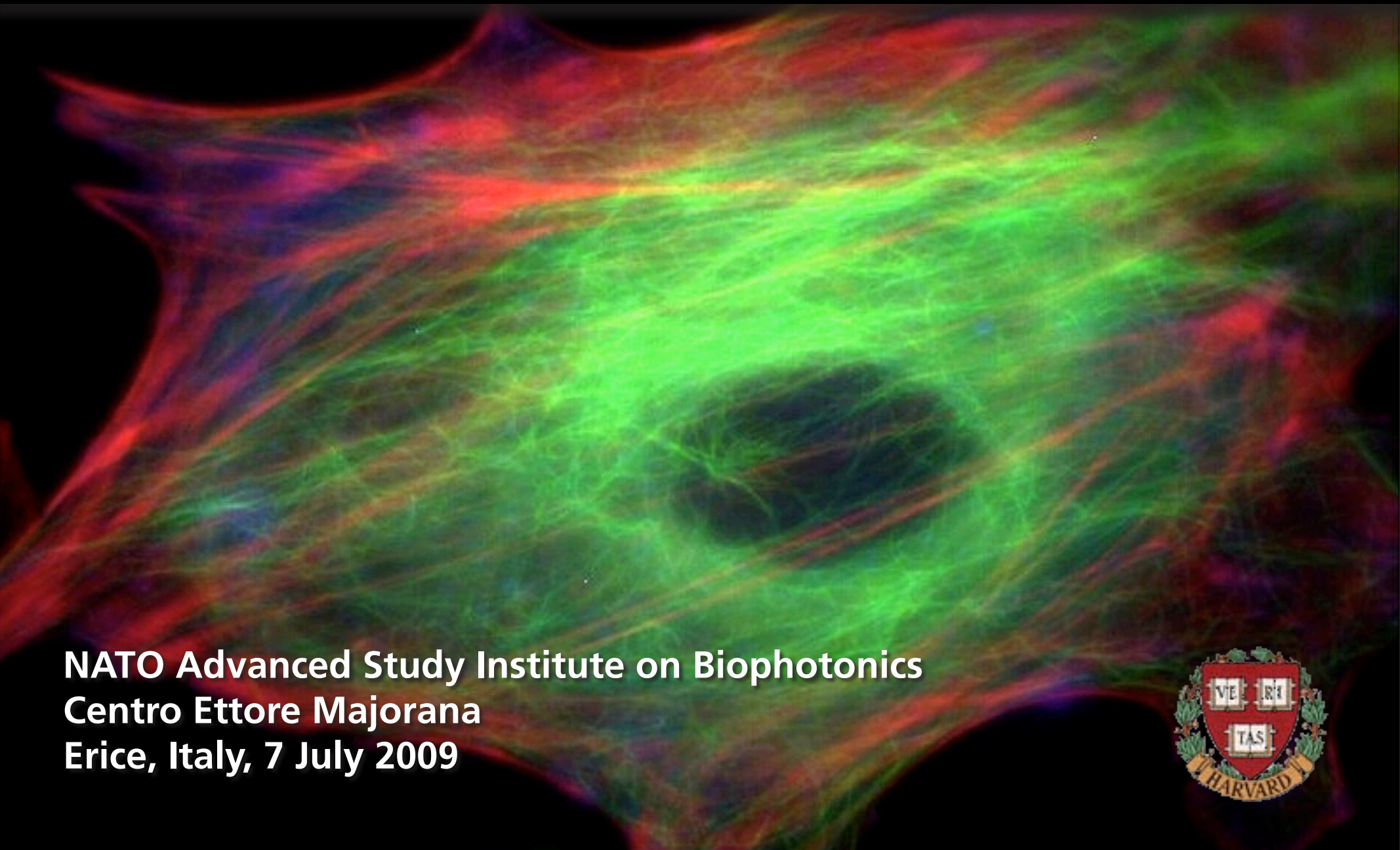
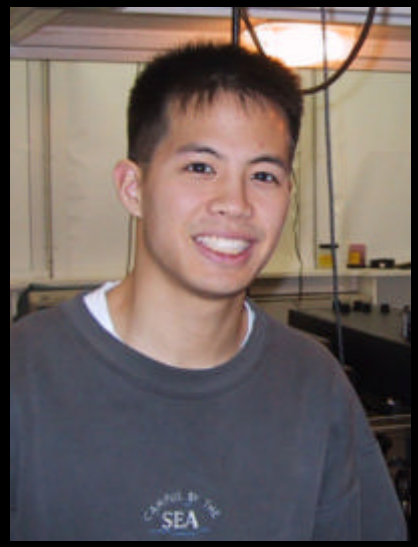
# Subcellular surgery and nanoneurosurgery using femtosecond laser pulses



NATO Advanced Study Institute on Biophotonics
Centro Ettore Majorana
Erice, Italy, 7 July 2009

Iva Maxwell



Sam Chung

Valeria Nuzzo

Alexander Heisterkamp

and also....

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

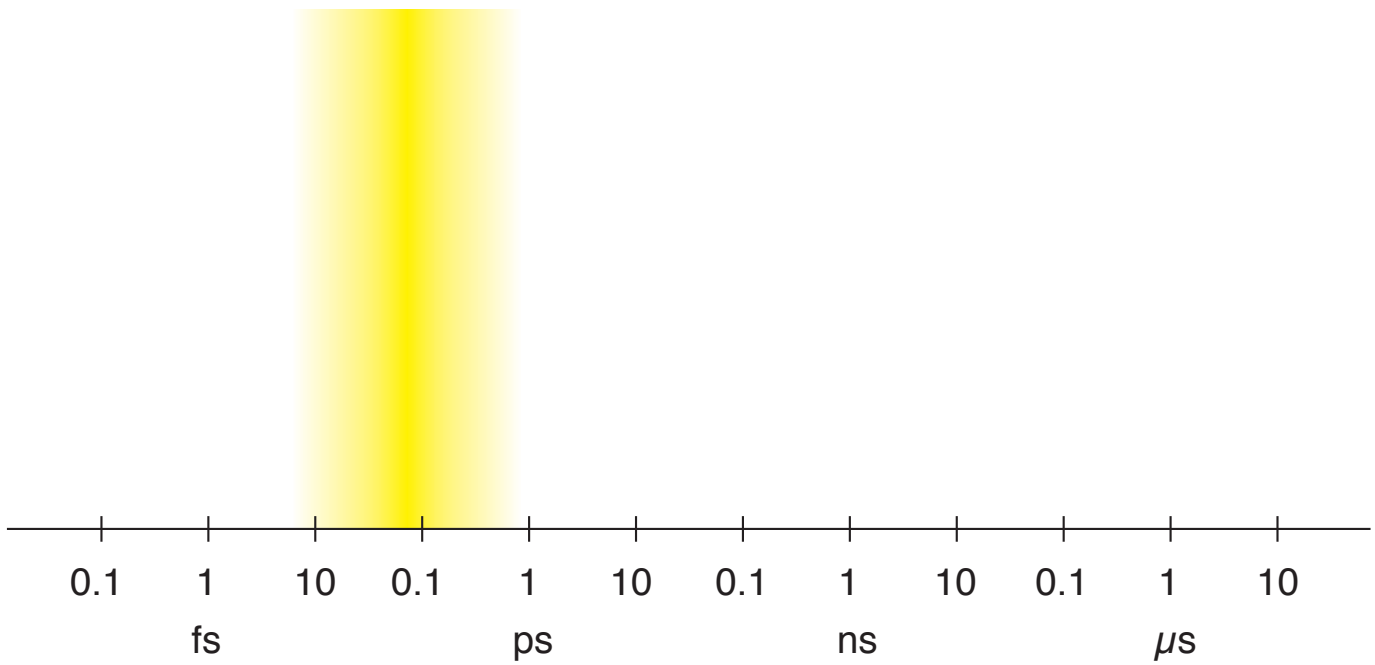
Prof. Don Ingber (Harvard Medical School)
Prof. Aravi Samuel (Harvard)
Prof. Chris Gabel (Boston University)
Dr. Damon Clark (Harvard University)
Prof. J.M. Underwood (UMass Worcester)
Prof. J.A. Nickerson (UMass Worcester)
Prof. Philip LeDuc (Carnegie Mellon)
Prof. Sanjay Kumar (UC Berkeley)

# Introduction

**why use femtosecond pulses?**

# Introduction

## relevant time scales



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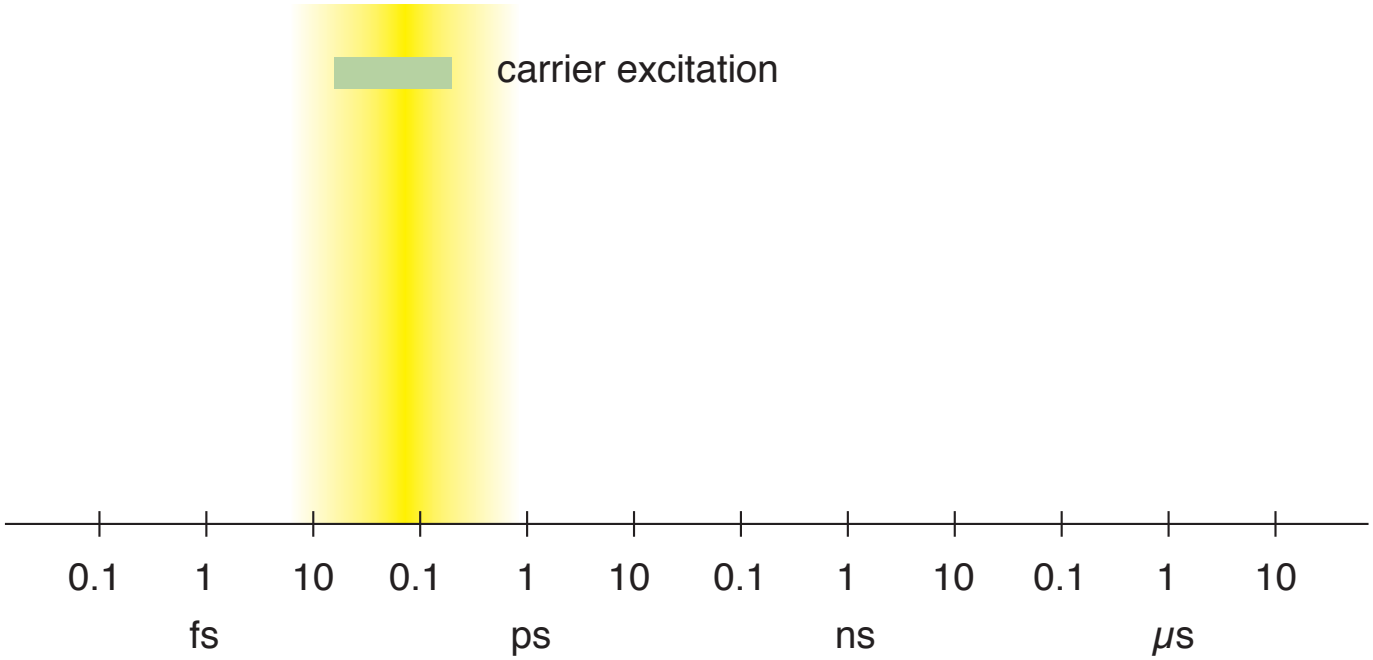

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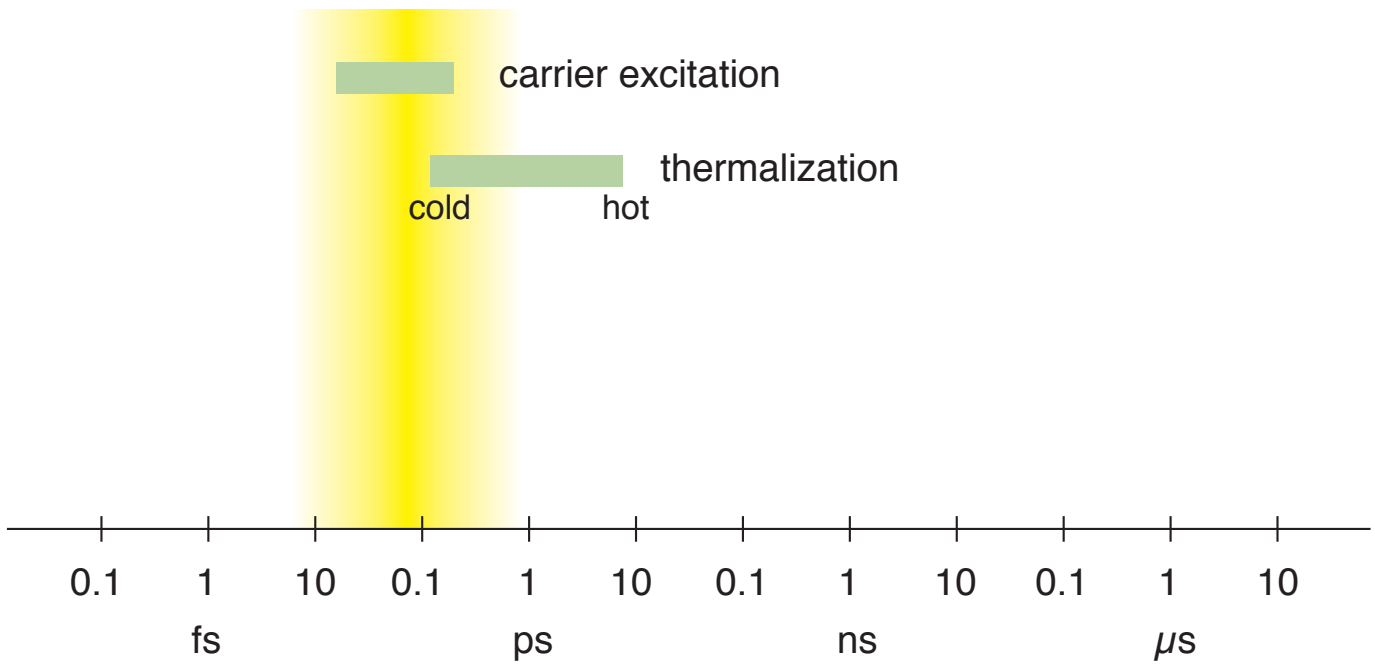

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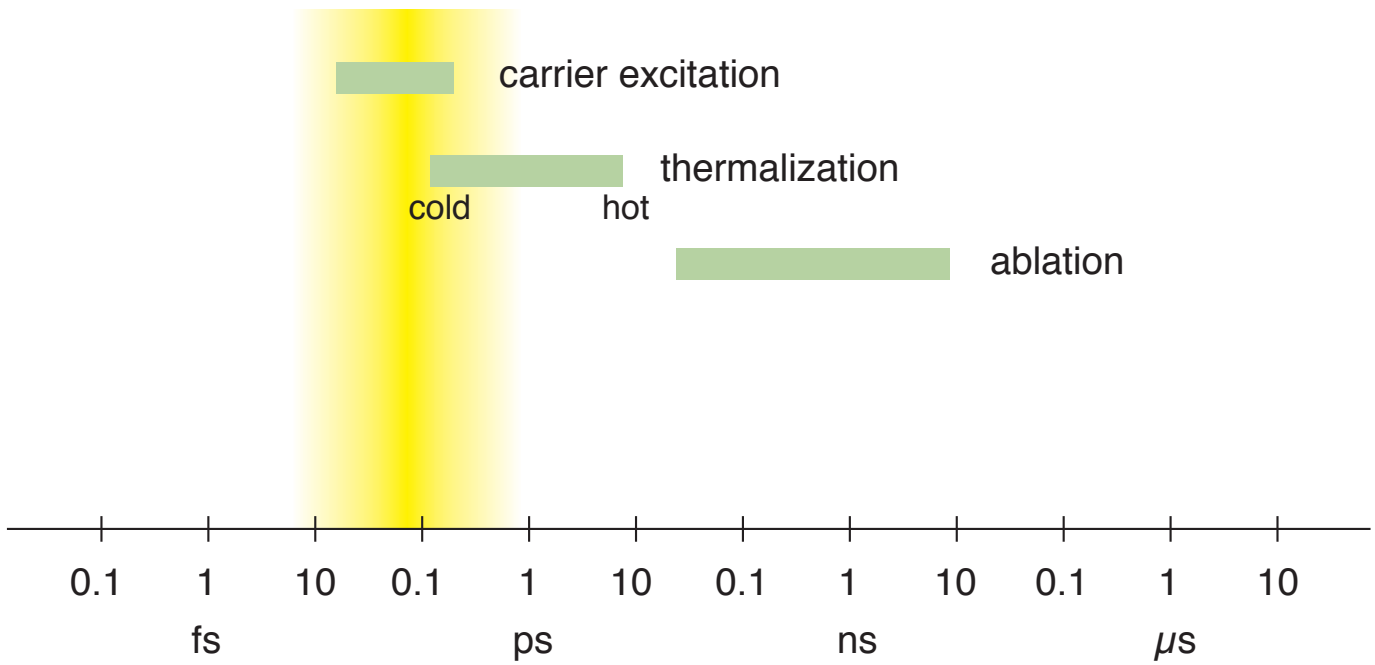

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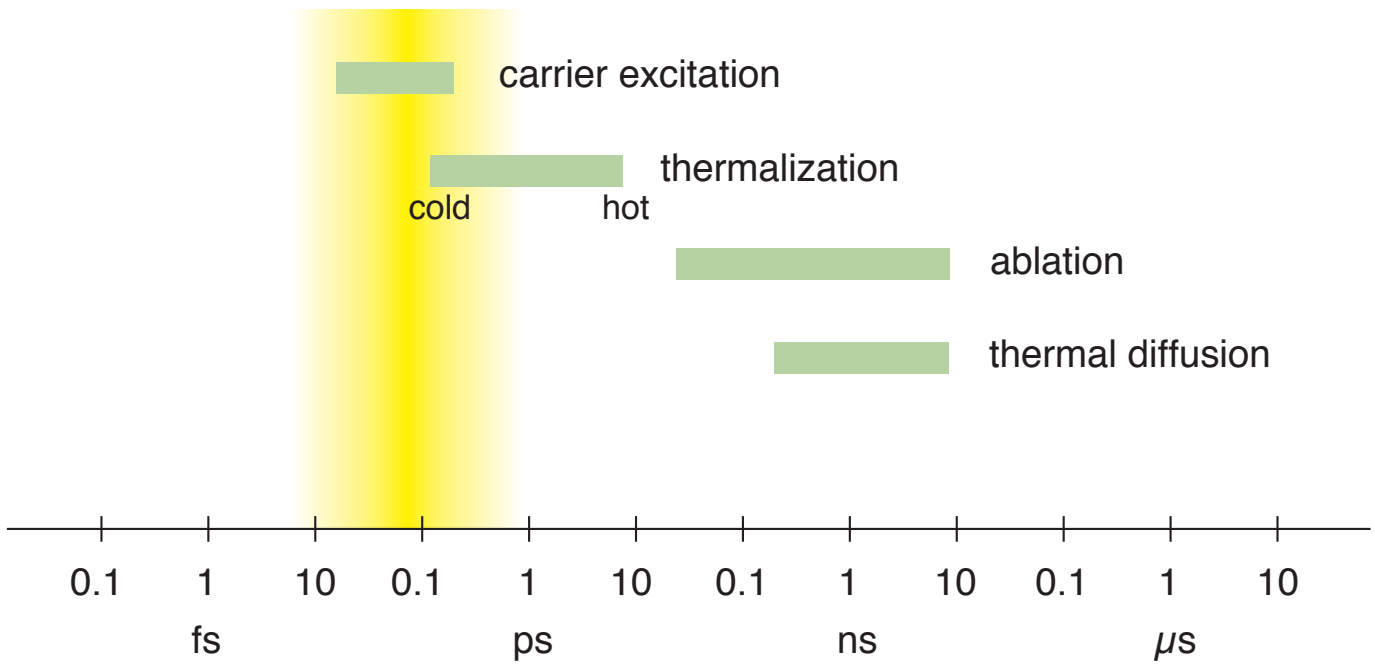

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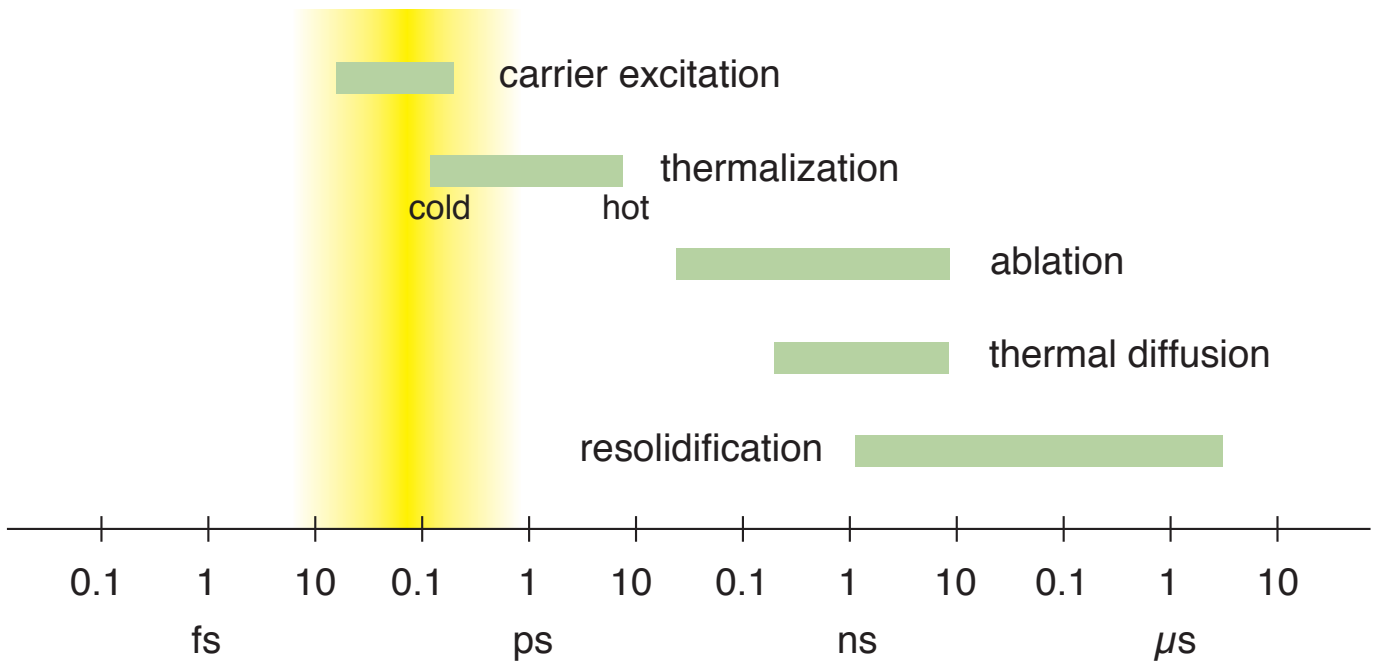

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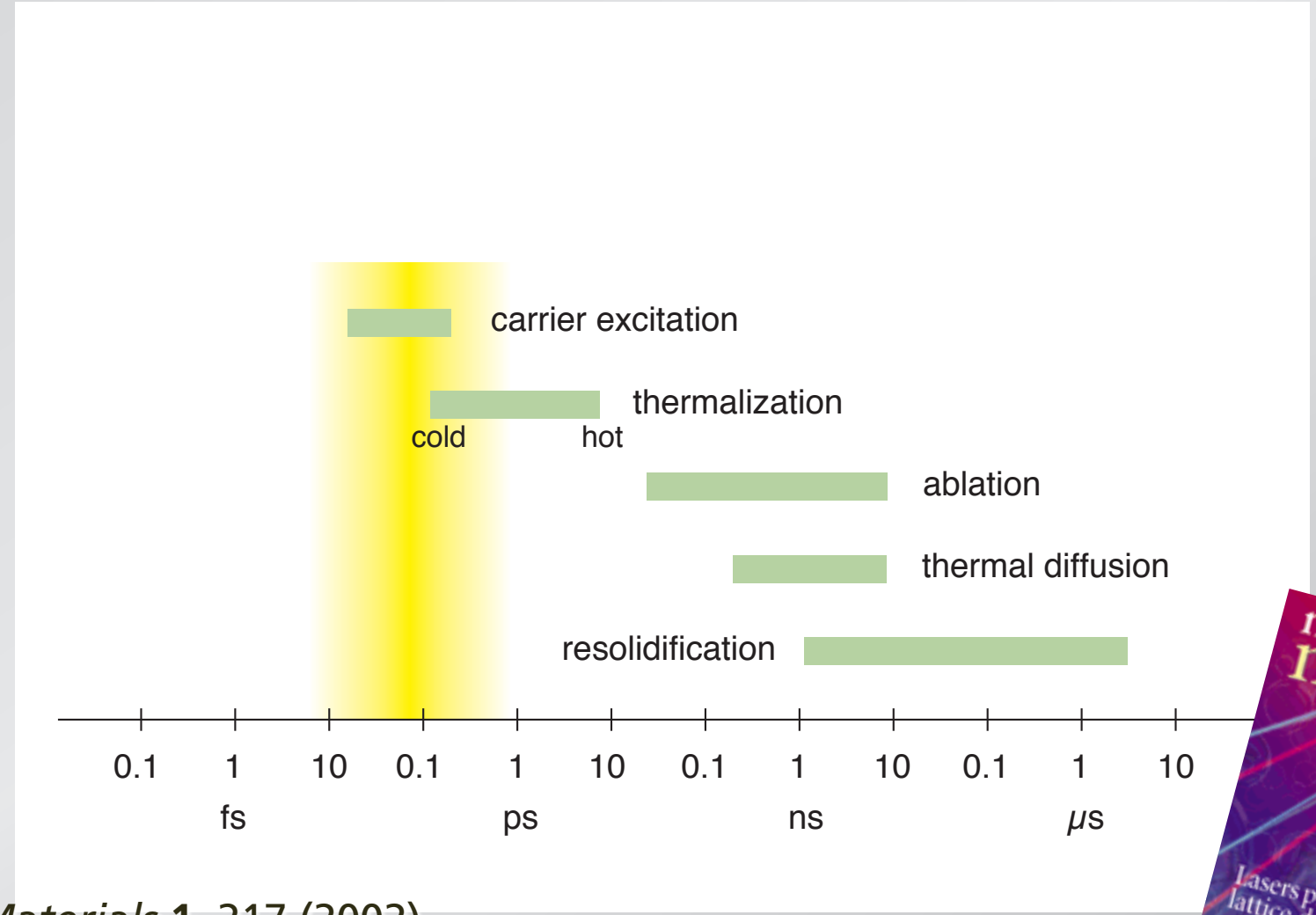
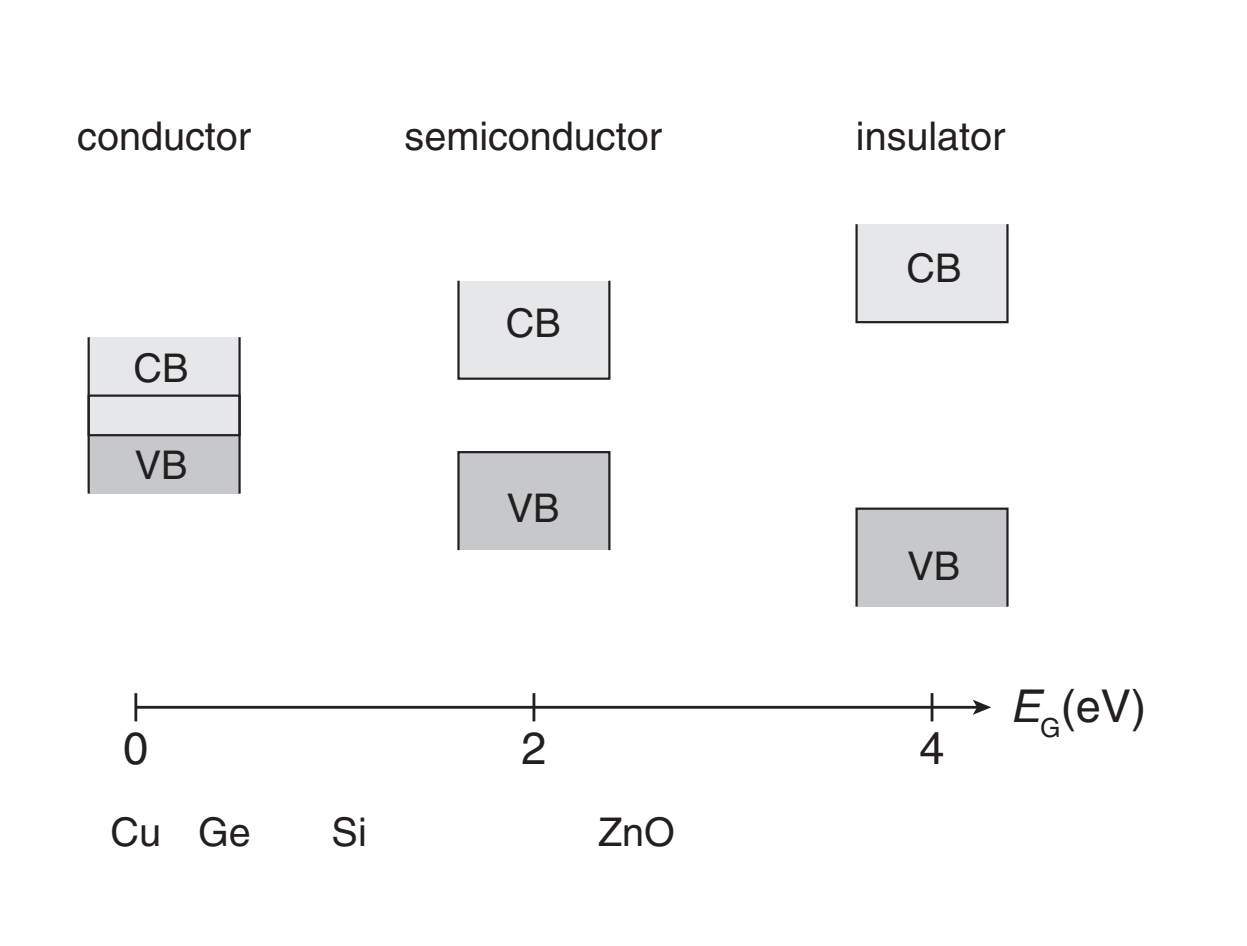




*Nature Materials* **1**, 217 (2002)

# Introduction

## gap determines interaction



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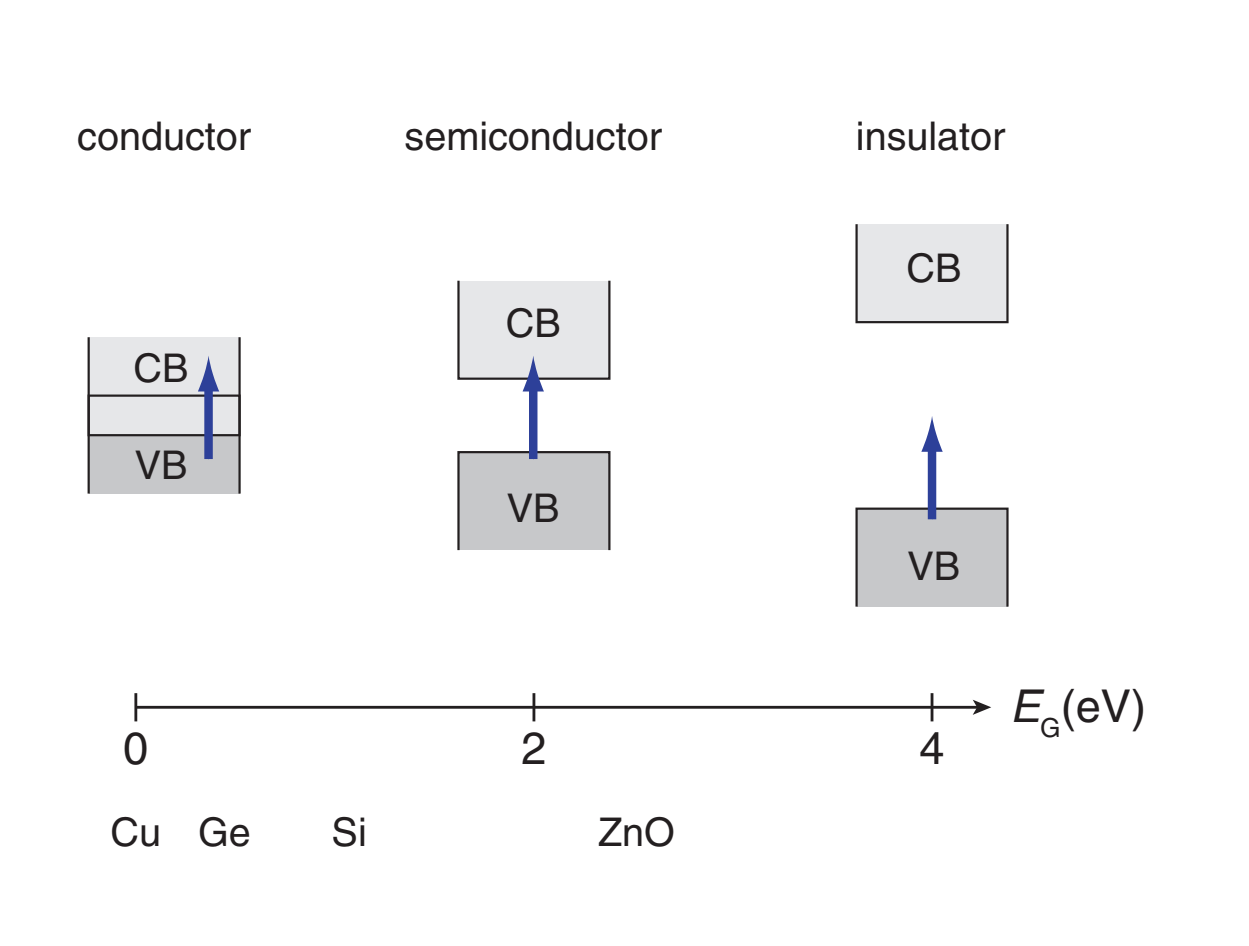

# Outline

- femtosecond materials interactions
- subcellular surgery
- nanoneurosurgery
- optofection

# Femtosecond materials interactions

photon energy < bandgap $\longrightarrow$ nonlinear interaction

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

# Femtosecond materials interactions

**Nonlinear polarization can drive new field:**

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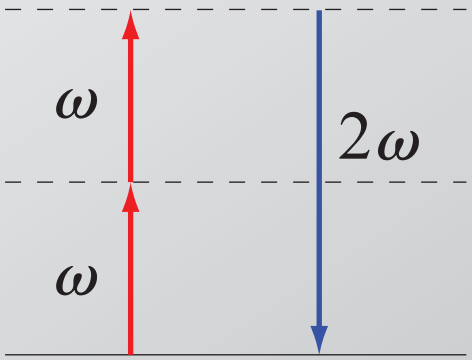

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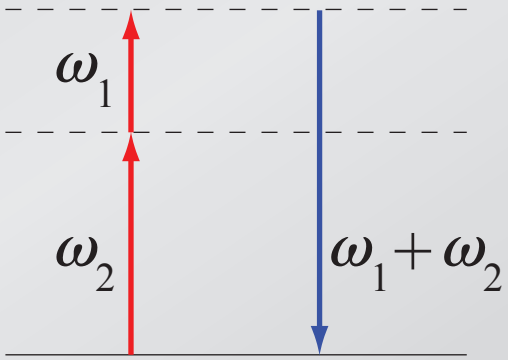


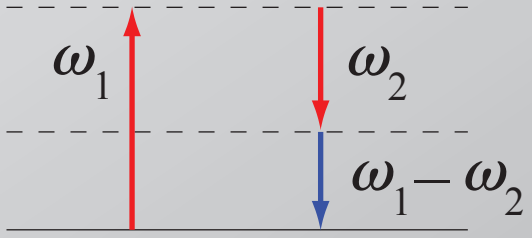

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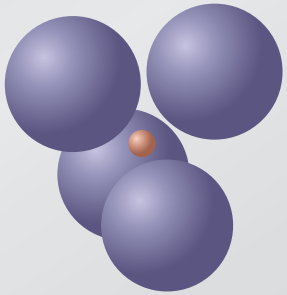

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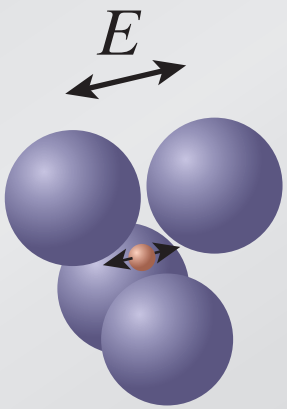

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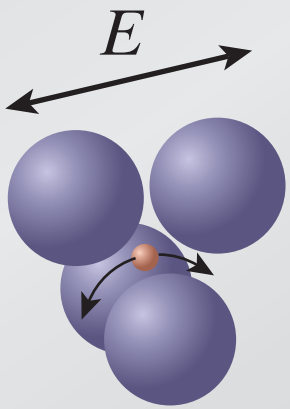

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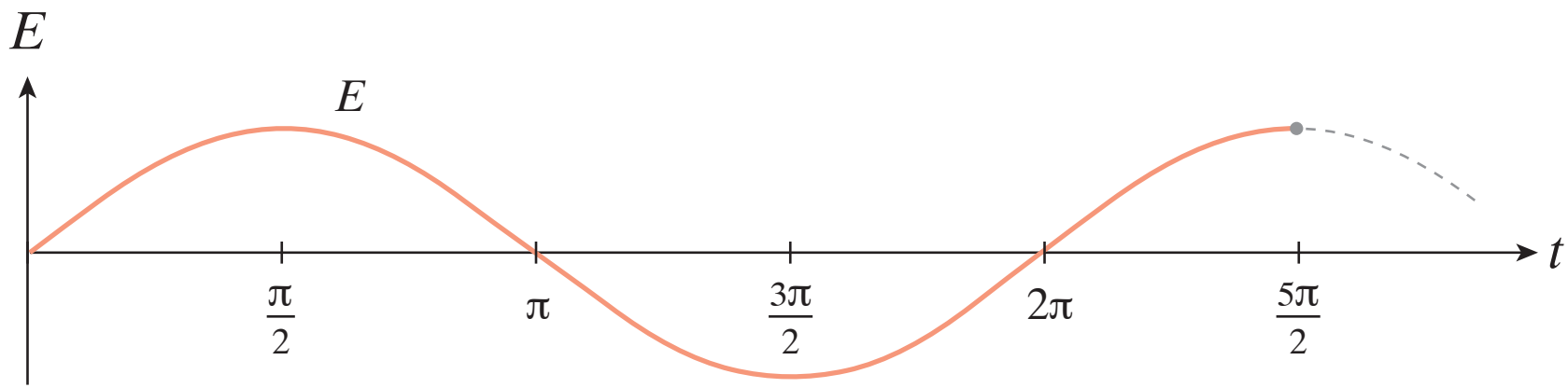

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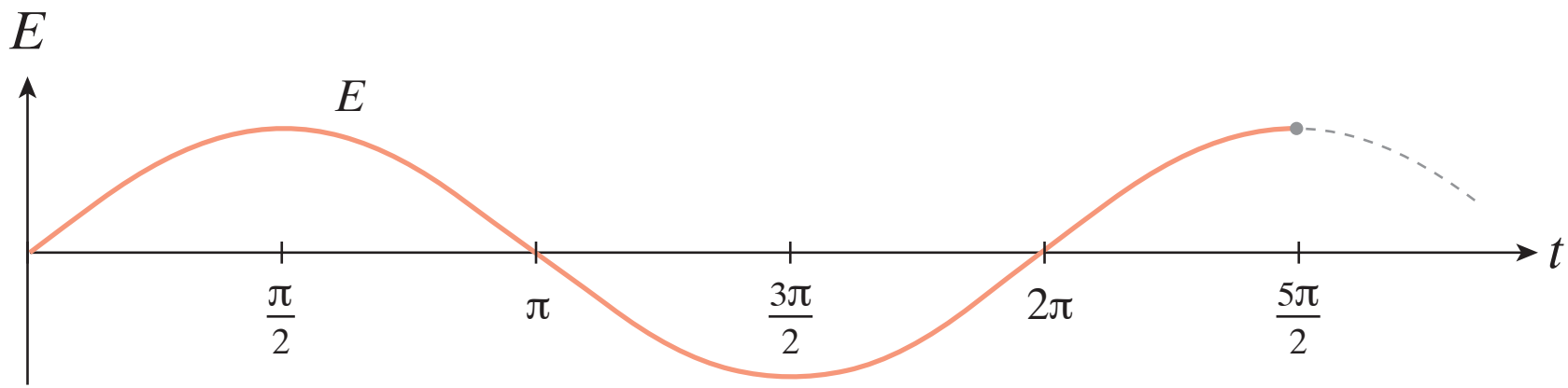


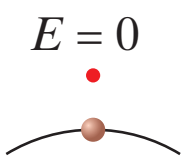

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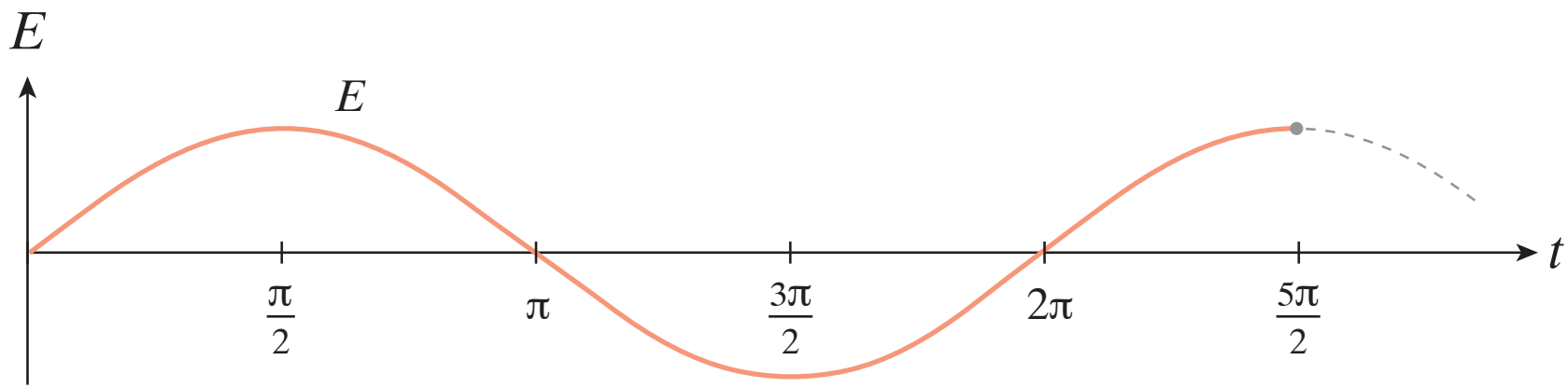


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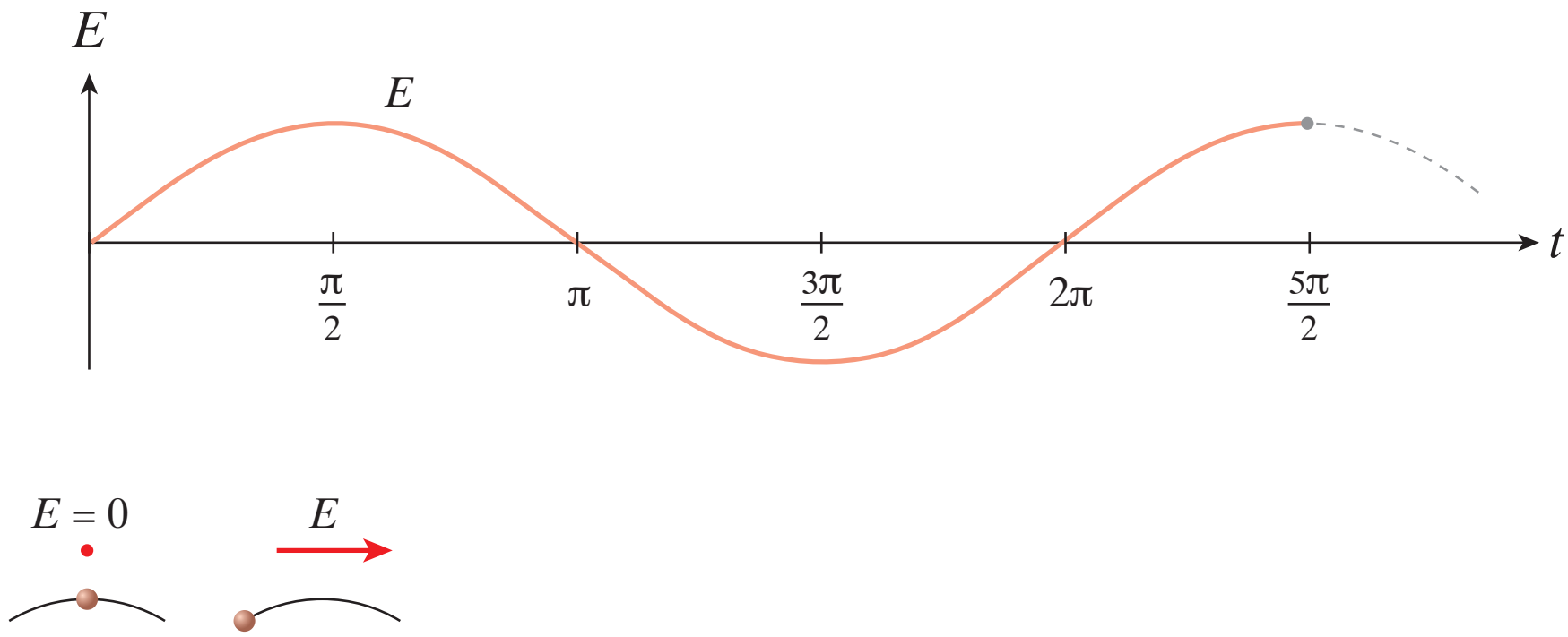

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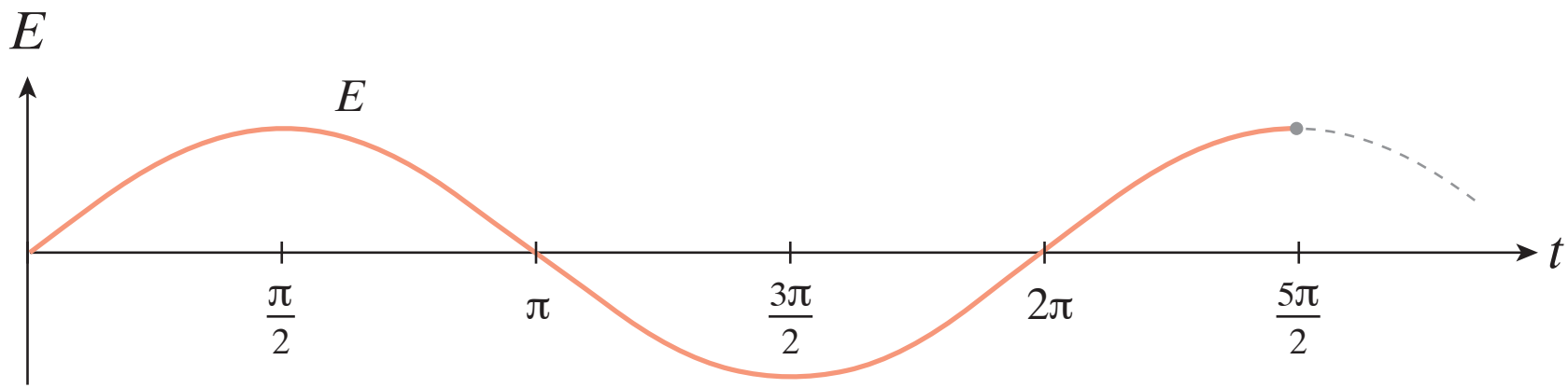


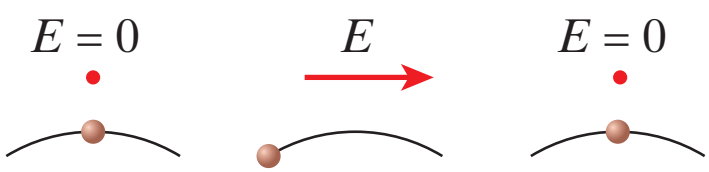

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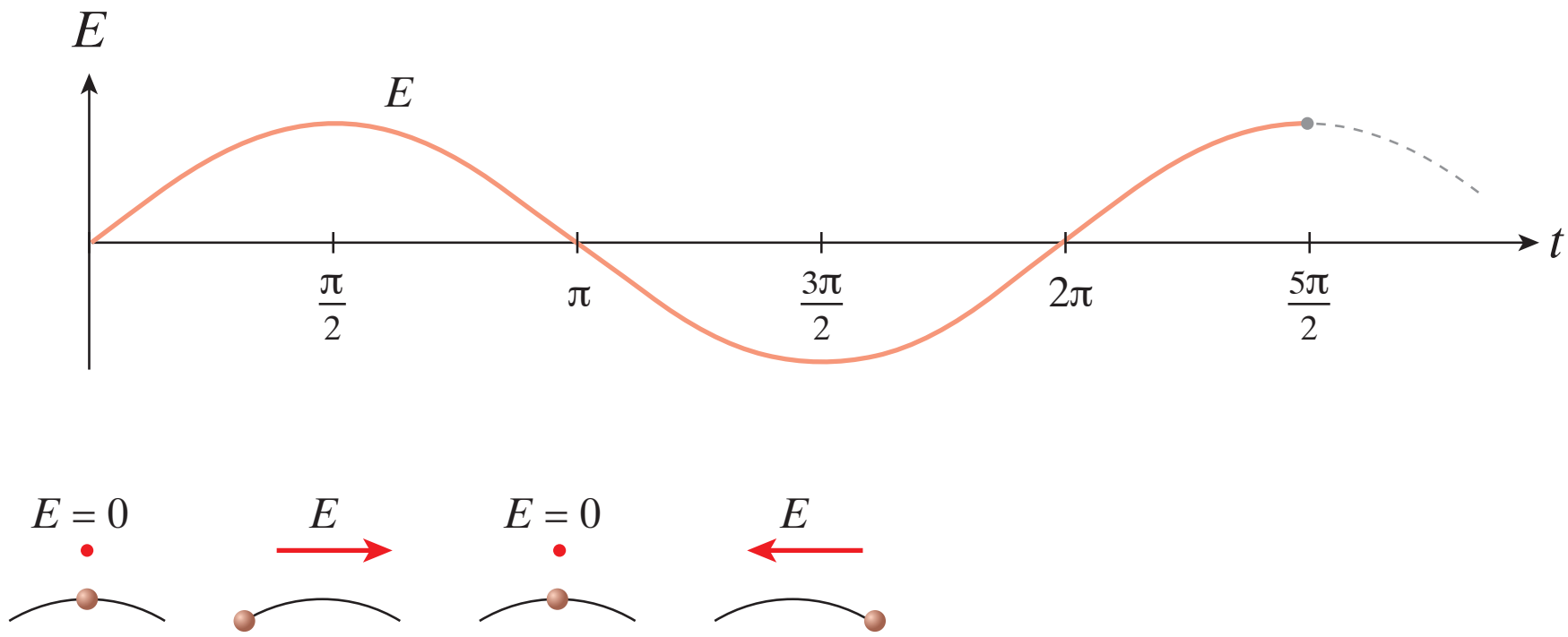

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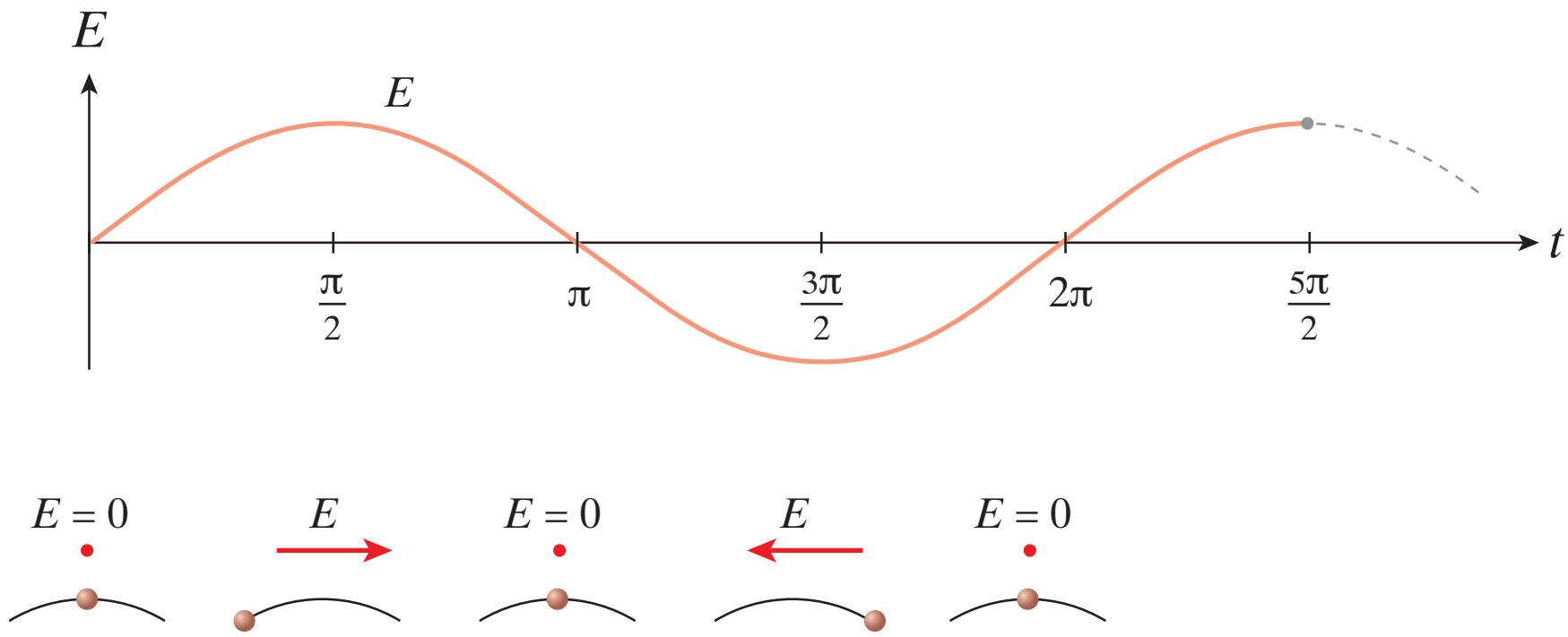

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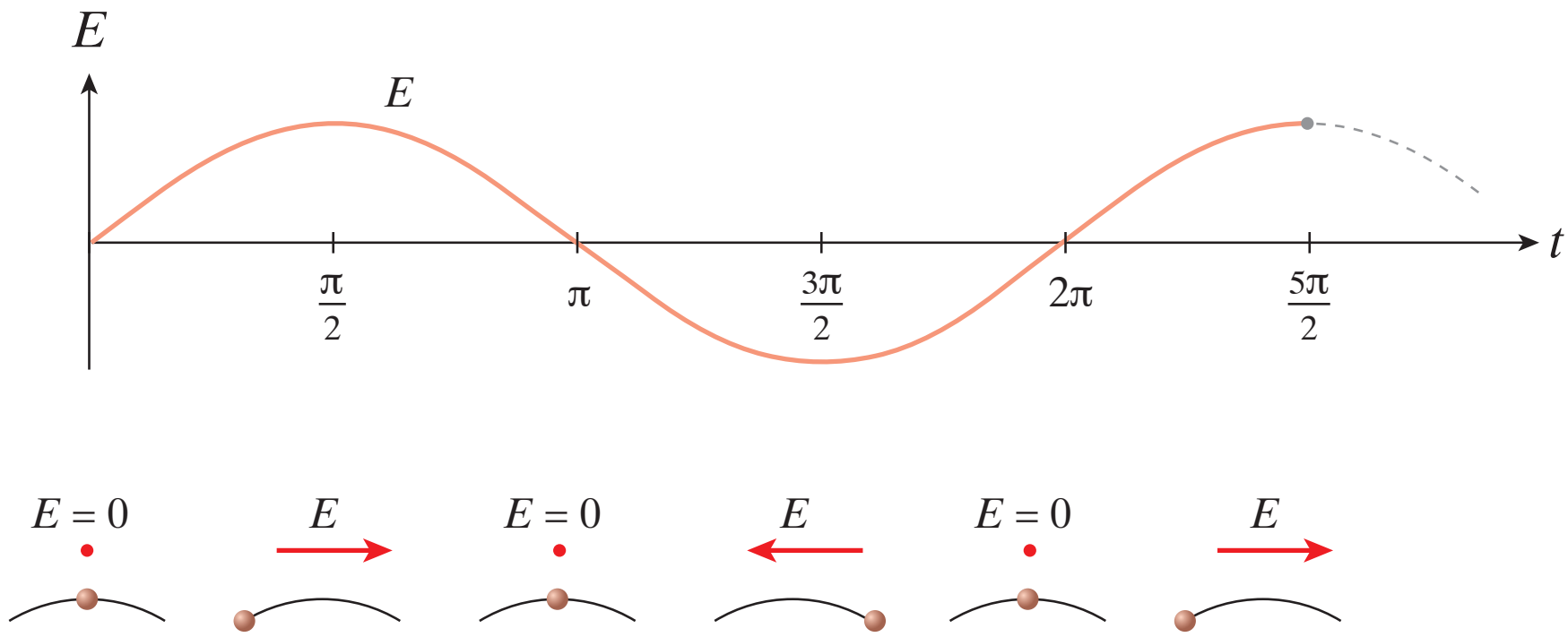

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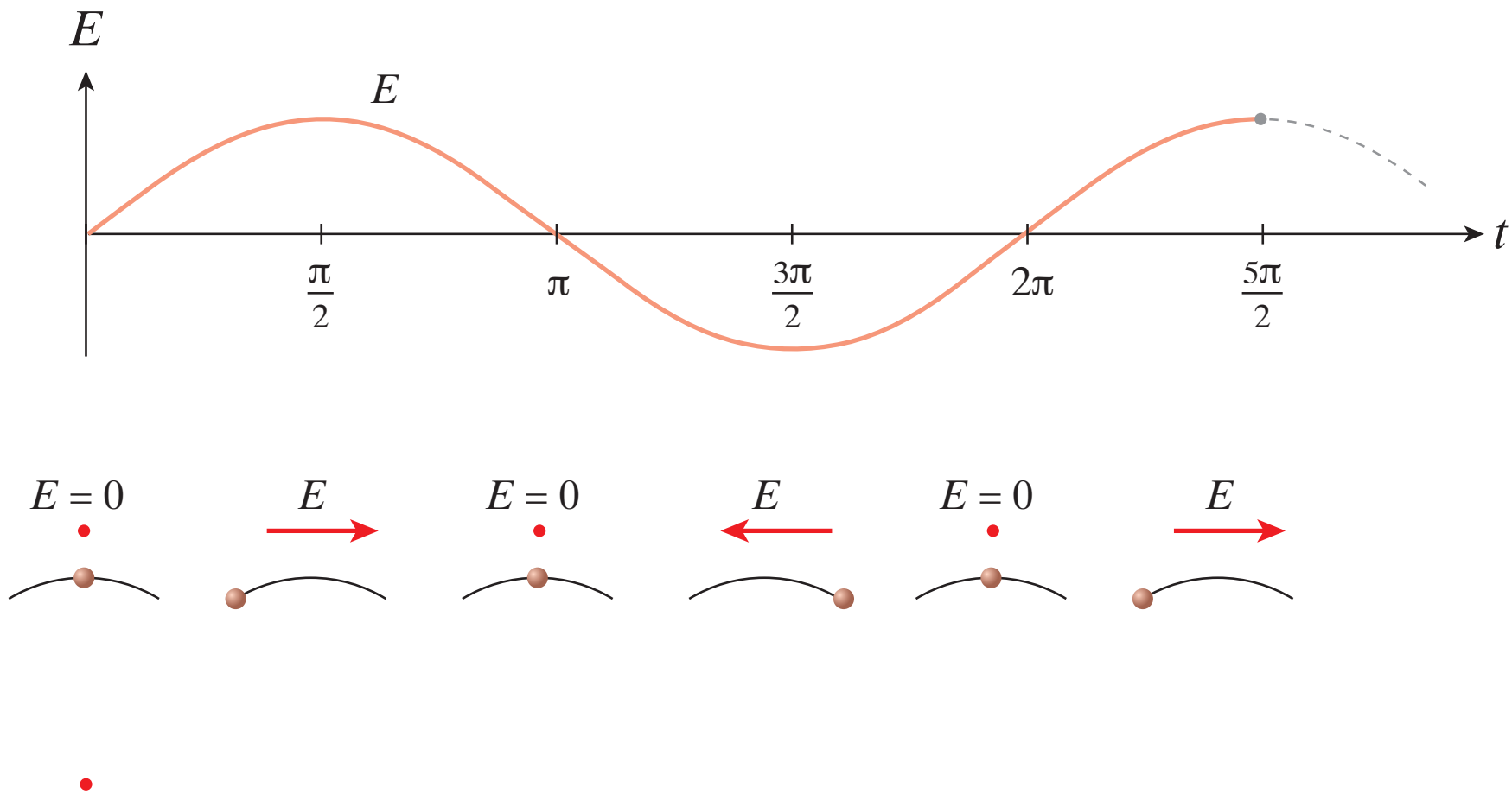

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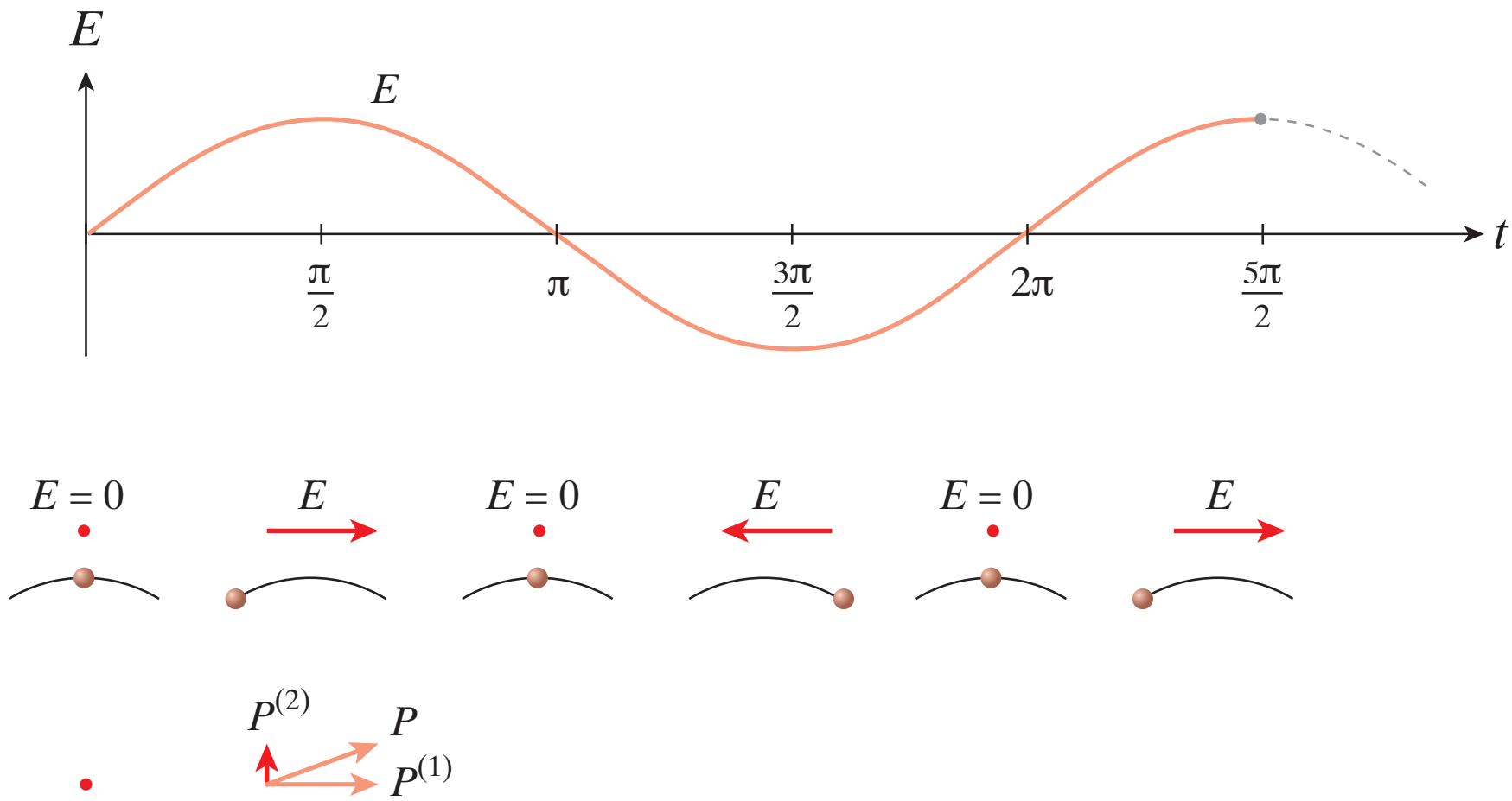

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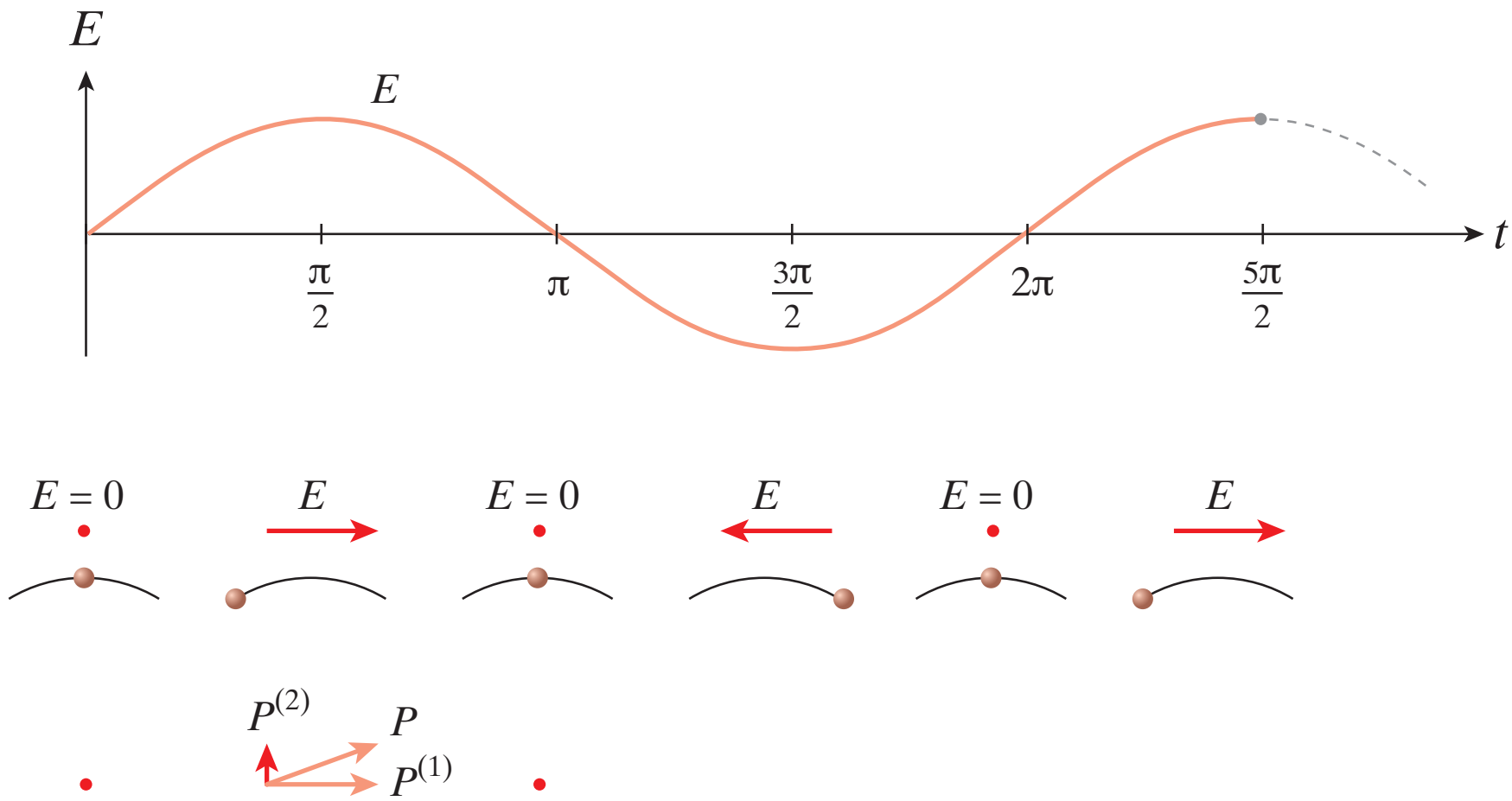

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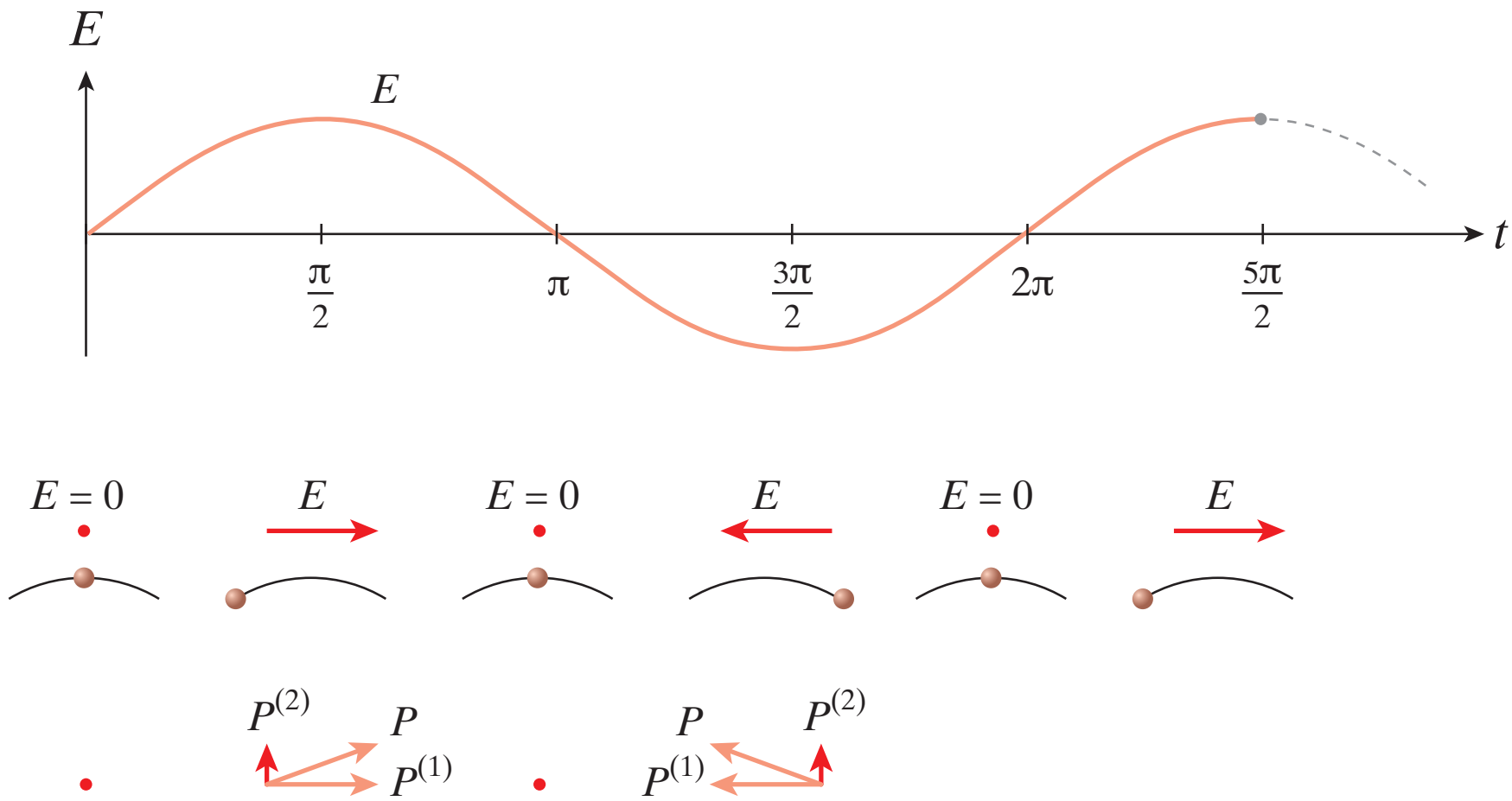

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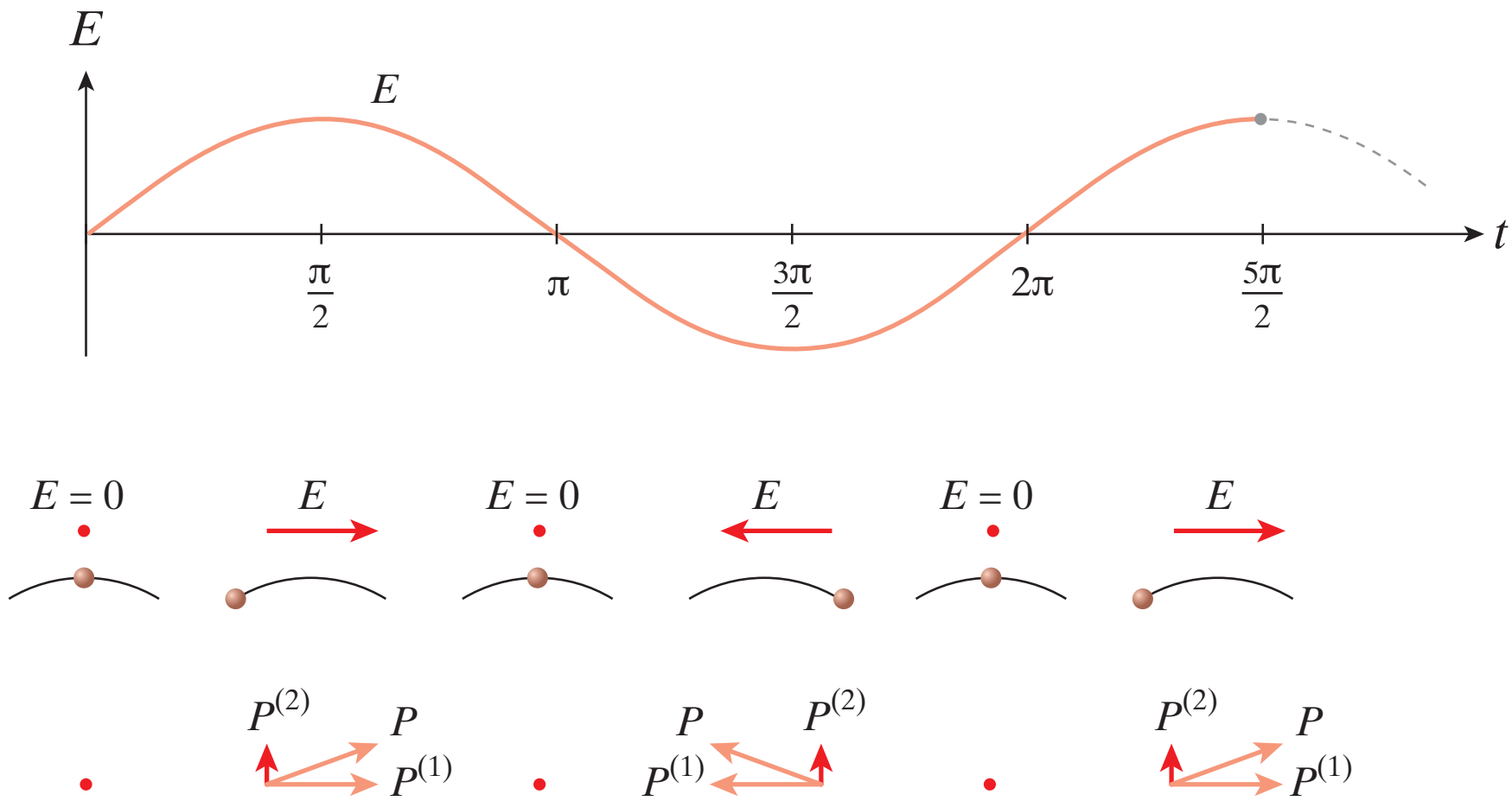

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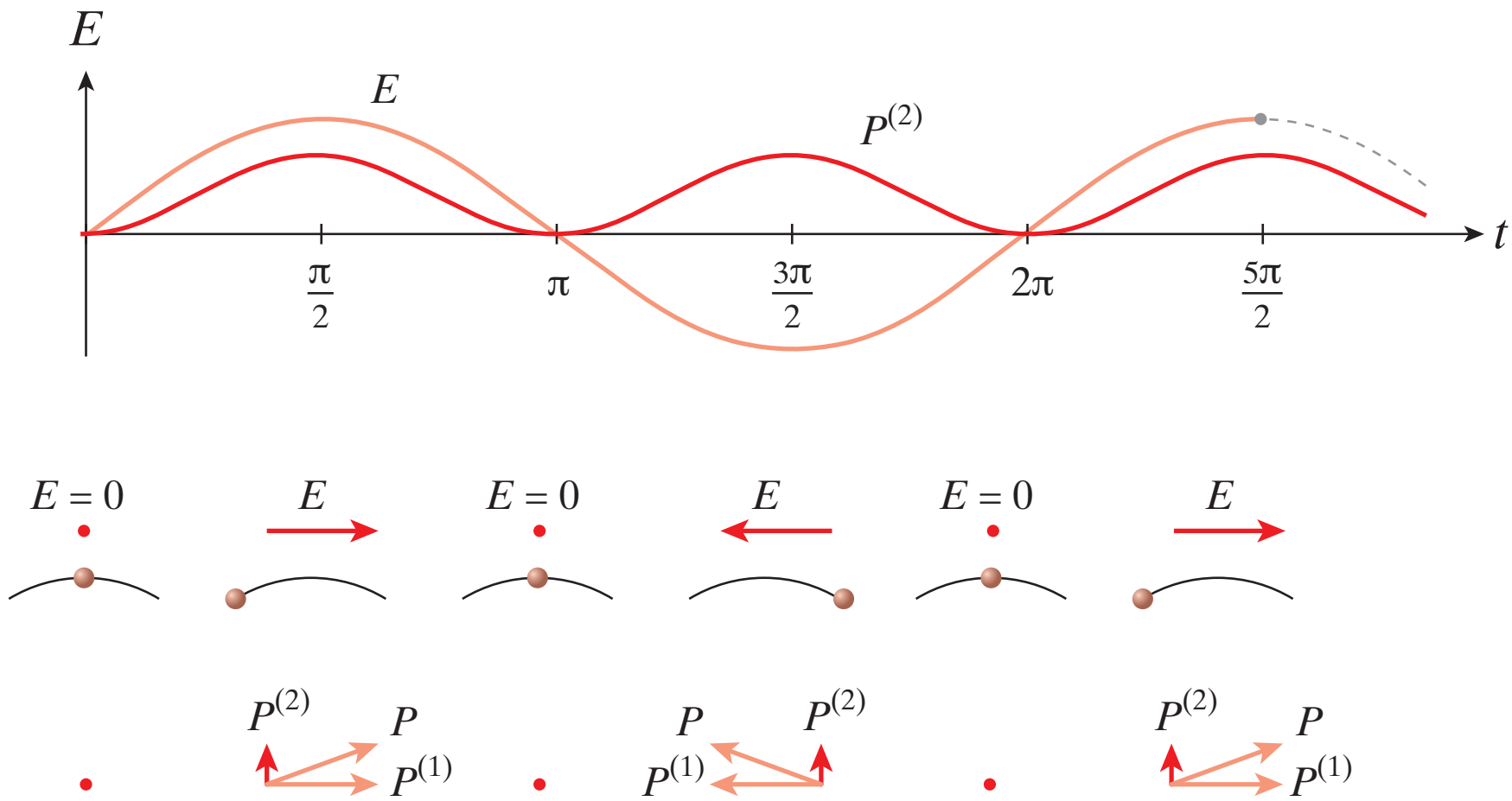

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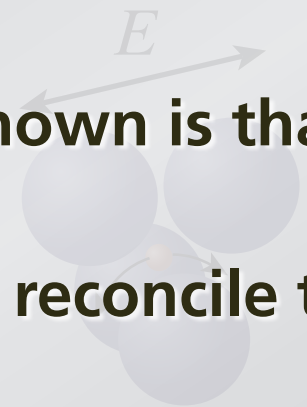
**Question to you:**




**The atomic arrangement shown is that of silicon, which is centrosymmetric. How can we reconcile this conceptual picture of SHG with the fact that $\chi^{(2)} = 0$ for centrosymmetric materials?**

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**3 frequencies, 3 terms + c.c.: complicated! In general**

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$$n = \sqrt{\epsilon} = \sqrt{1 + \chi_{eff}} \approx \sqrt{1 + \chi^{(1)}} + \tfrac{1}{2} \frac{\chi^{(3)} I}{\sqrt{1 + \chi^{(1)}}} = n_o + n_2 I$$

# Femtosecond materials interactions

**Intensity-dependent index of refraction:**

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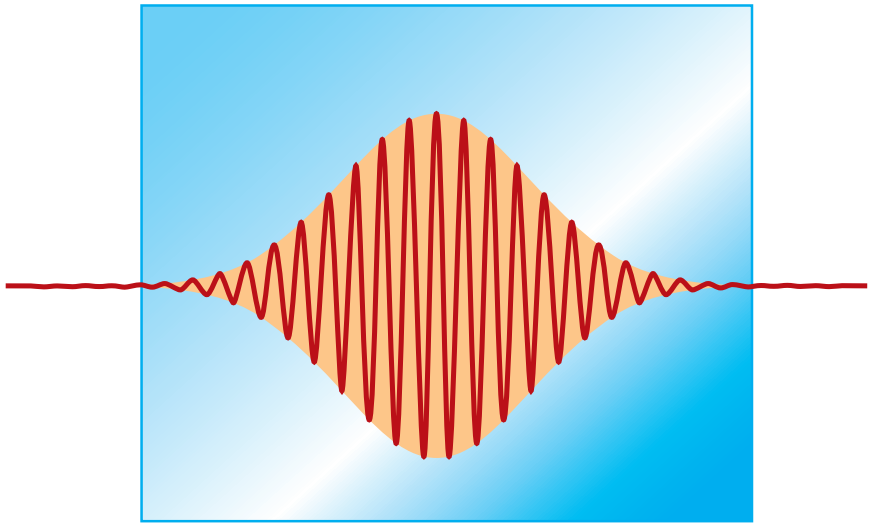

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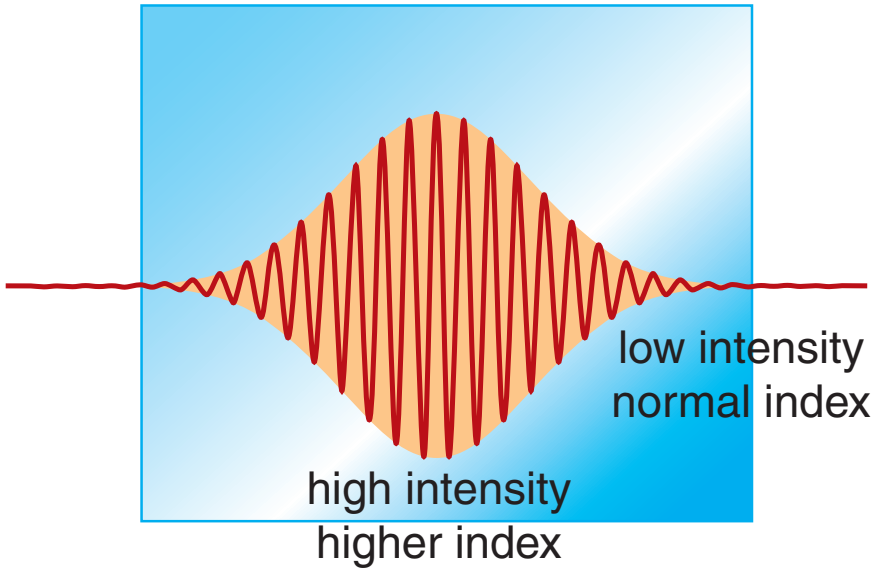

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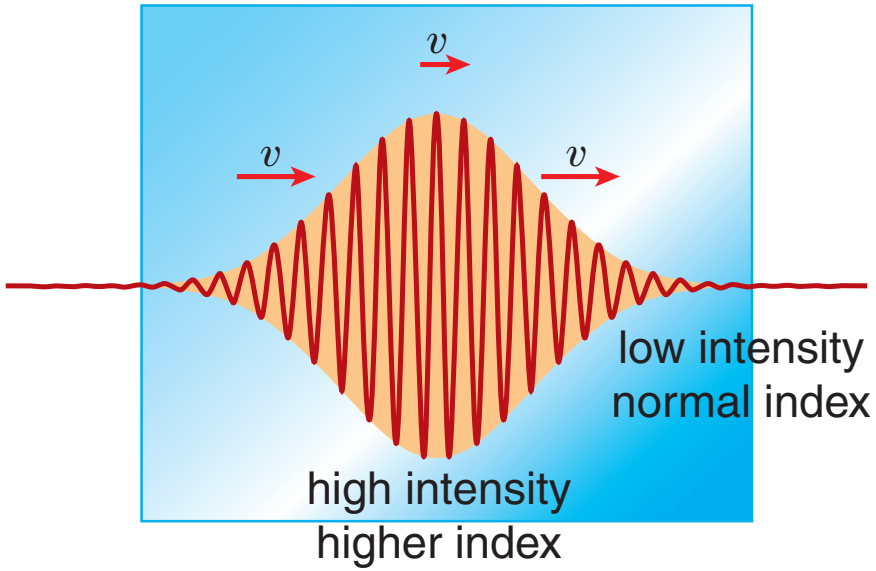
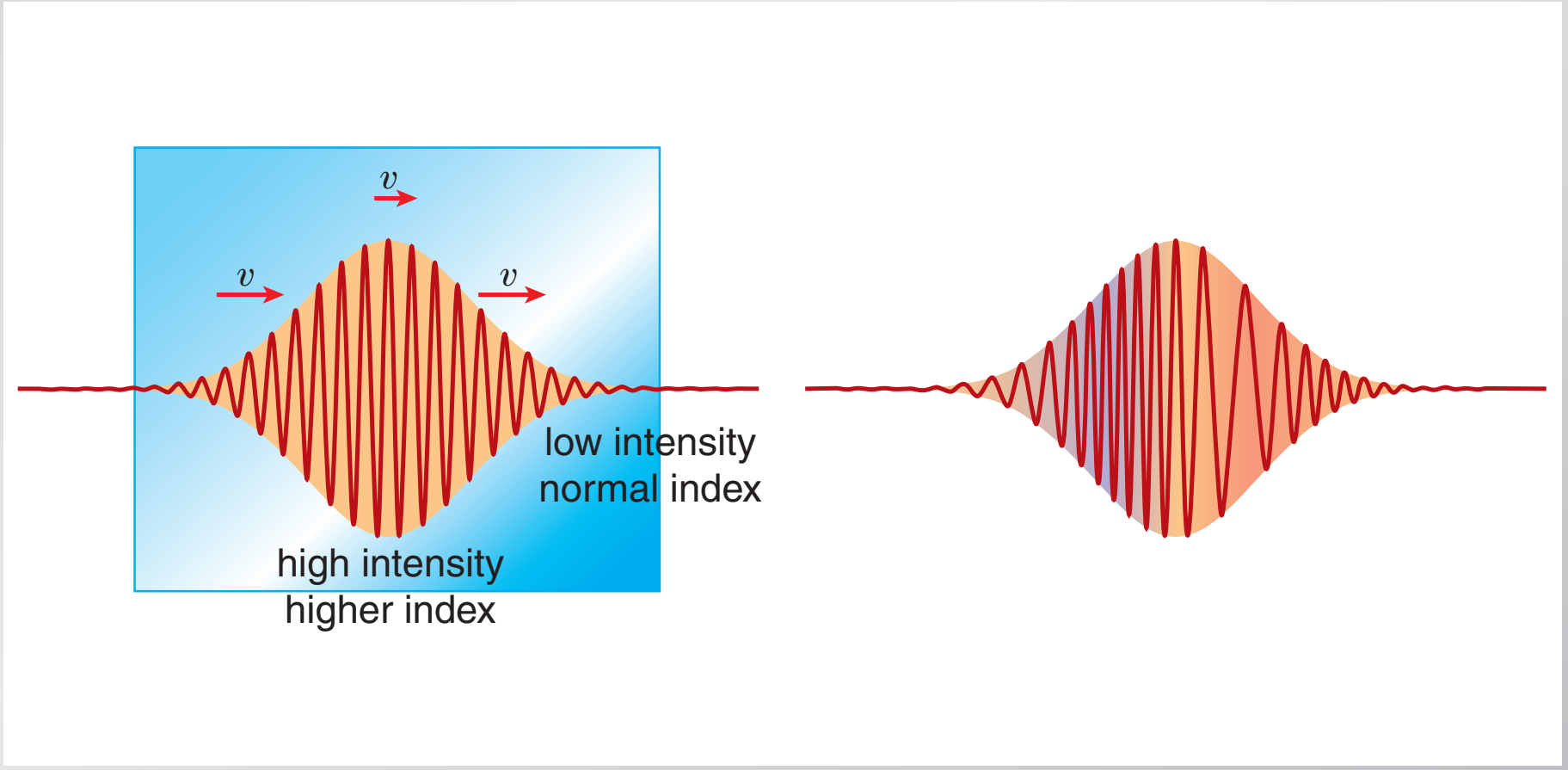


