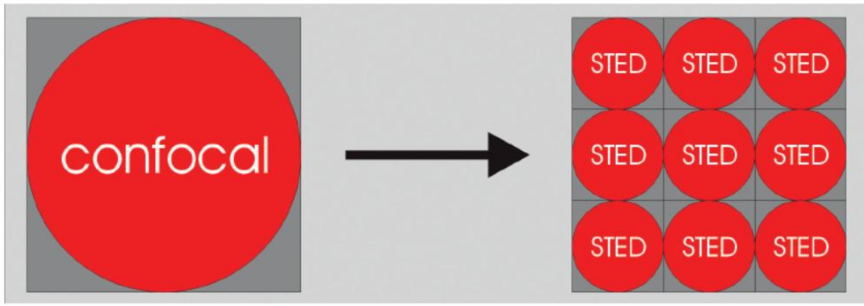
Stimulated Emission Depletion (STED) ï ʙʘʟʦɘʳ ʔ ʧʨɖʣʤʥʧ



The effective fluorescent area (green) decreases with increasing depletion laser power (red)



3-fold reduction of the scanning spot size in x and y yields 9-fold more accurate sampling

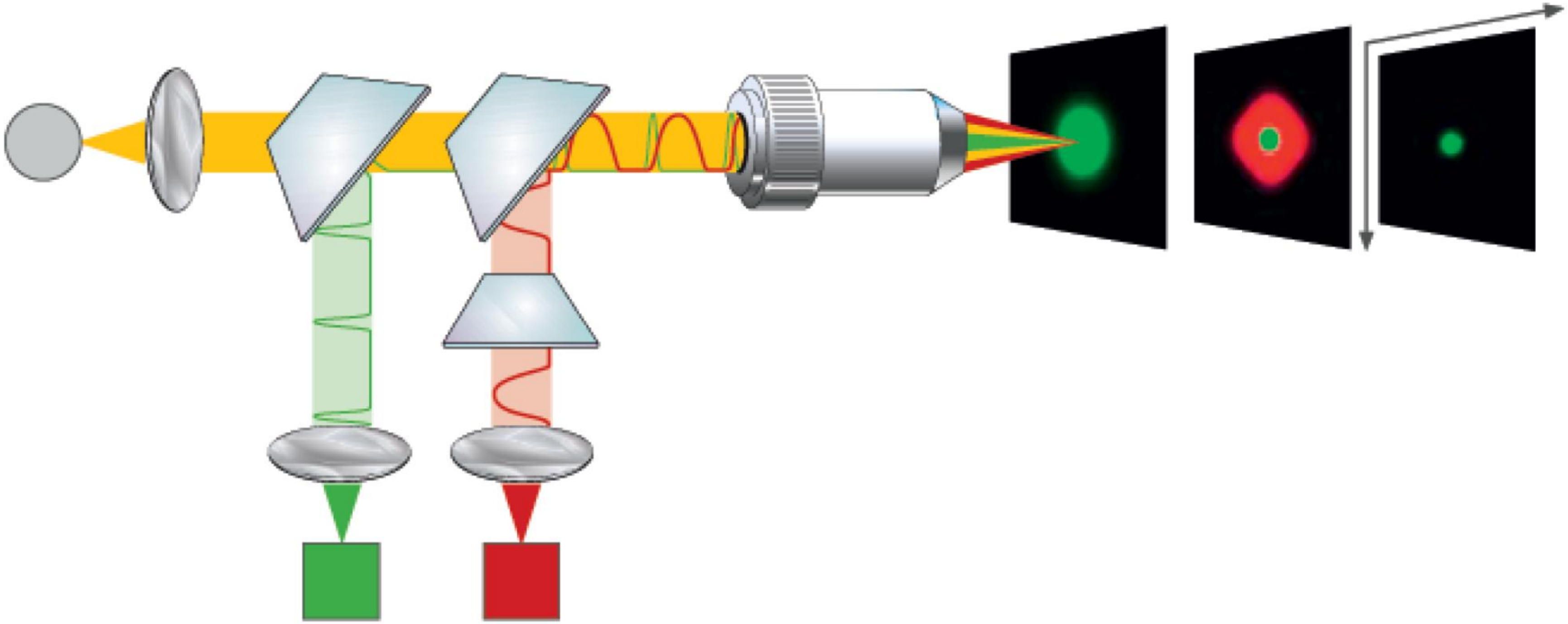


STED resolution:

