

# 3D CHARACTERIZATION OF TETHERED PARTICLE MOTION USING BIFOCAL IMAGING

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Single-molecule methods are powerful tools for studying the biophysics of biopolymers. We use a method called Tethered Particle Motion (TPM) where a single biomolecule (e.g. DNA) is used to tether a gold nanoparticle to a glass substrate. The system is placed in buffer; therefore the reporter particle exhibits confined Brownian motion. The motion and position distribution of the particle are influenced by the mechanical properties of the tether molecule. This methodology lends itself to multiplexing, that is, the placement of an array of molecules and reporter particles in order to achieve statistical reliability and single-molecule analysis.

The motion of the nanoparticles is observed in an epi-illumination dark field microscope and recorded using a CCD camera. Biophysical properties such as the persistence length of DNA can be determined by analyzing the position distribution of the nanoparticles and comparing them to Monte Carlo simulations of the experiment [1].

Our simulations have shown that using not only the projected motion as is usual in TPM experiments, but also the height information, makes it possible to distinguish which adaptation of the worm-like chain model correctly describes the position distribution of a particle tethered by a DNA molecule [1]. 3D positional information is necessary to distinguish between reduced motion as a consequence of a change in the persistence length of the DNA and a particle stuck to the substrate.

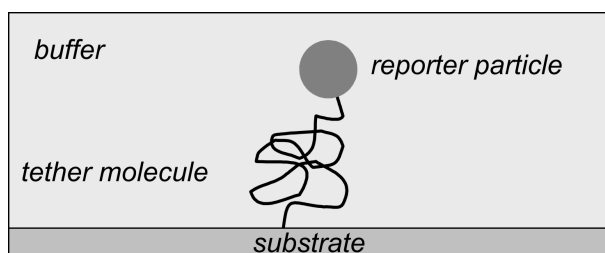


Figure 1: Principle of Tethered Particle Motion

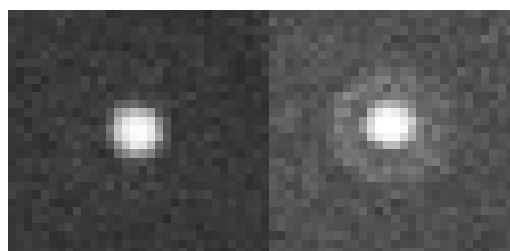


Figure 2: Simultaneously acquired focused and defocused image of the same nanoparticle

We use bifocal imaging to simultaneously collect a focused and defocused image on a single camera via a beamsplitter. The focused image is used to determine the projected position ( $x,y$ ) of the particles. In the defocused image, the height ( $z$ ) of the nanoparticle can be determined by comparing the defocused image to prerecorded calibration images with known defocus [2].

## REFERENCES

- [1] S. Brinkers et al., "The Persistence Length of Double Stranded DNA Determined Using Dark Field Tethered Particle Motion," *Submitted to the J. Chem. Phys.* (2008).
- [2] E.H. Toprak et al., "Three-Dimensional Particle Tracking via Bifocal Imaging," *Nano Letters*, **7**, 2043-2045 (2007).