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# Class Variance Based Instruction function for Microscope Auto-focus

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***Abstract***

*This paper proposes an auto-focus Instruction function. The function is derived from Ostu segmentation. It uses class variance of segmentation result to instruct auto-focus process. Compare with the exist functions, class variance function need a small calculation mount. It is immunity to jam coming from camera and other inspection. It responses in a wide object distance range. And the value changes in a large range. All this features make this function is most flexible for microscope real-time auto-focus process. This paper analyzes the function and compares it to some traditional function. All the data are based practical instrument.*

***Keywords****: auto-focus, Ostu, instruction function, optical microscope.*



## 1. Introduction

There appears more and more applications that use microscope to detect and analyze material. The general structure is to install the microscope camera on the eyepiece of the microscope which is connected with a computer, intake image from the microscope and then import it into the computer for processing and analysis. Microscope glass table is connected with the motor after transformation; it can control the plane and three-dimensional mobile through a microscope computer. Because the quality of the image is seriously affected by the focus process, auto-focus technology becomes a kernel issue to the performance of the whole system.

In these systems, the auto focusing is inevitably a challenge and at the same time, it is also an important factor in affecting the test result. When the microscope is defocus, the system should start a auto-focus process. And when the auto-focus process ended, the quality of microscope image should be fine. And the subsequent process treat the clear image, so the performance of the whole system can be guaranteed.

Most of existing microscope auto-focus system is passive auto-focus system. In these systems, the image is analyzed when a new position is arrived, if the image quality is low, then an auto-focus process is start. In the process, image is evaluated with instruction function, the instruction function can evaluate the clarity of the image, and the system drive the motor system move according the evaluation result. So the image can be clear, when the quality of the image is best, the auto-focus process end. In these systems, instruction function is the kernel facet for the performance of the auto-focus system.

Traditional instruction function is most migrated from digital camera auto-focus technology. Those functions include the variance of gray, gradient, texture feature, entropy and frequency domain information. But there is some different feature in microscope auto-focus system. This paper proposes a new instruction function that based on image segment. Because microscope images are mostly simple, so the segment result is better than macroscopic image and in the segment result, the distance of the two classes can indict the quality of the orient microscope image.

Ostu threshold segmentation is classical method based on the threshold of image segmentation methods, it uses the gray point that acquire largest class variance as the threshold to segment the image. If the image is clear, the segment result will be smooth and

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clear, and the class variance will be larger. While poor image quality will get lower class variance. So the class variance of segment result can indict the quality of image and it can be used to instruct the auto-focus process.

So we put forward that regarding the class variance of threshold partition algorithm as instruction function, its calculation is small, the range of object distance is wide, the range of value is large, the anti-interference ability is strong, which insures that the focusing process perform correctly and achieve the best results.

This method is applied to an optical microscope and automatic detection system, in which the sample is made to be glass to be observed.

## 2. Traditional Instruction Functions and Quality of those Functions

(1) The existing evaluation function

The existing automatic focusing instruction function mainly divided into two categories, which are spatial domain and frequency domain. They are all designed based on the basic cognition that the image edge of the best aiming point is the clearest.

There are variance [7], gradient [1-3], autocorrelation function, neighborhood information [3] and entropy [10] and other methods based on spatial domain.

The method based on variance is for obtaining the variance of pixel gray value (or brightness values) as function evaluation. Because of its accurate focus, the clear edges of the image and the most obvious contrast, its variance will reach the maximum point.

Gradients are very important concepts in the analysis of image edge. Because the edges of the image area are larger parts in the local gradient, the greater the gradient is, the clearer the edge is, so the gradient in the edge recognition has been widely used. The method based on the gradient is also based on the feature that the best focusing image edge is the clearest; calculate the average or maximum gradient as a guiding function. General gradient operators are sobel operator, laplace operator, Brenner function, Roberts operator, Prewitt operator [4] and so on. According to the different statistical methods, it can be divided into two ways, which are taking variance and taking average. According to the processing of operator results, there are many ways, such as taking squares, taking modulus and variance taking absolute value for operators.

