A 60-bp double-stranded DNA, custom manufactured by IBA, was labeled with ATTO647N fluorescent dye by classical NHS-ester chemistry. This labeled oligonucleotide was immobilized on a streptavidin-coated cover slide by a BSA-Biotin-linker, so that the fluorescent dye is bound by a DNA-“tether” of about 20 nm (Fig. 1). Using this setup for STED (Stimulated Emission Depletion) microscopy Kasper et al. could show that a reducing and oxidizing buffer system (ROXS) leads to an improvement in photostability of the fluorophores and fluorescence brightness decreases less during a series of scans (Fig. 2).

**Figure 1**: Scheme of the model used to estimate the influence of the linker length on resolution.

**Figure 2**: Fluorescence images of single ATTO647N-labeled 60-bp oligonucleotides immobilized under different aqueous buffer conditions in the absence (confocal) and presence of the STED beam (750 nm) in three successive scans (1-3). To visualize the effect of STED-beam-induced photobleaching, the image of the first scan is color encoded red and overlaid with the image of the third scan (“1 + 3”). Measurements were performed in PBS in the absence (a) and presence (b) of the ROXS. In (b) there is almost no difference between the first and third scan.
REFERENCE