Sub-cellular nanosurgery in live cells using ultrashort laser pulses

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motivation

femtosecond lasers for sub-cellular manipulation

- high penetration depth in tissues

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- nonlinear interaction

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- no damage outside focal region

motivation

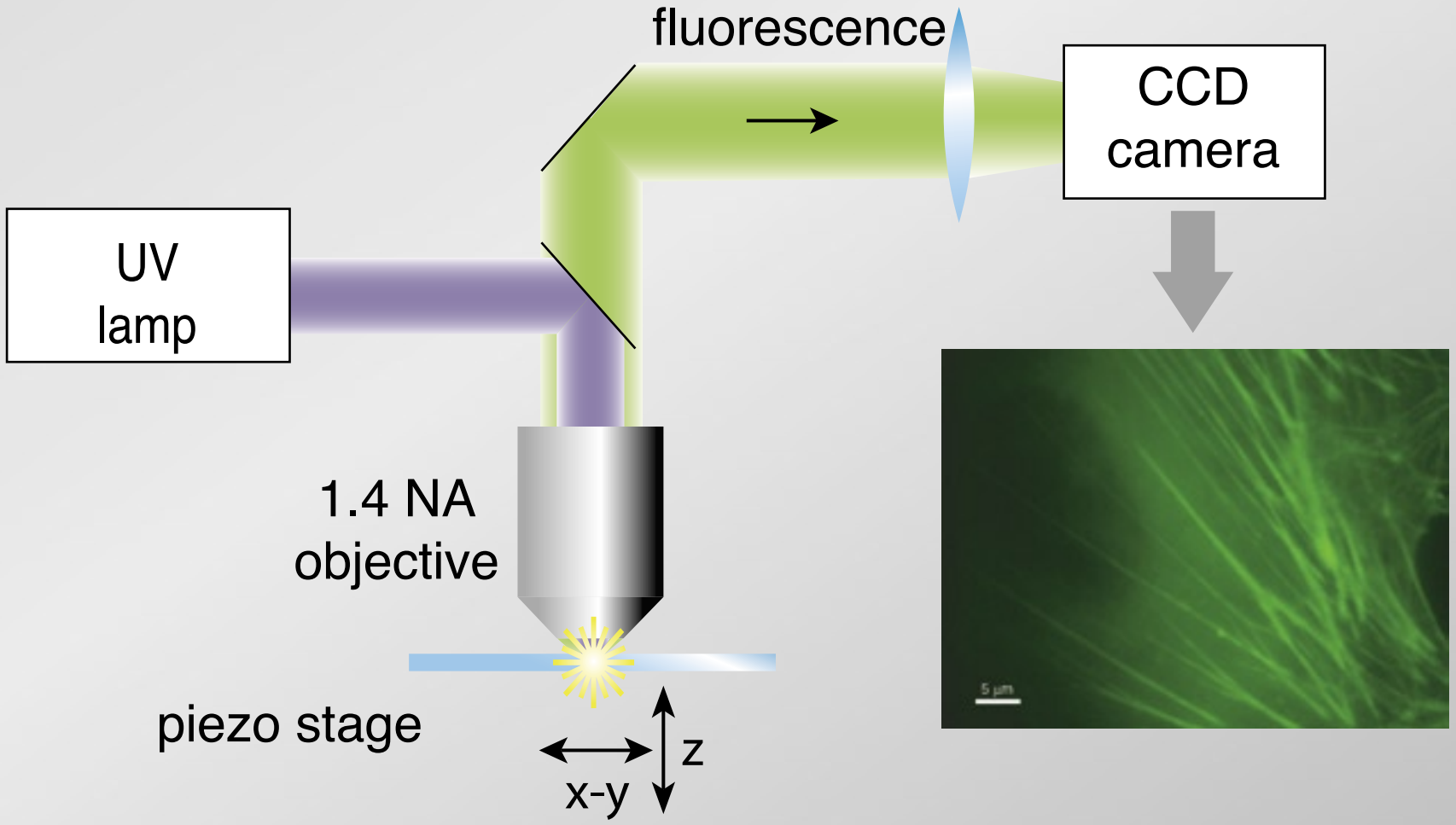
femtosecond lasers for sub-cellular manipulation

- high penetration depth in tissues
- nonlinear interaction
- no damage outside focal region
- easily integrated with high resolution microscopy

