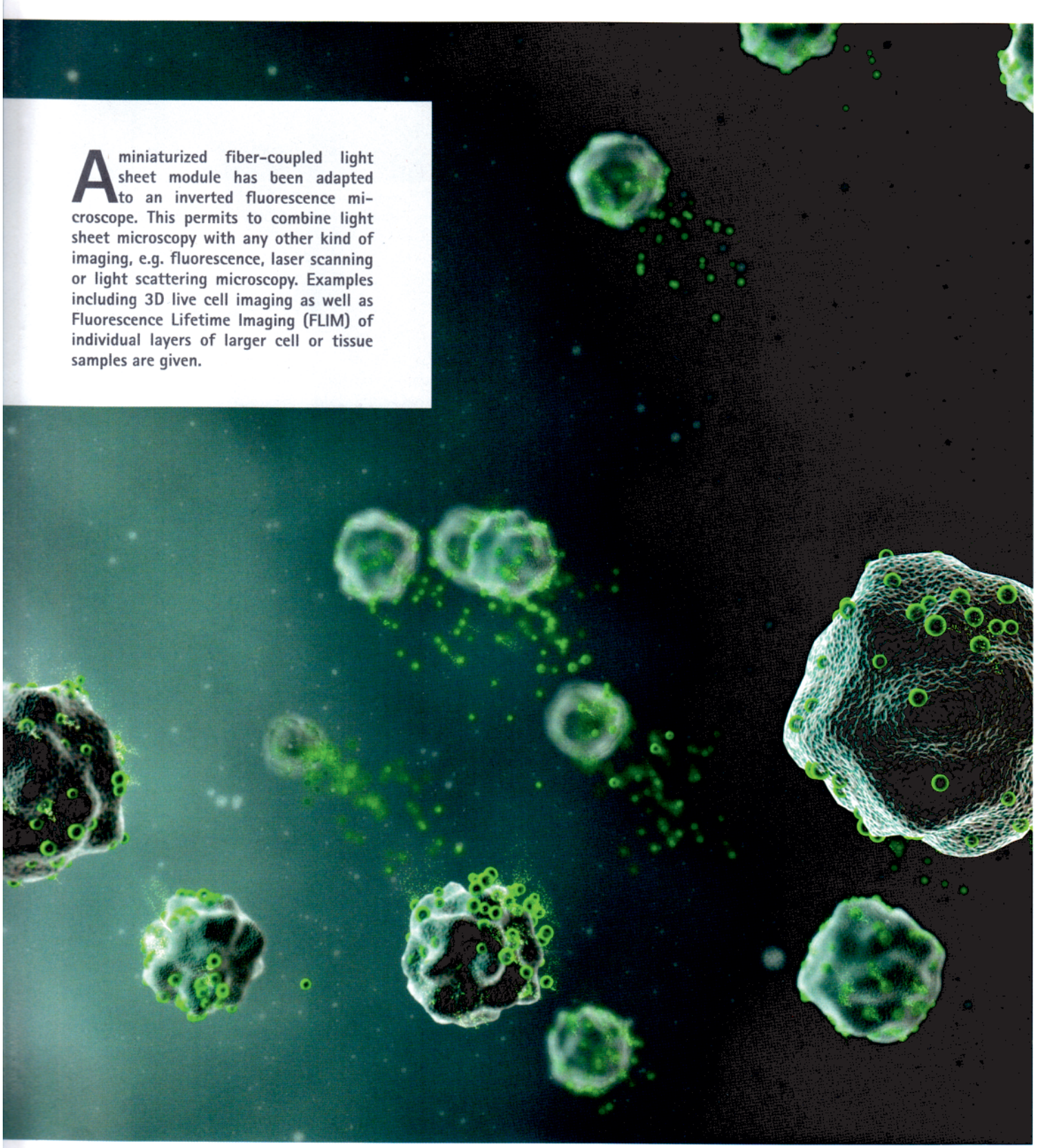
Light Sheet Module for 3D Imaging

A Miniaturized Device Permits 3D Resolution in Microscopy

Herbert Schneckenburger[1], Sarah Bruns[1], Verena Richter[1], Michael Wagner[1], Thomas Bruns[1]

A miniaturized fiber-coupled light sheet module has been adapted to an inverted fluorescence microscope. This permits to combine light sheet microscopy with any other kind of imaging, e.g. fluorescence, laser scanning or light scattering microscopy. Examples including 3D live cell imaging as well as Fluorescence Lifetime Imaging (FLIM) of individual layers of larger cell or tissue samples are given.



