



## Illumination device for light sheet based fluorescence microscopy of living cells

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### SUMMARY

An illumination device for light sheet based or selective plane illumination microscopy (SPIM) is described. Cultivated cells, in particular 3-dimensional multi-cellular spheroids [1] are located in a micro-capillary, and a light sheet for illumination is generated in an optical setup adapted to a conventional inverse microscope [2]. Layers of the sample of about 10µm or less in size are thus illuminated selectively and imaged by high resolution fluorescence microscopy. SPIM is operated at low light exposure (52mW/cm<sup>2</sup>) [3], even if a larger number of layers is imaged, and is easily combined with laser scanning microscopy (LSM). While Chinese hamster ovary cell spheroids expressing a membrane associated green fluorescent protein (CHO-pAcGFP1-Mem) are used for preliminary tests, the method may be further applied in cancer research, e.g. for the detection of cellular responses to chemotherapeutic drugs or oxidative stress.

### INTRODUCTION

Imaging of 3-dimensional samples, e.g. cell or tissue cultures, requires assessment of individual focal planes. For this purpose laser scanning microscopy or structured illumination are well established methods. However, for measuring each focal plane the whole sample is exposed to light, and upon recording of numerous focal planes the light dose sums up and may damage sensitive organisms like living cells [3]. Since this problem may be overcome by single plane illumination microscopy (SPIM), a SPIM device was developed and integrated into a conventional inverse microscope.

### MATERIALS AND METHODS

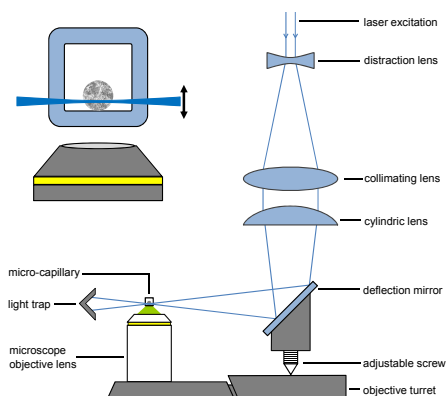


Figure 1. Illumination device for light sheet based fluorescence microscopy coupled to an inverse microscope (inlays: illumination of a spheroid within a micro-capillary).



For fluorescence microscopy micro-capillaries of borosilicate glass with rectangular shape and an inner edge length of 600-900µm are used. Culture medium or physiological buffer solution containing the cell spheroids is taken up by capillary forces and placed horizontally in a microscope, whereas no further fixation is necessary.

For illumination of the samples a collimated laser beam is expanded by a Galilei telescope, focused by a cylindrical lens and deflected onto the spheroid by a 90° mirror (Figure 1). A numerical aperture of 0.08 limits the waist of the illumination beam to about 10µm along the spheroid. By an adjustable screw the setup for beam deflection and focusing is coupled to the objective turret of the microscope. Therefore, the light sheet and the objective lens are moved simultaneously into vertical direction, and all planes of the spheroid are imaged without re-adjustment of the microscope. Fluorescence is commonly recorded by a 10x/0.30 or a 20x/0.50 microscope objective lens, a long pass filter for  $\lambda \geq 515\text{nm}$  and an integrating CCD camera with subsequent background discrimination from all images.

### RESULTS

Cell spheroids, consisting of Chinese hamster ovary cells which were permanently transfected with a plasmid encoding for a membrane associated green fluorescent protein (CHO-pAcGFP1-Mem), are grown for 5 days up to a diameter of 250-300µm. SPIM images of various layers of a cell spheroid recorded at distances of 20µm, 40µm, 60µm and 80µm from its edge are depicted in Figure 2a-d. An irradiance of 52mW/cm<sup>2</sup> and an exposure time of 1s is used for all images. Individual cells with brightly fluorescent membranes are well resolved. Due to scattering along the light paths of excitation (from top to bottom) and emission (perpendicular to the image plane) the images are becoming more diffuse with increasing light propagation. Irradiation from different sides may improve this in the future. Figure 3e shows a 3D-surface image reconstructed from a z-stack of images.

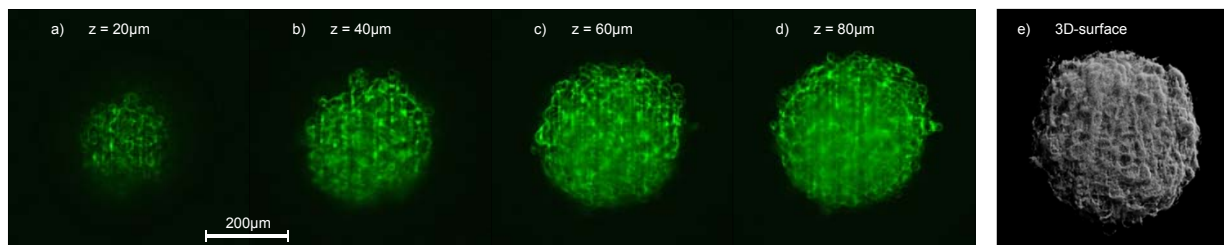


Figure 2. (a-d) SPIM images of various layers of 10µm thickness of a CHO-pAcGFP1-Mem cell spheroid recorded at distances of 20µm, 40µm, 60µm and 80µm from its edge with light incidence from top to bottom (excitation wavelength: 488nm; fluorescence detected at  $\lambda \geq 515\text{nm}$ ); (e) 3D-surface reconstruction from an image z-stack.

### SELECTED REFERENCES

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- [3] H. Schneckenburger, P. Weber, M. Wagner, S. Schickinger, V. Richter, T. Bruns, W.S.L. Strauss and R. Wittig, "Light exposure and cell viability in fluorescence microscopy," *J. Microsc.* 245, 311-318 (2012).