$$\Delta x \approx \frac{\lambda}{2n\sin\alpha\sqrt{1+\frac{I}{I_s}}}$$

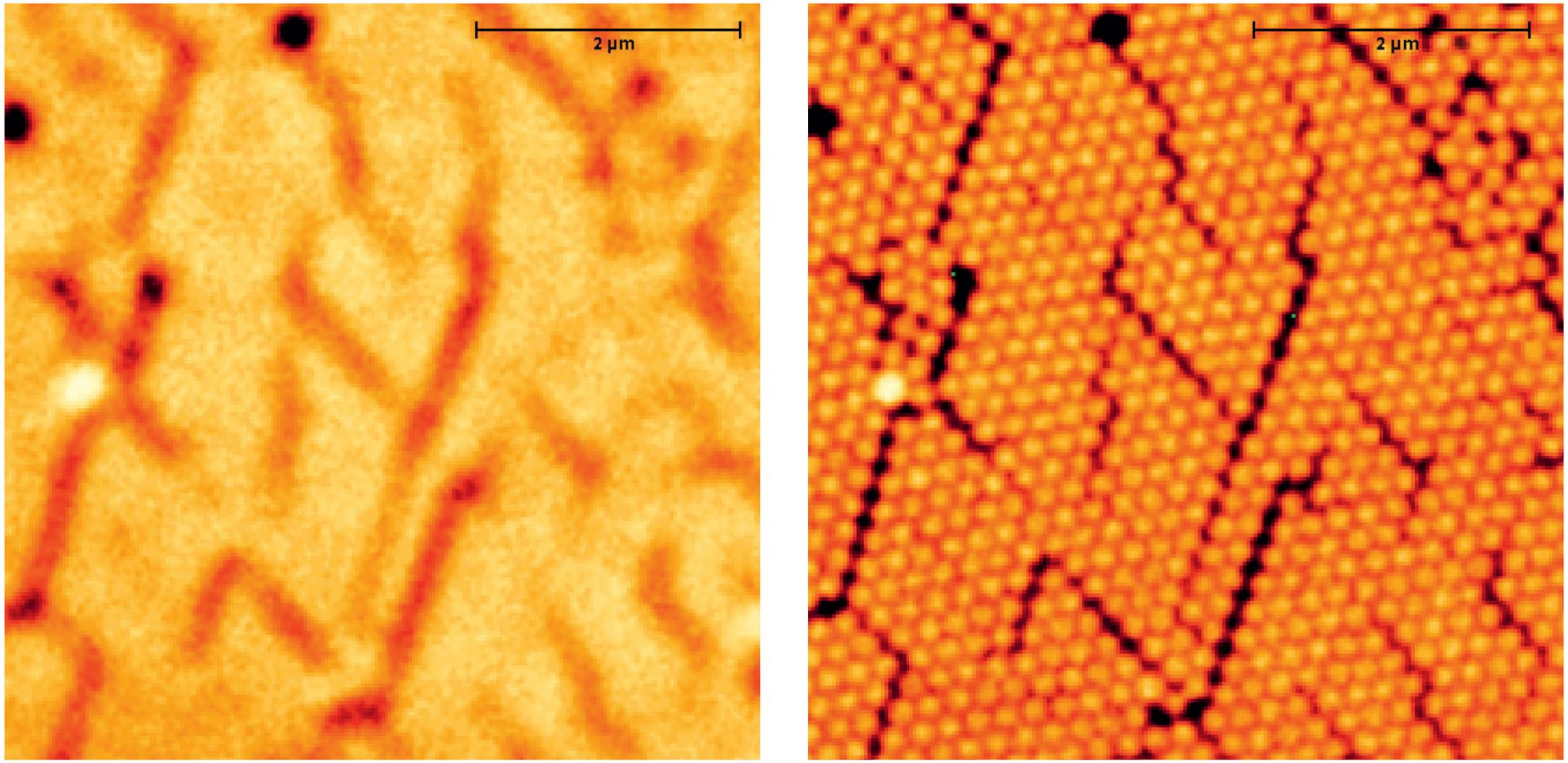
LEICA MICROSYSTEM WEB SITE

Stimulated Emission Depletion (STED)

