Counting method of wheat stripe rust spores based on shape factor and improved Harris corner detection

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Abstract: Wheat stripe rust is one of the most serious diseases which affect the yield and quality of wheat in China. It is an important guarantee to predict wheat stripe rust accurately for the quality and high yield of wheat. This can be realized by automatically monitoring the number of stripe rust spores in the air and analyzing the stripe rust combined with other data. Due to the problem of time consumption and low accuracy of traditional microscopic observation and molecular biological counting methods, an automatic counting method for adhesive spores was proposed. Firstly, the K-means clustering algorithm was applied to extract target object from complex background in the spore image, and mathematical morphology algorithm was carried out. Secondly, the geometric shape factor was applied to estimate whether the spores were adhered or not. The optimal geometric factor threshold was chosen and adhesive spores were tested by using an improved Harris algorithm to get much robust and accurate detection of corners. Finally, 30 wheat stripe rust images were randomly selected to verify the feasibility of the method, and compared with the average area method. The experimental results demonstrated that the average accuracy rate based on the improved Harris corner detection method was 91%, which was 26% higher than that of the average area method. The results showed that the improved Harris corner point detection algorithm could achieve a higher accuracy for the counting of wheat stripe rust spores.

Keywords: wheat stripe rust, adhesive spores, K-means, shape factor, Harris corner detection

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1 Introduction

Grain problem is one of the primary issues puzzling the global development. Wheat stripe rust is widespread and serious in China, which seriously restricts the overall economic and social benefits of Chinese grain industry (Li et al., 2002; Shang, 2008). It is important to prevent the occurrence of the disease and ensure wheat yield (Luo et al., 2008). And the number of wheat stripe rust spores in air is vital parameter to monitor whether wheat stripe rust happened or not. The prediction of wheat strip rust disease has a wide range of practical applications and has received increased attention.

Presently, the counts of the stripe rust spores are mostly carried out using microscopic observation or molecular biology counting (eg, Real-time Polymerase Chain Reaction PCR) in laboratory. The amount of slant spores of cephalosporium acremonium was measured through photoelectric turbidimetry by Zhang Ping (2002). The number of exact spores of slant was measured by plate colony-counting, and then the suspension was prepared into different concentrations of diluents. Finally, the absorbance values which were measured by spectrophotometer were substituted into the regression equation to calculate the number of unknown slant spores. The accuracy of microscope counting method is low and the cost of biological methods is high, and the turbidimetric operation is also complex. Besides, the three methods are time-consumption and laborious, so it is difficult to achieve real-time processing. Applying image processing technology to recognize and count wheat stripe rust spores can overcome the

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above-mentioned shortcomings and realize an automatic counting.

So far, there are little relevant studies and reports on spores recognition and counting by applying microscopic image processing technology, especially in the field of segmentation of adhesion targets. Lin et al. (2003) proposed an approach by applying computer image recognition technology to the automatic counting of poplar disease spores, and the recognition rate reached 98%, but the adherent spores were not considered. Huang et al. (2003) and Hu (2011) introduced morphological characteristics to the study of the identification of silkworm microbial disease, and the recognition rate was about 88%. Jin (2008) obtained the total number of bacteria in food by micro-image technology and BP-neural network technology, and the rapid detection system of total bacteria in food was established, but the method was time-consumption. Lee et al. (2013) realized the automatic counting and labeling of wheat stripe summer spores by taking K-means clustering and watershed segmentation algorithms into the images which were obtained from microscopic photographic techniques. However, the counting of multiple adhesive spores was ignored. Hu et al. (2015) presented the approaches and processing which can deal with the area counting of bunt smut winter spores of imported wheat, but the count would be severely reduced for multiple spore adhesion problems. Qi et al. (2015) proposed a method of automatic detection and counting of P.oryzae spores by applying improved watershed algorithm based on distance transformation and Gaussian filtering. However, the identification rate of adherent spores was only 75%.