# Femtosecond materials interactions

## Intensity-dependent index of refraction:

$$n = n_o + n_2 I$$



# Femtosecond materials interactions

## Intensity-dependent index of refraction:

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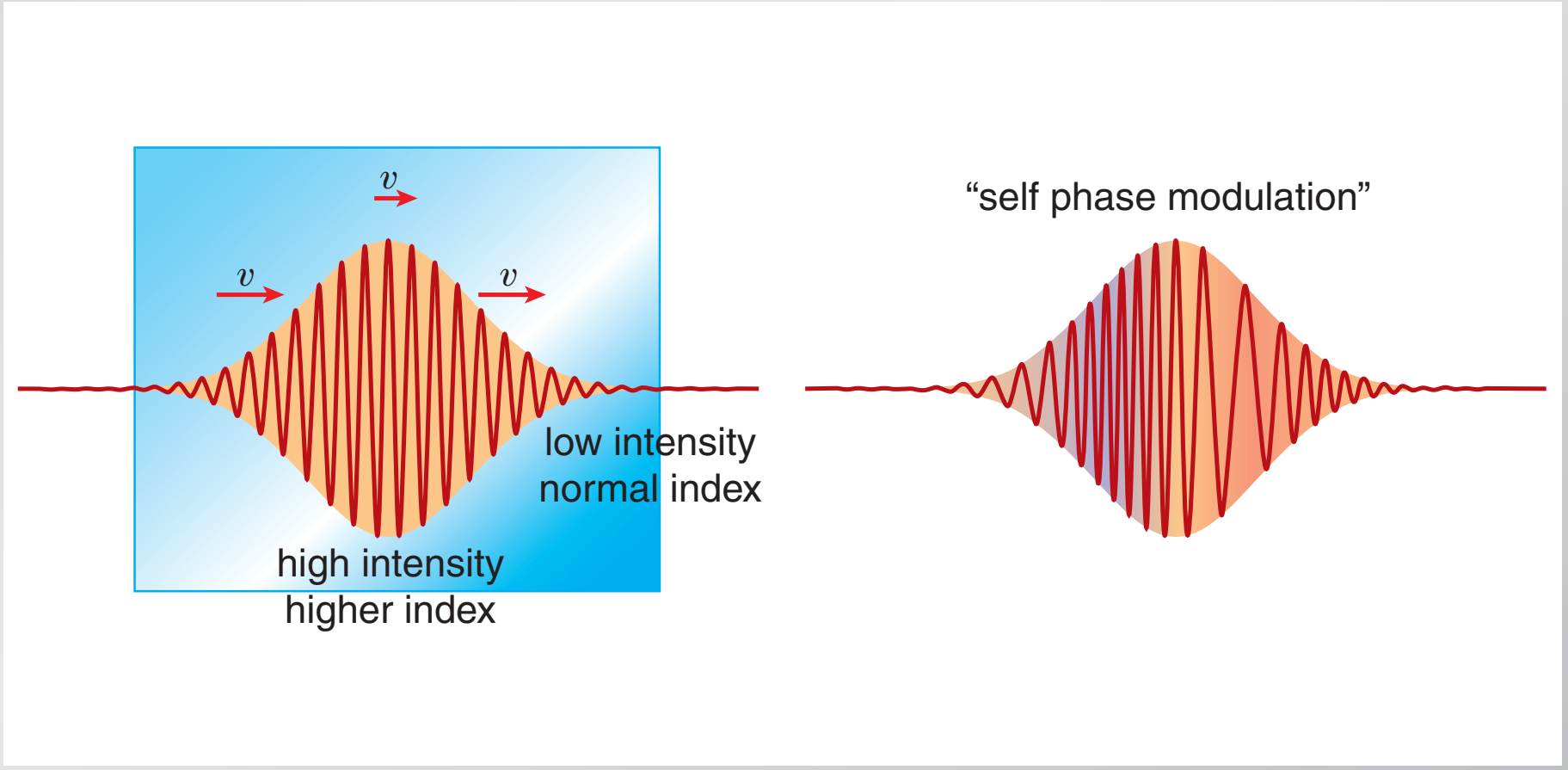
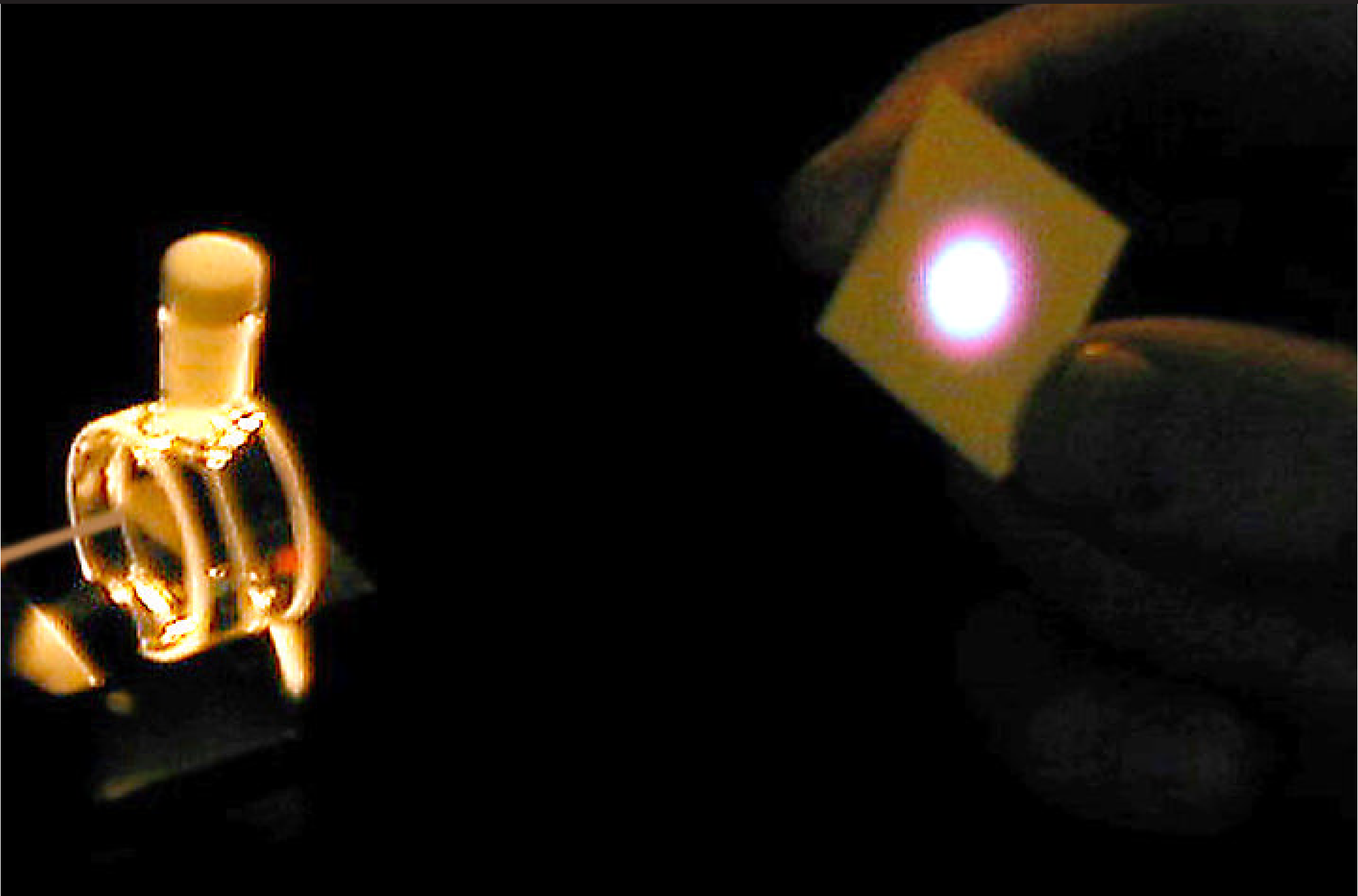


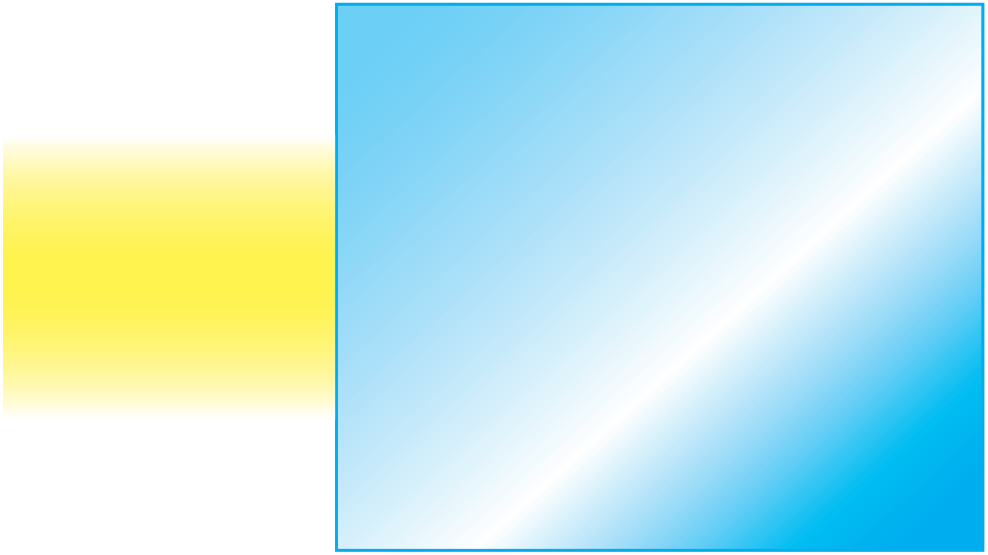
# Femtosecond materials interactions



# Femtosecond materials interactions

**Intensity-dependent index of refraction:**

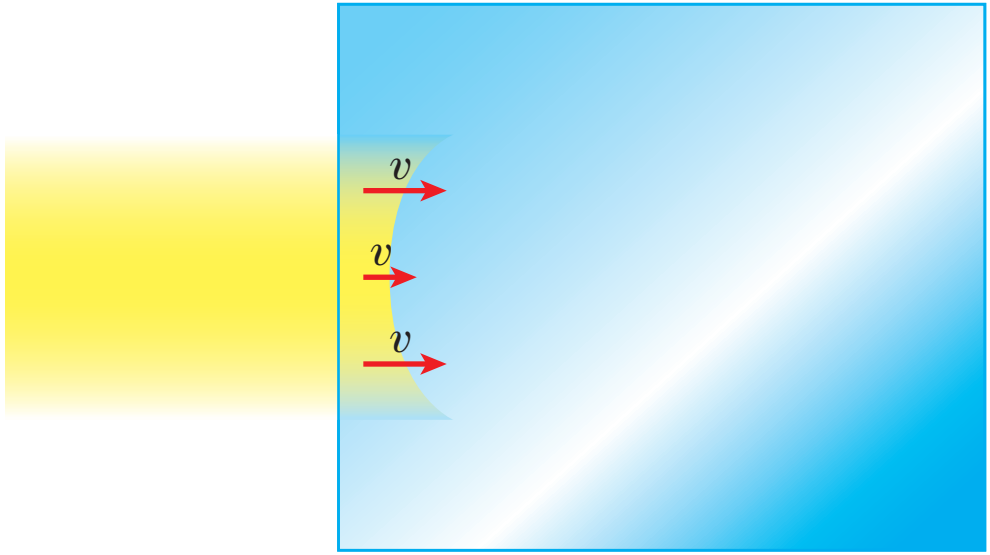
$$n = n_o + n_2 I$$



# Femtosecond materials interactions

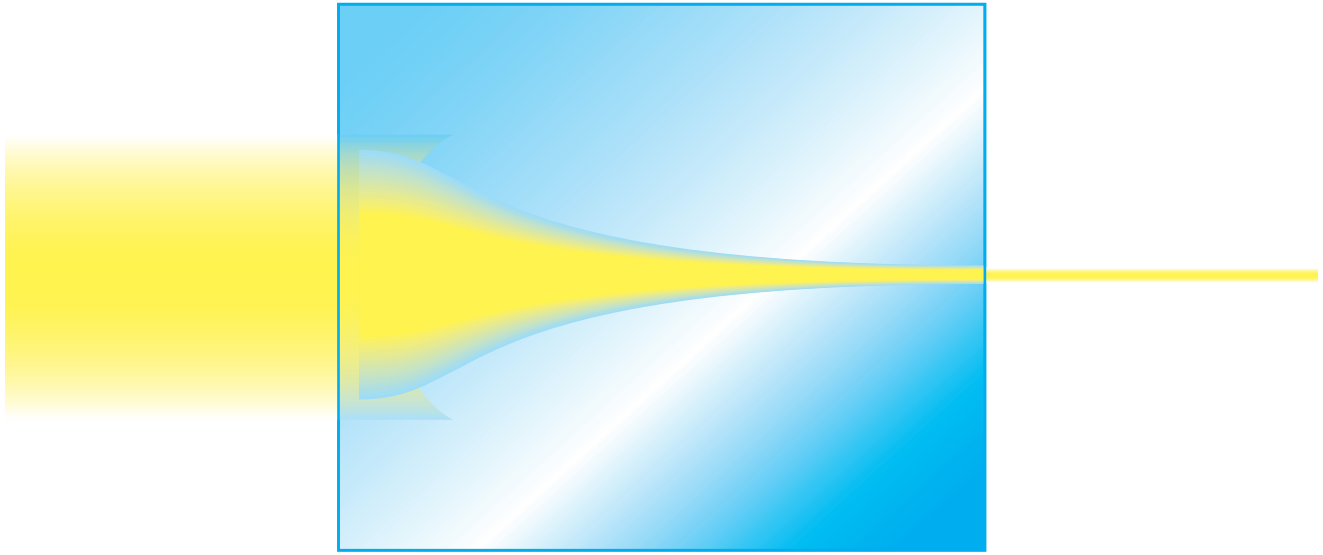
**Intensity-dependent index of refraction:**

$$n = n_o + n_2 I$$



# Femtosecond materials interactions

## self-focusing



# Femtosecond materials interactions

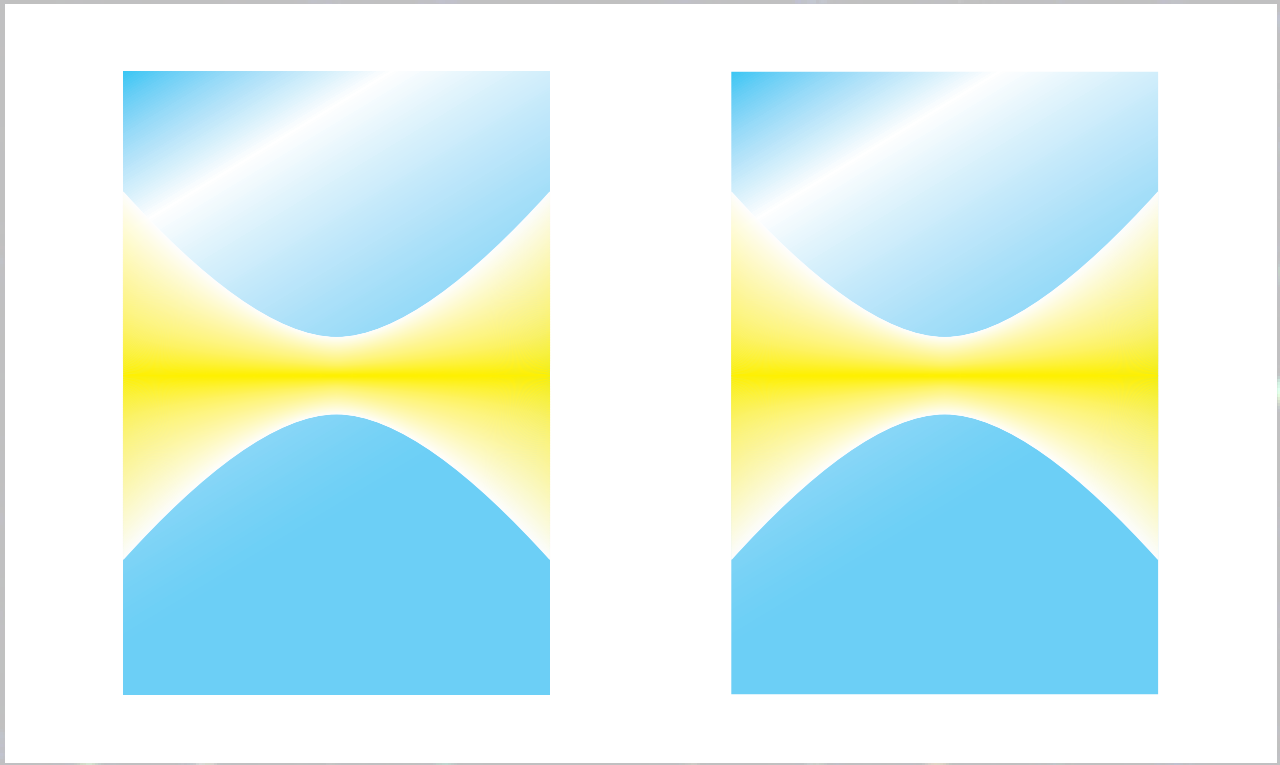
**but susceptibility is complex!**

| susceptibility | real part | imaginary part |
|---|---|---|
| linear | refraction | absorption |
| nonlinear | SHG, SFG, DFG, THG,… | multiphoton absorption |

$$\alpha = \alpha_o + \beta I + \gamma I^2 + \ldots$$

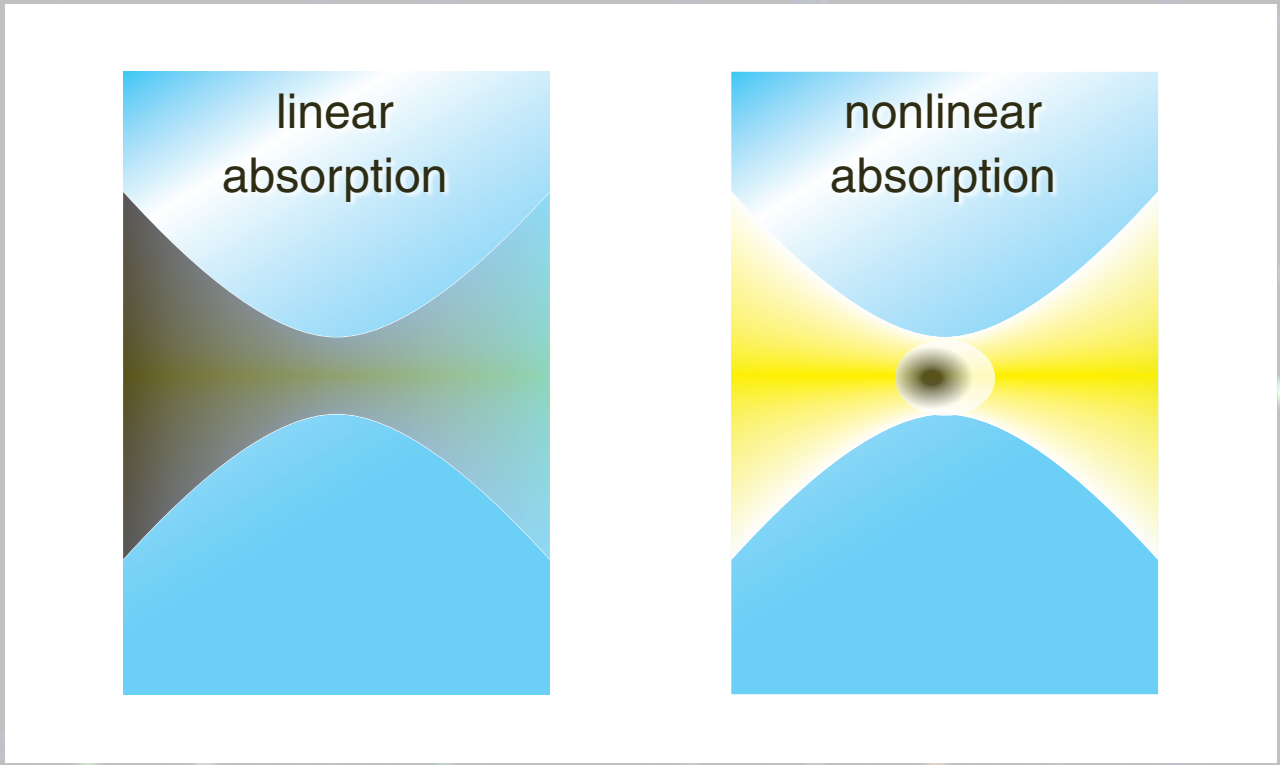
# Femtosecond materials interactions

nonlinear interaction provides bulk confinement



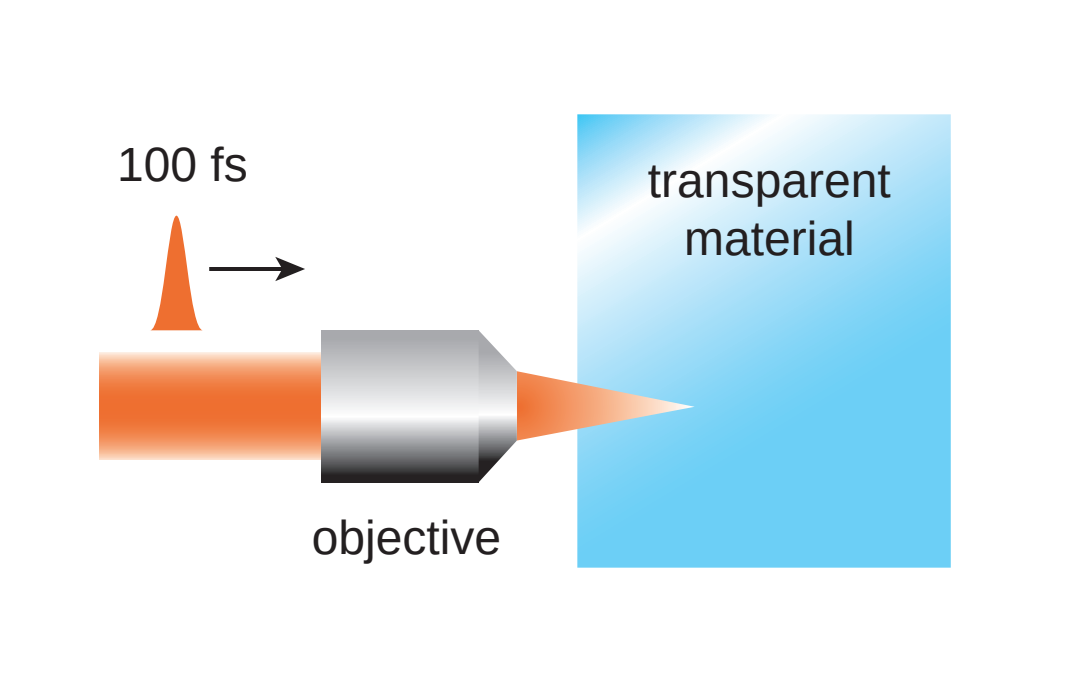
# Femtosecond materials interactions

## nonlinear interaction provides bulk confinement



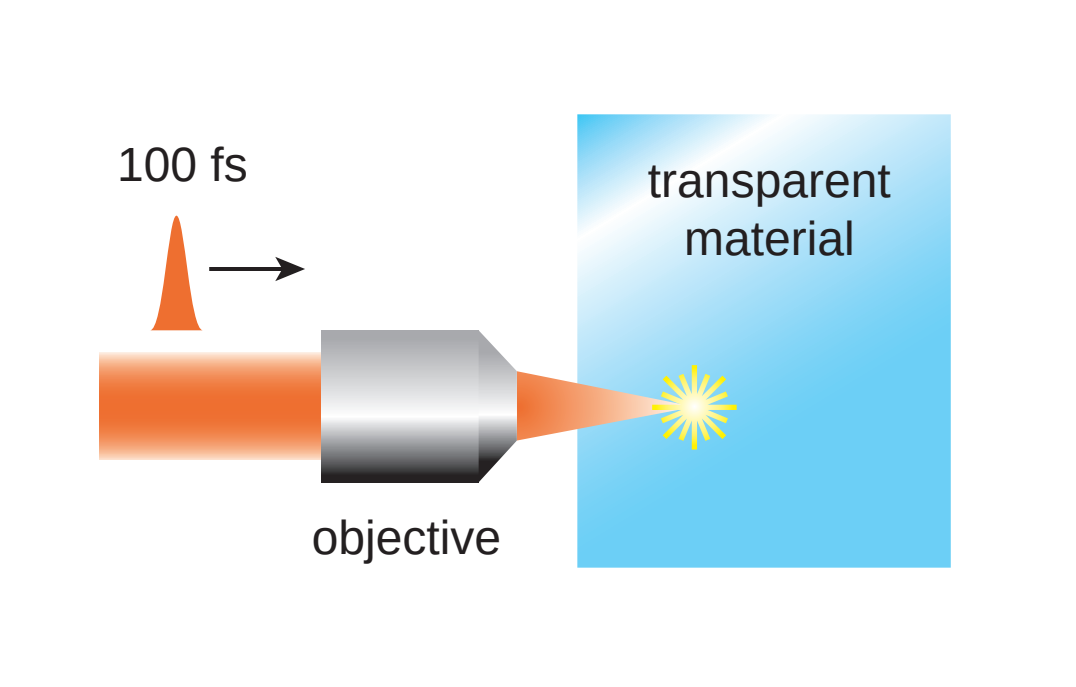
# Femtosecond materials interactions

**high intensity at focus…**



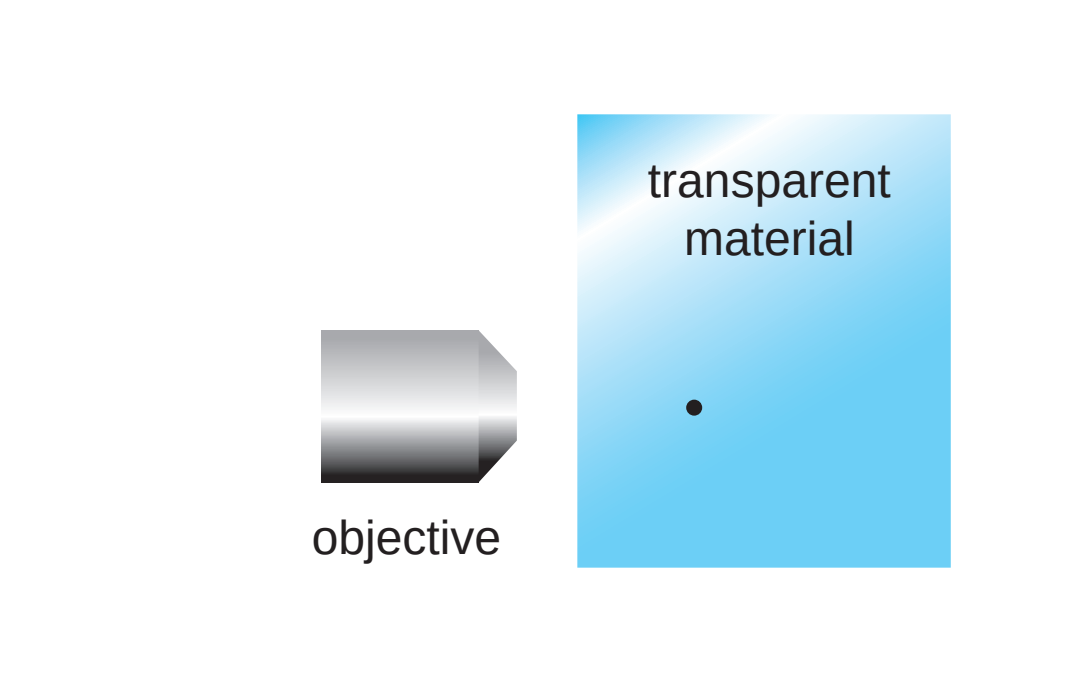
# Femtosecond materials interactions

**…causes nonlinear ionization…**

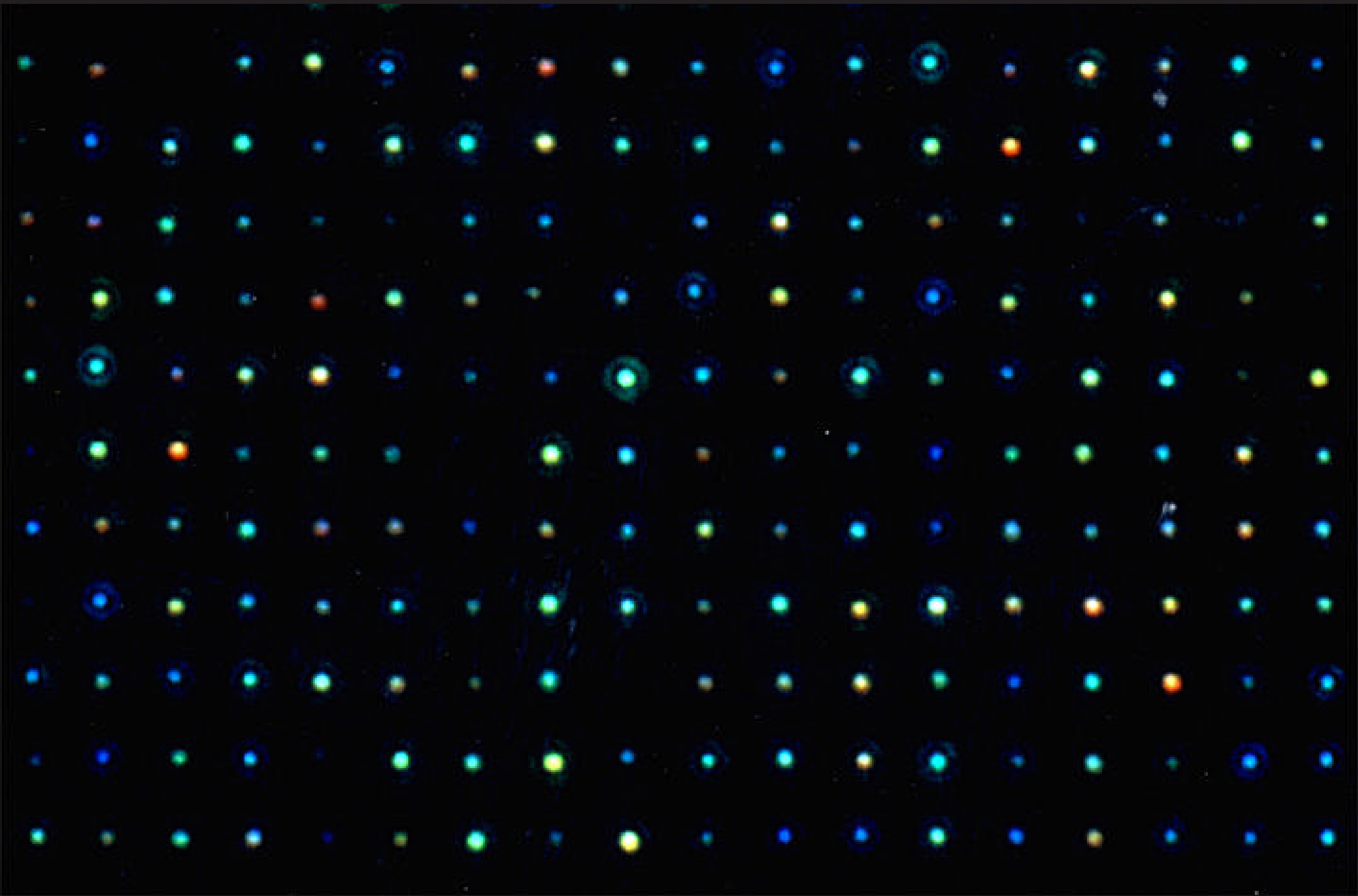


# Femtosecond materials interactions

**and ‘microexplosion’ causes microscopic damage…**



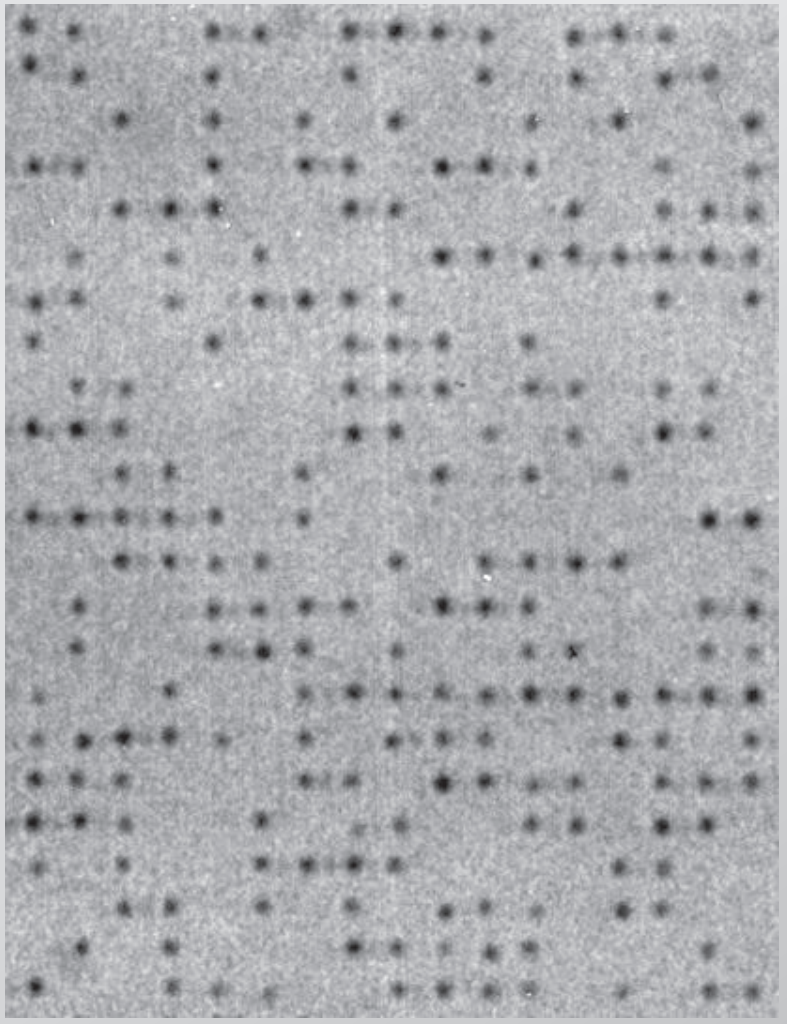
# Femtosecond materials interactions



# Femtosecond materials interactions

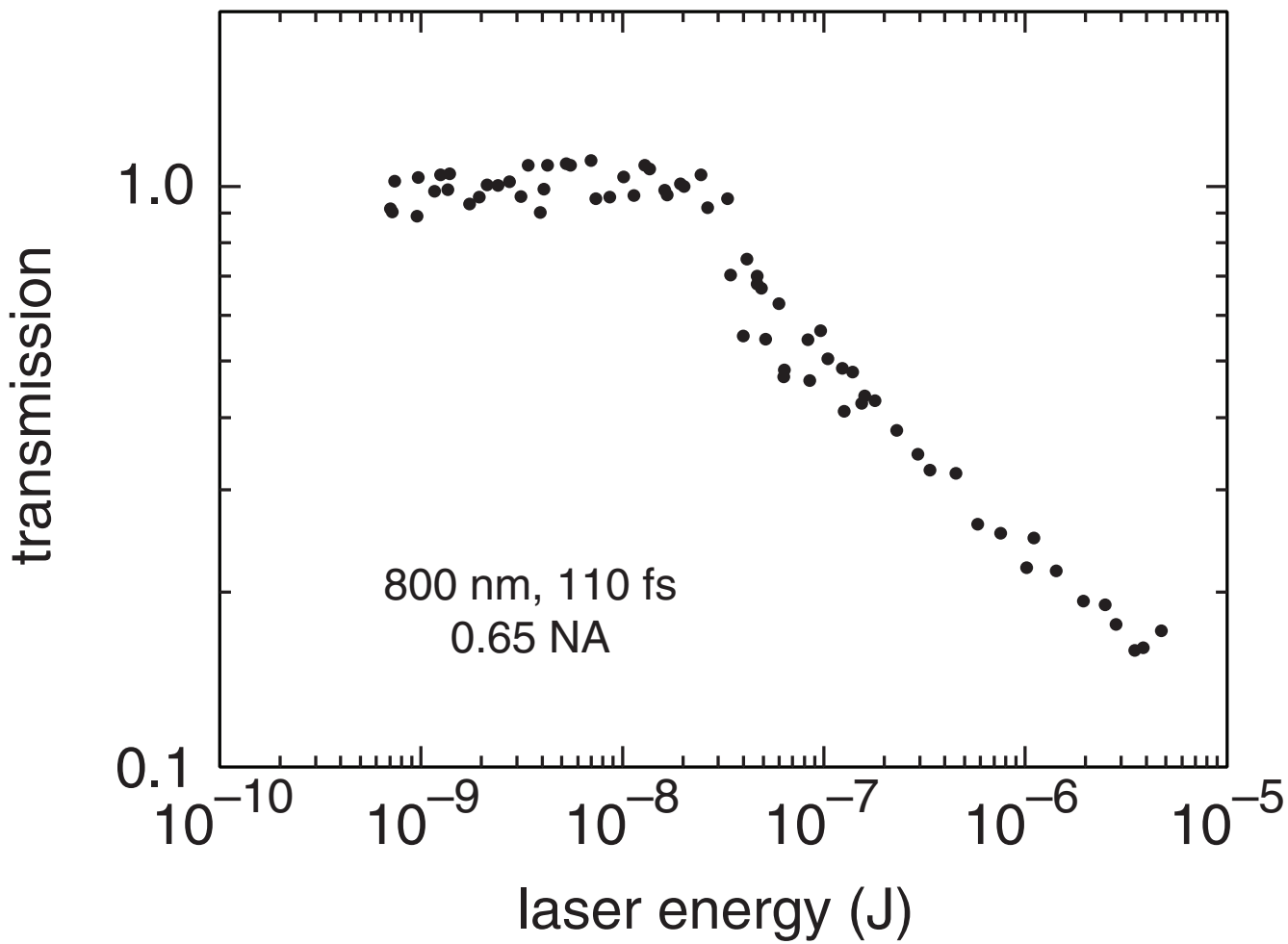
Some applications:

- data storage
- waveguides
- microfluidics



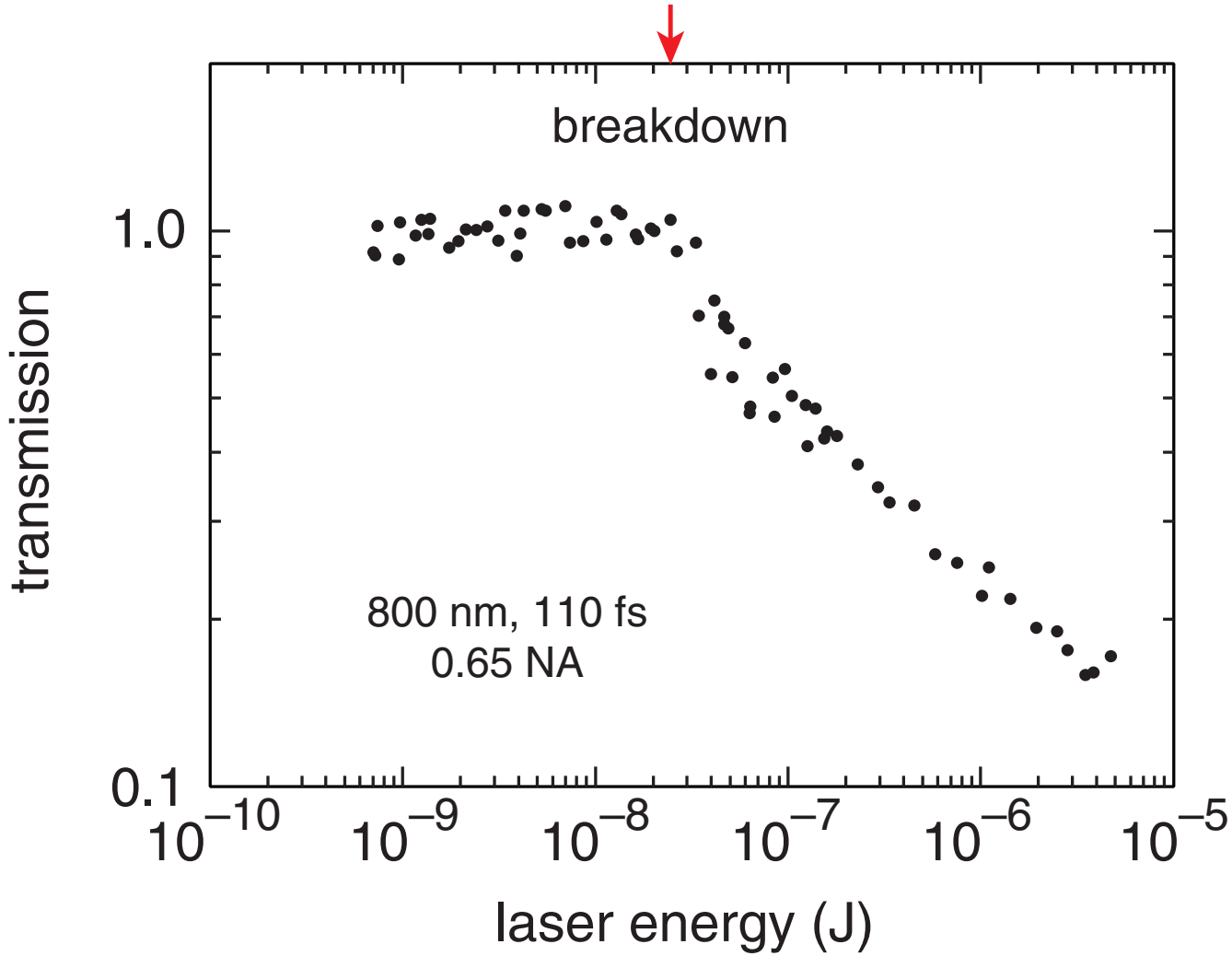
# Femtosecond materials interactions

## what is the threshold energy?



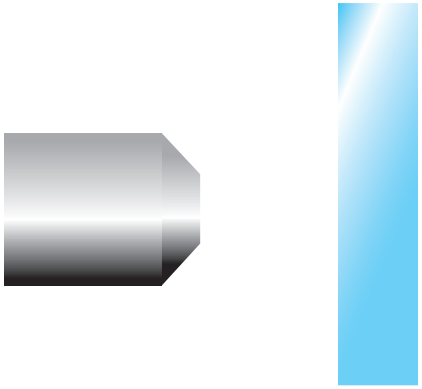
# Femtosecond materials interactions

## what is the threshold energy?



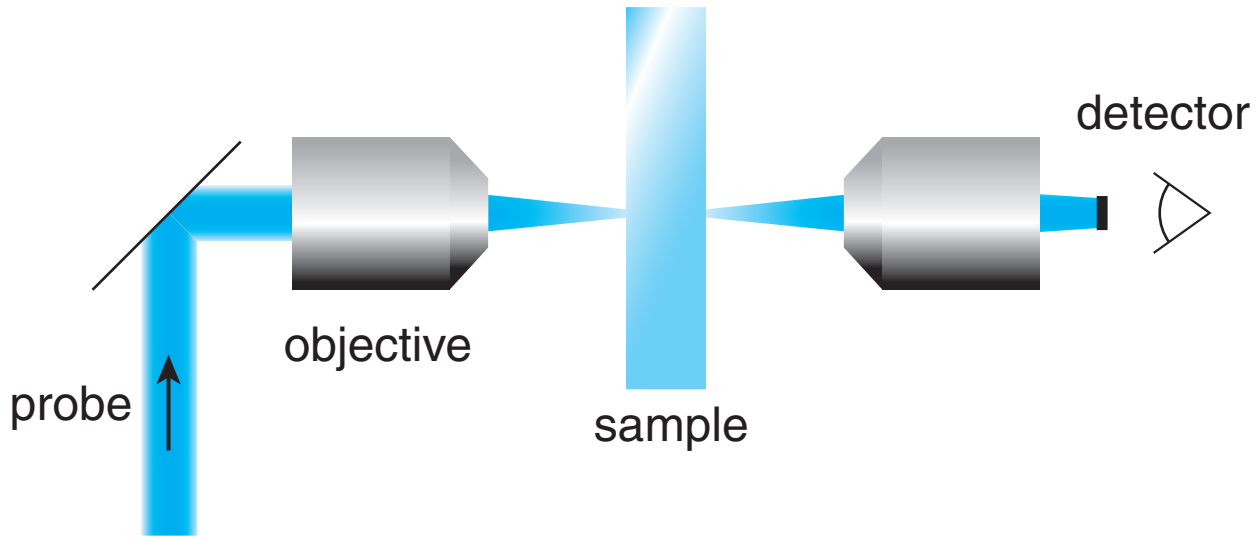
# Femtosecond materials interactions

## Dark-field scattering



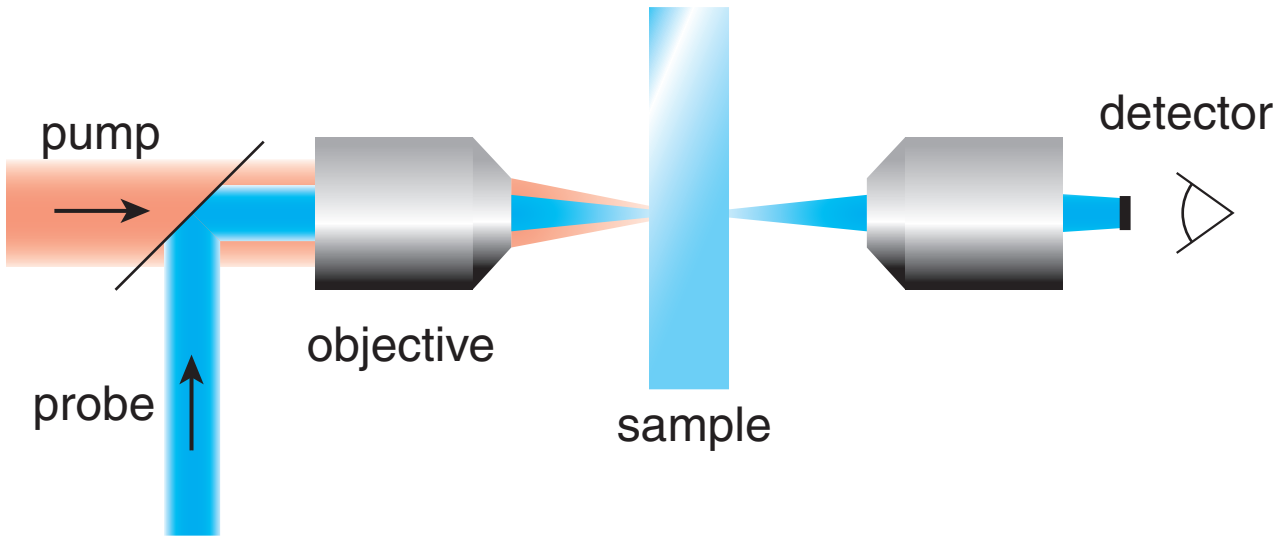
# Femtosecond materials interactions

**block probe beam…**



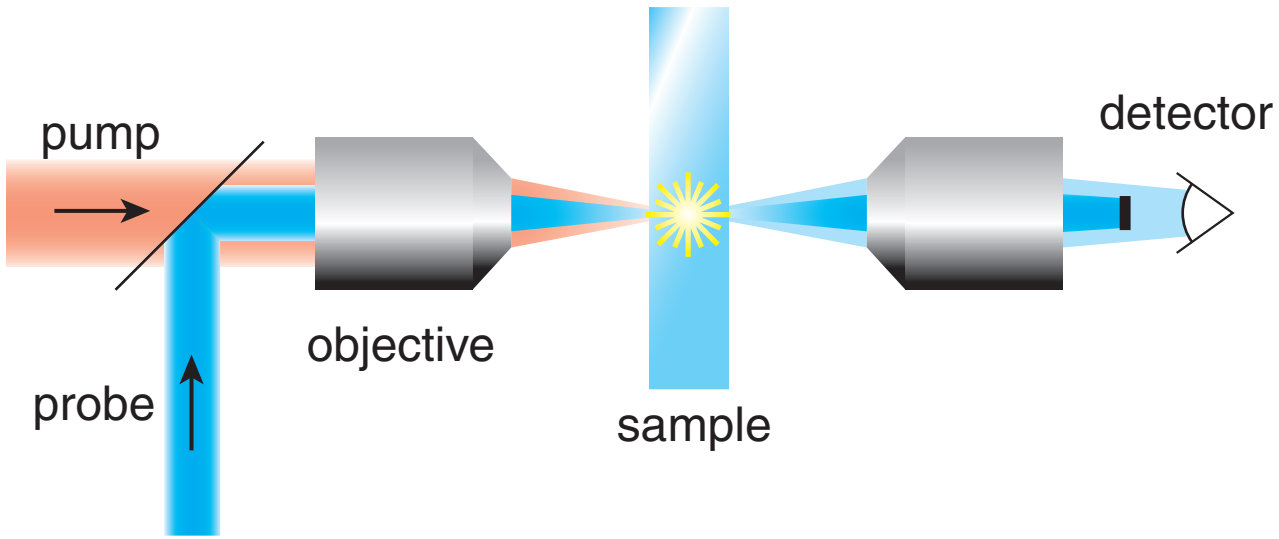
# Femtosecond materials interactions

## … bring in pump beam…



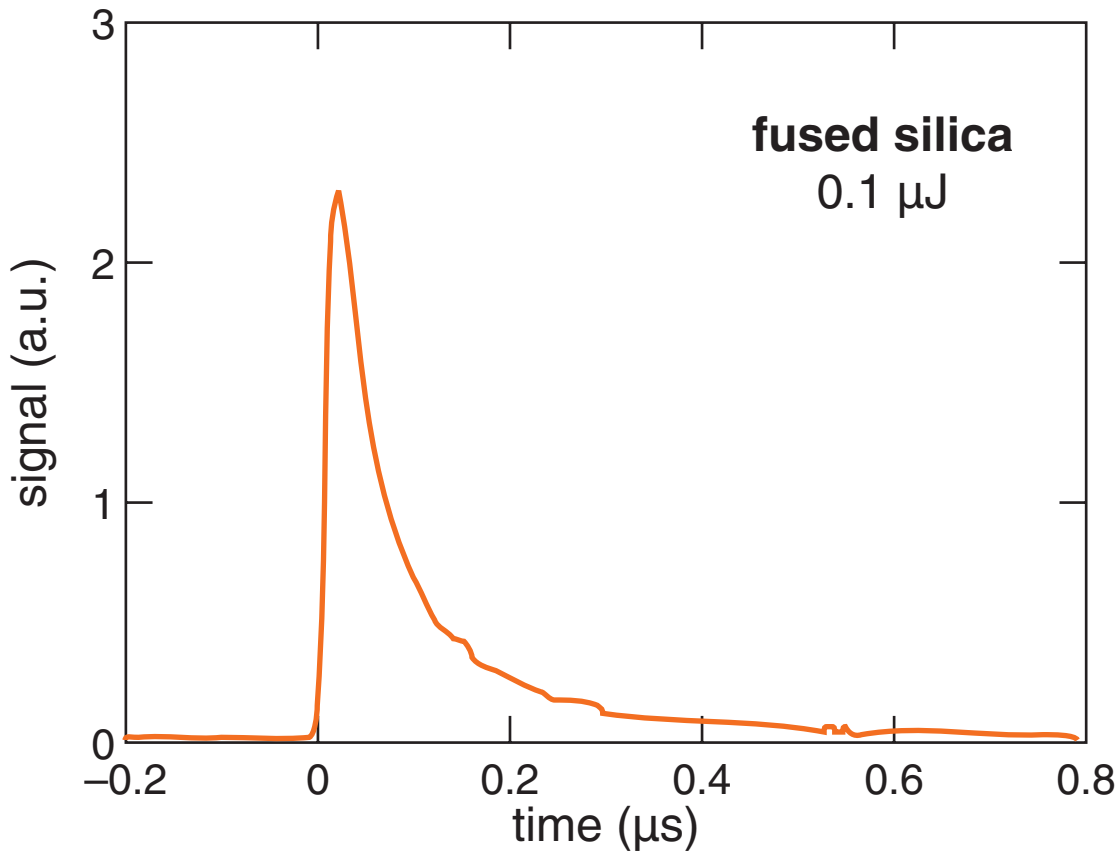
# Femtosecond materials interactions

## … damage scatters probe beam



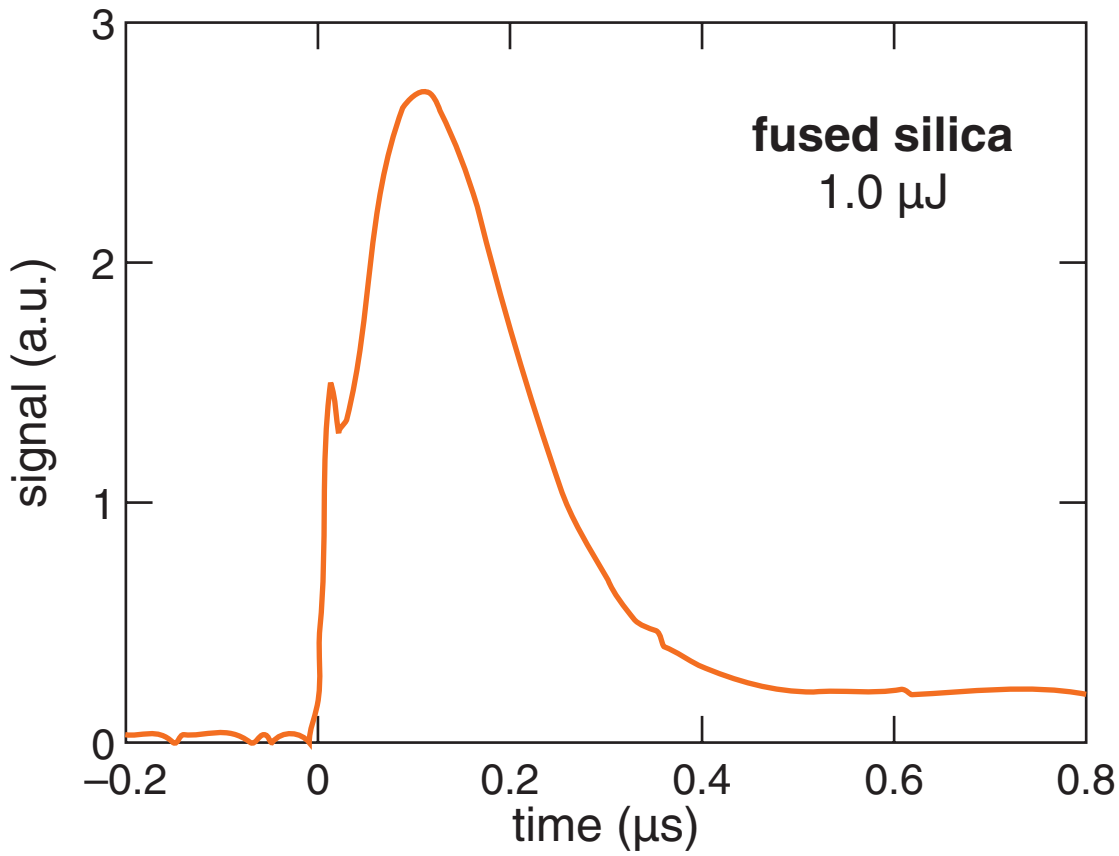
# Femtosecond materials interactions

## scattered signal



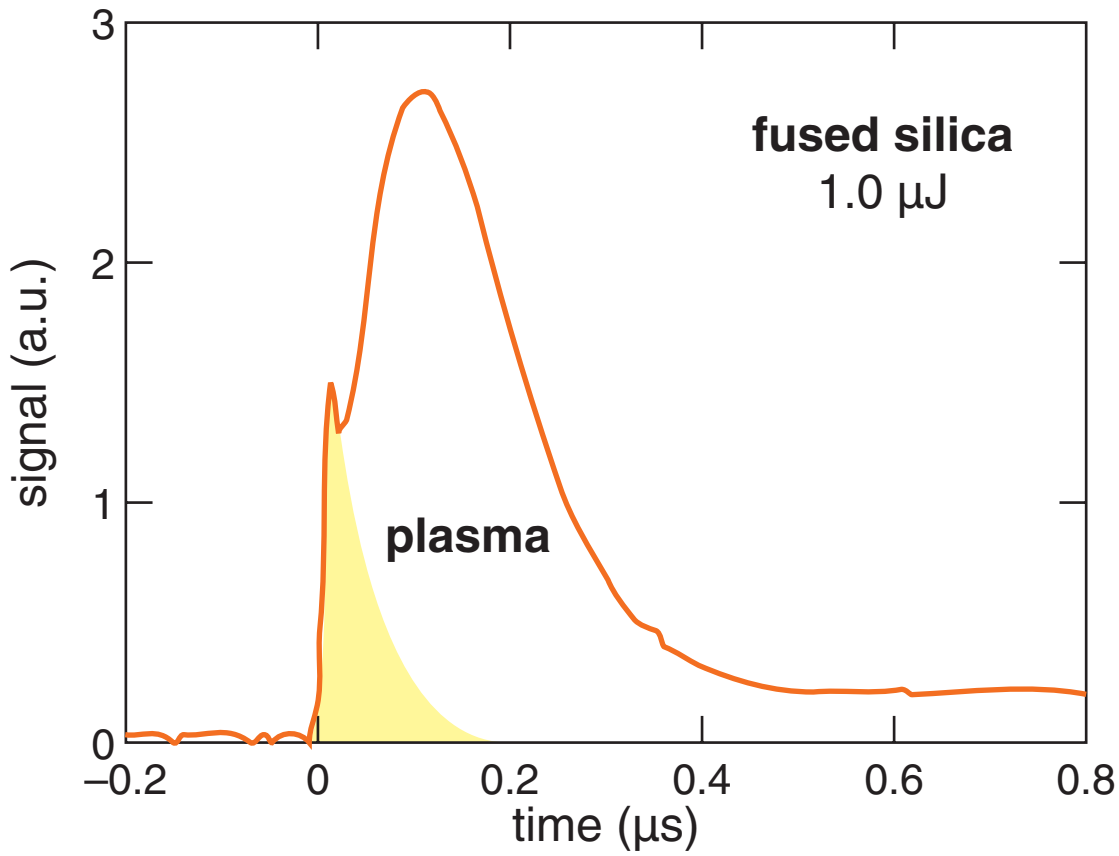
# Femtosecond materials interactions

## scattered signal



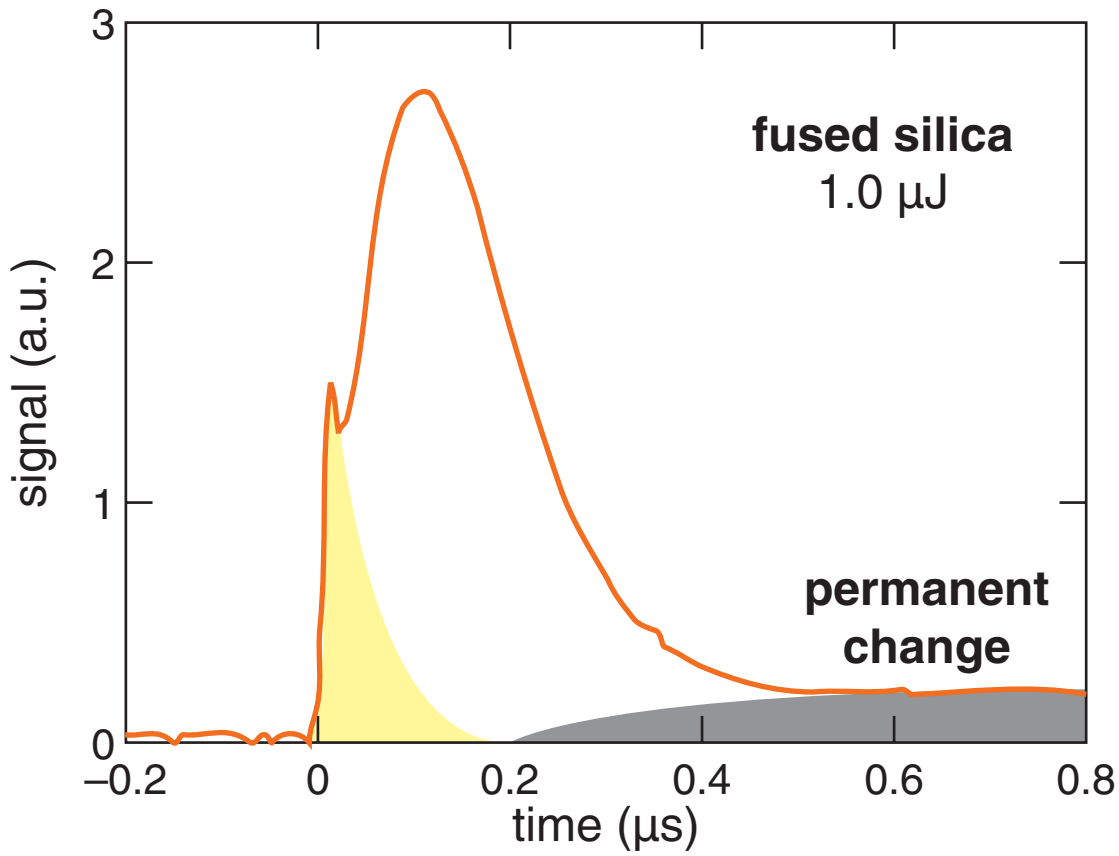
# Femtosecond materials interactions

## scattered signal



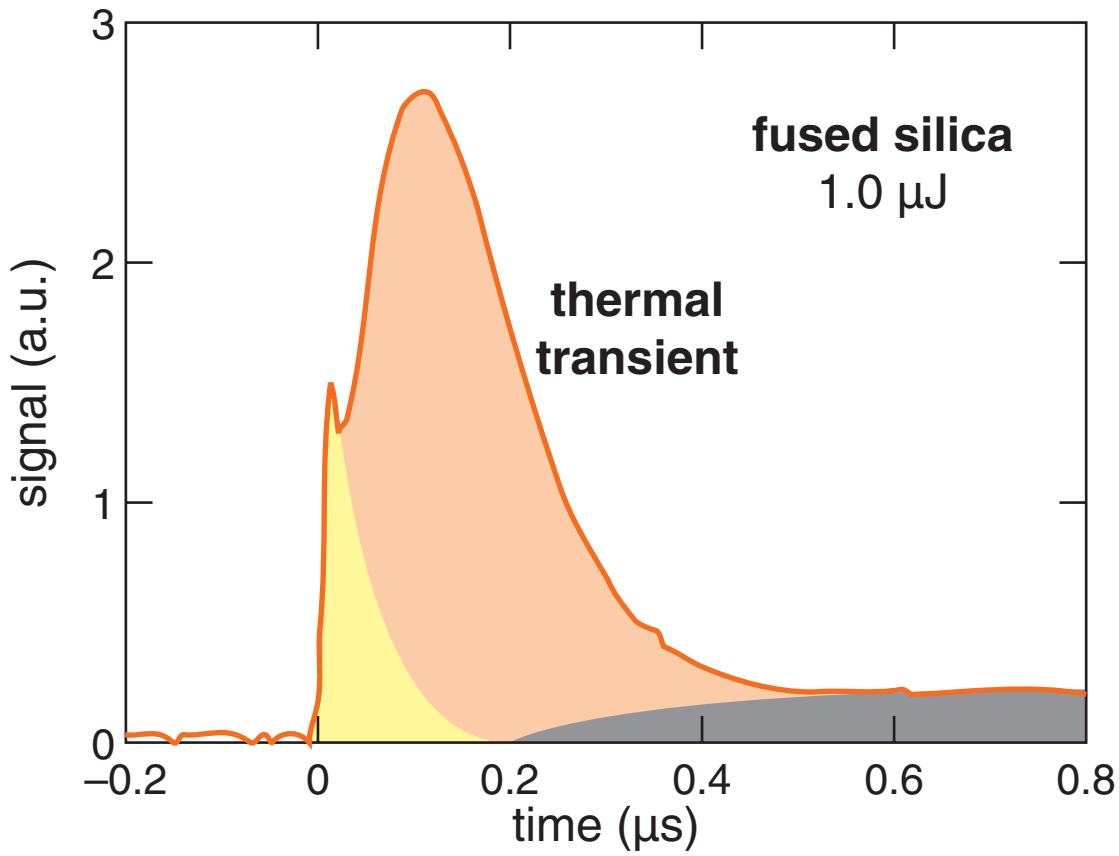
# Femtosecond materials interactions

## scattered signal



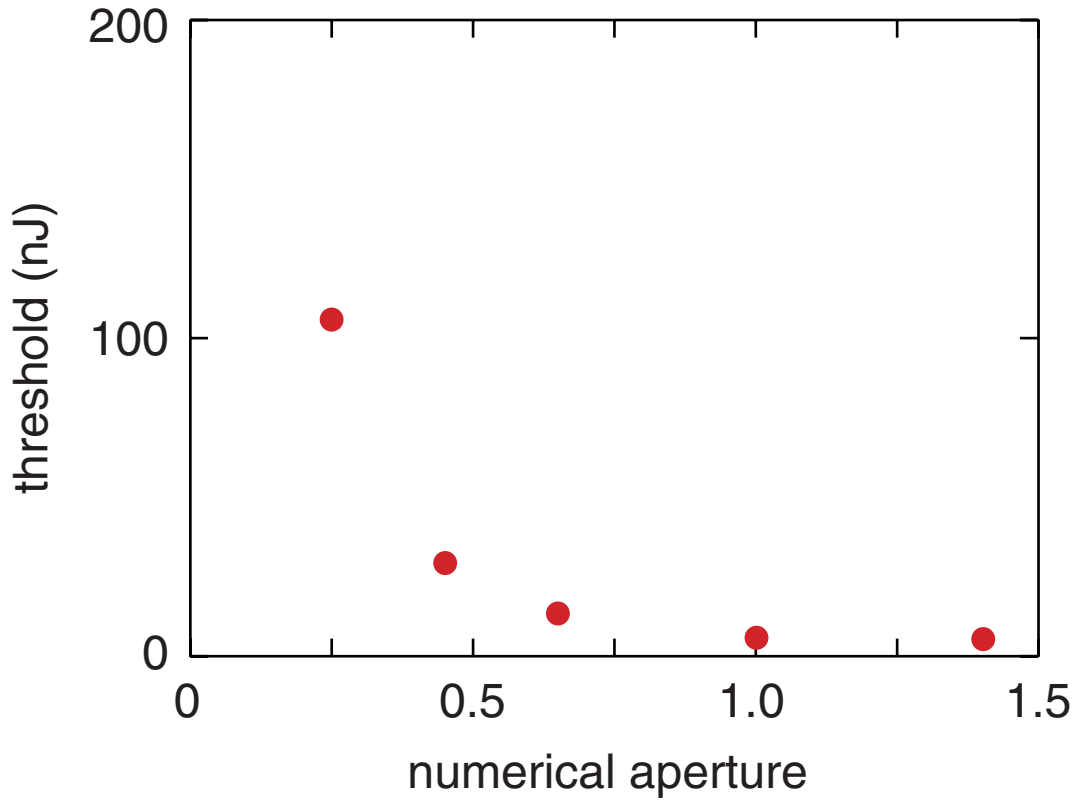
# Femtosecond materials interactions

## scattered signal



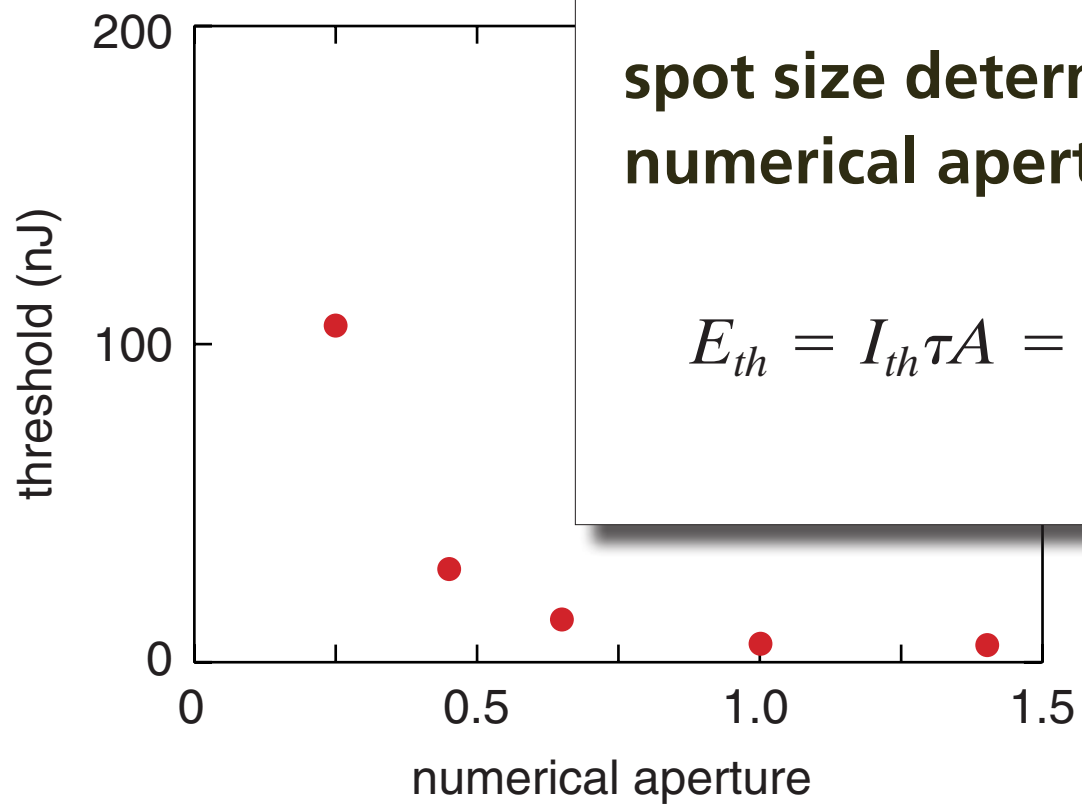
# Femtosecond materials interactions

## vary numerical aperture



# Femtosecond materials interactions

vary numeric




**intensity threshold:**

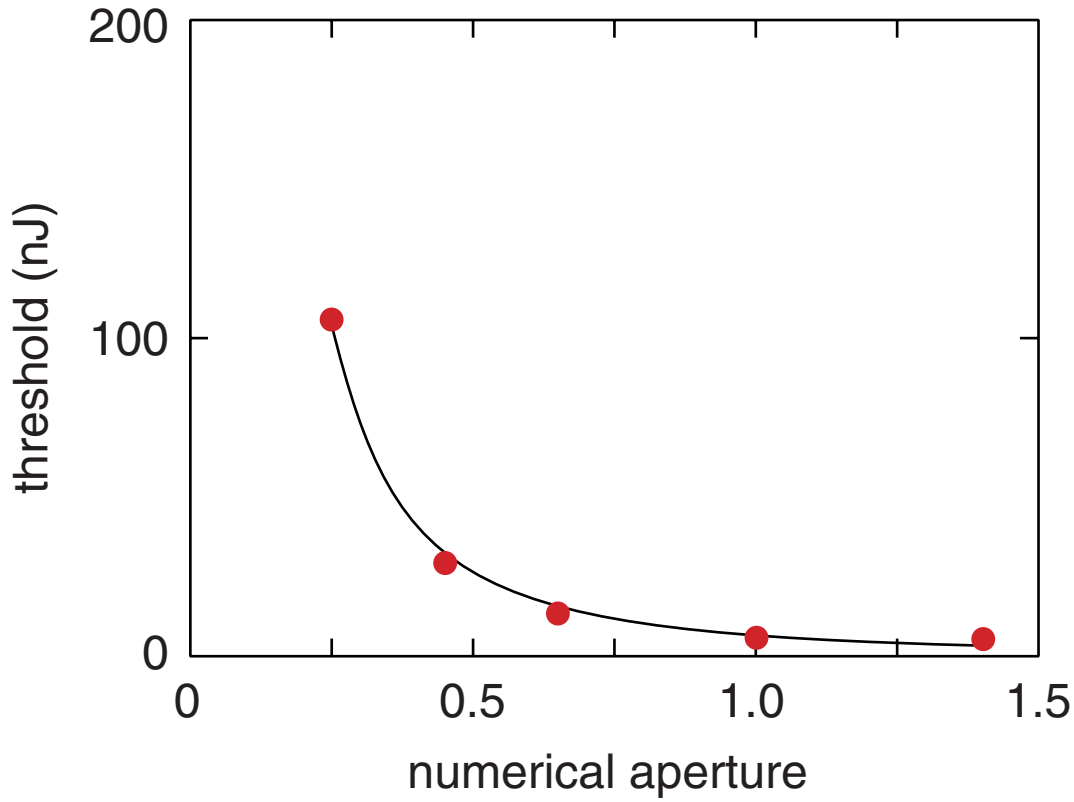
$$E_{th} = I_{th}\tau A$$

**spot size determined by numerical aperture:**

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

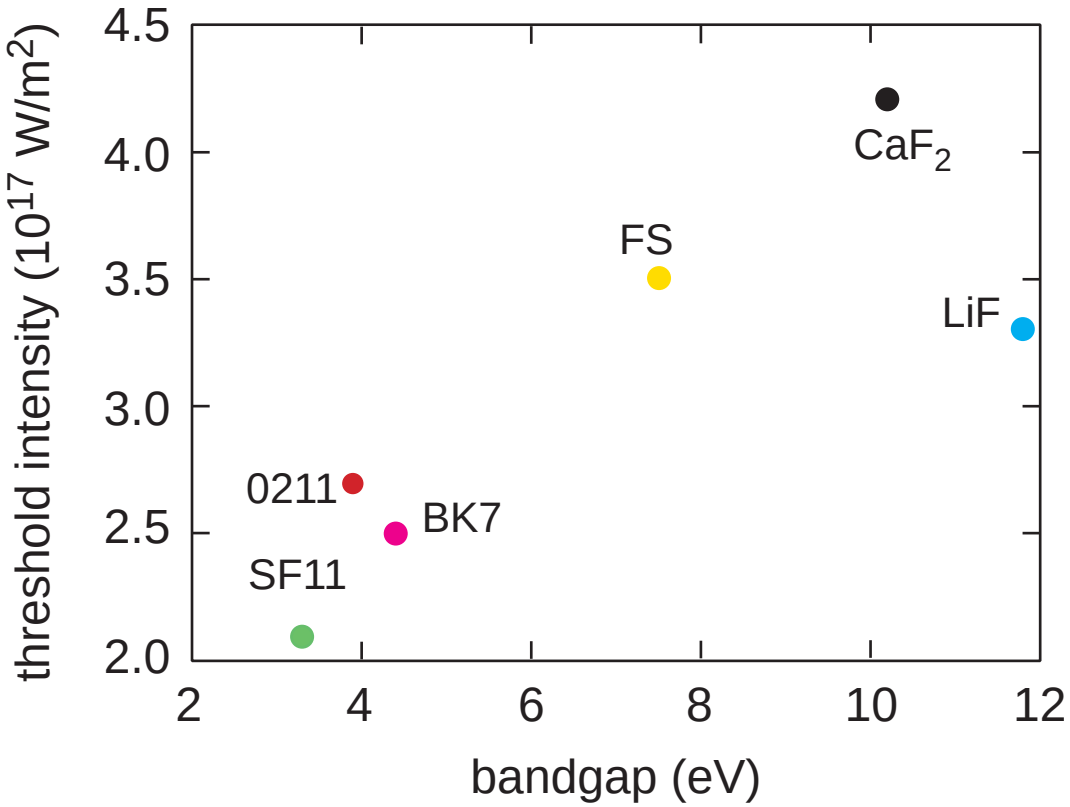
# Femtosecond materials interactions

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



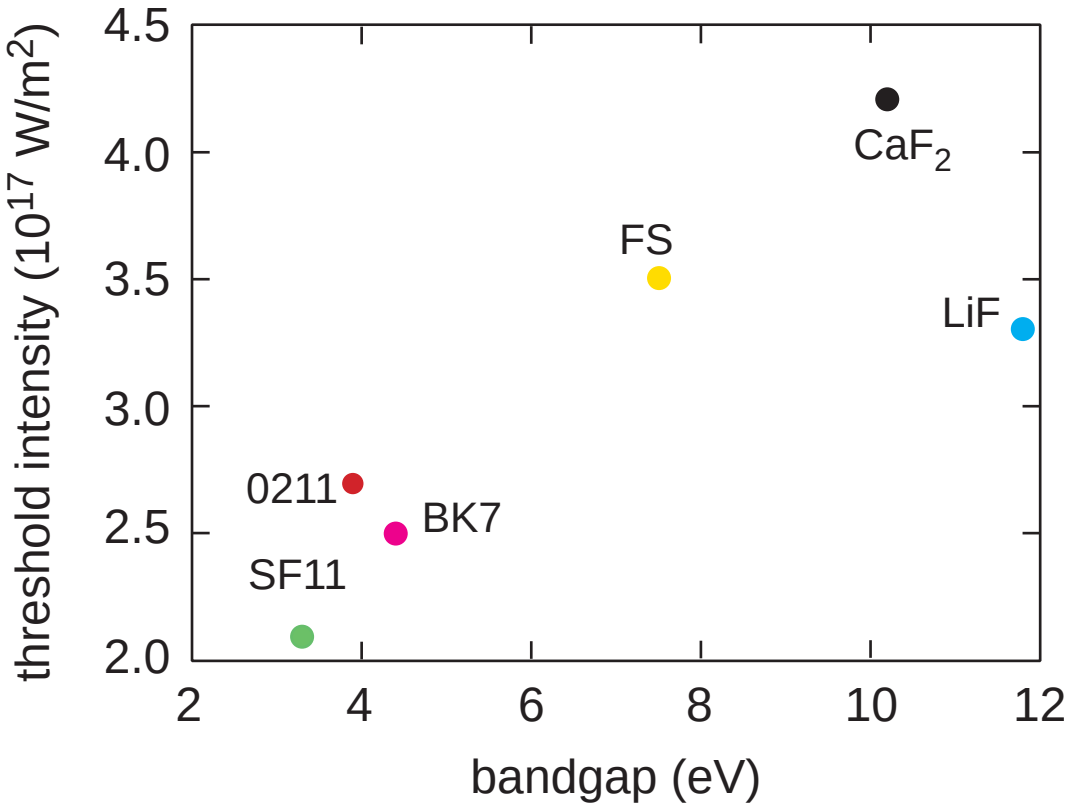
# Femtosecond materials interactions

## vary material…



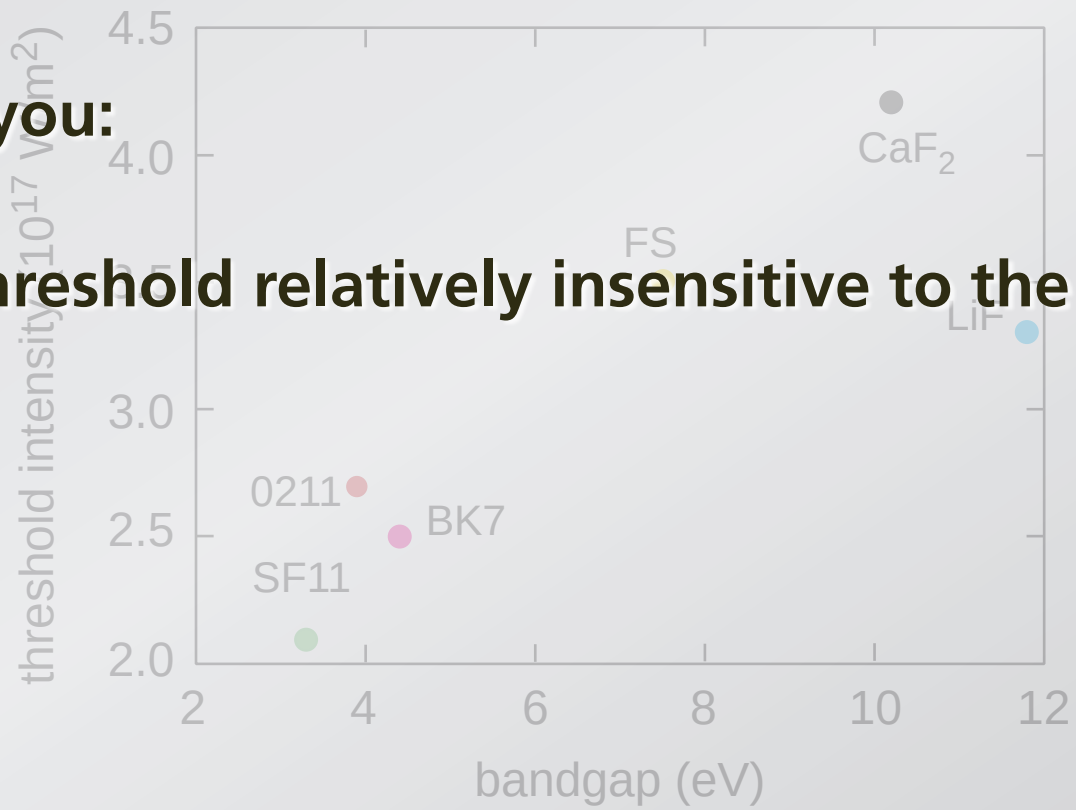
# Femtosecond materials interactions

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



# Femtosecond materials interactions

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

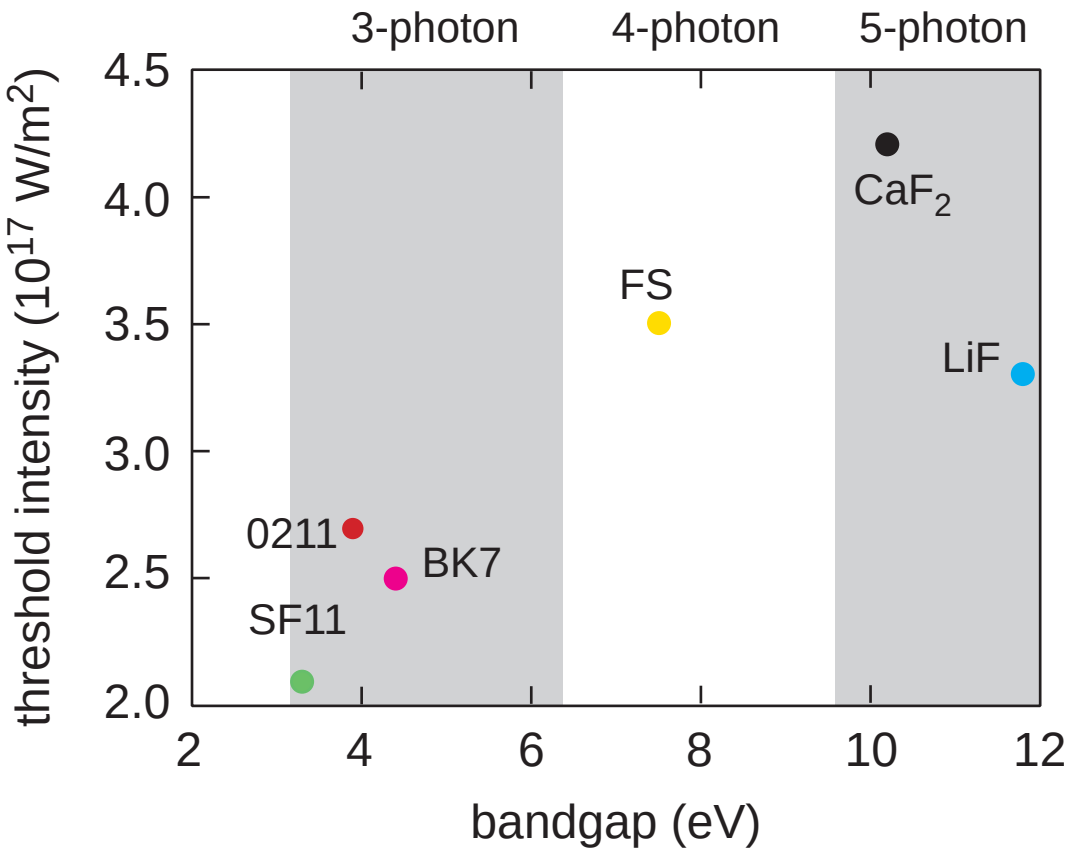



**Question to you:**

**Why is the threshold relatively insensitive to the band gap?**

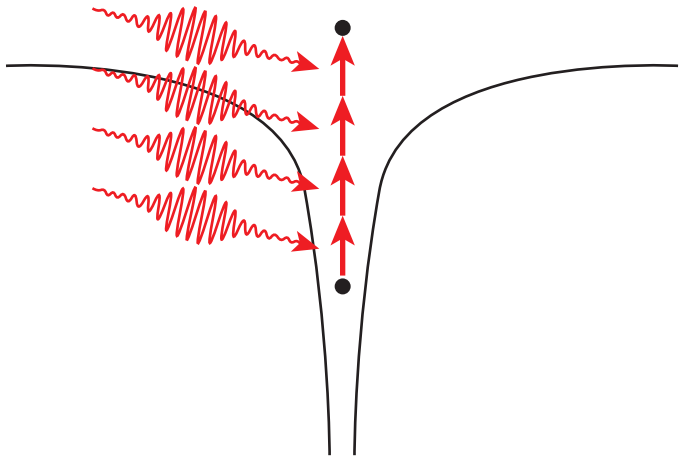
# Femtosecond materials interactions

**would expect much more than a factor of 2**



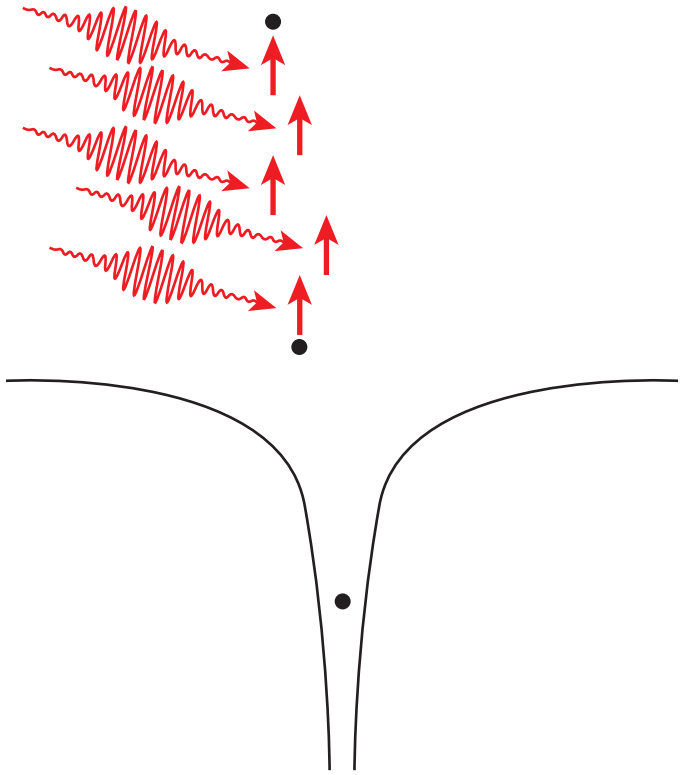
# Femtosecond materials interactions

**multiphoton excitation seeds free electrons…**



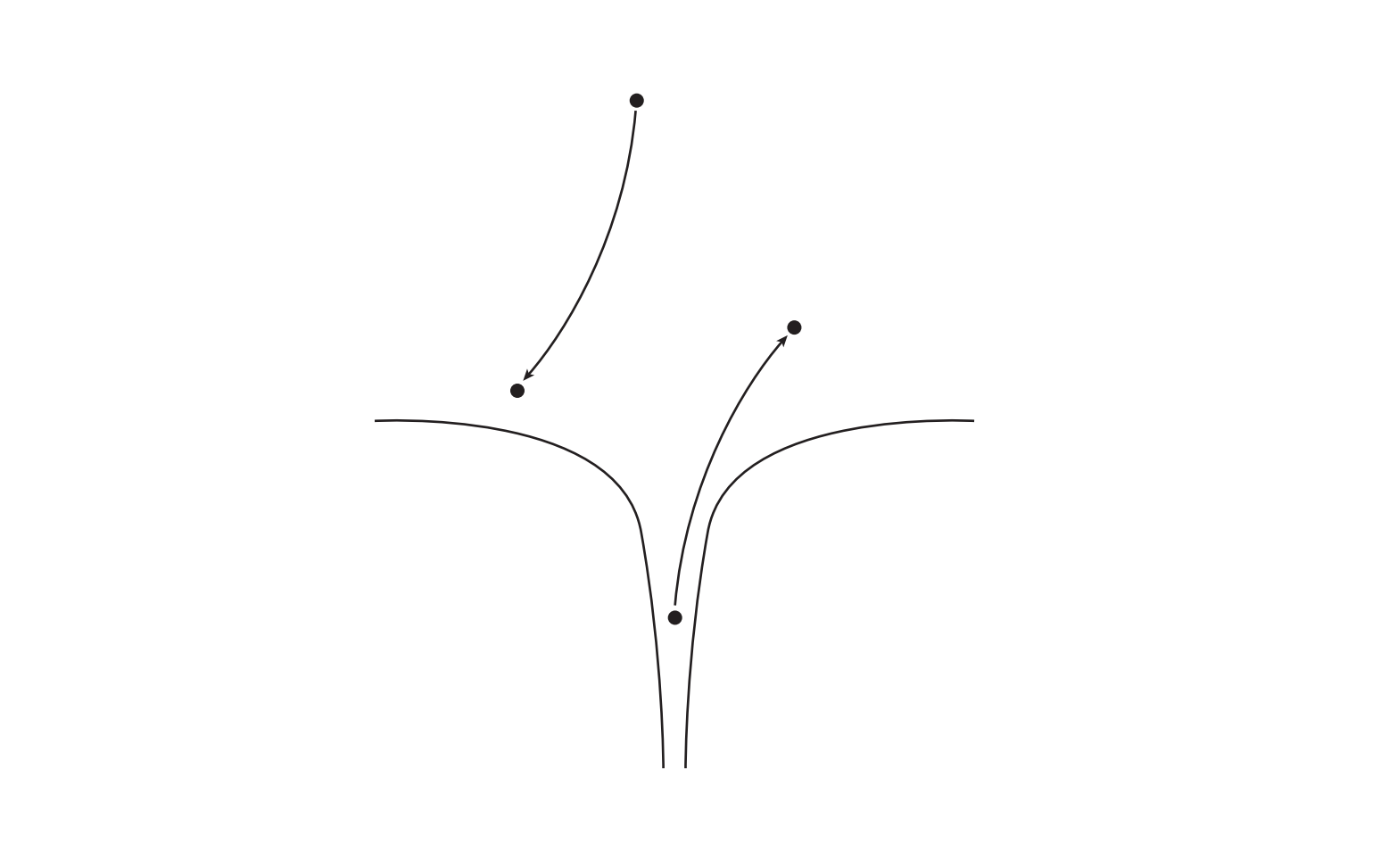
# Femtosecond materials interactions

…which can then absorb lineary.



