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Rapid Autofocus Method for Optical Microscopes Using Dual Photodetectors

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Abstract. Aiming at solving the problems of low-efficiency and high-cost of existing auto-focusing methods for microscopic imaging, an on-line auto-focusing method is proposed and developed using dual photodetectors. Firstly, two identical photodetectors are placed in symmetrical positions with equal distance away from the focal plane in the image space. One is in front, the other is behind the focal plane. The photodetectors convert the light intensity signals originating from the sample into two electric signals, and their difference is proportional to the amount of defocus. Using a pre-calibrated curve between the differential voltage signal and the amount of defocus, the actual distance of the current sample from the focal plane can be measured in real time. The sign of the differential signal determines the direction of defocus, i.e., before or behind the focal plane. The sign and magnitude of the differential signal is used to control the direction and number of steps of a motor, which drives the carriage to move toward the focal plane and bring the sample into sharp focus, realizing the on-line automatic focusing of the microscope. The results of experiments show that the system can achieve efficient and stable automatic focusing. Under 40X objective lens, the effective working range of the proposed method is ±40um.Within this working range, the time required to achieve auto-focusing is 0.90702s, with the majority time, i.e., 0.907s spent on mechanical movement while 0.02ms for sensing the defocus. The major advantage of the proposed method is its high efficiency defocusing sensing at an updating frequency of 50k Hz. Further, it is easy to implement and with a low cost. Therefore, it is reasonable to expect that the proposed autofocusing method would have wide applications include classroom teaching, laboratory microscopic imaging, and inline product quality inspection.

Introduction

In the fields of optical microscopic imaging as well as other precision optical instruments, autofocus method is essential to improve the scanning efficiency while maintaining high imaging quality of samples. At present, existing microscope autofocus methods can be broadly classified into two categories: pure image processing autofocusing methods and defocusing amount detection methods [1, 2]. The former category includes depth from focus (DFF) and depth from defocus (DFD) method. The depth of focus method achieves autofocus by searching the extreme values of the focus evaluation curve of a series of out-of-focus images. The key is to select the image sharpness evaluation function and the focus search algorithm. Ren Si gang et al. [3] proposed the sum of the absolute values of the grayscale differences of the image as the in-focus evaluation function, and adopted the hill climbing search strategy to improve the focusing efficiency, The system repeatability is up to 8um, and the precision is high but the speed is relatively slow. The defocus depth method uses the established optical system defocus model to obtain the depth information of the out-of-focus image to achieve autofocus. Soshiro Makise et al. [4] used the defocus depth method to extract the depth information of the target cells from the diffraction image to focus the cells with a time response of 20 ms. The defocus depth method is more suitable for objects with certain optical characteristics (such as cells), and the versatility is poor. The second category of autofocusing methods mainly includes: eccentric beam method, critical angle method, and differential focusing method[1]. Li Qingxiang and others of Tsinghua University [5] applied the eccentric beam method in the microscope,

and the focusing accuracy reached 0.1um in the range of ±500um, but this method requires strict external environment. The critical angle method of the focal point system designed by Hao Xianpeng et al, [6] has a precision of 15 nm with a linear range of ±4 um, and the structural optical path is relatively complicated. Harbin Institute of Technology Tan Jiu bin et al. [7] adopted the differential focus idea to obtain different axial information by increasing the number of cameras in the microscopic system to achieve accurate positioning. However, due to the time required to capture the images with at least several milliseconds of exposure time for each image, to transport the image, and to process the image, therefore such a method would have a low efficiency.

In summary, existing microscopic autofocus methods suffer from either low efficiency, or having a complicated structure, or having a high cost. To deal with these problems, this paper proposes an on-line fast autofocus method using dual photodetectors to construct a differential signal that is proportional to defocus amount. This online fast autofocus method is inexpensive, efficient, and convenient to use. Therefore, it is reasonable to expect that the proposed autofocusing method would have broad prospects in the applications of classroom teaching, laboratory microscopic imaging, and online product surface quality inspection.