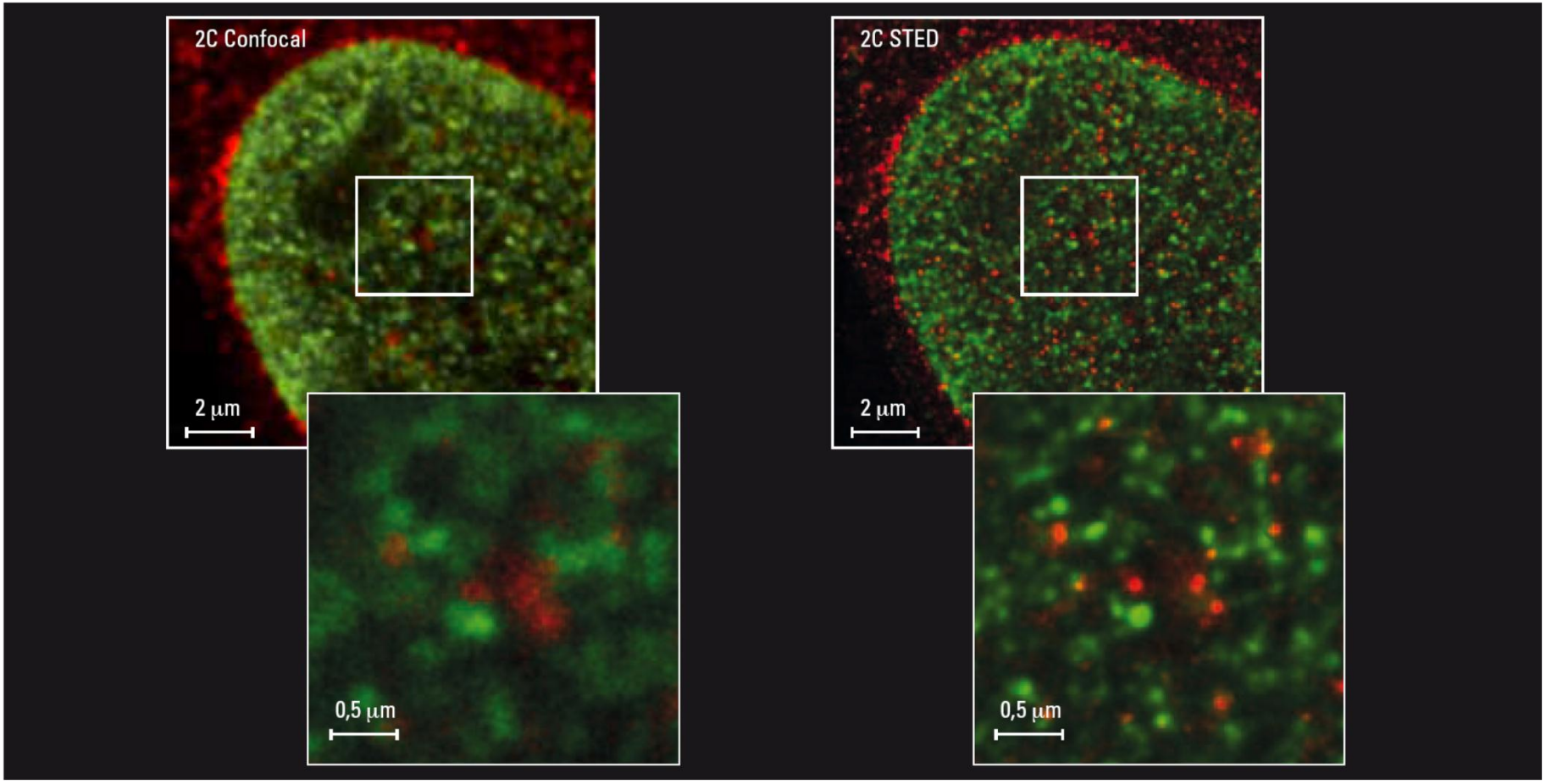


LEICA MICROSYSTEM WEB SITE

[illegible] (confocal) [illegible] STED ([illegible])
[illegible]



