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Counting Method for Potato Late Blight Spore Based on Integral Image

Lingxiao Su[1,2], Jianxing Li[1,2], Dongwei He[1,2], Ying Ma[1,2], Kan Luo[1,2], Fugui Tan[1,2], Hualiang Lin[1,2]

[1]Fujian Province Research and Development Center for Industrial Automation Technology, Fujian University of Technology, Fujian, China
[2]School of Information Science and Engineering, Fujian University of Technology, Fuzhou, China
Corresponding author: lijx@fjut.edu.cn

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Abstract. *This paper designs a potato late blight spores counting method based on integral image, aiming at the issues such as low gray scale contrast, uneven illumination and spore adhesion, which exist in the potato late blight spore images collected under electron microscope. Firstly, the acquired spore image is converted into grayscale image by grayscale processing, and the integral image principle is introduced to simplify the adaptive threshold segmentation method and the complex computation in the optimal threshold search process. Secondly, the binary image is filtered, logical operations are performed on the filtered image and the binary image, and then the morphological opening operation is performed. Finally, the watershed method based on distance transformation is designed for segmenting and counting the adherent spores. The experiment results demonstrate that the proposed method can segment and count the potato late blight fungus spores automatically and accurately in 30 microscopic potato late blight fungi spore images, and the average accuracy of spore counting archives 99.25%, it can provide technical support for the indoor resistance identification process aimed at potato late blight, and it can also provide technical reference for monitoring potato late blight in field.*
Keywords: Potato late blight spore, Integral image principle, Adhesion spores, Watershed method.

1. **Introduction.** With the important strategic development of industrialization of potato staple food proposed by the Ministry of agriculture of China, potato has become one of the four major crops in China. In its growth process, potato late blight is the primary disease which is threatening the development of potato production and breeding, it is the most serious disease causing loss of food crop yield, and it is known as the first enemy for potato production [1]. Potato late blight is a fungal disease, therefore, accurate counting for fungal spores is one of the indispensable links for potato late blight diseases control, disease analysis and evaluation accurately. At present it is usually the operator who completes the counting or the plate colony counting method to detect crop fungal spores, according to the morphological characteristics of the fungal spores under the microscope. Since the spores in the microscopic image are small in size, large in quantity and rich in impurities, the manual counting method is time-consuming and labor-intensive, besides it is easy to cause visual fatigue, and high error rate. The cost of the plate colony counting method is low, but the labor intensity is large and the detection takes an amount of time [2]. The above problems can be solved by using image processing technology, but spore microscopic

images have the following characteristics: (1) uneven-brightness spores and background causes complex changes in gray scale; (2) impurities in images; (3) spore overlap or stick. These characteristics make it difficult to segment the late blight fungus spores, and the traditional threshold segmentation method cannot meet the requirements [3-5]. Although the research on the spore counting method based on image processing technology is of great significance for late blight resistance identification and field disease monitoring. Although the use of image processing techniques for counting the spores of potato late blight has not been reported, some scholars have used image processing techniques to study the counts of other fungal spores. In [6], Deng et al. proposed a segmentation method based on color image for the characteristics of wheat smut fungus images. The method took the B component of the color image as the clustering object, and used the K-means clustering method to segment the fungal image, so that the distance between the pixels within the class and the local minimum were obtained. The segmentation method not only separated the background with uneven brightness, but also had less sensitivity to noise. Li et al. used a spore trap to capture wheat stripe rust uredospore and used microscopy techniques to obtain spore images. And the images were subjected to a series of processing such as scaled based on the nearest neighbor interpolation method, a segmentation process based on the C clustering method, a morphological operation modification, and a watershed segmentation to realize automatic counting and labeling of the summer spores[7]. Qi et al.[8] used the block background extraction method to illuminate compensate the spore image of the rice blast fungus obtained by the microscopic image system, and used the fuzzy C-means method to automatically determine the threshold of Canny edge detection on the gradient map; secondly, used mathematical morphology closed operation and the use of morphological feature parameters such as complexity and minimum circumscribed rectangle width to extract the binary image containing only spores. Finally, the improved watershed method using distance transform and Gaussian filtering was used to separate the adherent spores. The overall spore count accuracy rate achived 98.5%. Aiming at the problem of potato sticky image segmentation, Wang et al. [9] used the watershed method to initially segment the image, and then used the improved to MRF marker the correctly segment region and the over-segmented region, and calculated the closeness between the over-segmented region and the neighborhood. Finally, selected the neighborhood with the tightest degree is merged with it, and the test results showed that the correct rate is 95In summary, for spore images, there are some problems, such as image background noise, uneven illumination, uneven contrast, spore adhesion, the existing spore segmentation and counting methods can only solve the issue of low adhesion spores. However, the problem of uneven illumination and high spore adhesion has not been solved well. According to the characteristics of spore micrograph and the problems of uneven illumination and high spore attachment rate, this paper combines the integral image with the adaptive threshold method to segment spore micrograph roughly. The rest of this paper is arranged as follows: In section 2, the characters of microscopic image are discussed and analyzed, and the processing and analysis are presented. In section 3, the theoretical analysis of the second section is verified by experiments, the correctness and feasibility of the proposed method is verified. Finally, section 4 draws a conclusion for the work of this paper.

