Optical Engineering 44(11), 113603 (November 2005)

Rapid and simple automatic trapping-force calibration system for optical tweezers

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Abstract. The automation of a rapid and simple trapping-force calibration system is desired for optical tweezers to measure biological forces. One simple calibration method, the water-dragging-force method, is to calibrate the trapping force against a given water dragging force with an image-processing technique. However, the conventional image-processing technique is too slow because of the time it takes for image recording, transferring, storing, and retrieving. The pattern recognition technique of our automated calibration method is rapid and simple, because it directly processes the image signal without recording, storing, and retrieving any image. In an experiment, we have combined the dragging force method with the real-time pattern recognition technique to calibrate the relationship between the trapping force at a given laser power and a displacement of a bead. We demonstrate the calibration results of the trapping forces on two target beads 10 and 6.2 μm in diameter, separately, at two laser powers, 5 and 10 mW. Each calibration procedure is finished in 5 min at a pattern recognition rate of 10 Hz with a spatial resolution of 75 nm. We believe that this technique is reliable and rapid enough to be applied to biological force measurement. © *2005 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.2128632]

Subject terms: automation; rapid force calibration; optical tweezers; pattern recognition.

Paper 040927R received Dec. 3, 2004; revised manuscript received Apr. 27, 2005; accepted for publication Apr. 28, 2005; published online Nov. 11, 2005.

1 Introduction

Optical tweezers, working like a spring, have been widely used in capturing and manipulating micro particles since invented by A. Ashkin in 1986.[1] Typically, the springlike trapping force generated by optical tweezers is of the order of a piconewton (10^{-12} N), which is comparable with many biological forces. Consequently, optical tweezers are appropriate for measuring the biological force due to membrane tether, cell-cell interaction, antigen-antibody interaction, protein binding, etc.[2,3] For this purpose, it is crucial to calibrate the trapping force of optical tweezers at various laser powers for its application to biological force measurement.

Conventionally, there are several methods to calibrate the trapping force, such as the escape force method, dragging force method, equipartition method, power spectrum method, and step response method.[3–5] Among them, the dragging force method is one of the simplest in use.

In the dragging force method, the trapping force generated by an optical tweezers system on a trapped bead in water is calibrated against a given water dragging force. The magnitude of the water dragging force F_{drag} is adjustable by varying the speed v of the water flow relative to the trapped bead according to Stokes's law:

$$F_{\text{drag}} = 6\pi r \eta v, \tag{1}$$

where r is the bead radius and η is the coefficient of viscosity. As the speed of the water flow is increased, the water dragging force on the bead along with the water flow increases as well.

Experimentally, a target bead is first trapped at the center of the laser beam waist of the optical tweezers. Then, a water flow is applied to wash the bead. This induces a dragging force, which shifts the bead away from the center of the laser beam waist. The shift of the bead results in a spring-like trapping force F_{trap}, which is proportional to the displacement of the bead. As the shift of the bead is increased, the trapping force on the bead, which is opposite to the water dragging force, increases as well.

Consequently, the bead will be shifted to a new equilibrium position where the water dragging force is balanced by the trapping force of the optical tweezers arising from the bead shift. In other words, the magnitude of the trapping force associated with this particular bead shift is the same as that of the water dragging force, which can be derived from the given water speed according to Eq. (1). Therefore, the calibration of the trapping force as a function of the bead shift is obtained by gradually varying the speed of the water flow and simultaneously measuring the corresponding displacement of the bead shift.

