Enabling accurate photodiode detection of multiple optical traps by spatial filtering

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ABSTRACT

Dual and multiple beam optical tweezers allow for advanced trapping geometries beyond single traps, however, these increased manipulation capabilities, usually complicate the detection of position and force. The accuracy of position and force measurements is often compromised by crosstalk between the detected signals, this crosstalk leading to a systematic error on the measured forces and distances. In dual-beam optical trapping setups, the two traps are typically orthogonal polarized and crosstalk can be minimized by inserting polarization optics in front of the detector, however, this method is not perfect because of the de-polarization of the trapping beam introduced by the required high numerical aperture optics. Moreover, the restriction to two orthogonal polarisation states limits the number of detectable traps to two. Here, we present an easy-to-implement simple method to efficiently eliminate cross-talk in dual beam setups.[1] The technique is based on spatial filtering and is highly compatible with standard back-focal-plane photodiode based detection. The reported method significantly improves the accuracy of force-distance measurements, e.g., of single molecules, hence providing much more scientific value for the experimental efforts. Furthermore, it opens the possibility for fast and simultaneous photodiode based detection of multiple holographically generated optical traps.

Keywords: Optical tweezers, Detection, Photodiodes, Crosstalk, Spatial filtering, Signal processing, Optical manipulation

1. INTRODUCTION

Since the invention of single-beam optical tweezers,[2] numerous extensions[3,4] and completely new generations of optical tweezers[5–7] have been presented. These innovations broadened the manipulation capabilities and paved the way for break-through experiments within biophysics.[8,9] One particularly successful technique within single molecule biophysics are dual-beam optical tweezers, as these double traps allow for the manipulation of the two ends of linear molecules by using two trapped beads as handles.[10]

Dual-beam optical traps can conveniently be generated by using two laser beams with perpendicular polarization. Using beams in different polarisation states has advantages both for beam generation and detection. For beam steering control, the optical paths for the two beams can be split by polarization optics like, e.g., polarizing beam splitters, allowing for individual positioning of the two traps. On the detection side, polarisation optics can again be used to separate the signals, originating from the two individual traps, for separate detection using photodiodes.

However, one problem, that has often been overlooked, is that signal separation techniques based on polarization do not allow for a complete signal separation. This is mainly due to the use of high numerical aperture optics, which are required for efficient optical trapping and which unfortunately cause de-polarisation. Moreover, polarisation optics are not perfect. E.g., polarizing beam splitting cubes typically have an extinction ratio of only 1:100 for the reflected beam. This incomplete separation of signals leads to a systematic error called crosstalk. Crosstalk in dual-beam optical tweezers means that one detector is not measuring exclusively the signal from one trap, but a small fraction of the light from the other trap is also being detected. Crosstalk thus results into

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measuring partly the position and force acting on the second bead, when the aim is to exclusively measure the position and force acting on the first bead.

Recently, a few different methods have been presented which tackle this problem and vary in complexity, cost and efficiency.[11–13] Here we contribute to this topic by presenting a simple and easy-to-implement method that can be used in combination with polarisation based signal separation.[1] The method is based on spatial filtering and allows for reduced crosstalk levels as low as 0.2%, i.e., an improvement compared to the standard technique by a factor five. Moreover, the presented signal separation technique can be advantageously used for enabling fast and accurate detection by photodiodes in holographically generated traps, where traditionally cameras had to be used for detection, since a separation of multiple signals based on polarisation is not possible.

2. OPTICAL SETUP

2.1 Trapping Setup

Dual-beam optical tweezers in orthogonal polarisations were integrated into an inverted microscope (Leica DM IRBE) in the standard way.[14] Briefly, the linear polarized beam from a 1064nm CW laser (Spectra Physics J20I-BL-106C-02) was expanded to slightly overfill the back aperture of the objective (63X, NA=1.2, Leica HCX PL APO W CORR CS). Using a half-wave plate in combination with a polarizing beam splitter, this initial beam was split in a 50:50-ratio to generate two separate trapping beams. Beam steering capabilities were added to one of the trapping beams by means of a 1:1-telescope. The optical plane of the telescope's first lens, the 'steering lens', was optically conjugated with the back-focal-plane of the objective. In this arrangement, lateral displacement of the steering lens via piezo actuators (Newport Picomotor, 9066-X-P-M) resulted in lateral displacement of the trap focus. The two trapping beams were recombined using a second polarizing beam splitter and sent into the objective to form the two optical tweezers in the objective's focal plane. The trap foci were optimized by adjusting the objective collar to compensate for spherical aberration.[15]

As a generic sample, we mounted a perfusion chamber, formed by two #1.5 cover slips separated by double-sided sticky tape and filled with a dilute suspension of 0.96μm polystyrene beads (PS03N/9396, Bangs Laboratories) in Millipore water.

