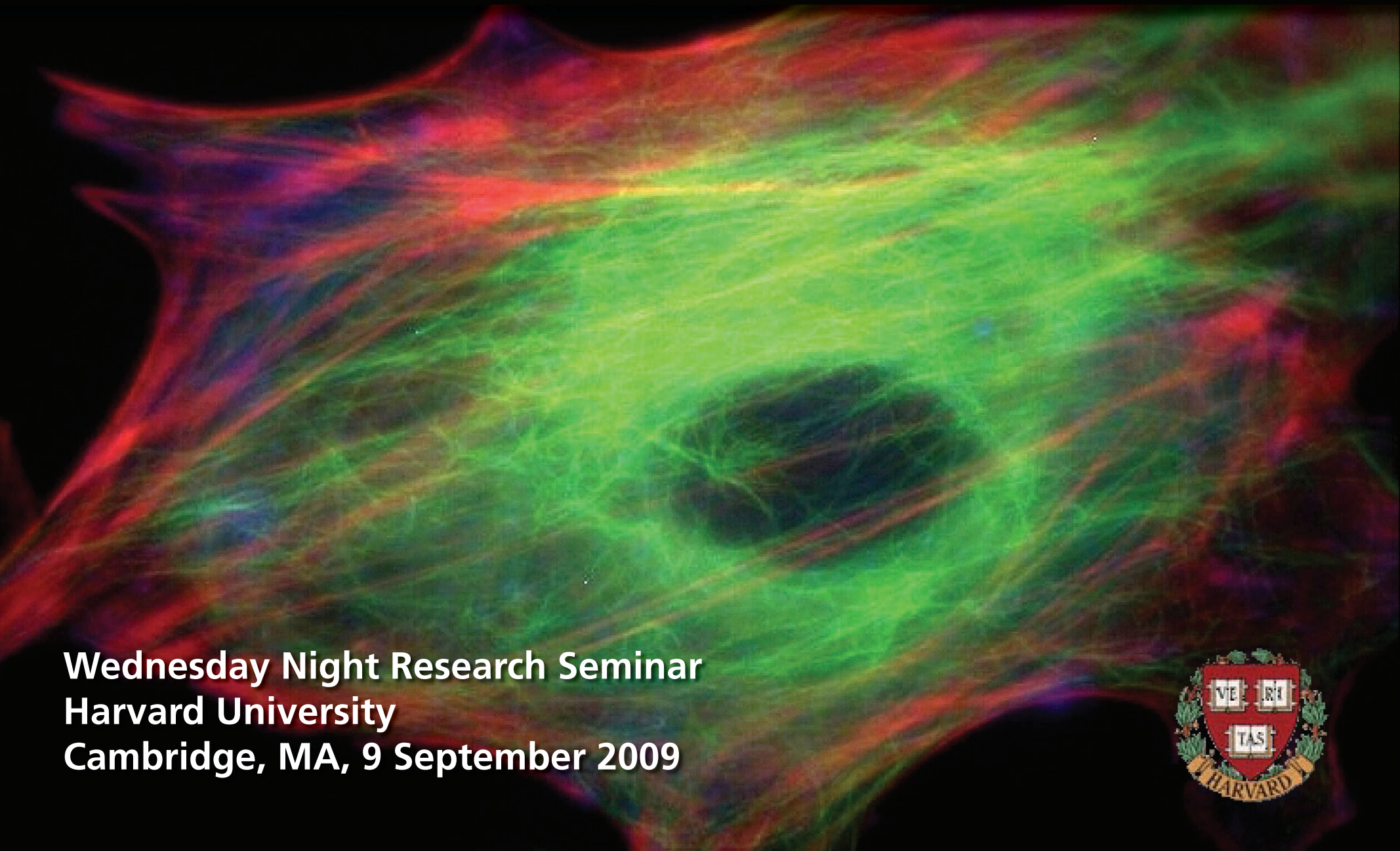
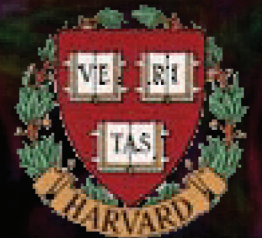


Manipulating cells using ultrashort laser pulses



Wednesday Night Research Seminar
Harvard University
Cambridge, MA, 9 September 2009

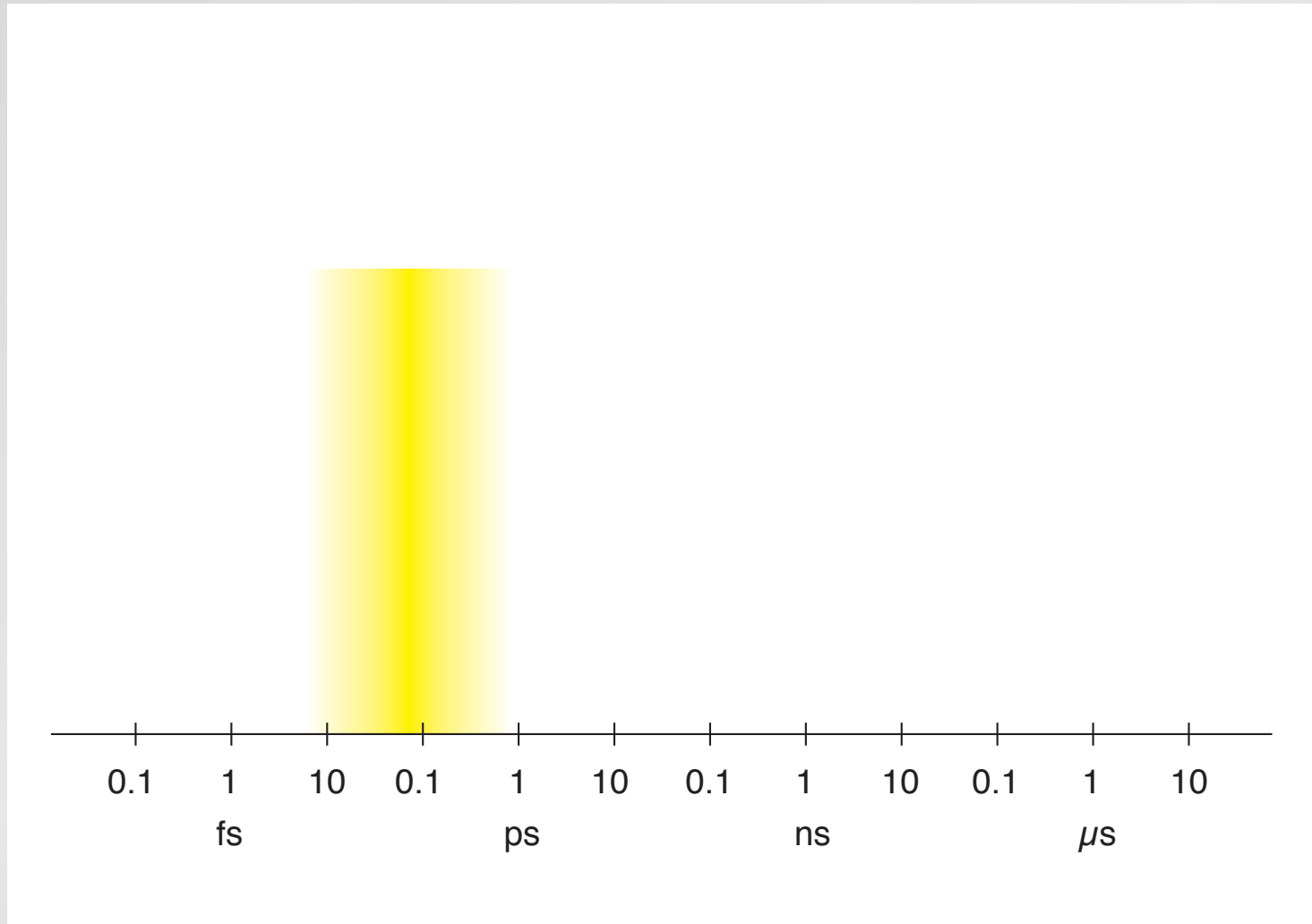


Introduction

why use femtosecond pulses?