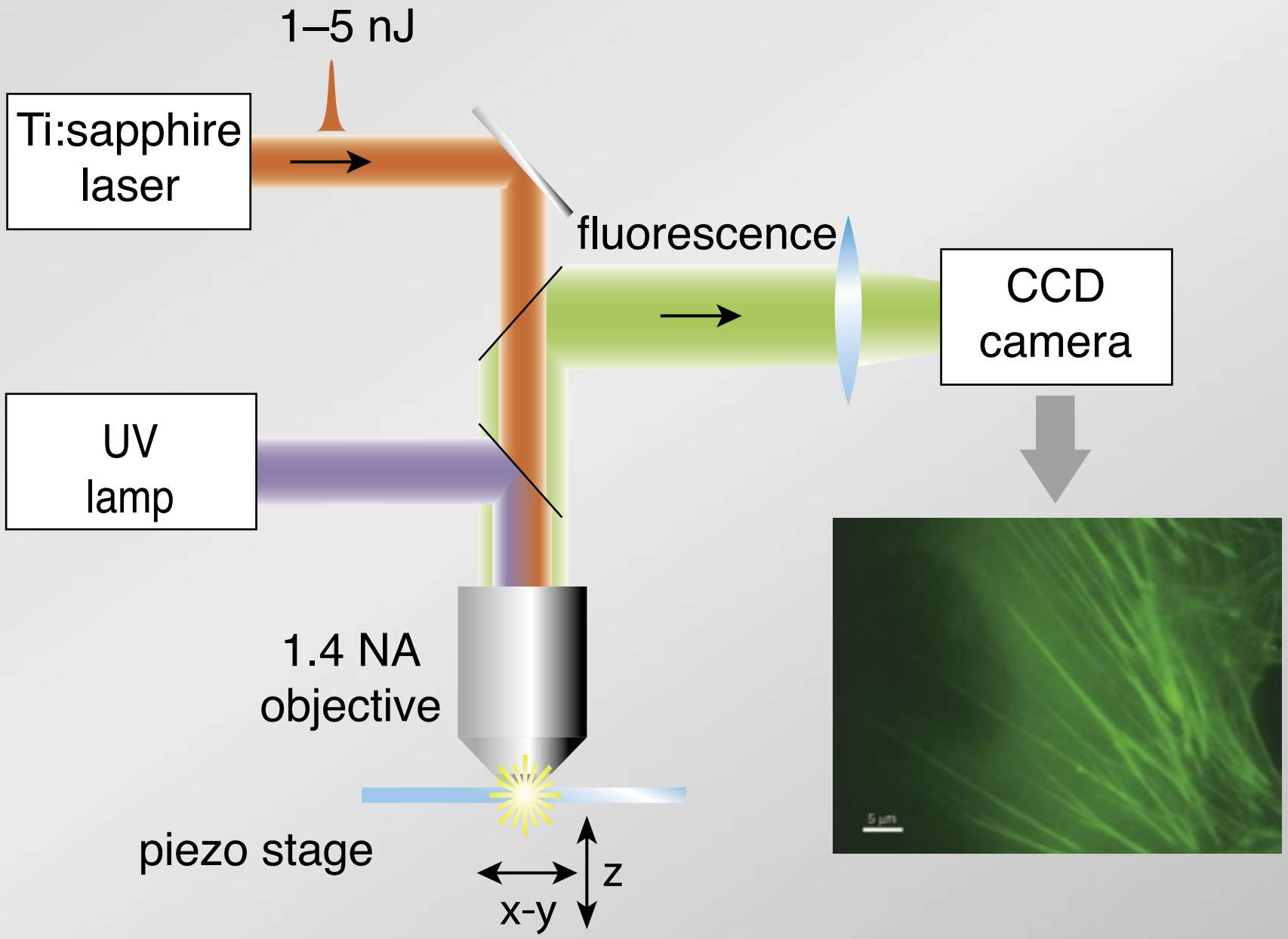
outline

- material ablation in cells
- nanosurgery in live cells
- stress fiber dynamics

setup

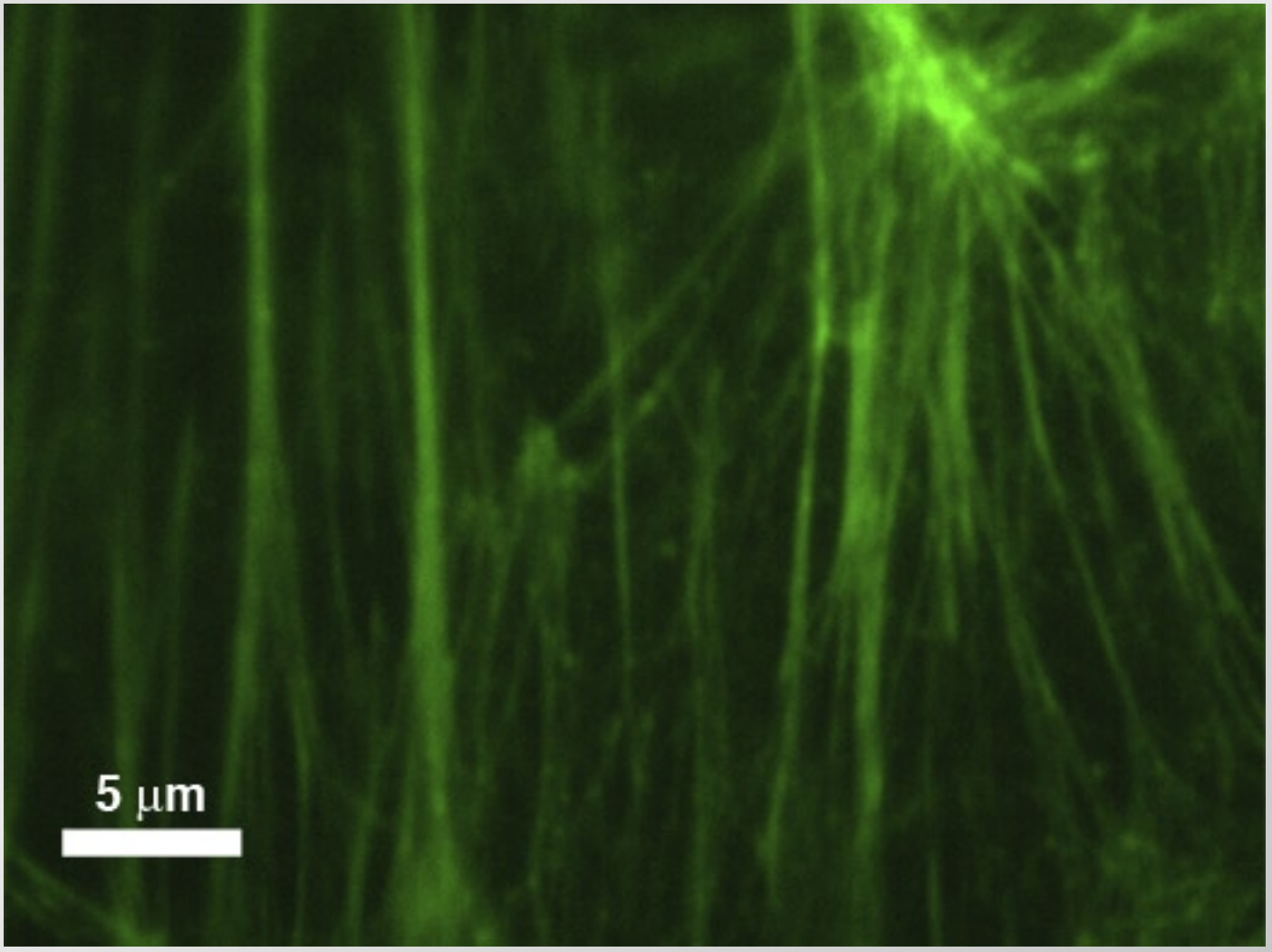

fluorescence
CCD camera
UV lamp
1.4 NA objective
piezo stage
x-y
z
5 µm

setup


1–5 nJ
Ti:sapphire laser
fluorescence
CCD camera
UV lamp
1.4 NA objective
piezo stage
x-y
z
5 µm

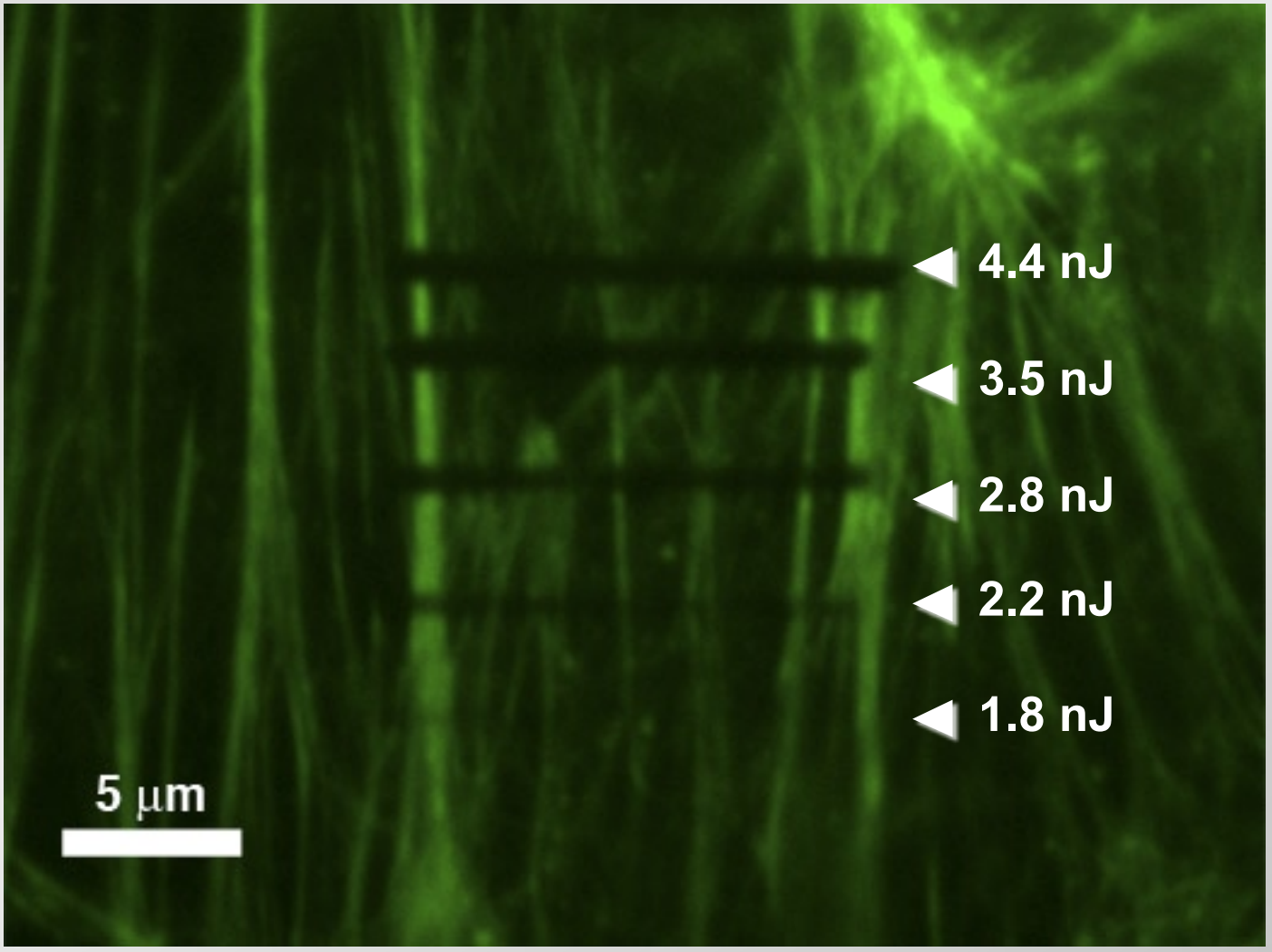
ablation in fixed cells

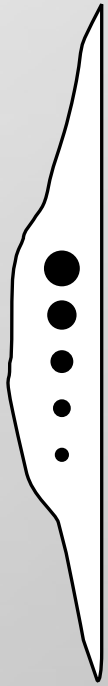
fluorescent actin network in a fixed cell



ablation in fixed cells

actin network after laser irradiation





ablation in fixed cells

q: material ablation or photobleaching?

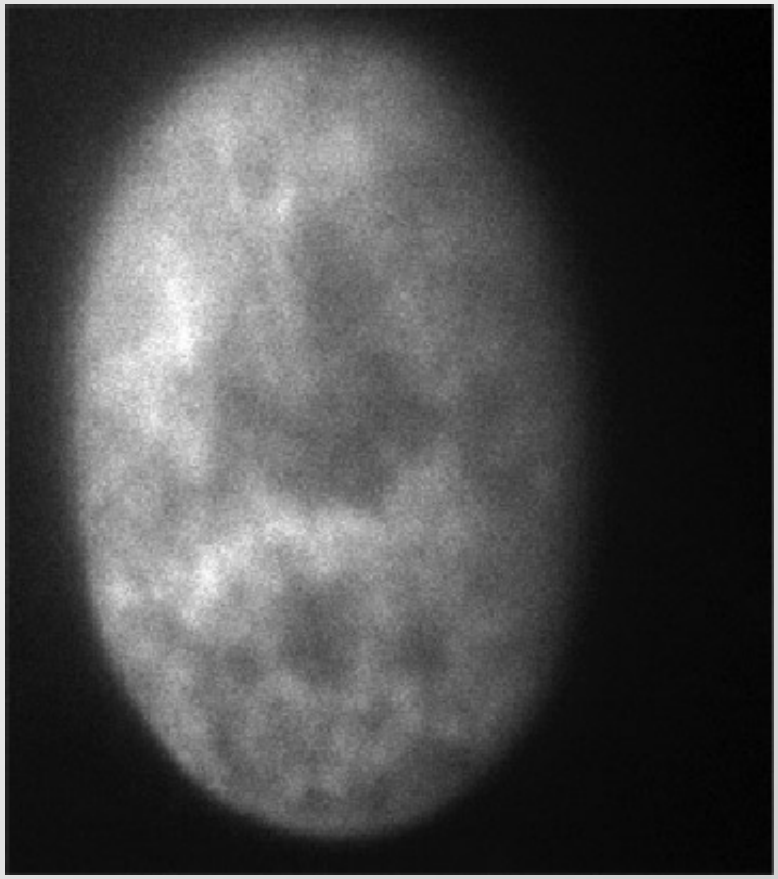
ablation in fixed cells

q: material ablation or photobleaching?

