Sub-cellular femtosecond laser ablation

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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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femtosecond lasers for sub-cellular manipulation

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

(confocal, MPM)

outline

- fixed cell experiments

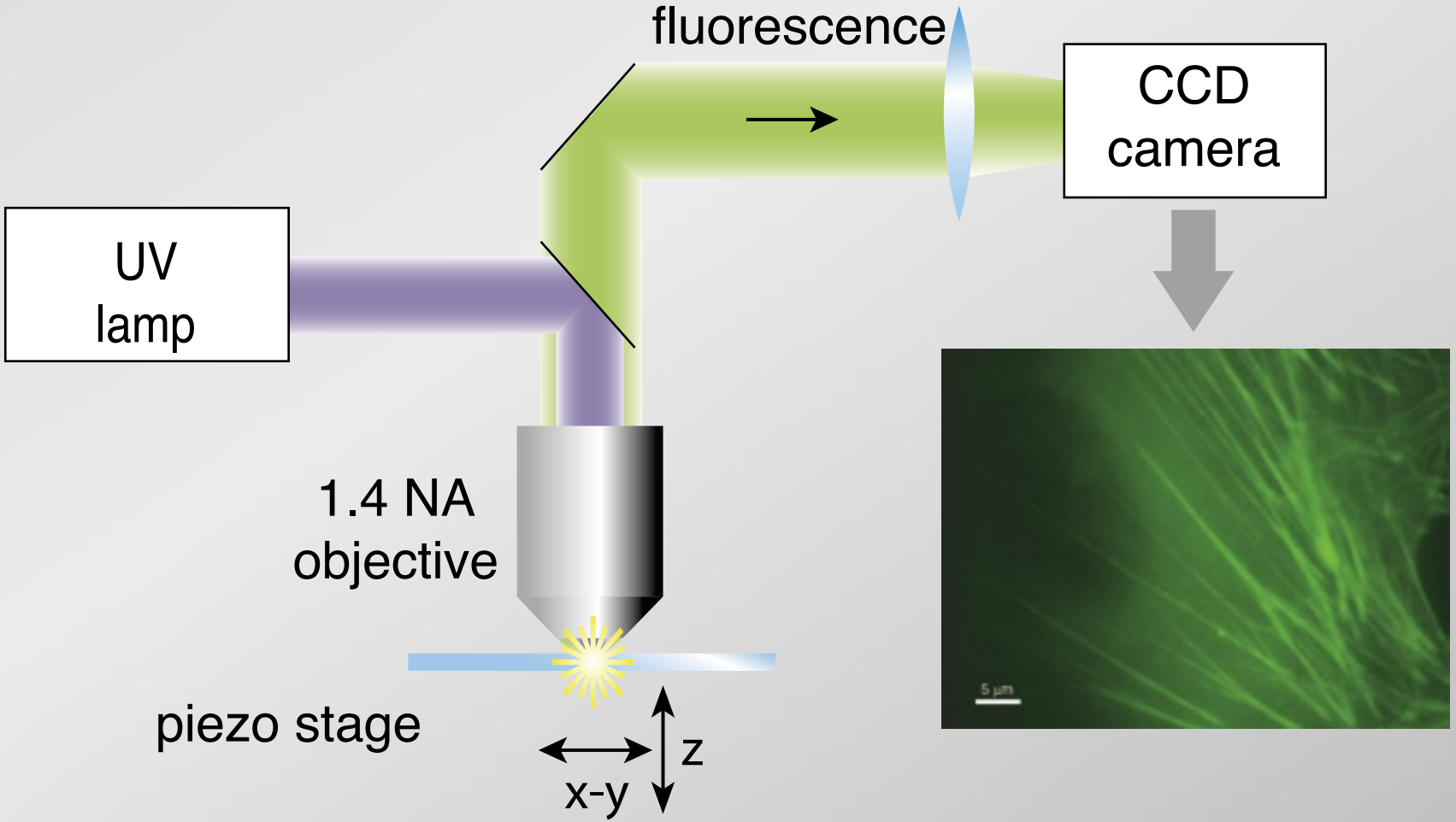
  show material ablation

- live cell experiments

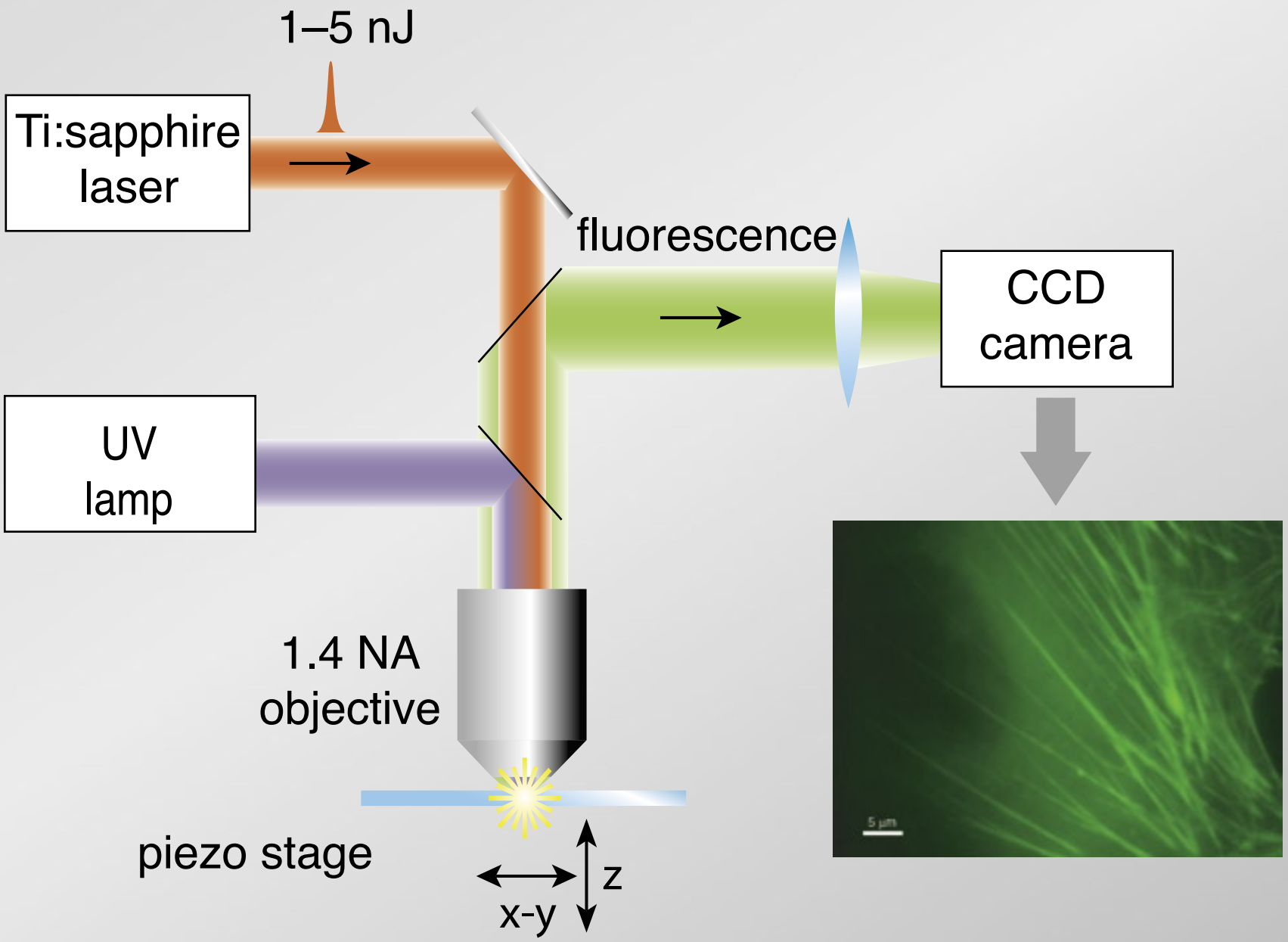
  nanosurgery in live cells

  cytoskeletal dynamics

setup

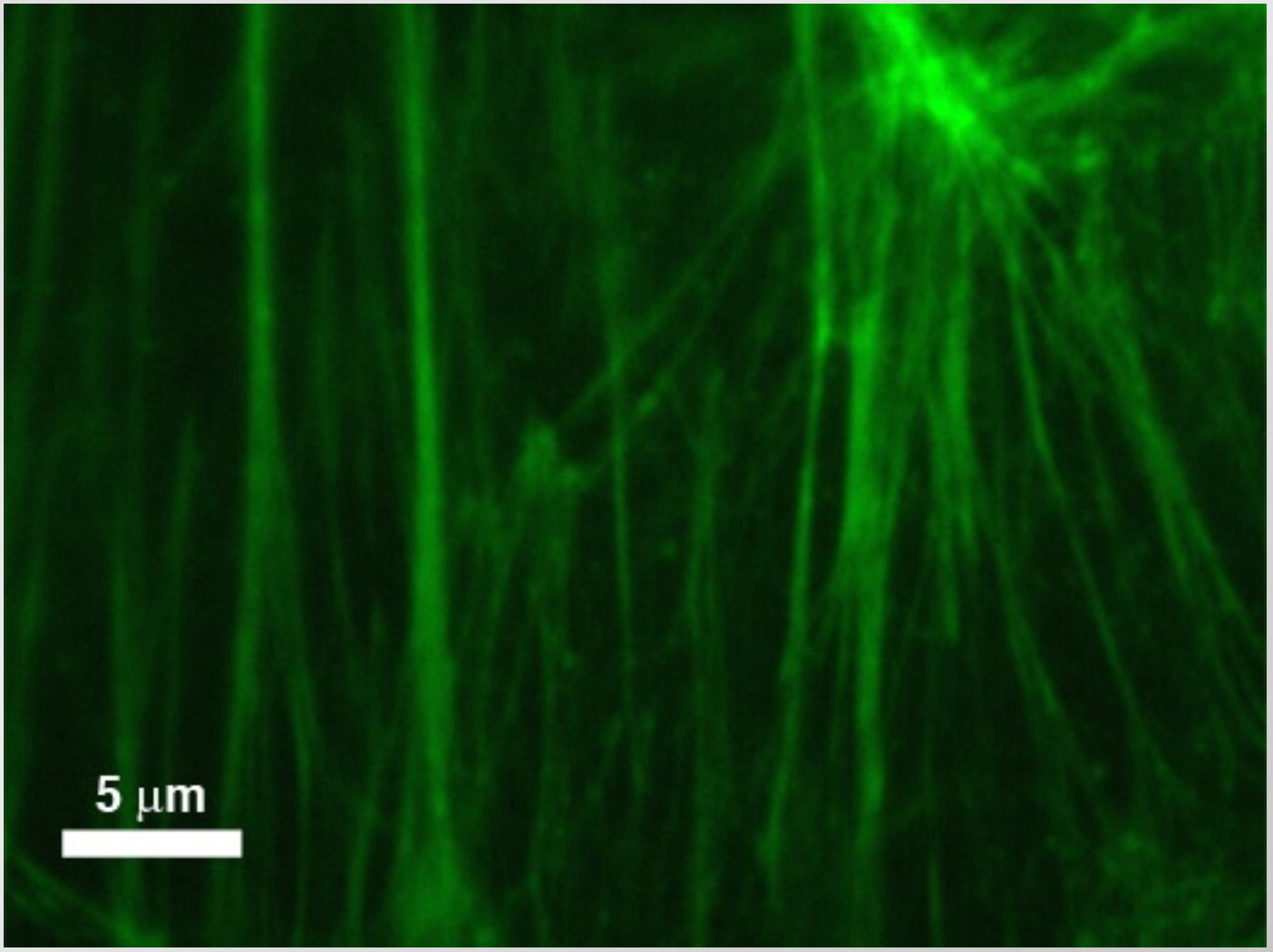

