LIGHT SHEET BASED FLUORESCENCE MICROSCOPES (LSFM, SPIM, DSLM) REDUCE PHOTOTOXIC EFFECTS BY SEVERAL ORDERS OF MAGNITUDE

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Most optical technologies (microscopy, optical tweezers [1], laser nanoscalpel [2]) are applied to two-dimensional cellular systems, i.e. they are used in a cellular context that is defined by hard and flat surfaces. However, physiological meaningful information relies on the morphology, the mechanical properties, the media flux and the biochemistry of a cell's context found in live tissue [3, 4]. A physiological context is certainly not found in single cells cultivated on cover slips. It requires the complex three-dimensional relationship of cells cultivated e.g. in an ECM-based gel or in naturally developing small embryos of flies or embryos and, of course, in tissue sections [4]. However, the observation as well as the optical manipulation of extended biological specimens suffers from at least two severe problems. 1) The specimens are optically dense, i.e. they scatter and absorb light. Thus, the delivery of the probing light and the collection of the signal light tend to become inefficient. 2) Many biochemical compounds apart from fluorophores also absorb light and suffer degradation of some sort (photo-toxicity), which induces malfunction or death of a specimen [4]. The situation is particularly dramatic in conventional and confocal fluorescence microscopy. Even though only a single plane is observed, the entire specimen is illuminated. 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 even 100-300 times more often than they are observed. Surprisingly, this can be avoided by a simple change of the optical arrangement. The basic idea is to use light sheets, which are fed into the specimen from the side and which overlap with the focal plane of a wide-field fluorescence microscope [5]. Thus, in contrast to an epi-fluorescence arrangement, which uses the same lens, our azimuthal arrangement uses two independently operated lenses for illumination and detection. Optical sectioning and no photo-toxic damage outside a small volume around the focal plane are its intrinsic properties. Light sheet based fluorescence microscopes (LSFM) take advantage of modern camera technologies, which provide them with a signal to noise ratio that is at least thirty times better than that of a confocal microscope [6]. LSFM can be combined with essentially every contrast and specimen manipulation tool to operate in a truly three-dimensional fashion. In a current application, they are used to record early zebrafish (*Danio rerio*) development *in vivo* and *in toto* from the early 32-cell stage until late neurulation with sub-cellular resolution and very short sampling periods (60-90 sec/stack) [7]. The recording speed is more than 30 Million voxels/sec or more than five very large frames/sec with a dynamic range of 12-14 bit. We follow the cell movements during gastrulation and reveal its development during the cell migration processes. We can show that an LSFM exposes an embryo to 200 times less energy than a conventional, 5,000-6,000 times less than a confocal and about one million times less than a two-photon fluorescence microscope. Based on this outstanding performance, we claim that our novel, truly three-dimensional approach will have a dramatic impact on developmental and cell biology as well as on biophysics [8].

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