

Study on Auto-focus Methods of Optical Microscope

Hongwei Shi, Yaowu Shi, and Xiaojie Li

Jilin University, College of Communication Engineering, ChangChun, 130025

Abstract. This paper describes the structure of the auto-focus system for microscope based on image treatment. The kernel of this system is the evaluation function. It gives two new image quality evaluation functions: color ratio and image difference. The image difference can be used to estimate that if the focus is seriously apart from the optimal position. Color ratio can provide 50 times of dynamic range while traditional evaluation functions only provide 2-3 times. It proposes a new type of grad: natural grad. Natural grad work better than traditional grad such as Sobel, Roberts, Prewitt, etc. It proposes the method based on the combination of the three function: First using image difference to estimate the foci, when it is enter the dynamic range, using natural grad. When entering near range, color ratio is used to search for the optimal position.

Keywords: Auto-focus; Natural Gradient; Image Difference; Color Ratio.

1. Introduction

As the development of camera and computer technology, applications based on the micro-image analysis are developed. These applications come from medicine, biological, food, geology, etc. In most of these applications, digital camera is fixed on the ocular of the microscope, and connected to computer. Images taken by the camera are transmitted to the computer. Software in the computer analyzes the images. In these systems good foci and clear image is necessary for the subsequent treatment. So an auto-focus system is needed, and the efficiency and impression of the auto-focus system directly impacts the performance of the whole system.

The existing auto-focus methods for microscope can be classified to two kinds: initiative methods^[1] and methods based on image treatment^[2,4]. In initiative methods object distance is measured by additional equipments and focus is adjusted according to the measurement. Because additional equipment is needed in this method, so it is rarely used in practical work. Most auto-focus methods are based on image treatment. In these methods, the evaluation function is the kernel of the system. It provides a measurement for clarity of the image taken from camera. When the quality of the image becomes too low, an auto-focus process is started. It searches for the optimal object distance that provides optimal clarity of image. These methods are developed according to auto-focus system of digital camera. But there are some differences between the two types of system. So when using these method in microscope, they don't work well.

In practical work, we can divide the auto-focus process into three stages according to the object distance. The first is the obviously defocus stage. The object distance has seriously apart away from the optimal position, so we can see nothing in the image. The second is the changing stage. The object distance is closed to the optimal position. We can see the outline of the observation object changes and decide the right direction of focusing. The third is the nearly focal stage. The object distance is near to the optimal position, the image is relative clear, and we just need to search for the best position in a small range.

The traditional evaluation functions include gradient^[1,2], information entropy, variance^[1,2,3] and high frequency band-pass^[3,4,7]. In practical applications, information entropy and variance cannot provide correct information for focus, high frequency band-pass method needs a large amount of calculation. Those methods provide a narrow range of value, which is disadvantageous to the precise focus.

⁺ Corresponding author. *E-mail address* qitiand@qq.com

This paper puts forward three evaluation functions: image difference method, gradient area method and color ratio method. We propose that estimating the obviously defocus stage with the image difference method, judging the searching direction with the gradient area method in the changing range, in the near range, color ratio method is used to search the optimal position to the focus.

2. Focus System Structure

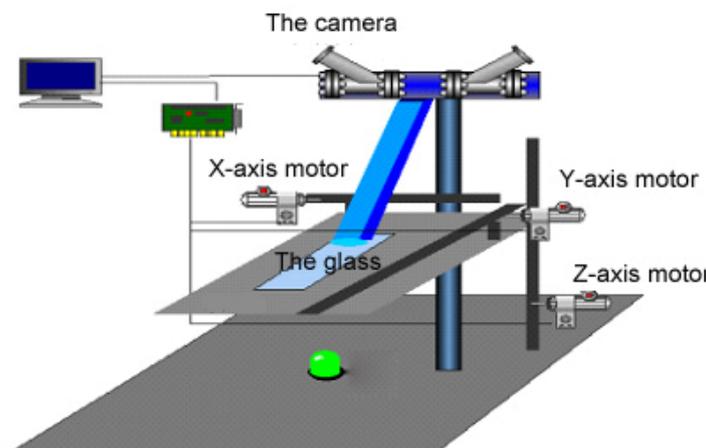


Fig.1 Auto-focus system structure diagram

As shown in the fig.1, the camera fixed on the ocular takes picture and transmits it to the computer. Step motor fixed on the shaft of the microscope to move the slide up and down to control the glass moving. Software in computer accepts image from camera, when the quality of the image is too low, it starts a auto-focus process to find the optimal position for object distance that provides clearest image.

