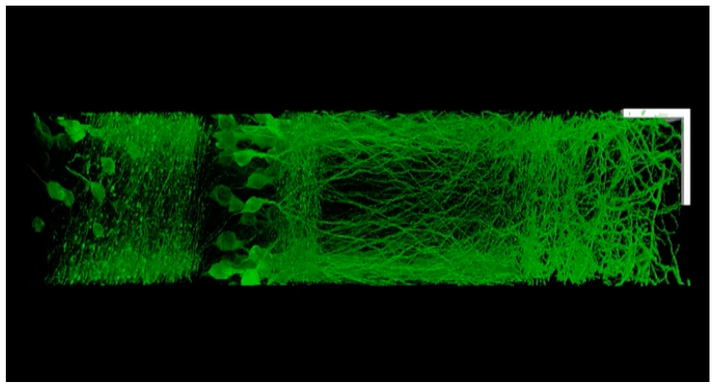
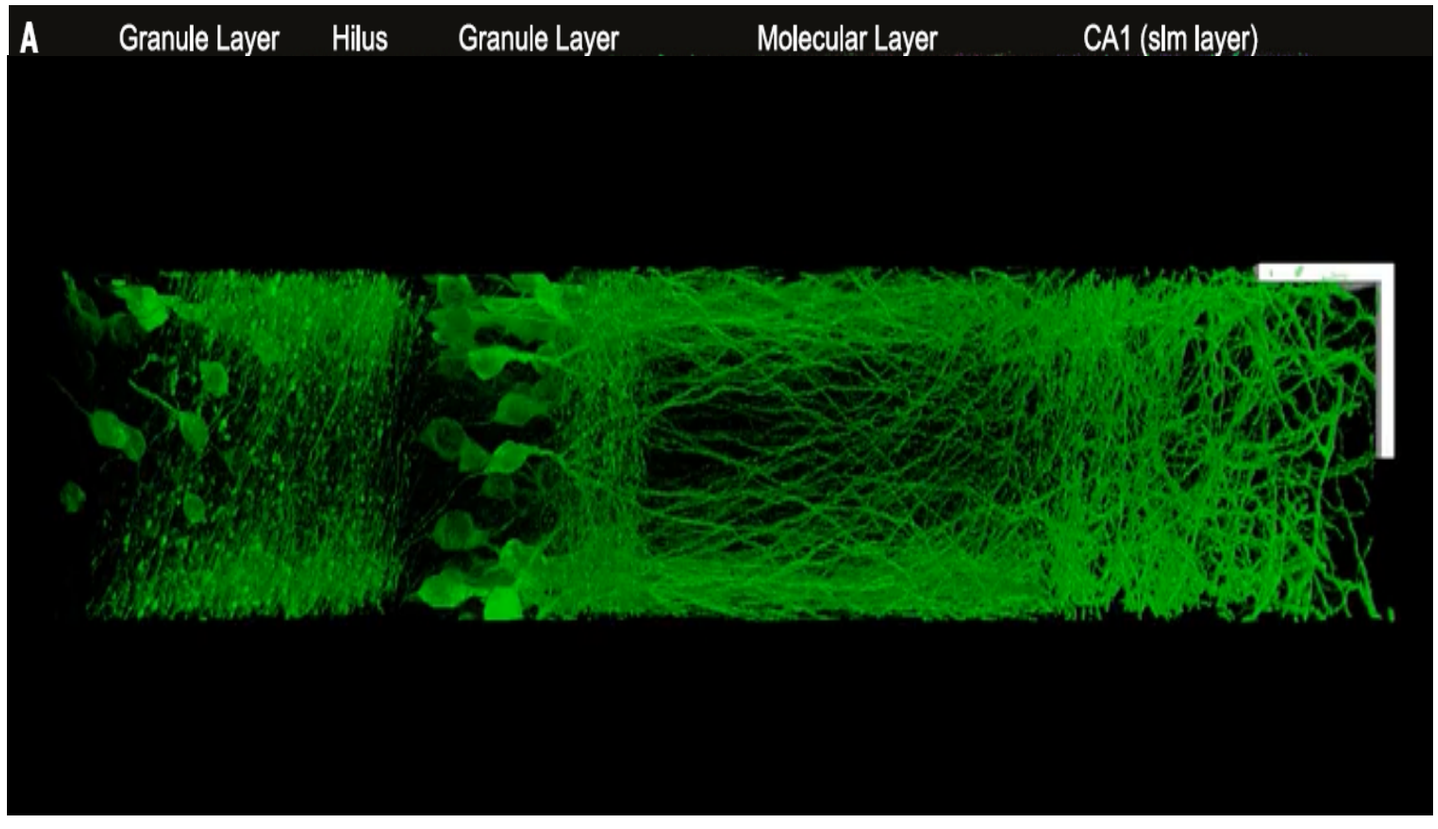
Expansion microscopy

Technical JC presentation 2015-04-11 Stephan Isringhausen



Expansion microscopy

Technical JC presentation 2015-04-11 Stephan Isringhausen



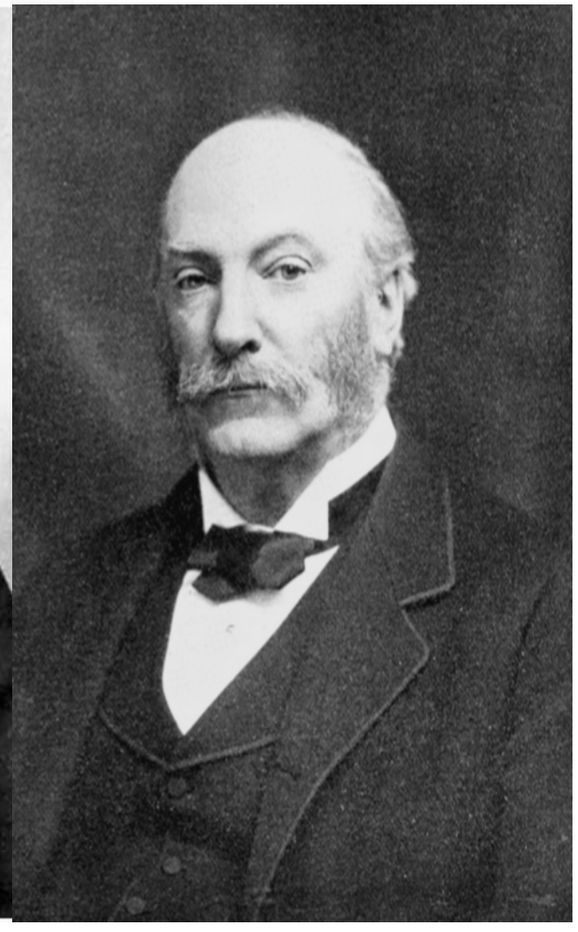
Back in the days…

Introduction - Background

Ernst Karl Abbe

1873

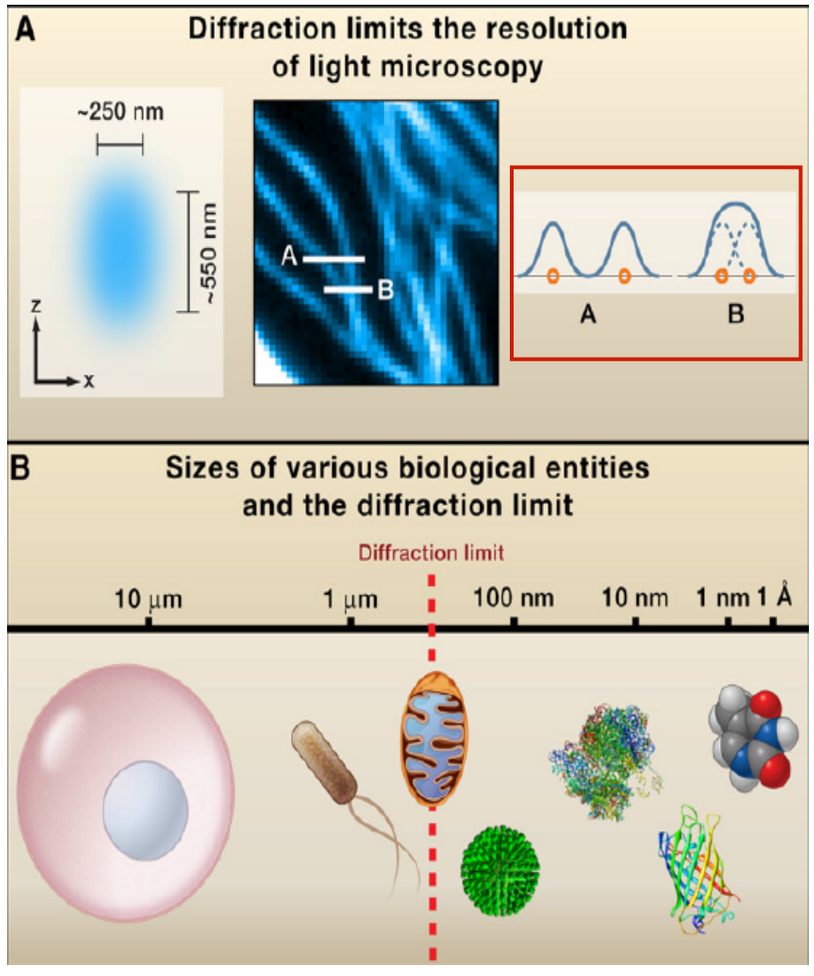
Lord Rayleigh



1896

$$d = \frac{\lambda}{2NA}$$

Lateral resolution~250nm



Huang et al., 2010, Cell, 143, 1047 – 57

Abbe E (1873) Beiträge zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung. *Archiv für mikroskopische Anatomy*.9: 413-420.

Superresolution microscopy

Superresolution Microscopy

The Nobel Prize in Chemistry 2014

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

The Nobel Prize in Chemistry 2014

Photo: A. Mahmoud

Eric Betzig

Prize share: 1/3

Photo: A. Mahmoud

Stefan W. Hell

Prize share: 1/3

Photo: A. Mahmoud

William E. Moerner

Prize share: 1/3

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of super-resolved fluorescence microscopy".*

http://www.nobelprize.org/

Different superresolution techniques:

a. Patterned illumination (STED, SIM/SSIM) – 'true' or deterministic response of **fluorophores**

b. Single-molecule switching (STORM, (F)PALM) – 'functional' or stochastic behavior of **fluorophores**

The struggle with SRM

- Expensive, specialized equipment required
- Long acquisition, high illumination intensities
- Often superficial

Clearing - Background

Werner Spalteholz, 1914

Ueber das Durchsichtigmachen von menschlichen und tierischen Praeparaten und seine theoretischen Bedingungen

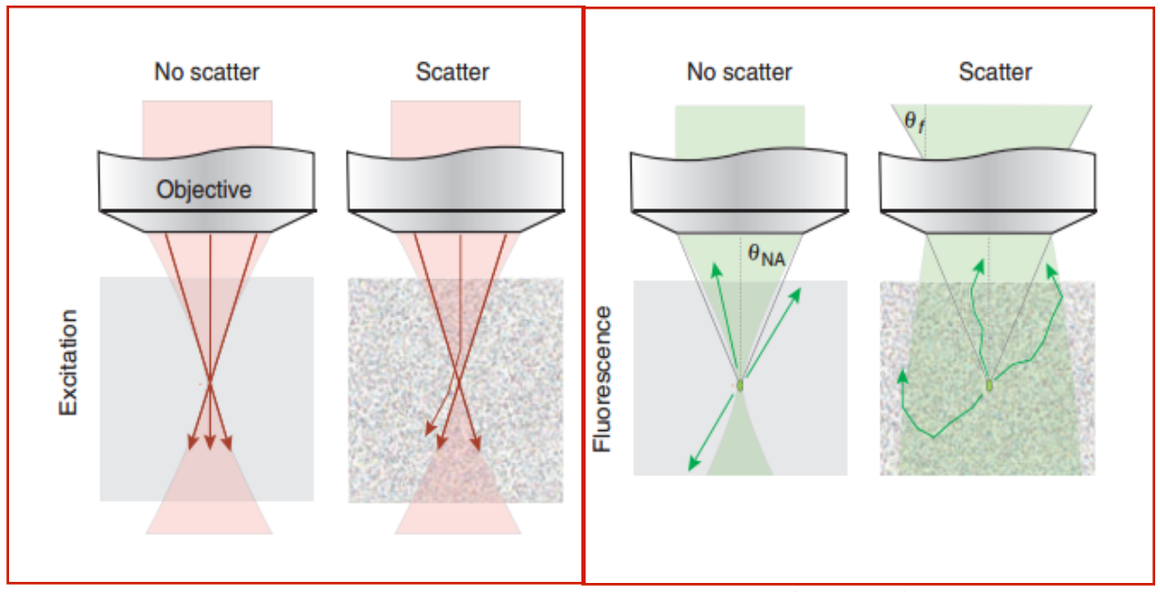


Figure 2 | Signal generation and fluorescence collection in clear tissue (no scatter) and in scattering tissue (scatter). In clear tissue all excitation light reaches the focus, but in scattering tissue, scattering (even by a small angle) causes light rays to miss the focus and be lost to signal generation. This leads to a roughly exponential decrease in excitation with depth. In clear tissue only fluorescence light rays initially emitted into the collection cone, determined by the objective's NA, can be detected, but in scattering tissue fluorescence light is (multiply) scattered and may even 'turn around'. Fluorescence light apparently originates from a large field of view but a larger fraction than in the nonscattering case is actually within the angular acceptance range θ_f of the objective.

DOI:10.1038/NMETH818

3D imaging and clearing

Cell

Resource

Whole-Brain Imaging with Single-Cell Resolution Using Chemical Cocktails and Computational Analysis

Etsuo A. Susaki,[1,2,3,4,14] Kazuki Tainaka,[1,3,4,14] Dimitri Perrin,[2,14] Fumiaki Kishino,[5] Takehiro Tawara,[6] Tomonobu M. Watanabe,[7] Chihiro Yokoyama,[8] Hirotaka Onoe,[8] Megumi Eguchi,[9] Shun Yamaguchi,[9,10] Takaya Abe,[11] Hiroshi Kiyonari,[11] Yoshihiro Shimizu,[12] Atsushi Miyawaki,[13] Hideo Yokota,[6] and Hiroki R. Ueda[1,2,3,4,*]

Cell

Resource

Whole-Body Imaging with Single-Cell Resolution by Tissue Decolorization

Kazuki Tainaka,[1,2,3,4] Shimpei I. Kubota,[1,4] Takeru Q. Suyama,[1] Etsuo A. Susaki,[1,2,3] Dimitri Perrin,[2] Maki Ukai-Tadenuma,[2] Hideki Ukai,[2] and Hiroki R. Ueda[1,2,3,*]

[1]Department of Systems Pharmacology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

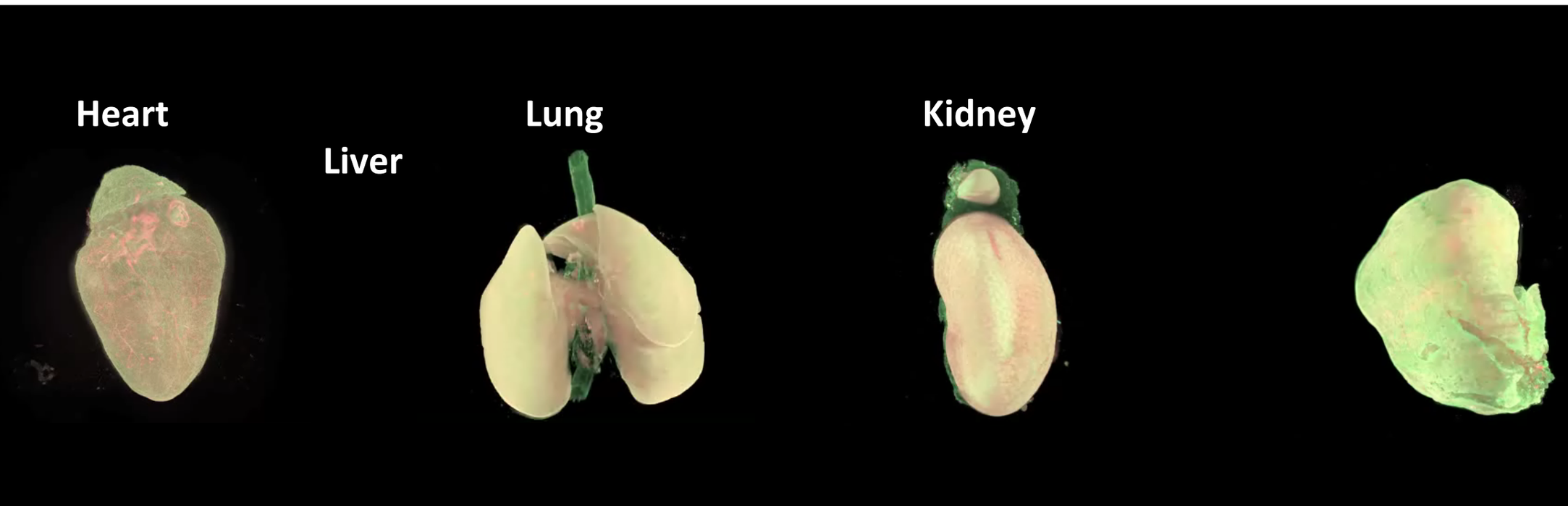
[2]Laboratory for Synthetic Biology, RIKEN Quantitative Biology Center, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan

[3]CREST, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

[4]Co-first author

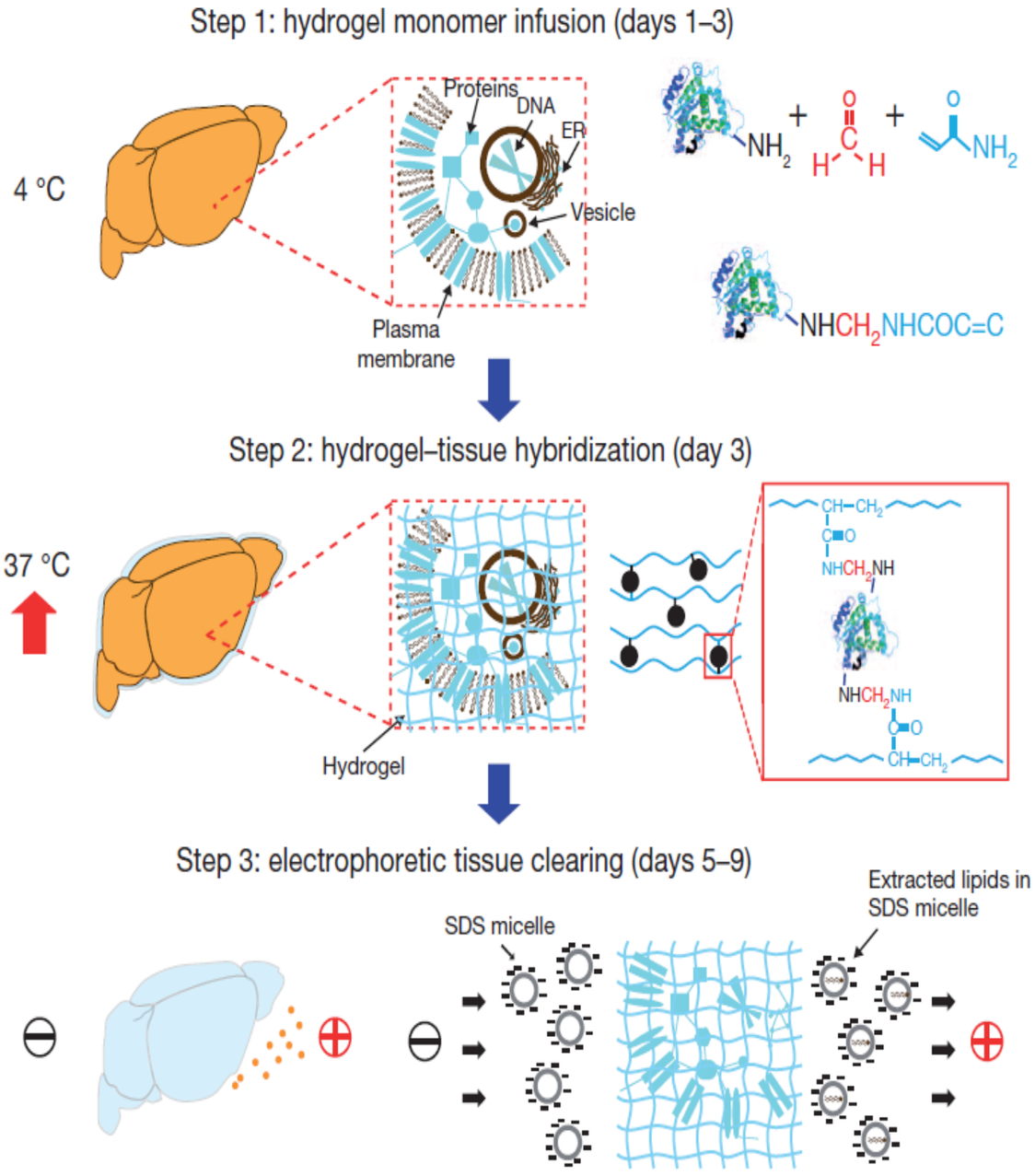
*Correspondence: uedah-tky@umin.ac.jp

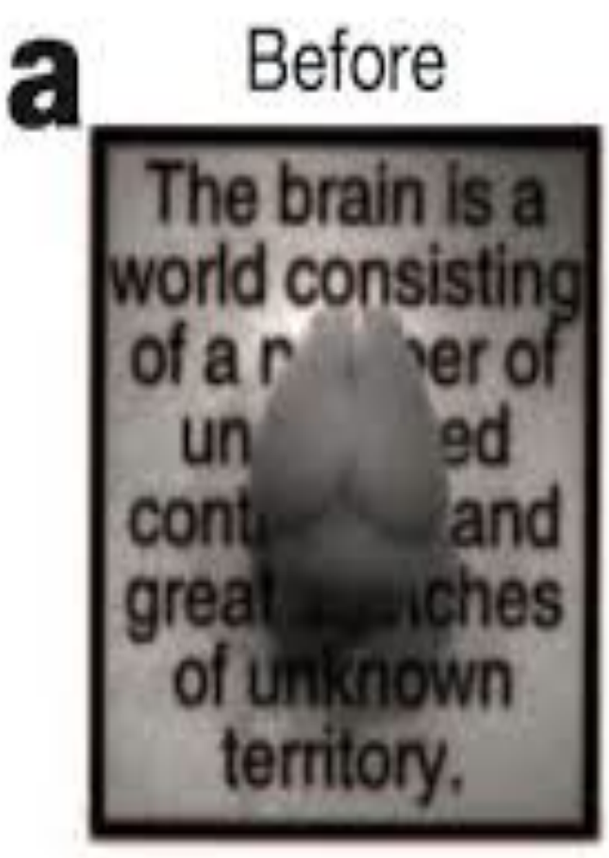
http://dx.doi.org/10.1016/j.cell.2014.10.034

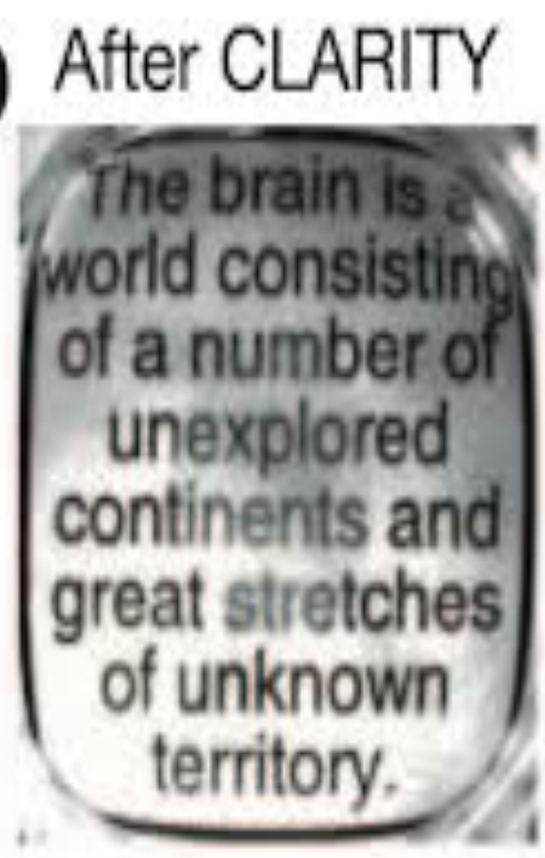


Clearing - Background

CLARITY (2013)







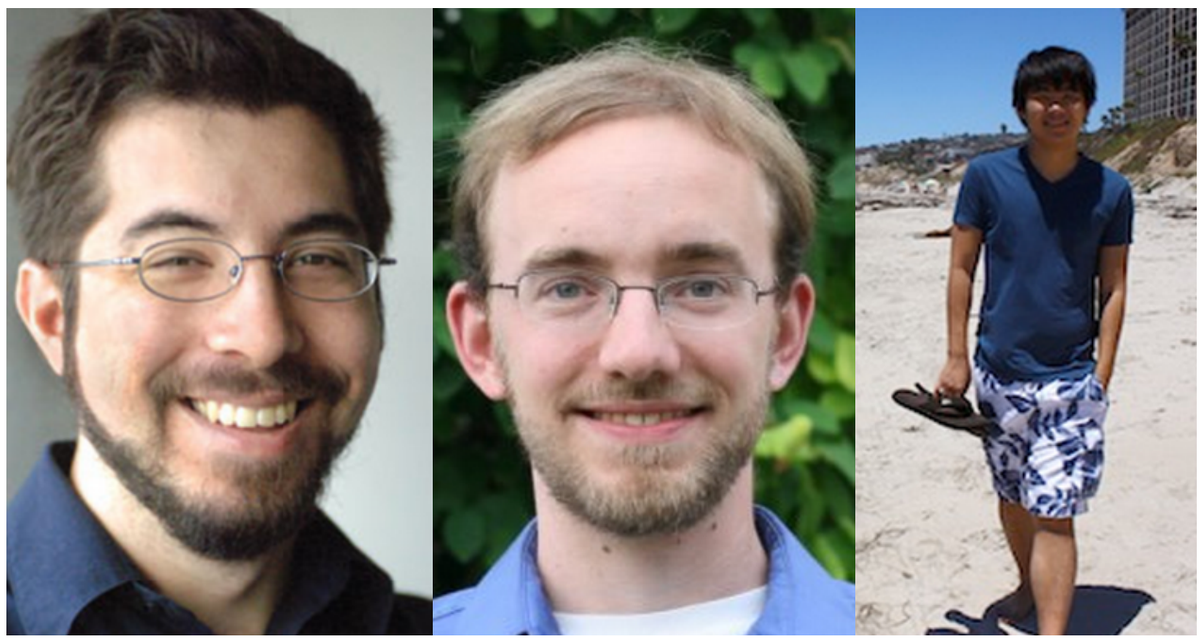
Summary

Advances in resolution

Numerous methods for making brains (organs) transparent

Limited clearing, limited speed for superresolution

Expansion Microscopy



http://syntheticneurobiology.org/people

OPTICAL IMAGING

Expansion microscopy

Fei Chen,[1*] Paul W. Tillberg,[2*] Edward S. Boyden[1,3,4,5,6†]

In optical microscopy, fine structural details are resolved by using refraction to magnify images of a specimen. We discovered that by synthesizing a swellable polymer network within a specimen, it can be physically expanded, resulting in physical magnification. By covalently anchoring specific labels located within the specimen directly to the polymer network, labels spaced closer than the optical diffraction limit can be isotropically separated and optically resolved, a process we call expansion microscopy (ExM). Thus, this process can be used to perform scalable superresolution microscopy with diffraction-limited microscopes. We demonstrate ExM with apparent ~70-nanometer lateral resolution in both cultured cells and brain tissue, performing three-color superresolution imaging of ~10^7 cubic micrometers of the mouse hippocampus with a conventional confocal microscope.

The idea

VOLUME 45, NUMBER 20 PHYSICAL REVIEW LETTERS 17 NOVEMBER 1980

Phase Transitions in Ionic Gels

Toyoichi Tanaka, David Fillmore, Shao-Tang Sun, Izumi Nishio,
Gerald Swislow, and Arati Shah
Department of Physics and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
(Received 13 June 1980)

The polymer network of a gel, under certain conditions, undergoes a discrete transition in equilibrium volume with changes in solvent composition or temperature. This Letter demonstrates that ionization of the gel network plays an essential role in the phase transition. The volume collapse is also observed when the *p*H within the gel is varied.

PACS numbers: 64.70.-p, 61.40.Km

Salt effects on the phase transition of ionic gels

Iwao Ohmine[a] and Toyoichi Tanaka

Department of Physics and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
(Received 10 June 1982; accepted 10 August 1982)

An ionized acrylamide gel is found to undergo a discrete phase transition in equilibrium volume upon varying the salt concentration in the solution. The salt concentration required for the transition depends strongly on the valency of the positive salt ion added to the solution. In certain cases the concentration at the transition is many thousand times larger for monovalent ions than for divalent ions. A simple theoretical consideration of the osmotic pressure of the ions can explain the phenomenon.

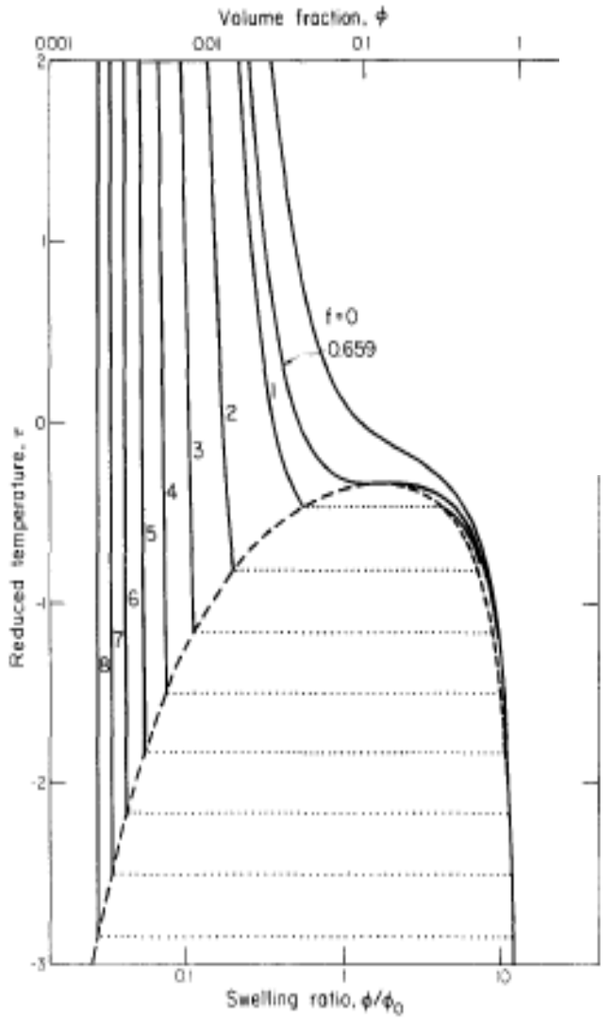


FIG. 3. The swelling ratio $\rho = \phi/\phi^*$ of the gel at which the total osmotic pressure on the gel is zero is calculated for various values of f, the net degree of ionization of the network (from Ref. 2). The values used are the same as for Fig. 2.

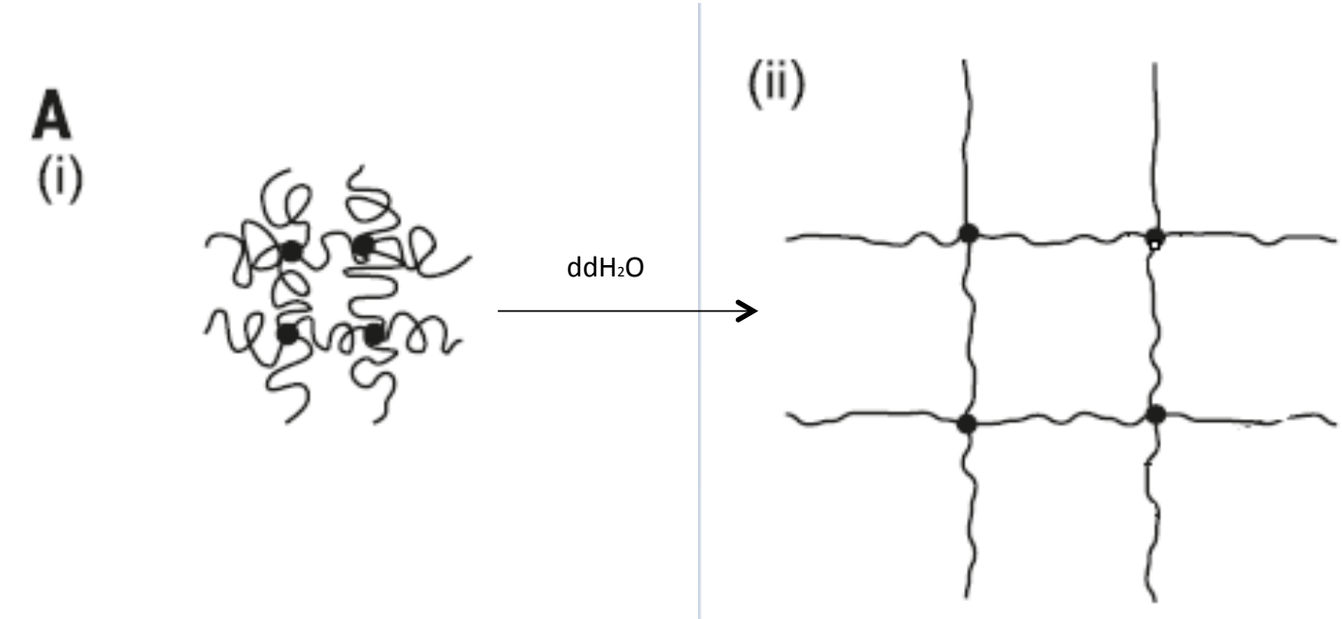
I. INTRODUCTION

The polymer network of partially ionized acrylamide gels undergoes a discrete and reversible volume transition with changes in temperature and solvent composition.[1,2] It has been shown that ionization of the polymer network plays an essential role in this phase transition. A discrete volume change as large as several 100-fold occurs if a small number of the acrylamide groups in the network is hydrolyzed into ionizable acrylic acid.[3]

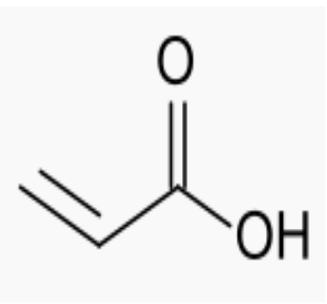
Sodium acrylate

Youtube clip: ‘Sodium Polyacrylate’

Sodium acrylate

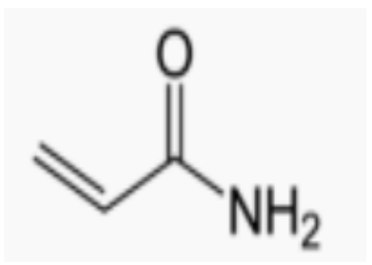


health.howstuffworks.com



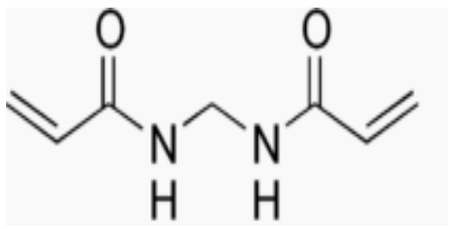
Sodium acrylate

+



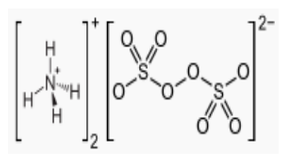
Acrylamide

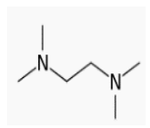
+

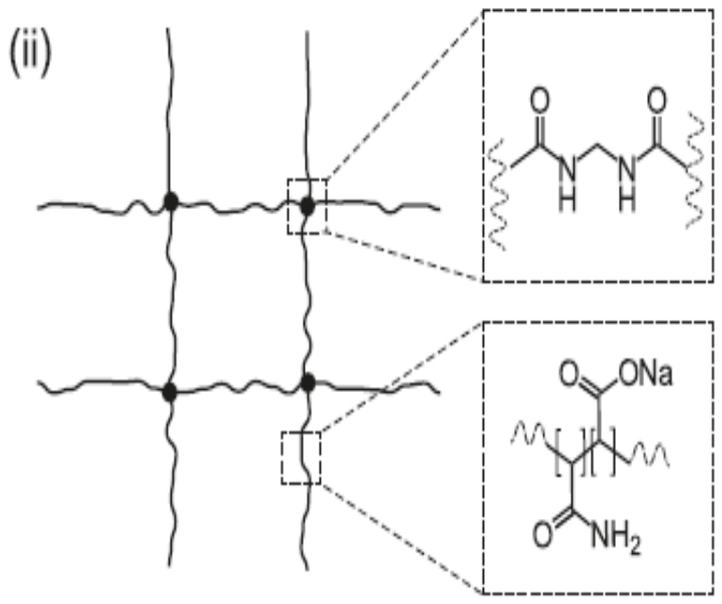


N-N'-methylenebisacrylamide

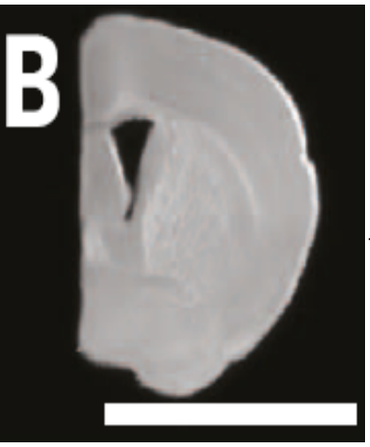
Polymerization



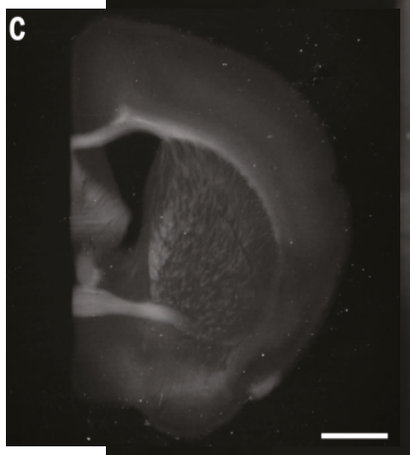




Pilot: Perm/fix brain tissue
Infuse Na-acrylate + acrylamide + X-linker
Protease K tissue digestion
Dialysis with ddH_2O