In the method of entropy, because of its defocused image is confusion, so the entropy increases gradually. As the edge of image is clear, the information is clear in the best aiming point, so the value of entropy is small.

The instruction function based on frequency domain has two types [11] which are based on fourier transform and wavelet transformation. Based on fourier transform, there are two ways which are taking expectation of amplitude and taking high frequency filtering. The method based on the wavelet is to make image wavelet decomposition, then calculate the sum of high frequency components absolute value and use it as a guide function. But because of the large amount of calculation and the complexity of the calculation, it is still at the research stage.

In the methods mentioned upper, frequency domain based instruction functions have an excellent performance except its large calculation mount. Actually all existing function need a large mount of calculation except function based on entropy. But in microscope auto-focus process, entropy function cannot correctly indicate the best position of object distance. So it need to search for a new instruction function that need little calculation mount and have strong anti-jam ability and can response in a wide object distance.

(2) Quality of instruction functions

Instruction function is the core of automatic focusing method, selecting the appropriate instruction function is the premise of designing the system of automatic focusing, makes the evaluation of instruction function an unavoidable problem. Some scholars have proposed a number of evaluation indexes [5-6], [8] of instruction function, which could evaluate and compare different instruction functions to select the best instruction function.

Using the existing camera system, by taking a series of images near the best aiming point to be the instruction function, we can get a curve that the function values change according to the focal length, the typical maxima instruction function curve is in Figure 1.

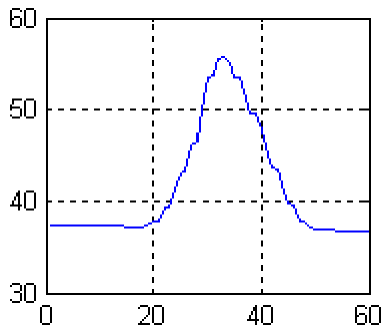


Figure 1. Sample for Instruction Function Curve

To measure the curve, you can develop a series of indicators to evaluate the instruction function, a common evaluation indicators are as follows:

1) Departure degree of extreme points $DU = \frac{1}{n}\sum |u_{\max} - u_0|$ $u_{\max}$ means extreme points; u0 represents the actual best aiming point, numerical methods is to take a number of different vision points, record the distance between instruction function's extreme point and the actual best aiming point, and then take the average.

2) The variation range of Q $C = \frac{\max(Q) - \min(Q)}{\bar{Q}}$. means the ratio of the variation range of the instruction function to its average.

3) Variation range of the object distance $U = u_1 - u_2$, u1 and u2 are defined as the most stable region separated from both sides, they are the values that move 0.2 toward the extreme.

4) The obvious degree of the peak (valley) value, $G = \frac{Ave(|f''(u)|)|_{|u-u_0|<u_d}}{\bar{Q}}$, u0 is defined as the extreme points, ud is a selected value, it means the average of the first derivative in a fixed range near the extreme points.

5) Anti interference ability of DN means the instruction function's resistance ability to the noise. It can be expressed in the number of local extreme points.

6) The variation range of evaluation value means taking several vision points, and calculate the evaluation value Qi of the best aiming points respectively.

$$K = \frac{\max(Q_i) - \min(Q_i)}{\bar{Q}}$$

7) Computation mount, this mount could not be given in mathematical form clearly, it can only be measured from the mathematical definition of the various methods.

In these indicators, the smaller extreme point deviation is premise of the application of the method, and the general instruction function was satisfied. In the case when the indicators are available, the most important factor on the system is the resistance ability. If the anti-interference ability is poor, guiding function curve will have many local extremes, it will lead to the error of search function, which leads to focus failure. To avoid this problem, we need to do a lot of detailed work for search function to judge the direction of current instruction function, this process can make the search procedure become very complicated. At the same time, it will cause the reliability to decrease. The variation range of Q value and the variation range of object distance will also have bad influence on system performance: the variation range of Q value is small, the ability of anti-interference is poor and the variation range of object distance is small, so focusing cannot be completed when defocus is serious.

