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Study of Microscope Autofocus Based on Weighted Light Intensity

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Abstract: Microscopes auto focusing is critically important for a great spectrum of scientific and industrial applications. Finding the best focus position for variety of surfaces under measurement proved to be challenging. In order to provide a rapid and accurate method for microscope focusing positioning, a laser focus deviate system was employed for microscope focusing position. The relationship between focusing sensitivity and the surface under measurement was investigated. First, the laser focus deviate technique is introduced in light of characteristic parameters such as the diameter of the focused spot, the laser wavelength and the distance from the optimal focus. Then, the focusing displacement versus spot position curve fitting is performed for a smooth isotropic surface to directly locate the best focusing position. Following, it is analyzed that when applied to anisotropic and rough surfaces, the centroid of the image spot after reflection/scattering from the surface will deviate from its original light intensity distribution, thus deviate from the calculated optimal focus position and cause focusing errors. Finally, a criterion of weighted light intensity value Q is introduced to achieve fast focusing of anisotropic surfaces in combination with the laser focusing deviate technique. The experimental results show that for a 20× microscope objective system, the focusing accuracy of this focusing positioning method is 1.7 μm on smooth surfaces; and is at the micro meter level for rough surfaces. It basically meets the requirements for rapid, stable and reliable microscope auto focusing needs.

Keywords: Laser Focus Deviation, Microscope Autofocus, Isotropic Surface, Anisotropic Surface, Weighted Light Intensity Q

1. Introduction

Microscopes are widely used in scientific research, ultra-precision manufacturing, and industrial automation. However, to acquire high quality and crispy clear microscope images, other than a good quality microscope optical system itself, the key is to let the microscope to work under its best focusing state. There are still a lot of research works being report in this field [1-4]. In many applications, the surface under observation may constantly varying its relative distance to the optical microscope, therefore requiring adjustment of focusing distance to maintain best focus. It will be best done through an auto focus system. In some applications, one may want to measure the height variation of the surface by adjusting the focus. Such scenario often accompany with high power microscopes. There are many approaches were proposed to tackle this autofocus requirement. These approaches can be categorized into the following groups based on the principles applied. 1) Analyzing images acquired using software algorithm to determine the best focus [5-20]; 2) based on auxiliary laser focusing spot deviation or spot diameter variation [21-24].

Auto focusing based on analyzing acquired images requiring software to acquire a large amount of images ranging across the best focusing position. The best focus position can then be found by analyzing these images. The algorithms used include gradient algorithm, edge algorithm etc. It requires the images within the entire field of view to have relatively clear picturesque characteristics [25]. To find the best focus position, requires massive data processing. It is time consuming and is not suitable for high speed auto focusing applications. Although numerous attempts and improvements were carried out [26-30], microscope auto focus still has a lot of room for improvementin areas where fast and precision focus are required. In addition to purely rely on software algorithm improvement, some hardware were added to speed up the process of reaching the best focus [31-32]. Above mentioned methods are suitable for applications where the image features are not sparse. In cases where the image is almost isotropic and homogeneous, such methods may fail to find the best focus, but in many real application scenarios, the surface under measurement may be a near isotropic, smooth, and homogeneous one. For isotropic, smooth, and homogeneous surfaces, a coaxiallycoupled laser focus deviation scheme will be suitable [21]. Such autofocus scheme has a relatively large dynamic range and by correlating the focus position with the centroid of laser focus spot, one can quickly determine the best focus position. Since the scheme relies on the image centroid of the laser focus spot, anything that may alter the centroid of spot will cause measurement error. For example, if the surface under measurement has non-uniform surface reflectivity or color, the intensity distribution of the image of the laser focus spot will deviate from its original distribution, thus, the focus position calculated based on the centroid of image will also deviate from the true center of the focus spot. Another way of determine the best focus position is to determine the waist position of a collimated laser beam through the same objective lens, however, due to the insensitivity of the waist diameter variation near focus area, the best focus position obtained from the smallest waist of laser beam may not be precise. Equation (1) shows the beam diameter of a collimated laser beam near its waist position [33].

$$d(z)=d_0\sqrt{1+\left(\frac{4\lambda z}{\pi d_0^2}\right)^2} \tag{1}$$

Here, d_0 is the waist diameter,λ is the laser wavelength, z is the distance away from the waist, d is the beam diameter near waist area. It can be seen that the beam diameter increases as |z| increases, however, the variation is relatively slow, thus, focus position variation is not very sensitive as z changes.

Above mentioned focus methods all require sampling multiple images in a relatively large focus range. They are not suitable for high speed focusing which is often demanded in production line. In this paper, a new focus scheme is described. A collimated laser beam which shares the microscope optical path is focused onto the surface under measurement. The scattered and reflected light from the surface goes back to the microscope system and forms a beam spot on an image sensor. The coarse focus position can be quickly determined by measuring the intensity center of the image spot. A weighted light intensity criterion Q will then be measured near the coarse focus position in small incremental steps. The best focus position can be determined from the peak of this weighted light intensity criterion Q.

