Influence of the Condenser on Sample Tracking via Forward Scattering Pattern Detection

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ABSTRACT

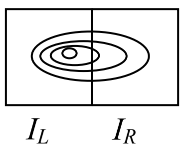
Sample tracking with a high spatial sensitivity is highly desired in force measurement with optical tweezers. However, the trick that sample tracking via forward scattering pattern detection would provide a higher sensitivity than that via regular image detection has never been investigated. In this paper, we systematically study the influences of the position and the numerical aperture of the condenser on sample tracking via forward scattering pattern detection. In our experiment, a 60X condenser is used to form the forward scattering pattern of a sample bead upon a CCD camera. As the bead is transversely shifted at a step size of 30nm by a PZT XYZ stage, we measure the magnitude of the corresponding shift of the forward scattering pattern when the 60X condenser of different angular apertures is placed at various locations along the optical axis. Our result shows that the most sensitive forward scattering pattern occurs when the condenser collimates the forward scattering light from the sample bead. We also find that the larger the numerical aperture is, the higher the sensitivity of forward scattering pattern detection will be.

Keywords: optical tweezers, sample tracking, forward scattering pattern, condenser, and numerical aperture

1. INTRODUCTION

In 1970s, Ashkin first introduced optical trapping of particles at micrometer–scale by radiation pressure [1]. Later in 1986, Ashkin further invented the first optical tweezers [2] to trap and manipulate small particles within tens of nm to tens of μm range with a single laser. The non-invasive and non-mechanical contacts features of optical tweezers have been widely used in biology, physics, and material science experiments ever since. Recently, Stelzer developed an optical-tweezers-based photonic force microscope (PFM) for three dimensional (3-D) morphological scans of a small object by monitoring the forward scattering pattern of the object[3, 4].

PFM is an advanced version of optical tweezers. It uses the trapped-bead as a probe for 3-D morphological scan. By scanning the probe bead over the surface of a sample and tracking the position of the probe bead, PFM is able to generate the 3-D morphology of the sample. To track the position of the scanning probe bead, the forward scattering pattern of the laser light is detected. While the bead has a relative displacement to the laser focus, the forward scattering pattern shifts as well. Therefore, the more sensitivity we distinguish the shift of the forward scattering pattern, the higher resolution we get the displacement of the bead to the laser focus. The concept to detect the bead displacement by forward scattering pattern is described in Fig. 1.



$$\text{Signal } x' \equiv \frac{I_R - I_L}{I_R + I_L}$$

Fig.1 The concept for the calculation of the shift x' of the forward scattering pattern .We use a program of LabVIEW application software to calculate the shift of the scattering pattern. Firstly, the program separates each frame of the taped images into left and right parts. Secondly, The intensities of left and right parts will be summed as I_L and I_R. At last, we calculate the shift x′ of each scattering pattern.

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Usually, a quadrant photo detector (QPD) or a CCD Camera placed behind the microscope condenser is used to detect the forward scattering pattern. However, the influence of condenser that collects the forward scattering is seldom been discussed. Therefore, in this report, we focus on the influence of condenser to the sensitivity to detect the bead forward scattering pattern. In section 2, we discuss the influence of the condenser position to the sensitivity of the bead displacement by detecting forward scattering pattern shifts. In section 3, we discuss the influence of the numerical aperture (N.A.) of the condenser to the sensitivity of the bead displacement by detecting forward scattering pattern shifts.

2. THE INFLUENCE OF THE POSITION OF THE CONDENSER ON SAMPLE TRACKING VIA FORWARD SCATTERING PATTERN DETECTION

In this experiment, we systematically study the influence of the condenser position to the sensitivity of the bead displacement by detecting forward scattering pattern shifts. We fixed a 1.1-μm-in-diameter polystyrene bead on the coverslip that is controlled by a PZT stage. Then we moved the coverslip to let the fixed bead scans across the laser focus. While the bead scans across the laser focus, the forward scattering pattern shifts corresponding to the displacement between the bead and the center of laser focus. We record a sequence of forward scattering patterns by a CCD camera and calculate their shifts corresponding to a sequence of displacements between bead and the center of laser focus. The sensitivity of the bead displacement by detecting forward scattering pattern shifts is defined to the slope between the shifts of forward scattering pattern and the bead's displacement. At last, we change several positions of the condenser and measure the corresponding sensitivities of the bead displacement by detecting forward scattering patterns. By comparing the corresponding sensitivities when the condenser is placed at various locations, we can find the optimized position for the condenser to track the bead wobbling in an optical-tweezers trap with a CCD camera.

