

Microscope Autofocus Method Based on Analog Image Video Signal Processing

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Abstract: The most important part of the automatic control microscope system is the auto focus technology. How to improve the accuracy and speed of the focus is the most noteworthy breakthrough point, in which the focus evaluation function is used to evaluate whether the image is correctly focused. Objective: through the evaluation of several kinds of focus evaluation functions, and according to the application of microscope focus technology in the research, we compare and select the focus method which is more easy to use image video signal for proper processing, and analyze and experiment the key technology. Methods: according to the high frequency component of image video signal, the class divergence of the difference between class samples, choosing the appropriate evaluation function and step size through the gradient value size, comparing the sharpness and edge of the focus image and the non-focus image to evaluate the quality and clarity of image video signal, selecting a high comprehensive auto focus algorithm to realize auto focus The gray difference method, Laplace operator and other evaluation functions. Results: in the end, the resolution of the image is 93.1%, and the success rate of auto focusing is 98.2%. The method based on analog image video signal processing improves the precision and speed of focusing, and it has been successfully applied to the auto focusing system of microscope.

1. Introduction

Microscope is a kind of common optical instrument widely used in many kinds of subjects. In order to process image and video signal quickly and accurately, the bottleneck technology based on analog image and video signal is auto focus.

With the development of related technology, the key problem to enhance the effectiveness of microanalysis system and meet the high accuracy measurement is how to complete the automatic focusing of microscope more efficiently. The key to realize auto focus technology is its efficient auto focus algorithm. The main difficulty of auto focus algorithm is to select proper focus

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evaluation function. At present, many scholars have done a lot of research on the focus assessment function, and given the strategies of recognition and system focus.

The high focusing precision of microscope can enhance the imaging effect and reduce the error rate of DNA sequencing. Zhang, X. et al proposed a new feedback method to improve the focusing condition to a high precision. The reference laser reflected by the sample is detected by two or more sensors around the common focus. Zhang, X. et al carried out on-line data processing after obtaining the signal of defocusing position to provide feedback for real-time focal plane locking of sample surface. The accuracy of this method is better than 1 / 10 of the target focal depth. In order to balance the optical aberration, a special optical feedback system needs to be designed, considering the non-thermal design, so that the DNA sequencing work can adapt to the temperature fluctuation. Zhang, X. et al used a new feedback method to improve the accuracy of focusing conditions, with high accuracy, but low reliability [1]. Podlech et al introduced a passive self-focusing method based on image analysis, which calculated Bayes spectral entropy (BSE). This method can be applied to optical microscope, combined with the specific structure of optical machine unit, and can analyze large samples of complex surface without secondary sampling. In order to prove the strength and robustness of the microscope system, podlech et al used the resolution map of the test mode of the U.S. Air Force in 1951 to determine the focus position of the microscope, and used the method and the optical microscope system to analyze a 100 line / mm grating, and verified the effectiveness of the method. The research method of podlech et al has a high stability, but the accuracy is slightly lacking [2]. Harada, M. et al proposed a scanning electron microscope (SEM) autofocus algorithm based on multi band pass filter. In order to obtain the best specific frequency response of various images, Harada, M. et al introduced multiple band-pass filters, used multiple band-pass filters to calculate two groups of focusing measures, and selected according to the reliability of one group of focusing measures. Through the pseudo image simulation, the SNR of the image which can accept the auto focusing precision is determined. Harada, M., et al have high accuracy, but lack of stability [3].

According to the high frequency components of image video signal, the average distance between different samples and the difference between different classes, this paper analyzes the effect of analog image video signal processing and auto focusing. In the process of focusing, function gradient correlation is used, and appropriate step and threshold are selected at the position far away from the focus. The advantages and disadvantages of gray difference method and high Laplace function method are evaluated.

2. Overview of Microscope

2.1. Microscope

(1) Application of microscope

Microscope has been widely used in social production, scientific research and many other disciplines, especially in biomedicine. However, at present, the manipulation of the microscope and the processing of image information are relatively complex, and some image videos are difficult to observe in close range, which limits the scope of application of the microscope. Based on the situation of difficult operation and fuzzy image, an automatic microscope control system which controls the microscope table by computer came into being. The computer can collect and process the image, and the high-efficiency focusing system improves the speed and accuracy of observation [4]. Through the observation of the object image through the microscope, the image is more clear, the resolution is high, and the observation position is convenient.