LEICA MICROSYSTEM WEB SITE



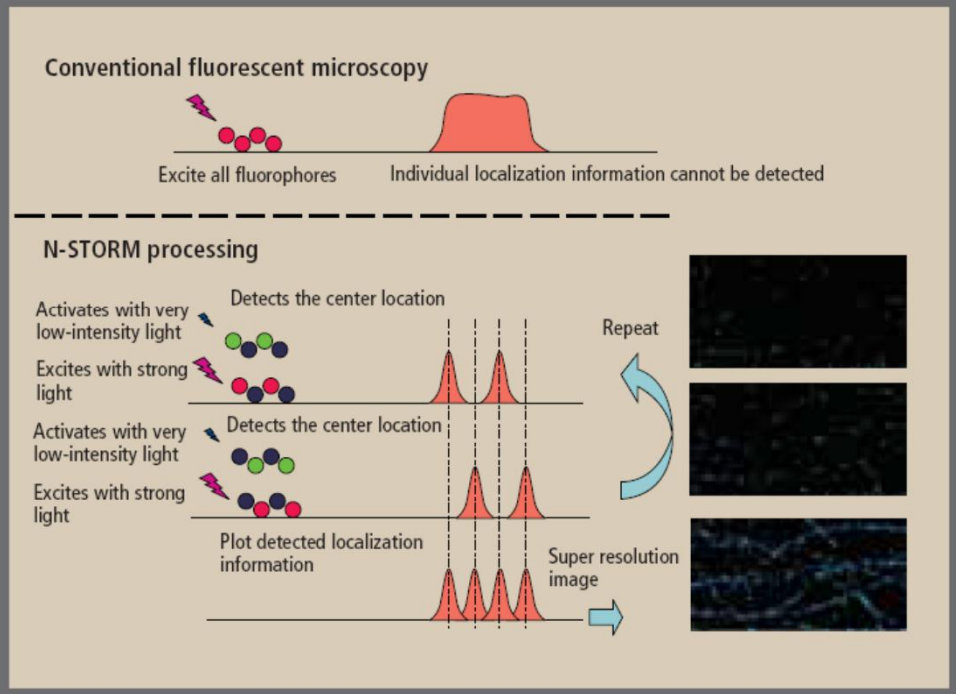
LEICA MICROSYSTEM WEB SITE

STochastic Optical Reconstruction Microscopy (STORM)

The Principle of N-STORM (STochastic Optical Reconstruction Microscopy)

STochastic Optical Reconstruction Microscopy (STORM) reconstructs a super resolution image by combining the high-accuracy localization information of each fluorophore in 3 spatial dimensions and multiple colors

N-STORM uses stochastic activation of relatively small numbers of fluorophor molecules using very low-intensity light. This low-level stochastic "activation" of discrete molecules enables high precision Gaussian fitting of each laterally. Additionally, taking advantage of an induced astigmatism via the special 3D-STORM optics, N-STORM localizes each molecule axially. Computationally combining molecular coordinates in 3 dimensions results in high contrast 3D images of the nanoscopic world with molecular specificity.



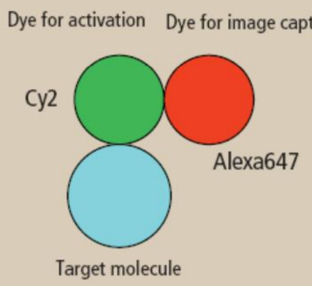
NIKON CORPORATION WEB SITE

[illegible] STORM

Dedicated fluorescent dyes

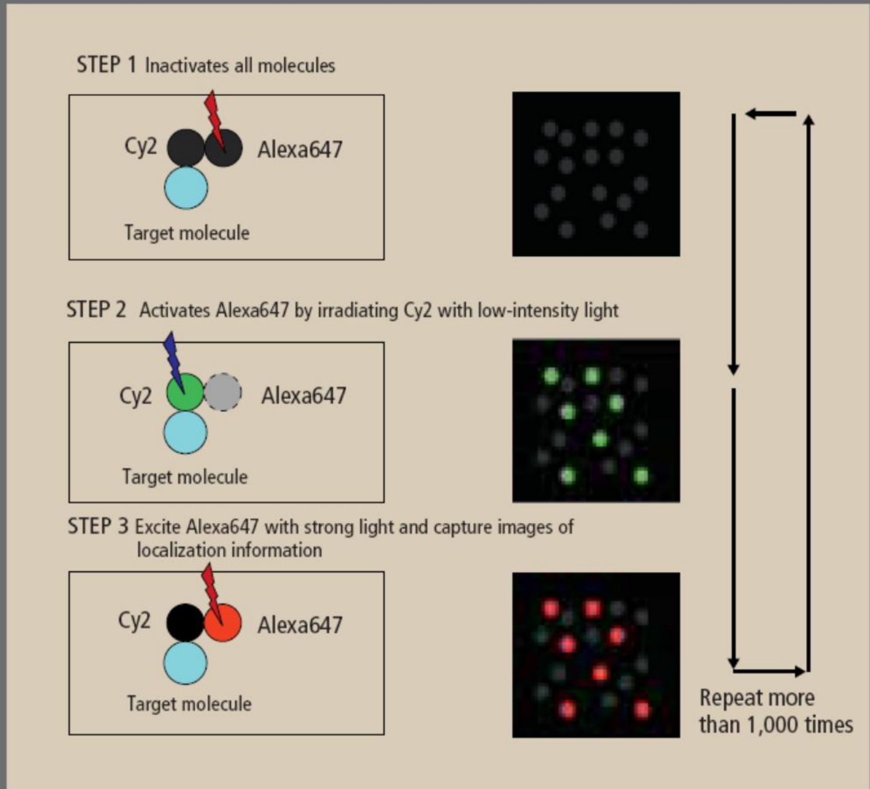
N-STORM uses dedicated fluorescent dye pairs containing an "activator" (relatively short wavelength excitation) and a "reporter" (relatively long wavelength excitation), which enables various color combinations, facilitating true multi-channel super resolution.