2. **Microscopic image processing and analysis.** In spore images, since color is very susceptible to illumination in nature, RGB varies greatly. On the contrary, gradient information can provide more essential information, and after converting three channels into one channel, the amount of computation greatly reduces. Therefore, in this paper, firstly, the color spore micro image is transformed into gray image by grayscale processing, and then the grayscale image is transformed into integral image considering the influence

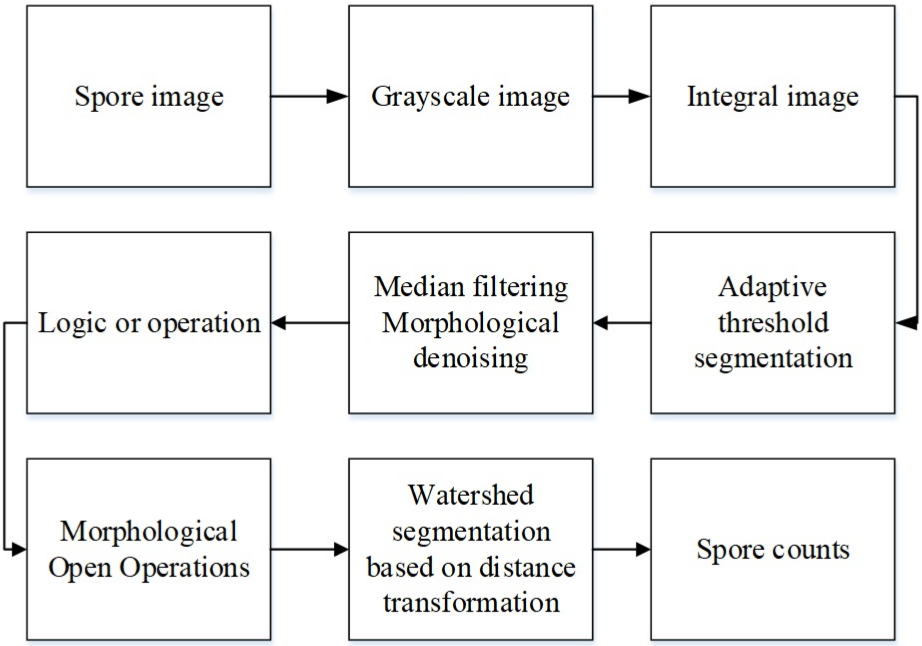

FIGURE 1. Flow chart of spore counts method

of uneven light and calculation speed. Secondly, the spore image is roughly segmented using an adaptive threshold segmentation method to segment the spores and background. Then the median filtering and morphological operations are used to image pretreat the spore binary image to obtain a binary image with only spores and complete spore shape.

