LIGHT SHEET–BASED FLUORESCENCE MICROSCOPY FOR QUANTITATIVE BIOLOGY

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KEY WORDS: SPIM, DSLM, LSFM, quantitative analysis, developmental biology, plant biology, Tribolium castaneum, Arabidopsis thaliana.

As long as we rely on epi-fluorescence microscopes, we are faced with serious challenges. Fluorophores and specimens are essentially wasted during the observation process, since all fluorophores and many endogenous organic compounds in the specimen are excited whenever we record a single plane. The situation becomes quite challenging when we perform complex biological experiments and observe the behavior of multiple targets in three dimensions as a function of time. Light sheet–based fluorescence microscopes (LSFM) rely on two main optical paths. The detection path consists of the detection microscope objective lens, a spectral filter, which discriminates the excitation light, a tube lens and a camera. The excitation path is perpendicular to the detection path. It directs a light sheet into the side of the specimen. The light sheet and the focal plane of the detection microscope objective overlap. Thus, only the fluorophores close to the focal plane of the detection lens are excited, whereas all fluorophores outside the focal plane receive no excitation light. This has enormous consequences. Fluorophores outside the thin volume close to the focal plane do not contribute out-of-focus light, which would blur the image and they are not photo bleached. For the same reason, potentially absorbing endogenous organic molecules are not degraded and specimens are less affected by photo toxicity. Since biological specimens survive long-term three-dimensional imaging at high spatio-temporal resolution, LSFM has become the tool of choice in plant and developmental biology. Light sheets have been known for more than 100 years, but so have light spots. Until lasers became available in the early 1960s, neither light spots nor light sheets were diffraction limited. Neither a confocal fluorescence microscope, which is based on the sequential illumination of the specimen by a diffraction-limited spot of light, nor a LSFM can be operated without a laser. It is essential for true optical sectioning. Laser light sheet-based devices, including a macroscope, had been built several times, but their capability to perform at a microscopic level was unknown until we described a diffraction-limited microscope, observed living biological samples and evaluated multiple-view imaging. LSFM developed from theta microscopy and a systematic evaluation of diffraction-limited microscopes with two to four lenses, in theory and practice. The properties of LSFM are illustrated with applications in the life sciences.