Sub-cellular nanosurgery in live cells using ultrashort laser pulses

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motivation

femtosecond lasers for subcellular manipulation

- high penetration depth in tissue

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- nonlinear interaction

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- no damage outside focal region

motivation

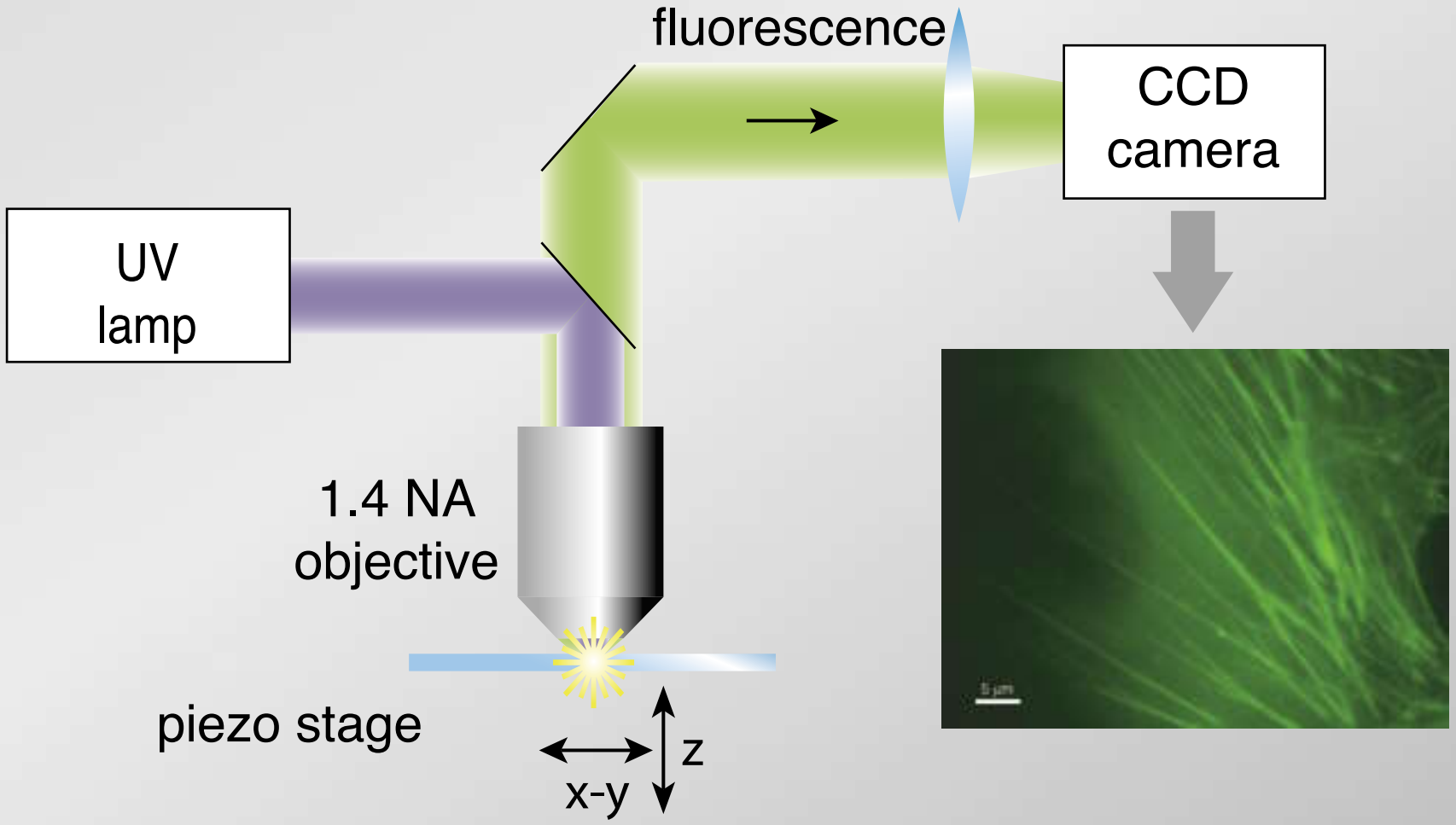
femtosecond lasers for subcellular manipulation

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

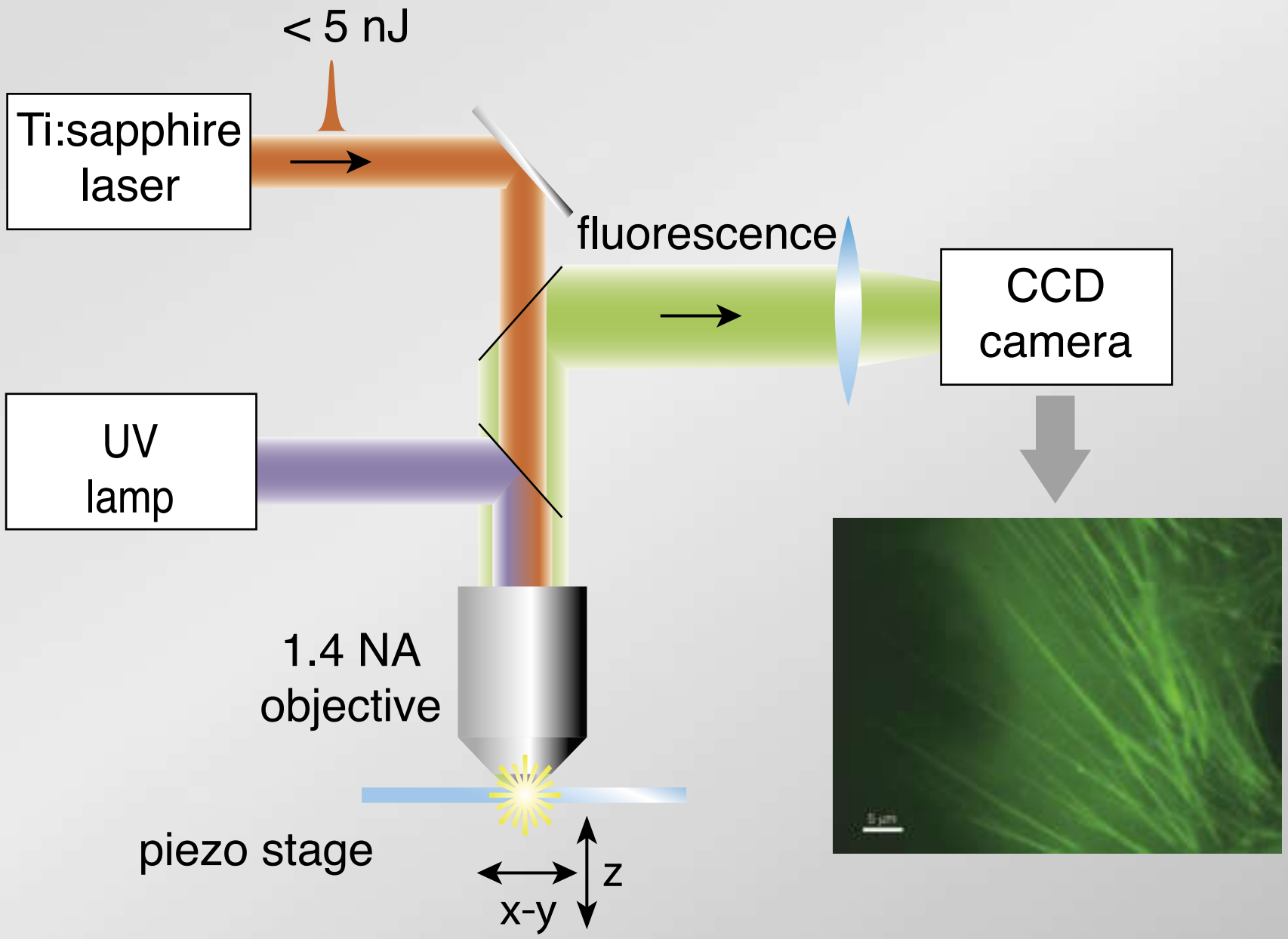
outline

- cellular nanosurgery
- kHz and MHz cutting in cells
- elucidating stress fiber dynamics

