Sub-cellular nanosurgery in live cells using ultrashort laser pulses

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femtosecond lasers for subcellular manipulation

• high penetration depth in tissue
motivation

femtosecond lasers for subcellular manipulation

- high penetration depth in tissue
- nonlinear interaction
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femtosecond lasers for subcellular manipulation

• high penetration depth in tissue

• nonlinear interaction

• no damage outside focal region
femtosecond lasers for subcellular manipulation

- high penetration depth in tissue
- nonlinear interaction
- no damage outside focal region
- easily integrated with high resolution microscopy
• cellular nanosurgery
• kHz and MHz cutting in cells
• elucidating stress fiber dynamics
cellular nanosurgery

1.4 NA objective

piezo stage

fluorescence

UV lamp

CCD camera
cellular nanosurgery

- Ti:sapphire laser
- < 5 nJ
- UV lamp
- 1.4 NA objective
- piezo stage
- fluorescence
- CCD camera
YFP fluorescent actin filaments in a live cell
cellular nanosurgery

10 seconds later
cellular nanosurgery

1 kHz, 3 nJ
fiber retraction vs. time after laser ablation
viscoelastic model of an actin stress fiber

\[ \Delta L = L_\infty \left( 1 - e^{-t/\tau} \right) + L_0 \]
cellular nanosurgery

modeling of tension release

![Graph showing retraction distance over time](image-url)
femtosecond nanosurgery gives:

access to subcellular structures

a measure of tension in actin stress fibers
• 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

![Graph showing energy/pulse (nJ) vs. exposure (ms)]
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
• cellular nanosurgery
• kHz and MHz cutting in cells
• elucidating stress fiber dynamics
stress fiber dynamics

use photobleached marker to unveil stress-fiber dynamics

76 MHz, 0.1 nJ, 10 s creating a marker
stress fiber dynamics

use photobleached marker to unveil stress-fiber dynamics

76 MHz, 0.5 nJ, 500 ms cutting
stress fiber dynamics

points along the same fiber have different retraction rates

![Graph showing retraction dynamics over time](image)
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
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 gels
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
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