IN VIVO CYTOSKELETON DYNAMICS REVEALED BY PULSED UV LASER NANOSURGERY

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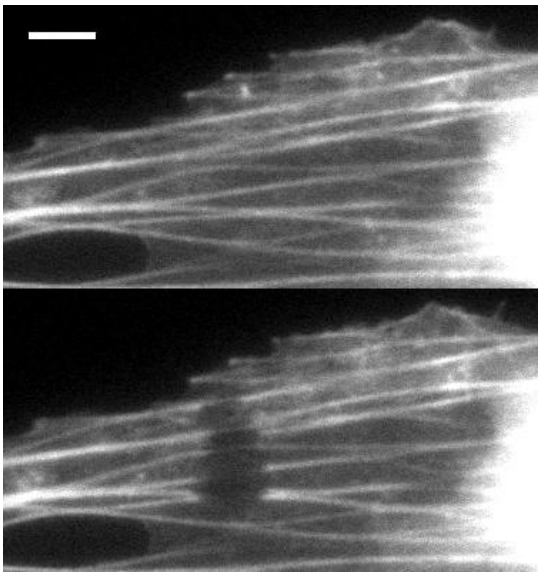
The organized behaviour of the cytoskeleton in general and the microtubule (MT) network in particular are necessary in fundamental biological functions such as the generation of cell shape, polarity, movement, morphogenesis, cell division and intracellular transport. MT dynamics have been extensively described *in vitro*, however, only a limited number of studies successfully characterized the MT dynamics in living cells due to fluorescence microscopy limitations when resolving single MTs.

Here we report on the manipulation of intracellular filaments and the precise measurement of cytoskeleton dynamics *in vivo* using laser nanosurgery. By optimizing the optical delivery of short UV laser pulses, we implemented a laser nanosurgery microscope[1] aiming to severe biological polymers with diffraction limit accuracy by inducing photoablation. We performed photoablation of microtubules in Ptk-2 cells and characterized the severing of MTs induced by the pulsed laser. We describe the accurate measurement of the dynamic instability parameters of MTs *in vivo* and *in situ* without compromising long term cell viability. Furthermore, we demonstrate the micromanipulation of Actin stress fibers in living cells (see Figure) and propose this application as a new method for the quantification of biological polymers dynamics in vivo.



A fluorescence micrograph sequence shows Actin-GFP fibers in Ptk-2 cells. Above, the unperturbed cell. Below, the cell after exposure to the UV laser, focussed down to diffraction limit on a restricted number of filaments. A few pulses are sufficient to induce the breakdown of the actin fibers and provoke mechanical retraction of the stress fibers. Scale bar 5 µm.

[1]: J. Colombelli, S.W. Grill, and E.H.K. Stelzer, "Ultraviolet diffraction limited nanosurgery of live biological tissues," *Rev. Sci. Instr.* **75** (2), 472-478 (2004).