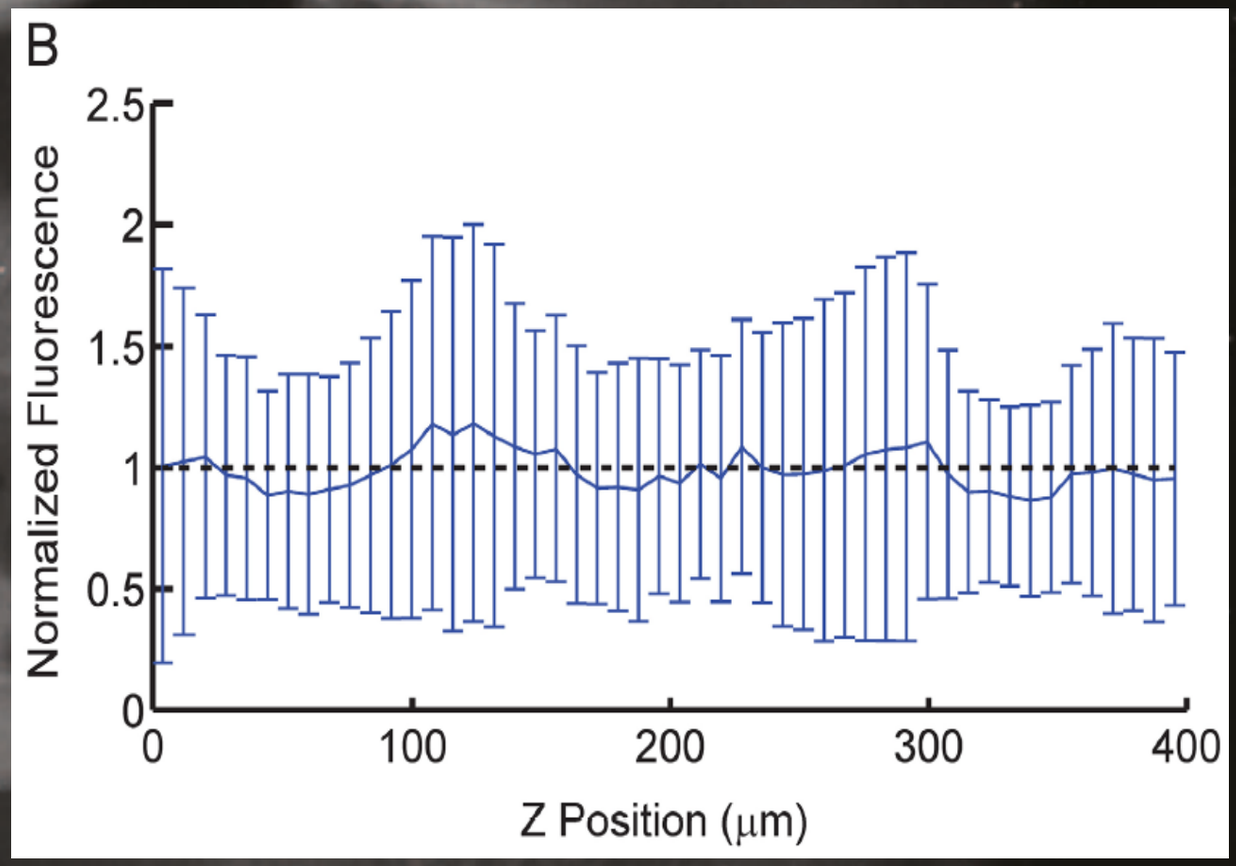
ddH_2O



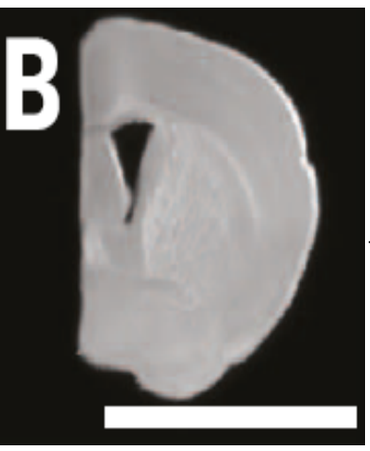
Scalebar 5mm

C

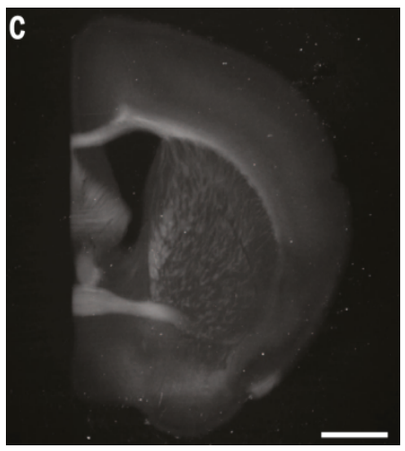
The control – uniform digestion



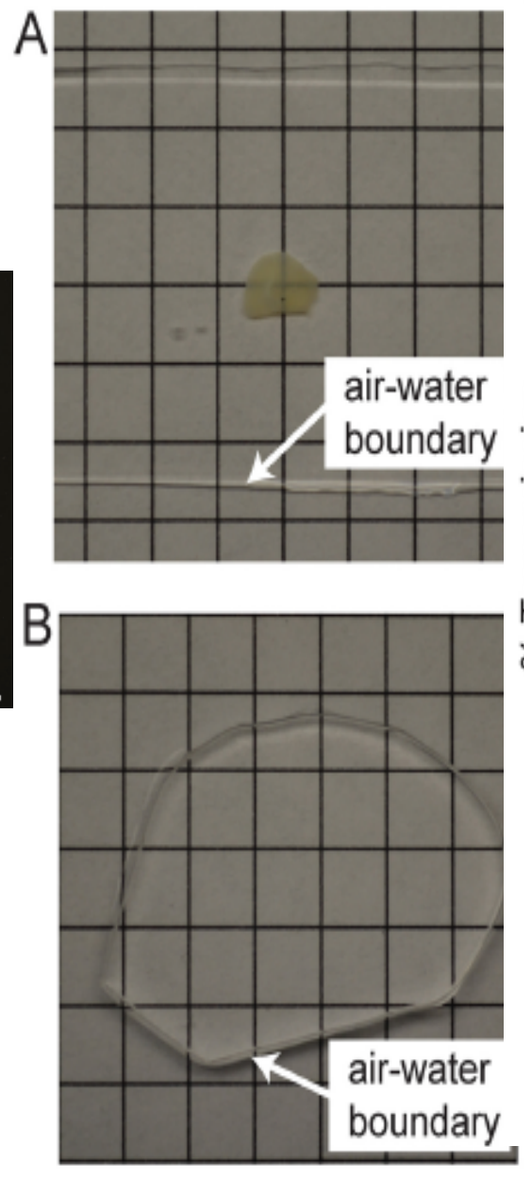
What about transparency?

