SEPARATION OF BALLISTIC AND DIFFUSIVE FLUORESCENCE PHOTONS IN CONFOCAL LIGHT-SHEET MICROSCOPY

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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]. While the phase of Gaussian or Bessel beams propagating through thousands of cells can be partly controlled holographically [2][3], the propagation of fluorescence light to the detector is difficult to control. With each scatter process a fluorescence photon loses information necessary for the image generation. Using Arabidopsis root tips we demonstrate that ballistic and diffusive fluorescence photons can be separated by analyzing the image spectra in each plane without a priori knowledge [4]. We introduce a theoretical model allowing to extract typical scattering parameters of the biological material. This allows to attenuate image contributions from diffusive photons and to amplify the relevant image contributions from ballistic photons through a depth dependent deconvolution. In consequence, image contrast and resolution are significantly increased and scattering artefacts are minimized especially for Bessel beams with confocal line detection.

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