

Precise autofocusing microscope with rapid response



Chien-Sheng Liu^{a,*}, Sheng-Hong Jiang^{b,1}

^a Department of Mechanical Engineering and Advanced Institute of Manufacturing with High-tech Innovations, National Chung Cheng University, No. 168, University Road, Minhsiung Township, Chiayi County 62102, Taiwan

^b Department of Mechanical Engineering, National Central University, No. 300, Jhongda Road, Jhongli City, Taoyuan County 32001, Taiwan

ARTICLE INFO

Article history:

Received 13 May 2014

Received in revised form

11 September 2014

Accepted 6 October 2014

Available online 25 October 2014

Keywords:

Geometrical fluctuations

Optical diffuser

Autofocusing microscope

Response

ABSTRACT

The rapid on-line or off-line automated vision inspection is a critical operation in the manufacturing fields. Accordingly, this present study designs and characterizes a novel precise optics-based autofocusing microscope with a rapid response and no reduction in the focusing accuracy. In contrast to conventional optics-based autofocusing microscopes with centroid method, the proposed microscope comprises a high-speed rotating optical diffuser in which the variation of the image centroid position is reduced and consequently the focusing response is improved. The proposed microscope is characterized and verified experimentally using a laboratory-built prototype. The experimental results show that compared to conventional optics-based autofocusing microscopes, the proposed microscope achieves a more rapid response with no reduction in the focusing accuracy. Consequently, the proposed microscope represents another solution for both existing and emerging industrial applications of automated vision inspection.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The requirement of automated optical inspection machines has been in great demand in recent years due to their good reliability and high throughput. In these machines, automated vision inspection is a critical operation in the manufacturing fields for on-line or off-line mass production lines [1]. For automated vision applications, such as liquid crystal display (LCD) plane inspection, laser repairing, pattern machining, and so forth, autofocusing technology is very important because it has to make sure that the image is in-focus [2,3]. As a result, many sophisticated autofocusing schemes have been proposed in recent decades. As discussed in [4], existing autofocusing microscopes can be broadly classified as either image-based (i.e., software) or optics-based (i.e., hardware). Image-based or software autofocusing microscope is based on the image sharpness identification or the image spatial frequency function [5]. These kinds of microscopes are cheap, reliable and robust, therefore they have many industrial applications [6–12]. Image processing algorithms for autofocusing are very basic and with recent advances of GPUs and other real-time processing technologies, fast calculations can be performed. However, this approach is rather time-consuming, because the focus position of the sample is adjusted by analyzing the sharpness of the image captured by an optical system and analyzing the image

sharpness needs more time compared to optics-based autofocusing microscopes [13,14]. For overcoming these problems, many optics-based or hardware autofocusing microscopes have been presented. By contrast, this kind of microscope uses a sensor to measure the distance between the lens and the sample, to correct for changes in the focal plane due to external factors such as vibrations, thermal drift, and so forth [15]. It has a more rapid response and achieves a higher focusing accuracy, therefore it is popularly used in many automated optical inspection and machining applications with a real-time requirement throughout industry [16–22].

For mass production lines, both the manufacturing and inspection processes should have a rapid response. To the best of the current authors' knowledge, the autofocusing market in the automated inspection and laser repairing of thin-film transistor liquid crystal display (TFT-LCD) is dominated by the company of WDI Wise Device Inc. The detailed structure and design of this autofocusing microscope is described in [23]. In order to satisfy the requirements of small size and low cost, a laser diode light source is used in this system.

In previous studies [24,25], the present group has proved that the geometrical fluctuations of the laser beam in a laser displacement sensor will introduce noise and degrade the measurement performance. And the laser displacement sensor can achieve a higher measuring accuracy by suppressing the geometrical fluctuations of the laser beam. The same method can also be used to improve the measuring accuracy of autofocusing microscopes. However, with the continuing trend toward high performance, it is necessary to improve the focusing response time of autofocusing

* Corresponding author. Tel.: +886 5 2720411; fax: +886 5 2720589.

E-mail addresses: imecsl@ccu.edu.tw (C.-S. Liu), ty20031224@hotmail.com (S.-H. Jiang).

¹ Tel.: +886 3 4267300; fax: +886 3 4254501.

microscopes yet further. Consequently, in the present study, the autofocusing performance of the microscope presented in [23] is further improved by using a high-speed rotating optical diffuser to suppress the effects of geometrical fluctuations of the laser beam. The performance of the modified microscope is verified experimentally using a laboratory-built prototype.

The remainder of this paper is organized as follows. Section 2 describes the structure of the optics-based autofocusing microscope proposed in [23]. Section 3 introduces the optics-based autofocusing microscope proposed in the present study. Section 4 describes the experimental characterization of the proposed microscope using a laboratory-built prototype and compares its performance with that of the microscope proposed in [23]. Finally, Section 5 presents some brief concluding remarks.