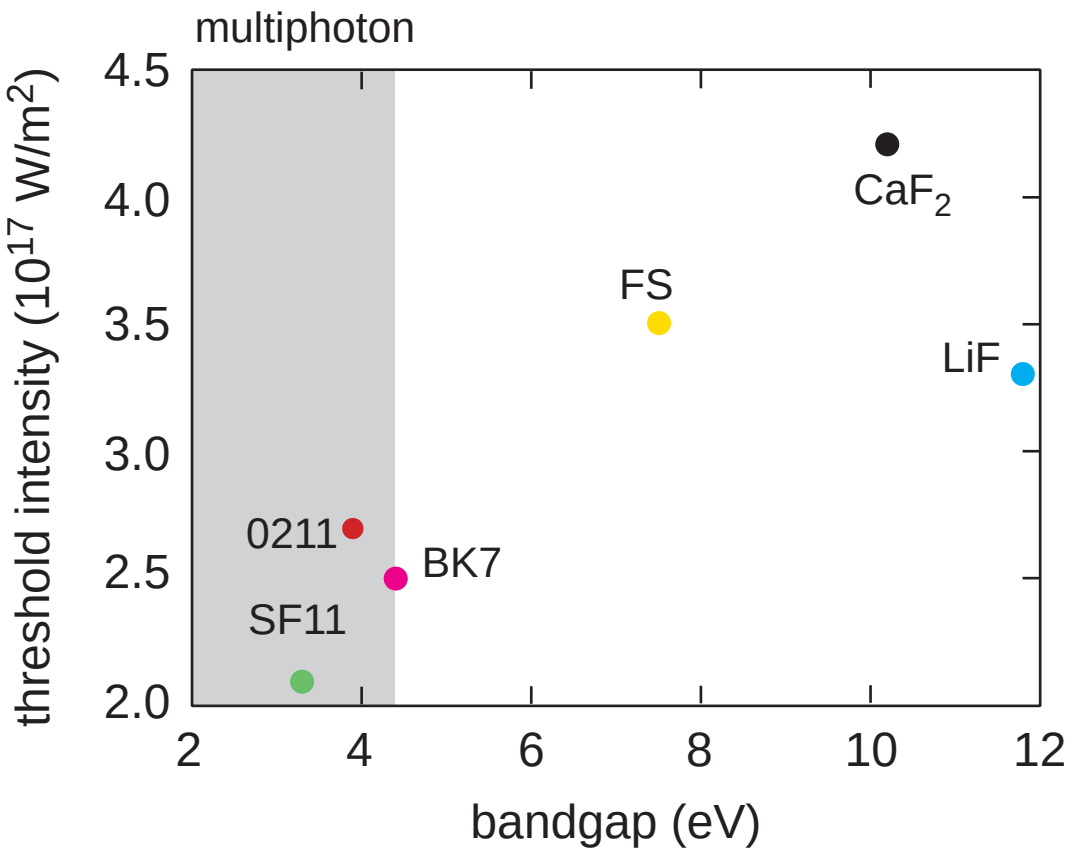
# Femtosecond materials interactions

**once excess energy large enough, impact ionization occurs**



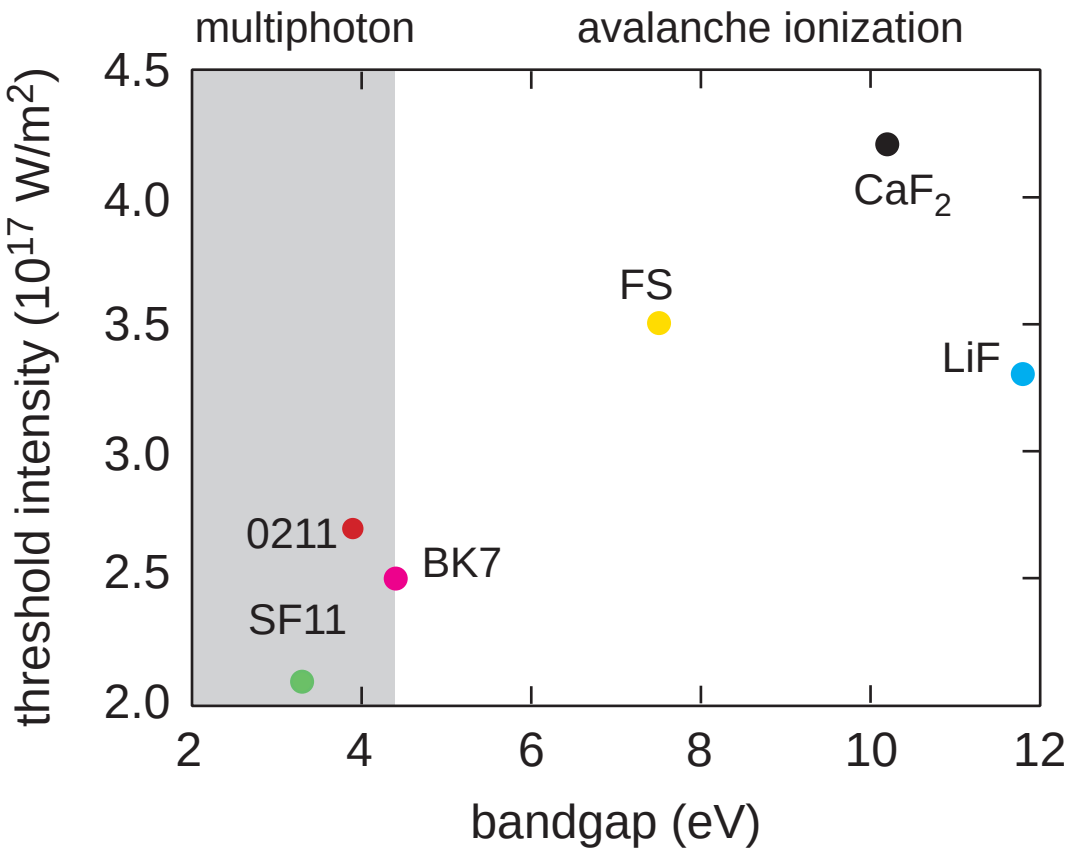
# Femtosecond materials interactions

**critical density reached by multiphoton for low gap only**



# Femtosecond materials interactions

## avalanche ionization important at high gap



# Femtosecond materials interactions

**what prevents damage at low NA?**

# Femtosecond materials interactions

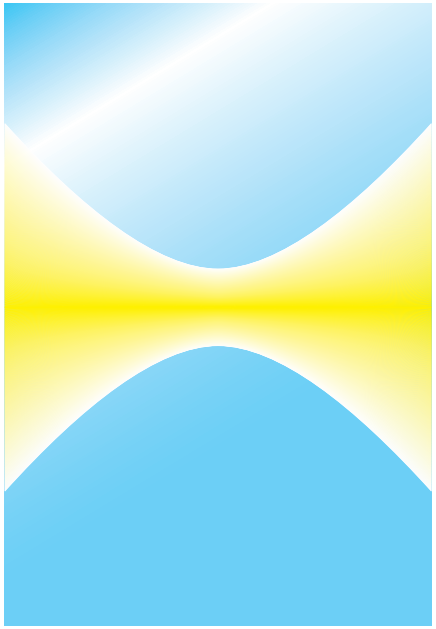
Competing nonlinear effects:

- multiphoton absorption
- supercontinuum generation
- self-focusing

# Femtosecond materials interactions

## why the difference?

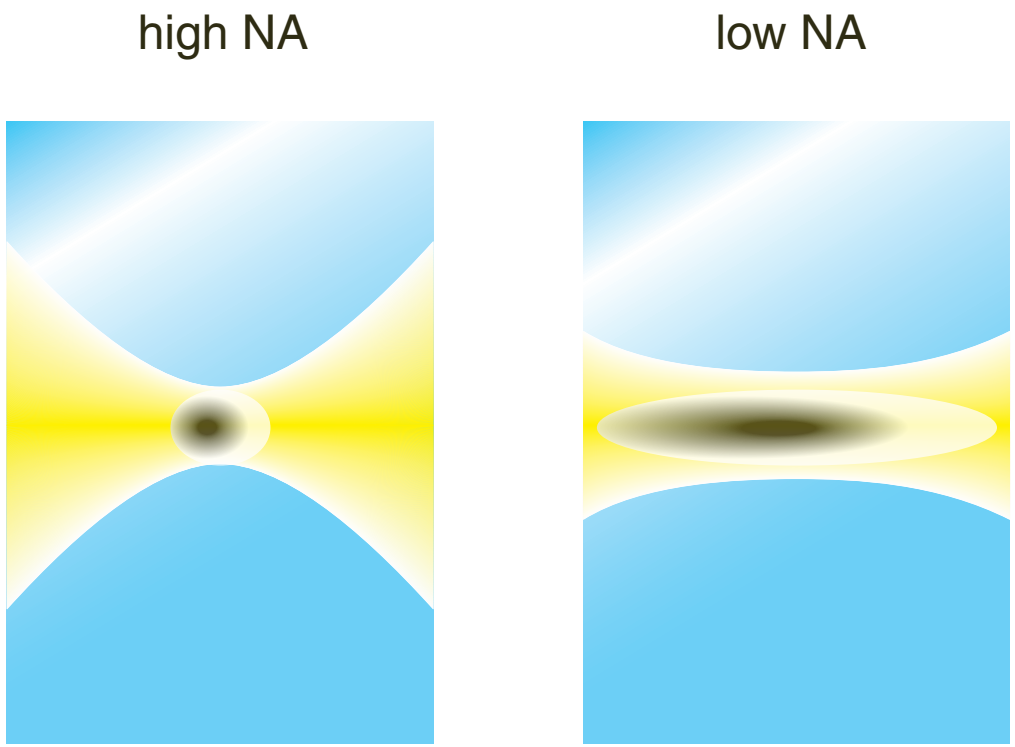
high NA



low NA

# Femtosecond materials interactions

## very different confocal length/interaction length

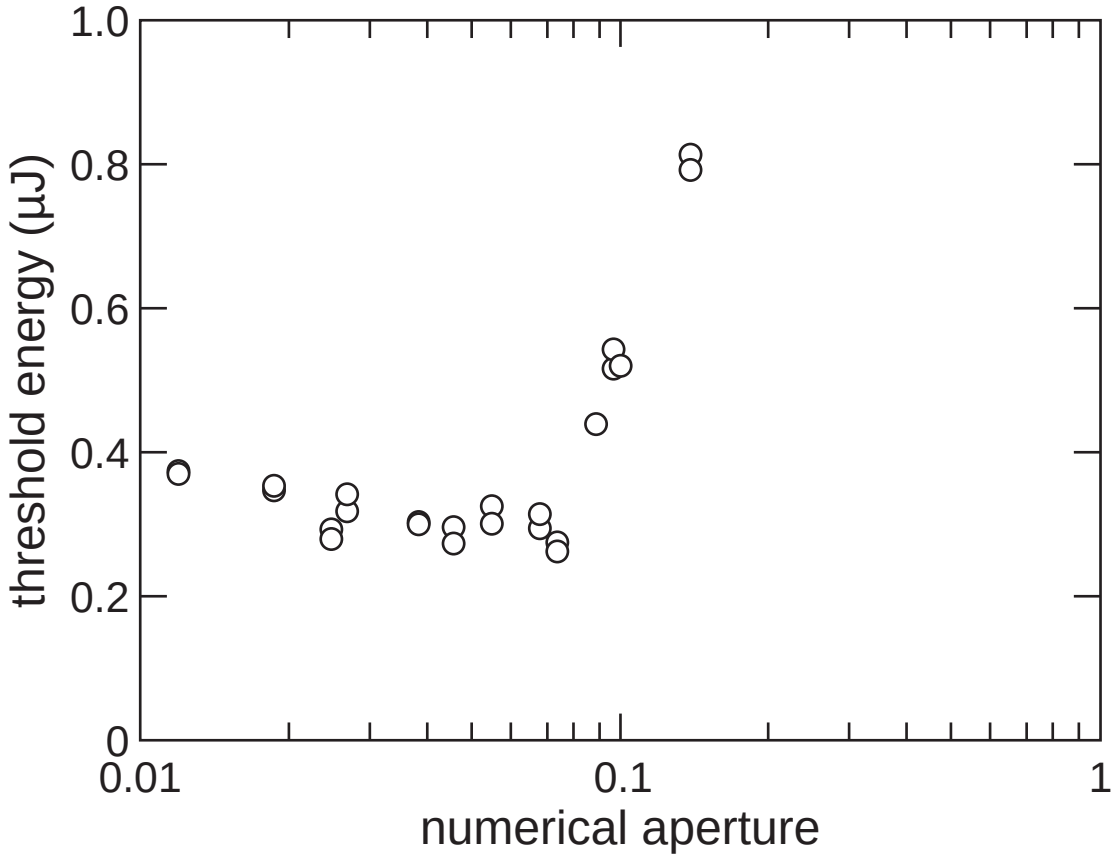


# Femtosecond materials interactions

high NA: interaction length too short for self-focusing

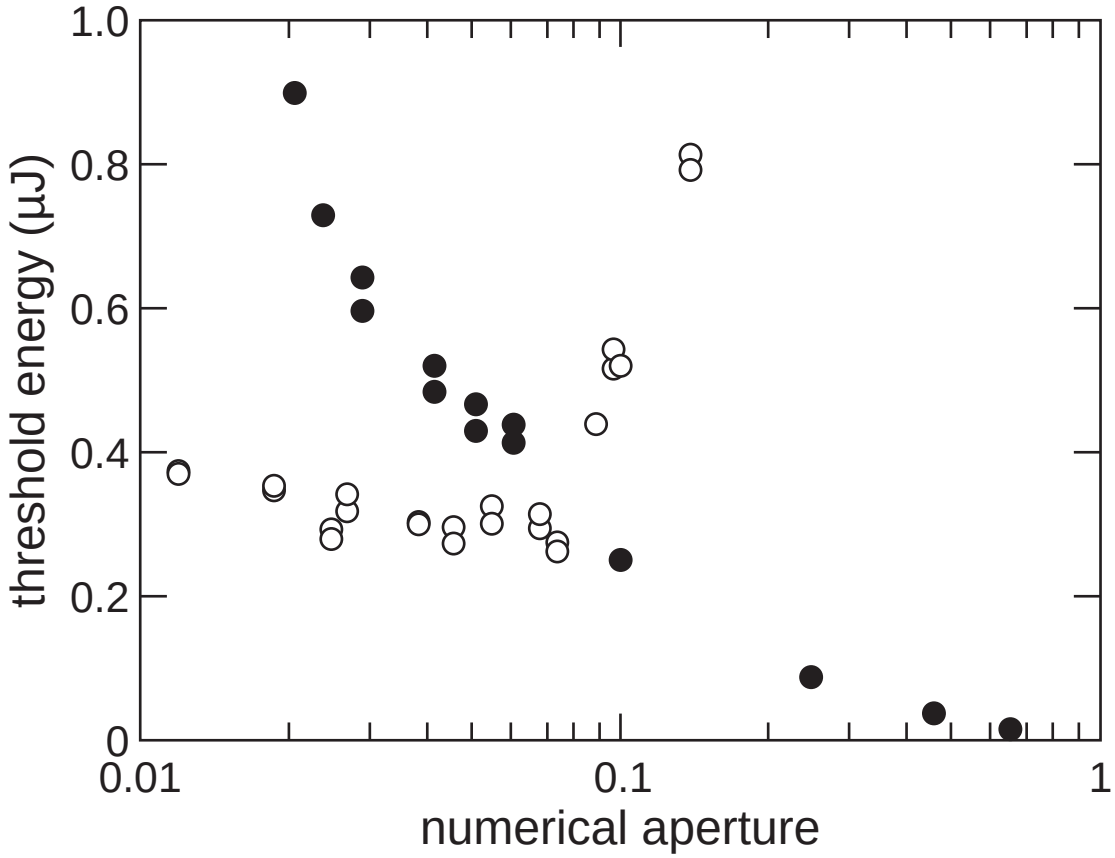
# Femtosecond materials interactions

## threshold for supercontinuum generation



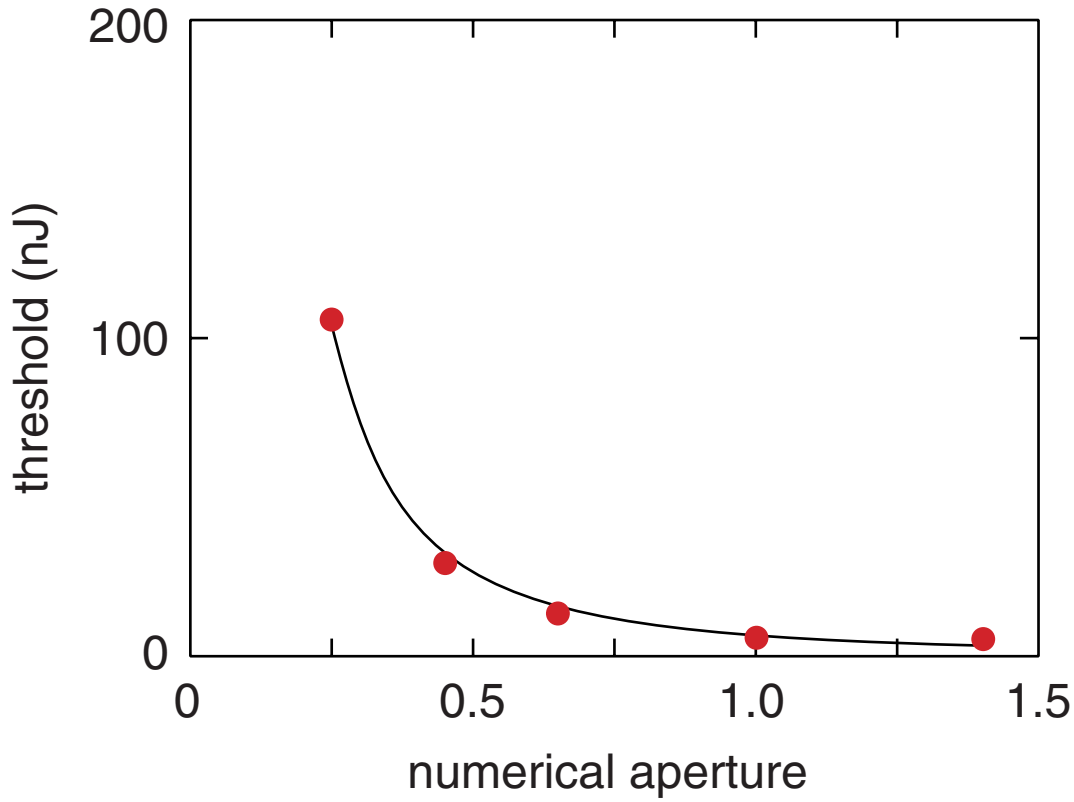
# Femtosecond materials interactions

## threshold for damage



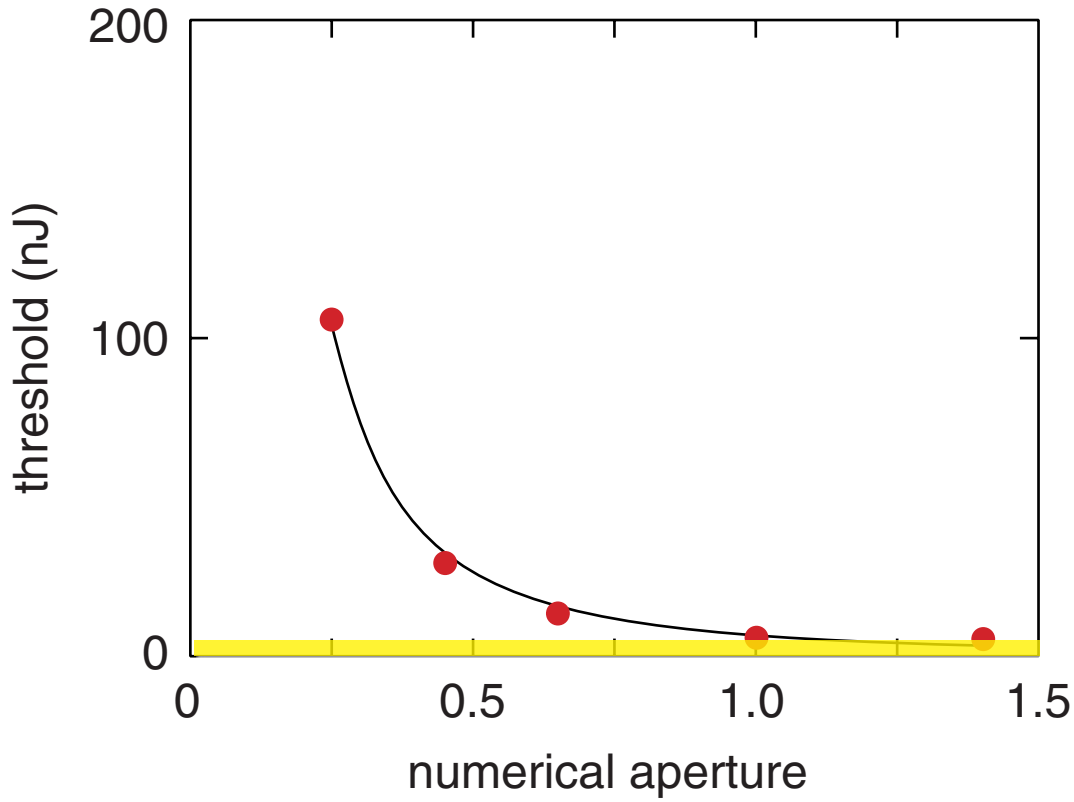
# Femtosecond materials interactions

**threshold decreases with increasing numerical aperture**



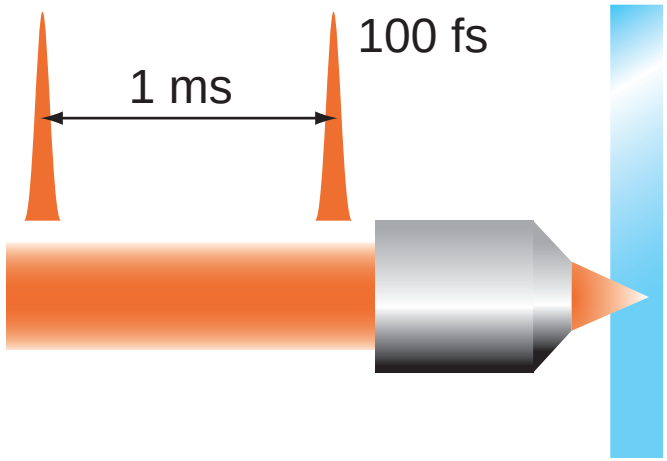
# Femtosecond materials interactions

**less than 10 nJ at high numerical aperture!**



# Femtosecond materials interactions

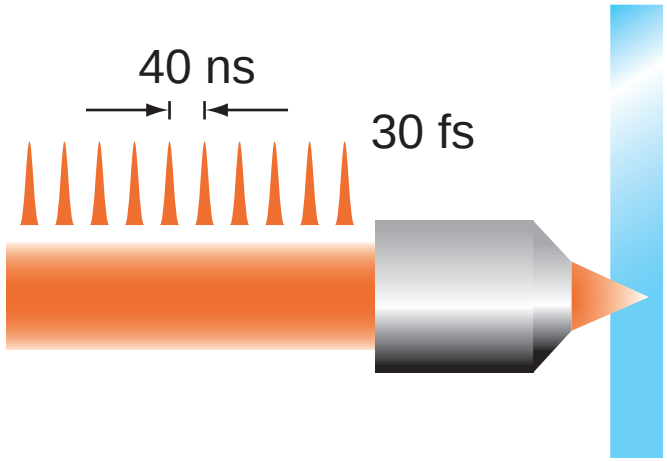
**amplified laser: 1 kHz, 1 mJ**




**heat diffusion time:** $t_{diff} \approx 1\ \mu s$

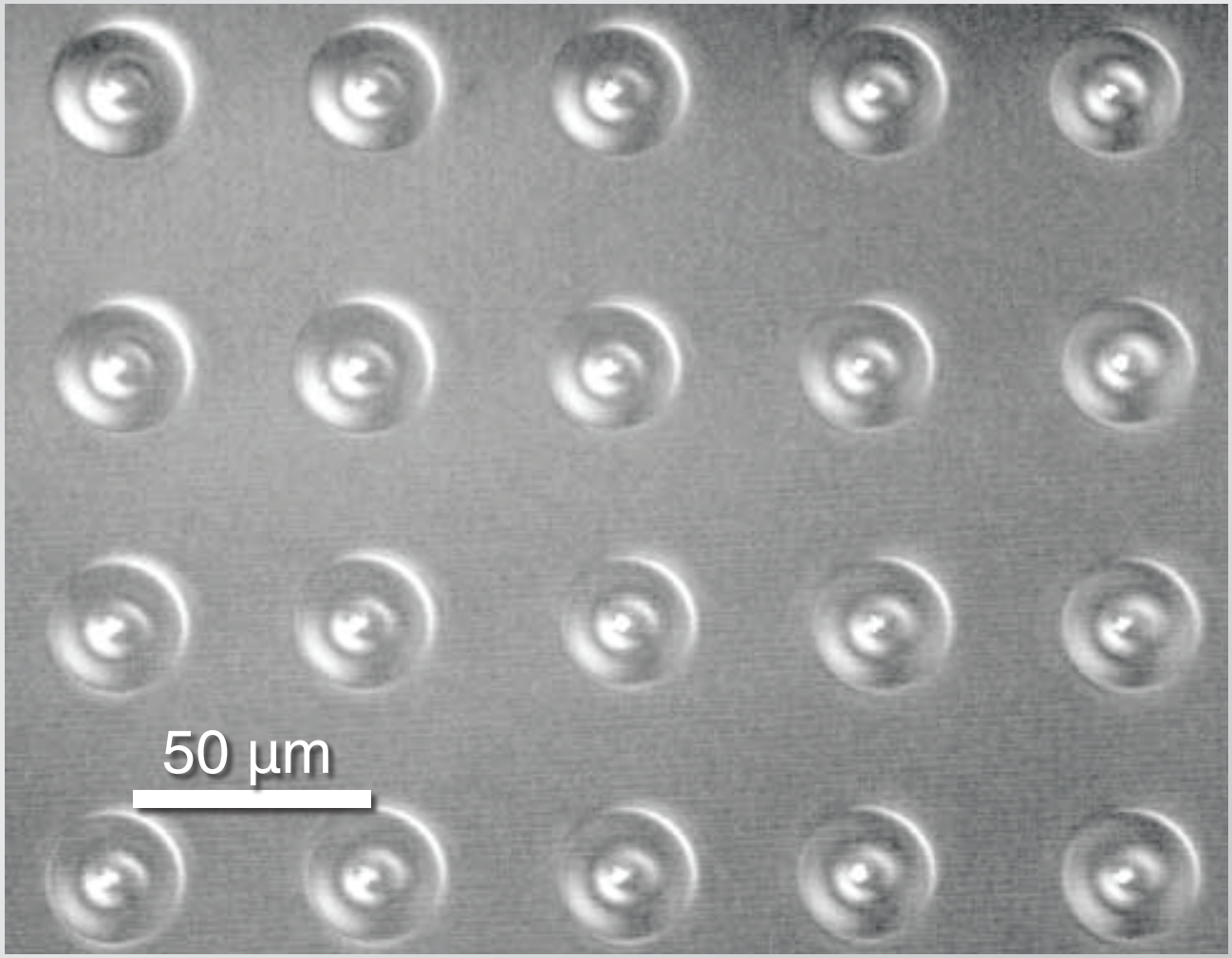
# Femtosecond materials interactions

**long cavity oscillator: 25 MHz, 25 nJ**




**heat diffusion time:** $t_{diff} \approx 1\ \mu s$

# Femtosecond materials interactions



# Femtosecond materials interactions

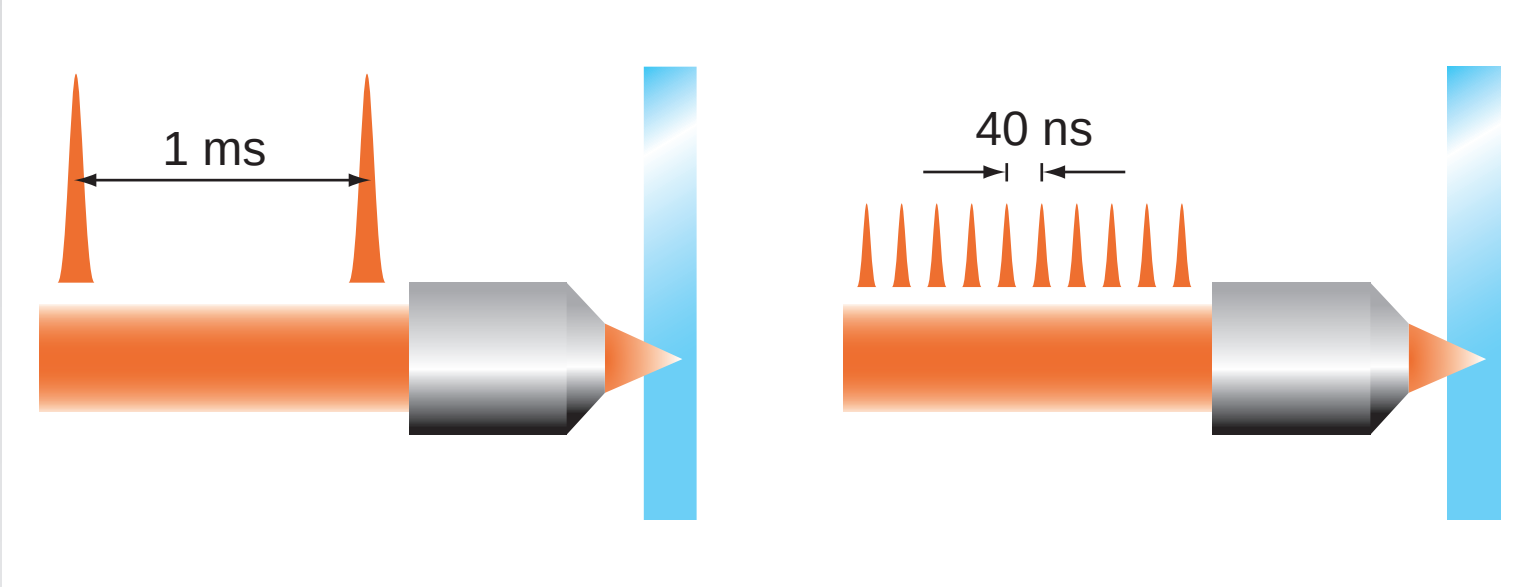
High repetition-rate micromachining:

- structural changes exceed focal volume
- spherical structures
- density change caused by melting

# Femtosecond materials interactions

amplified laser

oscillator

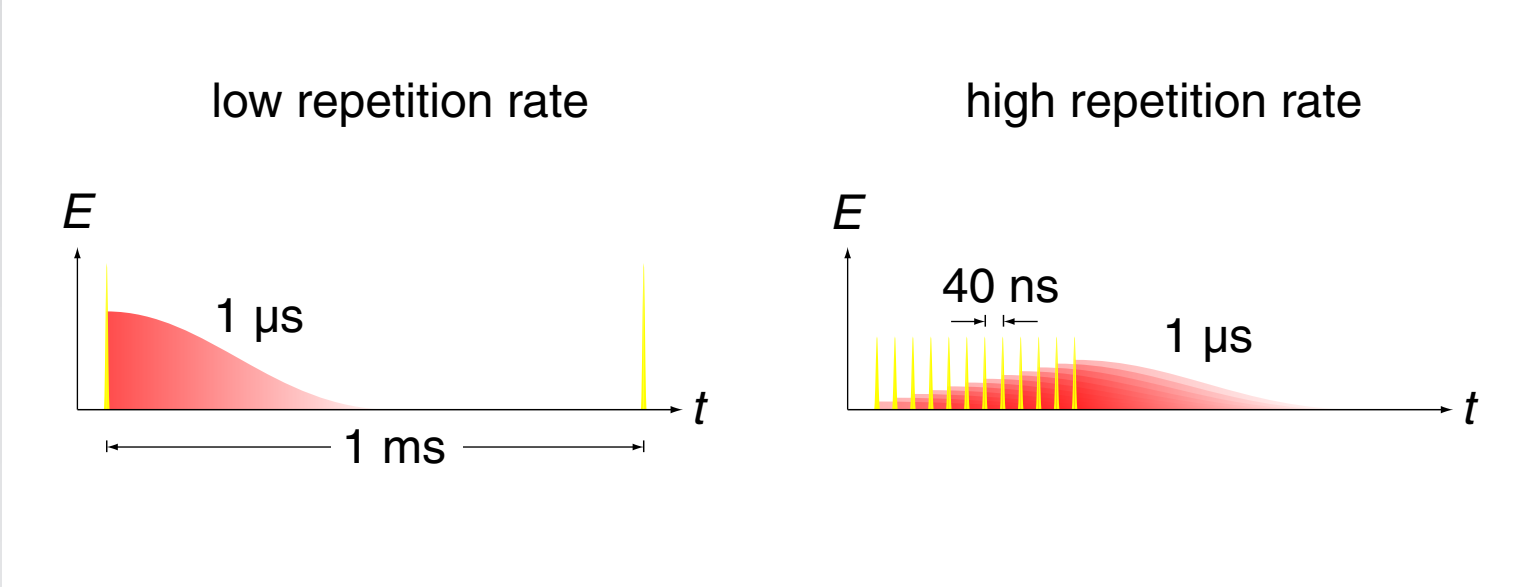



repetitive

cumulative

# Femtosecond materials interactions

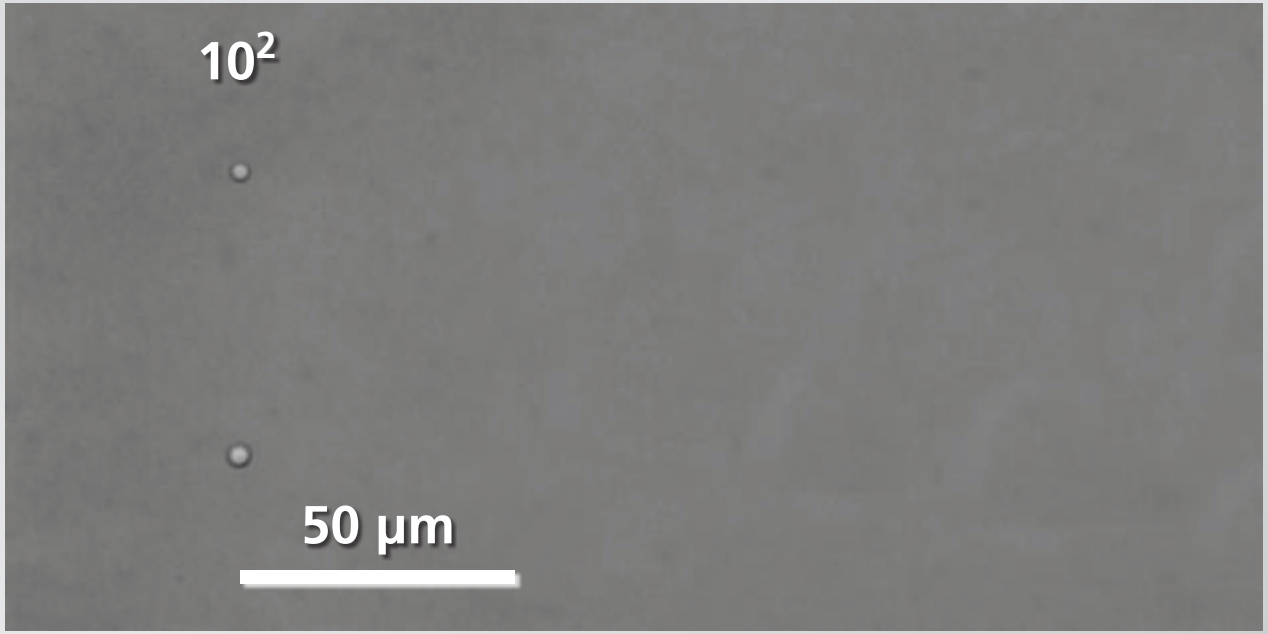
**amplified laser** | **oscillator**




**repetitive** | **cumulative**

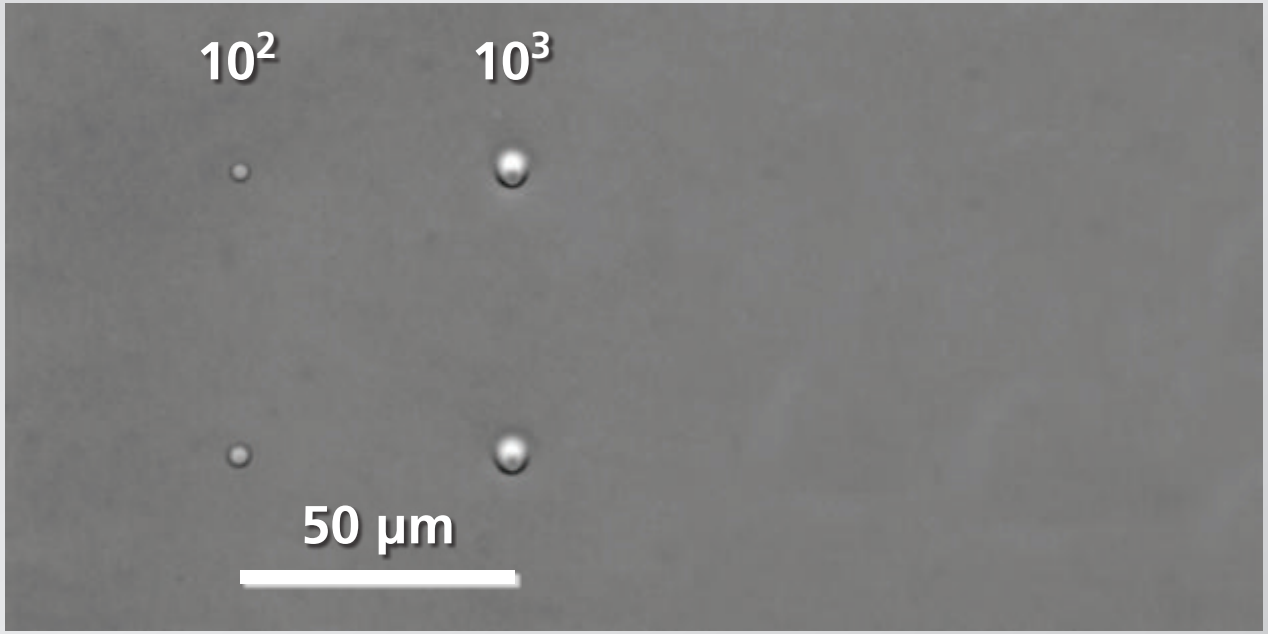
# Femtosecond materials interactions

the longer the irradiation…



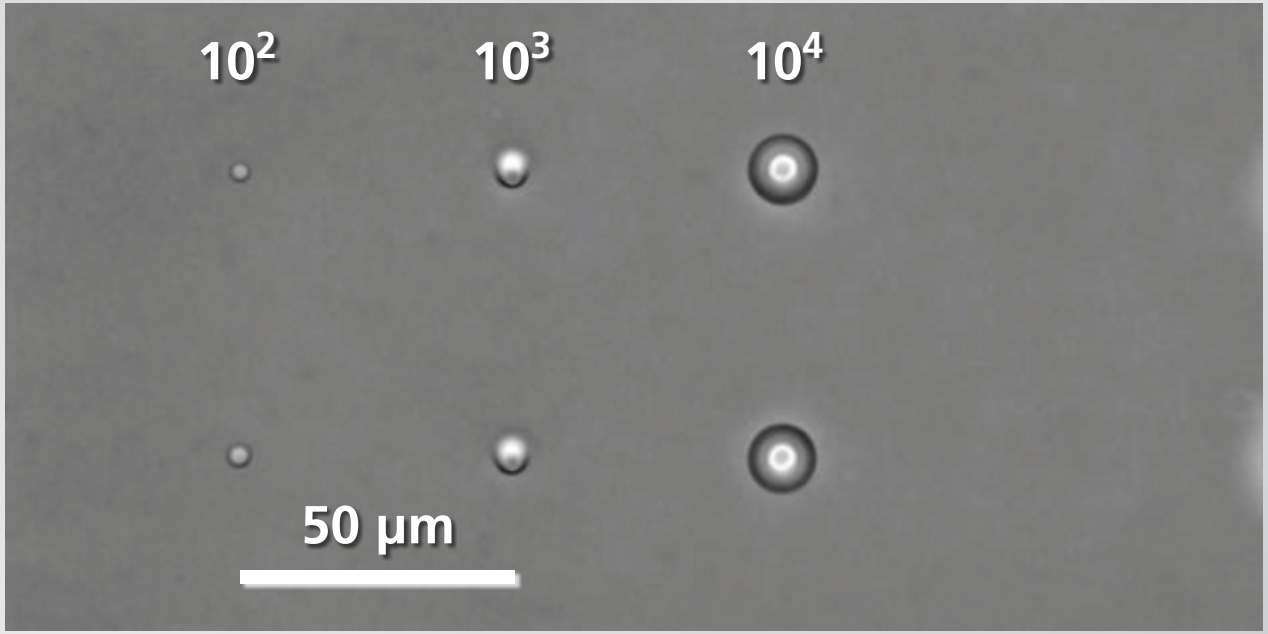
# Femtosecond materials interactions

the longer the irradiation…



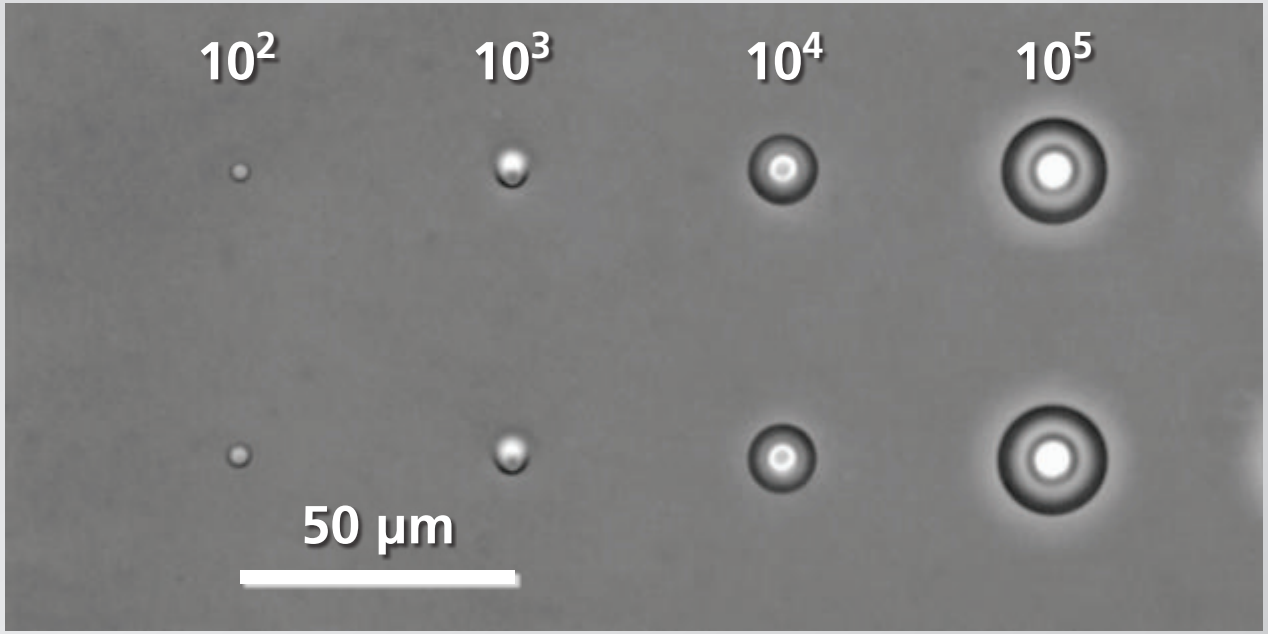
# Femtosecond materials interactions

the longer the irradiation…



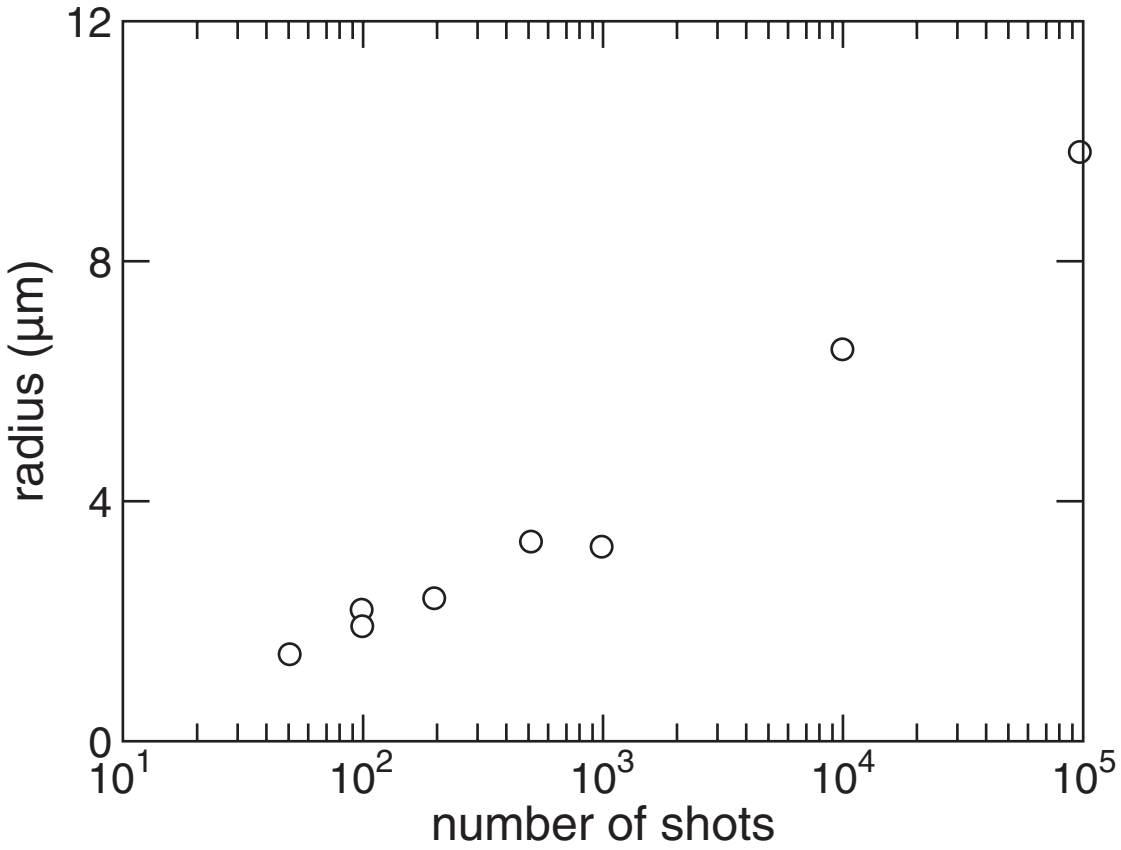
# Femtosecond materials interactions

the longer the irradiation…




… the larger the radius

# Femtosecond materials interactions



# Femtosecond materials interactions

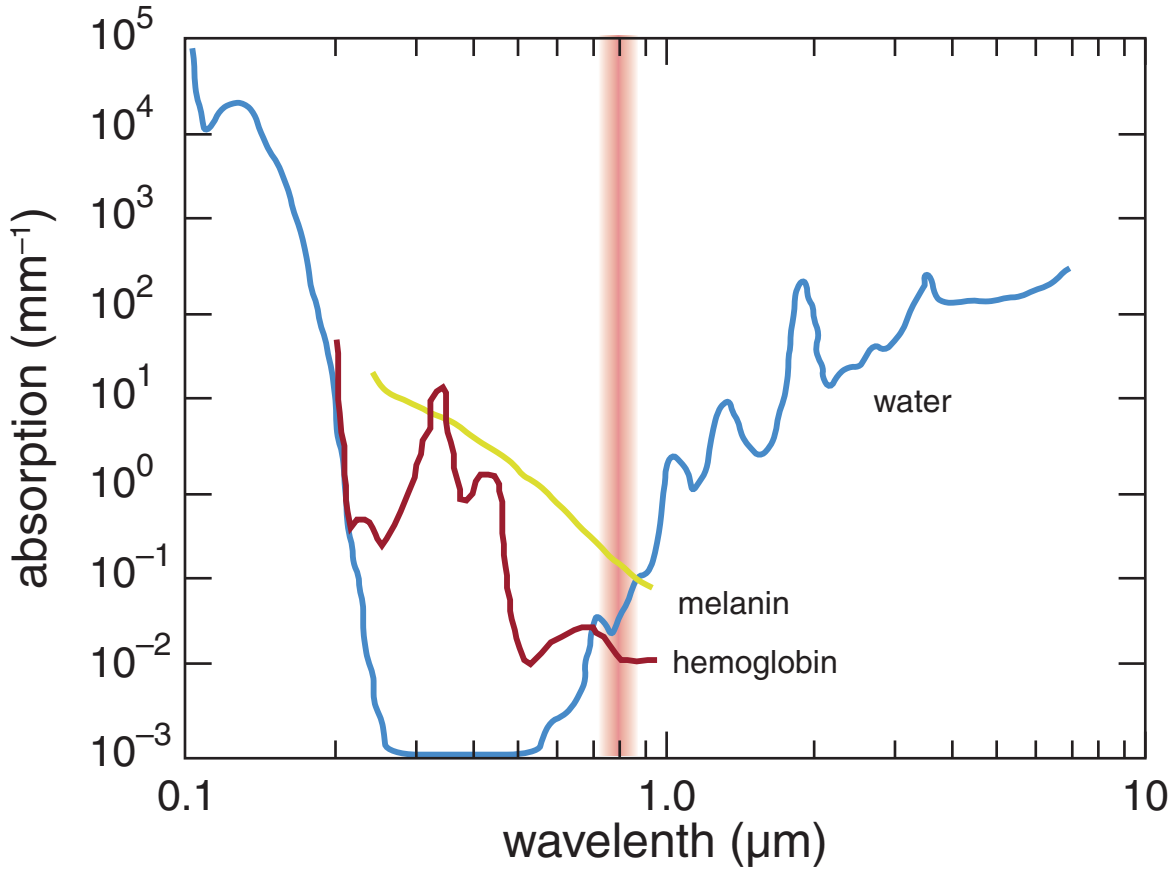
Points to keep in mind:

- threshold critically dependent on NA
- surprisingly little material dependence
- ablate in bulk with nJ pulses


*Nature Photonics* **2**, 219 (2008)

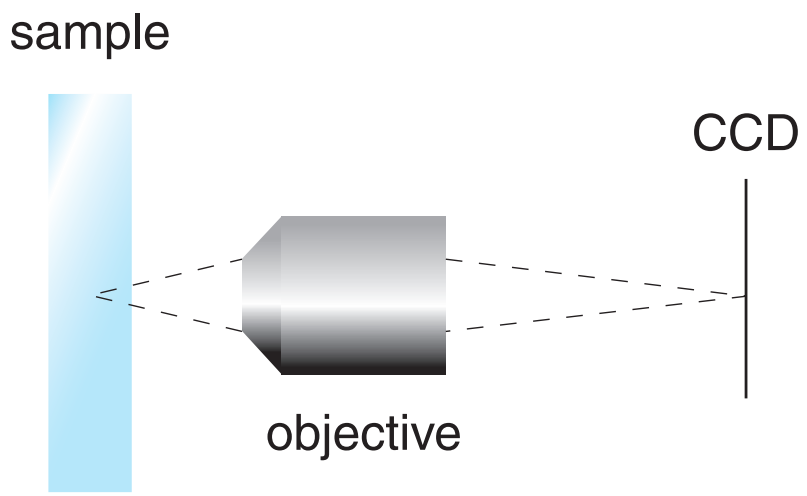
# Femtosecond materials interactions

## tissue is nearly transparent at 800 nm



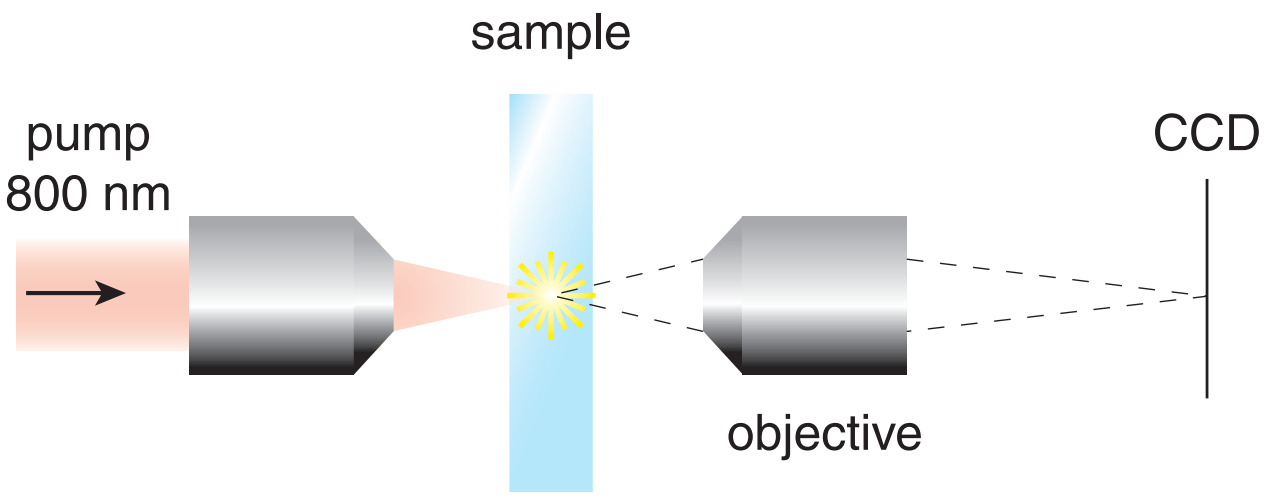
# Femtosecond materials interactions

## image dynamics; sample in focal plane



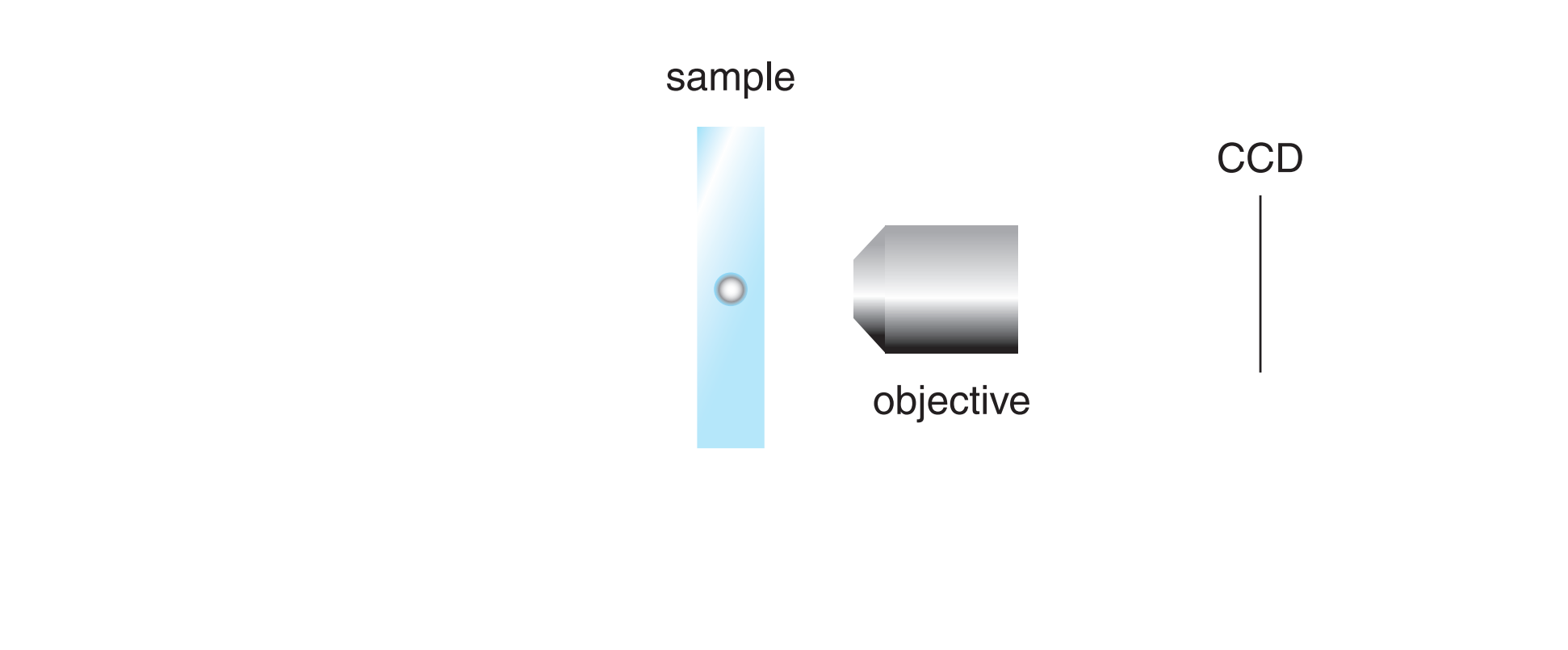
# Femtosecond materials interactions

## focus 800-nm pump pulse in focal plane



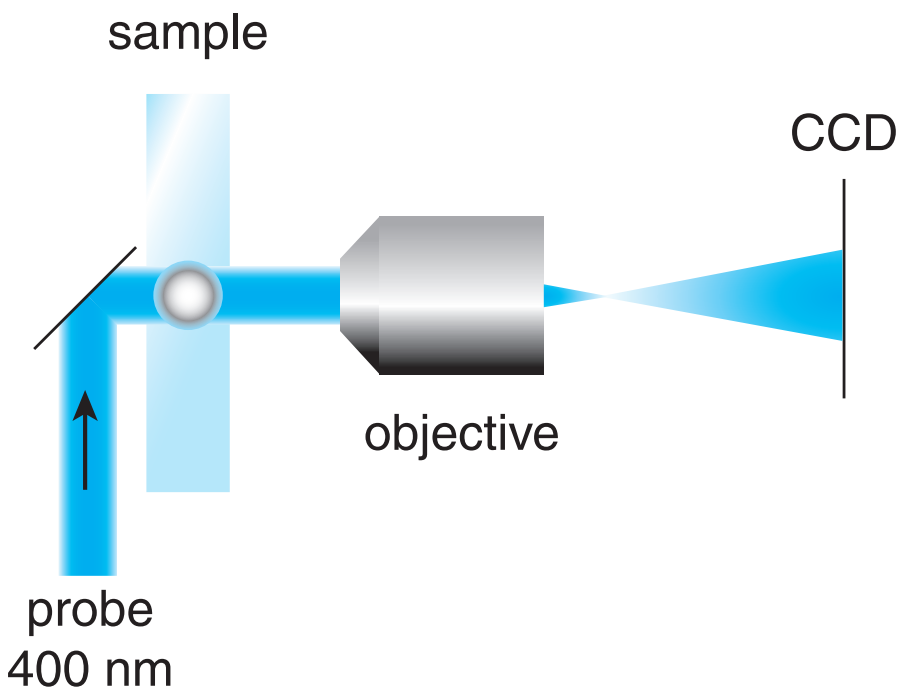
# Femtosecond materials interactions

## microexplosion launches expanding pressure wave



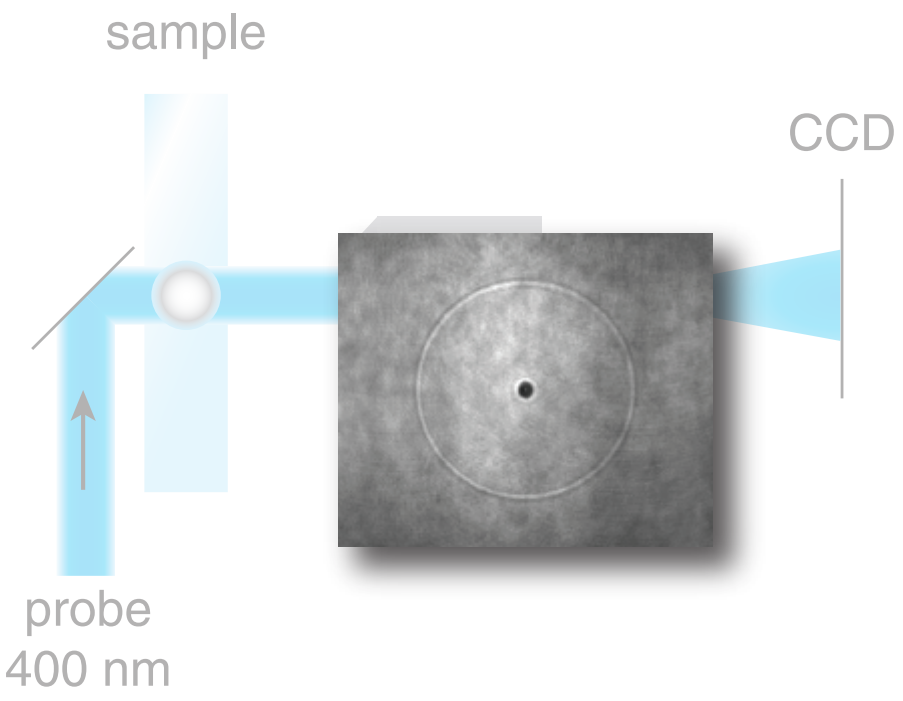
# Femtosecond materials interactions

## illuminate with 400-nm probe pulse



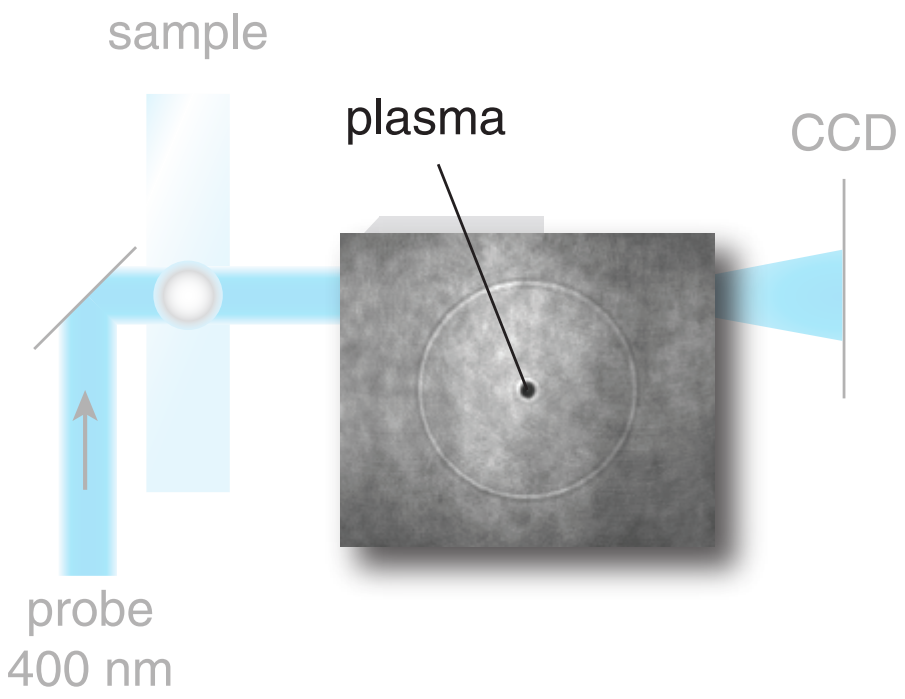
# Femtosecond materials interactions

## CCD records snapshot of dynamics



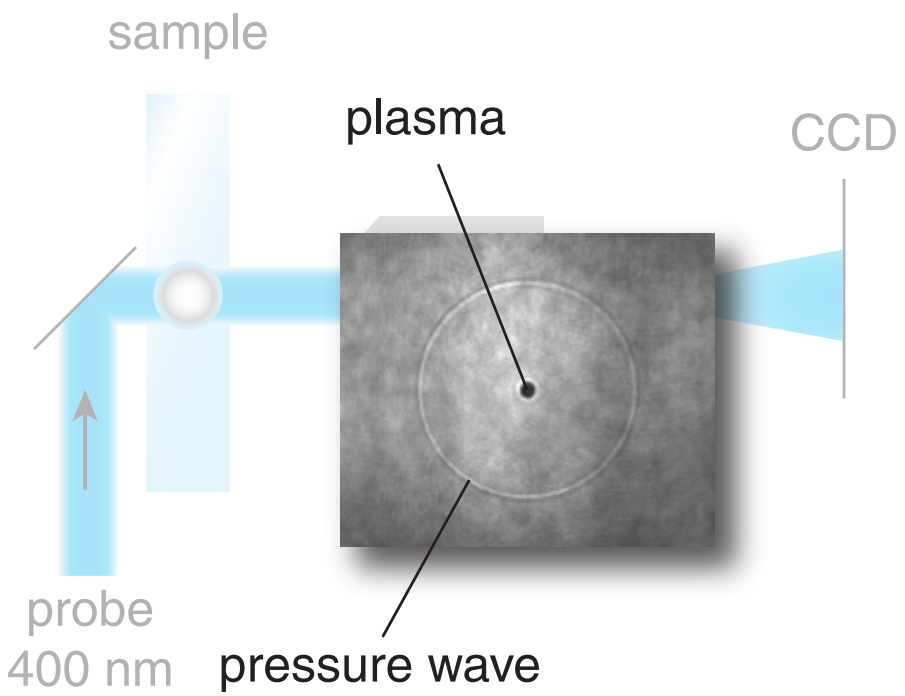
# Femtosecond materials interactions

## CCD records snapshot of dynamics



# Femtosecond materials interactions

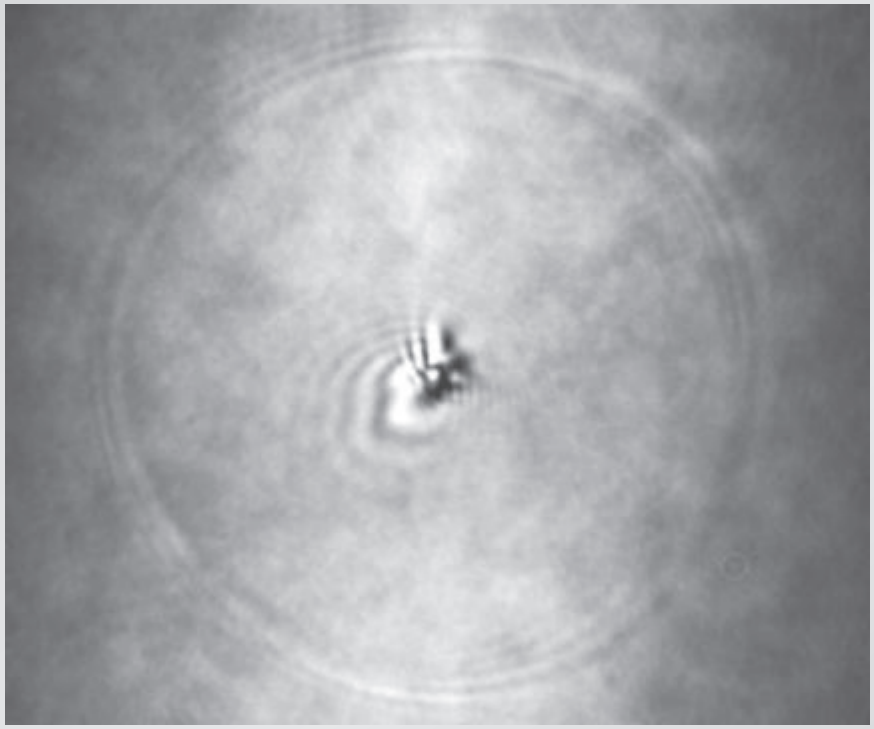
## CCD records snapshot of dynamics



# Femtosecond materials interactions

can see threefold symmetry of sapphire!

sapphire

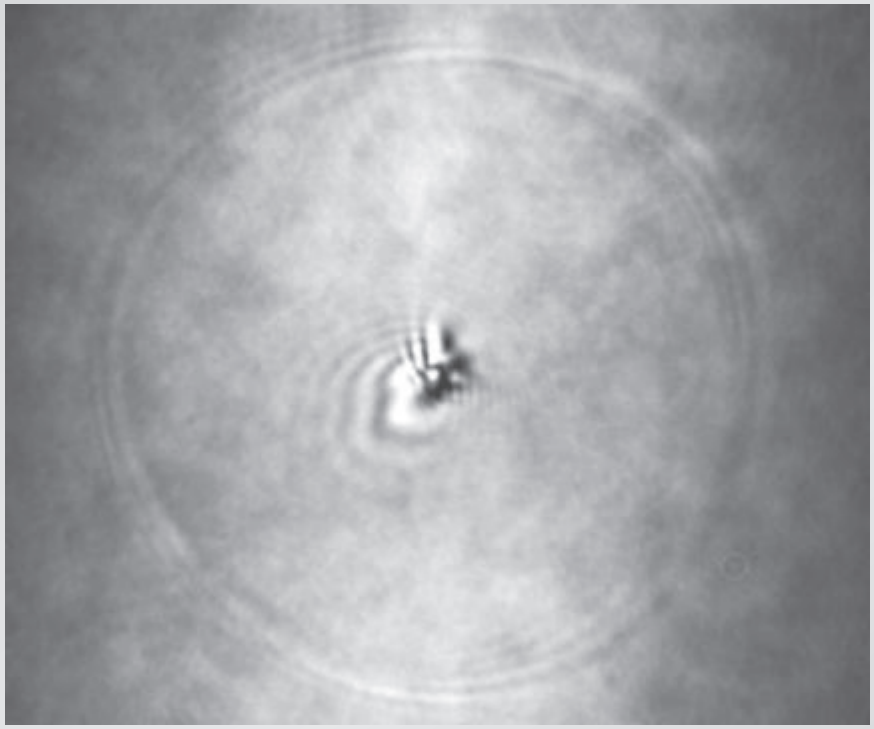


3 μJ pulse
3.8 ns delay
40 μm radius

# Femtosecond materials interactions

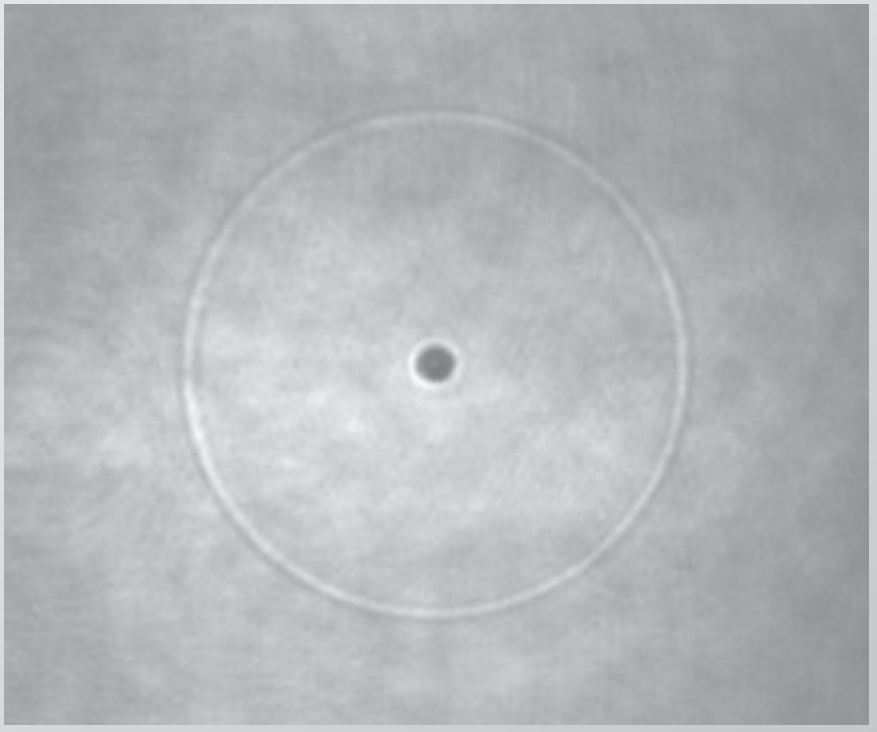
## water isotropic

### sapphire



3 μJ pulse
3.8 ns delay
40 μm radius

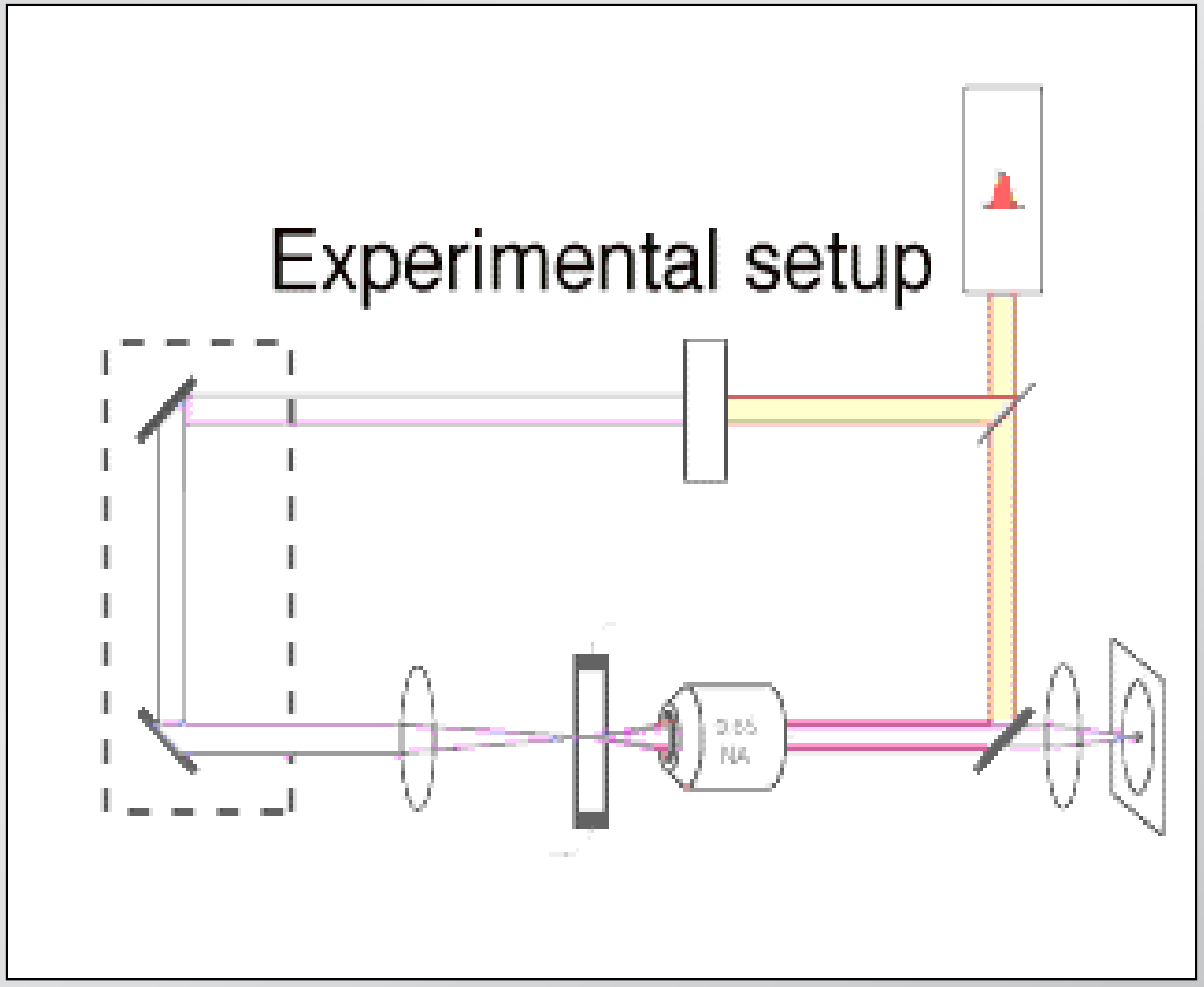
### water



1 μJ pulse
35 ns delay
58 μm radius

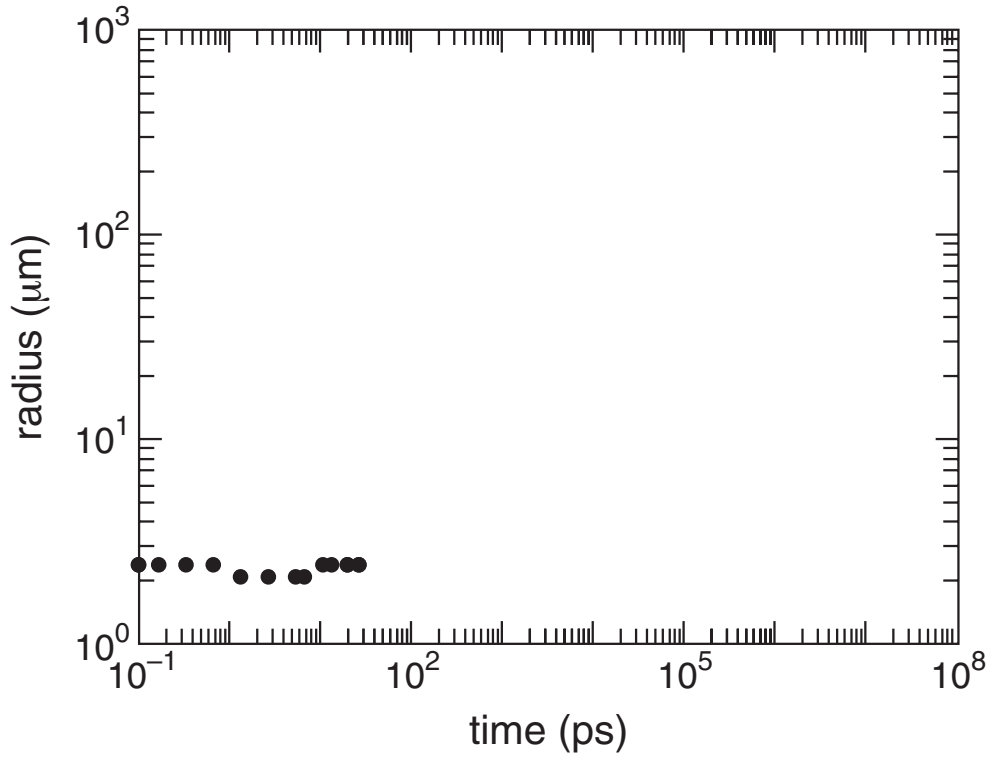
# Femtosecond materials interactions

**vary pump-probe delay to observe dynamics**



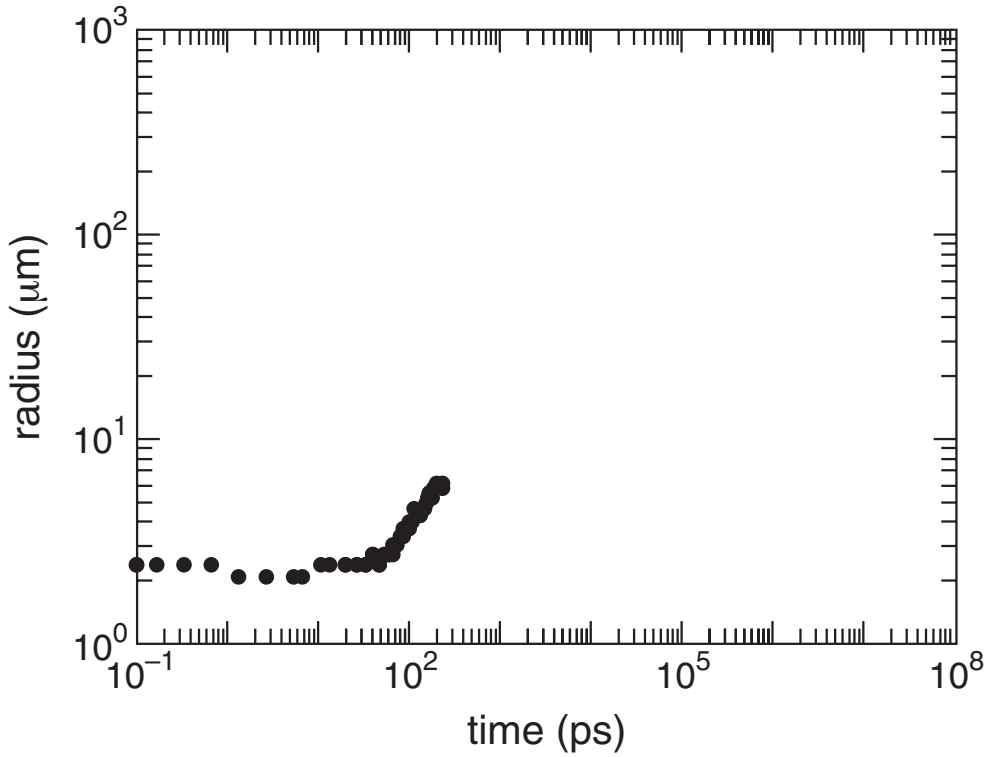
# Femtosecond materials interactions

## plasma remains at resolution limit for 5 ps



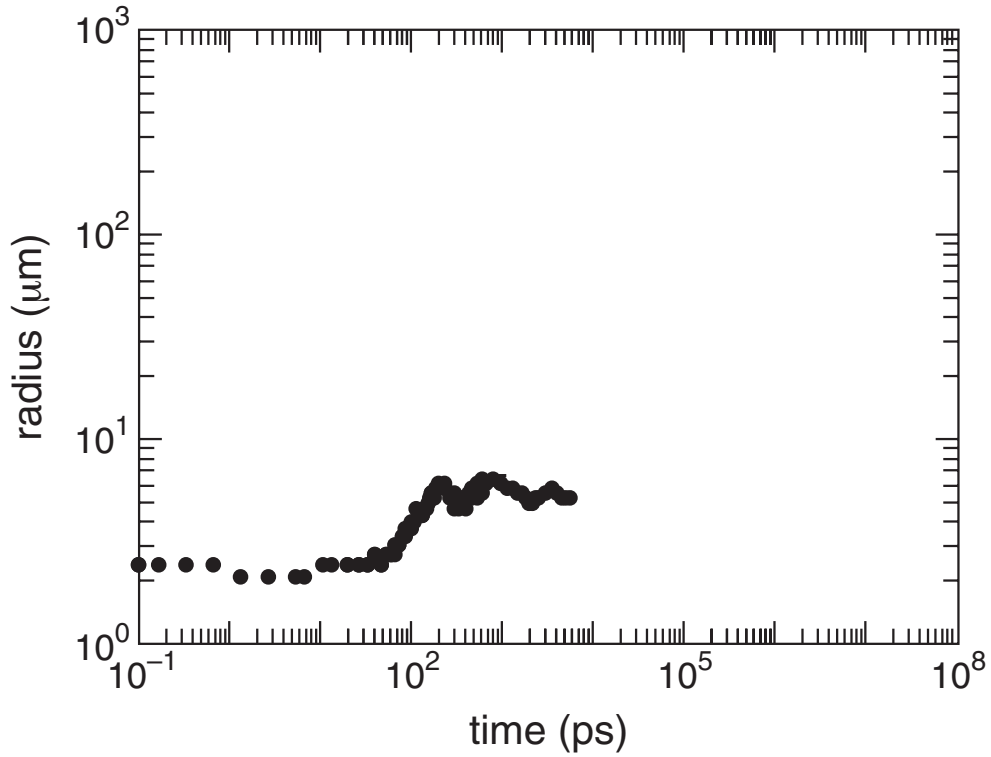
# Femtosecond materials interactions

## then expands supersonically



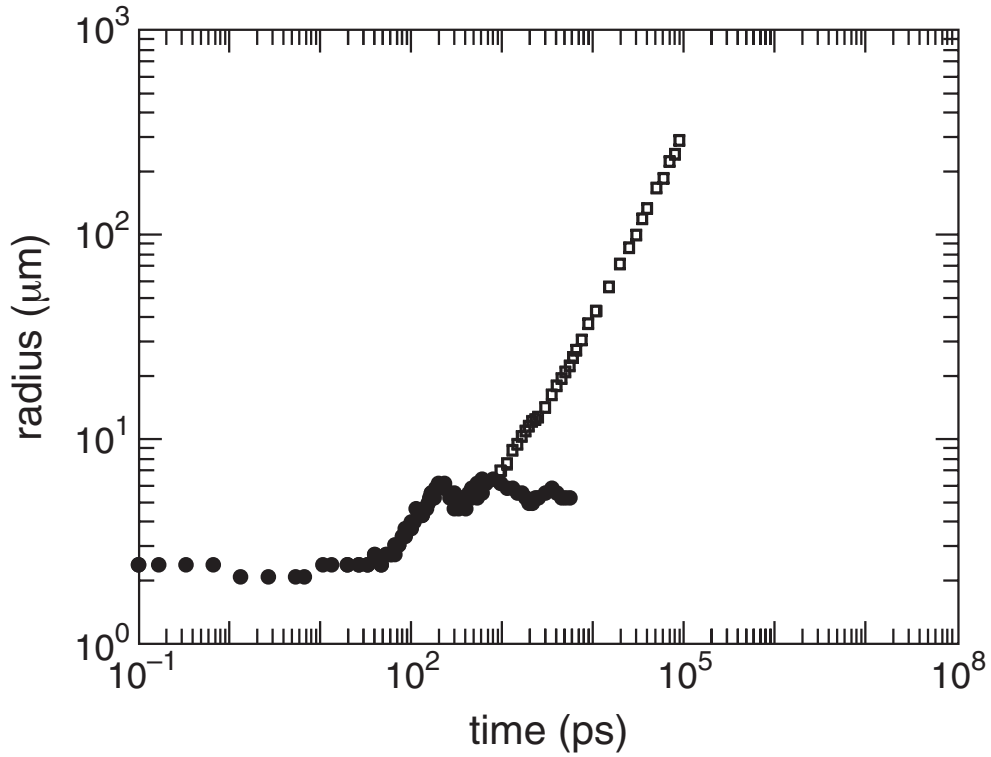
# Femtosecond materials interactions

## after 20 ps expansion slows and rings…



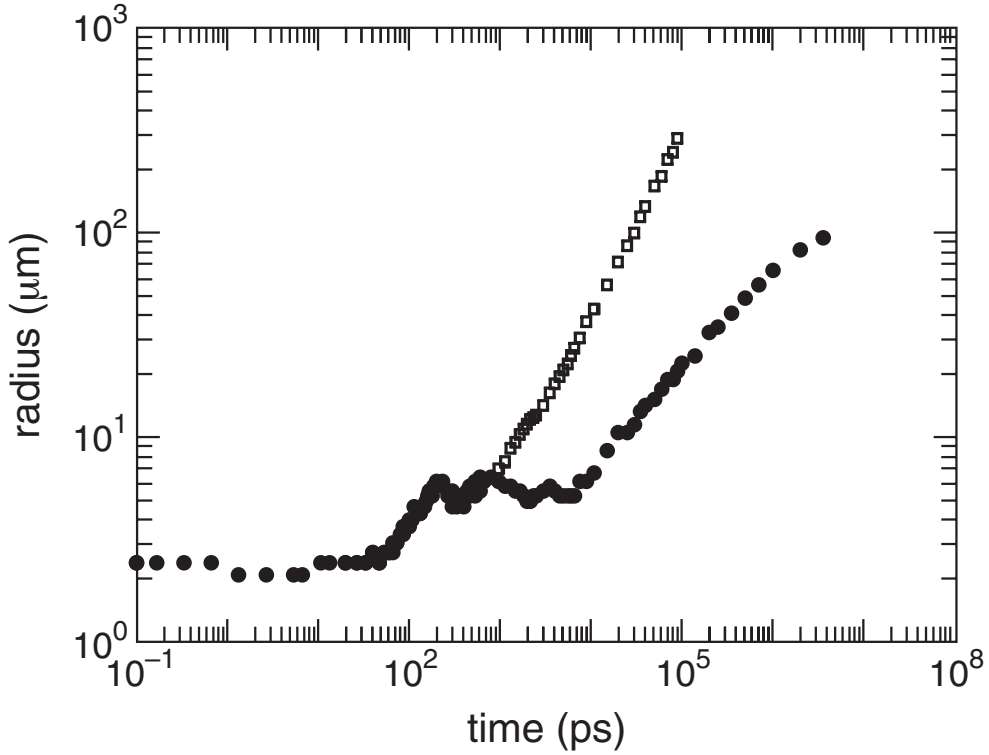
# Femtosecond materials interactions

## launching a pressure wave at the speed of sound



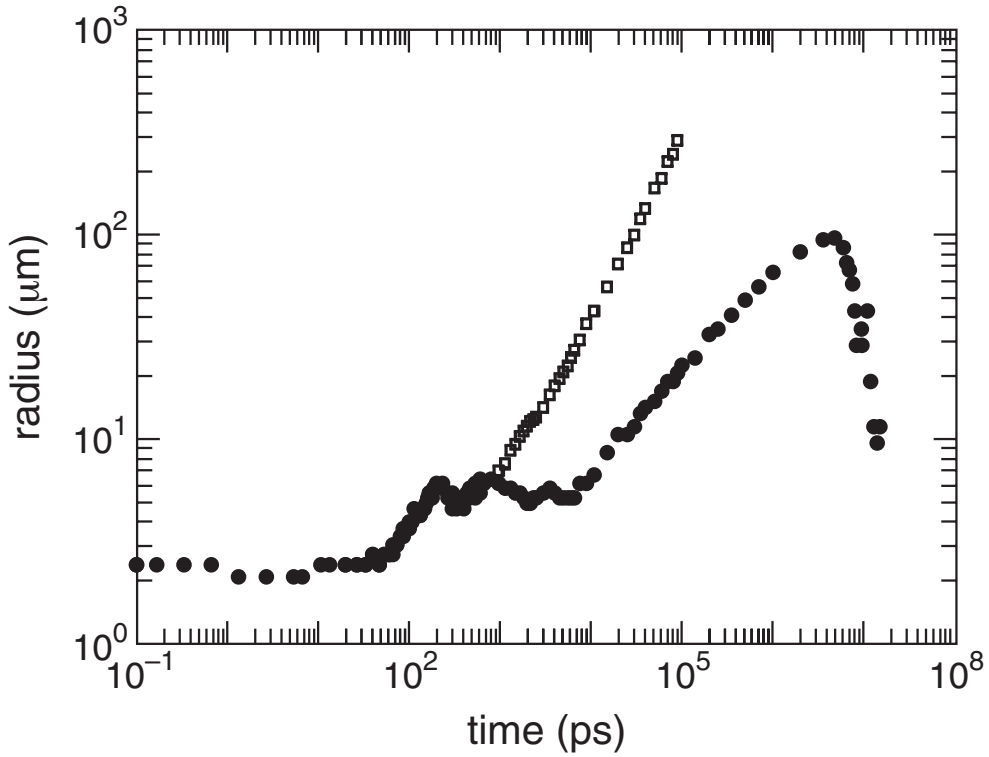
# Femtosecond materials interactions

## plasma forms an expanding cavitation bubble



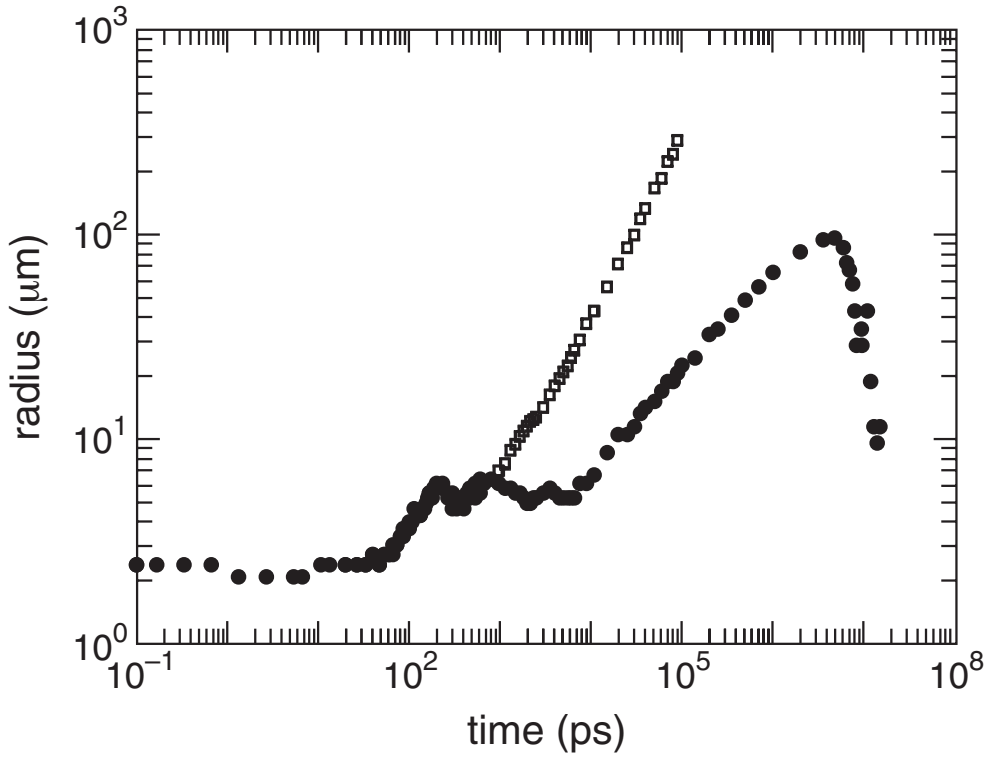
# Femtosecond materials interactions

## which collapses after 5 μs



# Femtosecond materials interactions

## pressure wave and cavitation bubble



# Femtosecond materials interactions

Damage caused by:

