Automation of an Optical Tweezers

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ABSTRACT

Optical tweezers is a newly developed instrument, which makes possible the manipulation of micro-optical particles under a microscope[1]. In this paper, we present the automation of an optical tweezers which consists of a modified optical tweezers, equipped with two motorized actuators to deflect a $1W$ argon laser beam, and a computer control system including a joystick. The trapping of a single bead and a group of *lactoacidofilus* was shown, separately. With the aid of the joystick and two auxiliary cursors superimposed on the real-time image of a trapped bead, we demonstrated the simple and convenient operation of the automated optical tweezers. By steering the joystick and then pressing a button on it, we assign a new location for the trapped bead to move to. The increment of the motion, $0.04\mu m$ for a 20X objective, is negligible. With a fast computer for image processing, the manipulation of the trapped bead is smooth and accurate.

The automation of the optical tweezers is also programmable. This technique may be applied to accelerate the DNA hybridization in a gene chip[2]. The combination of the modified optical tweezers with the computer control system provides a tool for precise manipulation of micro particles in many scientific fields.

Keywords: optical tweezers, DNA hybridization, gene chip

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1. INTRODUCTION

Optical tweezers is a powerful tool to manipulate a small particle by radiation force without physical contact. Since *Ashkin*[3~5] first demonstrated the trapping and acceleration of particles by radiation pressure in 1970, the single-beam optical tweezers has been used in a variety of applications, especially in the fields of molecular biology and biotechnology. Substantially, an optical tweezers generates a piconewton restoring force due to radiation pressure and works like a spring over a range of a few hundreds of microns in diameter. It is, therefore, suitable for the manipulation of micrometer-sized particles and various biological objects such as viruses, bacteria, cells, and chromosomes[6]. In the last decade, the optical tweezers has been further utilized to measure the forces generated by a single myosin[7] and a single kinesin[8] separately. The smaller the object[9,10] is, the stricter the requirement on the precise control of the optical tweezers becomes. Therefore, it is desired to have an automated optical tweezers.

The automated optical tweezers developed in this work consists of an optical system and a computer control system. It is capable of trapping and manipulating a single bead or a group of *lactoacidofilus*, separately. By steering a joystick and then pressing a button on it, we assign a new location for the trapped bead to move to. With a fast computer for image processing, the manipulation of the trapped bead is smooth, accurate, and programmable. We automatized this optical tweezers by deflecting the laser beam with two motorized actuators, which is controlled by the computer control system.

In this paper, we report the automation of an optical tweezers and the operation of the automated optical tweezers. This technique can be readily applied to the fields of biotech and biomedicine for precise manipulation and programmable control of small biological objects.

2. MODEL

Figure 1 shows the basic setup of an optical tweezers[11], which simply consists of a lens and a laser. The trapping force generated by the optical tweezers results from the interaction between the photons of the laser beam and the small object to be trapped. The mechanism of the trapping force is illustrated in Fig. 1, using a simple model of geometric ray optics[12]. Consider a spherical and transparent bead, which is placed behind the focus of a lens. The lens focuses a collimated and normally incident laser beam. As the diverging beam passes through the bead, each ray refracts twice. The double-refraction of the ray changes the propagating direction and the photons' momenta of the ray[13]. The rate of change of the photons' momenta is indeed the force exerted by the bead to deflect the ray. According to *Newton*'s third law of motion, an equal and opposite reaction force acts on the bead as well. The sum of the two reaction forces, arising from the pair of symmetrically diverging rays as drawn in Fig. 1, points toward the focus of the lens. To be more general, the net reaction force due to refraction always pulls the bead back to the point where the laser beam converges.

It can be shown that the converging point is an equilibrium position where the trapping force vanishes. Consequently, shifting the converging point leads the movement of the bead. The manipulation of the trapped object is performed simply

by tilting a 45° reflection mirror to deflect the incident angle of the collimated laser beam with respect to the lens. Traditionally, two 80-pitch fine screws are manually adjusted to tilt the mirror in a manual optical tweezers. In the automation of an optical tweezers in this work, we use two motorized actuators to tilt the mirror, which is controlled by a computer.