cellular nanosurgery

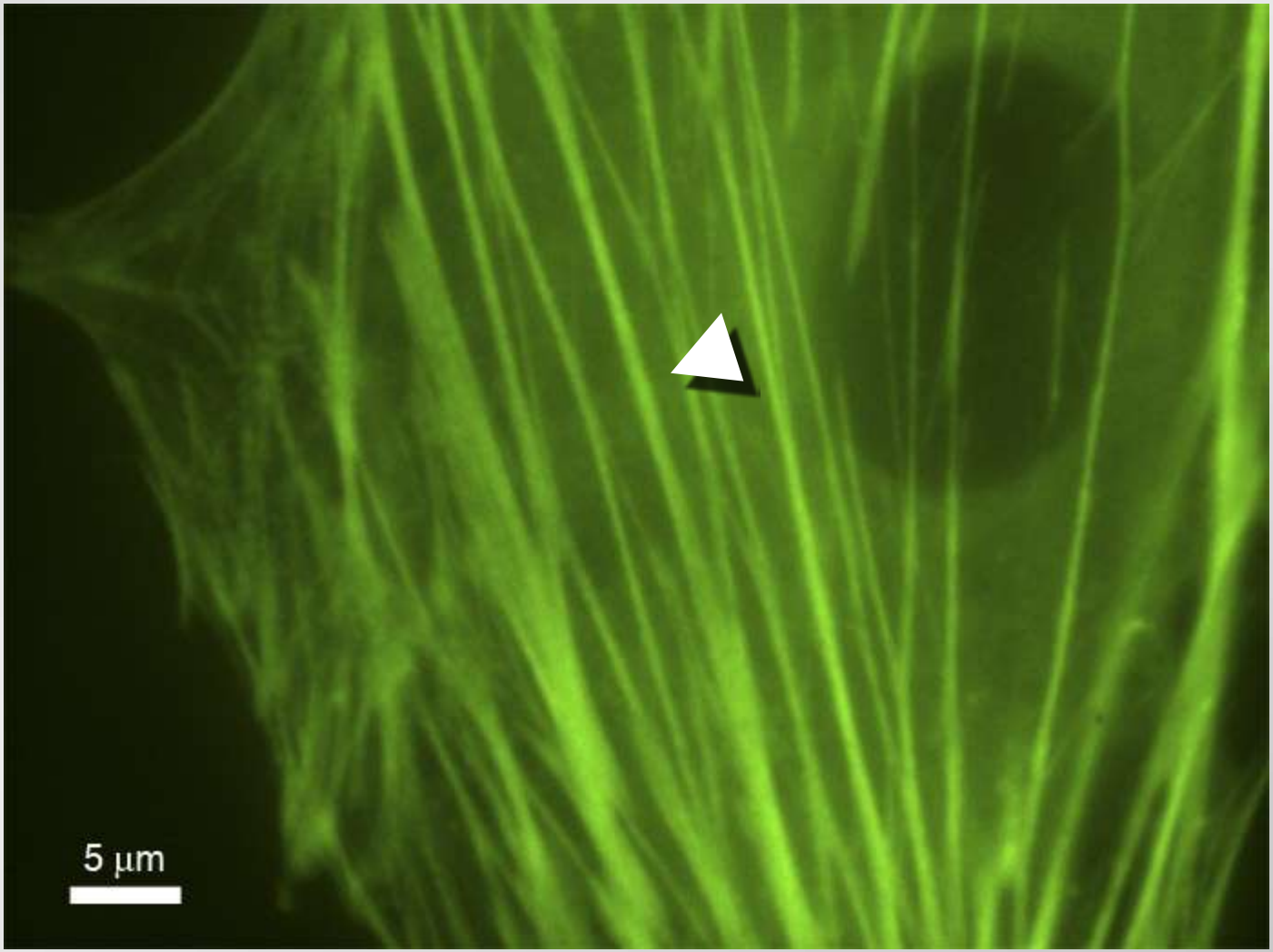


cellular nanosurgery



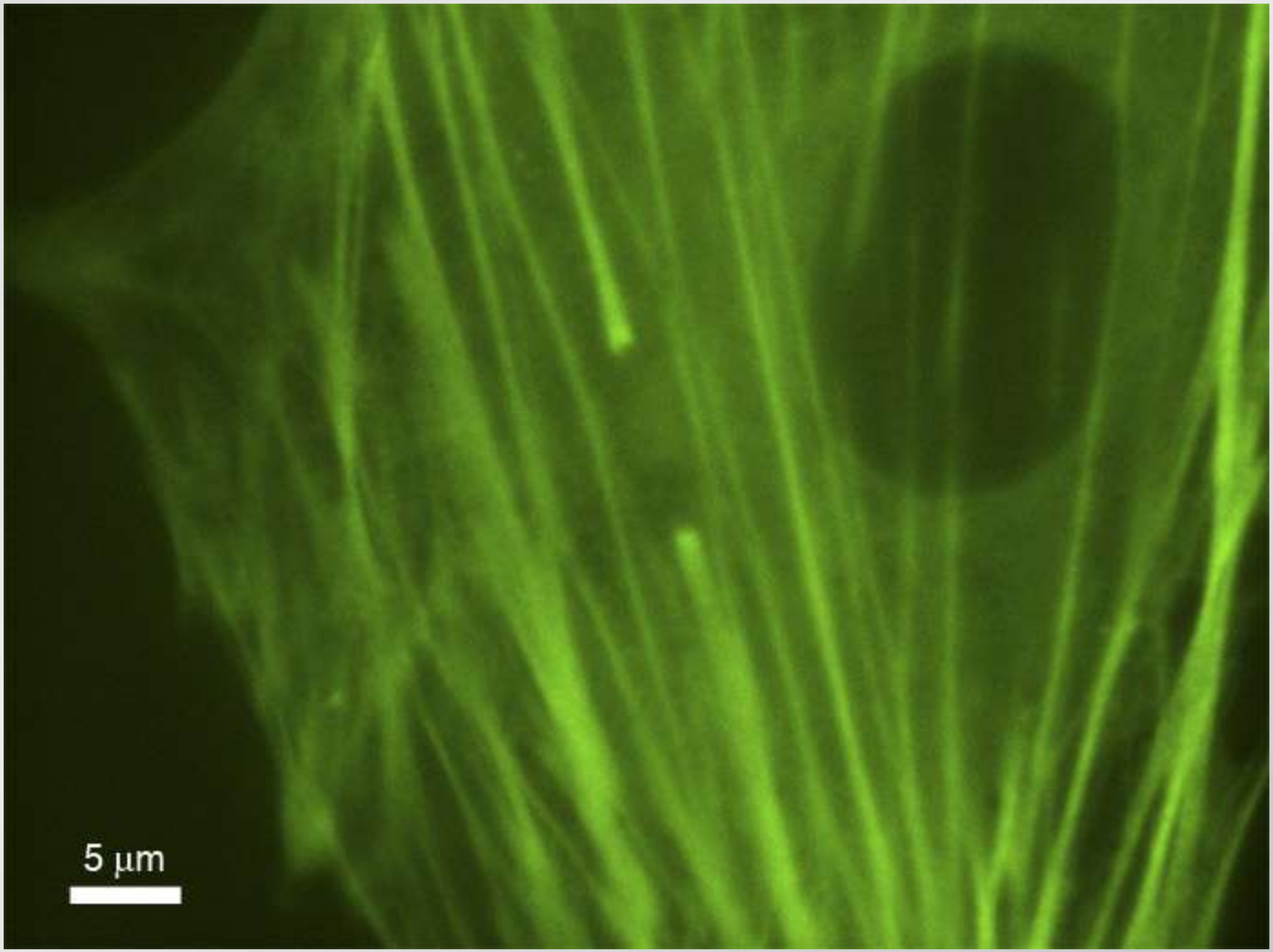
cellular nanosurgery

YFP fluorescent actin filaments in a live cell



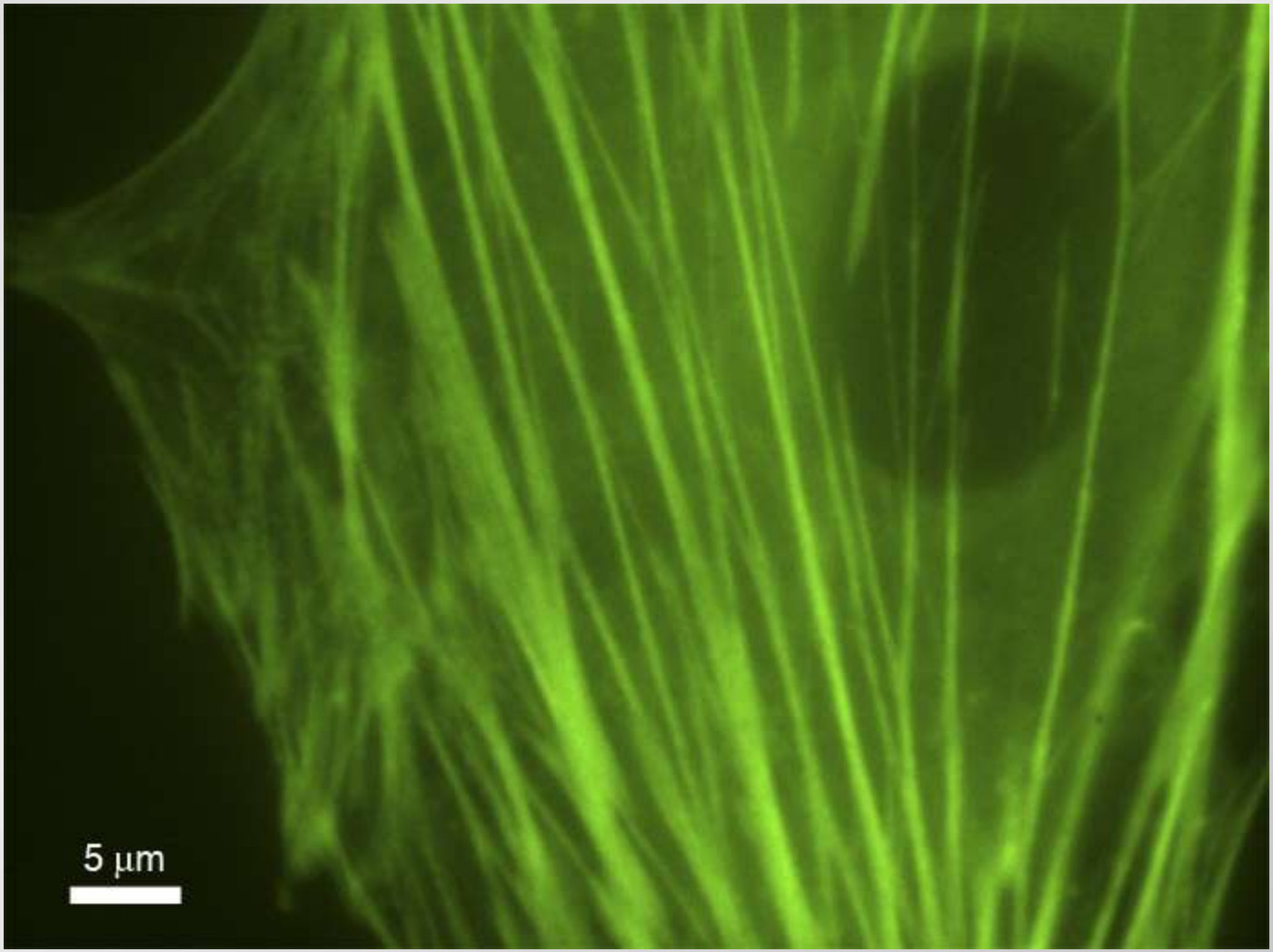
cellular nanosurgery

10 seconds later



