ABSTRACT
Multi-photon fluorescence microscopy (MPM) is a well-established method for high resolution, non-invasive investigations of biological tissue. The low probability of the two-photon excitation process ensures that the detected signal is only generated within the focal volume where the photon flux is high [1]; however, out-of-focus fluorescence has been demonstrated to be a limiting factor. We here explore annular beam shaping for MPM. Annular beams are expected to reduce background fluorescence, preferential for deep tissue imaging. Annular beams will create Bessel beams which will carry special characteristics, e.g. elongated focal volume [2]. In order to correctly implement annular beam shaping in MPM it is necessary to have full control on the shape and size of the focal volume, i.e. the point spread function (PSF).

In this study we investigate how the outer/inner diameter ratio of the beam affects the laser distribution. Simulations are performed by implementing the Fresnel–Kirchhoff diffraction integral in MATLAB. The simulations demonstrate that the focal area resembles that of an ordinary Gaussian beam for an inner radius of up to 40-50% of the full radius, when using an objective lens in the NA-range of 0.8 to 1, thus preserving axial resolution while lowering the out-of-focus signal (See Figure 1). When the inner radius of the beam exceeds this value, the focal volume becomes axially elongated. Measurements are presently undertaken on a custom-built TPM set-up to confirm the data experimentally. The study implies that annular beam-shaping using a medium inner radius can be applied for conventional TPM, particularly for deep tissue imaging.