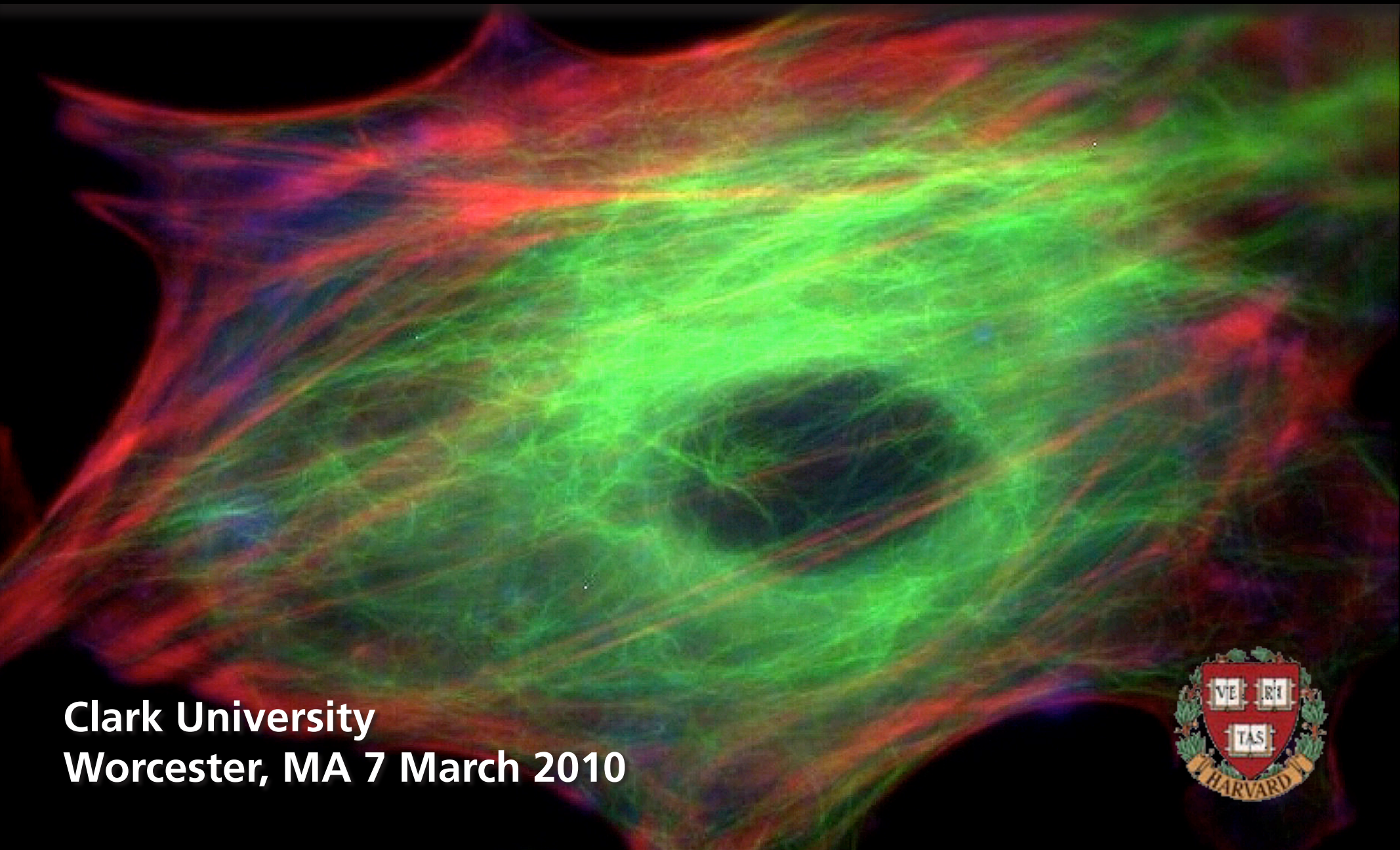
Subcellular surgery and nanoneurosurgery



Clark University
Worcester, MA 7 March 2010

Iva Maxwell

Sam Chung

Valeria Nuzzo

Alexander Heisterkamp

and also....

Dr. Eli Glezer
Prof. Chris Schaffer
Nozomi Nishimura
Debayoti Datta
Dr. Jonathan Ashcom
Jeremy Hwang
Dr. Nan Shen
Roanna Ruiz
Anja Schmalz
Prakriti Tayalia

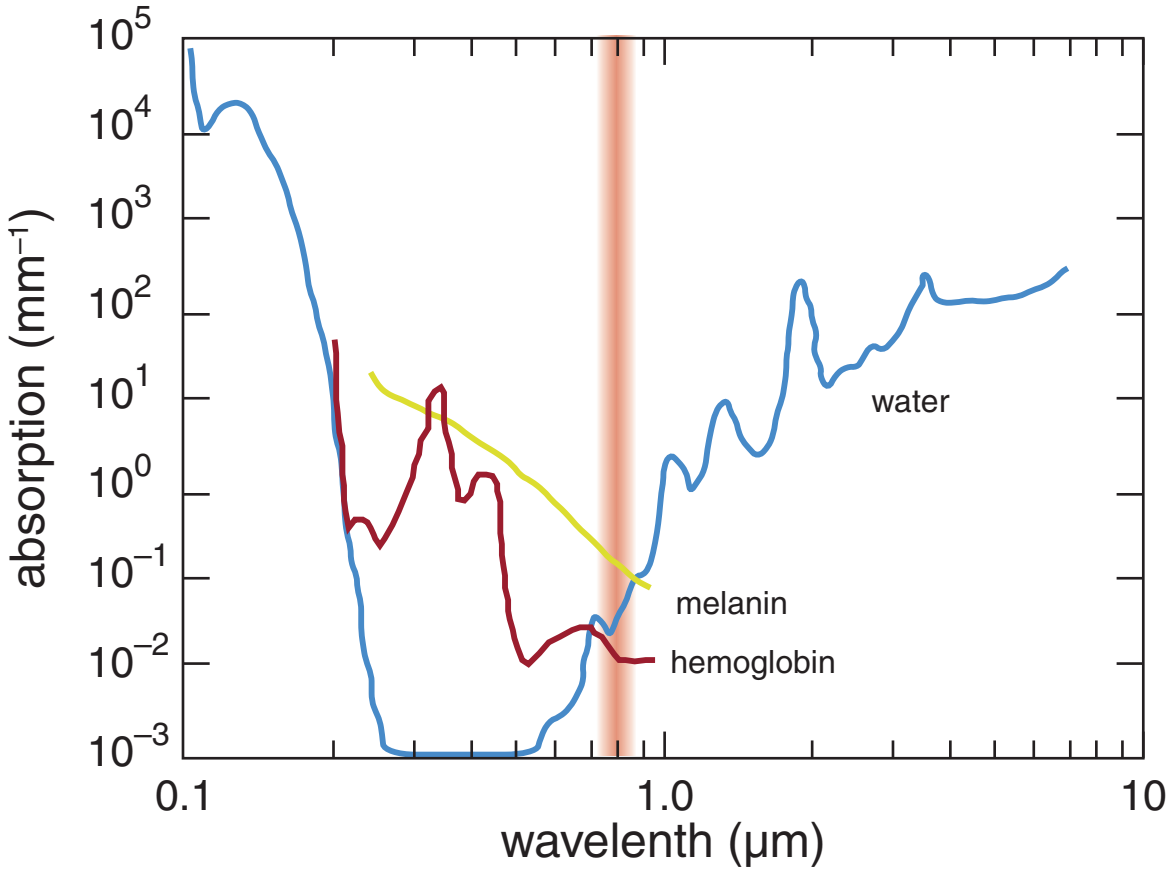
Prof. Don Ingber (Harvard Medical School)
Prof. Aravi Samuel (Harvard)
Prof. Chris Gabel (Boston University)
Dr. Damon Clark (Harvard University)
Prof. J.M. Underwood (UMass Worcester)
Prof. J.A. Nickerson (UMass Worcester)
Prof. Philip LeDuc (Carnegie Mellon)
Prof. Sanjay Kumar (UC Berkeley)

Introduction

why use femtosecond pulses?

Introduction

tissue is nearly transparent at 800 nm

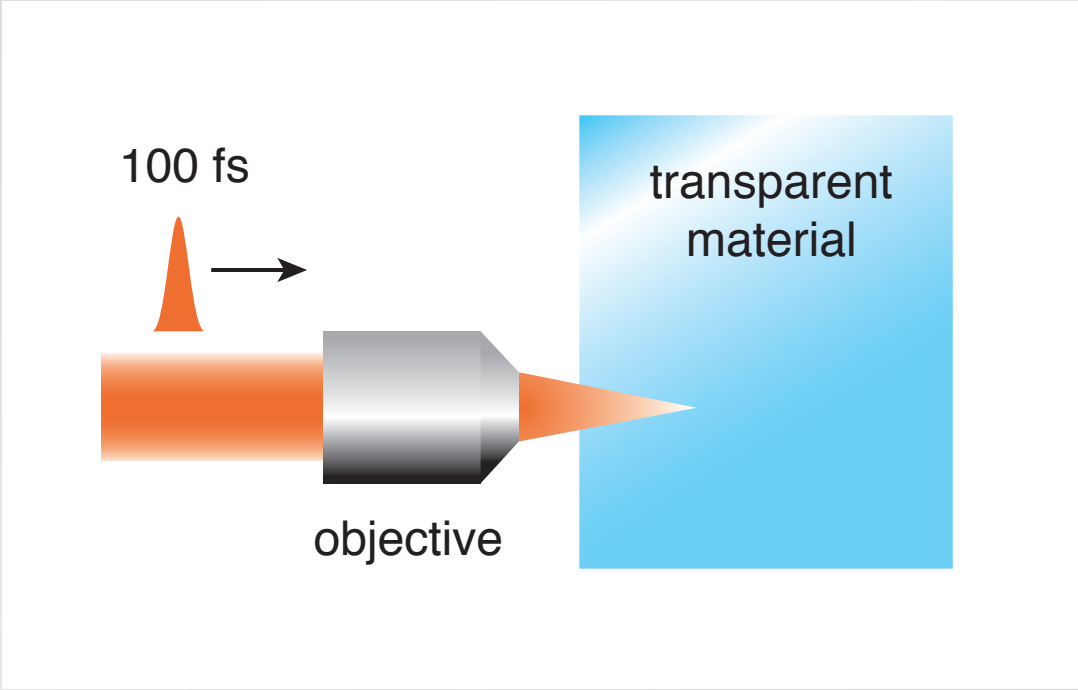


Outline

- femtosecond materials interactions
- subcellular surgery
- nanoneurosurgery

Femtosecond materials interactions

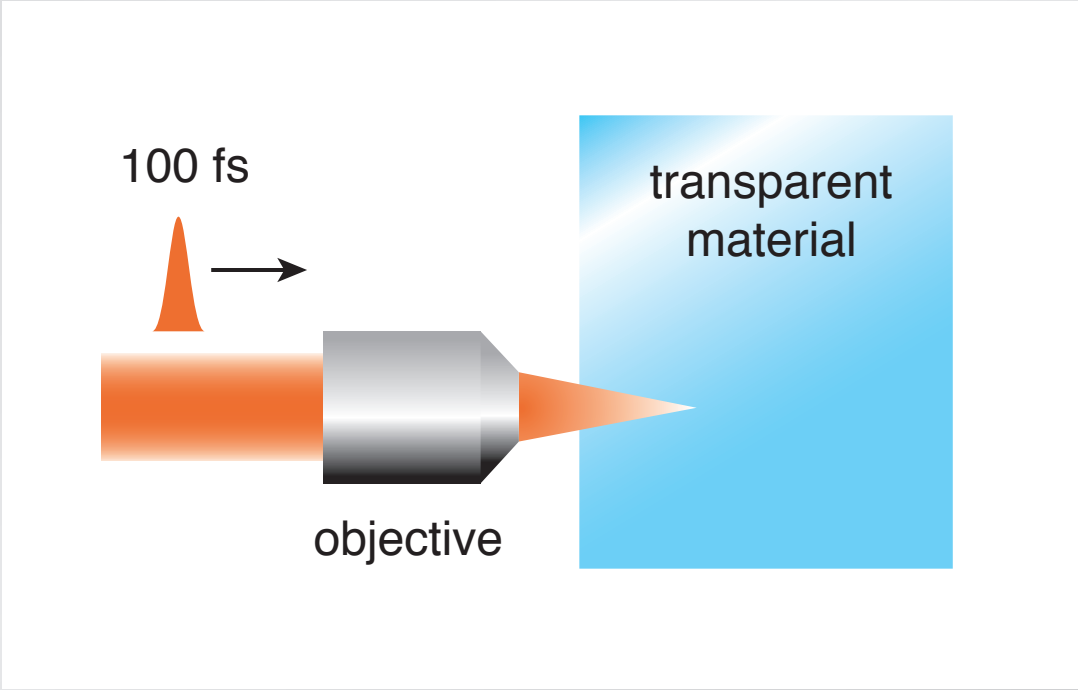
focus laser beam inside material



Opt. Lett. **21**, 2023 (1996)

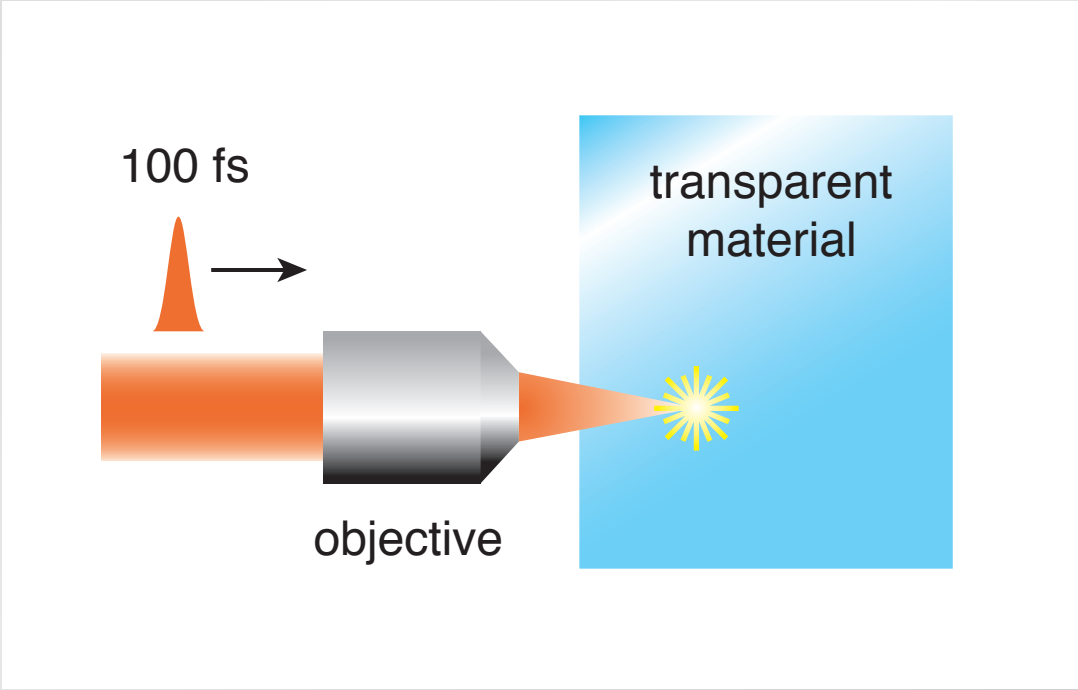
Femtosecond materials interactions

high intensity at focus…



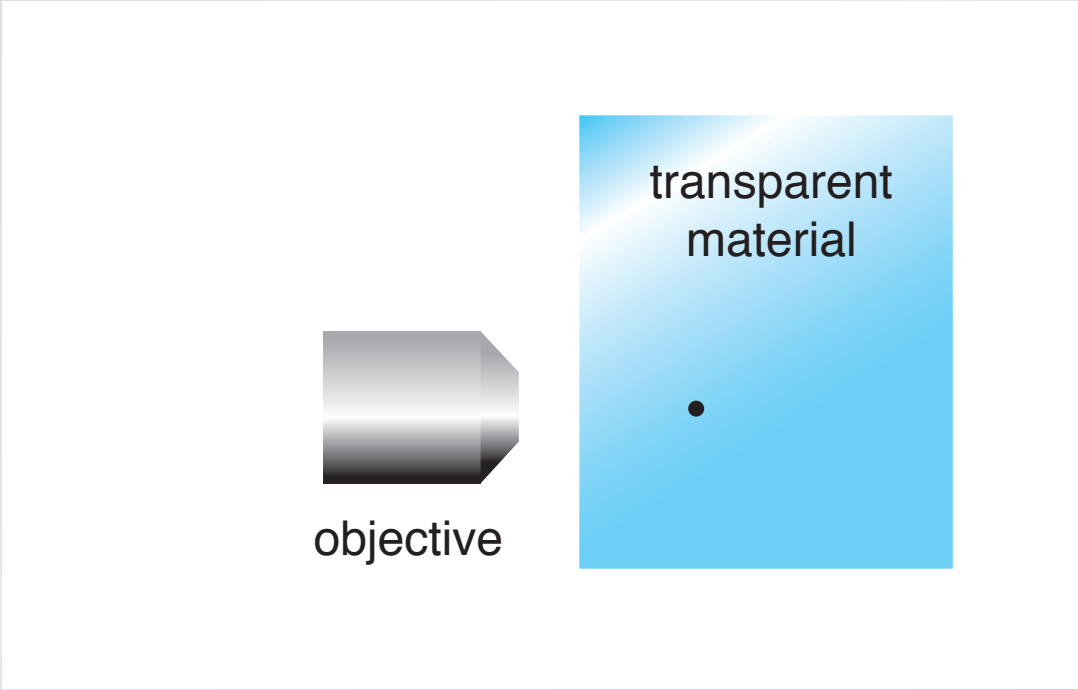
Femtosecond materials interactions

…causes nonlinear ionization…

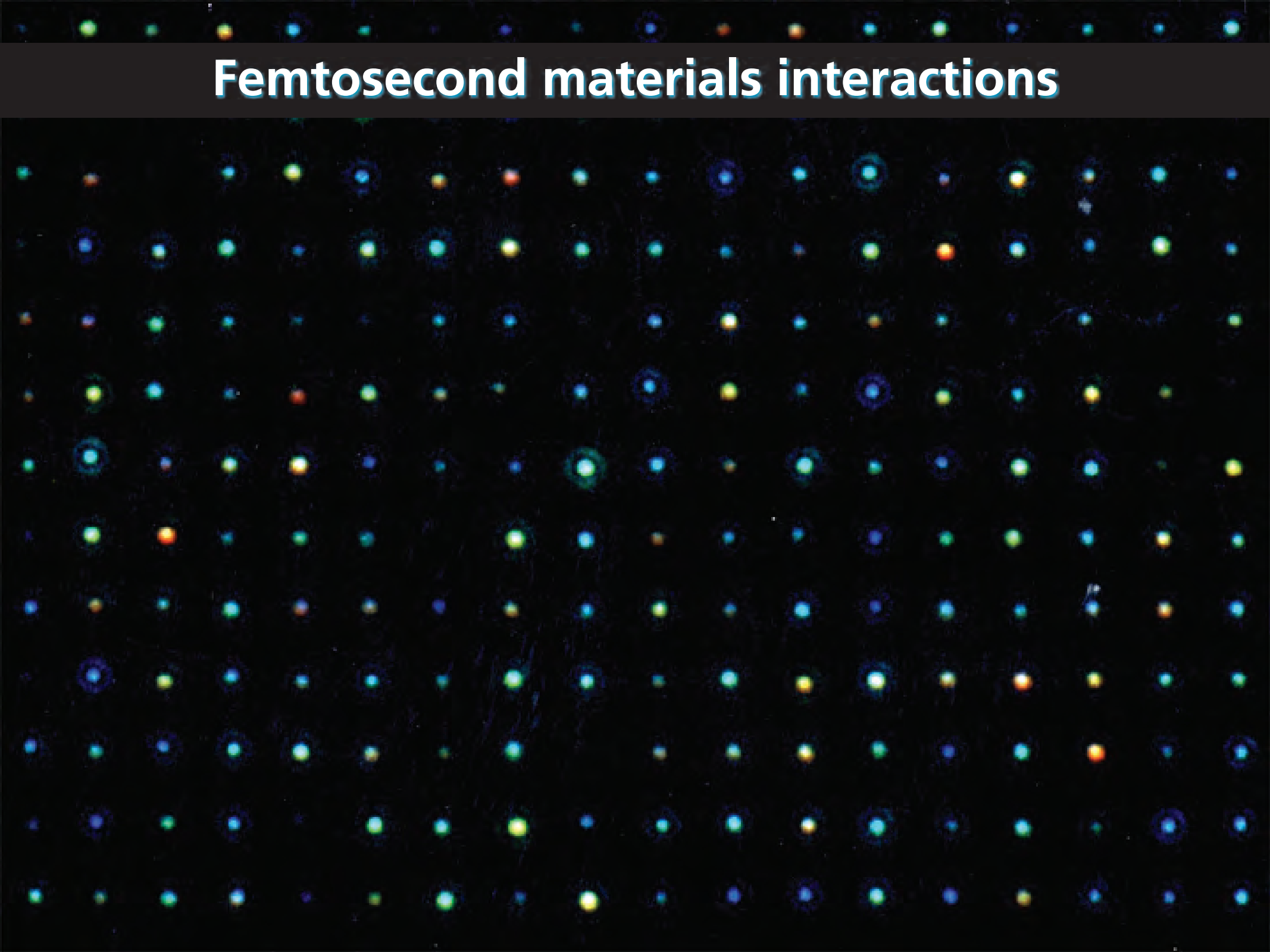


Femtosecond materials interactions

and ‘microexplosion’ causes microscopic damage…



Femtosecond materials interactions

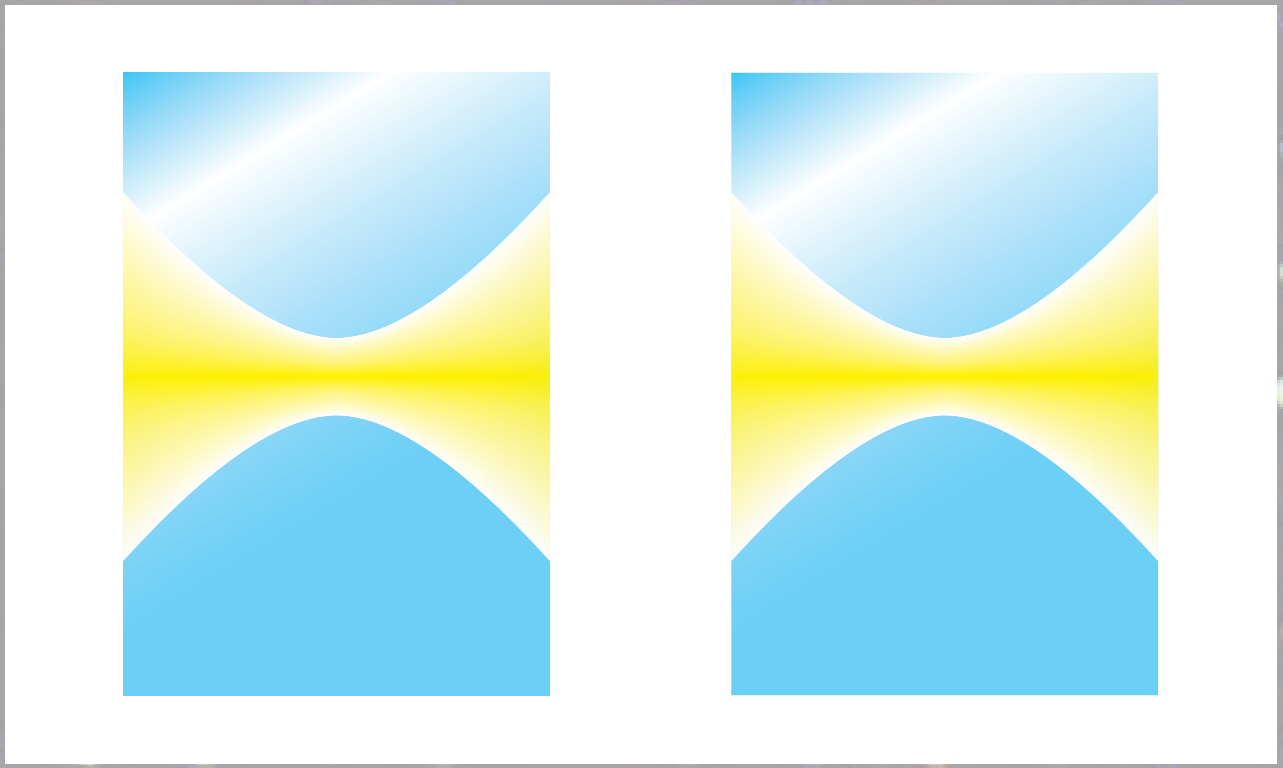


Femtosecond materials interactions

photon energy < bandgap $\longrightarrow$ nonlinear interaction

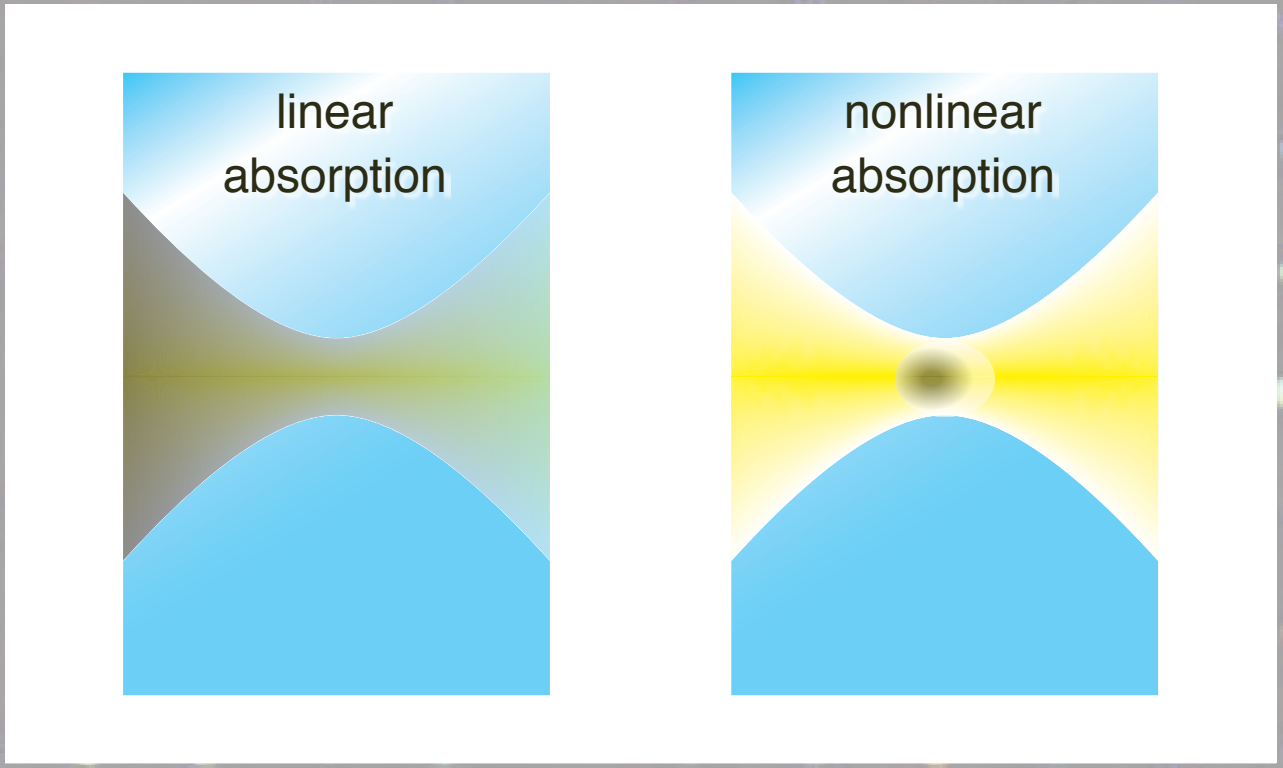
Femtosecond materials interactions

nonlinear interaction provides bulk confinement

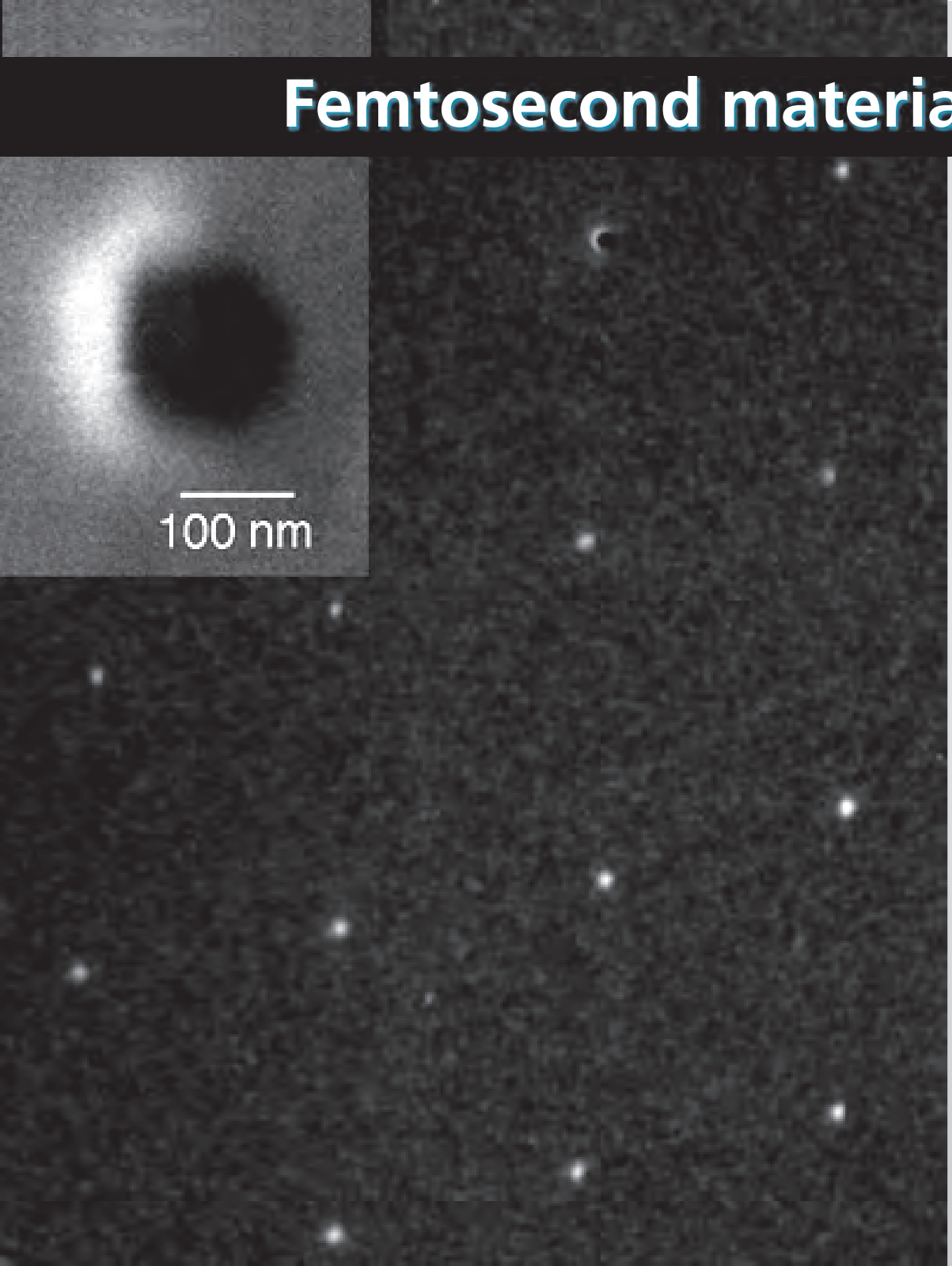


Femtosecond materials interactions

nonlinear interaction provides bulk confinement



Femtosecond materials interactions

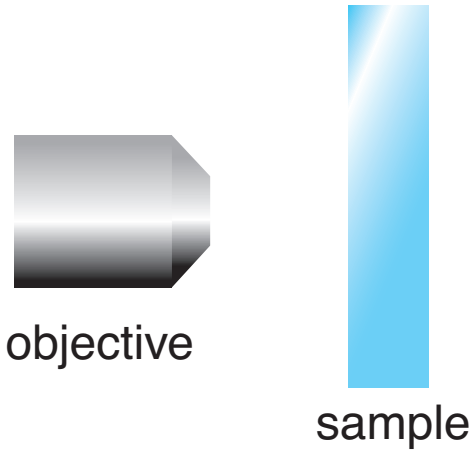


SEM & AFM:

- **100-nm cavities**
- **little colateral damage**

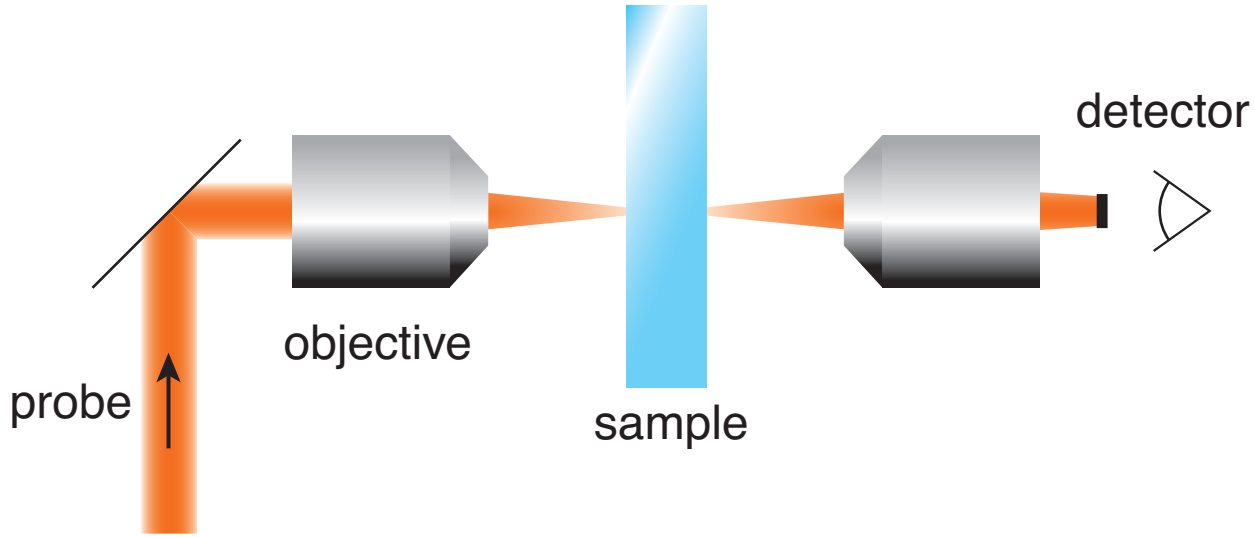
Femtosecond materials interactions

Dark-field scattering



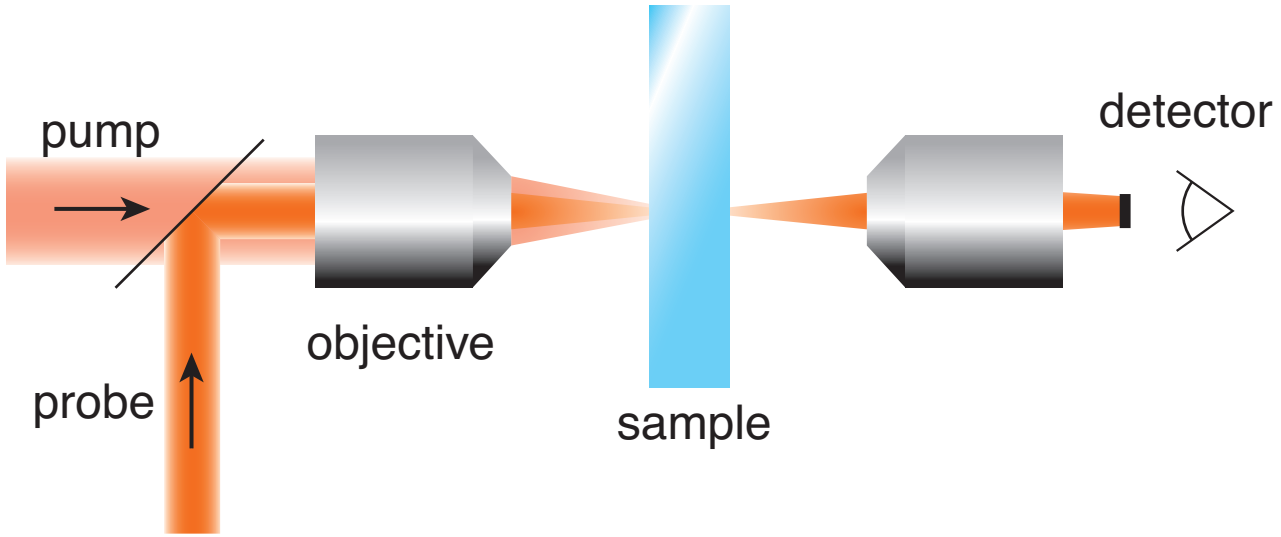
Femtosecond materials interactions

block probe beam…



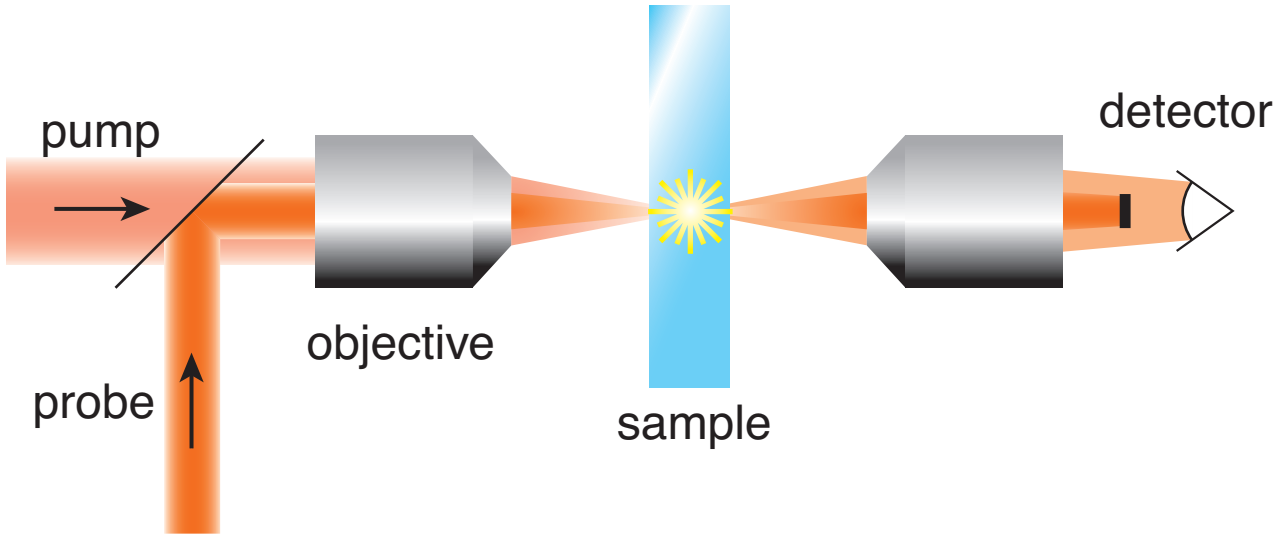
Femtosecond materials interactions

… bring in pump beam…



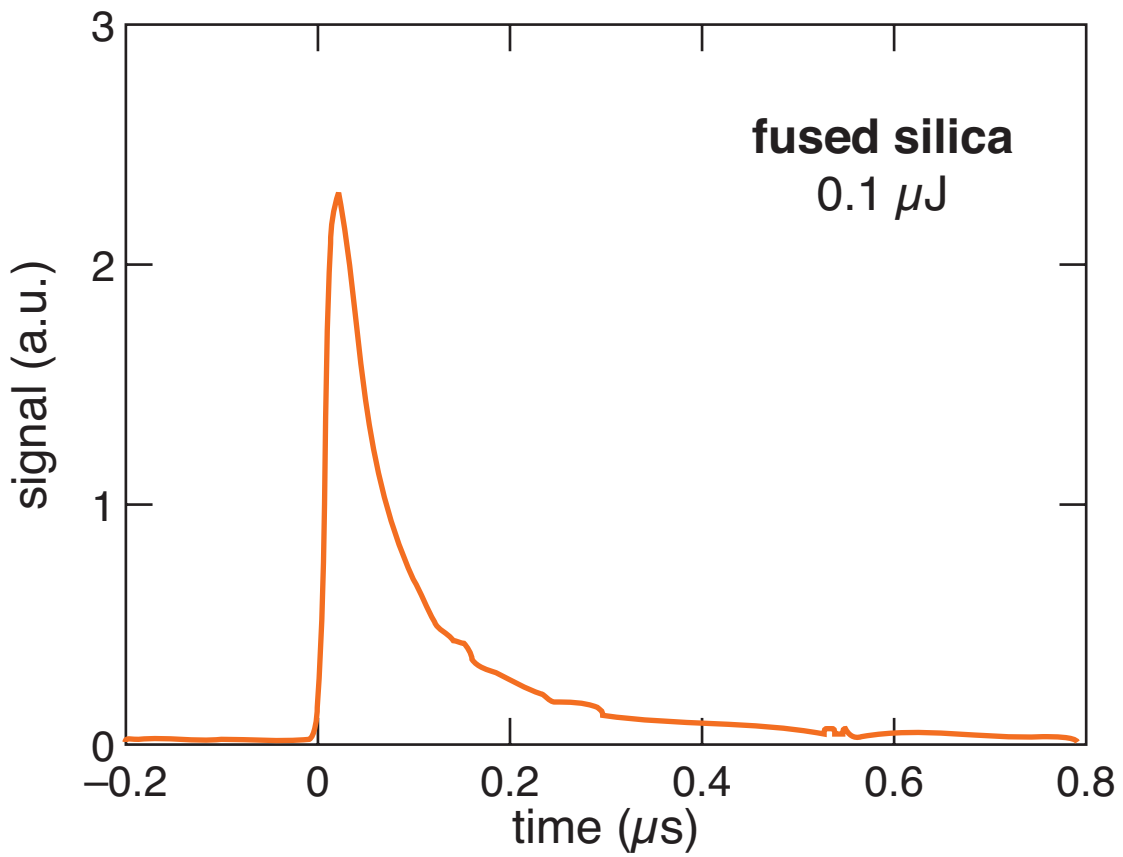
Femtosecond materials interactions

… damage scatters probe beam



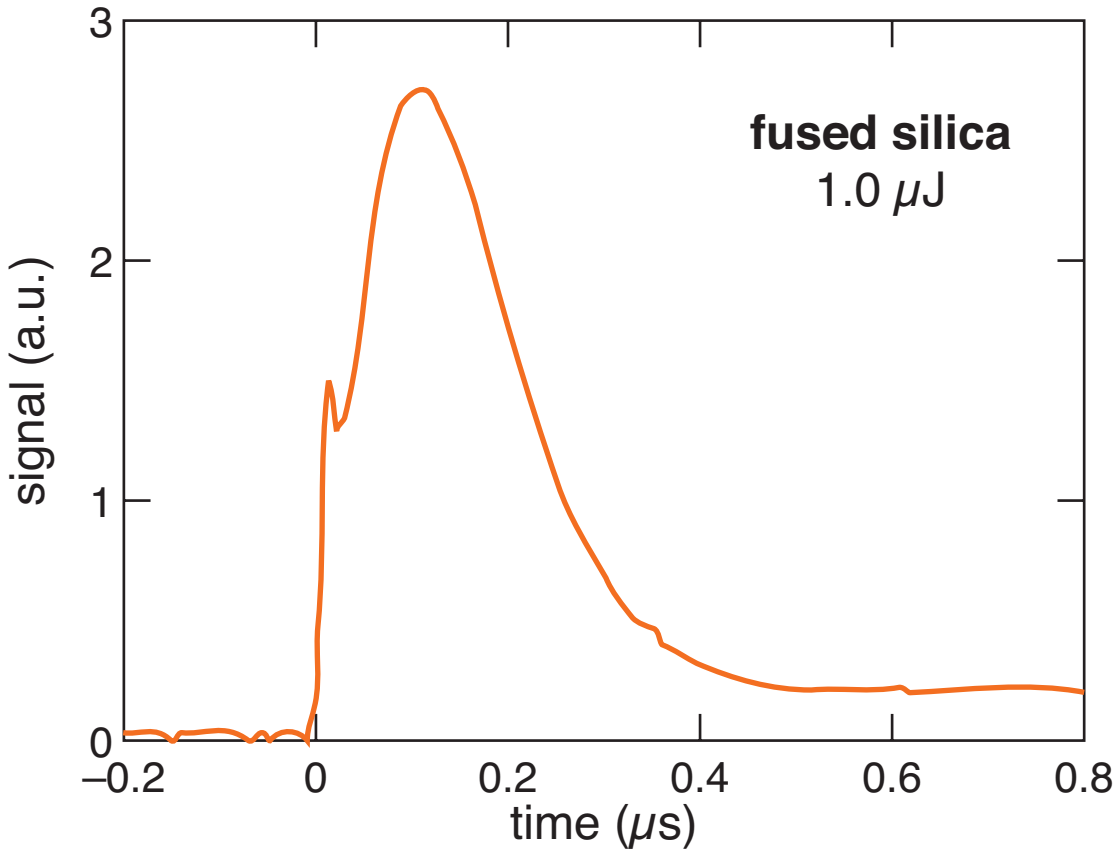
Femtosecond materials interactions

scattered signal



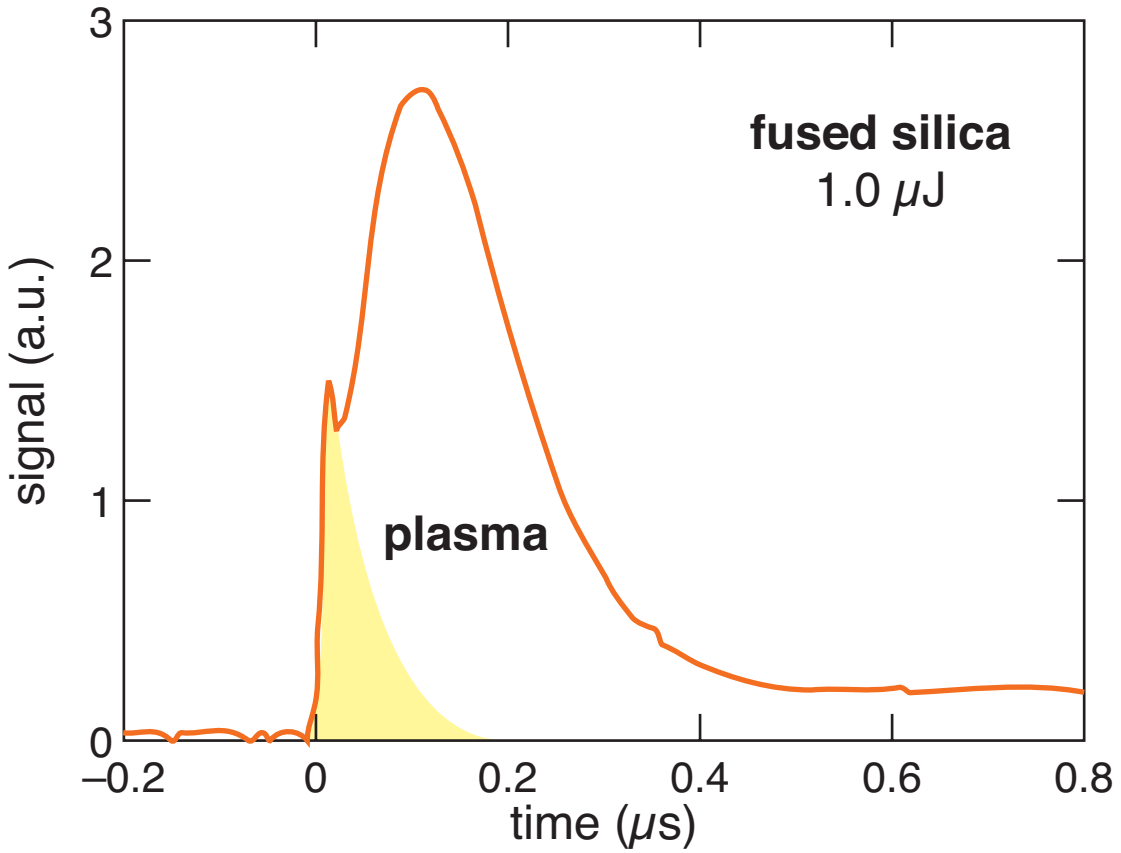
Femtosecond materials interactions

scattered signal



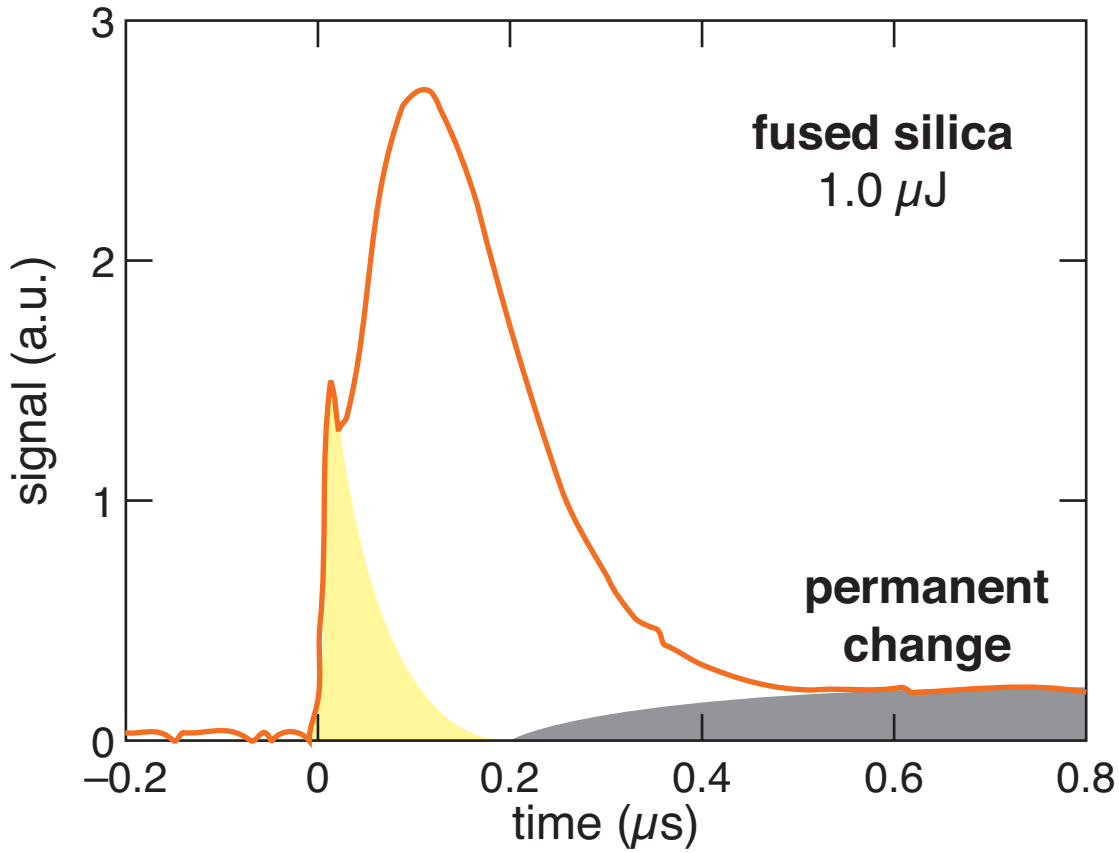
Femtosecond materials interactions

scattered signal



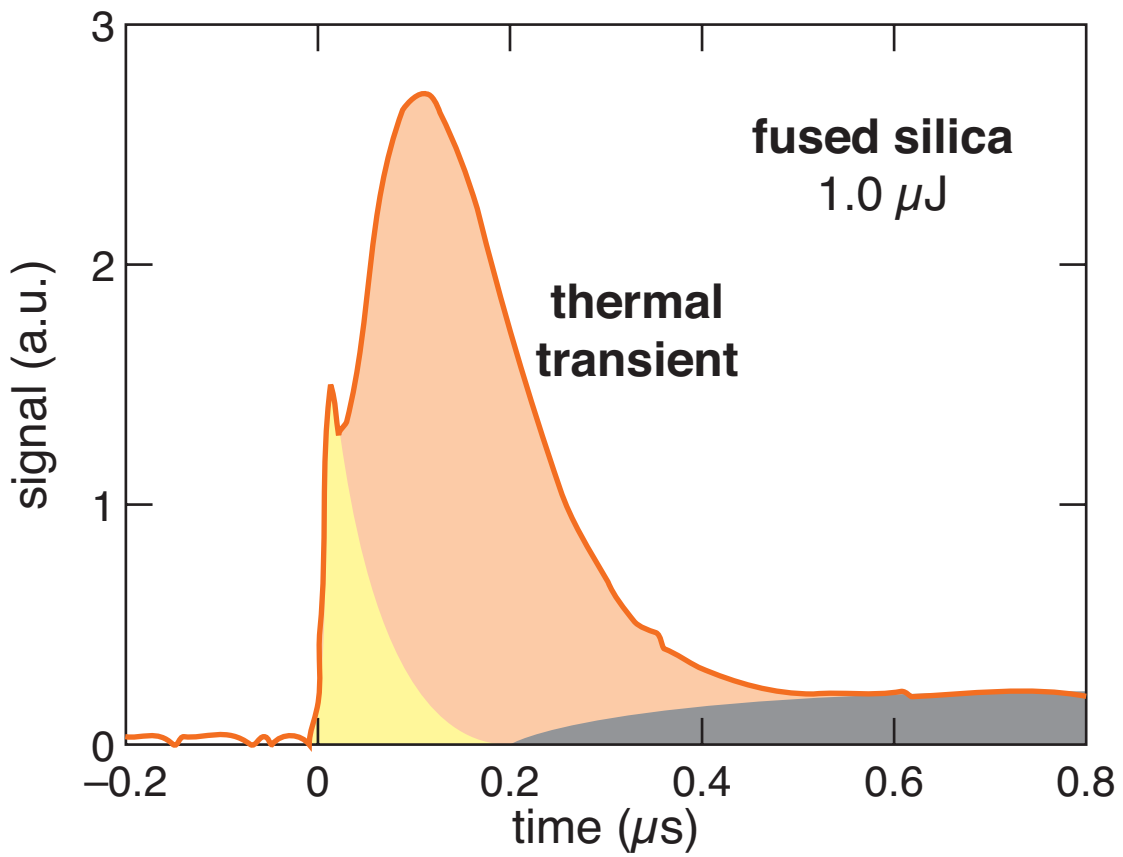
Femtosecond materials interactions

scattered signal



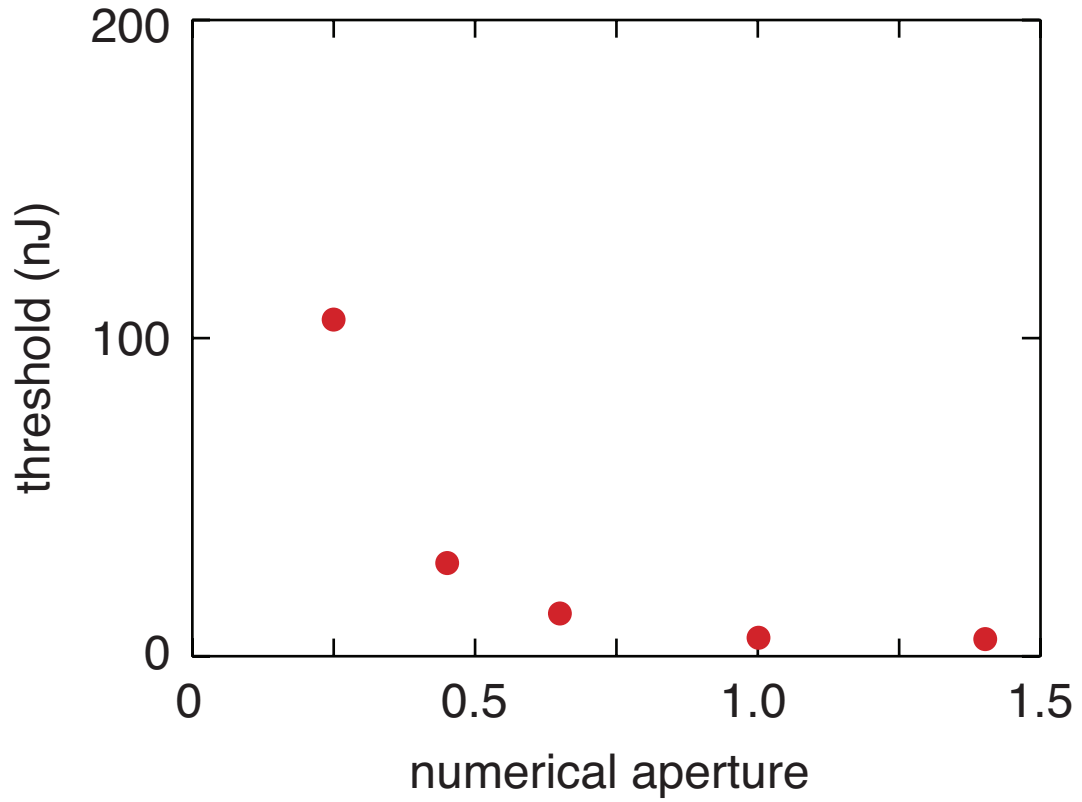
Femtosecond materials interactions

scattered signal



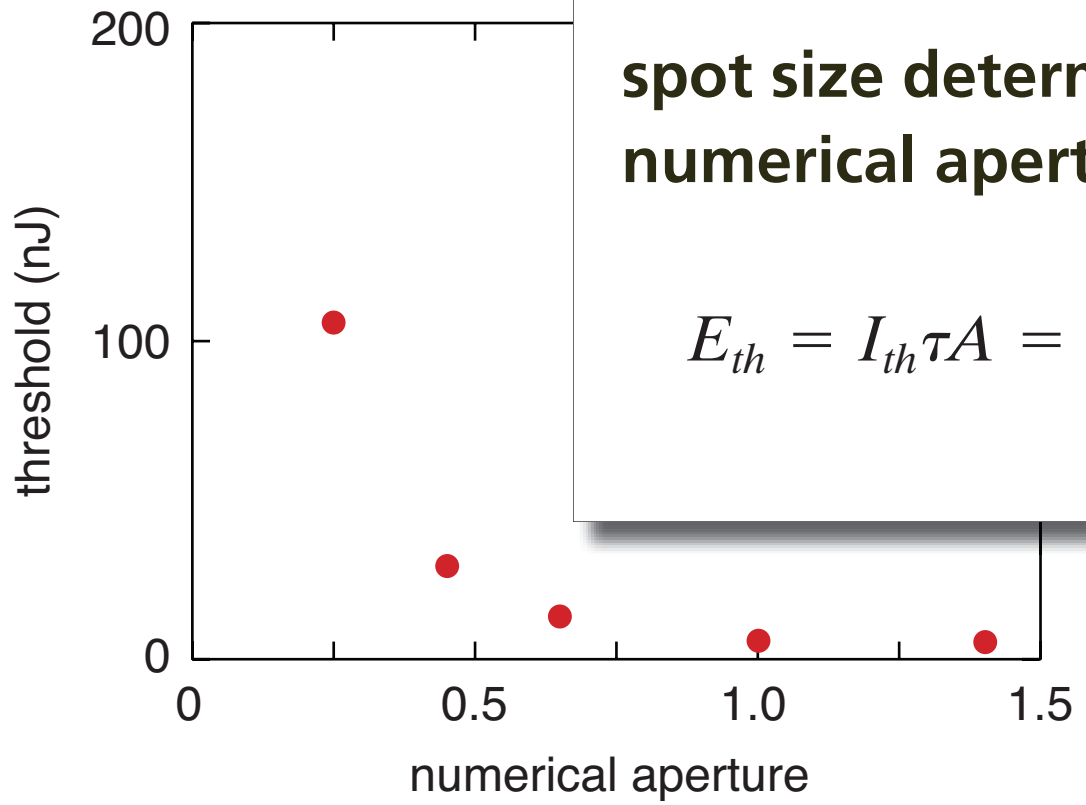
Femtosecond materials interactions

vary numerical aperture



Femtosecond materials interactions

vary numeric



intensity threshold:

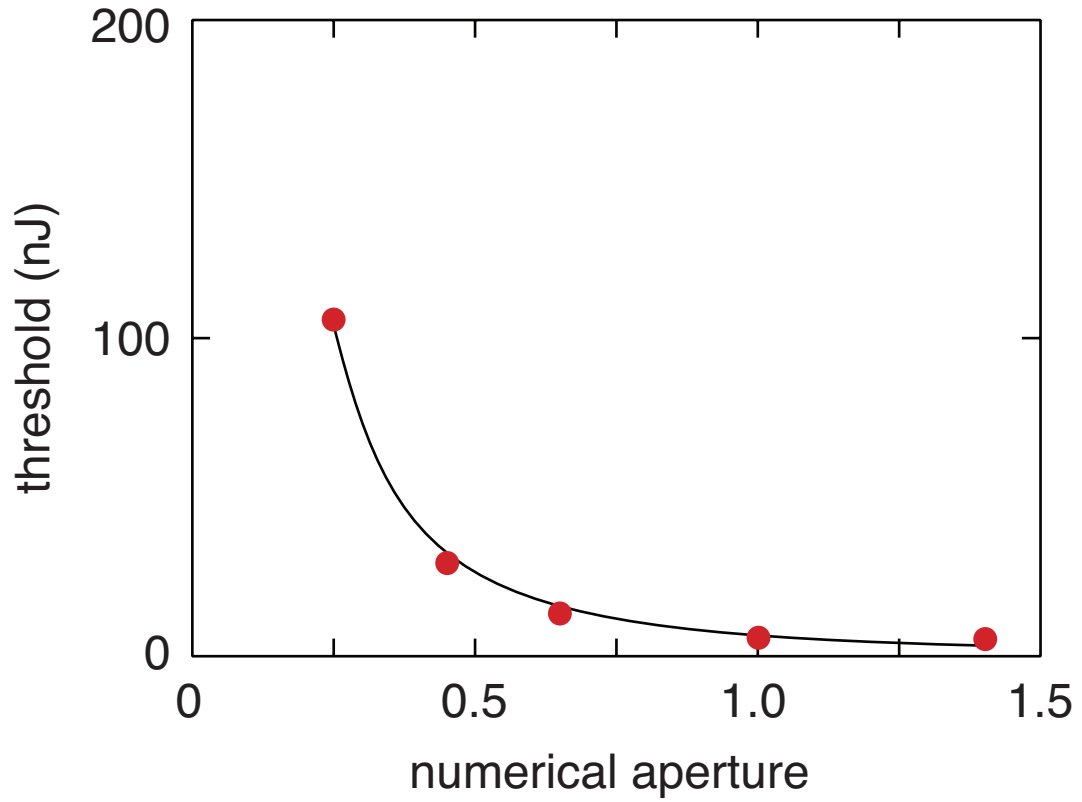
$$E_{th} = I_{th}\tau A$$

spot size determined by numerical aperture:

$$E_{th} = I_{th}\tau A = \frac{I_{th}\tau\lambda^2}{\pi(\mathrm{NA})^2}$$

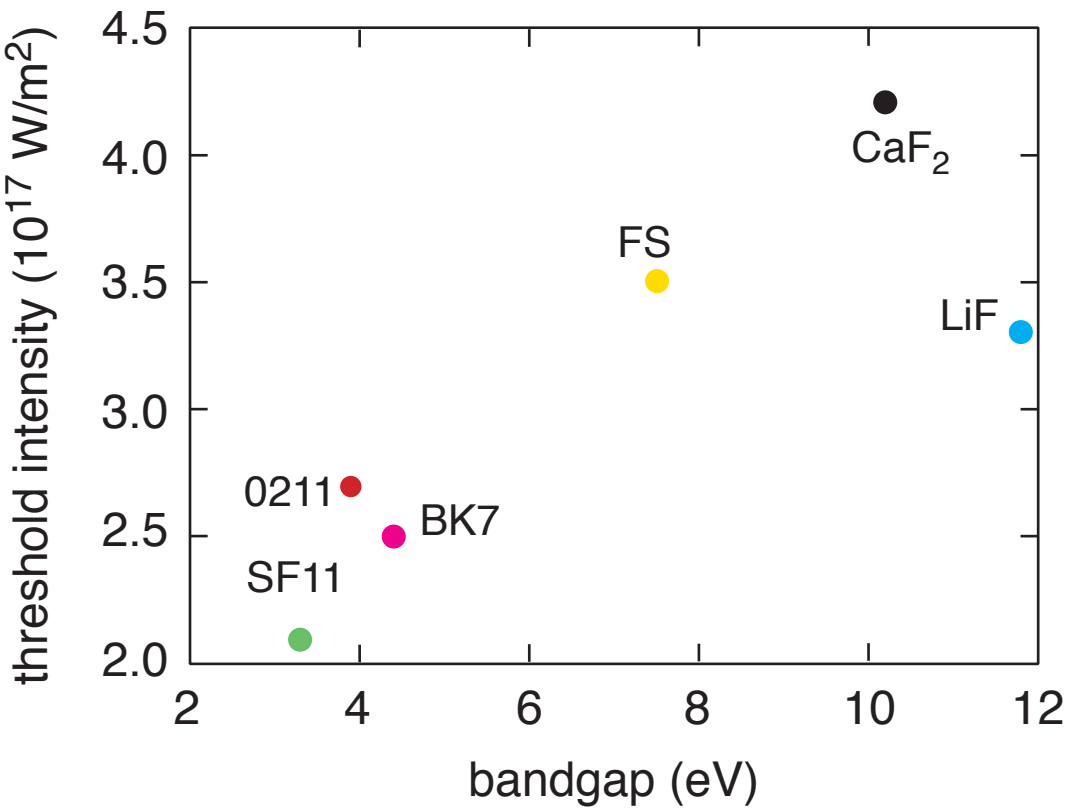
Femtosecond materials interactions

fit gives threshold intensity: I_{th} = 2.5 x 10^{17} W/m^2



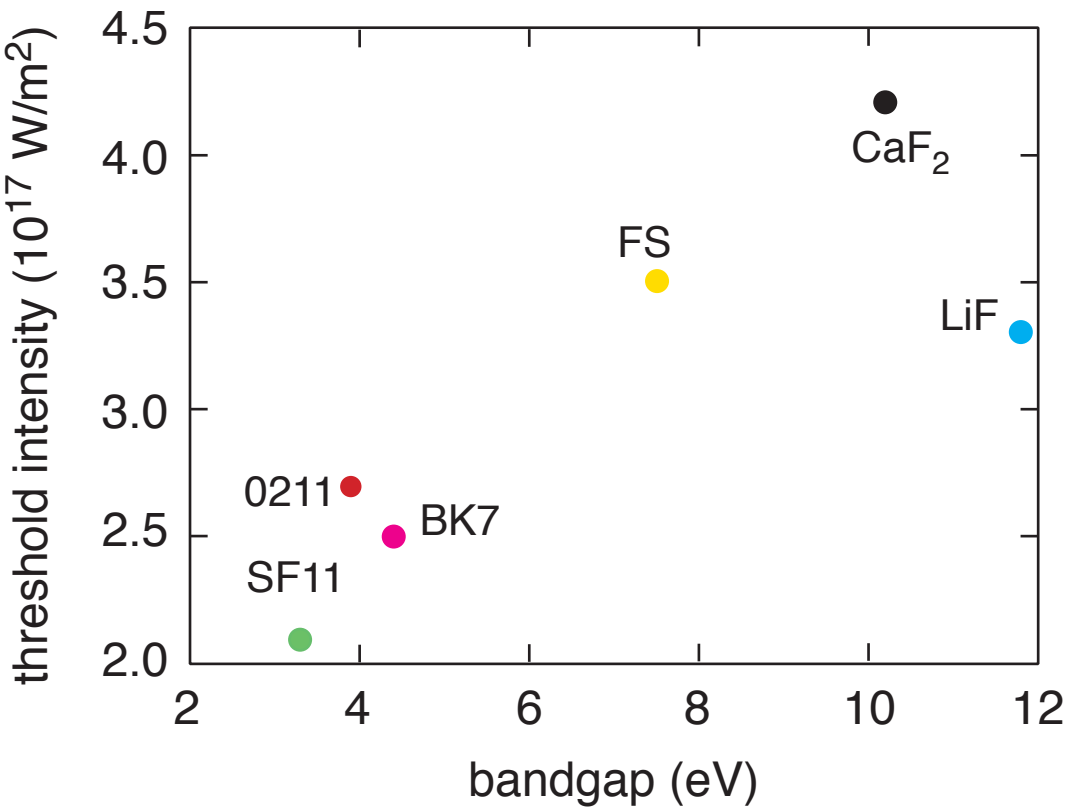
Femtosecond materials interactions

vary material…



Femtosecond materials interactions

…threshold varies with band gap (but not much!)



Femtosecond materials interactions

- nonlinear interaction
- disrupt matter inside bulk
- ablation at very low energy

Outline

- femtosecond materials interactions
- **subcellular surgery**
- nanoneurosurgery

Subcellular surgery

Q: can we ablate material on the subcellular scale?

Subcellular surgery

Requirements:

- submicrometer precision (in bulk)
- no damage to neighboring structures
- independent of structure/organelle type

Subcellular surgery

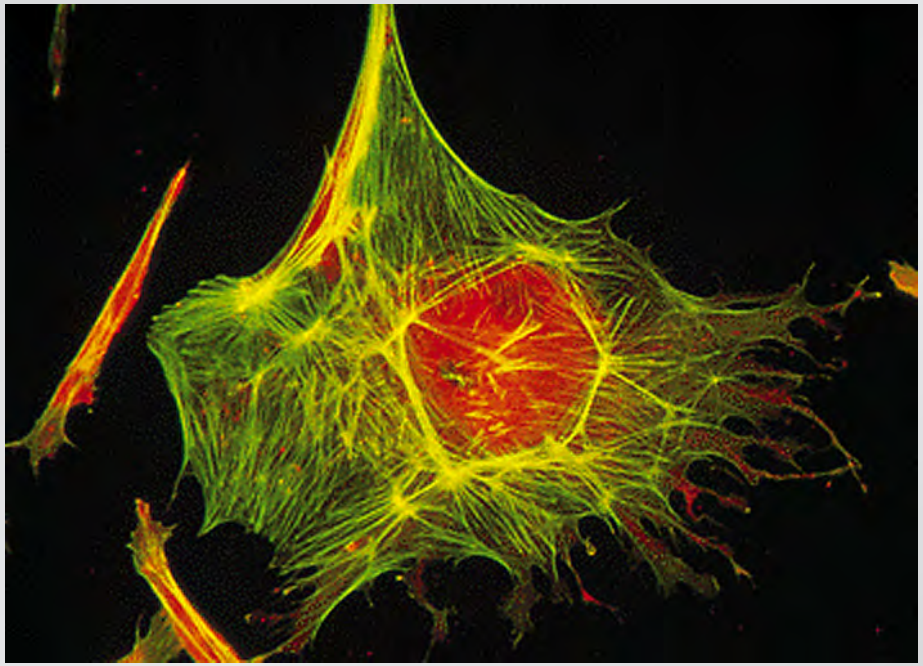
Cytoskeleton

- gives a cell its shape
- provides a scaffold for organelles
- responsible cell motion and attachment
- facilitates intracellular transport and signaling
- required for cell division

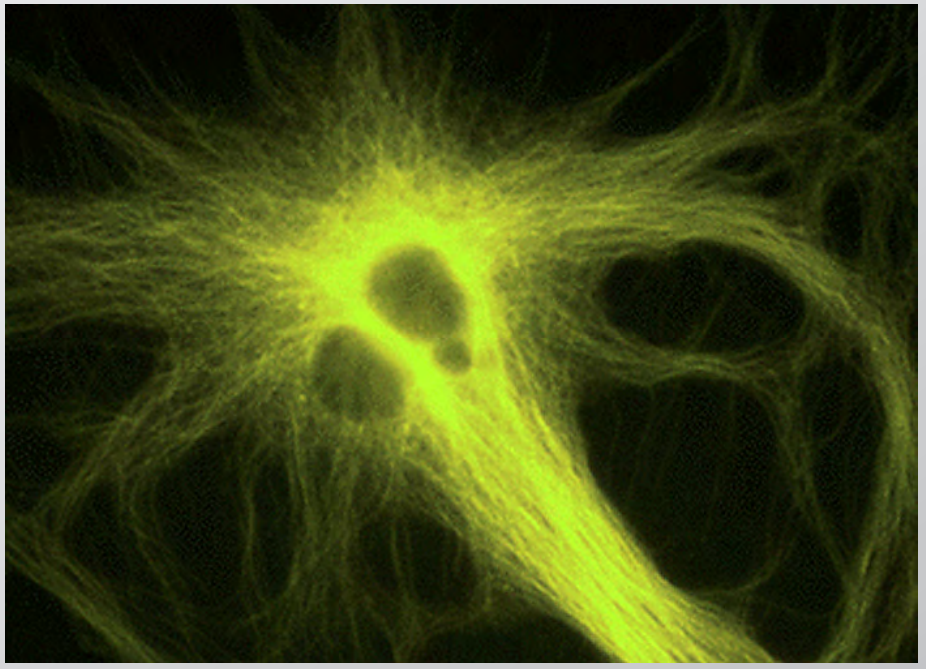
Subcellular surgery

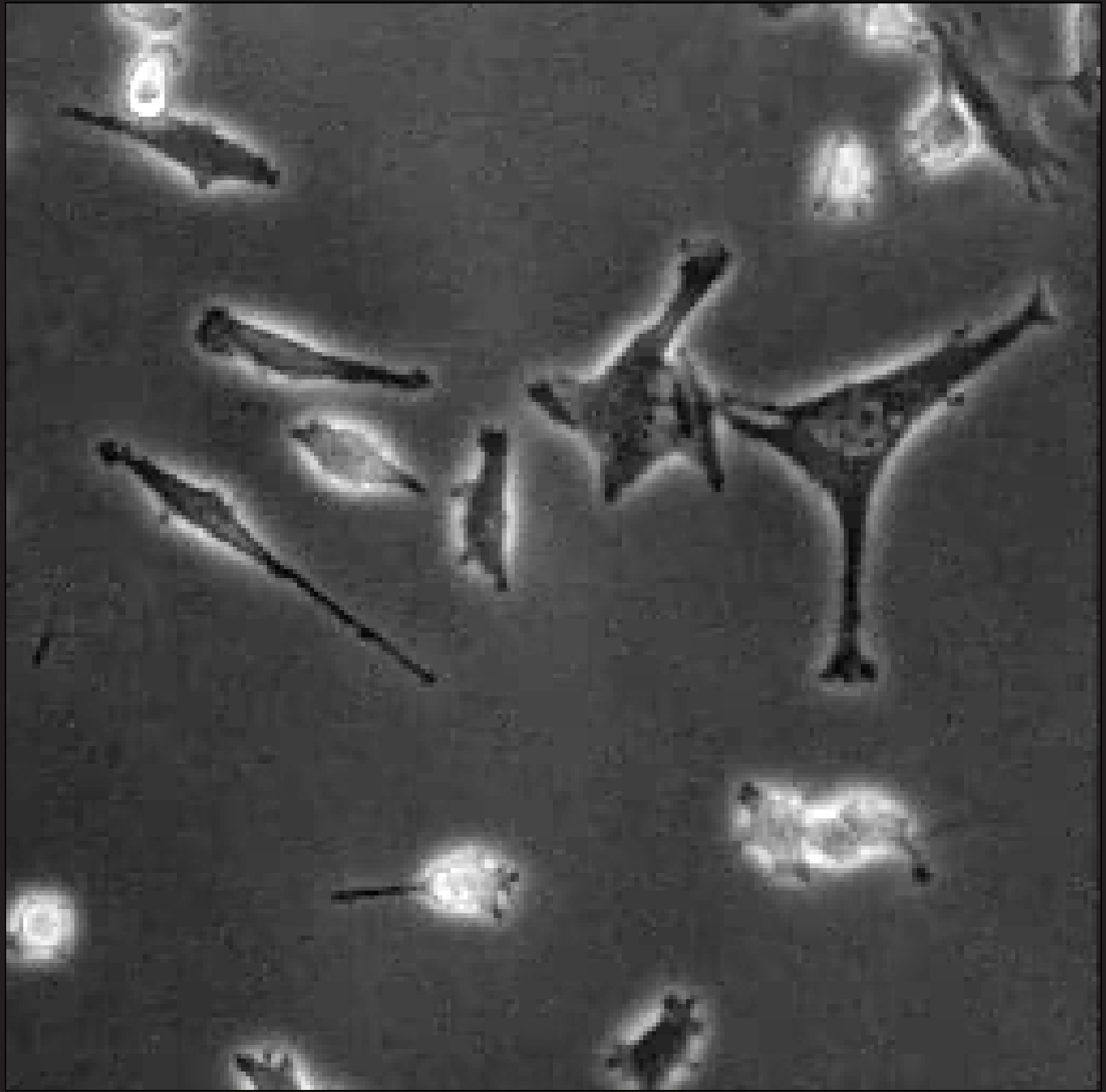
two components

actin fibers

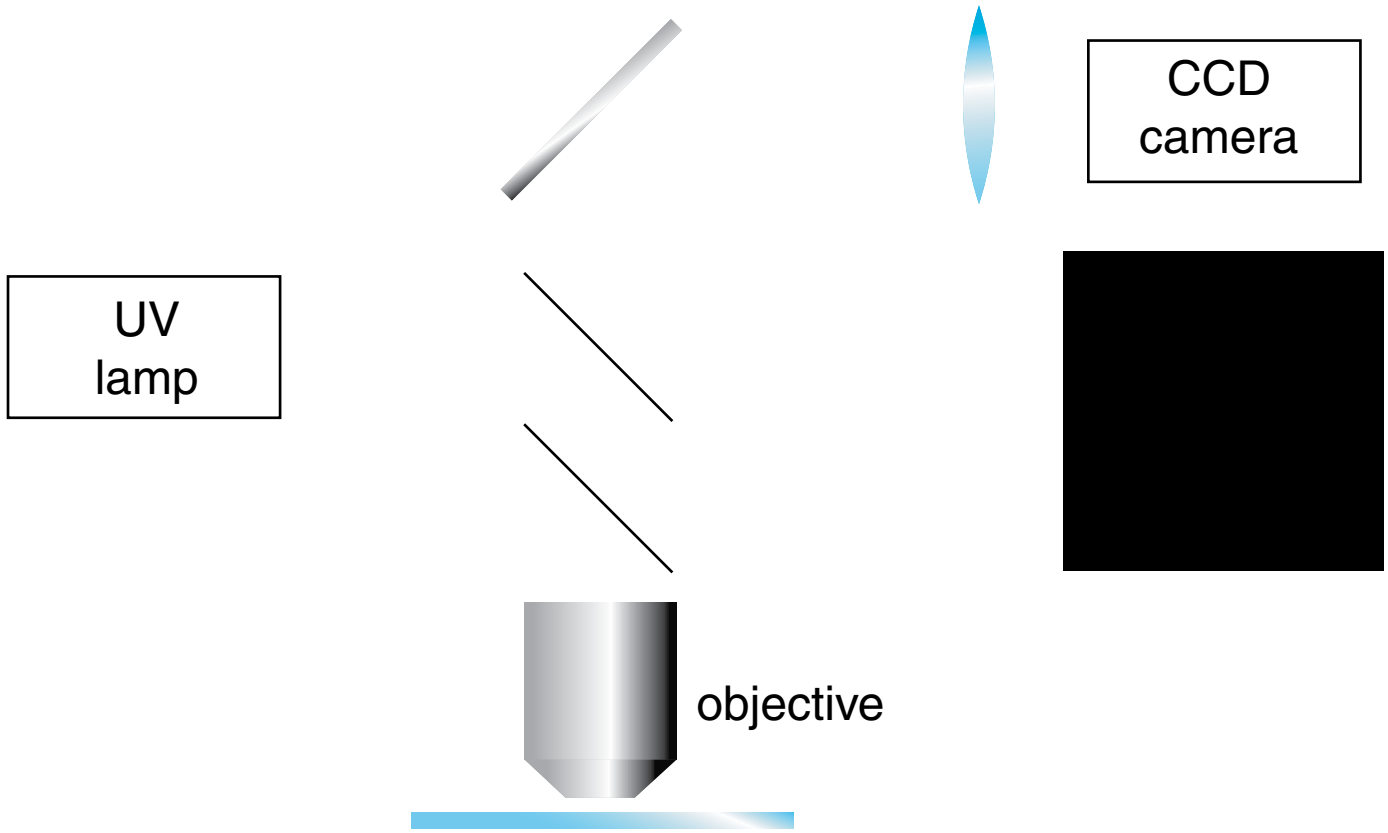


microtubules



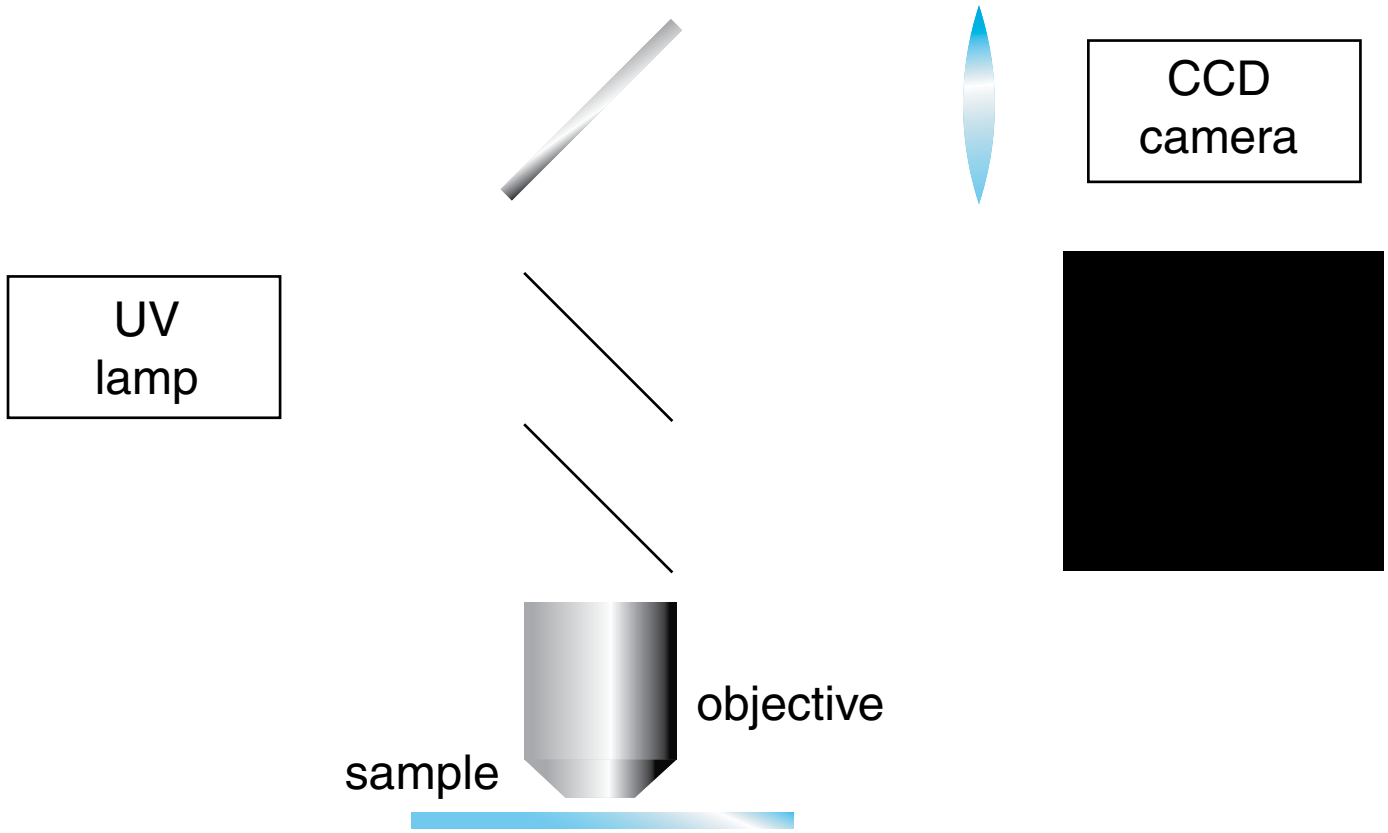


Subcellular surgery



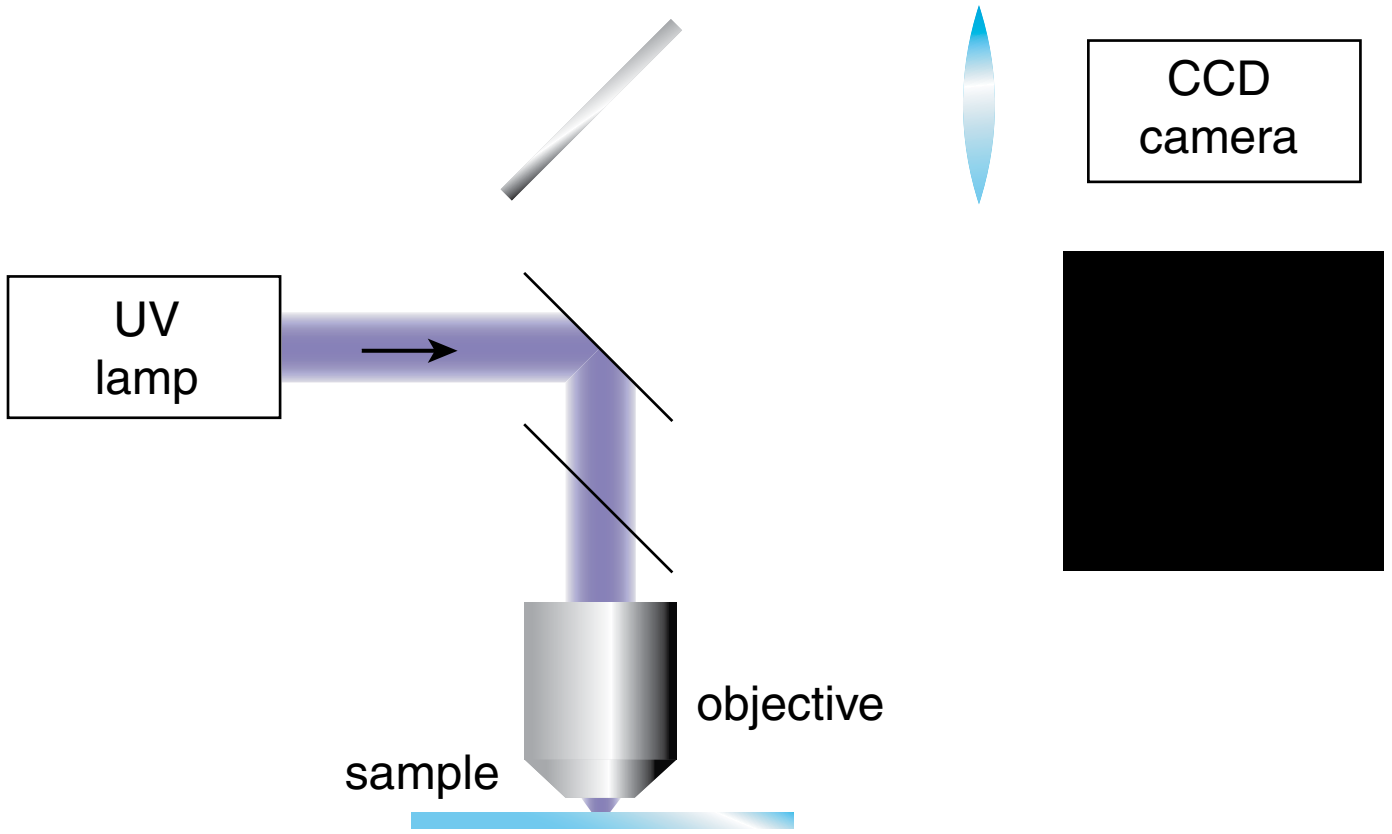
epi-fluorescence microscope

Subcellular surgery



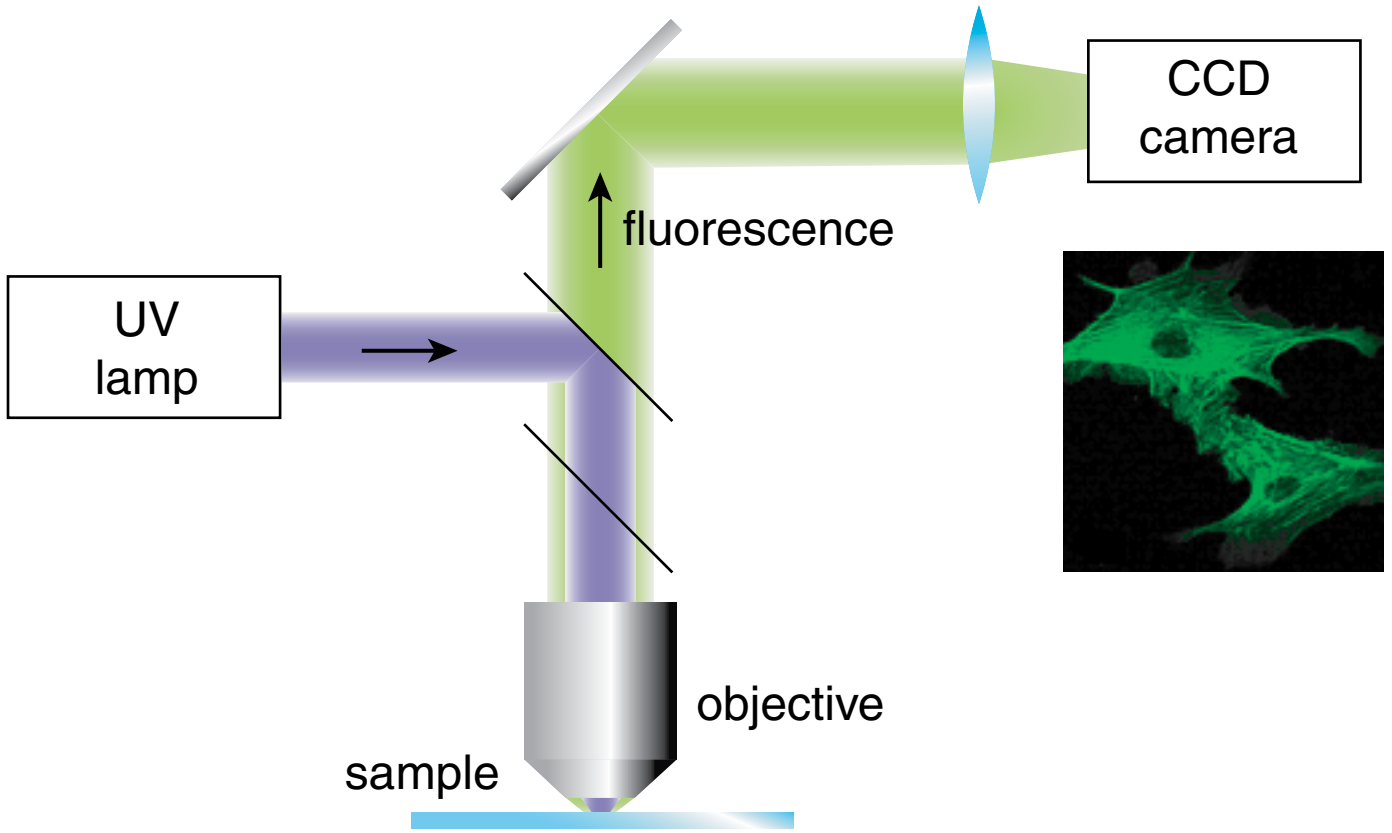
fluorescently label sample

Subcellular surgery



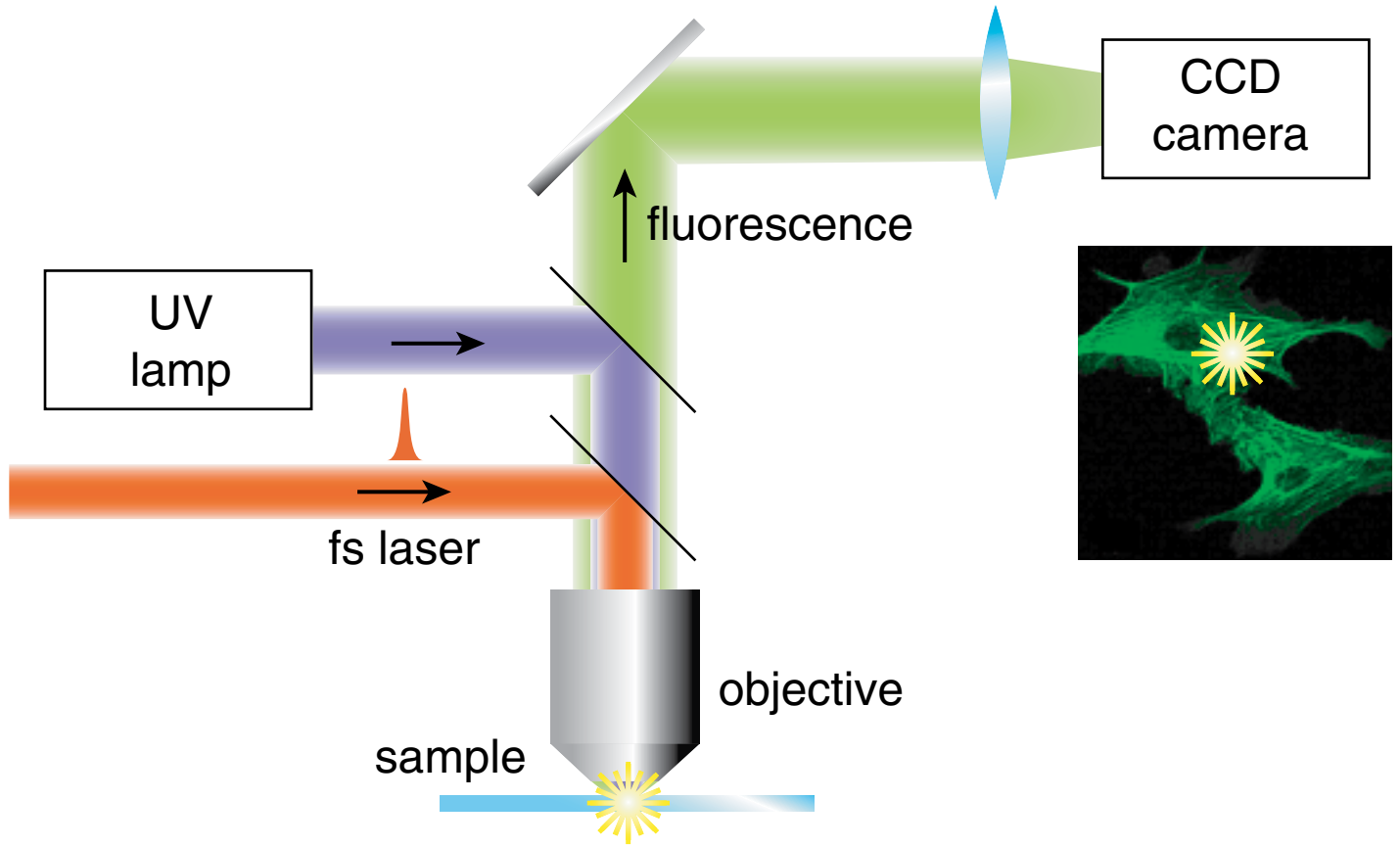
UV illumination…

Subcellular surgery



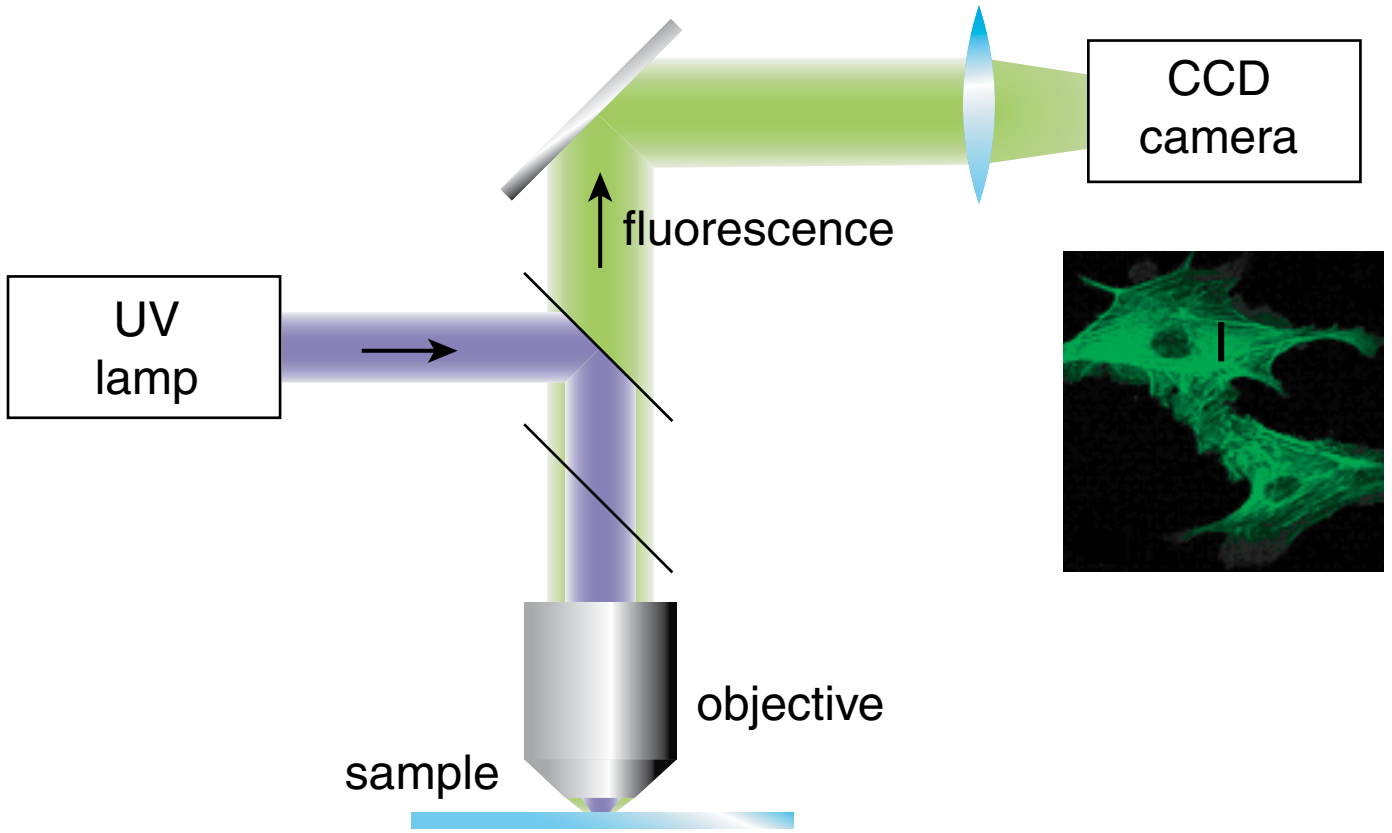
…causes fluorescence

Subcellular surgery



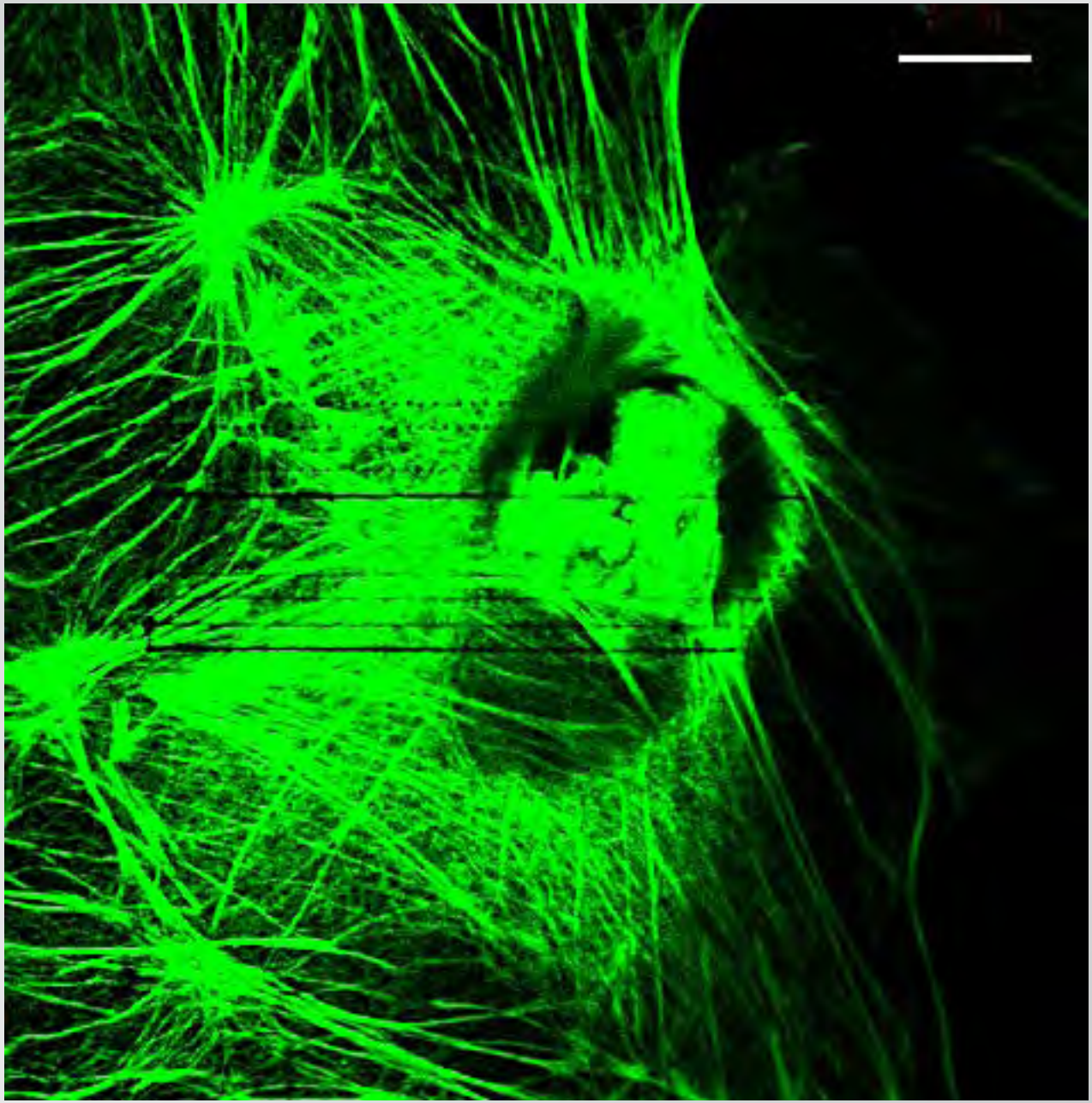
irradiate with fs laser beam

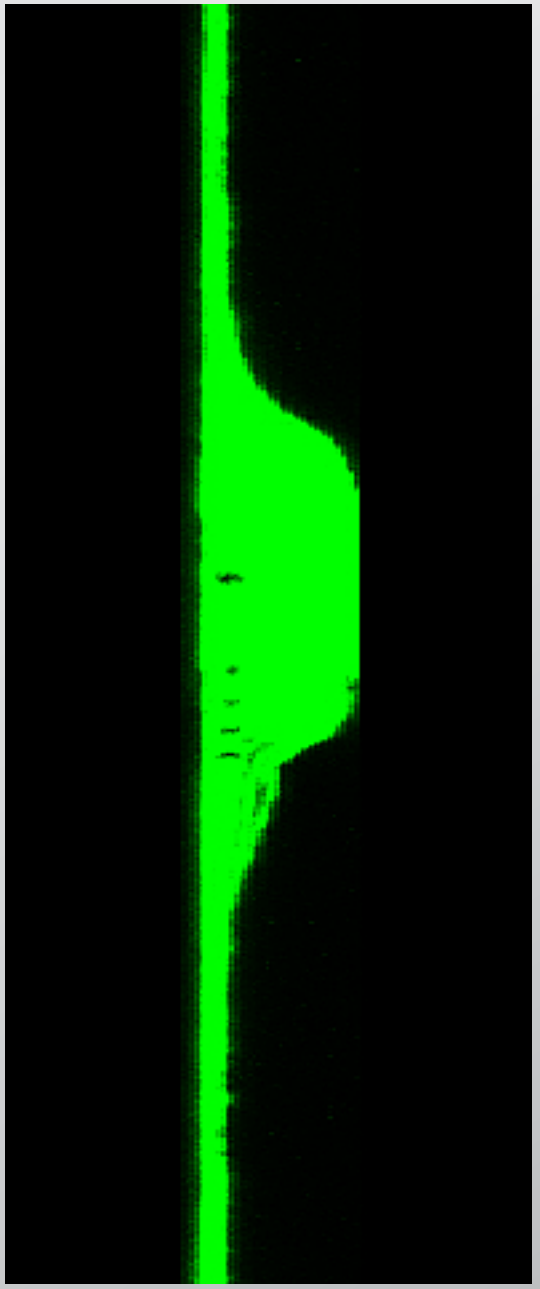
Subcellular surgery



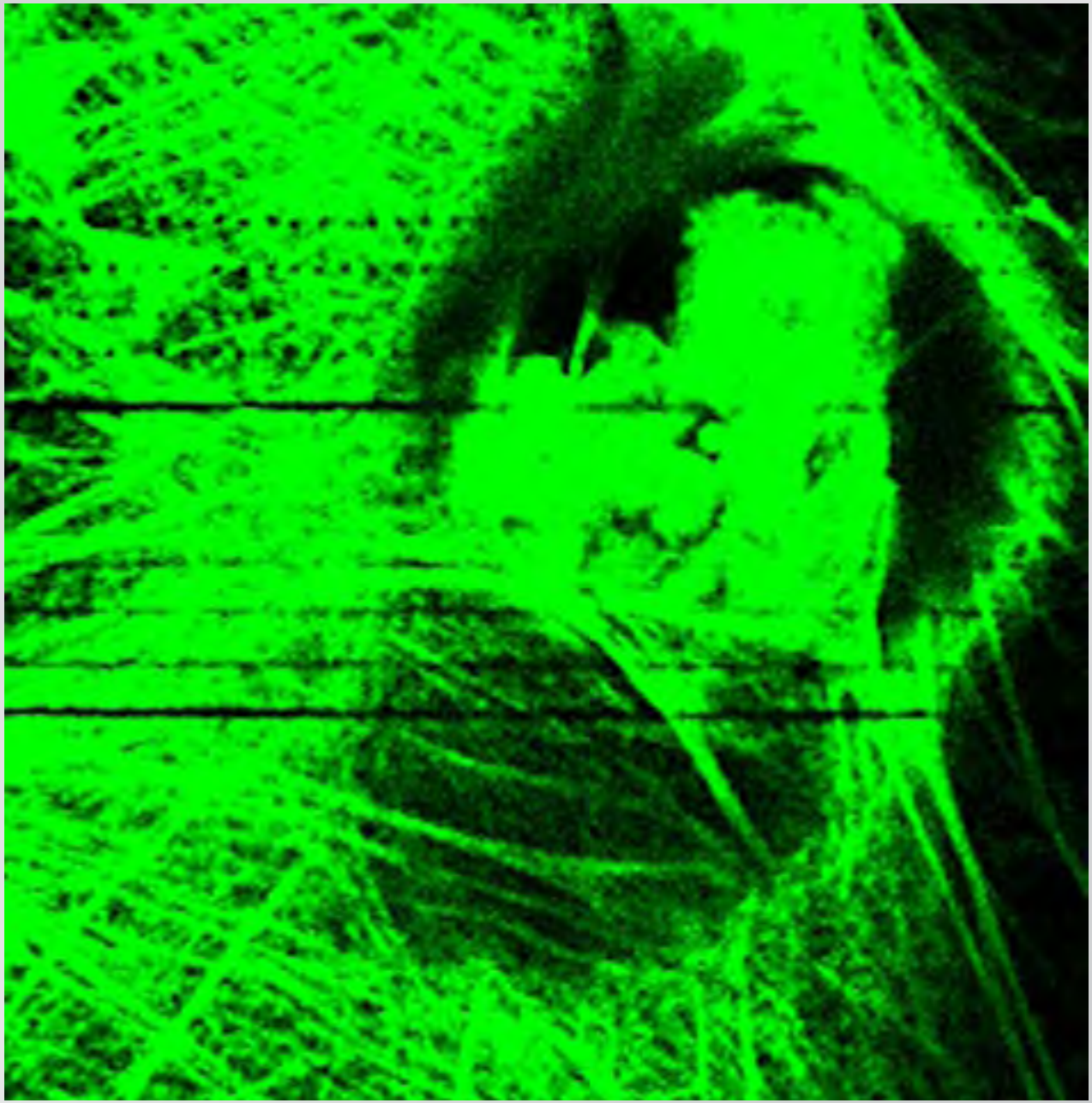
examine resulting ablation

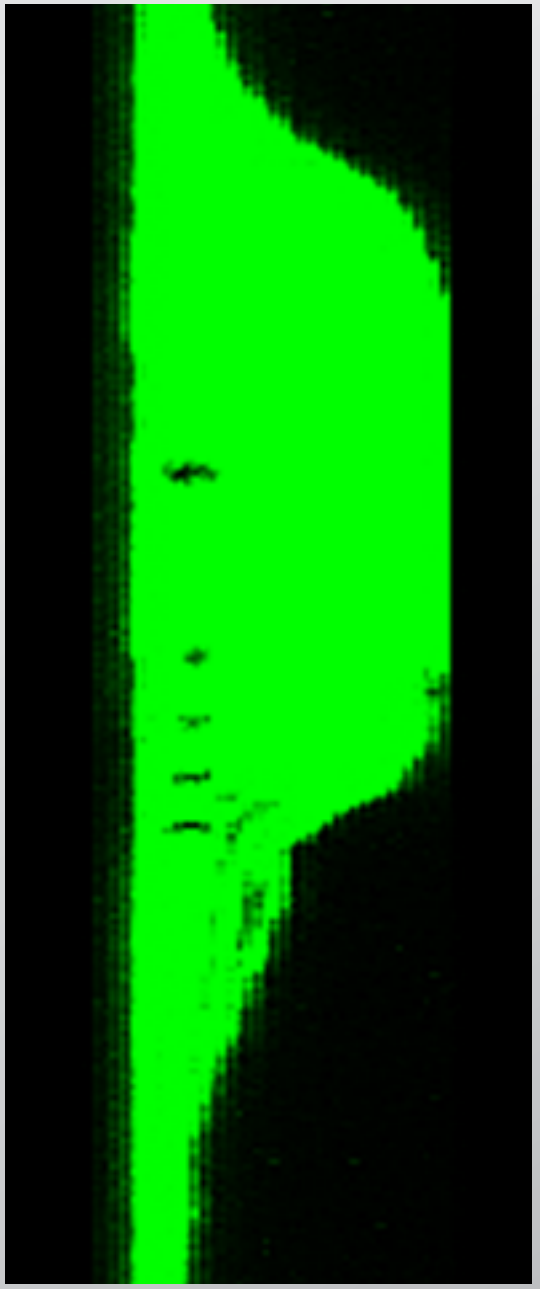
Subcellular surgery





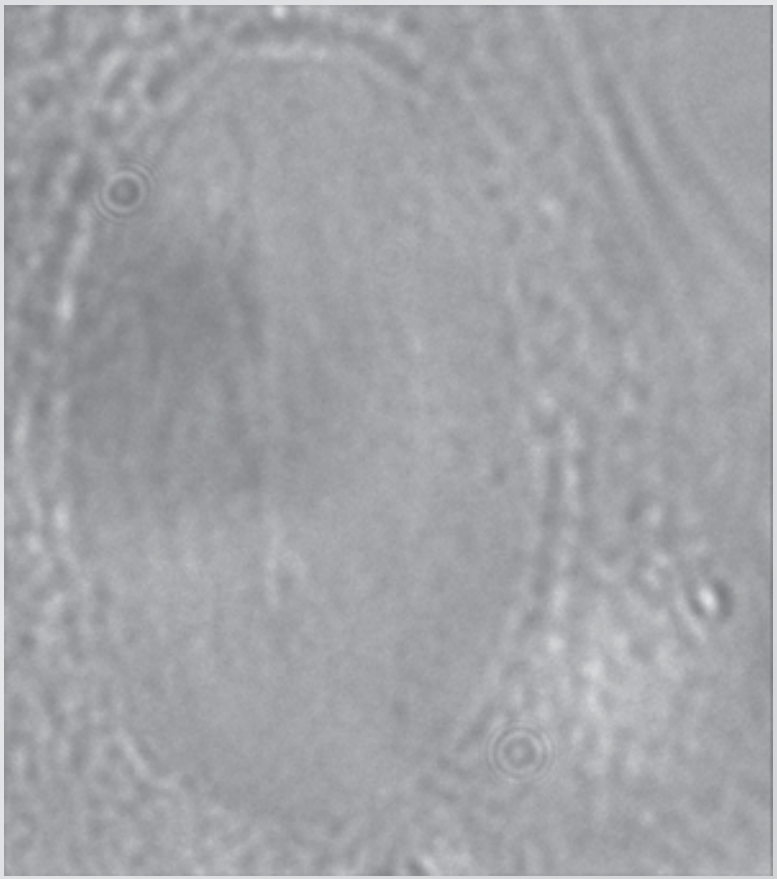
Subcellular surgery





Subcellular surgery

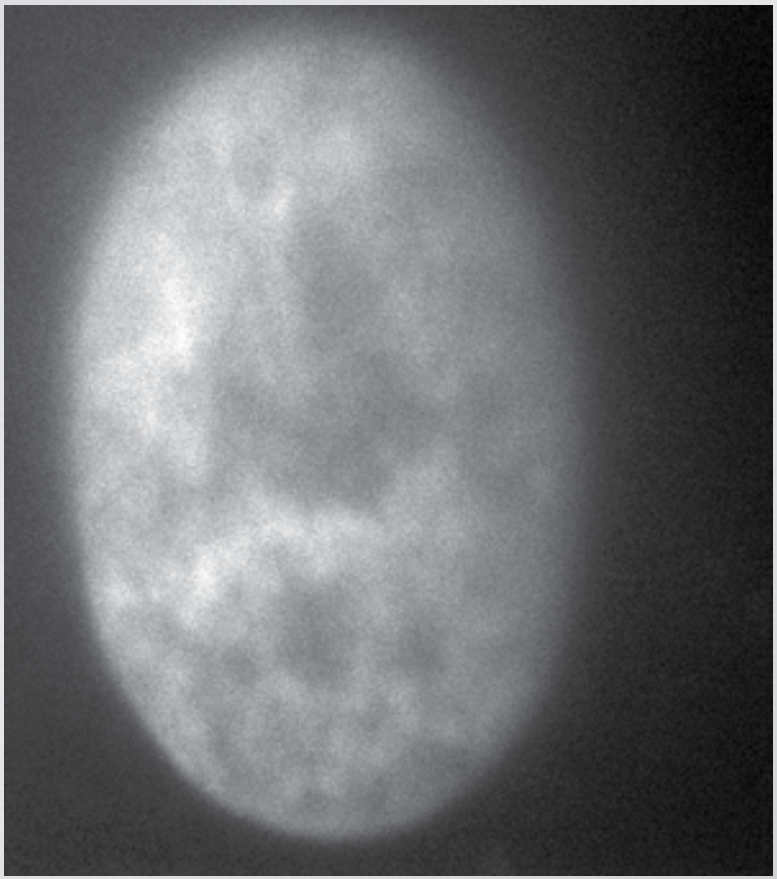
nucleus of fixed endothelial cell



white light microscopy

Subcellular surgery

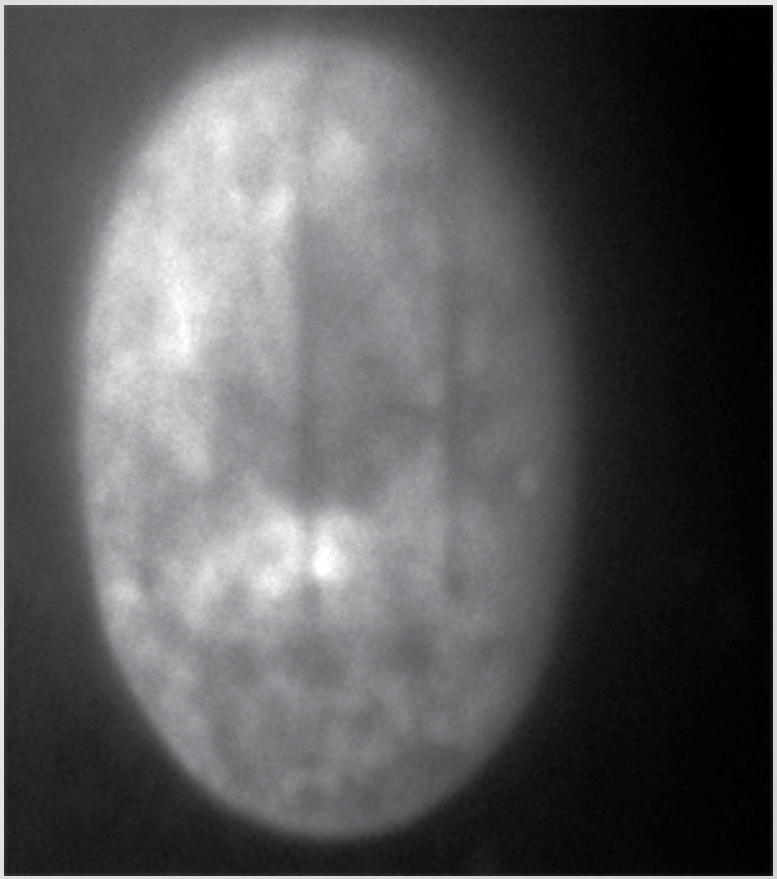
nucleus of fixed endothelial cell



fluorescence microscopy

Subcellular surgery

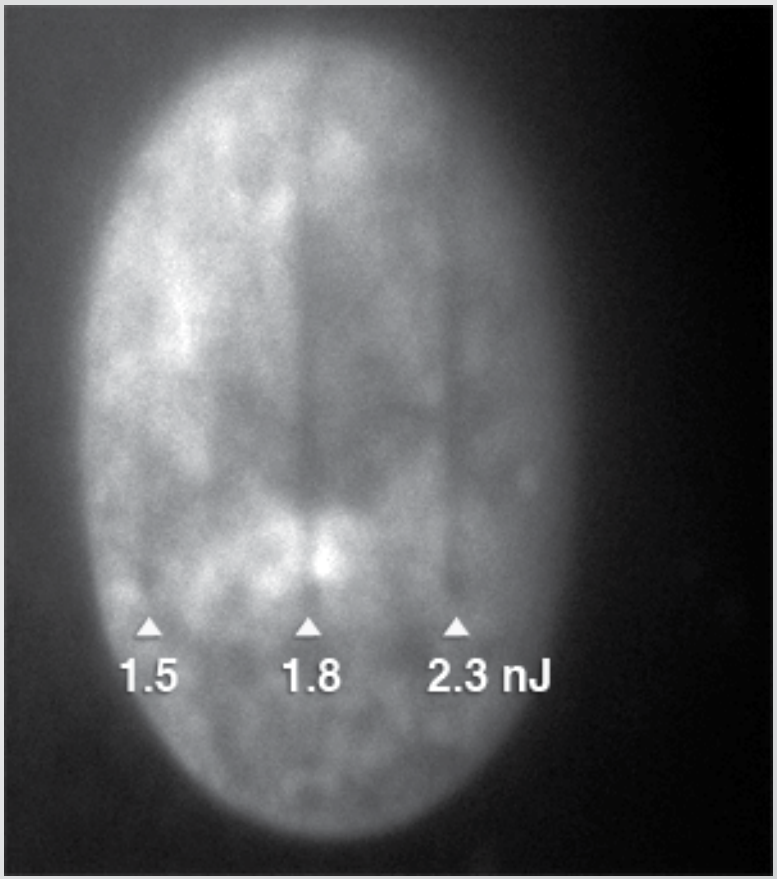
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

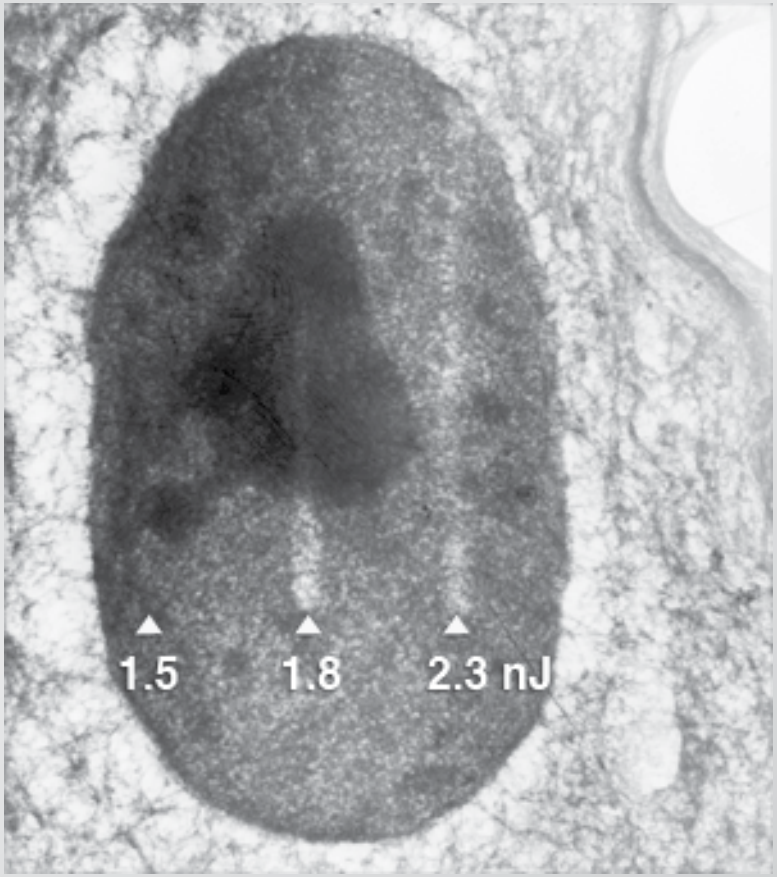
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

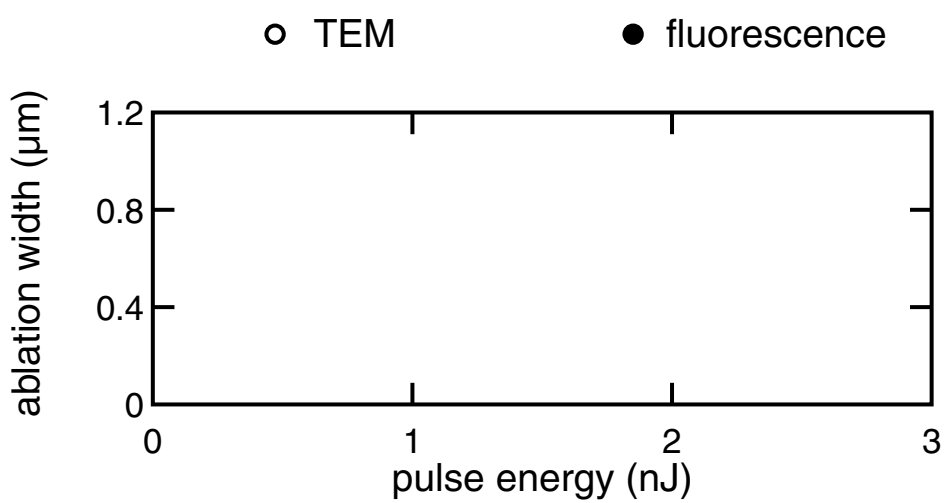
bleaching or ablation?



TEM image

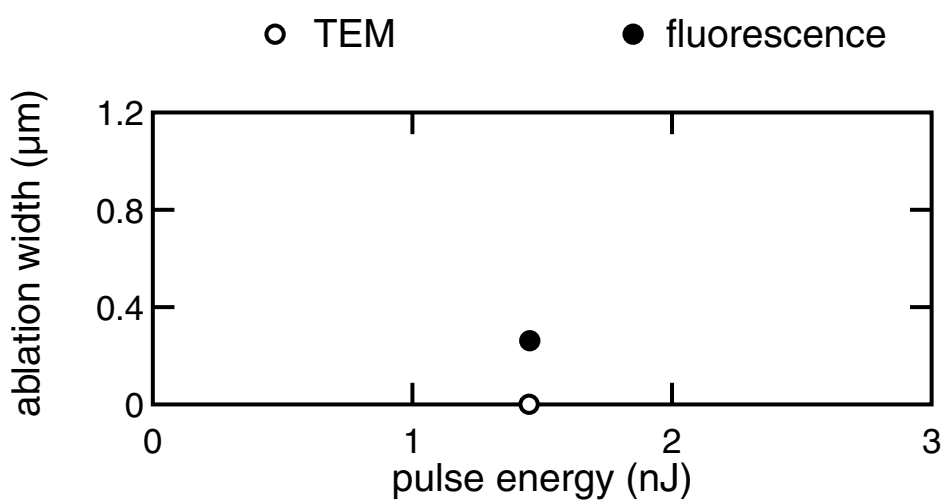
Subcellular surgery

three regions of interaction



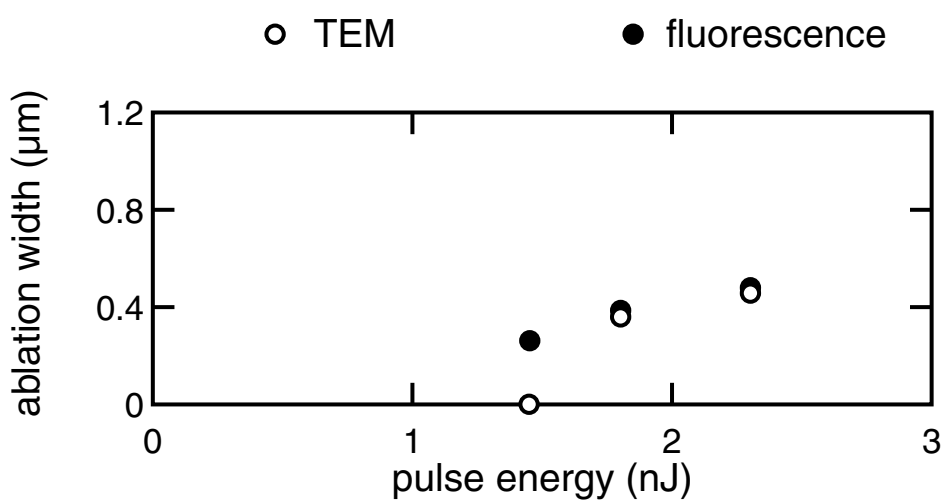
Subcellular surgery

three regions of interaction



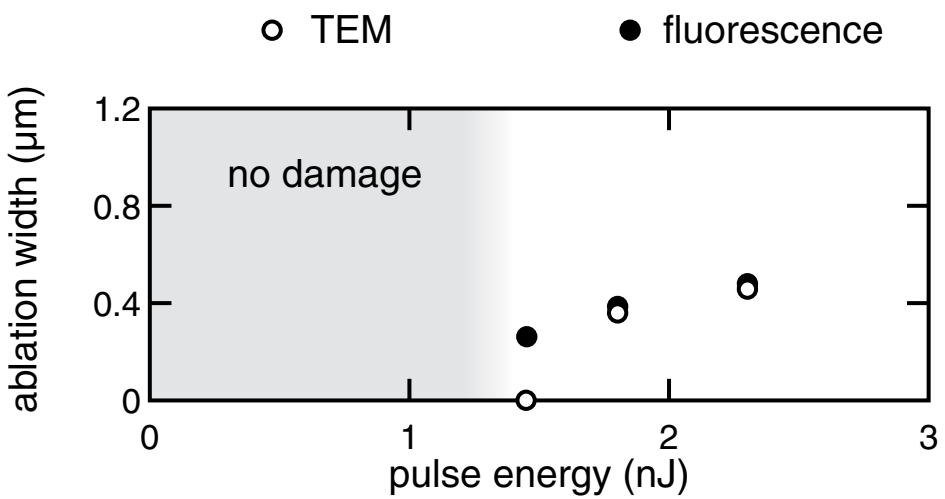
Subcellular surgery

three regions of interaction



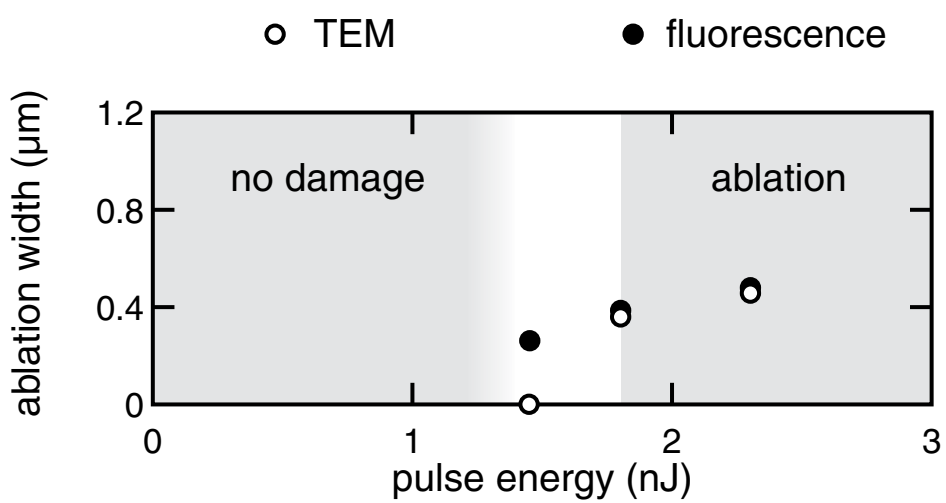
Subcellular surgery

three regions of interaction



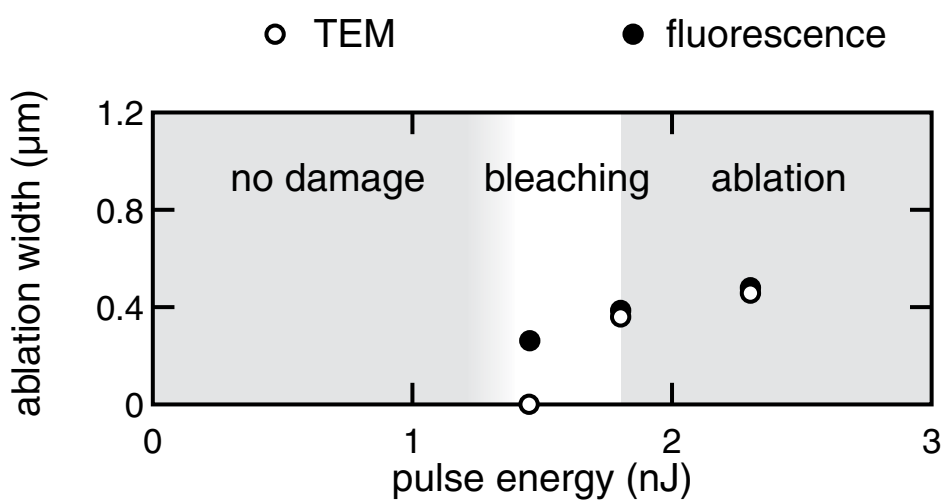
Subcellular surgery

three regions of interaction



Subcellular surgery

three regions of interaction



Subcellular surgery

Definitive proof of ablation

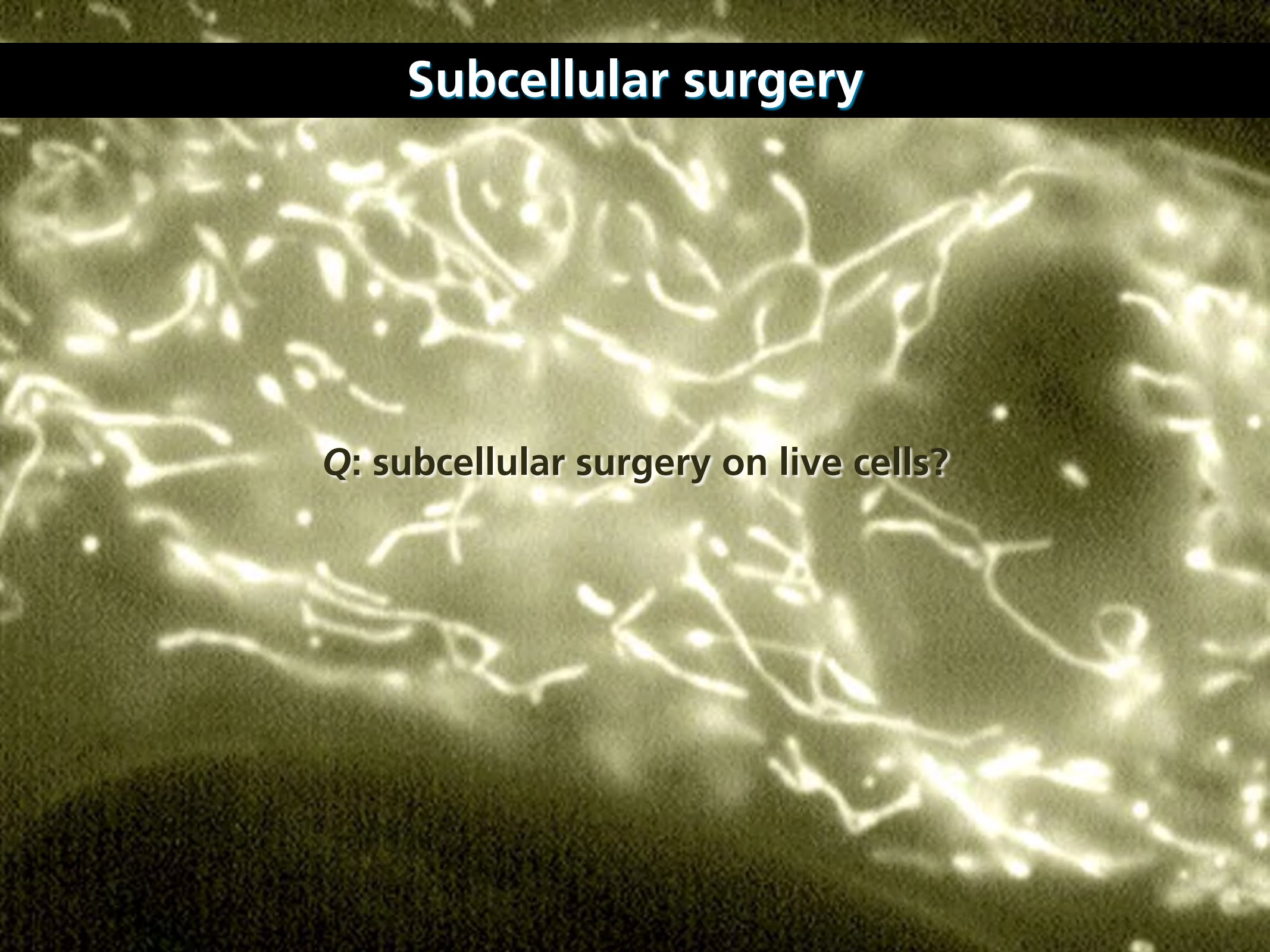
- ablation width as small as 100 nm
- ablation threshold varies slightly
- ablation threshold 20% above bleaching threshold

Subcellular surgery

Definitive proof of ablation

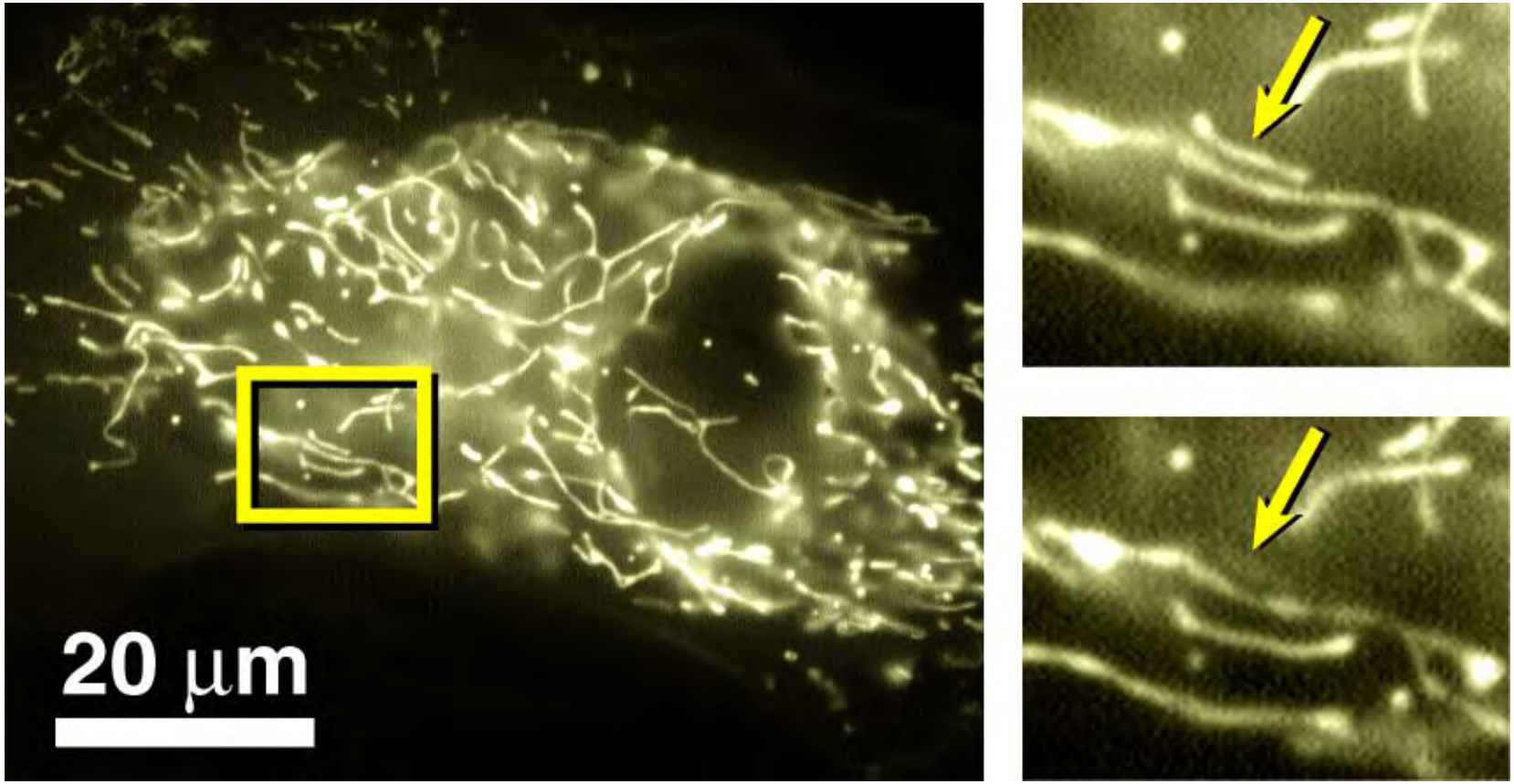
- ablation width as small as 100 nm
- ablation threshold varies slightly
- **ablation threshold 20% above bleaching threshold**

Subcellular surgery



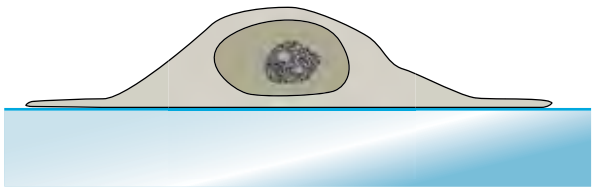
Q: subcellular surgery on live cells?

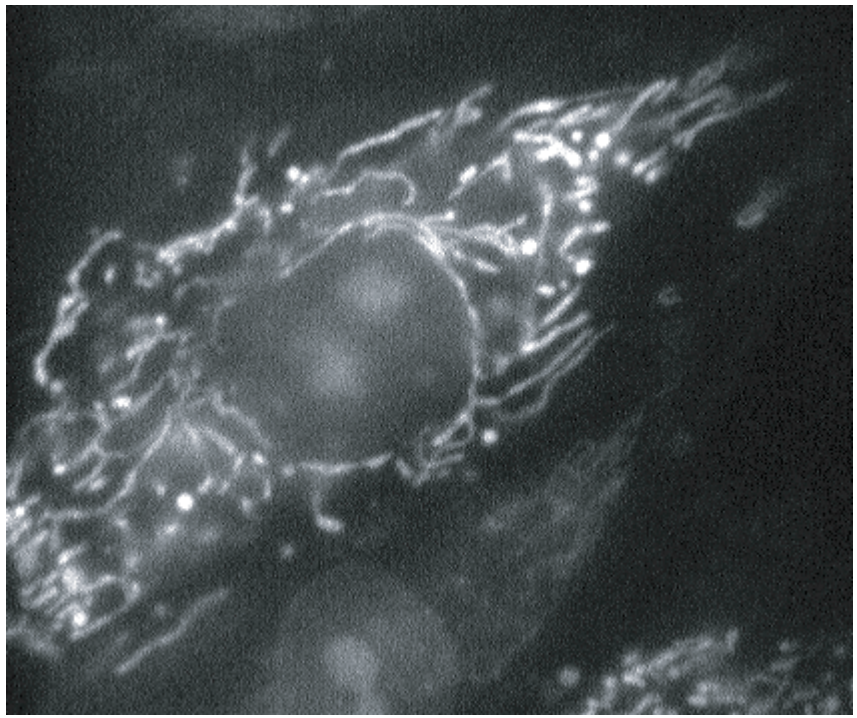
Subcellular surgery



Subcellular surgery

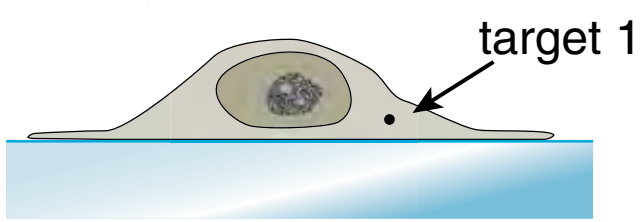
ethydium bromide test

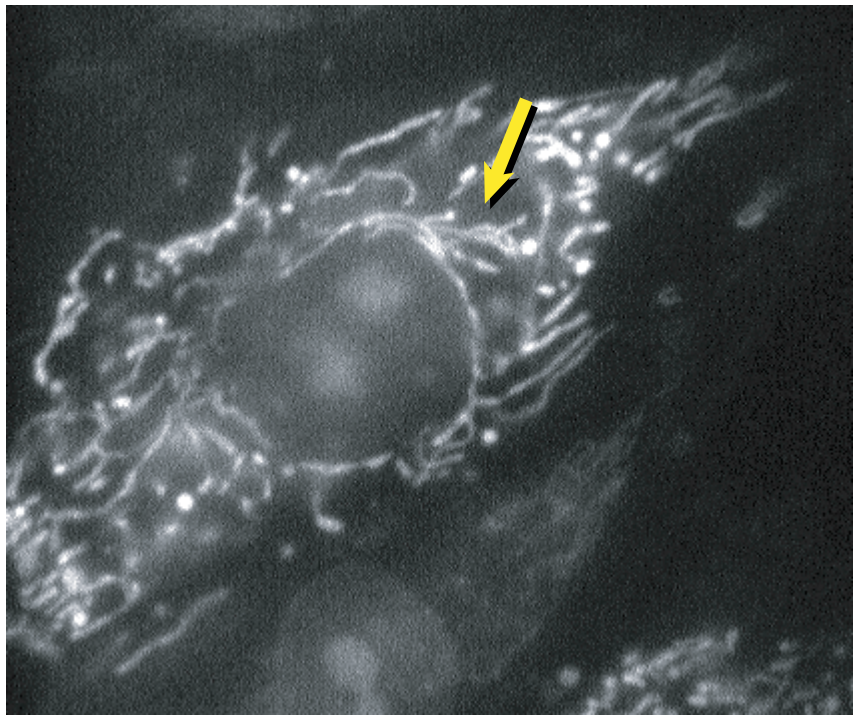




Subcellular surgery

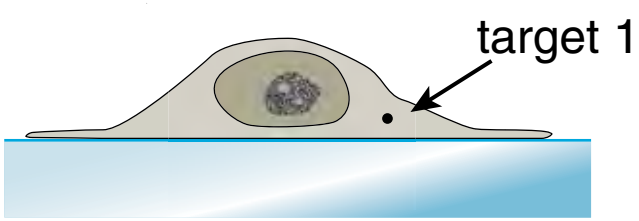
ethydium bromide test

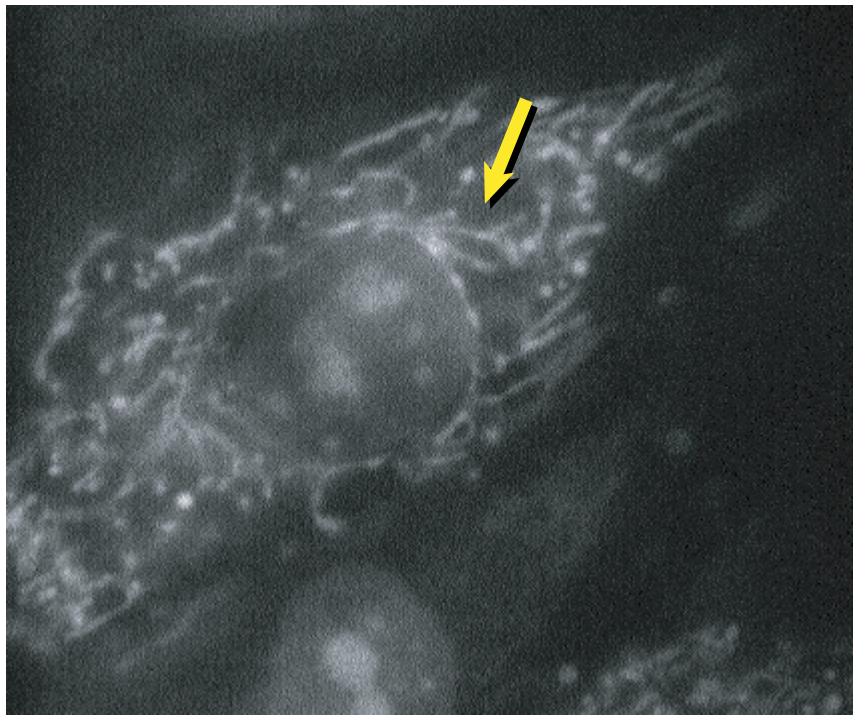




Subcellular surgery

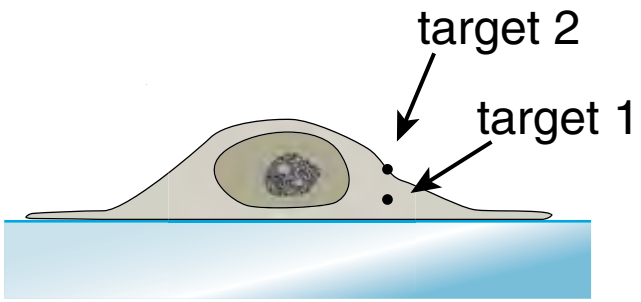
ethydium bromide test

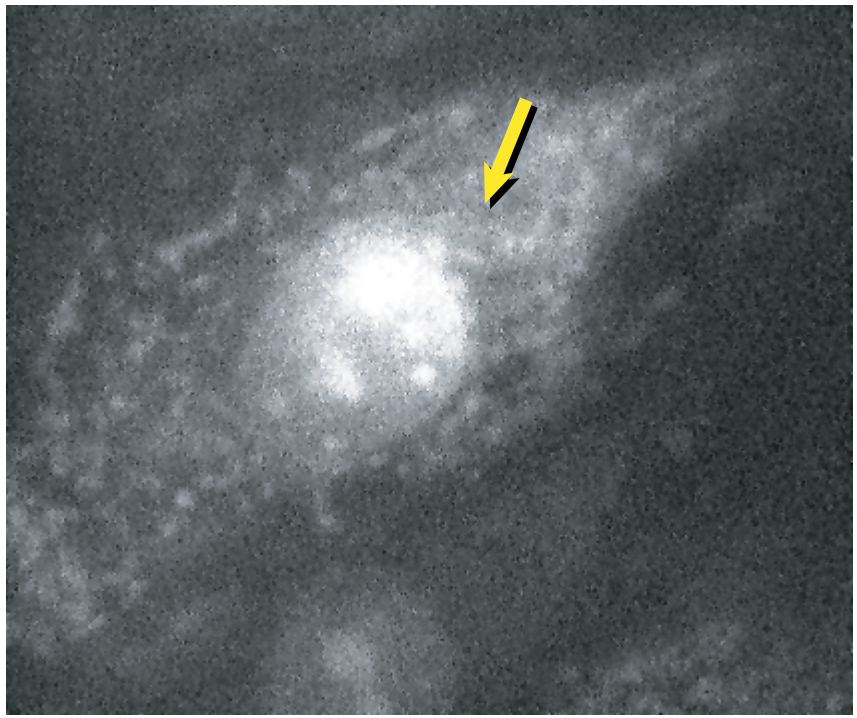




Subcellular surgery

ethydium bromide test



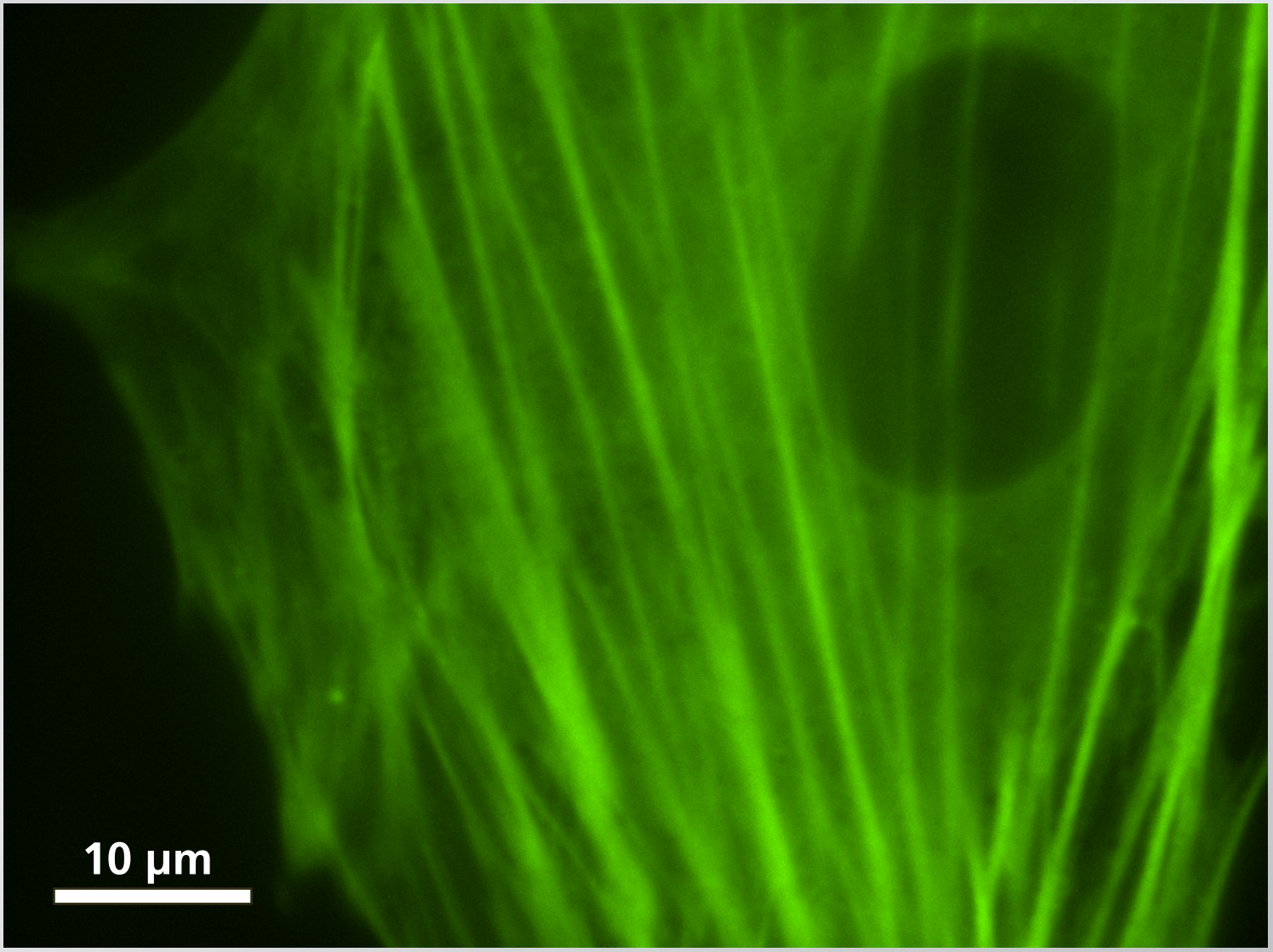


Subcellular surgery

Q: can we probe the dynamics of the cytoskeleton?

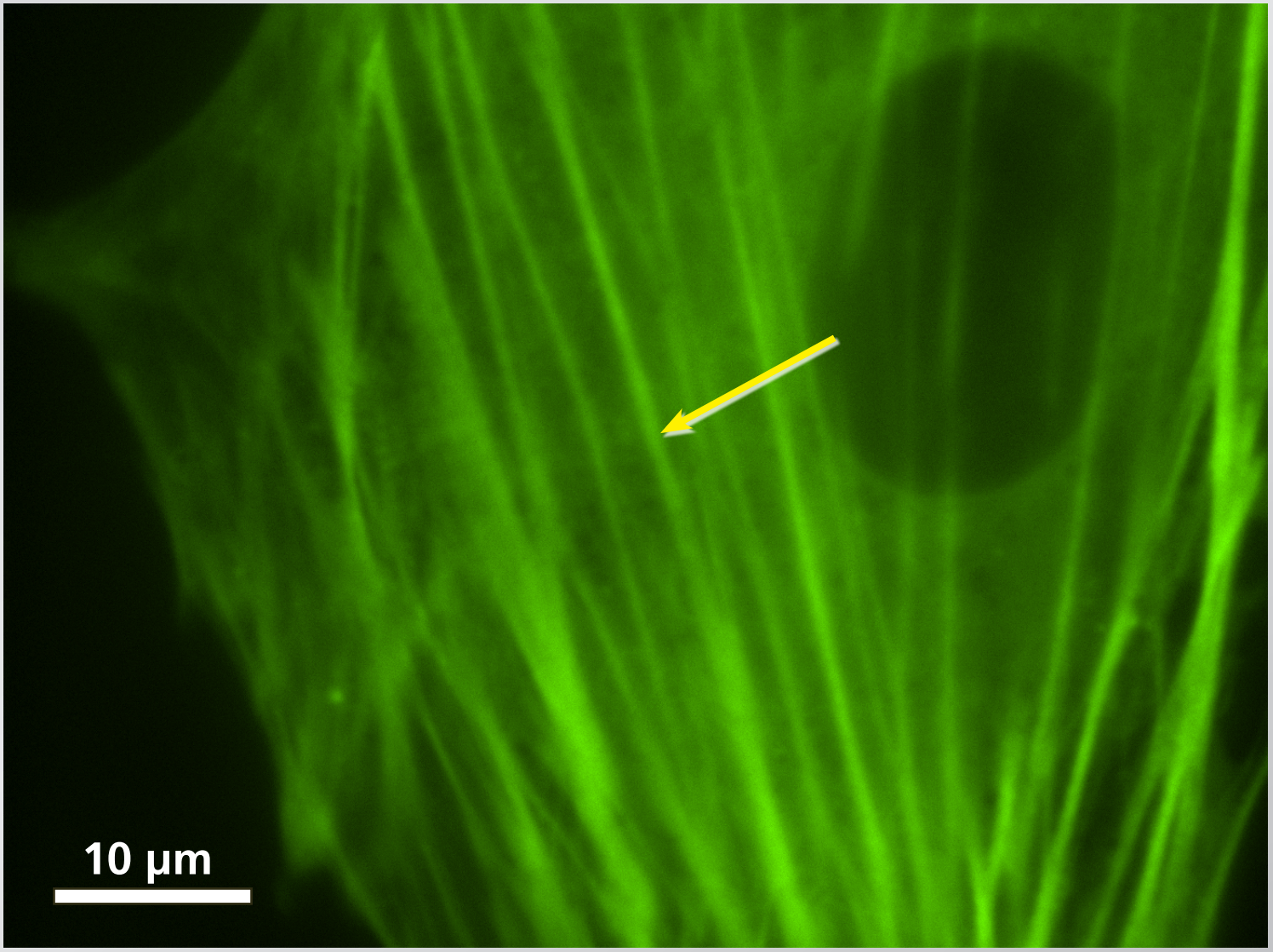
Subcellular surgery

YFP-labeled actin fiber network of a live cell



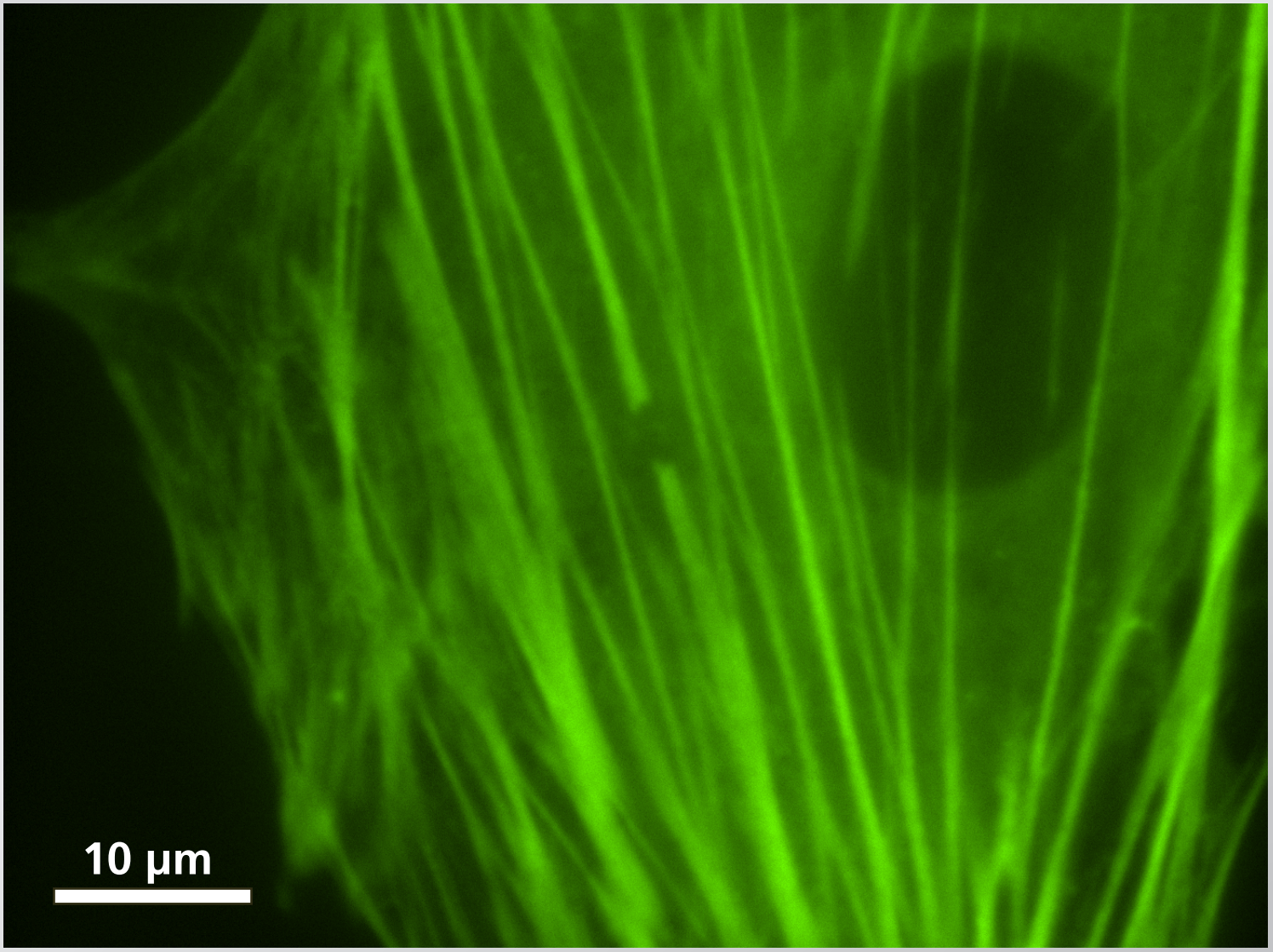
Subcellular surgery

cut a single fiber bundle



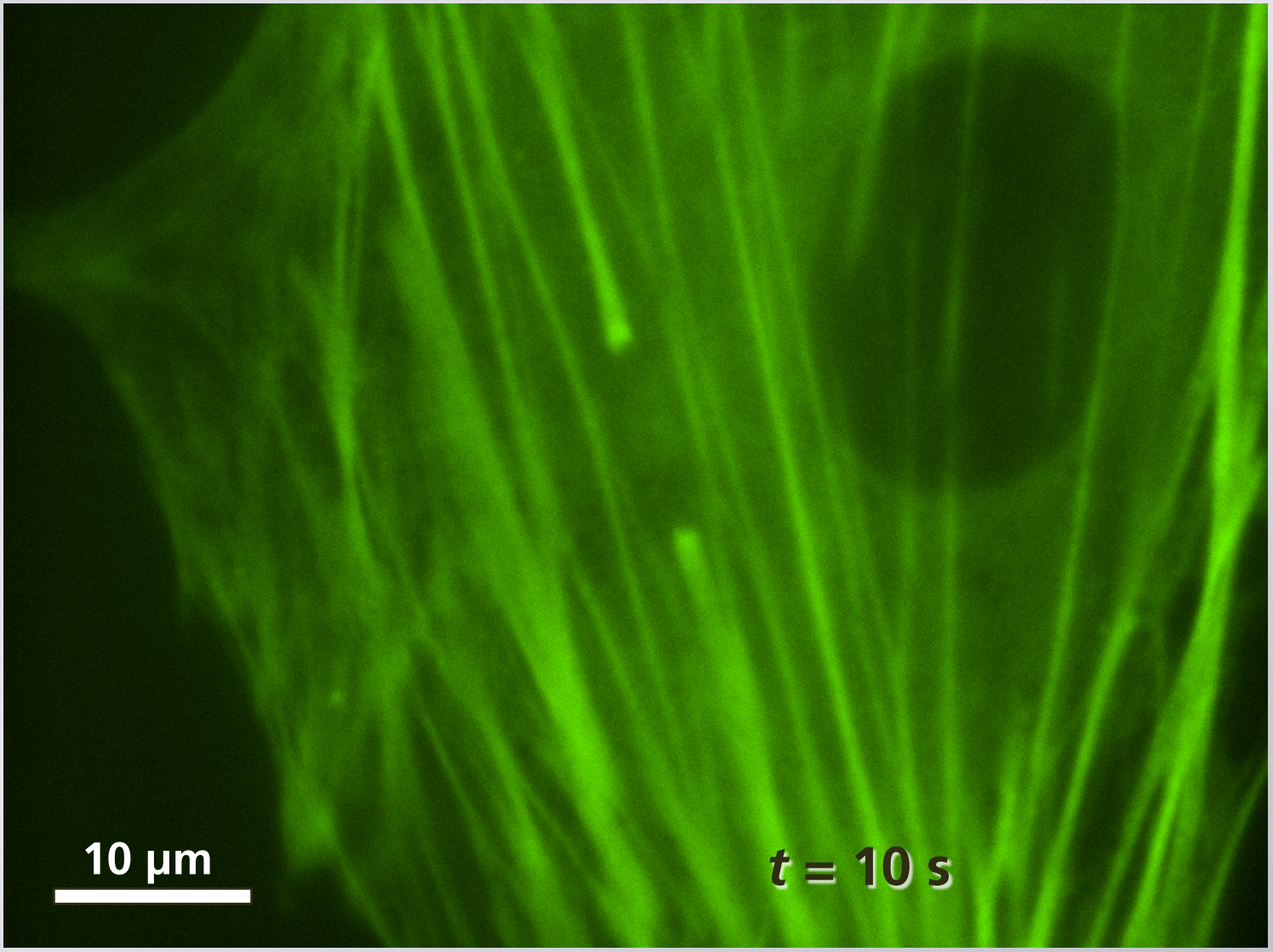
Subcellular surgery

cut a single fiber bundle



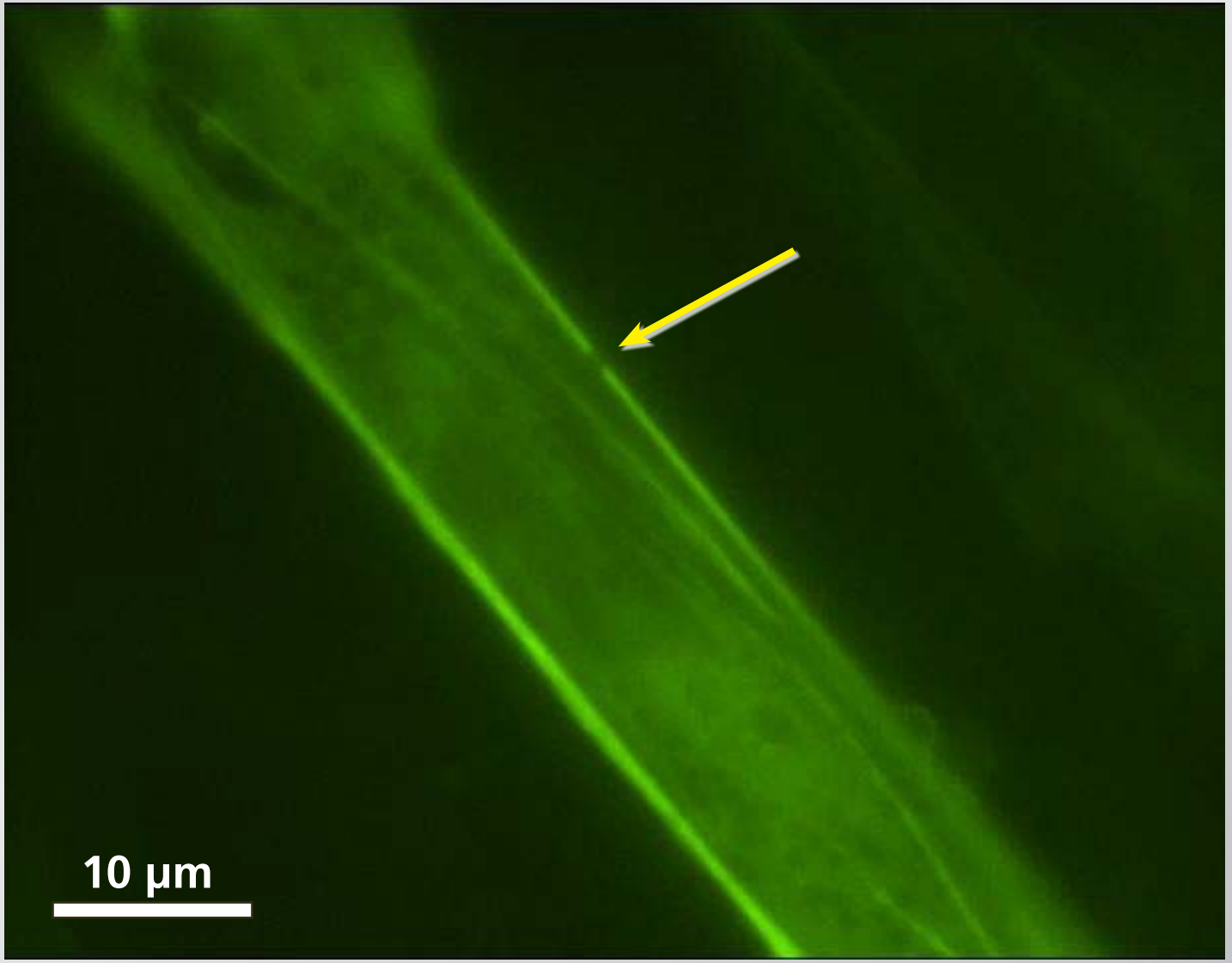
Subcellular surgery

gap widens with time



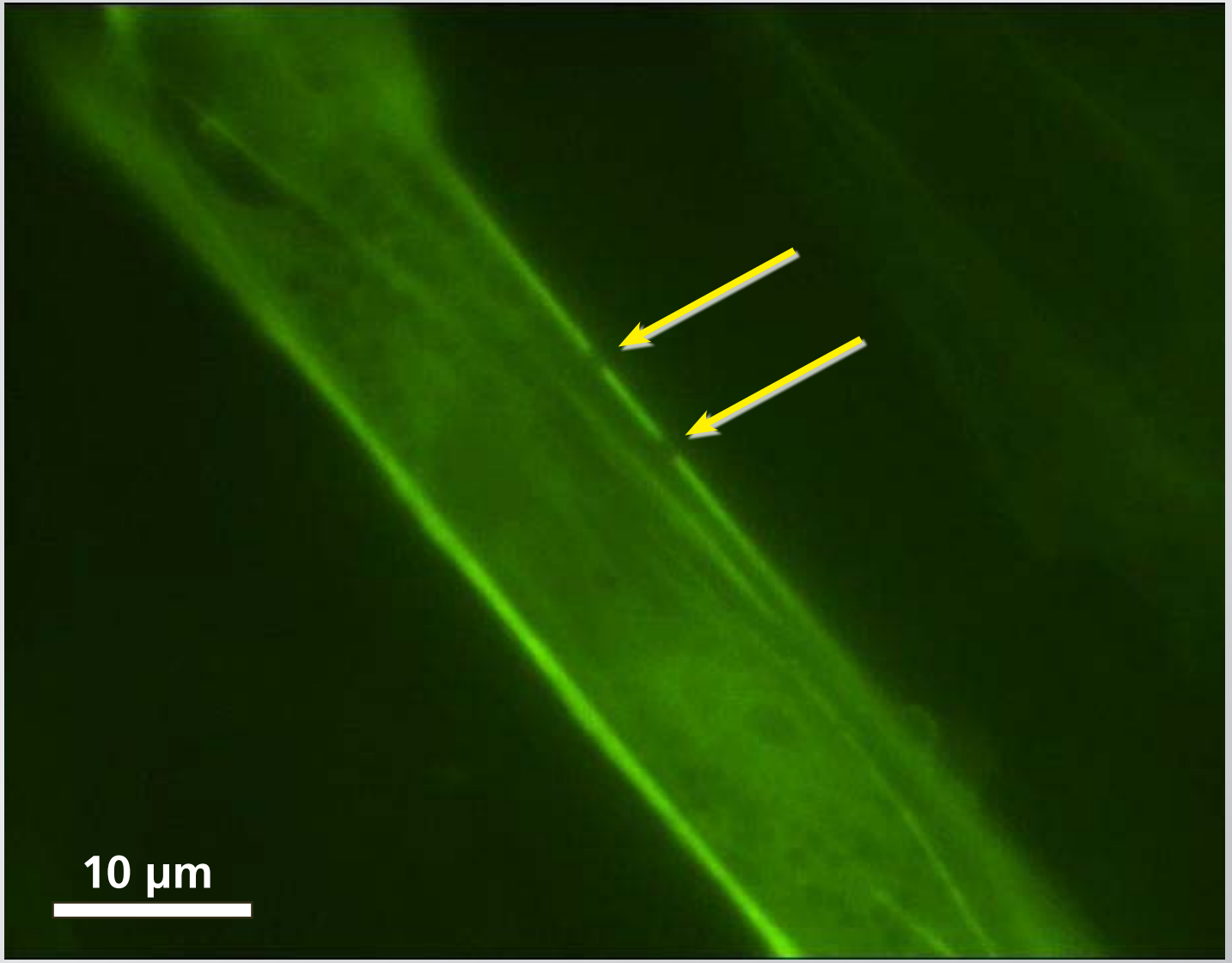
Subcellular surgery

retraction or depolymerization?



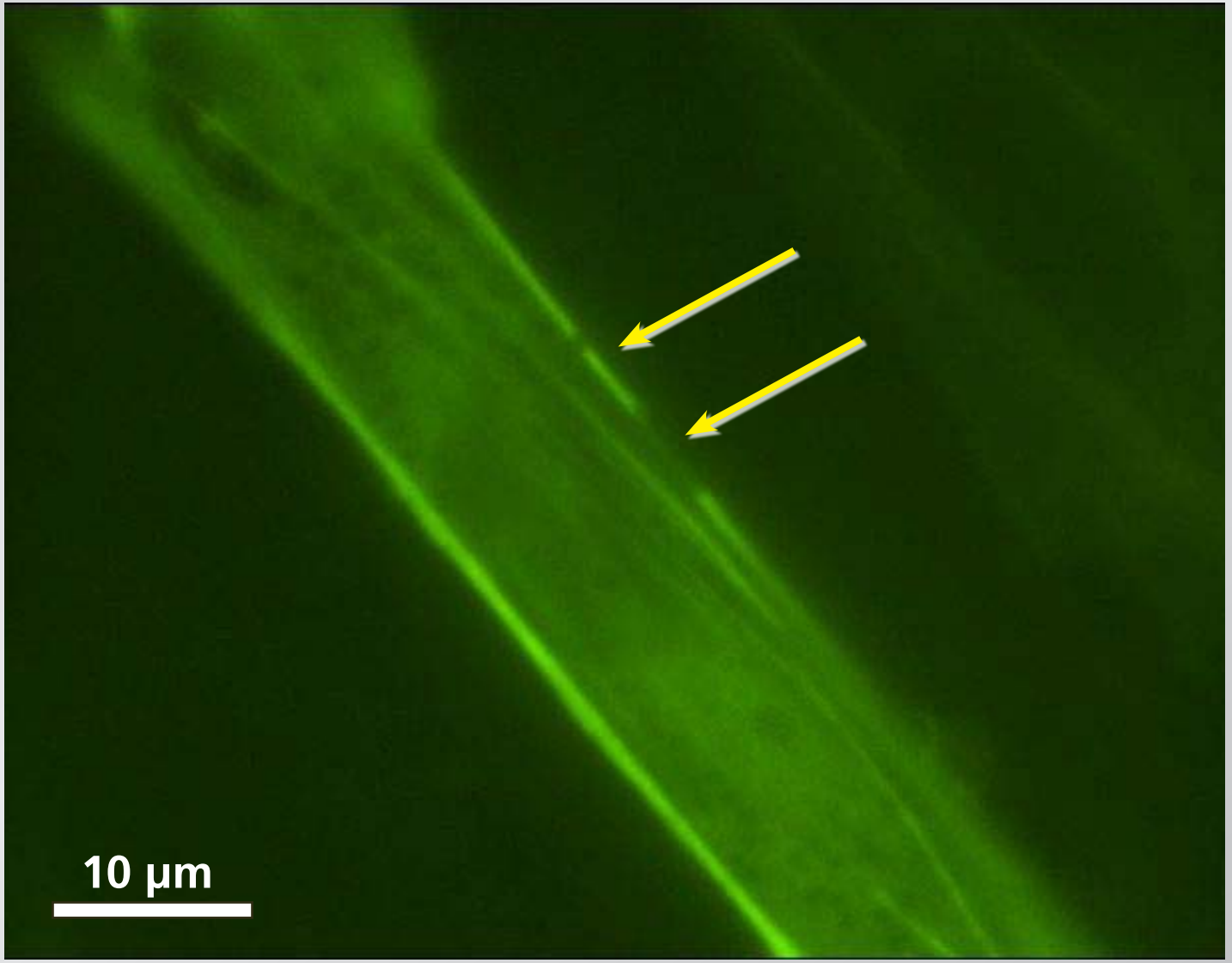
Subcellular surgery

retraction or depolymerization?



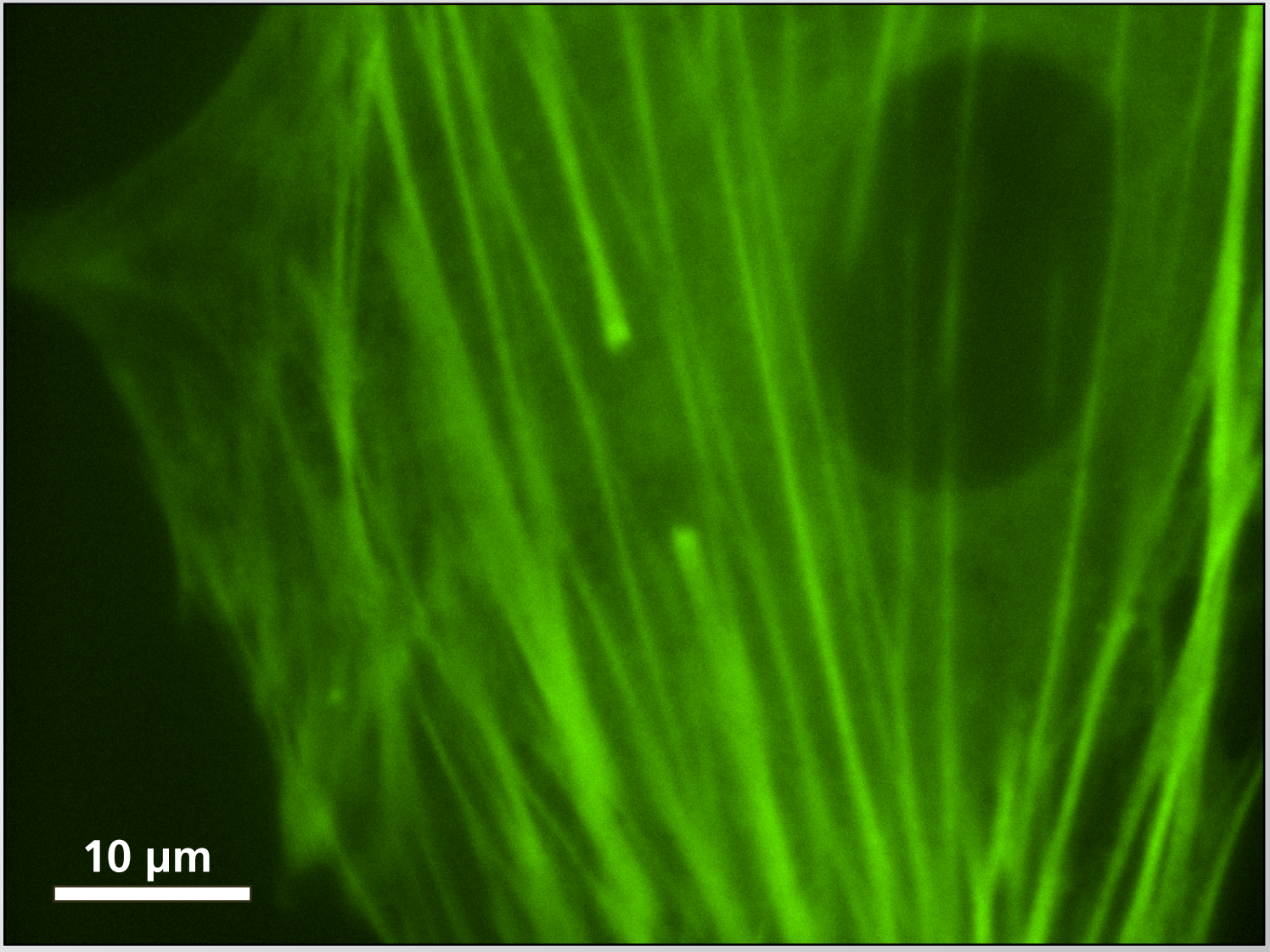
Subcellular surgery

retraction!

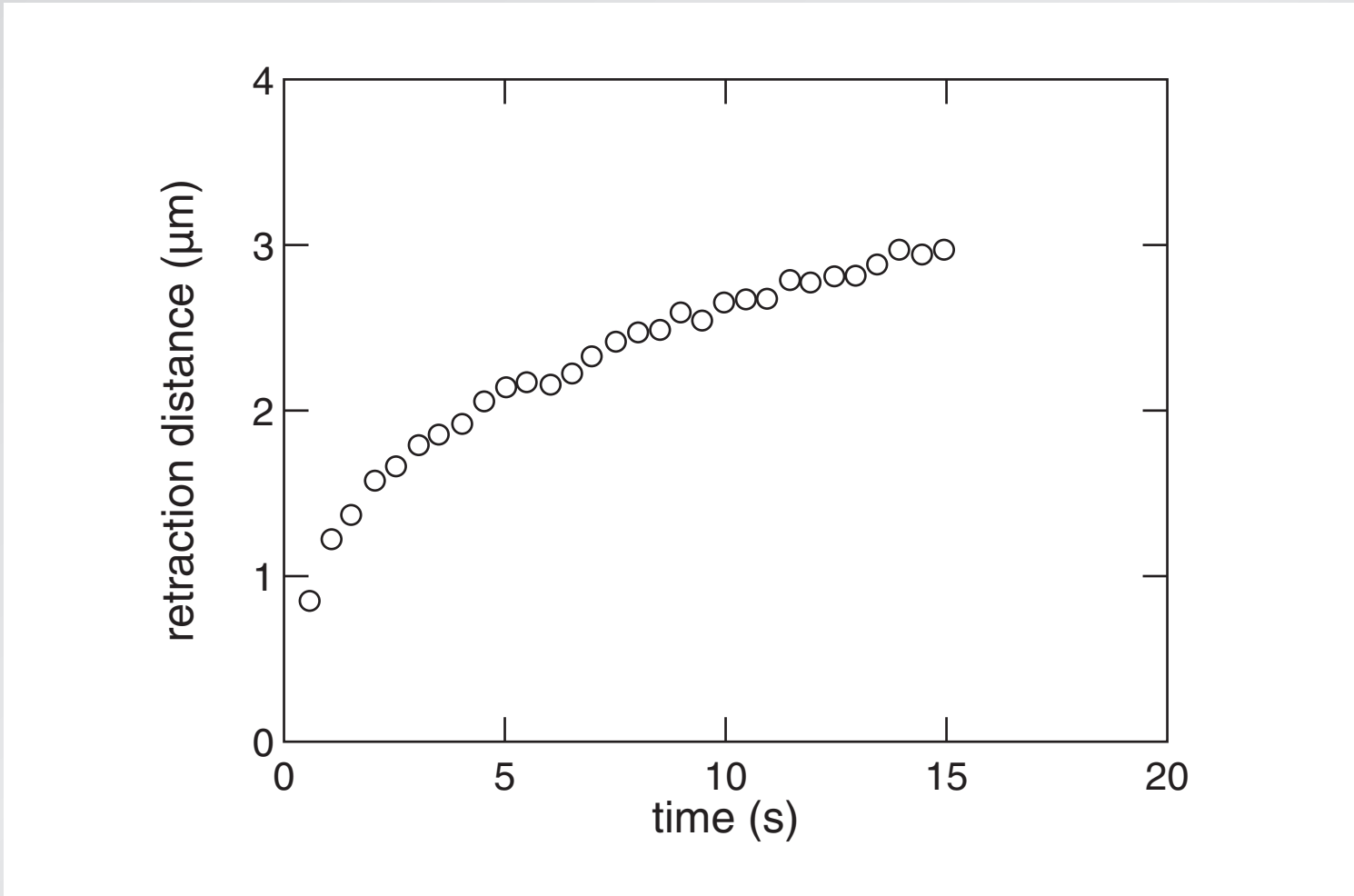


Subcellular surgery

dynamics provides information on *in vivo* mechanics

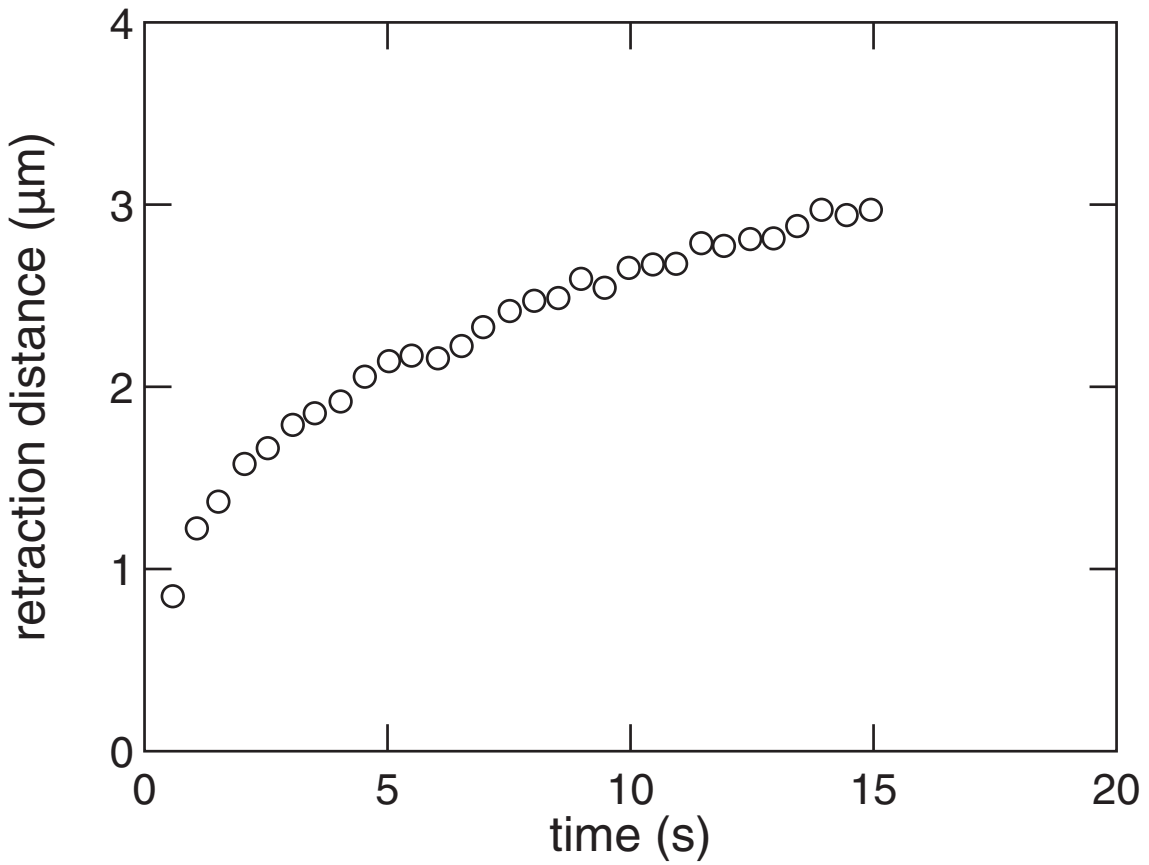


Subcellular surgery



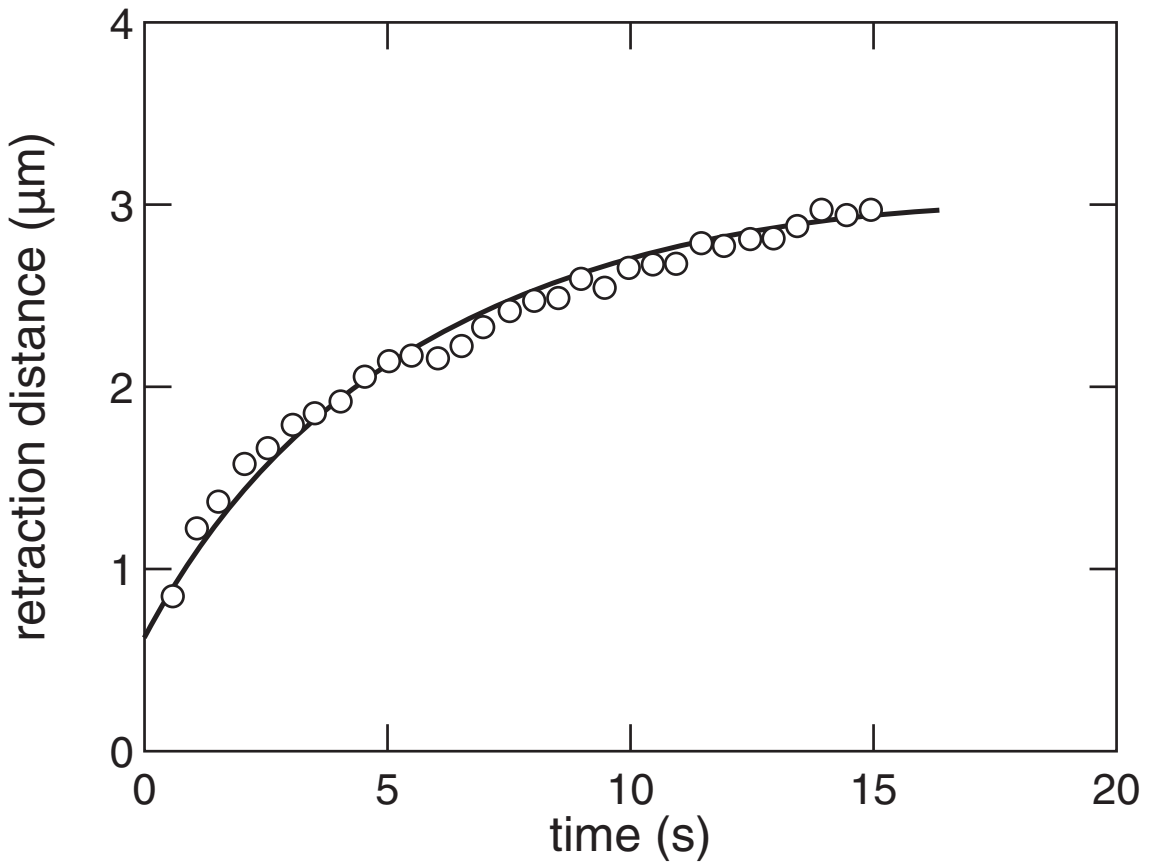
Subcellular surgery

overdamped spring: $\Delta L = L_{\infty}(1 - e^{-t/\tau}) + L_{o}$



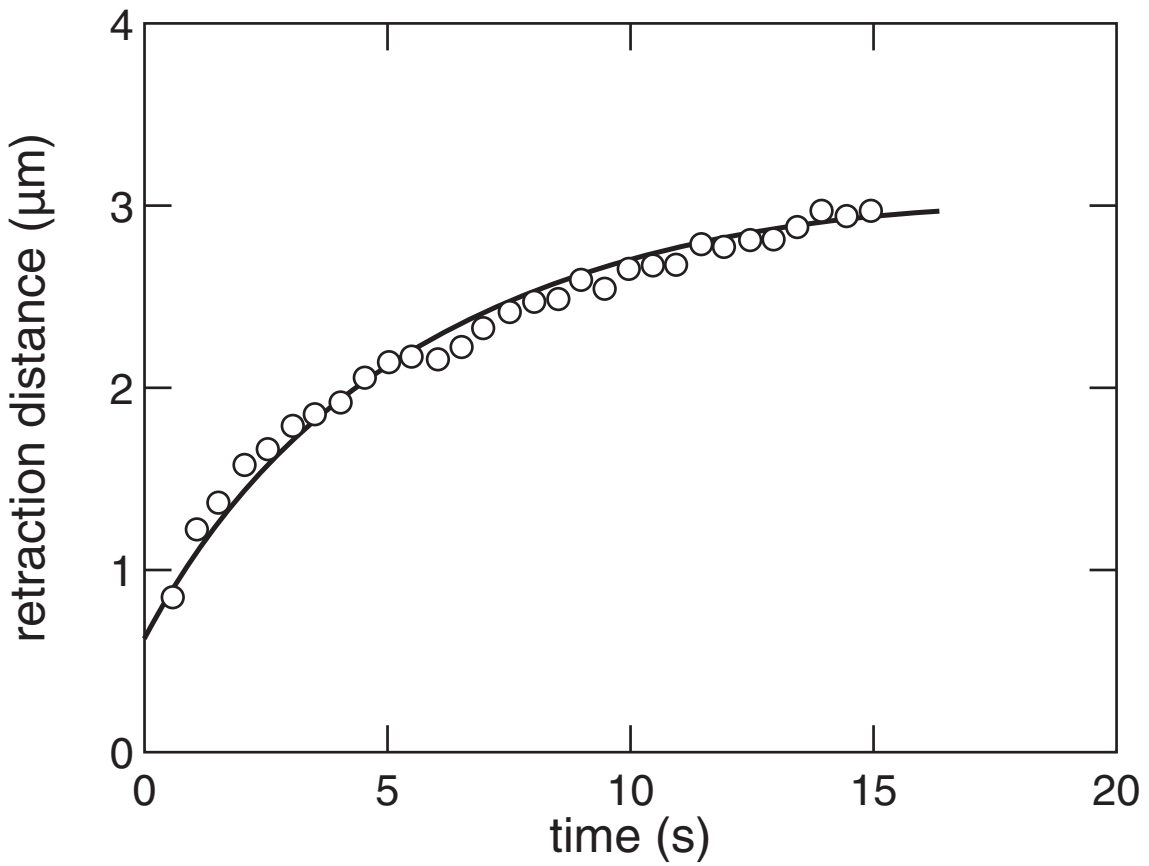
Subcellular surgery

overdamped spring: $\Delta L = L_{\infty}(1 - e^{-t/\tau}) + L_{o}$



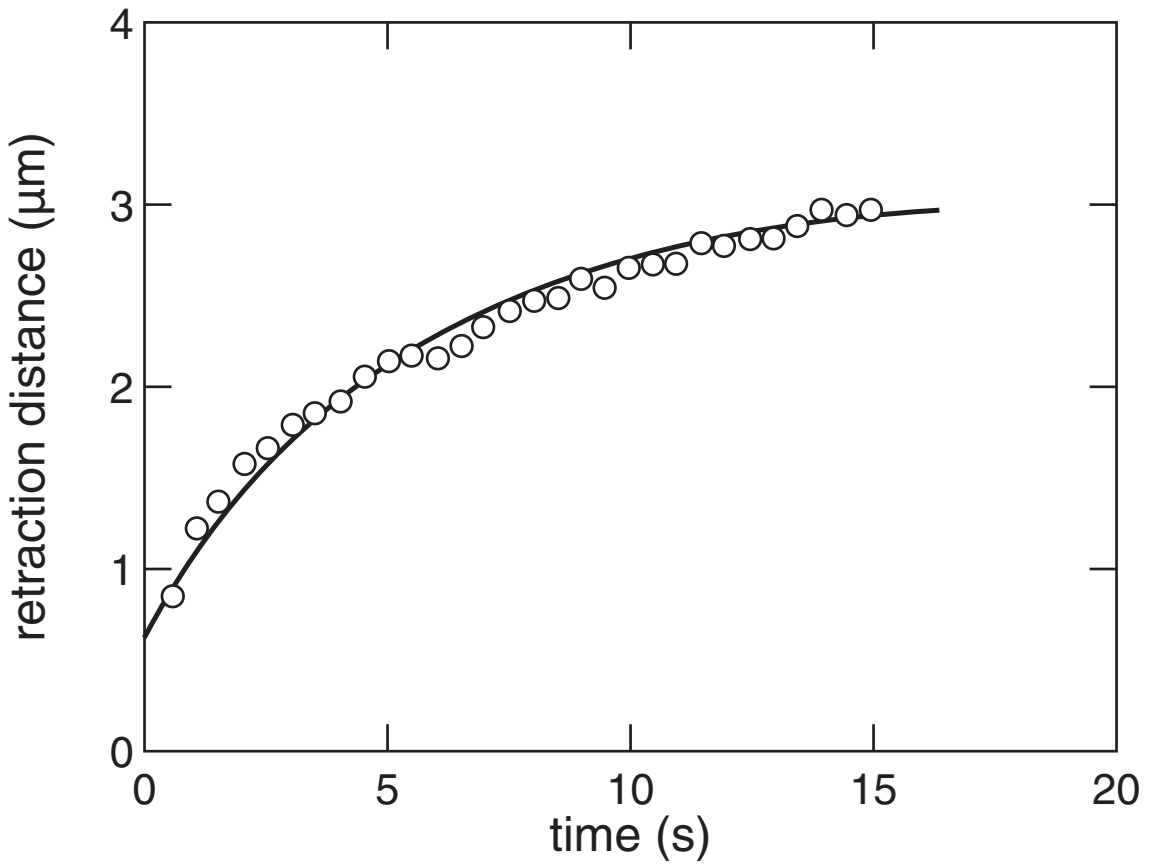
Subcellular surgery

L_o and τ independent of fiber width!



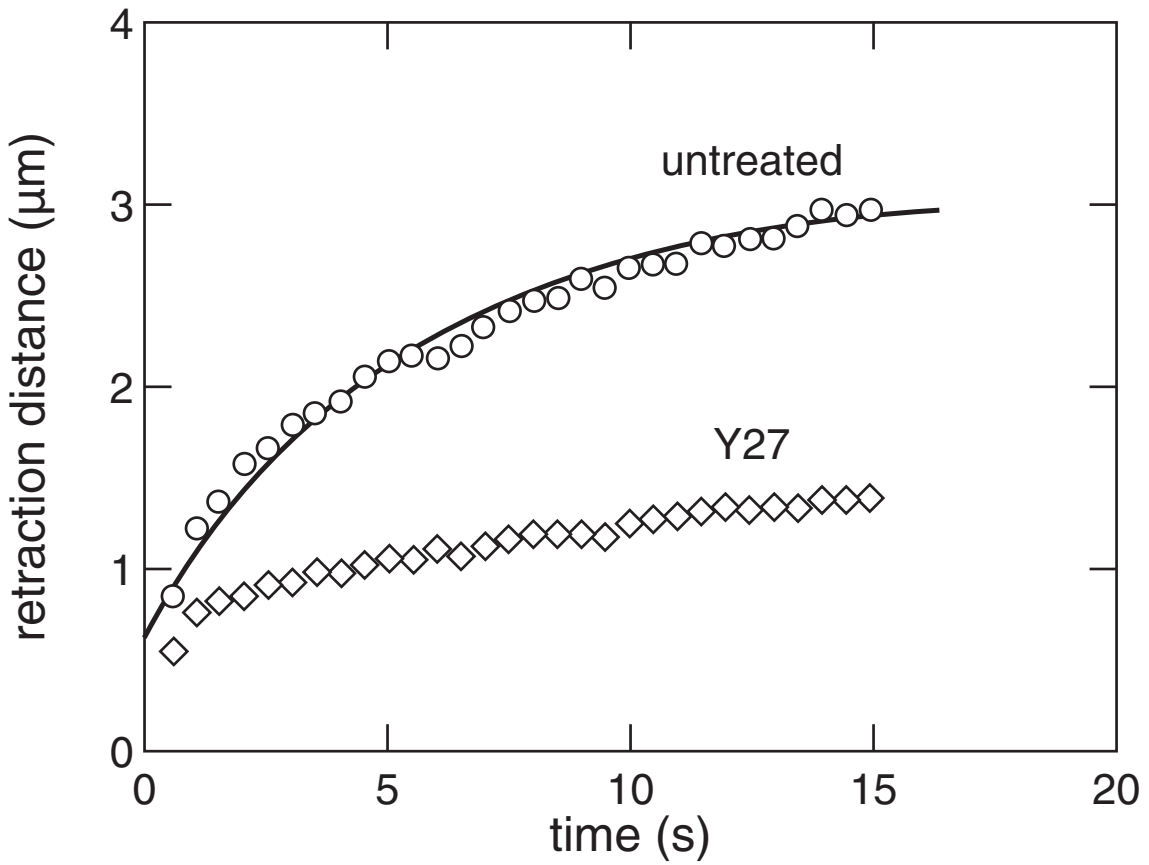
Subcellular surgery

tension in actin filaments is generated by myosin motors



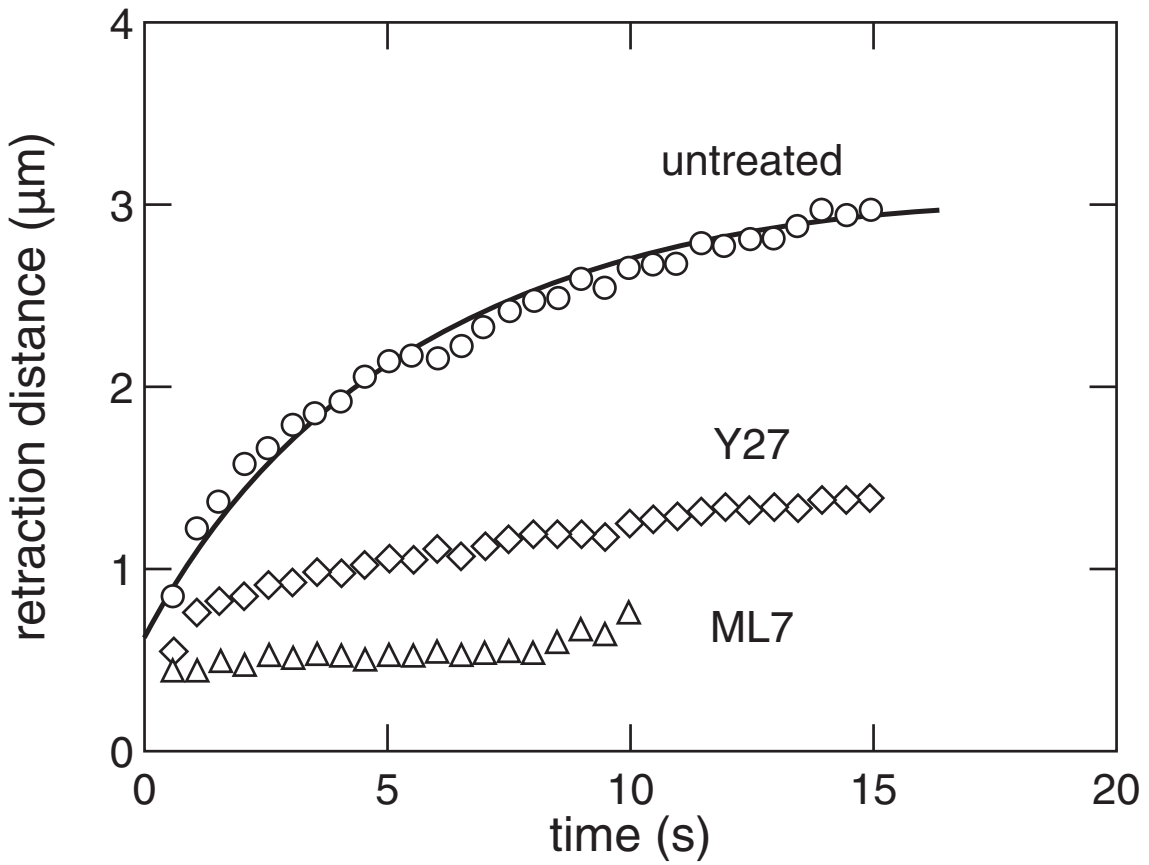
Subcellular surgery

Y27: inhibits some myosin activity



Subcellular surgery

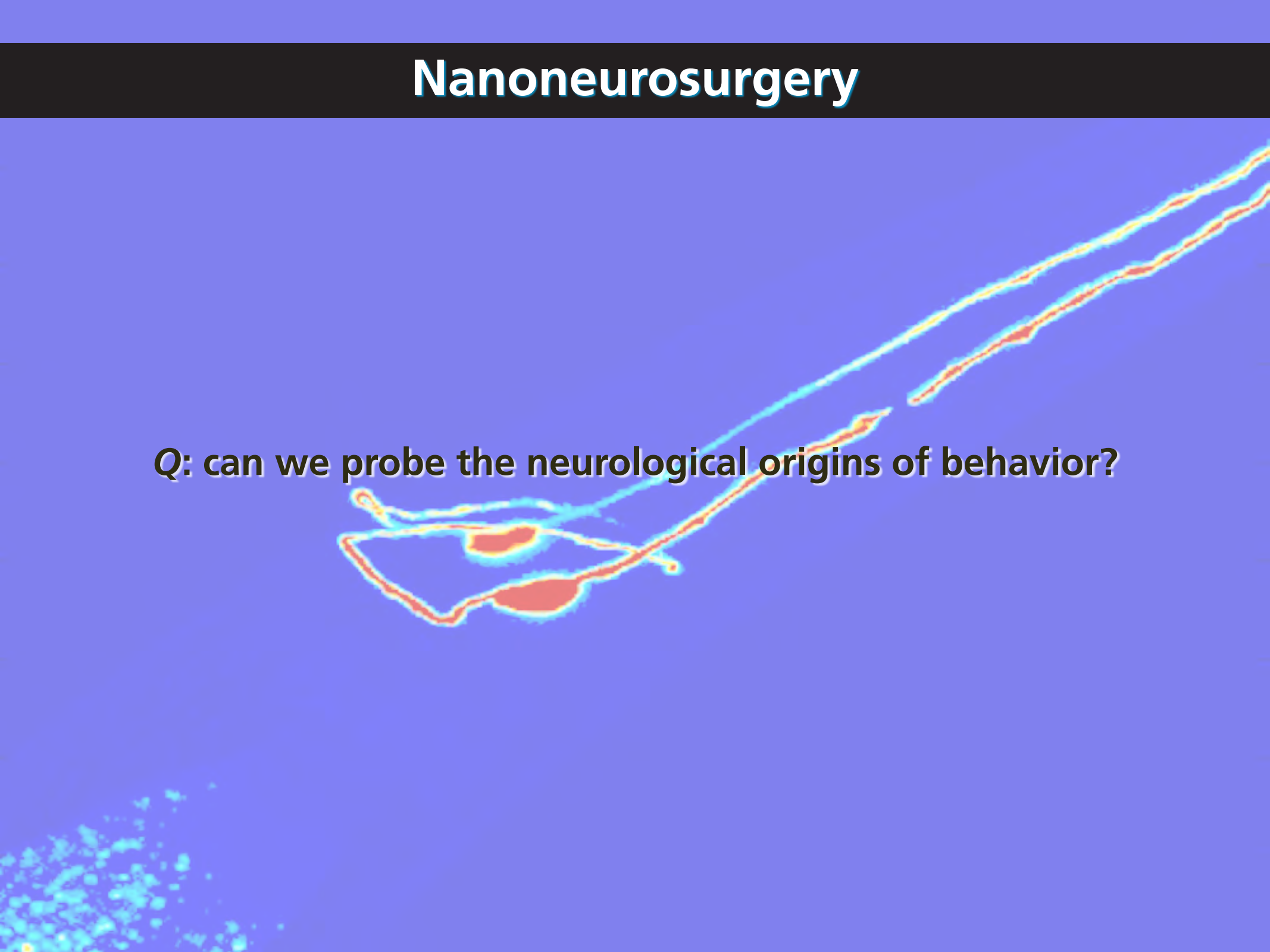
ML7: direct inhibitor of myosin activity



Outline

- femtosecond materials interactions
- subcellular surgery
- **nanoneurosurgery**

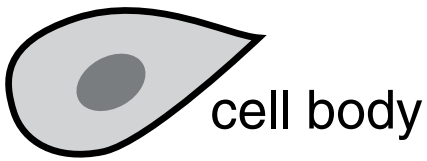
Nanoneurosurgery



Q: can we probe the neurological origins of behavior?

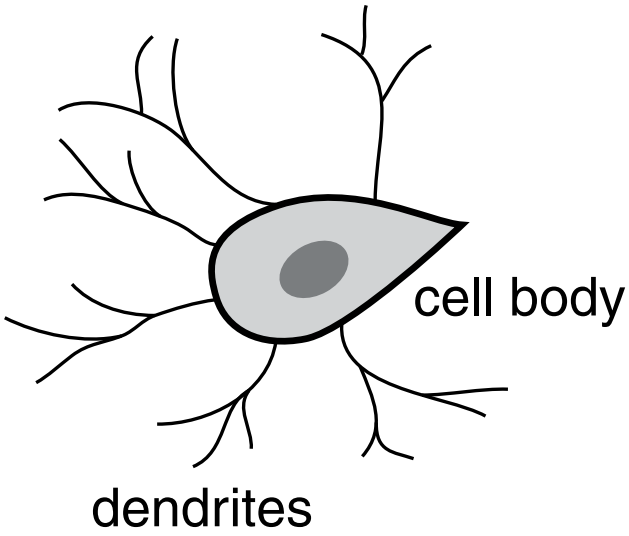
Nanoneurosurgery

neuron basics



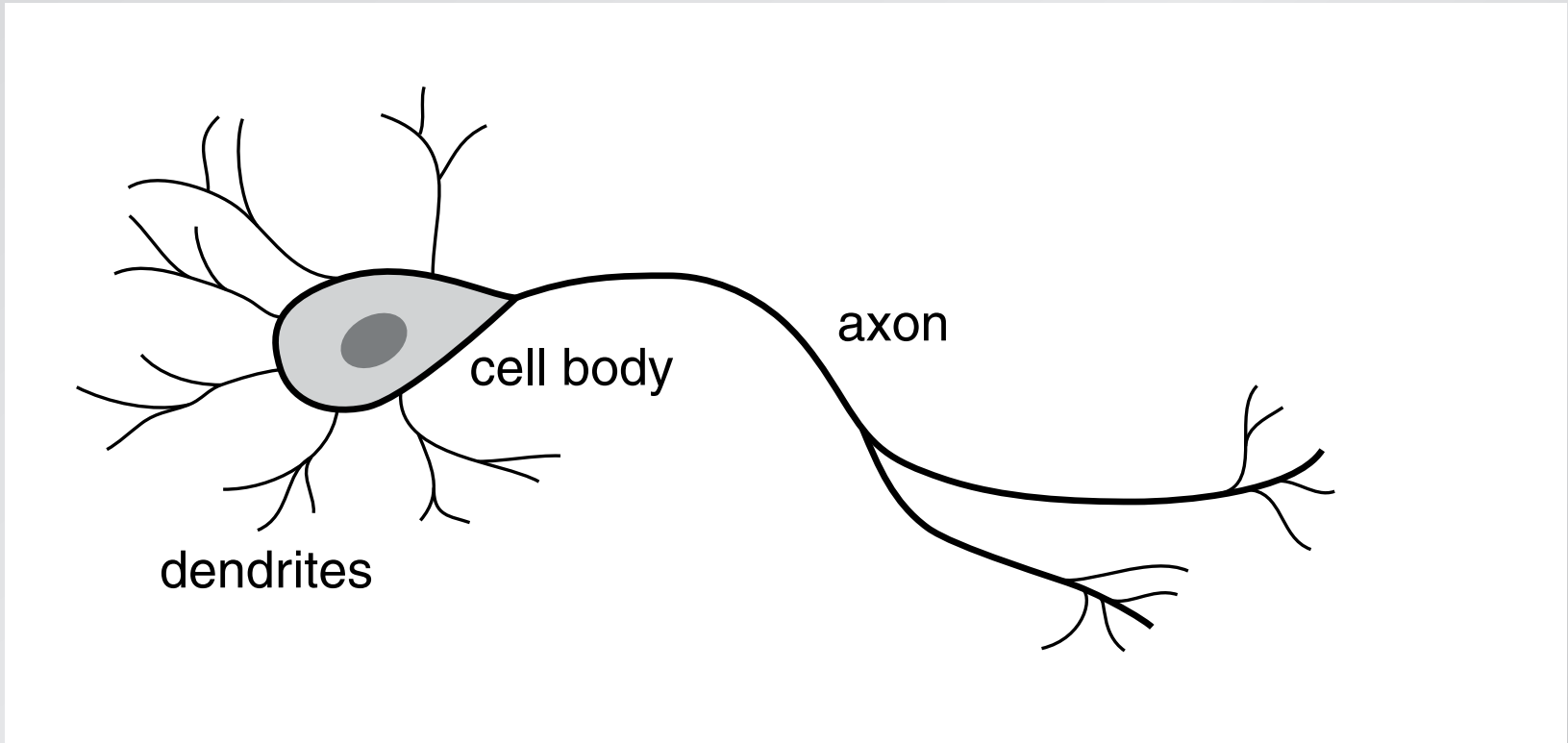
Nanoneurosurgery

neuron basics



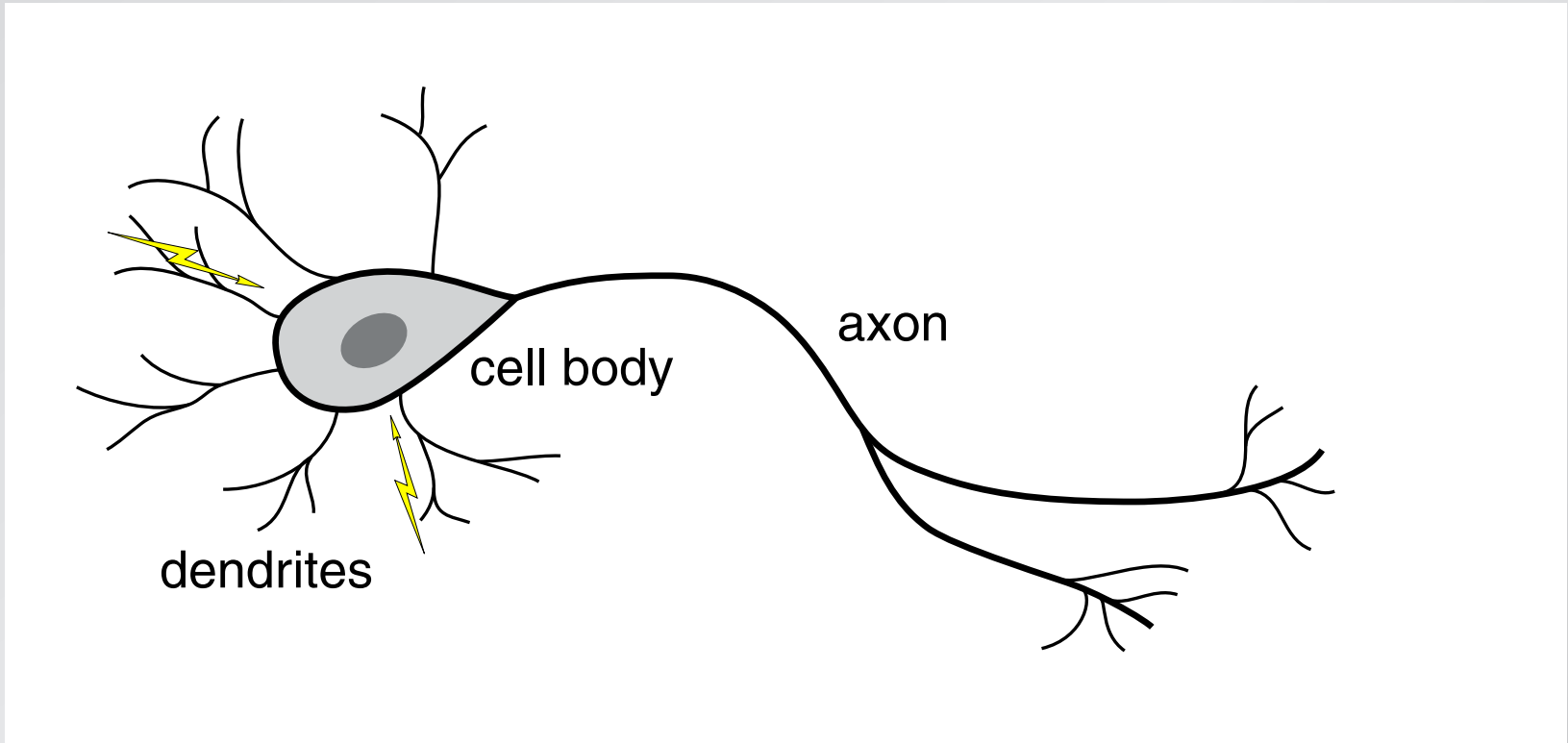
Nanoneurosurgery

neuron basics



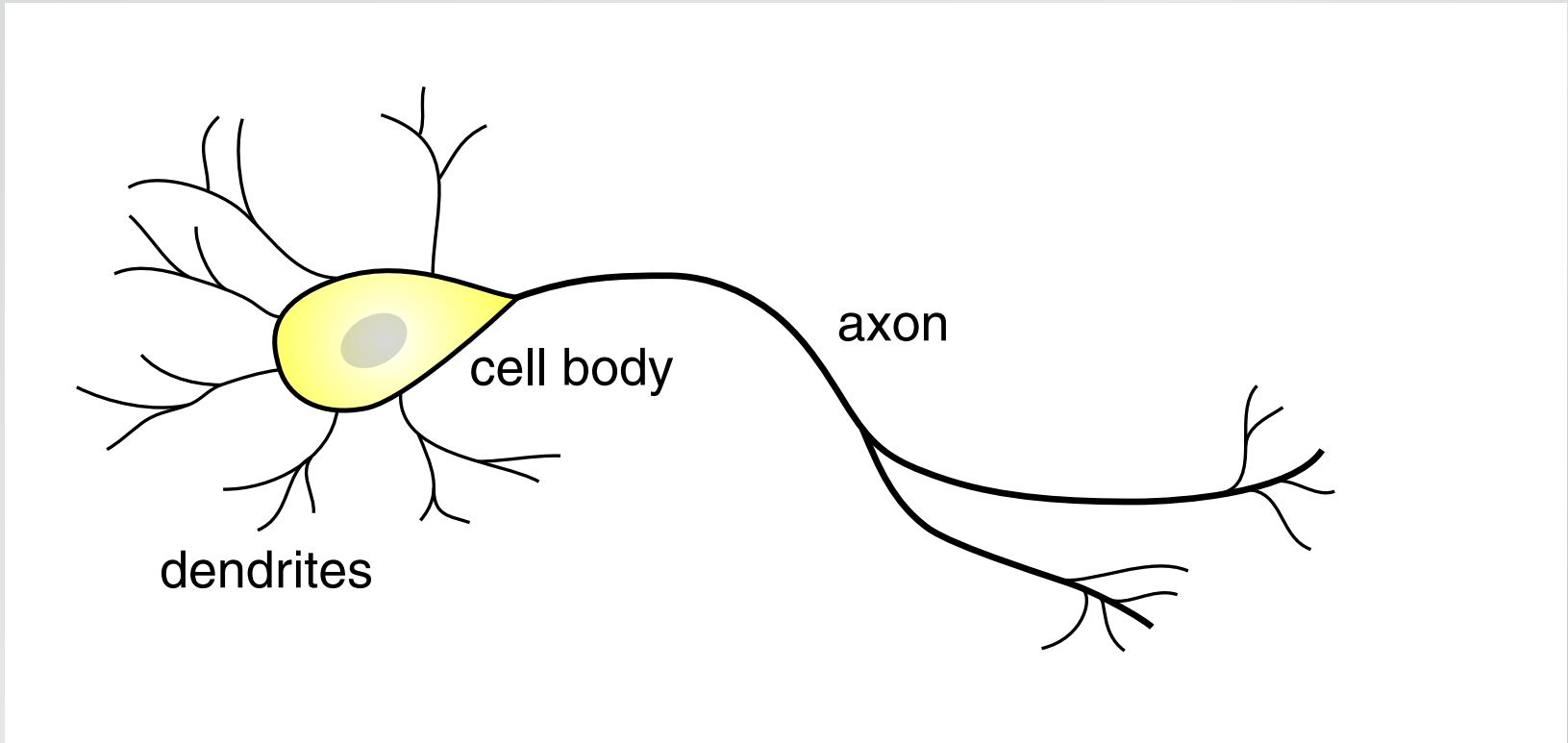
Nanoneurosurgery

neuron basics



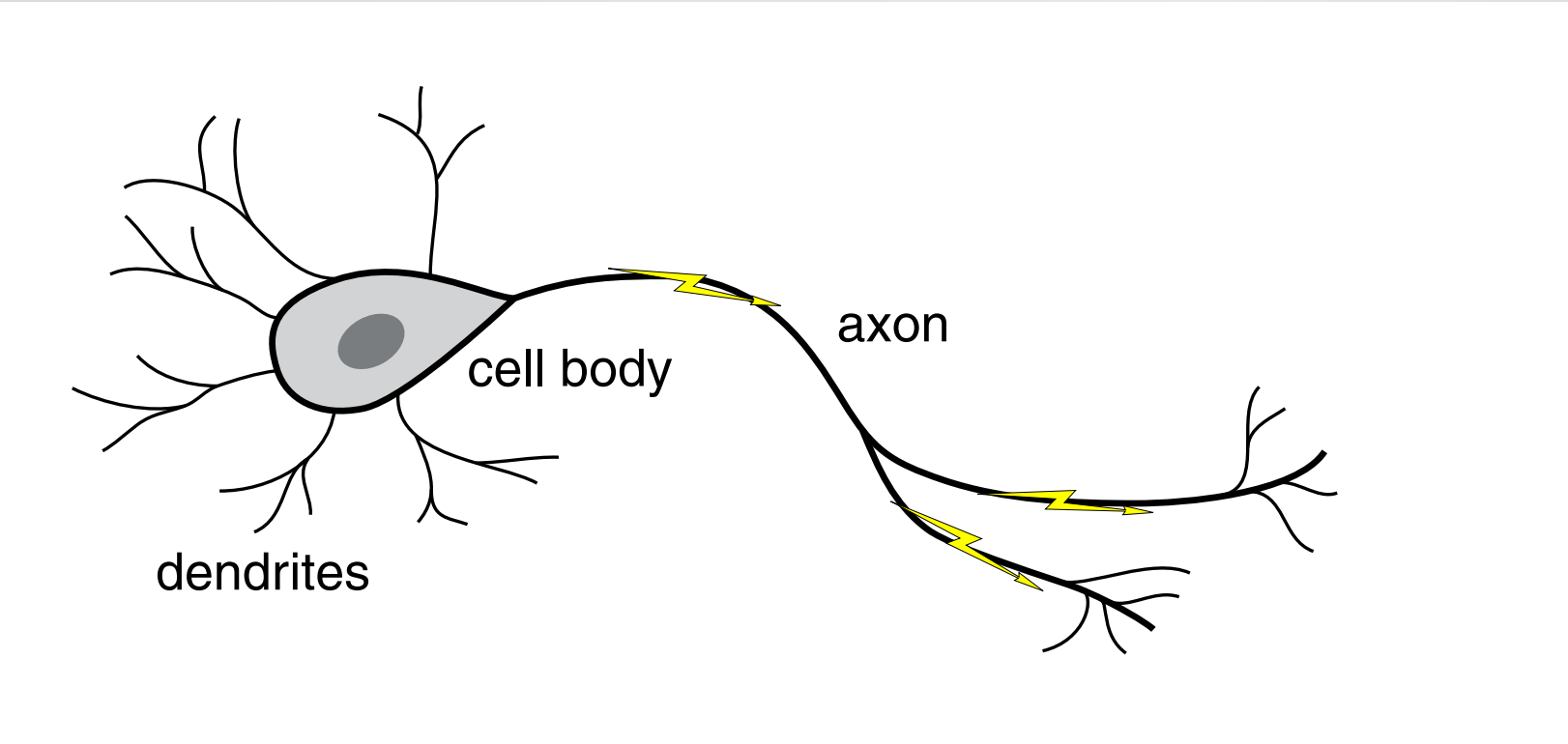
Nanoneurosurgery

neuron basics



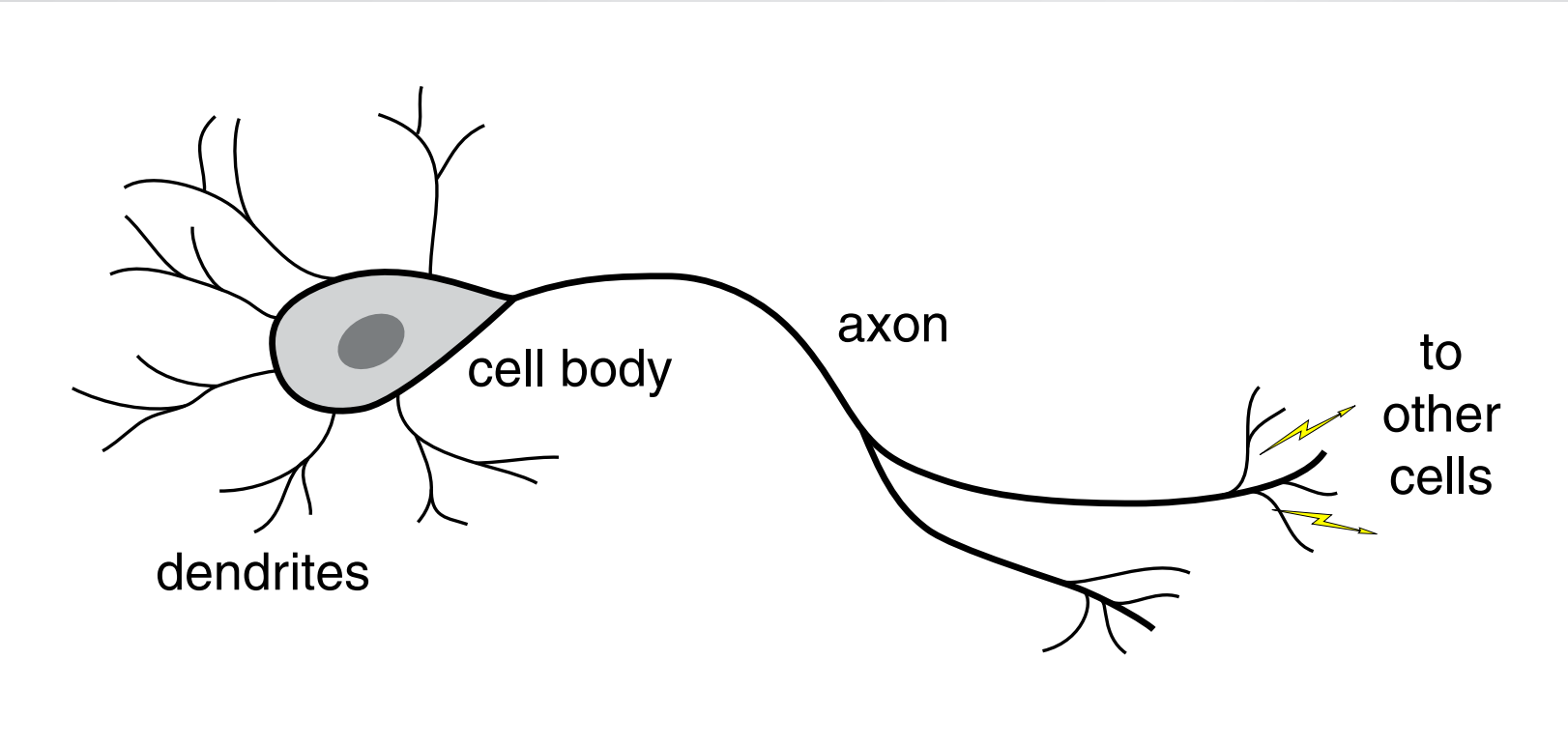
Nanoneurosurgery

neuron basics



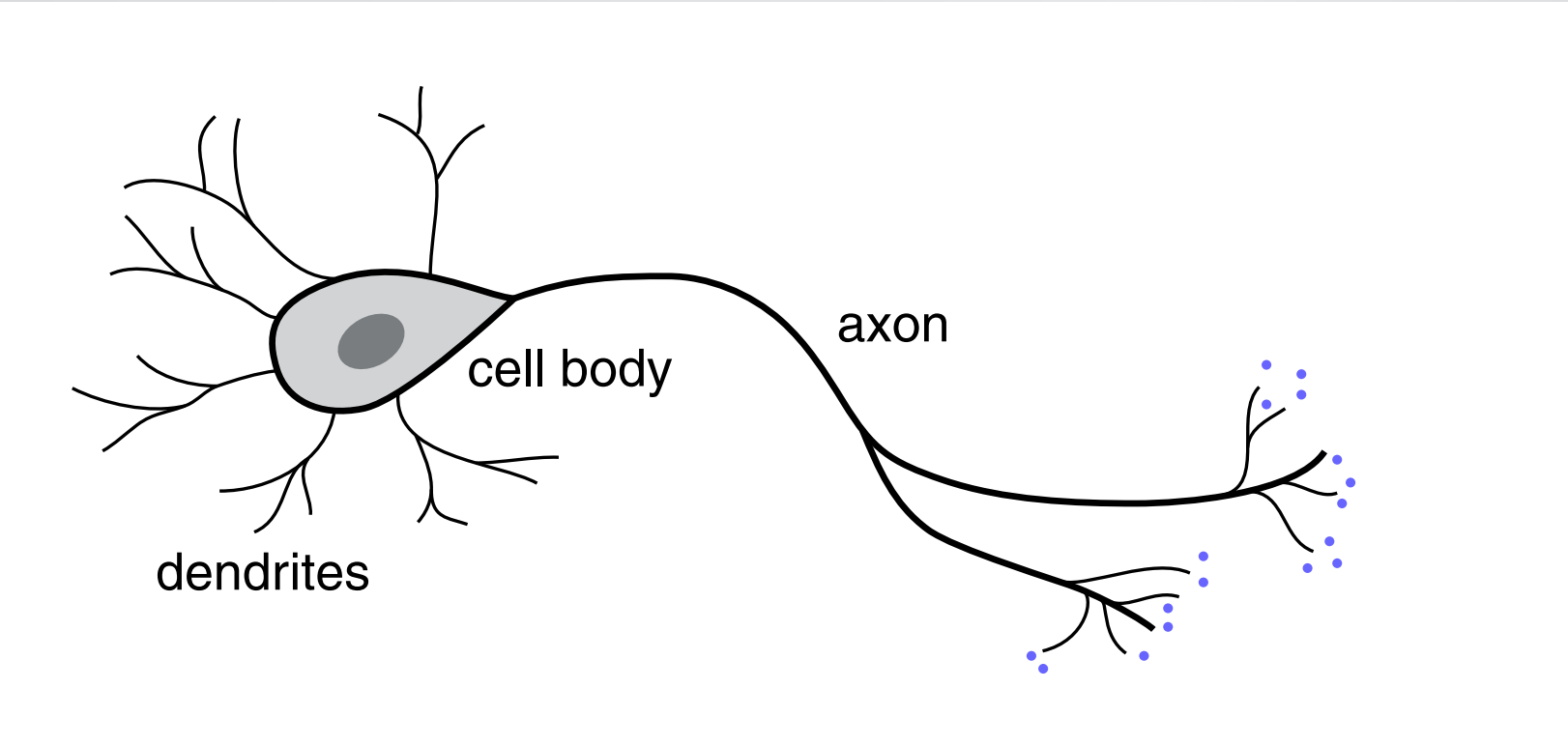
Nanoneurosurgery

neuron basics



Nanoneurosurgery

neuron basics



Nanoneurosurgery

Caenorhabditis elegans

Juergen Berger & Ralph Sommer
Max-Planck Institute for Developmental Biology

Nanoneurosurgery

Caenorhabditis elegans

- simple model organism
- similarities to higher organisms
- genome fully sequenced
- easy to handle

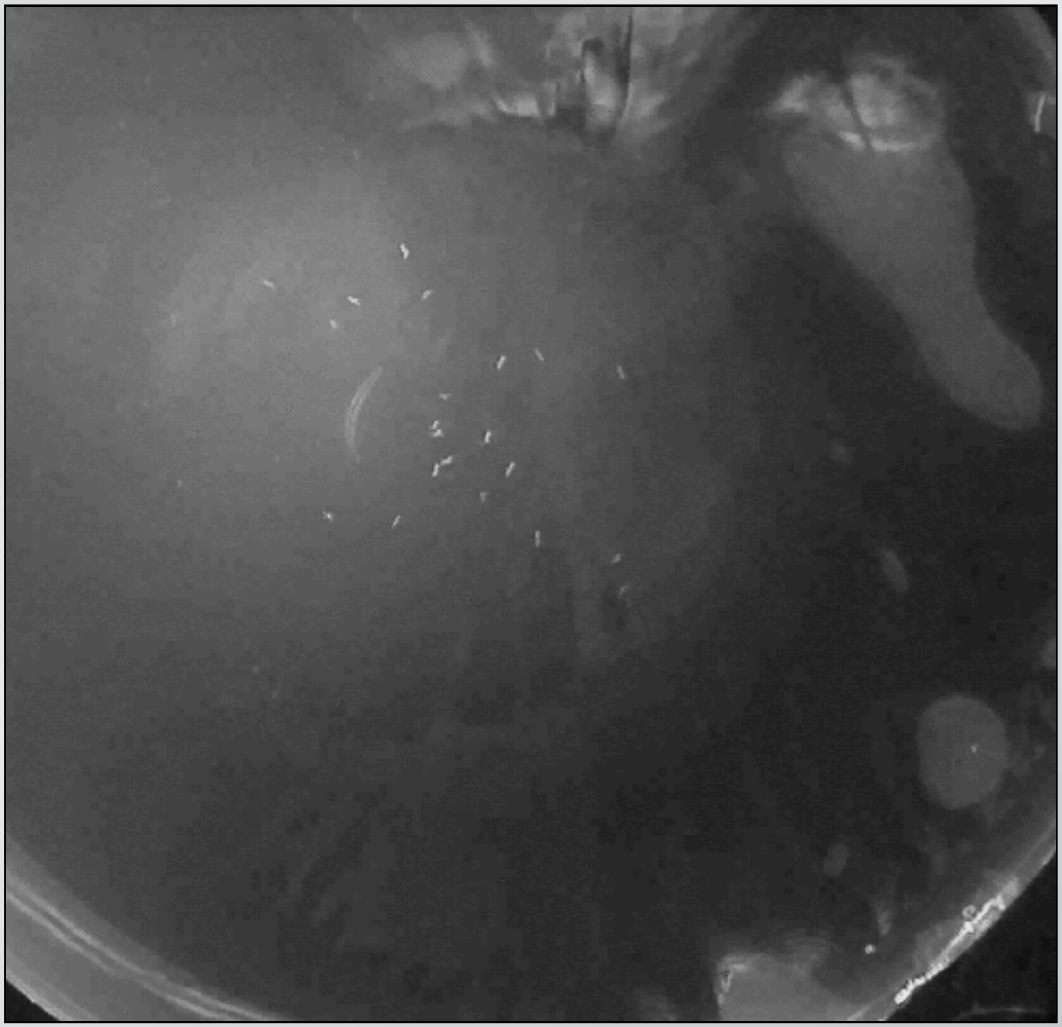
Nanoneurosurgery

Caenorhabditis elegans

- 80 µm x 1 mm
- about 1000 cells
- 302 neurons
- invariant wiring diagram
- neuronal system completely encodes behavior

Nanoneurosurgery

Caenorhabditis elegans

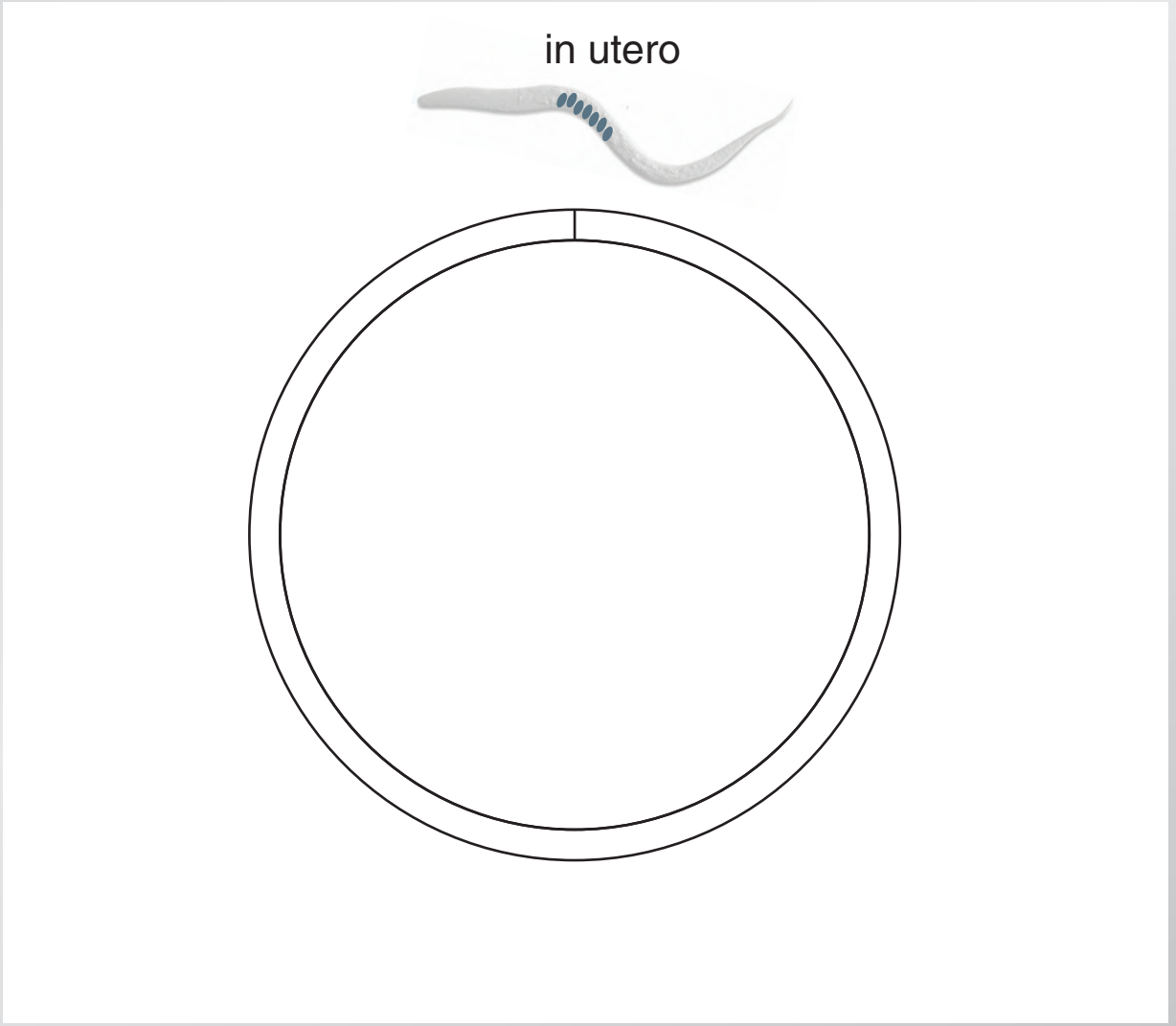


Nanoneurosurgery

Caenorhabditis elegans

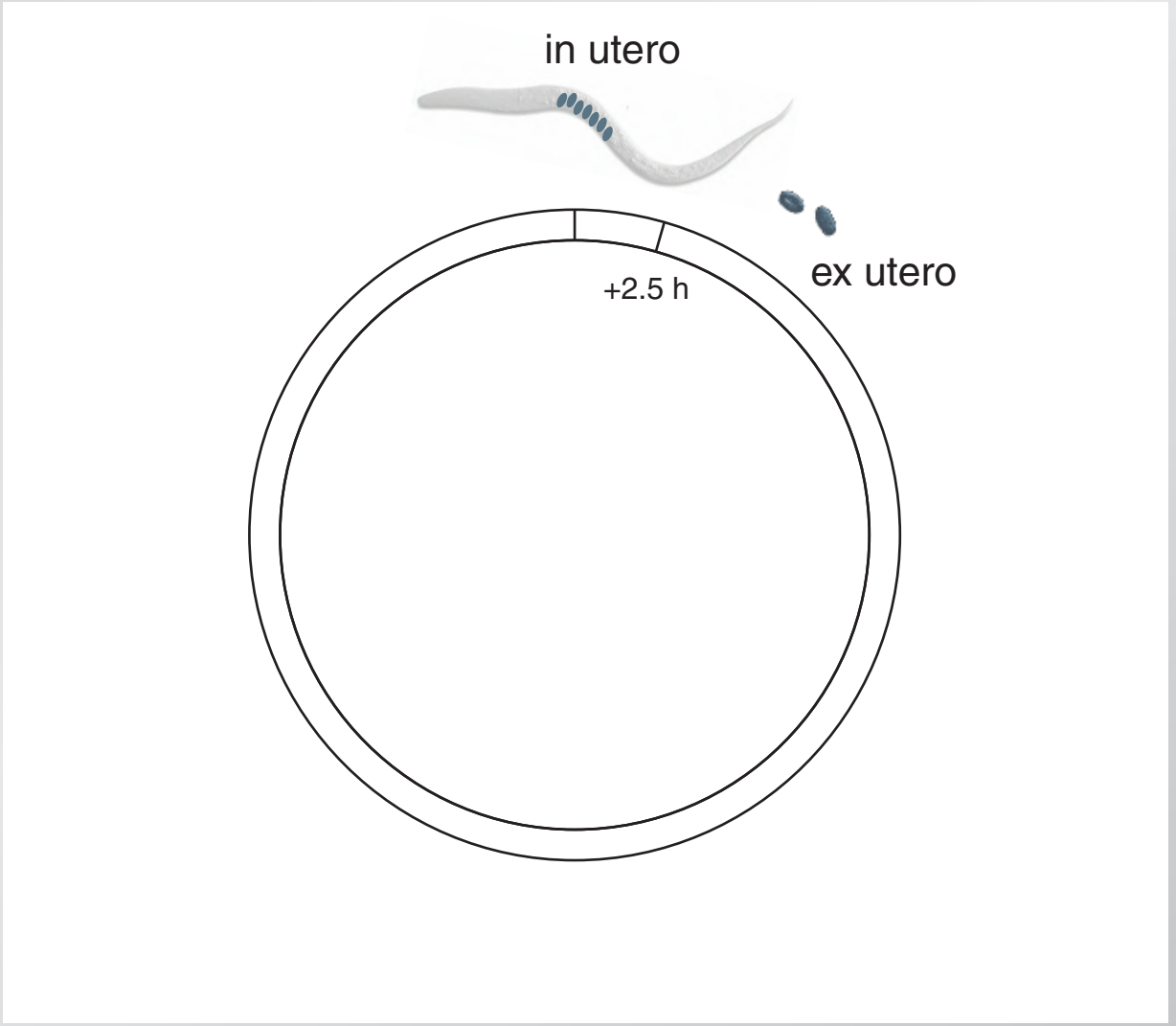
Nanoneurosurgery

C. elegans life cycle



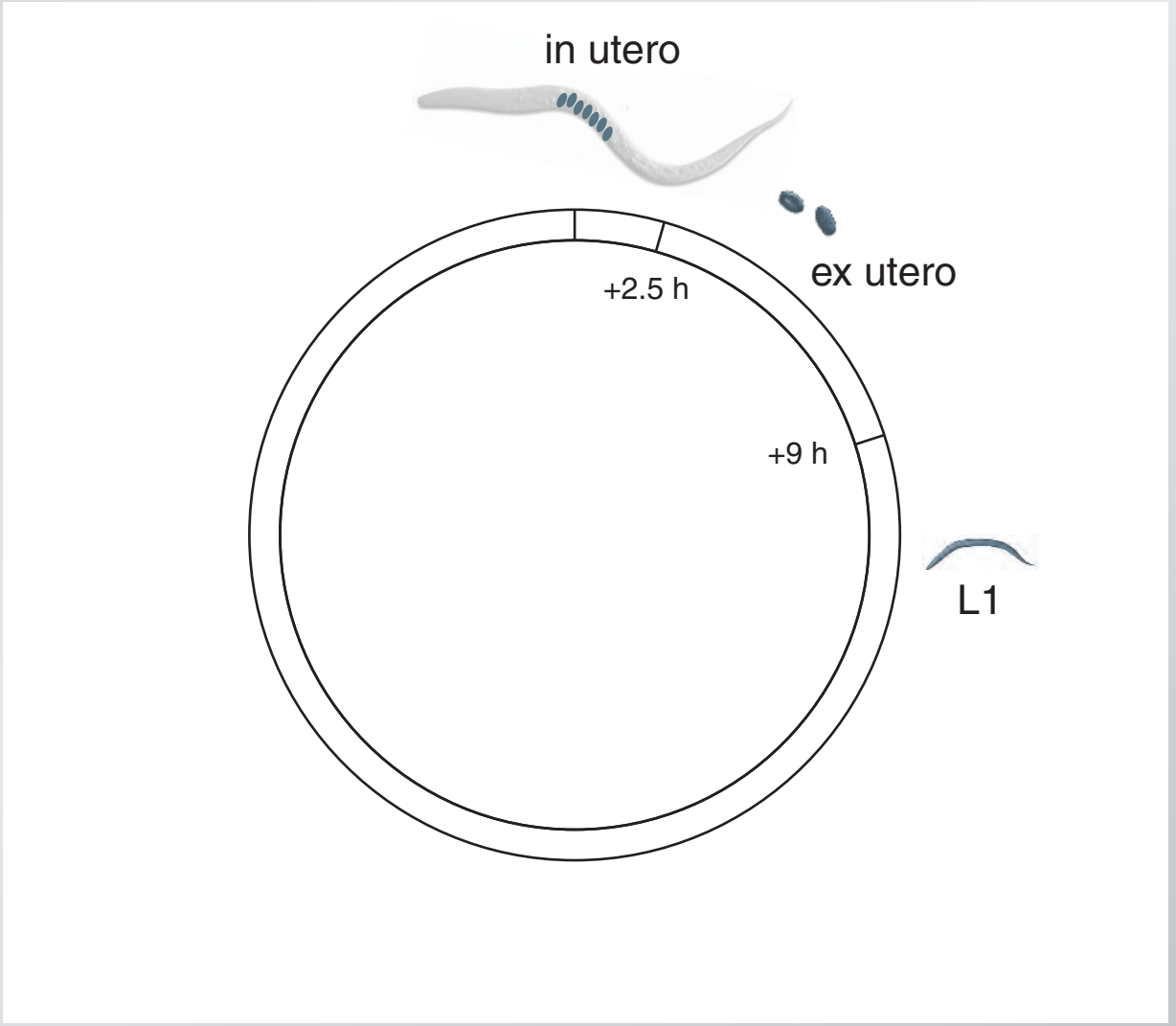
Nanoneurosurgery

C. elegans life cycle



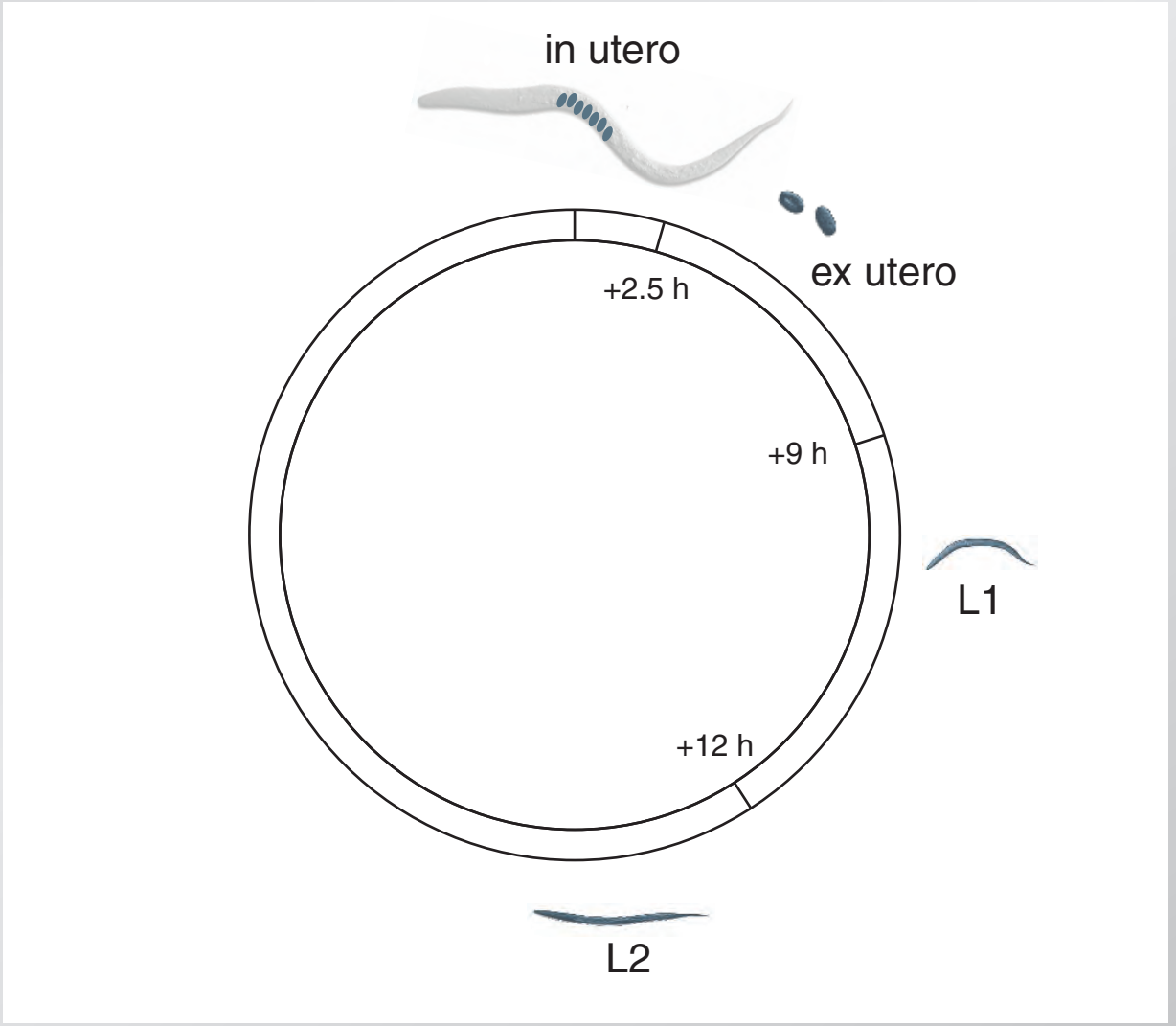
Nanoneurosurgery

C. elegans life cycle



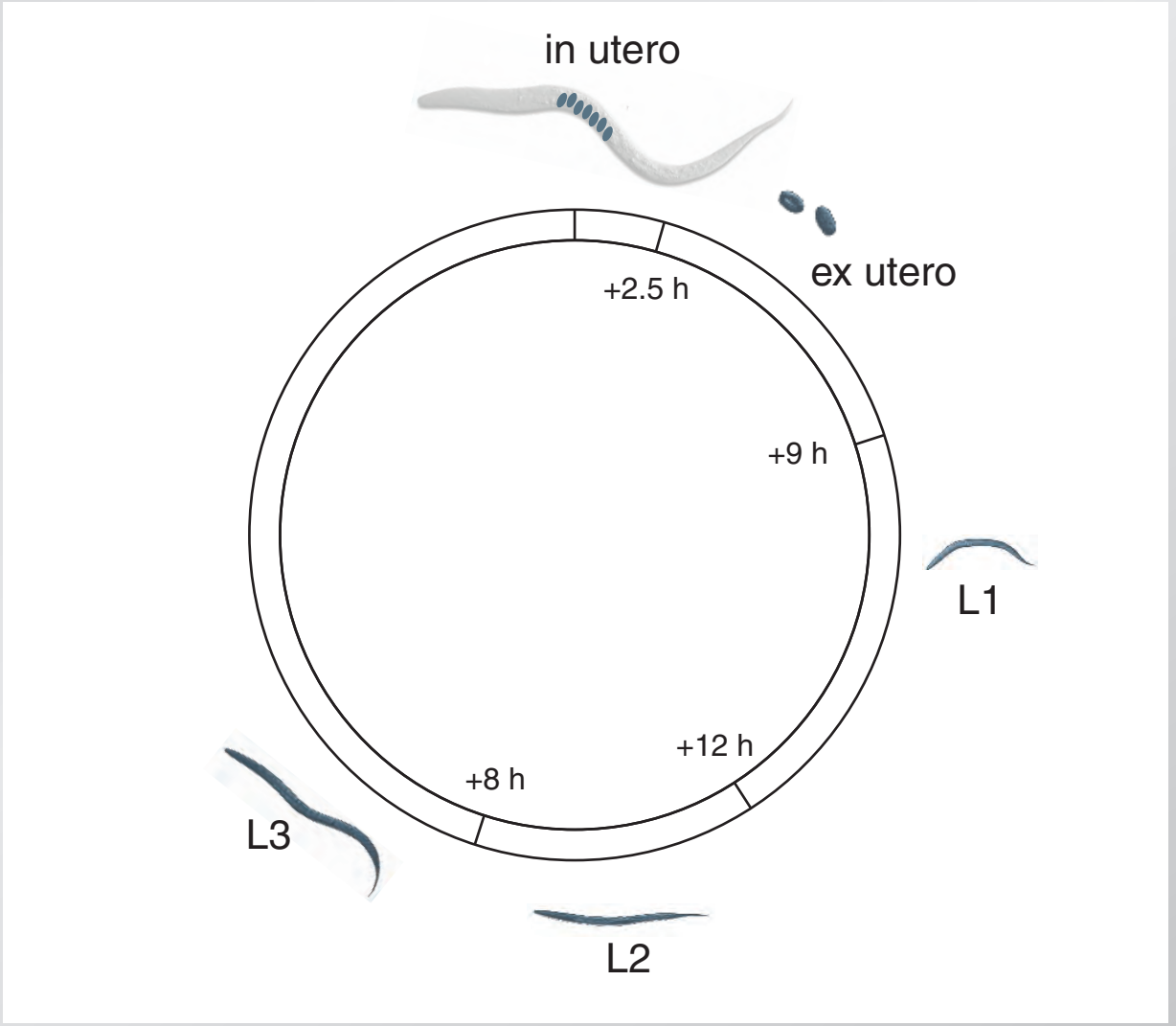
Nanoneurosurgery

C. elegans life cycle



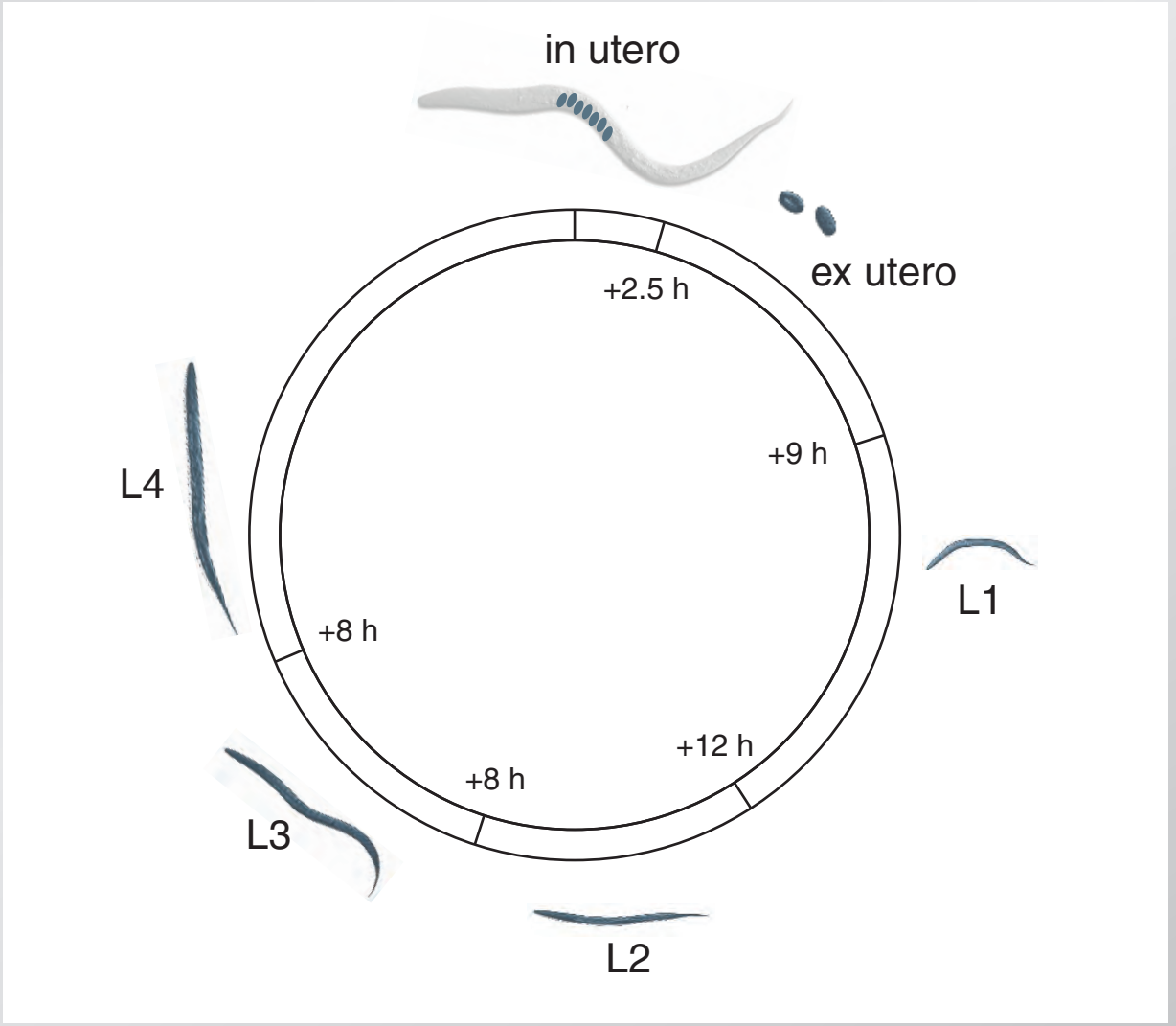
Nanoneurosurgery

C. elegans life cycle



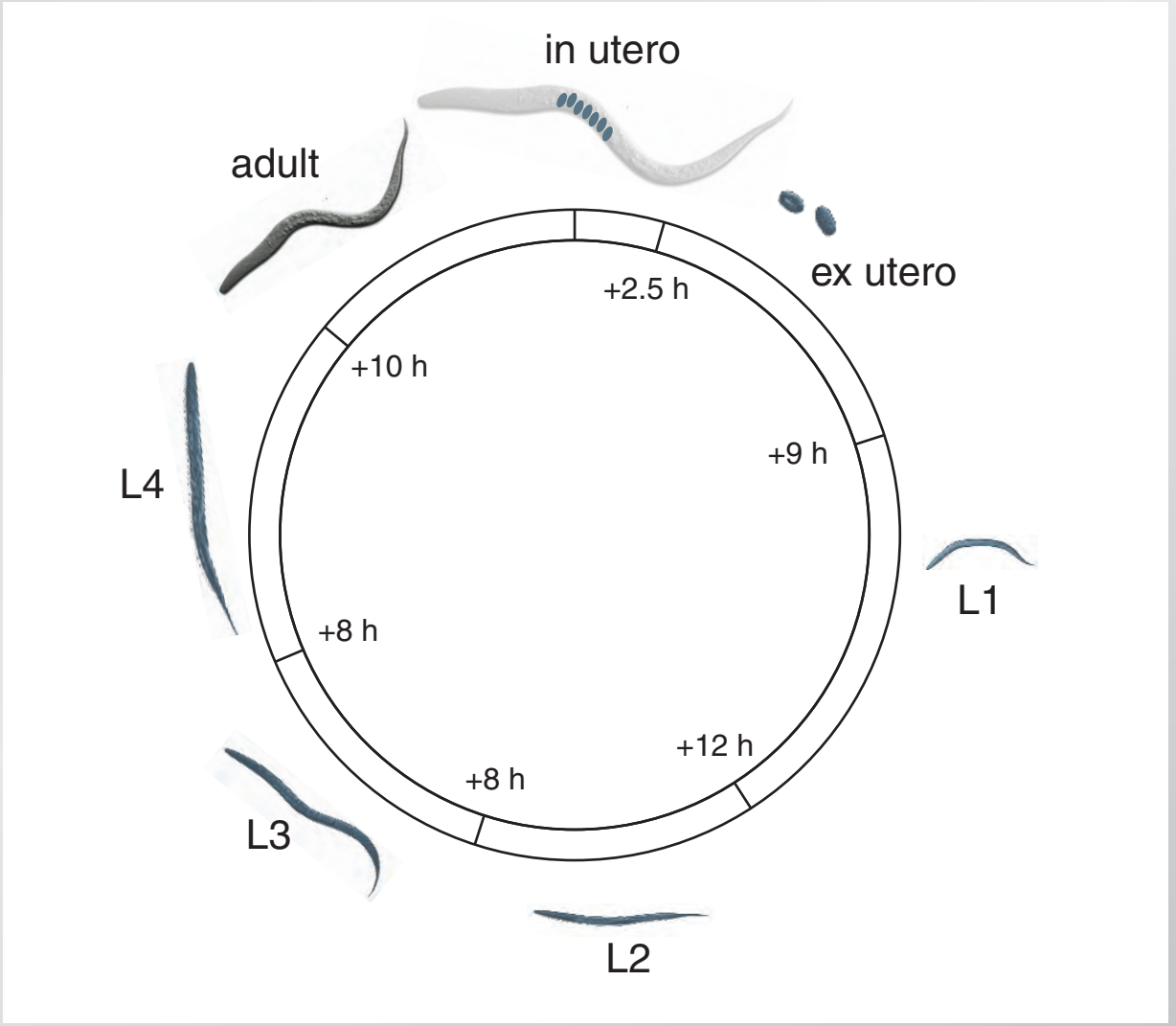
Nanoneurosurgery

C. elegans life cycle



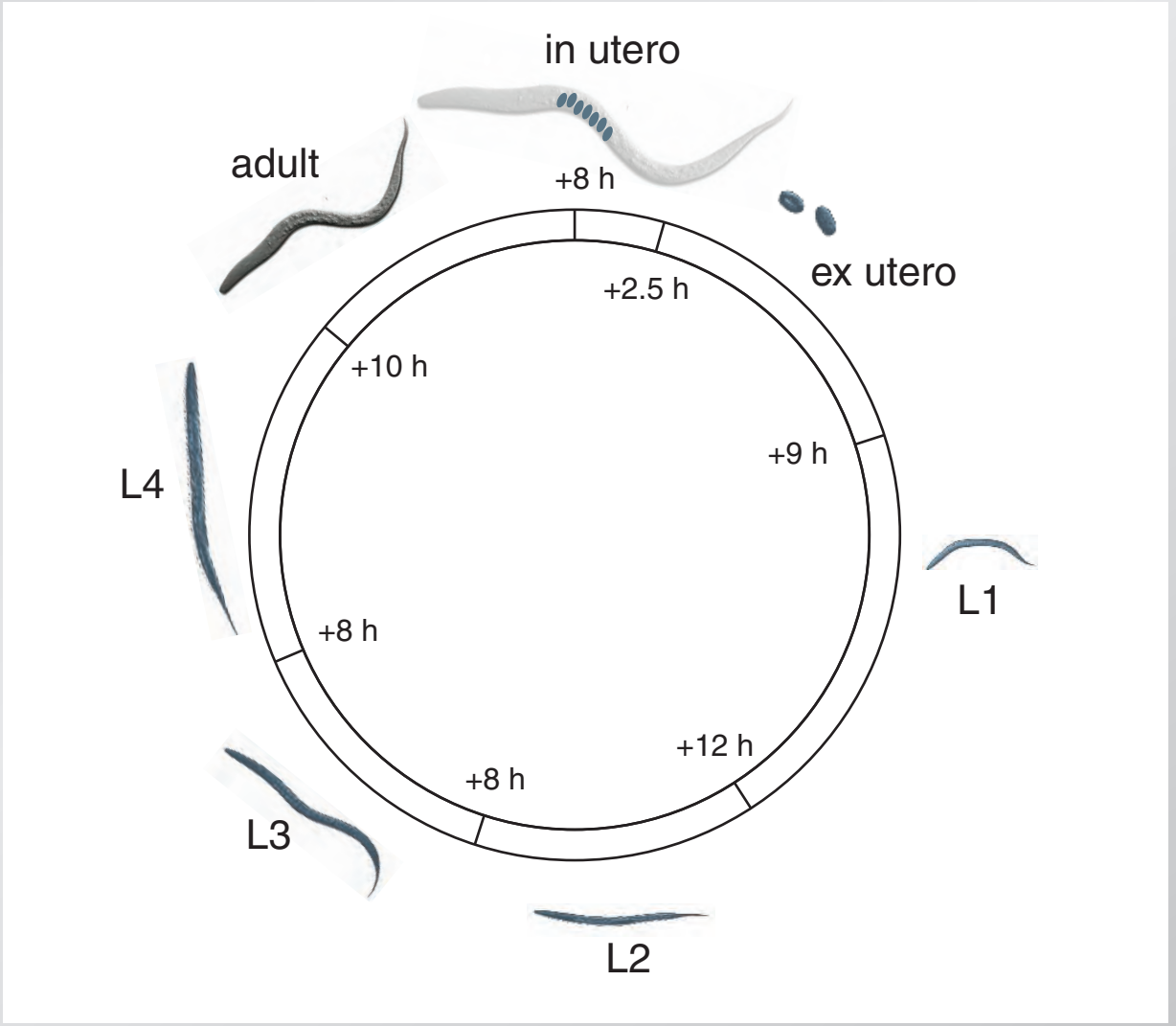
Nanoneurosurgery

C. elegans life cycle



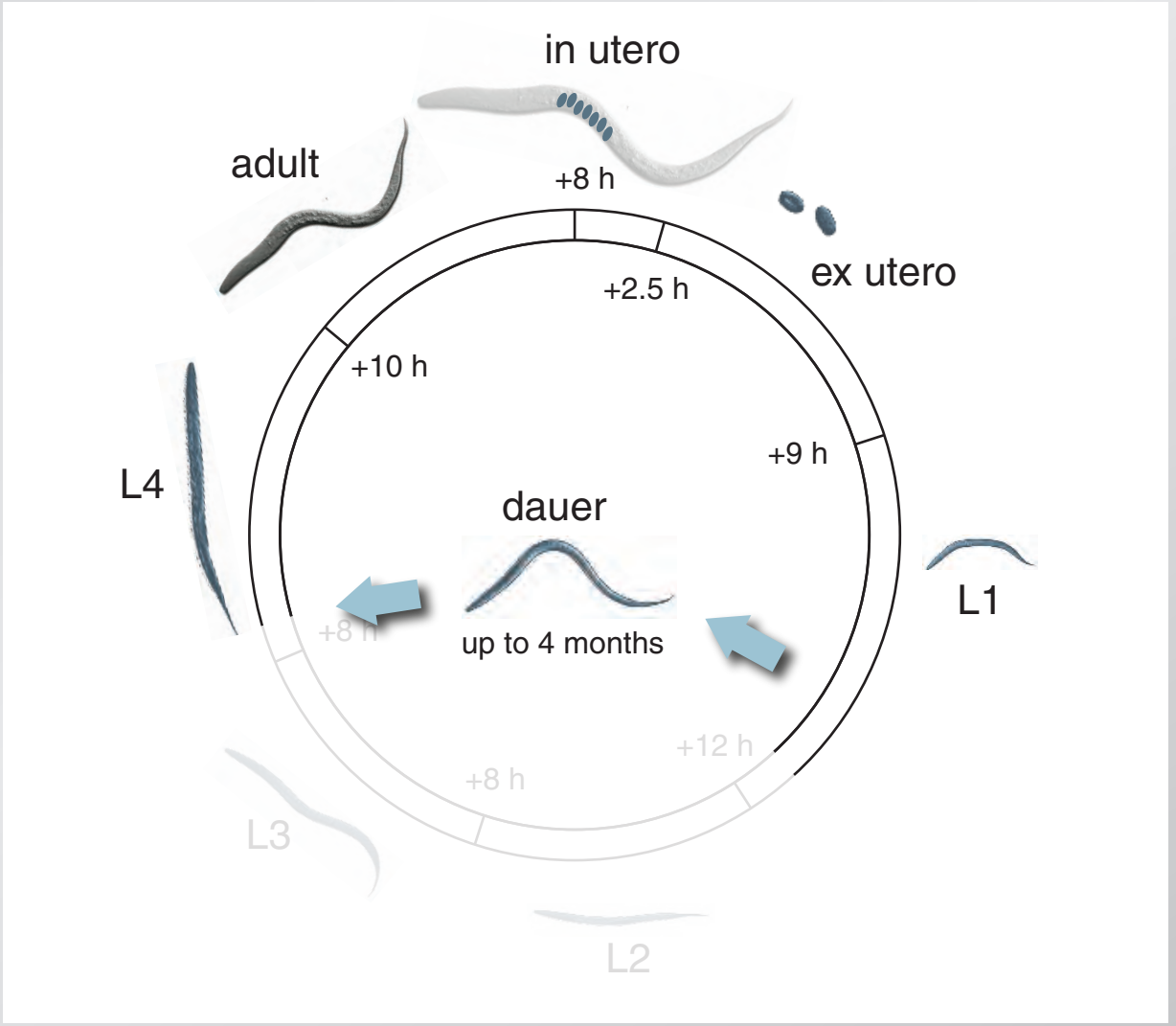
Nanoneurosurgery

C. elegans life cycle



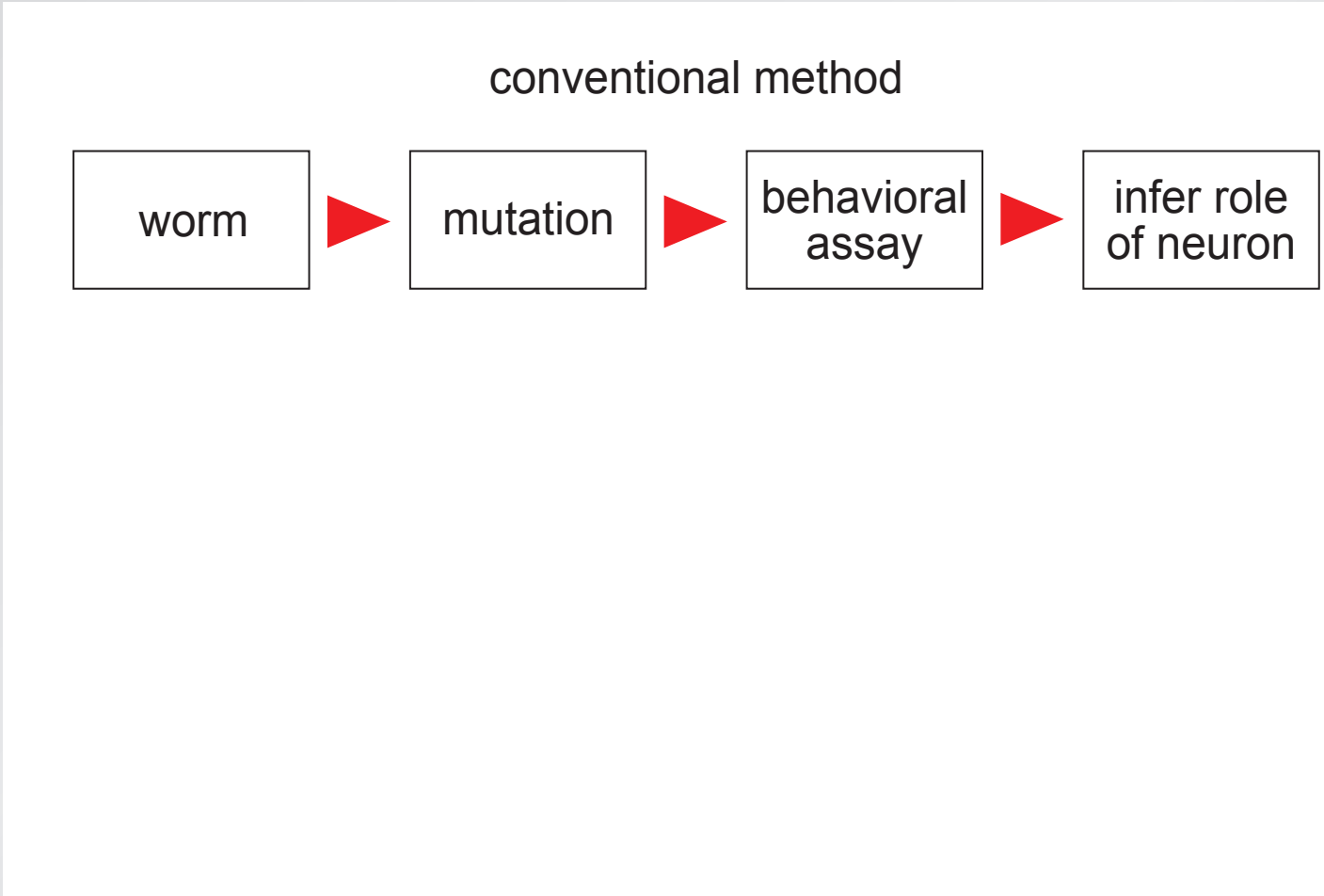
Nanoneurosurgery

C. elegans life cycle



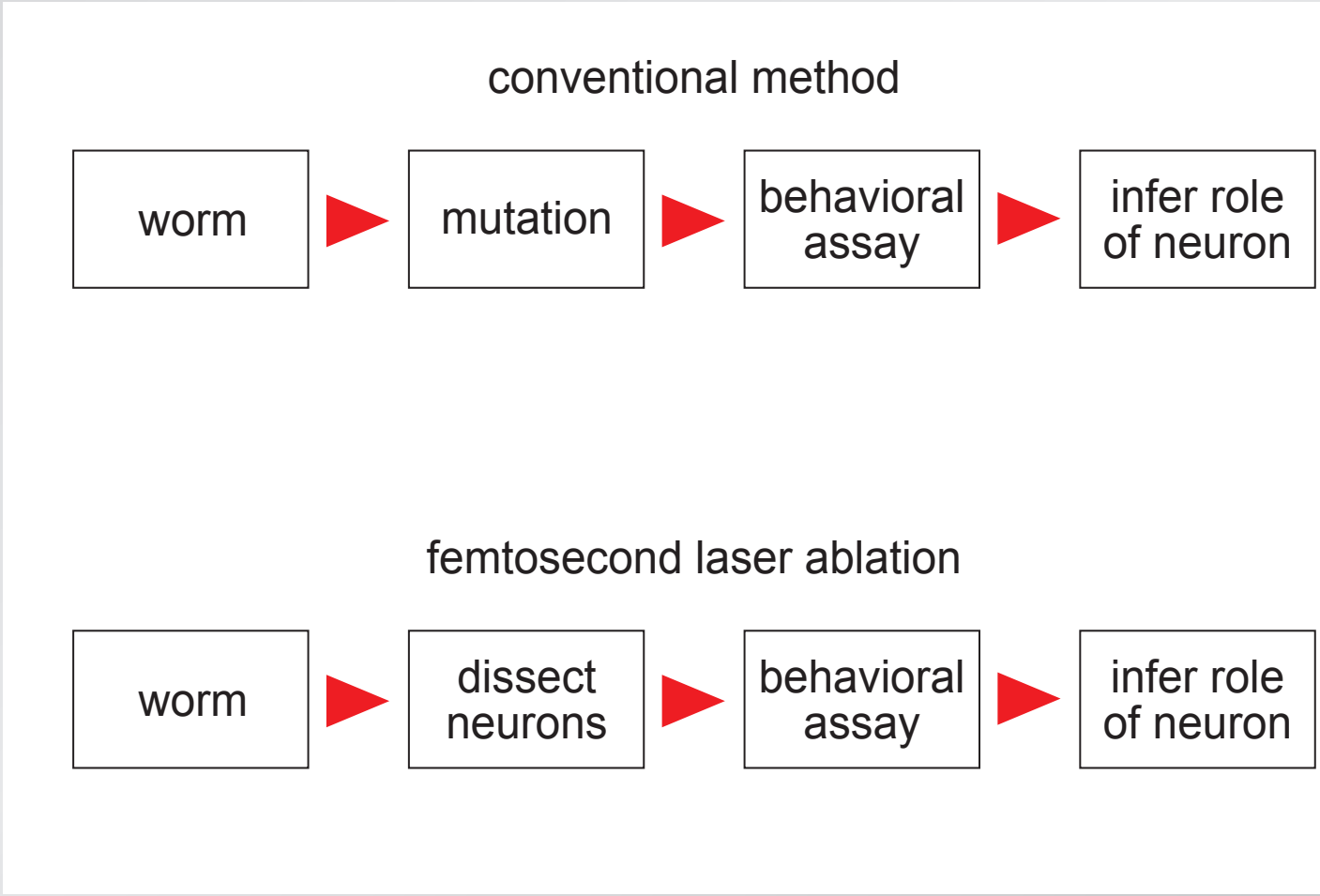
Nanoneurosurgery

Mapping behavior to neurons



Nanoneurosurgery

Mapping behavior to neurons



Nanoneurosurgery

ASH neurons

- responsible for chemical sensing
- ciliary projections extend through skin
- one on each side

Nanoneurosurgery

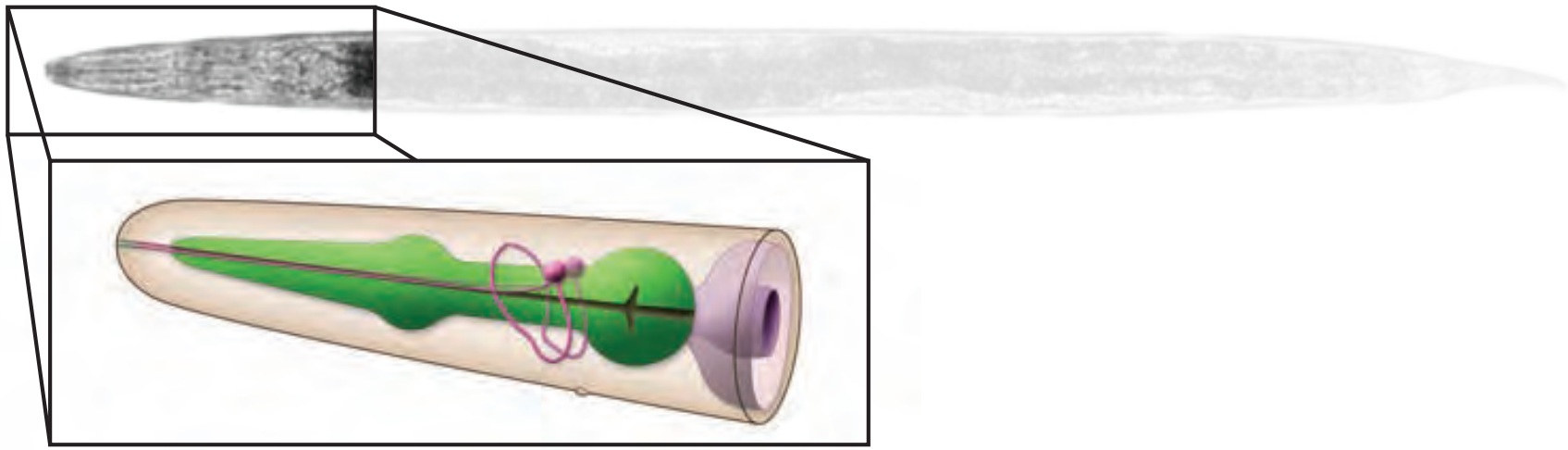
ASH neurons

Nanoneurosurgery

ASH neurons

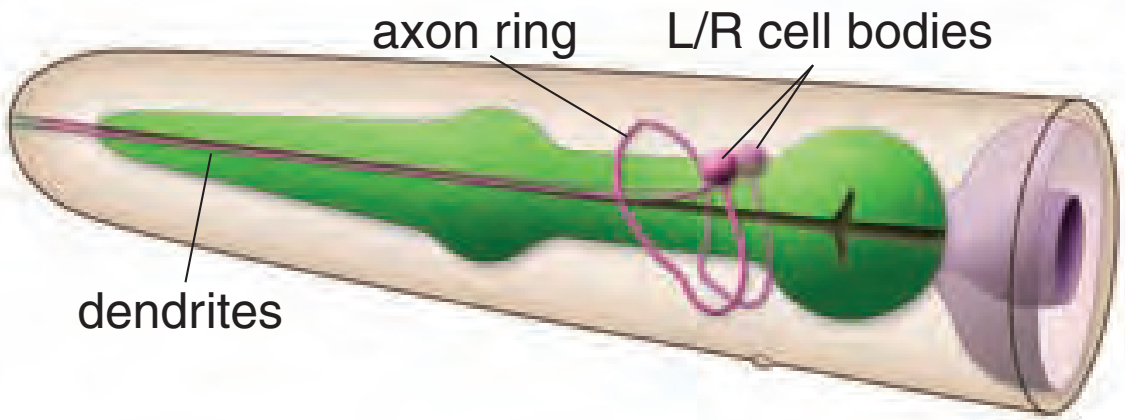
Nanoneurosurgery

ASH neurons



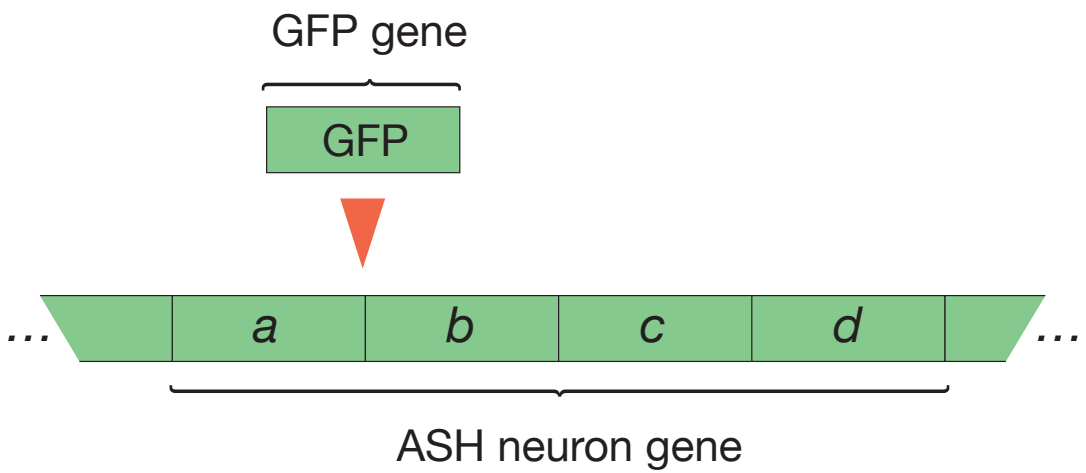
Nanoneurosurgery

ASH neurons



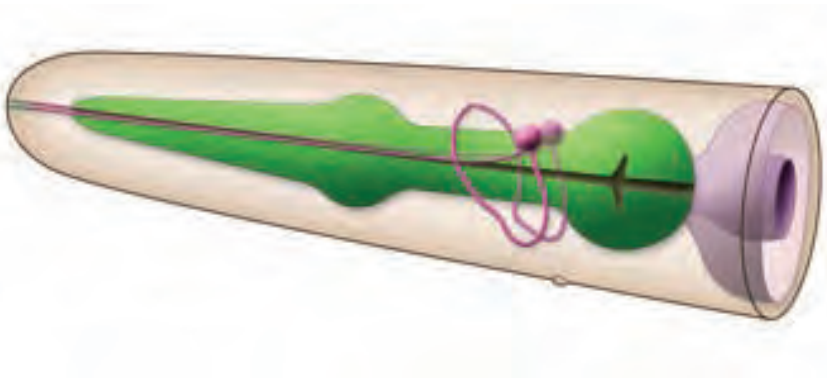
Nanoneurosurgery

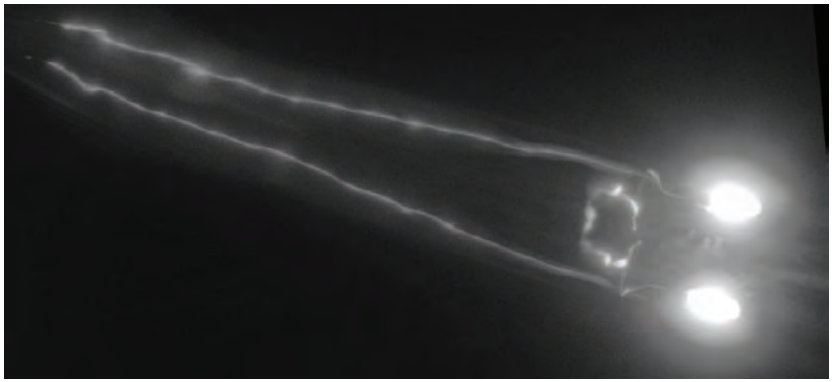
make ASH neurons express GFP



Nanoneurosurgery

make ASH neurons express GFP



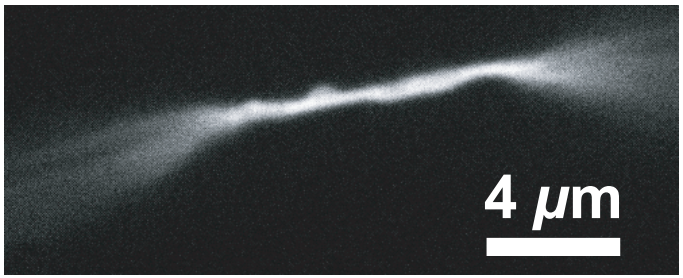


Nanoneurosurgery

GFP: absorbs UV, emits green

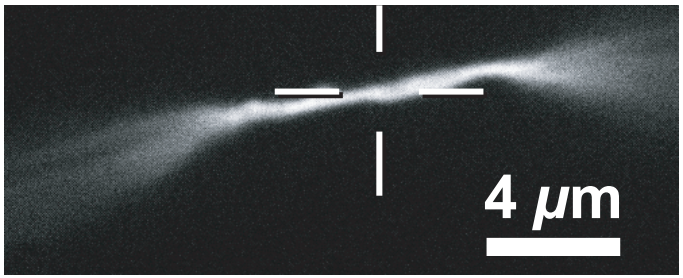
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



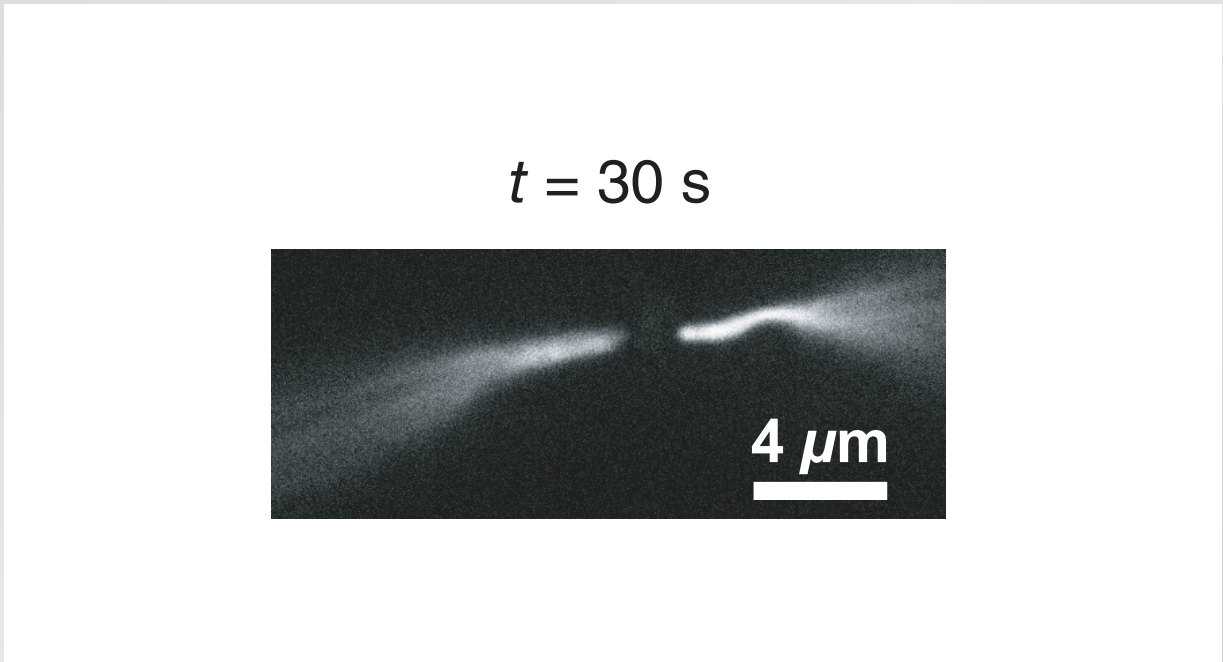
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



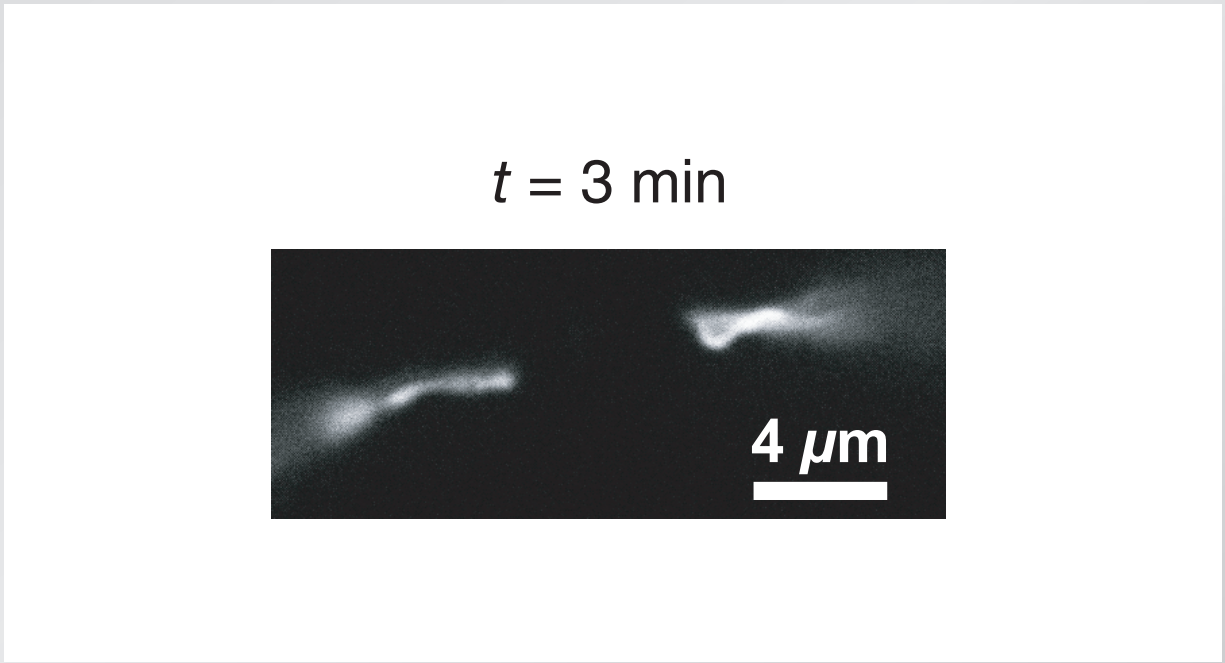
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



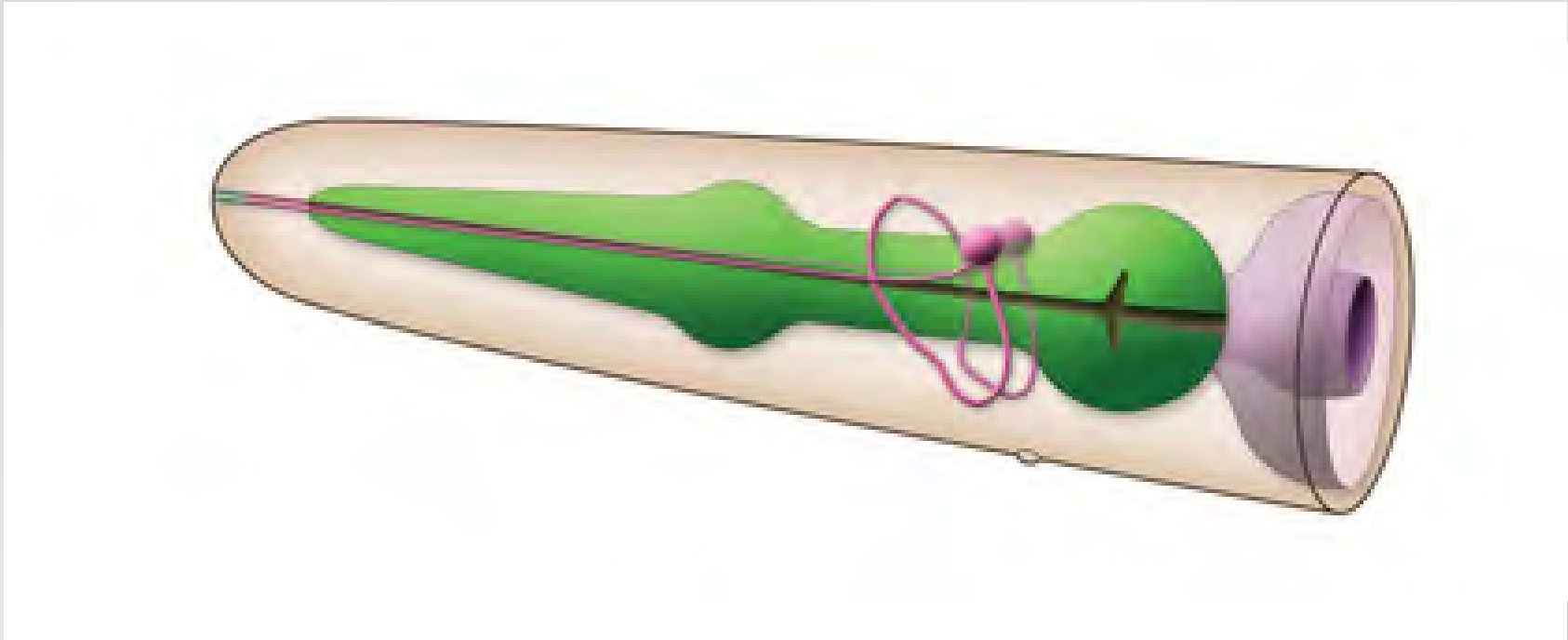
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



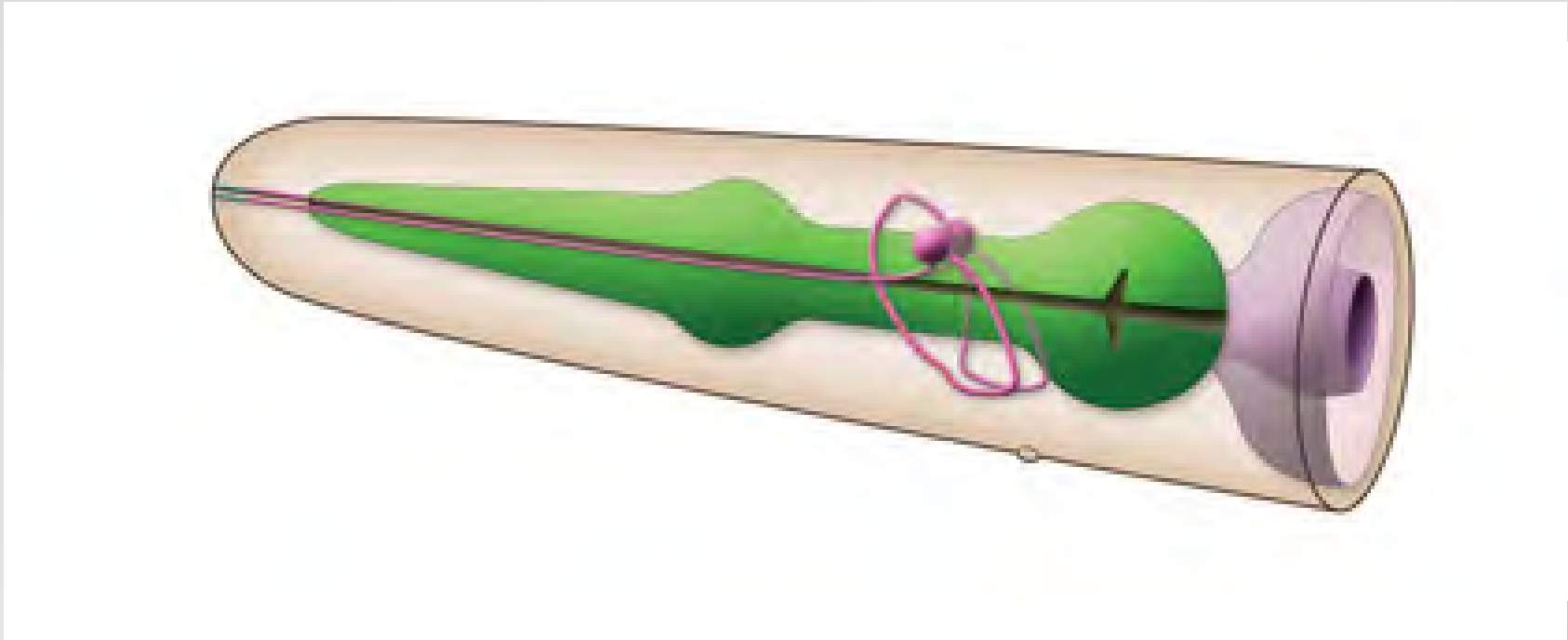
Nanoneurosurgery

ASH neurons



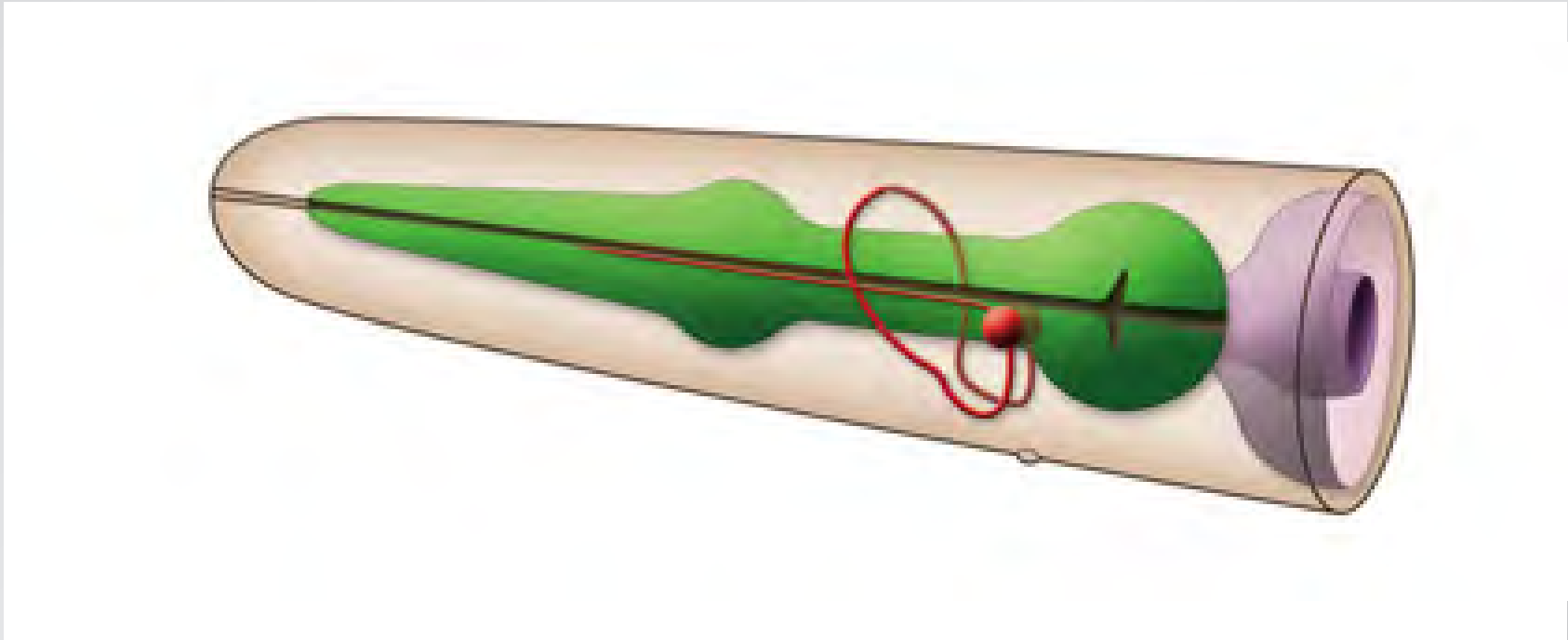
Nanoneurosurgery

ASK neurons



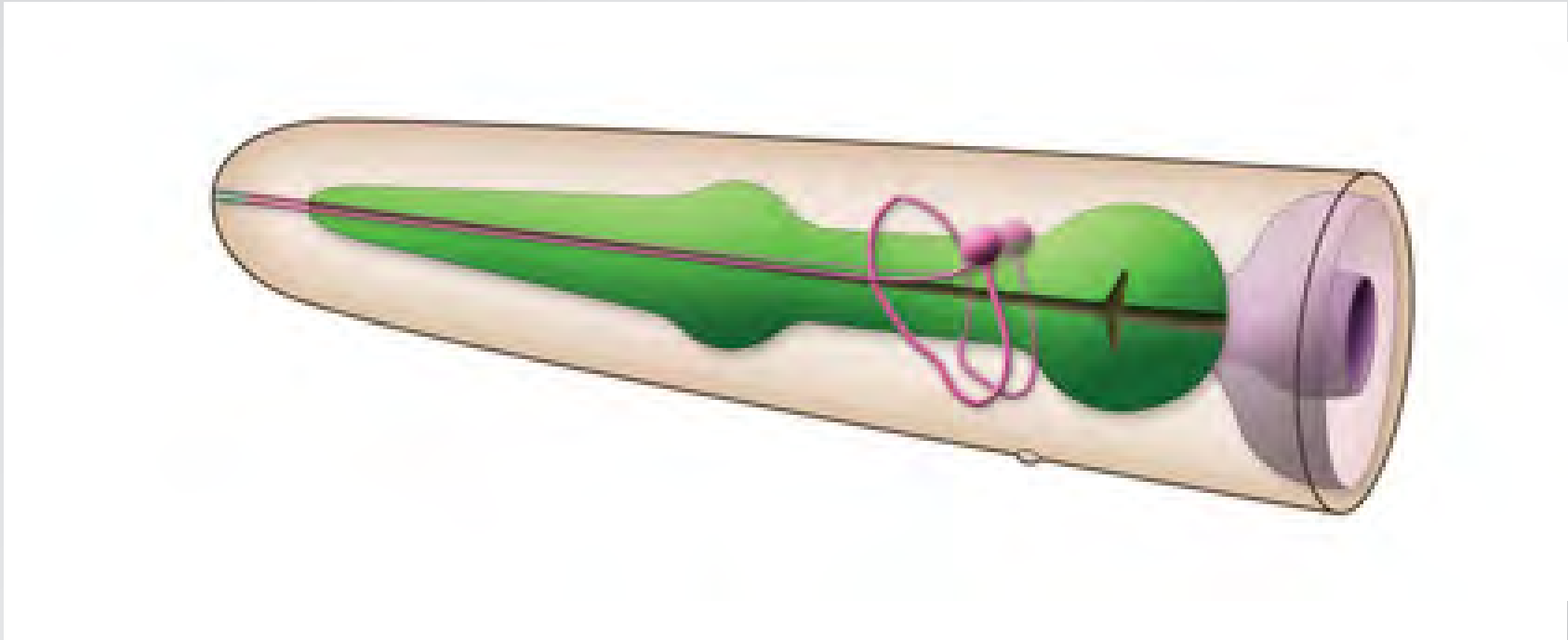
Nanoneurosurgery

AUA neurons



Nanoneurosurgery

ASI neurons

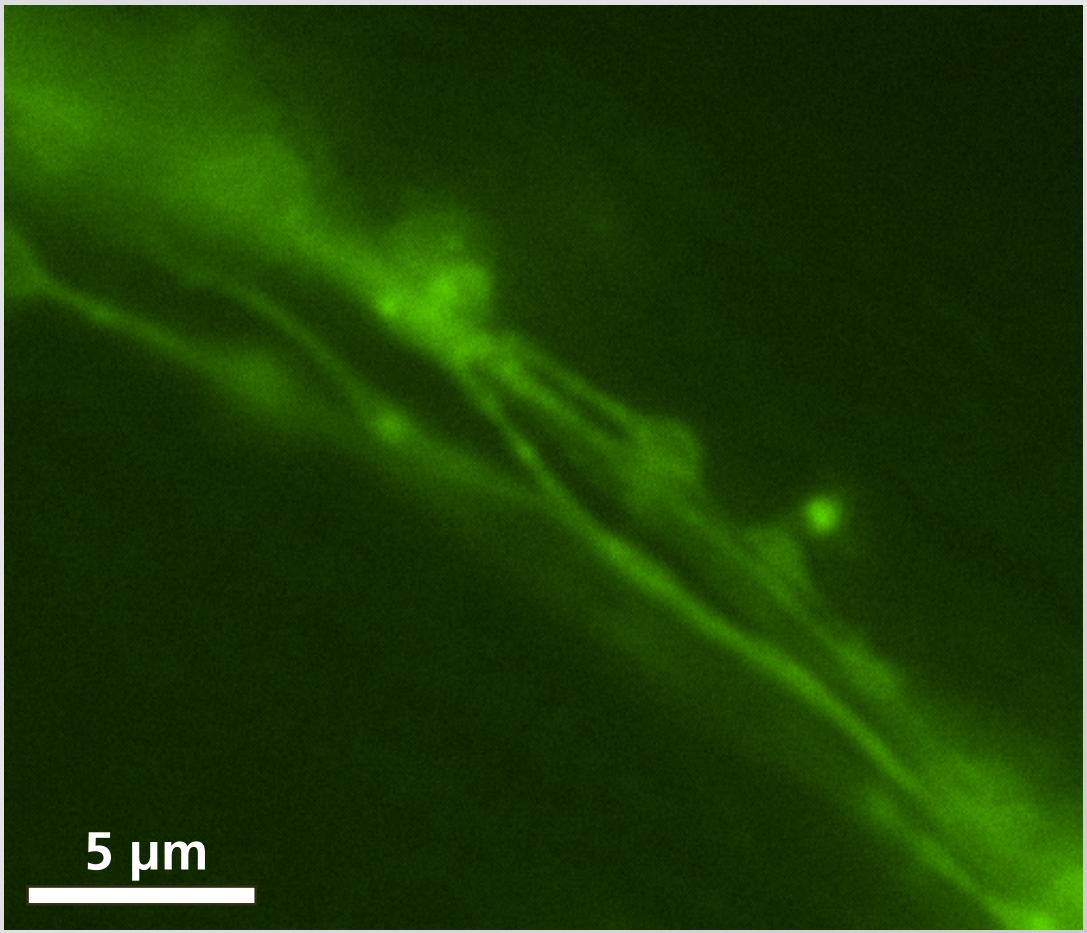


Nanoneurosurgery

need exquisite precision!