a: use electron microscopy to verify material ablation

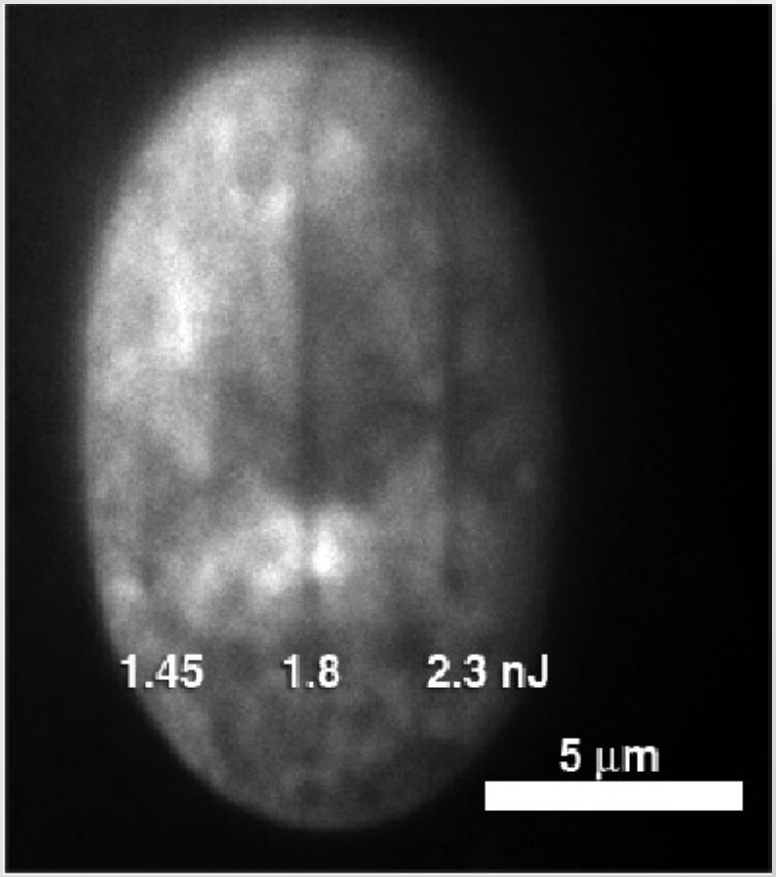
ablation in fixed cells

fluorescence image of a stained nucleus



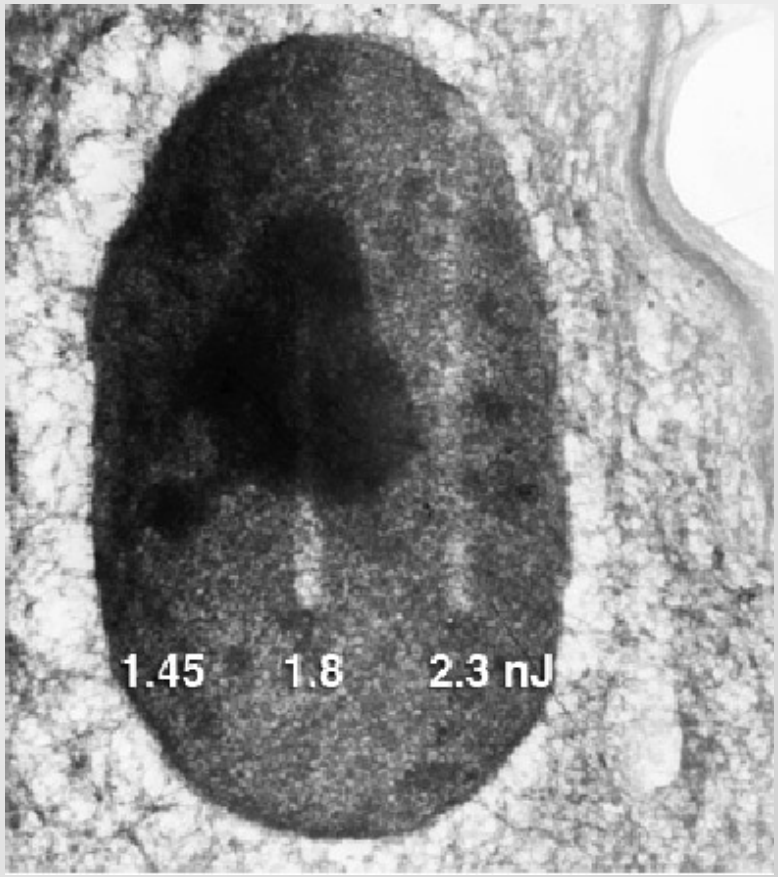
ablation in fixed cells

fluorescence image after laser irradiation



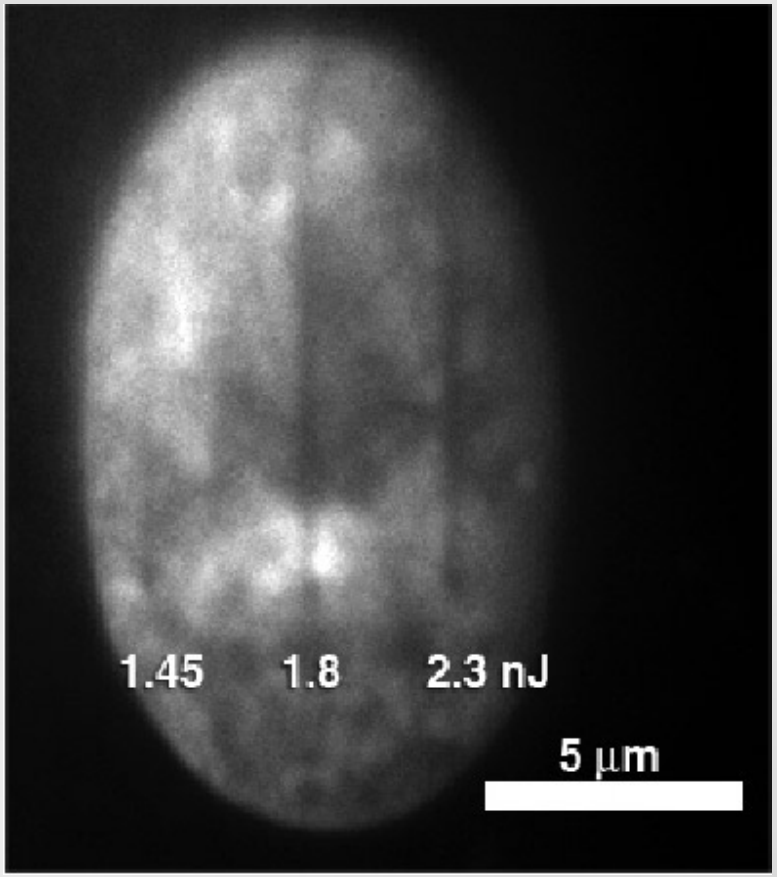
ablation in fixed cells

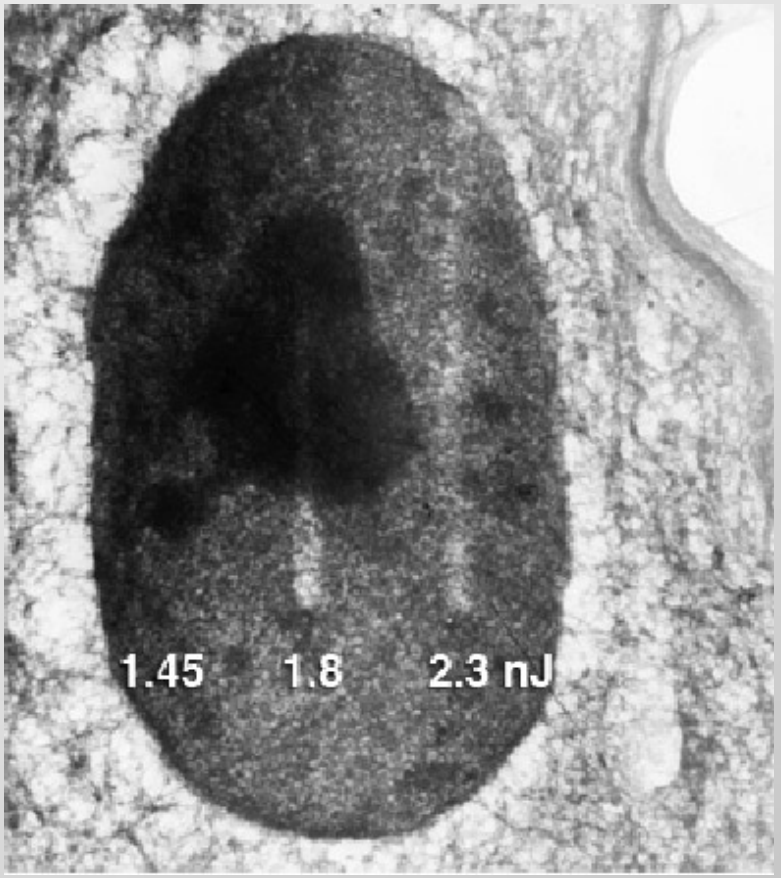
TEM image of the same nucleus



