

Fluorescence microscopy is an essential technique for live cell imaging. One of its drawbacks is a rather low diffraction limited spatial resolution, which is described by Abbe diffraction law. Therefore, in the last decade a lot of new methods improving spatial resolution were developed. One of them is dynamic saturation optical microscopy (DSOM) that is based on spatial monitoring of reversible transition kinetics between bright and dark states of fluorophores. The dark state is possible to obtain for example by using reversibly photoswitchable fluorescent proteins such as Dronpa and its variants. These proteins undergo reversible transition from fluorescent to nonfluorescent state after irradiation by blue and ultraviolet light. In my work I focus on employing the kinetics of controllable photoswitching of Dronpa in improving the overall image quality, including the spatial resolution. The experiments were performed on yeasts expressing selected proteins labelled with Dronpa. Firstly, photoswitching behaviour of Dronpa was confirmed. Secondly, experimental conditions were optimized by studying dependence of switching rate on laser intensities and on excitation wavelength and by studying protein photostability. Experiments were performed on different timescales and for various proteins. Using the optimal experimental conditions a DSOM image with two times better spatial resolution in focal plane than classical scanning confocal microscope was obtained. Moreover, background in form of out-of-focal plane fluorescence signal was suppressed as well as autofluorescence, leading to sharper images.