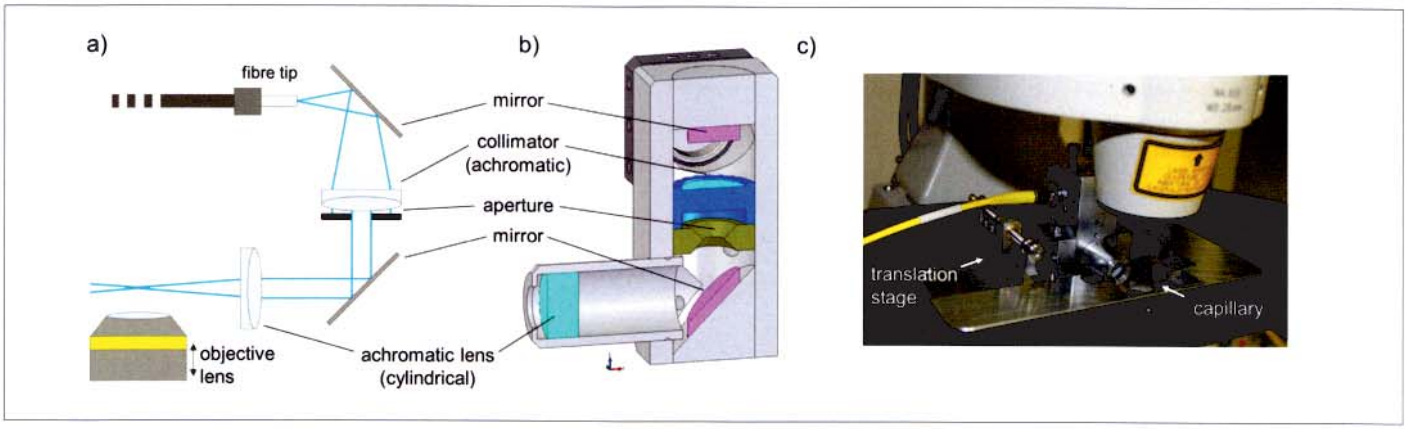


Fig. 1. Miniaturized module for light sheet illumination; (a) schematic (b) technical and (c) after adaptation to an inverted microscope. Reproduced from ref. 5 with modifications.

Introduction

In addition to established methods, e.g. confocal laser scanning or structured illumination microscopy, light sheet fluorescence microscopy (LSFM) has proven its potential as a valuable method for 3D imaging of biological specimens [1,2]. Samples are illuminated perpendicular to the detection path by a light sheet which is commonly created by a cylindrical lens or by scanning a laser beam. If either the light sheet or the sample is moved in axial direction, individual planes can be recorded successively with each plane being exposed only once to laser irradiation. This minimizes light exposure of the whole sample and reduces photobleaching and phototoxic damage to living cells or tissues [3,4], in particular in the case of long observation times.

Up to now most light sheet microscopes are extensive standalone instruments with well defined light sources and high resolution image detectors. In contrast to these systems it was our aim to miniaturize the optical setup and to combine LSFM with further methods of widefield or laser scanning microscopy including Spectral Imaging and Fluorescence Lifetime Imaging (FLIM). Therefore, we developed the fiber-coupled module depicted in figure 1 and adapted it to a conventional inverted microscope. This allows us to use any kind of light source and detection system including FLIM cameras and spectrometers.