ablation in fixed cells

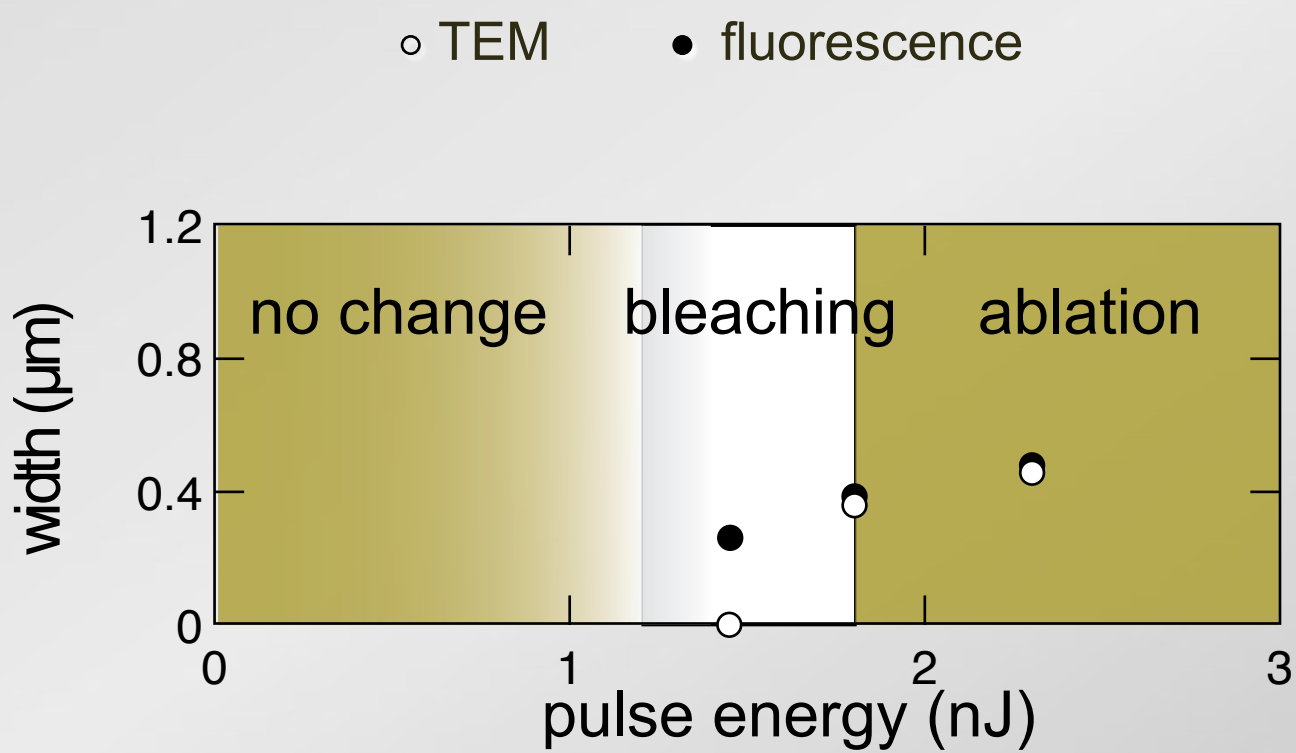
1.45 nJ shows photobleaching no ablation





ablation in fixed cells

define three regions of interaction



summary

definitive proof of sub-cellular material ablation

ablation widths as small as 250 nm

ablation threshold varies slightly

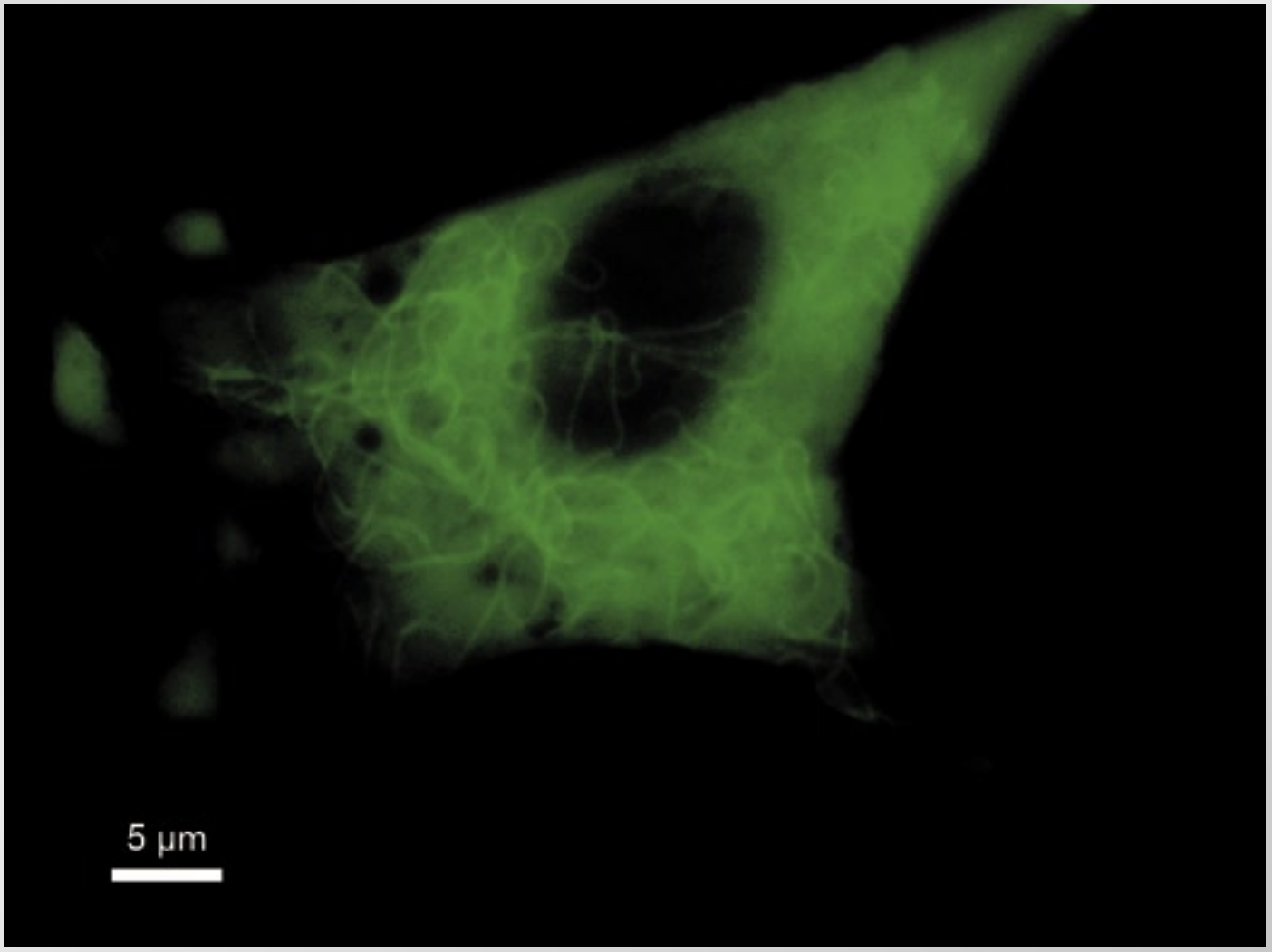
ablation threshold is 1.2 times that of photobleaching