In this study, after processing spore images through K-means clustering and morphological algorithm, the geometric shape factor was applied in estimating whether the spores were adhered or not. Then, the quantities of adherent spores were reached by measuring angular points by applying the improved Harris corners detection. Finally, the spores were identified and counted automatically.

2 Materials and equipment

In this study, the wheat stripe rust spores were grown in the laboratory of the College of Plant Protection, Northwest A&F University and mixed with the clear mineral oil at a volume of 1:10, and then the mixture was dropped onto the slides. Olympus biological electron microscope (BX63, UCMOS 14000 KPA Pro series true color high resolution with intelligent CCD microscope digital camera) was applied to capture spore images which were saved as 4096×3288 pixel size, JPEG format. The image acquisition device is shown in Figure 1.

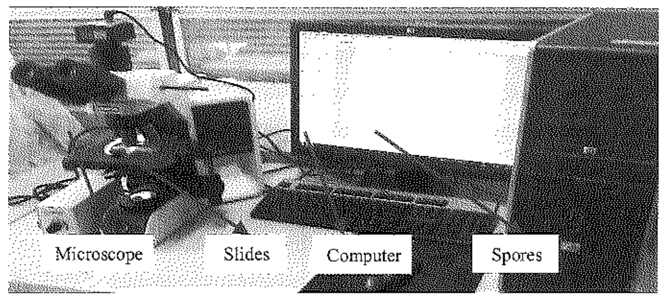


Figure 1 Image capture device

The image processing device is a desktop computer, which is configured as: CPU: Pentium (R) Dual-Core E5400 2.70 GHz; RAM: 3 G. All algorithmic programs run on MATLAB 2014a software.

3 Segmentation and counting of spores

3.1 Segmentation of spore images based on K-means algorithm

$L*a*b*$ color space is based on XYZ color space, and it is considered as an intermediate mode which can convert RGB color space to $L*a*b*$color space. RGB color space to XYZ color space conversion is presented in Equation (1), and the XYZ color space to $L*a*b*$ color space conversion is shown in Equation (2).

$$\begin{cases} X = 0.49\times R + 0.31\times G + 0.2\times B \\ Y = 0.177\times R + 0.812\times G + 0.011\times B \\ Z = 0.01\times G + 0.99\times B \end{cases} \tag{1}$$

$$\begin{cases} L* = 116\times f(Y) - 16 \\ a* = 500\times [f(\frac{X}{0.982}) - f(Y)] \\ b* = 200\times [f(Y) - f(\frac{Z}{1.183})] \end{cases} \tag{2}$$

In Equation (2), $f(t)$ is a function which can be described as follow.

$$f(t) = \begin{cases} t^{1/3} & t > 0.008856 \\ 7.787\times t + 0.138 & t \le 0.008856 \end{cases}$$

where, L^* is the brightness, a^* ranges from red to green, b^* ranges from blue to yellow. The values of a^* is $-128a \sim +127a$. $+127a$ represents red, $-128a$ represents green. The value of b^* is $-128b \sim +127b$. $+127b$ represents yellow, and $-128b$ represents blue. All colors are composed of these three interactive changed values.

K-means clustering algorithm is an indirect clustering algorithm based on the similarity measurement between samples, which belongs to the unsupervised learning algorithm, and its characteristics are fast processing speed, more intuitive and easy to implement. The algorithm improves the similarity between the same objects by applying iteration, and decreases gradually between the heterogeneous objects (Wang et al, 2009; Li et al, 2012; Rekik et al, 2007; Yusoff et al, 2013). The target is clustered into the k categories hypothetically, and the steps of K-means clustering algorithm are as follows:

Step 1: Select k categories initial clustering centers respectively: $\boldsymbol{Z}_1(1), \boldsymbol{Z}_2(1),\ldots, \boldsymbol{Z}_k(1)$, where k is the number of categories of clusters;