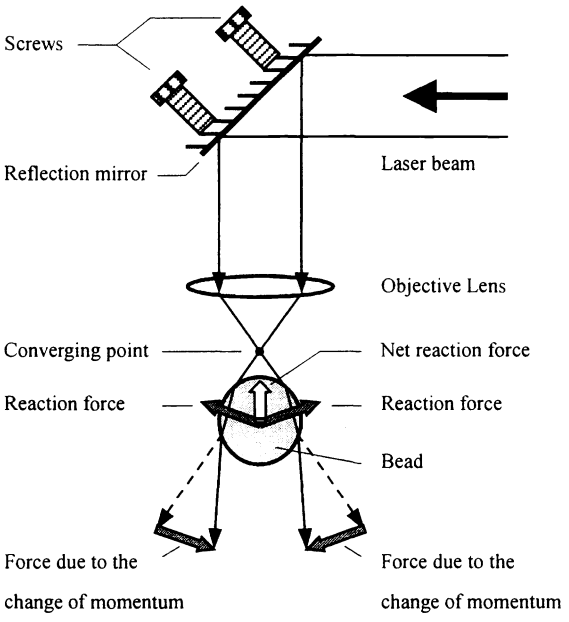


Fig. 1 The basic setup of an optical tweezers

3. SETUP

The automated optical tweezers system developed in this work can be classified into two sub-systems: namely, an optical system, including a modified optical tweezers and a monitoring and recording system, and a computer control system. Figure 2 shows the configuration of the automated optical tweezers and experimental setup.

3.1 OPTICAL SYSTEM

The optical system consists of a continuous-wave argon laser (LEXEL, 3500), a shutter, a periscope, two motorized actuators (Newport, 860A-1) and a motion controller (Newport, 860-C2), a beam expander, a dichroic mirror (CVI, AR1-1025-45-UNP), a 100X objective (Nikon, MSB01901, 100X/1.25, Oil) or a 20X objective (Nikon, MSB00201, 20X/0.40), a glass slide holder mounted on a XYZ stage, a condenser (Nikon, Abbe 1.25), a 300W halogen lamb, a filter (Newport, 10D20DM.5), a CCD camera, a monitor, and a VCR recorder.

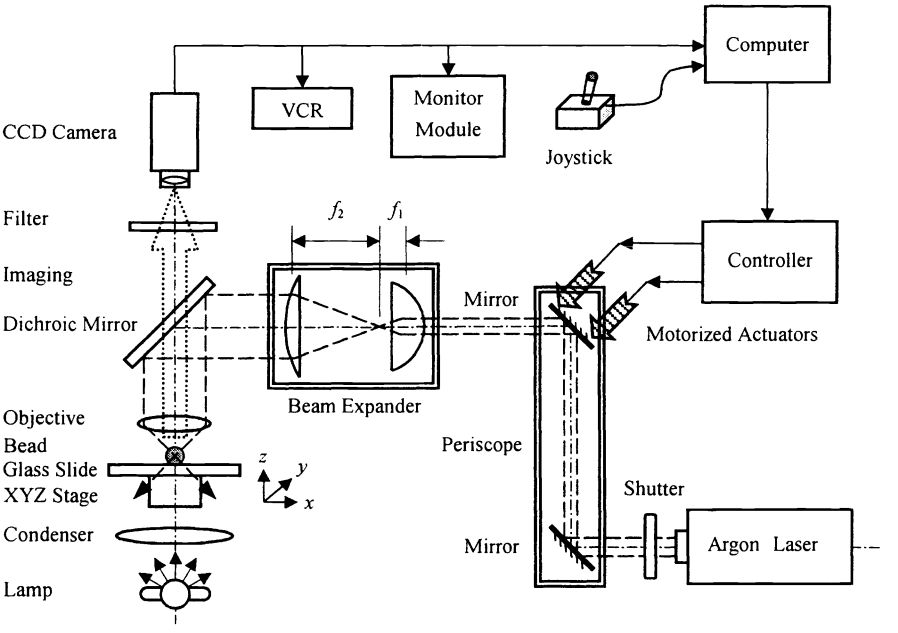


Fig. 2 The configuration of the automated optical tweezers and experimental setup

The argon laser is the light source for trapping. It outputs a laser beam up to $10W$ at two wavelengths of $488nm$ and $514nm$ with a beam waist of $3mm$ in diameter. Although harmful to most living objects due to high absorption, these two wavelengths are harmless to the beads ($10\mu m$~$100\mu m$-diameter spheres) and visible for demonstration. After passing through the shutter, the laser beam is raised by the periscope, a pair of two 45° reflection mirrors. Then, the laser beam is expanded by the beam expander, a pair of plano-convex lenses of focal lengths of $f_1 = 38.1mm$ (Newport, KPX079 AR.14) and $f_2 = 75.6mm$ (Newport, KPX088 AR.14), separately. Spaced the sum of their focal lengths apart, $f_1 + f_2$, these two lenses expand the beam from $3mm$ to $6mm$ in diameter so as to fill it in the entrance aperture of the objective. By adjusting the XYZ stage, we can move one of the beads, immersed in a drop of water upon the glass slide, to the focus of the beam. Thus, this bead is trapped. From now on, we shift the trapped bead by deflecting the laser beam without adjusting the XYZ stage. As shown in Fig. 2, this is achieved by tilting the top 45° reflection mirror of the periscope with the two motorized actuators. For reference, the actuator has an incremental motion capability to $\Delta\ell = 0.2\mu m$, giving an angular sensitivity to $\Delta\theta \cong 1\times10^{-5}\,rad.$ ($= 2arcsec$) in tilting a gimbal mount. The motion controller of the actuator provides a variable speed $v_{actuator}$ ranging from $50\mu m/sec$ to $250\mu m/sec$. It can be shown that the corresponding increment ΔL and travelling speed v_{trap} of the trapped bead on the glass slide are given by, respectively,

$$\Delta L(M) = \frac{160}{M}\frac{f_1}{f_2}\Delta\theta \qquad (1)$$

and

$$v_{trap}(M) \cong \frac{v_{actuator}}{\Delta \ell} \Delta L(M), \tag{2}$$

where M is the magnification of the objective. Consequently, the increments ΔL are $0.04\mu m$ and $0.008\mu m$ for the 20X (M = 20) objective and the 100X (M = 100) objective, respectively. The traveling speed v_{trap} of the trapped bead may vary from $10\mu m/sec$ to $50\mu m/sec$ for the 20X objective and from $2\mu m/sec$ to $10\mu m/sec$ for the 100X objective. Because these increments ΔL are beyond the resolution of the microscope, the motion of the trap is expected to be smooth and accurate.

The monitoring and recording system is necessary during manipulating the trapped bead. It is coupled to the optical tweezers via the dichroic mirror between the beam expander and the objective. The dichroic mirror has a high reflectance of $R \geq 99\%$ for $488nm \leq \lambda \leq 515nm$ and a high transmittance otherwise. It reflects the expanded argon laser beam to the objective while transmits to the CCD camera most of the illumination light, $\lambda < 488nm$ and $\lambda > 515nm$, emitted from the halogen lamp. The illumination light is first converged by the condenser and then scattered by the beads and its surroundings. The image of the sample is formed by the objective and projected onto the CCD camera. The video signal from the CCD camera is then connected to a monitor for observation and a VCR for recording. To avoid the undesired argon light back scattered from the sample to the camera, we insert the additional filter (Newport, 10D20DM.5, $R \geq 99\%$ for $488nm \leq \lambda \leq 515nm$) in front of the CCD camera. This results in an image with a reddish background and a bright spot, as will be shown in Fig. 3 and Fig. 4 in section 4. At this stage, the optical tweezers is semi-automatic; we need to push the buttons on the motion controller to start and stop the actuators.

3.2 COMPUTER CONTROL SYSTEM

In order to control the actuators automatically, we further setup a computer control system to regulate the motion controller. This computer control system consists of a personal computer (Pentium II $333MHz$ CPU, $64Mb$ SDRAM), an image acquisition board (National Instruments, IMAQ PCI-1408, variable scan rate 5 to 20 MHz), a graphical programming language (National Instruments, LabVIEW 5.0), a PC compatible joystick, and an analog-to-digital (A/D) converter card (National Instruments, Lab-PC+).

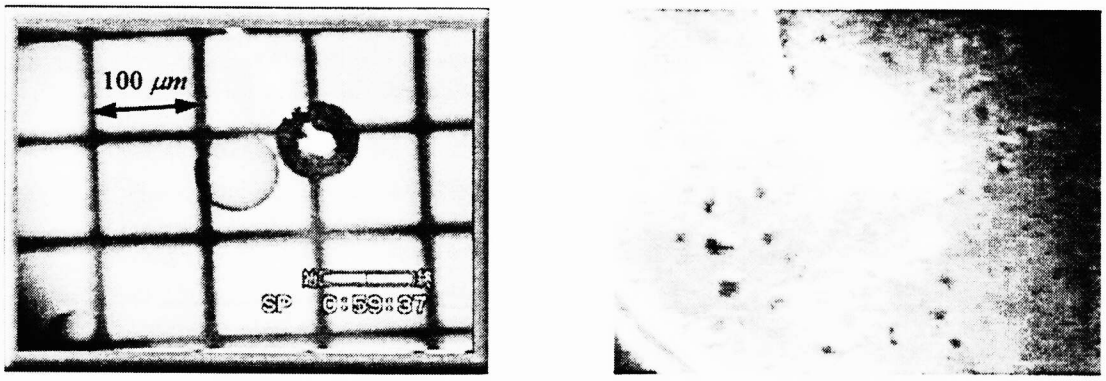
Firstly, we send the video signal of the sample image from the CCD camera to the personal computer via the image acquisition board. With a scanning rate up to 20 MHz and a typical size of 640 × 480 pixels for each frame, this image acquisition board transfers up to 30 monochromatic frames per second for real-time monitoring. Using LabVIEW5.0 for image acquisition, image processing, A/D data acquisition, and data analysis, we can easily locate the brightest spot in the image and display its coordinates on the computer screen.

Secondly, we use a PC compatible joystick to assign a destination for the trap to move to. This destination signal is sent, via the A/D converter card, to the computer and displayed on the screen as a striking crossed cursor. The distance between the cursor and the brightest spot is then calculated and converted into a voltage signal.

Lastly, this voltage signal is output, via the A/D converter card, to the motion controller to drive the actuators and deflet the angle of the laser beam. As a result, the trapped object will be moved to the new position and comes to a fully stop. Alternatively, a series of pre-assigned positions can be programmed into the computer in advance, which results in an automatic operation of the optical tweezers.

4. RESULTS AND DISCUSSION

Figure 3 shows two typical pictures of a trapped bead and a group of trapped *lactoacidofilus*, separately. The image of the beads and trap was formed by the 20X objective (Nikon, MSB00201, 20X/0.40). The brightest spot in the picture indicates the center of the trap produced by the argon laser with an output power of $1W$, whereas all the other bright but smaller spots are noises due to reflection. The dark bead which is out of focus tightly occupies the center of the trap, leaving the bright bead aside. It can be seen that the diameter of the beads is approximately $75\mu m$, which is calibrated with respect to the $100\mu m \times 100\mu m$ grids coated on the slide (Nakamura, A05-1220 OM-500N). Similarly, the image of the *lactoacidofilus* was formed by the 100X objective (Nikon, MSB01901, 100X/1.25, Oil). However, the argon laser was operated at a lower output power of $0.1W$. This is because the *lactoacidofilus*, only a few microns in length, is much smaller than the bead. Moreover, it is so small that a group of *lactoacidofilus* can be trapped at a time.



(a) Beads (b) *Lactoacidofilus*

Fig. 3 The images of (a) a trapped bead and (b) a group of trapped *lactoacidofilus*

Figure 4 illustrates the operation of the automated optical tweezers developed in this work. We used the 20X objective to have a relatively large field of view for the smaller beads to travel within. Note that there are two auxiliary

crossed cursors superimposed on top of the image: namely, a target cursor and a joystick cursor. The target cursor is automatically fixed over the trapped bead at the brightest spot. The joystick cursor is free to move with the steering of the joystick until we push a stop button on the stick. Once the moving joystick cursor is fixed at a new location, the trapped bead along with its target cursor starts to shift toward this destination. The numerical values of the coordinates of the two cursors are real-time processed by the computer and displayed on the screen. Accordingly, the two motorized actuators are independently driven by the motion controller to tilt the 45° reflection mirror, which is controlled by the computer. The spindle of each actuator is controlled to travel at a speed varying from up to $250\mu m/sec$ to $50\mu m/sec$, depending on the instantaneous distance between the two cursors. We observed that the target cursor and the trapped bead always followed the joystick cursor straightly. The trapped bead moved faster for longer separation between the two cursors and slower for shorter separation between the two cursors, and stopped at the assigned location. Programmably, it could be steered around, too.

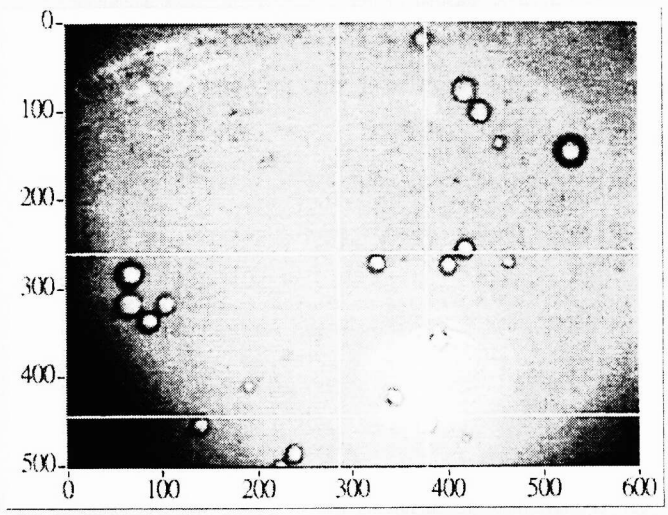


Fig. 4 The superposition of the image of a trapped bead and the two auxiliary cursors

Unfortunately, we also observed the bead pausing. Once in a while, the traveling bead encountered a very short pause and the real-time image on the computer screen was suspended simultaneously. Consequently, the observed travelling speed of the trapped bead was slower than the predicted according to Eq. (2). Nevertheless, we have improved this problem by deleting the sub-program for the real-time image display from the LabVIEW 5.0 main program. The manipulation of the trapped bead was smooth again, which was confirmed from another monitor and from the recording of the VCR. Therefore, we believe that the pause problem is due to insufficient computer speed. Using a fast computer with an efficient central processing unit (CPU) to enhance the image processing and real-time monitoring will solve this problem.

5. CONCLUSION

We automatized an optical tweezers for a precise and convenient manipulation of micrometer-sized objects. The operation of the automated optical tweezers, consisting of a modified optical tweezers, a monitoring and recording system, and a computer control system, is manual and programmbale. In short, we used two motorized actuators to deflect the laser beam and manipulate the trapped objects, while using the computer control system along with a joystick to control the actuators. We showed the trapping of a single bead and a group of *lactoacidofilus*, separately, by this optical tweezers using an argon laser. With the aid of a joystick and two auxiliary cursors superimposed on the real-time image of the trapped bead, we also demonstrated the operation of the automated optical tweezers. The increments for the bead to shift at a time, $0.04\mu m$ for the 20X objective and $0.008\mu m$ for the 100X objective, are beyond the resolution of the microscope and thus negligible. The manipulation of the bead was fairly smooth and accurate except for some occasional pauses due to insufficient computer speed. We believe that the pause problem can be easily solved with a fast computer.

The automated optical tweezers is designed to meet the future demand for manipulating smaller and smaller objects[14], especially in the fields of molecular biology and biotechnology[15]. This technique may be applied to accelerate the DNA hybridization in a DNA chip in our future work. The combination of a traditional optical tweezers with our computer control system also provides a tool for accurate manipulation of micro particles in many scientific fields.

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