fluorescence
CCD
camera
UV
lamp
1.4 NA
objective
piezo stage
z
x-y
5 µm

setup


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

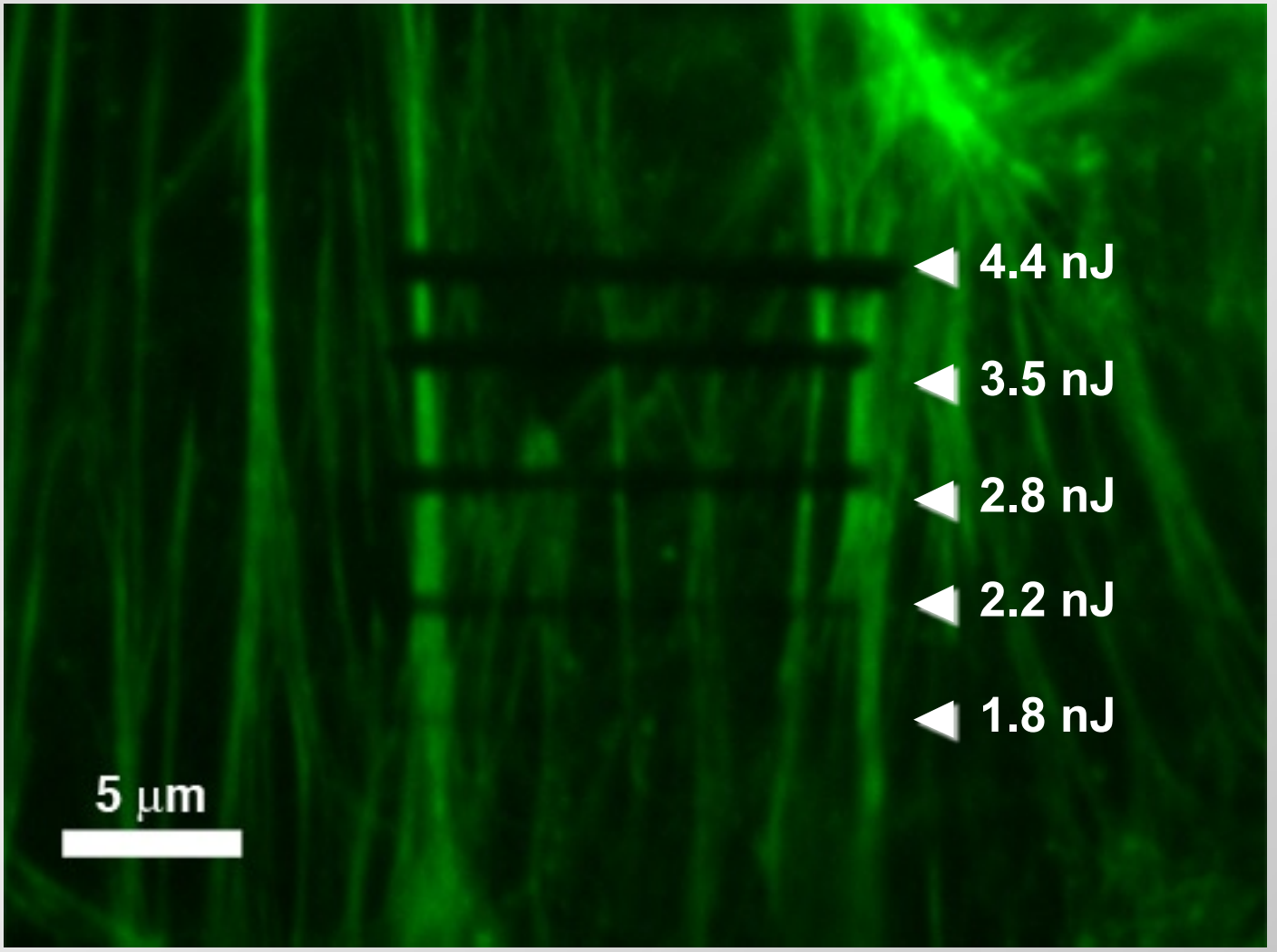
ablation in fixed cells

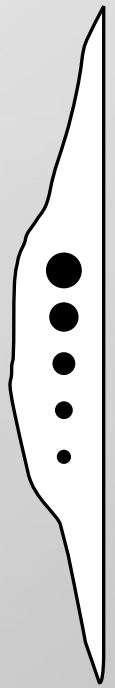
fluorescent actin network in a fixed cell



ablation in fixed cells

actin network after laser irradiation





ablation in fixed cells

q: material ablation or photobleaching?

ablation in fixed cells

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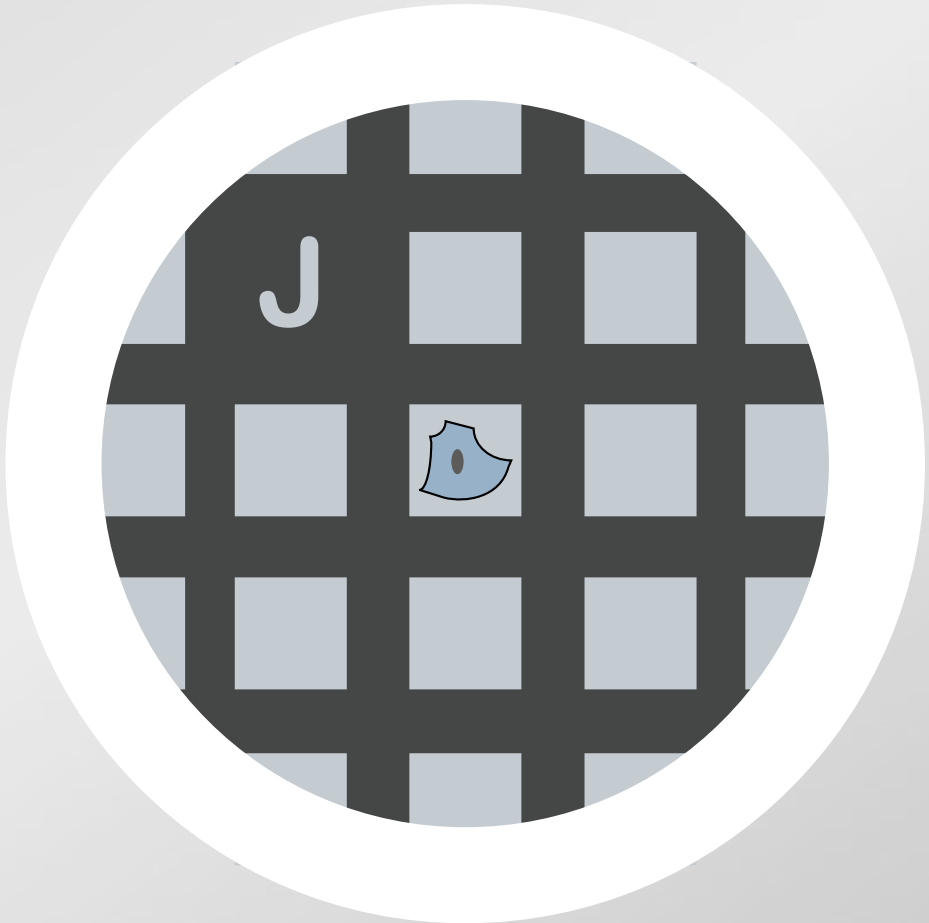
a: use electron microscopy to verify material ablation

