LIGHT SHEET-BASED FLUORESCENCE MICROSCOPY (LSFM) REDUCES PHOTOTOXIC EFFECTS AND PROVIDES NEW MEANS FOR THE MODERN LIFE SCIENCES

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Most optical technologies are applied to flat, basically two-dimensional cellular systems. However, physiological meaningful information relies on the morphology, the mechanical properties and the biochemistry of a cell’s context (Pampaloni 2007). A cell requires the complex three-dimensional relationship to other cells (Pampaloni 2010). However, the observation of multi-cellular biological specimens remains a challenge. Specimens scatter and absorb light, thus, the delivery of the probing light and the collection of the signal light become inefficient; many endogenous biochemical compounds also absorb light and suffer degradation of some sort (photo-toxicity), which induces malfunction of a specimen. In conventional and confocal fluorescence microscopy, whenever a single plane is observed, the entire specimen is illuminated (Verveer 2007). Recording stacks of images along the optical z-axis thus illuminates the entire specimen once for each plane. Hence, cells are illuminated 10-20 and fish embryos 100-300 times more often than they are observed (Keller 2008). This can be avoided by changing the optical arrangement. The basic idea is to use light sheets, which are fed into the specimen from the side and overlap with the focal plane of a wide-field fluorescence microscope. In contrast to an epifluorescence arrangement, such an azimuthal fluorescence arrangement uses two independently operated lenses for illumination and detection (Stelzer 1994; Huisken 2004). Optical sectioning and no photo-toxic damage or photo-bleaching outside a small volume close to the focal plane are intrinsic properties. Light sheet-based fluorescence microscopy (LSFM) takes advantage of modern camera technologies. LSFM can be operated with laser cutters (e.g. Colombelli 2009) and for fluorescence correlation spectroscopy (FCS, Wohland 2010). During the last few years, LSFM was used to record zebrafish development from the early 32-cell stage until late neurulation with sub-cellular resolution and short sampling periods (60-90 sec/stack). The recording speed was five four Megapixel large frames/sec with a dynamic range of 12-14 bit. We followed cell movements during gastrulation, revealed the development during cell migration processes and showed that an LSFM exposes an embryo to 200 times less energy than a conventional and 5,000 times less energy than a confocal fluorescence microscope (Keller 2008). Most recently, we implemented incoherent structured illumination in our DSLM (Keller 2010). The intensity modulated light sheets can be generated with dynamic frequencies and allow us to estimate the effect of the specimen on the image formation process at various depths in objects of different age.

Pampaloni F, Stelzer EHK, Leicht S, Marcello M (2010) MDCK cells are increased in aerobic glycolysis when cultured on flat and stiff collagen-coated surfaces rather than in physiological three-dimensional cultures, Proteomics, advanced online publication 26 July 2010, DOI: 10.1002/pmic.201000236.