To measure the corresponding displacement of the bead shift, a CCD camera or a quadrant photodetector (QPD) is usually used. In the QPD detection technique, a pattern,

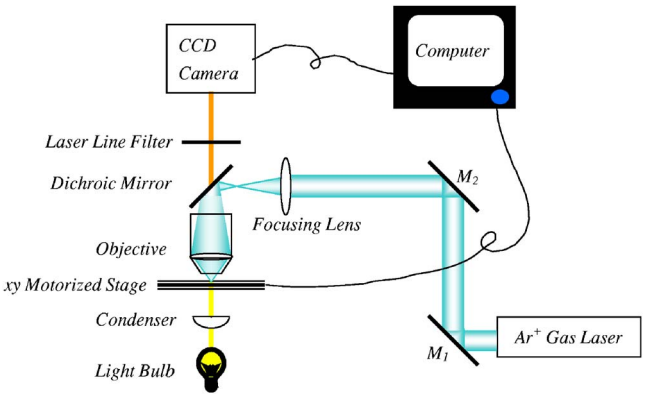

Fig. 1 The setup of the Ar^+-laser-based optical tweezers system.

which is created by a fluorescent bead or scattered light from a bead, is imaged on a QPD.[6–8] The movement of the pattern reflects and magnifies the real movement of the bead, thereby offering nanometer spatial resolution and fast temporal resolution in detecting the bead shift. However, a CCD camera is nowadays common and standard equipment on a commercial microscope. Its spatial resolution can also reach a few nanometers, as described in several articles.[2,9] It does not need another ultralow-noise amplifier such as is needed to process the signal from the QPD. Moreover, the CCD camera directly images the bead, so that, with an image-processing technique, we can measure the displacement of any bead in the field of view, whereas the QPD can only measure the pattern from a specific bead. Although the temporal resolution of CCD camera detection (a few tens of hertz) is not as good as those of QPDs (up to megahertz[7]), the few-hertz CCD camera temporal resolution is still sufficient for many experiments. Thus, a CCD camera detection technique is a simple and low-cost choice for measuring the bead shift. In this article, we demonstrate the way to measure and analyze the bead shift by using a common CCD camera and a commercial software application for real-time image processing.

Nowadays, some of the commercial image-processing software is real-time, convenient, and user-friendly. Unlike conventional image-processing techniques, which involve time-consuming image recording, transferring, storing, and retrieving, the pattern recognition technique of our commercial software application directly processes the image signal at a rate of 10 Hz. In this experiment, we combine the dragging-force method with the real-time pattern recognition technique to calibrate the relationship between the trapping force at a given laser power and the displacement of the bead shift.

2 Setup

The setup of our optical tweezers system is shown in Fig. 1. An Ar^+ gas laser (Lexel Laser 3500) provides a laser beam with two dominant wavelengths at 488 and 514 nm. First, the laser beam is directed into the objective (numerical aperture 1.25, 100×, oil-immersed; Nikon) of a microscope through two mirrors M_1 and M_2, a focusing lens, and a dichroic mirror (CVI laser mirrors AR1-1025-45). Note that the focal length of the focusing lens is so chosen that the laser beam fills the objective entrance aperture. In addition, the focusing lens is adjustable along the optical axis so that the laser beam comming out of the microscope will converge on the sample plane of the microscope to trap a target bead in water on top of a glass slide. The glass slide is placed on a dc motorized stage (Newport 860A motorizer with a 860-C2 motion controller), which provides a maximum speed as high as 1300 μm/s. According to Eq. (1), a maximum dragging force up to 122 pN is thus available for a 10-μm-diameter target bead. From the other end of the optical path, a light bulb illuminates the trapped bead via a condenser. This projects a bright-field image of the bead onto a CCD camera via the objective followed by the dichroic mirror and a laser line filter. Both of them reject the backscattered Ar^+ aser light from the trapped bead. Lastly, the bright-field image of the trapped bead is monitored by the CCD camera and transferred into a computer via an image grab board (National Instrument PCI-1409).

3 Program

In this experiment, the speed of the motorized stage and, as a consequence, the water dragging force are controlled by a water-flow-control subprogram via a data acquisition card (National Instrument PCI-6024E). In addition, the center coordinates of the trapped bead in each frame are located with a pattern recognition technique by an image-analysis subprogram, which results in the displacement of the bead shift. Both the water-flow-control subprogram and the image-analysis subprogram, constituting the image-processing program, coordinate the operation of the calibration procedure.

First, the image-analysis subprogram is established on the platform of two LabVIEW application programs: NI-Imaq and Imaq Vision. NI-Imaq is powerful in image acquisition and storage. Complementarily, Imaq Vision is efficient in image processing, especially in pattern recognition. Thus, the combination of NI-Imaq and Imaq Vision enables the real-time measurement of the displacement of the bead shift.

Second, the water-flow-control subprogram is responsible for driving the motorized stage of the microscope at various speeds. This induces a controllable water flow, which generates a desired water dragging force to calibrate the trapping force of optical tweezers. Consequently, this image-analysis plus water-flow-control program plays an important role in the automation of a real-time trapping-force calibration system for optical tweezers.

4 Method

As shown in Fig. 2, the flow chart of the image-analysis plus water-flow-control program for the automation of the trapping force calibration comprises four steps. The first step of this main program is to build a *learn template* for pattern recognition by using the image-analysis subprogram after the computer starts receiving the image signal from the CCD camera. For this purpose, as shown in Fig. 3(a), a region of interest in a picture frame containing the profile of the target bead image is manually assigned to the image-analysis subprogram as the learn template. Subsequently, this subprogram will create a description of the template image. Then this subprogram will continue to the matching step. During the matching step, the template description is used to search for the matched pattern in the subsequent

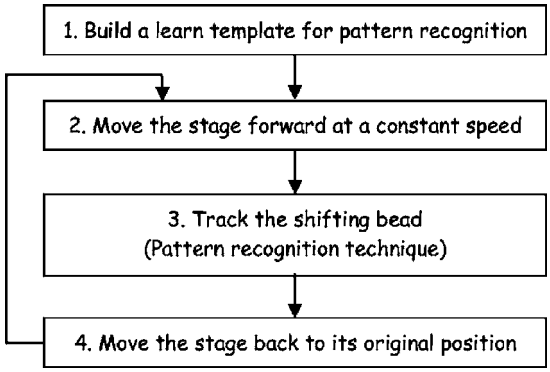

Fig. 2 The flow chart of the image-analysis plus water-flow-control program.

frames. As a result, the image-analysis subprogram will output the center coordinates of any pattern that matches the target bead's profile in the learn template, as shown in Fig. 3(b).

The second step of the main program is to move the stage forward at a constant speed by the water-flow-control subprogram. This subprogram manipulates the motorized stage via the analog output voltages from the LabVIEW data acquisition card. Thus, the movement of the stage induces an effective water flow relative to the trapped bead. Consequently, this step generates a water dragging force on the bead.

The third step is to track the shifting bead by the image-analysis subprogram in each of the subsequent frames taken by the CCD camera, utilizing the learn template technique from the first step. Then, the image-analysis subprogram outputs the center coordinates of the shifting bead in real time. As explained earlier, the trapped bead will be shifted to a new equilibrium position where the water dragging force is balanced by the resulting trapping force arising from the bead shift. When this equilibrium situation occurs, the main program stores only the magnitudes of the displacement of the bead shift and the corresponding water dragging force according to Eq. (1). Without storing and retrieving any image files, this image-analysis subprogram is rapid enough to track the shifting bead.

Finally, the fourth step is to move the stage back to its original position by the water-flow-control subprogram. Then, the main program repeats the second step but moves the stage forward at a new speed. Subsequent steps follow likewise. Repeating this procedure on varying the speed of the stage in the second step, the main program obtains the relationship between the displacement of the bead shift and the water dragging force.

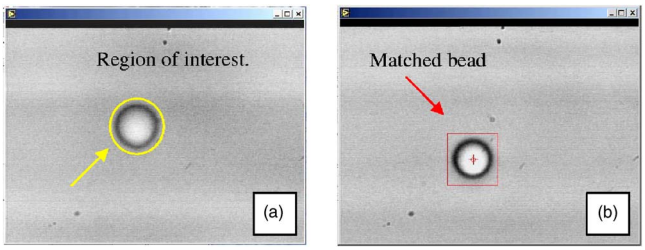

Fig. 3 Pattern recognition algorithm. (a) A yellow circle in a frame defines the region of interest containing a target bead as a learn template. (b) The bead within a red rectangle in a subsequent frame matches the target bead in the learn template.

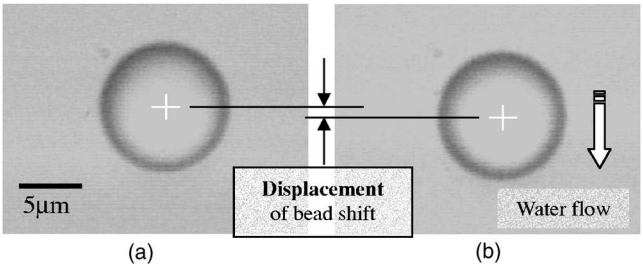

Fig. 4 Bead images when the motorized stage is (a) immobile and (b) mobile.

5 Results

Because there is no image storing and retrieving in the main program, we achieve a real-time image acquisition and pattern recognition rate up to 10 image frames per second in this experiment. As to the quality of the bead image in the frames, a 10-μm-diameter target bead occupies 133 pixels on a CCD camera chip. Therefore, a shift of the image on the CCD chip from one pixel to another is 75 nm (=10 μm/133 pixels). Since we use this CCD image to track the bead displacement, this results in a guaranteed spatial resolution of 75 nm per pixel for pattern recognition of the trapped bead while the motorized stage is driven at a constant speed. Because the bead is directly imaged on the CCD camera without introducing any other imaging technique such as DIC or phase contrast, the spatial resolution is not as good as those described in Refs. 2 and 9. However, the spatial resolution could be easily improved—for example, from 75 to 25 nm if we just magnify the image by 3×.

Figures 4(a) and 4(b) show the images of the same trapped bead when the motorized stage is immobile and mobile, respectively. The center coordinates of the bead are output to the front panel of the image-analysis plus water-flow-control program. Consequently, the displacement of the bead shift—the separation between the center of the trapped bead in Fig. 4(a) and in Fig. 4(b)—is obtained.

The speed of the motorized stage is slowly increased from zero to the speed at which the trapped bead is just washed off by the water flow. Simultaneously, the trapped bead is tracked over this period of time. As a result, the main program succeeds in automatically calibrating the relationship between the trapping force of the optical tweezers at a fixed laser power and the displacement of the bead shift, in 5 min.

In our experiment, the motorized stage was driven at 10 different speeds at each laser power. At each speed, 15 image frames were acquired for pattern recognition of the trapped bead at 10 Hz, which results in an averaged displacement of the bead shift. For repeatability, this process was executed 5 times for a total of 750 image frames. Specifically, we calibrated the trapping forces on two target beads 10 and 6.2 μm in diameter, separately, at two laser powers, 5 and 10 mW, alternately. In addition, we calcu-

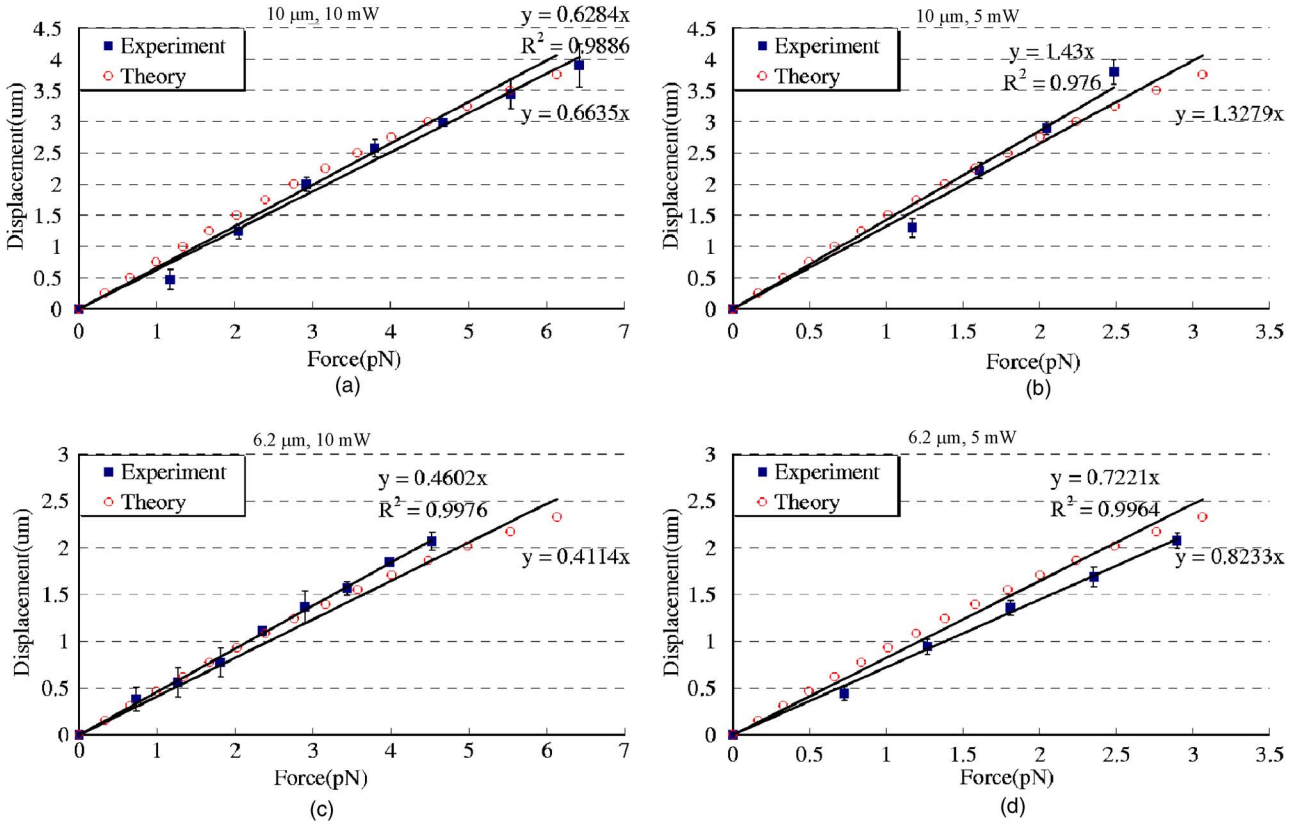

Fig. 5 The comparison of theoretical prediction and experimental data on a 10-μm-diameter bead at two laser powers, (a) 10 mW and (b) 5 mW. Similar comparison for a 6.2-μm-diameter bead at (c) 10 mW and (d) 5 mW.

lated the theoretical prediction under the same conditions. In this calculation, we assume that the temperature of water is 30°C, and the corresponding water-dragging coefficient η at this temperature is 0.7975.[10] Figure 5 shows the comparison between the experimental data and the theoretical results[3,11] of the displacements of the bead shift as a function of the trapping forces that correspond to 10 different water dragging forces.

6 Discussion

There are two features associated with the demonstrated automation technique for calibrating the trapping force of optical tweezers: linearity and rapidity. First, it is obvious that the measured displacement of the bead shift in Fig. 5 is linearly proportional to the trapping force, all the R^2 values for the experimental data being greater than 0.97. This confirms that optical tweezers do behave like an optical spring except for a small difference between the slopes of the experimental data and the theoretical predication. Second, the simplified calibration procedure can be executed automatically and rapidly in only 5 min at each laser power, as discussed in the following paragraphs. Therefore, we believe that the automated calibration technique as demonstrated in this work is reliable enough to be applied to biological force measurement.

In measuring biological force, it is sometimes beneficial to monitor the variation of the force as a function of time. For this purpose, it is desired to track the displacement of the bead shift in real time. Conventional image-processing techniques are too slow for this purpose, because they first record all the images of an event from a CCD camera with a recorder, then transfer a portion of the recorded images into a computer and stores them on its hard disk, and last, retrieve the stored image files into a computer buffer for image analysis frame by frame. Recall that at least 750 image frames are analyzed for the trapping force calibration at each laser power in this experiment. In view of the time it takes to record, transfer, store, and retrieve so many image frames, real-time force monitoring is impossible.

Our pattern recognition technique is much faster. This is because the image-analysis subprogram directly processes the image signal without storing and retrieving any of the images themselves. As described earlier, it grabs a single image frame at a time from the CCD camera into the computer buffer for pattern recognition. Then, it stores on the hard disk only the resulting center coordinates of the bead, without any image frame. Therefore, the bead can be tracked quickly and continuously with this pattern recognition technique, which enables real-time biological force monitoring.

On the other hand, it should be pointed out that, as shown in Fig. 5, the slope of the experimental data is slightly smaller than that of the theoretical calculation. Table 1 shows that the ratio of the experimental slope to the theoretical slope is 1.005 on average. This discrepancy may result from the unstable movement of the motorized stage,

Table 1 The slopes of the experimental data and the theoretical results in Fig. 5. The ratio of the two slopes is 1.005 on average.

Wavelength (μm)	Power (mW)	Slope		
		Exp.	Theor.	Exp./theor.
10	10	0.6284	0.6635	0.947
	5	1.43	1.3279	1.077
6	10	0.4602	0.4114	1.12
	5	0.7221	0.8233	0.877

the fluctuation of the laser power, and the errors in measuring the bead sizes and water temperature, leading to inaccuracy of the water-dragging coefficient.

7 Summary

In this experiment, we have demonstrated the automation of a rapid trapping force calibration system for optical tweezers by simply combining our pattern recognition technique with the water-dragging-force method. Once the main program of the calibration system is executed, the relationship between the trapping force at a given laser power and the corresponding displacement of the trapped bead is automatically calibrated in 5 min. Because there is no image storing and retrieving, the technique is rapid enough to track the trapped bead at a rate of 10 Hz with a spatial resolution of 75 nm. Therefore, we believe that our automated calibration system is reliable enough to be applied to real-time biological force measurement.

References

1. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, "Observation of a single-beam gradient force optical trap for dielectric particles," *Opt. Lett.* **11**(5), 288–290 (1986).
2. J. Dai and M. P. Sheetz, "Membrane tether formation from blebbing cells," *Biophys. J.* **77**, 3363–3370 (1999).
3. M. P. Sheetz, *Laser Tweezers in Cell Biology*, Academic Press, San Diego (1998).
4. K. Visscher, S. P. Gross, and S. M. Block, "Construction of multiple-beam optical traps with nanometer-resolution position sensing," *IEEE J. Sel. Top. Quantum Electron.* **2**(4), 1066–1076 (1996).
5. M. Capitanio, G. Romano, R. Ballerini, M. Giuntini, and F. S. Pavone, "Calibration of optical tweezers with differential interference contrast signals," *Rev. Sci. Instrum.* **73**(4), 1687–1696 (2003).
6. F. Quan, S. Ermilov, and D. Murdock, "Combining optical tweezers and patch clamp for studies of cell membrane electromechanics," *Rev. Sci. Instrum.* **75**(9), 2937–2942 (2004).
7. A. Rohrbach, C. Tischer, D. Neumayer, E.-L. Florin, and E. H. K. Stelzer, "Trapping and tracking a local probe with a photonic force microscope," *Rev. Sci. Instrum.* **75**(6), 2197–2210 (2004).
8. J. H. G. Huisstede, K. O. van der Werf, M. L. Bennink, and V. Subramaniam, "Force detection in optical tweezers using backscattered light," *Opt. Express* **13**(4), 1113–1123 (2005).
9. J. Gelles, B. J. Schnapp, and M. P. Sheetz, "Tracking kinesin-driven movements with nanometer-scale precision," *Nature (London)* **331**, 450–453 (1988).
10. R. C. Weast, *CRC Handbook of Chemistry and Physics*, 1st ed., p. F-19, Boca Raton, FL (1988).
11. A. Ashkin, "Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime," *Biophys. J.* **61**, 569–582 (1992).

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