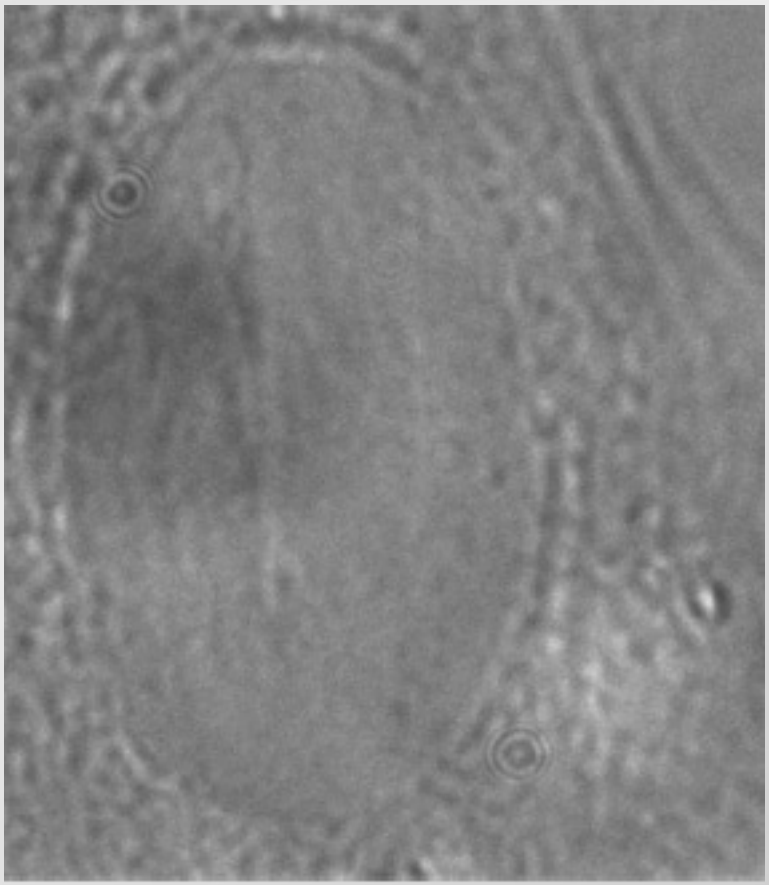
ablation in fixed cells

grow and fix cell onto TEM grids



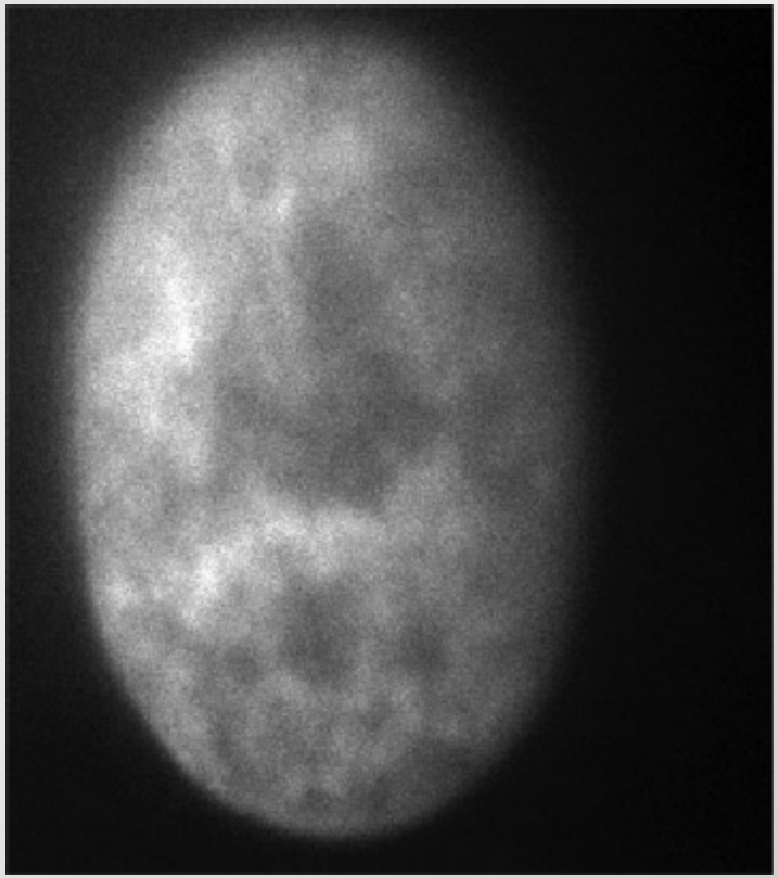
ablation in fixed cells

white light image of a nucleus



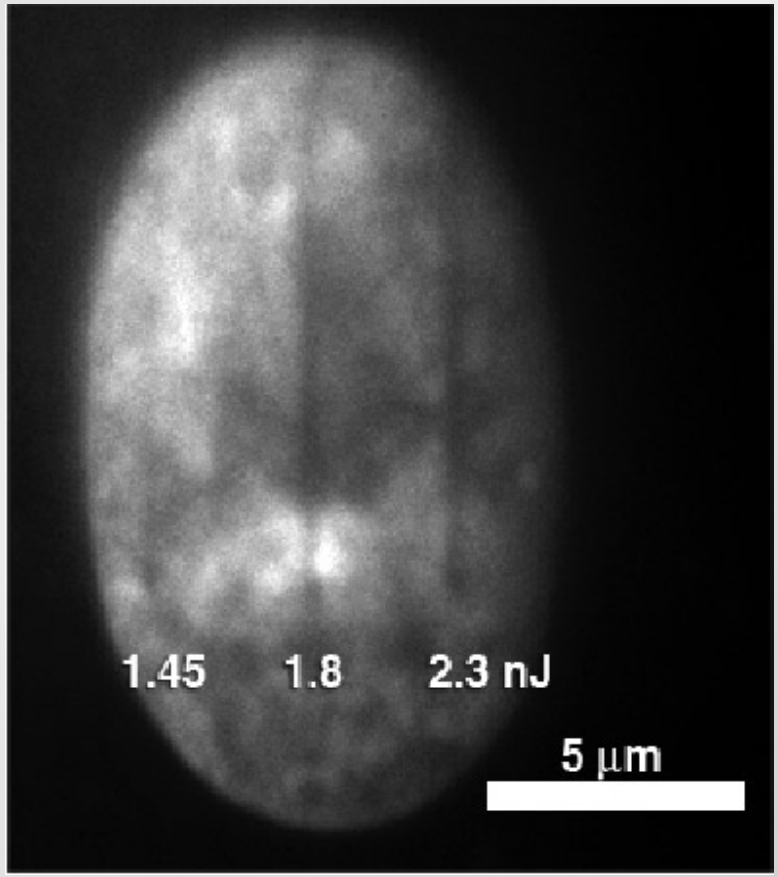
ablation in fixed cells

fluorescence image of a stained nucleus



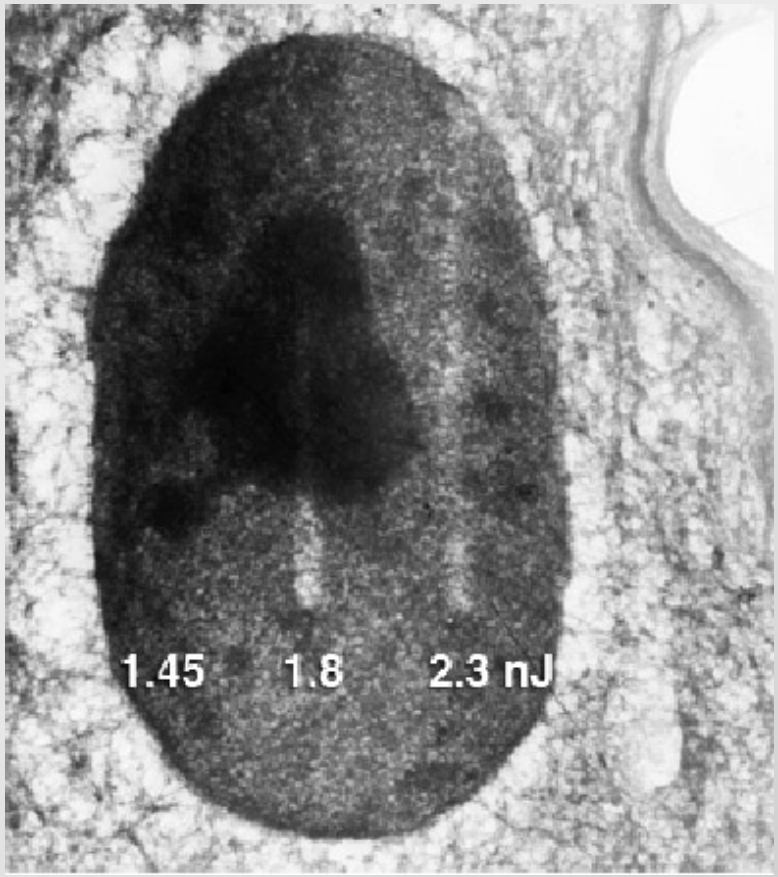
