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Three-dimensional microscope tracking system using the astigmatic lens method and a profile sensor

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Abstract

We developed a three-dimensional microscope tracking system using the astigmatic lens method and a profile sensor, which provides three-dimensional position detection over a wide range at the rate of 3.2 kHz. First, we confirmed the range of target detection of the developed system, where the range of target detection was shown to be $\pm 90\ \mu m$ in the horizontal plane and $\pm 9\ \mu m$ in the vertical plane for a 10× objective lens. Next, we attempted to track a motion-controlled target. The developed system kept the target at the center of the field of view and in focus up to a target speed of 50 μm/s for a 20× objective lens. Finally, we tracked a freely moving target. We successfully demonstrated the tracking of a 10-μm-diameter polystyrene bead suspended in water for 40 min. The target was kept in the range of approximately 4.9 μm around the center of the field of view. In addition, the vertical direction was maintained in the range of $\pm 0.84\ \mu m$, which was sufficiently within the depth of focus.

1 Introduction

Optical microscopes are powerful tools for observing minute objects, such as microorganisms, tissues, and cells. These microscopes are currently indispensable in the life sciences. They can magnify and visualize small objects that cannot be identified with the naked eye. However, the field of view and the depth of field are very narrow. Hence, it is difficult to observe freely moving objects over long durations. Therefore, a tracking technique that automatically maintains an object inside the field of view while keeping it in focus is desired.

Berg developed a three-dimensional tracking microscope for observing *E. coli* in 1971 [1]. It was the first tracking microscope. The object position was detected by using six optical fibers that collected scattered light from different positions. This tracking microscope has been used for research such as the motion of cells and bacteria [2].

Recently, tracking microscopes using high-speed cameras and real-time image processing techniques have been developed [3–5]. Tracking systems using high-speed cameras have a wide range for position detection compared with the first tracking microscope, but the computational cost of the image processing is huge. The algorithm for depth position detection is especially complicated.

In our research, we developed a three-dimensional microscope tracking system using the astigmatic lens method and a profile sensor. The astigmatic lens method is the most common tracking method used in current optical drive systems devised by Bricot et al. [6, 7], which provide three-dimensional target positioning. In the astigmatism lens method, a quadrant photodiode is usually used. Though this method can easily detect the three-dimensional target position, the detection area is quite narrow, especially in the transverse direction to the optical axis. A profile sensor is a type of image sensor that provides only projection profiles in two directions. Because of the small amount of data, the data acquisition rate is much higher than the normal image sensors. By combining the astigmatism lens method with a profile sensor, the high-speed detection of a three-dimensional target position can be achieved in a wide area. A wide detection area helps to track a rapidly moving target and to begin

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tracking a target that is not at the center. Herein, we demonstrate the three-dimensional tracking of motion-controlled and freely moving polystyrene beads in pure water using this system.

2 Experimental setup

Figure 1 shows the schematic diagram of the developed microscope tracking system. Light from a laser diode (LD) with a wavelength of 532 nm was illuminated around the target. The scattered light from the target was reflected by a dichroic mirror and was imaged on a profile sensor (Hamamatsu Photonics K.K. S9132, 2×2 mm area: 256×256 pixels) by an imaging lens. A cylindrical lens placed behind the imaging lens created the astigmatism. The three-dimensional position was estimated from the position and the shape of the target image. The detected projection profiles in the X and Y directions were fed to a PC. The PC controlled the microscope XY stage (OptSigma BIOS-Light, resolution: 0.1 μm) and the Z piezo stage (OptSigma SFAI-OBL-1R, resolution: 0.01 μm) attached to an objective lens so that the target was in the center of the field of view and in focus. Another camera (OptSigma STC-MCA5MUSB3) was attached to the camera port of the microscope (Olympus IX71) to record the microscopic observation. The size of the image sensor in the camera was 5.7×4.2 mm.

