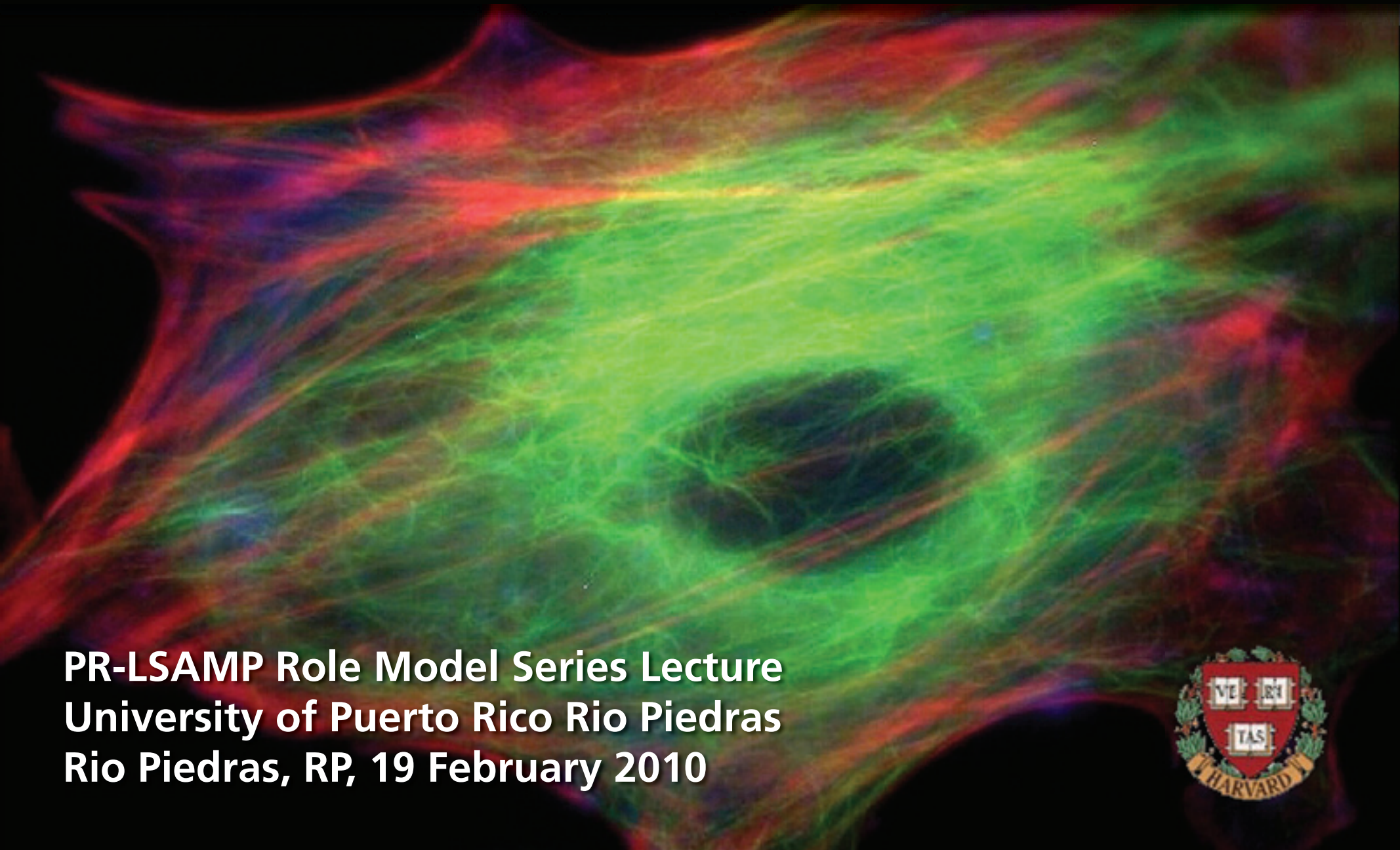
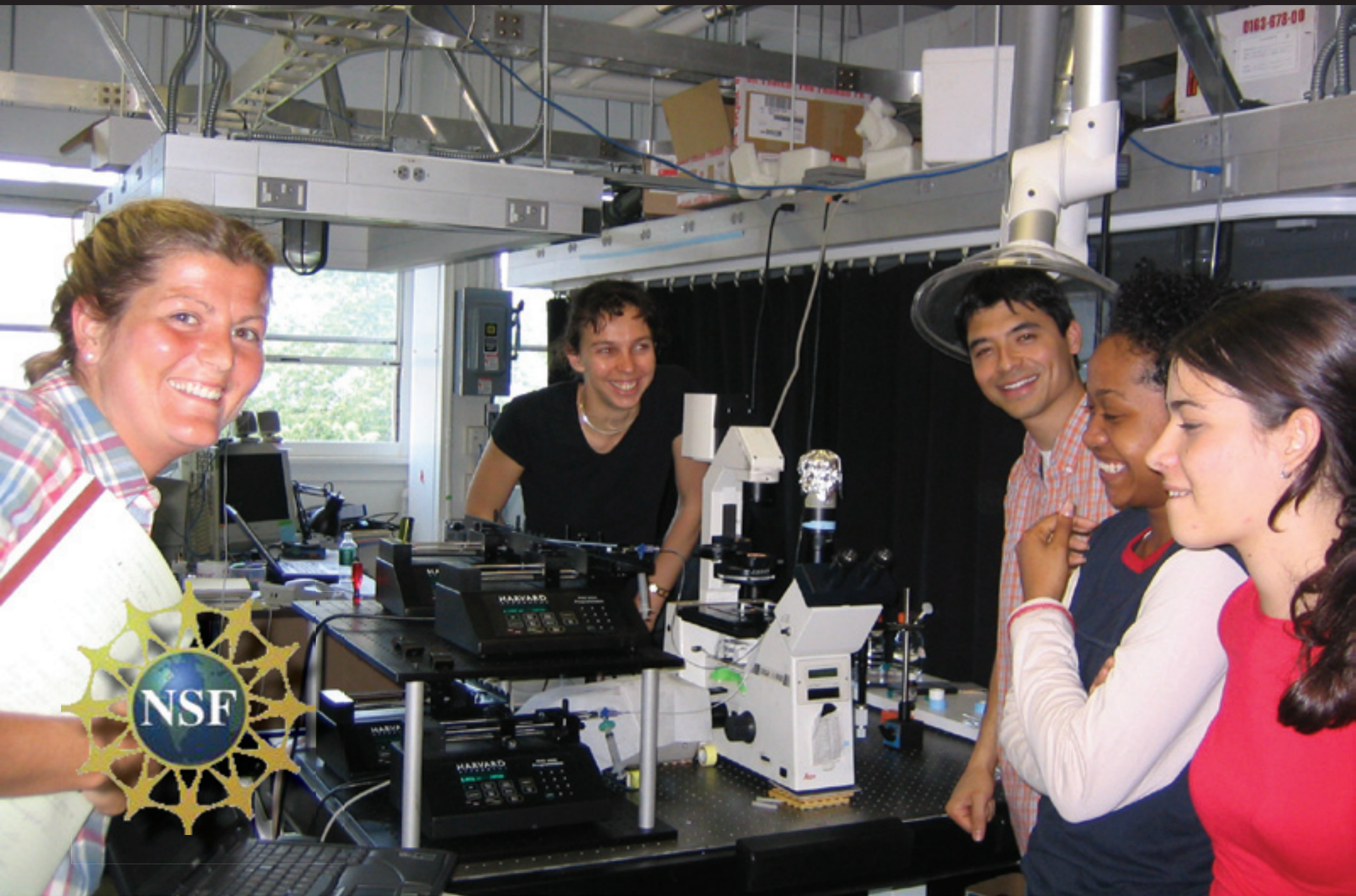
Nanosurgery with femtosecond lasers

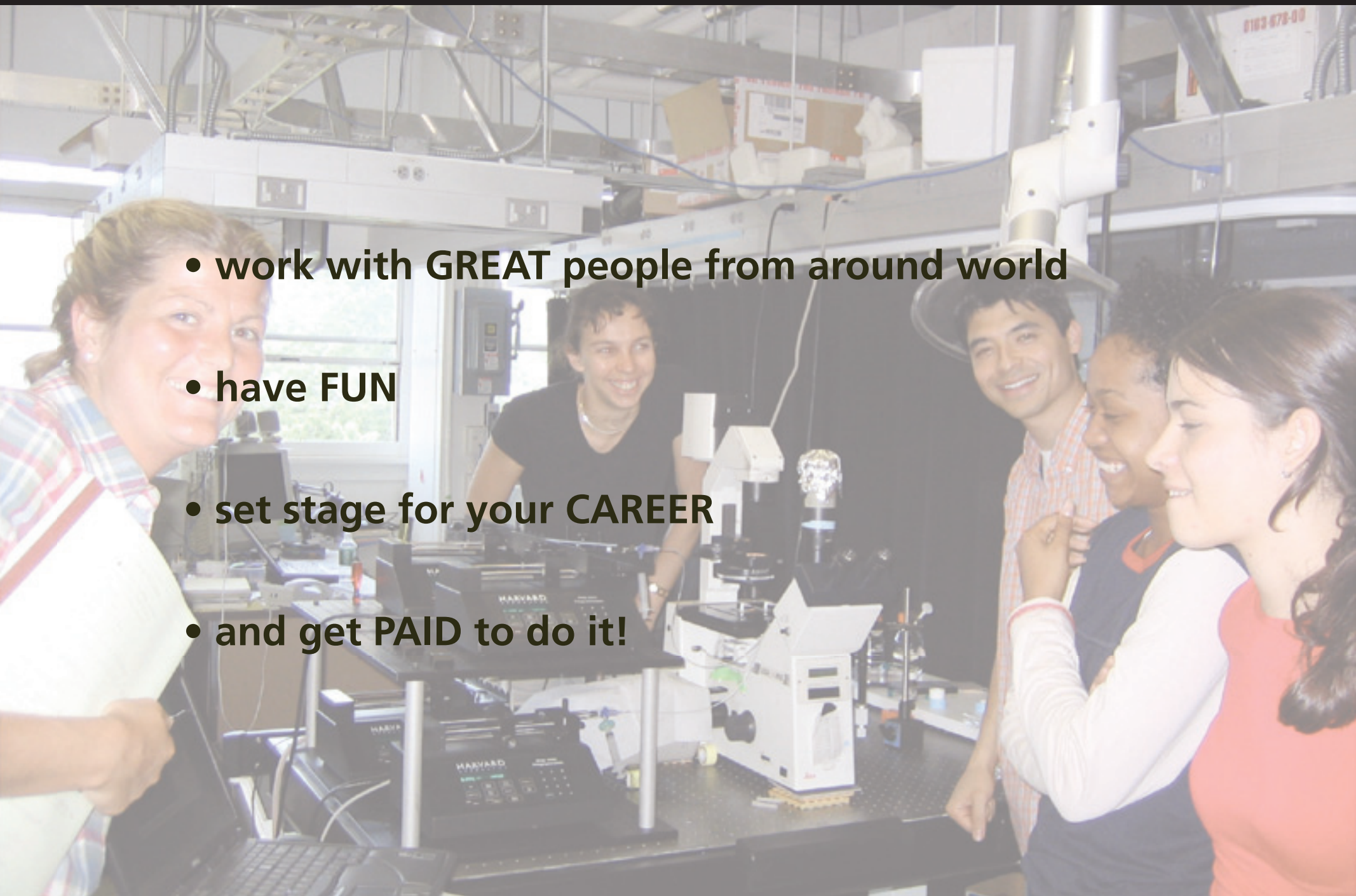


PR-LSAMP Role Model Series Lecture
University of Puerto Rico Rio Piedras
Rio Piedras, RP, 19 February 2010

REU @Harvard



REU @Harvard



- work with GREAT people from around world
- have FUN
- set stage for your CAREER
- and get PAID to do it!

REU @Harvard

REU @Harvard

Eric Martin

REU '09

University of California Santa Barbara

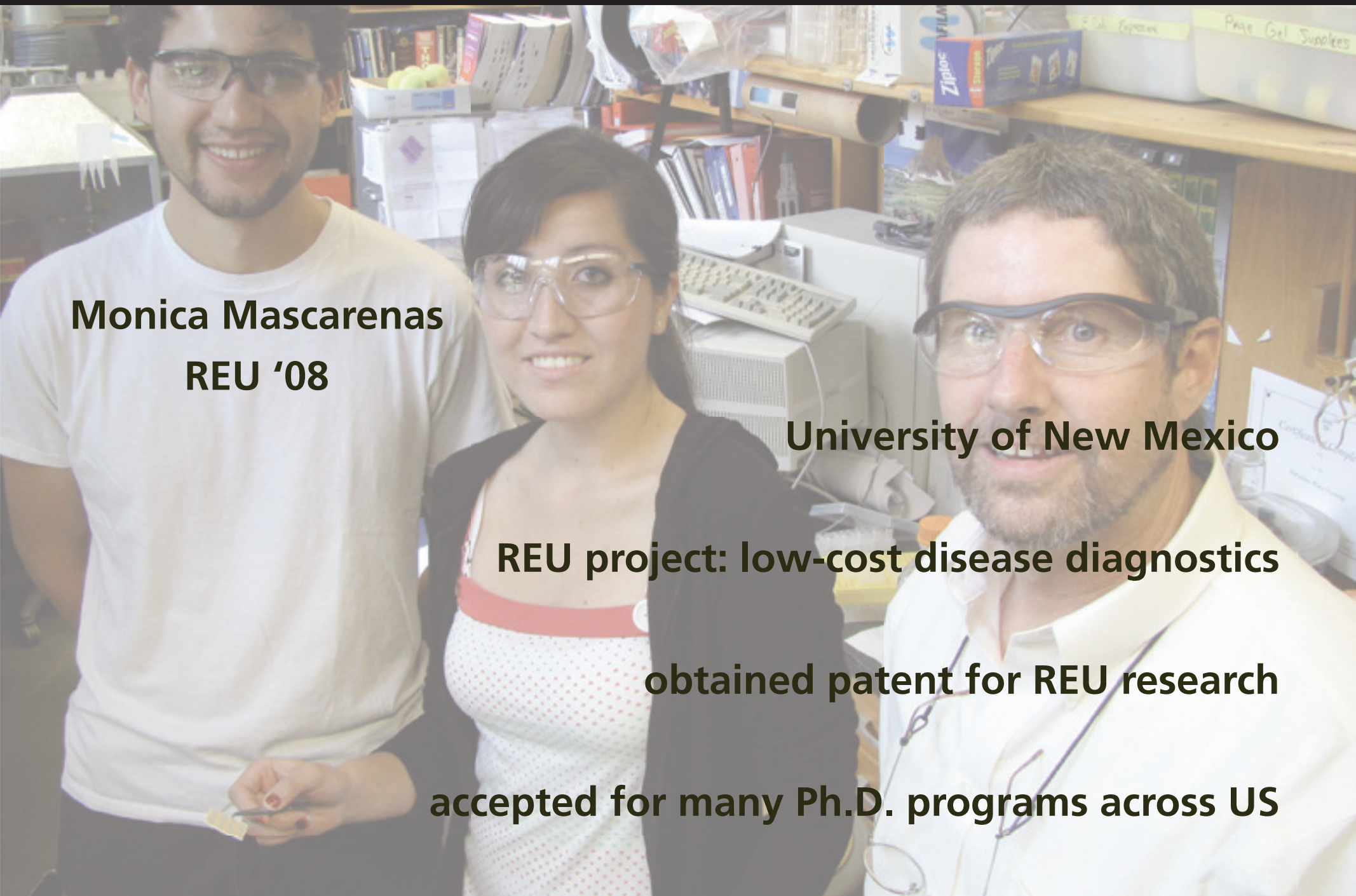
REU project: holographic microscopy

Steven Chu Award for REU research

accepted for Ph.D. program at Harvard

REU @Harvard

REU @Harvard



Monica Mascarenas

REU '08

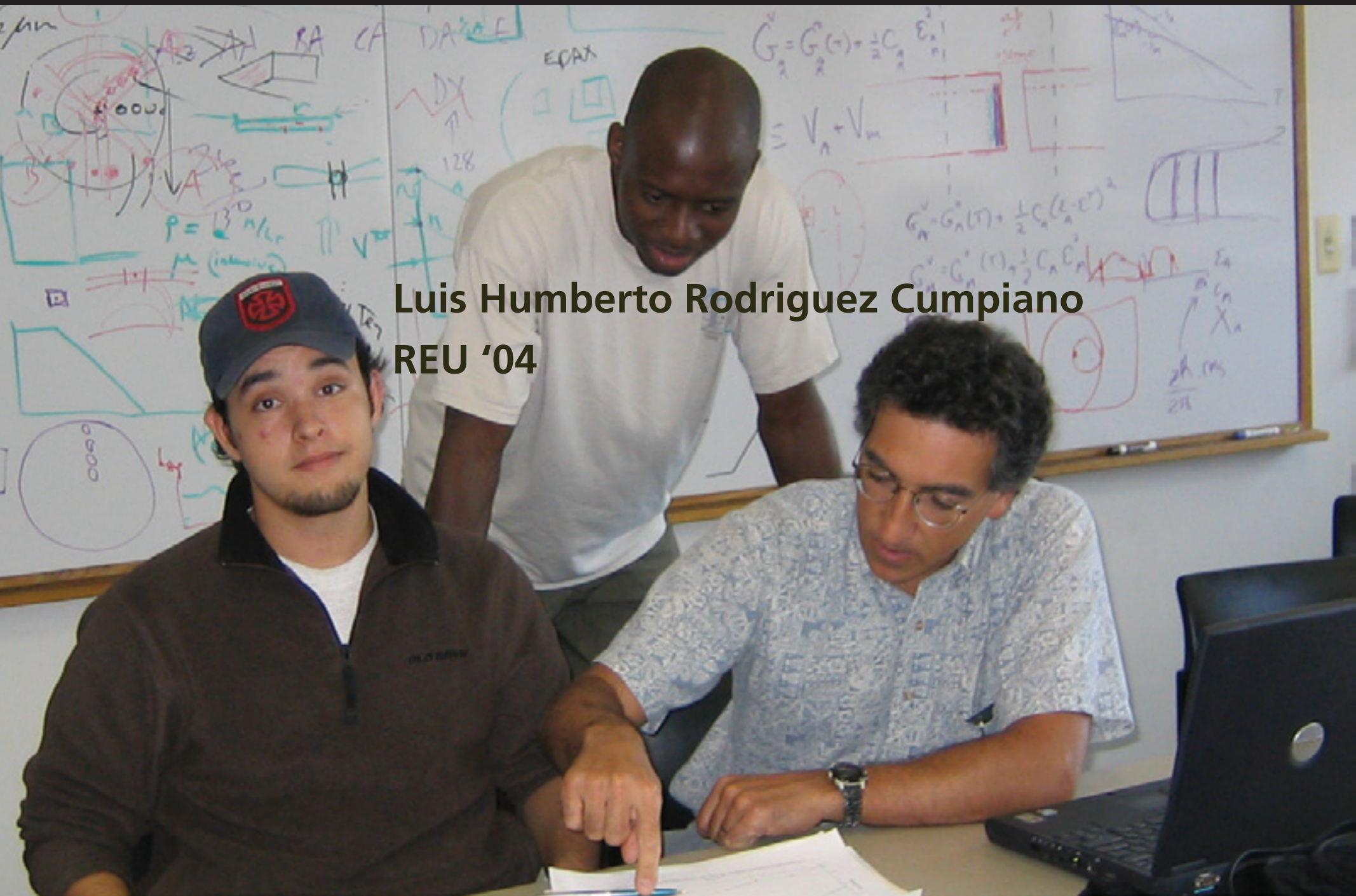
University of New Mexico

REU project: low-cost disease diagnostics

obtained patent for REU research

accepted for many Ph.D. programs across US

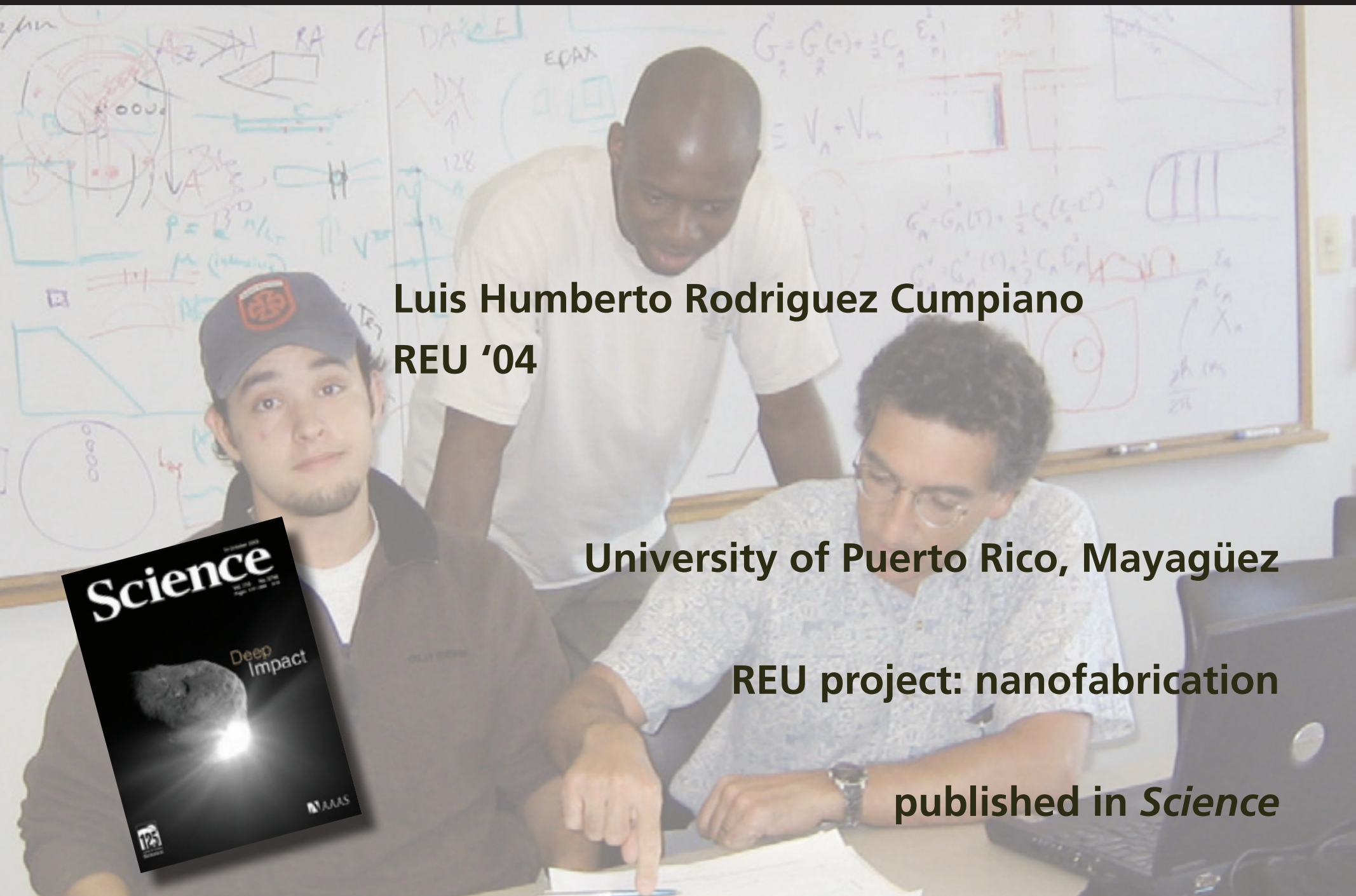
REU @Harvard



Luis Humberto Rodriguez Cumpiano

REU ‘04

REU @Harvard



Luis Humberto Rodriguez Cumpiano

REU '04

University of Puerto Rico, Mayagüez

REU project: nanofabrication

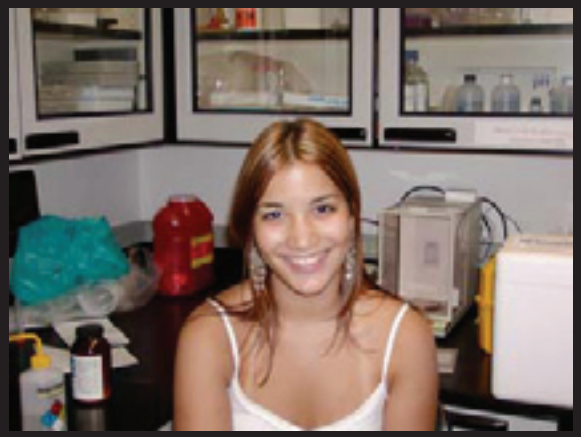
published in *Science*


YOU COULD BE ONE OF THEM!

Some Puerto Rican REUs from my group

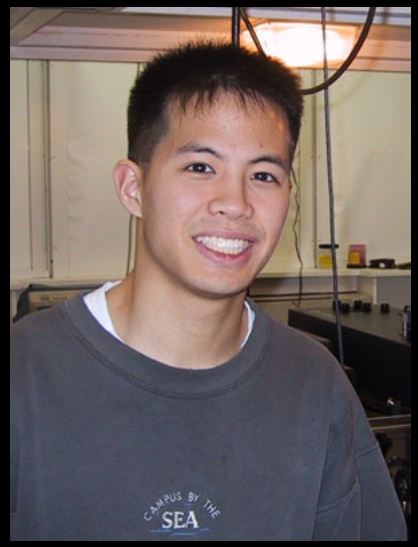
Jose Garcia
REU 1999
Femtosecond laser micromachining
Naval Research Labs Washington

Luis Valentin
REU 2000
Femtosecond laser micromachining
UPR Rio Piedras Ph.D. student



Niviann Blondet
REU 2004
Femtosecond laser nanosurgery
UPR School of Medicine

Iva Maxwell



Sam Chung

Valeria Nuzzo

Alexander Heisterkamp

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

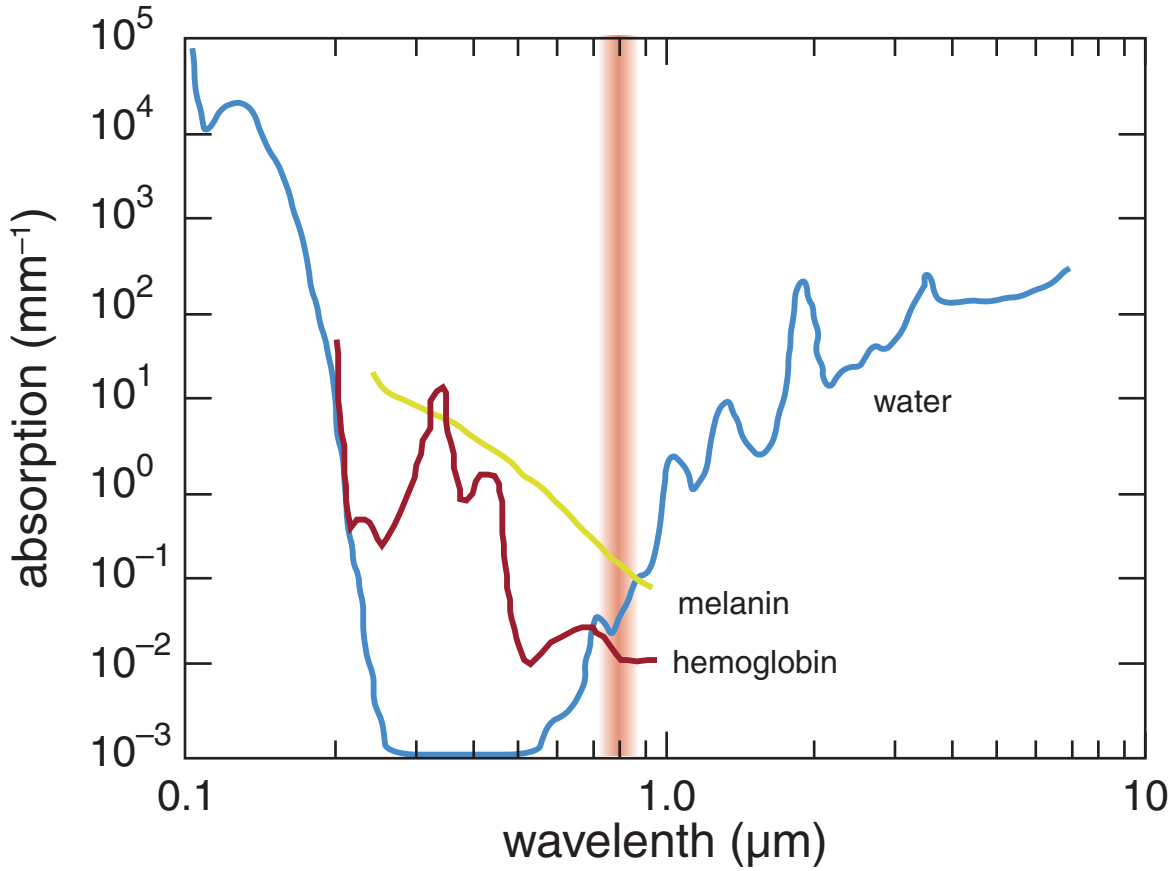
Prof. Don Ingber (Harvard Medical School)
Prof. Aravi Samuel (Harvard)
Prof. Chris Gabel (Boston University)
Dr. Damon Clark (Harvard University)
Prof. J.M. Underwood (UMass Worcester)
Prof. J.A. Nickerson (UMass Worcester)
Prof. Philip LeDuc (Carnegie Mellon)
Prof. Sanjay Kumar (UC Berkeley)

Introduction

why use femtosecond pulses?

Introduction

tissue is nearly transparent at 800 nm

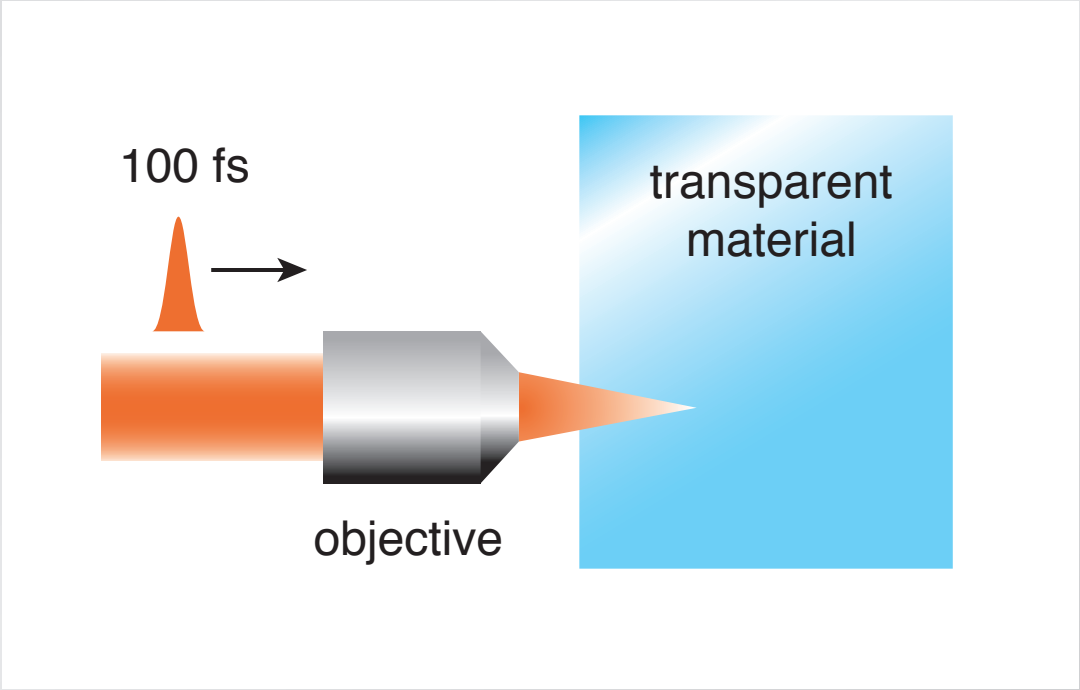


Outline

- femtosecond materials interactions
- subcellular surgery
- nanoneurosurgery

Femtosecond materials interactions

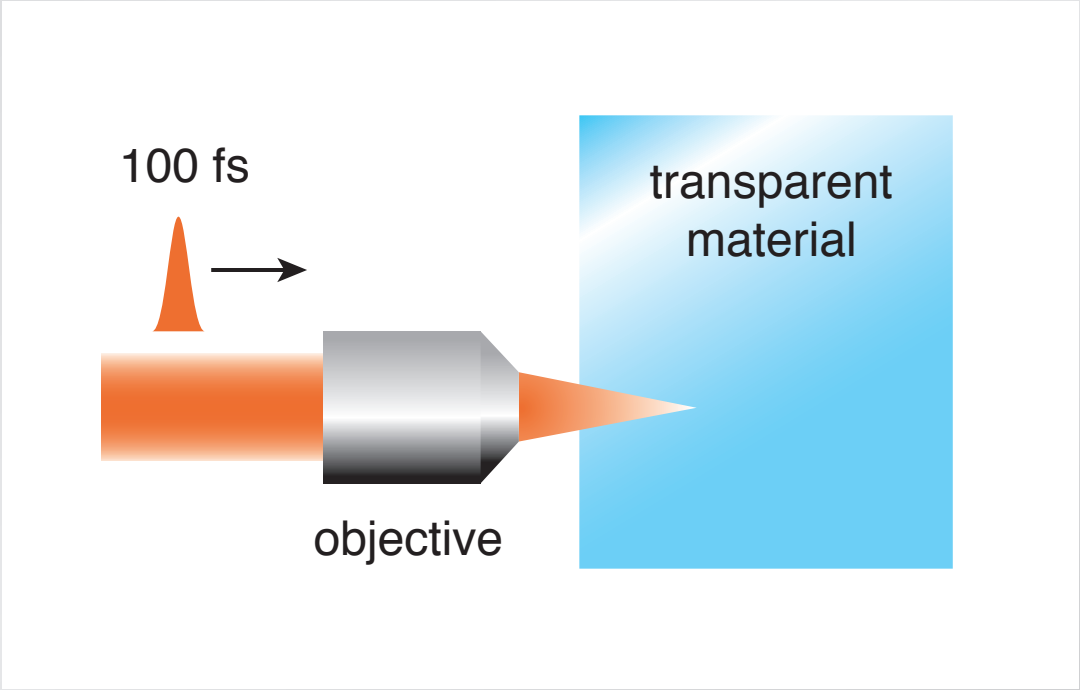
focus laser beam inside material



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

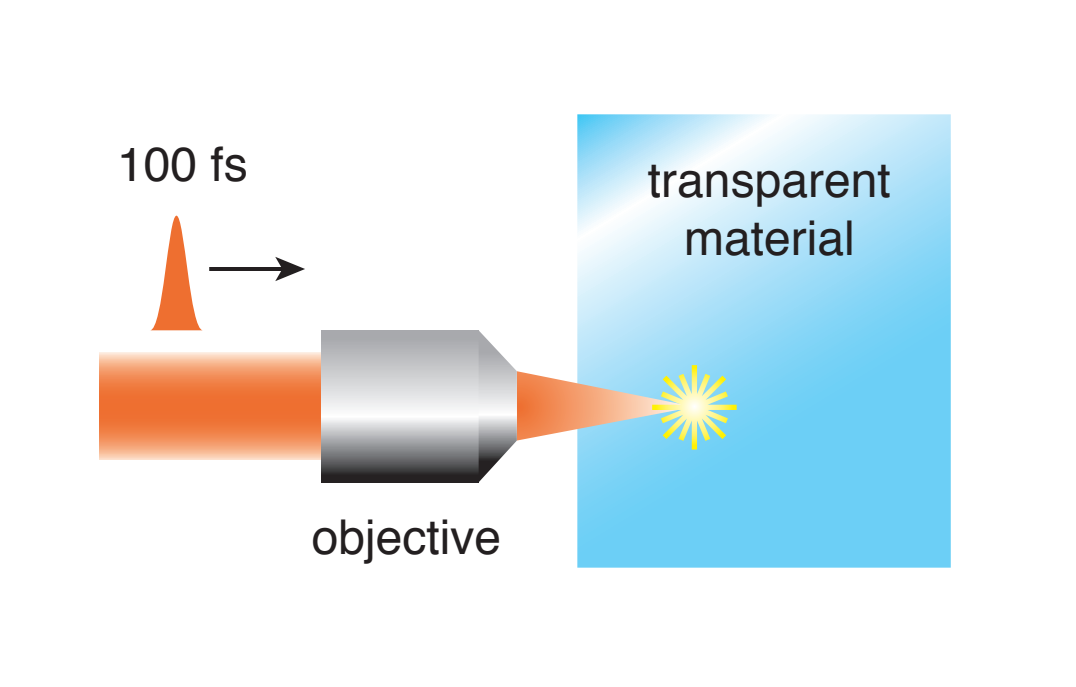
Femtosecond materials interactions

high intensity at focus…



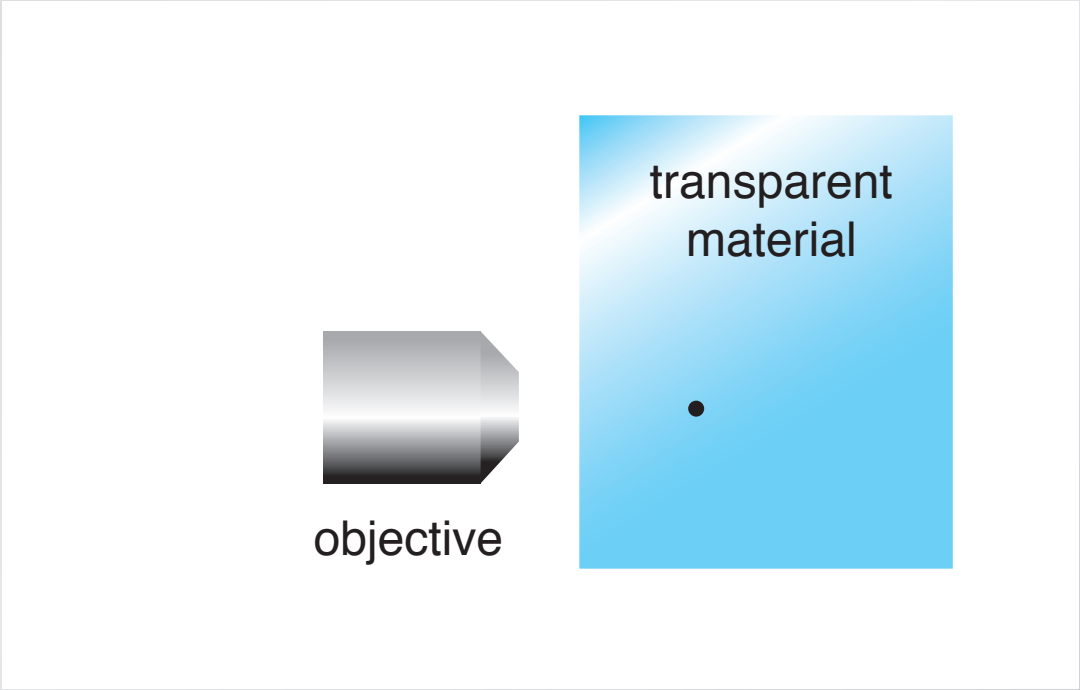
Femtosecond materials interactions

…causes nonlinear ionization…

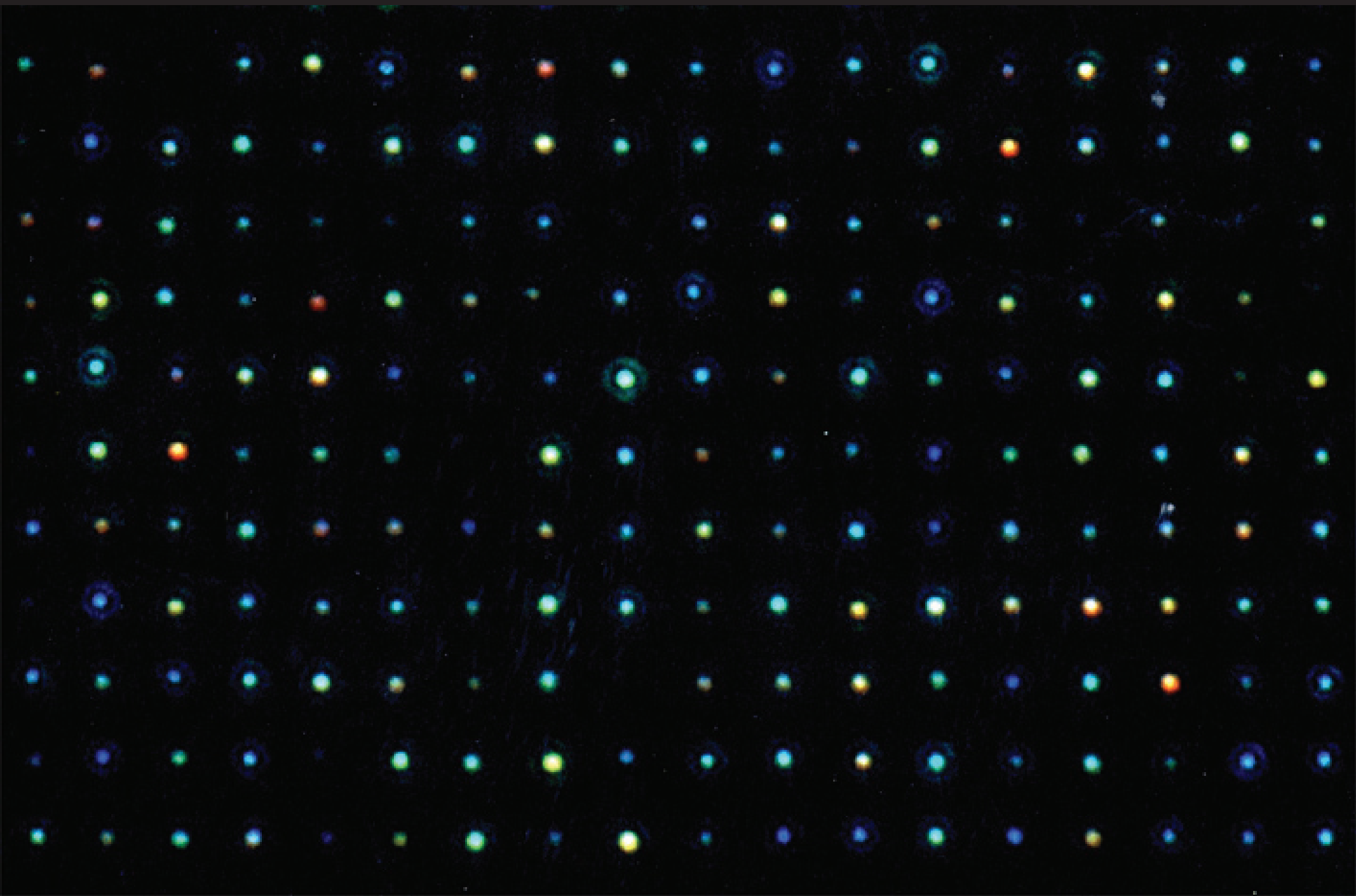


Femtosecond materials interactions

and ‘microexplosion’ causes microscopic damage…



Femtosecond materials interactions

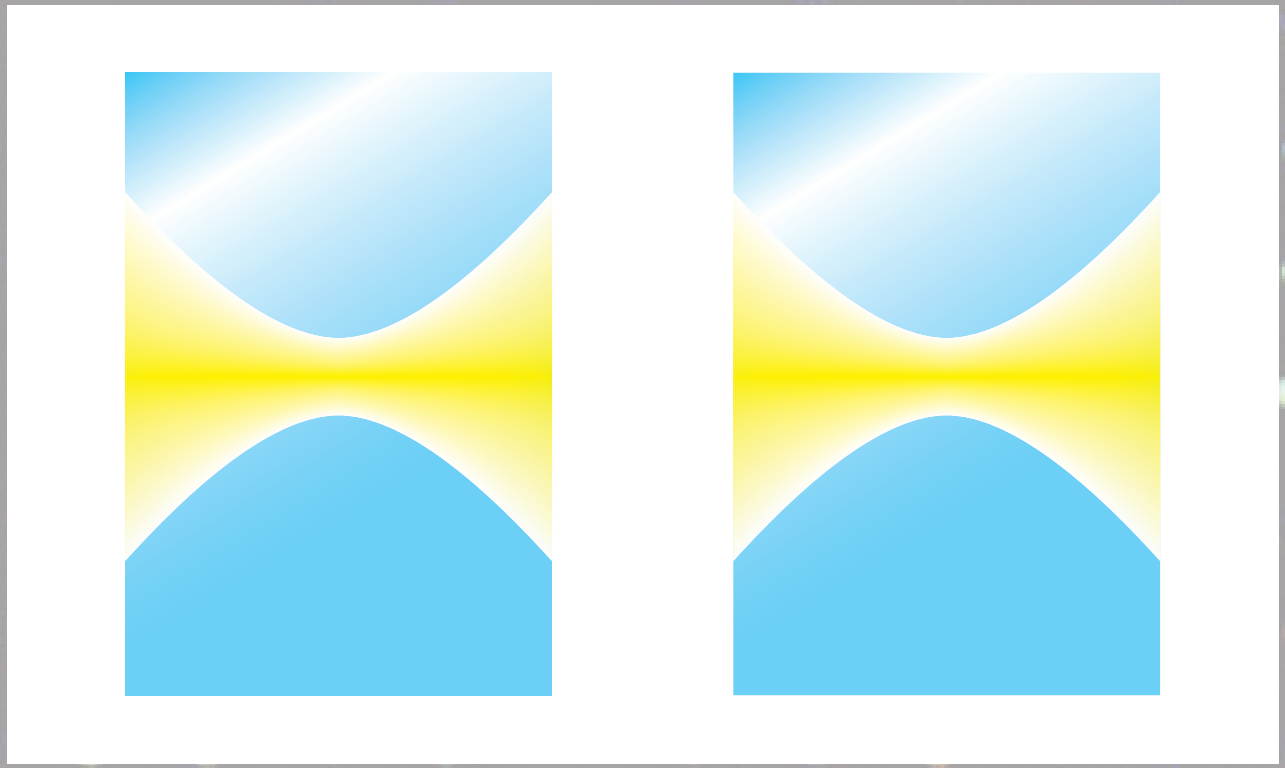


Femtosecond materials interactions

photon energy < bandgap $\longrightarrow$ nonlinear interaction

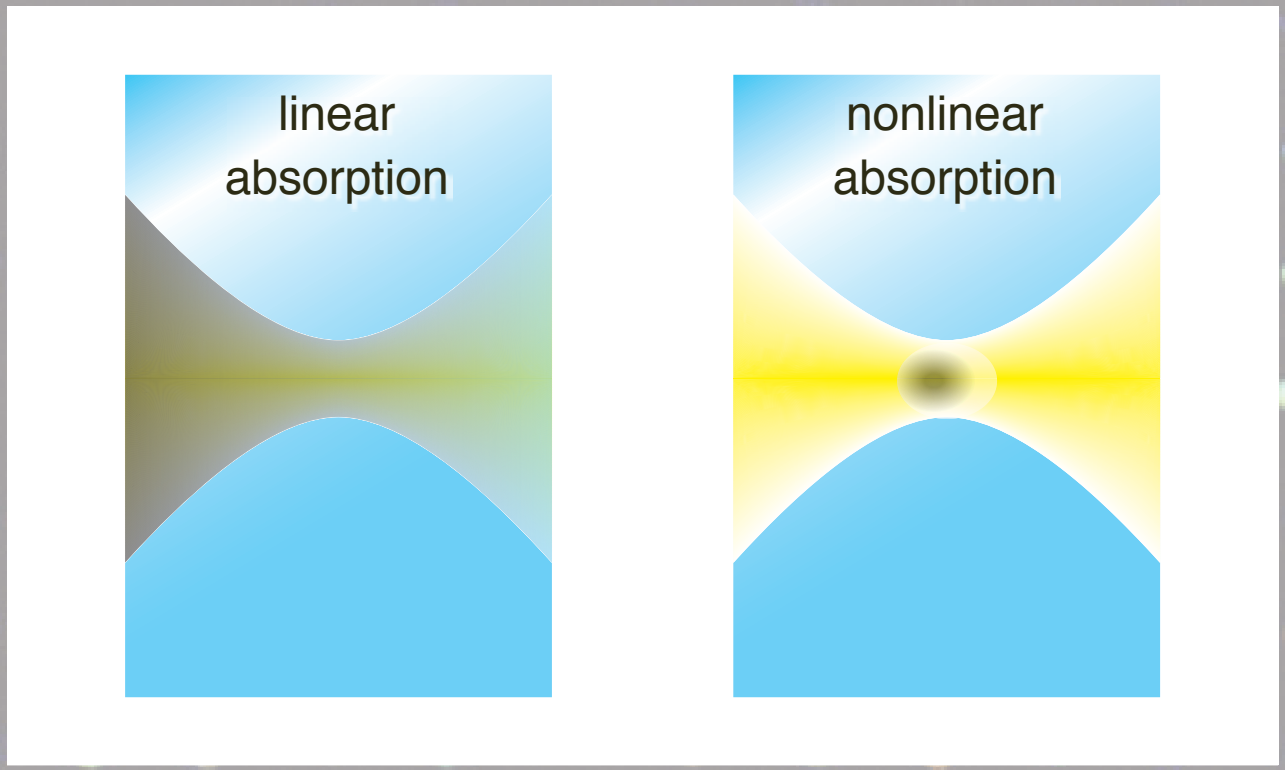
Femtosecond materials interactions

nonlinear interaction provides bulk confinement

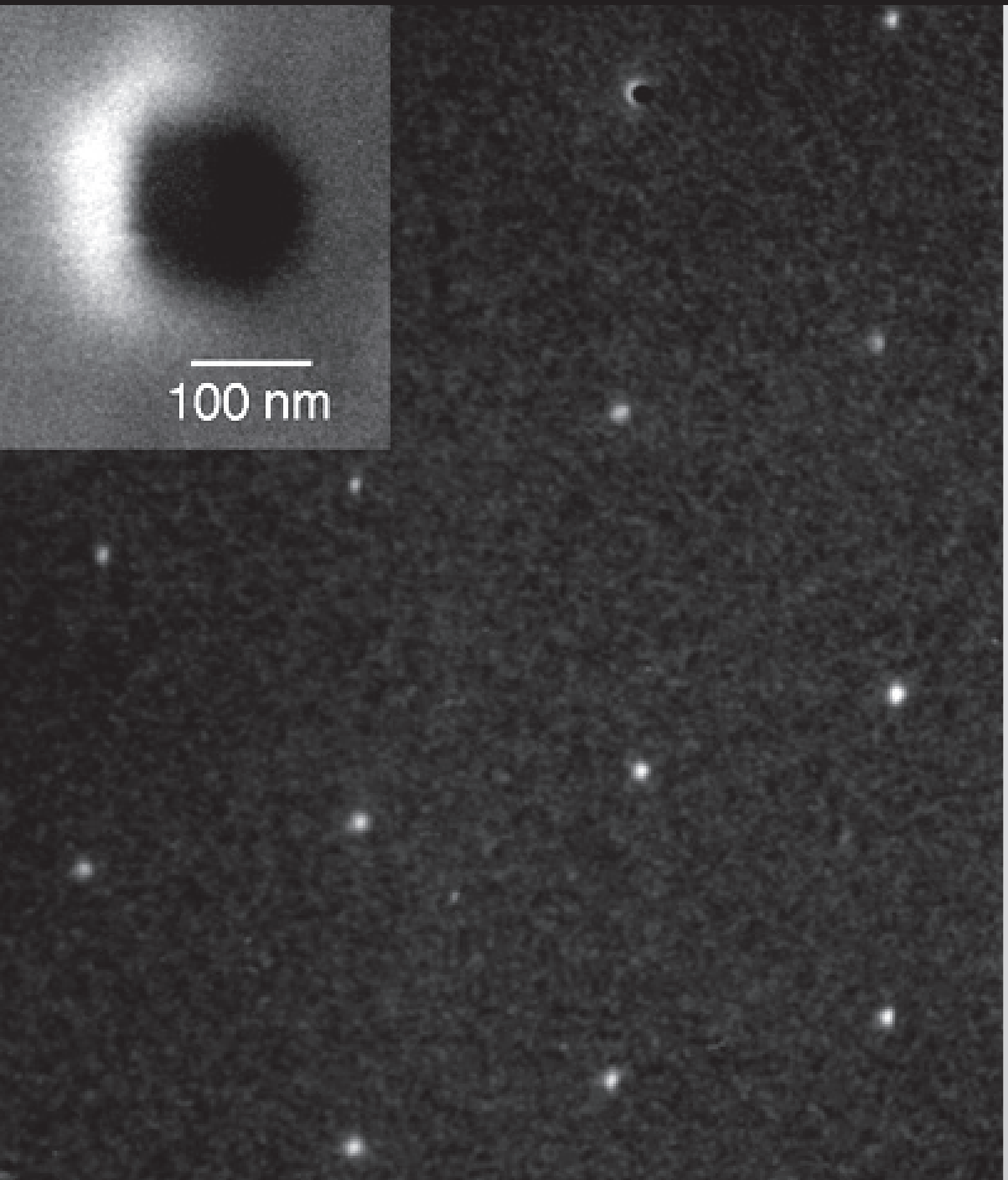


Femtosecond materials interactions

nonlinear interaction provides bulk confinement



Femtosecond materials interactions

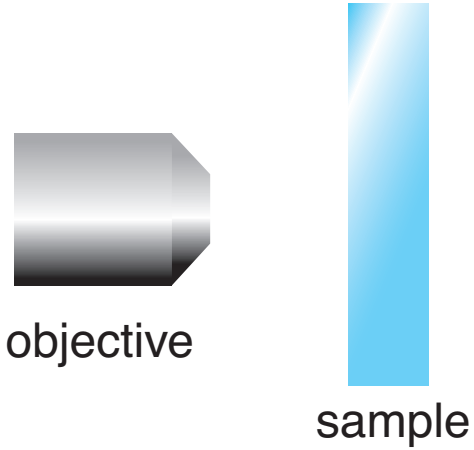


SEM & AFM:

- 100-nm cavities
- little colateral damage

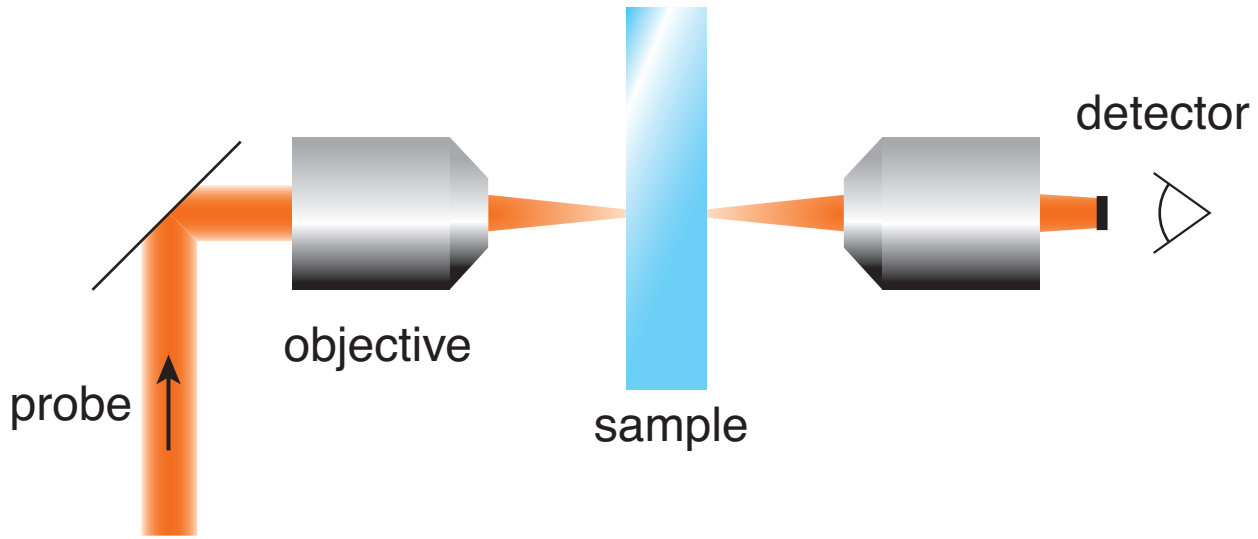
Femtosecond materials interactions

Dark-field scattering



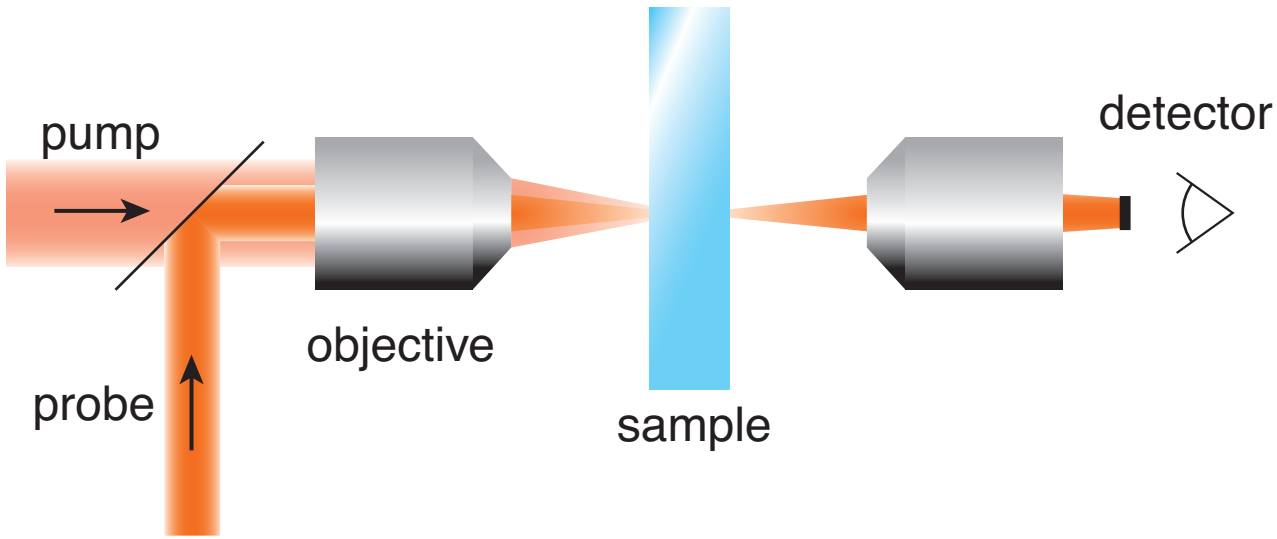
Femtosecond materials interactions

block probe beam…



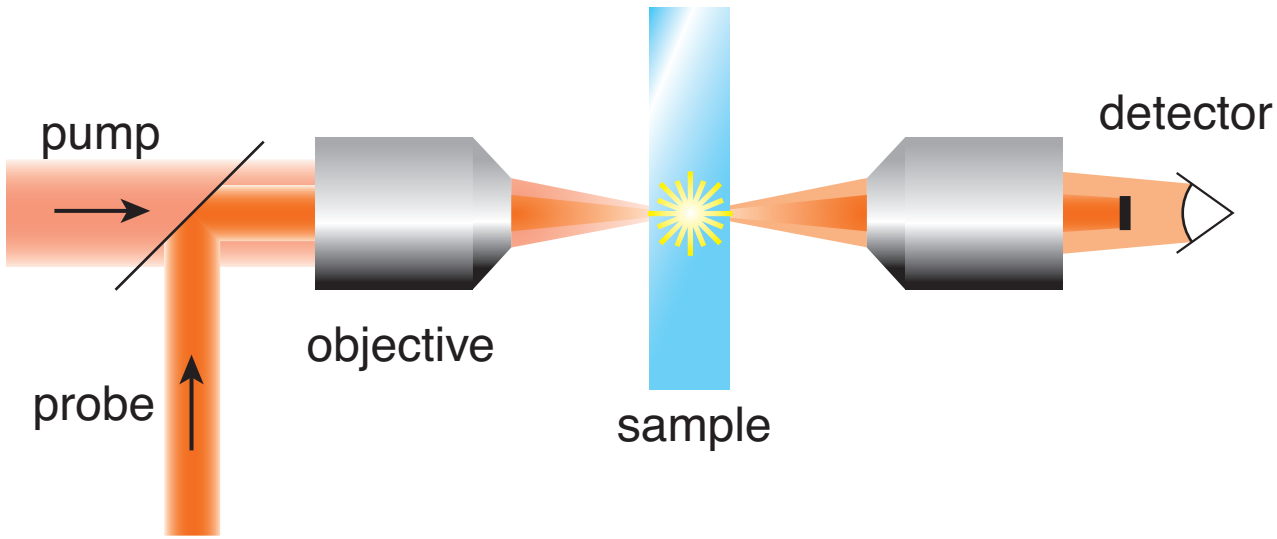
Femtosecond materials interactions

… bring in pump beam…



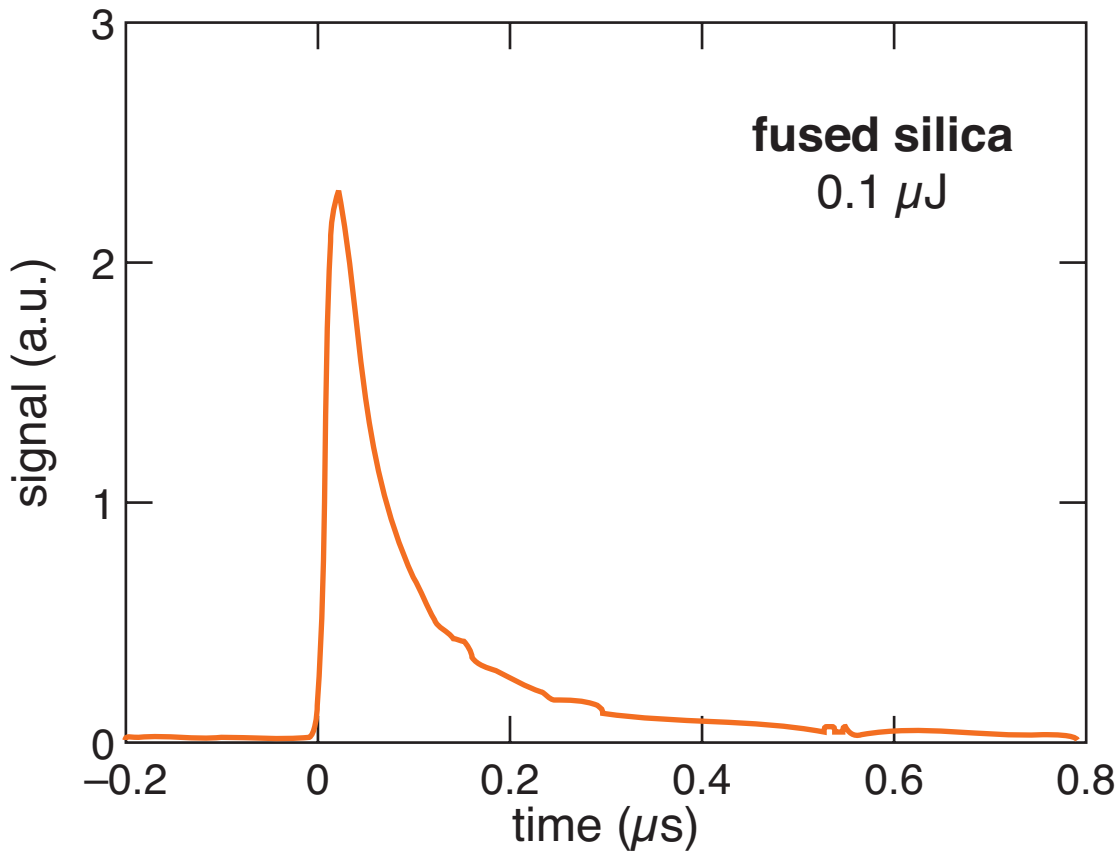
Femtosecond materials interactions

… damage scatters probe beam



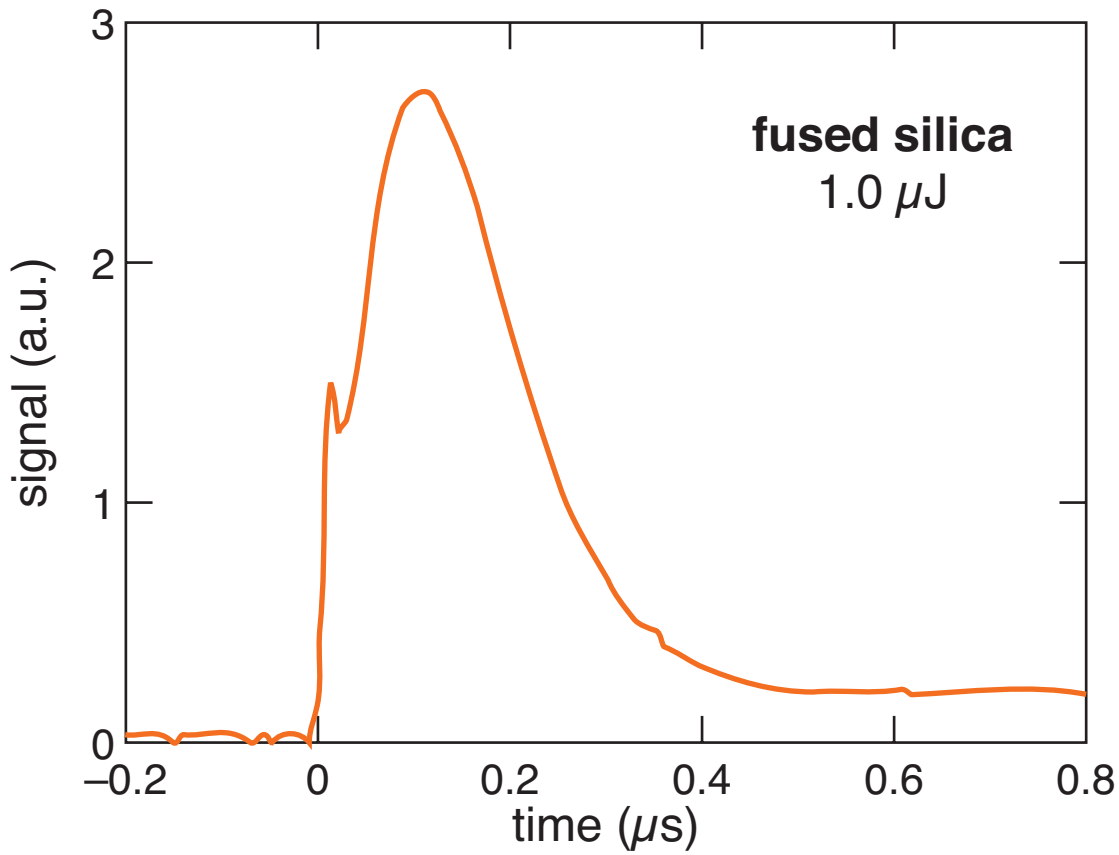
Femtosecond materials interactions

scattered signal



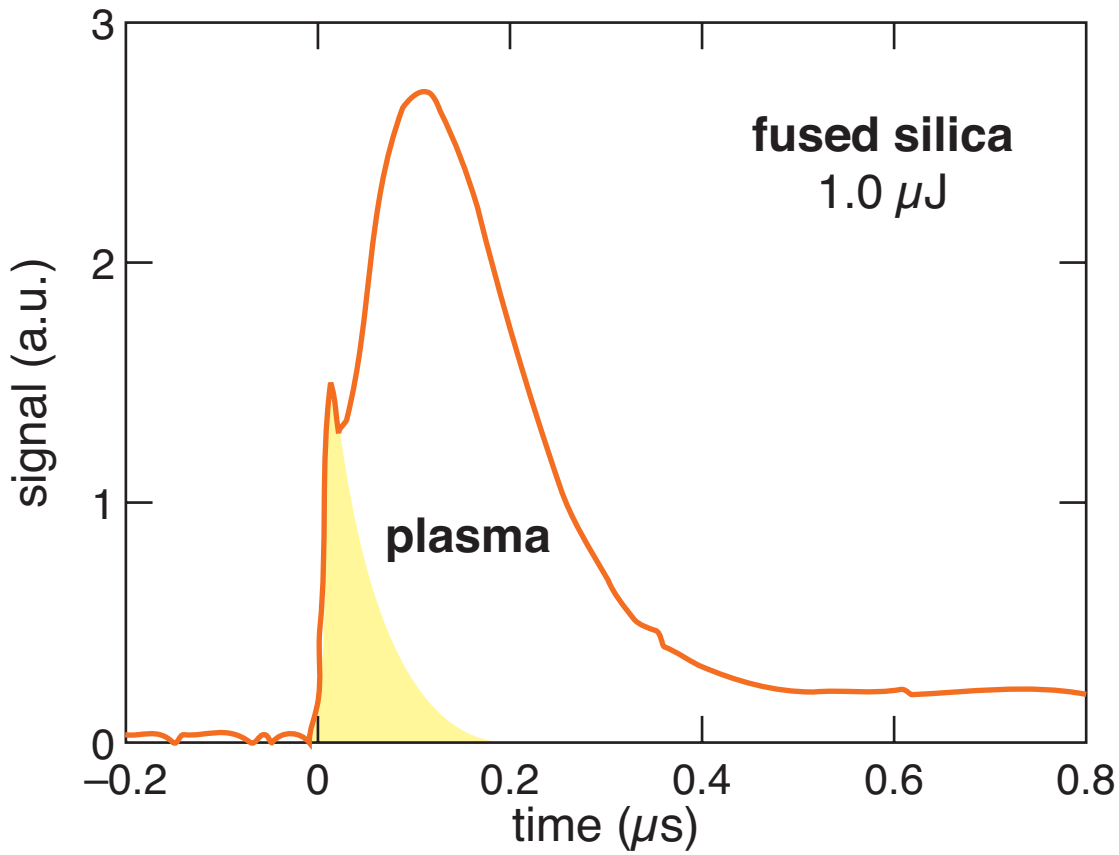
Femtosecond materials interactions

scattered signal



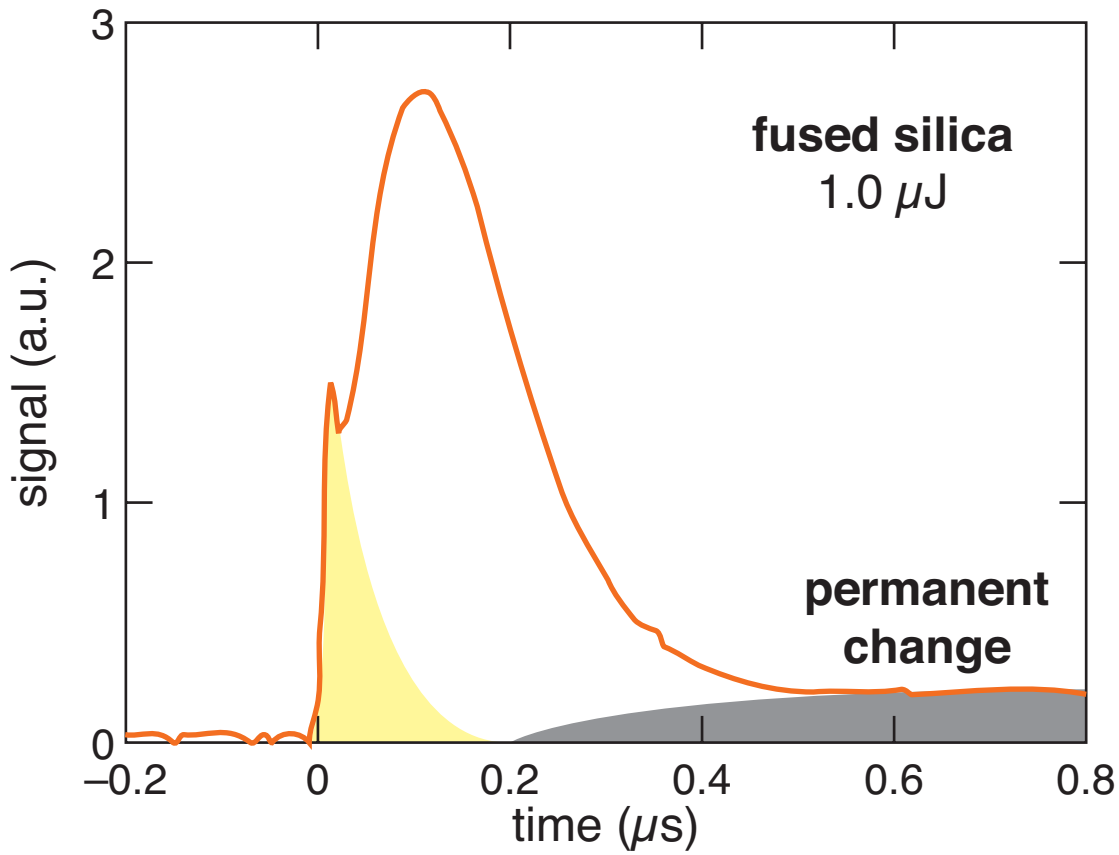
Femtosecond materials interactions

scattered signal



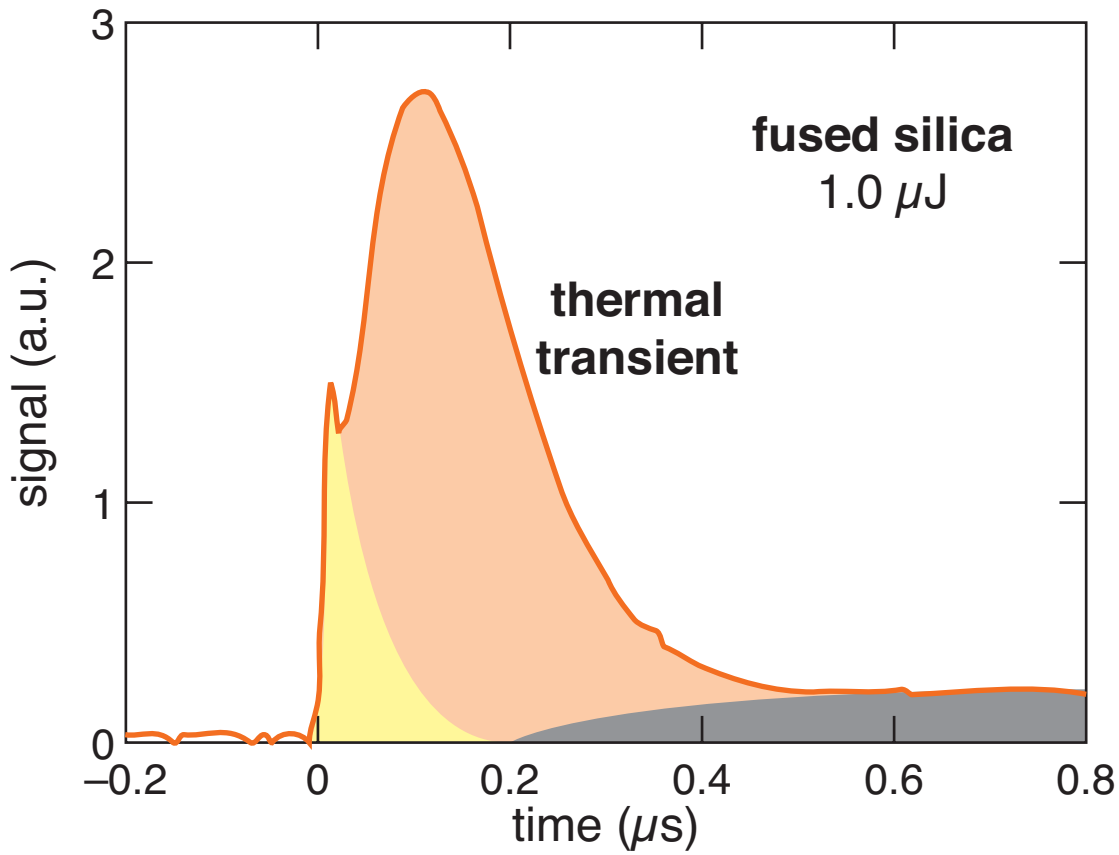
Femtosecond materials interactions

scattered signal



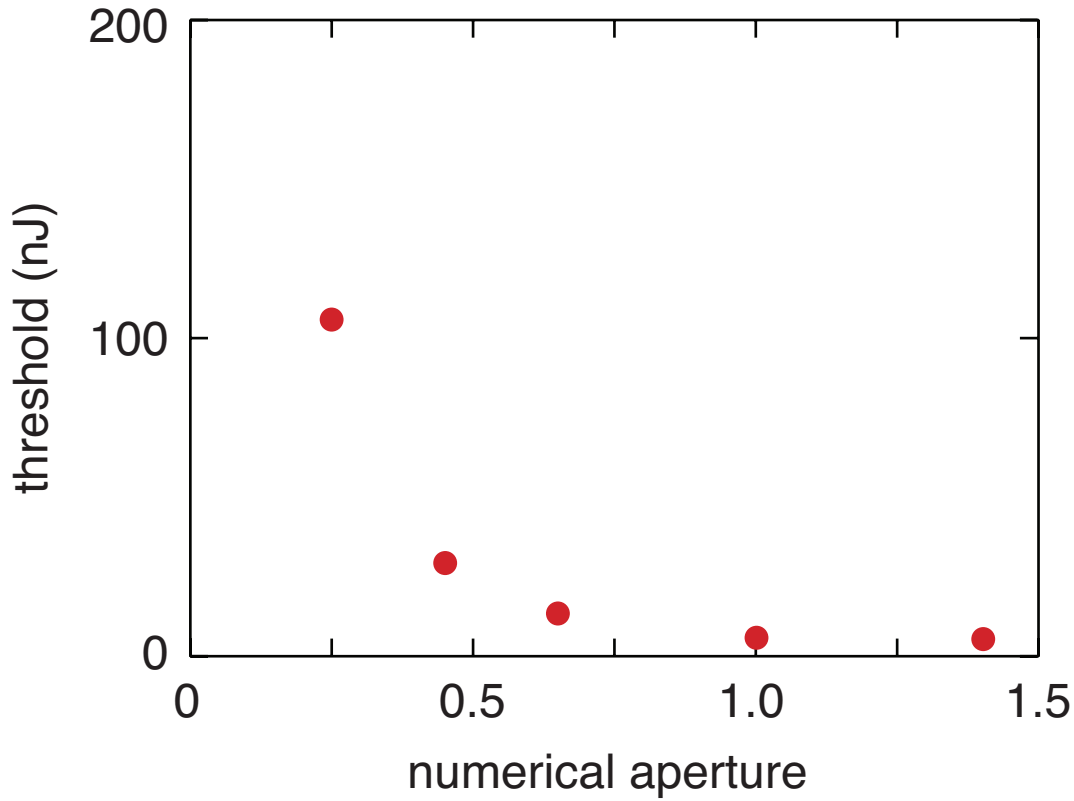
Femtosecond materials interactions

scattered signal



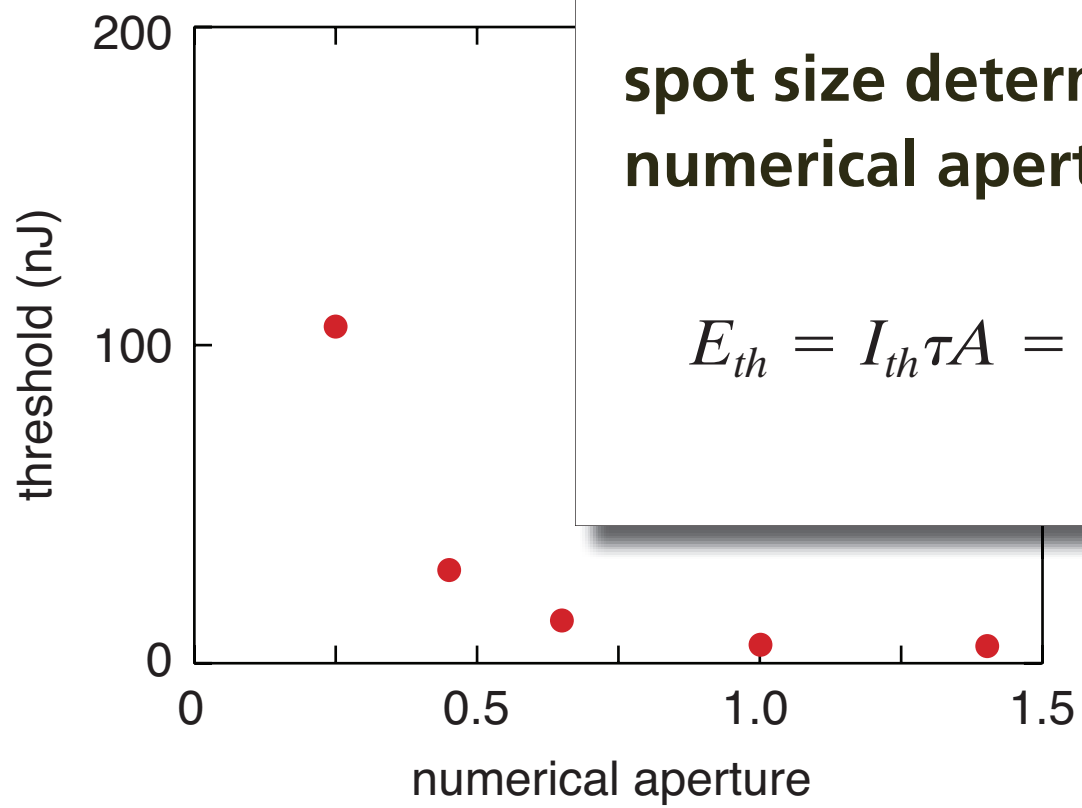
Femtosecond materials interactions

vary numerical aperture



Femtosecond materials interactions

vary numeric



intensity threshold:

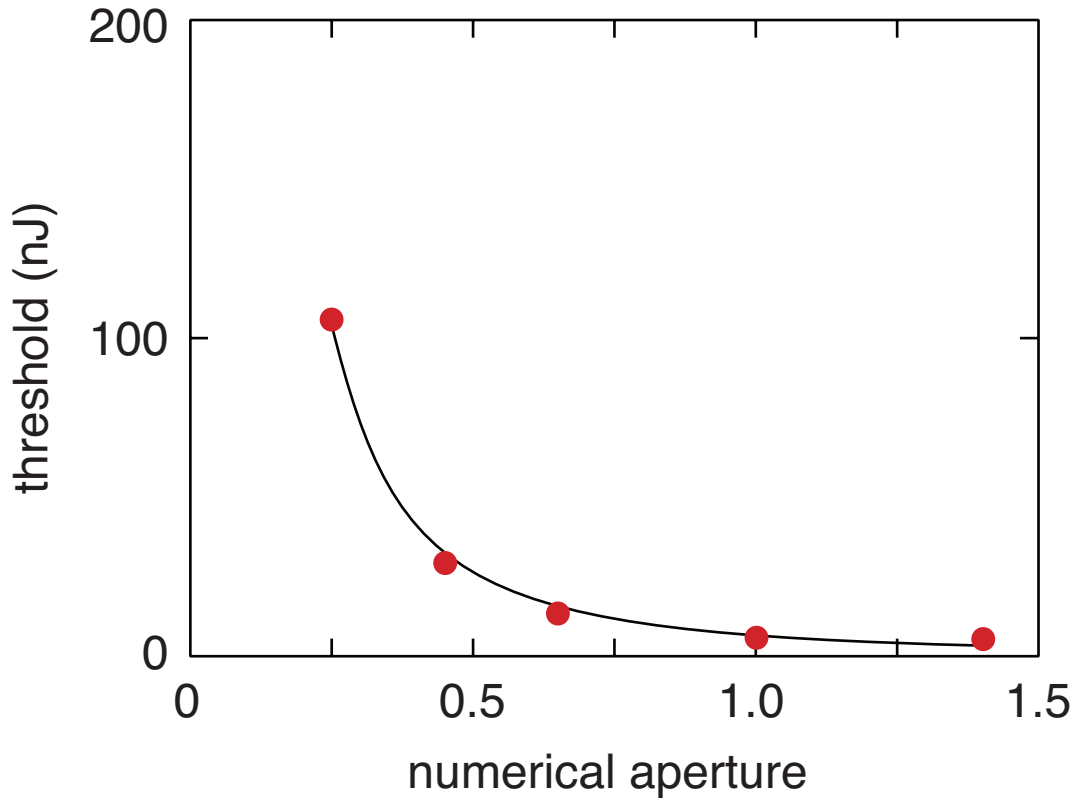
$$E_{th} = I_{th}\tau A$$

spot size determined by numerical aperture:

