

Light Sheet based Fluorescence Microscopy reduces phototoxic effects while spatial intensity modulation estimates the optical scattering properties of a biological specimen

Ernst H. K. Stelzer, Philipp J. Keller
EMBL-Heidelberg, Meyerhofstrasse 1, D-69117 Heidelberg, Germany
stelzer@embl.de

Three-dimensional, fluorescence, light sheet, structured illumination, developmental biology, cell biology, LSFM, SPIM, DSLM.

Most optical technologies are applied to two-dimensional cellular systems. However, physiological meaningful information relies on the morphology, the mechanical properties and the biochemistry of a cell's context. One requires the complex three-dimensional relationship of cells. However, the observation of multi-cellular biological specimens remains a challenge. 1) The specimens scatter and absorb light, thus, the delivery of the probing light and the collection of the signal light become inefficient. 2) Many biochemical compounds apart from fluorophores also absorb light and suffer degradation of some sort (photo-toxicity), which induces malfunction of a specimen. 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 100-300 times more often than they are observed. 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 which overlap with the focal plane of a wide-field fluorescence microscope. In contrast to an epi-fluorescence arrangement, an azimuthal fluorescence arrangement uses two independently operated lenses for illumination and detection. 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 was used to record early 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 the cell movements during gastrulation, revealed its development during cell migration processes and showed that an LSFM exposes an embryo to 200 times less energy than a conventional and 5,000-6,000 times less than a confocal fluorescence microscope. Most recently, we implemented structured illumination in our DSLM. The intensity modulated light sheets can be generated with various frequencies and estimates the effect of the specimen on the image formation process at various depths in the object.