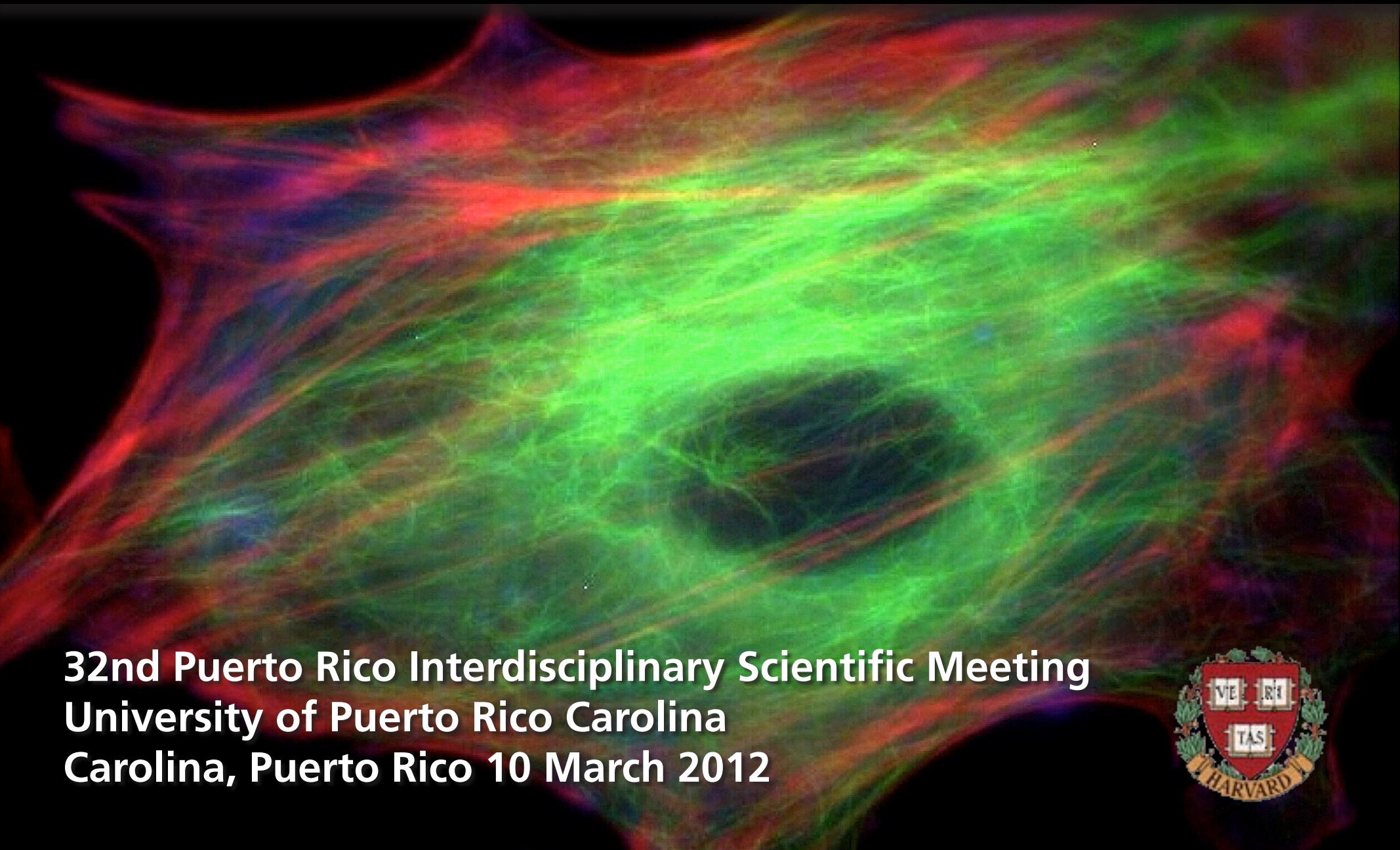
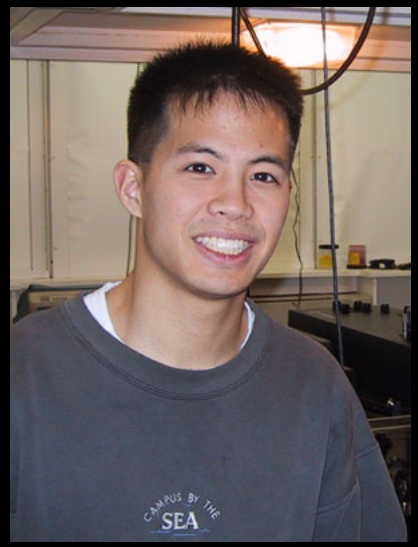
Subcellular surgery and nanoneurosurgery



32nd Puerto Rico Interdisciplinary Scientific Meeting
University of Puerto Rico Carolina
Carolina, Puerto Rico 10 March 2012

Iva Maxwell



Sam Chung

Valeria Nuzzo

Alexander Heisterkamp

eric_mazur

and also....

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

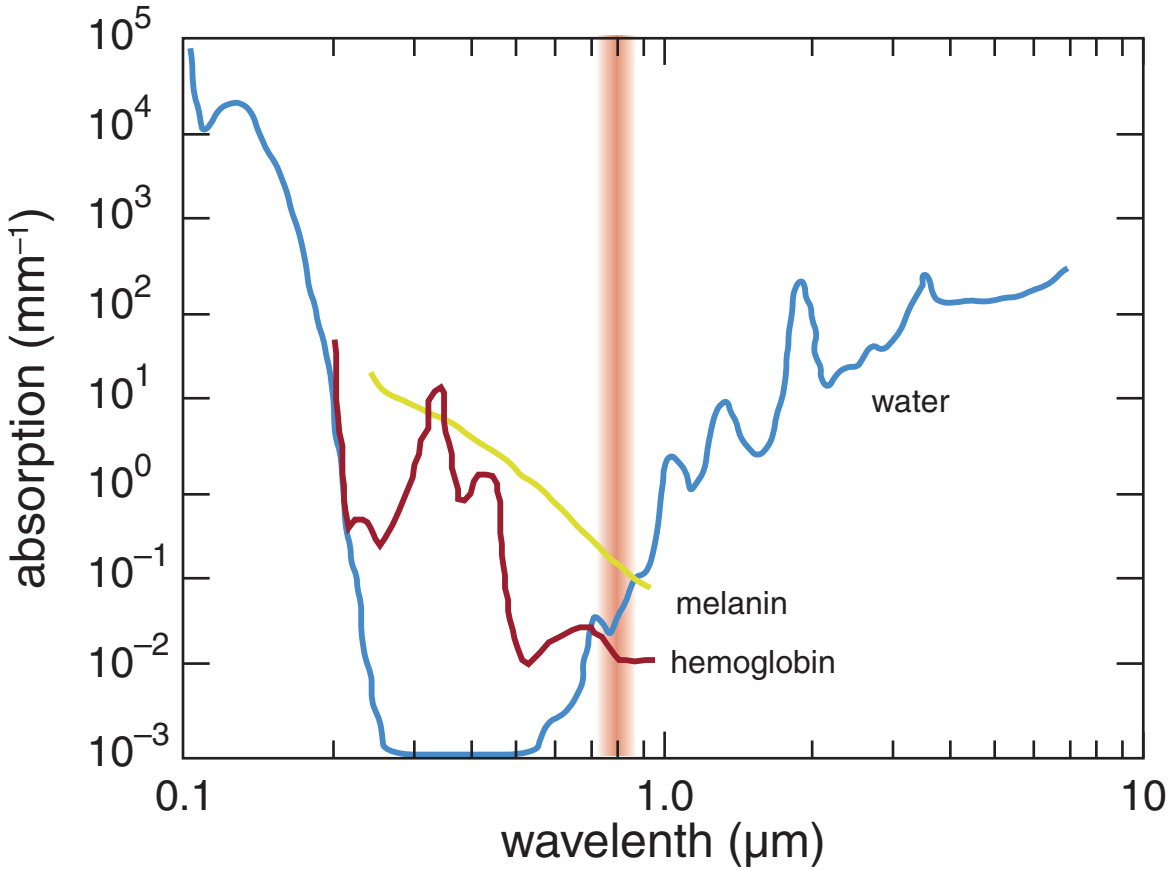
Prof. Don Ingber (Harvard Medical School)
Prof. Aravi Samuel (Harvard)
Prof. Chris Gabel (Boston University)
Dr. Damon Clark (Harvard University)
Prof. J.M. Underwood (UMass Worcester)
Prof. J.A. Nickerson (UMass Worcester)
Prof. Philip LeDuc (Carnegie Mellon)
Prof. Sanjay Kumar (UC Berkeley)

Introduction

why use femtosecond pulses?

Introduction

tissue is nearly transparent at 800 nm

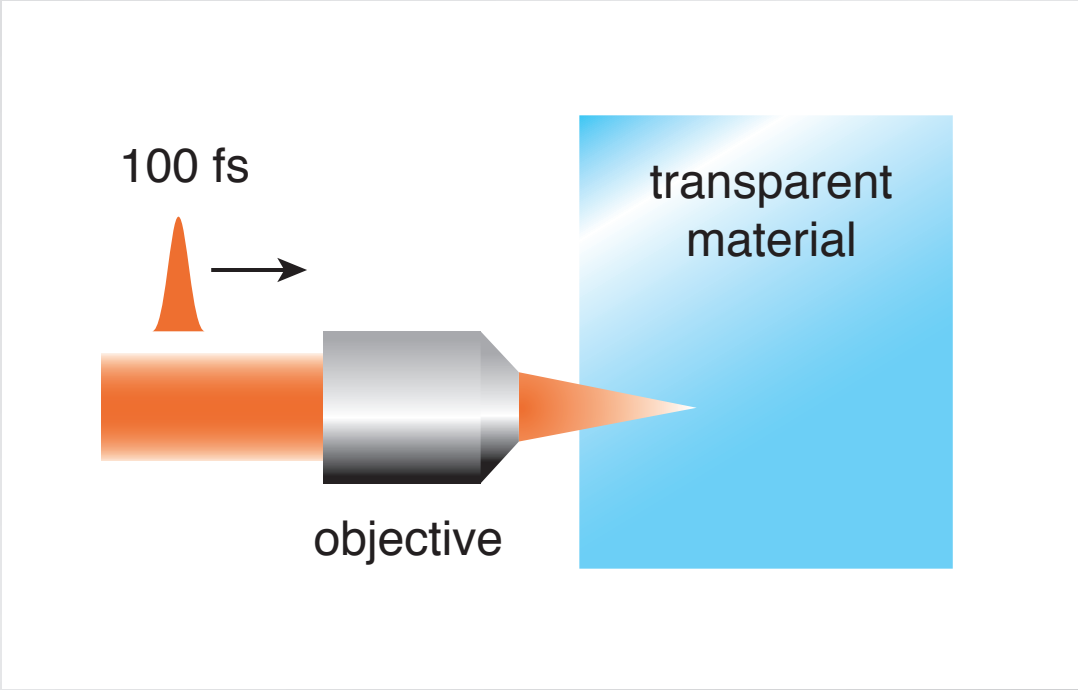


Outline

- femtosecond materials interactions
- subcellular surgery
- nanoneurosurgery

Femtosecond materials interactions

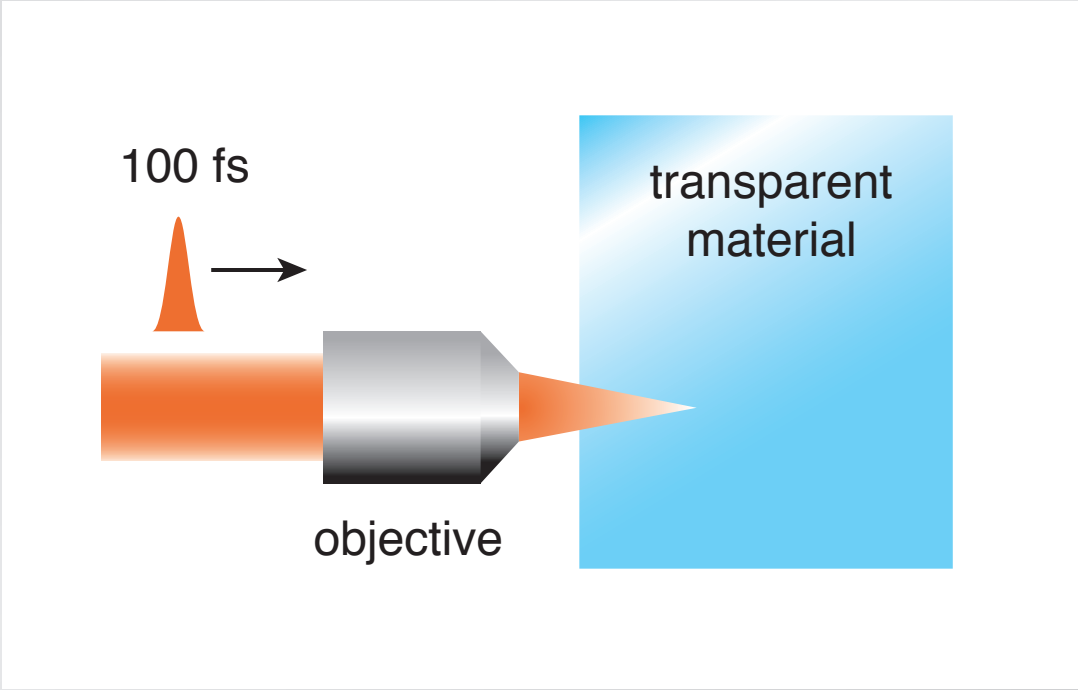
focus laser beam inside material



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

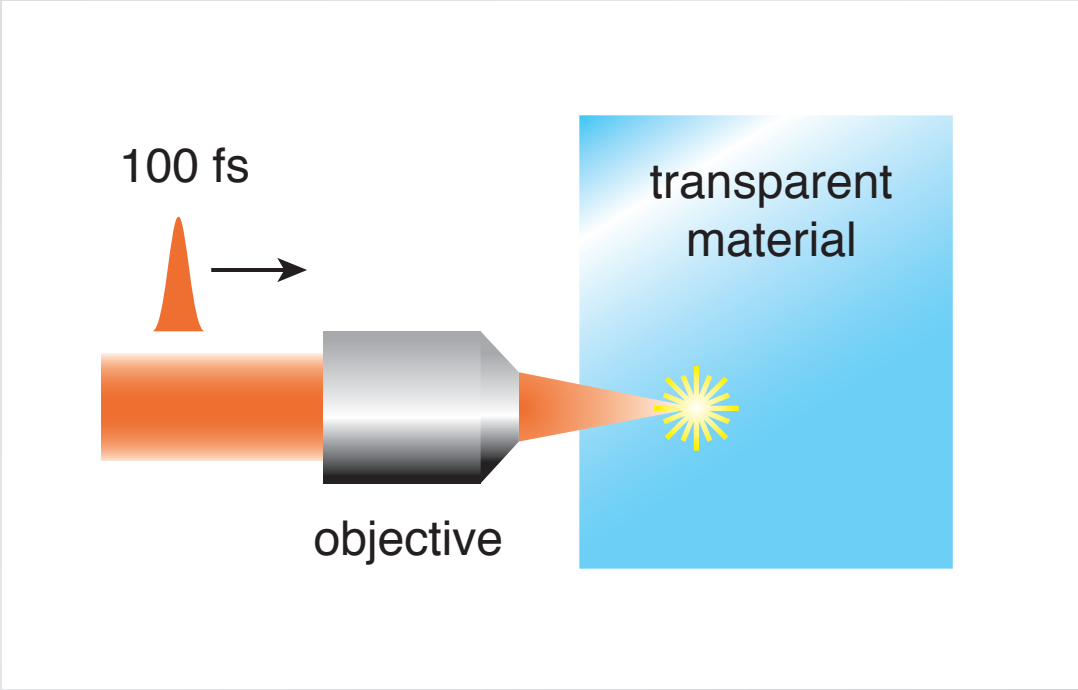
Femtosecond materials interactions

high intensity at focus…



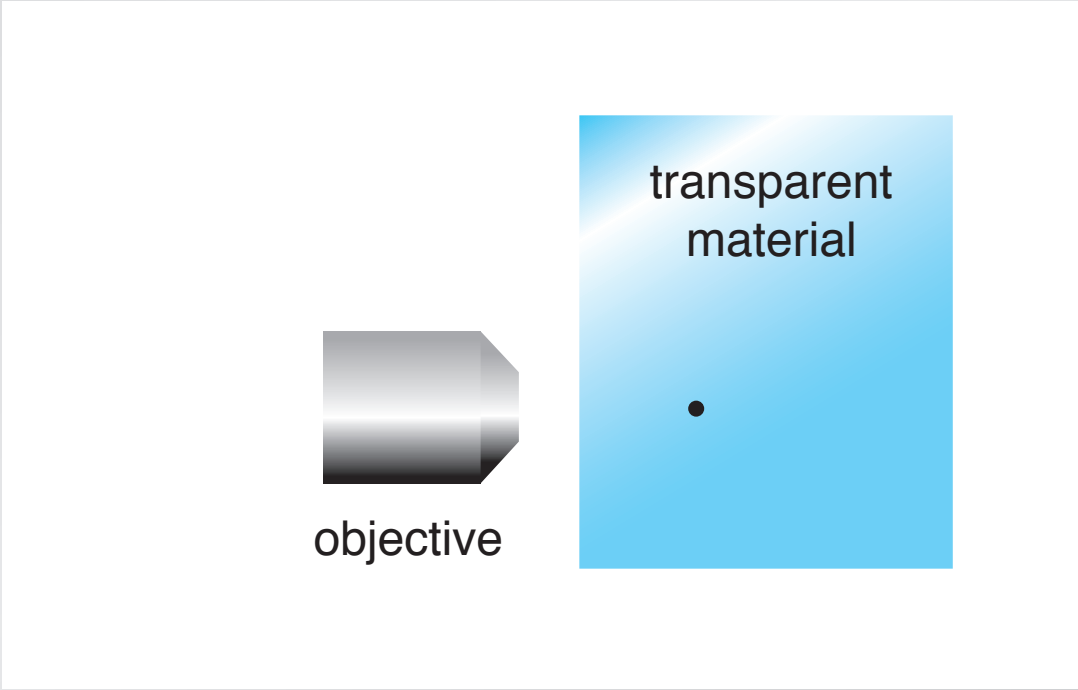
Femtosecond materials interactions

…causes nonlinear ionization…

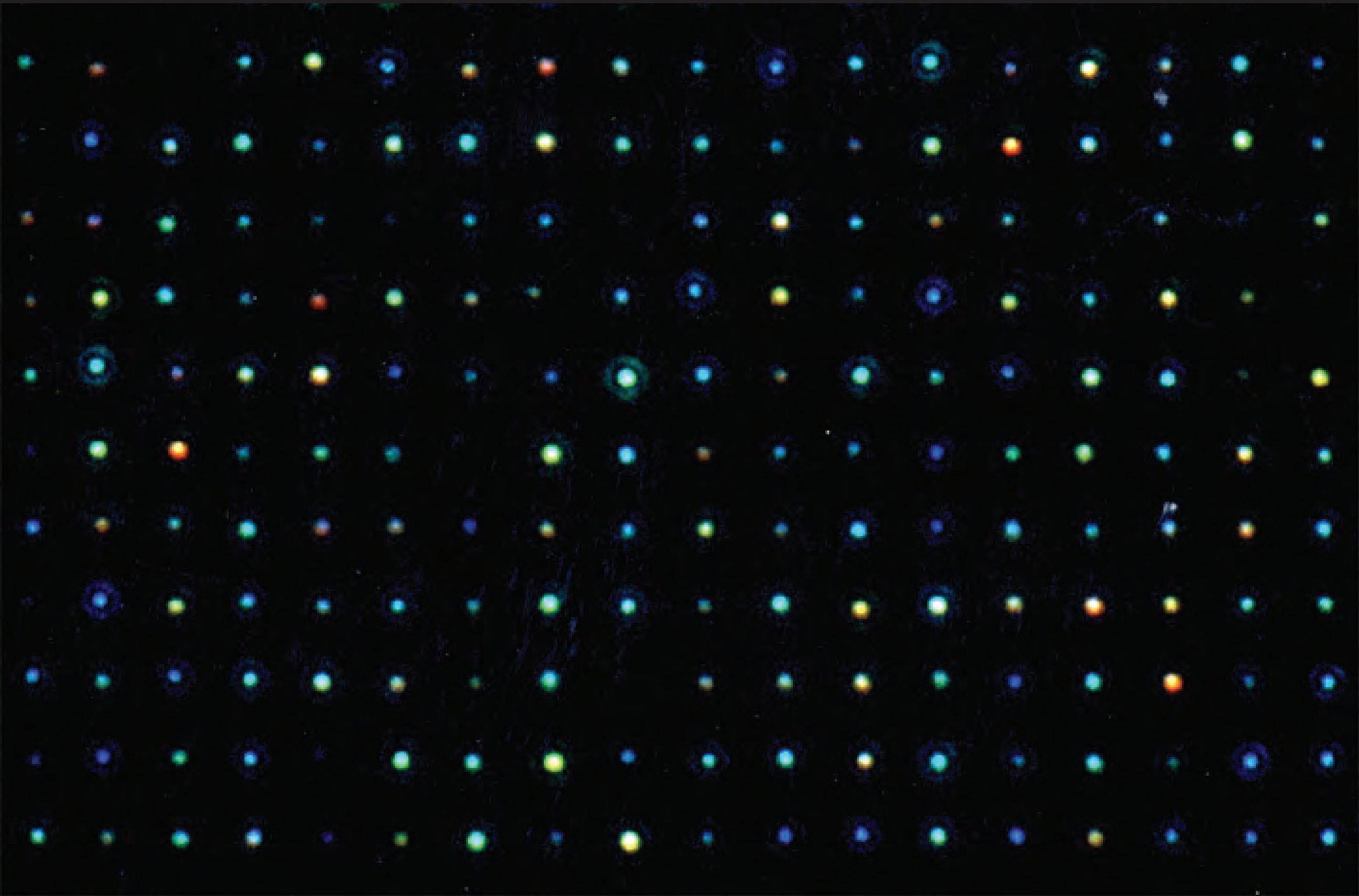


Femtosecond materials interactions

and ‘microexplosion’ causes microscopic damage…



Femtosecond materials interactions

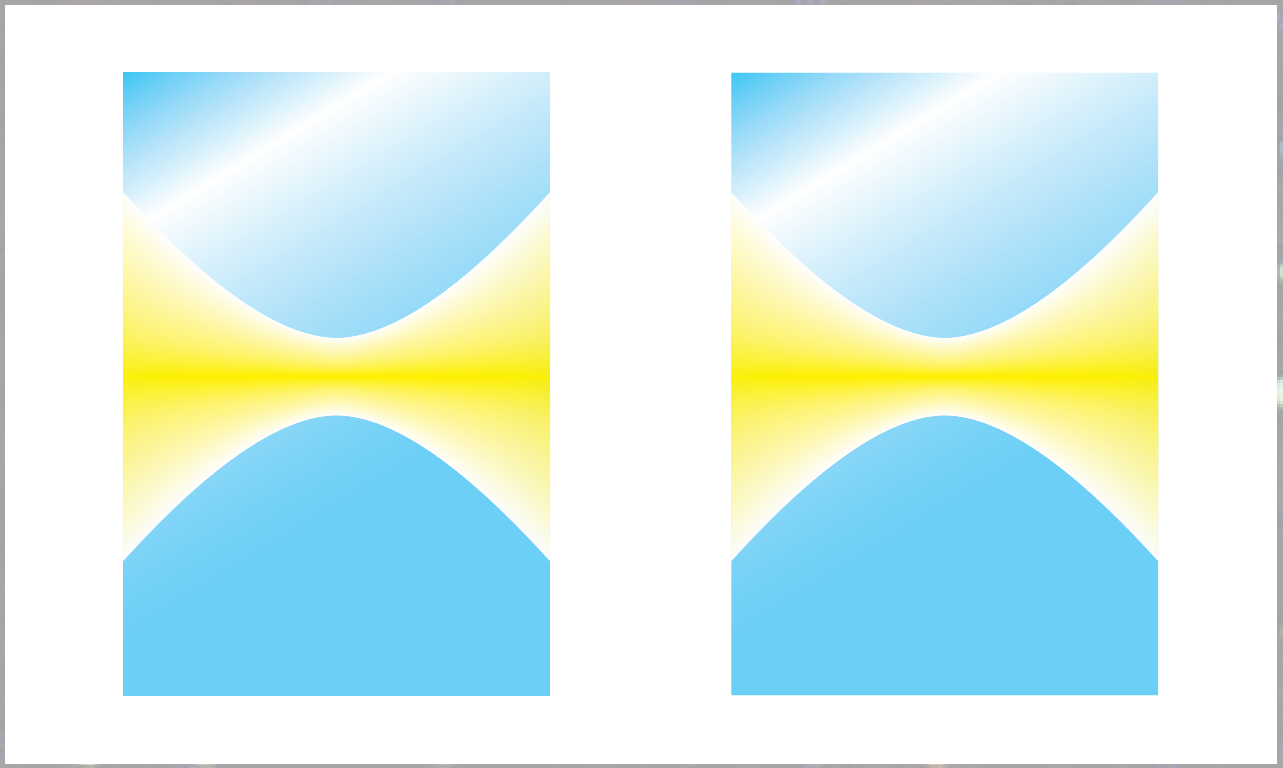


Femtosecond materials interactions

photon energy < bandgap $\longrightarrow$ nonlinear interaction

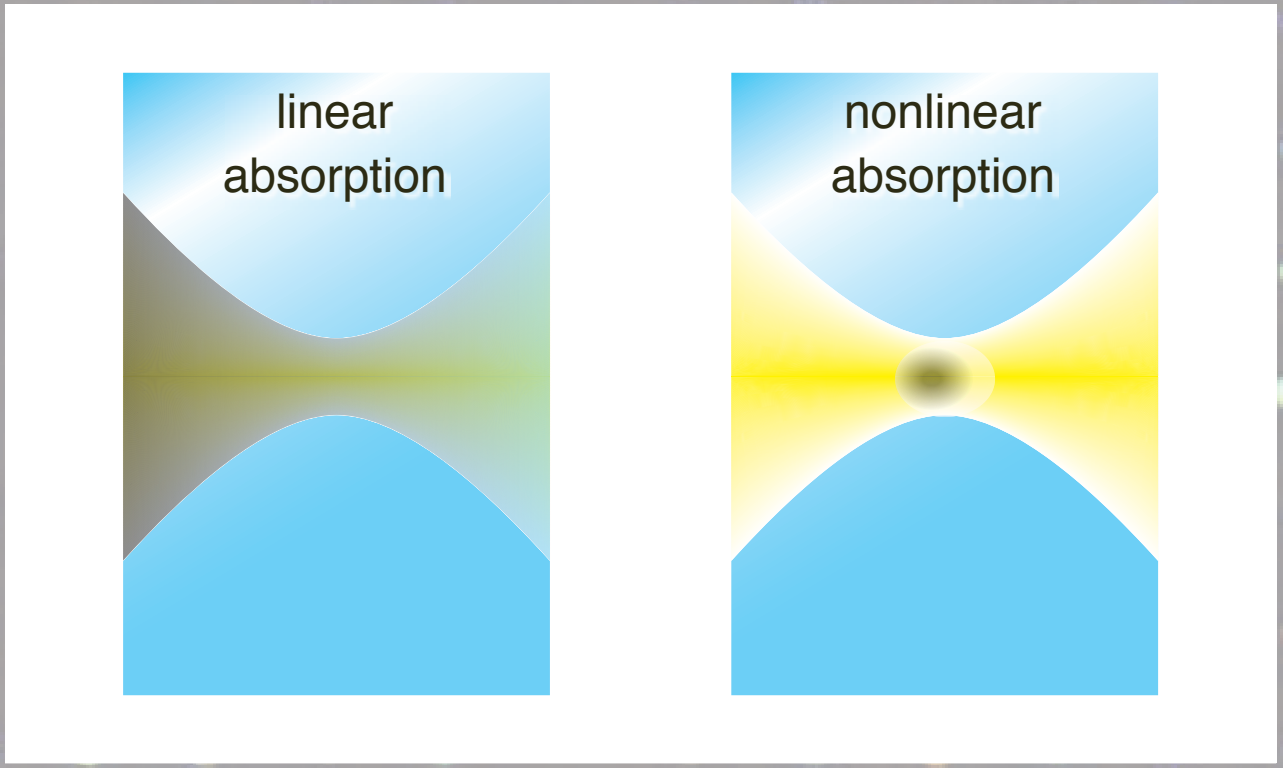
Femtosecond materials interactions

nonlinear interaction provides bulk confinement

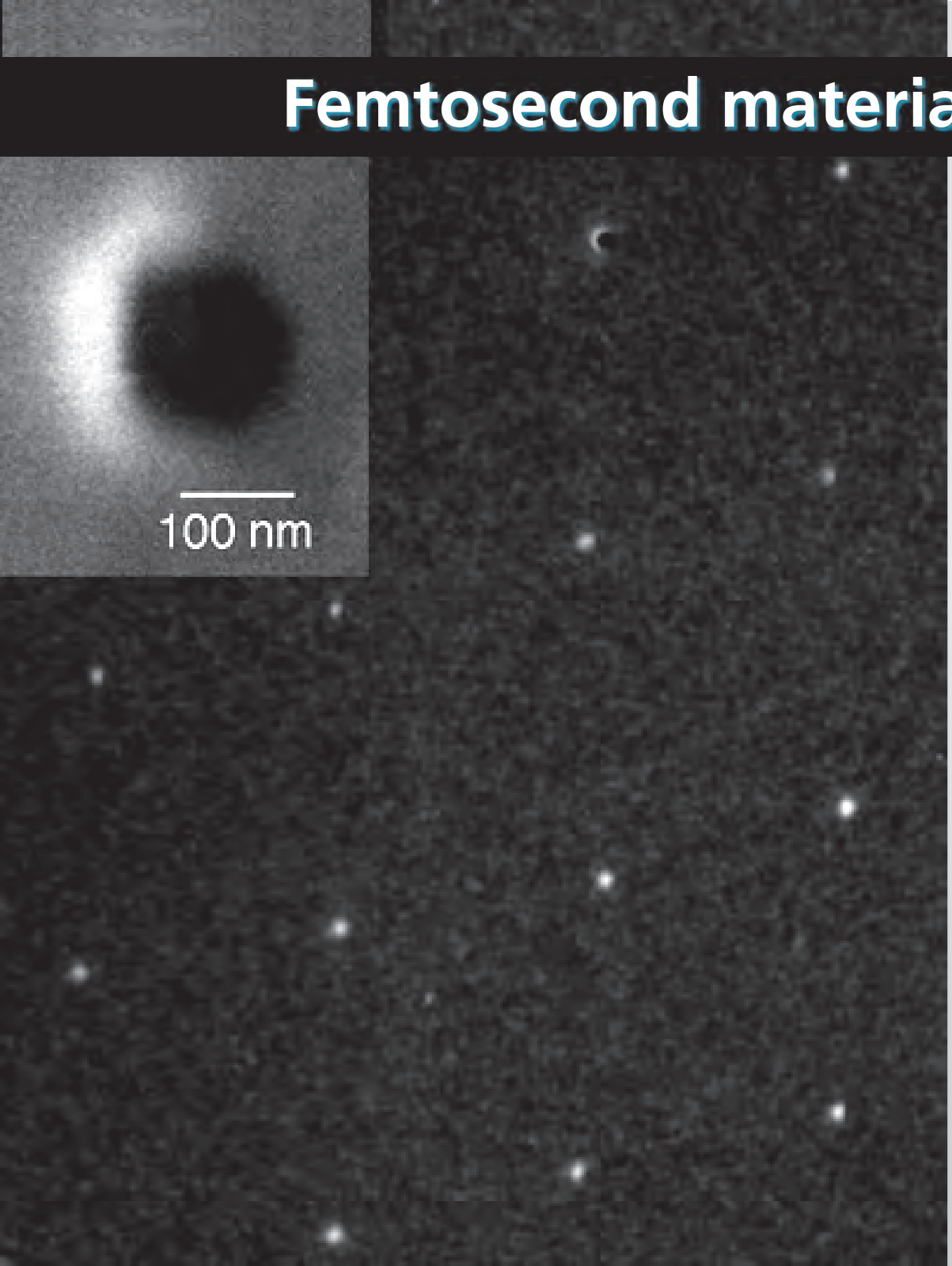


Femtosecond materials interactions

nonlinear interaction provides bulk confinement



Femtosecond materials interactions

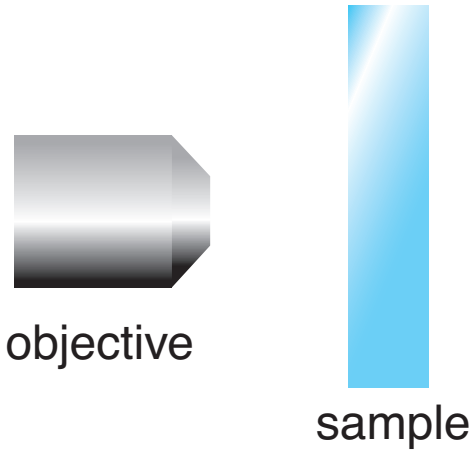


SEM & AFM:

- **100-nm cavities**
- **little colateral damage**

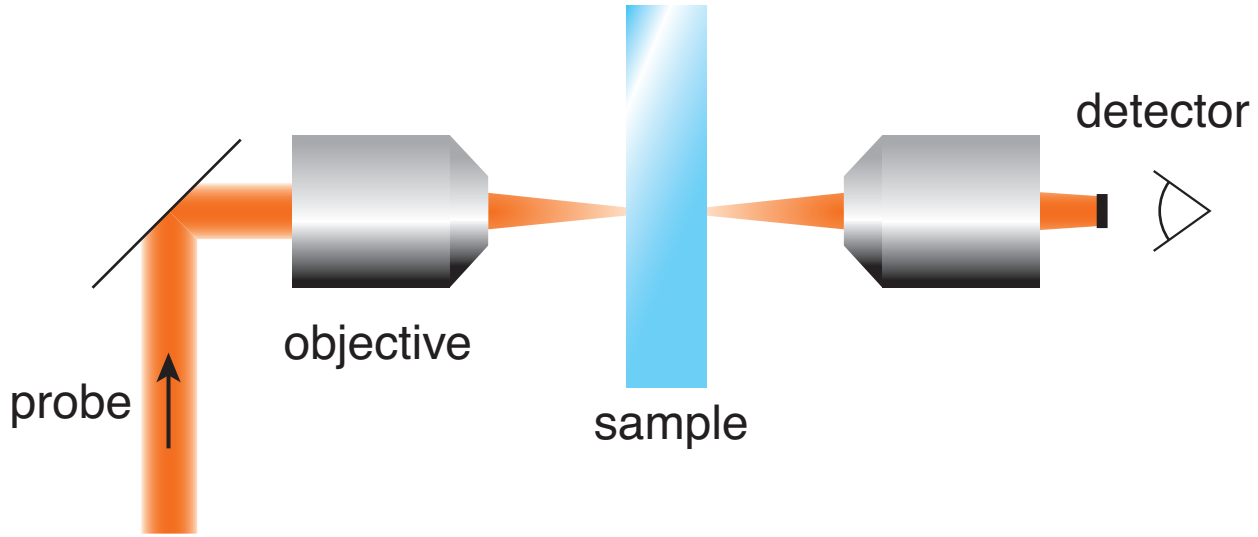
Femtosecond materials interactions

Dark-field scattering



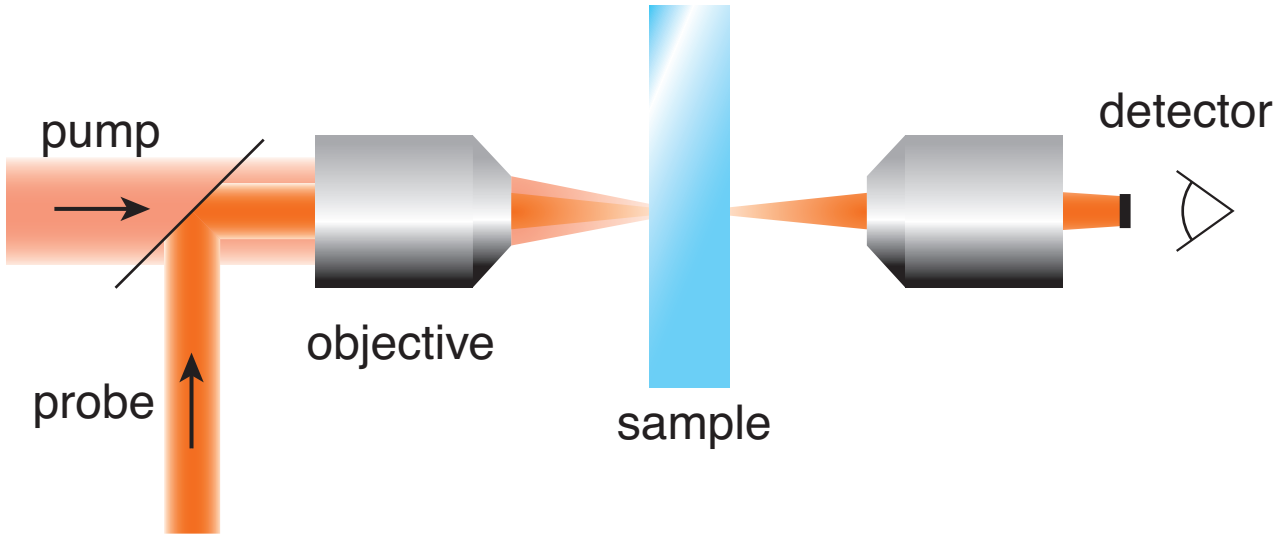
Femtosecond materials interactions

block probe beam…



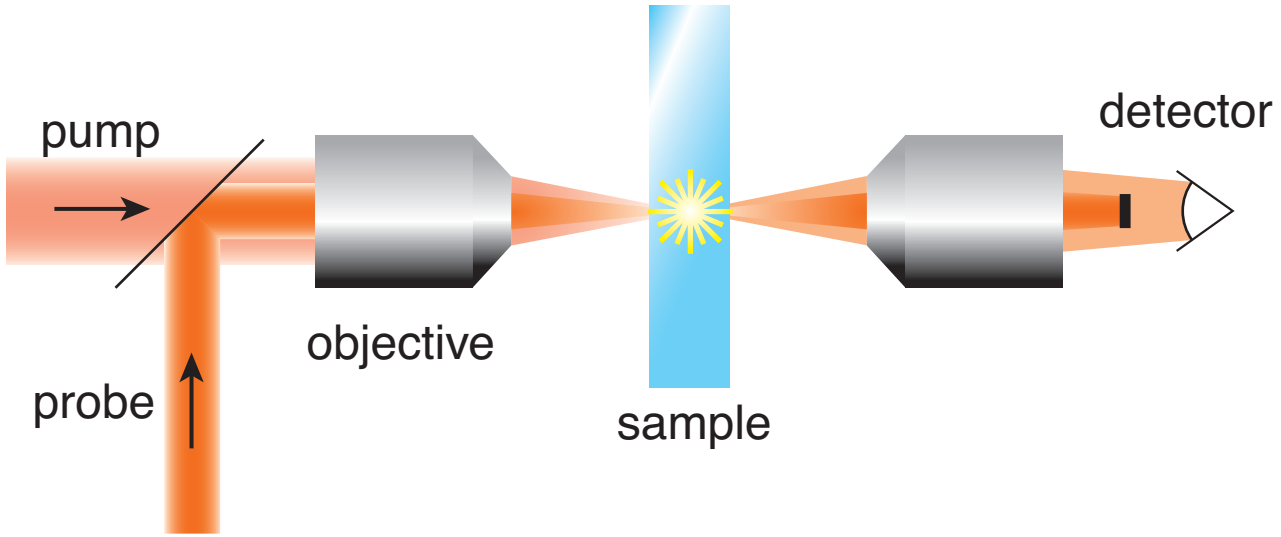
Femtosecond materials interactions

… bring in pump beam…



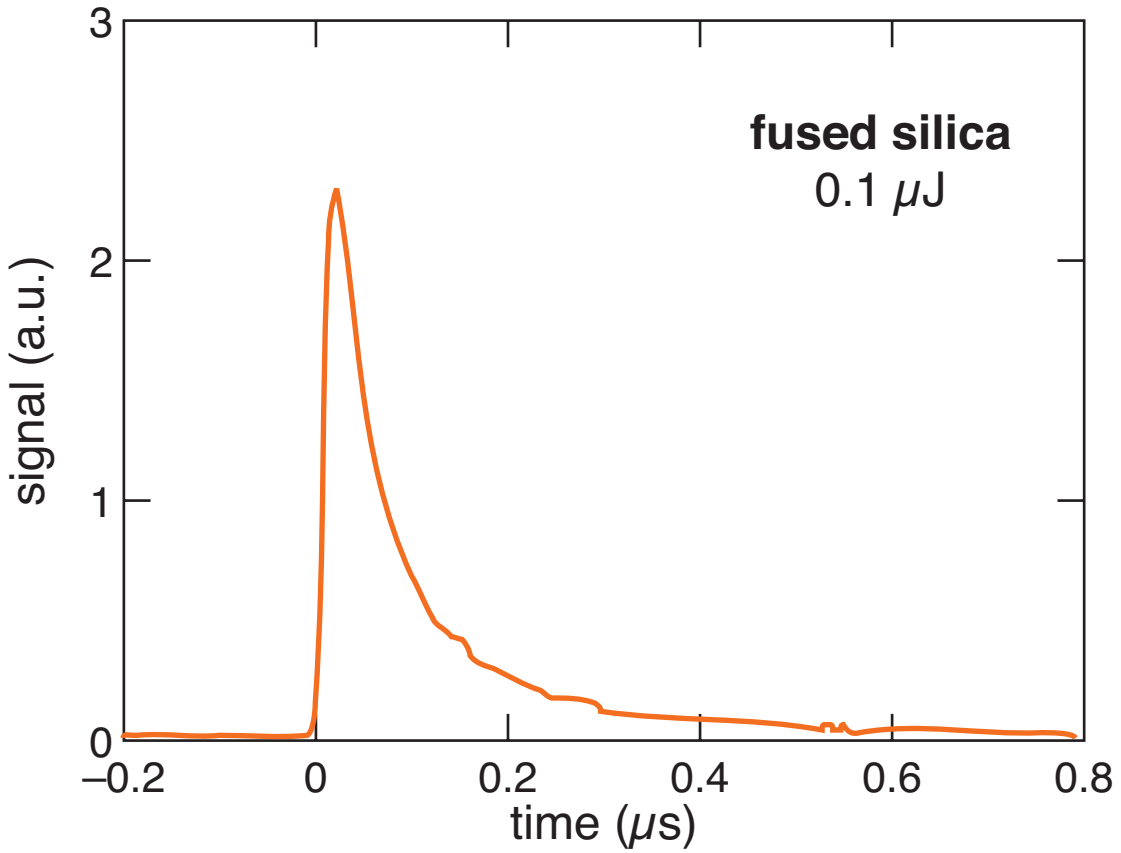
Femtosecond materials interactions

… damage scatters probe beam



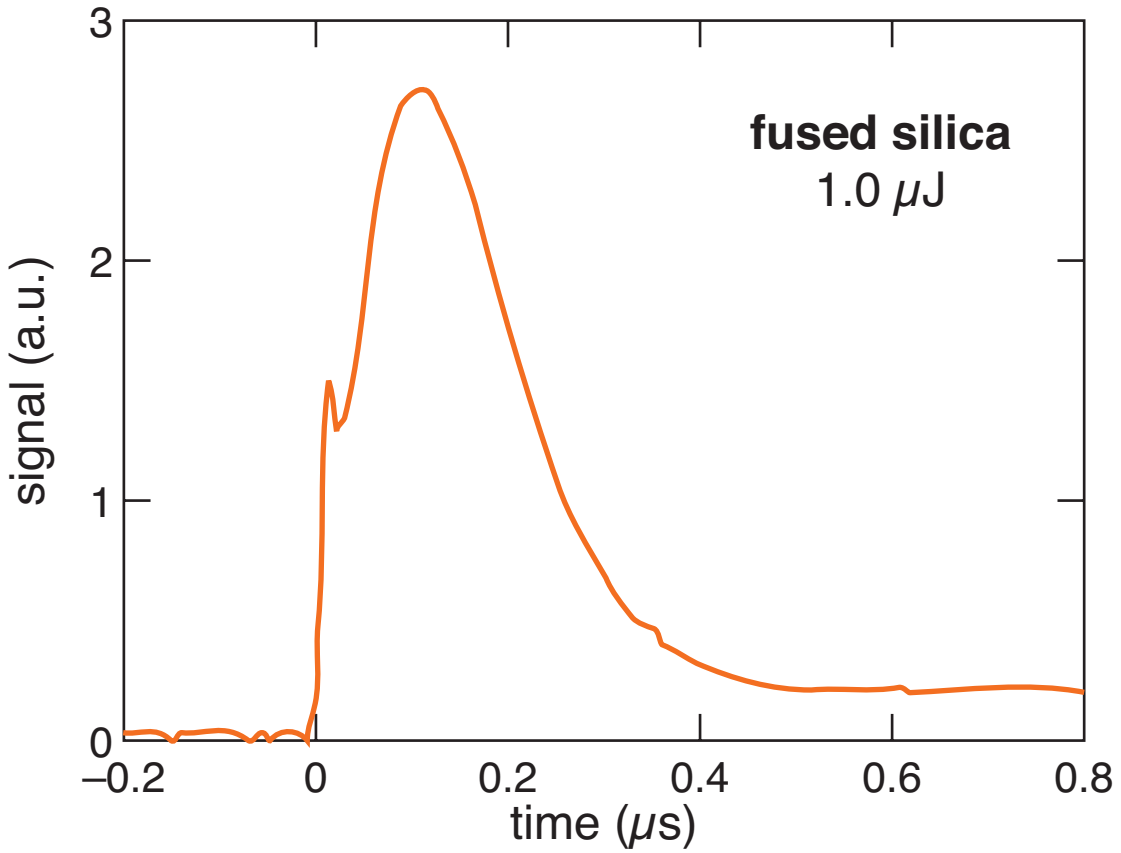
Femtosecond materials interactions

scattered signal



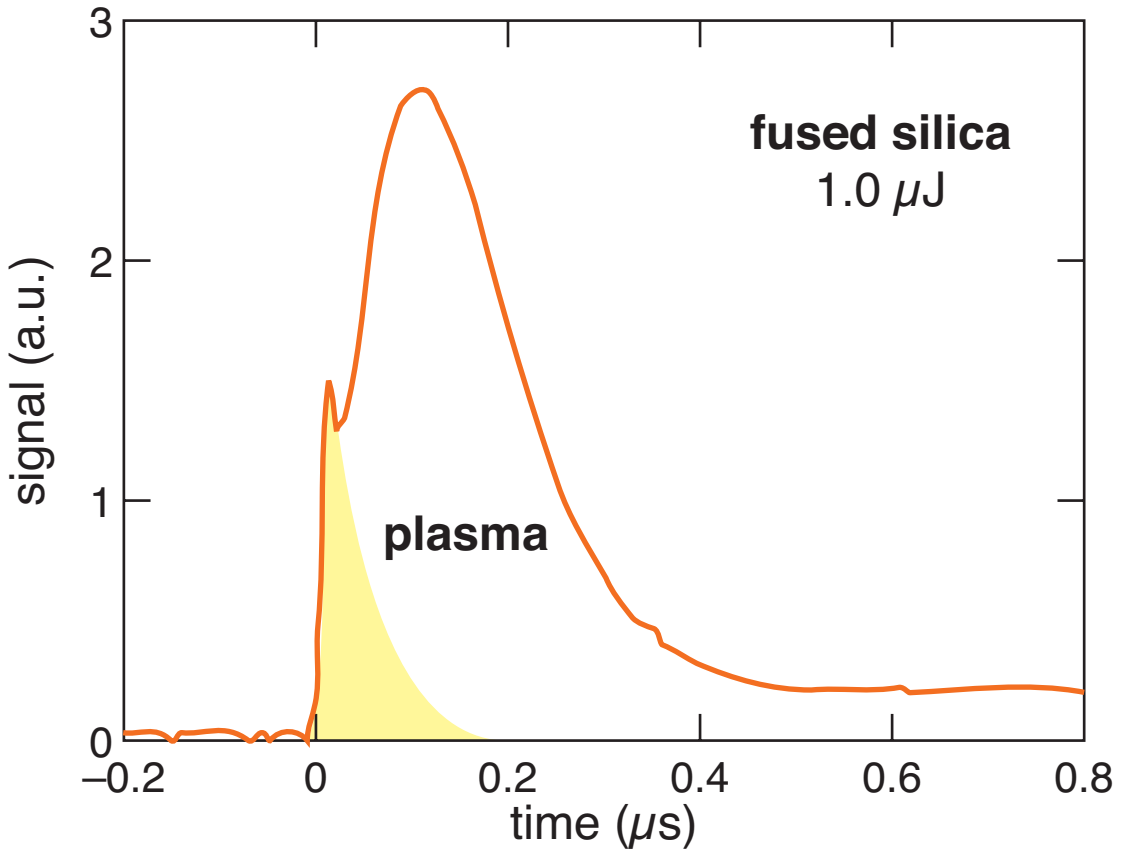
Femtosecond materials interactions

scattered signal



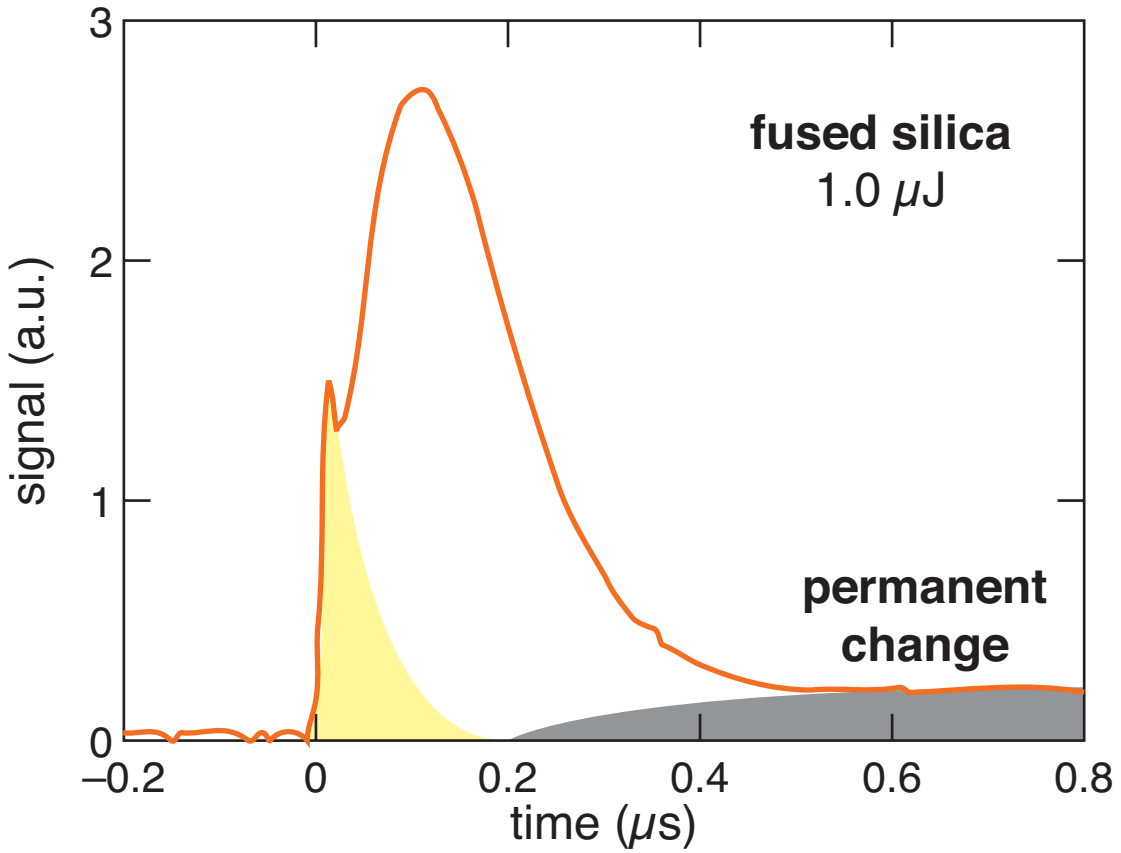
Femtosecond materials interactions

scattered signal



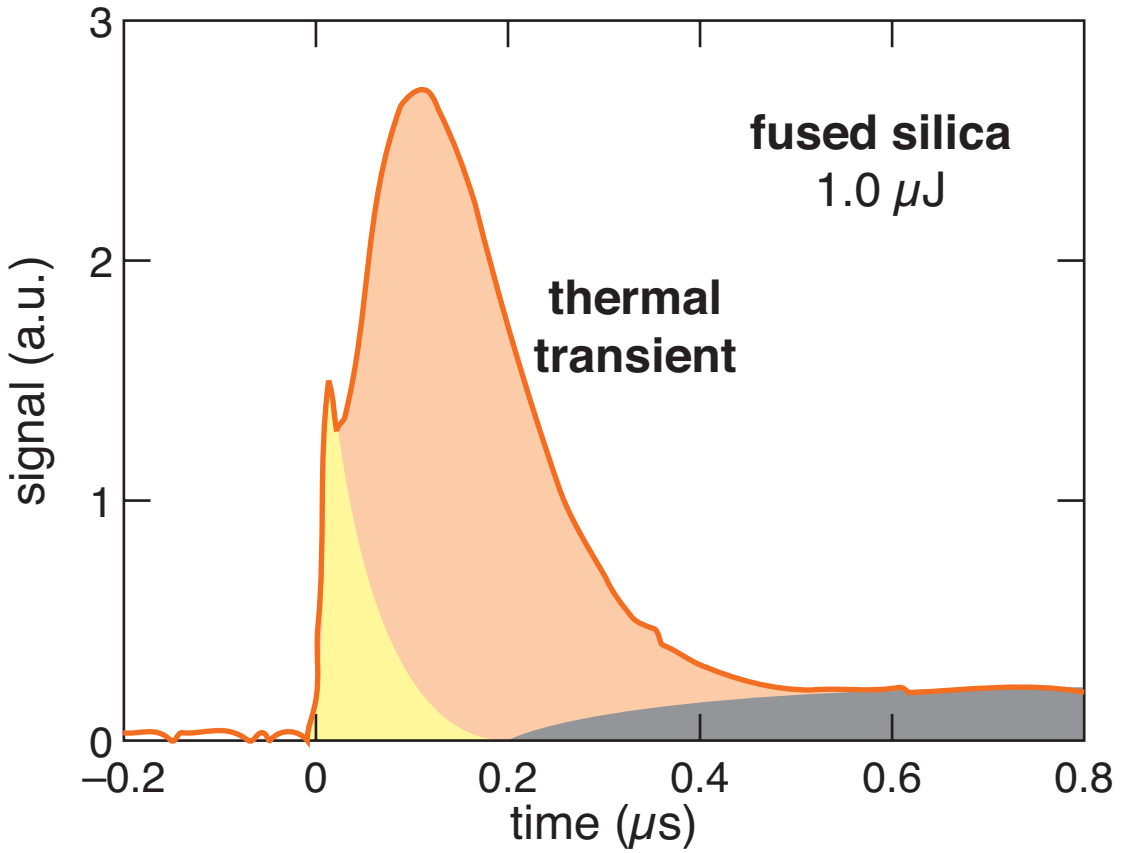
Femtosecond materials interactions

scattered signal



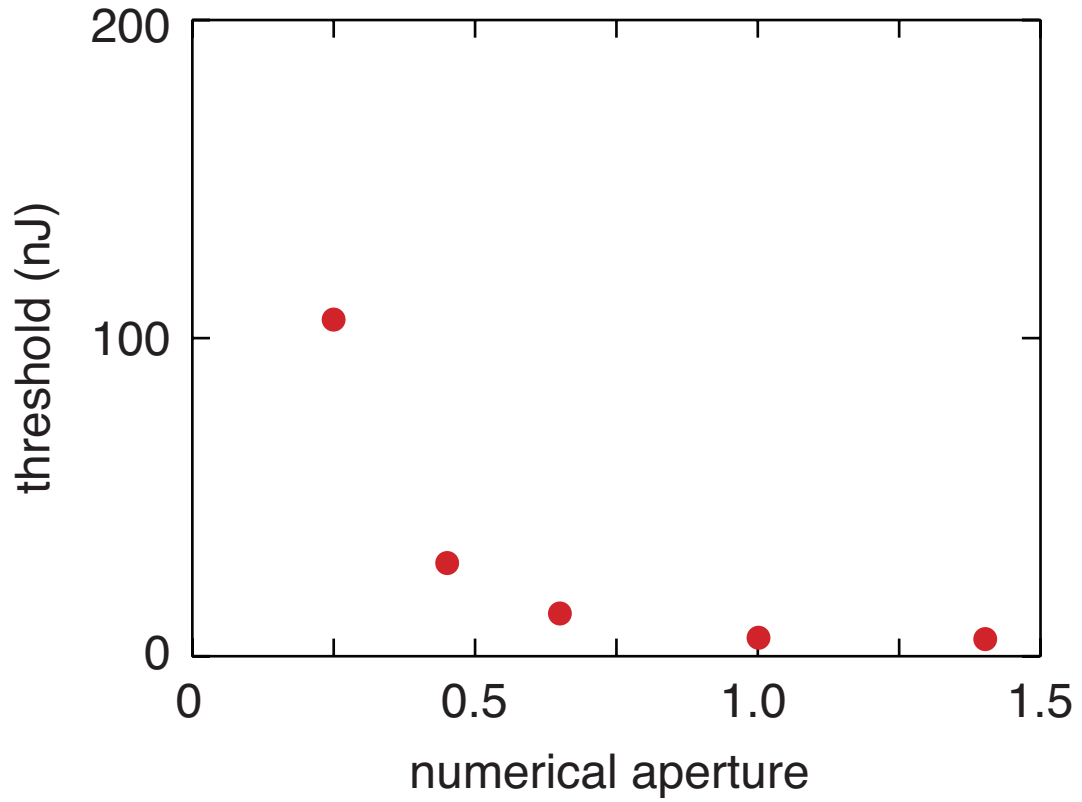
Femtosecond materials interactions

scattered signal



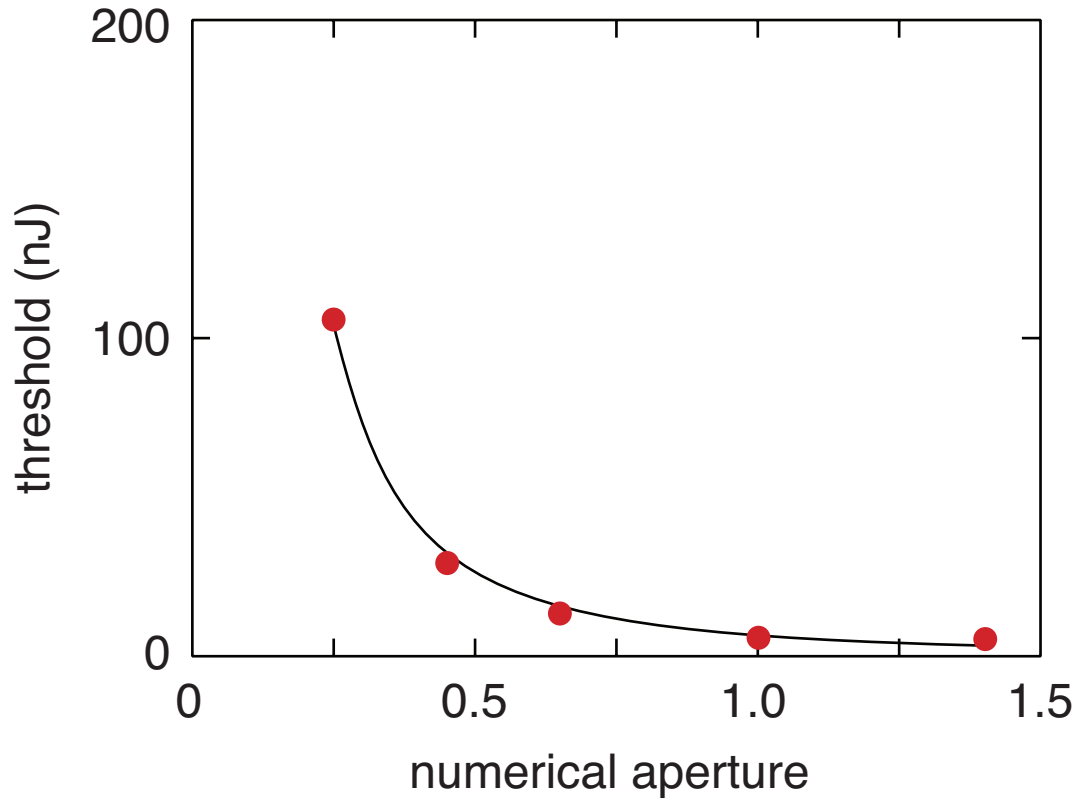
Femtosecond materials interactions

vary numerical aperture



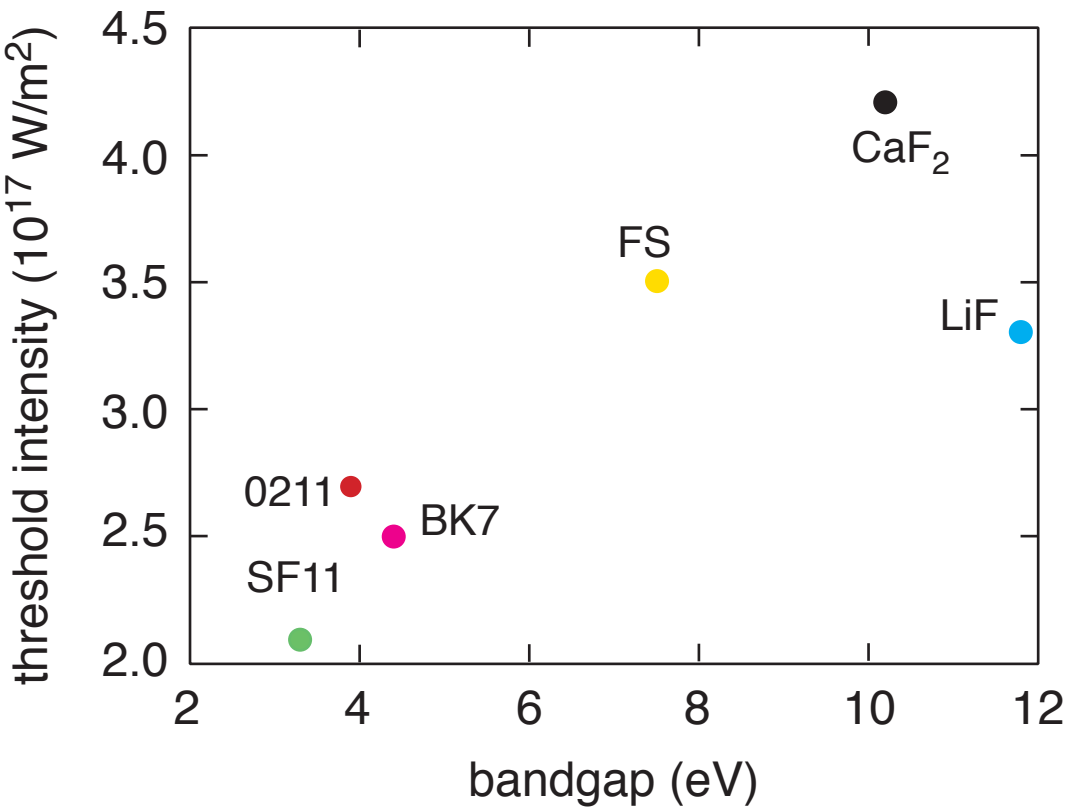
Femtosecond materials interactions

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



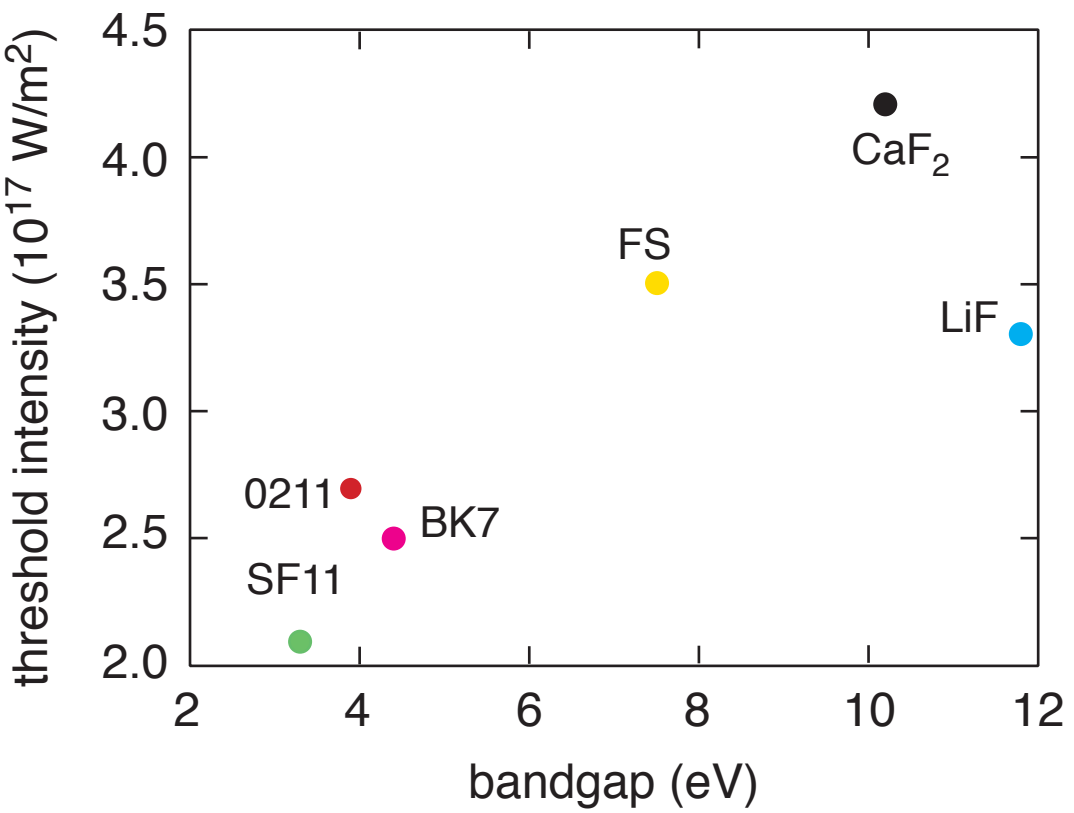
Femtosecond materials interactions

vary material…



Femtosecond materials interactions

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



Femtosecond materials interactions

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

Outline

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

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

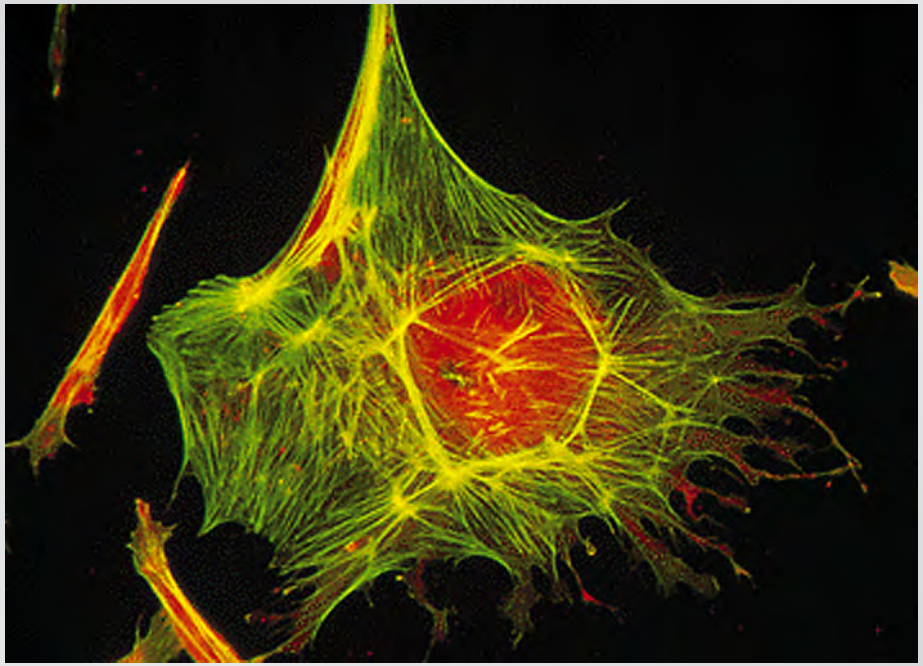
Cytoskeleton

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

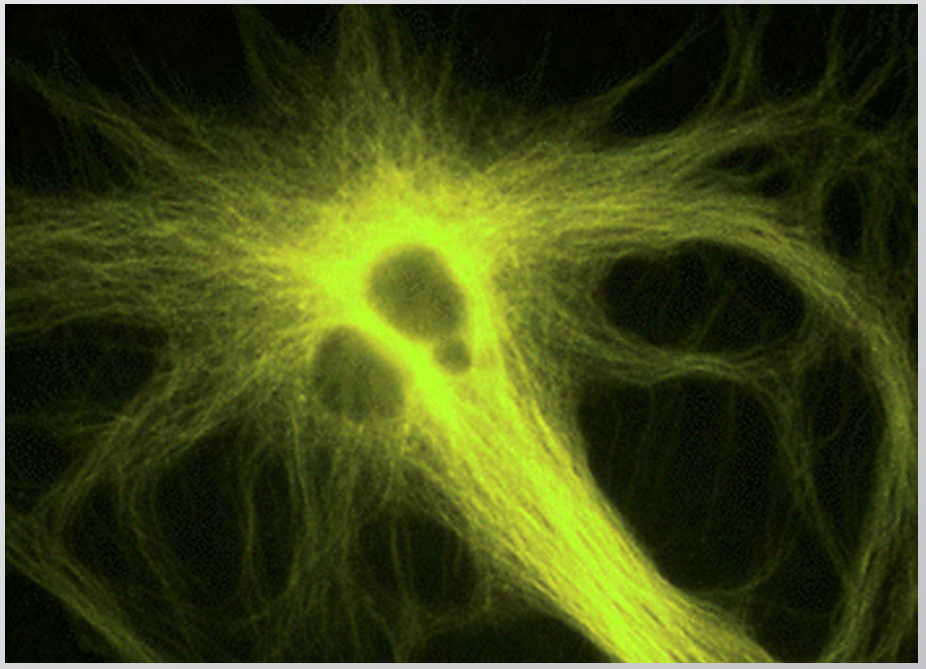
Subcellular surgery

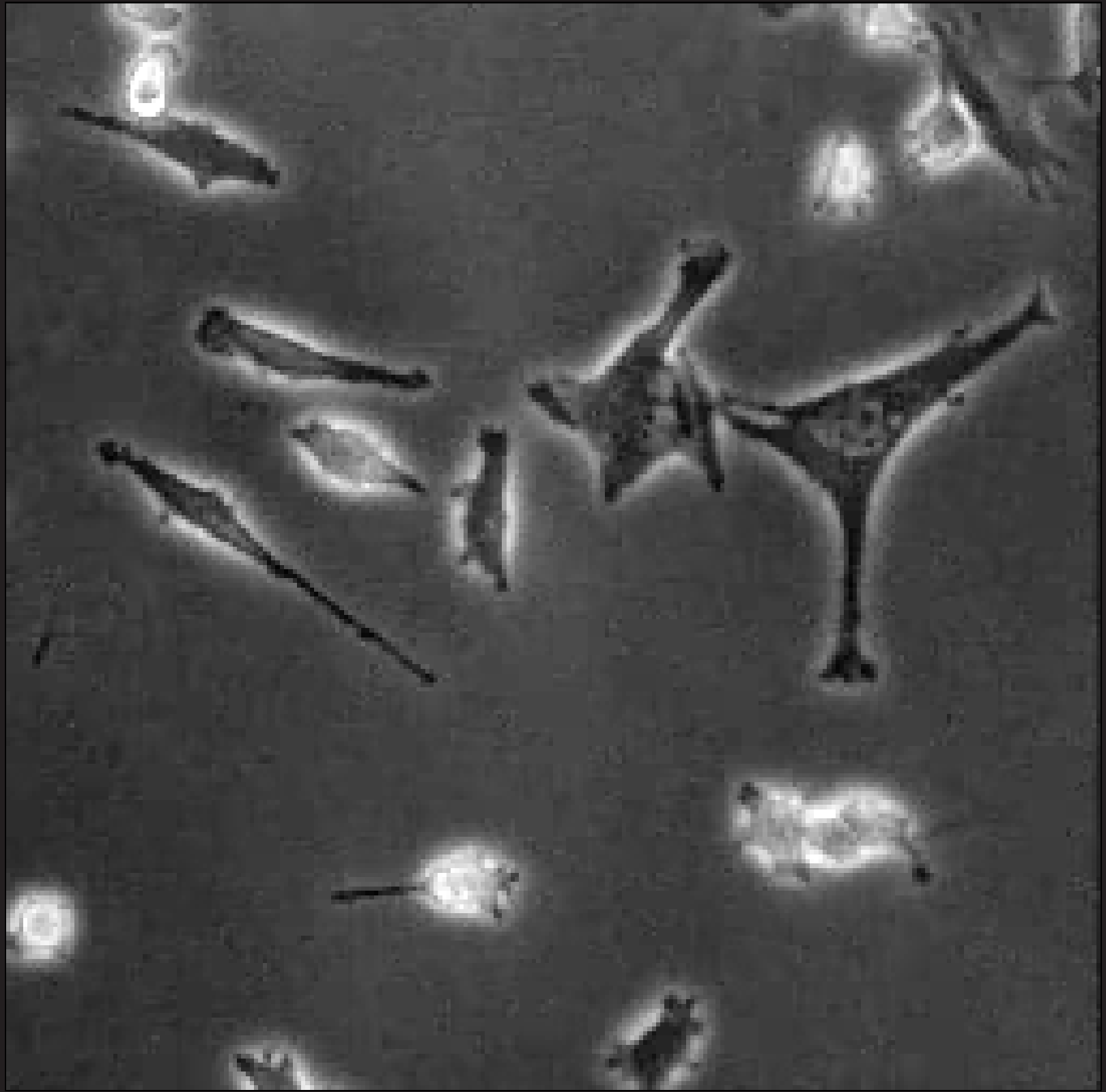
two components

actin fibers

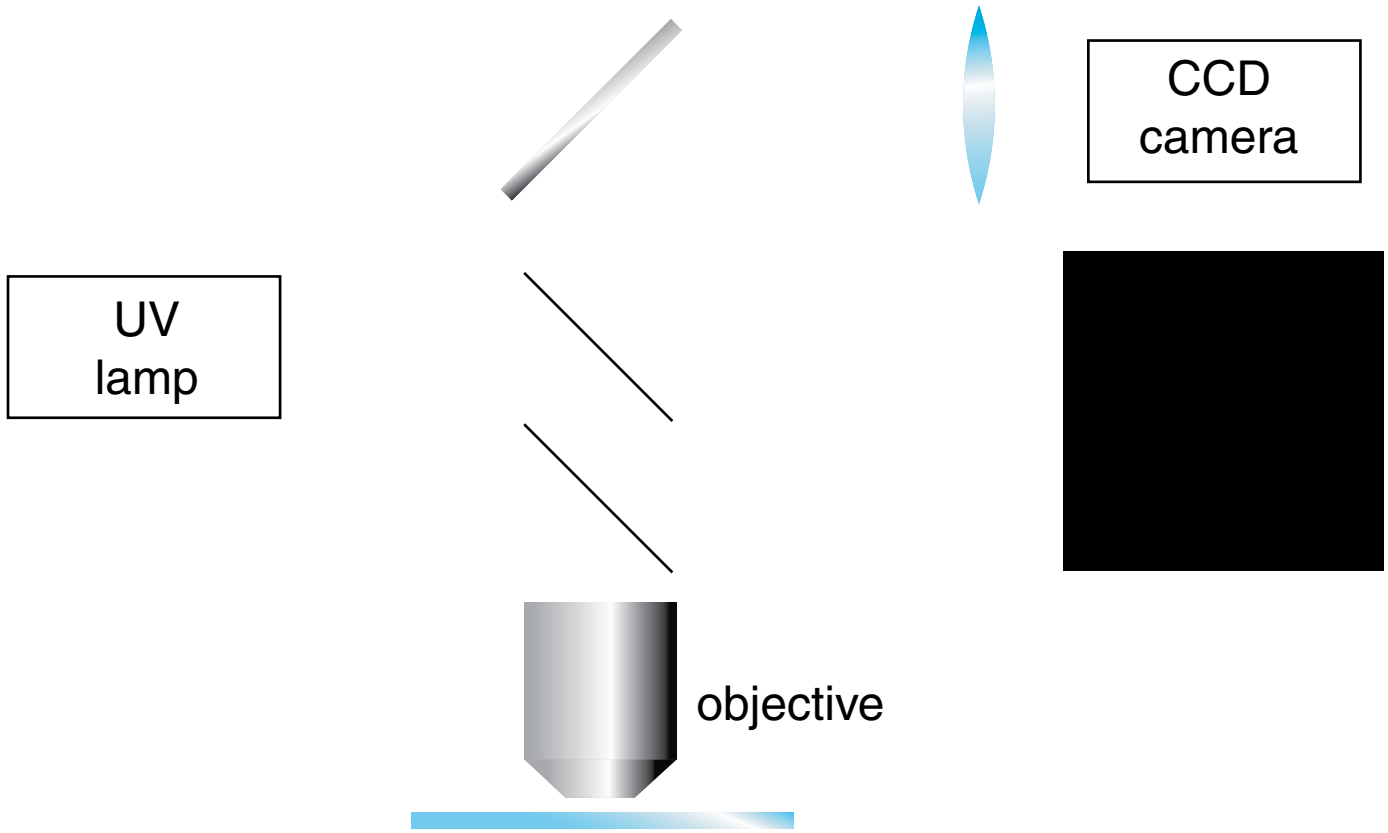


microtubules



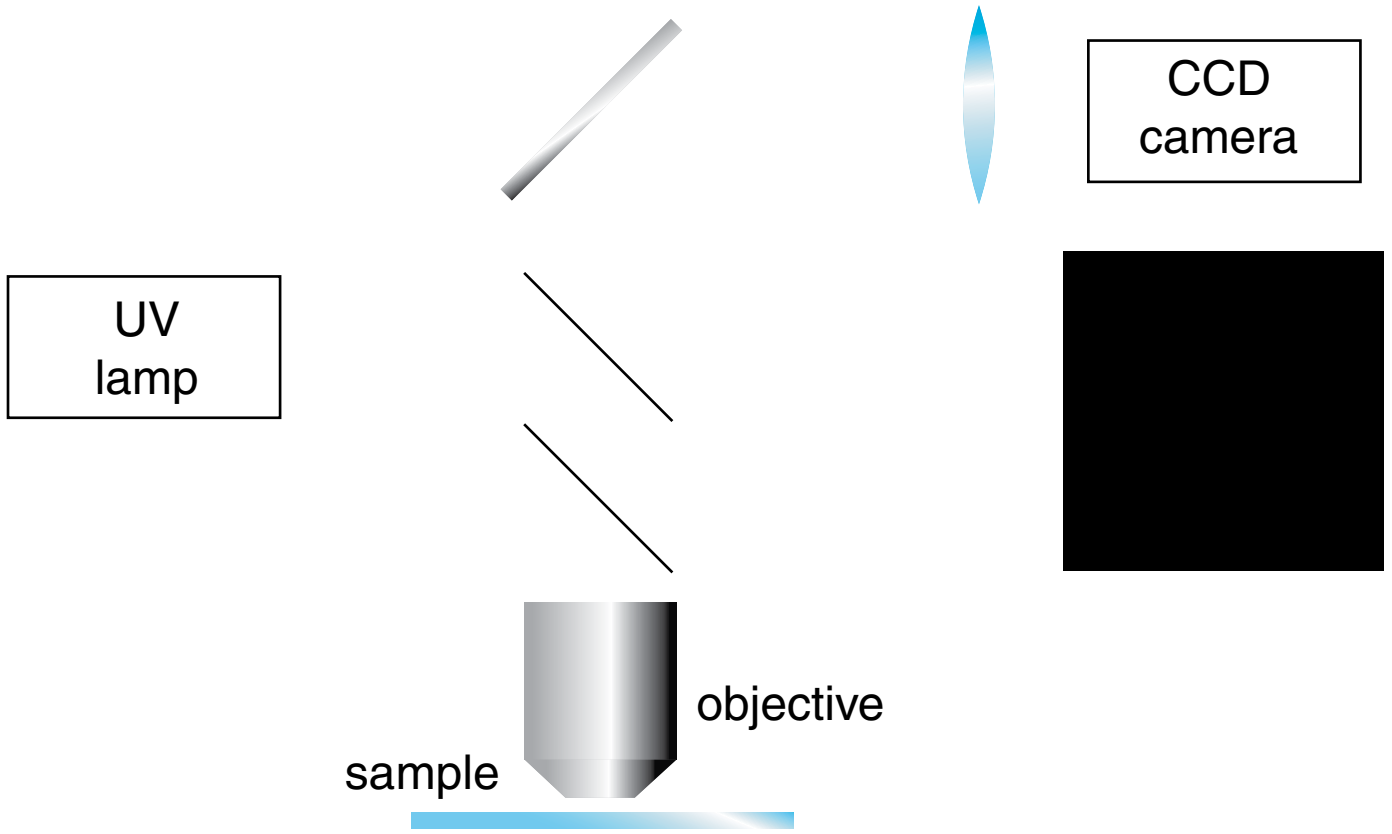


Subcellular surgery



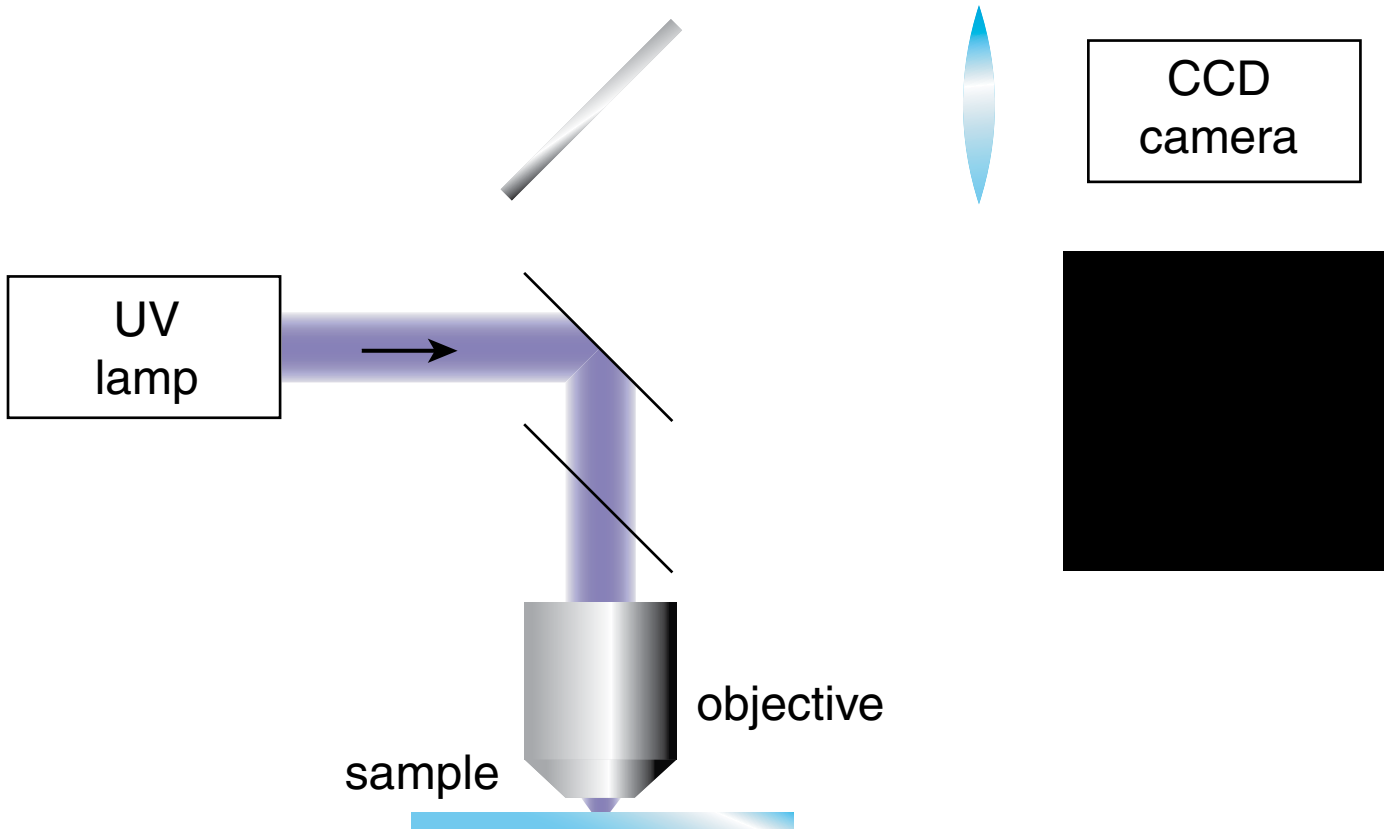
epi-fluorescence microscope

Subcellular surgery



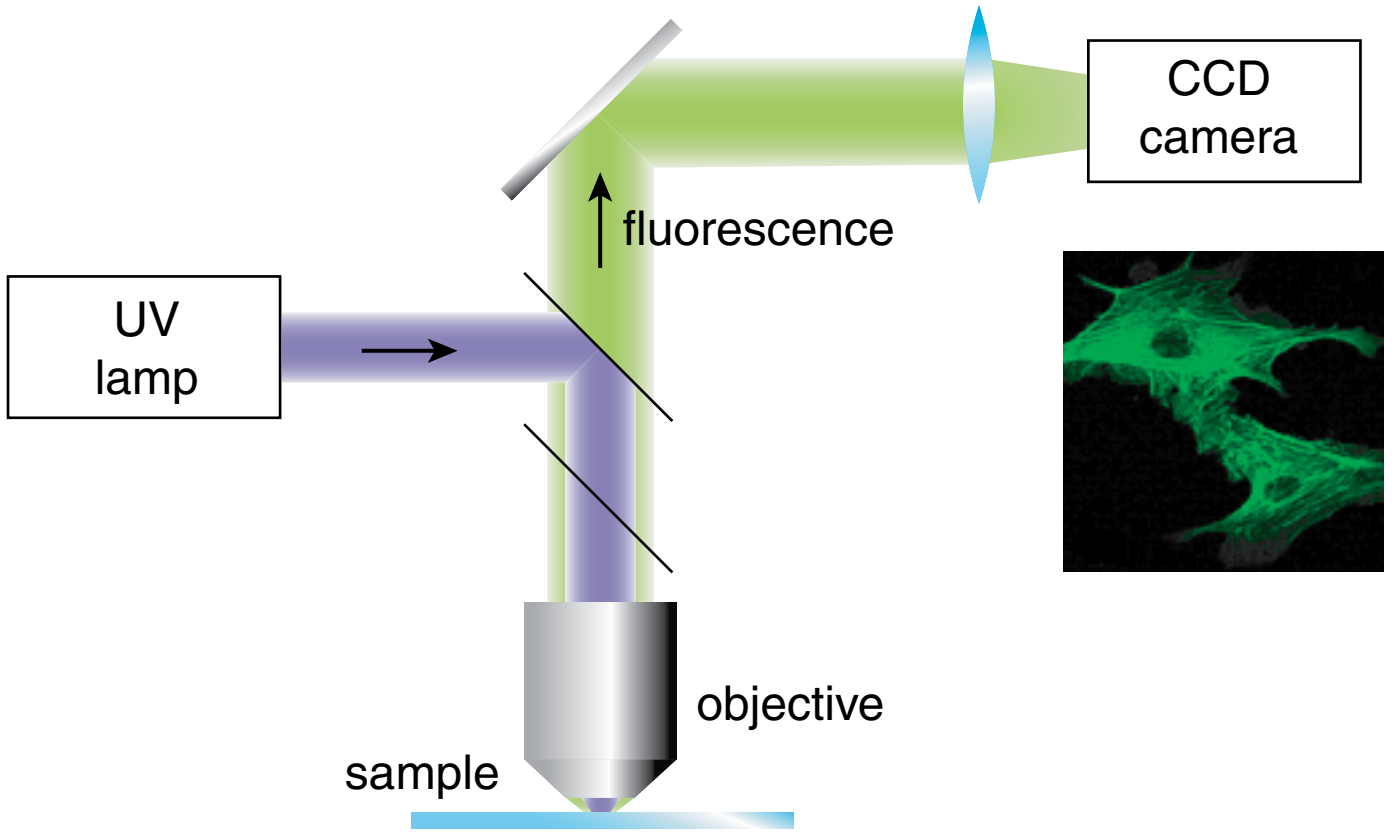
fluorescently label sample

Subcellular surgery



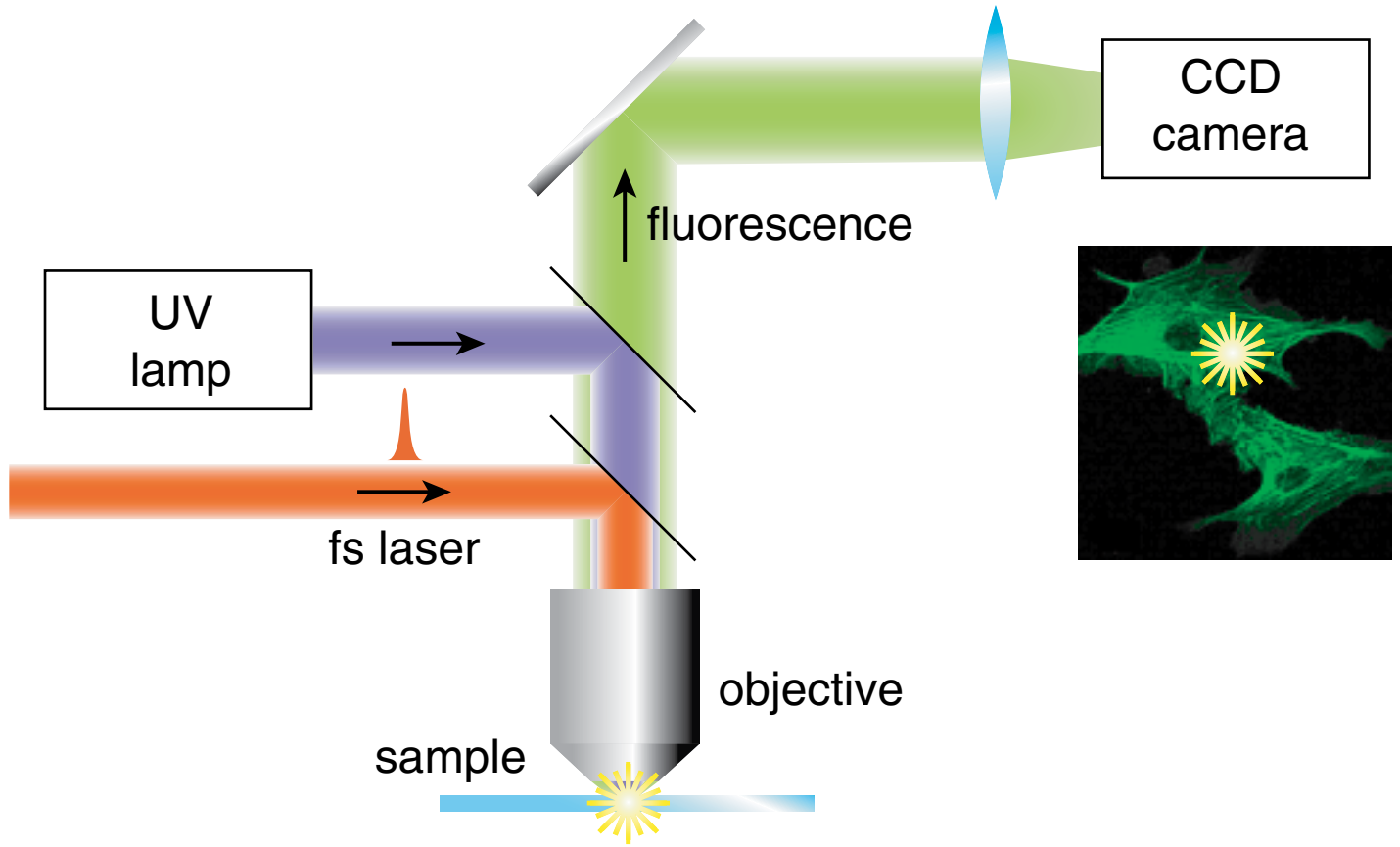
UV illumination…

Subcellular surgery



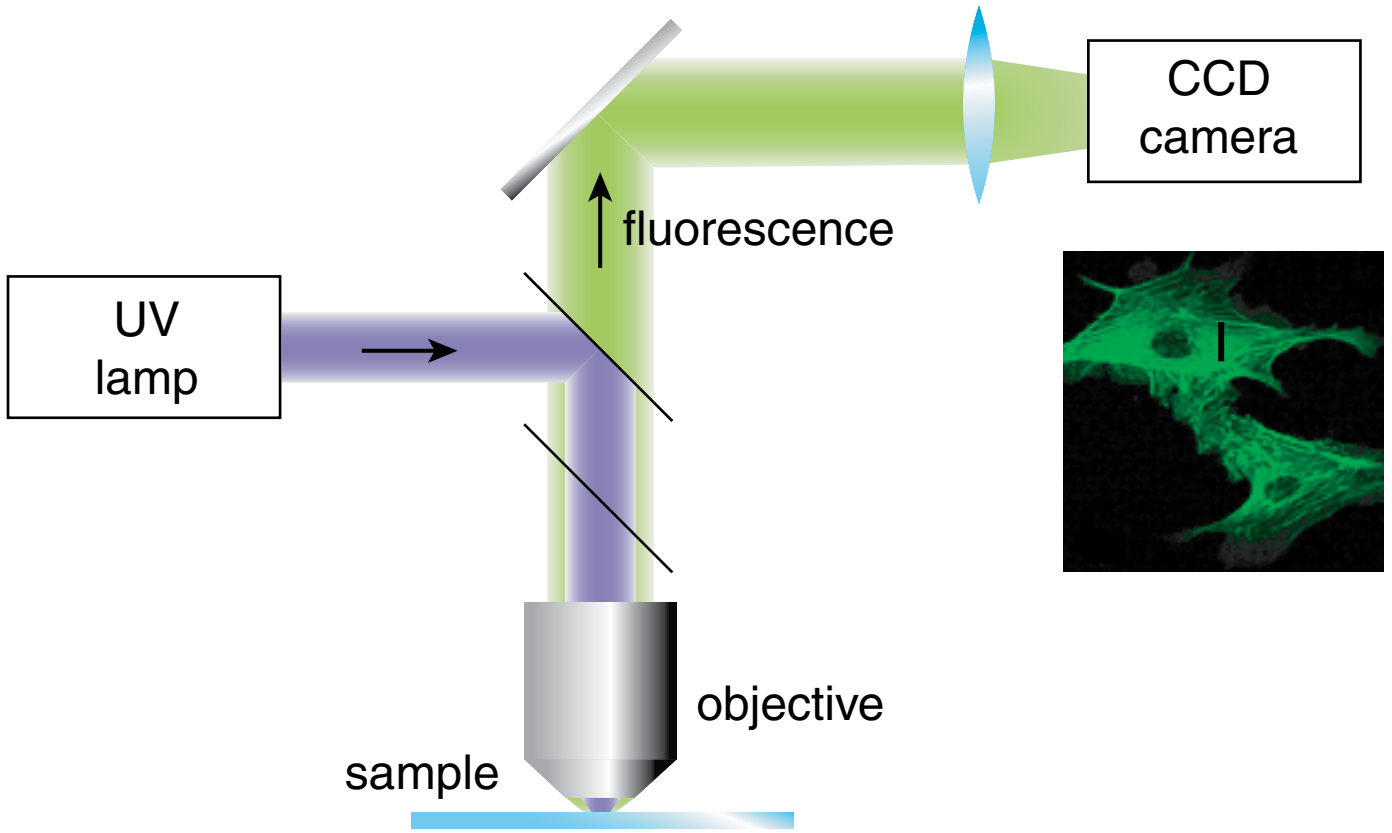
…causes fluorescence

Subcellular surgery



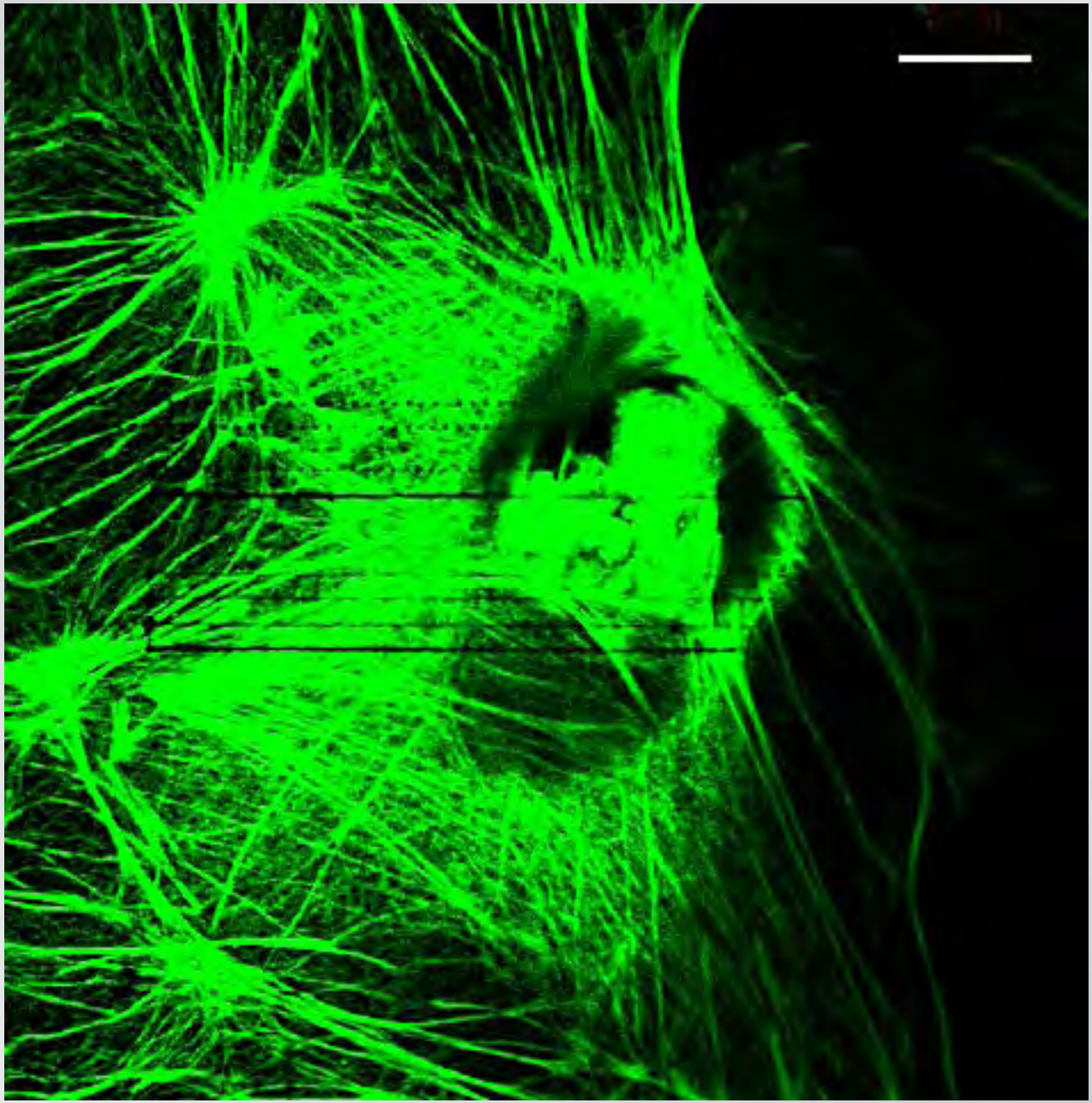
irradiate with fs laser beam

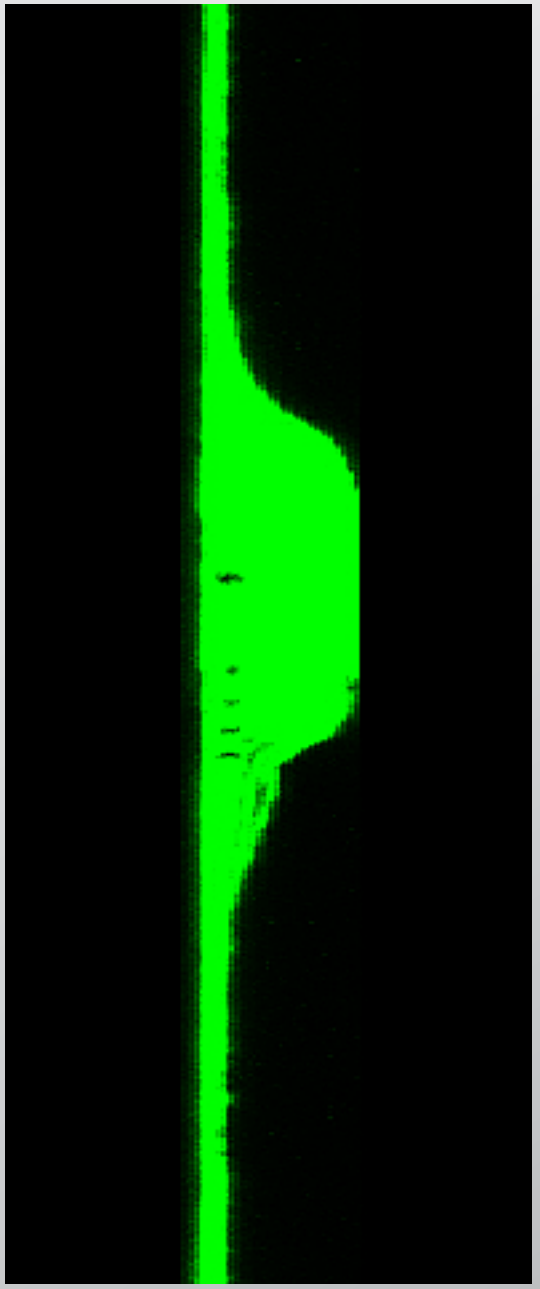
