LIGHT-SHEET MICROSCOPY IN SCATTERING MEDIA: HOW TO SELECT THE BEST EXCITATION PHOTONS AND FLUORESCENCE PHOTONS

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Image quality in light-sheet fluorescence microscopy is strongly affected by the shape of the illuminating laser beam inside embryos, plants or tissue [1]. We introduced Bessel beams into light-sheet microscopy [2] to maximize propagation stability and penetration depth. We introduced the concept of confocal-line detection [3] to block the fluorescence from the Bessel ring system to maximize image resolution and contrast even in the back parts of scattering media. We introduced the concept of sectioned Bessel beams, which excite minimal fluorescence out of the focal plane at maximal propagation stability [4]. Using a switchable amplitude mask, we showed how to adapt the length and position of Bessel beams to the local extent of the object, thereby increasing contrast and reducing fluorophore bleaching [5].
However, the propagation of fluorescence light to the detector is more difficult to control. With each scatter process, a fluorescence photon loses information necessary for the image generation. We showed that ballistic and diffusive fluorescence photons can be separated by analyzing the image spectra in each plane without a priori knowledge [6]. A theoretical model allows to extract typical scattering parameters of the biological material. From that we attenuated image contributions from diffusive photons and amplified the relevant image contributions from ballistic photons through a depth dependent deconvolution. In this talk I will show how image contrast and resolution are again significantly increased and scattering artefacts are minimized especially for Bessel beams with confocal-line detection.

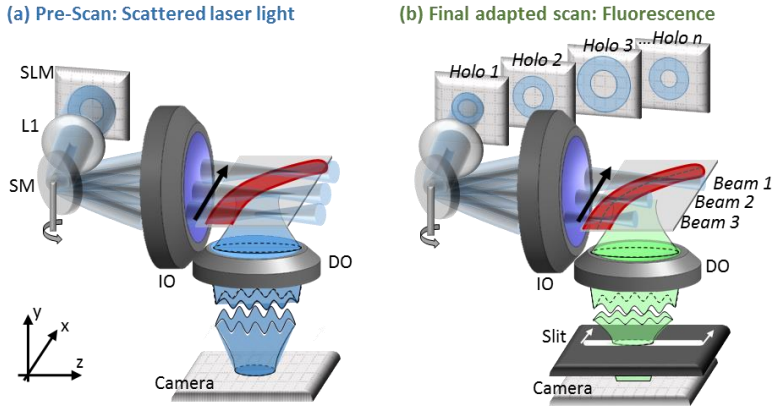

Fig. 1 Setup sketch for a light-sheet microscope using length-adapted Bessel beams.

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