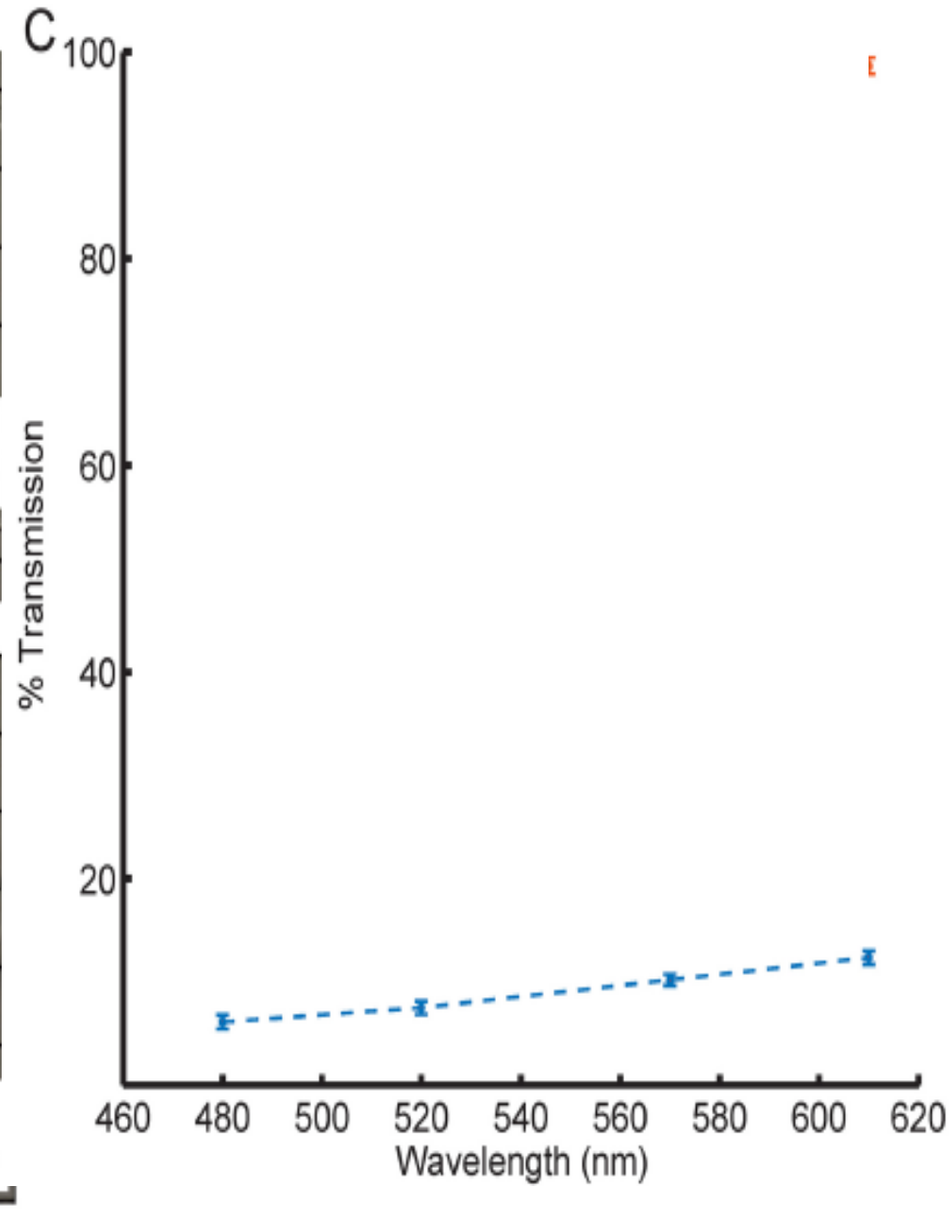


ddH2O



Scalebar 5mm





→ The sample is mostly water

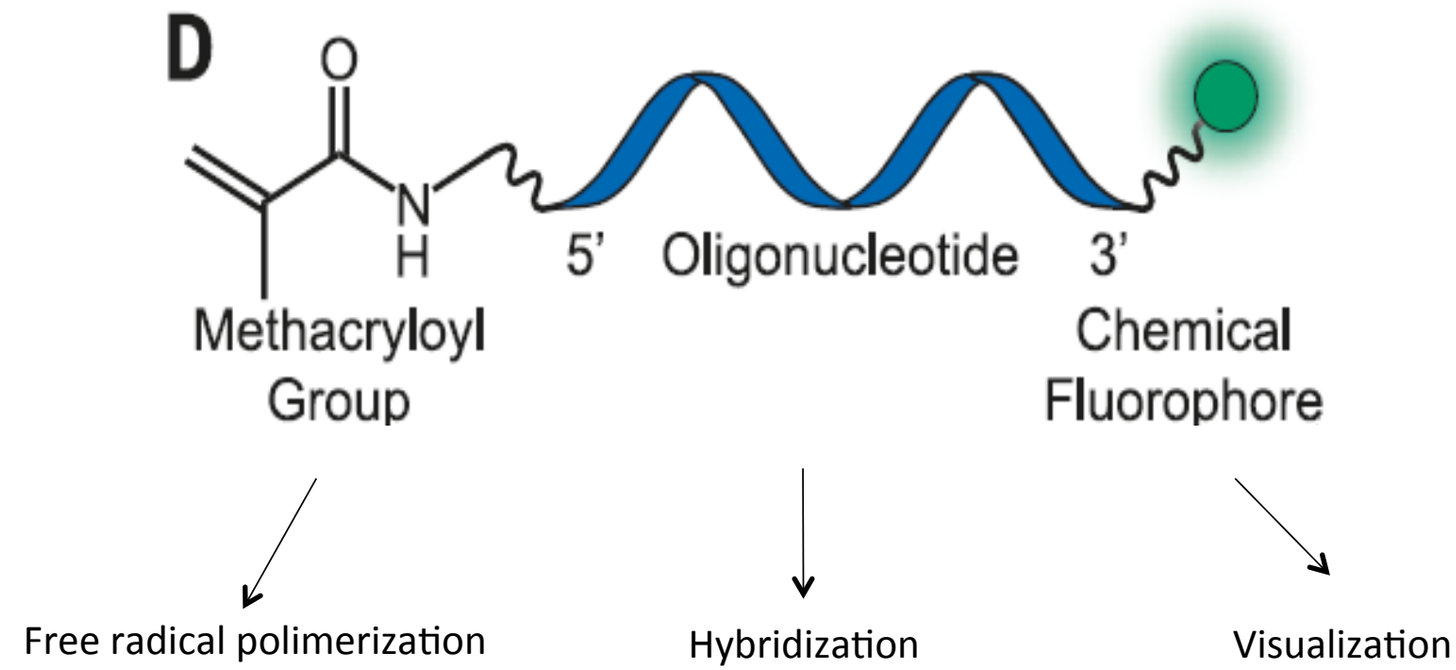
Progress

- ✓ Uniform digestion
- ✓ Clearing/transparency
- ✗ Digestion efficiency

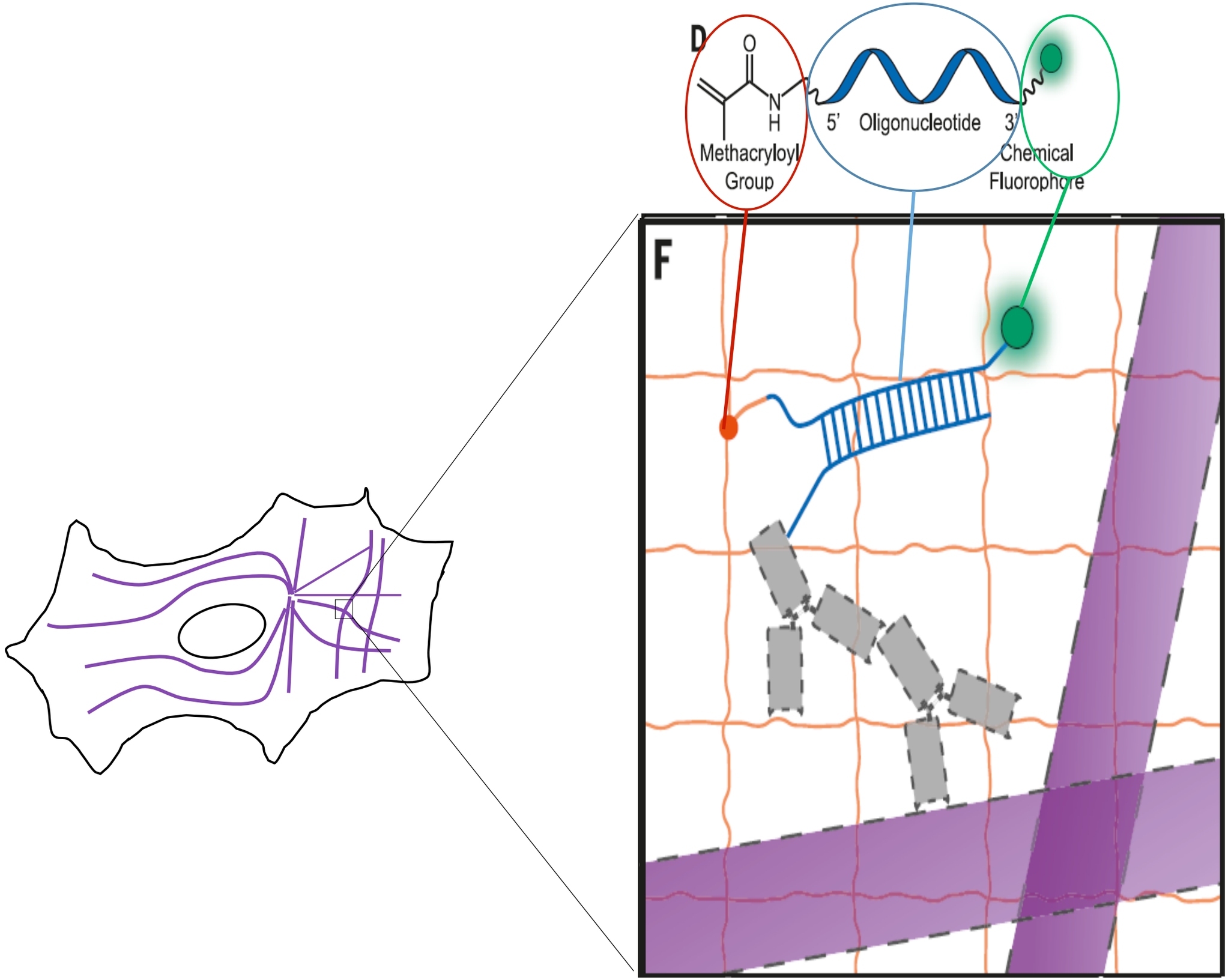
Fluorescent labeling strategy

→ Digestion is uniform, expansion looks uniform

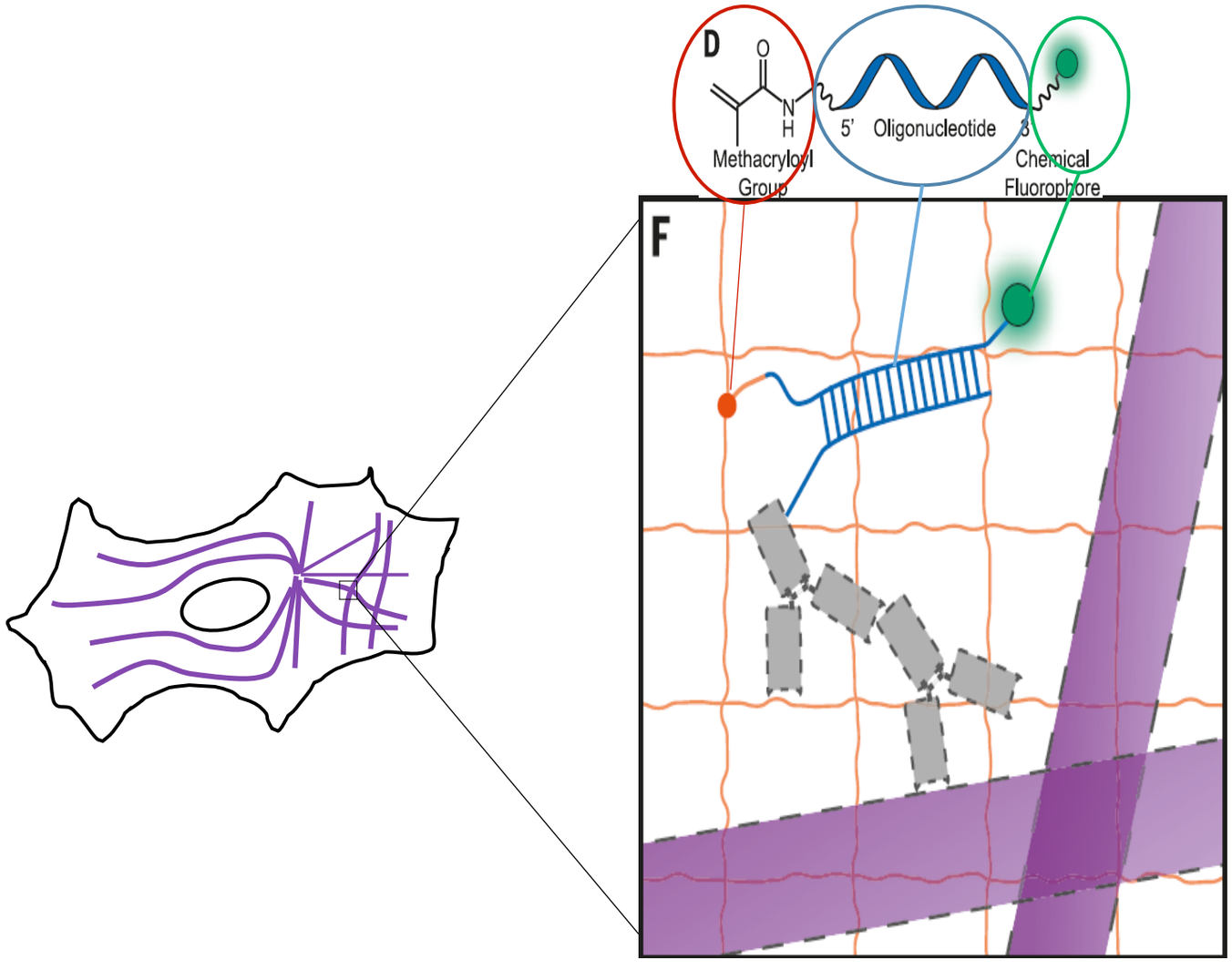
→ How to label proteins when everything is digested?



→ Fluorescent tag is targeted to biomolecule of interest, remains covalently anchored to polymer


D
O
N
H
Methacryloyl
Group
5'
Oligonucleotide
3'
Chemical
Fluorophore
F

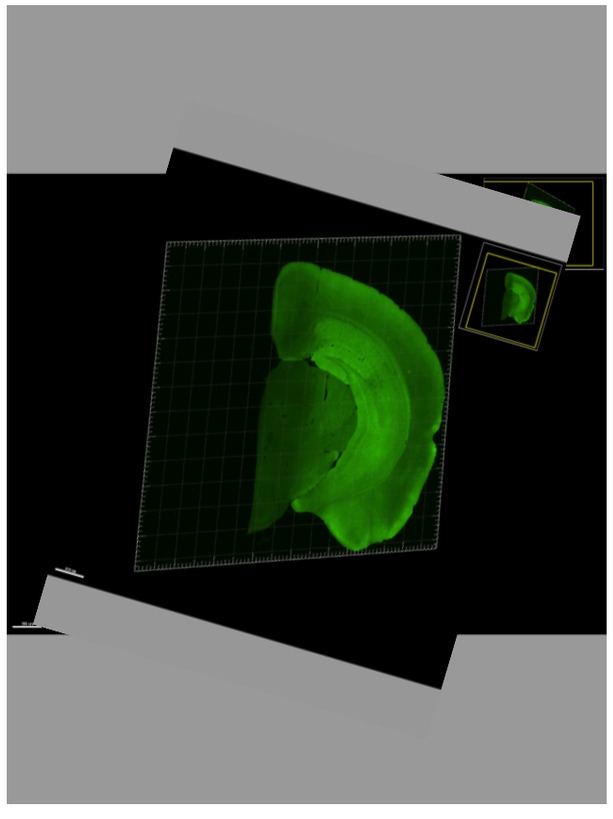
The control retention after gelation

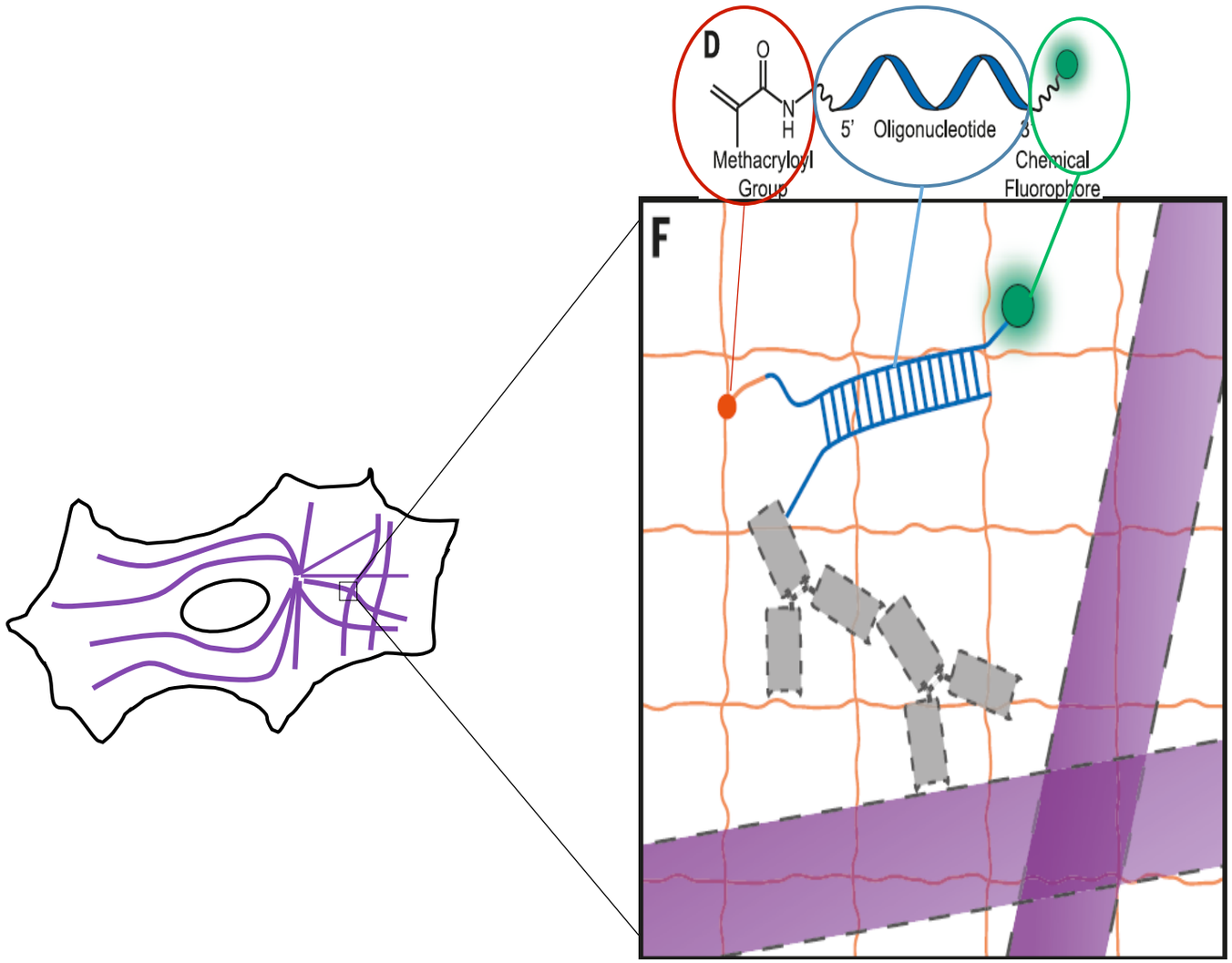


Supplementary Tables

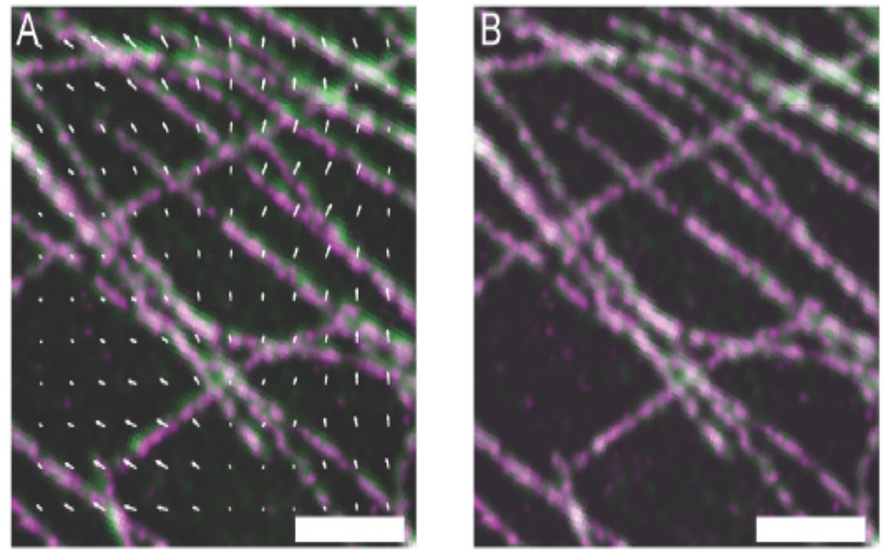
Table S1. Fluorescence retention during ExM chemical steps.

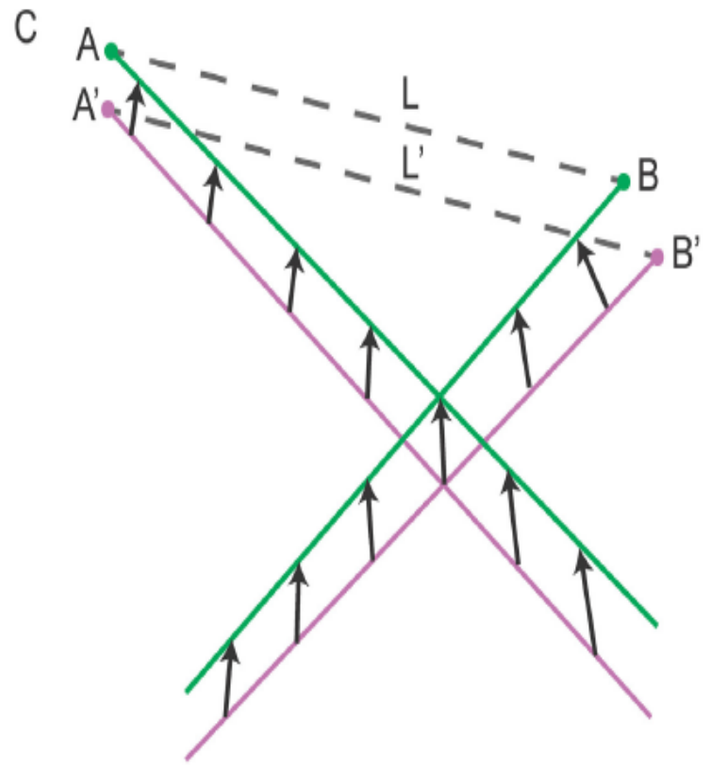
Fluorescence Retention After Gelation		
	Percent Retention	Standard Deviation (%)
Alexa 488	57.2	2.9 (n = 2 slices)
Atto 565	76.2	0.5 (n = 2 slices)
Atto 647N	58.5	2.8 (n = 2 slices)
Covalent Anchoring Efficiency During Gelation		
	Percentage Anchored	Standard Deviation (%)
Acrydite DNA	87.2	1.1 (n = 4 gels)





The control

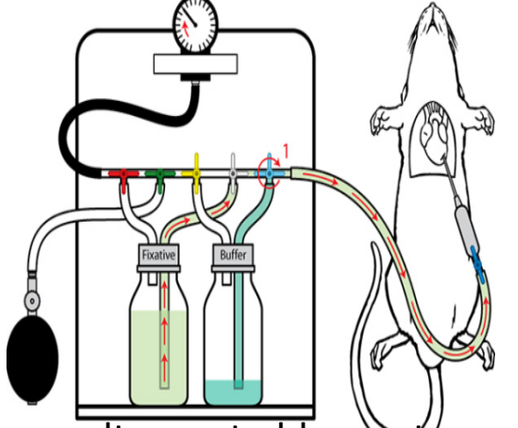




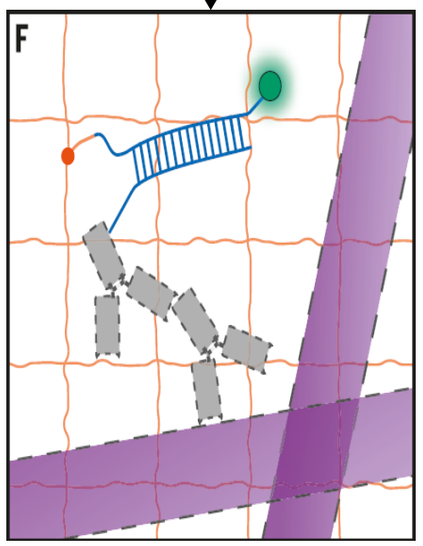
Non-rigid registration

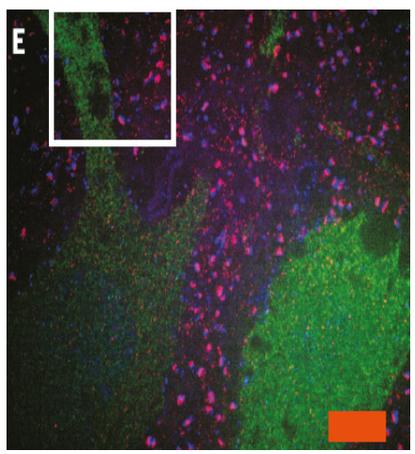
Workflow

I. Perfuse, fix, section
II. Staining
III. Polymer synthesis
IV. Digestion
V. Expansion
VI. Imaging



voltagegate.blogspot.com





Progress

- ✓ Uniform digestion
- ✓ Clearing/transparency
- ✓ Labeling
- ✓ Uniform digestion

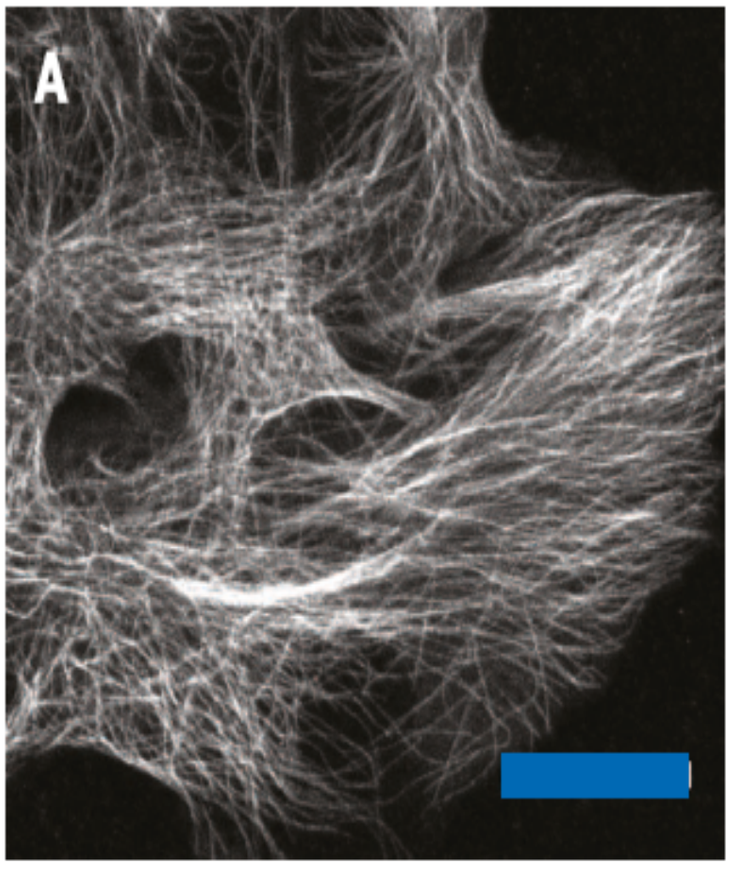
How well does it perform?