Subcellular surgery



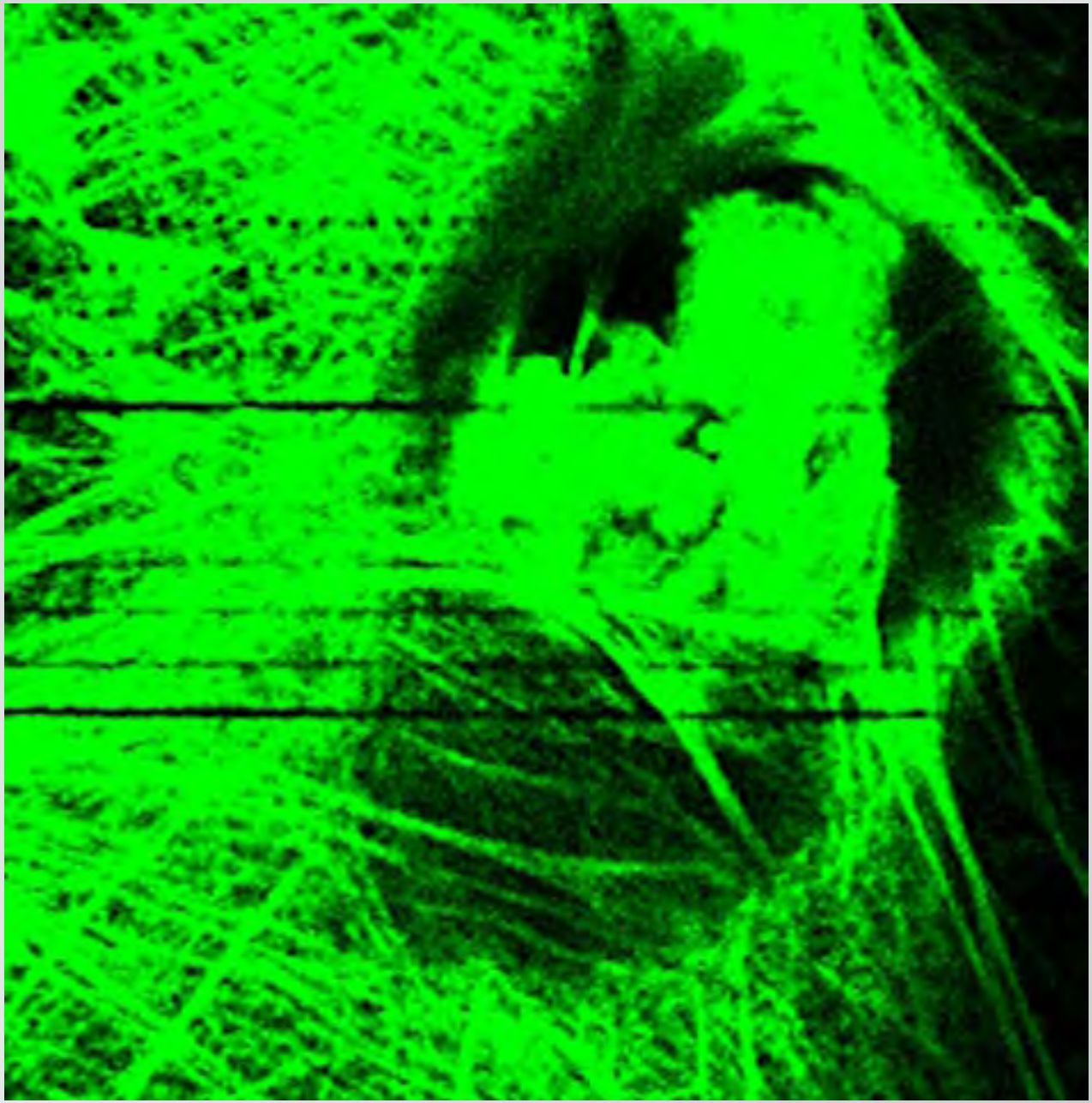
examine resulting ablation

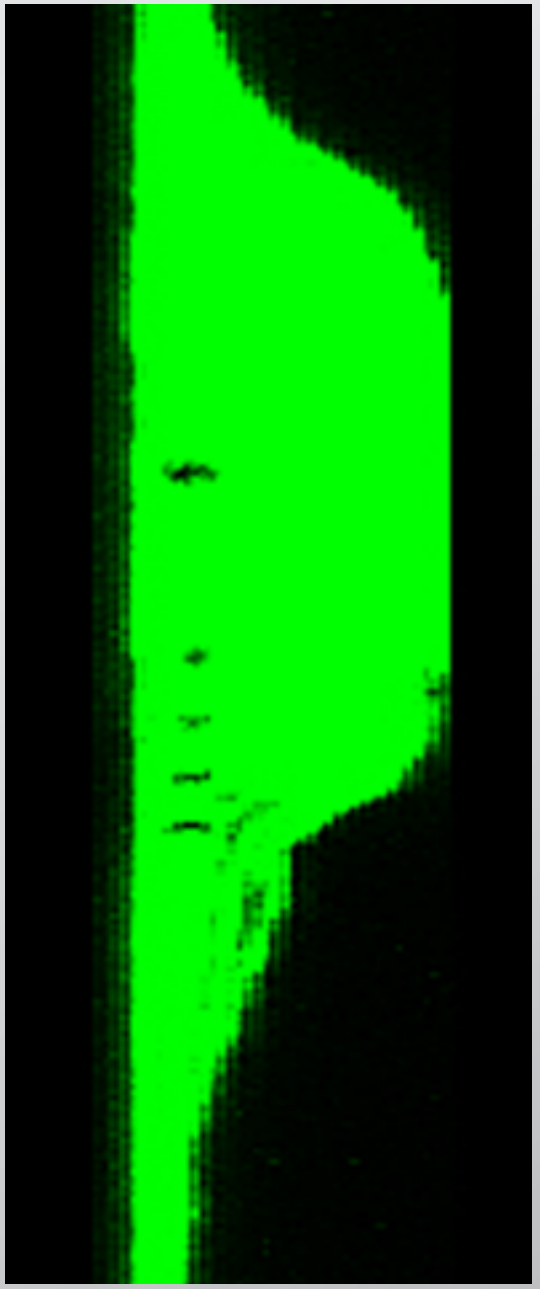
Subcellular surgery





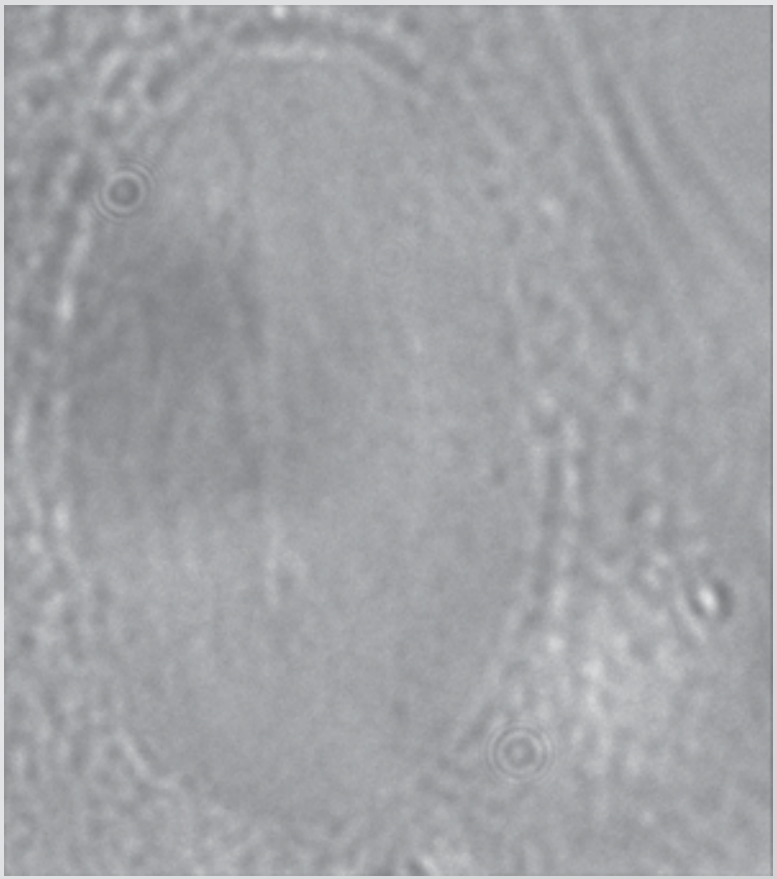
Subcellular surgery





Subcellular surgery

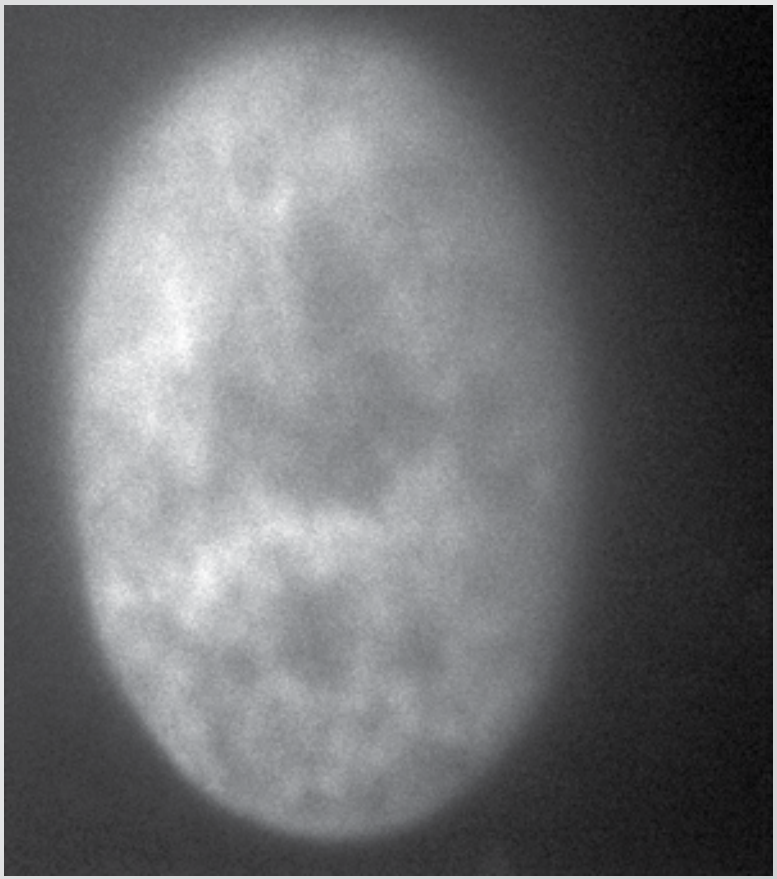
nucleus of fixed endothelial cell



white light microscopy

Subcellular surgery

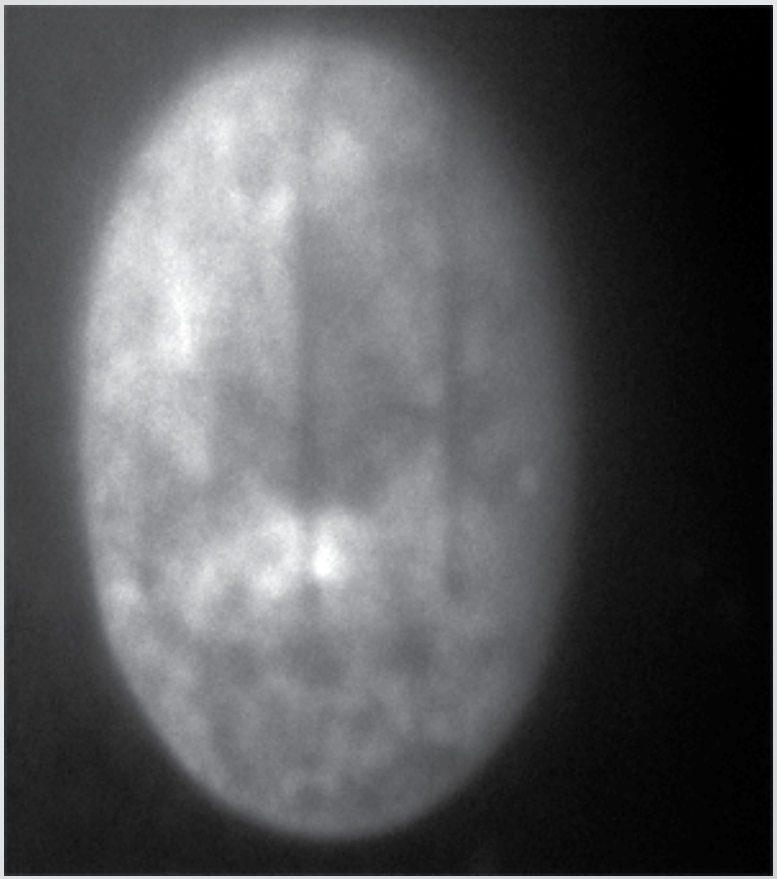
nucleus of fixed endothelial cell



fluorescence microscopy

Subcellular surgery

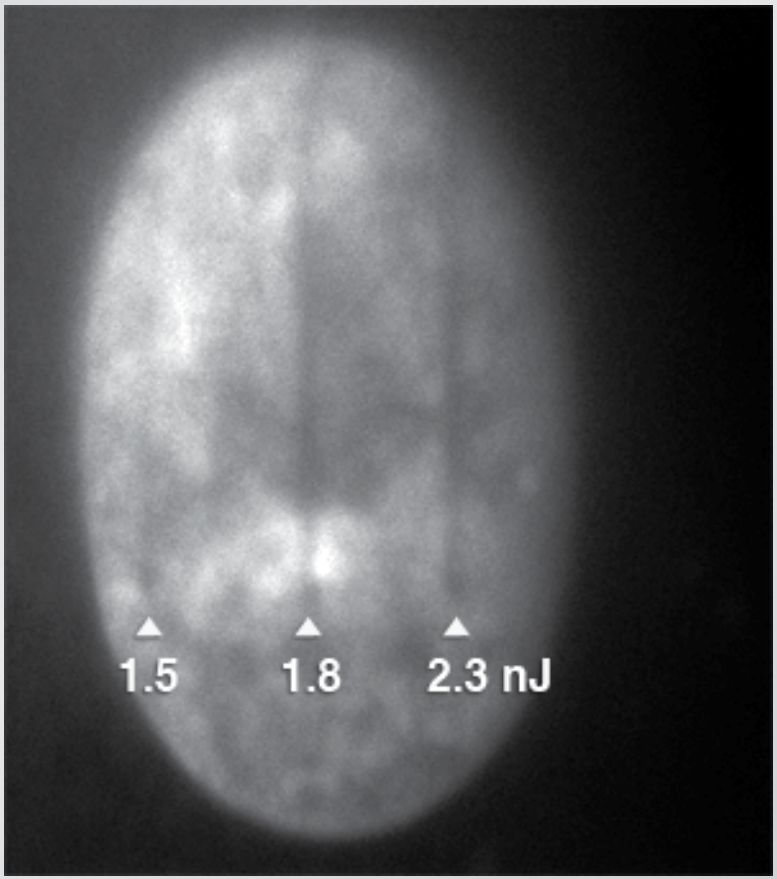
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

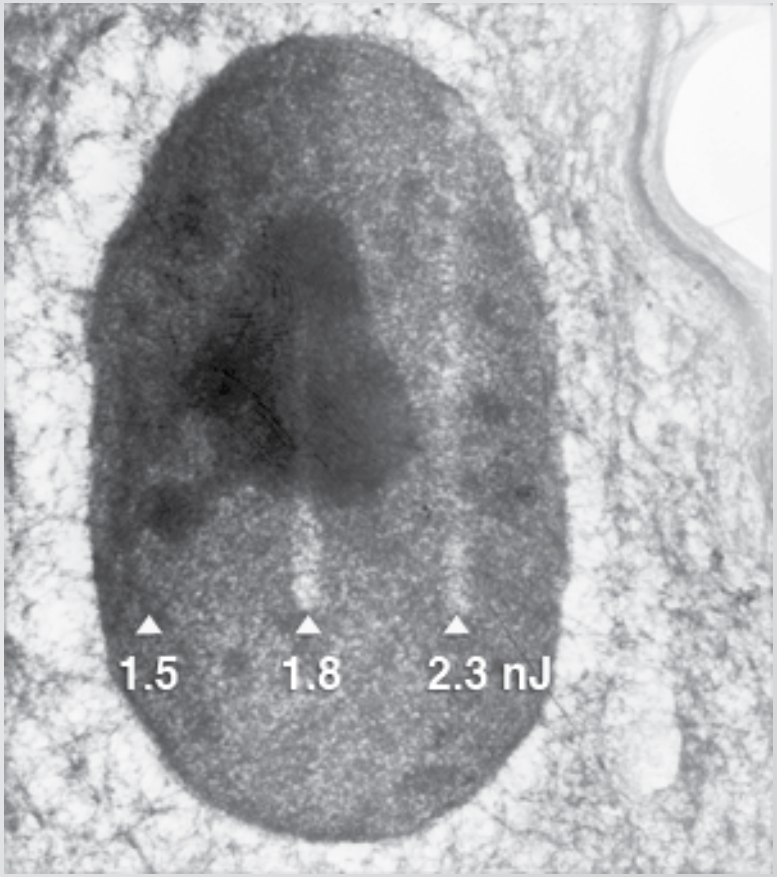
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

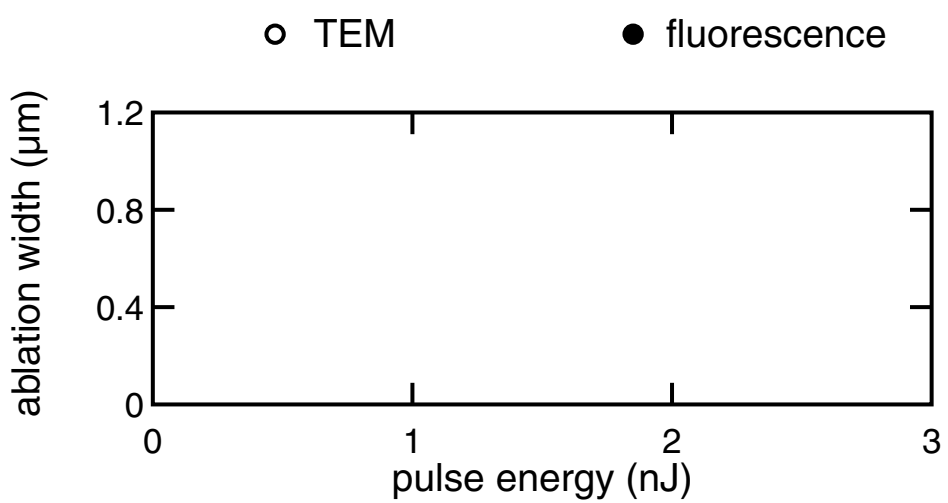
bleaching or ablation?



TEM image

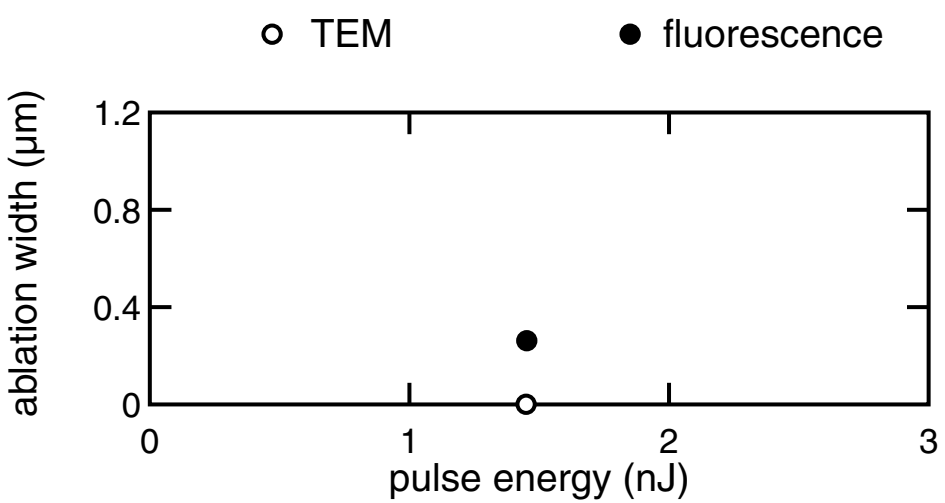
Subcellular surgery

three regions of interaction



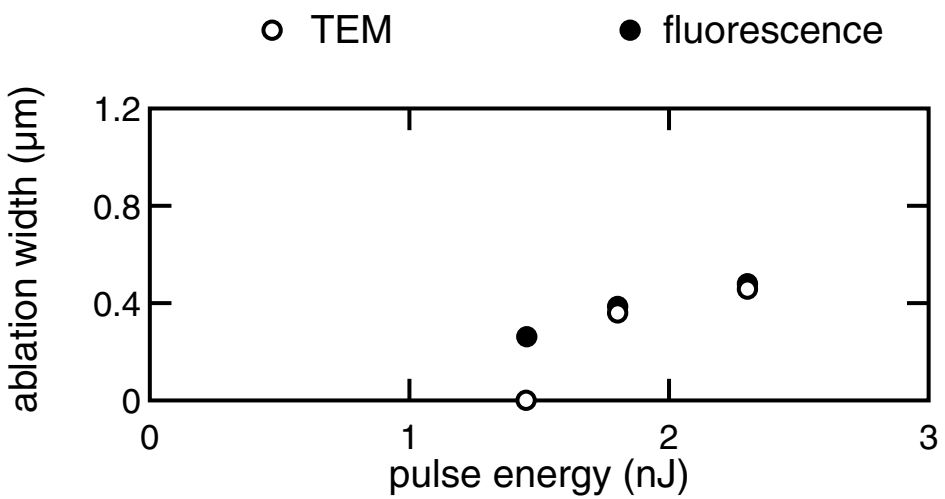
Subcellular surgery

three regions of interaction



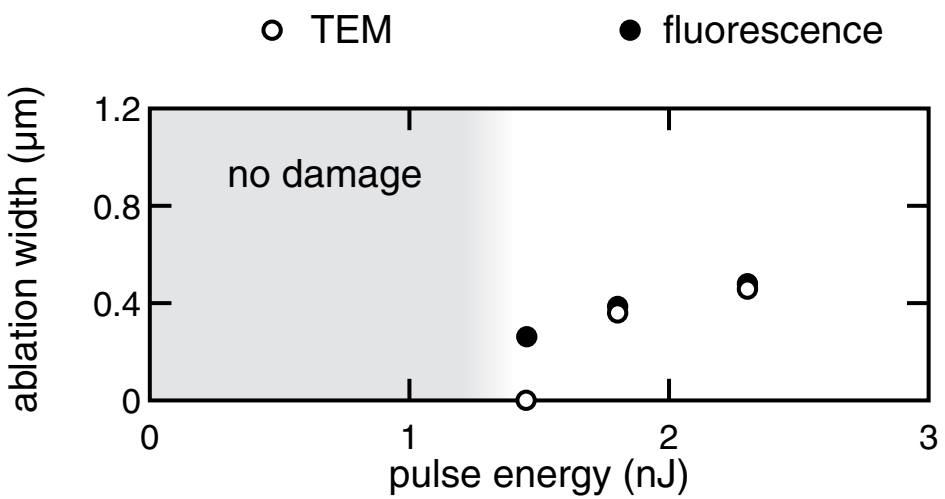
Subcellular surgery

three regions of interaction



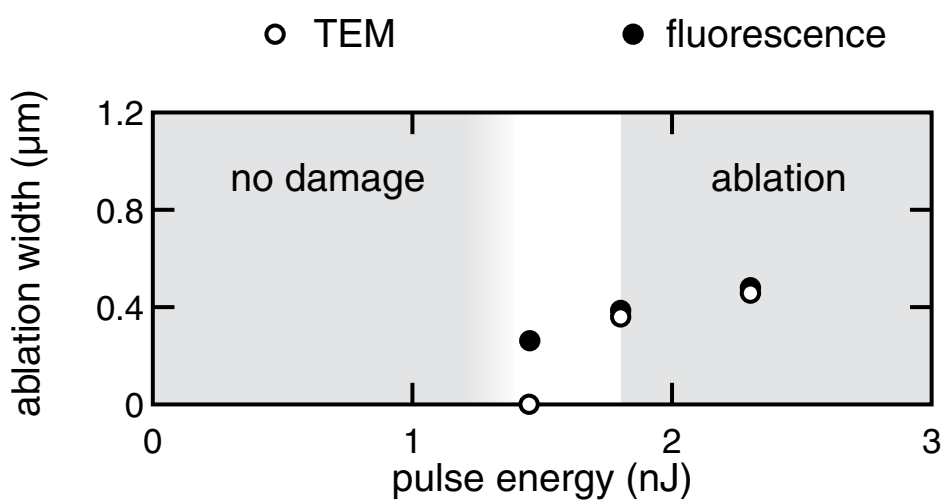
Subcellular surgery

three regions of interaction



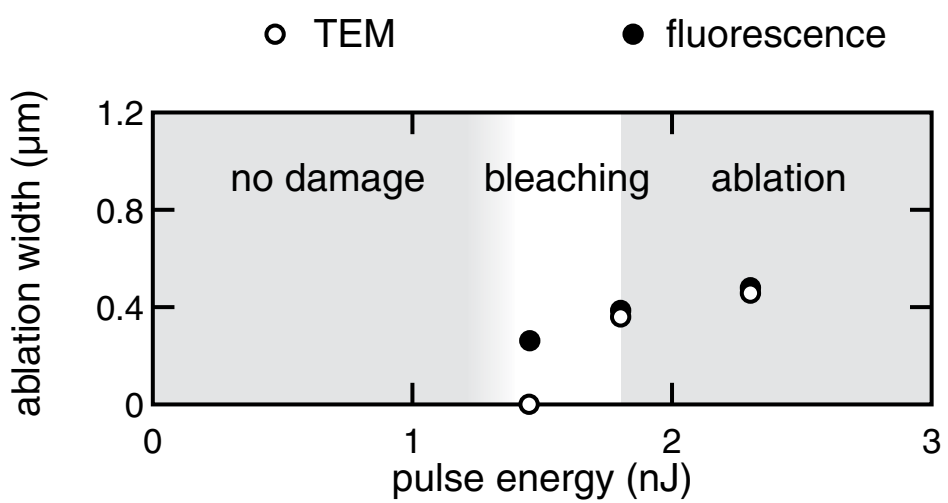
Subcellular surgery

three regions of interaction



Subcellular surgery

three regions of interaction



Subcellular surgery

Definitive proof of ablation

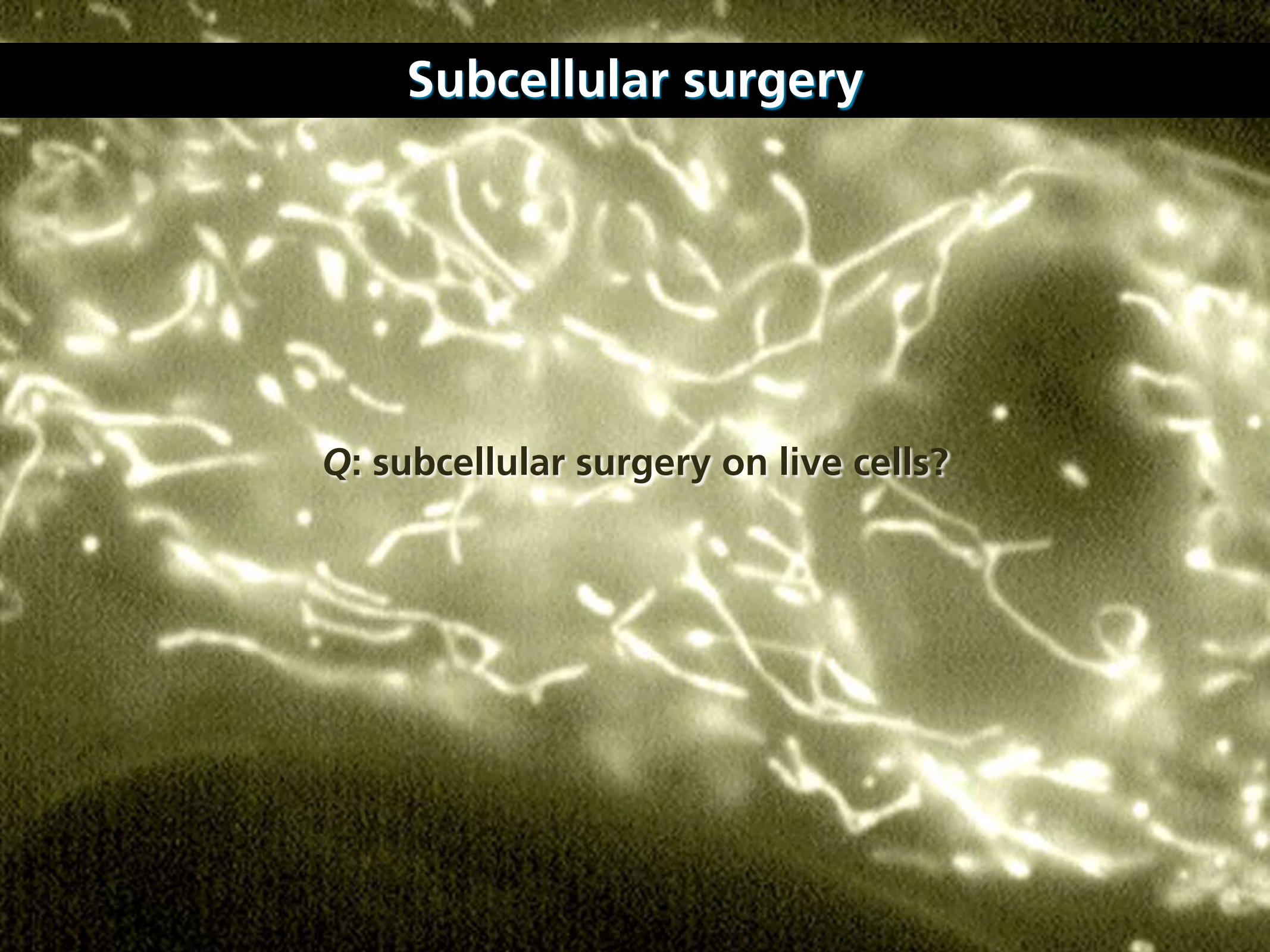
- ablation width as small as 100 nm
- ablation threshold varies slightly
- ablation threshold 20% above bleaching threshold

Subcellular surgery

Definitive proof of ablation

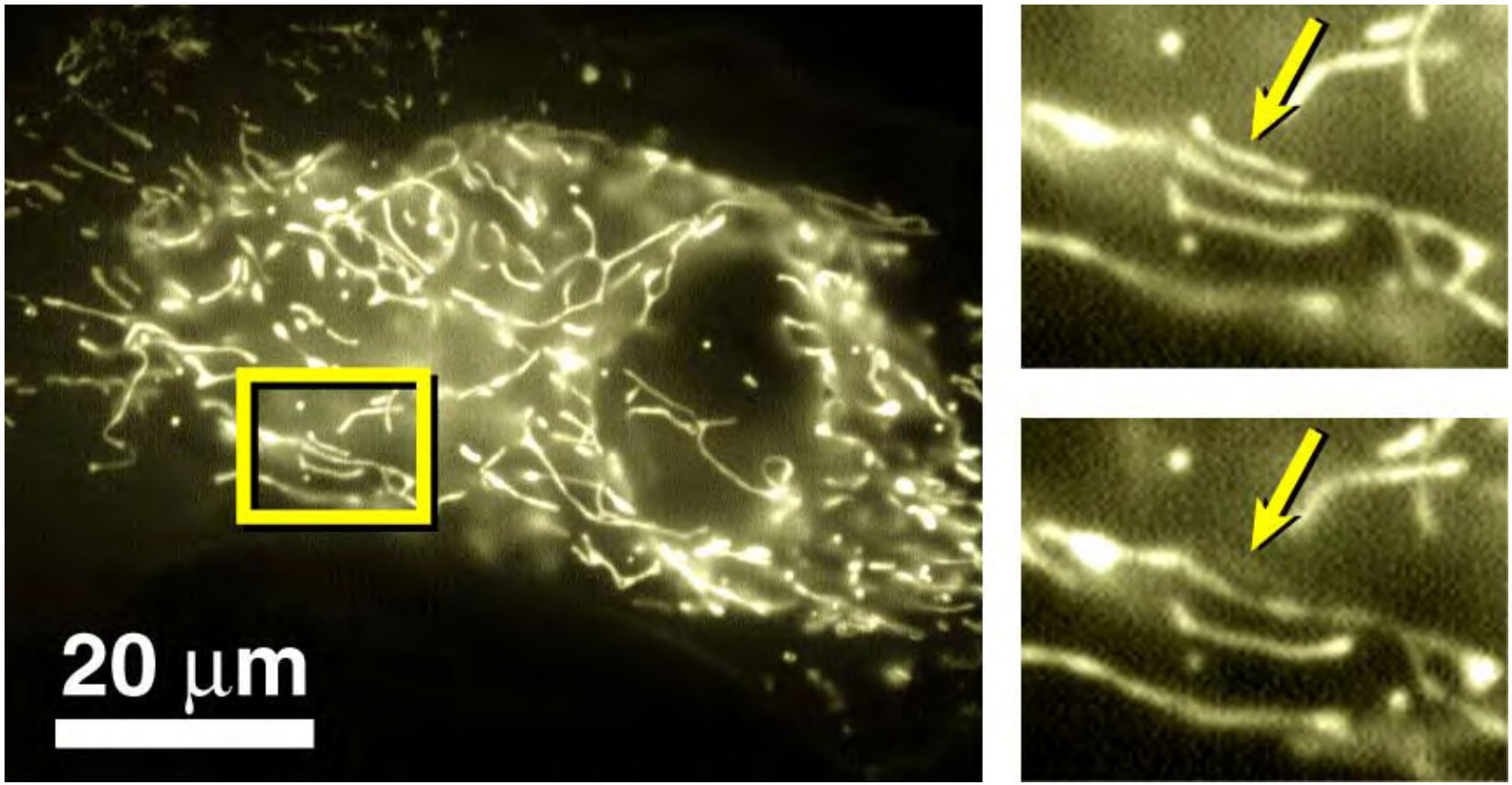
- ablation width as small as 100 nm
- ablation threshold varies slightly
- **ablation threshold 20% above bleaching threshold**

Subcellular surgery



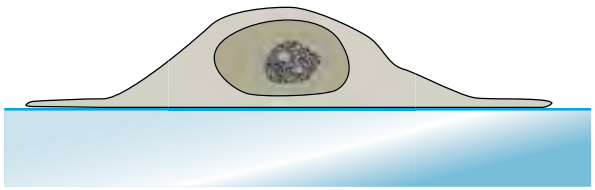
Q: subcellular surgery on live cells?

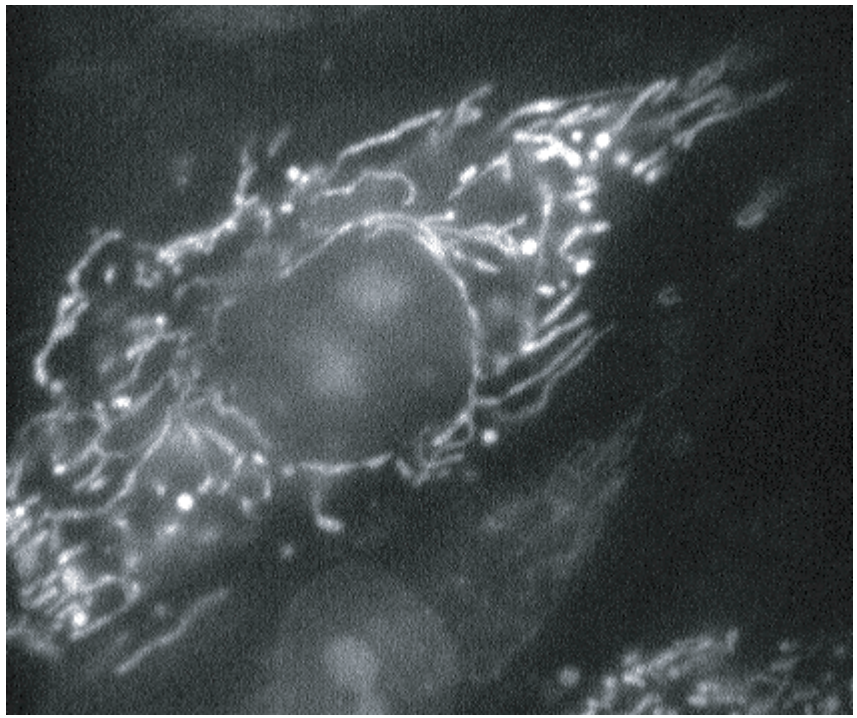
Subcellular surgery



Subcellular surgery

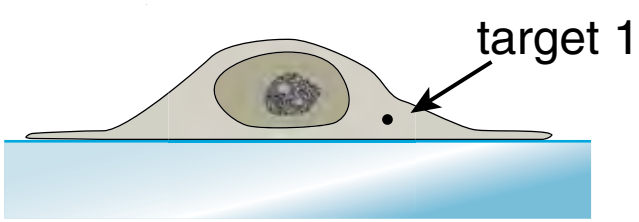
ethydium bromide test

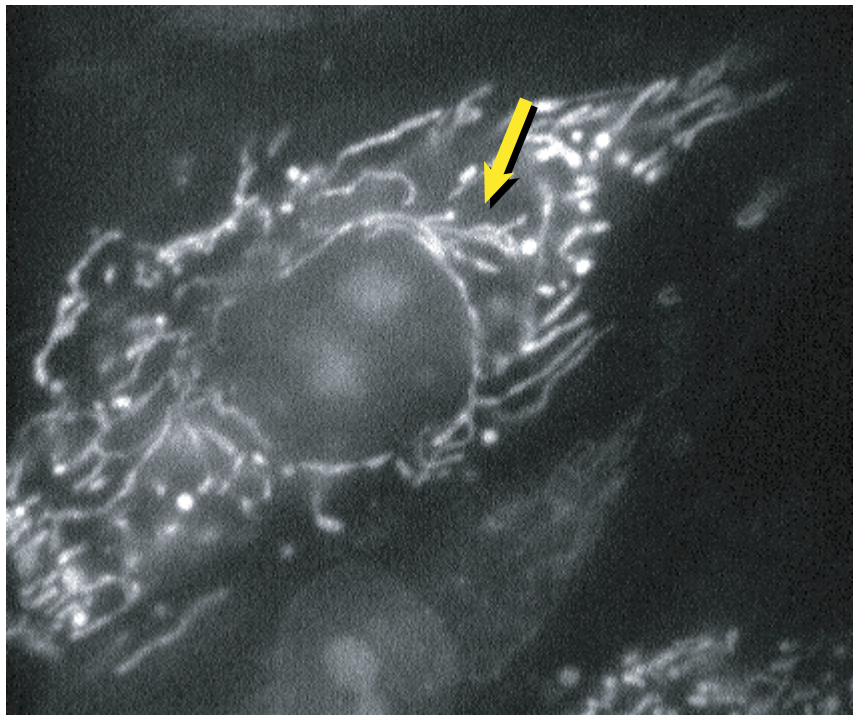




Subcellular surgery

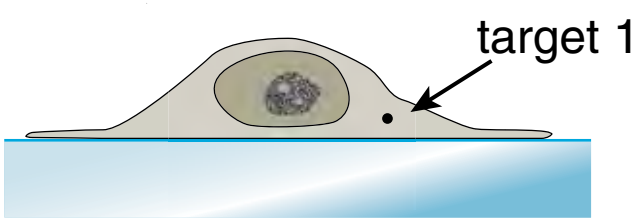
ethydium bromide test

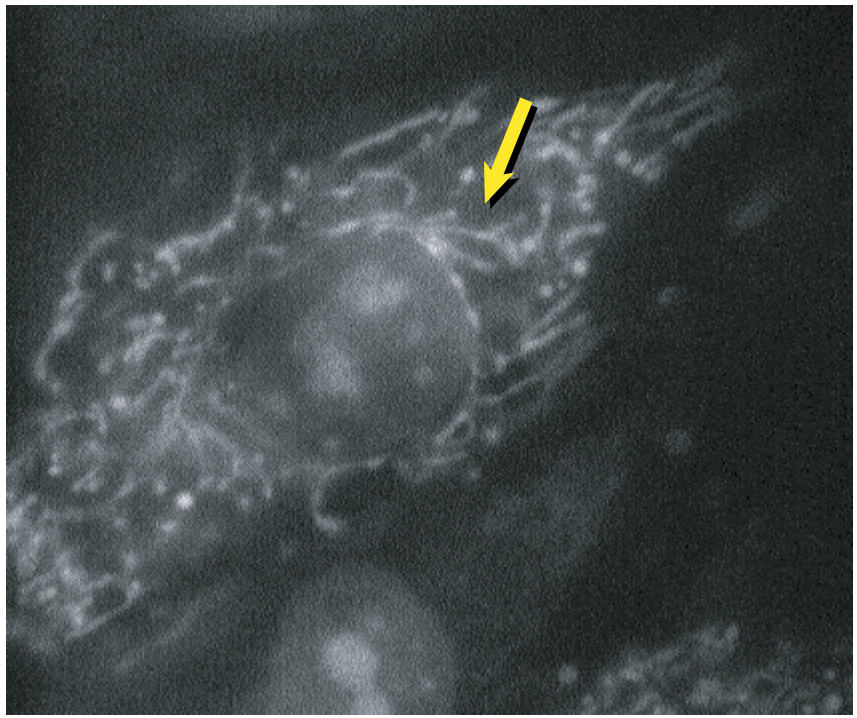




Subcellular surgery

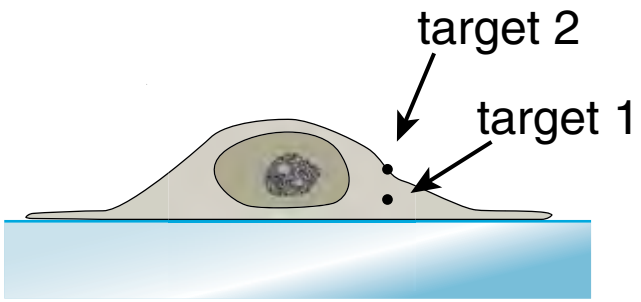
ethydium bromide test

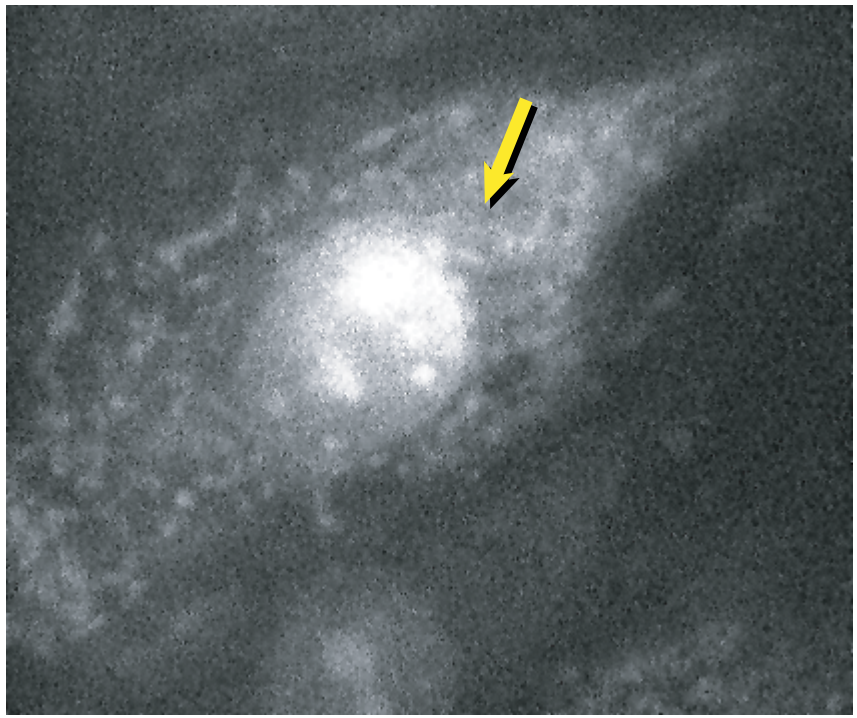




Subcellular surgery

ethydium bromide test



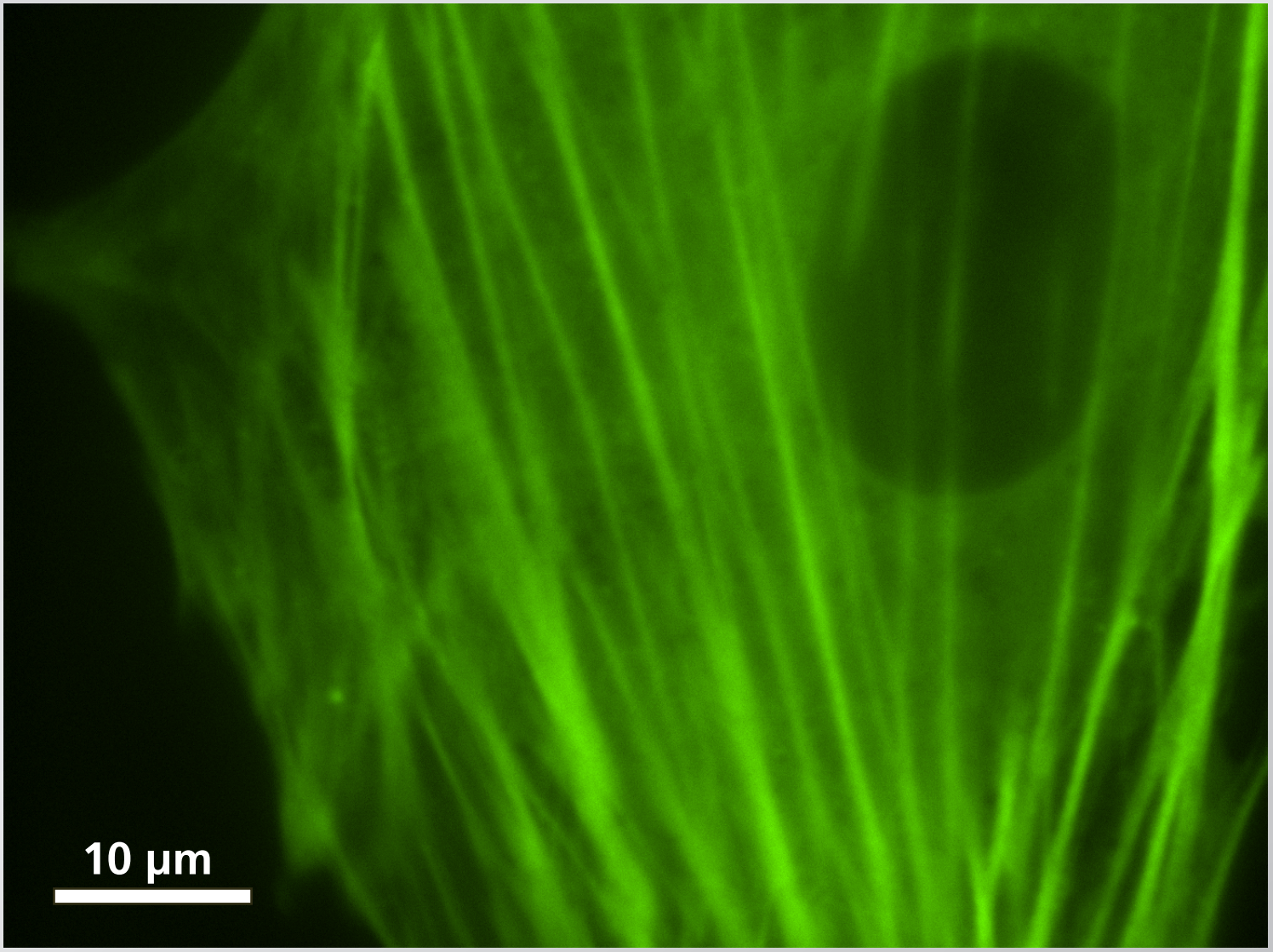


Subcellular surgery

Q: can we probe the dynamics of the cytoskeleton?

