ZERNIKE INSTITUTE COLLOQUIUM

Thursday, June 21st, 2012

16:00h, Lecture Hall: 5111.0080 **Coffee and cakes from 15:30h**

NANOSCOPY WITH FOCUSED LIGHT

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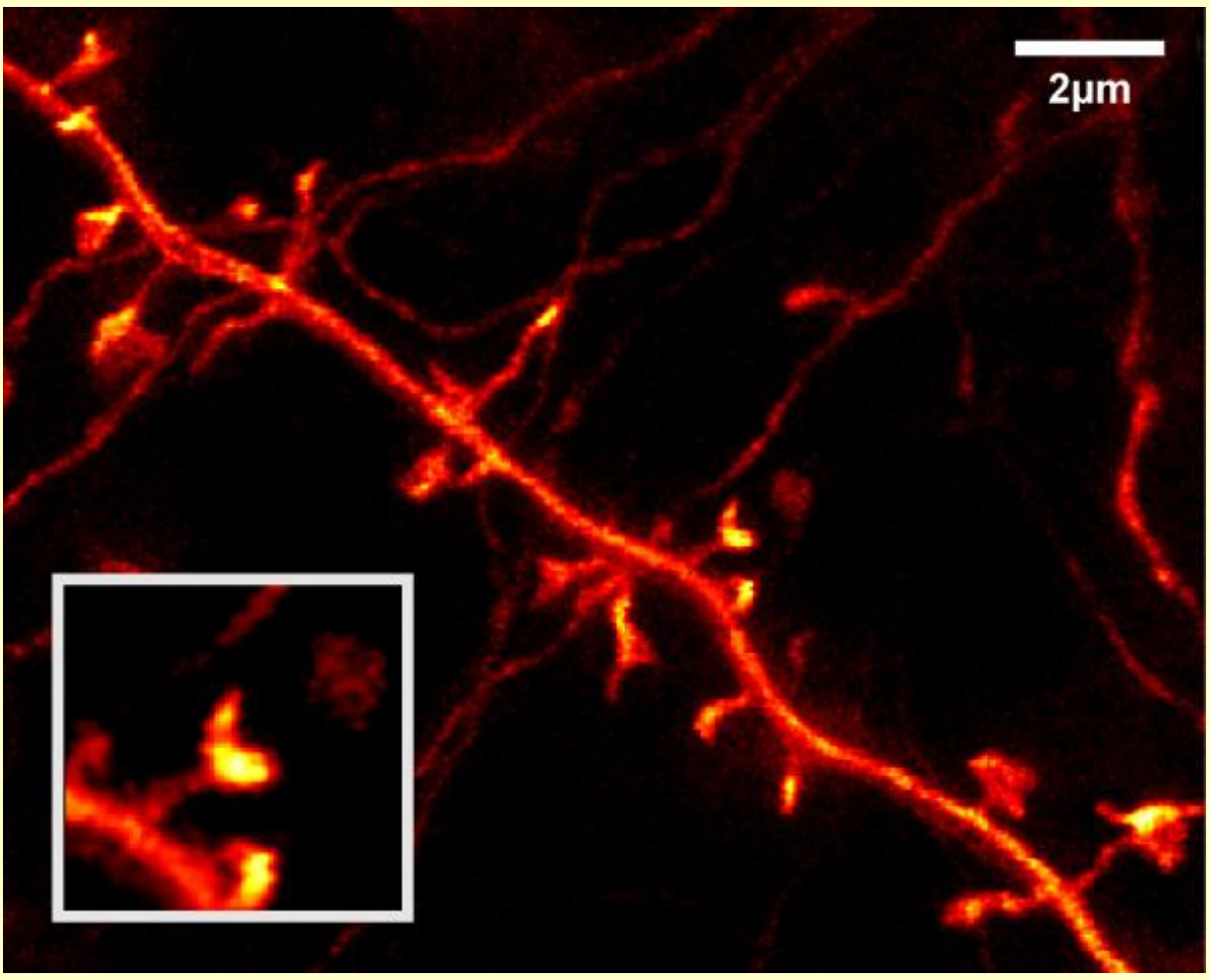
In STED microscopy[1], fluorescent features are switched off by the STED beam, which confines the fluorophores to the ground state everywhere in the focal region except at a subdiffraction area of extent $d \approx \lambda/(2NA\sqrt{1 + I/I_s})$.

In RESOLFT microscopy[2,3], the principles of STED have been expanded to fluorescence on-off-switching at low intensities *I*, by resorting to molecular switching mechanisms that entail low switching thresholds I_s. An I_s lower by many orders of magnitude is provided by reversibly switching the fluorophore to a long-lived dark (triplet) state[2-4] or between a long-lived 'fluorescence activated' and 'deactivated' state[2,5].



These alternative switching mechanisms entail an I_s that is several orders of magnitude lower than in STED. In imaging applications, STED/RESOLFT enables fast recordings and the application to living cells, tissues, and even living animals[6,7].

Starting from the basic principles of nanoscopy we will discuss recent developments[8,9] with particular attention to RESOLFT and the recent nanoscale imaging of the brain of living mice[7] by STED.

STED movie from a living mouse brain Neuron recorded from the molecular layer of the somatosensory cortex of a living transgenic mouse expressing YFP with resolution < 68 nm, showing moving dendritic spines.

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2. Hell, S. W. Toward fluorescence nanoscopy. *Nat Biotechnol* **21**, 1347-1355 (2003).
3. Hell, S. W., Jakobs, S. & Kastrup, L. Imaging and writing at the nanoscale with focused visible light through saturable optical transitions. *Appl Phys A* **77**, 859-860 (2003).
4. Hell, S. W. Far-Field Optical Nanoscopy. *Science* **316**, 1153-1158 (2007).
5. Hofmann, M., Eggeling, C., Jakobs, S. & Hell, S. W. Breaking the diffraction barrier in fluorescence microscopy at low light intensities by using reversibly photoswitchable proteins. *PNAS* **102**, 17565-17569 (2005).
6. Rankin, B. R. et al. Nanoscopy in a Living Multicellular Organism Expressing GFP. *Biophys J* **100**, L63 - L65 (2011).
7. Berning, S., Willig, K. I., Steffens, H., Dibaj, P. & Hell, S. W. Nanoscopy in a Living Mouse Brain. *Science* **335**, 551 (2012).
8. Grotjohann, T. et al. Diffraction-unlimited all-optical imaging and writing with a photochromic GFP. *Nature* **478**, 204-208 (2011).
9. Brakemann, T. et al. A reversibly photoswitchable GFP-like protein with fluorescence excitation decoupled from switching. *Nat Biotechnol* **29**, 942-947 (2011).

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