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How to measure separations and angles between intra-molecular fluorescent markers

HENRIK FLYVBJERG, KIM I. MORTENSEN, Tech Univ of Denmark, JONGMIN SUNG, Dept of Biochem and Dept of Phys, Stanford University, JAMES A. SPUDICH, Dept of Biochem, Stanford University School of Medicine — We demonstrate a novel, yet simple tool for the study of structure and function of biomolecules by extending two-colour co-localization microscopy to fluorescent molecules with fixed orientations and in intra-molecular proximity. From each color-separated microscope image in a time-lapse movie and using only simple means, we simultaneously determine both the relative (x,y)-separation of the fluorophores and their individual orientations in space with accuracy and precision. The positions and orientations of two domains of the same molecule are thus time-resolved. Using short double-stranded DNA molecules internally labelled with two fixed fluorophores, we demonstrate the accuracy and precision of our method using the known structure of double-stranded DNA as a benchmark, resolve 10-base-pair differences in fluorophore separations, and determine the unique 3D orientation of each DNA molecule, thereby establishing short, double-labelled DNA molecules as probes of 3D orientation of anything to which one can attach them firmly.

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