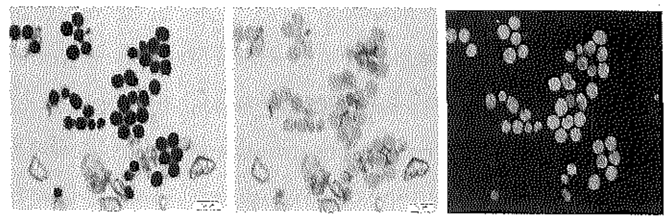
Step 2: Apply the following method to classify samples $\{\mathbf{Z}\}$ in the N^{th} iteration: for $i,j=1,2,\ldots,k$, $i \neq j$, if the relationship of inequality $\|\mathbf{Z}-\mathbf{Z}_j(n)\|<\|\mathrm{Z}-Z_i(n)\|$ is satisfied, the expression $\mathbf{Z} \in S_j(n)$ is right;

Step 3: Define the samples obtained from Step 2 as new clustering centers $\mathbf{Z}_j(n+1)$ which makes the values of $\sum_{j=1}^{k} \sum_{Z \in s_j(n)} \left\| Z - Z_j(n+1) \right\|^2$ minimum.

Step 4: For any $j=1, 2,\ldots, k$, if the relationship $\boldsymbol{Z}_j(n+1)=\boldsymbol{Z}_j(n)$ is met, the iterative process is completed; Otherwise, $n=n+1$, and jump to Step (2) and continue.

In this study, the original images were compressed to the size of 1024×822 pixels to improve the speed of the algorithm, and converted from *RGB* color space to $L^*a^*b^*$ color space. The image could be divided into spores and background (only need to extract the spore target areas, clustering number k=2) by applying the K-means algorithm, and then the spore targets would be separated from the image.

The image was processed by using K-means algorithm, and the results were shown in Figure 2. Figure 2a was an original image where there were adhesive spores with many complex impurities. Figure 2b was the background sub-image, and Figure 2c was the spore sub-image. It could be seen from these images that the active spores had been separated from the background and impurities, and the spores were well extracted.

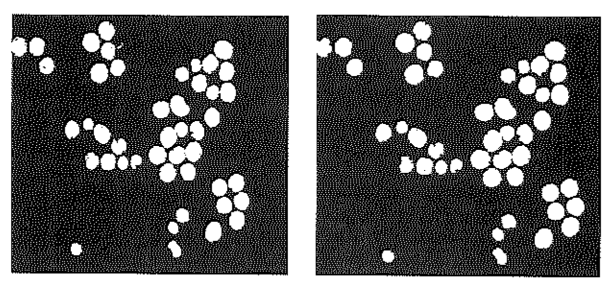


a. Original image b. Background sub-image c. Spore sub-image

Figure 2 Results of K-means clustering

3.2 Pre-processing of spores' images

The image segmentation using threshold (value was 0) was implemented in Figure 2c, and the result was shown in Figure 3a where existed holes and noise spots. In order to deal with the above problems, the open operation using 'disk' structure elements with a radius of 3 pixels was carried out to remove noise and got more smooth contour lines in Figure 3a, and then area filling was carried out to remove holes, as was shown in Figure 3b. It could be found that the holes and noises had been removed.



a. Segmentation result b. Morphological processing result

Figure 3 Results of pre-processing

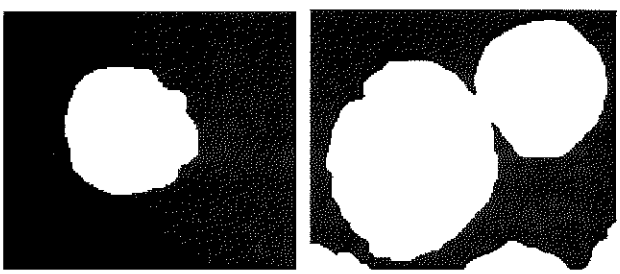
3.3 Detection of adhesive spores based on shape factor

