

Rhodamine-derived fluorescent dye with inherent blinking behavior for super-resolution imaging

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Super-resolution microscopy enables imaging of structures smaller than the diffraction limit. Single-molecule localization methods, such as dSTORM and PALM, reconstruct images by plotting the centroids of fluorescent point sources from a series of frames in which only a few molecules are fluorescing at a time. These approaches require less complex instrumentation than methods that depend on point-spread function engineering, and thus have gained growing popularity among scientists of diverse backgrounds. However, the relative simplicity of the instrumentation comes with a trade-off in probe requirements. These methods rely on the suppression/activation of the detectable on-state to achieve sparsely activated fluorophores. Additionally, chemical additives, complex illumination schemes, high irradiation intensities, or some combination are usually required to optimize the resulting images. Here, we report a rhodamine derivative which converts between a bright and a dark state in response to pH-dependent cyclization. At pH 7, only ~0.5% of the dye population is fluorescent. The spontaneous blinking occurs on timescales of seconds to minutes and can therefore be used for imaging approaches (dSTORM) without further sample treatment or complex illumination schemes. The ease-of-use as well as the brightness of this dye make it an excellent tool for super-resolution imaging techniques based on stochastic read-out.