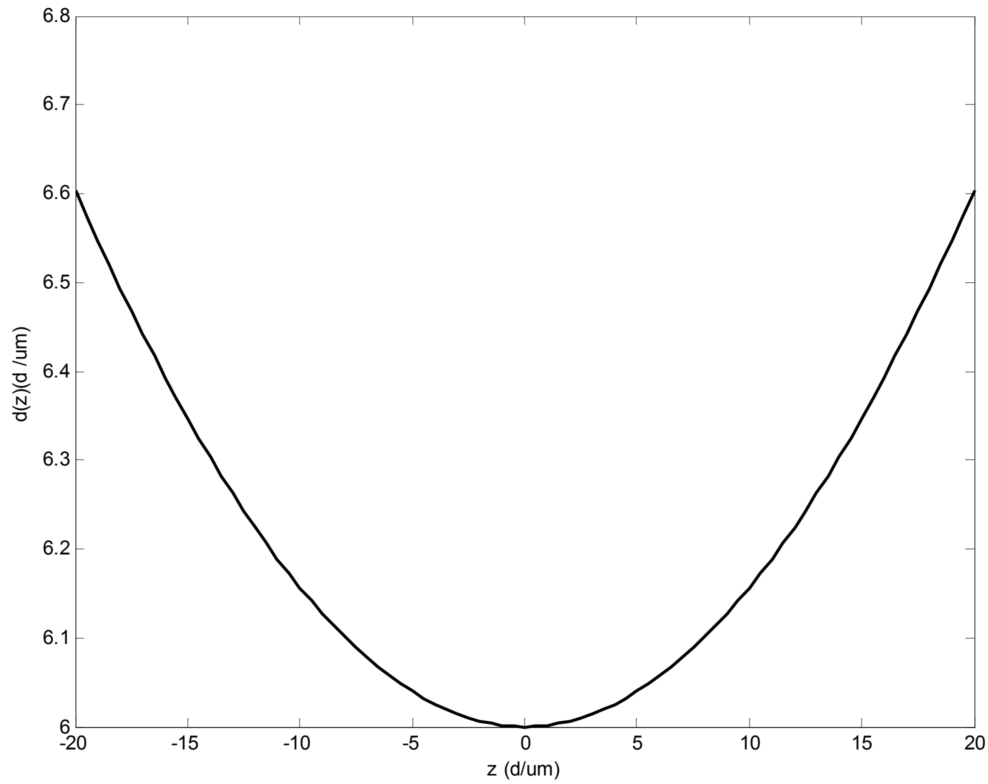

Figure 1. *Beam diameter d near the best focus position.*

2. Microscope Focus Based on Laser Focus Deviation Method

As described in laser focus deviation method [21], a collimated laser beam which is parallel to the microscope objective's optical axis, but slightly shifted laterally can be used to measure distance by measuring the lateral shift of the returned image spot on the image plane. Imbedding a laser focus deviation module into a microscope system, one can achieve autofocus for the microscope system. Figure 2 shows the schematic drawing of the optical path of such device.

As shown in Figure 2 (a), a collimated beam from a laser enters a beam splitter, the reflected beam travels parallel to the microscope optical axis with a small lateral shift. The beam is focused by the microscope objective onto the focal plane. If there is a surface placed right at this focal plane, then the scattered/reflected light will enter the objective again on the other side (as shown in Figure 2 (a)) and travel in opposite direction. After being split by the beam splitter, the transit beam passes through an imaging lens and form a spot image on the imaging plane. A CMOS camera placed at this image plane will record the spot position. Another path collects light reflected from the beam split and form an image on the microscope camera. The narrowband filter placed in front of the camera is to filter out the collimated laser beam.

If the surface under measurement has a small shift Δh along the microscope optical axis as shown in Figure 2 (a), then the spot from the laser beam will shift away from the microscope optical axis, and form a spot on one side of the optical axis. The relative distance and sign of the spot image to the microscope optical axis on the CMOS camera reflects the magnitude and direction of Δh. This distance are determined by microscope objective focal length f, imaging lens focal length F, collimating beam lateral shift δx, and Δh. Theoretically speaking, to obtain the best spot image, the CMOS photo sensitive plane shall satisfy the Scheimflug condition [34]. However, in the laser focus deviation optical layout, the angle between the collimating beam and the microscope principal optical axis is too small (in this experiment setup, the angle is 3.57°), if the Scheimflug condition should be met, the angle between the surface normal of the CMOS camera and the imaging beam will be greater than 89°, exceeding the CMOS camera working range. Fortunately, Figure 1 shows that the focused beam spot diameter changes slowly as the surface under measurement moves away from the best focus position. With ±20μm range of the surface shift, the diameter of the spot will vary less than 10%, therefore, if the surface under measurement shift away from the best focus position is small, well formed spot image still can be obtained without satisfying the Scheimflug condition.