## 3. The Instruction Function Based on Ostu Segmentation

(1) Ostu segmentation method

Image segmentation is a very important operation in the image processing, the aim is to divide image into black and white according to brightness or other indicators. In the method of image segmentation, the method based on threshold is fundamental: to set a threshold and determine the segmentation result according to the target value is greater than or less than a threshold. In this method, the determination of threshold is the central focus. There are many methods, in which the most famous and classic one is the Ostu method.

The idea of Ostu segmentation method is to make the maximum between-class variance as the criteria of threshold .Such as image I, in which $I(x,y)$ is pixel set, segmentation threshold is T, then the segmentation results U are:

$$U(x,y)=\begin{cases}1 & I(x,y)\ge T\\ 0 & I(x,y)<T\end{cases} \tag{1}$$

The between-class variance is defined as variance of gray value of pixel points which are divided into two kinds in the original drawing. As the gray scope of the image is 0-K (usually 0-255), $h(i)$ means the image histogram. $p_i$ is the ratio of the point whose gray is i , the image average gray is as follows:

The average gray is:

$$\bar{u}=\frac{\sum_{i=0}^{K} ih(i)}{\sum_{i=0}^{K} h(i)} \tag{2}$$

the average gray of the points whose Segmentation are 0 and 1 are:

$$u_0=\frac{\sum_{i=0}^{T-1} ih(i)}{\sum_{i=0}^{T-1} h(i)} \tag{3}$$

$$u_1=\frac{\sum_{i=T}^{K} ih(i)}{\sum_{i=T}^{K} h(i)} \tag{4}$$

the ratio of the points whose Segmentation are 0 and 1 are:

$$P_0=\frac{\sum_{i=0}^{T-1} h(i)}{\sum_{i=0}^{K} h(i)} \tag{5}$$

$$P_1=\frac{\sum_{i=T}^{K} h(i)}{\sum_{i=0}^{K} h(i)} \tag{6}$$

The definition of between-class variance is:

$$Var = P_0(u_0 - \bar{u})^2 + P_1(u_1 - \bar{u})^2 \qquad (7)$$

Choose the point which makes the between-class variance become the largest as threshold to do image segmentation.

(2) Focus instruction function based on Ostu segmentation.

Ostu segmentation is a very effective segmentation method for microscopic image processing. The image of best aiming point is the most clear, the result of Ostu method is the best, and its between-class variance is maximum. For out-of-focus image, because of its image is blur and gray distribution is dispersion, so the between-class variance is relatively small and we can use the between-class variance of Ostu segmentation as a instruction function for automatic focusing. Instruction function is defined as following:

$$Q = \max(Var_i) \qquad (8)$$

To calculate the between-class variance after the image segmentation, whose threshold is from 0 to K, take the maximum value. Because the purpose is to get the maximum between-cluster variance, there is no need to do image segmentation and obtain threshold. What we need to do is just to traverse one time for image, calculate the histogram of image h and then take it as calculation object. Compared with the existing method, the number of the element of H is 256 and its calculation is very small.

The curve of Ostu instruction function is as follows:

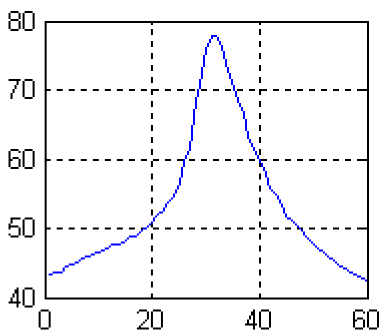


Figure 2. Curve of Ostu Instruction Function

From Figure 2, we can conclude the instruction function can take extreme value in the best aiming point, as the curve is smooth, so the interference of noise is negligible, and as its value range is close to 2 times, so it is suitable for focusing.