Finally, In order to solve the problem of a lot of spores and multi-spore adhesion, the improved watershed method based on distance transformation is designed to separate the adherent spores, and the spores are counted according to the markers of the connected regions. The flow chart of the proposed method is shown in Fig.1.

2.1. **The integral image.** In this paper, an adaptive threshold segmentation method is adopted to segment the fungal spore image, but the traditional adaptive threshold method needs to consider the number of iterations and takes a long time. In order to improve the efficiency, the adaptive threshold method must limit the number of iterations through the image. Therefore, an improved adaptive threshold method is proposed by combining the integral image method. Integral image (also known as a summed-area table) is a tool proposed to quickly calculate pixel integrals of an image [10-12]. For an image $G(x, y)$, its corresponding integral image $I(x, y)$ is defined as:

$$I(x, y) = \sum_{i=1}^{x} \sum_{j=1}^{y} G(x, y) \tag{1}$$

Where, x and y are the row and column coordinates of a certain pixel of the integral image $I(x, y)$, i and j are the row and column coordinates of the pixel points in the mapping function $G(x, y)$.

As the term suggests, the integral image needs to be integrated for each pixel value of each point, the value of each pixel equal to the sum of the values of all the pixels before that as shown in Fig.2.

The value of any point $I(x, y)$ in the integral image is the sum of all the pixels in the rectangular frame enclosed by the starting point of the upper left corner of the image

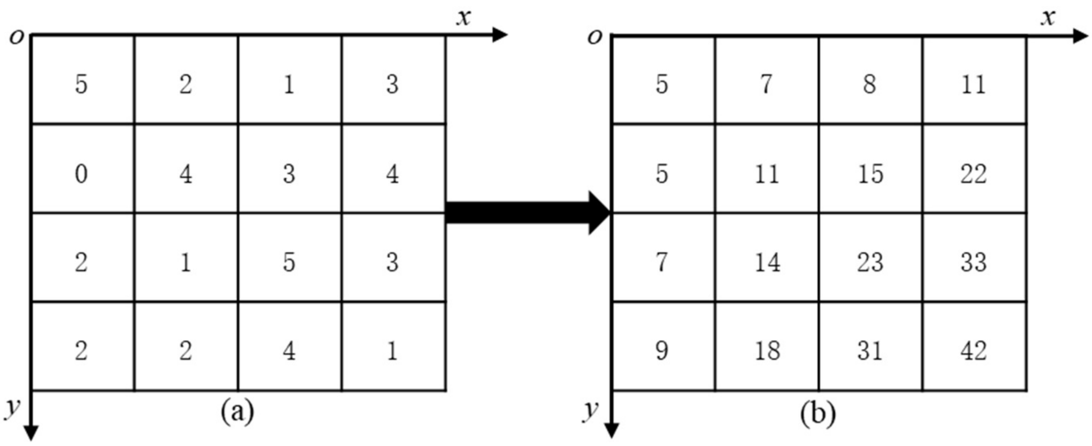

FIGURE 2. The computation of an integral image, (a) A simple image, (b) The integral image