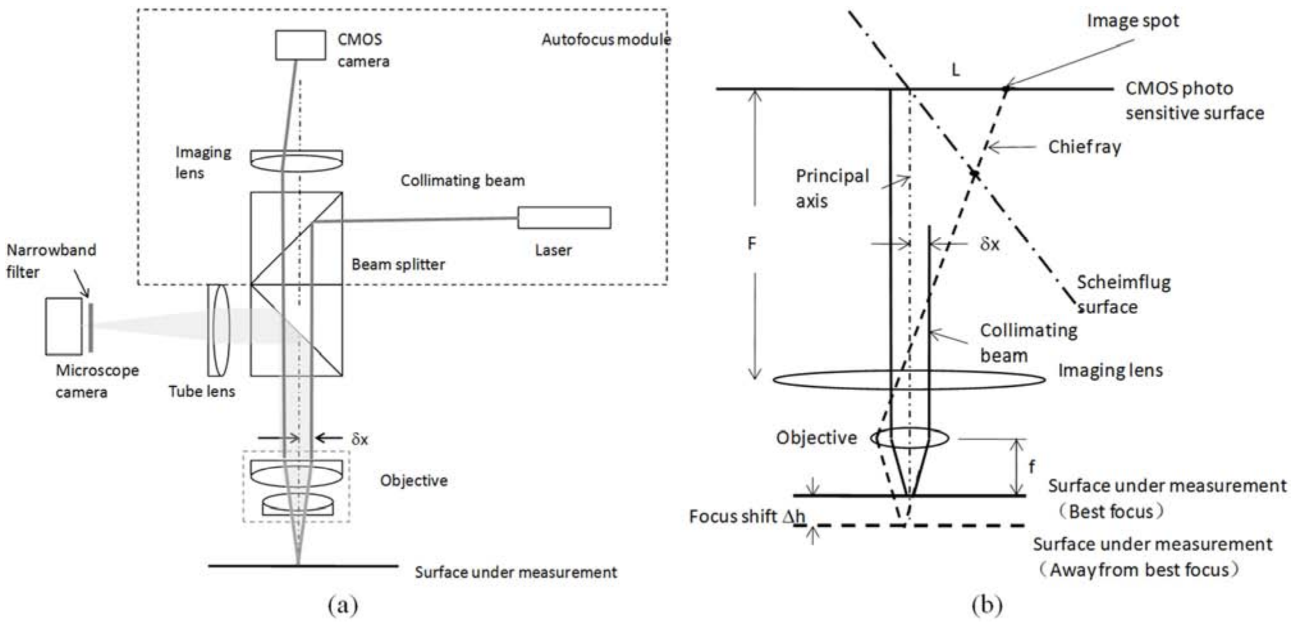

Figure 2. *(a) Schematics of optical layout of laser focus deviation; (b) Relationships betweenparameters.*

The focus shift can be determined by measuring the spot position on the CMOS camera. Therefore, by adding an auxiliary system, one can measure the focus shift of a surface under measurement in real time [35, 36] and adjust the focus position to maintain the best focus for the microscope system, realizing auto focusing. The auto focus module in Figure 2 can be embedded into a microscope system as an independent subsystem. By sharing the common optical path to realizing auto focusing for the microscope. For isotropic and smooth surfaces, such subsystem can accurately determine the best focus position for the microscope. However, in many applications, the surface under measurement may neither be isotropic, nor smooth. In such cases, the light intensity distribution of the spot image may deviate from its original distribution, thus affect the position accuracy as the position is calculated based on the centroid of the spot. Solely rely on centroid of the image spot to determine focus position is not suitable for anisotropic and rough surfaces. This paper discussed how to utilize weighted light intensity criterion Q to rapidly acquire the best focus position through coarse and fine focus positioning from both theoretical and experimental point of view.

3. Experiment Setup and the Effect of Non-isotropic and Rough Surfaces on Focus Position Accuracy

3.1. The Application on Isotropic and Smooth Surfaces

Figure 3 shows the experiment setup. A collimated laser with beam diameter of 1mm, wavelength of 650nm is used. The maximum output power is 5mW. The beam enters a 50/50 non-polarizing beam splitter. The transmitting portion enters the microscope optical path, the reflecting portion escapes from the side and away from the microscope system. After being focused by the microscope objective lens, the laser beam forms a focusing spot on the surface under measurement. The beam then being scattered and reflected back into the microscope objective. Passing through the beam splitter again and the reflecting portion enters the imaging lens and forms a bright spot on the digital camera. The objective lens used in this experiment is an infinity corrected 20x lens, the imaging lens has focal length of 100mm.

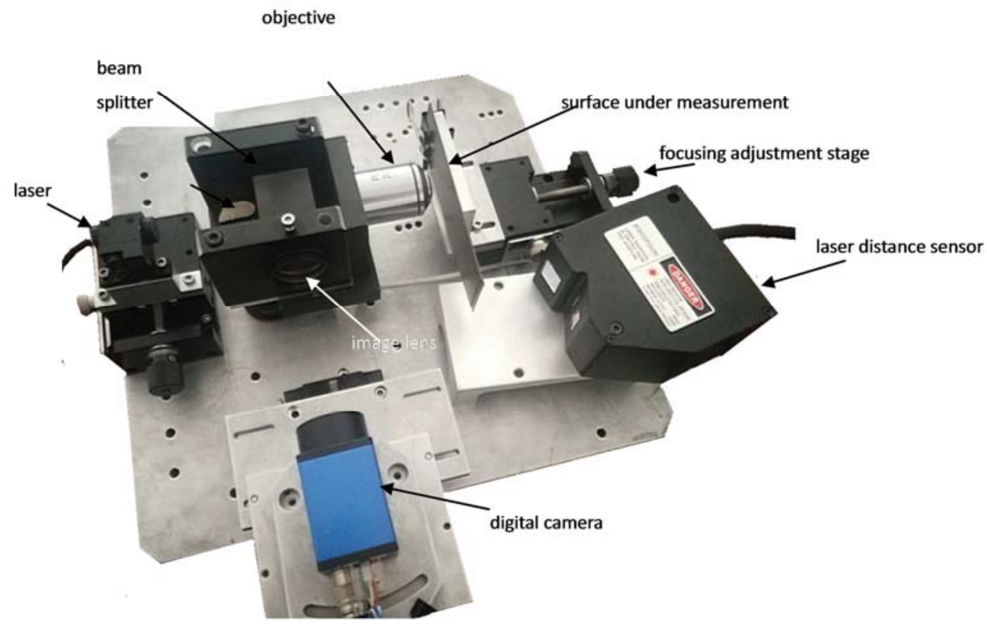

Figure 3. *Experiment setup.*

The displacement of the surface under measurement was measured using a laser displacement sensor. The displacement sensor (CTDS-CM10) was provided by CorhaiTechnologies. The resolution of the sensor is 0.1μm.

Figure 4 (a), (b) shows an image spot and its profile on the digital camera. The laser output power was set at 0.5mW.

