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 in a conventional inverse microscope is described. This microfluidic capillary approach for SPIM proves to be an essential advantage in sample preparation and opens up new perspectives of kinetic measurements in fluorescence diagnosis, e.g. upon application of low amounts of drugs with highly variable application times [1].

MATERIALS AND METHODS

For fluorescence microscopy a micro-capillary - holding the sample - is combined with microfluidics, which easily permits the application of nutrients, pharmaceutical agents or fluorescent dyes without moving or affecting the probe. 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 all applications in flowing media these capillaries are coated with fetal calf serum (FCS) to assure appropriate adhesion of cell spheroids [2] with diameters of 200-300µm.
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 irradiance of 50-100mW/cm² [3] and imaged by high resolution fluorescence microscopy (Figure 2). Especially when combined with selective plane illumination microscopy (SPIM) this microfluidic capillary approach proves to be an essential advantage in sample preparation [1].

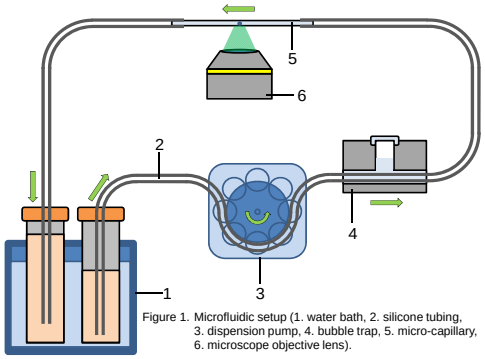

Figure 1. Microfluidic setup (1. water bath, 2. silicone tubing, 3. dispension pump, 4. bubble trap, 5. micro-capillary, 6. microscope objective lens).

Culture medium containing the agent or fluorescent dye is pumped through the micro-capillary at a temperature of 37 C 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). 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.

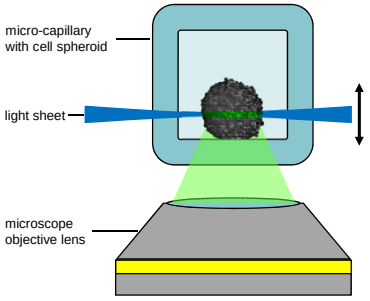

Figure 2. Illumination of a spheroid within a micro-capillary using light sheet based fluorescence microscopy coupled to an inverse microscope.

SELECTED APPLICATIONS

Spheroids incubated with fluorescent dye

Spheroids of chinese hamster ovary cells expressing a membrane-associated green fluorescent protein (CHO-pAcGFP1-Mem) are used, and the uptake of the fluorescent marker acridine orange (5µM in culture medium) via the microfluidic system is visualized.
Acridine orange is localized in the outer cell layers at the beginning of incubation and penetrates deeper into the spheroid with increasing incubation time (Figure 3).

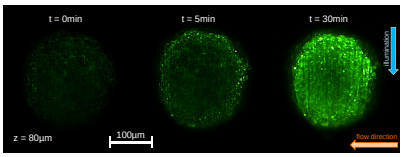

Figure 3. SPIM images of a single layer of the cell spheroid under flow.

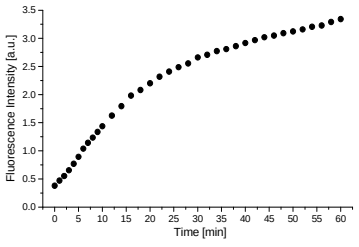

Figure 4. Time course of cellular uptake of acridine orange.

Oxidation of redox sensitive spheroids

SPIM measurements of intracellular redox states reveal information on the presence of reactive oxygen species, e.g. in tumors.
Spheroids of U251MG-L106 glioblastoma cells permanently transfected with the glutathione sensitive green fluorescent redox sensor Grx1-roGFP2 were used for measurements of the intracellular redox state. Oxidation of glutathione was induced by exposure to 50µM hydrogen peroxide (H_2O_2) in salt solution (EBSS) via the microfluidic system and predominantly found in the outer cell layer of the spheroid.
The uptake of H_2O_2 results in a decrease of fluorescence intensity excited at 470nm. The time course is depicted in Figure 5. This demonstrates the possibility of fast drug application combined with rapid detection of the presented McSPIM-system.

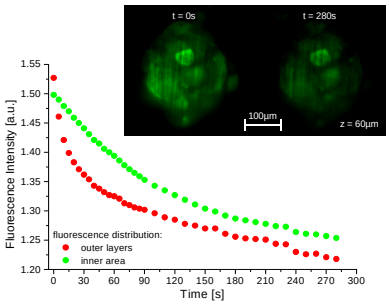

Figure 5. Time course and SPIM images of fluorescence decrease after H_2O_2 incubation (single layer of the cell spheroid under flow).

Spheroids incubated with chemotherpeutic drug

This microfluidic SPIM approach is used to examine uptake and intracellular localization of the chemotherpeutic drug doxorubicin in native MCF-7 human breast cancer cell spheroids. Within the first 400min of doxorubicin incubation (2µM in culture medium) under flow one can observe an increase of red and green fluorescence (images and spectrum depicted in Figure 6).

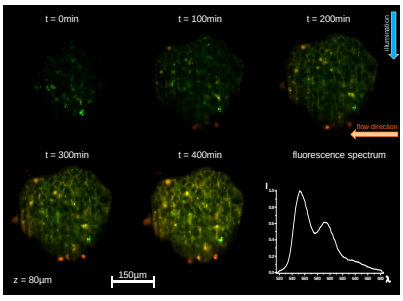

Figure 6. Time course of cellular uptake of doxorubicin via the microfluidic system. SPIM images of a single layer of a MCF-7 cell spheroid recorded at a distance of 80µm from its edge.

Within the outer cell layer doxorubicin is mainly localized in the nucleus (red fluorescence). For inner cell layers green doxorubicin fluorescence is dominant and localized in the cellular membrane.

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).
[2] H. Schneckenburger, M. Wagner, P. Weber, T. Bruns, V. Richter, W.S.L. Strauss and R. Wittig, "Multi-dimensional fluorescence microscopy of living cells," *J. Biophotonics* 4(3), 143-149 (2011).
[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).

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