Dyes for N-STORM



Dye for activation	Dye for image capturing
Alexa405	Alexa647
Cy2	Alexa647
Cy3	Alexa647

A dye for N-STORM consists of a shorter-wavelength dye for activation and a longer-wavelength dye for image capturing. Creation of two color super resolution images is possible with pairs of dye.



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[illegible] N-SIM and N-STORM

Specifications

	N-SIM	N-STORM
XY resolution	Approx. 100nm (up to 85nm: theoretical, in TIRF-SIM mode, 488nm excitation)	Approx. 20nm
Z-axis resolution	Approx. 300nm	Approx. 50nm
Image acquisition time	Up to 0.6 sec./frame (TIRF-SIM/2D-SIM) Up to 1 sec. (3D-SIM) (needs more 1-2 sec. for calculation)	10 minutes or more
Imaging mode	TIRF-SIM (TIRF XY super resolution) 2D-SIM (XY super resolution, up to 3μm deep) 3D-SIM (XYZ super resolution, up to 20μm deep)	TIRF-STORM 3D STORM
Multi-color imaging	Up to 5 colors	2 colors simultaneously
Compatible Laser	Standard: 488nm, 561nm Option: 457nm, 515nm, 532nm	405nm, 457nm, 561nm, 647nm
Compatible microscopes	Motorized inverted microscope ECLIPSE Ti-E Perfect Focus System Motorized XY stage with encoders Piezo Z stage	
Objectives	CFI Apo TIRF 100× oil (NA1.49) CFI Plan Apo IR 60× WI (NA1.27)	CFI Apo TIRF 100x oil (NA 1.49) CFI Plan Apo VC 100x oil (NA 1.40)
Camera	Andor Technology iXon DU897 EMCCD camera	
Software	NIS-Elements Ar/ NIS-Elements C (with confocal microscope A1)	NIS-Elements Ar/ NIS-Elements C (with confocal microscope A1) Both need the NIS-A STORM Analysis
Operation conditions	25 °C ± 0.5 °C	20 °C to 25 °C (± 0.5 °C)

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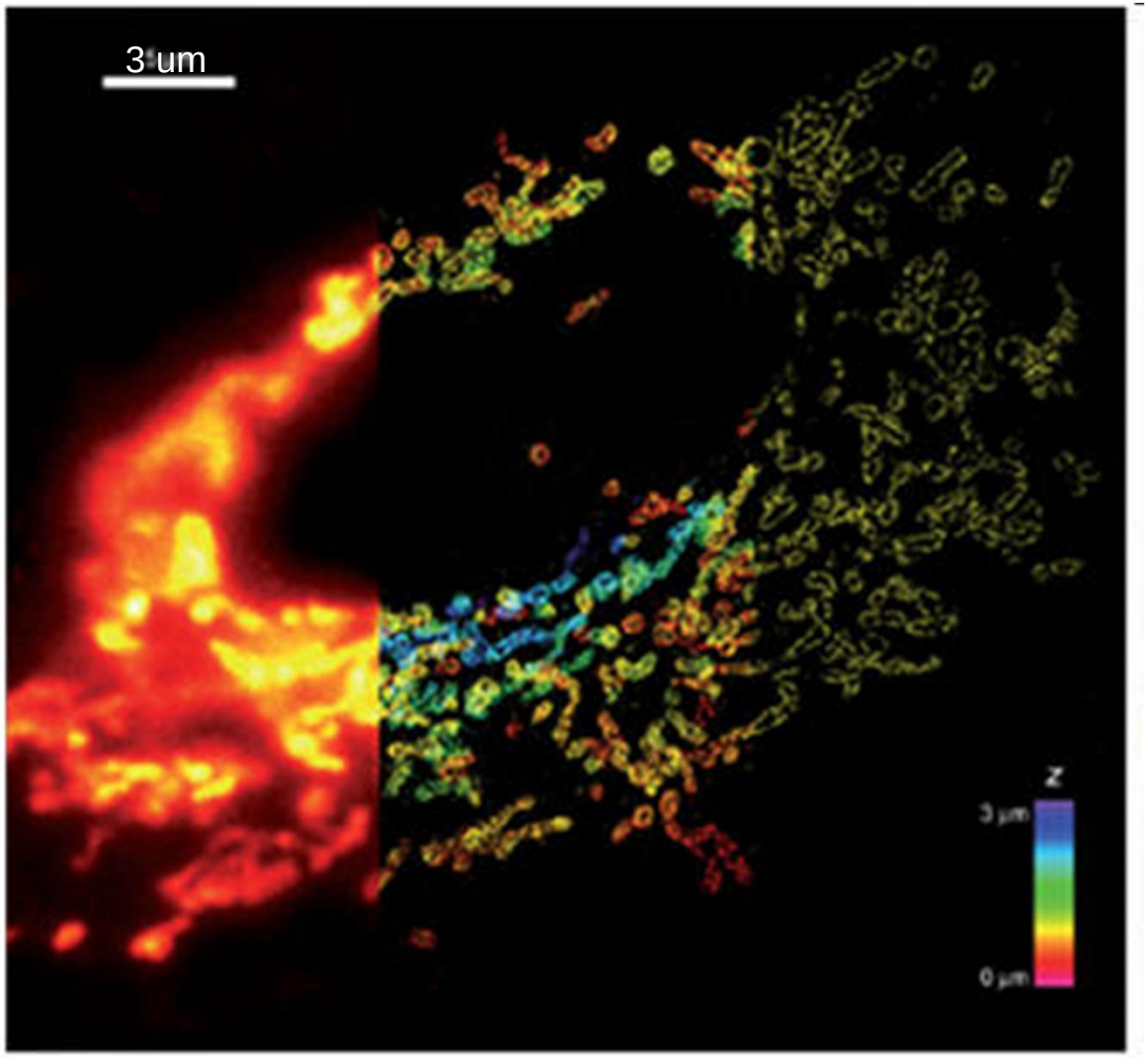
Nikon STORM System