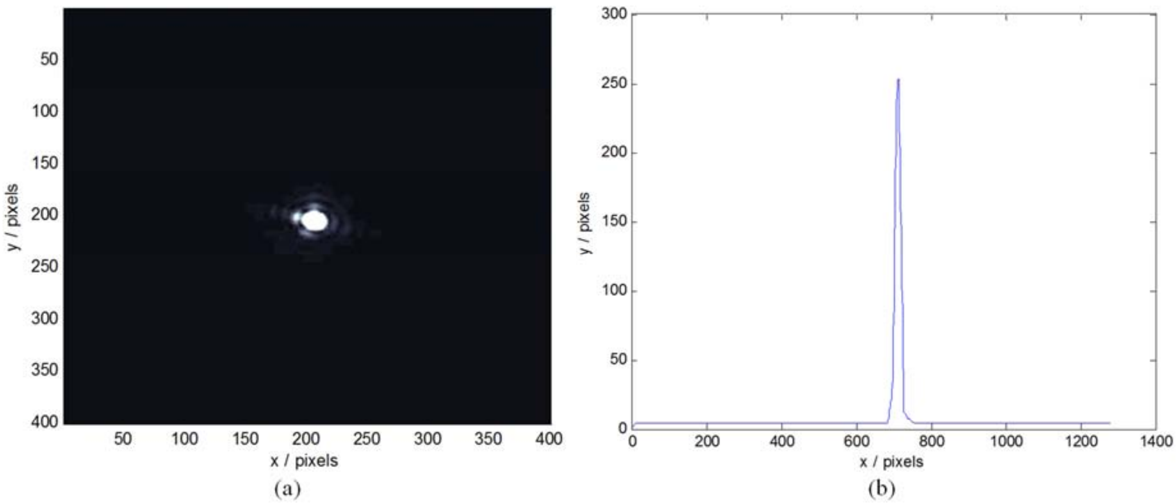

Figure 4. *(a) Spot intensity distribution; (b) Spot profile.*

From Figure 4 (a), it can be seen that the collimated laser beam after being focused on the surface under measurement forms a bright spot on the digital camera. The spot slightly deviates from an ideal circle. Its axis along x direction has a small stretch (as shown in the figure along horizontal direction). The x direction happens to be the incident collimating beam shifting from the microscope principal optics axis direction. Thus, the incident laser after objective and travelstowards the surface under measurement with a small angle. The value of the angle is determined by the lateral shift δx and the objective's focal length f.δx is 0.5mm, objective focal length is 8mm, and this small angle is 3.57°. Although the image spot on the digital camera is not an ideal round spot, it does not affect calculating the focus distance using centroid of the spot. Figure 5 shows at different focus position, the images of the spot on the digital camera. In this experiment, a region of interest (ROI) of 300x300 pixels is selected to excluding stray light from affecting the measurement. It also reduced data processing time. The surface under measurement is a smooth glass sample.

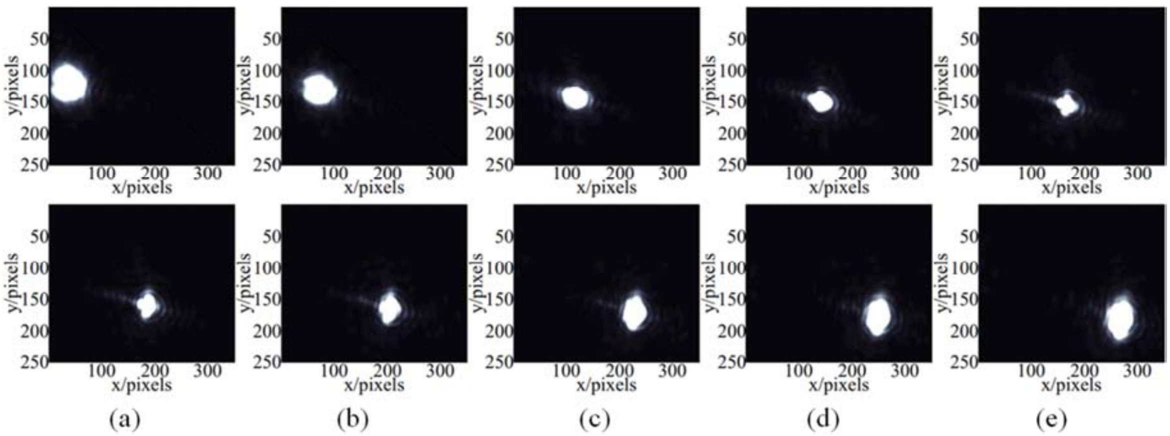

Figure 5. *Spot position on digital camera while focus shifts from far to near, focus gap between each spot from (a) to (j) is 10μm.*

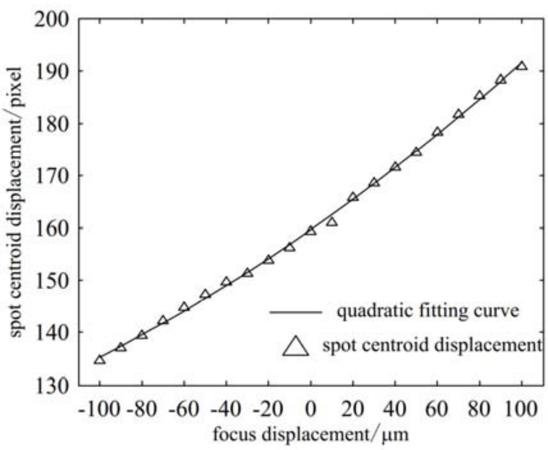

Figure 6. *Focus position vs. spot image.*

To ensure that the spot intensity is bright enough when focus deviates from the best focal position, the laser output power was set at 1mW. Compare to Figure 4, it is clear that at the same focus position (Figure 5 (e)), the spot diameter increased and its shape changed as well. Therefore, in real applications, the spot intensity must be maintained at a constant value by employee laser power auto adjusting for different surfaces under measurement, to ensure that focus position measurement will not be influenced by surface variations. The spot diameter on the digital camera reaches its minimum value while at the best focus position. Once deviates from the best focus position, other than the spot center position will shift, its diameter will also increase. The focus position of an isotropic and smooth surface can be easily determined by calculating the centroid of the spot image with a pre-calibrated lookup table of the focus position vs. centroid coordinates. Figure 6 is the centroid of a spot image vs. focus position. The solid line is its quadratic fitting curve.

