A light sheet module for 3D fluorescence microscopy of tumour cell spheroids

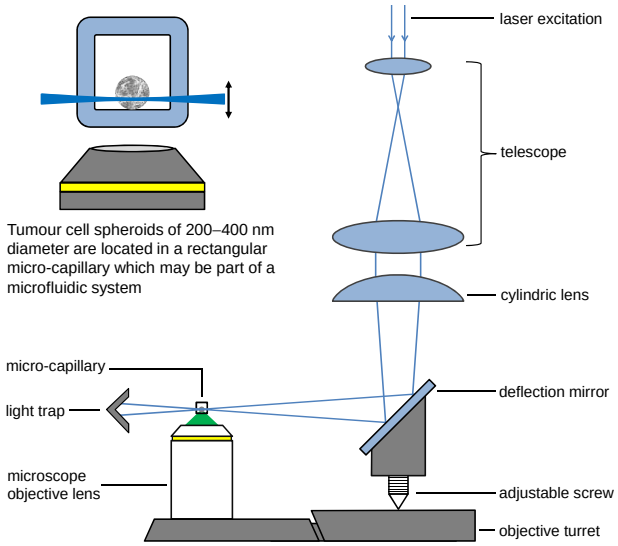
Herbert Schneckenburger[1,2,*], Sarah Schickinger[1], Michael Wagner[1], Petra Weber[1], Rainer Wittig[2], and Thomas Bruns[1]

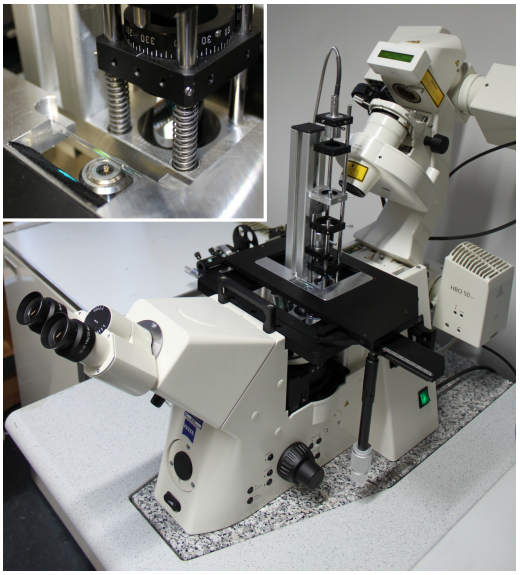
[1]Hochschule Aalen, Institut für Angewandte Forschung, Beethovenstr. 1, D-73430 Aalen, Germany
[2]Institut für Lasertechnologien in der Medizin und Messtechnik an der Universität Ulm, Helmholtzstr. 12, D-89081, Germany

SUMMARY

Light sheet or single plane illumination illumination microscopy (SPIM) represents a 3D method of low light exposure permitting long observation times and maintaining cell viability [1]. Here, a light sheet module is adapted to a conventional inverse microscope, permitting high flexibility and combination with further microscopic techniques, e.g. transillumination, LSM, spectral imaging, FLIM, or nanosecond ratio imaging. Combination with a microfluidic system [2] permits easy application of nutrients, fluorescent dyes, metabolites or drugs, e.g. in pharmaceutical test systems. The present technique is adapted to 3D cell systems [3] with an axial resolution around 5 µm and a focal depth up to 400 µm; by an additional microscope lens in front of the sample it can be easily modified, if higher resolution is needed.

METHOD

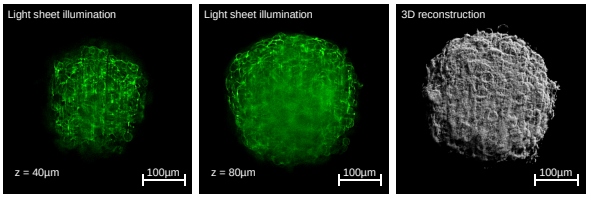




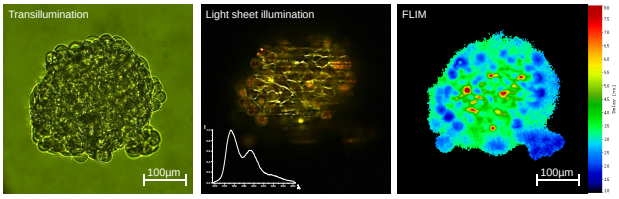
- z positions of light sheet and objective lens are synchronized
- The illumination device is fixed to the objective tower of the microscope
- All detection channels of the microscope can be used for imaging, spectroscopy, FLIM etc.

APPLICATIONS

- 3D imaging of CHO cell spheroids with membrane associated GFP
- Advantages: low light dose; preferential forward scattering
- Option: irradiation from different sides



- Spectral imaging and fluorescence lifetime imaging (FLIM) of a chemotherapeutic drug (doxorubicin) applied by microfluidics



SELECTED REFERENCES

[1] H. Schneckenburger et al.: “Light exposure and cell viability in fluorescence microscopy”, J. Microsc. 245 (2012) 311–318.
[2] T. Bruns et al.: “Preparation strategy and illumination of three-dimensional cell cultures in light sheet-based fluorescence microscopy”, J. Biomed. Opt. 17 (2012) 101518.
[3] H. Schneckenburger et al.: “Multi-Dimensional Fluorescence Microscopy of Living Cells”, J. Biophotonics 4(3) (2011) 143–149

This project is funded by the *Land Baden-Württemberg* as well as by the European Union, *Europäischer Fonds für regionale Entwicklung*.

* Correspondence to:
herbert.schneckenburger@htw-aalen.de
Tel: +49-7361-576-3401