The procedure for estimating the three-dimensional position was as follows. The image position (x, y) corresponded to the target position (X, Y). The shape of the image changed to a vertically long ellipse, a circular shape, and a horizontally long ellipse, depending on target position Z, as shown in Fig. 2. The image positions of the projection profiles in the x and y directions provided the target positions X and Y, respectively. The ratio of the peak intensities of the projection profiles provided target position Z. The frame rate of
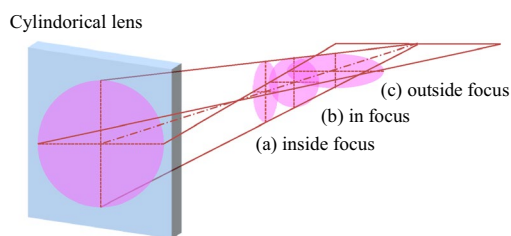

**Fig. 2** Representation of the astigmatic lens method

the profile sensor was 3.2 kHz. Position estimation could be carried out at almost the same rate.

For the experiment of tracking of a motion-controlled target, another XZ stage was mounted on the microscope XY stage, as shown in Fig. 3. The sample chamber was the XZ stage and the target was fixed on the sample chamber. We thus controlled the speed, direction, and movement distance of the target.

3 Results and discussion

First, we confirmed that the developed system could estimate the three-dimensional target position over a wide range. We used a polystyrene latex bead with a diameter of 45 μm as a target, which was fixed at the bottom of sample chamber. A 10× objective lens was used for observing the target. The field of view of the microscope was approximately 0.57×0.42 mm. However, the detection range of the target position in the XY plane was approximately 180×180 μm. Figure 4a, b shows a target image captured by the microscope camera and a screenshot of the software of the profile sensor, respectively. In Fig. 4b, the bottom and right graphs denote the projection profiles in the X and Y directions, respectively. In these figures, the bead was at the center of
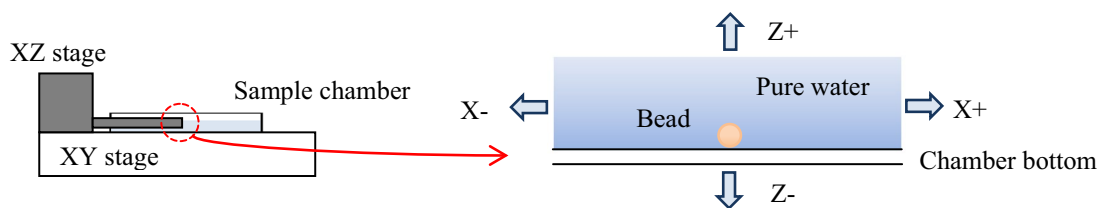
Fig. 1 Schematic diagram of the developed tracking system
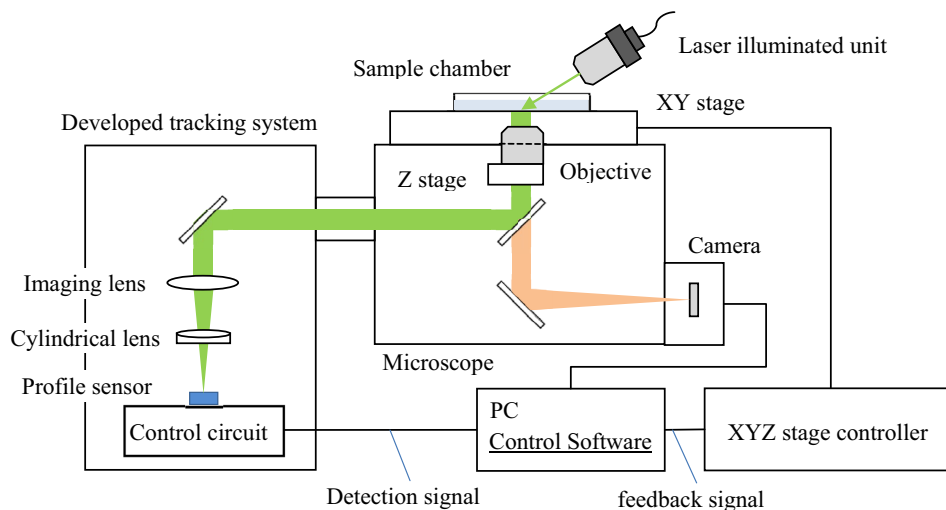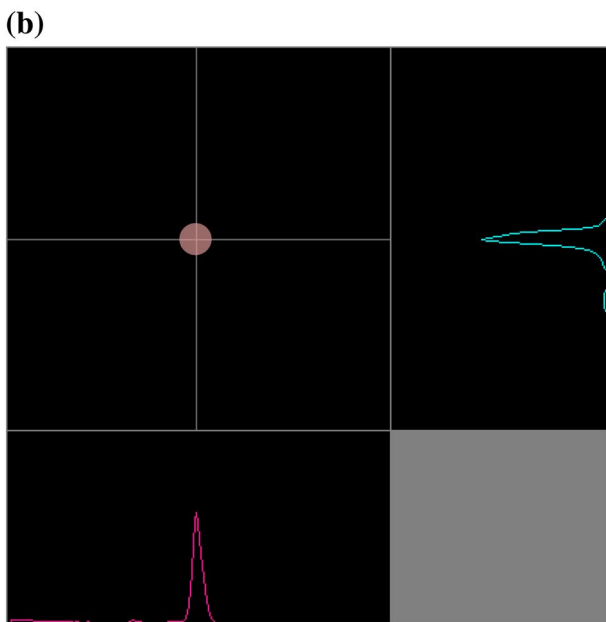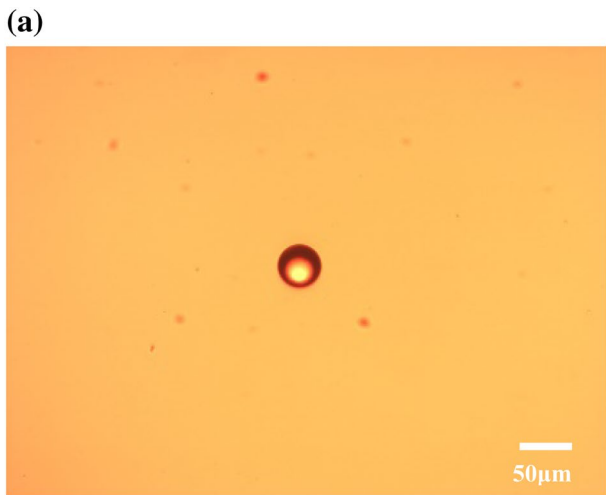
Fig. 3 Representation of the sample control system

