The stimulated emission depletion microscopy (STED) is a technique that, overcoming the diffraction barrier, allows distinguishing details of cellular structures and even molecular structures, never been visible before[1,2]. In a typical STED microscope, a laser beam inducing stimulated emission is superimposed with an excitation beam. Such a STED beam typically has a doughnut-shaped focal pattern featuring a zero-intensity point. When the intensity of the depletion beam at the doughnut crest saturates the stimulated emission transition, the spatial extent of the effective fluorescence signal is confined to subdiffraction dimensions. Scanning the co-aligned beams through the sample, or viceversa, yields images whose resolution is tuned by the intensity of the STED beam.

The wide use of STED microscopy has been encouraged by the introduction of a simplified and more economic STED microscope implementation that relies, for instance, on supercontinuum laser sources[3]. This solution is particularly attractive since it uses the same laser source for both excitation and STED beam, respectively. Moreover, it can be easily extended to multi-color and three-dimensional imaging with improved spatial resolution. Nevertheless the implementation of such a solution requires several experimental stratagems in order to achieve the maximum resolution. In particular, excitation and STED beams still require alignment in all spatial directions as well as in temporal dimension. Moreover a good quality of the “zero”-intensity point of the doughnut-shaped focal STED is mandatory. For this reason, we focused our attention on the following two key points to maximize the performances of our pulsed STED microscope: the temporal alignment between the excitation and the STED pulses and the polarization state of the STED beam. Hereby we present the results of such an optimization on a custom made STED microscope based on a supercontinuum laser source, showing the effects by imaging calibration fluorescent beads and biological samples. We also applied the recently developed technique g-STED[4] to highlight the advantages given by time gating detection for temporal alignment.