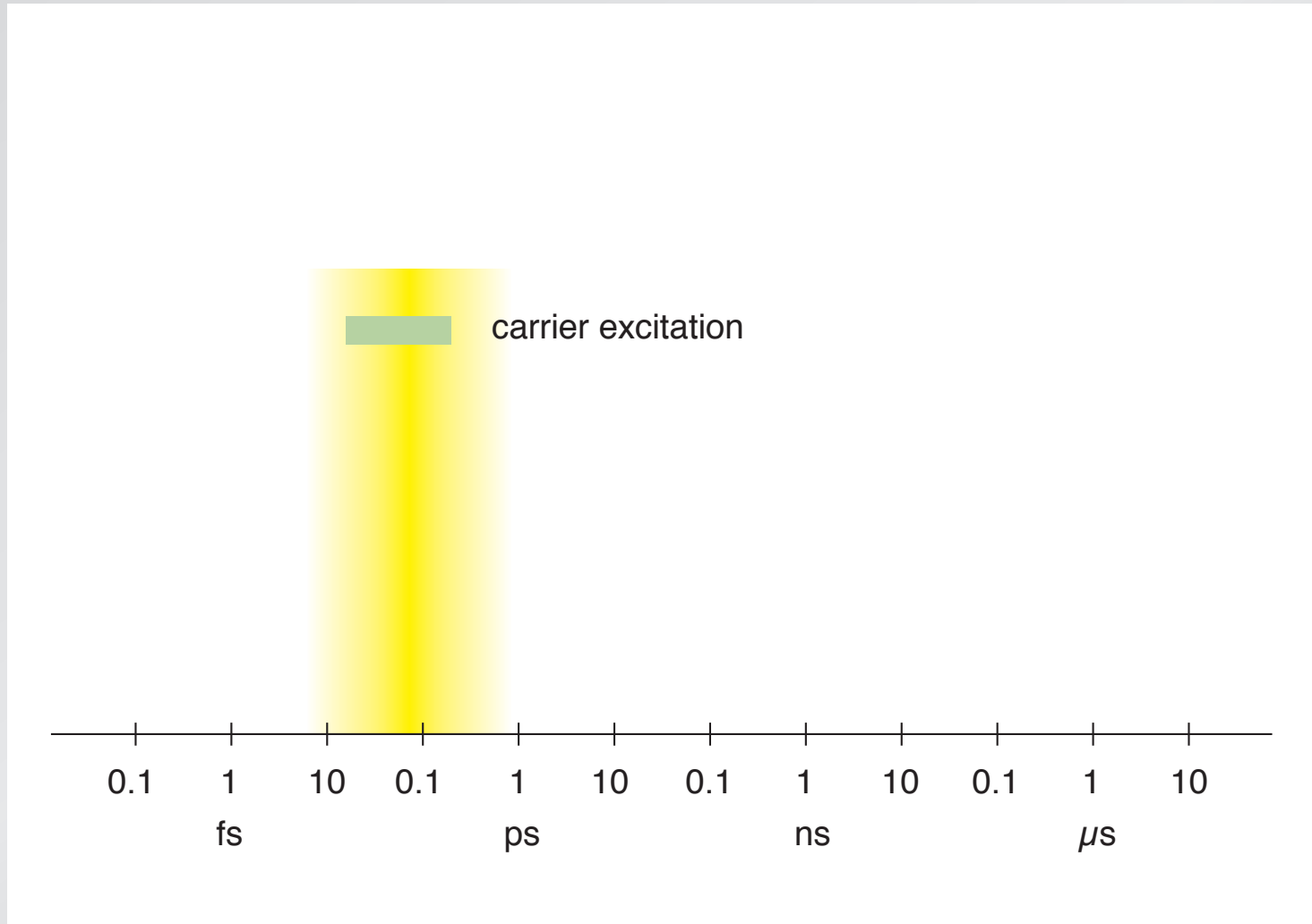
Introduction

relevant time scales



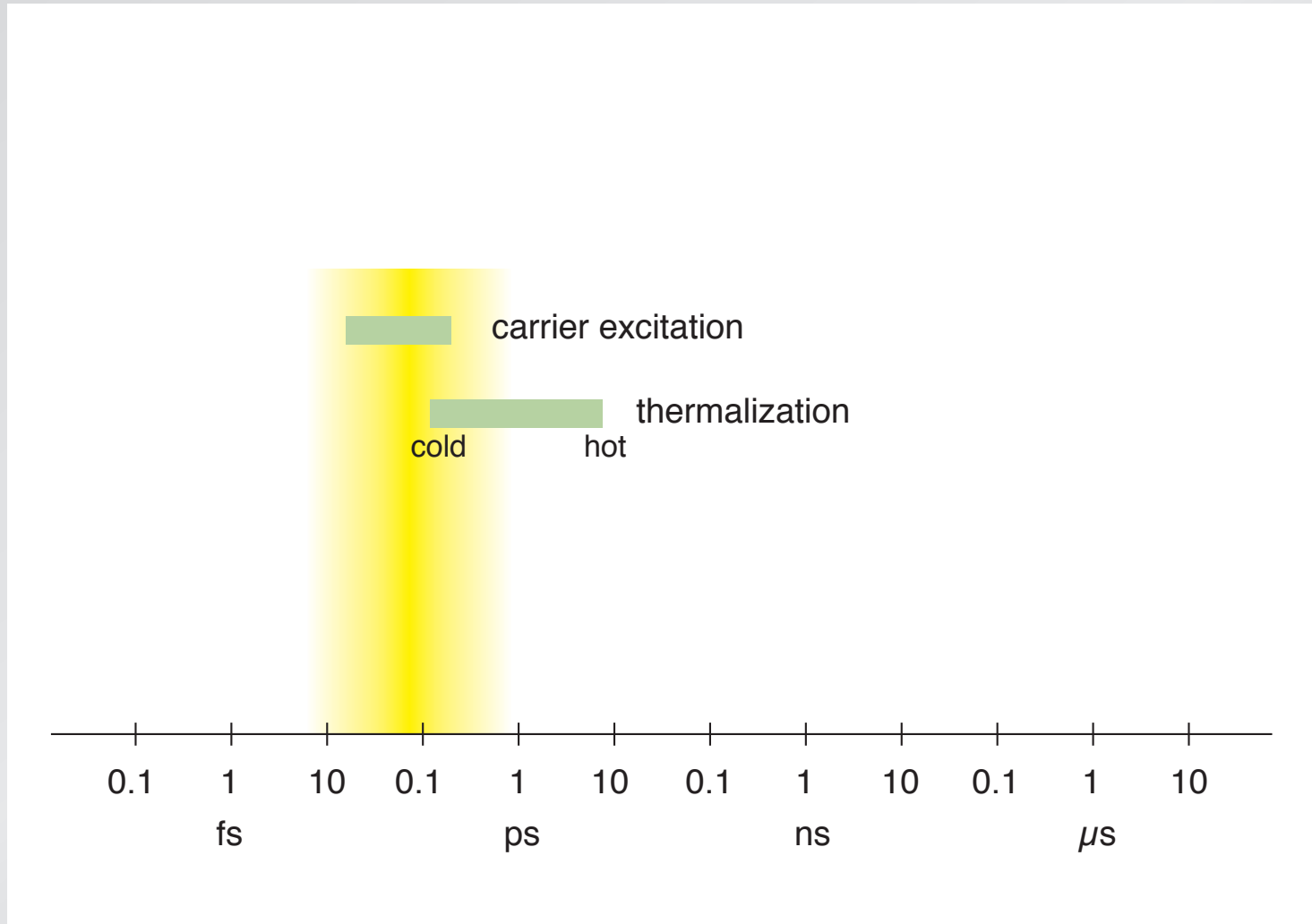
Introduction

relevant time scales



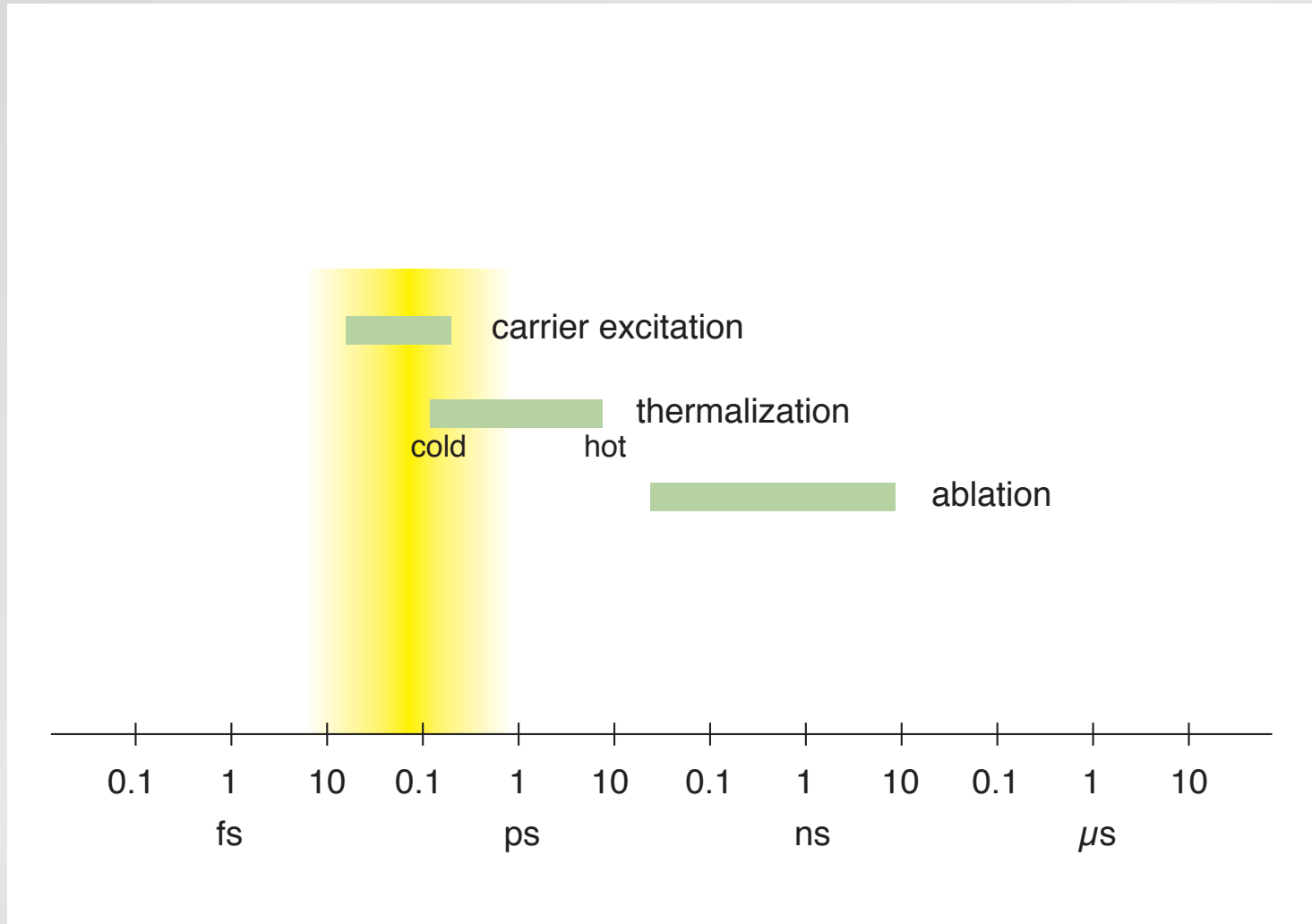
Introduction

relevant time scales



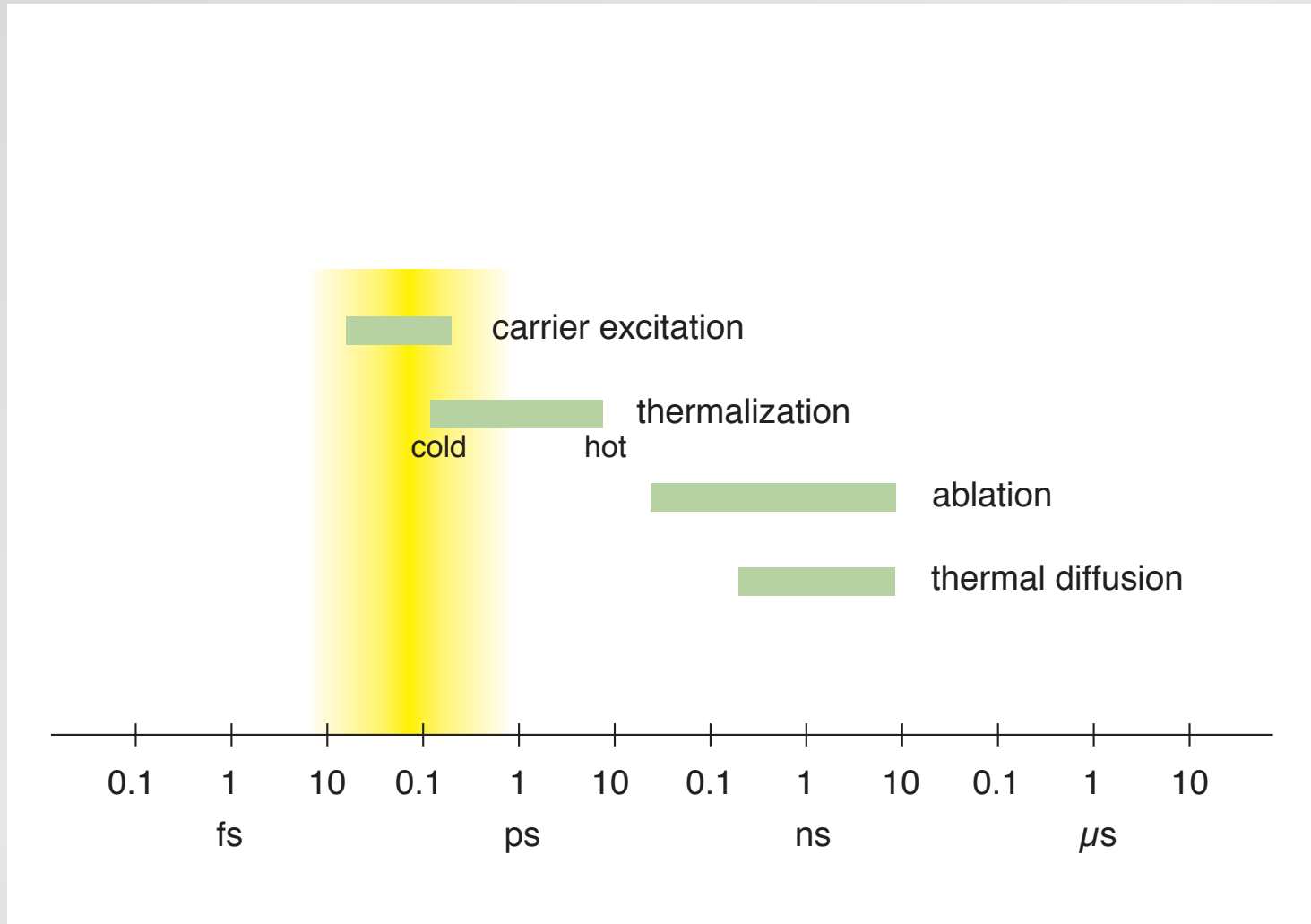
Introduction

relevant time scales



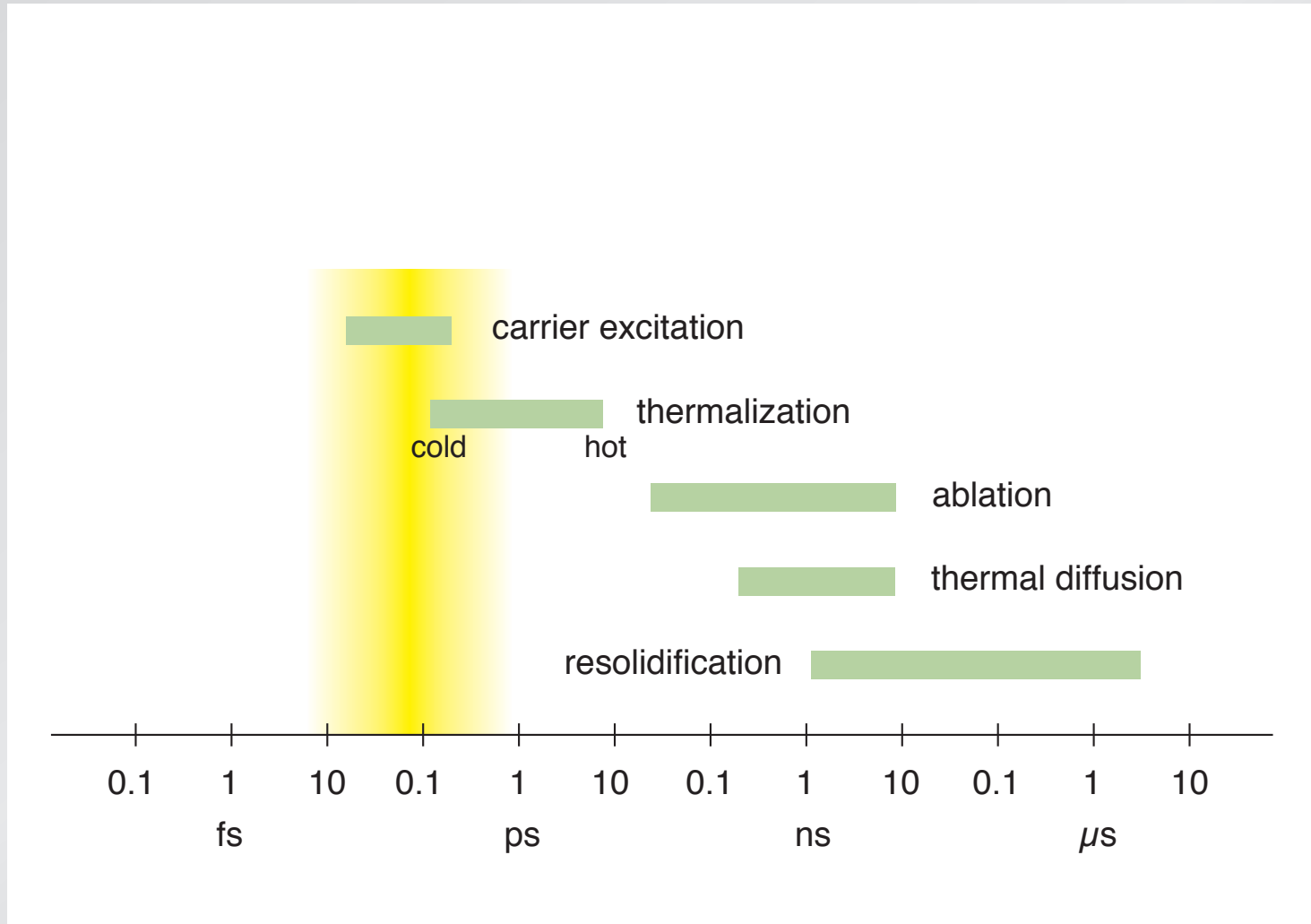
Introduction

relevant time scales



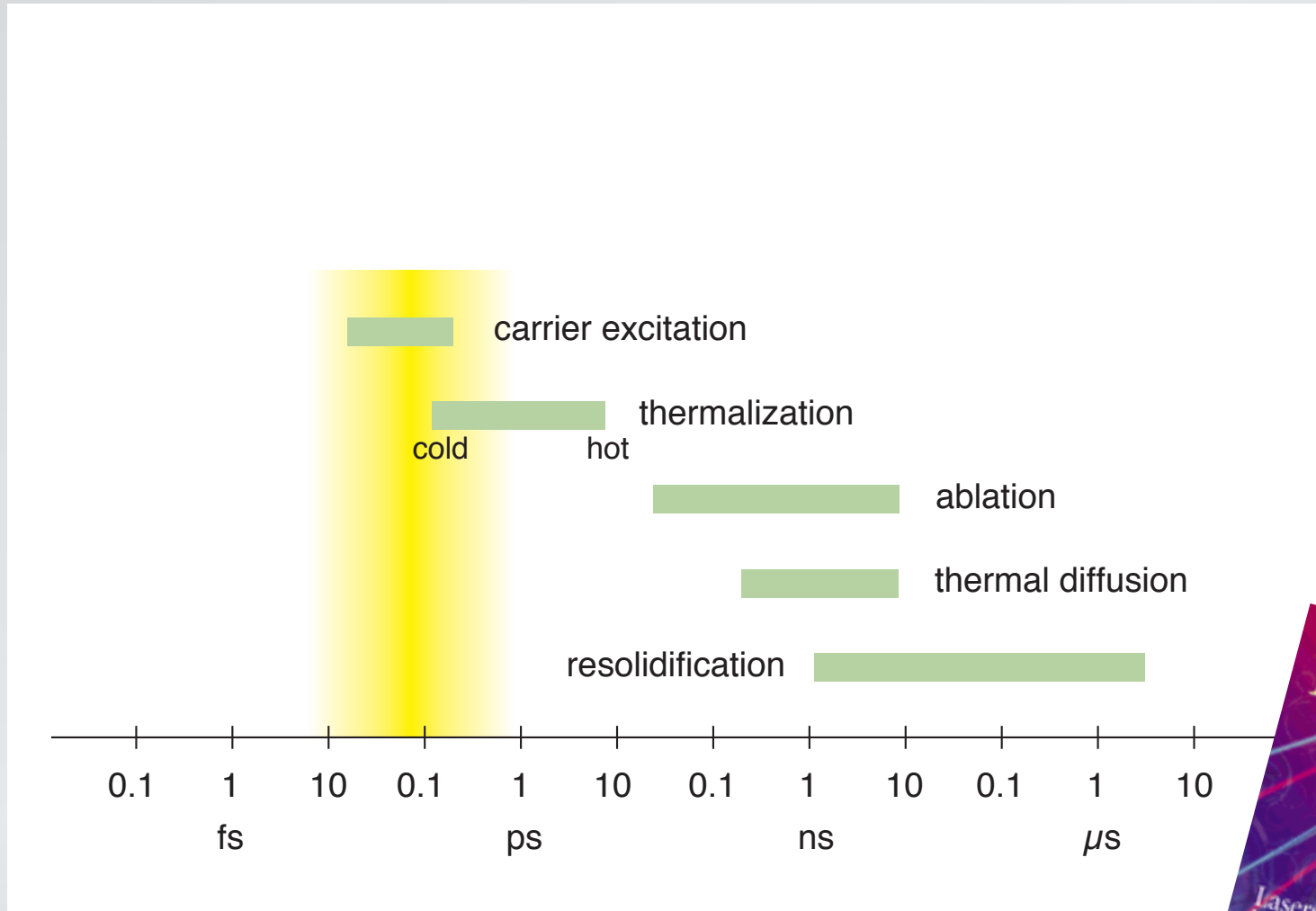
Introduction

relevant time scales

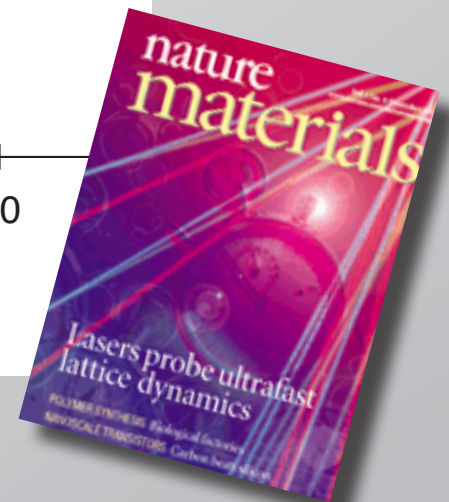


Introduction

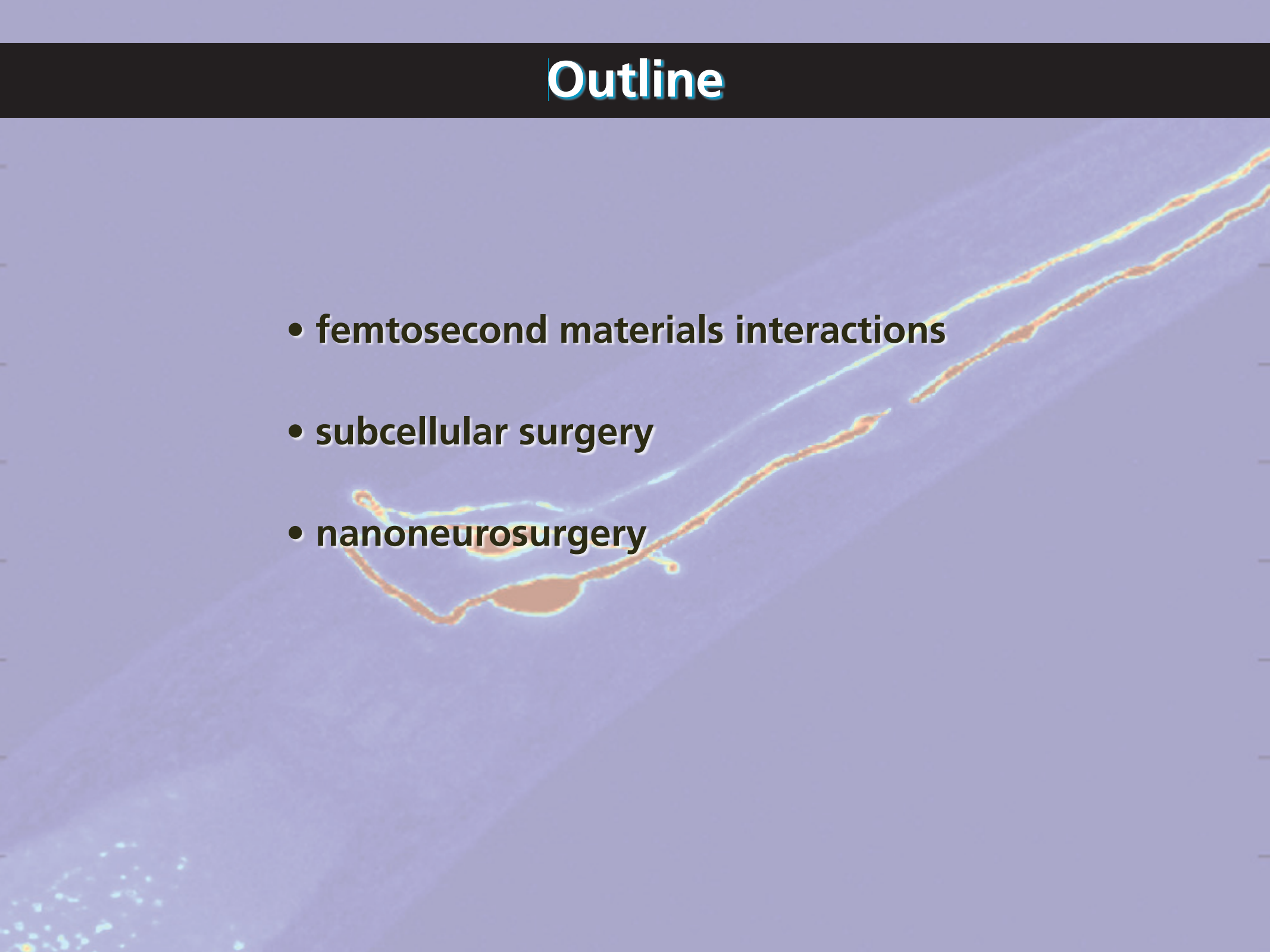
relevant time scales



Nature Materials 1, 217 (2002)



Outline

- femtosecond materials interactions
 - subcellular surgery
 - nanoneurosurgery
- 
- A microscopic image of a biological structure, possibly a neuron or a similar elongated cell, with a highlighted path in orange and yellow. The path starts from the bottom left, moves up and right, then down and right, and finally up and right towards the top right. The background is a light blue, textured surface.

Femtosecond materials interactions

Linear optics:

$$\vec{P} = \chi \vec{E}$$

Femtosecond materials interactions

Linear optics:

$$\vec{P} = \chi \vec{E}$$

