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Continuous and time-shared multiple optical tweezers for the study of single motor proteins

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Abstract

We present a comparison between continuous (CW) and time-shared (TS) multiple optical tweezers applied to the study of the interaction between a single motor protein (myosin) with its track (an actin filament). In the experimental assay, named "three-bead assay", a single actin filament is stretched between two beads trapped in a CW or in a TS double trap. The actin filament is presented to a single myosin molecule lying on a third bead attached to the coverslide. The CW double trap is obtained by splitting a single laser source into two orthogonally polarized beams, while the TS one is obtained by rapidly scanning a single laser beam with acusto-optic modulators. When using the CW traps, position detection of the left or right bead is obtained by means of a single quadrant detector photodiode (QDP) placed in the back focal plane of the condenser and selected with a polarizer; when using the TS traps, the position of multiple traps with the same QDP can be collected independently using triggered and synchronized generation and acquisition. The two techniques are thoroughly analysed and compared, evidencing advantages and disadvantages of each one.

Keywords: Position measurement; Polymer; Microscopy; Detection

1. Introduction

During the last decade, optical tweezers have become a powerful tool for manipulating single biomolecules and for investigating the mechanic and kinetic properties of proteins and biopolymers.

Since their development [1], optical tweezers have undergone many technical advances. Among these, multiple optical tweezers have been proved to be especially useful in experiments where polymers need to be manipulated at the single-molecule level [2].

Multiple optical tweezers can be divided into two major classes: time-shared (TS) and continuous (CW). The first class is obtained by rapidly moving a single optical trap between different positions in the sample plane; if the time taken to scan the different trap positions is much smaller than the diffusion time of the trapped particles, the laser beam works as stable multiple optical tweezers. Faster scans imply that more traps can be generated simultaneously and/or that more efficient trapping can be achieved. TS traps have been obtained by deflecting the laser beam using galvano mirrors [3], piezoelectric mirrors [4], or acousto-optic modulators (AOMs) [5–7]; depending on the technique used, scanning rates can reach respectively 10–50, 1–2, and 10–200 kHz. Traps generation is usually computer-controlled so that the number of traps, their position, and stiffness can all be modified in real time. TS traps are also easy to be constructed and aligned, since only one laser beam is needed.

CW multiple optical tweezers are obtained by simply dividing a beam into two or more optical paths and then recombining the beams before the objective [8]; alternatively, two or more laser sources can be combined together [6]. This approach is simple to be realized when two traps are needed, but becomes more complicated when more than two traps are required.

A double optical tweezers assay largely used to study interactions between a single myosin motor and a single actin filament is the "three-bead assay", developed by Finer et al. in 1994 [8]. In this assay, double optical tweezers are used to catch and stretch an actin filament

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between two trapped beads, creating a configuration usually named ''dumbbell''. The actin filament is presented to a third bead stuck to the coverslide surface, which on average carries one myosin molecule. Interactions between actin and myosin are detected from noise reduction in the bead position signal and from data analysis the mechanic and kinetic properties of the molecule are derived [9].

Myosin is a family of motor proteins almost ubiquitous in eukaryotic cells. The chemical energy contained in an ATP molecule is converted during a chemo-mechanical cycle in which the myosin motor produces its movement (working stroke). In particular, myosin II is a non-processive molecular motor responsible for muscle contraction. During the last decade, its properties at the single-molecule level have been investigated using both CW [8–13] and TS [14,15] double optical tweezers. The same experimental assay has been used to study cardiac V1 and V3 myosin [16], smooth muscle myosin [17,18], myosin I [19], and myosin V [20,21].

We have built a multiple optical tweezers setup that can work both as CW and TS double optical tweezers. CW traps are obtained by splitting an infrared laser source into two orthogonally polarized beams, while TS traps are obtained by using two crossed AOMs. Position detection of the CW or TS tweezers is achieved using a single quadrant detector photodiode (QDP) placed in the back-focal plane of the condenser. Separate detection of the TS traps is possible using an acquisition board synchronized and triggered to the generation signal, as explained in the next section.