- ionization/ablation
- pressure wave
- cavitation bubble
- thermal effects
- photochemical effects

# Outline

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

# 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

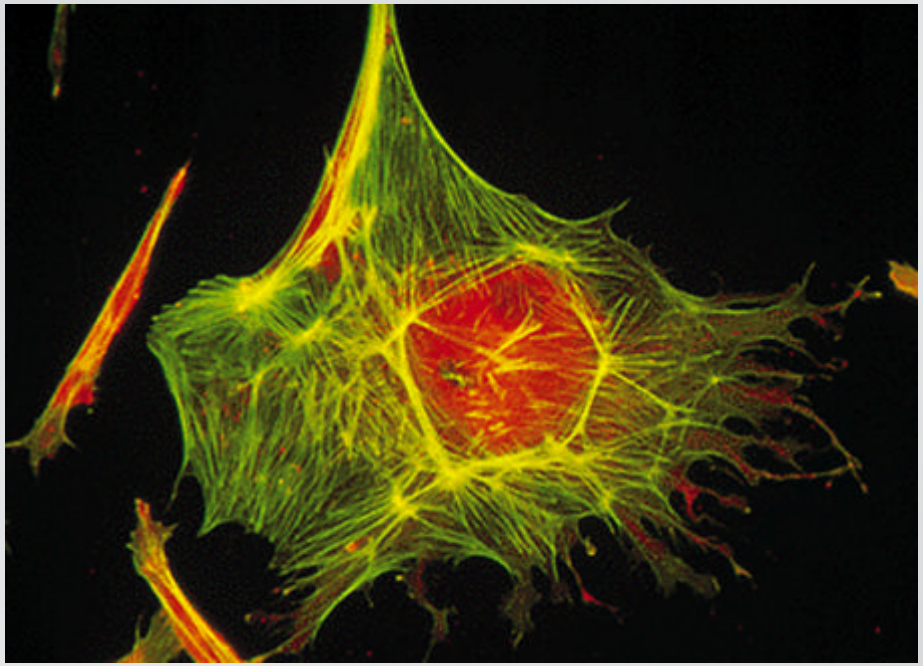
Cytoskeleton

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

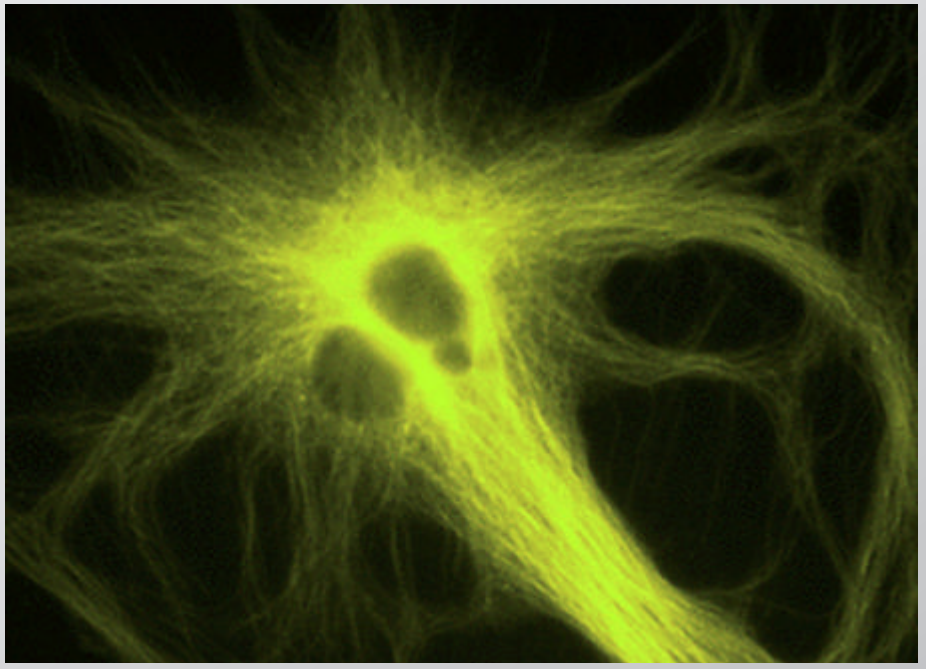
# Subcellular surgery

two components

actin fibers

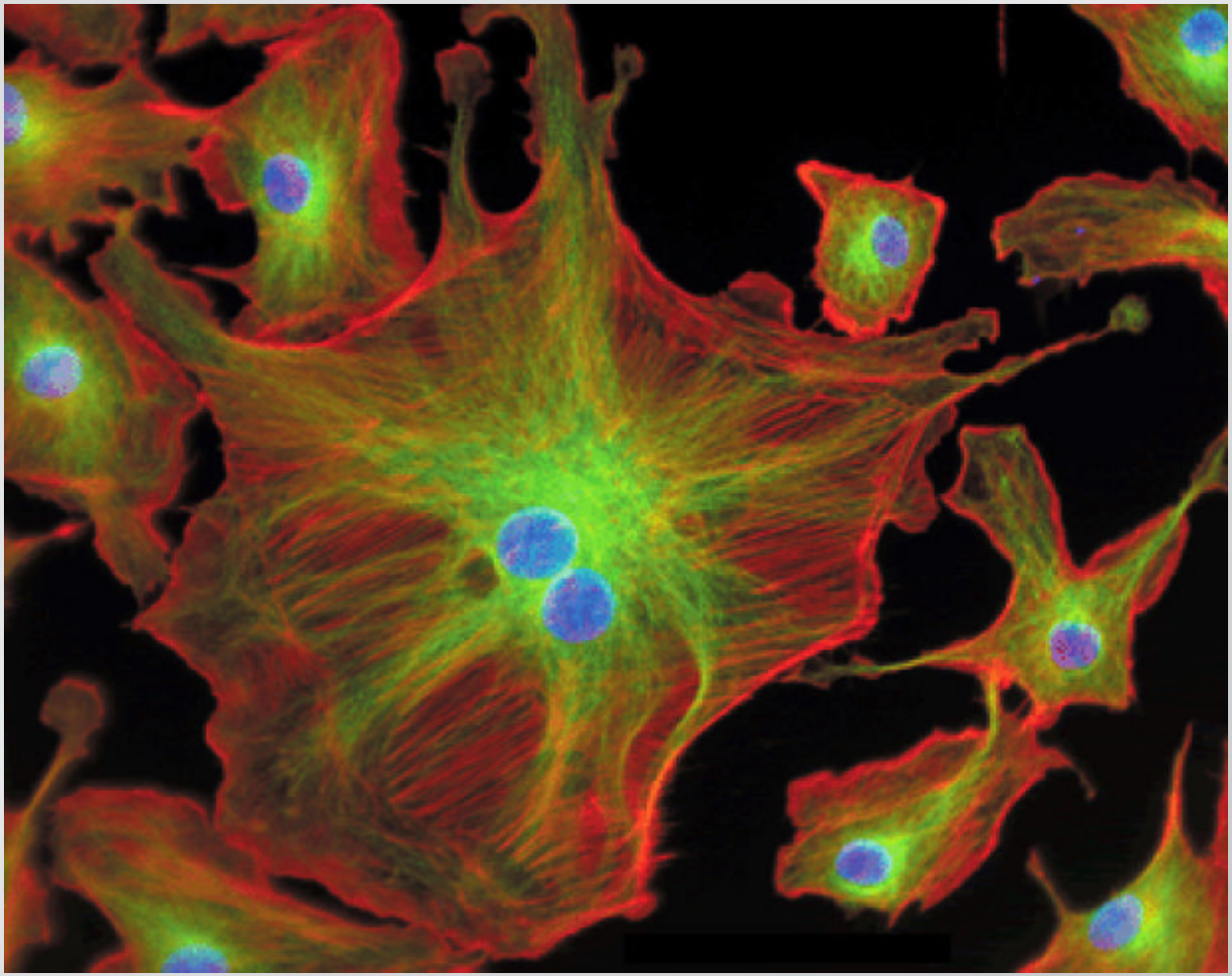


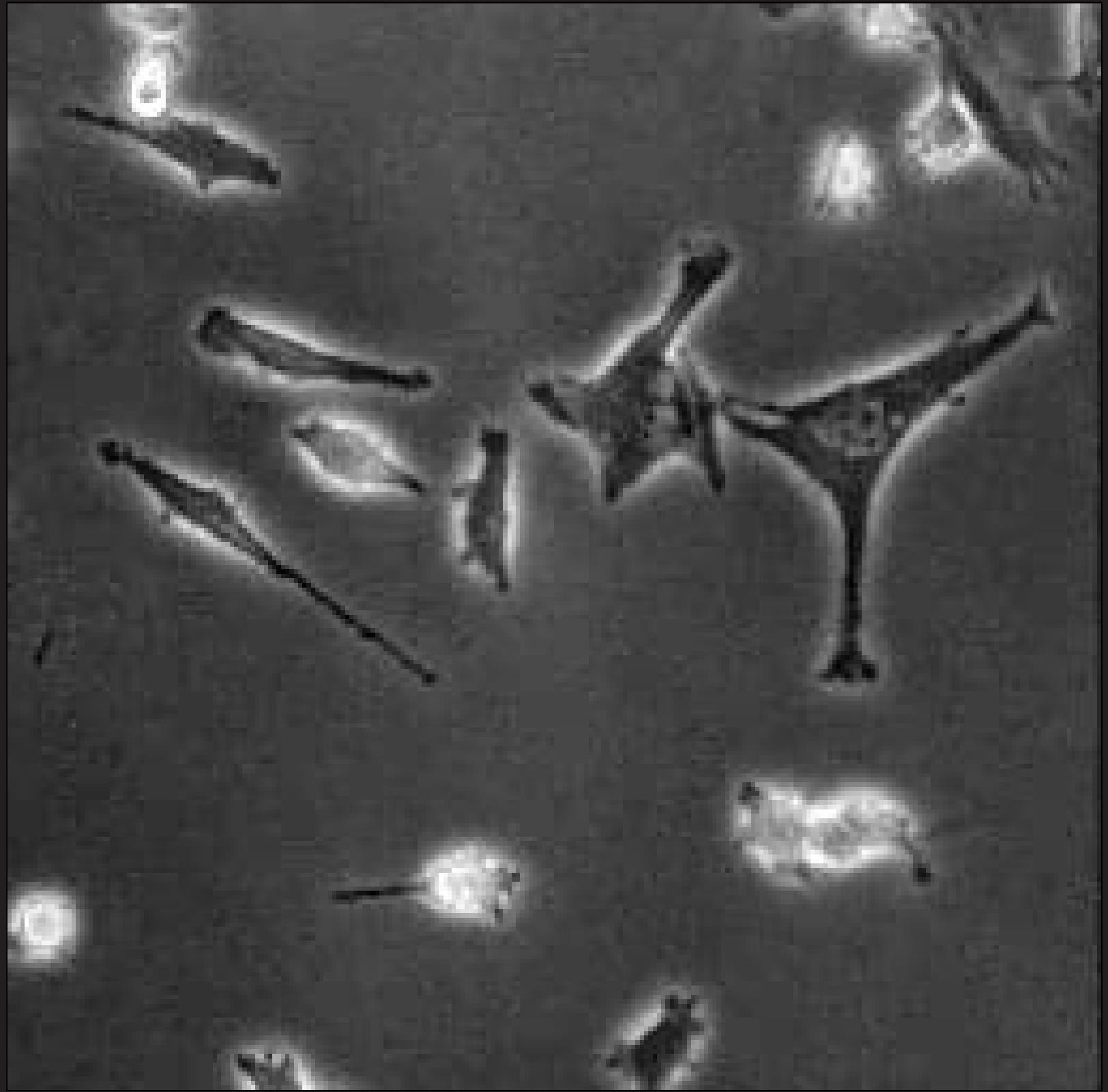
microtubules



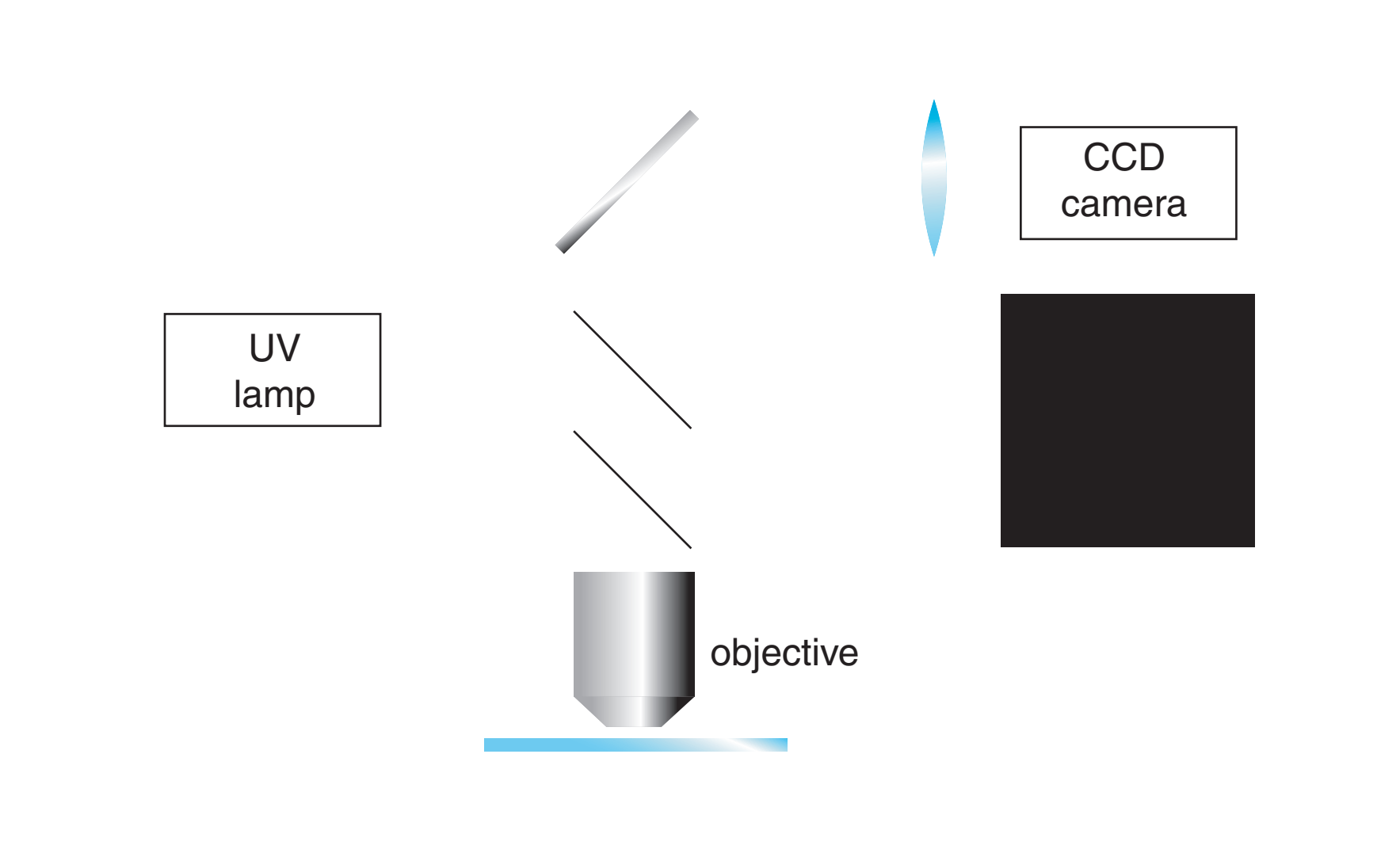
# Subcellular surgery

two components



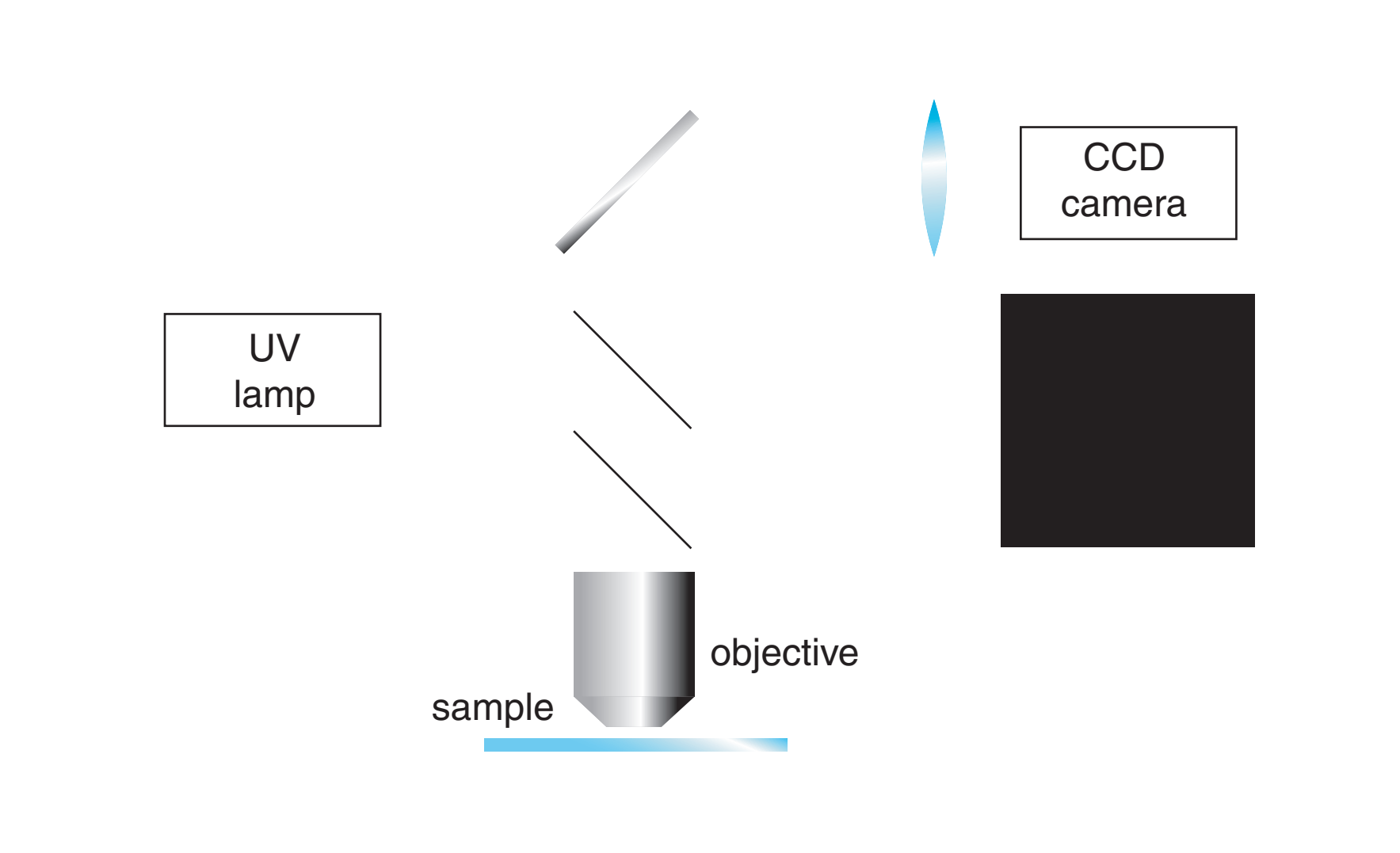


# Subcellular surgery



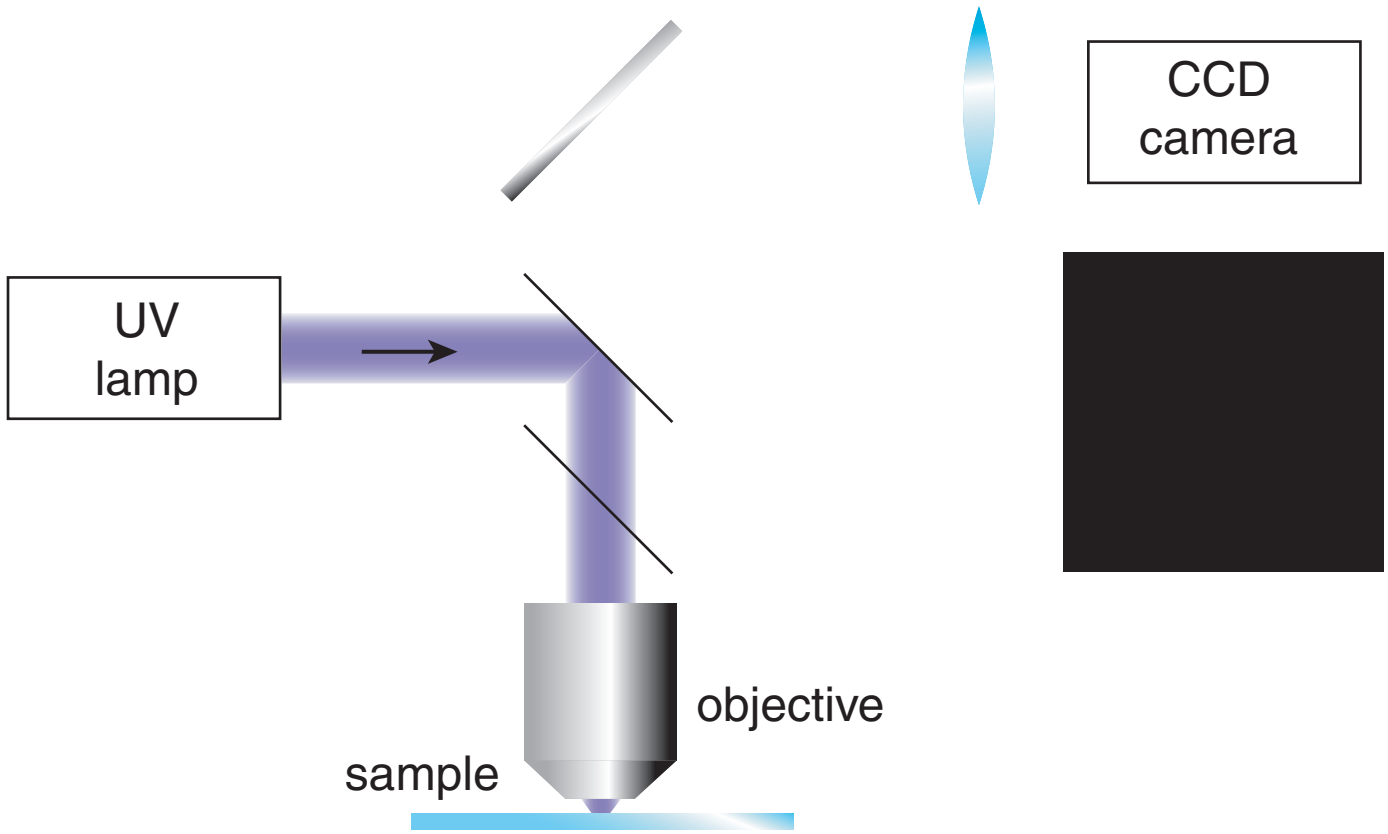

epi-fluorescence microscope

# Subcellular surgery



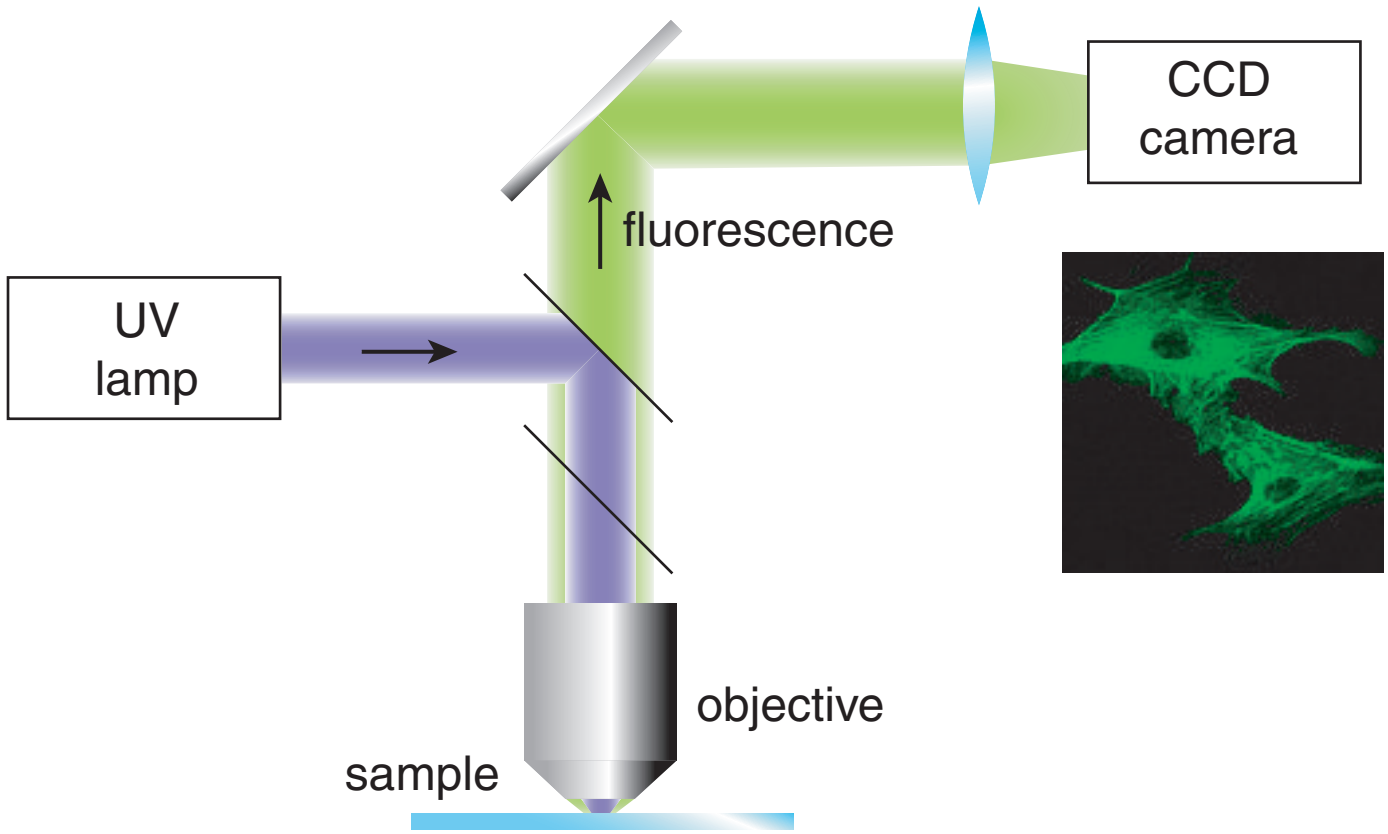

**fluorescently label sample**

# Subcellular surgery



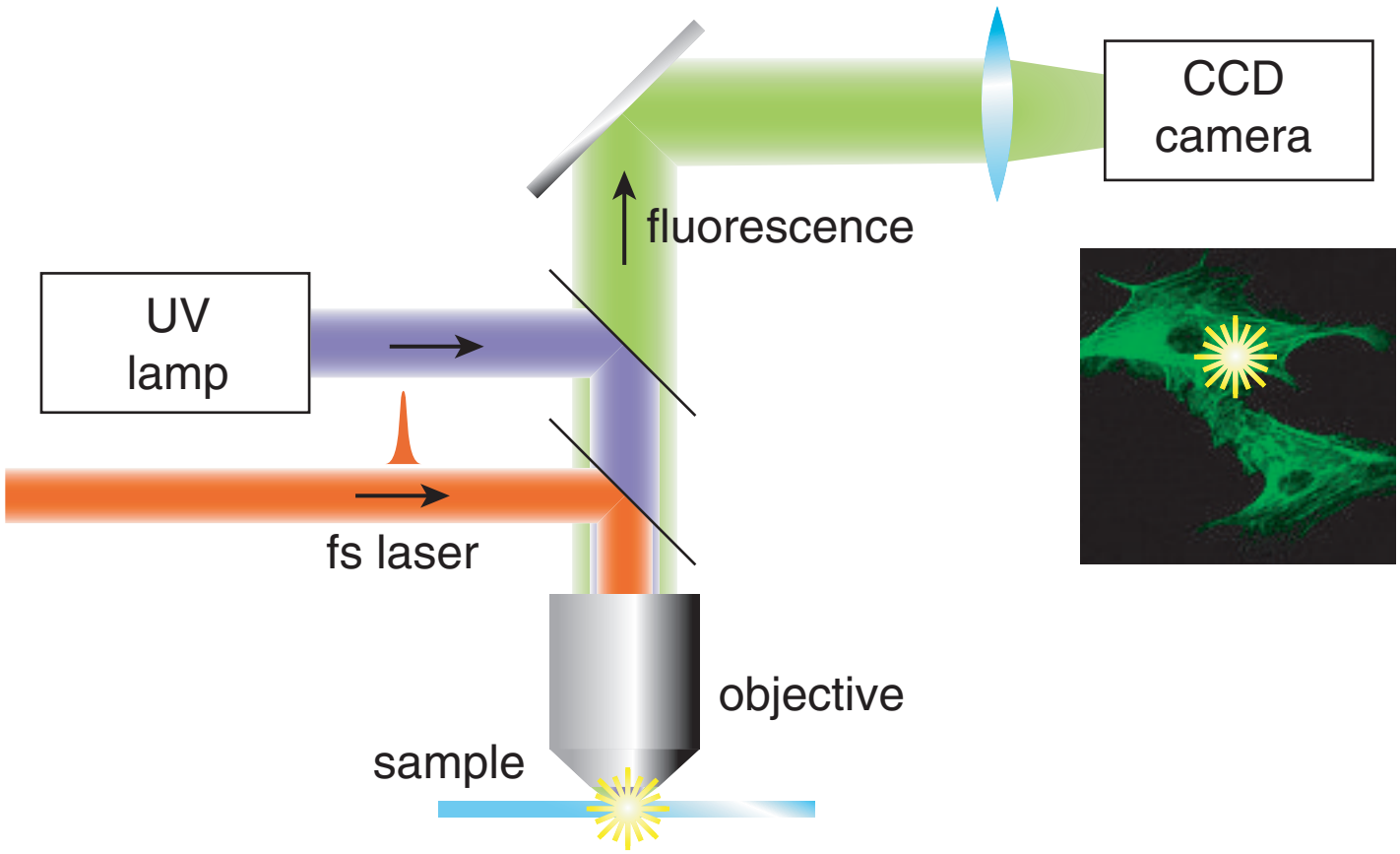

**UV illumination…**

# Subcellular surgery



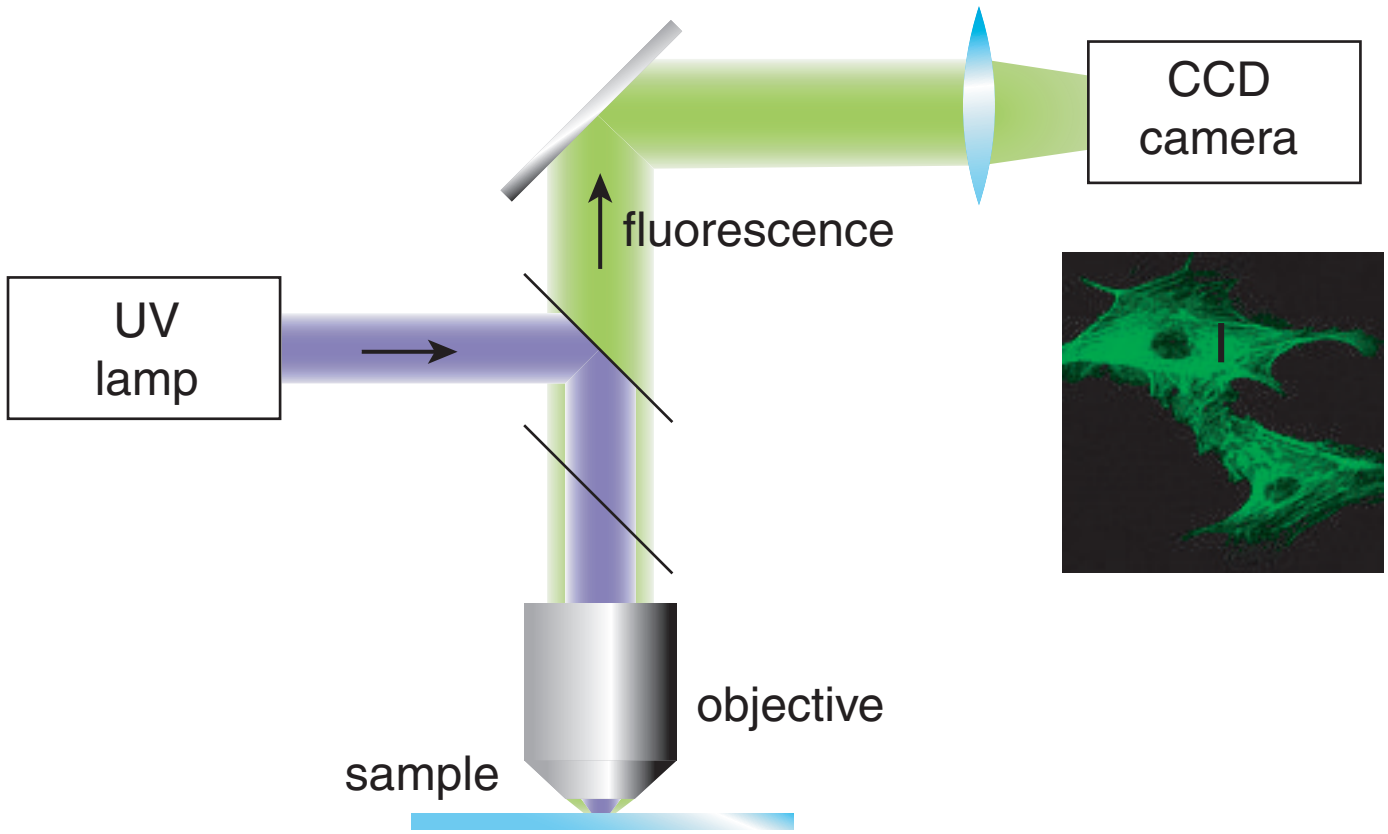

**…causes fluorescence**

# Subcellular surgery



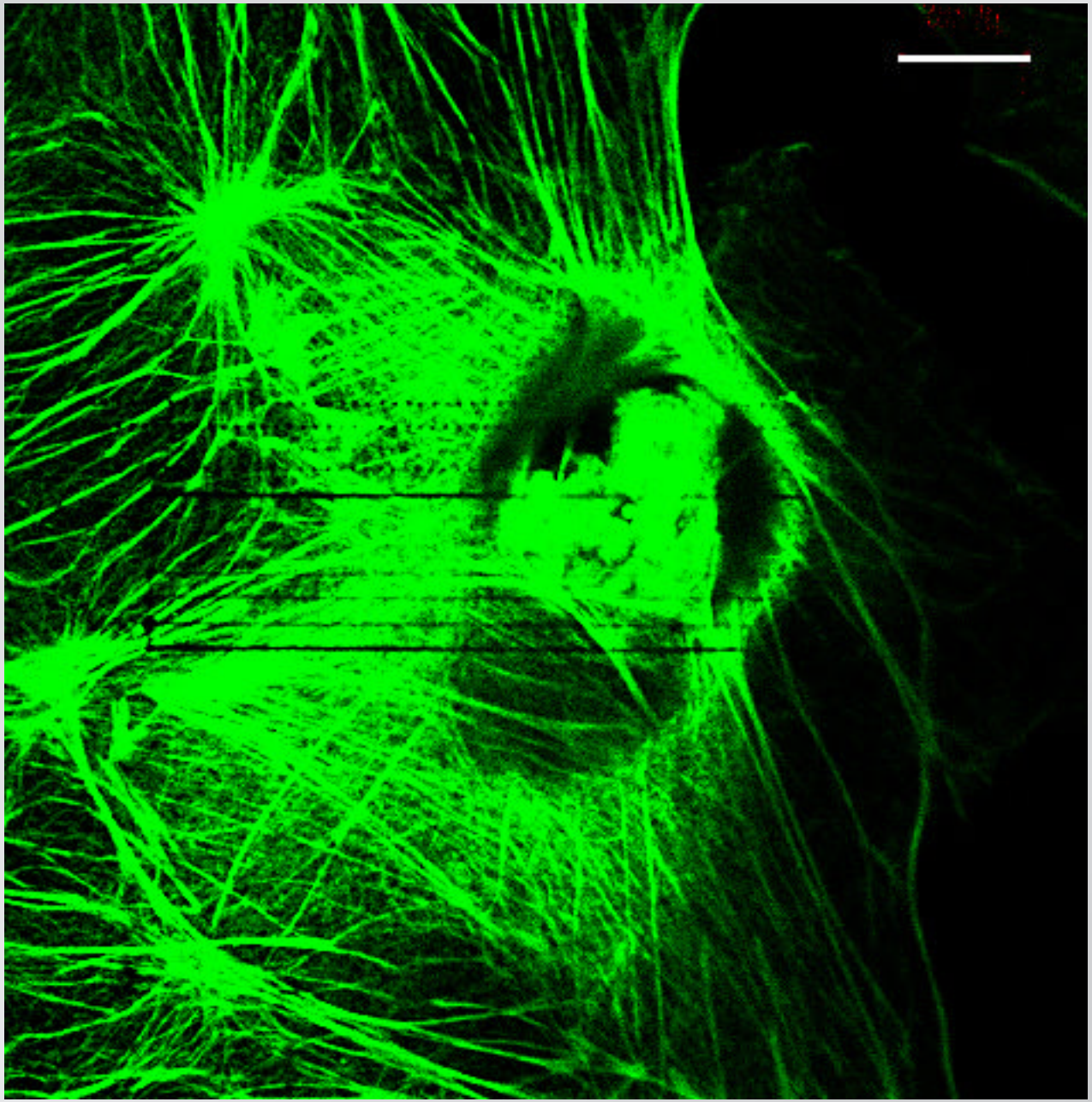

**irradiate with fs laser beam**

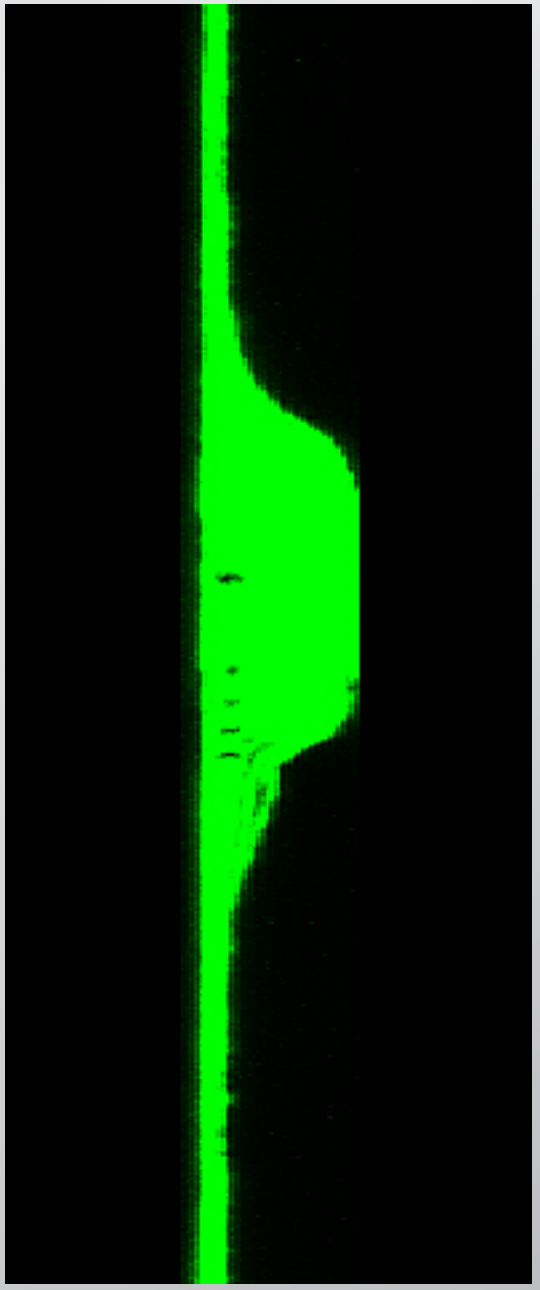
# Subcellular surgery



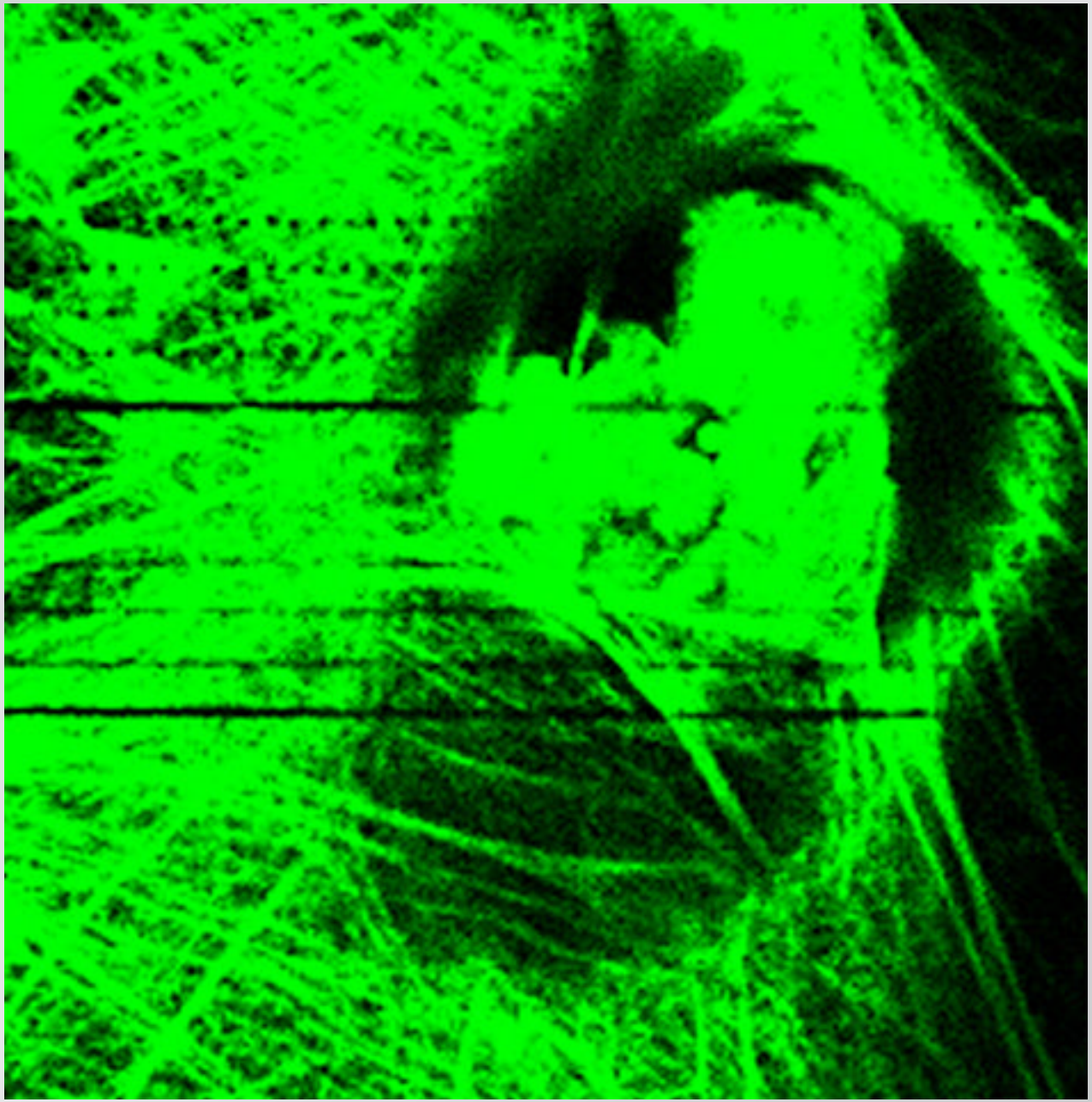

**examine resulting ablation**

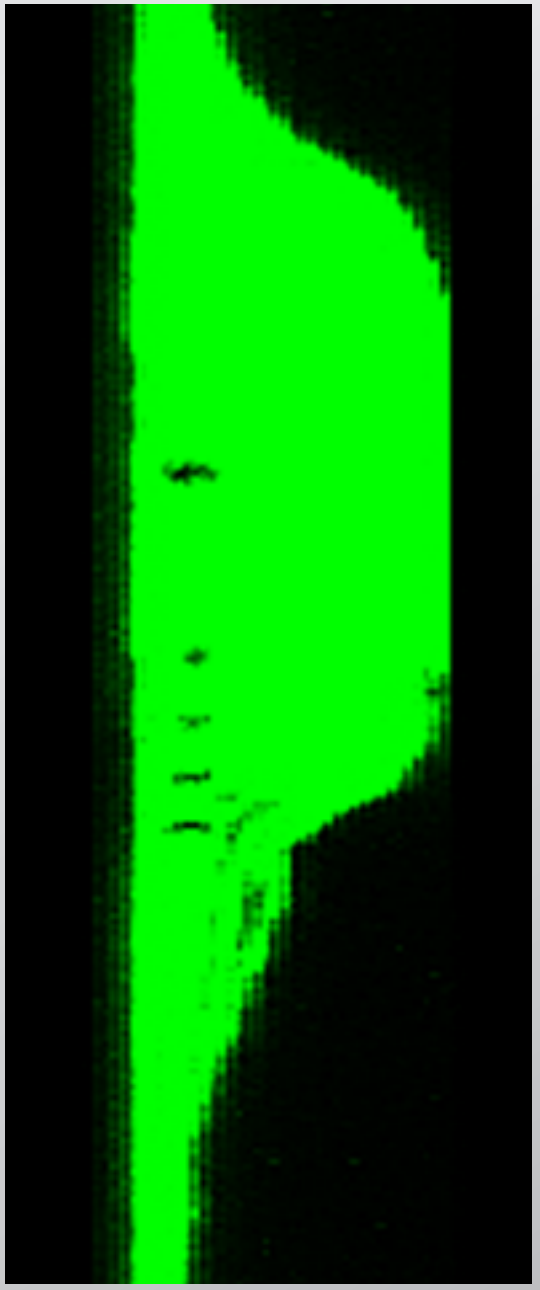
# Subcellular surgery





# Subcellular surgery





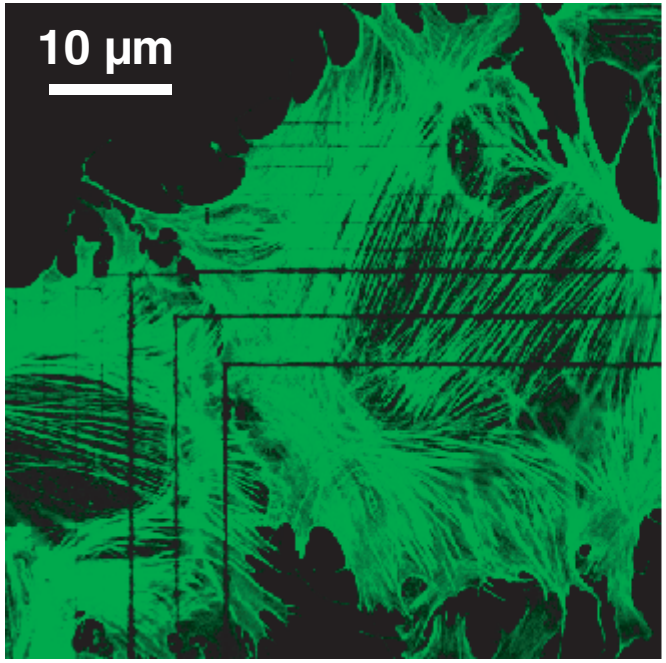
# Subcellular surgery

**Question to you:**

**Can we conclude from the disappearance of fluorescence that parts of the actin network have been ablated?**

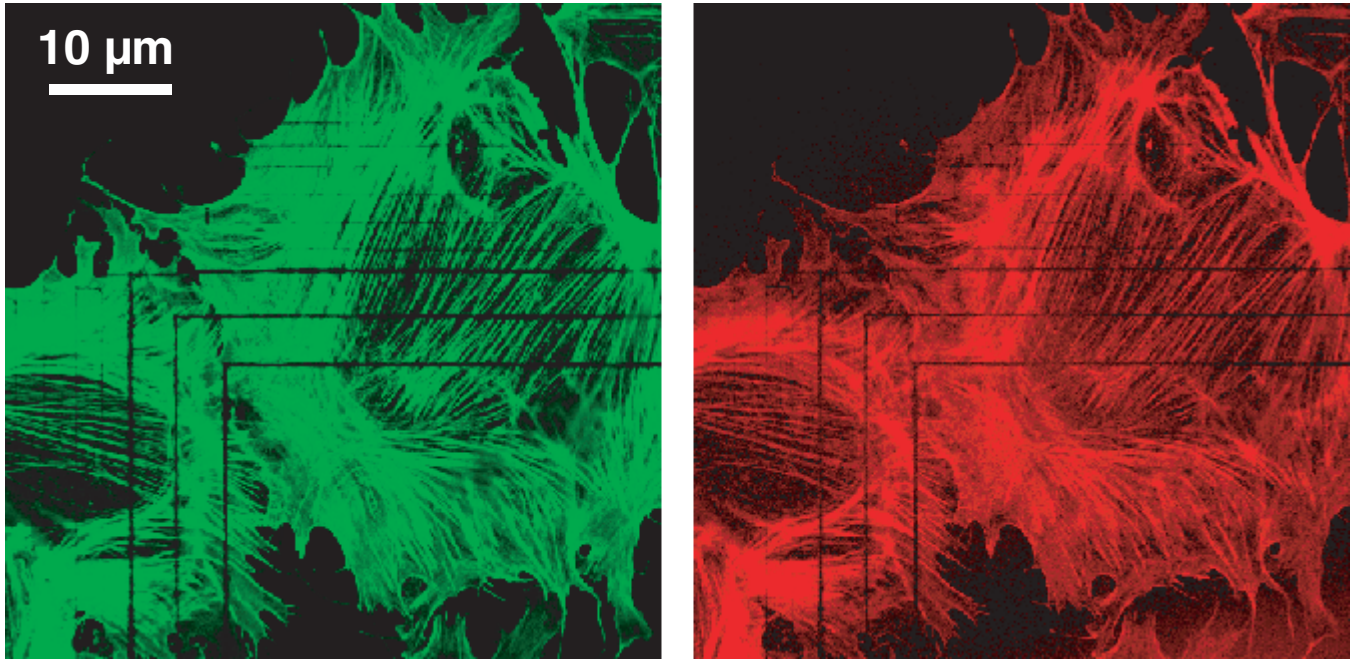
# Subcellular surgery

## ablation or bleaching?



# Subcellular surgery

## restain after exposure



# Subcellular surgery

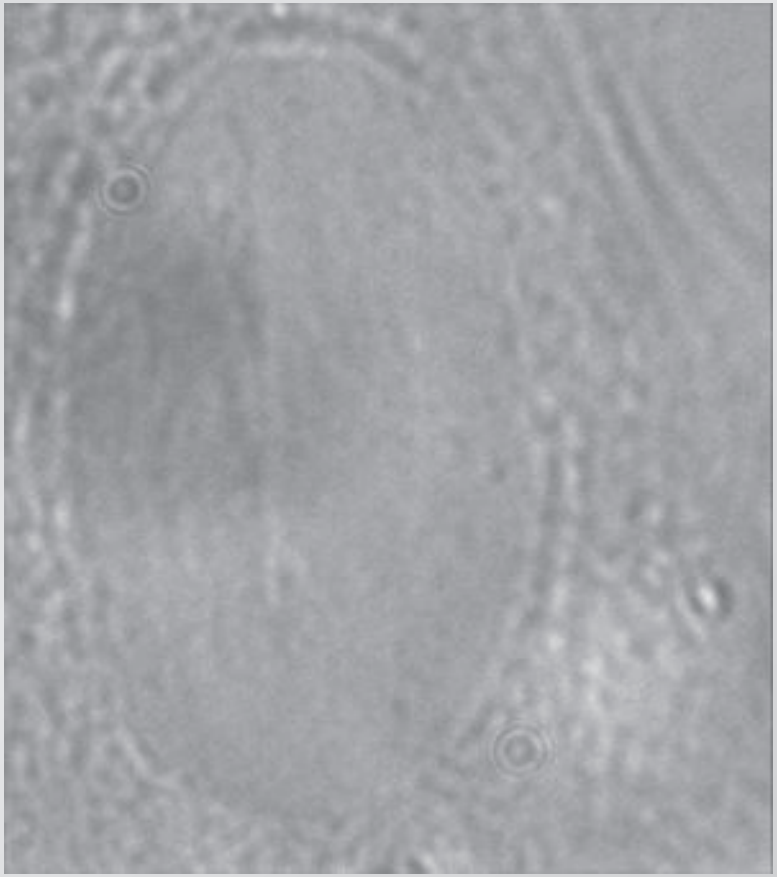
restain after exposure


**Question to you:**

**Why would restaining with the same dye be a bad idea?**

# Subcellular surgery

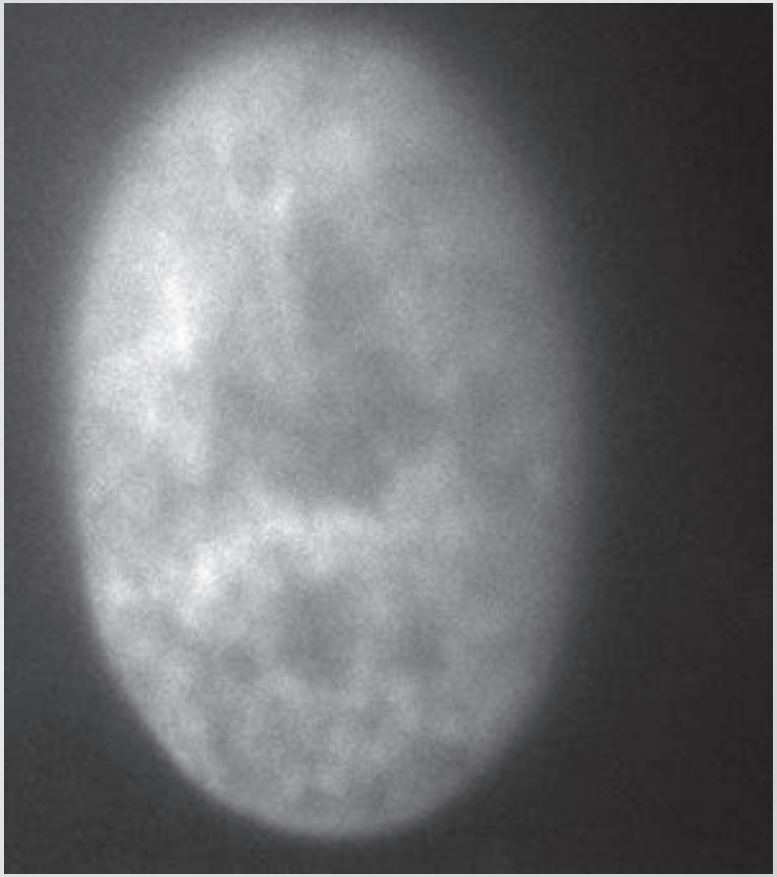
nucleus of fixed endothelial cell



white light microscopy

# Subcellular surgery

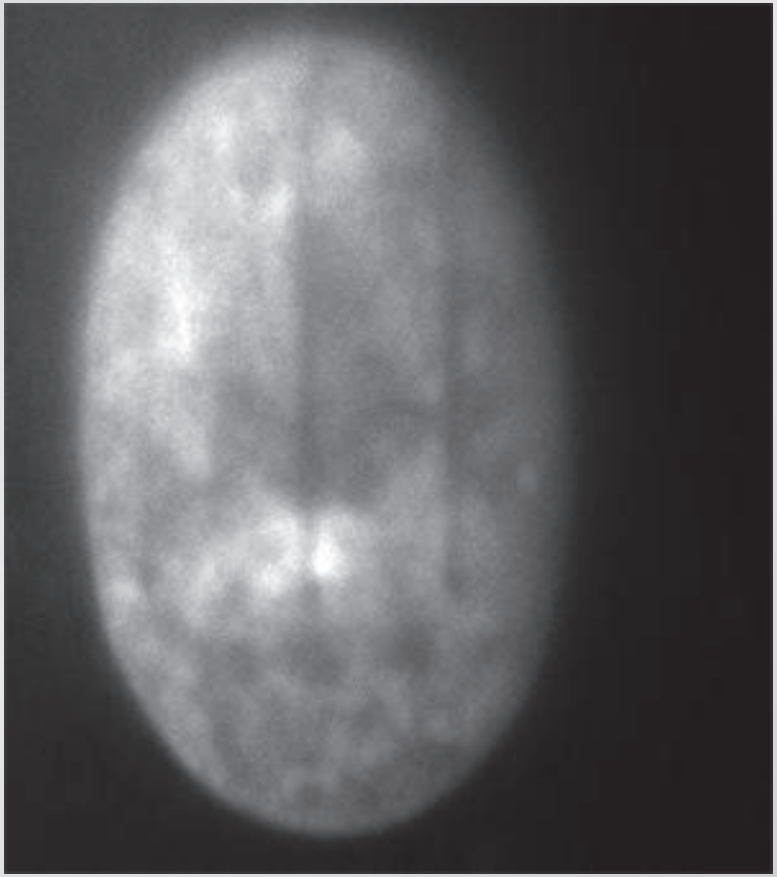
nucleus of fixed endothelial cell



fluorescence microscopy

# Subcellular surgery

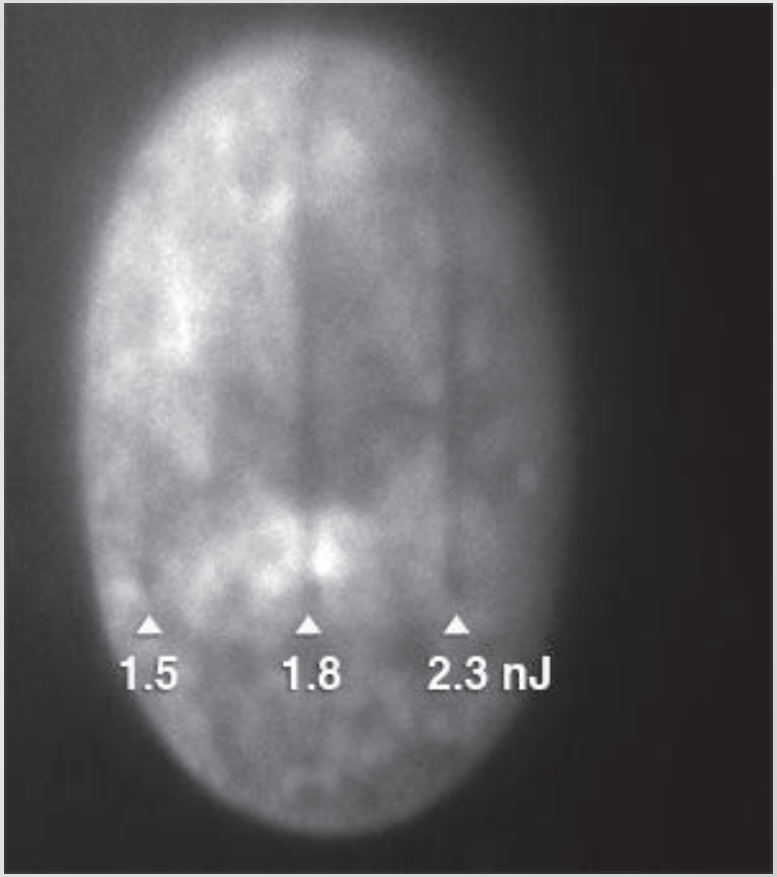
irradiate with fs laser



fluorescence microscopy

# Subcellular surgery

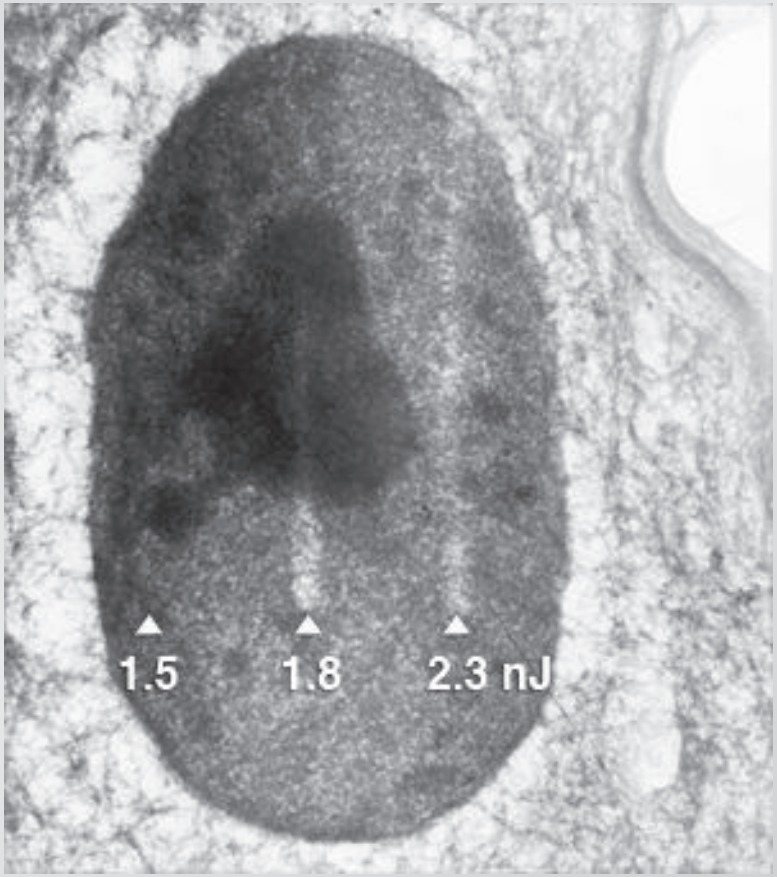
irradiate with fs laser




fluorescence microscopy

# Subcellular surgery

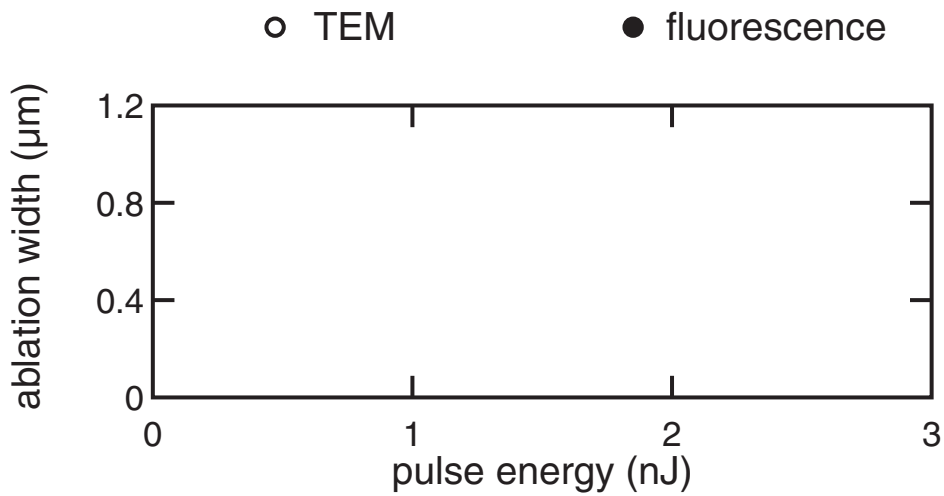
bleaching or ablation?




TEM image

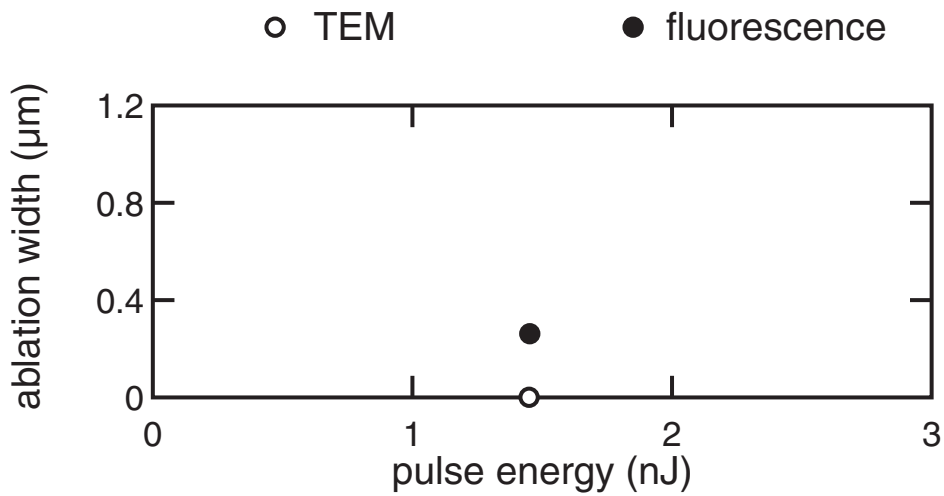
# Subcellular surgery

## three regions of interaction



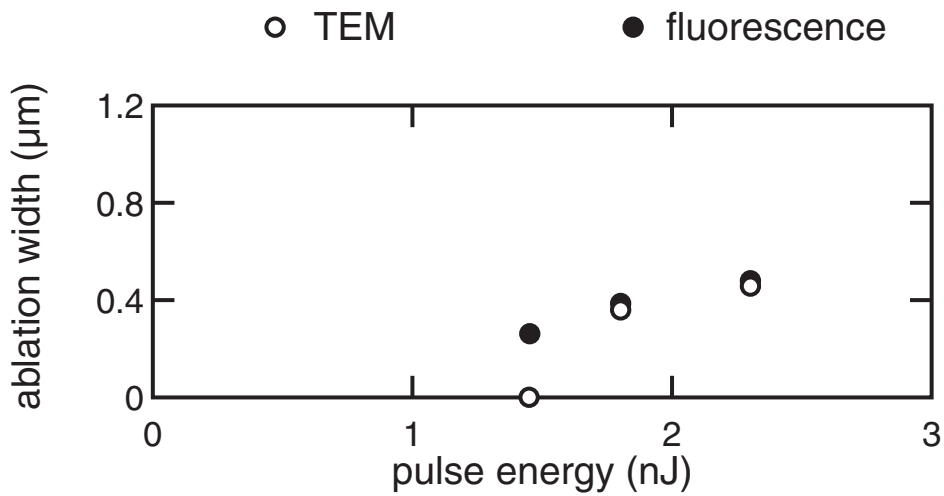
# Subcellular surgery

## three regions of interaction



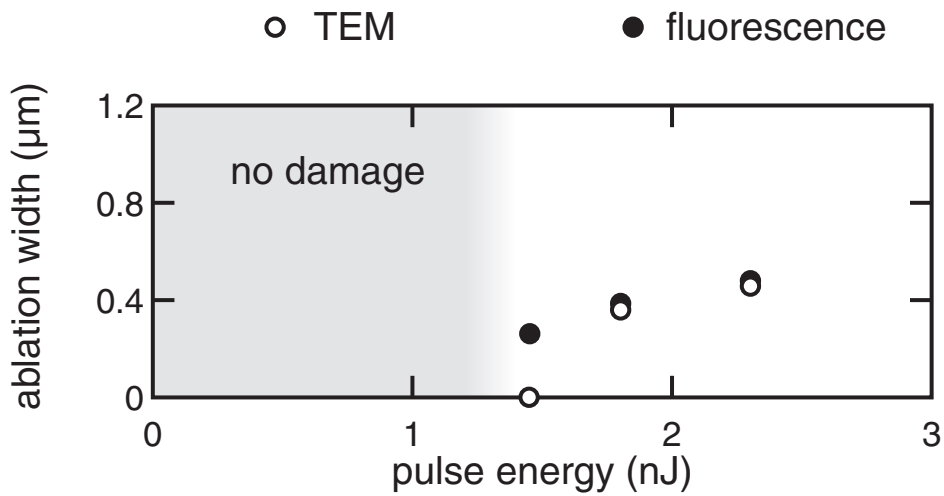
# Subcellular surgery

## three regions of interaction



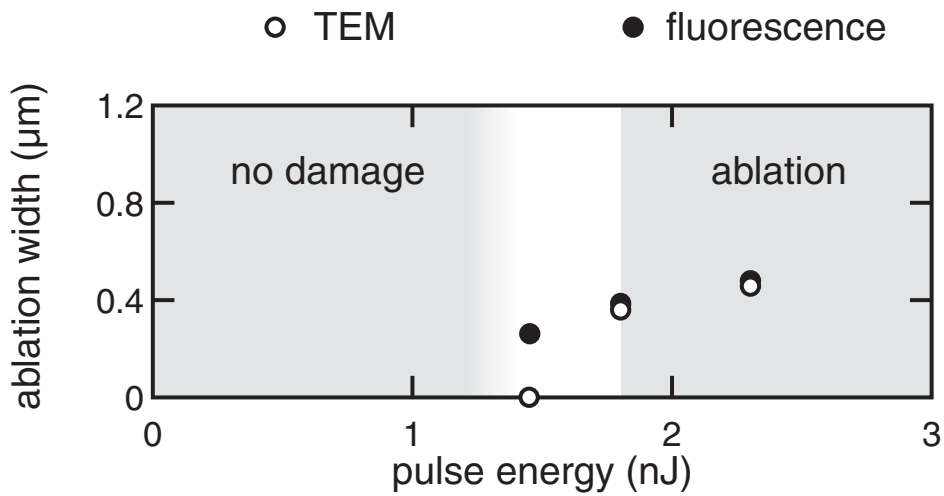
# Subcellular surgery

## three regions of interaction



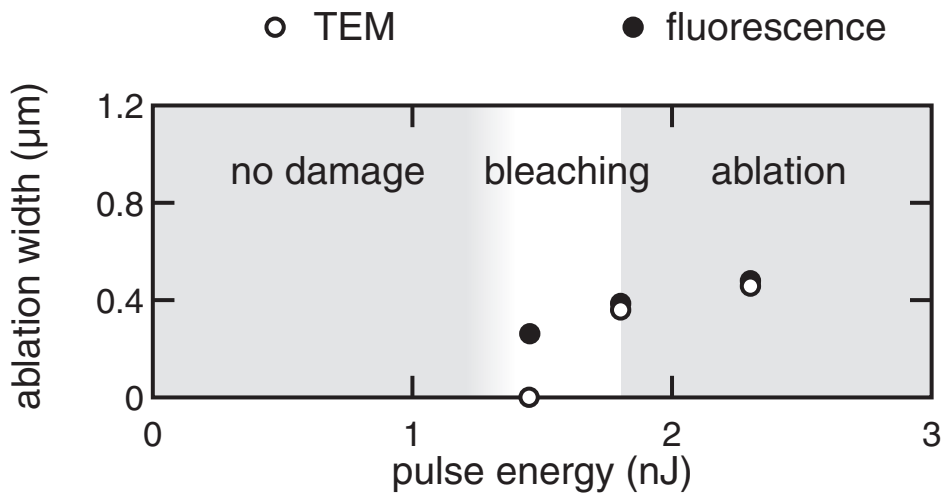
# Subcellular surgery

## three regions of interaction



# Subcellular surgery

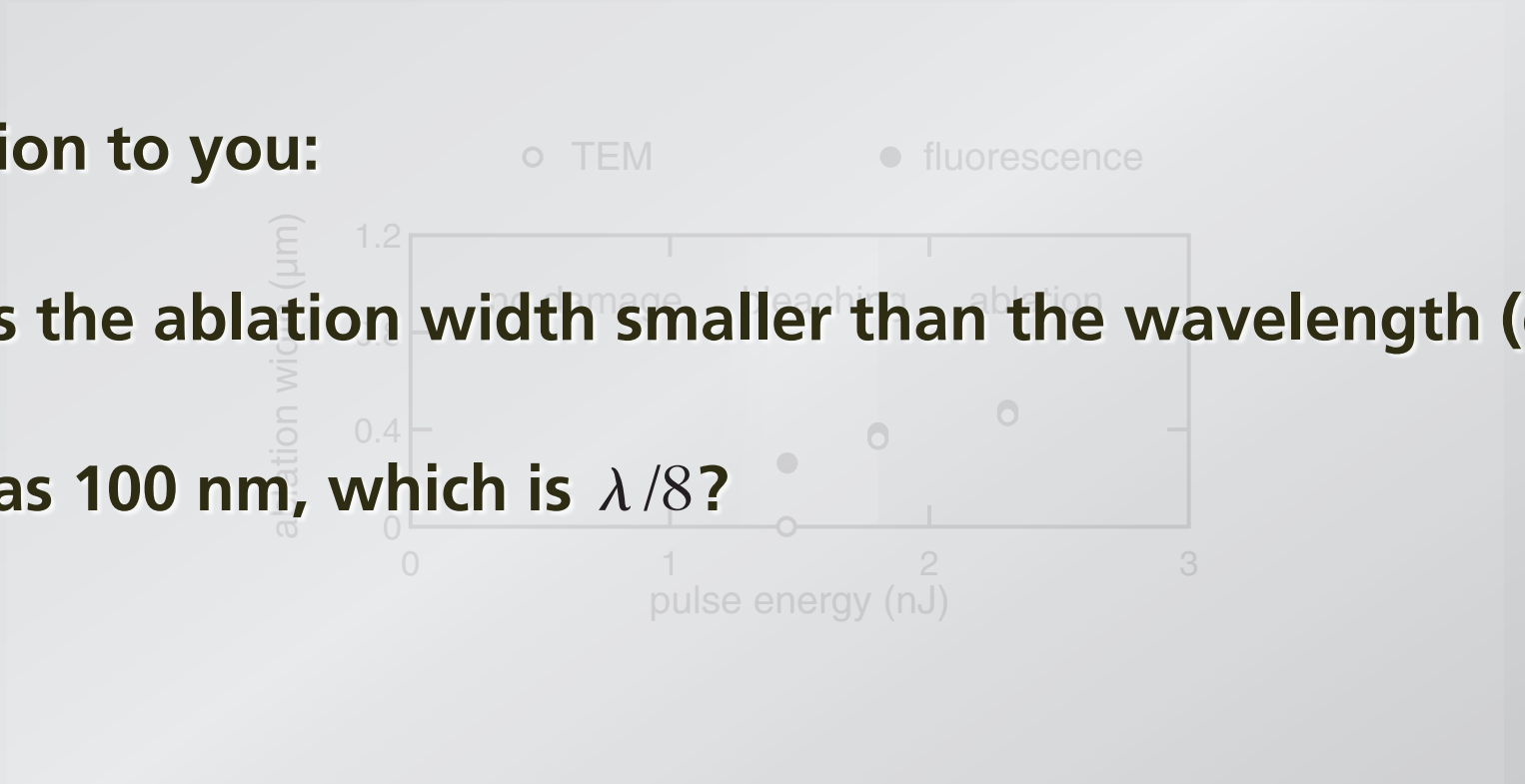
## three regions of interaction




*Opt. Express* **13**, 3690 (2005)

# Subcellular surgery

three regions of interaction




**Question to you:**

**Why is the ablation width smaller than the wavelength (even as small as 100 nm, which is $\lambda/8$?**

*Opt. Express* **13**, 3690 (2005)

# Subcellular surgery

Definitive proof of ablation

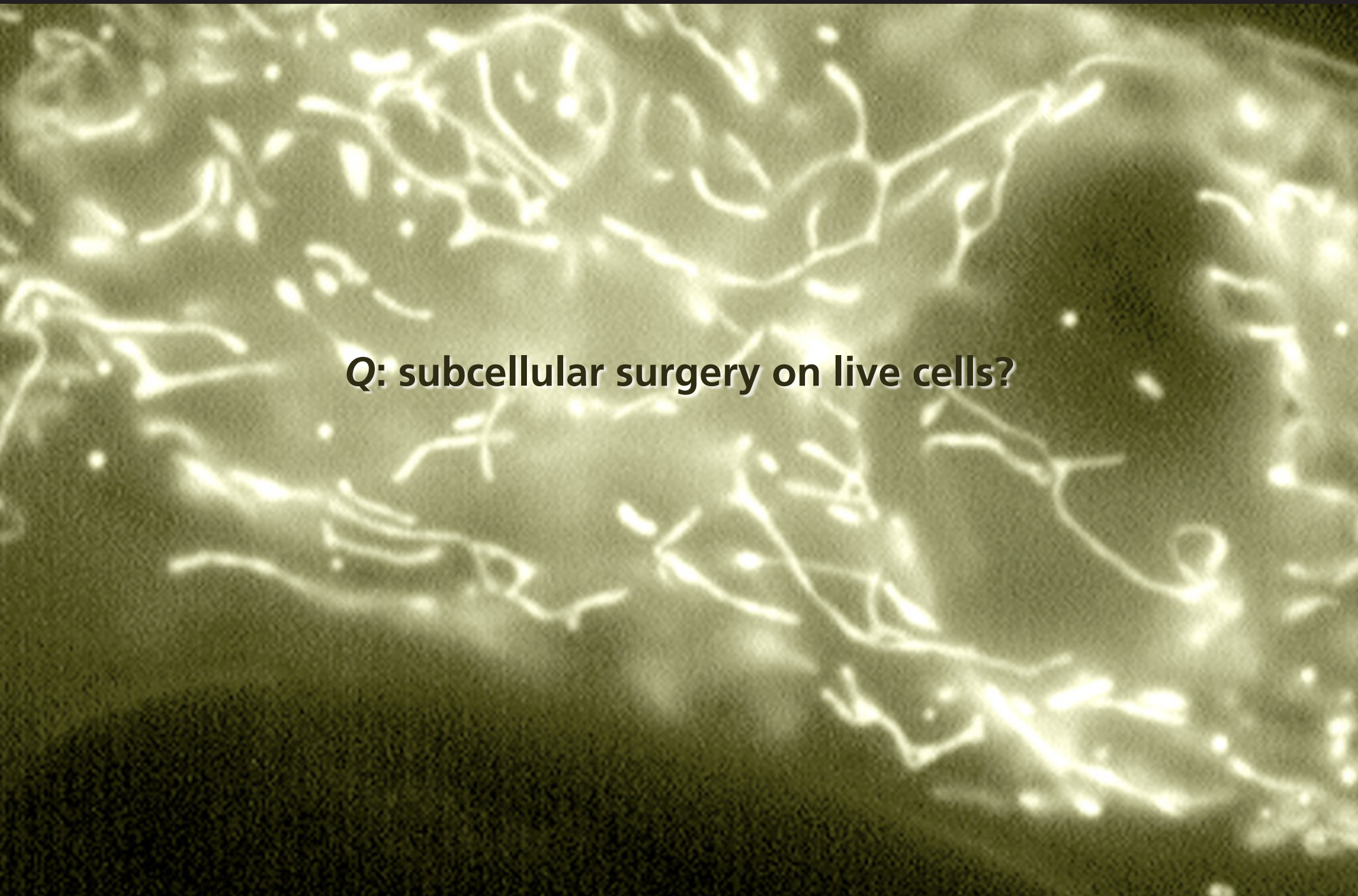
- ablation width as small as 100 nm
- ablation threshold varies slightly
- ablation threshold 20% above bleaching threshold

# Subcellular surgery

Definitive proof of ablation

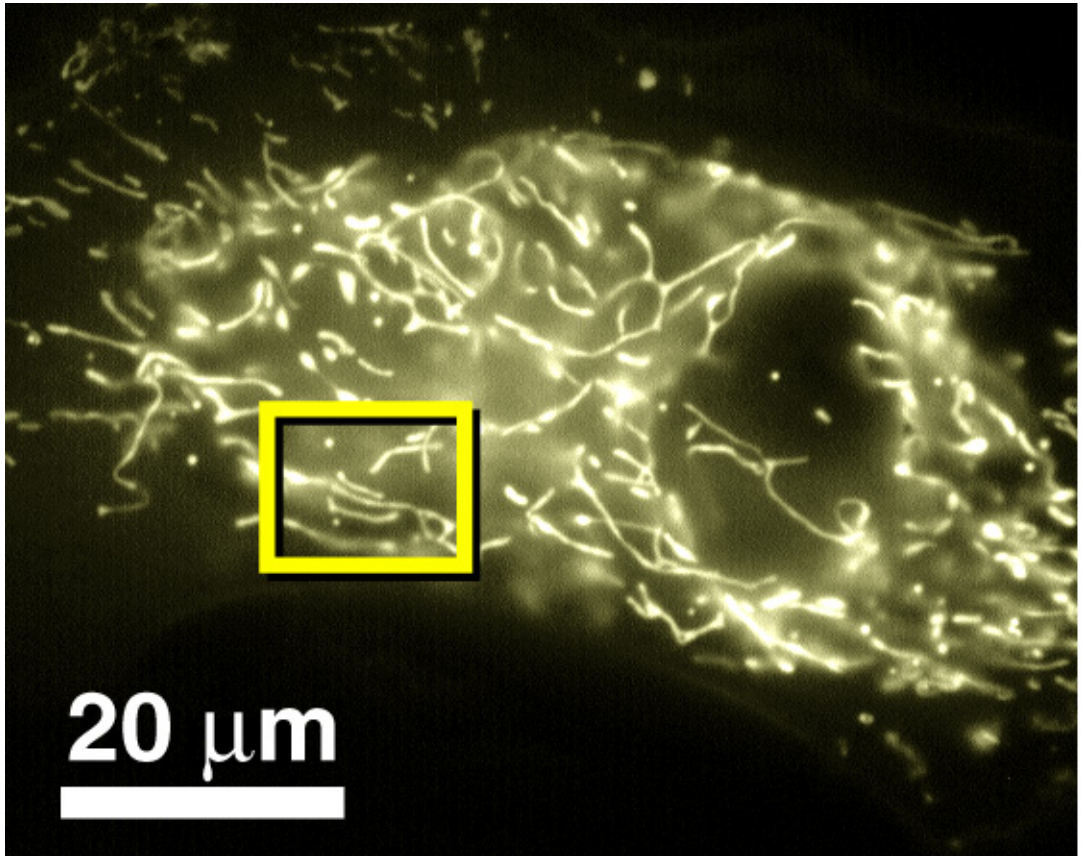
- ablation width as small as 100 nm
- ablation threshold varies slightly
- ablation threshold 20% above bleaching threshold

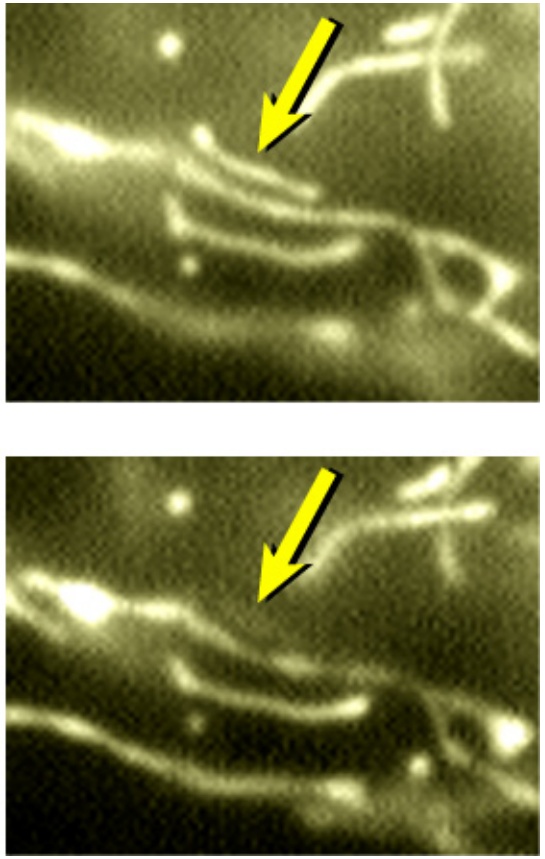
# Subcellular surgery



*Q*: subcellular surgery on live cells?

