In localization microscopy experiments, repeated photo-activation or photo-conversion of single fluorescent emitters during the acquisition results in images with multiple localizations per emitter [1]. This causes overestimation of the density of labeled molecules and overestimation of the resolution according to the FIRE concept [2]. For low labeling densities, localizations from the same emitter appear as clusters in the images and can straightforwardly be counted [3]. For high labeling densities however, further activations of an emitter become indistinguishable from activations of other nearby emitters.

We present a method for estimating how often each emitter is localized on average, without requiring prior knowledge about the spatial structure of the sample or the photophysics of the emitter. In our method the set of localizations is split into two subsets. Subsequently we take the Fourier transforms of the two images of the subsets and compute the cross-correlation at each spatial frequency. For intermediate spatial frequencies, the influence of multiple localizations of the same emitter dominates the cross-correlation. Therefore the decay of the correlation in this regime is fitted with a Gaussian model in order to extract the average number of times \( Q \) each emitter is localized from the fit parameters. We validate our approach with simulations and with experimental two-color STORM data.