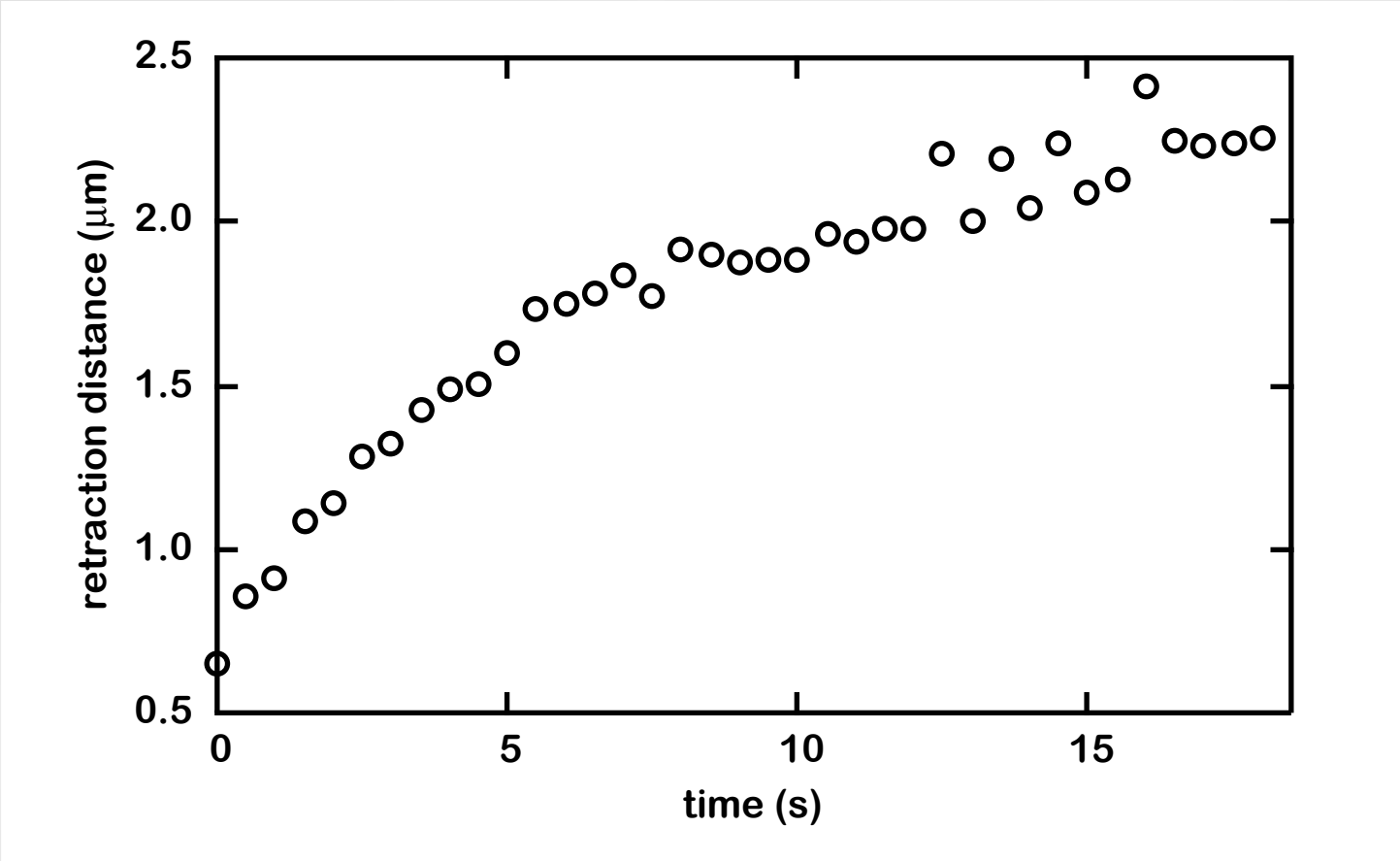
cellular nanosurgery

1 kHz, 3 nJ



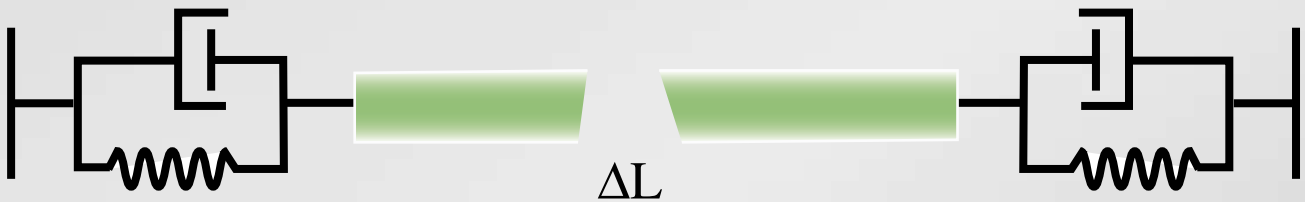
cellular nanosurgery

fiber retraction vs. time after laser ablation



cellular nanosurgery

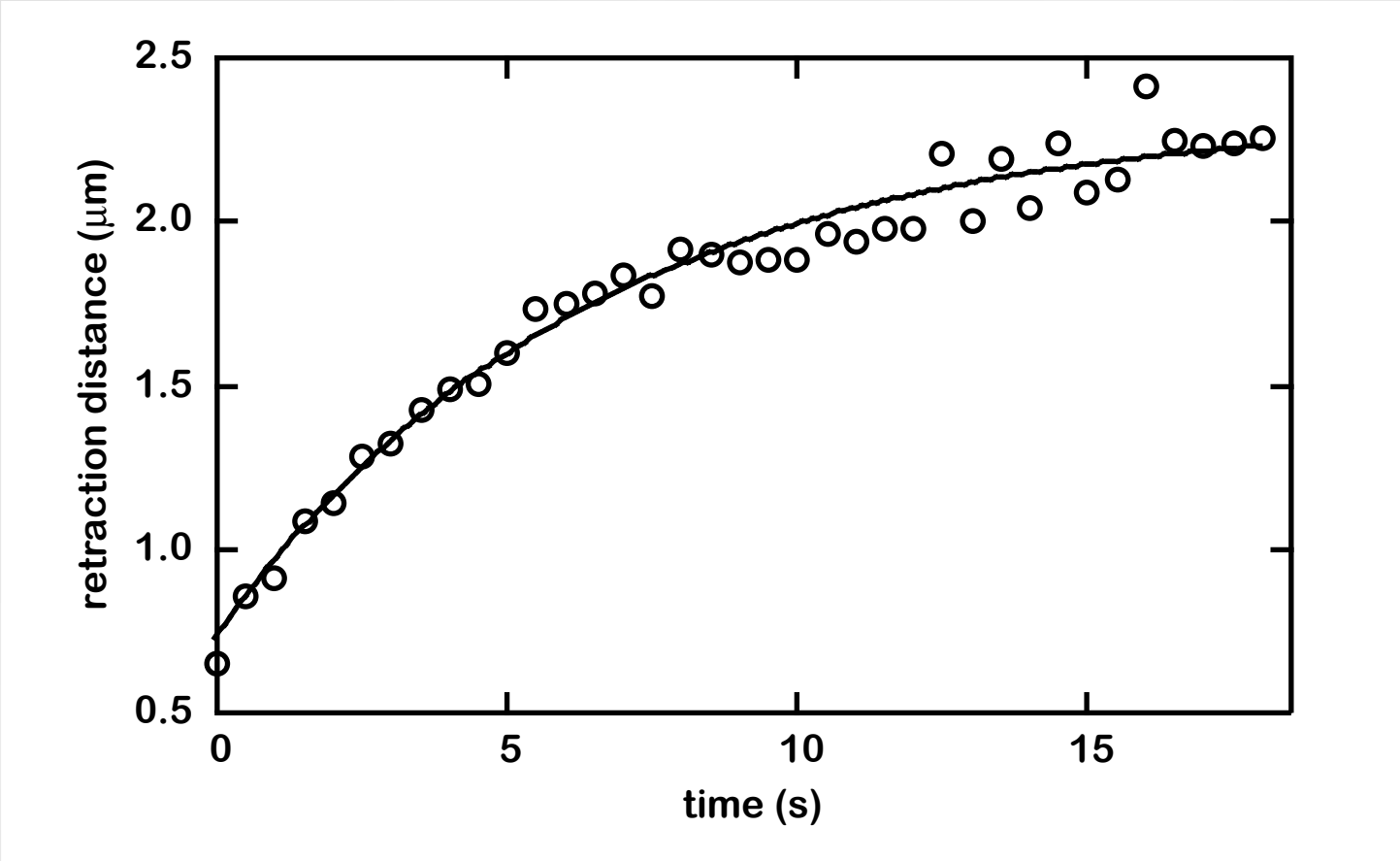
viscoelastic model of an actin stress fiber