(2) Microscope focusing requirements

The operation space and observation object of the microscope are relatively narrow, so it is

necessary to achieve the focusing process of ultra-high accuracy. If the focal length adjustment step is too large, it is likely that the worktable will exceed the proper focusing position, resulting in focusing error. On the contrary, choosing a too small step size for focusing can achieve correct focusing, but at the same time, it may consume more time and reduce system performance. The premise of getting high quality image information is fast and accurate auto focus. In this process, the current auto focus evaluation function is described and compared. In order to solve the problem of focusing speed, it is necessary to find an accurate focusing mode for analog image video signal processing and an auto focusing evaluation function to find the most appropriate sum of the absolute value of system level difference [5]. Aiming at the real problems in the process of auto focusing, the focusing evaluation function and search method are optimized to make the system more centralized and accurate. The focusing accuracy is related to the resolution of the micrograph and the selection of the focusing area. According to the characteristics of microscopic imaging, when the field of view is large, the target point near the edge of the field of view is far away from the optical axis, and the beam has a large inclination, which will cause astigmatism after being refracted by the lens, and the spots may interfere with the definition of the surrounding image. In order to reduce the influence of astigmatism on the focusing accuracy, the focusing area can be as close as possible to the center of the field of view.

2.2. Automatic Focus of Microscope

(1) Microscope focusing method

A microscope magnifies an object through the efficiency of an optical system: studying and recognizing the characteristics of an object from its microscopic form. With the development of instrument automation and intelligence, it is urgent to control the microscope system automatically. At present, microscope focusing methods can be divided into two categories:

1) Laser confocal method. According to the laser beam through the illumination pinhole, the point light source is obtained, all points in the focal plane of the specimen and the irradiated points on the specimen are scanned, the image is imaged at the observation hole, and acquired by the photomultiplier tube (PMT) behind the observation hole, and the fluorescent image is quickly formed on the computer display screen. This method is very complex and difficult to implement.

2) For example, absolute variance function, gray gradient operator function, gray difference method, energy spectrum function, Laplace operator and other evaluation functions are used[6]. This method requires a high level of hardware processing speed, so there are some limitations for its in-depth development. At present, through improving the focusing algorithm and changing the structure of the hardware circuit to achieve the rapid focus of the microscope, the accuracy of the focus of the microscope is improved.

(2) Focus algorithm selection

In order to meet the needs of time and accuracy, this paper studies the automatic focusing algorithm with high comprehensive degree. When focusing microscope automatically, the algorithm selects appropriate evaluation function and step size by using the amount related to the gradient of evaluation function, and uses gray difference method and large step size far from the focus, while Laplace selects smaller step size near the focus [7]. Thus, while focusing, the above functions can take advantage of the fast gray difference method and high accuracy Laplace function method, and change from fast coarse tuning to precise fine tuning by increasing or decreasing step size. Using manual operation to control the fine movement of precision instruments is prone to errors and low efficiency, so it is urgent that the operating arm can automatically and accurately complete the corresponding operation. However, due to the narrow operation space and short span, it is necessary to detect and distinguish observation samples under the microscope, so clear microscopic image is

defined as an important factor to correctly distinguish observation samples. The key to realize the automatic operation is to obtain the appropriate micro image directly and quickly, that is, the automatic focus of micro vision. Auto focus technology can be divided into active mode and passive mode.

1) The active mode is to measure the distance between the target and the lens by active light wave or sound wave, and then adjust the focal length. Due to the need for some other equipment (such as light wave or sound wave transmitting and receiving equipment), and the small observation range and object of the microscope, it is impossible to add the above equipment, so in most cases, the application of auto focus mode is passive.

2) Passive mode is to analyze the characteristics of the image itself to distinguish the focus direction. After repeated adjustment, a clear image can be obtained. It is an important problem to choose the right sharpness evaluation function and the right auto focus strategy.