(a)

(b)

Fig. 4 a Camera image of the target and **b** capture image obtained with the software of the profile sensor

Fig. 5 Peak positions of x and y in the projection profiles against the target positions X and Y, respectively

Fig. 6 Peak intensities of the projection profiles along the x and y directions against the depth direction target position Z

the camera image and the peak positions of the projection profiles were also at the center. Figure 5 shows the peak positions of the projection profiles when the target position changed in the XY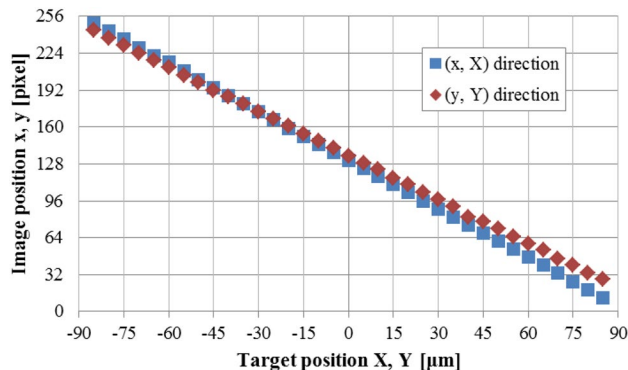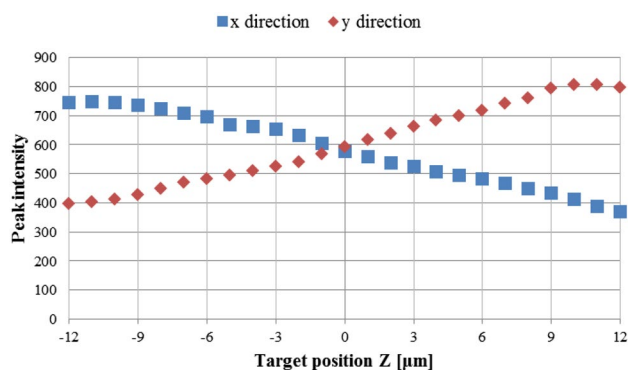 plane. The symbol filled square shows the image position x on a profile sensor, when the X of the target position was changed from −90 to 90 μm, and the Y of the target position was 0. The symbol filled diamond shows the image position y on a profile sensor, when the Y of the target position was changed from −90 to 90 μm, and the X of the target position was 0. We confirmed that the peak positions of the projection profiles corresponded to X and Y of the target position over the entire detection area. Figure 6 shows the peak intensities of the projection profiles in the x and y directions when the target position changed in the Z direction. The target was located at the center of
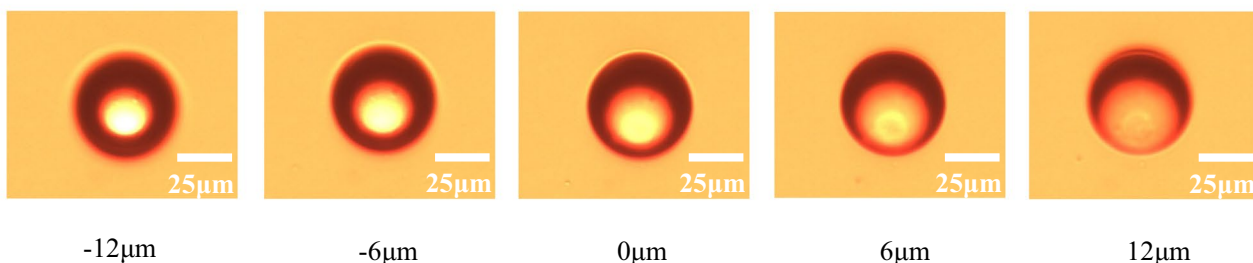
Fig. 7 Camera images of the target scanned in the depth direction

the field of view. Figure 7 shows the camera images of the target at $Z = -12, -6, 0, 6, 12$ μm. A slight blurring was observed. When the target was in focus, the peak intensities of the projection profile in the x and y directions were the same. By changing the target position to be out of focus, one projection profile became sharp and the peak intensity increased. In contrast, the other projection profile broadened and the peak intensity decreased. This was the effect of the astigmatism lens method and the profile sensor. It should be noted that the projection profile in the x and y directions is the summations along the y and x directions, respectively. Therefore, when a projection profile becomes sharp, the peak intensity increases. For normal image sensors, such a simple relation between the width and the peak intensity does not exist. Using a profile sensor, we can detect the shape of the image from the peak intensities of the projection profile in the x and y directions. Over the range of Z positions from -9 to 9 μm, the peak intensities of the projection profile in the X and Y directions linearly decreased and linearly increased with an increase of the Z target position. We could accurately estimate the Z target position from the ratio of the peak intensities. We confirmed that the target estimation could be performed over the range of -90 μm $< X < 90$ μm, -90 μm $< Y < 90$ μm, and -9 μm $< Z < 9$ μm for the 10× objective lens.

Next, we attempted tracking a motion-controlled target. As a sample, polystyrene beads of φ 10 μm fixed to the bottom of the sample chamber were used. The target motion was controlled by the XZ stage to which the sample chamber was attached. A 20× objective lens was used for observing the target. The field of view of the observation camera and the detection range of the profile sensor were 0.285×0.210 mm and 90×90 μm, respectively. Figure 8 shows the temporal changes in the target position x estimated from the profile sensor and the target position X from the control signal of the XZ stage. The target was moved from $X = 0$ to $X = 1000$ μm at a speed of 10 μm/s. The target was kept in the range of approximately 10.5 μm around the center of the field of view. We confirmed that the developed system kept the target at the center of the field of view and in focus up to a target speed of 50 μm/s for a 20× objective lens.
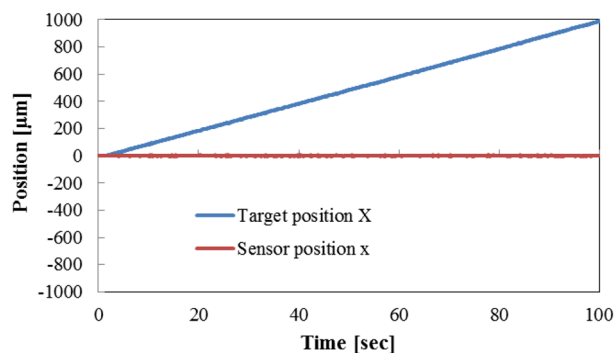

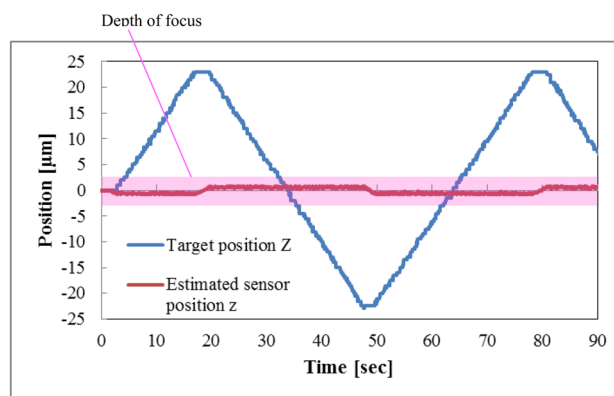**Fig. 8** Temporal changes of the target (fixed bead) position X and sensor position x

Fig. 9 Temporal changes of the target (fixed bead) position Z and estimated sensor position z

Figure 9 shows the temporal changes in the target position z estimated from the profile sensor and the target position Z from the control signal of the XZ stage. The target moved from $Z = -24$ μm to $Z = 24$ μm at the speed of 10 μm/s. In the Z direction, we set a dead zone for the tracking control to avoid oscillation of the objective lens. When the ratio of the peak intensities in the projection profile in the x and y directions was over 1.2 or under 0.8, which corresponded to $z = 0.93$ and 0.89 μm, the objective lens was moved, so that

the target was at the center of the focus. This did not affect the microscopic images, because the dead zone was smaller than the depth of focus. It was confirmed that the target was kept in focus.

Finally, we tracked a freely moving target. We used polystyrene latex beads with a diameter of 10 µm suspended in pure water. The polystyrene latex beads were randomly moving owing to Brownian motion. Figure 10a shows the trajectory of the target bead for 40 min. The trajectory was obtained from the control signals of the microscope's XY stage and Z stage. Figure 10b–d shows the temporal changes of the target position (x, X), (y, Y), and (z, Z), respectively, estimated from the profile sensor and from the control signal of the XZ stage. It was confirmed that the target was kept in the center of the field of view and the center of the focus for 40 min and the trajectory of the target beads and microscopic images could be recorded. The movement distance in the X, Y, and Z directions for 40 min were 70, 20, and 5 µm, respectively. The target was kept in the range of approximately 4.9 µm (X) and 2.4 µm (Y) around the center of the field of view. In addition, the vertical direction was maintained in the range of $-0.84 \leq z \leq 0.82$ µm. This was also sufficiently within the depth of focus.

4 Conclusion

We developed a three-dimensional microscope tracking system using the astigmatic lens method and a profile sensor. The range of target detection was -90 µm $< X < 90$ µm, -90 µm $< Y < 90$ µm, and -9 µm $< Z < 9$ µm when a 10× objective lens was used to observe the target. The developed system can keep the target at the center of the field of view and in focus up to a target speed of 50 µm/s for the 20× objective lens. Thus, by combining the astigmatism lens method and a profile sensor, the high-speed detection of a three-dimensional target position is achieved in a wide area. The wide detection area helps to track a rapidly moving target and to start tracking of a target that is not at the center.

Using the developed tracking system, we demonstrated the tracking of a 10 µm-diameter polystyrene bead suspended in water for 40 min. The use of the proposed method is limited to simple spherical targets such as Chlamydomonas. Fluorescence protein and beads and quantum dots are also applicable targets. By attaching or embedding a fluorescence marker, the developed tracking system can be applied to arbitrarily shape targets. The proposed method has another limitation where only one target can exist in the
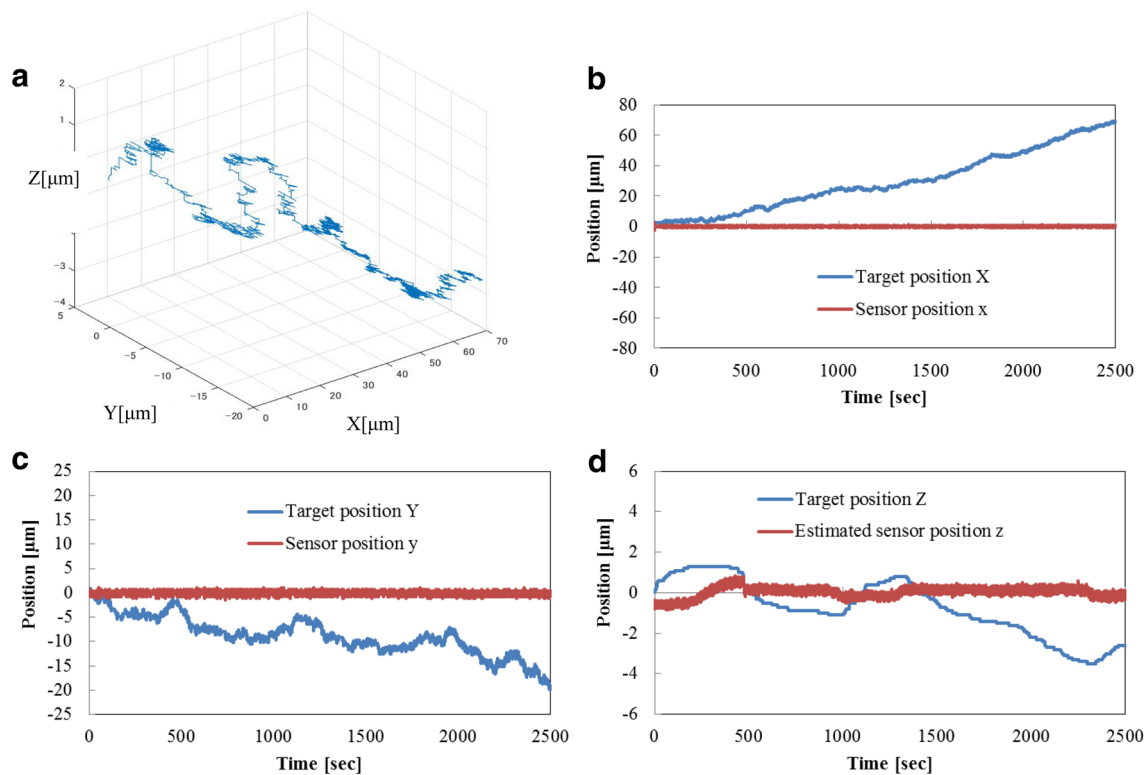

Fig. 10 a Trajectory of the target (floating bead), temporal change of b the target position X and sensor position x, c the target position Y and sensor position y, and d the target position Z and estimated sensor position z

detection area. For fluorescence markers, such a condition can be easily realized.

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