Comparison of Conventional and STORM Images of Mitochondria in a Mammalian Cell



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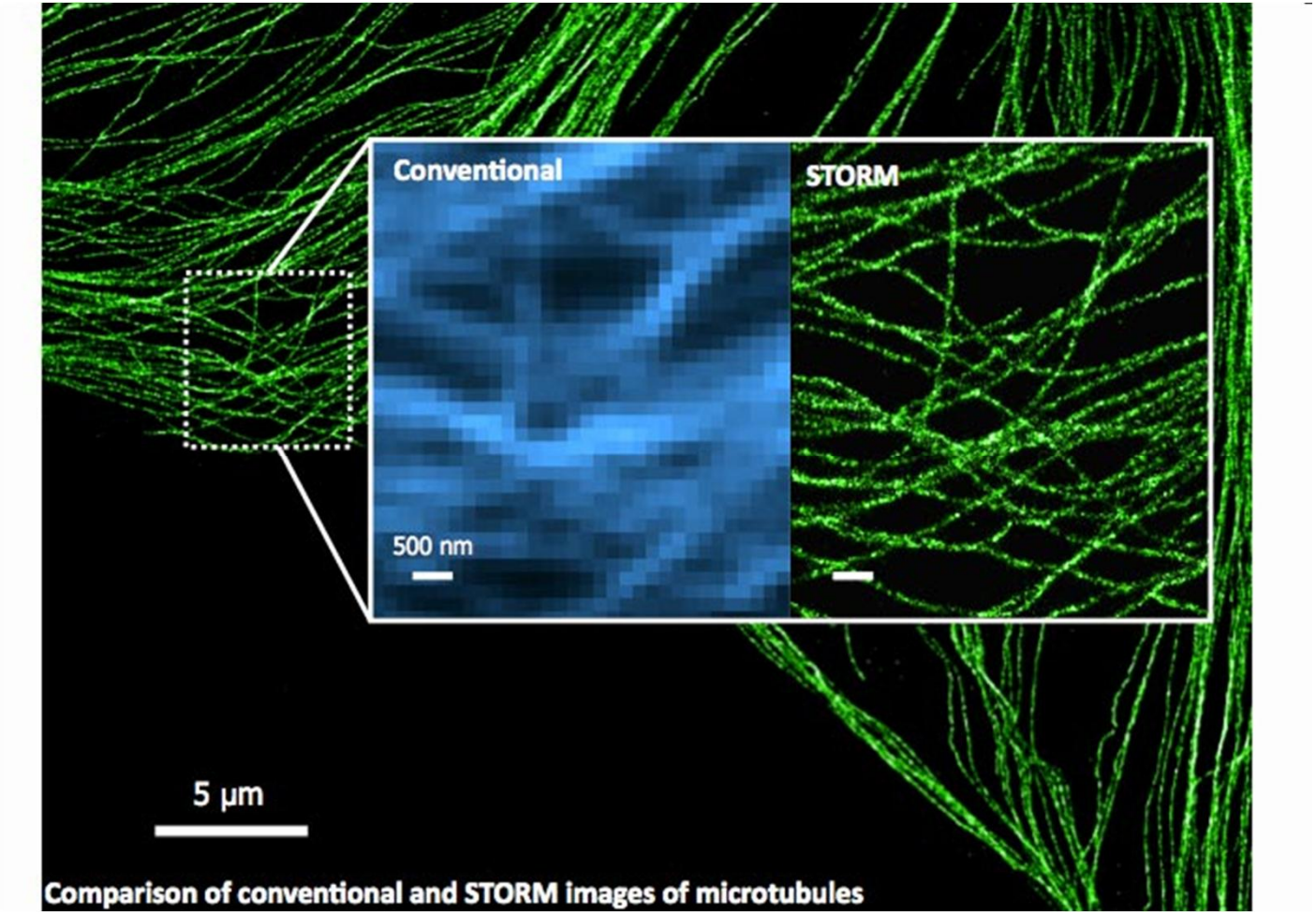
Comparison of Conventional and STORM Images of microtubules