3. Analog Image Video Signal Processing and Focusing Function

3.1. Analog Image Video Signal Processing

(1) Feature selection of analog image video signal

It can not only reduce the computational complexity of the learning algorithm, improve the learning ability of the algorithm, but also more easily obtain a simpler and clearer learning algorithm model [8]. With the development of information technology and the maturity of network technology, more and more scholars pay attention to the feature selection of analog image video signal. When selecting the features of analog image signals, there are often insufficient researches on the relationship between each category and the features of analog image signals, which makes the traditional methods of feature selection of analog image video signals difficult to solve. Because it only selects the feature from the direction of the whole category label set, it is difficult to complete the feature selection of analog image signal effectively.

Visually, it depends on whether the image is clear to judge whether it is focused. From the perspective of spatial domain, compared with defocused image, the gray level of focused image changes significantly and has sharp edges [9]. From the perspective of frequency domain, because defocusing is a low-pass filtering process, when defocusing occurs, the high-frequency component of the image will be smaller. Defocused images lack a lot of fine information compared with focused images. Defocused images contain more high frequency components correspondingly. The quality and clarity of images are closely related to the high frequency components of images.

(2) Simulation chart based on divergence analysis

The discrete degree of analog image signal categories can reflect the different average intervals between each group of samples. The larger the dispersion range between classes is, the larger the average interval between samples is, and the intra group dispersion can reflect the density of samples in a group. If the intra group divergence is small, the higher the density between samples in the same group [10]. Within class differences and between class samples can show the separability of samples from the above two aspects. Therefore, in order to classify the characteristics of analog image signals, we should try to minimize the dispersion of samples within the category and maximize the dispersion of samples between categories, so we take the function:

$$f(y) = y^{T} (S_{b} - S_{w}) y$$
⁽¹⁾

Suppose that the inter class scatter matrix S w and the inter class scatter matrix of the training set are respectively described by the covariance matrix of the corresponding samples:

$$S_{w} = \sum_{i=1}^{r} {}_{p}(C_{1}) / n \sum_{j=1}^{n_{j}} (x_{ij} - \bar{x_{i}}) (x_{ij} - \bar{x_{i}})^{T}$$
(2)

$$S_{b} = \sum_{i=1}^{r} {}_{P} (C_{i}) (\bar{x}_{i} - \bar{x}) (\bar{x}_{i} - \bar{x})^{T}$$
(3)

According to the definition, the above matrices are all positive semidefinite.

3.2. Focus Function Evaluation

(1) Image definition evaluation

From the perspective of spatial domain, the definition evaluation function of image generally includes the evaluation function based on gray difference, the evaluation function based on energy gradient and the evaluation function based on Laplace operator. Although the calculation of these evaluation functions is small and fast, their reliability is very poor.

From the perspective of frequency domain, by analyzing the spatial frequency of the image, we can get that the contour information of the image is reflected in the low-frequency segment, while the fine and accurate information is reflected in the high-frequency segment [11]. The method of frequency domain analysis is used to analyze the corresponding information contained in each part of the image video signal, and the high frequency component of the image is taken as the measurement of the definition evaluation function, so as to determine the clarity of the image video.

(2) Focus evaluation function:

1) Gray entropy method: take image entropy H(X)as the focus evaluation function of the algorithm:

$$H(X) = -\sum_{pi} \log pi \tag{4}$$

Where: p^i is the frequency of pixel display of gray value I in the image. The entropy values of images in different positions are different: the larger the defocus value is, the less clear the image is, and the higher the entropy of information is. When the focus is accurate, the image is clear and the information entropy reaches the minimum value. On the basis of this feature, the extremum of entropy function can be determined as the focus position of the system. This method is easy to implement, but the focusing accuracy is not enough.

Gray difference method: use the sum of the absolute gray value difference of the adjacent pixels of the image S(X) as a simple focus evaluation function:

$$S(X) = \sum_{x} \sum_{y} |f(x, y) - f(x, y-1)| + |f(x, y) - f(x+1, y)|$$
(5)

The difficulty of this method is very small, but the sensitivity near the focus is low. Evaluation function based on Laplace operator:

$$L(X) = \sum_{x} \sum_{y} \{ |\frac{\partial^2 f(x, y)}{\partial x^2}| + |\frac{\partial^2 f(x, y)}{\partial y^2}| \}^2$$
(6)

Laplace operator is selected to accumulate the results of the second order differential operation of the target region of the image as the standard. The larger the values of these standards change, the more obvious the gray value, gradient value or edge change, and the clearer the image. This method is difficult to operate, but the effect is very good, especially near the focus.