2.2 QPD detection using spatial filtering

For the interferometric, photodiode based position detection, a relay lens was used to image the back-focal-plane of the light-collecting condenser onto a quadrant photodiode (QPD) (Si-PIN photodiode, Hamamatsu S5981).[16] As the only modification to this standard detection, we added a pinhole in a plane that was conjugate to the sample plane. In a manner similar to confocal detection in microscopy, this added element restricts the imaging volume, i.e., the volume in the sample chamber from where light can reach the detector. Using xyz-micropositioners, the pinhole was positioned in this conjugate plane, where an image of the sample plane was formed, such that only light originating from one trap of interest could pass though the pinhole to the QPD, while the light from the second trap was blocked (Fig. 1A-C). This small addition to the optical system effectively suppresses crosstalk without the requirement of a specific polarisation.

3. RESULTS

3.0.1 Crosstalk suppression

After alignment of the pinhole to the trap of interest, we were able to measure the time-dependent position and the power spectrum of the trapped bead (Fig. 1E and 1F). Scanning a bead that was attached to the surface of the cover glass through the trap focus confirmed that the linear relation between bead position and QPD signal remained valid (Fig. 1D). This linear relation facilitates an easy calibration procedure due to a constant conversion factor between the travelled distance, measured in metre, and the photodiode voltage signal for small displacements of the bead from its equilibrium position.

Next, we investigated the crosstalk suppression due to spatial filtering. We used the quantity

$$\Psi = \frac{S_{\text{parasitic}}}{S_{\text{total}}} , \tag{1}$$

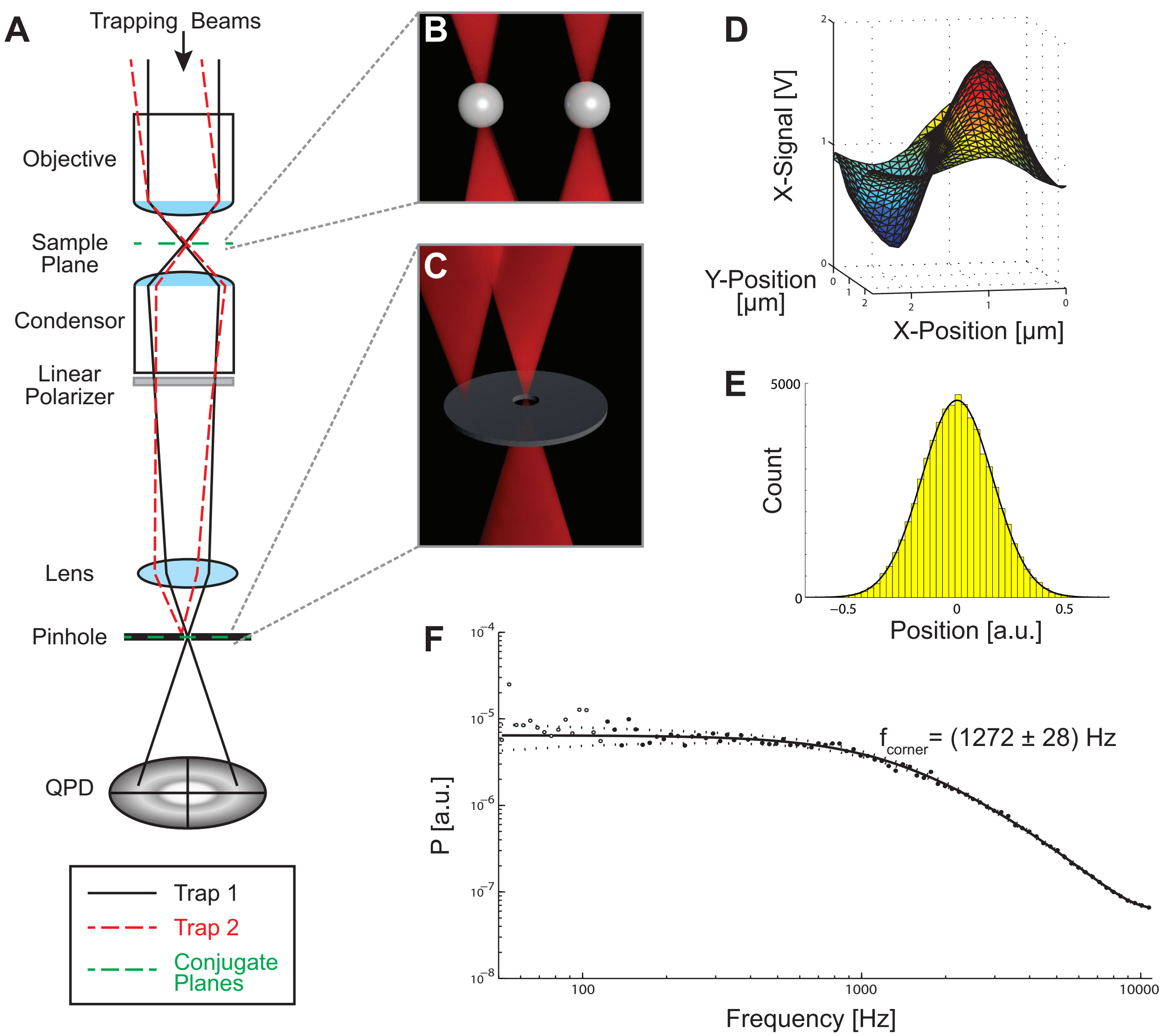

Figure 1. Dual-beam optical tweezers with photodiode based detection and signal separation by spatial filtering. A) Two trapping beams enter the objective and generate two optical traps in the sample plane. A condenser collects the light for QPD detection and a relay lens images the back-focal-plane of the condenser onto the QPD. Selective transmission of light is achieved by placing a pinhole in a plane that is conjugate to the sample plane. B) Illustration of the sample plane where two beads are trapped. C) Illustration of the conjugate plane where the pinhole is located. Only light originating from one trap of interest can reach the QPD. D) Relation between lateral bead position and QPD signal. The graph shows the QPD output voltage for the X-channel, while a bead was raster-scanned through the focus. The signal shows a minor dependence on y-displacement and a linear relation between x-displacement and the output voltage of the QPD x-channel, confirming a constant conversion factor for small bead displacements. E) Position histogram with Gaussian fit. F) Power spectrum of the captured time-series. The corner frequency f_{corner} was determined by a Lorentzian fit to the data (black dots). The fit is shown as solid black line with ± 1 standard deviation (dotted lines). Empty dots are data points outside the fitting range. Data analysis was done using the freely available power spectrum analysis tool by Hansen et al.[17]

as a measure of signal crosstalk from the blocked trap to the trap of interest. Here the parasitic signal $S_{\text{parasitic}}$ is the light intensity measured when only the blocked trap was switched on, and the total signal S_{total} denotes the intensity measured with both traps being active. It is expected that the amount of crosstalk will depend on the distance between the traps. The closer the traps are, the larger the crosstalk should be. We analyzed this dependency by changing the inter-trap distance between the two trap foci, while continuously recording the transmitted light intensities. The trap of interest was kept in place, while the position of the second trap was varied using the beam steering lens. The results for different pinhole sizes are shown in Fig. 2A. The measurements confirmed that the amount of crosstalk depends on the inter-trap distance and decreases faster for smaller pinholes. The diffraction limited spot size sets a limit to the minimal usable pinhole size.

The presented method can be combined with standard polarization based filtering. To investigate the added value of the presented technique, we compared the performance of this combined method with the standard polarisation based technique. For this purpose, we added a linear polarizer (Thorlabs LPNIR100) in front of the QPD to block the polarized light from the second trap.

With the polarizer but no pinhole installed, the crosstalk was at a constant level just above 1%, in accordance with earlier measurements where exclusively polarisation optics were used for crosstalk reduction.[11] As shown in Fig. 2B, the combination of spatial filtering and polarisation based signal separation allowed for crosstalk levels far below what is achievable with polarisation based filtering alone. Crosstalk levels were as low as 0.2% for a 20μm pinhole, providing a factor five improvement over the standard technique. Notably, the measurements were limited by electronics noise, and not by the method itself. So even lower crosstalk levels, close to zero, appear to be possible.

3.1 QPD detection for holographic optical tweezers

The presented method, which was originally developed for crosstalk reduction, will prove useful in the field of holographic optical trapping. Holographic optical tweezers enable convenient generation of multiple traps and have found wide-spread use in various research fields.[7] However, on the detection side, a remaining challenge is to separate the signals from the individual traps for separate photodiode based detection. Photodiode based detection is desirable due to its unrivaled bandwidth, spatial resolution and low cost.

Along the lines of the reported detection for dual traps, it will be possible to position a pinhole such that only light from one individual trap, out of an array of traps, reaches the QPD. The principle advantage over existing methods is that it will be possible to parallelize the detection to allow for simultaneous detection of multiple traps using photodiodes. Current and future efforts are aiming at implementing this combination of holographic optical tweezers and the reported detection technique.

4. SUMMARY

Here we have presented a method for fast and simultaneous photodiode based detection of multiple optical traps. It was experimentally shown that performing spatial filtering in a conjugate plane to the sample plane allows for efficient suppression of crosstalk from a nearby trap. Combining the method with established polarisation based signal separation further reduced crosstalk to levels as low as 0.2%. By parallelizing this method, i.e., using several pinholes to detect several traps simultaneously, we will be able to track the position and measure forces of multiple traps in holographic optical tweezers.

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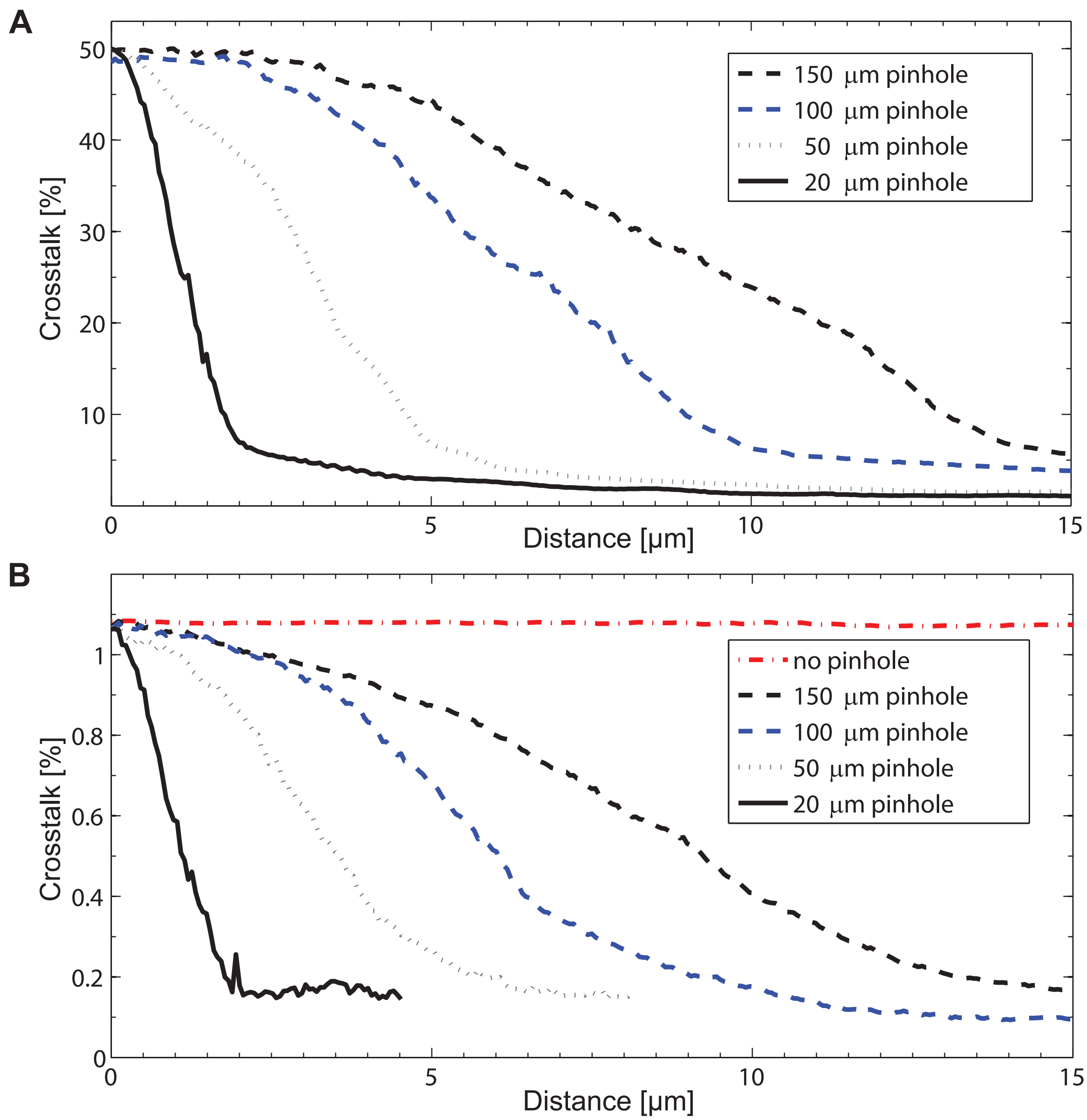

Figure 2. Crosstalk suppression. A) The dependence of crosstalk Ψ on inter-trap distance is shown for different pinhole sizes. Smaller pinholes allow for more effective crosstalk suppression at shorter inter-trap distances. B) Same as in A), but now for a combined spatial filtering and polarisation based signal separation. A factor five improvement over the standard technique, i.e., with polarisation only (dot-dash red line), can be achieved for small pinholes at trap-trap distances of more than 2μm (full black line). Figures adapted from Ref. 1.

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