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

cellular nanosurgery

modeling of tension release



cellular nanosurgery

femtosecond nanosurgery gives:

access to subcellular structures

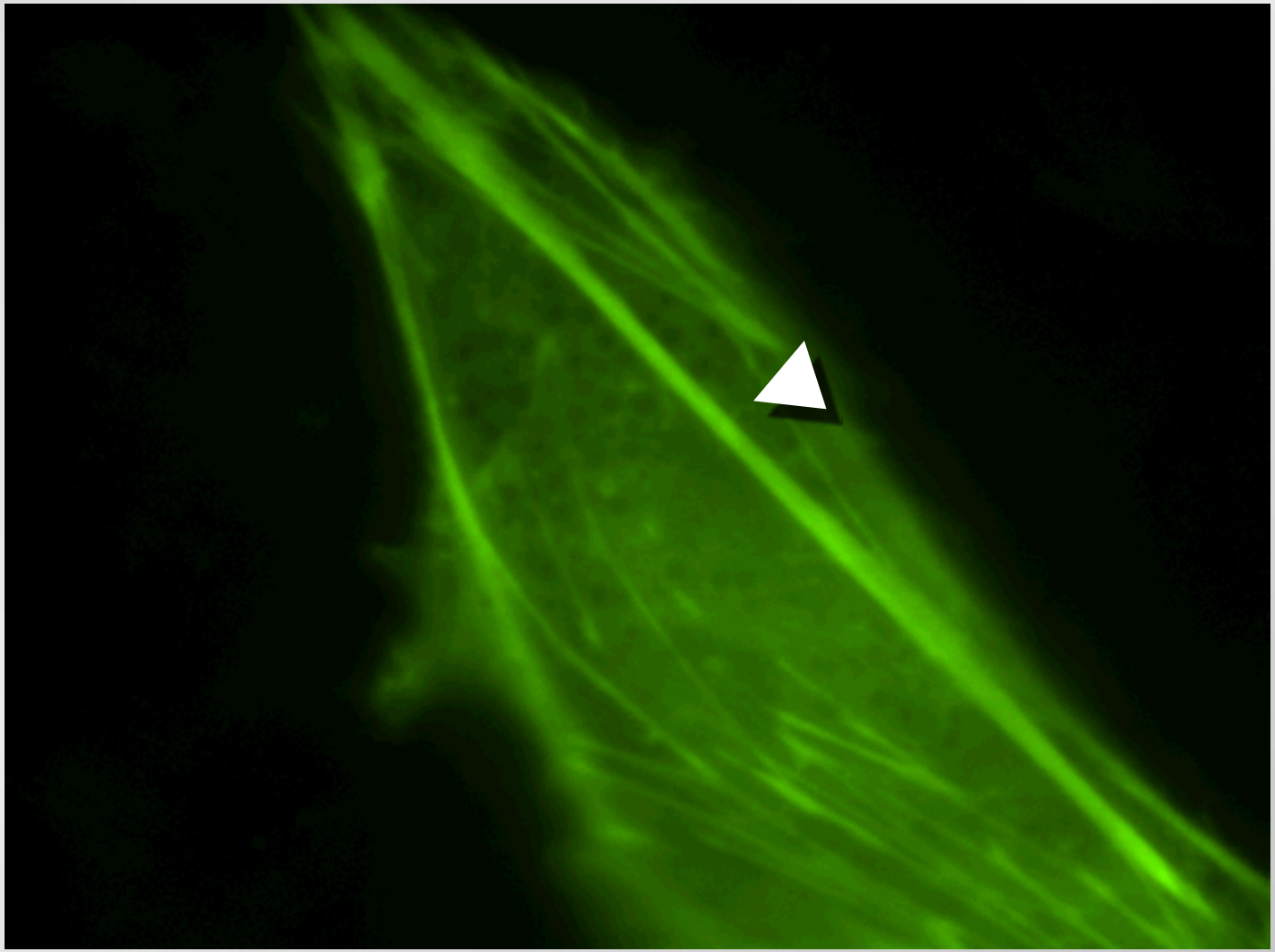
a measure of tension in actin stress fibers

outline

- cellular nanosurgery
- kHz and MHz cutting in cells
- elucidating stress fiber dynamics

