CELL MANIPULATION BY NON-LINEAR OPTICAL MICROSCOPY

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KEY WORDS: Non-linear microscopy, Laser surgery, Optical Trapping, Laser Induced Damage

1. LASER SURGERY AND OPTICAL MANIPULATION

We present a combination of nonlinear microscopy, laser nanosurgery and optical trapping applied to the 3D imaging and manipulation of intracellular structures in live cells. We use Ti:sapphire laser pulses for a combined nonlinear microscopy and nanosurgery on microtubules tagged with green fluorescent protein (GFP) in fission yeast (see figure 1A). The same laser source is also used to trap small round lipid droplets naturally present in the cell. The trapped droplets are used as handles (see figure 1B) to exert a pushing force on the nucleus, allowing for a displacement of the nucleus away from its normal position in the center of the cell (see figure 1C).

Figure 1: A) Nanosurgery on the fission yeast mitotic spindle. B) A scheme of the “scanning trap” procedure for displacing the cell nucleus. A granule is moved along a raster scanning trajectory in the y-z plane (small arrow), and simultaneously in the x-direction to push the nucleus (large arrow). C) Optically induced displacement of the nucleus. Left, image of the cell before the optical manipulation. Right, the cell after the manipulation.

2. CHROMOPHORE-ASSISTED LASER INACTIVATION

We used Ti:sapphire laser pulses to selectively induce damage on labeled plasma membrane on live cells. The cell damage was quantified by measuring the variation in the resting membrane potential during the laser irradiation. We measured the reduction velocity of the resting potential at different laser power and different dye concentration. We found that this reduction is likely due to an increase in the membrane permeability, evident by an decrease in cell input resistance. We believe that a full characterization of this damage process could be applied to laser inactivation of specific membrane protein.