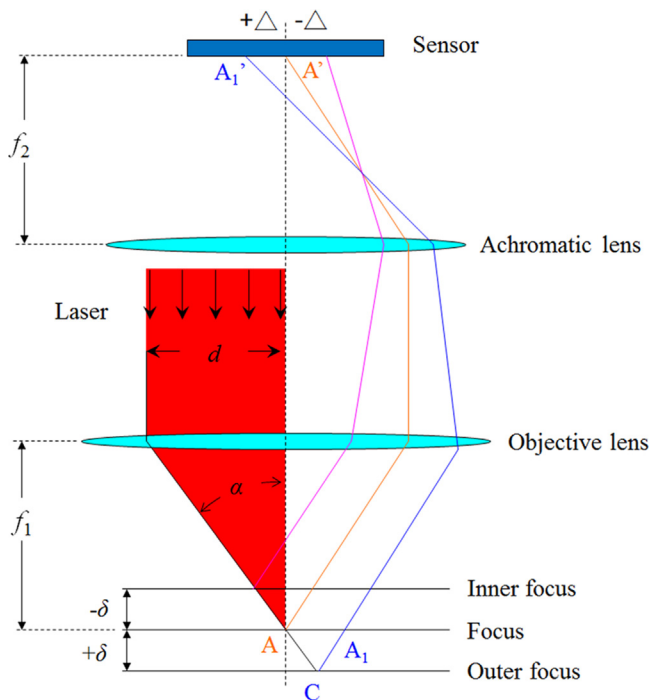


Fig. 1. Schematic illustration of optical path in a conventional optics-based autofocusing microscope [4,23].

2. Conventional optics-based autofocusing microscope

This section reviews the basic structure and measuring principle of the autofocusing microscope proposed in [23].

Fig. 1 presents a schematic illustration of the optical path within a conventional optics-based autofocusing microscope. It has been shown that if the image intensity remains constant over the entire sensor image, a linear relationship exists between the centroid of the image captured by the CCD sensor and the defocus distance, δ . Exploiting this linear relationship, an autofocusing capability can be achieved by driving the objective lens using a position feedback signal based on the centroid coordinates of the detected image [4,23].

Fig. 2 illustrates the basic structure of the conventional autofocusing microscope constructed for comparison purposes in the present study. As shown, the light beam emitted by the laser (Thorlabs HL6501MG, 658 nm) is expanded and collimated by means of a lens (Lens₁) and is then bisected by a knife such that its cross-section has the form of a semi-circle. The light beam is passed through a beam splitter (BS₁), a 45° red dichroic filter and an objective lens, and is then incident on the sample surface. The light is reflected from the surface and passes back through the objective lens, filter, BS₁, and a lens assembly (Lens₂), and is then incident on a CCD sensor (CCD₁).

In accordance with basic geometrical optics principles, the shape of the laser spot (or centroid (x_{centroid} , y_{centroid}) of the image) on the CCD sensor varies in accordance with the defocus distance δ . In the present study, the variation of coordinates (x_{centroid} , y_{centroid}) was calculated using a self-written autofocus-processing algorithm. An autofocusing capability was then achieved by using a position feedback signal derived from the computed image centroid coordinates to dynamically adjust the position of the objective lens via a linear motor. Real-time images of the sample were captured during the focusing process using an infinity-corrected optical system (Navitar Zoom 6000).

Fig. 3 illustrates the theoretical motion characteristics of the conventional autofocusing microscope (please refer to user's manual of ATF4 auto focus & scanning sensor, WDI Wise Device Inc.). As shown, if the initial defocus distance δ_1 is located outside of this linear range, the autofocusing procedure should commence by shifting the objective lens through a distance L , where L is equivalent to half the linear range. If the resulting defocus distance δ_2 is still located outside the linear range, the objective lens should be shifted through a further displacement L . This procedure is repeated until the defocus distance (e.g., δ_3) falls within the linear range, at which point the objective lens can be moved directly to the point of maximum focus [4]. Therefore,

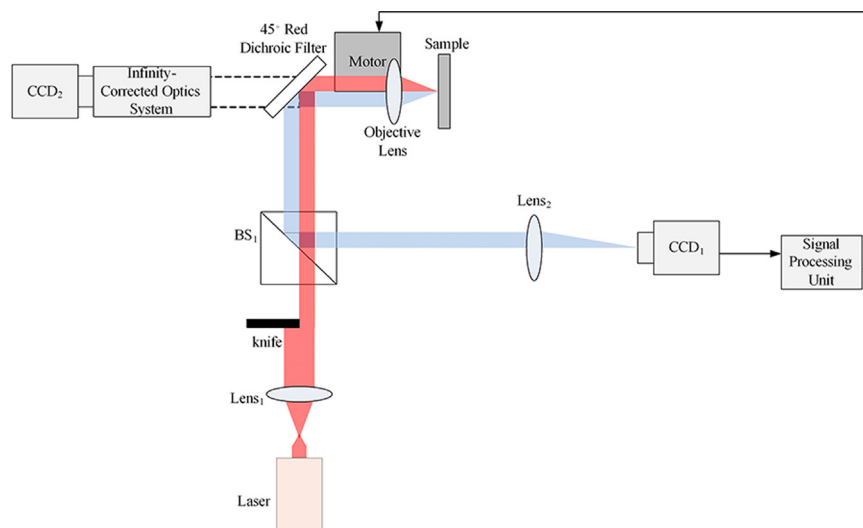


Fig. 2. Structure of a conventional optics-based autofocusing microscope.

the focusing response time of the autofocusing microscope depends on the linear curve of its motion characteristics and the number of times the objective lens is shifted.