Nonlinear polarization:

$$P = \chi^{(1)} E + \chi^{(2)} E^2 + \chi^{(3)} E^3 + \dots$$

Femtosecond materials interactions

Linear optics:

$$\vec{P} = \chi \vec{E}$$

Nonlinear polarization:

$$P = \chi^{(1)} E + \chi^{(2)} E^2 + \chi^{(3)} E^3 + \dots$$

and so:

$$P = P^{(1)} + P^{(2)} + P^{(3)} + \dots$$

Femtosecond materials interactions

Linear optics:

$$\vec{P} = \chi \vec{E}$$

Nonlinear polarization:

$$P = \chi^{(1)} E + \chi^{(2)} E^2 + \chi^{(3)} E^3 + \dots$$

and so:

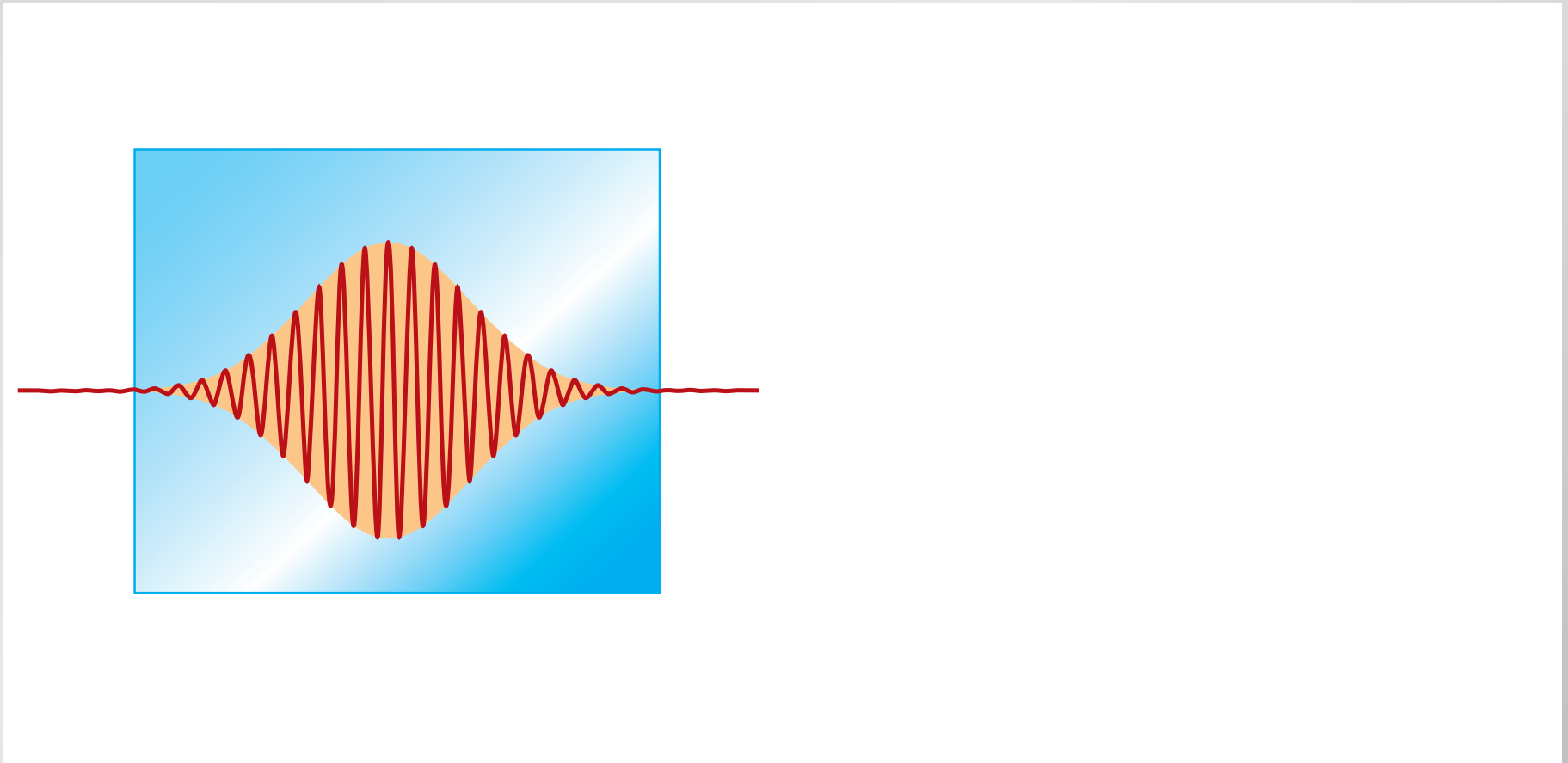
$$P = P^{(1)} + P^{(2)} + P^{(3)} + \dots$$

$$P^{(2)} \approx P^{(1)} \text{ when } E = E_{at} \approx \frac{e}{a}, \text{ and so } \chi^{(n)} \approx \frac{\chi^{(1)}}{E_{at}^{n-1}}.$$

Femtosecond materials interactions

Intensity-dependent index of refraction:

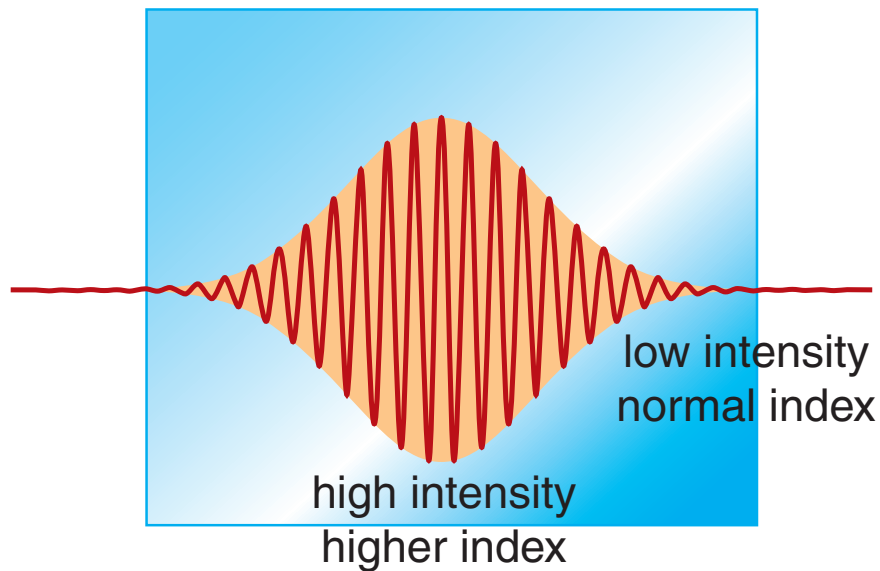
$$n = n_o + n_2 I$$



Femtosecond materials interactions

Intensity-dependent index of refraction:

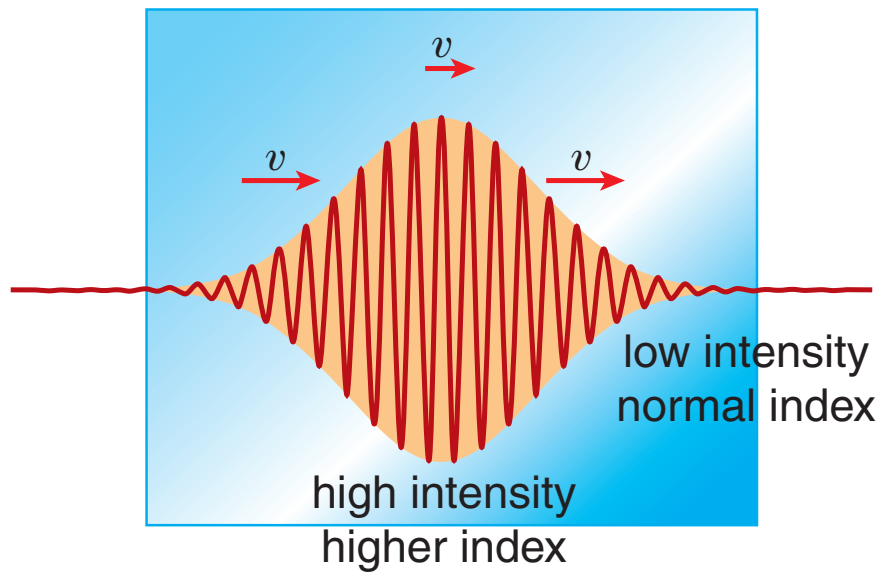
$$n = n_o + n_2 I$$



Femtosecond materials interactions

Intensity-dependent index of refraction:

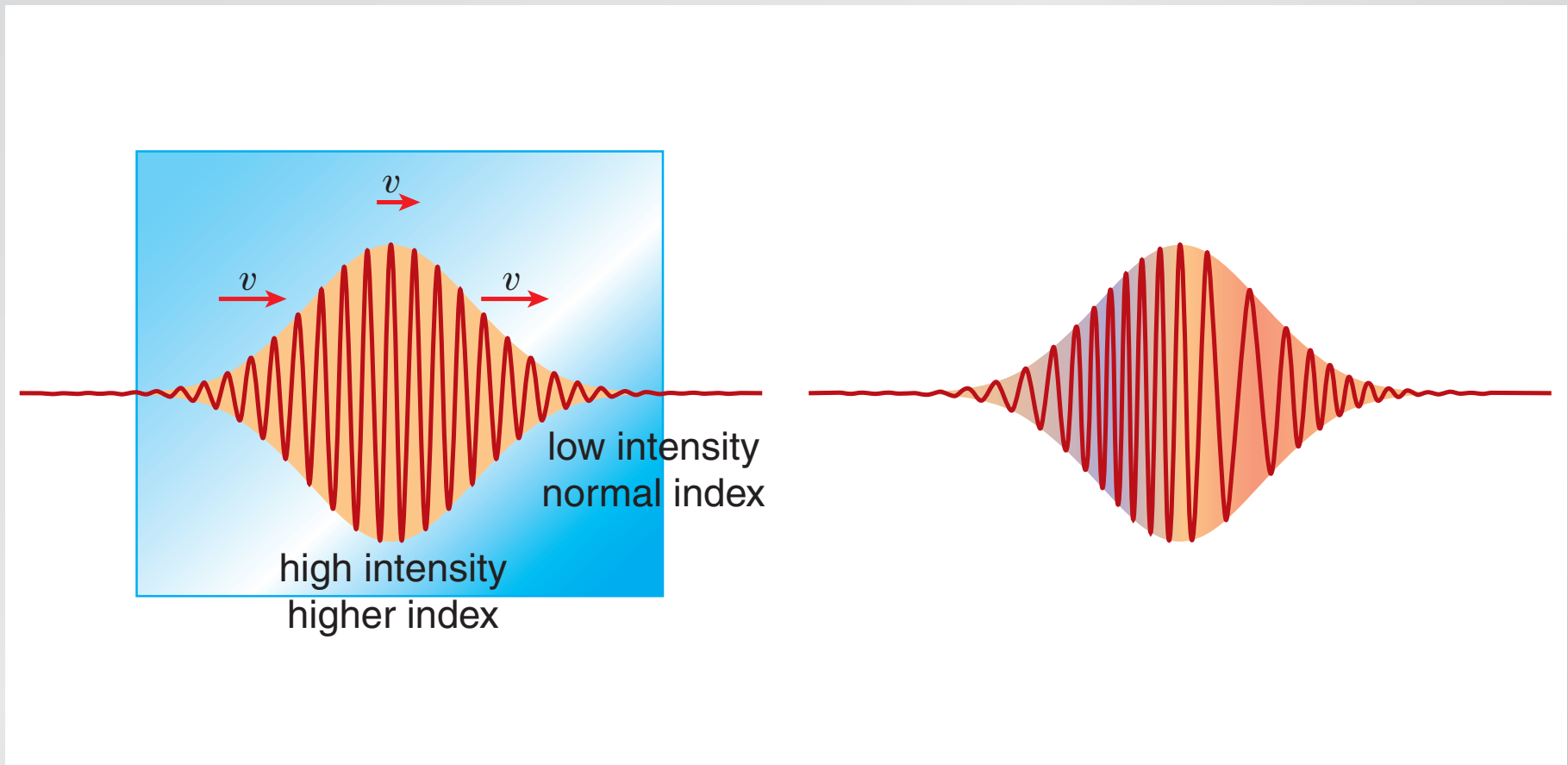
$$n = n_o + n_2 I$$



Femtosecond materials interactions

Intensity-dependent index of refraction:

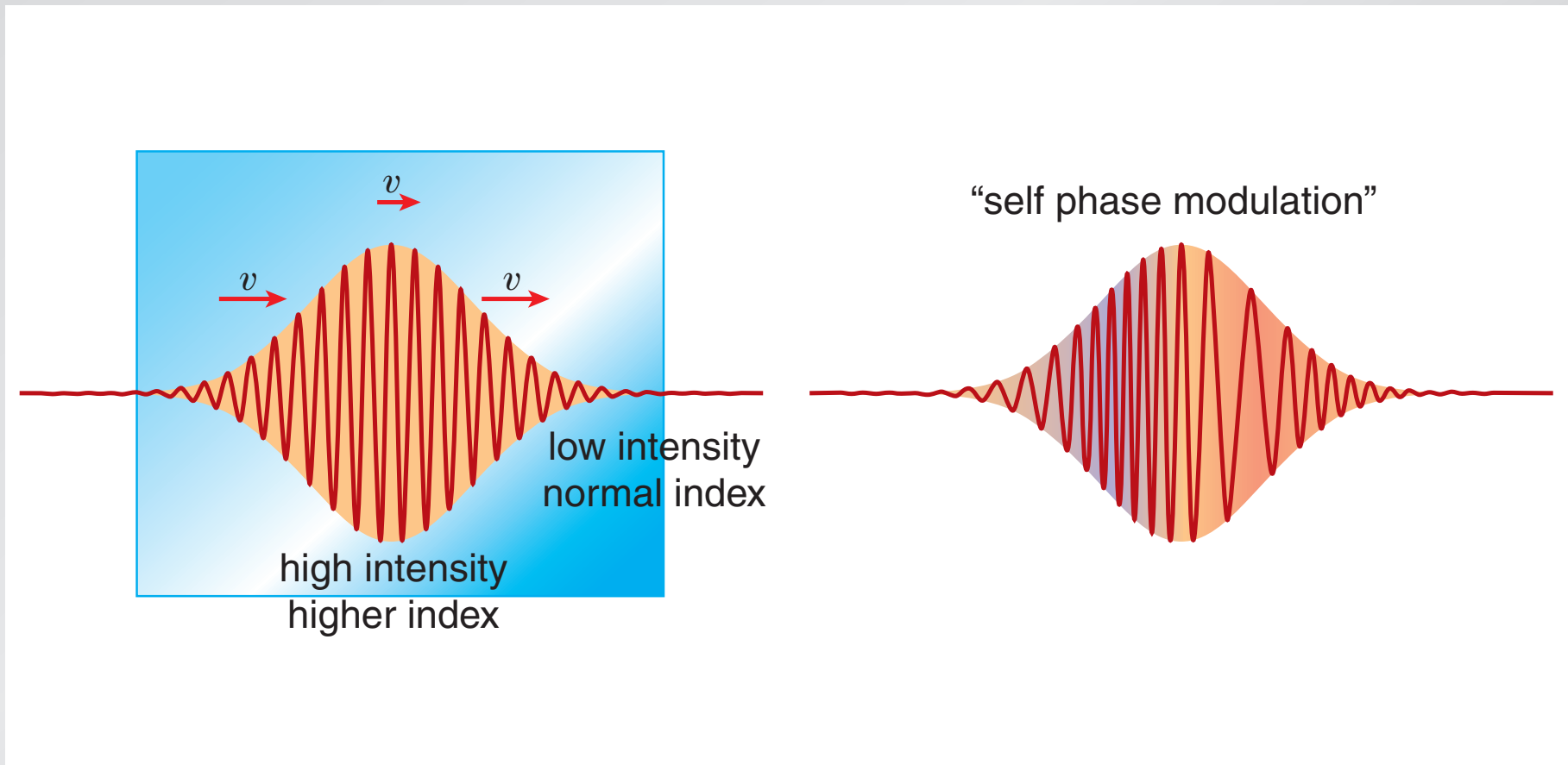
$$n = n_o + n_2 I$$



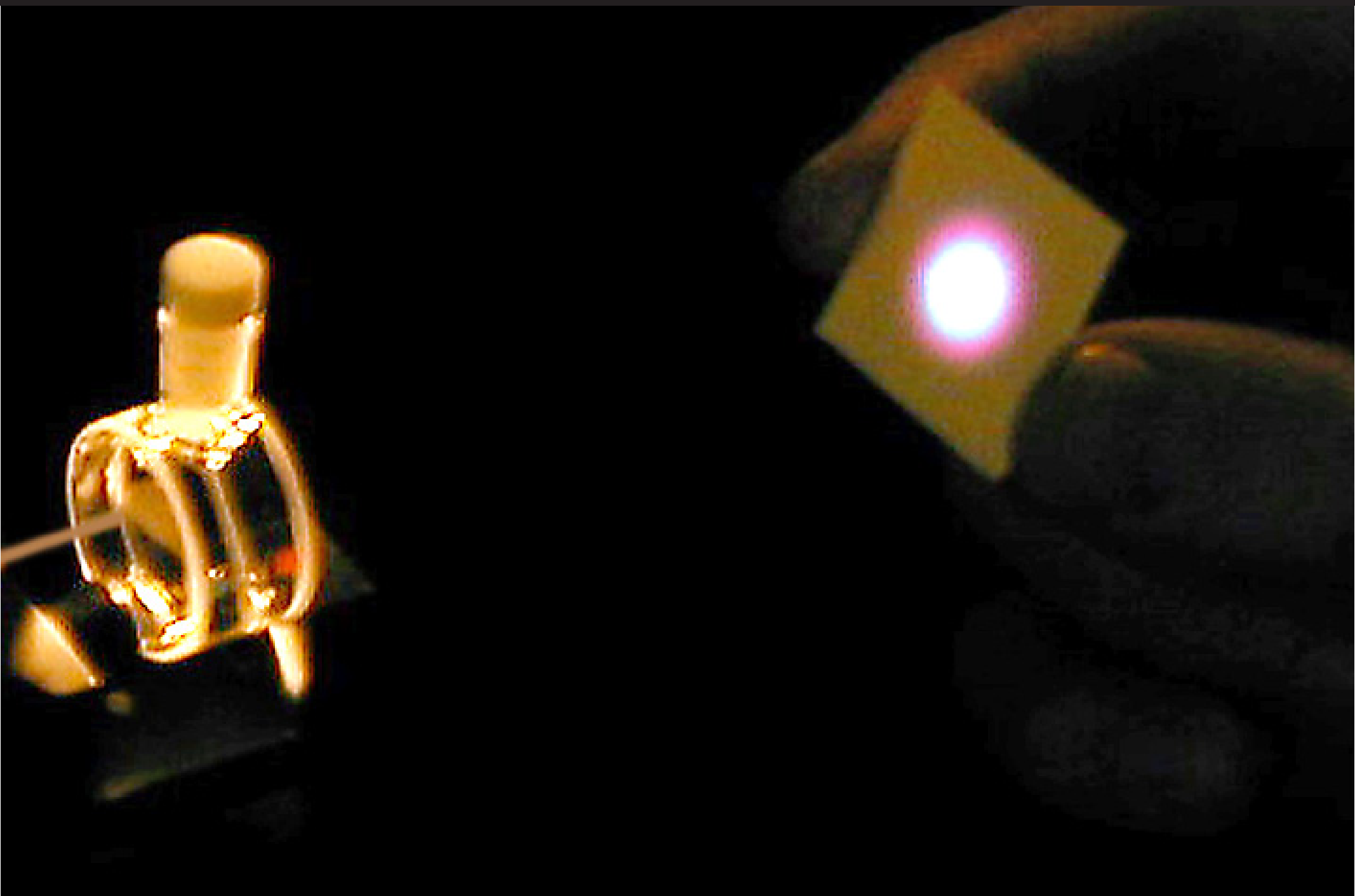
Femtosecond materials interactions

Intensity-dependent index of refraction:

$$n = n_o + n_2 I$$

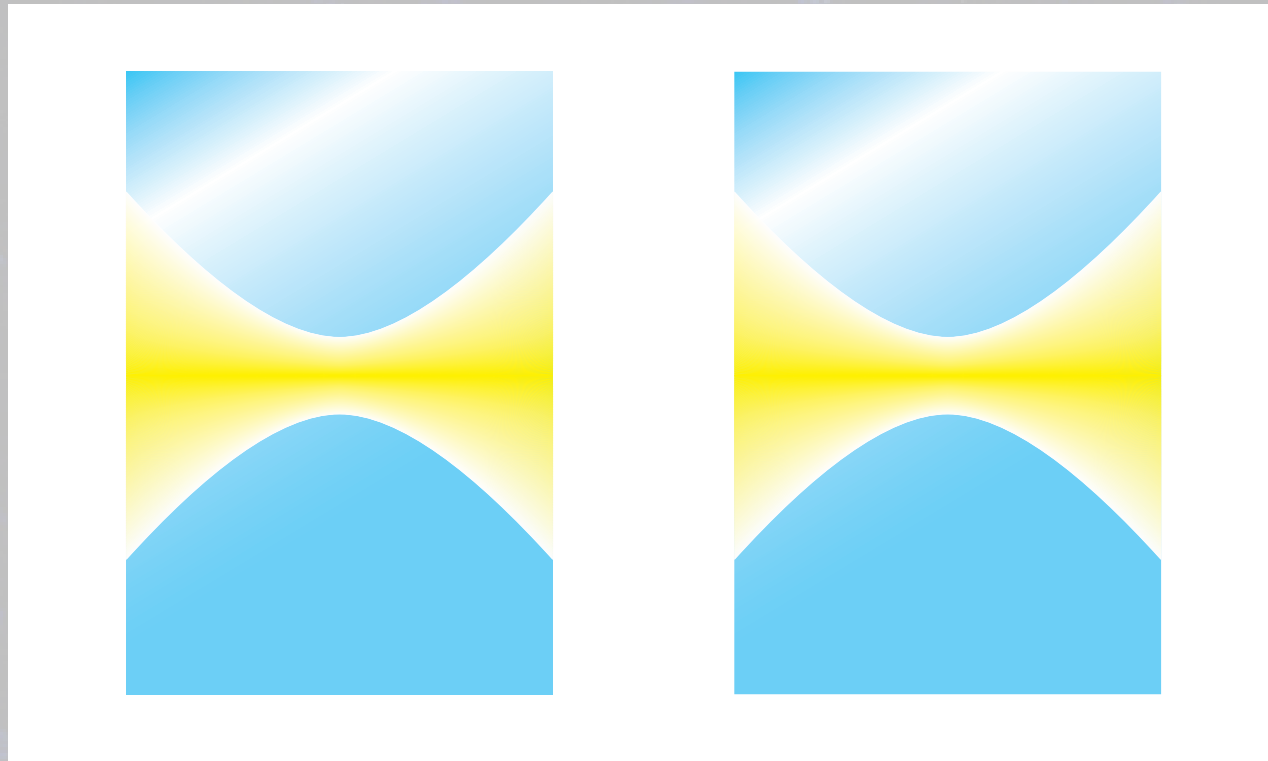


Femtosecond materials interactions



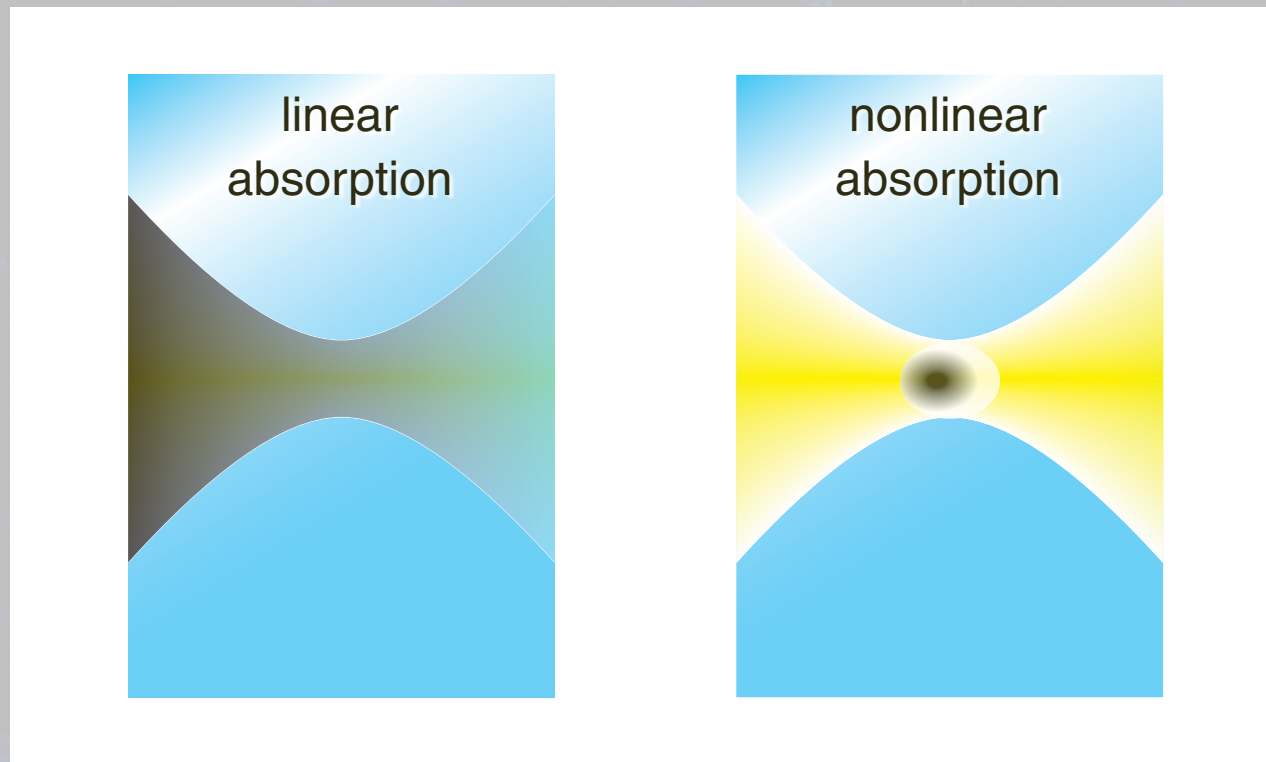
Femtosecond materials interactions

nonlinear interaction provides bulk confinement



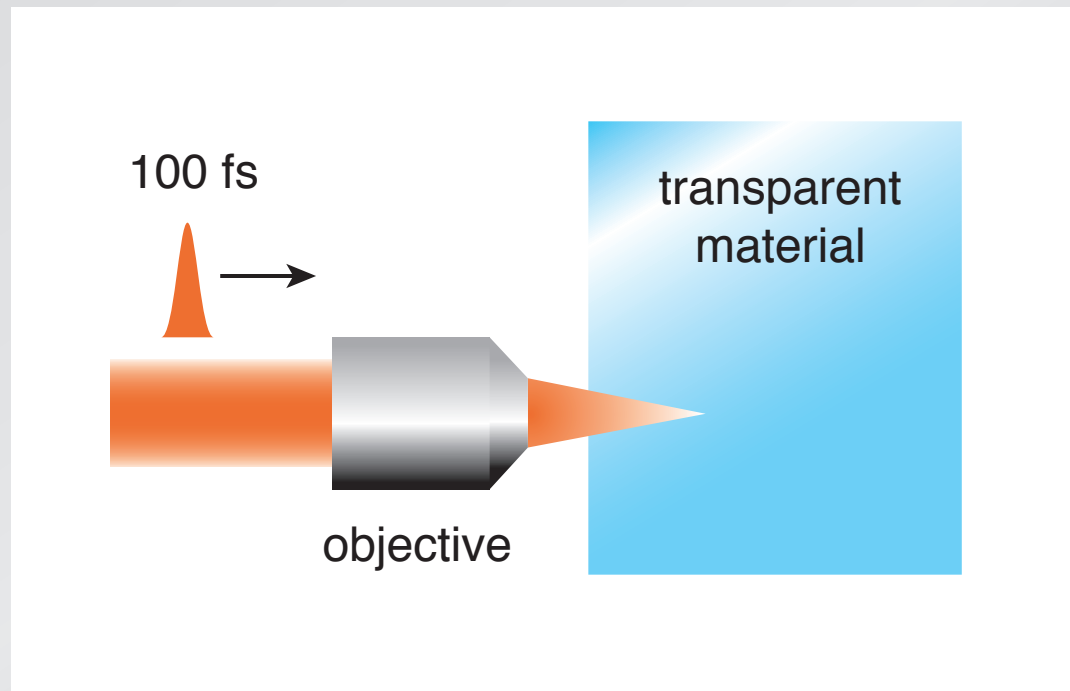
Femtosecond materials interactions

nonlinear interaction provides bulk confinement



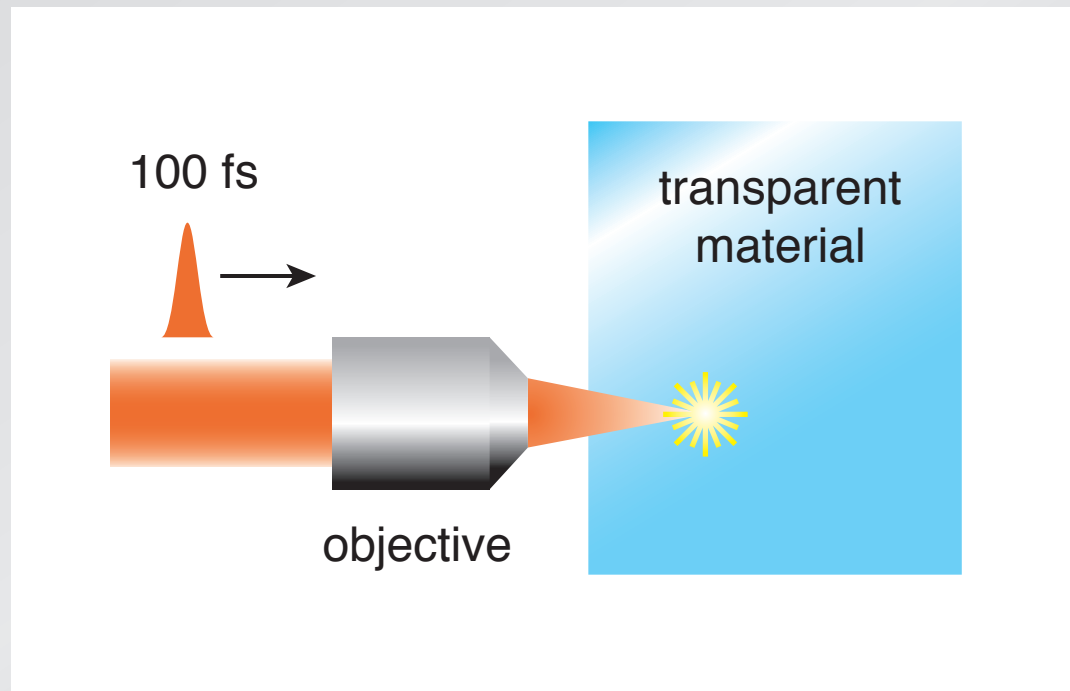
Femtosecond materials interactions

high intensity at focus...



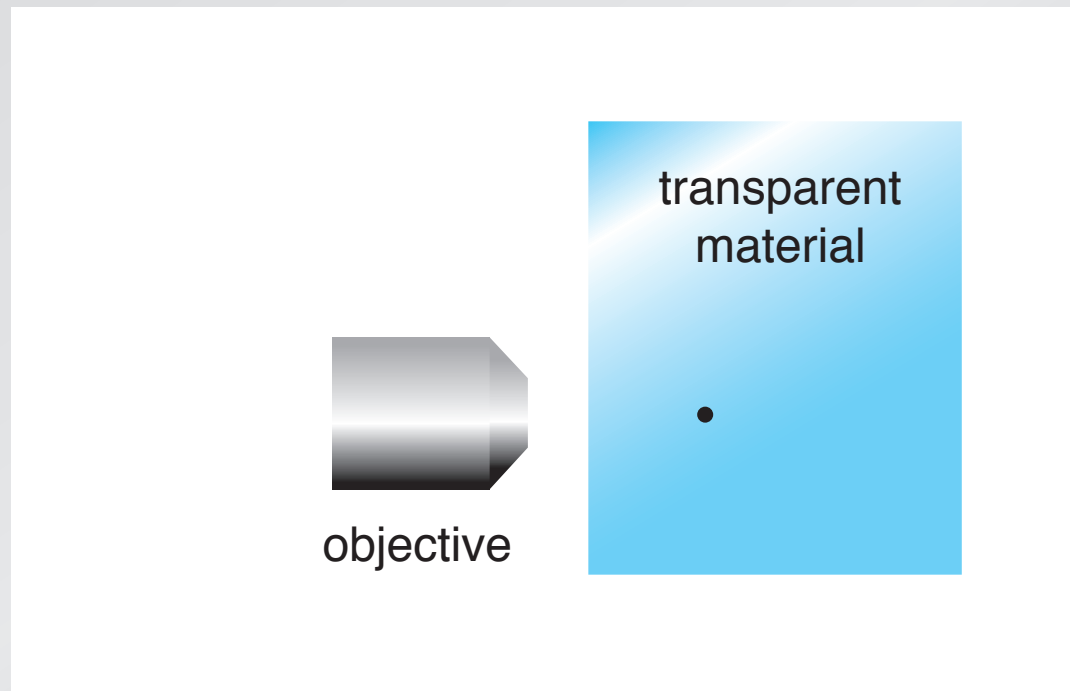
Femtosecond materials interactions

...causes nonlinear ionization...

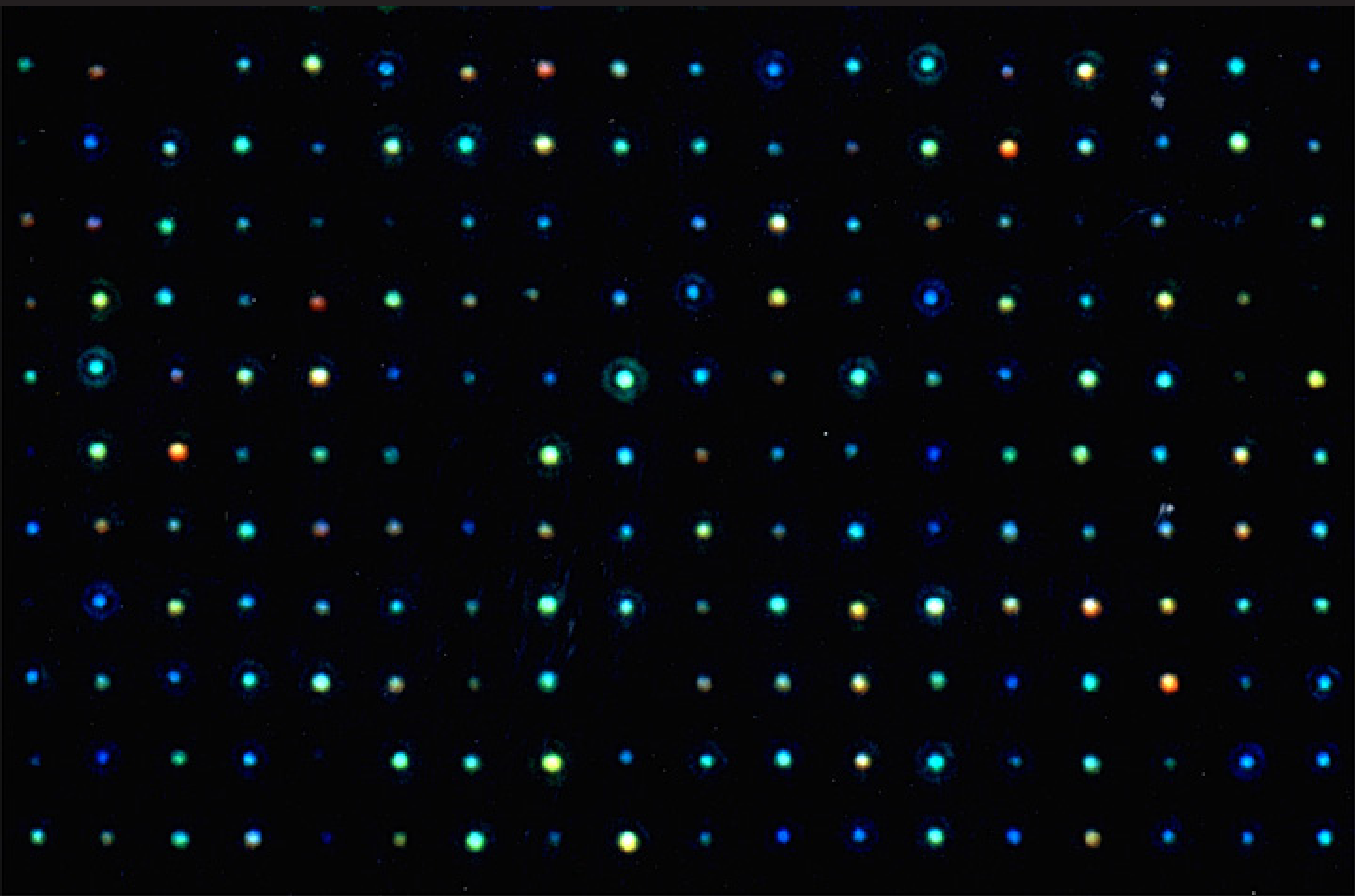


Femtosecond materials interactions

and 'microexplosion' causes microscopic damage...