ablation in fixed cells

fluorescence image after laser irradiation



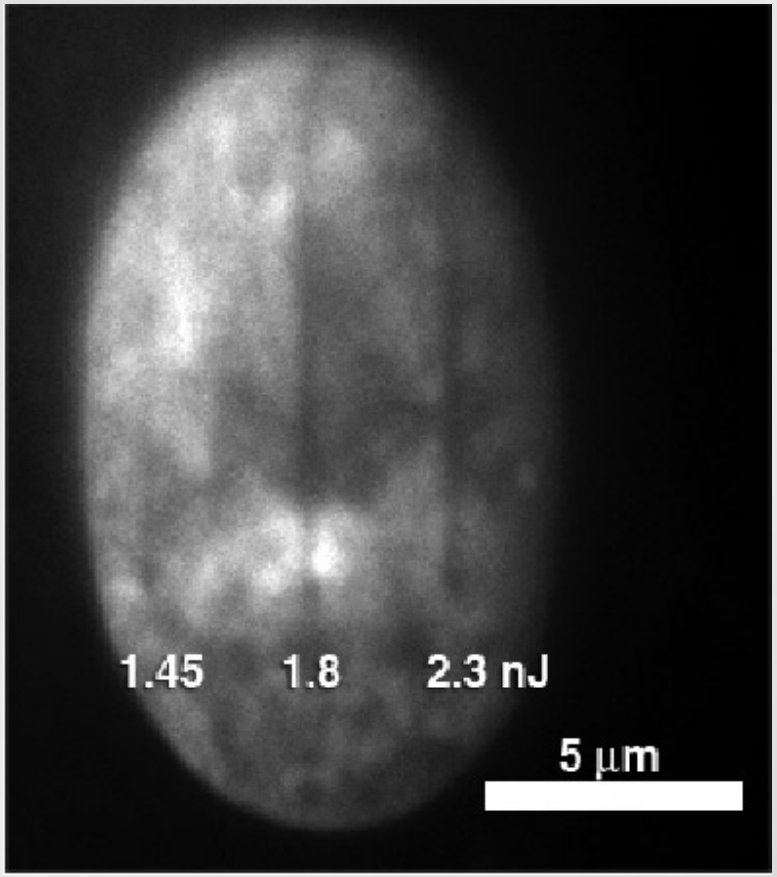
ablation in fixed cells

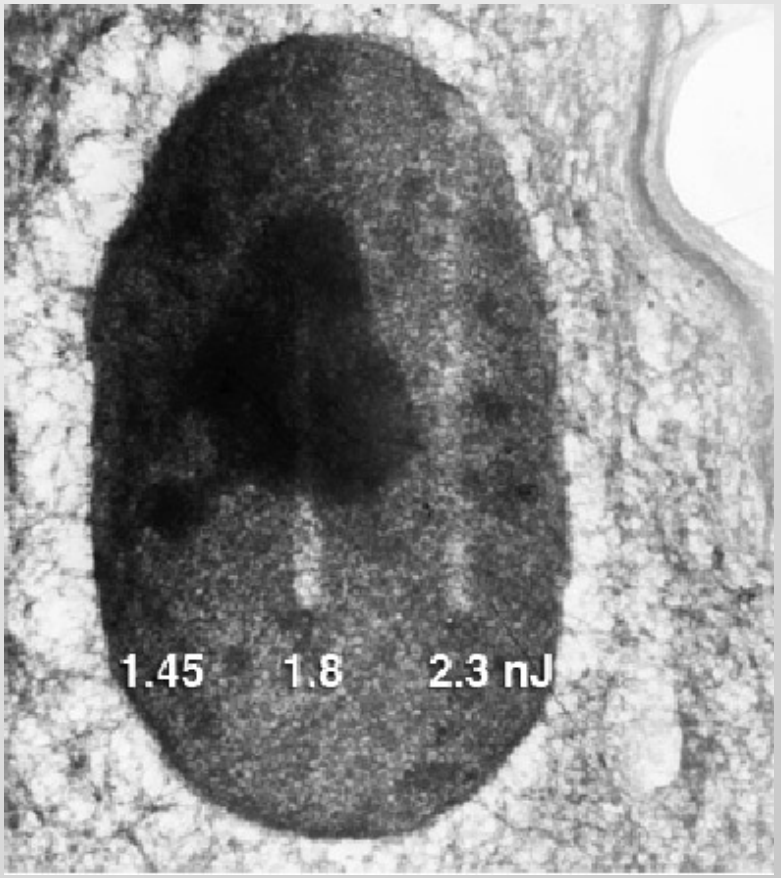
TEM image of the same nucleus



ablation in fixed cells

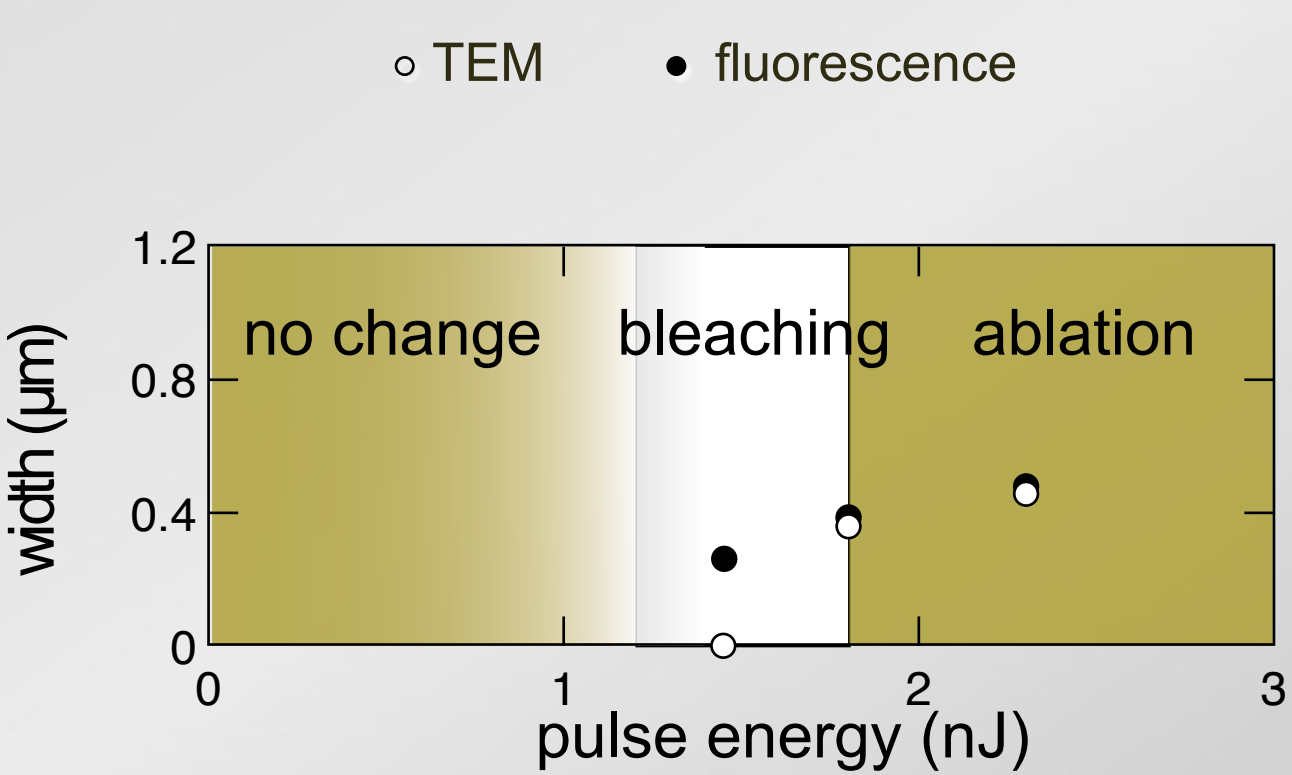
1.45 nJ shows photobleaching no ablation