3. Proposed optics-based autofocusing microscope

Fig. 4 illustrates the structure of the autofocusing microscope proposed in the present study, in which the conventional microscope presented in [23] is further developed via the addition of a high-speed rotating optical diffuser. (扩束器) Although the rotating optical diffuser is already employed to reduce the laser speckle in classic laser speckle metrology [26,27], to the best of our knowledge, none use the rotating diffuser to reduce the effects of geometrical fluctuations of the laser beam in the autofocusing microscope and improve its response time. As shown in Fig. 4, the light beam emitted from the laser light source is passed through a rotating optical diffuser (particle size: 1 μm , rotational speed: 12,000 rpm), and is expanded and collimated through a lens. Since the optical diffuser rotates at a high speed, the captured image is formed by the corresponding increase in the integration times of the

intensity distribution over the exposure time period. In other words, the effect of the geometrical fluctuations of the laser beam on the image centroid coordinates (x_{centroid} , y_{centroid}) is suppressed.

4. Experimental characterization of conventional and proposed autofocusing microscopes

The validity of the proposed autofocusing microscope was verified using a laboratory-built prototype (see Fig. 5). To demonstrate the practical feasibility of the proposed autofocusing microscope, an autofocusing experiment was conducted using a mirror sample given initial defocus distances, δ , ranging from $-300\ \mu\text{m}$ to $+300\ \mu\text{m}$. For comparison purposes with the same standard, the experiments were repeated using the conventional autofocusing microscope constructed in this study (see Fig. 6), and the linear motor was controlled by a constant speed. Table 1 summarizes the selected values of each design parameter. Fig. 7 presents a flow chart showing the basic steps in both the conventional and proposed autofocusing procedures, and in this study, the depth of the focus is set as 2 μm .

Fig. 8 shows experimental images of the laser spot on the CCD sensor given different values of the defocus distance in both the conventional and proposed autofocusing microscopes, respectively.

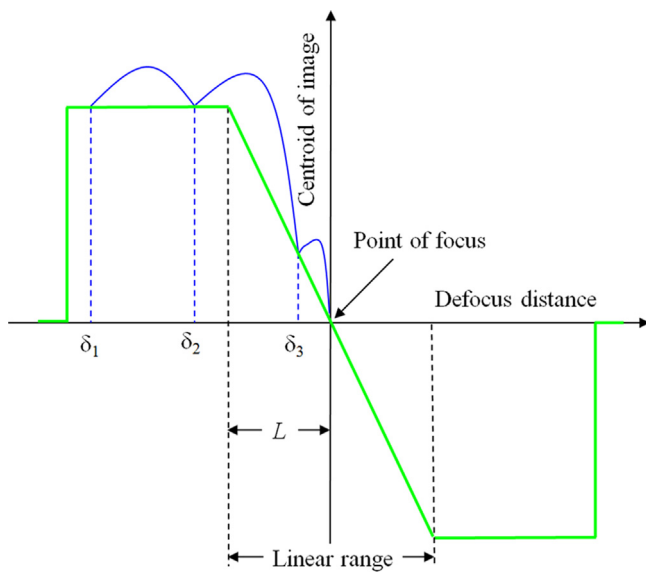


Fig. 3. Theoretical motion characteristics of a conventional autofocusing microscope.

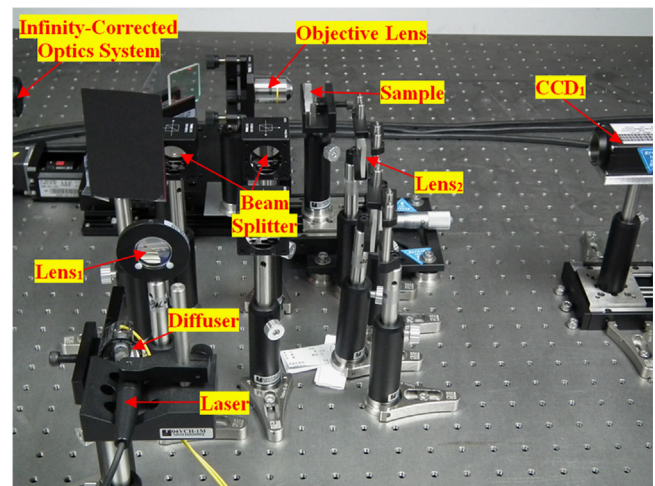


Fig. 5. Laboratory-built prototype of the proposed autofocusing microscope.

