Deep and Fast Imaging of Thick 3D Samples by means of Light Sheet Fluorescence Microscopy.

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The advent of fluorescence microscopy opened a new window in the direct investigation of biological processes that animate life. The key advantage of fluorescence microscopy is the ability to follow biological processes in their native state, with minimal perturbation and high specific molecular contrast. Within this framework, widefield epifluorescence and confocal microscopy have become a golden standard and nowadays play a key role in elucidating structure and function of different biological systems. However, when deeper and fast imaging of large samples is required (sample thickness > 150 µm), their limitations became clear. These techniques are, indeed, inherently limited by a tradeoff between spatial resolution, temporal resolution, and phototoxicity that prevent their use for fast imaging of thick samples.
In the last decade, Light Sheet Fluorescence Microscopy (LSFM) has emerged as a valuable alternative for the imaging of large specimens at high speed, with improved optical penetration, enhanced image contrast and reduced photo bleaching and phototoxic effects [1]. The core concept of LSFM lies in selective illumination of the sample by means of a thin sheet of light placed at the focal plane of a perpendicularly-oriented widefield detection system. In this optical configuration, fluorescence is generated only in a thin region of the sample that can be collected by a fast multi-array detector (CCD or sCMOS).
This simple but powerful combination of capabilities makes LSFM a useful imaging tool in many fields of life science, from developmental biology to in vivo 3D imaging of complex biological systems at high spatio-temporal resolution [2,3].

[1] E.H. Stelzer, “Light-sheet fluorescence microscopy for quantitative biology.” Nat Methods. 12(1):23-6; 2014
[2]J. Huisken, et al., " Optical sectioning Deep Inside Live Embryos by Selective Plane Illumination Microscopy," Science 305, 1007; 2004
[3] P.J., Keller, et al., Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. Science, 322(5904): p. 1065-9; 2008.