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PHOTON^n - PHOTONische Verfahren in neuen Dimensionen

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McSPIM - A Microfluidic Capillary Approach for Selective Plane Illumination Microscopy

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INTRODUCTION

A microfluidic system combined with an illumination device for light sheet based fluorescence measurements using a conventional inverse microscope is described, and first experimental results are shown [1]. This opens up new perspectives of kinetic measurements in fluorescence diagnosis with low amounts of drugs and short application times.

A micro-capillary - holding the sample - is combined with microfluidics, which easily permits the application of e.g. nutrients, pharmaceutical agents or fluorescent dyes without moving or affecting the probe. Spheroids of chinese hamster ovary (CHO) cells expressing a membrane-associated green fluorescent protein are used for preliminary tests, and the uptake of the fluorescent marker acridine orange via the microfluidic system is visualized to demonstrate its potential in cancer research, e.g. for the detection of cellular responses to anticancer drugs. The cell spheroids [2] are located in a micro-capillary, and a light sheet for illumination is generated in an optical setup adapted to a conventional inverse microscope. Layers of the sample of about 10µm thickness are thus illuminated selectively with low light exposure and imaged by high resolution fluorescence microscopy. Especially when applied to techniques like selective plane illumination microscopy (SPIM) this microfluidic capillary approach proves to be an essential advantage in sample preparation [1].

MATERIALS AND METHODS

For fluorescence microscopy micro-capillaries of borosilicate glass with rectangular shape and an inner cross section of 600µm x 600µm are used. The thickness of their walls is 120µm, thus similar to a conventional cover glass used in high aperture microscopy. For applications in flowing media capillaries coated with fetal calf serum (FCS) and containing cell spheroids are left for 4h in the incubator (37ºC, 5% CO_2) to assure appropriate adhesion. Cell spheroids with a diameter of 200-300µm consisting of chinese hamster ovary cells permanently transfected with a plasmid encoding for a membrane associated green fluorescent protein (CHO-pAcGFP1-Mem) are used. For visualizing the uptake of the fluorescent dye acridine orange, culture medium containing 5µM acridine orange is pumped through the micro-capillary at a temperature of 37ºC and a flow rate of 9µl/min (corresponding to a velocity of 25mm/min) using a dispension pump, a water bath, and an appropriate silicone tube with a trap for potential bubbles (Figure 1).

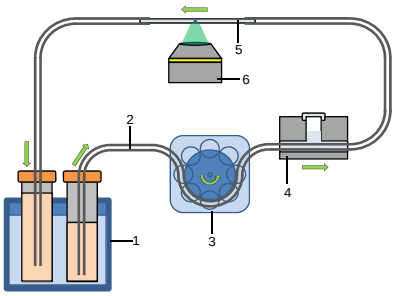

Figure 1. Microfluidic setup (1. water bath, 2. silicone tubing, 3. dispension pump, 4. bubble trap, 5. micro-capillary, 6. microscope objective lens).

For illumination of the sample under SPIM conditions collimated light of a laser diode operated at 470nm is shaped to a line sheet of 10µm waist and deflected onto the spheroid matching the focal plane of detection (Figure 2). An irradiance of 52mW/cm² is used [3]. Fluorescence is recorded by a microscope objective lens, appropriate filters and an integrating CCD camera with subsequent background discrimination from all images.
In general, fluorescence can be measured with any objective lens, even of high aperture and magnification, since the thickness of the micro-capillary is similar to a conventional cover slip (170µm) for which most microscope lenses are corrected. Therefore, moderate or even high axial resolution can be combined with high lateral resolution.

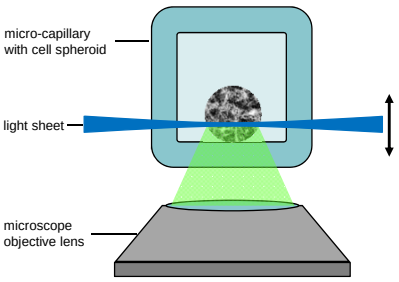

Figure 2. Illumination of a spheroid within a micro-capillary using light sheet based fluorescence microscopy coupled to an inverse microscope.

RESULTS

SPIM images of a single layer of a CHO-pAcGFP1-Mem cell spheroid recorded at a distance of 40µm from its edge are depicted in Figure 3. The images show the uptake of acridine orange over the time (pumped into the micro-capillary) by the multi-cellular spheroid, where its fluorescence is compared with the intrinsic fluorescence of the spheroid at t = 0min. The fluorescent marker – causing a pronounced increase of intensity (as depicted in Figure 4) – is localized in the outer cell layers at the beginning of incubation and penetrates deeper into the spheroid with increasing incubation time.

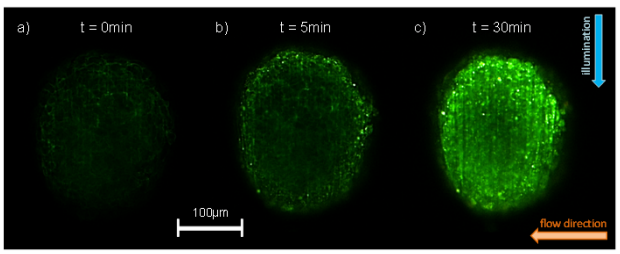

Figure 3. Uptake of acridine orange (5µM in culture medium) by a multi-cellular spheroid in a microfluidic system. Fluorescence intensity is recorded within a single layer of the spheroid at a distance of 40µm from its edge after 0min (a), 5min (b) and 30min (c) of application (flow rate: 9µl/min; flow temperature: 37°C; microscope objective lens: 10x/0.3; detection range: $\lambda \geq 515$nm).

Although a low flow rate (as used here) would be advantageous for cost-efficient drug screening, flow rates up to 1440µl/min (corresponding to velocities up to 4000mm/min) revealed to be possible under the present experimental conditions without detachment of the spheroids from the capillary. Pumped liquids can be either collected in a recipient (open system) or fed back to their source (closed loop). In the latter case a total liquid volume of 200-300µl is required, independently from pump velocity.

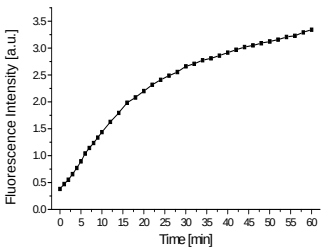

Figure 4. Time course of fluorescence intensity during uptake of acridine orange by the multi-cellular spheroid in a microfluidic system presented in Figure 3.

SELECTED REFERENCES

[1] T. Bruns, S. Schickinger, R. Wittig and H. Schneckenburger, "Preparation strategy and illumination of 3D cell cultures in light sheet based fluorescence microscopy," *J. Biomed. Opt*. 17, 101518 (2012).
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