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

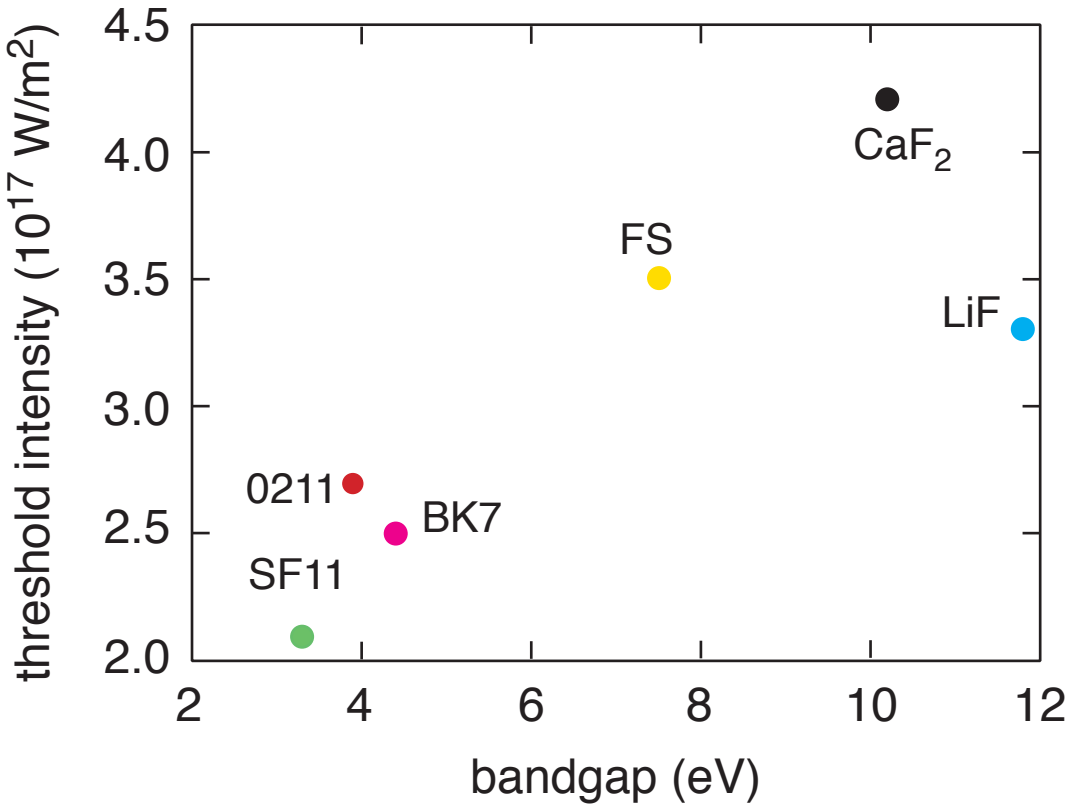
Femtosecond materials interactions

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



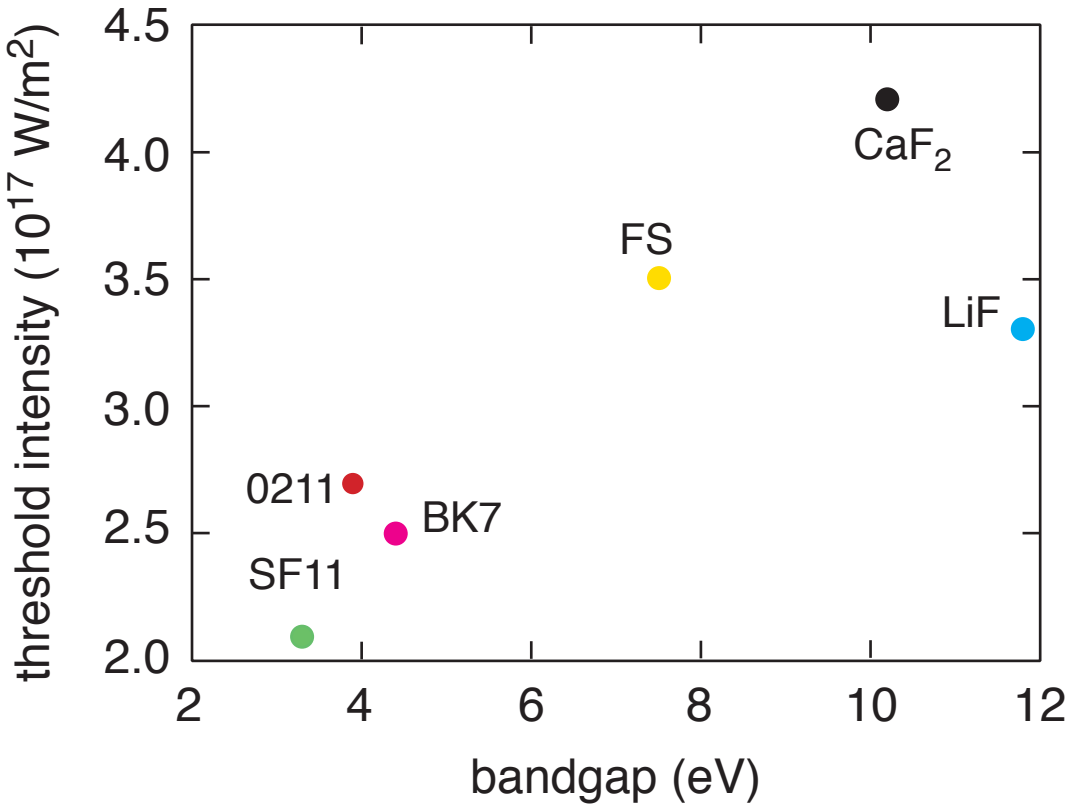
Femtosecond materials interactions

vary material…



Femtosecond materials interactions

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



Femtosecond materials interactions

- 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

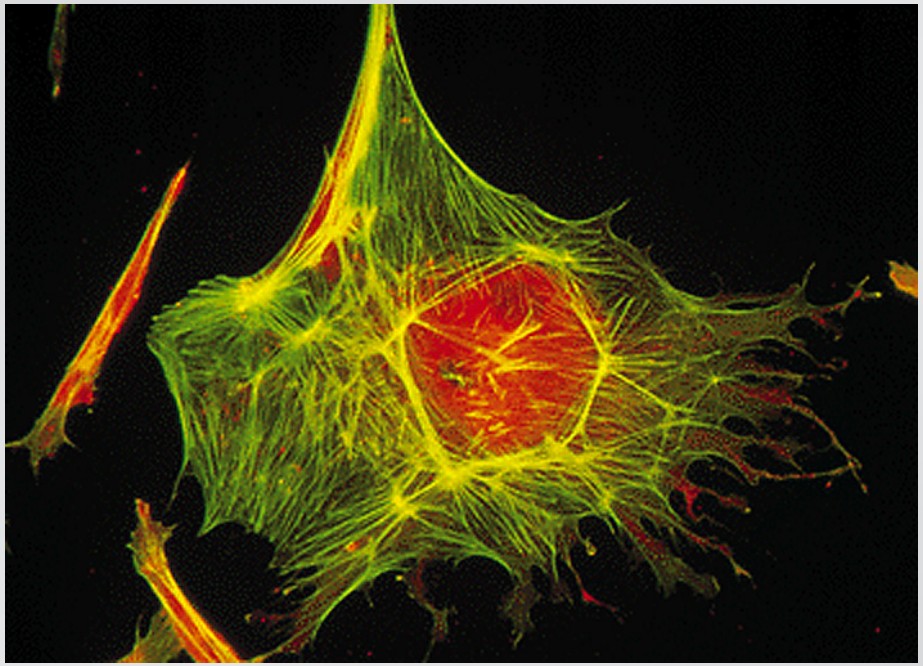
Cytoskeleton

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

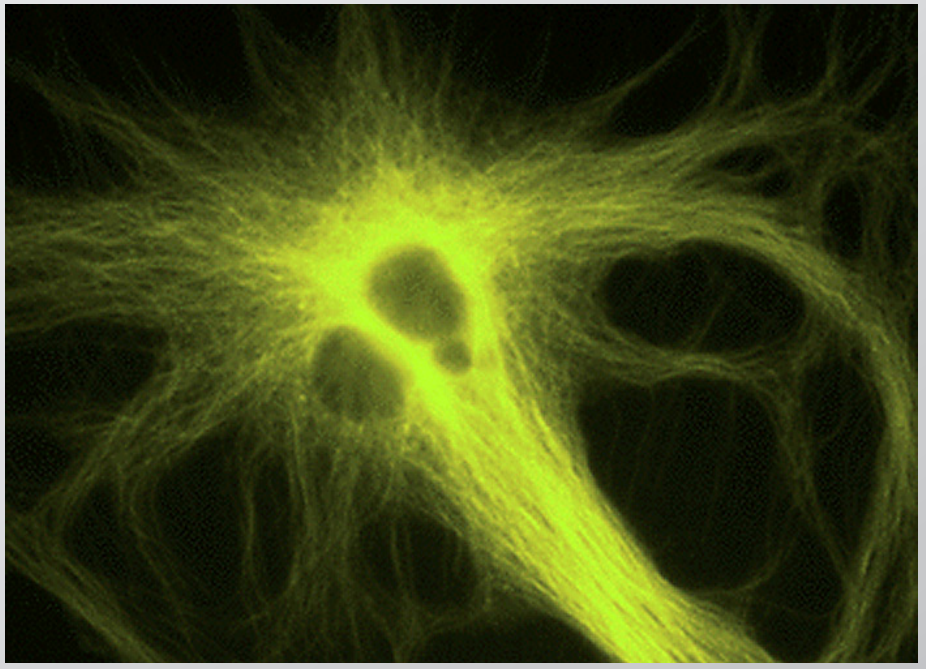
Subcellular surgery

two components

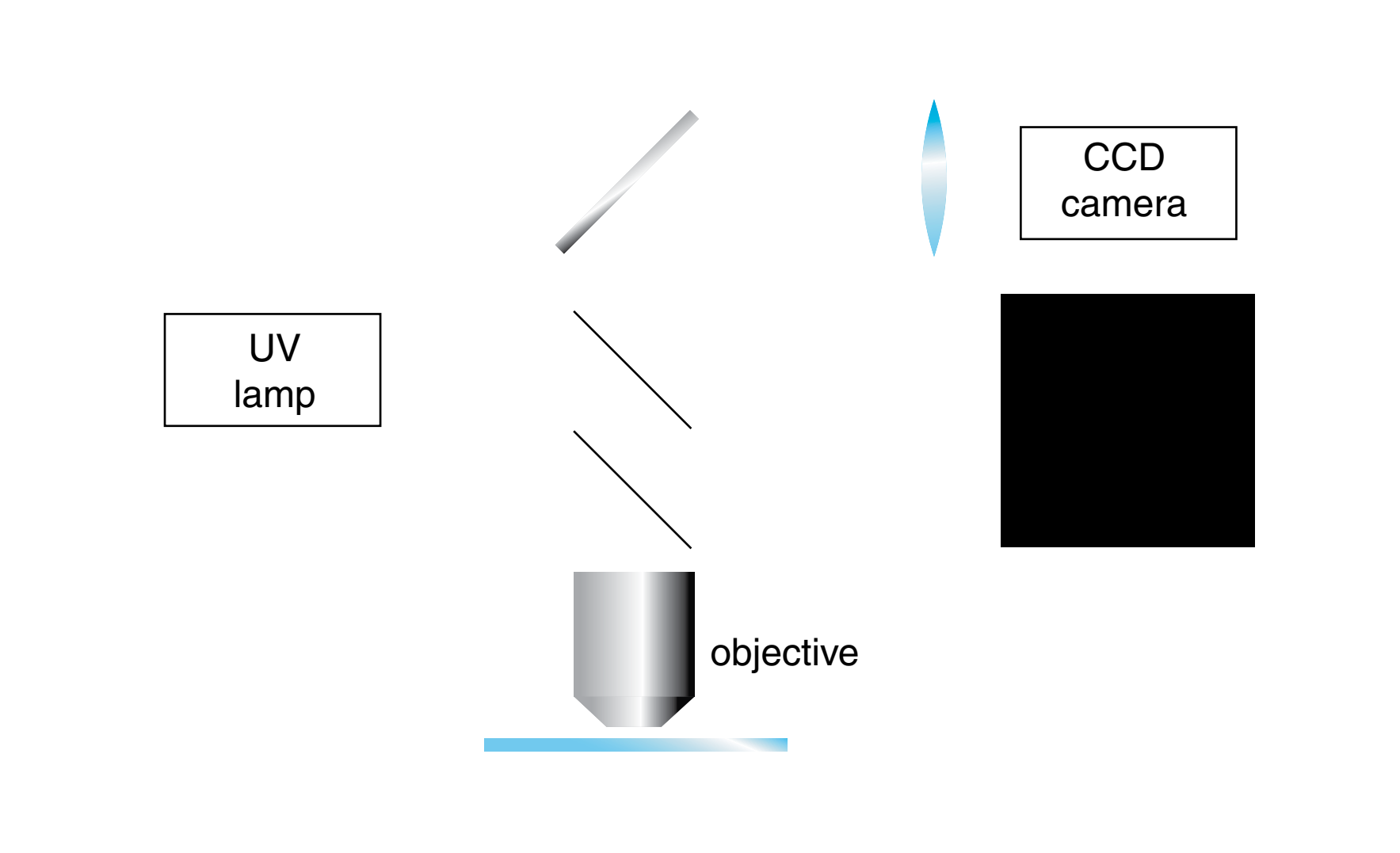
actin fibers



microtubules

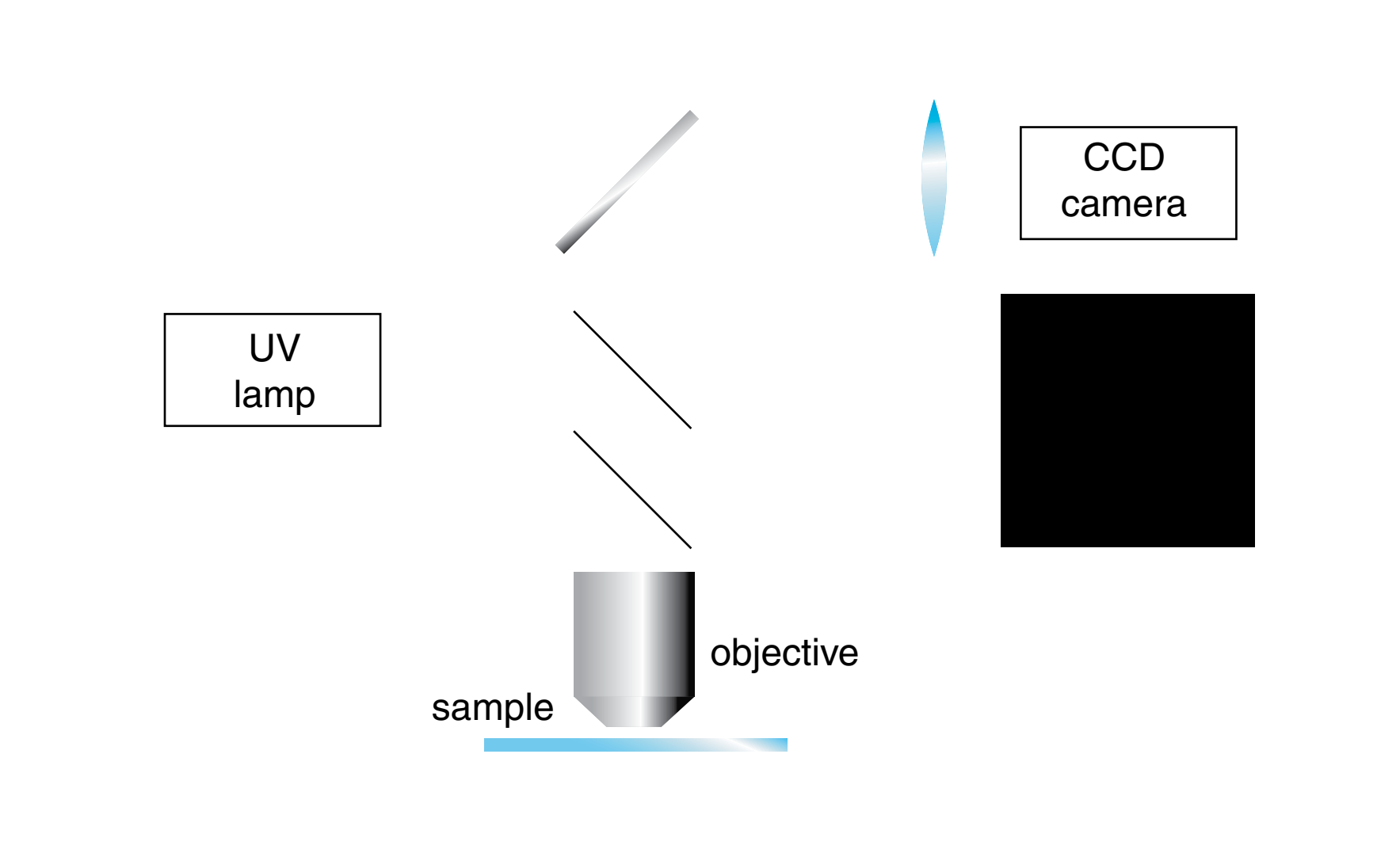


Subcellular surgery



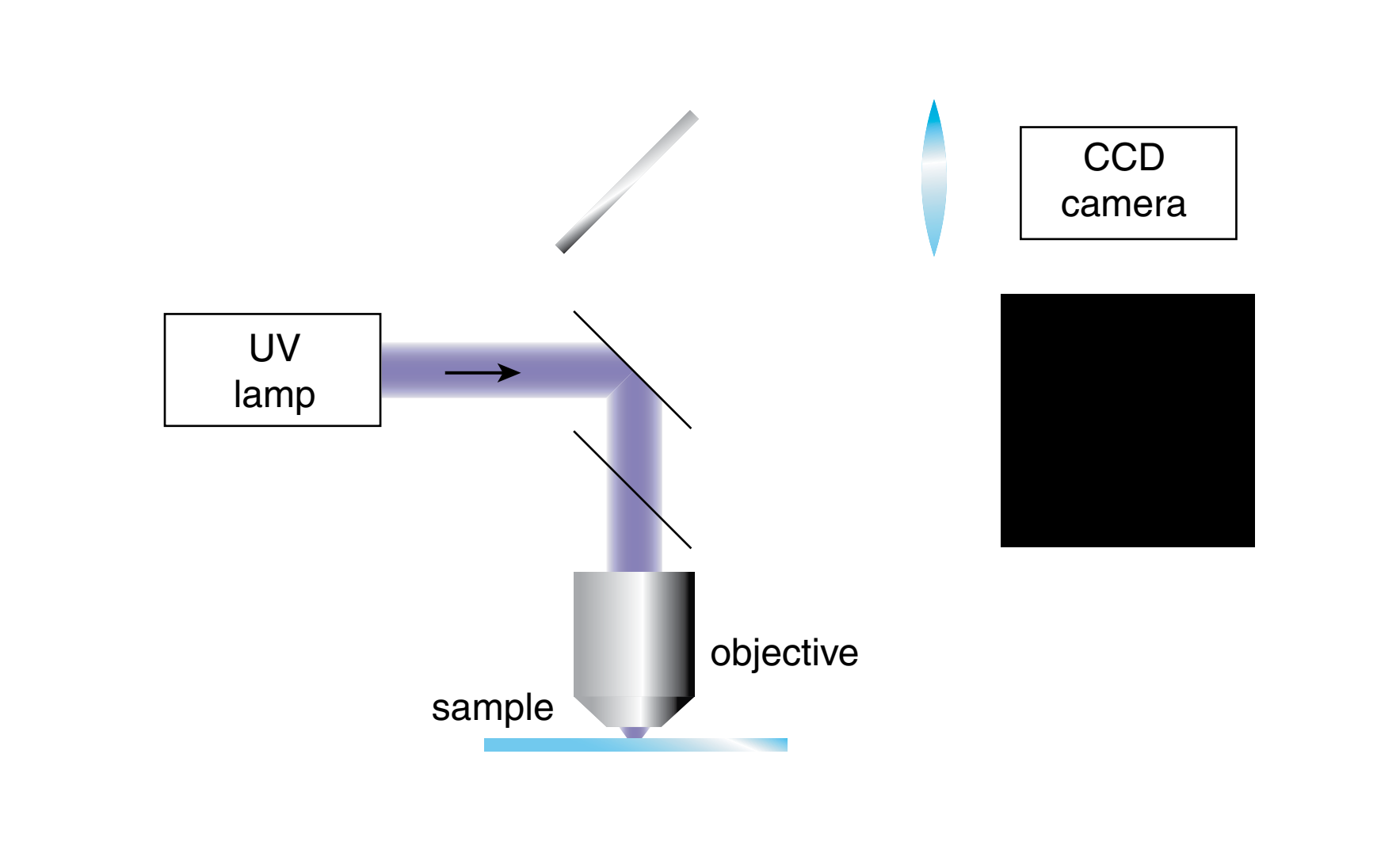
epi-fluorescence microscope

Subcellular surgery



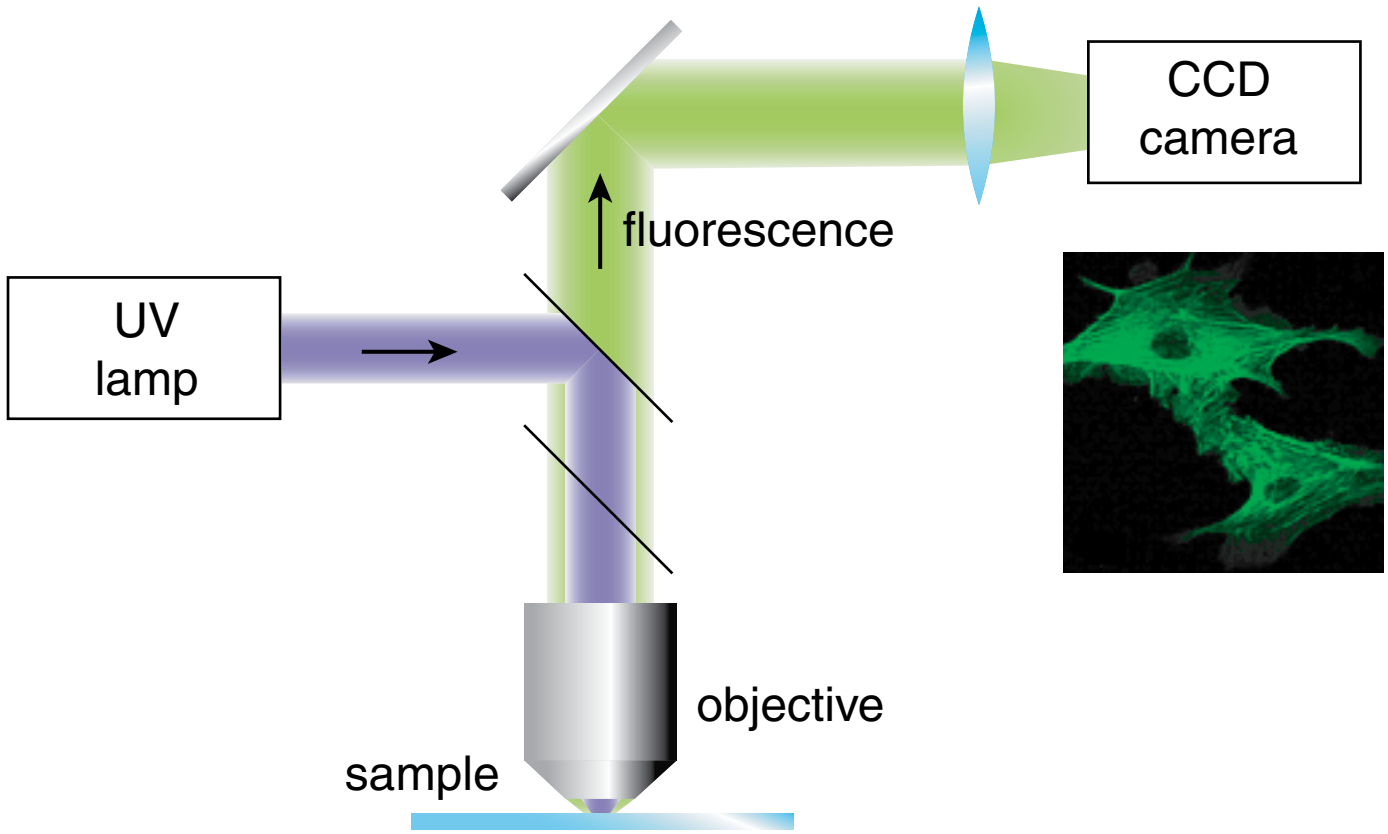
fluorescently label sample

Subcellular surgery



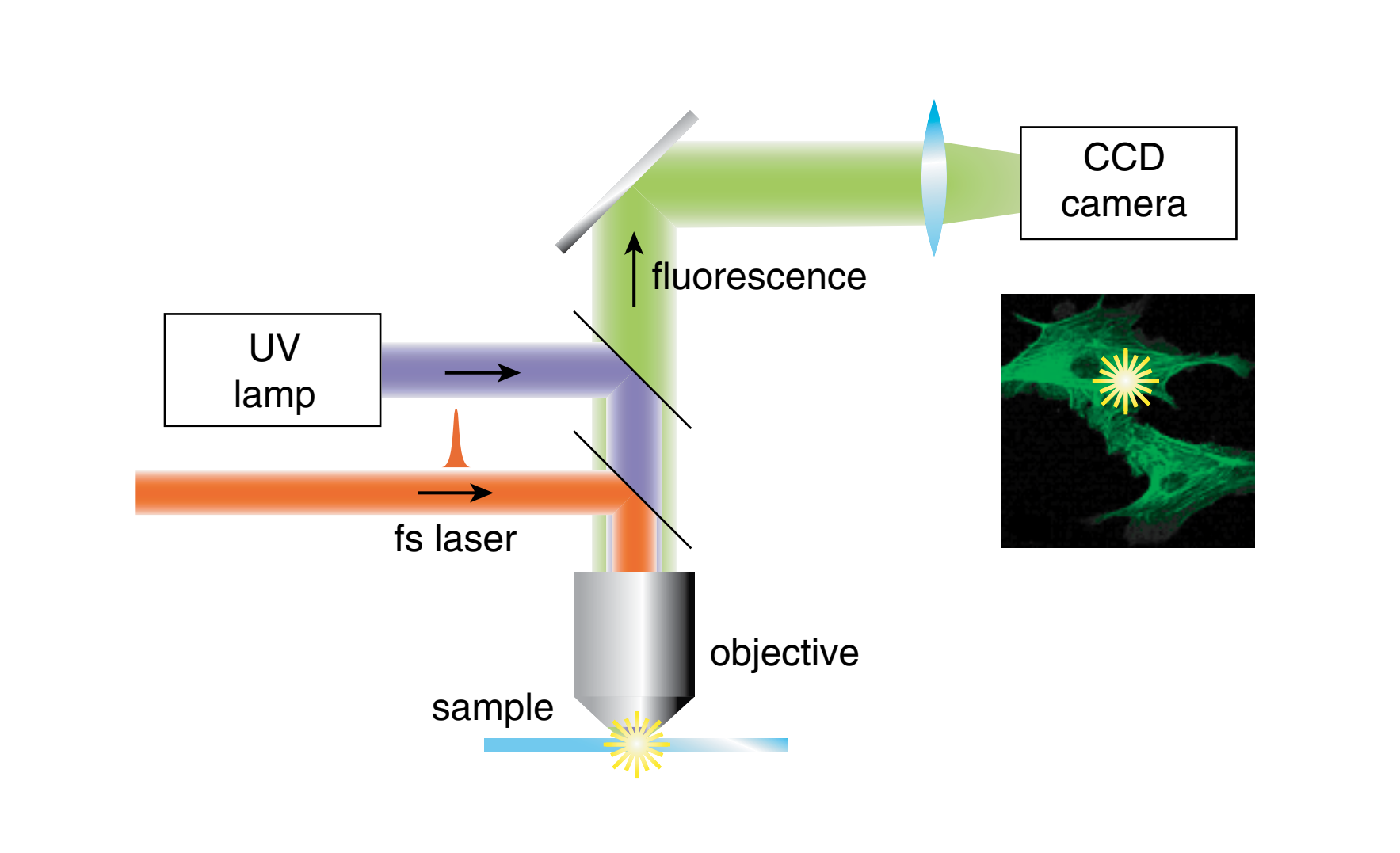
UV illumination…

Subcellular surgery



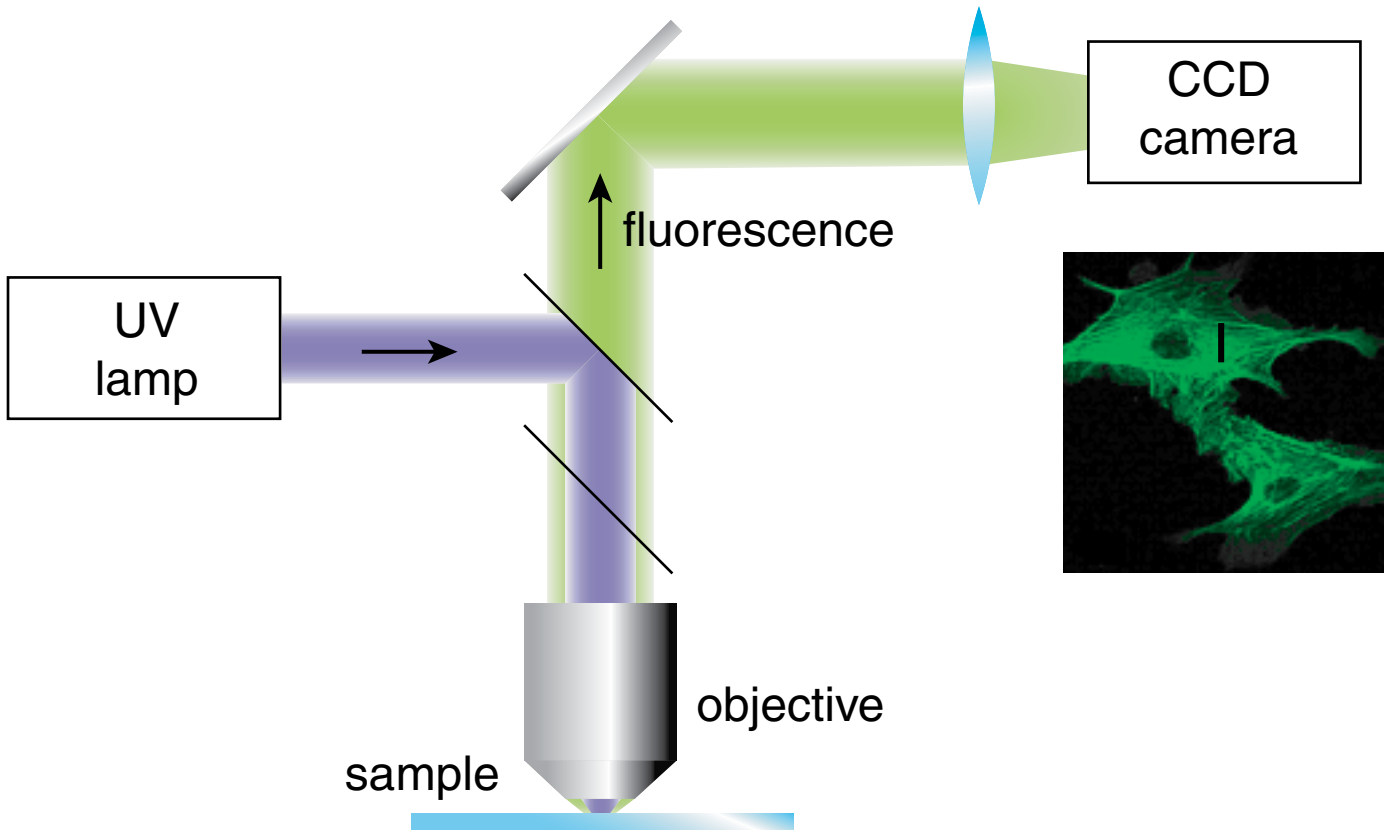
…causes fluorescence

Subcellular surgery



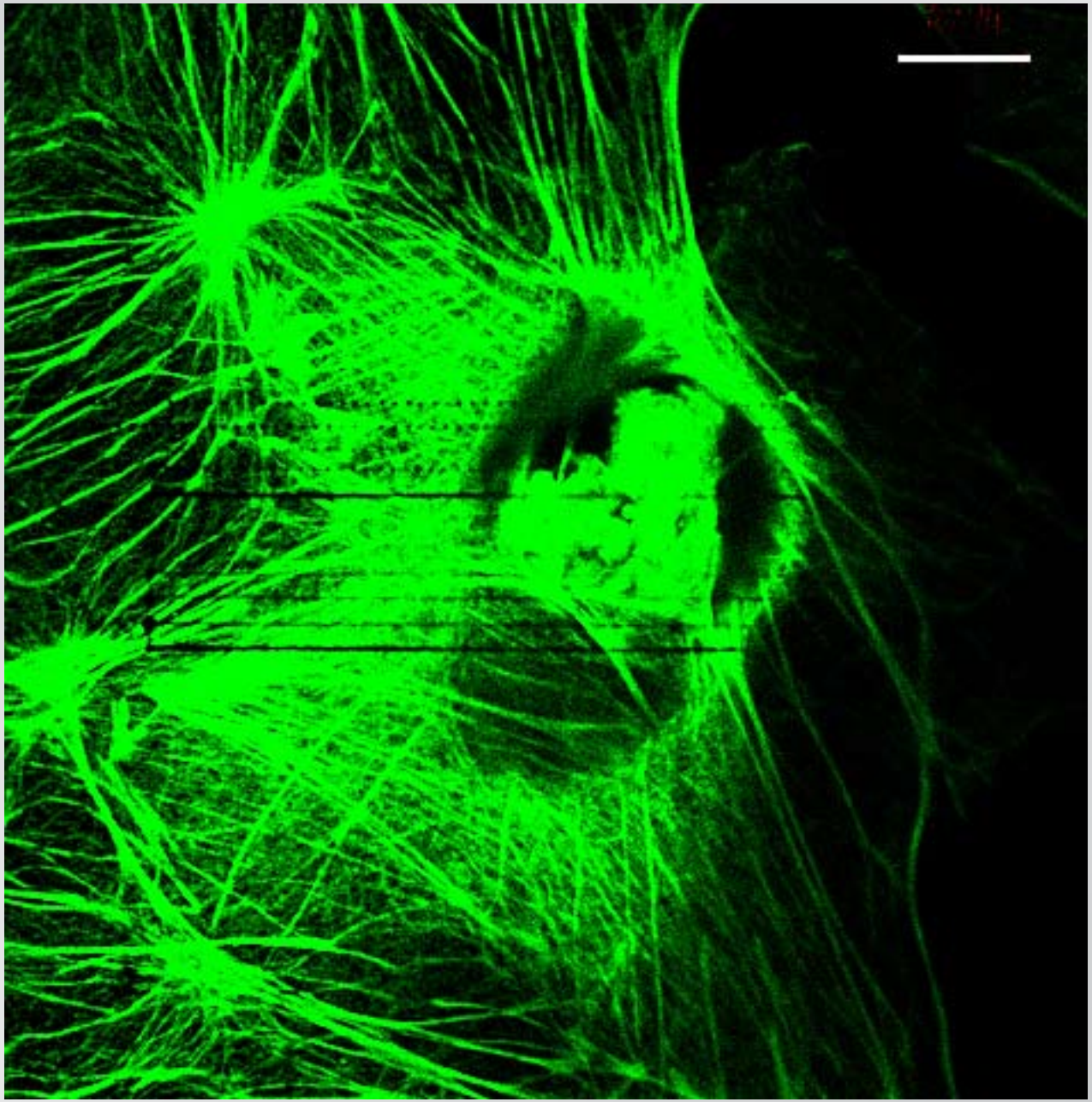
irradiate with fs laser beam

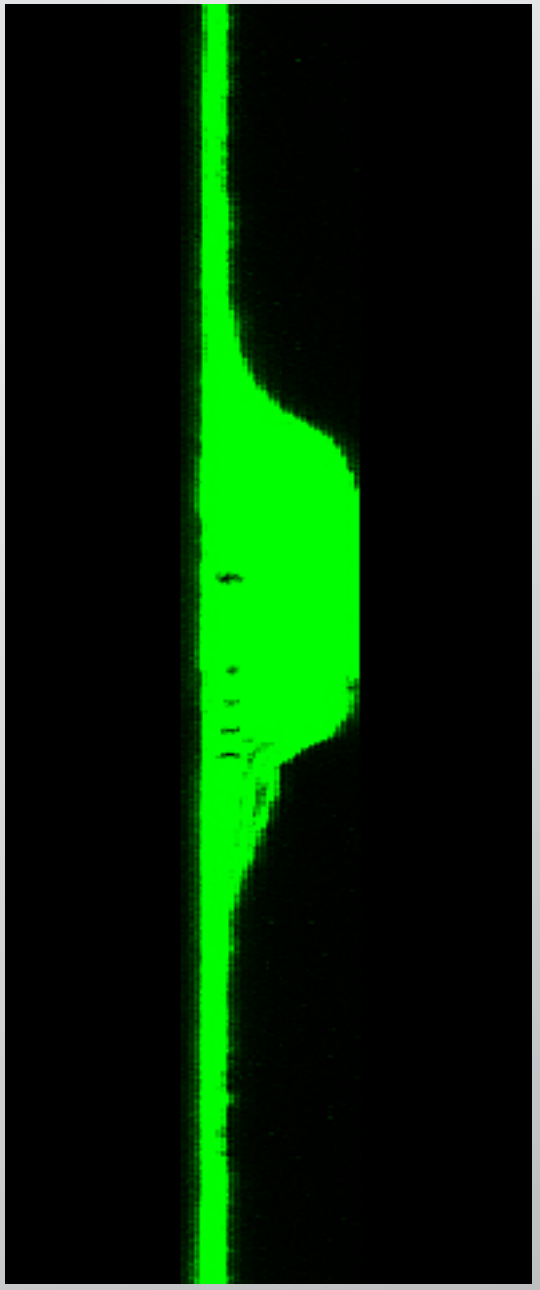
Subcellular surgery



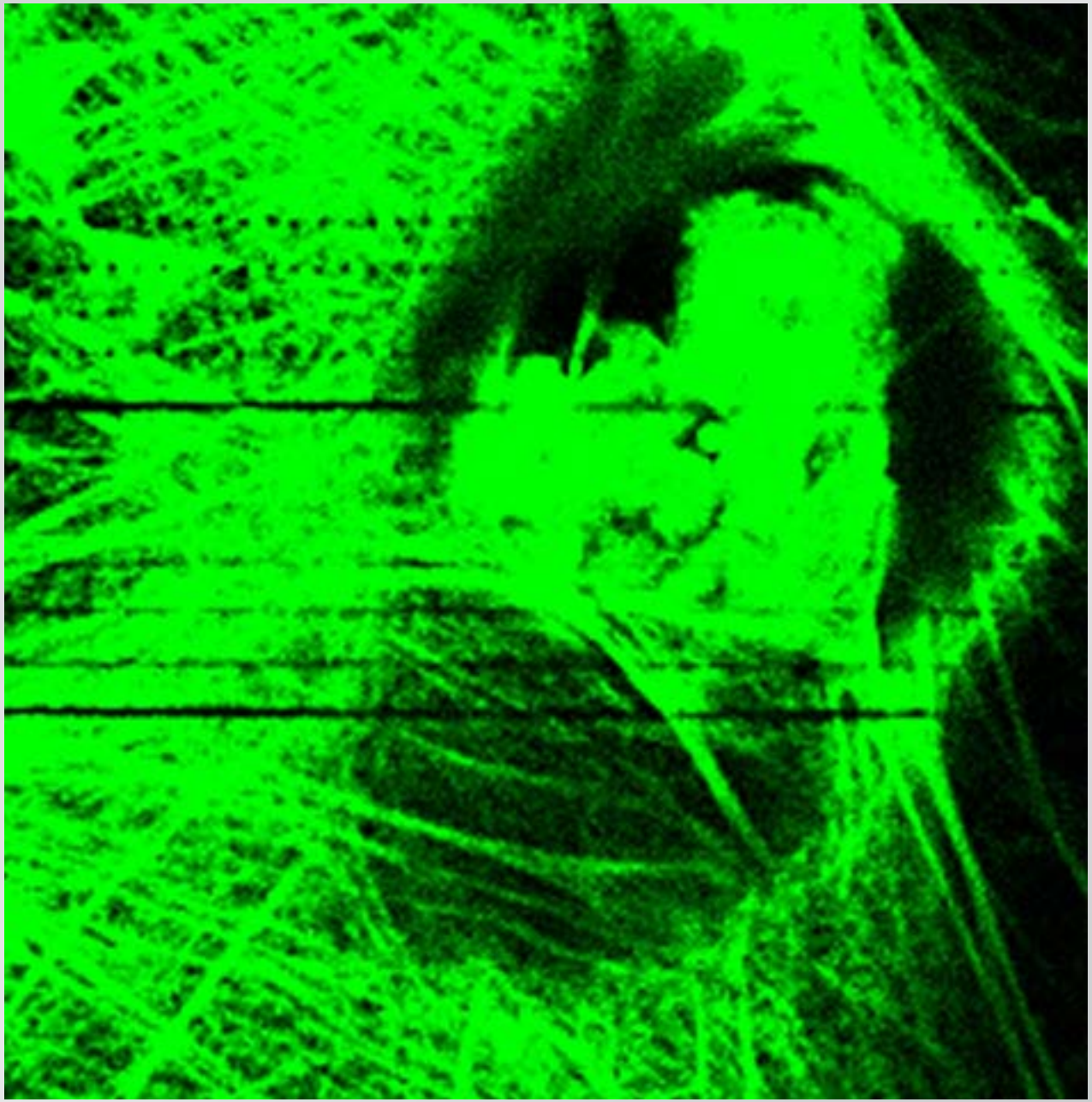
examine resulting ablation

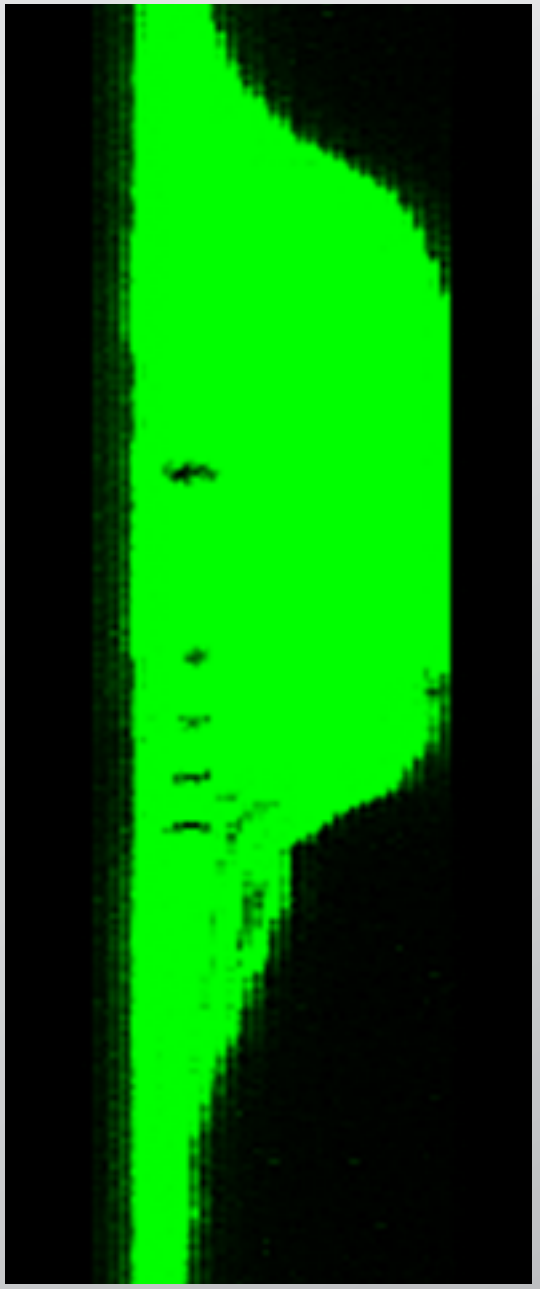
Subcellular surgery





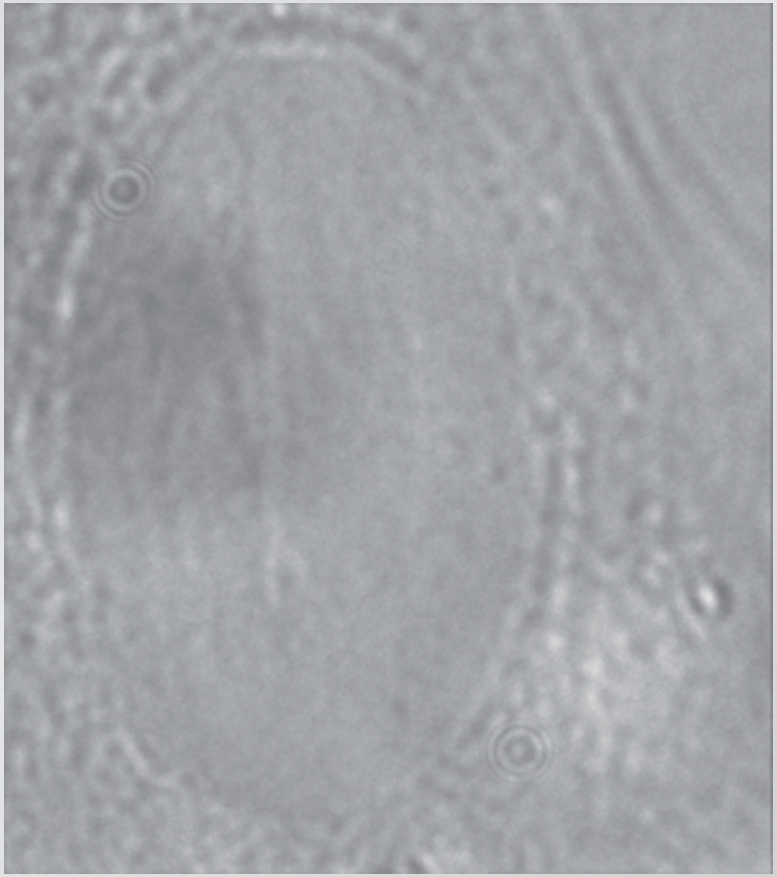
Subcellular surgery





Subcellular surgery

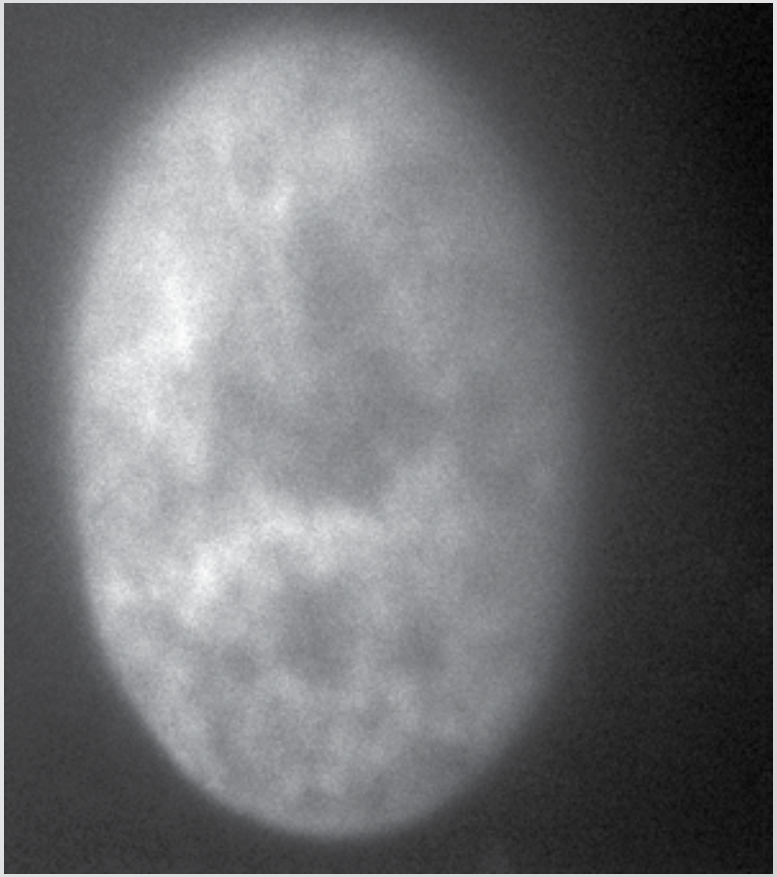
nucleus of fixed endothelial cell



white light microscopy

Subcellular surgery

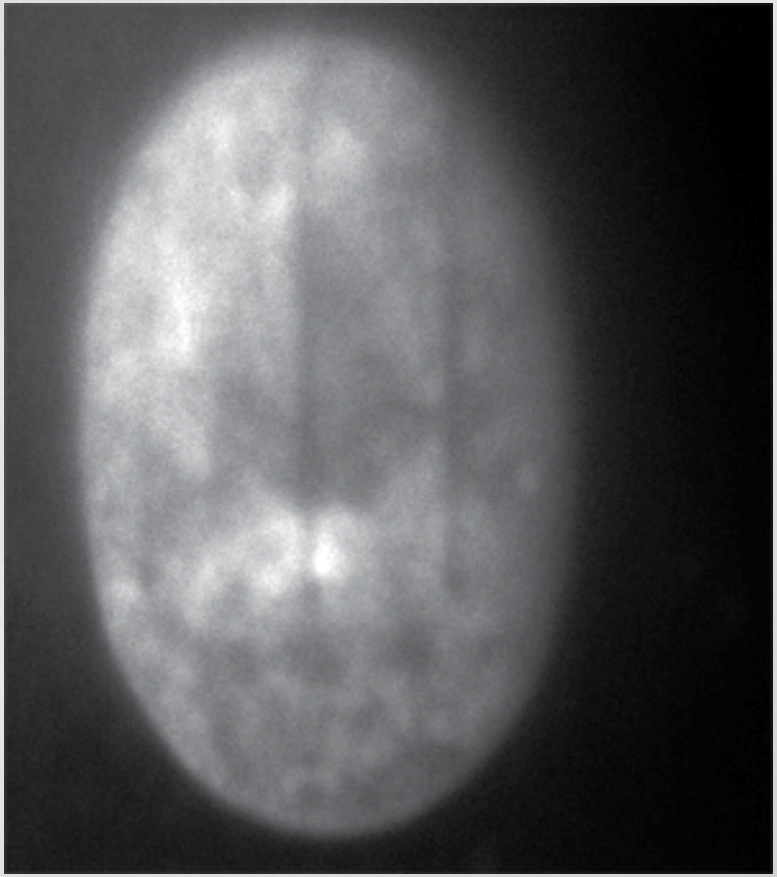
nucleus of fixed endothelial cell



fluorescence microscopy

Subcellular surgery

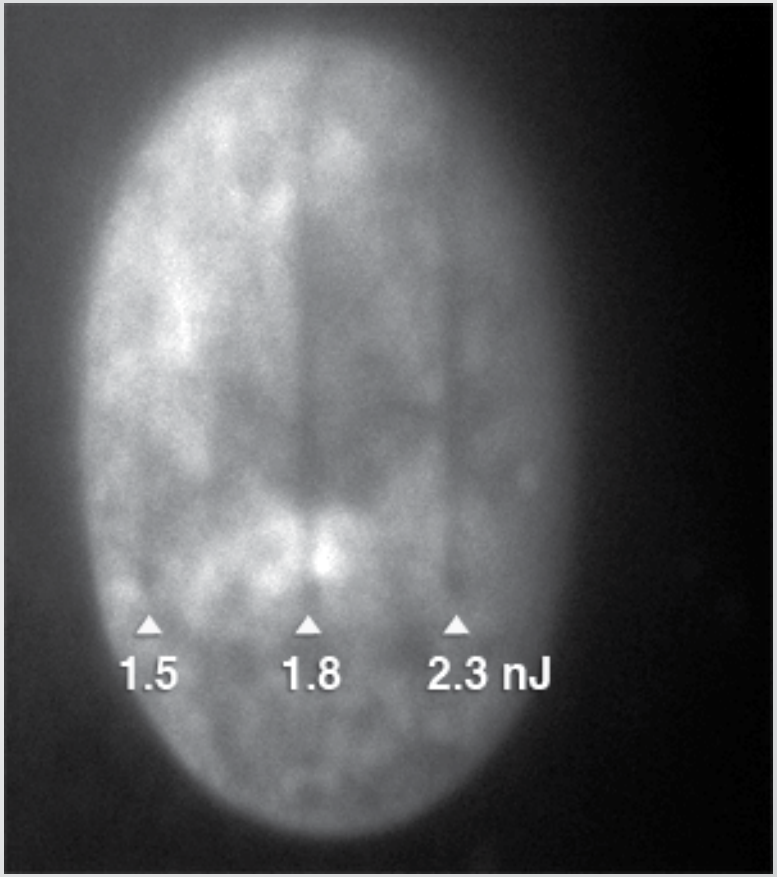
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

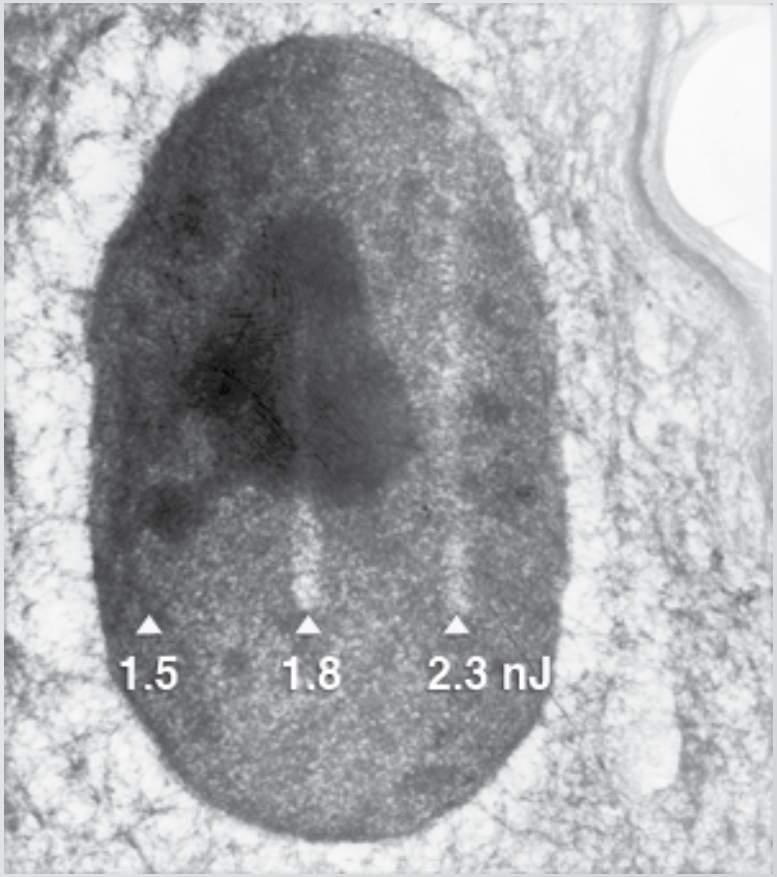
irradiate with fs laser



fluorescence microscopy

Subcellular surgery

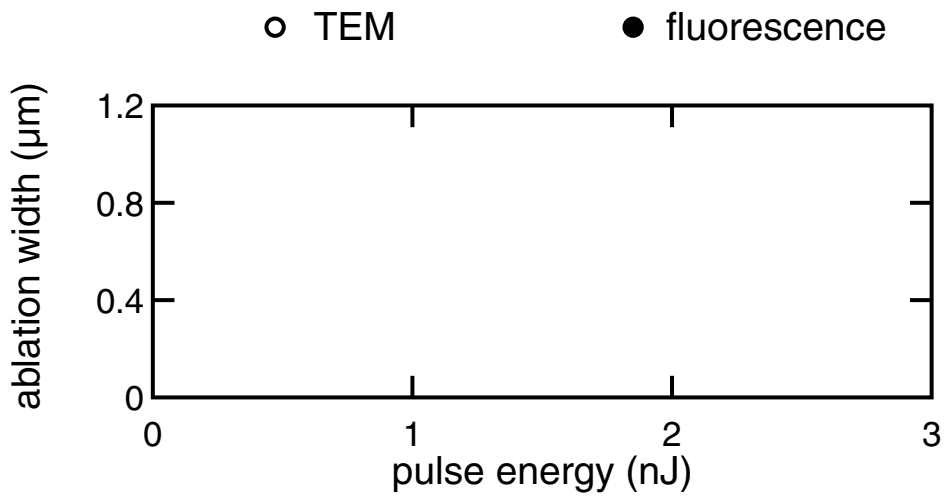
bleaching or ablation?