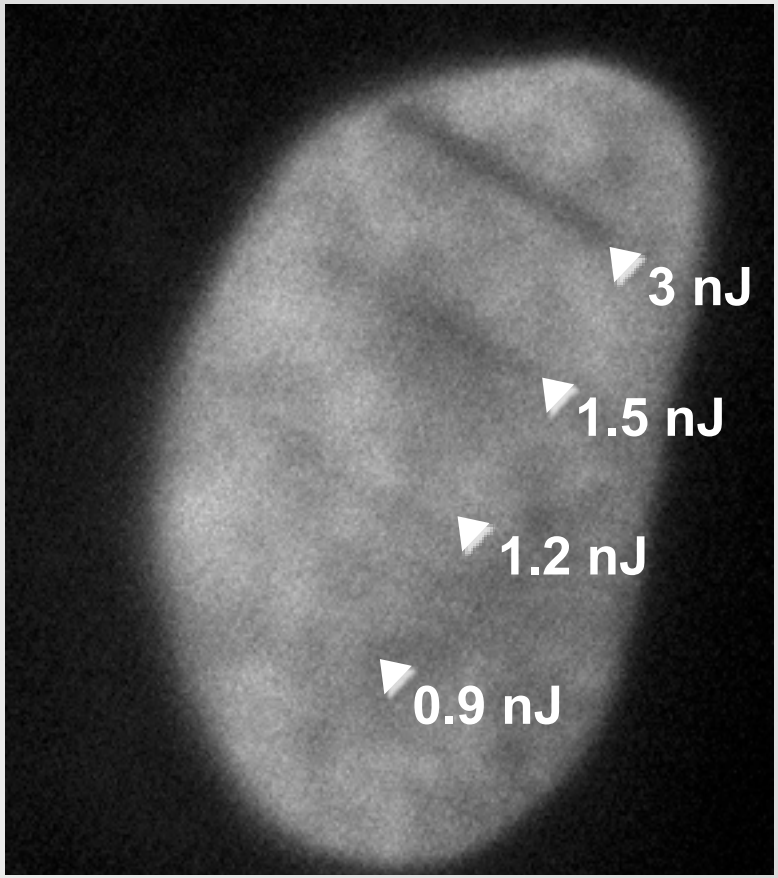
ablation in fixed cells

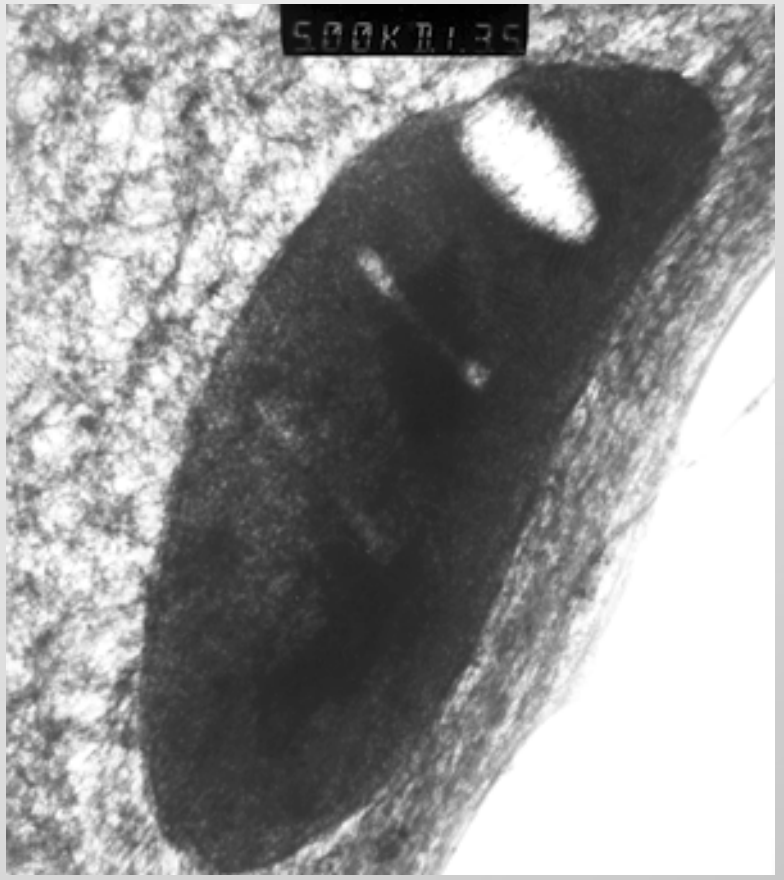
define three regions of interaction



ablation in fixed cells

no interaction below 1 nJ





ablation in fixed cells

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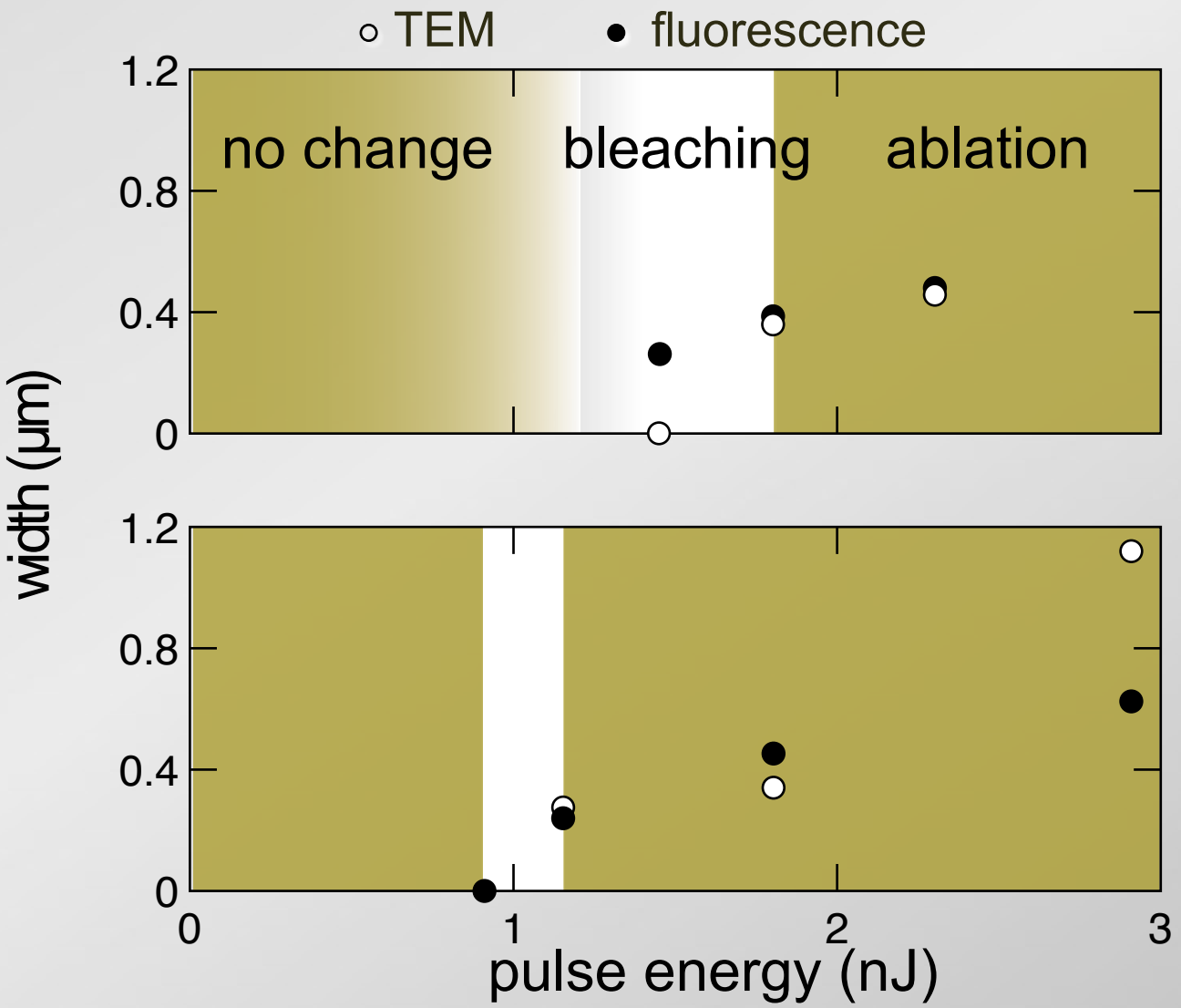

