Sub-cellular nanosurgery in live cells using ultrashort laser pulses

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ECBO/CLEO
Munich, Germany, 13 June 2005
femtosecond lasers for sub-cellular manipulation

- high penetration depth in tissues
motivation

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
• material ablation in cells
• nanosurgery in live cells
• stress fiber dynamics
setup

1.4 NA objective

piezo stage

UV lamp

fluorescence

CCD camera
**setup**

- **Ti:sapphire laser**
- **1–5 nJ**
- **fluorescence**
- **1.4 NA objective**
- **piezo stage**
- **UV lamp**
- **CCD camera**
ablation in fixed cells

fluorescent actin network in a fixed cell
ablation in fixed cells

actin network after laser irradiation

5 μm
q: material ablation or photobleaching?
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

1.45  1.8  2.3 nJ
ablation in fixed cells

1.45 nJ shows photobleaching no ablation
ablation in fixed cells

define three regions of interaction

- TEM
- fluorescence

<table>
<thead>
<tr>
<th>Pulse Energy (nJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
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<table>
<thead>
<tr>
<th>Width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.4</td>
</tr>
<tr>
<td>0.8</td>
</tr>
<tr>
<td>1.2</td>
</tr>
</tbody>
</table>

- No change
- Bleaching
- Ablation
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
• material ablation in cells
• nanosurgery in live cells
• stress fiber dynamics
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
• material ablation in cells
• nanosurgery in live cells
• stress fiber dynamics
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

![Graph showing retraction distance 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

cut 1

cut 2 - delayed

5 μm
stress fiber mechanics

release of tension release

![Graph showing retraction distance (µm) over time (s). The graph displays a curve indicating the retraction process following a tension release event. The x-axis represents time in seconds, ranging from 0 to 30, and the y-axis represents retraction distance in micrometers, ranging from 0.5 to 3.5.]
stress fiber mechanics

release of tension release

![Graph showing retraction distance (µm) over time (s) for cuts 1 and 2.](image)
fs laser sub-cellular ablation is:

verified by TEM

used for live cell nanosurgery

a tool to study stress fiber mechanics
stress fiber mechanics

effect of myosin motor inhibition on stress fibers tension
stress fiber mechanics

effect of myosin motor inhibition on stress fibers tension

![Graph showing the effect of myosin motor inhibition on stress fibers tension. The x-axis represents time (s), and the y-axis represents retraction distance (µm). The graph compares control and inhibited conditions, with error bars indicating variability.](image-url)