TEM image

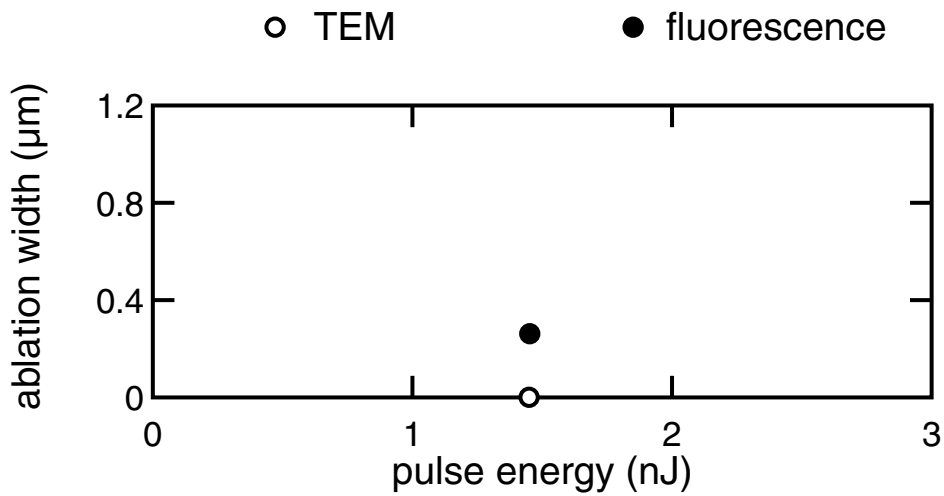
Subcellular surgery

three regions of interaction



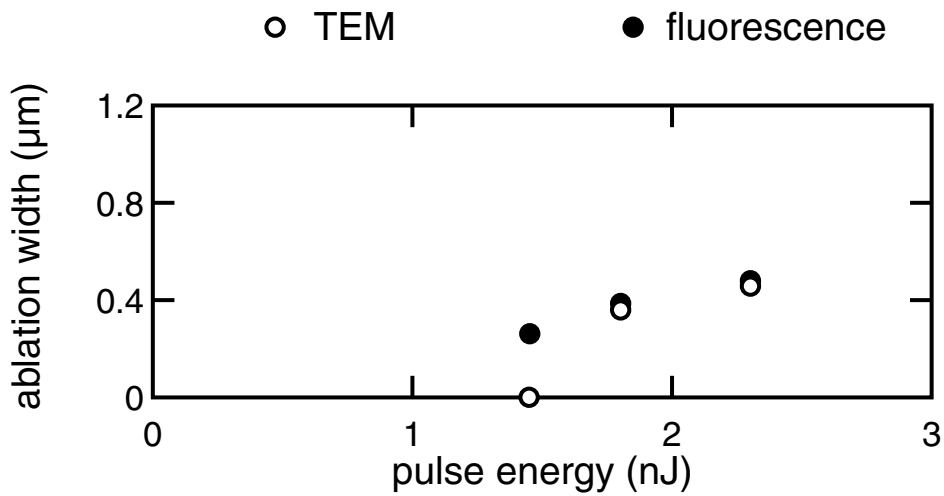
Subcellular surgery

three regions of interaction



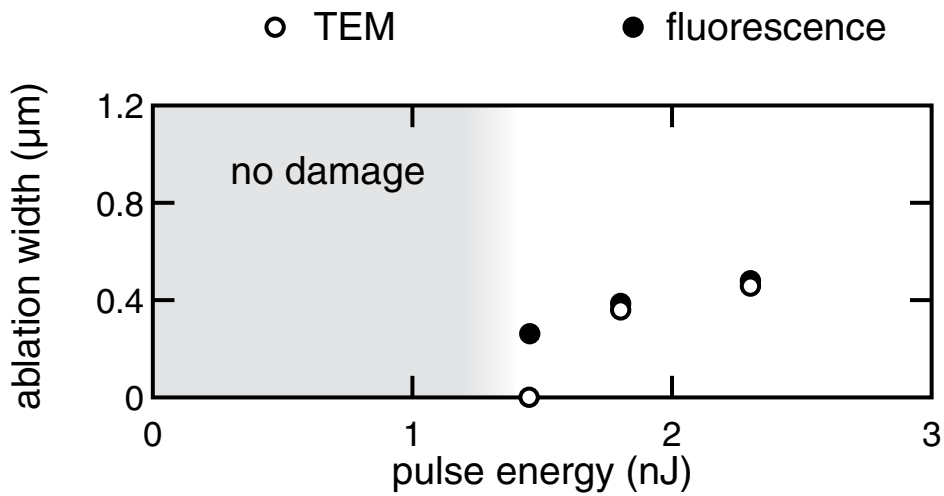
Subcellular surgery

three regions of interaction



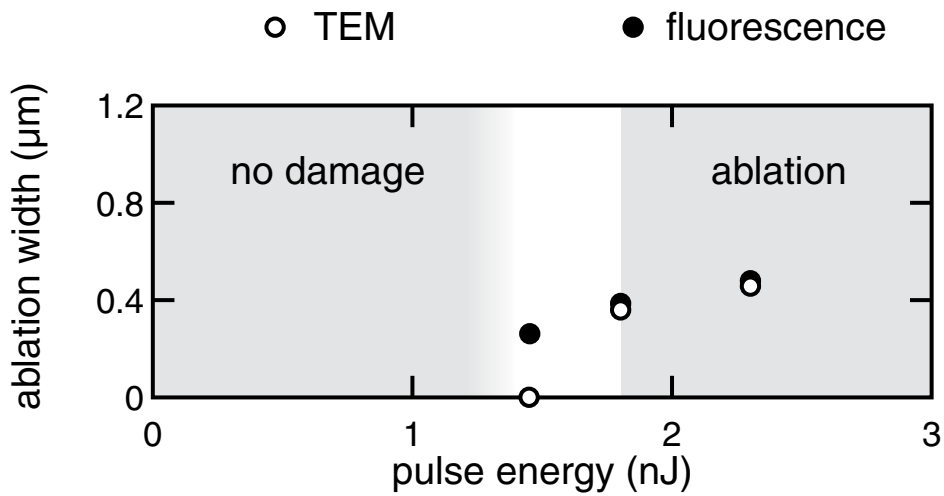
Subcellular surgery

three regions of interaction



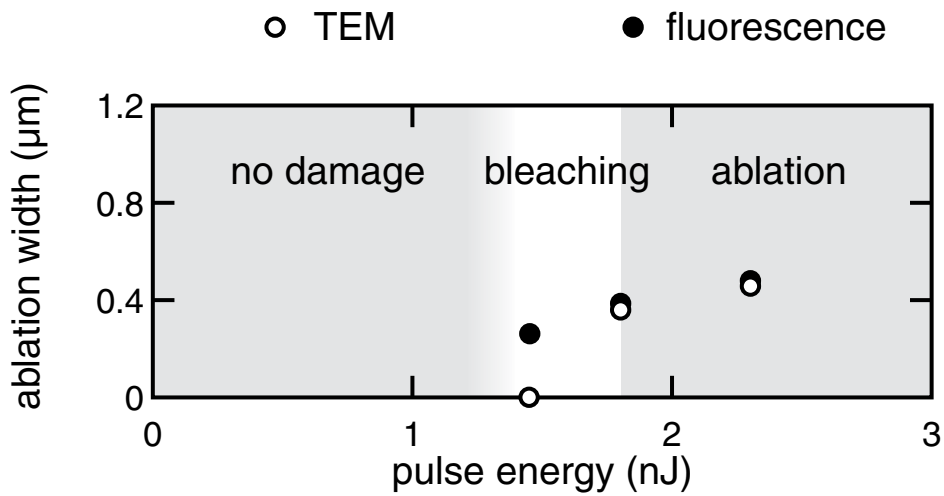
Subcellular surgery

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

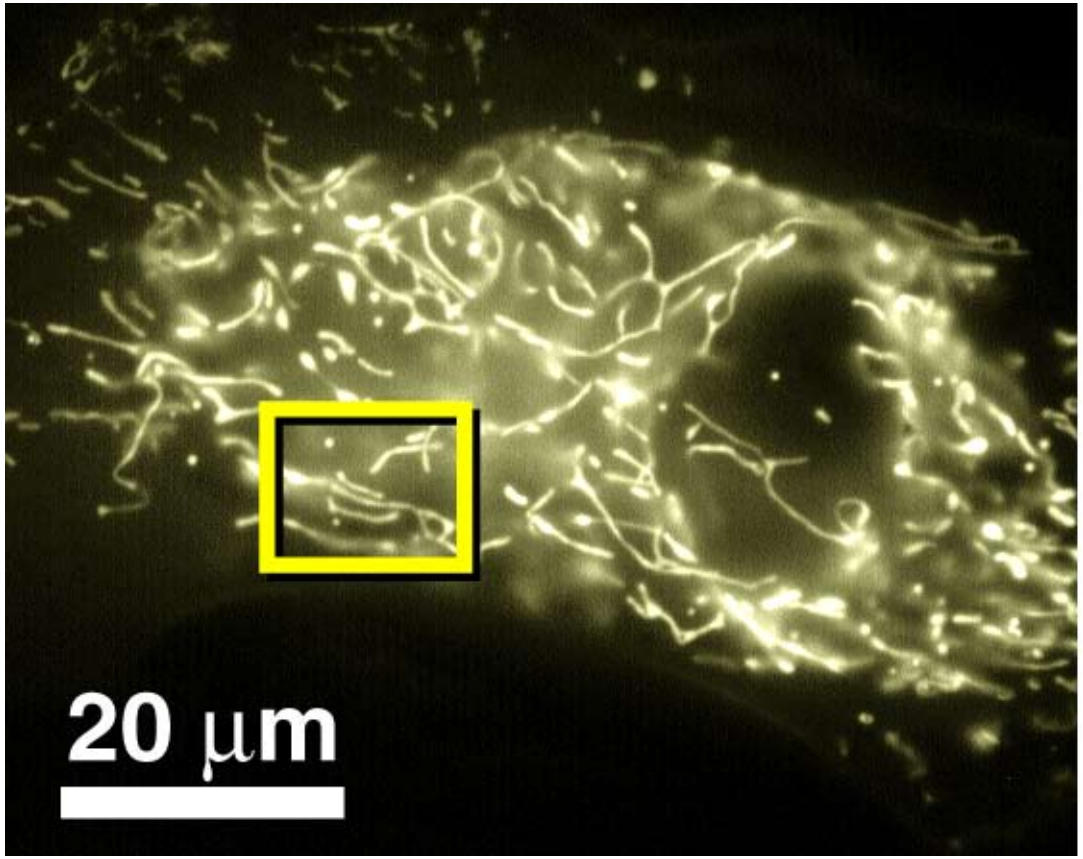
Definitive proof of ablation

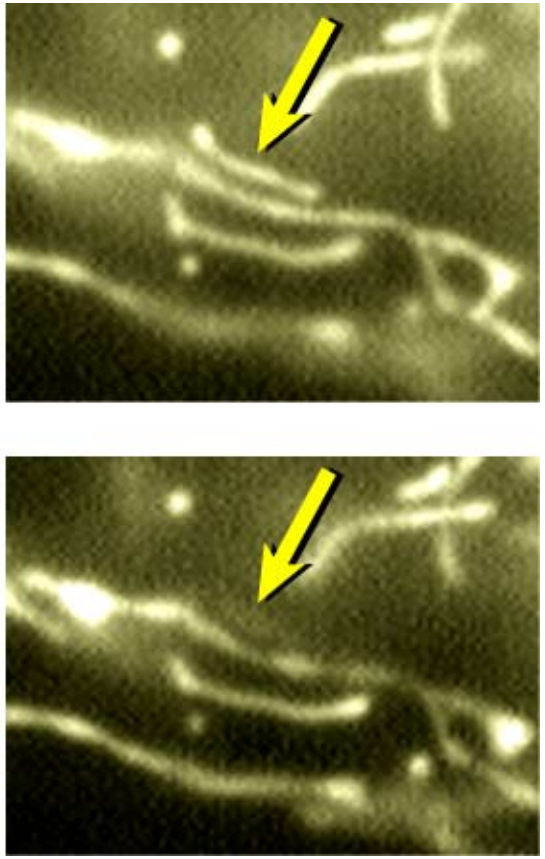
- ablation width as small as 100 nm
- ablation threshold varies slightly
- ablation threshold 20% above bleaching threshold

Subcellular surgery

Q: subcellular surgery on live cells?

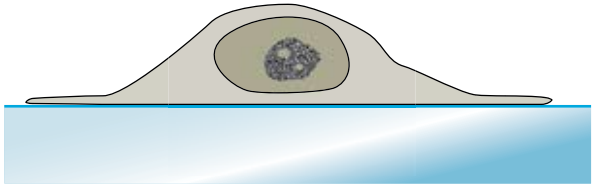
Subcellular surgery

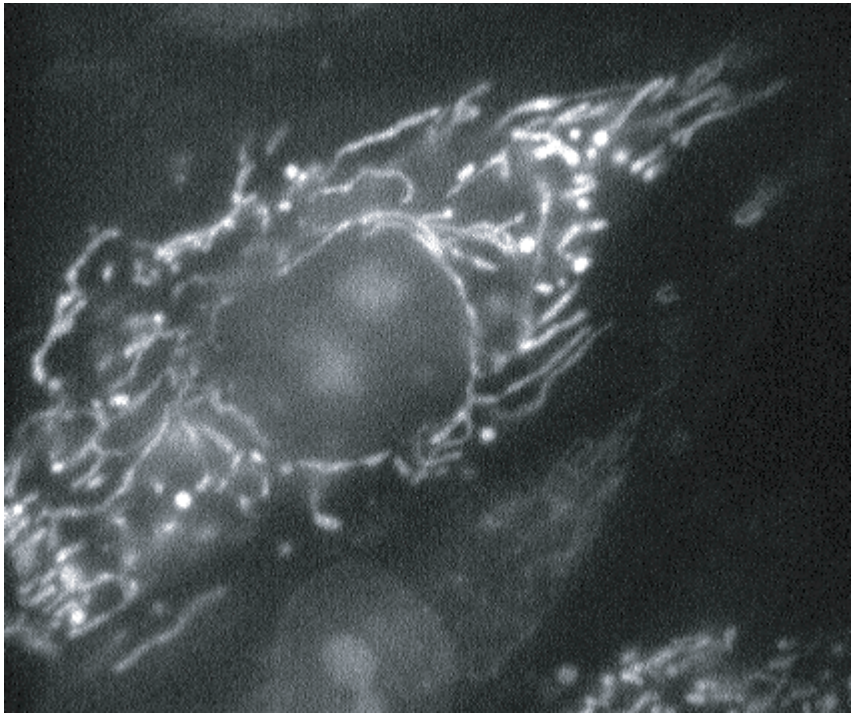




Subcellular surgery

ethydium bromide test





Subcellular surgery

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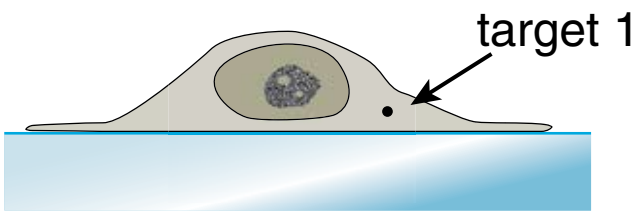
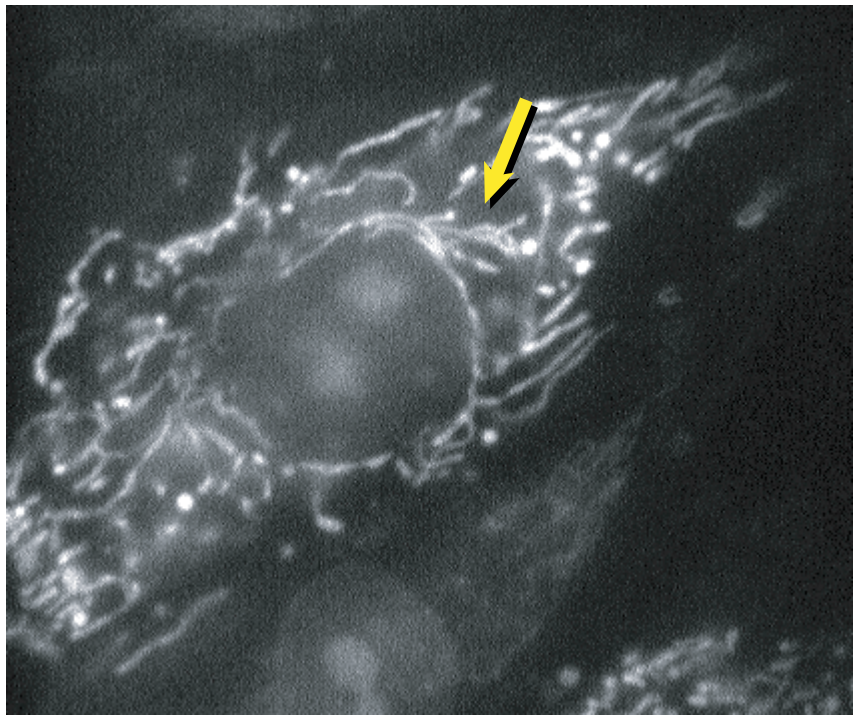
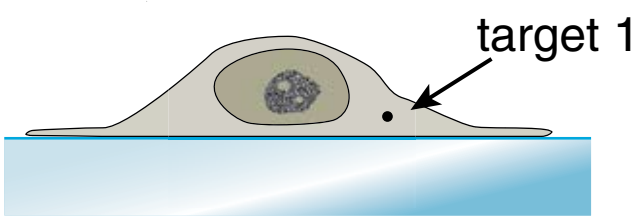
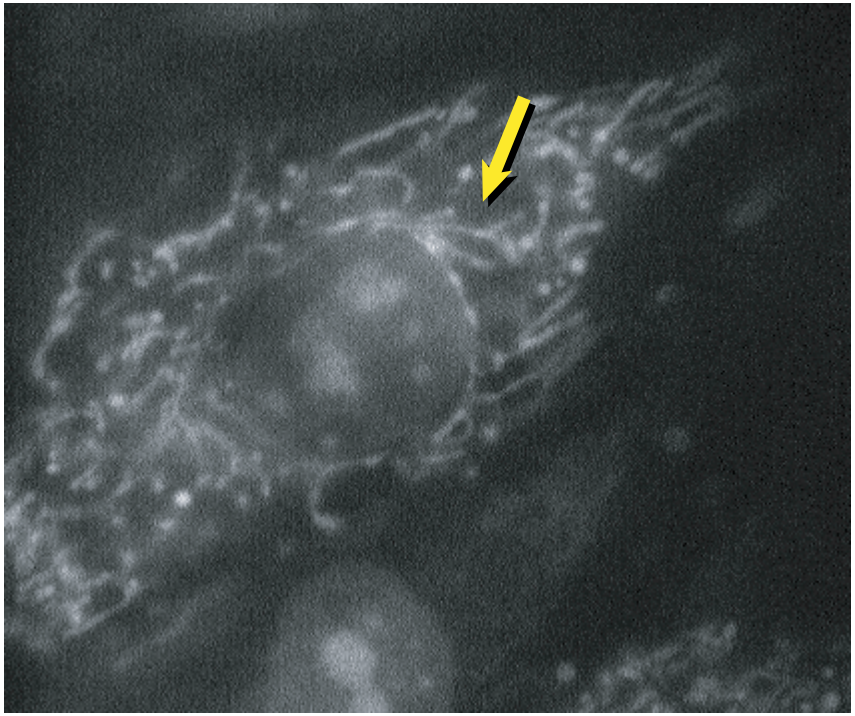




Subcellular surgery

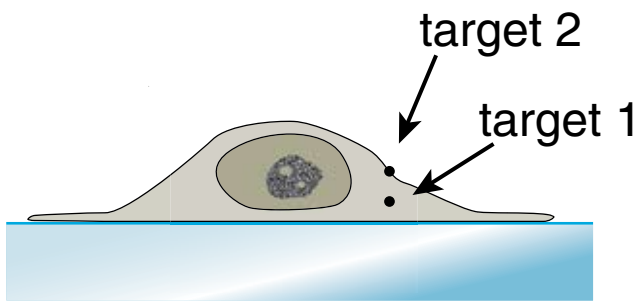
ethydium bromide test

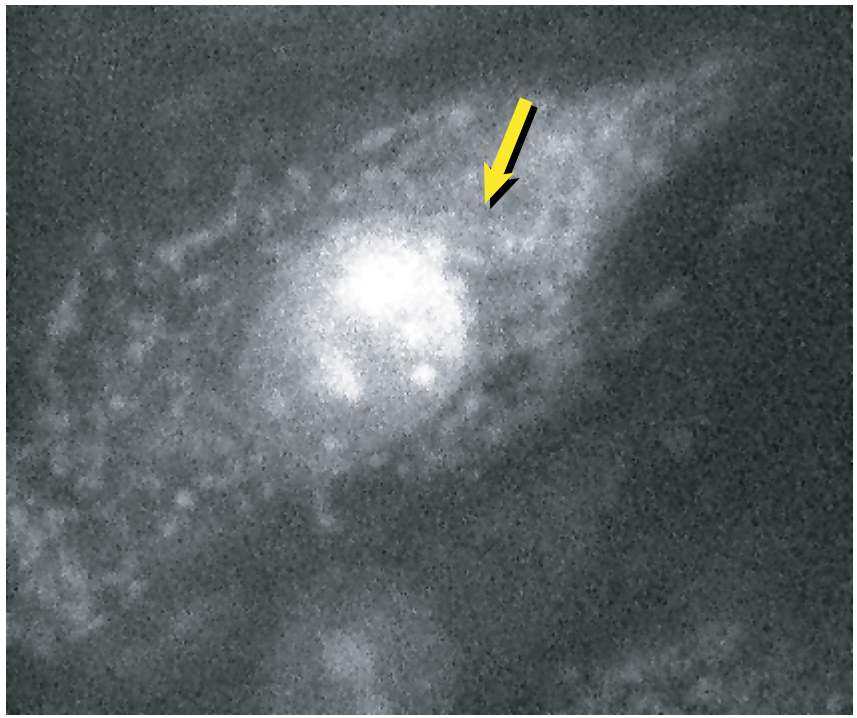




Subcellular surgery

ethydium bromide test



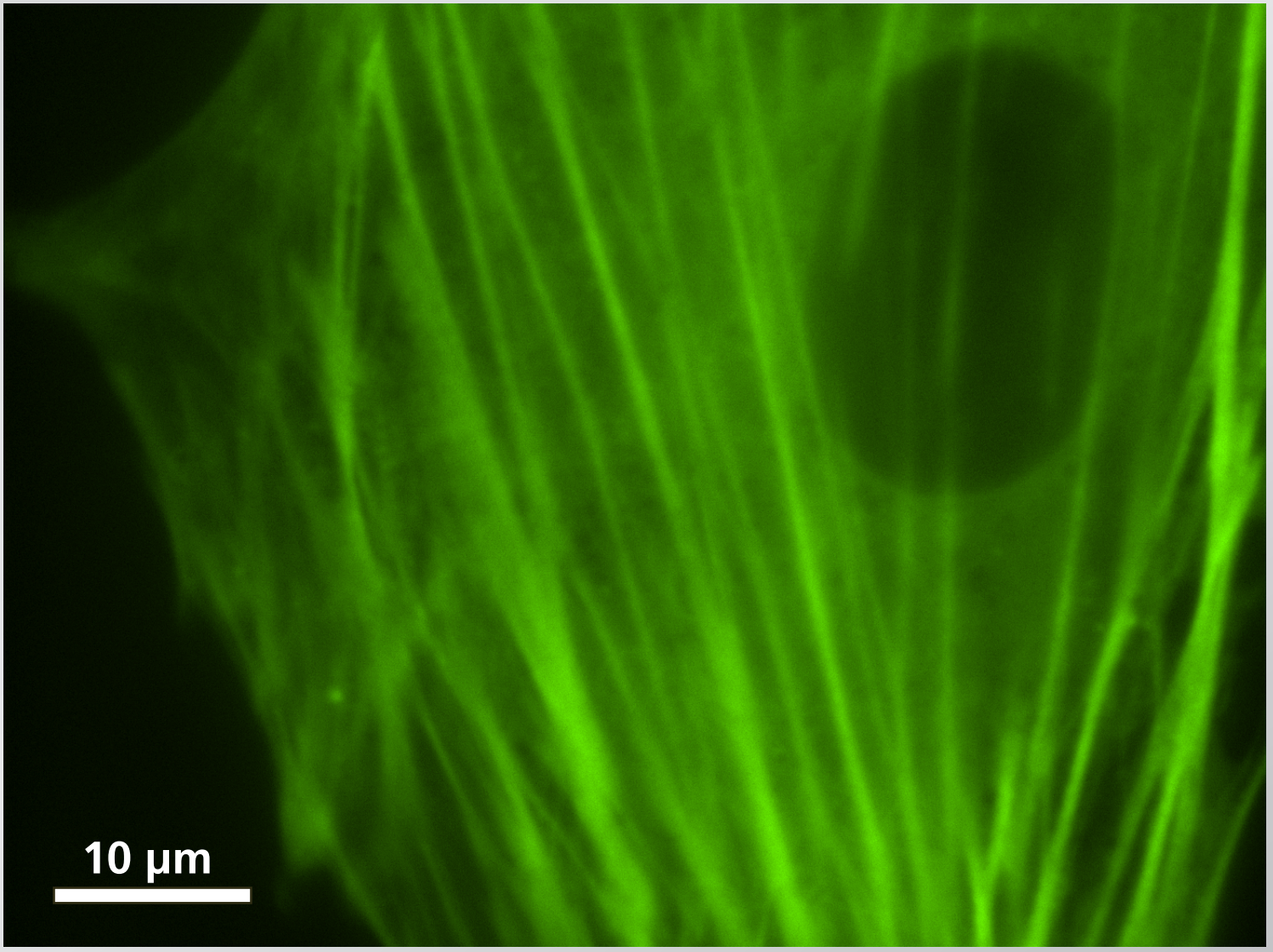


Subcellular surgery

Q: can we probe the dynamics of the cytoskeleton?

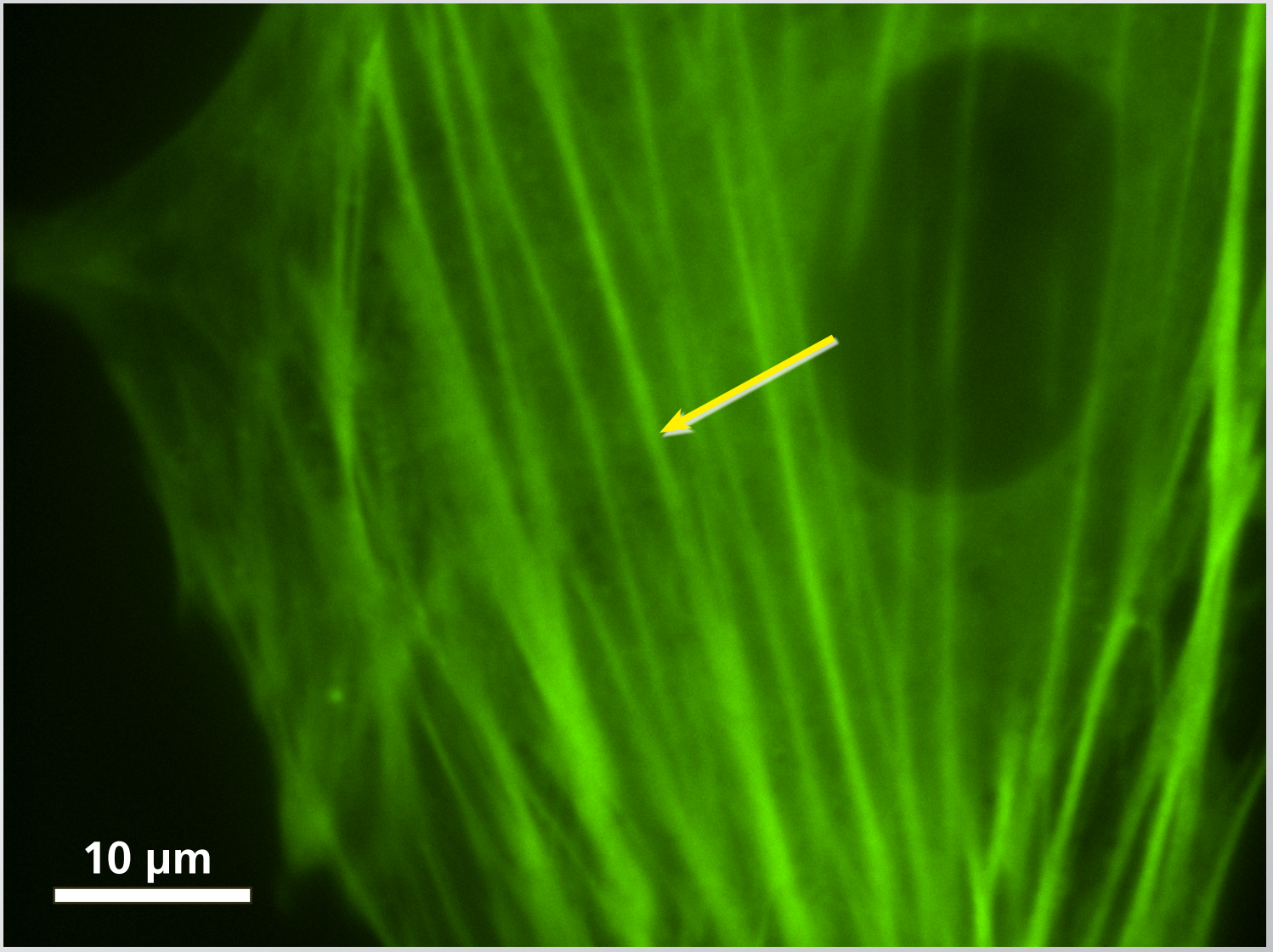
Subcellular surgery

YFP-labeled actin fiber network of a live cell



Subcellular surgery

cut a single fiber bundle



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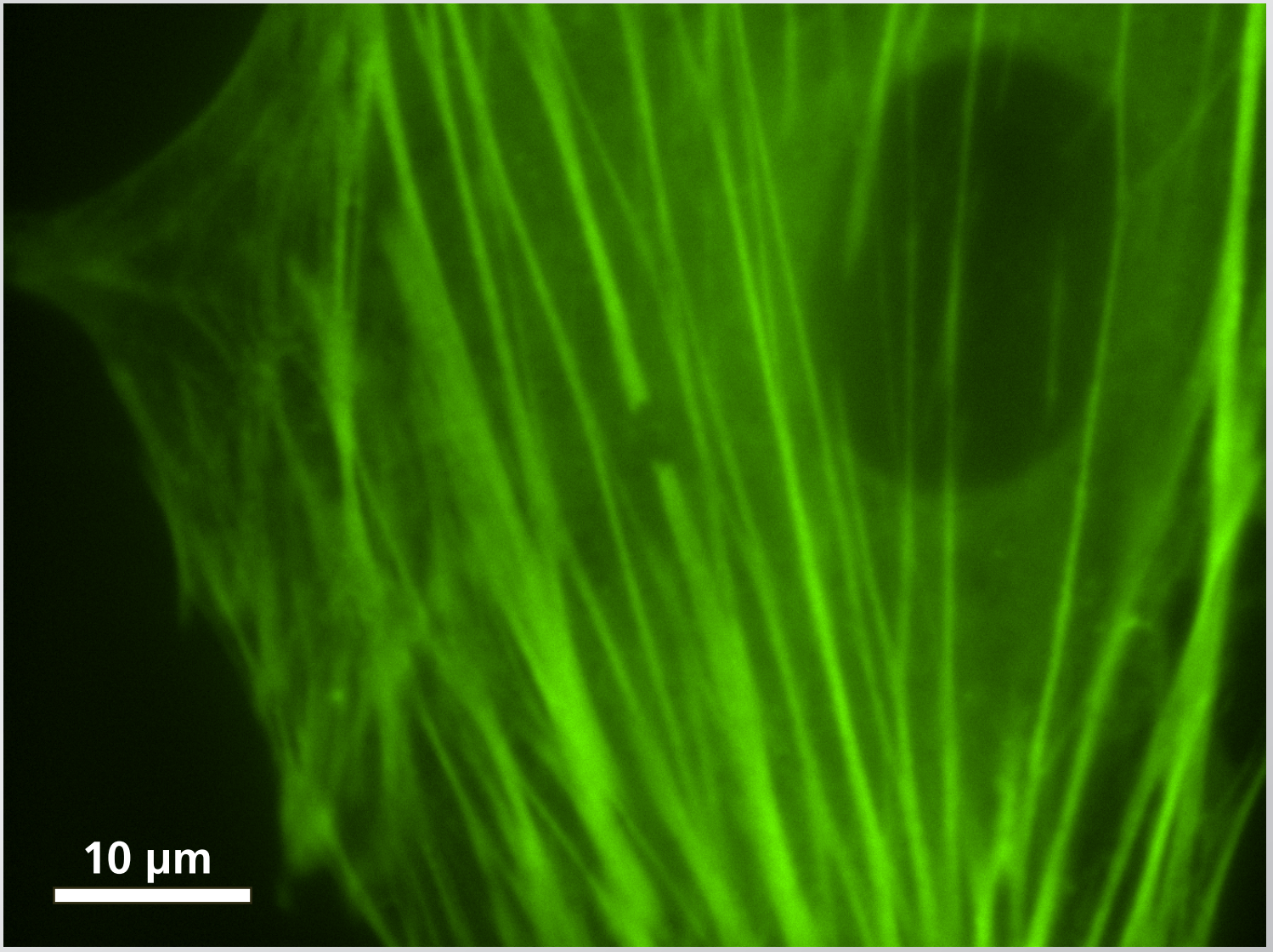
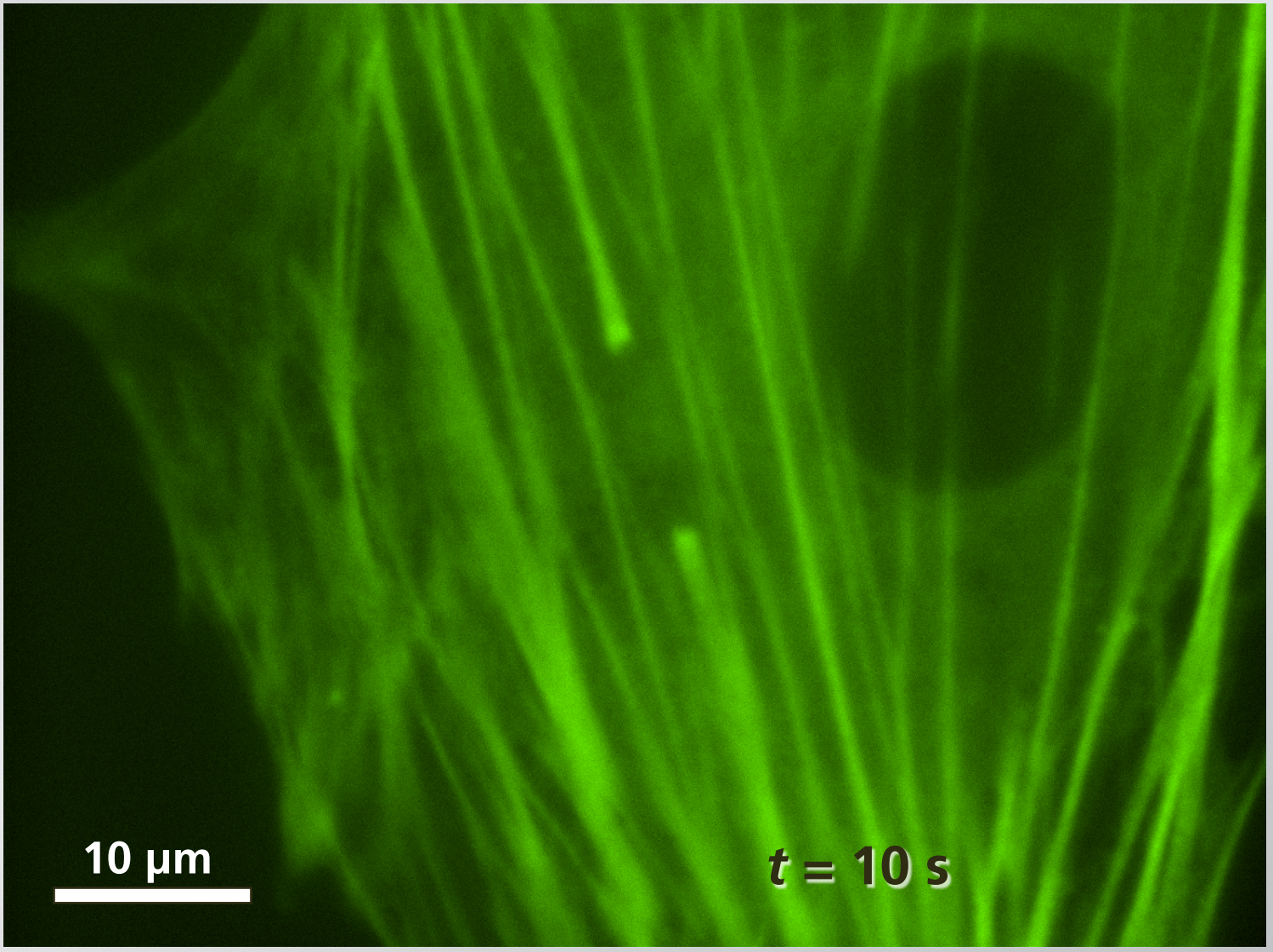


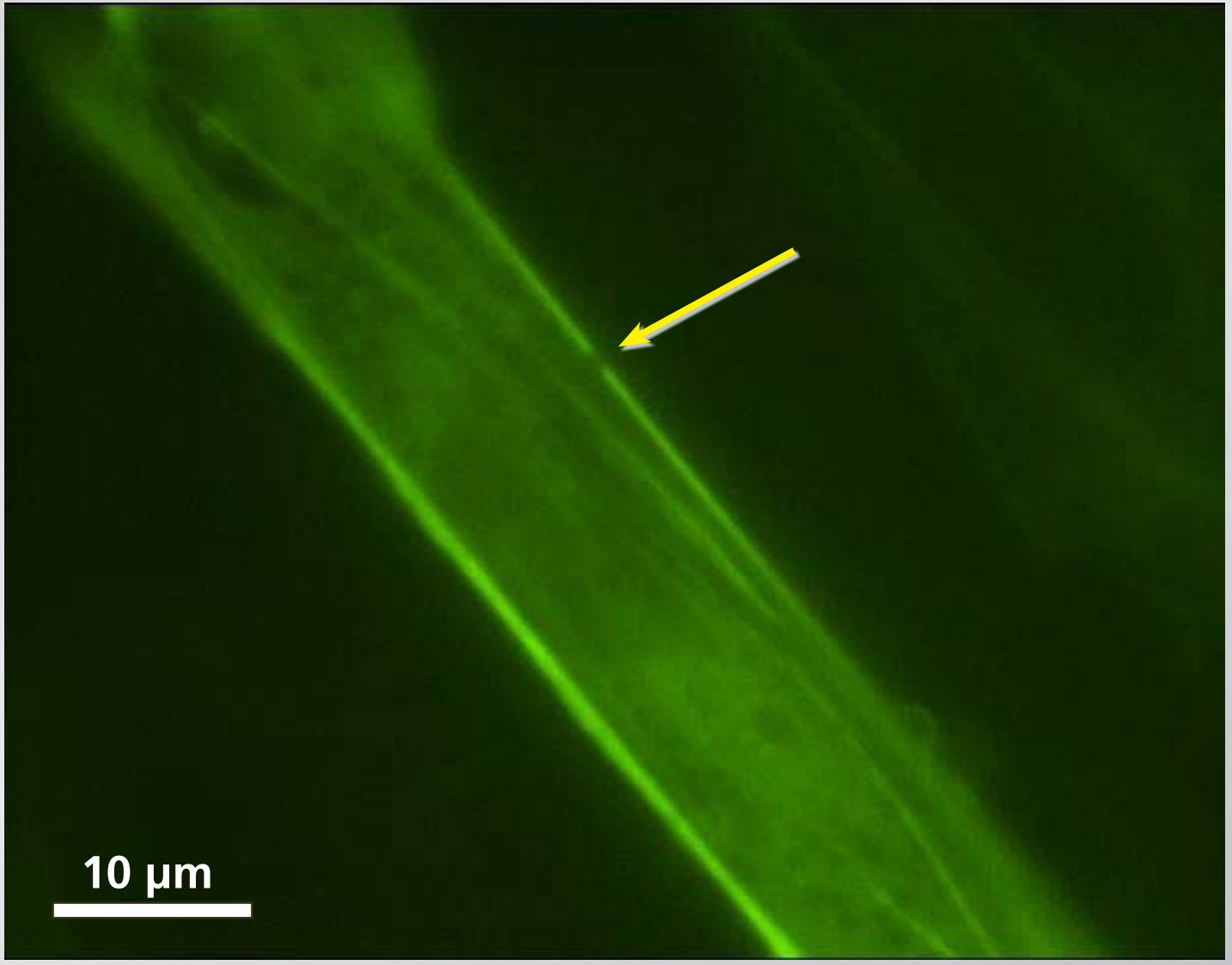
Subcellular surgery

gap widens with time



Subcellular surgery

retraction or depolymerization?



Subcellular surgery

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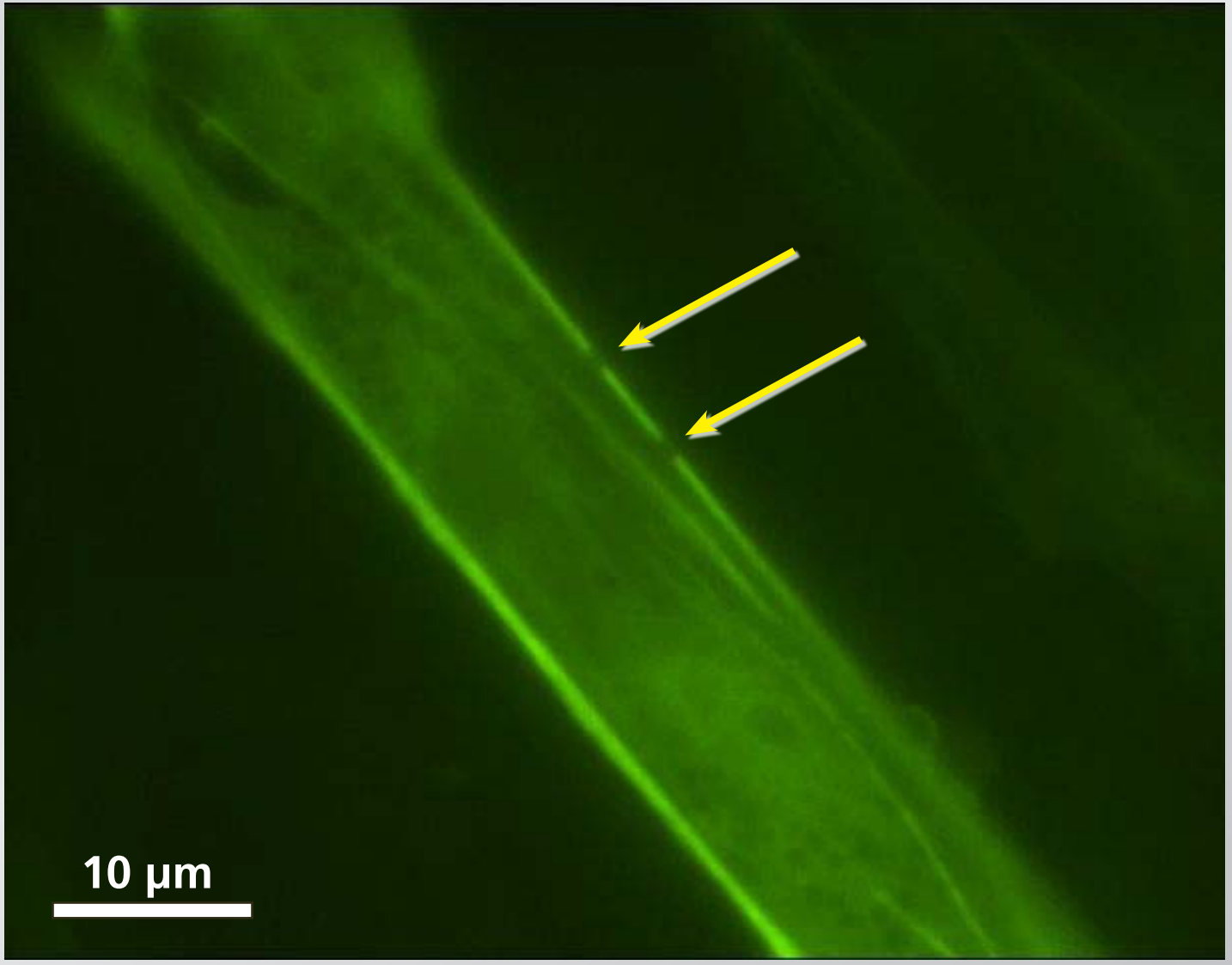
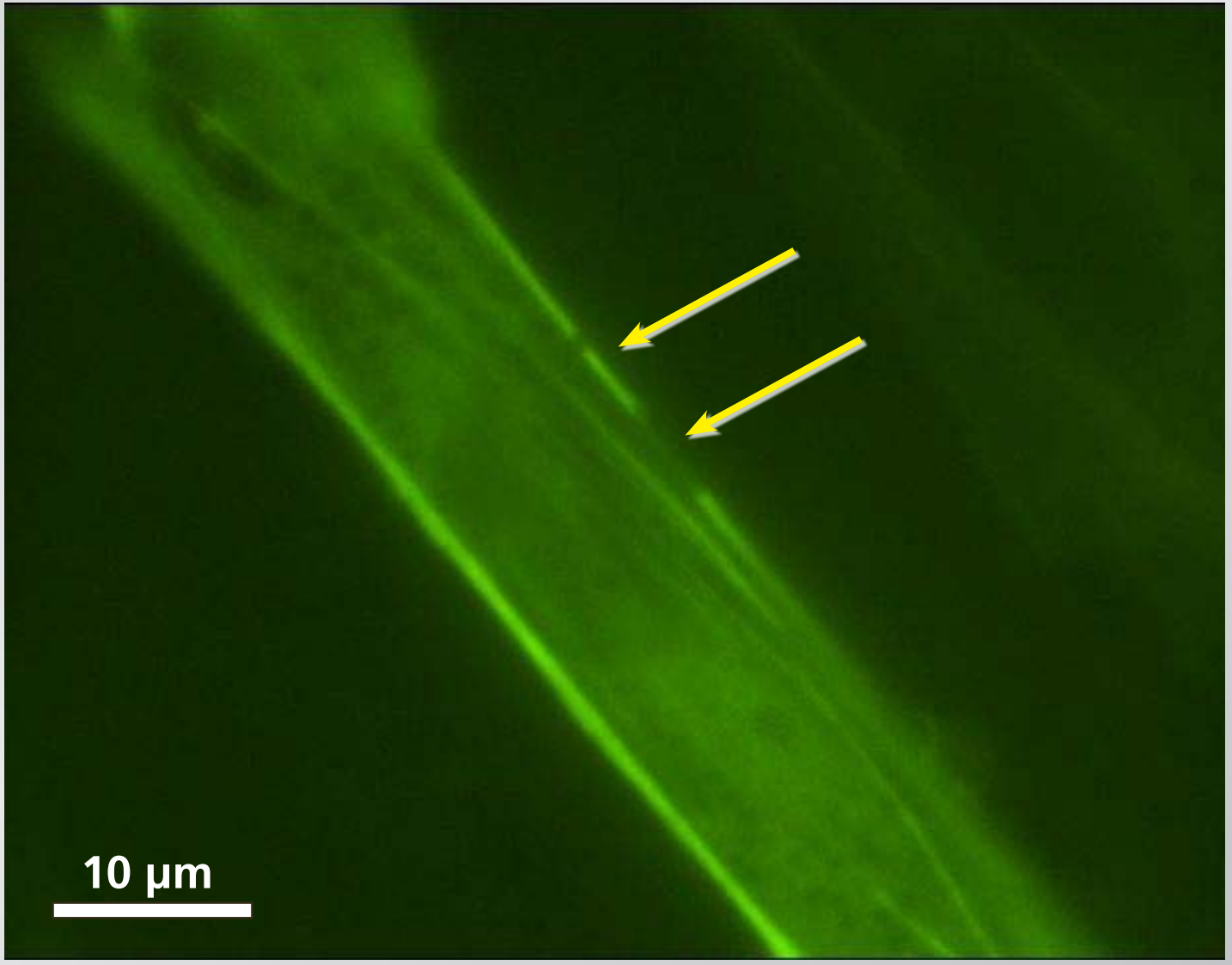


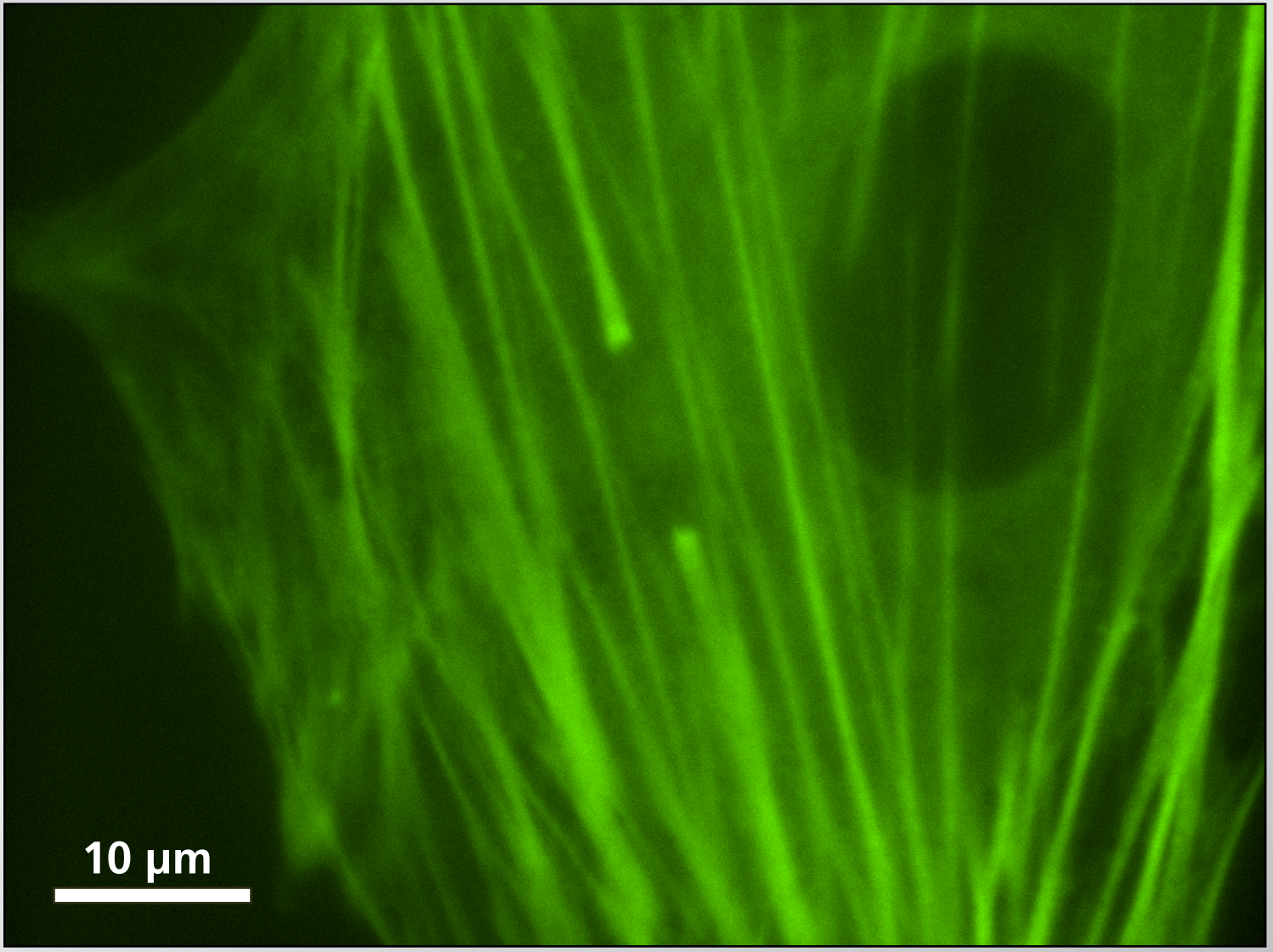
Subcellular surgery

retraction!