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Multi-color STORM image of microtubules (green) and clathrin-coated pit (red) in a cell

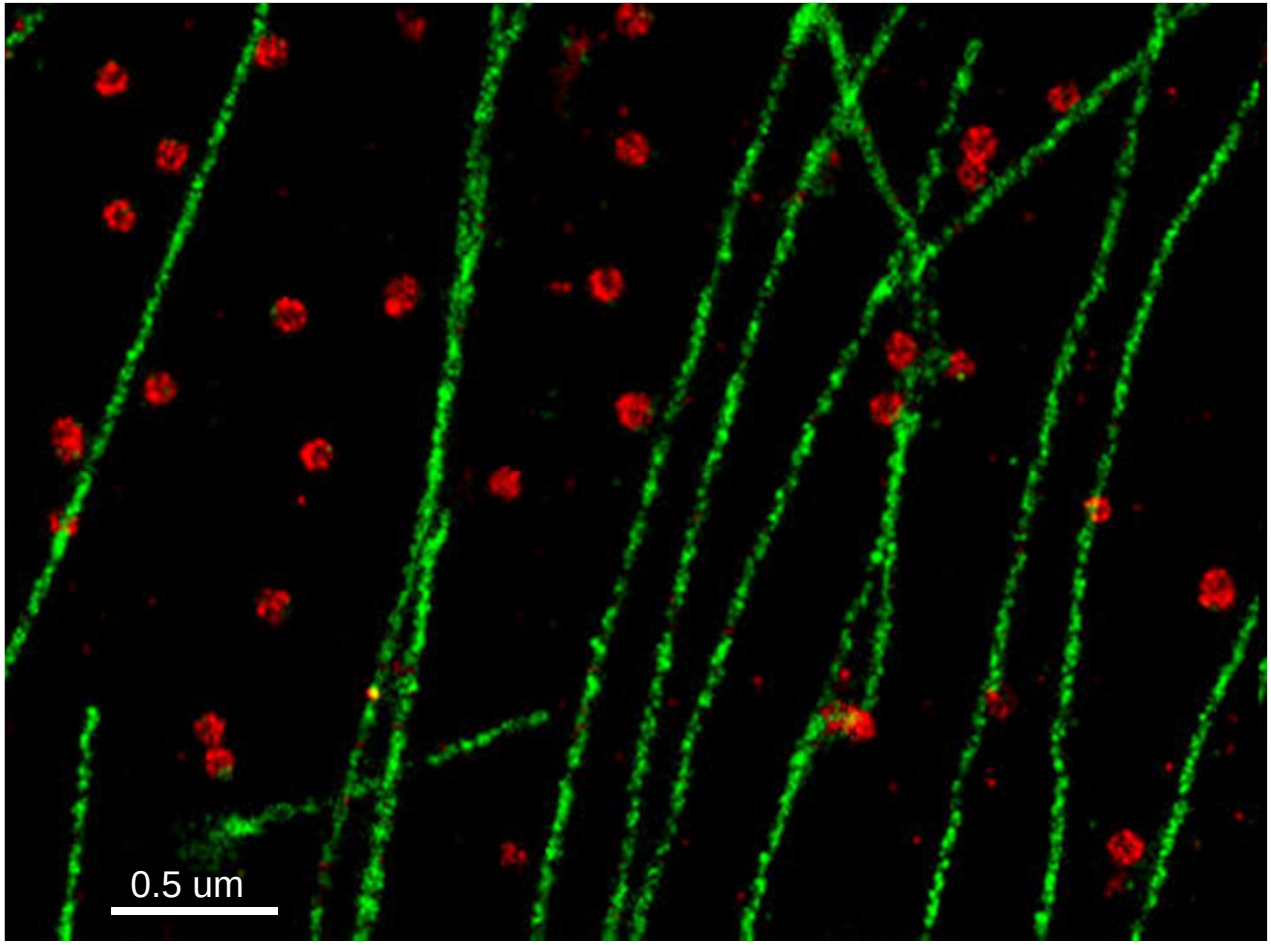

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Image courtesy of Zhuang Research Group, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA.

