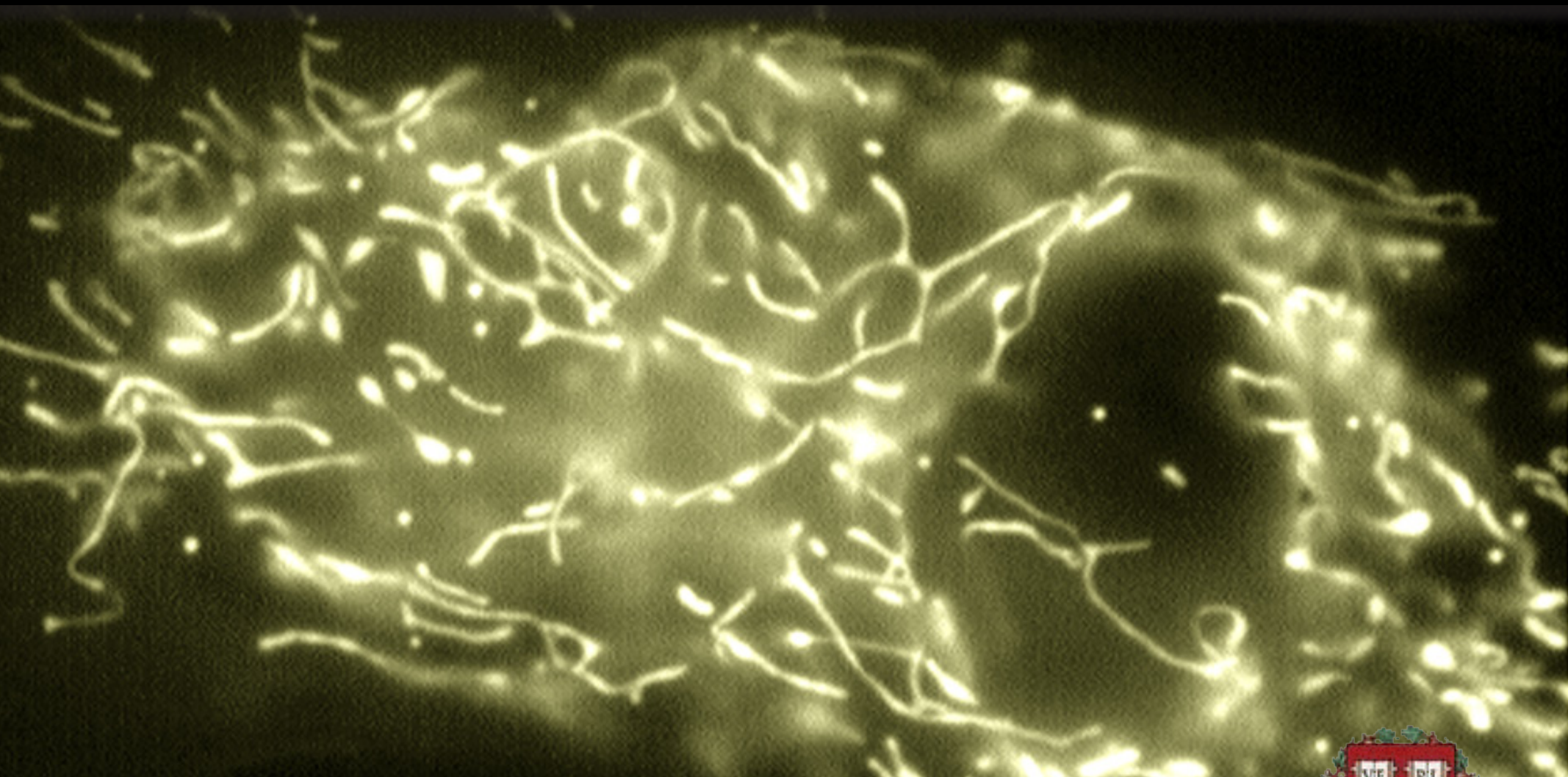


Subcellular surgery and nanoneurosurgery

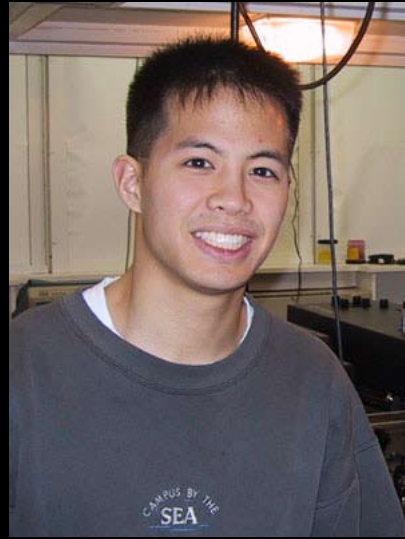


Benemérita Universidad Autónoma de Puebla
Puebla, Mexico, 29 May 2009





Iva Maxwell



Sam Chung



Prakriti Tayalia



Alexander Heisterkamp

and also....

Nozomi Nishimura

Chris Schaffer

Nan Shen

Deb Datta

Jonathan Kamler

Prof. Donald Ingber (Harvard Medical School)

Prof. Phil LeDuc (Carnegie Mellon University)

Prof. Sanjay Kumar (UC Berkeley)

Prof. Aravi Samuel (Harvard University)

Prof. Jean Underwood (UMass Worcester)

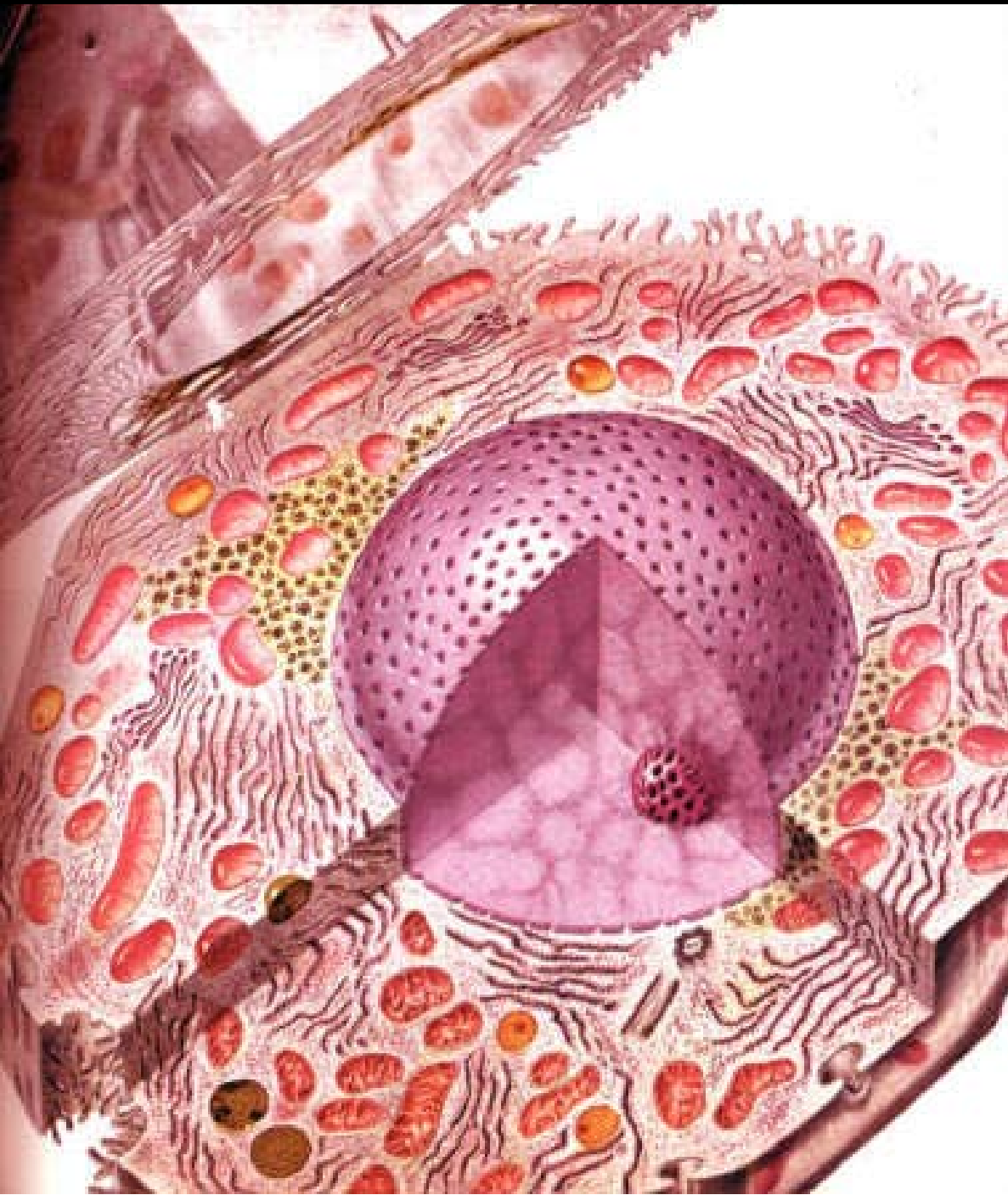
Prof. Jeffrey Nickerson (UMass Worcester)

Introduction

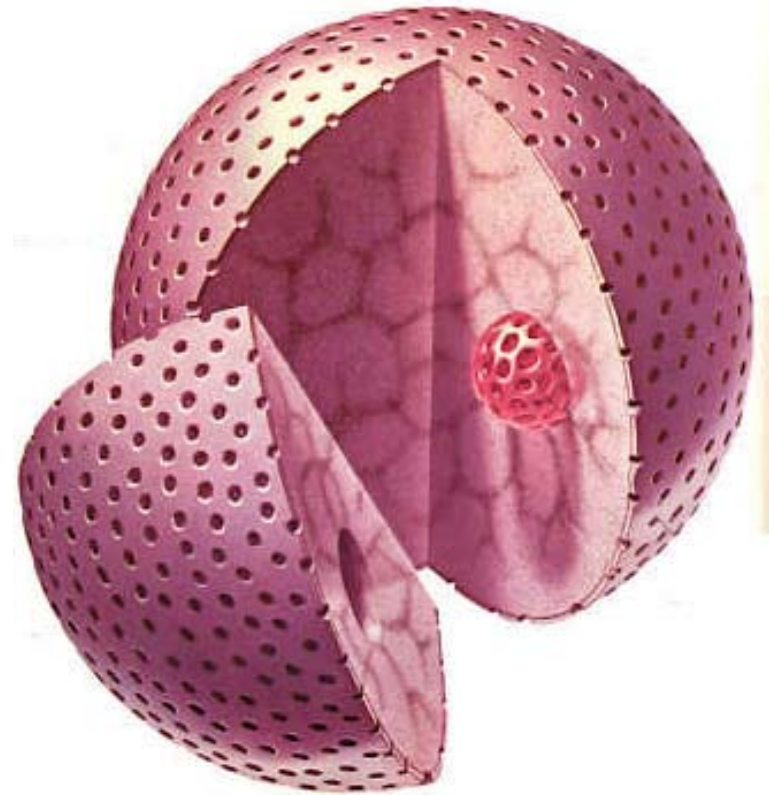
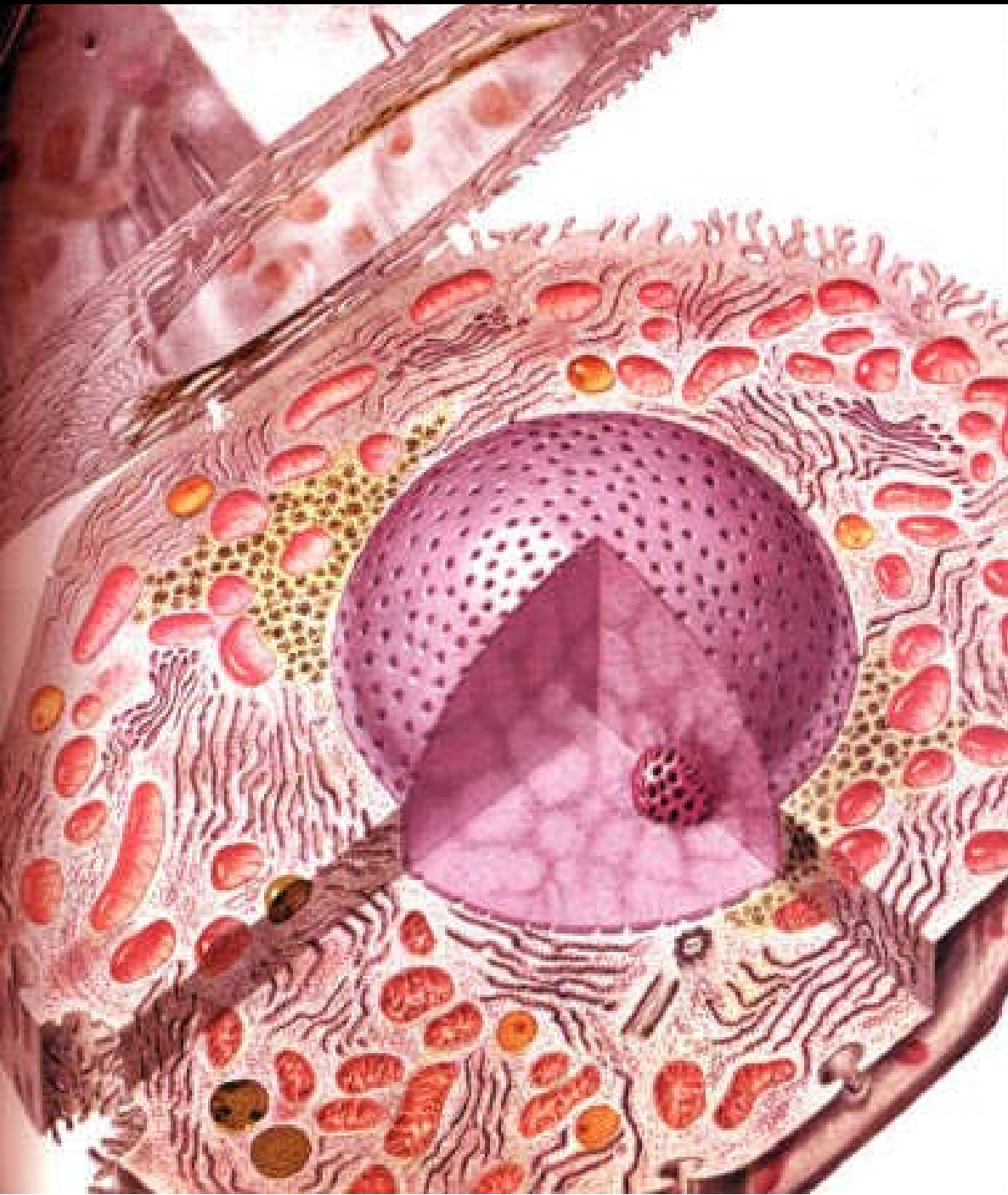
living systems require interdisciplinary tools

A scanning electron micrograph (SEM) showing several red, spherical cells. The cells have a textured, granular surface and contain numerous small, light-colored, spherical inclusions. The cells are arranged in a cluster, with some overlapping. The background is black.

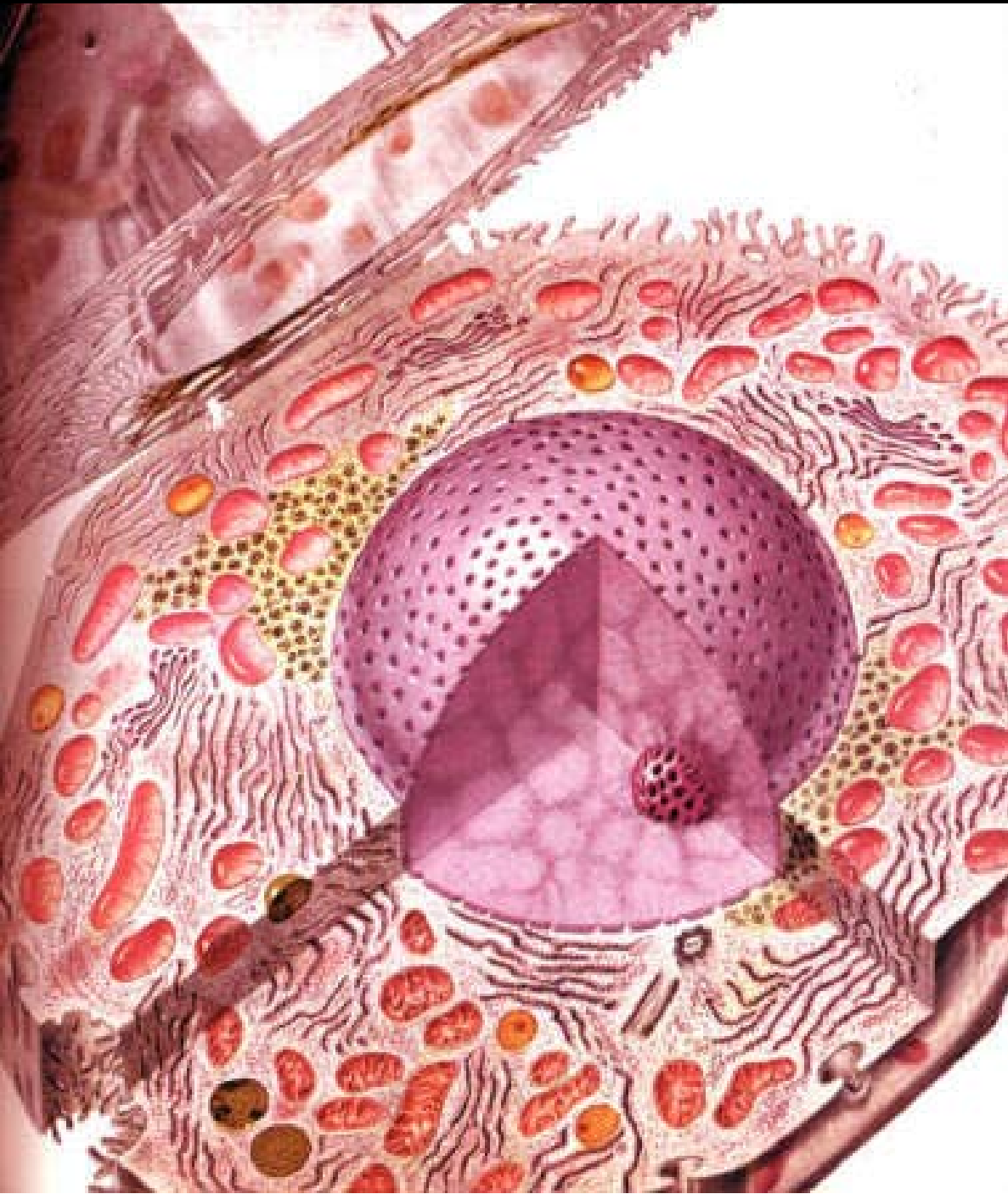
Introduction



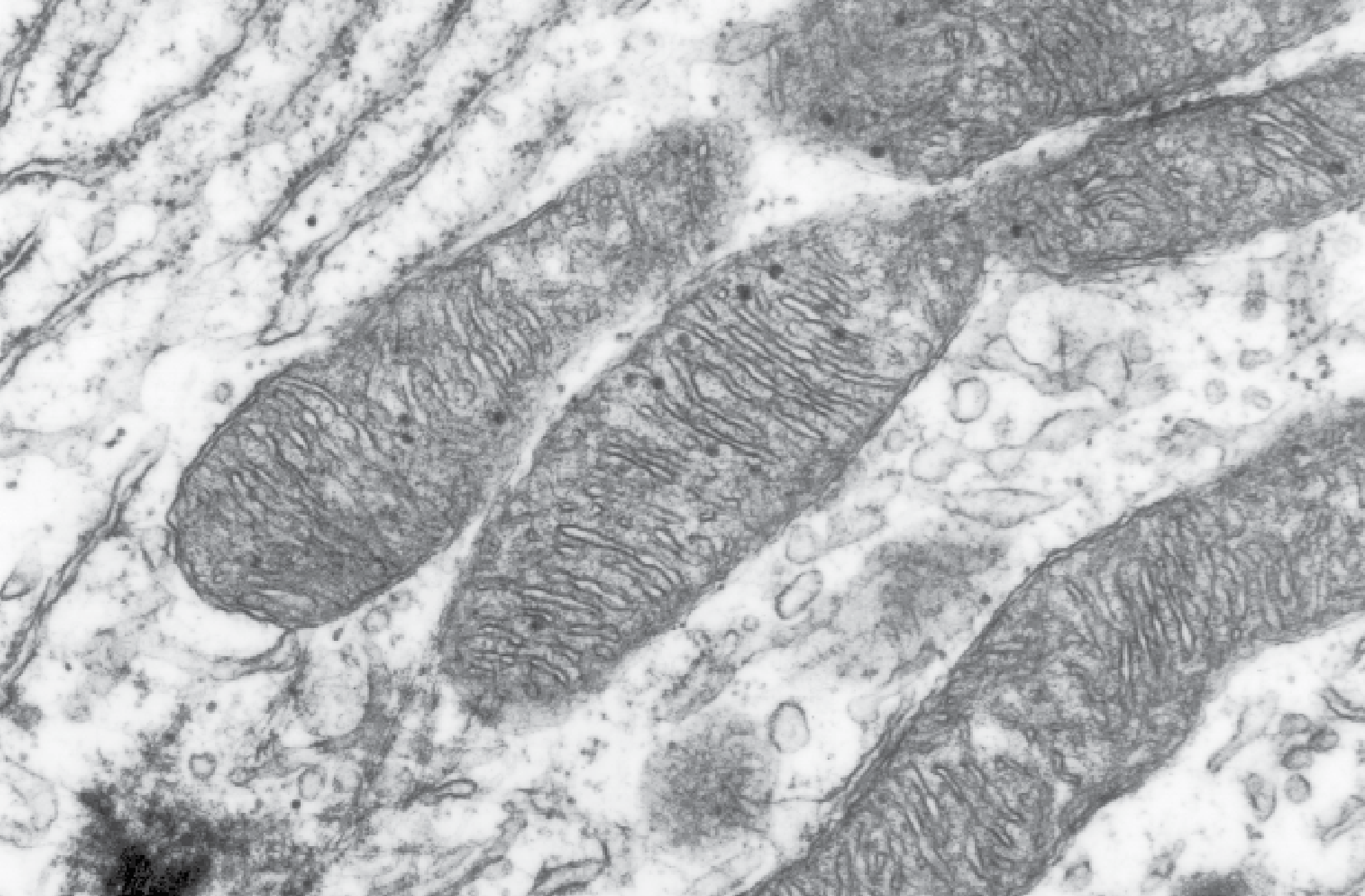
Introduction



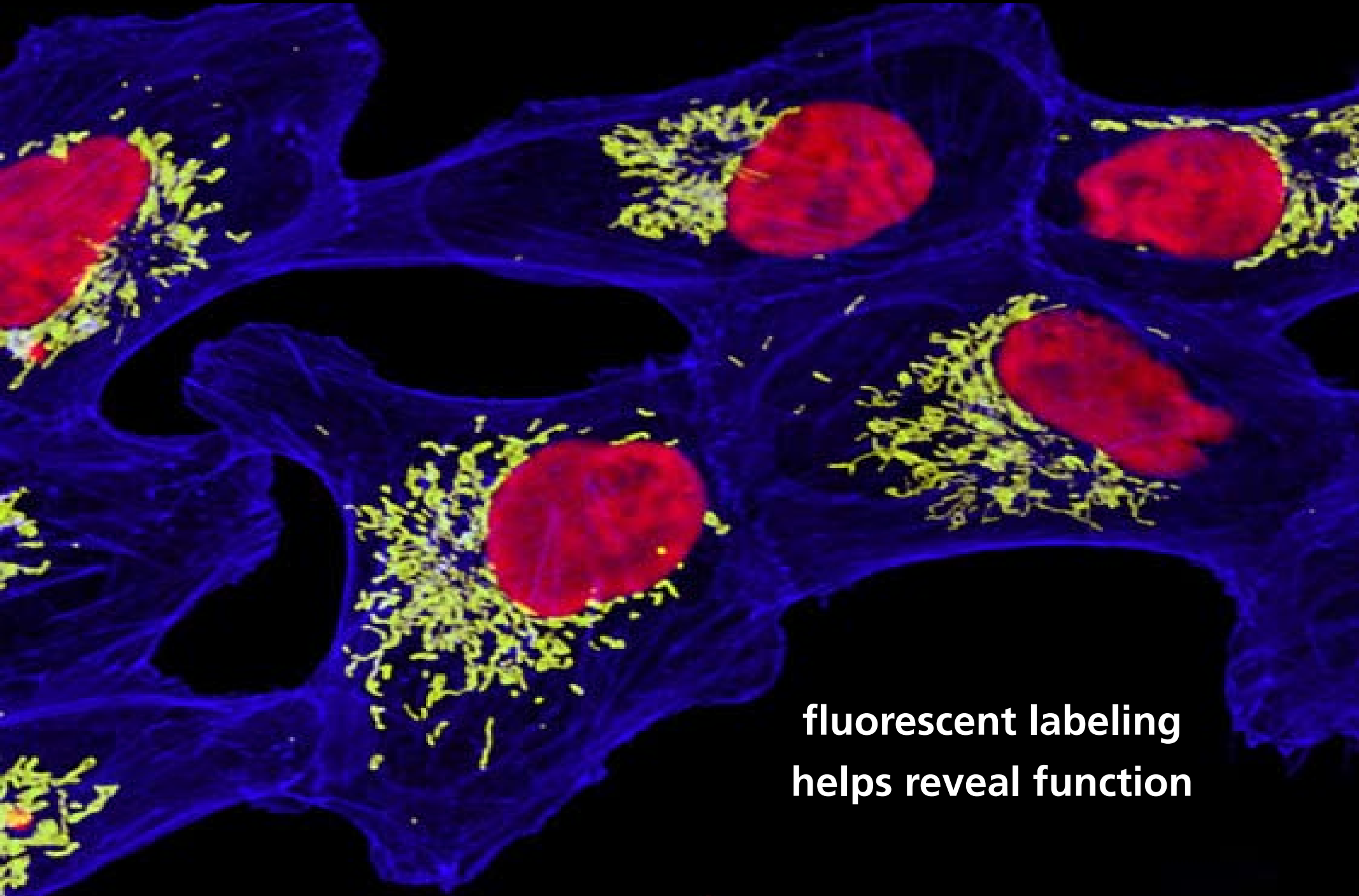
Introduction



Introduction

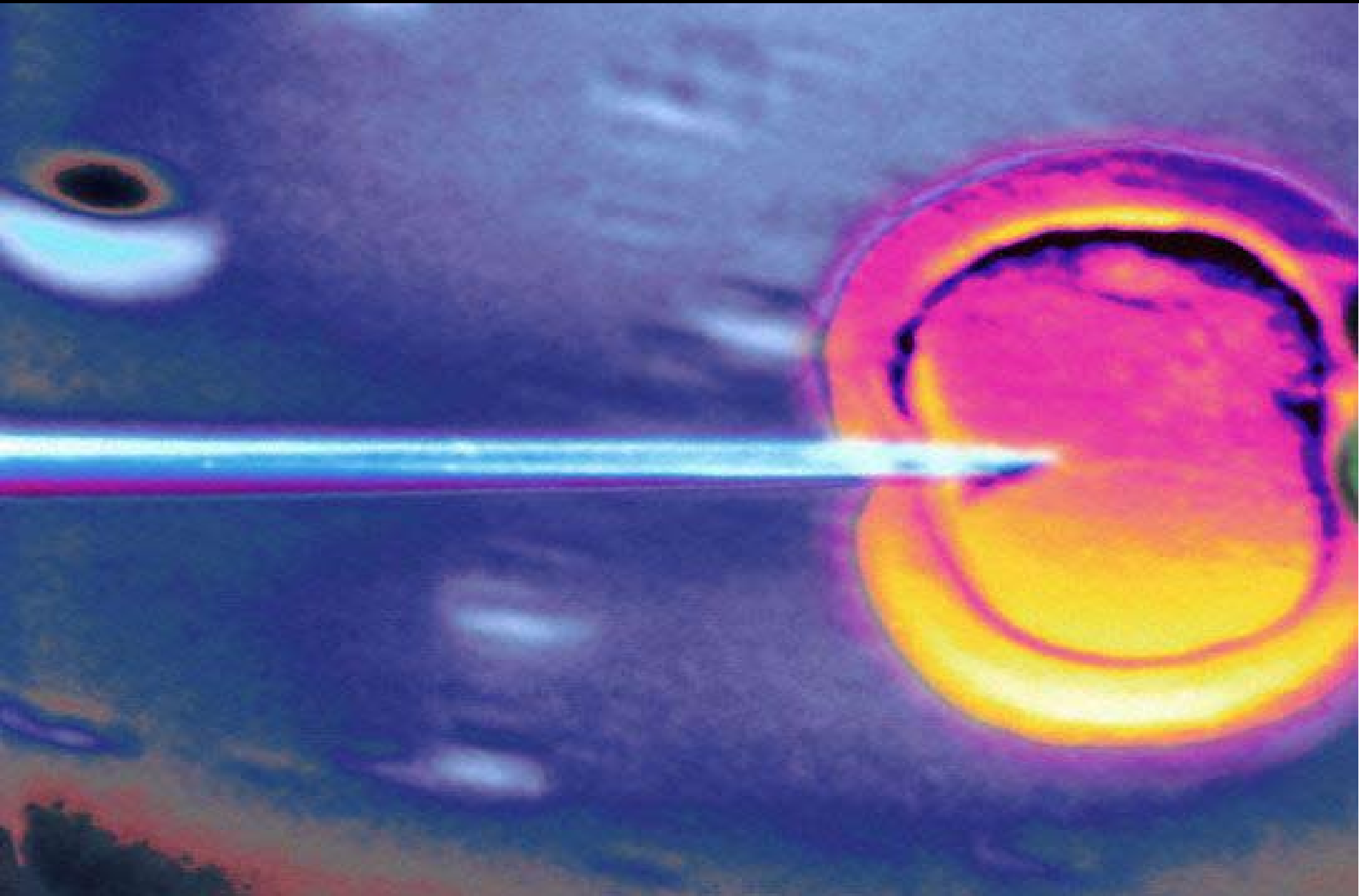


Introduction



fluorescent labeling
helps reveal function

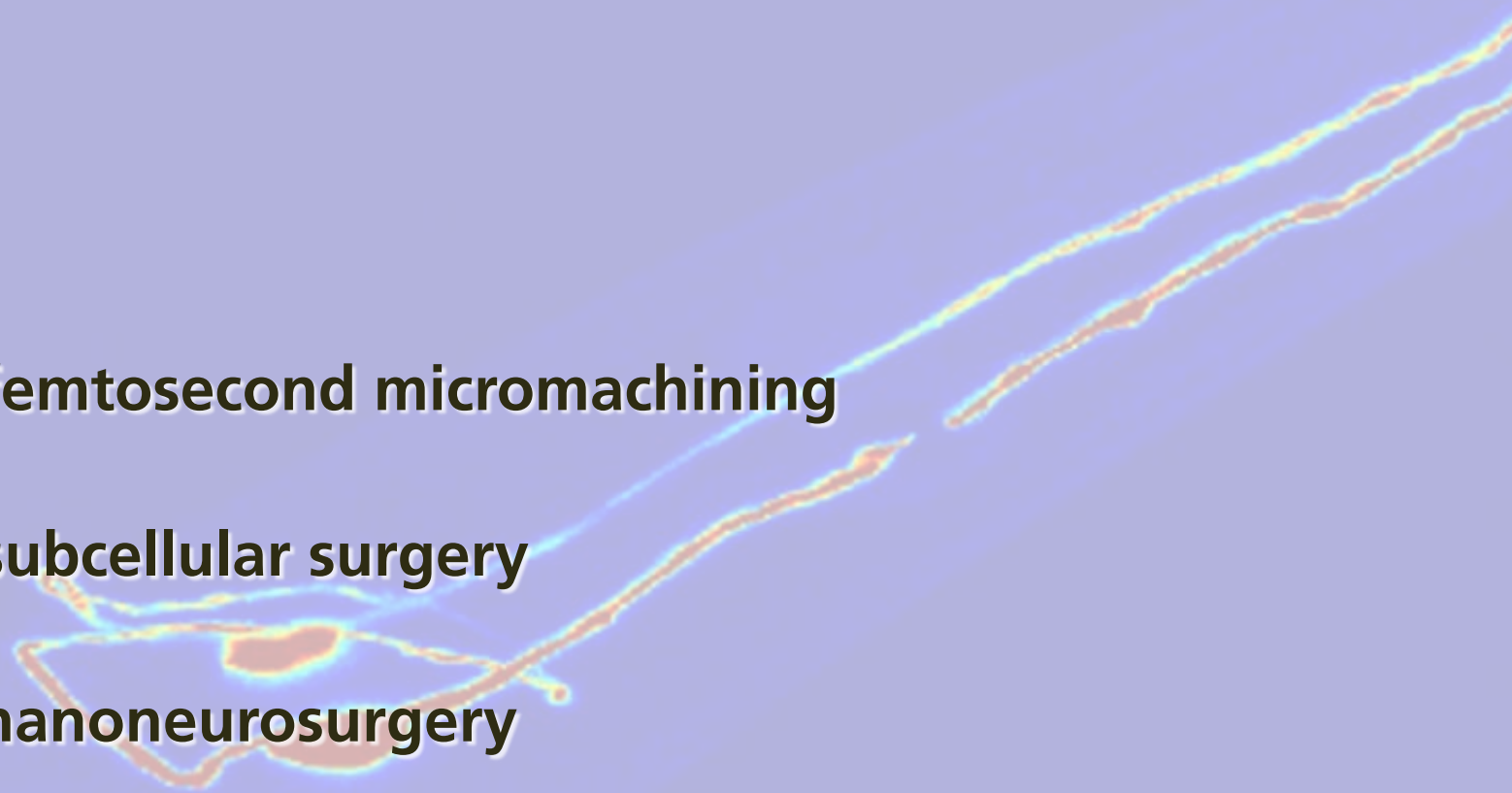
Introduction



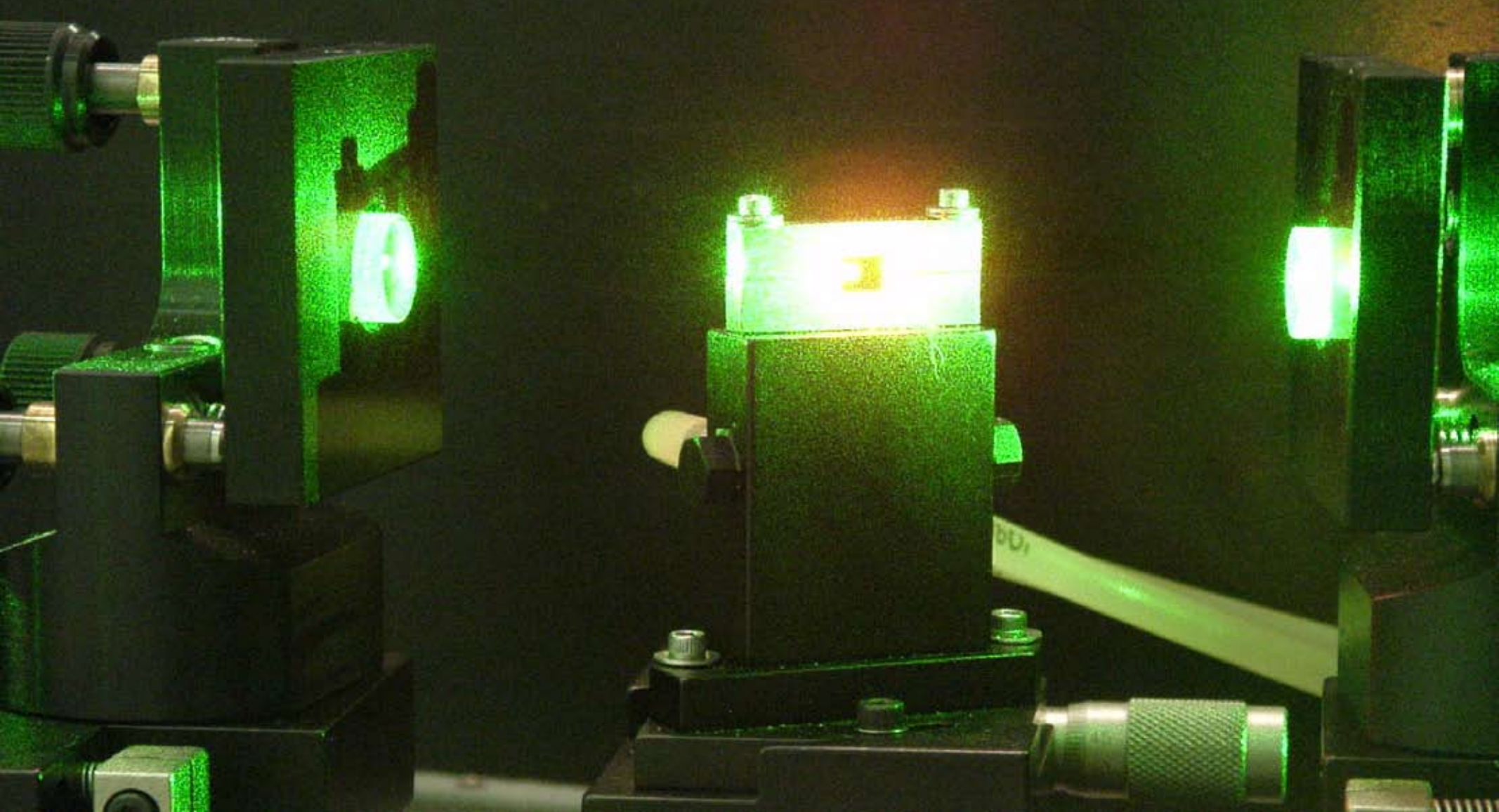
Introduction

- **standard biochemical tools: species selective**
- **fs 'nanosurgery': site-specific**

Outline

- femtosecond micromachining
 - subcellular surgery
 - nanoneurosurgery
- 

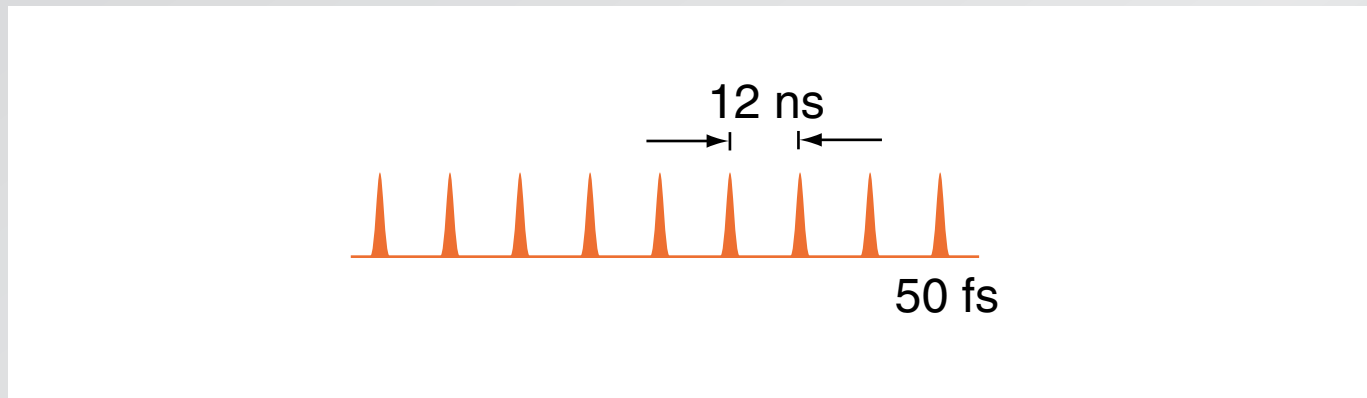
Femtosecond micromachining



Ti: sapphire laser

Femtosecond micromachining

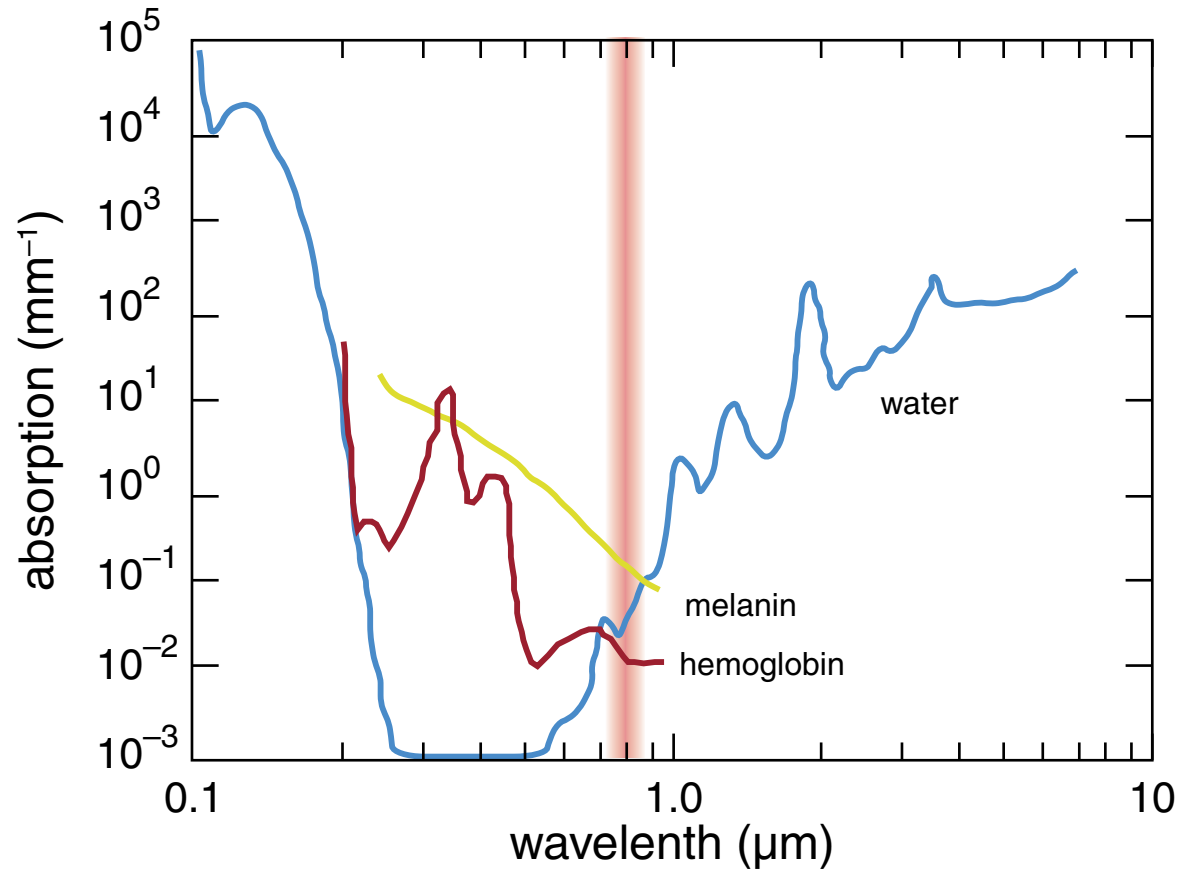
Ti:sapphire lasers



pulse duration:	50 fs	repetition rate:	80 MHz
average power:	1 W	peak power:	10^{10} W
energy per pulse:	1 mJ	wavelength:	800 nm

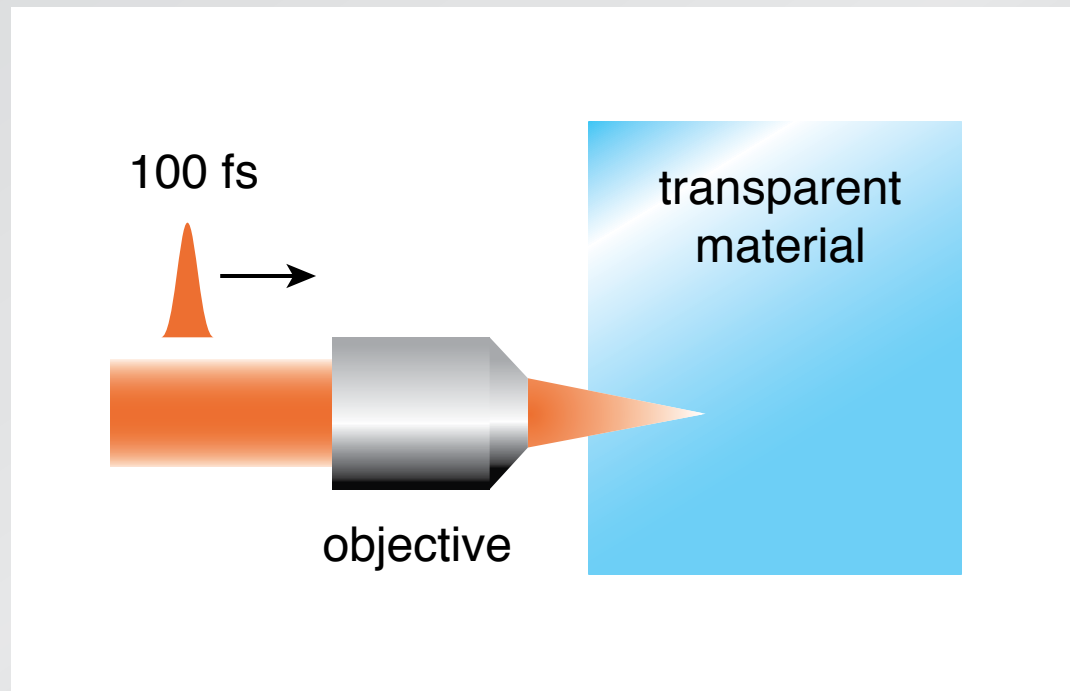
Femtosecond micromachining

tissue is nearly transparent at 800 nm



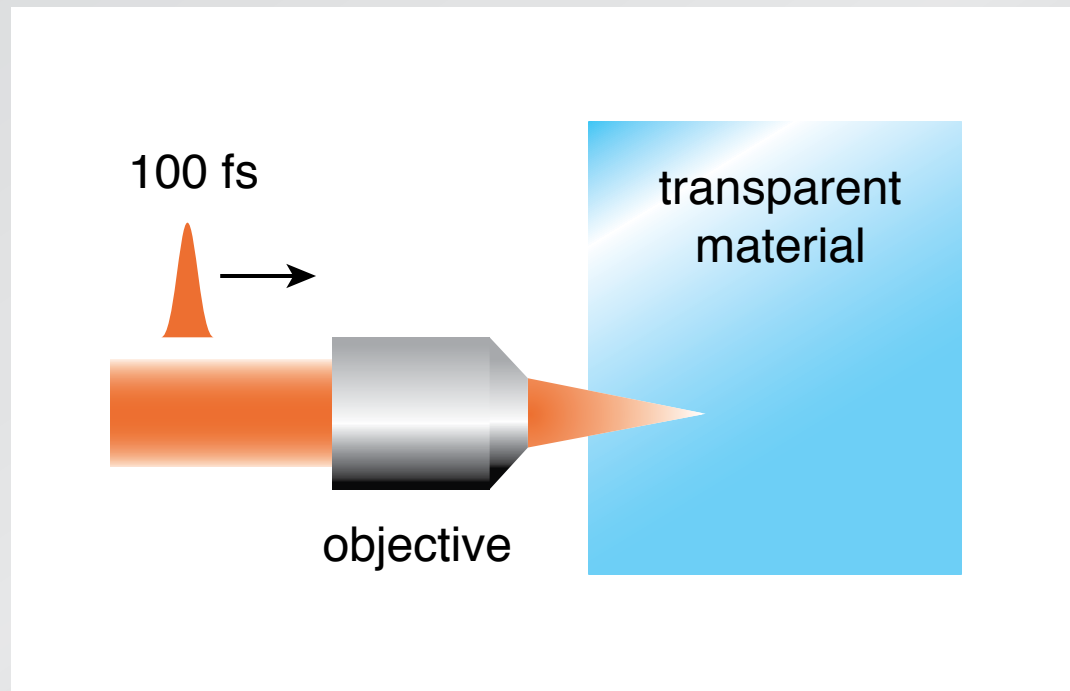
Femtosecond micromachining

focus laser beam inside material



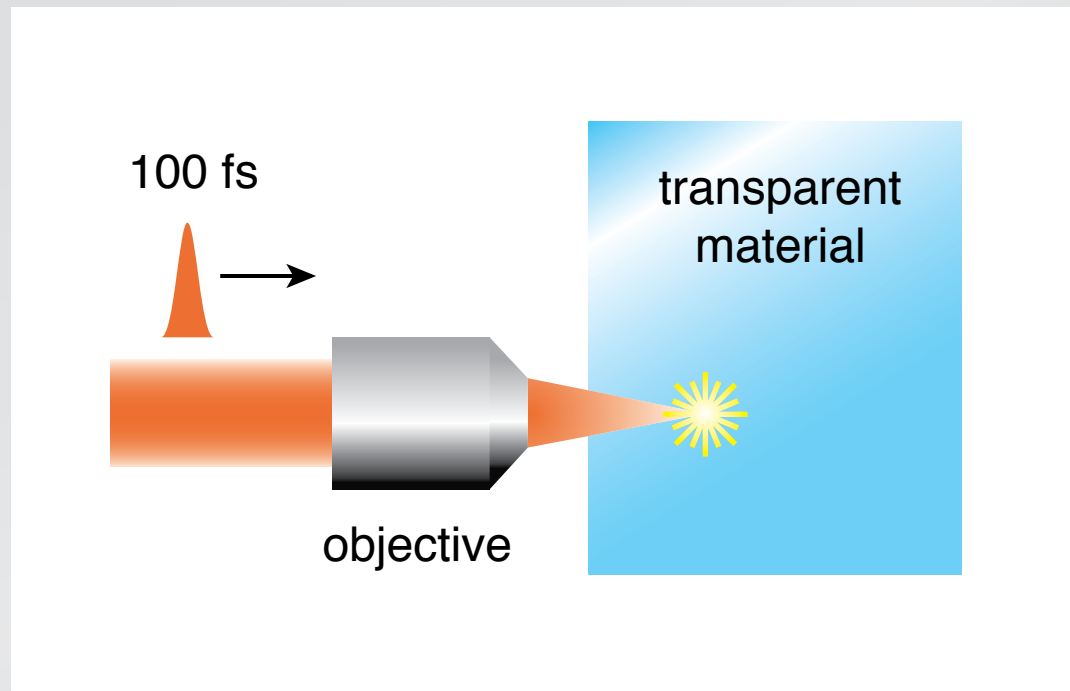
Femtosecond micromachining

high intensity at focus...



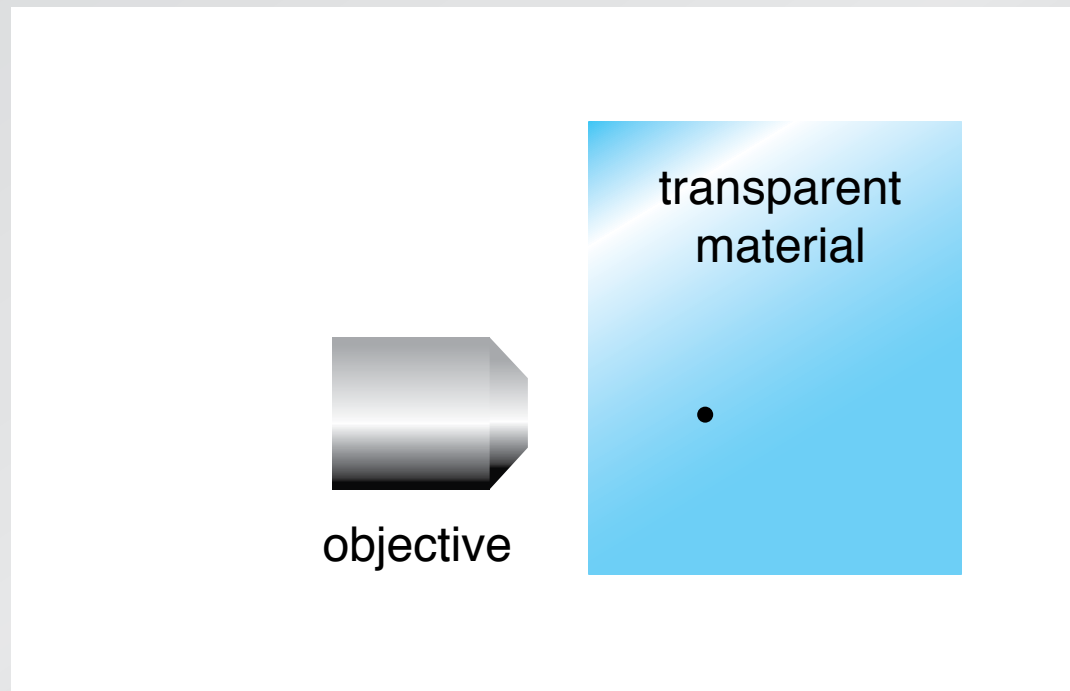
Femtosecond micromachining

...causes nonlinear ionization...

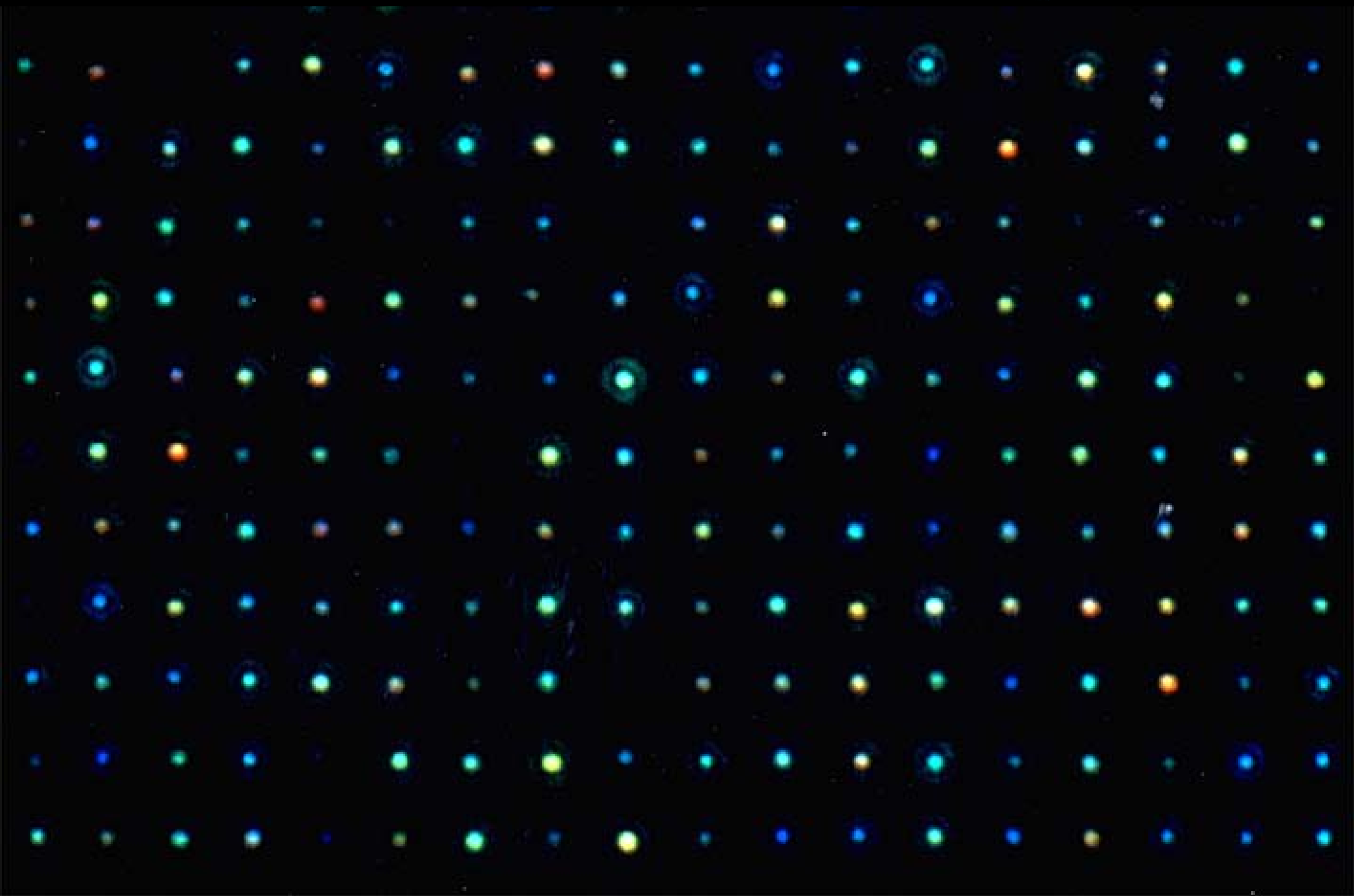


Femtosecond micromachining

and 'microexplosion' causes microscopic damage...



Femtosecond micromachining

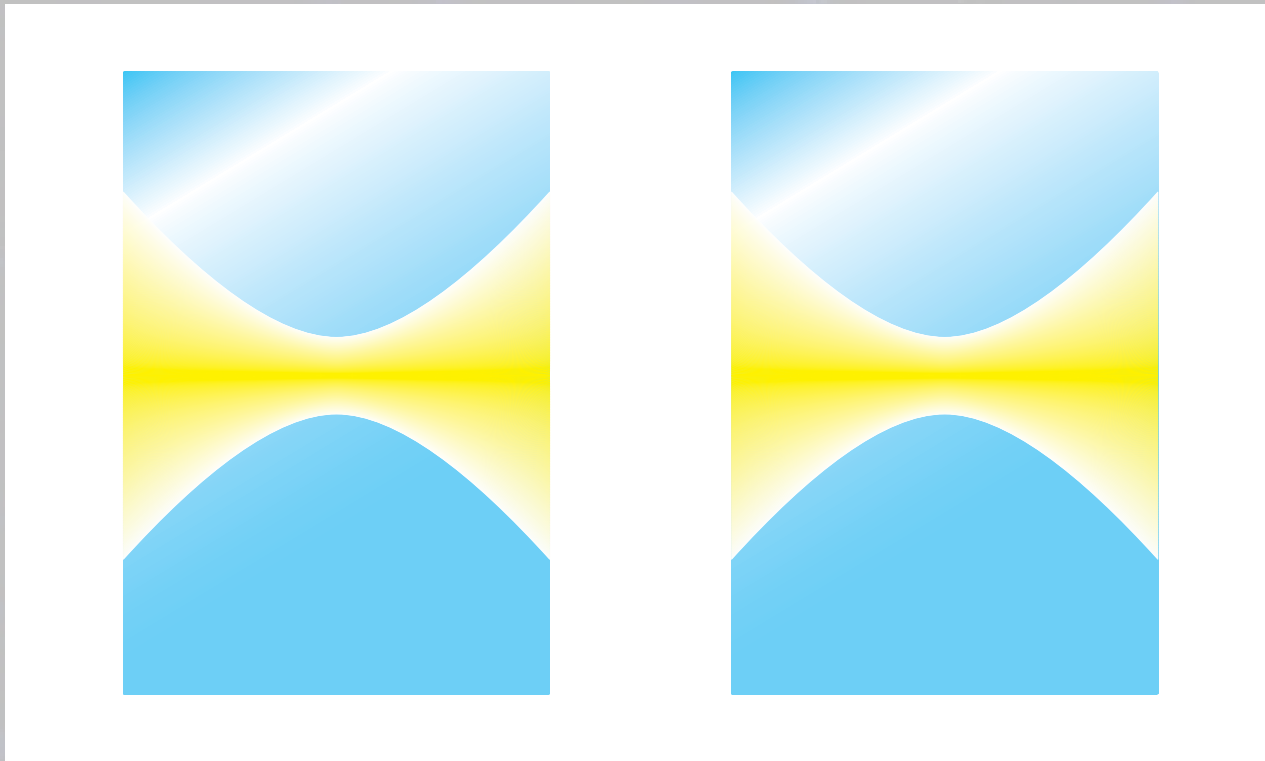


Femtosecond micromachining

photon energy $<$ bandgap \longrightarrow nonlinear interaction

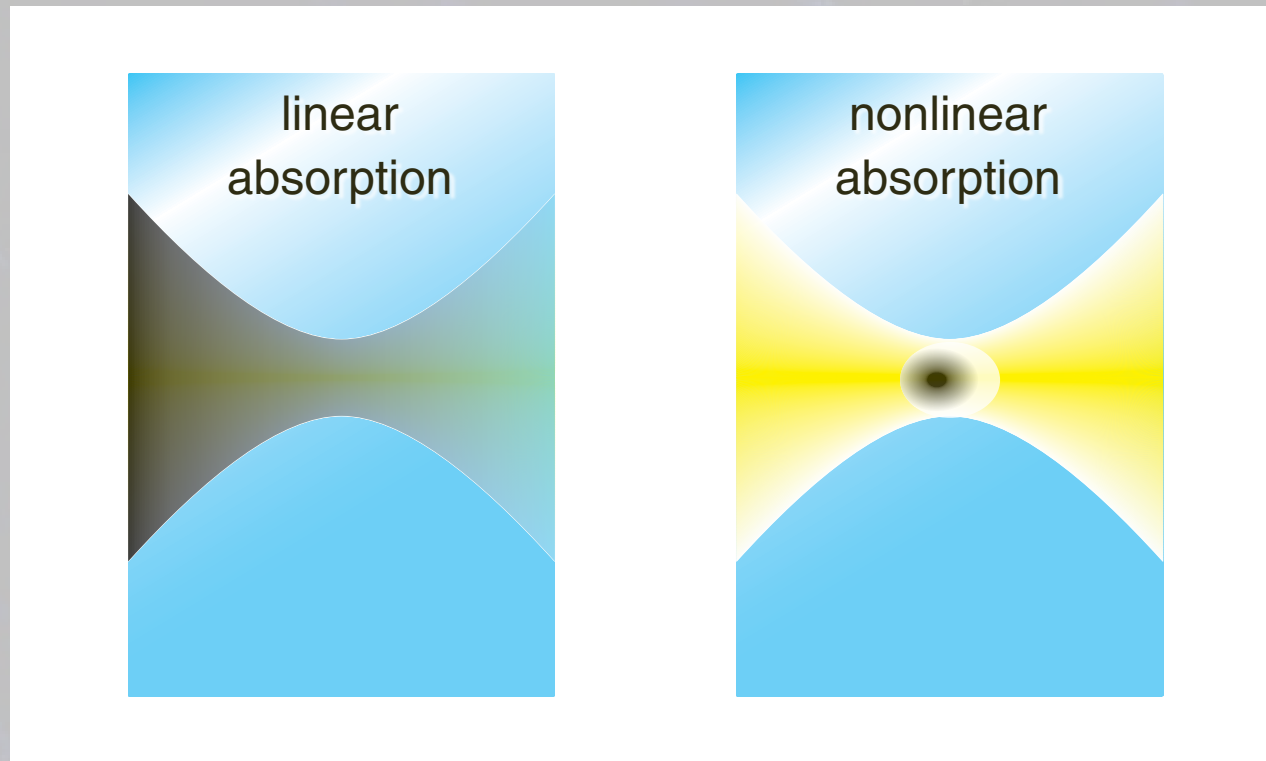
Femtosecond micromachining

nonlinear interaction provides bulk confinement

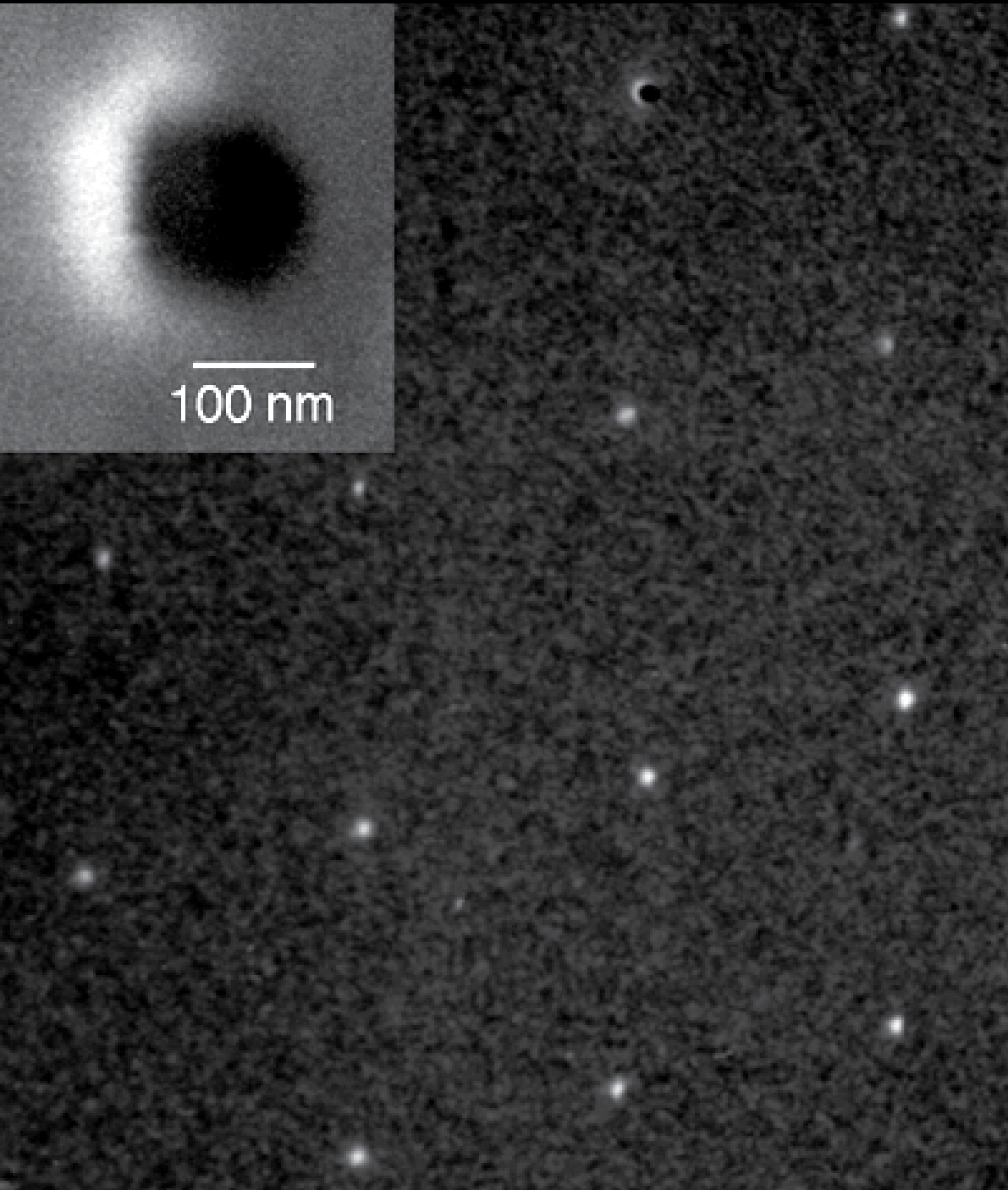


Femtosecond micromachining

nonlinear interaction provides bulk confinement



Femtosecond micromachining

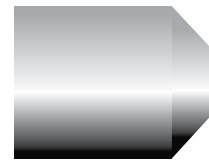


SEM & AFM:

- 100-nm cavities
- little colateral damage

Femtosecond micromachining

Dark-field scattering



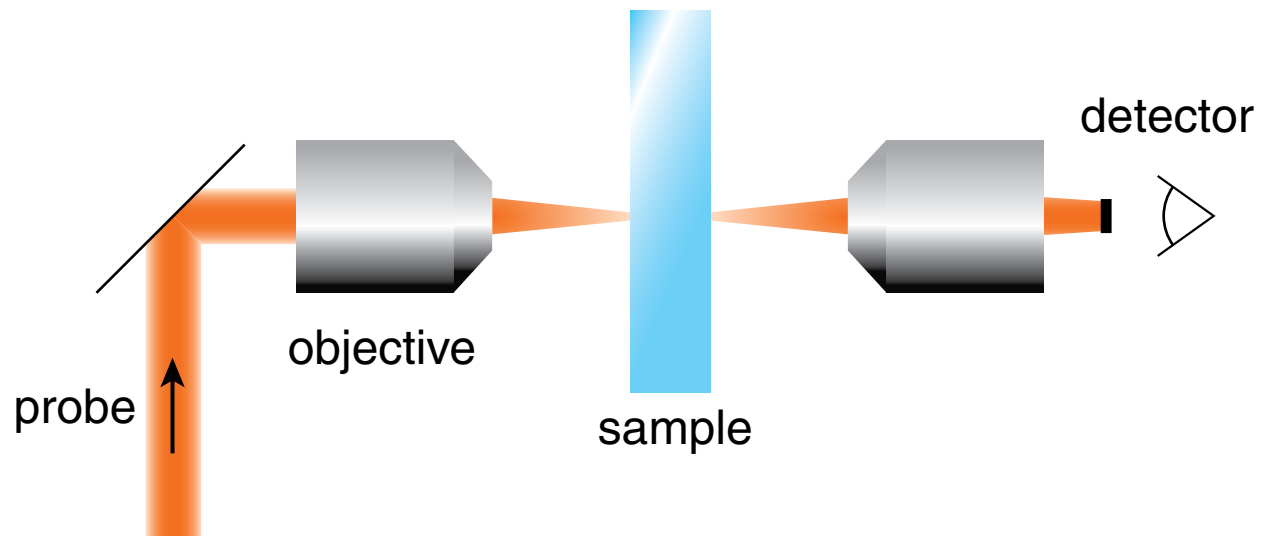
objective



sample

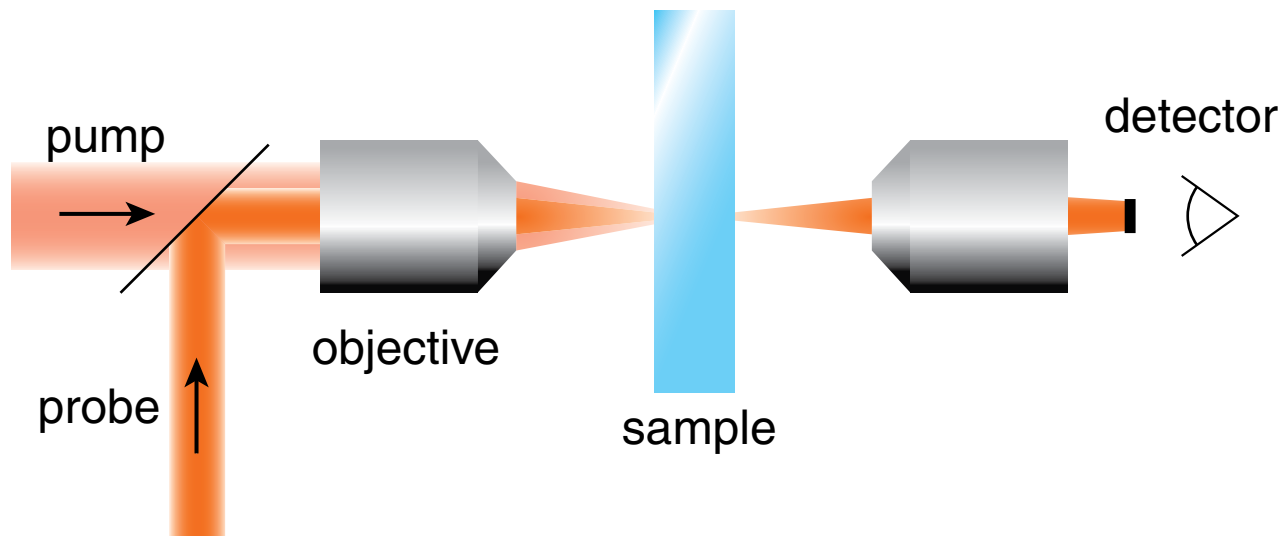
Femtosecond micromachining

block probe beam...



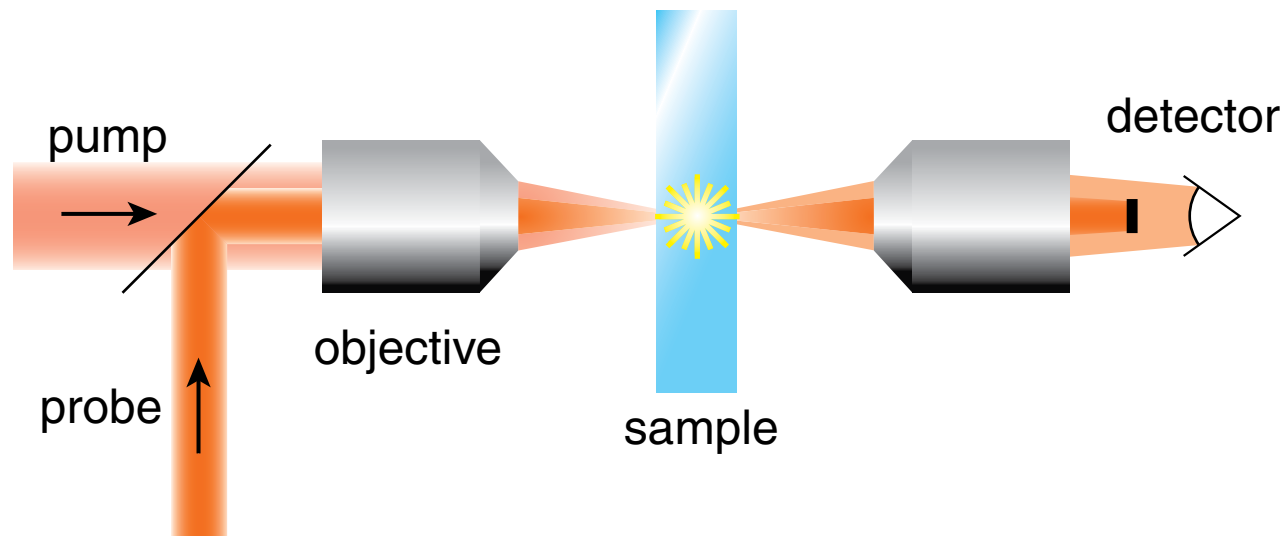
Femtosecond micromachining

... bring in pump beam...



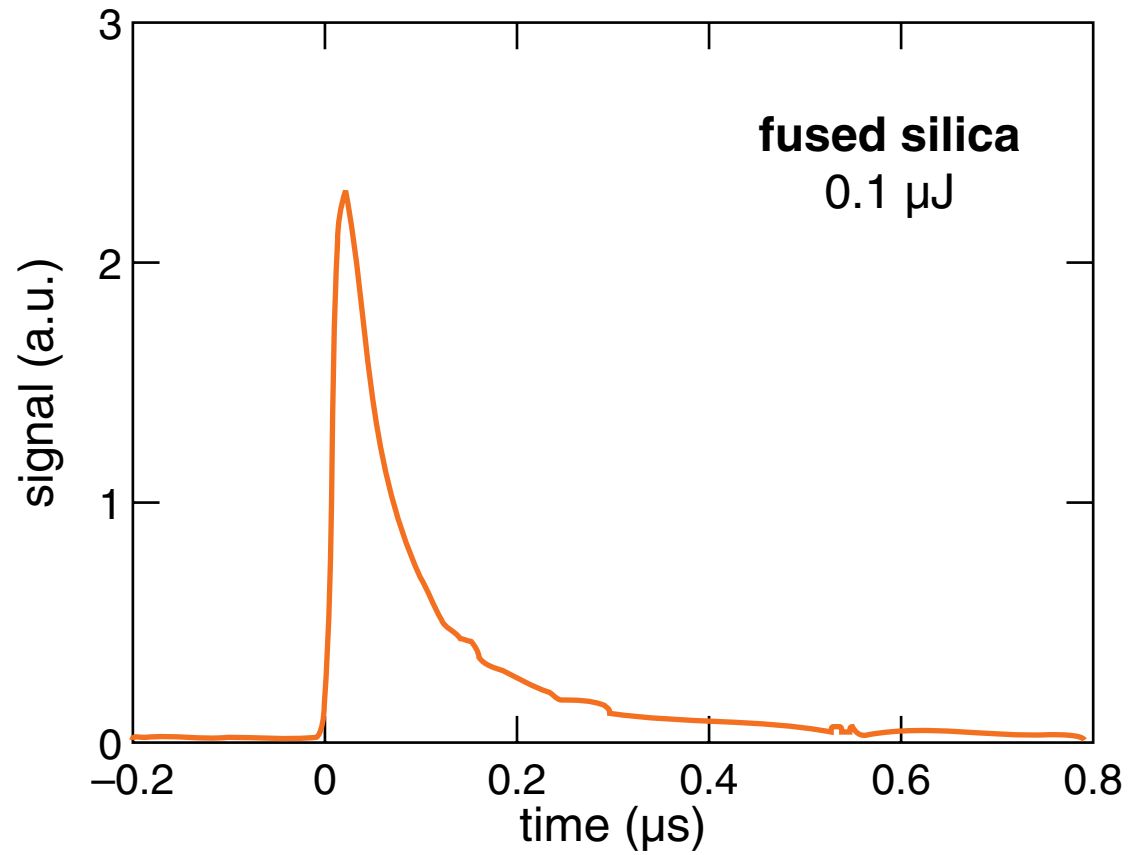
Femtosecond micromachining

... damage scatters probe beam



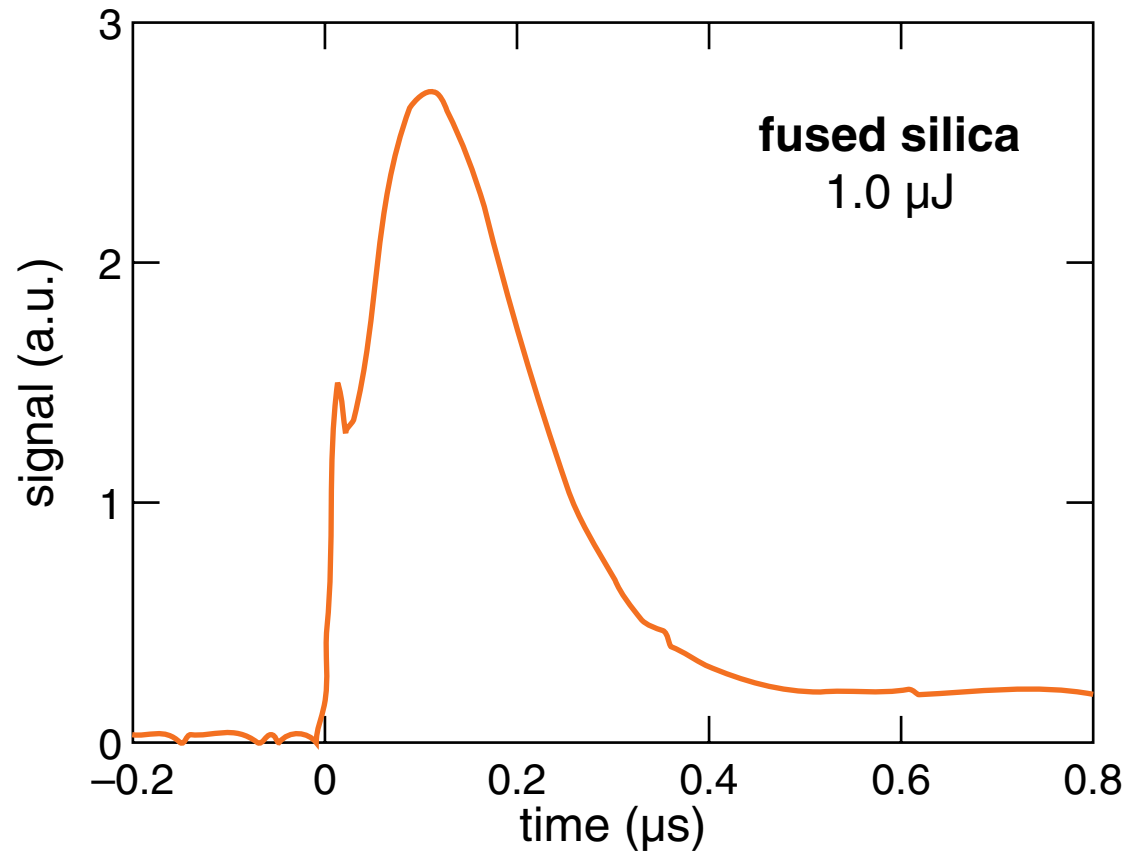
Femtosecond micromachining

scattered signal



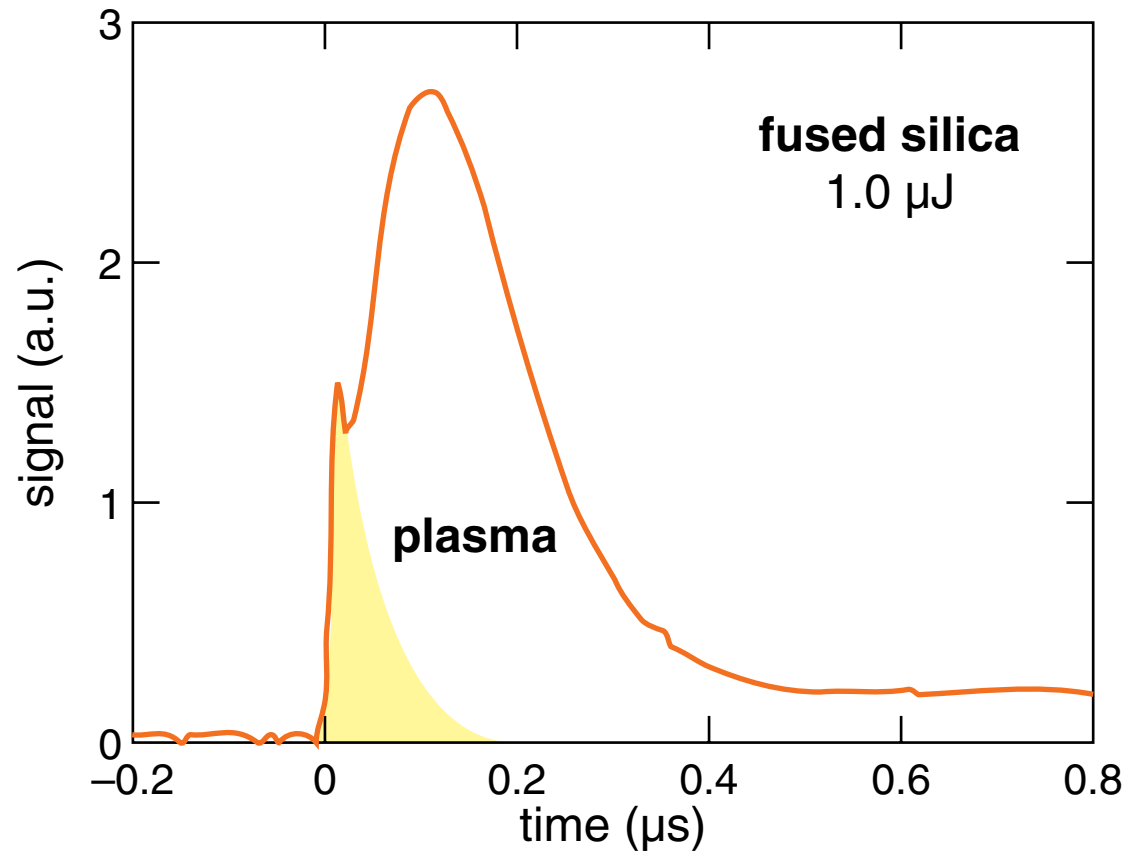
Femtosecond micromachining

scattered signal



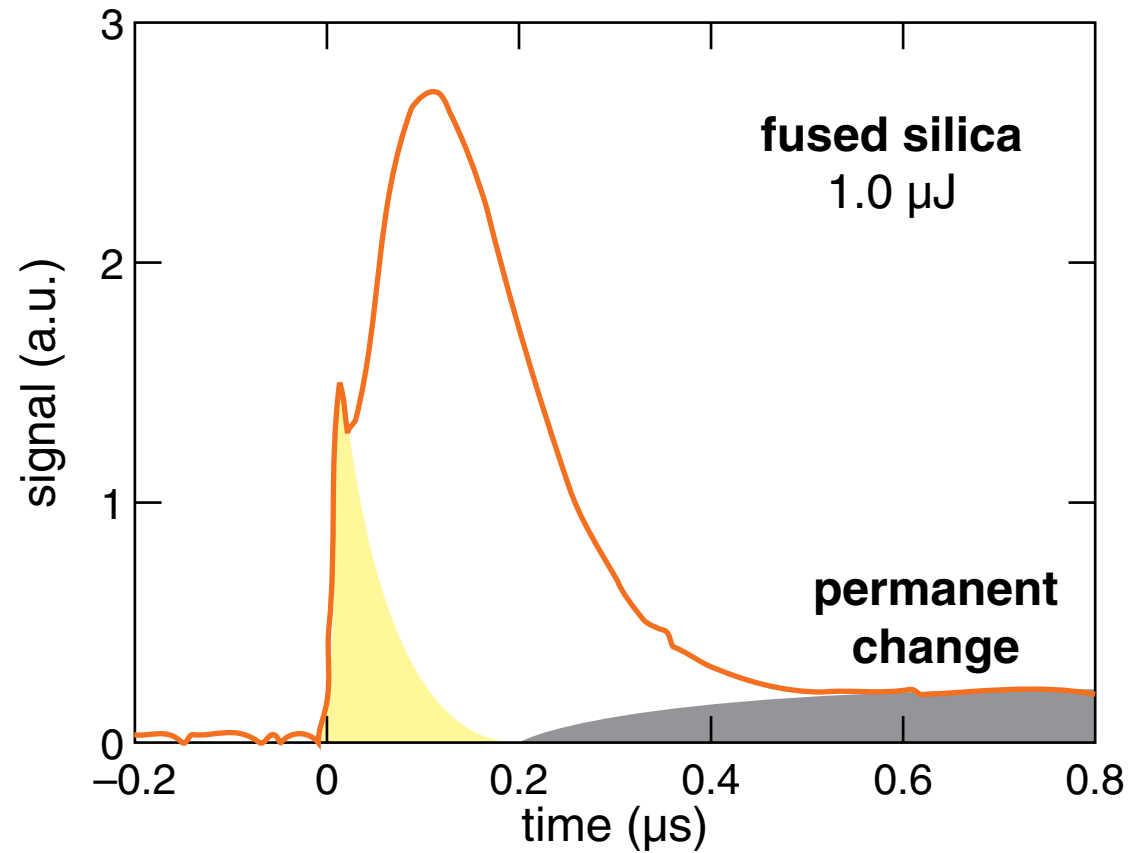
Femtosecond micromachining

scattered signal



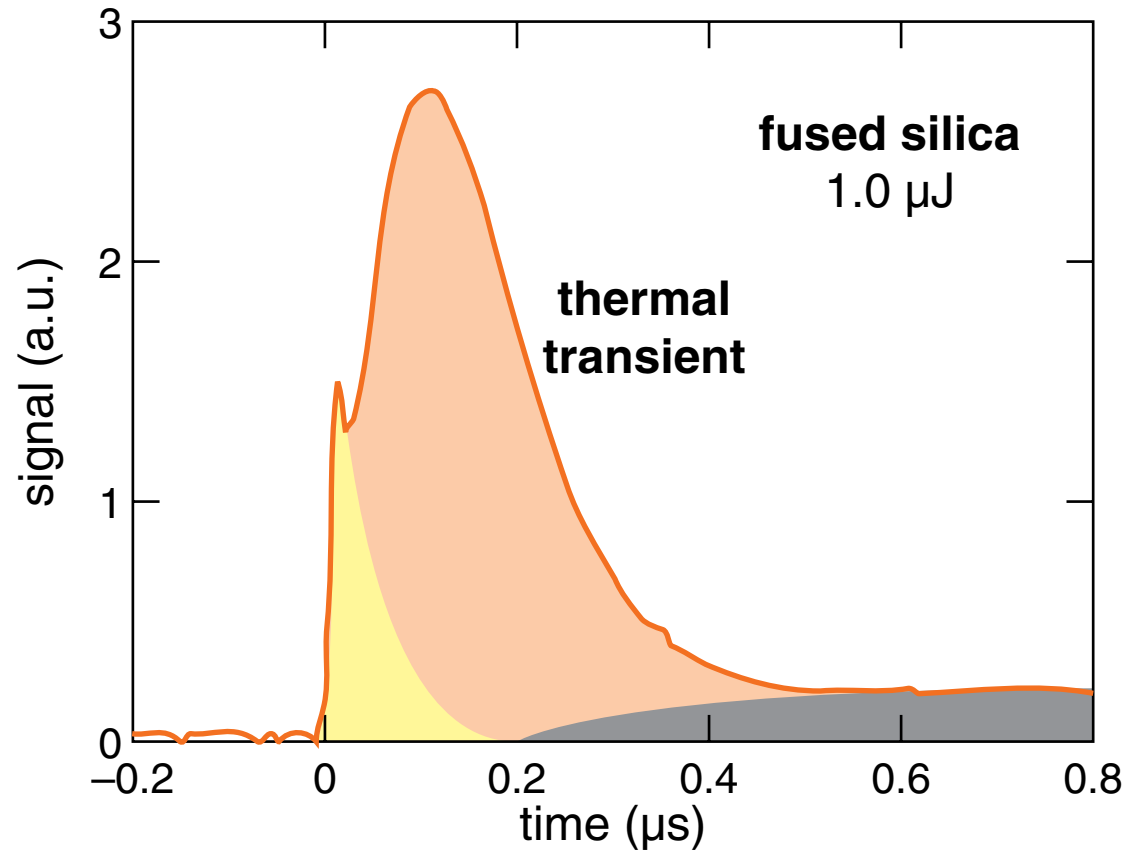
Femtosecond micromachining

scattered signal



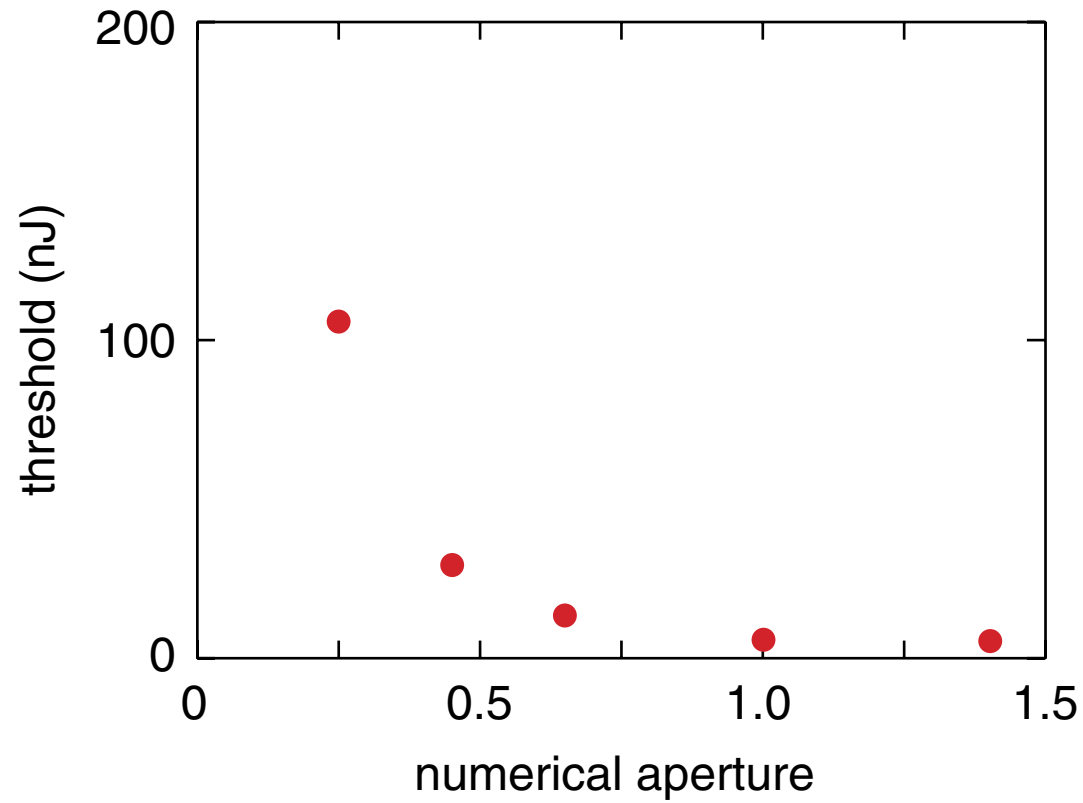
Femtosecond micromachining

scattered signal



Femtosecond micromachining

vary numerical aperture



Femtosecond micromachining

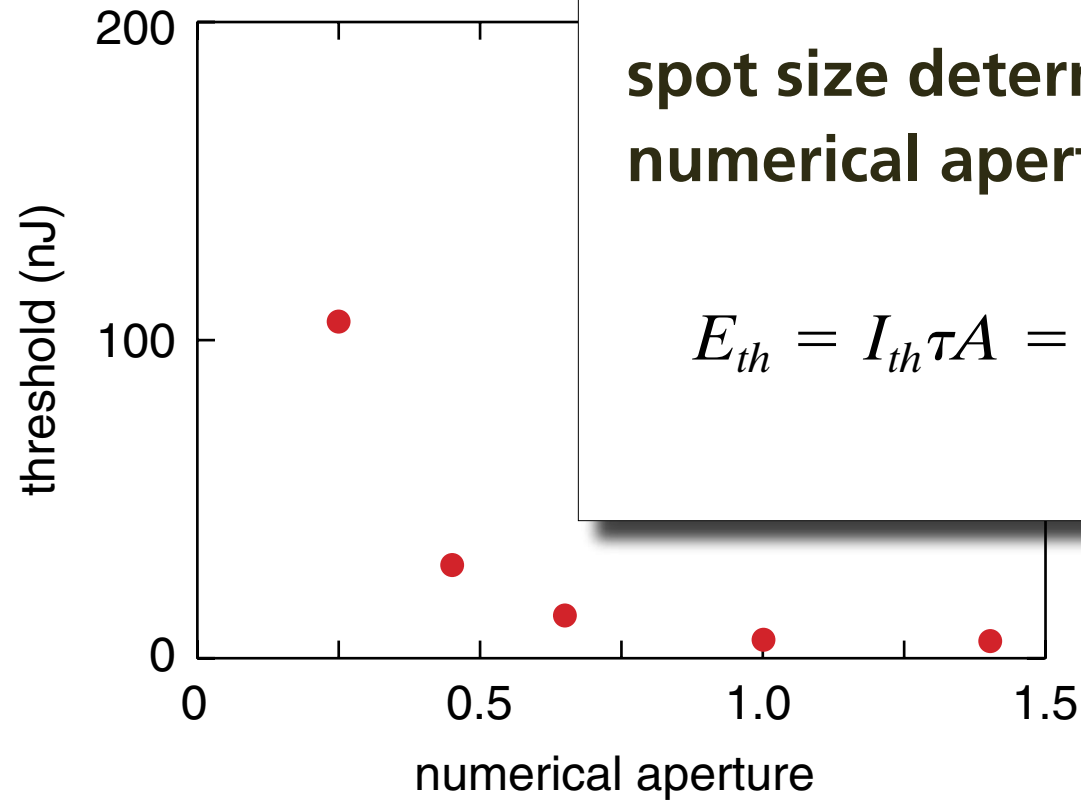
vary numerical

intensity threshold:

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

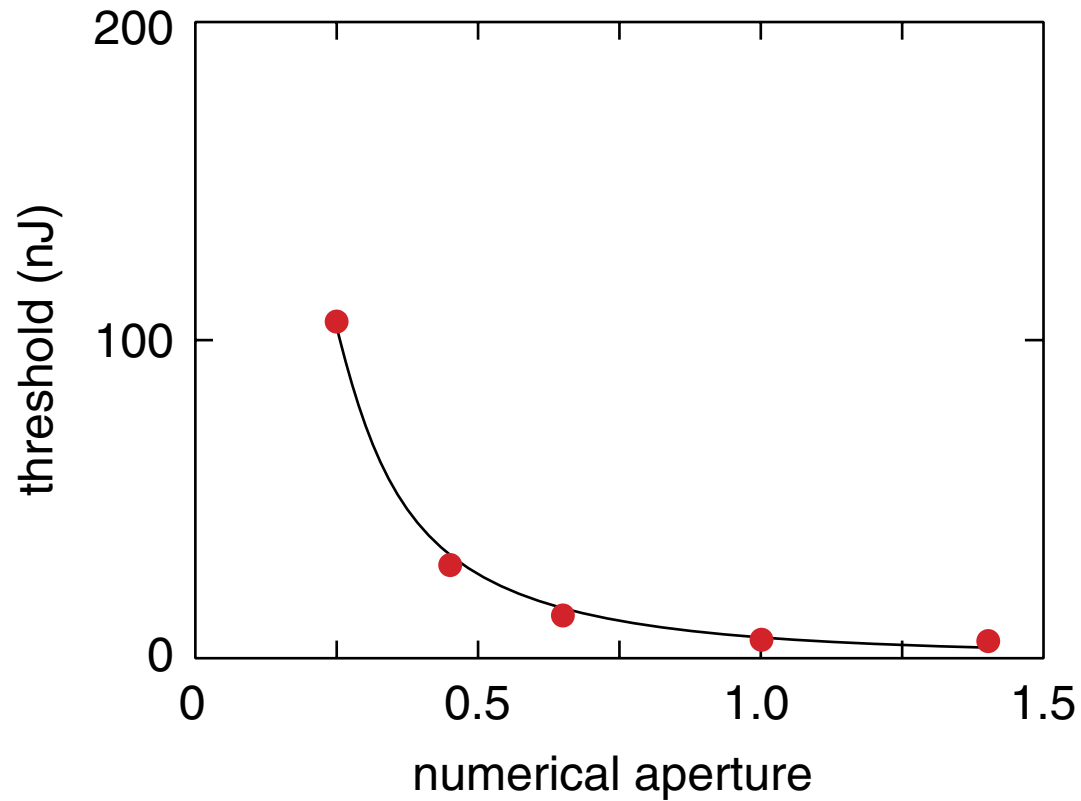
spot size determined by
numerical aperture:

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



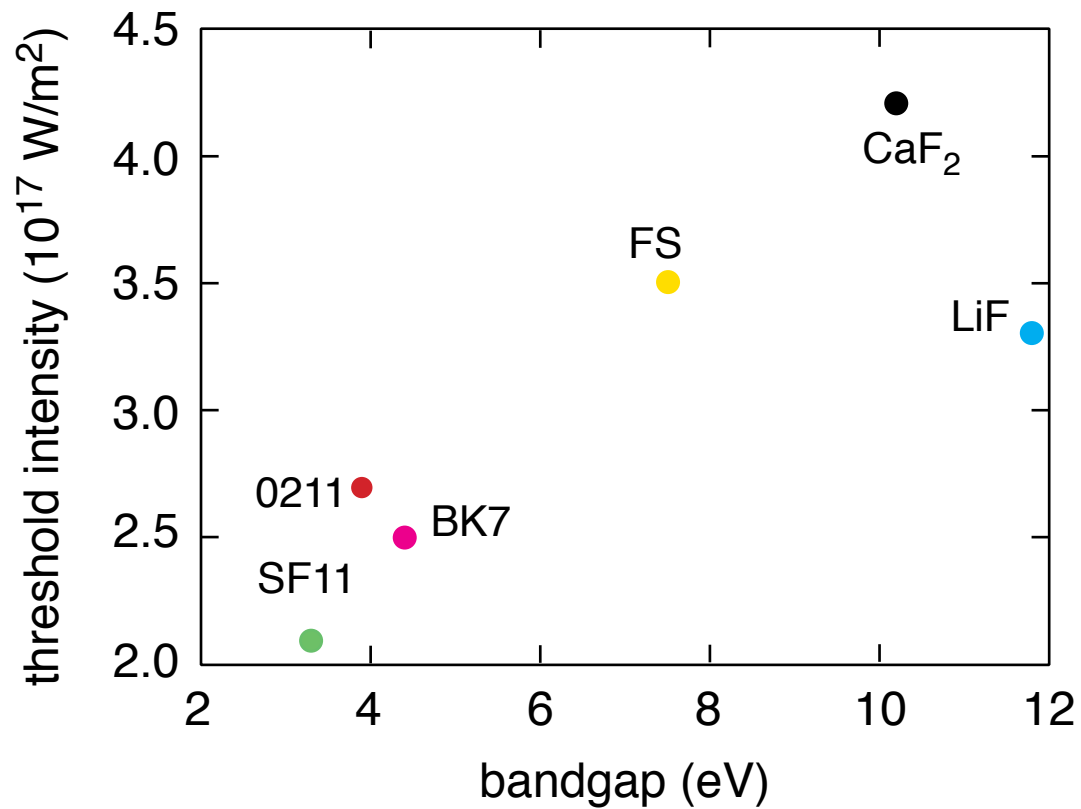
Femtosecond micromachining

fit gives threshold intensity: $I_{th} = 2.5 \times 10^{17} \text{ W/m}^2$



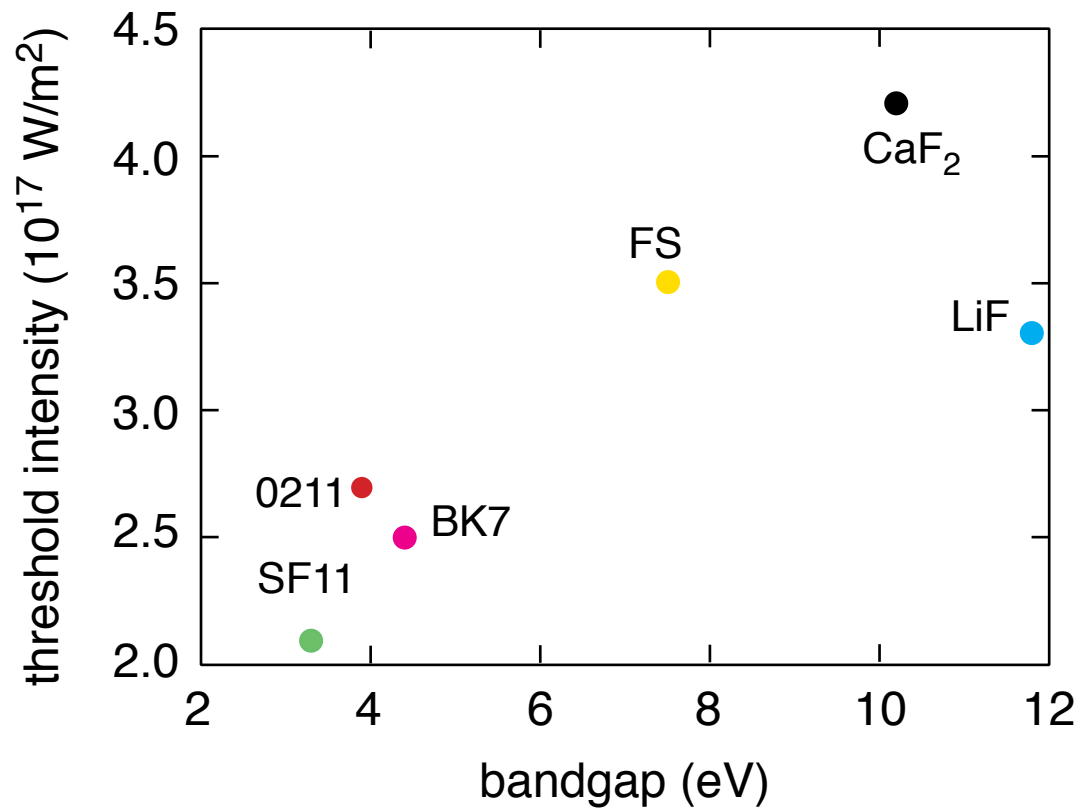
Femtosecond micromachining

vary material...



Femtosecond micromachining

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



Femtosecond micromachining

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

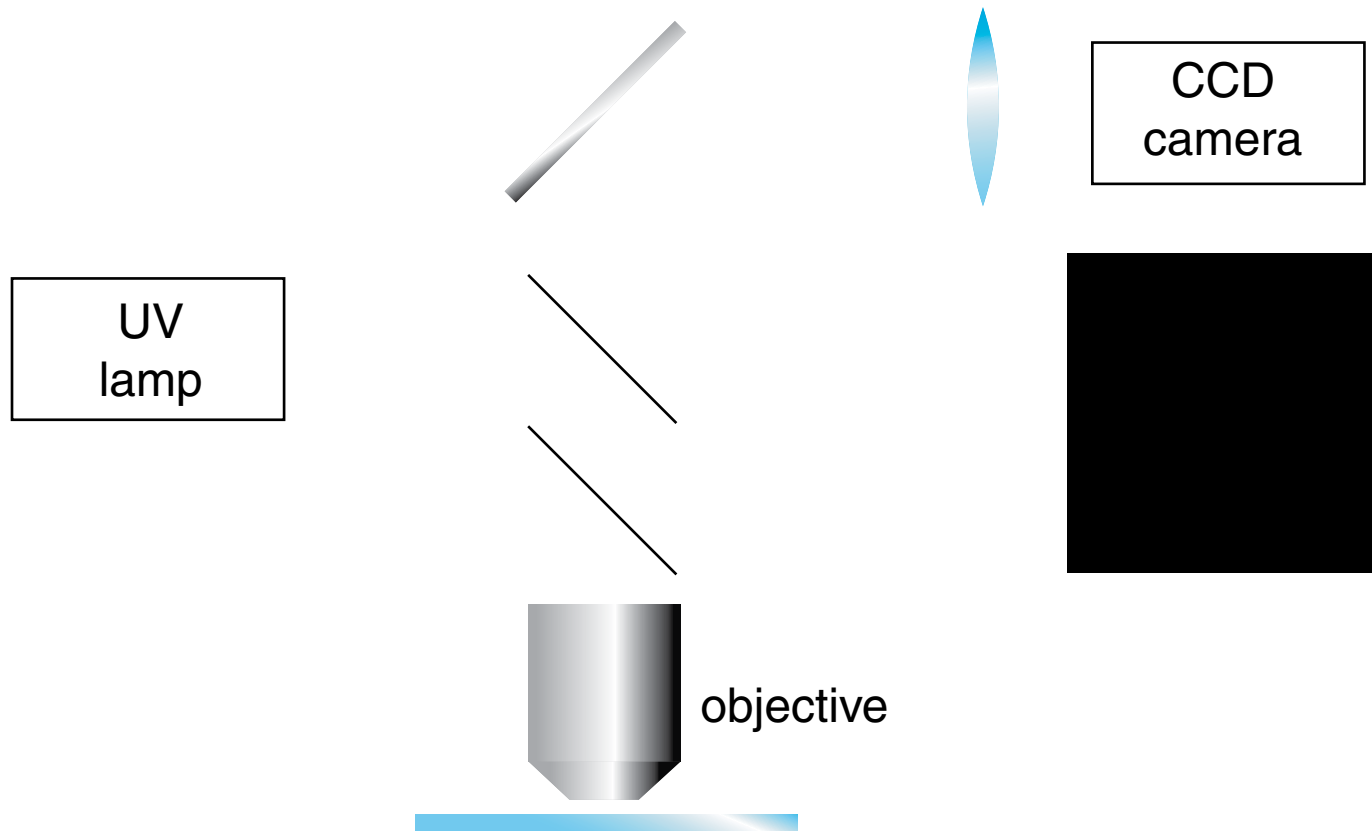
Outline

- femtosecond micromachining
 - **subcellular surgery**
 - nanoneurosurgery
- 

Subcellular surgery

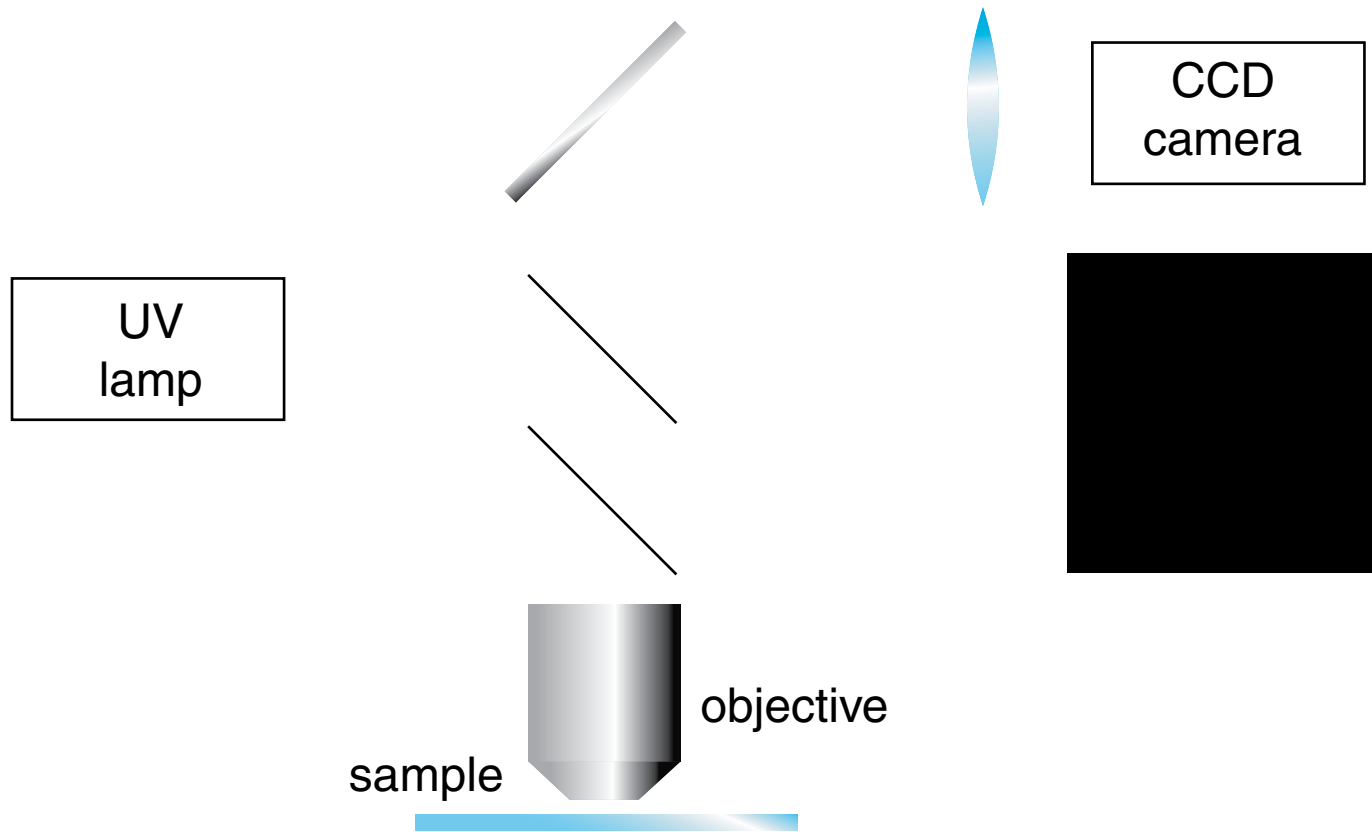
Q: can we ablate material on the subcellular scale?

Subcellular surgery



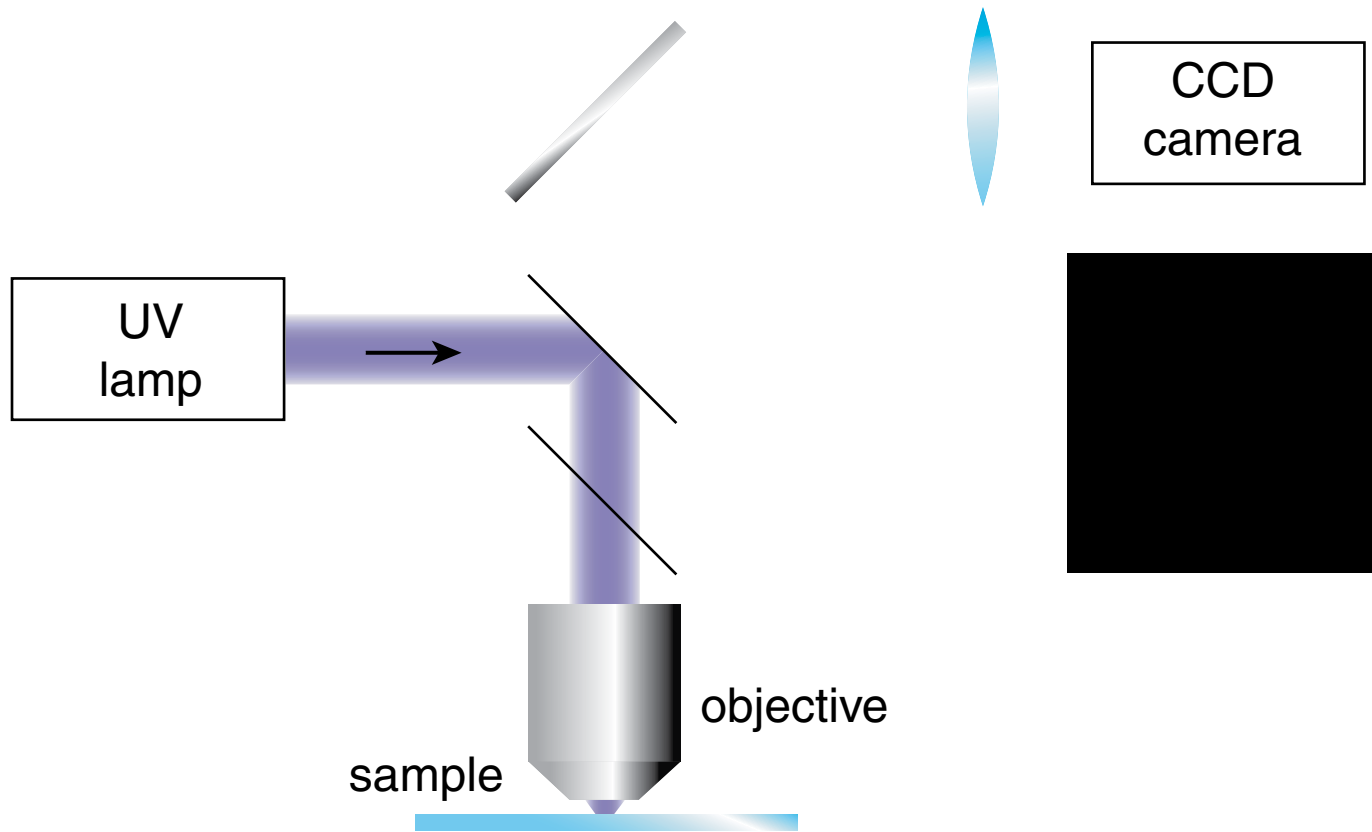
epi-fluorescence microscope

Subcellular surgery



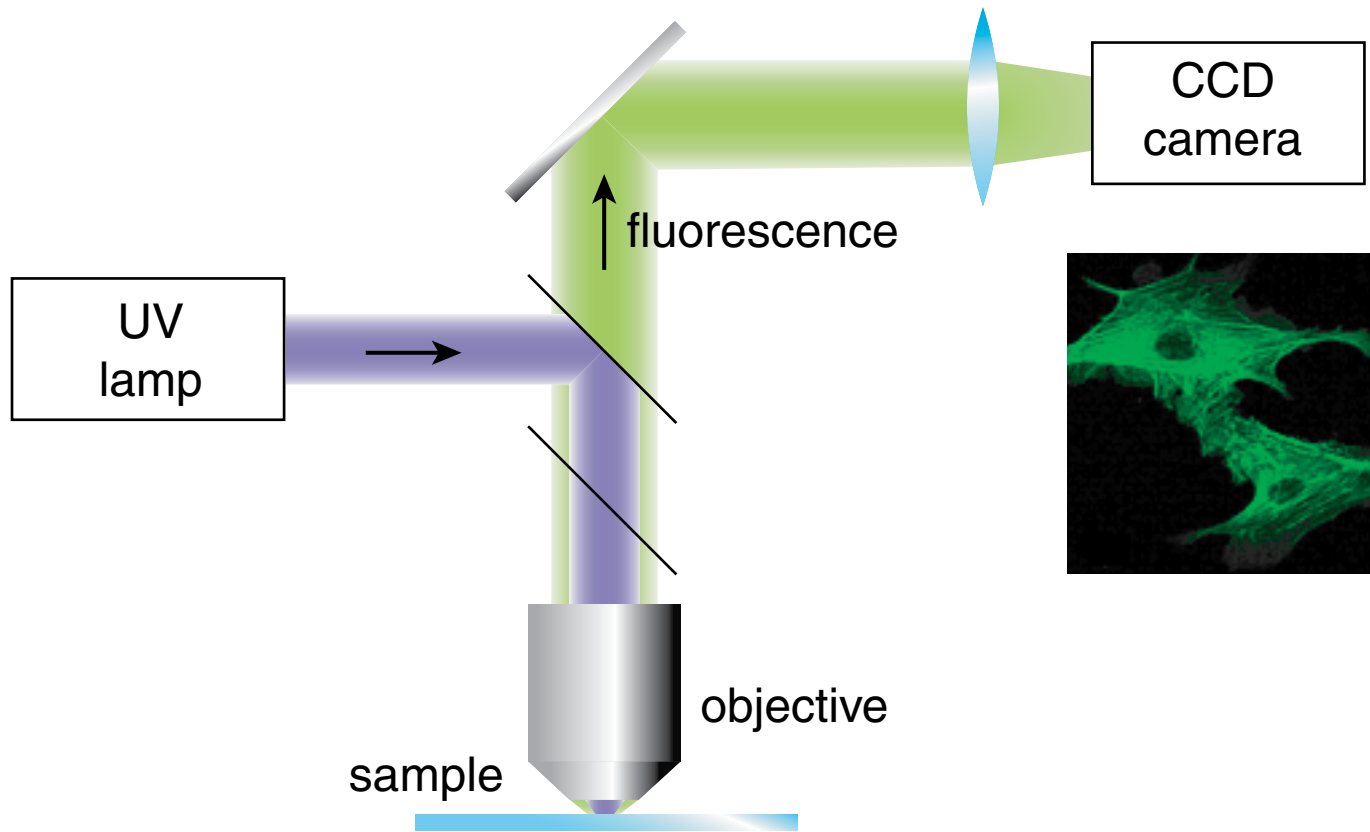
fluorescently label sample

Subcellular surgery



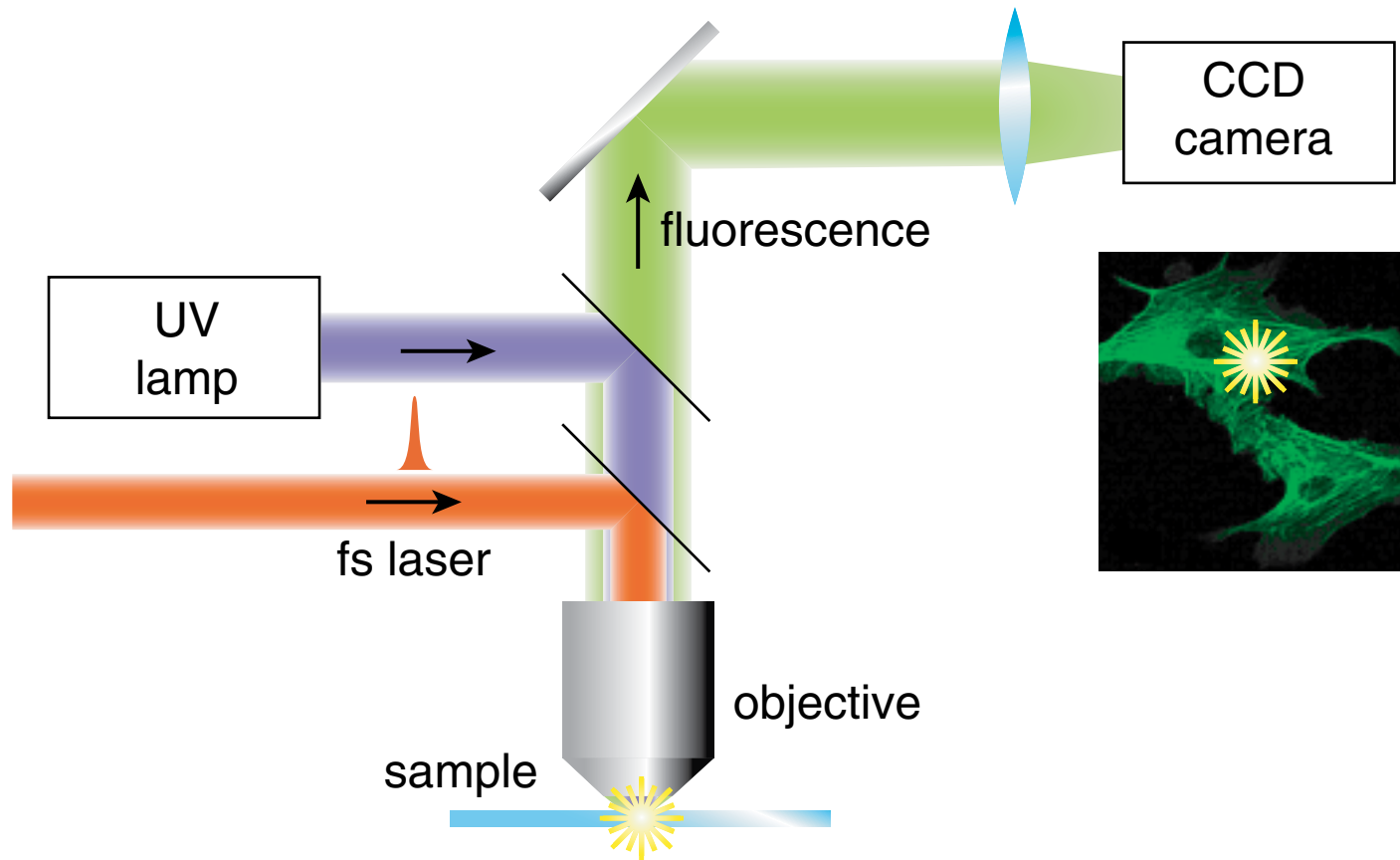
UV illumination...

Subcellular surgery



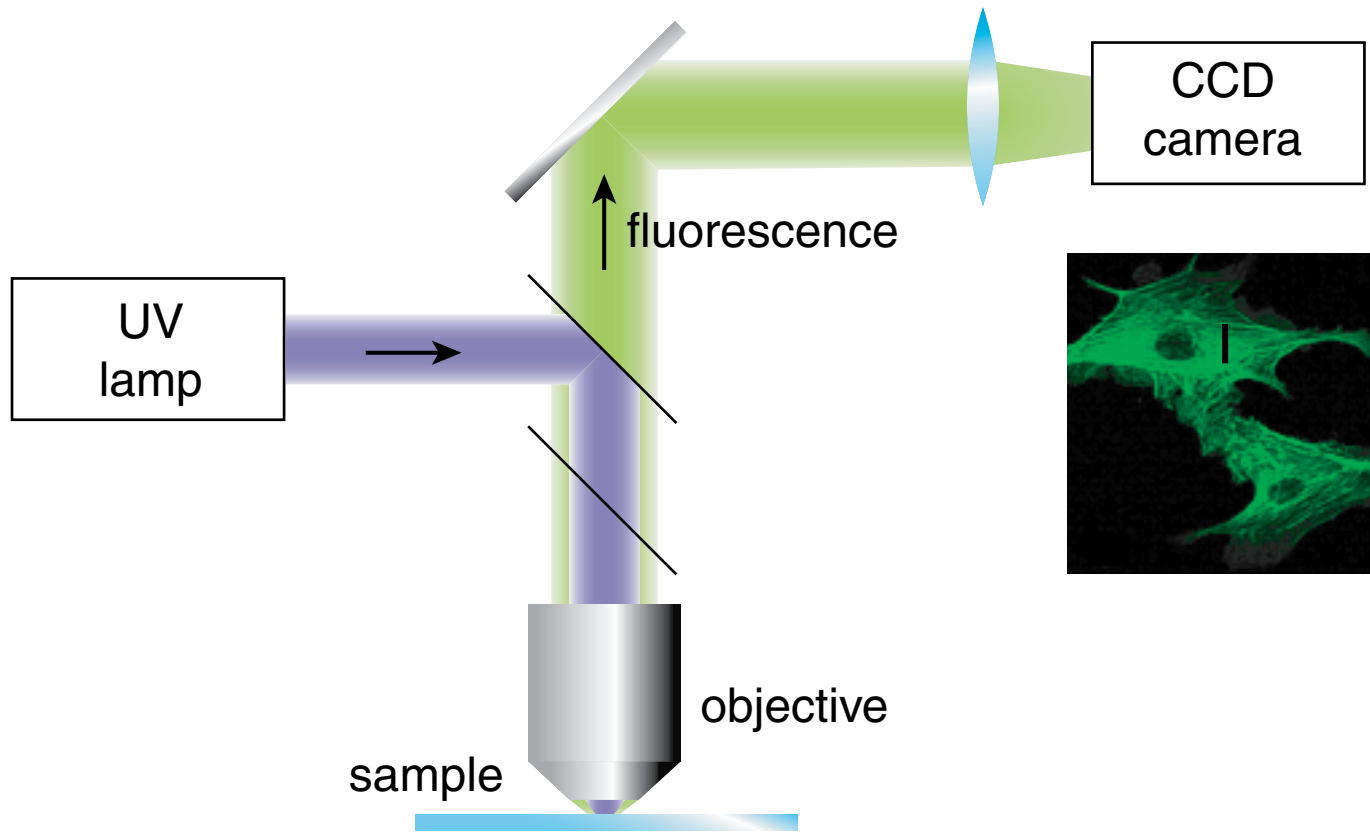
...causes fluorescence

Subcellular surgery



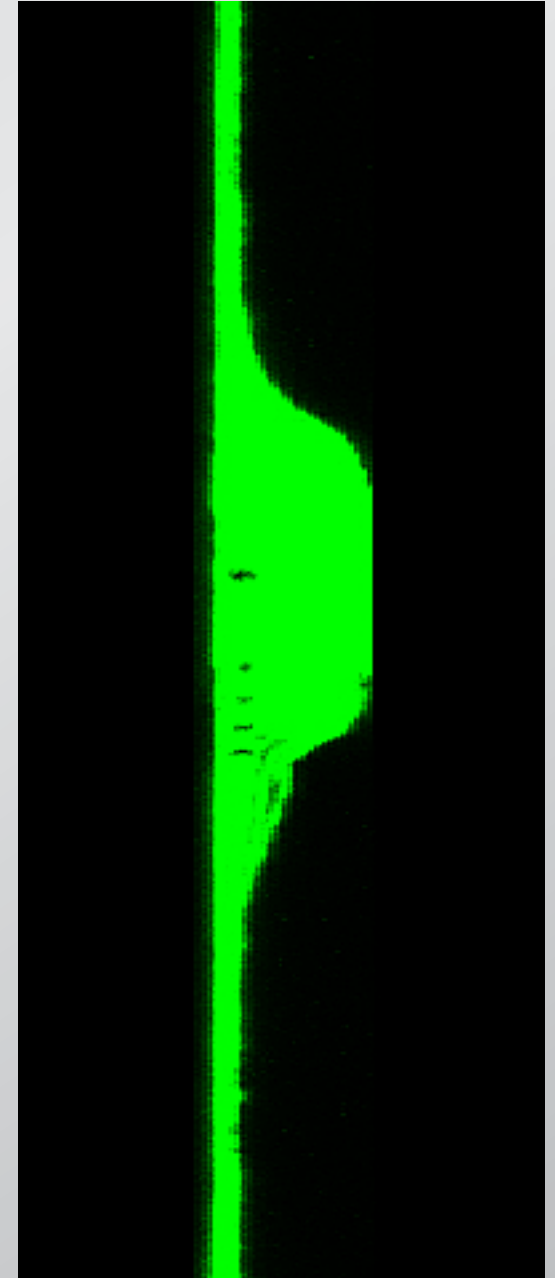
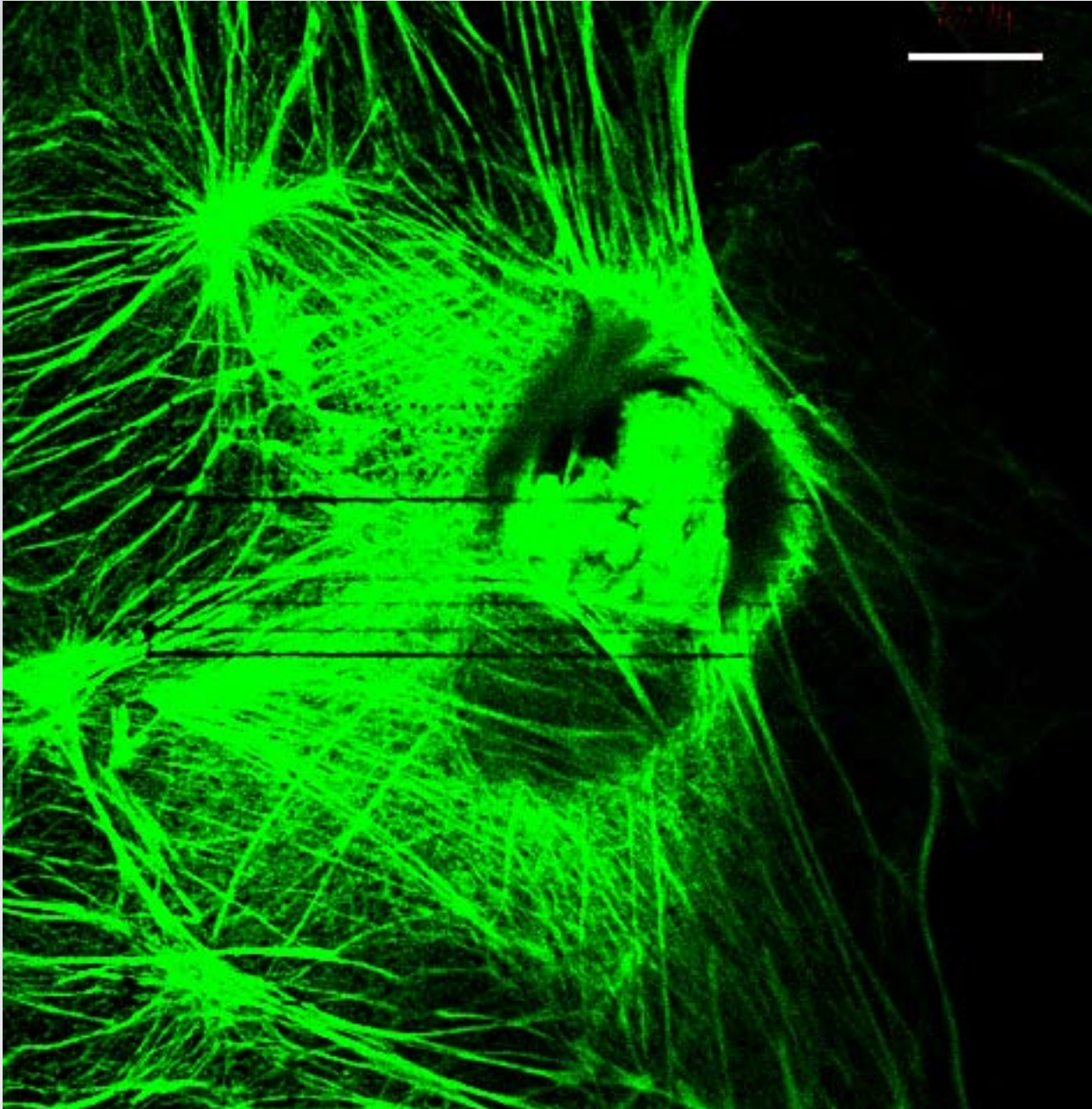
irradiate with fs laser beam

Subcellular surgery

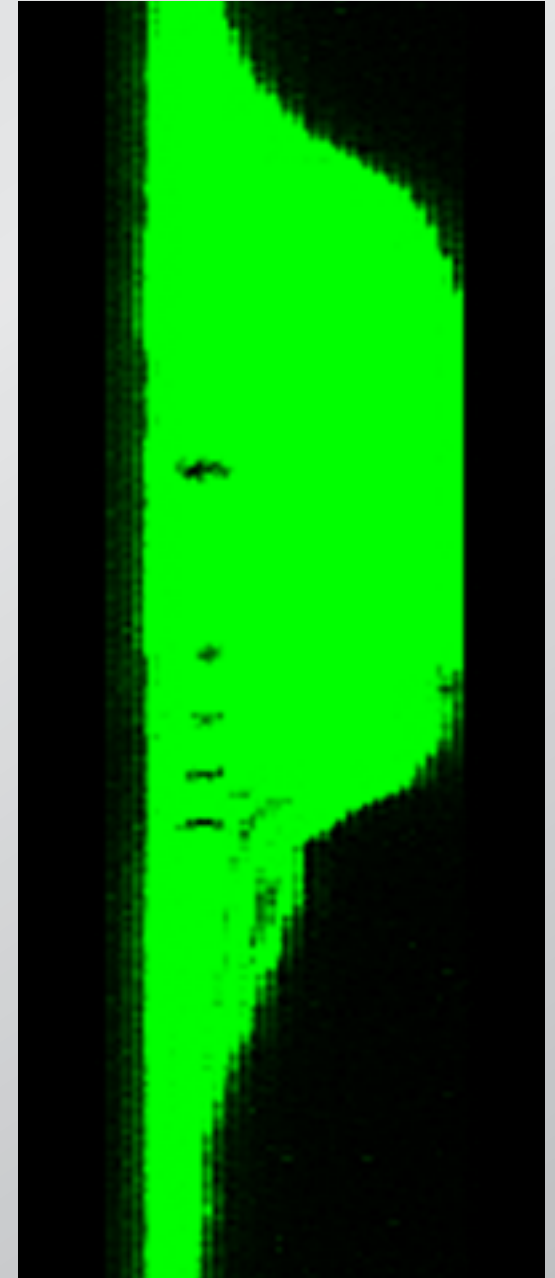
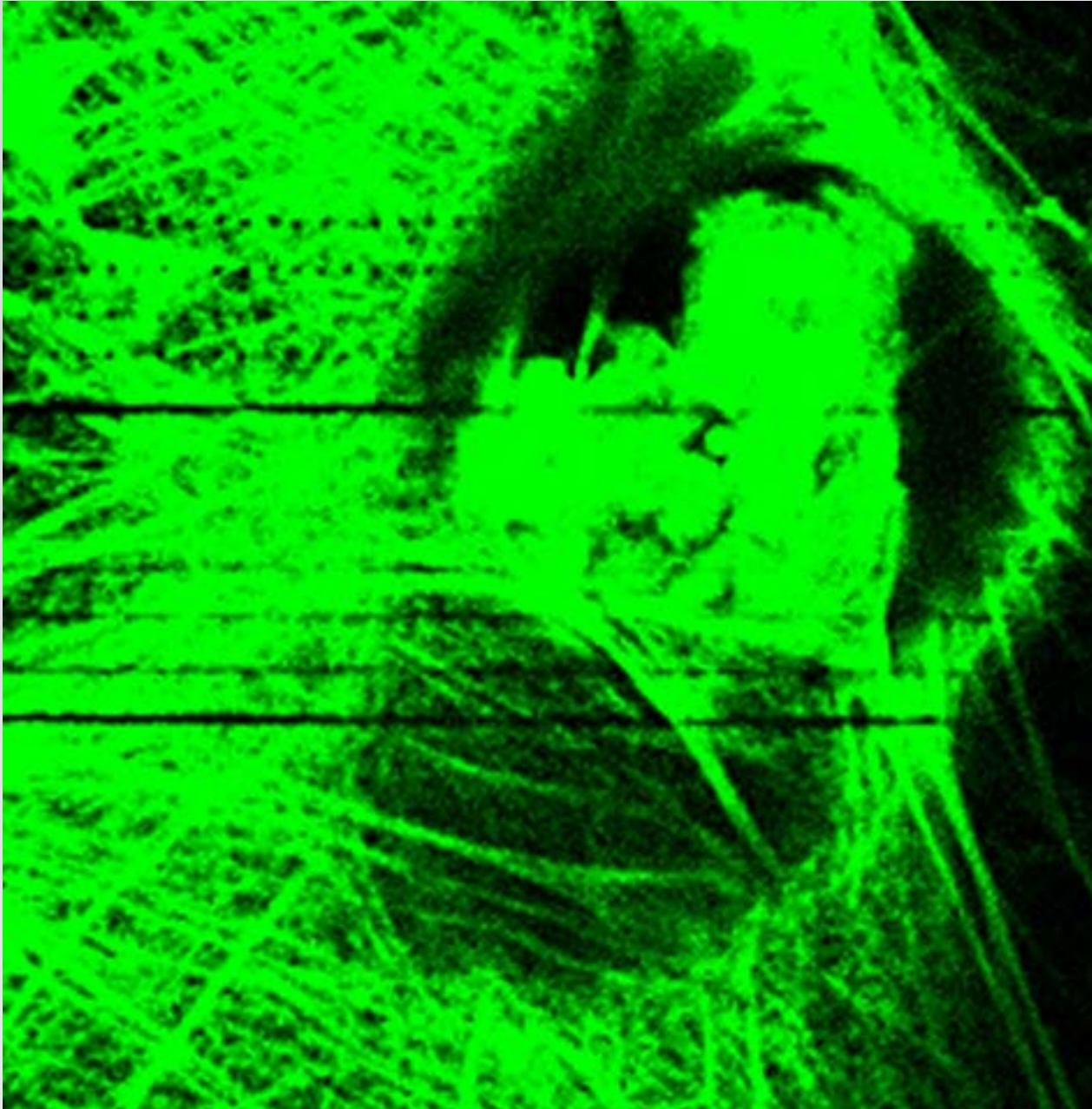


examine resulting ablation

Subcellular surgery

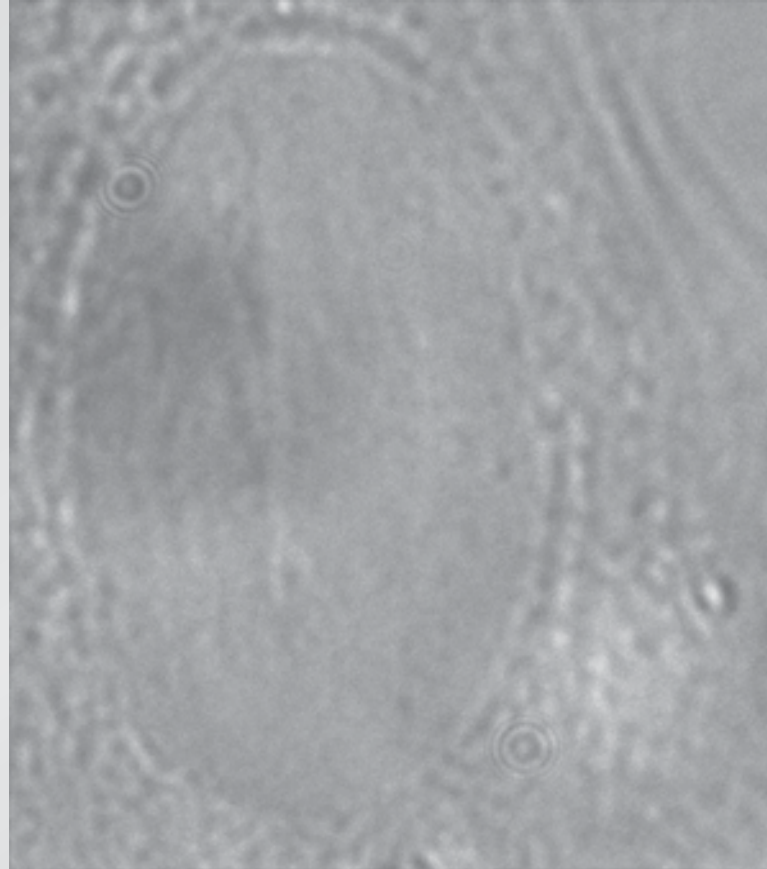


Subcellular surgery



Subcellular surgery

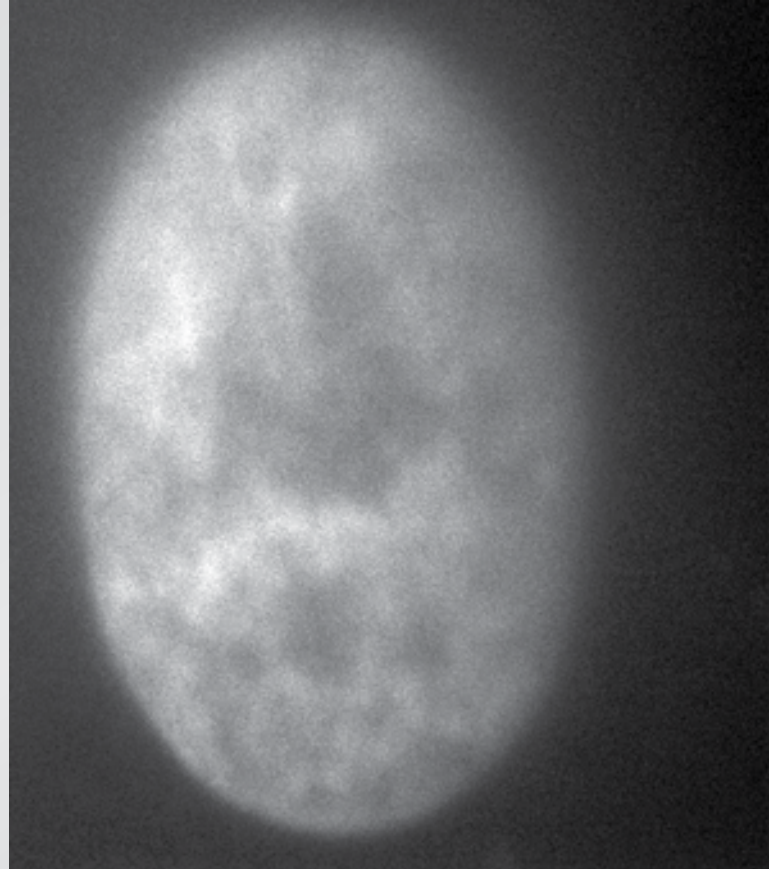
nucleus of fixed endothelial cell



white light microscopy

Subcellular surgery

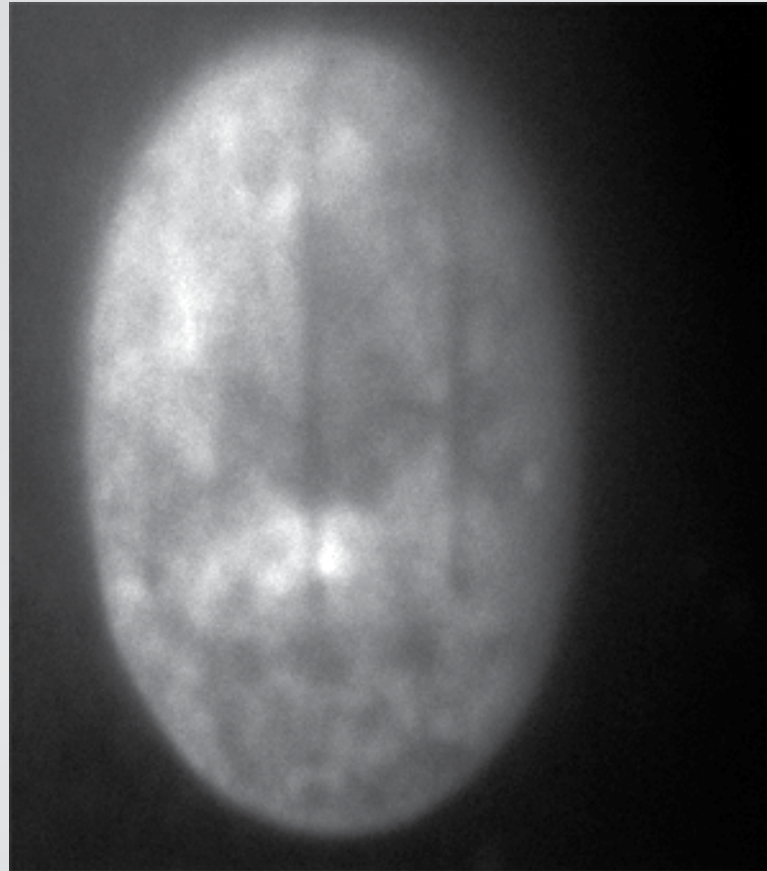
nucleus of fixed endothelial cell



fluorescence microscopy

Subcellular surgery

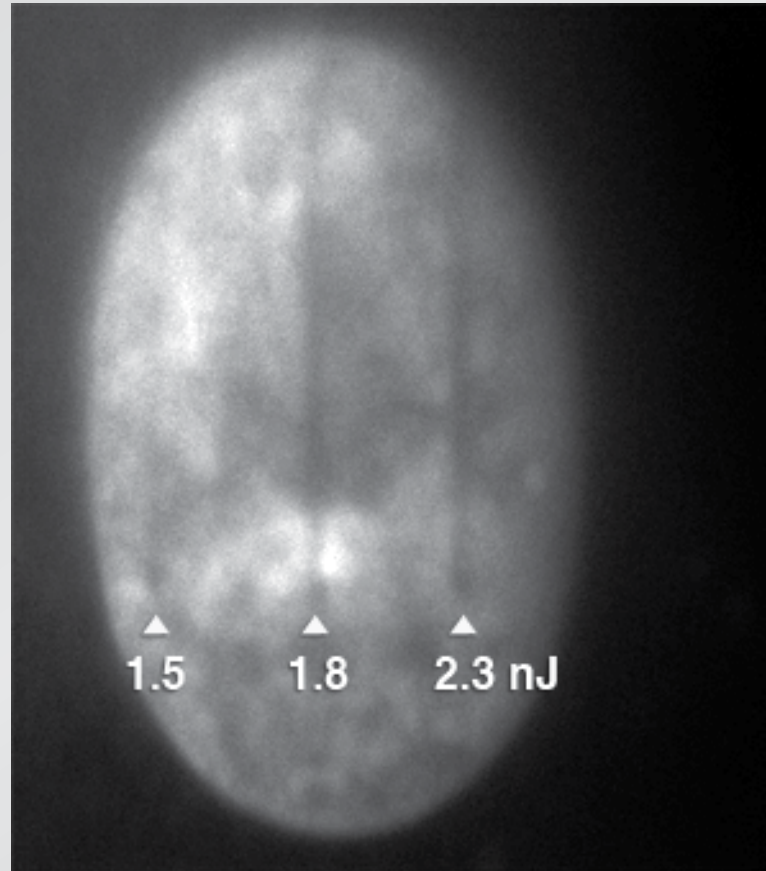
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

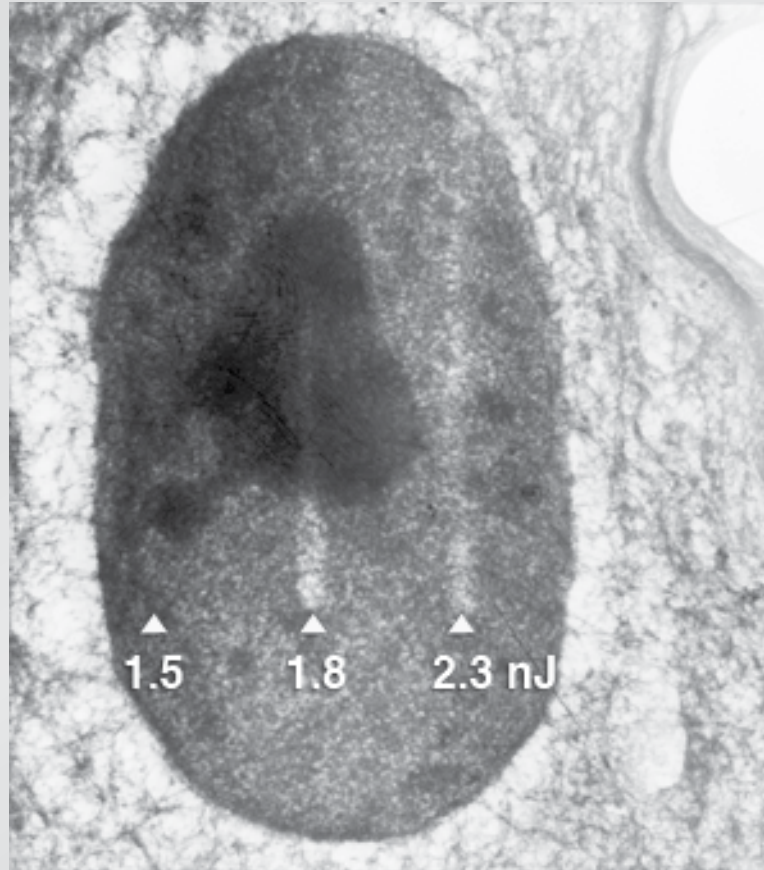
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

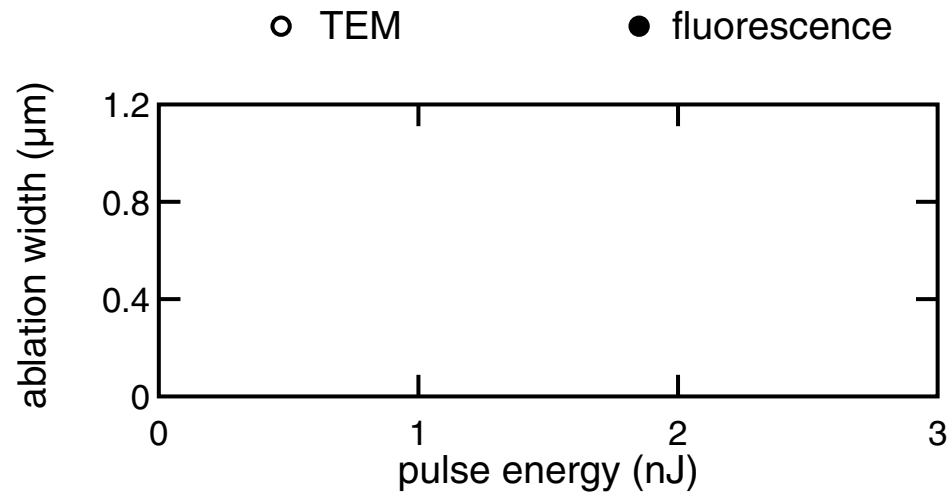
bleaching or ablation?



TEM image

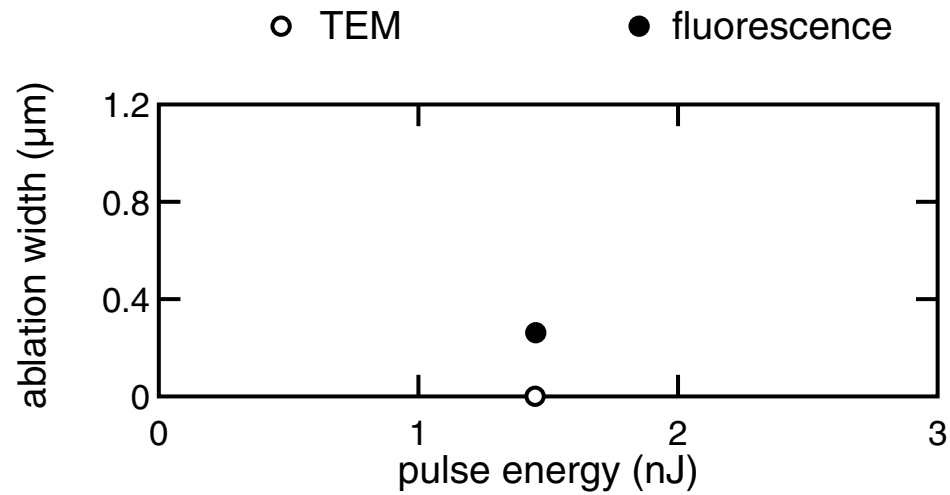
Subcellular surgery

three regions of interaction



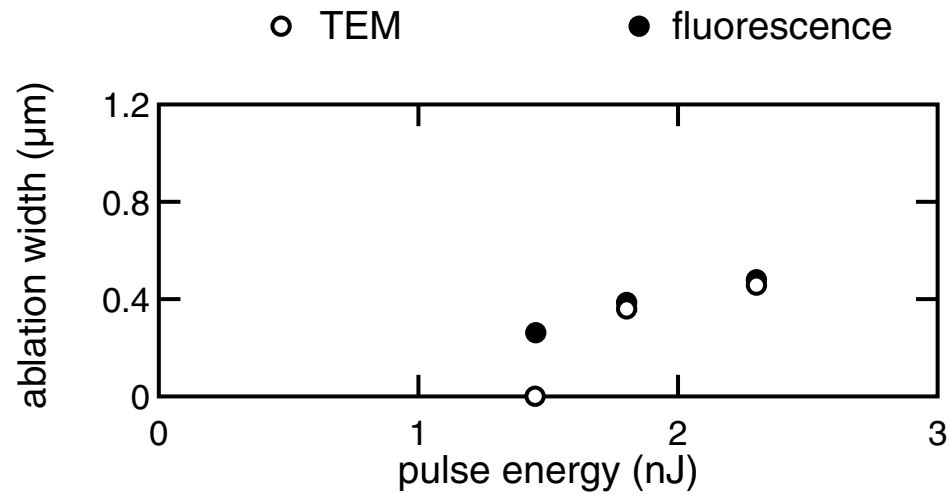
Subcellular surgery

three regions of interaction



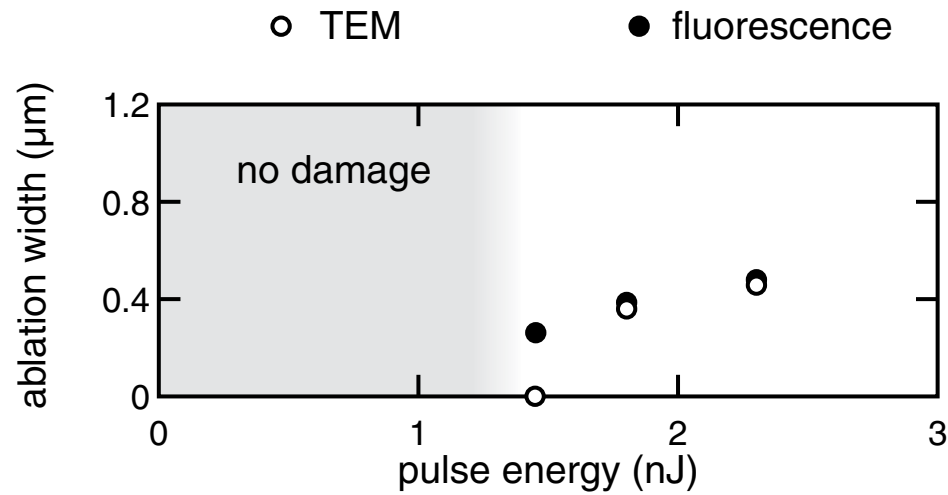
Subcellular surgery

three regions of interaction



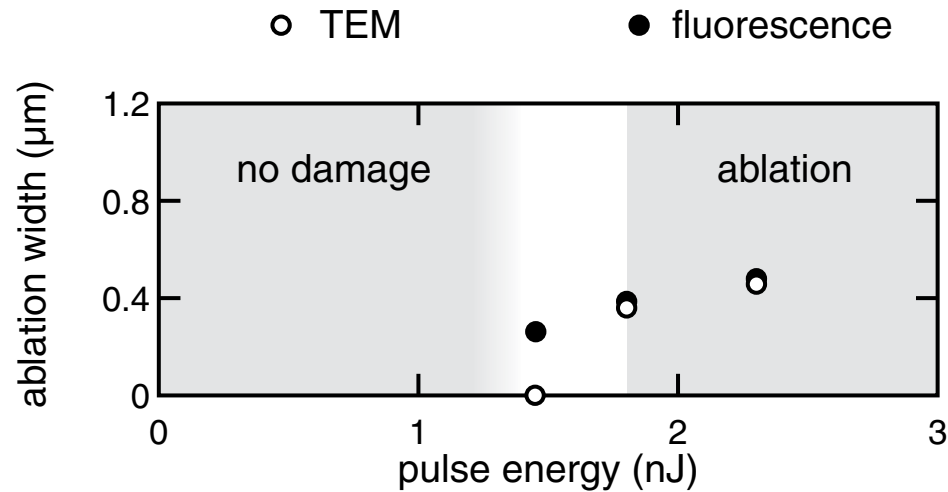
Subcellular surgery

three regions of interaction



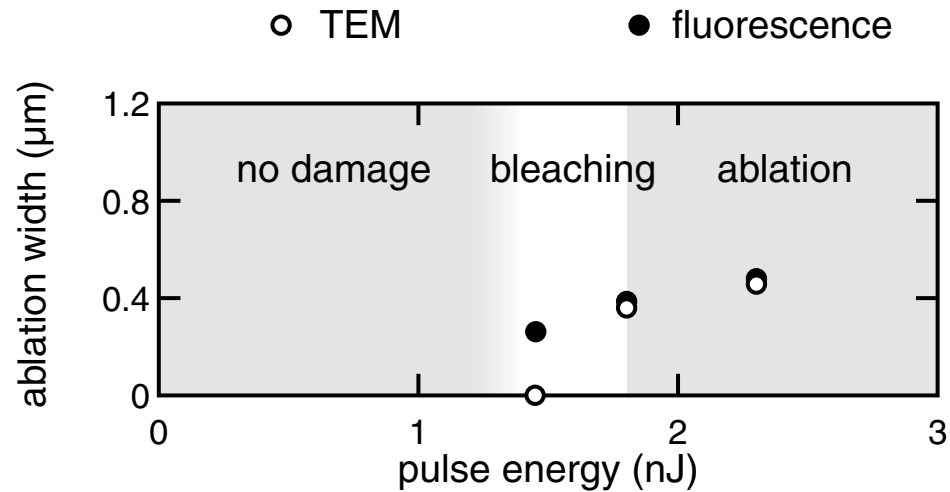
Subcellular surgery

three regions of interaction



Subcellular surgery

three regions of interaction

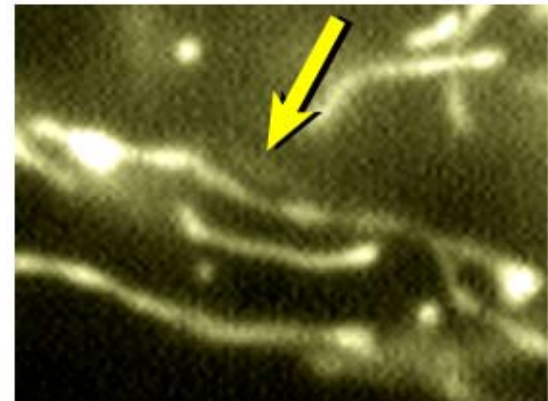
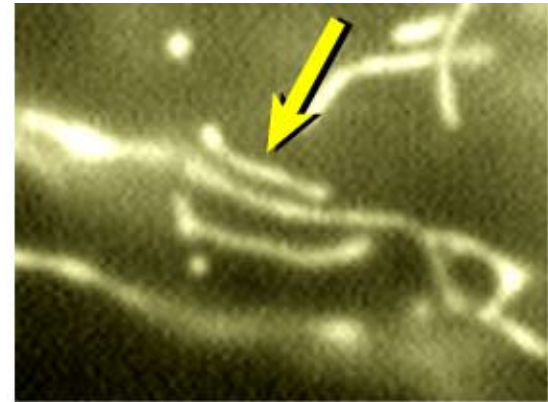
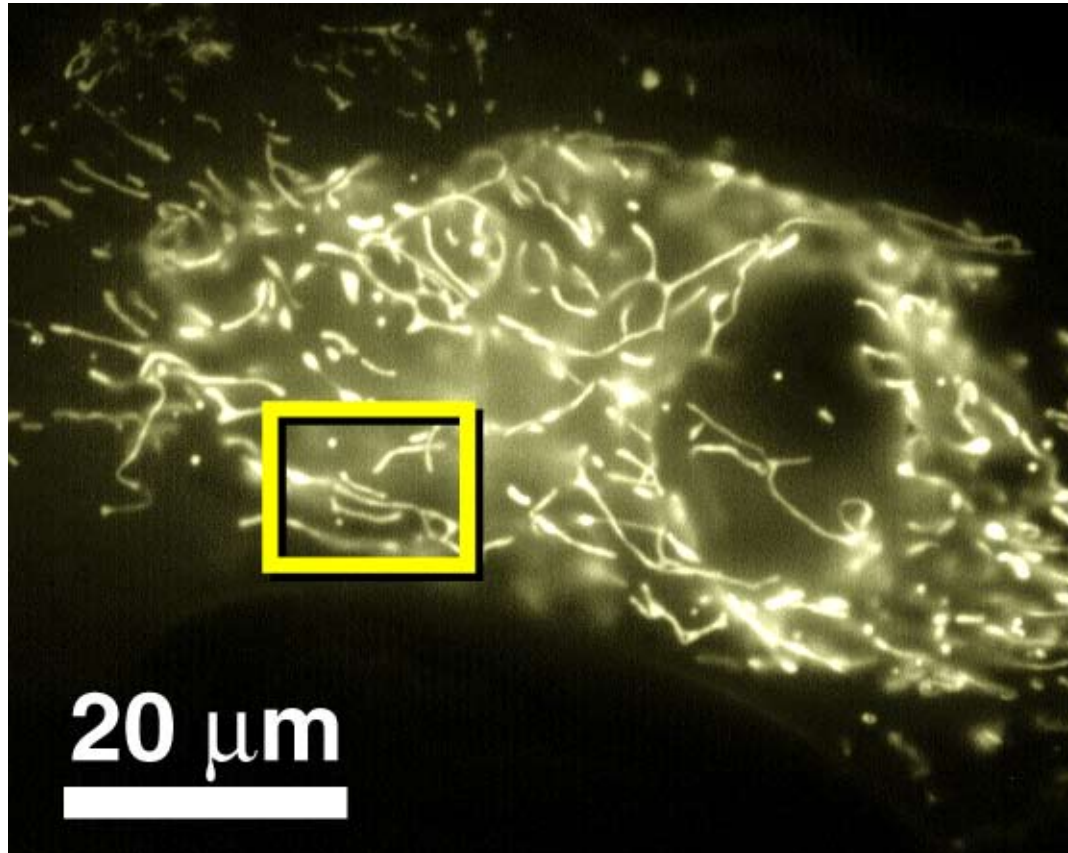


Subcellular surgery

Q: subcellular surgery on live cells?

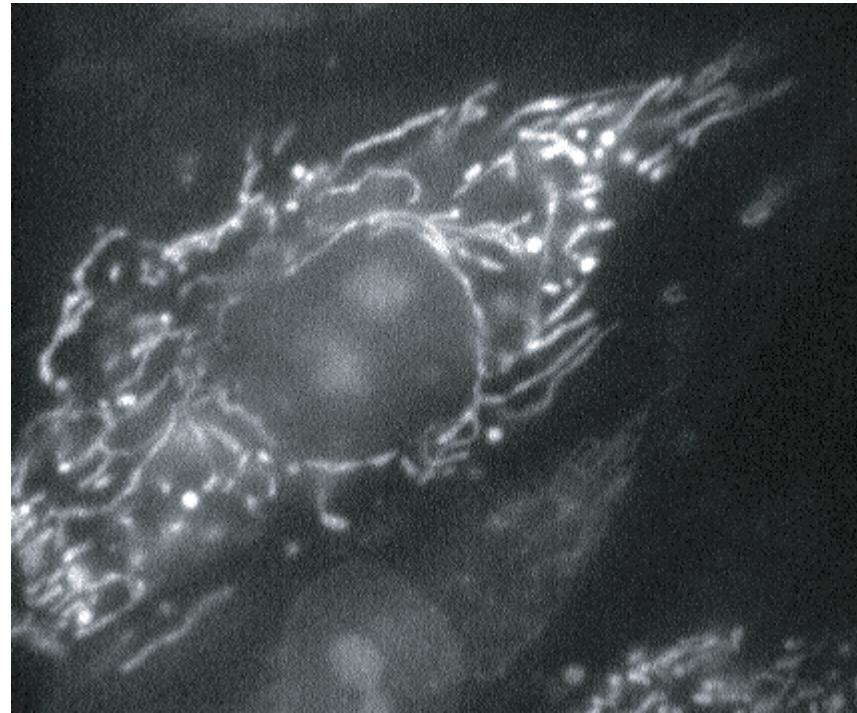
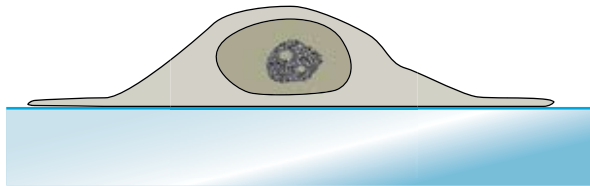
A fluorescence microscopy image of a cell. The cytoskeleton is highlighted in bright yellow/green, showing a complex network of filaments and microtubules. The nucleus is visible as a darker, more rounded structure on the right side of the cell. The overall image has a dark background, making the glowing structures stand out.

Subcellular surgery



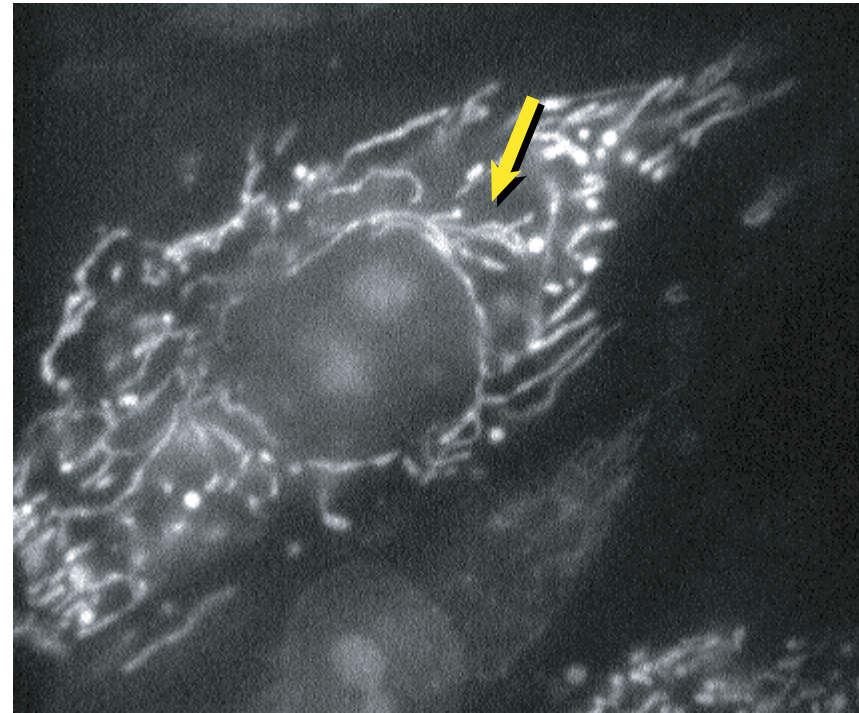
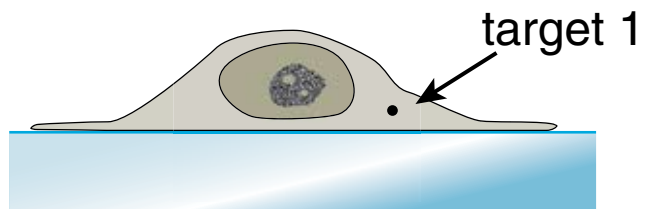
Subcellular surgery

ethyidium bromide test



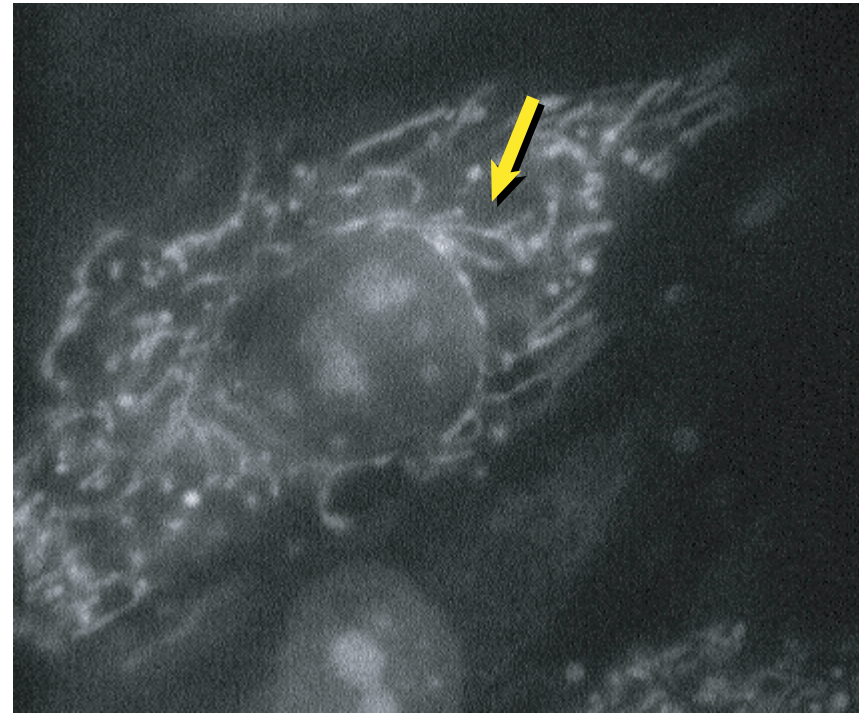
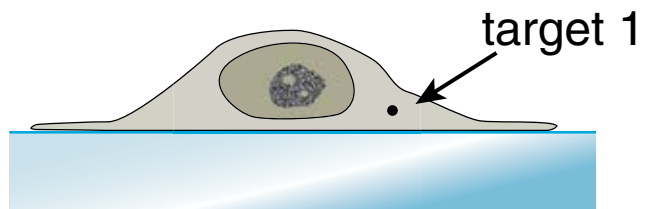
Subcellular surgery

ethyidium bromide test



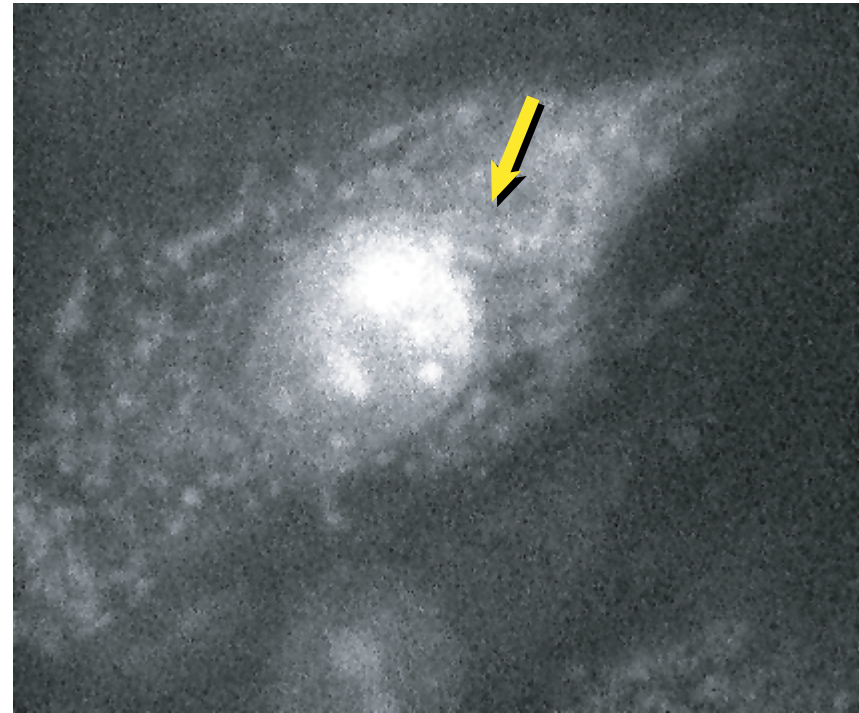
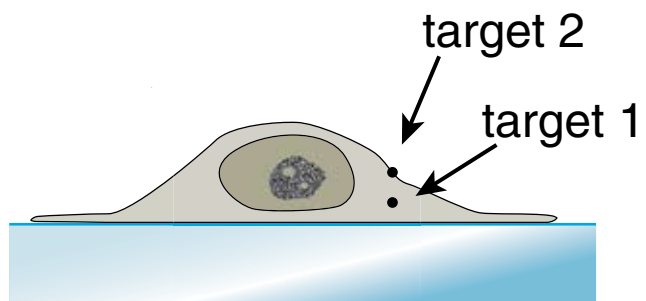
Subcellular surgery

ethyidium bromide test



Subcellular surgery

ethyidium bromide test

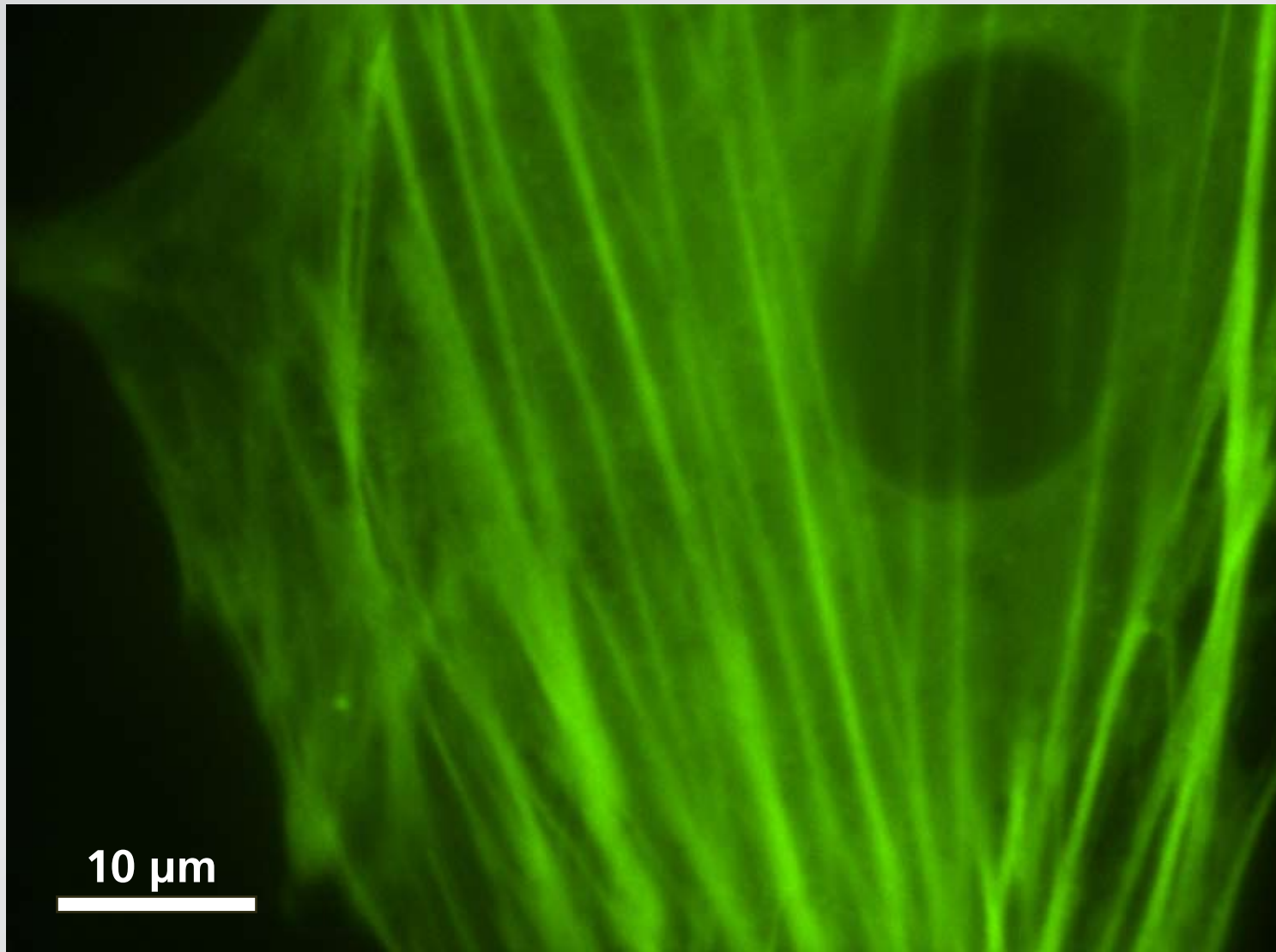


Subcellular surgery

Q: can we probe the dynamics of the cytoskeleton?

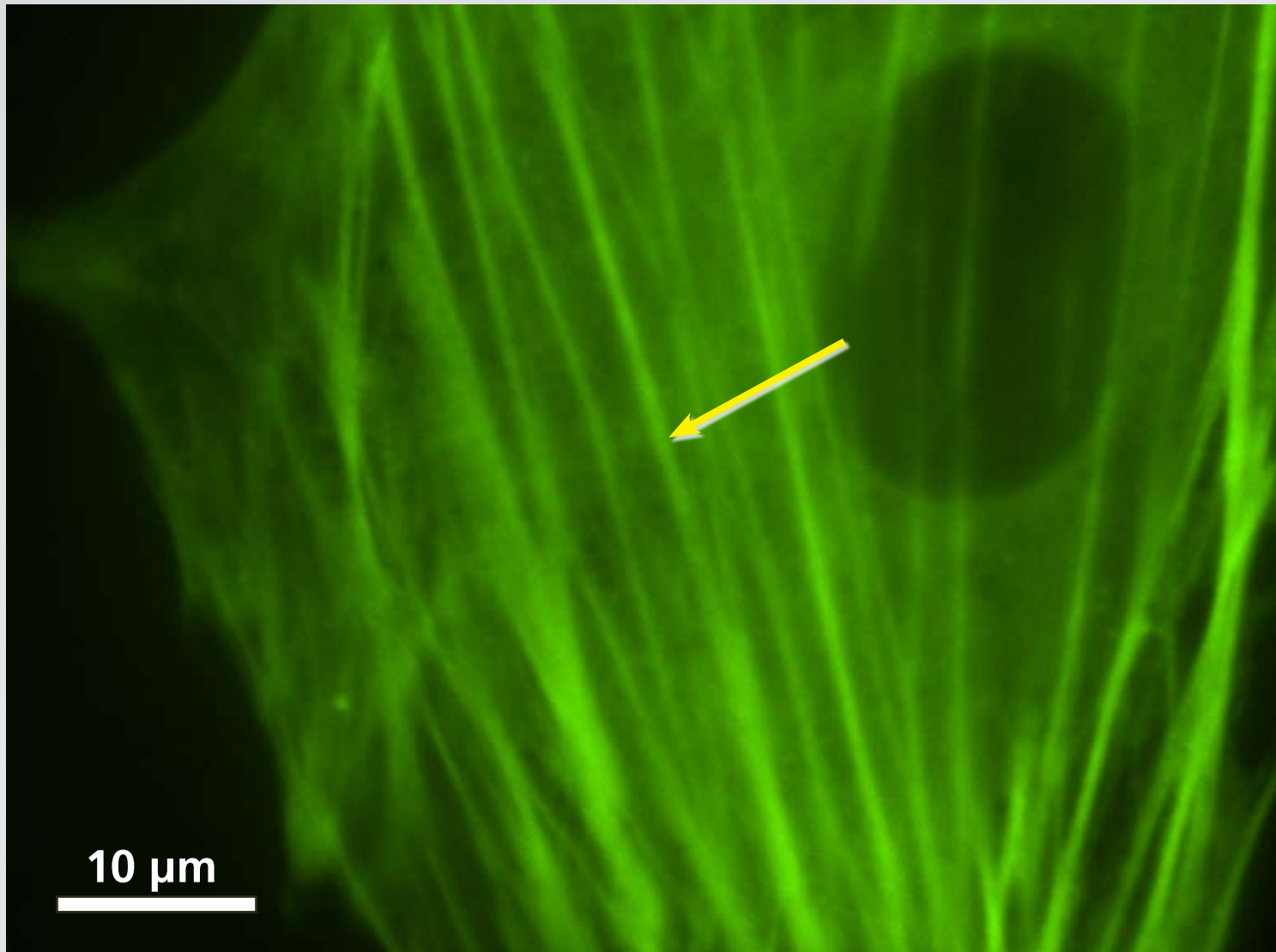
Subcellular surgery

YFP-labeled actin fiber network of a live cell



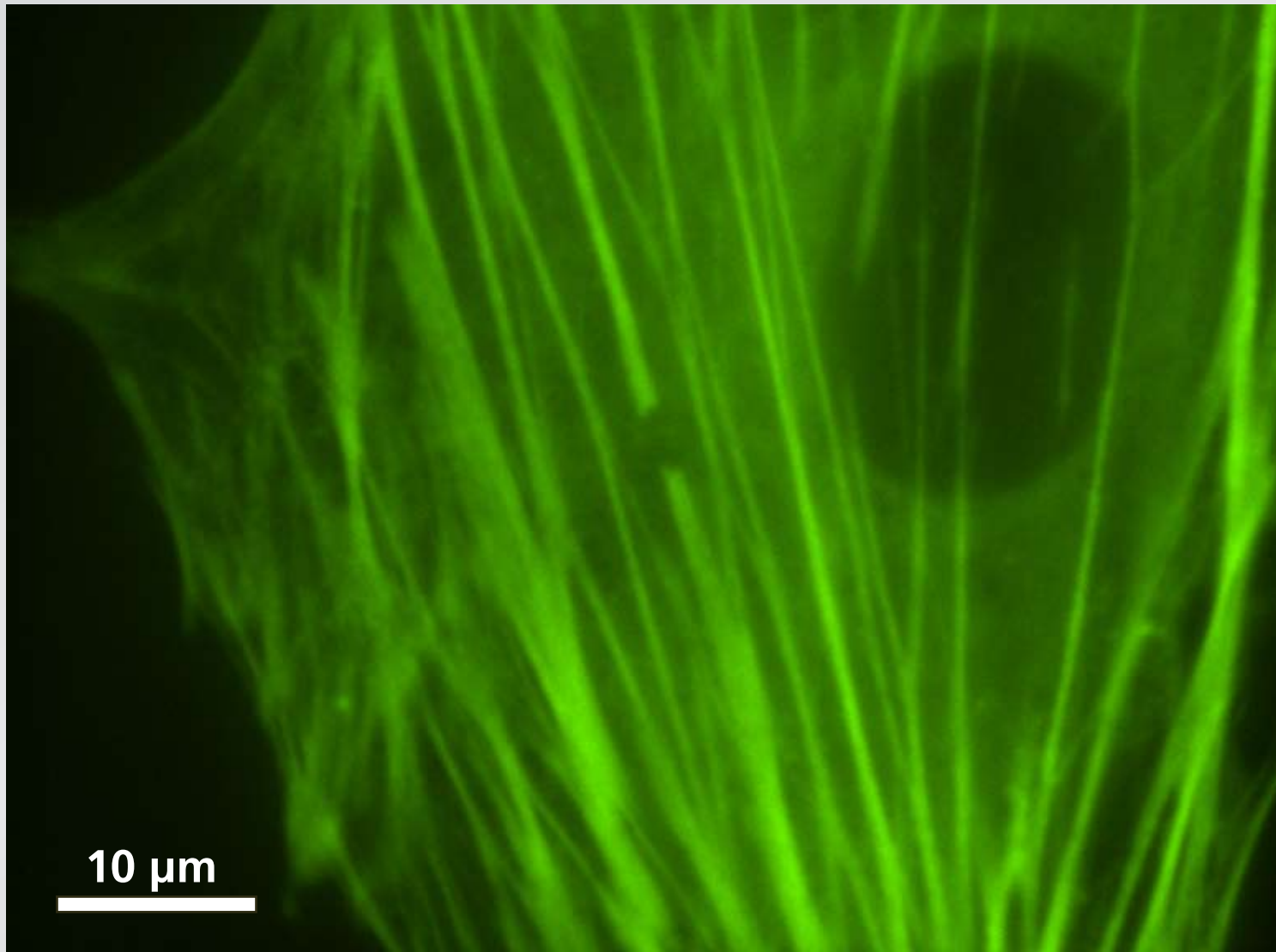
Subcellular surgery

cut a single fiber bundle



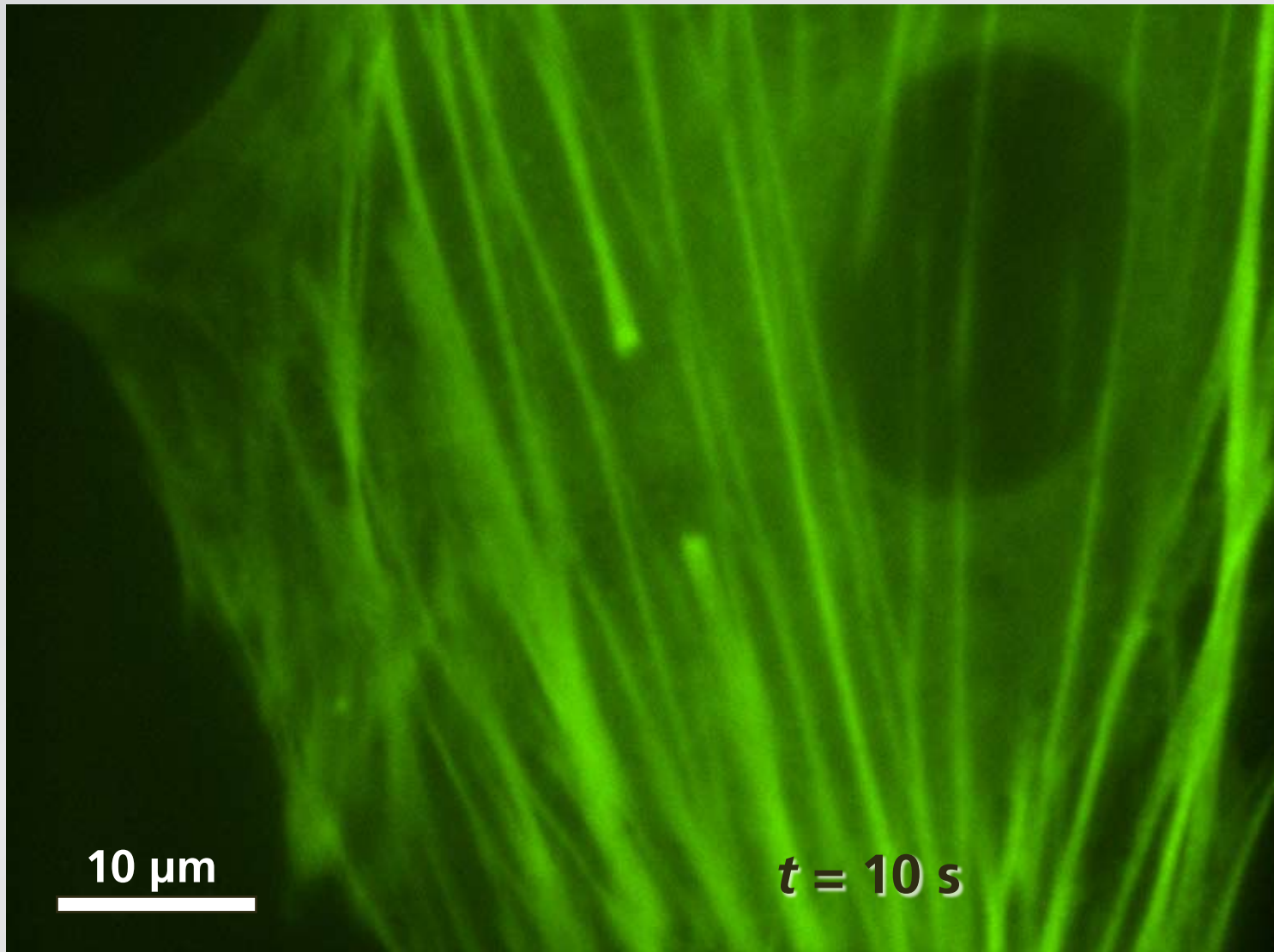
Subcellular surgery

cut a single fiber bundle



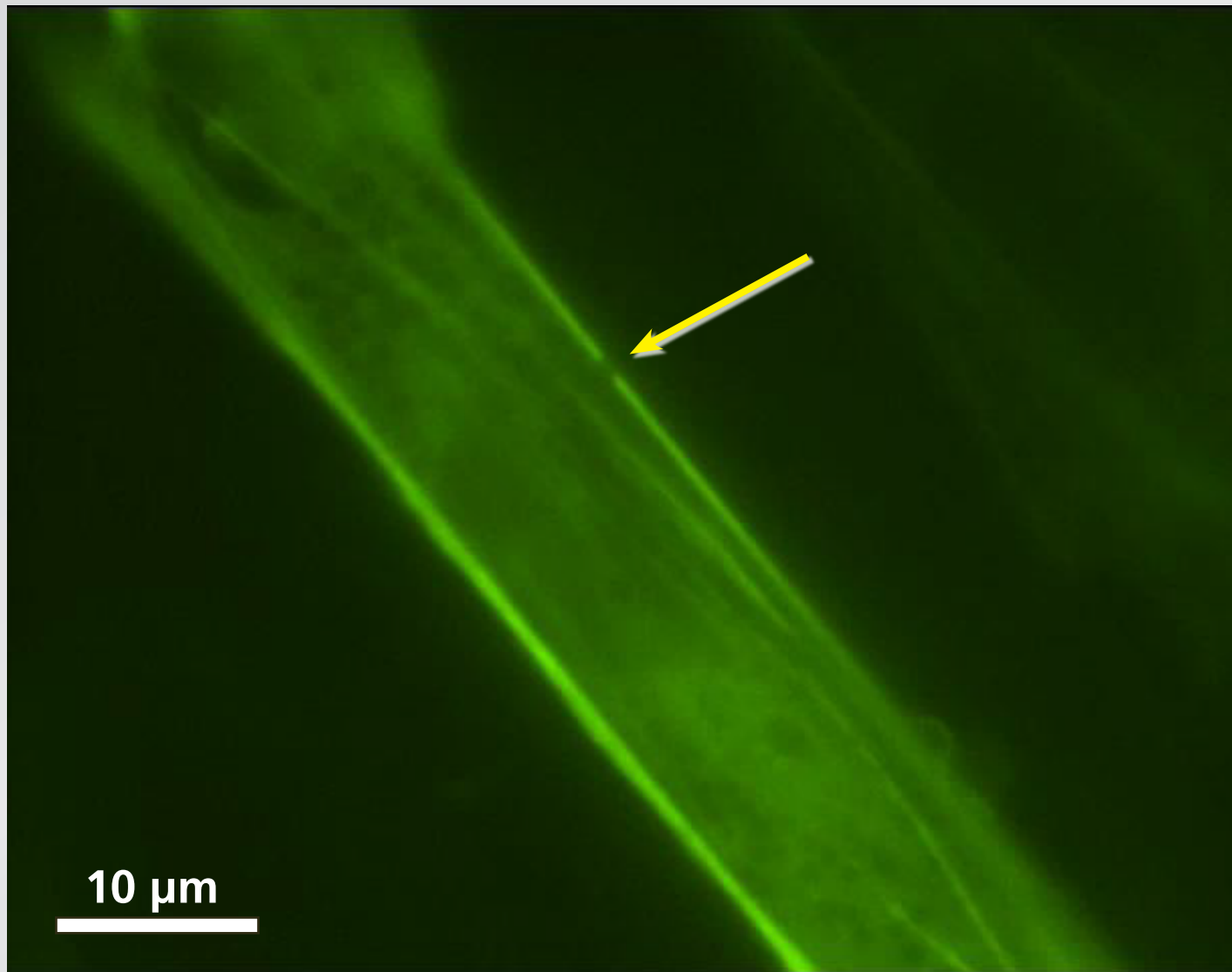
Subcellular surgery

gap widens with time



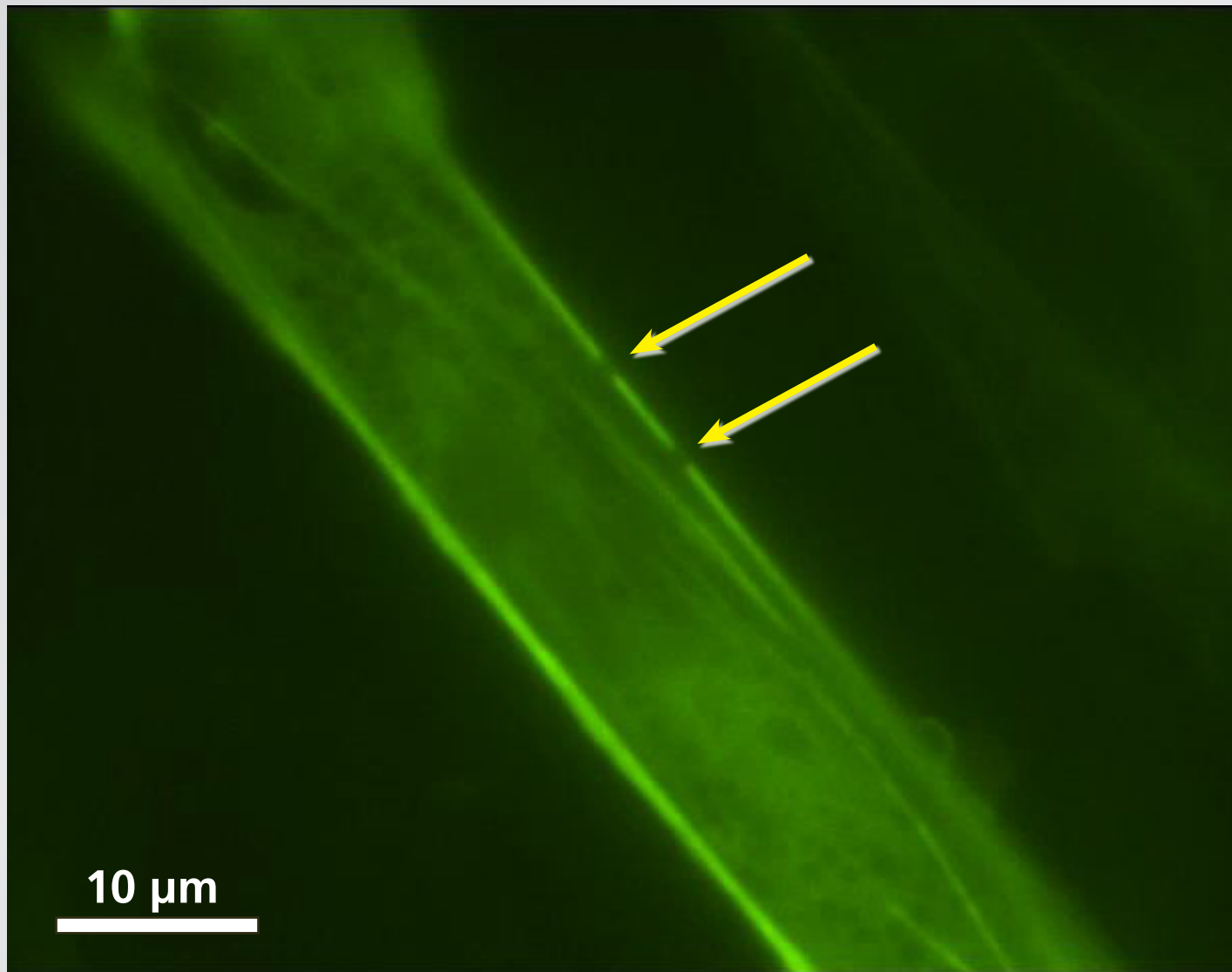
Subcellular surgery

retraction or depolymerization?



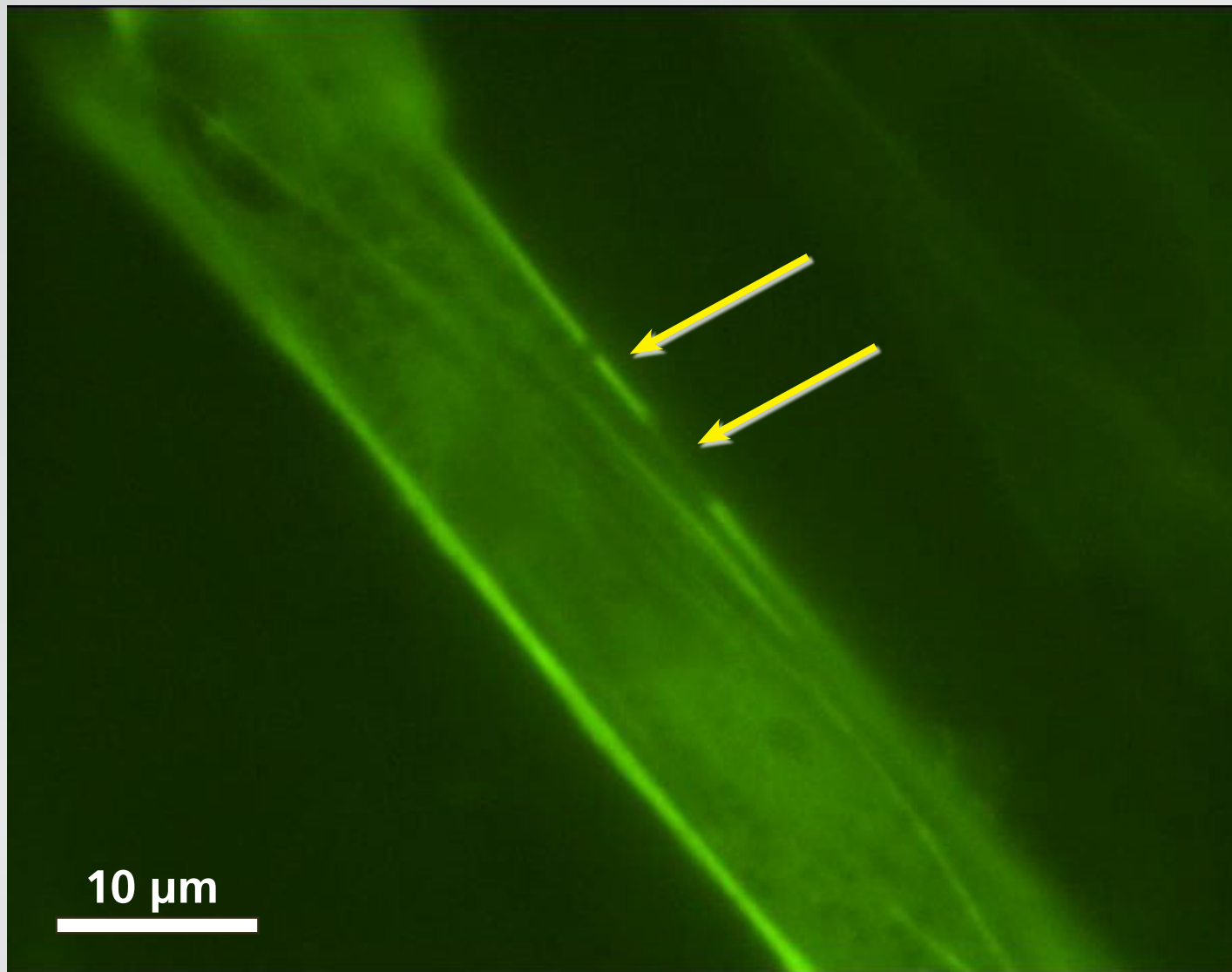
Subcellular surgery

retraction or depolymerization?



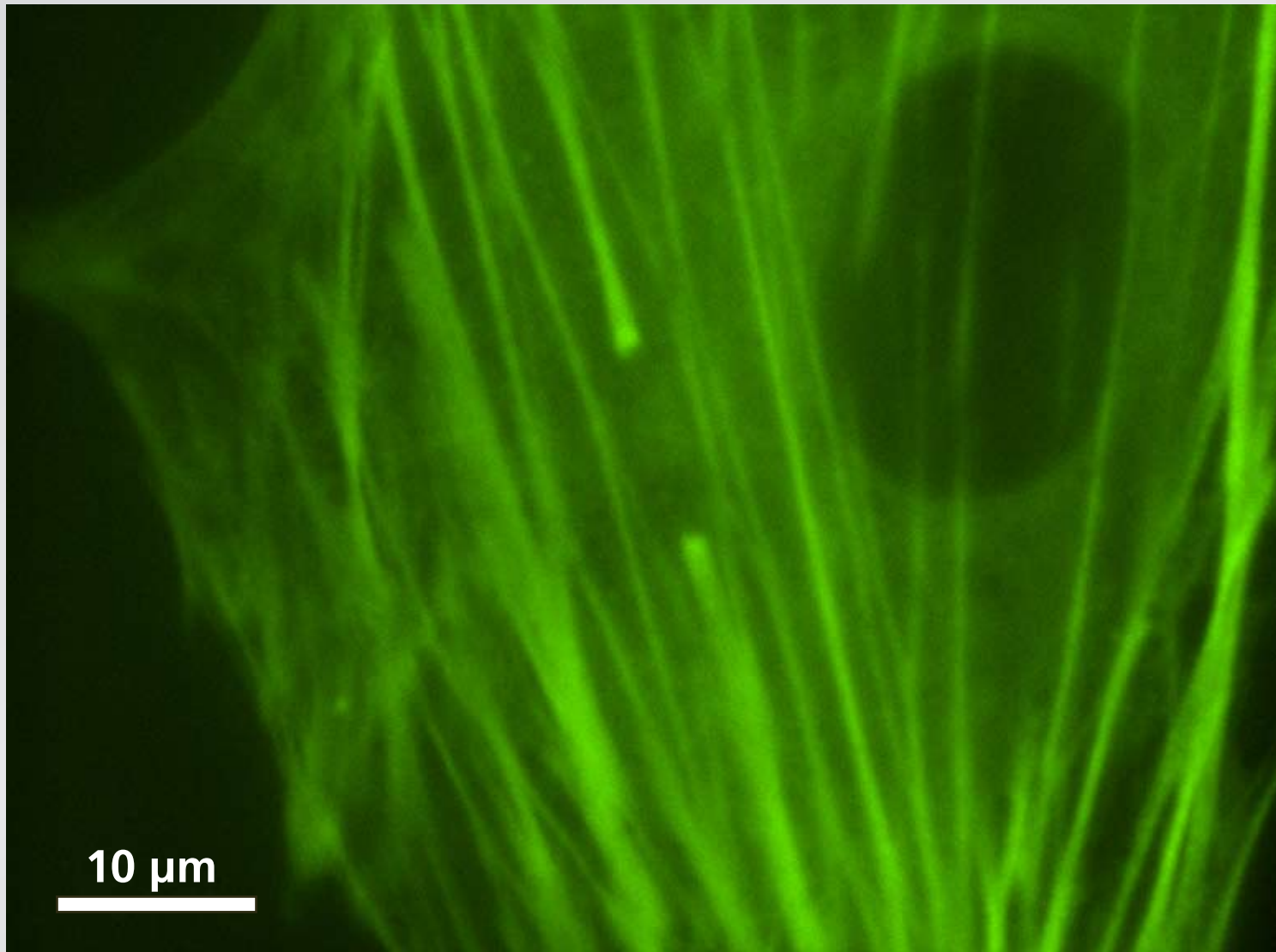
Subcellular surgery

retraction or depolymerization?



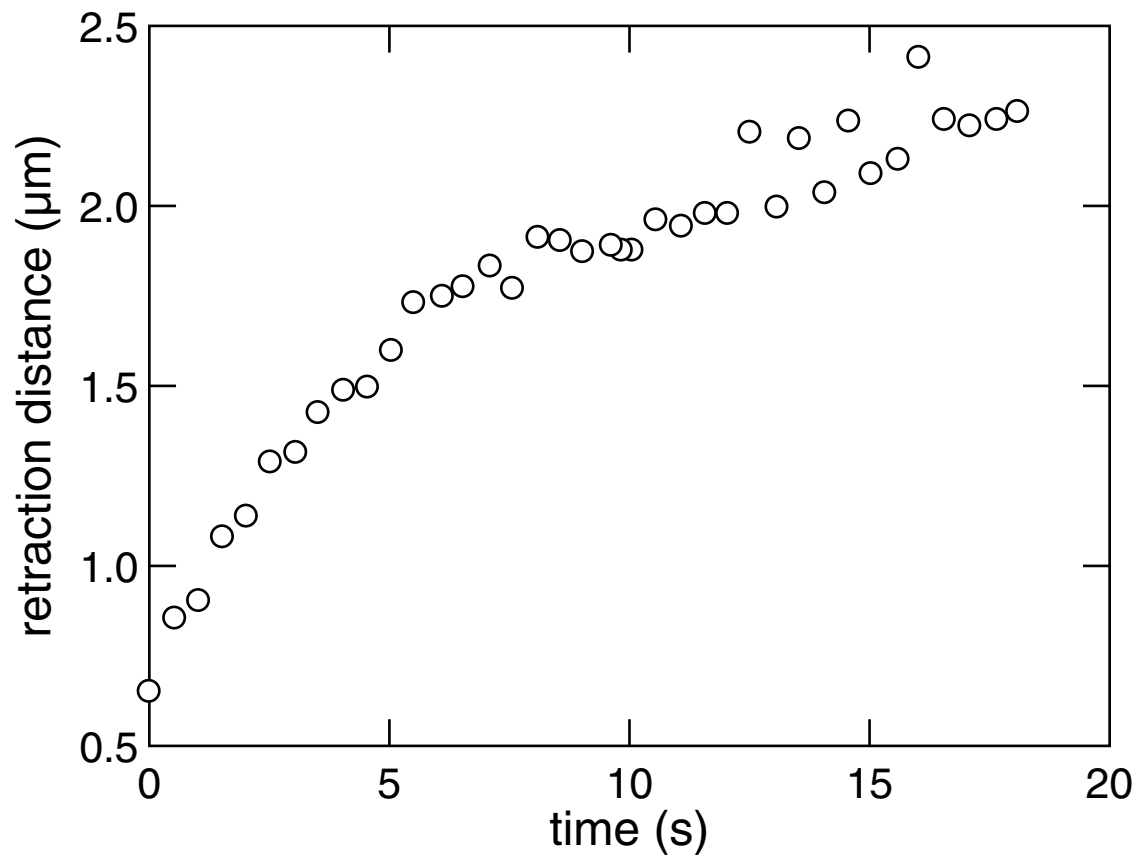
Subcellular surgery

dynamics provides information on *in vivo* mechanics



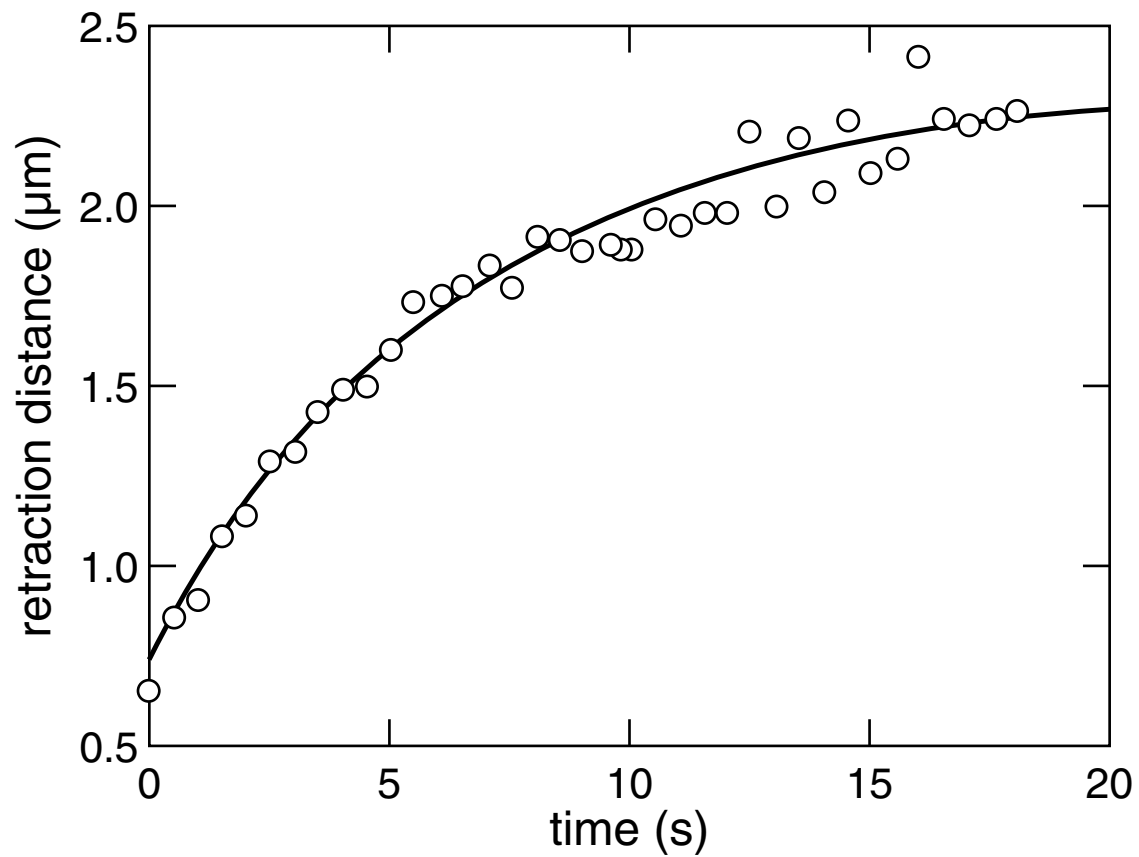
Subcellular surgery

overdamped spring: $\Delta L = L_{\infty}(1 - e^{-t/\tau}) + L_0$



Subcellular surgery

overdamped spring: $\Delta L = L_{\infty}(1 - e^{-t/\tau}) + L_0$



Outline

- femtosecond micromachining
 - subcellular surgery
 - **nanoneurosurgery**
- 

Nanoneurosurgery

Q: can we probe the neurological origins of behavior?



Nanoneurosurgery

Caenorhabditis elegans



Juergen Berger & Ralph Sommer
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Nanoneurosurgery

Caenorhabditis elegans

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

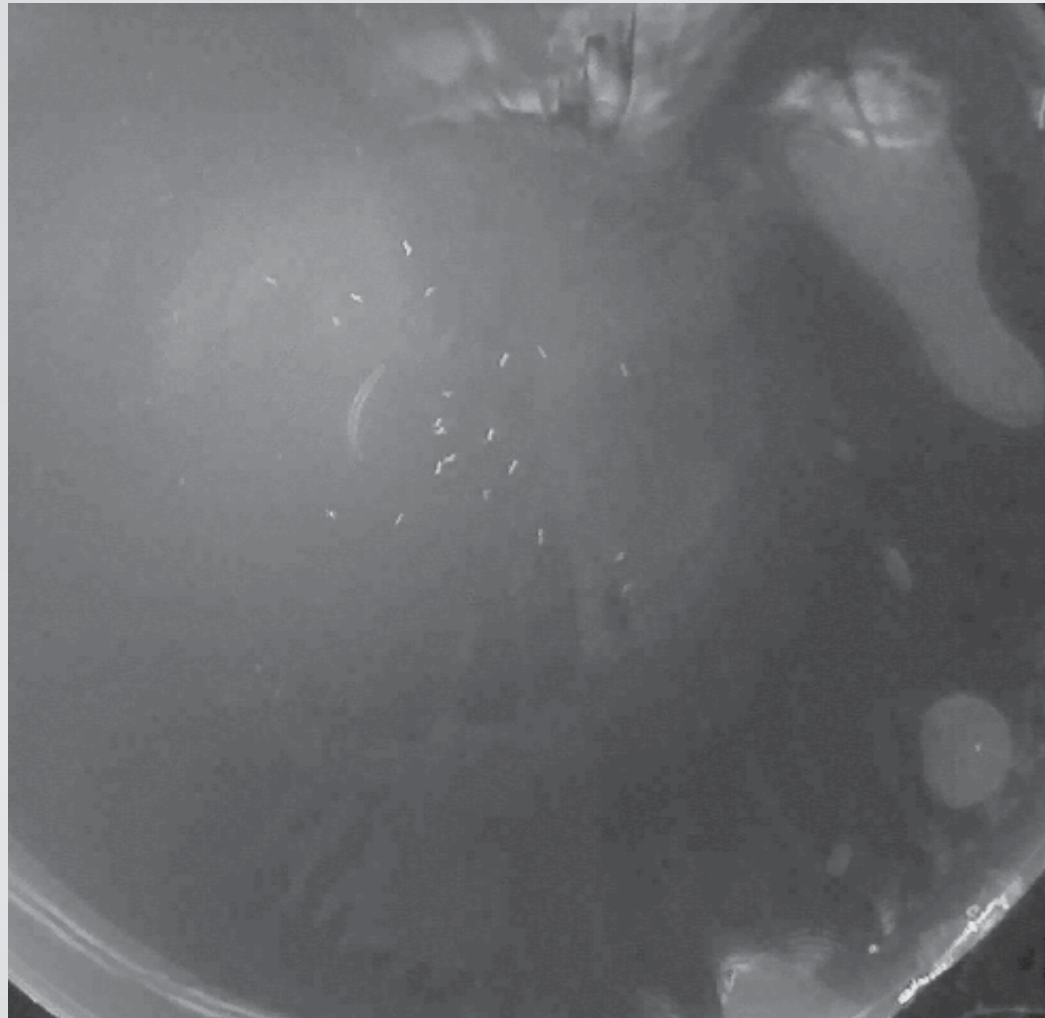
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Caenorhabditis elegans

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

Nanoneurosurgery

Caenorhabditis elegans



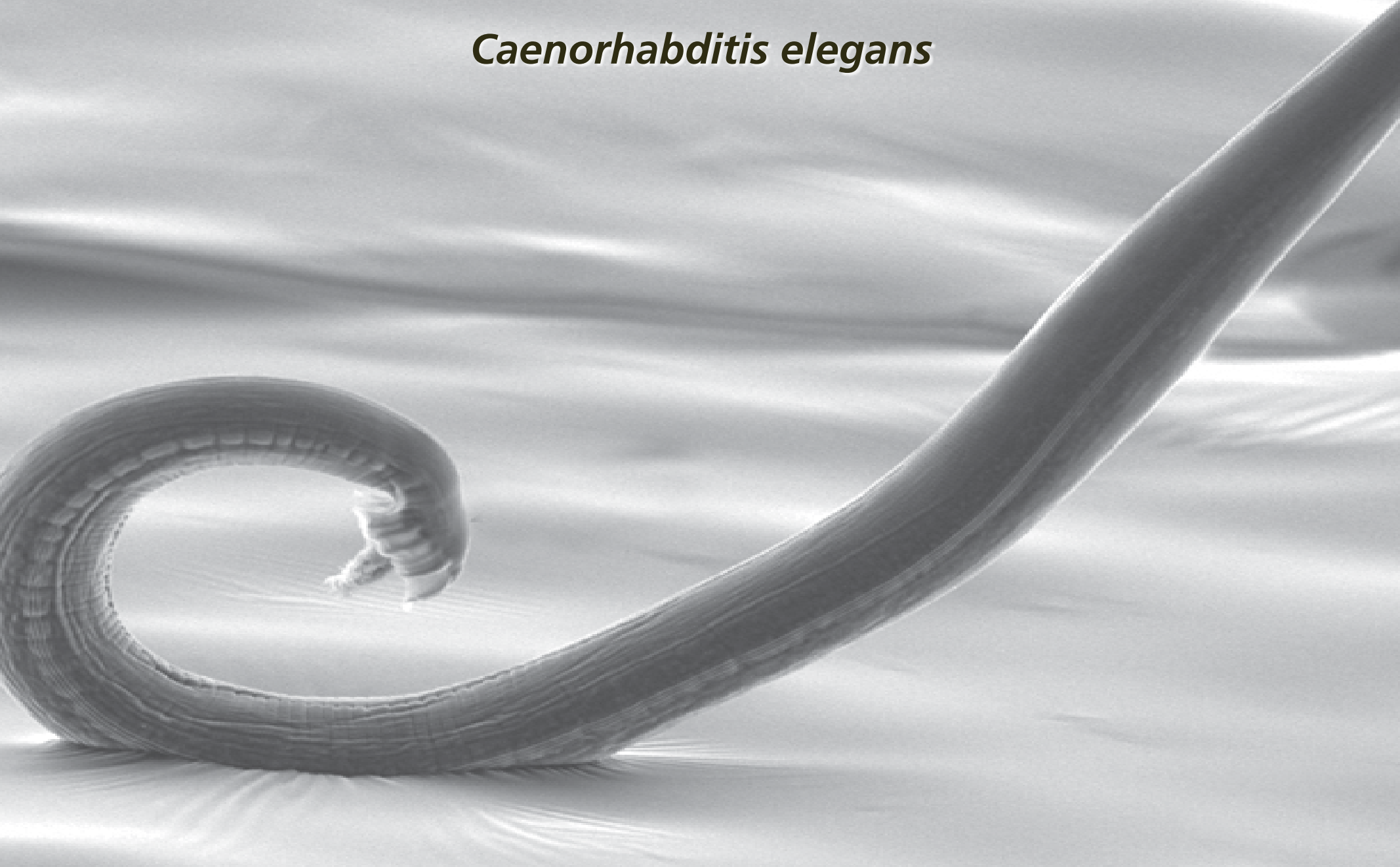
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Caenorhabditis elegans



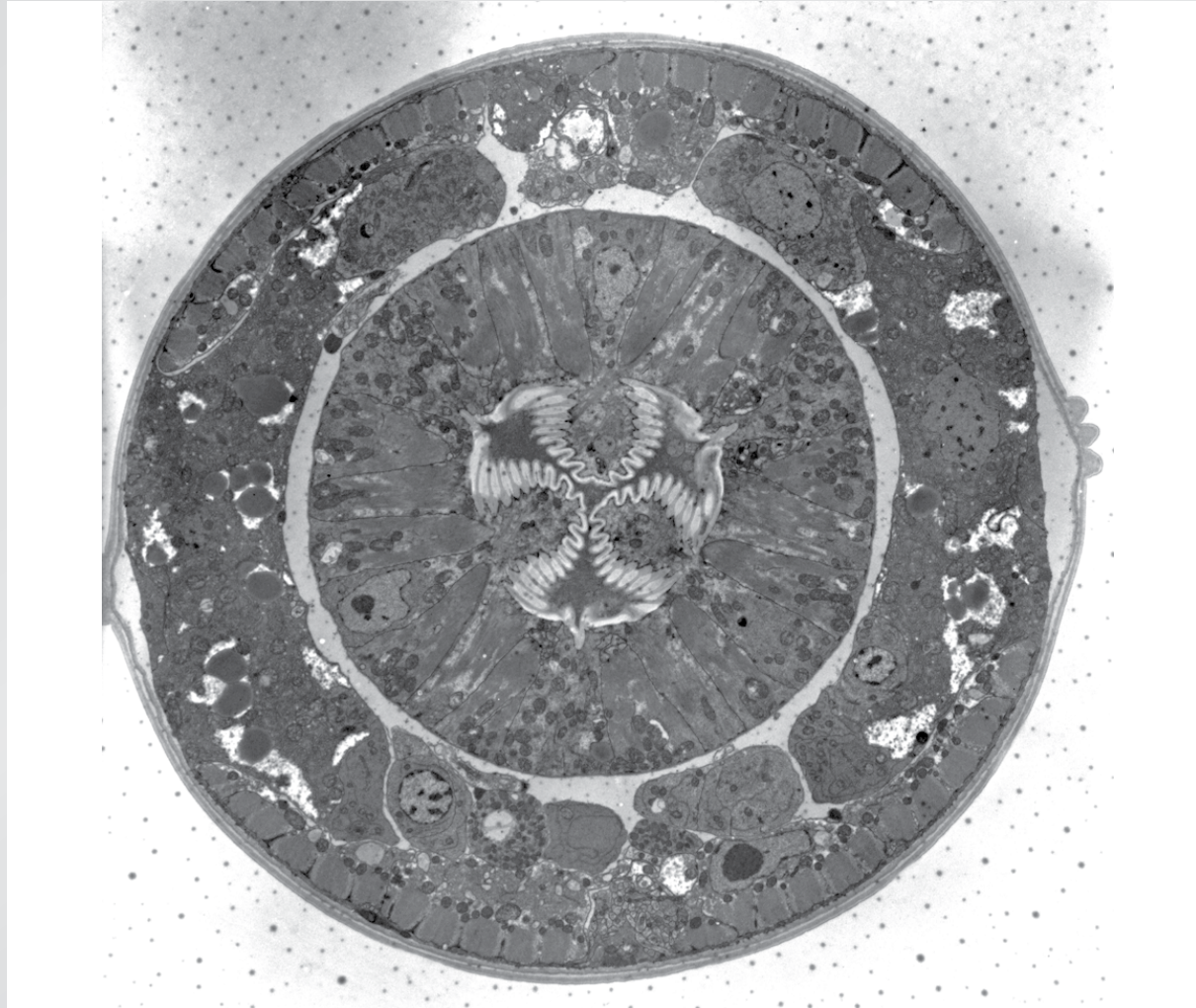
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Caenorhabditis elegans



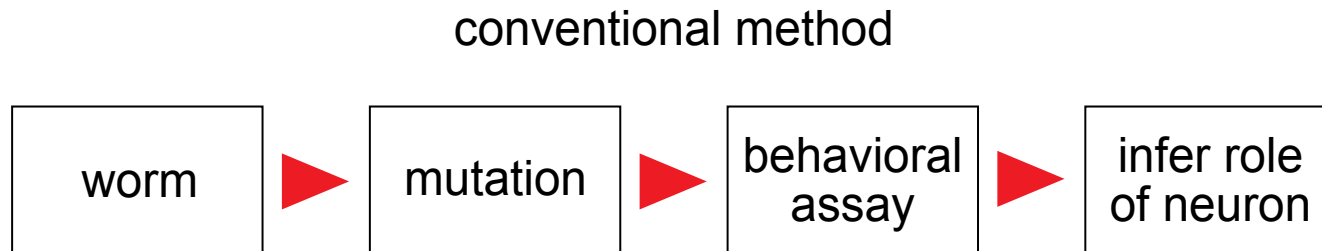
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Caenorhabditis elegans



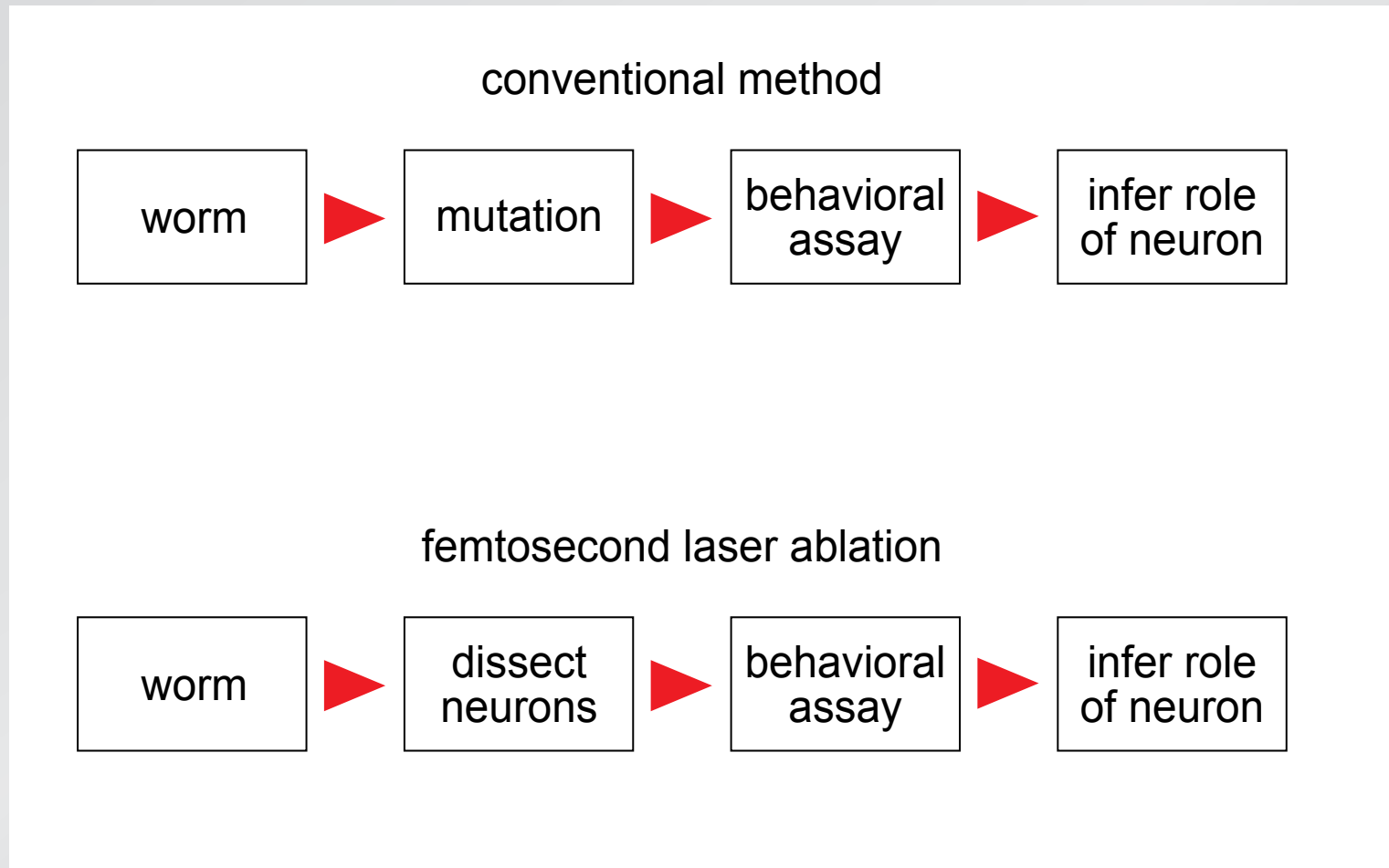
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Mapping behavior to neurons



Nanoneurosurgery

Mapping behavior to neurons



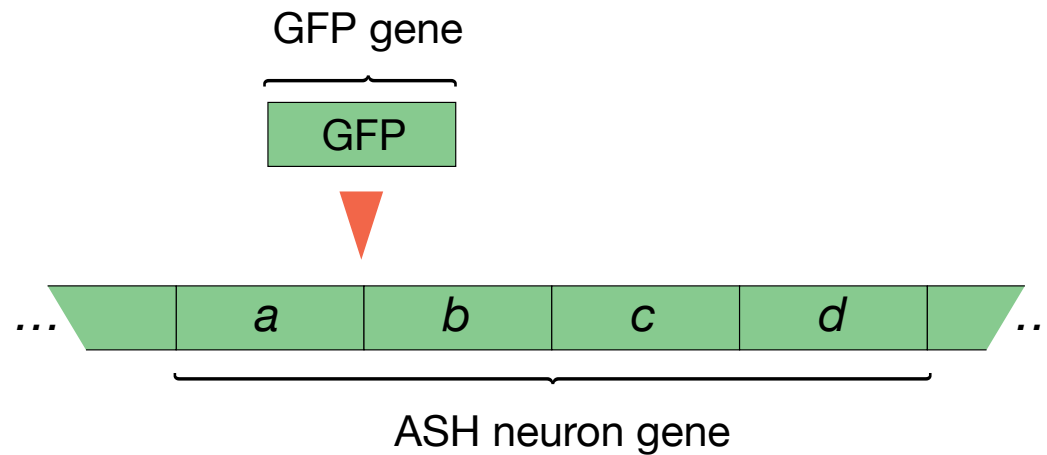
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ASH neurons

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

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Make ASH neurons express GFP



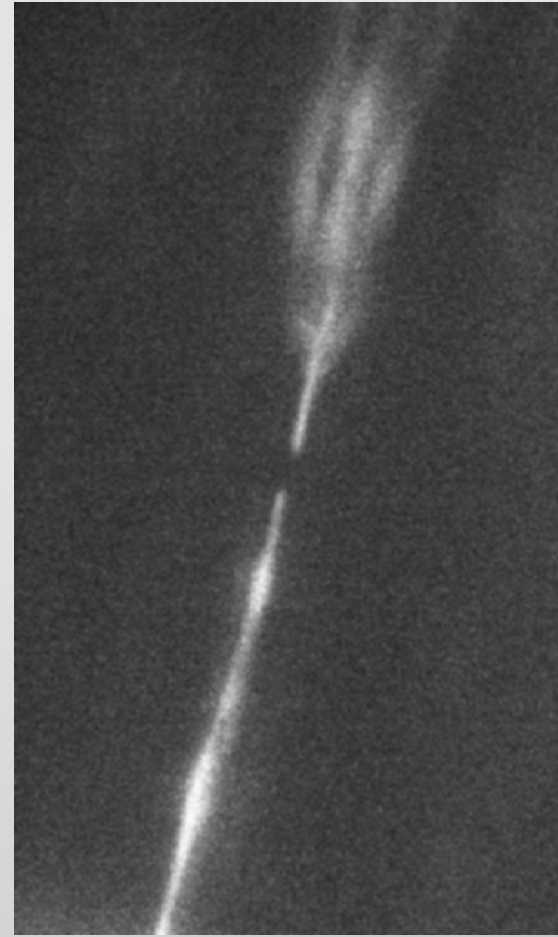
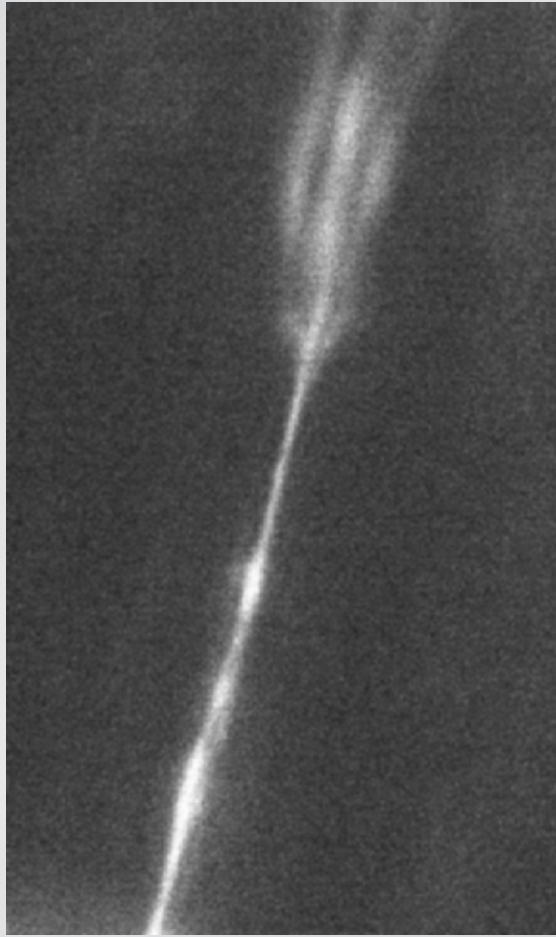
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GFP: absorbs UV, emits green



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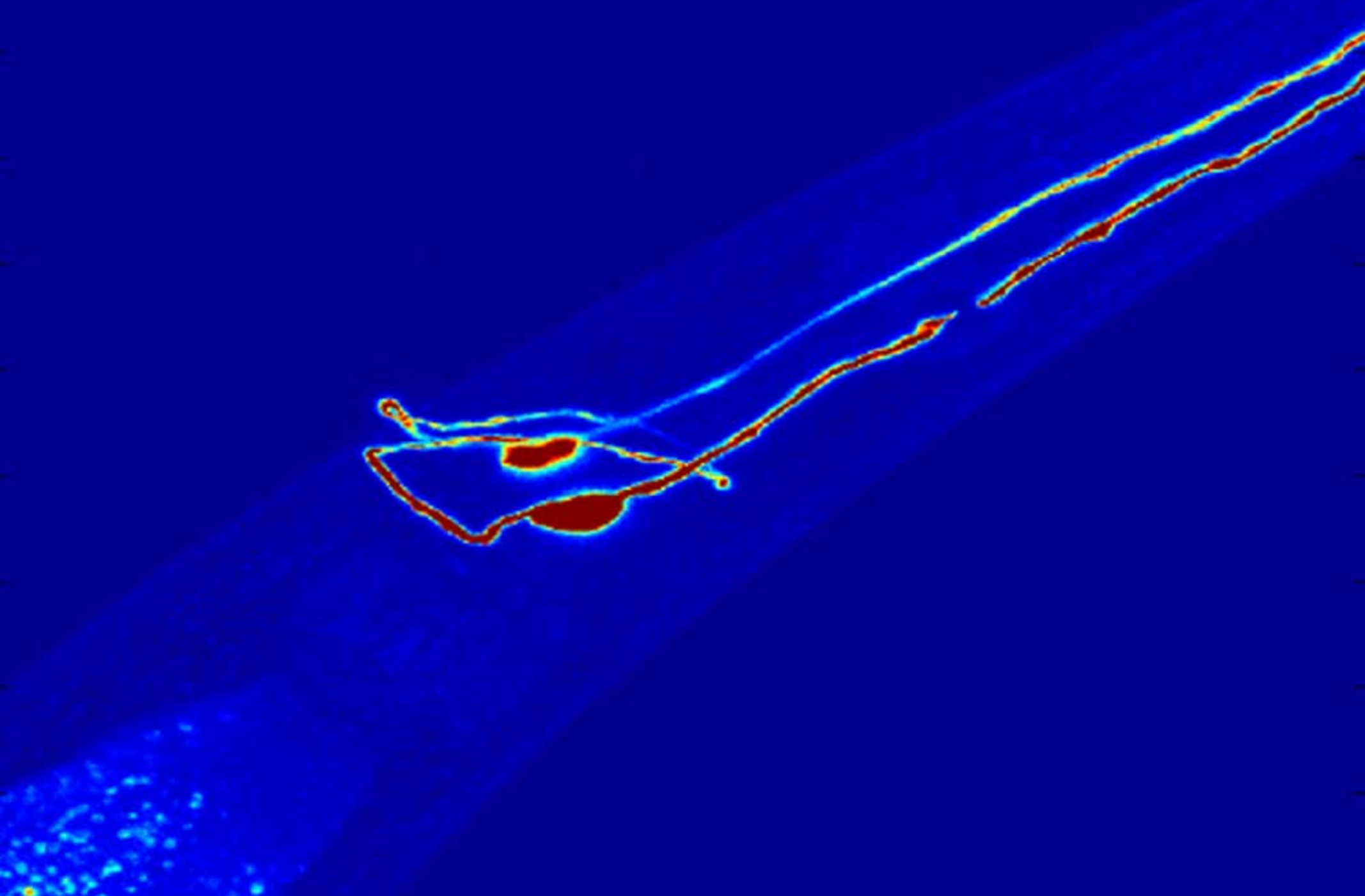
minutes after surgery with 14 nJ pulses



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revive worm, reimage 1 day later

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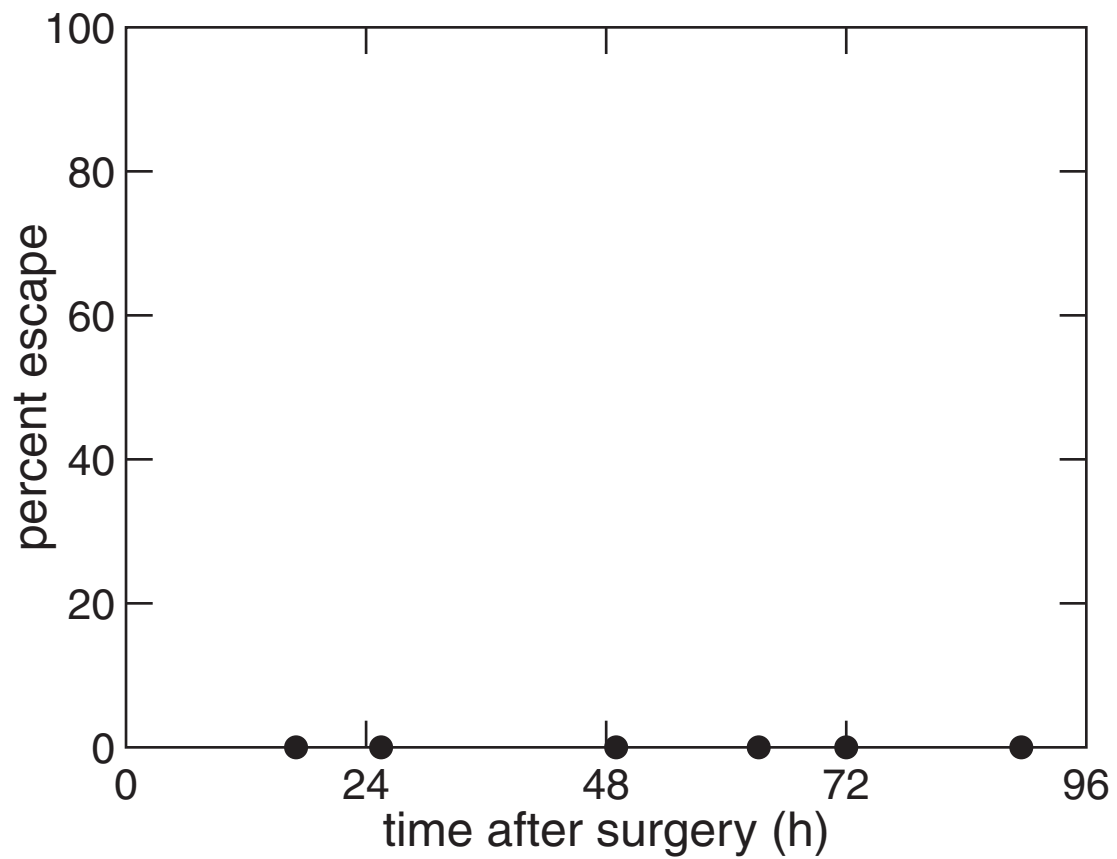
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osmolarity assay



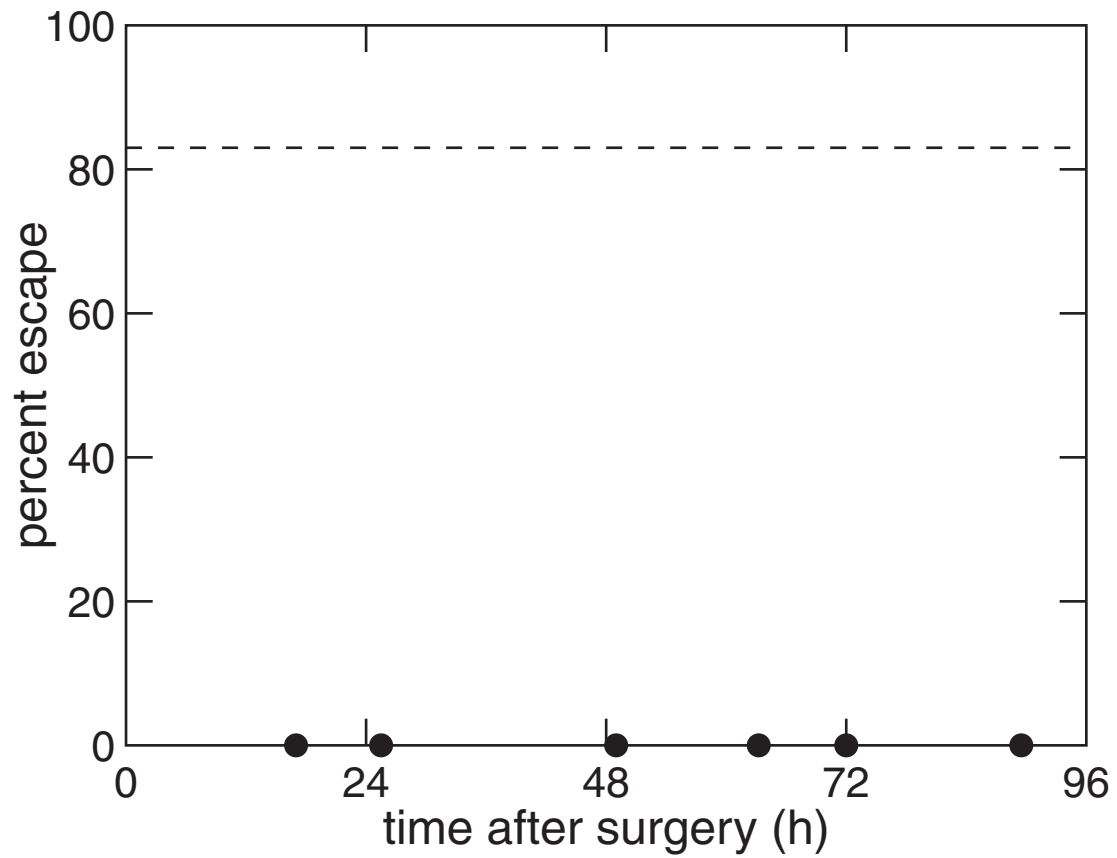
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escape rate after 'mock' surgery



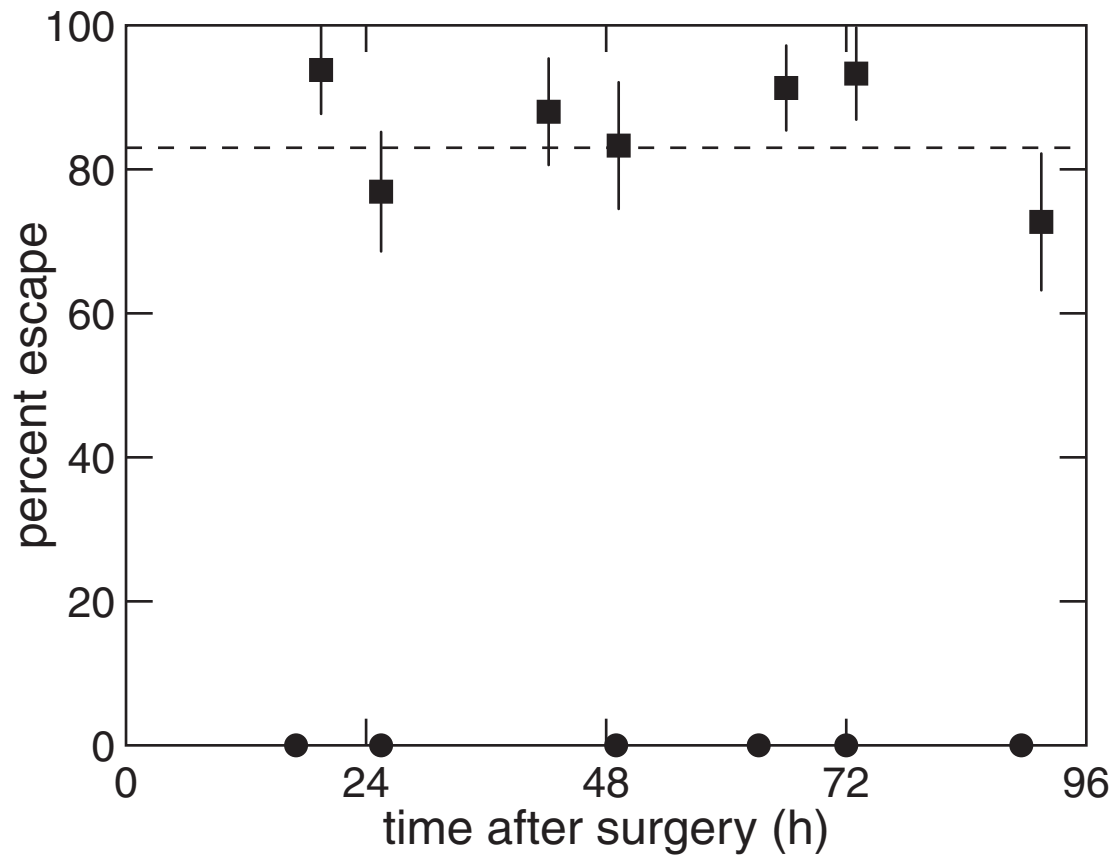
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escape rate of ASH-lacking mutant



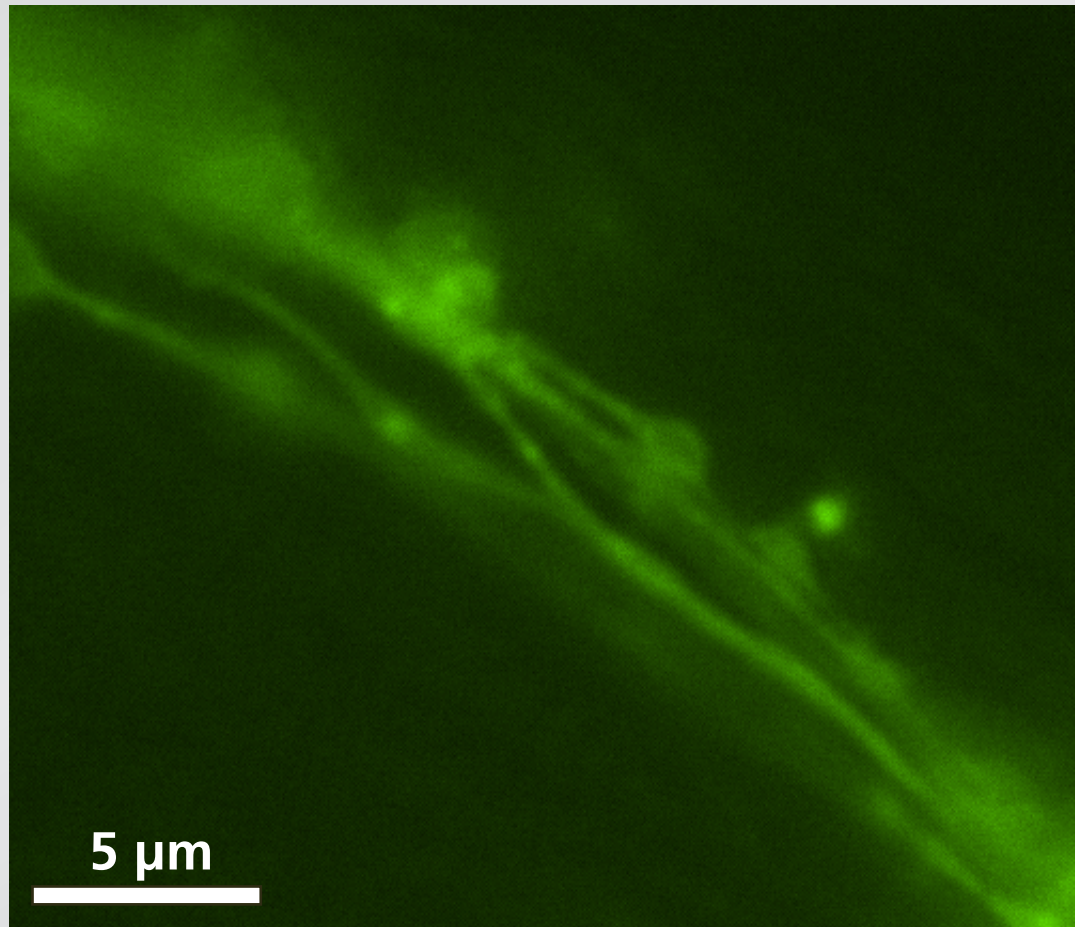
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escape rate after ASH-ablation surgery



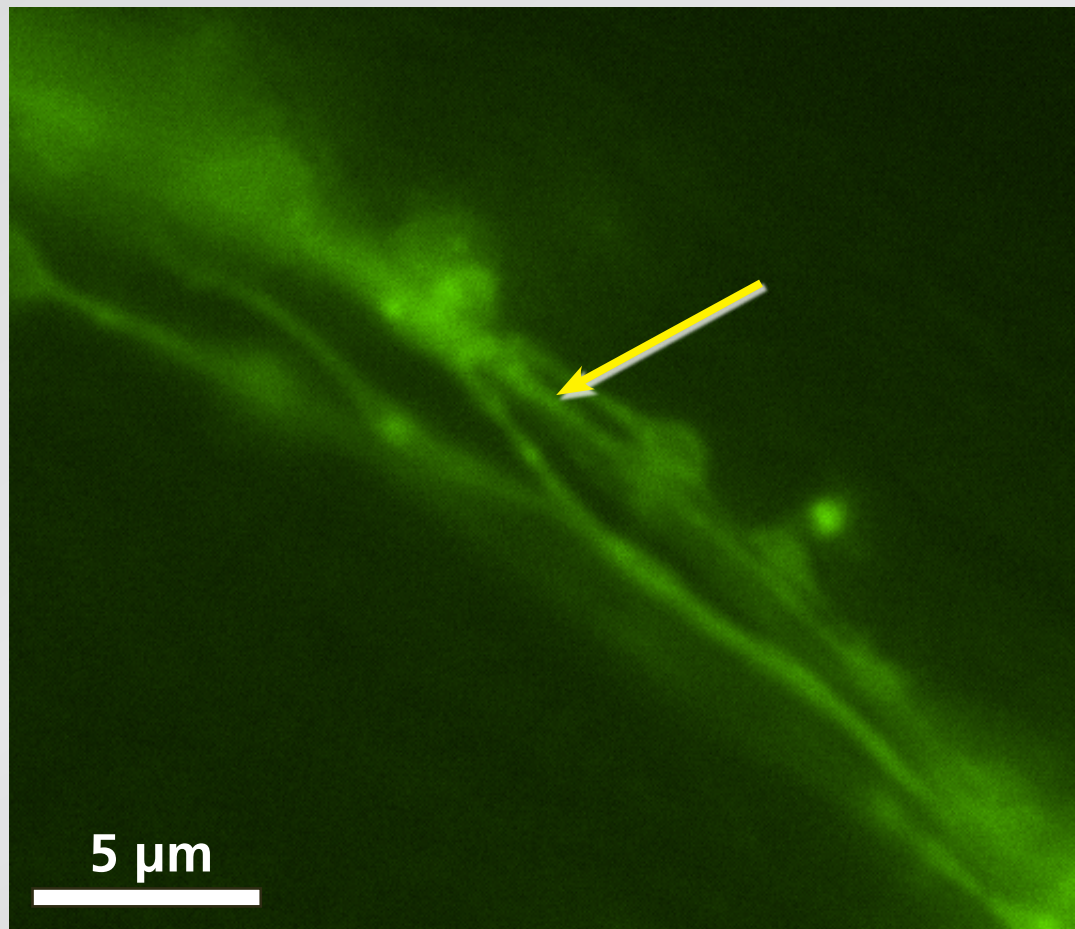
Nanoneurosurgery

cut single dendrite in amphid bundle



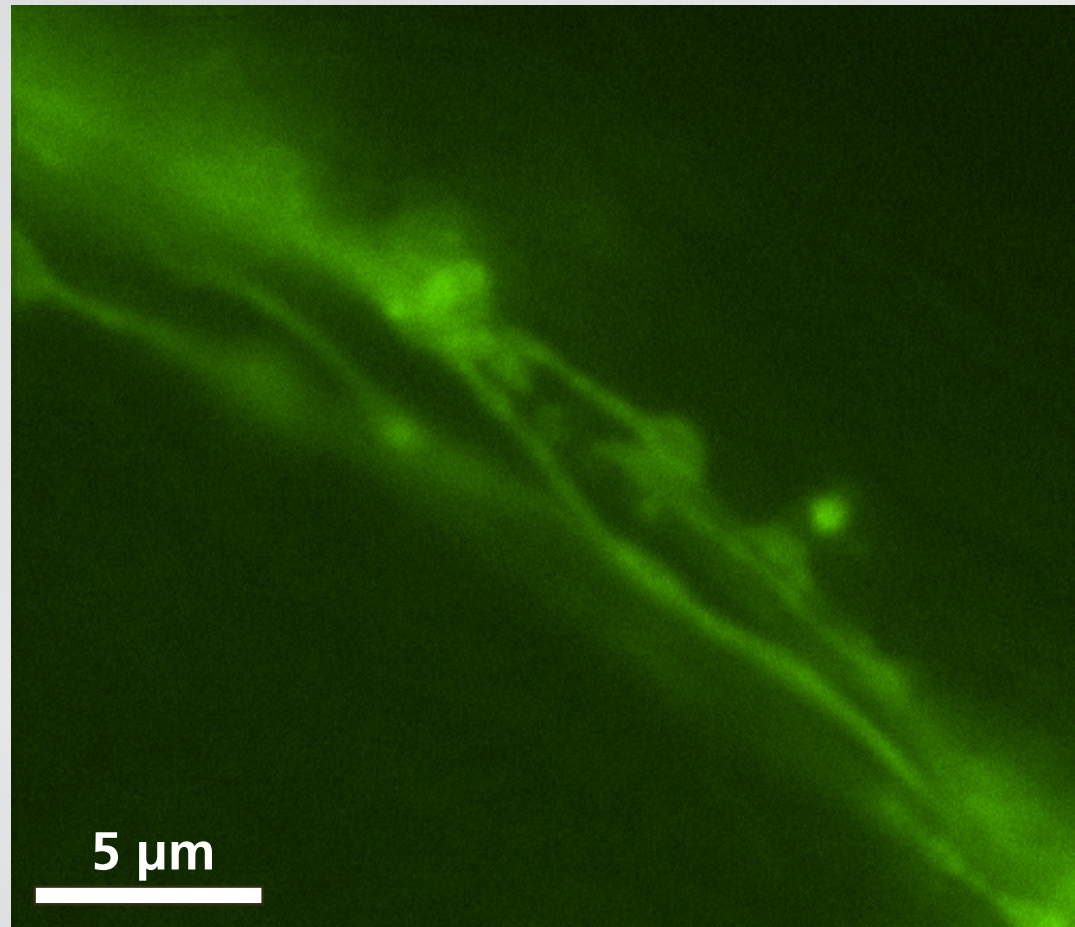
Nanoneurosurgery

cut single dendrite in amphid bundle



Nanoneurosurgery

cut single dendrite in amphid bundle

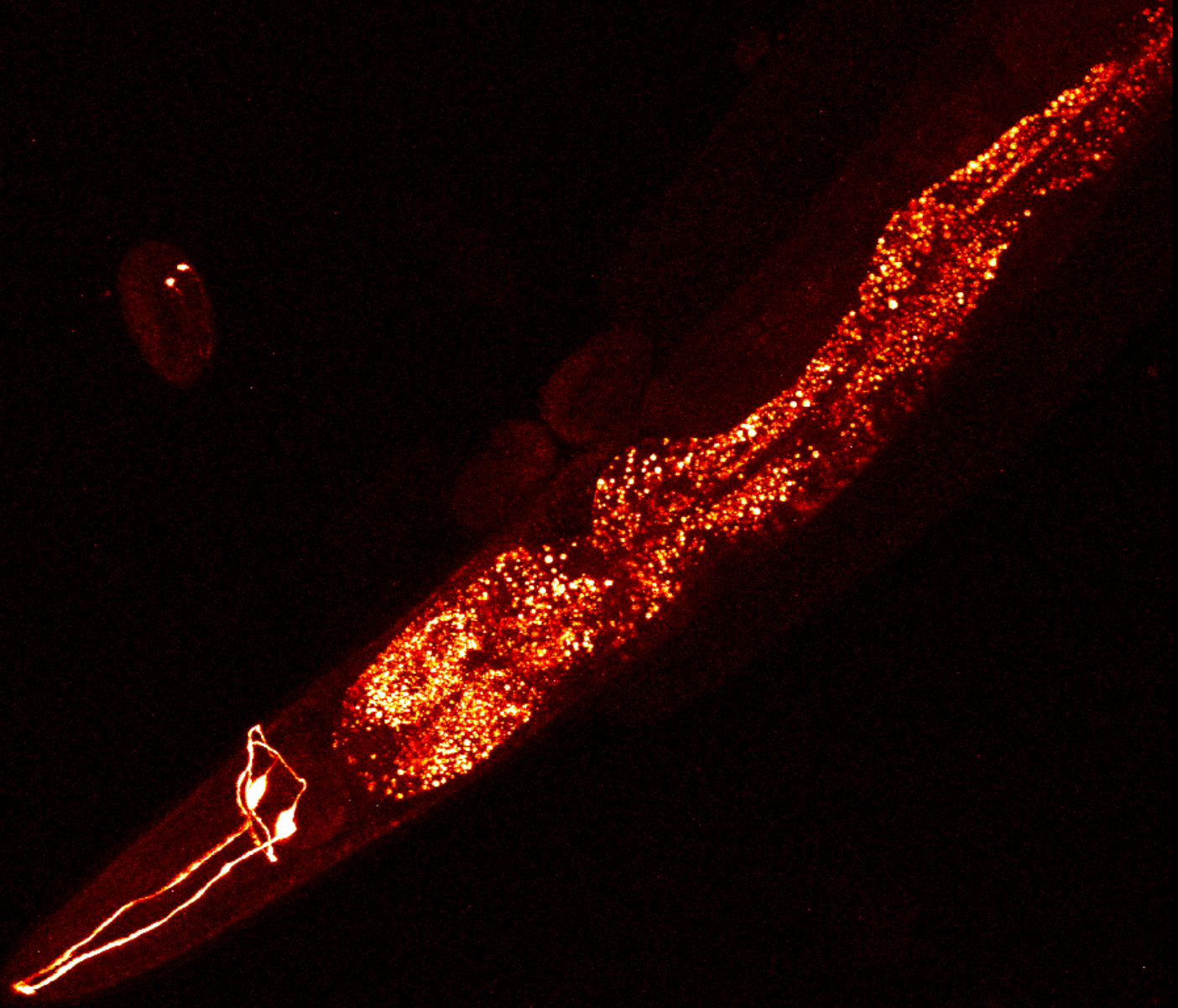


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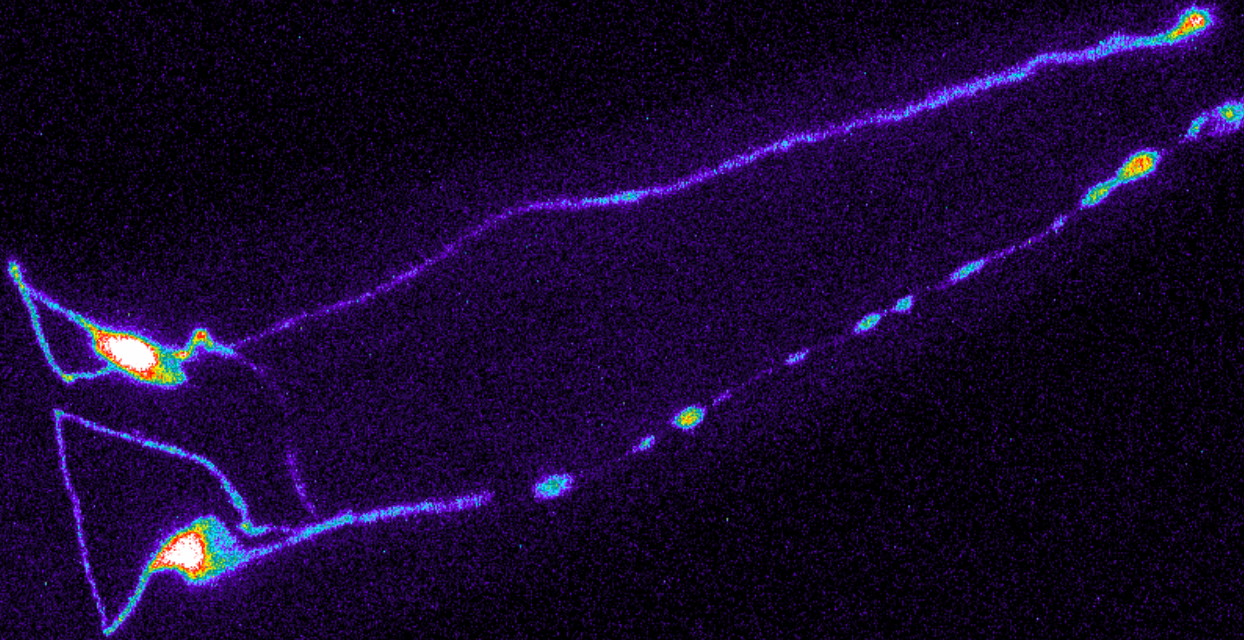
AFD neurons

- responsible for temperature sensing
- ciliary projections
- one on each side

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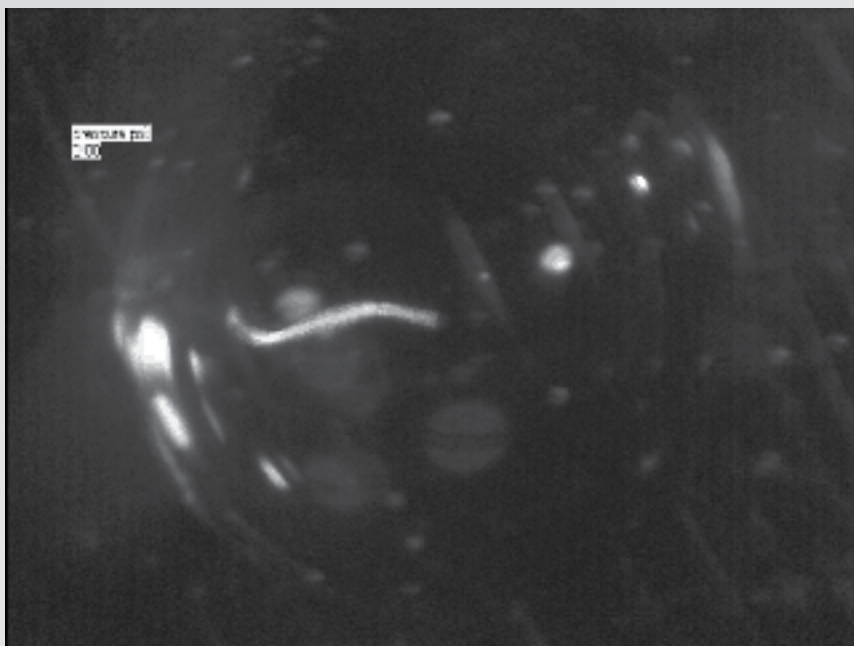


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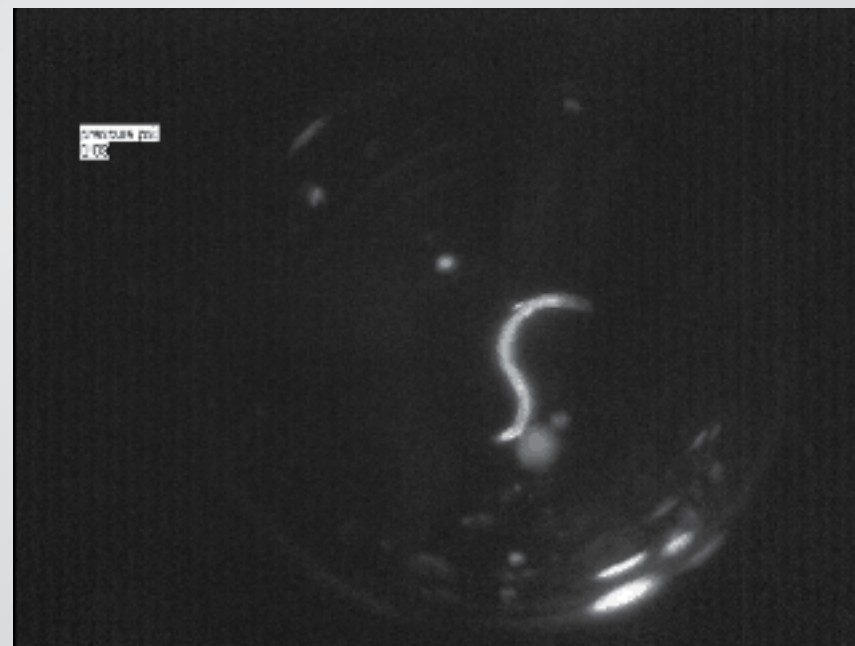


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surgery results in quantifiable behavior changes



before



after

Nanoneurosurgery

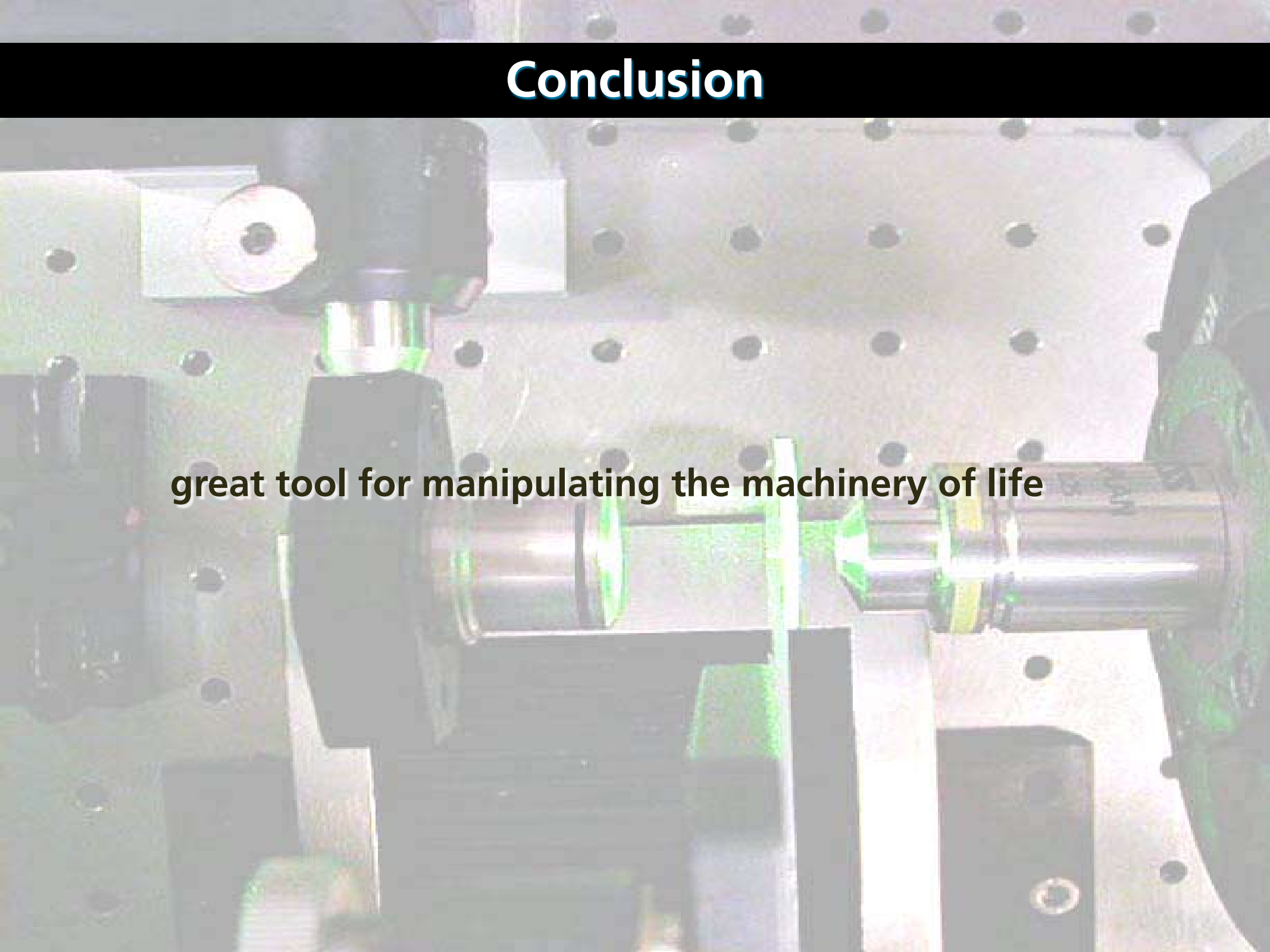
temperature sensing occurs at tip of dendrite

Summary

- **manipulate on subcellular, submicrometer scale**
- **penetrate in bulk without compromising viability**
- **study cell structure and mechanics**
- **study neurobiological basis of behavior**

Conclusion

great tool for manipulating the machinery of life





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