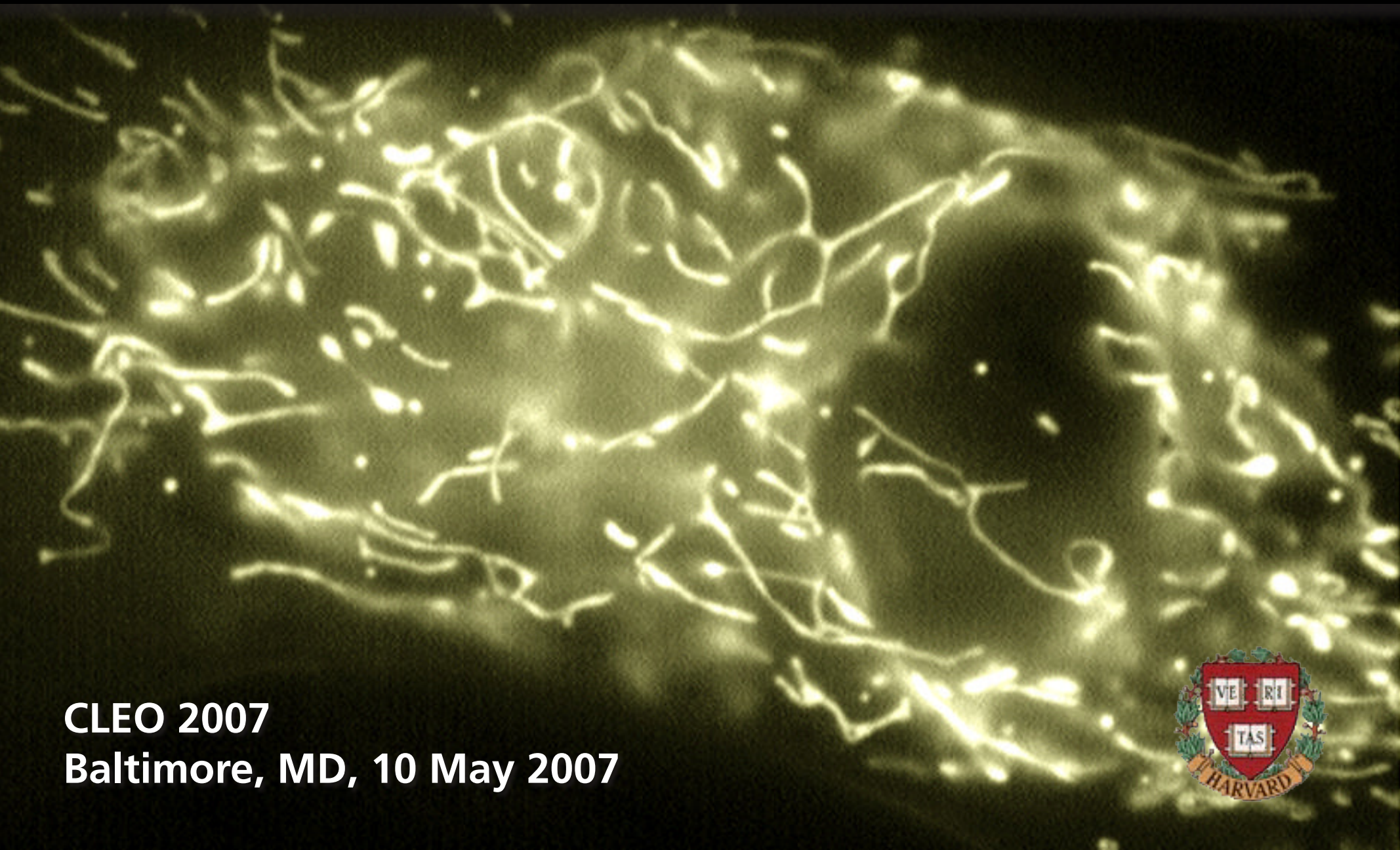
Subcellular surgery and nanoneurosurgery



CLEO 2007
Baltimore, MD, 10 May 2007

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

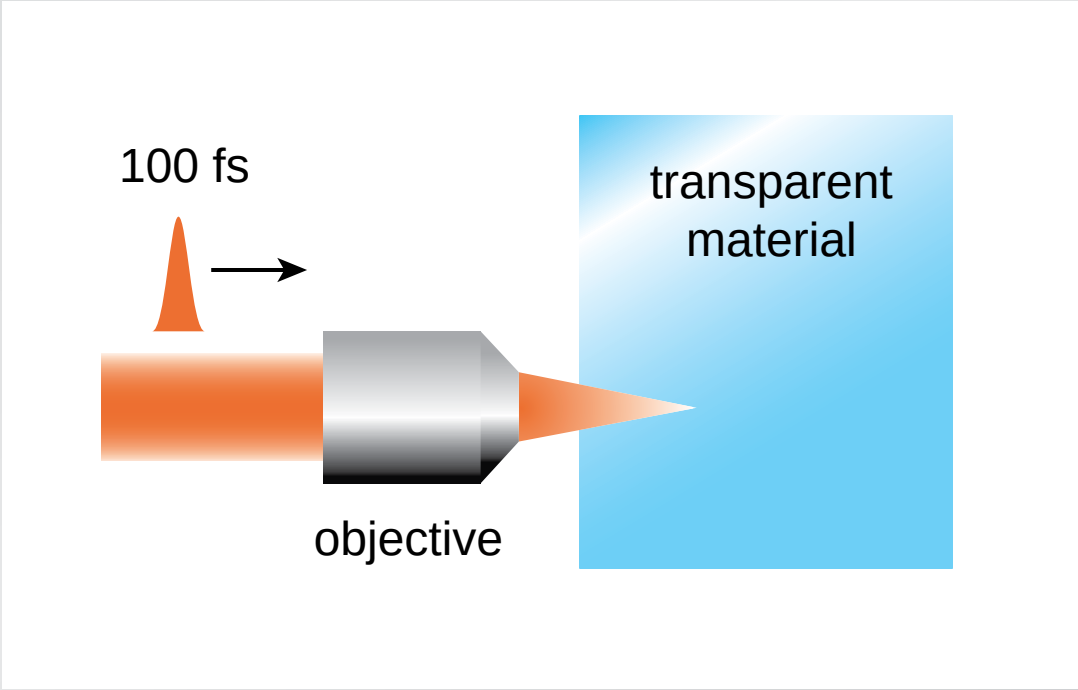
- standard biochemical tools: species selective
- fs ‘nanosurgery’: site-specific

Outline

- femtosecond micromachining
- subcellular surgery
- nanoneurosurgery

Femtosecond micromachining

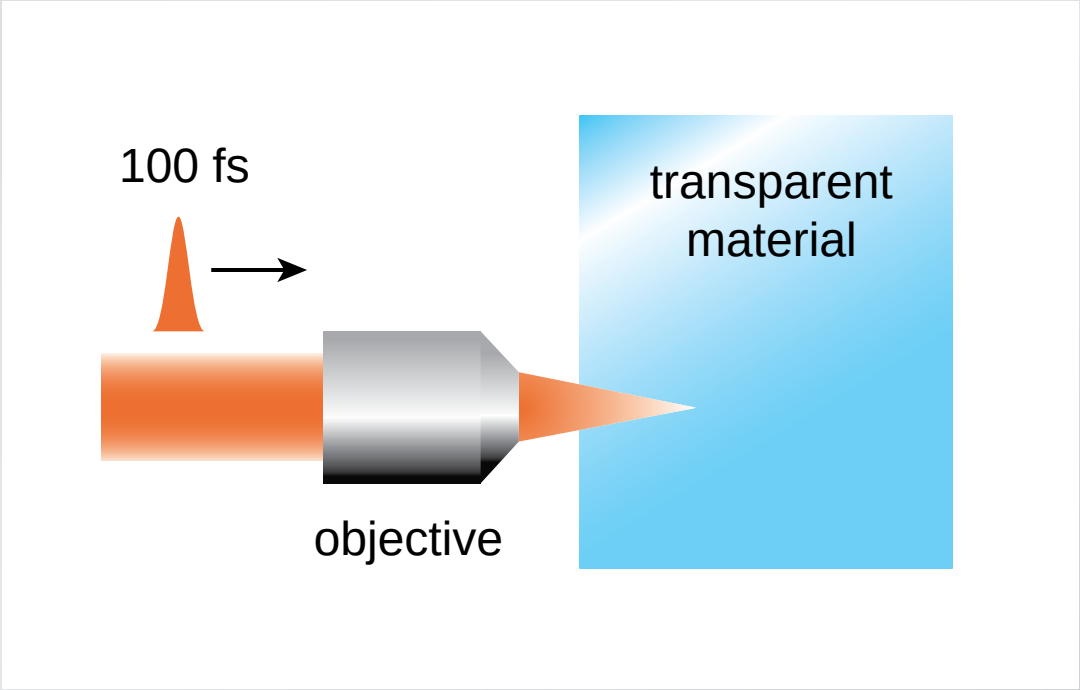
focus laser beam inside material



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

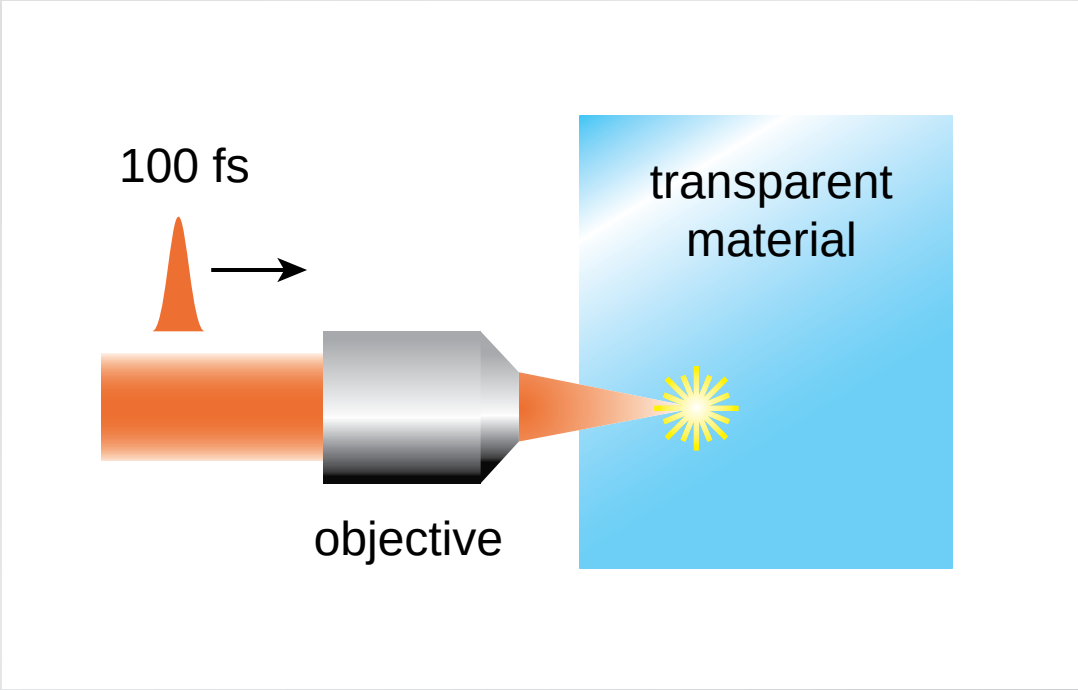
Femtosecond micromachining

high intensity at focus…



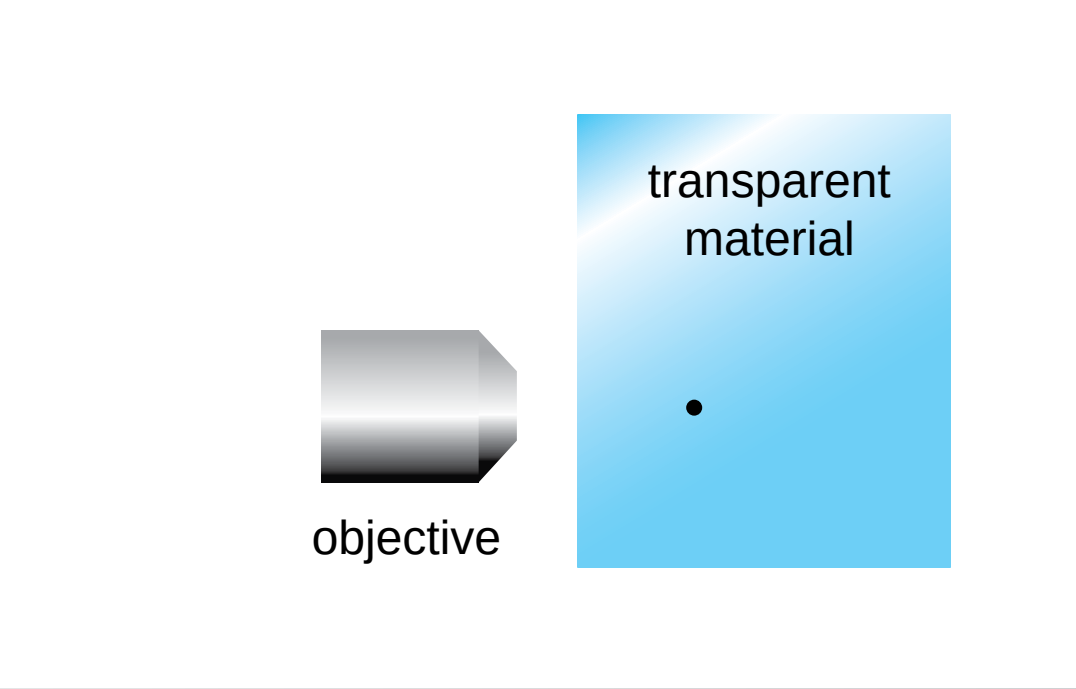
Femtosecond micromachining

…causes nonlinear ionization…

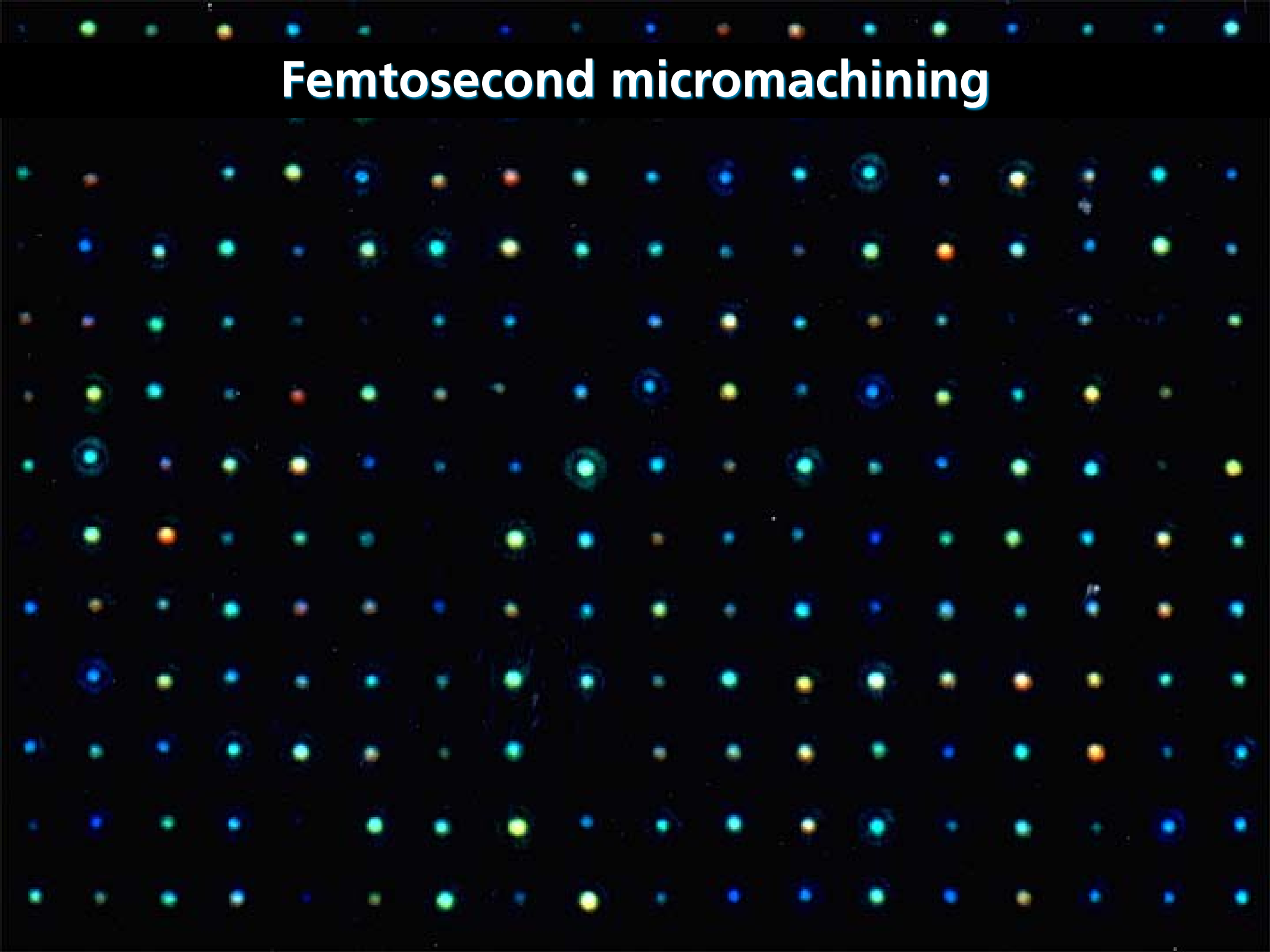


Femtosecond micromachining

and ‘microexplosion’ causes microscopic damage…



Femtosecond micromachining

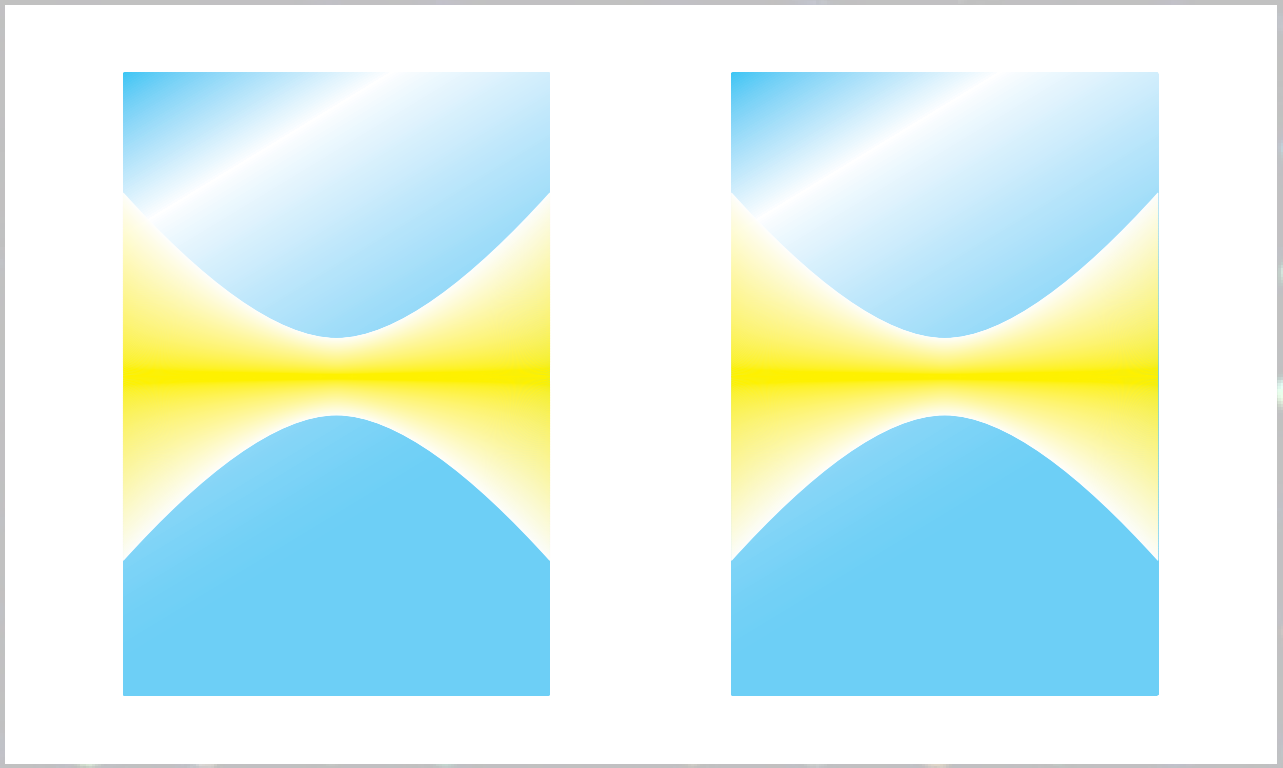


Femtosecond micromachining

photon energy < bandgap $\longrightarrow$ nonlinear interaction

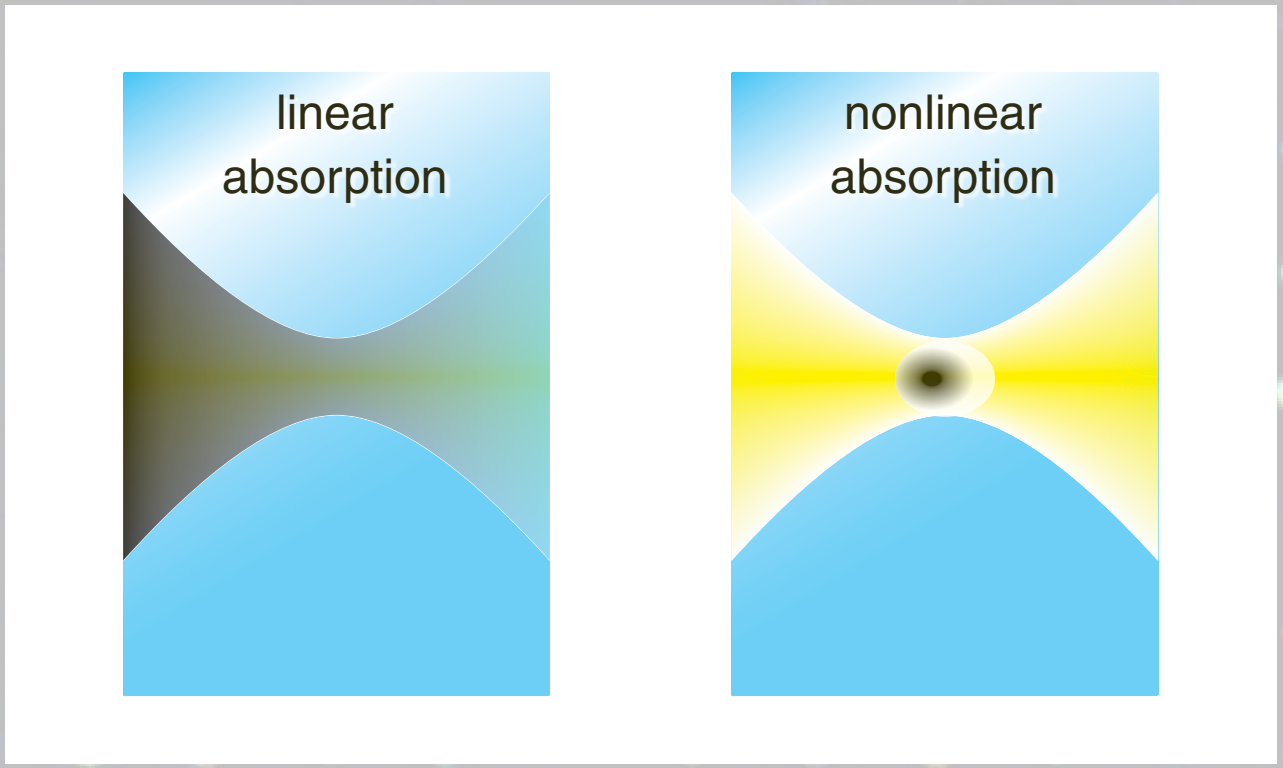
Femtosecond micromachining

nonlinear interaction provides bulk confinement

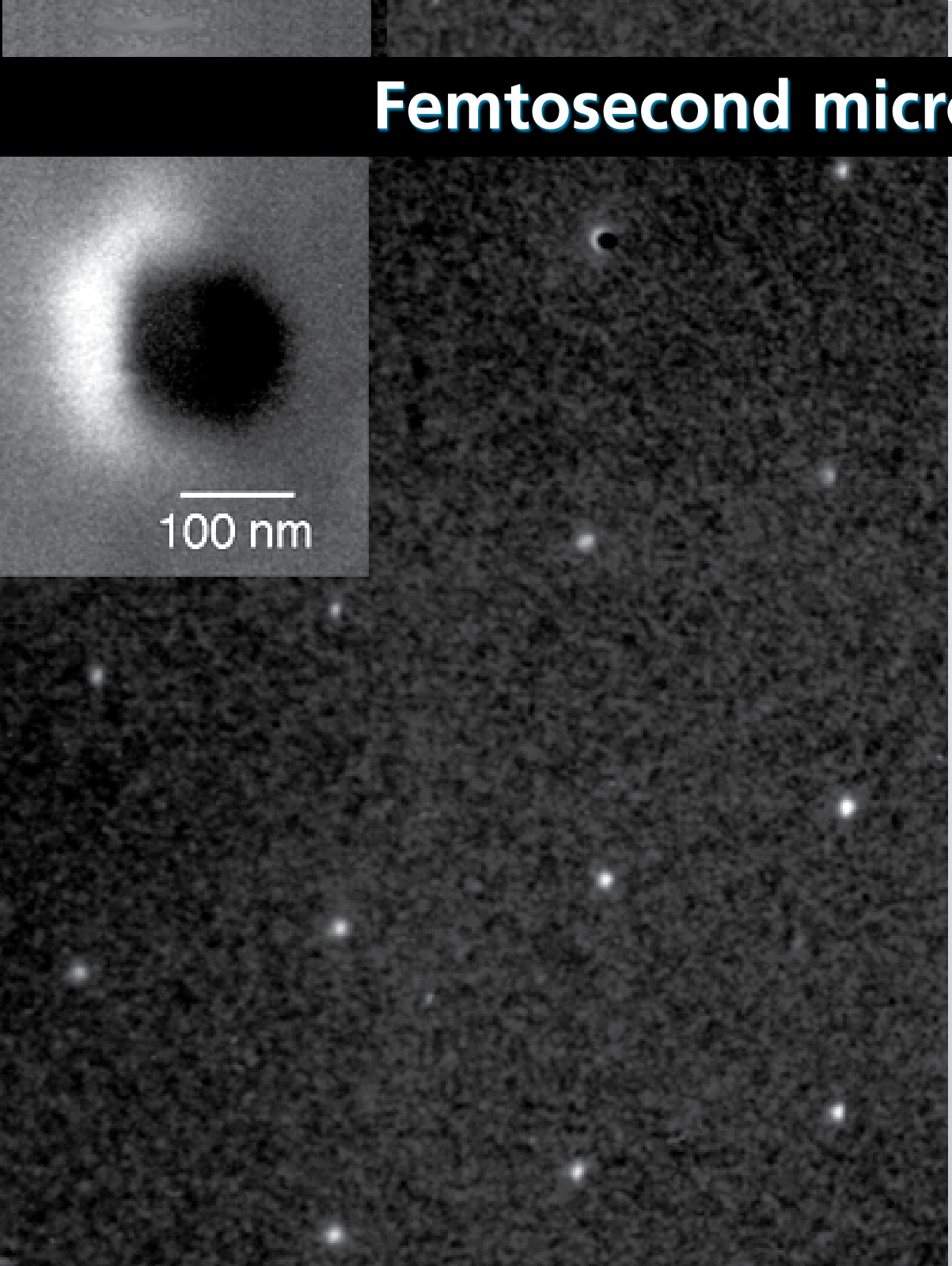


Femtosecond micromachining

nonlinear interaction provides bulk confinement



Femtosecond micromachining



SEM & AFM:

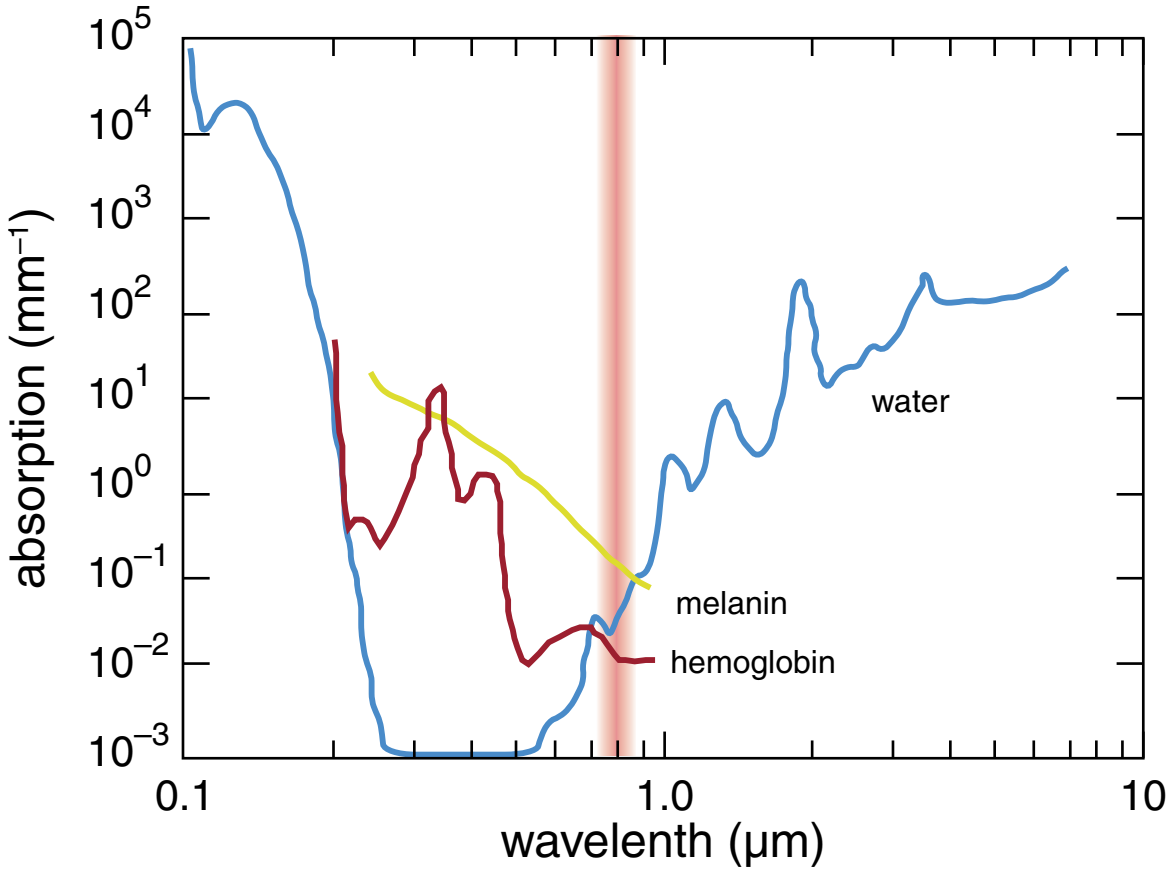
- 100-nm cavities
- little colateral damage

Femtosecond micromachining

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

Femtosecond micromachining

tissue is nearly transparent at 800 nm



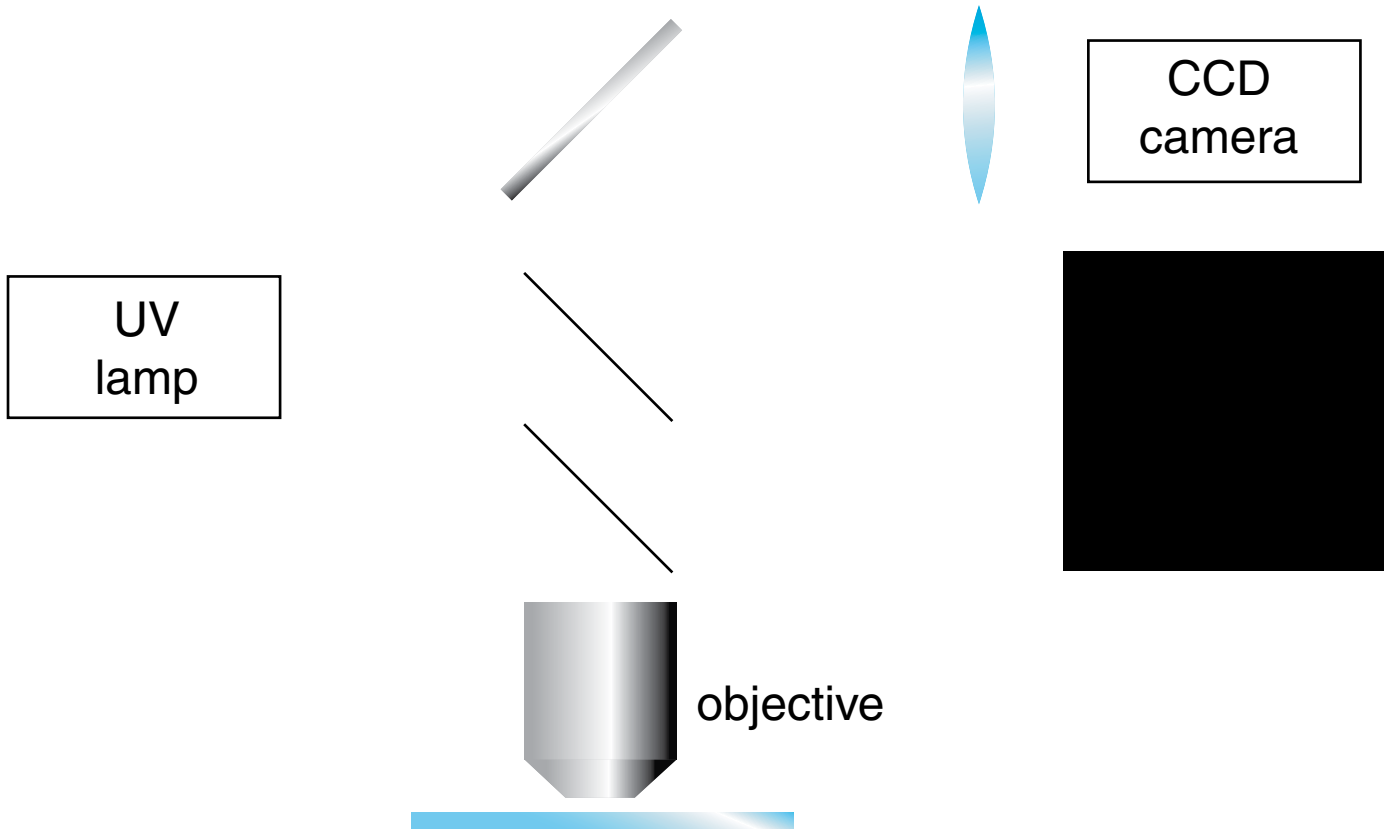
Outline

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

Subcellular surgery

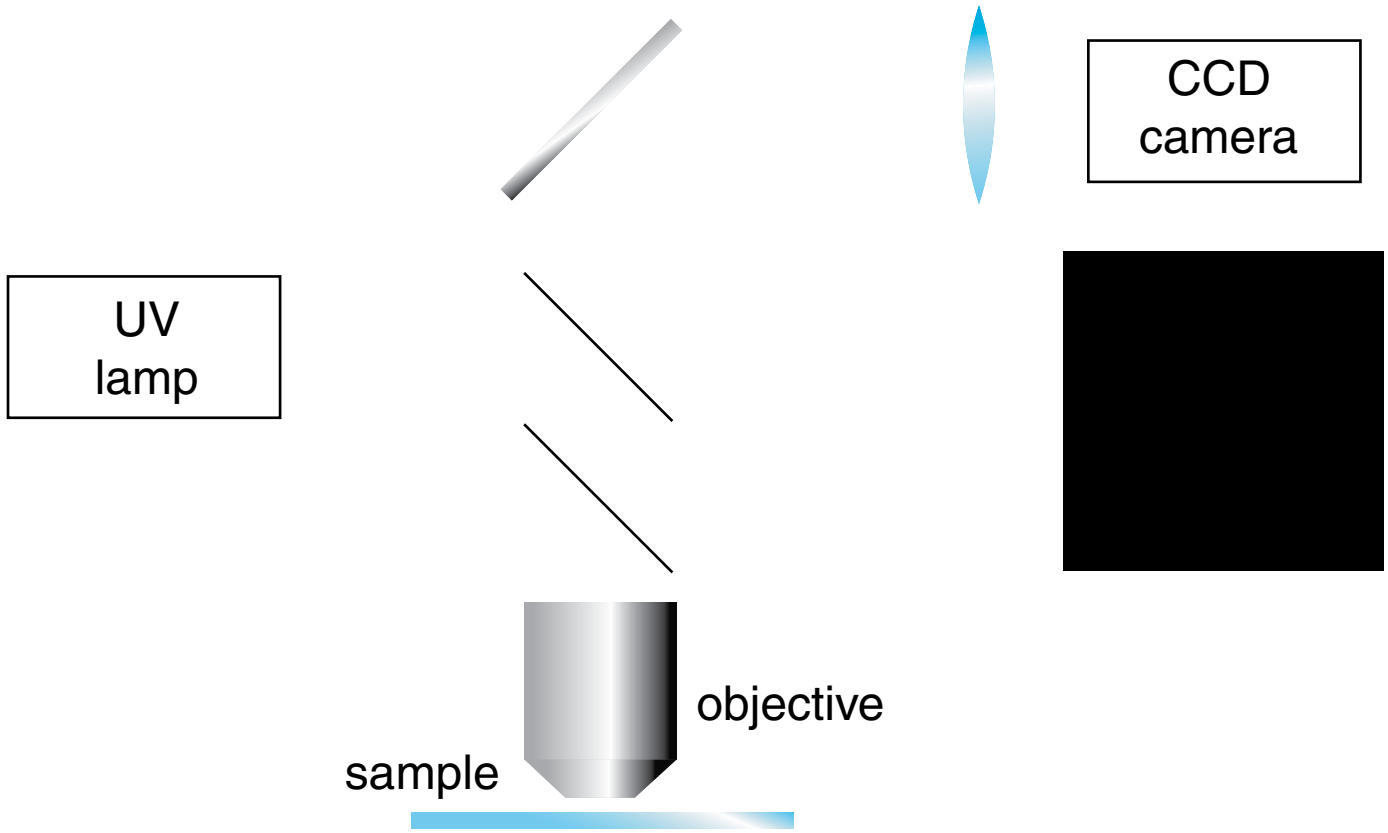
Q: can we ablate material on the subcellular scale?

Subcellular surgery



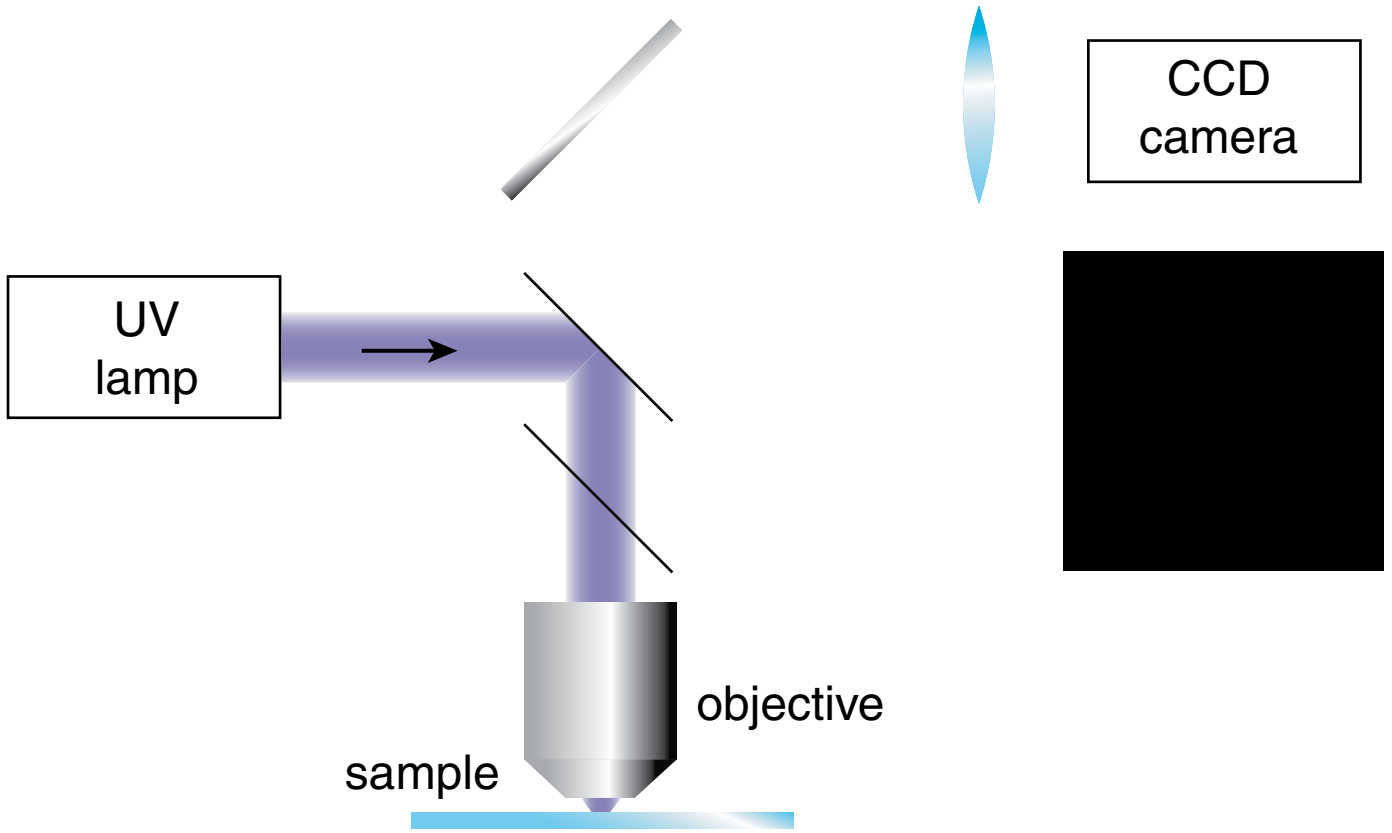
epi-fluorescence microscope

Subcellular surgery



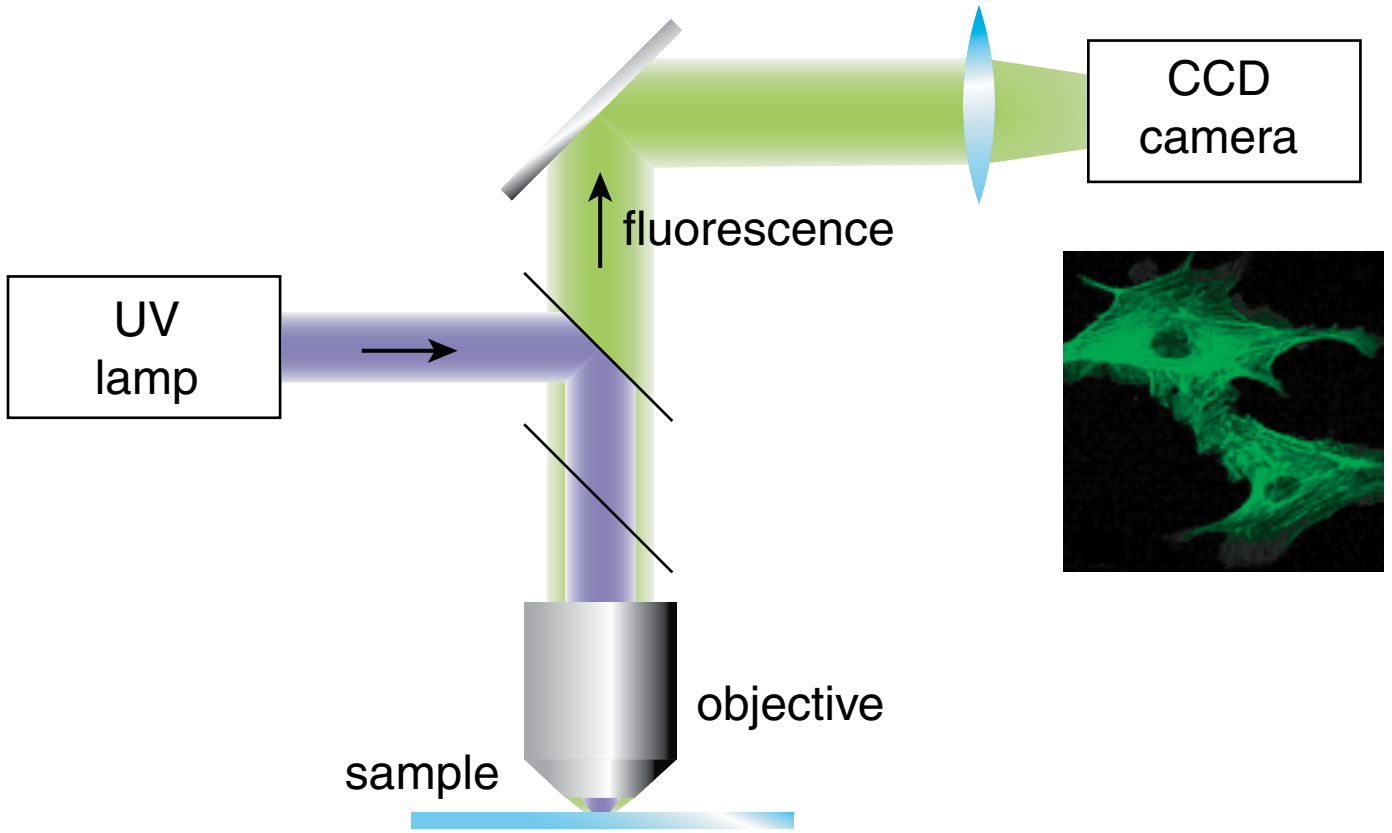
fluorescently label sample

Subcellular surgery



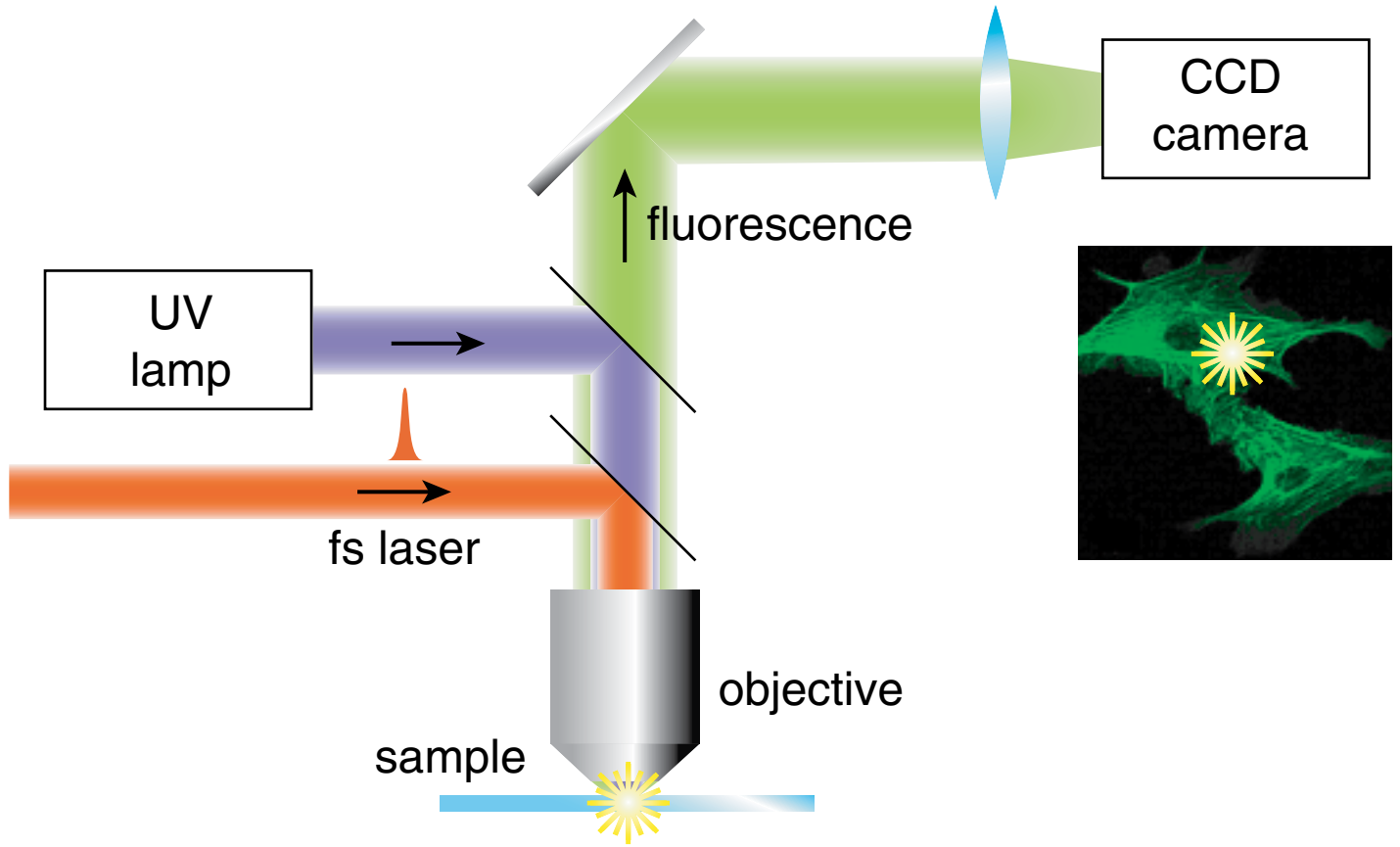
UV illumination…

Subcellular surgery



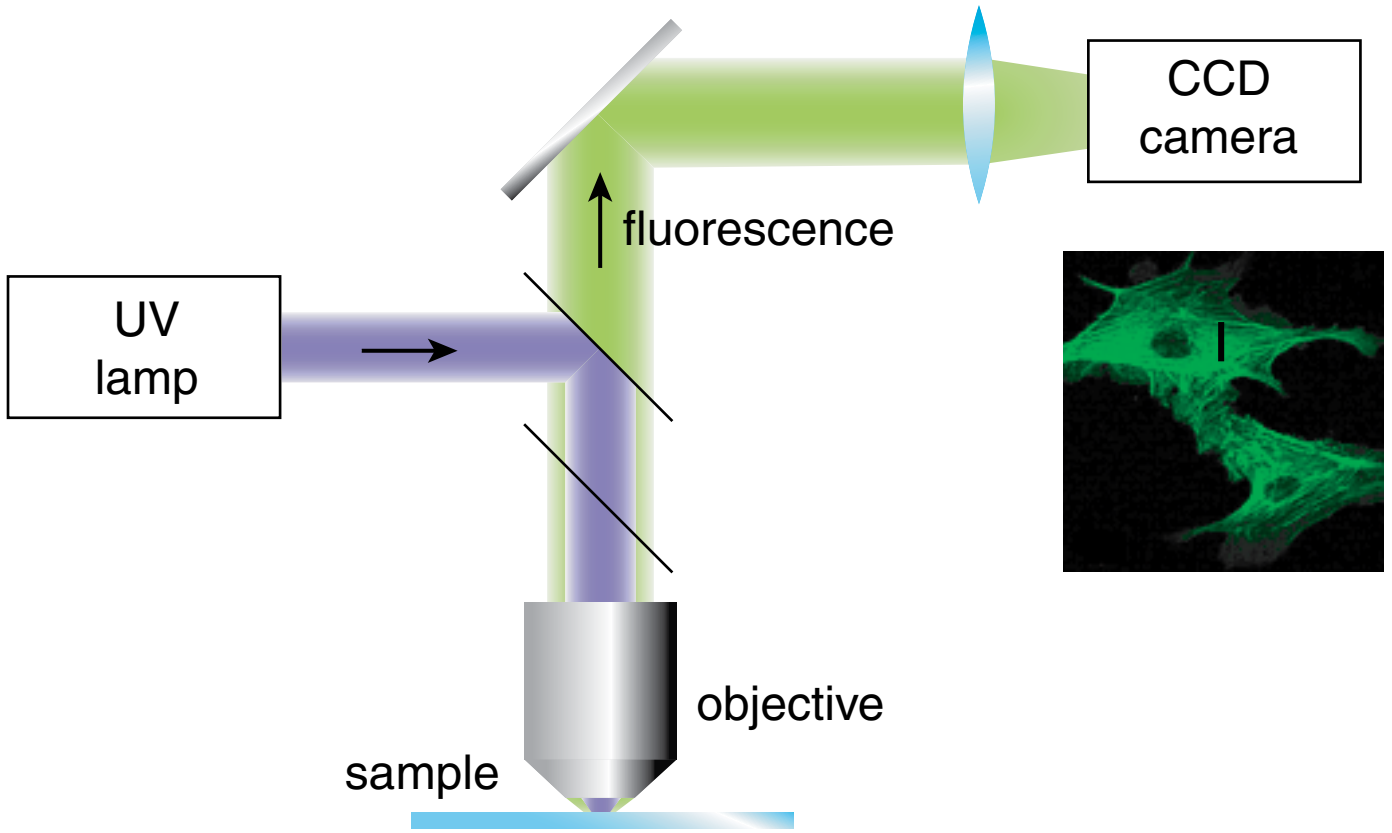
…causes fluorescence

Subcellular surgery



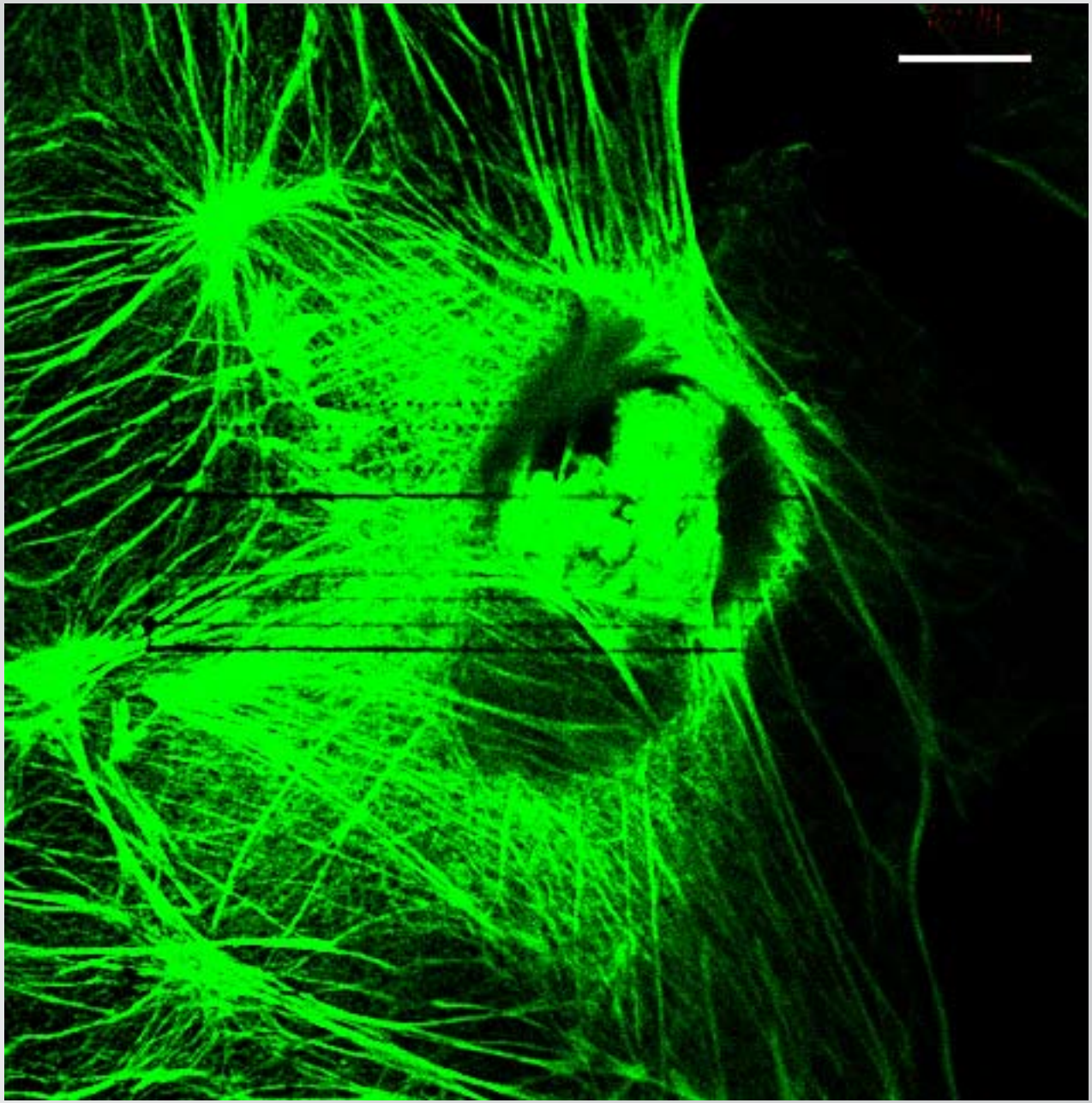
irradiate with fs laser beam

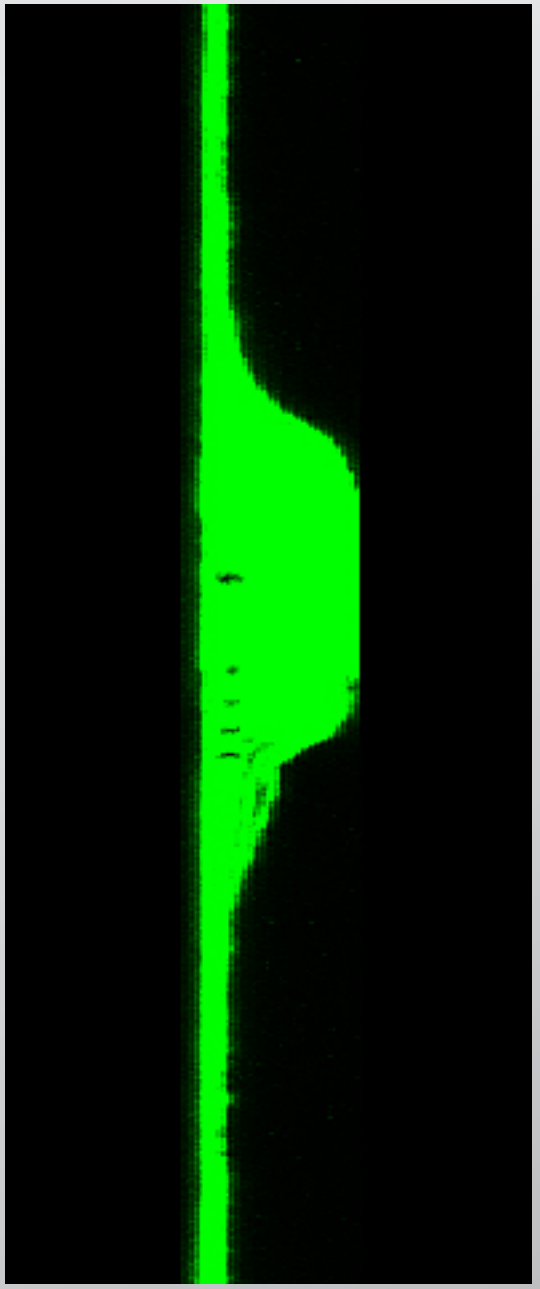
Subcellular surgery



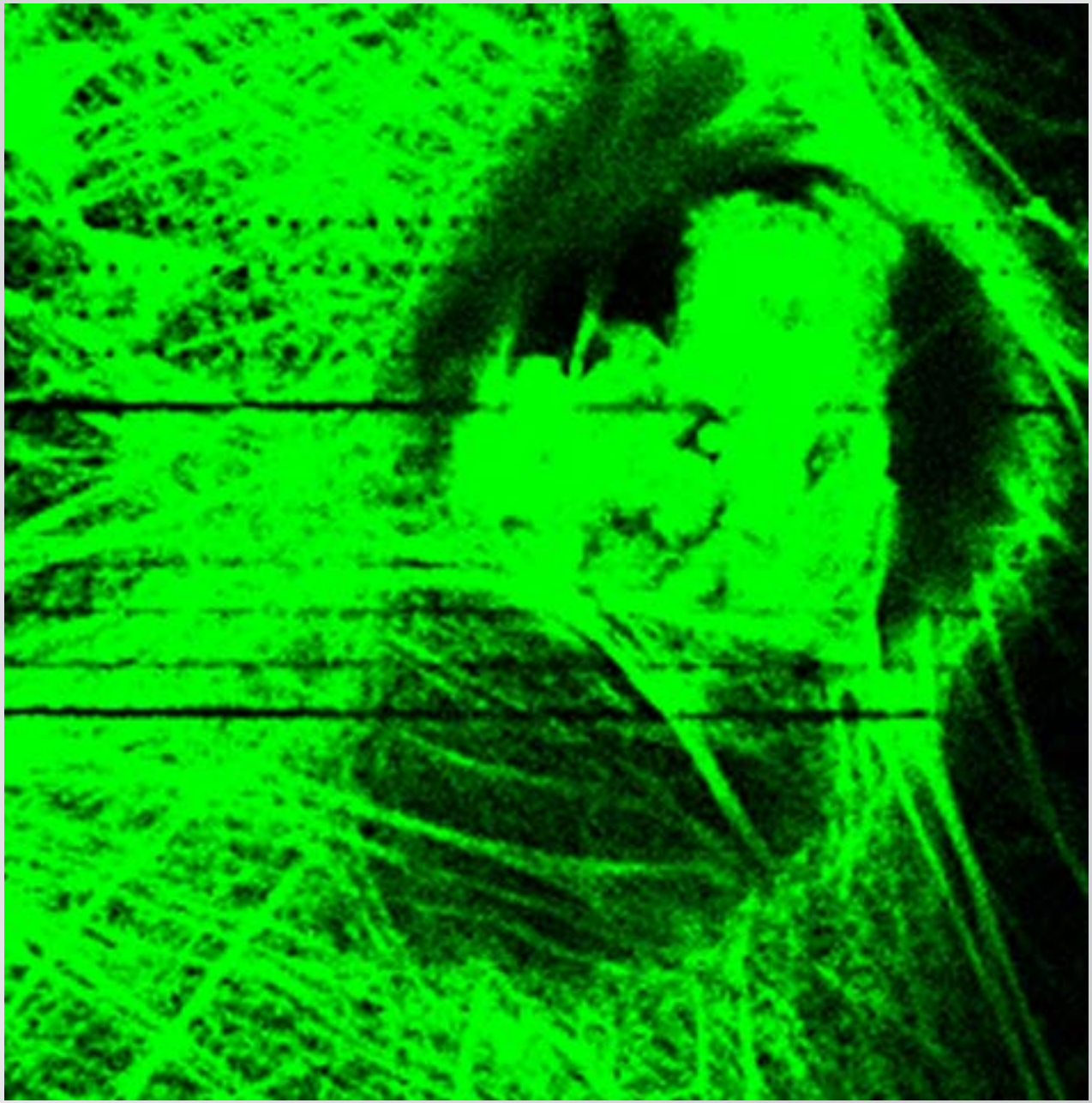
examine resulting ablation

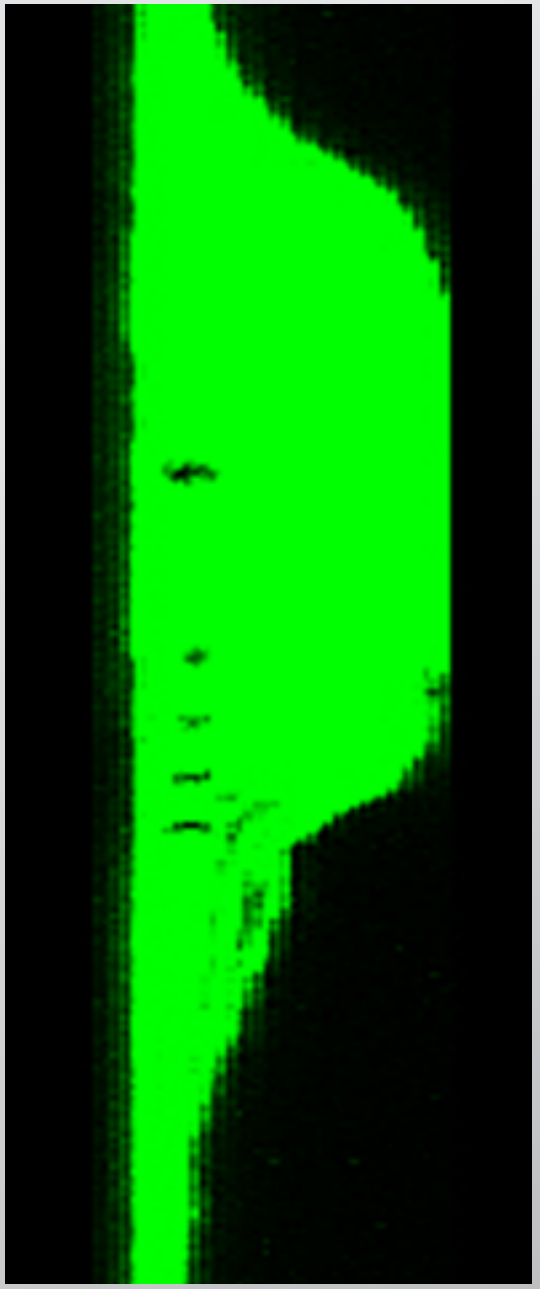
Subcellular surgery





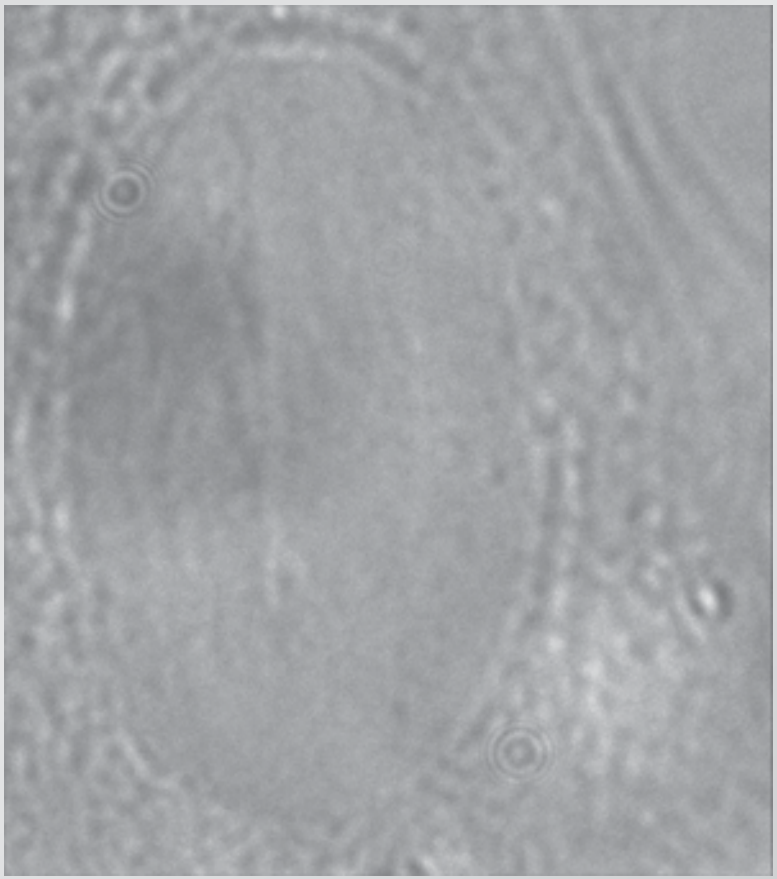
Subcellular surgery





Subcellular surgery

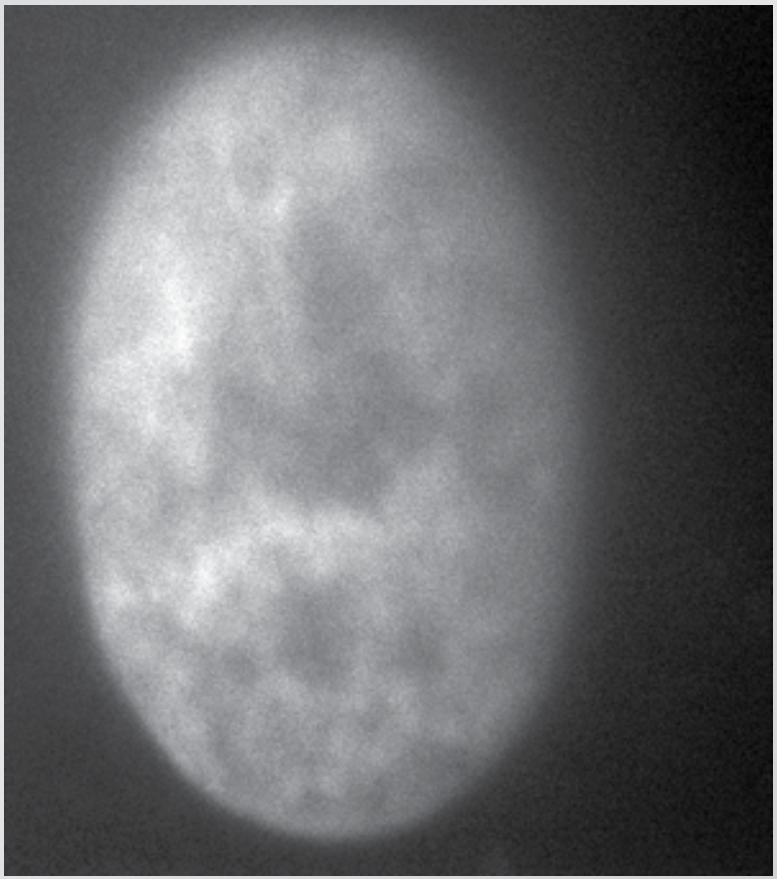
nucleus of fixed endothelial cell



white light microscopy

Subcellular surgery

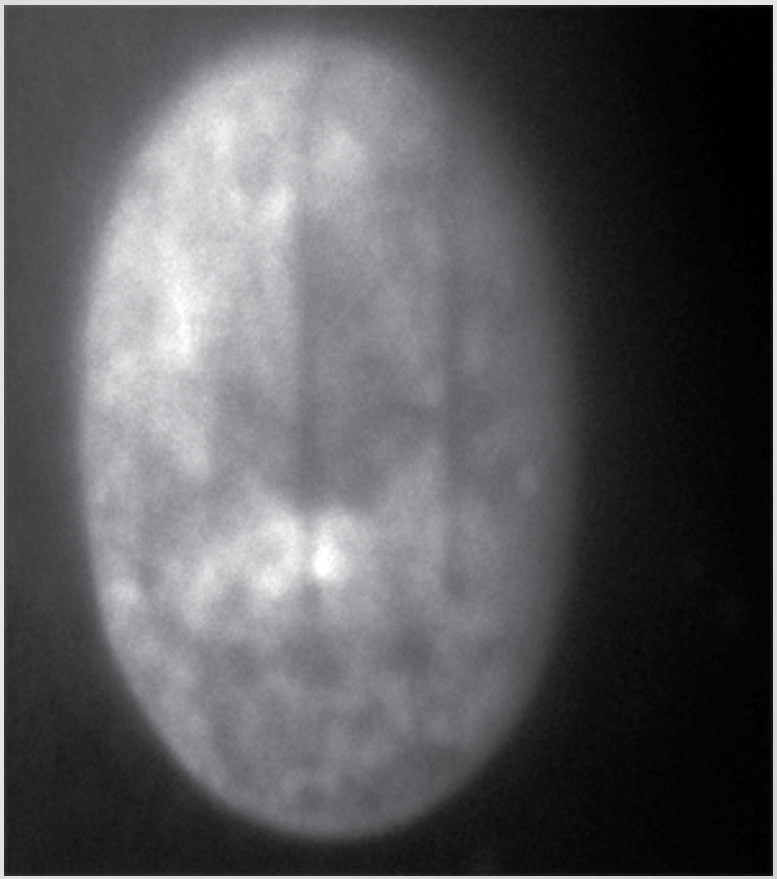
nucleus of fixed endothelial cell



fluorescence microscopy

Subcellular surgery

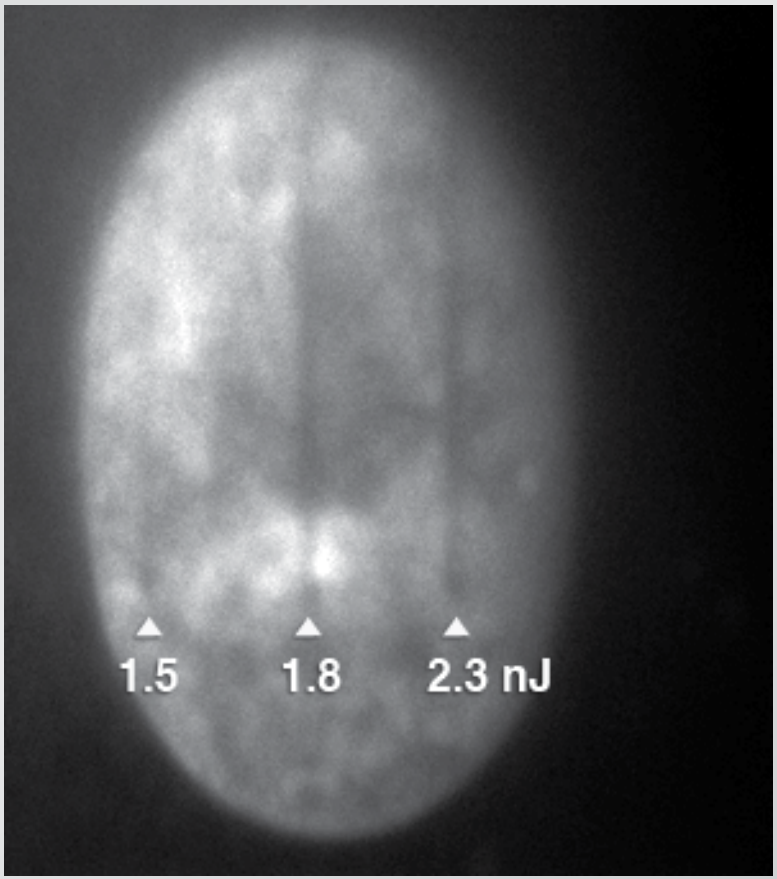
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

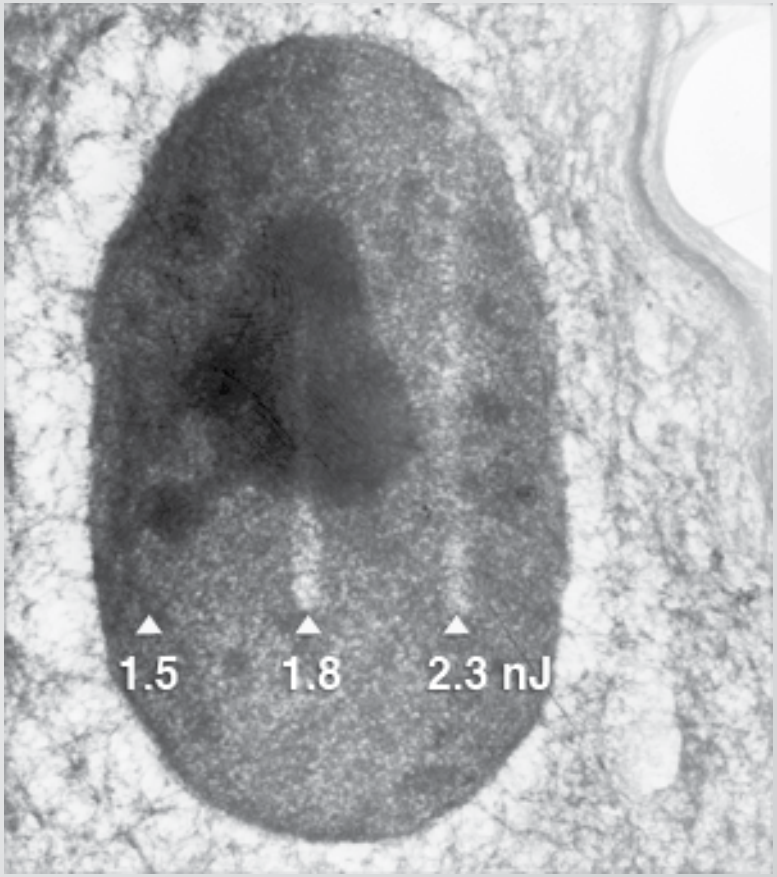
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

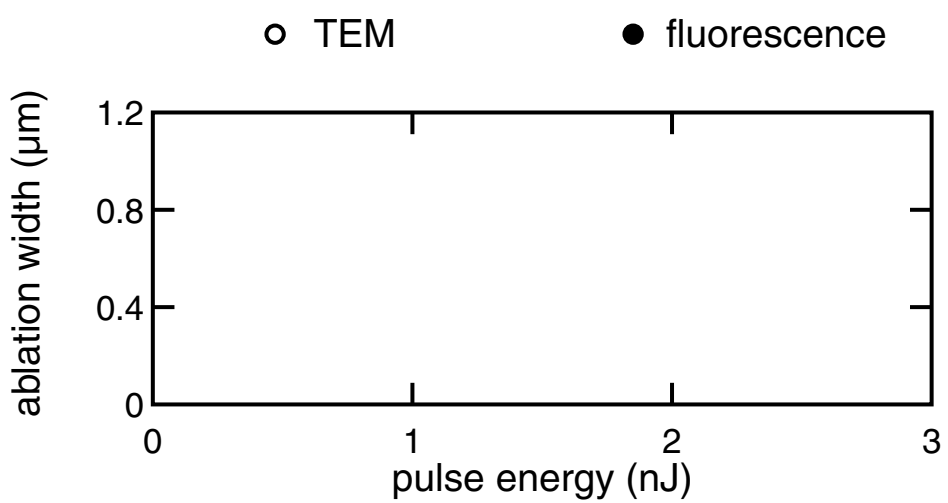
bleaching or ablation?



TEM image

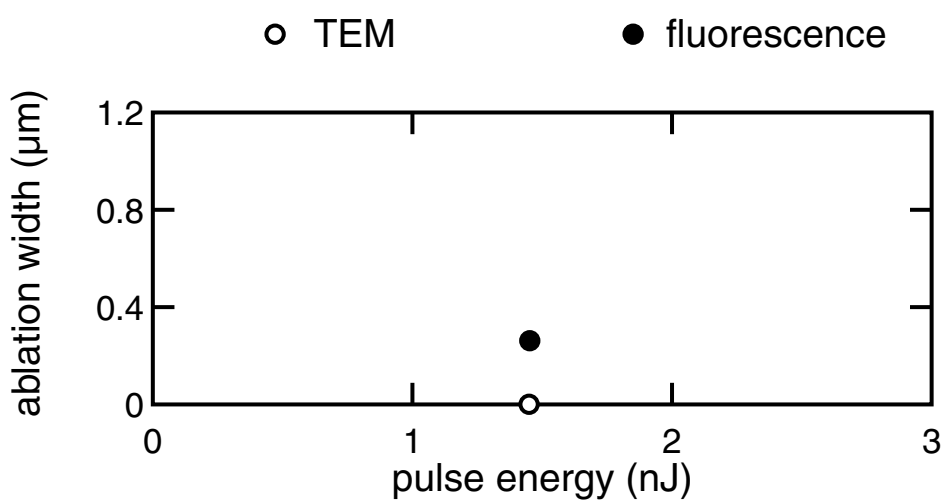
Subcellular surgery

three regions of interaction



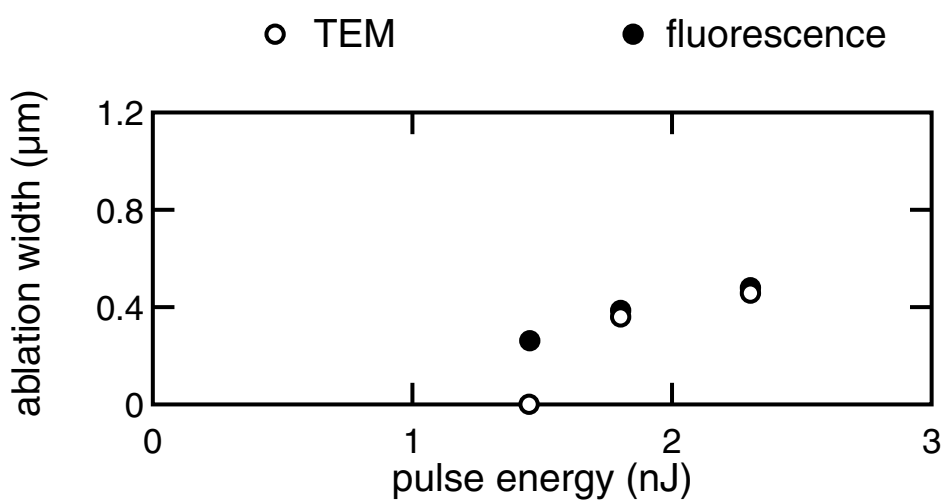
Subcellular surgery

three regions of interaction



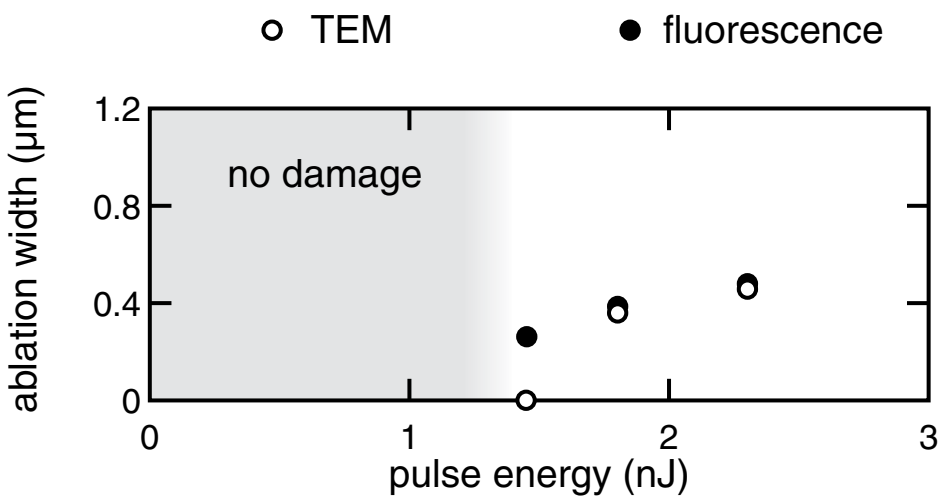
Subcellular surgery

three regions of interaction



Subcellular surgery

three regions of interaction



Subcellular surgery

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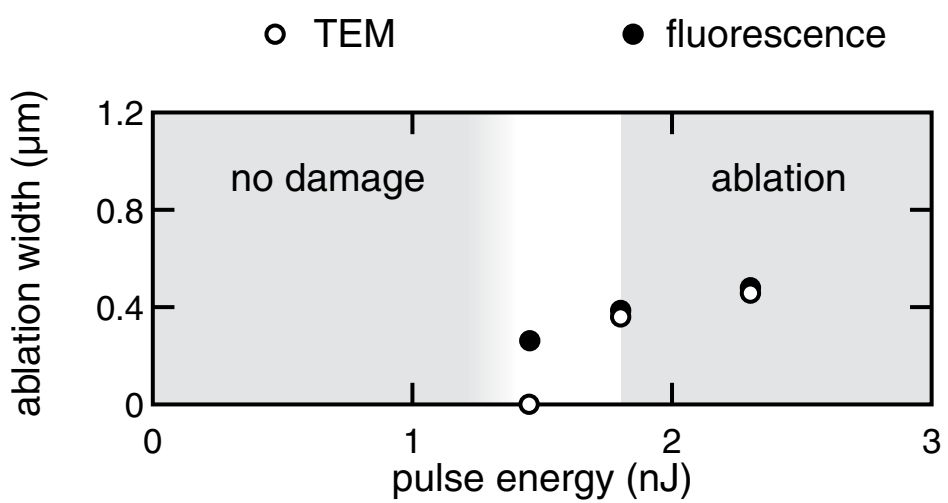
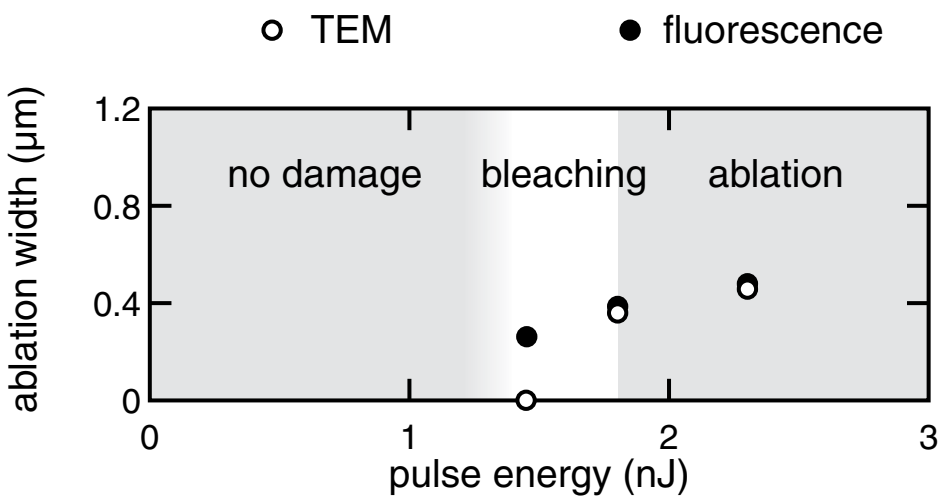
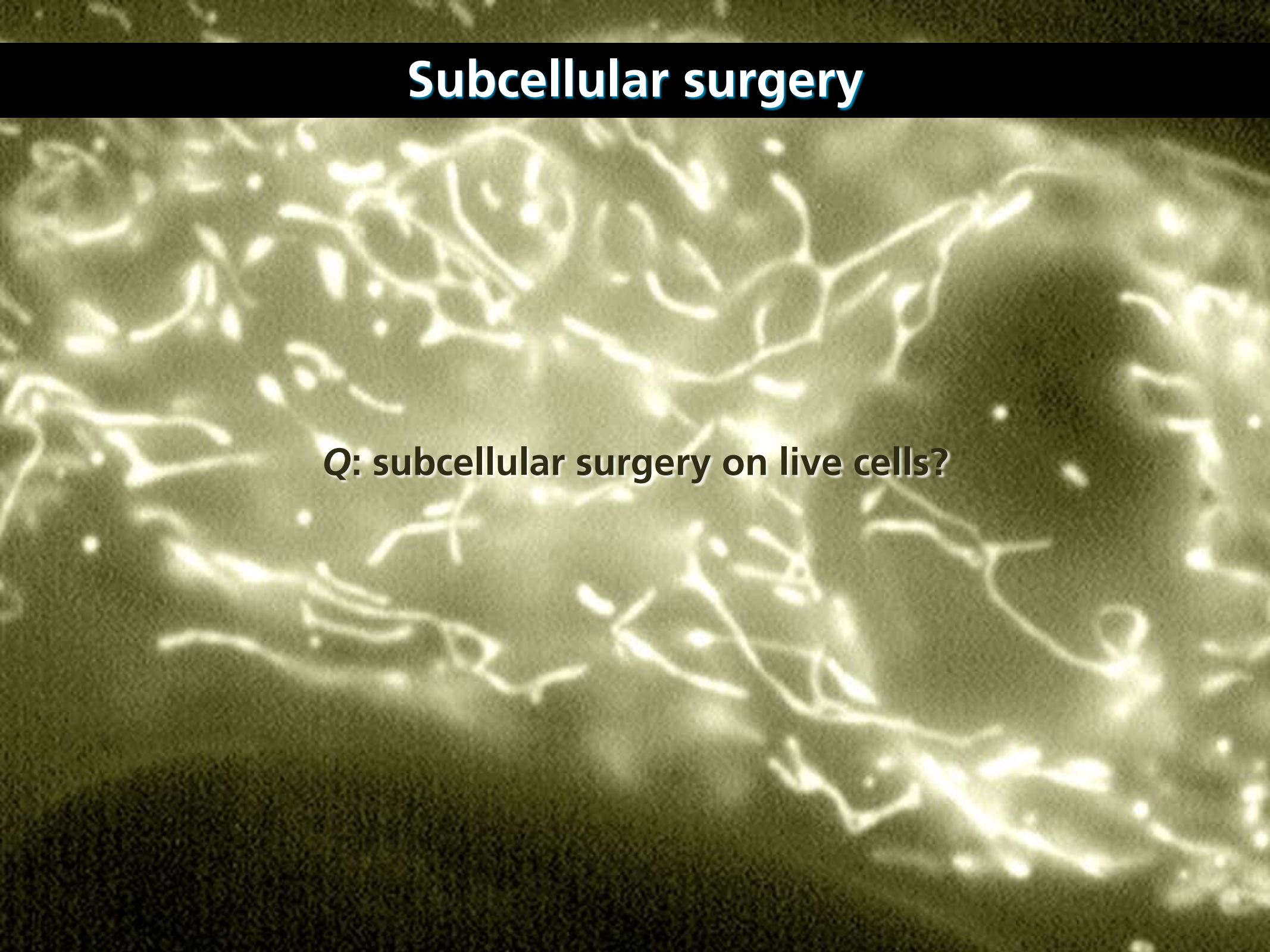


Subcellular surgery

three regions of interaction

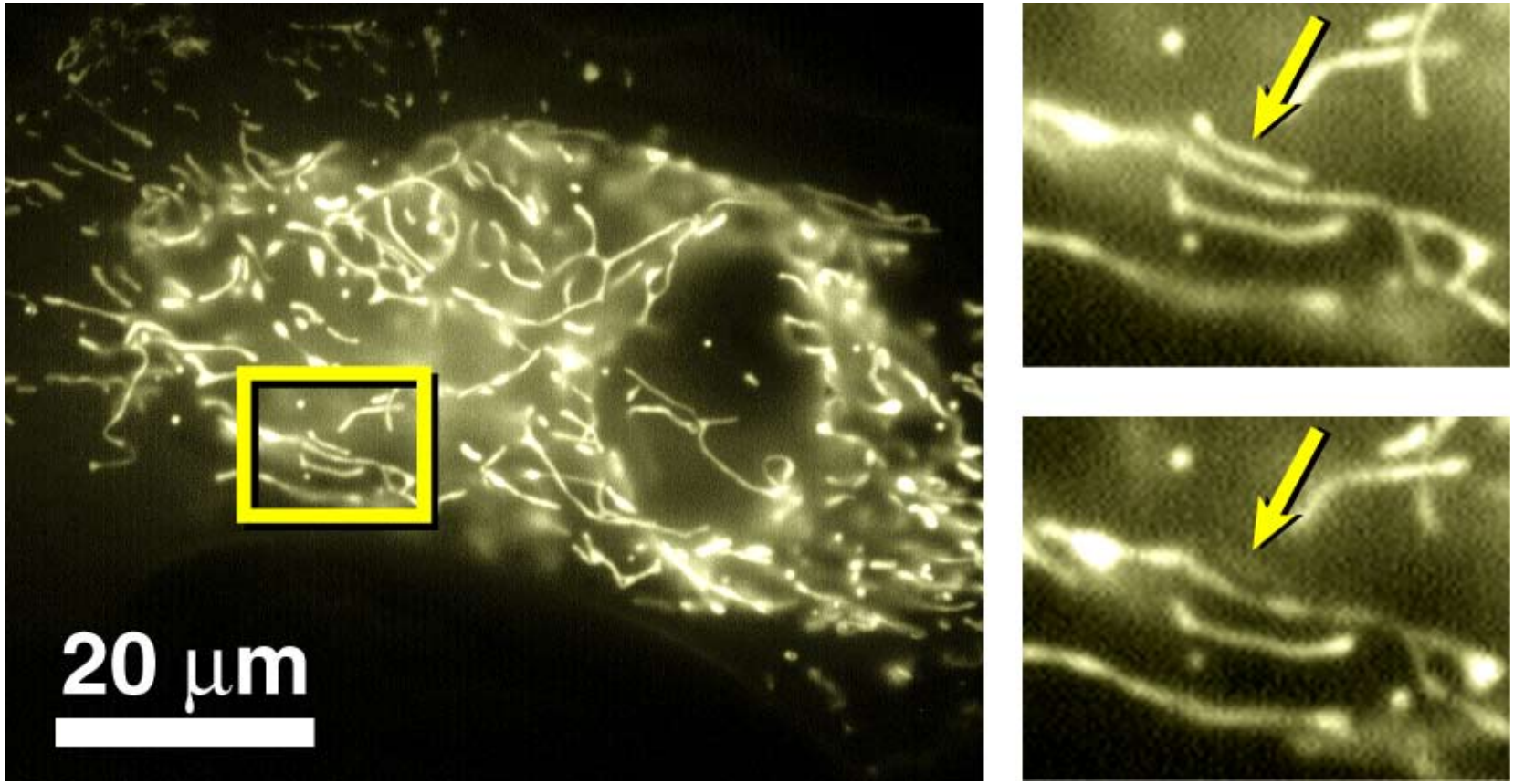


Subcellular surgery



Q: subcellular surgery on live cells?

Subcellular surgery

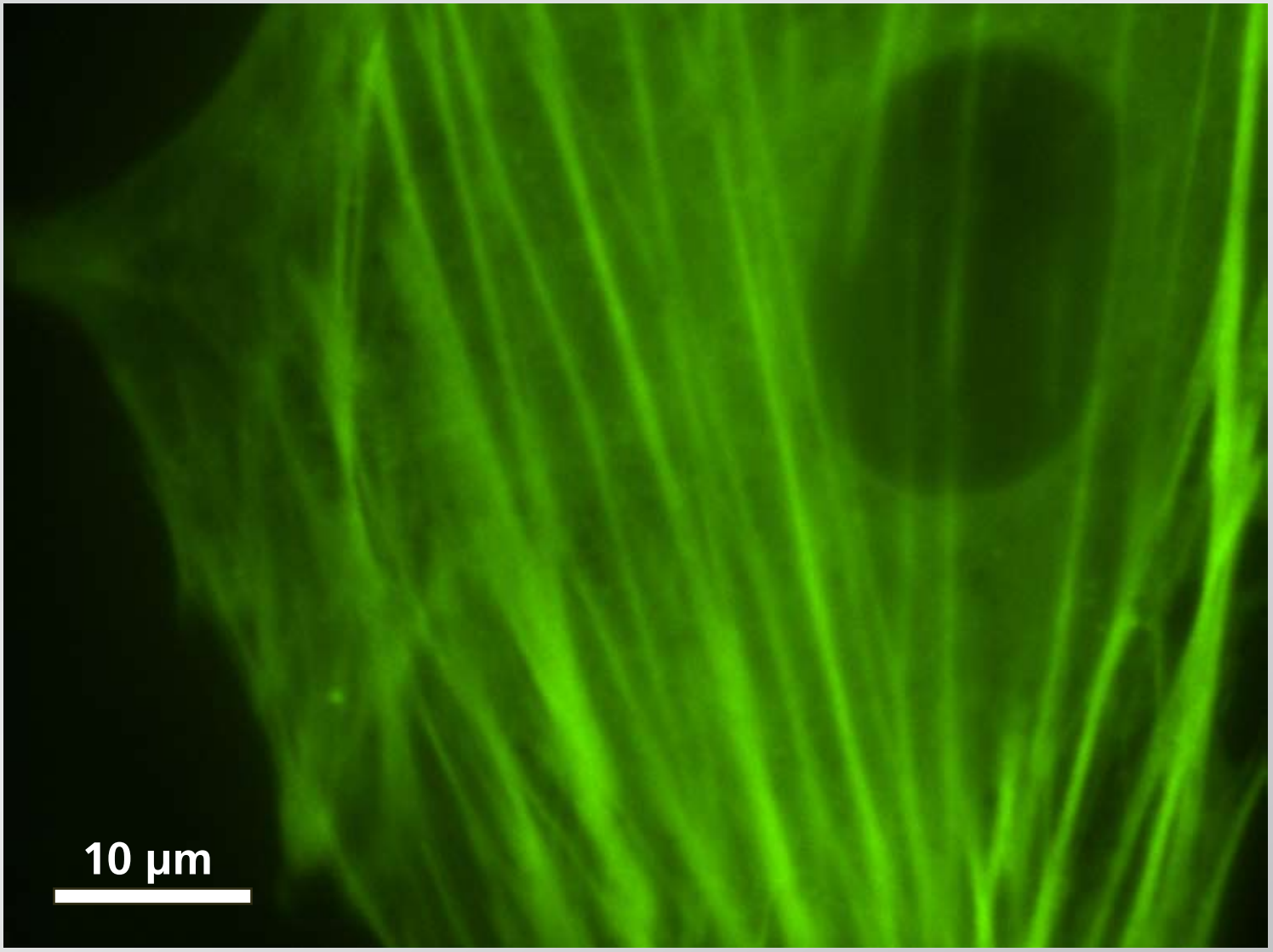


Subcellular surgery

Q: can we probe the dynamics of the cytoskeleton?

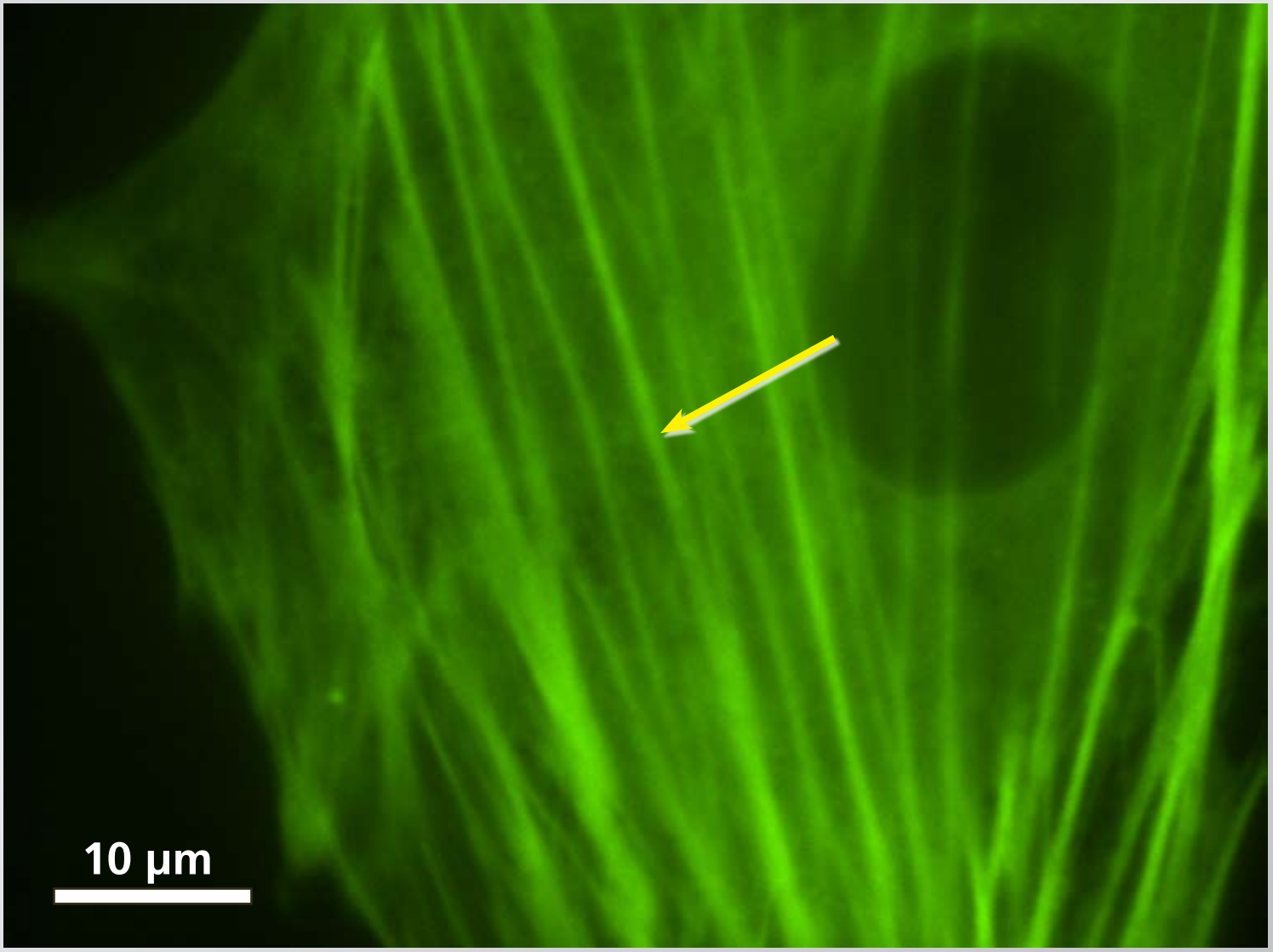
Subcellular surgery

YFP-labeled actin fiber network of a live cell



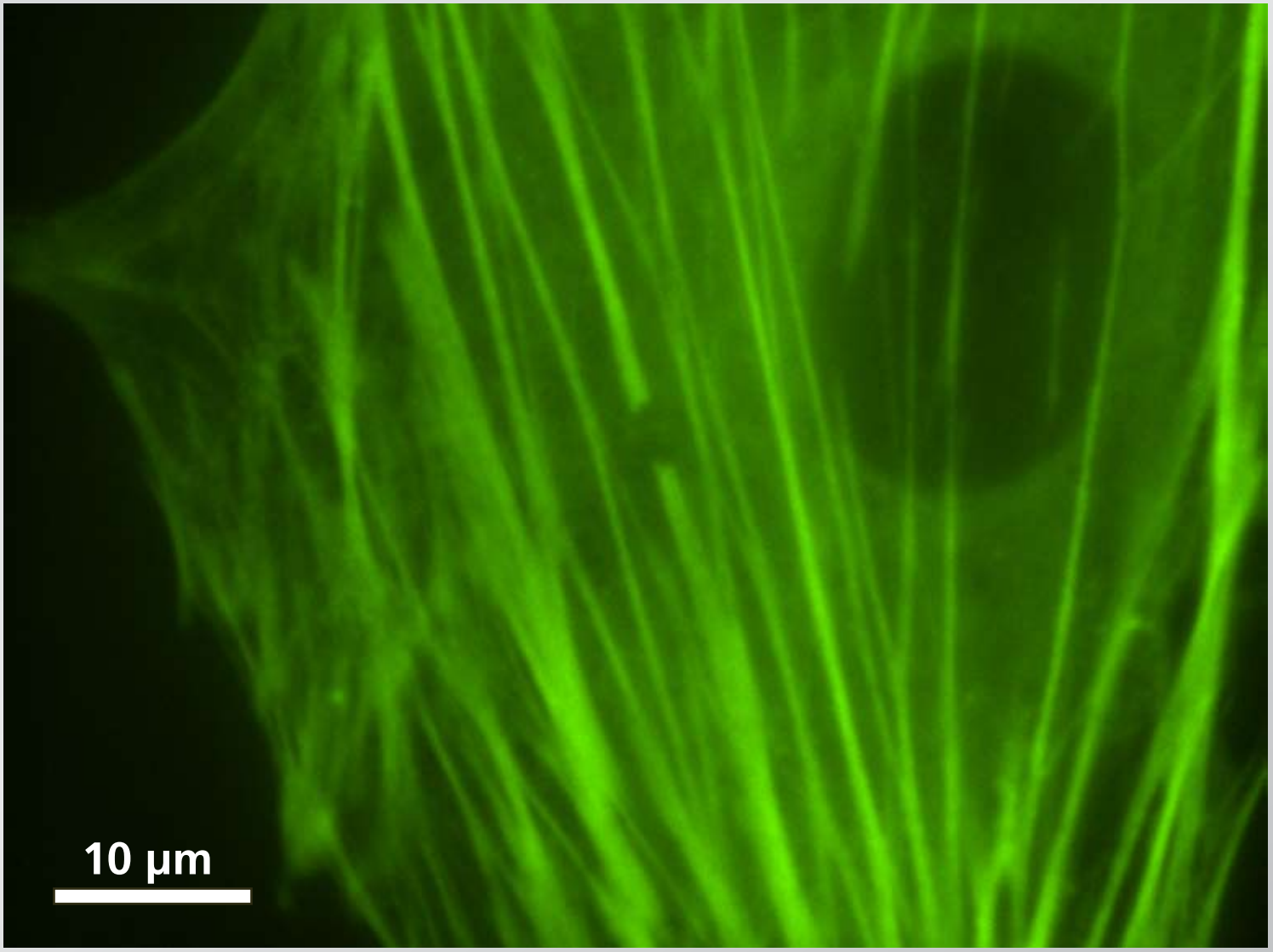
Subcellular surgery

cut a single fiber bundle



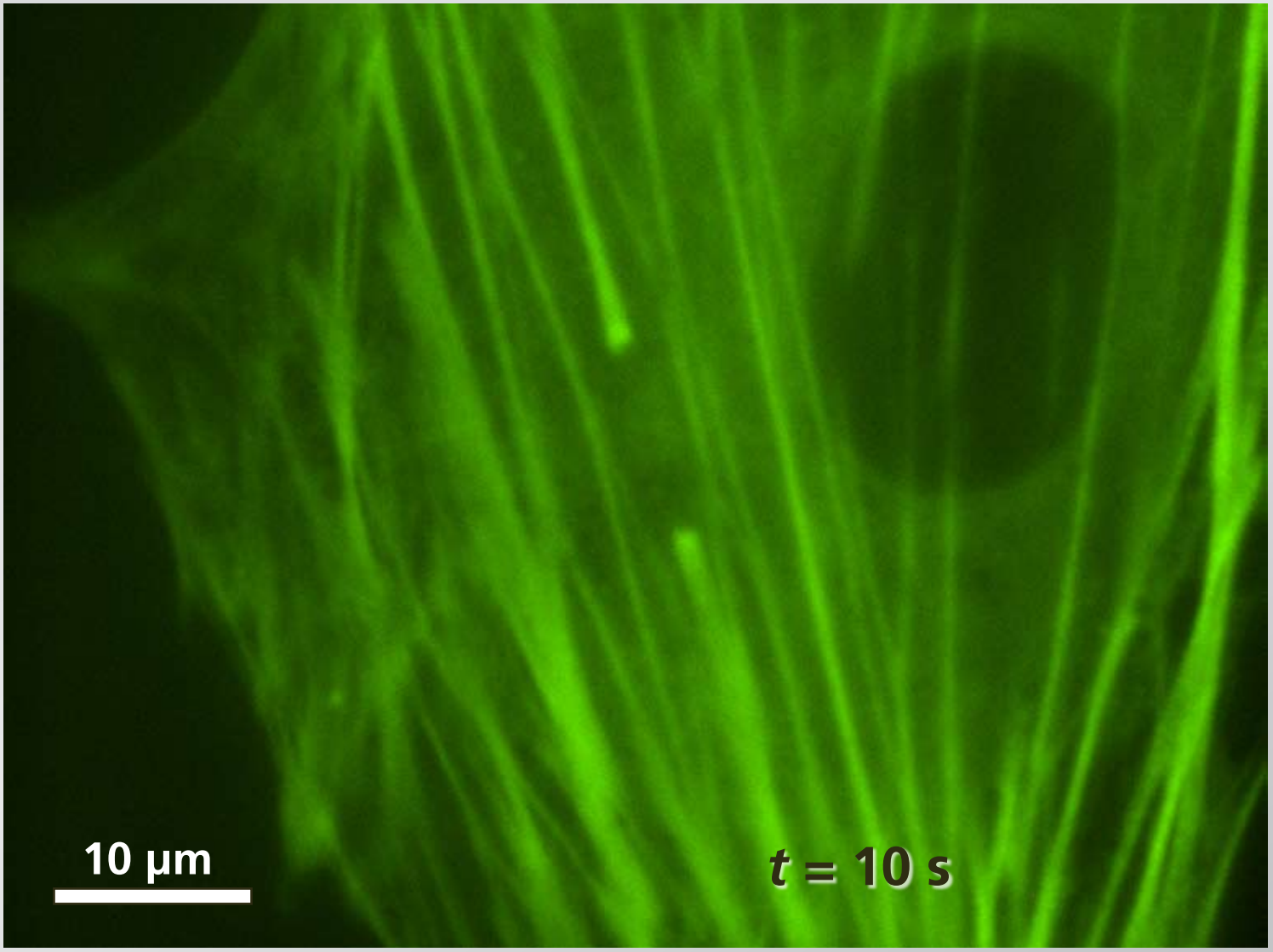
Subcellular surgery

cut a single fiber bundle



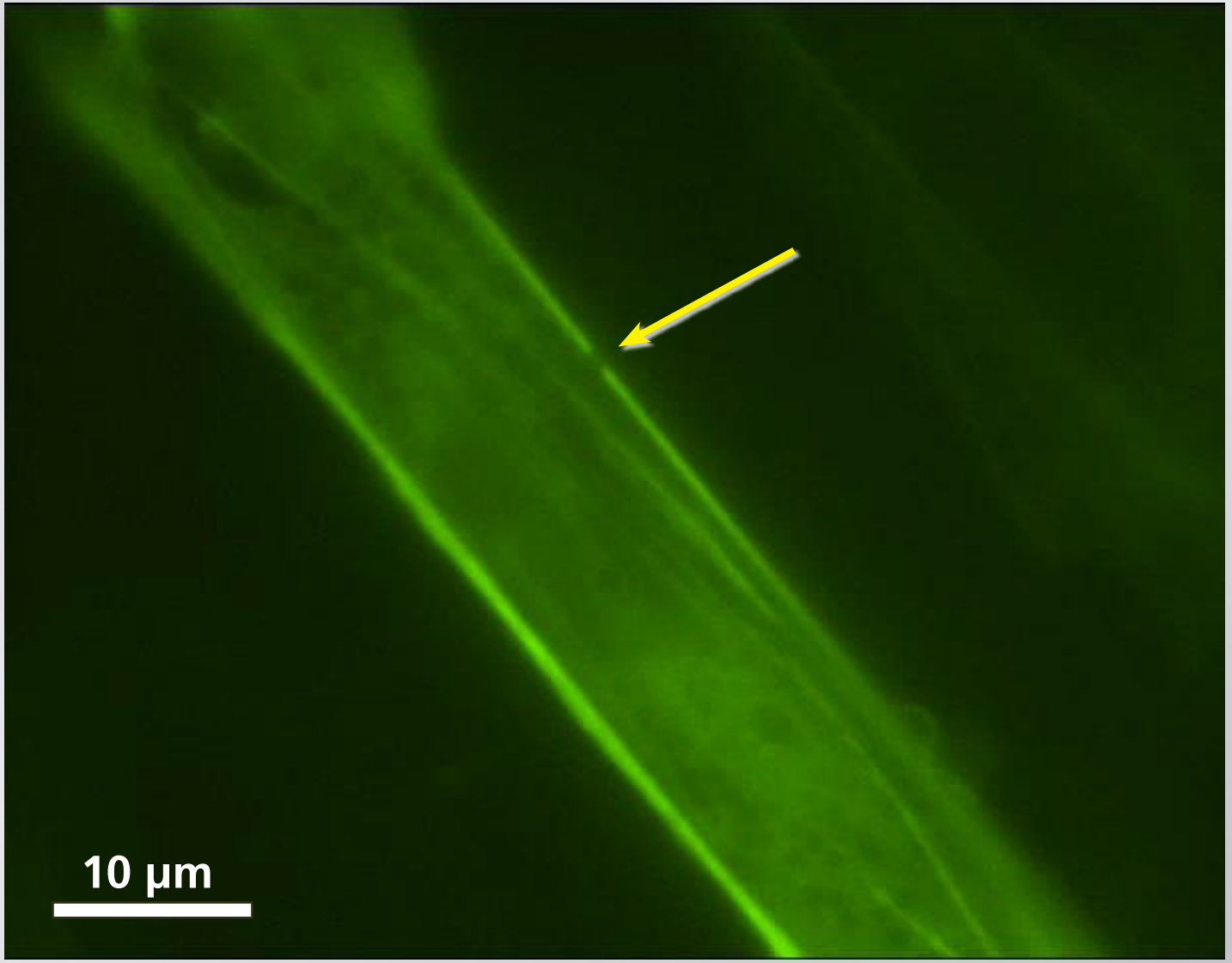
Subcellular surgery

gap widens with time



Subcellular surgery

retraction or depolymerization?



Subcellular surgery

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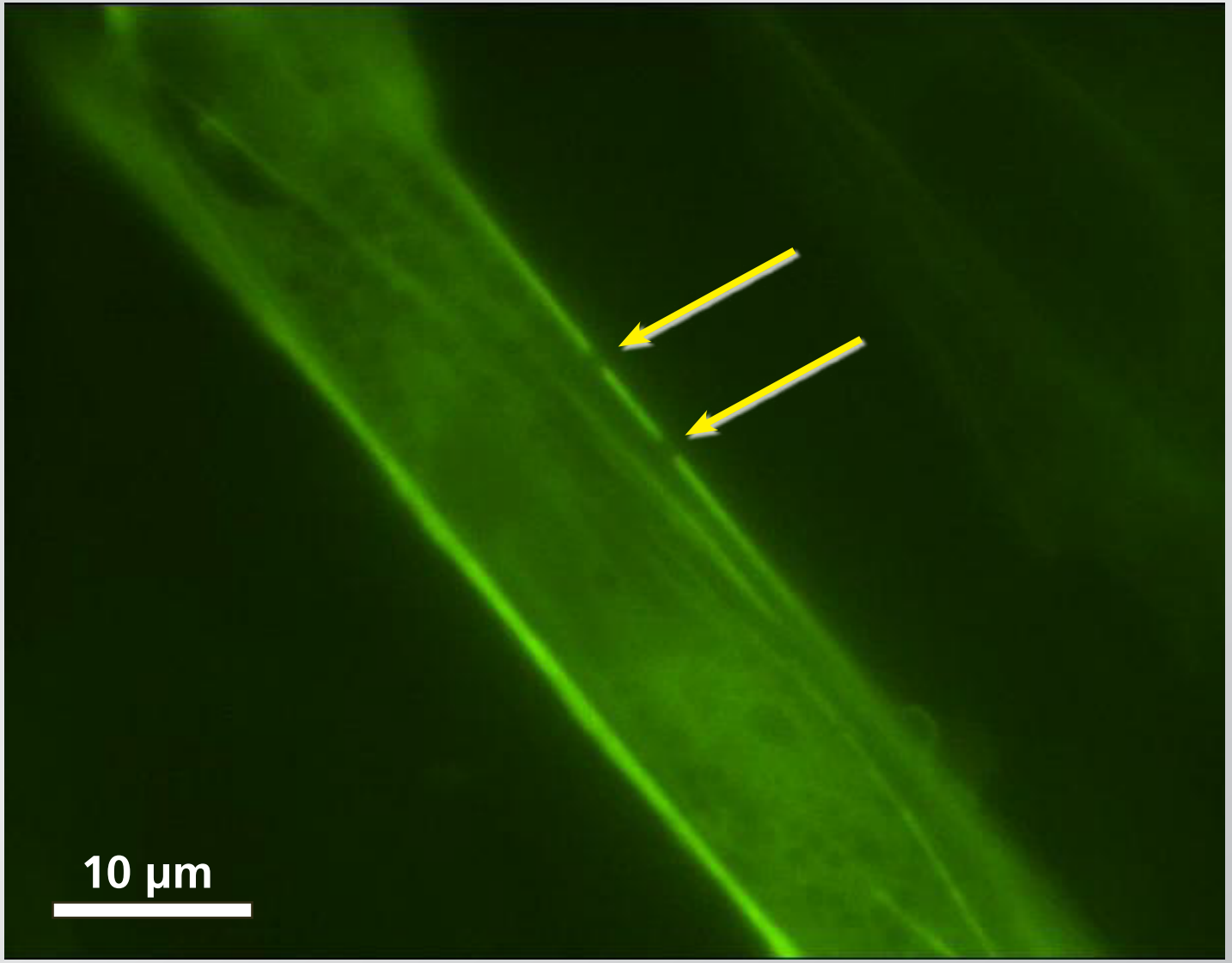

Subcellular surgery

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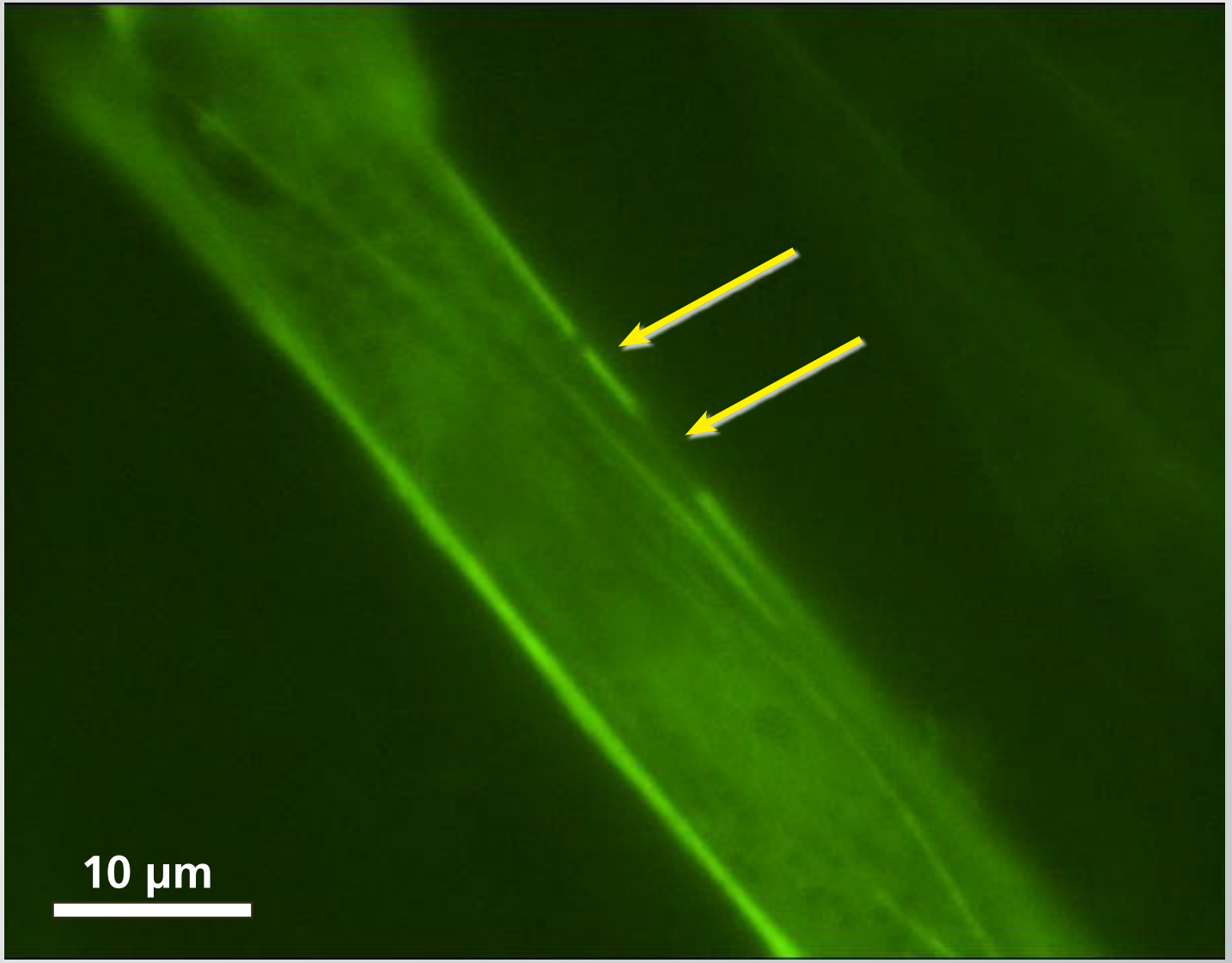
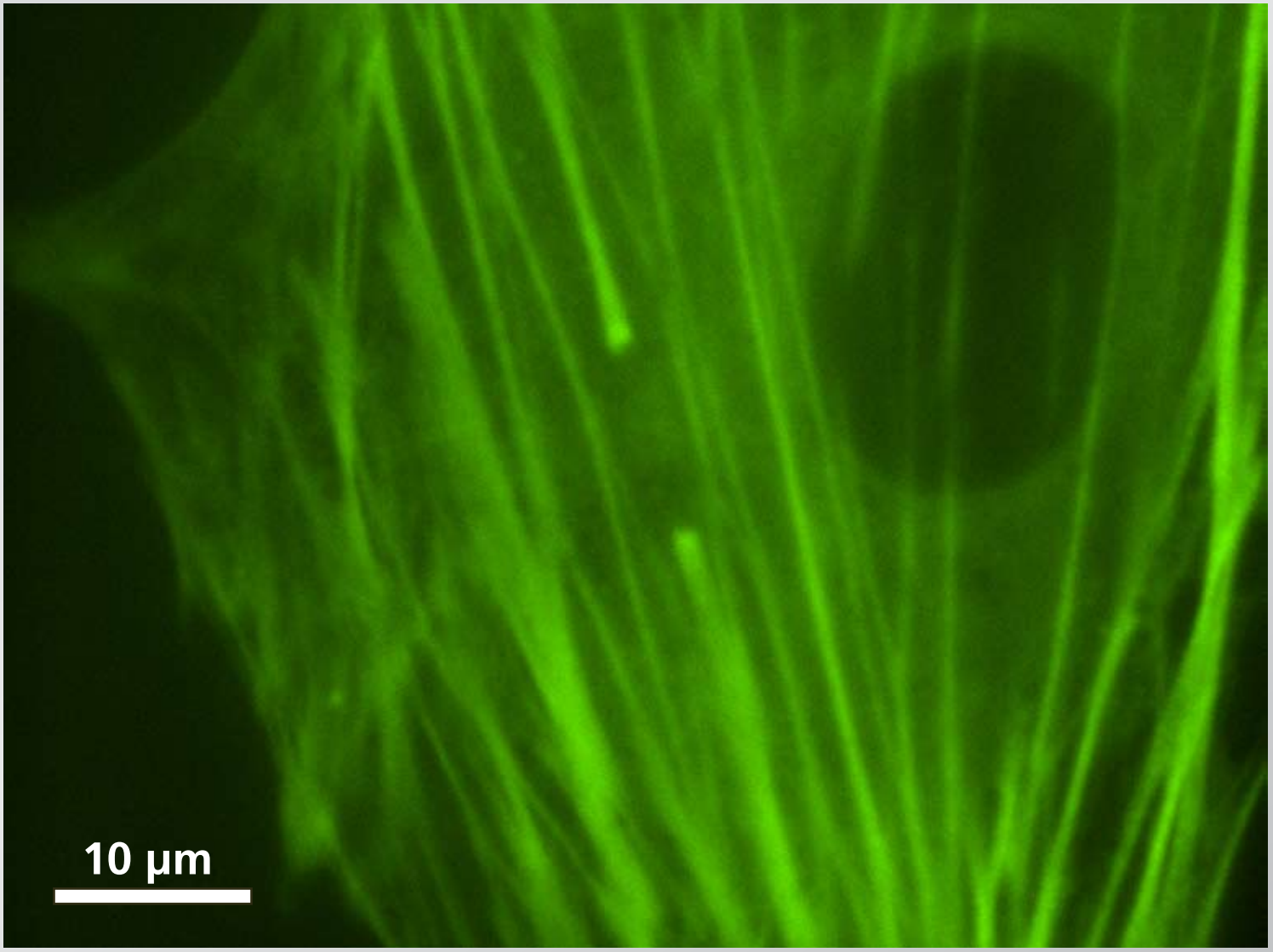


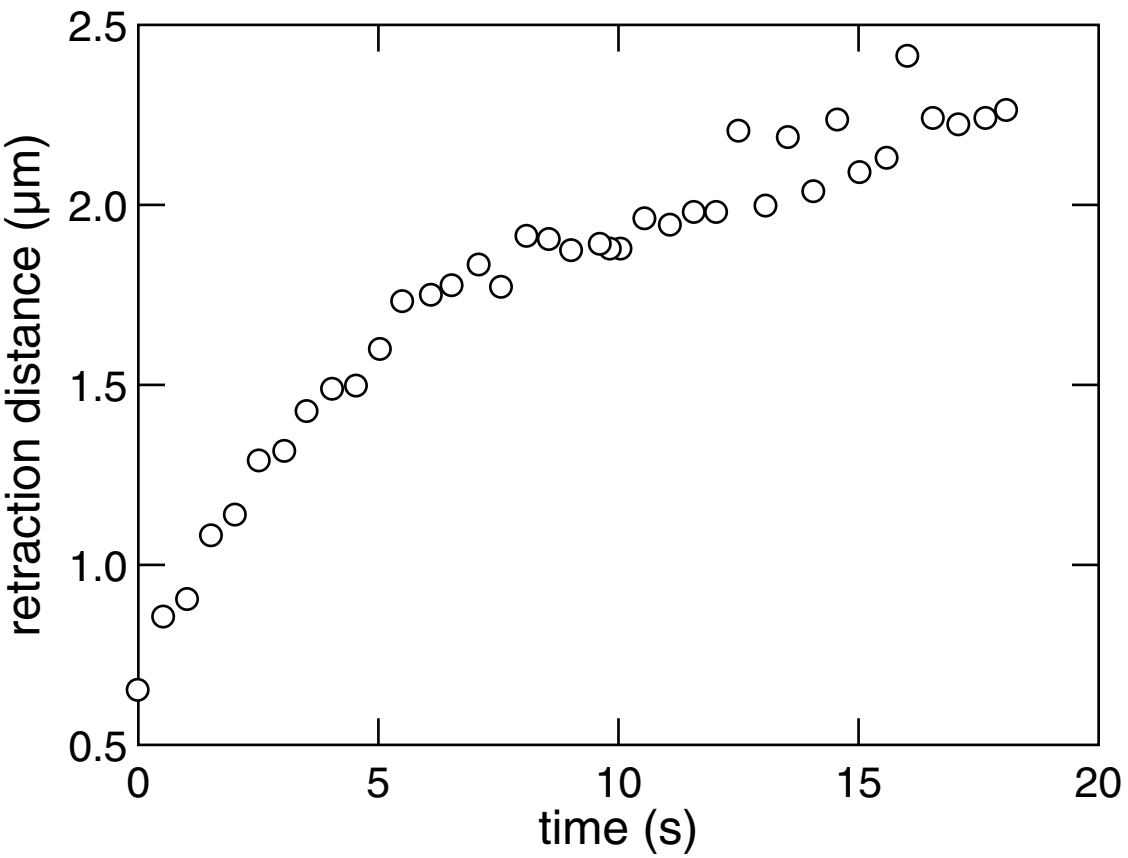
Subcellular surgery

dynamics provides information on *in vivo* mechanics



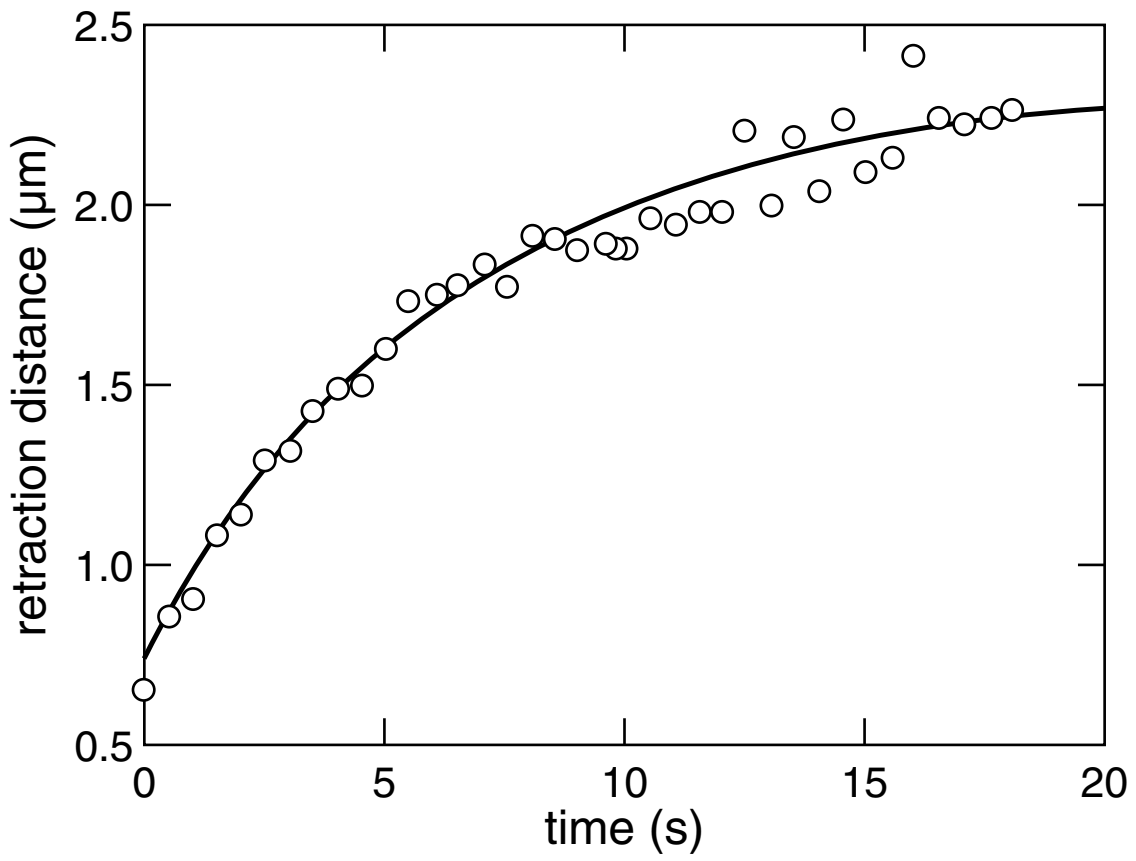
Subcellular surgery

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



Subcellular surgery

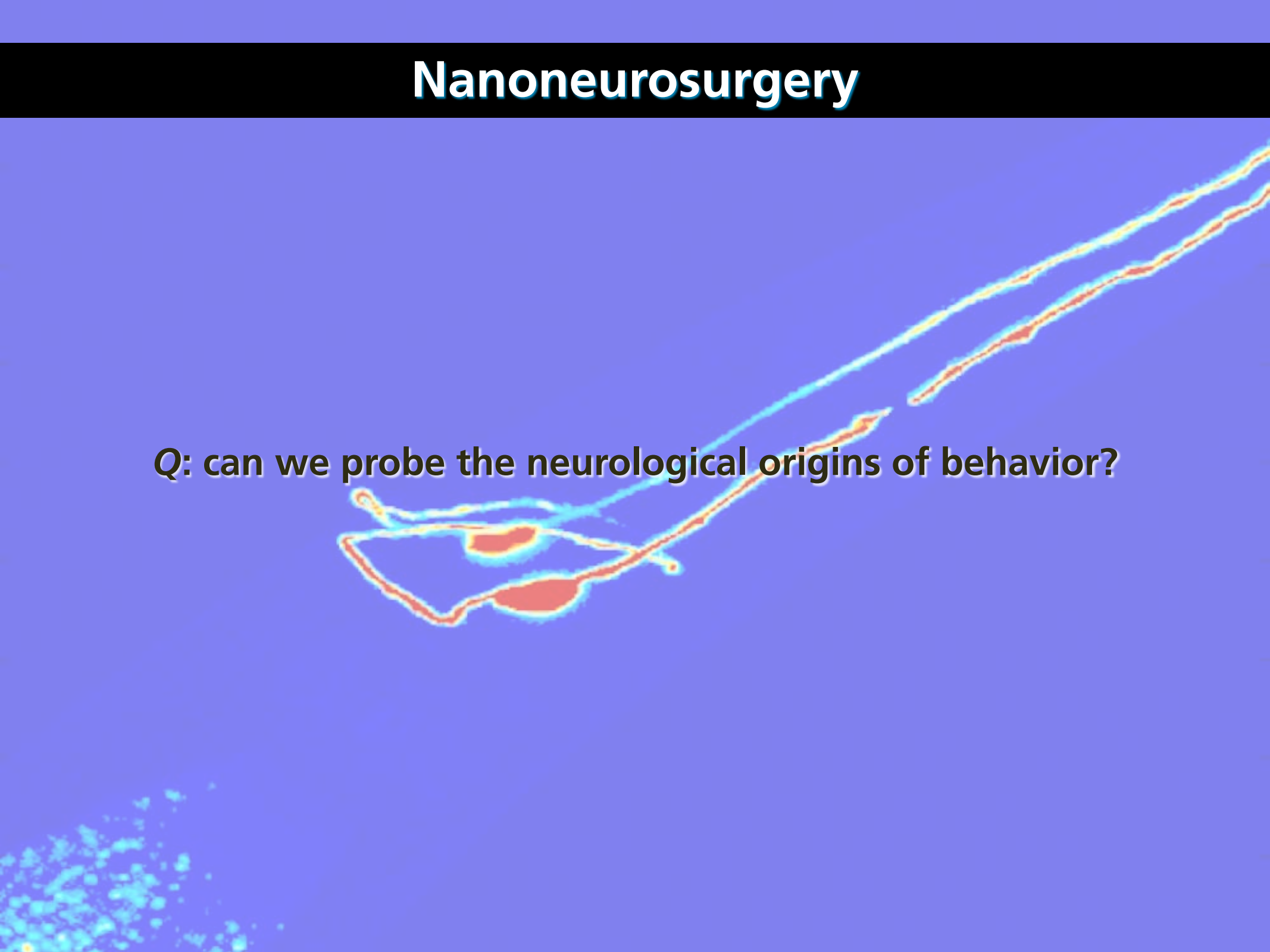
overdamped spring: $\Delta L = L_\infty(1 - e^{-t/\tau}) + L_o$



Outline

- femtosecond micromachining
- subcellular surgery
- nanoneurosurgery

Nanoneurosurgery



Q: can we probe the neurological origins of behavior?

Nanoneurosurgery

Caenorhabditis elegans

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

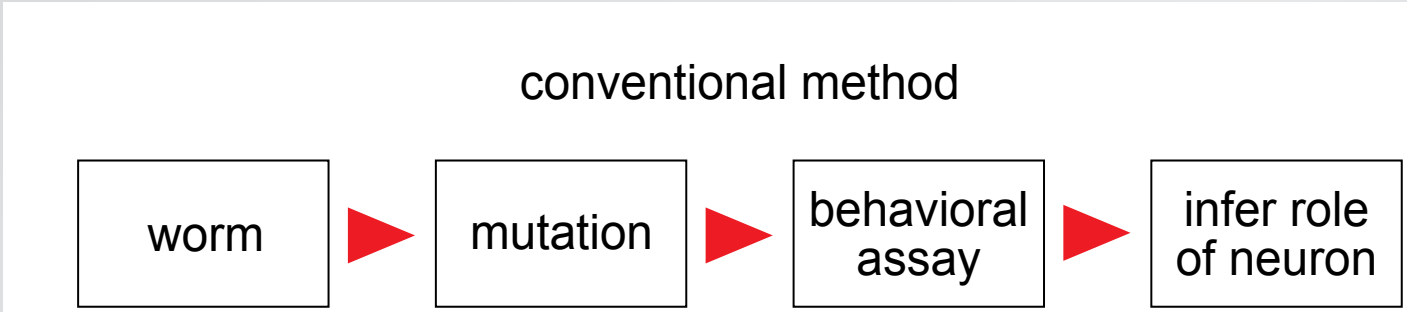
Nanoneurosurgery

Caenorhabditis elegans

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

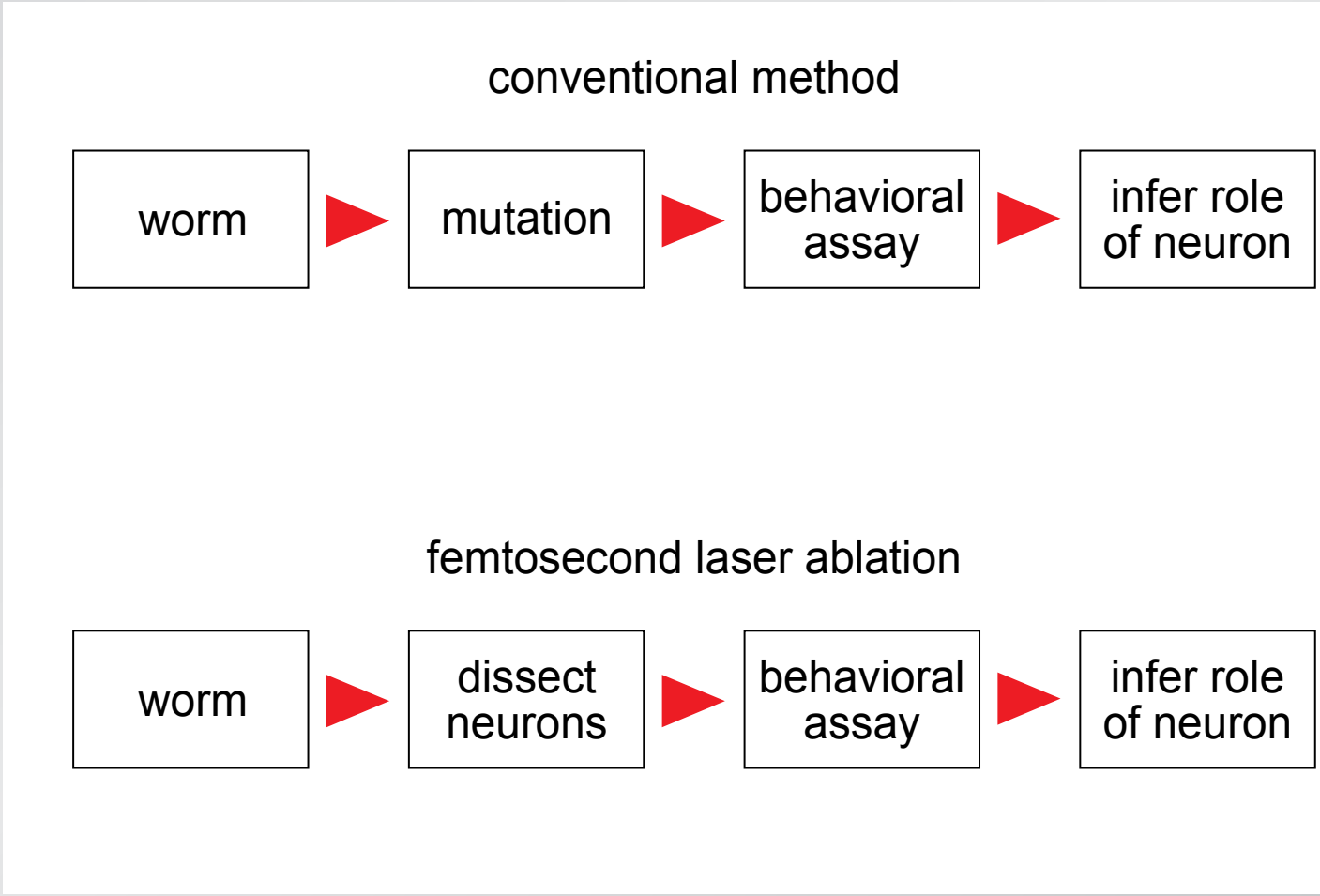
Nanoneurosurgery

Mapping behavior to neurons



Nanoneurosurgery

Mapping behavior to neurons



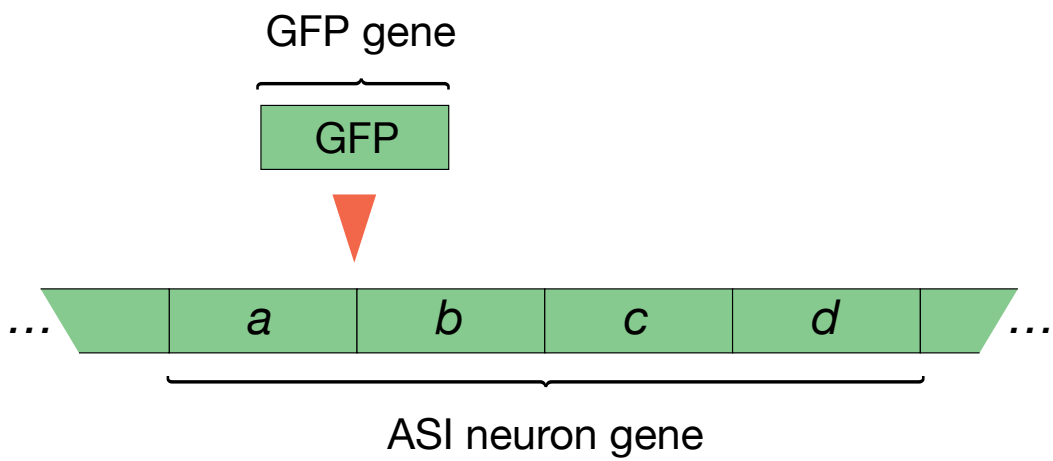
Nanoneurosurgery

ASI neurons

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

Nanoneurosurgery

Make ASH neurons express GFP

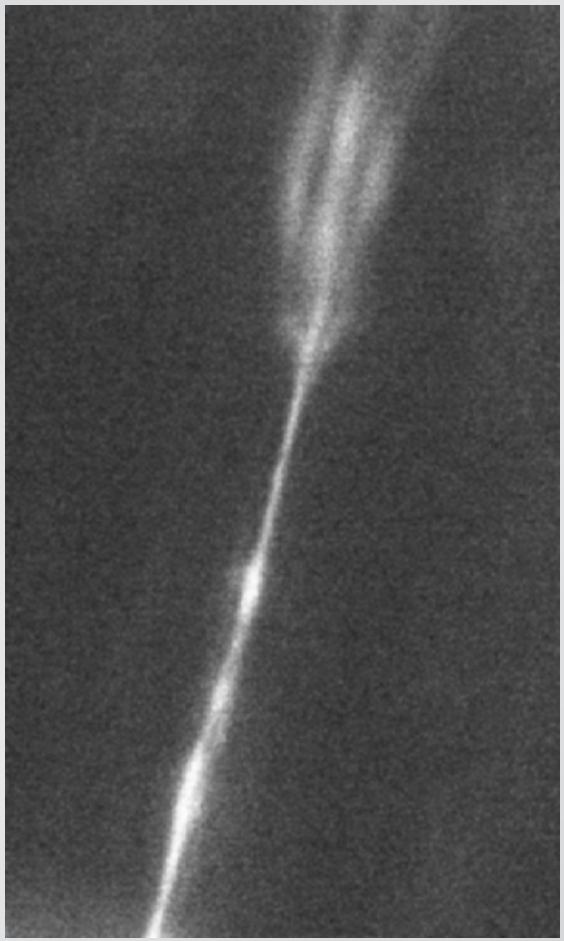


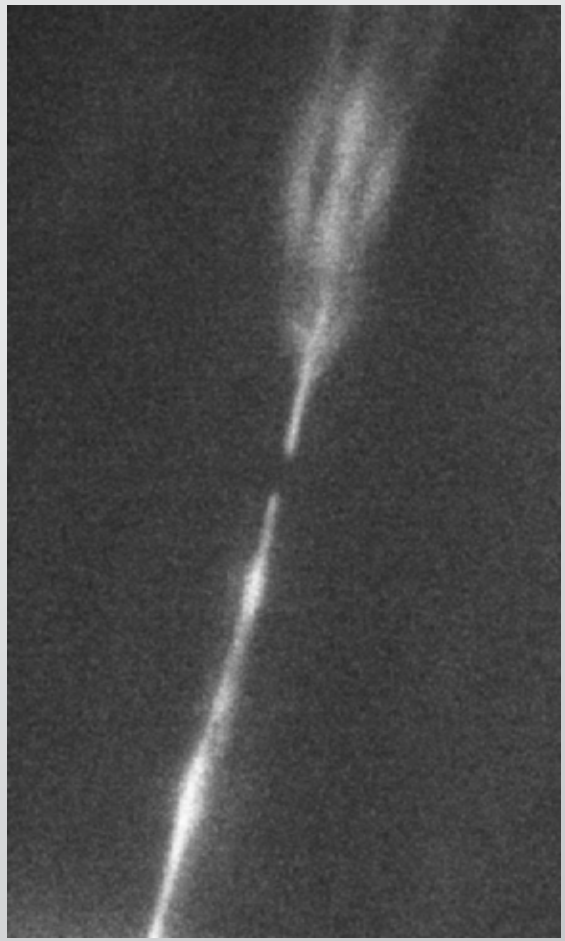
Nanoneurosurgery

GFP: absorbs UV, emits green

Nanoneurosurgery

minutes after surgery with 14 nJ pulses

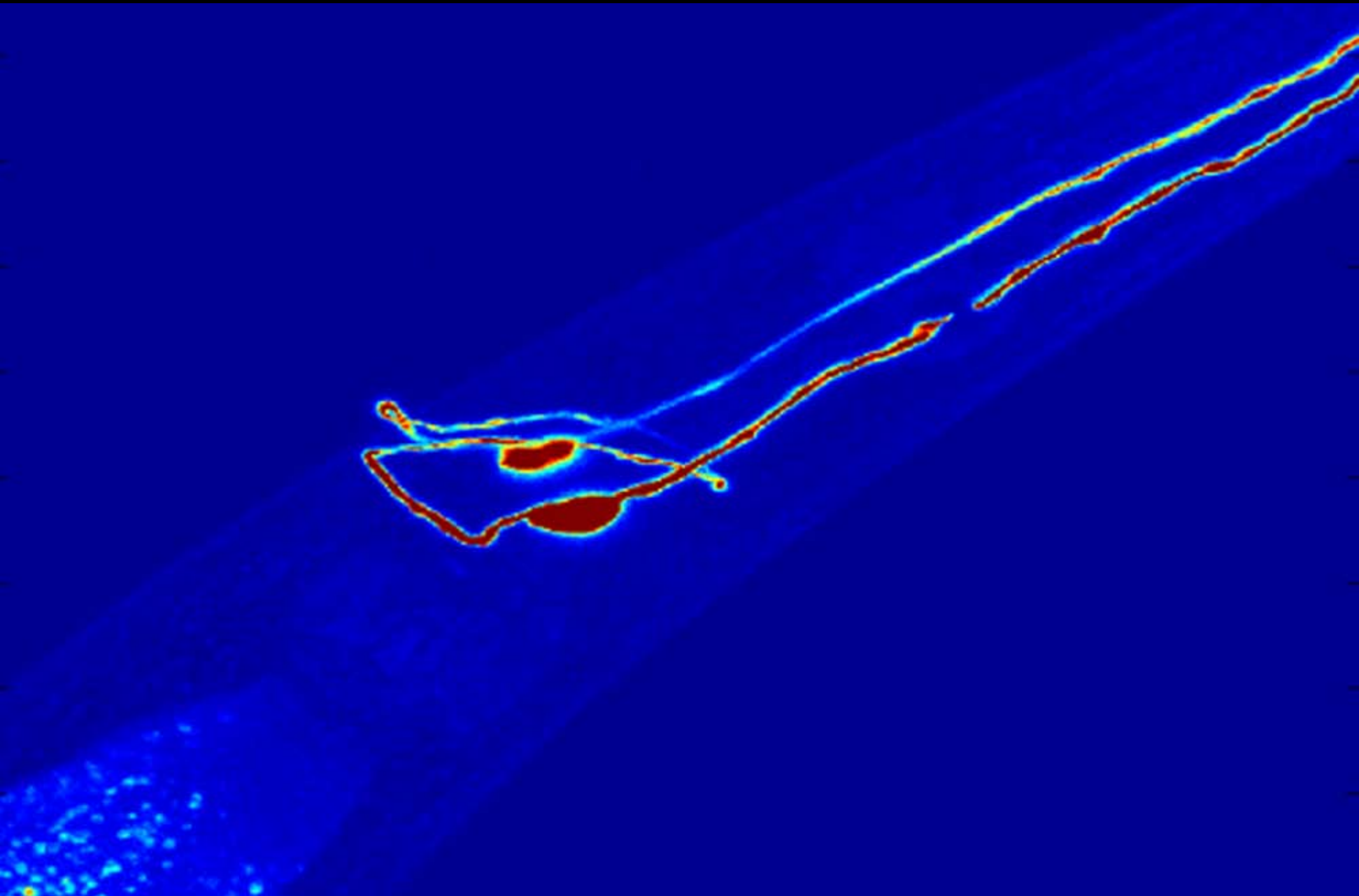




Nanoneurosurgery

revive worm, reimage 1 day later

Nanoneurosurgery

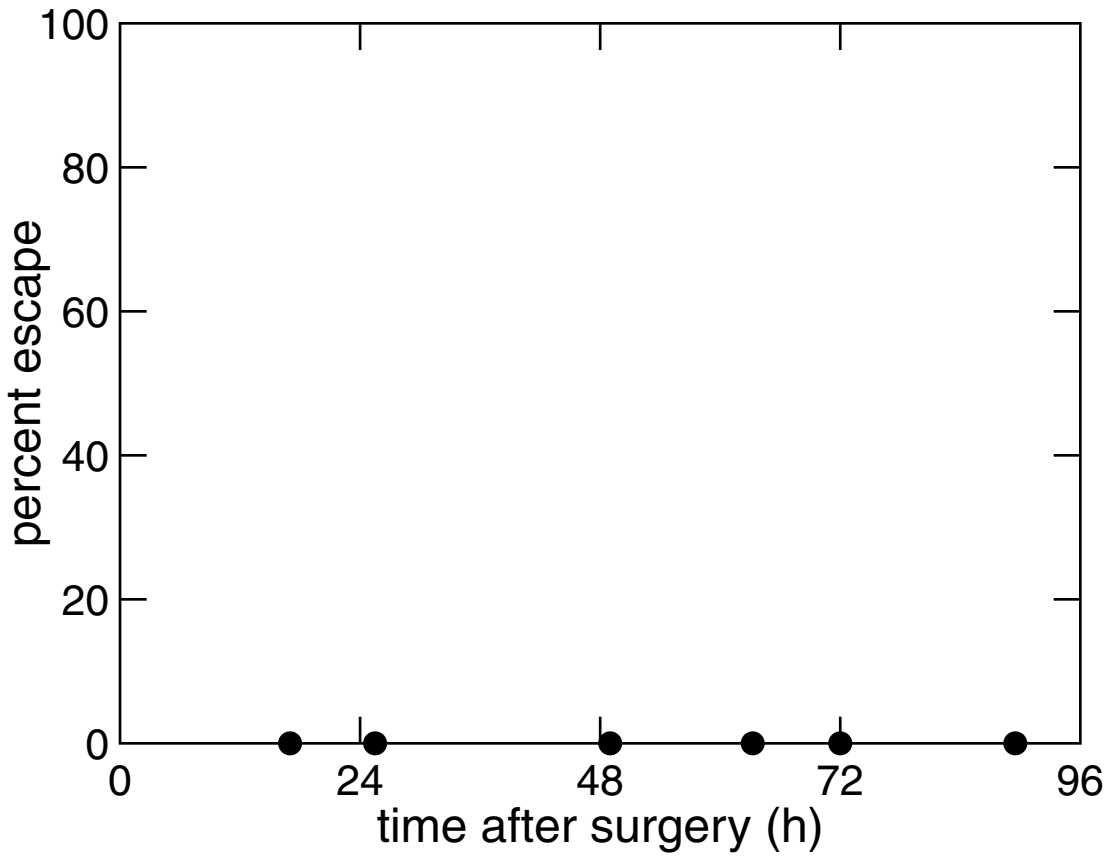


Nanoneurosurgery

osmolarity assay

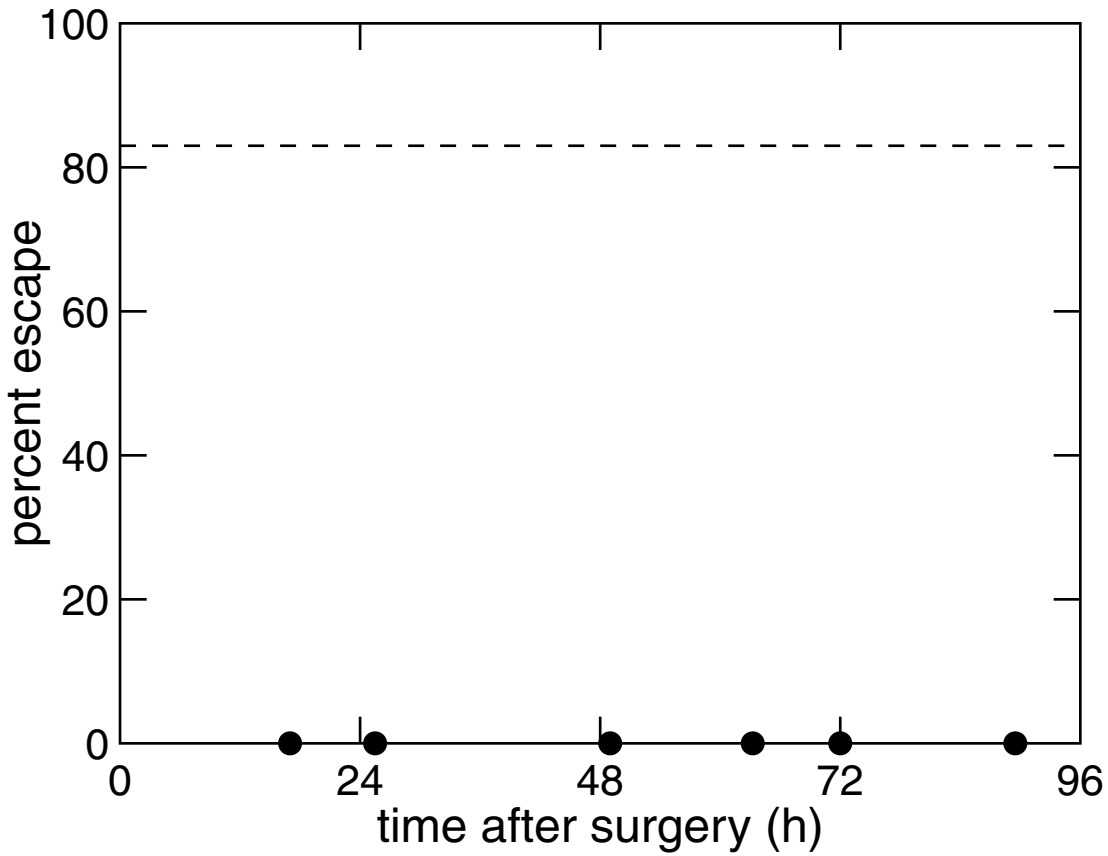
Nanoneurosurgery

escape rate after ‘mock’ surgery



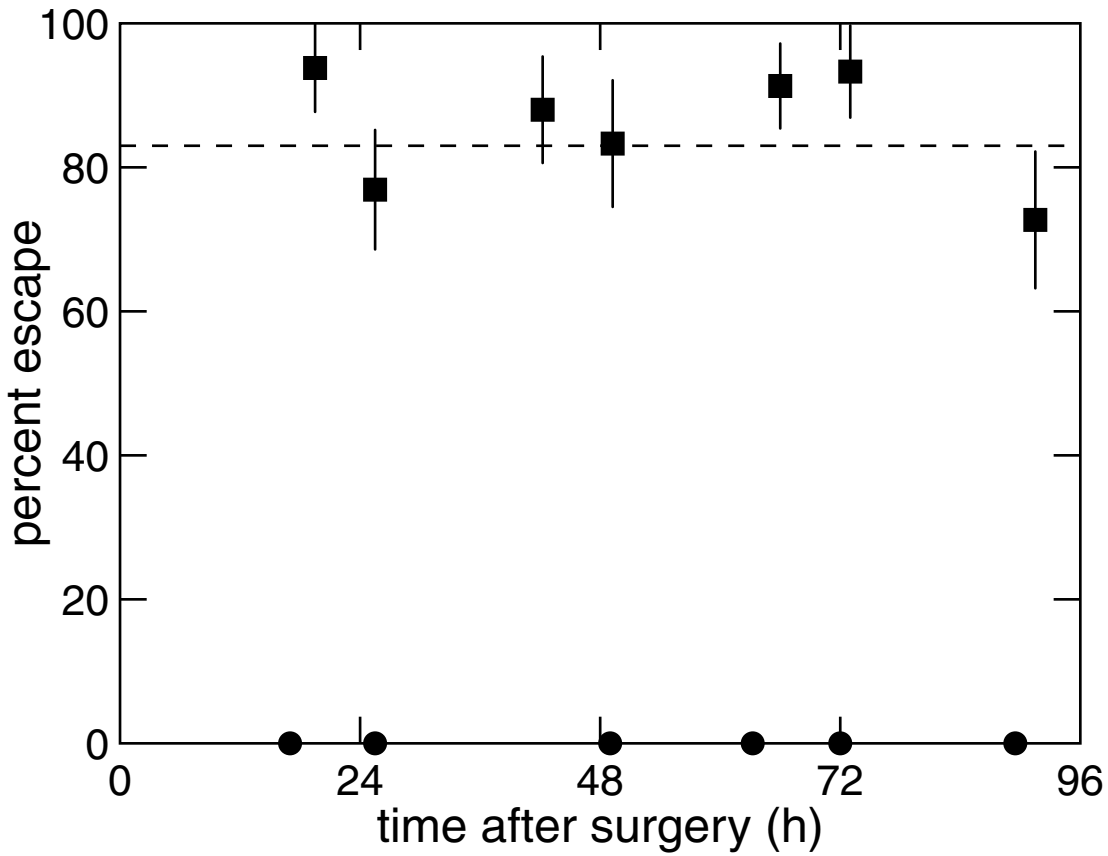
Nanoneurosurgery

escape rate of ASH-lacking mutant



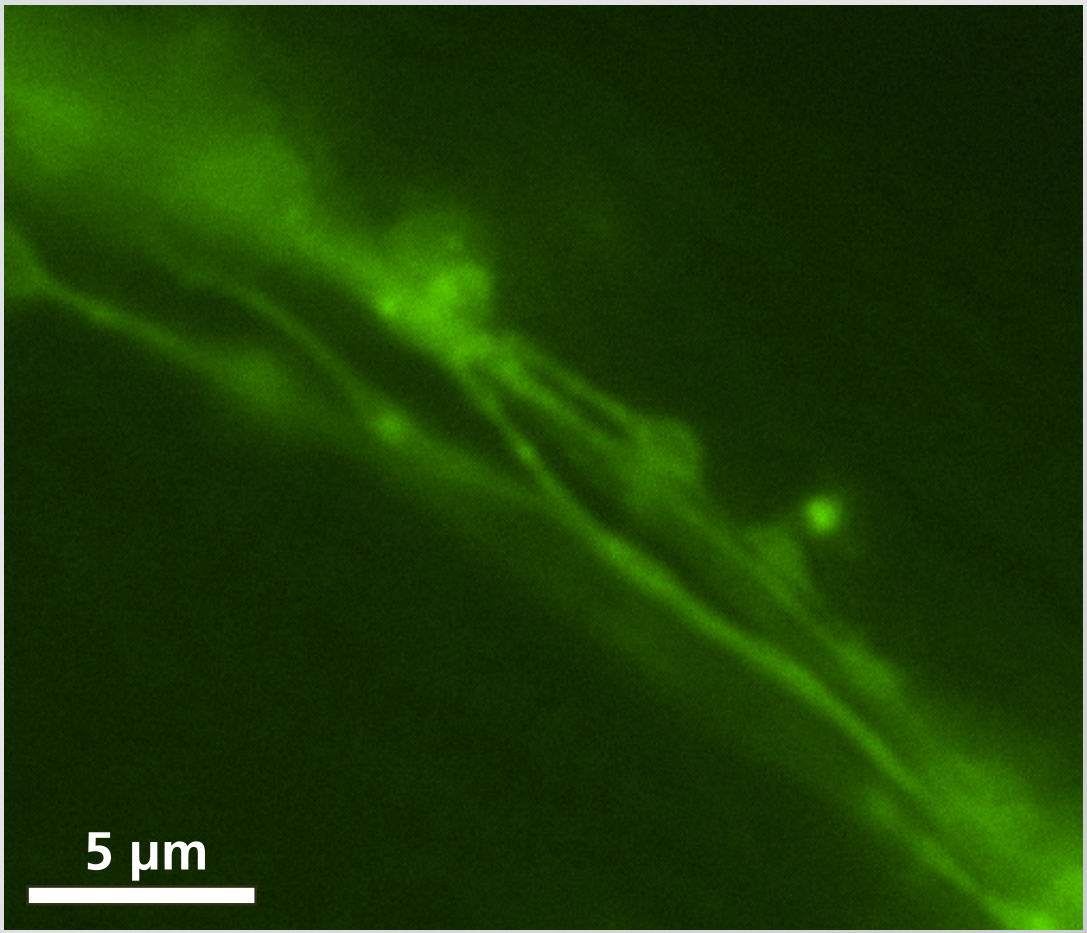
Nanoneurosurgery

escape rate after ASH-ablation surgery



Nanoneurosurgery

cut single dendrite in amphid bundle



Nanoneurosurgery

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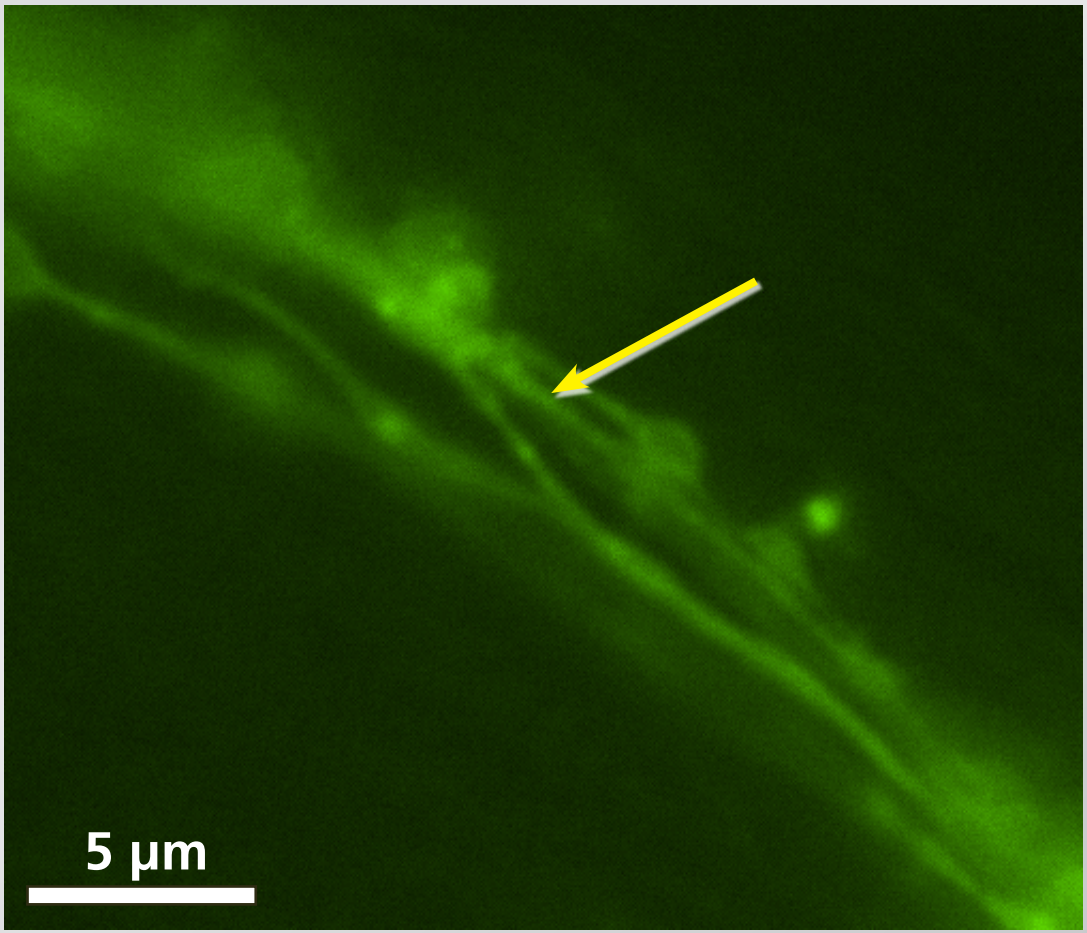
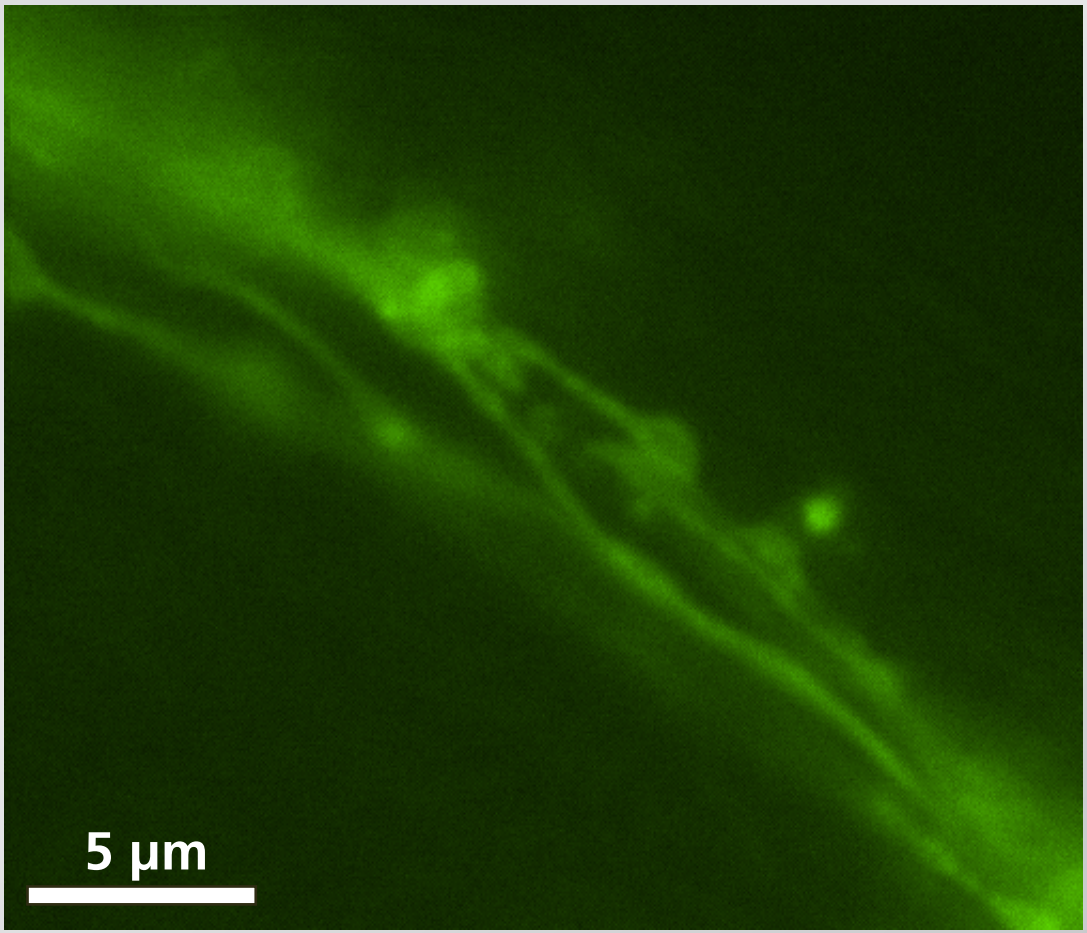


Nanoneurosurgery

cut single dendrite in amphid bundle

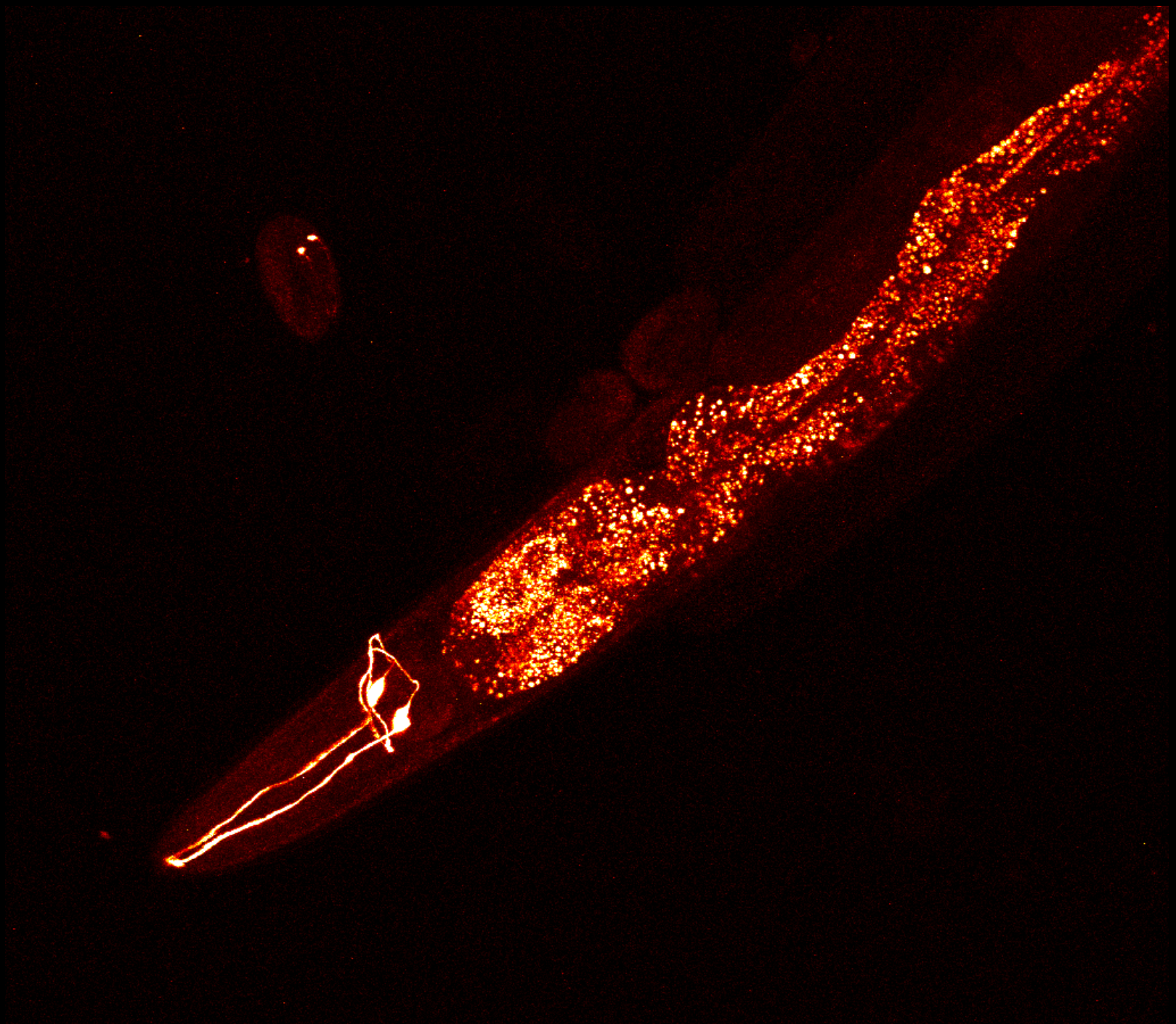


Nanoneurosurgery

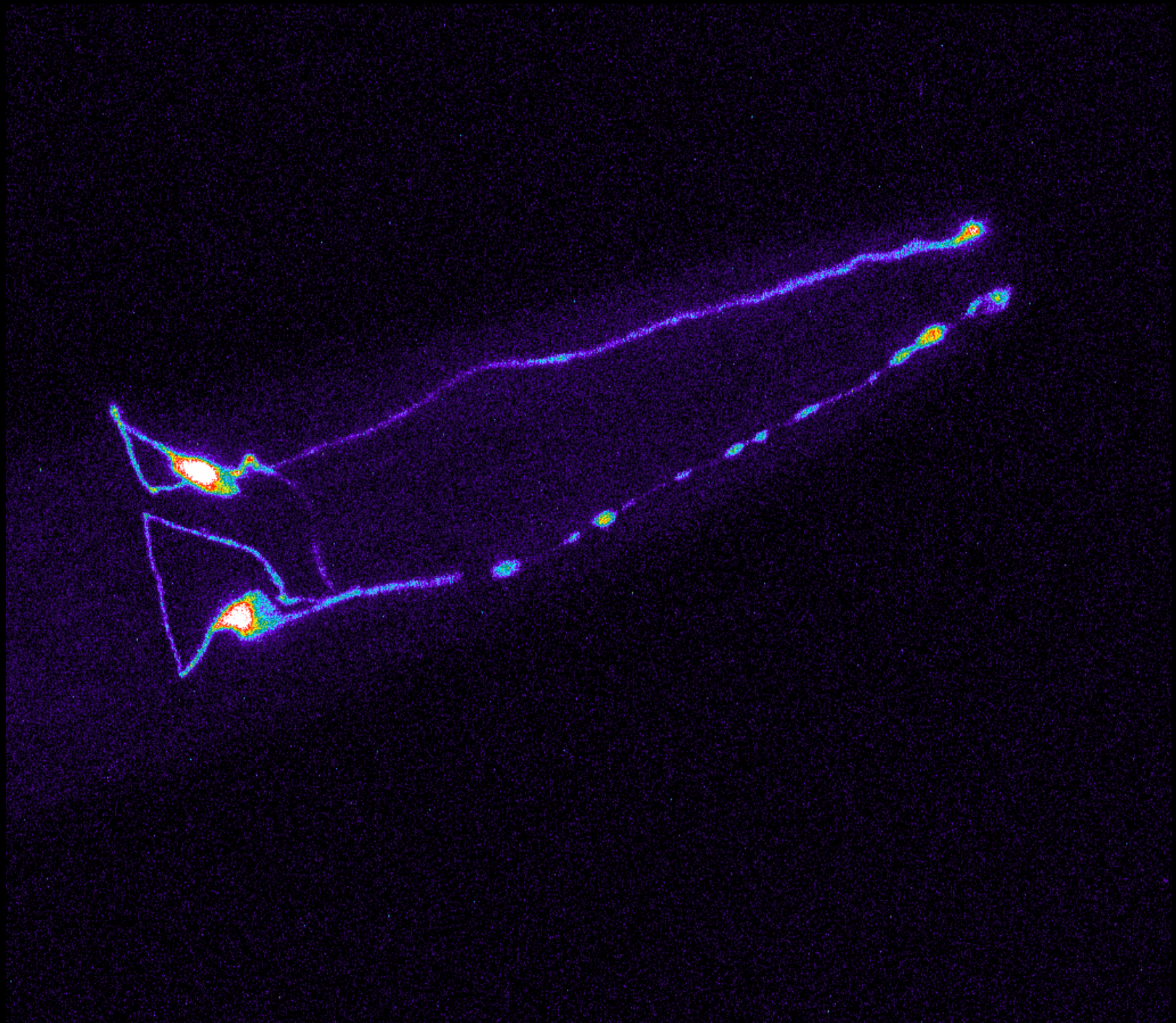
ASD neurons

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

Nanoneurosurgery

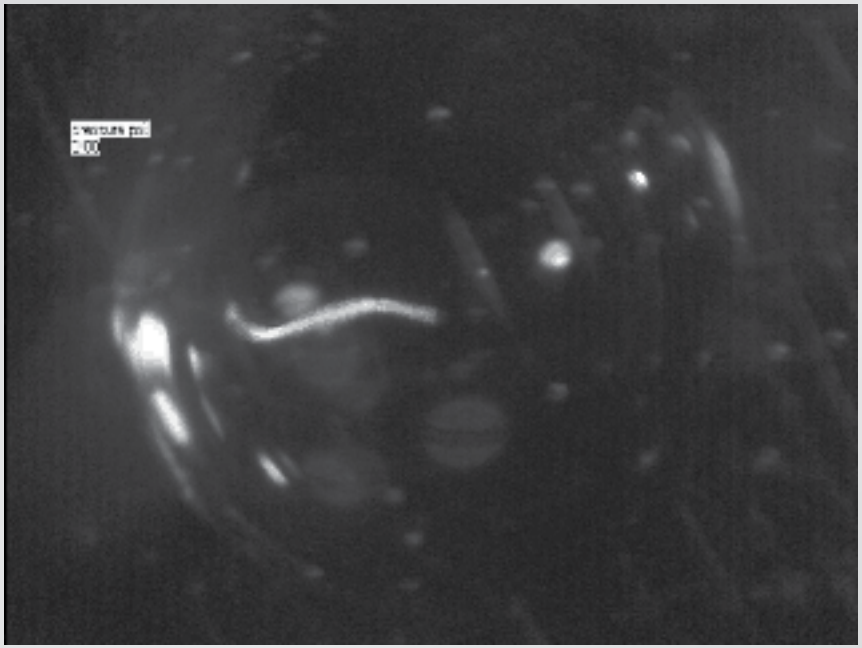


Nanoneurosurgery

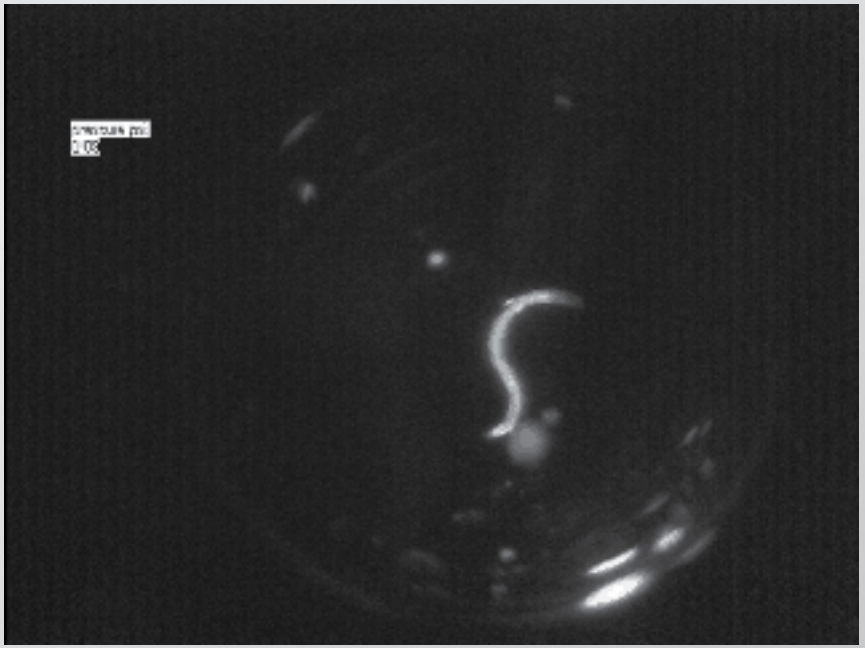


Nanoneurosurgery

surgery results in quantifiable behavior changes



before



after

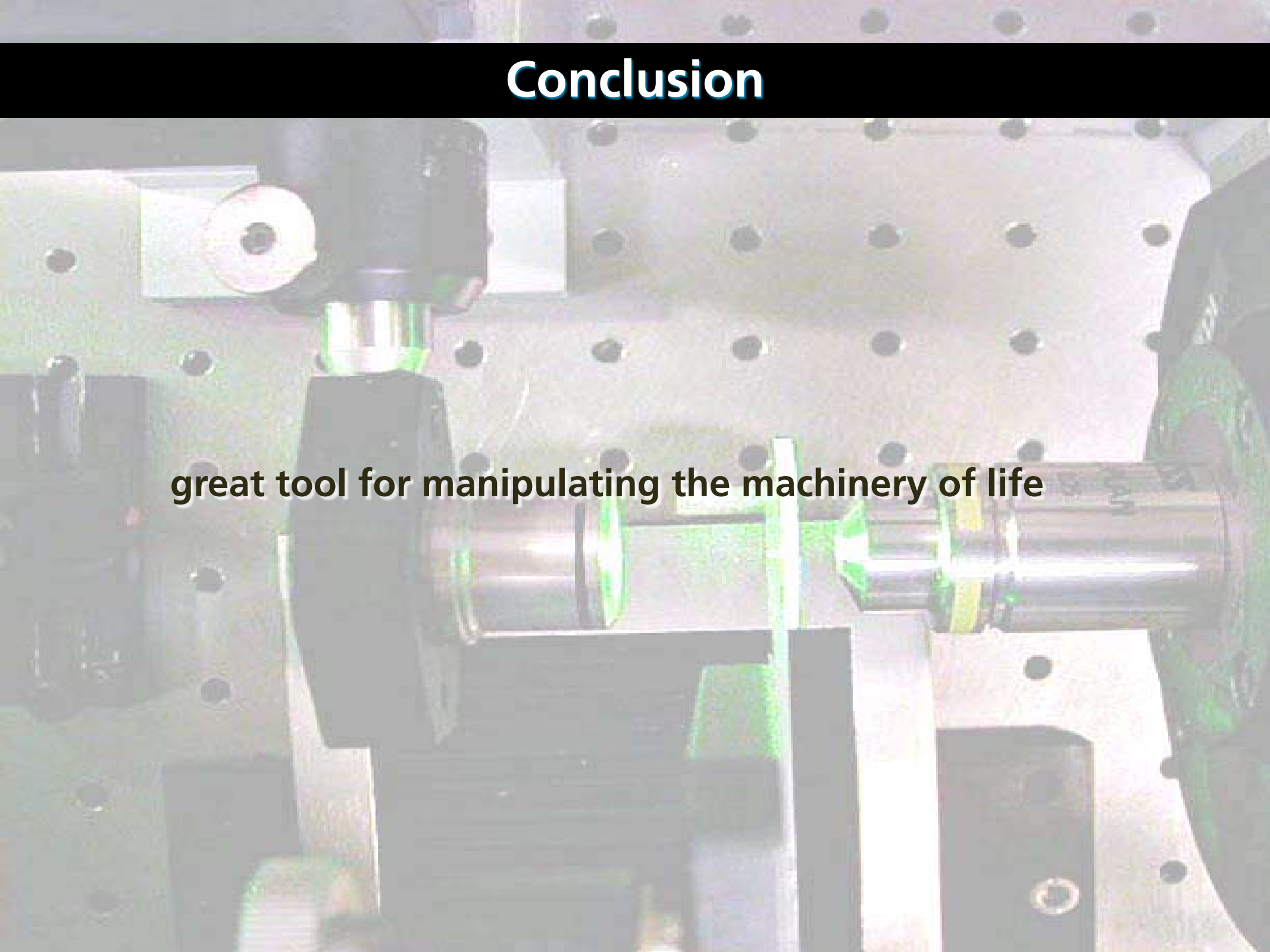
Nanoneurosurgery

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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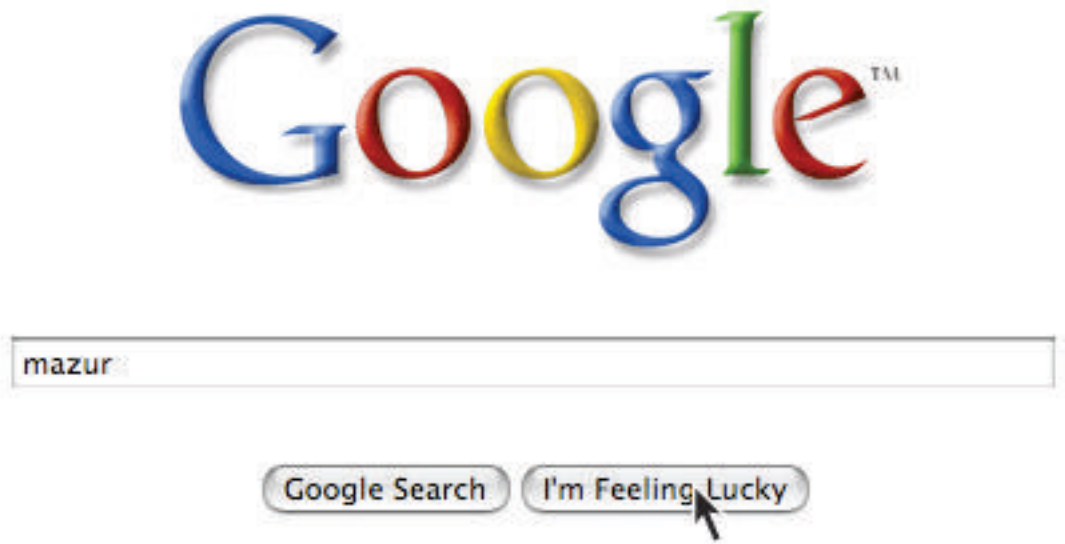
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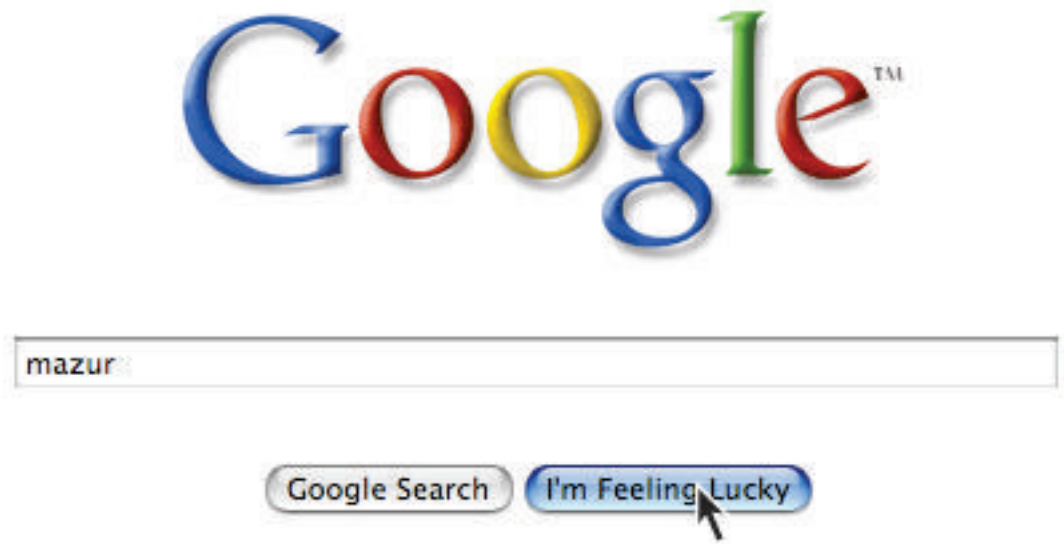
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