2.1 Setup

The setup of the experiment for the influence of condenser's position on sample tracking (which is the probe bead tracking) via forward scattering pattern detection system consists of a He-Ne laser, a lens, a dichroic beamsplitter, an objective lens, a glass slide, a PZT XYZ stage, a condenser, a polarizor, two CCD cameras, and a computer. As shown in Fig. 2, the lens (L1) slightly diverges the He-Ne laser beam via the dichroic beamsplitter (DM1) into the 100x objective lens (OL). This objective lens in turn focuses the incident beam to illuminate a bead on the glass slide on top of the PZT XYZ stage with a precision within 30*nm*. A CCD camera (CCD1) is placed on the image plane of the objective lens to monitor the target bead. On the other side of the glass slide, the 60X condenser lens (CL) is used to form a forward scattering pattern of the bead upon another CCD camera (CCD2). The intensity of forward scattering pattern on the CCD2 may be adjusted by the polarizor (PL), which is located in front of the CCD2.

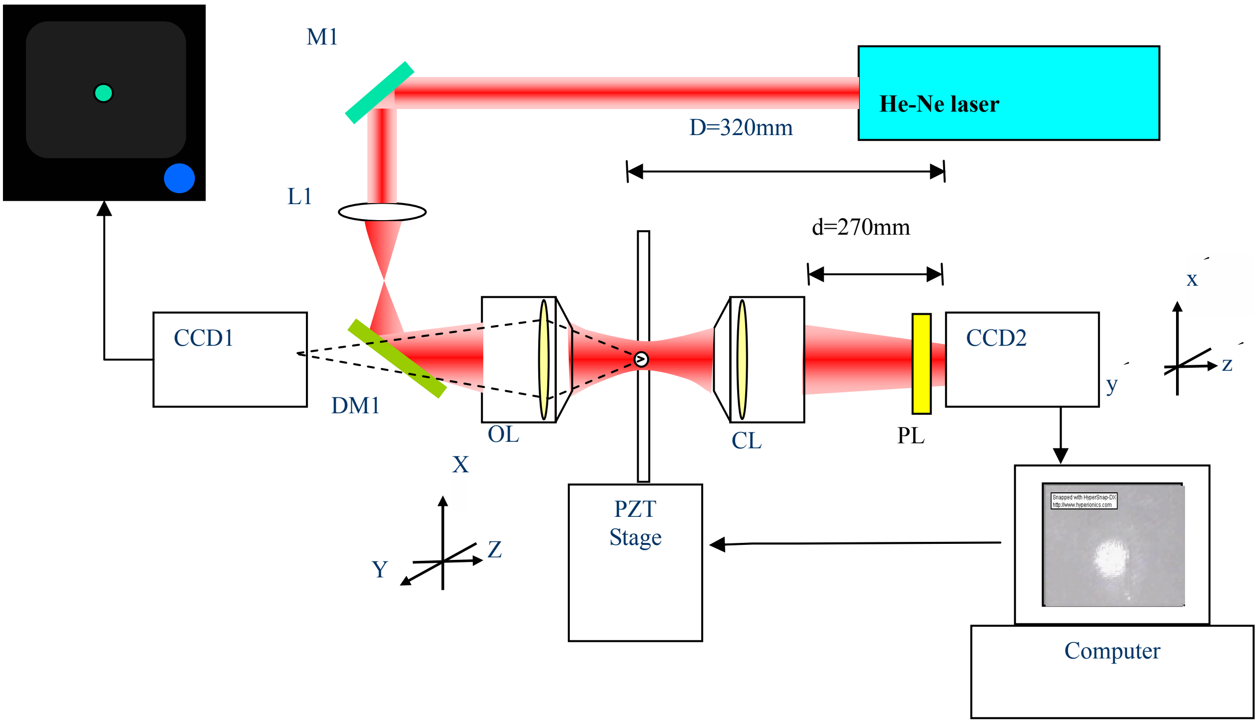
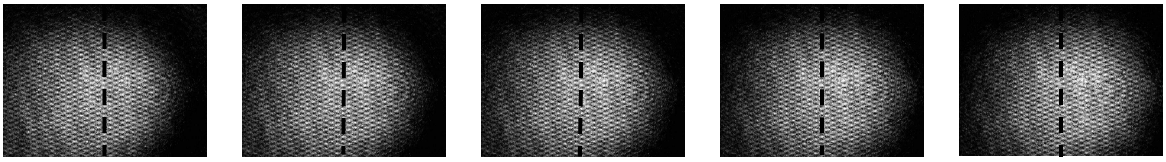