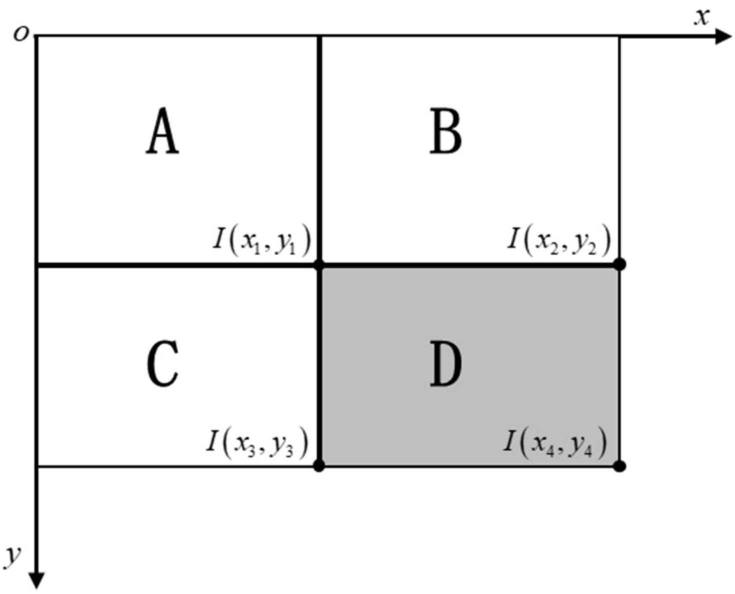

FIGURE 3. Calculation of region integral of integral image

$G(x, y)$ and the current point $I(x, y)$. In order to speed up the operation, the integral map can be obtained by the shortcut method shown in Eq. (2).

$$I(x, y) = G(x, y) + I(x - 1, y) + I(x, y - 1) + I(x - 1, y - 1) \tag{2}$$

Given the integral image $I(x, y)$, the pixel integral of any rectangular area on image $G(x, y)$ can be quickly calculated by integral image $I(x, y)$, as shown in Fig.3. Suppose that $I(x_1, y_1)$ is represented by $R(A)$, $I(x_2, y_2)$ is represented by $R(A)+R(B)$, $I(x_3, y_3)$ is represented by $R(A)+R(C)$, and $I(x_4, y_4)$ is represented by $R(A)+R(B)+R(C)+R(C)$. Where the notation $R(*)$ represents the region integral, therefore, the integral of the D region can be calculated by the Eq. (3).

$$R(D) = I(x_4, y_4) - I(x_3, y_3) - I(x_2, y_2) + I(x_1, y_1) \tag{3}$$

2.2. **Adaptive Threshold Segmentation.** Due to the limitation of equipment, non-uniform illumination of spore microscopic images greatly effects the segmentation of

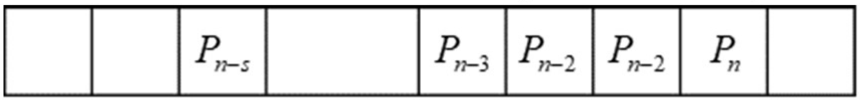

FIGURE 4. Diagram of traversal image

spores, which raises the difficulty of spore counting. Thus the threshold should be adaptive, accounting the change in brightness caused by uneven illumination and compensates for illumination. At the same time, in order to eliminate uneven illumination, maintain the computational speed and improve the counting accuracy of fungal spores, the threshold method must limit the number of iterations. To solve the above problem of segment the potato fungus spores from microscopy image, this paper proposes an adaptive threshold method based on integral image. In this paper, the Wellner adaptive filtering threshold method is adopted, the two-dimensional smoothing value of the rectangular region as template is proposed to replace the one-dimensional smoothing value [11]. Thus, the problem of one-dimensional smoothing directionality is solved. The basic idea of this method is to calculate a moving average value based on traversing images: if a pixel is obviously lower than this average value, it is set to black, otherwise it is set to white. Suppose P_n is the gray value at the point n in the image, where we assume that the image is a single line which is connected in order by all the rows (as shown in Fig. 4).

Notate $f_s(n)$ as the sum of the gray values of the s points before the n point, and then it can be derived as follows:

$$f_s(n) = \sum_{i=0}^{s-1} P_{n-1} \tag{4}$$

Then use Eq. (2), the binary image($T(n)$) is drerived, where $T(n)$ is 1 (black) or 0 (white) depending on whether it is lower than the $t\%$ of the average gray value of its previous s points. Where t is the initial value of the adaptive threshold.

$$T(n) = \begin{cases} 1, P_n < (\frac{f_s(n)}{s})(\frac{100-t}{t}) \\ 0, \ others \end{cases} \tag{5}$$

The traditional method has the problems of the sequence dependence of pixel scanning and the uneven distribution of neighborhood samples in all directions, so moving average cannot represent the neighborhood pixels of each step well. In order to solve the above problems, an improved method is proposed, that is, for any pixel point (x, y) , the average value of energy under the fixed size sxs window is calculated with this point as the center. Where s is the size of the window.