Experimental Setup

For illumination various lasers, e.g. diode lasers with fixed wavelengths or tunable photonic crystal fiber lasers can be used in combination with a single mode fiber. The light sheet module is mounted on the microscope positioning stage and consists of a collimating lens, a 4 mm aperture and an achromatic cylindrical lens of 25 mm focal length, resulting in a numerical aperture $A_N = 0.08$. For a wavelength $\lambda = 470$ nm and a refractive index $n = 1.33$ in the micro-capillary holding the sample [5], a beam waist $\delta = \lambda/A_N = 5.9$ µm and a depth of focus $L = n\lambda/A_N^2 \approx 100$ µm are thus attained. This allows single cell layers of a 3-dimensional assembly to be imaged in a field of at least 100 µm diameter. A small chromatic focal shift of -60 µm between wavelengths of 470 nm and 600 nm can be easily corrected by micro-positioning of a translation stage (fig. 1). Fluorescence detection occurs with any kind of microscope objective lens, in the present case with a 10x/0.30, 20x/0.50 or 40x/0.60 long distance lens, permitting sub-cellular resolution according to the Rayleigh criterion. For 3D imaging the light sheet and the detection lens can be shifted simultaneously in axial direction using a mechanical coupler between the illumination unit and the z-stage of the objective turret as described earlier [6]. This coupler permits to move the illumination unit (and therefore the light sheet) and the detection lens by a different feed factor to compensate for the so-called fish tank effect describing the difference of optical pathways in media with different refractive indices, e.g. cell (with the surrounding medium) and air. For adjustment of the light sheet the focusing cylindrical lens can be rotated by 90° to visualize the beam waist and to adjust its position by moving the illumination unit via the translation stage back and forth in horizontal direction [5]. Finally, the cylindrical lens is rotated back to its initial position for illumination of the samples.

Results

Experiments of various cell cultures with membrane associated Green Fluorescent Protein [5],

with a genetically encoded redox sensor [7], or with a cytostatic drug accumulating in breast cancer cells [6] have been reported previously. A further example of 3D imaging of a multi-cellular tumor spheroid is given in figure 2. Glioblastoma cells exposed for 3h to the cytotoxic agent rotenone (1 µM) were incubated with CellTox Green Dye (1 µM, 2h), and necrotic cells were detected by LSFM. While figure 2a shows a single cell layer, figure 2b shows a 3D-reconstruction of all detection planes.

A further example is given for the chemotherapeutic agent doxorubicin at 96h after incubation (fig. 3). This drug accumulates in multi-cellular spheroids within 48h, emits red fluorescence and is cytotoxic at incubation times above 24h [8]. Concomitantly, a degradation product is formed which can be distinguished from doxorubicin by its fluorescence spectrum (spectral band around 560 nm compared to 600 nm) and lifetime (3.5 ns compared to 1.8 ns). Therefore, a combination of LSFM with Spectral Imaging and Fluorescence Lifetime Imaging (FLIM) gives additional valuable information.

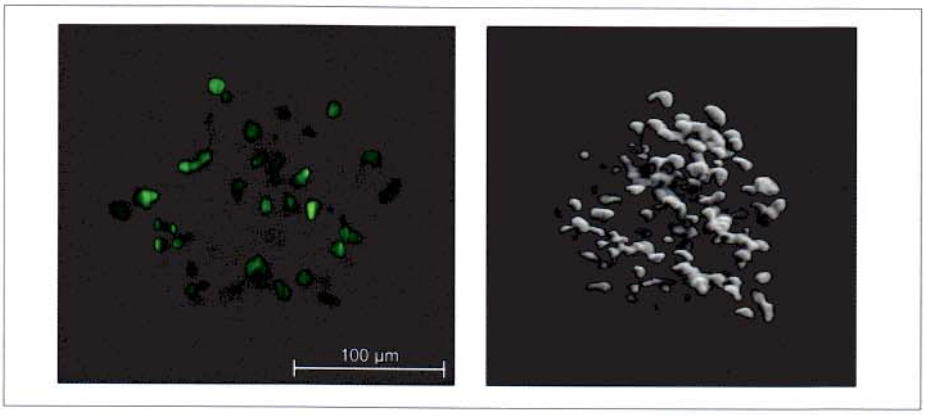


Fig. 2. CellTox Green Dye staining necrotic cells of a multi-cellular tumor spheroid after inhibition of the respiratory chain by rotenone; (a) single cell layer and (b) 3D reconstruction from 35 layers with Δz = 5 µm (excitation wavelength: 470 nm; detection range: ≥ 515 nm).