Principle of Differential Signal of Dual Photodetectors

The differential focusing principle of double photodetectors is based on the axial-response property of general microscopic imaging technology. The conjugate relation among the light source, the sample point and the detector is satisfied [8, 9]. Fig. 1 shows the differential focusing principle of double photodetectors. The two photodetectors in the figure are placed at the positions away from the focal plane of image space ΔM ($M>0$) meanwhile with the equidistant deviation from the central optical axis ΔR ($R>0$) respectively, which forms the two-way detection for the feature optical flow of the sample. When the target object is located at the focal plane, the energy of the optical signals received by the two photodetectors are the same, that is, the differential focusing electric signal is zero. When the target object deviates from the focal plane, the energy of the optical signals received by the two photodetectors are different. If the target object moves upward from the focal plane for a distance, the convergent image points will fall behind the focal plane, then the energy of the optical signals received by detector 1 will be greater than that received by detector 2. On the contrary, the energy of the optical signals received by detector 1 will be less than that received by detector 2 [10]. According to the mapping relationship between the calibrated differential focusing electrical signal and the focal shift, the current focal shift can be quickly obtained from the differential electric signal output by the signal processing module.

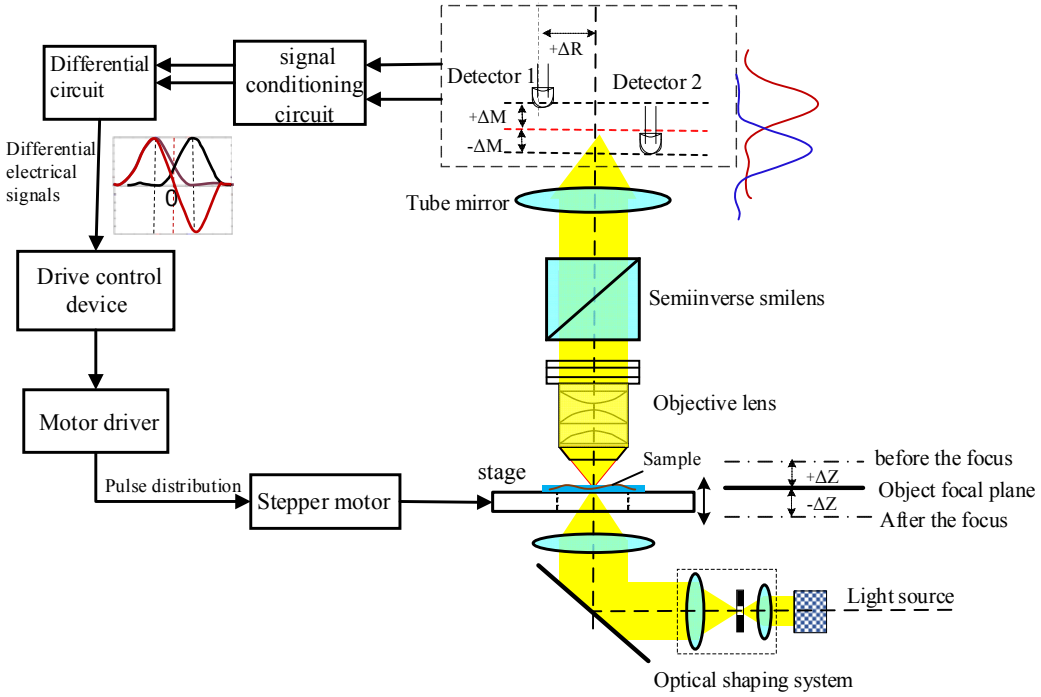

Figure 1. Illustration of the proposed autofocus system using dual-photodetectors to construct differential signal

The differential focusing system based on double photodetectors is composed of photoelectric detection module, signal processing module and driving control module. The working process of the system is as follows: Firstly, two optical signals of the sample are converted into two electric signals by two photodetectors with the equal distance in front of and behind the focal plane of image space meanwhile with the equidistant deviation from the central optical axis. Secondly, two electric signals enter differential circuit after the conditioning of signal processing circuit, then the differential electric focal shift signal can be obtained and amplified. Thirdly, the amplified the differential electric focal shift signal is converted into control pulse signal by the controller and be sent to the actuator. Finally, the motor drives the stage to the focal plane, so that the magnitude of the differential signal, in other words, the focal shift, approaches zero gradually, thus realizing the auto-focusing online of the microscope.