Cell culture (HEK 293)

Cell culture (HEK 293)

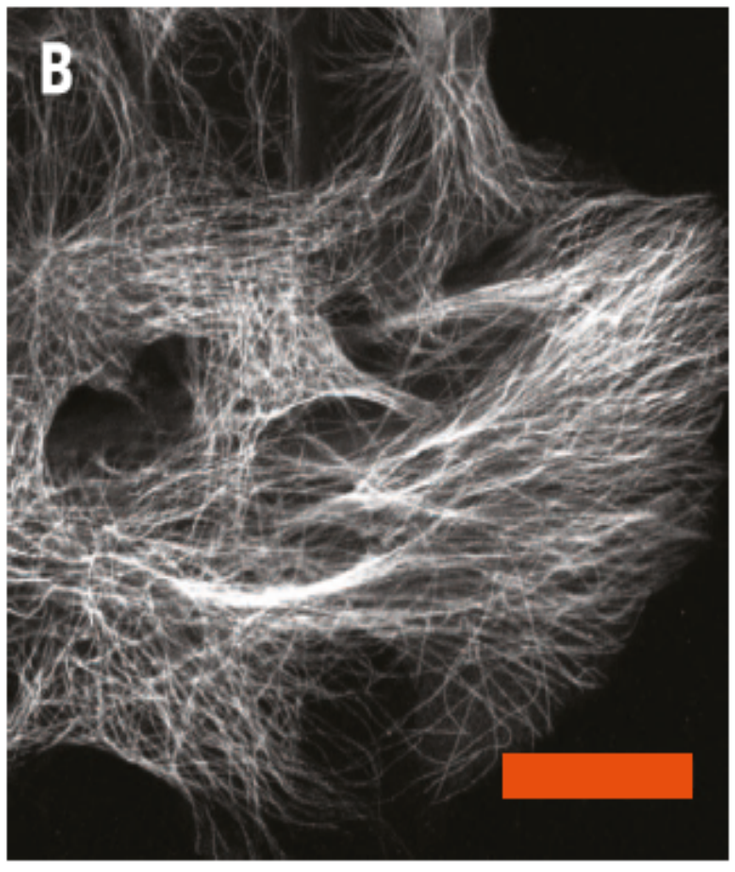
Expansion microscopy physically magnifies, with nanoscale isotropy

Confocal



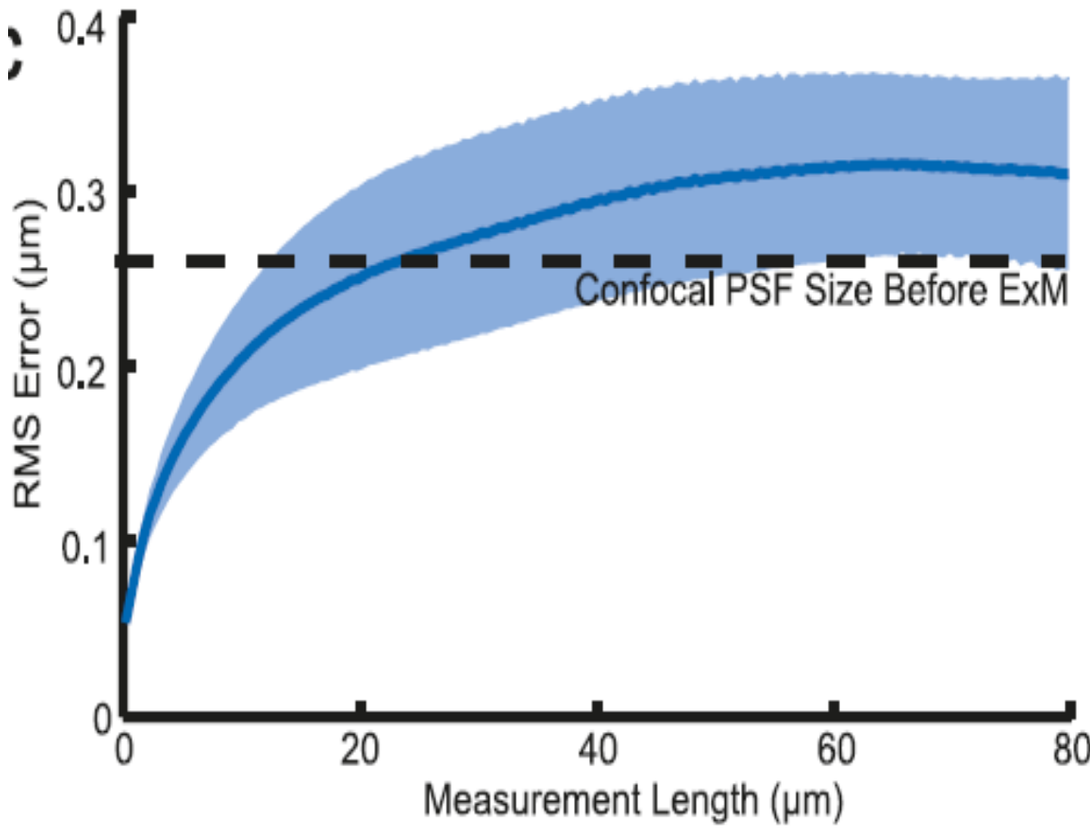
Scale bar: 20um

Post-expansion confocal



20um (81um)

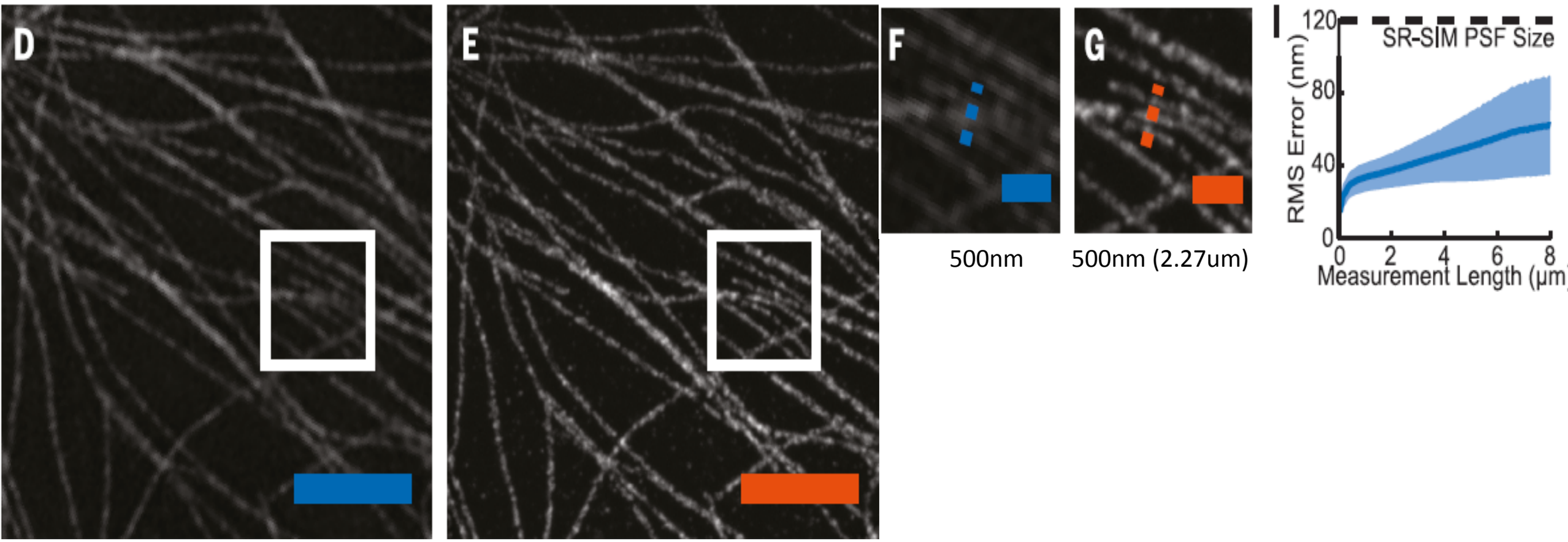
Errors in length



Cell culture (HEK 293)

SR-SIM image of microtubules

SR-SIM (structured illumination) **Post-expansion confocal**



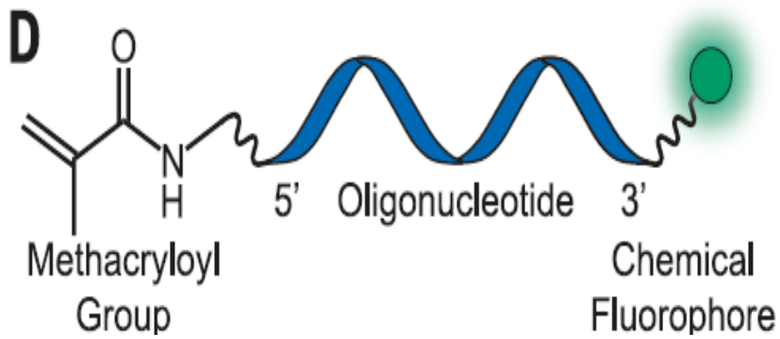
Scale bar: 2um

2um (9.1um)

500nm

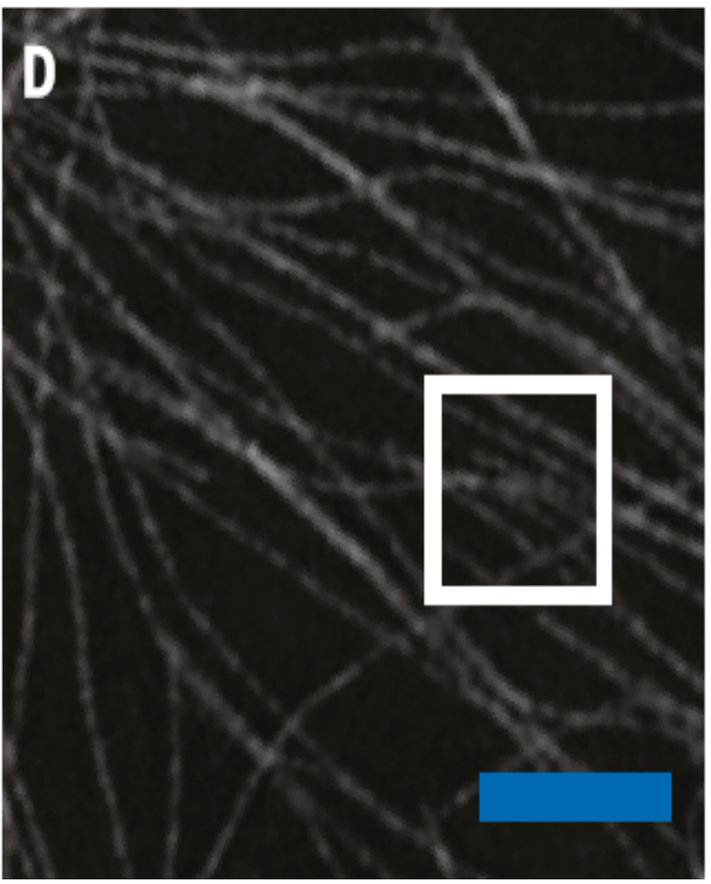
500nm (2.27um)

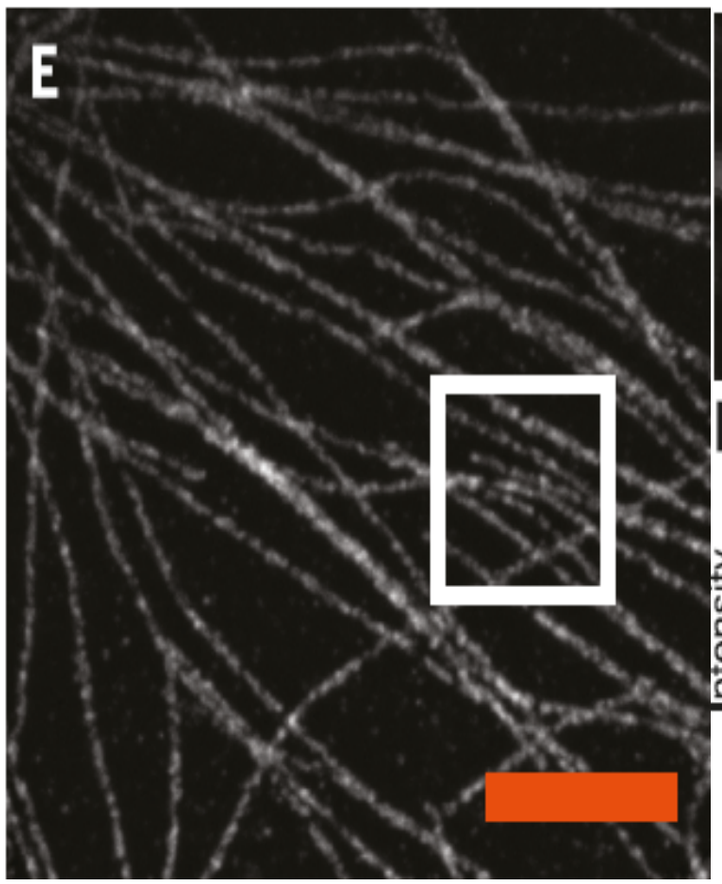
Cell culture (HEK 293)



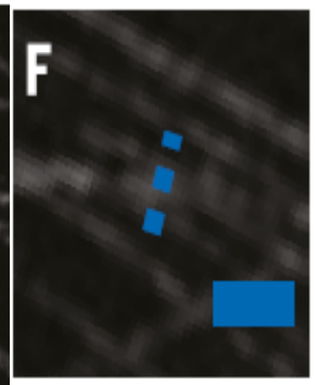
SR-SIM image of microtubules

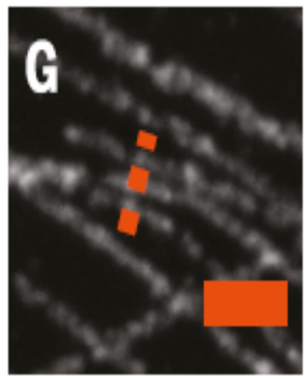
SR-SIM (structured illumination) **Post-expansion confocal**

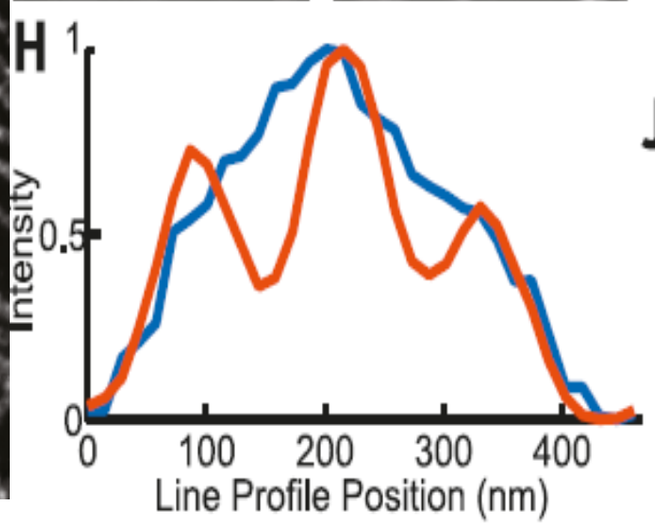




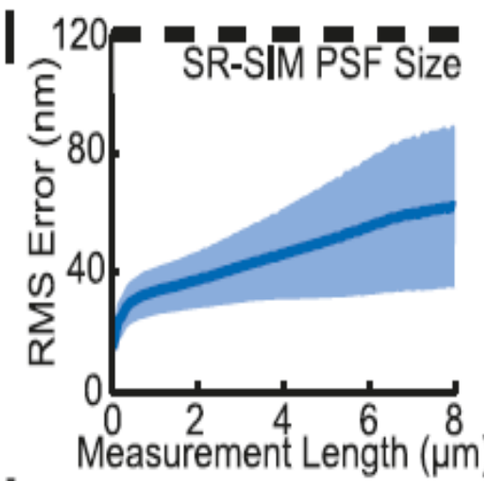
Scale bar: 2um

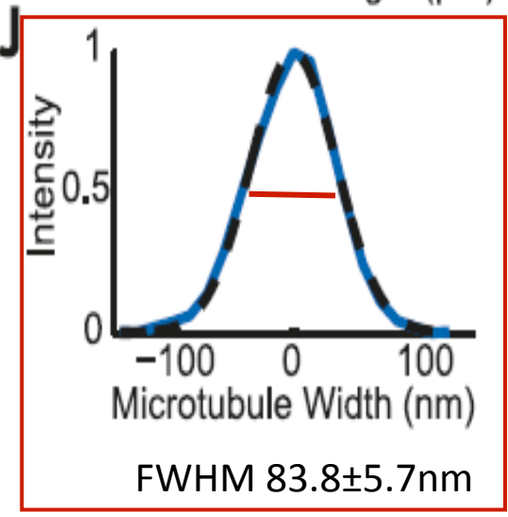






2um (9.1um)



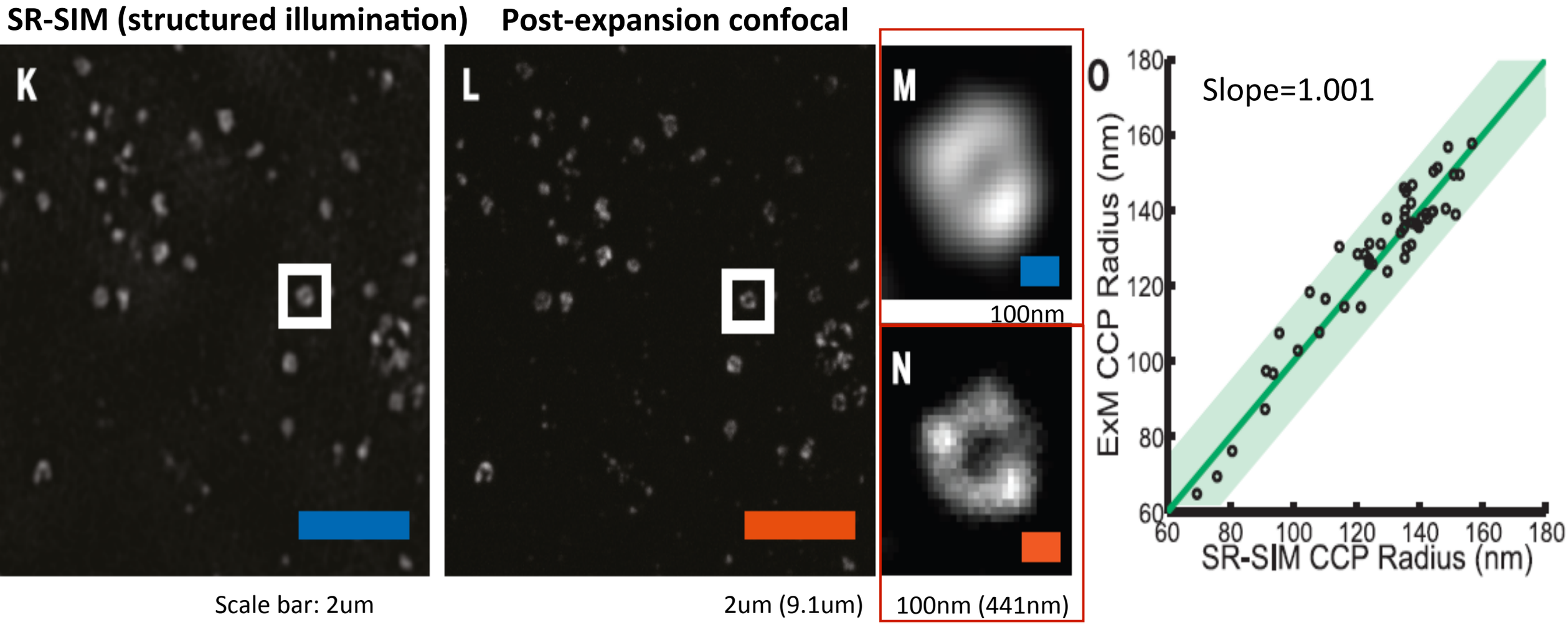


FWHM 83.8±5.7nm

Effective resolution: ~60nm

Cell culture (HEK 293)

Clathrin-coated pits (CCPs) in HEK293 cells



Scale bar: 2um

2um (9.1um)