outline

- material ablation in cells
- nanosurgery in live cells
- stress fiber dynamics

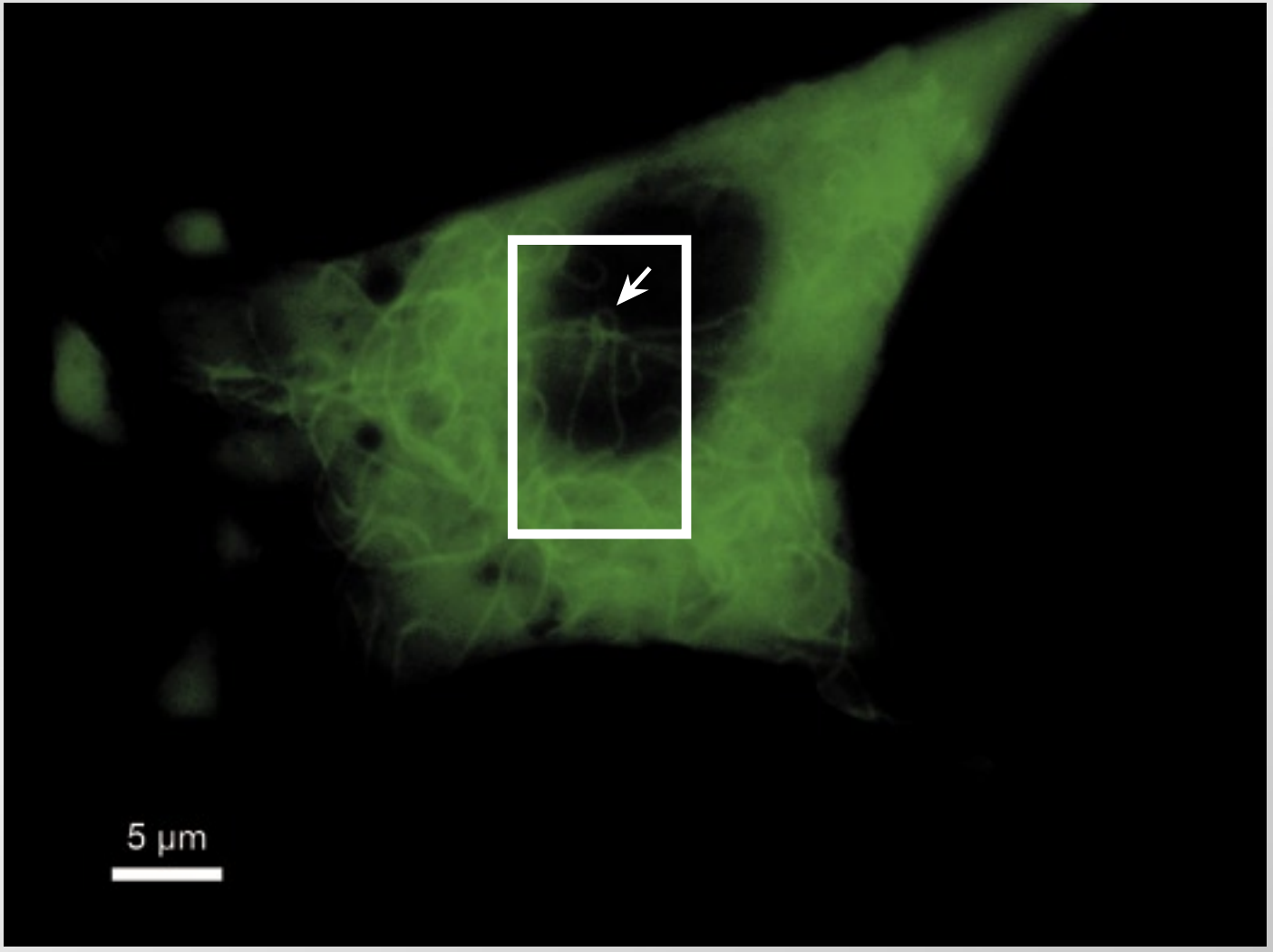
nanosurgery in live cells

GFP-labeled microtubules in live endothelial cells



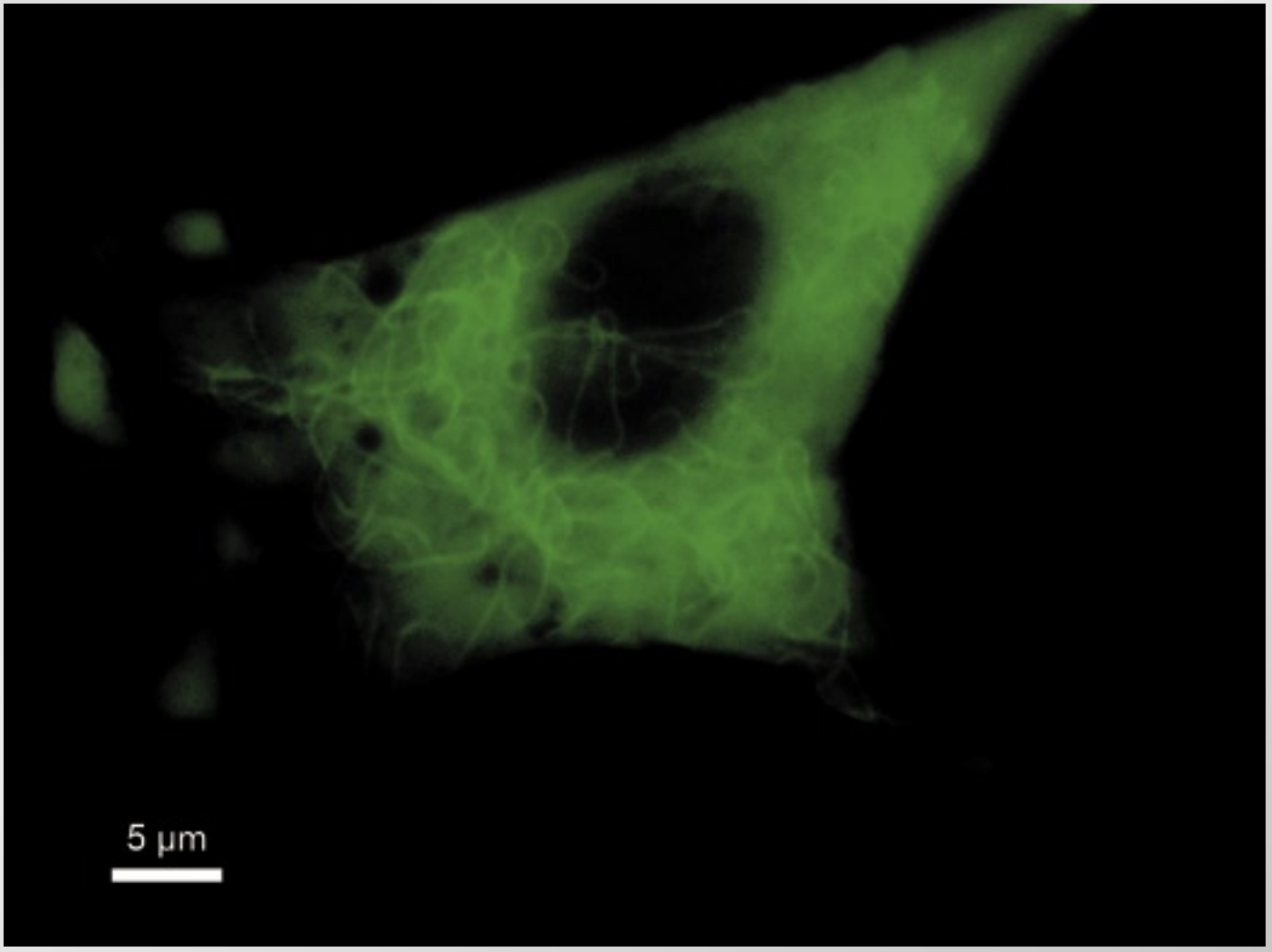
nanosurgery in live cells

cutting microtubules in live cells



nanosurgery in live cells

cutting microtubules in live cells



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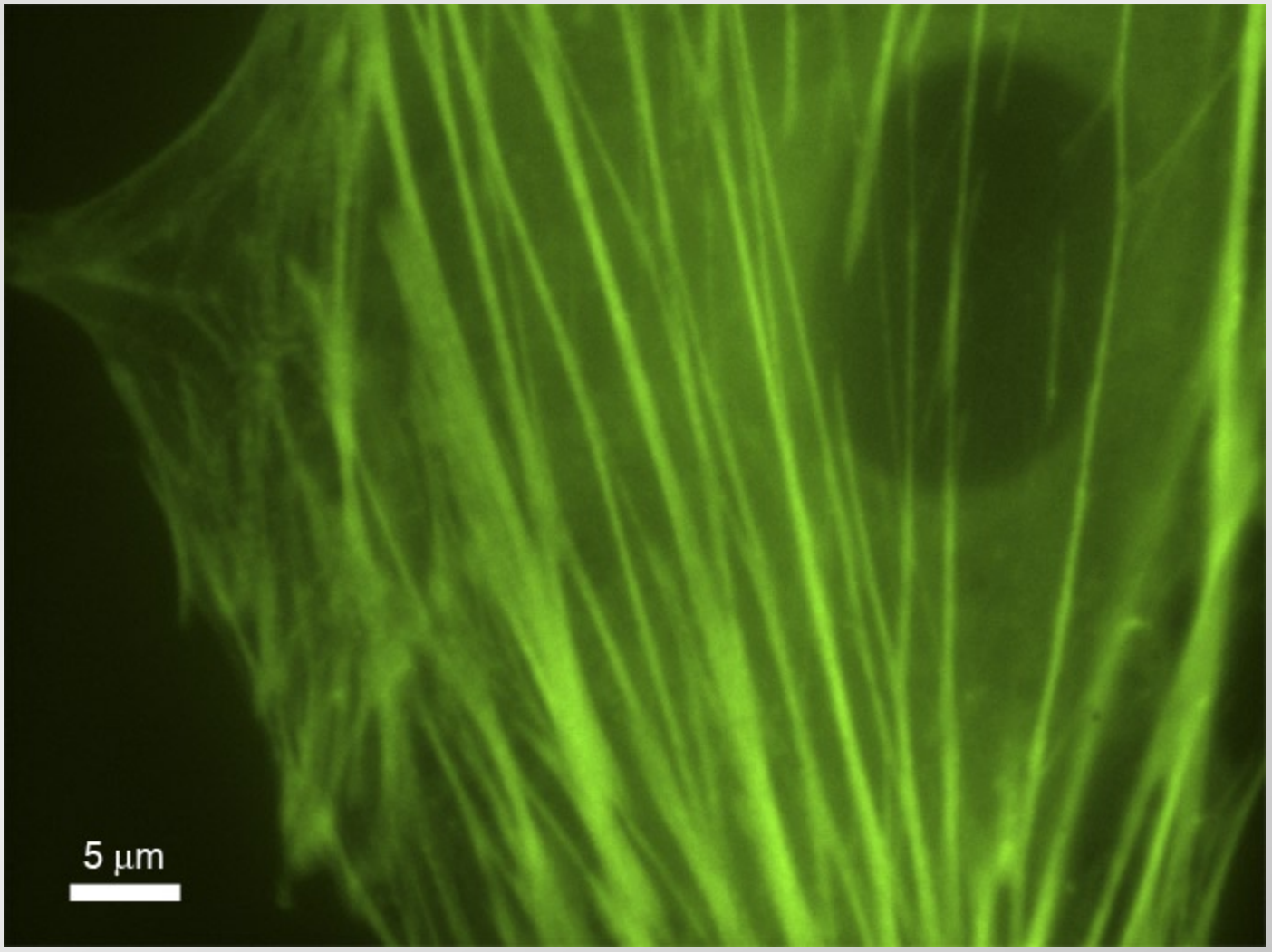
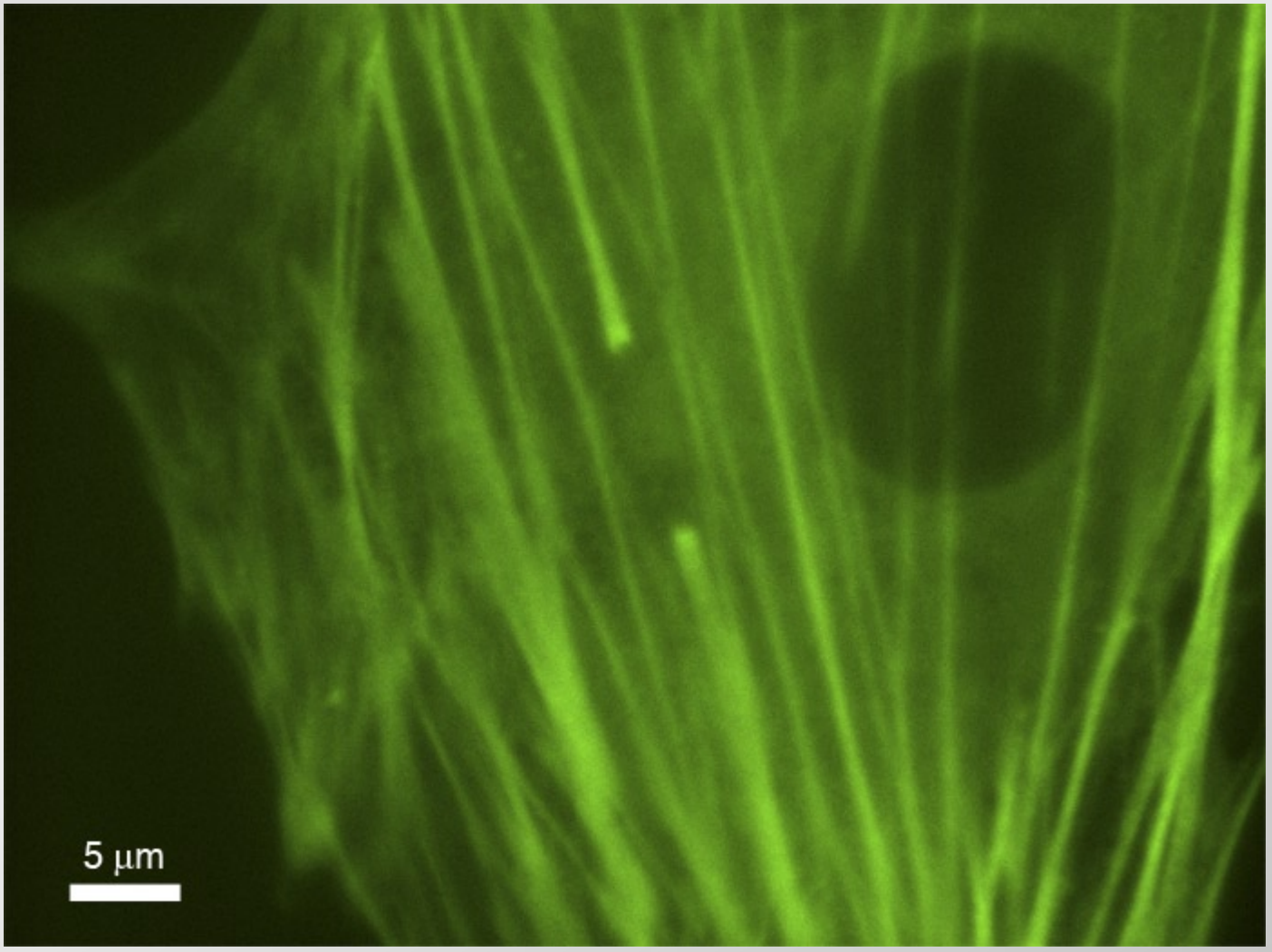
nanosurgery in live cells