The quadratic fitting curve is:

$$y = 0.0004x^2 + 0.2818x + 159.7159 \tag{2}$$

Here, the unit for y is microns and for x is pixels. Equation (2) indicates that within ±100μmrange, the curve is almost linear, with its linear coefficient equal to 0.2818. The measurement sensitivity can be estimated with this linear coefficient: 1/0.2818=3.55μm/pixel. If consider 1/10 of sub-pixel processing, the system resolution will reach 0.35μm. This can meet the requirement for auto focusing for a 20x objective lens.

The repeatability of the spot displacement and radius can be checked by laterally (normal to the collimated laser beam) shift the surface under measurement with very small distances. Since the small displacement in lateral direction will not alter the distance between the surface under measurement and the laser deviation system in a noticeable way, the displacement variation can be attributed to repeatability of the system. The repeatability value can be used to estimate the measurement accuracy. Table 1 is the measurement results at 30 different best focus location. The spot radius is calculated using the half maximum width of the spot intensity on the digital camera.

Table 1. *Repeatability of spot displacement and spot radius (unit: pixel).*

Serial No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Spot Disp.	830	829	829	830	830	829	829	830	830	830	829	830	829	830	830
Spot Rad.	14.23	14.05	14.07	14.21	14.23	14.12	14.25	14.25	14.21	14.25	14.21	14.21	14.16	14.23	14.18

Serial No.	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Spot Disp.	830	830	829	830	830	830	830	830	830	830	830	829	830	830	829
Spot Rad.	14.18	14.16	14.14	14.23	14.18	14.21	14.18	14.18	14.18	14.14	14.16	14.12	14.21	14.18	14.12

The average spot displacement is at 829.7/pixel and the standard deviation is σ_a=0.5/pixel; the average spot radius is at 14.181/pixel and the standard deviation is σ_b=0.05/pixel.

FromFigure 6, it can be estimated that the standard deviation of the focus repeatability is: 0.5×3.55 < 1.7μm. The value can be considered the focus accuracy of such setup on isotropic and smooth surfaces.

3.2. Anisotropic Surfaces

To evaluate the applicability of such method for anisotropic surfaces, radius of 26 pixels with Gaussian distribution is simulated for collimated laser beam using Matlab 2017b. Figure 7 (a) and (b) shows the simulated light spot and its intensity profile.

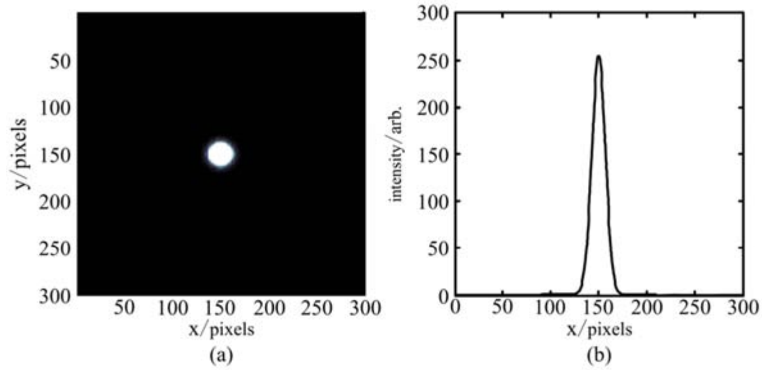

Figure 7. *Simulated light spot (a) and its intensity profile (b).*

Anisotropic background surfaces are simulated with a radius of 4 pixels dot array on a 300x300 pixel surface, and a radius of 32 pixels dot array on a 960x960 pixel surface. Thebrightness of the background is set at 1 and the dot brightness is set at 0.2, The pitch distancesof these dot arraysare 20 and 120 pixels respectively as shown in Figure 8 (a) and (b).

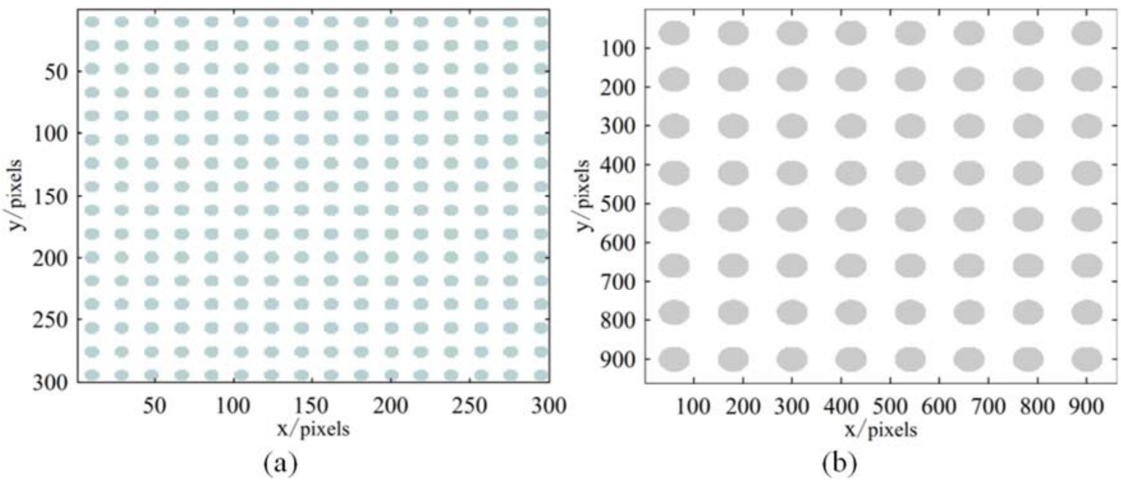

Figure 8. *Dot array with normalized brightness of 0.2, (a) 300x300 pixels with dot radiusof 4 pixels, (b) 960x960 pixels with dot radius of 32 pixels.*