We have compared the two techniques in a three-bead assay. We show that, using CW traps, the stability of the system is within 1 nm and the working stroke of the molecule can be determined with sub-nanometer accuracy, along with its kinetic properties. On the other hand, in the TS configuration, the dumbbell oscillates at the switching frequency with amplitude that depends on the traps stiffness and position and on the switching frequency. This oscillation does not change the kinetics of the molecule significantly, but must be taken into account when evaluating the working stroke using back focal plane detection.

2. The experimental apparatus

The experimental apparatus (Fig. 1) was set up around a custom made optical microscope. The mechanical structure was designed in order to obtain high mechanical stability and to allow using custom optics. Two linear manual translators (M-014 Physik Instrumente) and a piezoelectric stage (P-527.2 CL Physik Instrumente), allow gross (25 mm stroke) and fine (1 nm resolution) movements of the sample in the xy plane, respectively. The objective (Nikon Plan-Apo 60X, NA 1.2, WD 0.2 mm, water immersion) is mounted on a piezoelectric translator that provides fine z movements (P-721.20 Physik Instrumente, 100 μm stroke, 1 nm minimum displacement). The condenser (Olympus U-AAC, Aplanat, Achromat, NA 1.4, oil immersion) is used both to illuminate the sample and to collect the forward scattered laser light for position detection.

The apparatus is provided with three light sources. A halogen lamp (Schott KL 1500 LCD, 150 W) supplies illumination for bright-field microscopy. The sample image is acquired on two different CCD cameras. One of them (CCD 200X, Hamamatsu C3077) is used for wide-field magnification, while the other (CCD 2000X, Ganz ZC-F11C3) collects a high-magnification image that serves as the basis for an optical feedback system, which prevents thermal drifts by stabilizing the sample position within 1 nm [12,22].

A laser beam at 532 nm wavelength, coming from a Nd:YAG duplicated laser (Coherent, VERDI V-10), provides illumination for fluorescence microscopy. The fluorescence image of the sample is projected onto an intensified CCD camera (Hamamatsu C2400).

Multiple optical tweezers were integrated within the optical and fluorescence microscope. The trapping beam, coming from a Nd:YAG laser (Spectra-Physics Millennia IR, $\lambda = 1064$ nm), passes through an optical isolator (OI) to prevent back-reflections of the trapping light into the laser cavity and through a telescope to collimate and expand the beam. A $\lambda/2$ waveplate and a polarizing beam splitter (PBS) divide the beam into two beams with mutually orthogonal polarizations: the undeflected beam is used for the TS multiple optical tweezers, while both ones are used for the CW double optical tweezers.

2.1. TS multiple optical tweezers

The undeflected beam passes through two crossed AOMs (A & A DTS-XY 250), placed in a conjugate point of the aperture diaphragm of the objective. The modulators are driven by a two-channel digital synthesizer (DDS, A & A AA.DDS.XX) that supplies two sinusoidal signals with frequencies between 60 and 90 MHz and amplitudes from 0 to 30 V. These signals drive the first-order Bragg diffracted beam emerging from the two AOMs. The beam angular deflection is proportional to the frequency, while the beam intensity is proportional to the squared amplitude of the driving signal. The beam angular deflection from the AOMs is converted into a linear displacement of the optical trap in the focal plane of the objective. By alternatively switching the signals between two frequencies, we caused the optical trap to switch between two positions within the focal plane of the objective. Moreover, by alternatively switching the signal amplitude between two values, we independently regulated the stiffness of the two optical traps. The rising time of the DDS is few nanoseconds, while the bandwidth of the AOMs limits the time needed to deflect the beam to ~5 μs. This is fast enough to realize TS double optical tweezers. In fact, if the switching time is much less than the diffusion time of the trapped particle (about 10 ms for a 1 μm diameter sphere to