Femtosecond materials interactions



Outline

- femtosecond materials interactions
 - **subcellular surgery**
 - nanoneurosurgery
- 
- A microscopic image of a biological structure, possibly a cell or tissue, with a blue background. A prominent, elongated, and somewhat irregular structure is highlighted in orange and yellow, running diagonally across the frame. This structure has several distinct, rounded protrusions or lobes. The background shows faint, parallel lines, suggesting a layered or fibrous structure.

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

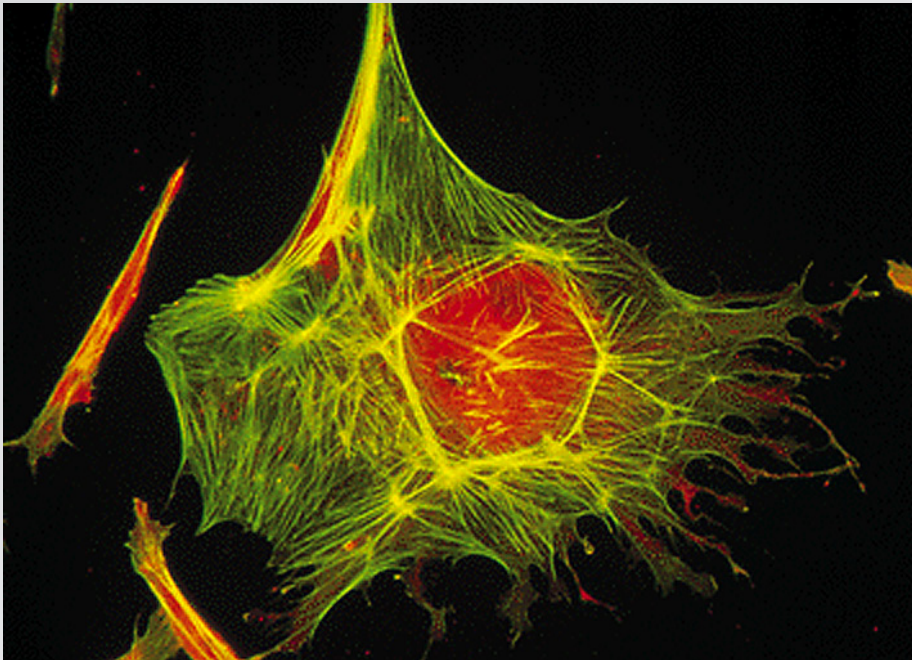
Cytoskeleton

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

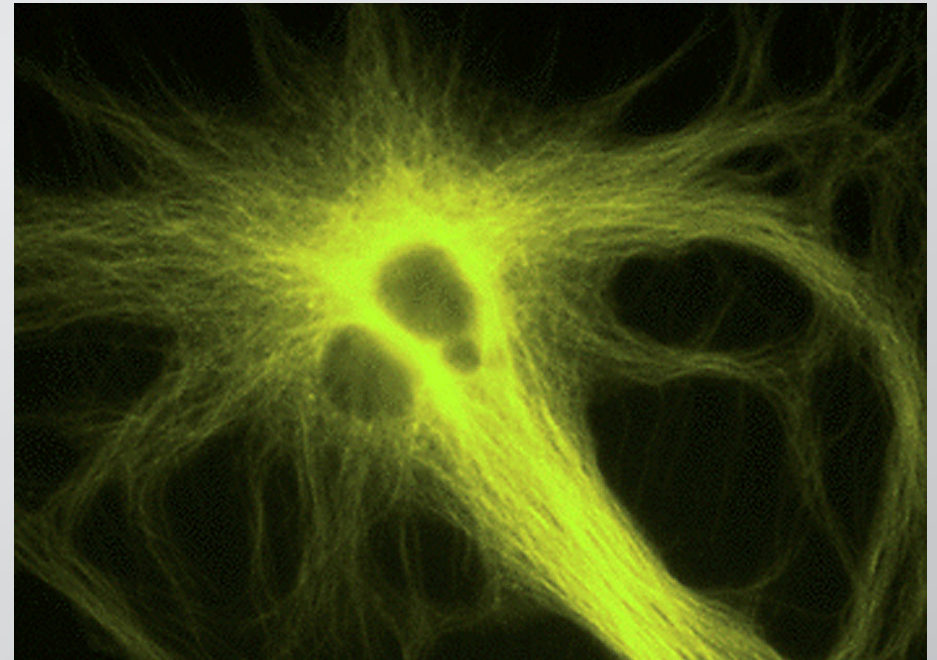
Subcellular surgery

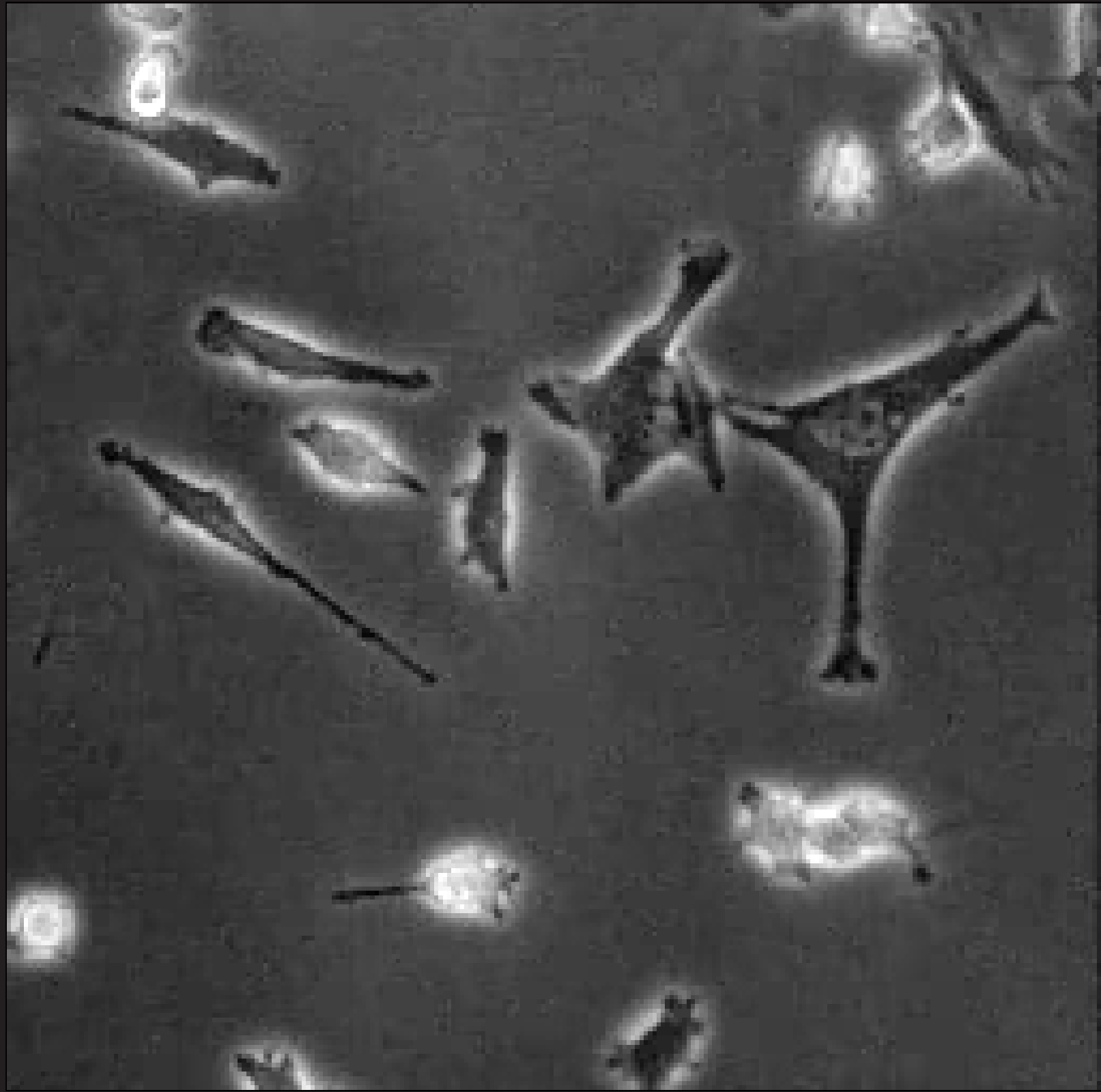
two components

actin fibers



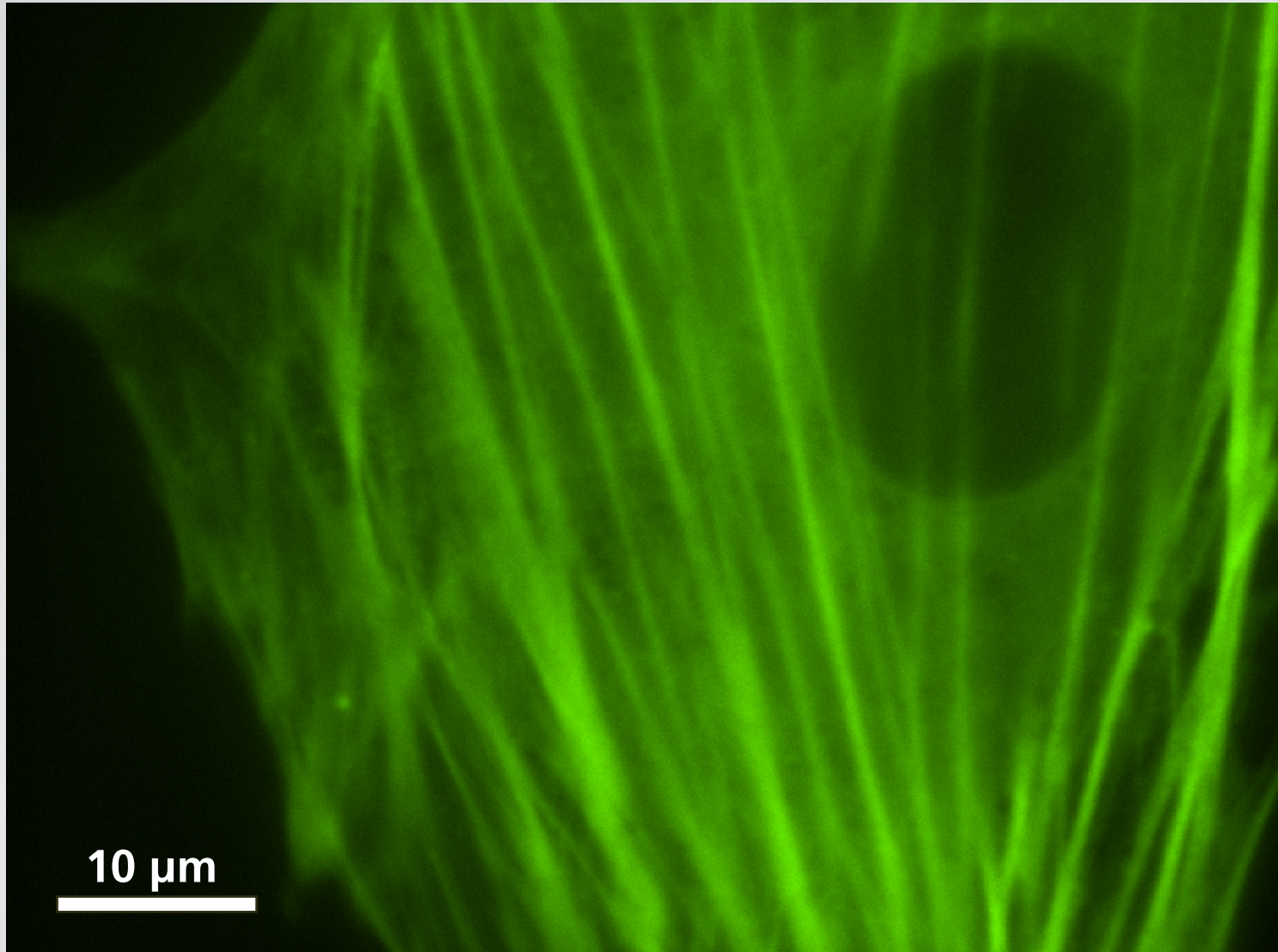
microtubules





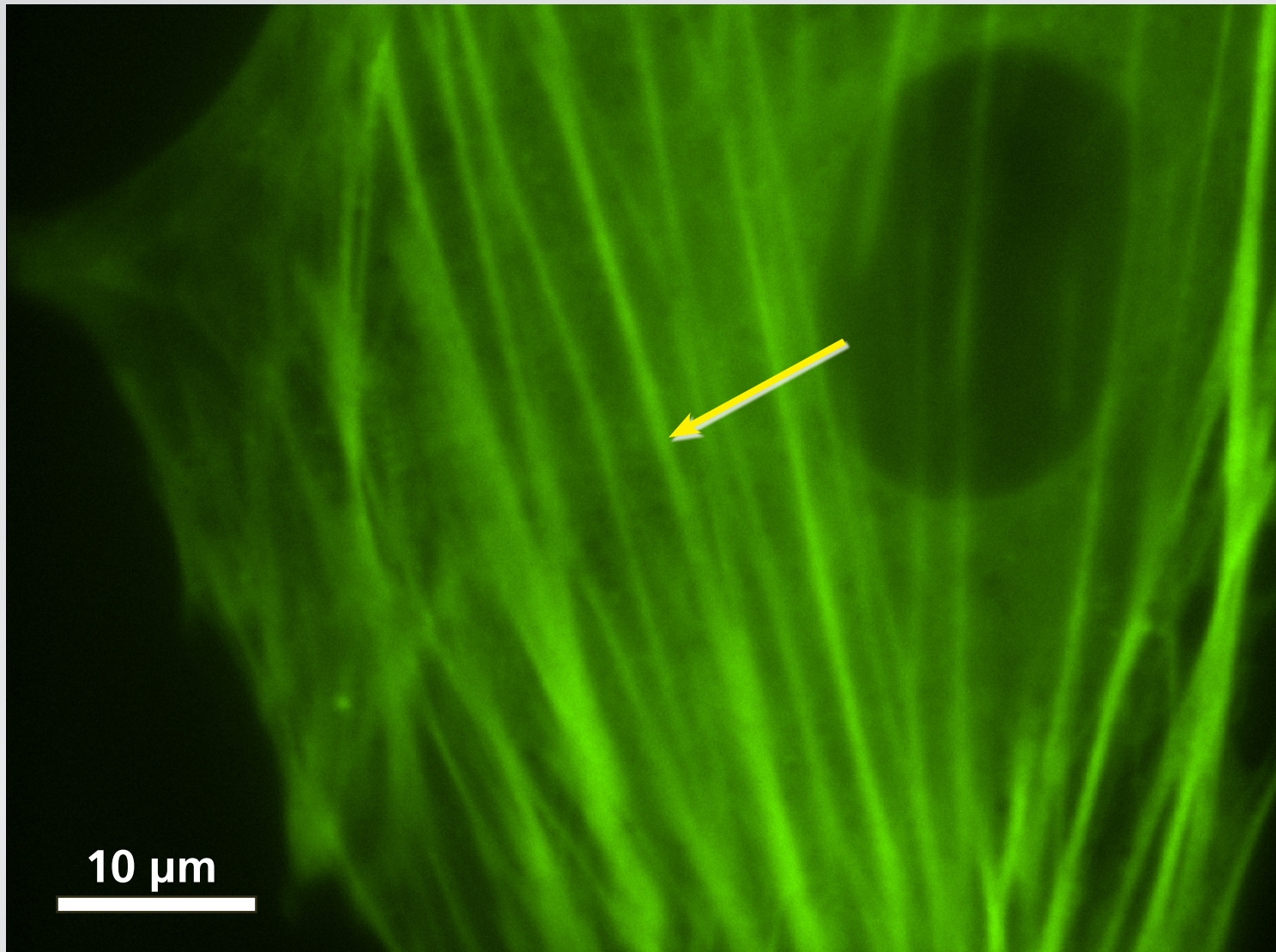
Subcellular surgery

YFP-labeled actin fiber network of a live cell



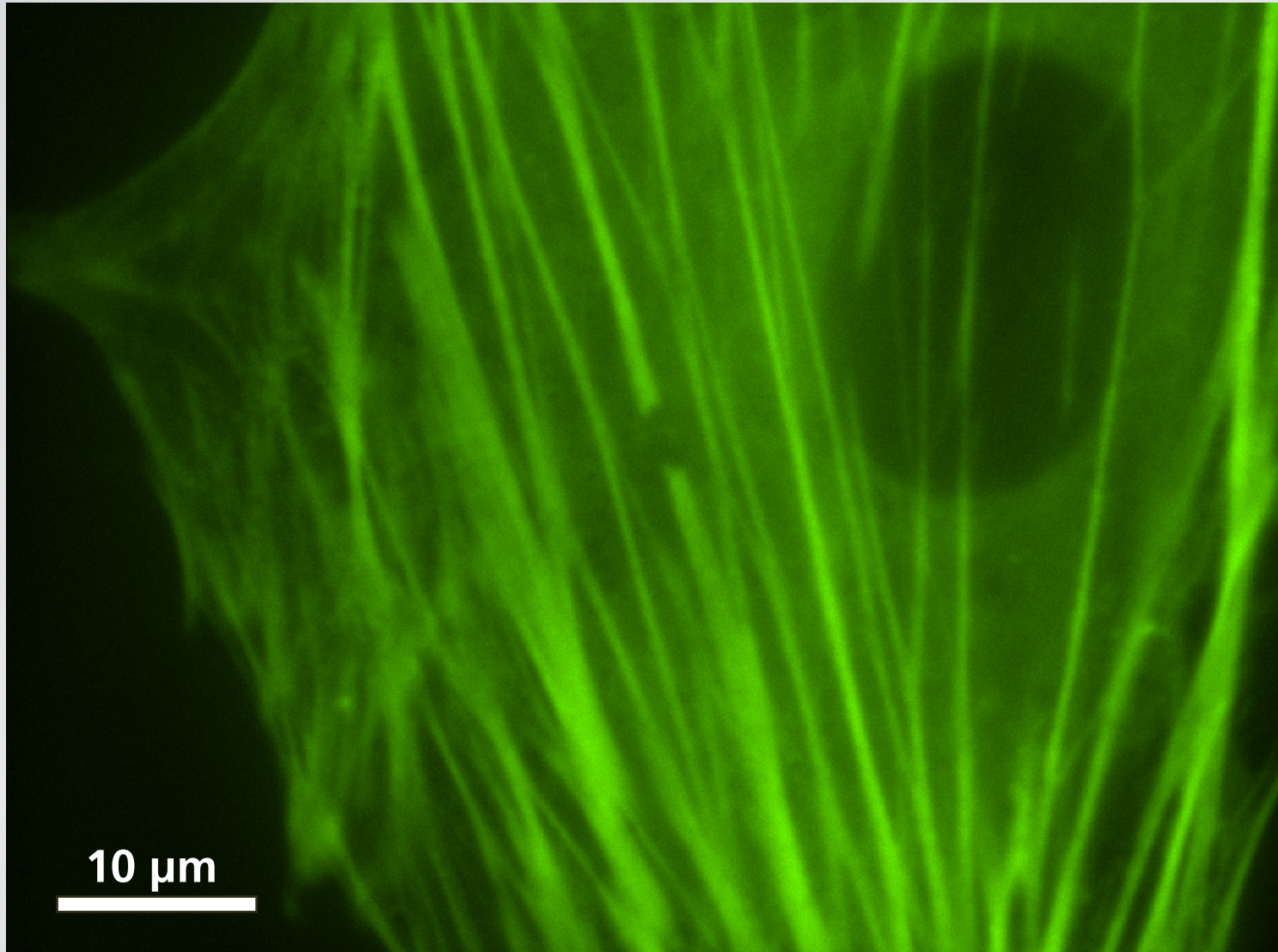
Subcellular surgery

cut a single fiber bundle



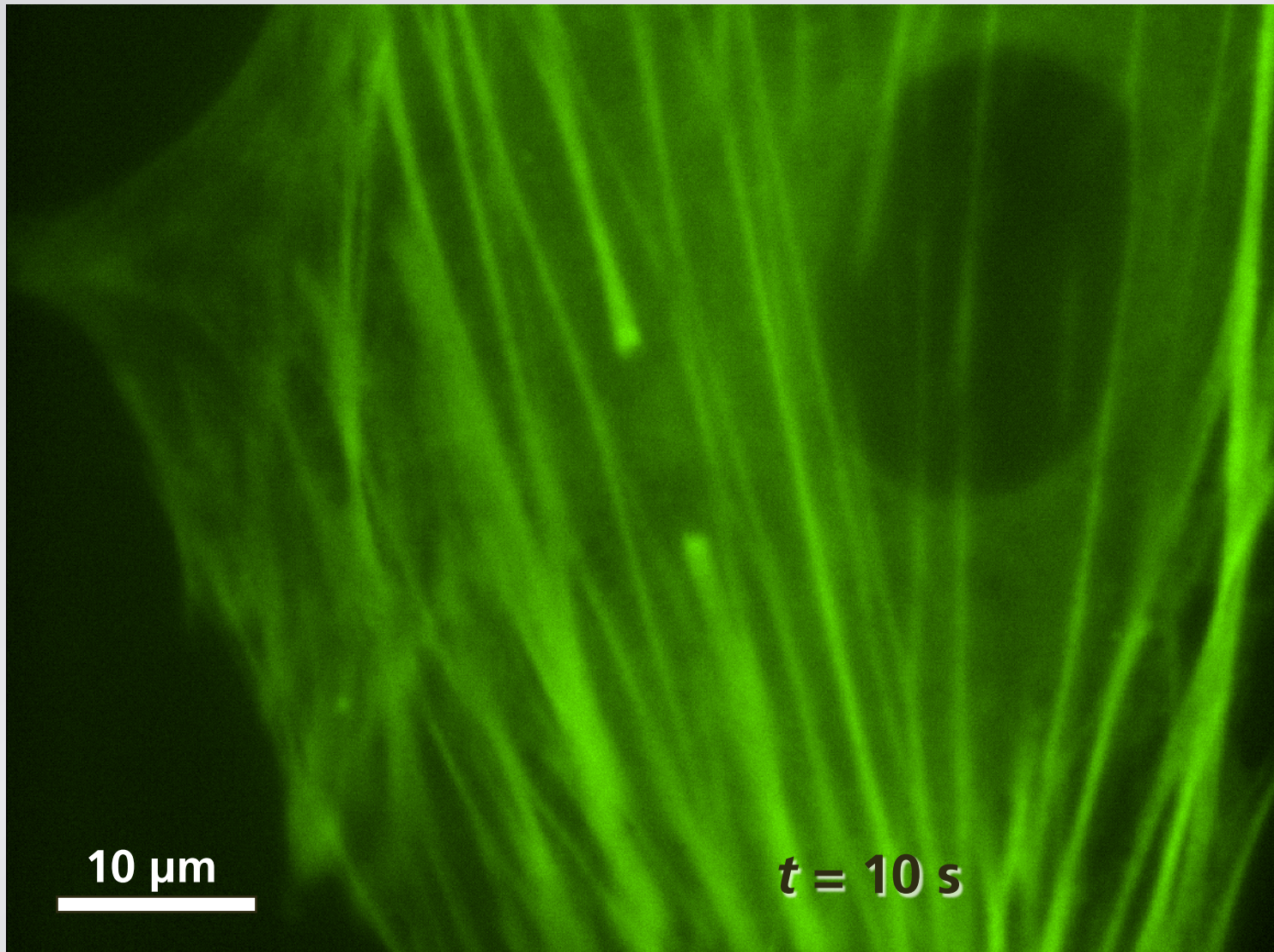
Subcellular surgery

cut a single fiber bundle



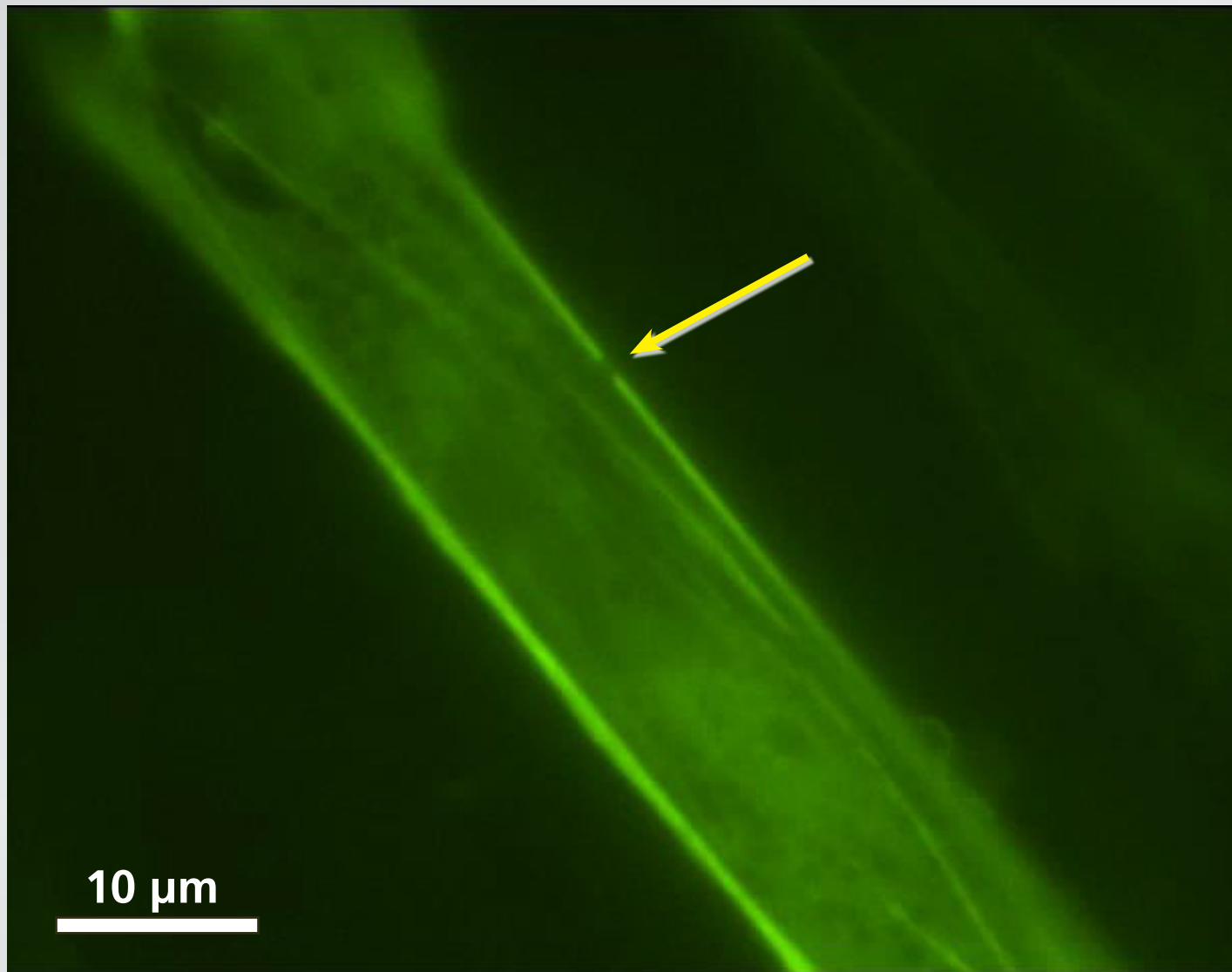
Subcellular surgery

gap widens with time



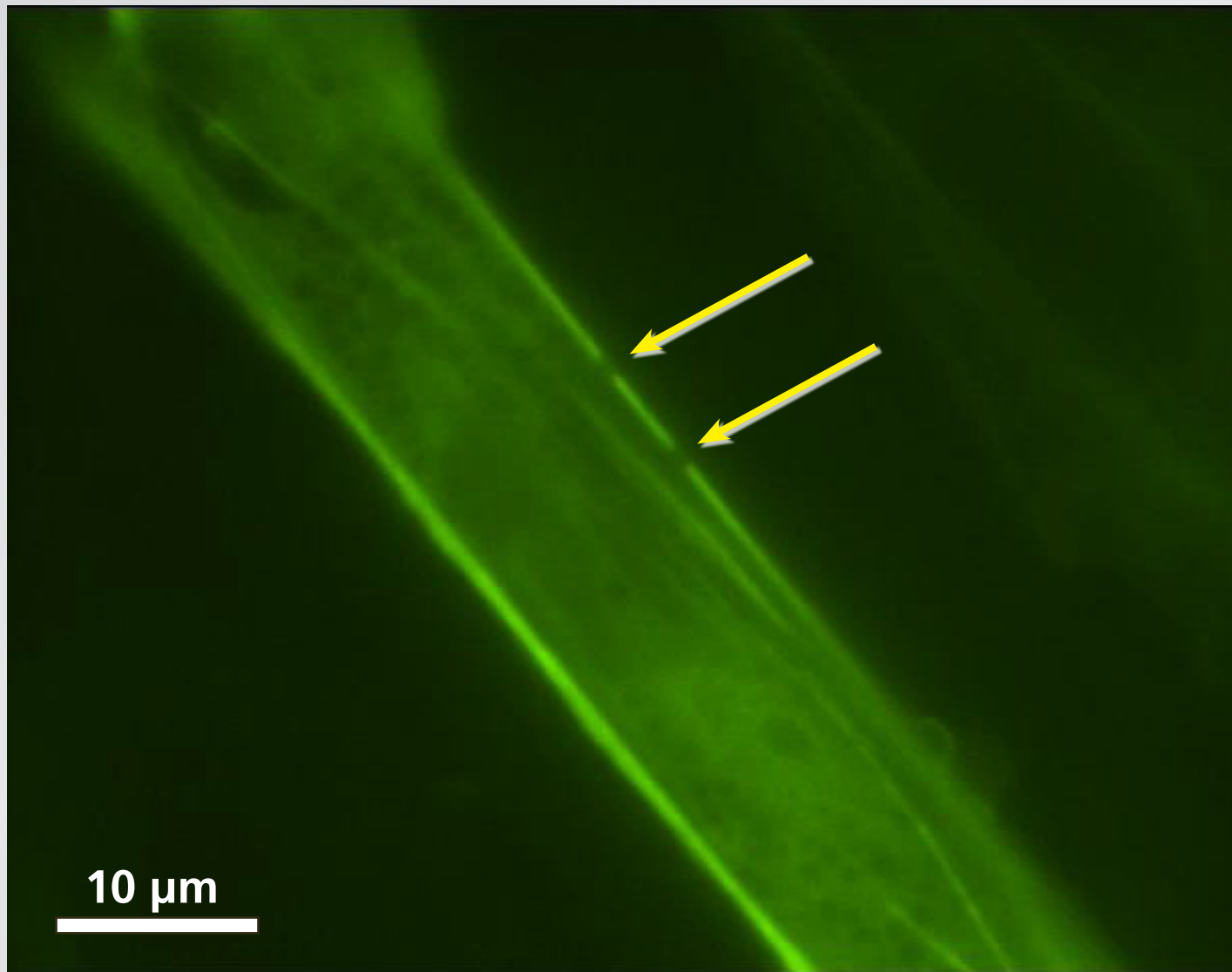
Subcellular surgery

retraction or depolymerization?



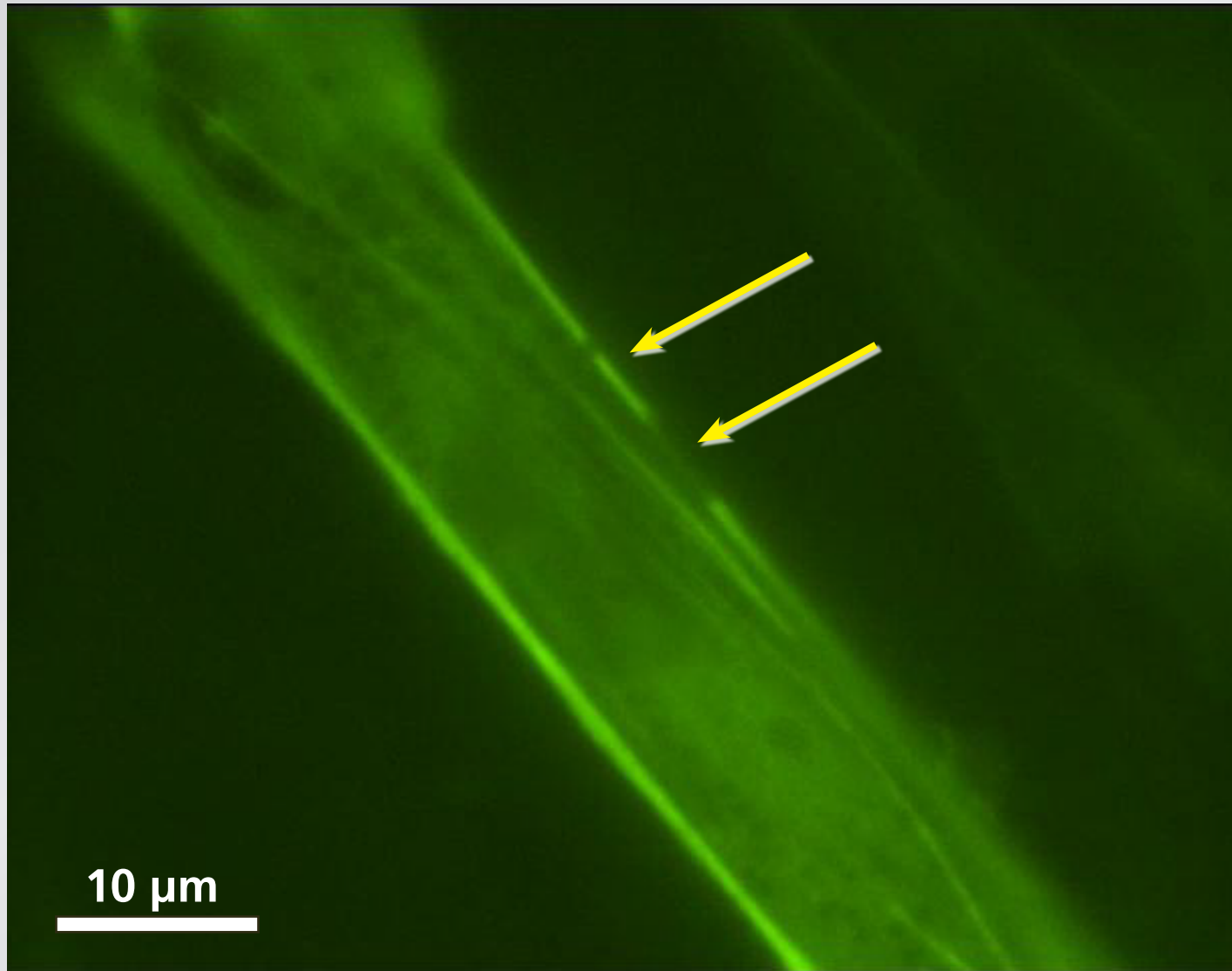
Subcellular surgery

retraction or depolymerization?



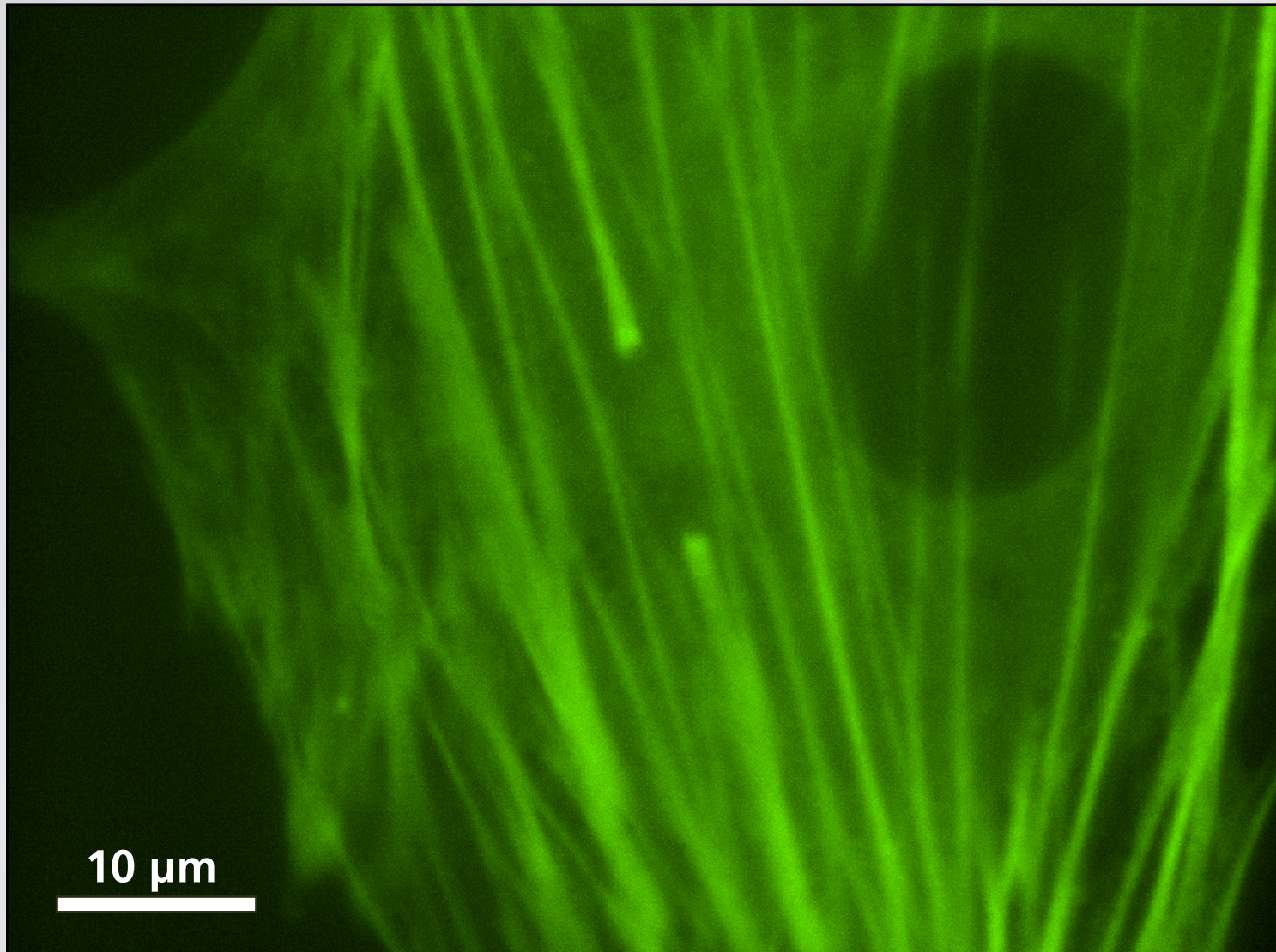
Subcellular surgery

retraction!