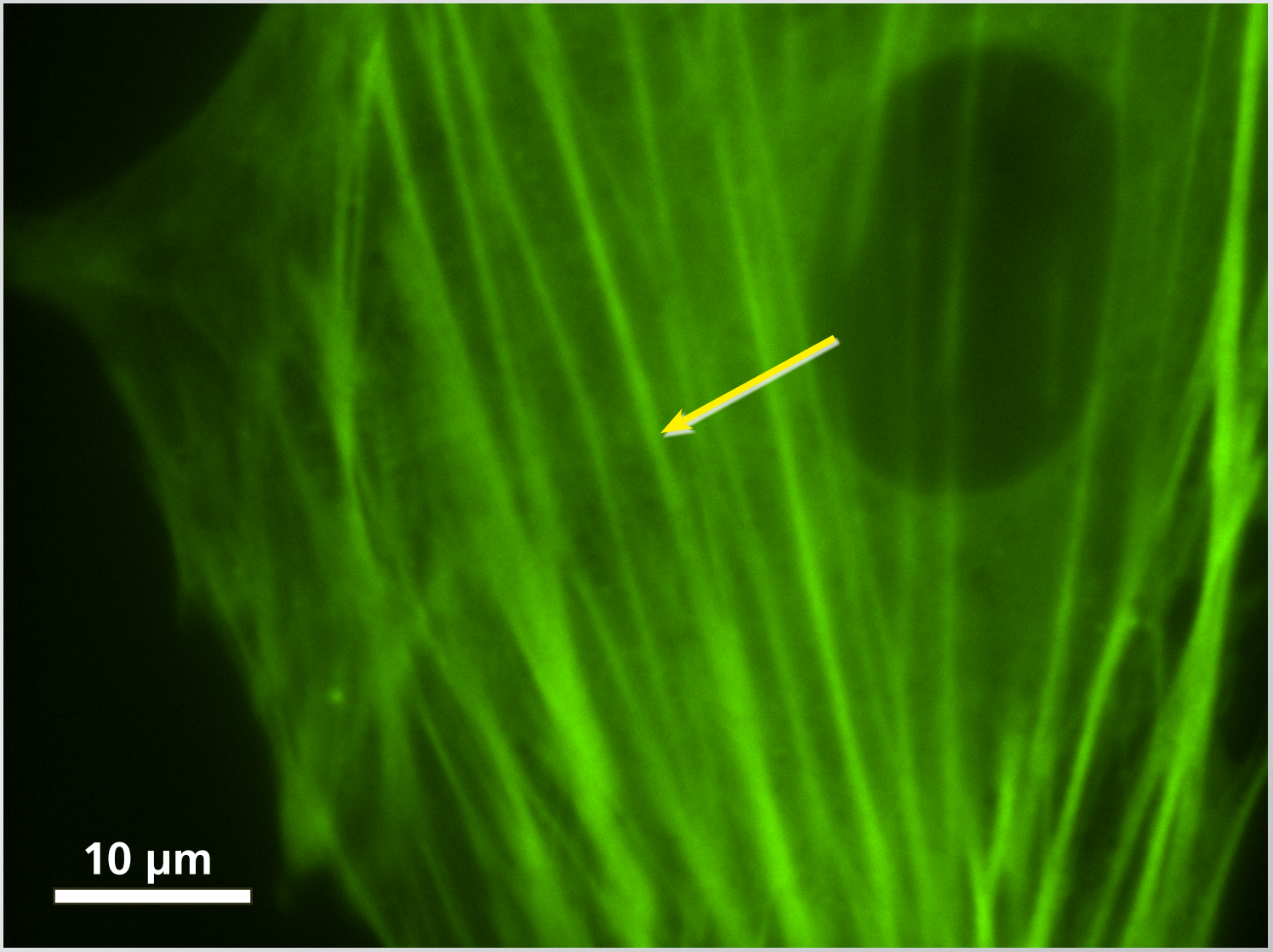
Subcellular surgery

YFP-labeled actin fiber network of a live cell



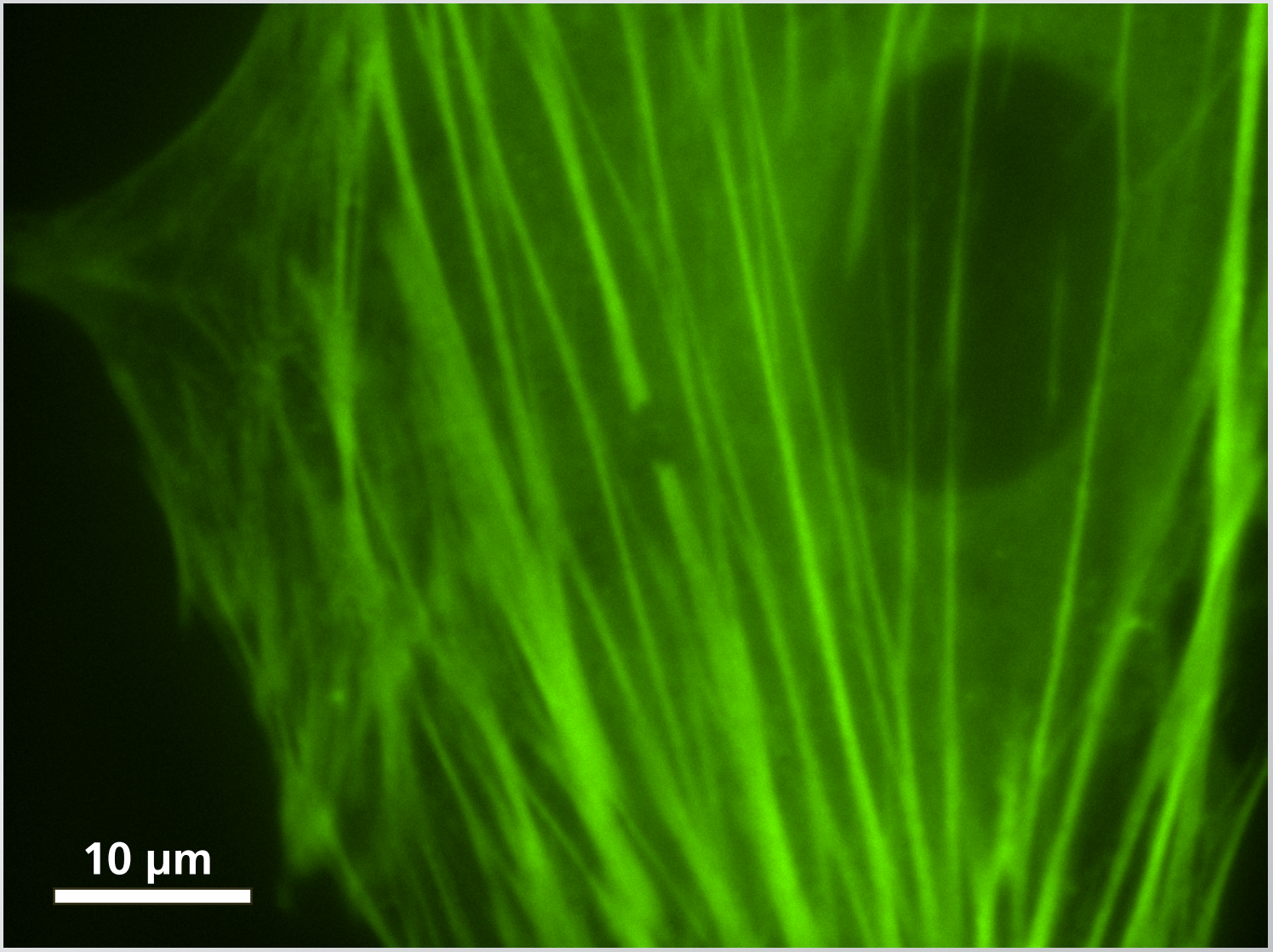
Subcellular surgery

cut a single fiber bundle



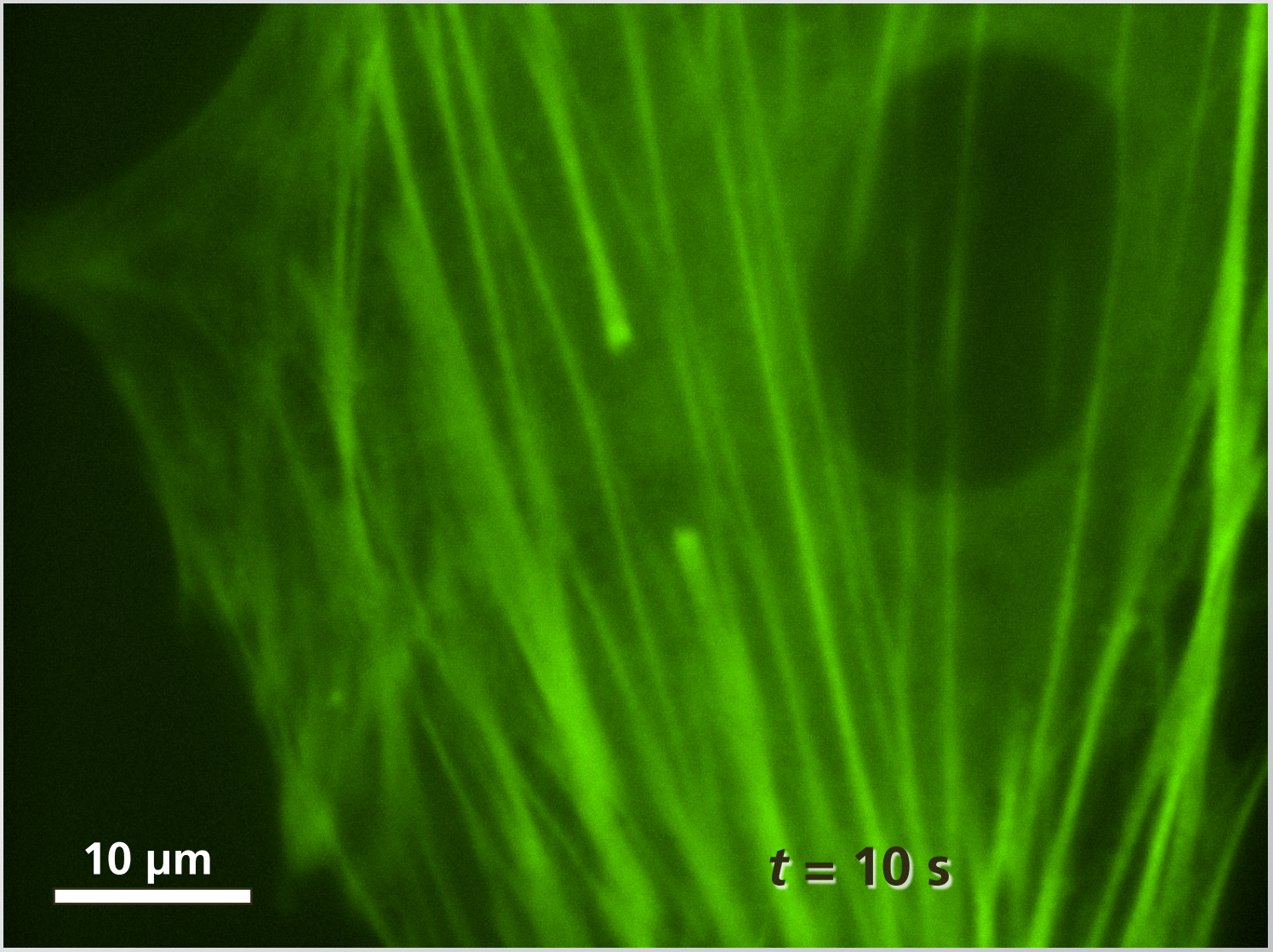
Subcellular surgery

cut a single fiber bundle



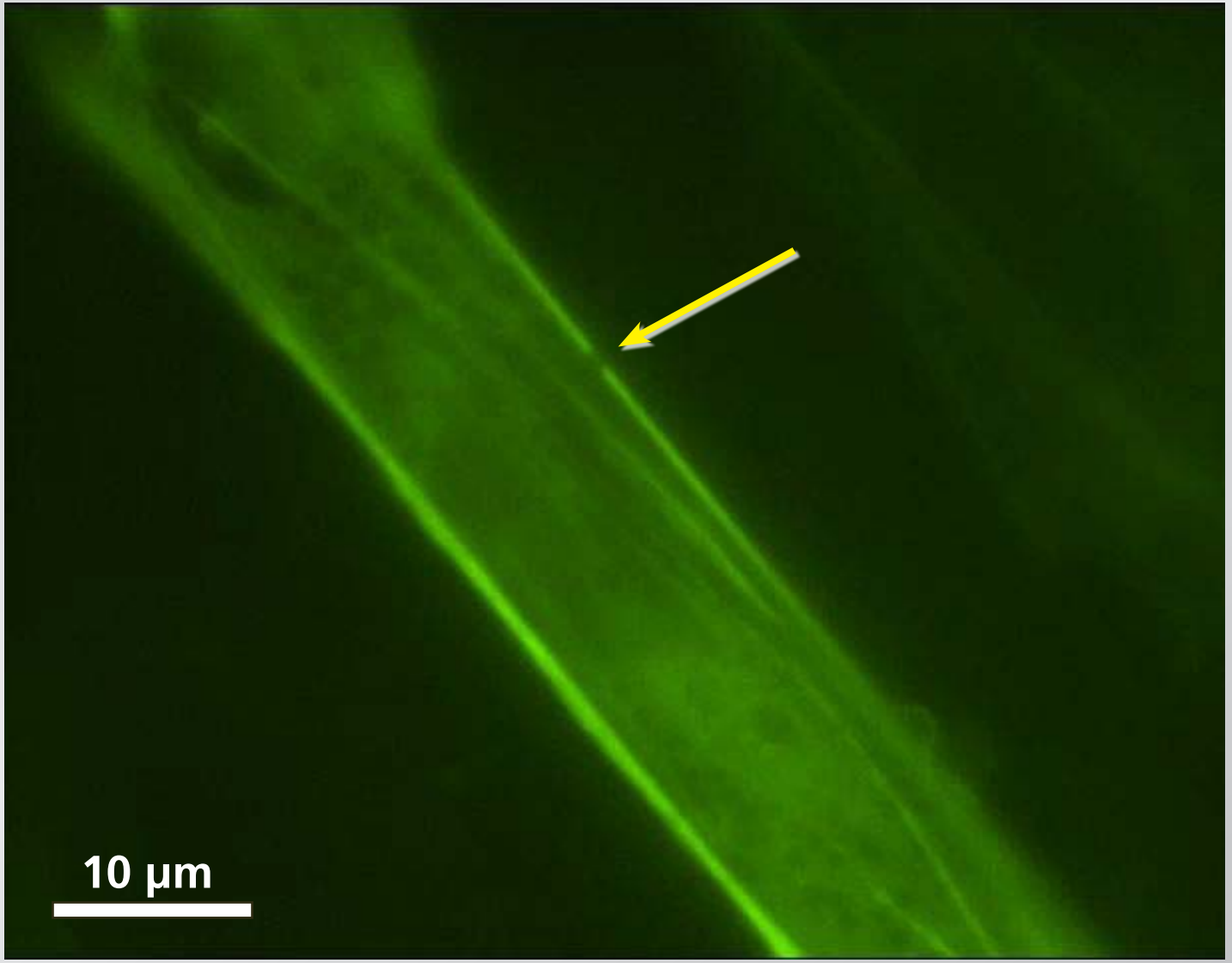
Subcellular surgery

gap widens with time



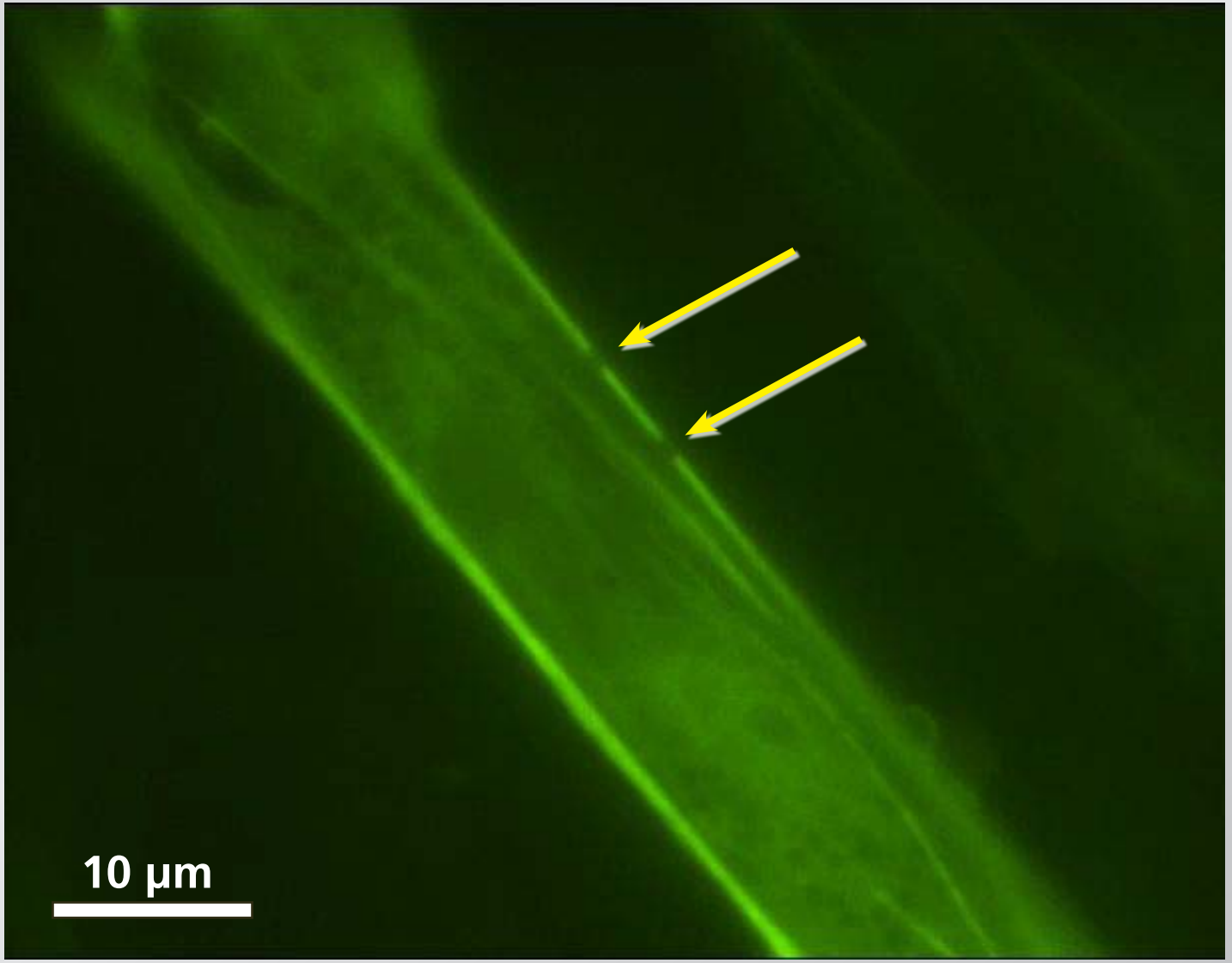
Subcellular surgery

retraction or depolymerization?



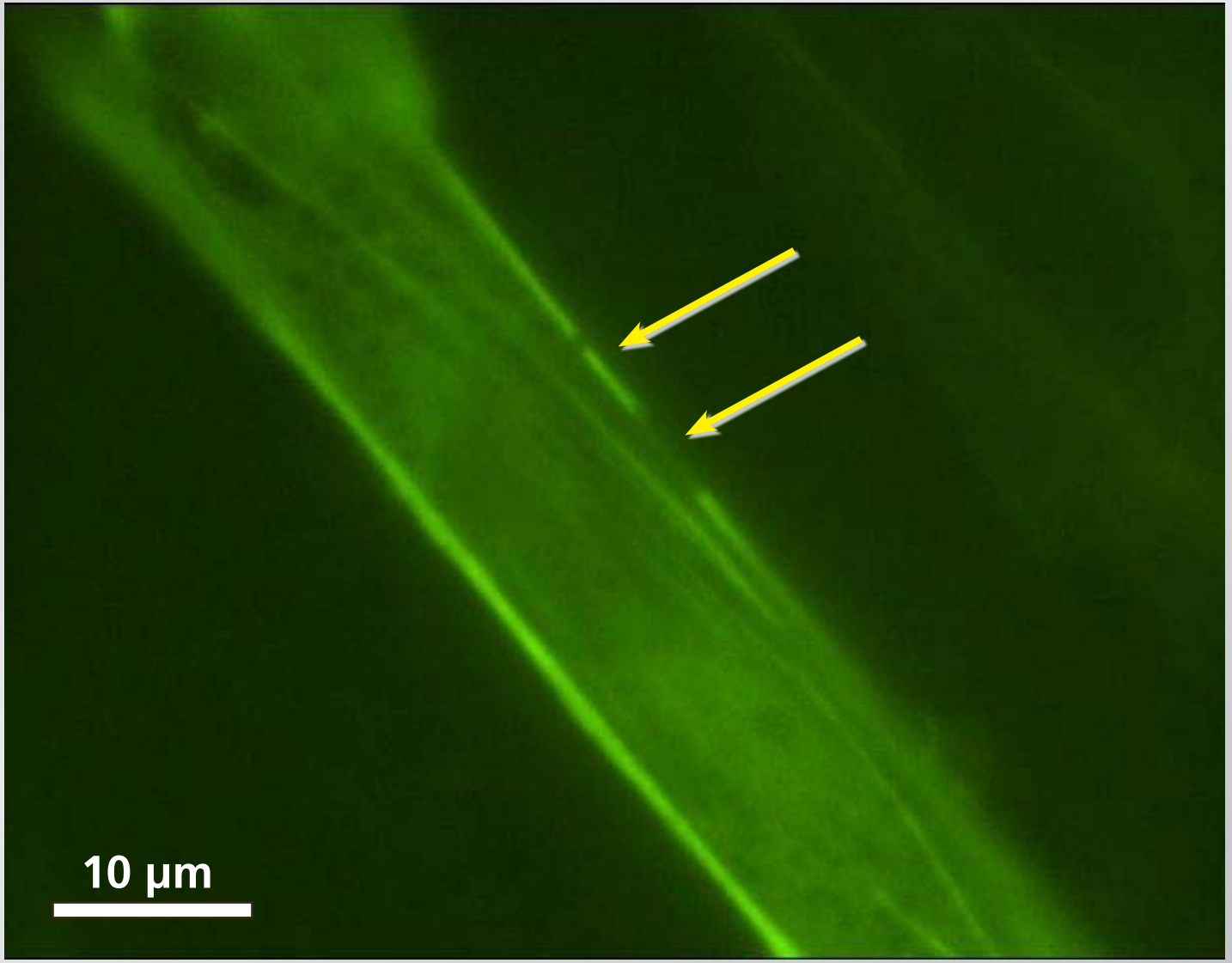
Subcellular surgery

retraction or depolymerization?



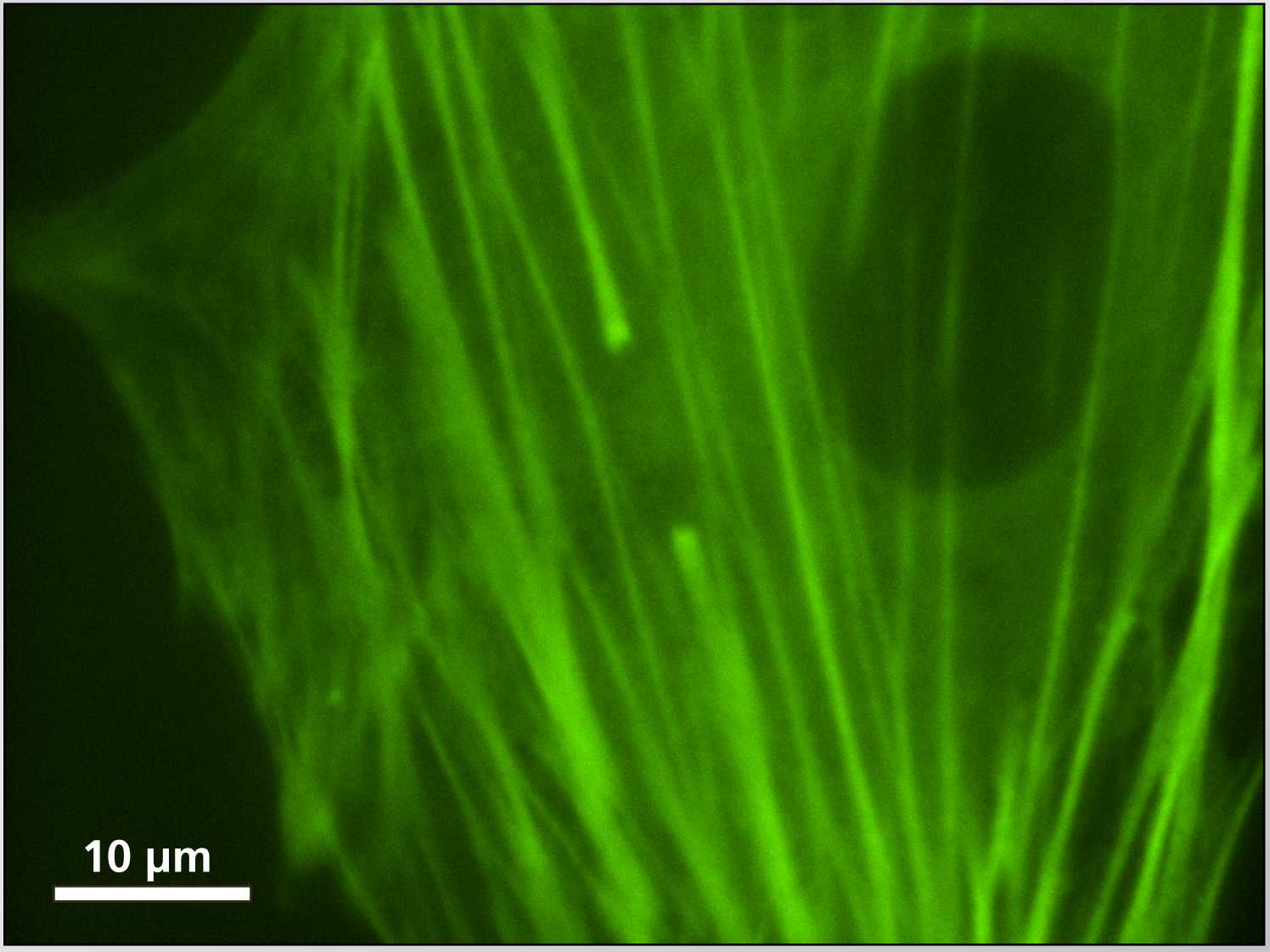
Subcellular surgery

retraction!

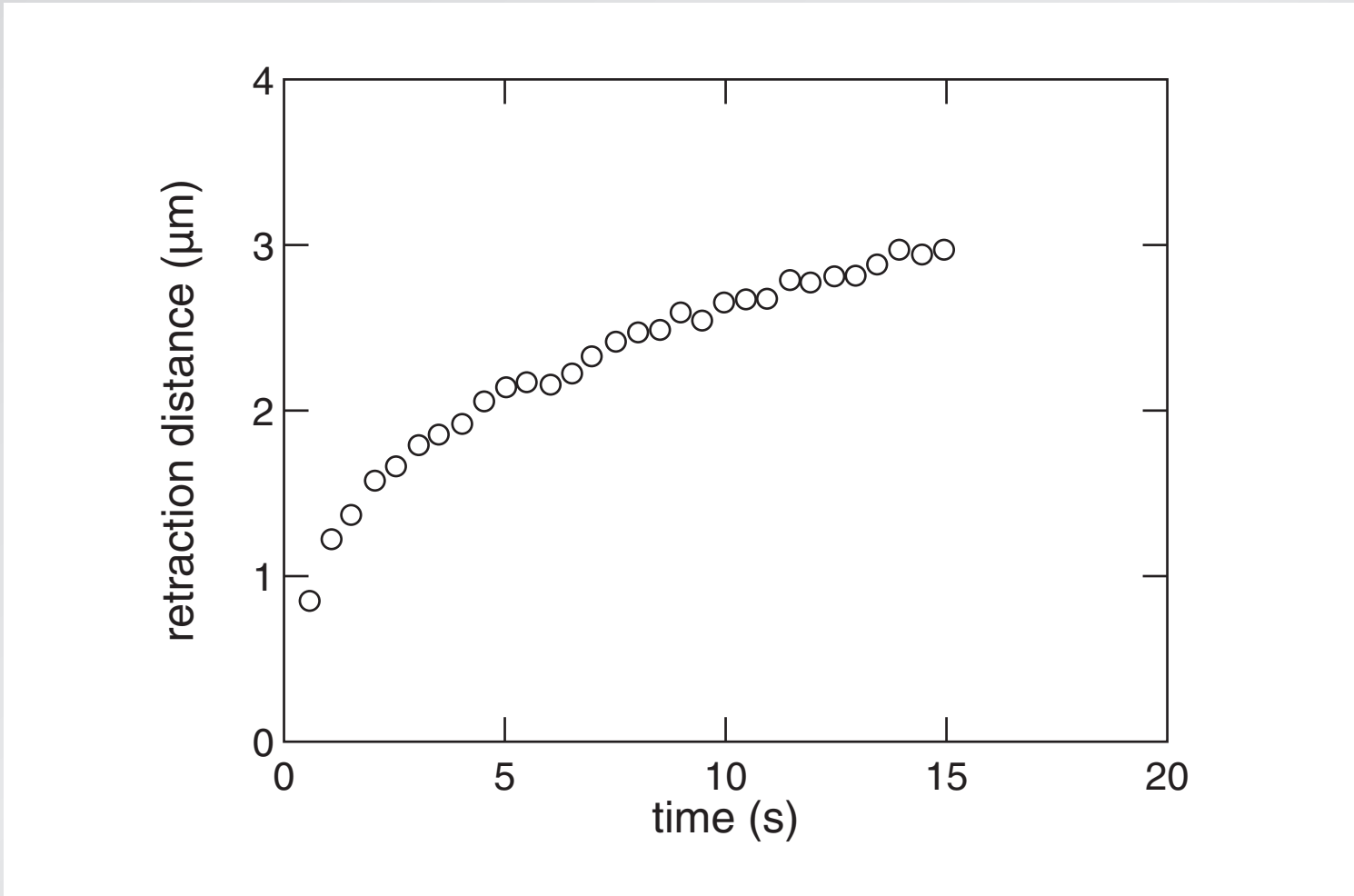


Subcellular surgery

dynamics provides information on *in vivo* mechanics

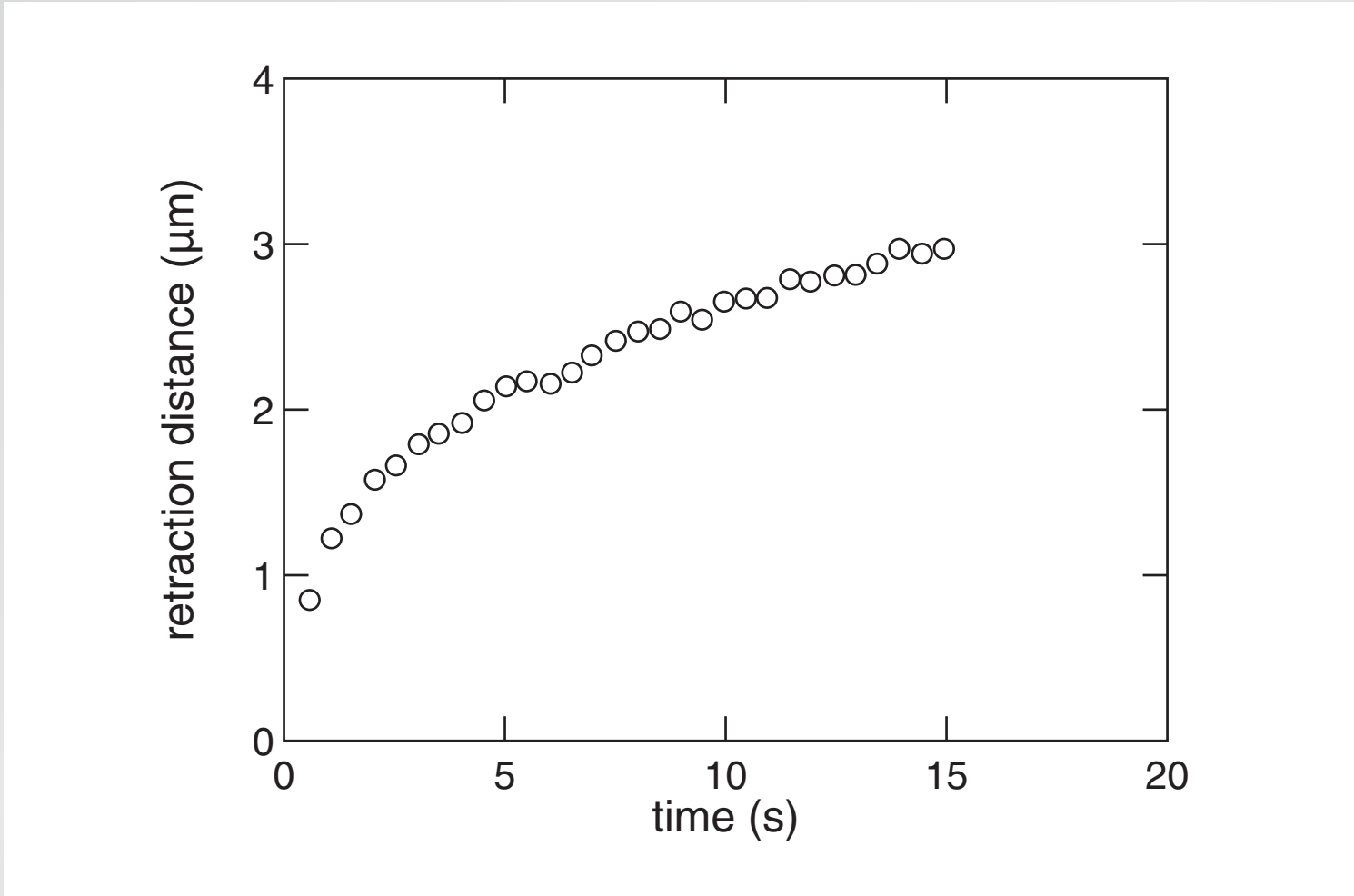


Subcellular surgery



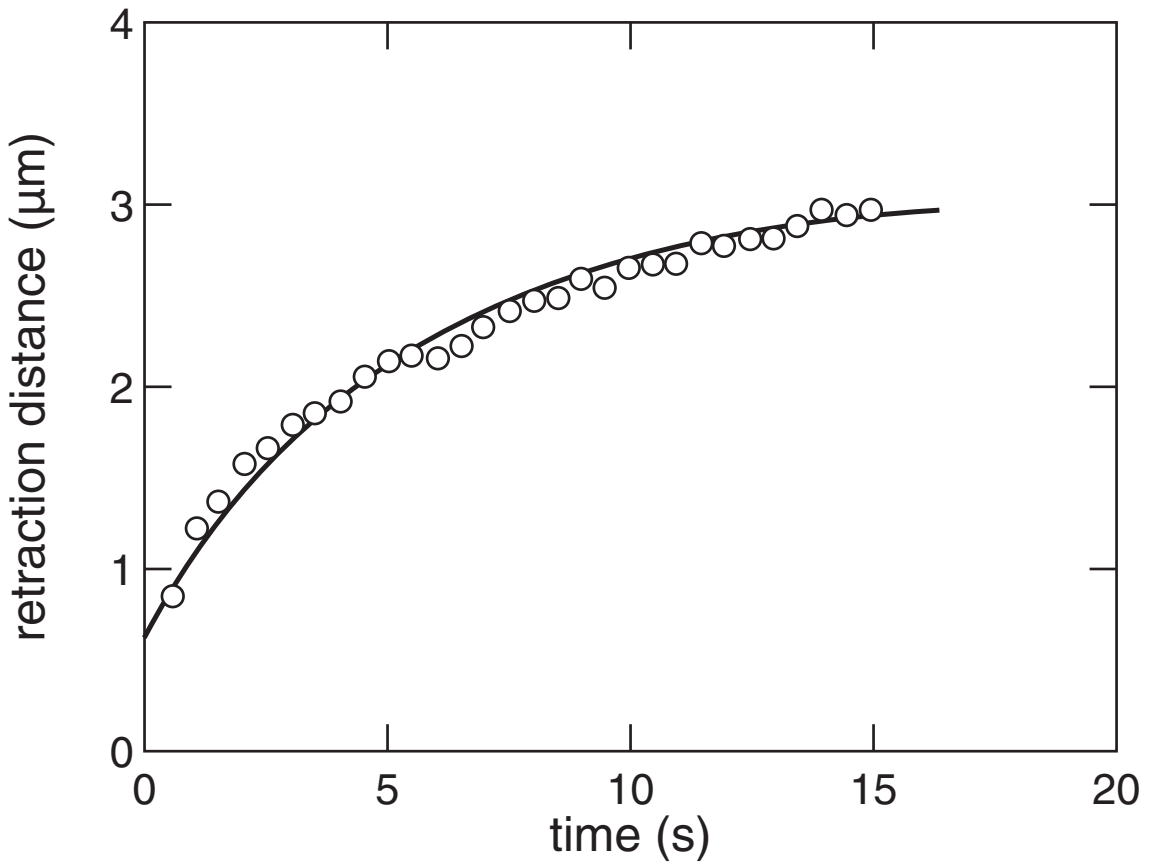
Subcellular surgery

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



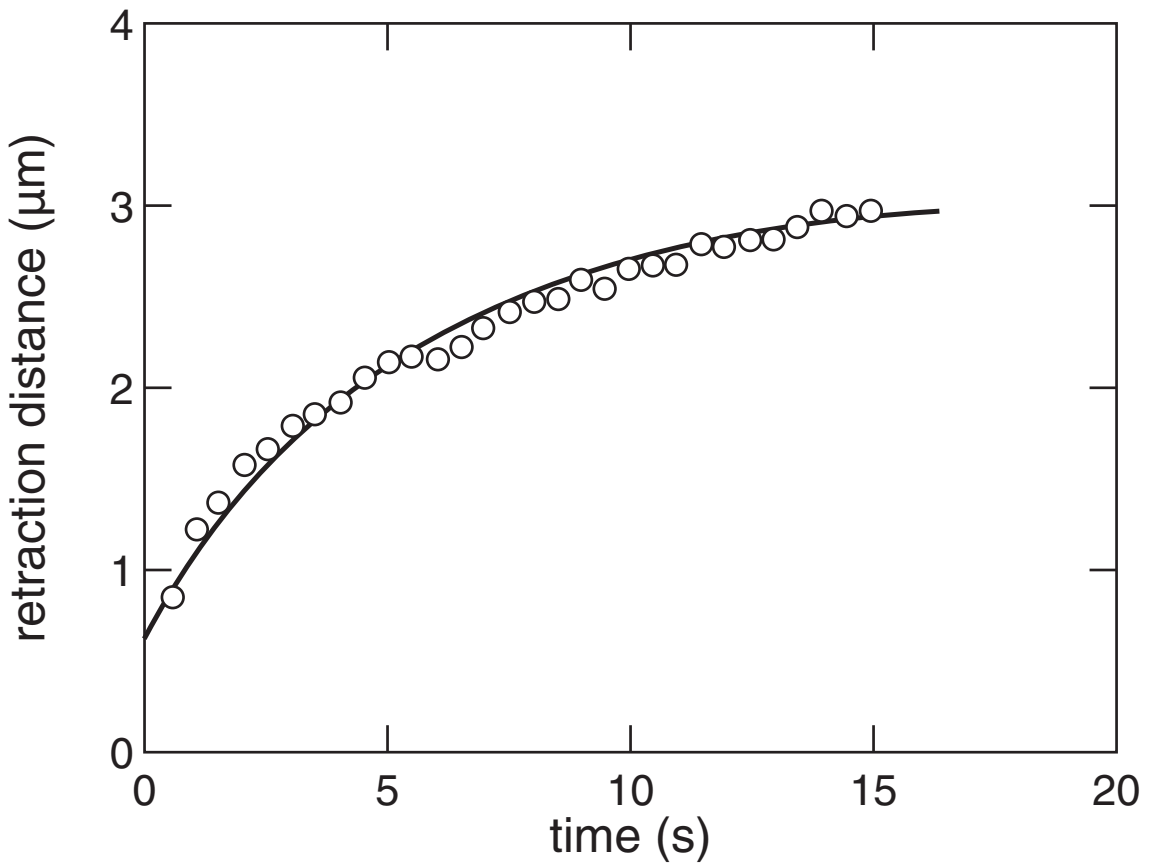
Subcellular surgery

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



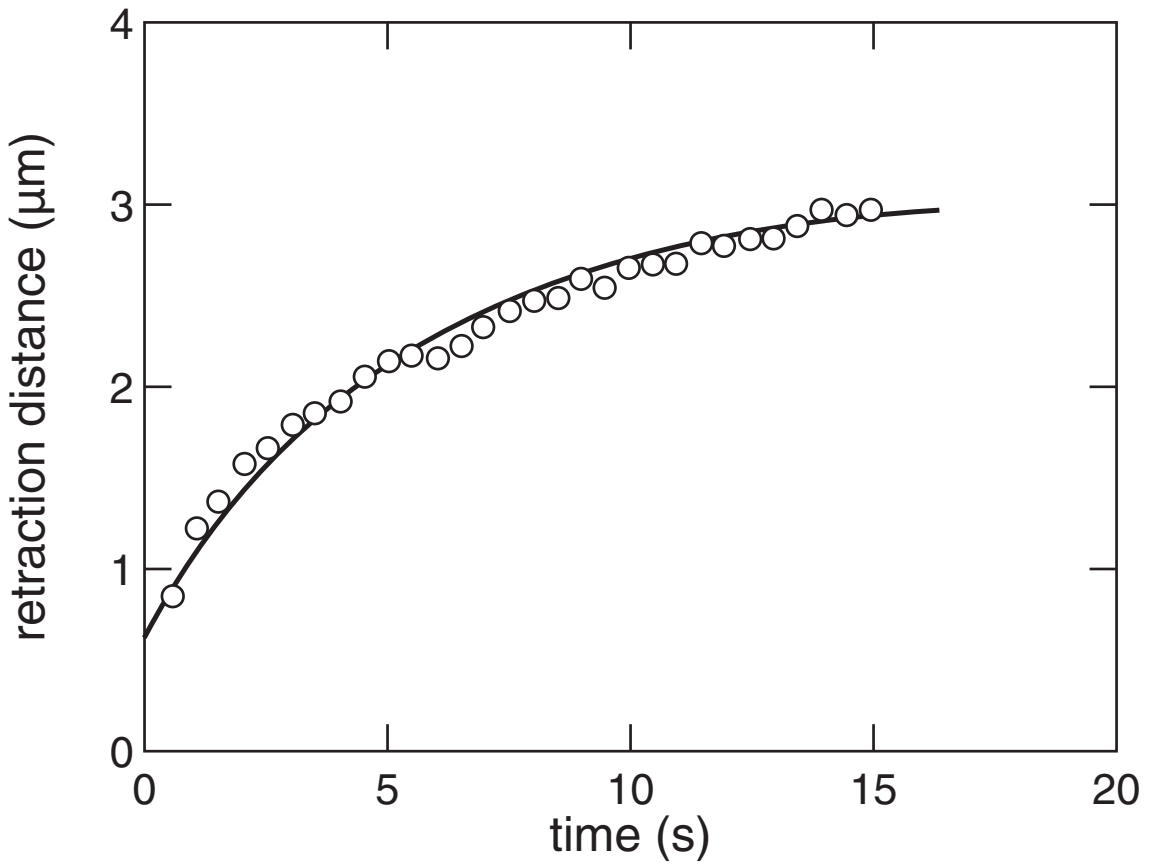
Subcellular surgery

L_o and τ independent of fiber width!



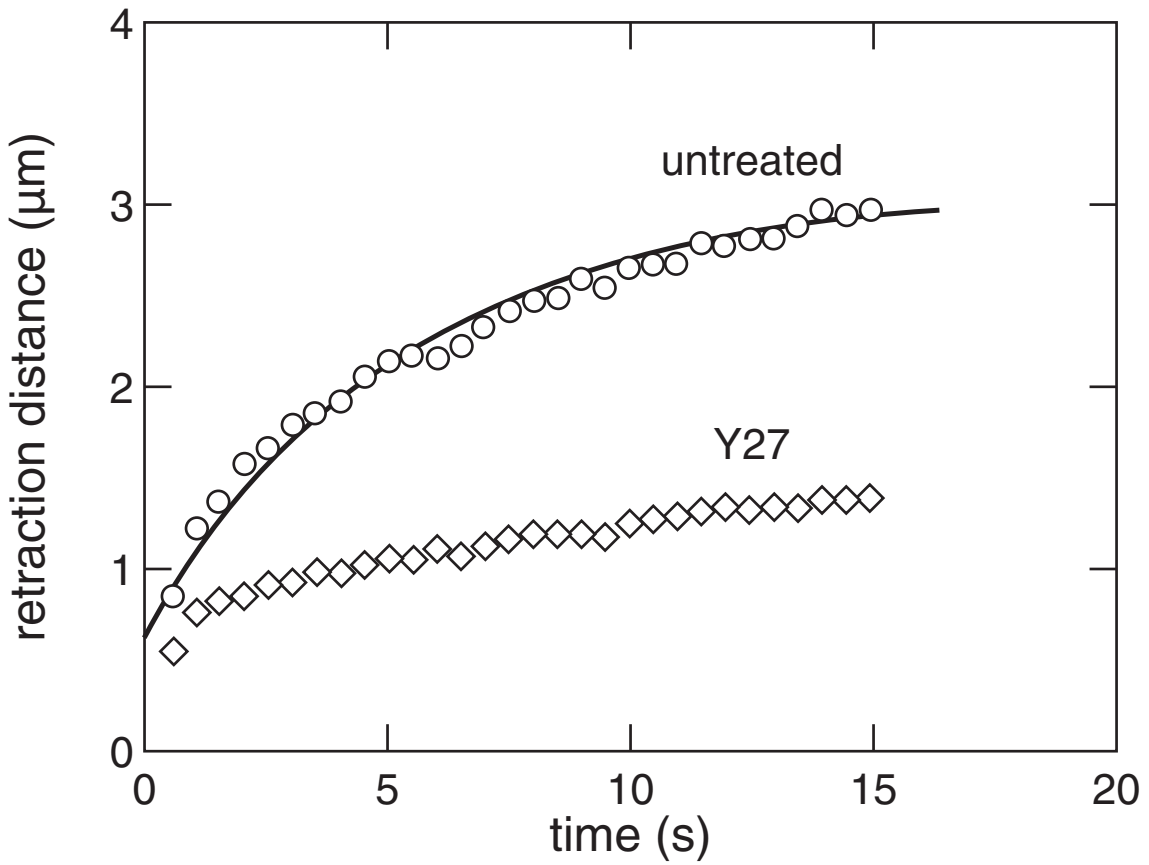
Subcellular surgery

tension in actin filaments is generated by myosin motors



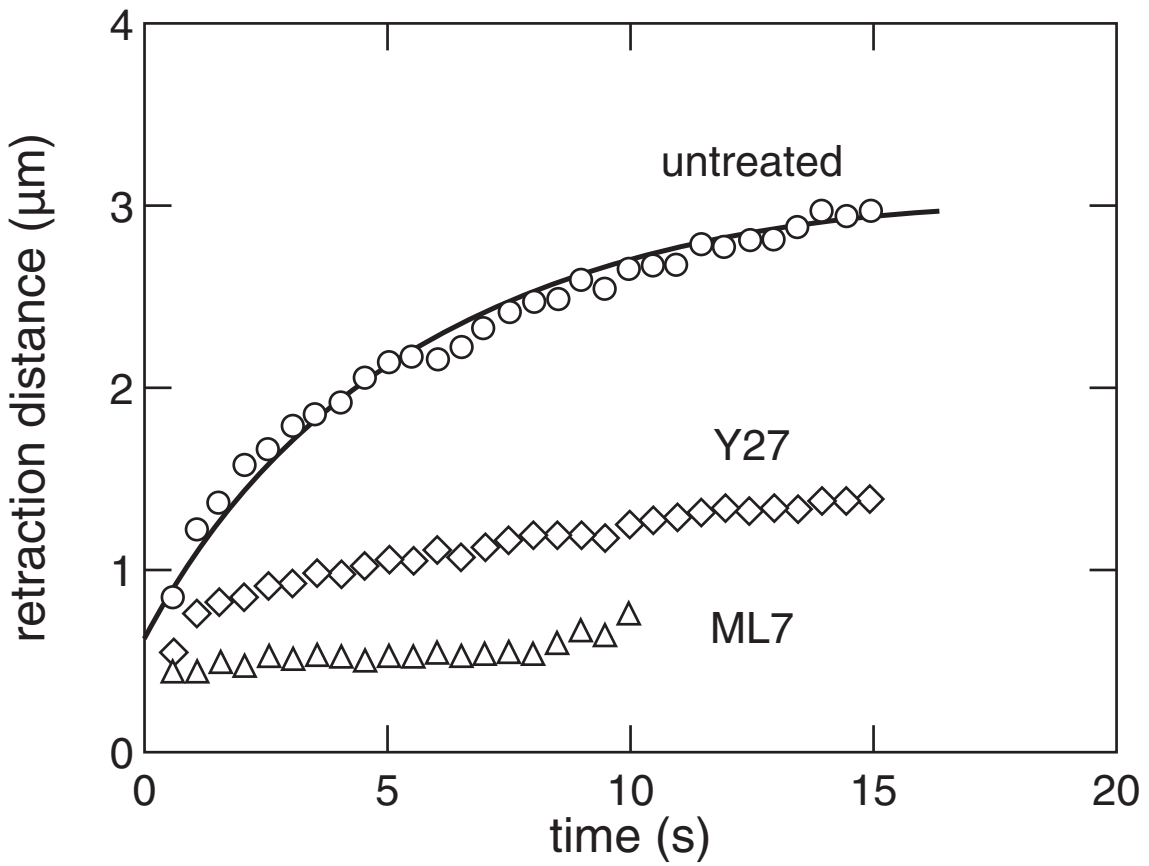
Subcellular surgery

Y27: inhibits some myosin activity



Subcellular surgery

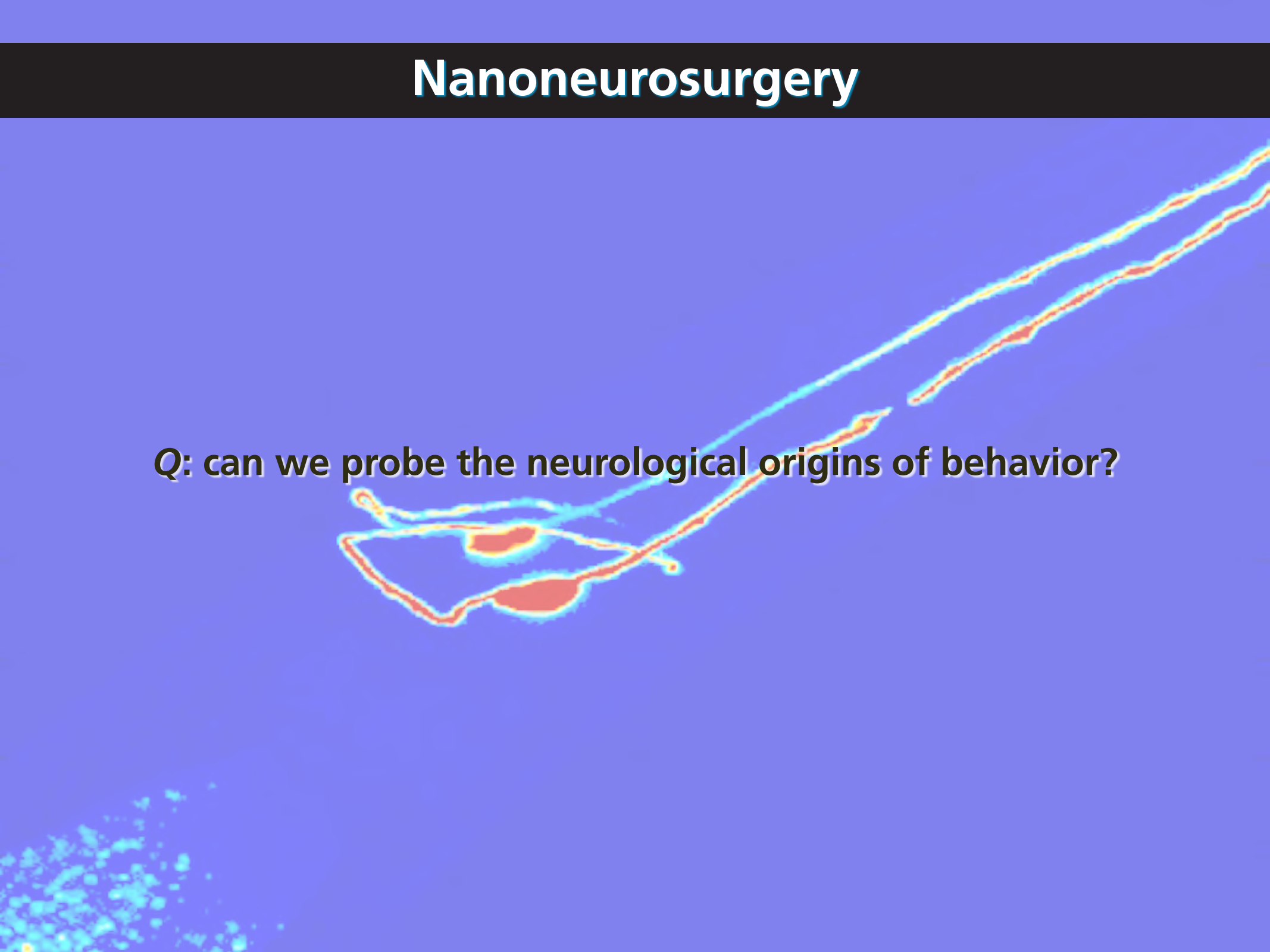
ML7: direct inhibitor of myosin activity



Outline

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

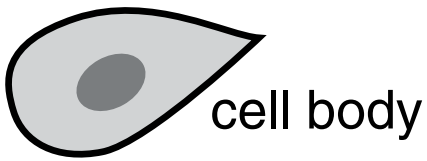
Nanoneurosurgery



Q: can we probe the neurological origins of behavior?