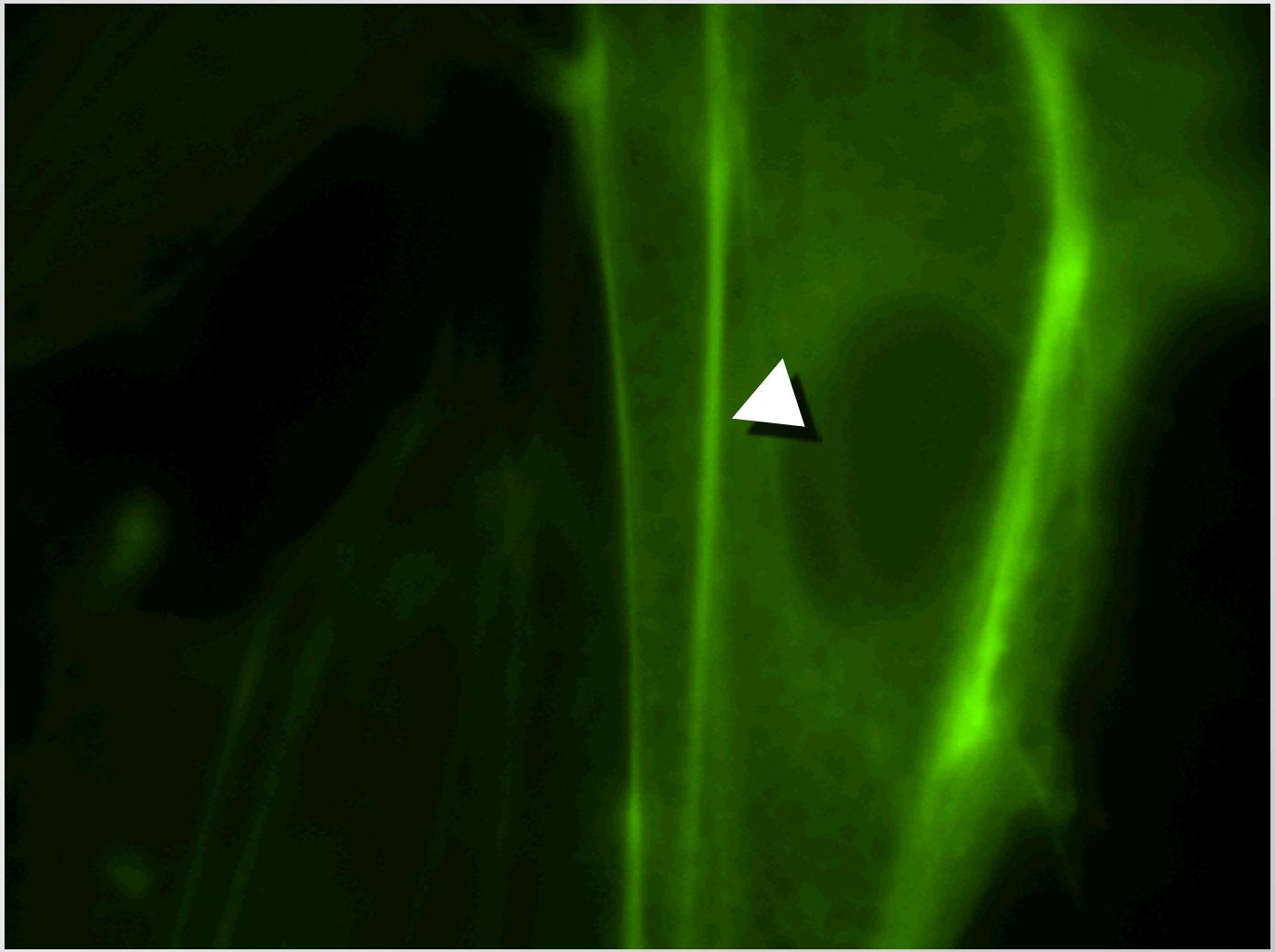
kHz and MHz cutting

14 kHz, 3 s , 1 nJ

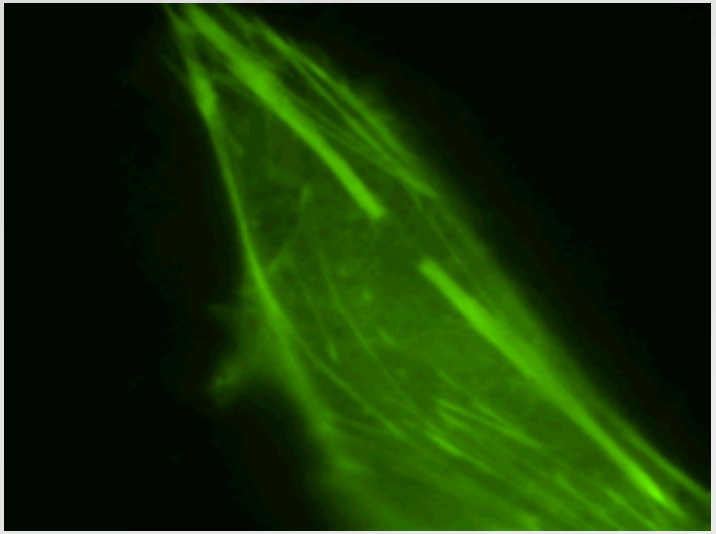


kHz and MHz cutting

76 MHz, 3 s, 1 nJ

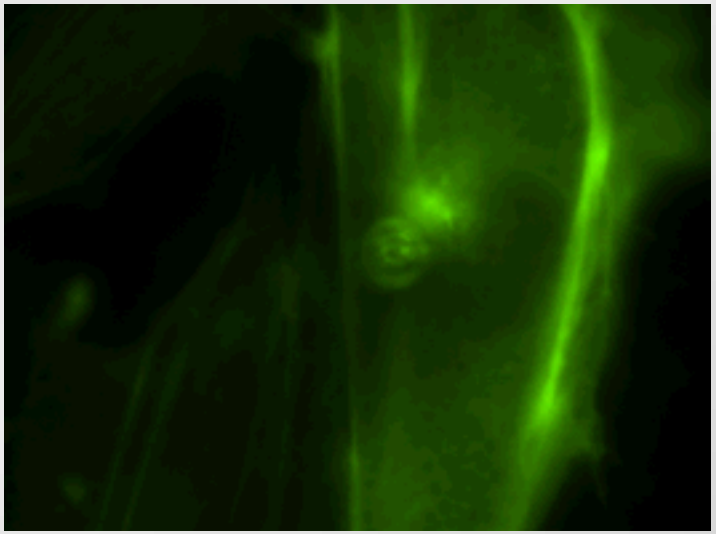


kHz and MHz cutting



14 kHz
3 s
1 nJ at focus

Cutting

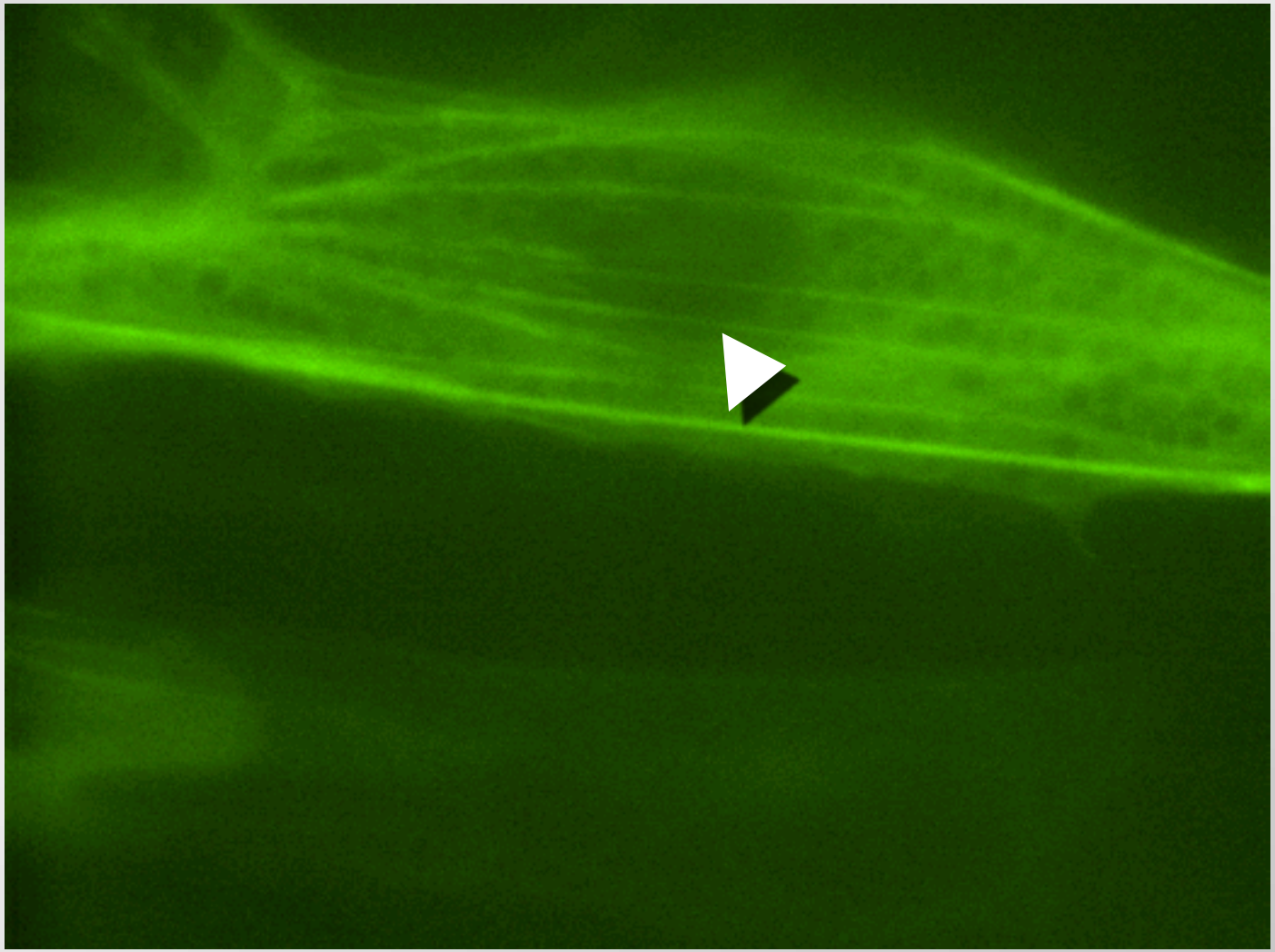


76 MHz
3 s
1 nJ at focus

Damage

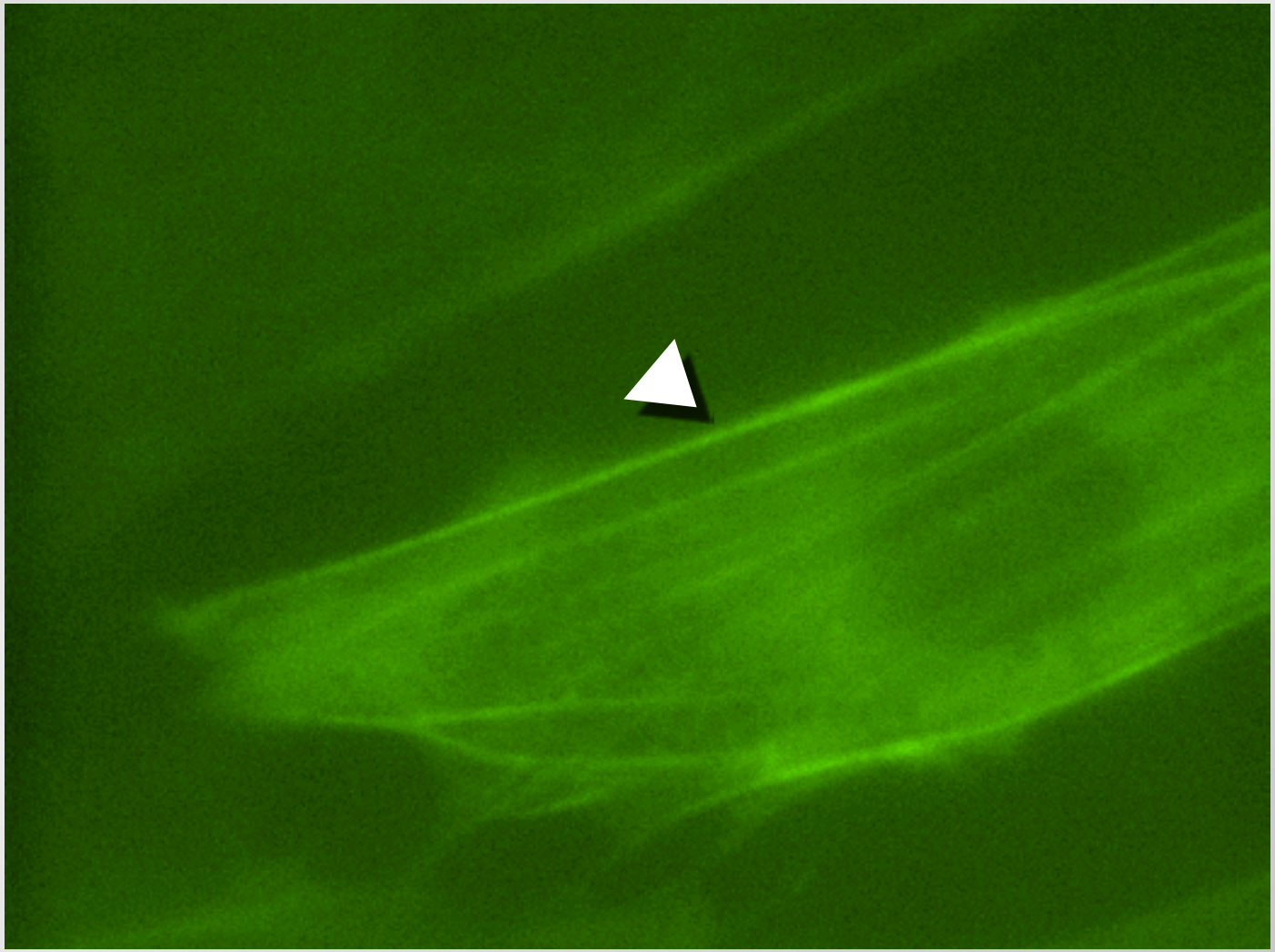
kHz and MHz cutting

14 kHz, 3 s , 0.5 nJ

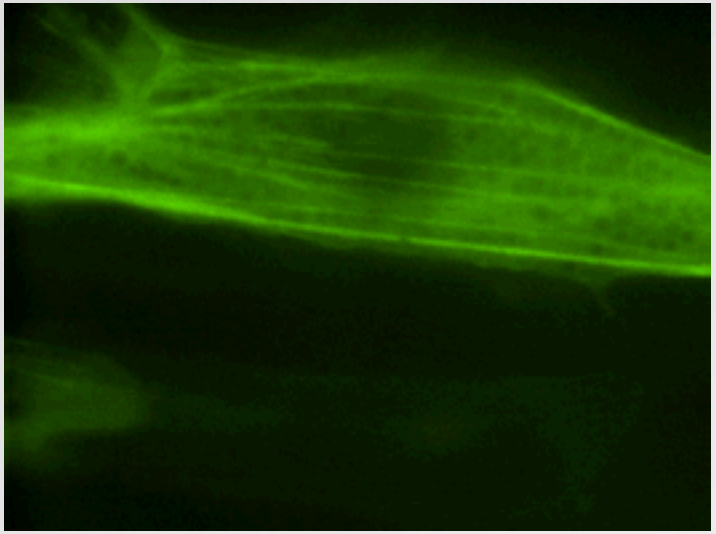


MHz nanosurgery

76 MHz, 500 ms, 0.5 nJ