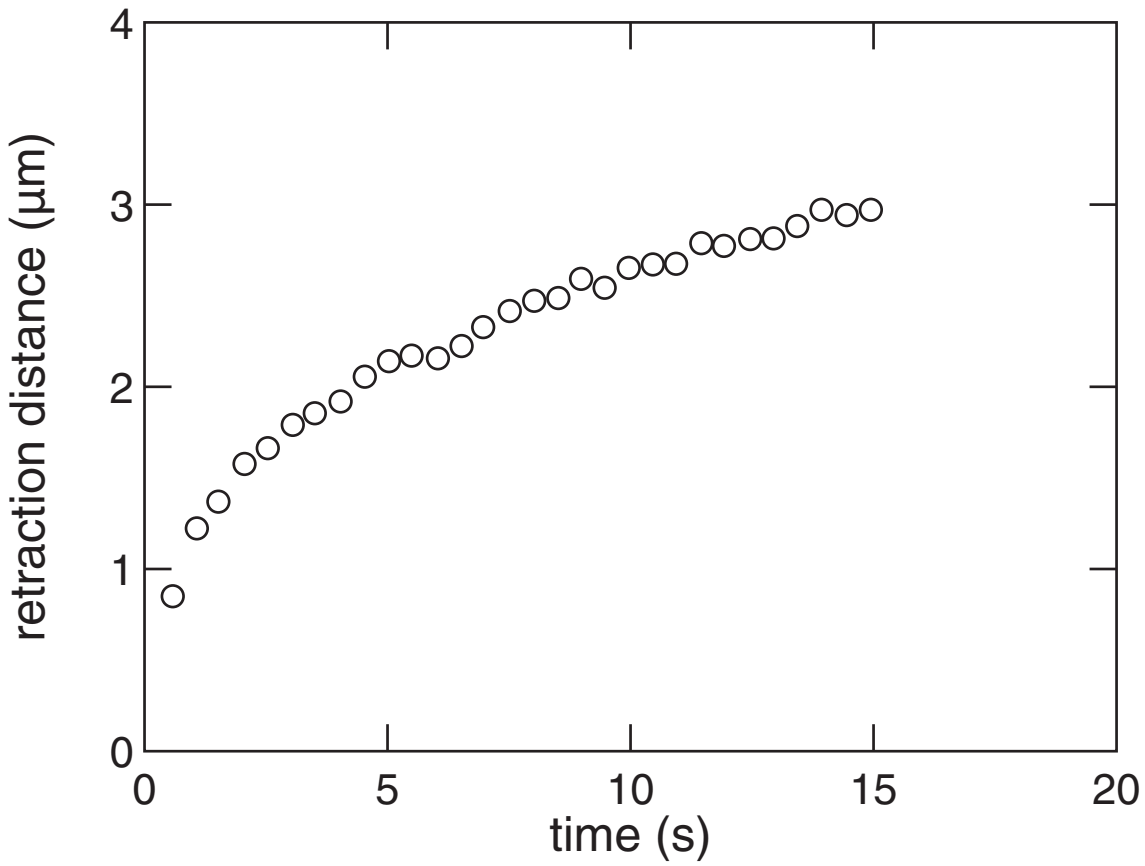


Subcellular surgery

dynamics provides information on *in vivo* mechanics

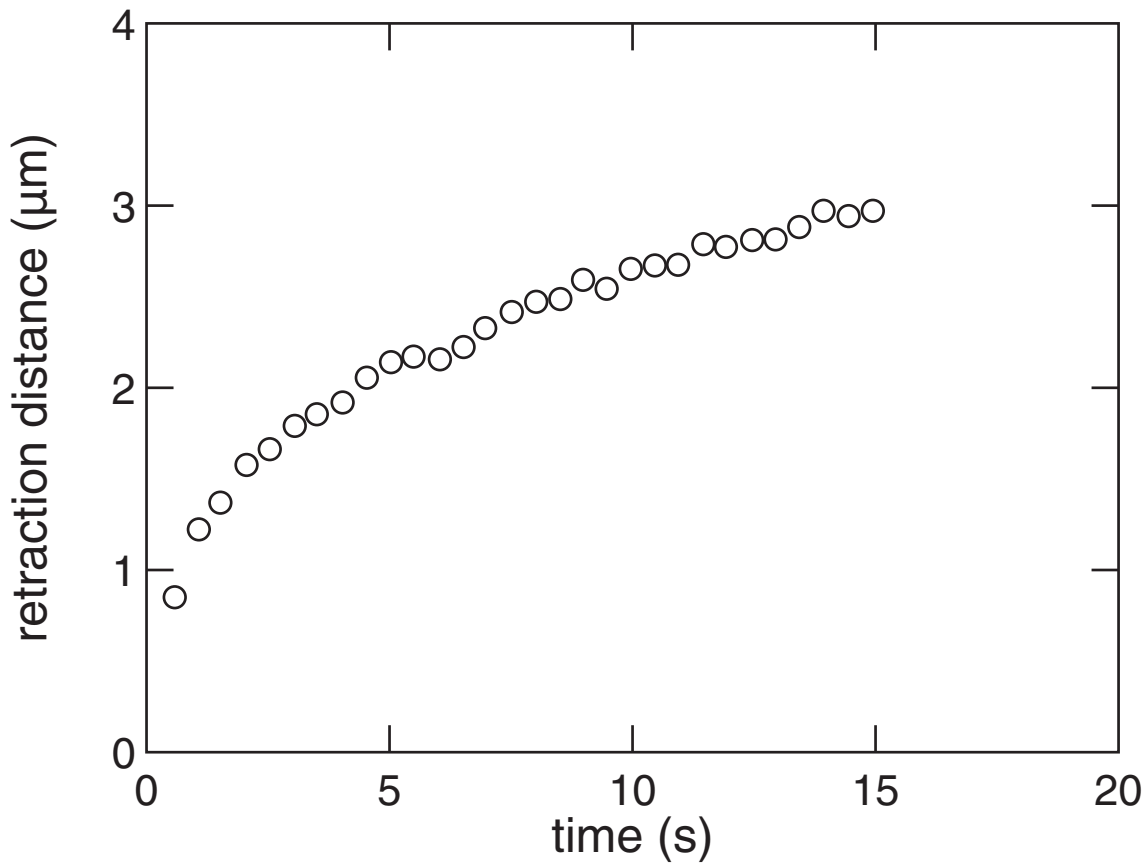


Subcellular surgery



Subcellular surgery

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



Subcellular surgery

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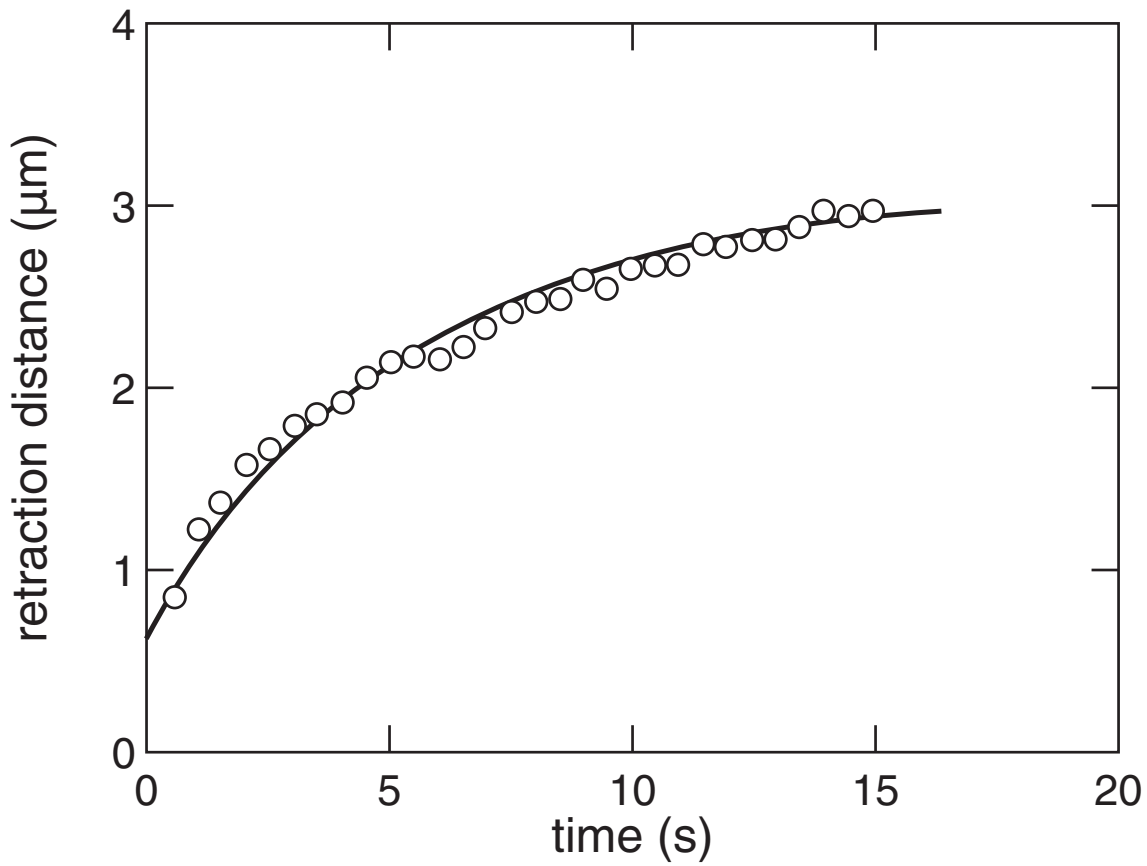
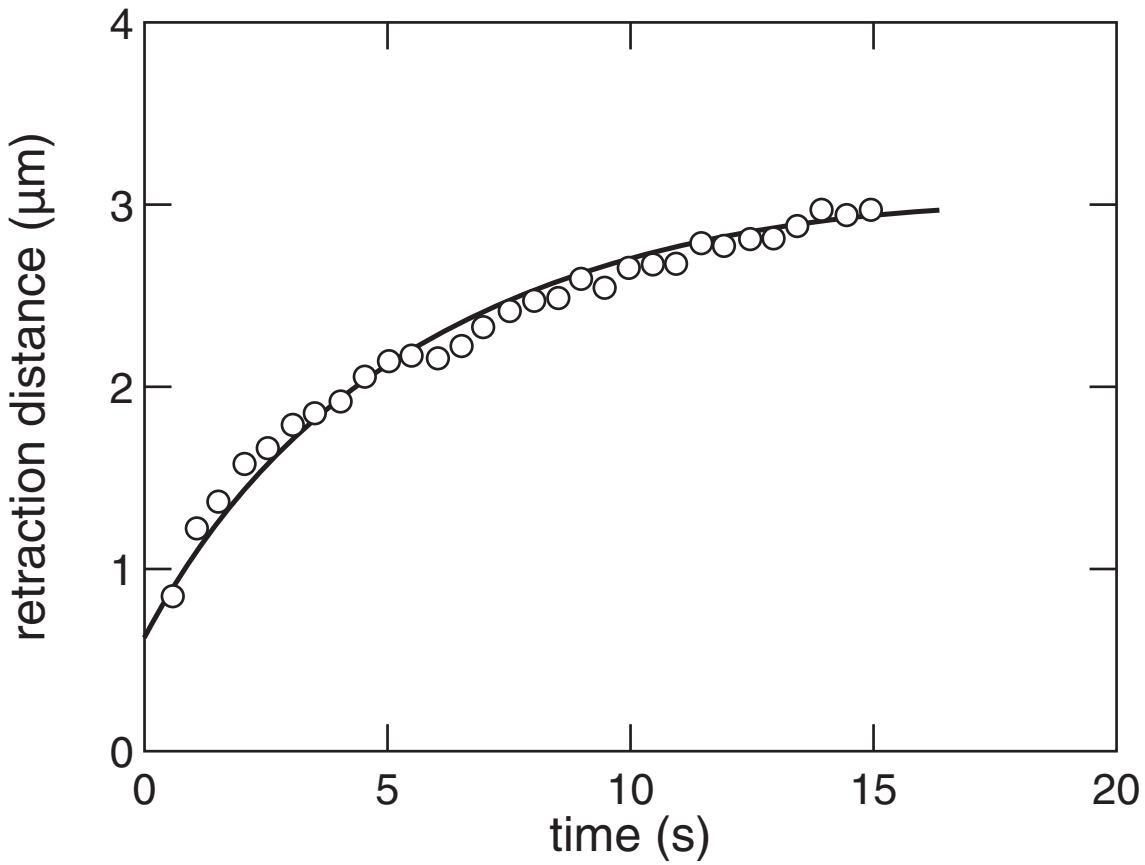


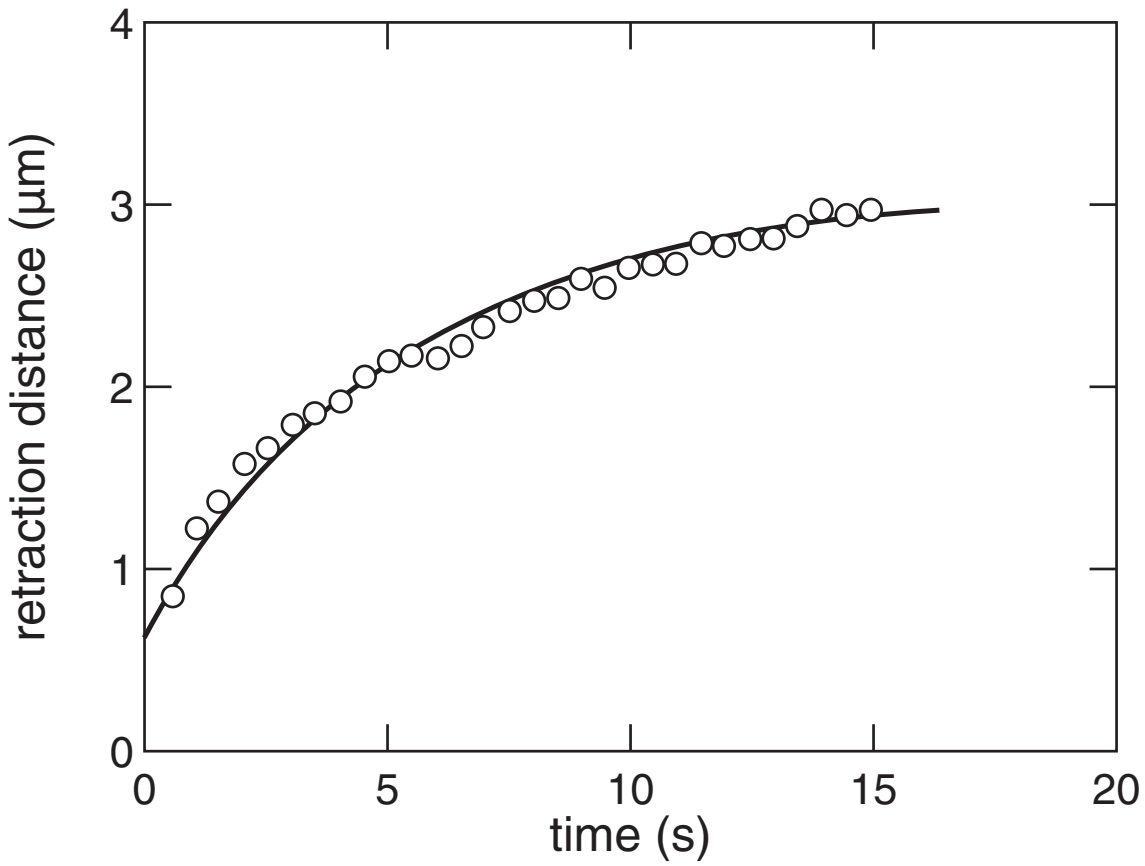
Subcellular surgery

L_o and τ independent of fiber width!



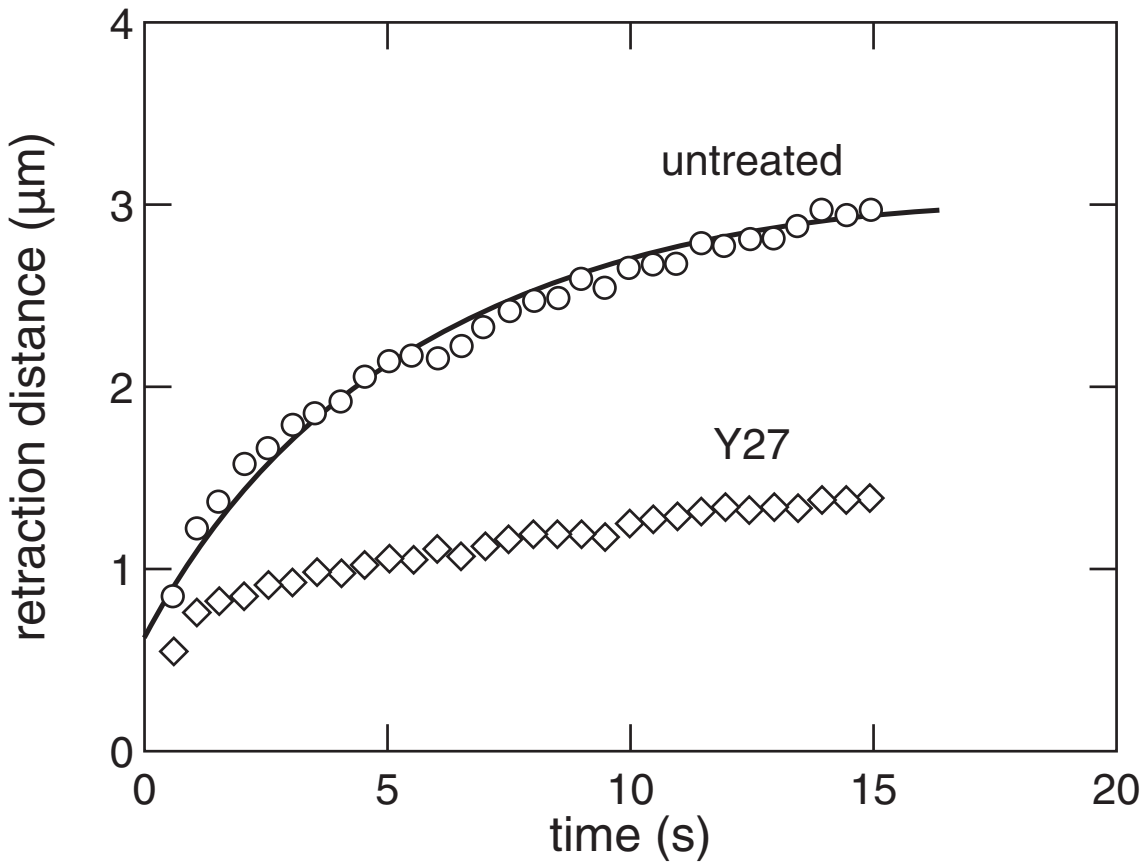
Subcellular surgery

tension in actin filaments is generated by myosin motors



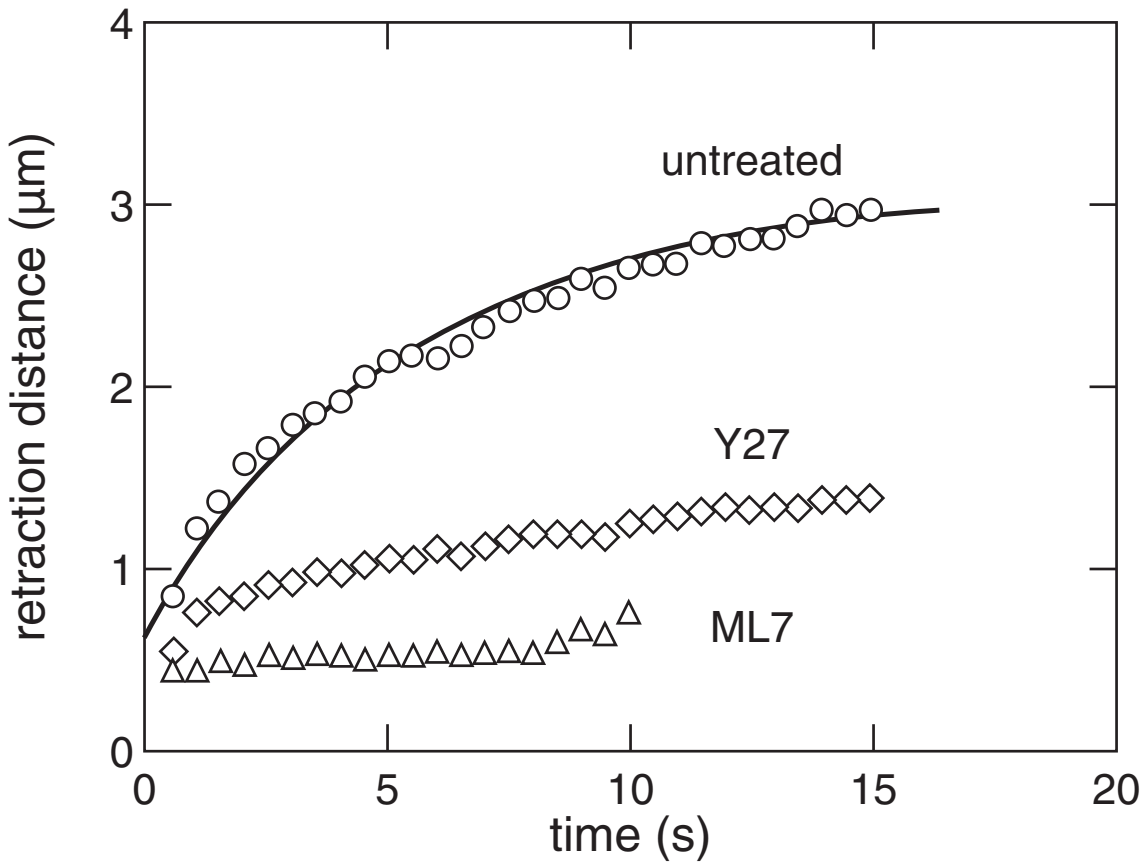
Subcellular surgery

Y27: inhibits some myosin activity



Subcellular surgery

ML7: direct inhibitor of myosin activity



Outline

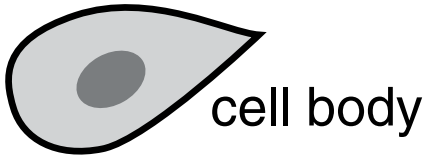
- femtosecond materials interactions
- subcellular surgery
- nanoneurosurgery

Nanoneurosurgery

Q: can we probe the neurological origins of behavior?