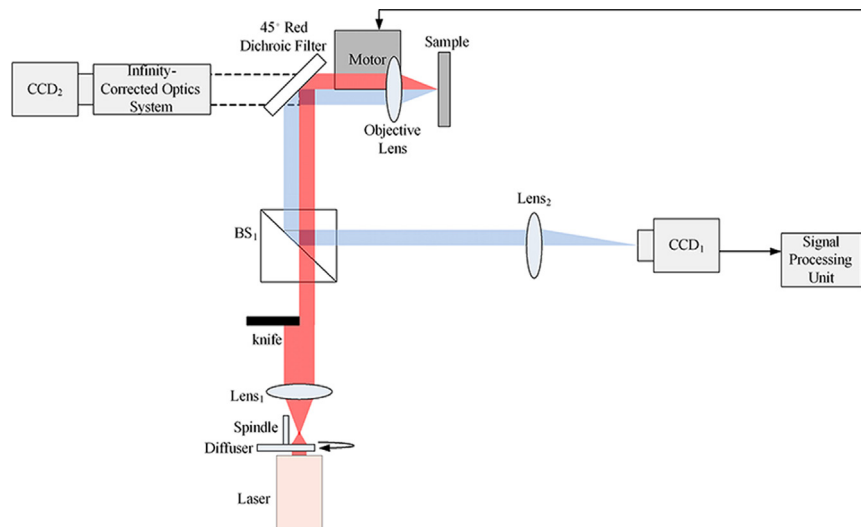


Fig. 4. Structure of the proposed autofocusing microscope.

As shown, when using the conventional autofocusing microscope, the laser spot images have an obvious interference pattern. Therefore, it will introduce the noise and the pixel intensity varies markedly from one pixel to another. By contrast, in the proposed

autofocusing microscope, it is evident that the rotating optical diffuser eliminates the interference pattern and improves the uniformity of the laser spot shape. It is noted that, in the conventional autofocusing microscope, the shape of the laser spot image in focus is not a semi-circle also due to the manufacturing tolerances and misalignment laboratory-built prototype. By contrast, in the proposed autofocusing microscope, the shape of the laser spot image in focus is like a circle and it can be attributed to the reason that the optical diffuser rotates at a high speed and vibrates, so the captured image is formed by the average of numerous instances of different pixel intensities over the exposure time period. As a result, it looks like a circle.

Fig. 9 illustrates the variation over time (3 hours) of the image centroid position with the defocus distance in the conventional autofocusing microscope and the proposed autofocusing microscope, respectively. As shown in Fig. 9, the linear relationships between the image centroid coordinate (x_{centroid}) and the defocus distance δ is given by

$$\text{centroid}(x_{\text{centroid}}) = -0.5607\delta, \quad (1)$$

In this study, we only consider the image centroid coordinate (x_{centroid}). Fig. 9 shows that in both conventional and proposed autofocusing microscopes, the image centroid position (x_{centroid}) varies linearly with the defocus distance δ over a certain defocus

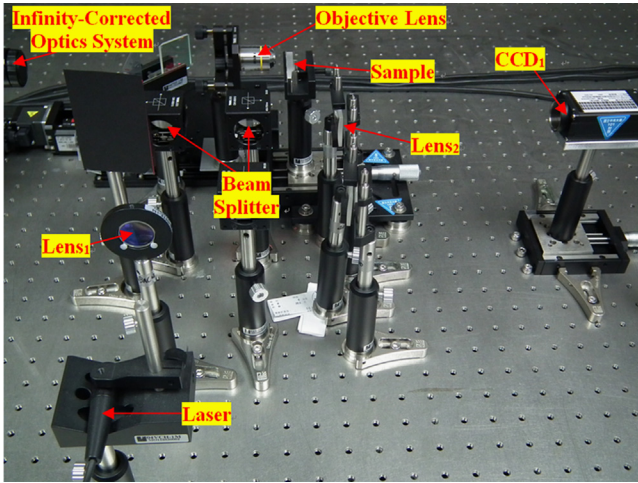


Fig. 6. Laboratory-built prototype of a conventional autofocusing microscope.

Table 1
Design parameters of conventional and proposed autofocusing microscopes, respectively.

Variable	Proposed microscope	Conventional microscope
Laser	Thorlabs HL6501MG, 658 nm	Thorlabs HL6501MG, 658 nm
Effective focal length of lens ₂ f_2	104.5 mm	104.5 mm
Focal length of objective lens f_1	18.0 mm	18.0 mm
Optical diffuser	Edmund, 0.5° diffusing angle	