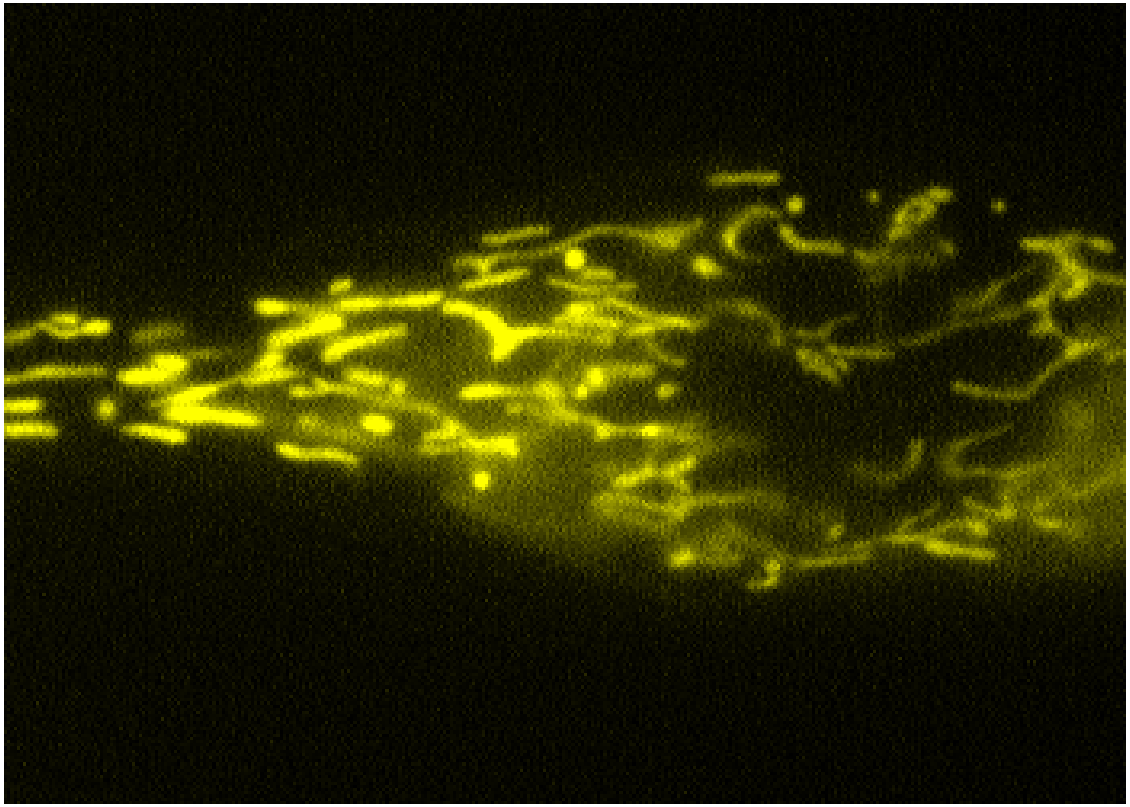
# Subcellular surgery



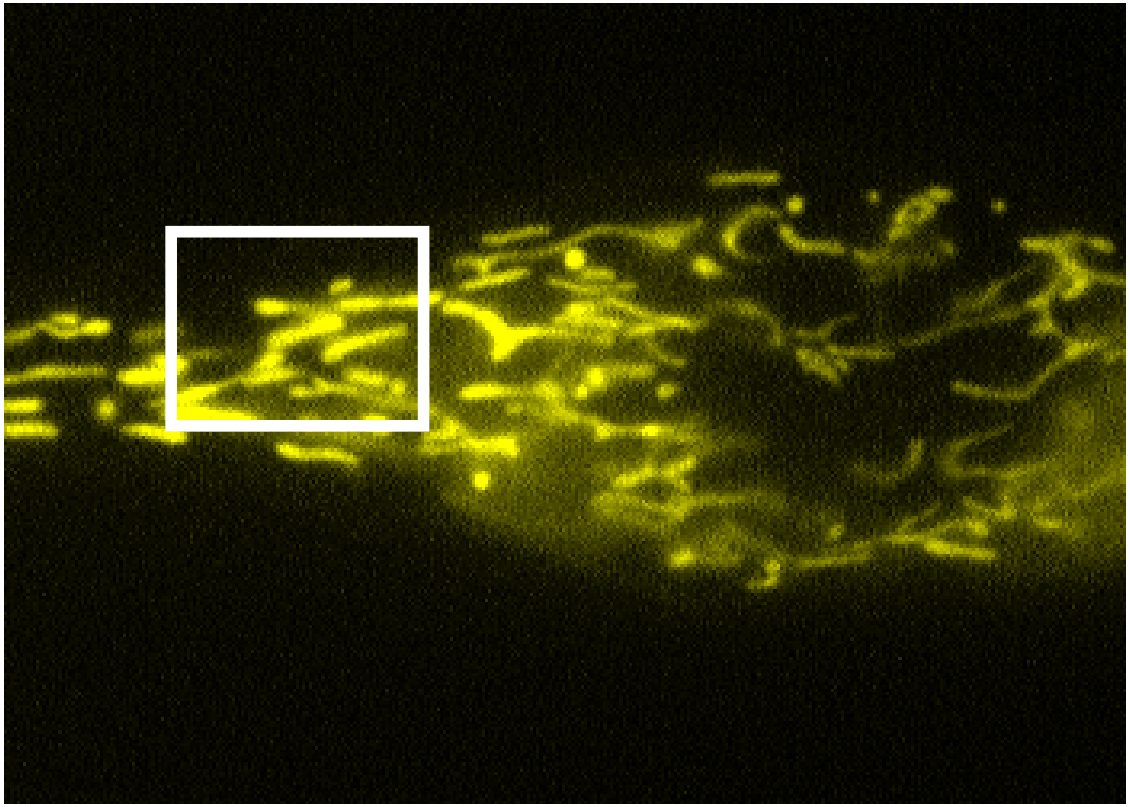




2 nJ/pulse
1 s exposure

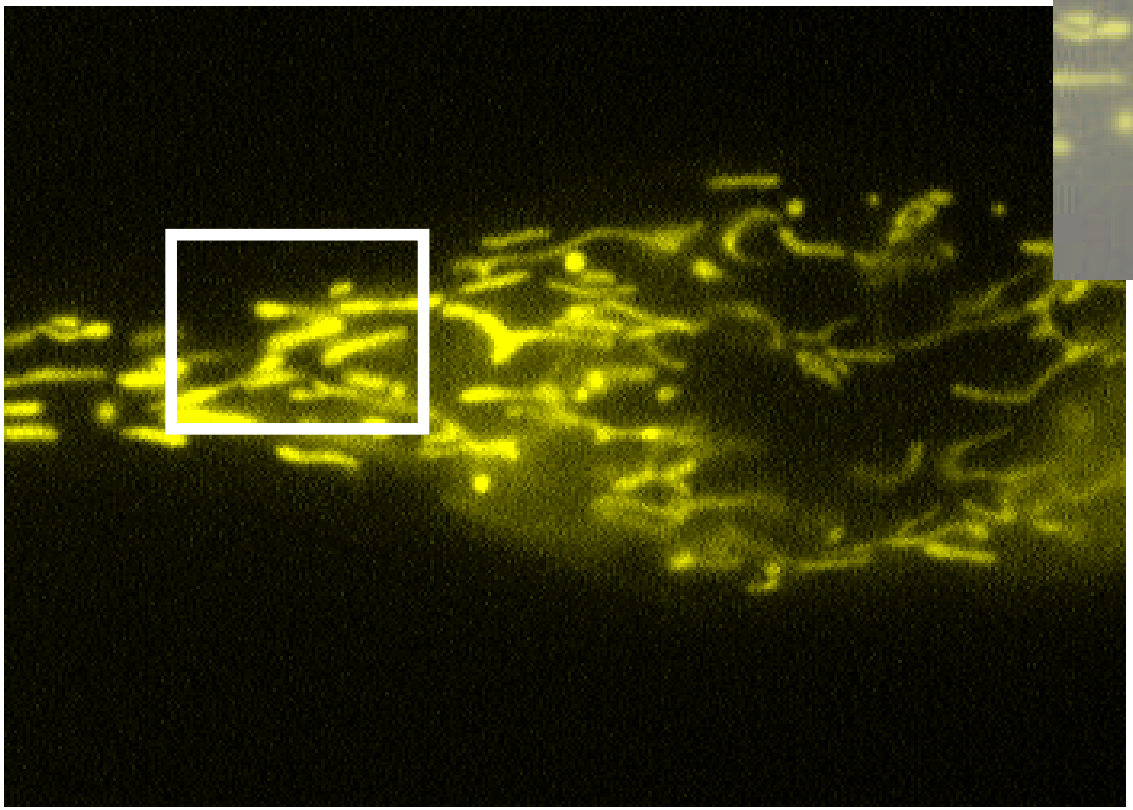
# Subcellular surgery

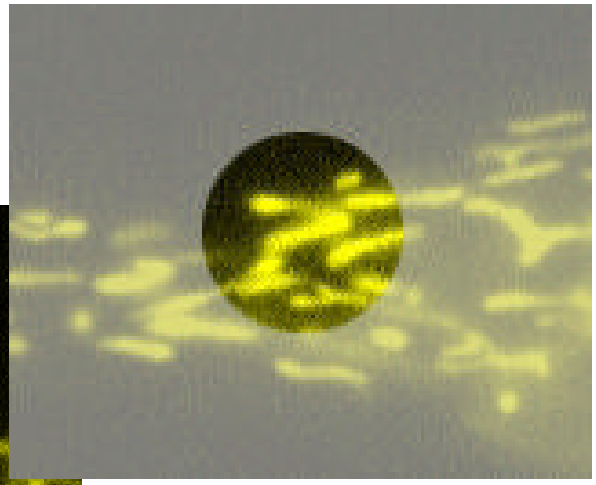


# Subcellular surgery



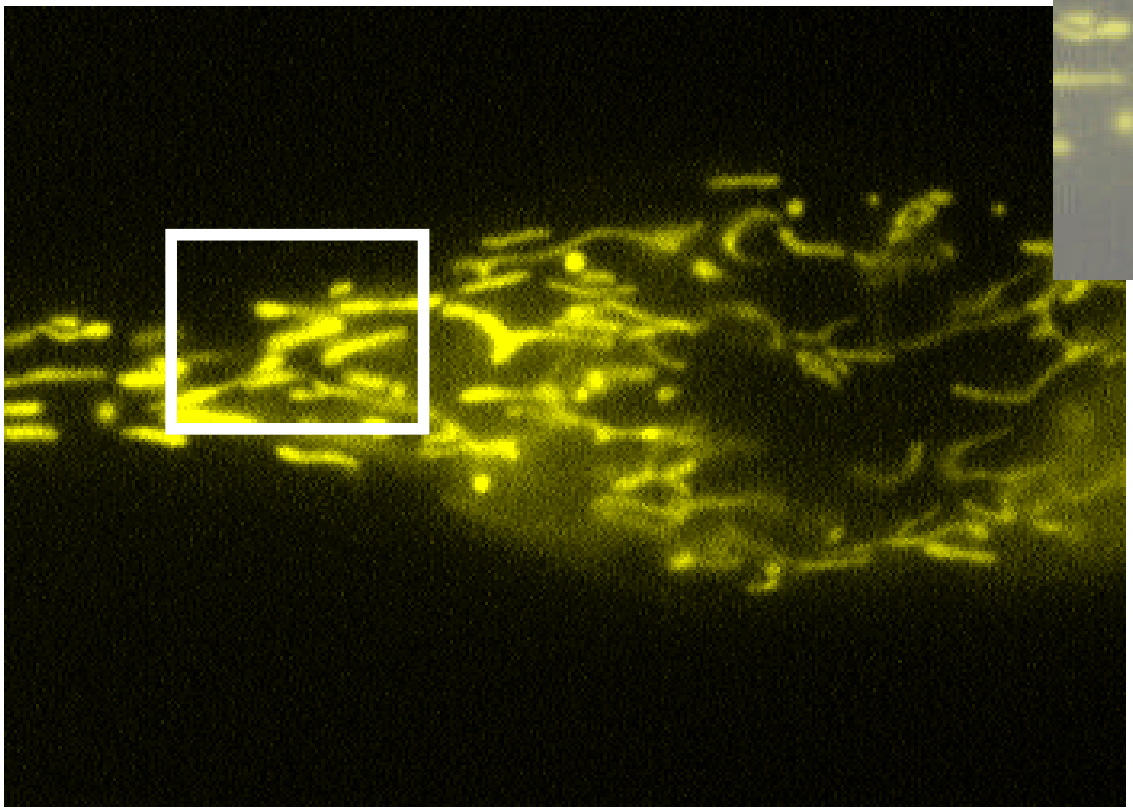
# Subcellular surgery

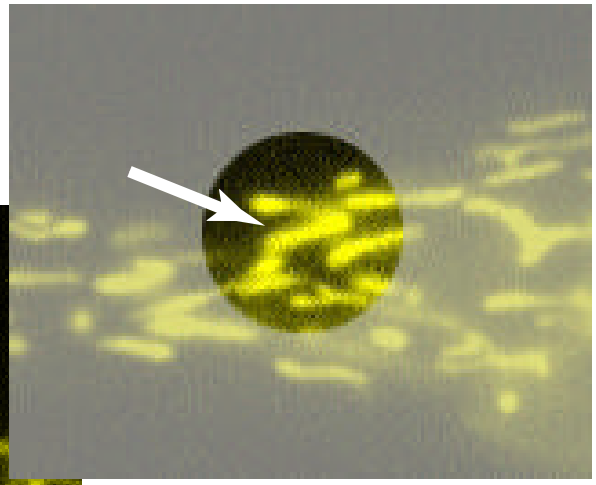




before

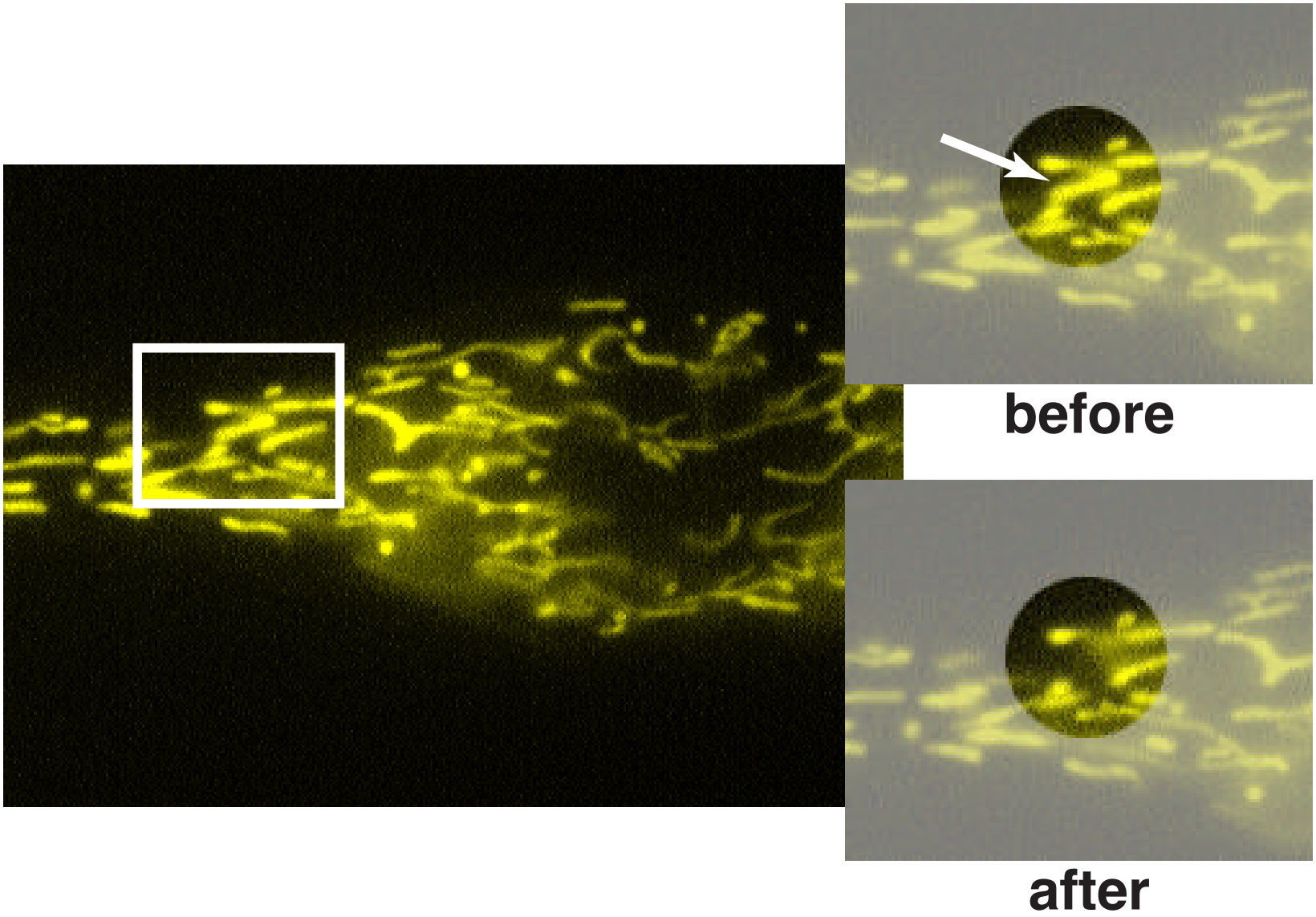
# Subcellular surgery



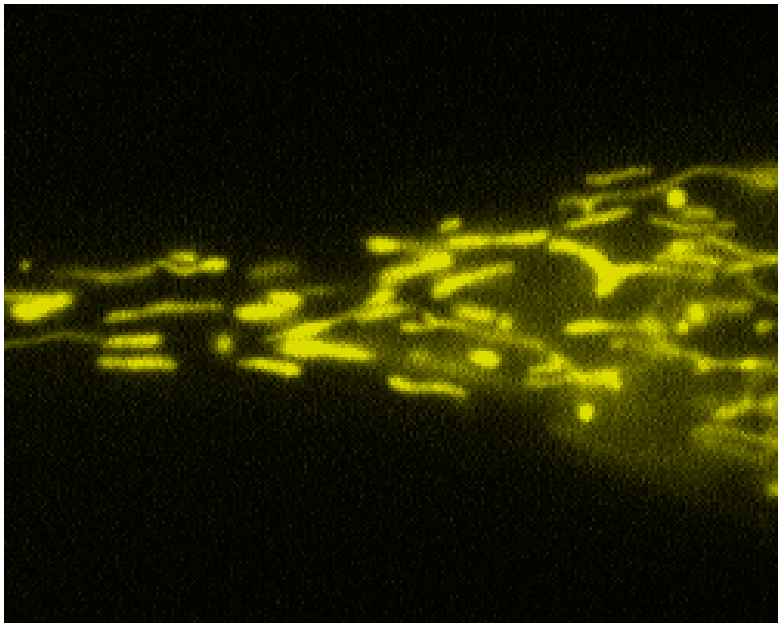


before

# Subcellular surgery

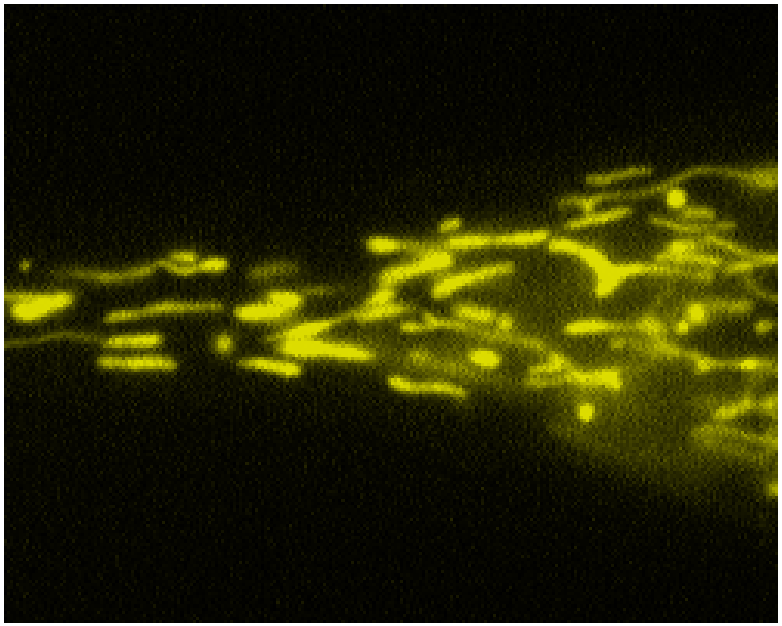


# Subcellular surgery

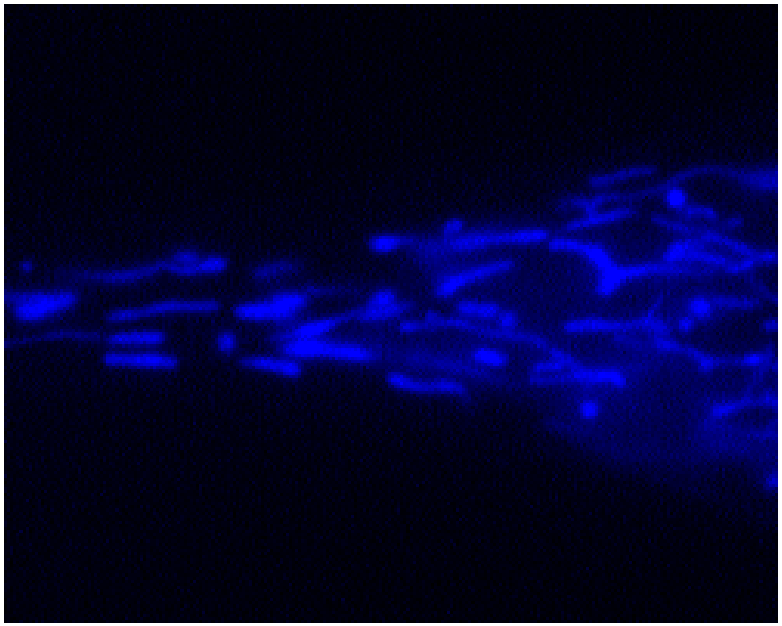


before

# Subcellular surgery

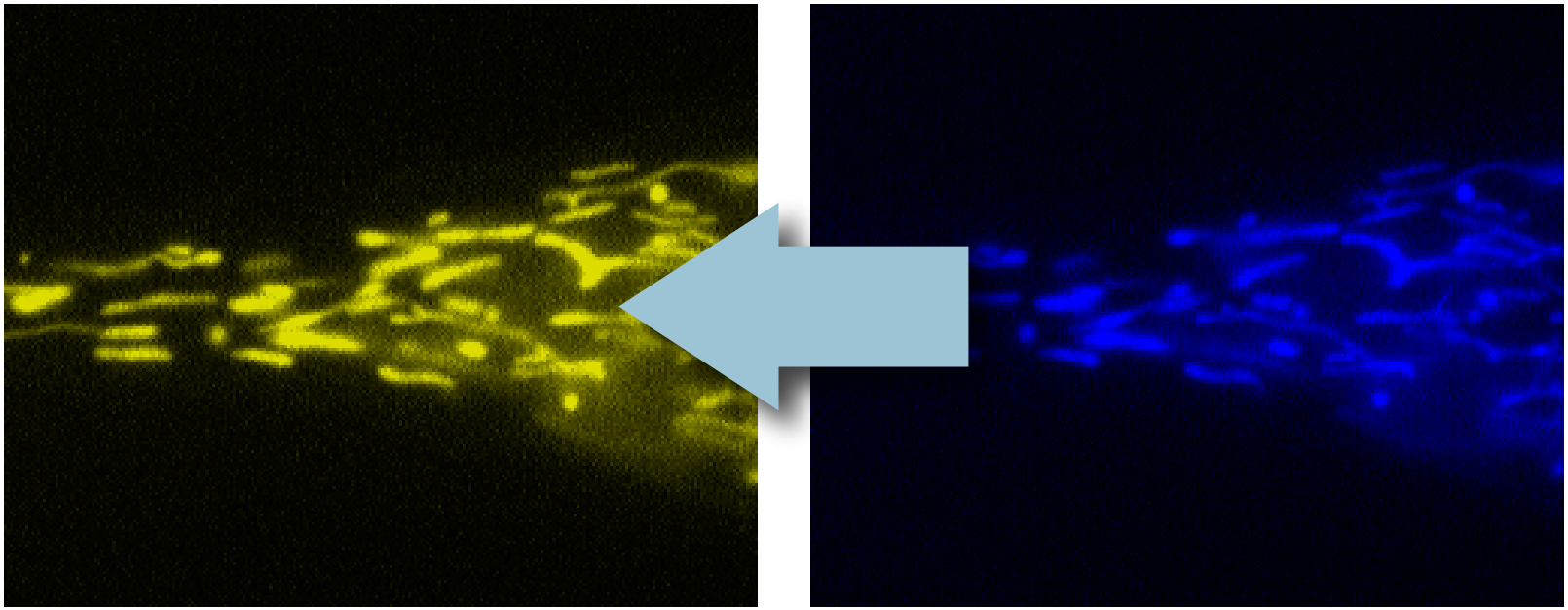


before



after

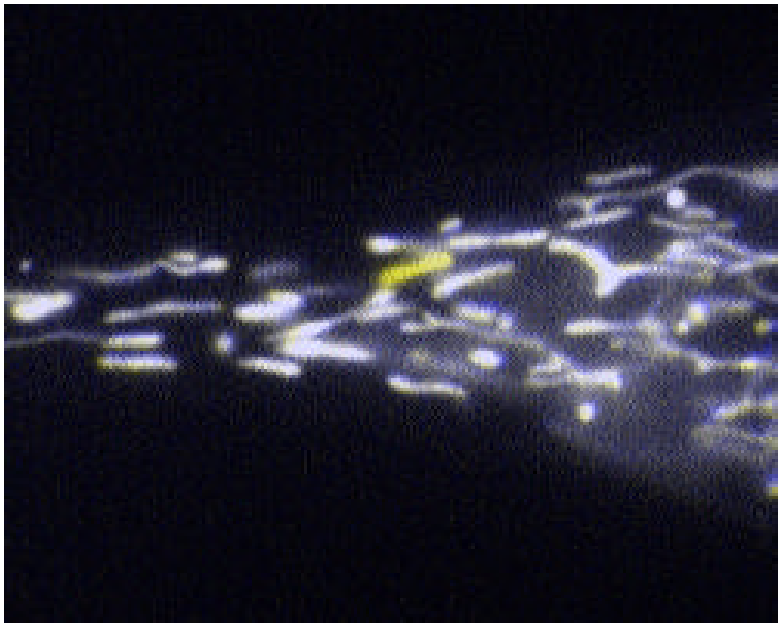
# Subcellular surgery



before

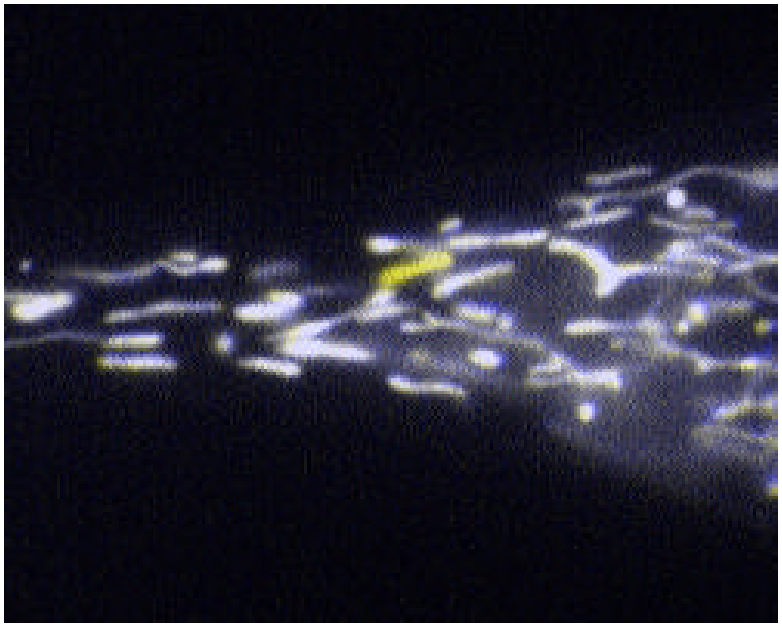
after

# Subcellular surgery

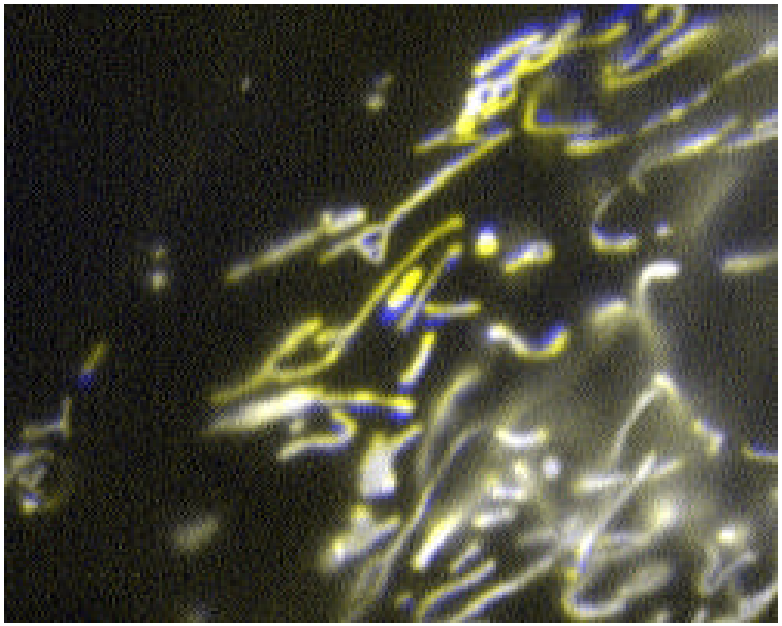


2 nJ

# Subcellular surgery



2 nJ

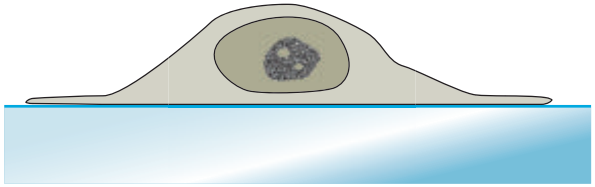


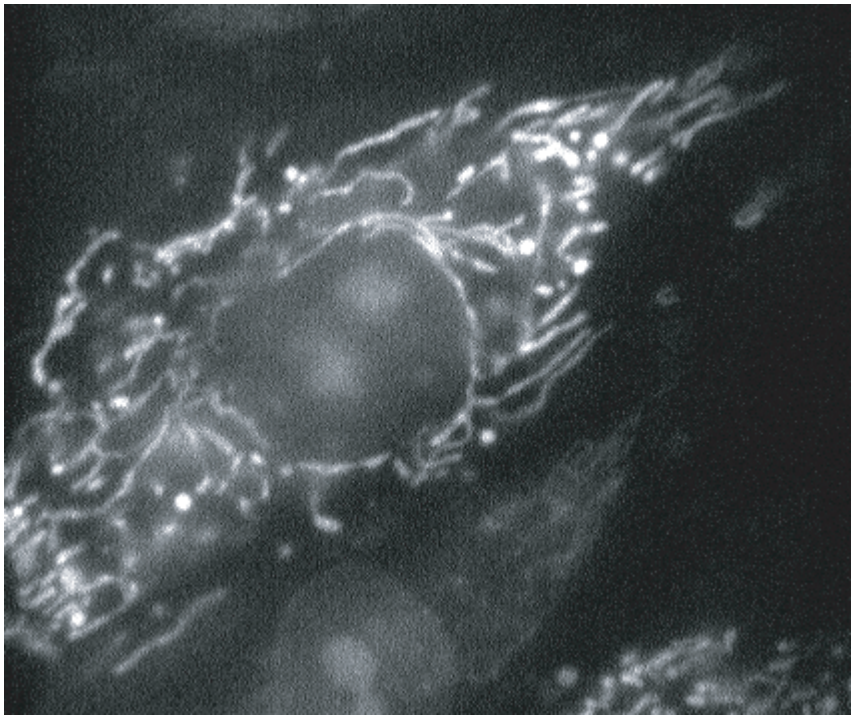
4 nJ

*Med. Chem. Biosyst.* **2**, 17 (2005)

# Subcellular surgery

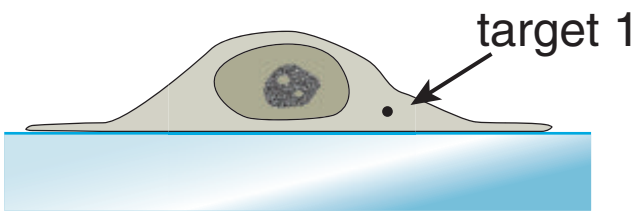
## ethydium bromide test

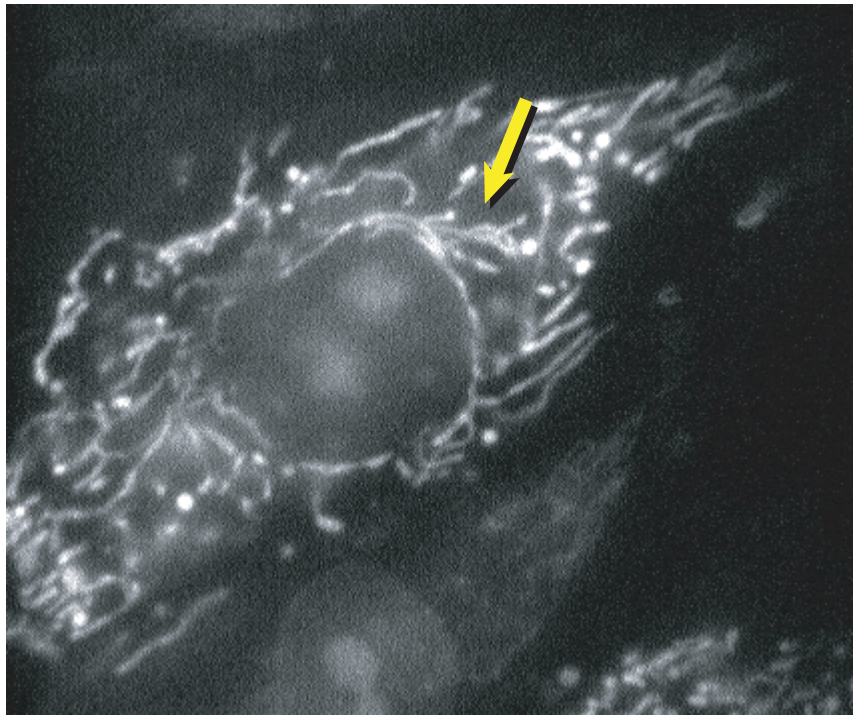




# Subcellular surgery

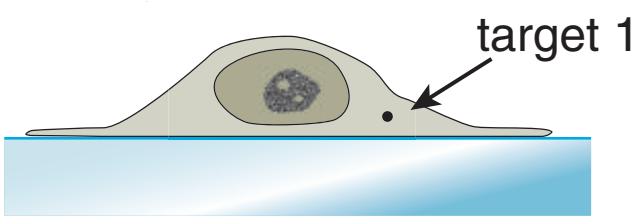
## ethydium bromide test

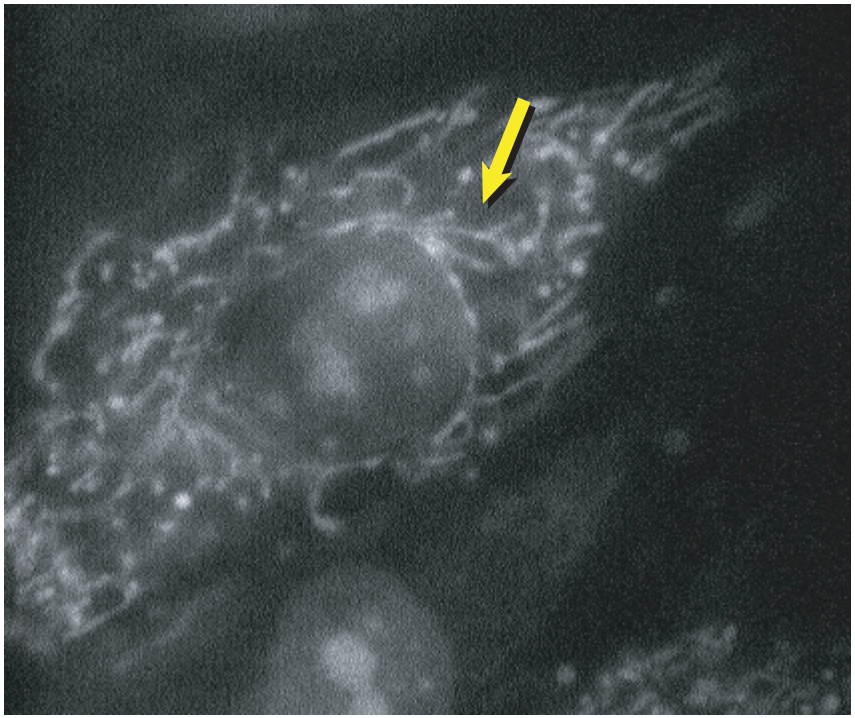


# Subcellular surgery

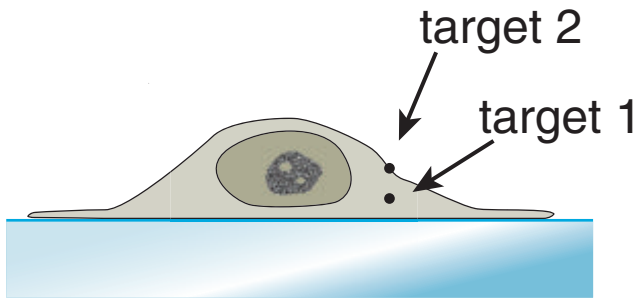
## ethydium bromide test

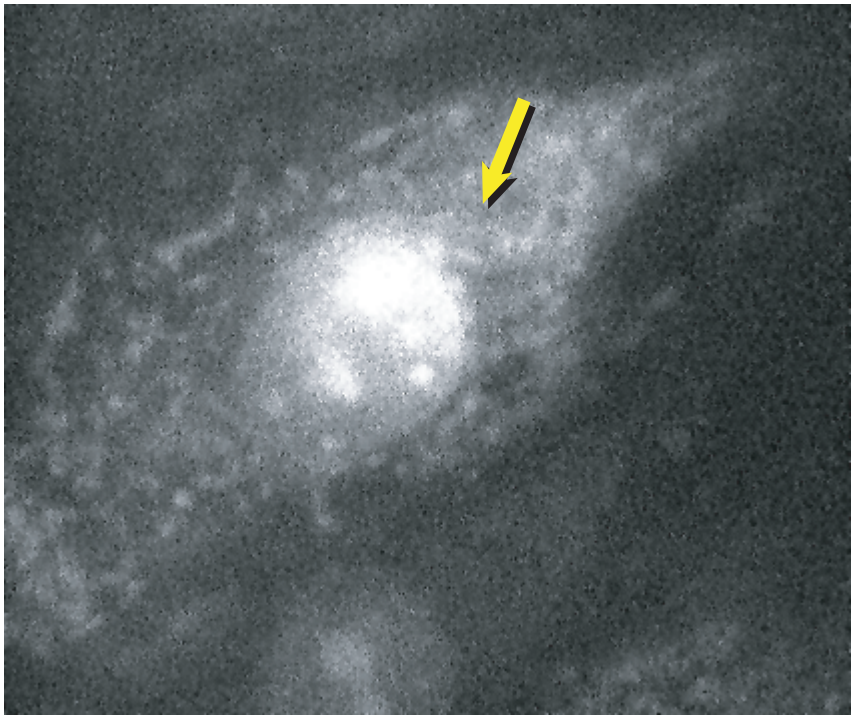


# Subcellular surgery

## ethydium bromide test



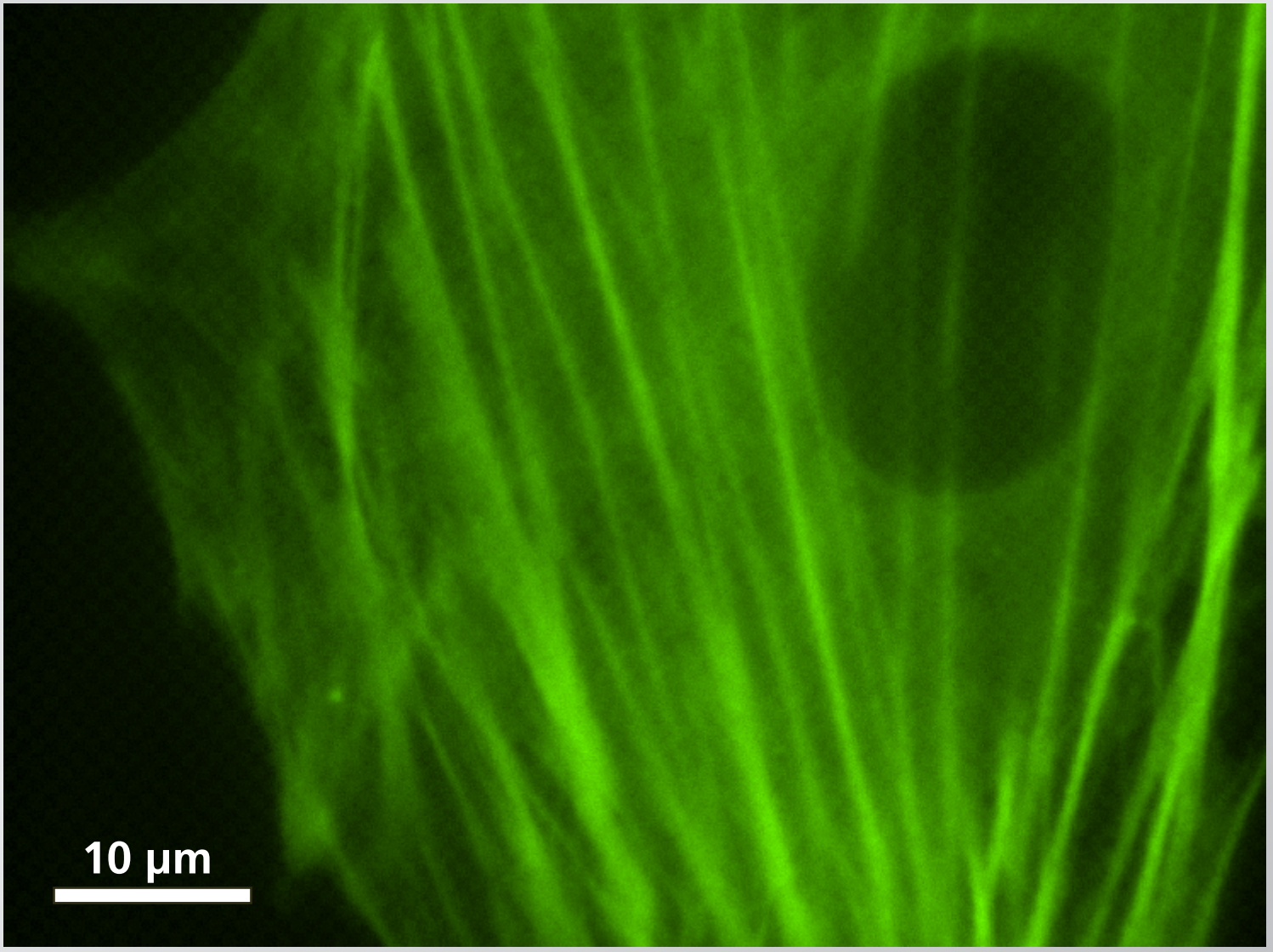



# Subcellular surgery

*Q*: can we probe the dynamics of the cytoskeleton?

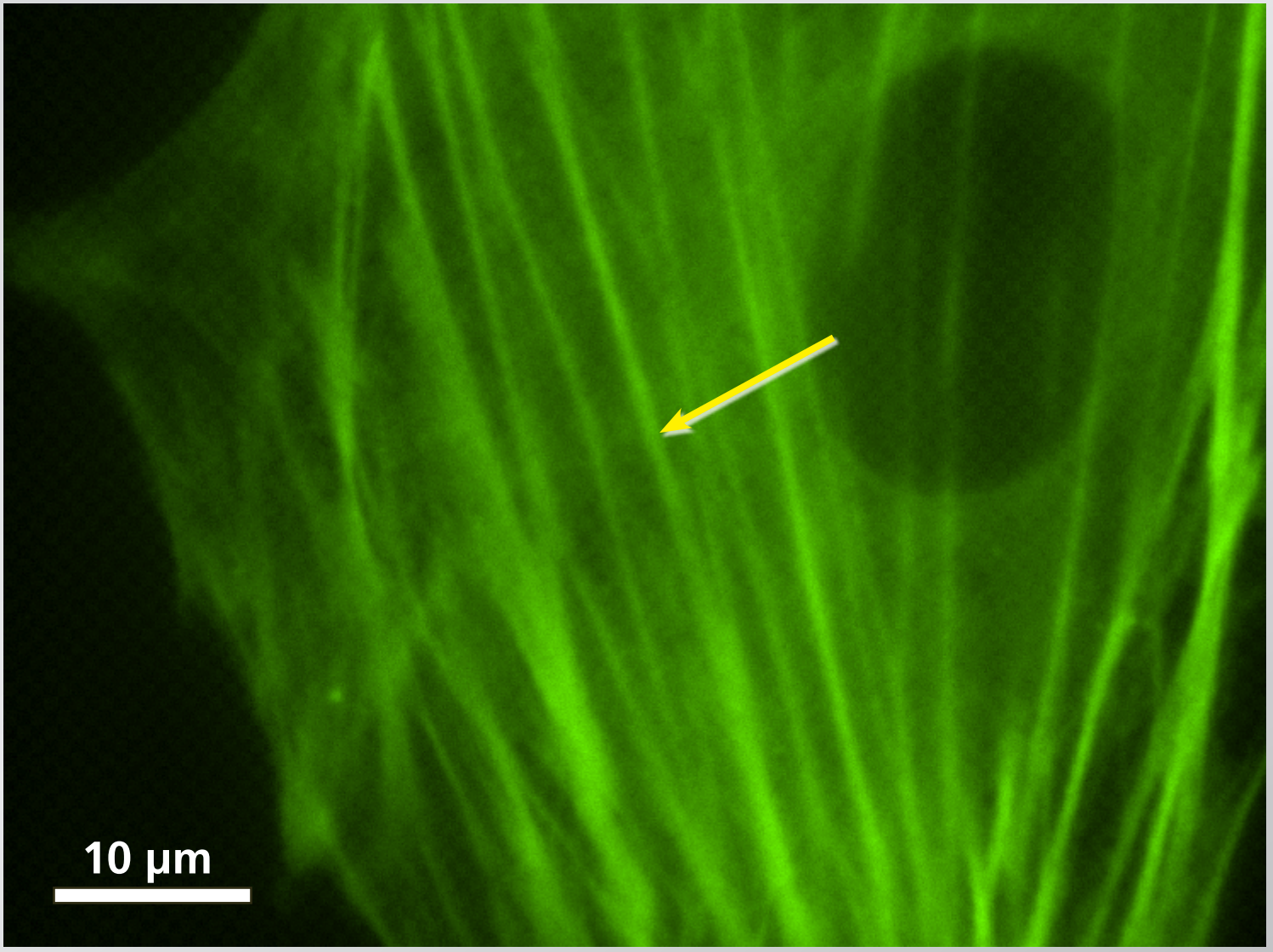
# Subcellular surgery

## YFP-labeled actin fiber network of a live cell



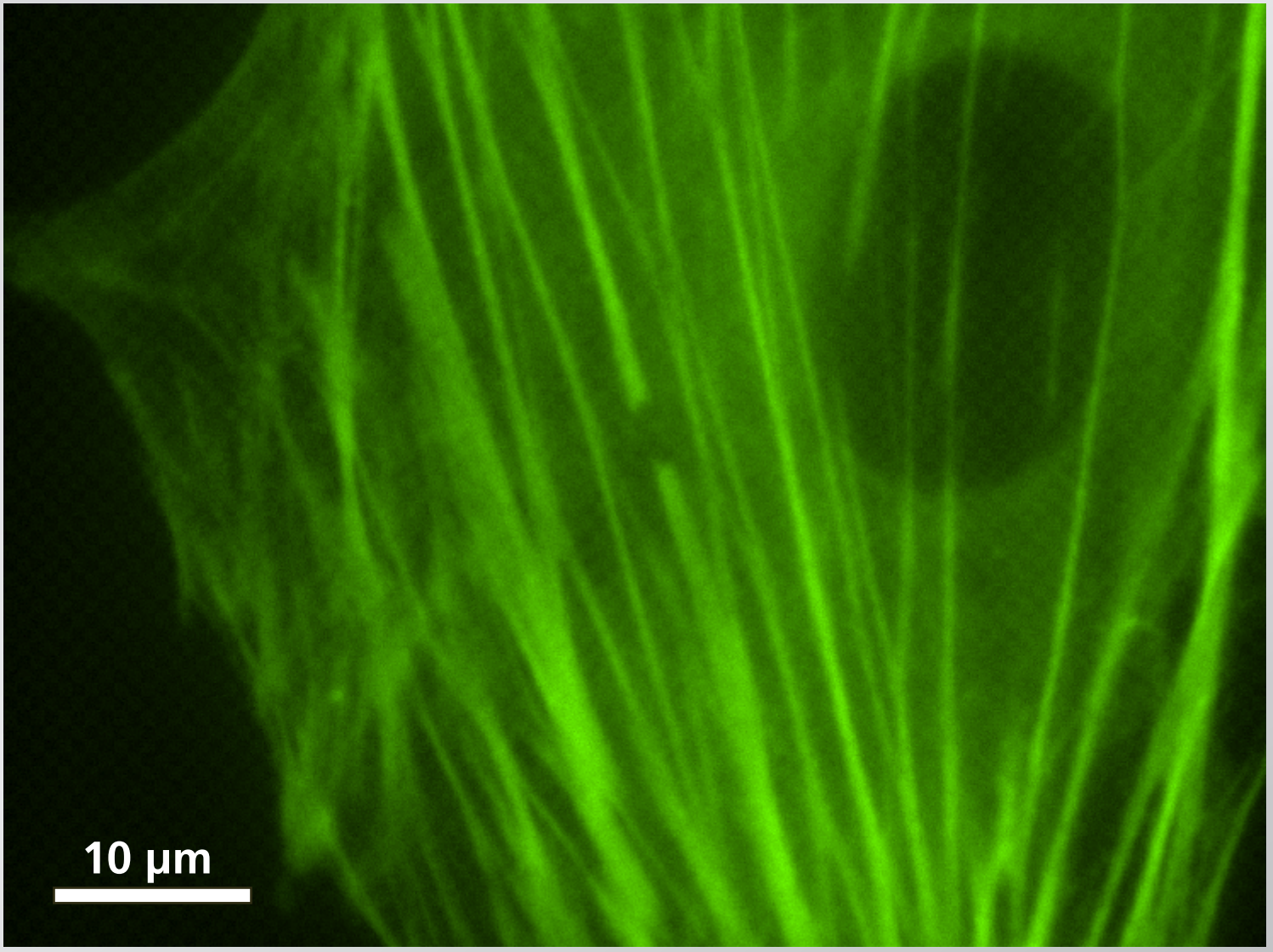
# Subcellular surgery

cut a single fiber bundle



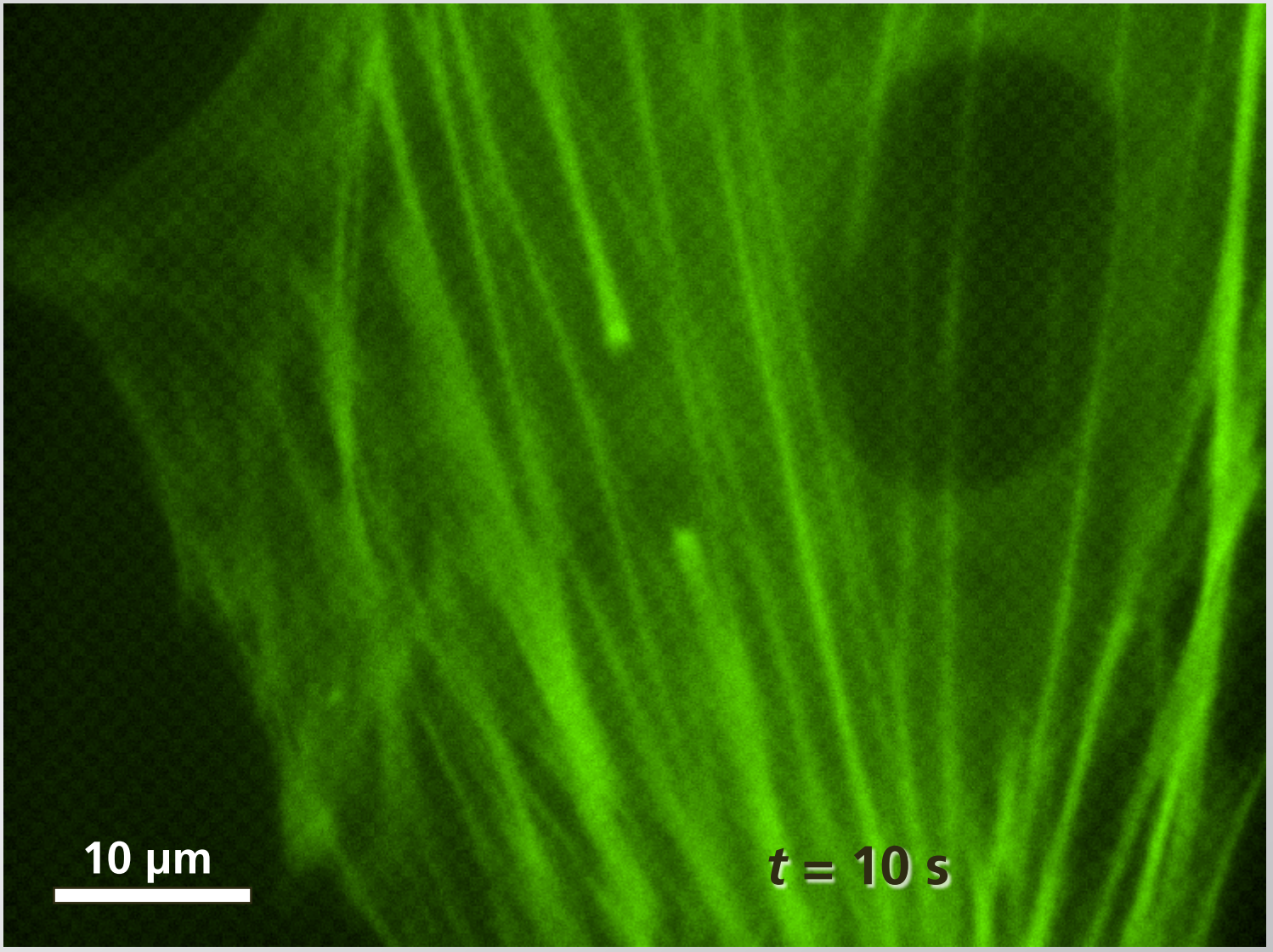
# Subcellular surgery

cut a single fiber bundle



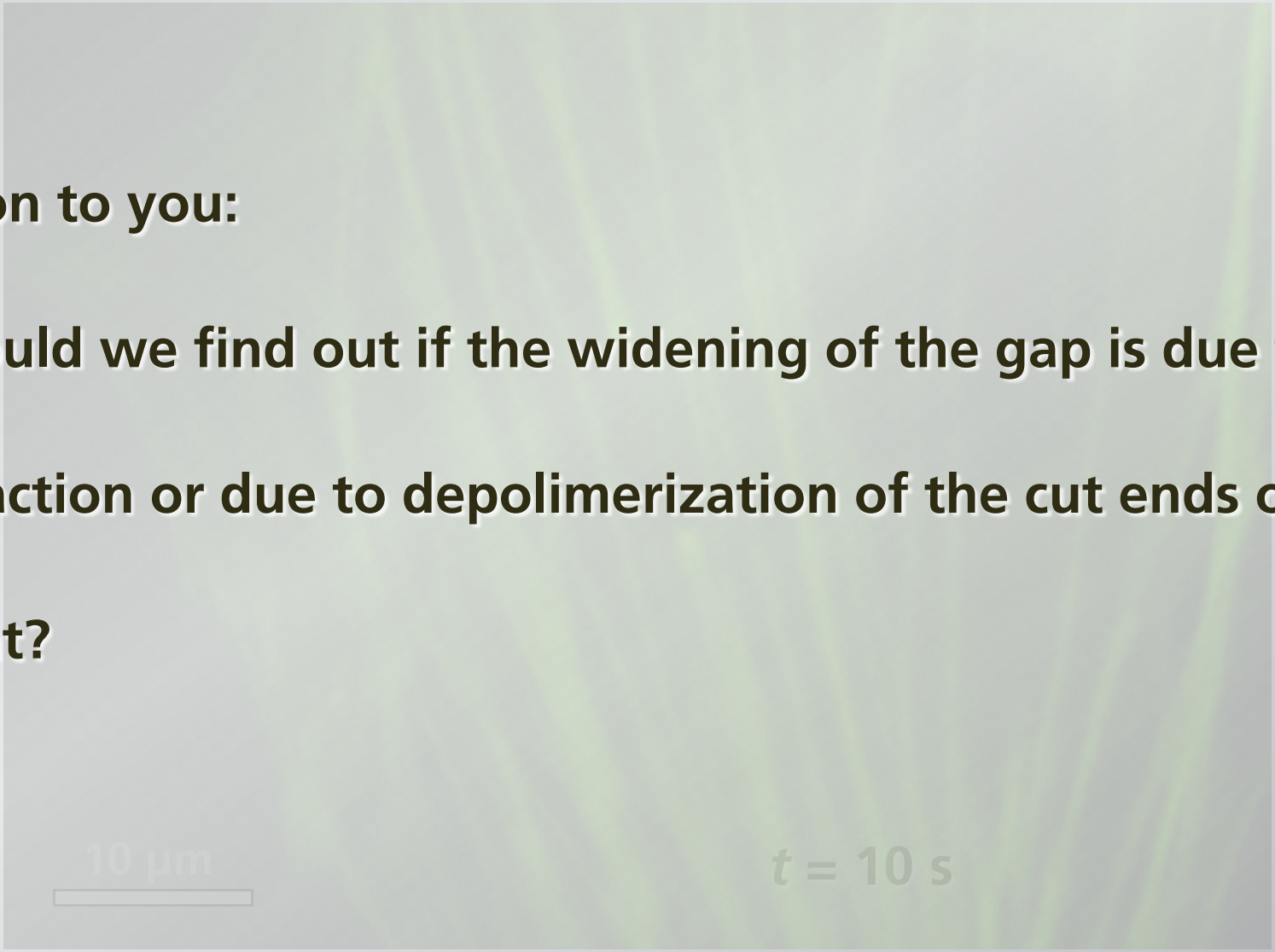
# Subcellular surgery

gap widens with time



# Subcellular surgery

gap widens with time

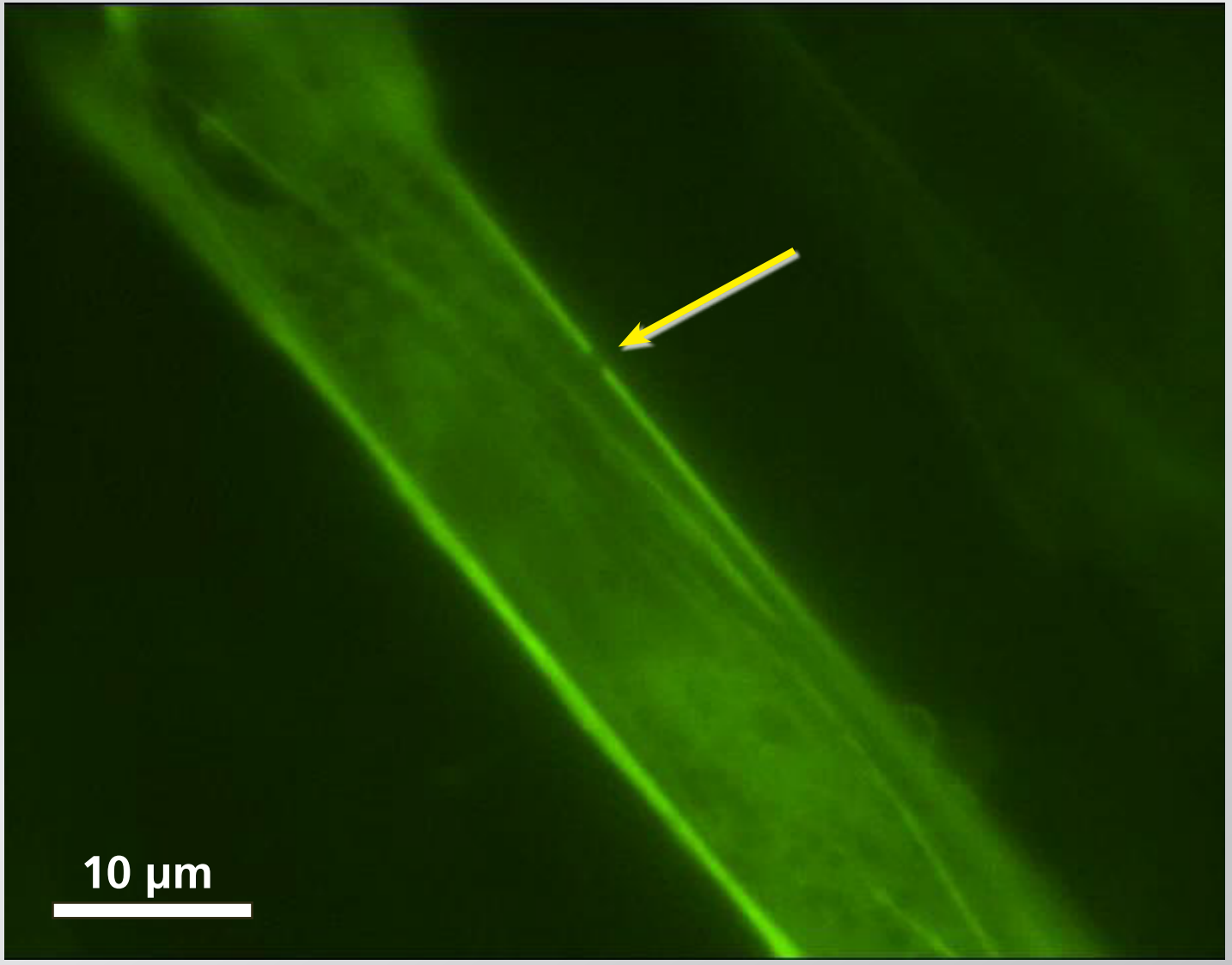



**Question to you:**

**How could we find out if the widening of the gap is due to elastic retraction or due to depolimerization of the cut ends of the filament?**

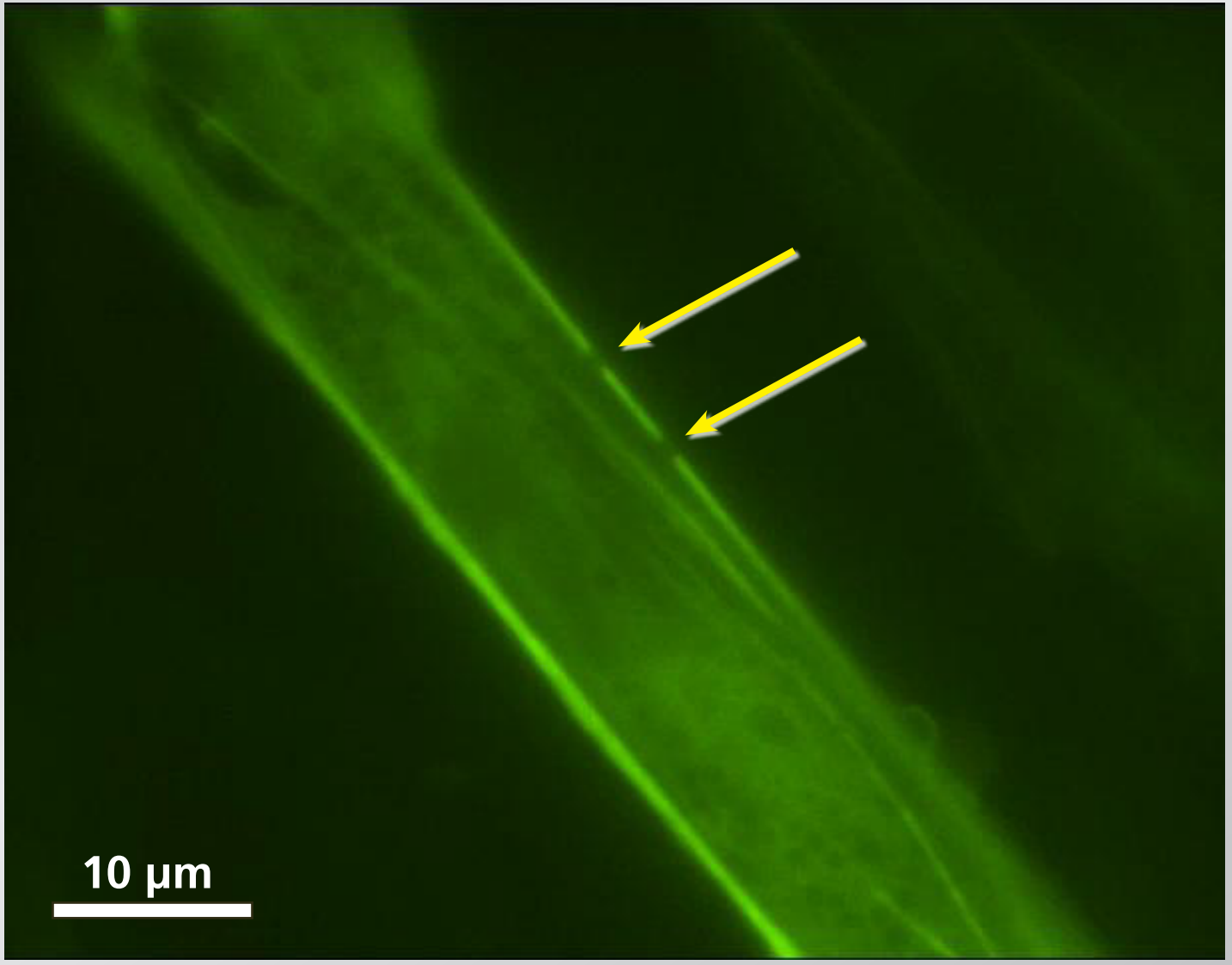
# Subcellular surgery

retraction or depolymerization?



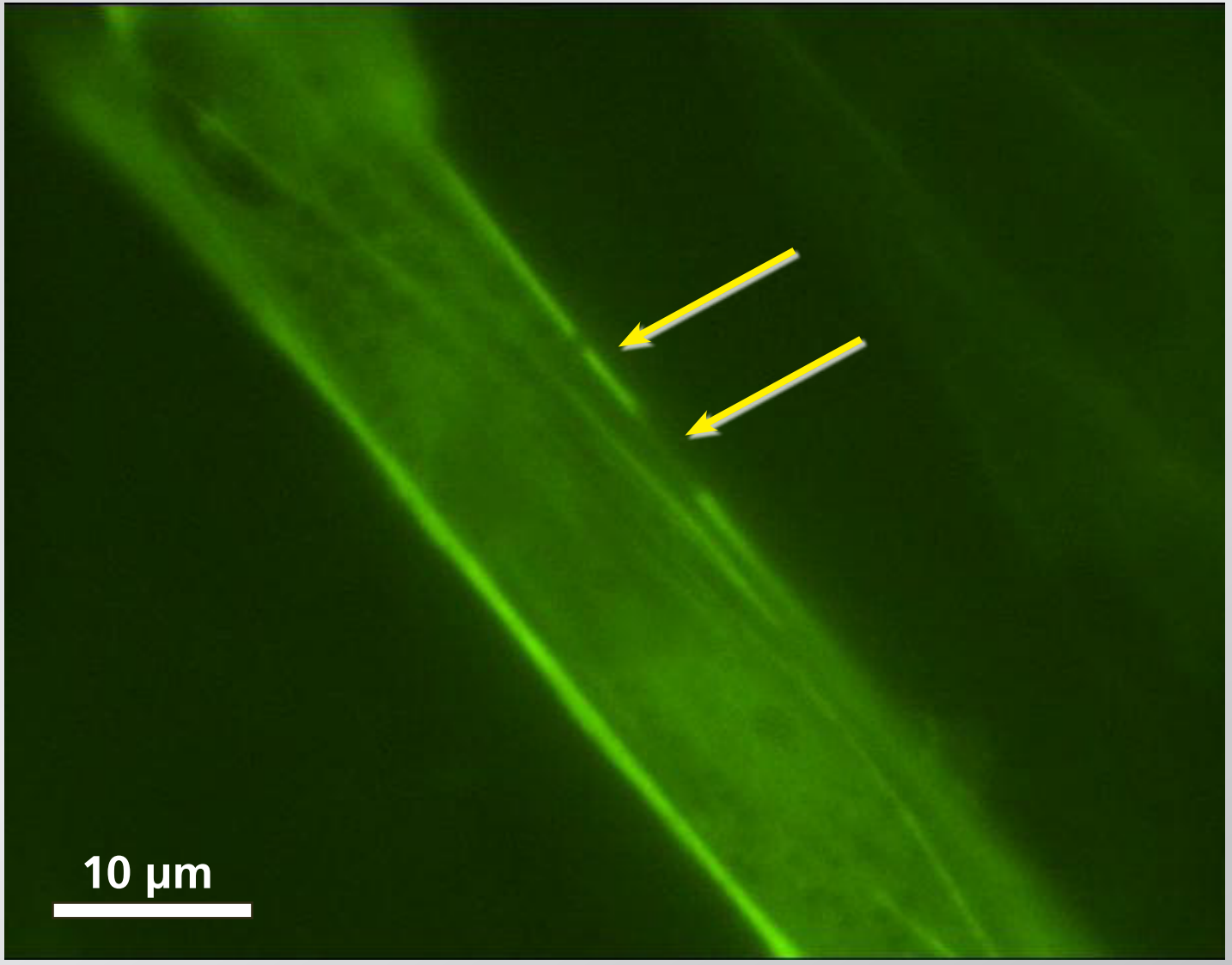
# Subcellular surgery

retraction or depolymerization?



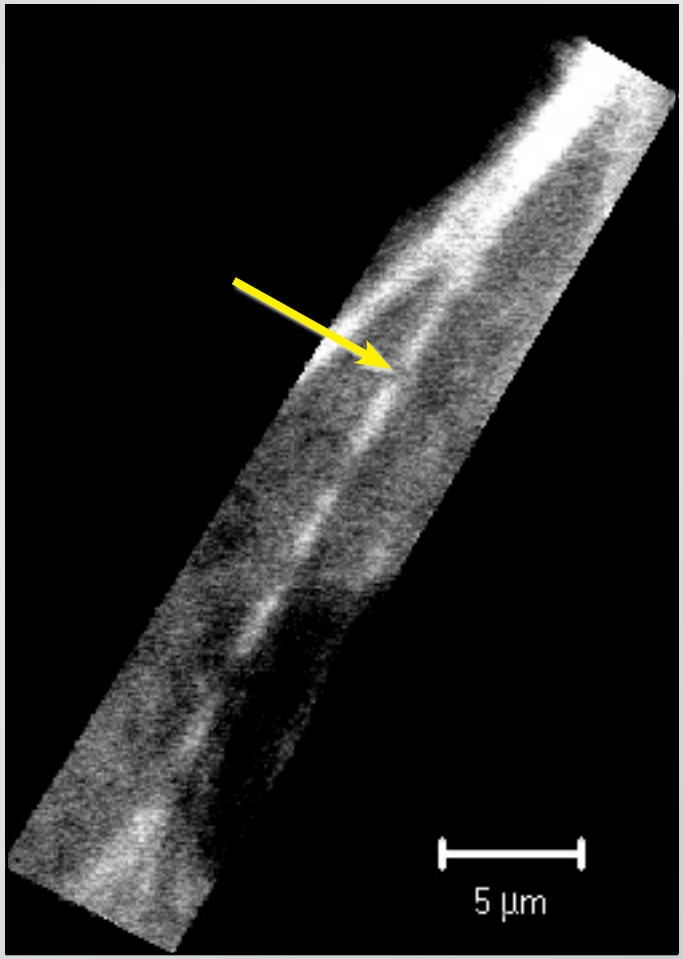
# Subcellular surgery

retraction!



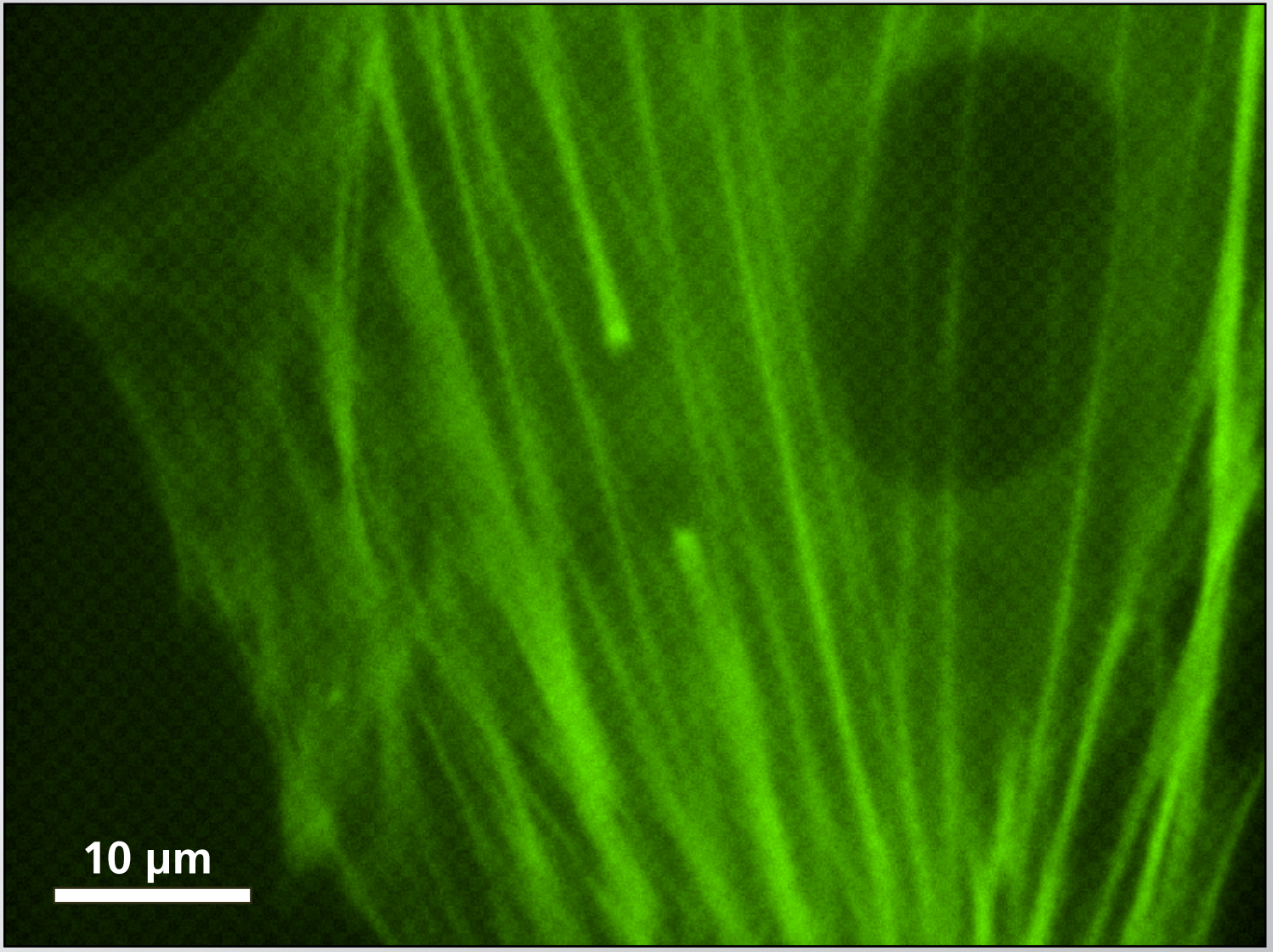
# Subcellular surgery

**retraction!**

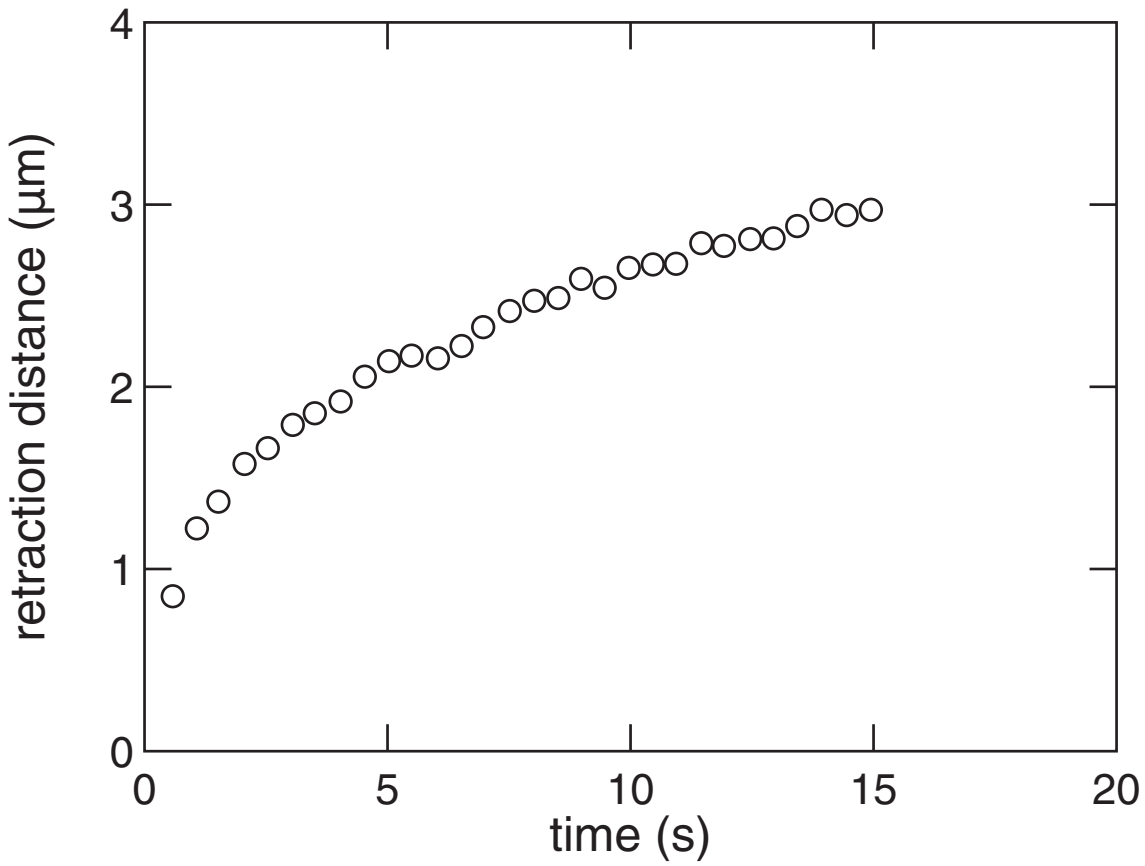


# Subcellular surgery

**dynamics provides information on *in vivo* mechanics**

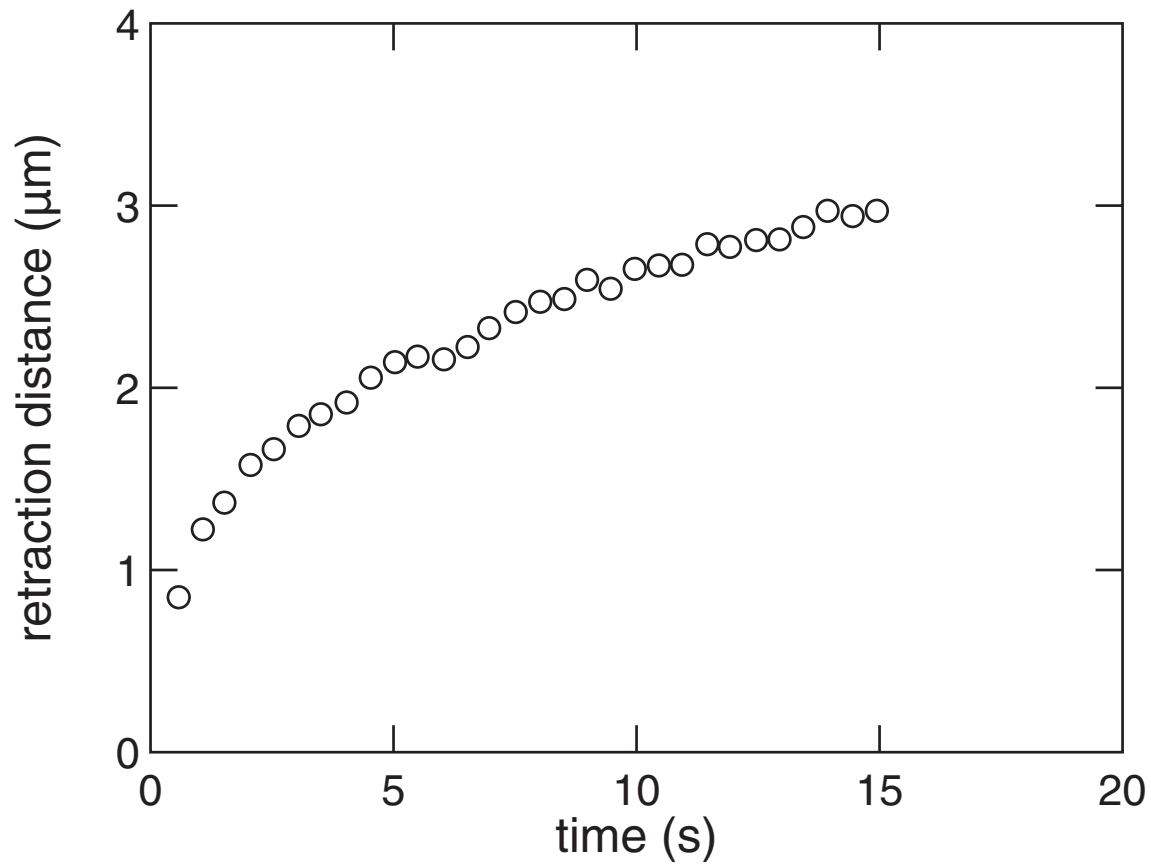


# Subcellular surgery



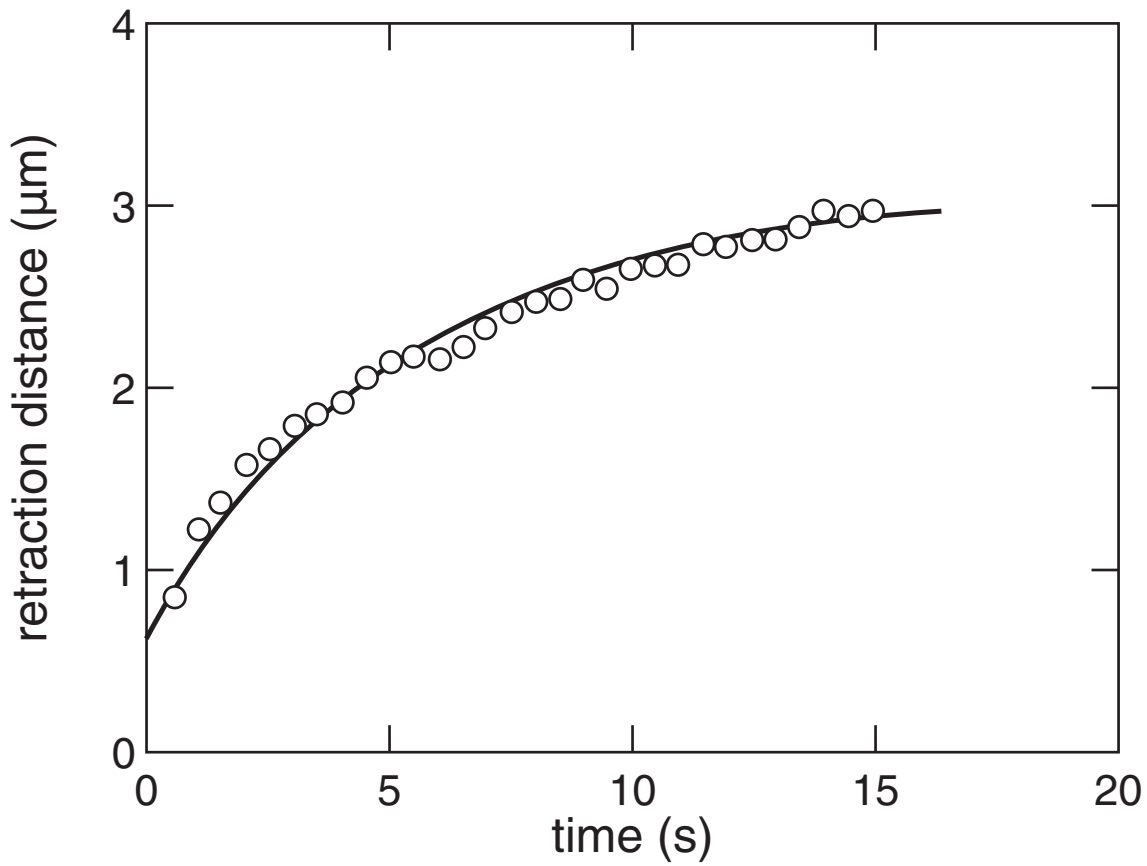
# Subcellular surgery

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



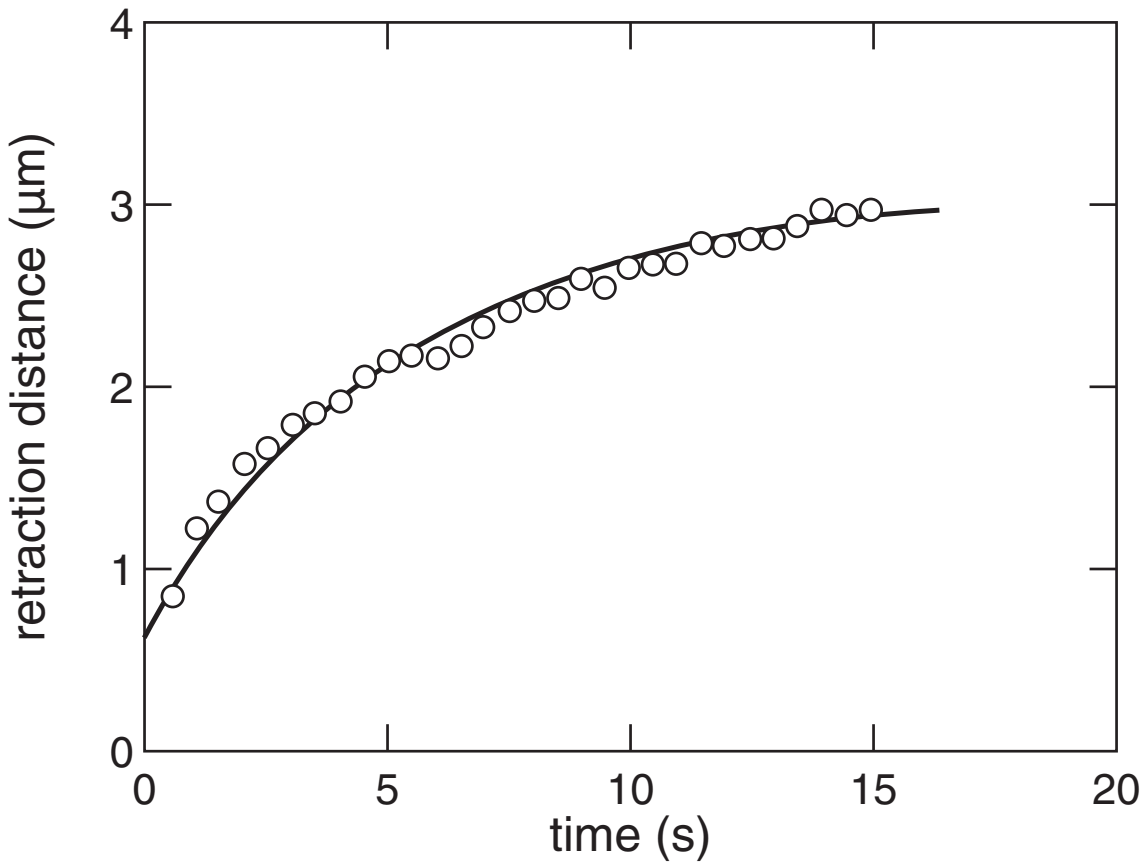
# Subcellular surgery

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



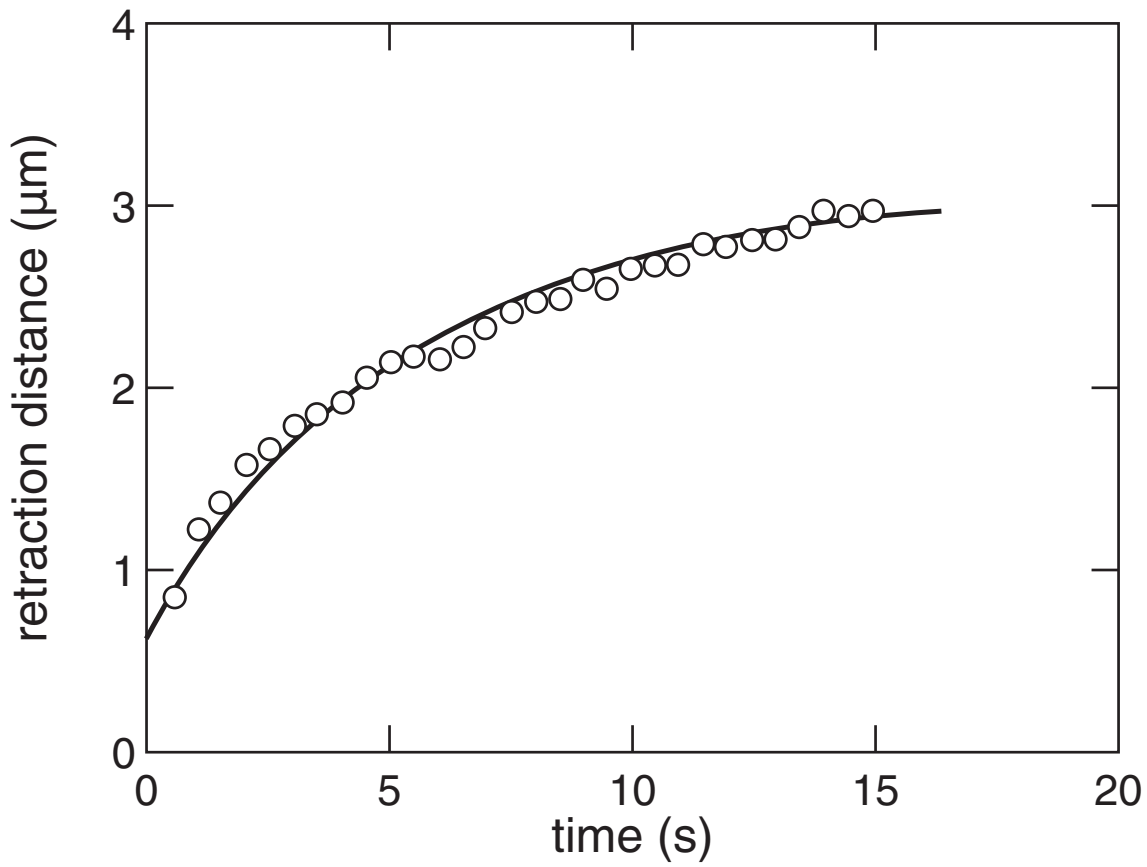
# Subcellular surgery

$L_o$ and $\tau$ independent of fiber width!