YFP fluorescent actin filaments in a live cell



nanosurgery in live cells

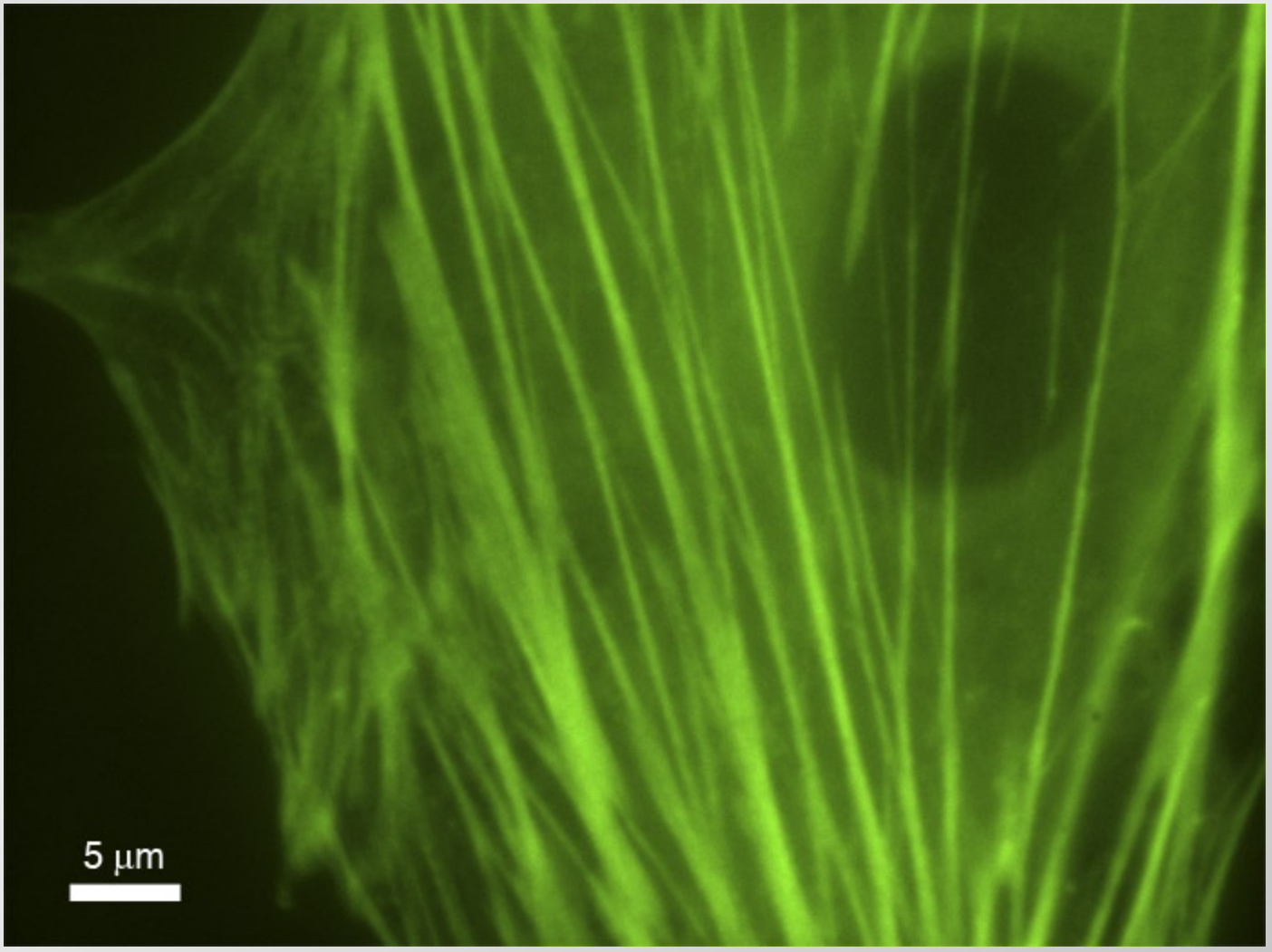
10 seconds later



cell mechanics

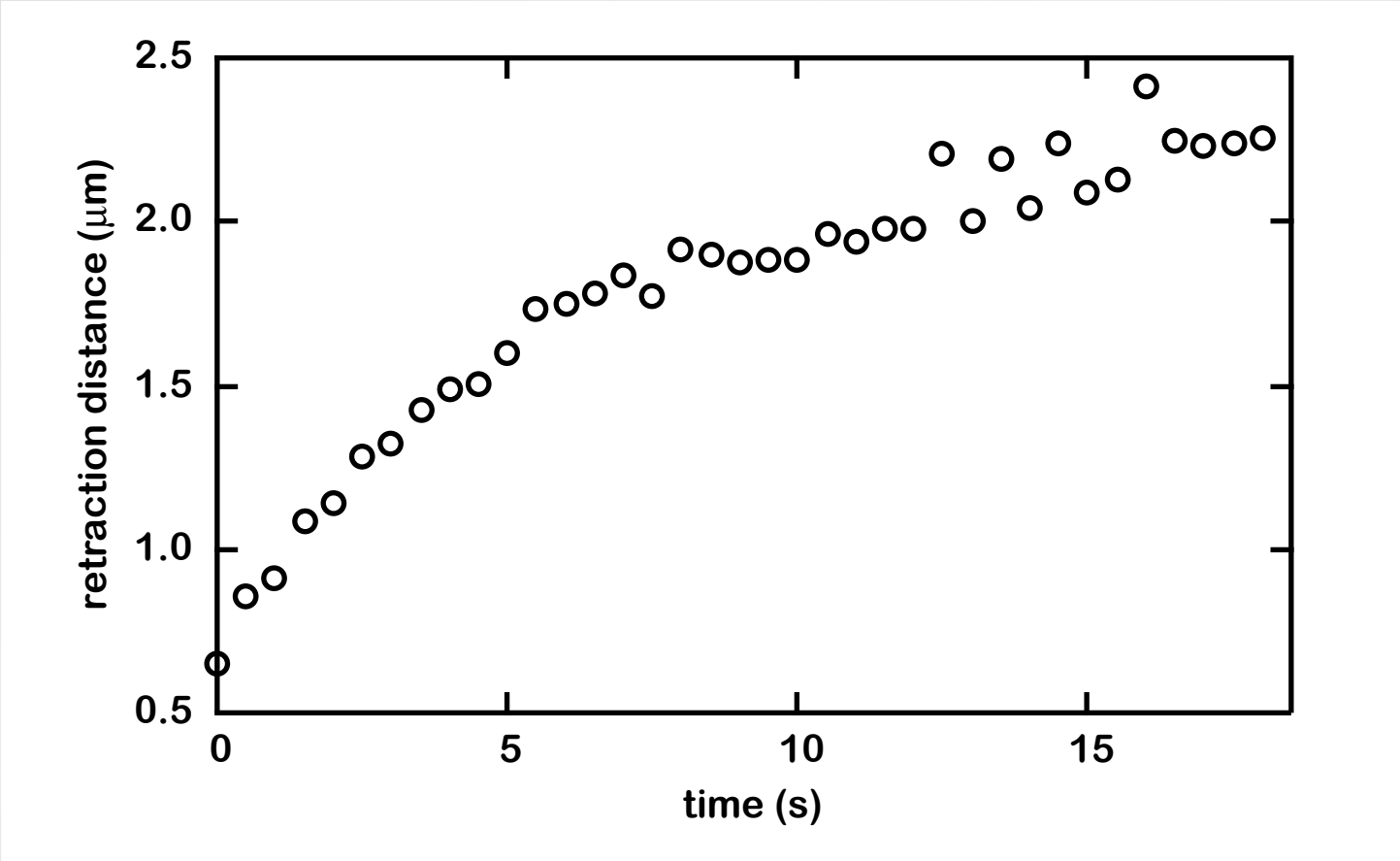
- cells are thought to be tensegrity structures
- tensegrity is balance of tension and compression
- actin bundles bear tension