The shape factor is a characteristic description of target, and it can be extracted effectively according to the calculation and statistics of a certain type of specific shape factor. There are single and adhesive spores in preliminary processing spore images, which could be distinguished by using shape factors. The principle—the boundary profile of the adhesive spores is more complex than single spore's—can be applied to judge the types of spores. The shape factor (*SF*) can be reached through the operation of essential attributes of original shape. The *SF* is defined as follow:

$$SF=4\pi S/C^2 \tag{3}$$

where, S is the pixel values of connected domain area; C is the pixel values of connected domain perimeters (Li et al, 2015).

As is shown in Equation (3), SF ranges from 0 to 1. The highest value of the shape factor is one if the target to be detected is a standard circle. Graph boundary would become complex because of many adhesive spores. And the value of SF will be reduced accordingly, thus it can be applied in differentiating adhesive spores. As were shown in Figure 3, Figure 3a was a single spore, and its shape factor was 0.8469; Figure 3b was adhesive spores, and its shape factor was 0.5526. It could be concluded that SF can be applied to distinguish single and the adhesive spores preferably.



a. Single spore b. Adhesive spores

Figure 3 Geometric shape factor

Finally, the threshold SF_0 and maximum area S_{max} were obtained through comparing the results of experiments, and they could be the basis to determine whether there were adhesive spores or not. That is to say that this area is considered as an adhesive region if the regional data satisfy the inequalities of $SF_i<SF_0$, $S_i>S_{\max}$. The parameters $SF_0=0.7$ and $S_{\max}=6600$ were selected by the comparison of many tests.

3.4 Corner detection of spores based on improved Harris algorithm

Corner point refers to the point of abrupt change on the edge of image, and can also be regarded as the point of intersection of two-side boundary. It contains feature points of two main directions in the neighborhood. And the corner points have characteristic with rotation invariance and scale invariance (Liu et al, 2013; Panchal et al, 2016).

Suppose $I(x,y)$ is the spores image to be detected, and a whole process of the Harris corner detection algorithm is as follows:

Step1: Calculate the gradient I_x and I_y of $I(x, y)$ at the x and y directions respectively, as are shown in Equation (4).

$$I_x = \frac{\partial I}{\partial x} = I \otimes \begin{bmatrix} -1 & 0 & 1 \end{bmatrix},\ I_y = \frac{\partial I}{\partial y} = I \otimes \begin{bmatrix} -1 & 0 & 1 \end{bmatrix} \quad (4)$$

Step2: Calculate the product of all gradients, as is shown in Equation (5).

$$I_x^2 = I_x \cdot I_x;\ I_y^2 = I_y \cdot I_y;\ I_{xy} = I_x \cdot I_y \quad (5)$$

Step3: All product terms in the above Equation (5) are carried out Gauss weighting with Gaussian function w, and then generate matrix **M** which contains the elements of A, B, and C; as are shown in Equation (6):

$$\begin{cases} A = g\left(I_x^2\right) = I_x^2 \otimes \omega; \\ B = g\left(I_y^2\right) = I_y^2 \otimes \omega; \\ C = g\left(I_{xy}\right) = I_{xy} \otimes \omega; \\ M = \begin{bmatrix} A & C \\ C & B \end{bmatrix} \end{cases} \quad (6)$$

Step4: Count the Harris response values of all pixels, and limit the threshold;

$$R = \{R : \det M - \alpha(traceM)^2 < t\} \quad (7)$$

in Equation (7), t is the threshold value, and R is the response value. If R is less than the threshold t, R will be set to zero.

Step5: Reach the maximum value of the 5*5 neighborhood by non-maximal suppression method, and the maximum is the corner point of the image.

Due to the strong detection sensitivity, the effect of Harris corner detection algorithm in actual operation is not ideal, and excessive false corners are always detected, as is shown in Figure 4a. The number of corners which are detected will be less with the decrease of sensitivity.

