A LIGHT SHEET MODULE FOR 3D FLUORESCENCE MICROSCOPY OF TUMOUR CELL SPHEROIDS

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ABSTRACT
Light dose is an important parameter for maintaining cell viability in 3D microscopy, in particular, if long observation times are required. For this reason, light sheet based or selective plane illumination microscopy (SPIM) seems to be an ideal tool, since only selected parts of a cell or tissue sample (e.g. those in the focal plane of the microscope) are illuminated. However, present light sheet microscopes (e.g. SPIM microscope, Carl Zeiss Jena; Germany) revealed to be complex instruments of limited flexibility requiring special sample preparation and comparably large sample chambers.

Therefore, we describe a miniaturized (and low cost) alternative which is easily adapted to a conventional inverse microscope, and which can be combined with further techniques, e.g. epi-illumination or laser scanning microscopy. For SPIM measurements a laser beam is imaged to a light sheet of about 8 mm diameter and 10 µm waist which is optimized for a single layer of a multi-cellular spheroid (smaller beam waists can be generated, but are presently not needed). The beam waist and the microscope objective lens can be shifted simultaneously in vertical direction, so that all illuminated parts of the sample are automatically in the focus of the microscope. Illumination from different sides and sample rotation are additional options. A main feature of this setup is that cell spheroids are located in rectangular micro-capillaries of 600−900 µm diameter and surrounded by stationary or flowing liquid media. By coupling to a microfluidic system small quantities of agents (e.g. fluorescent dyes, pharmaceutical agents) can thus be applied easily.

At least two different laser sources can be used simultaneously, e.g. for energy transfer (FRET) or ratio microscopy. An innovative device includes two different pulsed laser diodes with a nanosecond time delay between one another as well as prompt and delayed (by a fibre bundle) detection of fluorescence images on two parts of a time-gated camera. This permits quasi continuous registration of two fluorescence images in a “shake hand” mode and online measurement of ratio images.

Present applications include:
- measurements of the uptake of fluorescent dyes which are specific for certain organelles;
- ratio imaging of an intrinsic redox sensor for detection of oxidative stress;
- measurement of the uptake and cellular interaction of cytostatic drugs as a function of various metabolites (e.g. cholesterol).

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