nanosurgery in live cells



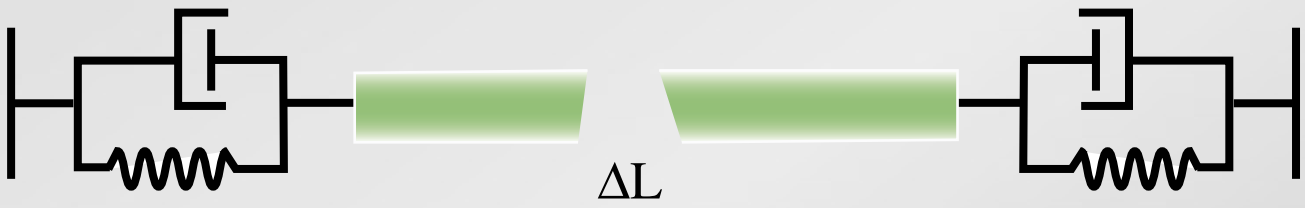
stress fiber mechanics

fiber retraction vs. time after laser ablation



stress fiber mechanics

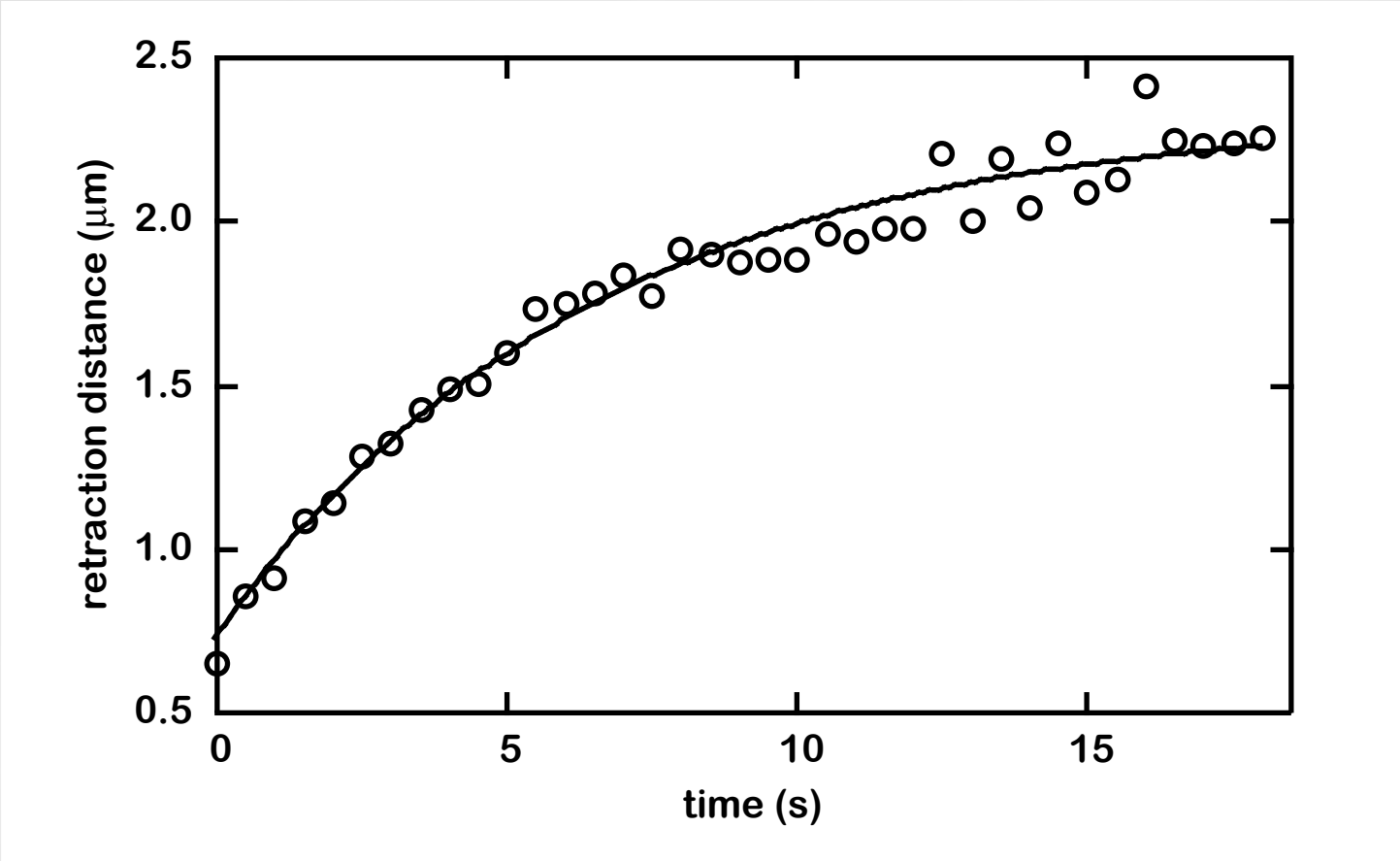
viscoelastic model of an actin fiber bundle