## 4. The Comparison of Ostu Focus Function and the Traditional Function

(1) Implementation methods for comparison

First, we concern the calculation mount, it is difficult to compare the calculation mount of different function. But according to the definitions, except entropy function, other methods are needed to traverse the image more than two times, the computation is much larger than the Ostu function, which is the most obvious advantages of Ostu function.

With the actual microscope image system, it take a total of 12 different locations in the four slides and get 60 pictures in equal space, which centers on the best aiming point, position number is a-l [12]. We can get twelve sequences evaluating the pictures by each instruction function and measure the evaluating indicator for each sequence and get the average of the indicator as the indicator of the method. Due to the variety of gradient methods are almost the same except the difference of the operator, we select the typical Sobel gradient, whose calculation method is average method and0-order area method (the sum of points ‘area whose average are greater than the gradient average), the following is the comparison of variance method and the typical high frequency band-pass method [13].

(2) Result of comparison

Table 1. The Evaluation Result of Instruction Function

| | single peak | DU | C | U | DN | G | K |
|---|---|---|---|---|---|---|---|
| Ostu method | 91.6% | 2.0833 | 0.8913 | 29.9 | 1. 1667 | 0.0710 | 2.8979 |
| Sobel 0-order area method | 100% | 2.3333 | 0.2104 | 16.6667 | 14. 3333 | 0.0198 | 0.0174 |
| Sobel gradient mean value method | 100% | 1.6667 | 0.3140 | 26.5 | 10. 8333 | 0.0320 | 1.1041 |
| variance | 50% | 3.6667 | 0.1914 | 48.3 | 11. 9167 | 0.0138 | 1.4215 |
| frequency bandpass method | 91.6% | 3.0833 | 0.3177 | 33.5 | 4. 1667 | 0.0227 | 0.8997 |

Ostu method and frequency bandpass method generates double peaks, but the peaks are near the best aiming point. Variance method generates a large number of multi-peak values, so it does not have practical means. Single peak based on gradient methods is better.

One of the most noteworthy observations is the anti-interference ability (DN) indicator. Because it directly related to the performance of focusing system, indicator of the Ostu method is 1.667, the frequency bandpass method is 4.1667, it is the smallest among the four methods, while the other three methods are more than 10. This means the local extreme of Ostu method is the smallest, its curve is smoother and it is the best search method.

From the deviation degree of extreme point (DU), every method has certain deviation. But, the deviation of single position may has errorous artificial judgment about the best aiming point. So it allows a certain deviation, of which the deviation of Sobel average method is the minimum. In addition to square variance, the deviation of Ostu method is the minimum among other three methods.

From the range of function value (C), the range of Ostu methods is the biggest, that is, it changed obviously in sensitive area, which is suitable for focusing.

From the range of object distance (U), frequency bandpass method is the maximum, the Ostu method is the second, it responses in the large object range.

From the obvious degree of peak (G), the value of Ostu method is the maximum, which means Ostu peak is the most obvious in the peak point.

From the range of extreme (K), Sobel 0-order area method is the minimum, which means its extreme is stable. The value of K in Ostu method is maximum, which explains the value range of extreme is larger. Because it is for segmentation and it's value have a serious effect on image content, so it shows different extreme in different positions, which have an unfavorable influence on the process of judging whether to enter the focusing. But with the same slide, Ostu function value is stable basically in each position. We could determine a threshold, which means we could start focusing at the time of detecting.

The above results showed, Ostu method's performance is good, especially when the range is large and strong anti-interference ability. In addition, the calculation is small, which is suitable for practical applications.