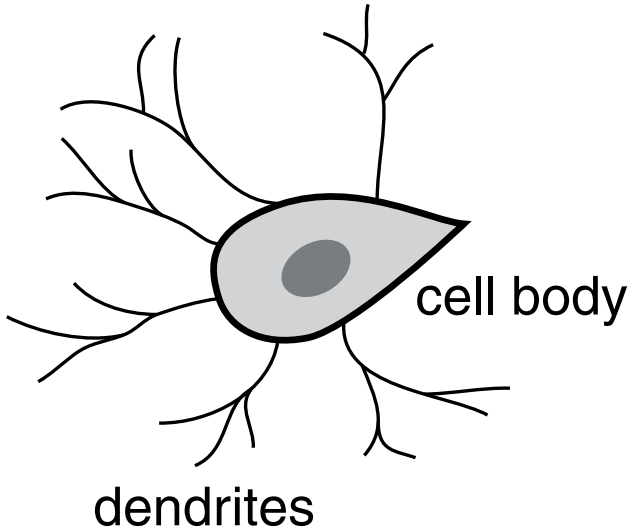
Nanoneurosurgery

neuron basics



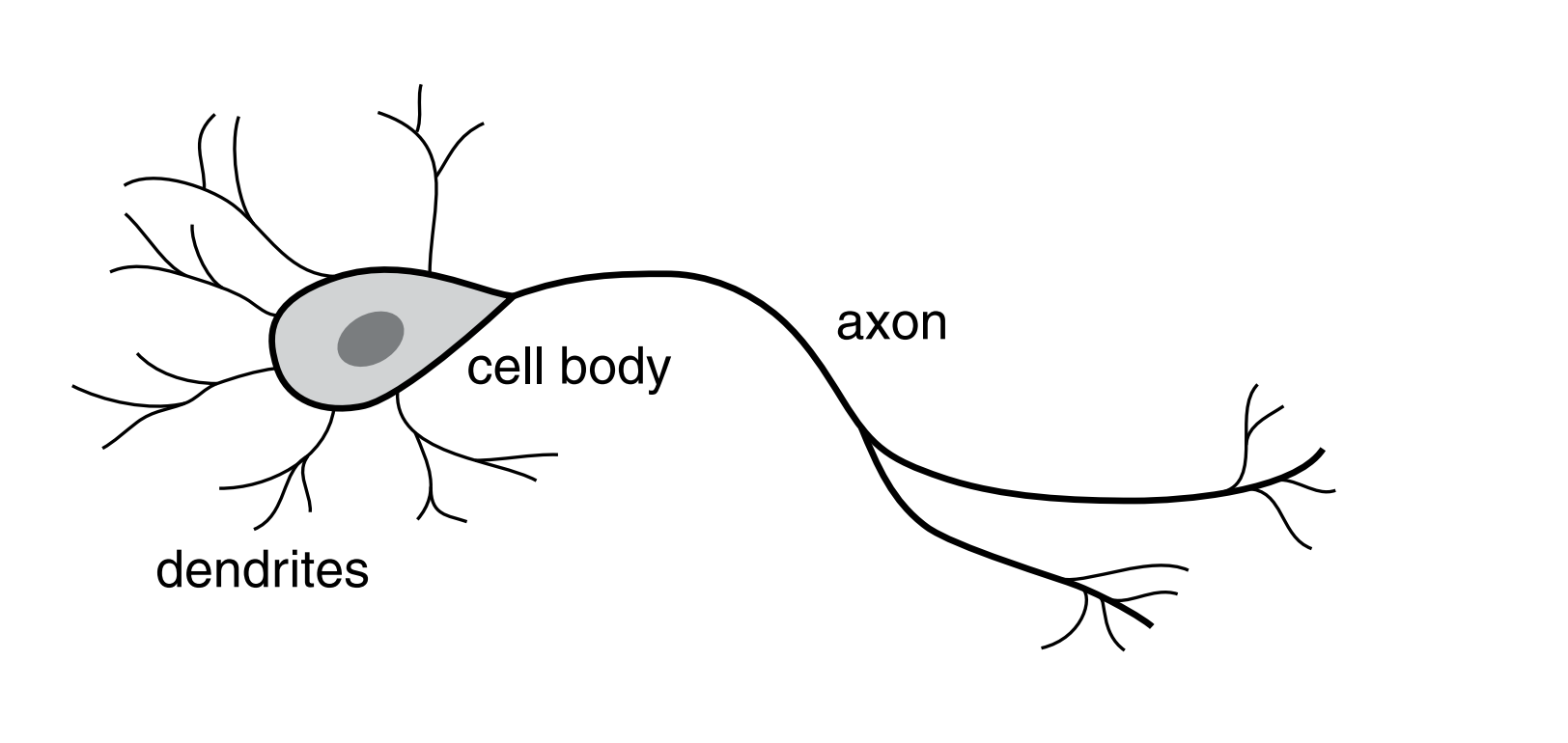
Nanoneurosurgery

neuron basics



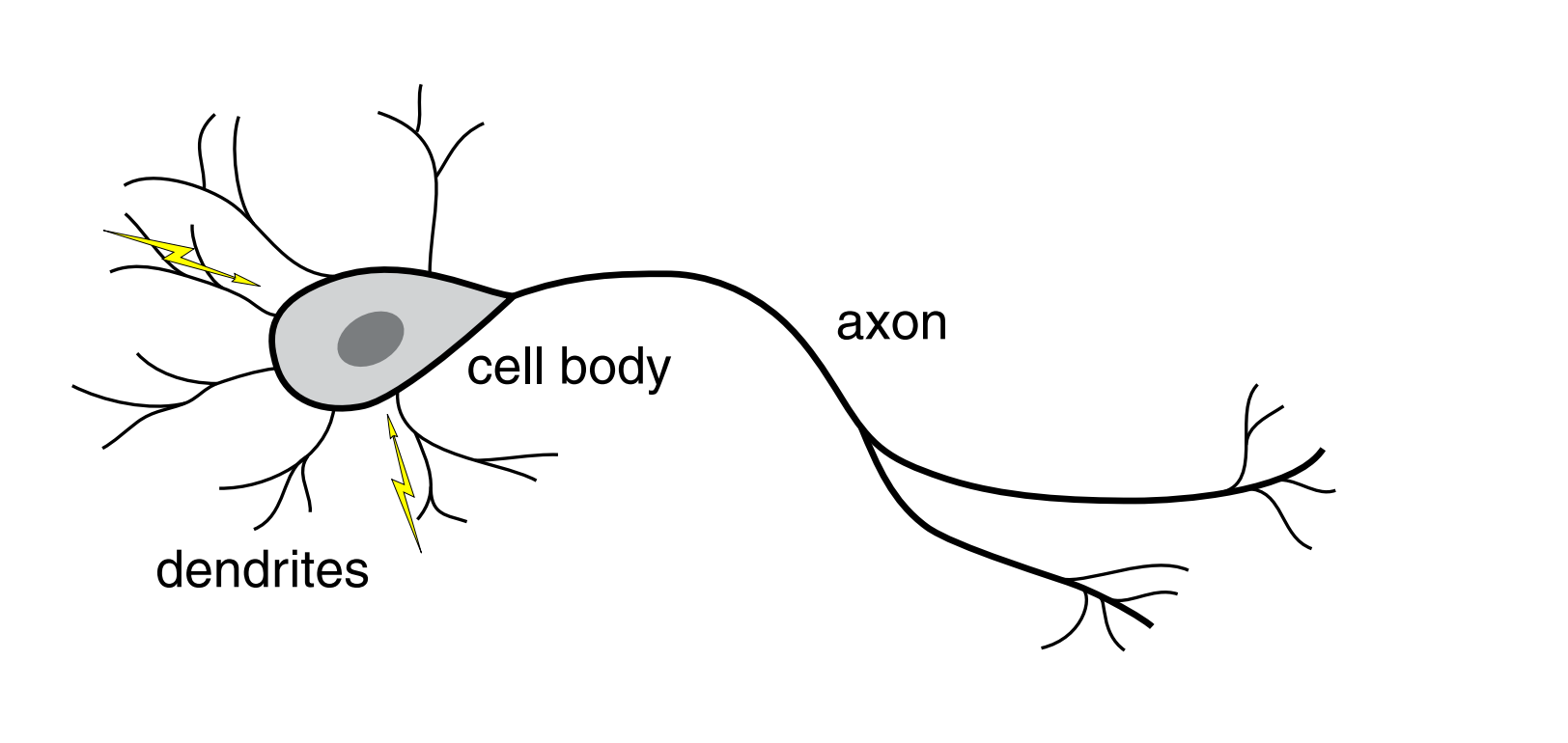
Nanoneurosurgery

neuron basics



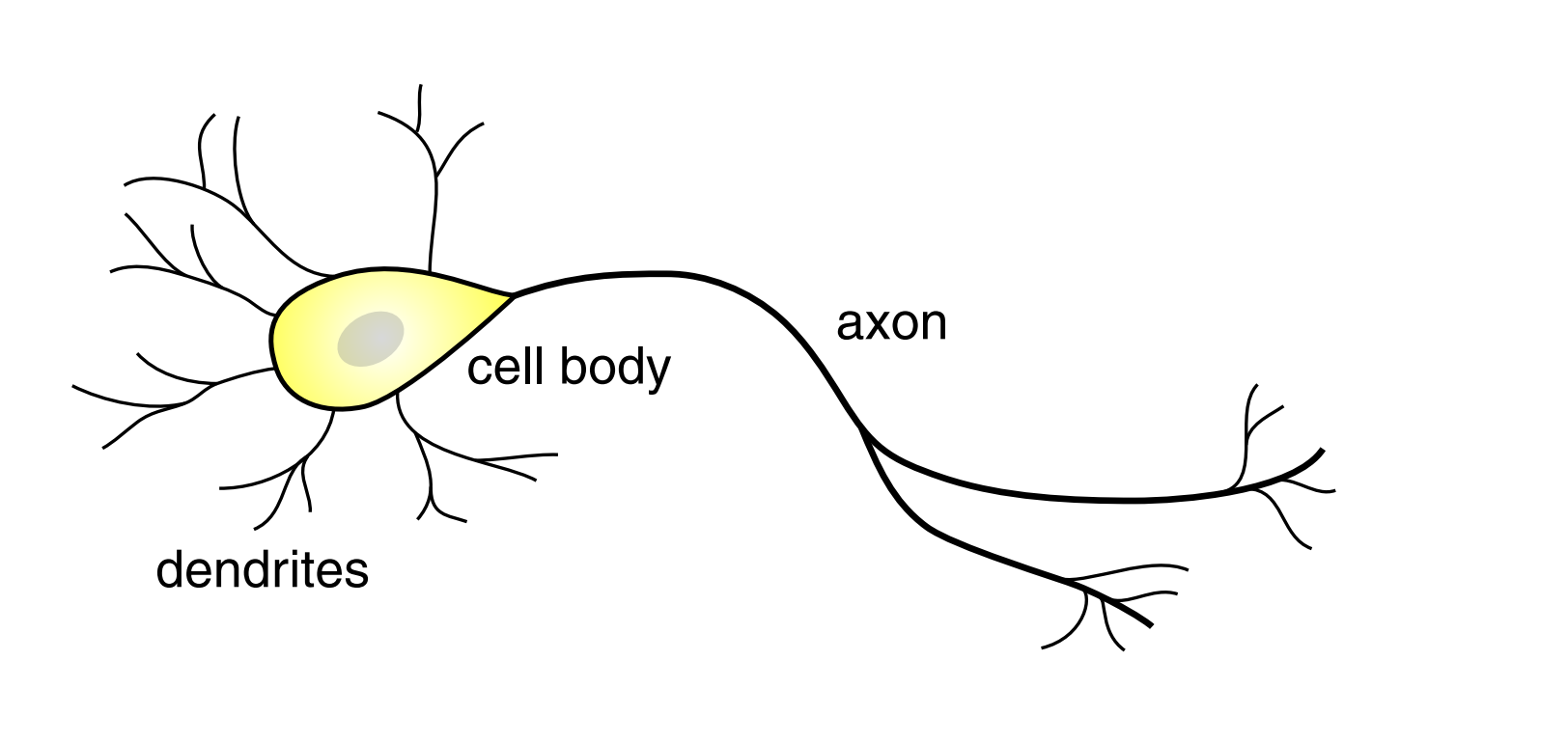
Nanoneurosurgery

neuron basics



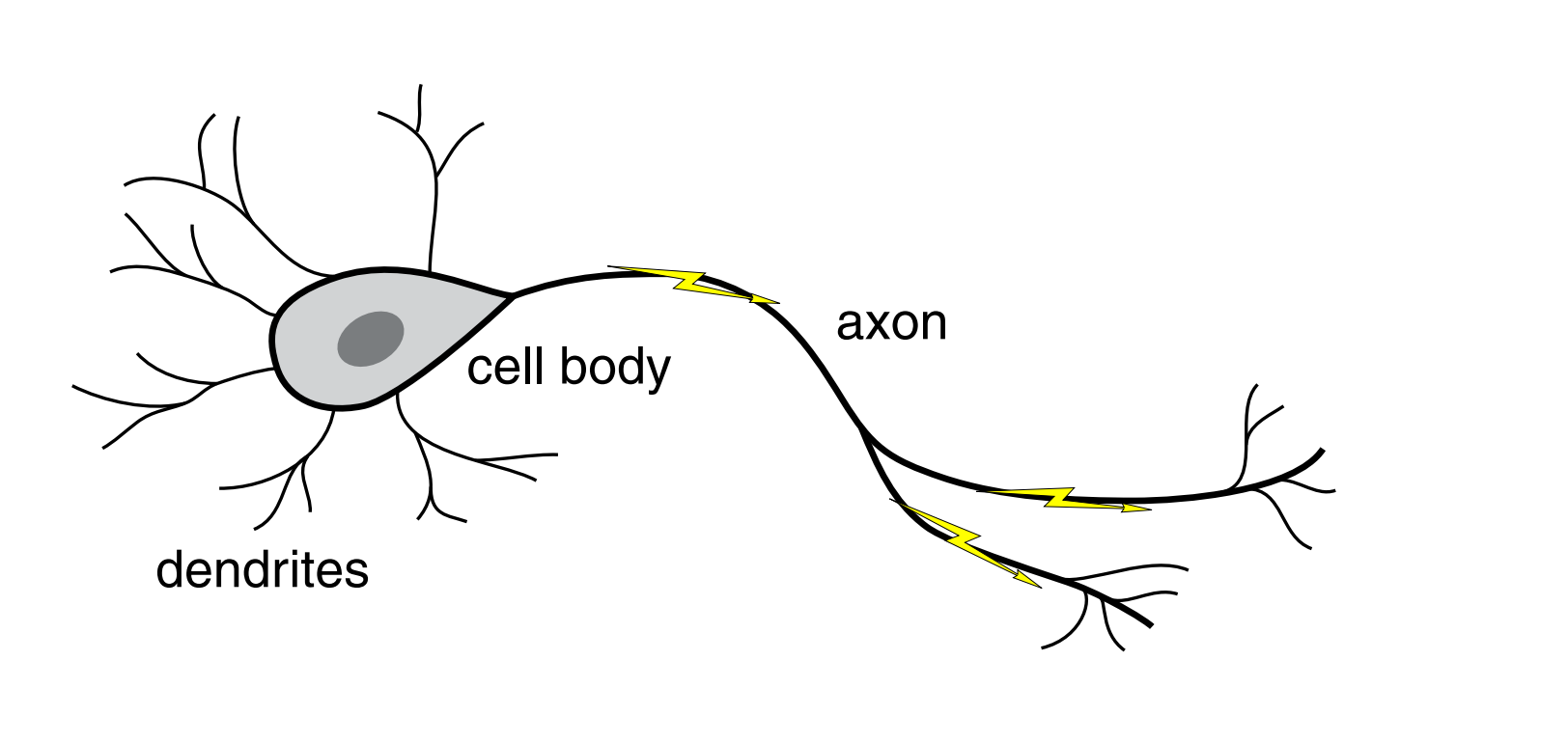
Nanoneurosurgery

neuron basics



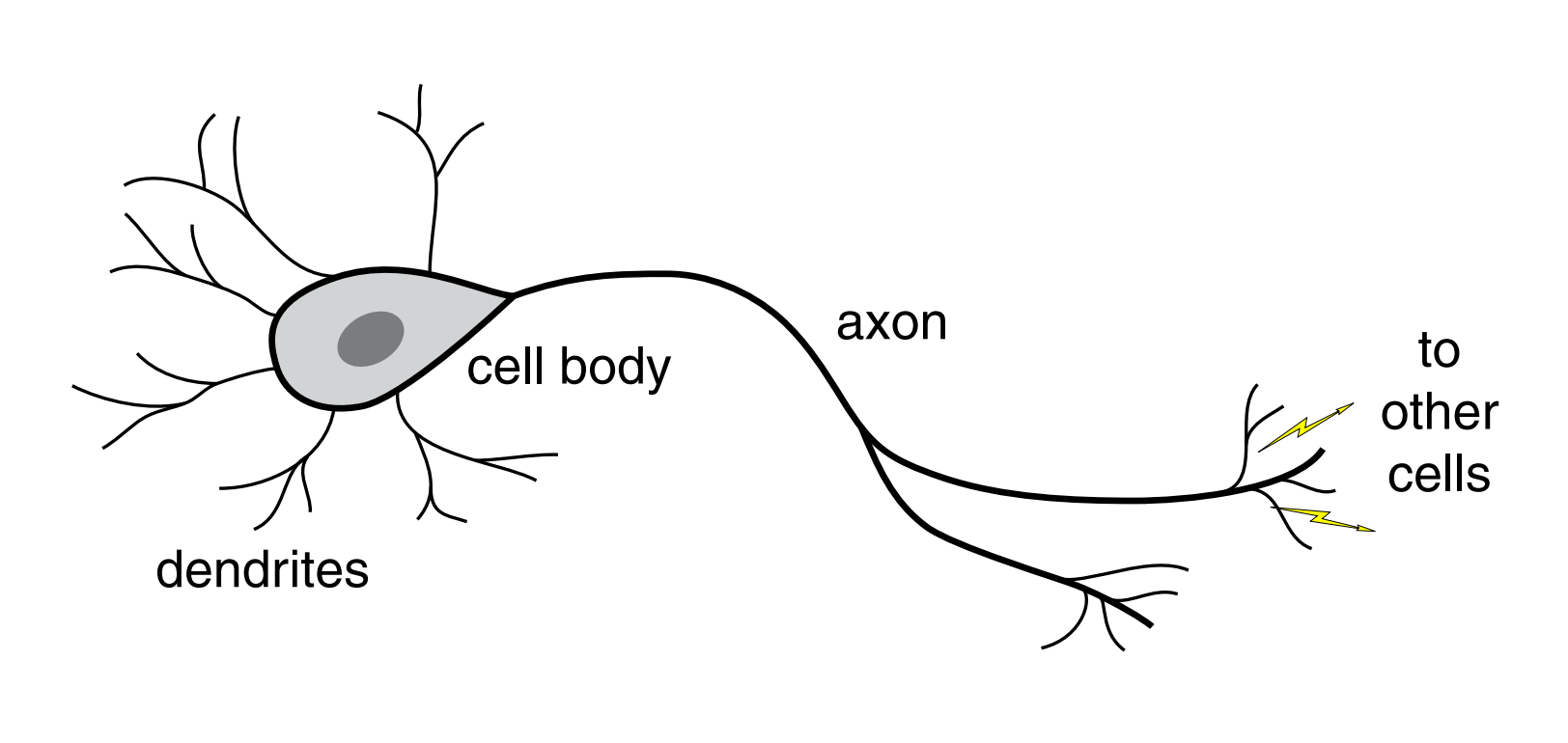
Nanoneurosurgery

neuron basics



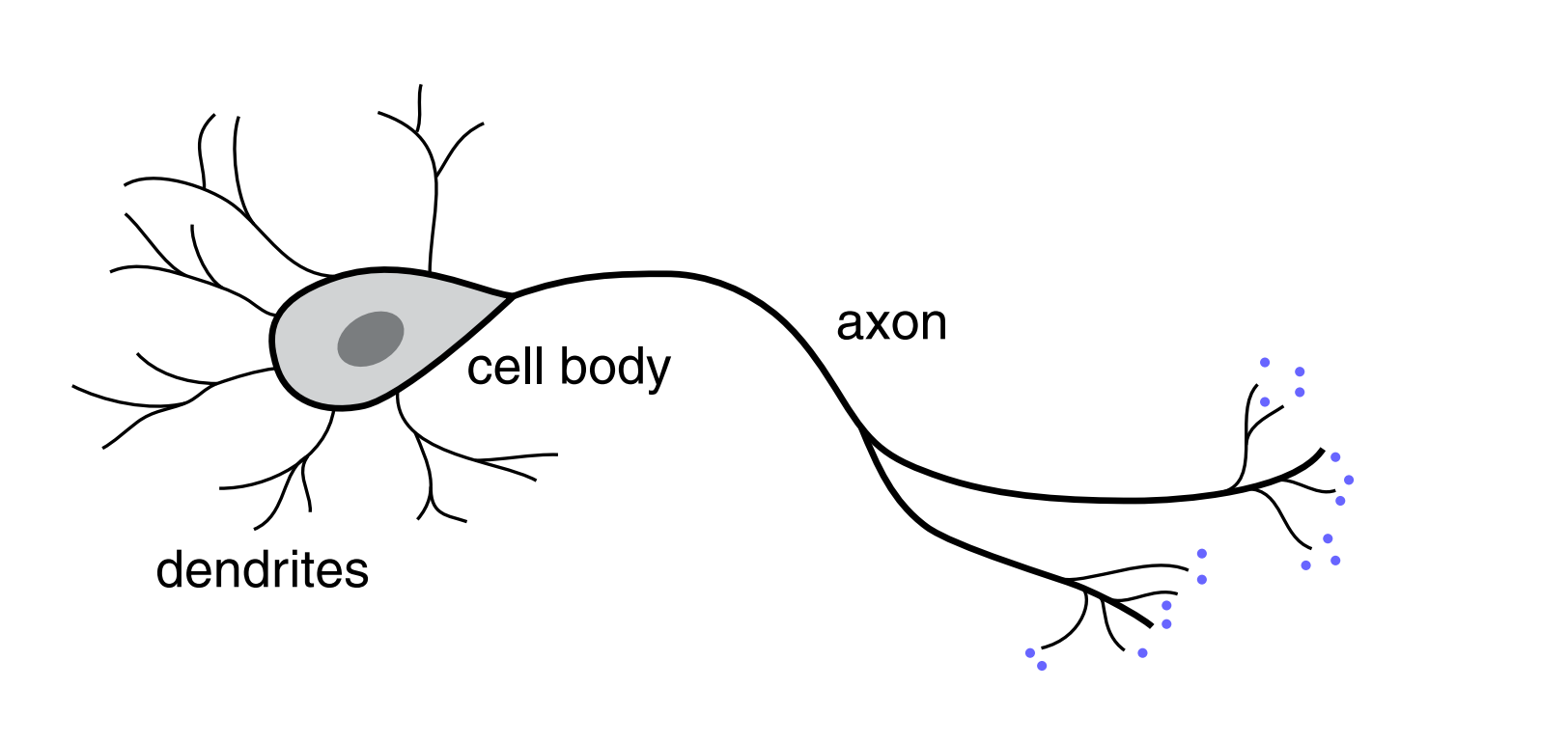
Nanoneurosurgery

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

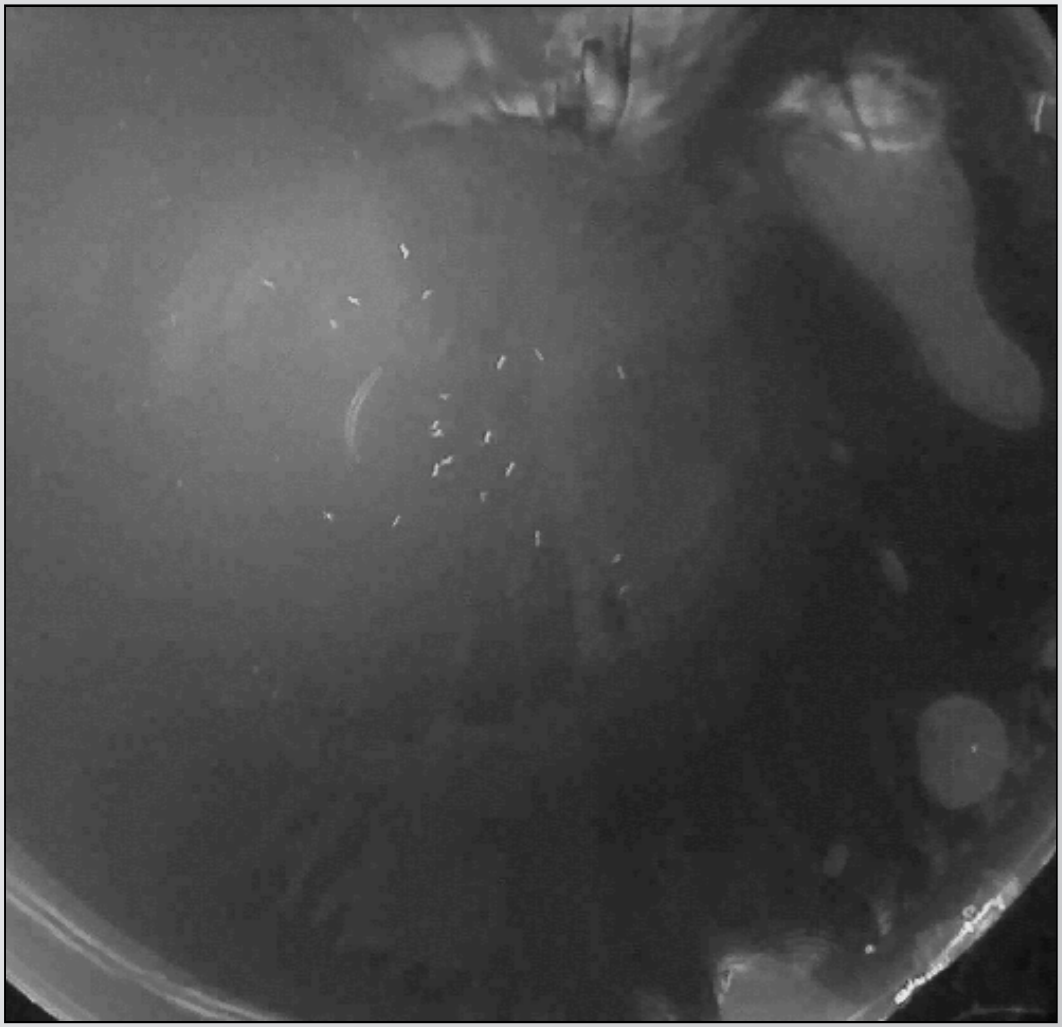
Nanoneurosurgery

Caenorhabditis elegans

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

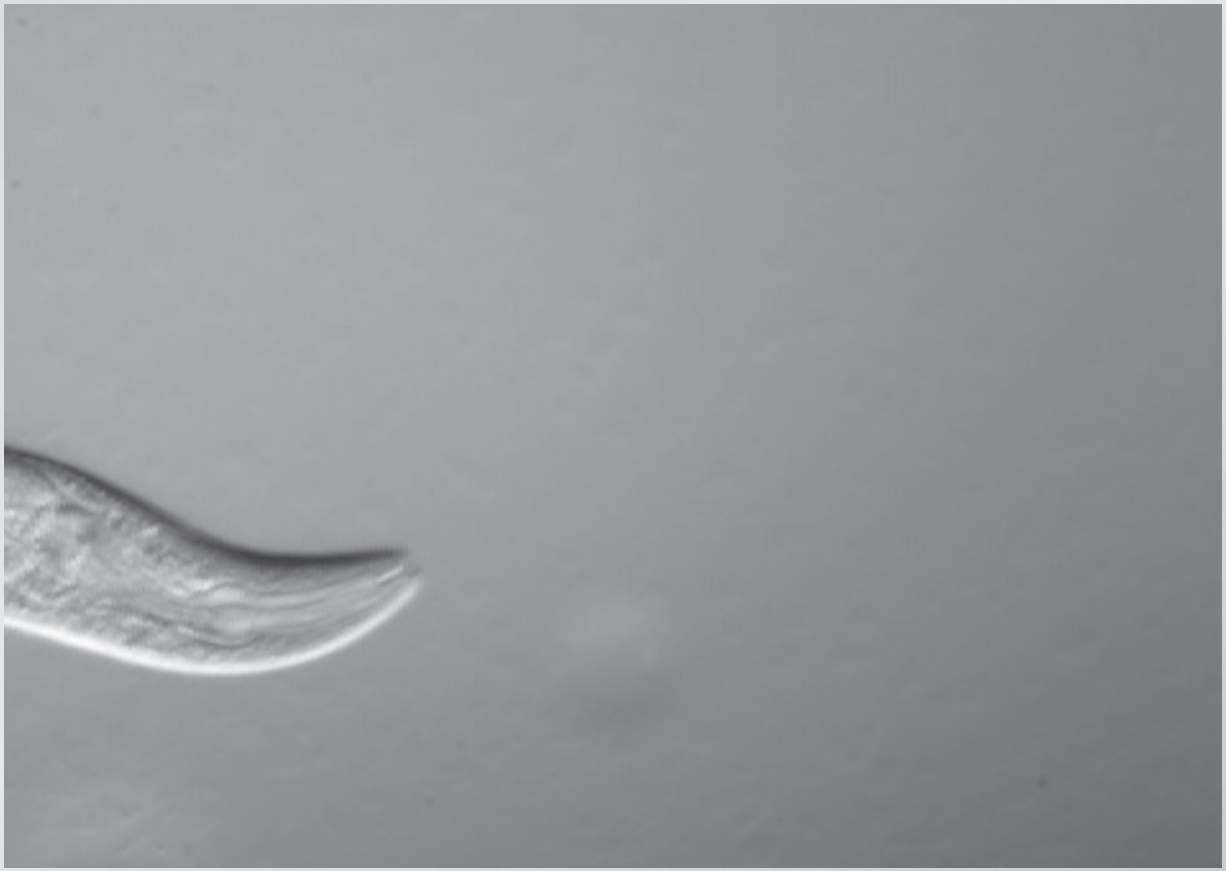
Nanoneurosurgery

Caenorhabditis elegans



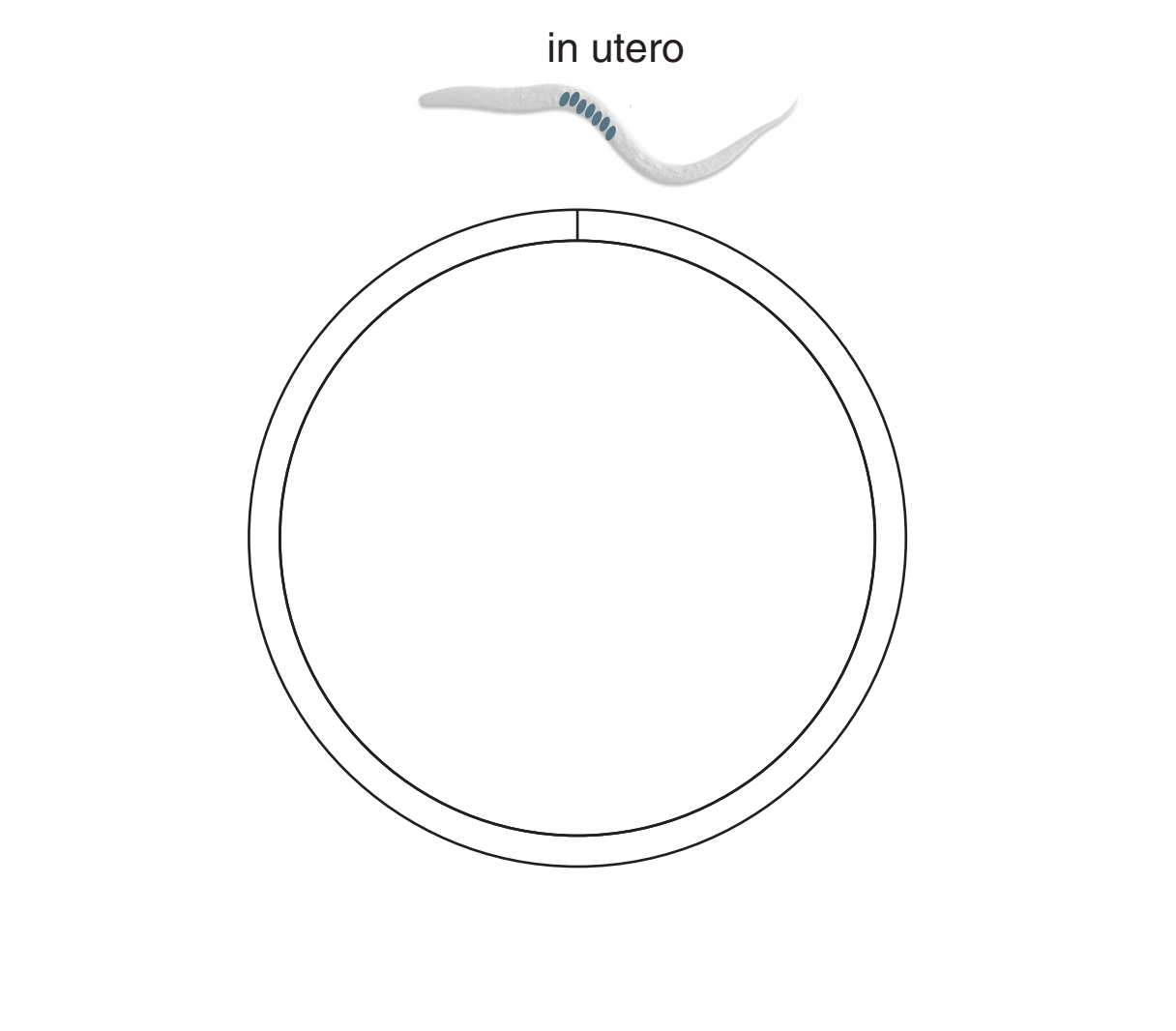
Nanoneurosurgery

Caenorhabditis elegans



Nanoneurosurgery

C. elegans life cycle



Nanoneurosurgery

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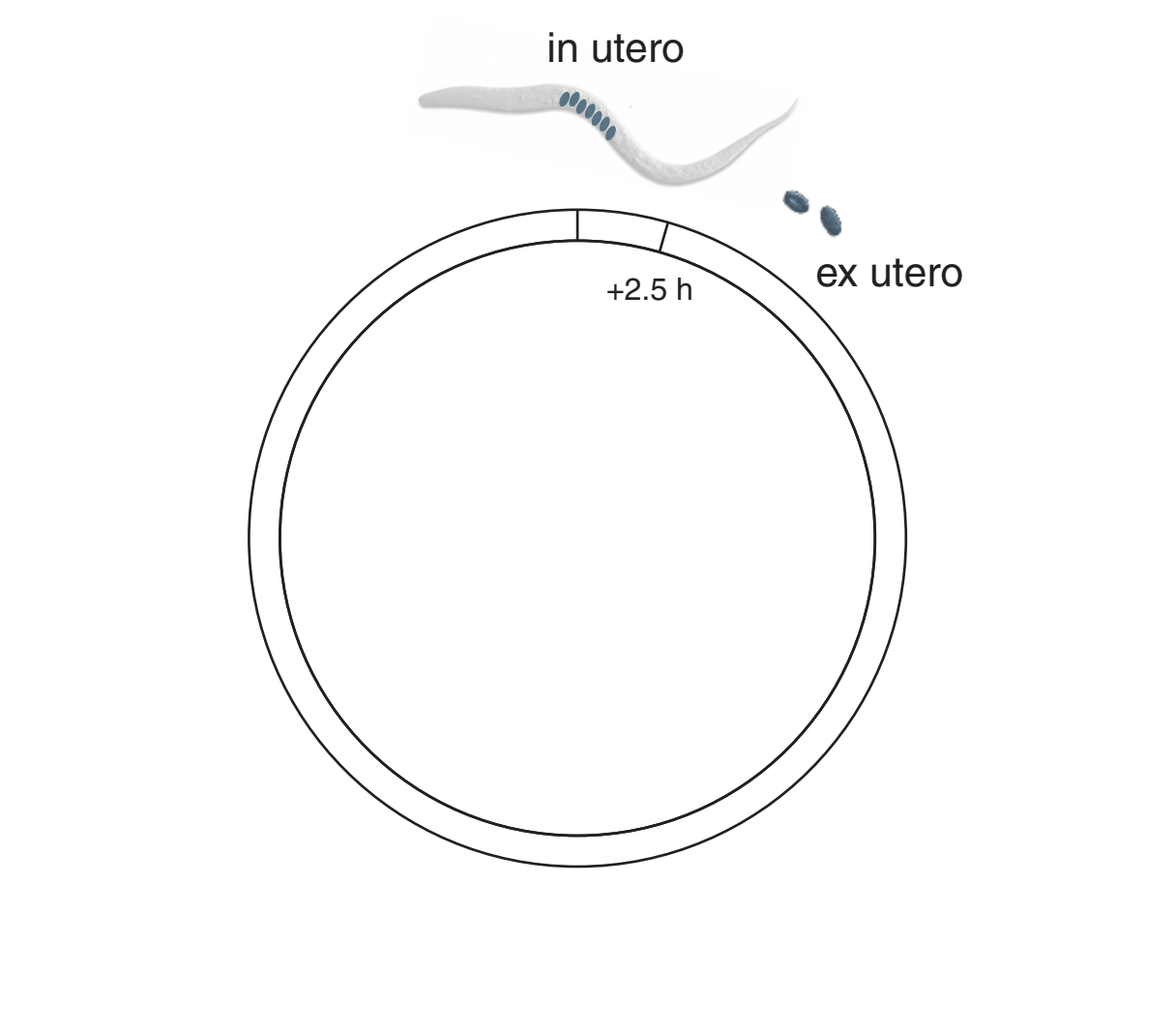

Nanoneurosurgery

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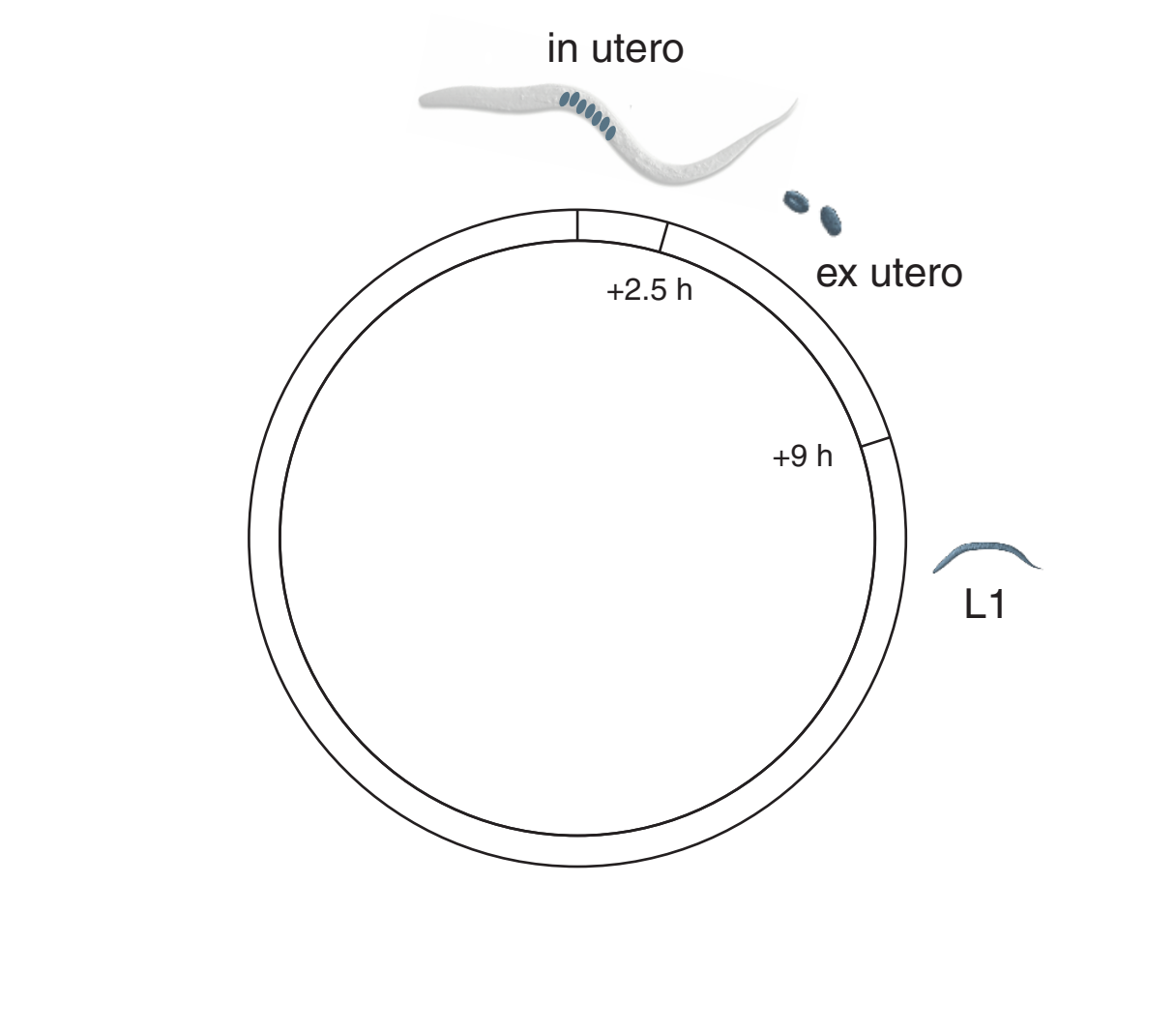
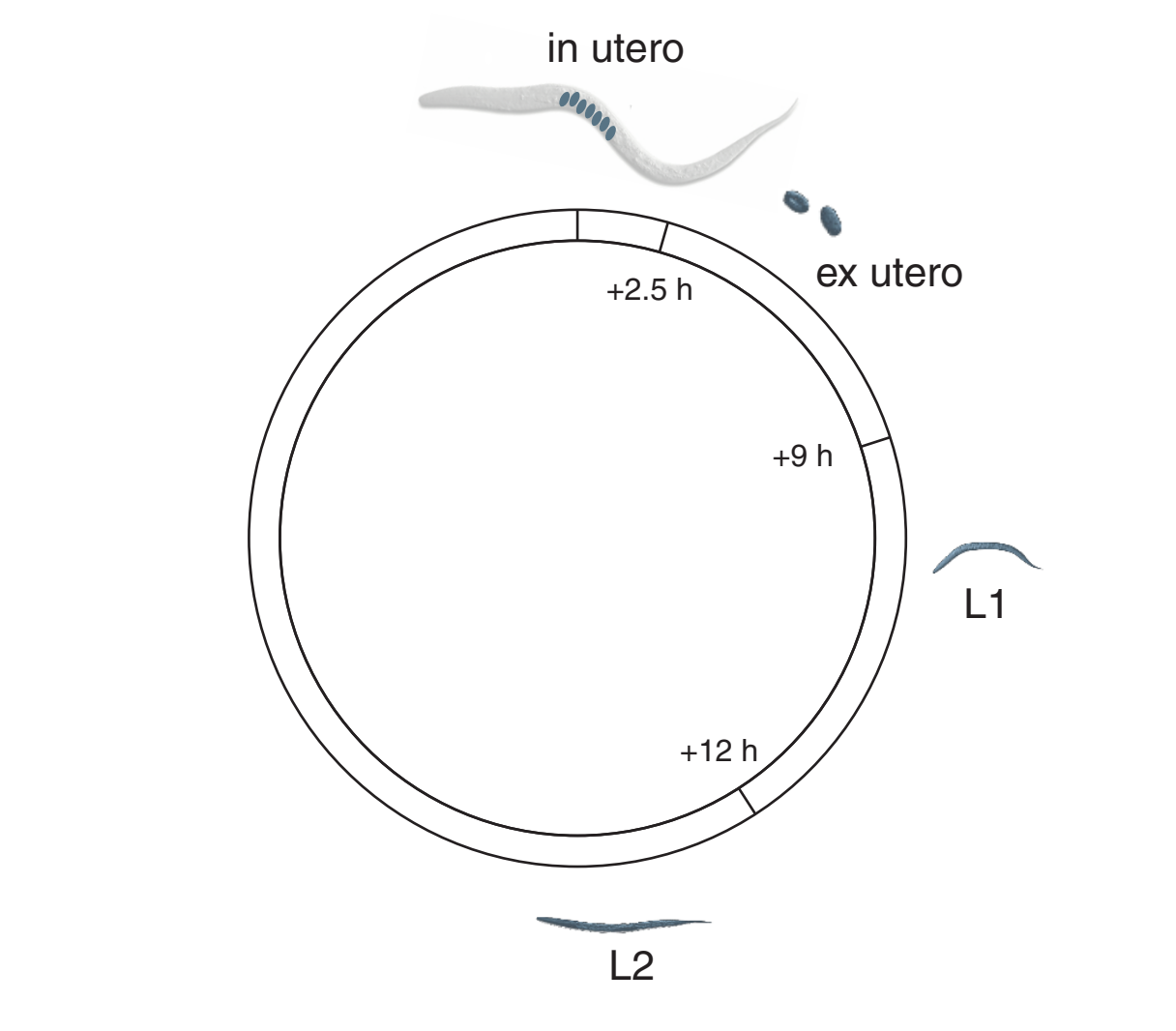


Nanoneurosurgery

C. elegans life cycle



Nanoneurosurgery

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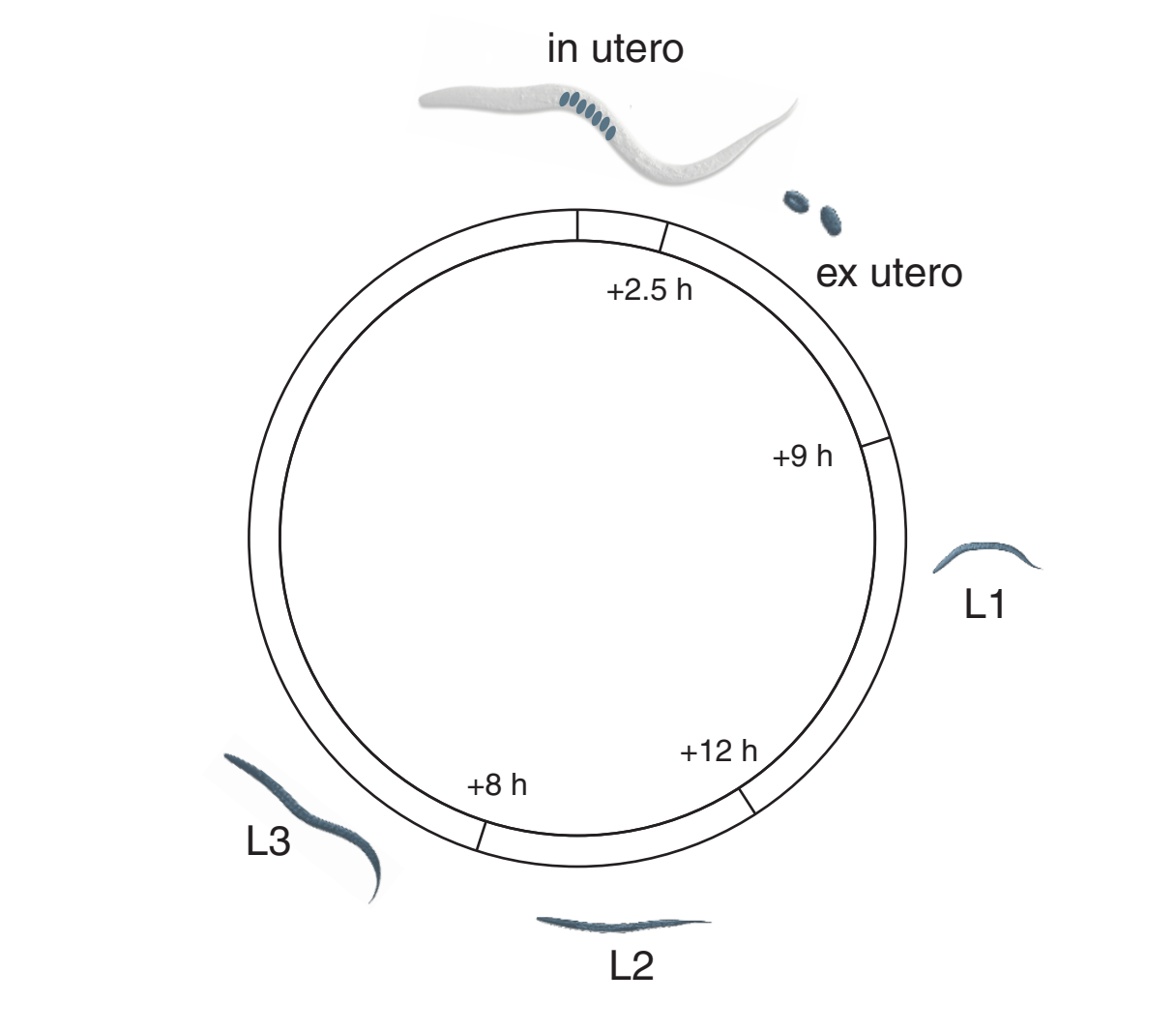
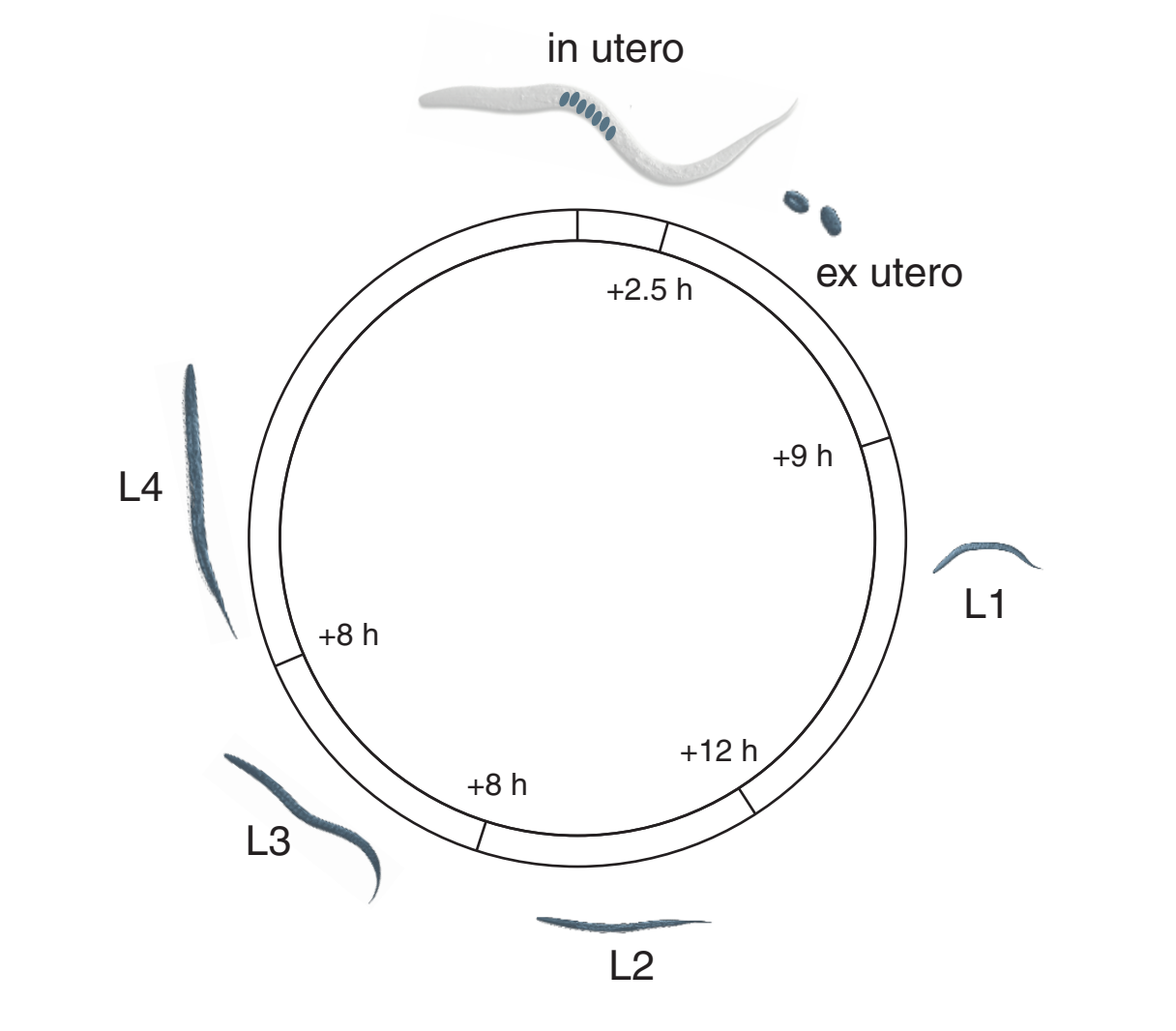


Nanoneurosurgery

C. elegans life cycle



Nanoneurosurgery

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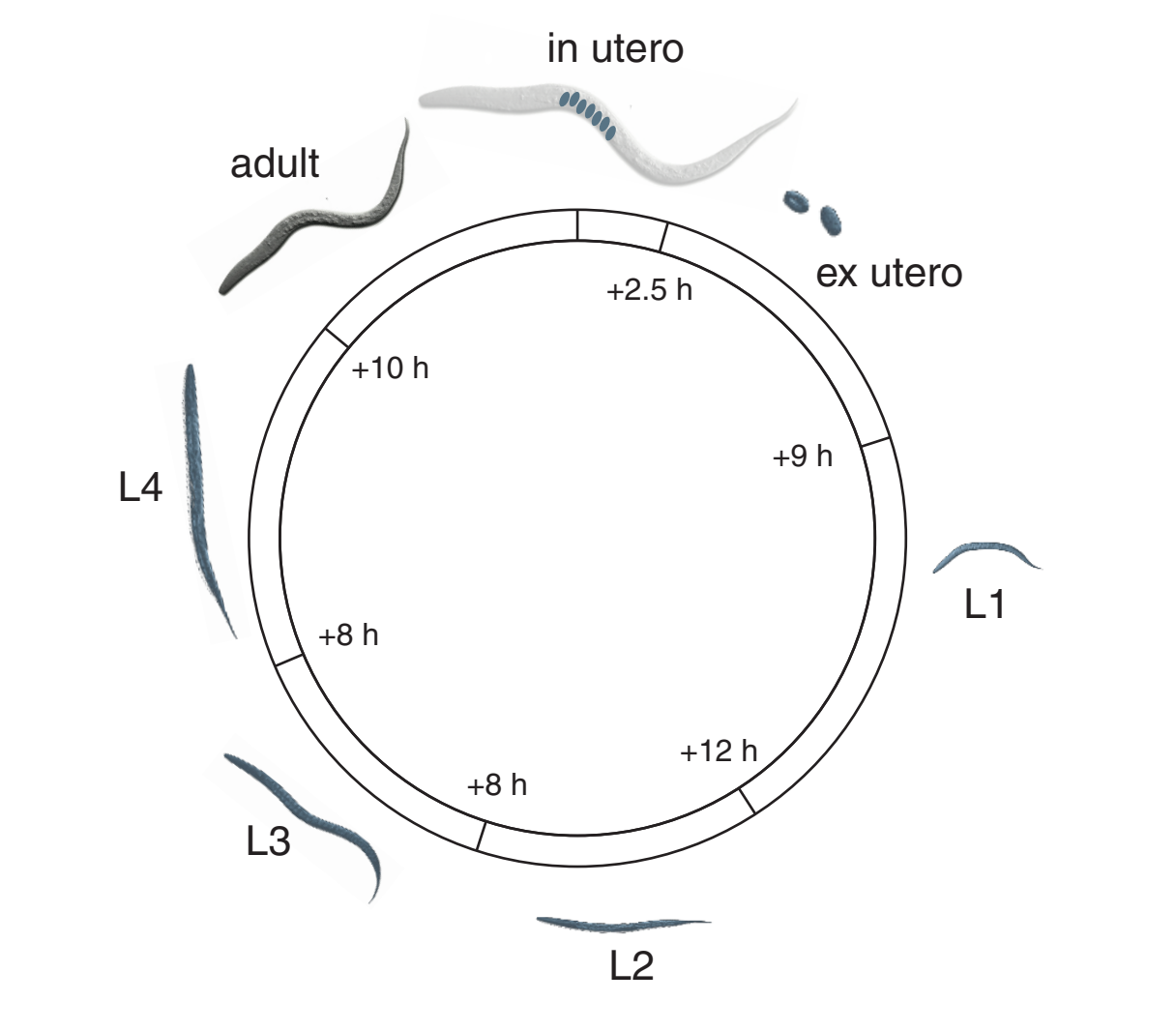
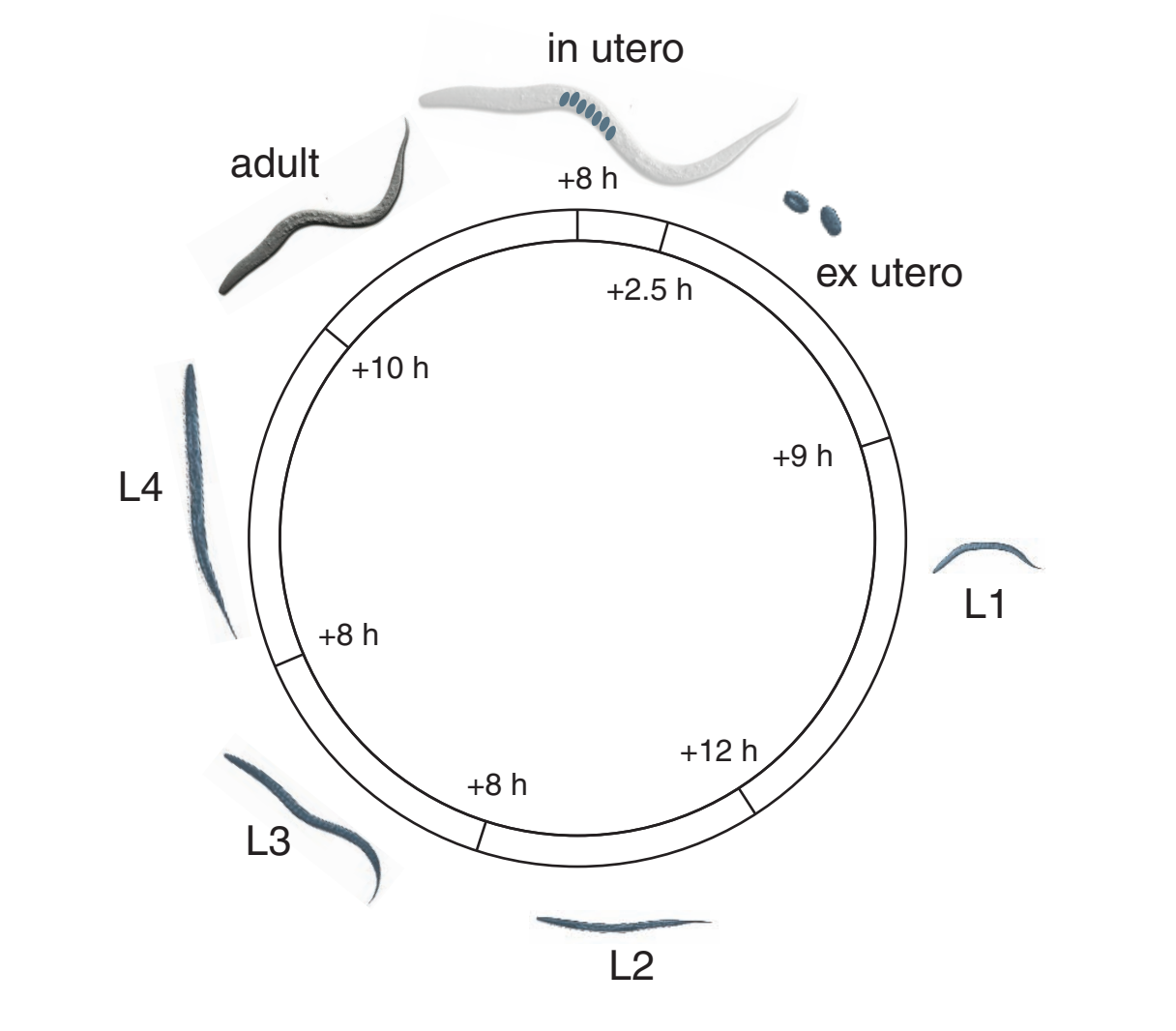


Nanoneurosurgery

C. elegans life cycle



Nanoneurosurgery

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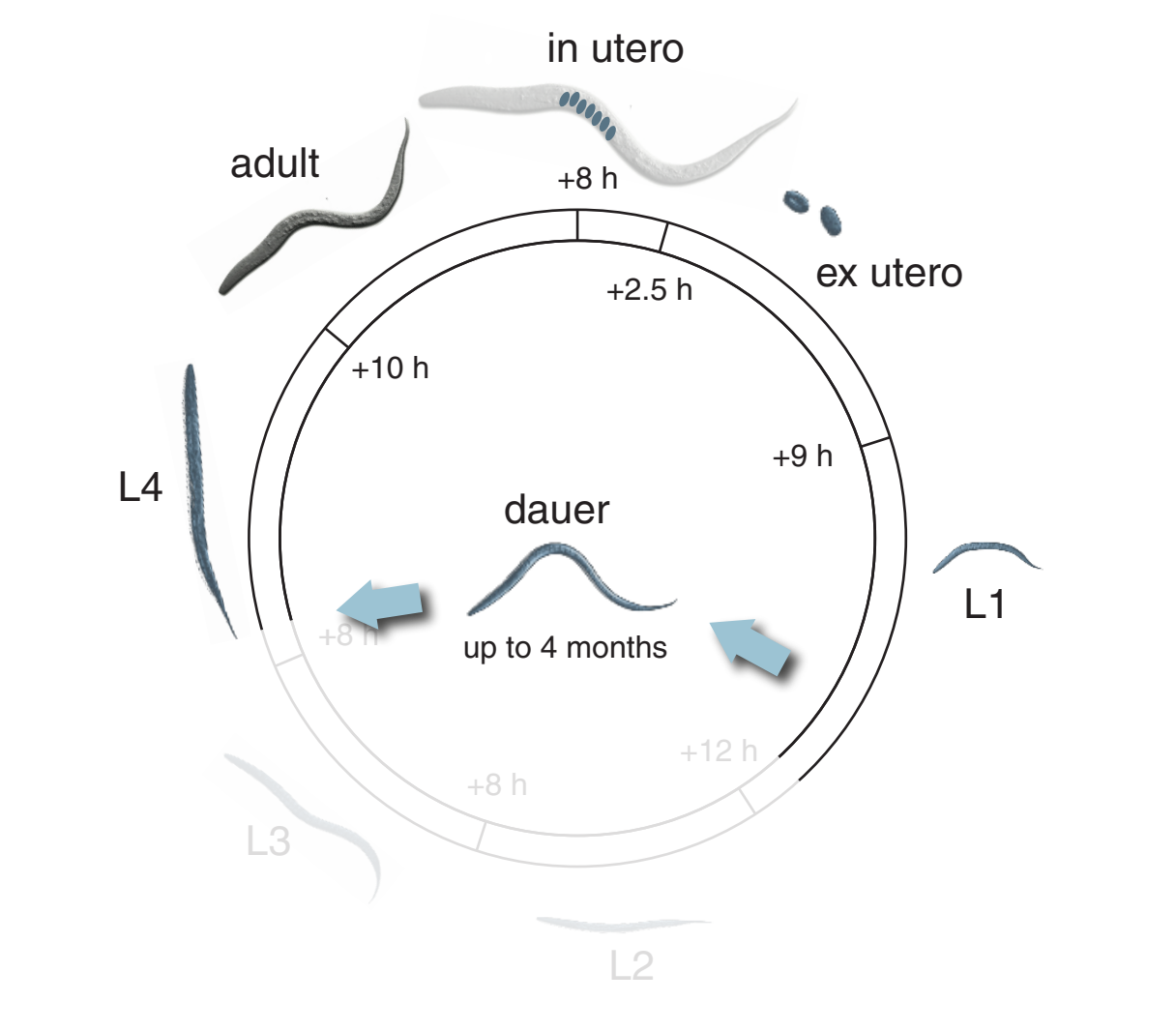
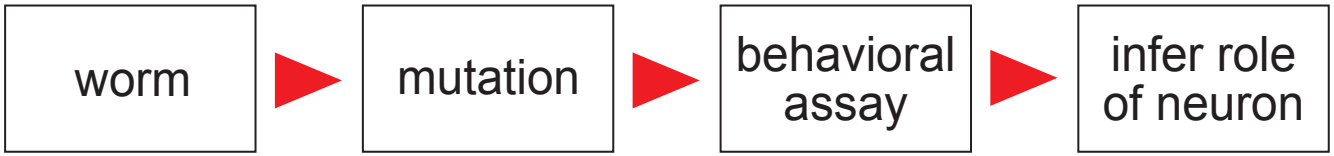


Nanoneurosurgery

Mapping behavior to neurons

conventional method



Nanoneurosurgery

Mapping behavior to neurons

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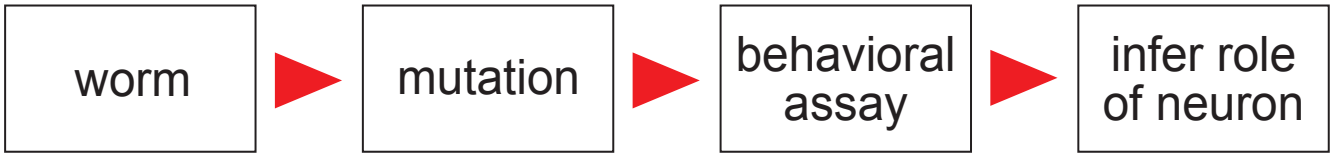
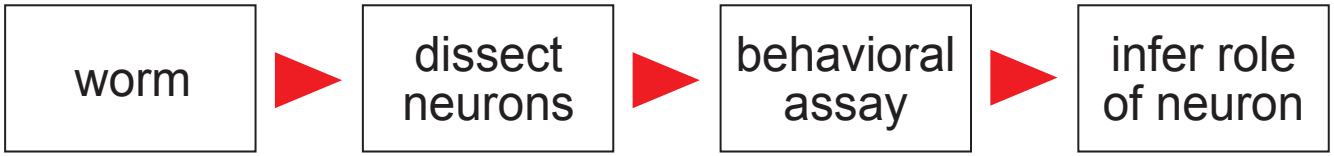


femtosecond laser ablation



Nanoneurosurgery

ASH neurons

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

Nanoneurosurgery

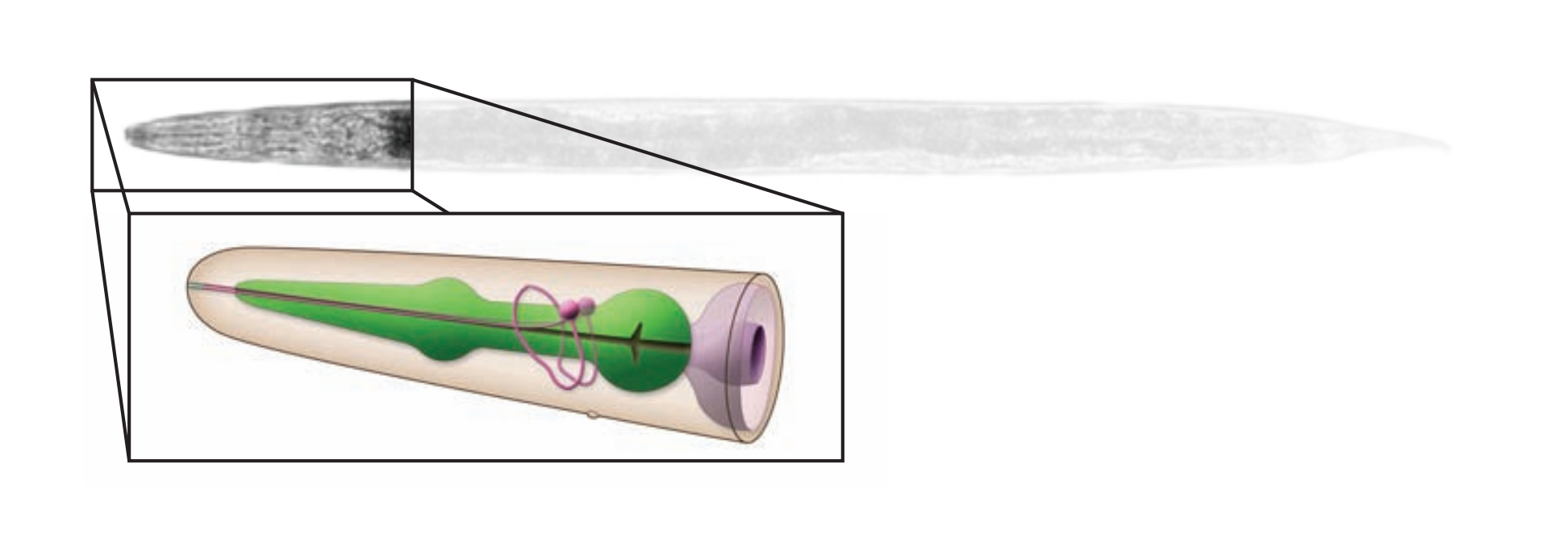
ASH neurons

Nanoneurosurgery

ASH neurons

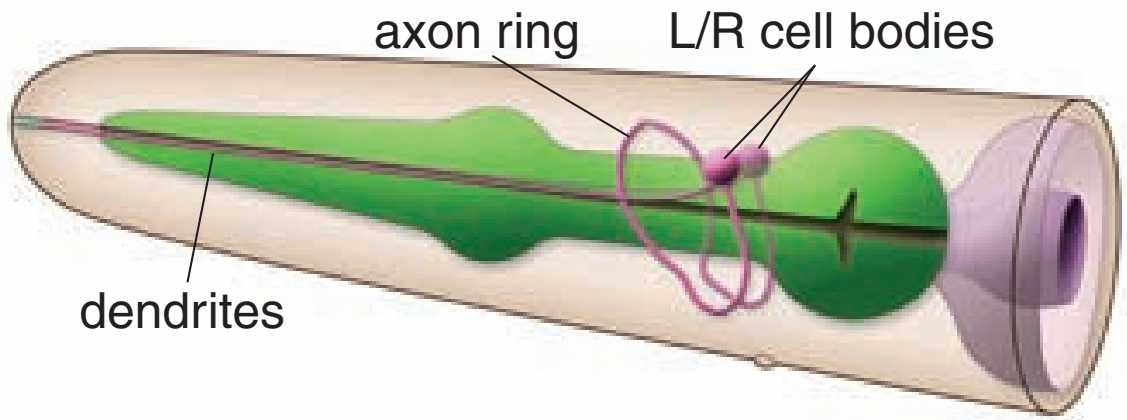
Nanoneurosurgery

ASH neurons



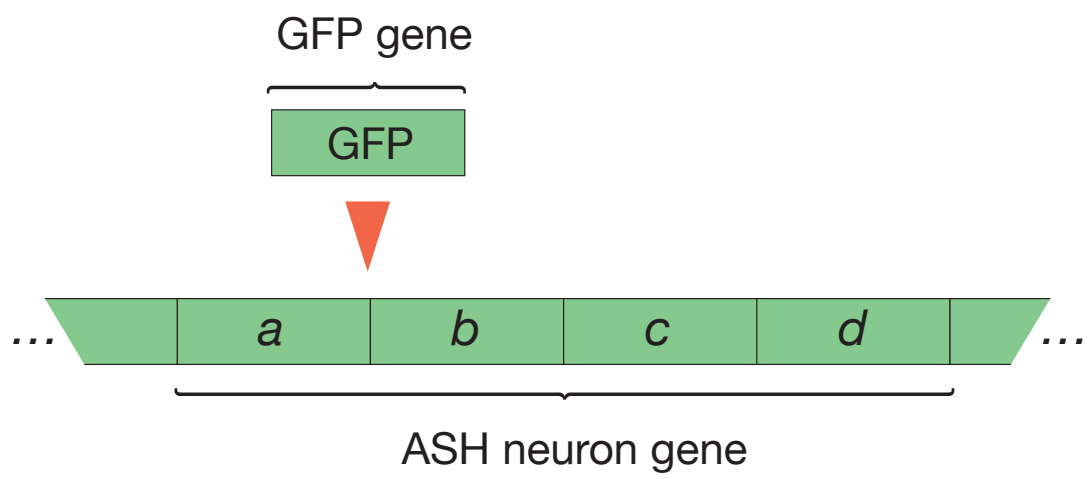
Nanoneurosurgery

ASH neurons



Nanoneurosurgery

make ASH neurons express GFP



Nanoneurosurgery

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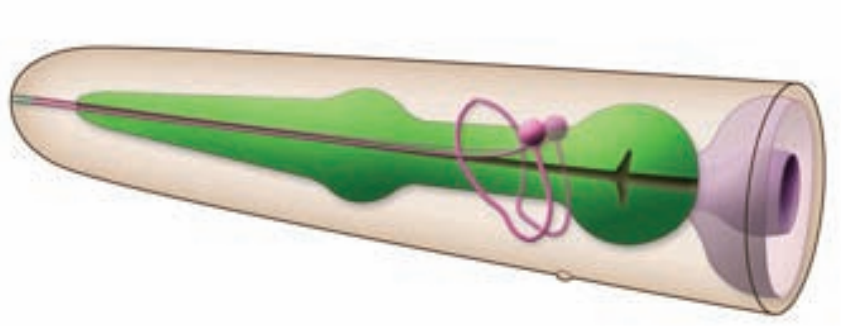
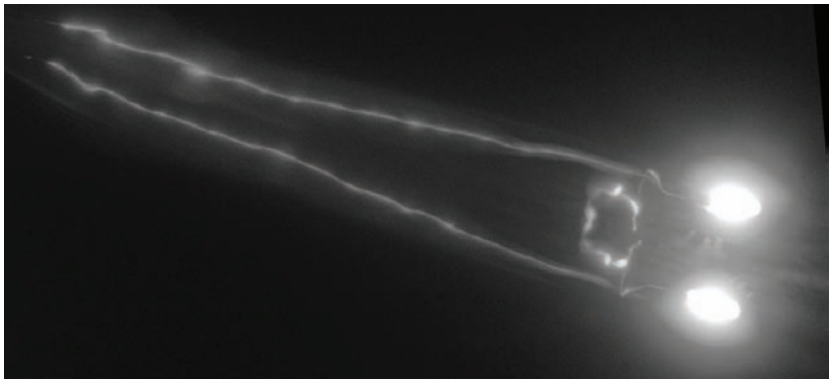