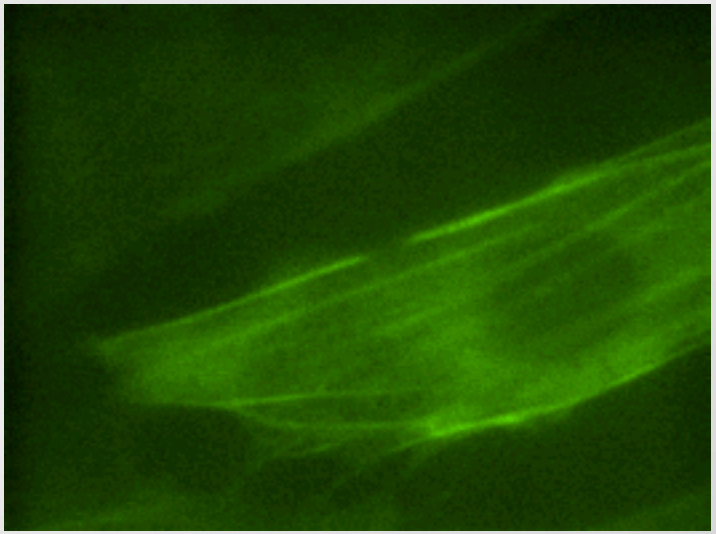


kHz and MHz cutting



14 kHz
3 s
0.5 nJ at focus

Loss of fluorescence

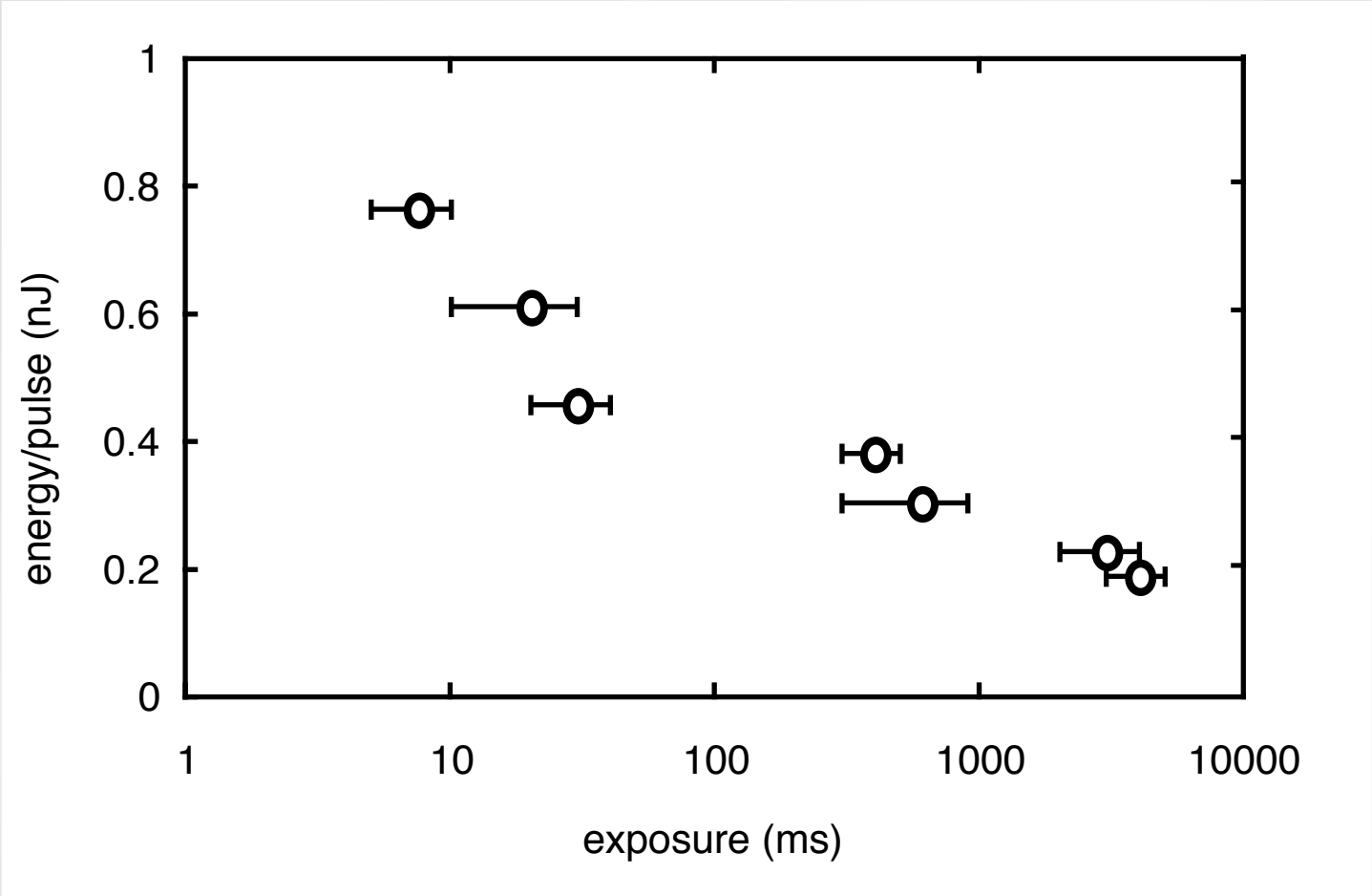


76 MHz
500 ms
0.5 nJ at focus

Cutting

kHz and MHz cutting

MHz threshold irradiation for actin cutting



kHz and MHz cutting

both kHz and MHz pulses suitable for nanosurgery

- kHz: pulse energy determines fiber cutting threshold

  no additional damage due to prolonged exposure

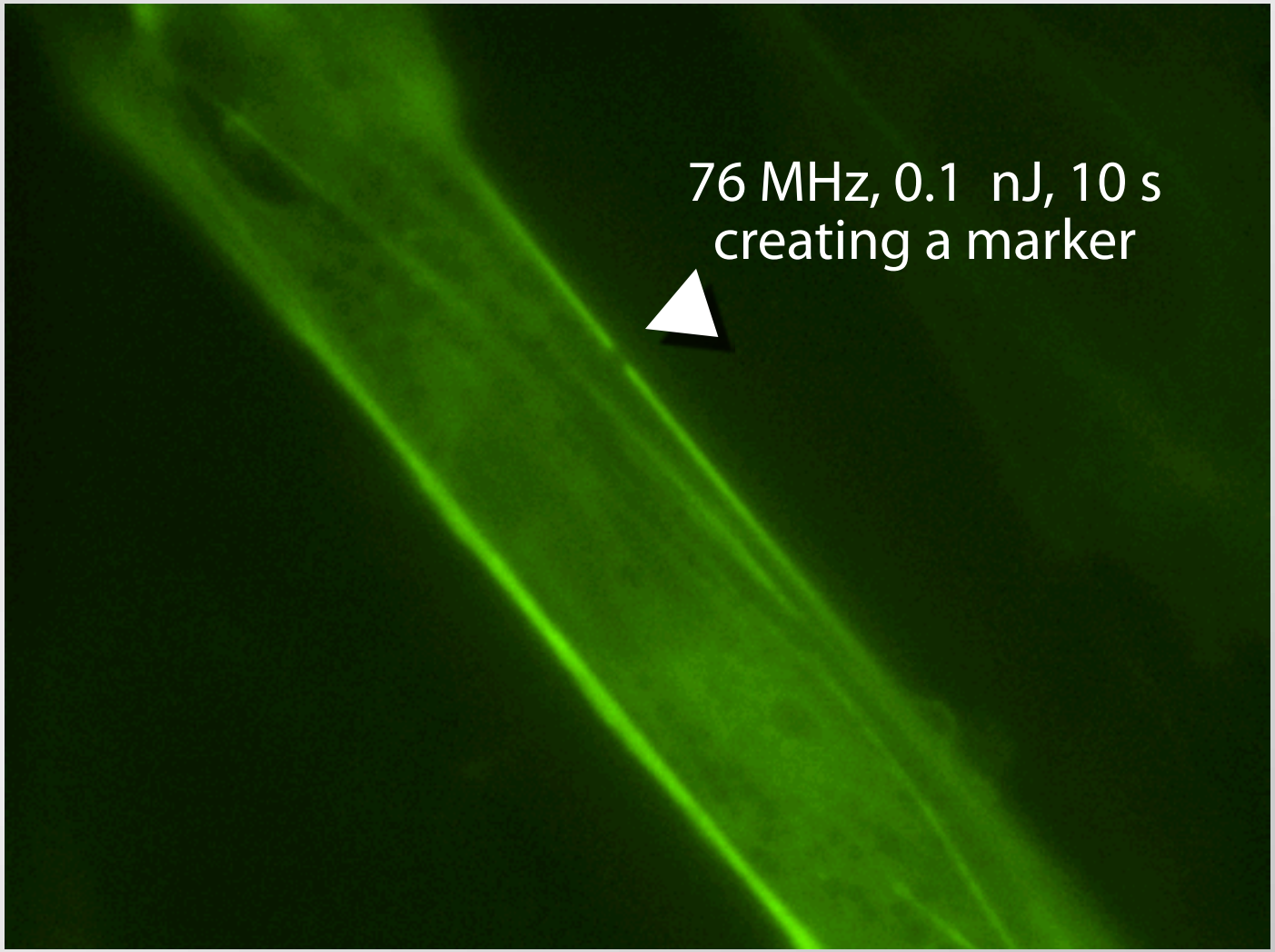
- MHz: pulse energy and exposure determine threshold

