PERFORMANCE OF SYNTHETIC FLUOROPHORES FOR SINGLE-MOLECULE LOCALIZATION MICROSCOPY: SCREENING AND IMAGE ANALYSIS

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Single-molecule localization microscopy (SMLM) overcomes the diffraction limit by analyzing an image sequence of blinking fluorophores for extracting the positions of individual fluorophores. Based on these positions a high-resolution image of the fluorophore distribution in the sample can be reconstructed. The spatial resolution of the reconstructed image is limited by the precision of the position estimates and the density of localized fluorophores. The quality of the reconstructed image relies chiefly on photo-physical and photo-chemical properties of the fluorophore, which have been studied for a number of fluorophores earlier, see for instance [1,2].

In this study we investigate major photo-physical and -chemical properties of a large number of synthetic fluorophores under typical imaging conditions in SMLM. For this purpose, we immunostained the nuclear pore protein NUP153 in Vero cells by secondary antibody labeling, imaged these fixed cells with a commercial GSDIM microscope by Leica and analyzed the reconstructed SMLM images and the event lists of fluorophore localizations. We screened the synthetic fluorophores in different buffer solutions: an imaging buffer containing glucose oxidase (Glox) enzyme and β-mercaptoethylamine (MEA), an oxygen-depleting thiol-containing buffer that improves the performance of many fluorophores decisively [3,4]; phosphate-buffered saline (PBS); and for some fluorophores Mowiol as well.

We analyze the localization events to estimate key parameters such as the duration and the number of detected photons per blink event, the photo-bleaching rate and the number of localizations per nuclear pore complex. We sort the reconstructed images for increasing contrast and sharpness and aim to combine the criteria into an overall performance index for each fluorophore and imaging buffer. This method allows us to investigate a large number of fluorophores and compare their suitability for SMLM. We found numerous fluorophores applicable with the GloxMEA buffer and some in Mowiol. We also found fluorophores that perform well in PBS and may be applicable in live cell microscopy. For all buffers, we found spectrally different fluorophores, particularly useful for multi-color imaging.

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