STED Microscopy of DNA Damage Markers*

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STED (stimulated emission depletion) is a specialized form of immunofluorescence microscopy that overcomes the resolution limitations by diffraction. 1873 Ernst Abbe defined the diffraction limit [1] as:

$$\Delta d = \frac{\lambda}{2n \sin \alpha}$$

λ wavelength of light, n refractive index, α semiaperture angle of the lens.

With λ being typically between 400 and 700 nm and α < 70° this means, that classical light microscopy is limited to 200 nm resolution at its best.

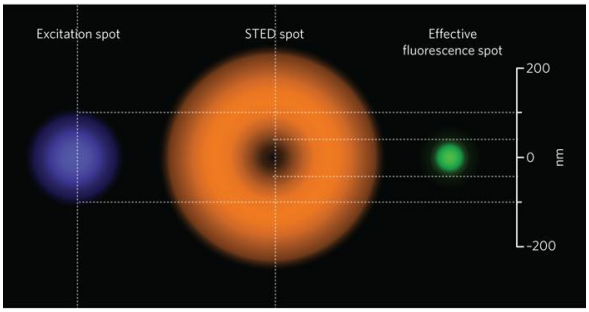

Figure 1: Coupling the excitation beam (blue) with a doughnut-shaped STED beam (orange) shrinks the fluorescence area (green) below the diffraction limit (200 nm). [2]

However, STED microscopy [3] makes use of the RESOLFT (reversible saturable optical fluorescent transitions) concept to reach below this barrier. A usual excitation beam is coupled with a doughnut-shaped STED beam depleting all of the fluorescence except of the emission of the very centre of the focus (Figure 1). By scanning with this sharpened spot across the sample images can be recorded with resolutions below the diffraction barrier.

53BP1 (p53 binding protein 1) shares common features with the commonly used DNA damage marker γH2AX [4]. Both are phosphorylated by ATM in response to ionising radiation (IR) and form IR induced foci (IRIFs) which colocalize.

Here, we show a biological application for the high resolution STED technique.

Results and Discussion

Recent work in our group [5] indicated that high resolution 4 Pi microscopy can provide a more precise insight in IRIF organisation. We tried to further improve this effort by making use of the STED microscopy. 53BP1 foci, which appear as large, homogeneous spots in confocal microscopy (Figure 2 A, red) show distinct substructure in STED microscopy (green). In our biological sample we were able to reach a resolution of 60 nm (Figure 2 B), well below the diffraction limit.

Future work will possibly be able to give a much deeper insight in IRIF organisation, DNA damage response factor recruitment and repair complex formation, especially by performing multi colour STED.

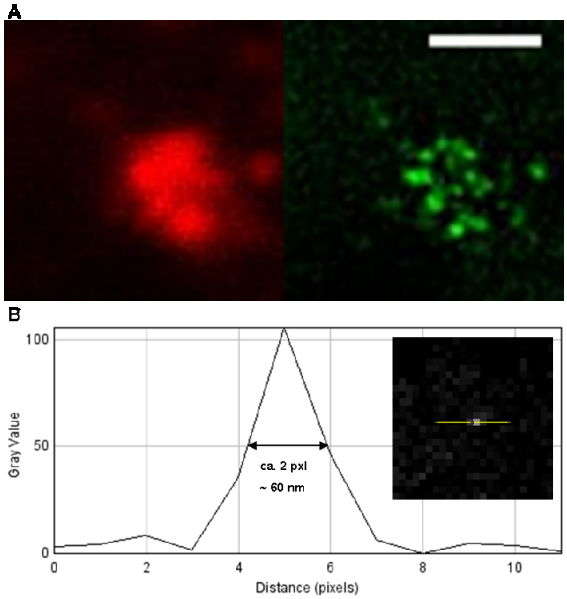

Figure 2: (**A**) MEF wt cells irradiated with Ne ions, 9.6 MeV/u, 460 keV/µm at UNILAC, fixed 1 h post irradiation. Red: 53BP1 confocal; green: 53BP1 STED. Scalebar 1 µm. (**B**) Focus measurement, full width half maximum ~ 60 nm.

References

[1] Abbe, E. (1873); Beitrage zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung. *Arch. Mikr. Anat.* 9: 413–420.

[2] Alison Abbott (2009); Microscopic marvels: The glorious resolution. *Nature* 459, 638-639.

[3] Hell, S.W. et al (2006); Fluoreszenzmikroskopie ohne Beugungsgrenze. *BIOspektrum*, 12. Jahrgang

[4] Noon, A.T. et al. (2011); 53BP1-mediated DNA double strand break repair. *DNA Repair* 10, 1071-1076.

[5] Splinter, J. et al. (2010); Biological dose estimation of UVA laser microirradiation utilizing charged particle-induced protein foci. *Mutagenesis* 1-9.

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