(3) Comparative analysis of focusing evaluation function

The traditional auto focus algorithm is to search in a whole range after selecting a certain focus evaluation function, that is, to search at both ends of an area in the focus range of the system, so as to get the focus position [12]. The key problem of this method is that it is difficult to match the speed and accuracy. At present, the gradient value of focus evaluation function is selected, and the proper evaluation function and step size are the core methods of the algorithm. The results of many studies show that the gradient value of focus evaluation function decreases when it is far away from the focus, but increases significantly when it is close to the focus. Based on this observation. In this paper, we choose to set the threshold to determine whether the real-time position is close to the focus. Laplace function method and small step size are used near the focus, while gray difference method and large step size are used far away from the focus. Therefore, the Laplace function method can be used in a small area, which can effectively avoid the shortage of "long focusing time", and make the algorithm reach the conditions of focusing accuracy and speed at the same time. The form of the focused evaluation function is as follows:

$$F(X) = \begin{cases} S(X)(\eta < \eta_0) \\ L(X)(\eta \ge \eta_0) \end{cases}$$
(7)

In the formula: S(X) is the focus evaluation function corresponding to the gray difference method; L(X) is the evaluation function corresponding to the Laplace function method; η is a function related to the shaving of the evaluation function, which is defined as follows:

$$\eta = \frac{F(n) - F(n-1)}{F(n)} \tag{8}$$

Where: n represents the current position in the focusing process, η_0 represents a threshold; F(n) represents the value of the evaluation function corresponding to different positions. The denominator of formula (6) is set as F(n), to ensure the consistency of threshold η_0 at different image focus points.

4. Experimental Design

4.1. Research Object and Experimental Design

The performance of the new algorithm is verified by a large number of autofocus experiments, and compared with the traditional autofocus algorithm (gray difference method / Laplace function method). In the study of threshold verification, 60 images were randomly divided into three groups, 20 images in each group, from defocus to focus, to test the step size and threshold size and degree in the function formula. Through focusing evaluation function curve, the focusing algorithm is compared with traditional autofocusing algorithms.

The image processing technology is used to determine whether the observation sample is on the focal plane. The movement of the console in different directions. In the process of motion, the value of image focus evaluation function at the corresponding object position is recorded and calculated. The object to evaluate the maximum value of the function is the focus plane. Therefore, the key problem of auto focus is to build a reliable and fast focus evaluation function.

Taking the analog image video signal as a reference, this paper selects its characteristics, evaluates the effectiveness of this method, and makes relevant research. All analog image video signals are divided into three groups. The methods and traditional methods in this paper are used to select the characteristics of the analog image video signal. Calculate accuracy, false alarm rate

(judge normal state as fault state) and missed diagnosis rate (judge fault state as normal state), and describe the results obtained.

4.2. Observation Index and Effect Evaluation Standard

(1) Requirements for focusing evaluation function:

1) Unbiased and unimodal peak position,

2) The focal plane position corresponding to the global maximum,

3) No local extremum,

4) High SNR.

(2) Function and energy spectrum methods:

Contrast the sharpness corresponding to the changing step size and the operational speed, and analyze the change of details.

(3) Analog image video signal:

Accuracy, false alarm rate and missed diagnosis rate.

4.3. Evaluation Standard of Observation Effect

When the objective flow is changed to a clear position far away from the image, the microscopic image changes from the non-image area to the image sensitive area, with little change in the details. Therefore, the sharpness evaluation function can adjust the step distance and speed up the operation. On the one hand, it can reflect the change of details well, on the other hand, it can reduce the interference of noise. When the microscopic image changes from imaging sensitive area to imaging transparent area, the details will change significantly. Therefore, using a small increase and decrease step distance and a better single peak definition evaluation function, not only can significantly find the changes in the details, but also can reduce the number of searches, so as to make the focus more accurate.

Function evaluation: Good: the focus evaluation function curve is smooth, almost no local maximum value, good unimodal, better meet the requirements of image focus evaluation function. Good: the curve of focus evaluation function is relatively smooth, basically there is no local maximum value, and the single peak is good, which better meets the requirements of image focus evaluation function. Poor: the curve of focus evaluation function is rough, with local maximum value, and single peak is poor, which cannot meet the requirements of image focus evaluation function.

