Two-color STED microscope for the analysis of stress-induced interaction between mitochondrial chaperone Mortalin and the plasma membrane attack complex

F. Rocca[1], N. Mazkereth[2], J. Krüger[1], C. Geisler[1], A. Egner[1] and Z. Fishelson[2]

[1)] Department of Optical Nanoscopy, Laser-Laboratorium Göttingen e.V., Hans-Adolf-Krebs-Weg 1, D-37077 Göttingen, Germany
[2)] Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, P.O.B 39040 Ramat Aviv, Tel Aviv 69978, Israel

E-mail: francesco.rocca@llg-ev.de

When addressing questions relevant to biological or medical cell research with fluorescence microscopy, the ability to distinguish between different molecular species with high spatial resolution is a major benefit: it allows gaining knowledge about the species' relative spatial organization and their interaction on the molecular scale. We therefore designed a super-resolution STED microscope [1] for robust and precise co-localization measurements with the ability to acquire two channels quasi-simultaneously with minimal crosstalk. Capitalizing on the microscope's sub-diffraction resolution of 35 nm in both channels, we investigated the postulated interaction of a mitochondrial chaperone (Mortalin/GRP75) and a stress-inducing membrane attack complex (MAC/C5b-9) at the cell plasma membrane [2], thereby contributing to the understanding of cancer cell's high resistance to complement-dependent cytotoxicity. It is well known that MAC complex is a key component in the immune system's task to dispose the body's own defective cells. Once MAC clusters are formed on the target cell's membrane, the membrane is disrupted leading to cell death. The mitochondrial chaperon Mortalin, which is overexpressed in cancer cells, plays an essential role in this cell resistance against MAC cytotoxicity. Our nanoscopical study gained valuable insights into the relocation of the Mortalin at the plasma membrane of human leukemia cancer cells K562 in response to MAC-induced stress conditions [3]. The spatial distribution of Mortalin on the cell surface is a time-dependent process, juxtaposition of fluorescence-labeled Mortalin and MAC at the plasma membrane region has been identified already within minutes after MAC attack. The analysis of Mortalin distribution in the vicinity of the mitochondria in treated cells shows a more diffused pattern relative to control cells, proposing exit of Mortalin from the mitochondria in response to MAC-induced stress. In summary, our data demonstrated that cells can sense MAC activation at the plasma membrane and in response send Mortalin to this region to deactivate it.

[1] S.W. Hell, "Far-Field Optical Nanoscopy" *Science* **316**, 1153-1158 (2007)
[2] Z. Fishelson et al., "Monoclonal Antibody-C3b Conjugates: Killing ofK562 Cells and Selection of a Stable Complement Resistant Variable" *Molecular Immunobiology* **29**, 771-781 (1992)
[3] N. Mazkereth et al., "Complement Triggers Relocation of Mortalin/GRP75 from Mitochondria to the Plasma Membrane" *Immunobiology* **221**, 1395-1406 (2016)