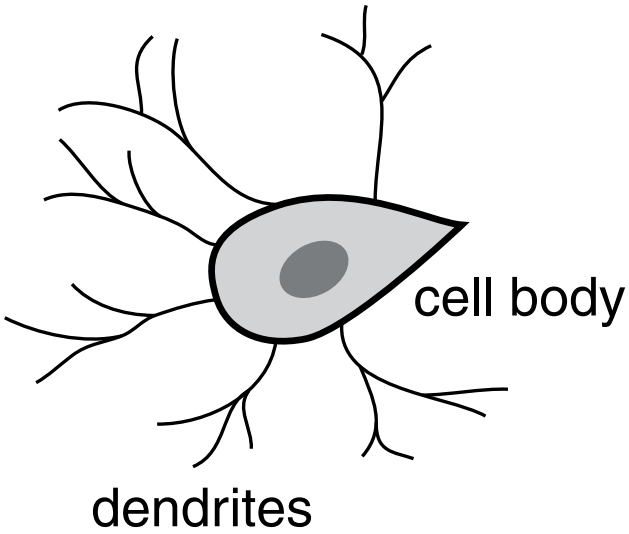
Nanoneurosurgery

neuron basics



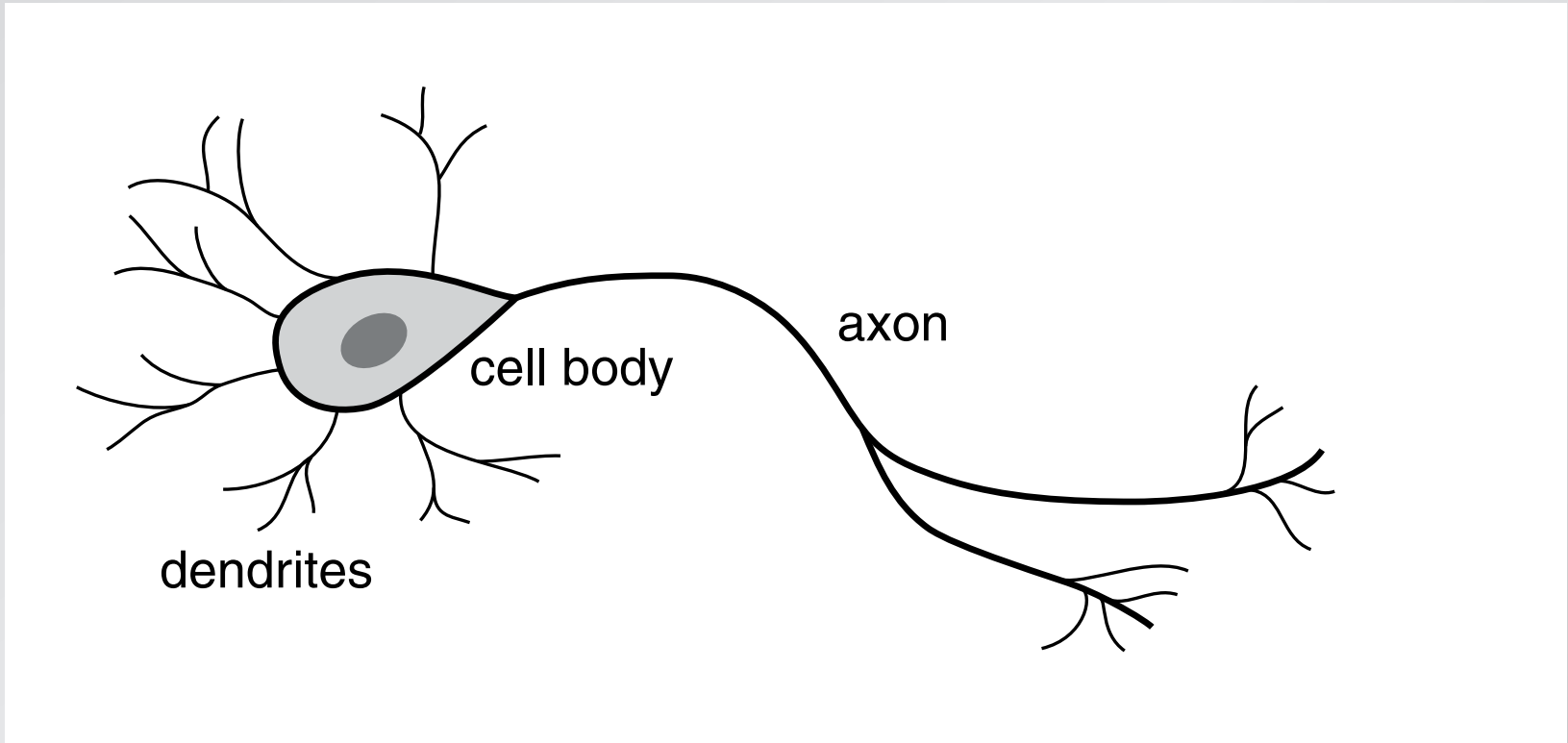
Nanoneurosurgery

neuron basics



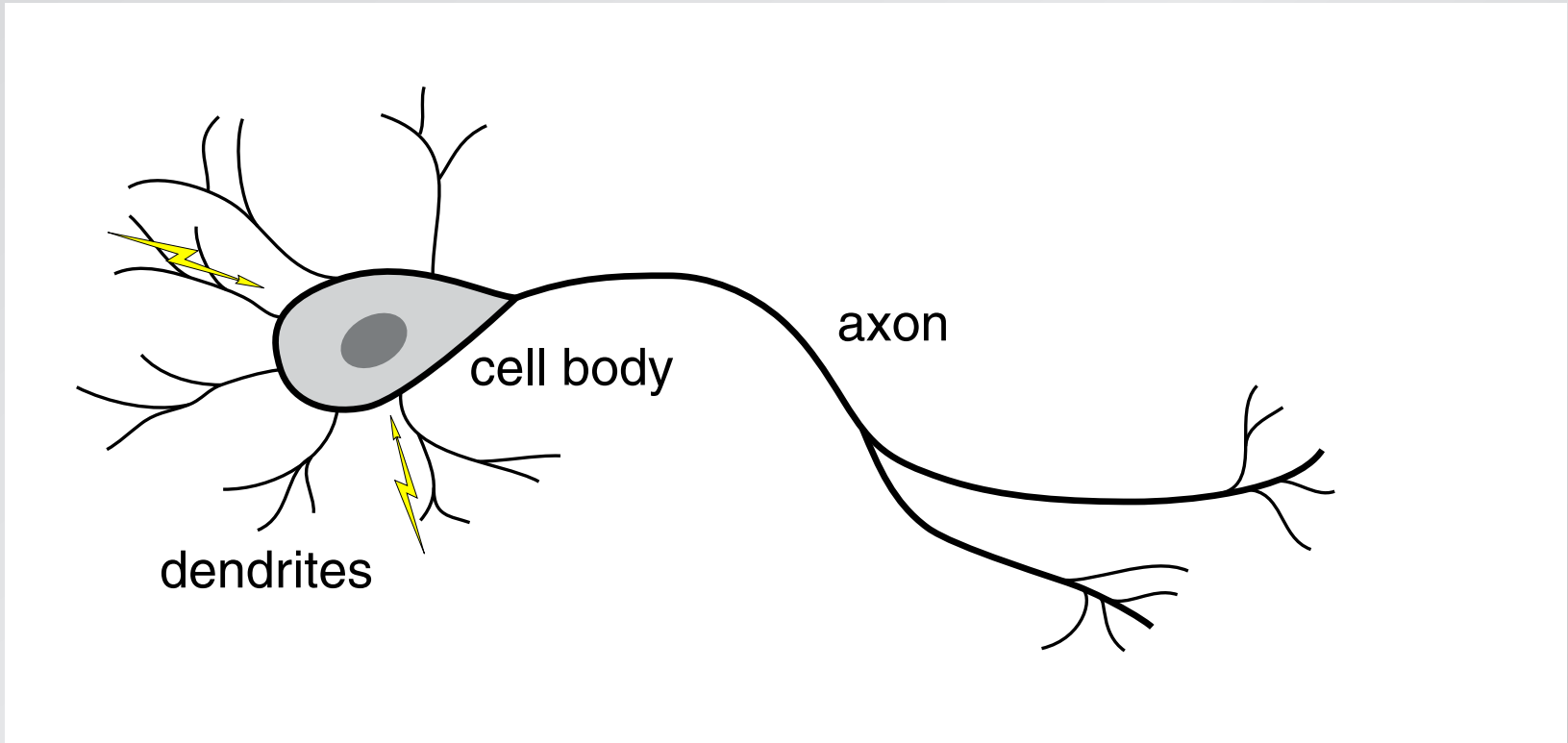
Nanoneurosurgery

neuron basics



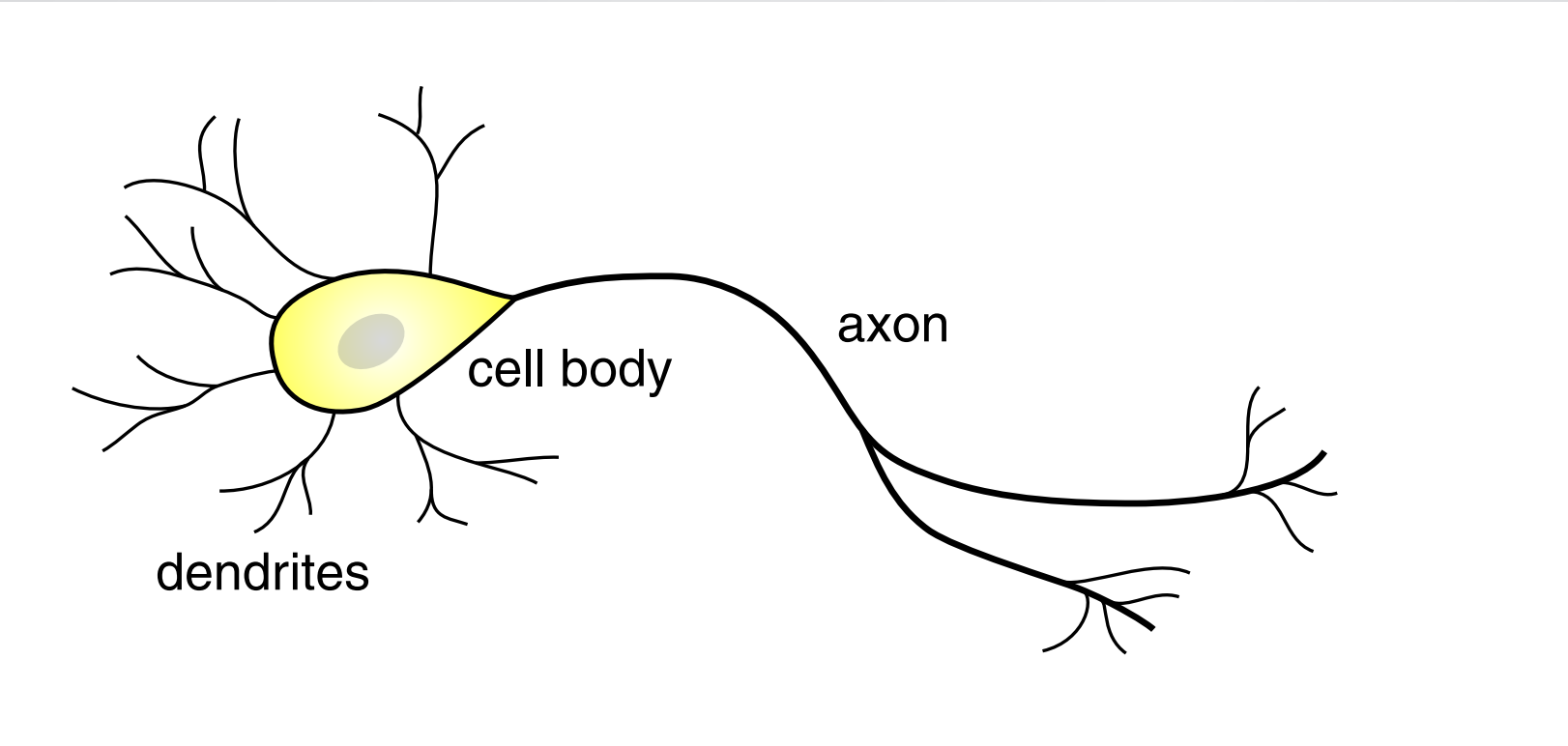
Nanoneurosurgery

neuron basics



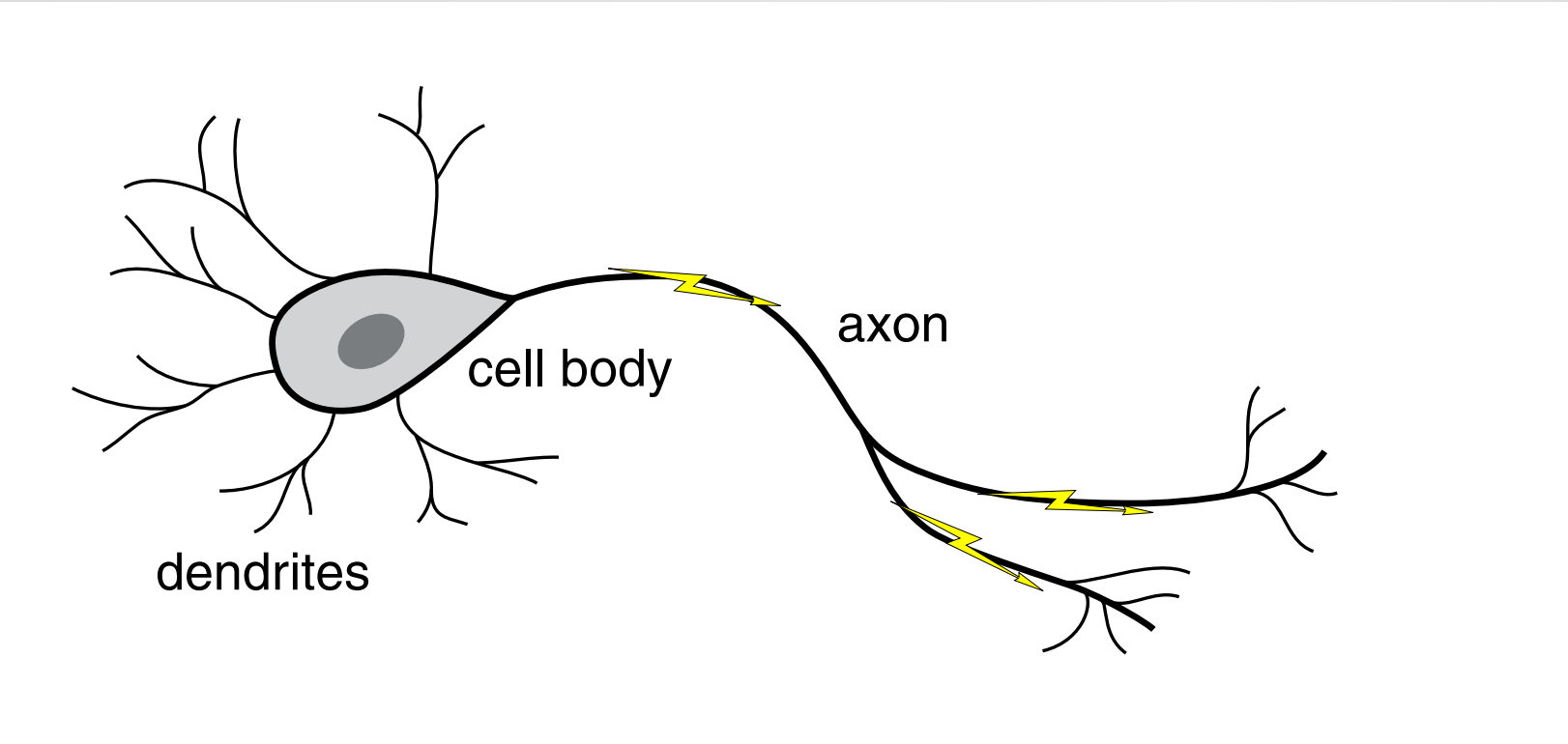
Nanoneurosurgery

neuron basics



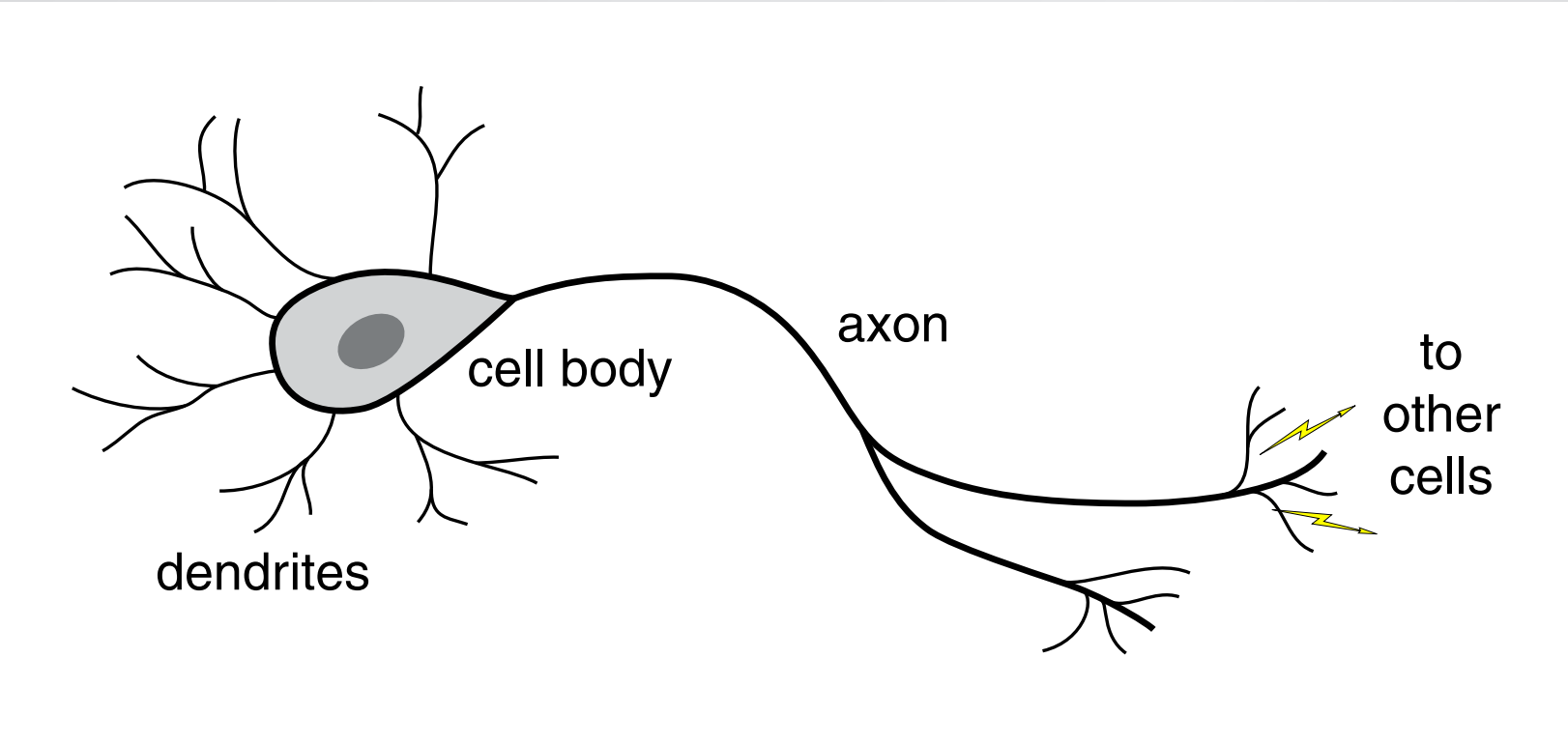
Nanoneurosurgery

neuron basics



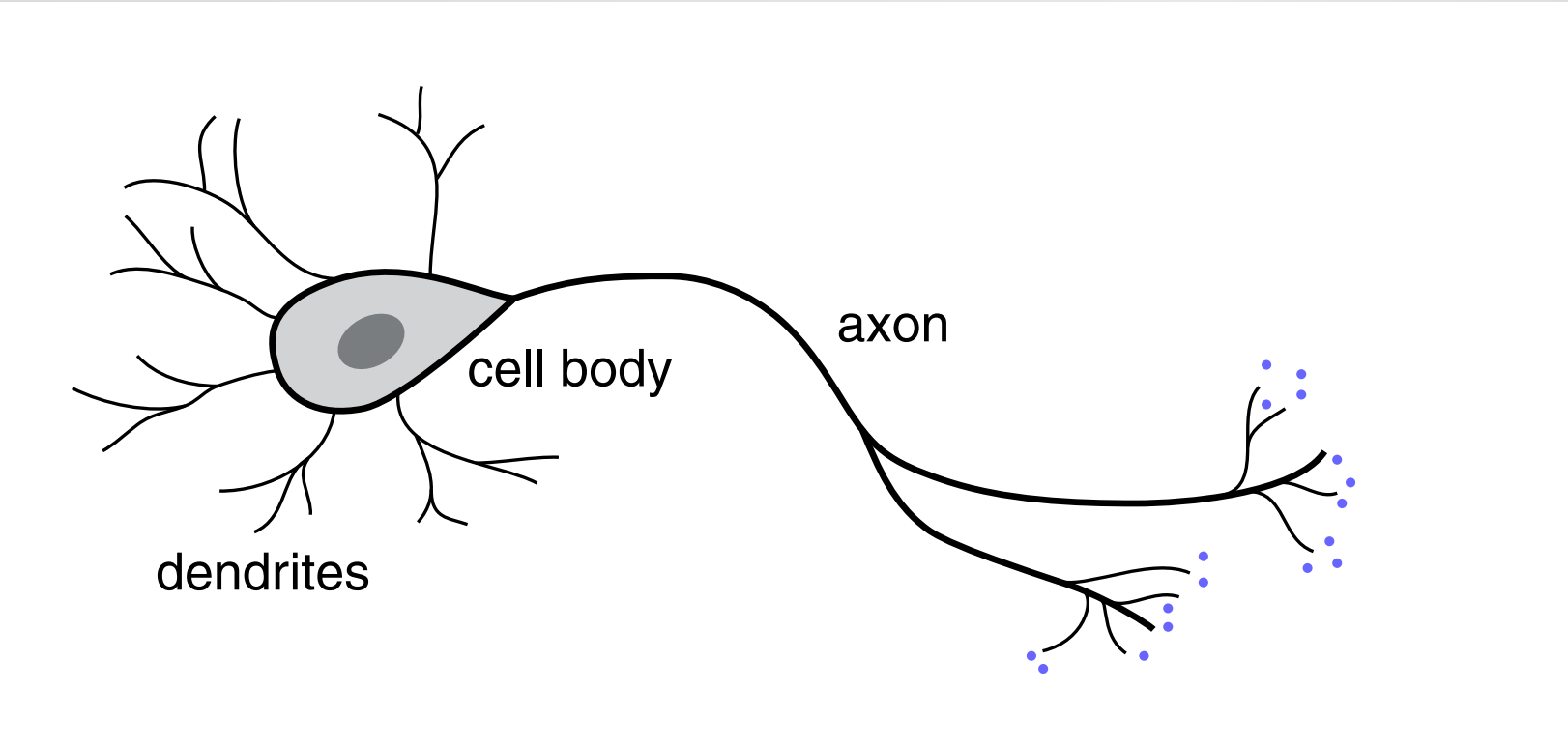
Nanoneurosurgery

neuron basics



Nanoneurosurgery

neuron basics



Nanoneurosurgery

Caenorhabditis elegans

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

Nanoneurosurgery

Caenorhabditis elegans

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

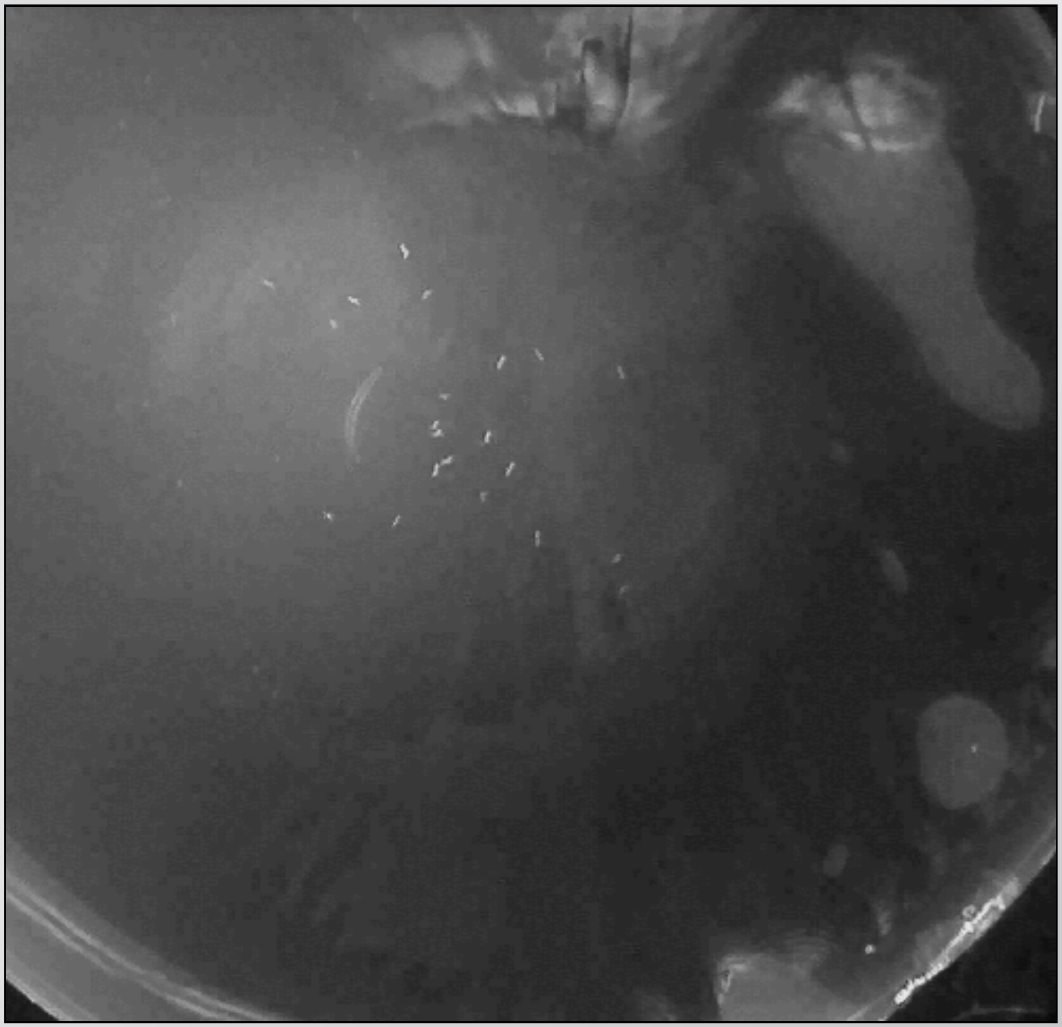
Nanoneurosurgery

Caenorhabditis elegans

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

Nanoneurosurgery

Caenorhabditis elegans

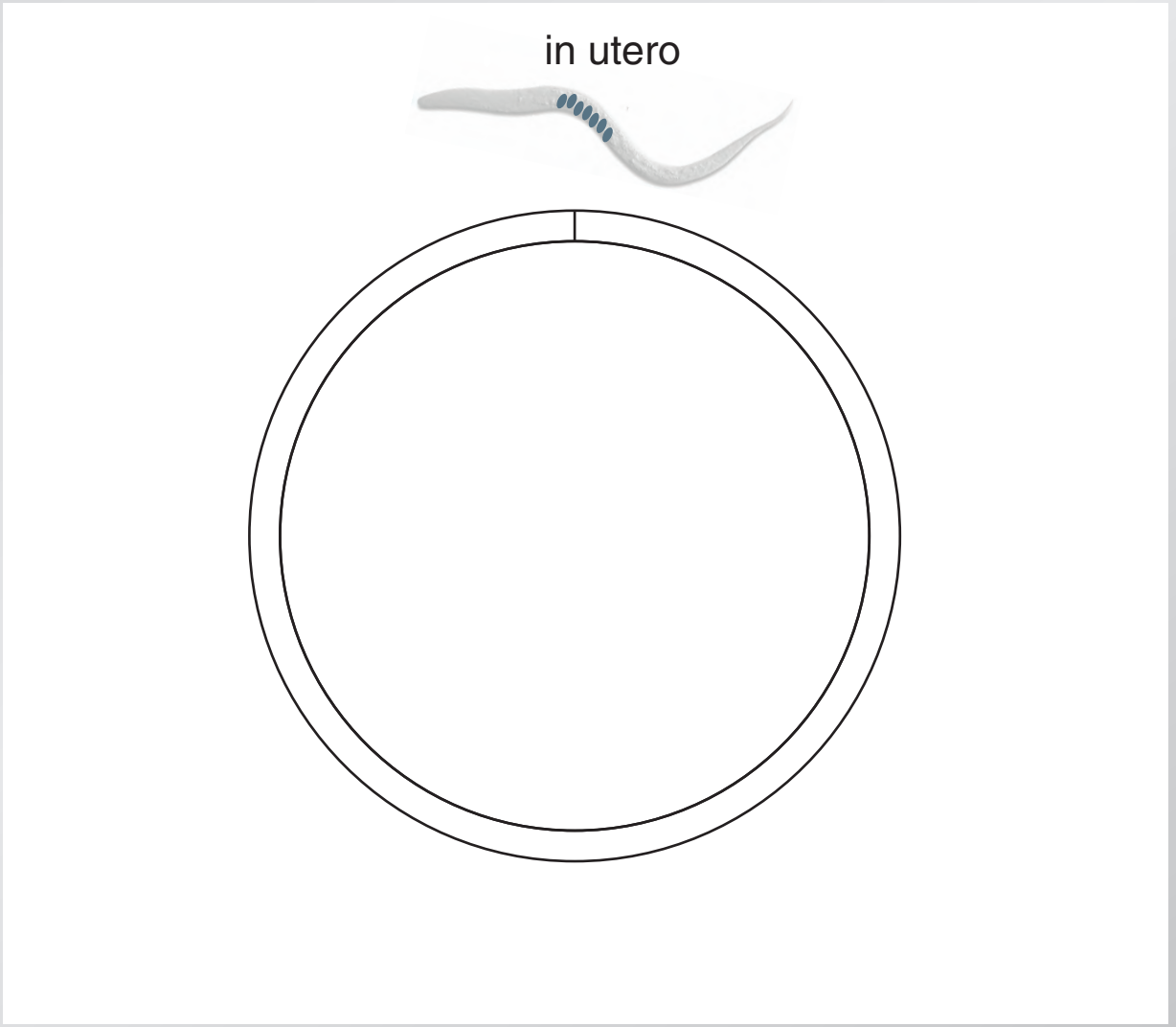


Nanoneurosurgery

Caenorhabditis elegans

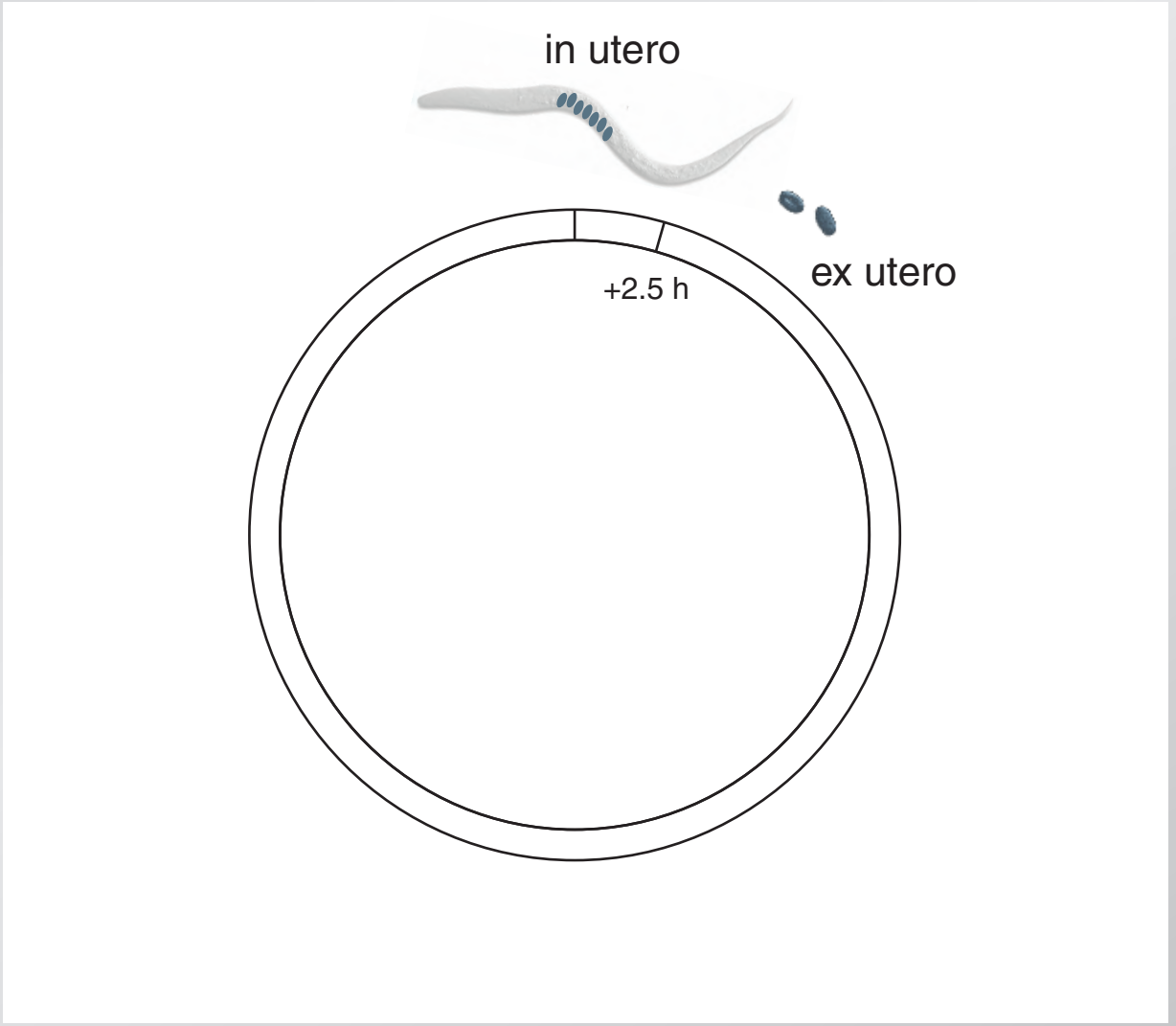
Nanoneurosurgery

C. elegans life cycle



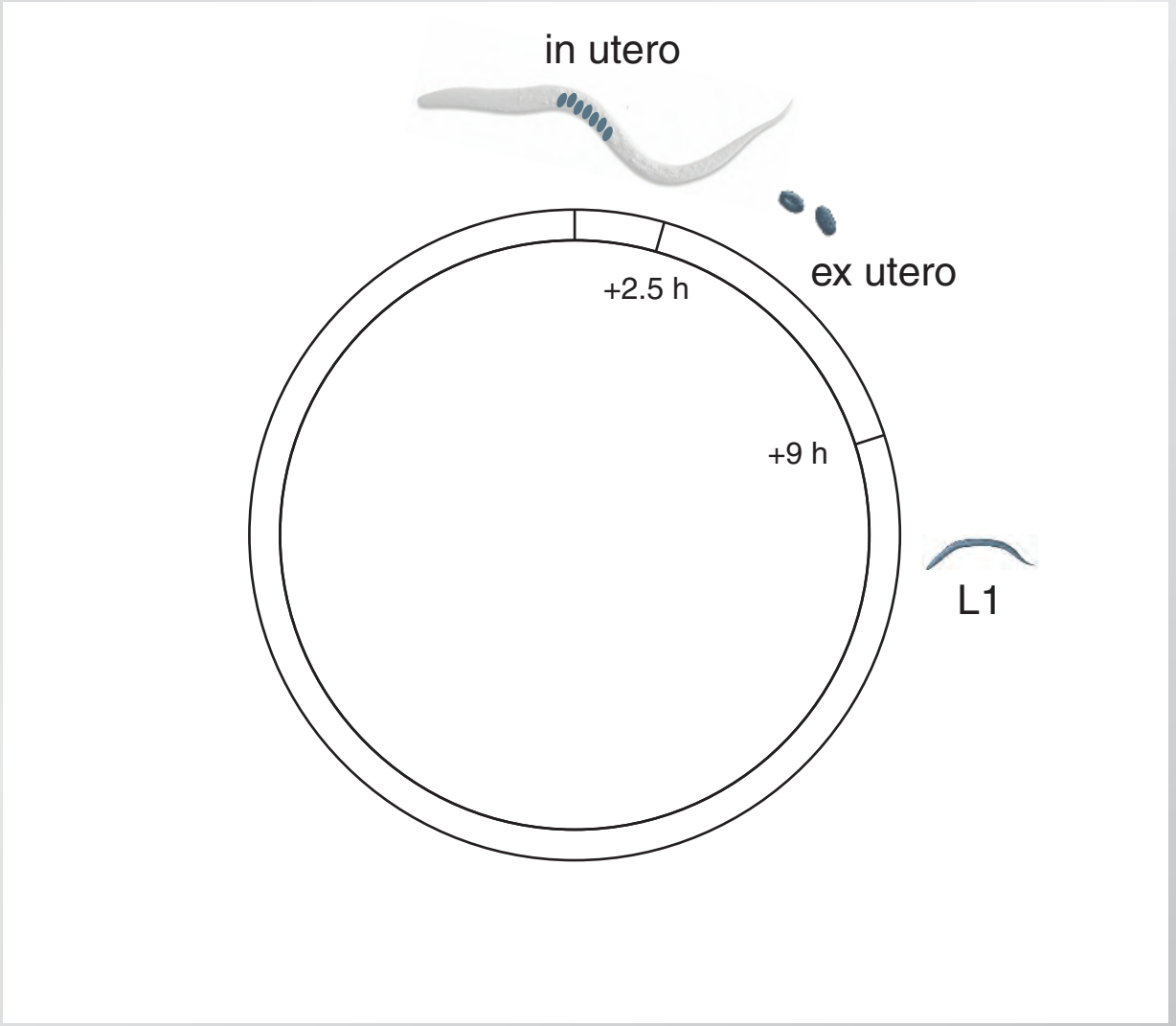
Nanoneurosurgery

C. elegans life cycle



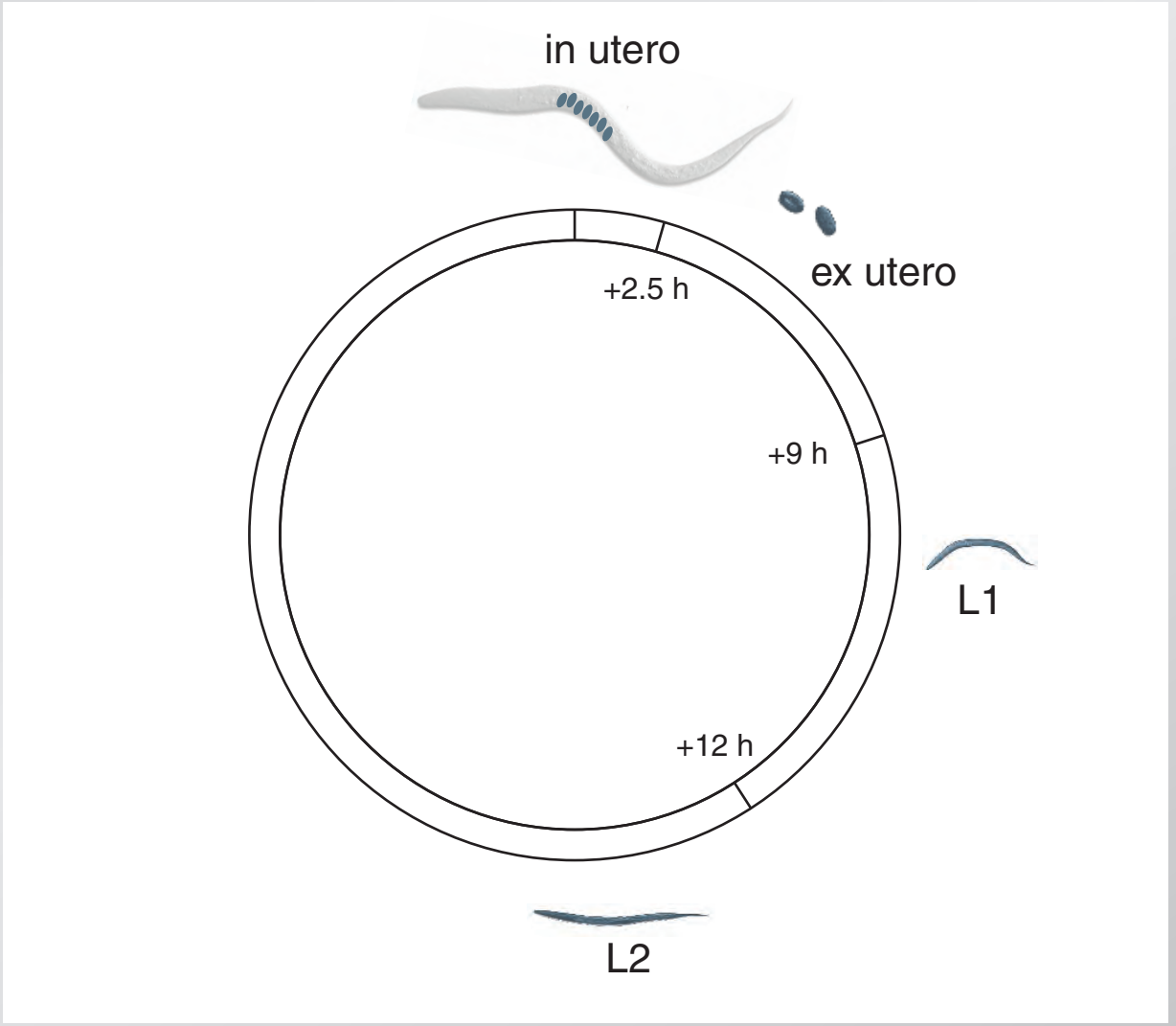
Nanoneurosurgery

C. elegans life cycle



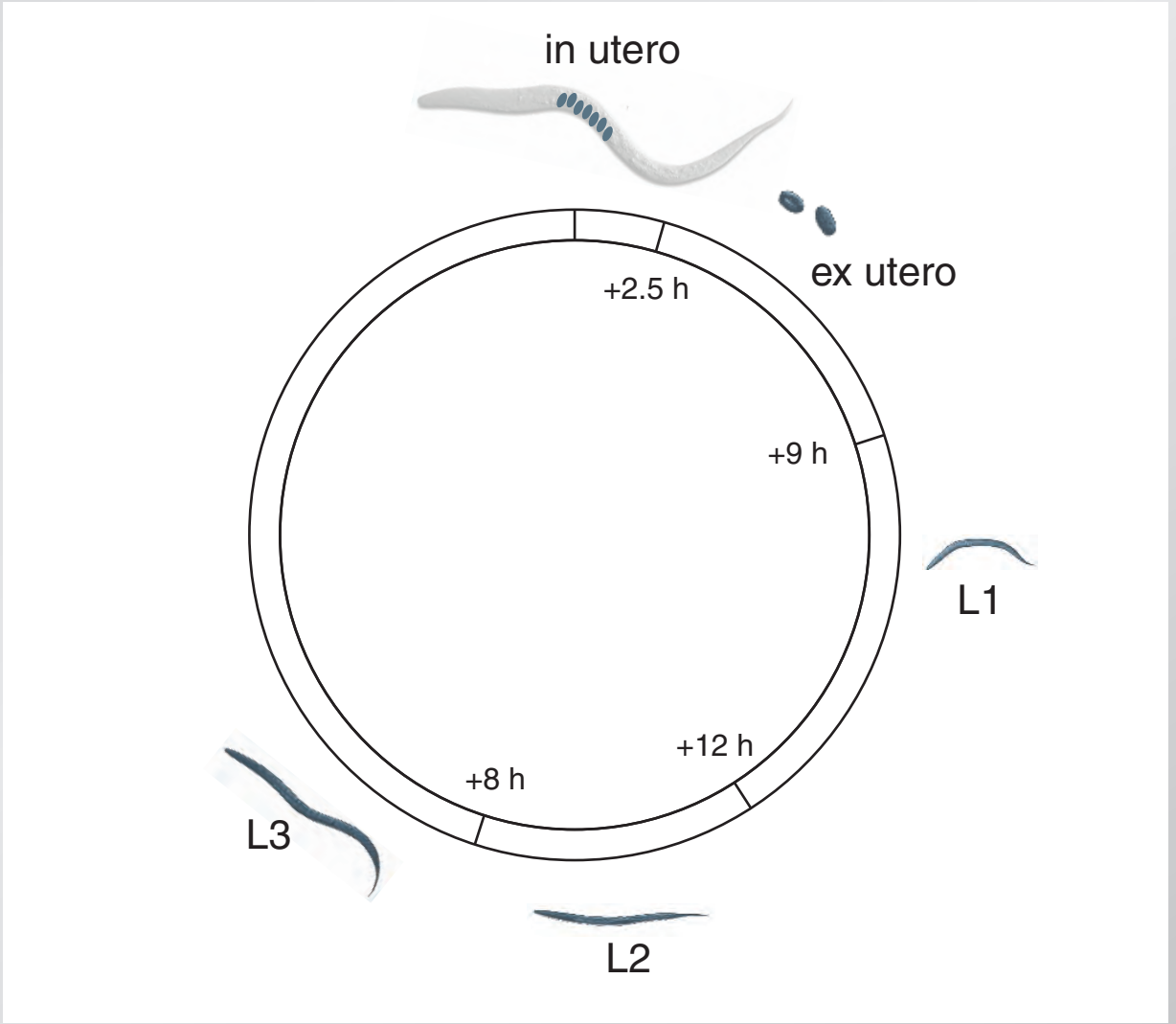
Nanoneurosurgery

C. elegans life cycle



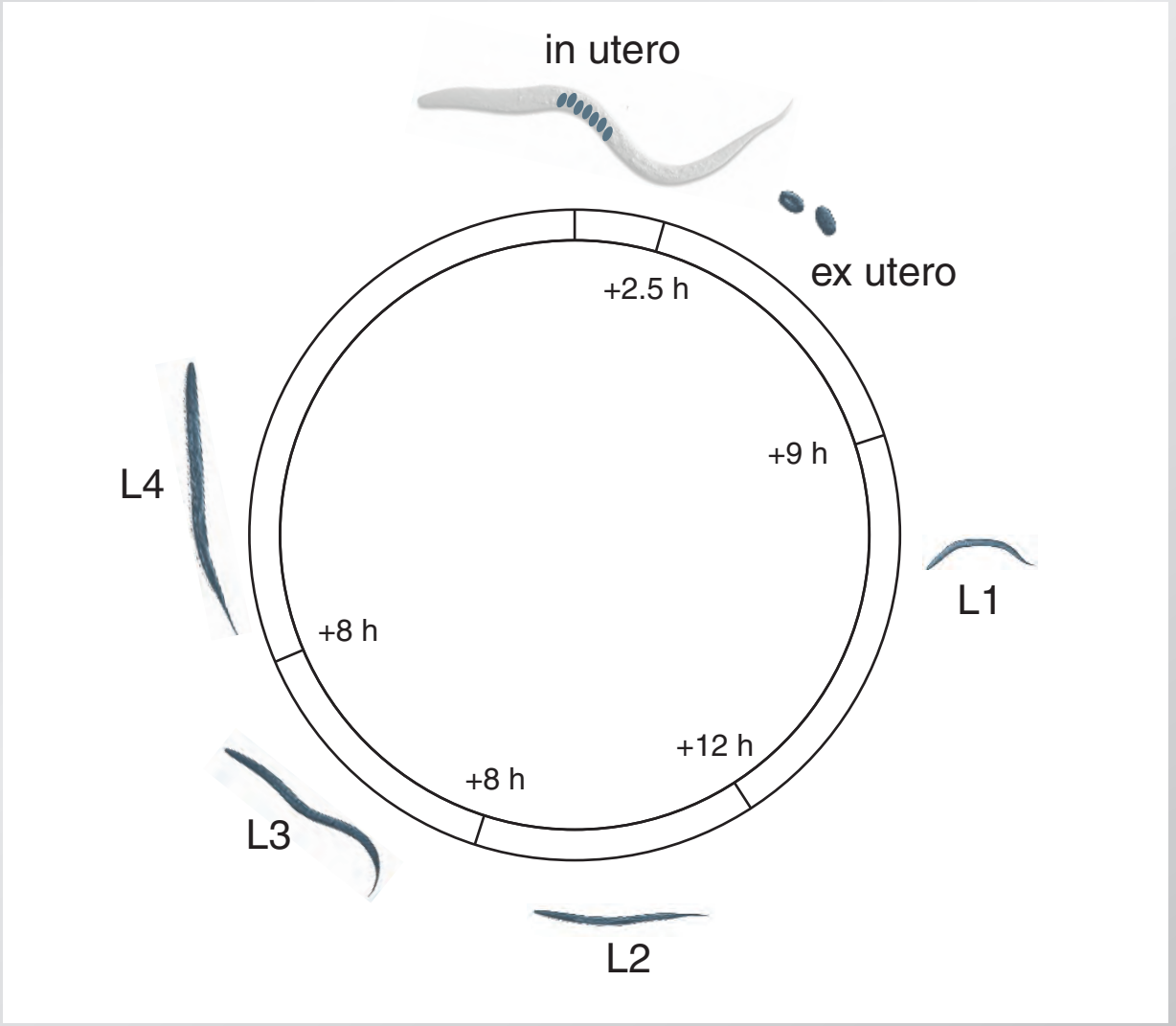
Nanoneurosurgery

C. elegans life cycle



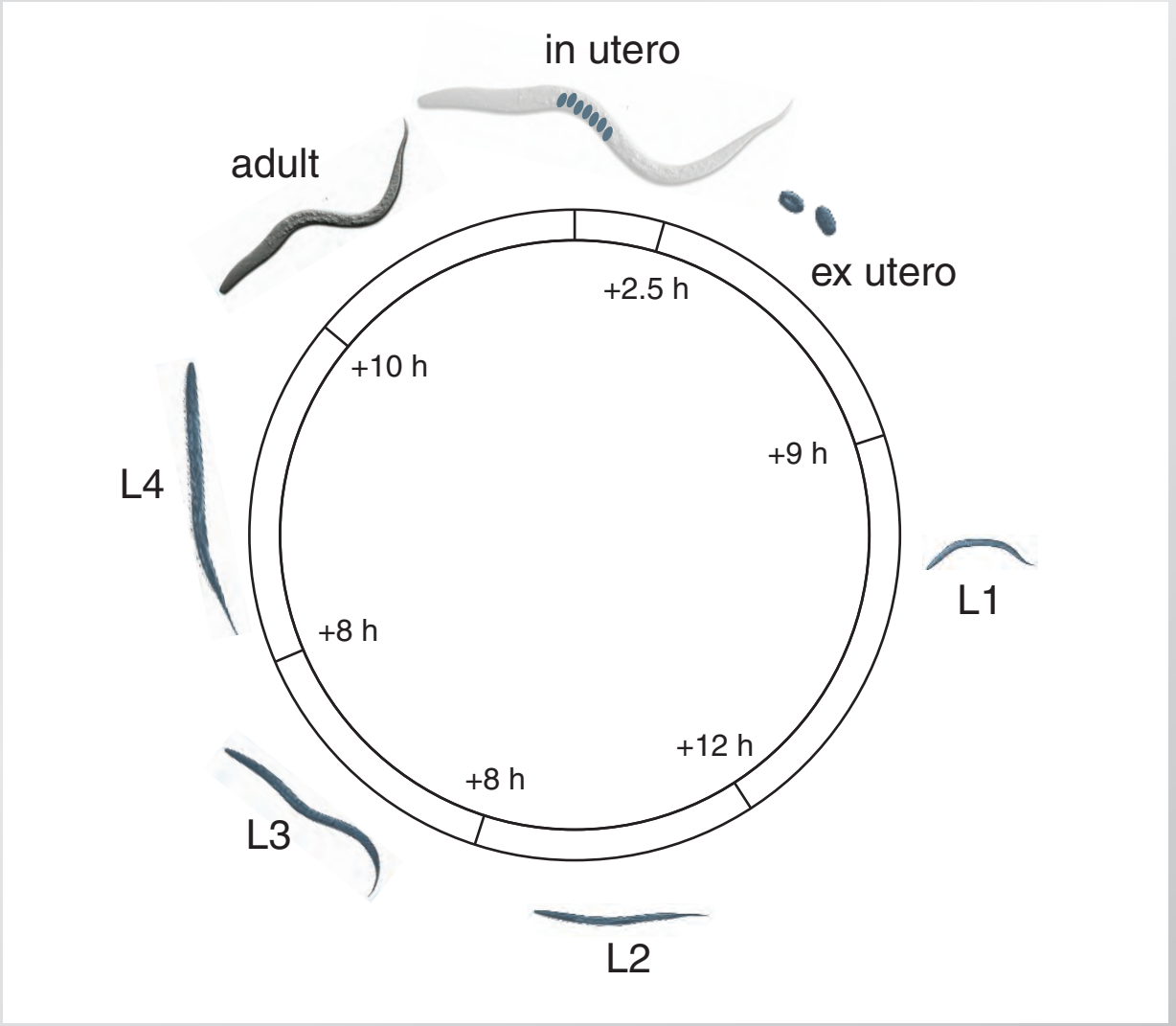
Nanoneurosurgery

C. elegans life cycle



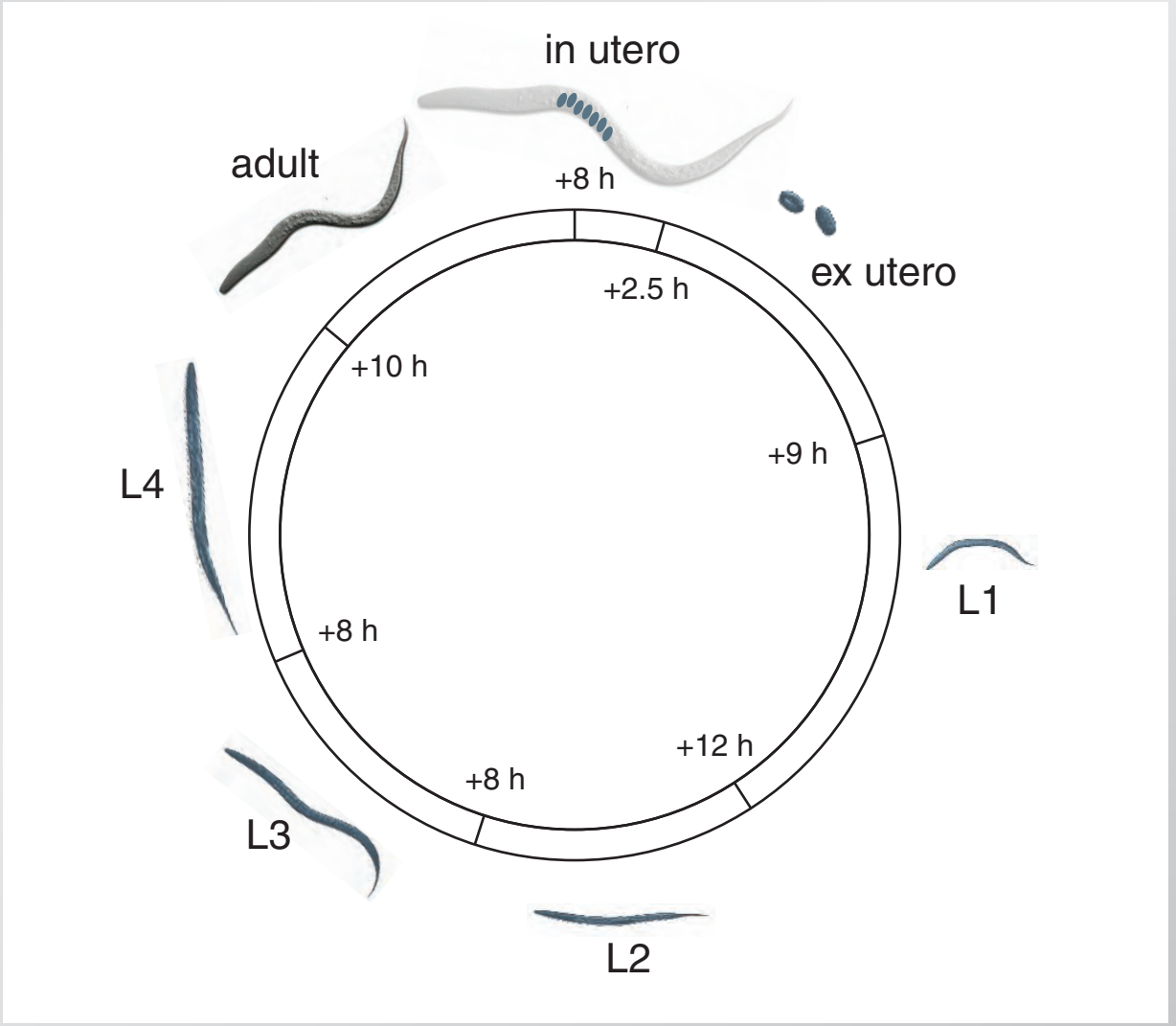
Nanoneurosurgery

C. elegans life cycle



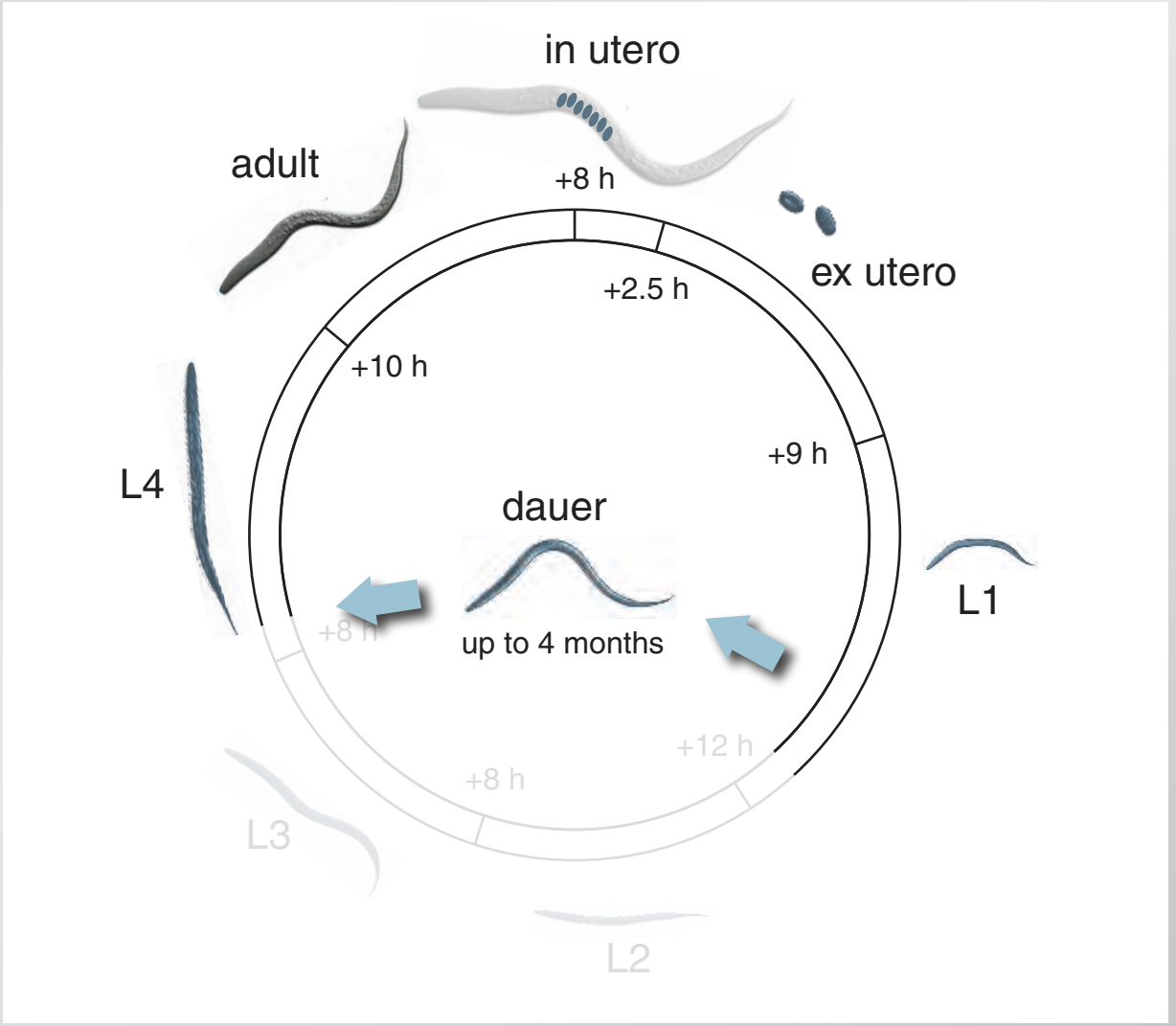
Nanoneurosurgery

C. elegans life cycle



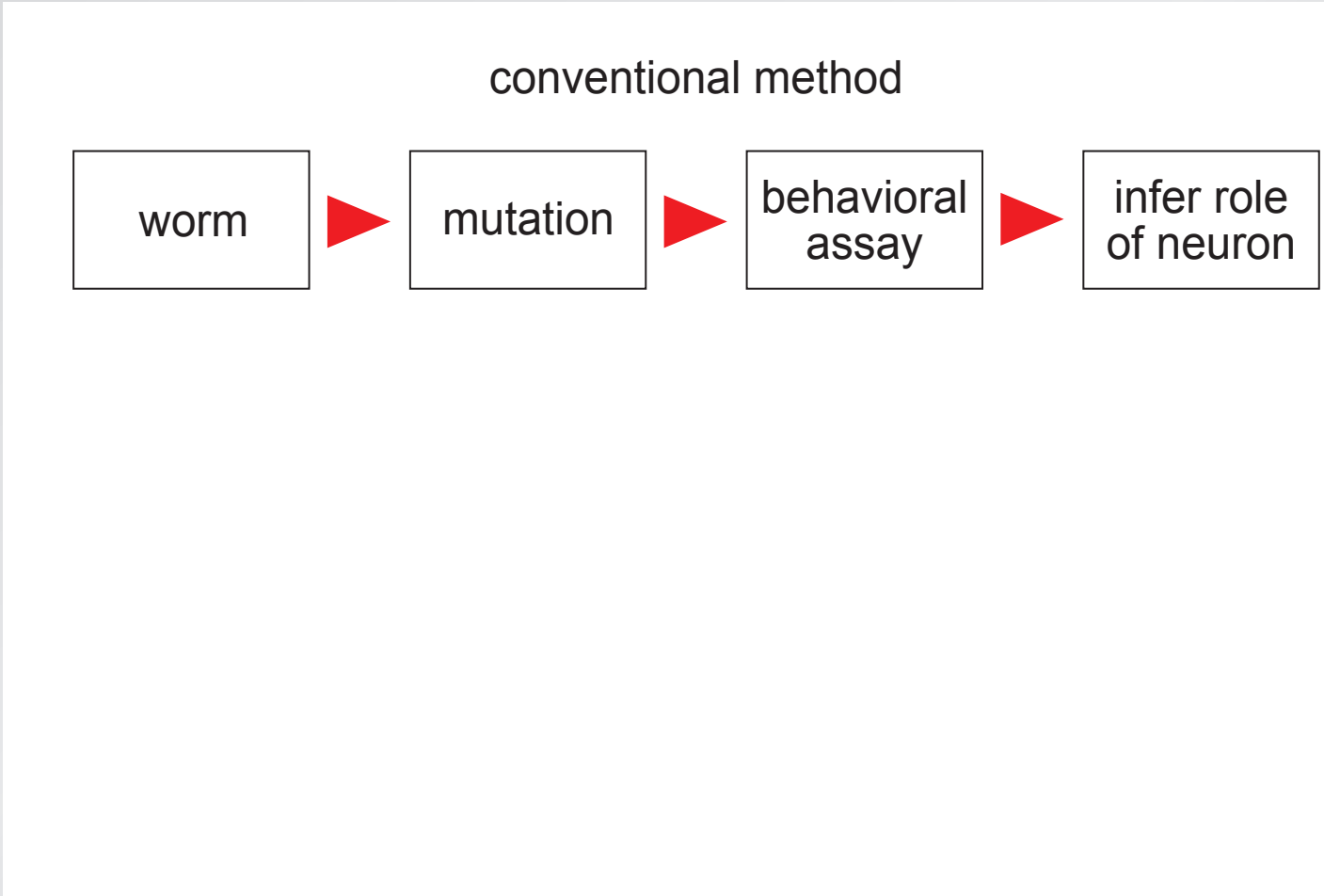