summary

definitive proof of sub-cellular material ablation

ablation widths of 250 nm at 2 nJ

ablation threshold varies slightly

ablation threshold is 1.2 times that of photobleaching

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  show material ablation

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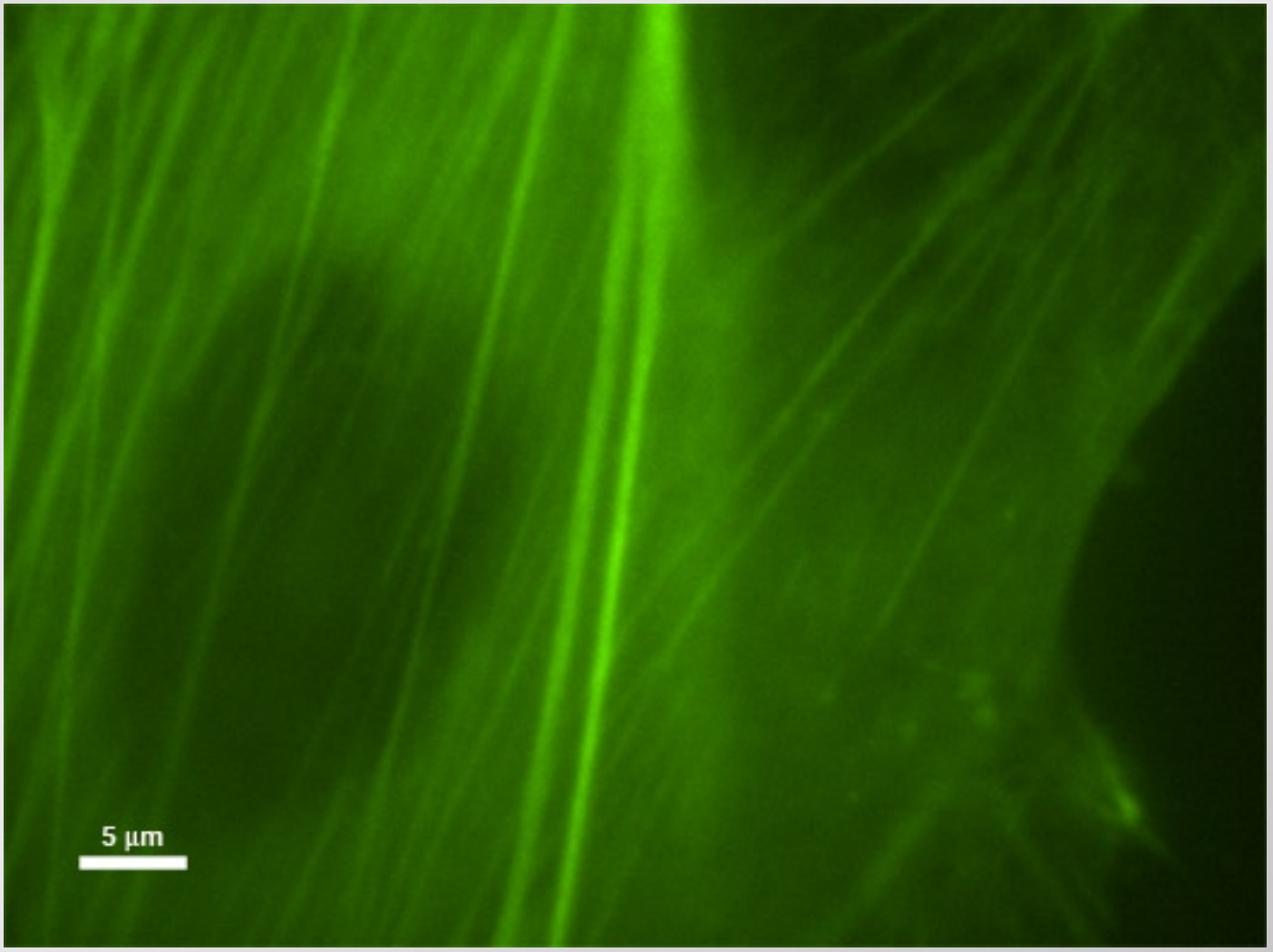
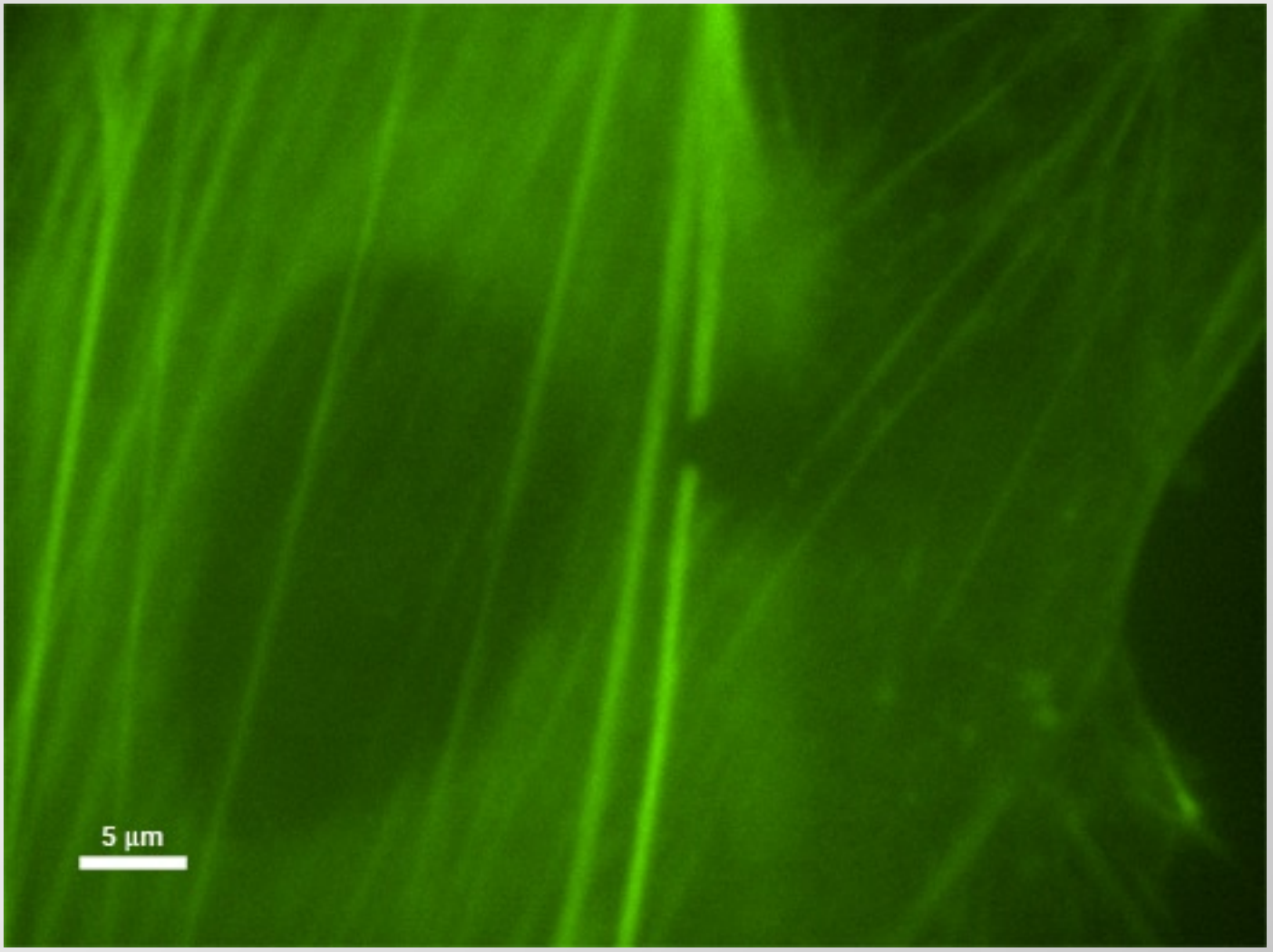
ablation in live cells