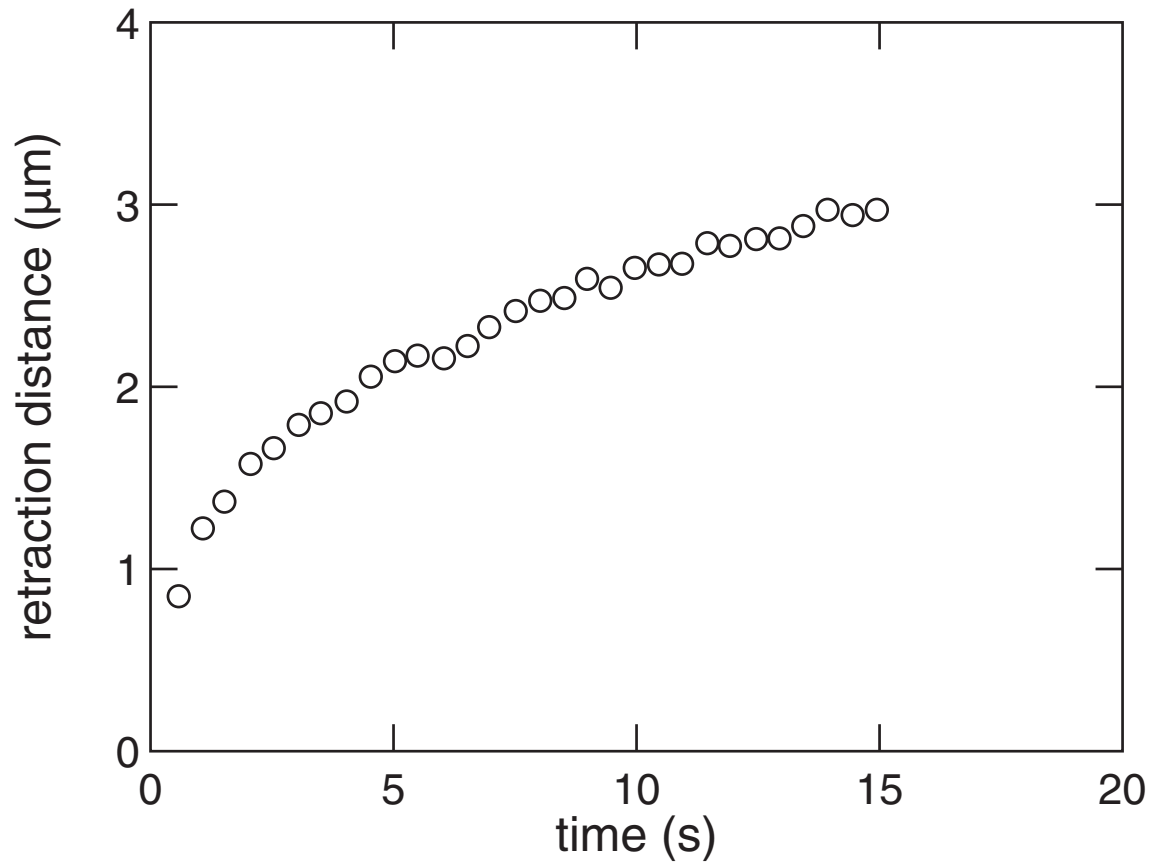


Subcellular surgery

dynamics provides information on *in vivo* mechanics

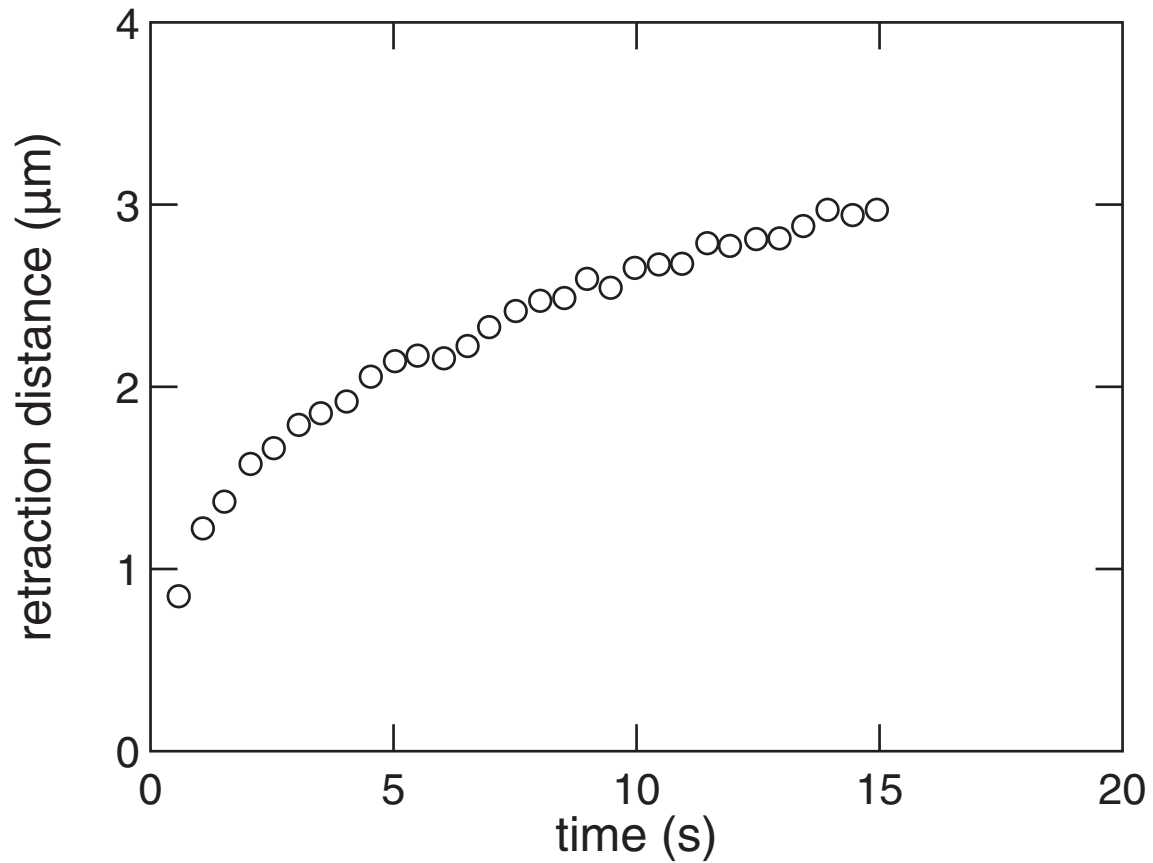


Subcellular surgery



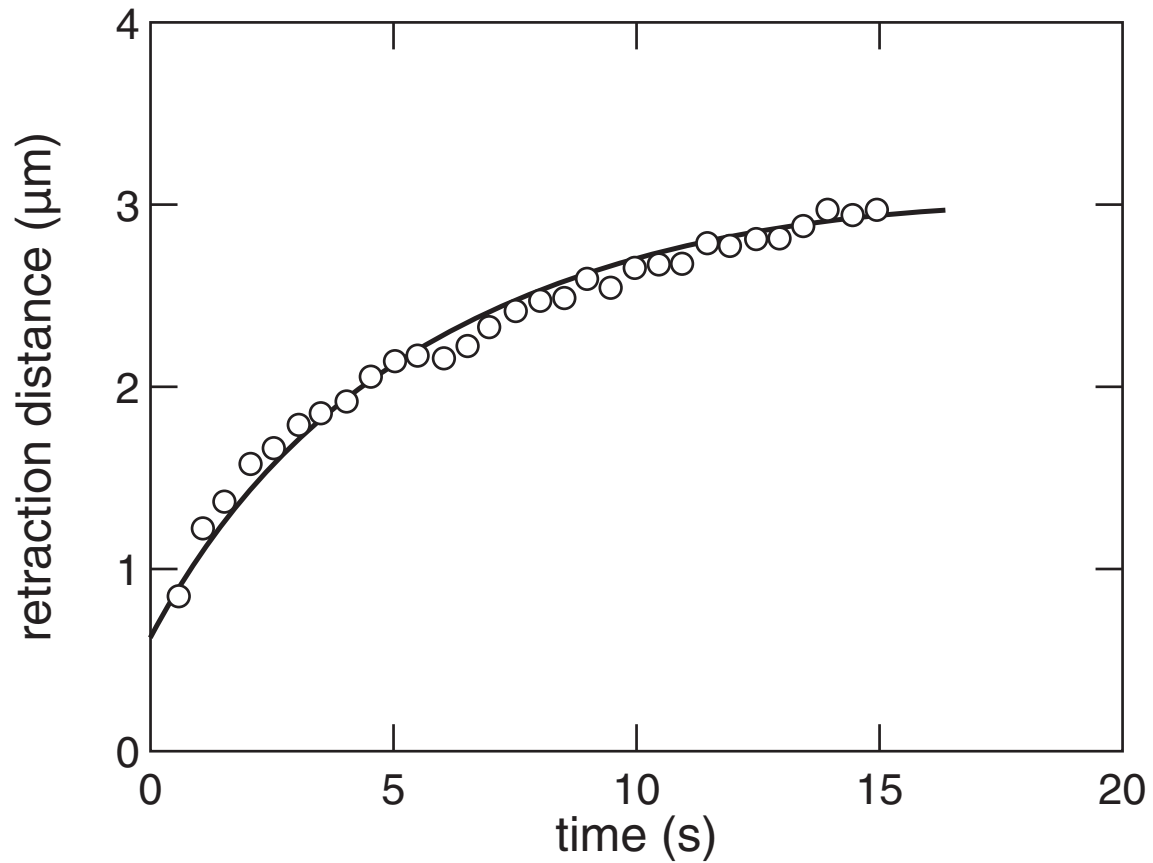
Subcellular surgery

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



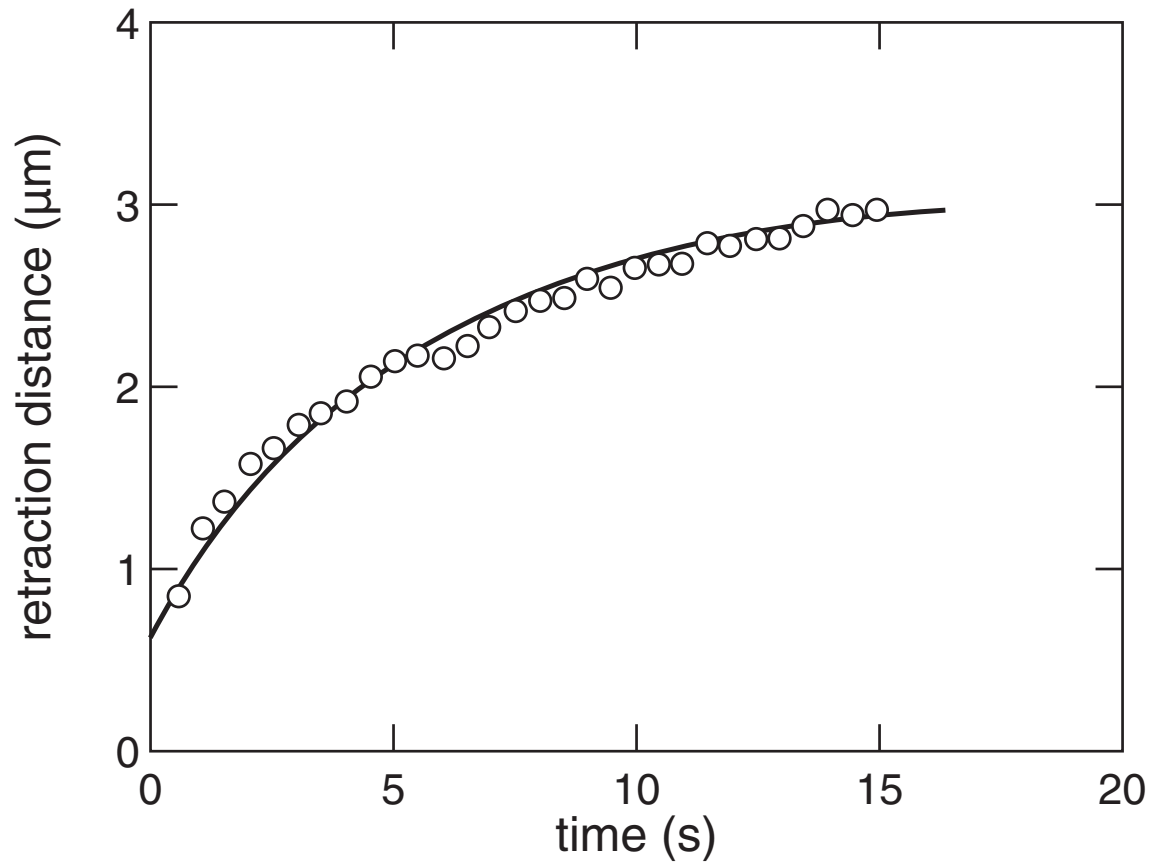
Subcellular surgery

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



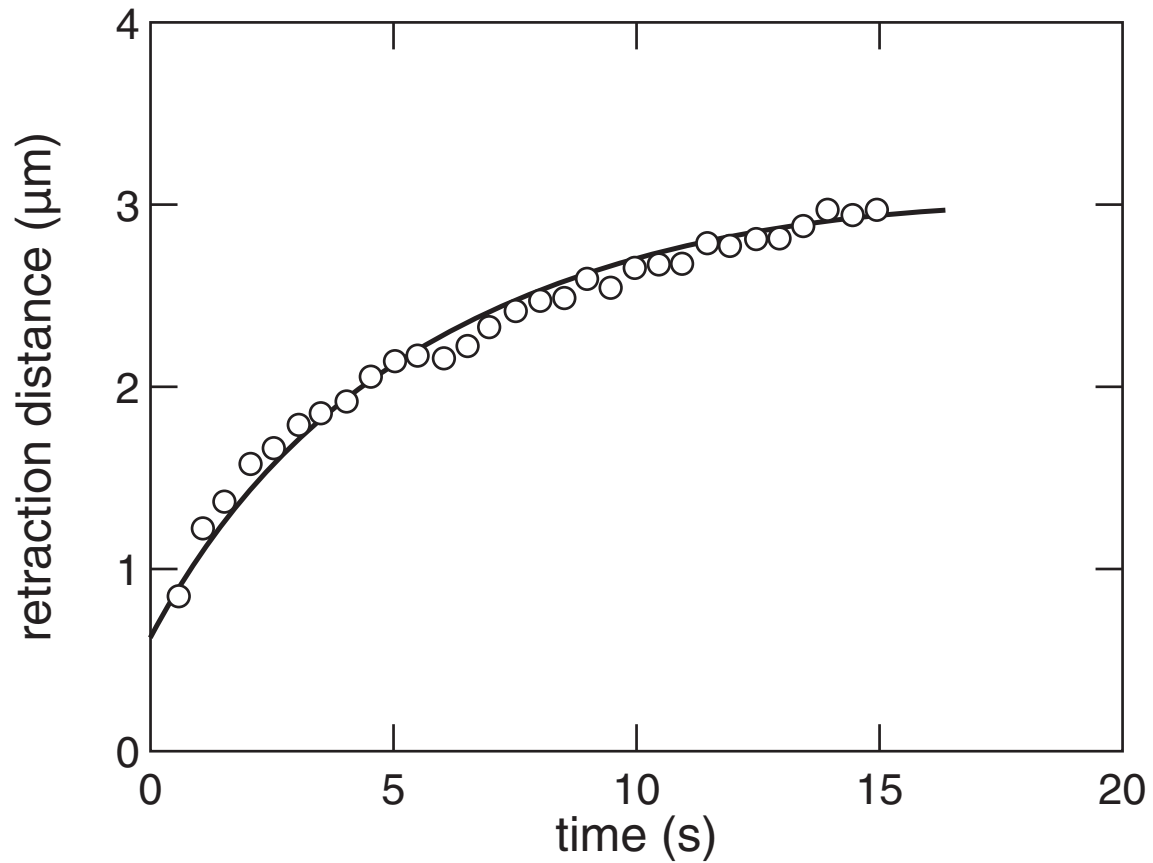
Subcellular surgery

L_0 and τ independent of fiber width!



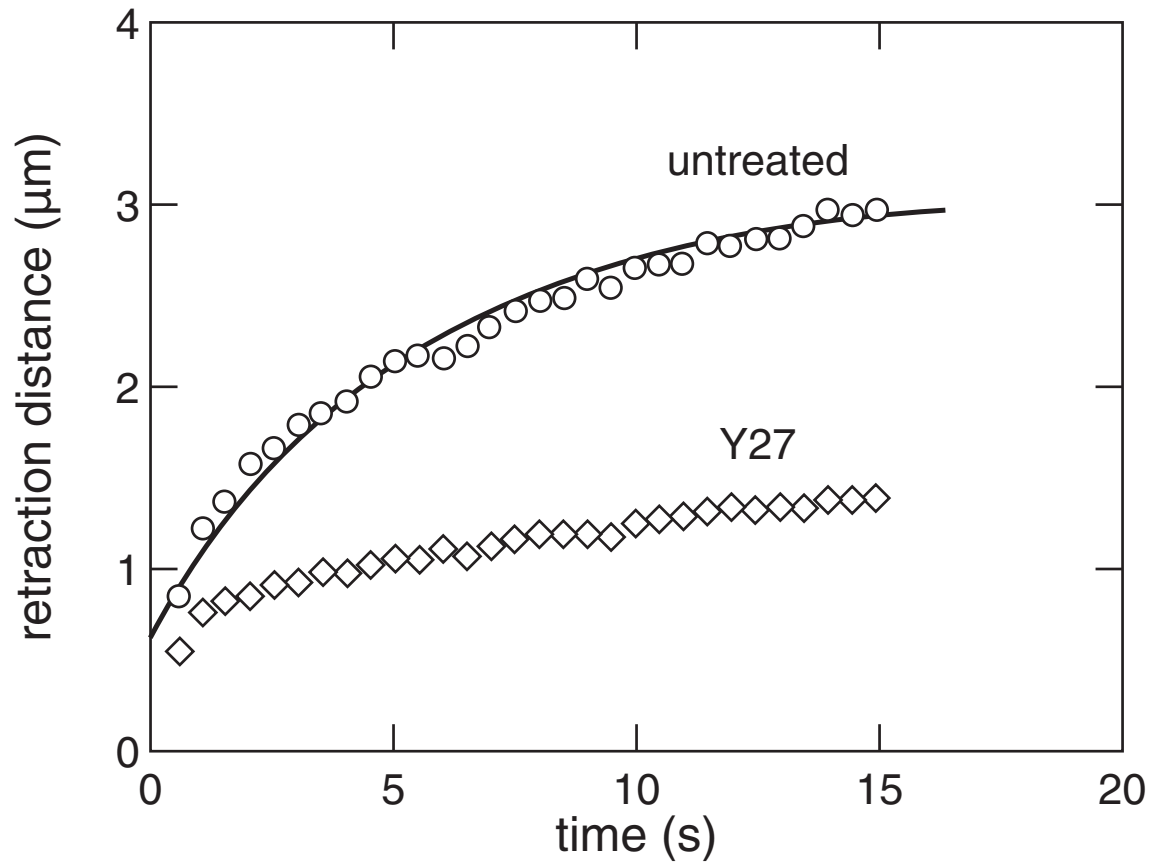
Subcellular surgery

tension in actin filaments is generated by myosin motors



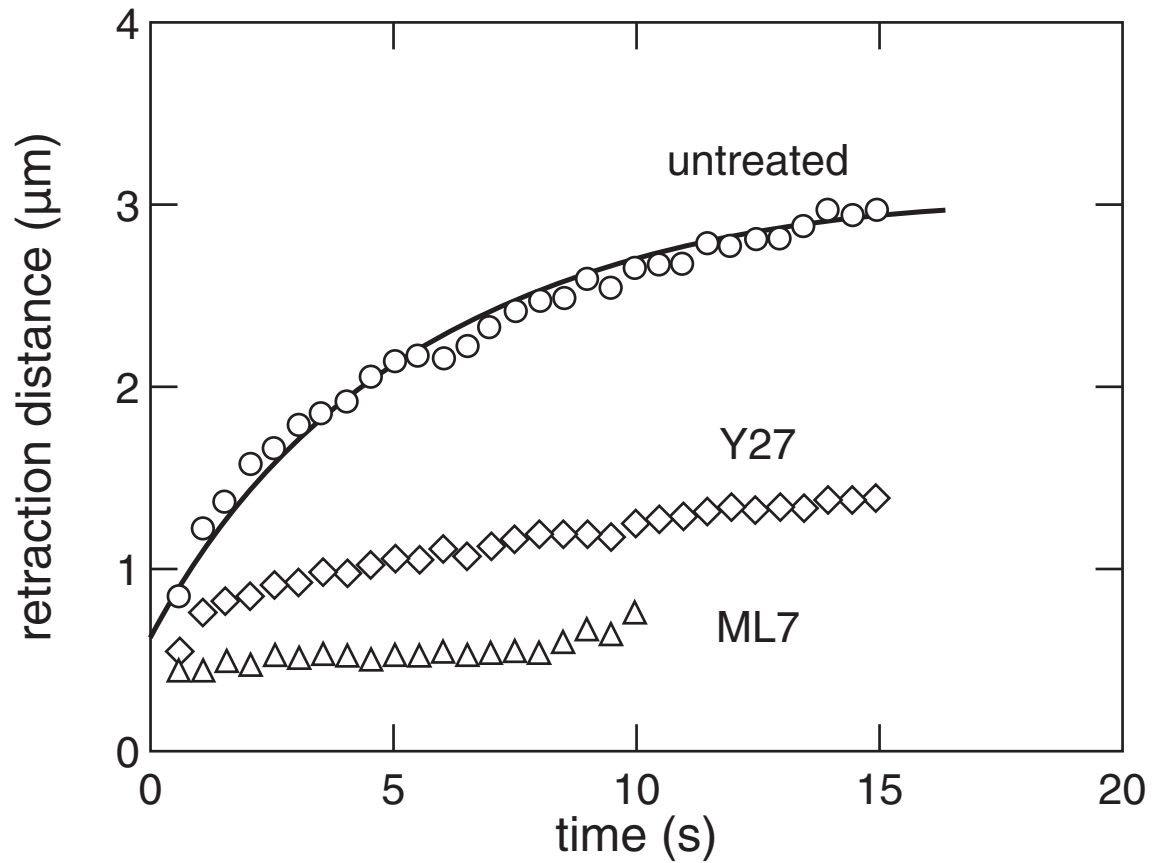
Subcellular surgery

Y27: inhibits some myosin activity



Subcellular surgery

ML7: direct inhibitor of myosin activity



Outline

- femtosecond materials interactions
 - subcellular surgery
 - **nanoneurosurgery**
- 
- A microscopic image of a biological structure, possibly a neuron, with a highlighted path in orange and yellow. The path starts from the bottom left, moves up and right, then down and right, then up and right, and finally up and right towards the top right. The background is a light blue, textured surface.

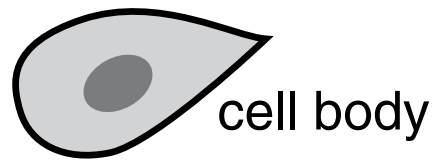
Nanoneurosurgery

Q: can we probe the neurological origins of behavior?