3. Image difference method

In the obviously defocus stage, we can see nothing in the image, and cannot decide the correct direction of focus, so we should search in a wide range at both directions. So we need a guideline to estimate whether it is in this range. Traditional evaluation functions cannot provide a stable value, so we cannot use them to determine whether it is seriously apart away from optimal position.

Because we can see nothing, the images taken at different positions in this range show little difference. So the image difference can be defined as the maximum difference of single pixel in gray image or color distance of single pixel in color image. In the range that the object distance seriously apart away from the optimal position, the difference of two image taken from different positions remains in a narrow range. Fig 2 shows the image difference of a focus process.

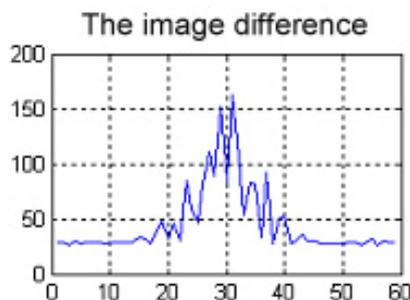


Fig.2 Image difference change to the object distance

As shown in fig 2, when the object distance remains in the seriously defocus stage, the image difference is stable in a narrow range. We calculate the value of different positions in the slide, the value is stable in 33-38. So we can calculate the value in auto-focus process, when the value is smaller than 36, we search in a

wide range in both direction until we get a image difference bigger than 40, it enter changing range. Then other evaluation function can be used to determine the focus direction.

4. Natural Gradient And Gradient Area Method

Gradient is the most general concept used in auto-focus. The concept of gradient is proposed in the study on image edge. The edge is very important in image treatment, and the edge part show high gradient in the image.

The common gradient operators include the Sobel operator, Roberts operator, Prewitt operator, the Kirsch operator, and the Robinson operator etc. Among them, the Sobel operator and the Roberts operator are more typical.

Sobel operator, Horizontal h:

-1	0	1
-2	0	2
-1	0	1

Vertical v:

-1	-2	-1
0	0	0
1	2	1

Roberts operator, Horizontal h:

-1	0
0	1

Vertical v:

0	1
-1	0

The anti-jamming ability of Sobel operator is better than the Roberts operator, because it uses the average of several parts.

In practical, we observed that in some cases Sobel operator is sensitive to the interference, so we propose the operator based on the definition of gradient:

Assume the image is a two dimensional function, namely $V = f(x, y)$, we define the gradient value following:

$$grad(f(x, y)) = \sqrt{\left(\frac{\partial f}{\partial x}\right)^2 + \left(\frac{\partial f}{\partial y}\right)^2} \quad (1)$$

$\frac{\partial f}{\partial x}$ is the Horizontal gradient, and $\frac{\partial f}{\partial y}$ is the Vertical gradient.

Suppose the image have a continue gradient distribution, gradient value change a little in a small area, then we can measure the gradient value of a position with kinds of methods, because of the existence of noise in images, it is more accurate to take the average of several independent measure.

The following is the direct measurement of horizontal gradient :

- a) $f(i+1, j) - f(i, j)$
- b) $f(i, j) - f(i-1, j)$

$$c) \frac{f(i+1, j) - f(i-1, j)}{2}$$

We can also define the same measurement of the following definition:

$$d) \frac{f(i+1, j+1) - f(i, j)}{\sqrt{2}}$$

$$e) \frac{f(i, j) - f(i-1, j-1)}{\sqrt{2}}$$

$$f) \frac{f(i, j) - f(i+1, j-1)}{\sqrt{2}}$$

$$g) \frac{f(i-1, j+1) - f(i, j)}{\sqrt{2}}$$

$$h) \frac{f(i+1, j+1) - f(i-1, j-1)}{2\sqrt{2}}$$

$$i) \frac{f(i+1, j-1) - f(i-1, j+1)}{2\sqrt{2}}$$

In the definitions above, a), b), d), e), f), g) are based on the single dx (distance of nearest pixels), while c), h), i) are based on 2dx and more strong to noise, Taking the average of the three operator, we can define the horizontal gradient as follows:

$-\frac{\sqrt{2}}{4}$	0	$\frac{\sqrt{2}}{4}$
$-\frac{1}{2}$	0	$\frac{1}{2}$
$-\frac{\sqrt{2}}{4}$	0	$\frac{\sqrt{2}}{4}$

Multiplied by a ratio, the operator can be transformed to the following for calculation:

-1	0	1
$-\sqrt{2}$	0	$\sqrt{2}$
-1	0	1

Likewise, the vertical gradient template can be defined as follows:

-1	$-\sqrt{2}$	-1
0	0	0
1	$\sqrt{2}$	1

As we see, Sobel operator and Prewitt operator is the approximate of it. We call this gradient operator as the natural gradient operator.