In order to obtain robust corner detection results, the Harris corner algorithm is improved. Suppose that eigenvalues of a got matrix **M** satisfy the inequations $\lambda_1 \geq \lambda_2 \geq 0$, the relationship between trace and determinant of matrix **M** is as follows:

$$\begin{cases} \det M = \prod_i \lambda_i \\ traceM = \sum_i \lambda_i \end{cases} \quad (8)$$

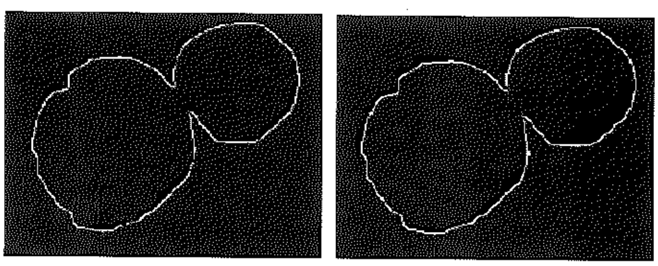
When the relationship of $\lambda_1=\lambda$; $\lambda_2=k\lambda(0\leq k\leq 1)$ are satisfied, there is the Equation (9).

$$R = \lambda_1\lambda_2 - \alpha(\lambda_1 + \lambda_2)^2 = \lambda^2(k - \alpha(1+k)^2) \quad (9)$$

When the value of R is not less than 0, there will be the inequations $0 \le \alpha \le \frac{k}{(1+k)^2} \le 0.25$. Suppose the value of k is smaller, the relation $R \approx \lambda^2(k-\alpha), \alpha < k$ can be obtained.

It can be concluded that with the increases of α value, the response value detected could be reduced, and the sensitivity of corner detection would be cut down, the number of corner points also would be lowered accordingly, thus the robustness of corner detection was improved. Parameter value α=0.23 was chosen after many tests in this study.

The result by using improved Harris corner detection method was shown in Figure 4b. It can be seen that the false corners points other than the adhesive points had been well suppressed by setting the sensitivity parameter to suppress non-maximum of the corner points, so that the number of detection would conform to the actual number of corner points. The number of spores could be the final count because there is a direct quantitative relationship between the number of corner points obtained and the number of spores. Therefore, this method can be applied to spore counting.



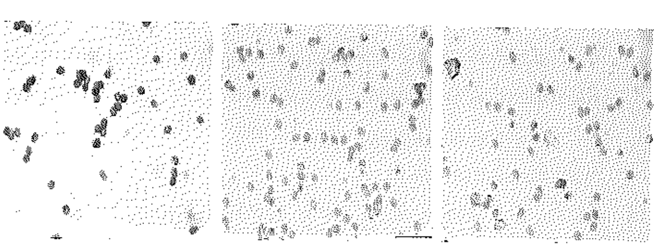
a. Detected corner points　　b. Detected corner points

Figure 4　Corner points detection

4　Results and analysis

4.1　Results

There were some spore images which were tested, as were shown as follows.



a. Detected image　　b. Detected image　　c. Detected image

Figure 5　Original spore images

Thirty images which contain 622 wheat stripe rust spores were tested by using the above algorithm. The test results of 20 images which basically include no more than three adhesive spores were shown in Table 1, and the test results of 10 images which include more than three adhesive spores were shown in Table 2. The average area method was regarded as the comparison algorithm, and the parameter—average area was calculated and chosen by contrast. In addition, the different magnification of the microscopic image has different parameters. For 200 times spore micrograph, the parameter 400 pixels was selected, and for 400 times image, the parameter was 800 pixels. The counting results were shown as Table 1 and Table 2.

