# Shifting the paradigm in modern light microscopy: Light Sheet-based Fluorescence Microscopy (LSFM, SPIM, DSLM)

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Specimens scatter and absorb light. Thus on one hand, the delivery of the probing light and the collection of the signal light (e.g. fluorescence) become inefficient, when relevant almost mm-thick specimens are investigated. On the other hand, not only fluorophores, but many endogenous biochemical compounds absorb light and suffer degradation of some sort (photo-toxicity), which can induce a 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 using 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 epi-fluorescence arrangement, an azimuthal arrangement uses at least two independently operated lenses for illumination and detection (Stelzer 1994; Huisken 2004). A SPIM employs a cylindrical lens to generate a light sheet. A collimated laser beam is focused into the plane of the detection lens along one direction while the other direction remains collimated (Engelbrecht & Stelzer, 2006; Greger et al., 2007). Although this approach is relatively simple and straightforward it suffers from the low quality of the cylindrical lens and the inefficiency of the illumination system (Breuninger et al., 2007). The major advantage of a digital scanned laser light sheet-based fluorescence microscope (DSLM; Keller et al., 2008) is that it relies entirely on cylindrically symmetric optics and hence provides a very good optical quality. In addition, a DSLM employs a minimal number of optical components and does not suffer from excessive wavefront aberrations. In general, optical sectioning and no photo-toxic damage or photo-bleaching outside a small volume close to the focal plane are intrinsic properties of light sheet-based fluorescence microscopy (LSFM). It takes advantage of modern camera technologies and can be operated with laser cutters (e.g. Colombelli 2009) as well as in fluorescence correlation spectroscopy (FCS, e.g. Wohland 2010). We have also successfully evaluated the application of structured illumination in a LSFM (SPIM, Breuninger et al., 2007; DSLM, Keller 2010). We also designed and implemented a wide-field frequency domain fluorescence lifetime imaging (FLIM/FRET, Greger 2011) setup. More recently, we applied LSFM for investigations in plant biology (Maizel 2011), three-dimensional cell biology as well as emerging model organisms and developed new scanning schemes based on novel optical arrangements that allow us to take full advantage of very high resolution light microscopy. The development of LSFM draws on many previous developments. In particular, confocal theta fluorescence microscopy played a very important role. About a dozen papers on theta microscopy describe its properties, its relationship to 4Pi microscopy and that of LSFM (single & two-photon, annular/Bessel beams, (a)symmetric arrangements) theoretically as well as practically.

1. http://www.researcherid.com/rid/A-7648-2011

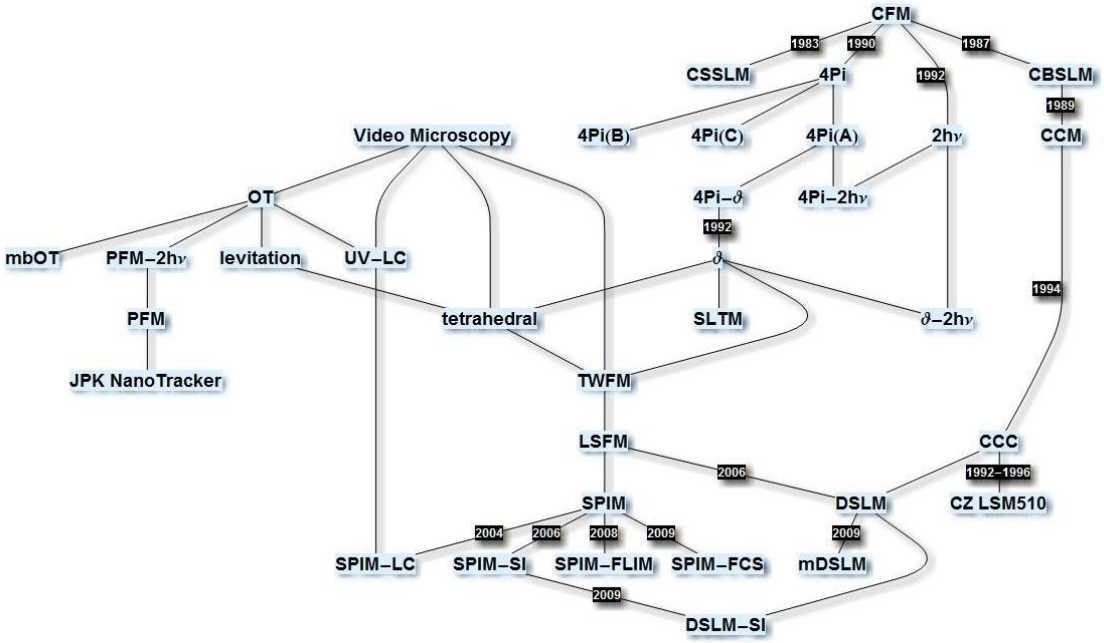
CFM
1983
1990
1987
CSSLM
4Pi
1992
CBSLM
1989
Video Microscopy
4Pi(B)
4Pi(C)
4Pi(A)
2hν
CCM
OT
4Pi–ϑ
4Pi–2hν
1992
mbOT
PFM–2hν
levitation
UV–LC
ϑ
1994
PFM
tetrahedral
SLTM
ϑ–2hν
JPK NanoTracker
TWFM
LSFM
CCC
2006
1992–1996
SPIM
DSLM
CZ LSM510
2004
2006
2008
2009
2009
SPIM–LC
SPIM–SI
SPIM–FLIM
SPIM–FCS
mDSLM
2009
DSLM–SI

**Figure 1.**