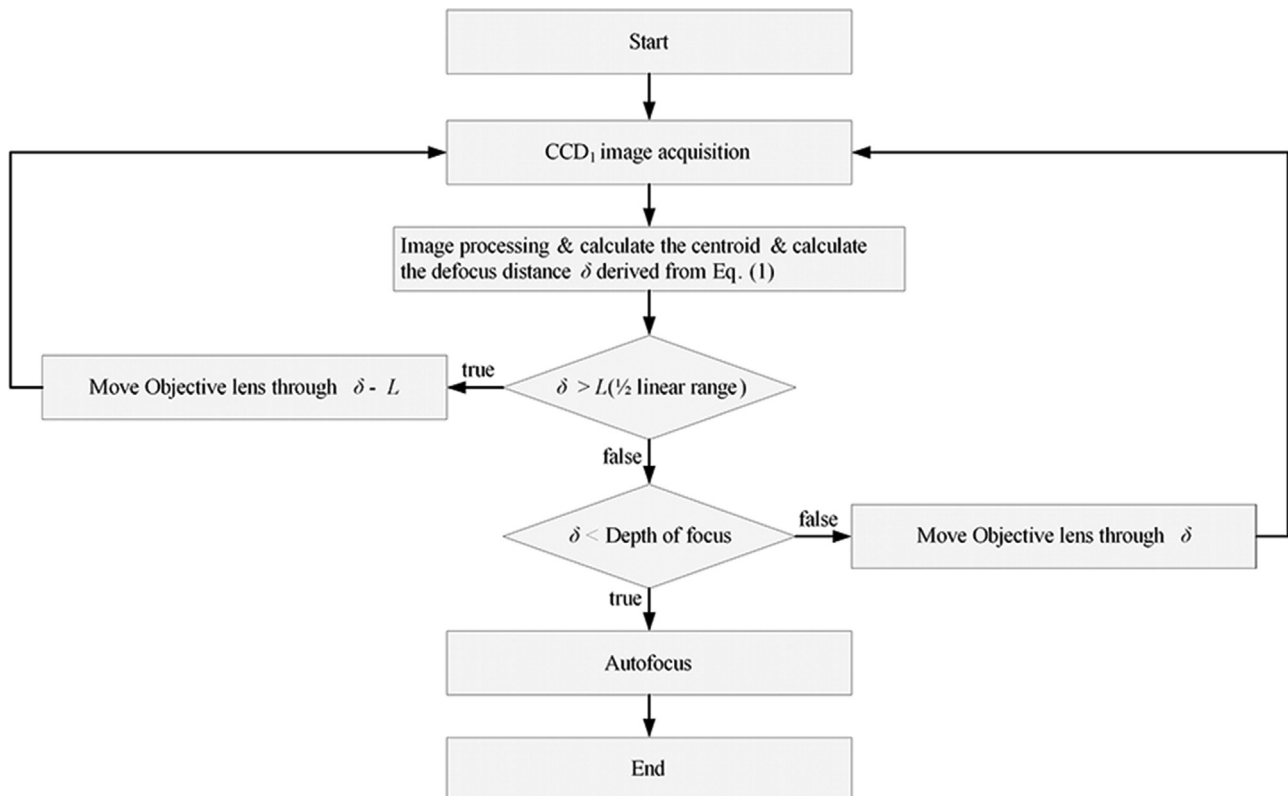


Fig. 7. Flow chart of autofocus-processing algorithm in a conventional and the proposed autofocusing microscopes.

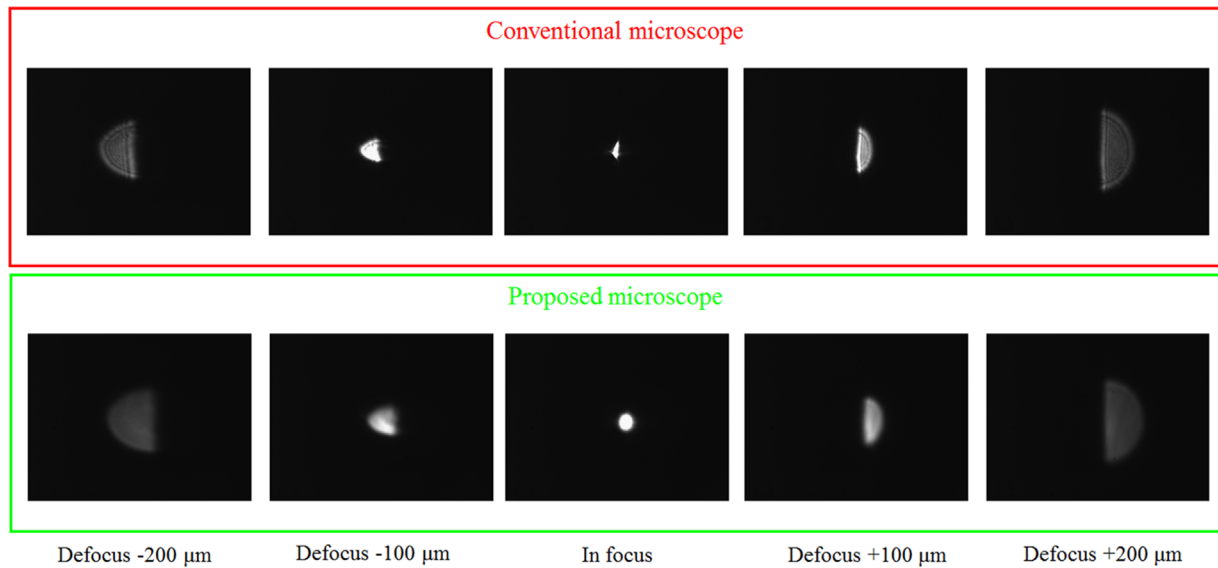


Fig. 8. Experimental images of laser spot on CCD sensor surface given different values of defocus distance in conventional and proposed microscopes.