Nanoneurosurgery

C. elegans life cycle



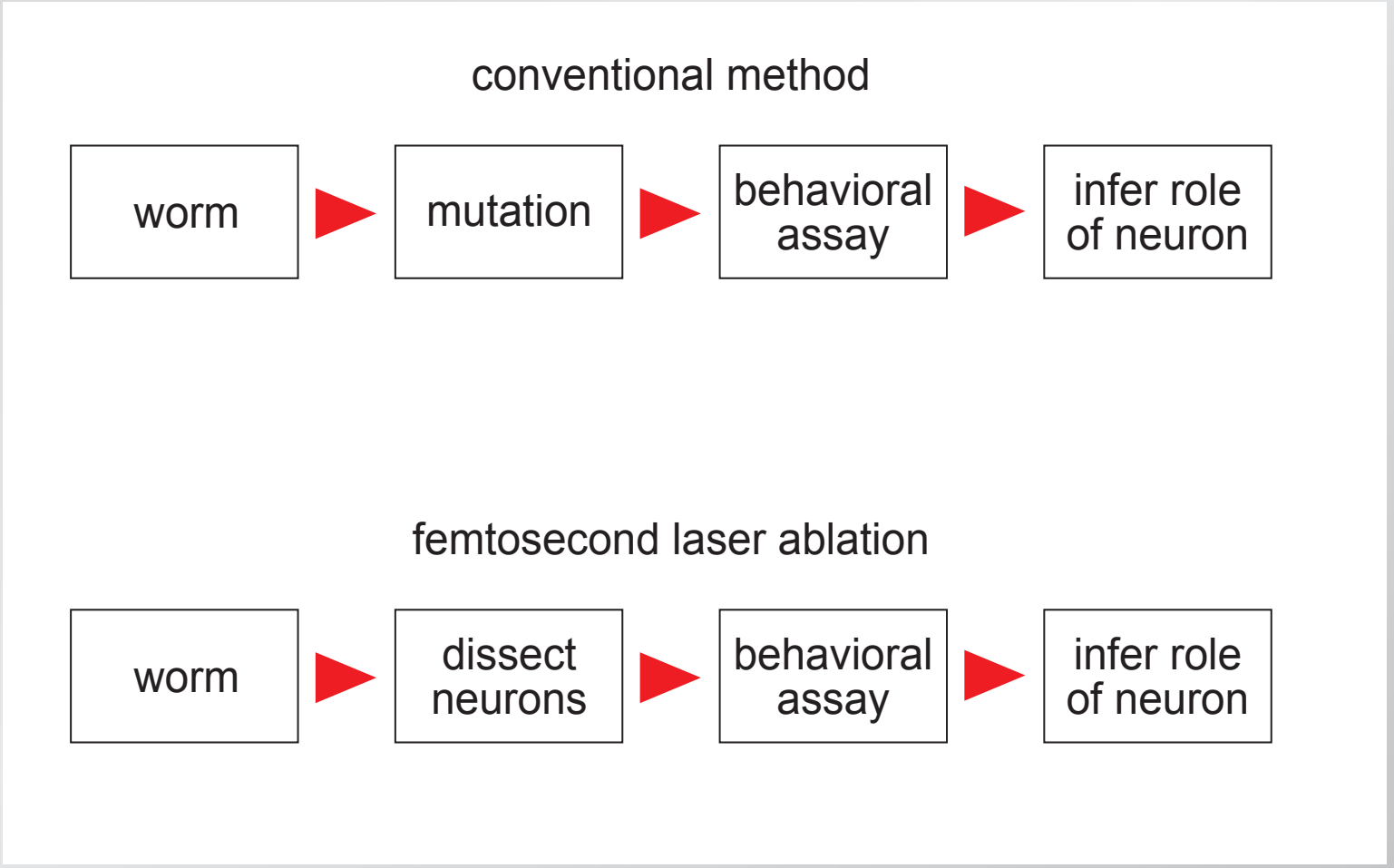
Nanoneurosurgery

Mapping behavior to neurons



Nanoneurosurgery

Mapping behavior to neurons



Nanoneurosurgery

ASH neurons

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

Nanoneurosurgery

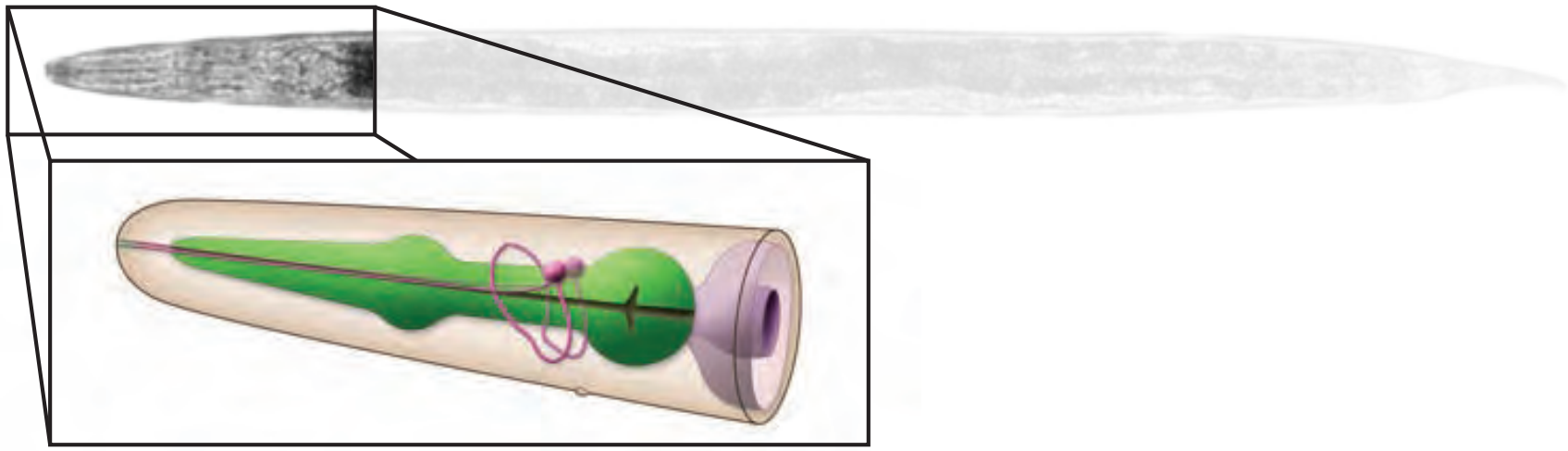
ASH neurons

Nanoneurosurgery

ASH neurons

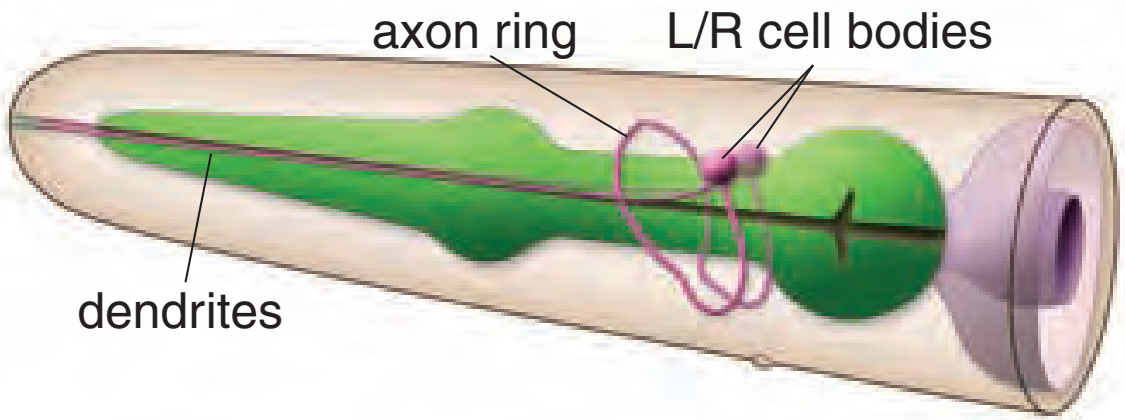
Nanoneurosurgery

ASH neurons



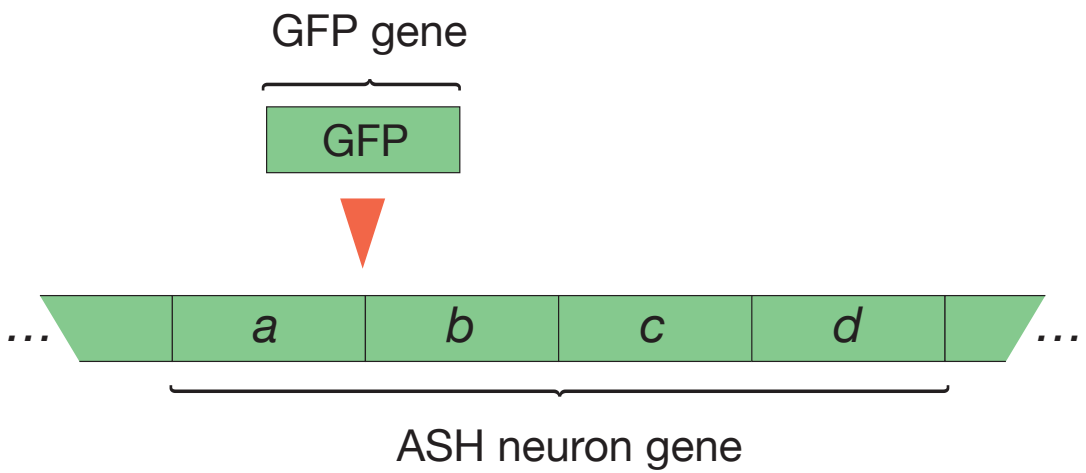
Nanoneurosurgery

ASH neurons



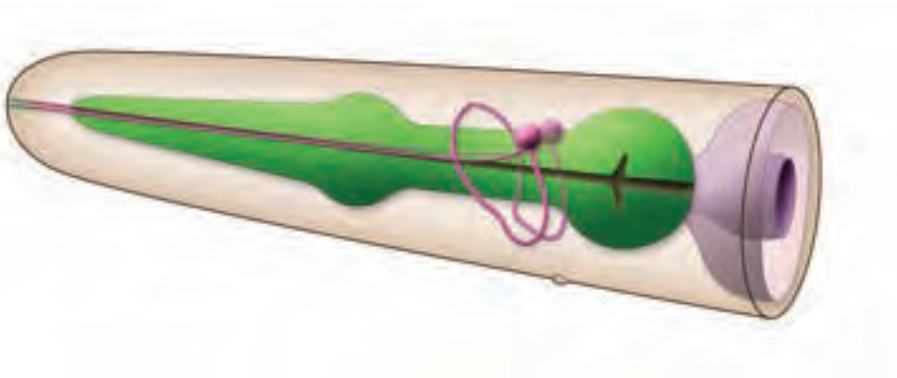
Nanoneurosurgery

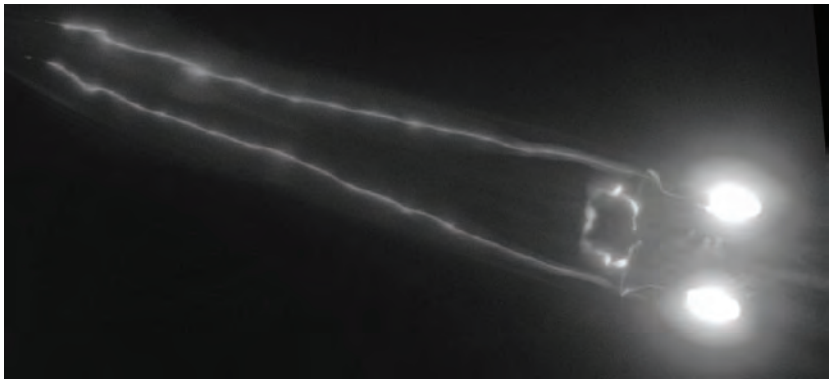
make ASH neurons express GFP



Nanoneurosurgery

make ASH neurons express GFP



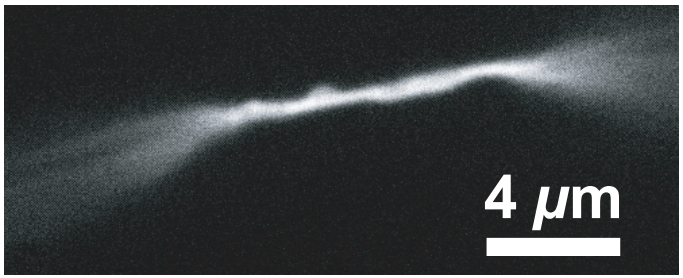


Nanoneurosurgery

GFP: absorbs UV, emits green

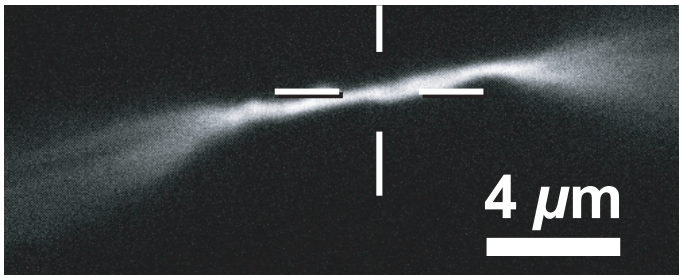
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



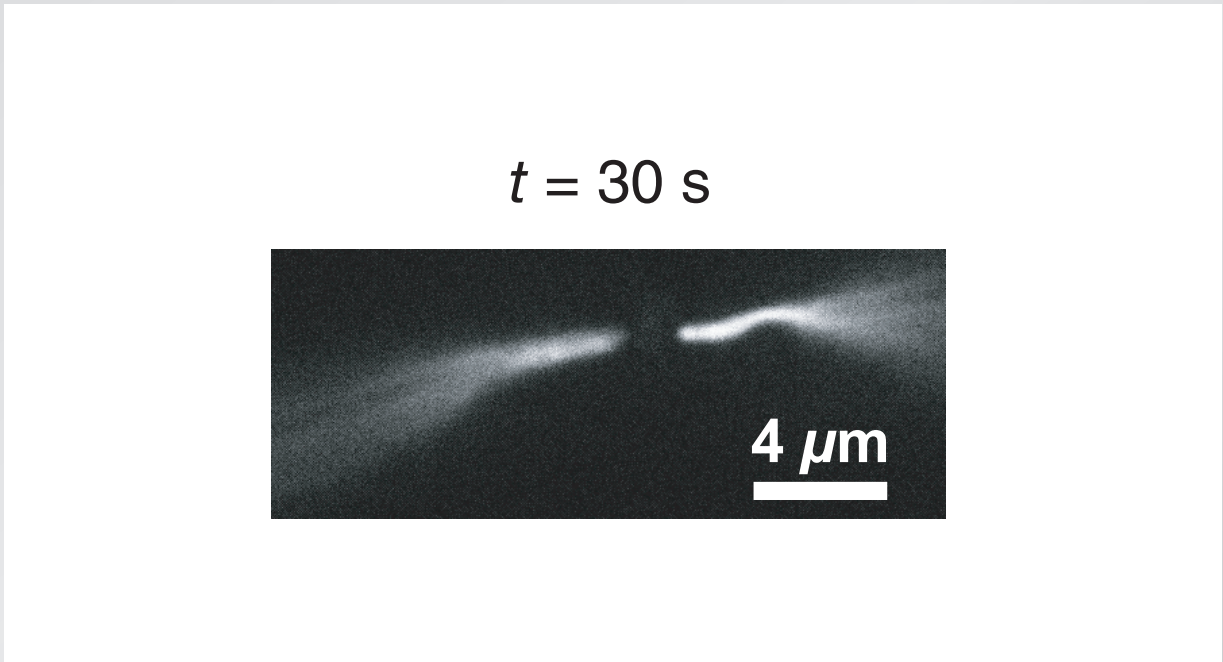
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



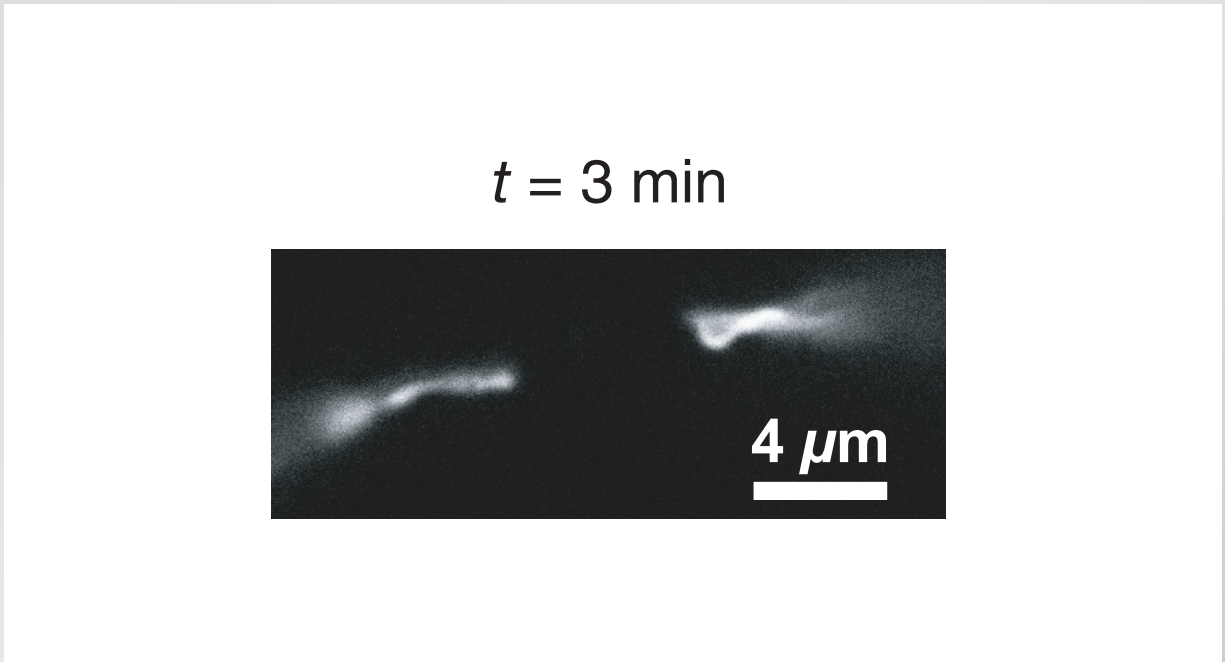
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



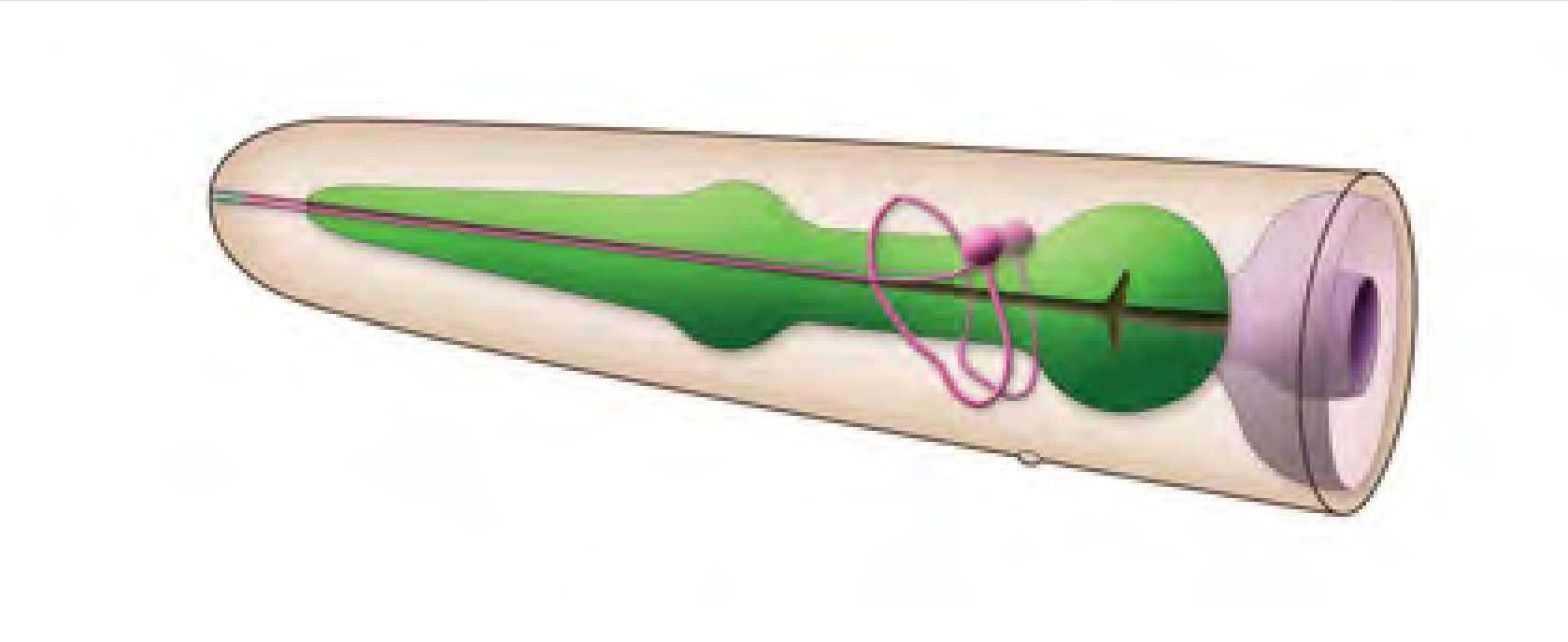
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



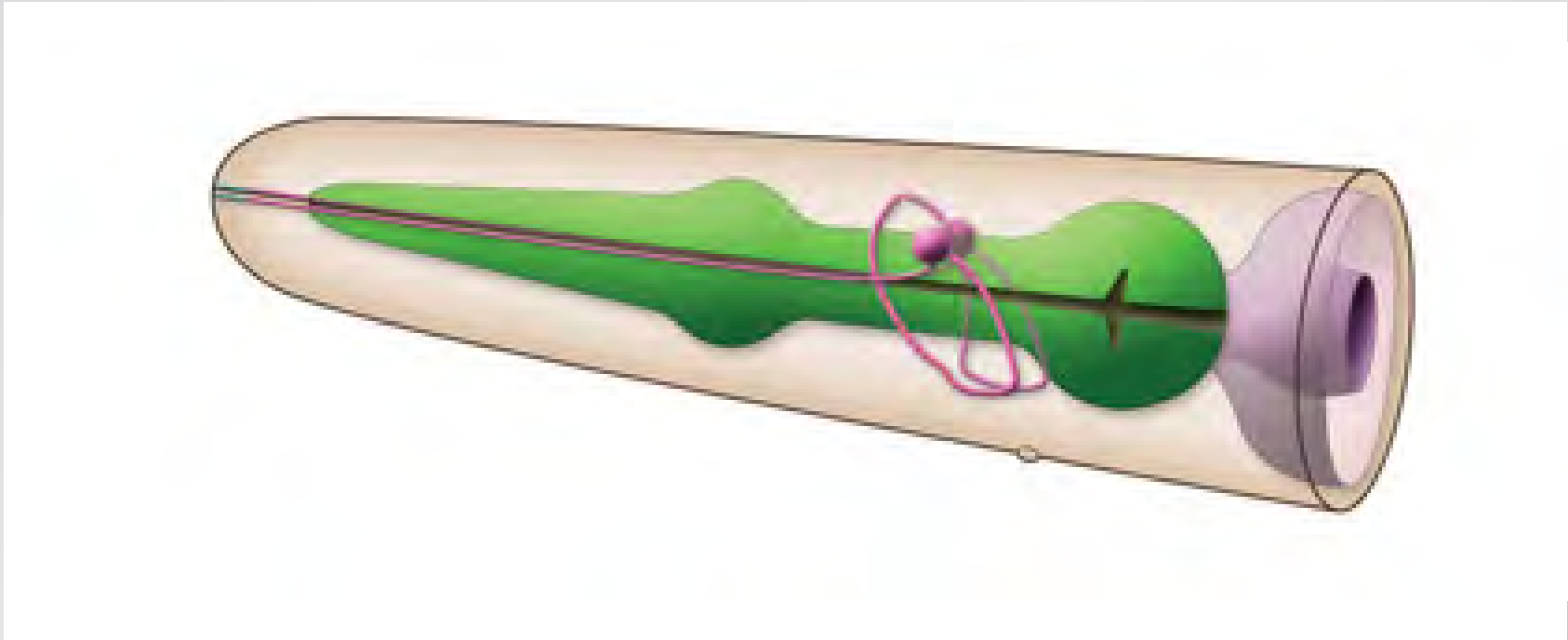
Nanoneurosurgery

ASH neurons



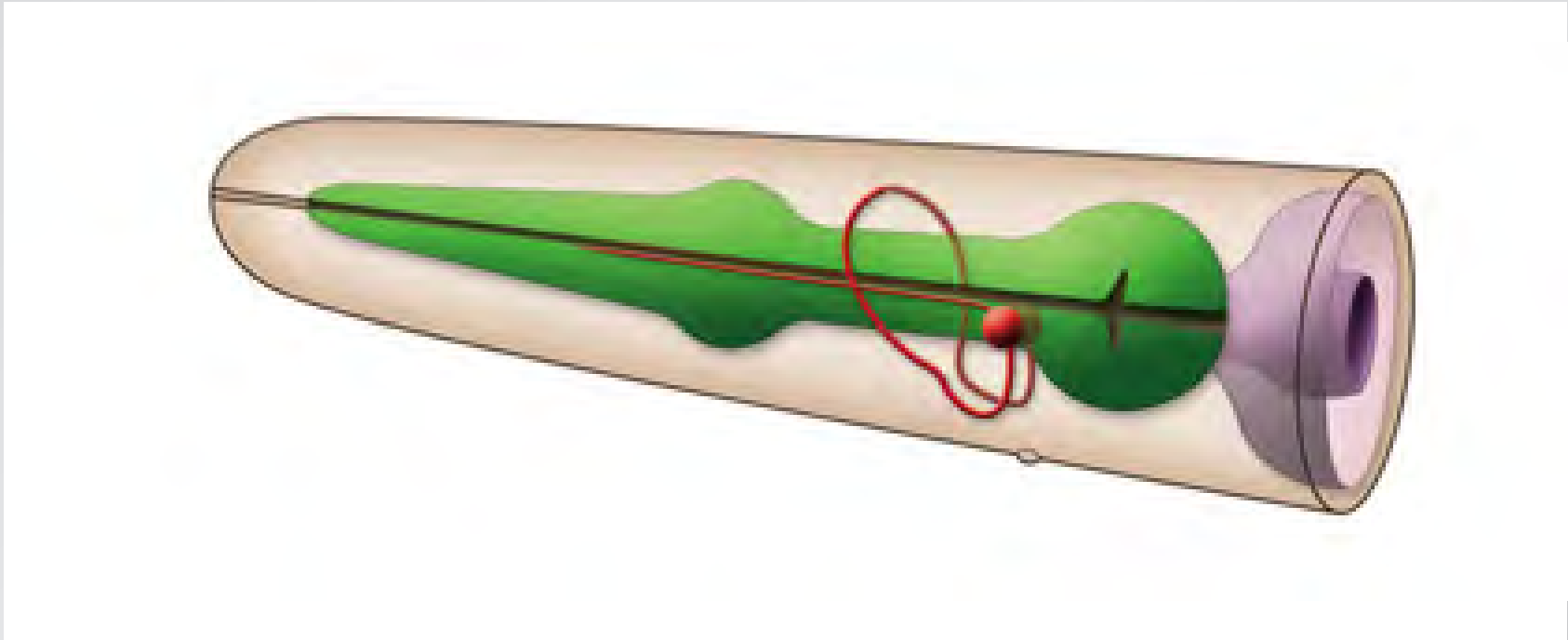
Nanoneurosurgery

ASK neurons



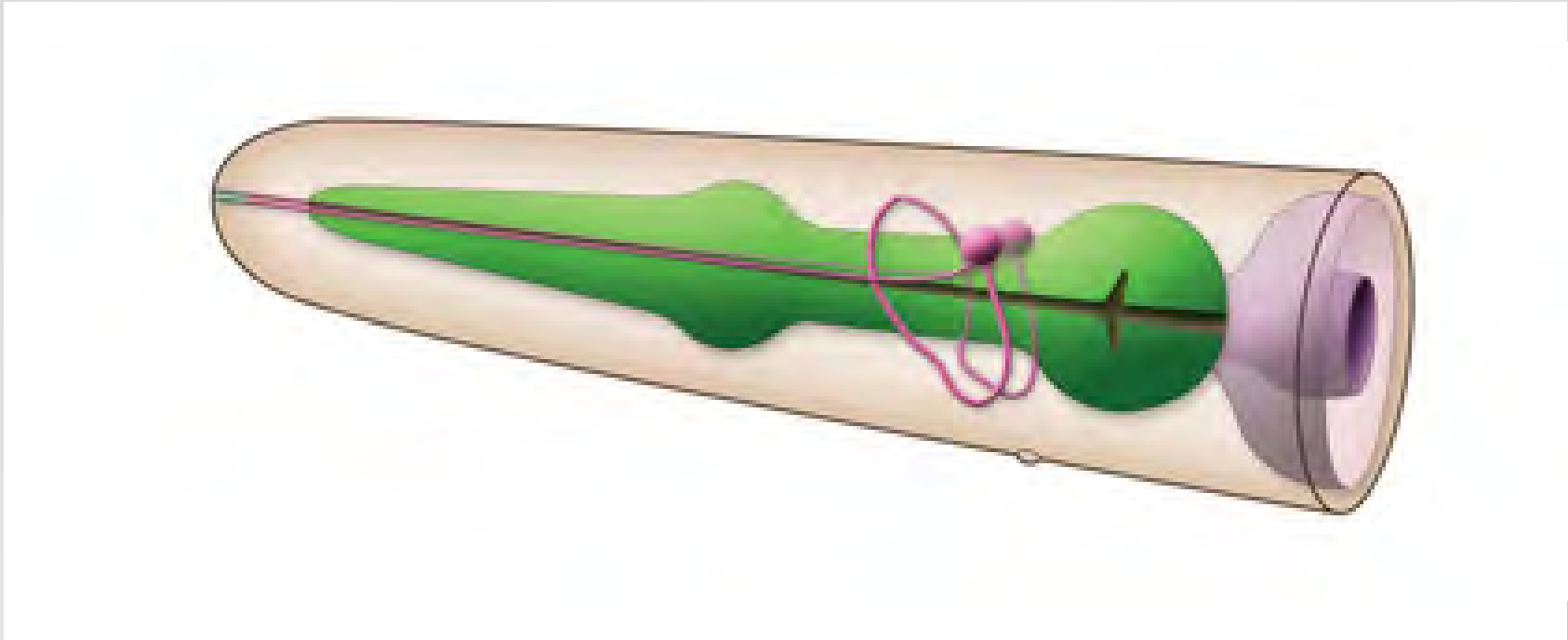
Nanoneurosurgery

AUA neurons



Nanoneurosurgery

ASI neurons

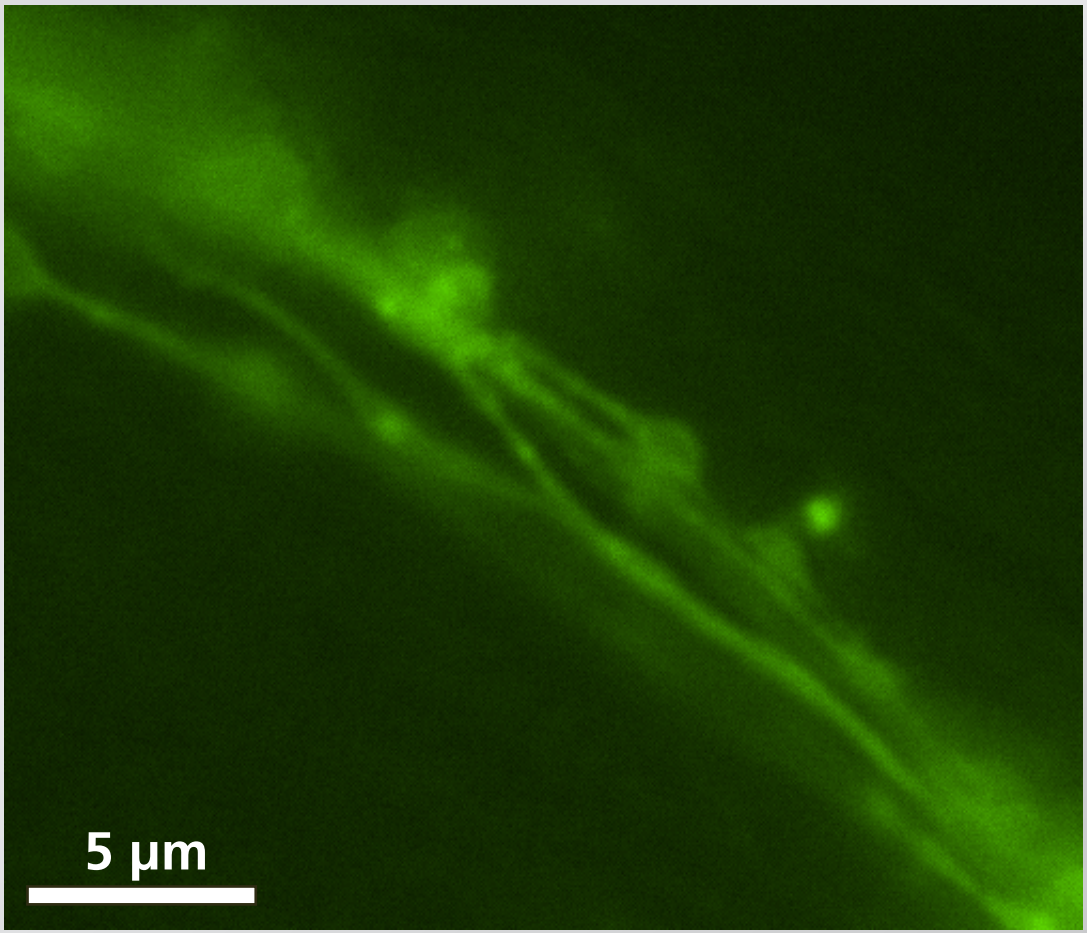


Nanoneurosurgery

need exquisite precision!

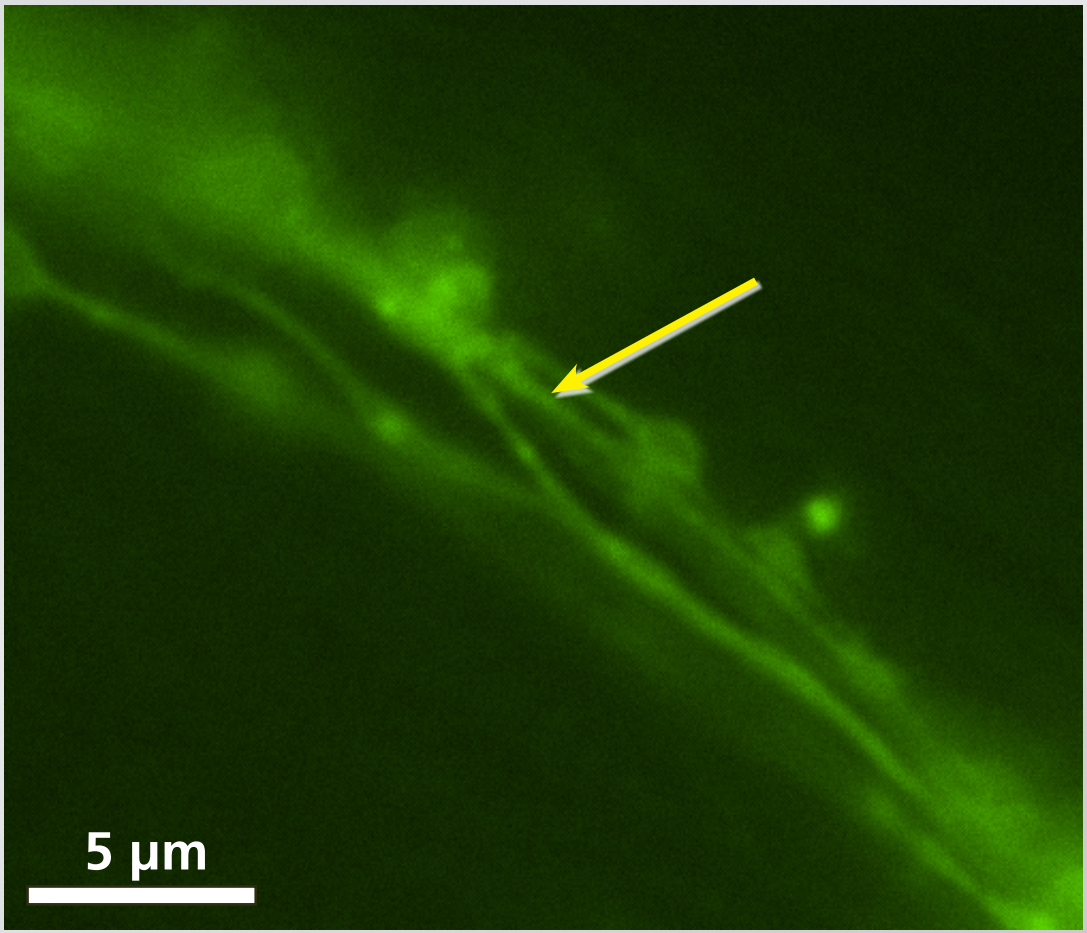
Nanoneurosurgery

DiO-stained bundle of dendrites



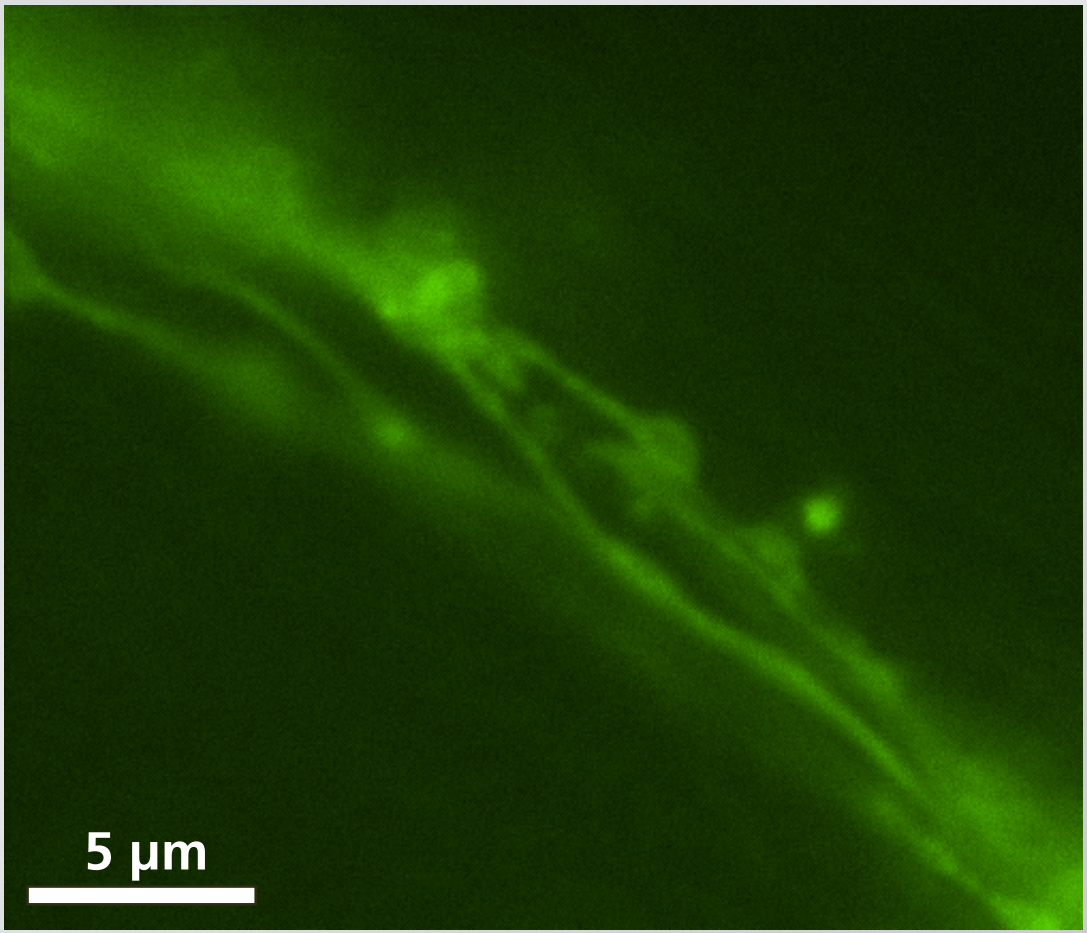
Nanoneurosurgery

cut single dendrite in bundle (3 nJ)