Overlapping the simulated spot in Figure 7 (a) on to the dot arrays in Figure 8 (a) and (b) to form combined images. The combined images simulate the spot images on digital camera. The centroid ofthe spot image represents the measured displacement. Due to the dot array, the spot centroid of x of the combined image will vary as the spot overlaps the dot array at different locations. This simulates the displacement error. Figure 9 (a) is a group of 20 combined spot images. From left to right and from top to bottom, each with the dot array matrix shifting to up left by one pixel. Figure 9 (b) represents a group of 25 combined spot images, also from left to right and top to bottom, each with the dot array shifting to up left by 5 pixels.

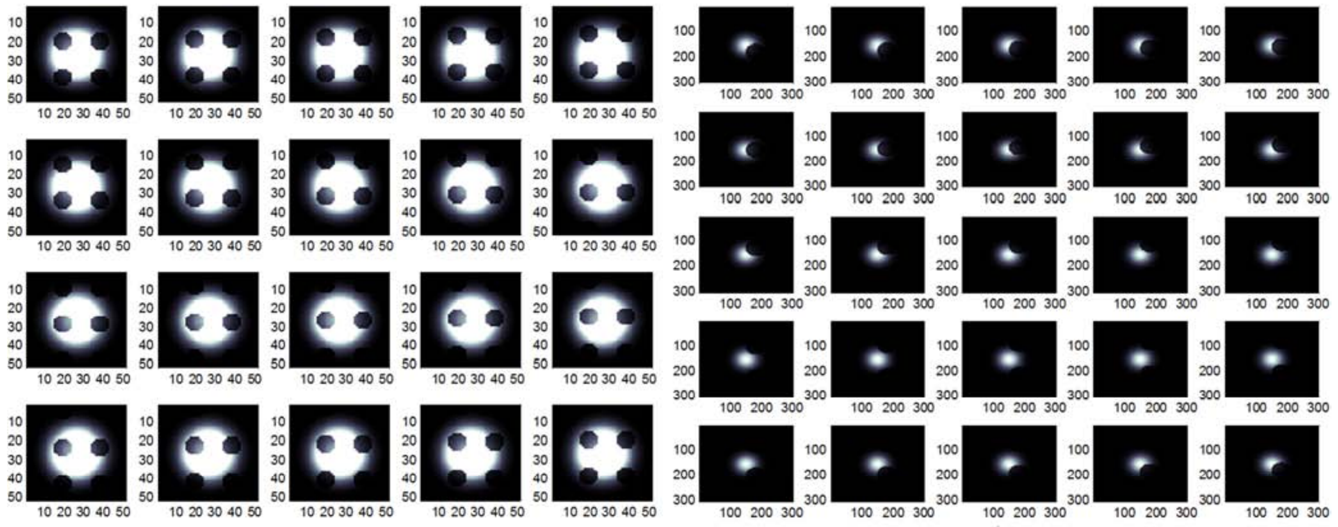

Figure 9. *(a). Combined images of simulated spot and dot array, dot shifting to up left by 1 pixel; (b). Combined images of simulated spot and dot array, dot shifting to up left by 5 pixel.*

Figure 10 shows the centroidinx of the spot image varies as the dot array shifts.

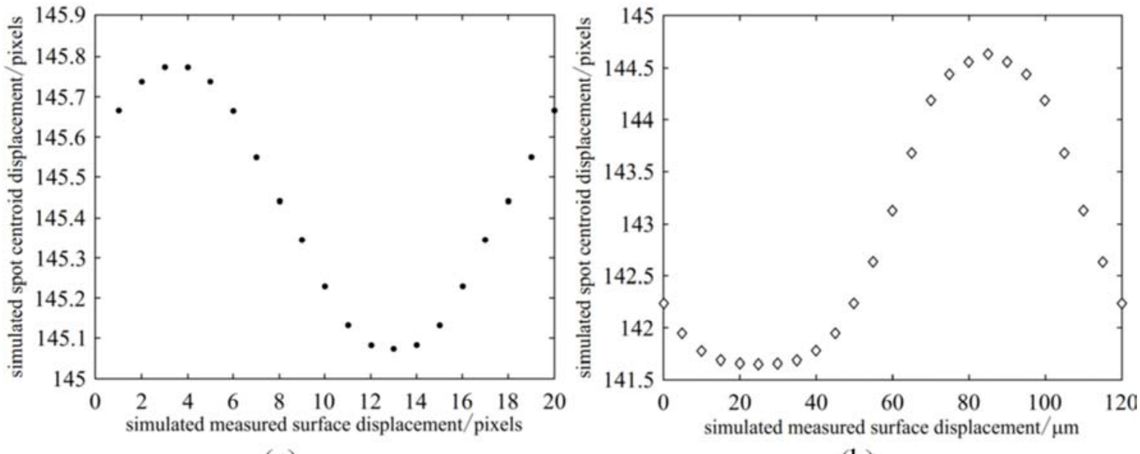

Figure 10. *(a) dot radius of 4, pitch of 20 pixels, (b) dot radius of 32, pitch of 120 pixels.*

Figure 10 showed that as the dot array shifts to up left, the spot centroid of combined image varies periodically along the x direction. The period matches the pitch distance of the dot array as expected. The amount of variation of the centroid depends on the dot radius and the dot pitches. The maximum variation in Figure 10 (a) and (b) are 0.7 and 3.2 pixels. It is clear that anisotropic surfaces will cause focus measurement error.