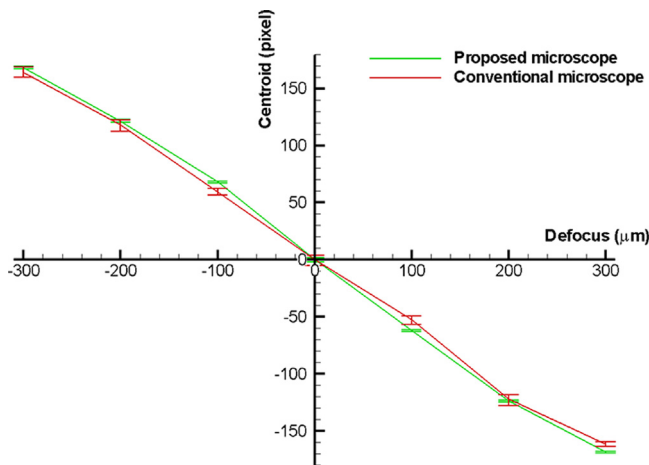


Fig. 9. Experimental results for variation over time of image centroid position with defocus distance in conventional and proposed microscopes, respectively.

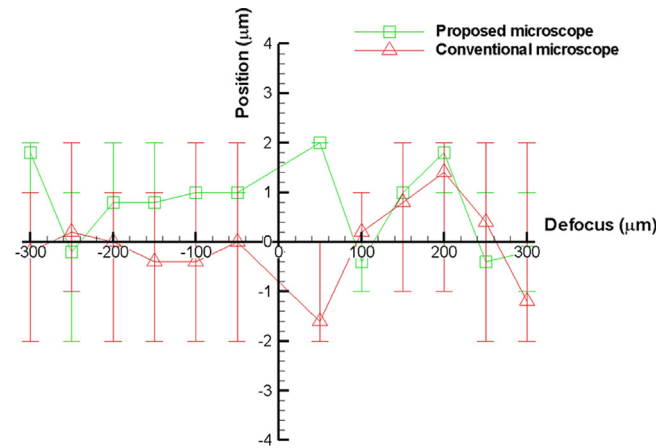


Fig. 10. Experimental results for variation of the autofocusing position with defocus distance in conventional and proposed microscopes, respectively.

range. In other words, the experimental results are consistent with the theoretical results presented in Section 2. The resolution of the autofocusing microscopes (i.e., the variation of the sensed defocus distance/the centroid (x_{centroid})) can be obtained experimentally as $1.78 \mu\text{m}/\text{pixel}$. (Note that Eq. (1) was obtained by applying a linear curve fitting technique to the experimental results presented in Fig. 9 using Microsoft Office Excel.) However, due to the geometrical fluctuations of the laser beam, the centroid (x_{centroid} , y_{centroid}) of the image captured by the CCD sensor varies over time. As shown, the error bar of the centroid position in the conventional autofocusing microscope varies significantly over the considered time frame (3 hours) and the maximum variation of the centroid position for the focal point is around 3.68 pixel. Therefore, the maximum experimental measuring accuracy is equal to $6.6 (=3.68/0.5607) \mu\text{m}$. (II) By contrast, in the proposed autofocusing microscope, the error bar of the centroid position varies only slightly over time and the maximum variation of the centroid position for the focal point is around 0.72 pixel (maximal). Therefore, the maximum experimental measuring accuracy is equal to $1.3 (=0.72/0.5607) \mu\text{m}$. Significantly, the experimental results indicate that the proposed autofocusing microscope has a smaller non-linearity and improved measuring accuracy. In other words, the measuring performance of the

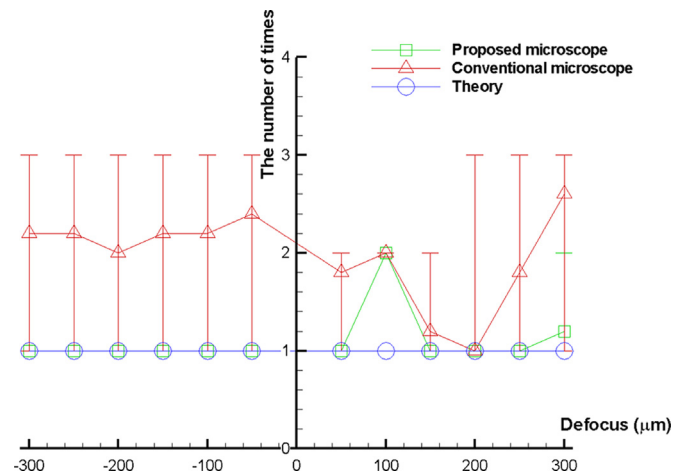


Fig. 11. Experimental results for variation of the number of objective lens movement times with defocus distance in conventional and proposed microscopes, respectively.

proposed autofocusing microscope is significantly improved because the rotating optical diffuser reduces the variation of the image centroid position.