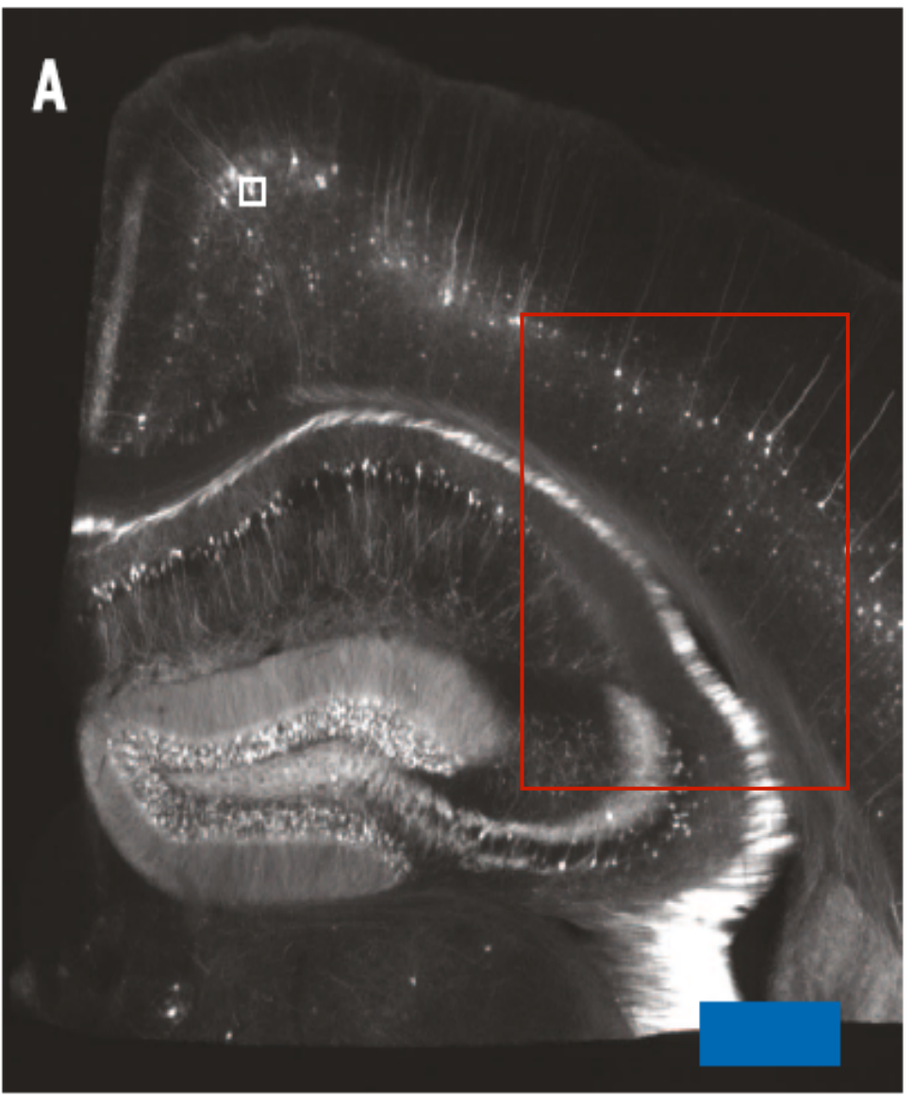
100nm (441nm)

Fixed brain tissue

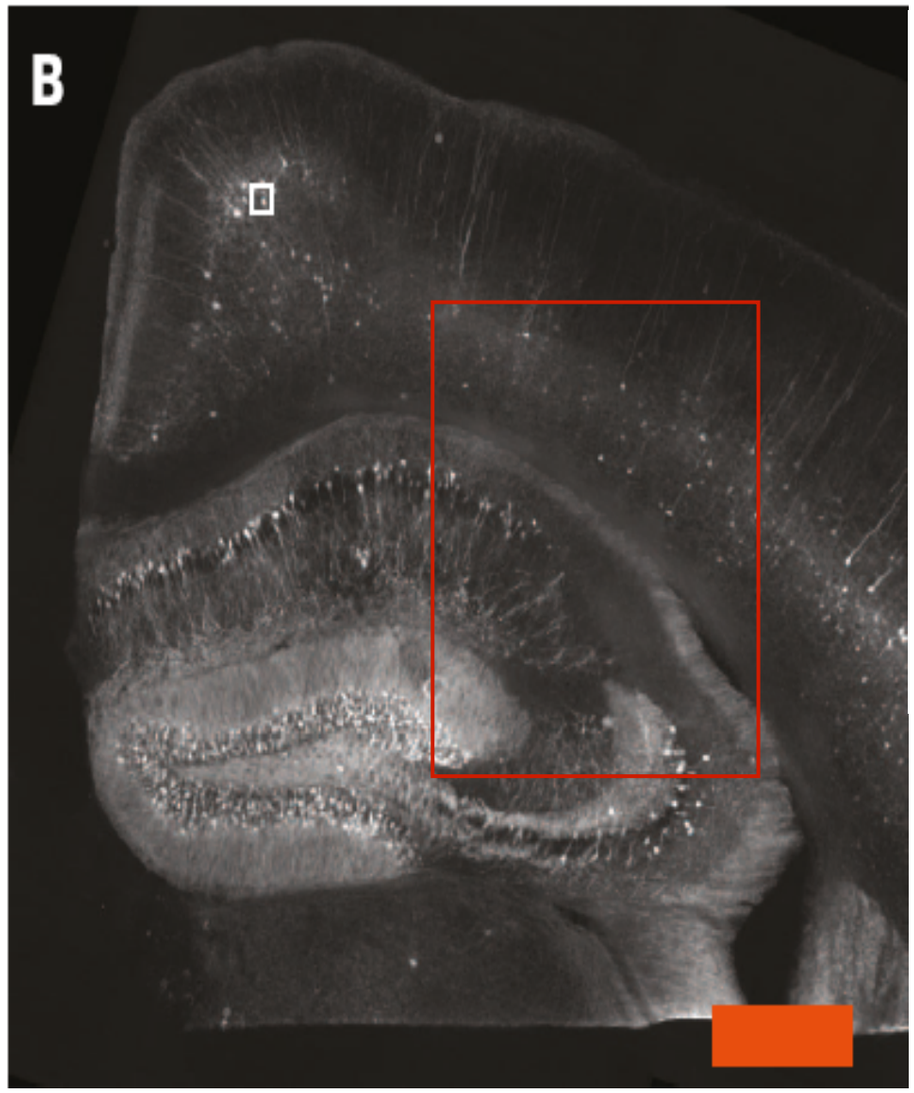
Fixed brain tissue

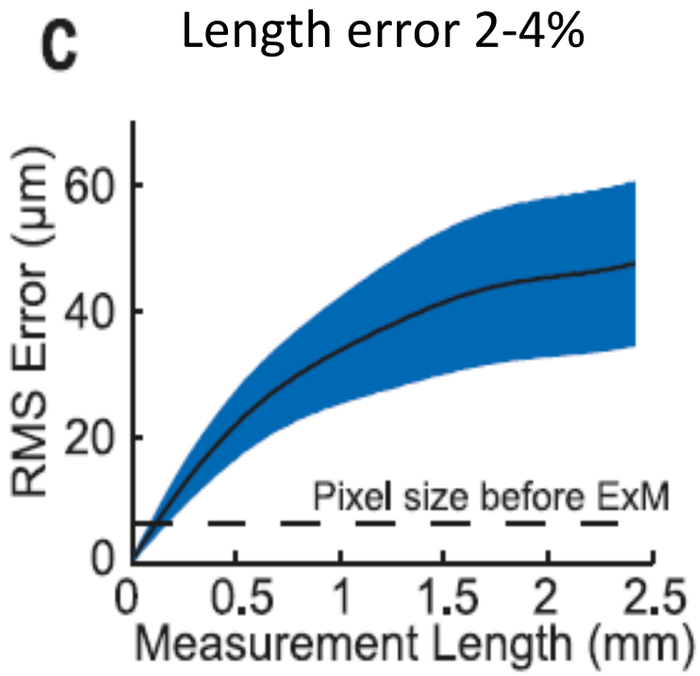
Thy1-YFP-H mice

Widefield fluorescence



Post-ExM Widefield

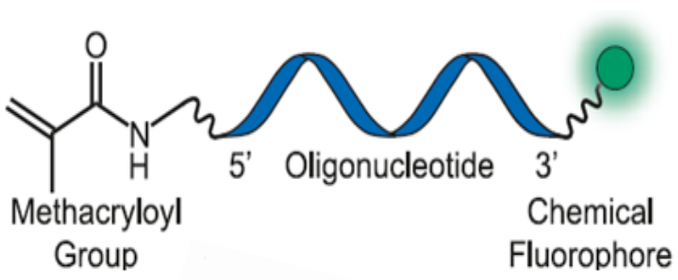


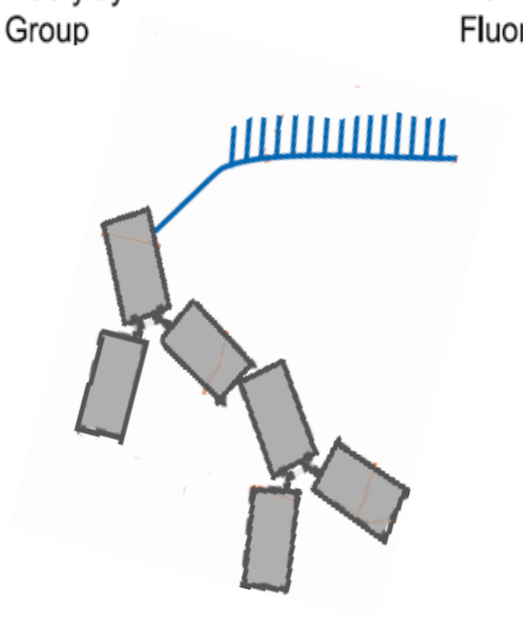


Length error 2-4%

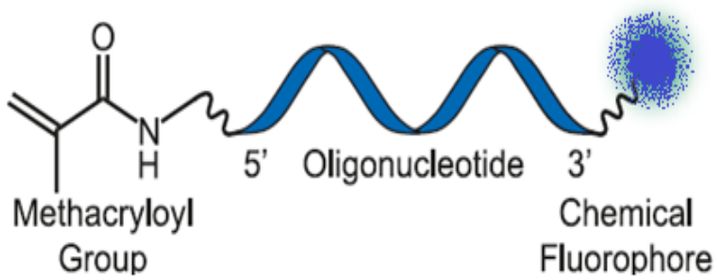
Multicolor ExM

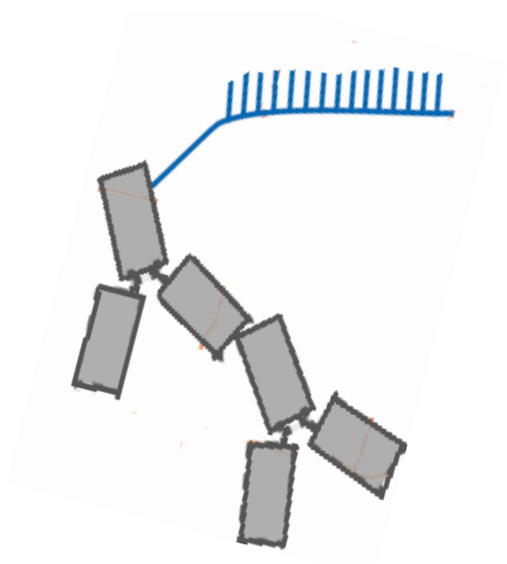
Anti-GFP, AF488



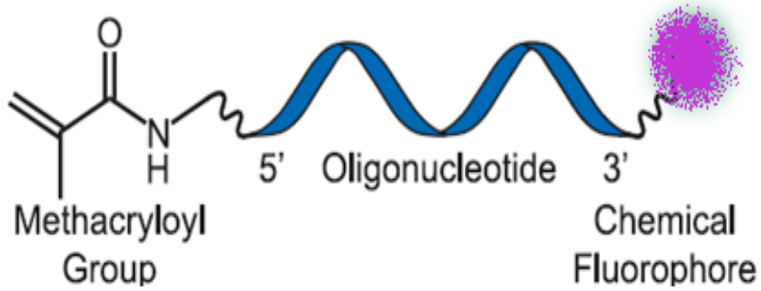


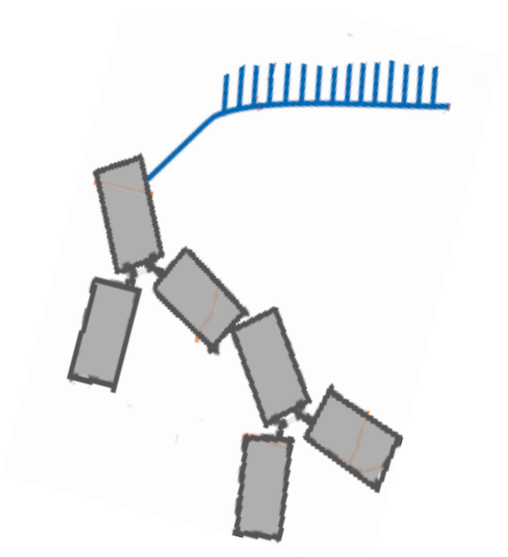
Anti-Bassoon, Atto565





Anti-Homer1, Atto647N

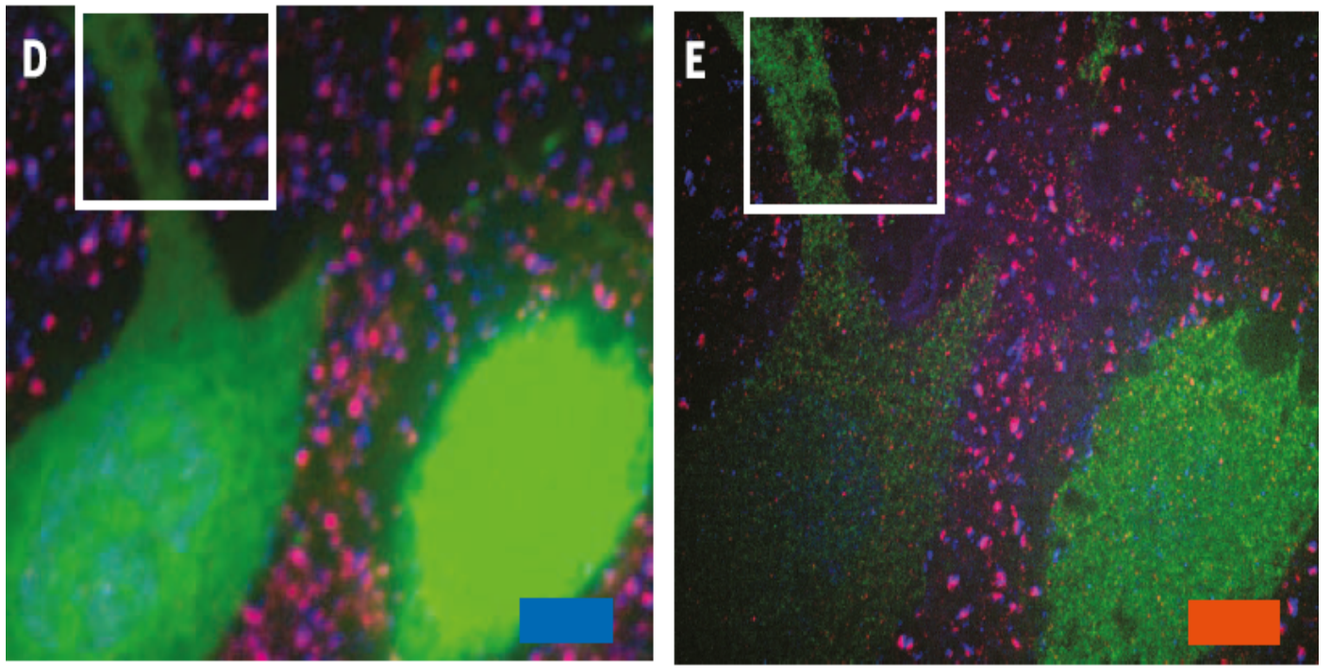




Fixed brain tissue

Confocal

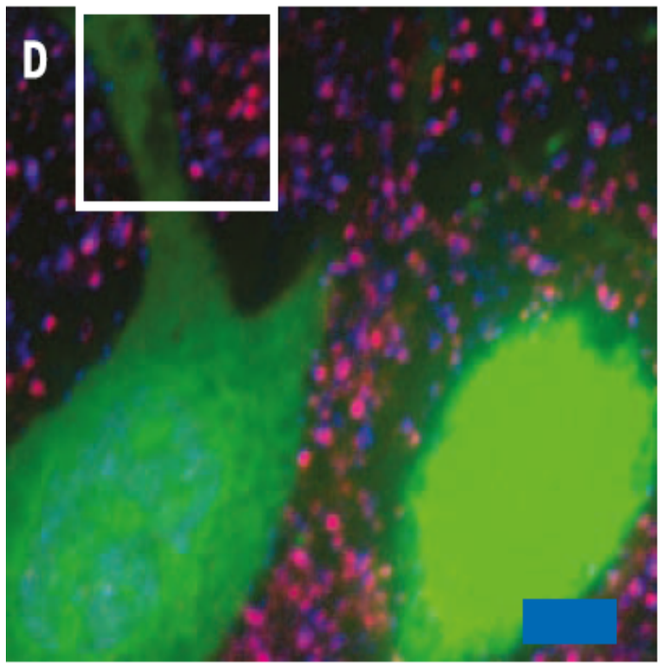
Post-expansion confocal



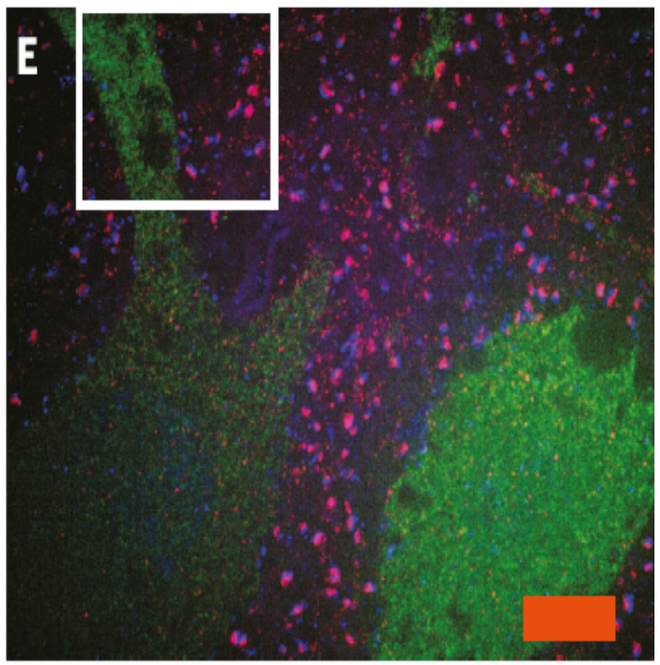
Thy1-YFP
Homer1(presynaptic)
Bassoon(postsynaptic)