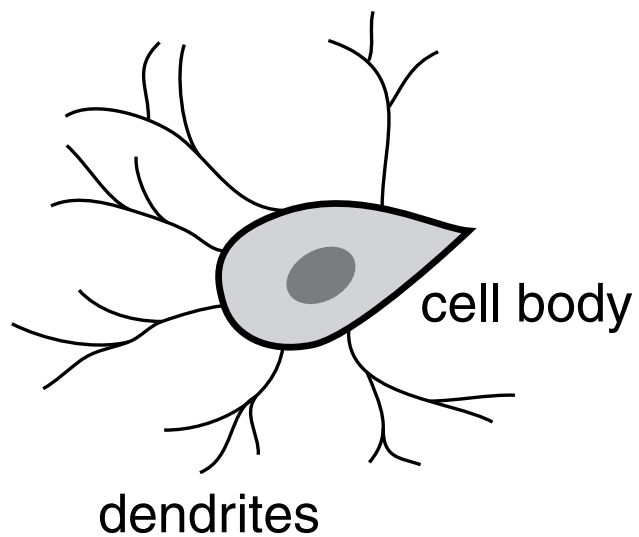
Nanoneurosurgery

neuron basics



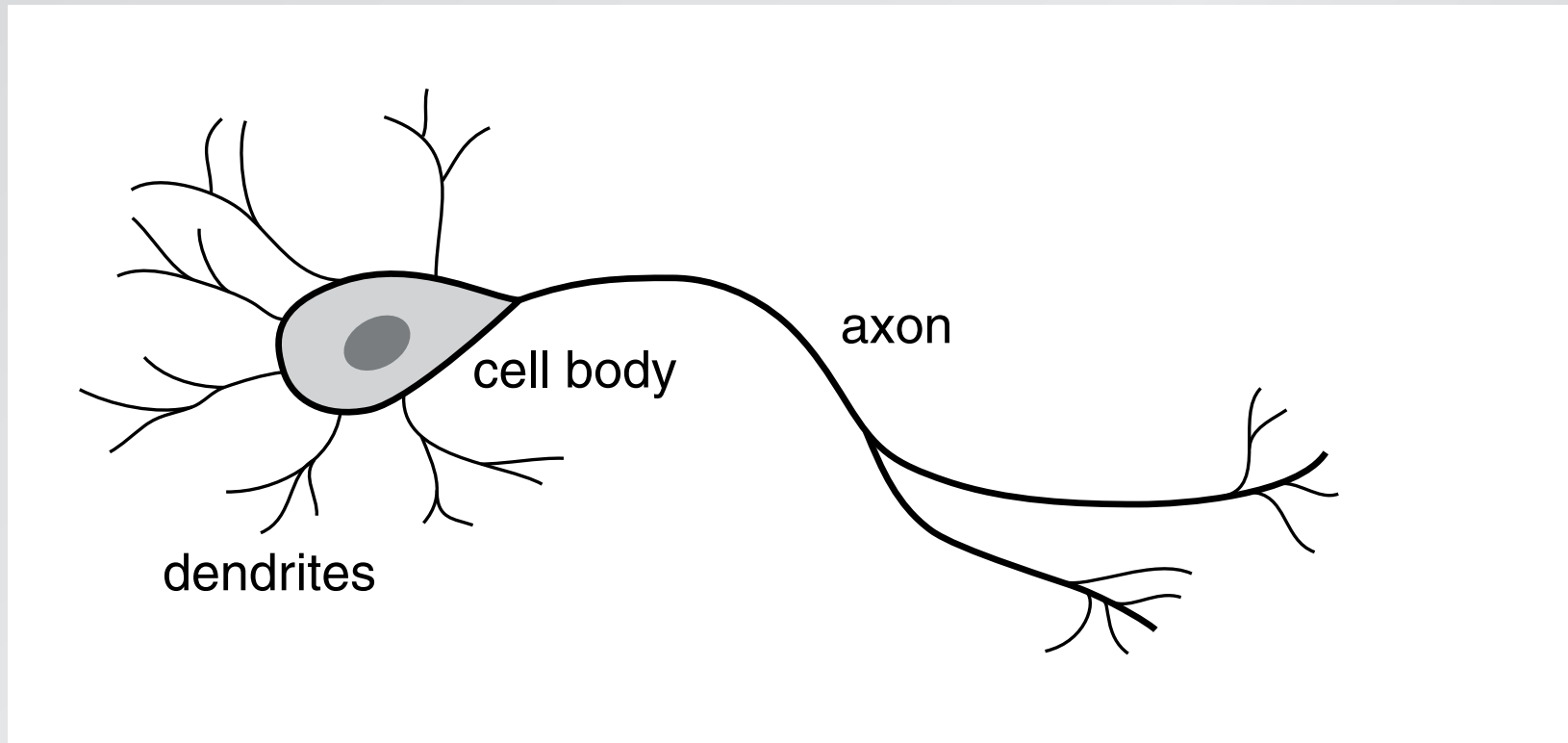
Nanoneurosurgery

neuron basics



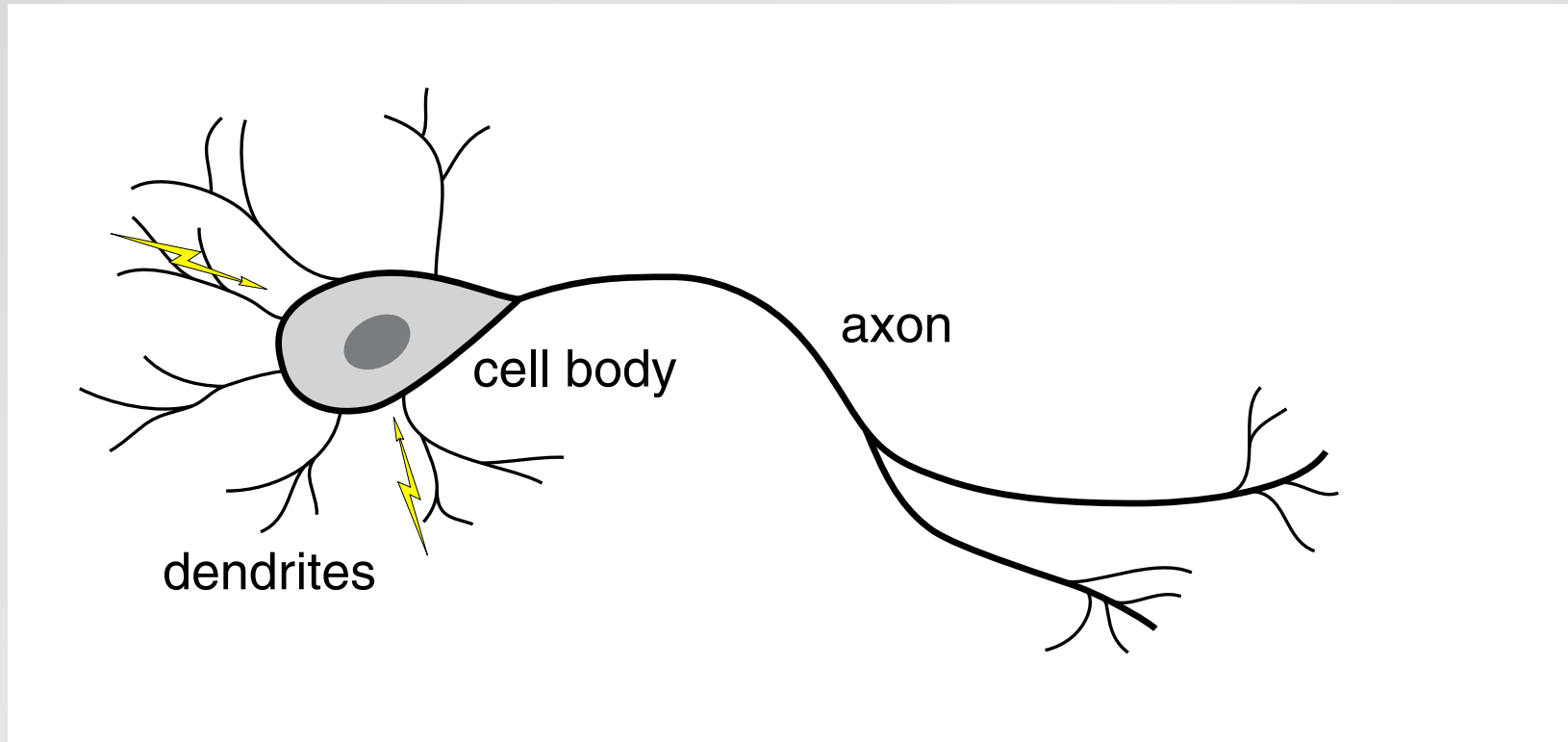
Nanoneurosurgery

neuron basics



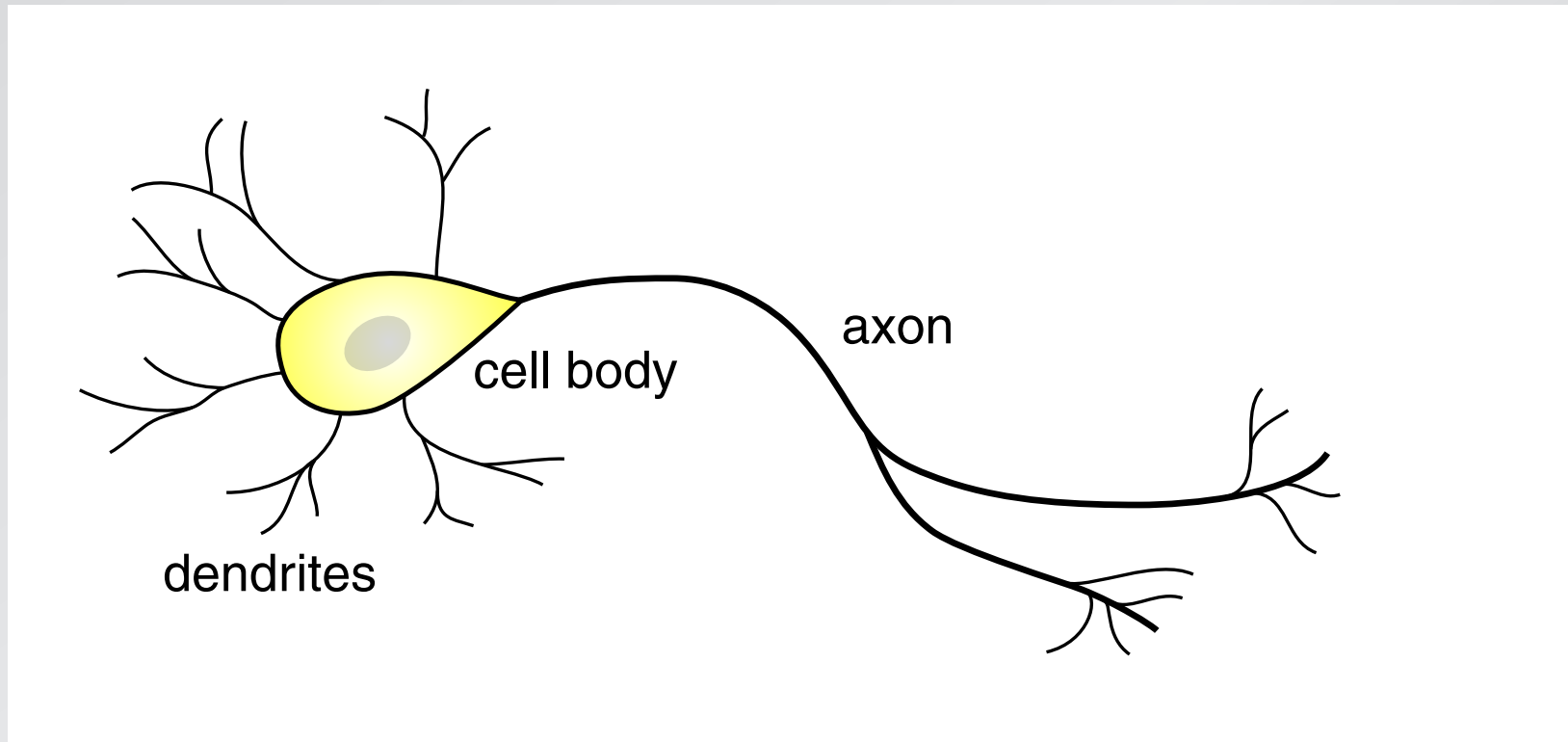
Nanoneurosurgery

neuron basics



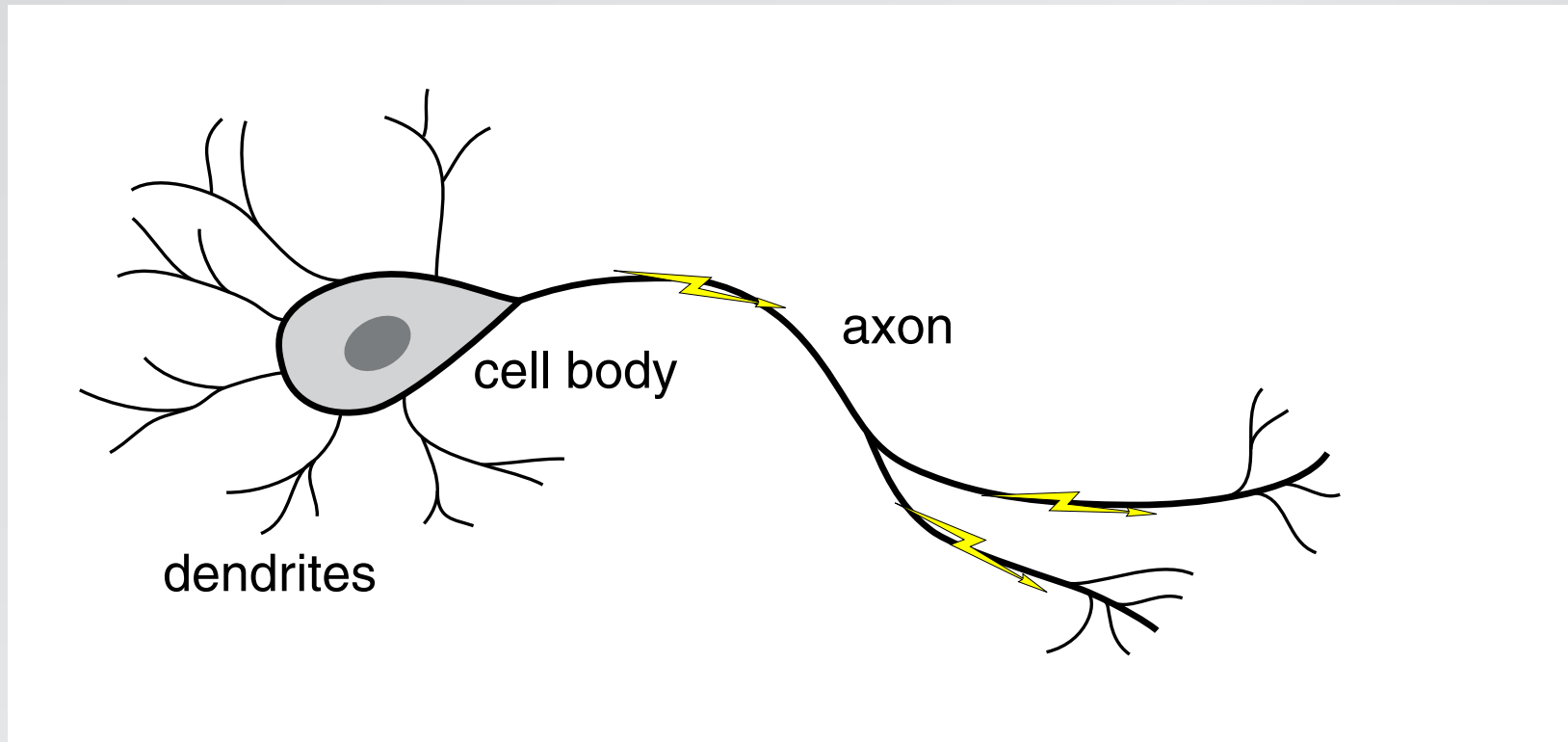
Nanoneurosurgery

neuron basics



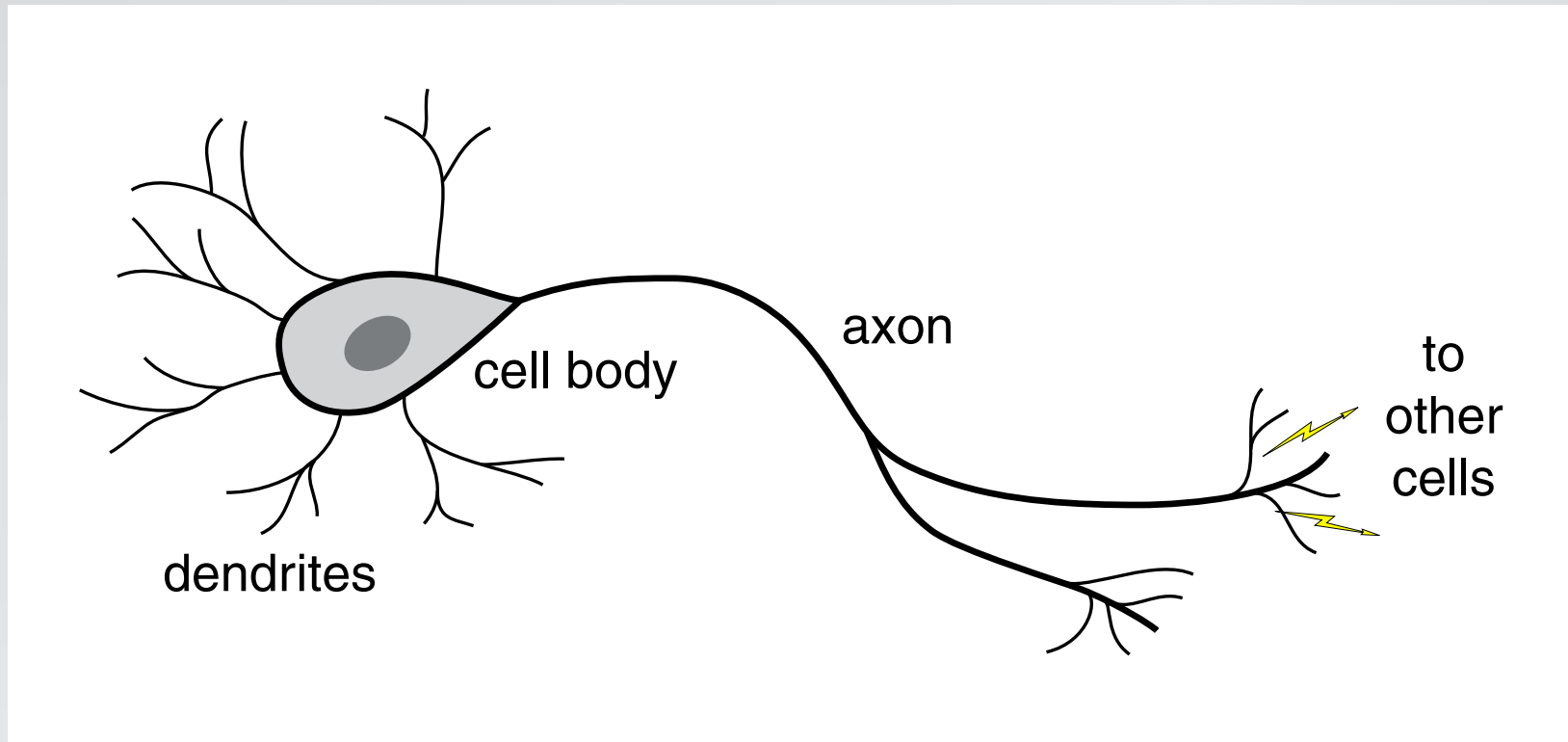
Nanoneurosurgery

neuron basics



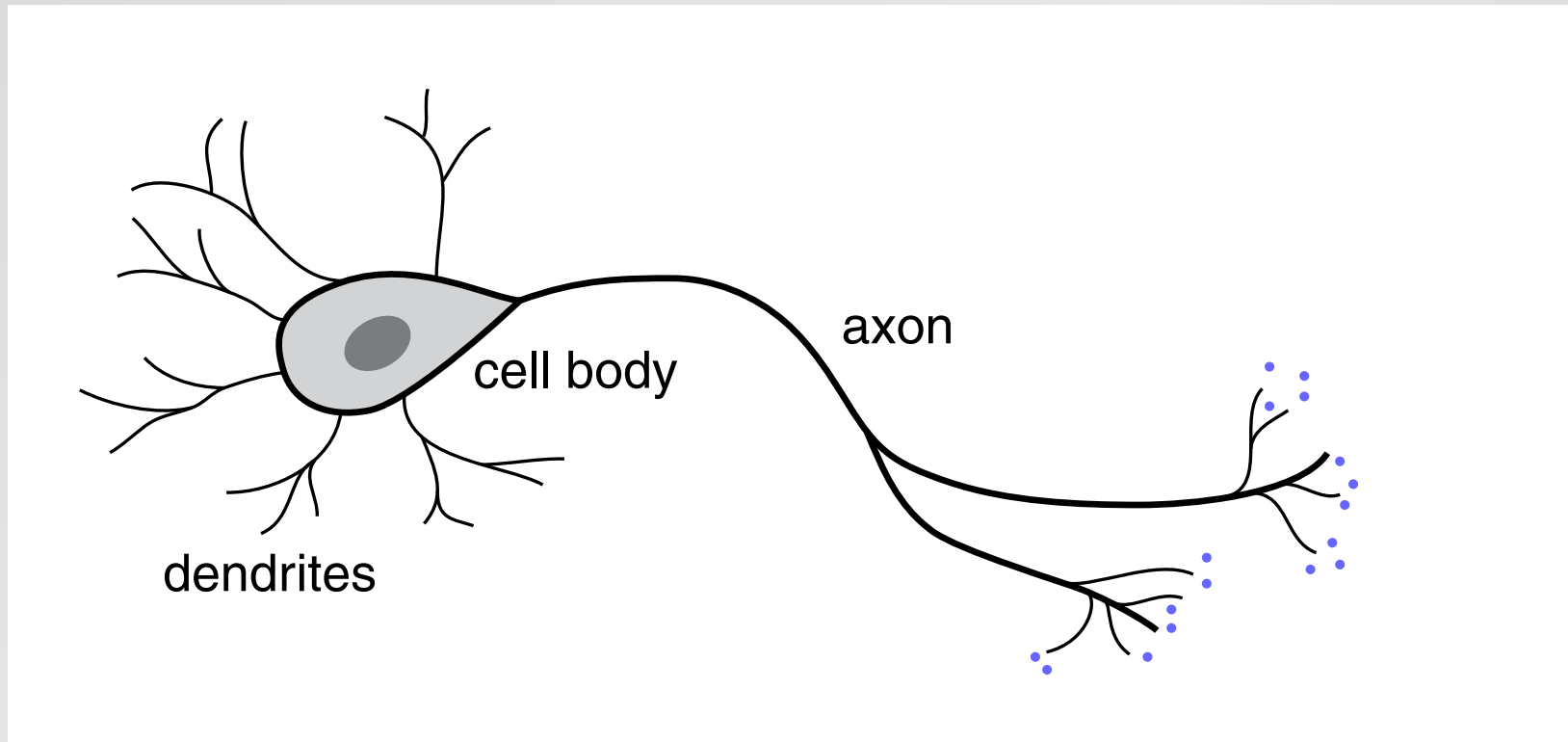
Nanoneurosurgery

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

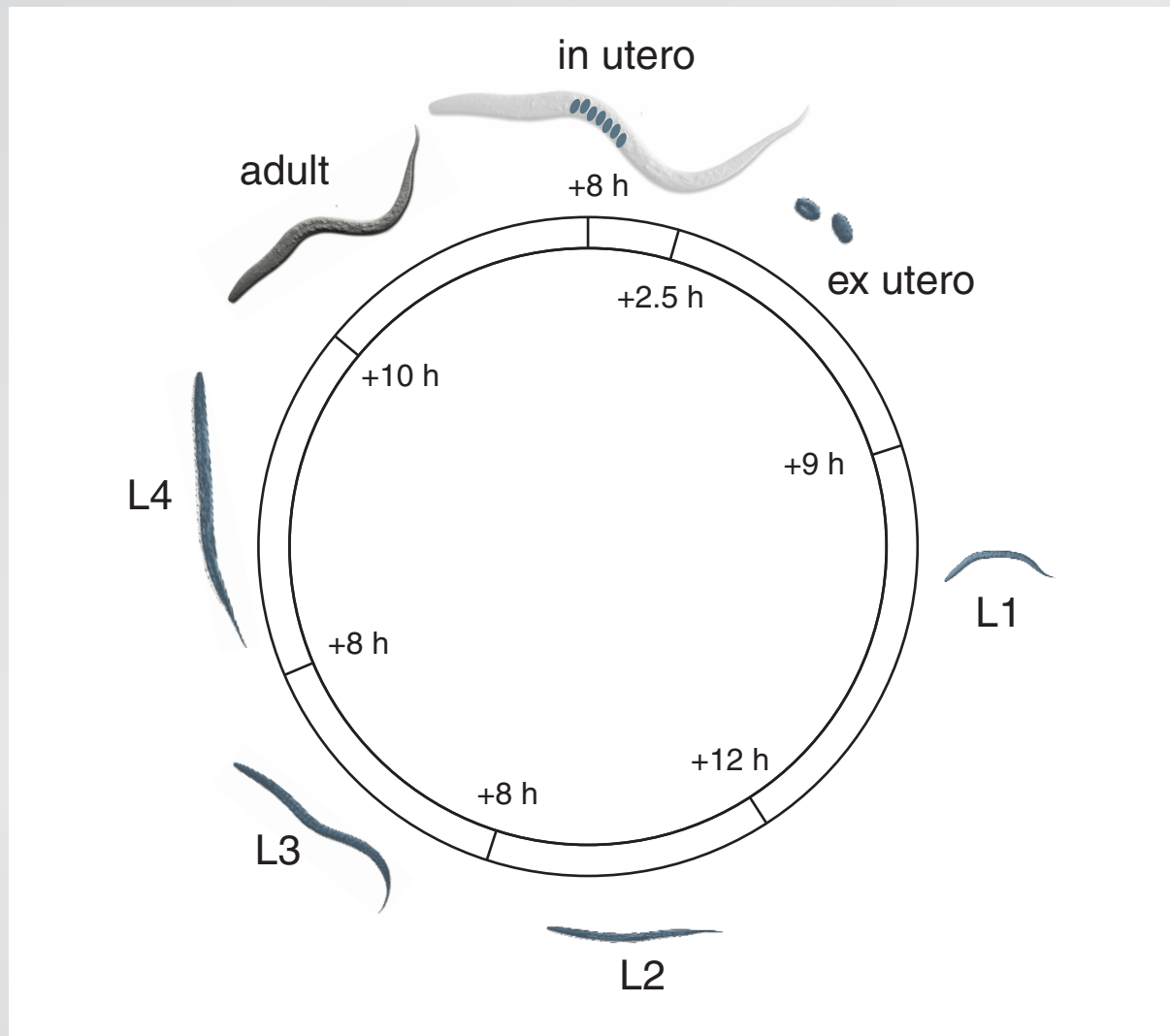
Nanoneurosurgery

Caenorhabditis elegans



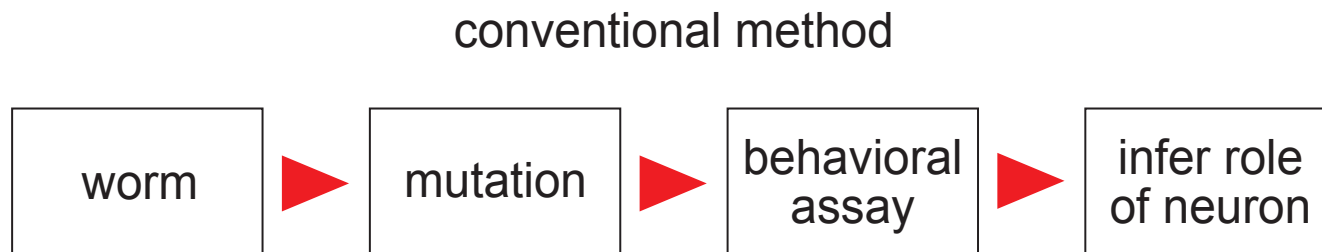
Nanoneurosurgery

C. elegans life cycle



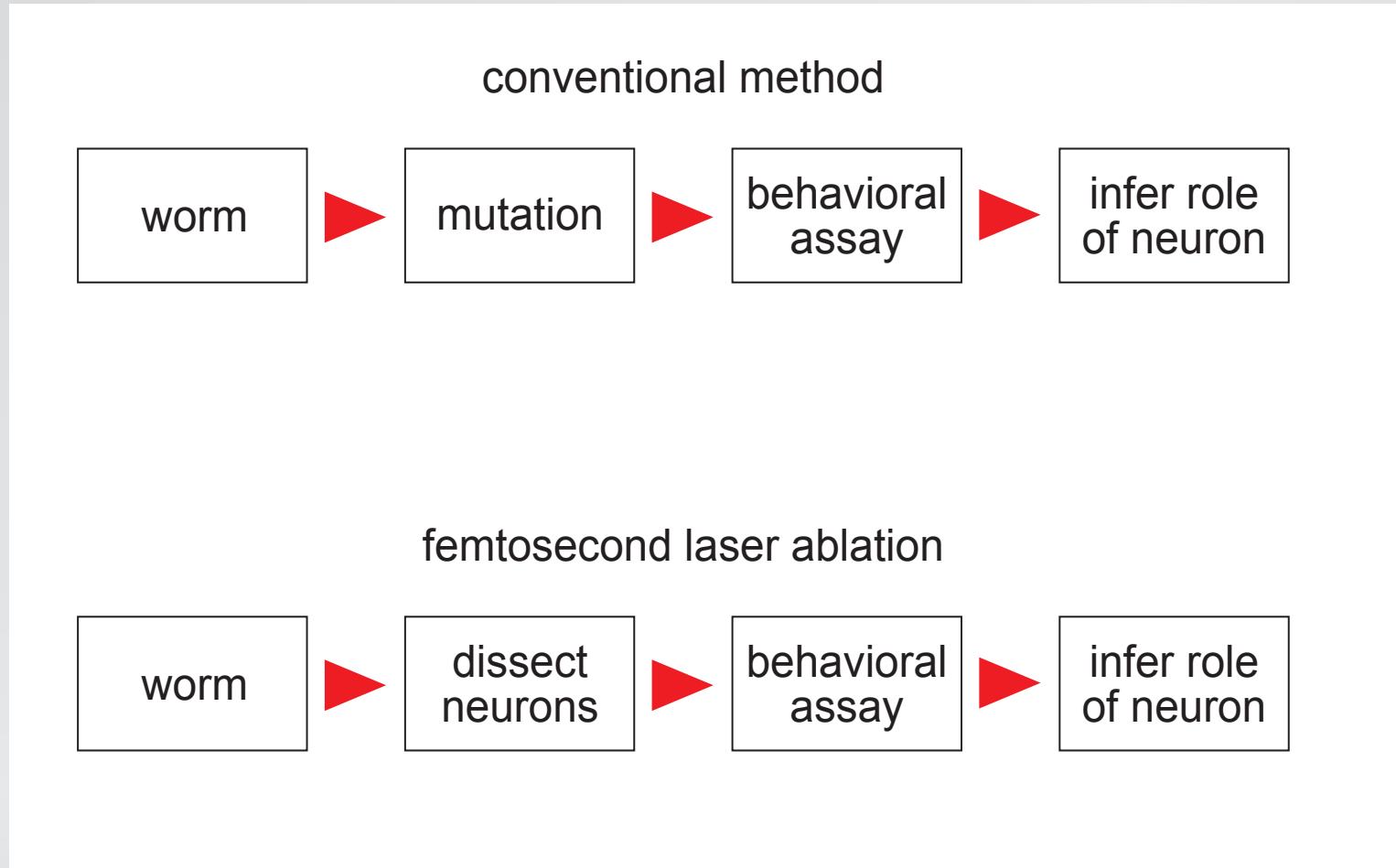
Nanoneurosurgery

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



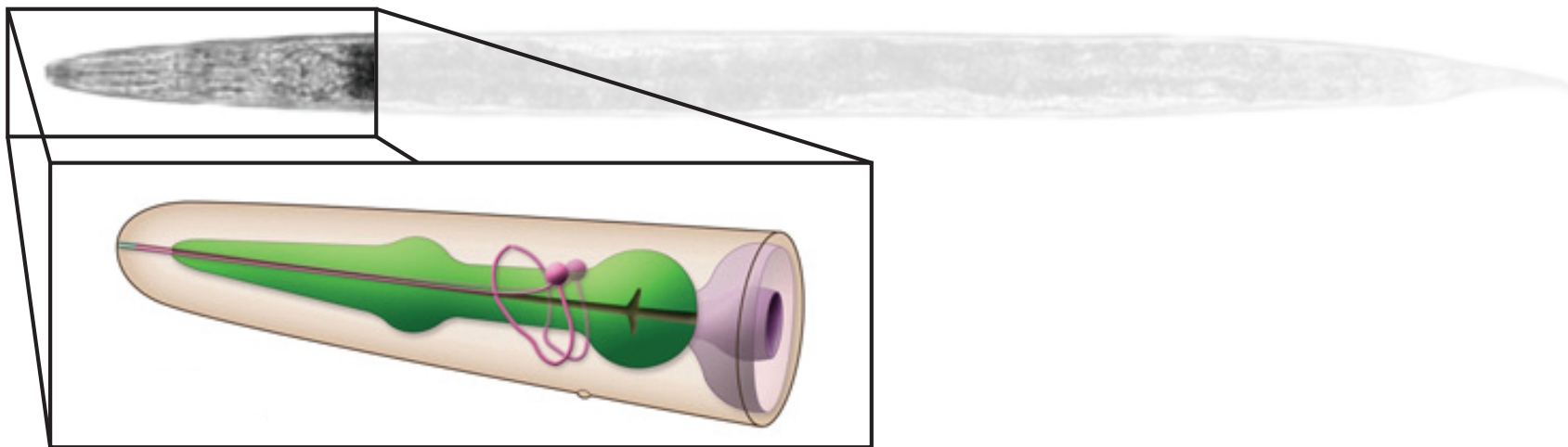
Nanoneurosurgery

ASH neurons



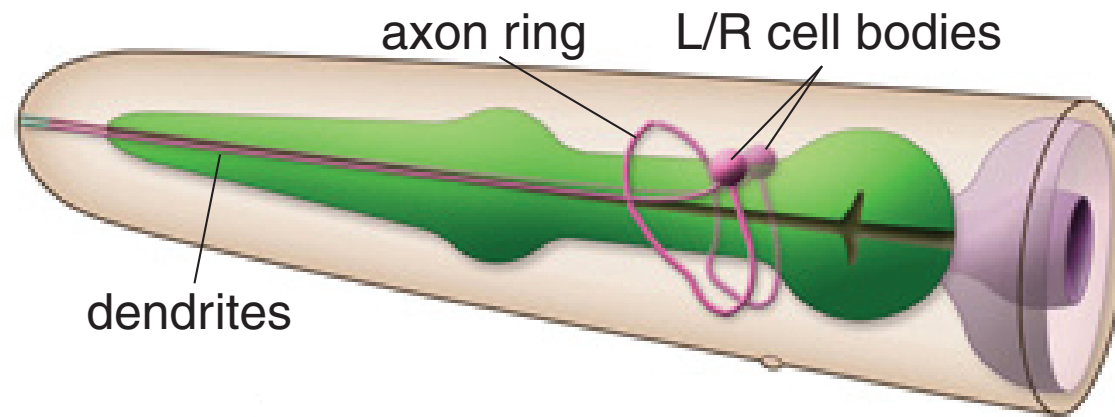
Nanoneurosurgery

ASH neurons



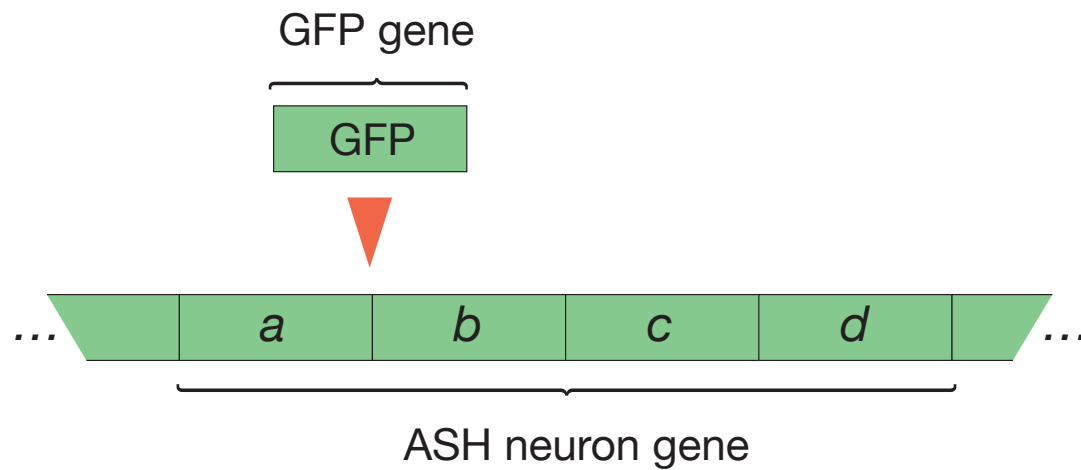