Experimental Process and Results Analysis

Platform Construction and Implementation

Differential focusing system is composed of photoelectric detection module, signal conditioning module, driving control module and microscopic imaging module. Regarding the hardware part, it is necessary to do the work of selecting the type and designing mechanical structure. As for software technology, we need to do three key technical work and motor driving algorithm well. Fig. 2 is the device diagram of the differential focusing experimental platform.

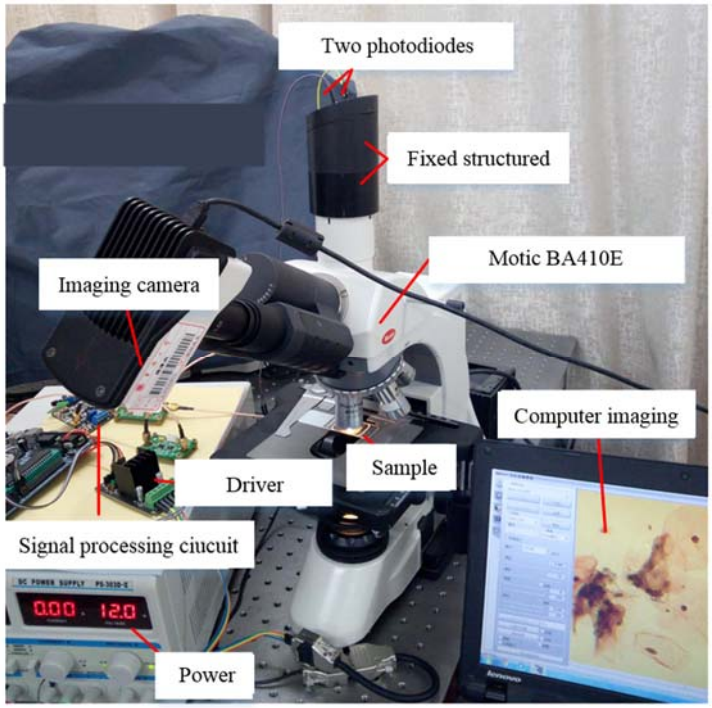

Figure 2. Device Diagram of Differential Focusing Experimental Platform

Calibration Work before the Experiment

(1) Determination of the position of two photodetectors in image space

As for the placement of two photodetectors, there are two key technical problems to be solved in this design. The first problem to be solved is where the two photodetectors should be placed. The second problem to be solved is how to put the two photodetectors.

First, let's discuss where two photodetectors should be placed. The selection of the image defocus amount ΔM and the lateral offset ΔR is not arbitrary. The amount of the image defocus amount of the detector directly affects the clear range of the microscopic imaging, the linear working range, etc. Therefore, The reasonable choice of ΔM and lateral offset ΔR of the image defocus amount is essential to the focusing accuracy of the system. According to the principle of differential focusing introduced above, The distance ΔM of the photodetector before and after the image square focal plane and the distance ΔR of the photodetector the left and right center optical axis are tested experimentally. Under the 40x objective lens, different ΔM were adjusted for the test experiment and the variation of ΔM was selected from 0-7 mm. According to the test results: the axial response curve shifts to the right with the increases of ΔM , while the detection curve basically stabilizes when ΔM increases to 5-7 mm. The deviation distance ΔM=5 mm is selected finally in combination with the curve response. The same experimental test of ΔR is carried out , adjust the axial defocus distance ΔM is fixed and the adjustment ΔR increases horizontally from 0-4mm. The test results show that the system imaging effect is best when ΔR=3mm.

After determining the offsets ΔM and ΔR of the two photodetectors in the image plane, it is necessary to further determine how the two photodetectors will be placed. As shown in Fig. 3, the detection planes of the two photodetectors should be perpendicular to the optical axis of the system. When the target sample is in the focal plane, the light intensity received by the two photodetectors should be the same. that is, the defocusing differential electric signal is zero. At this time, the position relationship between the two photodetectors is considered to be an ideal state.