Nanoneurosurgery

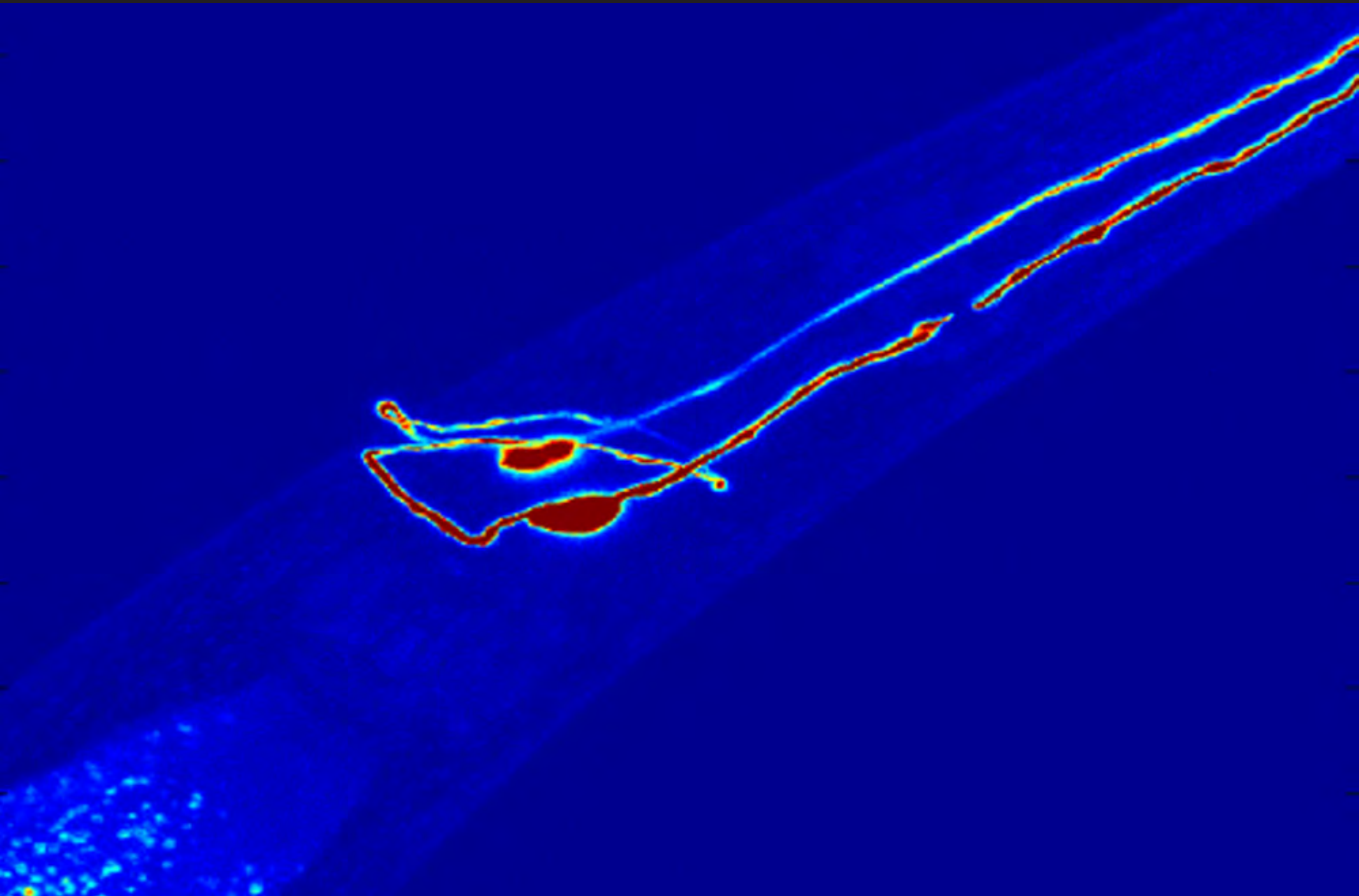
no damange to neighboring dendrites



Nanoneurosurgery

revive worm, reimage 1 day later

Nanoneurosurgery

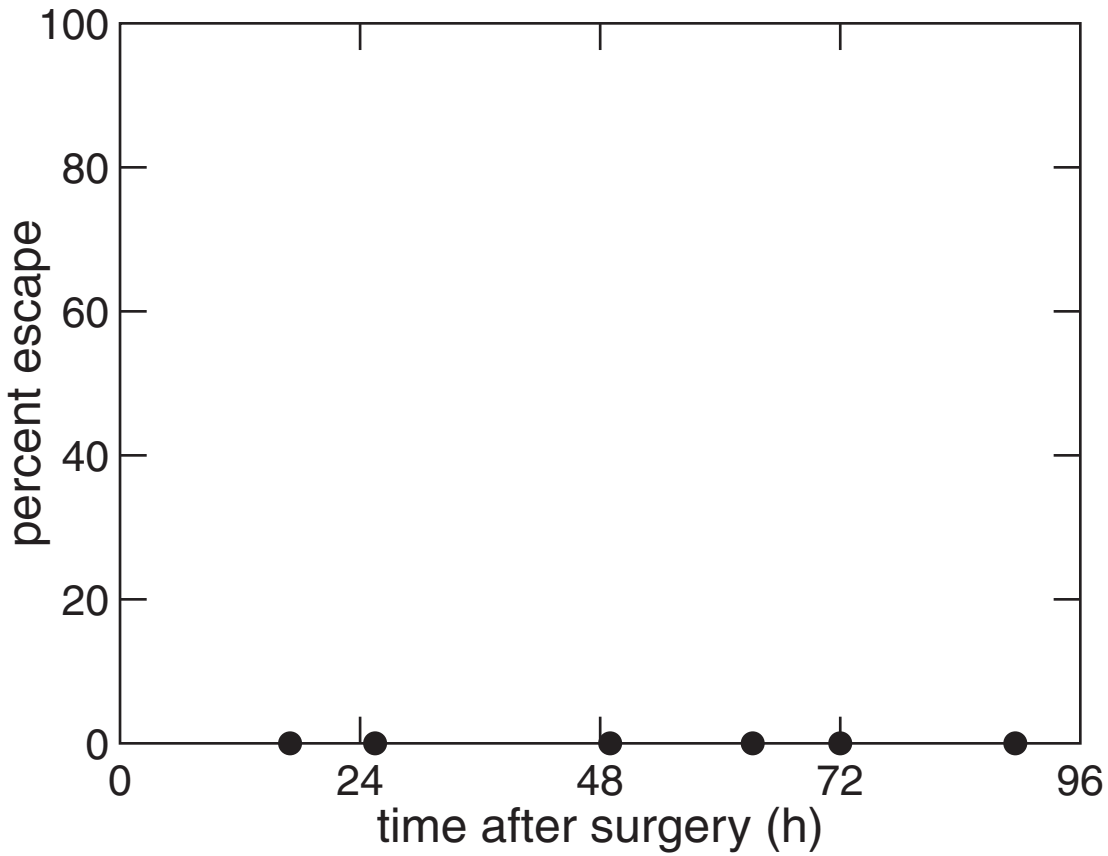


Nanoneurosurgery

osmolarity assay

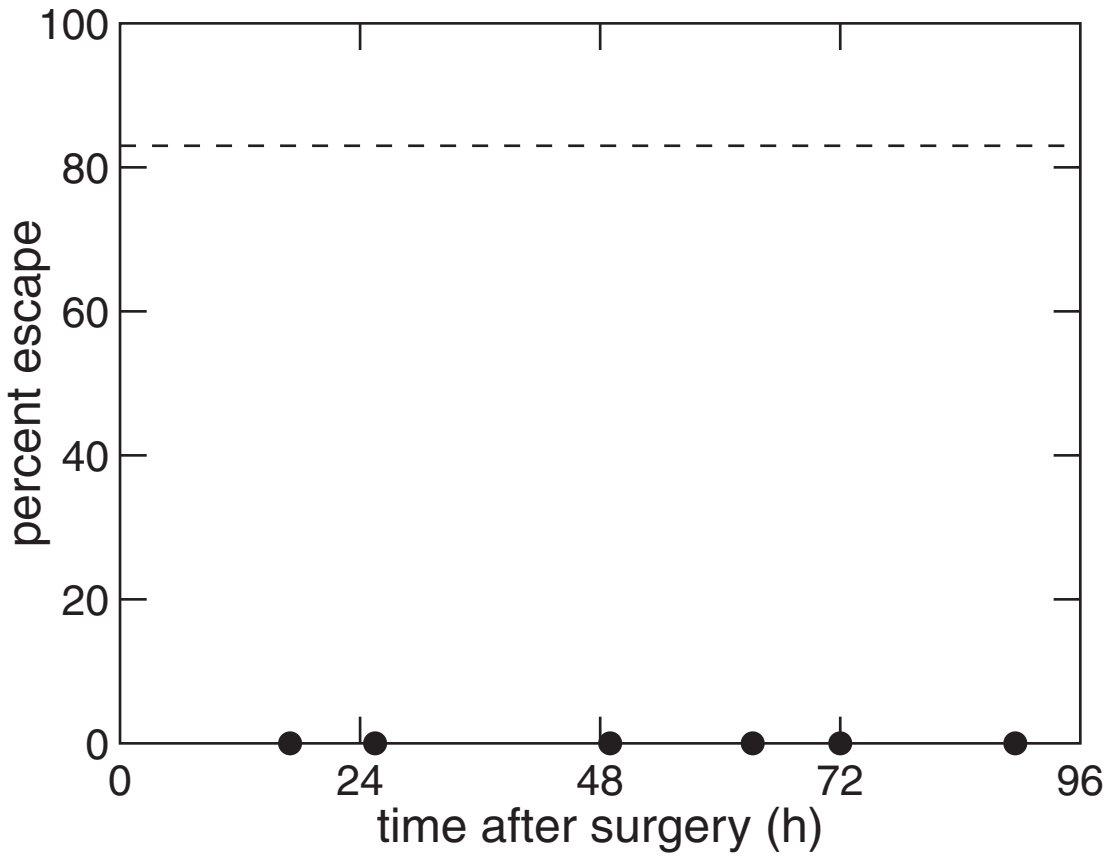
Nanoneurosurgery

escape rate after ‘mock’ surgery



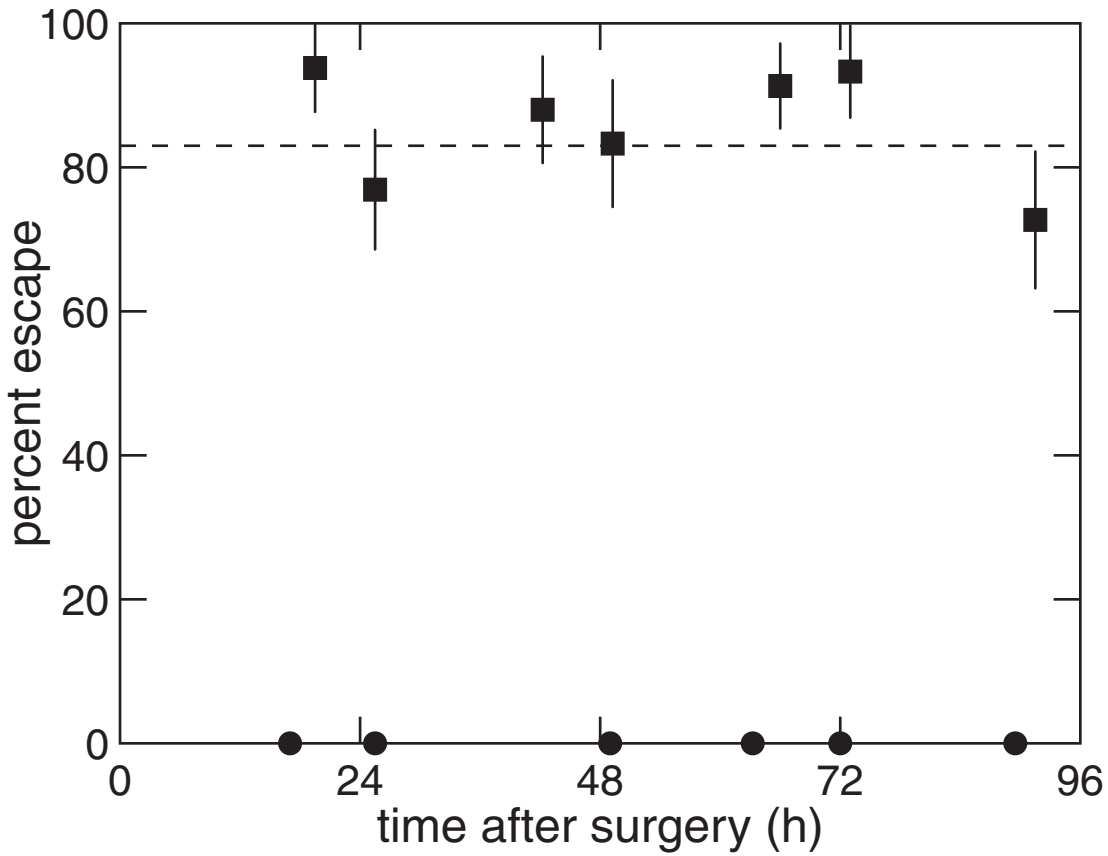
Nanoneurosurgery

escape rate of ASH-lacking mutant



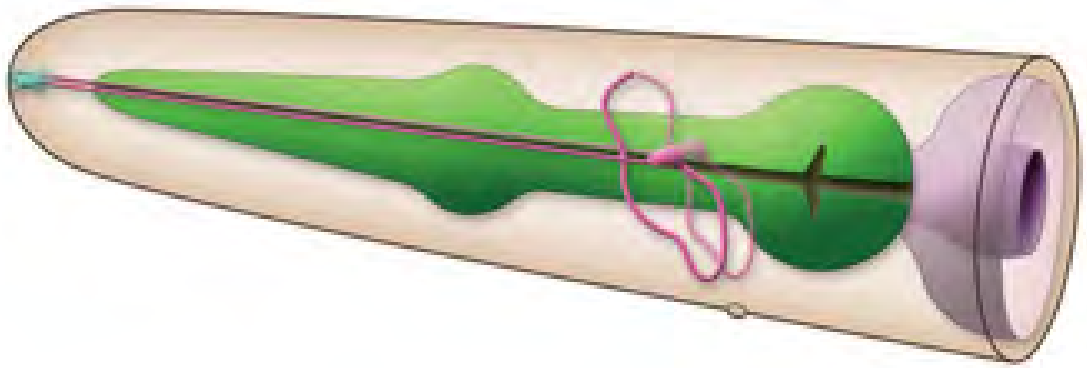
Nanoneurosurgery

escape rate after ASH-ablation surgery

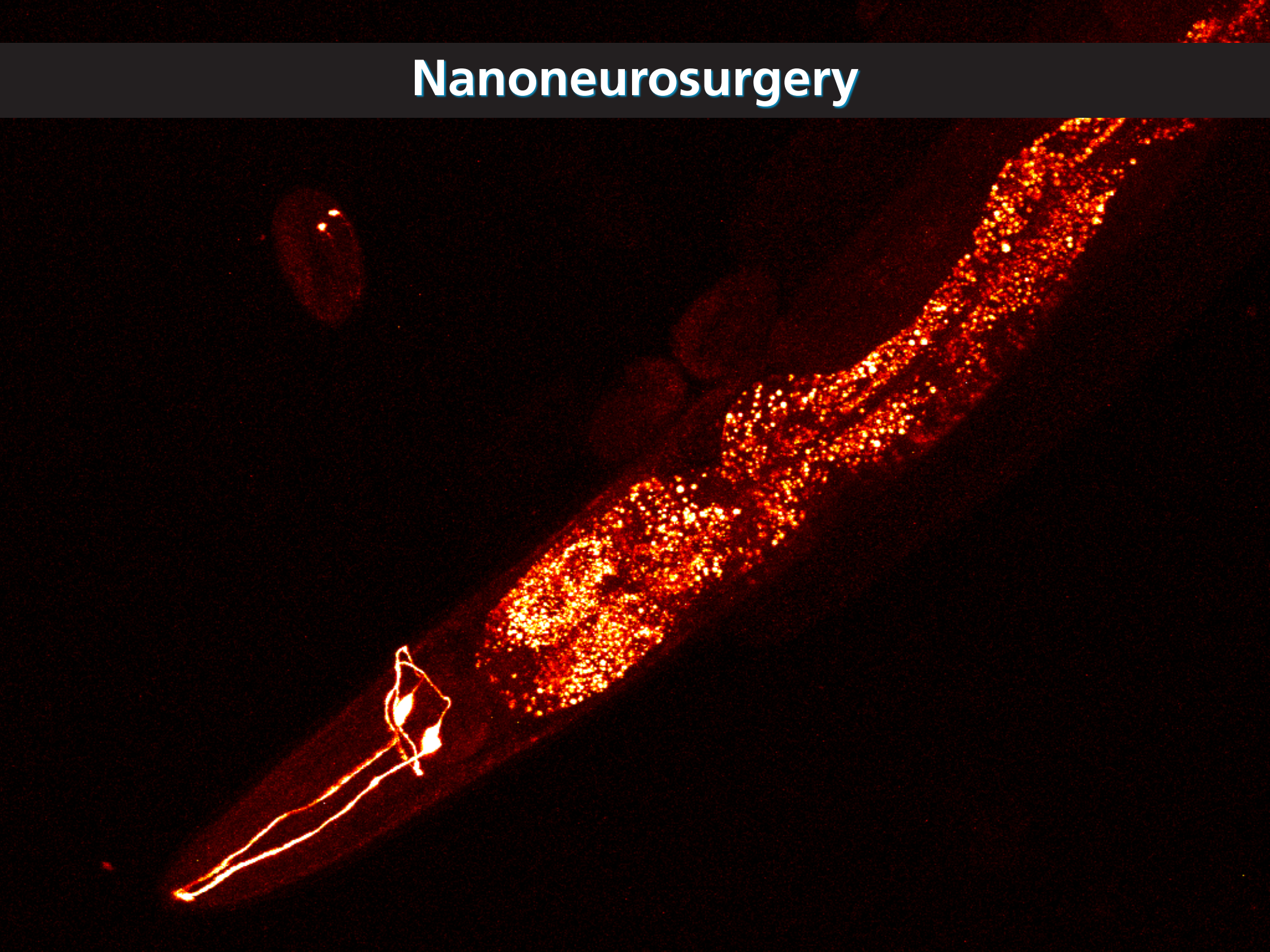


Nanoneurosurgery

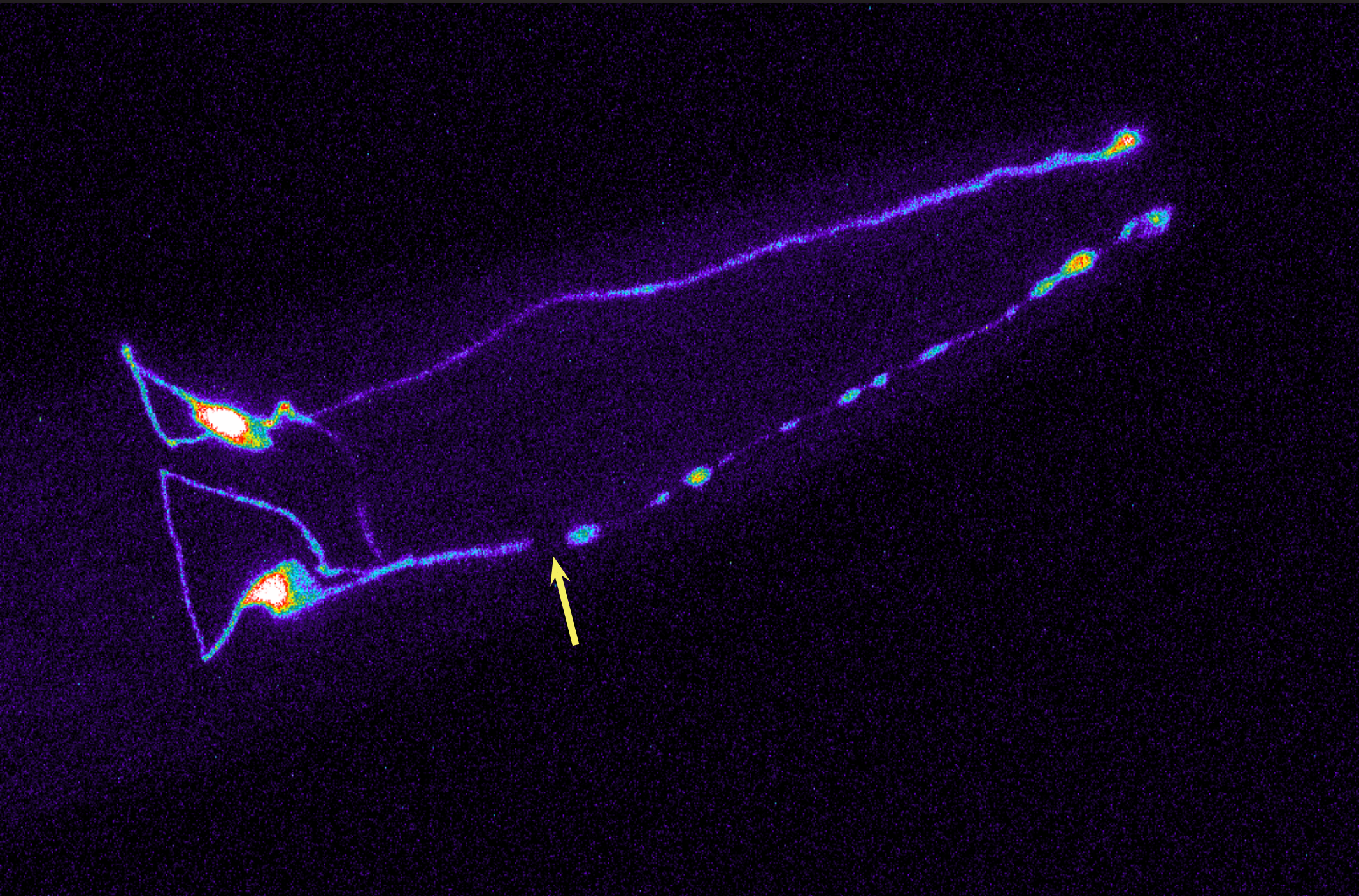
AFD neurons (temperature sensors)



Nanoneurosurgery



Nanoneurosurgery

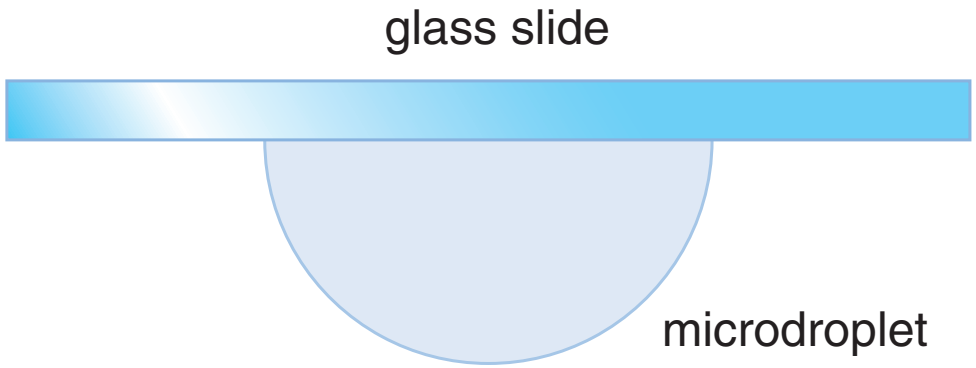


Nanoneurosurgery

Q: where does the ASH sense temperature?

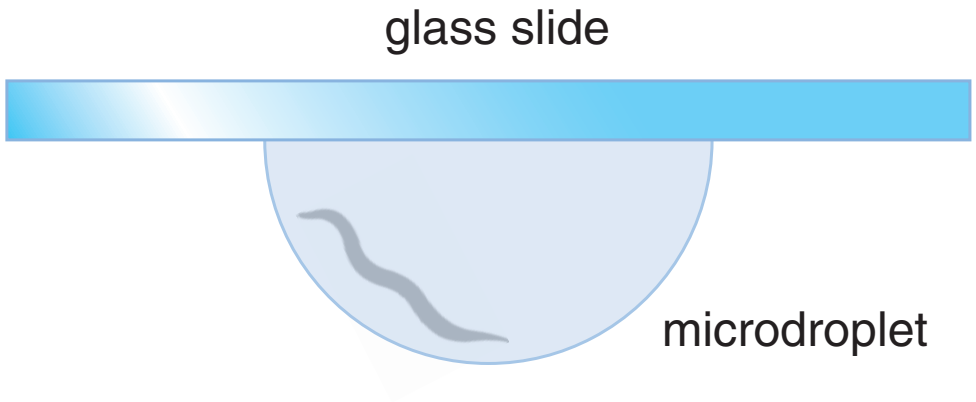
Nanoneurosurgery

microdroplet assay



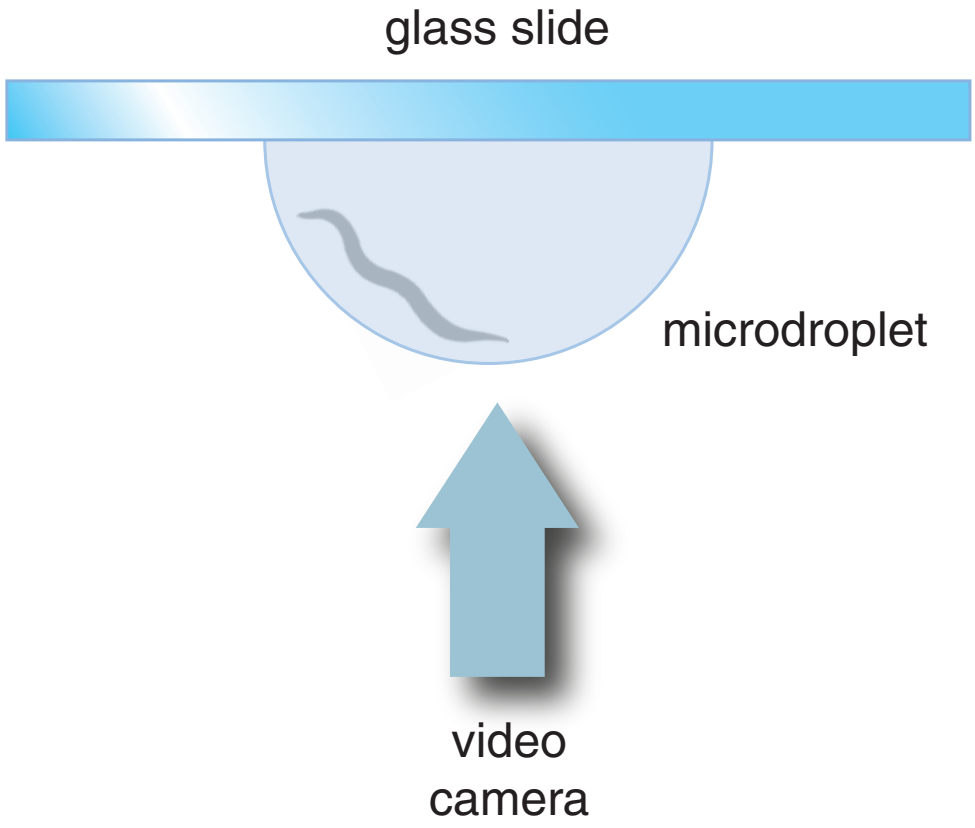
Nanoneurosurgery

microdroplet assay



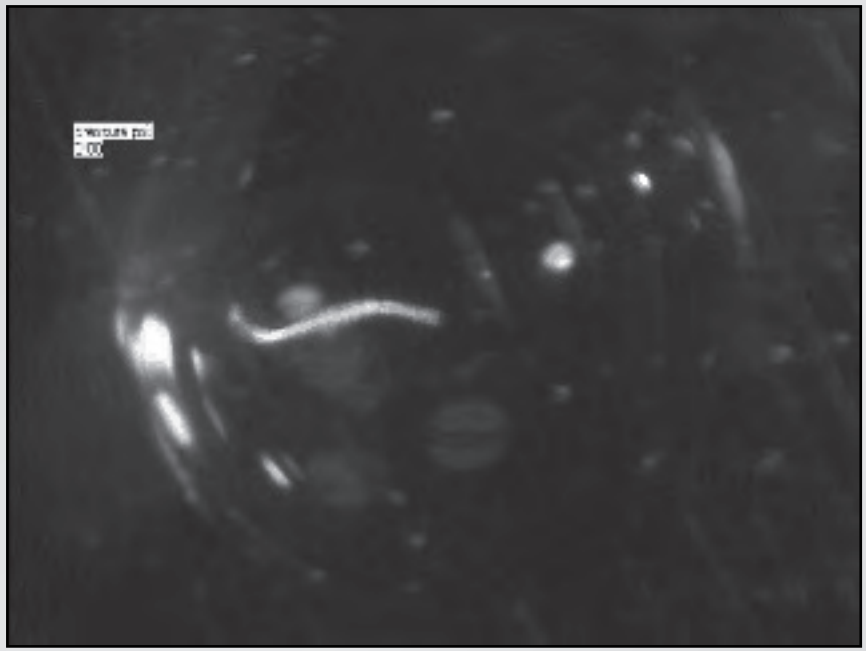
Nanoneurosurgery

microdroplet assay

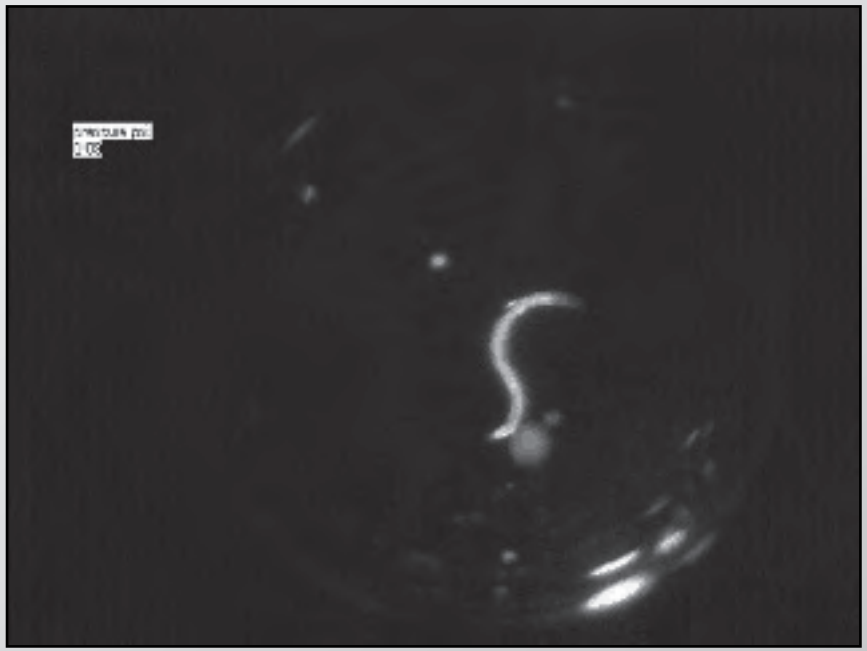


Nanoneurosurgery

surgery results in quantifiable behavior changes



before



after

Nanoneurosurgery

temperature sensing occurs at tip of dendrite

Cell transfection

*Hershey Foods Technical Center, PO Box 805, Hershey, Pennsylvania 17033, USA
e-mail: whurst@hersheys.com
†Department of Anthropology, University of Texas, Austin, Texas 78712-1086, USA

1. Hester, T. R. & Shafer, H. J. in *Archaeological Views from the Countryside: Village Communities in Early Complex Societies* (eds Schwartz, G. M. & Falconer, S. E.) 48–63 (Smithsonian Institution, Washington DC, 1994).
2. Valdez, F. Jr *The Prehistoric Ceramics of Colha, Northern Belize.* Thesis, Harvard Univ. (1987).
3. Powis, T. G. & Hurst, W. J. *Proc. 66th Annu. Meeting Soc. Am. Archaeol.* (New Orleans, 2001).
4. Coe, S. D. & Coe, M. D. *The True History of Chocolate* (Thames & Hudson, London, 1996).
5. Tozzer, A. M. *Landa's Relación de Las Cosas de Yucatán* (Kraus Reprint, New York, 1941).
6. Potter, D. R. in *The Colha Project, Second Season, 1980 Interim Report* (eds Hester, T. R., Eaton, J. D. & Shafer, H. J.) 173–184 (Center for Archaeological Research, San Antonio, Texas; Centro Studi Ricerche Ligabue, Venice; 1980).
7. Potter, D. R. in *Archaeology at Colha, Belize, 1981 Interim Report* (eds Hester, T. R., Shafer, H. J. & Eaton, J. D.) 98–122 (Center for Archaeological Research, San Antonio, Texas; Centro Studi Ricerche Ligabue, Venice; 1982).
8. Hurst, W. J., Martin, A. J. Jr, Tarka, S. M. Jr & Hall, G. D. *J. Chromatogr.* **466,** 279–289 (1989).
9. Hall, G. D., Tarka, S. M. Jr, Hurst, W. J., Stuart, D. & Adams, R. E. W. *Am. Antiquity* **55,** 138–143 (1990).
10. Stuart, D. *Antiquity* **62,** 153–157 (1988).
11. Turner, B. L. & Miksicek, C. H. *Econ. Bot.* **38,** 179–193 (1984).

Supplementary information accompanies this communication on *Nature*'s website.
Competing financial interests: declared none.

Cell biology

Targeted transfection by femtosecond laser

The challenge for successful delivery of foreign DNA into cells *in vitro*, a key technique in cell and molecular biology with important biomedical implications, is to improve transfection efficiency while leaving the cell's architecture intact. Here we show that a variety of mammalian cells can be directly transfected with DNA without perturbing their structure by first creating a tiny, localized perforation in the membrane using ultrashort (femtosecond), high-intensity, near-infrared laser pulses. Not only does this superior optical technique give high transfection efficiency and cell survival, but it also allows simultaneous evaluation of the integration and expression of the introduced gene.

Previous techniques that have been developed for transfection of cells with DNA[1] include carrier-mediated transfer[2] and transfer by plasma-membrane permeabilization[3], as well as direct transfer[4], but the efficiency of delivery by these methods is low. Moreover, none allows trans-

mediated by intense near-infrared femtosecond laser pulses. Cells were suspended inside a sterile miniaturized cell chamber in 0.5 ml culture medium containing 0.2 μg plasmid DNA vector pEGFP-N1 (4.7 kilobases) encoding enhanced green fluorescent protein (EGFP)[7]. Transmission images of cells were obtained at low power (<5 μW), and the near-infrared laser beam was then focused (under the same microscope) on the edge of the membrane of a target cell, which was exposed to an enhanced mean laser power of 50–100 mW for 16 ms so that transfection could occur. More than 200 cells of each type were targeted in each of 18 replicate experiments; it took 10–15 s to prepare for the transfection of each cell.

We assessed the integration and expression efficiency of the EGFP gene *in situ* by time-lapse two-photon fluorescence imaging[8] at a mean laser power of <1 mW over a period of 72 h, as well as by two-photon fluorescence-lifetime imaging (TPFLIM)[9]. Figure 1 shows that diffraction-limited focusing of intense femtosecond near-infrared laser pulses selectively facilitates transfection of the target cells, but not of the adjacent cells. Expression of EGFP in the transfected cells is also demonstrated by TPFLIM, and the measured fluorescence lifetime of about 2.4 ns is consistent with that reported for cells expressing EGFP[10]. ... type, the transfection ... invariably

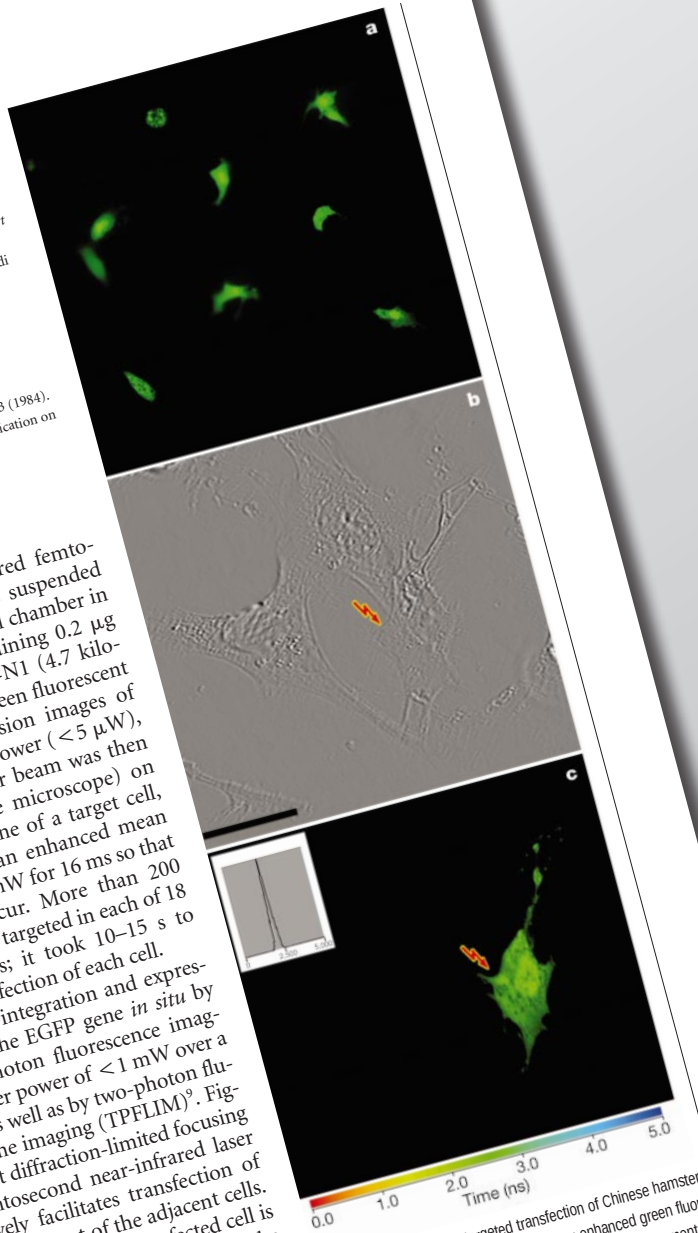
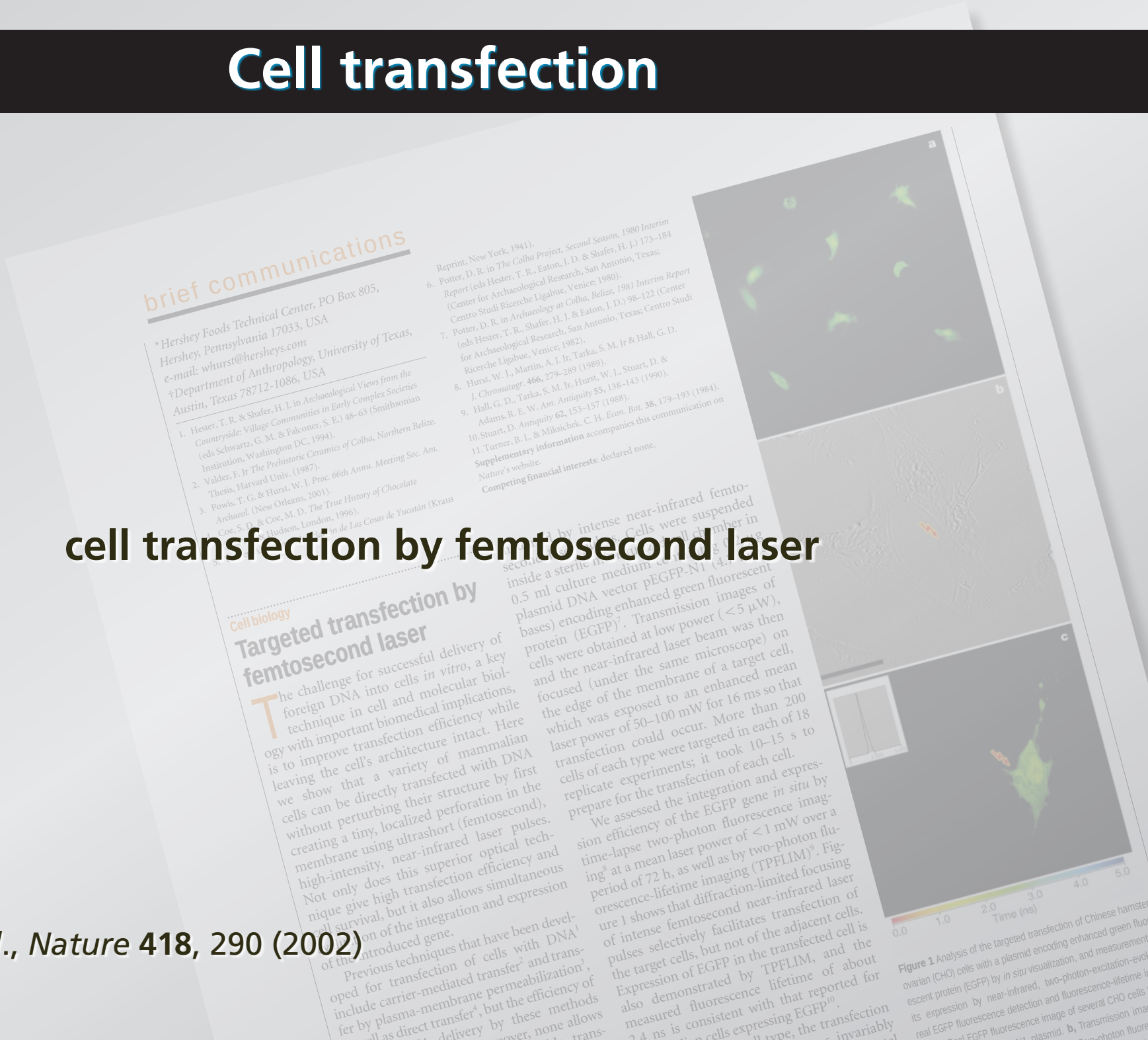


Figure 1 Analysis of the targeted transfection of Chinese hamster ovarian (CHO) cells with a plasmid encoding enhanced green fluorescent protein (EGFP) by *in situ* visualization, and measurement of its expression by near-infrared, two-photon-excitation-evoked real EGFP fluorescence detection and fluorescence-lifetime ... EGFP fluorescence image of several CHO cells ... plasmid. **b,** Transmission ... two-photon fluores...

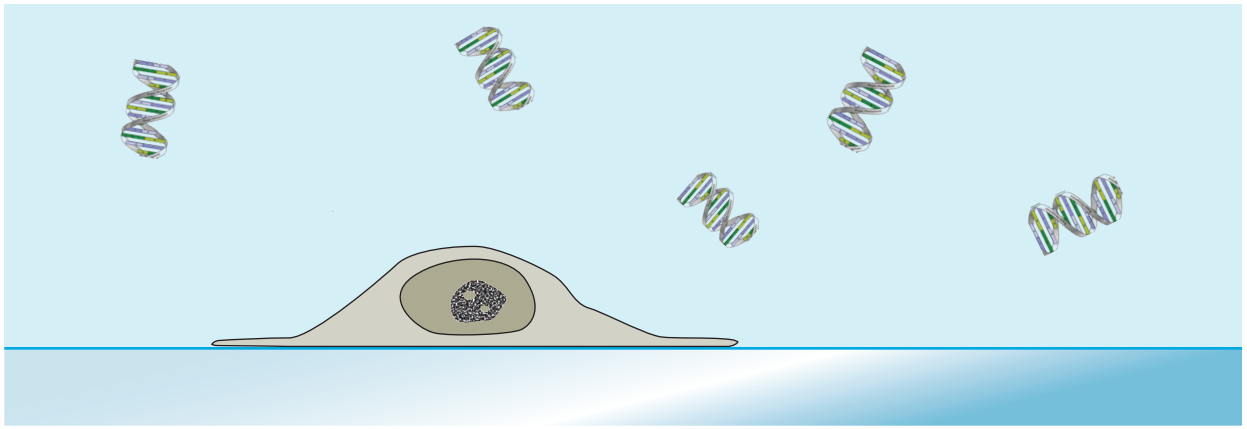
Cell transfection

cell transfection by femtosecond laser

Tirlapur, *et al.*, *Nature* **418**, 290 (2002)

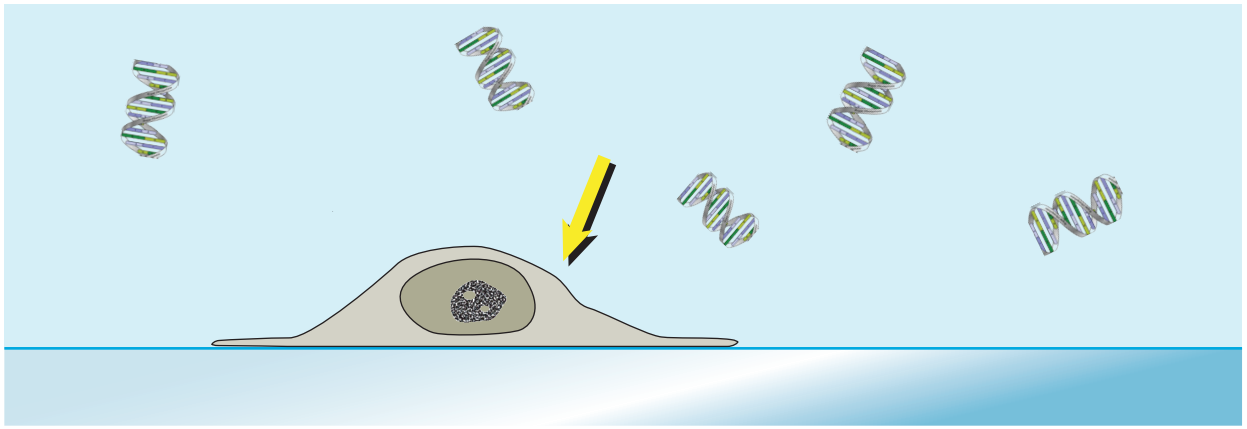


Cell transfection



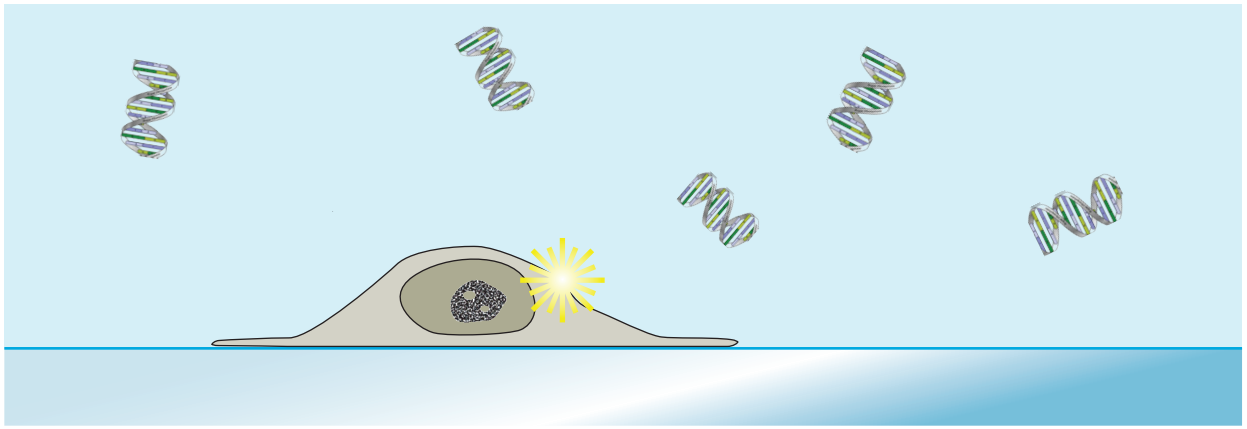
Tirlapur, *et al.*, *Nature* **418**, 290 (2002)

Cell transfection



Tirlapur, *et al.*, *Nature* **418**, 290 (2002)

Cell transfection



Tirlapur, *et al.*, *Nature* **418**, 290 (2002)

Cell transfection

	Toxicity	Efficiency	Throughput	Specificity
Goal	VL	H	H	L

Cell transfection

	Toxicity	Efficiency	Throughput	Specificity
Goal	VL	H	H	L
Naked DNA	VL	L	H	L
Polymer/lipid	M	M	H	H
Viral transfection	M	H	H	H
Electroporation	H	H	H	L

Cell transfection

	Toxicity	Efficiency	Throughput	Specificity
Goal	VL	H	H	L
Naked DNA	VL	L	H	L
Polymer/lipid	M	M	H	H
Viral transfection	M	H	H	H
Electroporation	H	H	H	L

Cell transfection

	Toxicity	Efficiency	Throughput	Specificity
Goal	VL	H	H	L
Naked DNA	VL	L	H	L
Polymer/lipid	M	M	H	H
Viral transfection	M	H	H	H
Electroporation	H	H	H	L
Laser poration	VL	H	VL	L

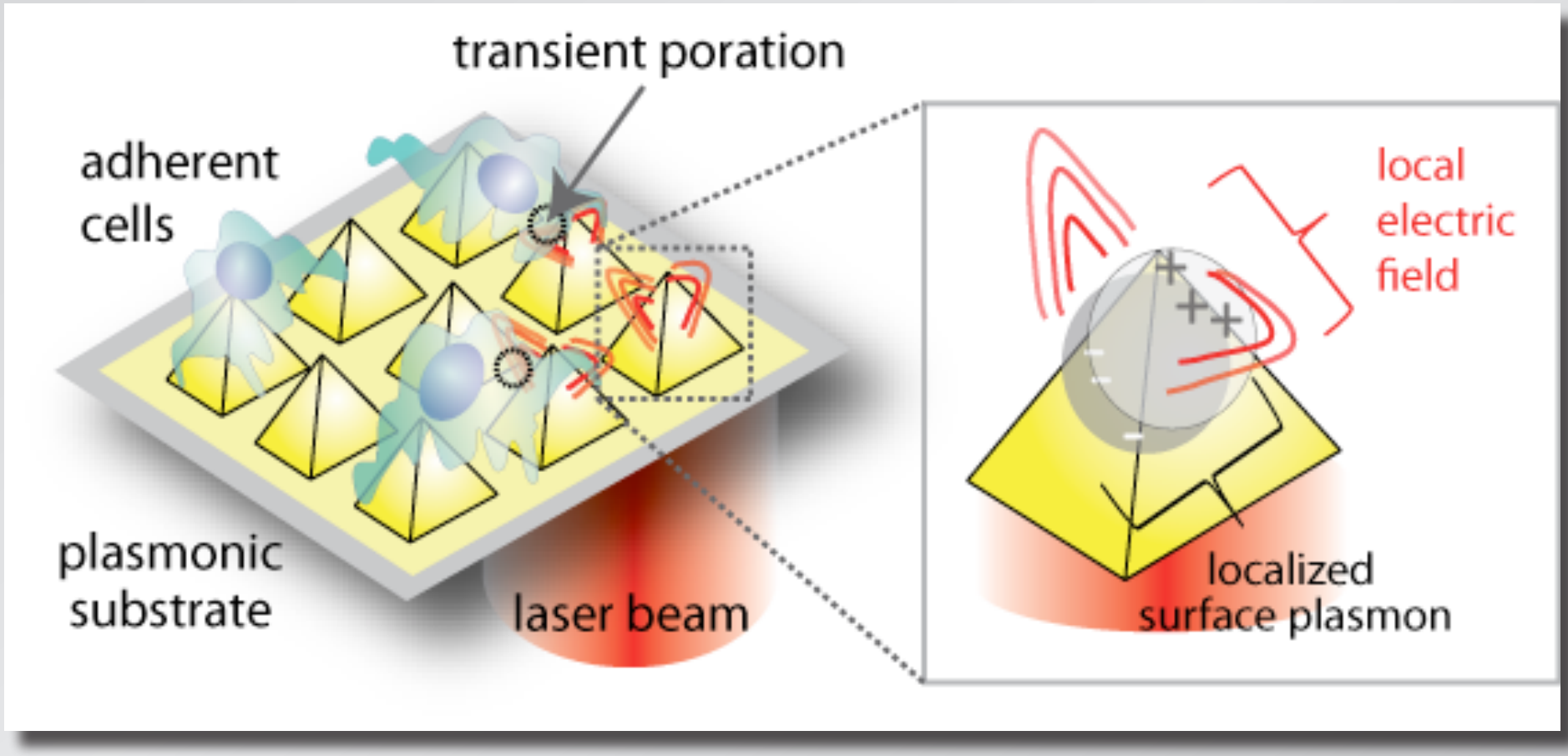
Cell transfection

	Toxicity	Efficiency	Throughput	Specificity
Goal	VL	H	H	L
Naked DNA	VL	L	H	L
Polymer/lipid	M	M	H	H
Viral transfection	M	H	H	H
Electroporation	H	H	H	L
Laser poration	VL	H	VL	L