Nanoneurosurgery

ASH neurons



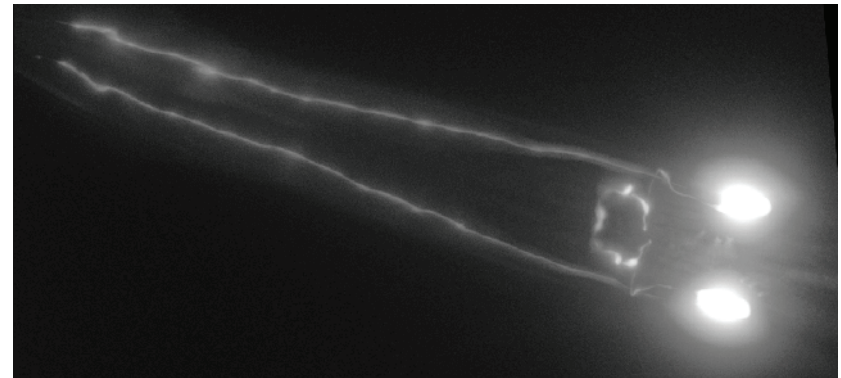
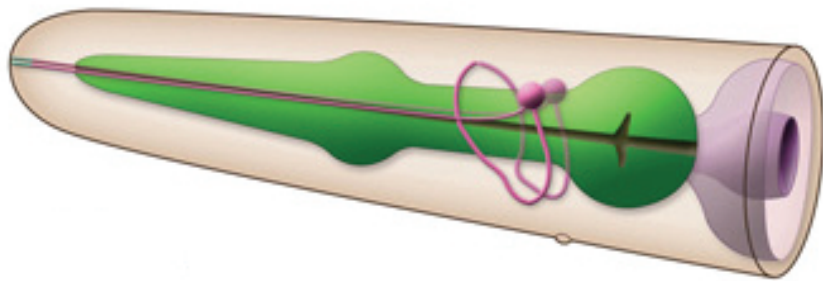
Nanoneurosurgery

make ASH neurons express GFP



Nanoneurosurgery

make ASH neurons express GFP



Nanoneurosurgery

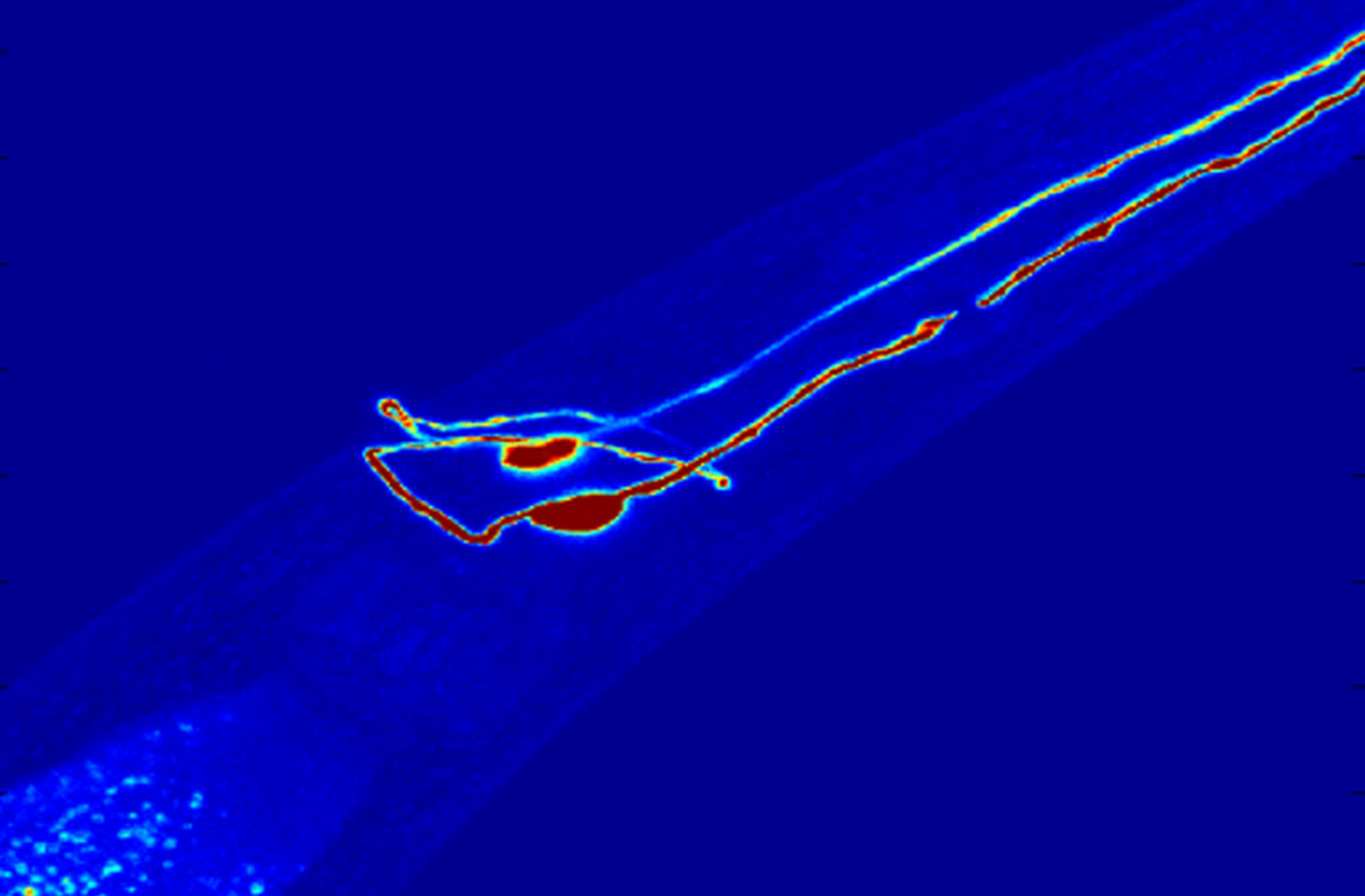
GFP: absorbs UV, emits green



Nanoneurosurgery

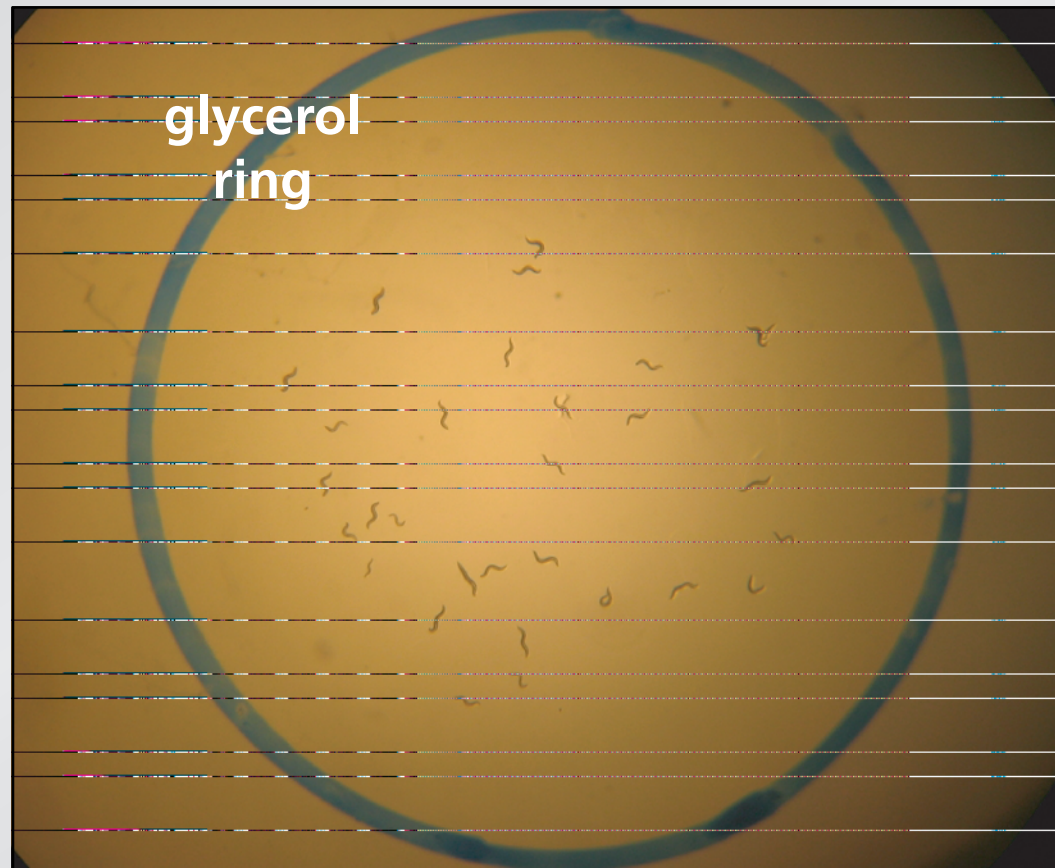
revive worm, reimage 1 day later

Nanoneurosurgery



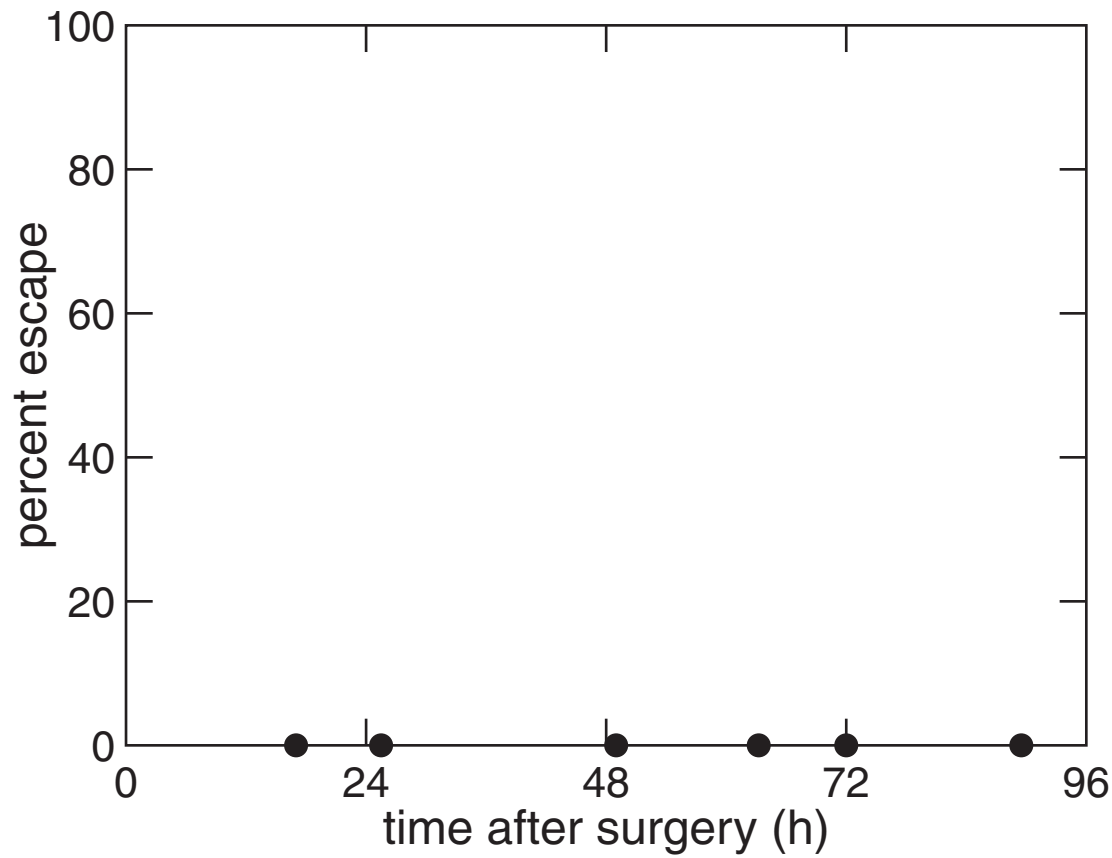
Nanoneurosurgery

osmolarity assay



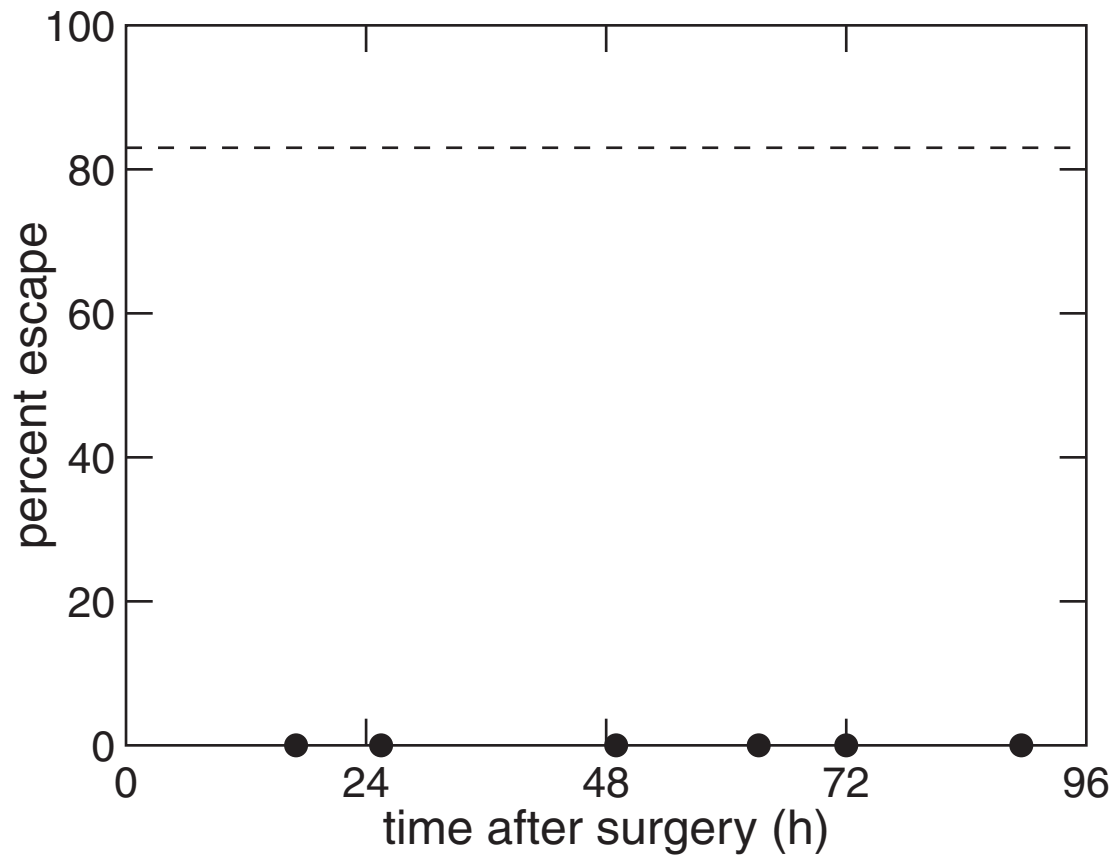
Nanoneurosurgery

escape rate after 'mock' surgery



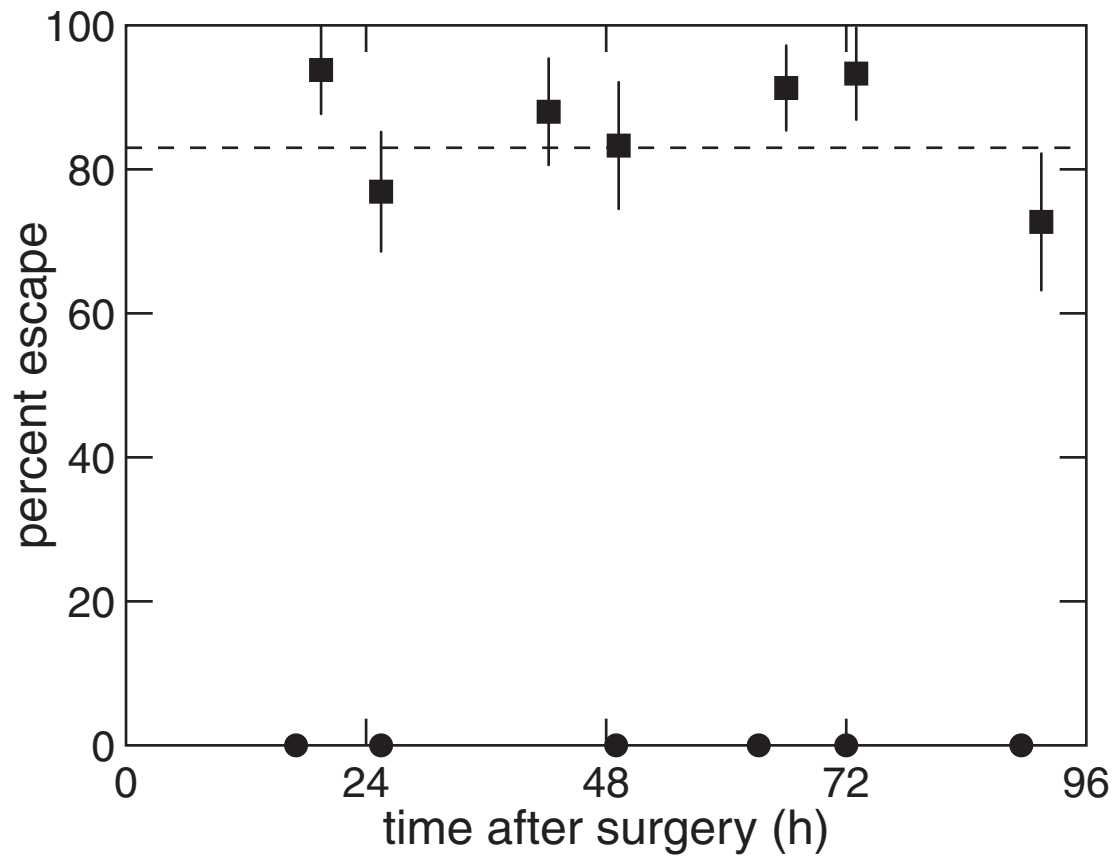
Nanoneurosurgery

escape rate of ASH-lacking mutant



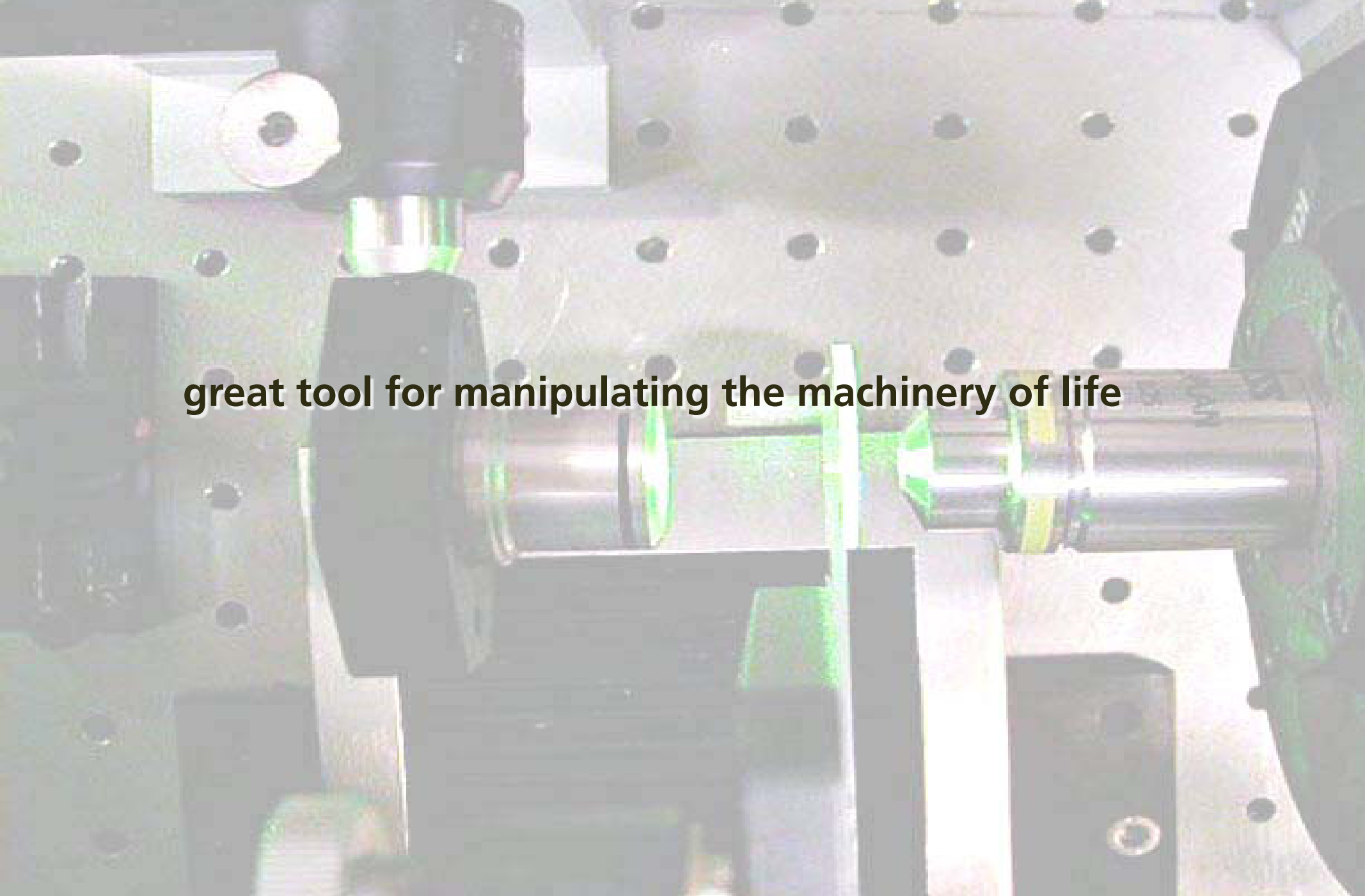
Nanoneurosurgery

escape rate after ASH-ablation surgery



Conclusion

great tool for manipulating the machinery of life







Open House

Thursday September 24 @ 6:30 pm

LISE 3rd floor lobby



Funding:

National Science Foundation

for a copy of this presentation:

<http://mazur-www.harvard.edu>

http://twitter.com/eric_mazur