Fig.2 The setup of the experiment for the influence of position of the condenser on sample tracking via forward scattering pattern detection

2.2 Method

In this experiment, we fixed a 1.1-μm-in-diameter polystyrene bead on the coverslip that is controlled by a PZT stage. The detailed operation procedure is listed as the followings:

1. We focus the optical tweezers to a single polystyrene bead and place the CCD2 at a distance of 320 mm (D=320mm) away from the target bead.
2. The position of the condenser is adjusted so that the condenser and the objective lens are confocal .Then the forward scattering laser light is collimated. The distance between the condenser and CCD2 is 270mm (d=270mm) at this position.
3. We move the target bead in x direction with the PZT XYZ stage at a step size of 60nm. In the mean time, we tape the forward scattering patterns of the bead at every single step, as shown in Fig. 3.
4. We increase the distance between the condenser lens and CCD2 by 5μm for several experiments. In every experiment, we repeat step 3.
5. We calculation the shift x' of each scattering pattern according to the concept that detect the bead displacement by forward scattering pattern is described in Fig. 1.



x=-120nm x=-60nm x=0nm x=60nm x=120nm

Fig.3 The scattering patterns of the bead at every single step of movement in *x* direction. The step size of the PZT XYZ stage is set to 60*nm*.

2.3 Results

We define the displacement of bead is *x* and the corresponding shift of the scattering pattern is x′. According to the concept as shown in Fig. 1, Fig. 4 shows the relationship between the shift x′ and x. As shown in Fig. 4, within the region nearby the center of laser spot, we can find the shift x′ is a linear function of x. So we calculate the slopes of each curve's in the linear region. Note that the higher the slope represents the higher detecting sensitivity of the bead's displacement.

Next, we define Δd = 0 where the position of the condenser is confocal with the objective lens. For Δd < 0, the condenser moves toward the bead and diverges the forward scattering light, and vice versa. Fig. 5 shows the different slopes of the curves obtained in Fig.4 at different condenser position. The result reveals the position dependence of the condenser on sample tracking via forward scattering pattern detection. As shown in Fig. 5, the maximum slope occurs when Δd = 0, which indicates the condenser is confocal with objective lens. Fig. 6 shows the various forward scattering patterns when the condenser is placed at different positions.

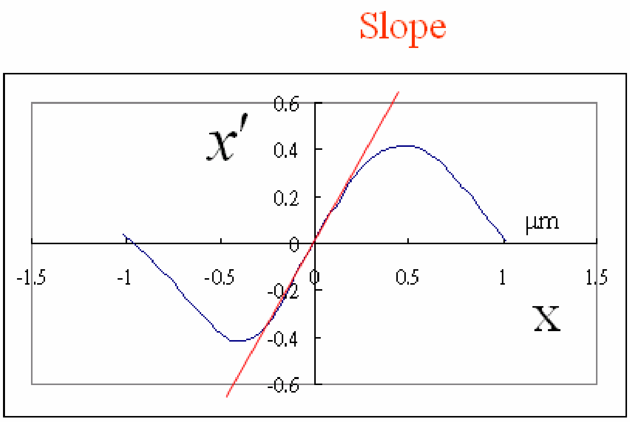


Fig.4 The shift of the scattering pattern x′ versus the displacement of the bead x. The slope within the linear region nearby the center of laser spot represents the sensitivity to detect the bead's displacement by forward scattering pattern.

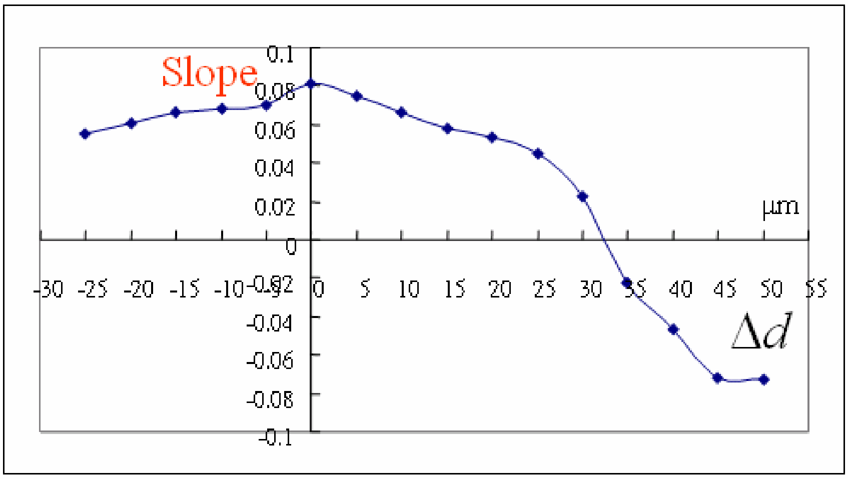


Fig.5 The different slopes of the curves obtained in Fig.4 at different condenser position. The maximum slope occurs when Δd = 0, which indicates the highest sensitivity to detect the bead's displacement by forward scattering pattern occurs when the condenser is confocal with the condenser.

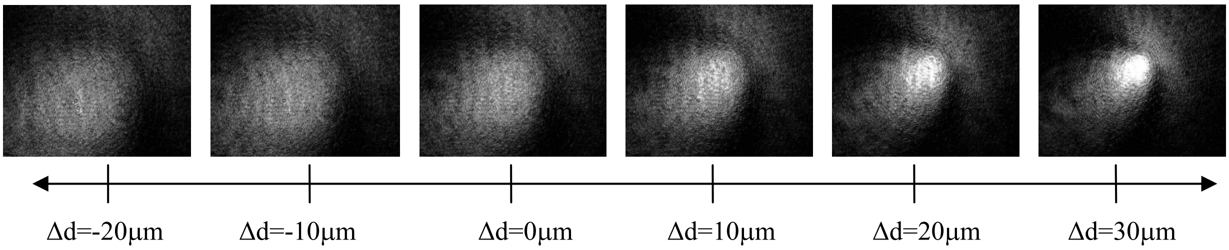


Fig.6 Various forward scattering patterns with the condenser at different positions.

2.4 Conclusion

We experimentally prove that the forward scattering pattern is affected strongly by the position of the condenser as shown in Fig.6. We also find the highest sensitivity to detect the bead's displacement by forward scattering pattern shifts occurs when the condenser and objective lens are confocal, then the condenser collimates the forward scattering light from the bead in a focused laser beam. This is actually agrees with several reports. Therefore, when we construct a PFM system afterwards, we know the best position for the condenser to obtain the most sensitive signal response to the wobbling of the small object from a quadrant photo detector.

3. THE INFLUENCE OF NUMERICAL APERTURE OF THE CONDENSER ON SAMPLE TRACKING VIA FORWARD SCATTERING PATTERN DETECTION

The light-gathering capability of the condenser is called the numerical aperture (N.A.). In this experiment, we study the influence of the numerical aperture of condenser to the sensitivity of the bead's displacement by detecting forward scattering pattern shifts. As the methods described in section 2, we also fixed a 1.1-μm-in-diameter polystyrene bead on the coverslip and scanned the bead across laser spot to measure the corresponding forward scattering pattern shifts. The sensitivity of the bead displacement by detecting forward scattering pattern shifts is defined to the slope between the shifts of forward scattering pattern and the bead's displacement as described in Fig.4. Then we change the numerical aperture of our condenser and record the corresponding detecting sensitivity of the bead's displacement. By comparing the corresponding sensitivities with different numerical aperture of our condenser, we can find the optimized numerical aperture for the condenser to track the bead with a CCD camera.