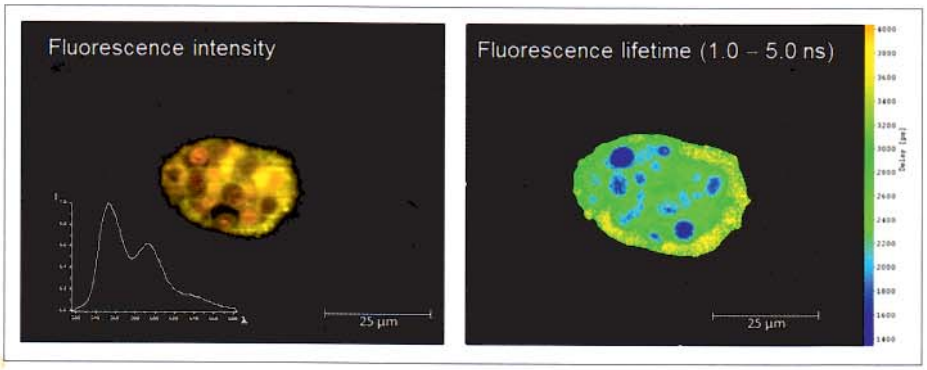


Fig. 3. Multi-cellular spheroid of breast cancer cells 96h after incubation with the cytostatic agent doxorubicin (6 µM); (a) fluorescence intensity and spectrum, (b) fluorescence lifetime in picoseconds (excitation wavelength: 470 nm; detection range: ≥ 515 nm). Reproduced from ref. 8 with modifications.

Discussion

The main purpose was to develop a miniaturized and inexpensive light sheet module which is comparably easy to handle and which can be adapted to inverted microscopes of various manufacturers. It appears ideal for imaging 3D cell cultures as well as small organisms, e.g. in developmental biology. While lateral resolution is the same as for conventional wide-field microscopes, axial resolution corresponds to the waist of the light sheet of about 6 µm and permits to select individual cell layers. For an improved axial resolution the light sheet may be focused into the back focal plane of a microscope objective lens with higher numerical aperture [9], resulting in a smaller beam waist, but also in a considerably reduced depth of focus.

Chromatic aberration is comparably small and can be reduced further, if instead of a cylindrical lens a spherical mirror with astigmatic distortion is used for focusing. If the light source is positioned outside the optical axis of this mirror, the meridional ray is defocused in the sagittal image plane, so that a light sheet of a few mm width is generated [5]. This "mirror system" has also been used in combination with a light emitting diode mounted in front of a 10 µm slit and replacing the exit field of the illuminating fiber. Therefore, a laser is not necessary, but may be advantageous for light sheet fluorescence microscopy. Use of an appropriate sample holder is an important issue for LSFM. Often rectangular micro-capillaries with a few hundred micrometers inner diameter fulfill the requirements of good optical quality and low quantities of reagents or culture media. For observing specimens from different sides a sample holder for a rotatable cylindrical micro-capillary inserted in and optically coupled to the rectangular capillary has been suggested [10]. Samples can be recorded in a static (liquid or solid) as well as in a dynamic (microfluidic) environment [6].

Conclusion

The present light sheet illumination module is a versatile and low cost alternative to extensive stand-alone light sheet fluorescence microscopes.

Acknowledgment

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Affiliation
[1]Institute of Applied Research,
Aalen University, Aalen, Germany

Contact
Prof. Dr. Herbert Schneckenburger
Institute of Applied Research
Aalen University
Aalen, Germany
herbert.schneckenburger@hs-aalen.de

More on Light Sheet Microscopy: http://bit.ly/IM-LSFM
More about 3D Imaging: http://bit.ly/IM-3D
[1] All references: http://bit.ly/IM-Schneckenburger