Among auto-focus process, there are several methods derived from gradient method: the average gradient method, the maximum gradient method, the gradient area method. The average gradient has good anti-interference ability, but has small value range; the maximum gradient method has large value change range but it is too sensitive to noise.

In defocus position, the edge become fuzzy and the edge area become bigger. In edge detecting, we calculate gradient of all pixels, and consider the pixels of which the gradient is higher than a threshold. Then the count of these points can be a evaluation for auto-focus process. Gradient area is defined as follows:

$$S_a = \iint_{grad(x,y)>t} 1dxdy \quad (2)$$

In the definition, the threshold t can be defined as a value between mean value and maximum value. a is a coefficient, generally the value range is 0-0.5. Then t is calculated as follows:

$$t = \overline{Grad} + a * (Grad_{max} - \overline{Grad}) \quad (3)$$

The discrete form of above as follows:

$$S_a = count(V |_{grad(i,j)>t}) \quad (4)$$

In practice, we set a to 0,0.2 and 0.4. The value is maximum at optimal position When a is set to 0.2 or 0.4. Their value is sensitive to diffraction phenomenon. But when a is set to 0, the value is minimum at optimal position. It can provide the information of best focus point.

The 0 order Sobel gradient area method is more sensitive to noise than natural gradient area. Fig 3 shows contrast of the two methods at two points.

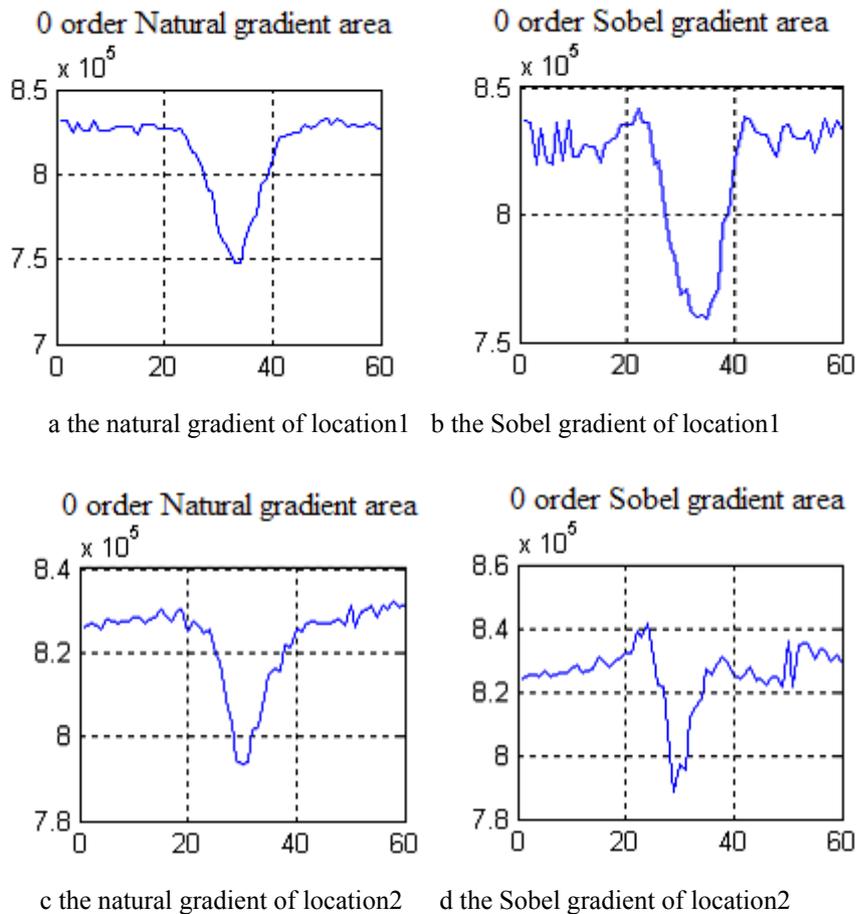


Fig.3 The 0 order gradient area method based on Sobel and Natural gradient operator.

From fig 3, we can see that 0 order natural gradient area provide more smooth curve than Sobel gradient.