3D STORM Image of Microtubules in a Cell

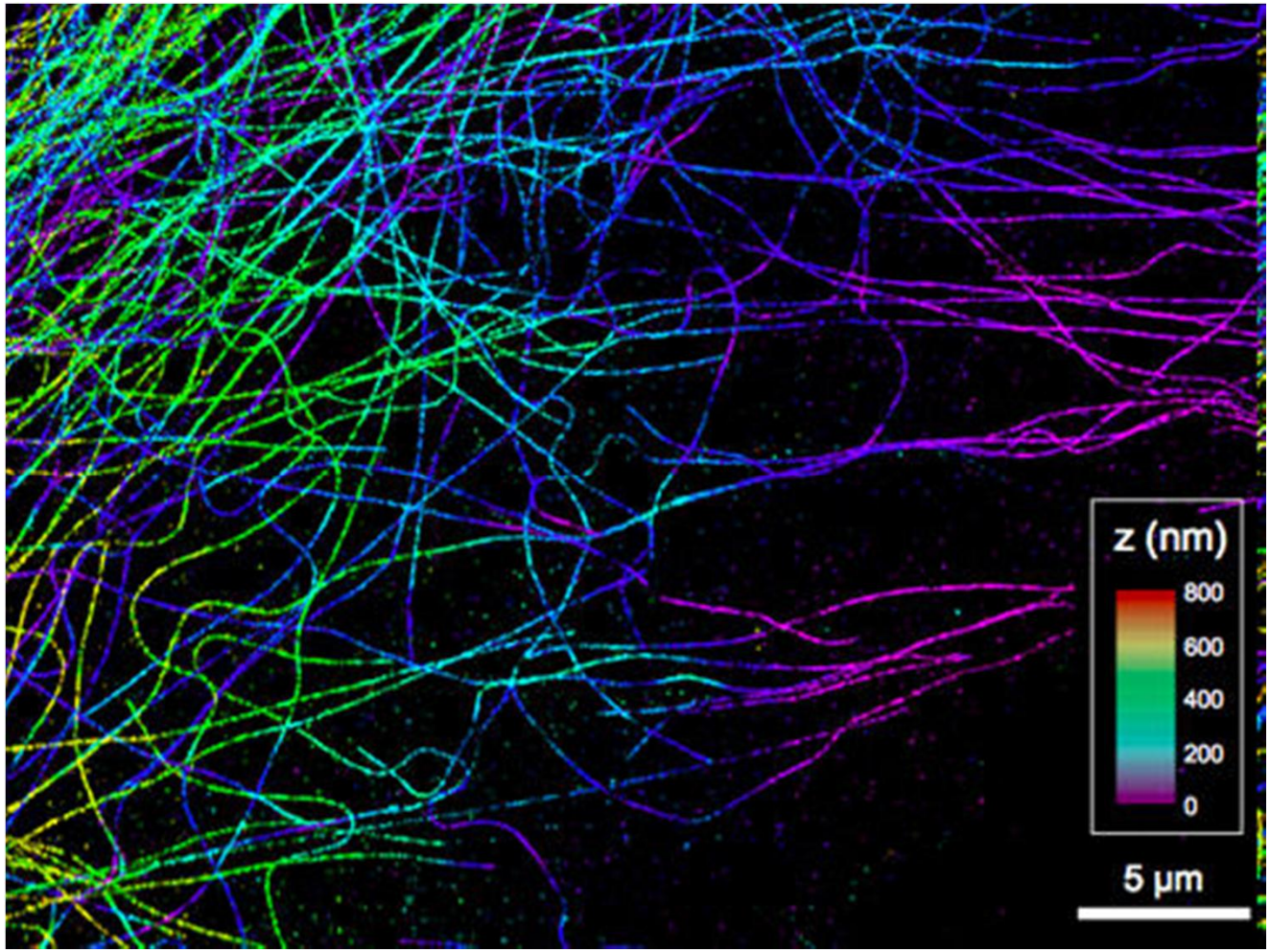

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Image courtesy of Zhuang Research Group, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA.