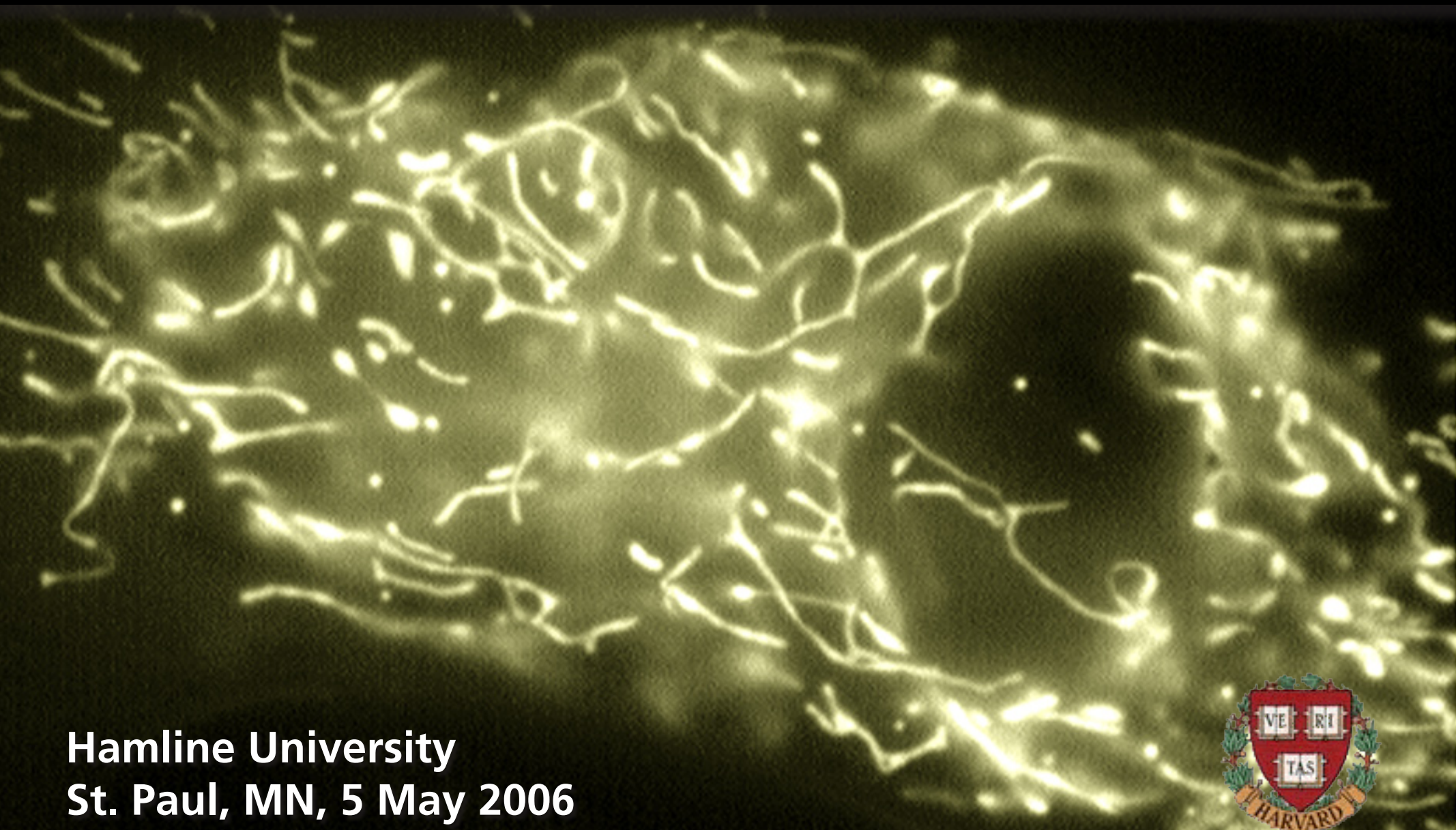


Subcellular surgery and nanoneurosurgery



Hamline University
St. Paul, MN, 5 May 2006





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)

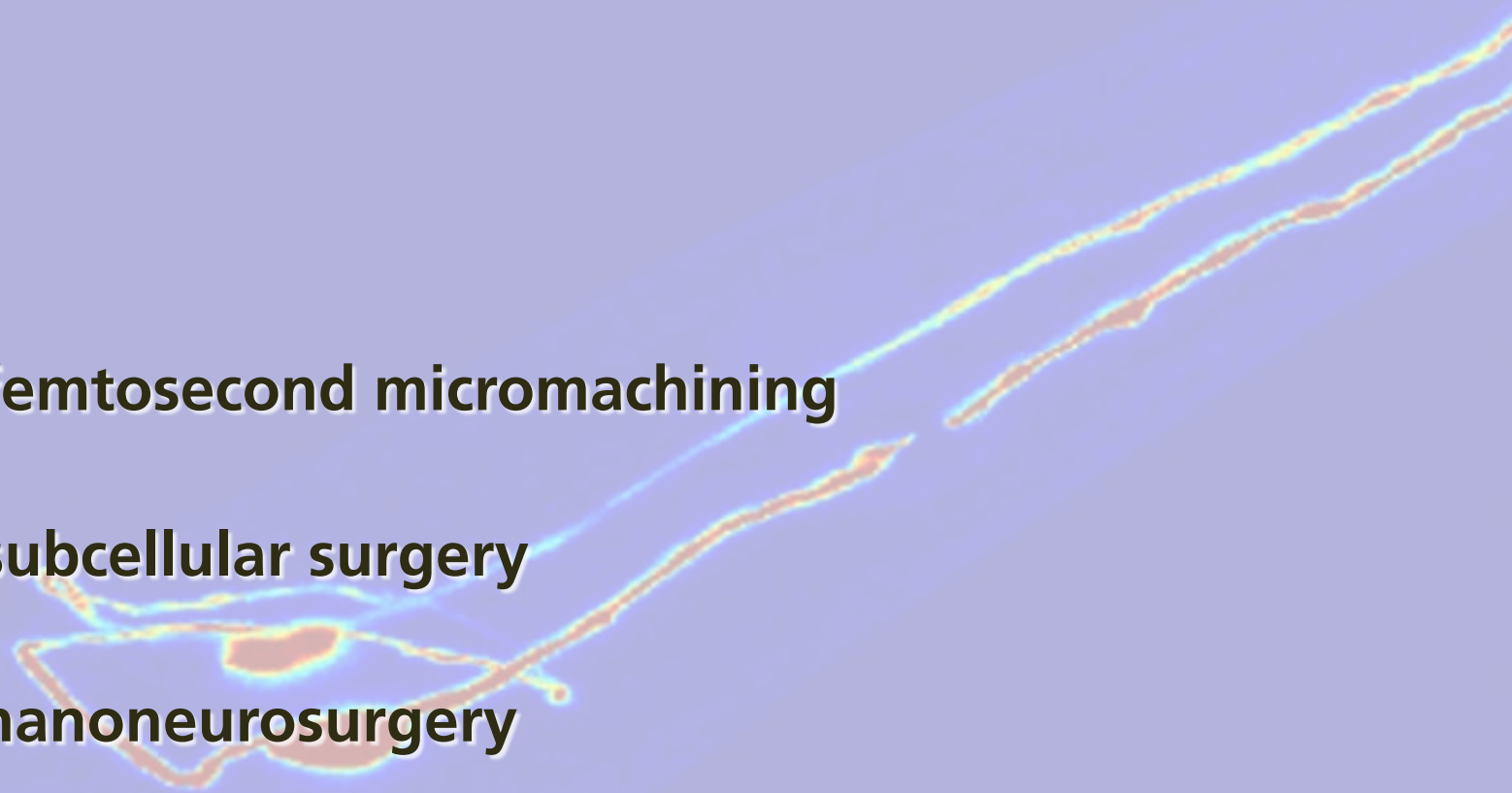
Prof. Jeffrey Nickerson (UMass Worcester)

Introduction

living systems require interdisciplinary tools

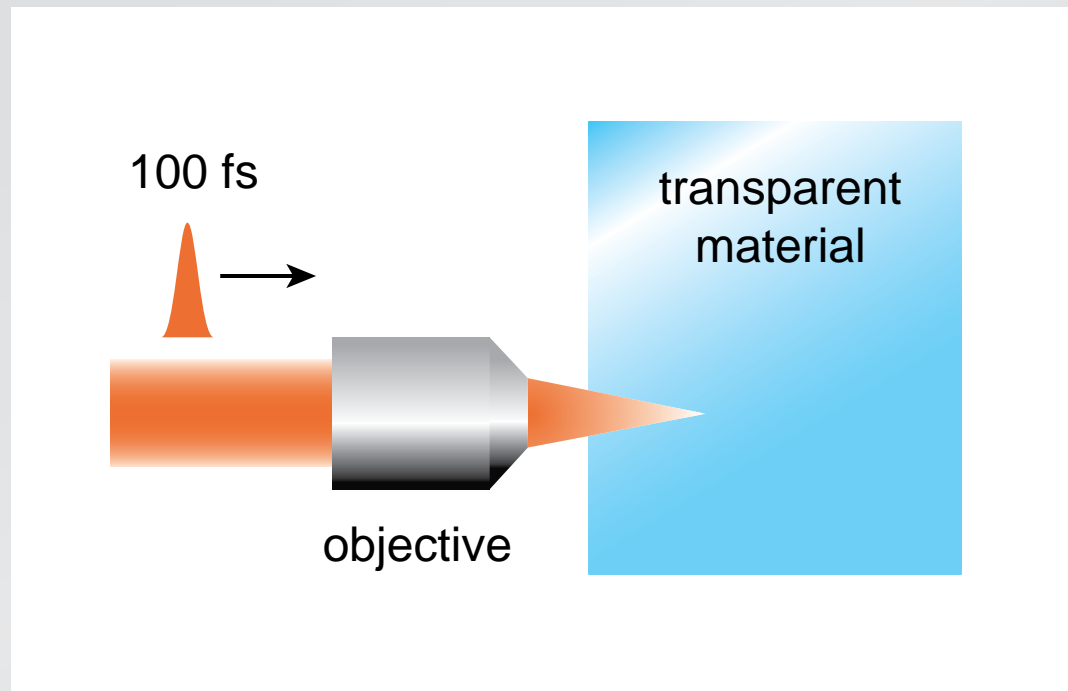
A microscopic image showing several red, spherical cells with a textured surface. The cells are arranged in a cluster, with some overlapping. Small white specks are scattered across the surface of the cells. The background is black.

Outline

- femtosecond micromachining
 - subcellular surgery
 - nanoneurosurgery
- 

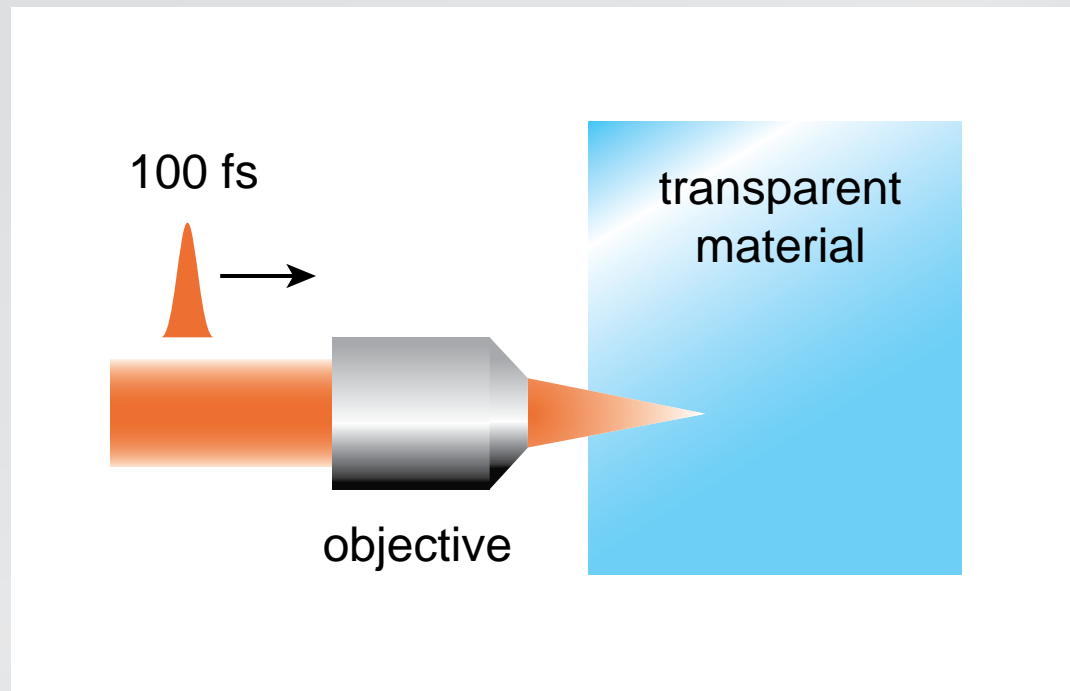
Femtosecond micromachining

focus laser beam inside material



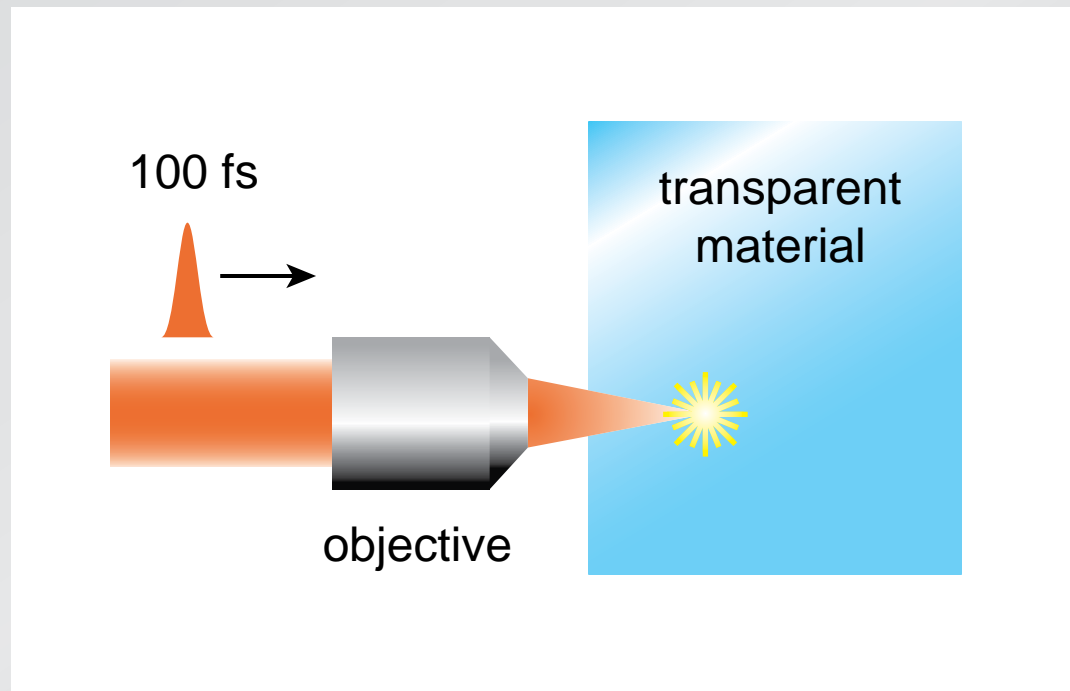
Femtosecond micromachining

high intensity at focus...



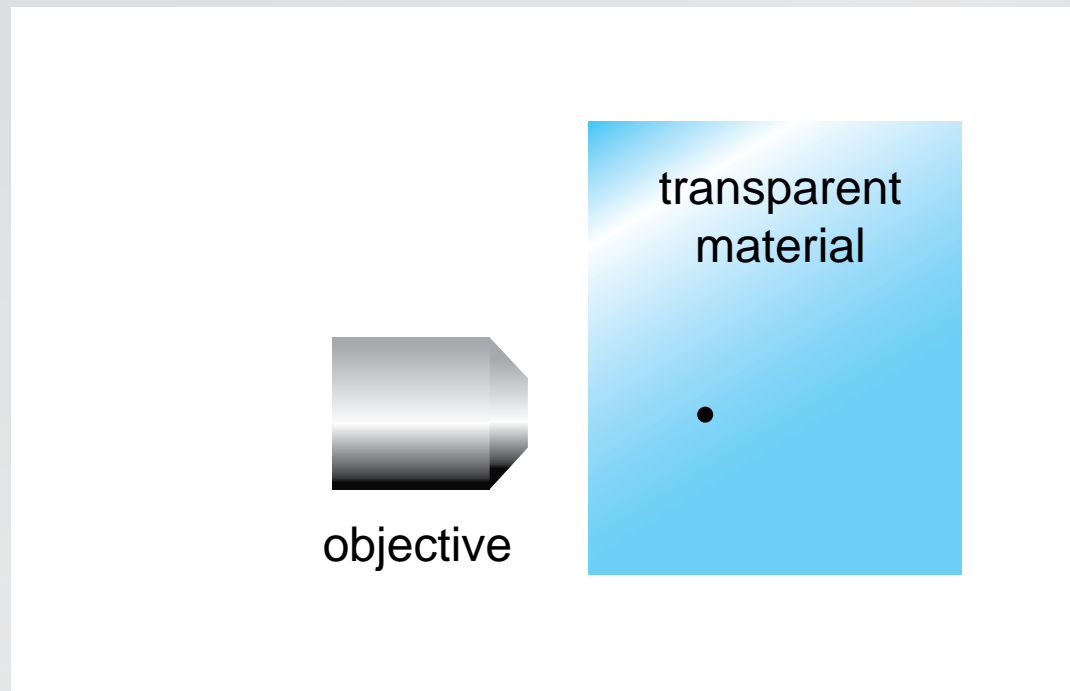
Femtosecond micromachining

...causes nonlinear ionization...

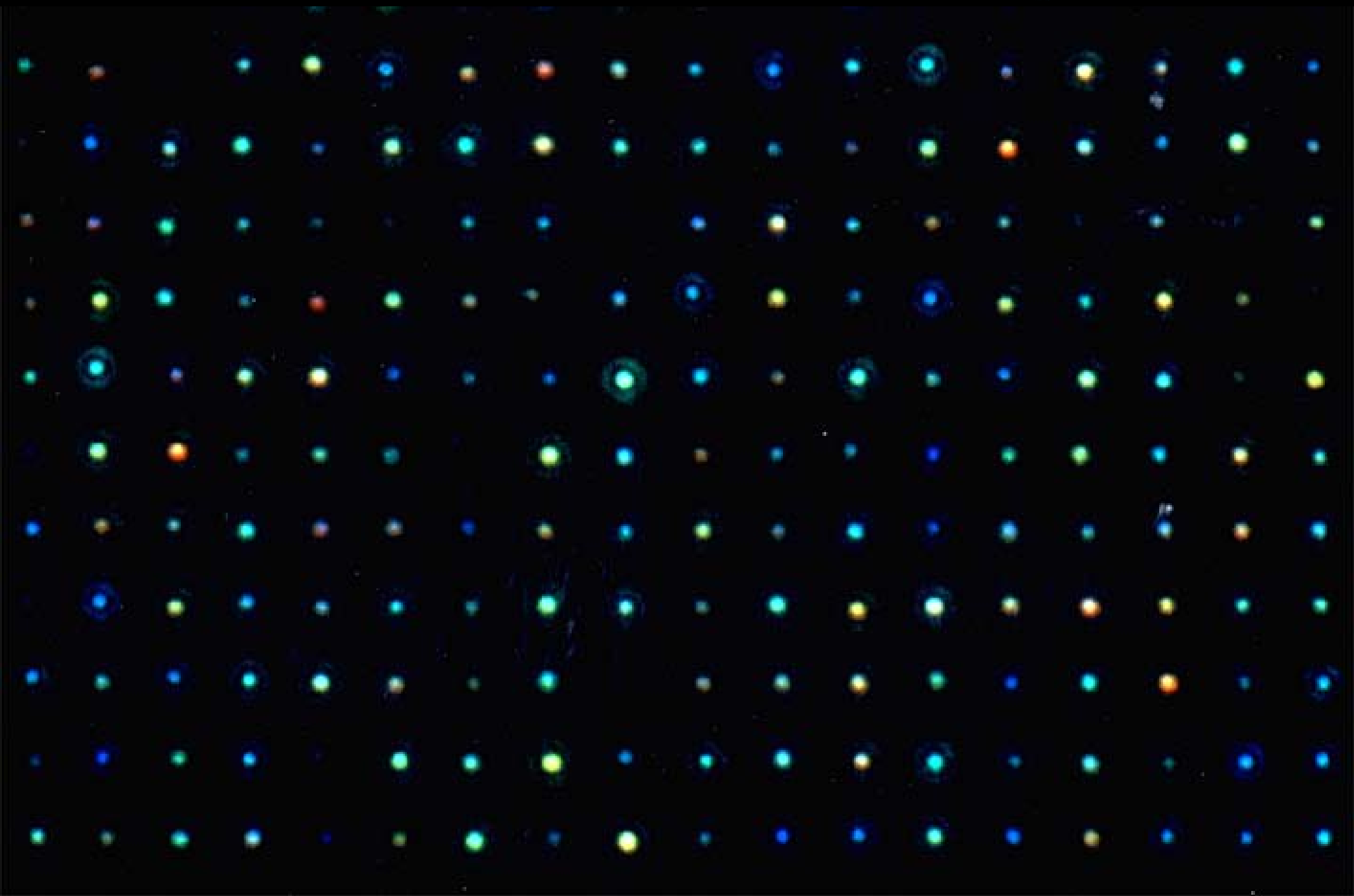


Femtosecond micromachining

and 'microexplosion' causes microscopic damage...



Femtosecond micromachining

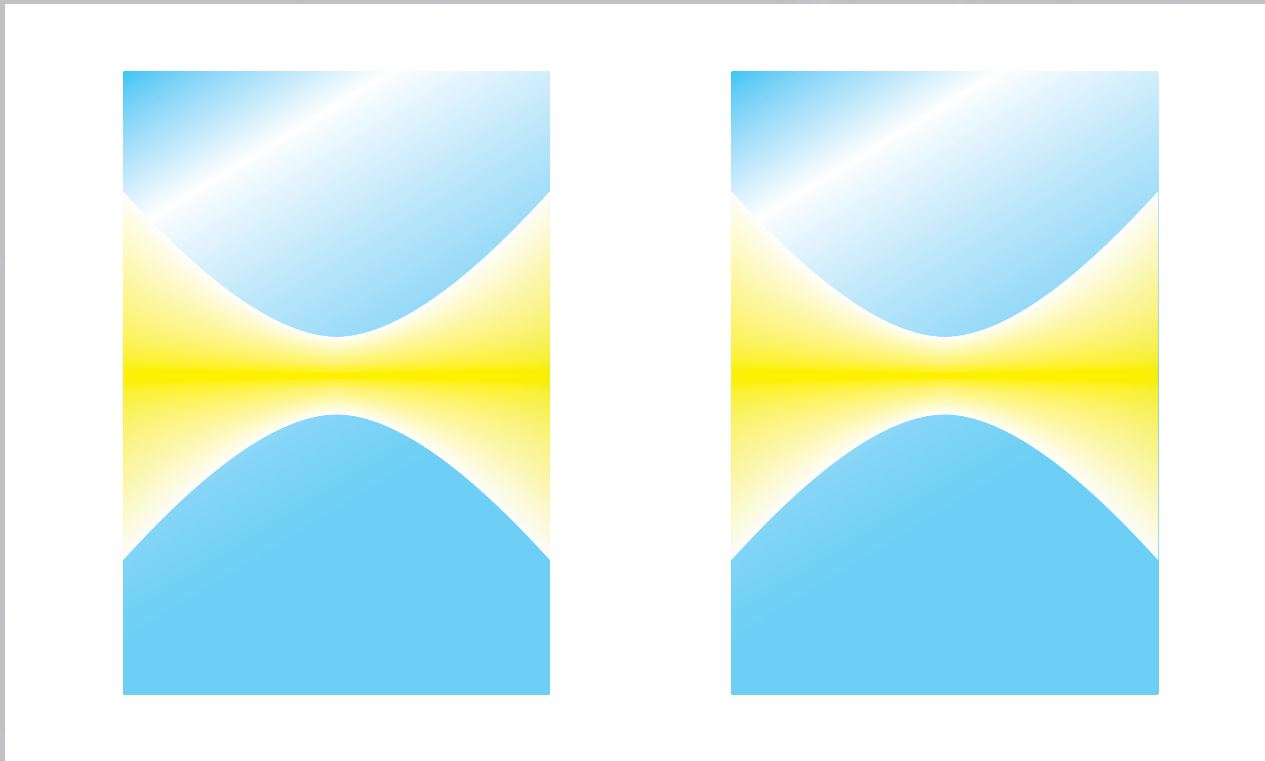


Femtosecond micromachining

photon energy $<$ bandgap \longrightarrow nonlinear interaction

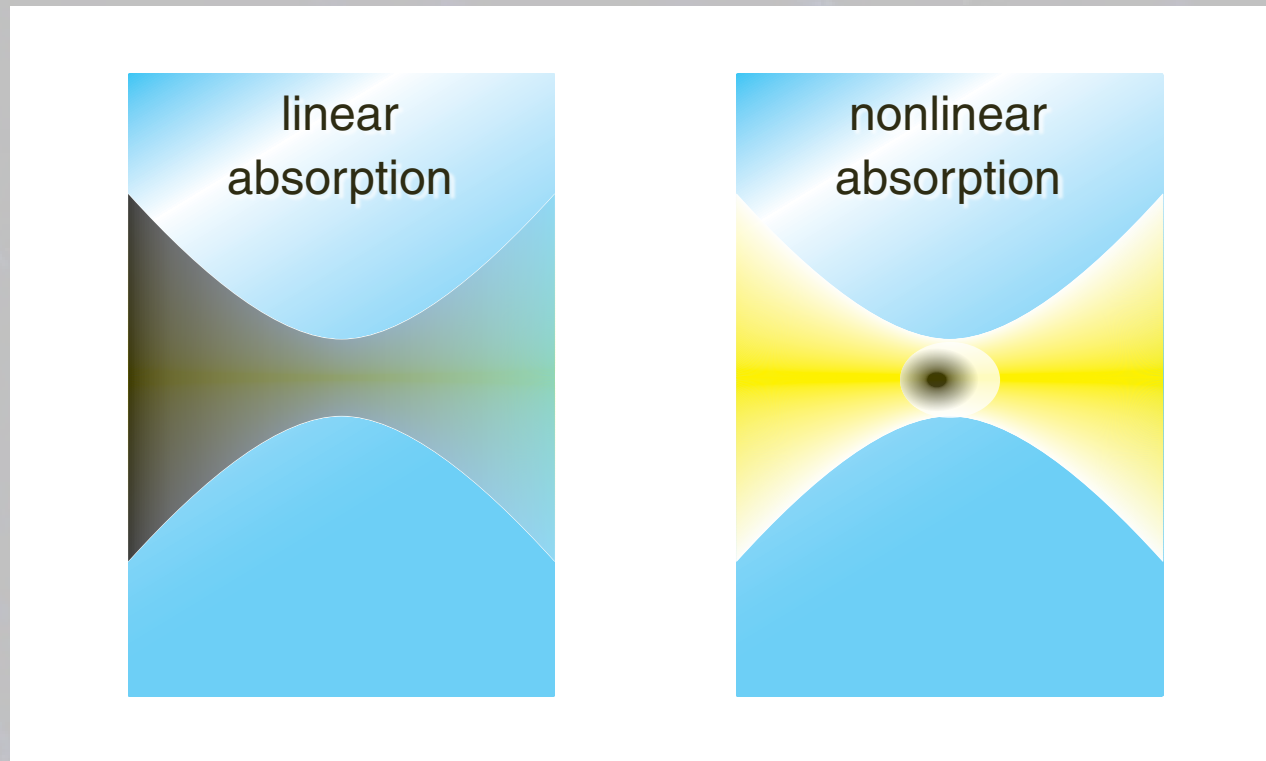
Femtosecond micromachining

nonlinear interaction provides bulk confinement

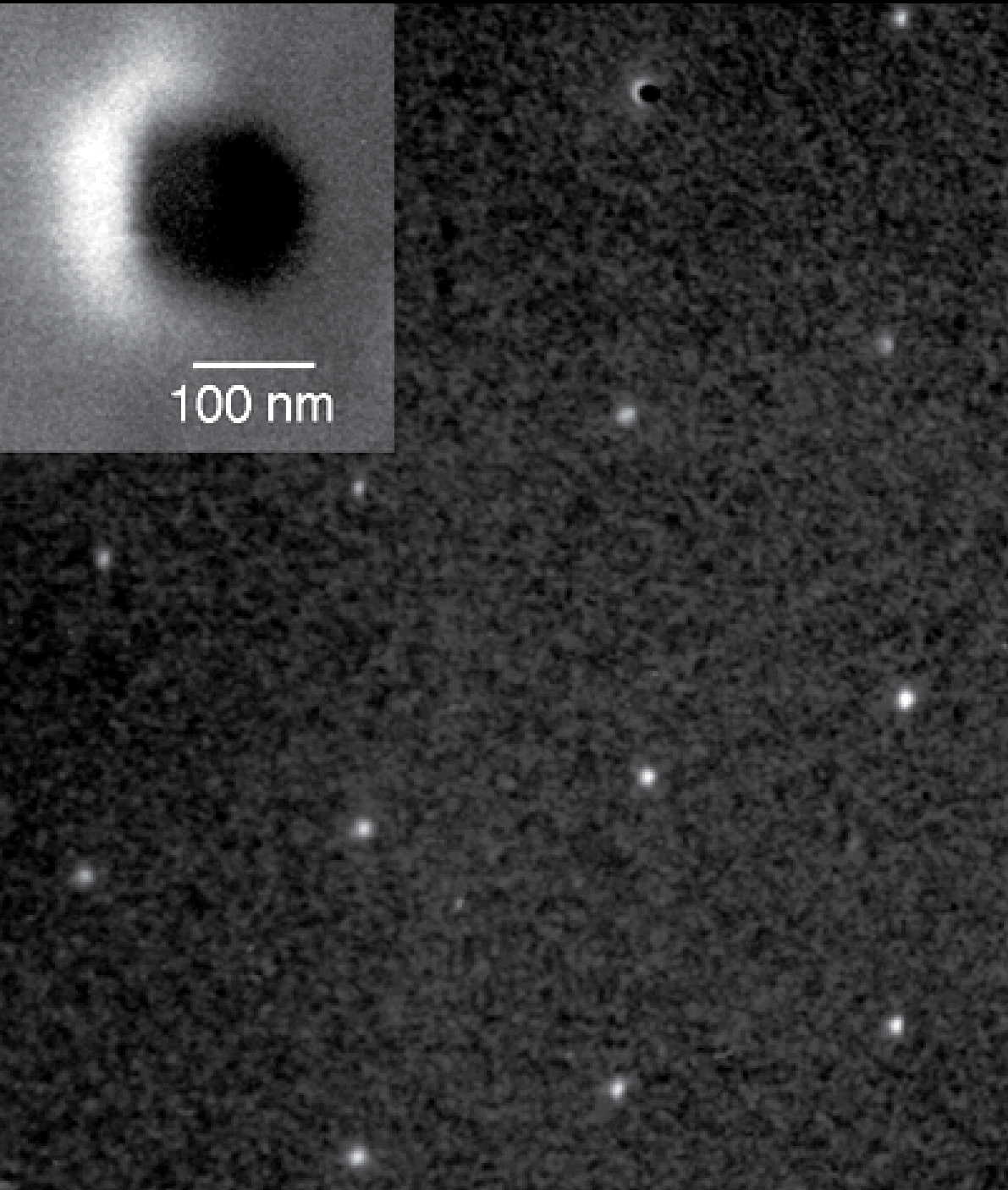


Femtosecond micromachining

nonlinear interaction provides bulk confinement



Femtosecond micromachining

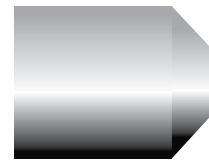


SEM & AFM:

- 100-nm cavities
- little colateral damage

Femtosecond micromachining

Dark-field scattering



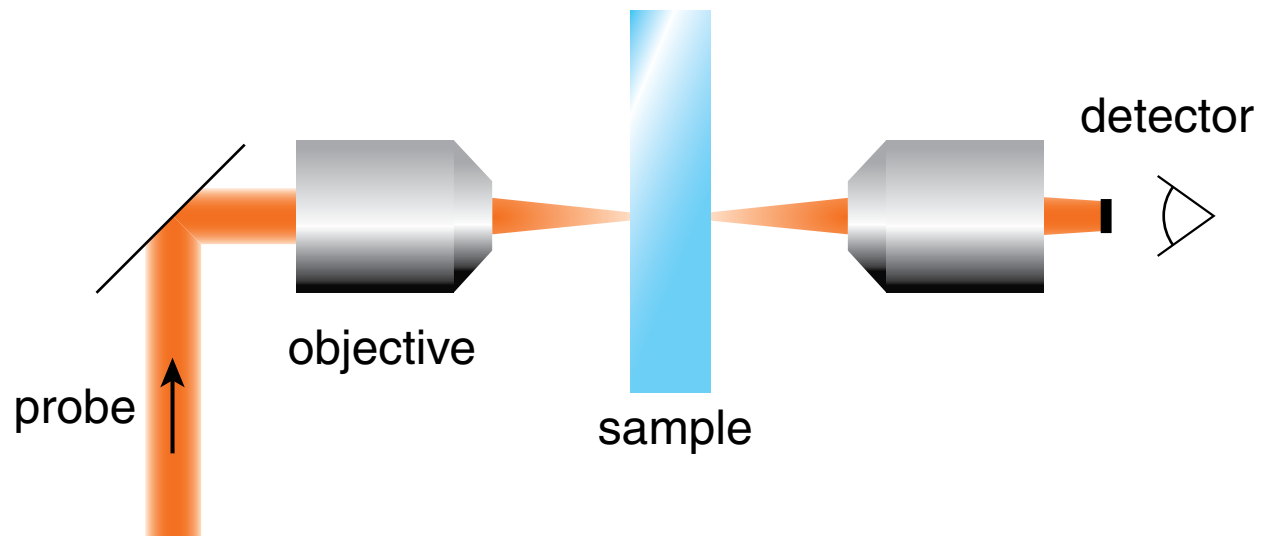
objective



sample

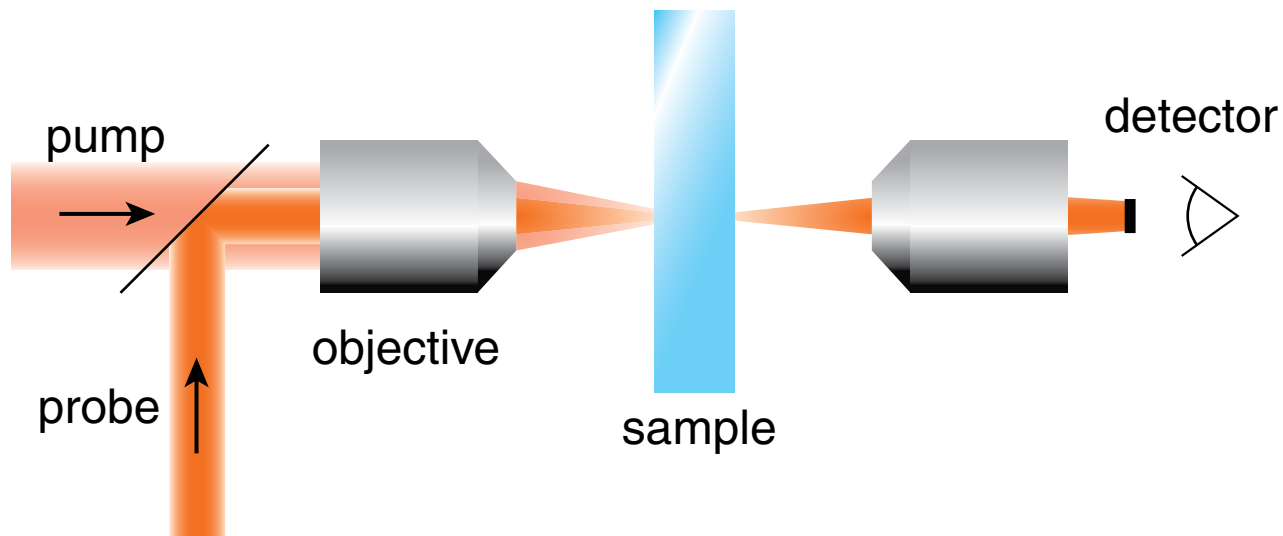
Femtosecond micromachining

block probe beam...



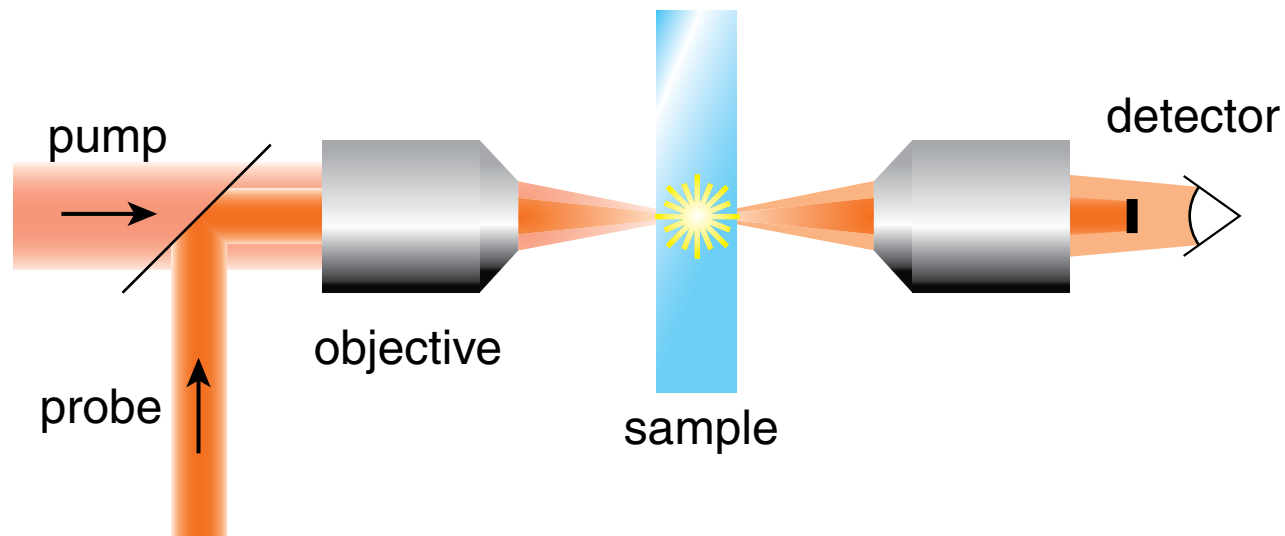
Femtosecond micromachining

... bring in pump beam...



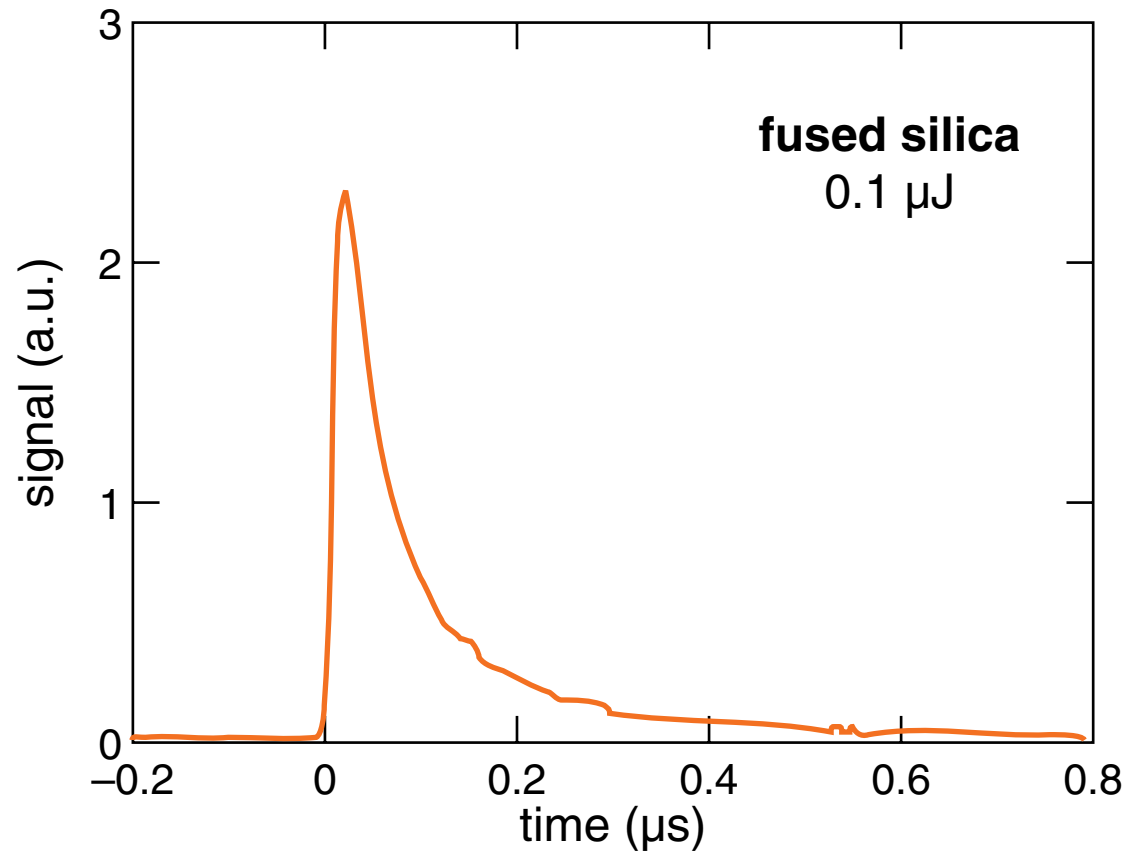
Femtosecond micromachining

... damage scatters probe beam



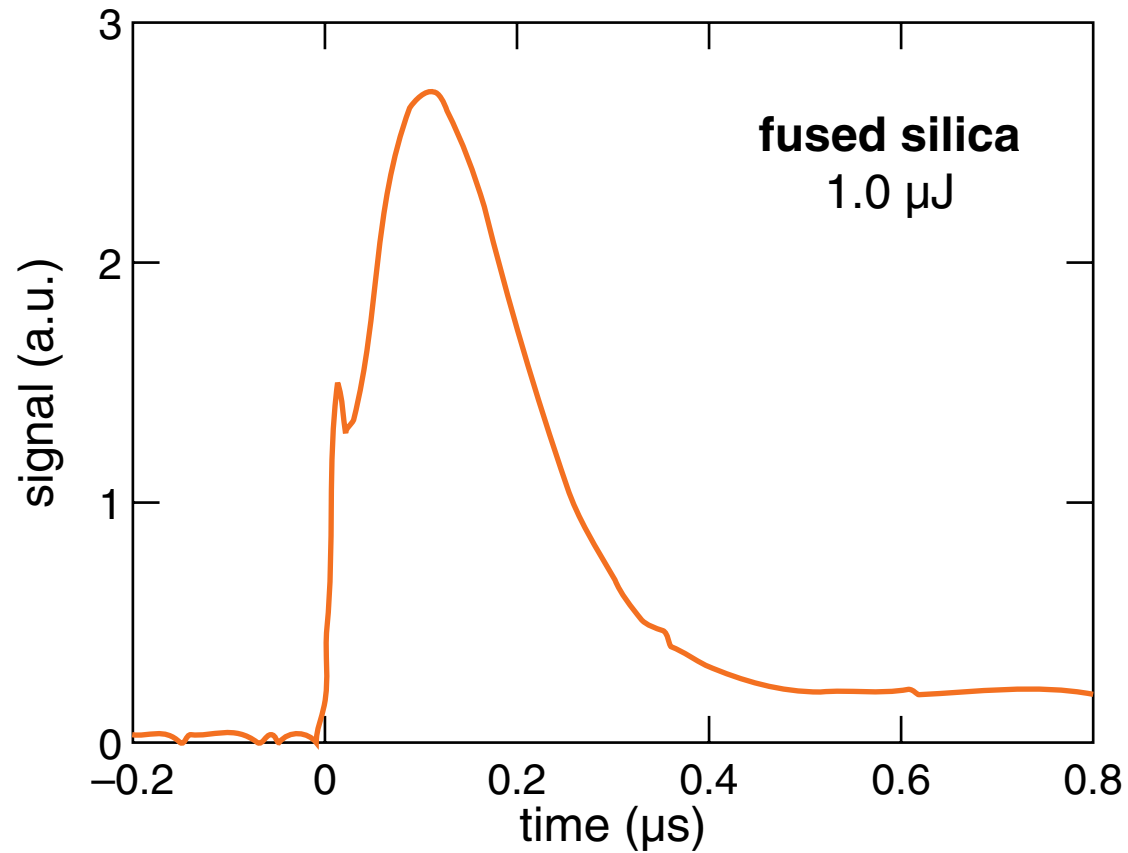
Femtosecond micromachining

scattered signal



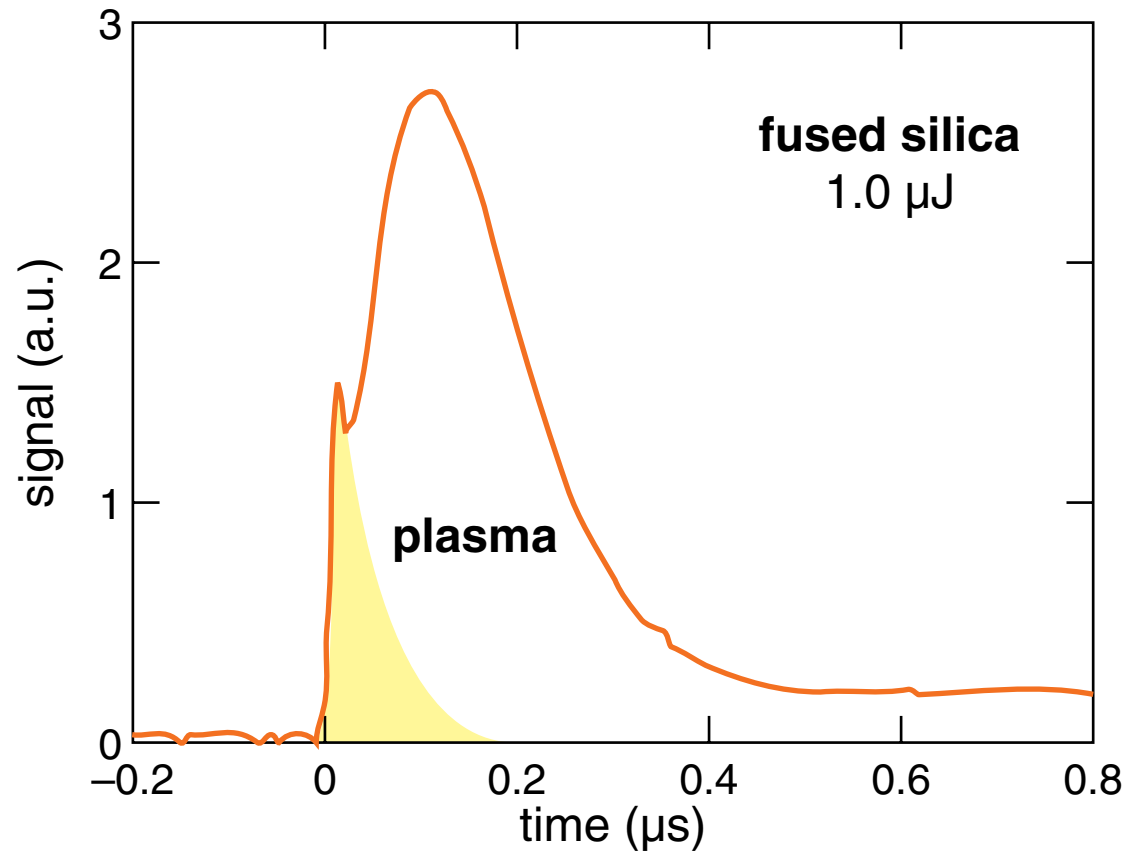
Femtosecond micromachining

scattered signal



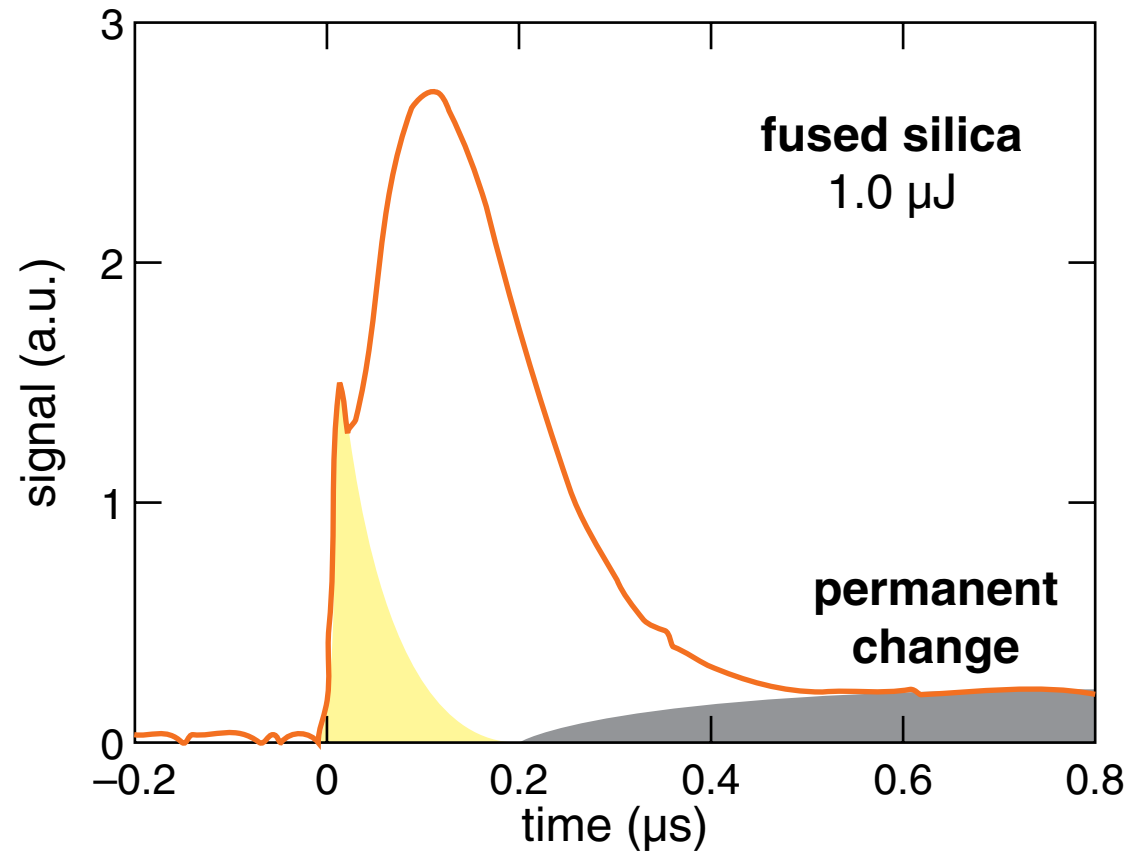
Femtosecond micromachining

scattered signal



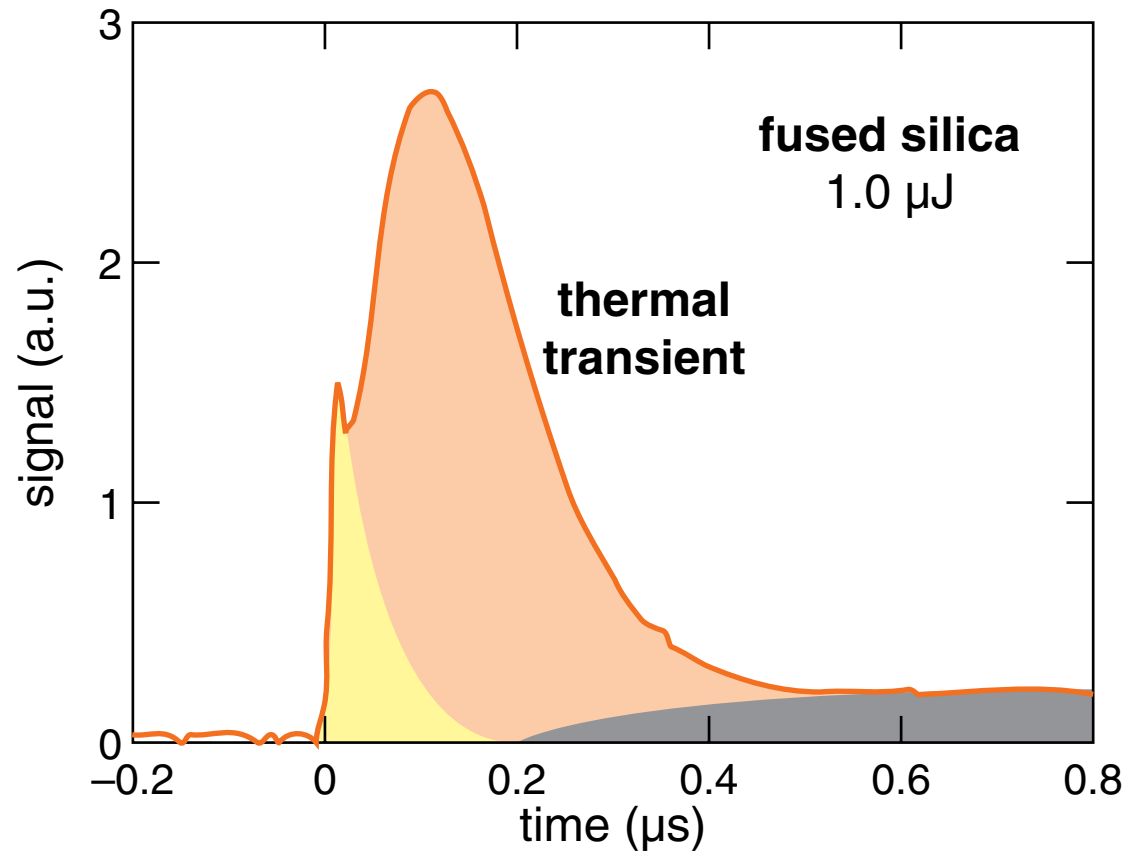
Femtosecond micromachining

scattered signal



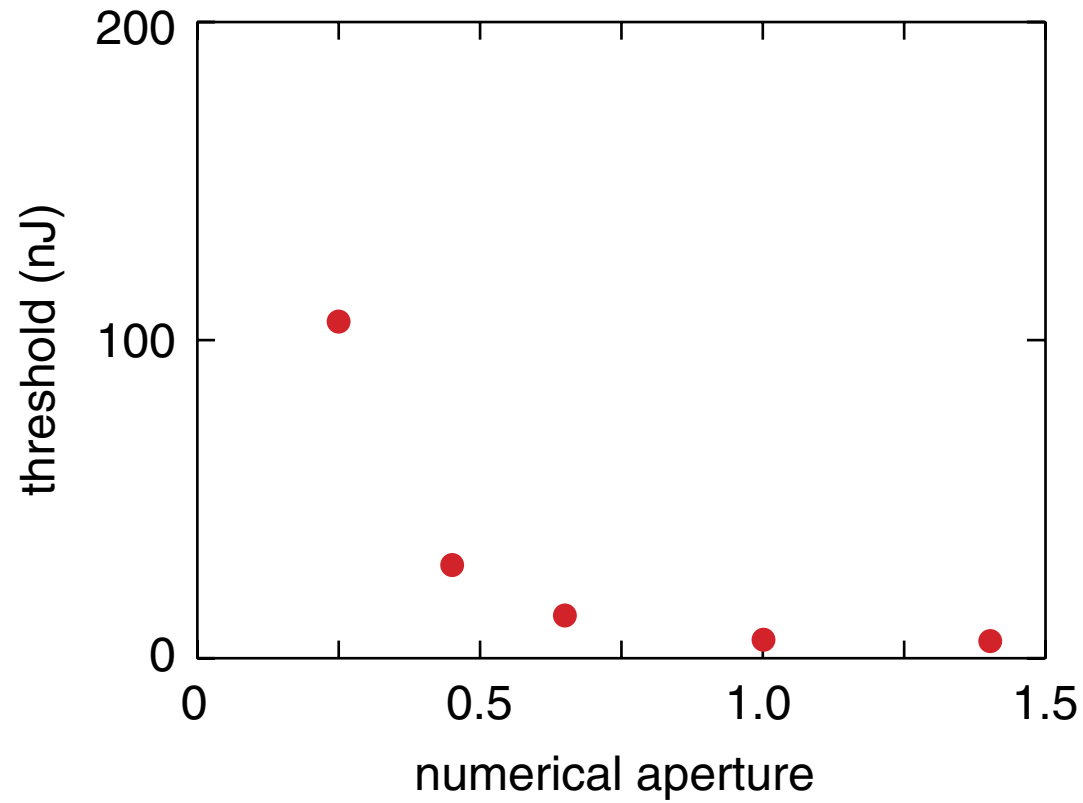
Femtosecond micromachining

scattered signal



Femtosecond micromachining

vary numerical aperture



Femtosecond micromachining

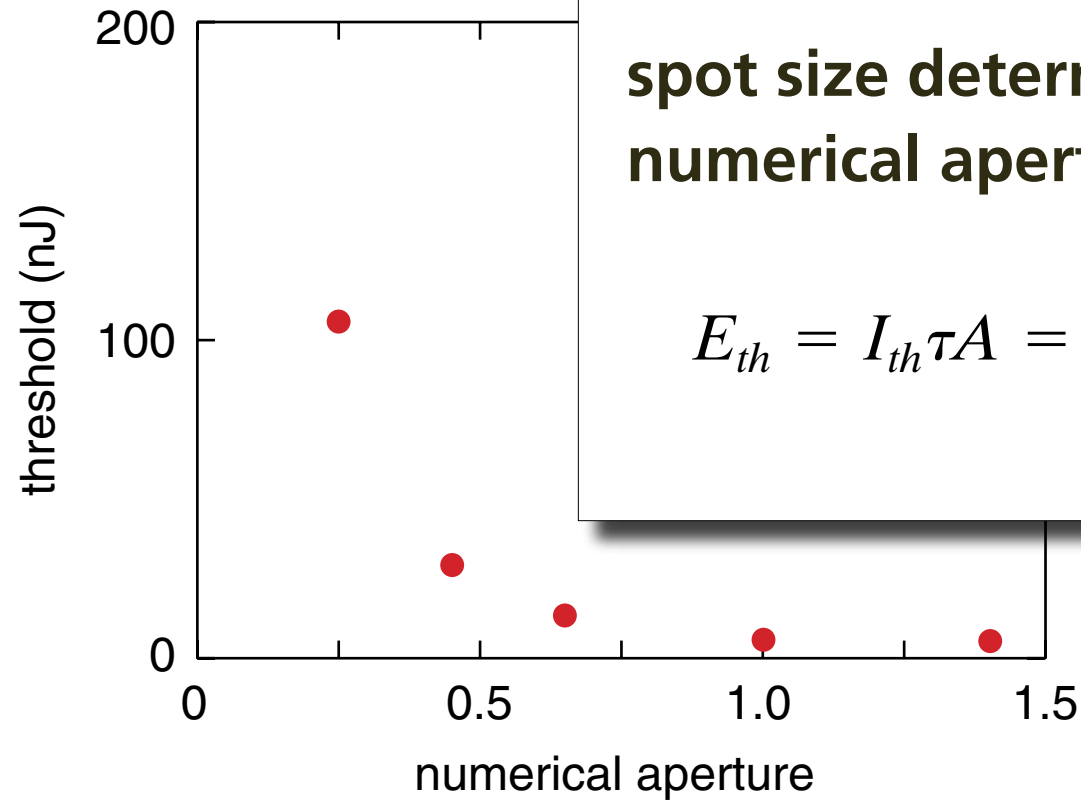
vary numerical

intensity threshold:

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

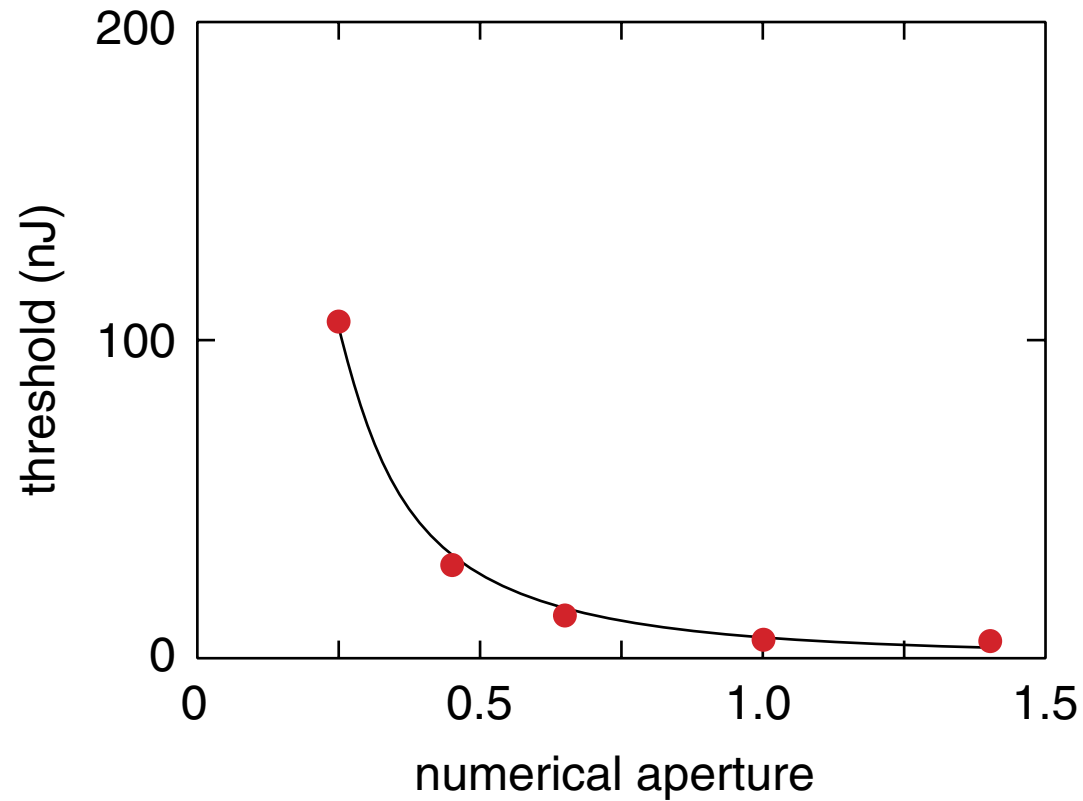
spot size determined by
numerical aperture:

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



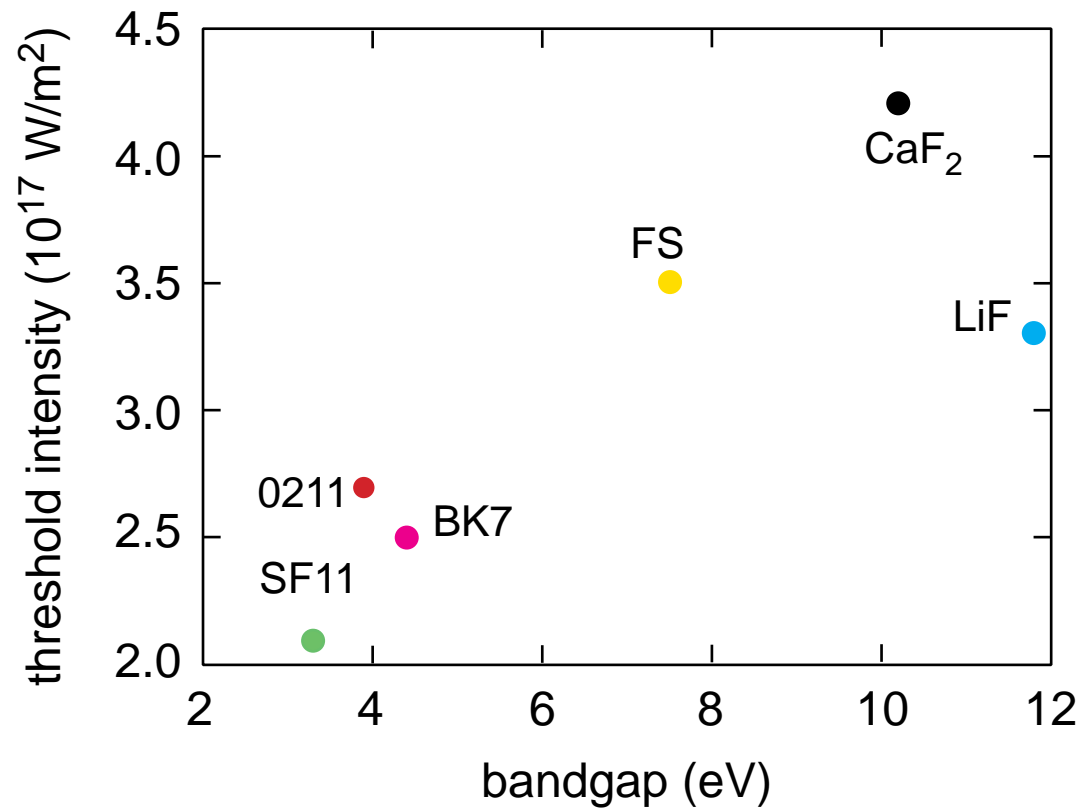
Femtosecond micromachining

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



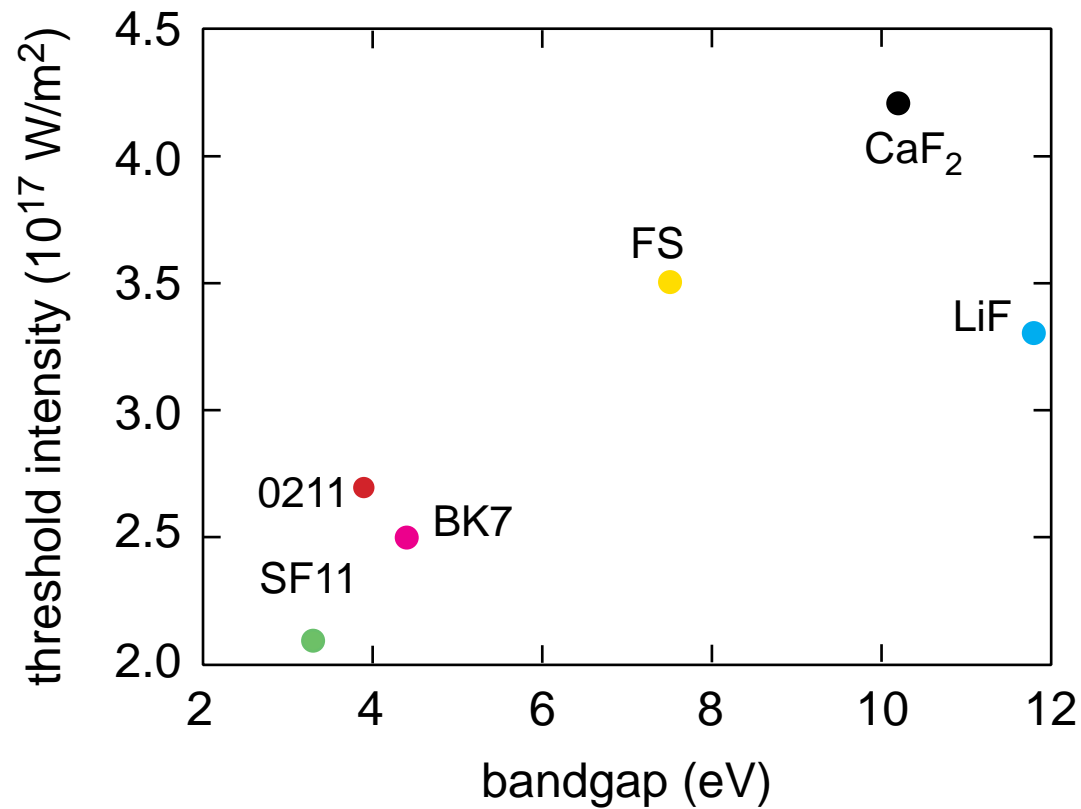
Femtosecond micromachining

vary material...



Femtosecond micromachining

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

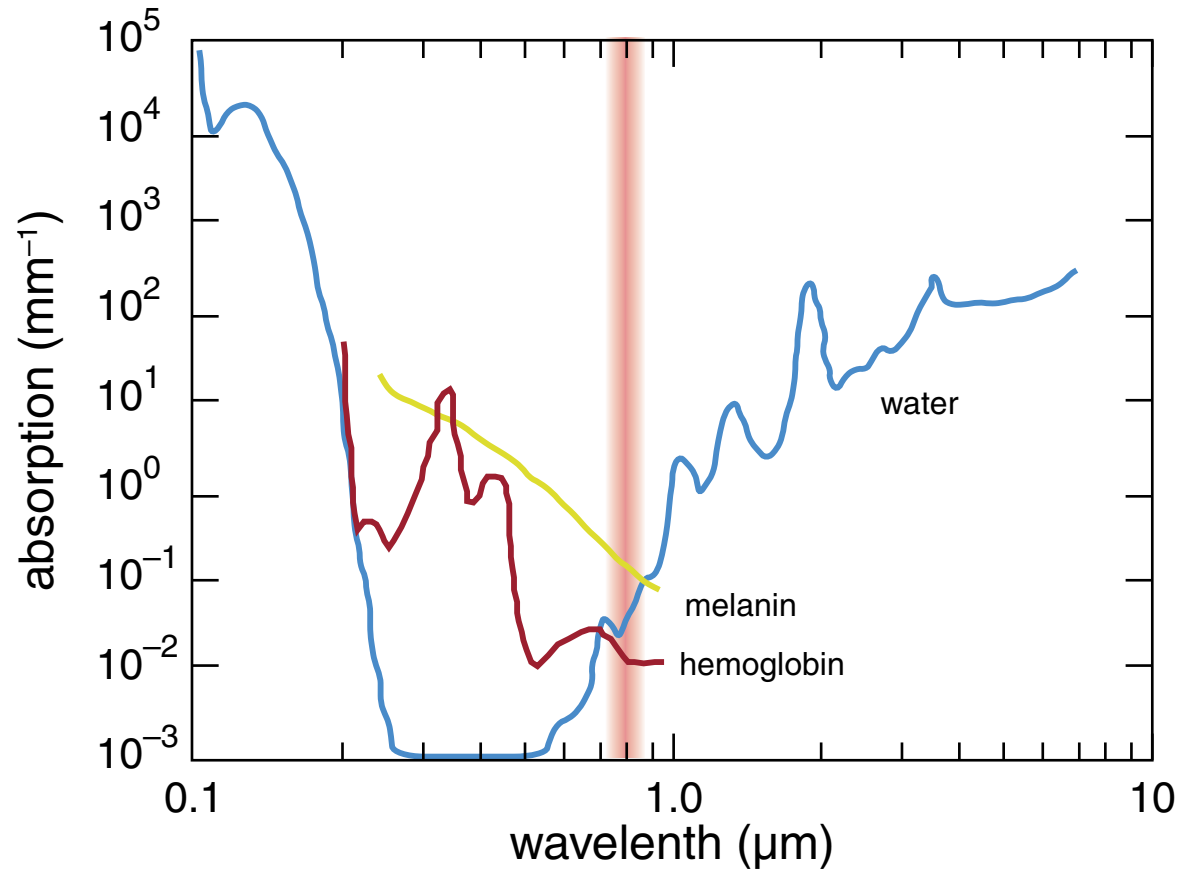


Femtosecond micromachining

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

Femtosecond micromachining

tissue is nearly transparent at 800 nm



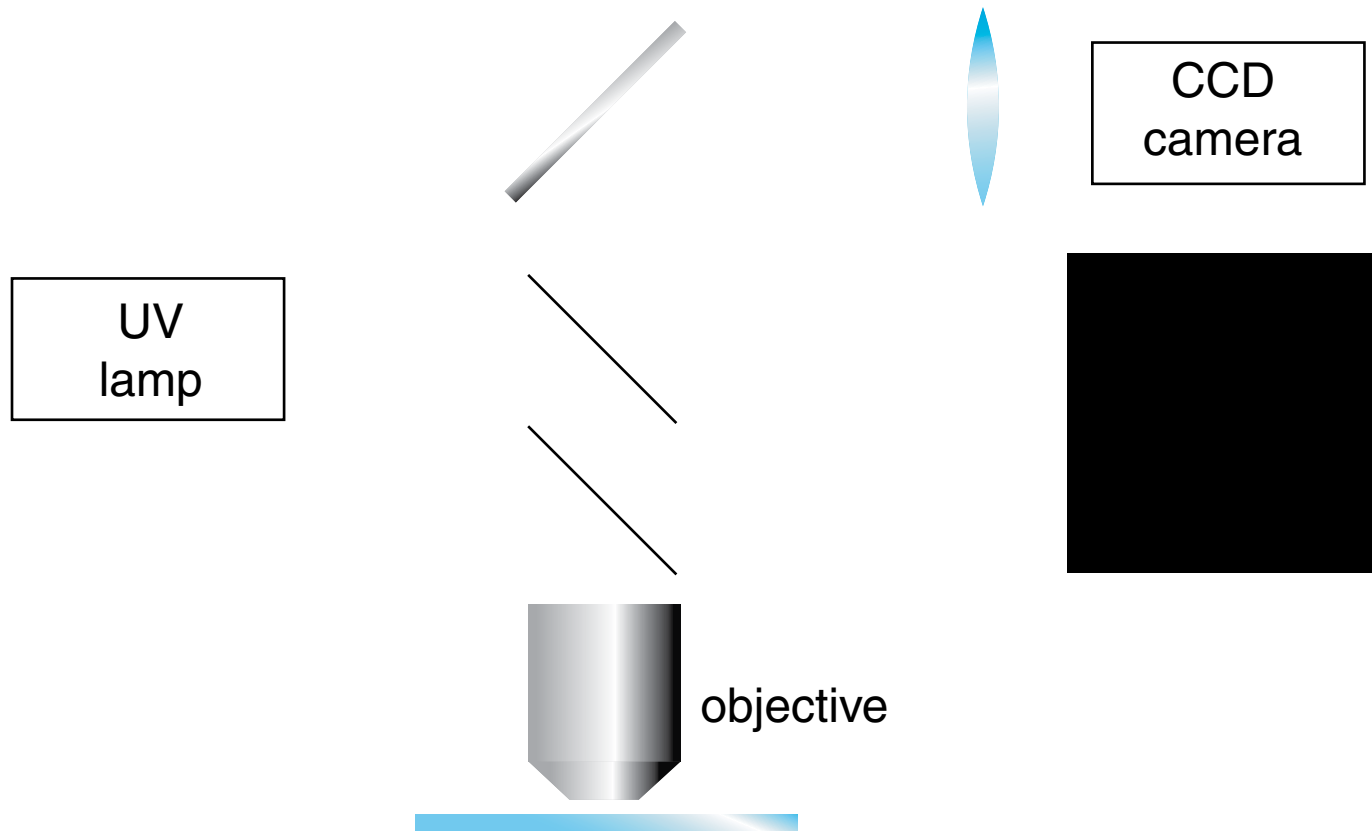
Outline

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

Subcellular surgery

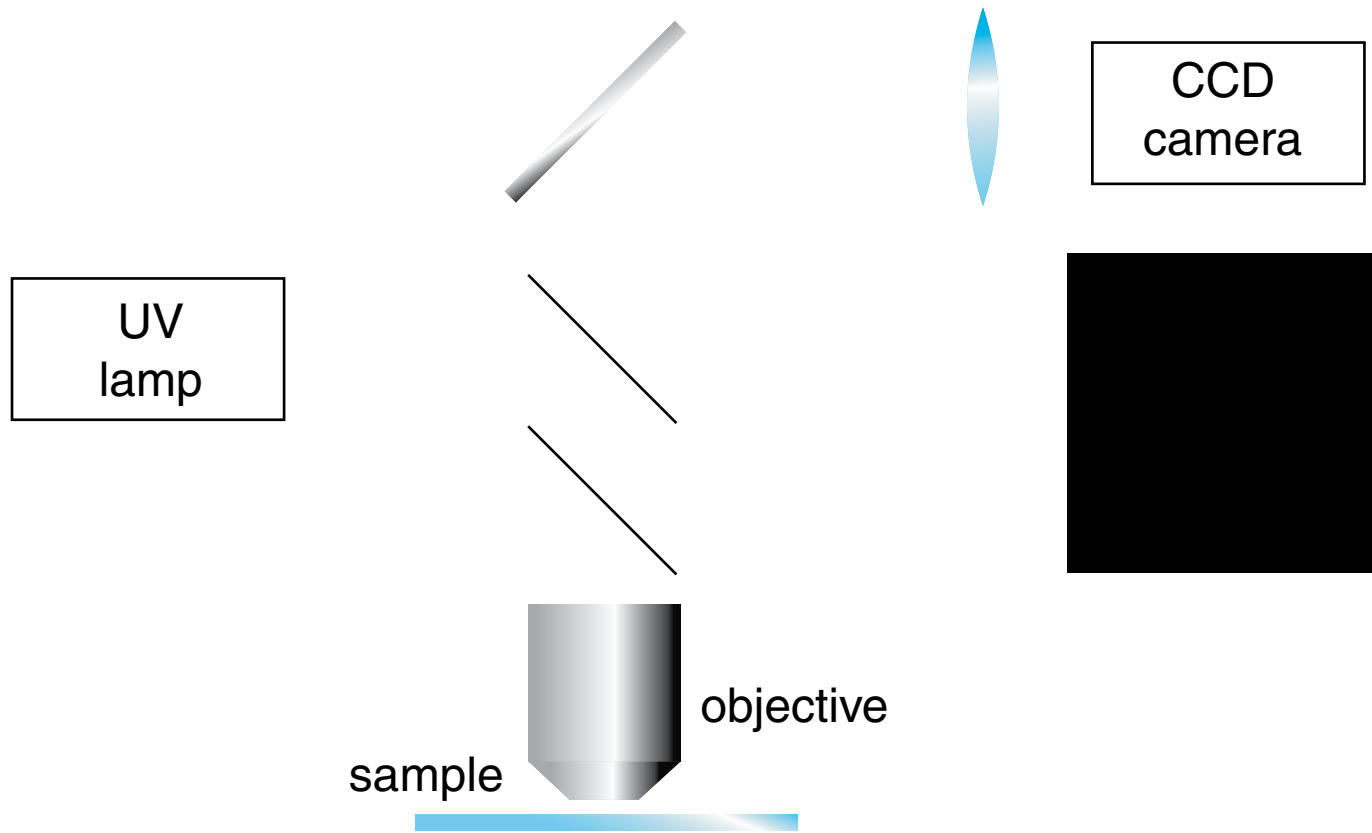
Q: can we ablate material on the subcellular scale?

Subcellular surgery



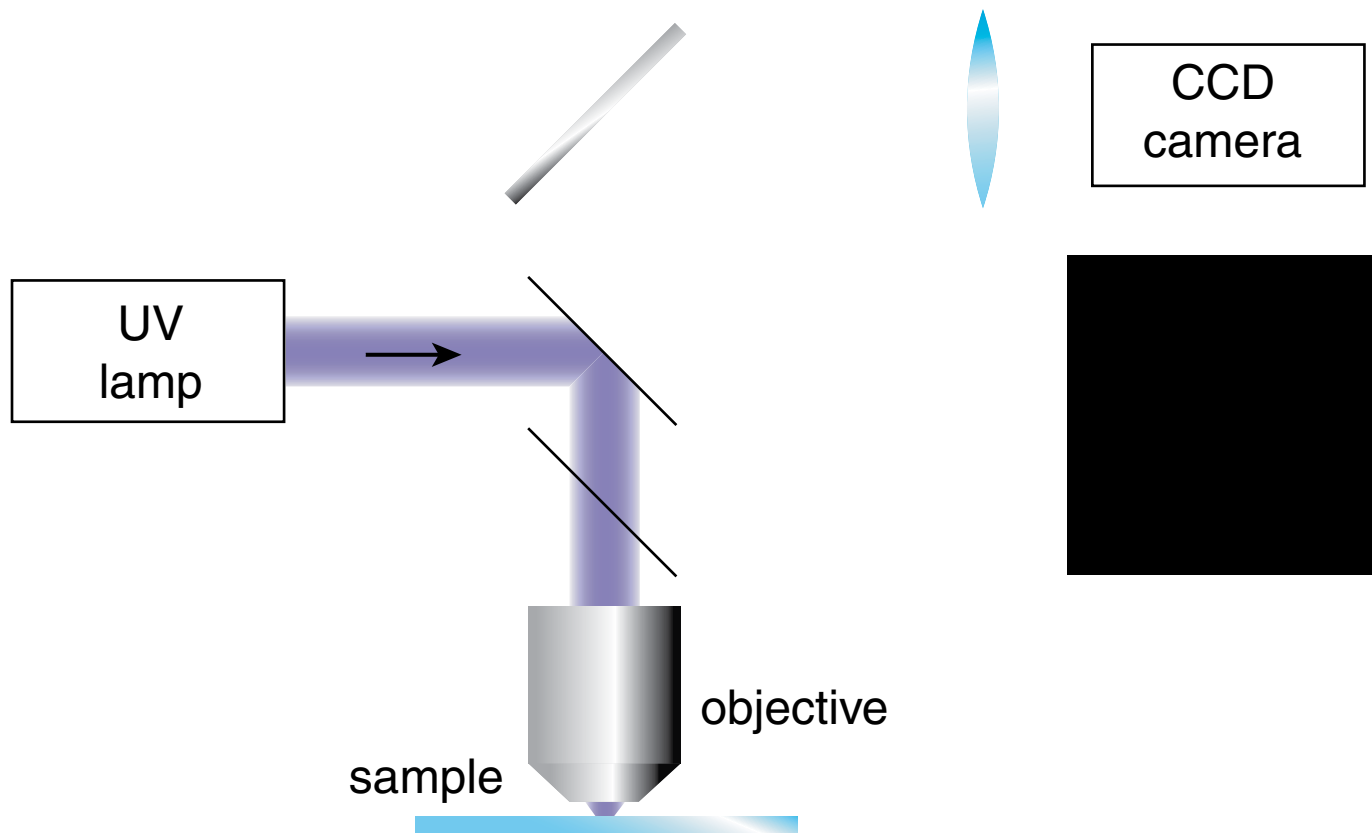
epi-fluorescence microscope

Subcellular surgery



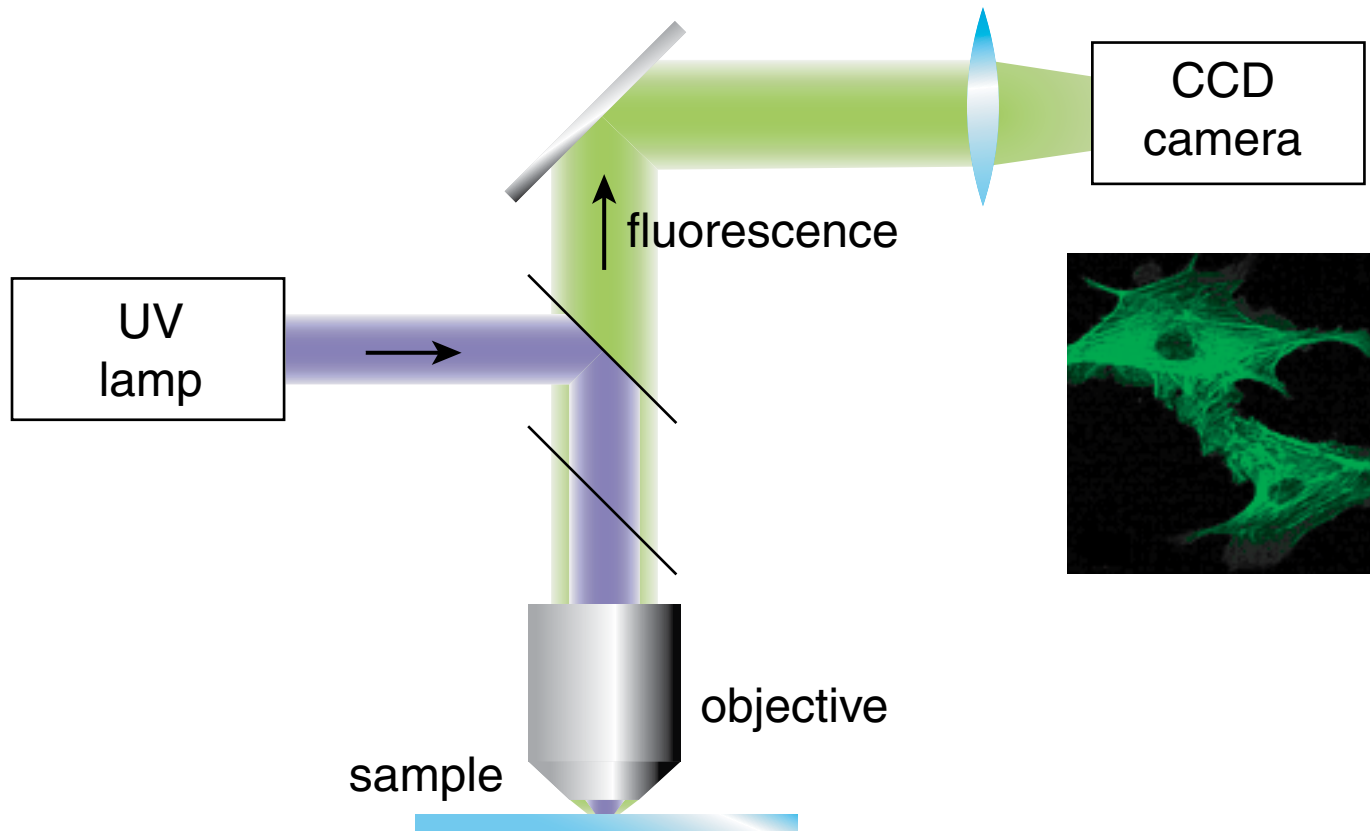
fluorescently label sample

Subcellular surgery



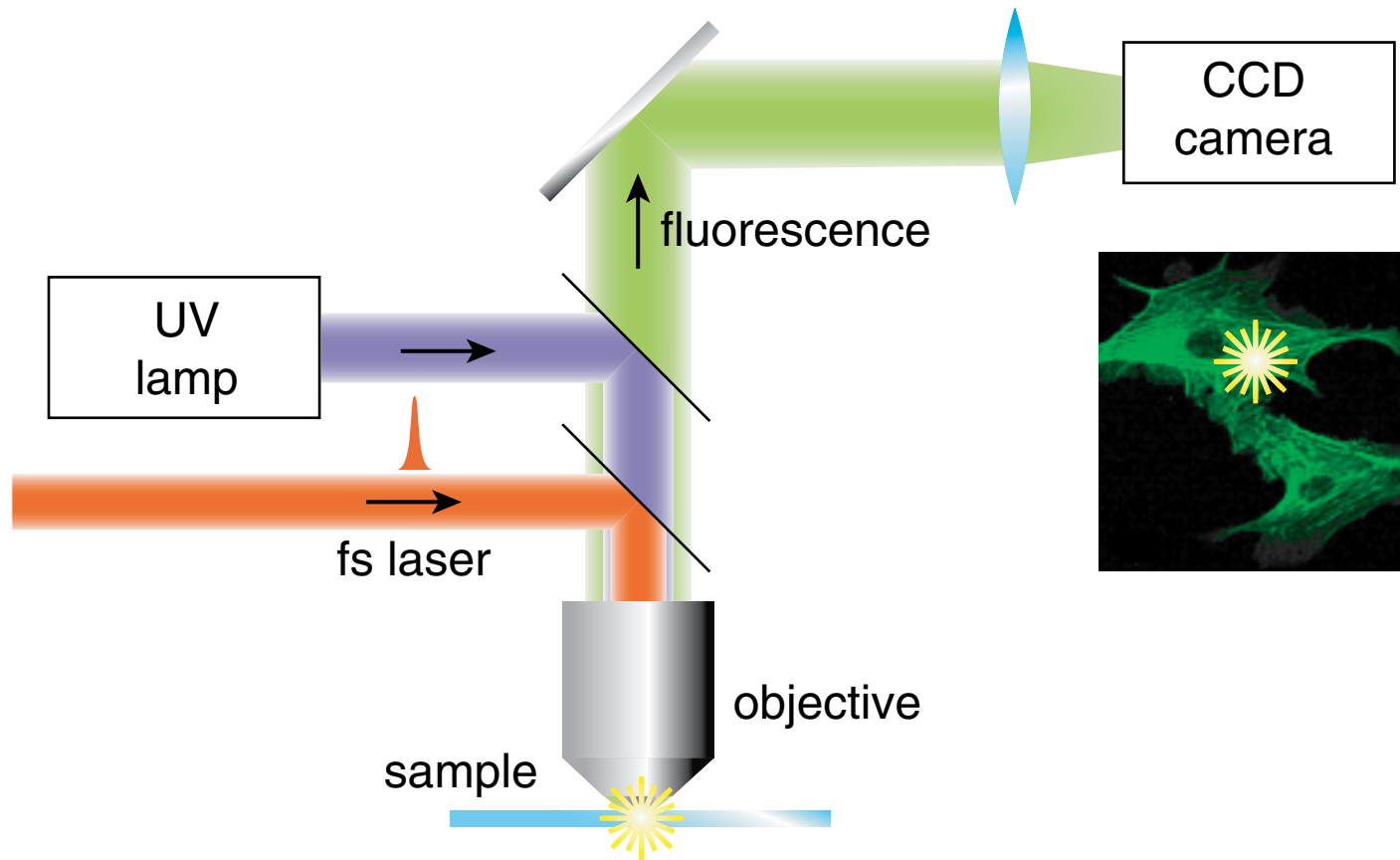
UV illumination...

Subcellular surgery



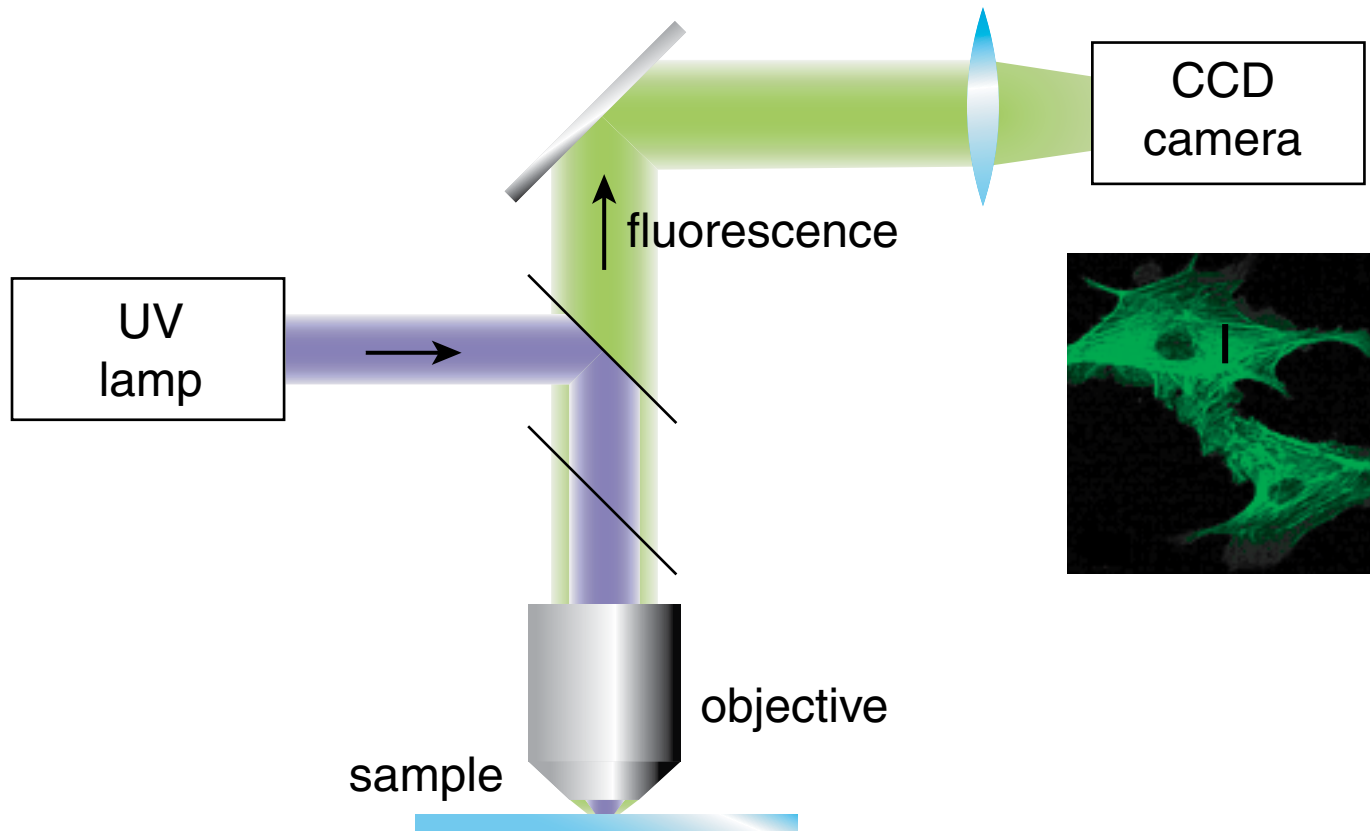
...causes fluorescence

Subcellular surgery



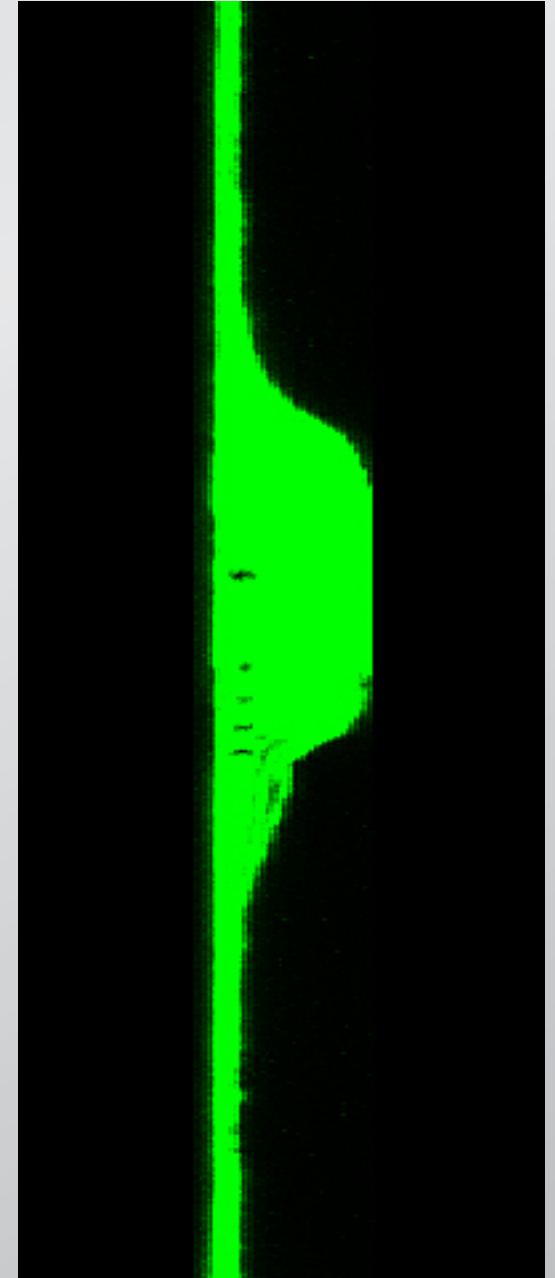
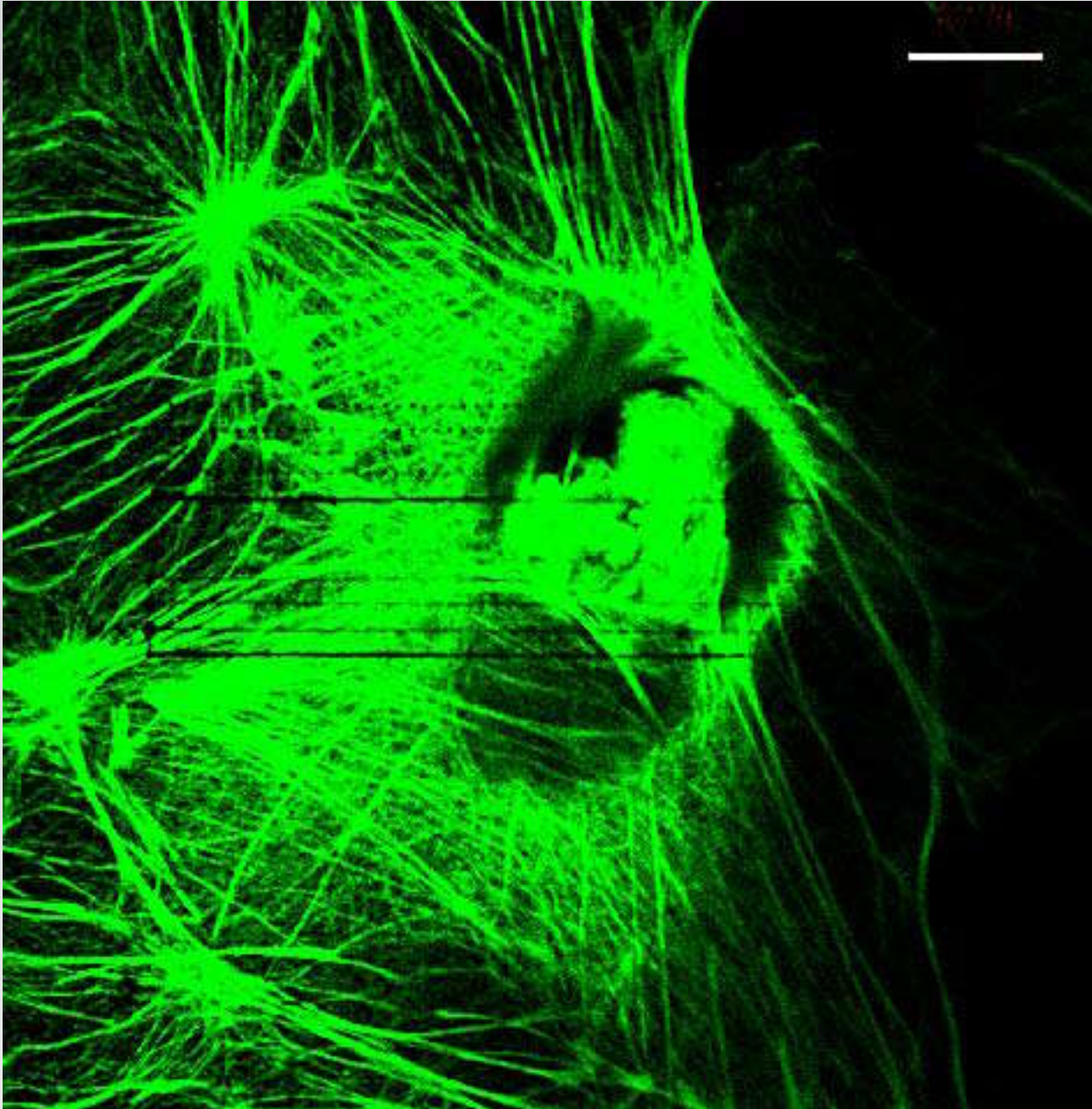
irradiate with fs laser beam

Subcellular surgery

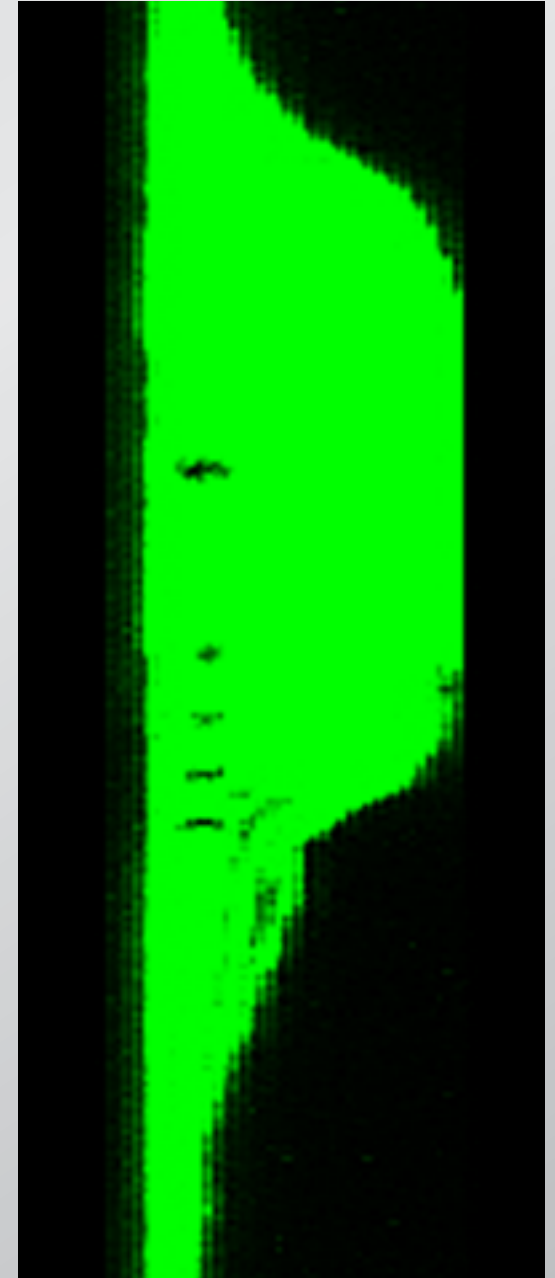
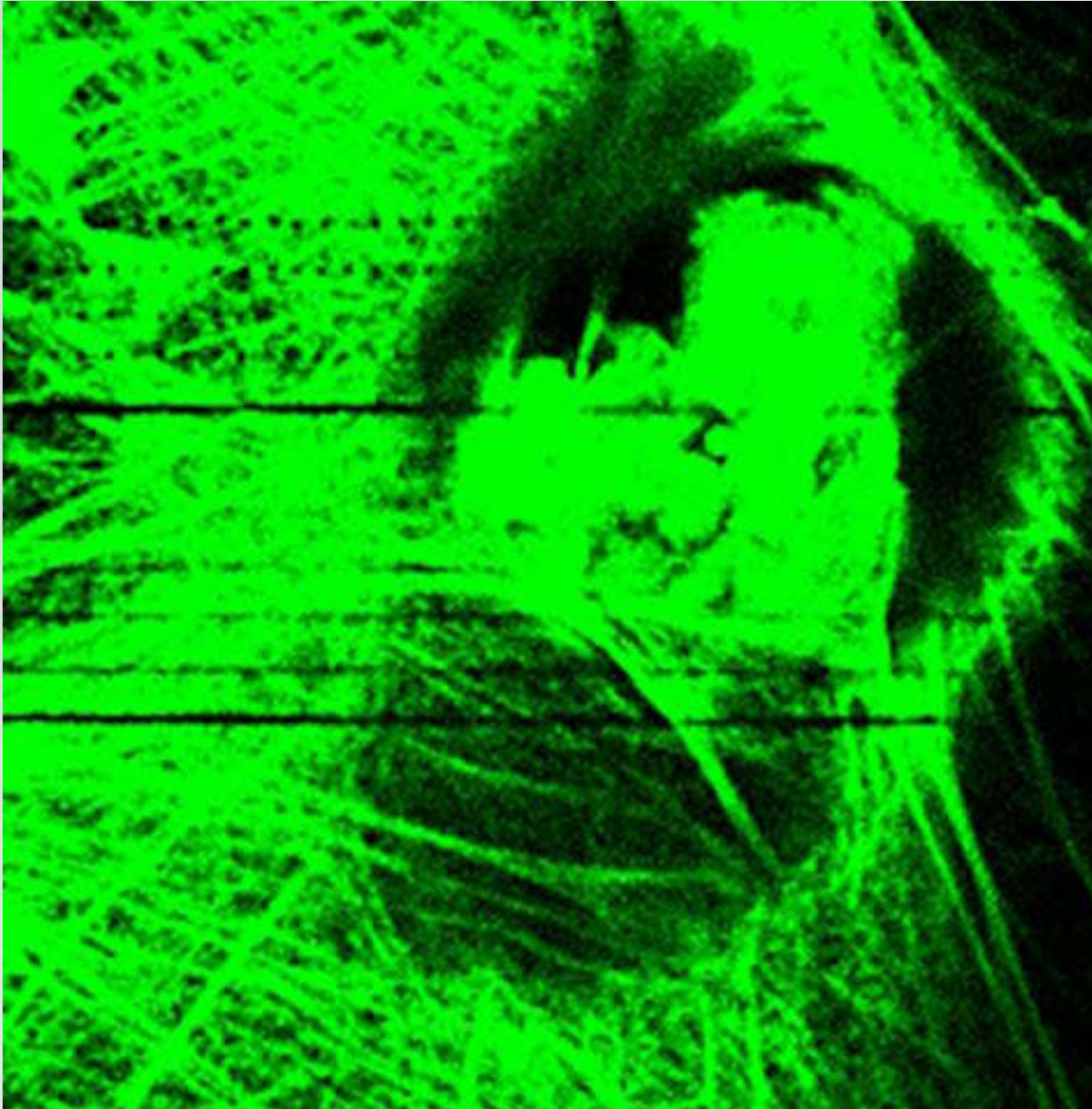


examine resulting ablation

Subcellular surgery

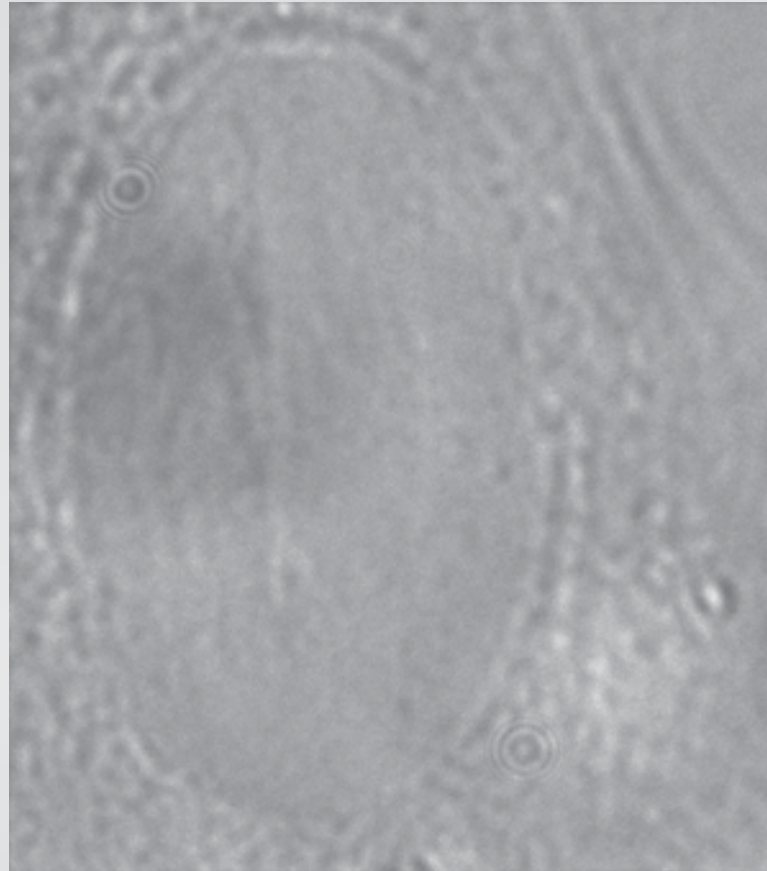


Subcellular surgery



Subcellular surgery

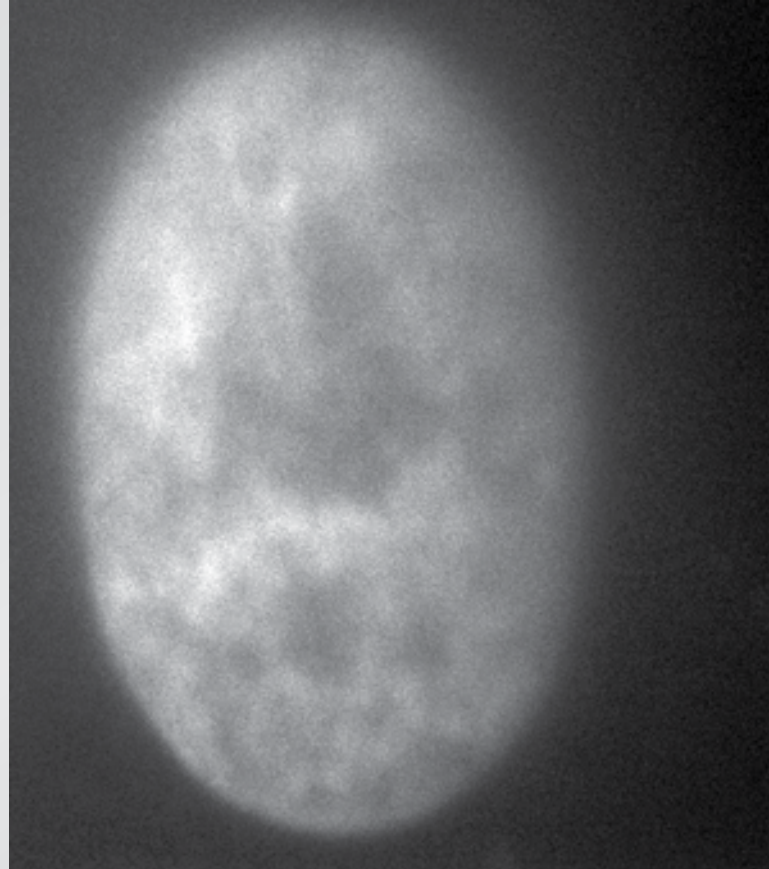
nucleus of fixed endothelial cell



white light microscopy

Subcellular surgery

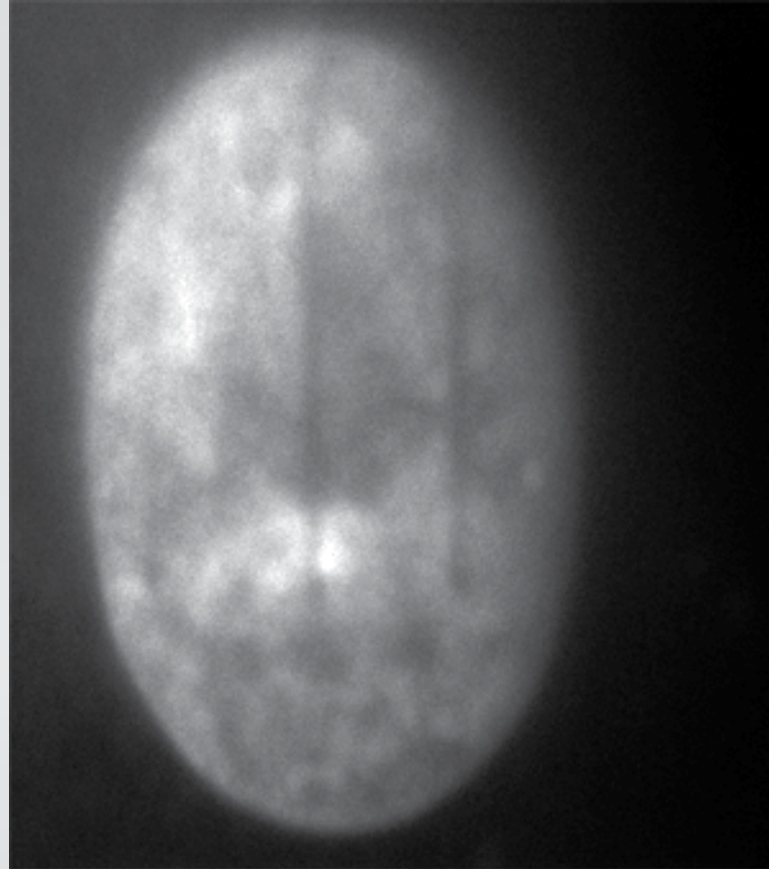
nucleus of fixed endothelial cell



fluorescence microscopy

Subcellular surgery

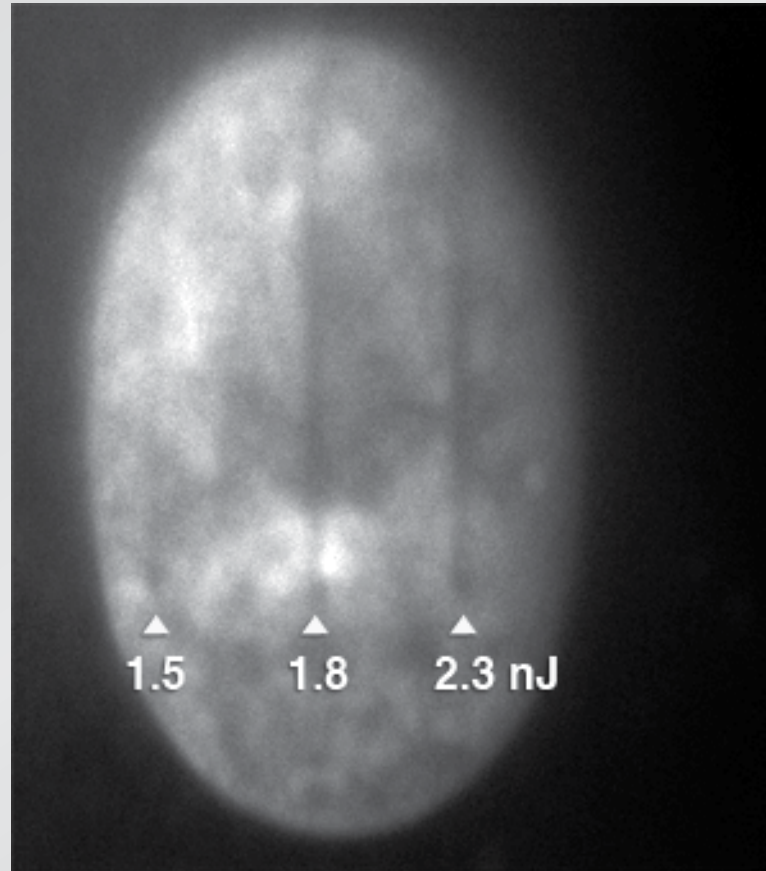
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

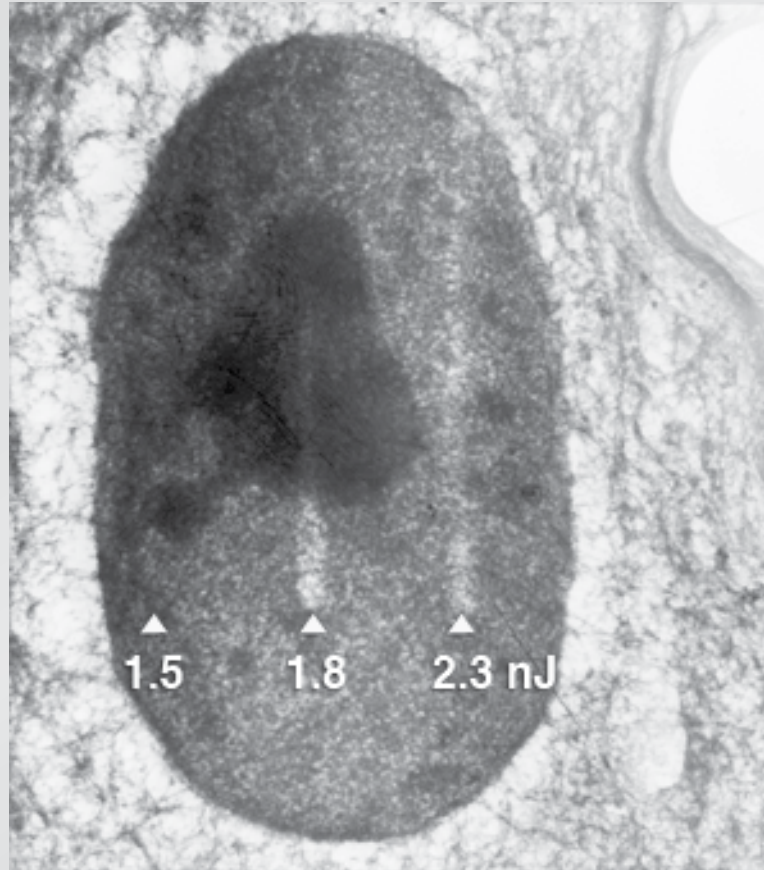
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

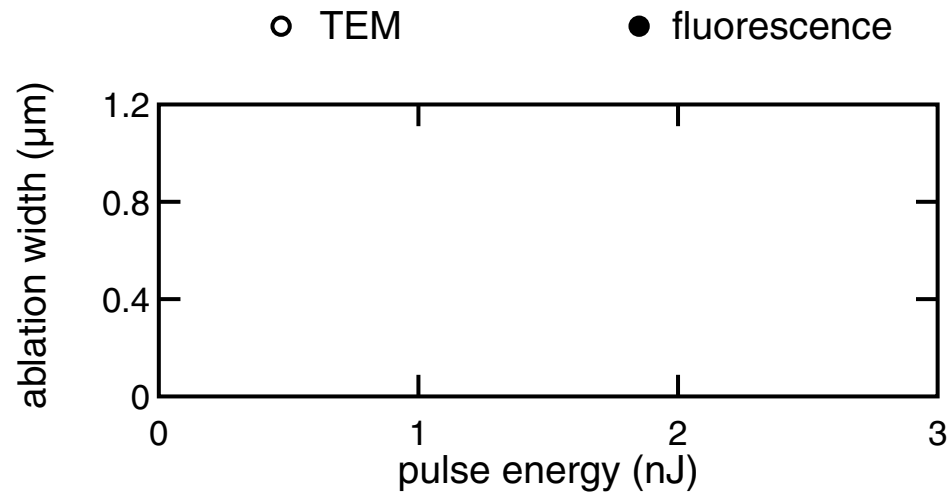
bleaching or ablation?



TEM image

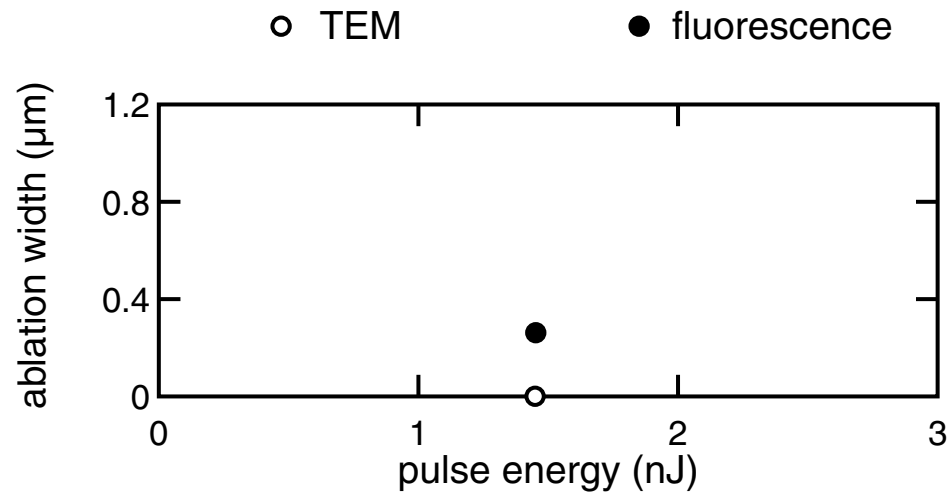
Subcellular surgery

three regions of interaction



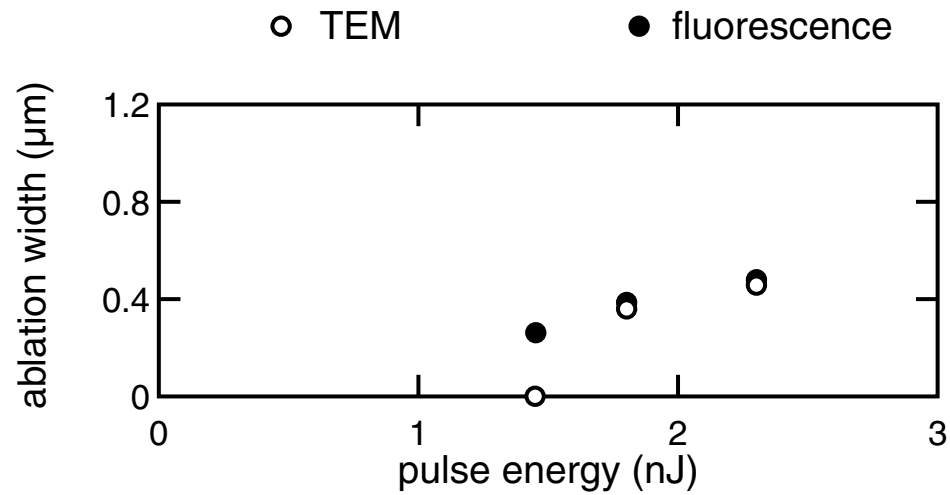
Subcellular surgery

three regions of interaction



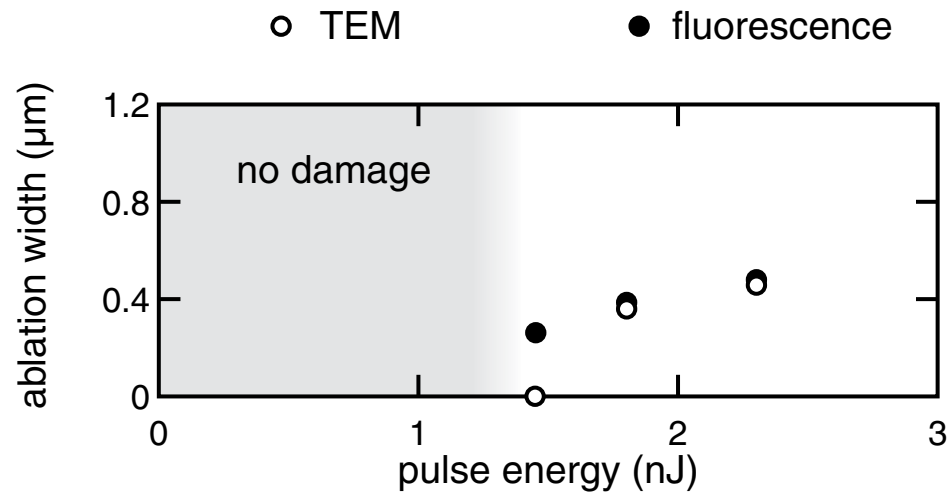
Subcellular surgery

three regions of interaction



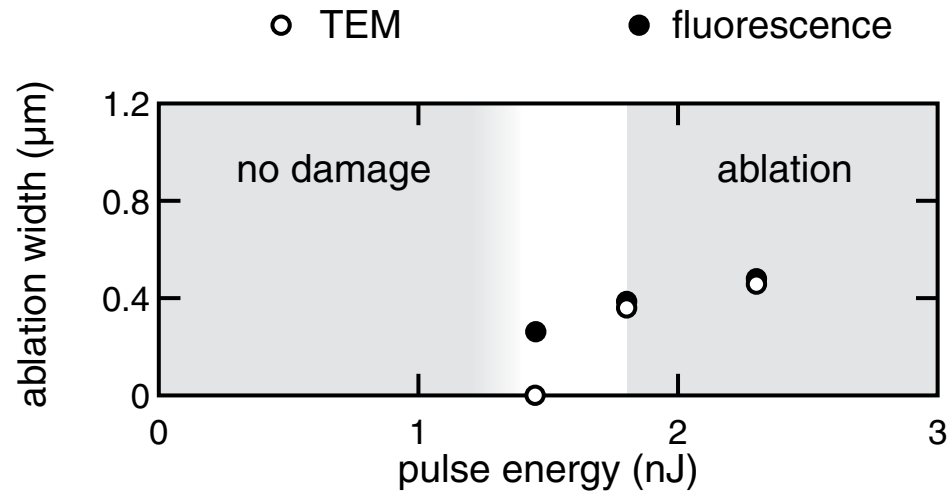
Subcellular surgery

three regions of interaction



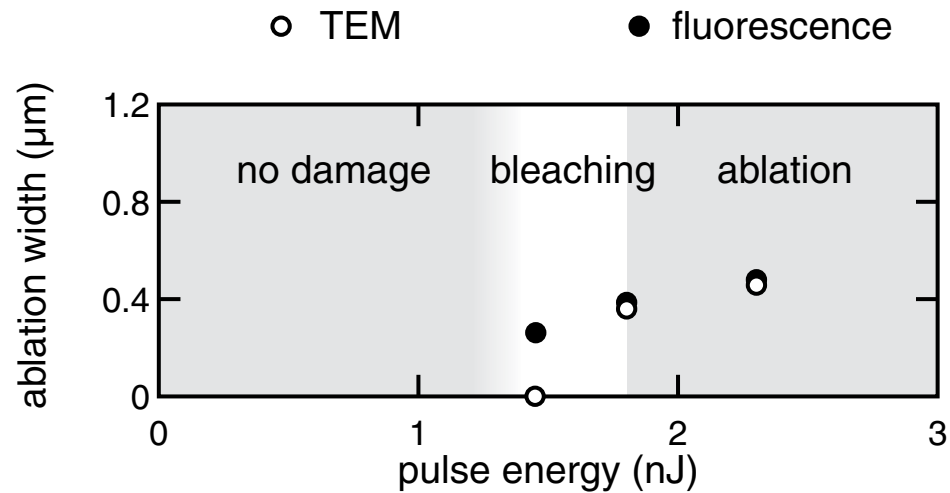
Subcellular surgery

three regions of interaction



Subcellular surgery

three regions of interaction

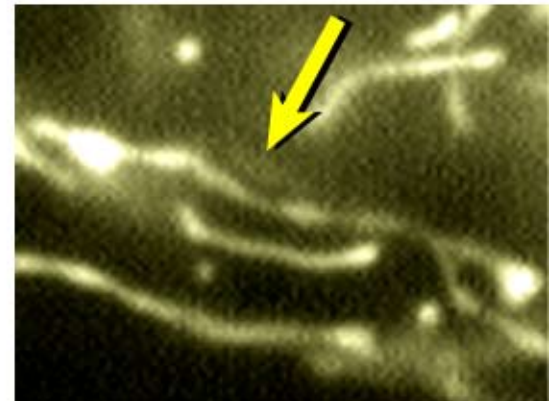
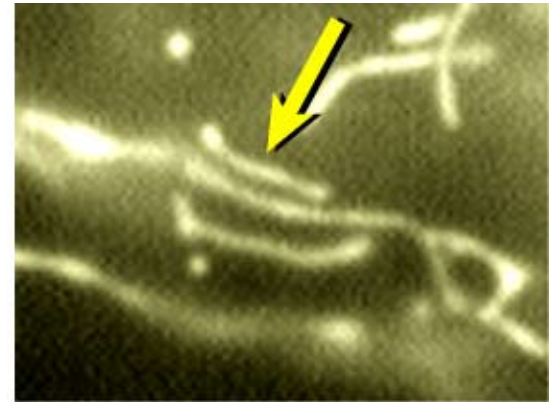
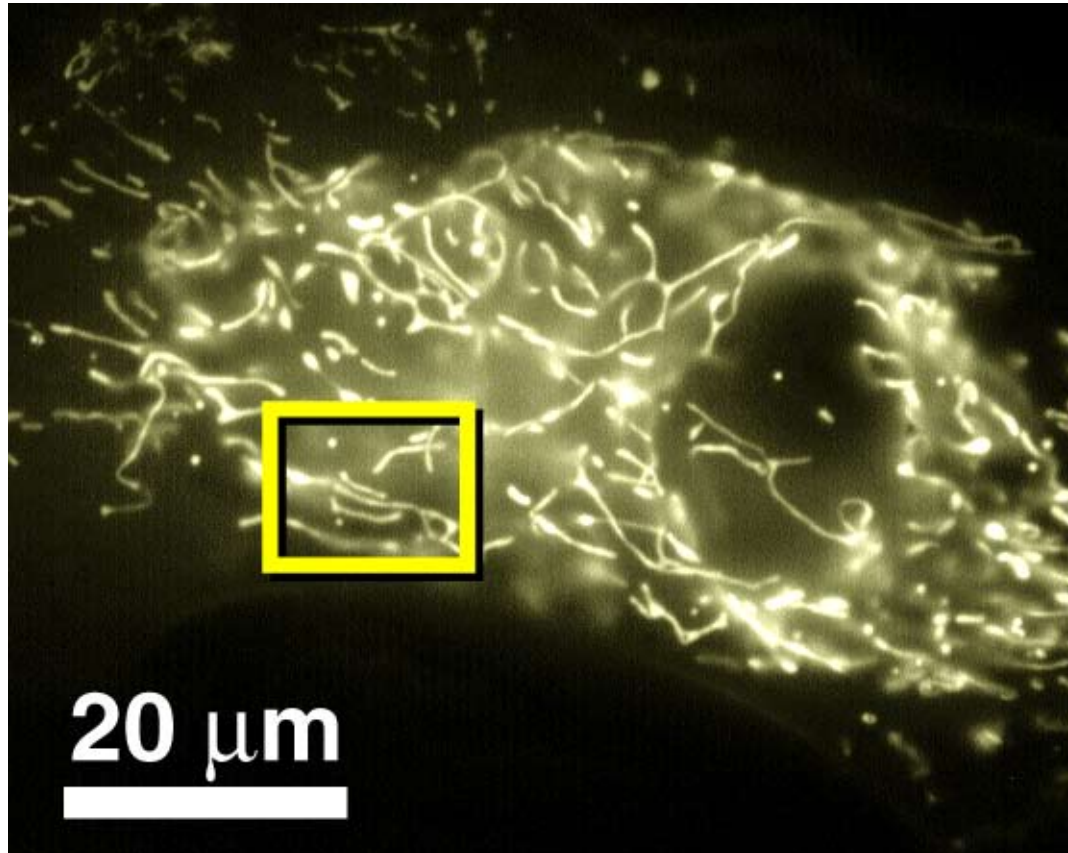


Subcellular surgery

Q: subcellular surgery on live cells?

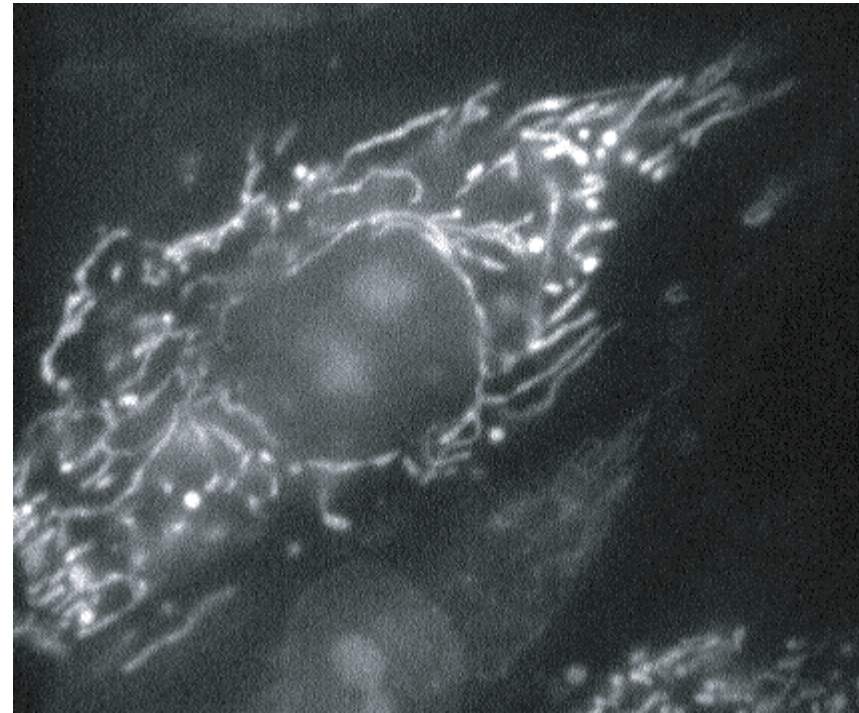
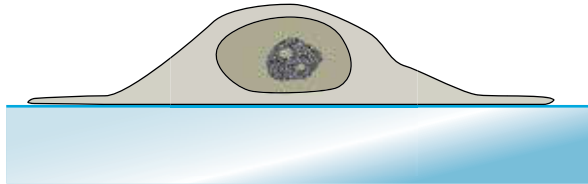
A fluorescence microscopy image showing a complex network of bright yellow-green filaments within a cell. The filaments are interconnected, forming a dense, branching structure that fills most of the cell's volume. The background is dark, highlighting the intricate pattern of the filaments.

Subcellular surgery



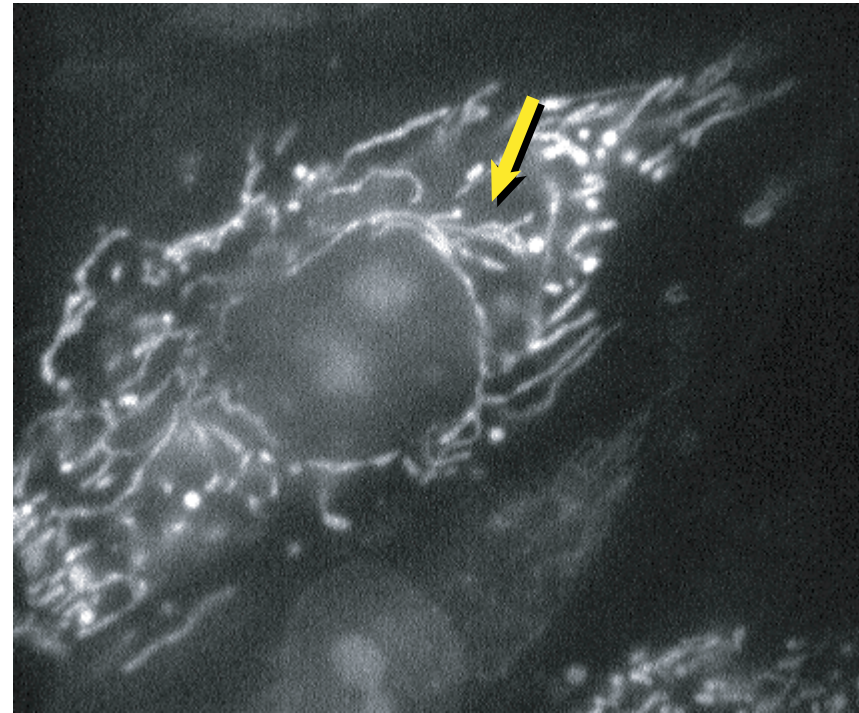
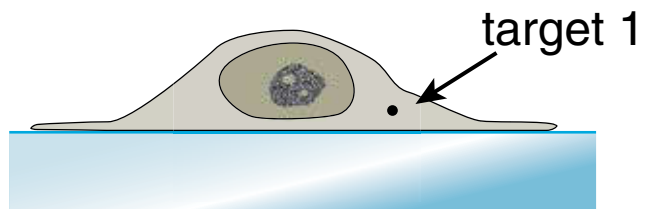
Subcellular surgery

ethyidium bromide test



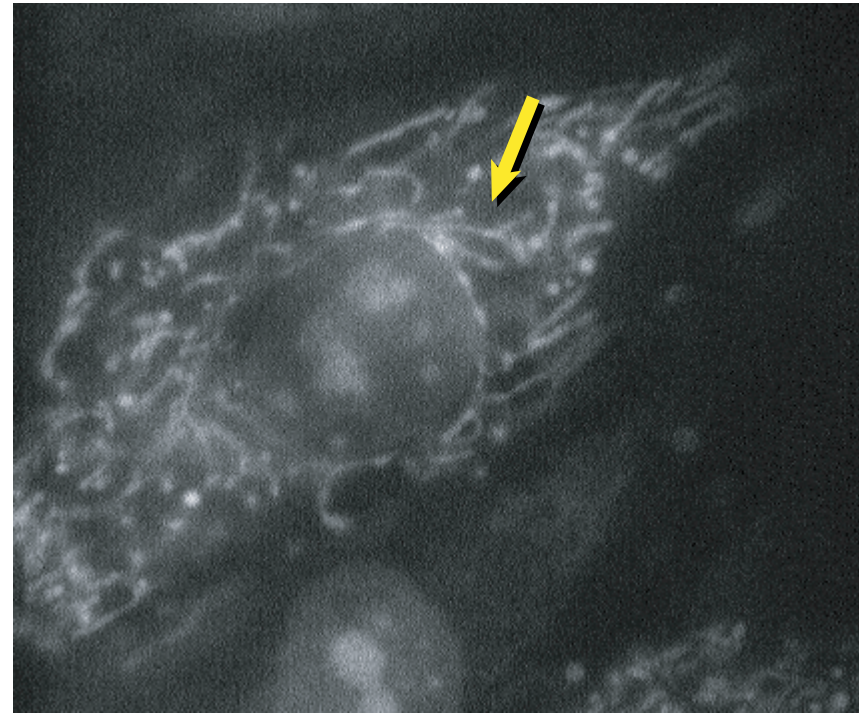
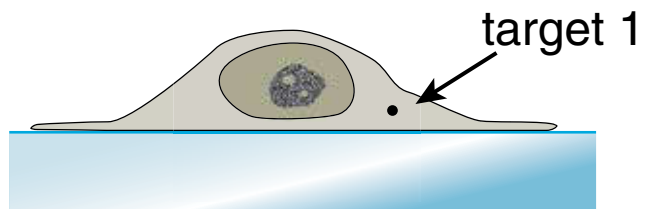
Subcellular surgery

ethyidium bromide test



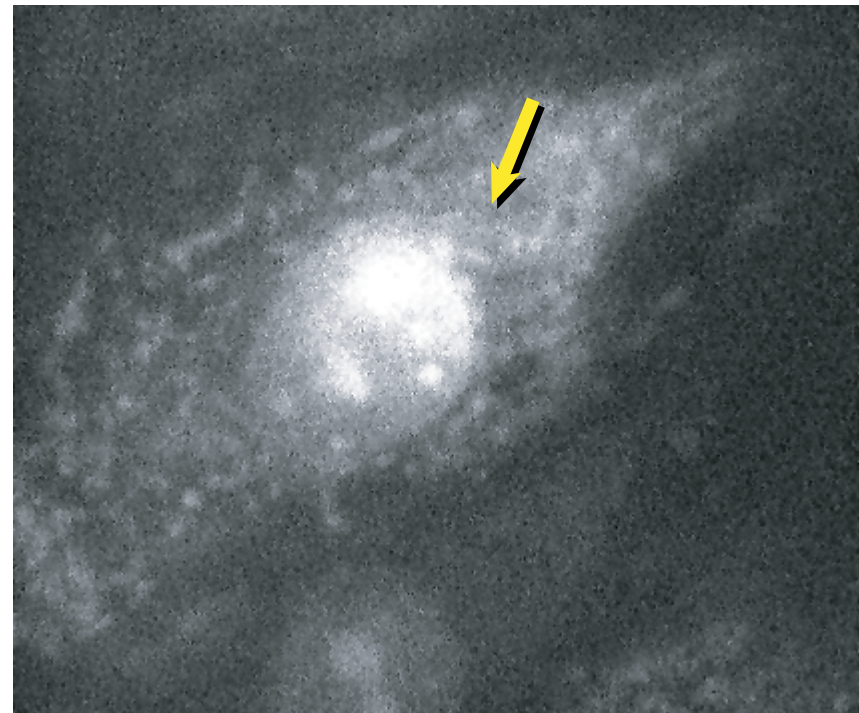
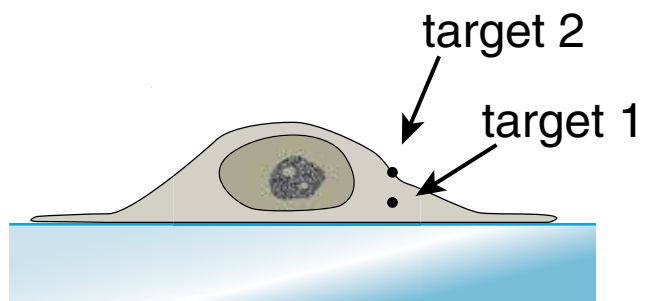
Subcellular surgery

ethyidium bromide test



Subcellular surgery

ethyidium bromide test

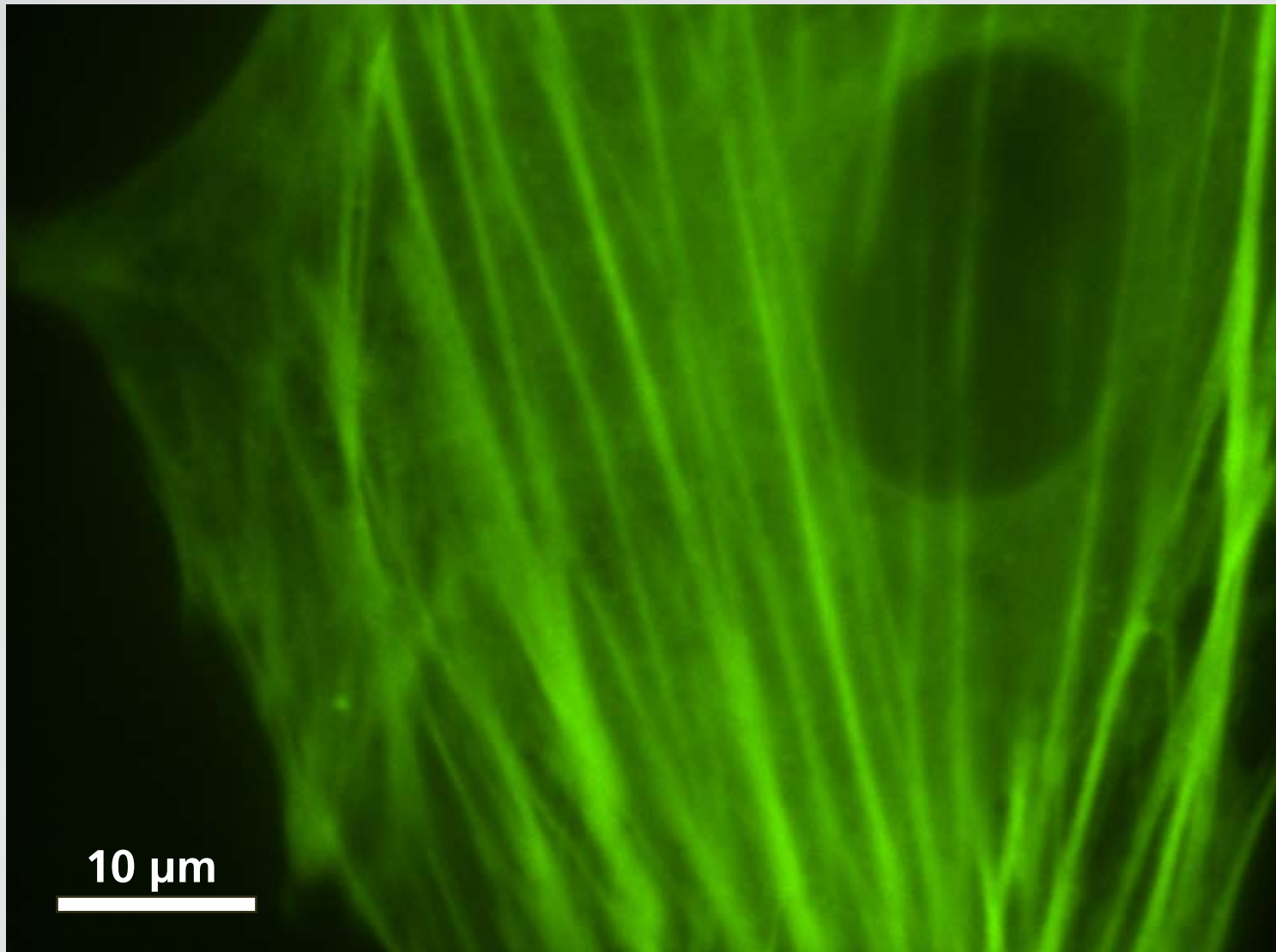


Subcellular surgery

Q: can we probe the dynamics of the cytoskeleton?

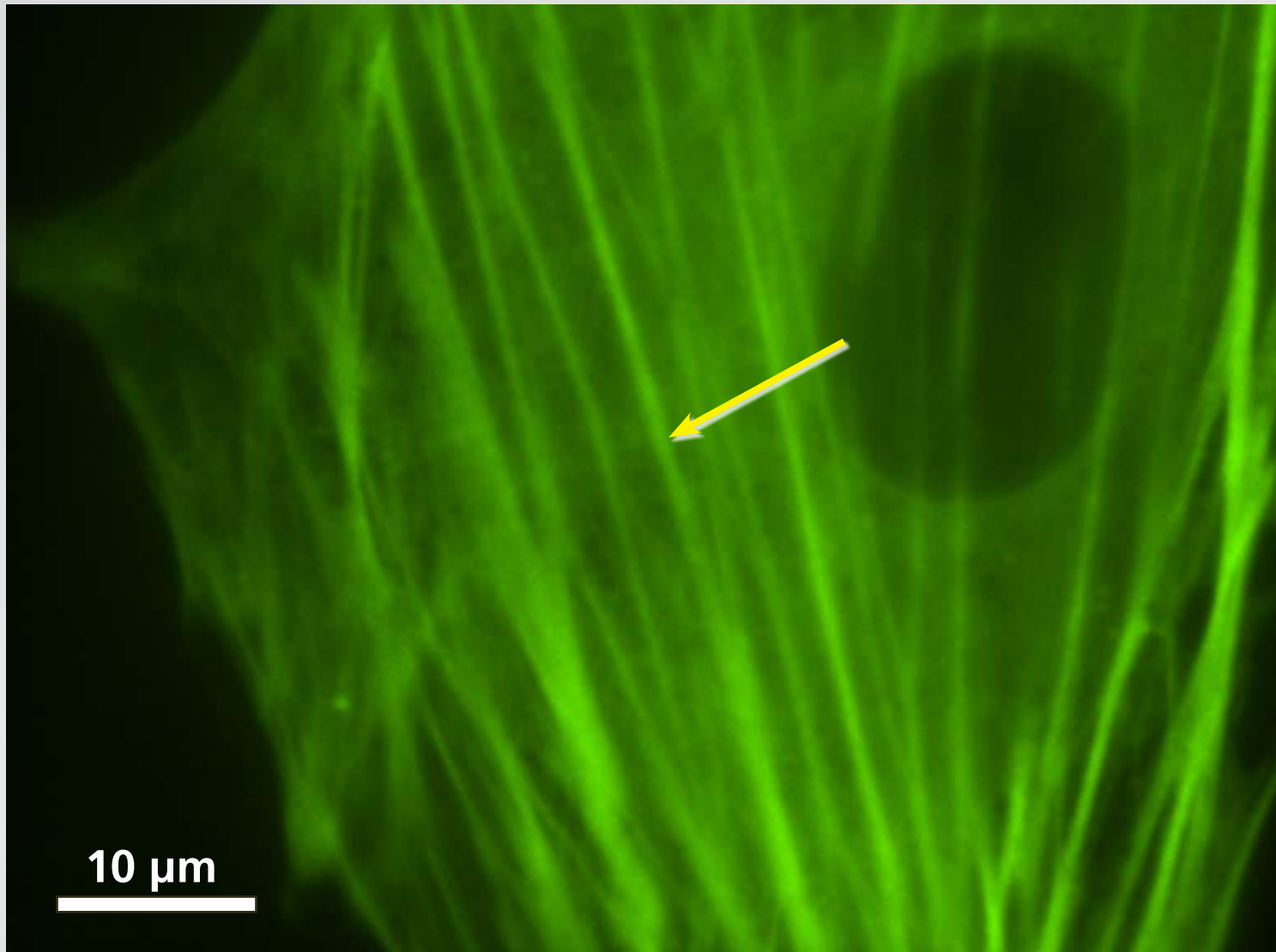
Subcellular surgery

YFP-labeled actin fiber network of a live cell



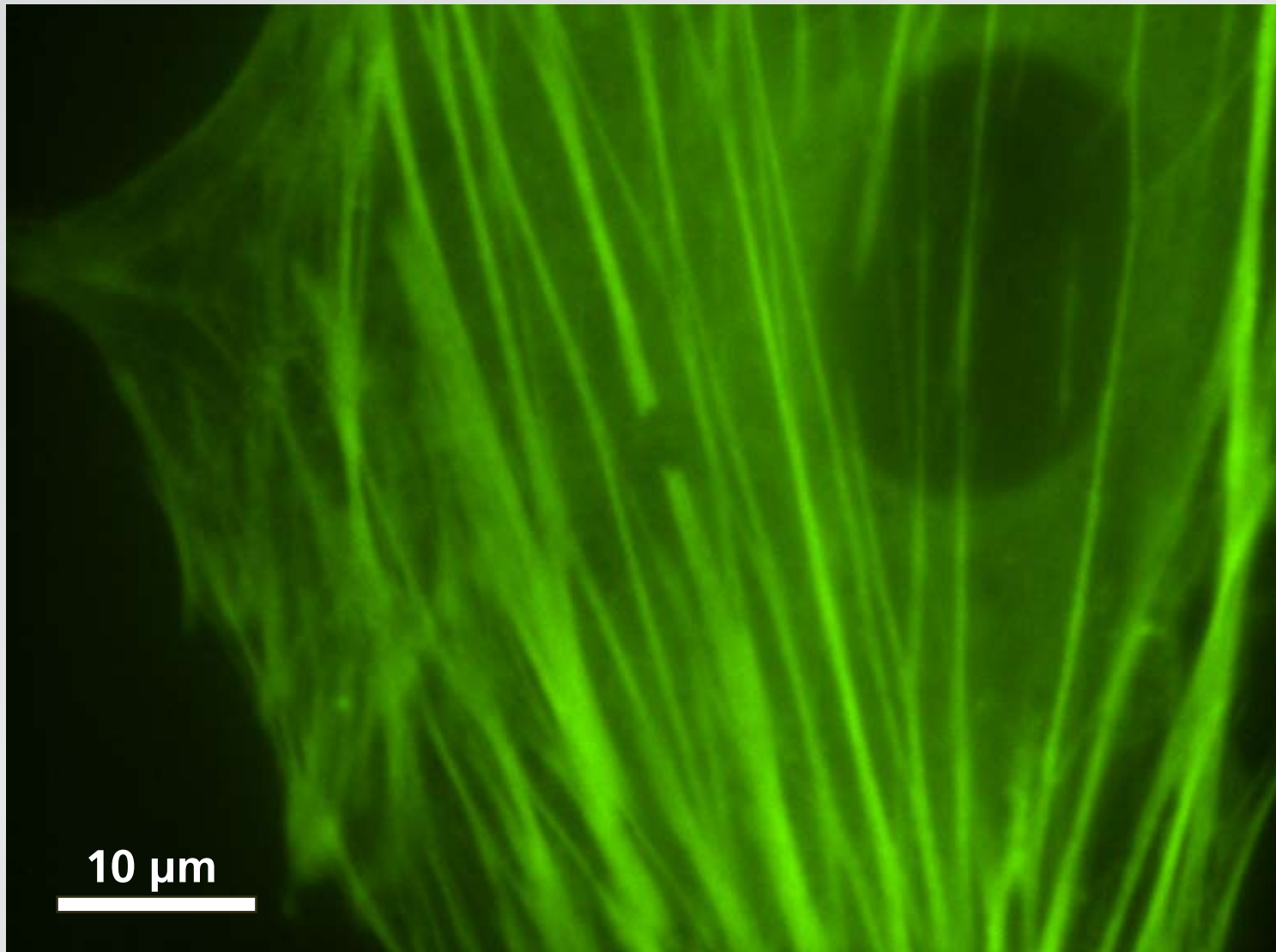
Subcellular surgery

cut a single fiber bundle



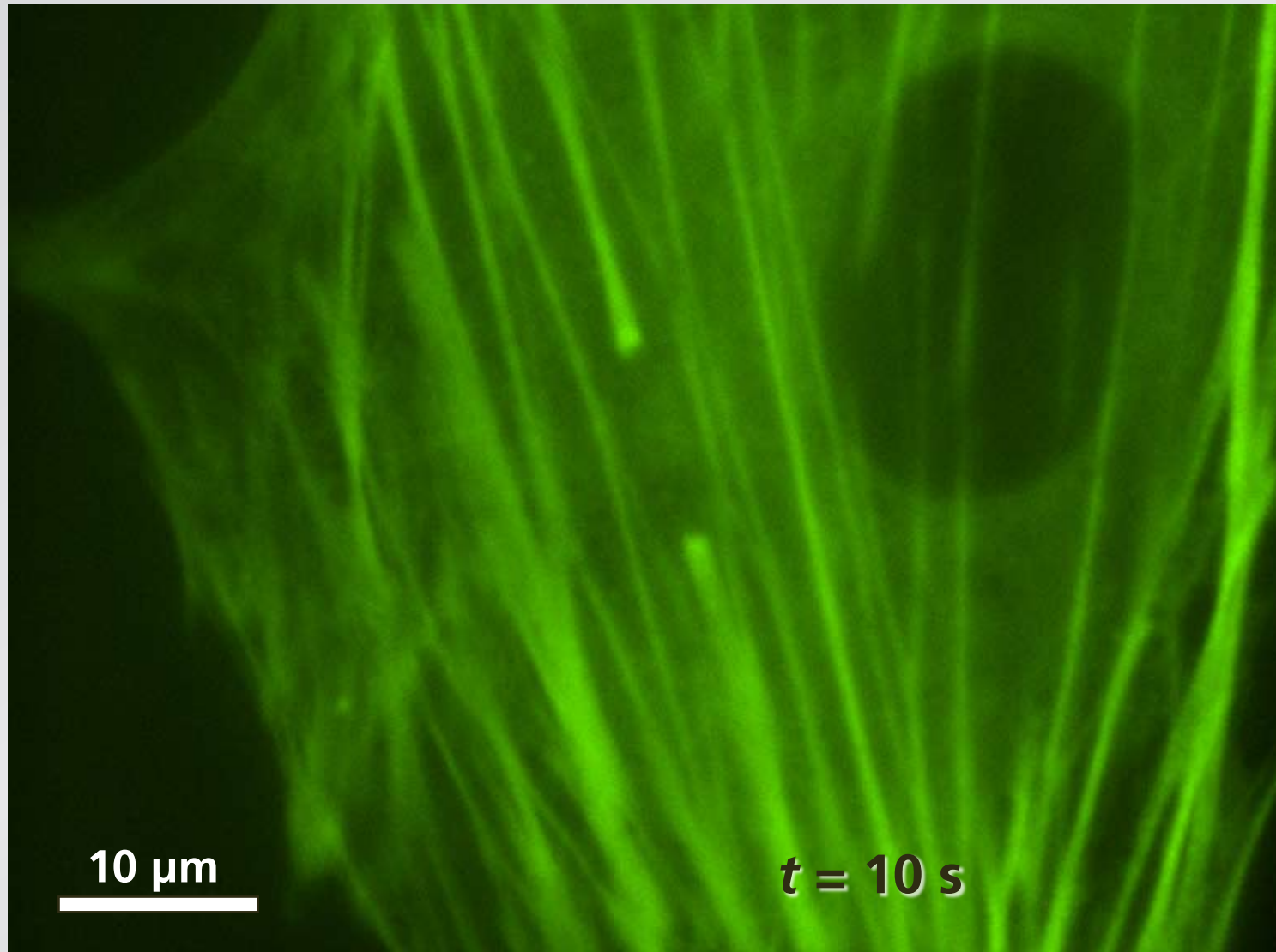
Subcellular surgery

cut a single fiber bundle



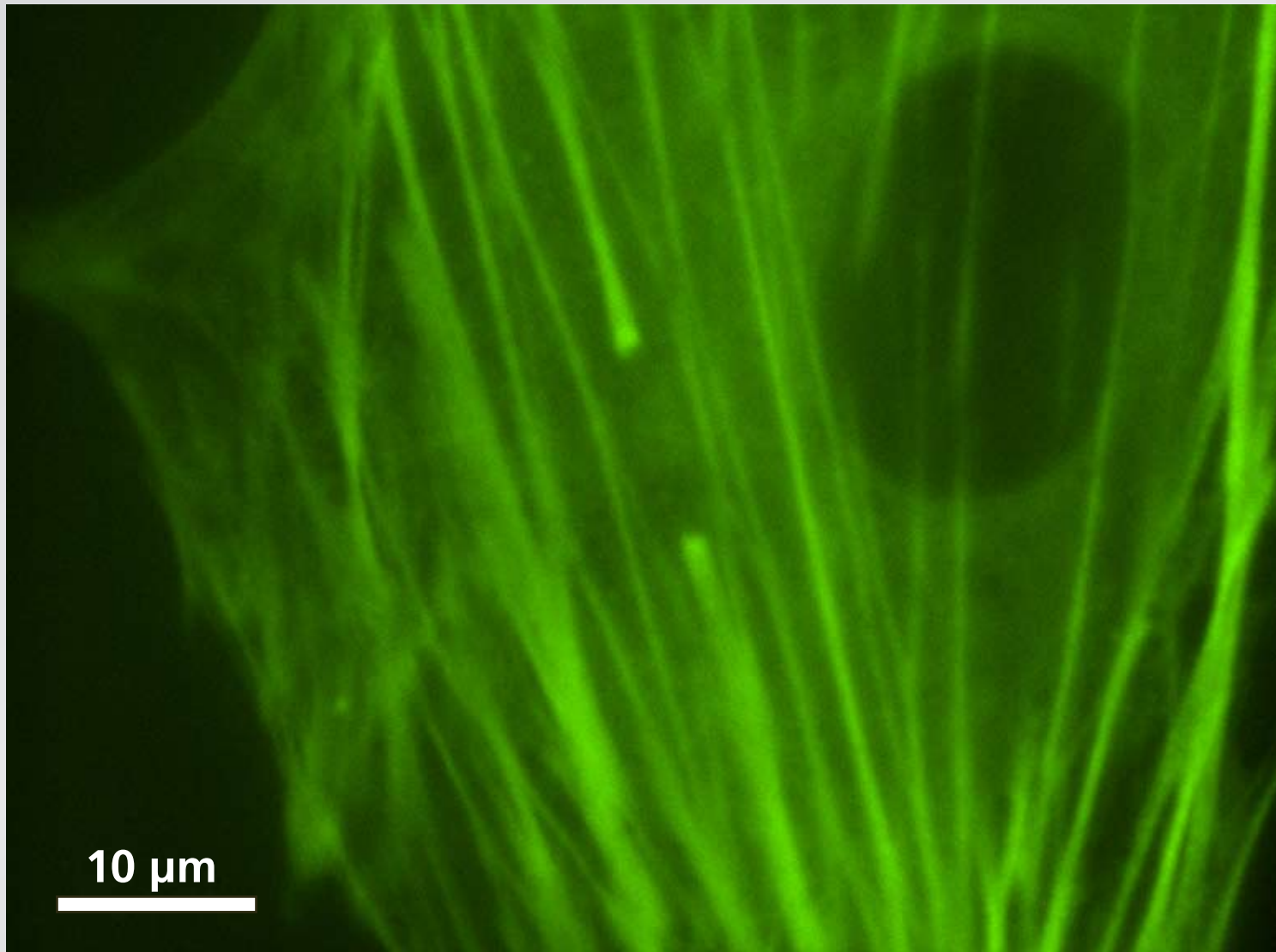
Subcellular surgery

gap widens with time



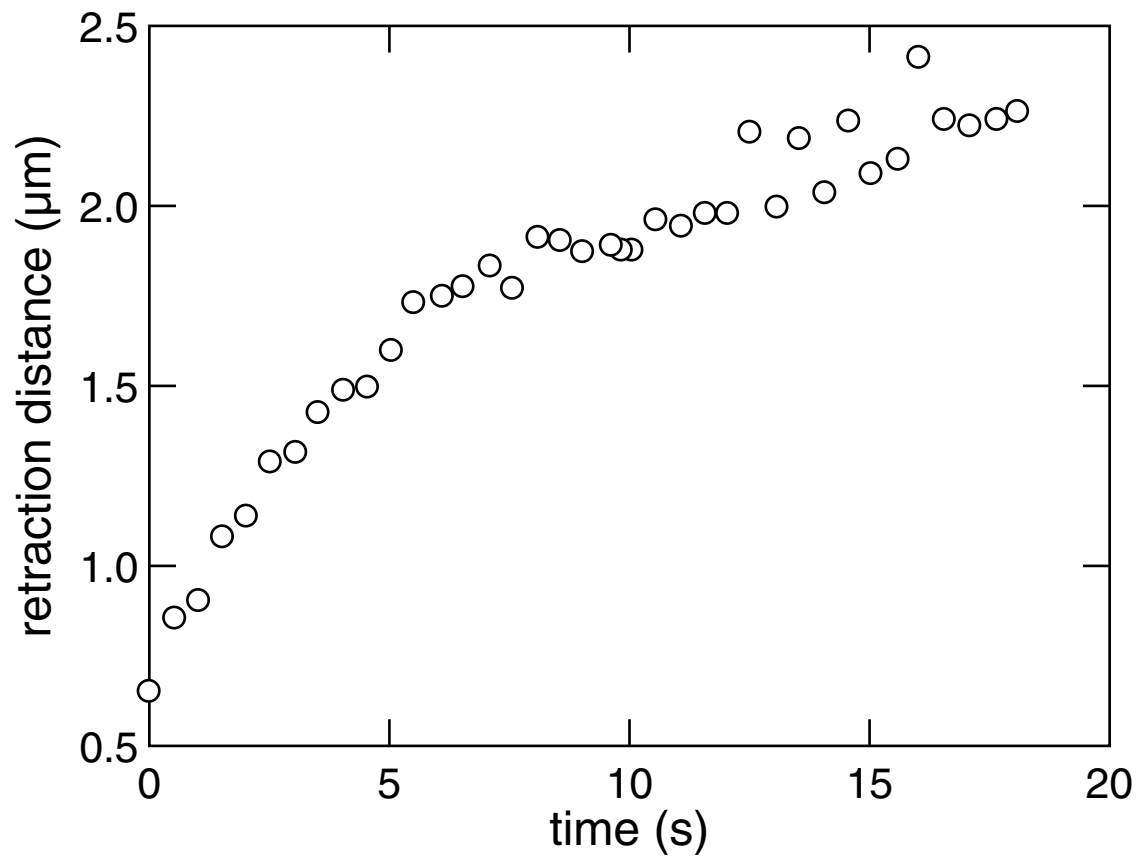
Subcellular surgery

dynamics provides information on *in vivo* mechanics



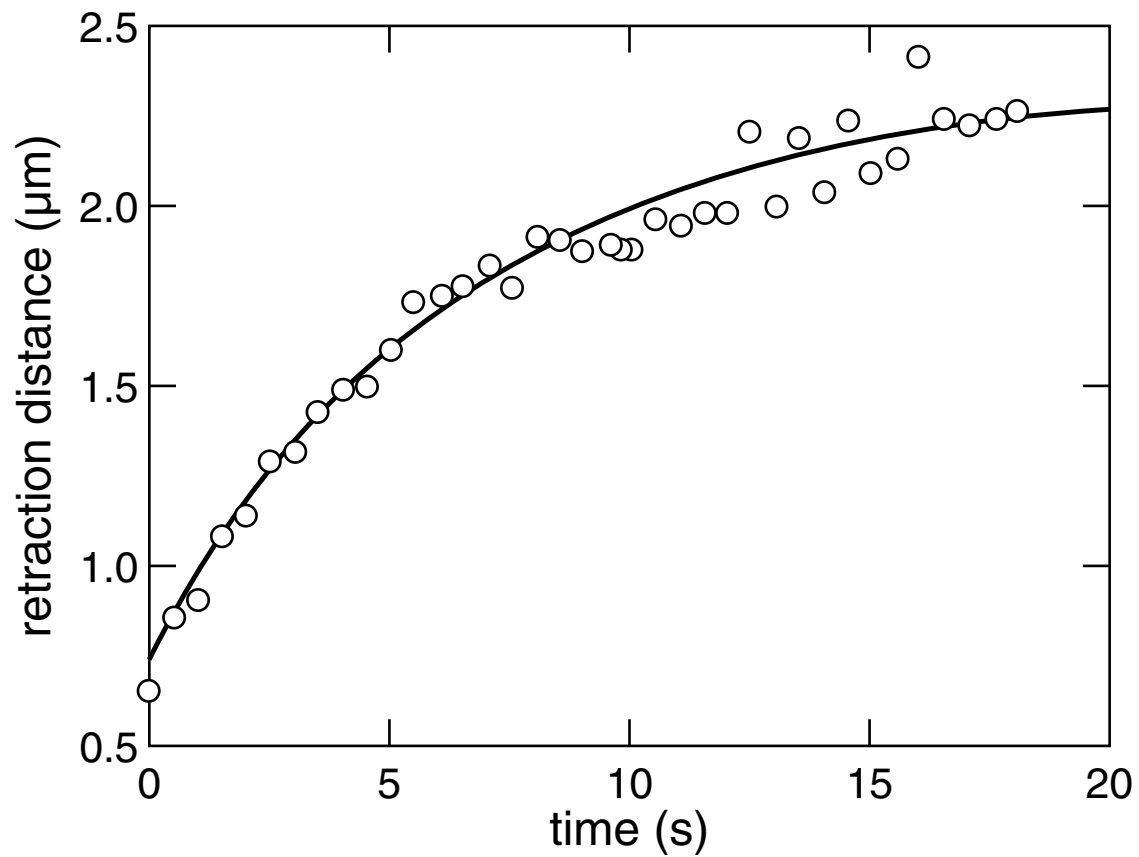
Subcellular surgery

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
Max-Planck Institute for Developmental Biology

Nanoneurosurgery

Caenorhabditis elegans

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

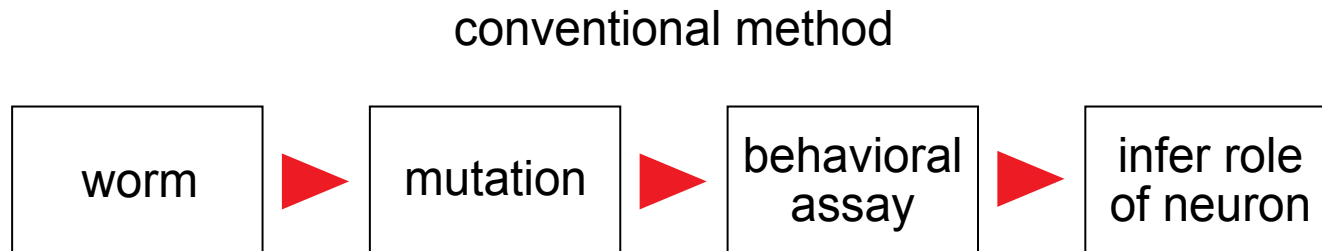
Nanoneurosurgery

Caenorhabditis elegans

- 80 μm x 1 mm
- about 1300 cells
- 302 neurons
- invariant wiring diagram
- neuronal system completely encodes behavior

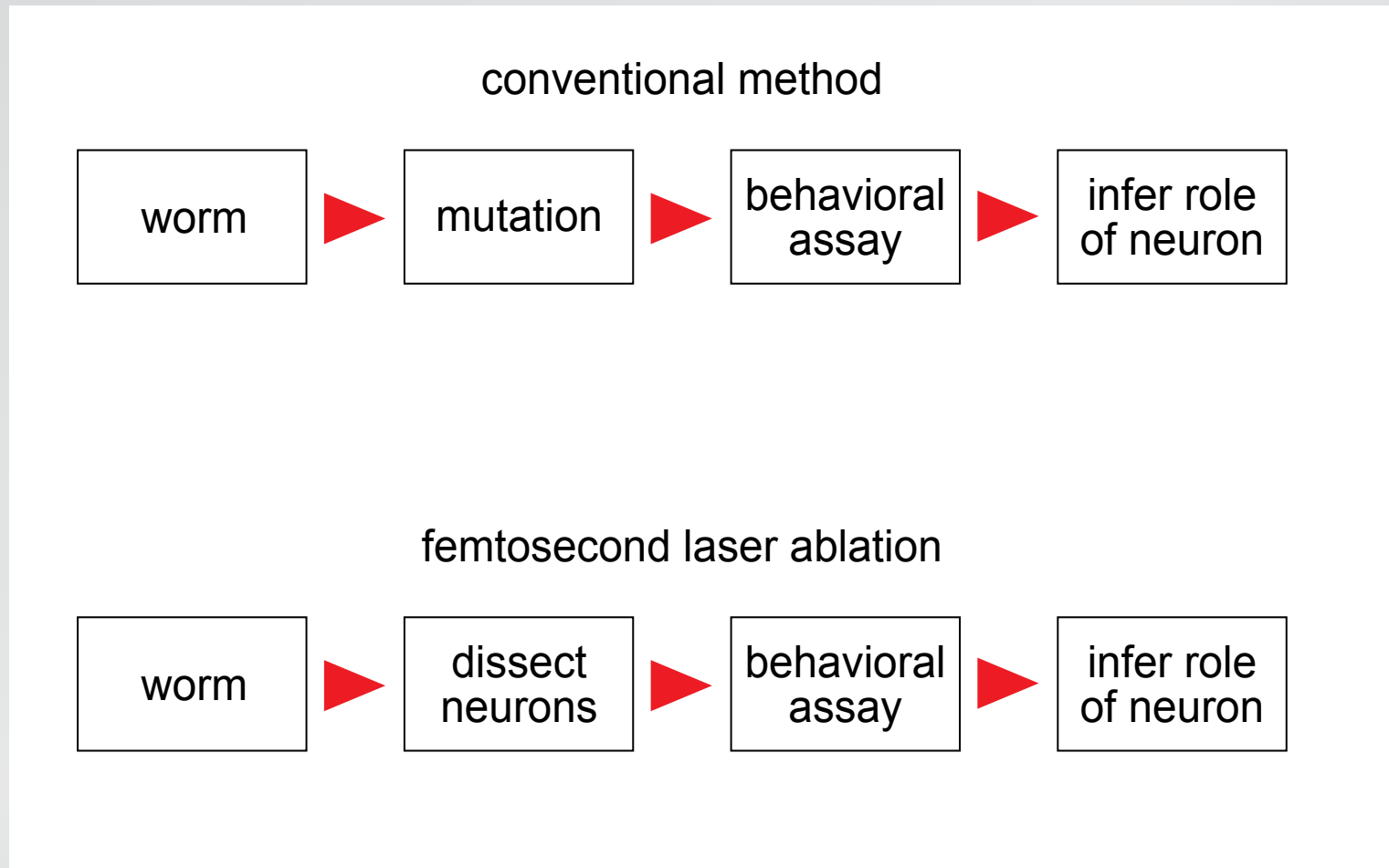
Nanoneurosurgery

Mapping behavior to neurons



Nanoneurosurgery

Mapping behavior to neurons



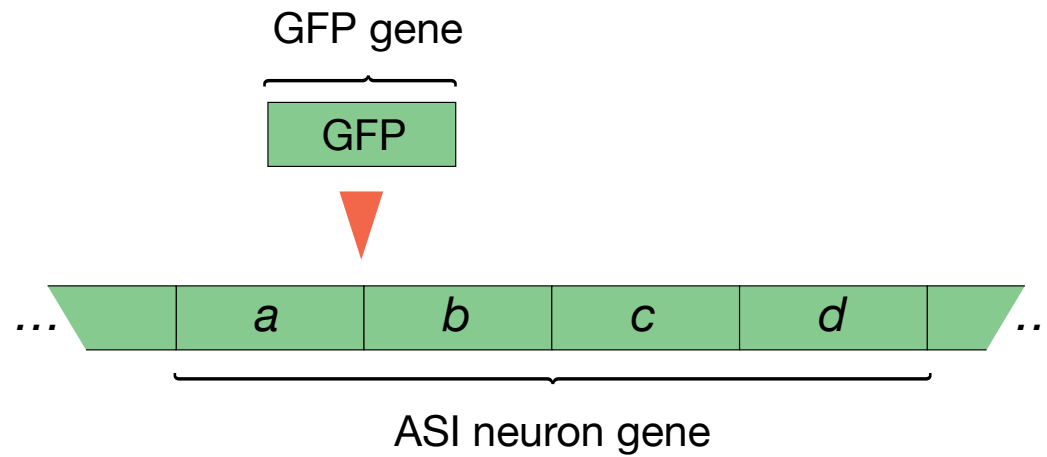
Nanoneurosurgery

ASI neurons

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

Nanoneurosurgery

Make ASI neurons express GFP



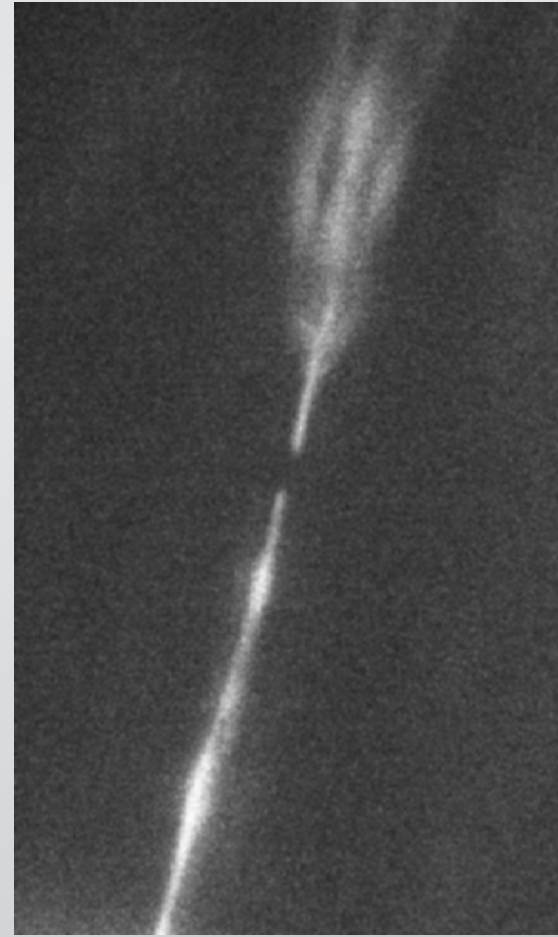
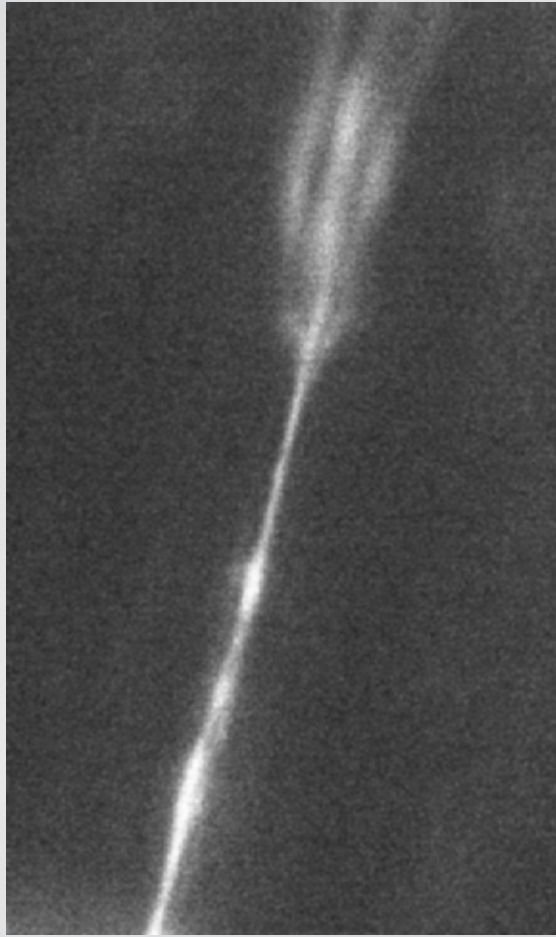
Nanoneurosurgery

