

Two-Photon Optical Force Correlation Spectroscopy Using Optical Tweezers

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Optical tweezers are used to apply piconewton forces on micron-sized objects in liquid media, and to measure forces on that order in colloidal, biological, and biomolecular systems. Forces may be applied directly on an element naturally present within the system under observations, such as bacteria, sperm, or specific organelles in a living eukaryotic cell. Alternately, the trapping force can be applied to implanted microspheres (beads) used as probes within the system, for example in the study of movement along a cell membrane, or handles by which individual macromolecules are manipulated. The position of the probe can be measured using video-based imaging techniques, or by evaluating its position relative to the trap center using scattered light. Unfortunately, methods based on scattered light are restricted to clean samples in which non-specific scattering from the probe environment is negligible. This normally precludes their use in intracellular measurements, one of the most appealing biophysical applications of optical tweezers techniques.

In the present work we develop an optical force correlation spectroscopy based on a fluorescent probe, in which the trapped particle acts as a light source rather than a scatterer. The optical trap is based on a continuous-wave infrared laser that excites a fluorescence bead by a two-photon absorption process [1]. This probe is located in equilibrium at a geometric origin defined by the optical tweezers, and the emitted intensity changes as the bead is pulled away from the origin. We monitor these displacements by measuring the emitted intensity. In order to address short time scales an averaging method is required, for which we use the auto-correlation function. The method is thus most useful for detecting forces that have a dominant characteristic time.

The normalized auto-correlation function of a bead trapped in the optical tweezers is shown in Fig. 1. The bead executes a damped Brownian motion within the trap volume. It can be seen immediately that the damping time is shorter for increasing laser power, corresponding to a stiffer trap. We can adapt the auto-correlation function for conventional scattering of a particle localized in a harmonic potential and subject to a Gaussian illumination [2] with a small modification. Since in our case the emitted intensity is proportional to the square of the excited intensity, the equation is applicable by considering a Gaussian whose width is narrower by a factor of $\sqrt{2}$. The dependence of the damping time to the inverse of the intensity is approximately linear, as predicted by the Langevin equation.

In order to study the force correlation in the presence of an external drive, we scan the stage back and forth with a sawtooth function. The drag force and transverse displacement of the bead from the trap center take the form of an asymmetric square wave. During each cycle the bead takes two positions and the emitted light intensity is different in each part of the cycle. Direct observation of the intensity reveals little

information, as changes at the msec time scale are buried in noise. We have shown (Fig. 2) that optical force correlation spectroscopy can be used to measure characteristic time scales and relative displacements.

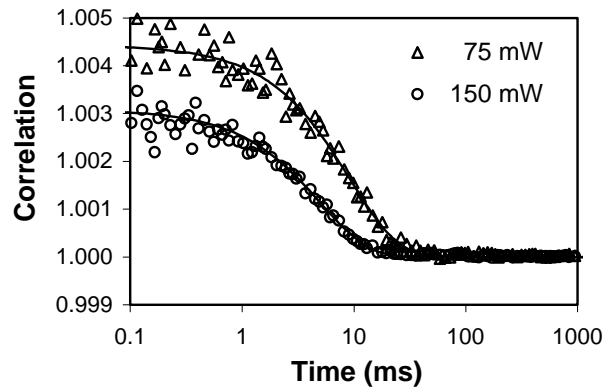


Figure 1: Thermal fluctuation of a bead within the optical tweezers as measured by the auto-correlation of the emitted fluorescence at indicated laser powers. Solid lines are fits to the theoretical function for conventional scattering of a particle localized in a harmonic potential

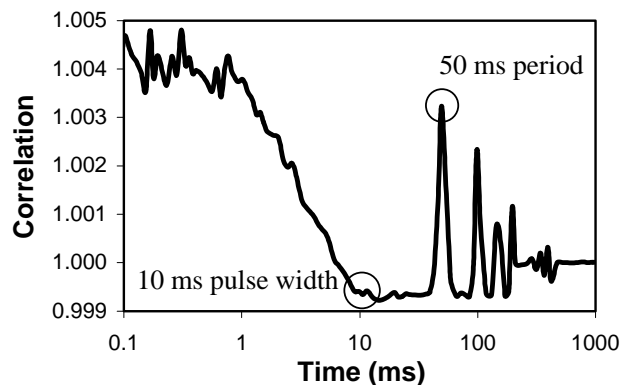


Figure 2: Auto-correlation of the emitted intensity of a 1 μm fluorescent bead trapped in the optical tweezers in the presence of an applied sawtooth drag force with frequency 20 Hz and a duty ratio of 1:5.

References

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2. A. Meller, R. Bar-Ziv, T. Tlusty, E. Moses, J. Stavans and S.A. Safran, "Localized dynamic light scattering: A new approach to dynamic measurements in optical microscopy", *Biophys J.* **74**:1541-1548, 1998.