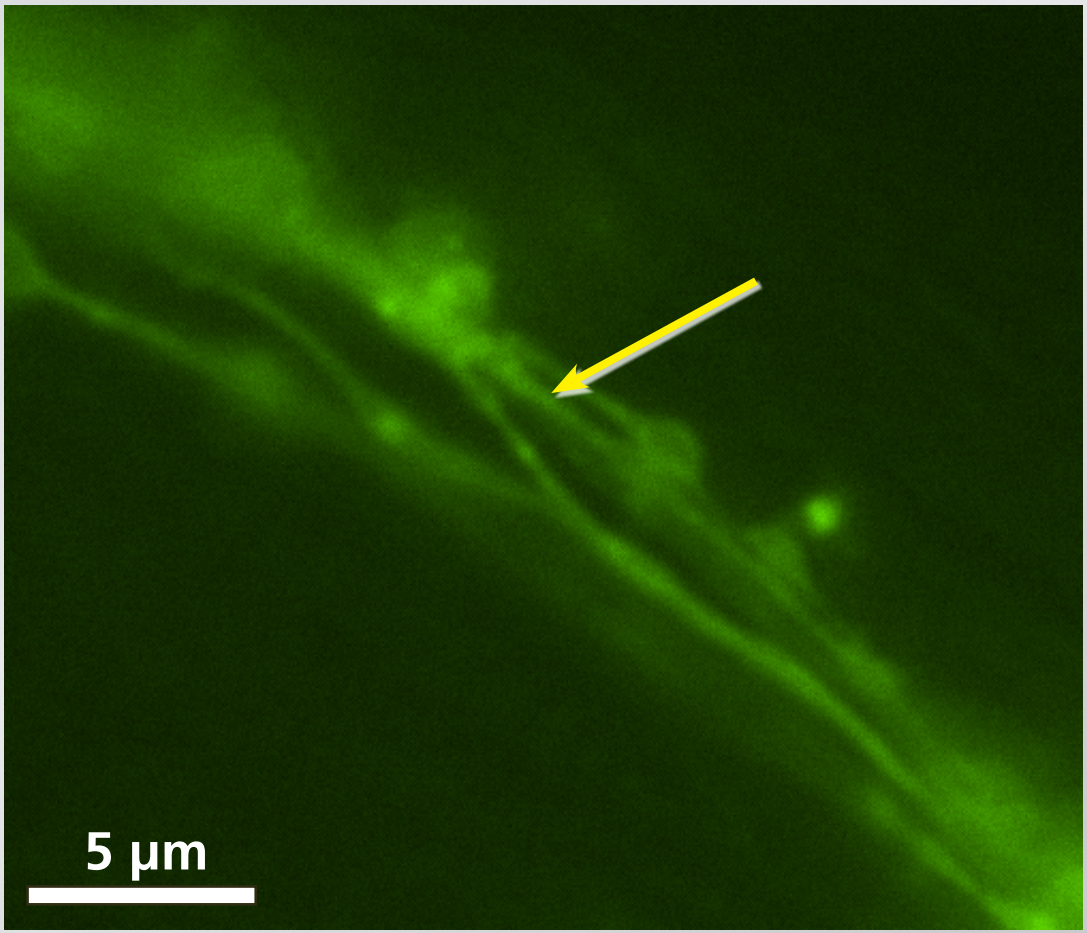
Nanoneurosurgery

DiO-stained bundle of dendrites



Nanoneurosurgery

cut single dendrite in bundle (3 nJ)



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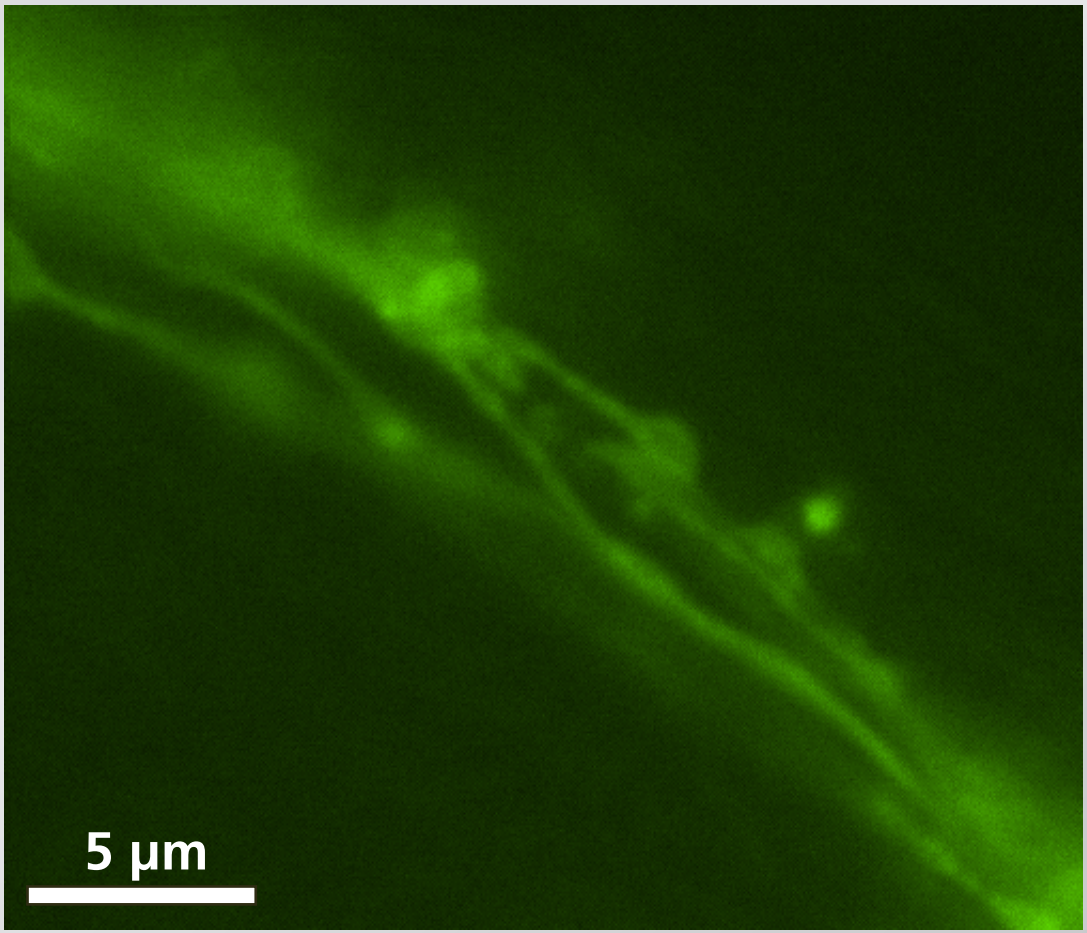
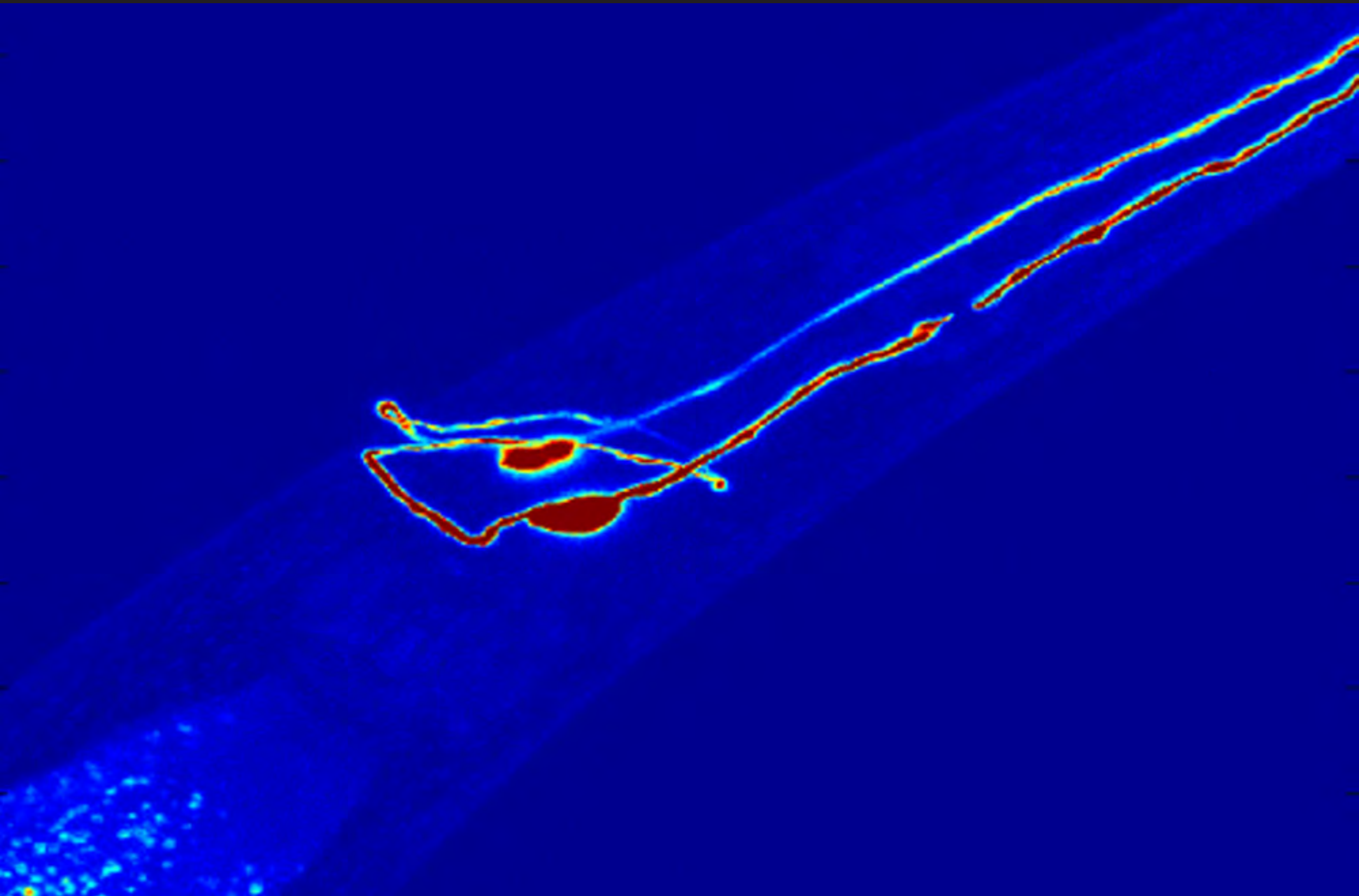
no damange to neighboring dendrites



Nanoneurosurgery

revive worm, reimage 1 day later

Nanoneurosurgery

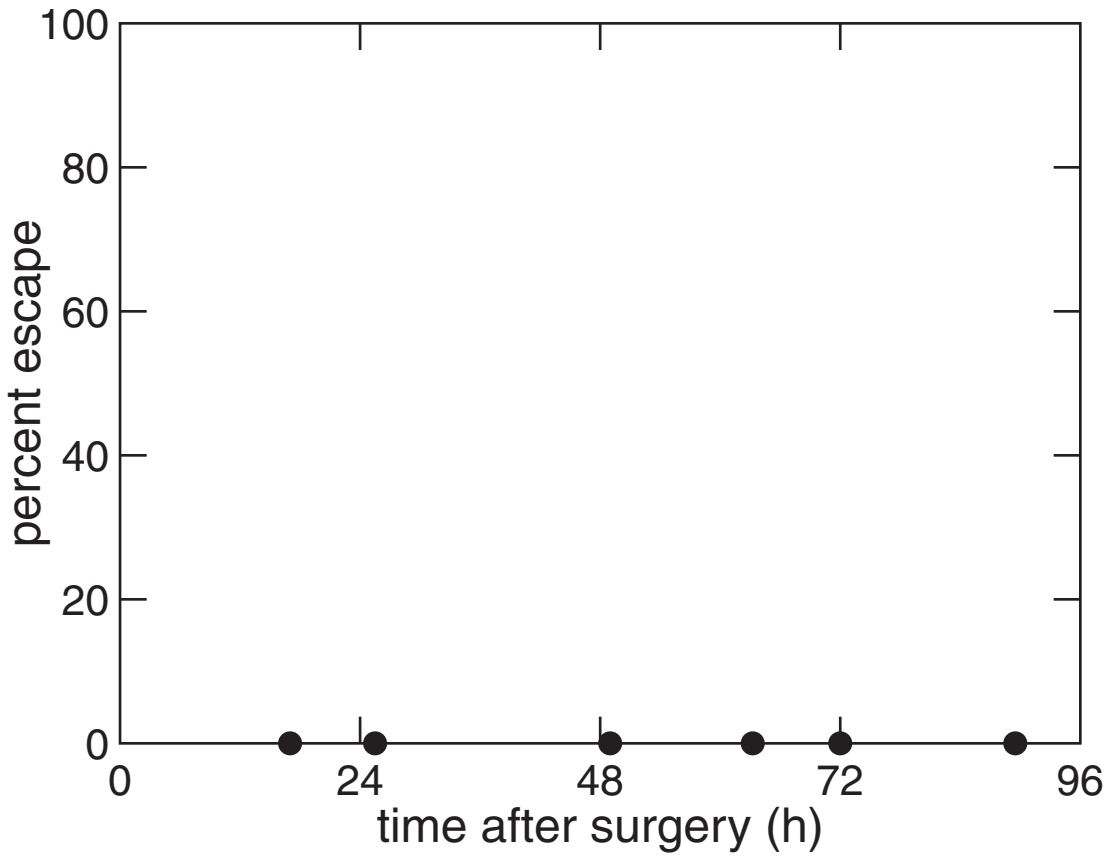


Nanoneurosurgery

osmolarity assay

Nanoneurosurgery

escape rate after ‘mock’ surgery



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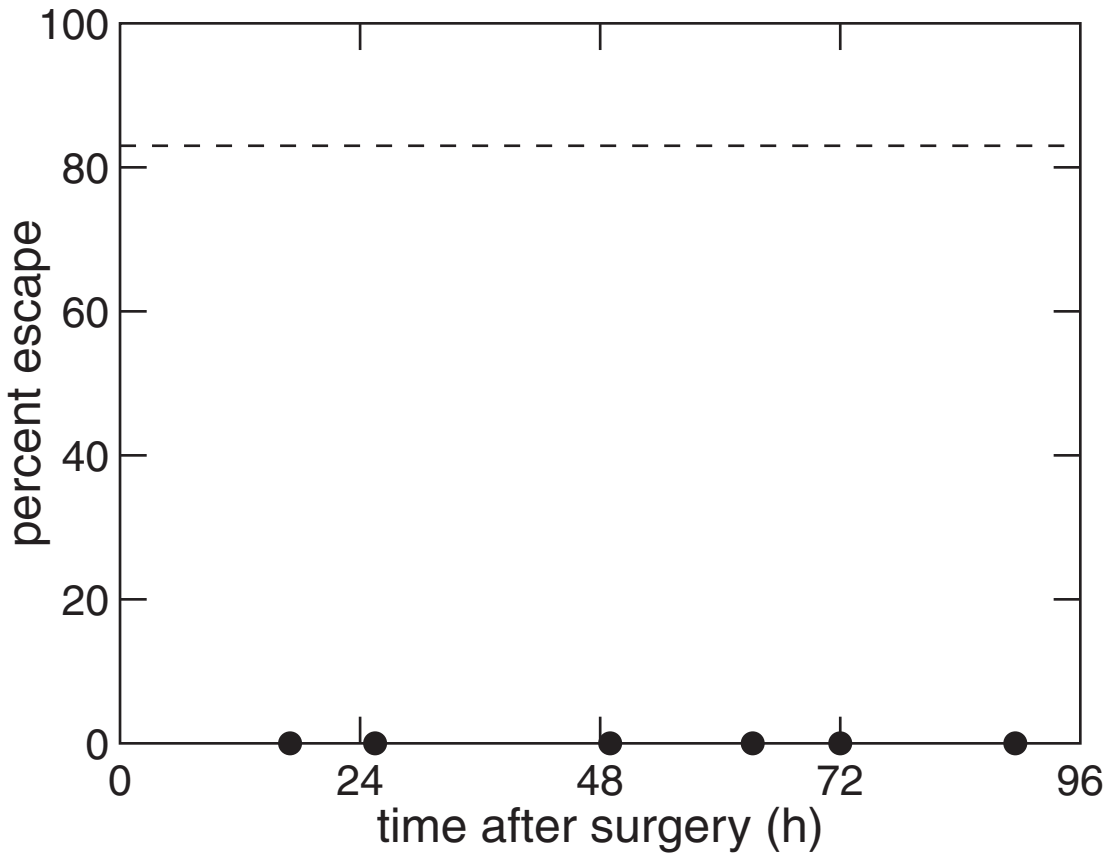
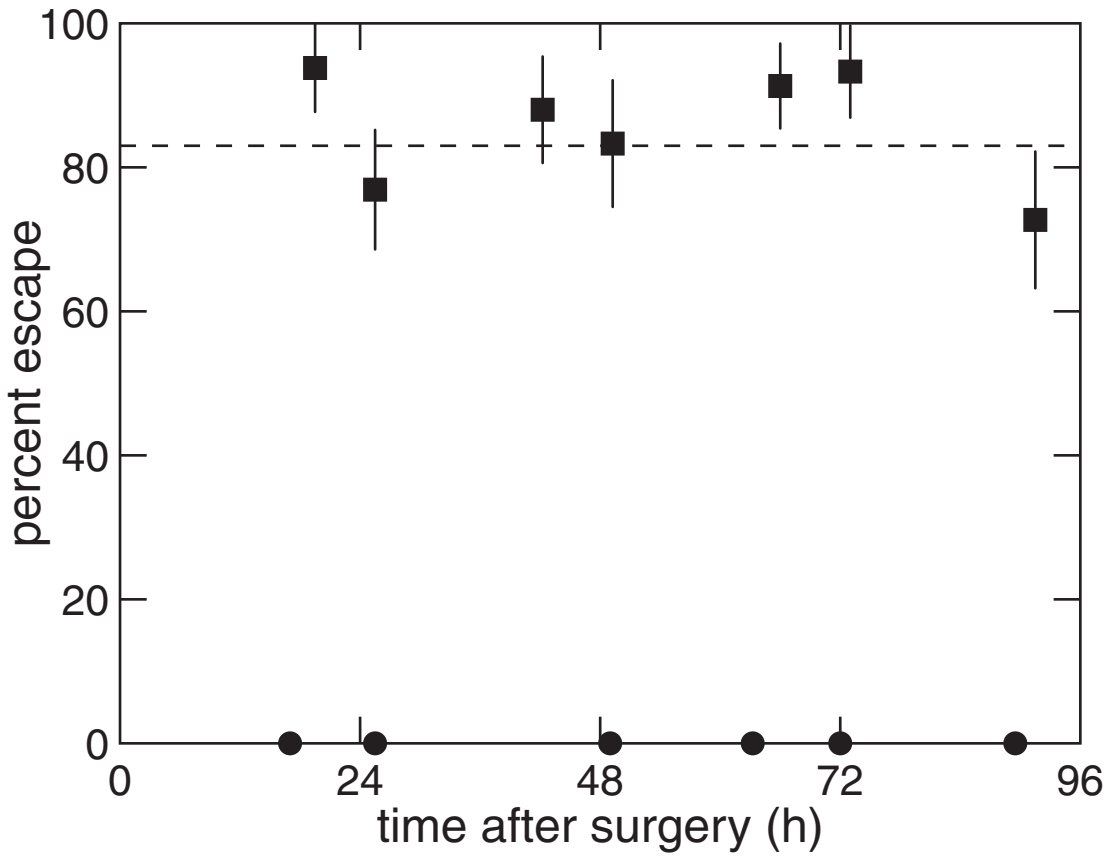
escape rate of ASH-lacking mutant



Nanoneurosurgery

escape rate after ASH-ablation surgery



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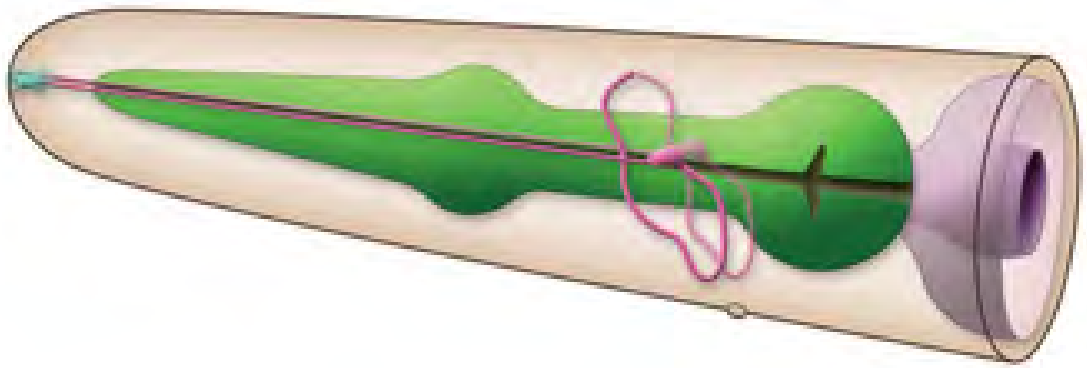
AFD neurons (temperature sensors)



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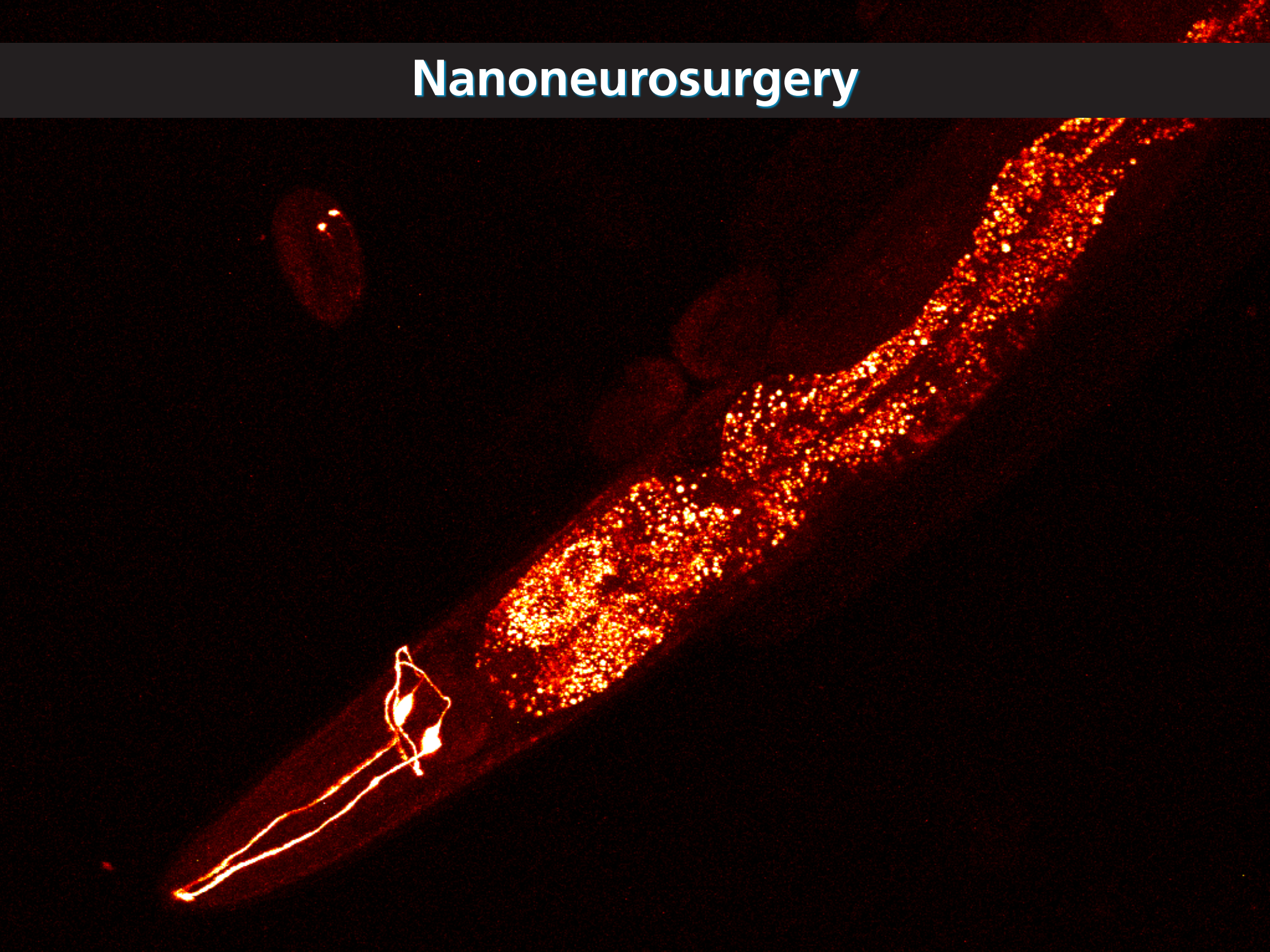
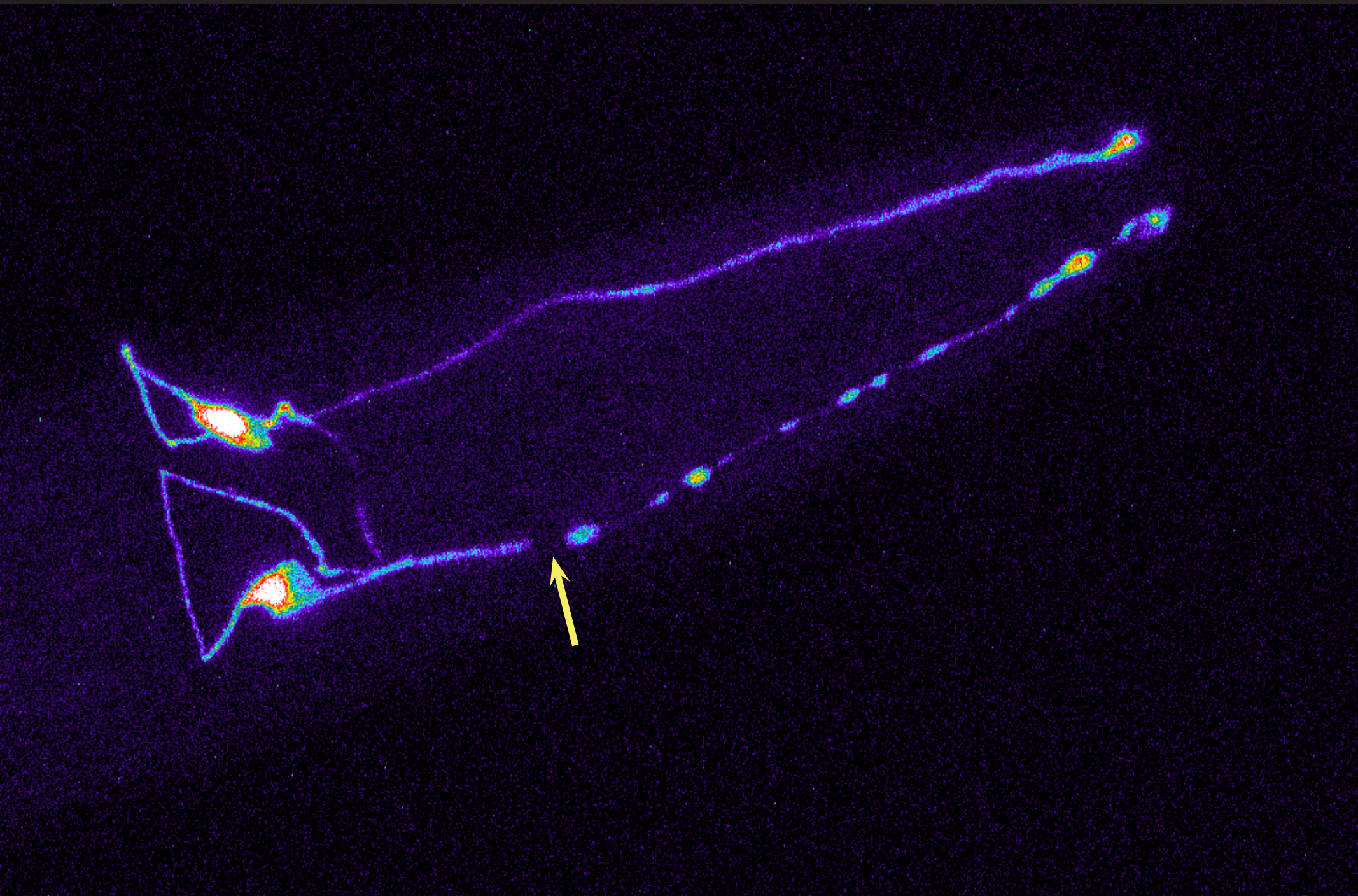


Nanoneurosurgery



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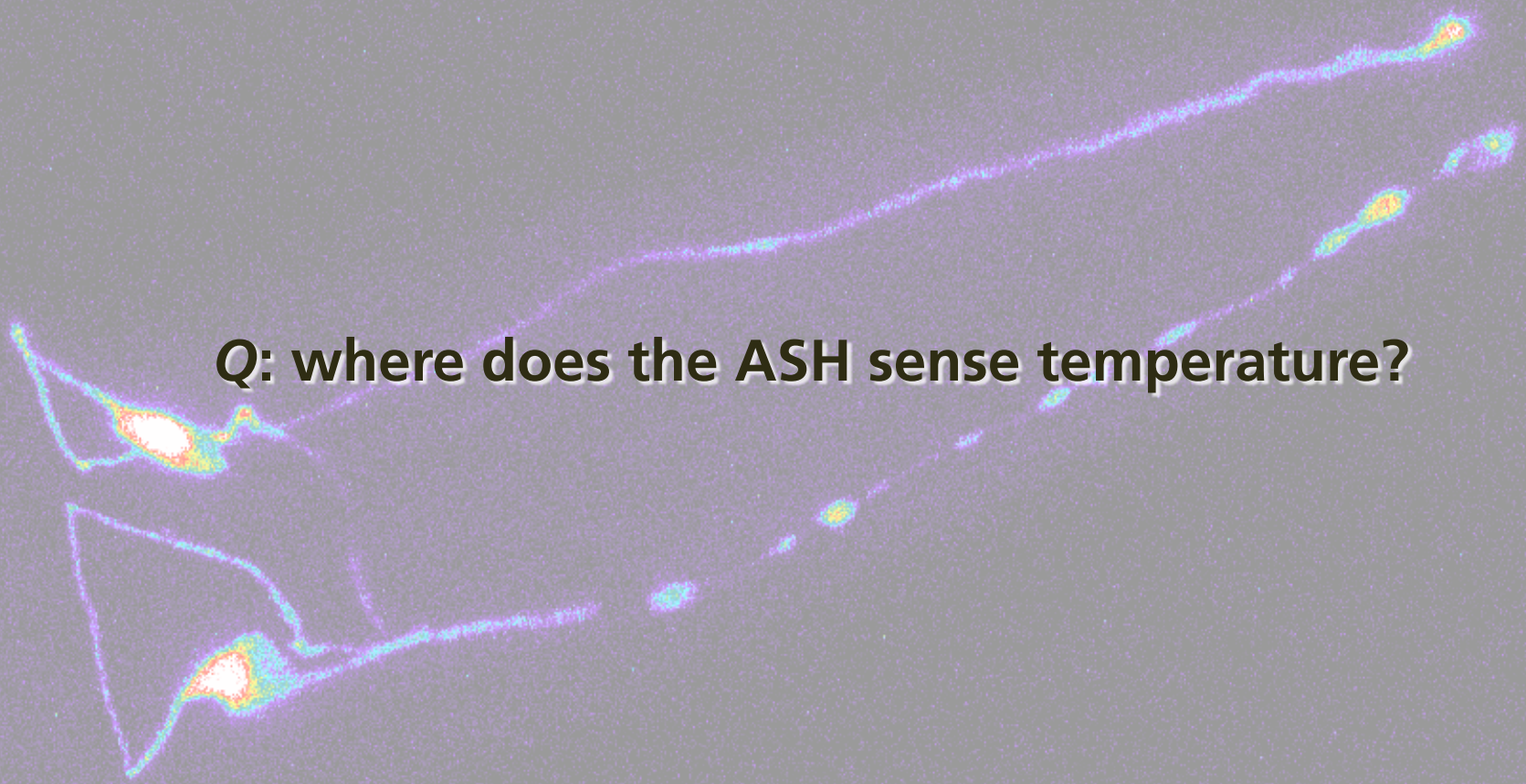
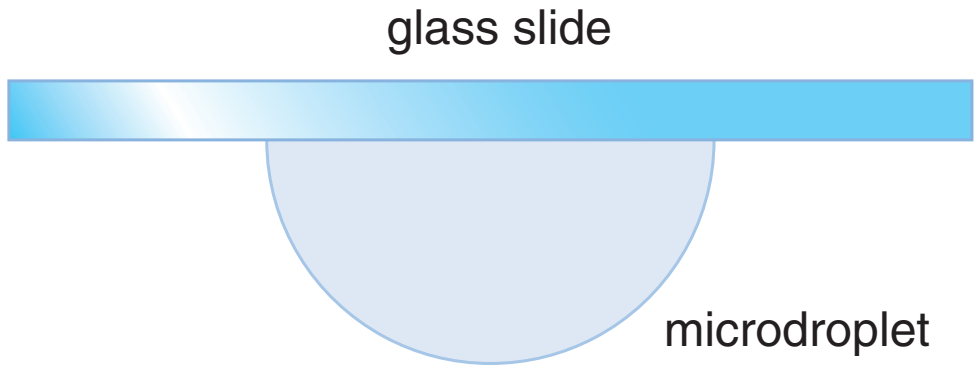


Q: where does the ASH sense temperature?

Nanoneurosurgery

microdroplet assay



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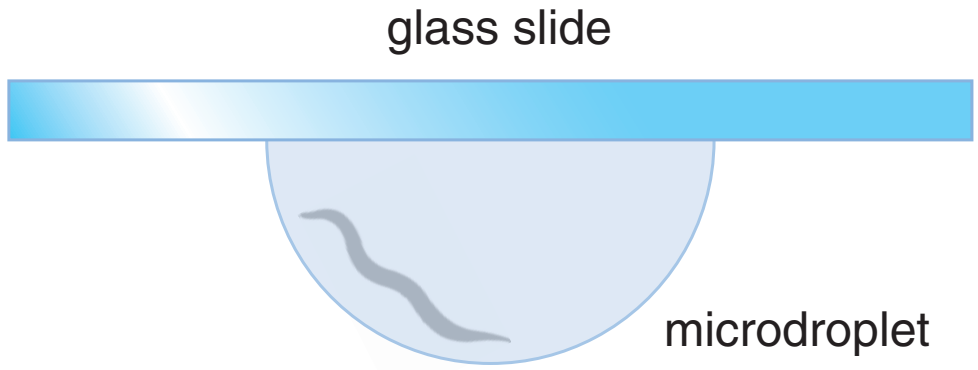
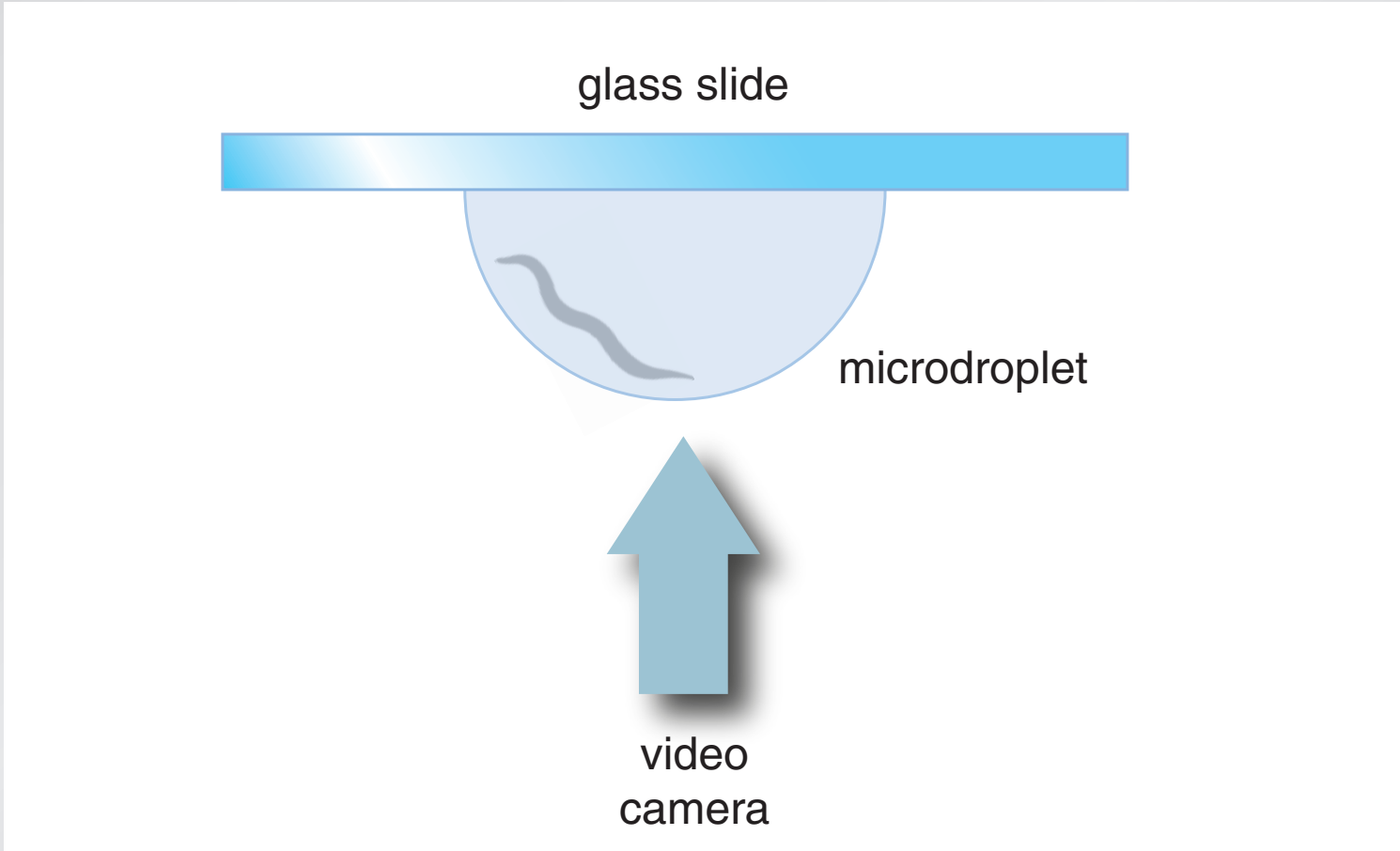
microdroplet assay



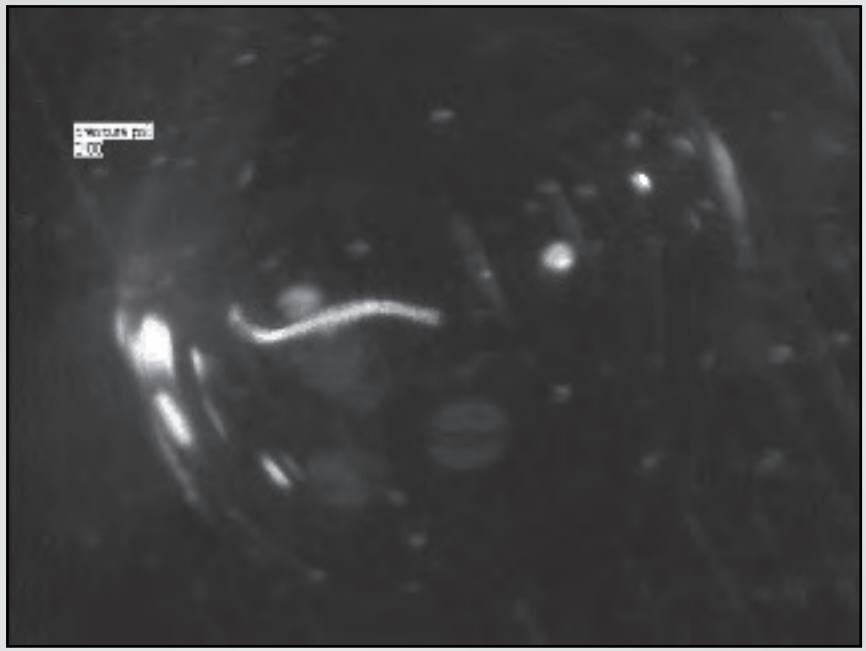
Nanoneurosurgery

microdroplet assay

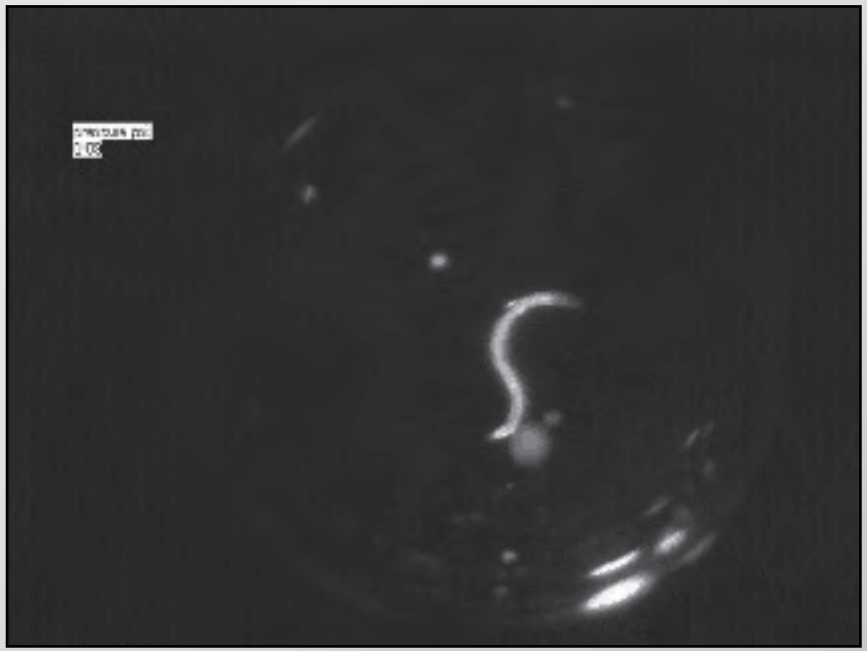


Nanoneurosurgery

surgery results in quantifiable behavior changes



before

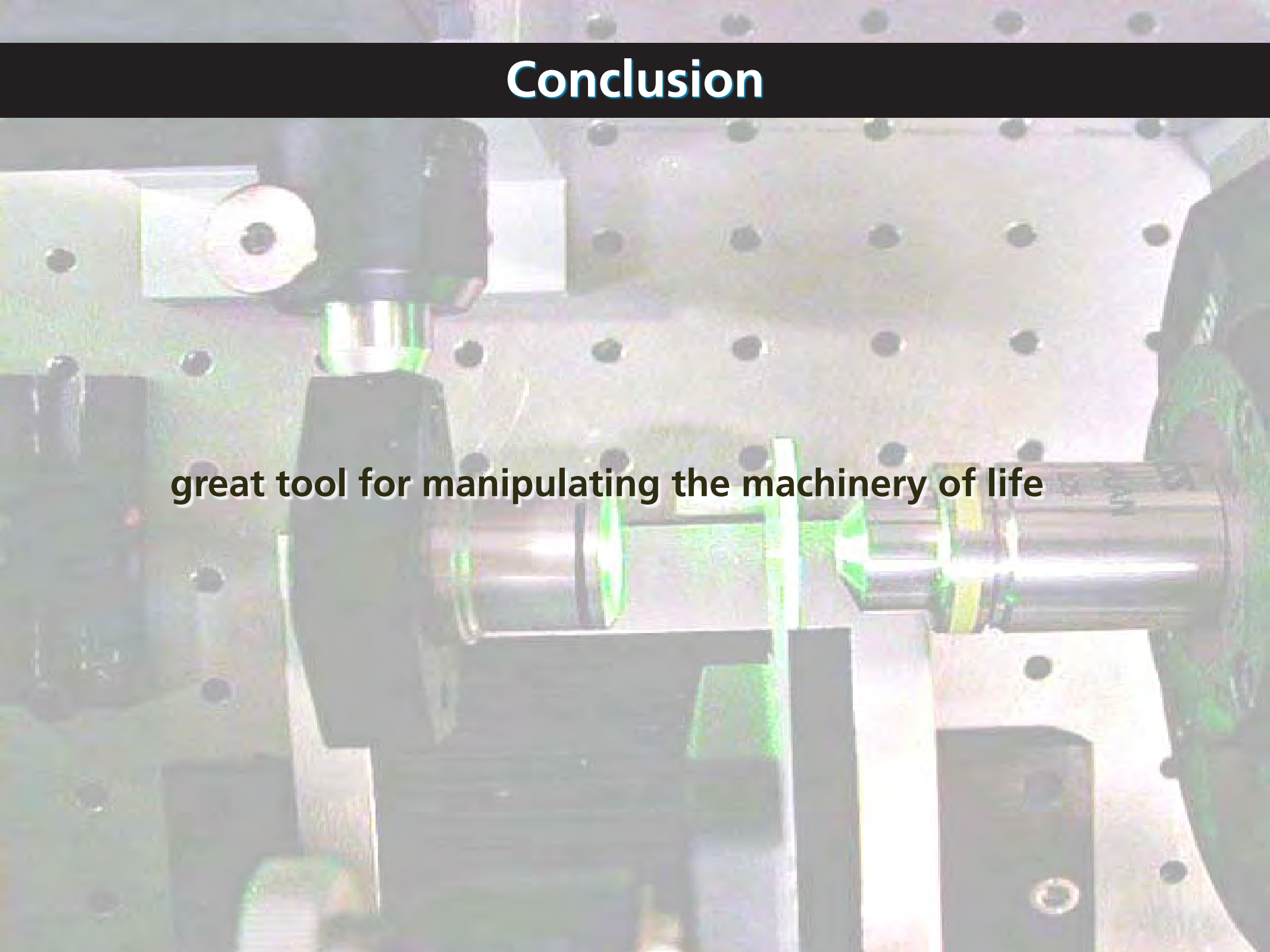


after

Nanoneurosurgery

temperature sensing occurs at tip of dendrite

Conclusion



great tool for manipulating the machinery of life

Funding:

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