Cell transfection

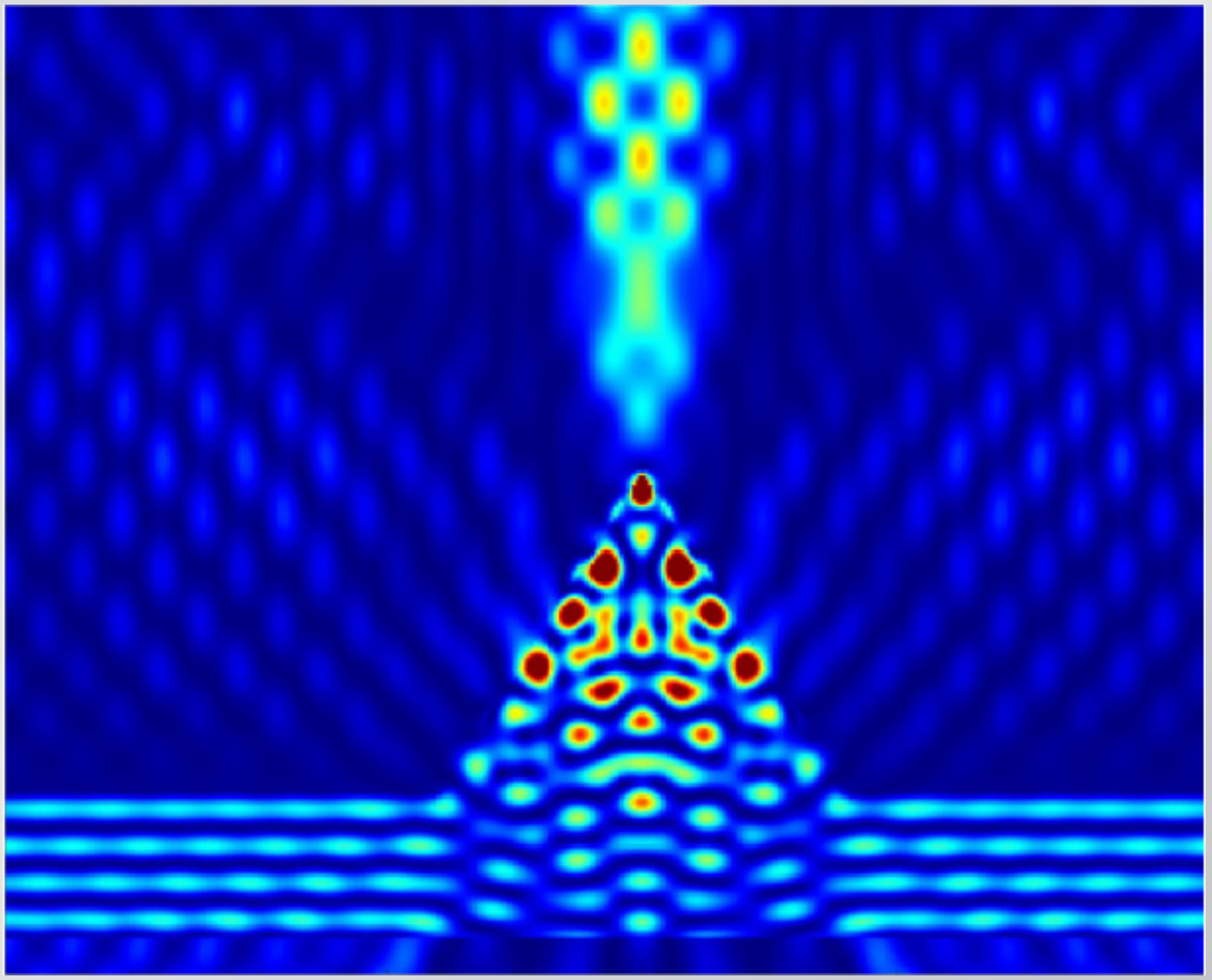
use structured plasmonic substrate

Cell transfection

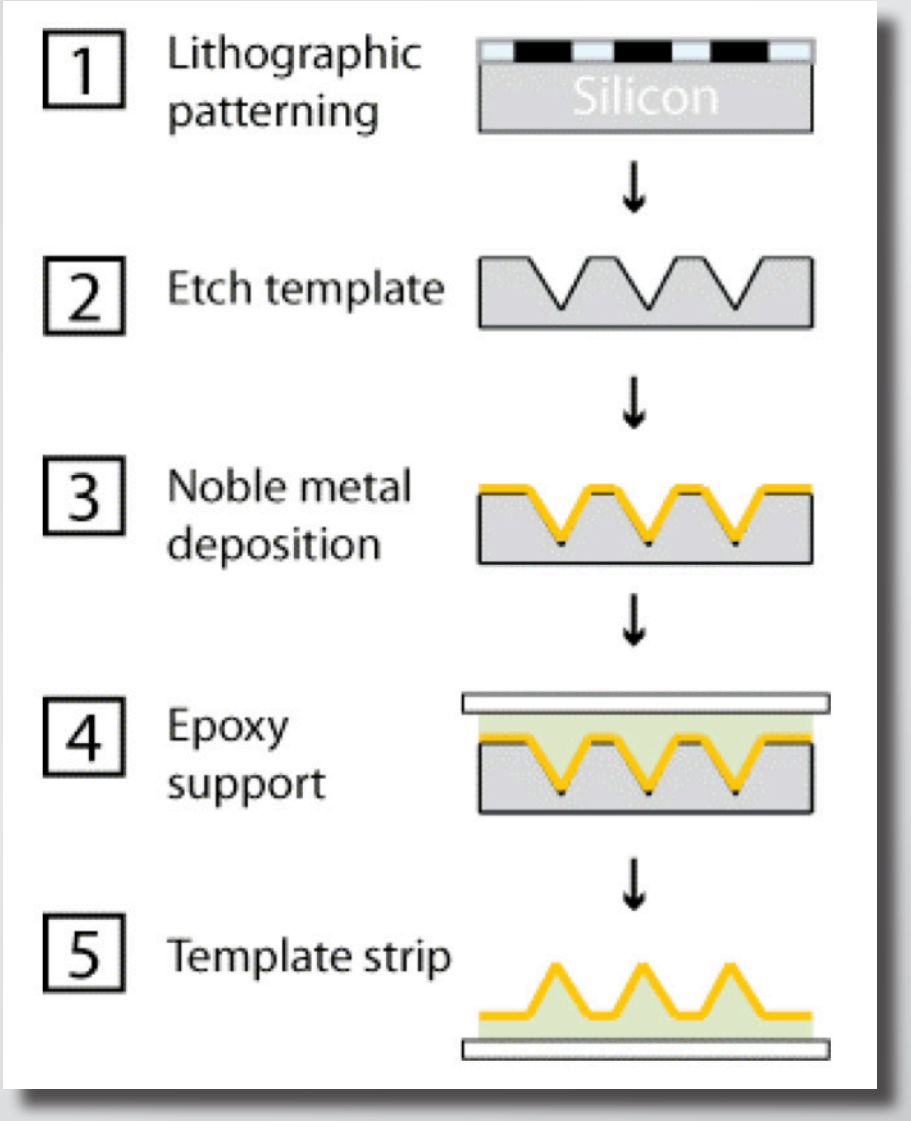


Cell transfection

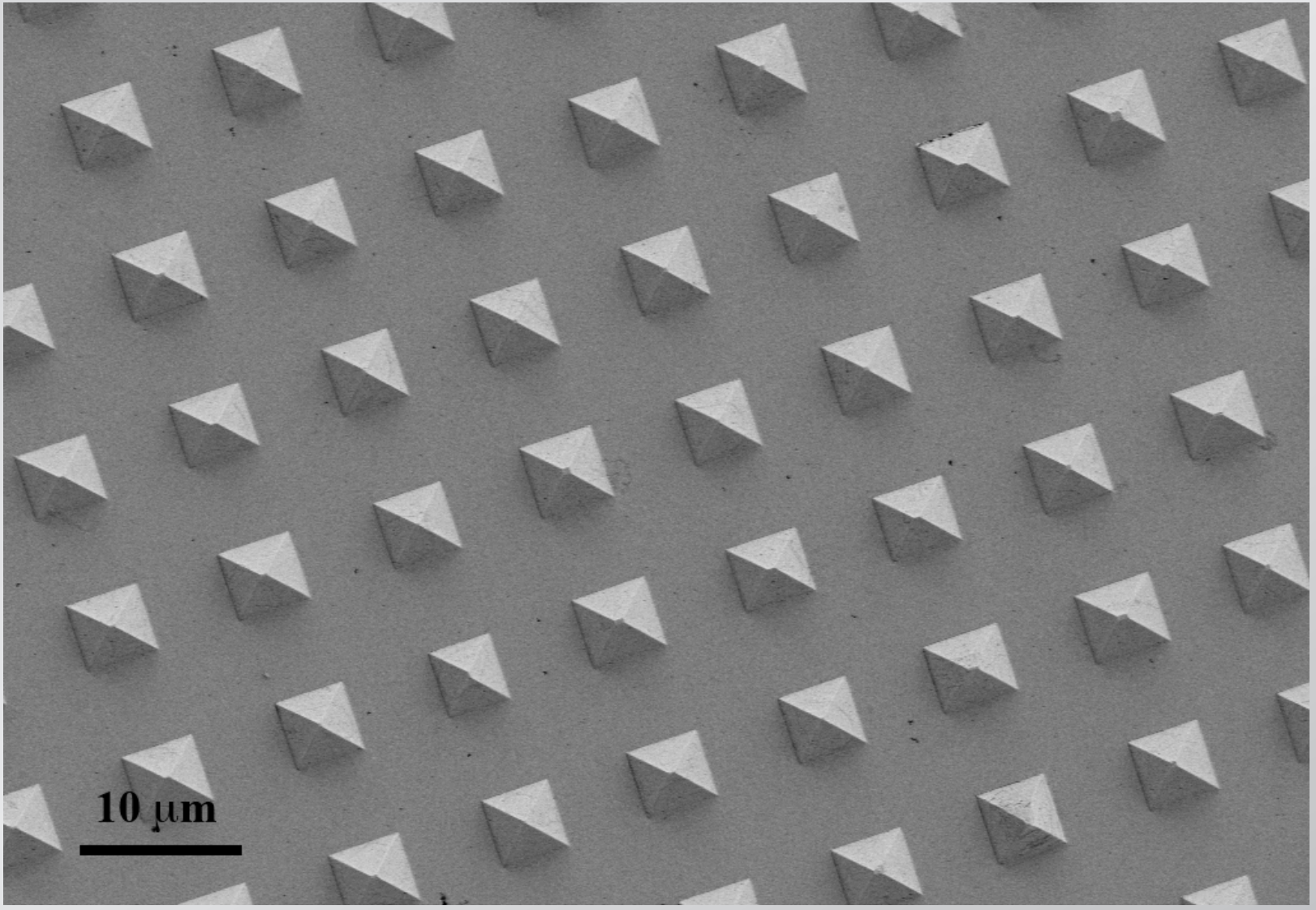
field enhancement at tip



Cell transfection

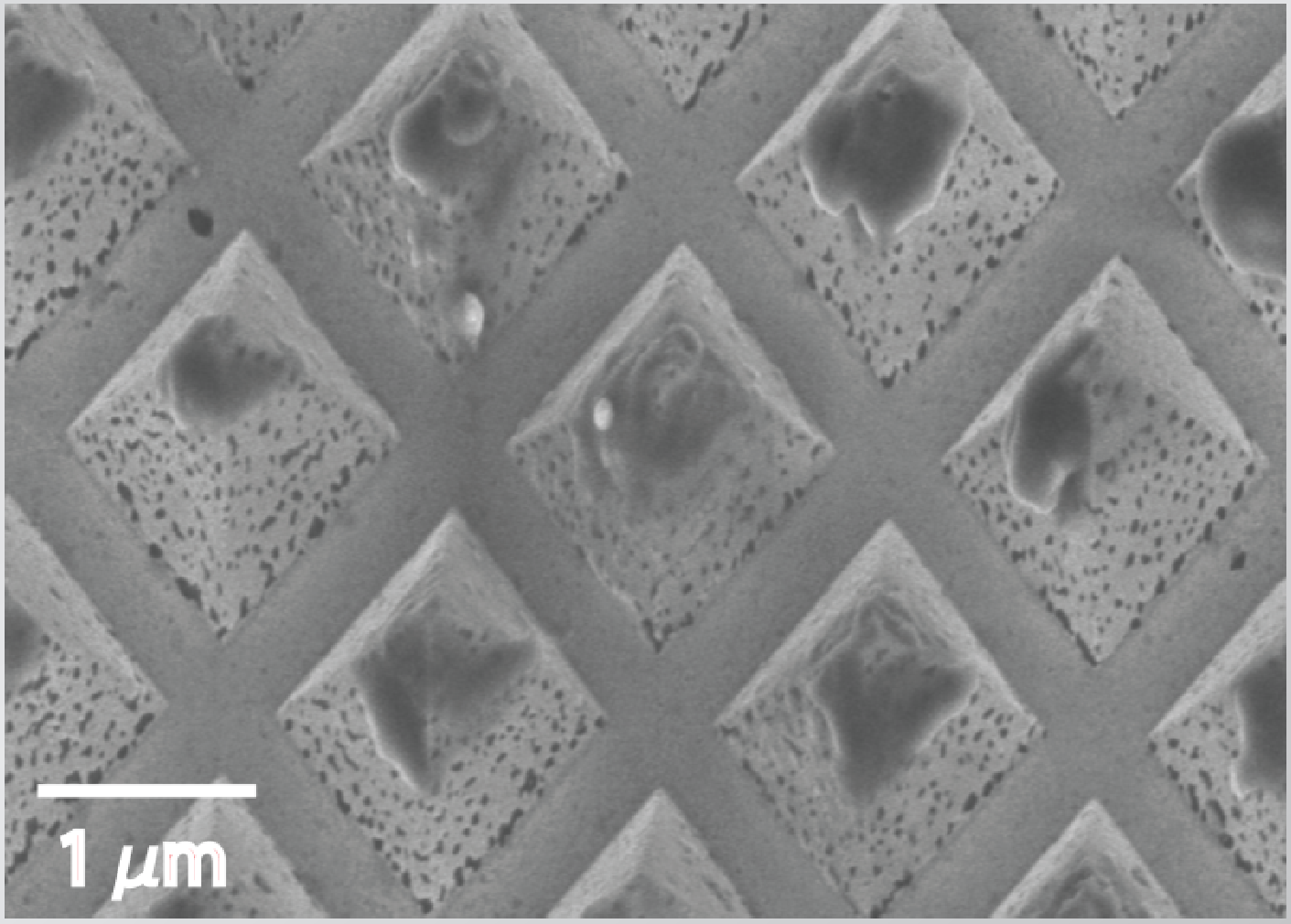


Cell transfection



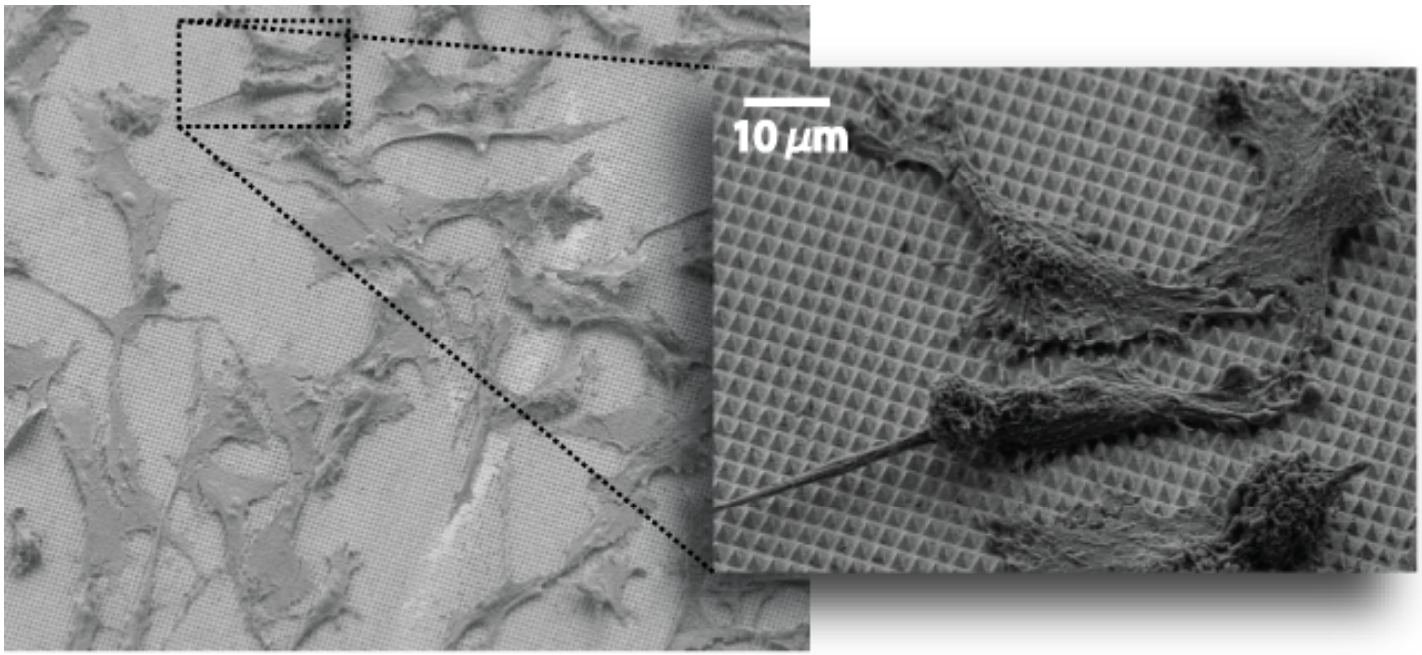
Cell transfection

two-photon polymerization enhancement



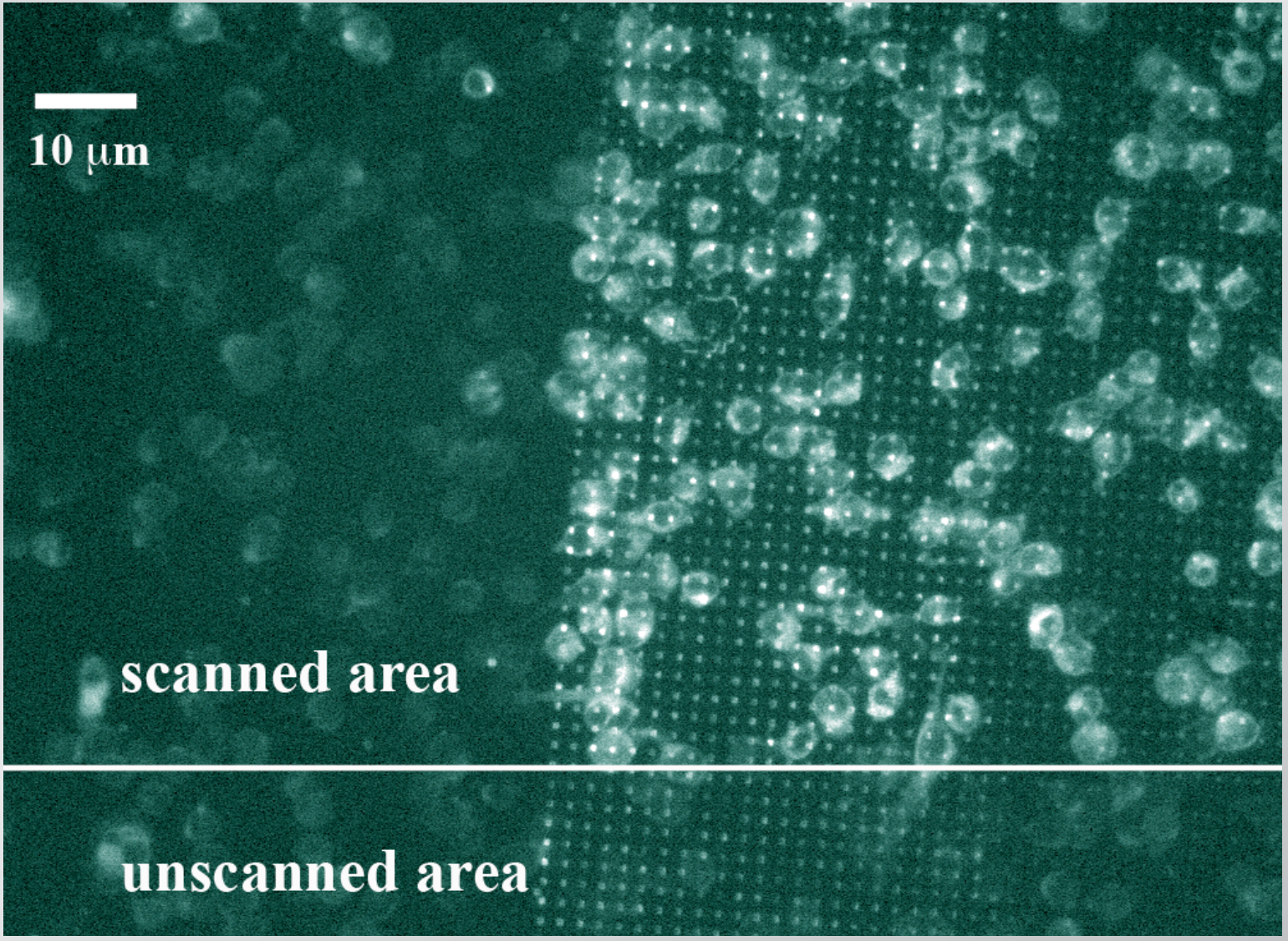
Cell transfection

attachment of TE cells on pyramid arrays

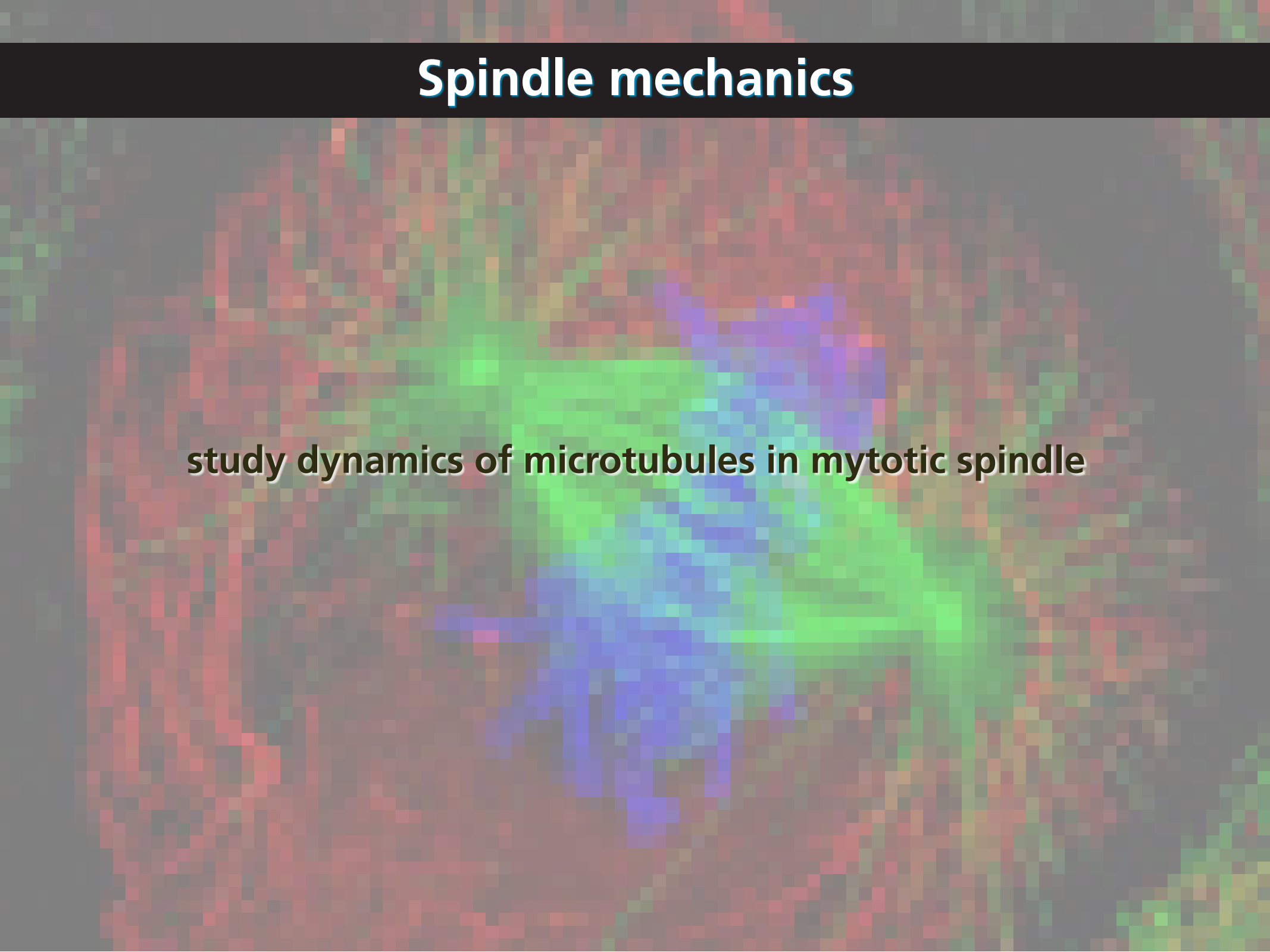


Cell transfection

only exposed cells on pyramids take up dye

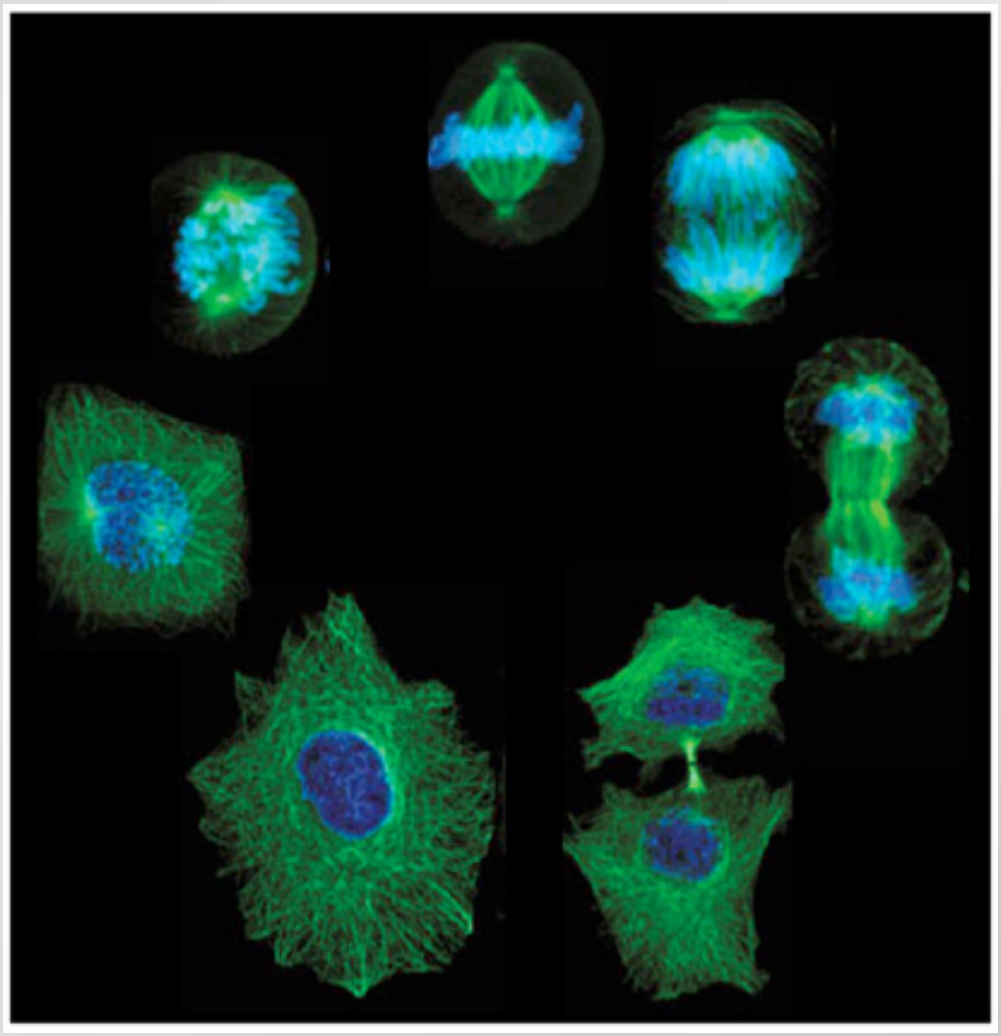


Spindle mechanics



Spindle mechanics

spindle forms during cell division



Spindle mechanics

spindle forms during cell division

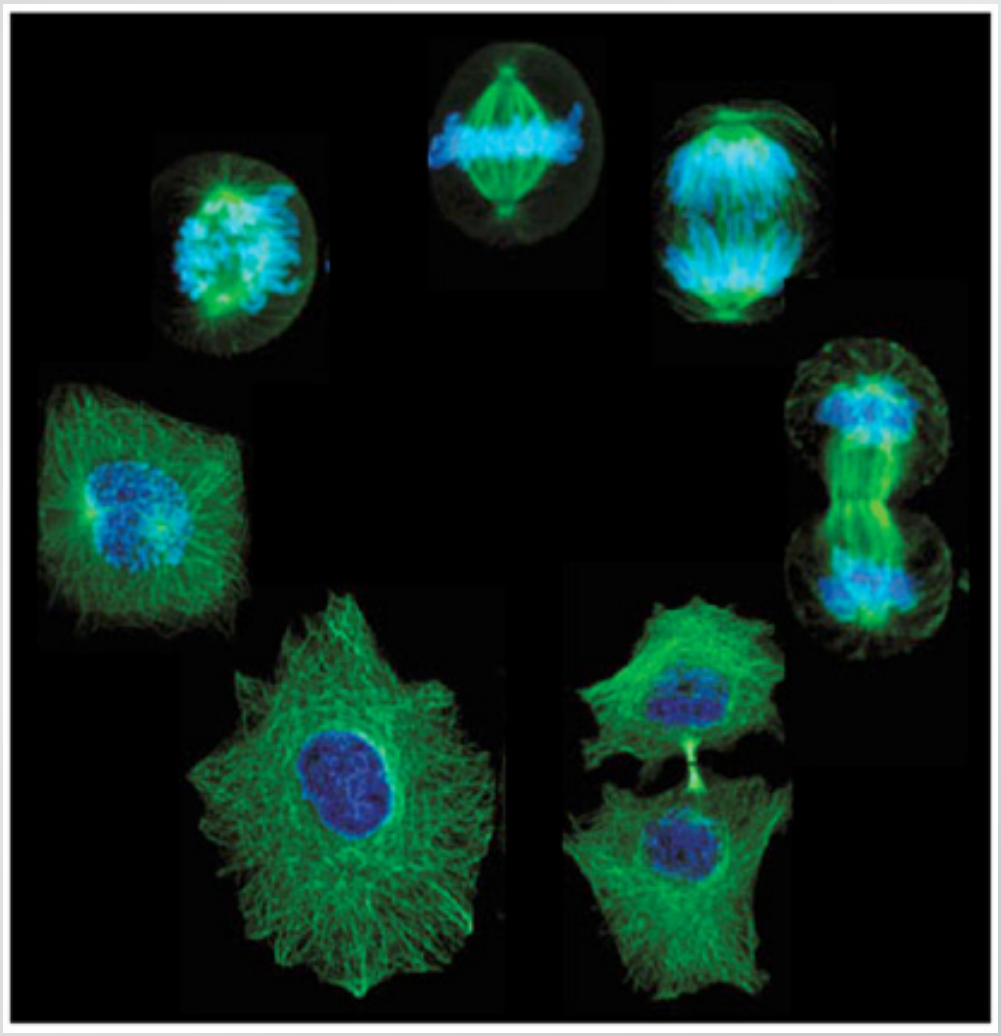
metaphase

prophase

anaphase

telophase

interphase

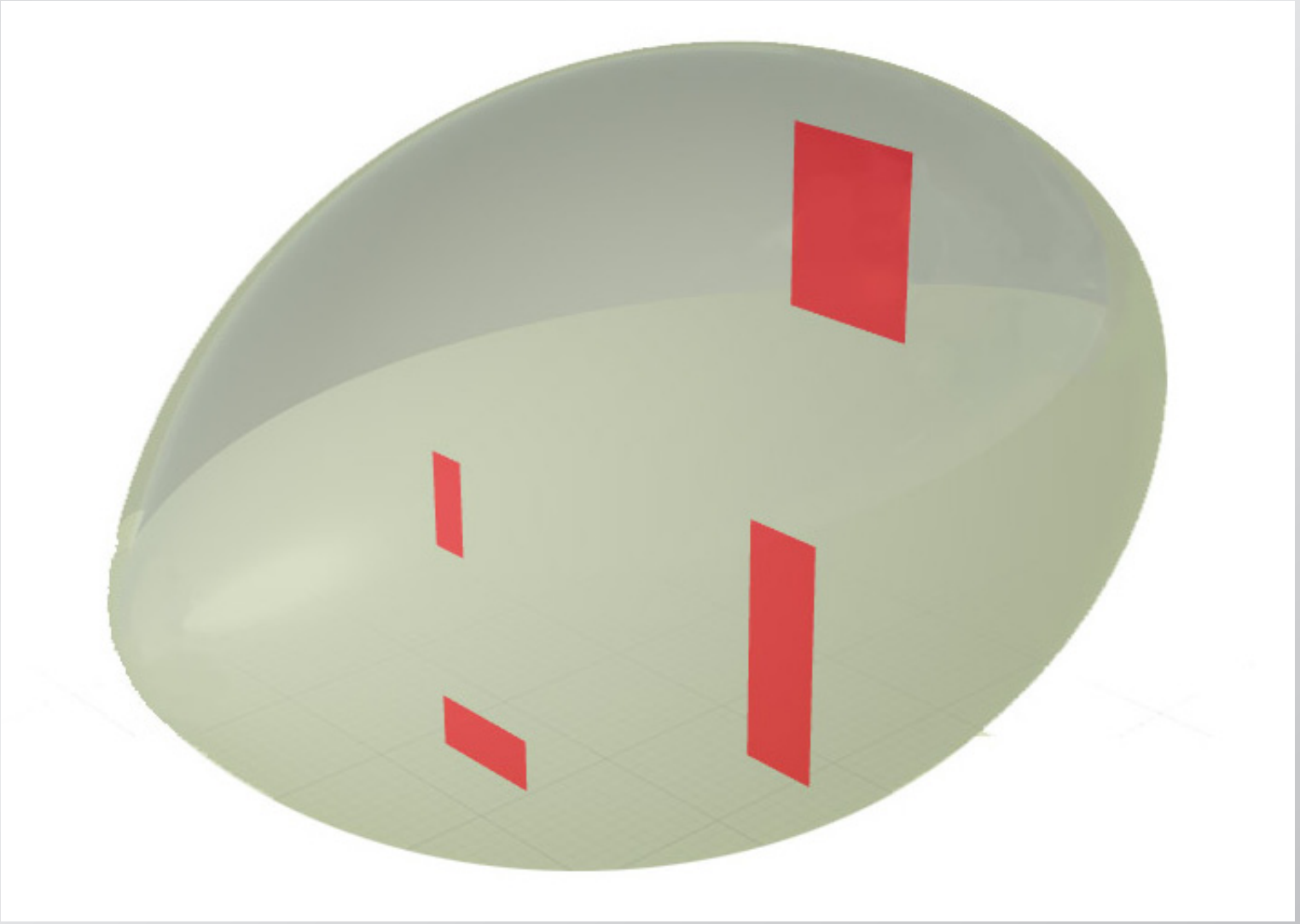


Spindle mechanics

can we determine polarity and length of microtubules?

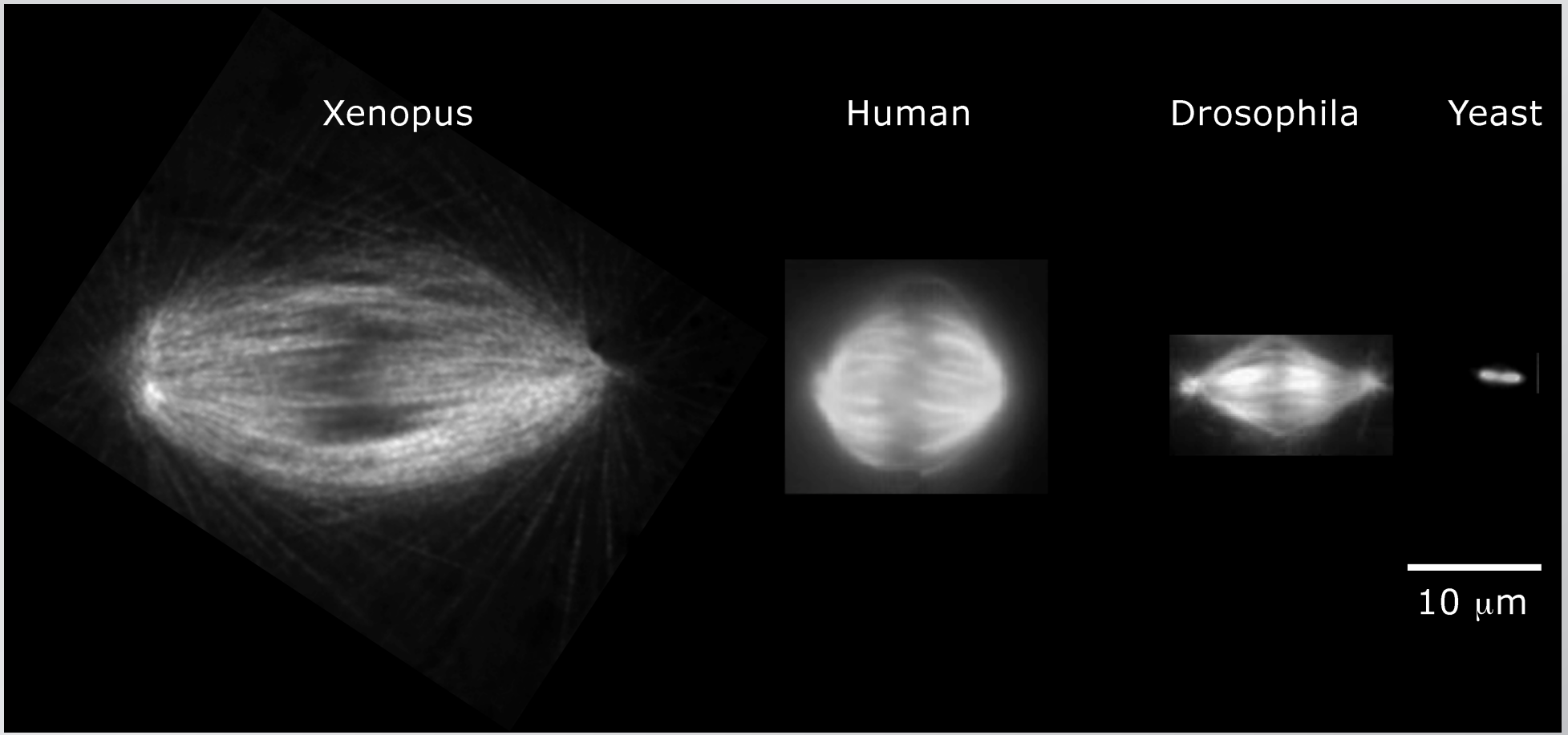
Spindle mechanics

observe depolymerization dynamics after planar cut(s)



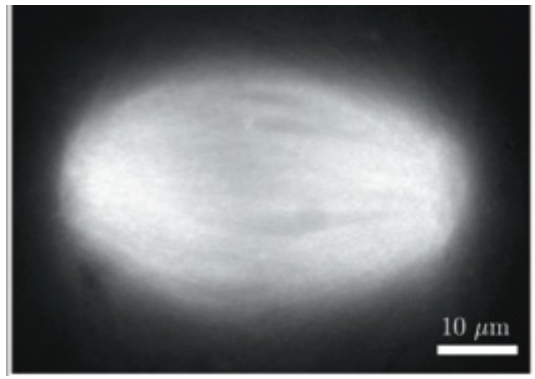
Spindle mechanics

spindles from frog egg extract



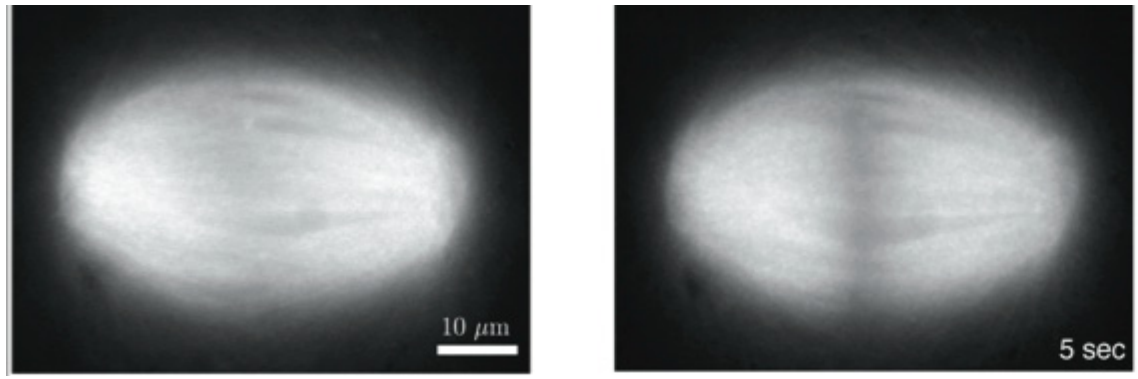
Spindle mechanics

direct observation of depolymerization wave



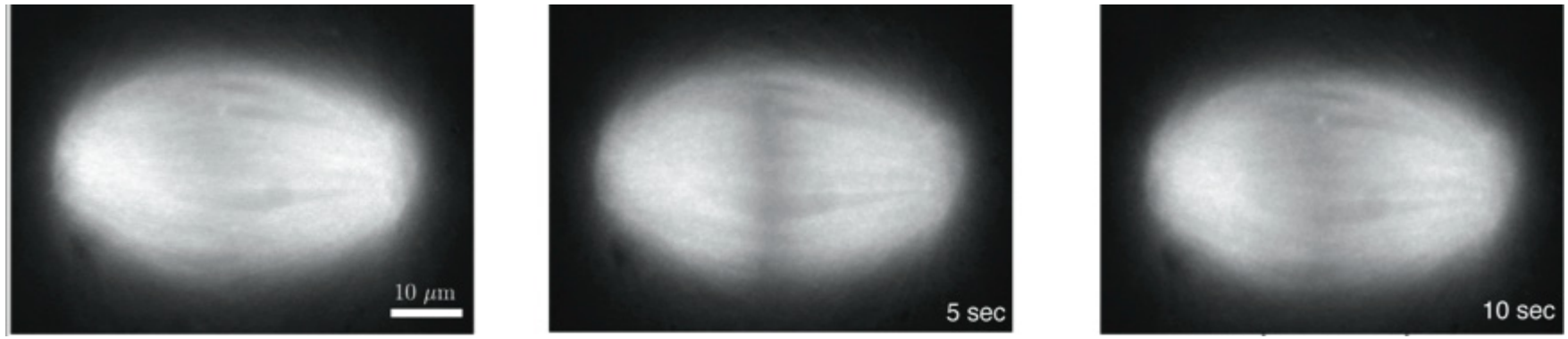
Spindle mechanics

direct observation of depolymerization wave



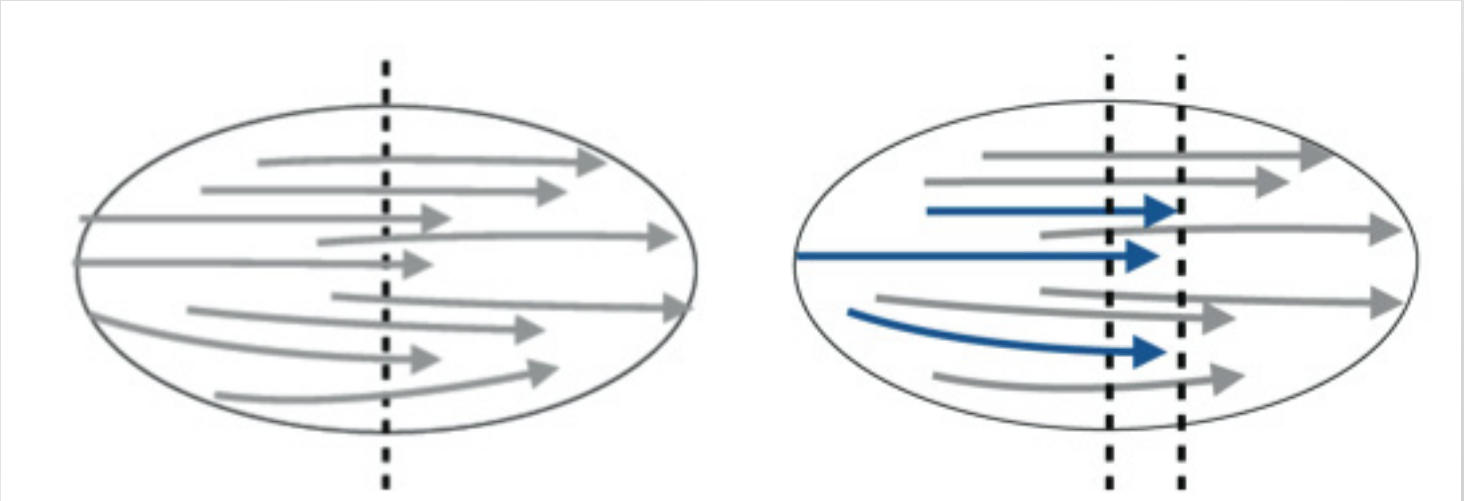
Spindle mechanics

direct observation of depolymerization wave



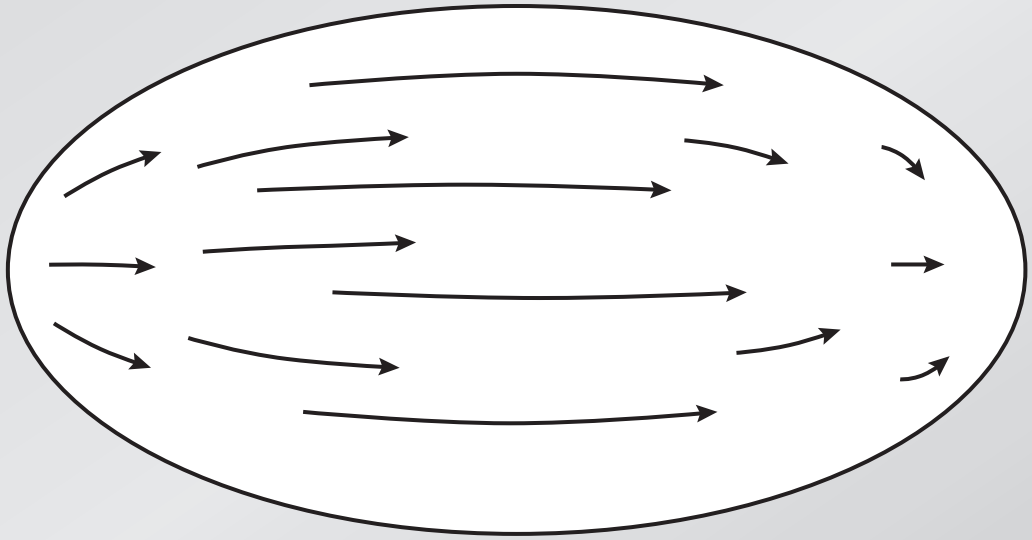
Spindle mechanics

double cuts provide information on mean length



Spindle mechanics

spindle organization

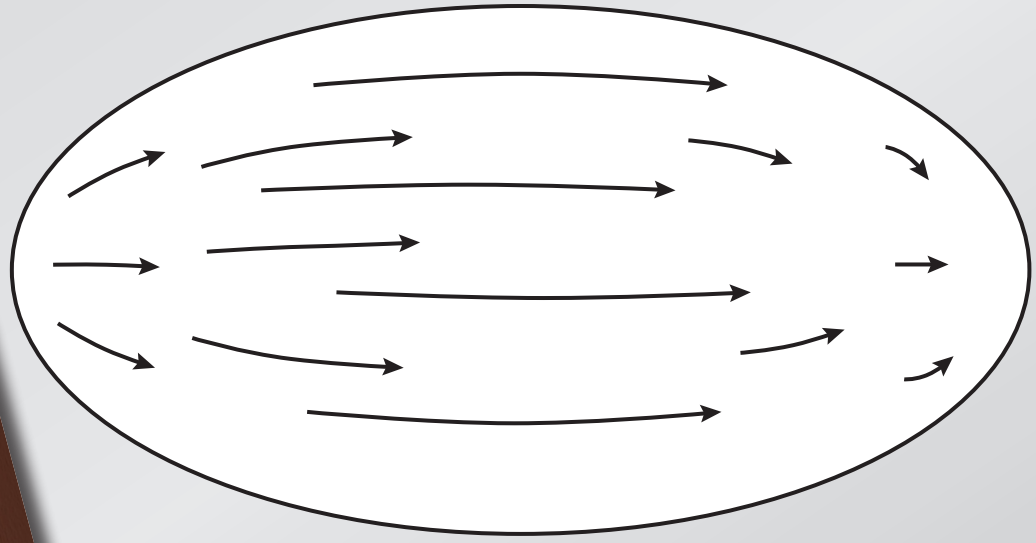


70% 50% 30%

polarity & length distributions varies across cell

Spindle mechanics

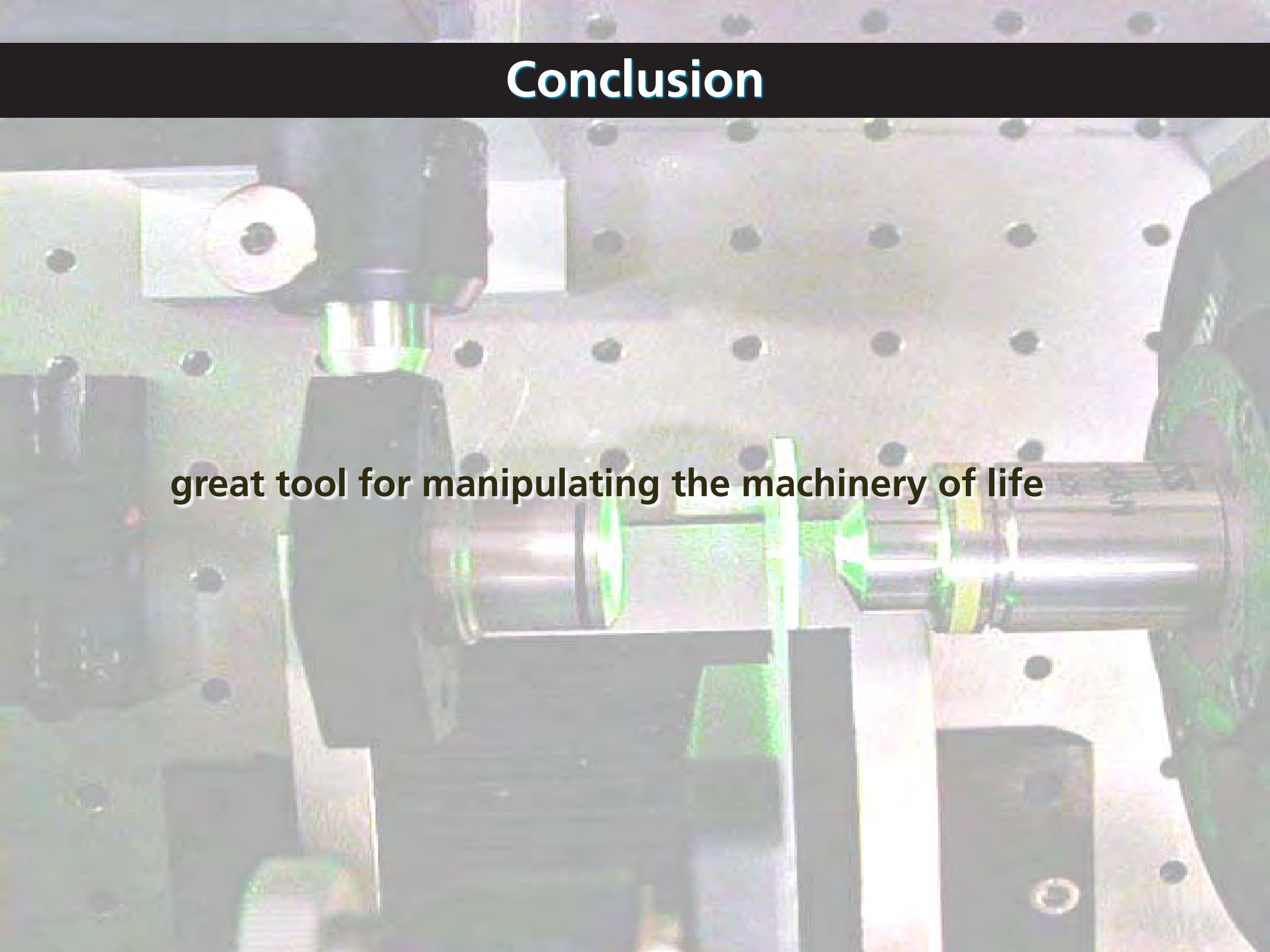
spindle organization



70% 50% 30%

length distributions varies across cell

Conclusion



great tool for manipulating the machinery of life

Funding:

National Science Foundation

for more information and a copy of this presentation:

http://mazur.harvard.edu

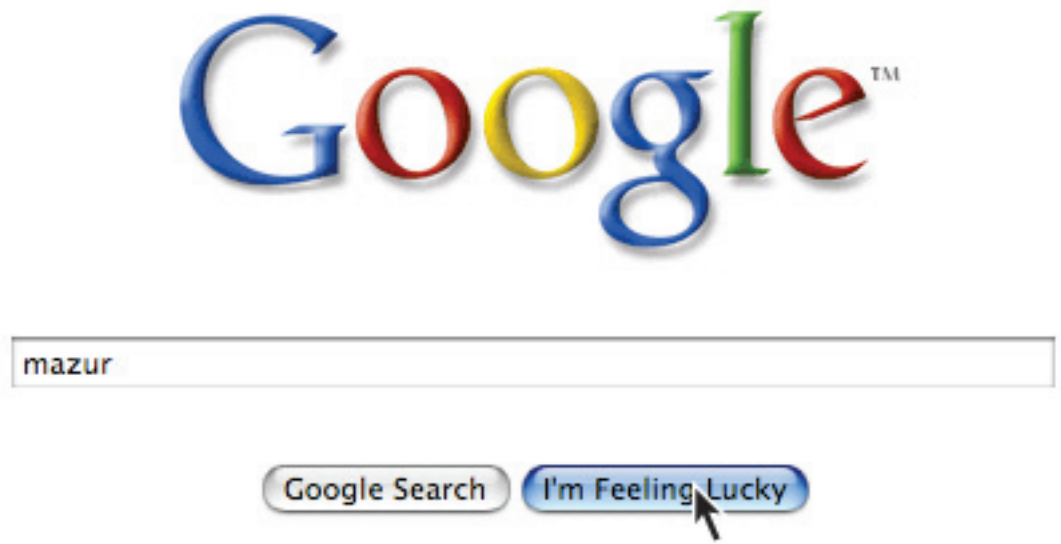
Follow me! **eric_mazur**

Google Search I'm Feeling Lucky

mazur

Google Search I'm Feeling Lucky


Google
mazur
Google Search
I'm Feeling Lucky


Google
mazur
Google Search
I'm Feeling Lucky

Funding:

National Science Foundation

for more information and a copy of this presentation:

http://mazur.harvard.edu

Follow me! **eric_mazur**