5. Color Ratio Method

At the optimal foci of the microscope, there will be obviously color information caused by the refraction at the edge of the observation object, at the edge toward the light source it shows a thick edge of light with high frequency, while at the edge depart from the light source, it shows a thick edge of light with low frequency. For example, we use a yellow lamp as light source, at the edge towards the light source, the edge is green while at the edge depart from light source, the edge is red. The two edges are most clear and obvious at the best focus point. This character can be used to guiding the search process of the best focus point.

We define the color ratio as the ratio of two colors of a pixel in the colored image. The single point color ratio is defined as follows:

$$C_{rg}(i, j) = \begin{cases} \frac{r(i, j)}{g(i, j)} & (r(i, j) \geq g(i, j)) \\ \frac{g(i, j)}{r(i, j)} & (r(i, j) < g(i, j)) \end{cases} \quad (5)$$

The color ratio is a value that always not less than 1. The maximum color ratio in a image can be used as evaluation for clarity of the image. Fig 4 shows the changes of color ratio with the object distance.

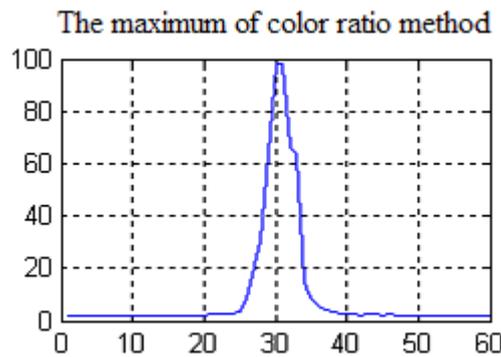


Fig. 4 The changes of color ratio to the object distance

The peak point is the optimal point of the object distance. In this point the image is clearest and the color character is most obvious. When objects distance depart this point in either direction, the value falls sharply. The maxim of the value is 50 times of the low value. That is larger than any traditional quality measurement method.

The value just change in a small object distance range, so we should use other quality function in widely searching process, and when it is closed to the optimal point, the color character is be useful to find the optimal point. When we find a single point with obvious color character, we can label a region around this point, and just calculate the value of this region in following searching.

6. Focusing Process

We discuss three methods for auto-focus system, they can be used in different stage in auto-focus process. First, we use the image difference method to determine whether it is in seriously defocus stage, if it is then search in a wide range on both directions until it enter the changing stage. In the changing stage, we use 0 order natural gradient area method to determine the search direction, calculate the color ratio at the same time. When it is into near stage then we search the optimal point of color ratio. The best focus point is just the maximum point of the color ratio.

7. Conclusion

In view of the auto-focusing process of optical microscope, this paper analyzes the system structure and point sout that kernel of auto-focus system is the evaluation function. This paper proposes two new evaluation functions: the image difference method and the color ratio method. The image difference method

has stable threshold value, can be used to determine significant defocus. Color ratio method has more wide value range than any other methods, but has a small responsive object distance. It can be used for nearly focus searching. This paper proposes the concept of natural gradient based on the analyzes of the traditional gradient method. Natural gradient works better than traditional gradient operator. This paper proposed the combination with three evaluation functions in auto-focus process.

8. References

- [1] JIA Xiao-yan, XIAO Ze-xin, DENG Sh-i chao, Research on auto-focusing method based on focusing evaluation function, *OPTICAL TECHNIQUE*, Vol. 33 Supp1 [7-9]
- [2] Wang Chao, Jiang Yuanda, Zhai Guangjie, Cai Shijie Study on auto focusing algorithm form microscope base on digital image processing, *Chinese Journal of Science Instrument*, 2009-6[1290-1294]
- [3] WANG Xin, AN Zhiyong, YANG Ruining, The Research of CCD Camera Auto-focusing Technology Based on Image Definition Criterion. *Journal of Changchun University of Science and Technology (Natural Science Edition)* 2008-3[12-14]
- [4] JIANG Haihua, Research of microscope auto-focusing technology based on image definition criterion function. *OPTICAL TECHNIQUE*, Vol 34 Supp1 [284-285]
- [5] Jiang Gang-yi, Huang Da-jiang, Wang Xu, Yu Mei, Overview on Image Quality Assessment Methods. *Journal of Electronics & Information Technology*. 2007 Vol.32No.1 [219-226]
- [6] HU Tao, CHEN Shi zhe, LIU Guodong, PU Zhaobang. Selection of Auto-focus Function in Micro Visual System. *SEMICONDUCTOR OPTOELECTRONICS*. 2006 Vol. 27 No. 2
- [7] Cao Maoyong. Sun Nongliang. Yu Daoyin Study on Clarity-evaluation-function of Out-of-focus Blurred Image. *Chinese Journal of Science Instrument*. 2001 Vol 22 No.3 [259-268]