To verify the simulated results, a card with printed dots was used as the surface under measurement. Figure 11 (a) is an image taken when a collimated laser focuses on the surface. Figure 11 (b) is its 3D intensity image.

It can be seen from Figure 11 that black dots on the surface affected the laser spot intensity distribution. Therefore, using mass center to calculate spot centroid position will introduce error. Figure 12 (a) and (b) showed the laser spots at the same focus position, when the surface is slight shifted laterally (normal to the laser beam direction). The centroid in x direction of the two spot are 210.70/pixel and 201.78/pixel. It is obvious that the dark dot affected the centroid in x of the spot when using mass center algorithm. In Figure 12 (a), the dark dot showed up at the left side of the spot, thus, causing the centroid in x to be shifted to right, similarly, in Figure 12 (b), the dark dot showed up on the right side of the spot, causing the centroid in x to be shifted to left.

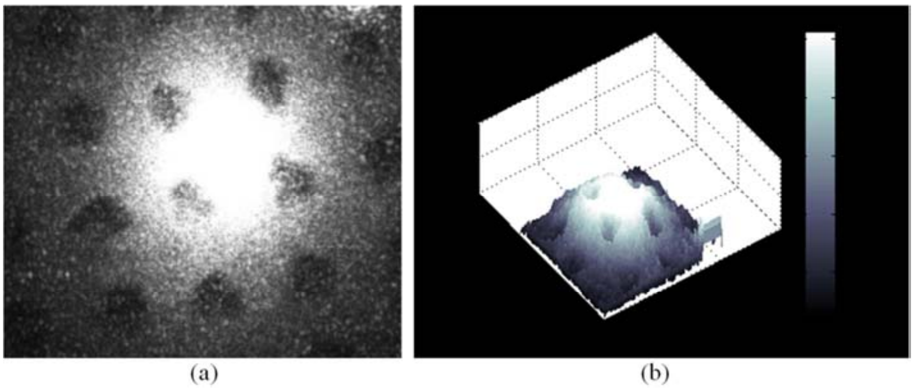

Figure 11. *(a) Laser spot with dots, (b) 3D laser spot intensity.*

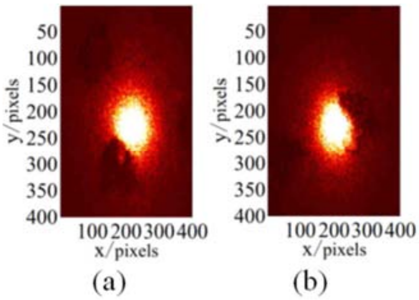

Figure 12. *Spot images of same focus position, different location on surface (a), (b).*

If one acquire the spot image and calculate its centroid while randomly move surface under measurement laterally, the centroid results will vary as shown in Figure 13.

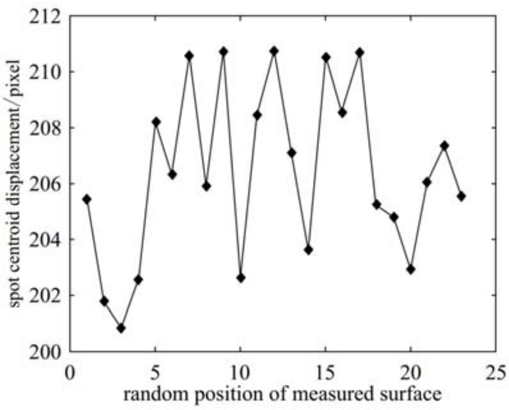

Figure 13. *Centroid in x of laser spot with different surface locations.*

Figure 13 indicated that due to the anisotropic nature of the card surface, the centroid in x measured at different surface location can vary up to 9.9 pixels. According to 3.1, the corresponding focus error will be 9.9x3.55=35.15μm, which is obviously too great of an error for auto focusing a 20x objective microscope system.

4. Focus Based on Weighted Light Intensity Criterion Q

In real applications, one can calibrate laser deviation method bycalculating the spot centroid in x at different distances of an isotropic and smooth target surface. Focus position can then be determined directly by measuring the centroid in x [21]. Thus, rapid auto focus can be realized using this method for smooth and isotropic surfaces. As indicated in previous section, when applying for anisotropic and rough surfaces, The focus error maybe too great to be useful for auto focusing applications. To overcome this problem, searching for maximum weighted light intensity value Q to determine best focus position was proposed. The procedure is described as follows:

a. Coarse focus position is determined by using laser focus deviation method.

b. Within the proximity region of the coarse focus position, scan the region in small steps to obtain the focus spot and calculate the weighted light intensity value Q, find the maximum value Q and its corresponding focus position.

