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Development of a highly efficient Bessel beam light sheet microscope

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Lightsheet fluorescence microscopy (LSFM) is currently one of the most efficient types of optical microscopy exposing the sample to a minimal photon dose. In LSFM, the sample is illuminated by an extremely thin "sheet" of light along the focal plane from which fluorescence is detected, such that only those areas of the sample from which fluorescence is detected are exposed. In the majority of cases, thus sheet of light is created by a cylindrical lens, leading to an extended focal region along the axis parallel to the cylindrical axis and an hour glass shaped focal spot perpendicular to this axis. In order to minimize the thickness of the region from where fluorescence is detected the cylindrical focus is often scanned through the sample, which reduces the overall efficiency of this method. An alternative scheme is Bessel beam microscopy. Here, an extended focus of a pencil lead like shape is created by focusing a thin ring of laser light into the back focal plane of a microscope objective lens. This leads to extended foci as small as 300 nm diameter with up to several 100 µm length, where the central focus is surrounded by concentric rings with a much lower peak intensity. To further minimize contributions from the out-of-focus concentric rings, multi-photon fluorescence excitation is often used. Scanning this pencil lead shaped beam across the sample leads to the currently most homogeneous fluorescence excitation across the detection plane. We have developed and constructed a highly efficient Bessel beam light sheet microscope. The Bessel beam is created by a custom-made double-sided conical lens (an axicon). We will present the otical system layout and demonstrate its performance with recent volumetric image data of biological samples collected with this system. A short outlook to future applications will also be provided.

Category

Other

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