# LIGHT SHEET MODULE APPLIED IN 3D LIVE CELL MICROSCOPY

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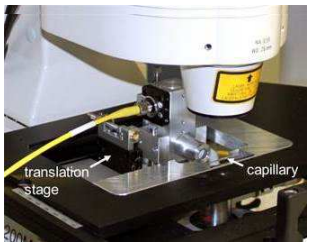
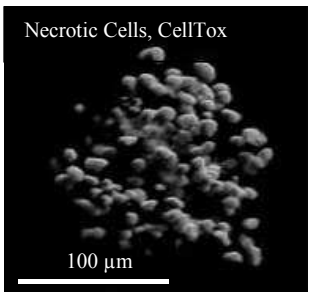



Innovative fiber-coupled add-ons for light sheet-based fluorescence microscopy (LSFM) with commercial inverted microscopes were described previously [1]. The light sheet is generated either by an achromatic cylindrical lens or by a spherical mirror with astigmatic distortion. Presently the "lens system" is further validated and applied to various samples including 3D multicellular spheroids and small organisms. Samples are commonly located in rectangular glass capillaries or in rotatable cylindrical capillaries optically coupled to the rectangular capillary [2].




Applications include autofluorescence measurements of 3D multicellular tumour spheroids with the possibility to probe cell metabolism upon glycolysis or oxidative phosphorylation. Necrosis or apoptosis in 3D cell systems are assessed by application of appropriate dyes or a Förster Resonance Energy Transfer (FRET) based sensor system [3]. Also uptake of a chemotherapeutic drug, e.g. doxorubicin, in 3D breast cancer cells is presently studied by LSFM and correlated with its cytotoxicity. In addition, light-sheet based fluorescence microscopy proved to be an appropriate method for imaging islets of Langerhans and their glucose dependent metabolism (*), as well as for imaging small organisms like ticks or copepods under various detection angles [2].

The present LSFM modules are characterized by a low numerical aperture ($A_N$ = 0.08–0.12) for illumination, thus creating a beam waist around 5 µm and a focal depth around 100 µm. This appears ideal for fluorescence microscopy of single or multiple layers of cell spheroids with subsequent 3D imaging. The light sheet and the detection lens can be moved simultaneously in vertical direction, and the "fish tank effect" is corrected adequately. In comparison with stand-alone LSFM systems the present setup is characterized by its high flexibility (e.g. combination with spectral or fluorescence lifetime imaging microscopy), low sample volumes (thus saving expensive drugs or reagents) and the possibility of rotating the samples for reduction of light scattering and for observation from any side.

References

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(*) in cooperation with U. Kraushaar, NMI Reutlingen, Germany.