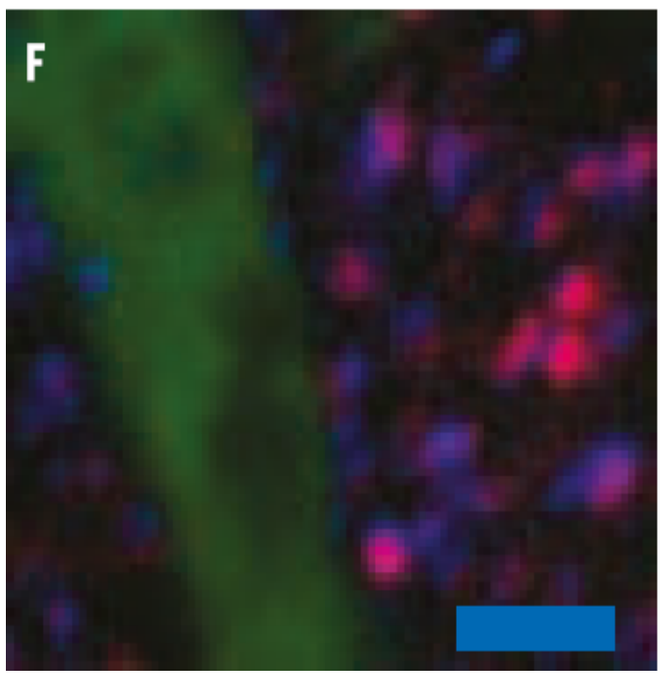
Fixed brain tissue

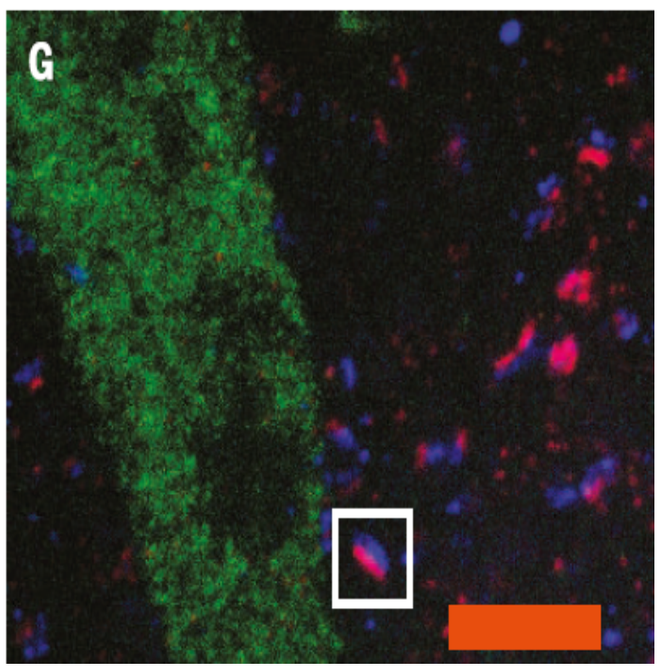
Confocal



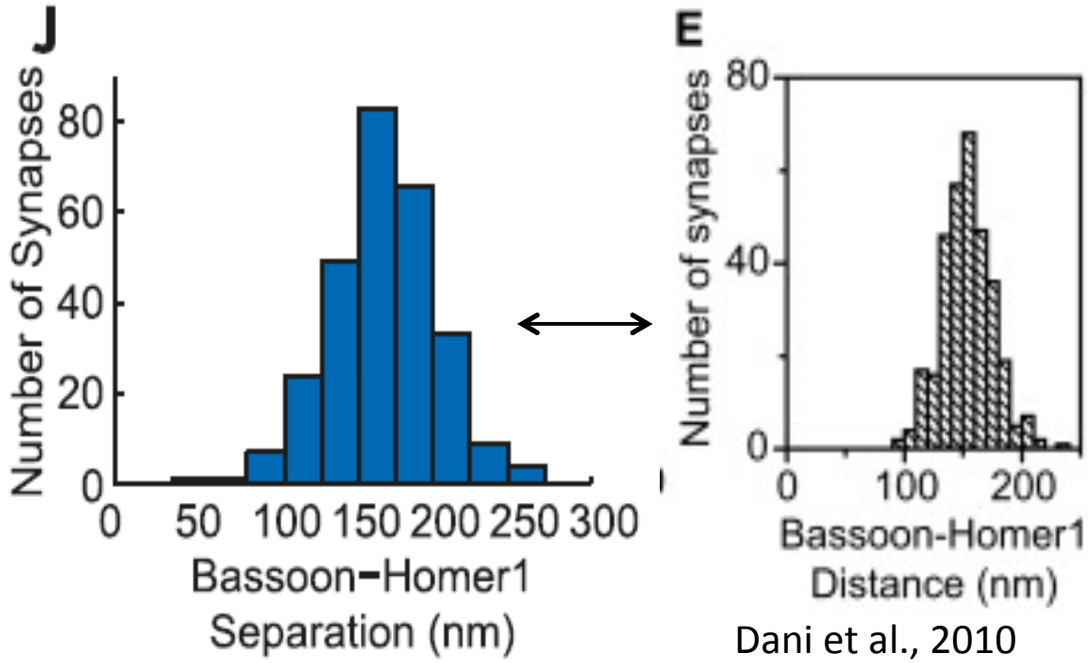
Post-expansion confocal



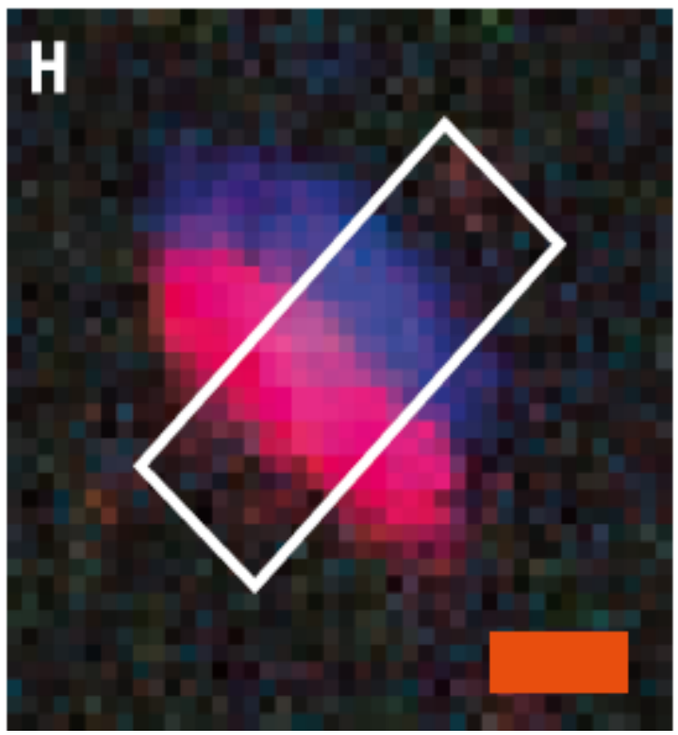




(F and G) Details of boxed regions in (D) and (E), respectively.



Dani et al., 2010



(H) Single representative synapse highlighted in (G).

3D superresolution

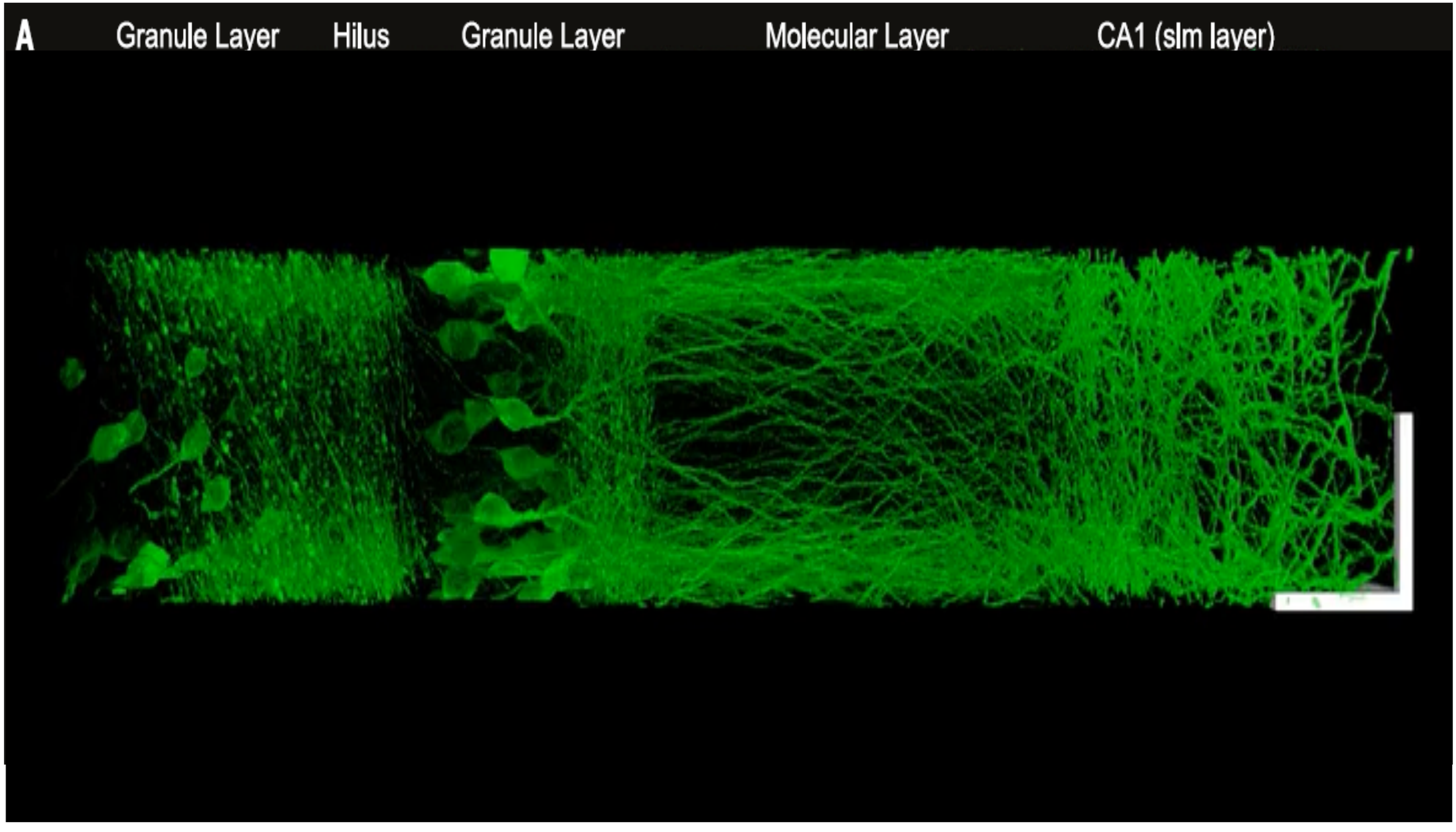
3D superresolution

500x180x100um volume image of adult Thy1-YFP-H mouse

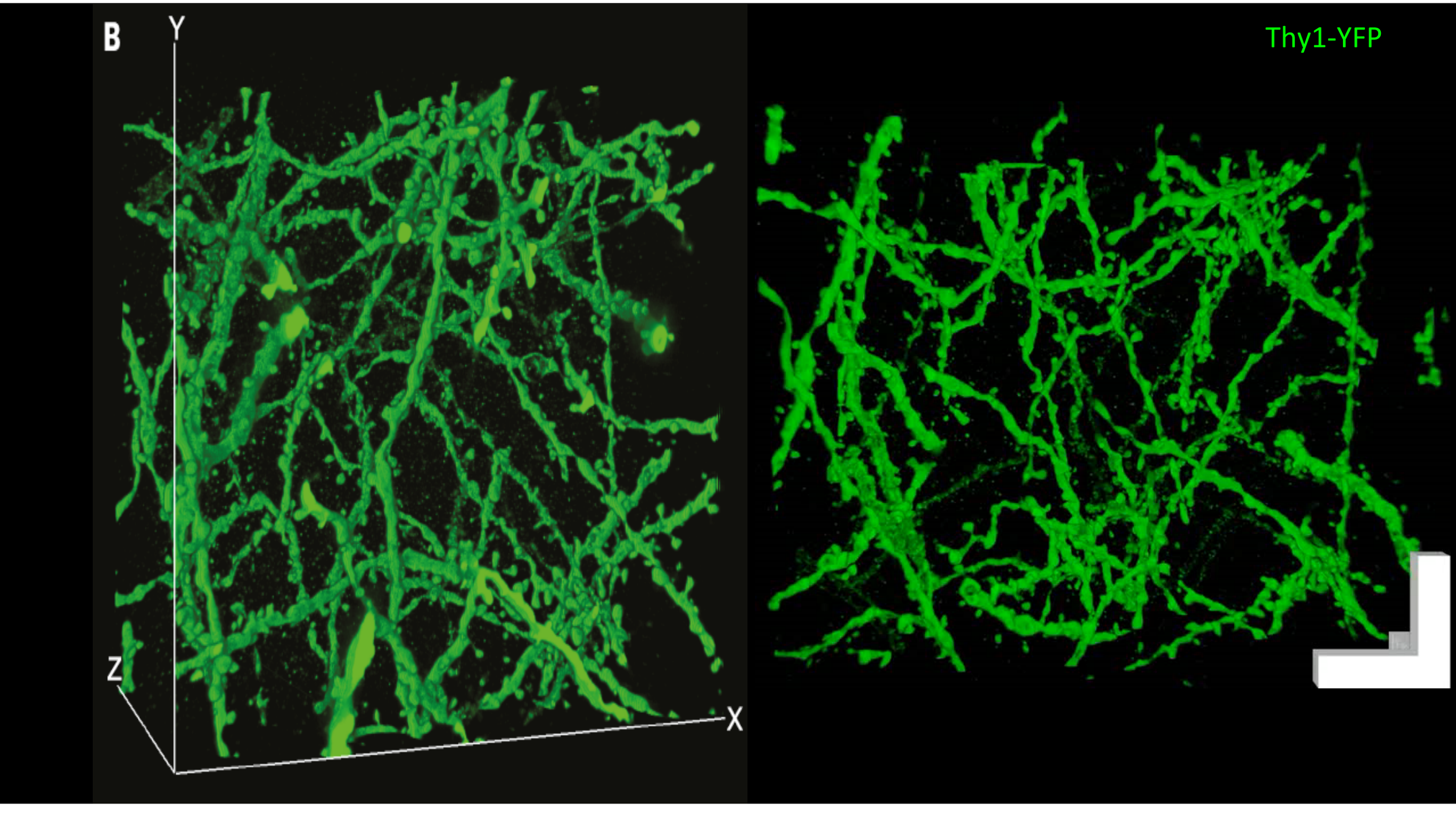
Thy1-YFP
Homer1(presynaptic)
Bassoon(postsynaptic)

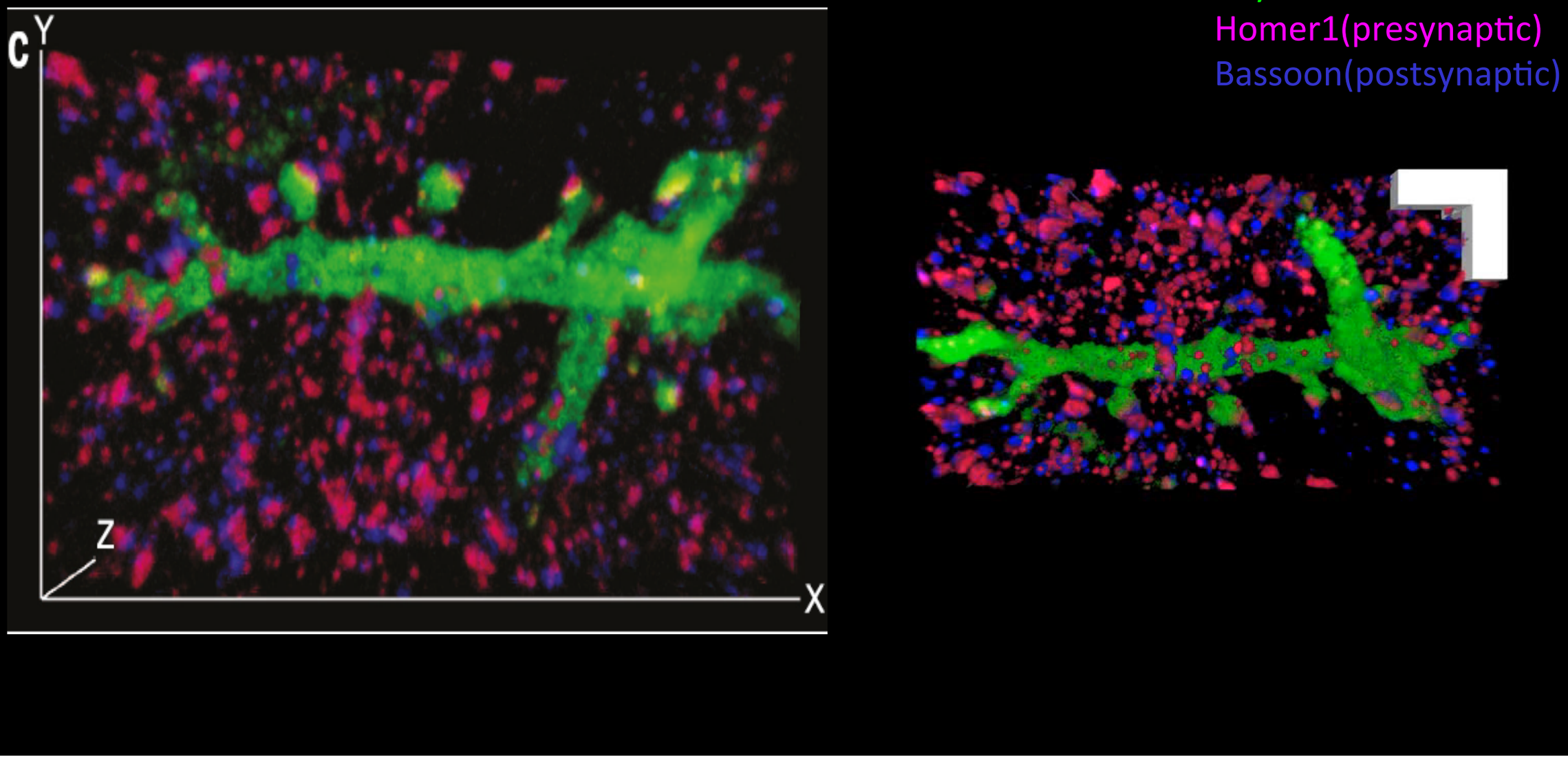
Lateral Resolution ~70 nm, axial resolution 200 nm

3D superresolution



CA1 stratum lacunosum moleculare (slm)





‘Focusing on a dendrite in CA1 slm, we observed the postsynaptic protein Homer1 to be well localized to dendritic spine heads, with the presynaptic molecule Bassoon in apposition’

