Sub-cellular femtosecond laser ablation

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femtosecond lasers for sub-cellular manipulation

- high penetration depth in tissues
femtosecond lasers for sub-cellular manipulation

• high penetration depth in tissues

• nonlinear interaction
femtosecond lasers for sub-cellular manipulation

- high penetration depth in tissues
- nonlinear interaction
- no damage outside focal region
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)
• fixed cell experiments
  show material ablation

• live cell experiments
  nanosurgery in live cells
  cytoskeletal dynamics
setup

- UV lamp
- 1.4 NA objective
- piezo stage
- fluorescence
- CCD camera

Diagram showing the setup with UV lamp, objective, piezo stage, and CCD camera.
setup

1–5 nJ

Ti:sapphire laser

fluorescence

1.4 NA objective

CCD camera

UV lamp

piezo stage

x-y z
ablation in fixed cells

fluorescent actin network in a fixed cell
ablation in fixed cells

actin network after laser irradiation

4.4 nJ
3.5 nJ
2.8 nJ
2.2 nJ
1.8 nJ

5 μm
q: material ablation or photobleaching?
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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

TEM
fluorescence

no change
bleaching
ablation

pulse energy (nJ)

width (µm)
ablation in fixed cells

no interaction below 1 nJ
Ablation in fixed cells

define three regions of interaction

- TEM
- Fluorescence

-no change
- bleaching
- ablation

width (μm)
pulse energy (nJ)
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
definitive proof of sub-cellular material ablation

ablation widths of 250 nm at 2 nJ

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ablation threshold is 1.2 times that of photobleaching
- fixed cell experiments show material ablation
- live cell experiments nanosurgery in live cells cytoskeletal dynamics
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
• fixed cell experiments
  show material ablation

• live cell experiments
  nanosurgery in live cells
  cytoskeletal dynamics
ablation in live cells

YFP fluorescent actin filaments in a live cell
ablation in live cells

10 seconds later
• tensegrity structures are a balance of tension and compression

• cells are thought to be tensegrity structures

• actin bundles bear tension
live cell nanosurgery
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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Harvard Materials Research Science and Engineering Center
National Science Foundation
National Institutes of Health

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