5. Analysis and Discussion of Experimental Results

5.1. The Influence of Step Size in Laplace Operator

During the experiment, first adjust the microscope to the near focus manually, then change the objective current, so that the focus position of the minimum beam spot calculated according to the objective current can continuously approach the determined working distance of the sample, and determine the focus position For the minimum beam spot of the electron beam, the peak value of the microscopic image of the microscope is obtained in turn with each change of the focus position of 10 PM as the adjustment step, which is used to verify various image definition evaluation functions. The results are shown in Figure 1.

From the above experimental results, it can be seen from Figure 1 that the perfect auto focus evaluation function of Laplacian operator can give a significant peak value at the most ideal focus position of a micro sample with certain surface roughness and texture change, and has good anti

noise and anti-interference ability and performance. The contrast experiment in the accurate focusing process shows that the improved Laplace operator has a good effect on focusing feasibility and focusing results, and it is suitable for the automatic focusing system of microscope. In the past, the utility of step size was often ignored in the auto focus evaluation function. Since the Laplacian operator is second-order difference, the noise in the image needs to be eliminated by proper step size. The results show that the auto focusing effect is improved significantly after adding step length, the single peak and unbiasedness of the evaluation function curve are good, the change trend near the focal plane is very significant, and the sensitivity is high. It should be noted that the larger the step, the better the focusing effect. Generally speaking, for the sequence image with slight noise interference, the step size is smaller, while for the sequence image with obvious noise and brightness change interference, the step size is larger. The next step is how to obtain excellent step size and threshold parameters according to the characteristics of micro image and apply them to the full-automatic microscope.



Figure 1. The influence of step size in Laplace operator

5.2. The Influence of Threshold in Laplace Operator

By adjusting the threshold value, the peak value of microscopic image of the microscope is obtained in turn, which is used to detect three groups of image definition evaluation functions. The results are shown in Figure 2:



Figure 2. The influence of threshold in Laplace operator

It can be seen from Figure 2 that the analysis table of the research results (indicating that the threshold value changes little with different images, and the value range is t = 450-650. The curve operators of the focus evaluation function obtained from a sequence of images basically coincide with each other, and the optimal focus position obtained from the three focus evaluation functions of the sequence of images is not completely consistent. The curves of the above three evaluation functions are stable, with little change near the focal plane, poor focusing effect, and the peak value and sensitivity of the Laplace operator curve with threshold control are significant. The focus evaluation curve is divided into two parts. The solid line indicates that this part is gray difference

method). It can be seen from the figure that the curve can almost reach the single peak condition of focusing evaluation function, and the curve changes greatly at the peak.

5.3. Research Curve of Auto Focus Definition Evaluation Function after Normalization

In order to select a better focusing algorithm that meets the high requirements, the focusing effects of different positions of the focusing algorithm in this paper and the traditional auto focusing algorithm are recorded and compared, and the results are shown in Figure 3:



Figure 3. Experimental curve of auto focus sharpness evaluation function

It can be seen from Figure 3 that the focus evaluation function is a reference to measure whether an image is focused. The final resolution of the image is 93.1%, and the success rate of auto focusing is 98.2%. Its setting basis is that the focused image covers more fine information than the defocused image. The correct focus can be judged visually by whether the image is clear or not. From the aspect of spatial domain, the gray level change of focus image is more significant than that of defocus image, and the edge is clear. From the aspect of frequency domain, when the image contrast is small, the high-frequency component of the image is also small; when the image contrast is slightly large, the high-frequency component of the image is increased, and the details are relatively increased. Compared with defocused image, focused image has more fine information and contains more high frequency components correspondingly. However, if you want to compare the sharpness of an image, you must find a value that can be directly compared with a microscope so that the microscope can compare the quality differences between multiple images. The value should be able to reflect the quality of the image at different focusing degrees. It is better to adjust this data to the maximum or minimum value at the most ideal focus. In addition, in other ranges, these values should be able to change monotonously depending on the position of the evepiece. That is to say, the distribution of the sequence set in different focal points should be at least necessarily continuous. The application results show that the algorithm can complete auto focusing more quickly and accurately than the traditional focusing algorithm.

5.4. Effect Analysis of Auto Focus Definition Evaluation Function

In order to analyze the best focusing method, the evaluation functions such as gray-scale difference method, Laplace operator and the comprehensive method are used to record and evaluate the stage data. The results are shown in Figure 4.