$$\begin{aligned} &E(x,y) = mean(\textstyle\sum_{i=x-s/2}^{x+s/2} \sum_{j=y-s/2}^{y+s/2} G(i,j)) = \\ &= \tfrac{1}{s^2}(I(x+s/2, y+s/2)) - I(x-s/2, y+s/2)) \\ &-I(x+s/2, y--s/2)) + I(x-s/2, y-s/2)) \end{aligned} \tag{6}$$

In this paper, 120 of the image width is taken as s and 15 as t. First of all, the average of linear time is calculated by integral image. That is to say, the integral image of the first pass is calculated according to the input image. Secondly, the average value of energy is calculated by the integral value of each pixel in the constant window. Finally, the current pixel value is compared with the average value of energy $E(x, y)$. If the value of the current pixel is less than $t\%$ of this average, set it to black, otherwise it will be set to white.

2.3. **The watershed segmentation.** The binary image obtained by image preprocessing can preserve the shape and edge information of fungal spores well. Which provides a powerful guarantee for segmentation of adherent spores based on the watershed method of distance transformation, and prevents over-segmentation and under-segmentation of watershed method.

The separation of adhesion spores is a key step in the automatic counting of potato late blight spores based on integral images, and it is also one of the difficulties and focuses of automatic counting of potato late blight spores. Because the method of the watershed method is relatively fast, and has a good segmentation effect on the weak edges in the image. However, considering that the water-shed method is sensitive to noise, it is easy to lead to over-segmentation or under-segmentation. Therefore, a watershed segmentation method based on distance transform is designed to separate and label the adhesion spores in the image pre-processed binary image. The watershed segmentation method is a mathematical morphology segmentation method based on topological theory [5] [13-15]. The basic principle is to regard the image as a topological topography of geology.

Literature [16] [17] proposed watershed segmentation of adherent cells based on distance topographic transformation. The distance transform method in this paper uses the Euclidean distance transform method. The pixel of the foreground target is 1, and the pixel of the background is 0. Assuming that the background pixel set is $formunal$ and the foreground target pixel set is $formunal$, then all pixel points $I(i,j)$ in the binary image set are calculated according to Eq. (7).

$$d(i,j) = min\{D_E(i,j),(x,y),(x,y) \in B\} \tag{7}$$

$$D_E(i,j),(x,y),(x,y) = \sqrt{(i-x)^2 + (j-y)^2} \tag{8}$$

Where a_{ij} is the pixel size of the binary image $I(i,j)$, (i,j) and (x,y) are the pixel position coordinates of the binary image , is Euclid distance formula. After the distance is transformed, the local minimum value is extracted from the distance image using the extended minimum value transformation function, and is marked to obtain a foreground target mark image. Assuming that the last marked image is I_m:

$$I_m = I_m^{fn} + I_m^{bm} \tag{9}$$

Where I_m^{fn} is the foreground mark, and I_m^{bm} is the background mark. Then the gradient image is modified with a minimum value calibration, so the local minimum value only exists where the binary marker image is 1 [18]. Use I_m to represent the image after calibration:

$$I_{cm} = impose\ min(I_c|I_m) \tag{10}$$

Where the Eq. (10) is used to modify the image gradient magnitude, so that the gradient magnitude image has a minimum value in the foreground image and the background image of the marker. The initial gradient image is I_c and the last marker image is I_m. Then the watershed transformation is performed in I_{cm} by the Eq. (11):

