A SIMPLE, VERSATILE METHOD FOR GFP-BASED SINGLE MOLECULE LOCALIZATION MICROSCOPY

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KEY WORDS: Superresolution microscopy, photoactivated localization microscopy, fluorescent labels, yeast

Single molecule localization-based superresolution microscopy methods, such as PALM or STORM, have been breakthrough techniques of the last years. Until now however, they require special fluorescent proteins to be cloned or high-affinity antibodies to be generated for specific labeling. On the other hand, many laboratories will have most of their constructs in GFP form and entire genomes are available as functional GFP-fusion proteins. Here, we report a method that makes all these constructs available for superresolution microscopy by targeting GFP with tiny, high-affinity antibodies coupled to blinking dyes. It thus combines the molecular specificity of genetic tagging with the high photon yield of organic dyes and minimal linkage error, as demonstrated on microtubules and living neurons. Due to their small size, these antibodies can penetrate the yeast cell wall, which enabled us to image >25 different proteins in S. cerevisiae with a resolution of 20 nm. We show that in combination with GFP-libraries, virtually any known protein can immediately be used in superresolution microscopy and that high-throughput superresolution imaging using simplified labeling schemes is possible.