## **Supplementary Movie 1.**

**Two-photon activation STED in mouse brain slice.** 1PA (left) vs. 2PA (right) STED recordings of actin in cortical layer V neurons within a paraformal dehyde-fixed brain slice (compare Fig. 4a,b and Supplementary Fig. 5). Nine steps of  $\Delta z = 200$  nm were recorded, and this recorded sequence is displayed repeated 3 times within the movie.