The numerical aperture (N.A.) is the product of light-gathering angle and refractive index n: N.A.=$n\sin\theta$. The θ is the diverging angle of the forward scattering light after laser focus as shown in Fig.7. Therefore, to alter the numerical aperture, we can simply change the diverging angleθ. Since the beam spot size on the back focal plane (BFP) of the condenser is $2f_{cl}\sin\theta$, we can control the N.A. of the condenser by controlling the back aperture of the condenser.

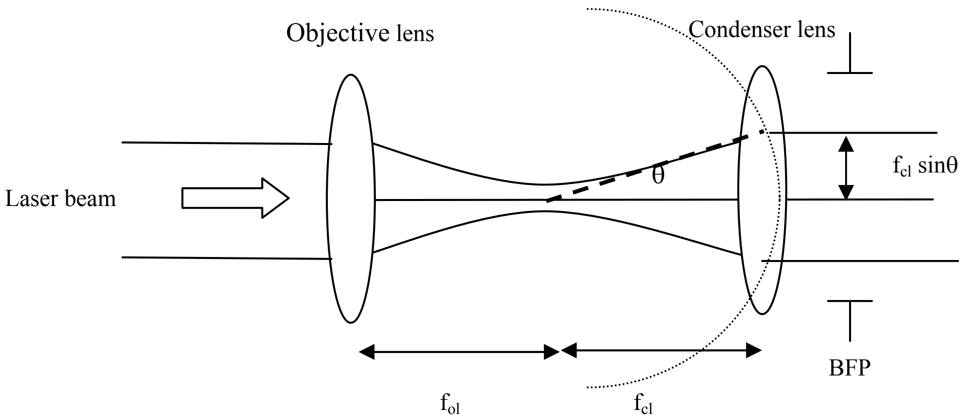


Fig.7 Focus geometry of the laser beam through the objective lens and the condenser lens. The objective lens and the condenser lens are confocal. The divergence angle of the forward scattering light is θ. The focal length of the condenser is f_{cl}. The beam spot size on the BFP of the condenser is $2f_{cl}\sin\theta$.

However, the condenser which we use is a general microscope objective lens, so the back focal plane of the condenser is inside its mechanical body. Therefore, we can not image the BFP of the condenser by a CCD camera directly. So we image the BFP of the condenser to the CCD camera by using an imaging lens. The magnification of the imaging lens is 1 for simplicity.

To control the back apeture of the condenser in our experiment, we use another substitution method by image processing program. When we alter the processing region of the forward scattering pattern in our program, it equavalently means to alter the back apeture of the condenser. So, it is very convienet for us to study the influence of the condenser N.A. to the sensitivity of the bead's displacement by forward scattering pattern. .

3.1 Setup

The setup of the experiment for the influence of condenser's numerical aperture on sample tracking via forward scattering pattern is shown in Fig 8. The imaging lens is placed between the condenser lens and CCD camara(CCD2) . The intervals between the condenser lens(CL), the imaging lens and the CCD camera are 2-folds of the focal length of the imaging lens. Therefore the imaging lens image the back focal plane of the condenser by 1-fold on the CCD Camera.