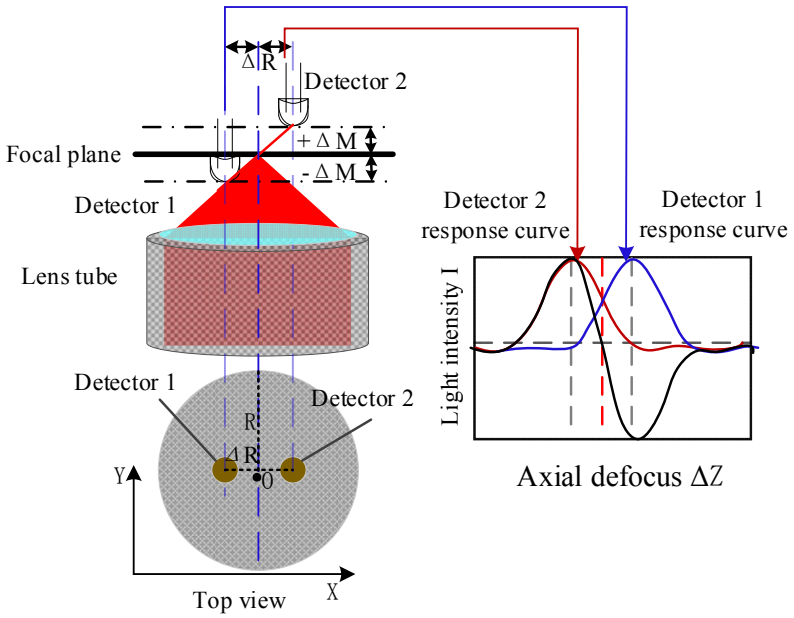

Figure 3. Brief diagram of position relationship between two photodetectors

(2) Calibration of mapping relationship between defocus differential voltage signal and defocus

After the position of the two photodetectors in the image space is determined (ΔM =5mm, ΔR=3mm), the mapping relationship between the defocused differential voltage signal and the defocus amount needs to be calibrated. In this paper, the mapping relationship curves between differential voltage signal and axial defocus amount is plotted under the experimental conditions of ΔM =5mm, ΔR=3mm and 40X objective lens. Firstly, the experimental platform of differential focusing is initialized, than, adjust the target object to the focal plan so that the differential voltage signal output by the differential amplifier circuit is zero.

Table 1. Differential voltage signals corresponding to interval sampling

Differential voltage signal ΔU（mv）	Raster feedback values	Defocusing amount ΔZ（um）
0.054	-1600	-80
0.057	-1400	-70
0.06	-1200	-60
0.053	-1000	-50
0.056	-800	-40
0.041	-600	-30
0.027	-400	-20
0.01	-200	-10
0	0	0
-0.018	200	10
-0.039	400	20
-0.056	600	30
-0.073	800	40
-0.076	1000	50
-0.073	1200	60
-0.07	1400	70
-0.068	1600	80

In the range of vertical (+ 80um), the carrier station is moved at equal intervals of 10um, and the differential voltage signal corresponding to each defocusing position of the carrier station is recorded. The specific defocusing value can be accurately measured by grating. The following 15 groups of data are collected in the experiment. Motic BA410E microscope was used in the experimental platform. The defocusing of 20 gratings was 1 um. The experimental data are shown in Table 1.

Based on the above experimental data, the mapping curve between differential electric signal and axial defocusing is drawn, and the linear equation is fitted, as shown in Fig. 4.

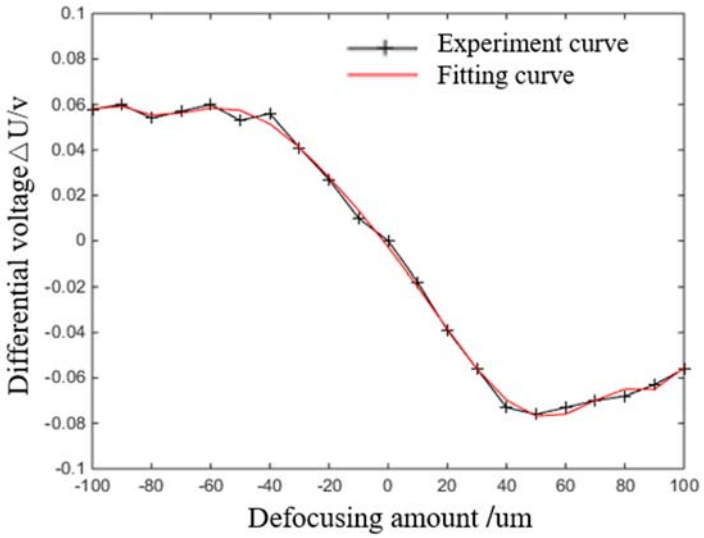

Figure 4. Mapping Curve of Differential Voltage ΔU and Defocus ΔZ

The linear region equation obtained from the fitted curve is as follows:

$$K = \frac{\Delta U}{\Delta Z} \tag{1}$$

$$\Delta U = -0.0021 \cdot \Delta Z \tag{2}$$

From the calibration curve, it can be seen that there is a linear relationship between the defocus differential voltage signal and the axial defocus. The direction and size of the current object's distance from the focal plane can be quickly and accurately converted from the defocus differential voltage signal. The system has a linear working range of about 80 microns, which effectively improves the automatic focusing range of the system. On the basis of the existing experimental conditions and platforms, the linear region equation obtained from the curve fitting of the above experimental data shows that the sensitivity of the measured system can reach 2.1 mv/um, that is, the variation of the unit defocus will make the differential amplifier circuit change 2.1 mv.

Experimental Results

Focus Efficiency Performance Test. Focusing time is mainly composed of voltage A/D acquisition and motor drive control. The time consumed in data transmission process can be neglected.

1) AD acquisition time. The acquisition time mainly depends on the frequency of the signal, the maximum is 50Hz, i.e. the period is 20ms. If 1000 points are sampled in a period, the sampling time Tad= 20ms/1000 = 20mus; that is, (1/fad)* t = 20mus.

2) Motion control time. The time consumed in motion control depends on the motor control algorithm. Motion efficiency is controlled by motor speed, control algorithm threshold and delay time function, which can be recorded as T machine and measured.

The step angle of the two-phase four-wire eight-beat stepper motor is 0.9 degrees [11], and the grating value obtained by sending a pulse feedback from the motor is 20. Experiments were carried out under the experimental conditions of 40X objective, ΔM=5mm and sample defocusing Z=30um. The experimental data of 10 groups were recorded as follows:

Table 2 Test data of autofocus efficiency

number	Motor control algorithm efficiency（s）
1	0.91
2	0.89
3	0.97
4	0.86
5	0.89
6	0.97
7	0.93
8	0.84
9	0.89
10	0.92
Average efficiency	0.907

The 10 sets of experimental data were analyzed and processed. The specific results are as follows:

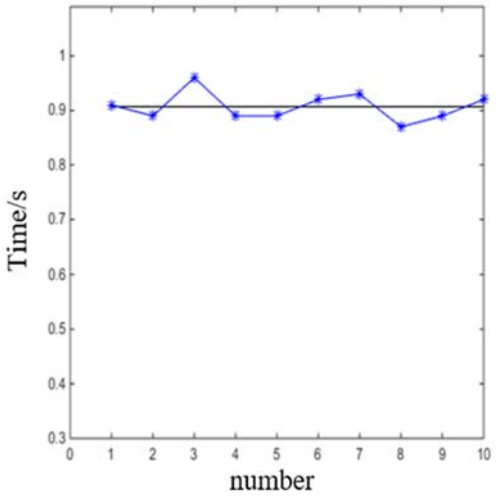

Figure 5. Fluctuations in focus time

The experimental results show that the efficiency of motor control drive is about 0.907s. In summary, under the above experimental conditions, at the defocusing position of 30 um, the time required for the system to achieve automatic focusing is as follows: Tm=0.00002s+0.907s=0.90702s

Conclusion

This paper proposes an on-line microscopic auto-focusing method, it employs dual photodetectors to construct differential signal which is proportional to the amount of defocus. The system consists of two parts: a defocusing detector and motor drive control module. The experimental results show that the system can achieve efficient and stable auto-focus. Under 40X objective lens, the effective working range of the proposed method is ±40um.Within this working range, the time required to achieve auto-focusing is 0.90702s, with the majority time, i.e., 0.907s spent on mechanical movement while 0.02ms for sensing the defocus. Compared with existing autofocusing methods, the major advantage of the proposed method is its fast defocusing detector, which measures direction and magnitude of defocus at a frequency of 50k Hz. This speed is at least three-order of magnitude higher than that of pure image-processing based autofocus detection methods. Further, it is easy to implement and with a low cost. Therefore, it is reasonable to expect that the proposed autofocusing method would have wide applications include classroom teaching, laboratory microscopic imaging, and inline product quality inspection.

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