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

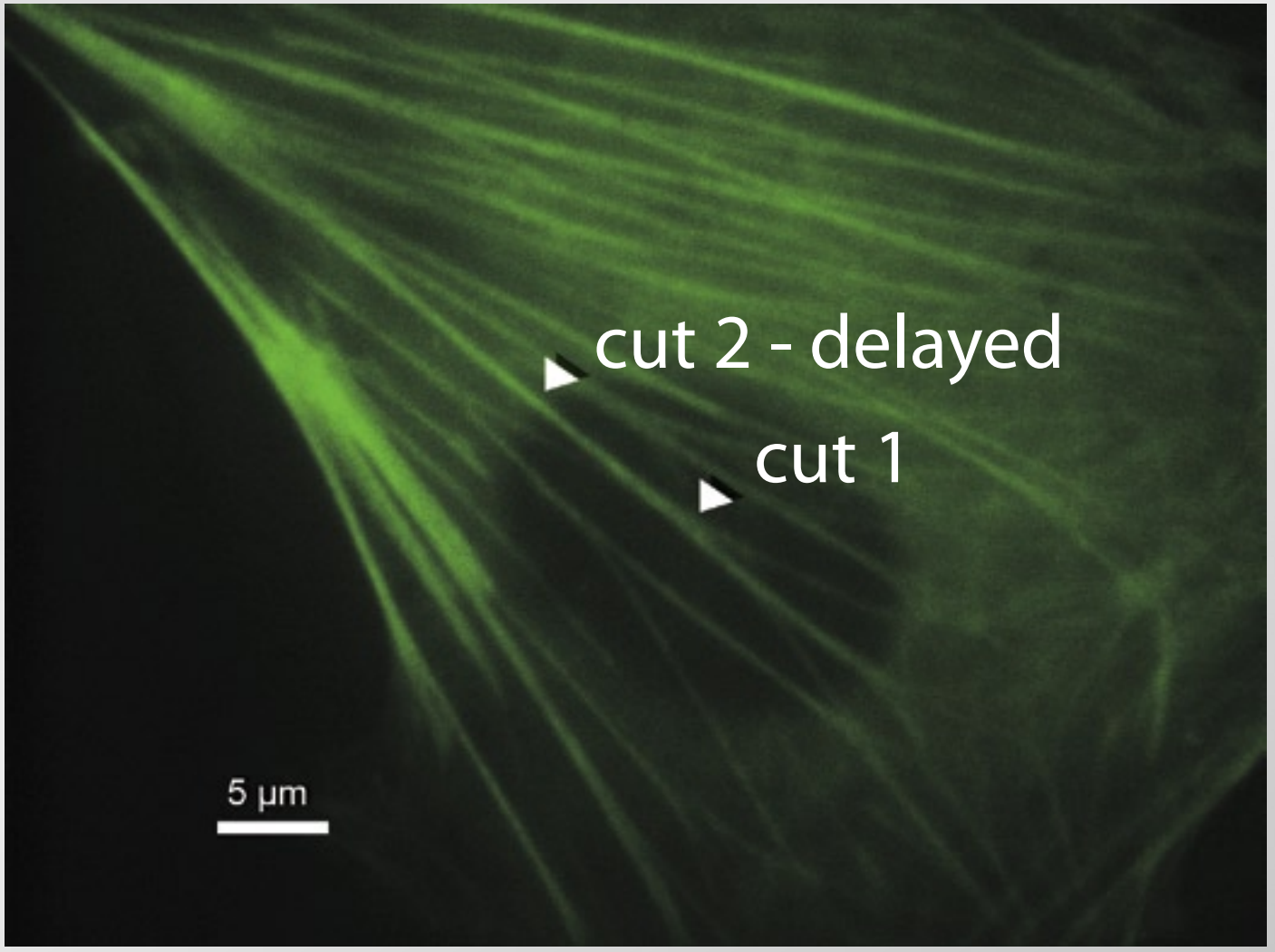
stress fiber mechanics

modeling of tension release



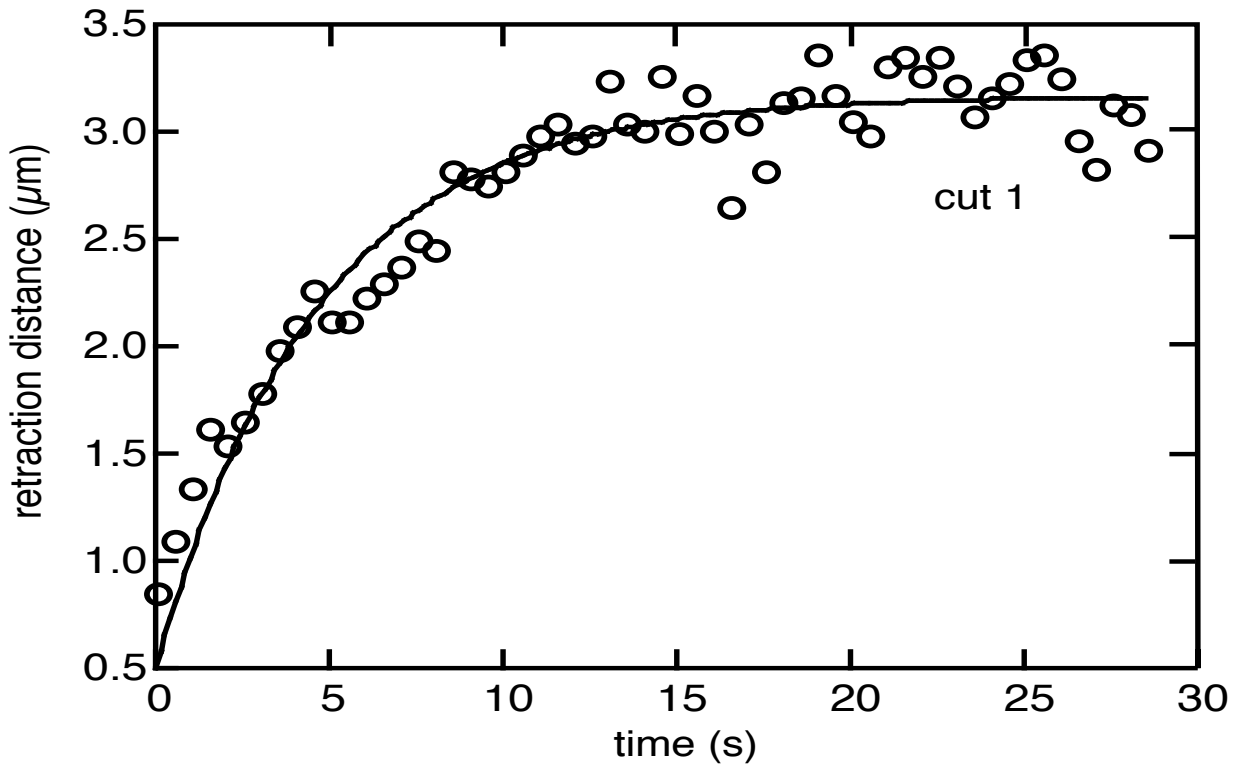
stress fiber mechanics

2 cuts along the same fiber



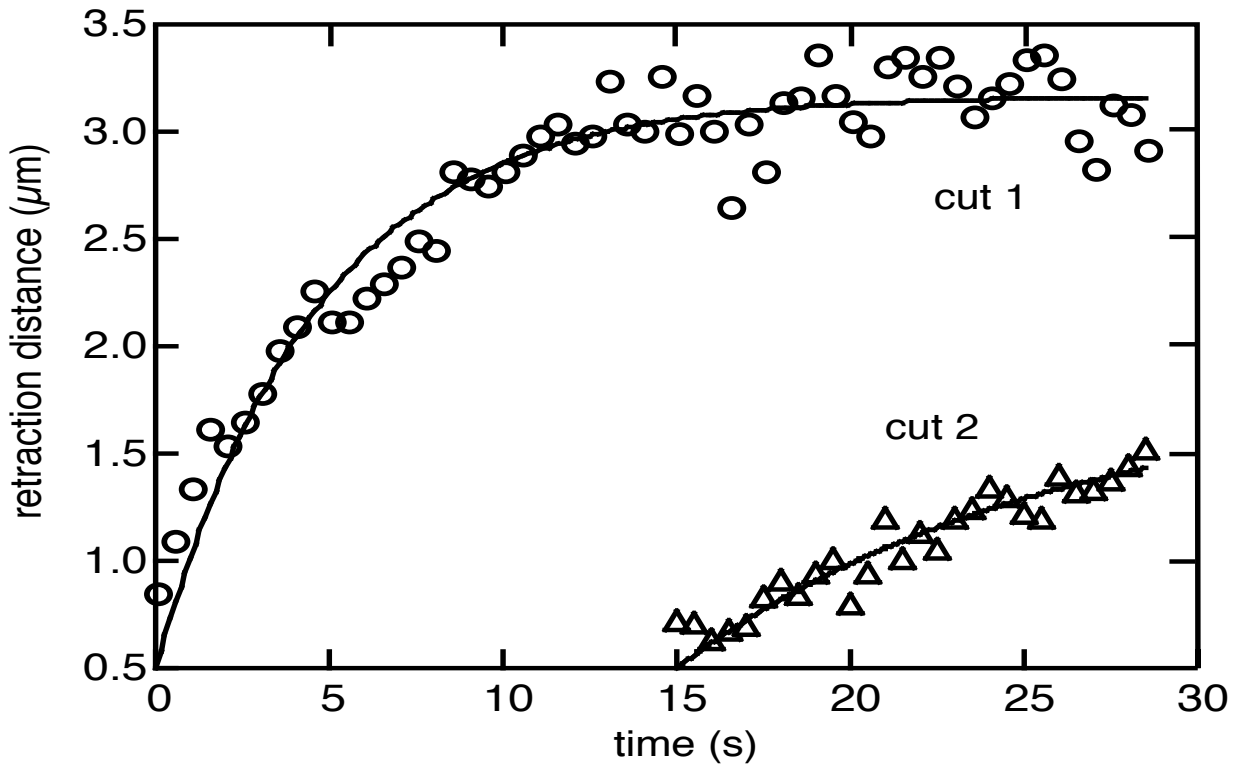
stress fiber mechanics

release of tension release



stress fiber mechanics

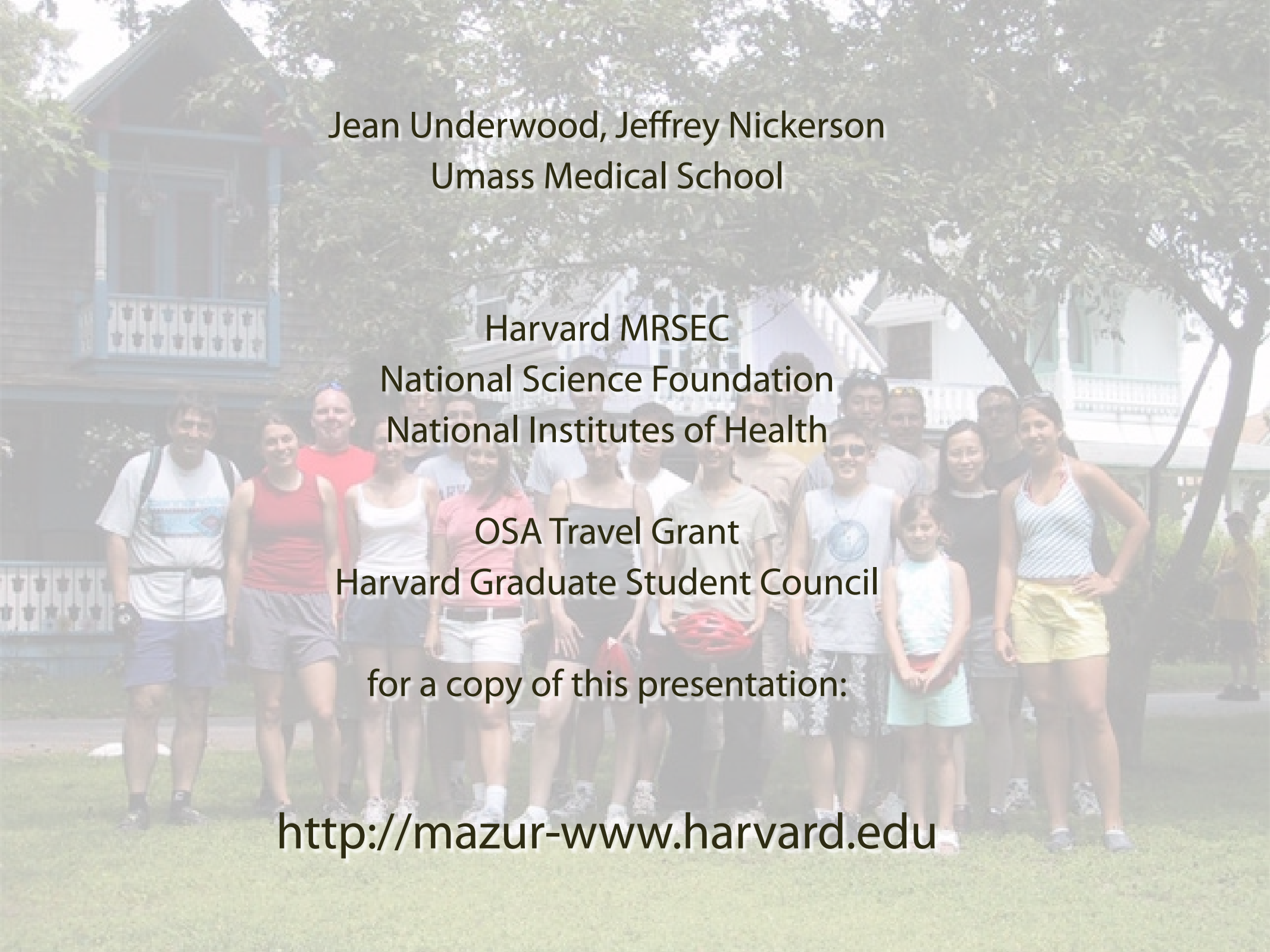
release of tension release



summary

fs laser sub-cellular ablation is:

- verified by TEM
- used for live cell nanosurgery
- a tool to study stress fiber mechanics



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stress fiber mechanics

effect of myosin motor inhibition on stress fibers tension

stress fiber mechanics

effect of myosin motor inhibition on stress fibers tension