

RESOLUTION IN THE APOTOME AND THE CONFOCAL LASER SCANNING MICROSCOPE: COMPARISON

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The essential feature of the confocal laser scanning microscope (cLSM) is the generation of optical sections by the removal of out-of-focus light. About ten years ago structured illumination microscopy (SIM) was introduced as an alternative method for obtaining optical sections from biological specimen [1]. Here we compare the resolution of the ApoTome (commercial SIM by Zeiss) to that achieved by a cLSM (Zeiss LSM 510).

If fluorescent beads are used as test objects, the ApoTome will achieve a lower axial resolution than the cLSM. In contrast to that, its lateral resolution scores slightly better and matches the resolution of standard widefield-epifluorescence.

If subresolution homogeneous fluorescent layers are used as test objects, the ApoTome will achieve a higher axial resolution than the cLSM. The ApoTome's axial resolution is homogeneous over the field-of-view while that of the cLSM changes markedly.

Finally, the anisotropy of the ApoTome's resolution was found to be negligible for standard applications while its capability to resolve fine structures within stained tissue slices is limited to one or two cell layers and thus worse than in the cLSM.

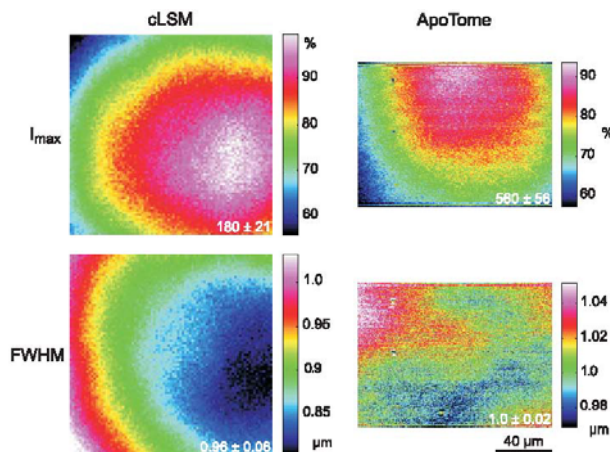


Fig. 1 Extract of SIPcharts [2]. a thin uniform fluorescent layer was recorded in a 3D-image stack, axial step size 100 nm, and subsequently processed to obtain SIPcharts (ApoTome: VH-grid, cLSM pinhole diameter: 1 A.U.). The maximum intensity has a circular-symmetric shape in both imaging modes. The resolution is rather homogeneous in the ApoTome. There remain stripe artefacts in the ApoTome-images. Objective: 63x/1.4 Plan-Apochromat.

REFERENCES

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