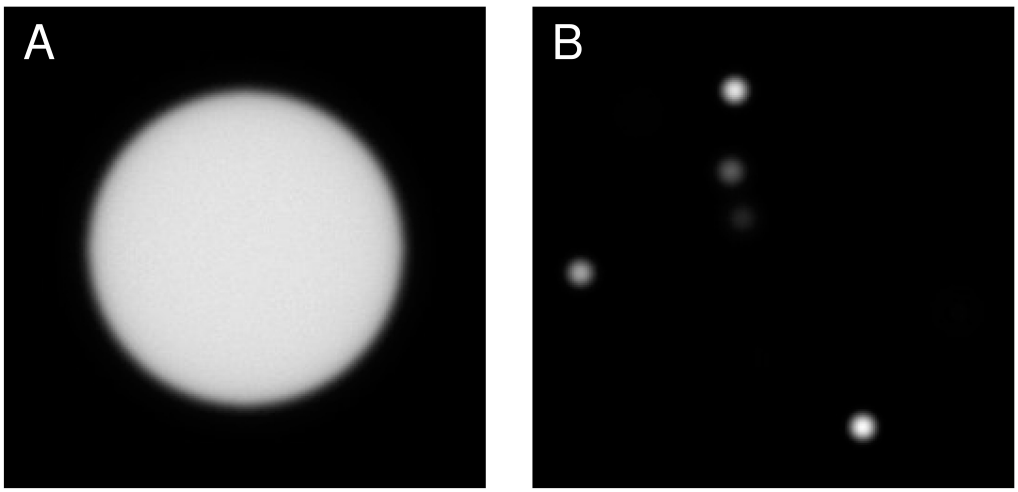
Supplementary Figure 1 Reduction of photobleaching and phototoxicity by CLEM depends of fluorophore distribution.



(**a,b**) Artificially generated images of cells with different fluorophore distribution patterns were subject to a computer program that simulates the photo-statistic properties of the fluorescence microscope as well as the CLEM decision and illumination strategy and calculates the effect of CLEM. Here two extreme examples of simulated fluorophore distributions are shown: (**a**) uniformly distributed intracellular fluorescence (single optical section shown) and (**b**) ten small, bright fluorescent spots distributed randomly in a cell (single optical section shown). To simulate typical optical and photo-statistical properties of the microscope, Poisson noise was superimposed on the convolution product of the 3D-images and the confocal point spread function (PSF). Subsequently, the illumination strategy of non-CLEM and CLEM was simulated, the exposure time and accumulated excitation light dose were calculated for every pixel (compare with Fig 2E and F of the main text). Reduction of photobleaching and phototoxicity was calculated by multiplying the accumulated light dose with the fluorophore distribution. This results in a reduction of photobleaching and phototoxicity by a factor of 2 and 10, respectively, for A and B. It shows that the effect of CLEM technology depend on the distribution of fluorophores.