$$I = watershed(I_{cm}) \tag{11}$$

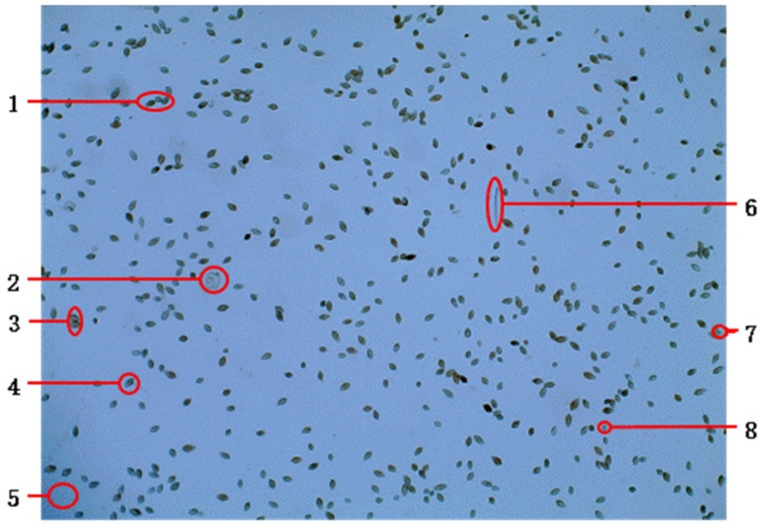

FIGURE 5. Spore image of potato late blight fungus, 1 and 3 are adhesion spores, 4 and 8 are single spores, 6 is hyphae, 5 is noise, 2 and 7 are impurities

3. **Experimental results and analysis.** In order to verify the accuracy and efficiency of the proposed potato late blight fungi spores count method, 30 microscopic images with potato late blight fungus spores were tested. These microscopic images used in this paper were provided by the Potato Cultivation Laboratory of Fujian Agriculture and Forestry University. The 30 spore micro images have a resolution of 1536 ﬃ 2048 pixels, a gray level of 256, with BMP format and RGB color space mode. The image processing device in the experiment is a desktop integrated computer, which has Intel coreTM i7-4790S processor, the main frequency is 3.2GHz and the memory is 8GB. The spore micrograph contains spores (adhesive spores and individual spores), hyphae, noise and impurities, which is shown in Fig.5.

3.1. **Threshold segmentation results.** The global threshold method, the intra-class variance threshold method, the iterative threshold method, and the integral image based adaptive threshold method proposed in this paper were used to segment the spores microscopy image (Fig. 5). The segmentation results are shown in Fig. 6.

As can be seen from Fig.5, the real spore image is affected by factors such as illumination and equipment precision during the production process, there are many characteristics including gray unevenness, low contrast and edge blurring, and identifying the spore microscopic images becomes more and more difficulty. From the partial enlarged view at the lower left part of Fig.6(a) and Fig.6(b), we can find that the global threshold method (Fig.6(a)) and the intra-class variance threshold method (Fig.6(b)) can extract the spore region from the background, but they cannot suppress the background noise caused by uneven illumination; As can be seen from the partial enlargement at the lower left of Fig.6(c) and the partial enlargement at the upper left of Fig.6(c), although the iterative threshold method can suppress the background noise caused by uneven illumination in Fig.6(c), the spores that are segmented out due to uneven gray scale contrast may have edge incompleteness or void in the center, which may cause difficulties in subsequent pretreatment. Meanwhile, the proposed method can segment the spores from the microscopic image more accurately (in Fig. 6(d)), this method proposed method can suppress the background noise caused by uneven illumination and the uneven gray scale contract,

