

MINFLUX TRACKING OF FLUORESCENT MOLECULES

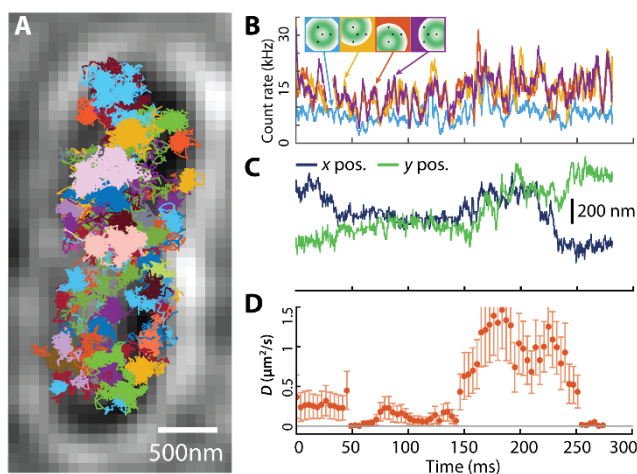
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The operation principle and challenges of MINFLUX applied to single molecule tracking (SMT) are presented, and the resulting performance is compared to state of the art camera tracking results. By probing the emitter with adapted illumination profiles featuring an intensity zero, MINFLUX minimizes the number of emitted photons needed for precise localization [1]. Proof of concept measurements on static emitters reduced the number of photon detections by 22-fold at equal localization precision compared to widespread camera-based methods. For an emitter at a given photon emission rate, this enhanced photon efficiency enables an increase in the spatio-temporal resolution of the localization, e.g. allowing tracking at higher temporal resolution with equal localization precision.

Using MINFLUX, the temporal resolution in single molecule tracking experiments of mEos2 fused to ribosomal subunits could be increased by 100-fold compared to published camera tracking performances employing fluorescent proteins. Using the available photons more effectively, the number of localizations per trace was enhanced by more than an order of magnitude, permitting a 3-fold improvement in the diffusion coefficient estimation precision. Since the experimental results were mainly limited by emitter blinking, we expect future MINFLUX implementations to further improve the spatio-temporal resolution in SMT applications.



(A) Transmission image of a bacterium overlaid with 77 tracks of 30S ribosomal protein subunits fused to the photoconvertible fluorescent protein mEos2. (B) Low pass filtered count rate of the four doughnut shaped exposures. The central (blue) doughnut produces a lower count rate, indicating that a single molecule is indeed well centered while tracking. (C) Extracted x and y coordinates of the trajectory. (D) Apparent diffusion constants D for a sliding window of 35 ms with their approximated error bars.

[1] Balzarotti, F., Eilers, Y., Gwosch, K. C., Gynnå, A. H., Westphal, V., Stefani, F. D., Elf, J., Hell, S. W. (2016), “Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes”, *Science*, aak9913