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Theoretical comparison of real-time single-particle tracking techniques

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One of the main challenges in studying single biomolecules in a native or near-native environment is their constant diffusion. An approach to solving this problem is real-time single particle tracking (SPT). In this study, we used statistical calculations and dynamic simulations to compare the orbital, Knight's Tour and MINFLUX localization methods, in the context of fluorescence-based and interferometric scattering (iSCAT) approaches. While the Knight's Tour method can track the fastest diffusion, MINFLUX achieves the greatest precision. The relative success of iSCAT vs fluorescence is strongly dependent on the particle size, and the photophysical properties and density of the fluorophores.

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