Table 1　Test results of spore count of wheat stripe rust (Three and below spores adhesion)

No.	Spores number	Mean area		Improved Harris angular points detection	
		Test number	Accuracy, %	Test number	Accuracy, %
1	6	3	50	6	100
2	38	33	92	38	100
3	52	48	92	51	98
4	21	16	76	22	95
5	7	5	71	8	98
6	10	6	60	9	88
7	5	4	80	5	100
8	9	8	88	9	100
9	20	33	35	19	95
10	16	23	69	16	100
11	20	13	65	18	89
12	12	19	41	12	100
13	12	21	25	13	92
14	13	21	38	12	92
15	22	17	77	20	90
16	43	39	91	45	95
17	18	13	72	18	100
18	23	17	73	23	100
19	21	18	85	21	100
20	53	64	79	52	98
Statistics	421	—	68	—	97

As was shown in Table 1, for no more than three adhesive spores, the counting accuracy which applied to improved algorithm was between 89% and 100%, and the average accuracy rate was 97%, which had been increased by 29% than the average area method. It could be found in Table 2 for more than three adhesive spores that the counting accuracy of the improved algorithm was between 38% and 96%, and an average accuracy rate was

78% which had been raised 21% than the average area method. In general, according to the statistical data of Table 1 and Table 2, the average accuracy rate of the improved Harris corner detection for wheat stripe rust spores was 91% which increased 26% than the average area method. Consequently, it proved that the test method based on improved Harris algorithm could be applied to the counting of the spores of wheat stripe rust exactly.

Table 2 Test results of spore count of wheat stripe rust (More than three spores adhesion)

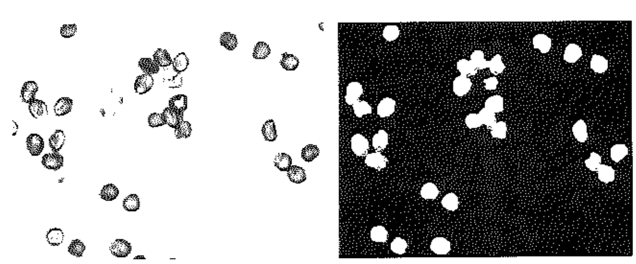
No.	Spores number	Mean area		Improved Harris angular points detection	
		Test number	Accuracy,%	Test number	Accuracy,%
1	14	28	50	18	72
2	22	29	68	33	50
3	15	24	40	16	93
4	15	22	53	17	87
5	25	32	68	20	80
6	28	35	75	31	90
7	26	26	100	42	40
8	26	45	26	27	96
9	13	20	46	14	92
10	17	27	41	14	82
Statistics	201	—	57	—	78

4.2 Analysis

(1) It can be seen from the above data that the improved algorithm can perform well, and satisfy the requirements by agricultural applications.

(2) The *SF* was effective to describe a single spore and adherent spores.

(3) The improved Harris algorithm was applied to detect corner points of adhesive spores, and the accuracy rate was lower, as were shown in Figure 6. The numbers of detected corner points were more than the actual, because the edge of the image was not smooth enough and the result was influenced by impurities.



a. Original image b. Results of corner detection

Figure 6 Improved Harris corner detection

5 Conclusions

In order to realize rapid and automatic counting of spores, the counting algorithm based on image K-means clustering algorithm and improved Harris corner detection method was proposed. Using the above methods, the following conclusions could be conducted:

(1) The proposed method could achieve accurate count of wheat stripe rust spores. And the average accuracy of the proposed algorithm was 91%, which was increase by 26% than average area method. Thus, the algorithm is feasible for the counting of wheat stripe rust spores.

(2) The shape factor was used to distinguish adhesive spores, and the results indicated that the shape factor could be applied to judge adhesive spores effectively.

(3) The proposed method can not only be applied in wheat stripe rust spores, but also in the segmentation of adhesive targets, as fruits, cells and particles.

(4) Given that it was limited by the condition of the equipment, like impurities on the spores or slide glass, it was still difficult to achieve high accuracy detection of adhesive spores. In addition, how to improve the accuracy of counting for more than three spores adhesions will still need further study in future.

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