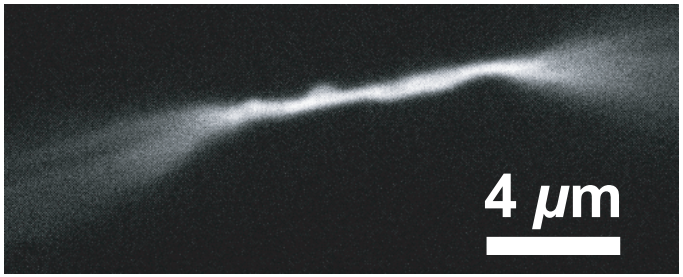


Nanoneurosurgery

GFP: absorbs UV, emits green

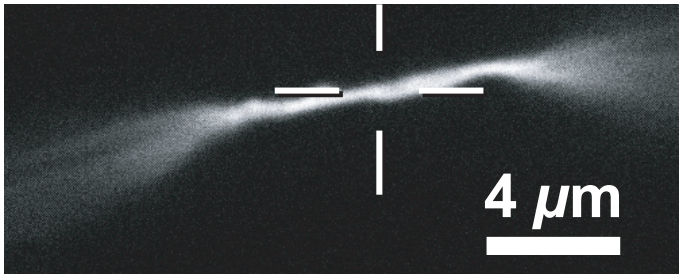
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



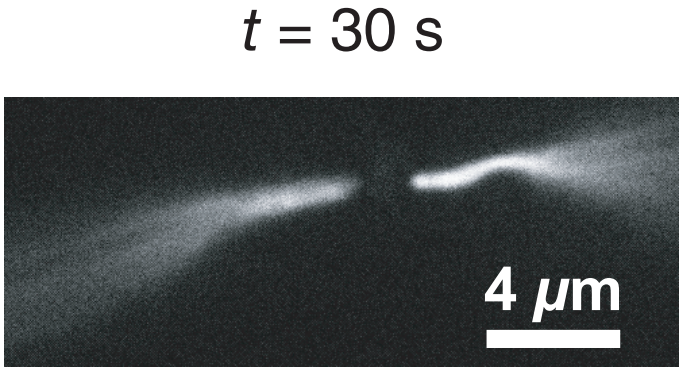
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



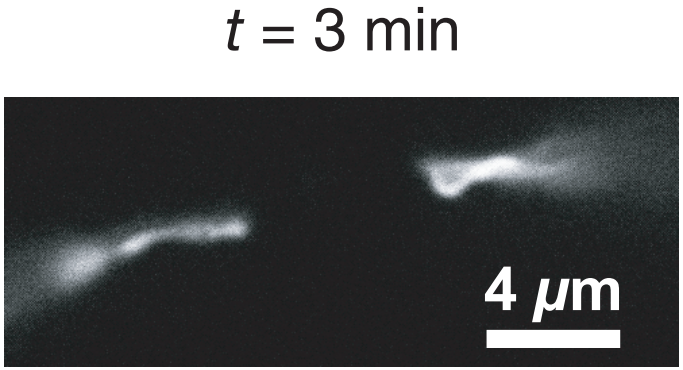
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



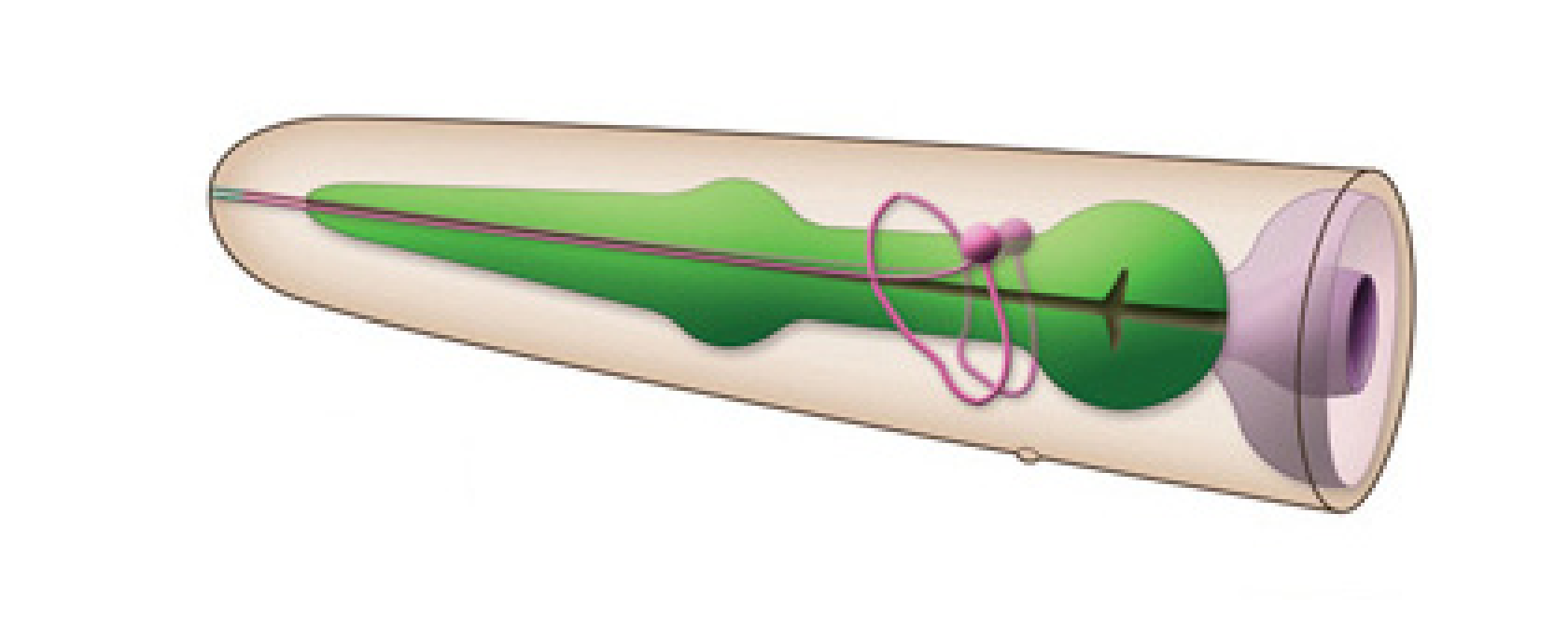
Nanoneurosurgery

retraction of cut dendrite (6 nJ)



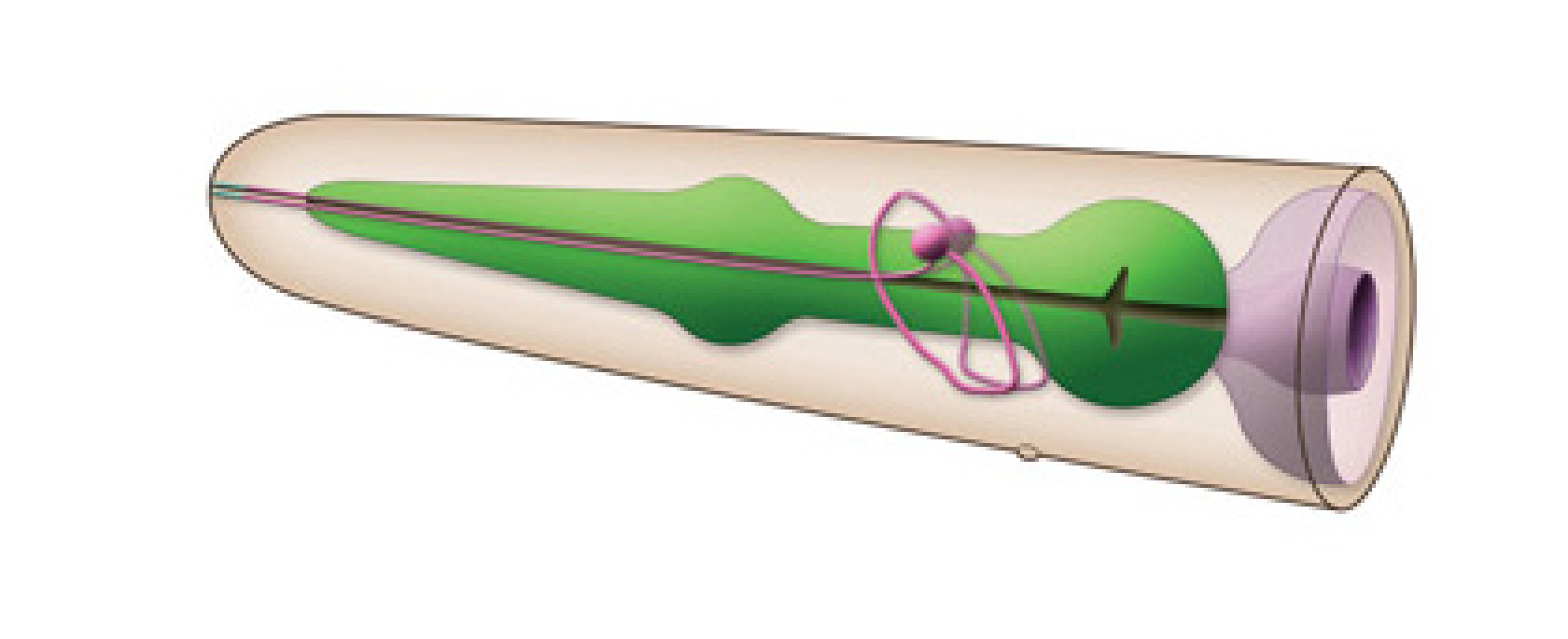
Nanoneurosurgery

ASH neurons



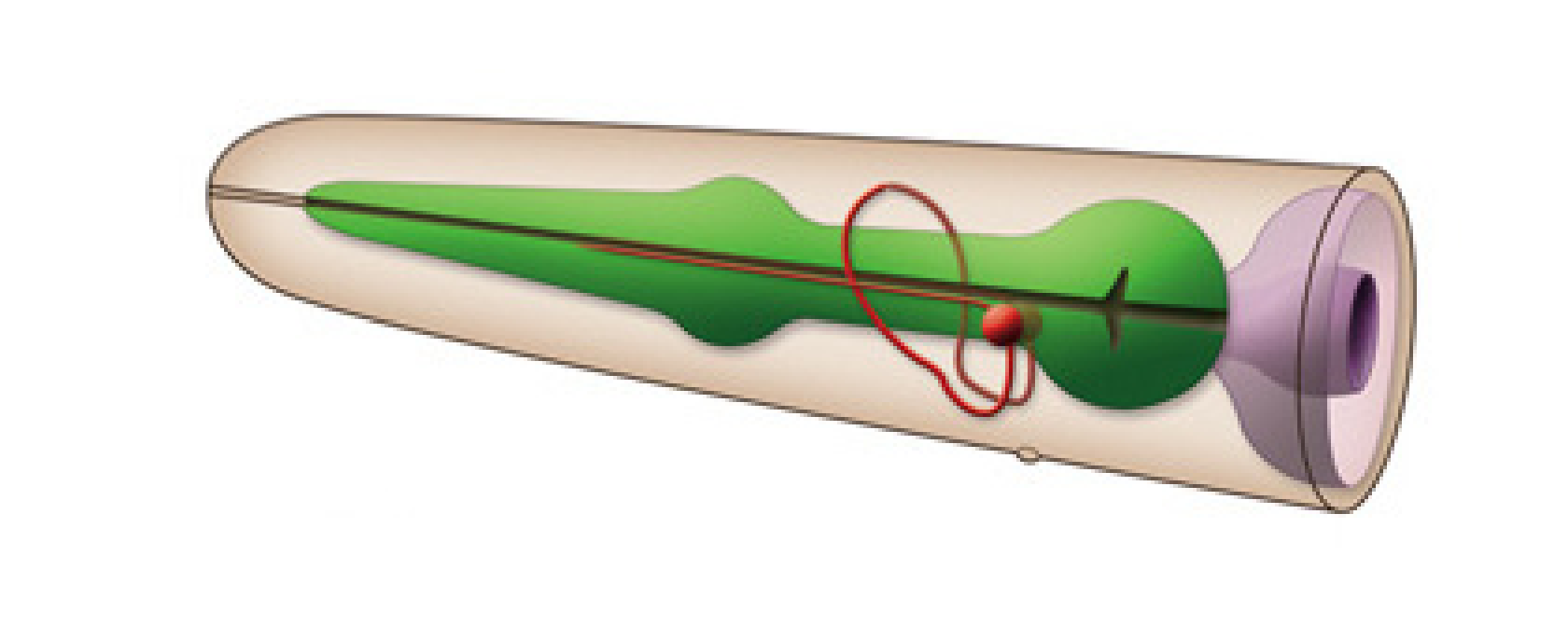
Nanoneurosurgery

ASK neurons



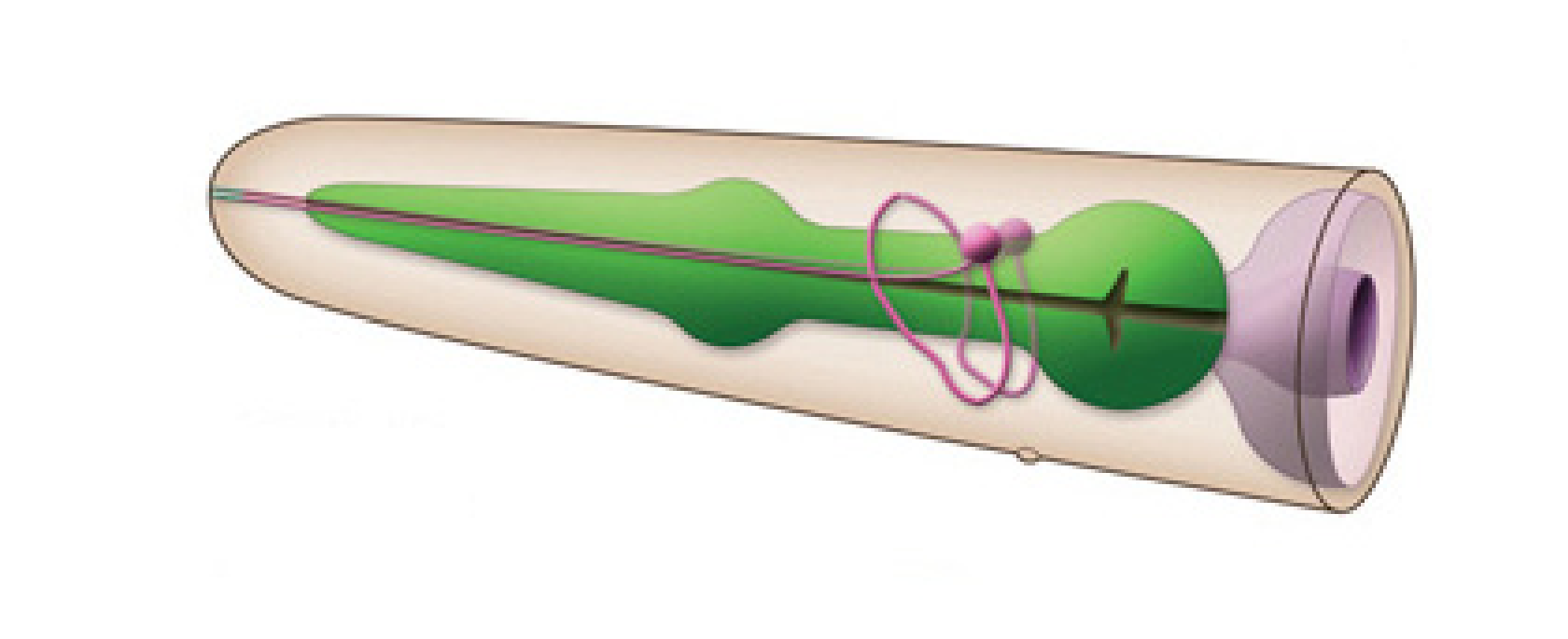
Nanoneurosurgery

AUA neurons



Nanoneurosurgery

ASI neurons

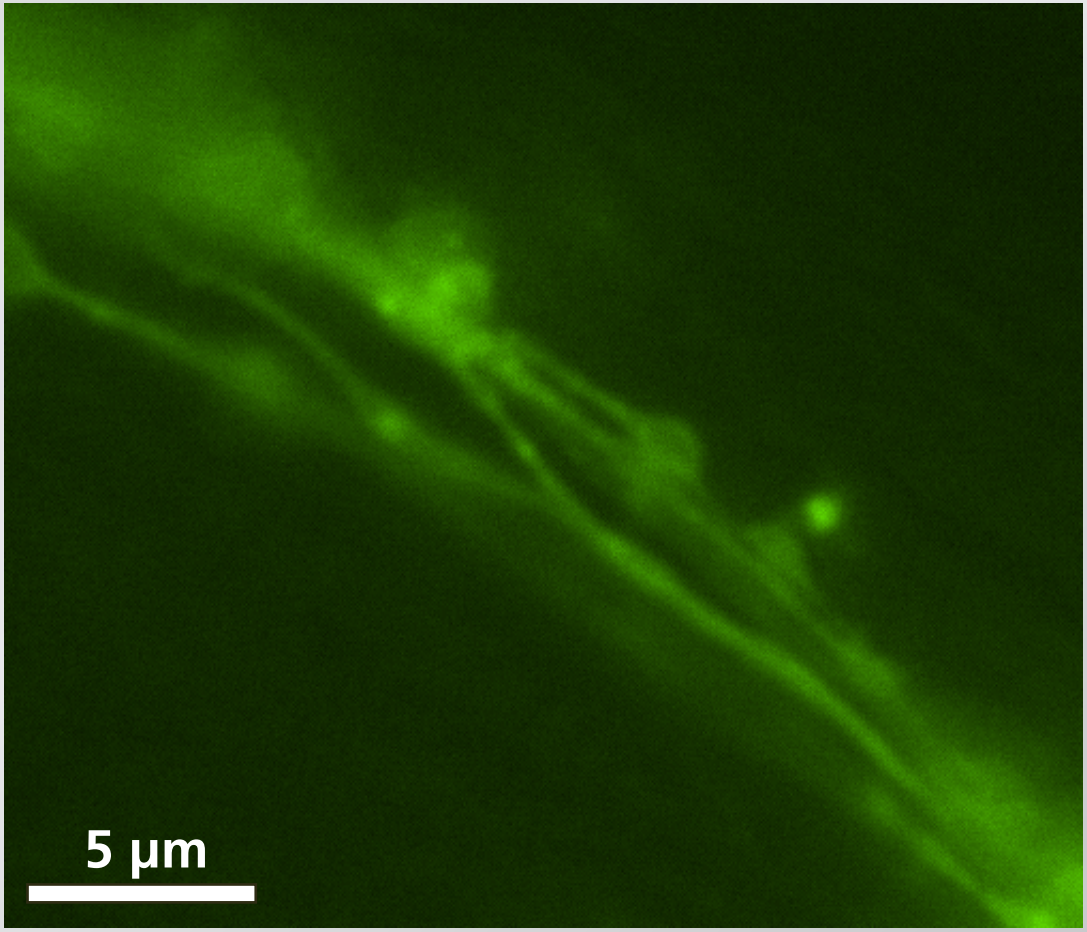


Nanoneurosurgery

need exquisite precision!

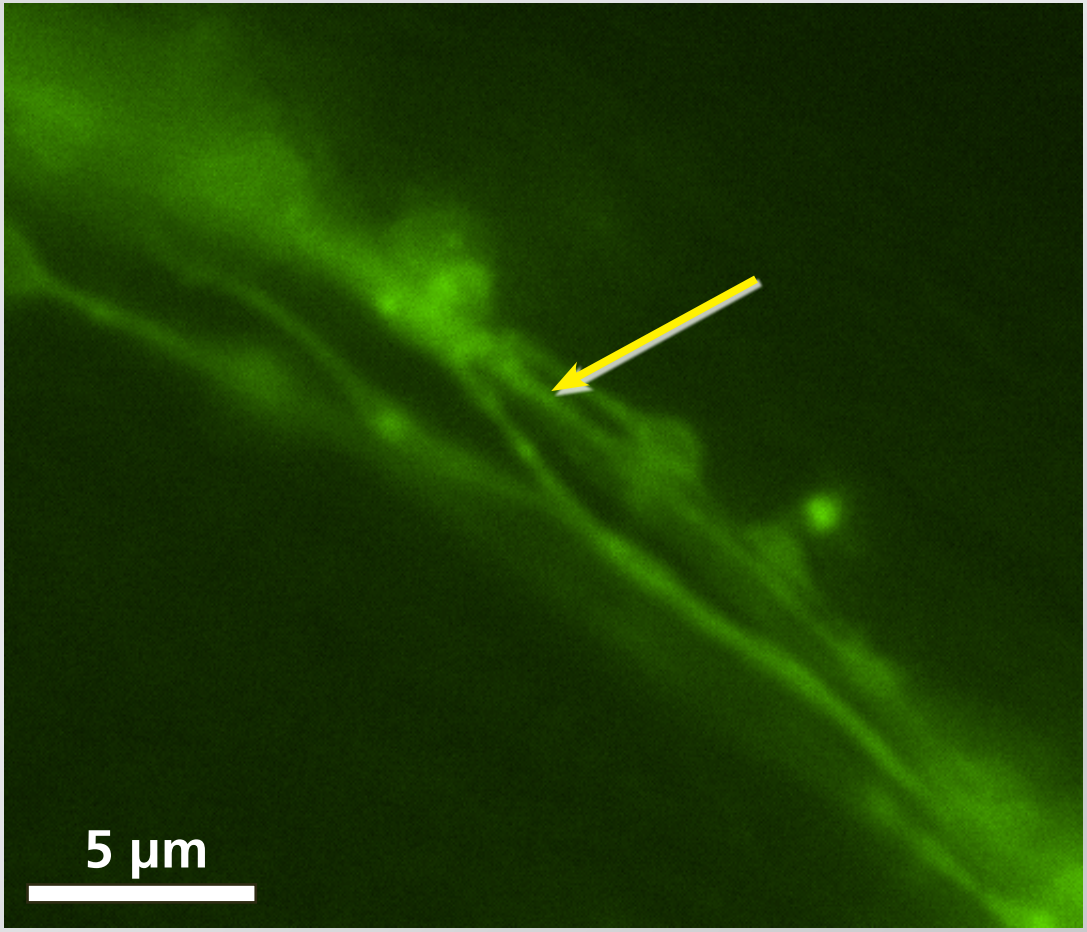
Nanoneurosurgery

DiO-stained bundle of dendrites



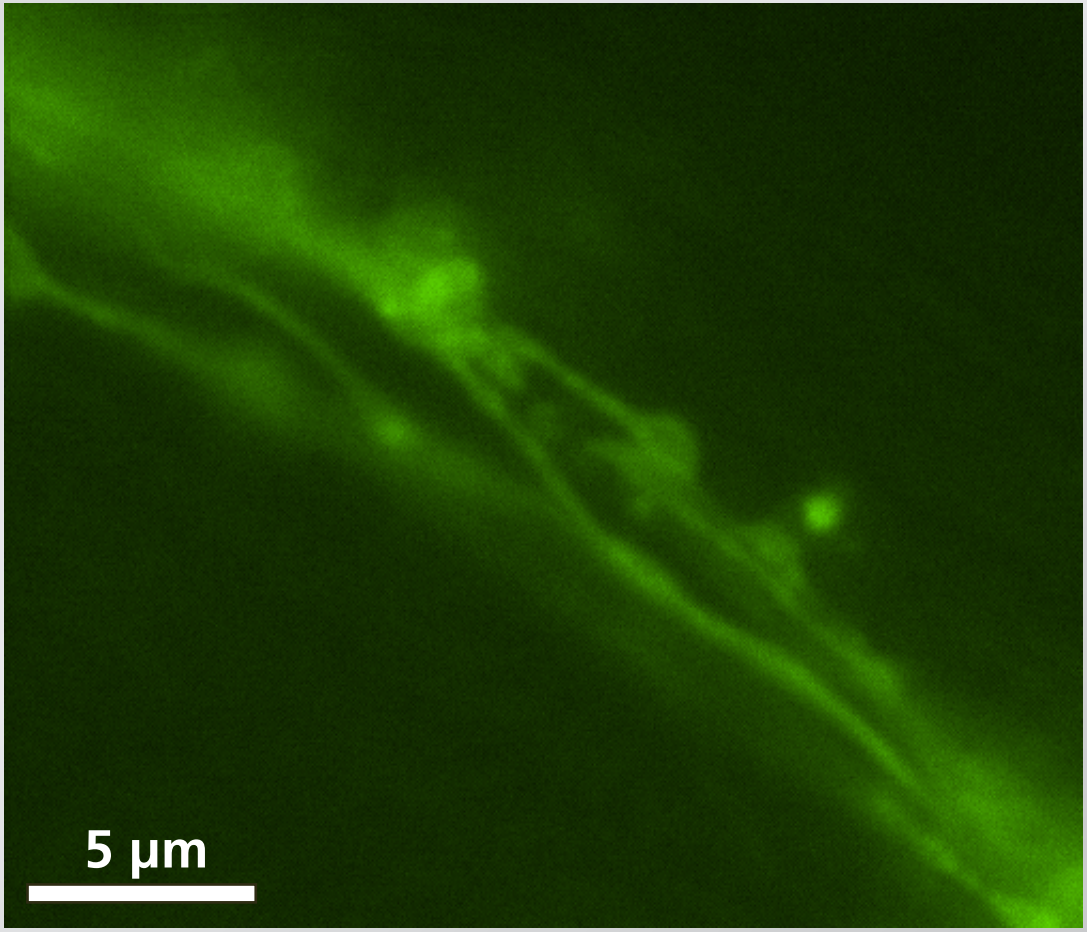
Nanoneurosurgery

cut single dendrite in bundle (3 nJ)



Nanoneurosurgery

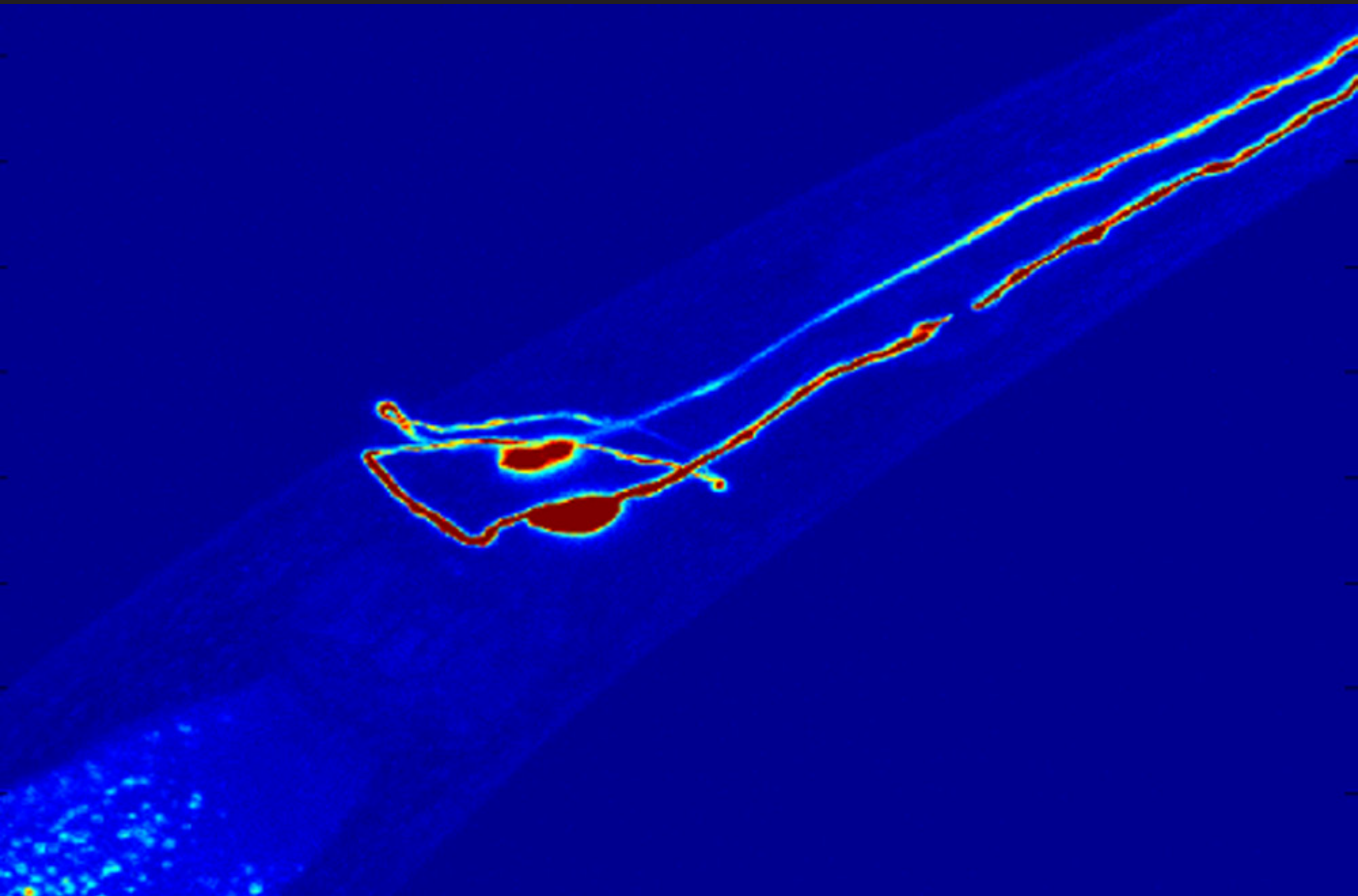
no damange to neighboring dendrites



Nanoneurosurgery

revive worm, reimage 1 day later

Nanoneurosurgery

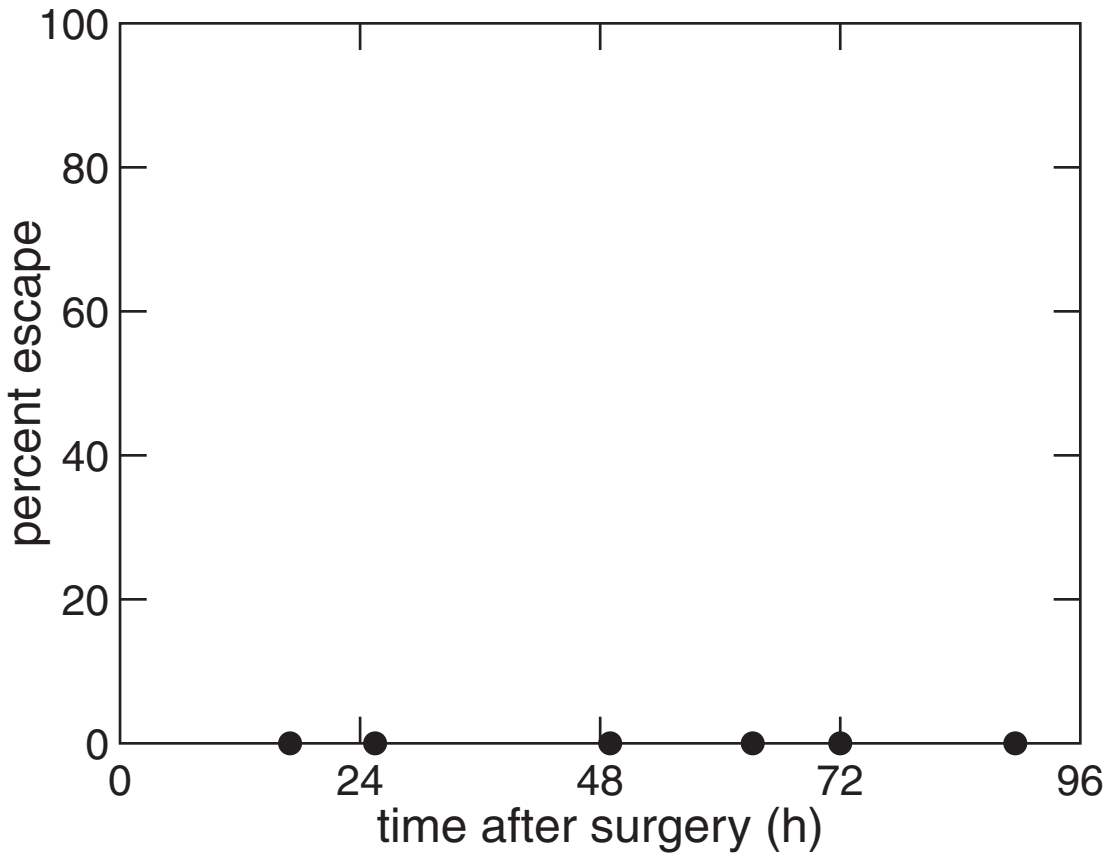


Nanoneurosurgery

osmolarity assay

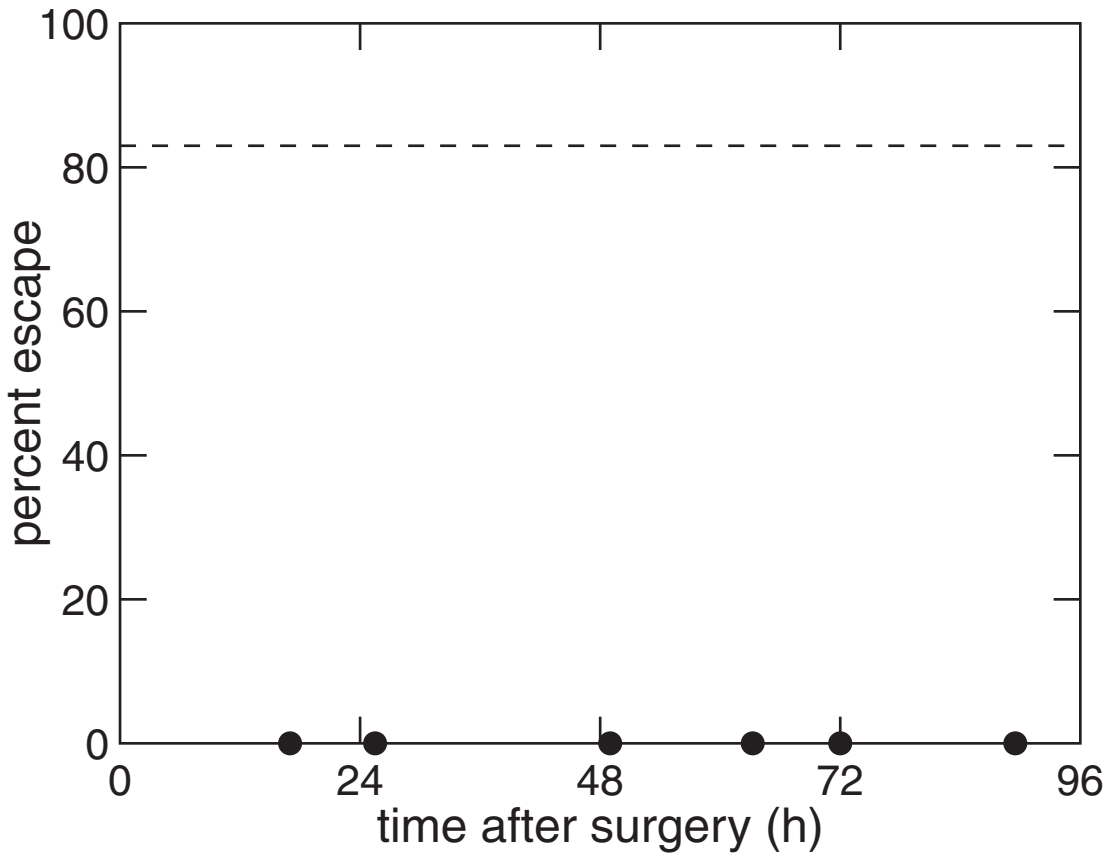
Nanoneurosurgery

escape rate after ‘mock’ surgery



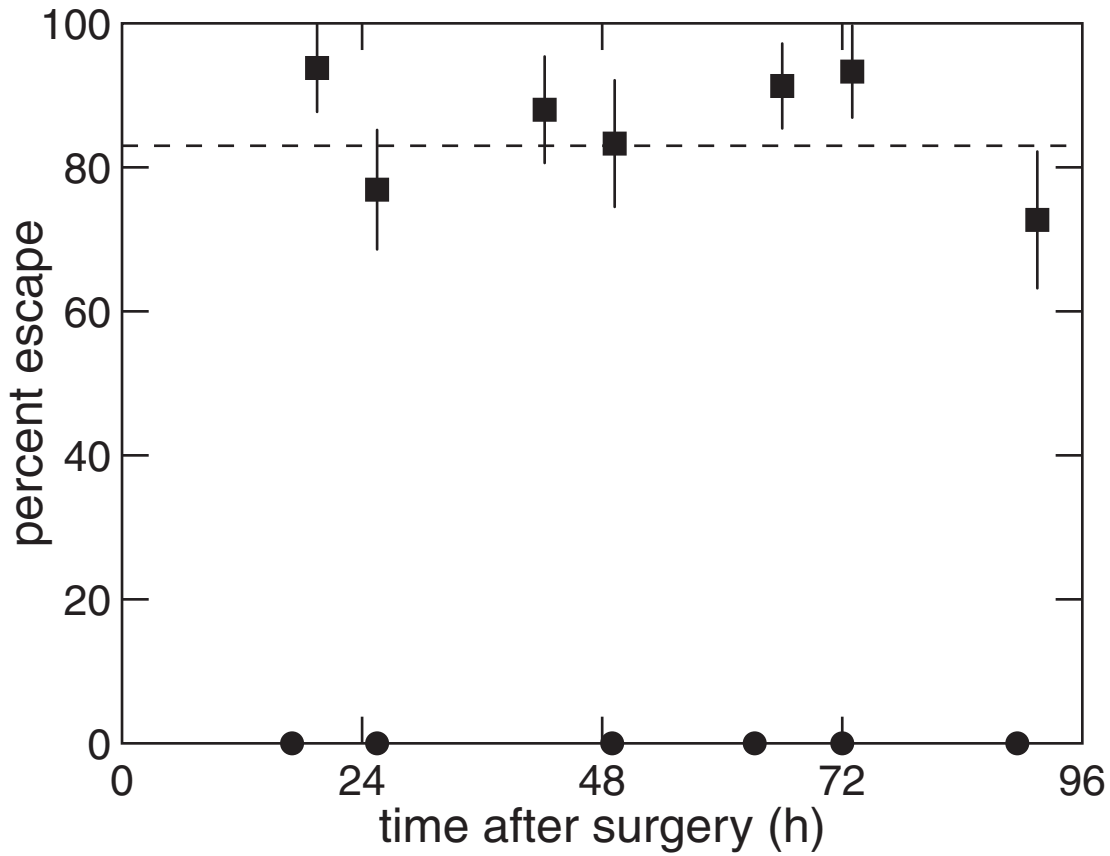
Nanoneurosurgery

escape rate of ASH-lacking mutant



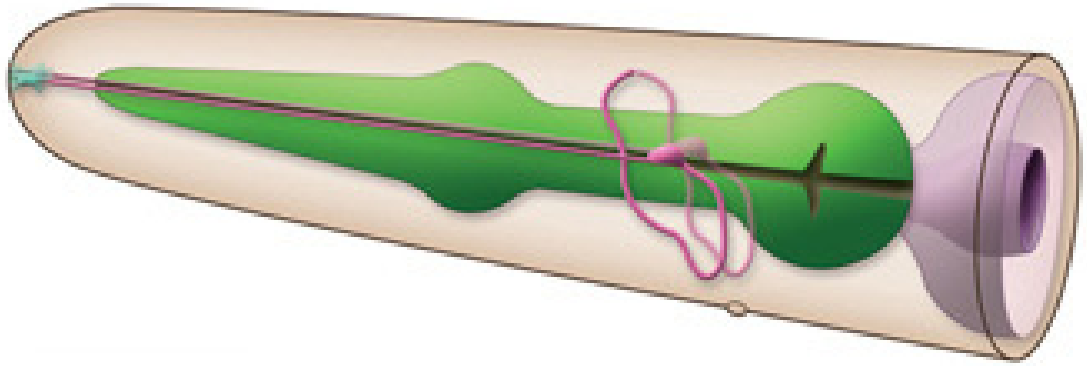
Nanoneurosurgery

escape rate after ASH-ablation surgery

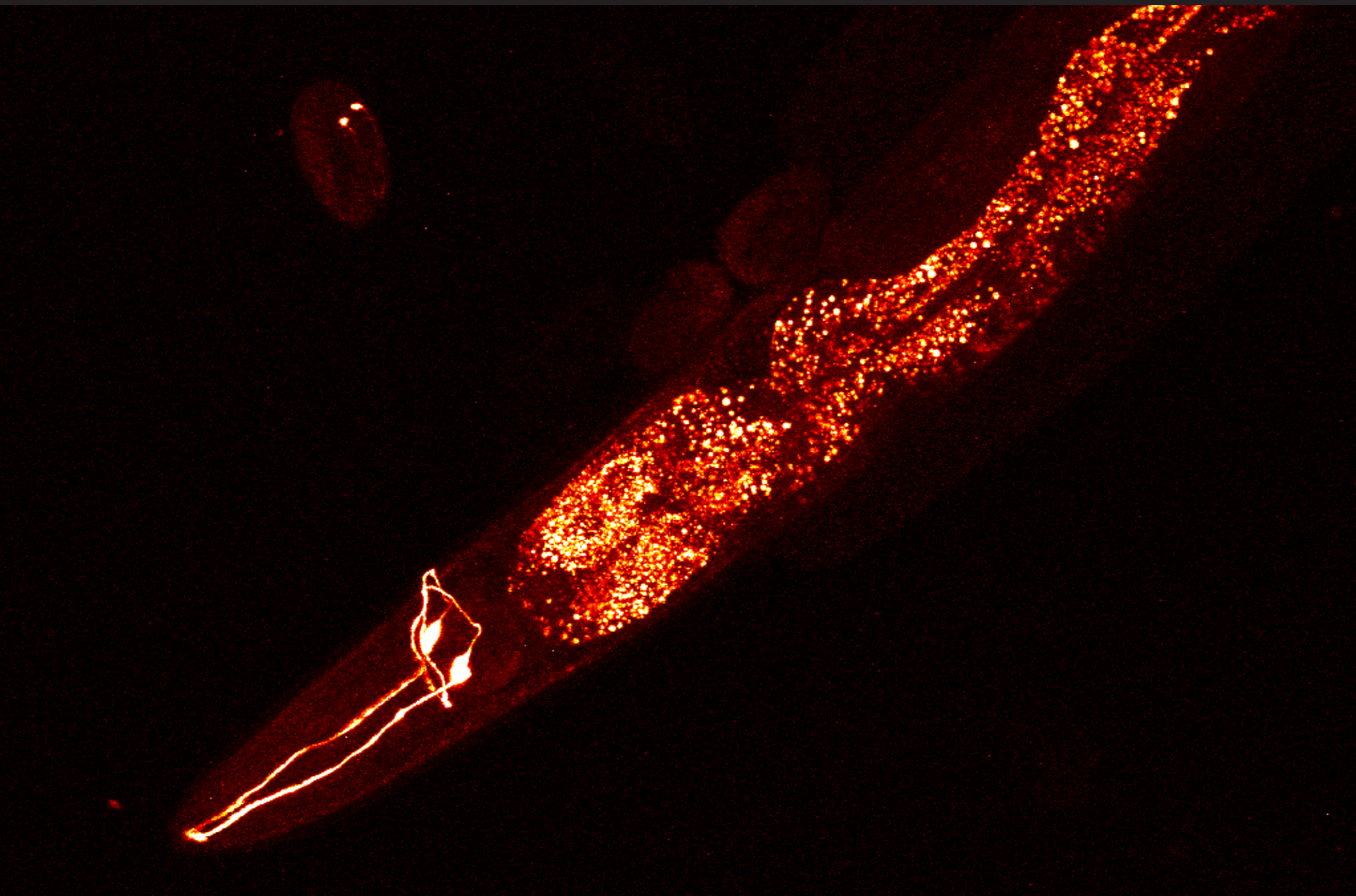


Nanoneurosurgery

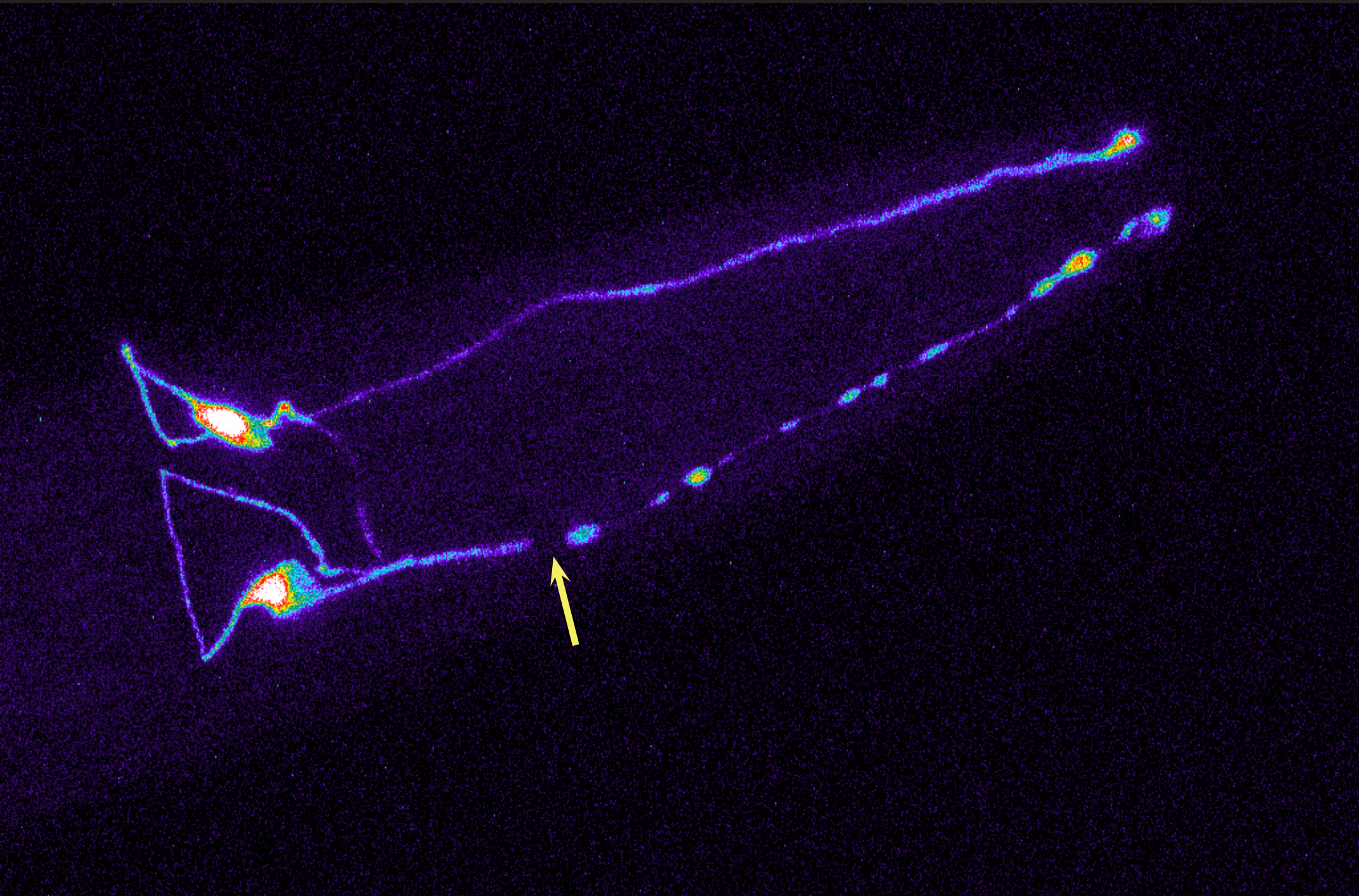
AFD neurons (temperature sensors)



Nanoneurosurgery



Nanoneurosurgery

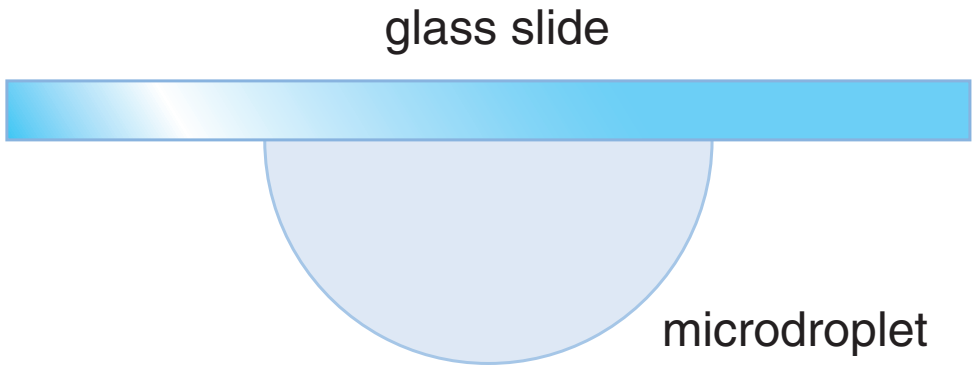


Nanoneurosurgery

Q: where does the ASH sense temperature?

