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Resolution in the ApoTome and the CLSM: a comparison

The ability of the confocal laser scanning microscope (cLSM) to remove out-of-focus light and thereby produce optical sections, making 3D-reconstruction of an acquired image stack possible, led to its unprecedented triumphal march in life sciences. Remaining unchallenged for decades, this unique capability has now been claimed to be a feature of a new method of light microscopy called structured illumination, of which the ZEISS ApoTome is a commercially available representation. Advertising it as “evolution in fluorescence microscopy”, the manufacturer promises a thickness of 1 Airy Unit (A.U.) for an optical section along with improved lateral resolution.

In this thesis, the performance of the ApoTome in terms of resolution has been compared to the cLSM by the use of thin, homogeneous fluorescent layers and fluorescent beads. It was found that the axial full-width-at-half-maximum (FWHM) as a measure of resolution in case of fluorescent layers was $d_{z,apo} = 1.33 \pm 0.05 \, \mu m$ for $\lambda_e = 516 \, nm$ and $d_{z,apo} = 1.46 \pm 0.07 \, \mu m$ for $\lambda_e = 565 \, nm$, imaged with a 63x/1.4 oil immersion objective, which corresponds to a pinhole diameter (PD) of $\approx 2 \, A.U.$ in the cLSM, and is far away from the promised resolution. Green fluorescent beads imaged with a 40x/1.2 water immersion objective however revealed a lateral resolution of $d_{r,apo} = 296 \pm 39 \, nm$, which corresponds to standard wide-field microscopy resolution and is surpassed by the cLSM by its dependency on $\lambda_i$ rather than on $\lambda_e$, and for small pinholes additionally by a factor of 1.4 due to an overlap of the illumination point spread function (PSF) with the detection PSF. In the axial direction, $d_{z,apo} = 786 \pm 49 \, nm$, which is about 0.8 A.U. and 15 – 40 % worse than acquired with the cLSM at a PD of $\approx 1 \, A.U.$, though the performance of the cLSM can still be improved by closing the pinhole to a PD $< 0.25 \, A.U.$.. The structural factor $S = d_z/d_r$ in the ApoTome was $S = 2.65 \pm 0.51$ and thus in good agreement with theoretical and experimental knowledge.