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Digital Scanned Laser Light Sheet Microscopy (DSLM)

The idea behind DSLM is to generate a "plane of light" with a laser scanner that rapidly moves a μm -thin beam of laser light vertically and horizontally through the specimen. The fluorophores in the illuminated plane emit fluorescence light, which is subsequently detected by a camera-based conventional widefield arrangement. The detection axis is oriented perpendicular to the illumination axis, such that the illuminated plane coincides with the in-focus plane of the detection system (figure 1).

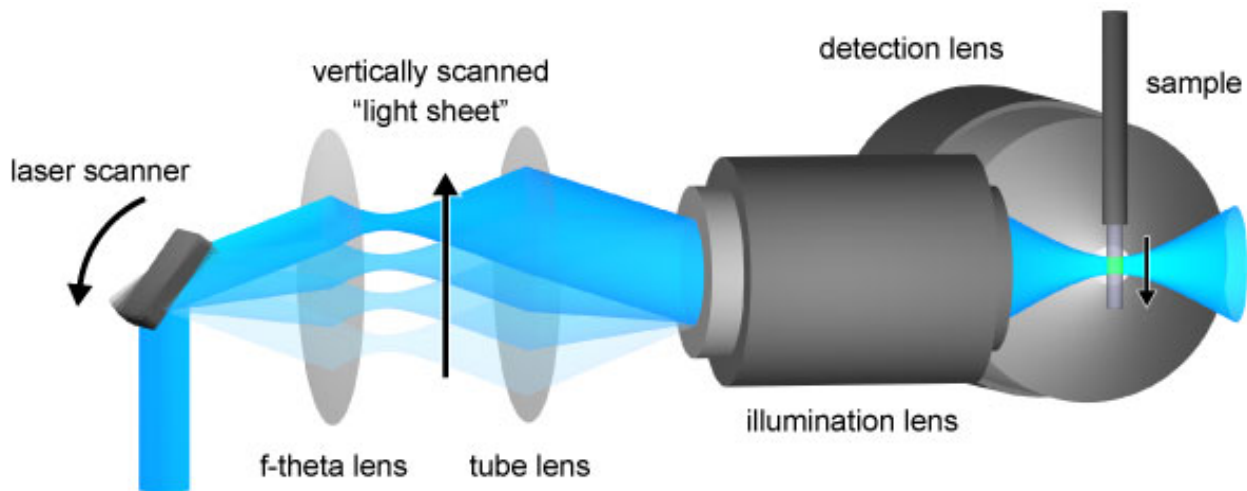


Figure 1: Digital Scanned Light Sheet Microscopy

DSLM does not only provide the usual advantages of light sheet-based microscopy, i.e. optical sectioning, low photo-bleaching and low photo-toxicity as well as a high signal-to-noise ratio due to the concept of light detection by CCD cameras, but also several new key features introduced by the laser scanning approach: DSLM allows performing three-dimensional high-speed imaging without moving the observed specimen itself, since the entire scanned light sheet can be displaced along the detection axis within fractions of a millisecond using the laser scanner. Thus, exceptionally high imaging speeds can be achieved, which are ultimately only limited by the frame rate of the camera. DSLM furthermore provides an excellent image quality and an illumination efficiency close to 100%, since no beam-shaping apertures are required to define the geometrical properties of the illuminating light sheet. Finally, DSLM presents a very powerful approach to structured illumination. The laser intensity can be easily modulated while scanning the specimen, thereby generating structured illumination profiles with digitally adjustable properties, which allow enhancing the imaging contrast deep inside highly light-scattering specimens.

For more details on DSLM and its application in vertebrate imaging please see Keller et al. 2008b.