It can be seen from Figure 4 that the comprehensive method has the best effect. This algorithm combines the advantages of fast gray difference method and high-precision Laplace function method. After improving the auto focus algorithm, it successfully completes the change from fast coarse tuning to accurate fine tuning. At present, there is no rigorous algorithm theory to prove, but most of the studies prove the effectiveness, function and value of the algorithm. The autofocus



algorithm has been successfully applied to the micro image automatic analysis system.

Figure 4. Analysis of the effect of auto focus sharpness evaluation function

5.5. Analysis of the Comparison Results between the Methods in this Paper and the Traditional Methods

Compare the processing method based on the analog image video signal with the microscope autofocus method based on the sorting and evaluation of the digital image, record its corresponding multiple indicators, and analyze the advantages and disadvantages of the two methods applied with the microscope autofocus. The results are shown in Table 1:

Table 1. The comparison results between the new methods and the traditional methods

Group	Accuracy(%)	False Alarm Rate(%)	Missed Diagnosis Rate(%)
Traditional Method	81	2.8	3.1
New Methods	87	2.1	1.6

It can be seen from Table 1 that the accuracy, false alarm rate and missed diagnosis rate of the experimental method in this paper are better than those of the traditional method. Compared with the automatic focusing method of microscope based on digital image processing and analysis, this research method does not need to transform the analog image video signal into digital image, but quickly obtains the analog signal of each point, records and judges whether the image conforms to the focusing reference standard in real time, adjusts the system to put it in the correct focusing state, and completes the automatic focusing. This greatly optimizes the image processing method and reduces the auto focus time. In the specific research process, the optimal feature subsets in each cardinal range can be calculated separately. Considering the scale and classification effect, we can get the best feature subset. According to the sequence, the research can calculate the feature vector as the input of the classifier. In order to meet the needs of analog image and video signal processing, this paper studies the key technologies of high-speed and high-precision microscope auto focusing system. At present, most of the automatic focusing methods used in microscope are passive.

5.6. Main Indicators of Three Analog Video Formats

By comparing the three methods of obtaining picture and video signals, such as mtsc-m, PAL-D and SECAM, a more convenient way for scientific application is obtained. The results are shown in Table 2:

Analog Video System	MTSC-M	PAL-D	SECAM
Frequency/Hz	35	27	27
Lines / Frame	520	620	620
Brightness Bandwidth/MHz	4.3	5.8	5.6

Table2. Main parameters of three analog video formats

It can be seen from Table 2 that the microscopic image of a microscope is usually a secondary electronic image. When the detector collects the secondary electronic signal, the microscope focus image of the object is obtained after transformation and arrangement. Because the secondary electron signal can only be displayed from the very thin layer on the surface of the object, the sharpness of the micrograph is closely related to the concentration of microelectronics on the sample surface, that is, the smaller the incident electron beam is, the clearer the micrograph of the microscope is. According to the imaging principle of the microscope, when the incident electron beam spot focused on the surface is the minimum, the microscopic image of the microscope is the most clear. At the same time, adjusting the objective current will increase the incident electron beam spot, which will make the microscopic image unclear and the larger the deviation, the more blurry. Compared with the fuzzy image, the edge of the clear image is very clear, there are more details, the gray level of adjacent pixels changes more in the spatial range, and the high-frequency components of the spectrum are more sufficient in the frequency domain. Therefore, the definition of the evaluation function should be based on the characteristics of fairness, monotony and uniqueness, and set the appropriate definition evaluation function to determine the clarity of the image.

6. Conclusion

In this paper, 60 simulated images are randomly divided into three groups, 20 images in each group, from defocus to focus, by describing the feature selection of simulated image video signal, simulated image based on divergence analysis, microscope auto focus method and focus algorithm selection, evaluation and judgment of image definition, focus evaluation function (gray difference method, evaluation function based on Laplace operator), etc, A more comprehensive and applicable auto focusing method is selected.