The definition for weighted light intensity criterion Q is as follows: located the centroid of the spot with mass center algorithm, draw a circle around the centroid with a radius of R, calculate the ratio of pixel intensity and its absolute radius for all pixels within the circle.

$$Q = \sum_{(x,y)\in R} \frac{I(x,y)}{\sqrt{(x^2+y^2)}} \tag{3}$$

Here, I(x,y) is the pixel intensity at (x,y), $\sqrt{x^2+y^2}$ is the distance of the pixel to centroid, unit is pixel. Figure 14 is the Q vs. focus shift for a isotropic surface. Three radius R is used, R=25 is defined where all pixels with intensity greater or equal to 1/2 of the maximum value are used to calculate the R. To checked the insensitivity of R for determining best focus position, two R values of R=30 and R=20 are also used. Figure 14 shows that with different R values, the Q curves varies only slight at the bottom, but almost identical for the peak positions. The peak position determines the best focus position. Thus, using Q criterion to determine the best focus is relatively insensitive to R selection, indicating that using Q to determine best focus is robust.

Figure 14 showed that for isotropic surfaces, weighted light intensity value Q has a prominent peak, which corresponds to best focus position, in this case, it is at -30μm. Thus, Q can be utilized to accurately determine the best focus position.

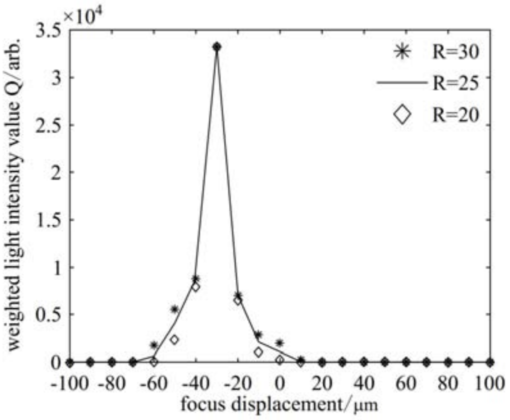

Figure 14. *Q vs. focus displacement for anisotropic glass surface.*

To determine whether weighted light intensity can also be applied for anisotropic and rough surfaces, two target surfaces were used, one is a card with doted surfaces as shown in Figure 12, and the other one is a piece of brass metal with its surface coated with a black paint and the surface is anisotropic and rough, as shown in Figure 15.

With coarse focus position being determined using laser focus deviation method, within ±100 mrange of the coarse focus position, recording spot images on the digital camera with focus step size of 10m. Calculating the weighted light intensity value of Q for each acquired images. Figure 16 (a) and (b) is the results for the two target surfaces. The radius R for calculating the Q values is set at 25 pixels. Figure 16 (a) shows that the best focus position for surface with dark dots is at -20m, while for surface with black paint is at 10m. The Q value drops rapidly as the focus position deviates from its best focus position. The experiment results indicated that the weighted light intensity criterion Q can be used to precisely determine the best focus position even for anisotropic and rough surfaces. It is noticed that for isotropic surface, the Q curve appears more symmetrical around the peak, while for anisotropic surfaces, the Q curve appear less symmetrical. This indicates that the anisotropic nature of the surface still has some negative influence on determining the best focus positions.

Figure 15. *Surface coated with black paint.*

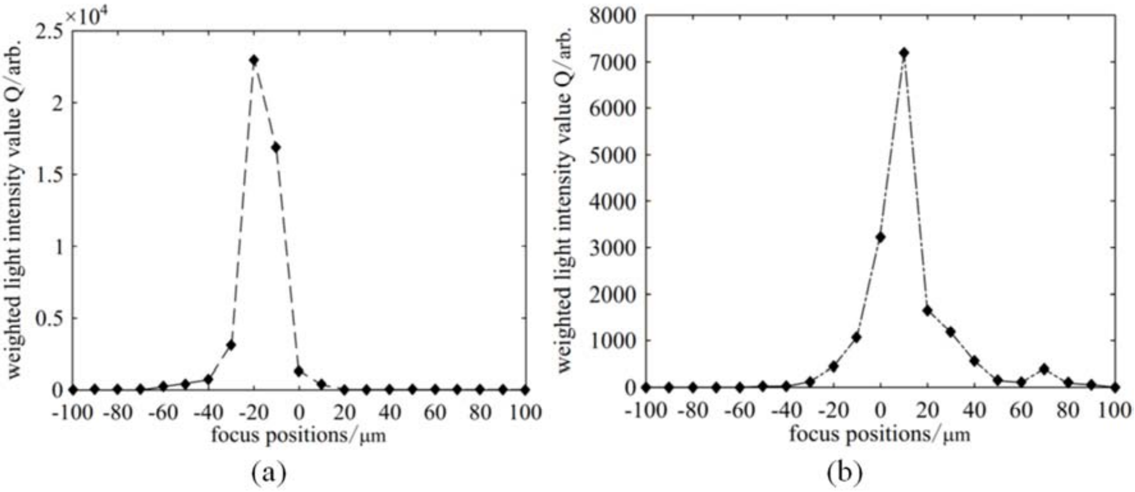

Figure 16. *Weighted light intensity value Q vs. focus positions, (a) for surface with dark dots, (b) for surface with black paint.*

5. Summary

Using mass center of laser focus spot image based on laser focus deviation method can precisely determine the best focus position for isotropic and smooth surfaces. For anisotropic and rough surfaces, such method will introduce large errors for determining the best focus position. However, a new criterion of weighted light intensity Q was introduced to assist in determining the best focus position based on laser focus deviation method. Results indicated that for anisotropic and rough surfaces, the weighted light intensity criterion Q greatly reduced the focusing errors. Thus, the laser focus deviation method along with the Q criterion can be employed for auto focusing application for anisotropic and rough surfaces. Though, laser deviation method coupled with weighted light intensity Q is able to quickly locate the best focus for anisotropic and roughness surface, the focusing accuracy is not as great as that for smooth surfaces. This can be seen from the asymmetric nature of the Q curve for anisotropic and roughness. Future work will look into the cause of this asymmetric of Q for anisotropic and rough surfaces and provide further corrections to improve the focus accuracy.

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