outline

- cellular nanosurgery
- kHz and MHz cutting in cells
- elucidating stress fiber dynamics

stress fiber dynamics

use photobleached marker to unveil stress-fiber dynamics



stress fiber dynamics

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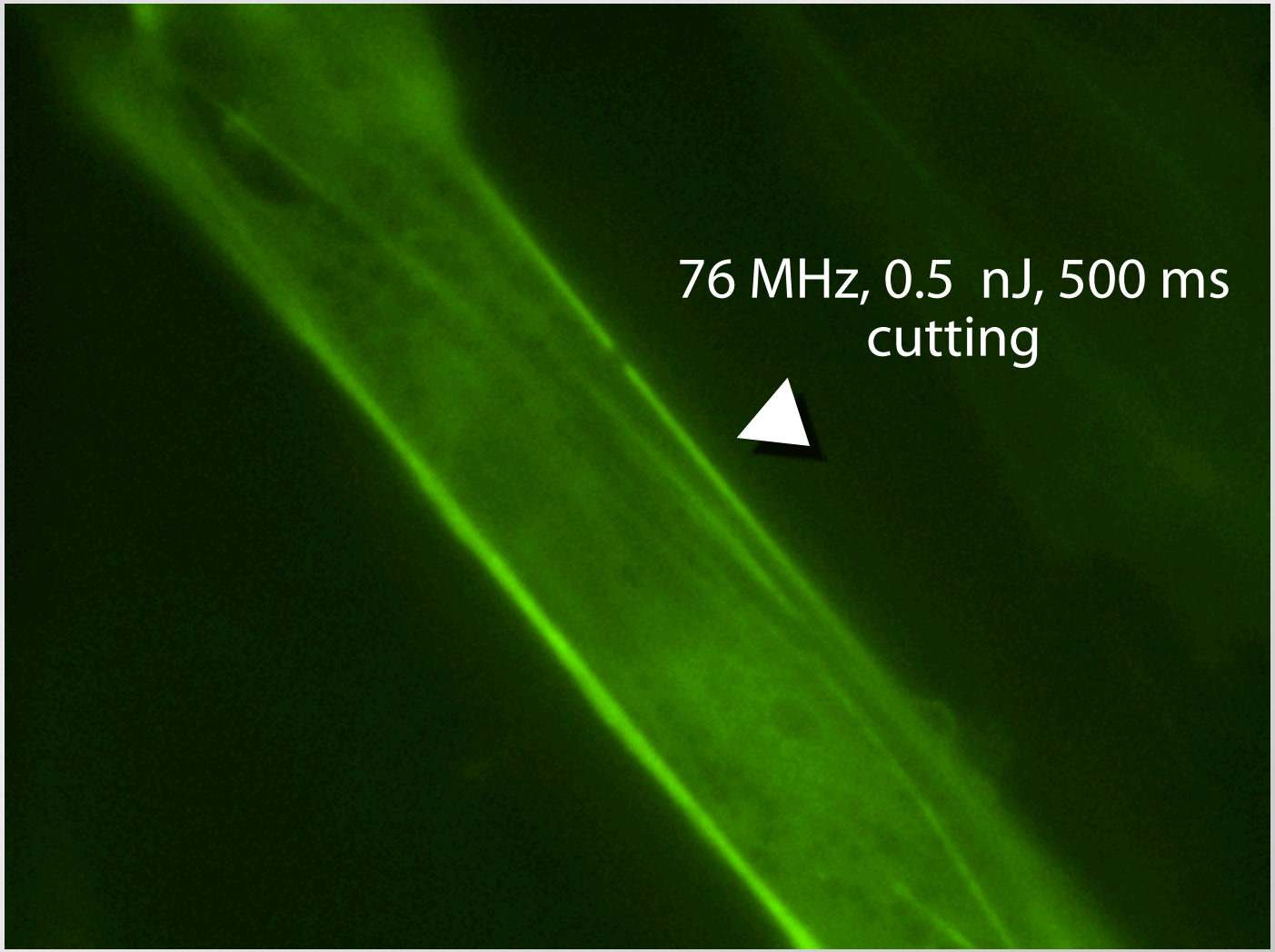
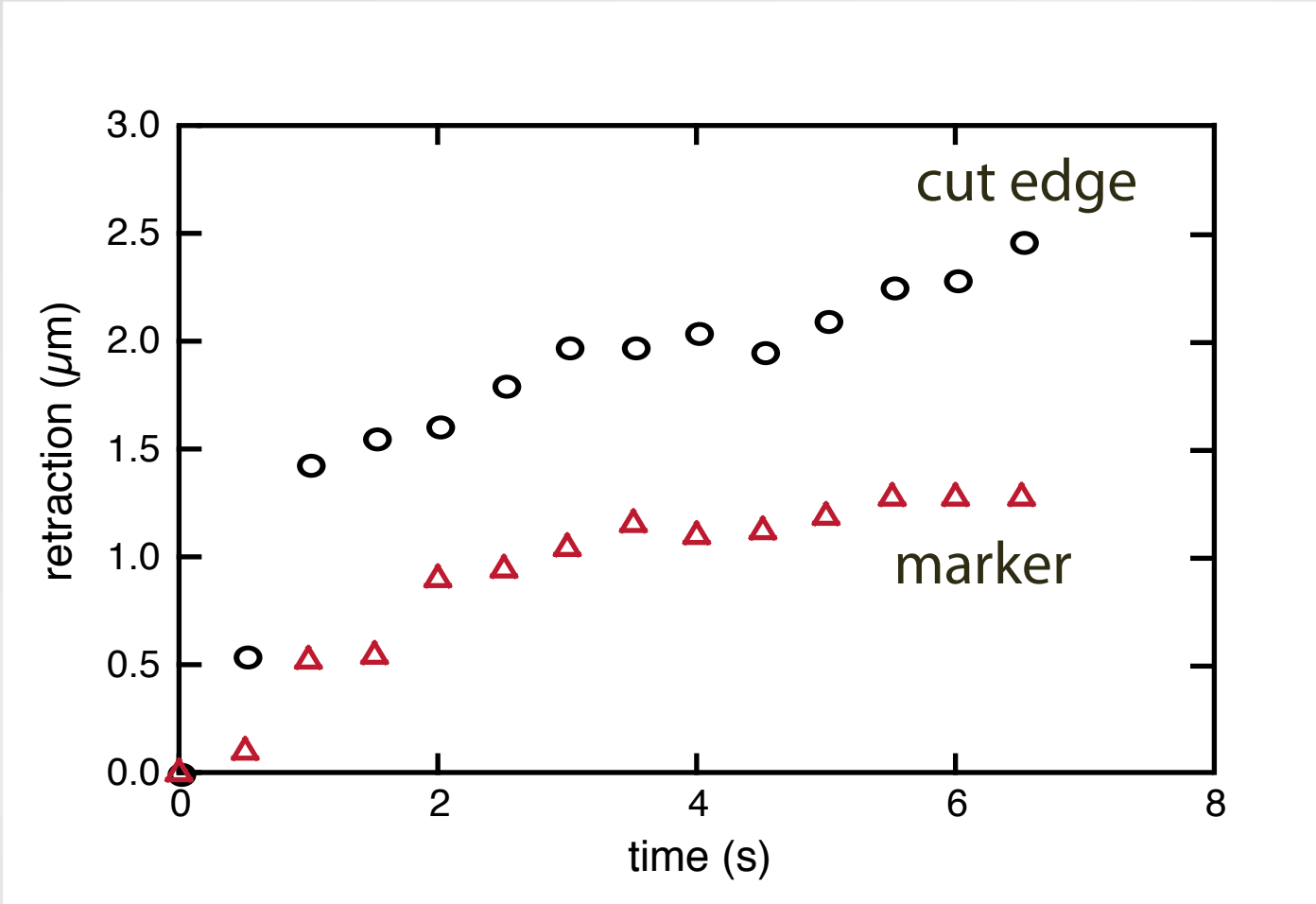