cutting YFP actin in live endothelial cells



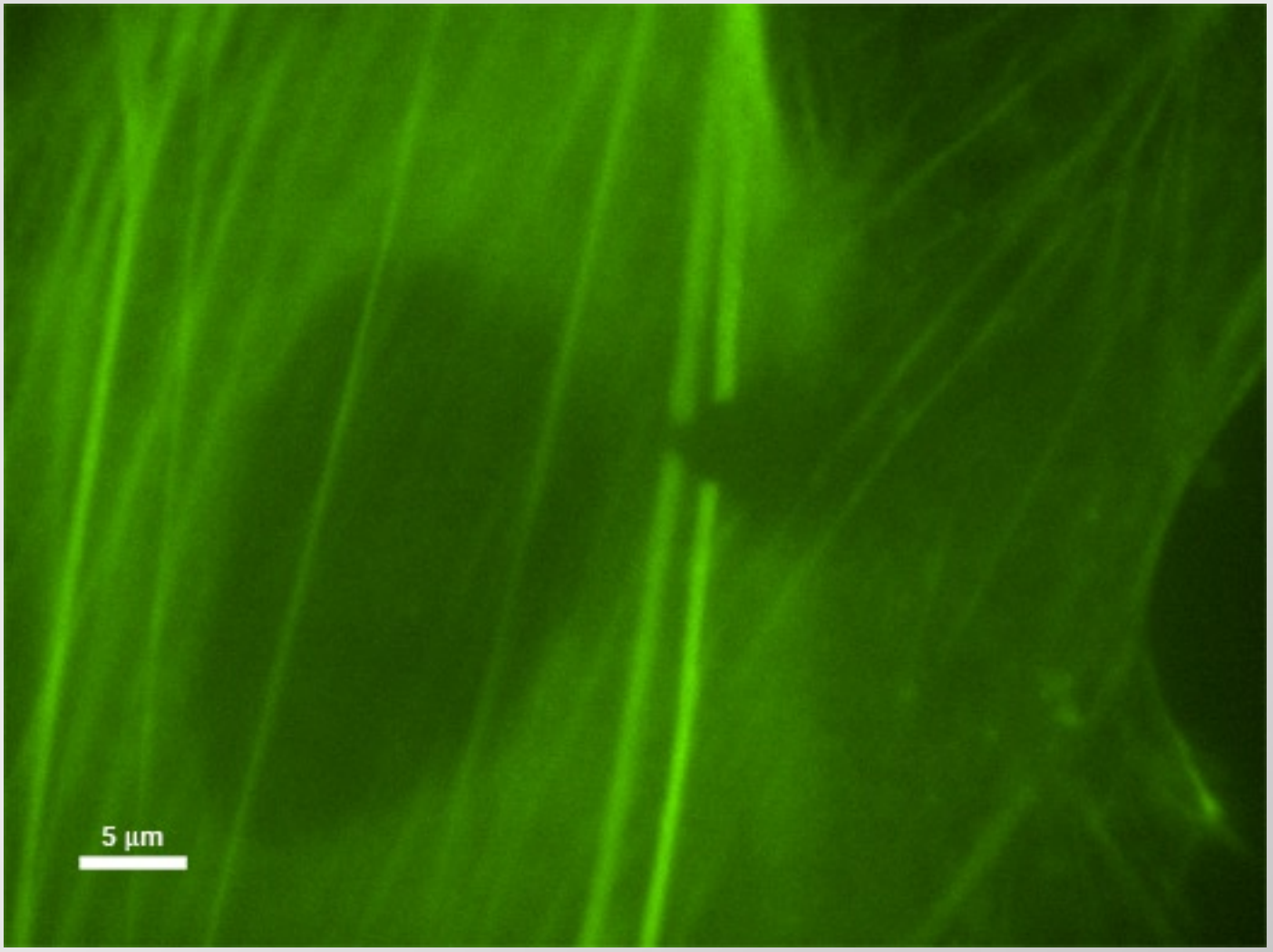
ablation in live cells

severing a single actin bundle with 4.3 nJ



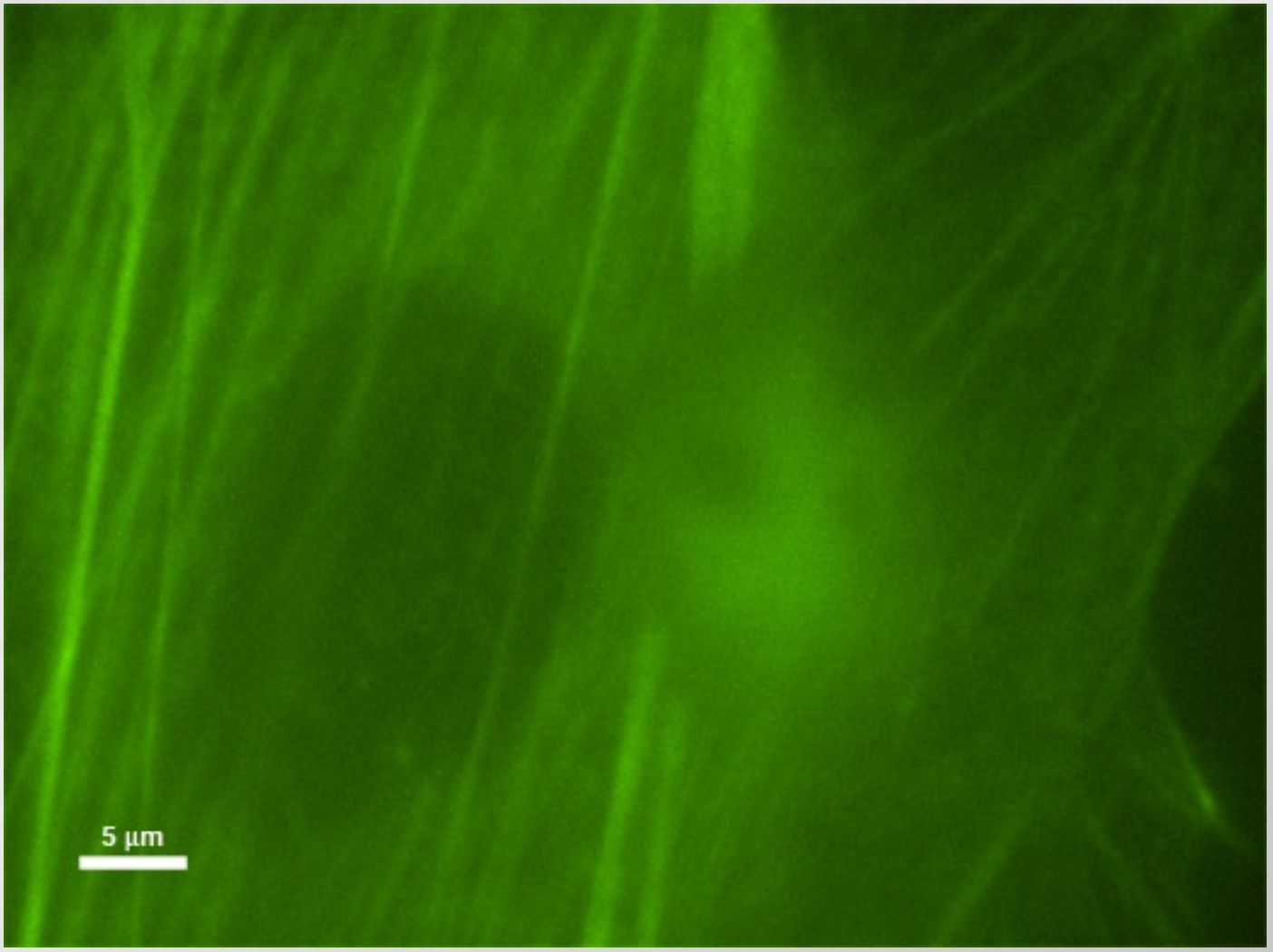
ablation in live cells

moving cell to sever parallel bundle



ablation in live cells

10 mins later cell is alive



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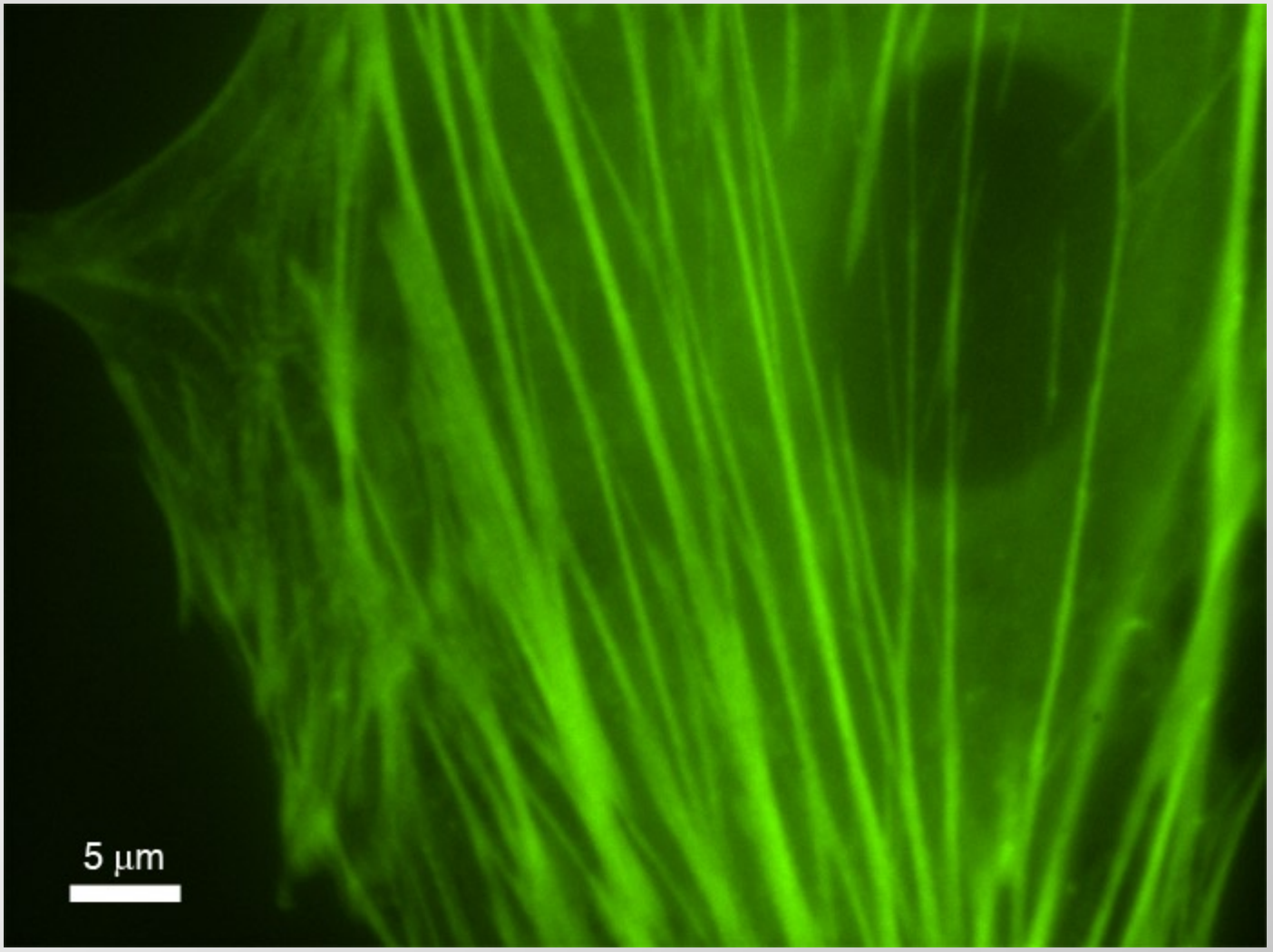
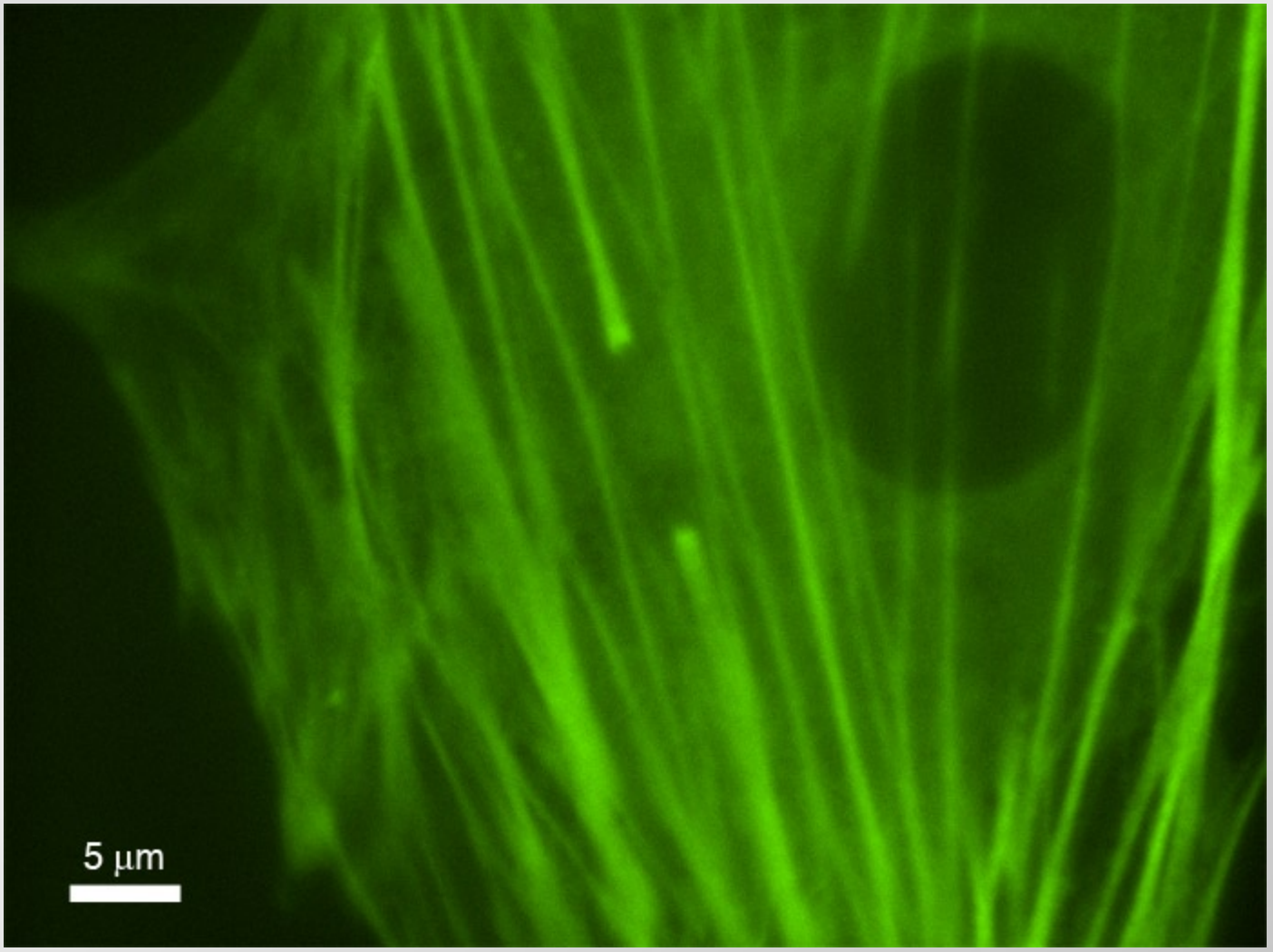
ablation in live cells

YFP fluorescent actin filaments in a live cell



ablation in live cells

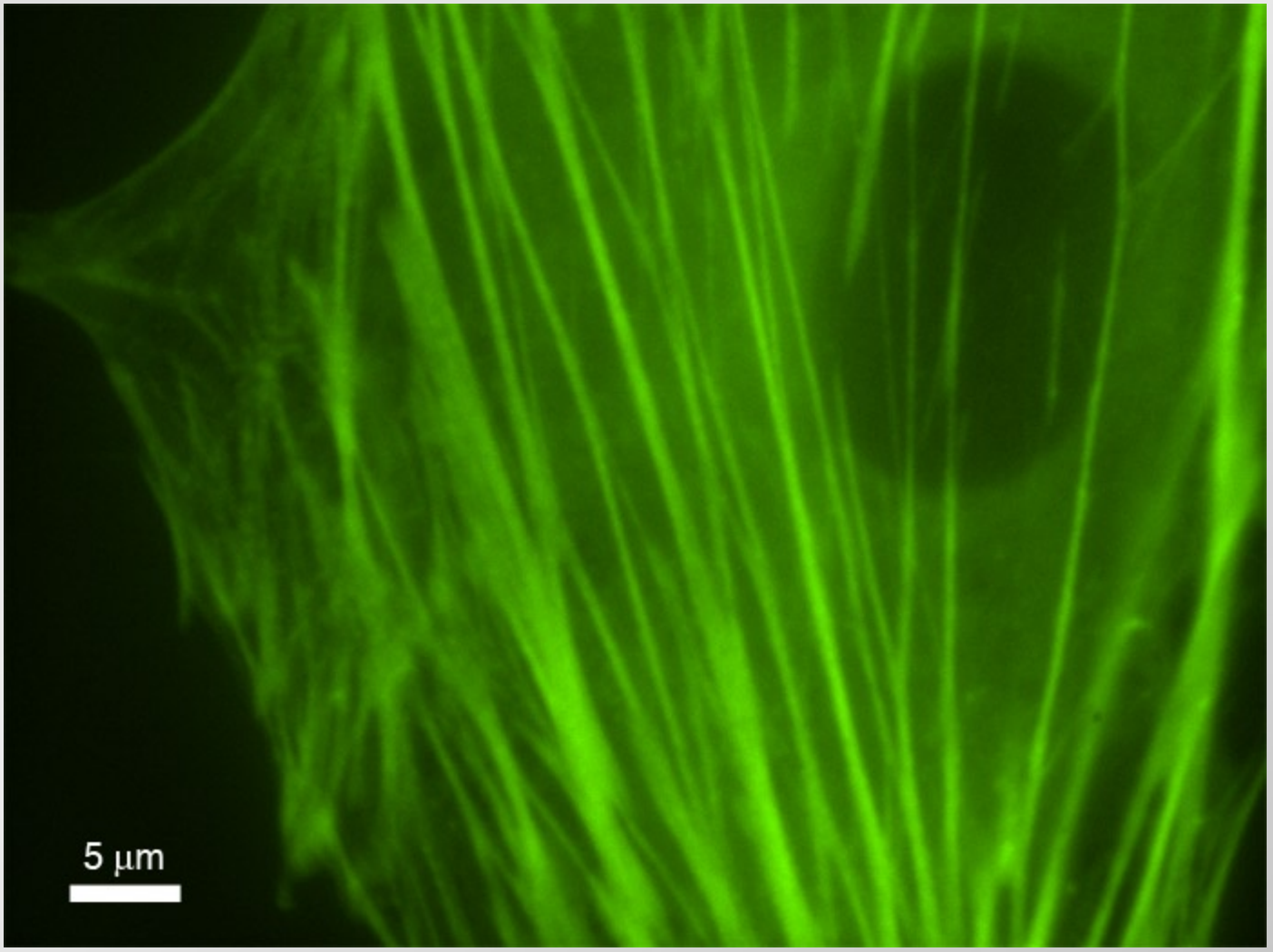
10 seconds later



cellular tensegrity

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

live cell nanosurgery



summary

fs laser sub-cellular ablation

is verified by TEM

has 1.2 times the photobleaching threshold

probes real time cell dynamics

elucidates viscoelastic properties of stress fibers

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Umass Medical School

Harvard Materials Research Science and Engineering Center
National Science Foundation
National Institutes of Health

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