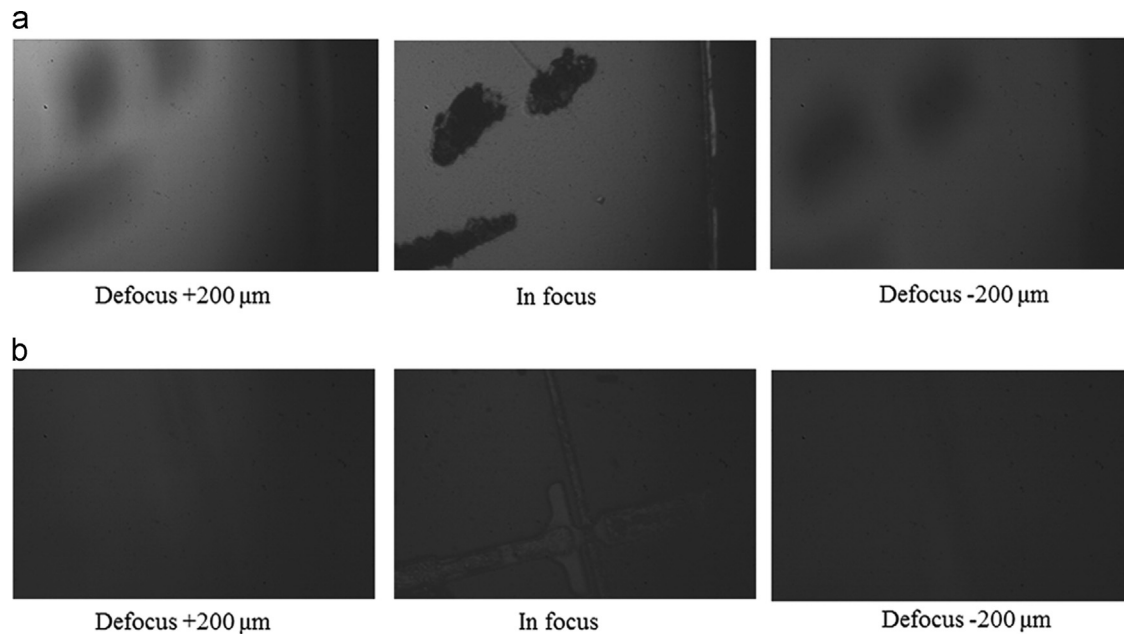


Fig. 12. Experimental images obtained during the autofocus procedure: (a) sample of mirror and (b) TFT array.

Fig. 10 shows the experimental results obtained for the variations of the measured autofocus position as a function of the initial defocus distance in the conventional and proposed autofocusing microscopes, respectively. The measured autofocus position is the final position of the objective lens relative to its ideal focusing position. Fig. 11 shows the experimental results obtained for the variations of the number of times that the objective lens is shifted as a function of the initial defocus distance in the conventional and proposed autofocusing microscopes, respectively. The experiments were repeated five times for every initial defocus distance. As shown in Fig. 10, in both the conventional and proposed autofocusing microscopes, the autofocusing performance can achieve a focusing accuracy of $\leq 2 \mu\text{m}$. However, as shown in Fig. 11, it can be seen that the numbers of times that the objective lens is shifted (or the focusing times) are obviously different in both the conventional and proposed autofocusing microscopes. As shown, every initial defocus distance falls within the linear range and in theory, the objective lens can be moved directly to the point of maximum focus. Therefore, the number of times that the objective lens is shifted equals 1 theoretically. In the conventional autofocusing microscope, the focusing times are greater due to the effects of geometrical fluctuations of the laser beam and greater non-linearity. By contrast, in the proposed autofocusing microscope, as a result of the effects of geometrical fluctuations of the laser beam is suppressed by using a high-speed rotating optical diffuser and has a smaller non-linearity, the focusing times are improved. As shown, a significant asymmetry (between negative and positive defocus distances) is observed for the number of the objective lens movement times in the conventional autofocusing microscope as a result of the manufacturing tolerances and misalignment laboratory-built prototype. By contrast, in the proposed autofocusing microscope, the asymmetry is suppressed and it can also be attributed to the reason that the high-speed rotating optical diffuser decreases the non-linearity and improves the stability of the microscope. As shown in Fig. 11, the averages and the standard deviations of the numbers of the objective lens movement times for the proposed autofocusing microscope and the conventional autofocusing microscope are (1.1 and 0.3), and (2.0 and 0.8), respectively. In a previous study [4], we have proved the response time of the microscope improves with a decreasing number of the objective lens movement times. As a consequence, the focusing response of the proposed

autofocusing microscope is significantly improved due to its decreasing number of the objective lens movement times.

Fig. 12 presents a sequence of real-time images captured by the infinity-corrected optical system during the autofocus procedure. Compared to other defocus images, the images in focus are clear. It proves the autofocusing function of the proposed autofocusing microscope is done well.

5. Conclusions

This study has proposed a novel optics-based autofocusing microscope with a rapid response and no reduction in the focusing accuracy. In the proposed design, the variation of the image centroid position as a result of the effects of geometrical fluctuations of the laser beam is decreased by using a high-speed rotating optical diffuser. The performance of the proposed microscope has been evaluated by means of experimental investigation using a laboratory-built prototype. The experimental results have shown that compared to the conventional autofocusing microscope, the proposed microscope has a more rapid response time and a comparable focusing accuracy of less than $2 \mu\text{m}$. As a result, the microscope provides a promising solution for a wide range of automated inspection applications.

Acknowledgments

The authors gratefully acknowledge the financial support provided to this study by the National Science Council Taiwan under Grant no. NSC 102-2221-E-194-023 and the Ministry of Science and Technology, Taiwan under Grant no. MOST 103-2221-E-194-006-MY3.