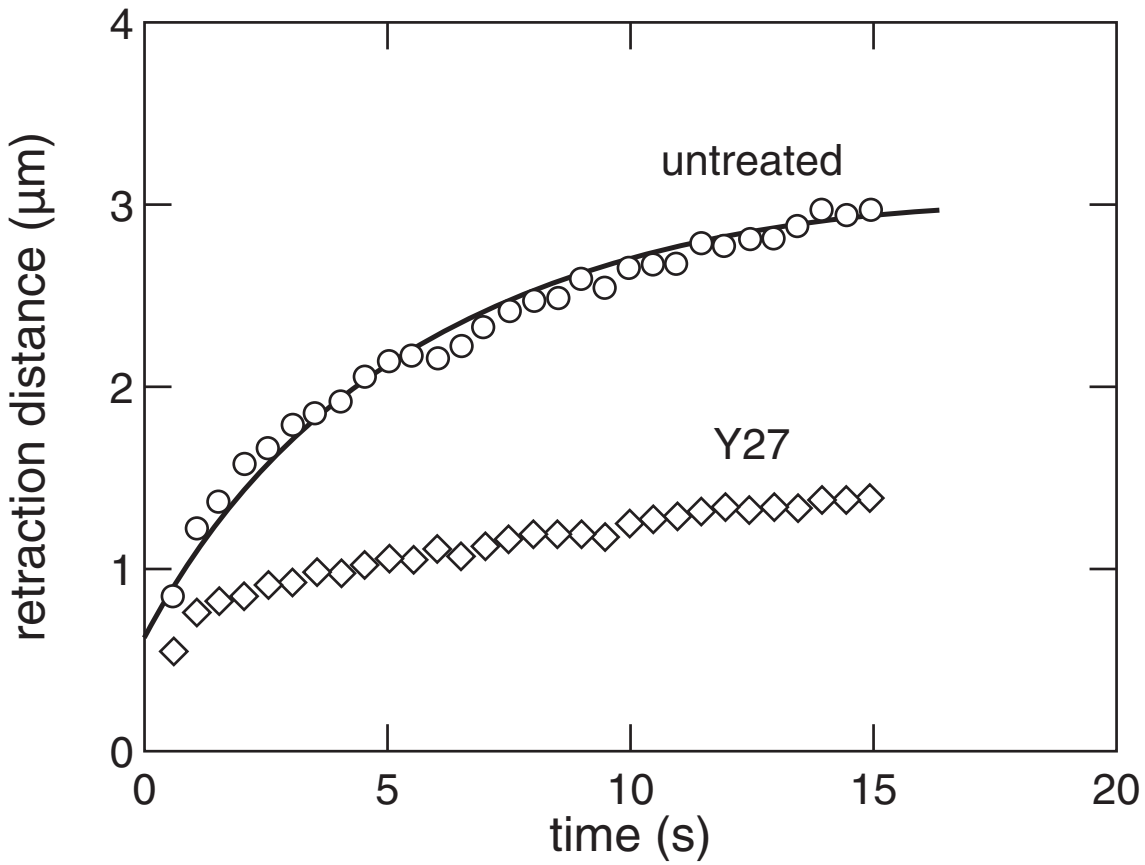
# Subcellular surgery

**tension in actin filaments is generated by myosin motors**



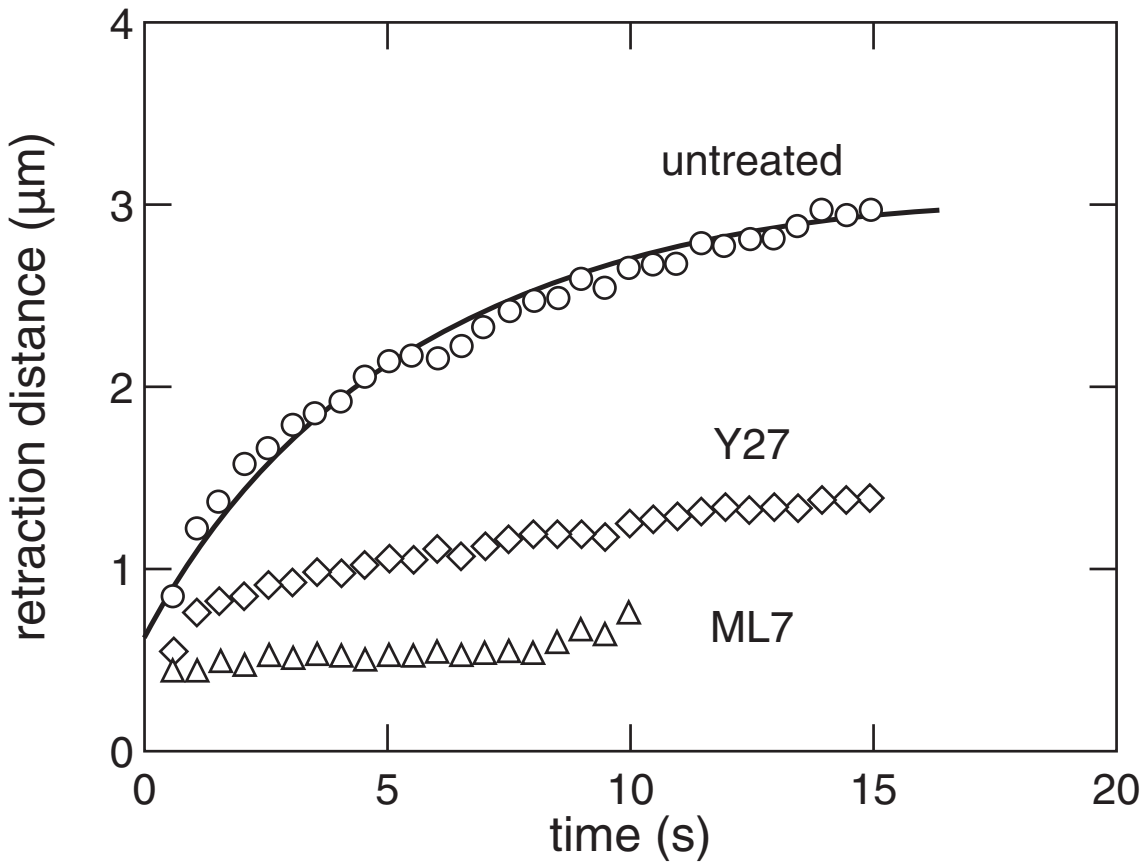
# Subcellular surgery

## Y27: inhibits some myosin activity



# Subcellular surgery

## ML7: direct inhibitor of myosin activity



# Subcellular surgery

*Q*: what is interplay between ECM and actin network?

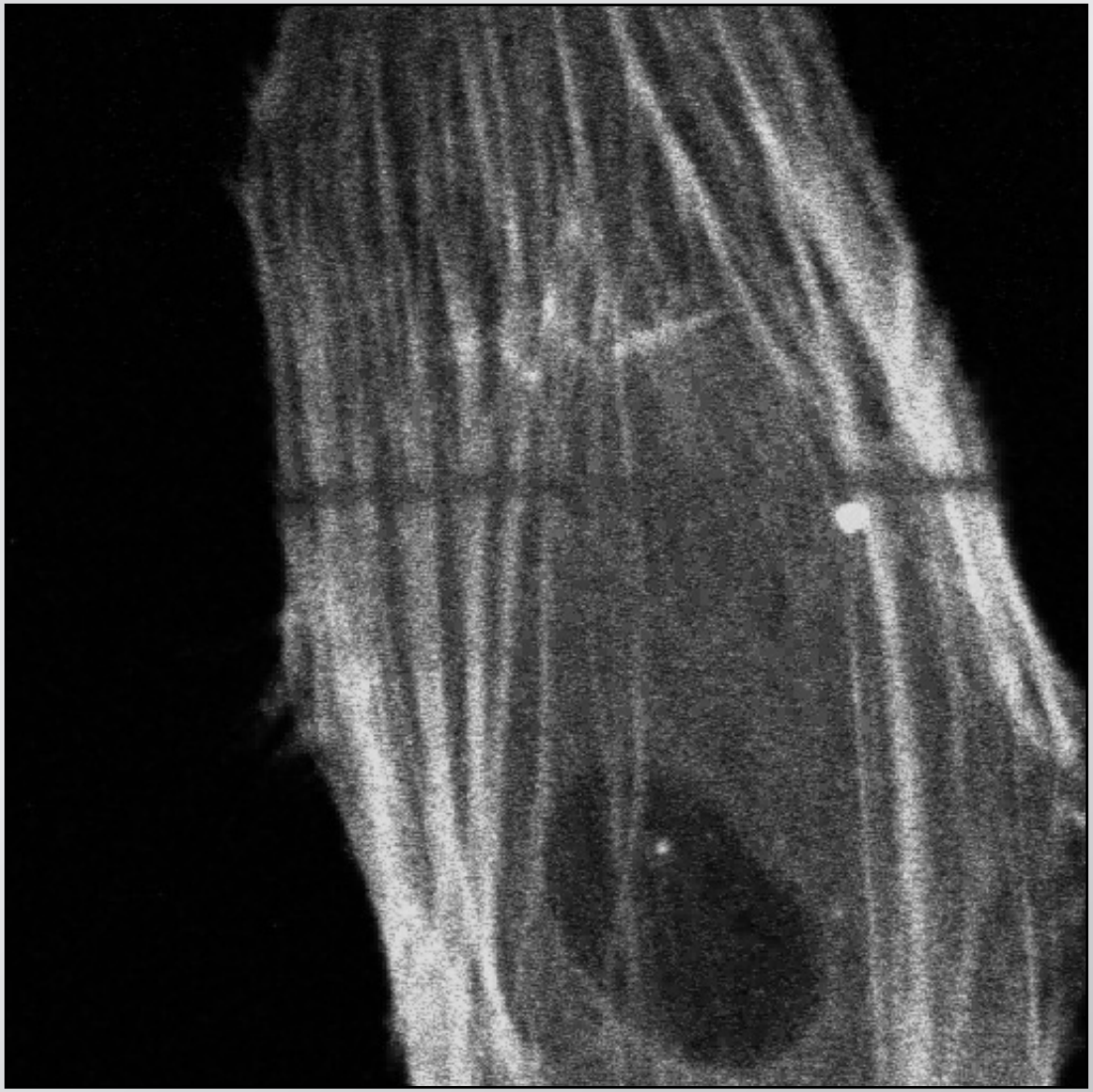
# Subcellular surgery

Connection between single stress fiber and extra-cellular matrix:

- stress fibers exert forces on ECM
- force balance between cell and ECM

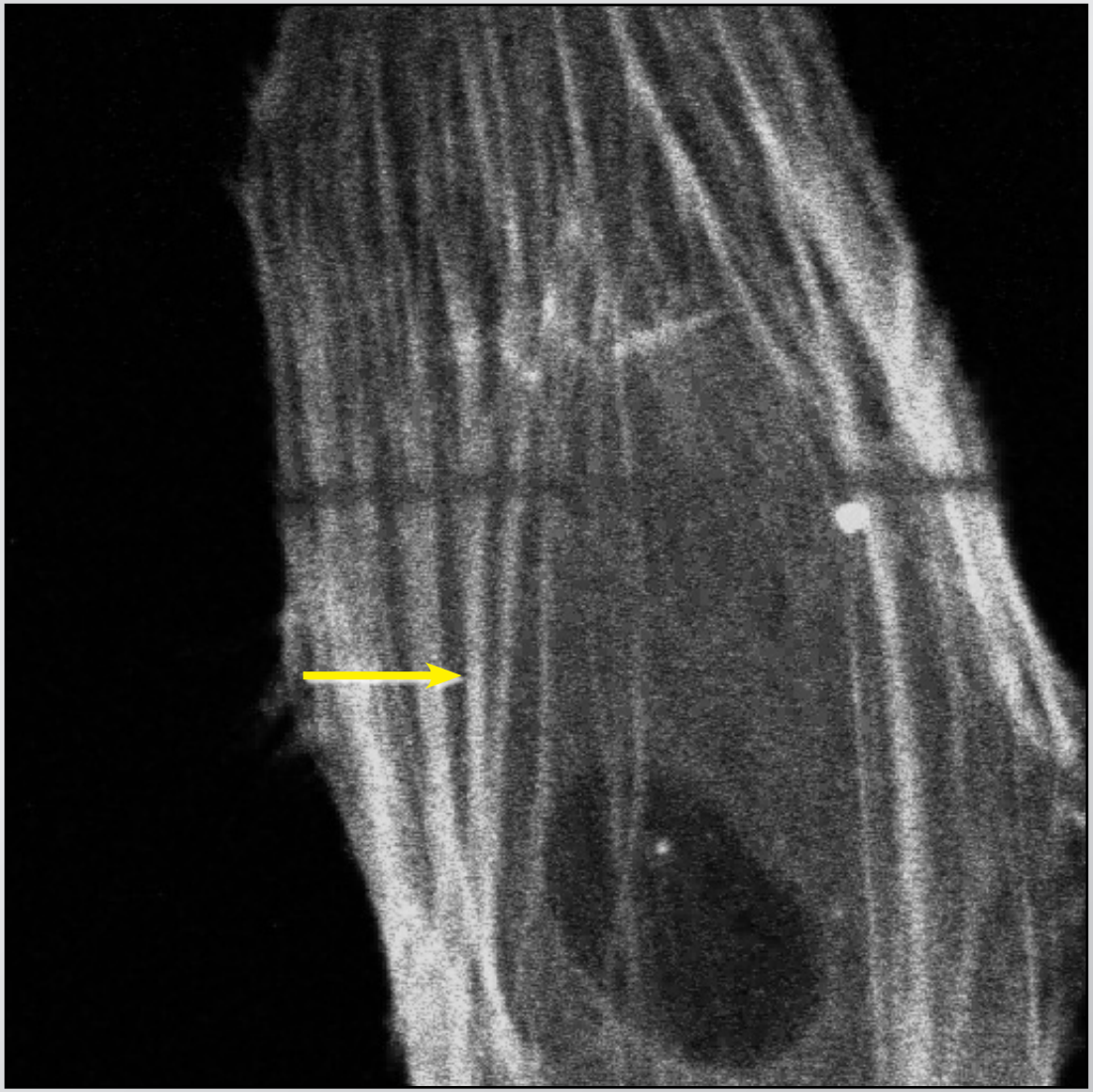
# Subcellular surgery

on stiff substrate no observable changes fiber surroundings…



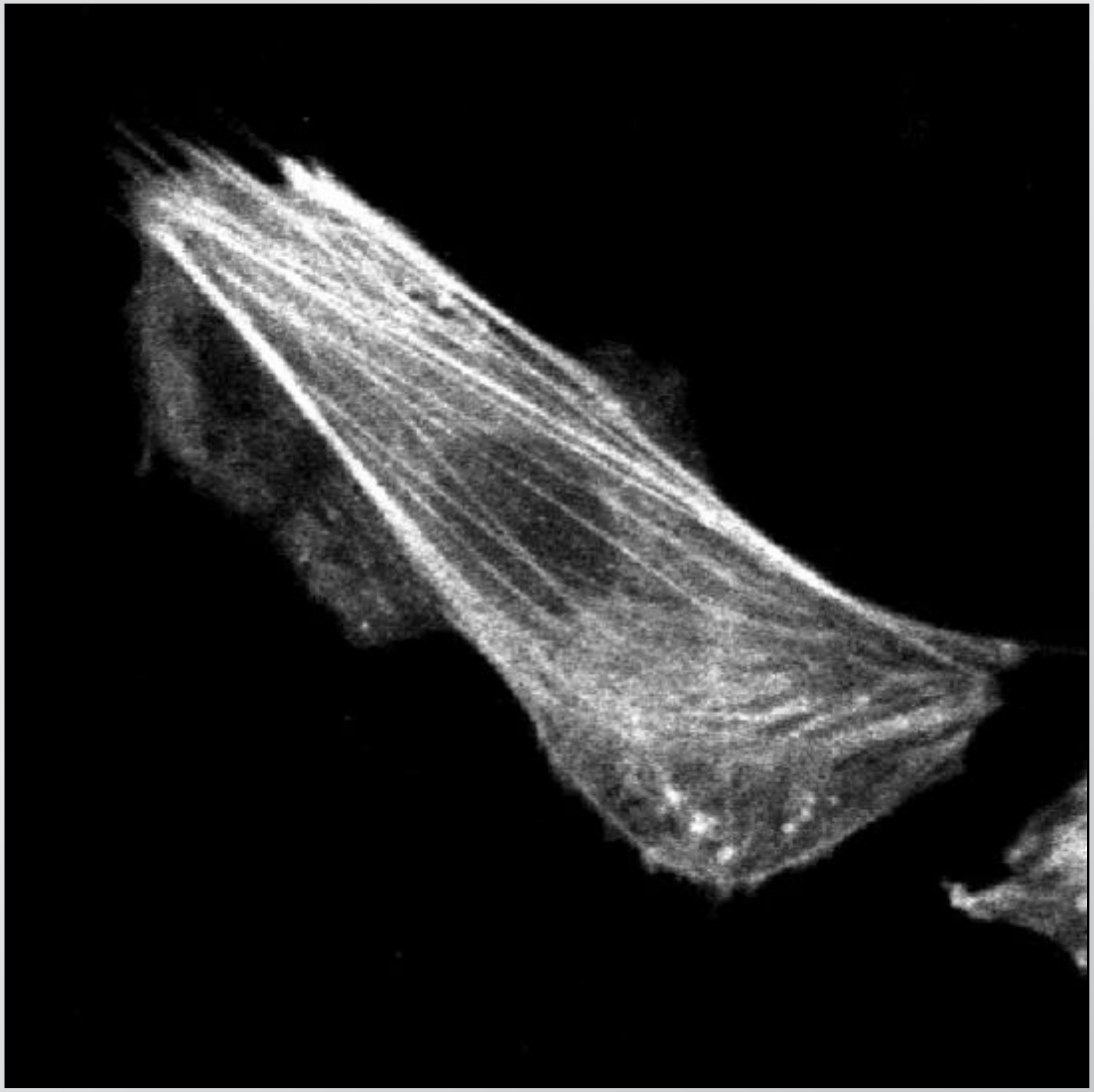
# Subcellular surgery

on stiff substrate no observable changes fiber surroundings…



# Subcellular surgery

…even when many fibers are cut



# Subcellular surgery

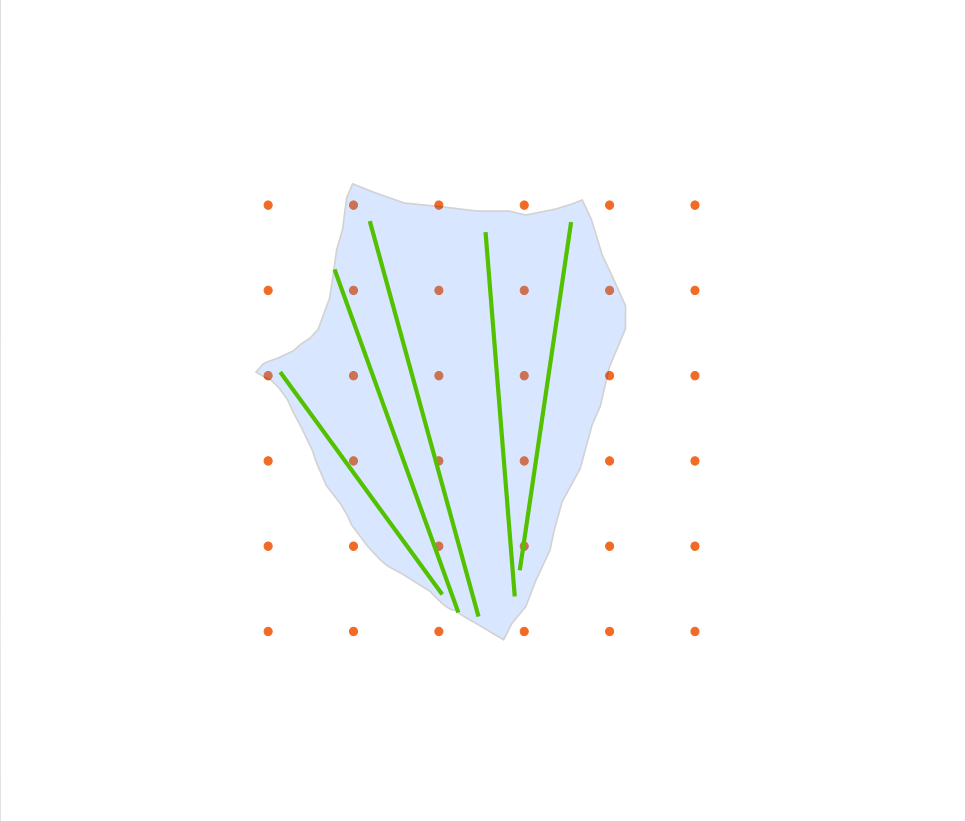
ECMs of living cells are compliant

(and cells exhibit physiologically more

relevant functions on flexible substrates)

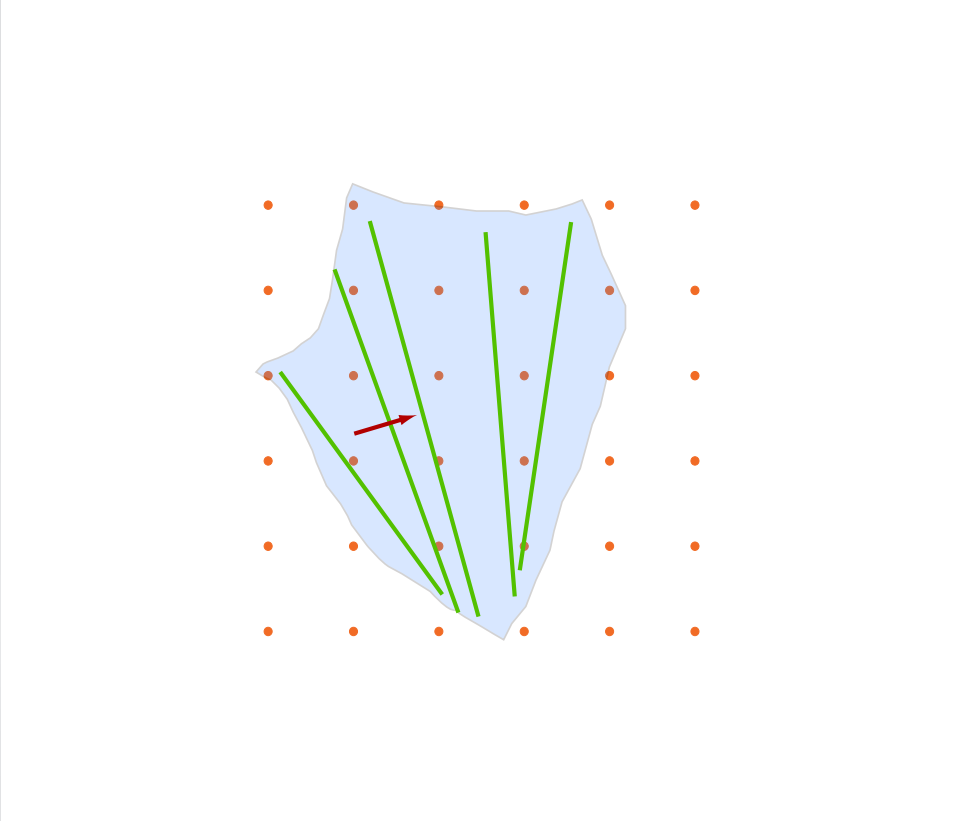
# Subcellular surgery

visualize motion of flexible ECM after cutting



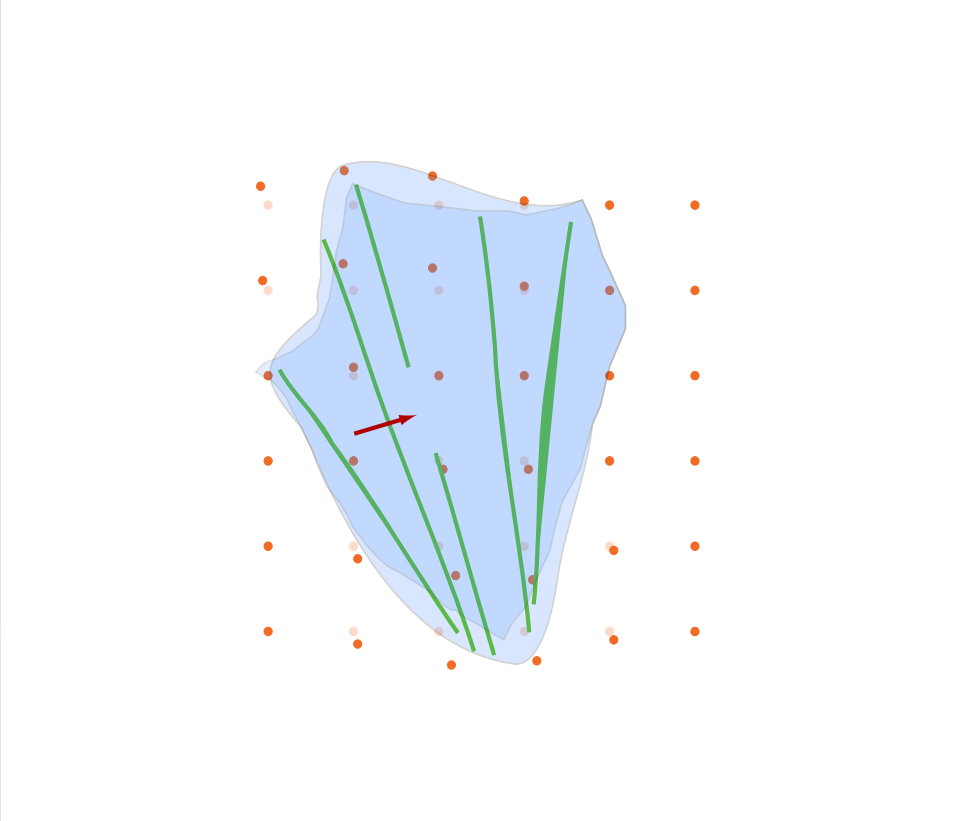
# Subcellular surgery

## traction force microscopy



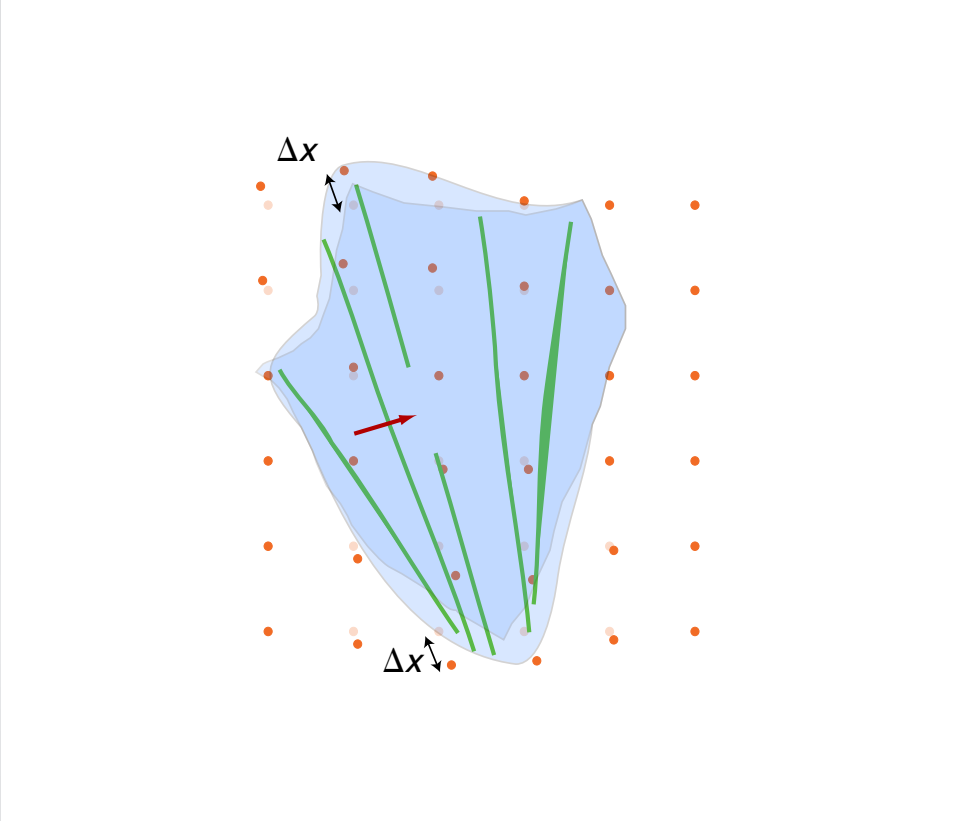
# Subcellular surgery

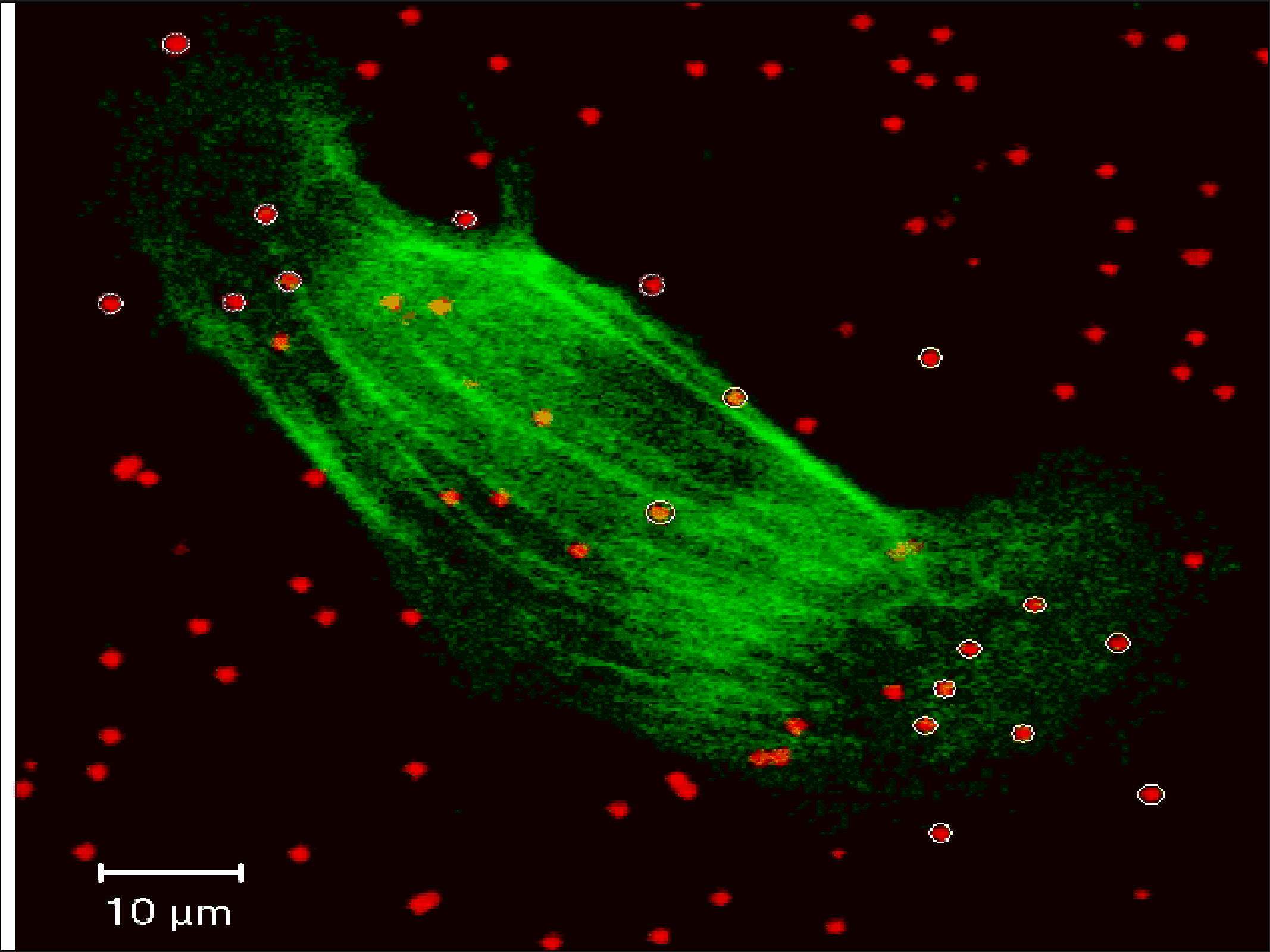
## traction force microscopy



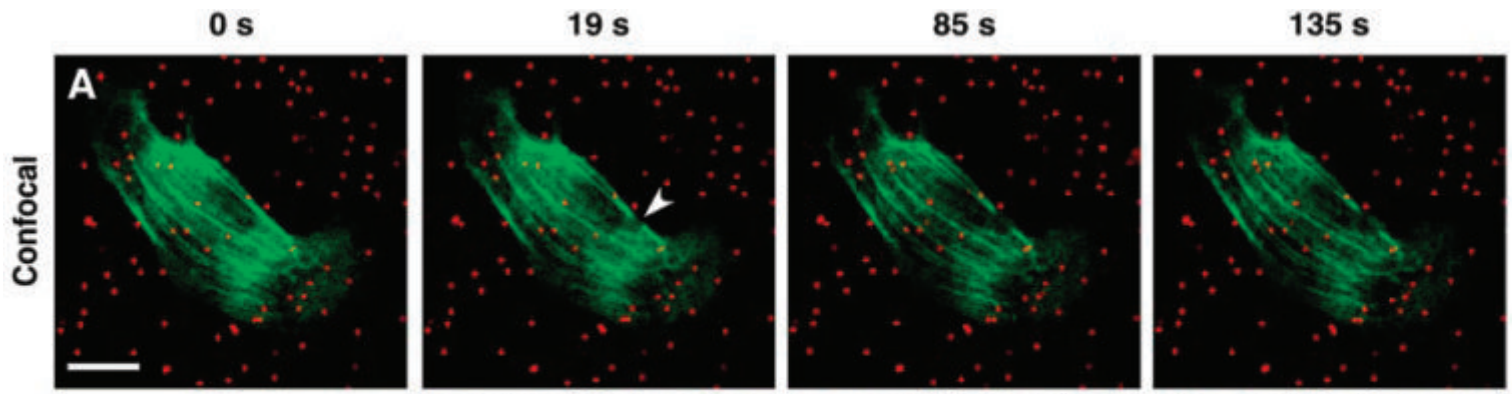
# Subcellular surgery

## measure bead displacement

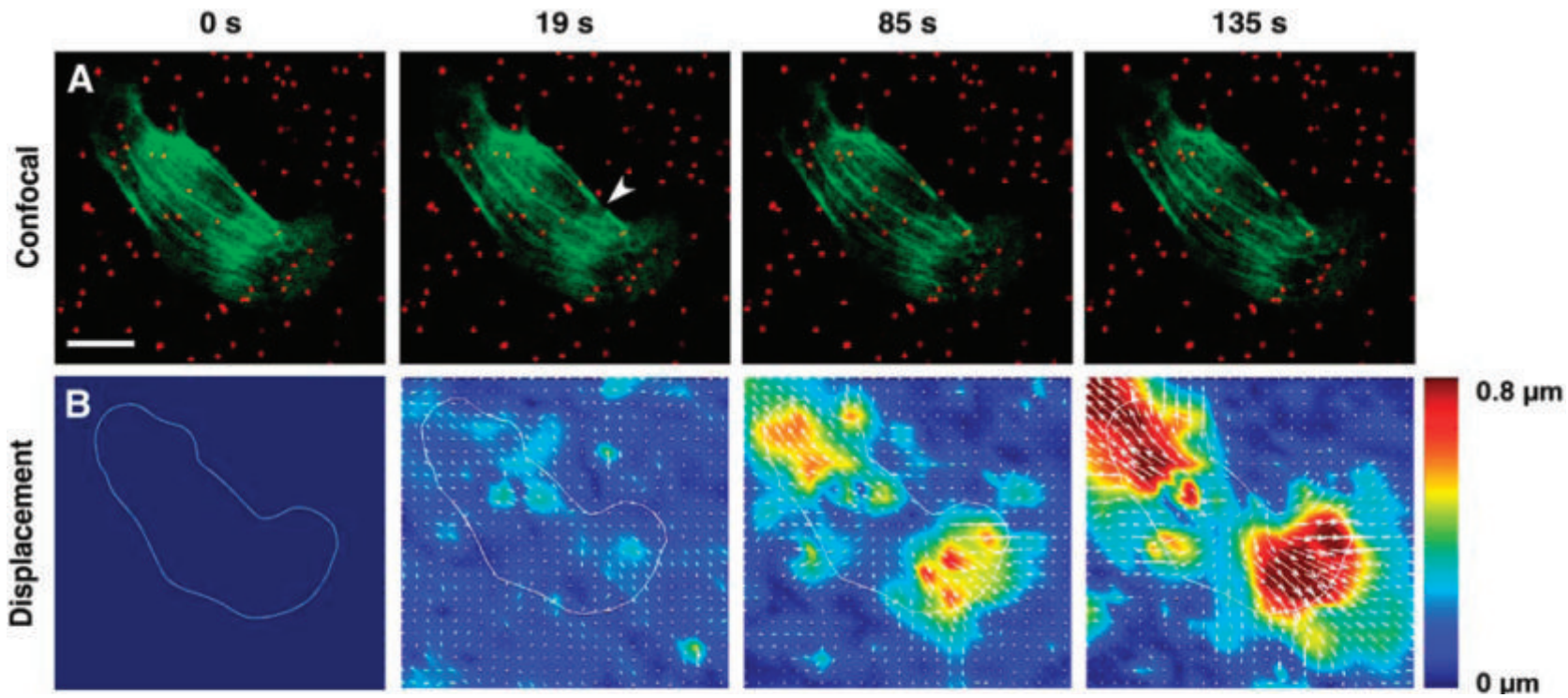



10 µm

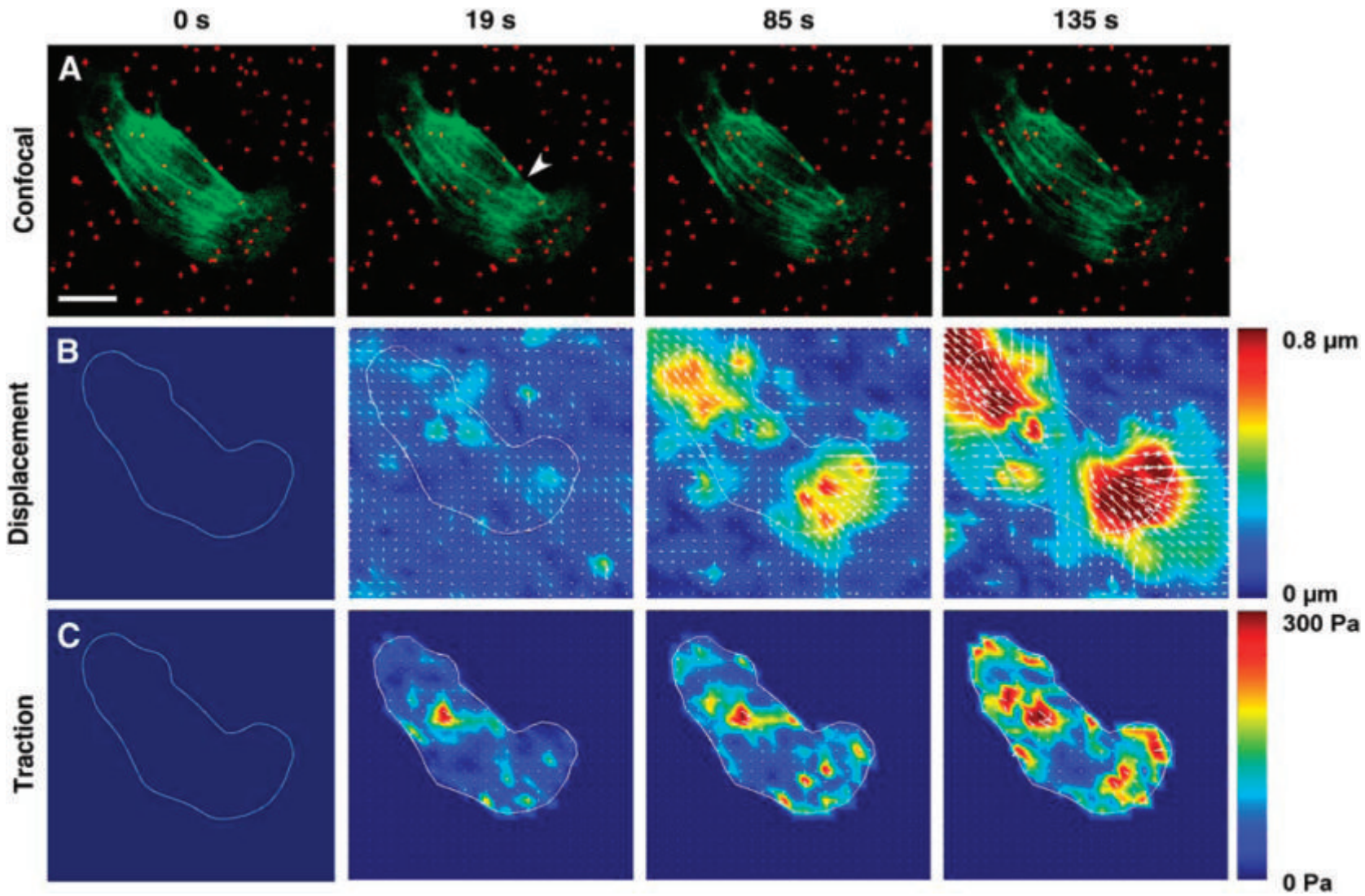
# Subcellular surgery



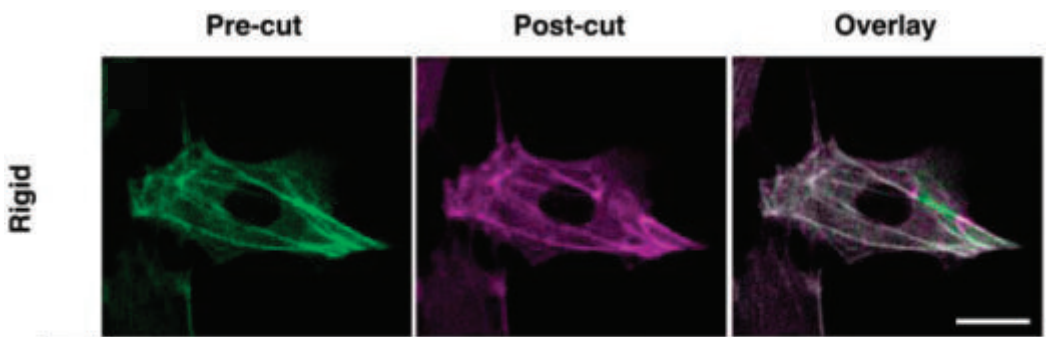
# Subcellular surgery



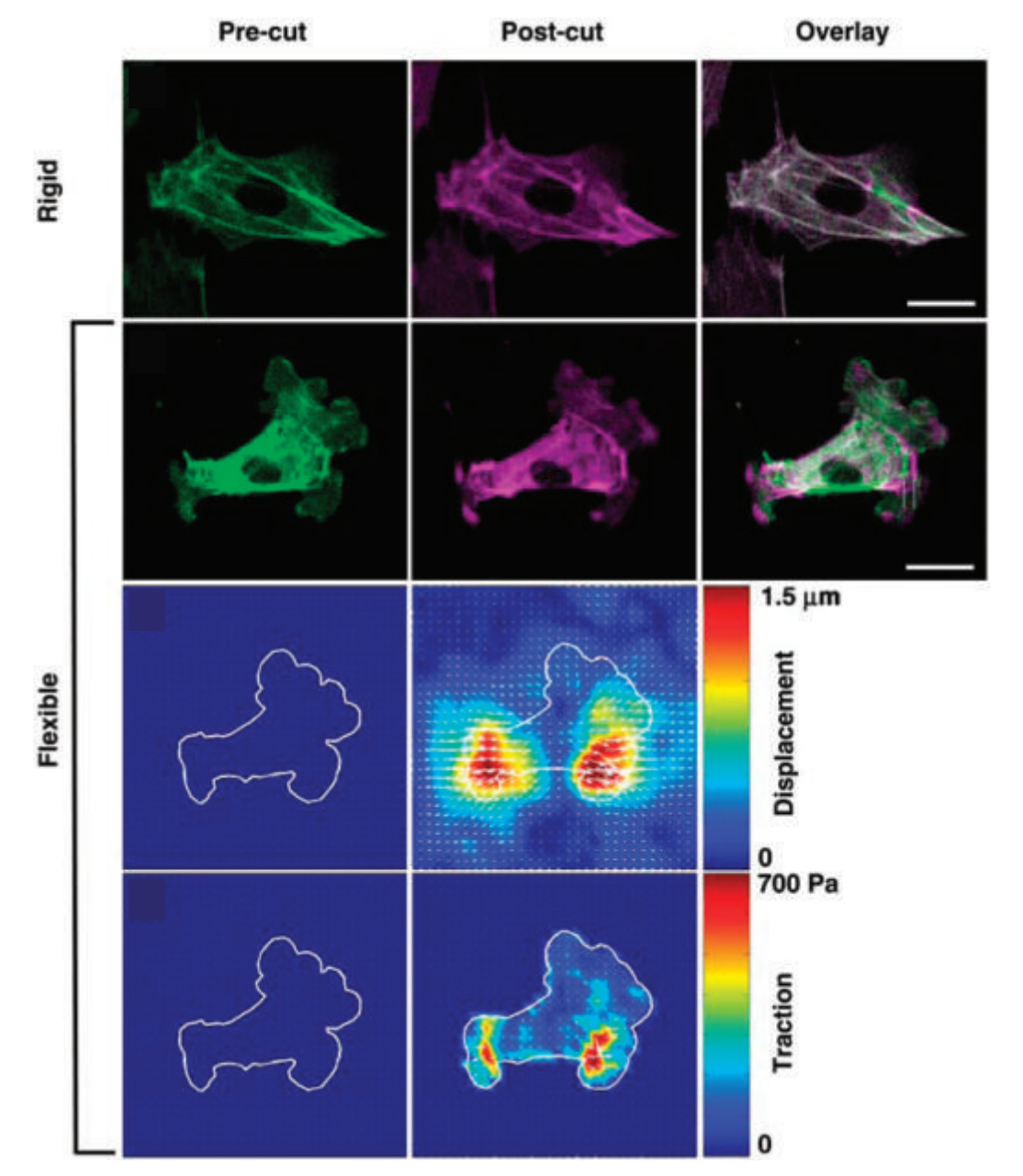
# Subcellular surgery



# Subcellular surgery



# Subcellular surgery



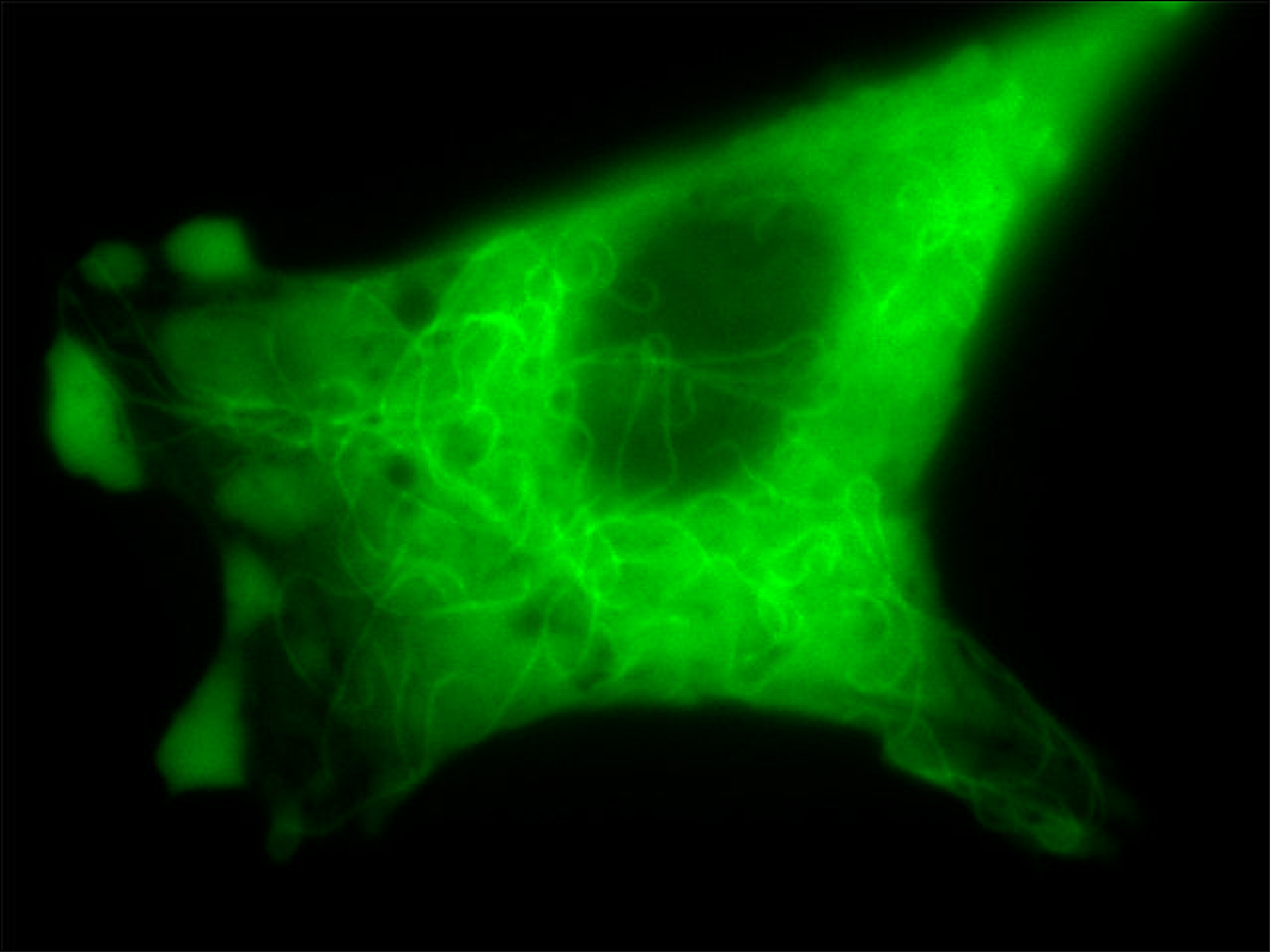
# Subcellular surgery

Stiff substrate:

- no change in cell shape
- no observable change in neighboring fibers

Compliant substrate:

- change in cell shape (displacements of about 1 μm)
- large scale structural rearrangement within cell
- force transfer to ECM of about 180 Pa



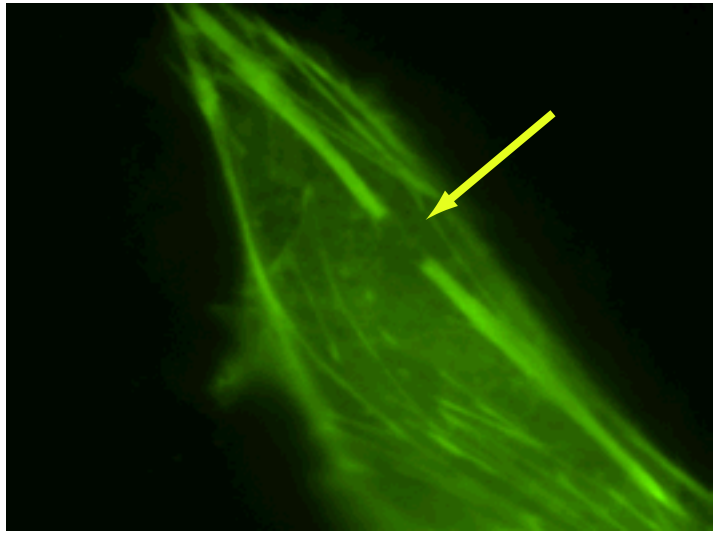
# Subcellular surgery

can oscillators be used for surgery?

# Subcellular surgery

3 s exposure, 1 nJ pulse energy

14 kHz

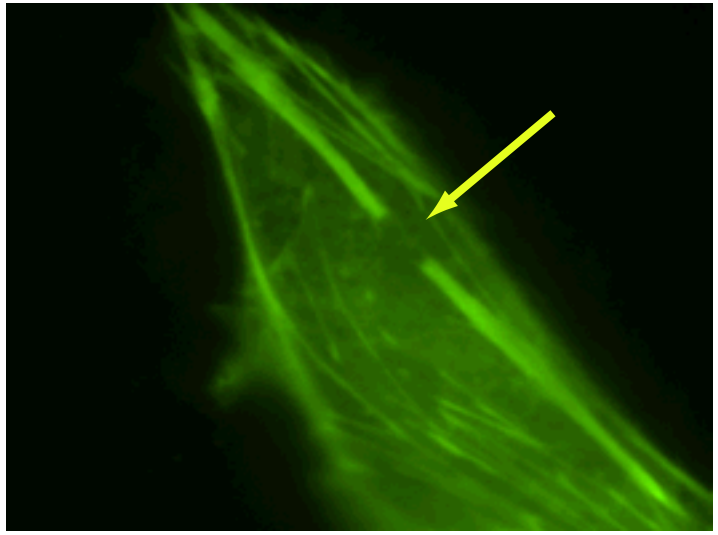


cutting

# Subcellular surgery

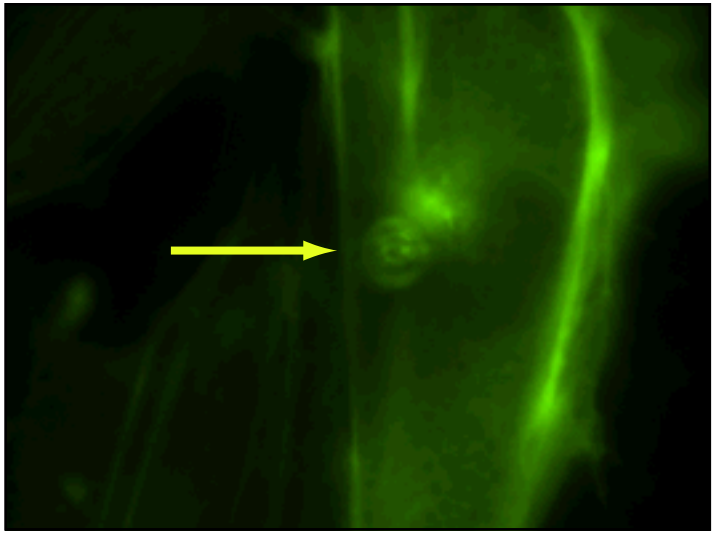
3 s exposure, 1 nJ pulse energy

14 kHz



cutting

76 MHz

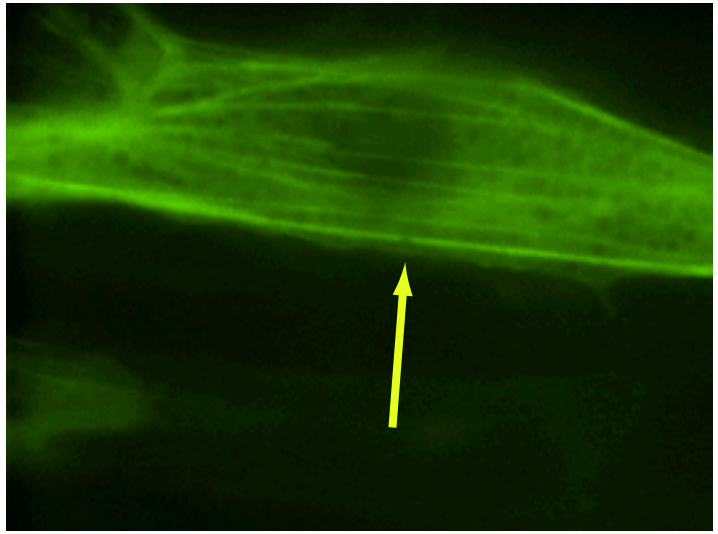


excessive damage

# Subcellular surgery

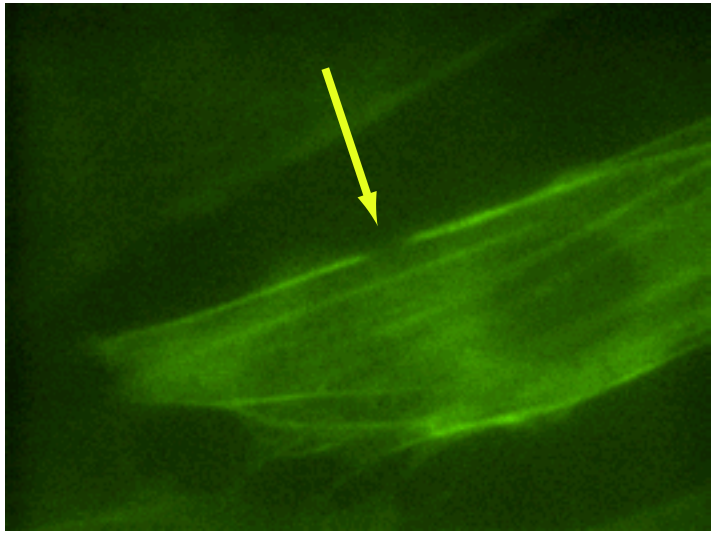
3 s exposure, 0.5 nJ pulse energy

14 kHz



cutting

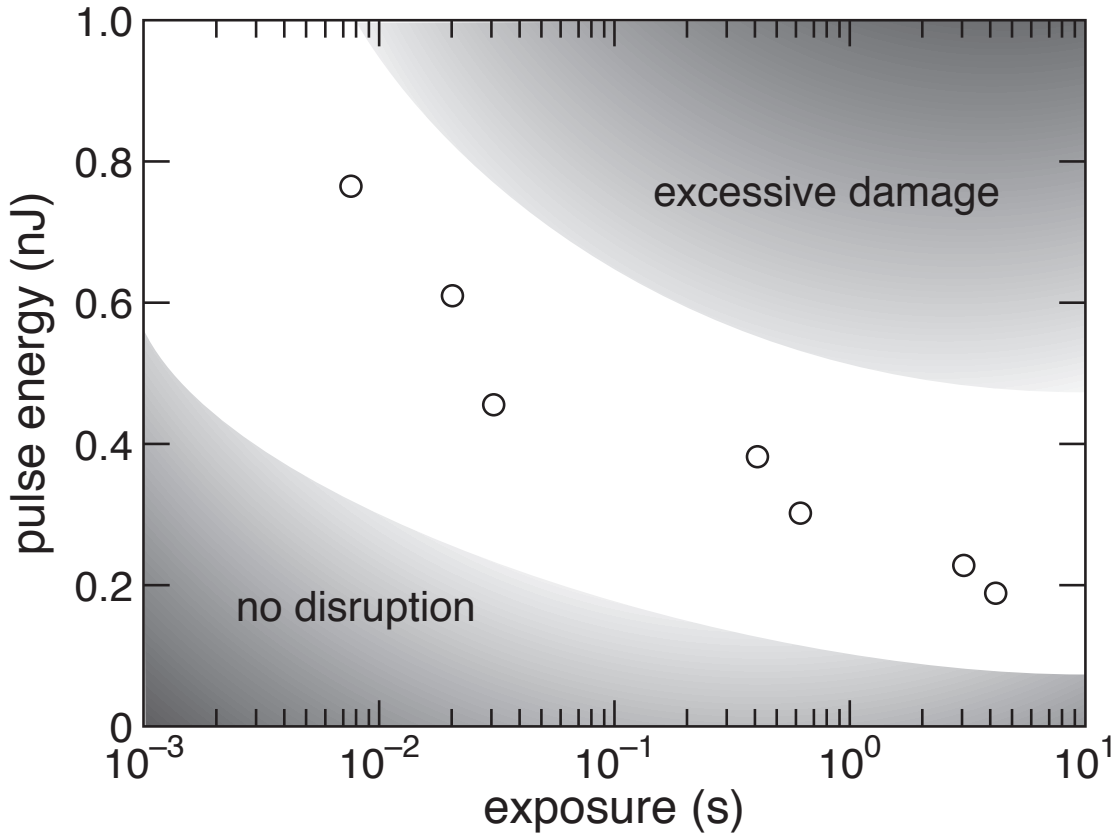
76 MHz



cutting

# Subcellular surgery

## MHz threshold for actin cutting



# Subcellular surgery

kHz surgery:

- threshold determined by pulse energy
- no additional damage from prolonged exposure

# Subcellular surgery

kHz surgery:

- threshold determined by pulse energy
- no additional damage from prolonged exposure

MHz surgery:

- threshold determined by pulse energy and exposure

# Subcellular surgery

kHz surgery:

- threshold determined by pulse energy
- no additional damage from prolonged exposure

MHz surgery:

- threshold determined by pulse energy and exposure

both kHz and MHz suitable for nanosurgery!

# Outline

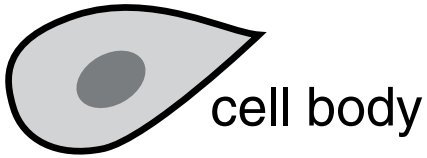
- femtosecond materials interactions
- subcellular surgery
- **nanoneurosurgery**
- optofection

# Nanoneurosurgery

*Q*: can we probe the neurological origins of behavior?

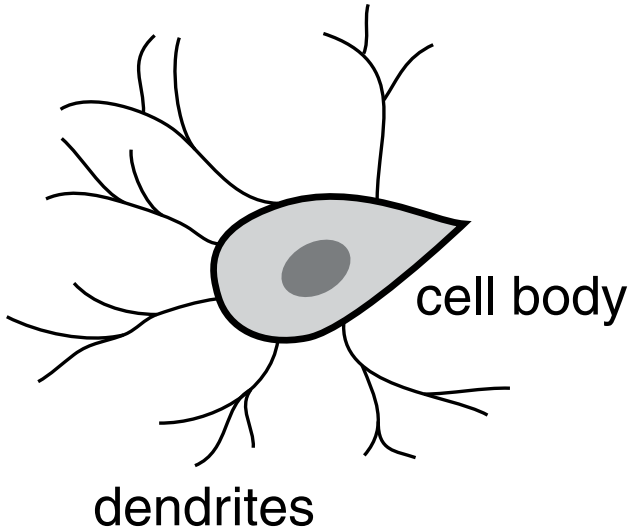
# Nanoneurosurgery

## neuron basics



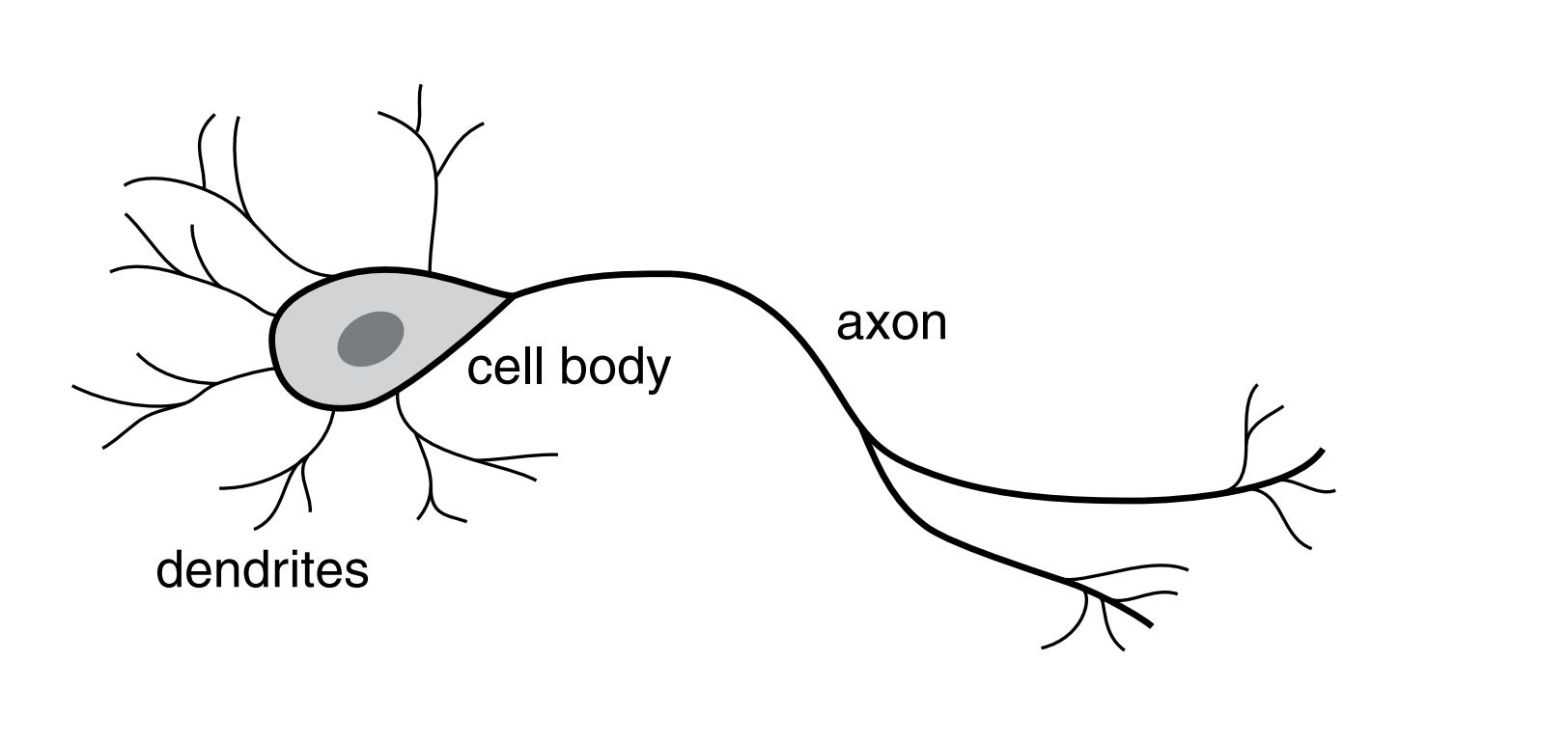
# Nanoneurosurgery

## neuron basics



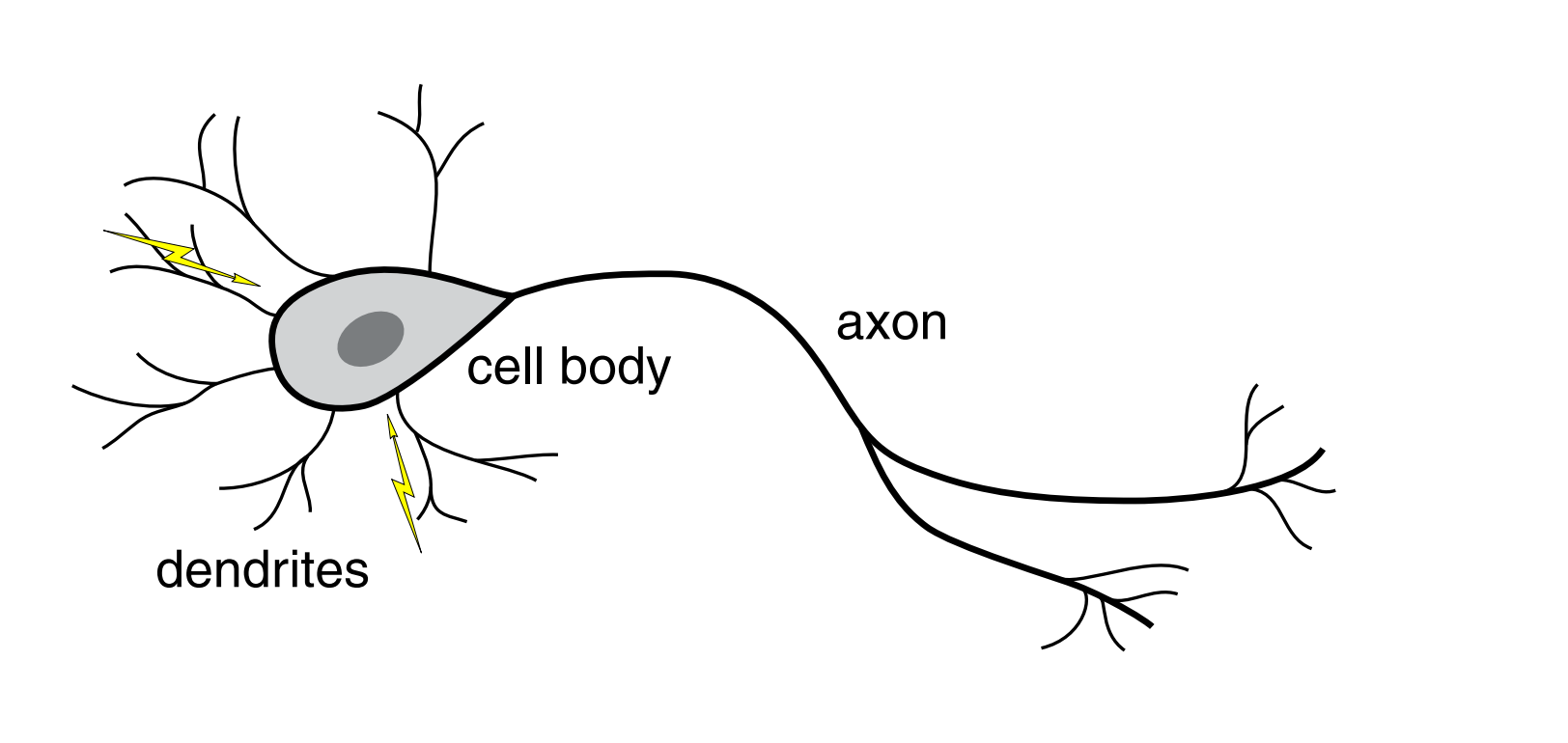
# Nanoneurosurgery

## neuron basics



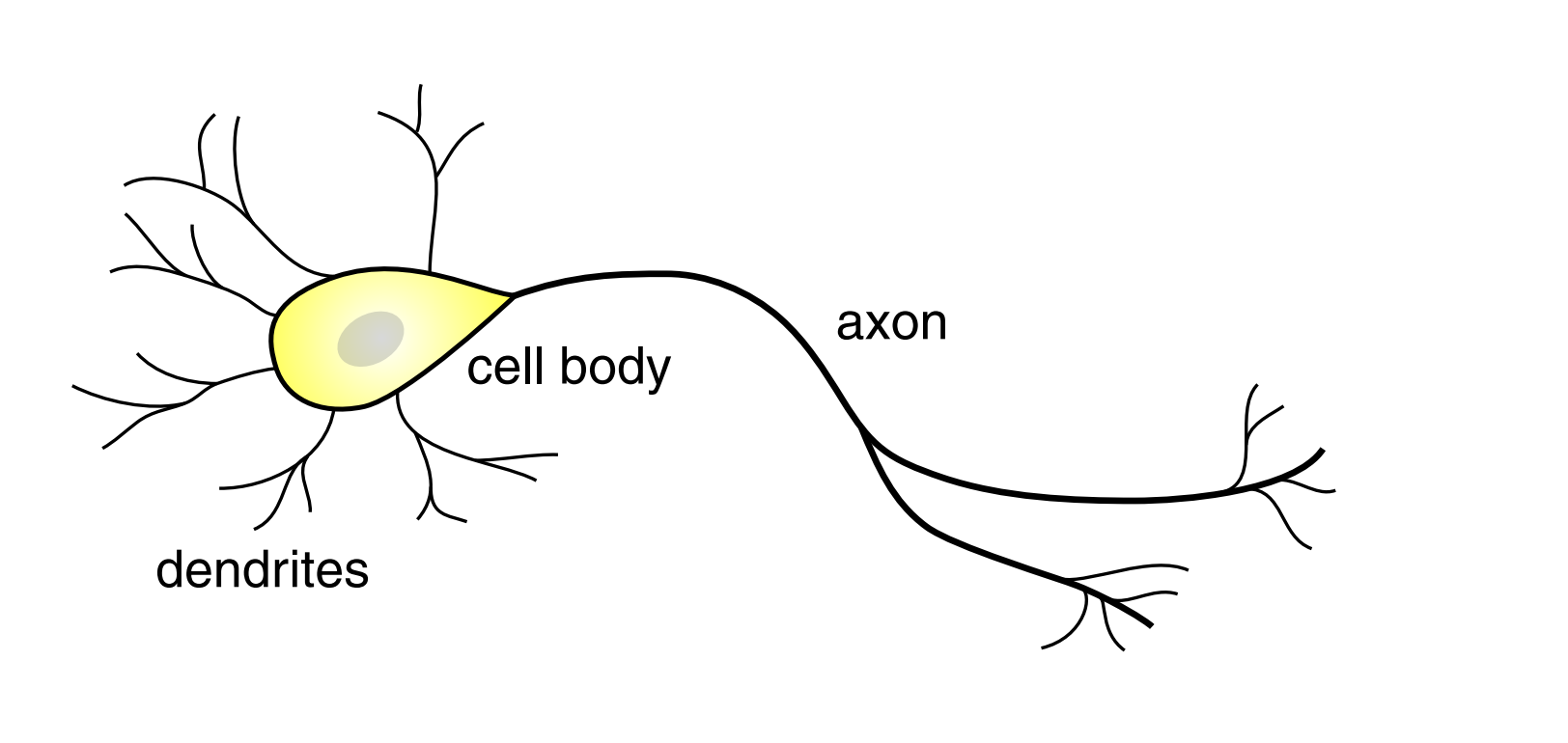
# Nanoneurosurgery

## neuron basics



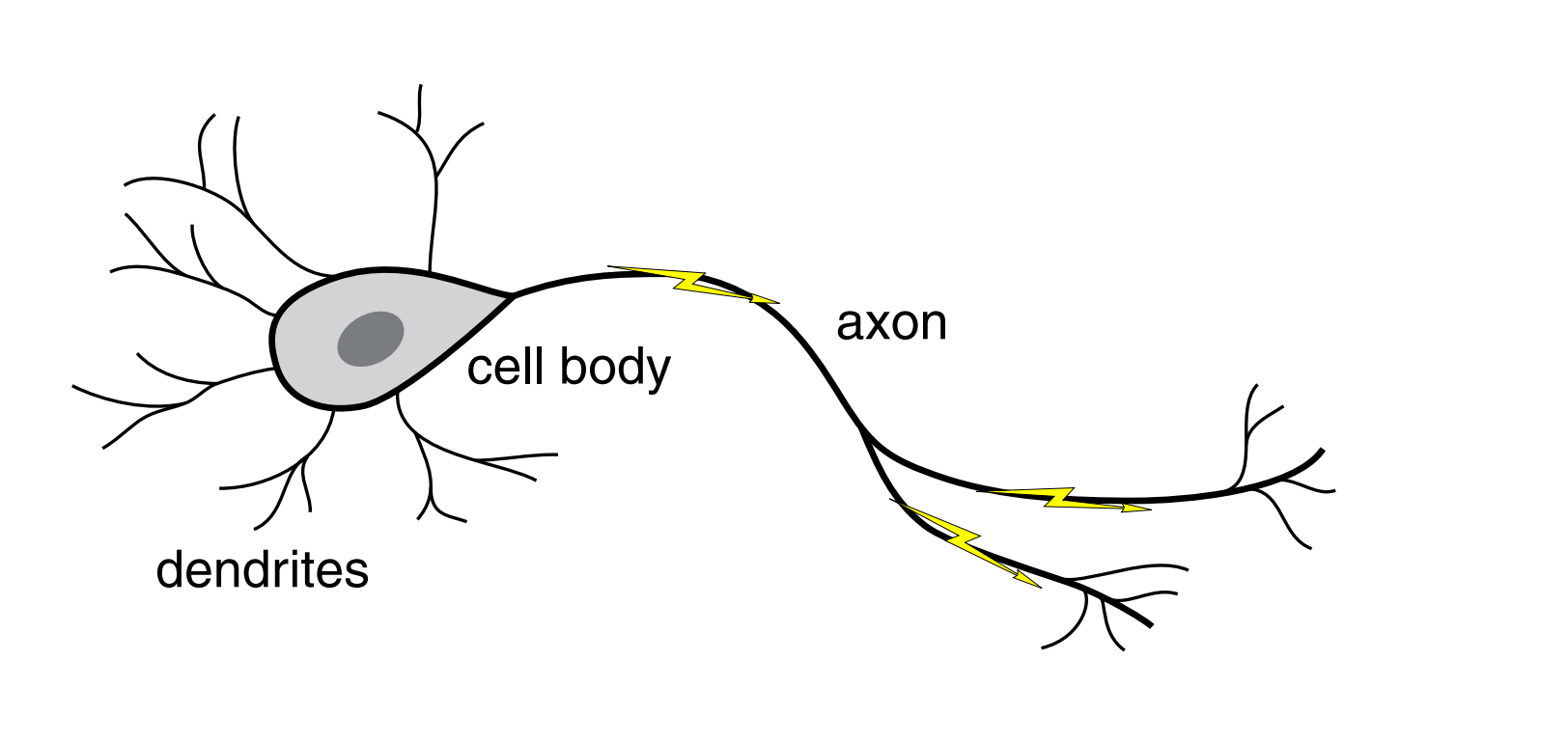
# Nanoneurosurgery

## neuron basics



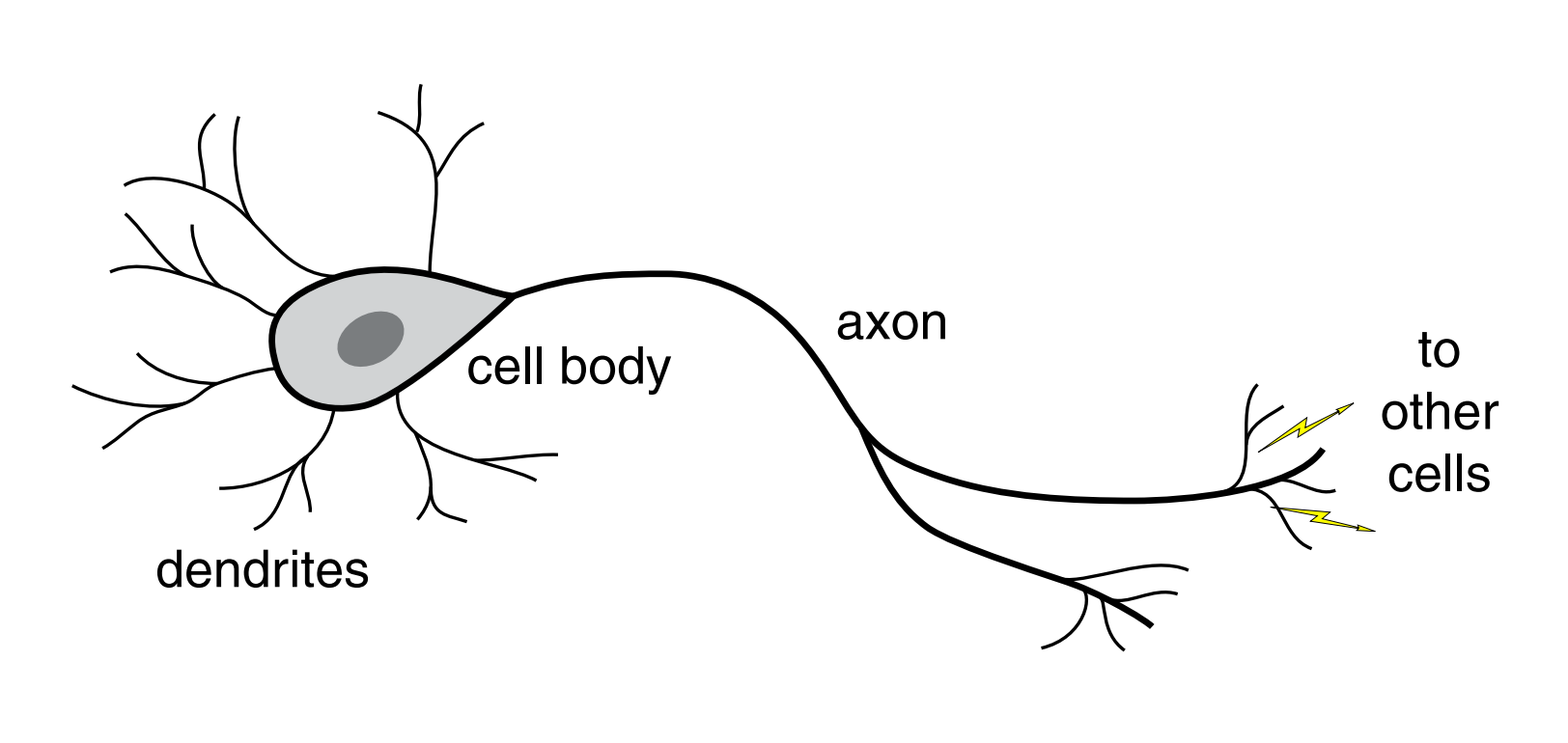
# Nanoneurosurgery

## neuron basics



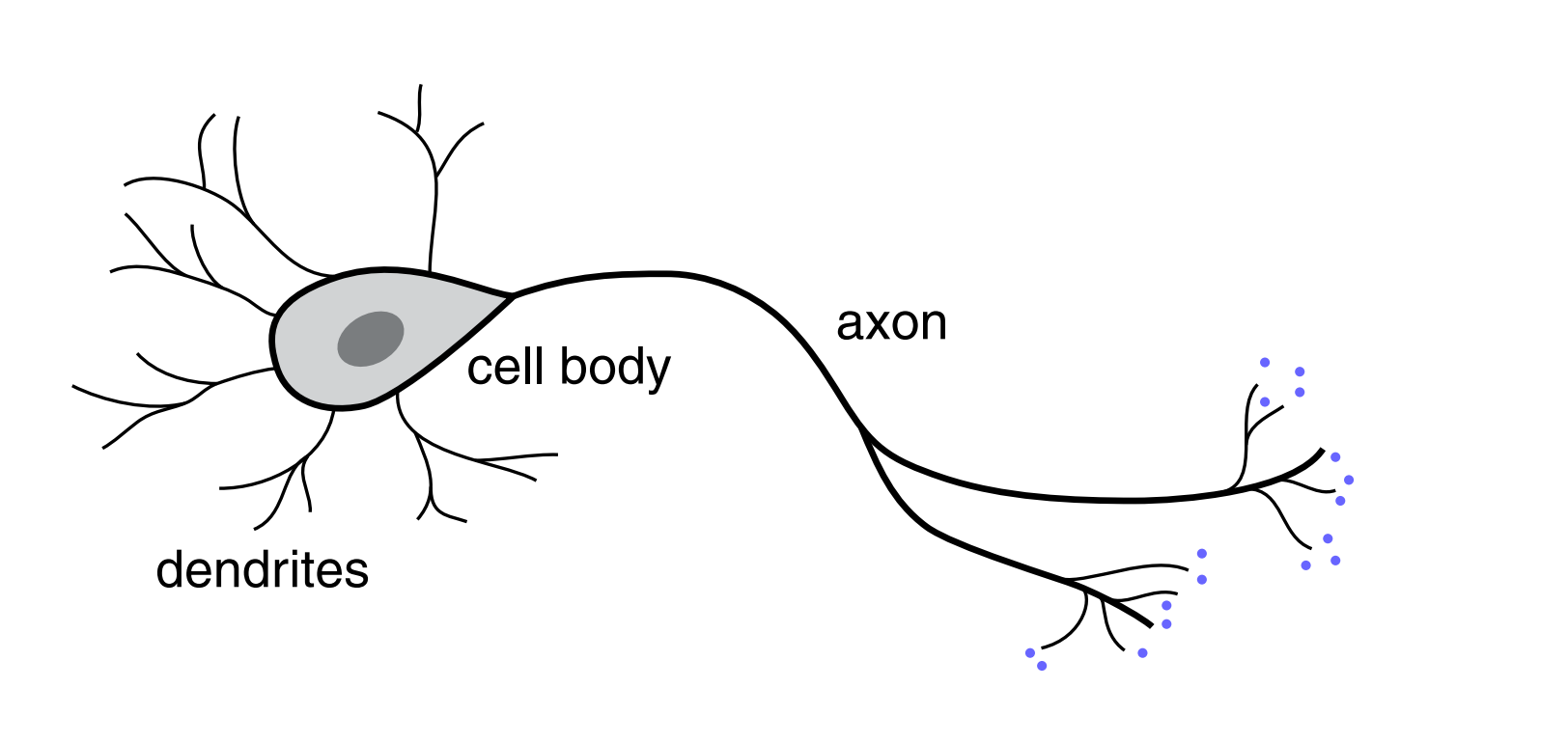
# Nanoneurosurgery

## neuron basics



# Nanoneurosurgery

## neuron basics



# Nanoneurosurgery

*Caenorhabditis elegans*

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

# Nanoneurosurgery

*Caenorhabditis elegans*

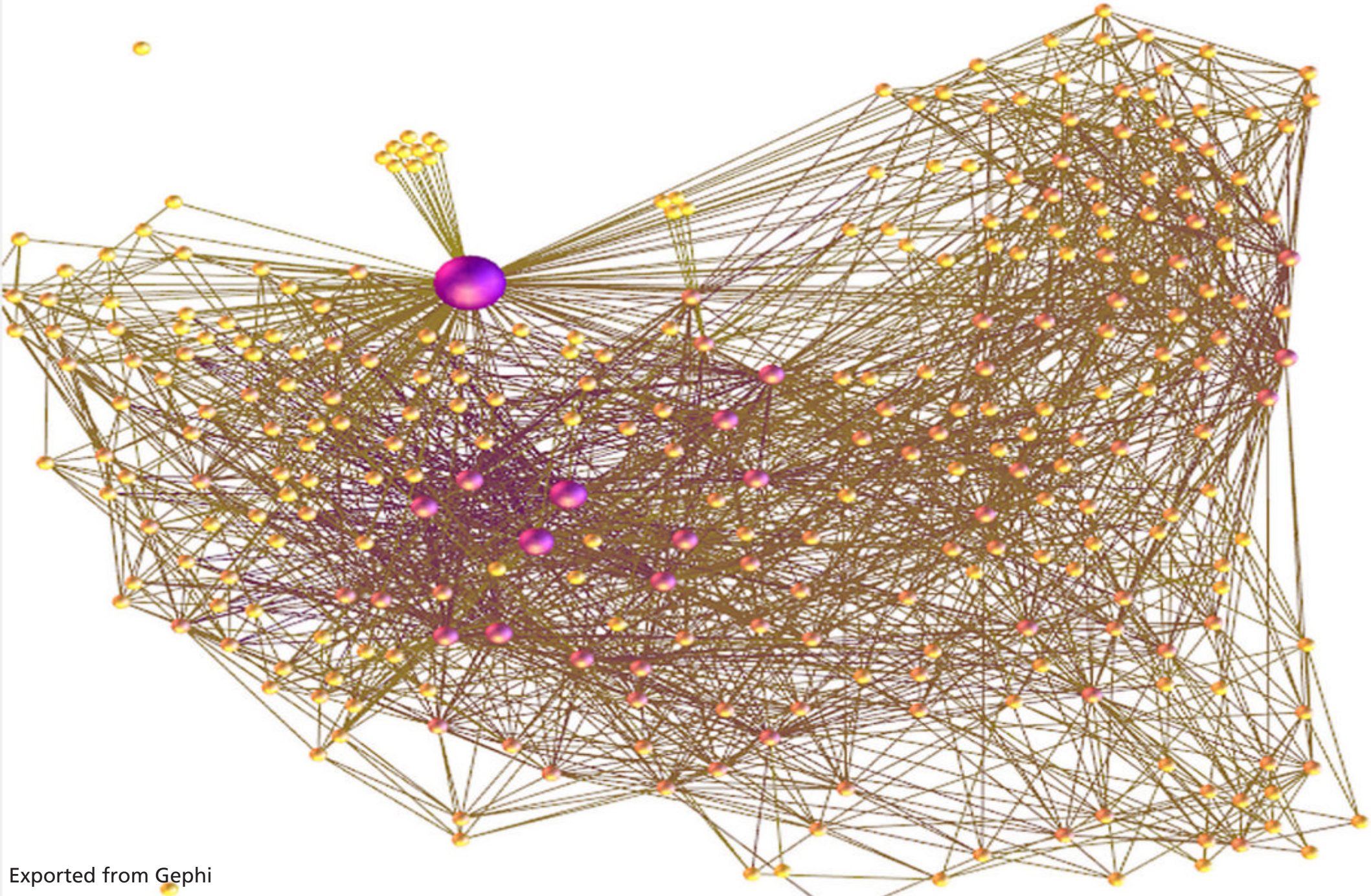
- simple model organism
- similarities to higher organisms
- genome fully sequenced
- easy to handle