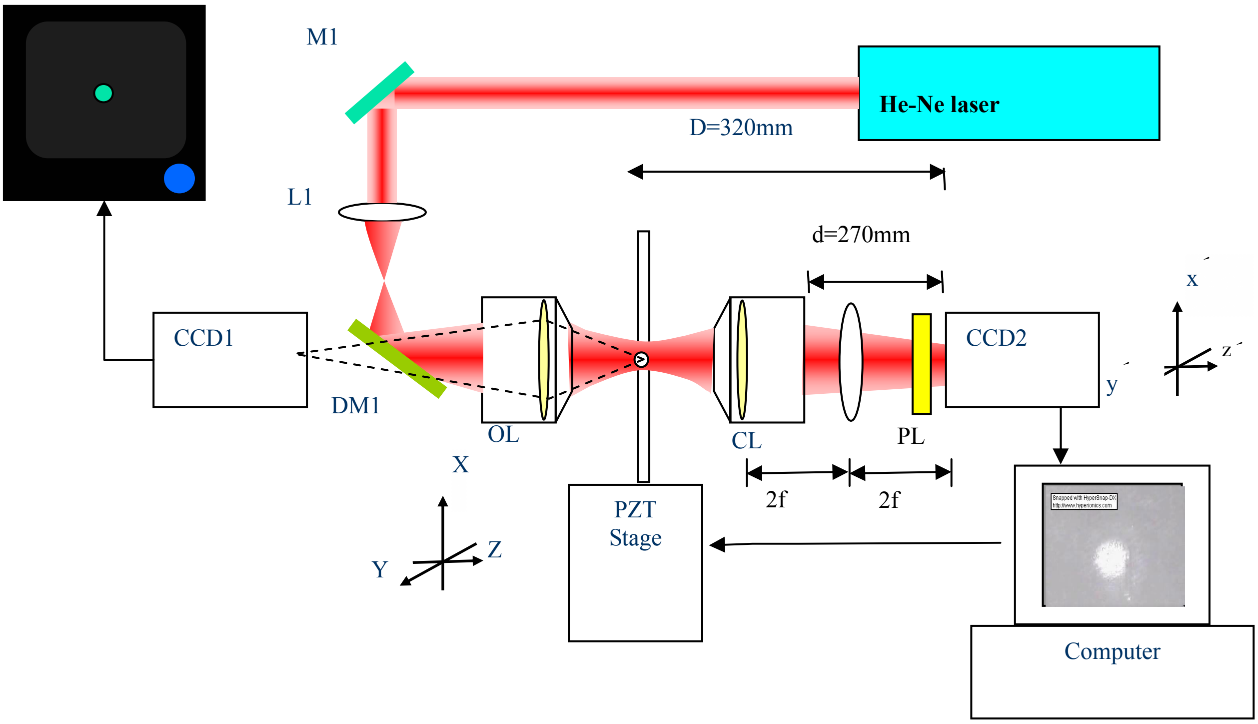


Fig.8 The setup of the experiment for the influence of condenser's numerical aperture on sample tracking via forward scattering pattern

3.2 Method

In this experiment, we fixed a 1.1-μm-in-diameter polystyrene bead on the coverslip. The detailed operation procedure is listed as the followings:

1. We focus the optical tweezers to a single polystyrene bead and place the CCD2 at a distance of 320 mm (D=320mm) away from the target bead.
2. The position of the condenser lens is adjusted so that the condenser lens and the objective lens are confocal .Then the forward scattering laser light is collimated. The distance between the condenser lens and CCD2 is 270mm (d=270mm) at this position.
3. we image the BFP of the condenser to the CCD camera by using an imaging lens. The magnification of the imaging lens is 1 for simplicity.
4. We move the target bead in x direction with the PZT XYZ stage at a step size of 60nm. In the mean time, we tape the forward scattering patterns of the bead at every single step.
5. We use a program of LabVIEW to calculate the shift x' of each scattering pattern according to the concept that detect the bead displacement by forward scattering pattern is described in Fig. 1 .
6. We use a program of LabVIEW to alter the processing region of the forward scattering pattern for several times and repeat step 5. The different processing region of the forward scattering pattern represents the different numerical aperture of the condenser.

3.3 Results

We define the displacement of bead is *x* and the corresponding shift of the scattering pattern is x′ according to the concept as shown in Fig. 1. In addition, the condenser and objective lens are confocal. Fig. 9 shows 6 curves of the shift x′ as a function of x, which each curves represents a different numerical aperture for condenser. We also calculate the slopes of each curve's within the region nearby the center of laser spot where the shift x′ is a linear function of x linear function. And Fig. 10 shows the slopes within the linear region of each curve's obtained in Fig.9, which reveals the numerical aperture dependence of the condenser on tracking the bead via forward scattering pattern detection. As shown in Fig. 10, the higher the value of the numerical aperture, the higher the slopes. As a result, the higher the numerical aperture of the condenser, the higher the sensitivity to track the bead via forward scattering pattern.