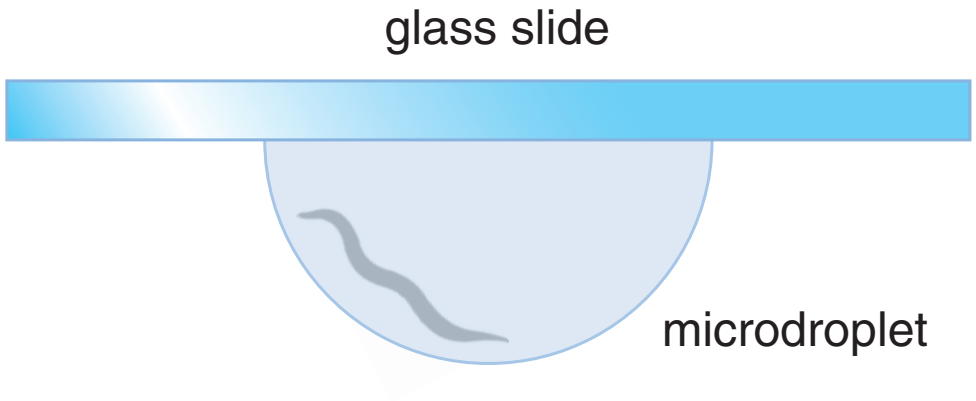
Nanoneurosurgery

microdroplet assay



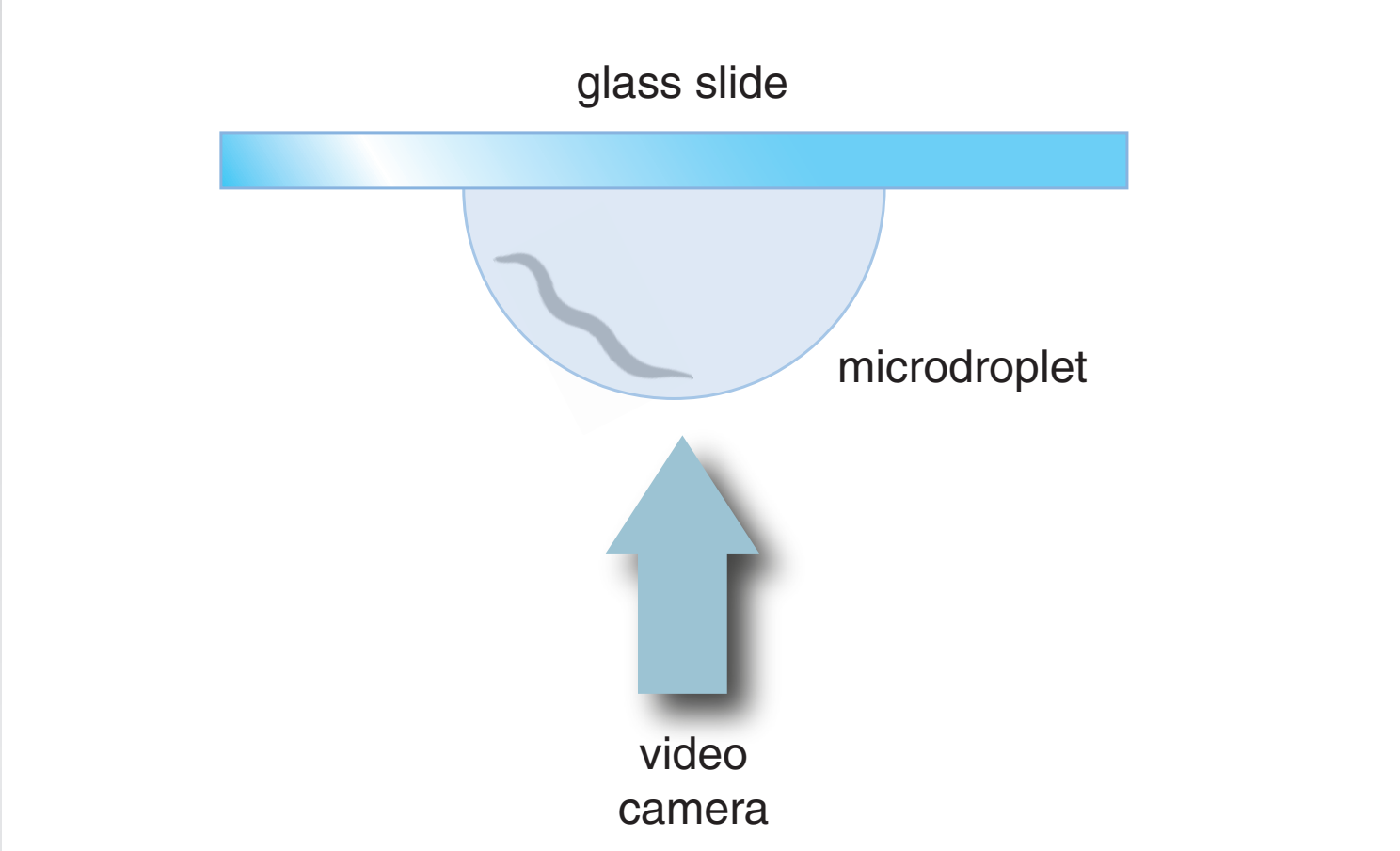
Nanoneurosurgery

microdroplet assay



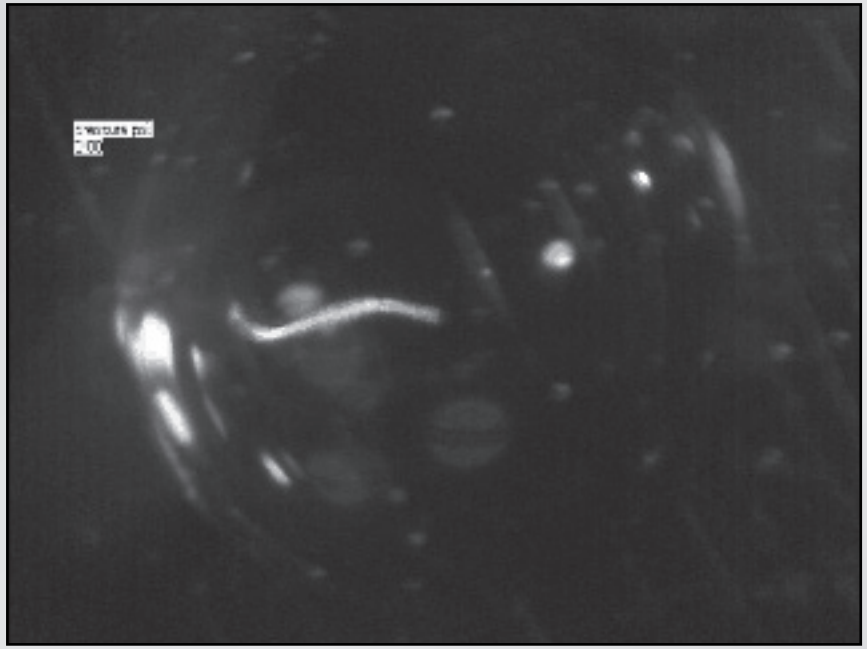
Nanoneurosurgery

microdroplet assay

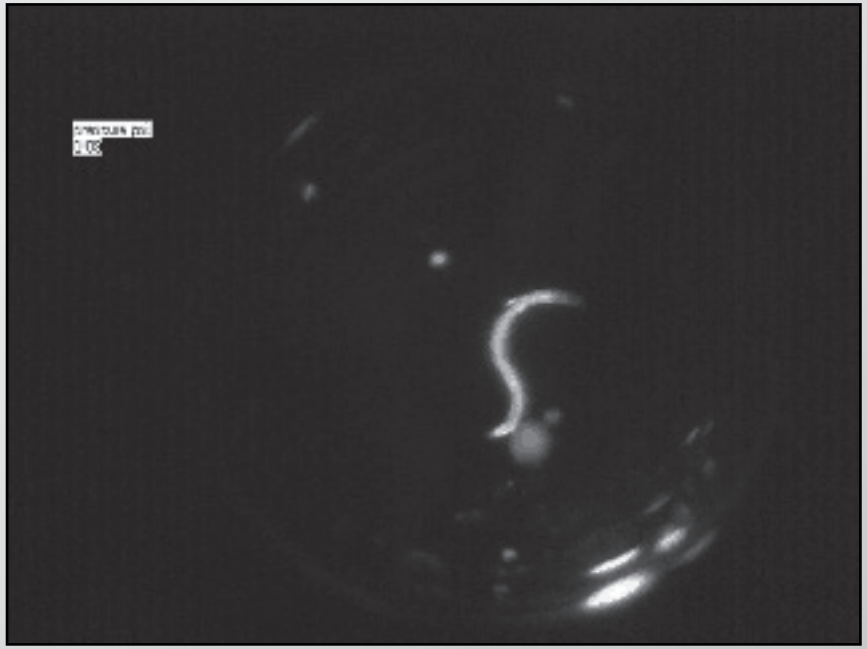


Nanoneurosurgery

surgery results in quantifiable behavior changes



before

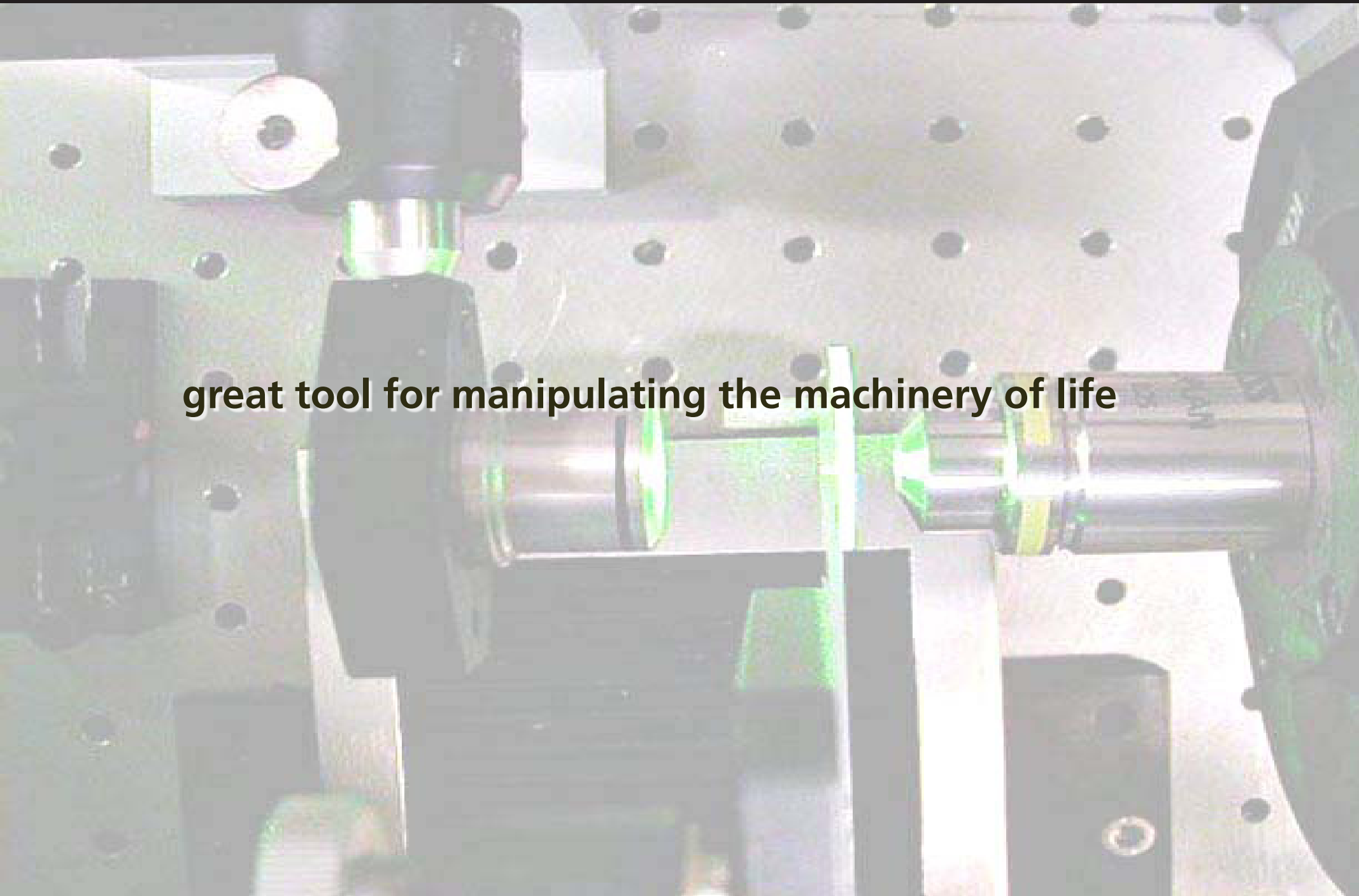


after

Nanoneurosurgery

temperature sensing occurs at tip of dendrite

Conclusion



great tool for manipulating the machinery of life

Funding:

National Science Foundation

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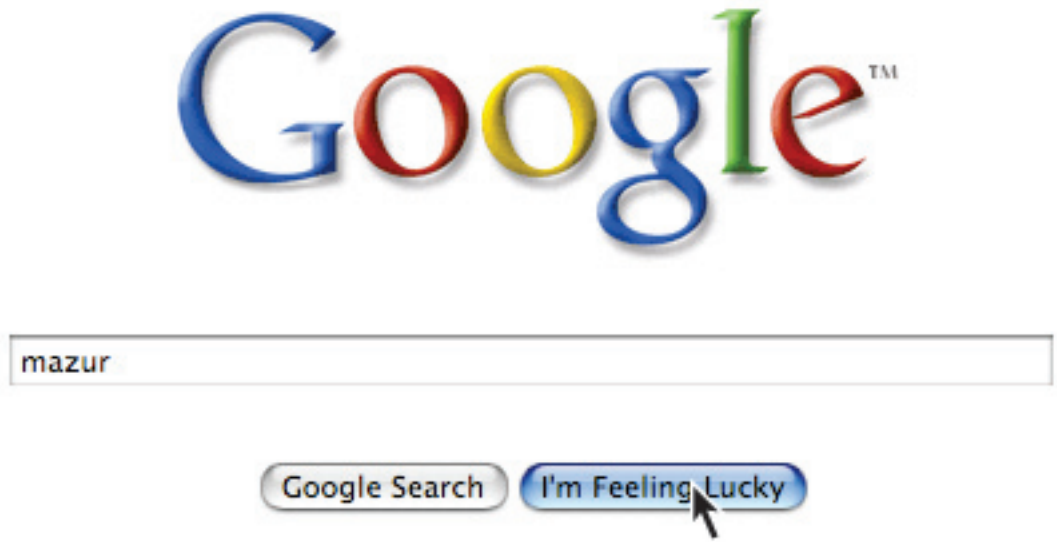
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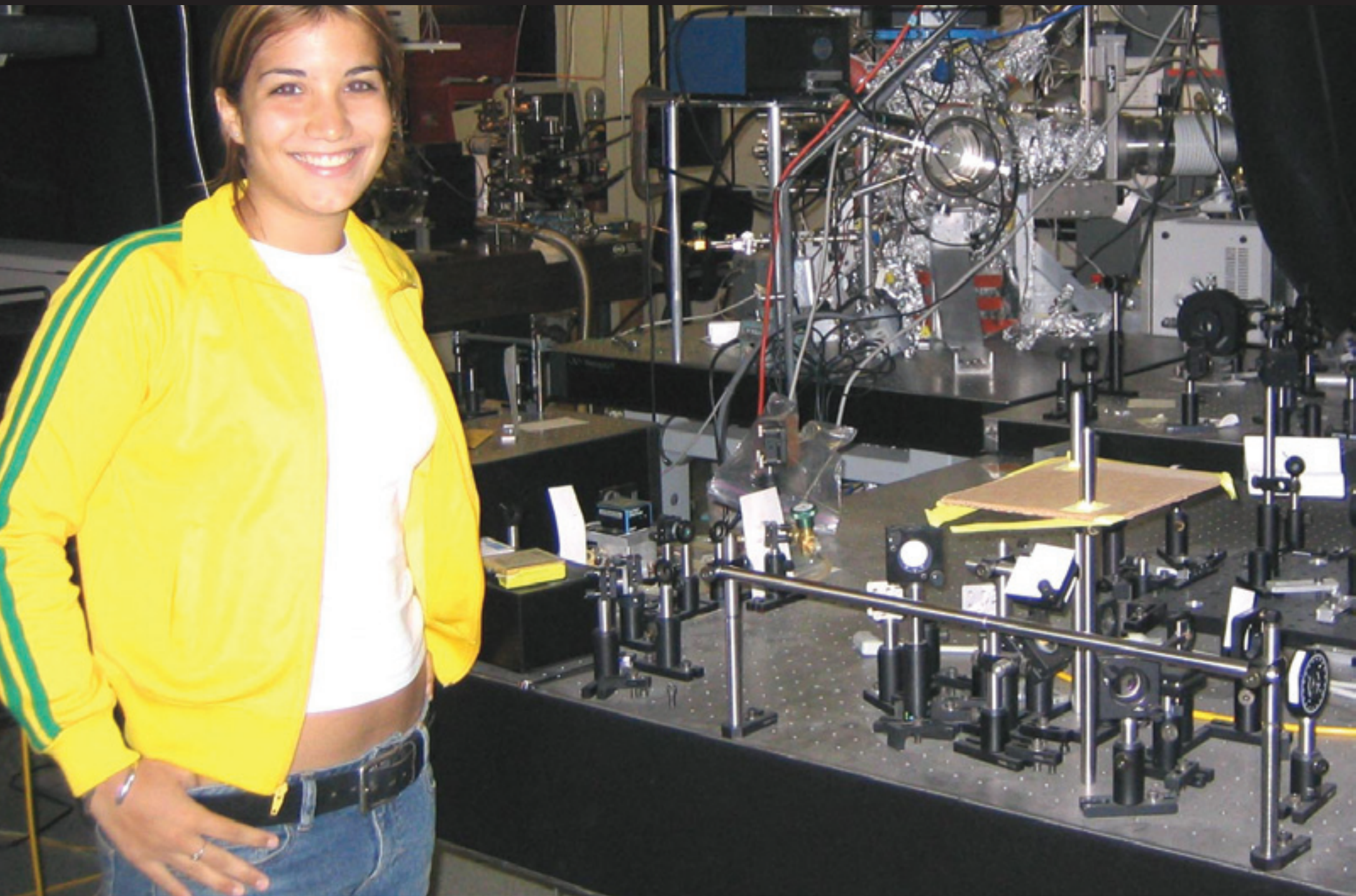
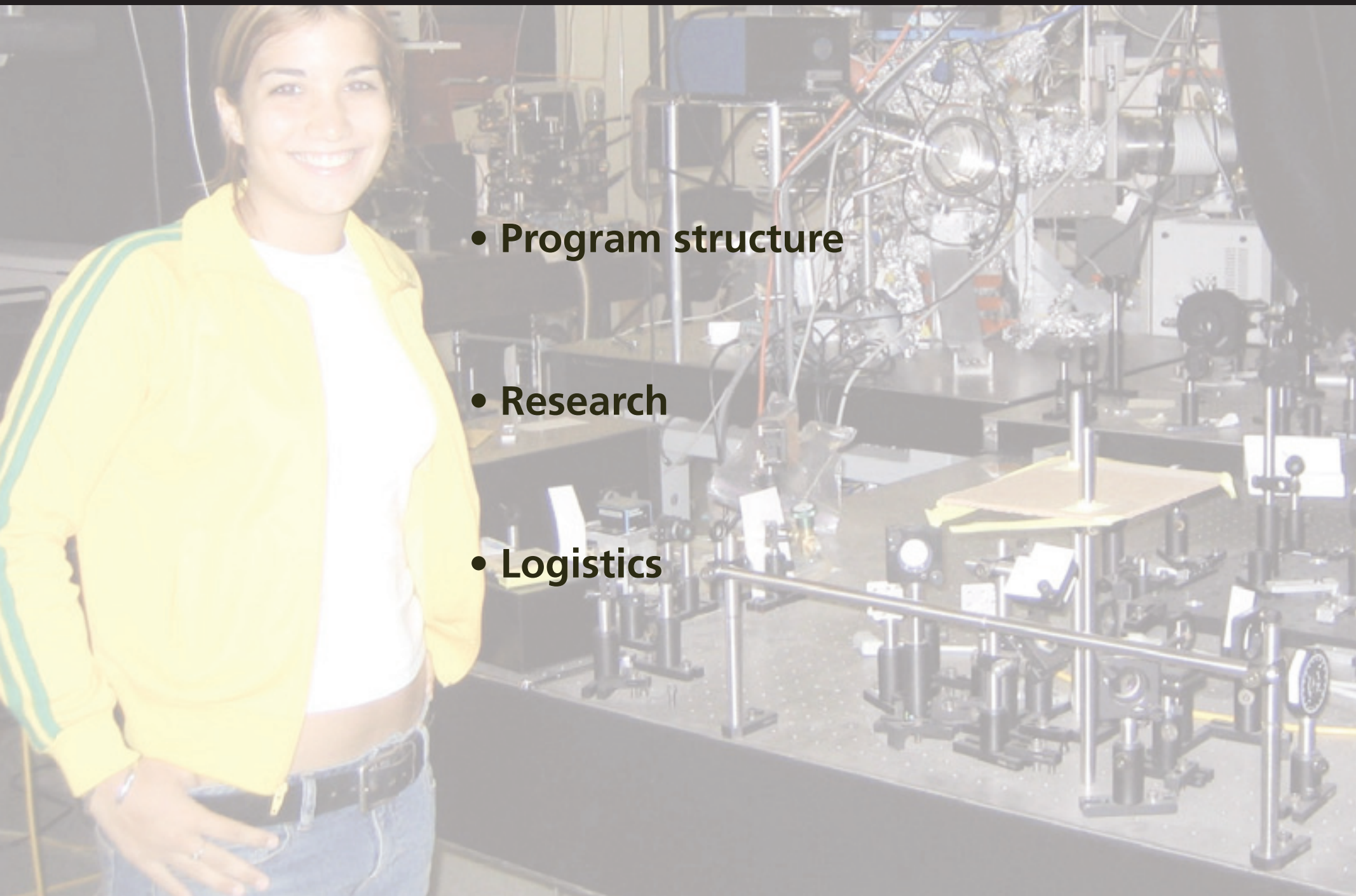
Research Experience for Undergraduates @Harvard University

University of Puerto Rico
Humaco, Puerto Rico, 18 February 2010

Outline



Outline



- Program structure
- Research
- Logistics

Program structure

Calendar

week 1:	orientation & lab tours
week 2–8:	research
	weekly faculty seminars
week 9:	final project presentation
week 10:	campus-wide symposium

Program structure

Final project

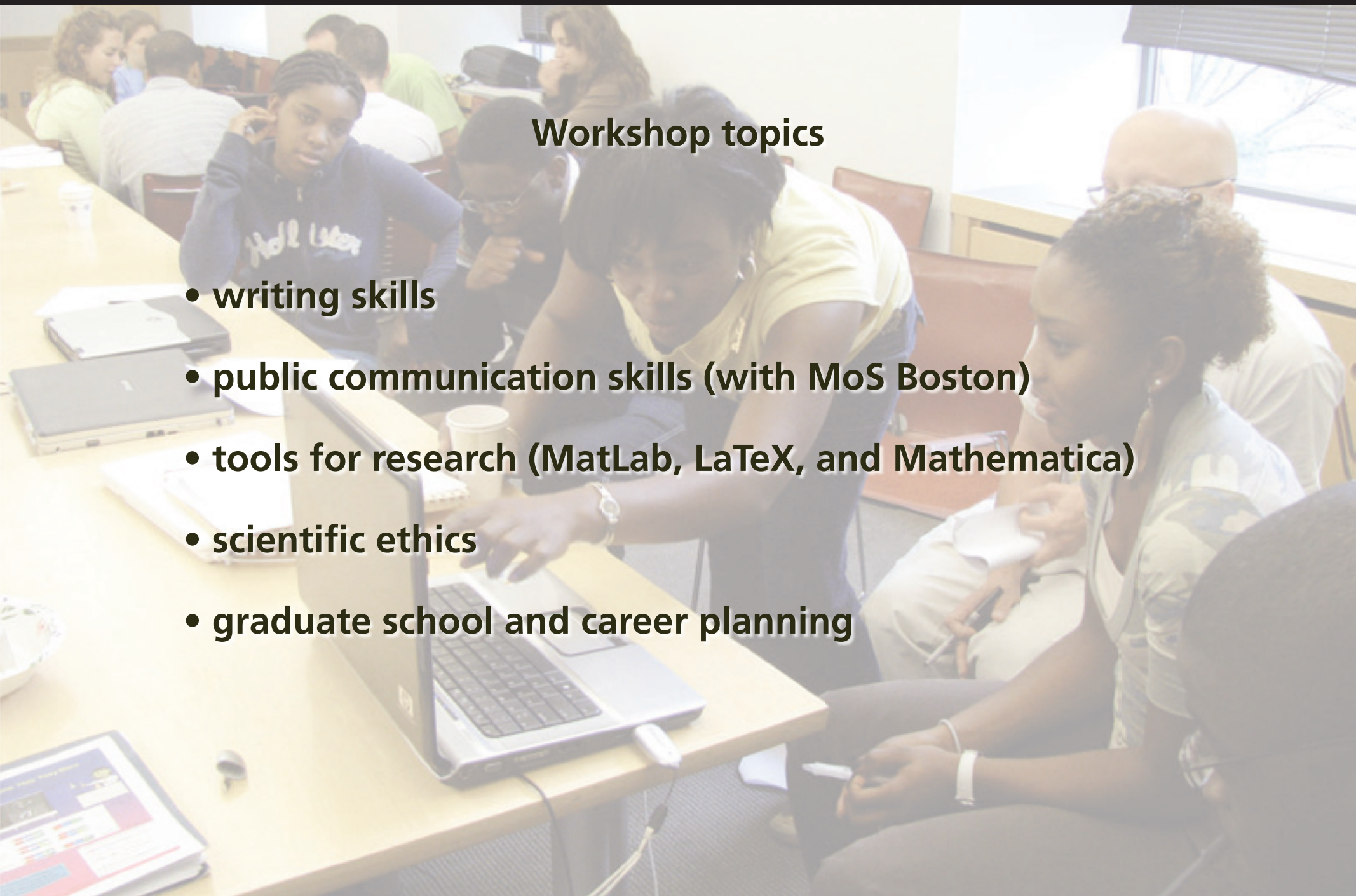
- 10-min oral presentation
- plus choice of: video, paper, or poster

Program structure

Professional development

- weekly workshops
- post-program conference travel support
- support letters for applications

Program structure



Workshop topics

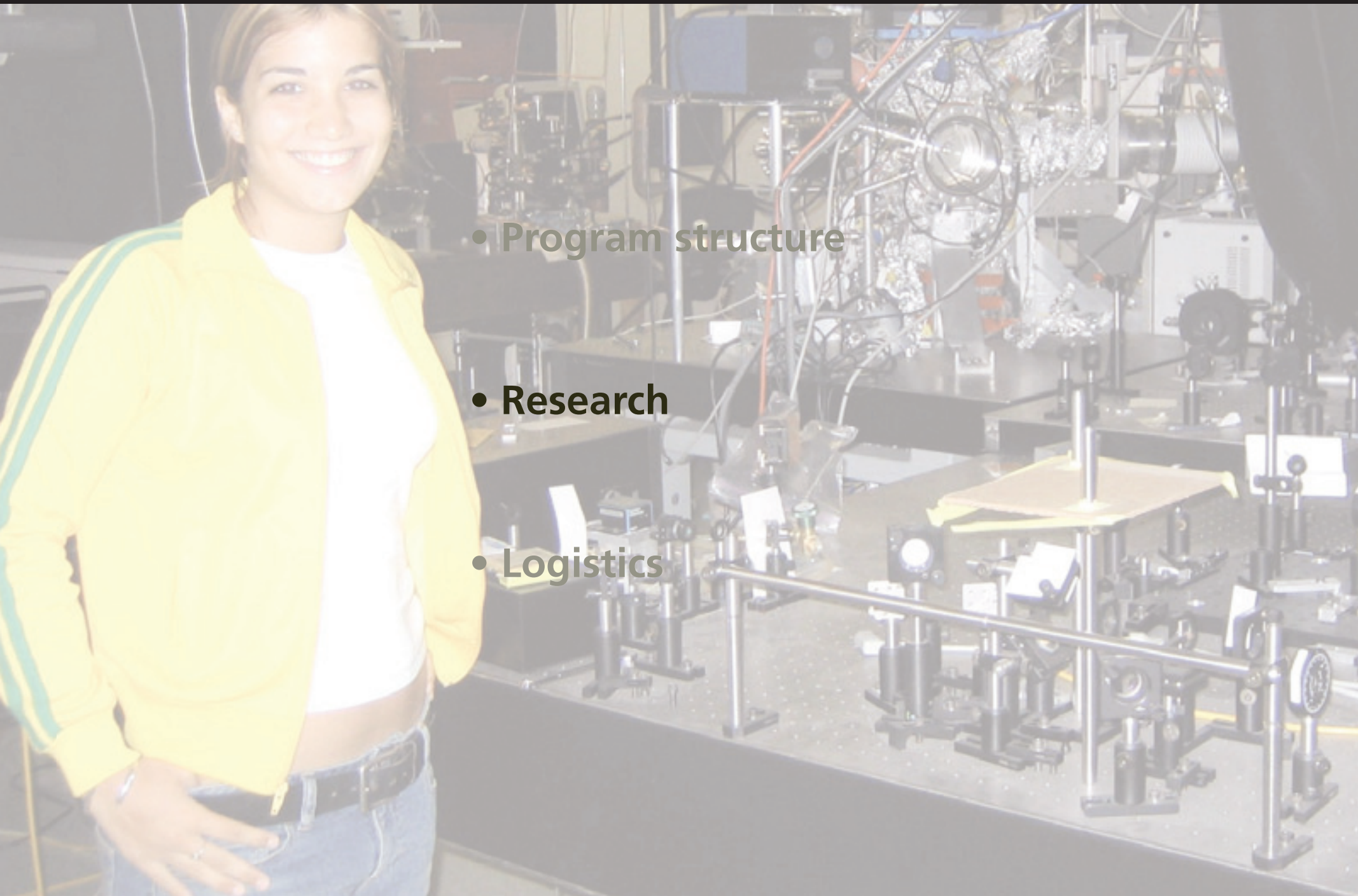
- writing skills
- public communication skills (with MoS Boston)
- tools for research (MatLab, LaTeX, and Mathematica)
- scientific ethics
- graduate school and career planning

Program structure

Social program

- excursions
- BBQ's and potlucks
- sports, dancing, and cultural activities

Outline



- Program structure
- Research
- Logistics

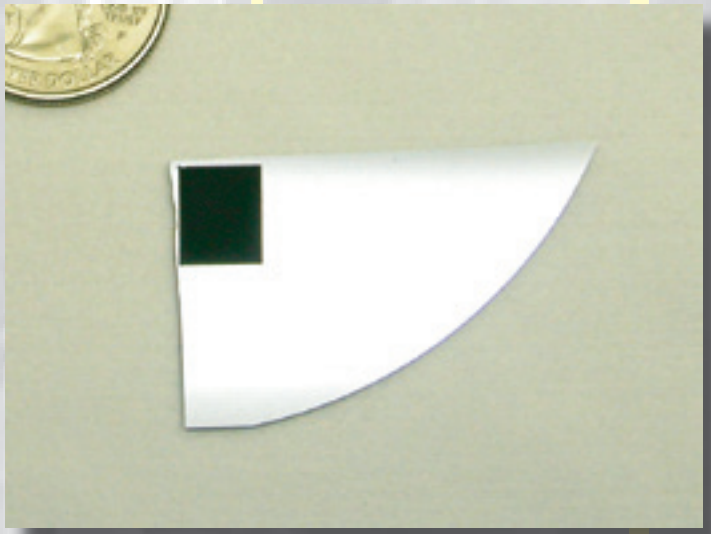
Research

Research Themes

- Nanotechnology
- Materials Science
- Biomaterials
- Solar energy harvesting
- Scientific computing
- Microrobotics

Research

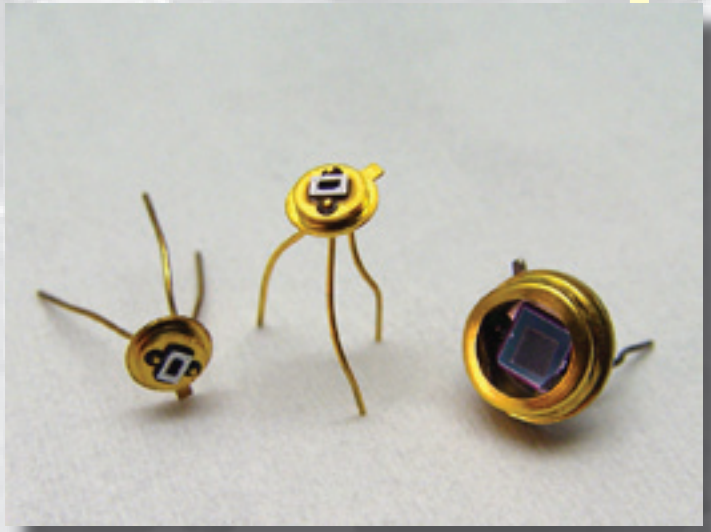
Black silicon



novel form of silicon

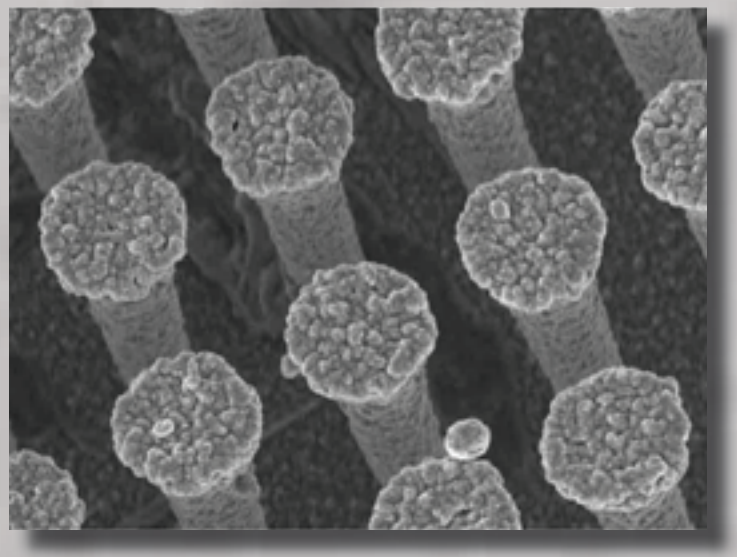
more efficient solar cells

build & characterize devices



Research

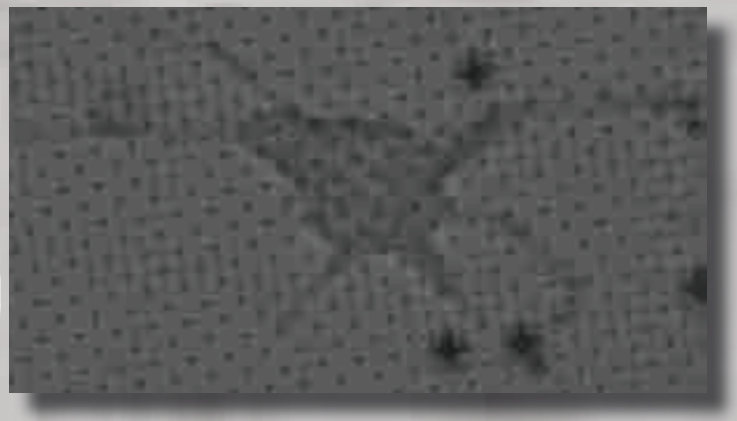
Biomaterials for cell adhesion



control cell attachment/detachment

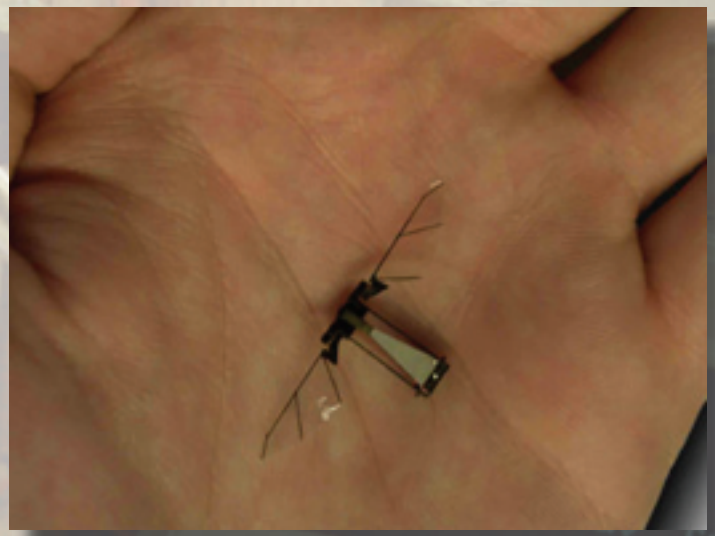
tissue engineering, medical implants

fabricate “nanograss” observe cell spreading



Research

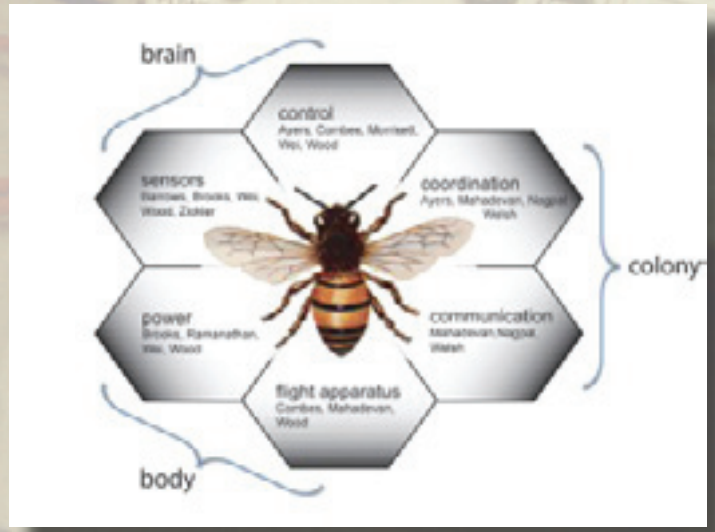
Robobees



autonomous insect-like microrobots

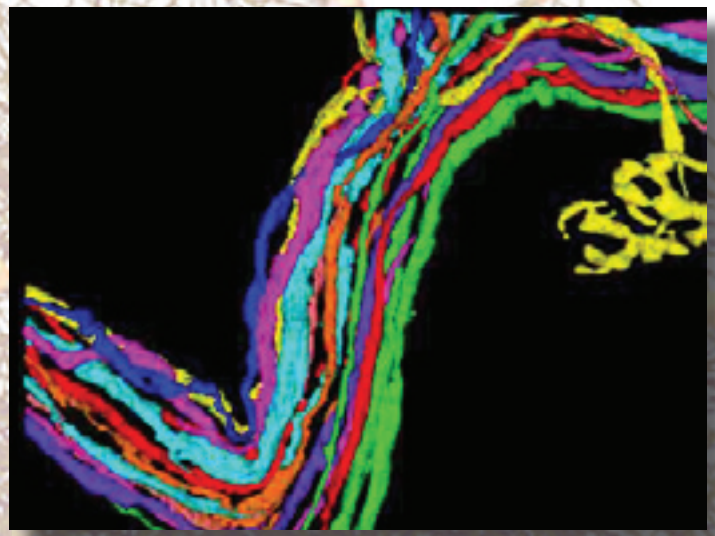
search and rescue, pollination

build & characterize devices



Research

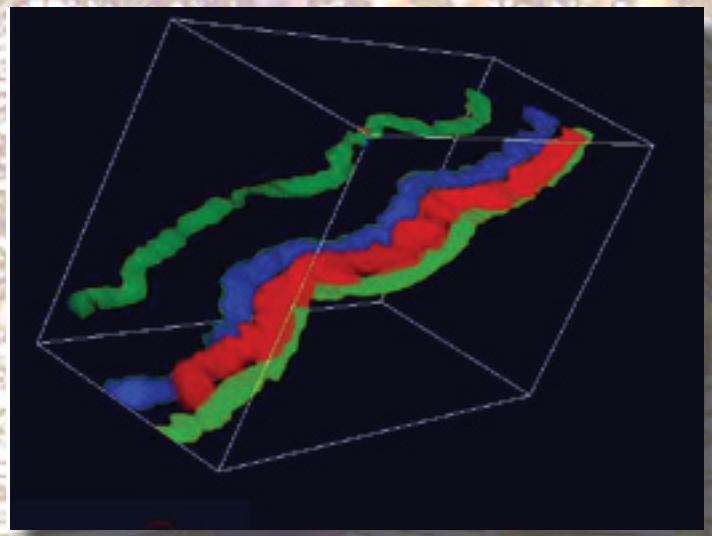
Scientific computing



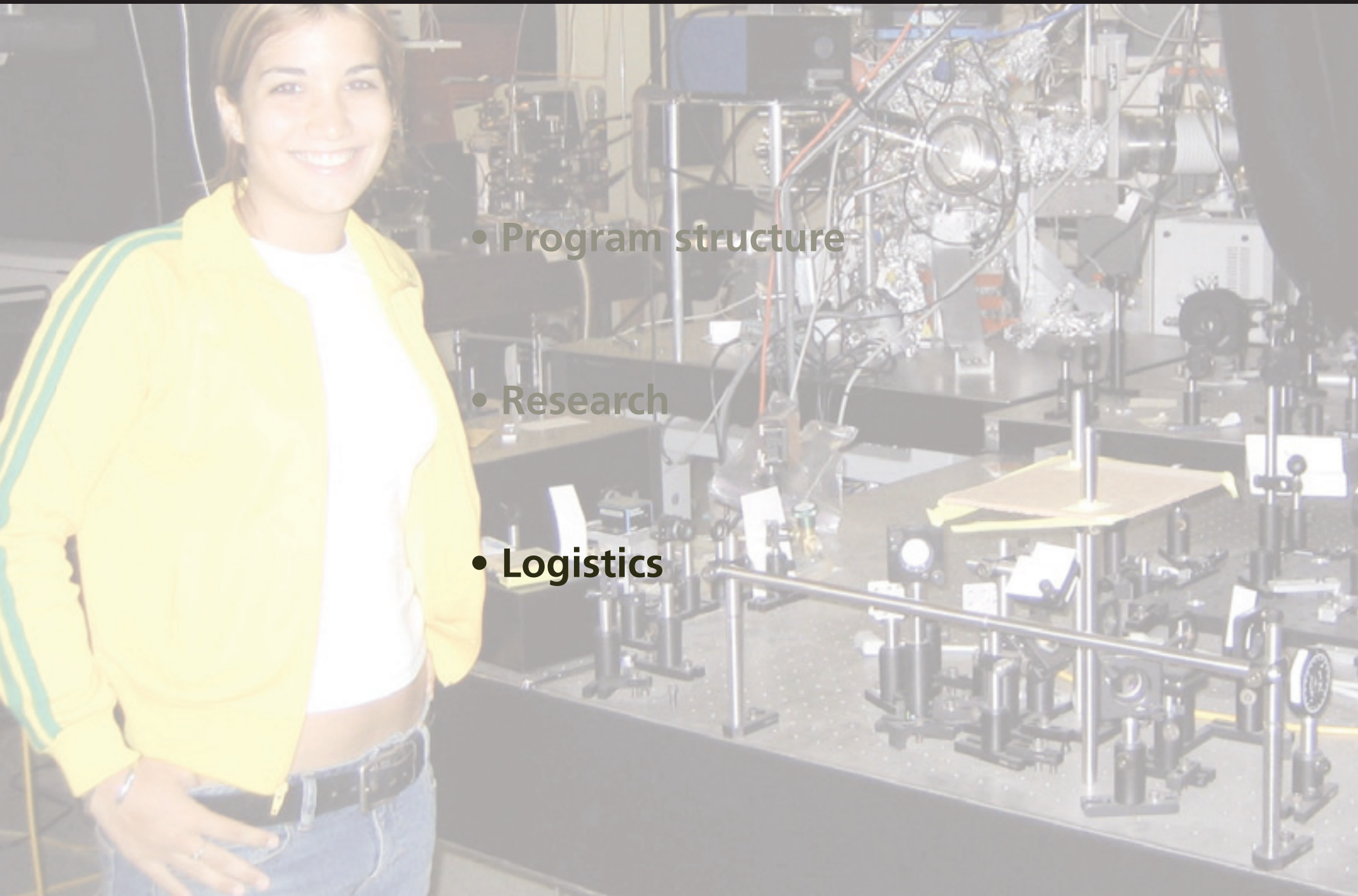
use GPUs to visualize complex systems

biology, astrophysics, quantum chemistry

obtain 3D structure of neurons



Outline



- Program structure
- Research
- Logistics

Logistics

Program dates

June 6 – August 14, 2010

Logistics

Applying

Where: reusite.seas. harvard.edu/application

Need: 2 recommendation letters & transcript

Deadline: February 28

Selection: mid-April

Logistics

Applying

Where: reusite.seas. harvard.edu/application

Need: 2 recommendation letters & transcript

Deadline: February 28

Selection: mid-April

NO PREVIOUS EXPERIENCE REQUIRED!

Logistics

Financial

$4000 stipend

$350 travel reimbursement

free university housing

option to purchase mealplan

“I keep in touch with my mentors, and they have continued to support me through graduate school decisions. … I was lucky to be in a lab environment where investment in the student was just as important as investment in the research project.”

“I had a very memorable summer […]. I made great friends [… and] developed a great relationship with my professor who continues to consult me in regards to my future endeavors.”

“I feel strongly that the REU has been my best academic research experience to date, and […] I decided that research is my passion, and that I want to pursue it regardless of my future career goals.”


YOU COULD BE ONE OF THEM!

Apply by February 28

reusite.seas.harvard.edu/application

notification by April 15

Program dates: June 6 – August 14

http://twitter.com/eric_mazur