Summary

Imaging of large 3D structures with nanoscale precision

⇒ Large tissue imaging with great accuracy

⇒ Axial & lateral resolution improved by same factor

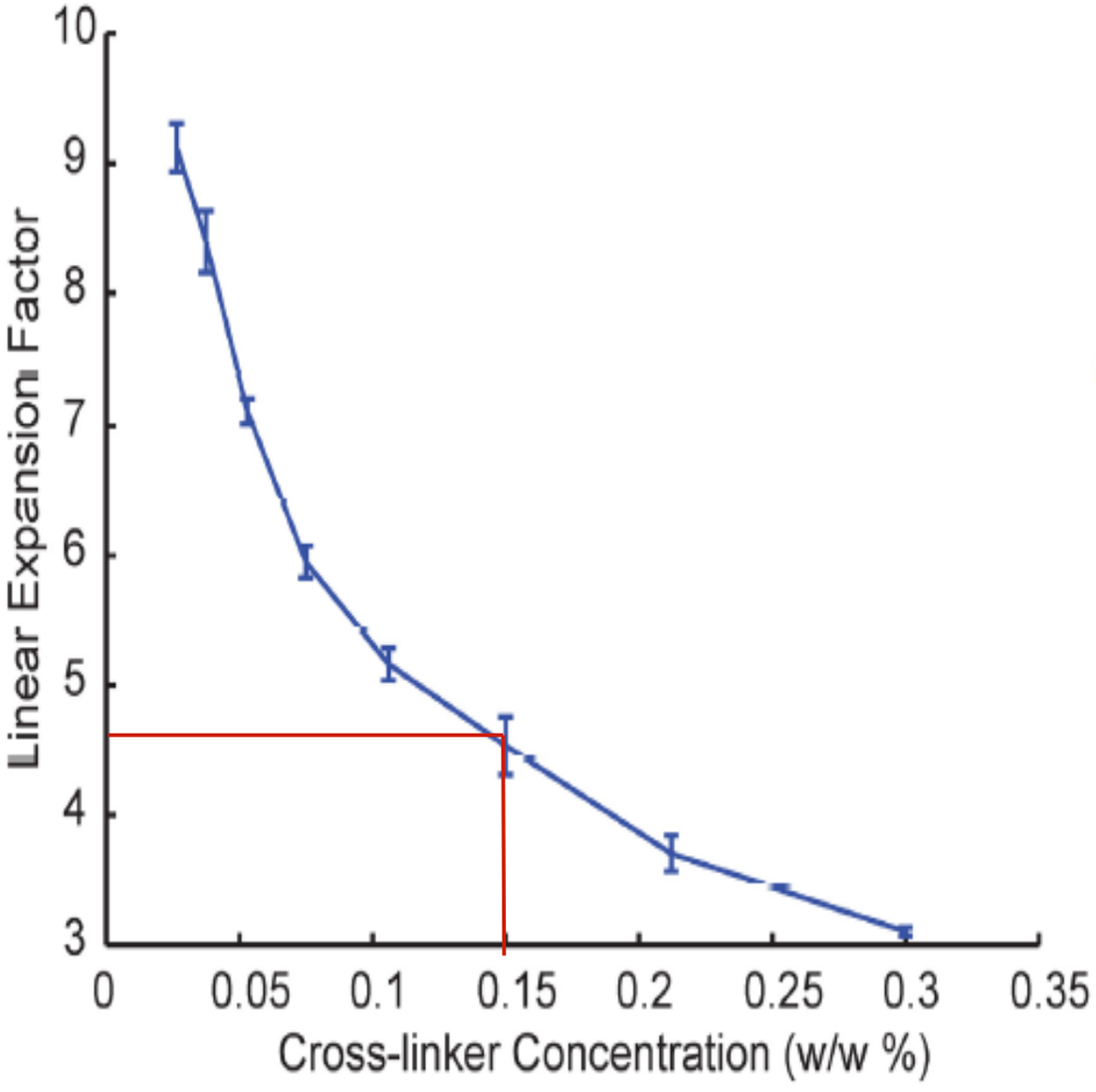
⇒ Improved mechanical error

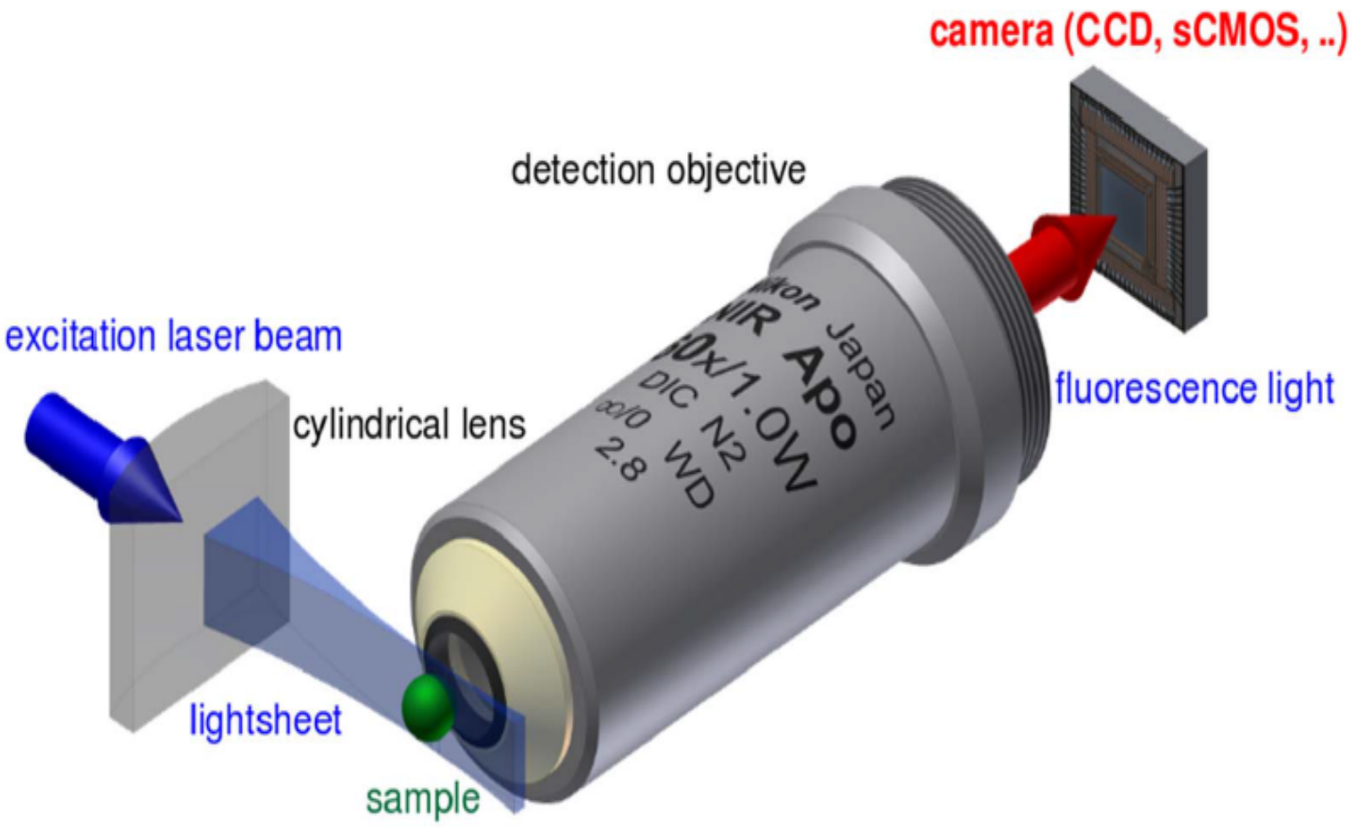
Works with conventional microscopes and fluorophores

Harsh sample treatment – imaging of a ‘ghost’

⇒ Combine with light sheet microscopy

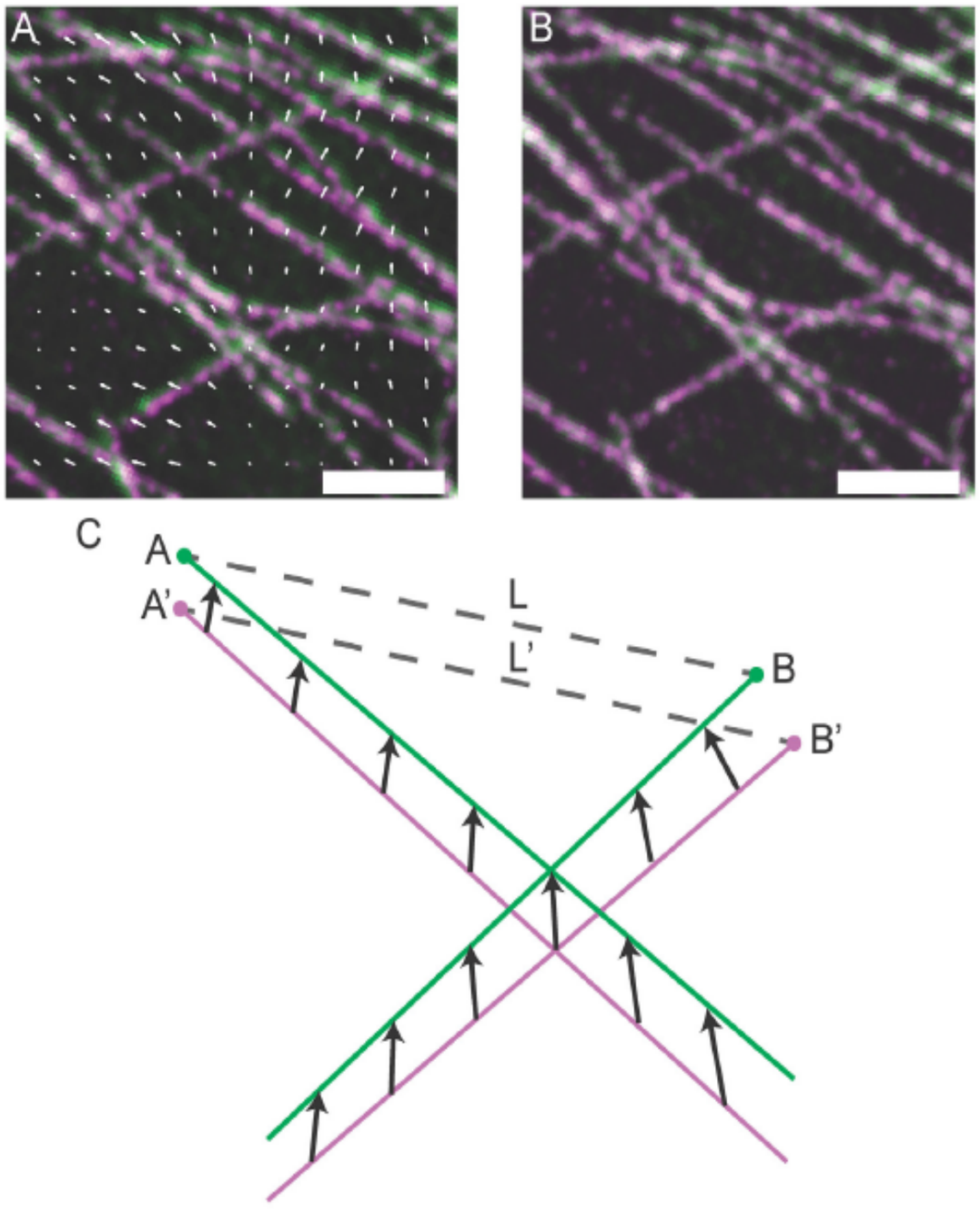
Outlook

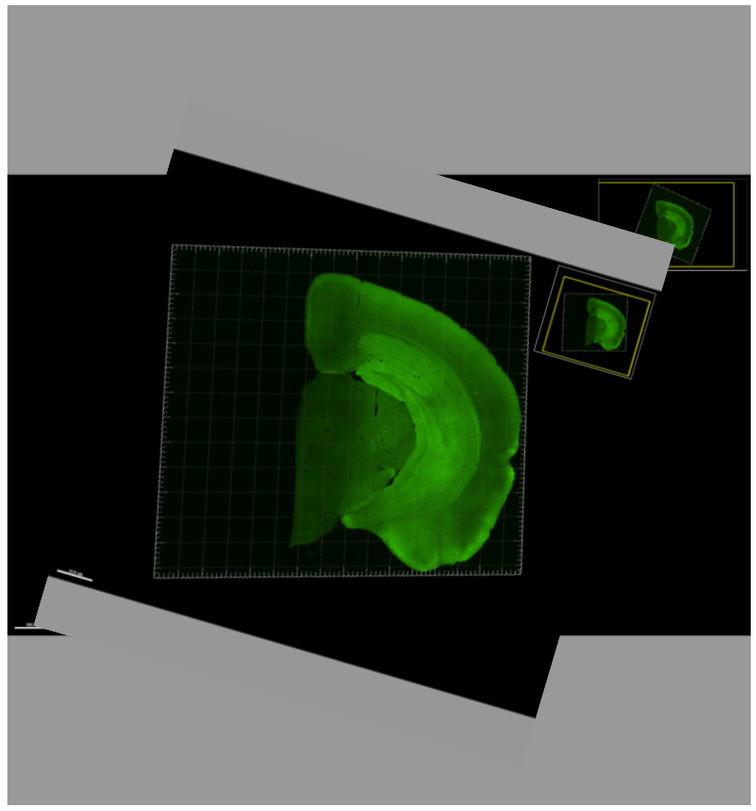


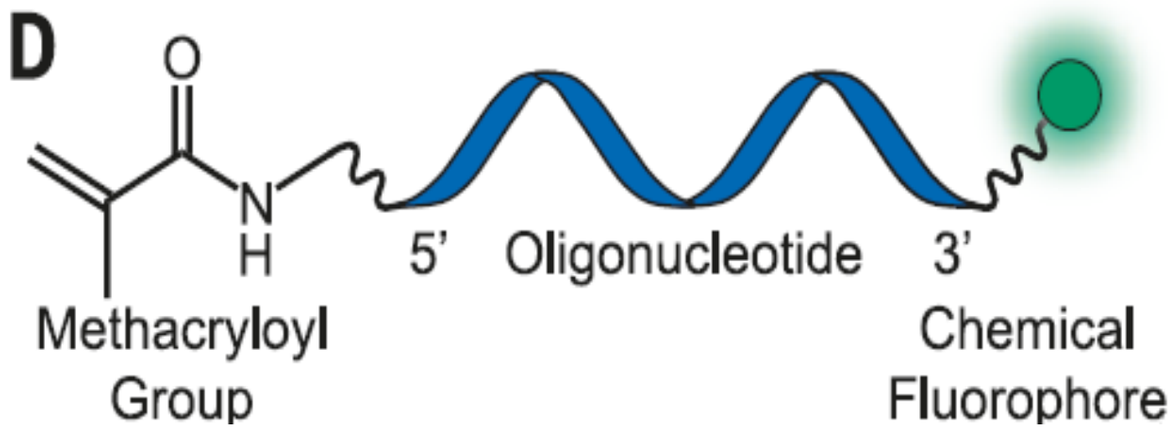


http://en.wikipedia.org/wiki/Light_sheet_fluorescence_microscopy

Criticism


A
B
C
A
A'
L
L'
B
B'




D
O
N
H
5'
Oligonucleotide
3'
Methacryloyl
Group
Chemical
Fluorophore

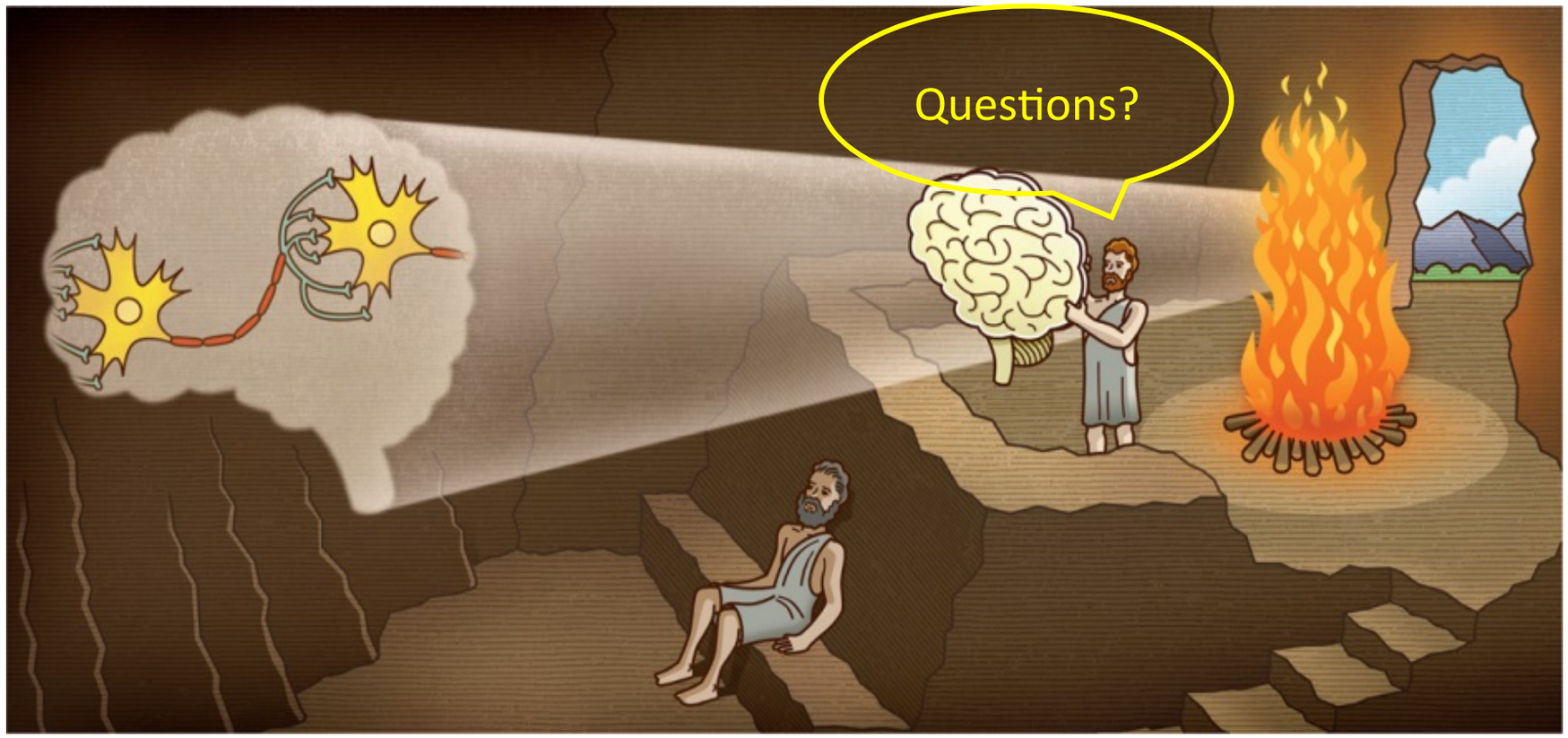


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ILLUSTRATION: PETER AND MARIA HOEY