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Characterization of Surface-Tethered Particles by TIRFM ARIVALAGAN GAJRAJ, SETH BLUMBERG, MATTHEW PENNINGTON, JENS-CHRISTIAN MEINERS, University of Michigan — Tethered particle experiments track the Brownian motion of a microsphere to obtain information about intramolecular processes involving the tethering biopolymer. While these experiments are very powerful techniques that yield insight into intra-molecular dynamics, accurate quantitative analysis can be a limiting factor. For instance, most of these experiments suffer from incomplete information about the out of plane trajectory of the microsphere. Also, tethered-particles generally exhibit a large variation in behavior from molecule to molecule. Further complications can arise from electrostatic and hydrodynamic interactions of the surface with the microsphere. To address these complications we have extensively characterized the temporal and spatial trajectories of DNA tethers obtained from a stroboscopically illuminated TIRF microscope. To eliminate visual bias, we have developed automatic acquisition and selection criteria. Our results permit a comparison to theoretical models for tethered particle behavior and allow a more sophisticated understanding of large- scale biopolymer conformations such as those associated with DNA looping.

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