References

- [1] Petrucci P, Riesenberger R, Kowarschik R. Optimized coherence parameters for high-resolution holographic microscopy. *Appl Phys B* 2012;106:339–48.
- [2] Chang HC, Shih TM, Chen NZ, Pu NW. A microscope system based on bevel-axial method auto-focus. *Opt Lasers Eng* 2009;47:547–51.
- [3] Lamadie F, Bruel L, Himbert M. Digital holographic measurement of liquid-liquid two-phase flows. *Opt Lasers Eng* 2012;50:1716–25.

- [4] Liu CS, Hu PH, Lin YC. Design and experimental validation of novel optics-based autofocusing microscope. *Appl Phys B* 2012;109:259–68.
- [5] Hsu WY, Lee CS, Chen PJ, Chen NT, Chen FZ, Yu ZR, Kuo CH, Hwang CH. Development of the fast astigmatic auto-focus microscope system. *Meas Sci Technol* 2009;20:045902-1–9.
- [6] Chen CY, Hwang RC, Chen YJ. A passive auto-focus camera control system. *Appl Soft Comput* 2010;10:296–303.
- [7] Bueno-Ibarra MA, Alvarez-Borrego J, Acho L, Chavez-Sanchez MC. Fast autofocus algorithm for automated microscopes. *Opt Eng* 2005;44:063601-1–8.
- [8] Bezzubik VV, Ustinov SN, Belashenkov NR. Optimization of algorithms for autofocusing a digital microscope. *J Opt Technol* 2009;76(10):603–8.
- [9] Lee JH, Kim YS, Kim SR, Lee IH, Pak HJ. Real-time application of critical dimension measurement of TFT-LCD pattern using a newly proposed 2D image-processing algorithm. *Opt Lasers Eng* 2008;46:558–69.
- [10] Brazdilova SL, Kozubek M. Information content analysis in automated microscopy imaging using an adaptive autofocus algorithm for multimodal functions. *J Microsc* 2009;236:194–202.
- [11] Wright EF, Wells DM, French AP, Howells C, Everitt NM. A low-cost automated focusing system for time-lapse microscopy. *Meas. Sci. Technol.* 2009;20:027003-1–4.
- [12] Kim T, Poon TC. Autofocusing in optical scanning holography. *Appl Opt* 2009;48:H153–9.
- [13] Moscaritolo M, Jampel H, Knezevich F, Zeimer R. An image based autofocus algorithm for digital fundus photography. *IEEE Trans Med Imag* 2009;28:1703–7.
- [14] Abdullah SJ, Ratnam MM, Samad Z. Error-based autofocus system using image feedback in a liquid-filled diaphragm lens. *Opt Eng* 2009;48:123602-1–9.
- [15] Pengo T, Munoz-Barrutia A, Ortiz-De-Solorzano C. Halton sampling for autofocus. *J Microsc* 2009;235:50–8.
- [16] Liu CS, Lin YC, Hu PH. Design and characterization of precise laser-based autofocusing microscope with reduced geometrical fluctuations. *Microsyst Technol* 2013;19:1717–24.
- [17] Fan KC, Chu CL, Mou JI. Development of a low-cost autofocusing probe for profile measurement. *Meas Sci Technol* 2001;12:2137–46.
- [18] Zhang Z, Feng Q, Gao Z, Kuang C, Fei C, Li Z, Ding J. A new laser displacement sensor based on triangulation for gauge real-time measurement. *Opt Laser Technol* 2008;40:252–5.
- [19] Jung BJ, Kong HJ, Jeon BG, Yang DY, Son Y, Lee KS. Autofocusing method using fluorescence detection for precise two-photon nanofabrication. *Opt Express* 2011;19:22659–68.
- [20] Tanaka Y, Watanabe T, Hamamoto K, Kinoshita H. Development of nanometer resolution focus detector in vacuum for extreme ultraviolet microscope. *Jpn J Appl Phys* 2006;45:7163–6.
- [21] Rhee HG, Kim DI, Lee YW. Realization and performance evaluation of high speed autofocusing for direct laser lithography. *Rev Sci Instrum* 2009;80:073103-1–5.
- [22] Wang Y, Kuang C, Xiu P, Li S, Hao X, Liu X. A lateral differential confocal microscopy for accurate detection and localization of edge contours. *Opt Lasers Eng* 2014;53:12–8.
- [23] Weiss A, Obotnine A, Lasinski A. Method and apparatus for the auto-focusing infinity corrected microscopes, U.S. patent 7700903. 2010.
- [24] Liu CS, Jiang SH. A novel laser displacement sensor with improved robustness toward geometrical fluctuations of the laser beam. *Meas Sci Technol* 2013;24:105101-1–8.
- [25] Liu CS, Lin KW. Numerical and experimental characterization of reducing geometrical fluctuations of laser beam based on rotating optical diffuser. *Opt Eng* 2014;53:122408-1–8.
- [26] Yang SW, Lin CS, Lin SK. 3D surface profile measurement of unsymmetrical microstructure using Fizeau interferometric microscope. *Opt Lasers Eng* 2013;51:348–57.
- [27] Liu CS, Lin CH, Sun YN, Ho CL, Hsu CC. True color blood flow imaging using a high-speed laser photography system. *Opt Eng* 2012;51:103201-1–9.