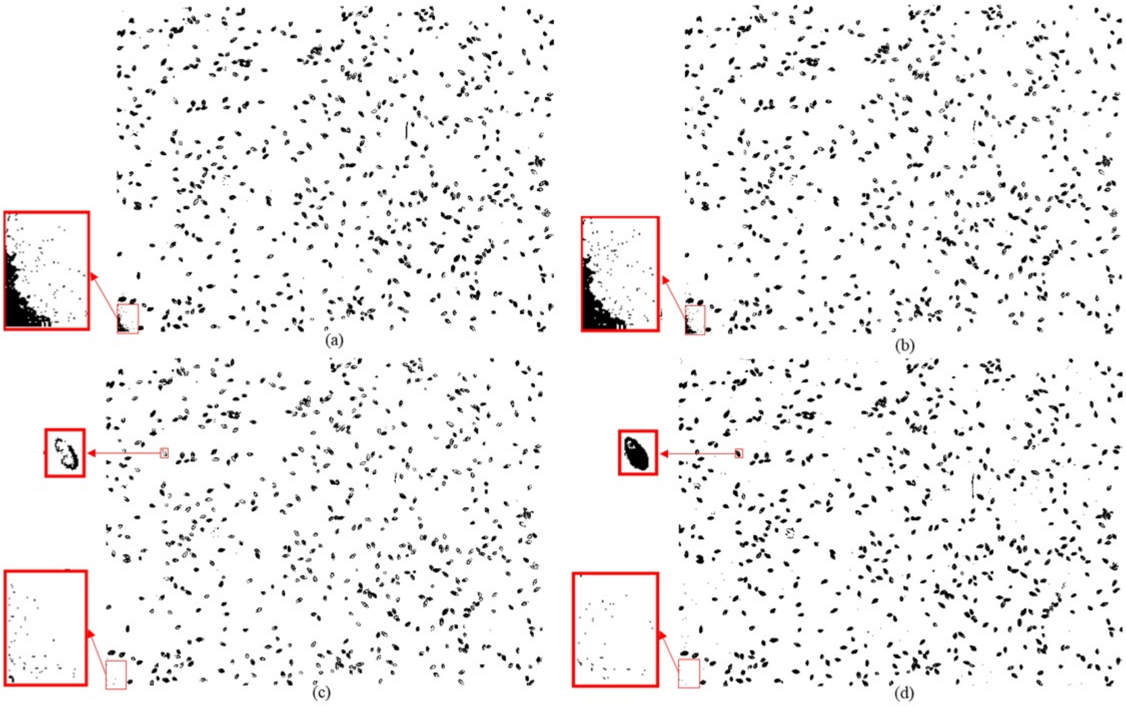

FIGURE 6. Comparison of segmentation results, (a) Global threshold method, (b) Intra-class variance threshold method, (c) Iterative threshold method, (d) Method in this paper

besides it can suppress the incomplete spore edge or central cavity phenomenon. Fig. 7 gives the results of image pretreatment, we can find that the impurity particles in the image are removed, the voids are filled, and the spore binary image is substantially restored to its original form. In Fig. 7(a), large impurity particles in the image are filtered by median filtering. In Fig. 7(b), the hollow phenomenon of the spores after median filtering is solved by the morphological expansion corrosion treatment. In Fig. 7(c), the spores are restored to their original shape by a logical or operation. In Fig. 7(d), the complete edge information of the spores is obtained by the morphological opening operation. Therefore, the binary image obtained by image preprocessing can preserve the shape and edge information of fungal spores, which provides a strong guarantee for the segmentation of adherent spores.

3.2. **Segmentation results of adhesion spores.** After the pre-processed binary image is processed by the watershed segmentation method based on the distance transform, the adhesion spores in the image can be segmented easier. Fig. 8 is a binary image derived from Fig. 7(d) by a watershed segmentation method based on distance transformation. After the segmentation process, most of the adhesion spores in the image can be accurately separated. However, the closely adhesion spores, especially the four-adhesion issue, cannot be well segmented. In Fig. 8 (b) shows the result after color marking of the 8-connection area in Fig. 8 (a). The connected region labeling method is used to accurately count the binary images after the watershed method, and the binary images are marked with different colors, it has obvious visual effect.

In order to further illustrate the effectiveness of the proposed method, the method proposed in this paper is compared with the method in literature [5], the method in literature [19] and the method in literature [3], which is shown in Fig. 9.