## 5. Practical Test

In the practical test, this paper establish a auto-focus system with class variance instruction function, take the hill-climbing algorithm as program search algorithm , that is, search towards a larger step, when the evaluation value began to decline, return, reducing step until it reduced to 1. When it began to decline, the focus is the best. Step sequence is: 10, 6, 4, 2, 1. Please note this sequence is designed based on our current system, it is not suitable for all systems.

Programming as described above, and tested, Figure 3 shows Value of Ostu function within an auto-focus process.

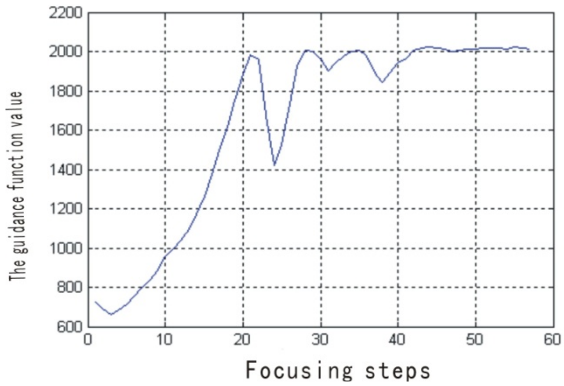


Figure 3. Value of Ostu Function within an Auto-focus Process

As the figure shown, at the beginning of focusing, search in one direction and turn to the opposite when the value of instruction function is decreasing. If the instruction function value is decreasing in the process of searching, change the direction until the number of steps is 1. At the same time, if the value of instruction functions decrease, it will complete the search and return to the maximum point in the process. As the system circumstance is different, we can adjust the step sequence and the speed of stepping motor to improve the search speed.

In testing process, we investigate the relationship of the focusing time and the initial defocus position, take several defocus distances and 5 positions at each distance, then take the carrier glass table to a specific defocus point and focus, record the focusing time and the number of steps after it was completed. Figure 4 shows time and steps cost by auto-focus process at different initial position:

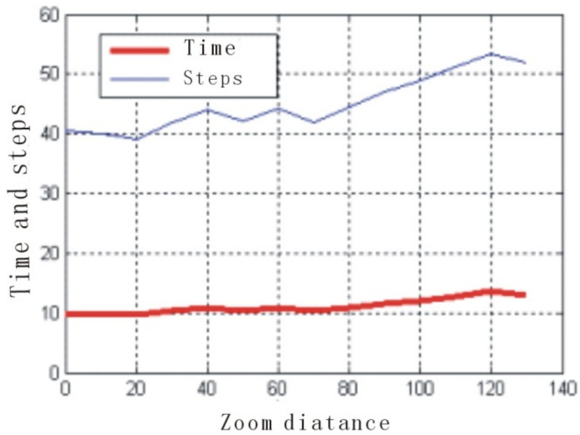


Figure 4. Time and Steps Cost by Auto-focus Process at Different Initial Position

As the figure shown, when initial defocusing distance increase, the growth of focusing time and part is slight, which means the system is not sensitive to the initial defocus position; we can find the point quickly as long as it is in the response range. During test, we also notice that the amount of time only cost in the movement, computing and communications basically don't take time.

## 6. Conclusion

This paper discusses the instruction function for microscope auto-focus and establish a auto-focus system with class variance instruction function. It analyses the traditional instruction function of automatic focusing system and expands the evaluation method of instruction function. By identifying the meanings of each indicator and selection announcements in evaluation method, this paper points out indicators of anti-jamming ability has a big impact on system performance. Poor anti-interference ability may result in the complexity of programming and poor focusing effect.

This paper proposes that the class variance of Ostu segmentation can be used to instruct auto-focus process. Compared to existing instruction functions, the new instruction function need little calculation mount. It responses in a wide object distance range. And have a strong anti-jam ability and a wide range of function value.

This paper establishes a practical auto-focus system based on class variance, and tests the performance of the system. This system can variable in a wide object distance range, and the init point for auto-focus process has little affect to the system.

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