# Nanoneurosurgery

*Caenorhabditis elegans*

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

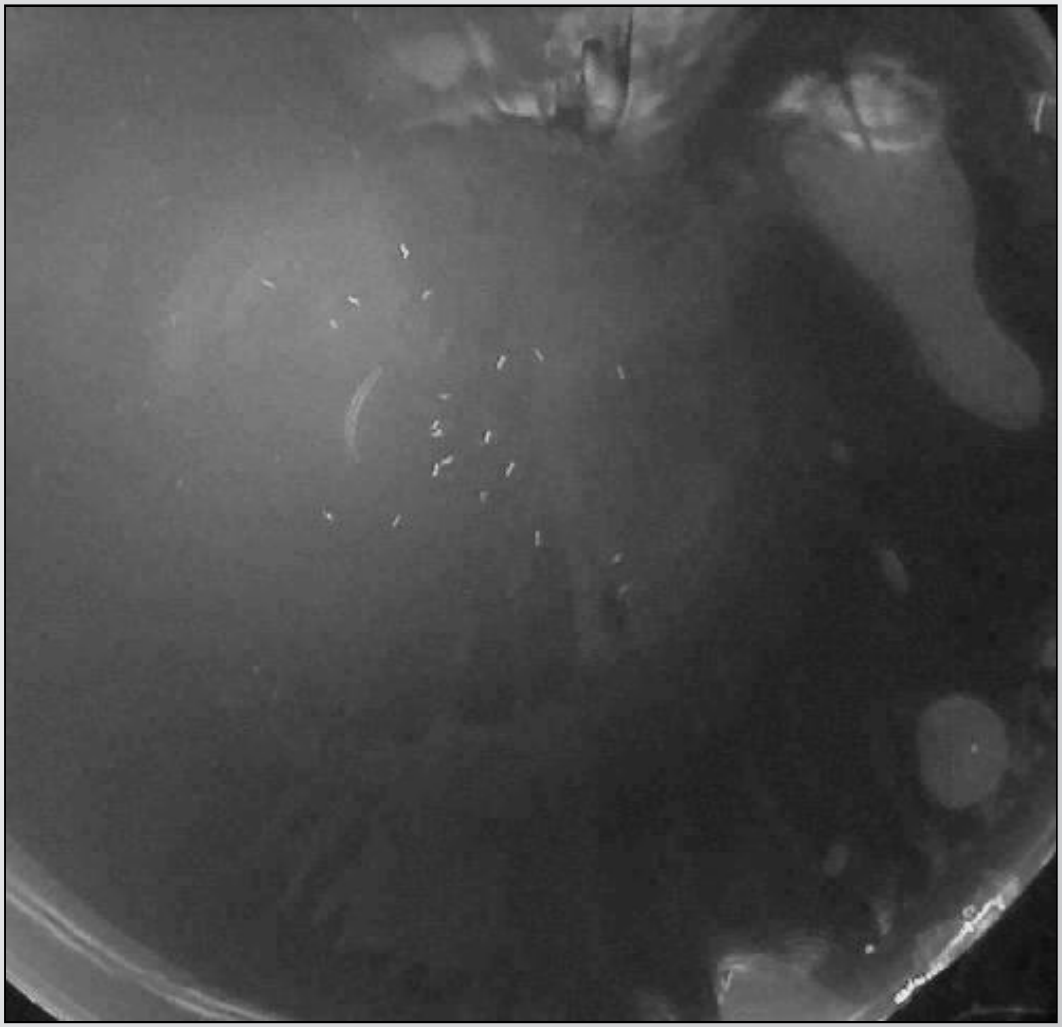
# Nanoneurosurgery



Exported from Gephi

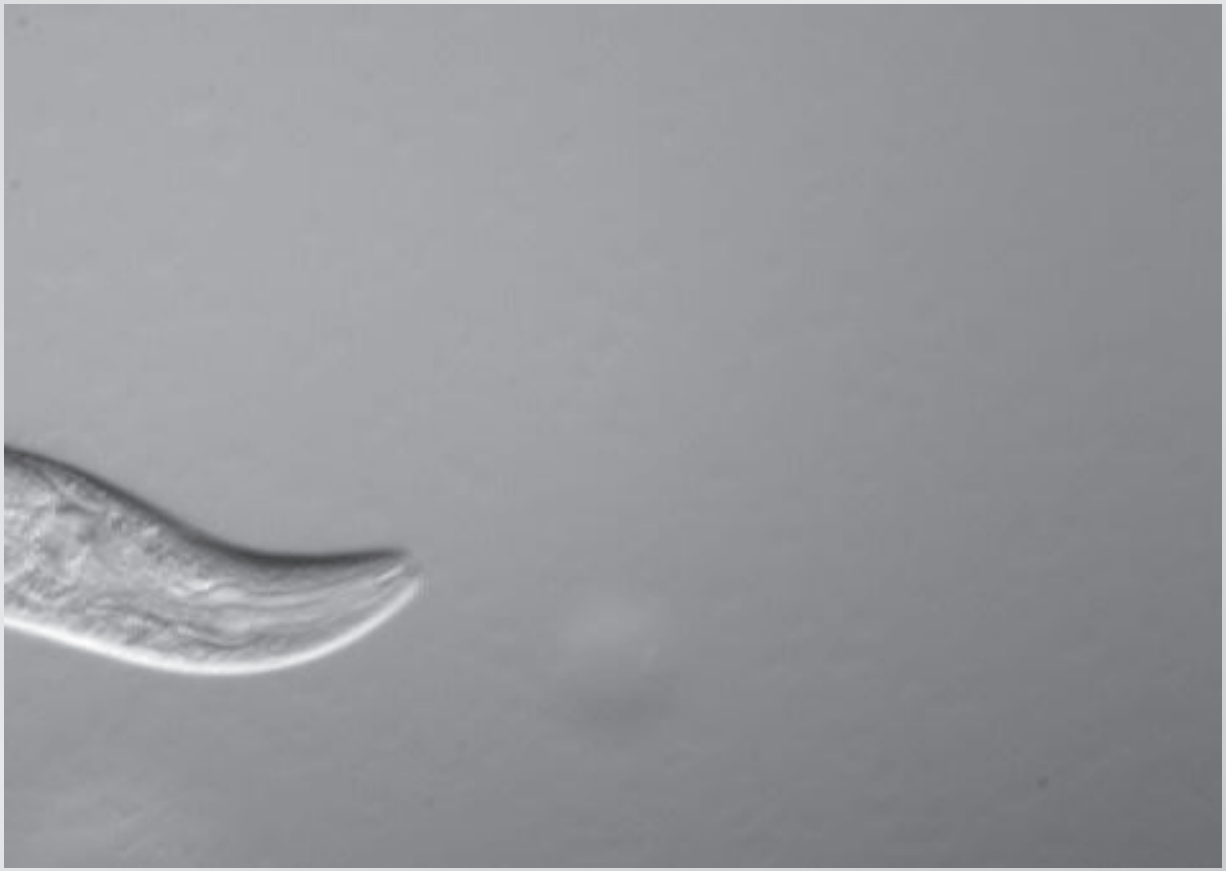
# Nanoneurosurgery

*Caenorhabditis elegans*

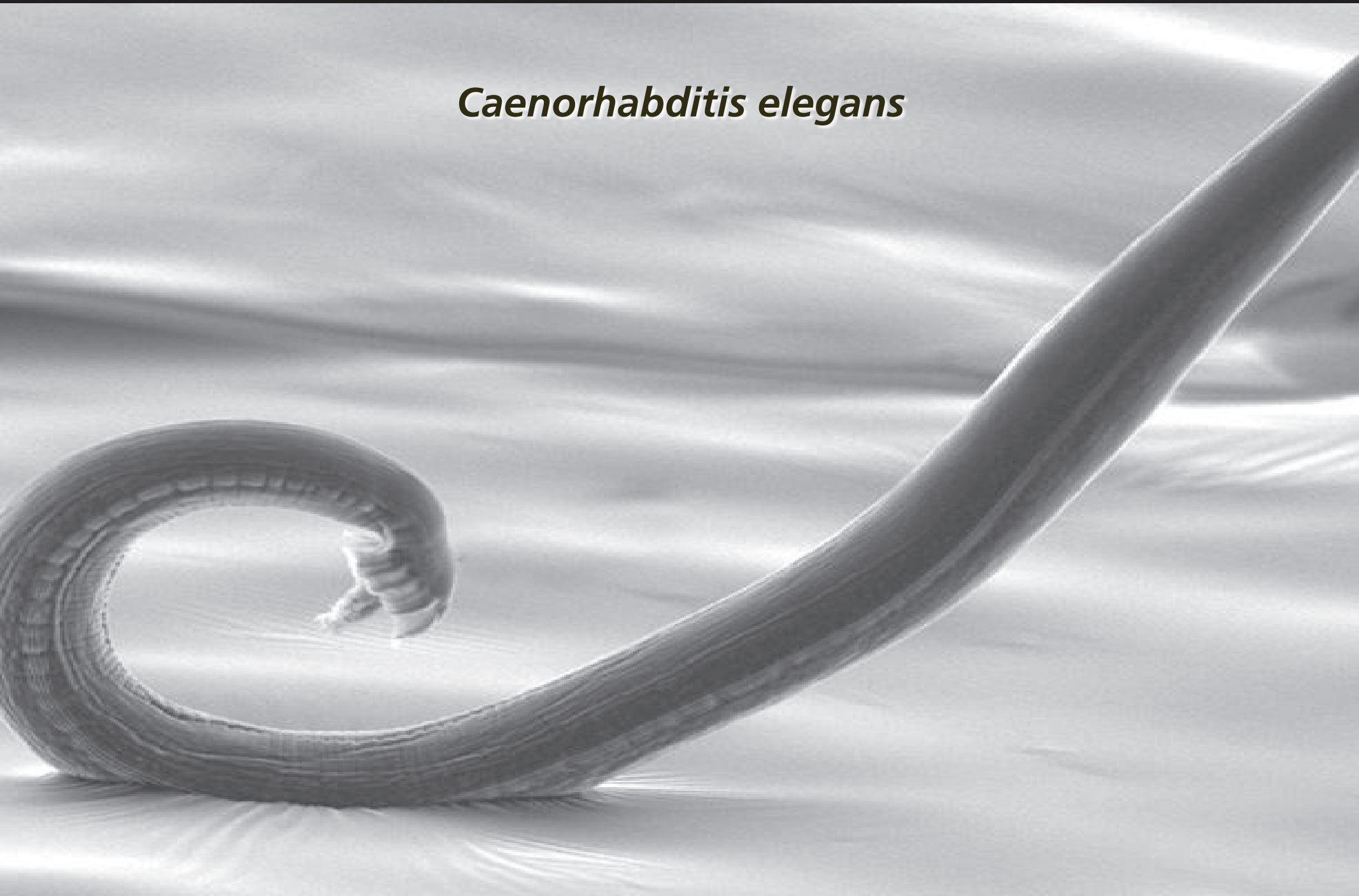


# Nanoneurosurgery

*Caenorhabditis elegans*

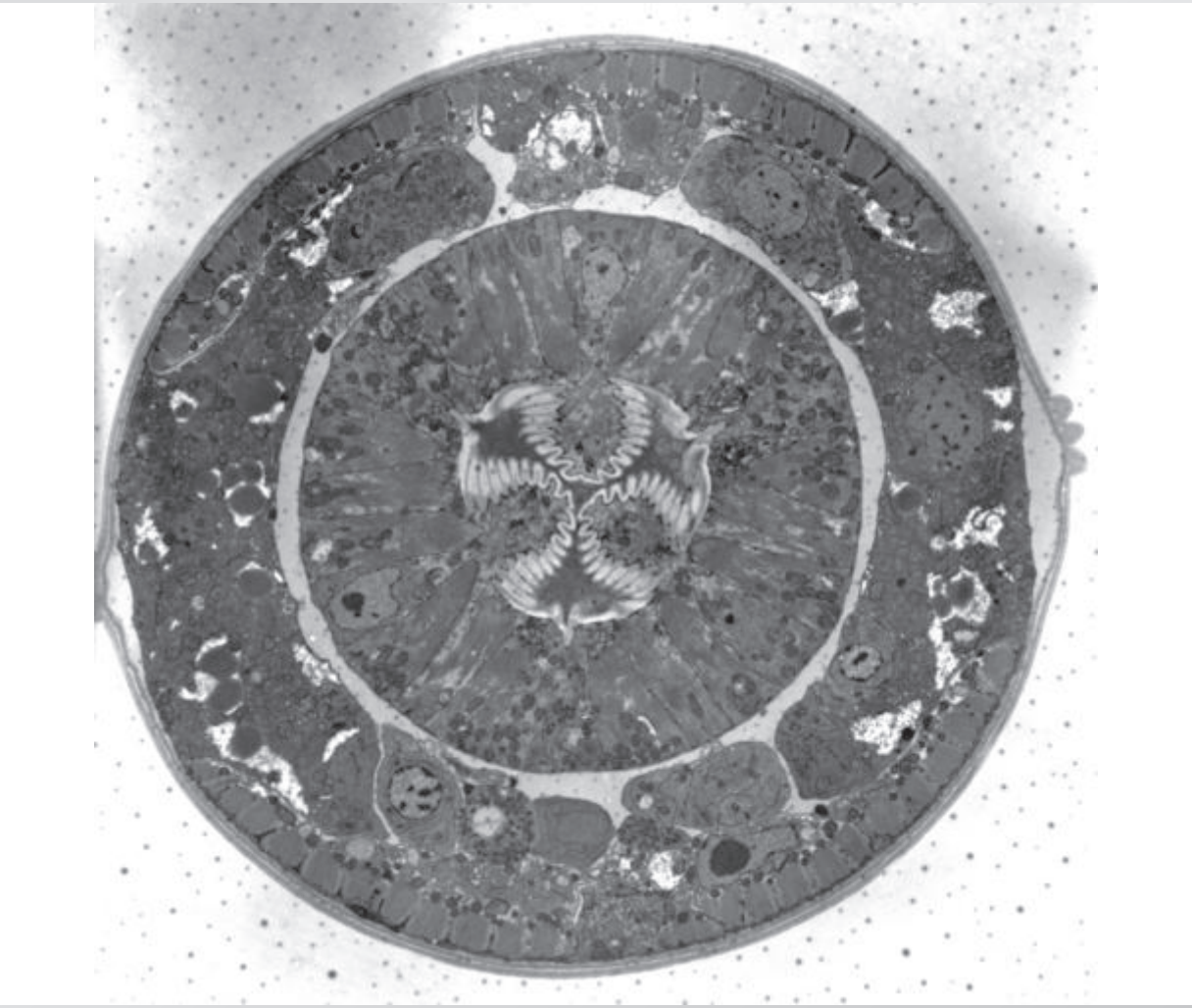


# Nanoneurosurgery



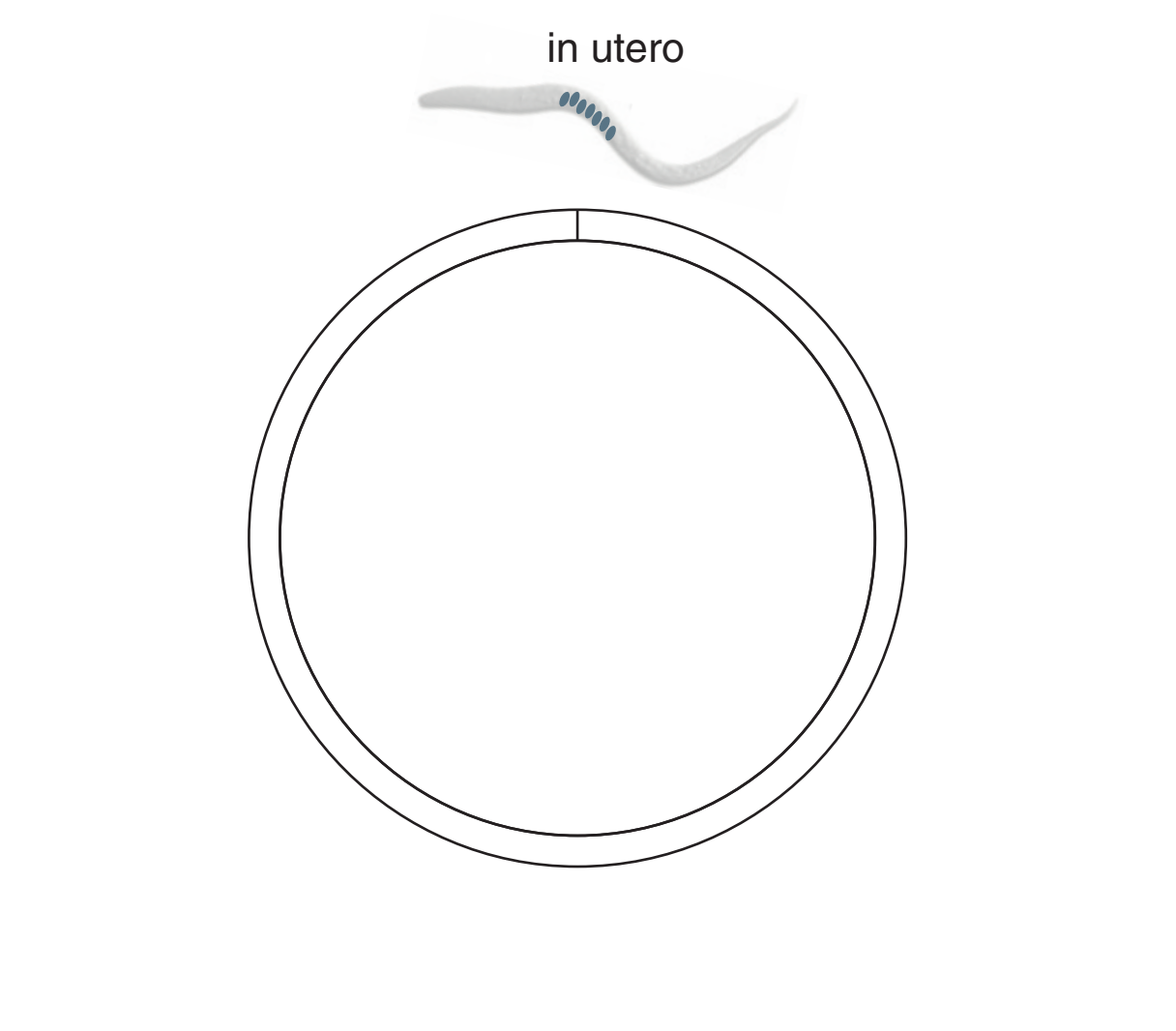
# Nanoneurosurgery

*Caenorhabditis elegans*



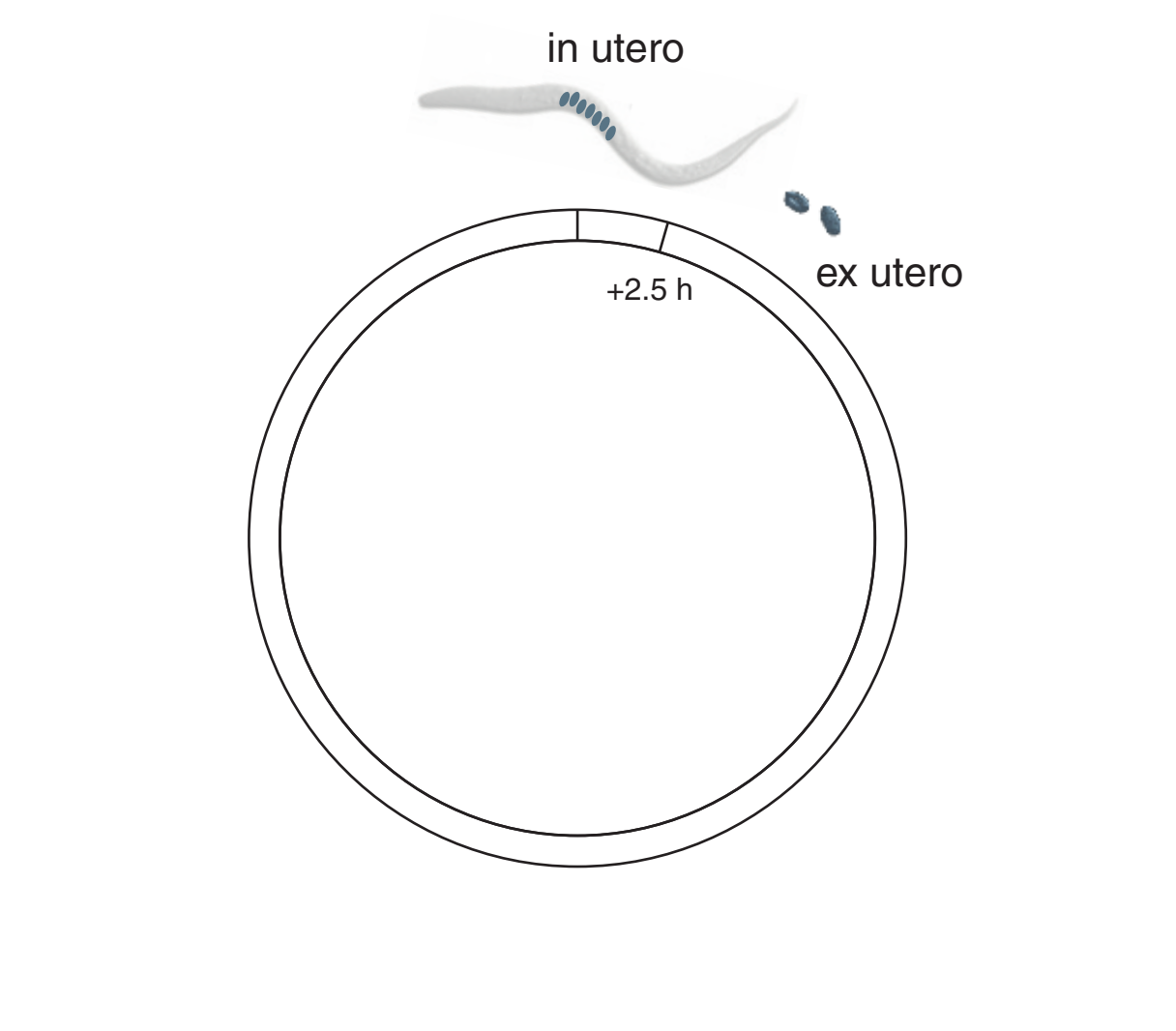
# Nanoneurosurgery

## *C. elegans* life cycle



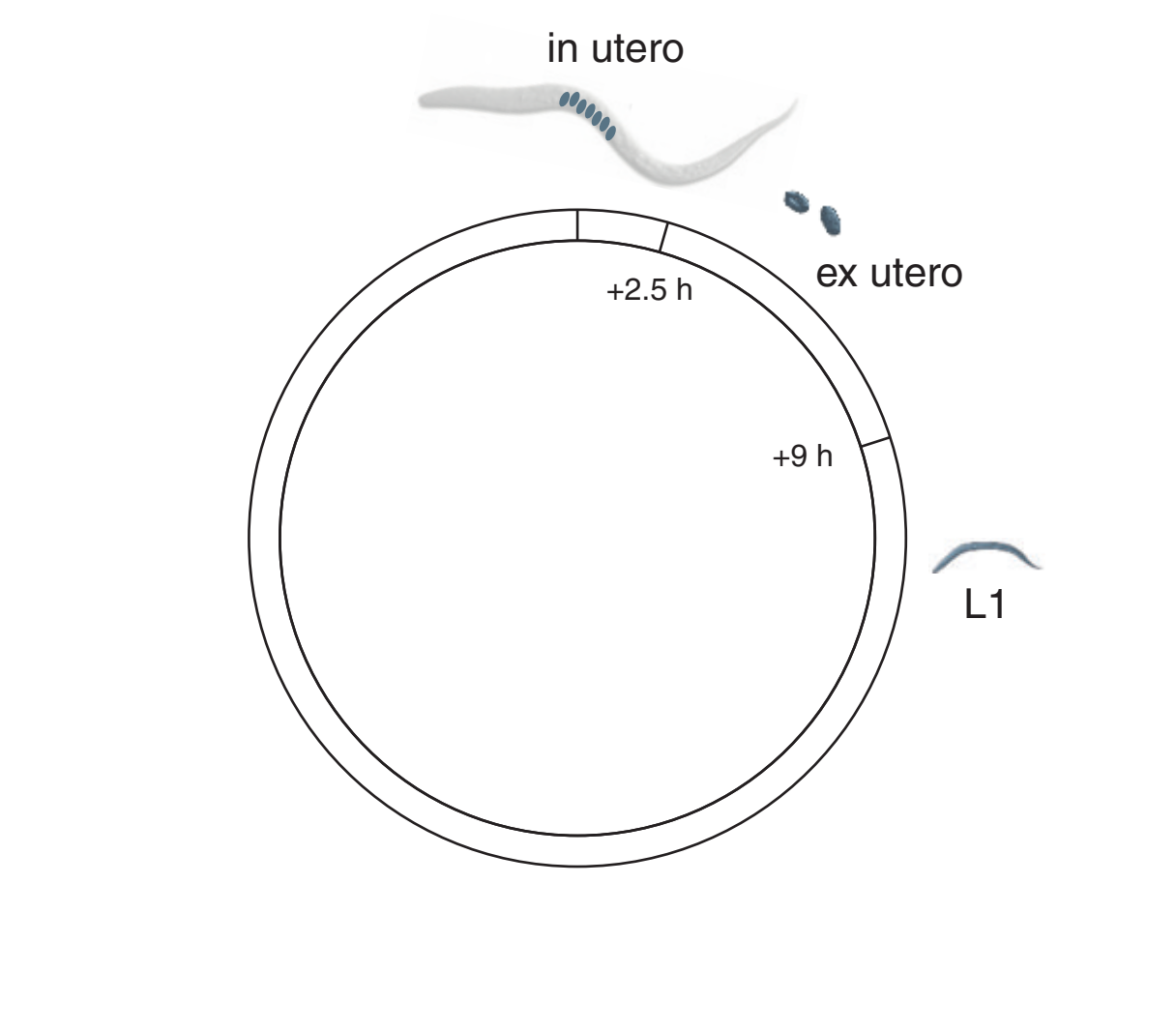
# Nanoneurosurgery

## *C. elegans* life cycle



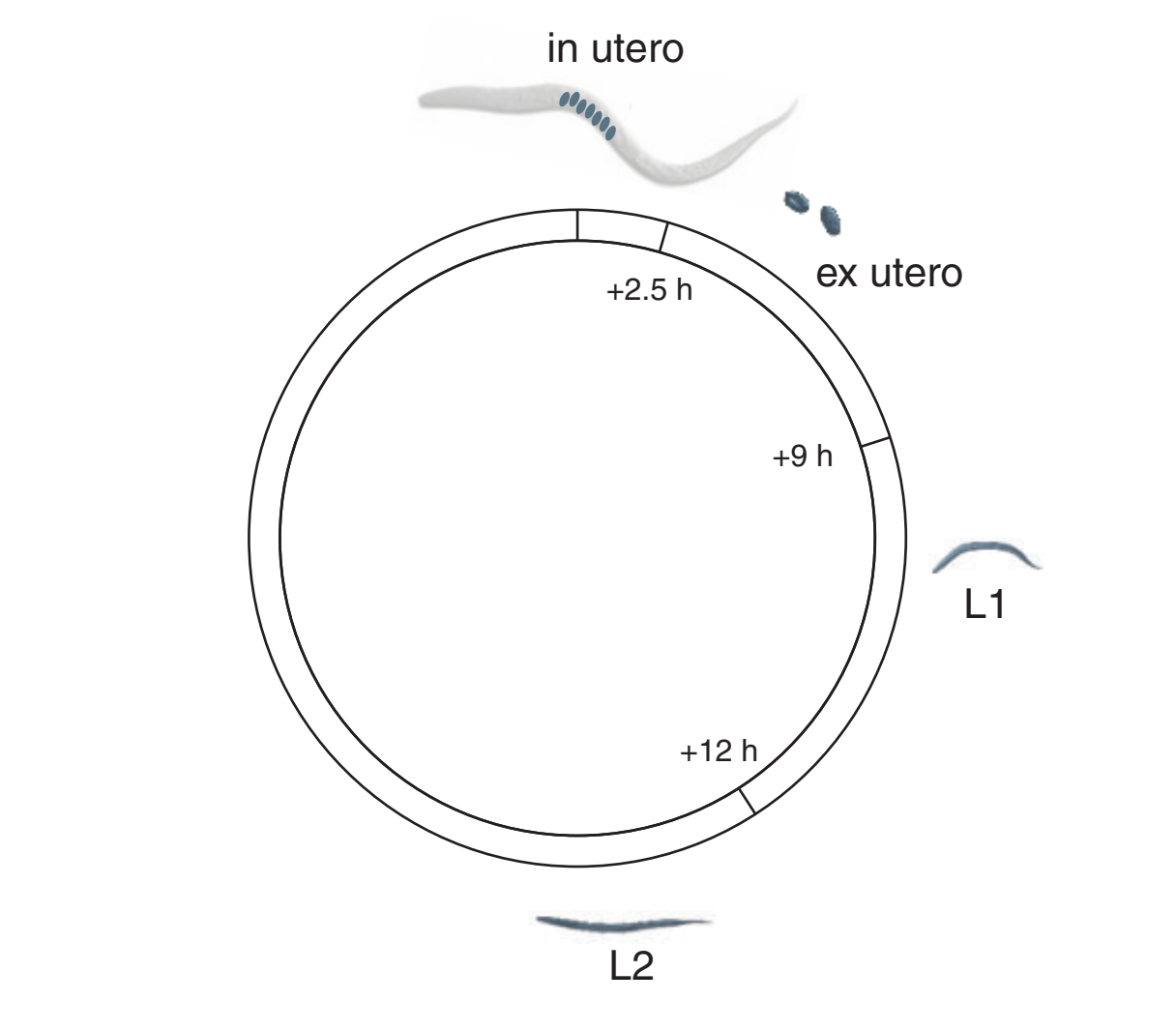
# Nanoneurosurgery

## *C. elegans* life cycle



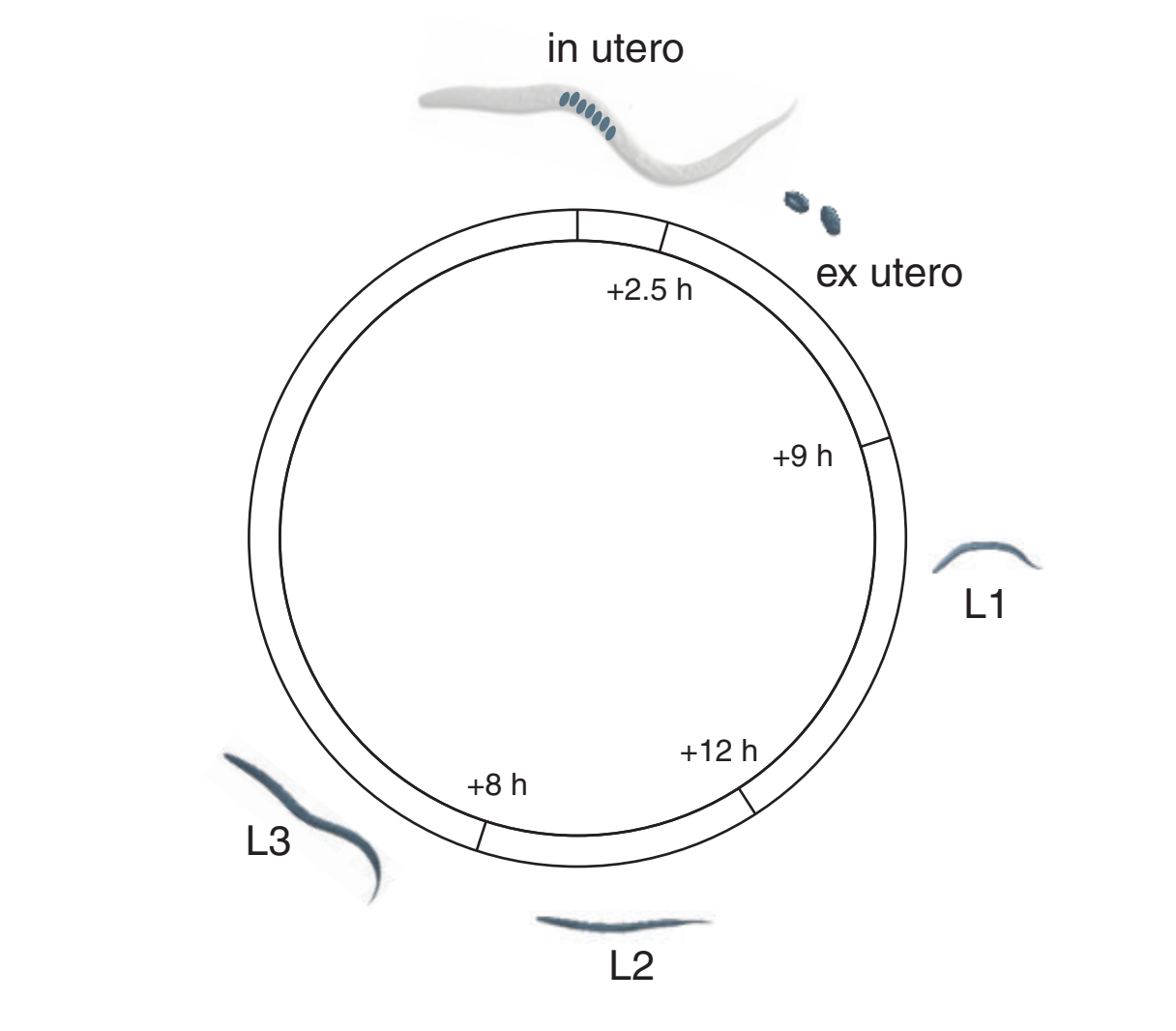
# Nanoneurosurgery

## *C. elegans* life cycle



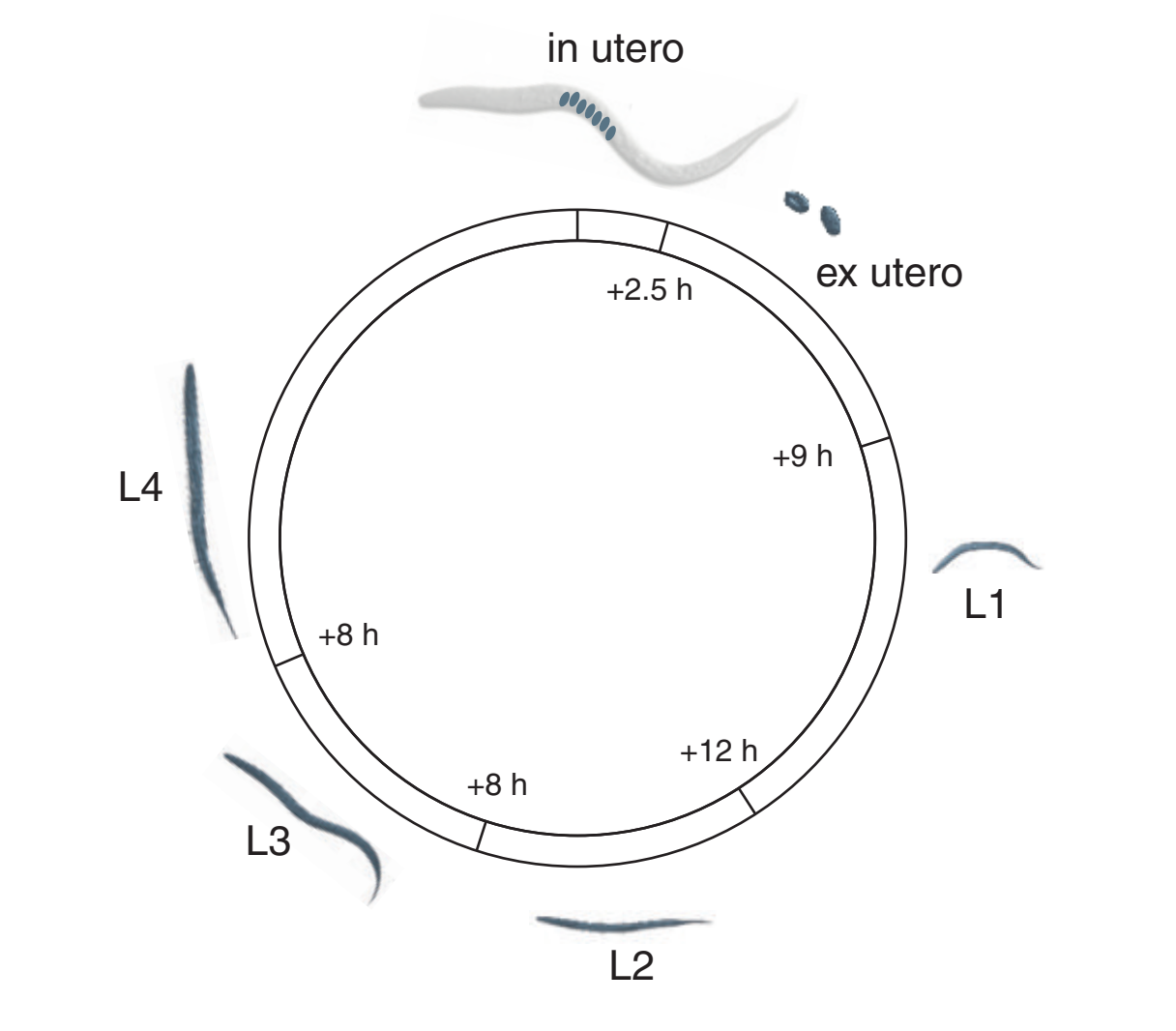
# Nanoneurosurgery

## *C. elegans* life cycle



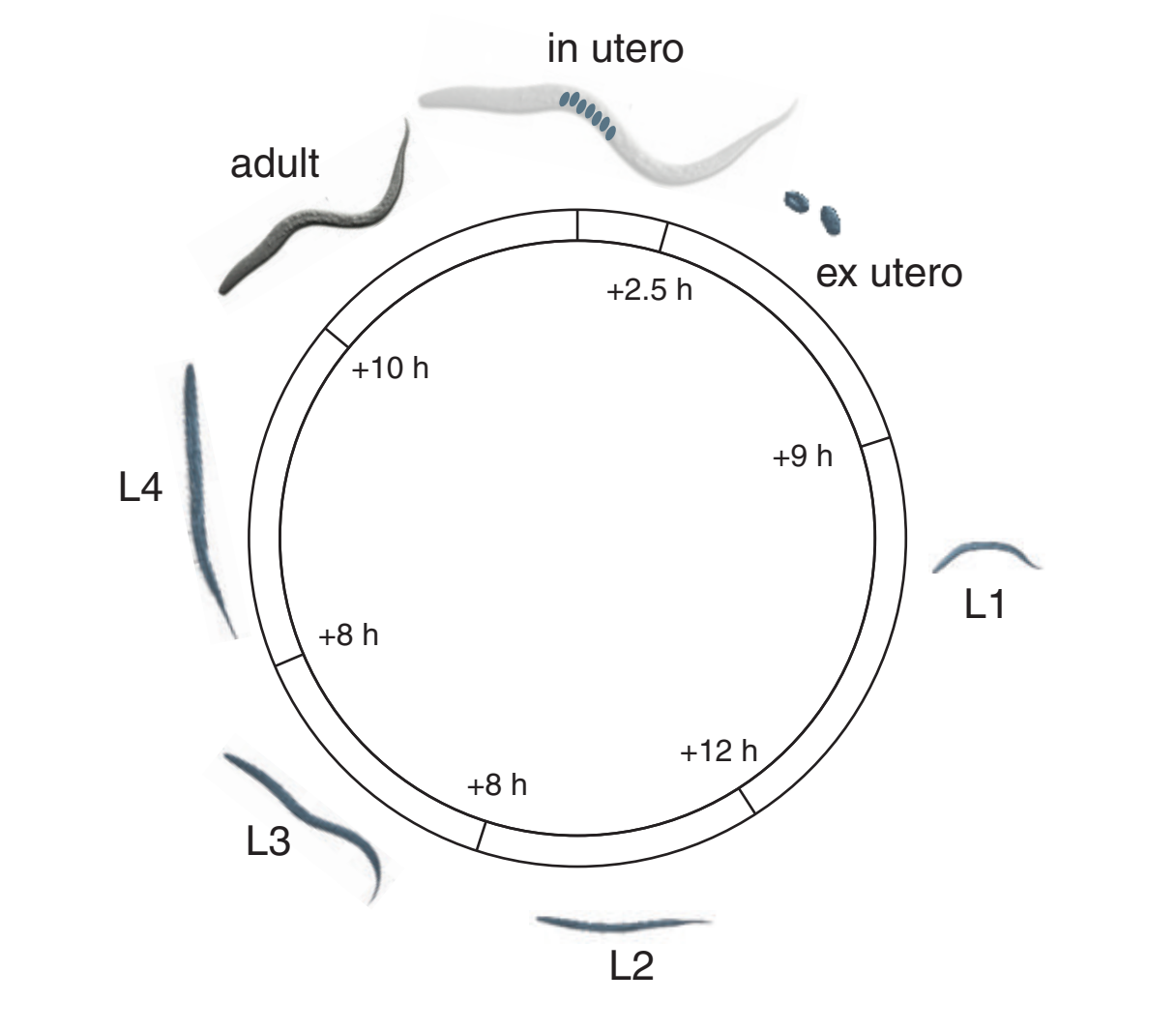
# Nanoneurosurgery

## *C. elegans* life cycle



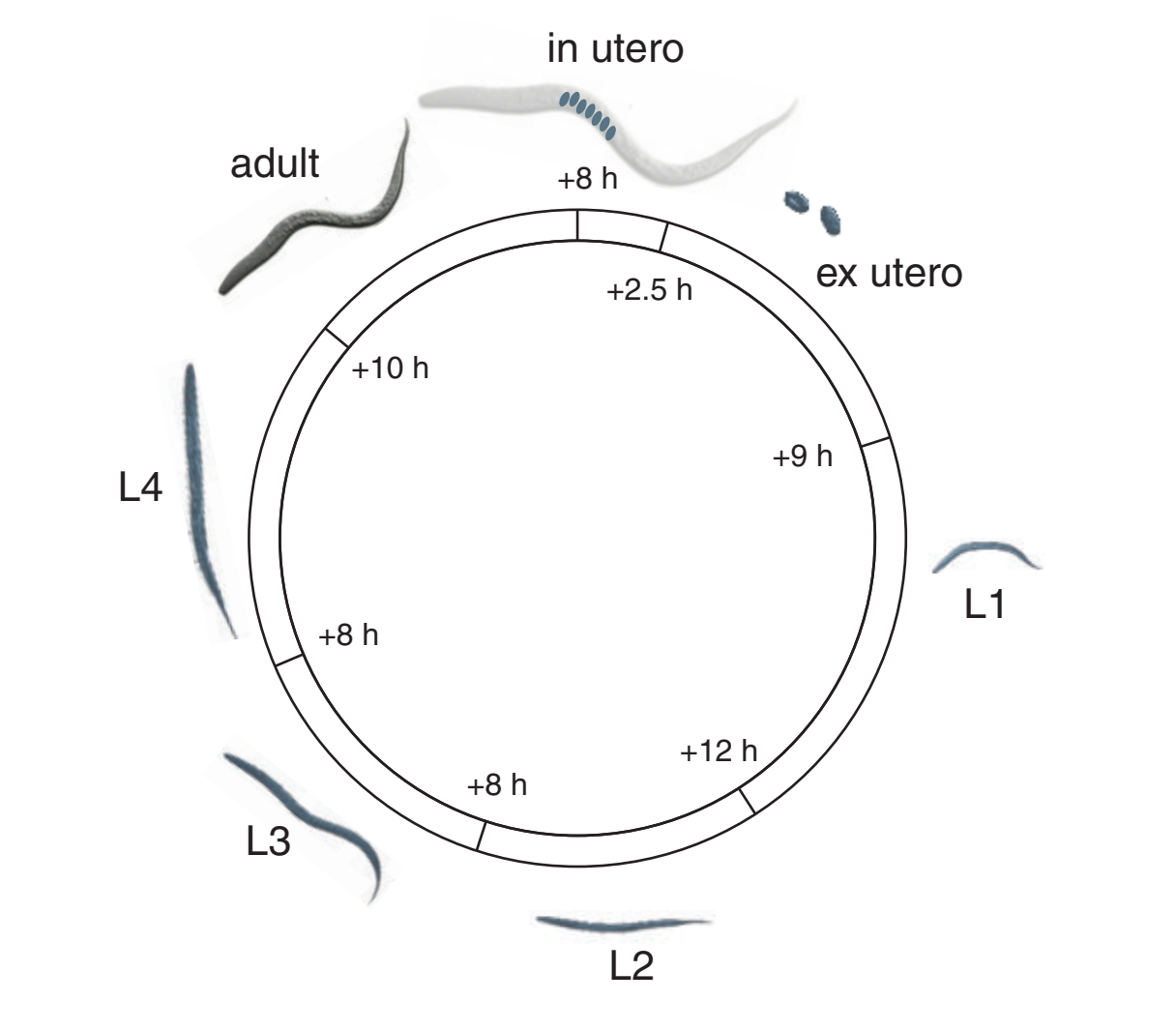
# Nanoneurosurgery

## *C. elegans* life cycle



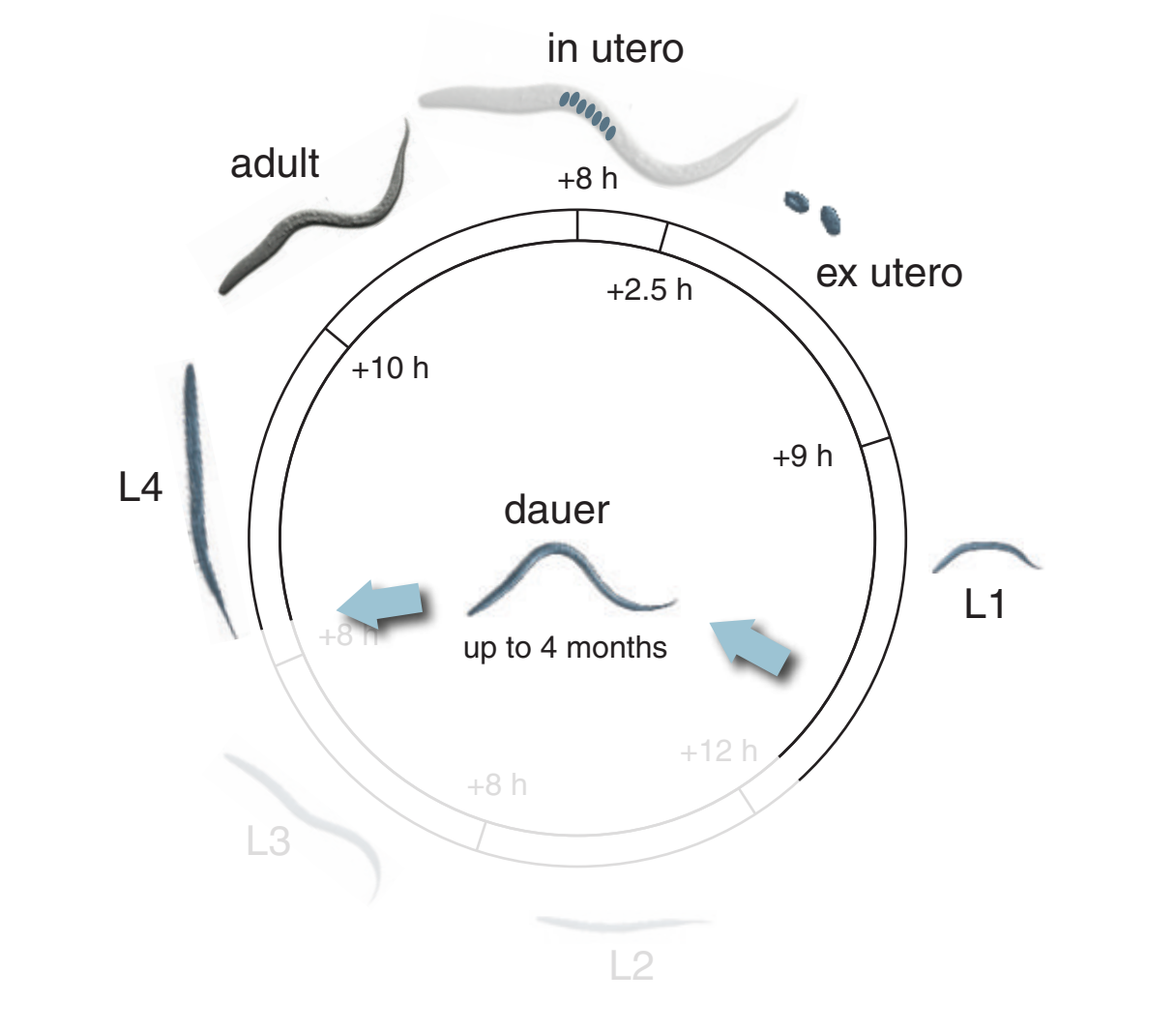
# Nanoneurosurgery

## *C. elegans* life cycle



# Nanoneurosurgery

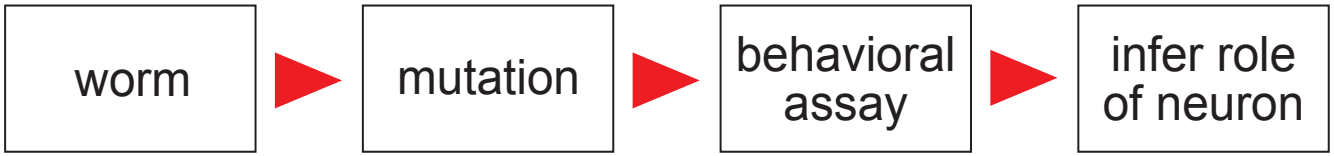
## *C. elegans* life cycle



# Nanoneurosurgery

## Mapping behavior to neurons

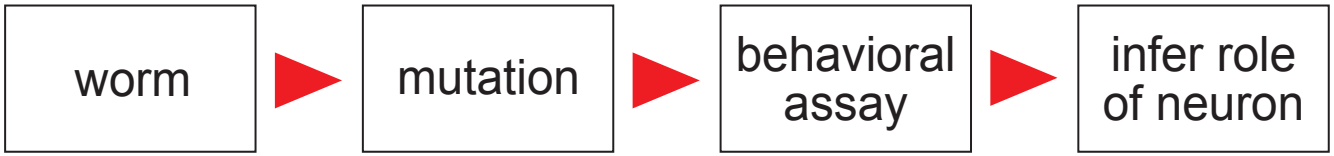
conventional method



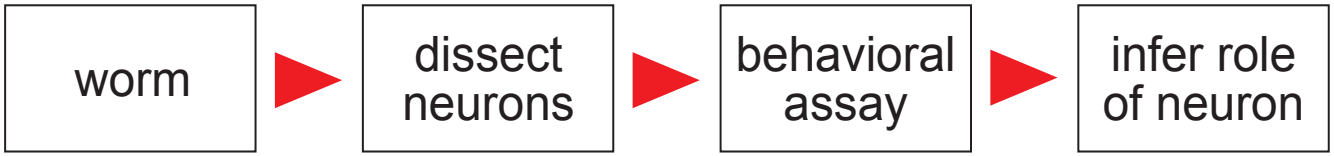
# Nanoneurosurgery

## Mapping behavior to neurons

conventional method




femtosecond laser ablation



# Nanoneurosurgery

## ASH neurons

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

# Nanoneurosurgery

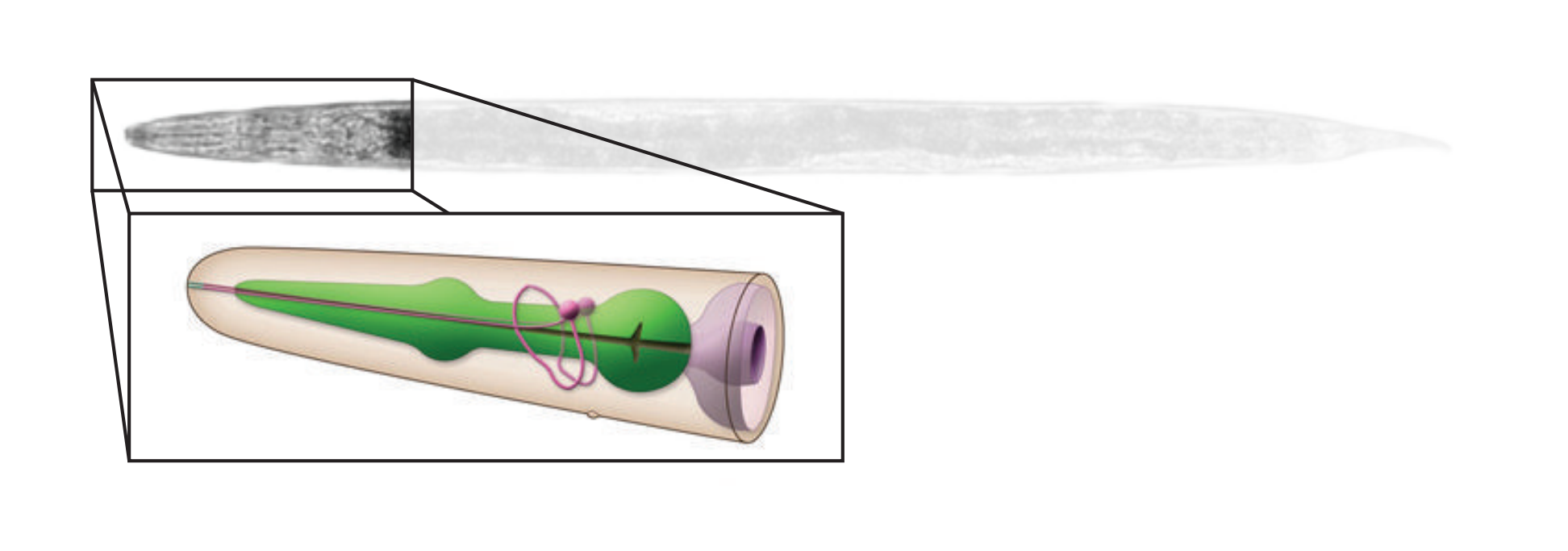
## ASH neurons

# Nanoneurosurgery

## ASH neurons

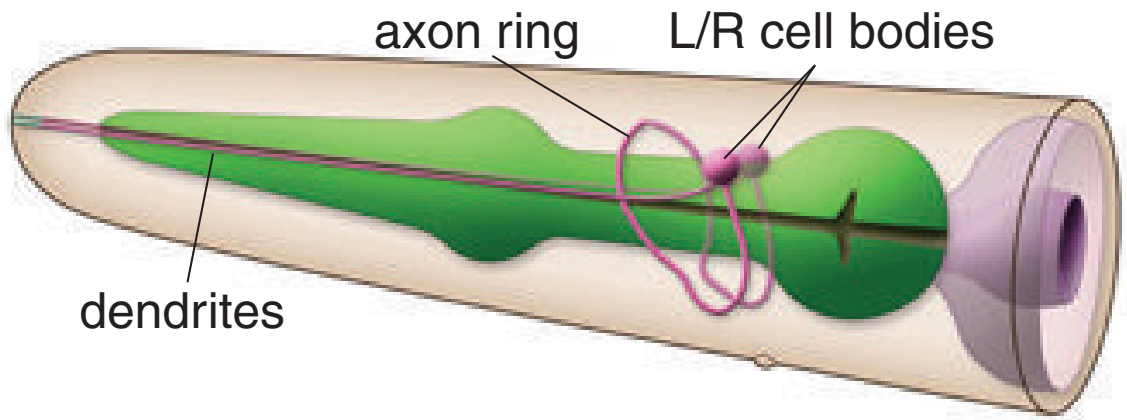
# Nanoneurosurgery

## ASH neurons



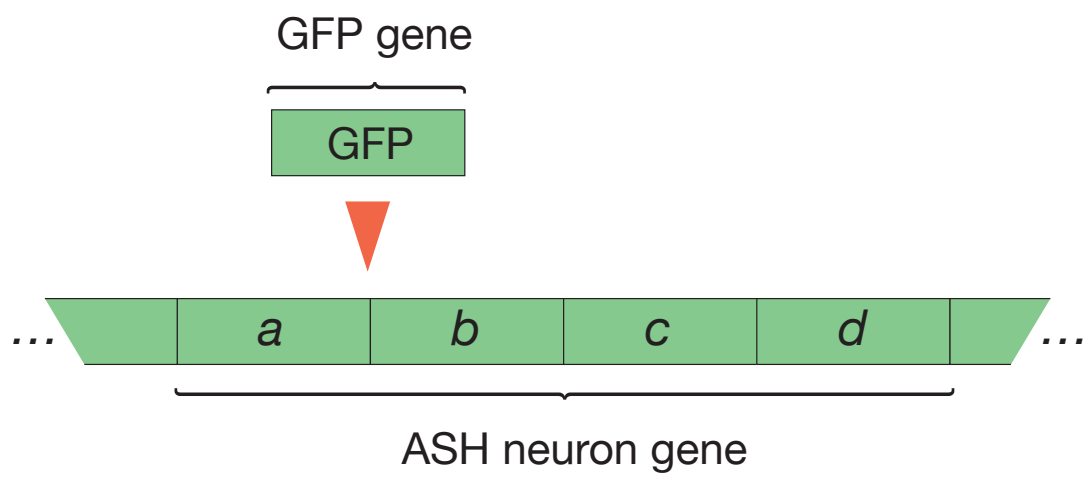
# Nanoneurosurgery

## ASH neurons



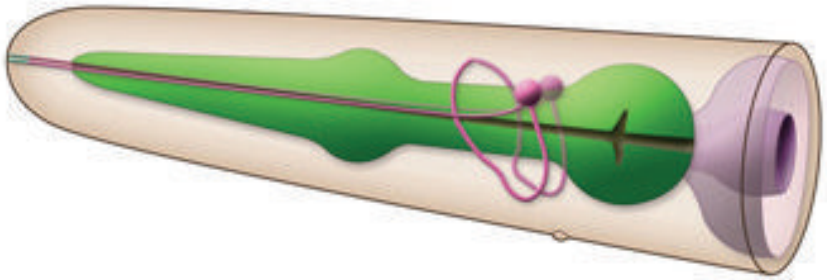
# Nanoneurosurgery

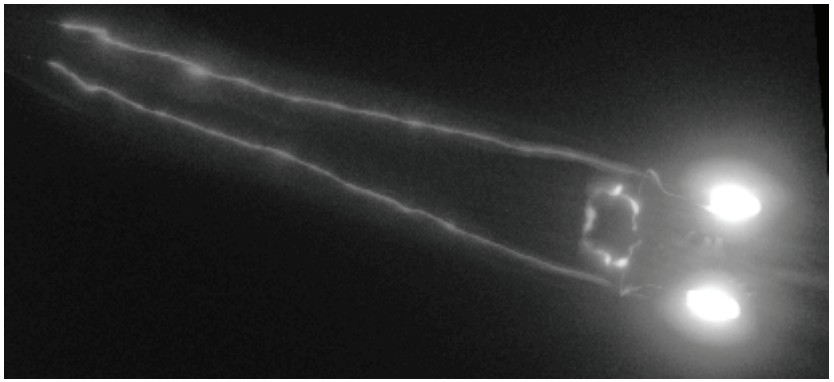
## make ASH neurons express GFP



# Nanoneurosurgery

**make ASH neurons express GFP**



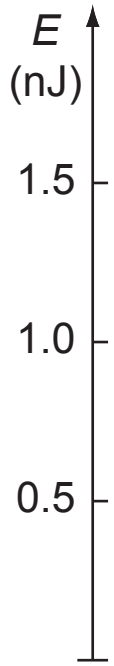


# Nanoneurosurgery

GFP: absorbs UV, emits green

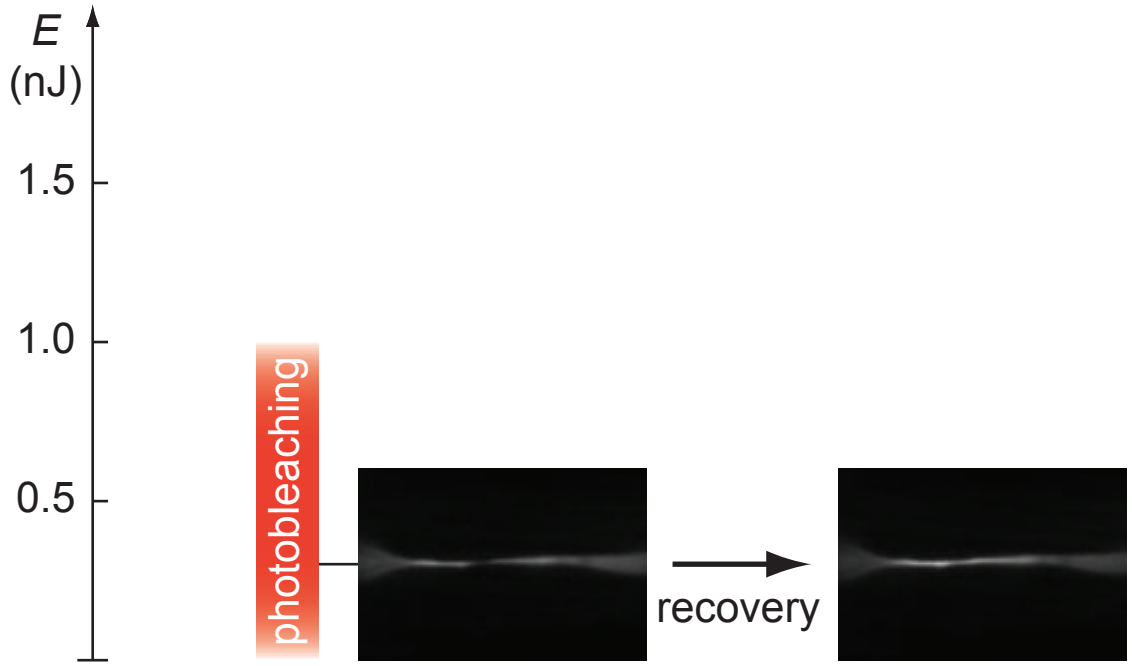
# Nanoneurosurgery

## axon ablation



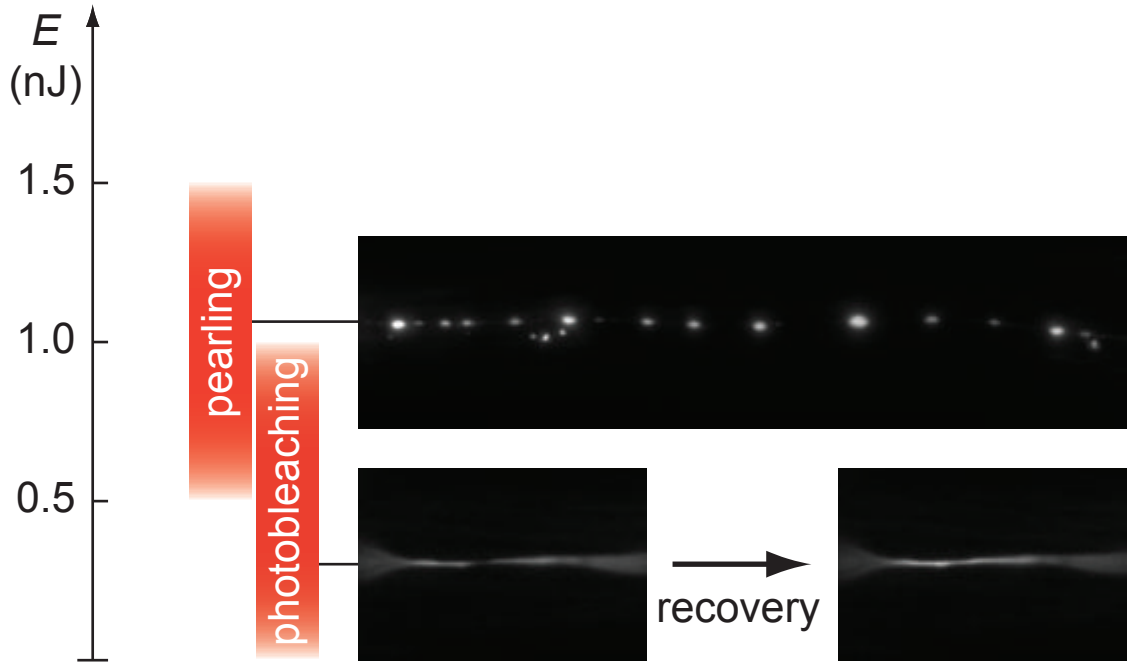
# Nanoneurosurgery

## axon ablation



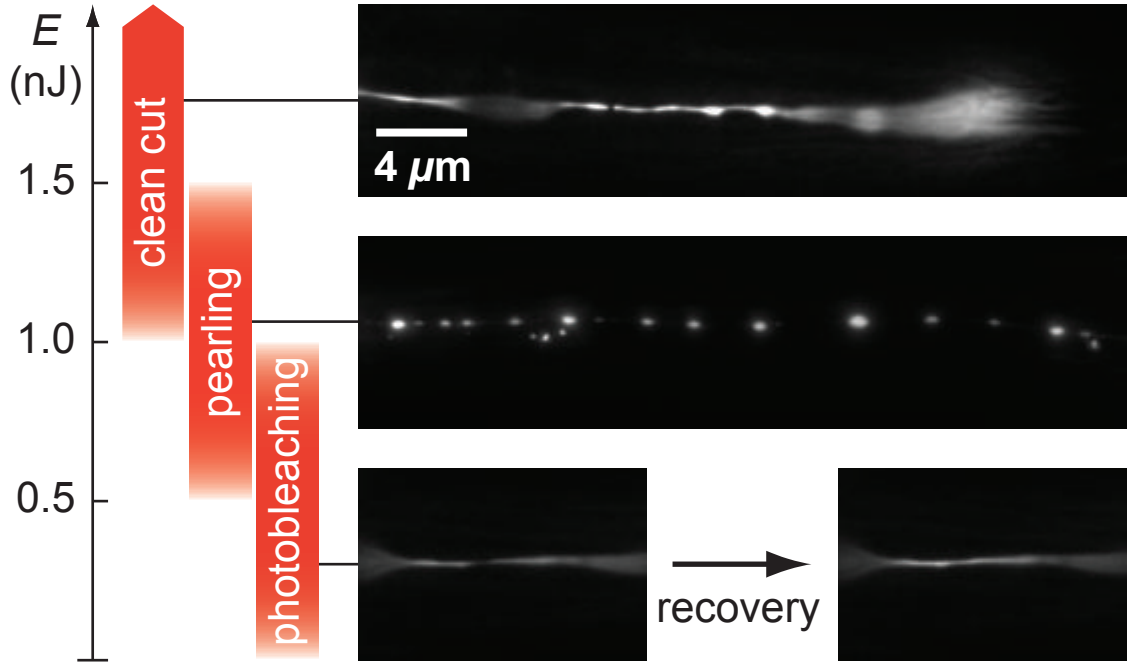
# Nanoneurosurgery

## axon ablation



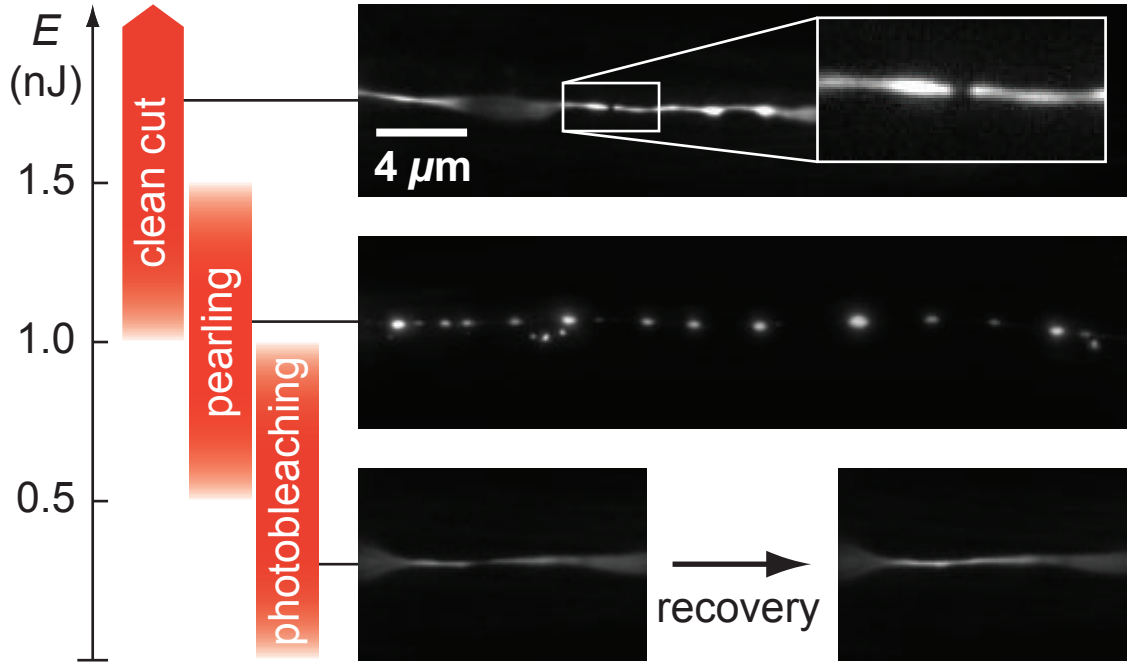
# Nanoneurosurgery

## axon ablation



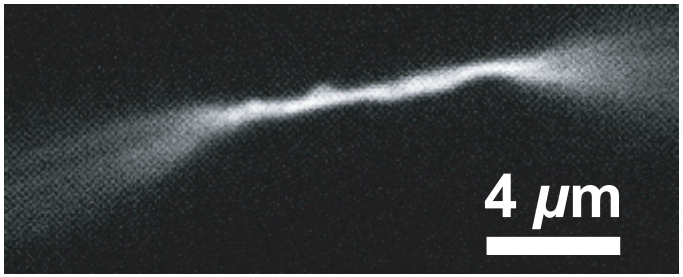
# Nanoneurosurgery

## axon ablation



# Nanoneurosurgery

retraction of cut dendrite (6 nJ)



# Nanoneurosurgery

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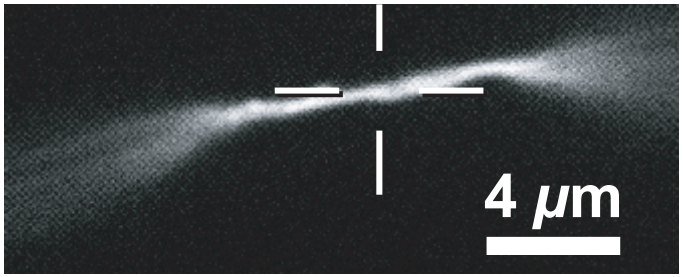
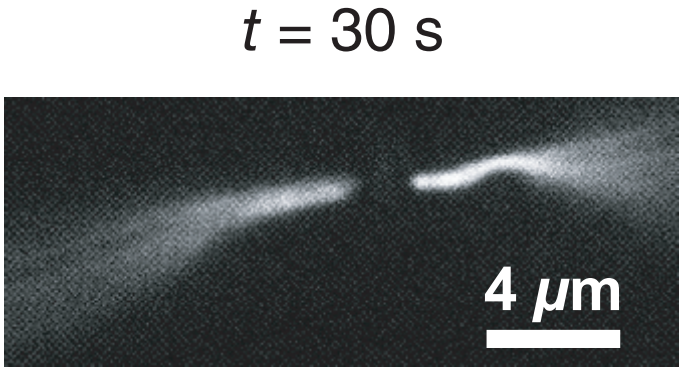


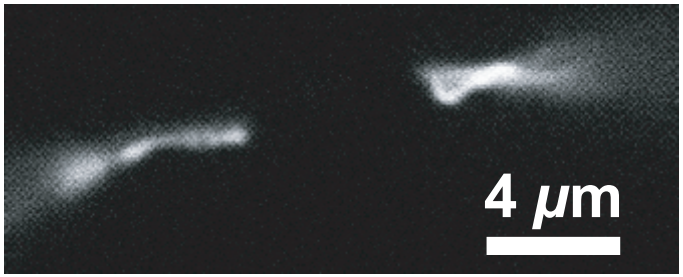
# Nanoneurosurgery

## retraction of cut dendrite (6 nJ)



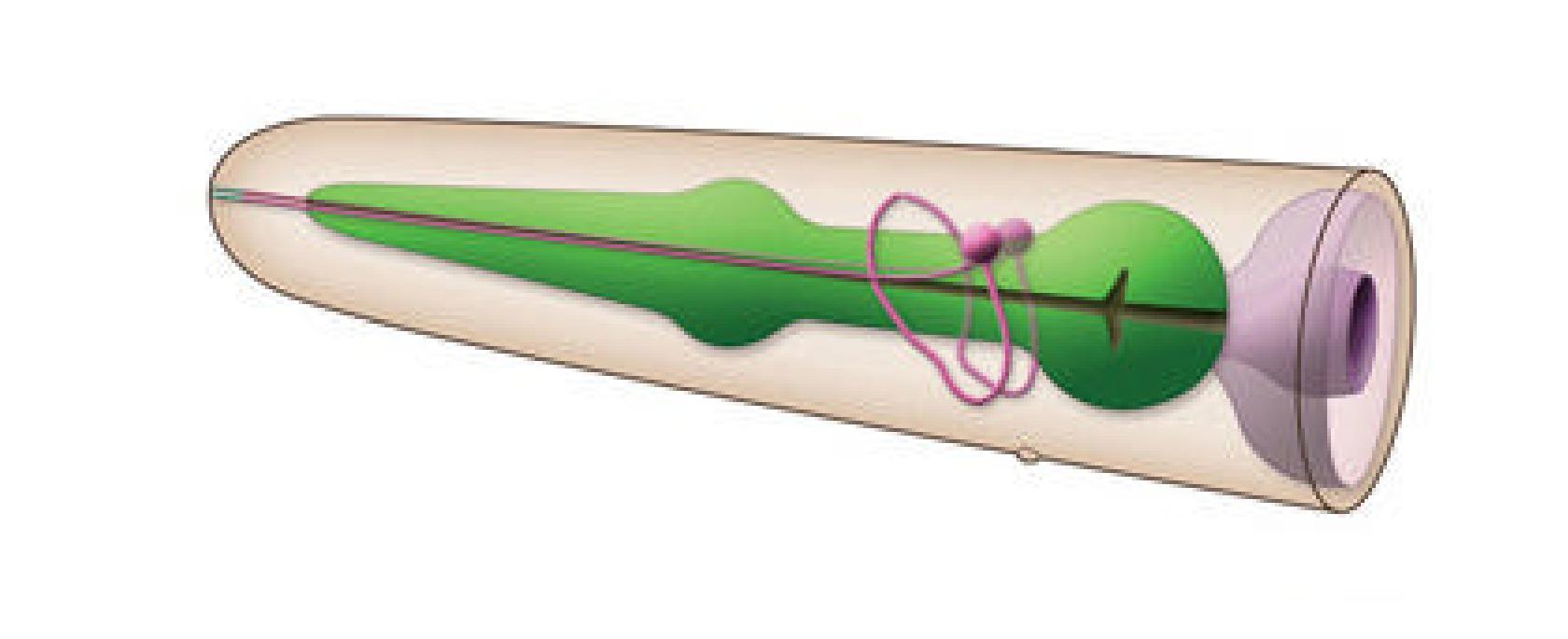
# Nanoneurosurgery

## retraction of cut dendrite (6 nJ)



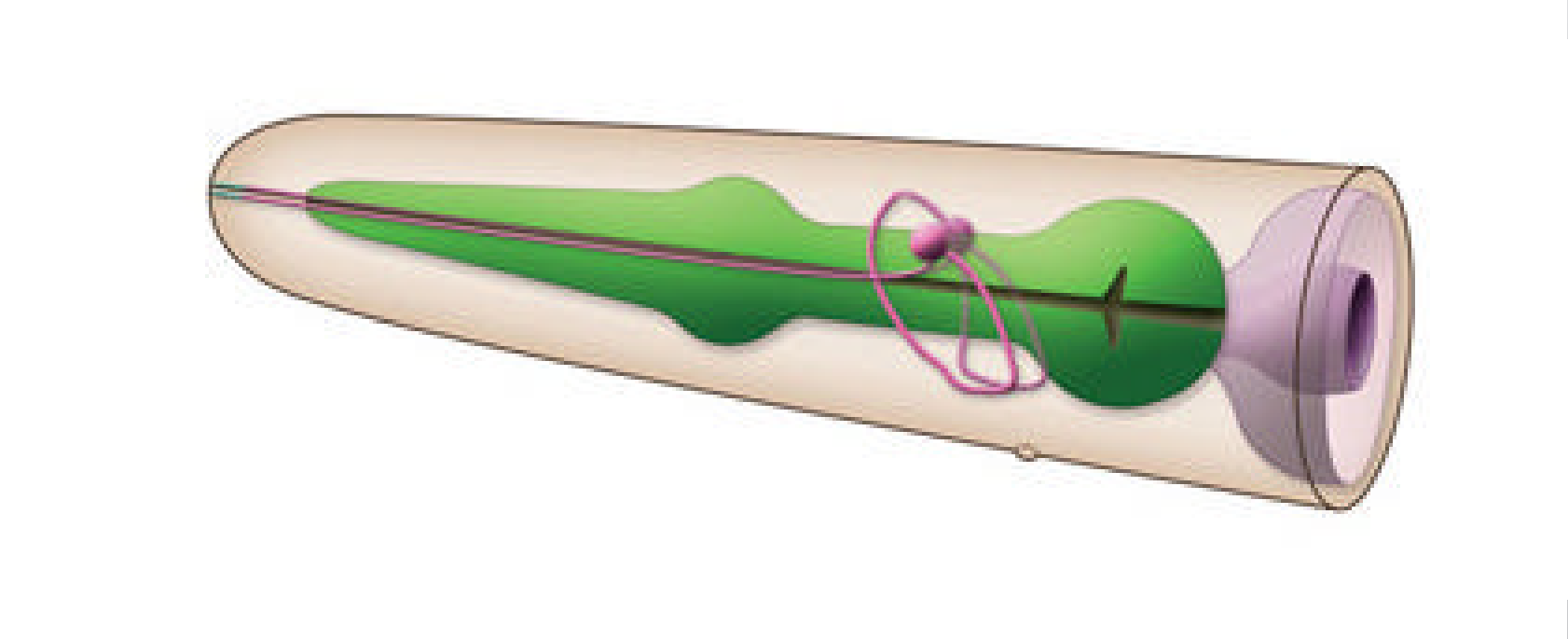
# Nanoneurosurgery

## ASH neurons



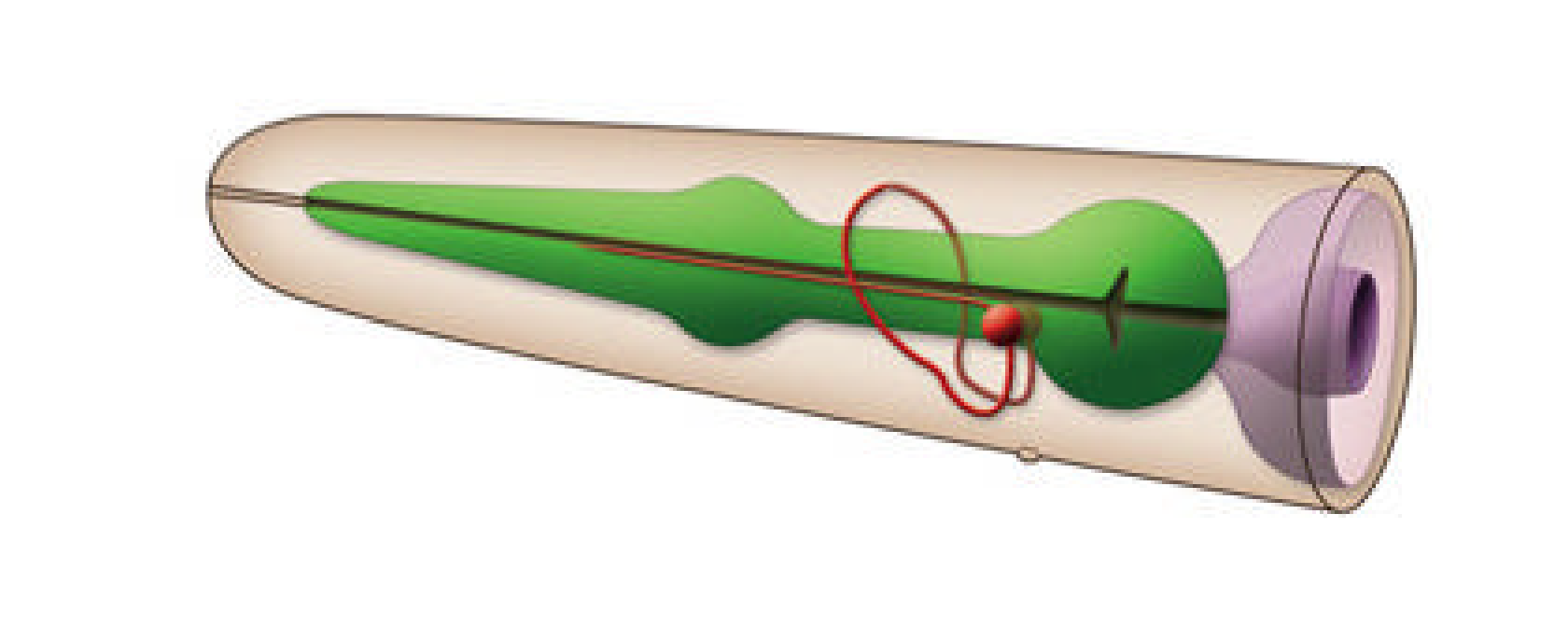
# Nanoneurosurgery

## ASK neurons



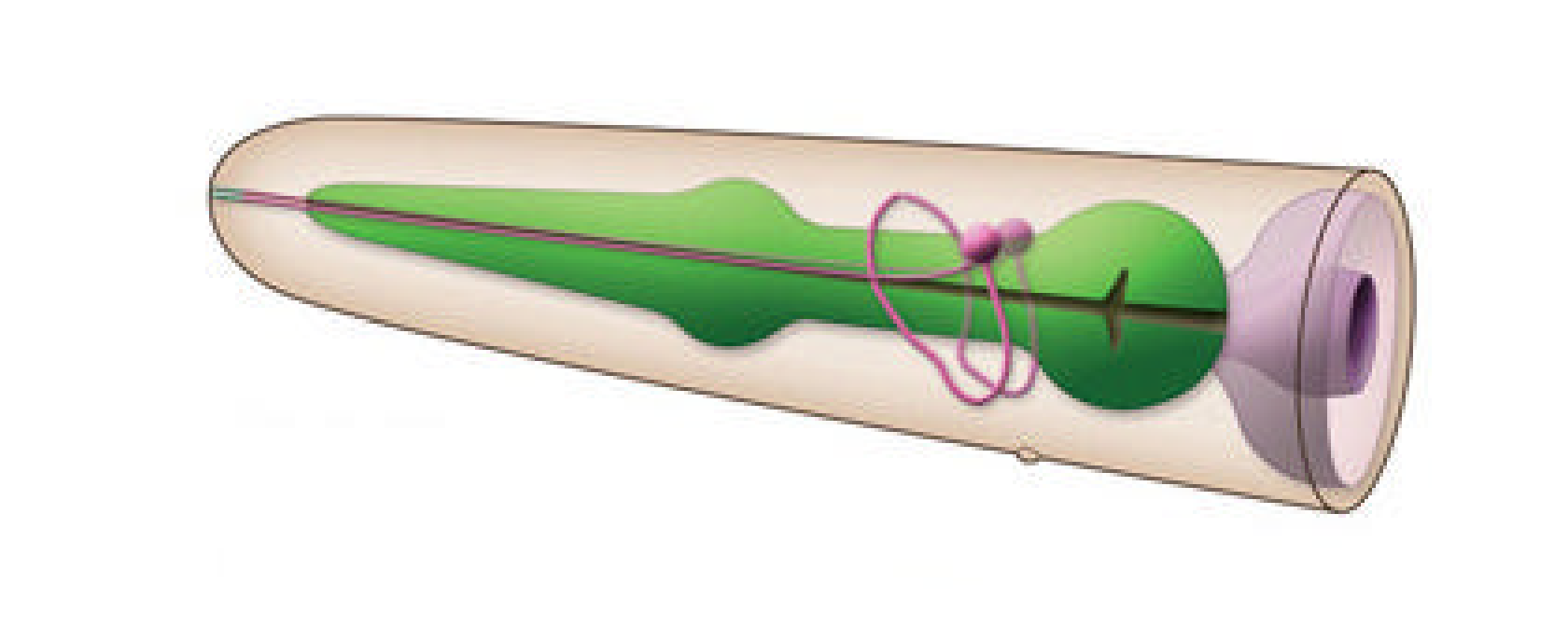
# Nanoneurosurgery

## AUA neurons



# Nanoneurosurgery

## ASI neurons

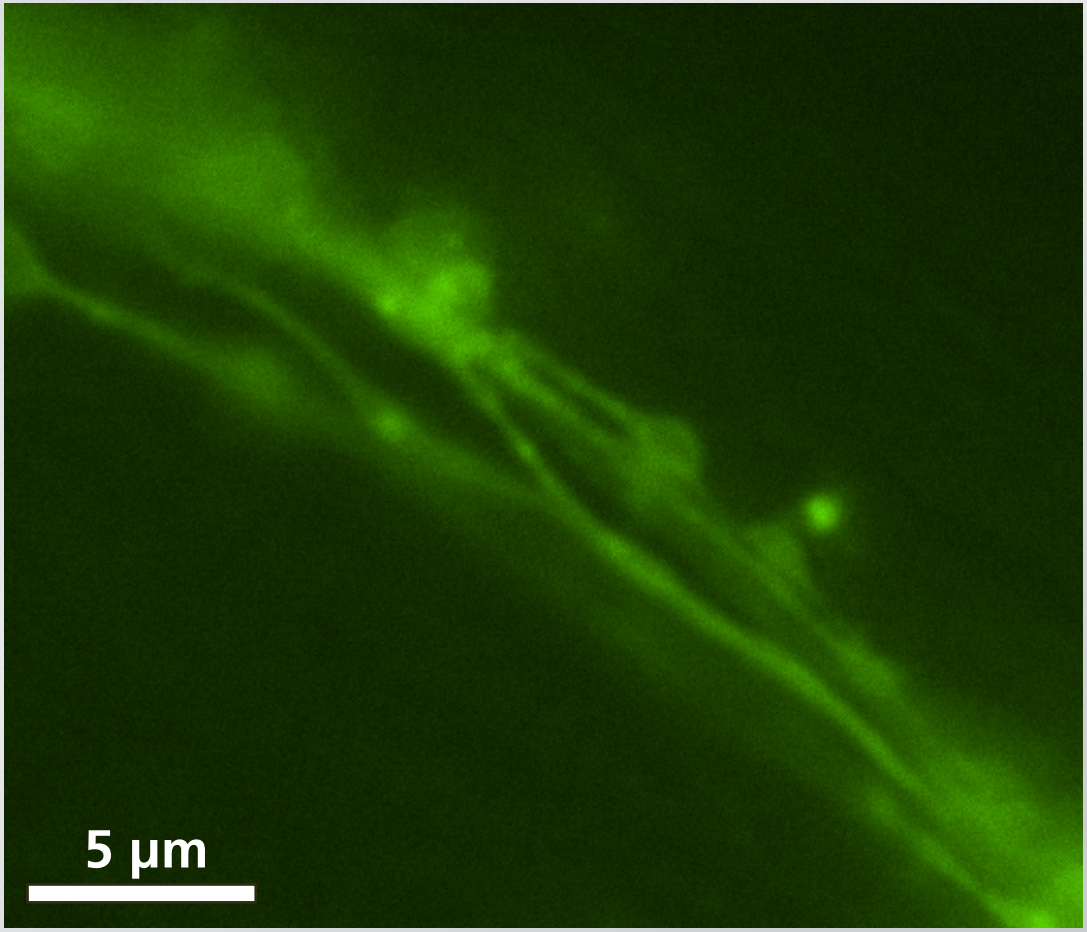


# Nanoneurosurgery

need exquisite precision!

