3D FLUORESCENCE MICROSCOPY OF LIVING CELLS AT LOW LIGHT EXPOSURE

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ABSTRACT

Modern methods of 3D fluorescence often provide excellent resolution, but require very high light exposure exceeding non-phototoxic light doses [1] considerably. This holds in particular for non-linear methods, e.g. stimulated emission depletion microscopy (STED), or methods based on single molecule detection. Although light exposure is generally lower in conventional laser scanning or wide-field microscopy (using e.g. structured illumination), each focal plane examined in 3D microscopy requires illumination of the whole sample. Therefore, upon recording of several planes the light dose sums up and may damage cells and organisms. This problem is overcome by single plane illumination microscopy (SPIM) where a light sheet is created perpendicular to the observation path, so that only the plane under investigation is exposed to light. An illumination device for SPIM has presently been developed and adapted to a conventional inverse microscope with 3-dimensional samples, e.g. multi-cellular spheroids, located in a micro-capillary. Variable layers of about 10 µm or less in diameter are thus selected and imaged by high resolution microscopy. While Chinese hamster ovary (CHO) cells expressing a membrane-associated green fluorescent protein are used for preliminary tests, the method may be further developed for applications in cancer research, e.g. for the detection of responses to anticancer drugs in 3D cell models.

In addition, at a non-phototoxic light dose of 1–2 J/cm², 3D topology of cells growing on a substrate is determined with nanometre precision by Variable-Angle Total Internal Reflection Fluorescence Microscopy (VA-TIRFM). Cells cultivated on transparent slides and incubated with a fluorescent membrane marker are illuminated under various TIR angles, while cell-substrate distances are calculated from about 10 fluorescence images. Differences of cell-substrate interactions between U251-MG glioblastoma cells (a: homogeneous tight adhesion) and less malignant derivatives (b: multifocal contacts) are depicted in Figure 1.

Figure 1. Cell-substrate distances in the range of 0–500 nm using the fluorescence marker laurdan (excitation wavelength: 391 nm; image size: 140 µm × 140 µm).

REFERENCE