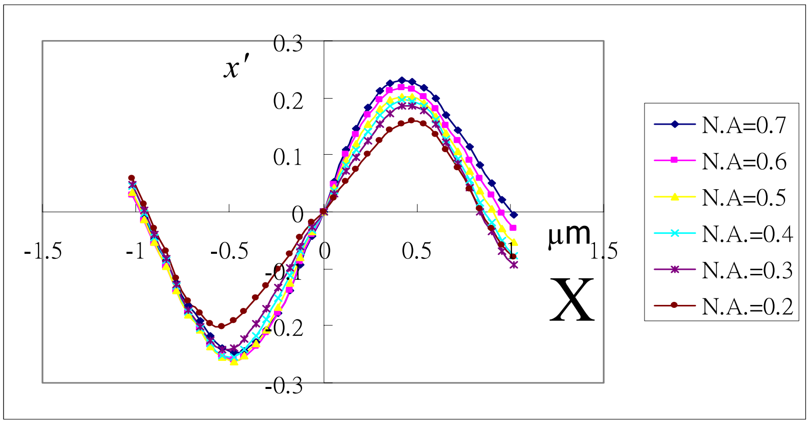


Fig.9 6 curves of the shifts of the scattering pattern x′ versus the displacement of the bead x under different numerical aperture of the condenser.

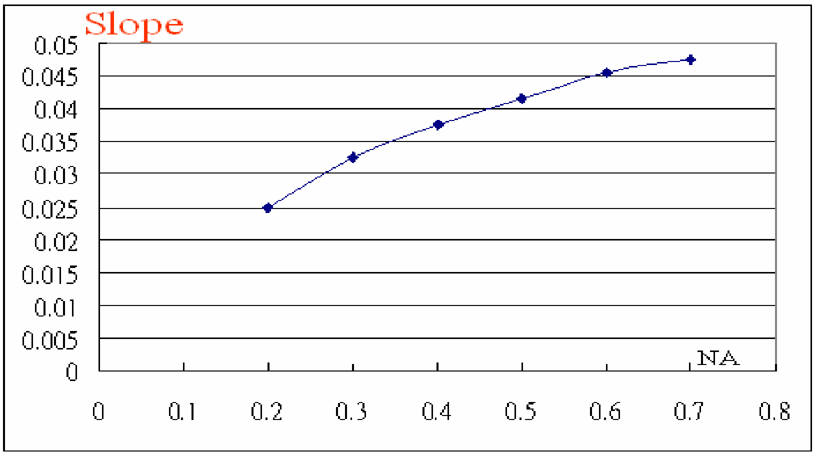


Fig.10 The influence of the numerical aperture of the condenser to the sensitivity to track the bead via forward scattering pattern. As it shows, the e higher the numerical aperture of the condenser, the higher the sensitivity.

3.4 Conclusion

We experimentally prove that the forward scattering pattern is affected strongly by the numerical aperture of the condenser. We also conclude that the value of numerical aperture is higher; the higher the sensitivity to track the bead via forward scattering pattern. This is actually agrees with several reports. Therefore, when we construct a PFM system afterwards, we know the best numerical aperture for the condenser to obtain the most sensitive signal response to the wobbling of the small object from a quadrant photo detector.

4. SUMMARY

In this report, we study on the influence of condenser on sample tracking via forward scattering pattern in two parts, the position and the numerical aperture of condenser. In the first experiment, we conclude the best forward scattering pattern occurs when the condenser and objective lens are confocal, and the condenser collimates the forward scattering light from the bead in a focused laser beam. In the second experiment, we conclude that sensitivity to track the bead via forward scattering pattern increase with the higher value of the condenser N.A. Therefore, when we construct a PFM system afterwards, we know the optimized position and numerical aperture for the condenser to obtain the most sensitive signal response to the wobbling of the small object via observing the forward scattering pattern.

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