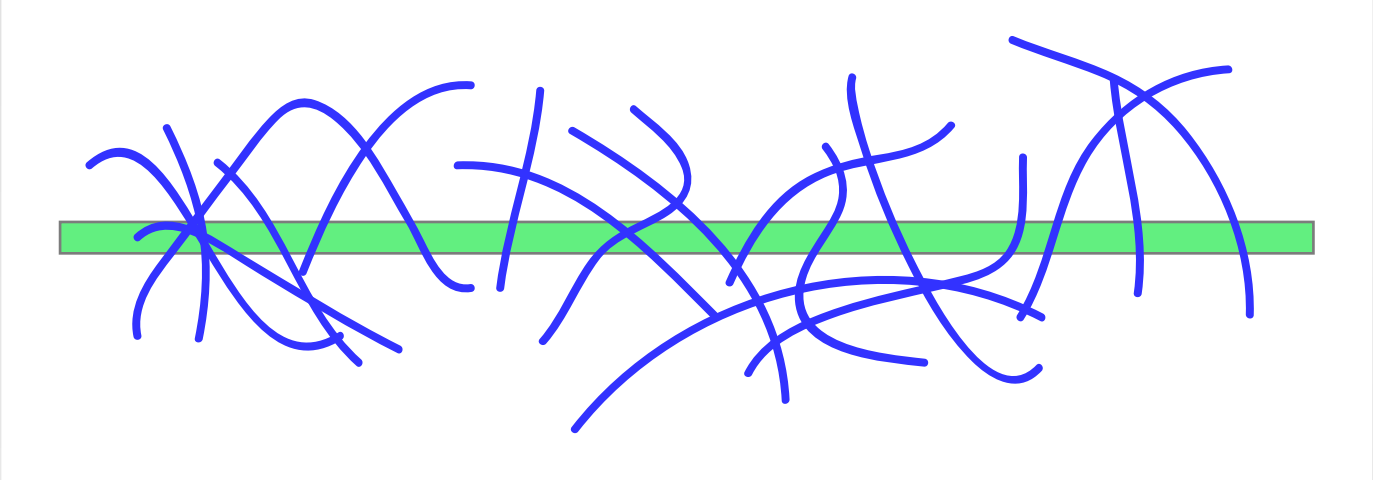
stress fiber dynamics

points along the same fiber have different retraction rates



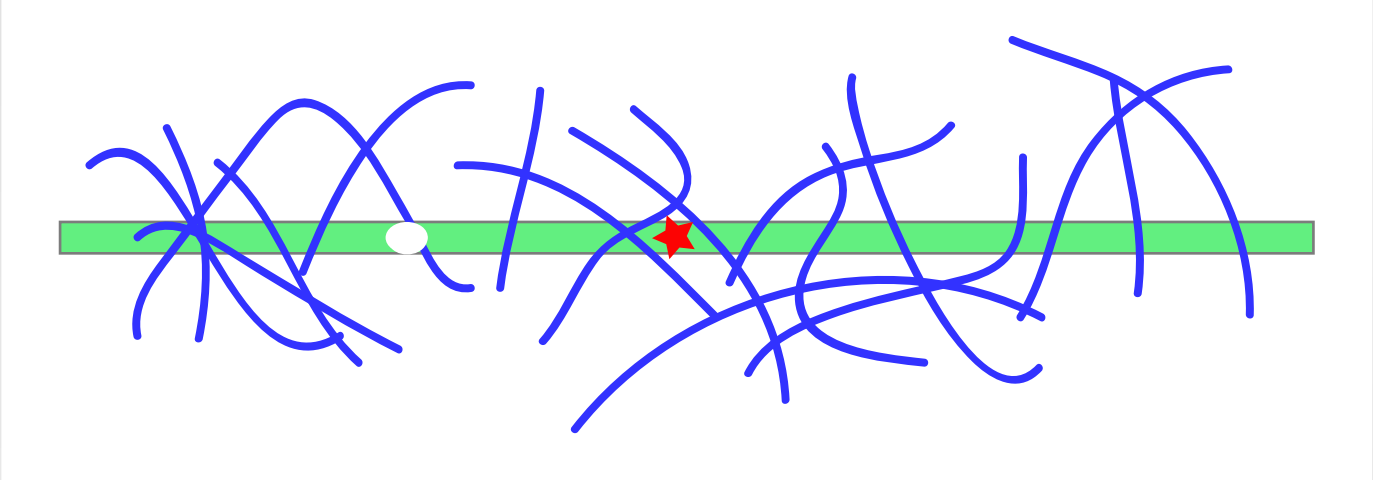
stress fiber dynamics

actin bundles are embedded in a complex network



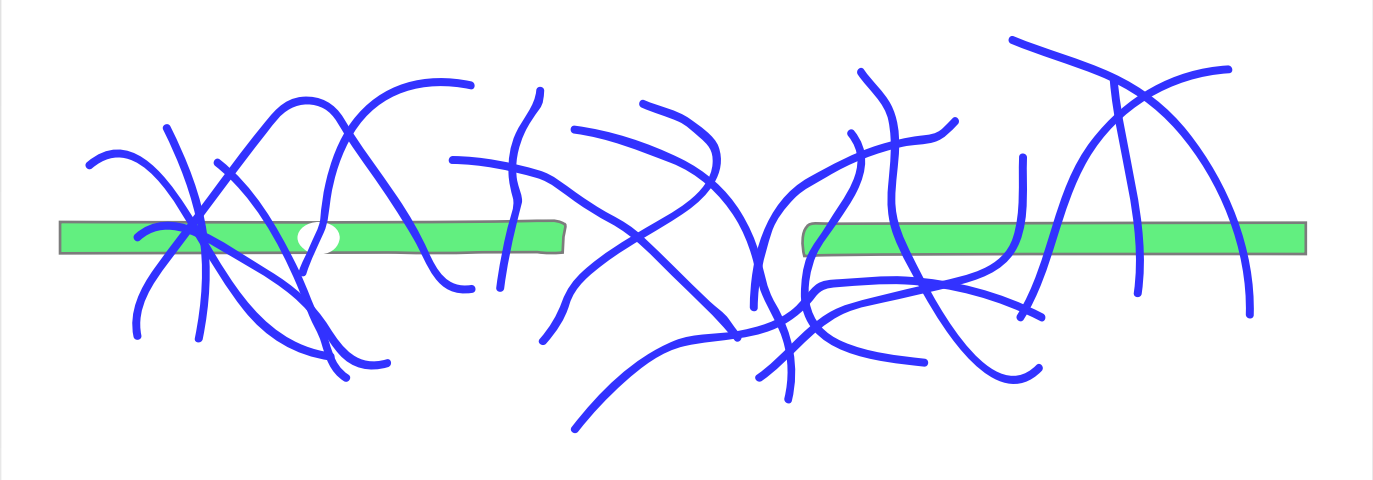
stress fiber dynamics

actin bundles are embedded in a complex network



stress fiber dynamics

actin bundles are embedded in a complex network



stress fiber dynamics

MHz nanosurgery

- enhanced versatility in studying fiber dynamics
- low energy, long exposure: loss of fluorescence
- high energy, short exposure: cutting

outlook

second harmonic generation imaging of collagen gells

outlook

MHz imaging and disruption

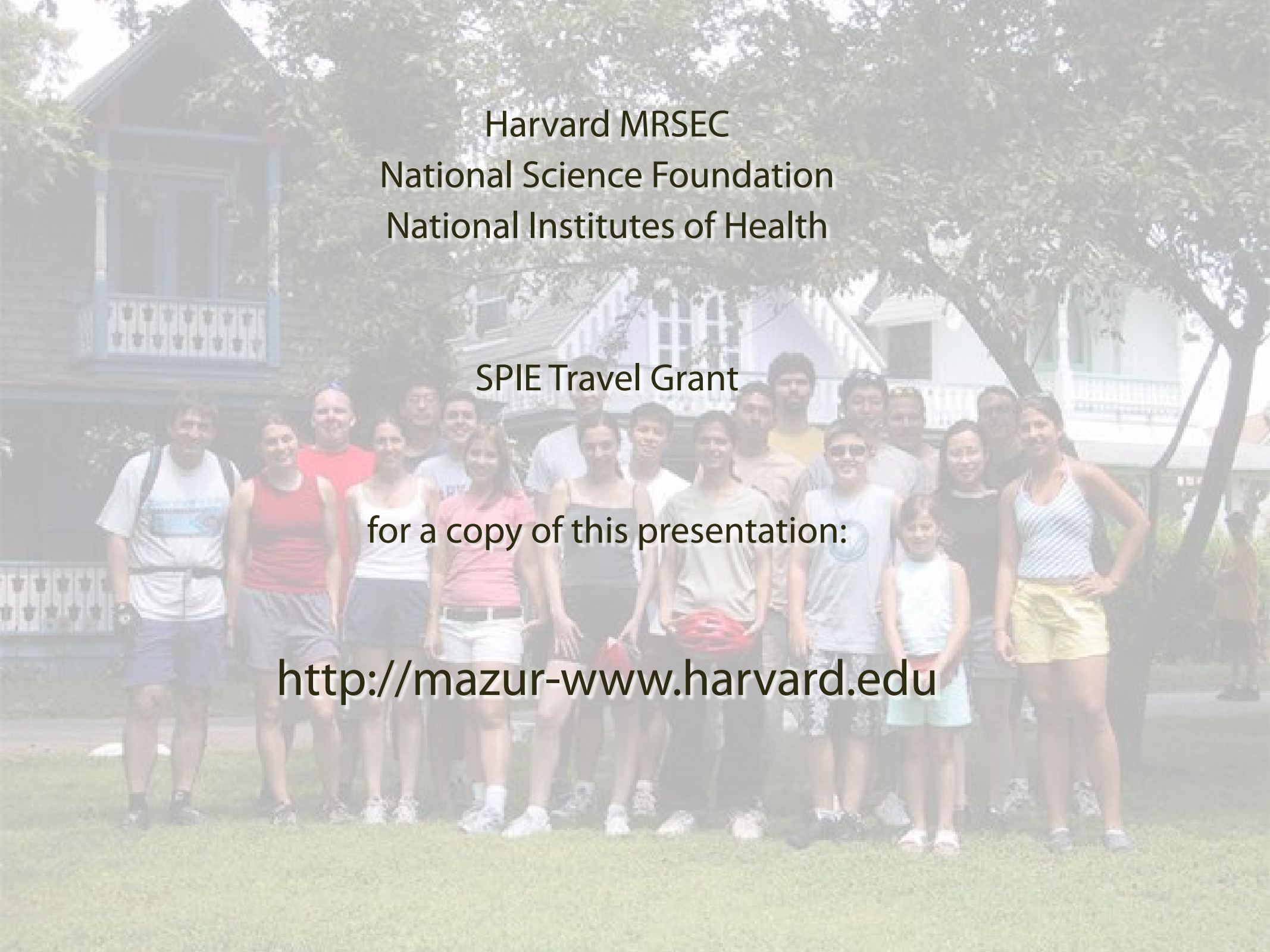
- image extracellular environment through SHG
- image stress fibers through MPM
- use MHz for versatile subcellular nanosurgery

achieve *in vivo,* real time study of cell mechanics

summary

femtosecond nanosurgery:

- **can be achieved with kHz and MHz lasers**
- **pulse energy and exposure need to be optimized**
- **is a versatile tool for studying stress fiber dynamics**
- **has further potential for unraveling biology problems**



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