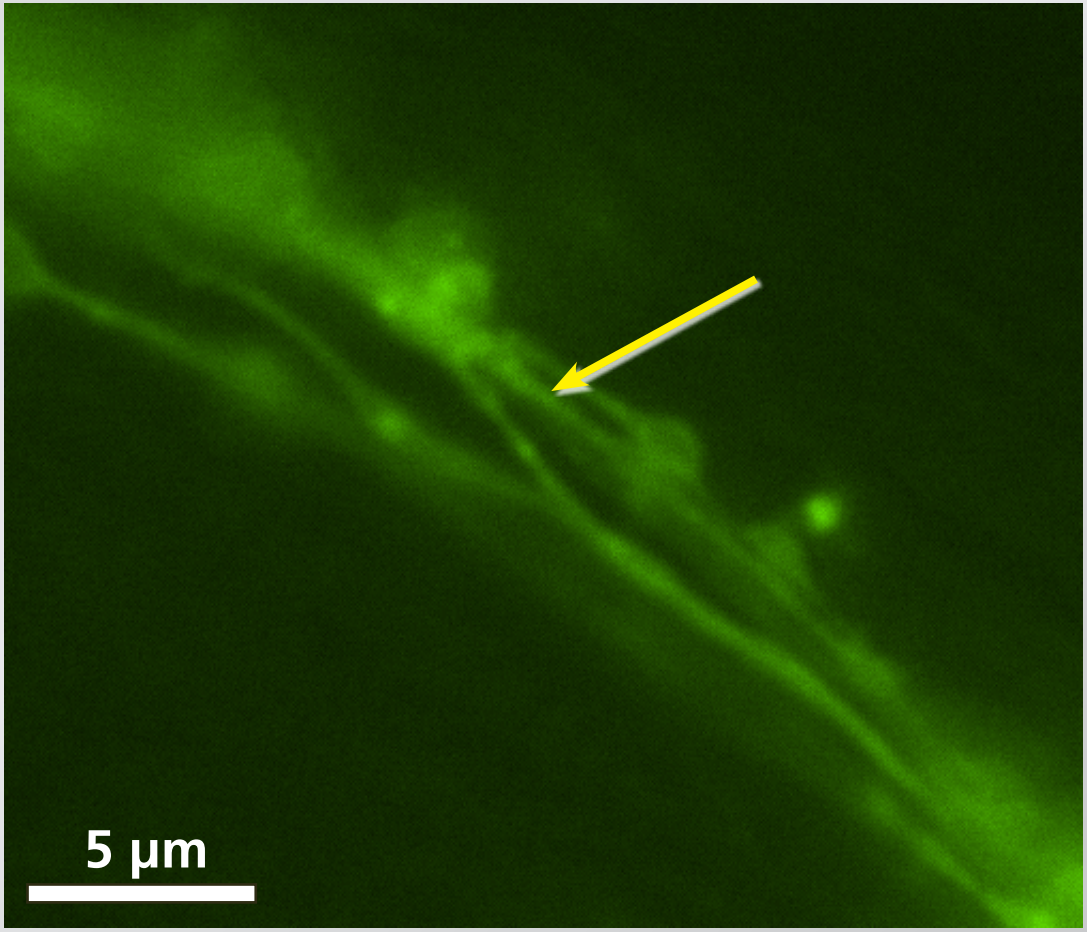
# Nanoneurosurgery

## DiO-stained bundle of dendrites



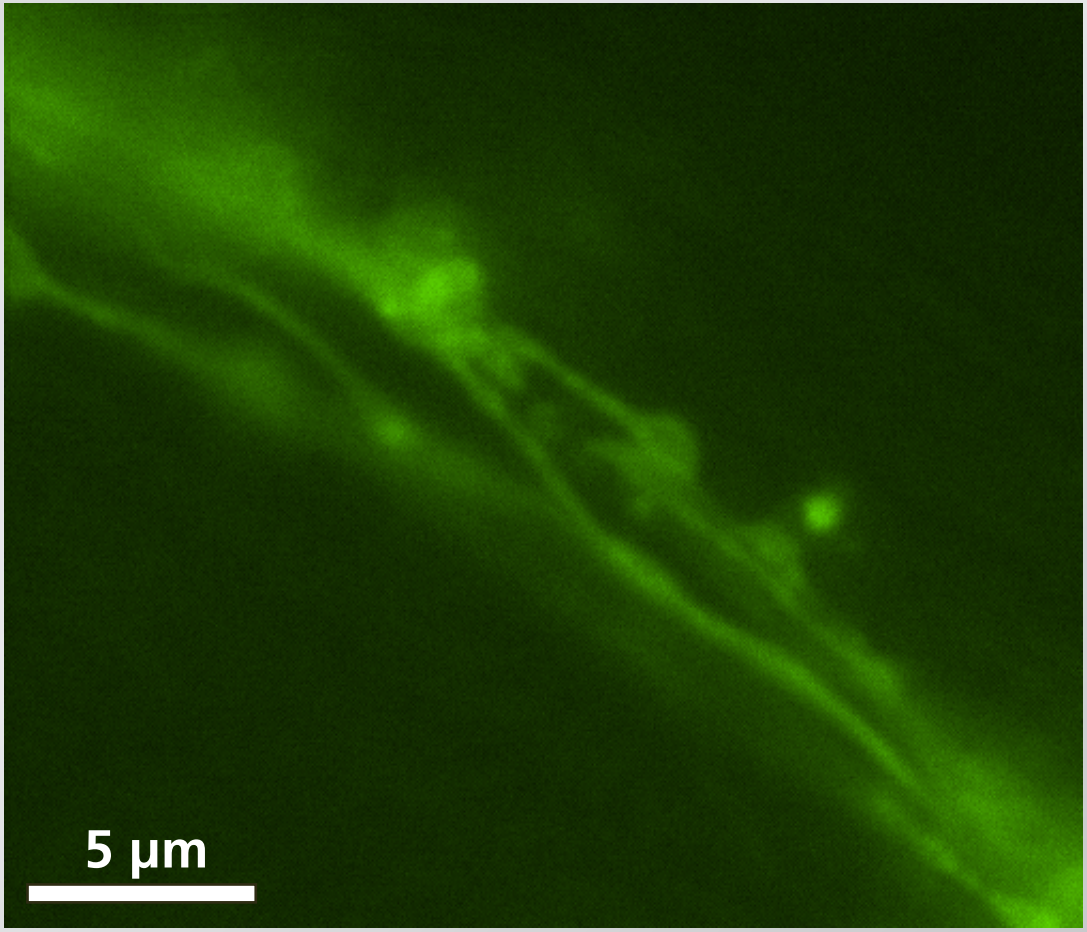
# Nanoneurosurgery

cut single dendrite in bundle (3 nJ)



# Nanoneurosurgery

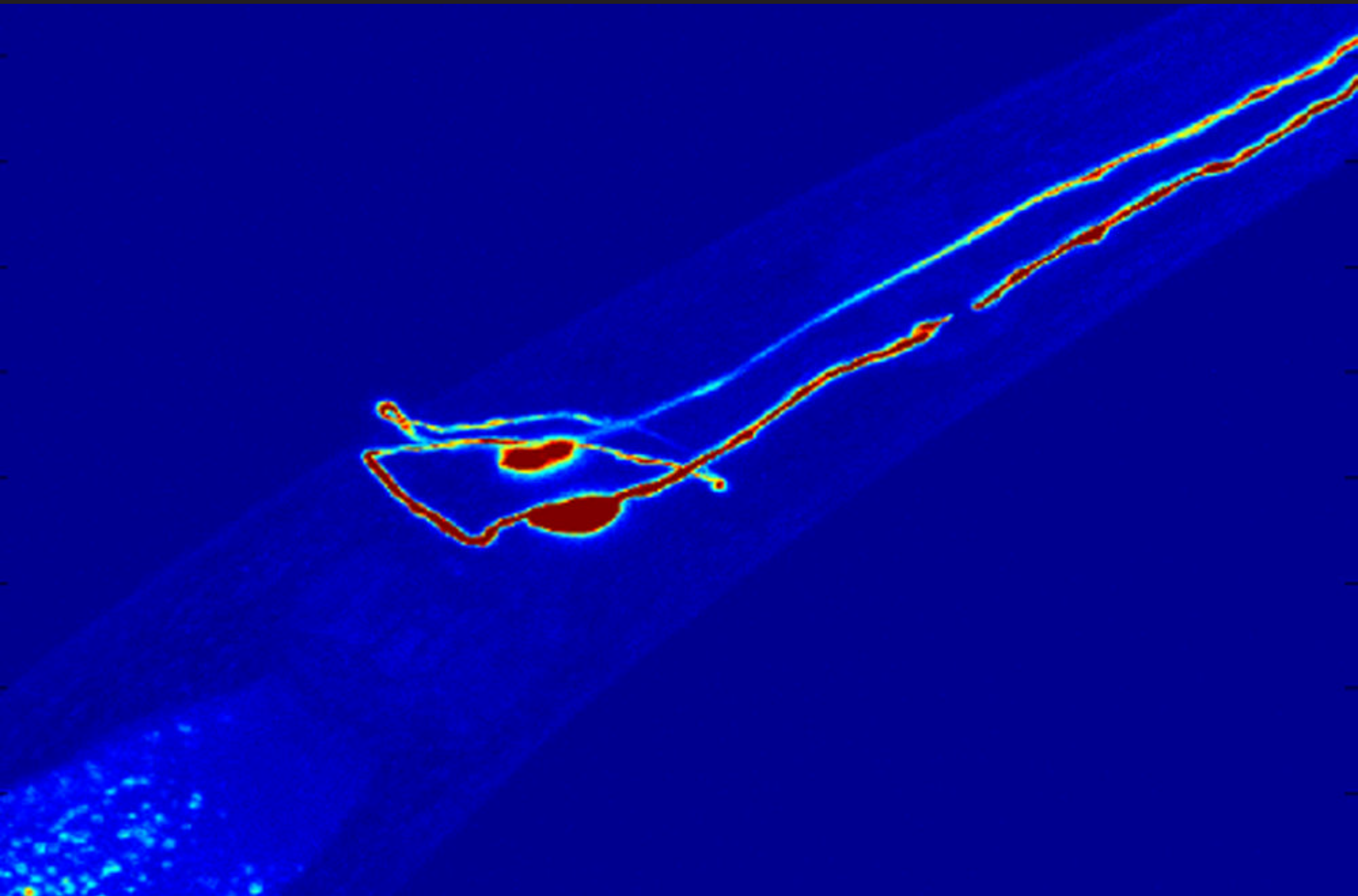
no damange to neighboring dendrites



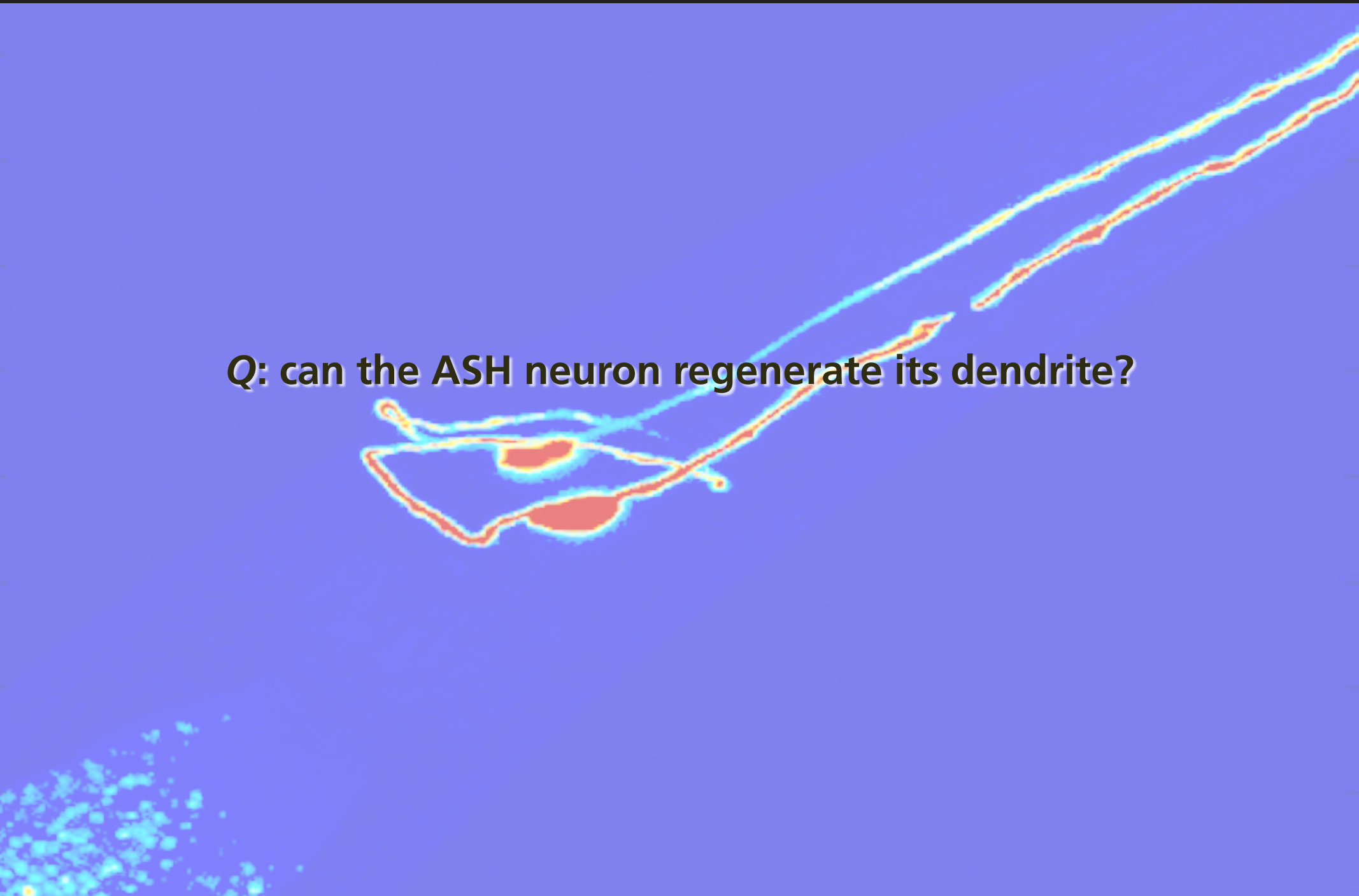
# Nanoneurosurgery

revive worm, reimage 1 day later

# Nanoneurosurgery



# Nanoneurosurgery



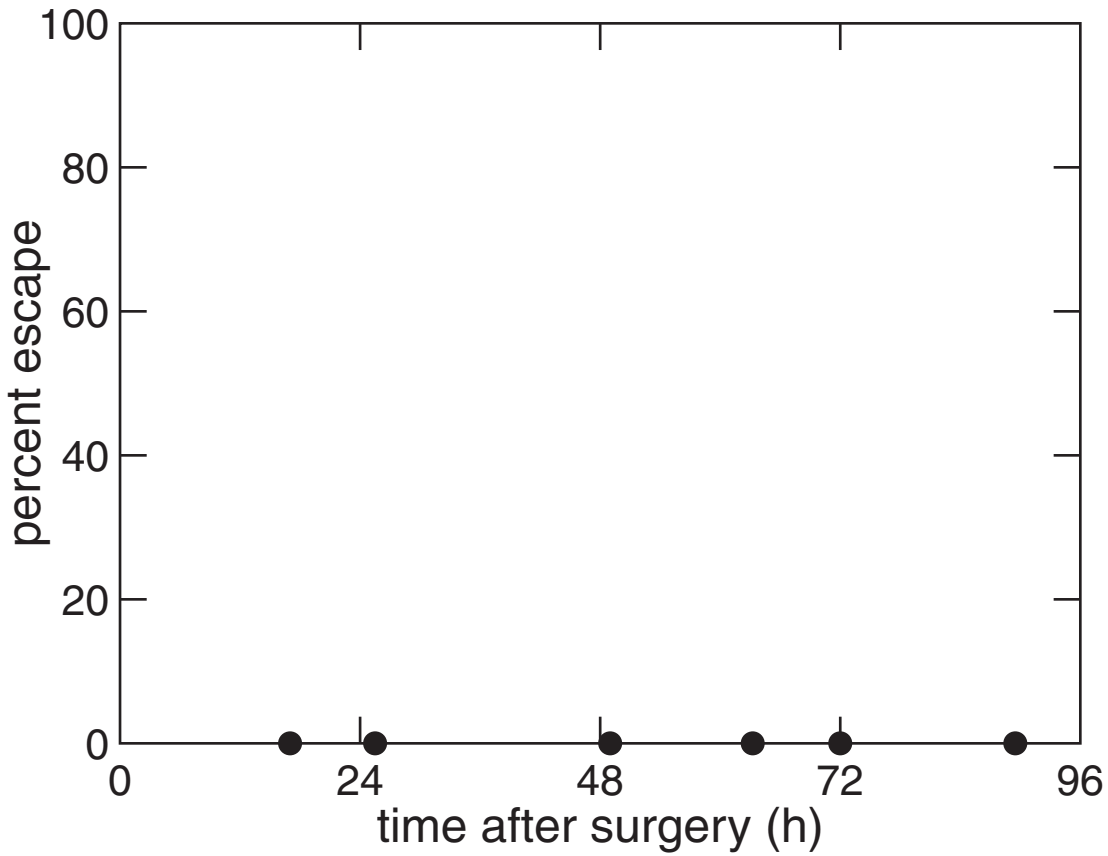
*Q*: can the ASH neuron regenerate its dendrite?

# Nanoneurosurgery

## osmolarity assay

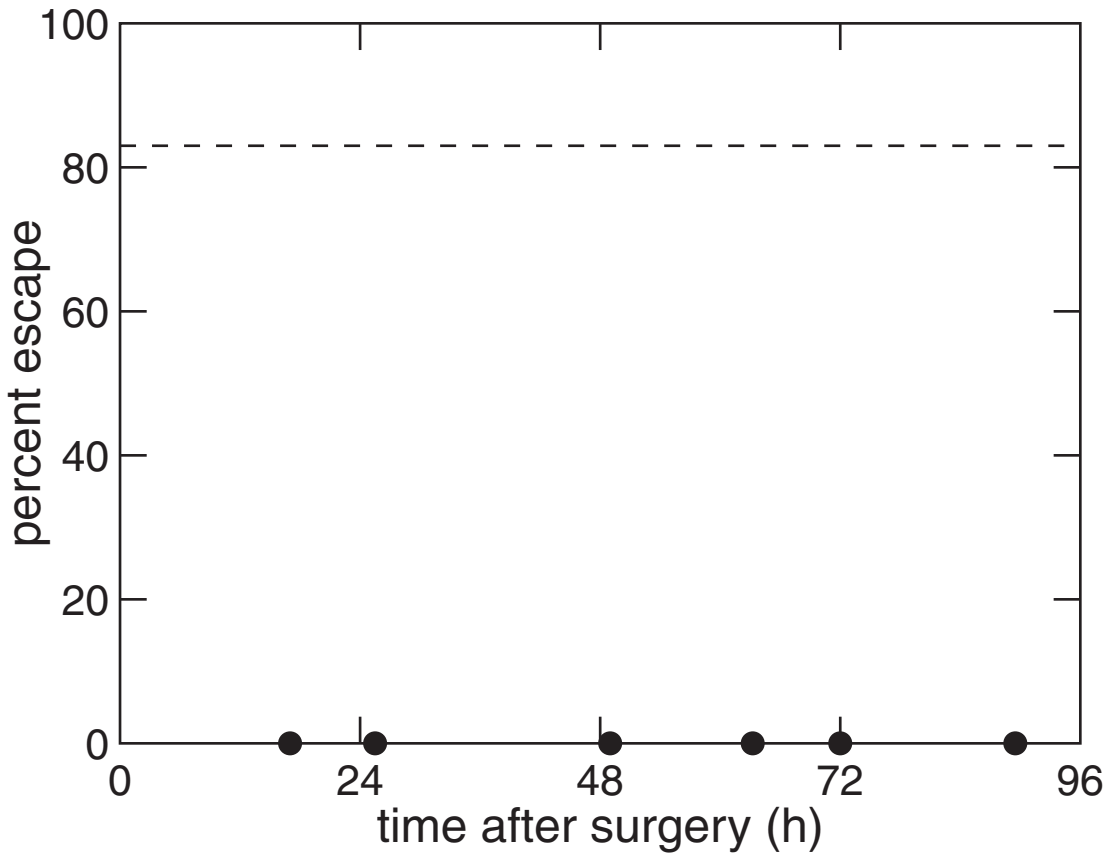
# Nanoneurosurgery

## escape rate after ‘mock’ surgery



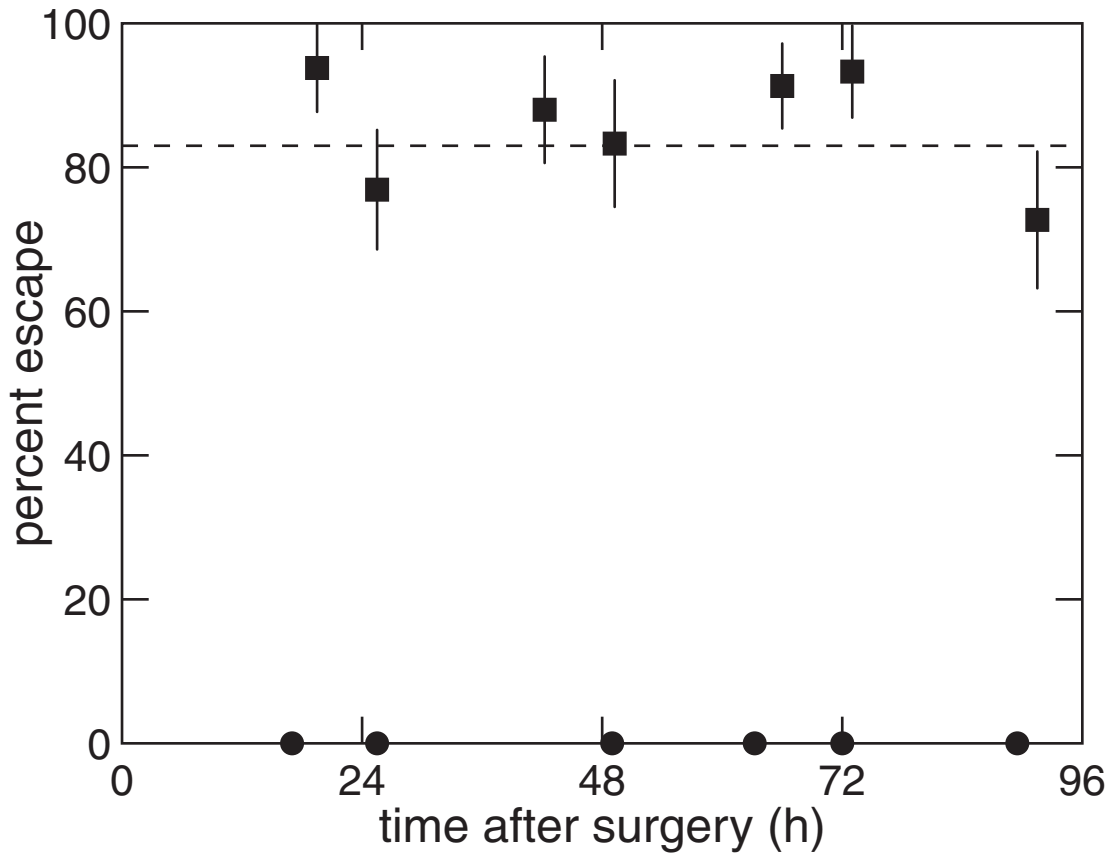
# Nanoneurosurgery

## escape rate of ASH-lacking mutant



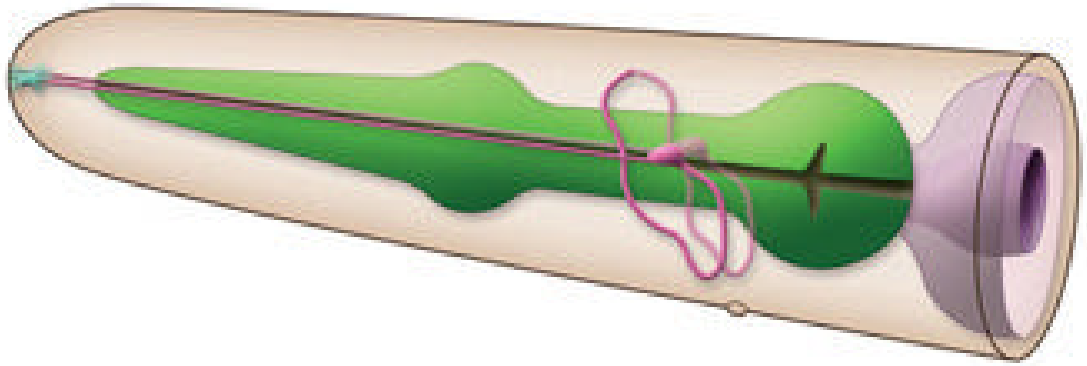
# Nanoneurosurgery

## escape rate after ASH-ablation surgery

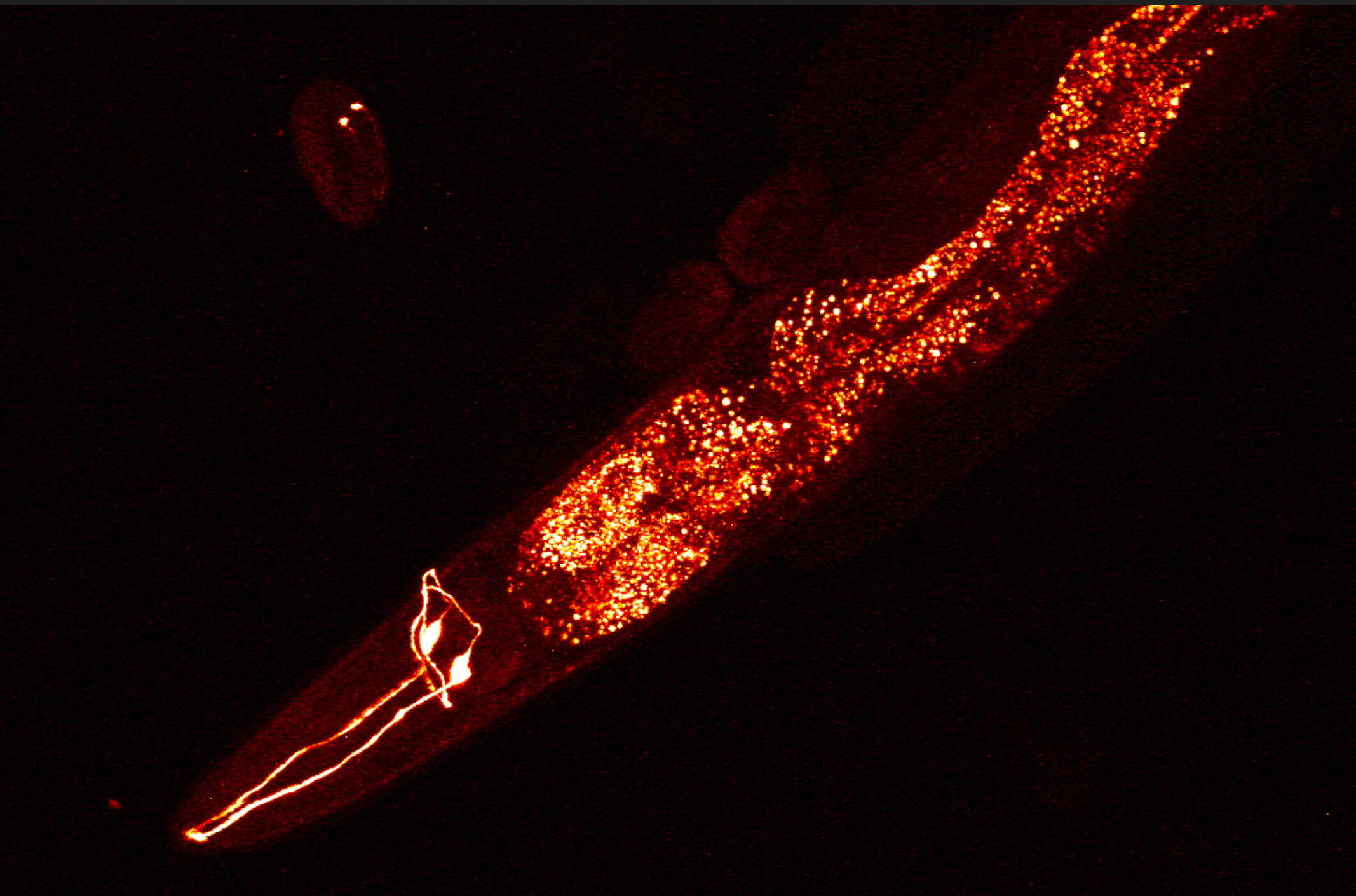


# Nanoneurosurgery

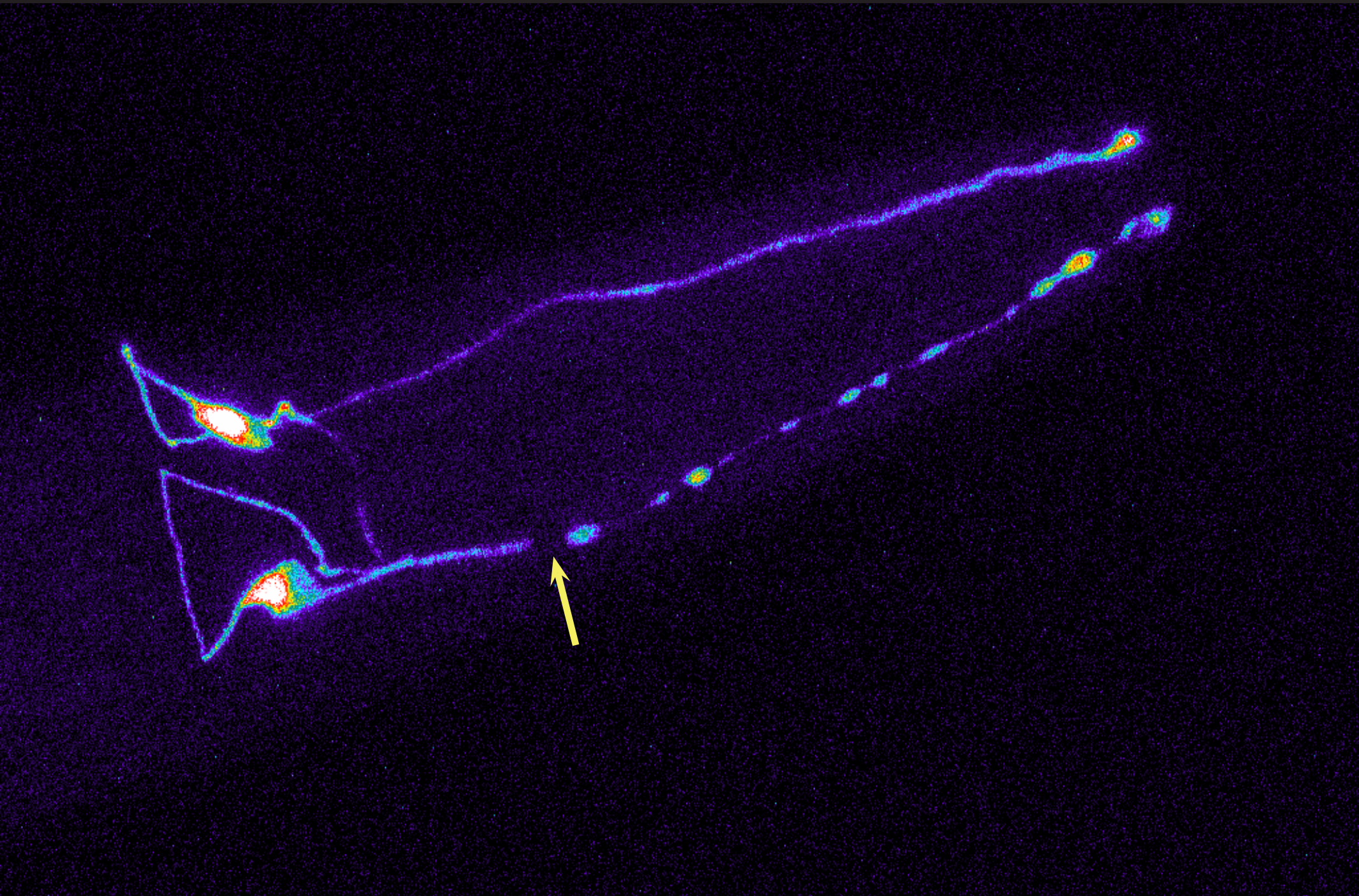
## AFD neurons (temperature sensors)



# Nanoneurosurgery



# Nanoneurosurgery

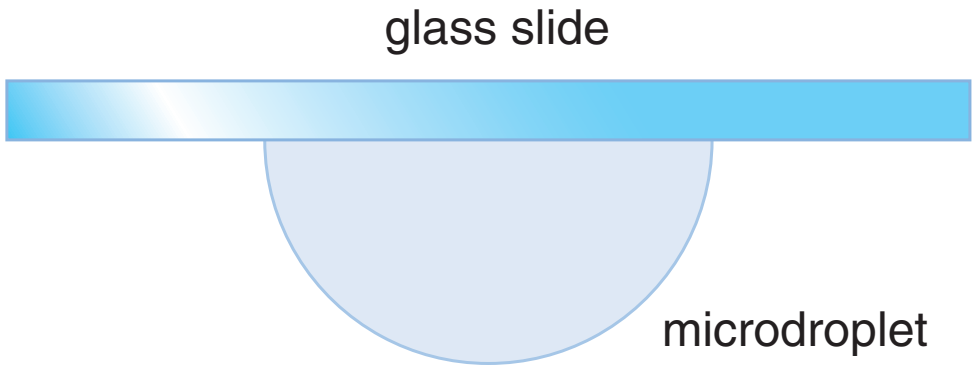


# Nanoneurosurgery

*Q*: where does the ASH sense temperature?

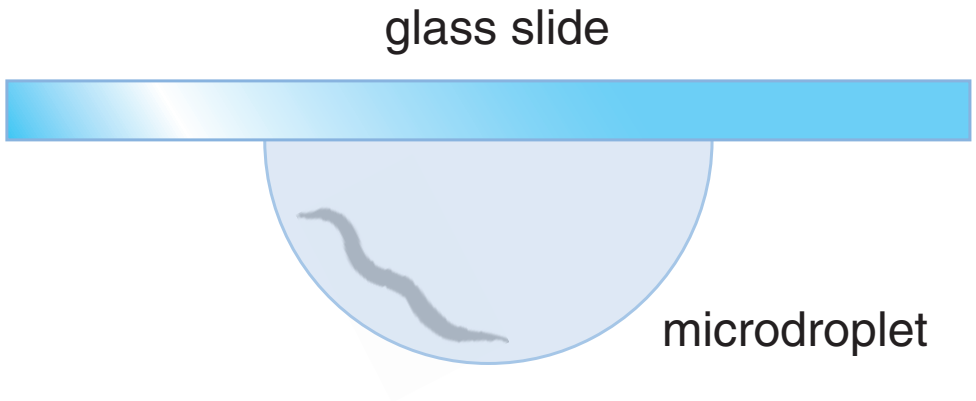
# Nanoneurosurgery

## microdroplet assay



# Nanoneurosurgery

## microdroplet assay



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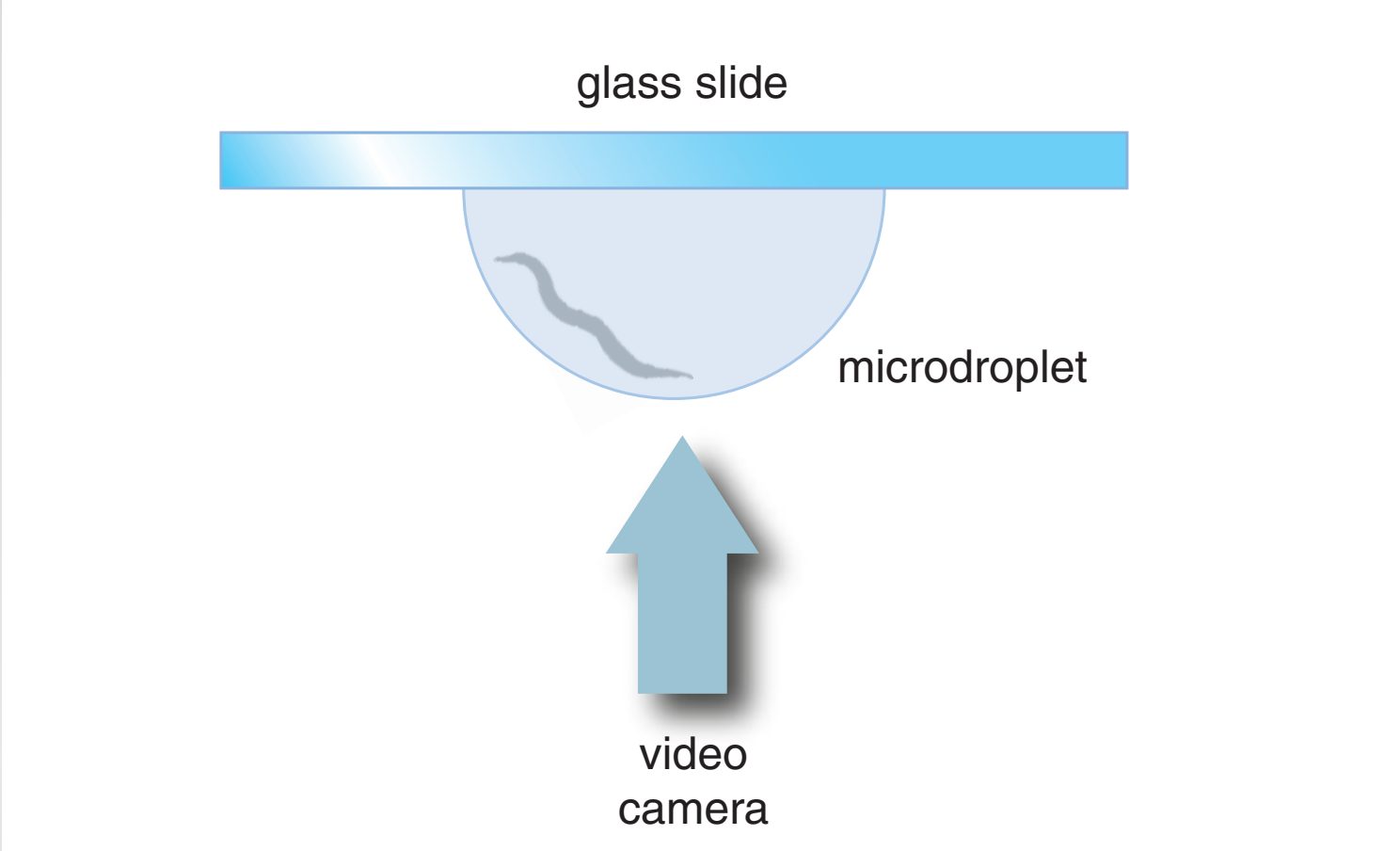
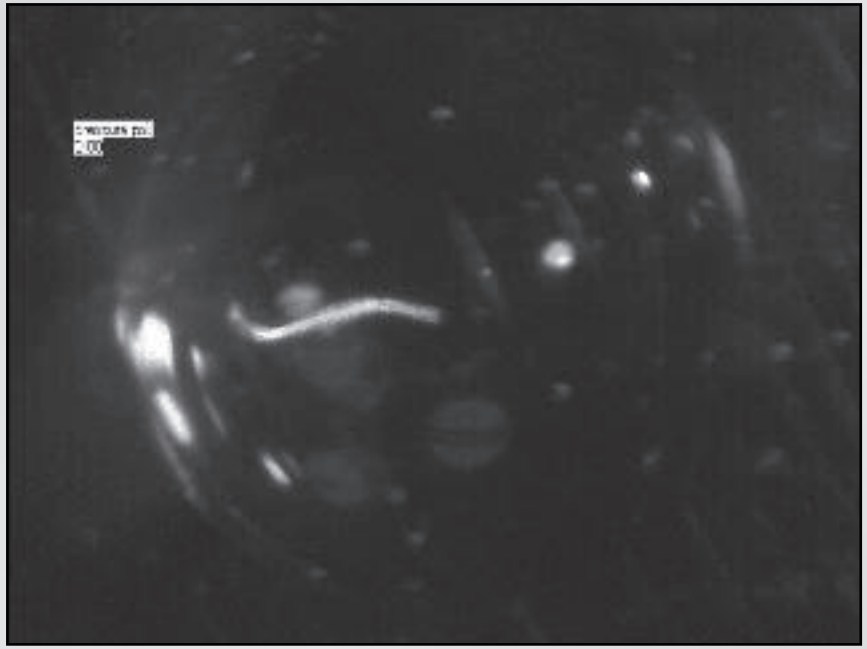
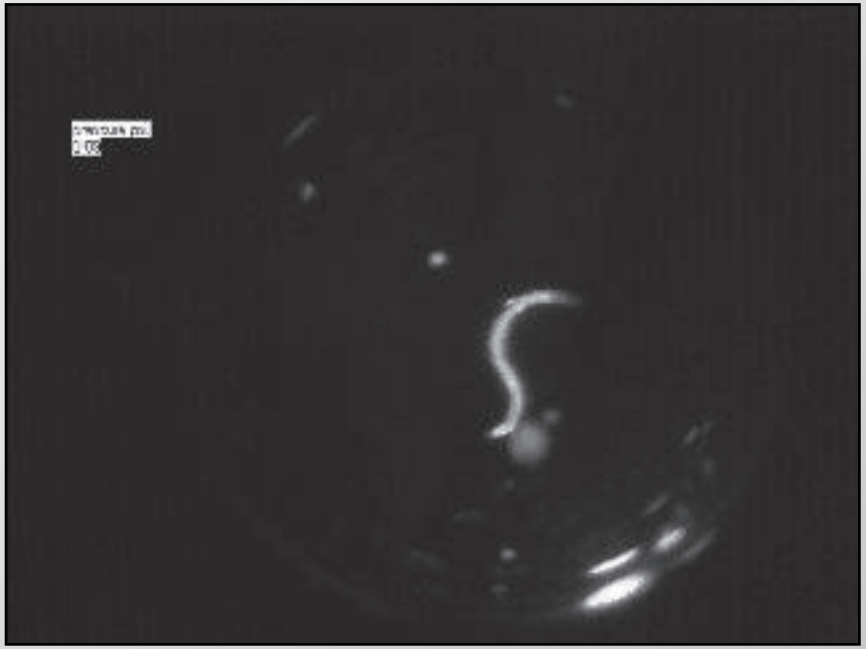


# Nanoneurosurgery

**surgery results in quantifiable behavior changes**



**before**



**after**

# Nanoneurosurgery

## data acquisition and analysis

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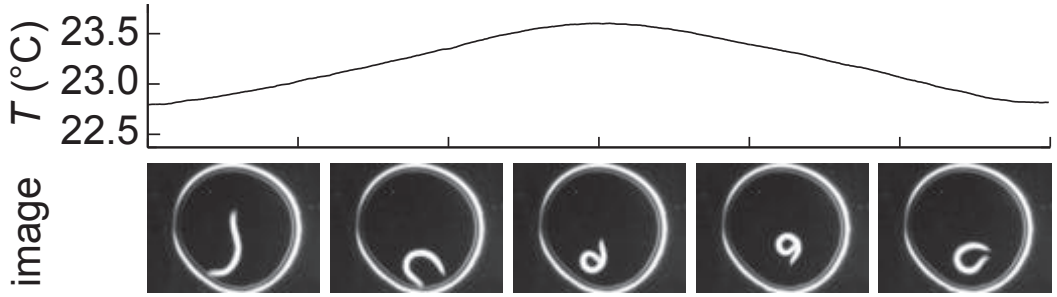

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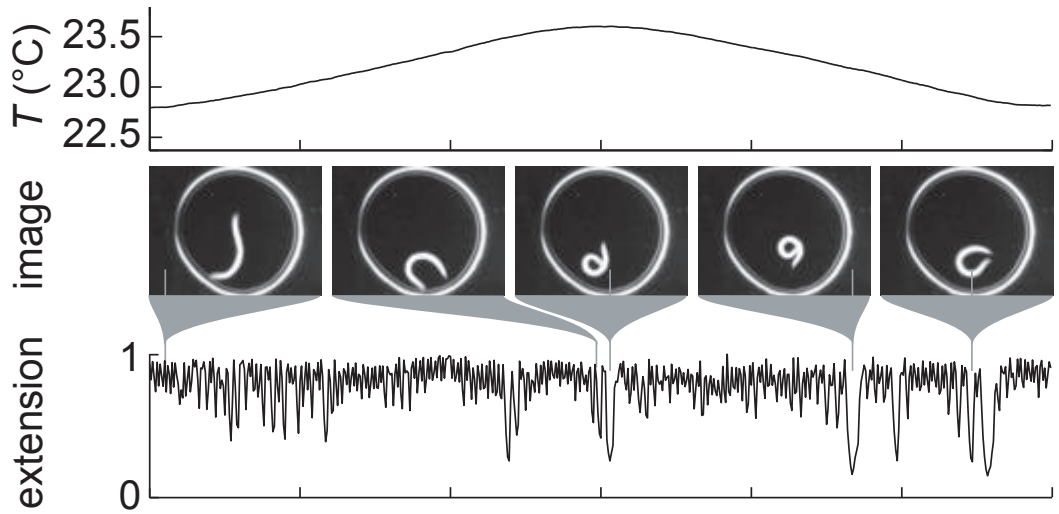

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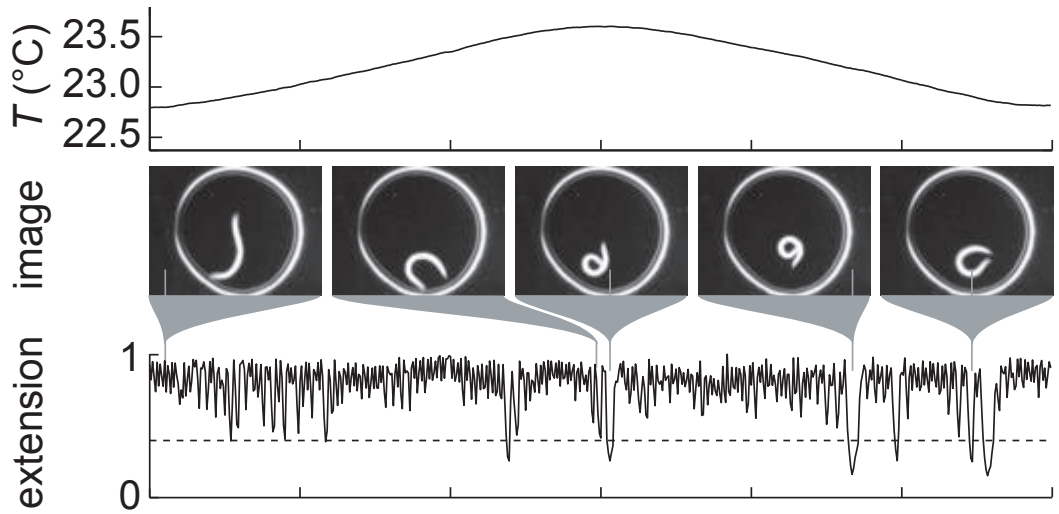

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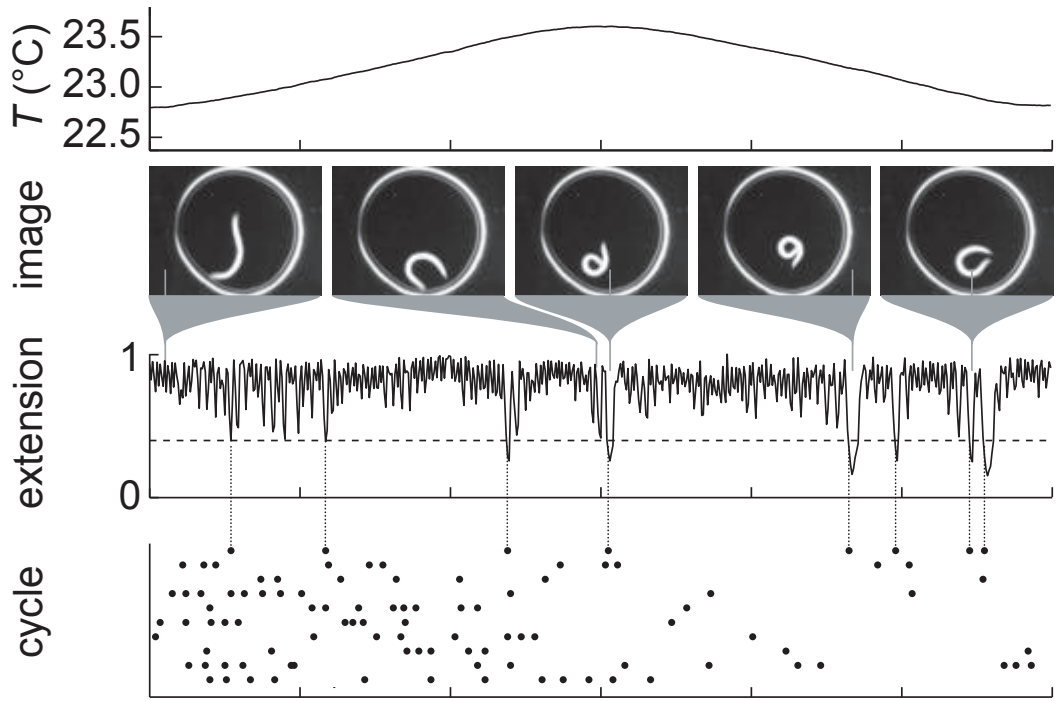

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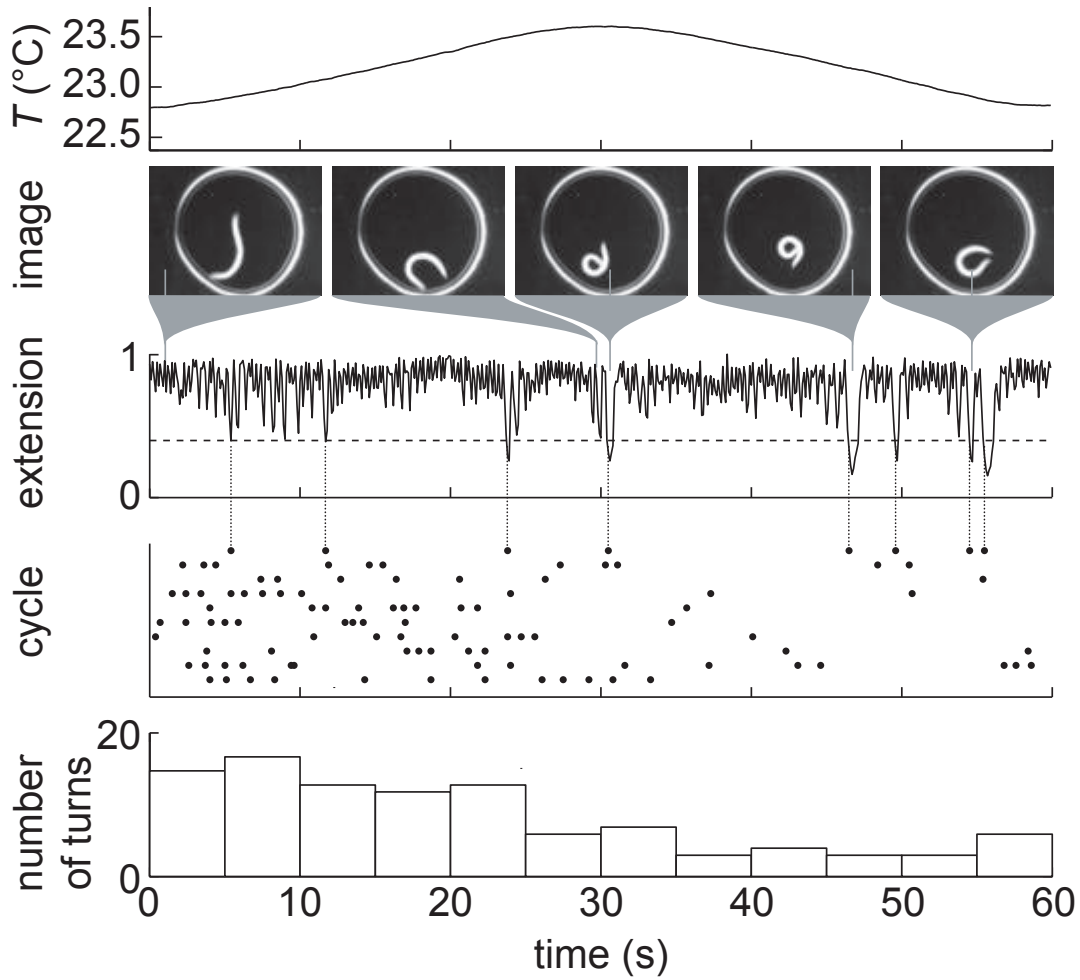

# Nanoneurosurgery

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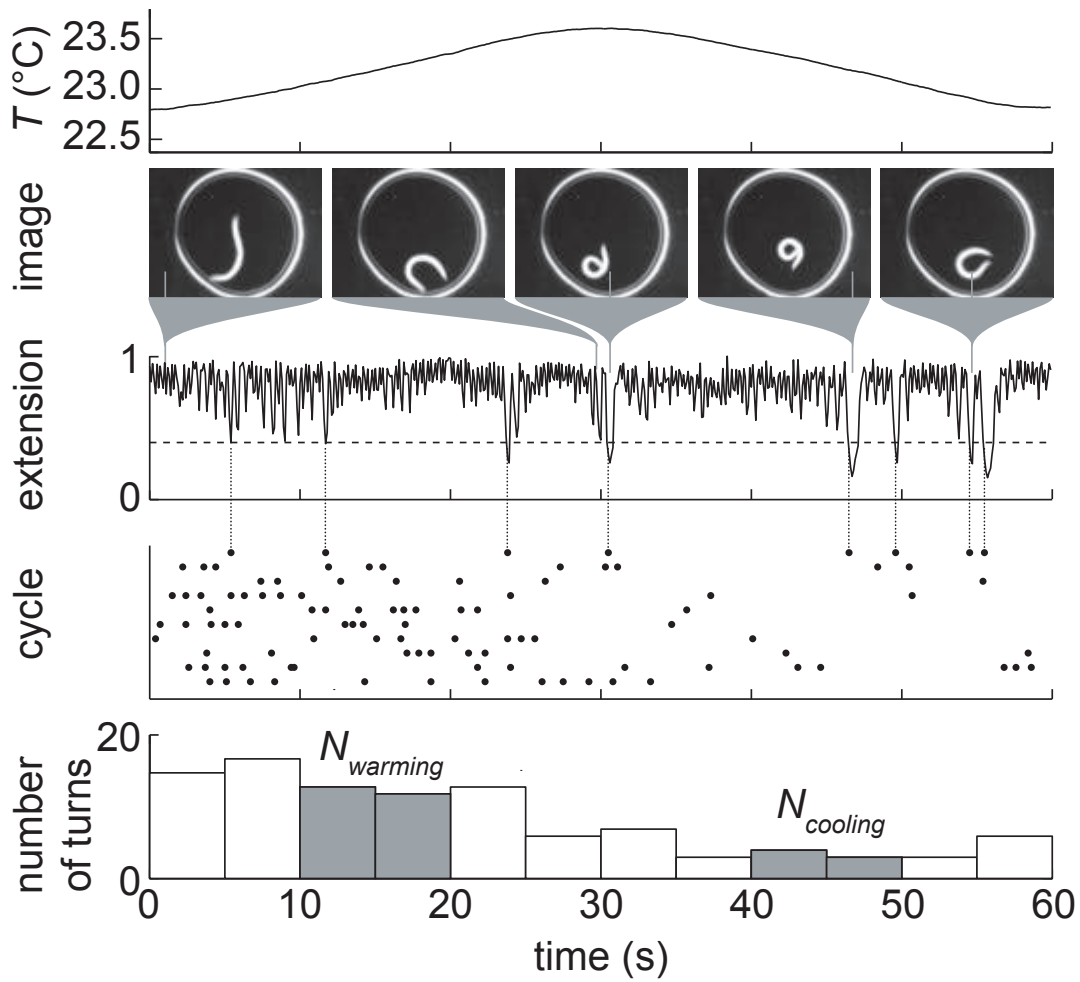

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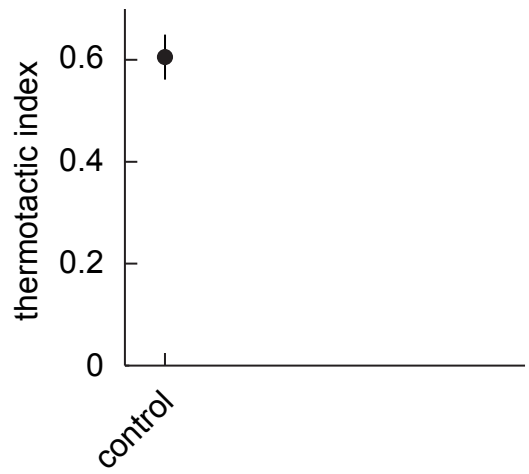

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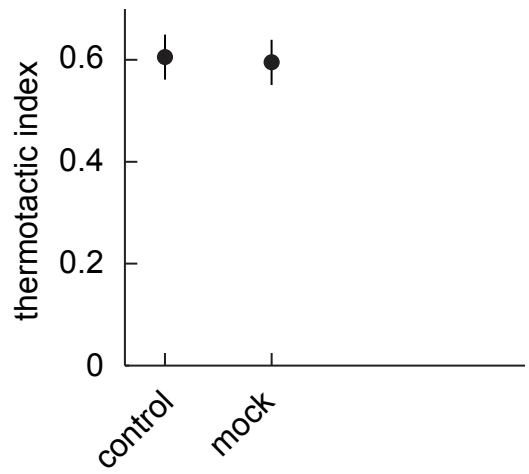

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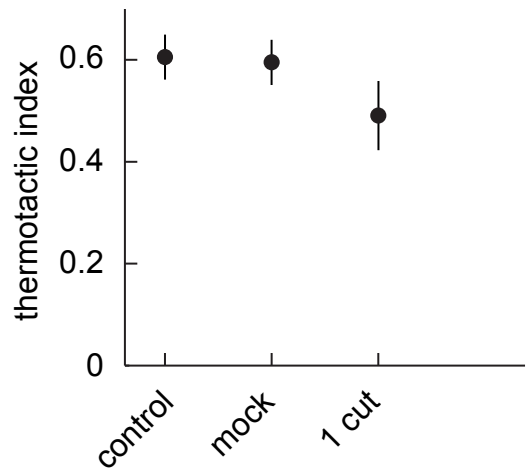

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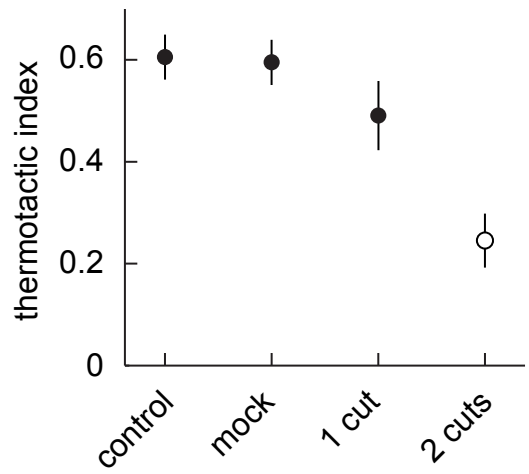

# Nanoneurosurgery

temperature sensing occurs at tip of dendrite

# Nanoneurosurgery

regeneration of HSN axon (regulates egg-laying)

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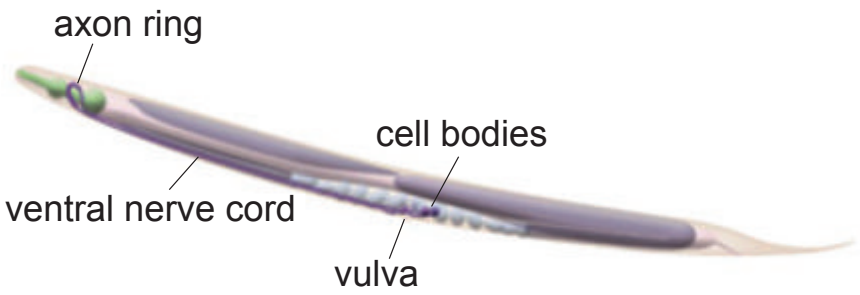

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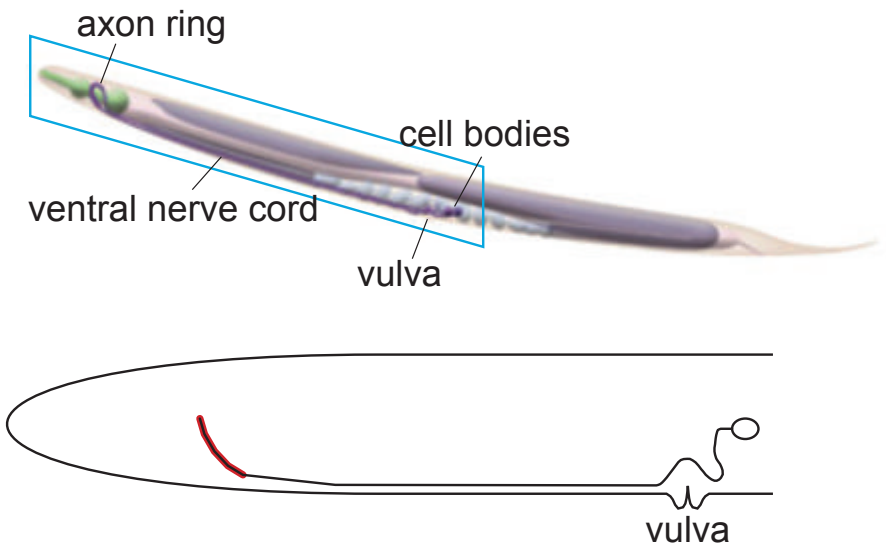

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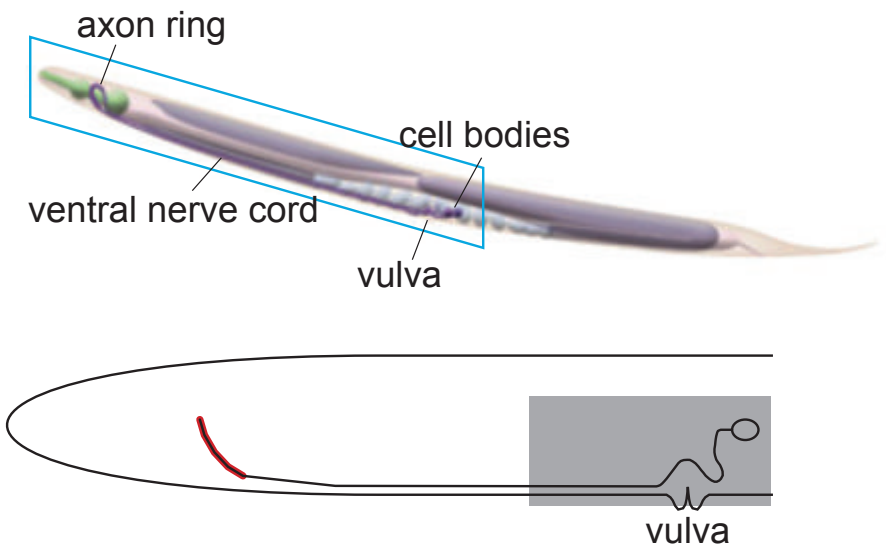

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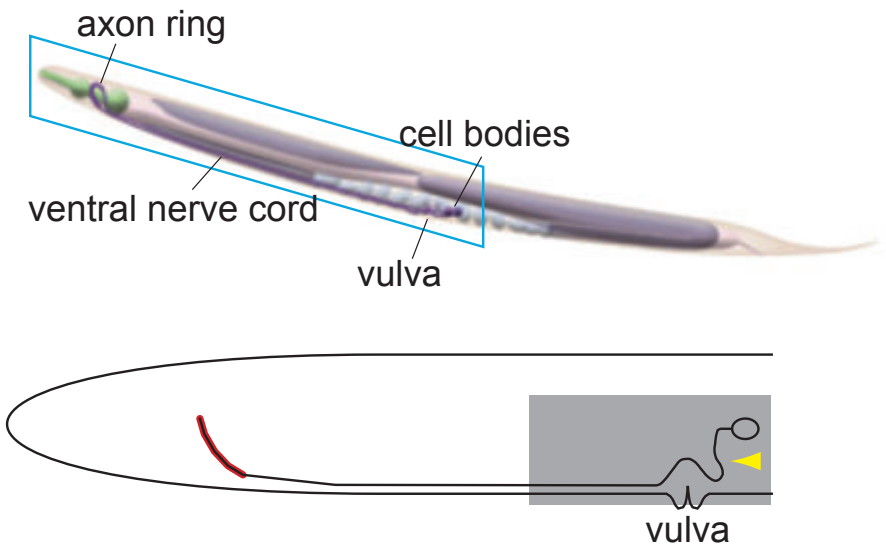

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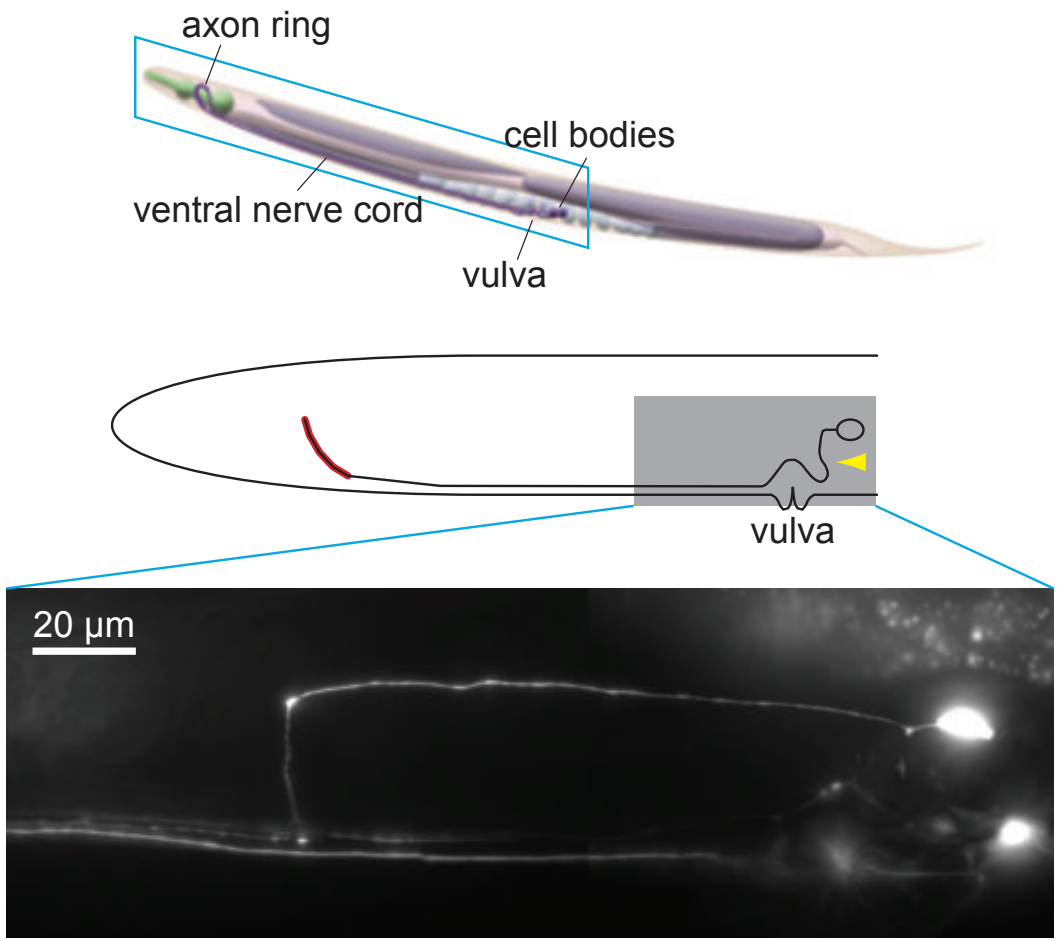

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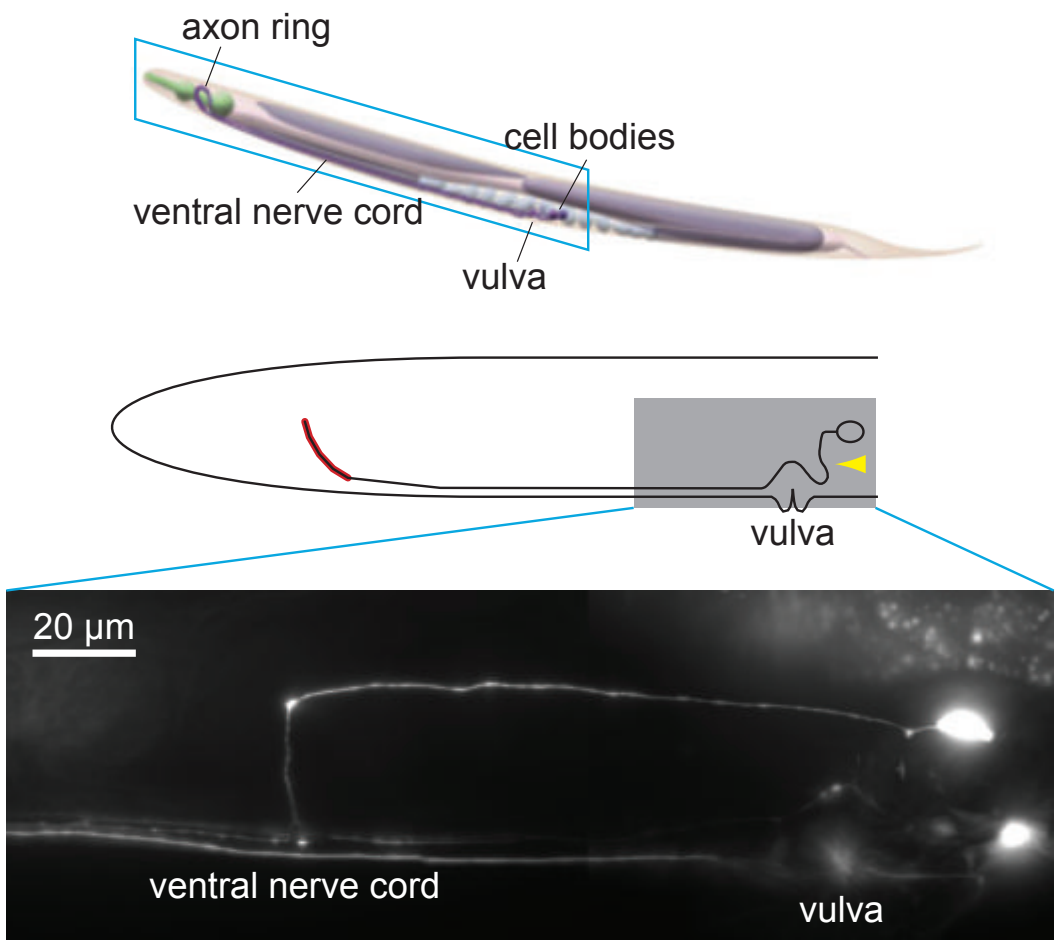

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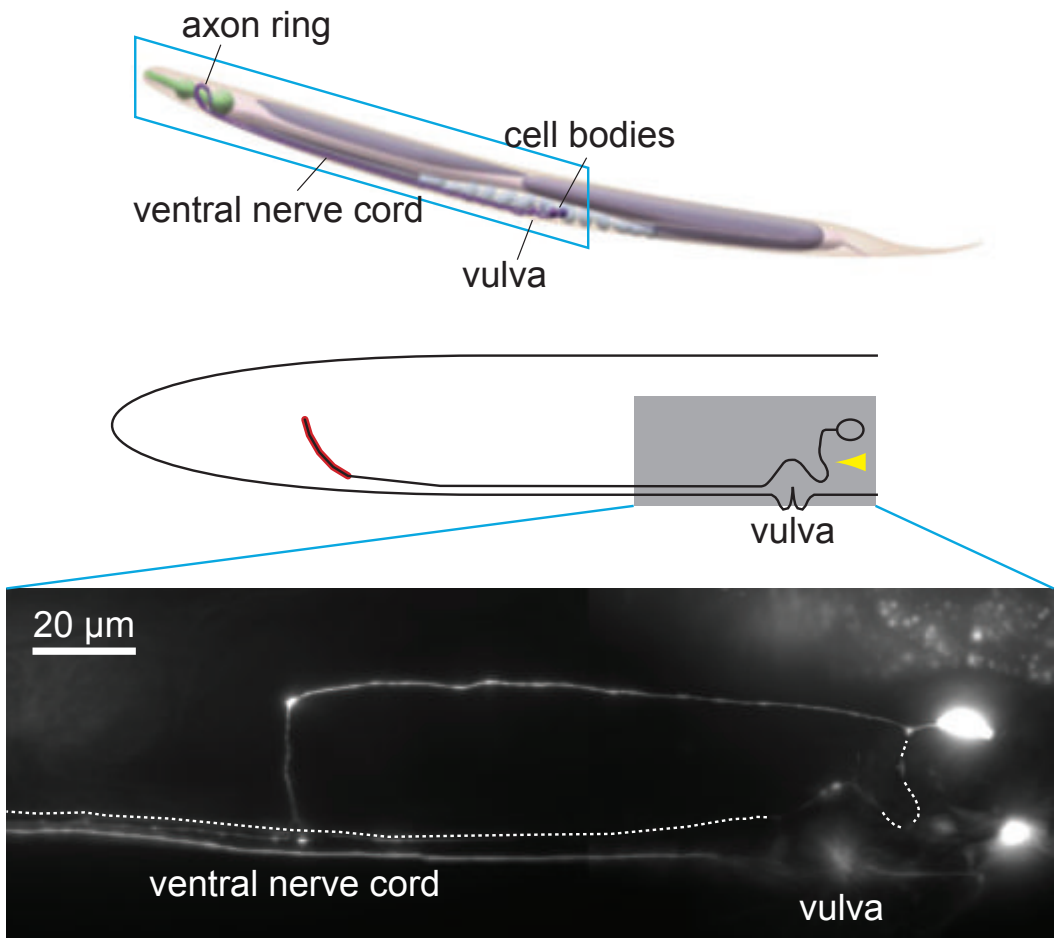

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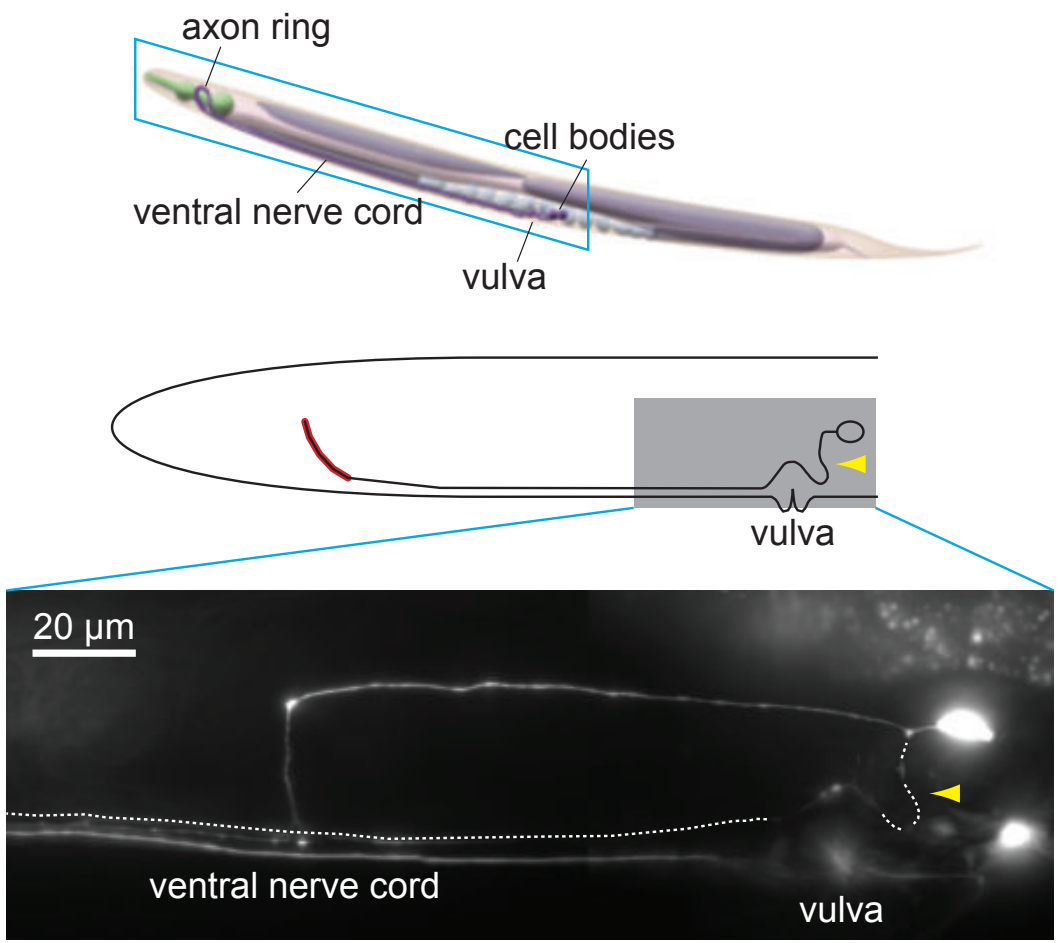

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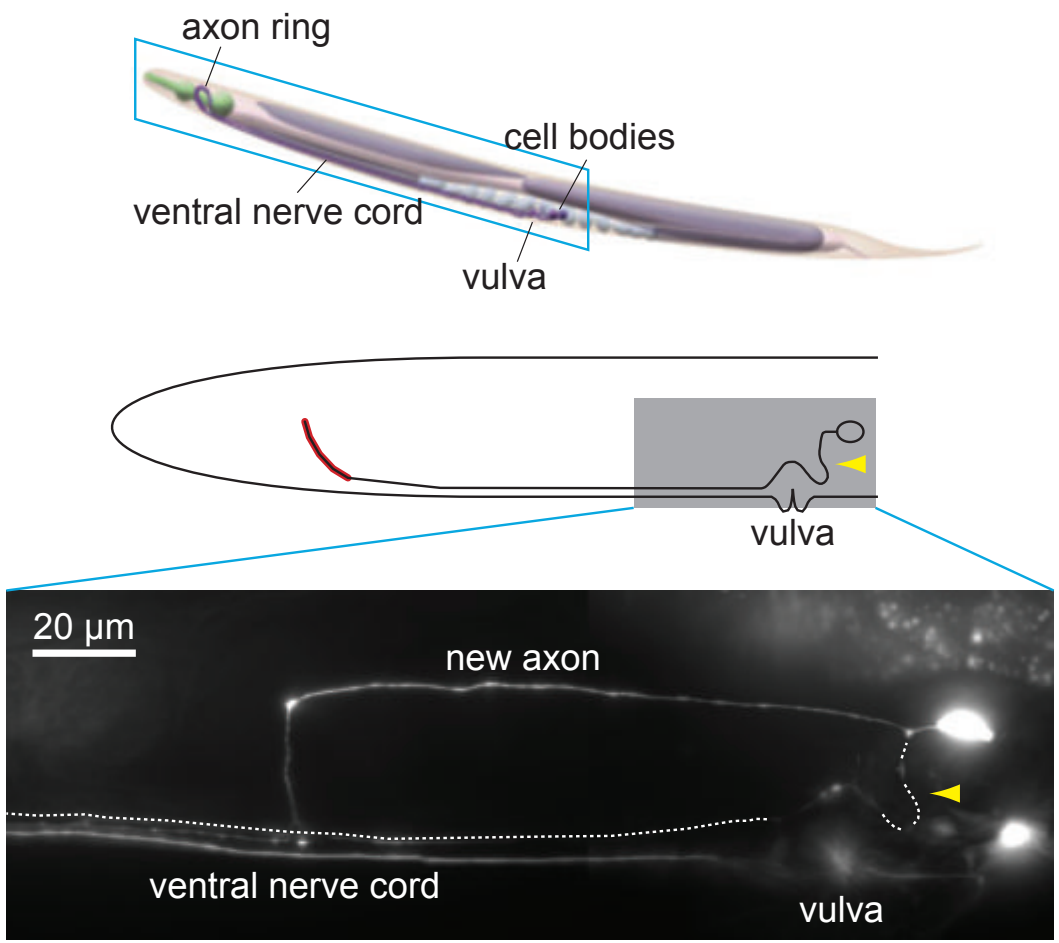
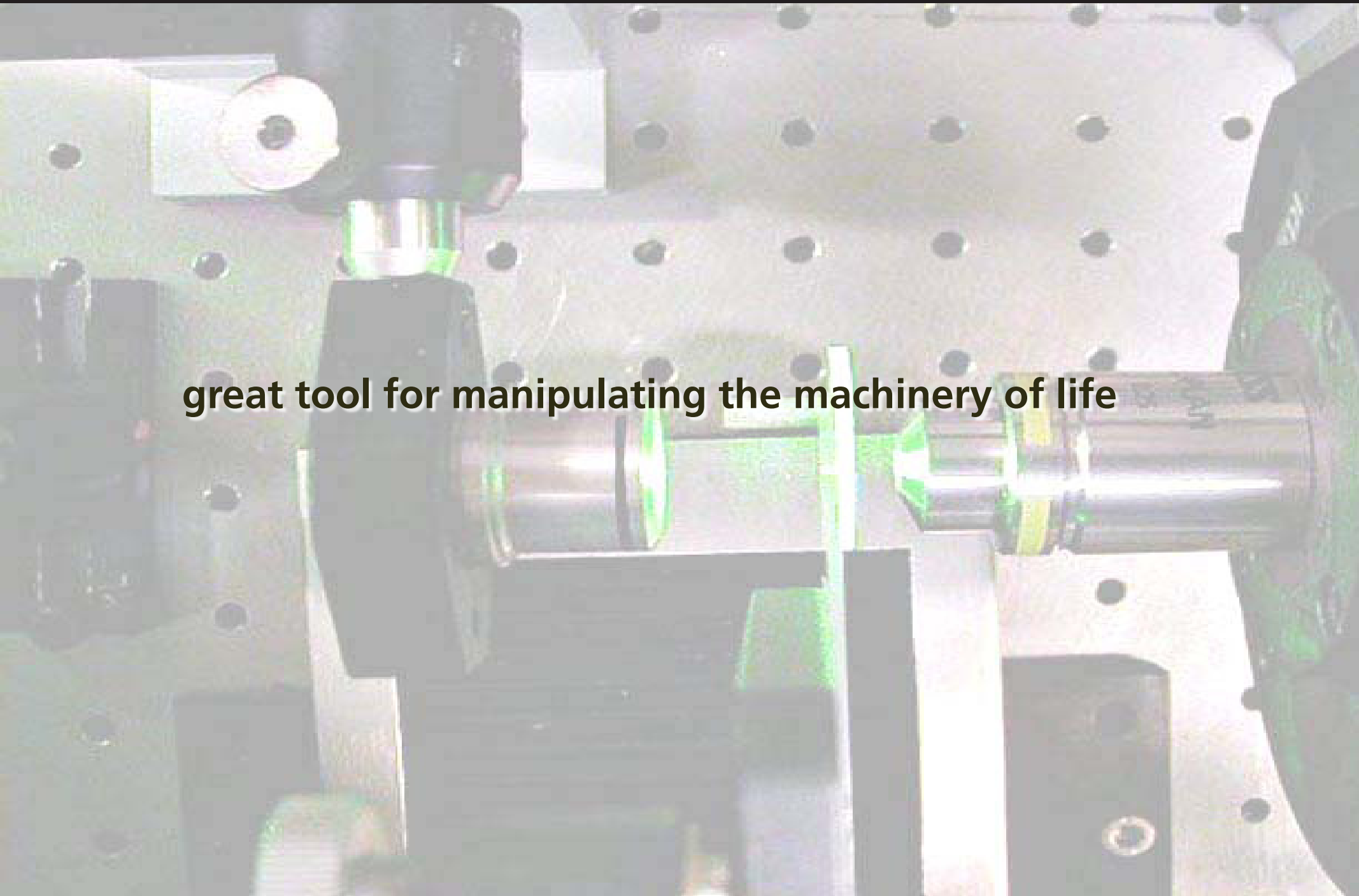


# Summary

- 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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**National Science Foundation**

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