Circumventing the limit imposed by diffraction is a major issue in the instrumental development to realize finer resolutions in biological samples. With STED microscopy [1], we exploit the molecular transitions of the fluorescent marker to image well below the Rayleigh criterion. Despite the major resolution improvement demonstrated, the technique isn't easily accessible to a wider community of biologists, mainly due to its apparent complexity, cost and limited tunability of the existing commercial systems. We have implemented a tunable, compact and easy to use STED microscope around a commercially available confocal microscope. The employment of a pulsed supercontinuum source (SC-450 PP-HE, Fianium) offers high tunability in terms of choice of fluorescent probes that can be used while enhancing the simplicity for the end user. The depletion beams’ intensity profiles are shaped with phase plates for the improvement of the lateral and axial resolution [2]. These beams are coupled to a commercially available confocal microscope (Nikon Ti-E) through a customized scan head that allows us to follow the dynamics of living cells. Thus intensity images with resolution below the diffraction limit can be obtained as easily as in a classic confocal mode.

We will present the current state of development of our setup and discuss its future coupling with complementary functionality. Pulsed excitation in combination with photon counting cards readily enables time resolved fluorescence studies as well as co-localization using fluorescence lifetime as the discriminating parameter [3]. Since this system is primarily dedicated to investigation of the fundamental molecular phenomena involved in Alzheimer’s disease, imaging neurons and its membrane activity is of major interest. In this regard, we will describe the different ways to specifically follow membrane activity while preserving lateral resolution.

REFERENCES