

Fluorescent proteins (FPs) are the workhorses of biological molecular imaging. Important imaging modalities (such as polarization microscopy or FRET imaging) exploit anisotropic optical properties of fluorescent proteins. In this thesis, we present the results of our polarization microscopy and X-ray diffraction experiments on FP crystals, as well as mathematical interpretation of these results, yielding information on the directionality of one- and two-photon absorption within the investigated fluorescent protein molecules. For the anisotropy of one-photon absorption, we determine the transition dipole moment (TDM) orientations in three representative fluorescent proteins. Validation with available quantum mechanical predictions values and an experimentally determined TDM orientation of the GFP gives confidence to the results obtained. For the two-photon absorption, we first test our hypothesis that two-photon absorptivity tensors of representative FPs exhibit vector-like behaviour and then examine the applicability of this simplification as a basis for the interpretation of our two-photon polarization microscopy data.