GFP: absorbs UV, emits green



Nanoneurosurgery

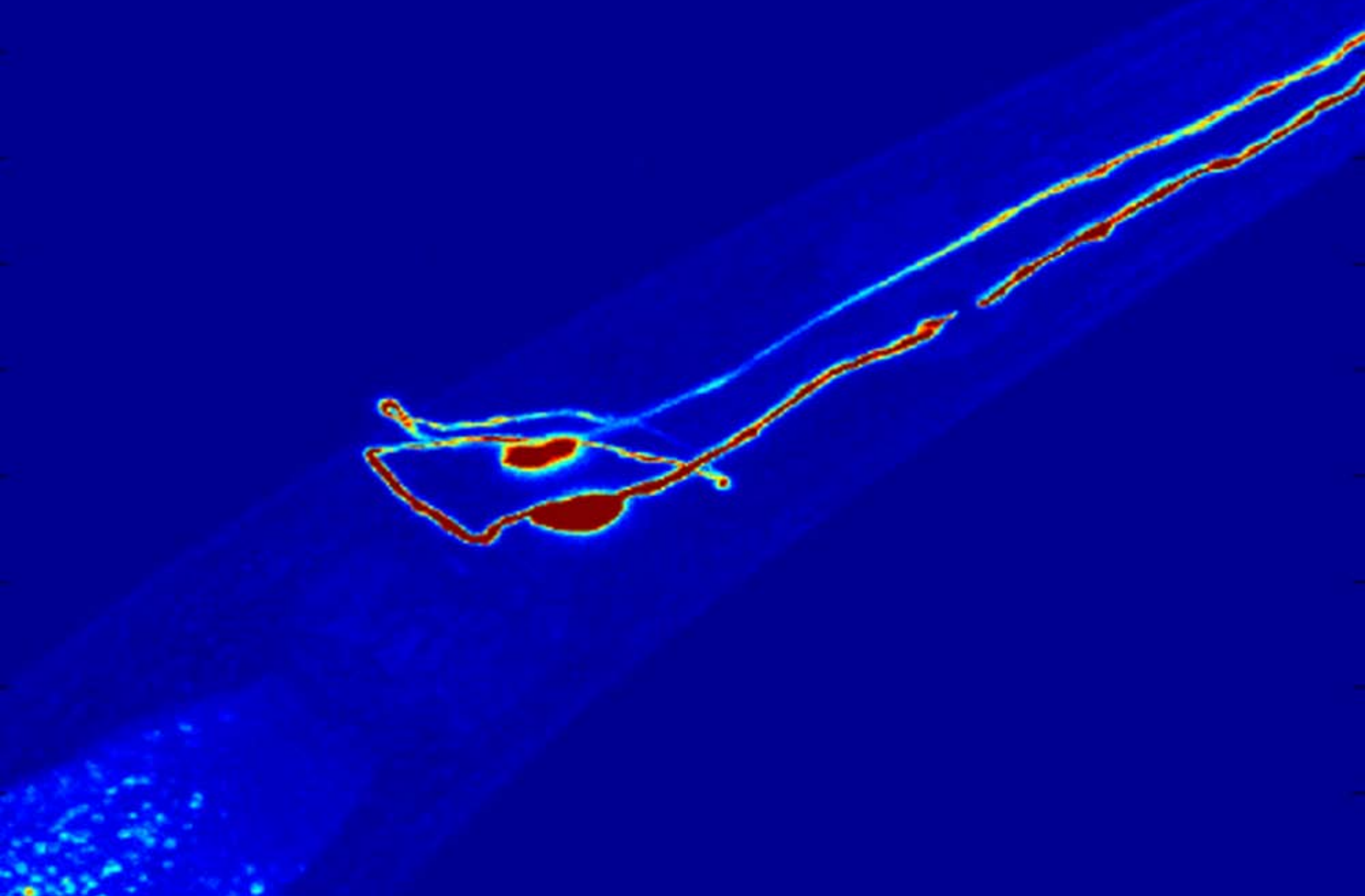
minutes after surgery with 14 nJ pulses



Nanoneurosurgery

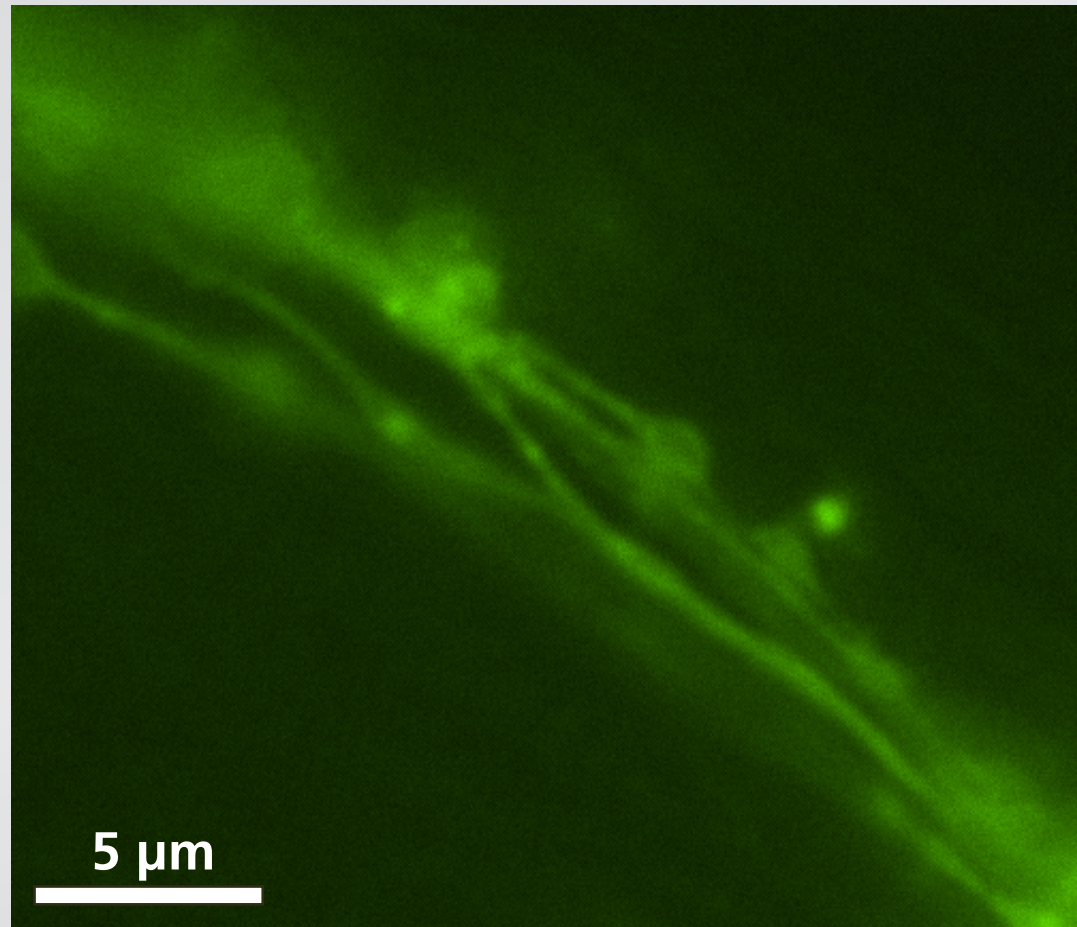
revive worm, reimage 1 day later

Nanoneurosurgery



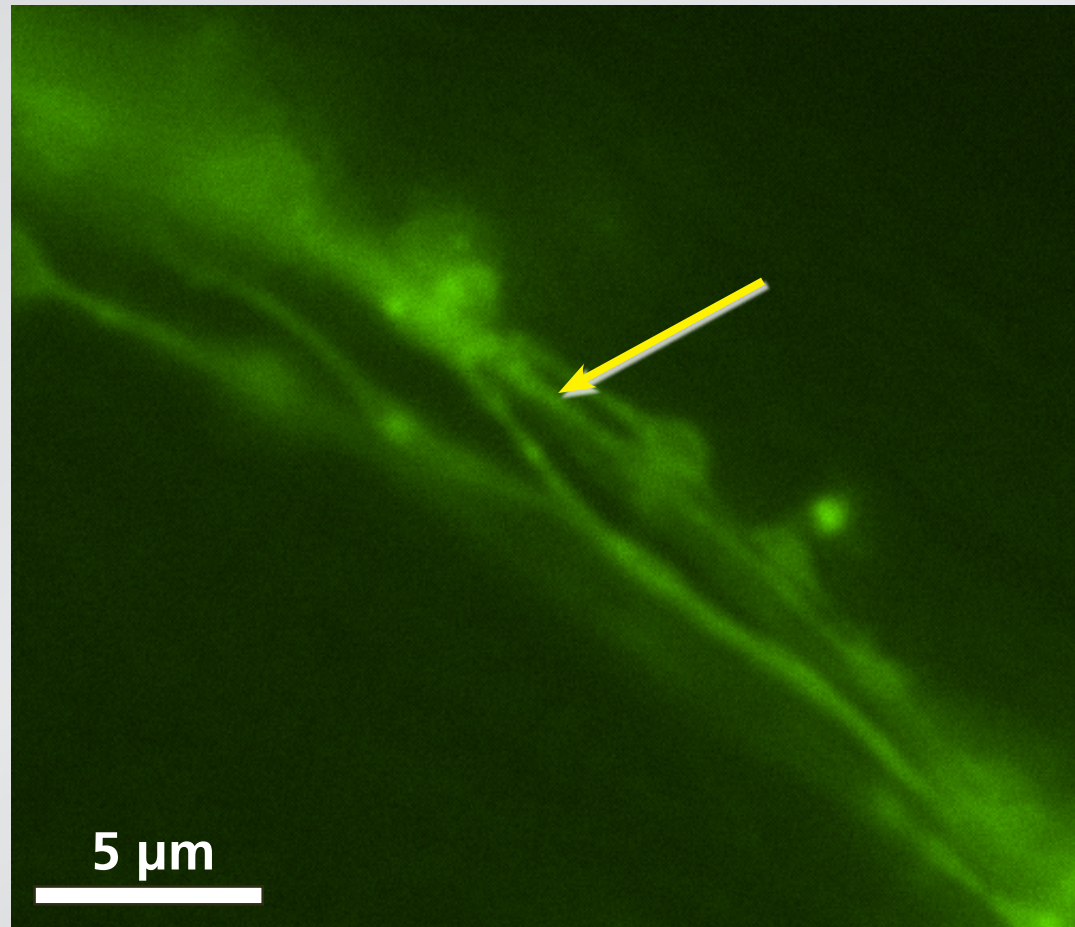
Nanoneurosurgery

cut single dendrite in amphid bundle



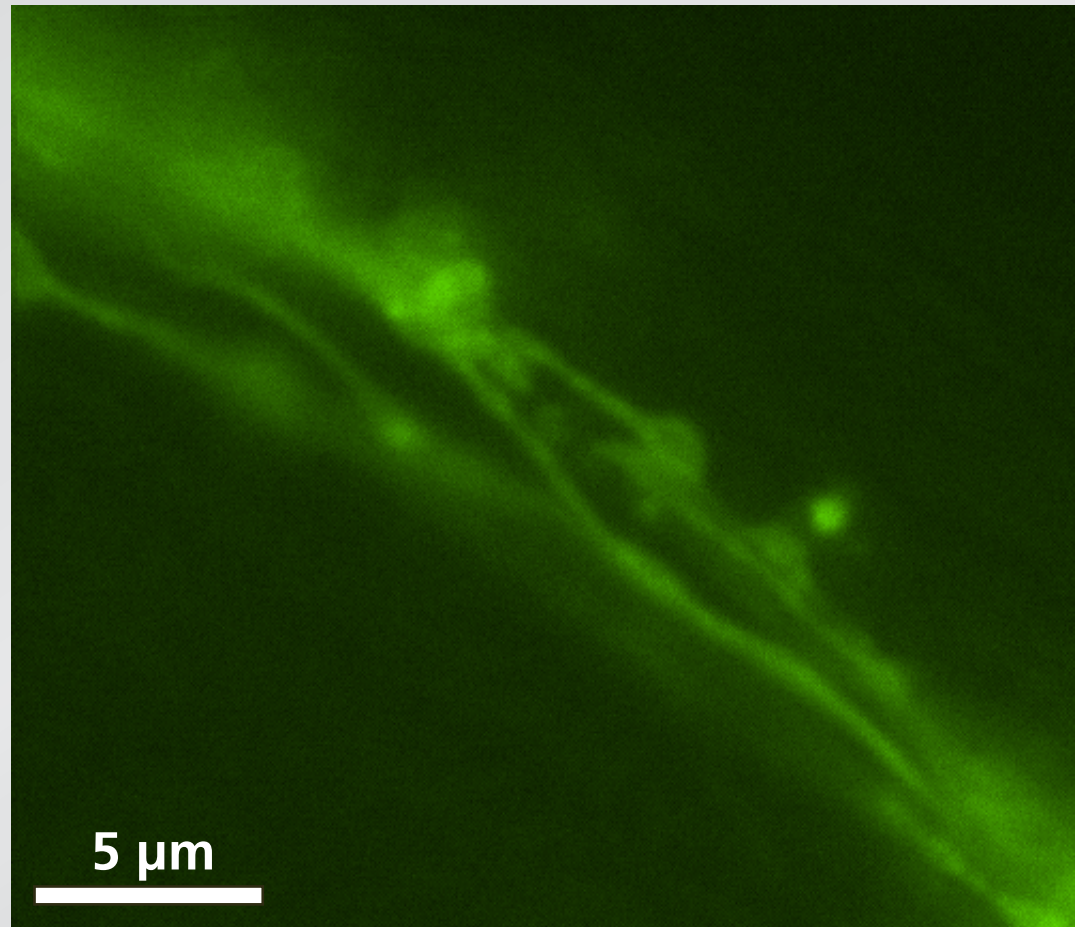
Nanoneurosurgery

cut single dendrite in amphid bundle



Nanoneurosurgery

cut single dendrite in amphid bundle

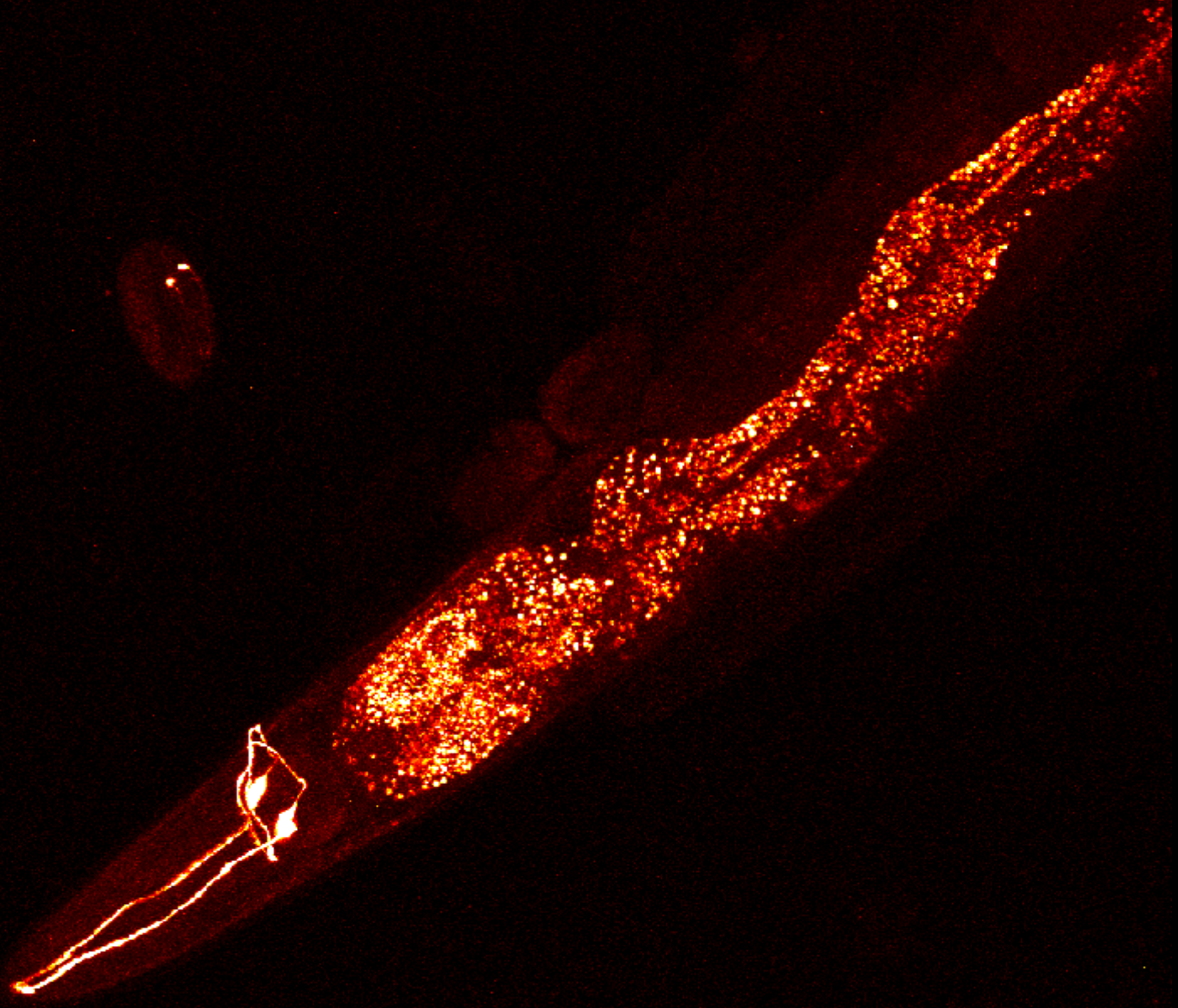


Nanoneurosurgery

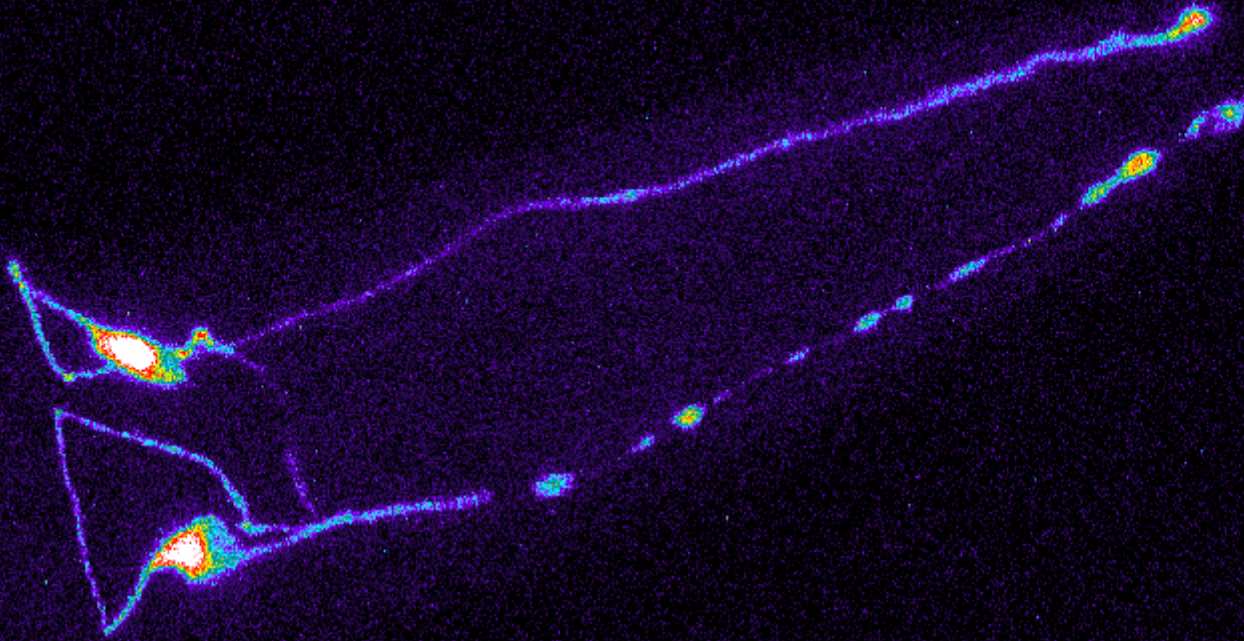
ASD neurons

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

Nanoneurosurgery



Nanoneurosurgery



Nanoneurosurgery

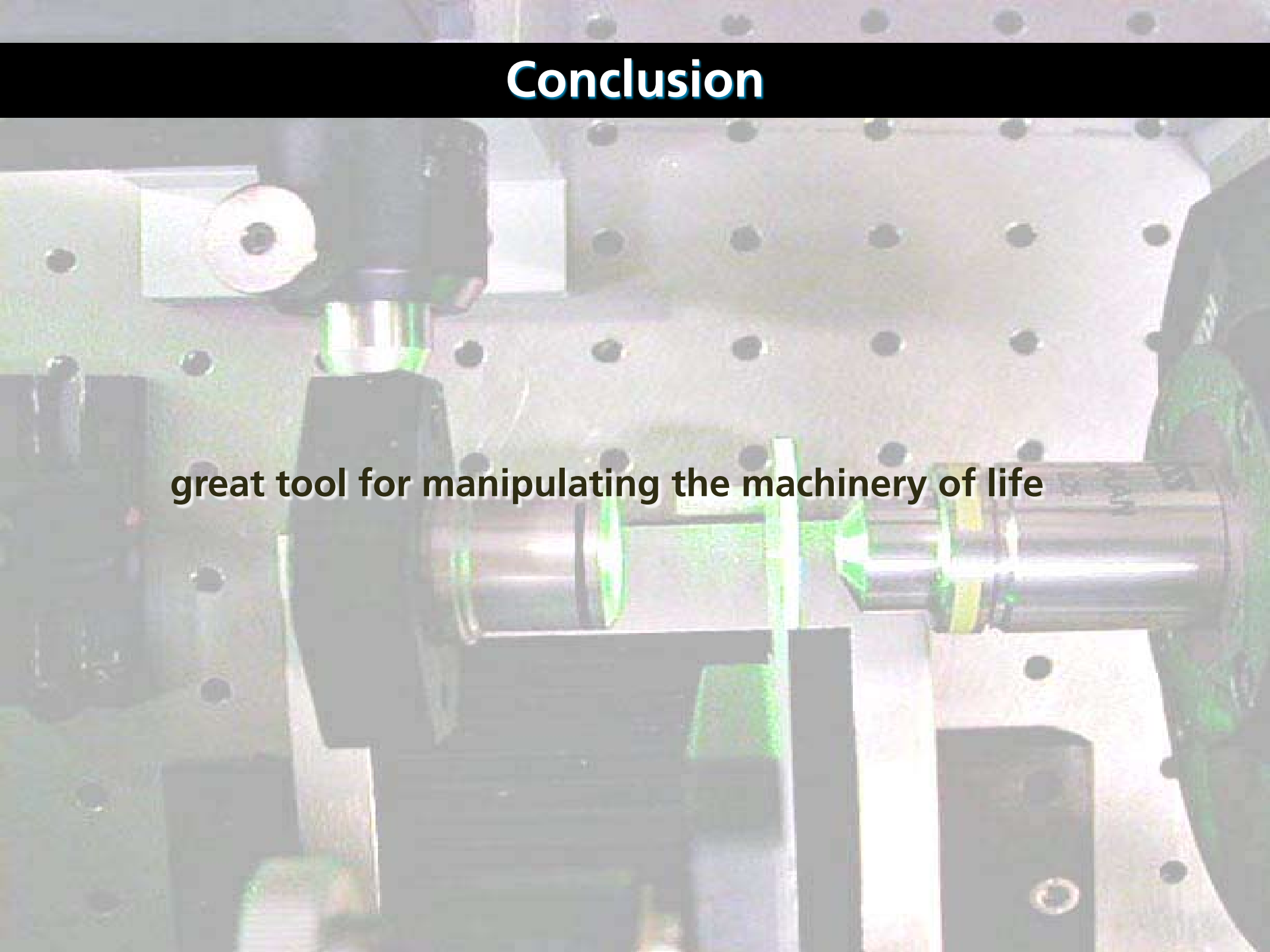
temperature sensing occurs at tip of dendrite

Summary

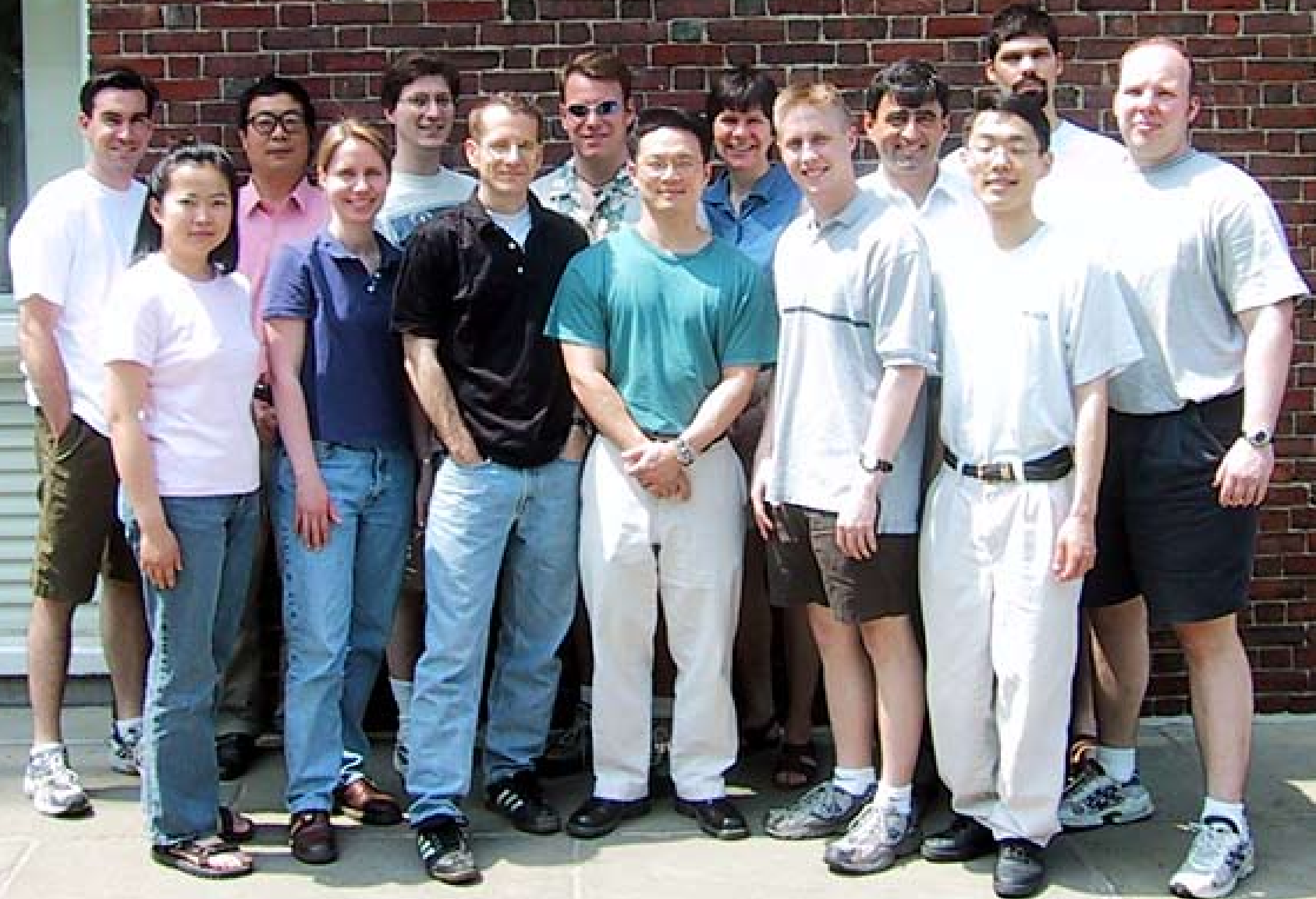
- **manipulate on subcellular, submicrometer scale**
- **penetrate in bulk without compromising viability**
- **study cell structure and mechanics**
- **study neurobiological basis of behavior**

Conclusion

great tool for manipulating the machinery of life



GORDON MCKAY
LABORATORY OF
APPLIED SCIENCE



GORDON VICKAY
LABORATORY OF
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