An automatic focusing algorithm with high comprehensive degree is selected and verified by many experiments. In this paper, the comprehensive focus method is good, the focus evaluation function curve is smooth, there is almost no local maximum value, the unimodal is good, and the condition of image focus evaluation function is better achieved; the curve of gray difference method and Laplace algorithm focus evaluation function is relatively smooth, basically there is no local maximum value, the unimodal is good, and the image focus evaluation function is better achieved Pieces. Finally, the resolution of the image is 93.1%, and the success rate of auto focus is 98.2%. The method based on the analog image video signal processing improves the focus accuracy and speed, and the auto focus system has a strong advantage in the focus accuracy and focus speed.

Although many experts have done a lot of research on the analog image video signal processing and microscope autofocus methods and made good achievements, there are still some problems to be solved in terms of the limitations of the research and acquisition of microscope autofocus methods in this paper. Moreover, the method proposed in this study occupies a more advanced application range. Microscope autofocus technology is an extended application of traditional optical autofocus technology in the field of microscope. It is of great significance for the further development of analog image video signal processing and microscope autofocus. Comparing the sharpness, edge and other features of the focus image and the non-focus image, the video signal quality of this study is better and the definition is higher, and the evaluation functions such as gray-scale difference method and Laplace operator of automatic focus are realized, the efficiency is improved by 50%.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

References

- [1] Zhang, X., Zeng, F., Li, Y., & Qiao, Y. (2018). "Improvement in Focusing Accuracy of Dna Sequencing Microscope with Multi-position Laser Differential Confocal Autofocus Method", Optics Express, 26(2), pp.887.
- [2] Podlech, & Steffen. (2016). "Autofocus by Bayes Spectral Entropy Applied to Optical Microscopy", Microscopy & Microanalysis, 22(01), pp.199-207. DOI: 10.1017/S1431927615015652
- [3] Harada, M., Obara, K., & Nakamae, K. (2017). "A Robust Sem Auto-focus Algorithm Using Multiple Band-pass Filters", Measurement Science & Technology, 28(1), pp. 015403. DOI: 10.1088/1361-6501/28/1/015403
- [4] Liang, H., Lu, K., Liu, X., & Xue, J. (2019). "The Auto-focus Method for Scanning Acoustic Microscopy by Sparse Representation", Sensing and Imaging, 20(1), pp.1-16.
- [5] Jiang, M., Zhang, N., Zhang, X., Gu, J., & Li, F. (2017). "Applications of Hybrid Search Strategy in Microscope Autofocus", Guangdian Gongcheng/Opto-Electronic Engineering, 44(7), pp.685-694. DOI: 10.3969/j.issn.1003-501X.2017.07.004
- [6] Marturi, N., Tamadazte, B., Sounkalo Demb & é, & Piat, N. (2016). "Visual Servoing-Based Depth-estimation Technique for Manipulation Inside Sem", IEEE Transactions on Instrumentation and Measurement, 65(8), pp.1847-1855.
- [7] Liu, Y, Yu, M, Cui, L, Jiang, G, Wang, G, & Fan, S. (2016). "Disparity Servoing Based Fast Autofocusing Method for Stereomicroscope", optica applicata, 46(4), pp. 651-663.
- [8] Yang, S. J., Berndl, M., Michael Ando, D., Barch, M., Narayanaswamy, A., & Christiansen, E., et al. (2018). "Assessing Microscope Image Focus Quality with Deep Learning", BMC Bioinformatics, 19(1), pp.77.
- [9] Miyamoto, A., & Kawahara, T. (2017). "Optimization of Cd-sem Imaging Sequence to Achieve High-speed and Multi-point Measurement", Ieej Transactions on Electronics Information & Systems, 137(10),pp. 1379-1386.
- [10] Miyamoto, A., & Matsuoka, R. (2016). "Automatic Generation of Imaging Sequence for Cd-sem Using Design Data", Electronics & Communications in Japan, 99(9), pp.62-71.
- [11] Fu, S. F., Sun, P. F., Lu, H. Y., Wei, J. Y., & Chou, J. Y. (2016). "Plant Growth-promoting Traits of Yeasts Isolated from the Phyllosphere and Rhizosphere of Drosera Spathulata Lab", Fungal Biology, 120(3), pp.433-448.
- [12] Jos é E Belizário, Sangiuliano, B. A., Perez-Sosa, M., Santos, B. V., & Gláucia Machado-Santelli. (2016). "Advances in the Integration of Optical and Mass Spectrometry Molecular Imaging Technologies: from Omics Data to Molecular Signature Discovery", Discovery Medicine, 20(112), pp.393-401.