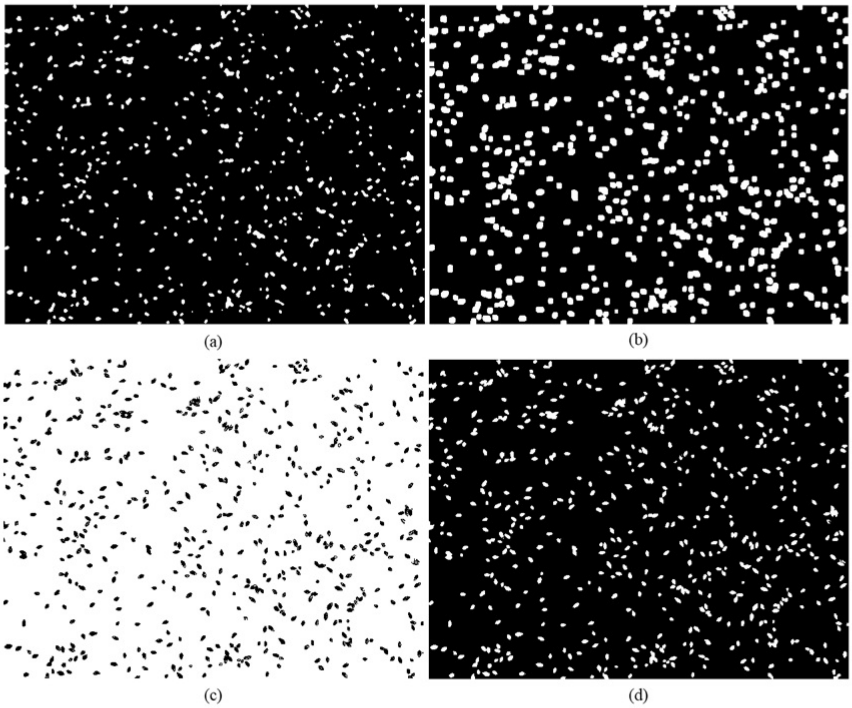

Figure 7. Comparison of segmentation results, (a) Global threshold method, (b) Intra-class variance threshold method, (c) Iterative threshold method, (d) Method in this paper

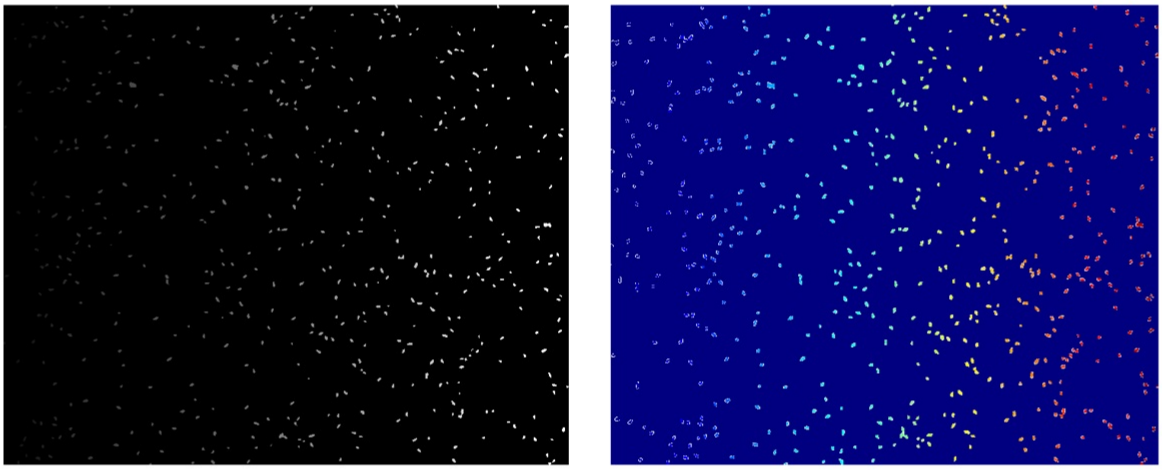

Figure 8. Watershed method based on distance transformation, (a) Segmentation result, (b) RGB color image

From Fig. 9, it can be seen that although the method in literature [5] eliminates over-segmentation or under-segmentation, it cannot segment the adhesive spores, the k-means clustering method and Otsu method cannot completely preserve the boundary of the spores, and they cannot obtain ideal segmentation effect on the adhesion spores. The proposed method can segment the adhesive spores and preserve the boundary information, with good segmentation result.

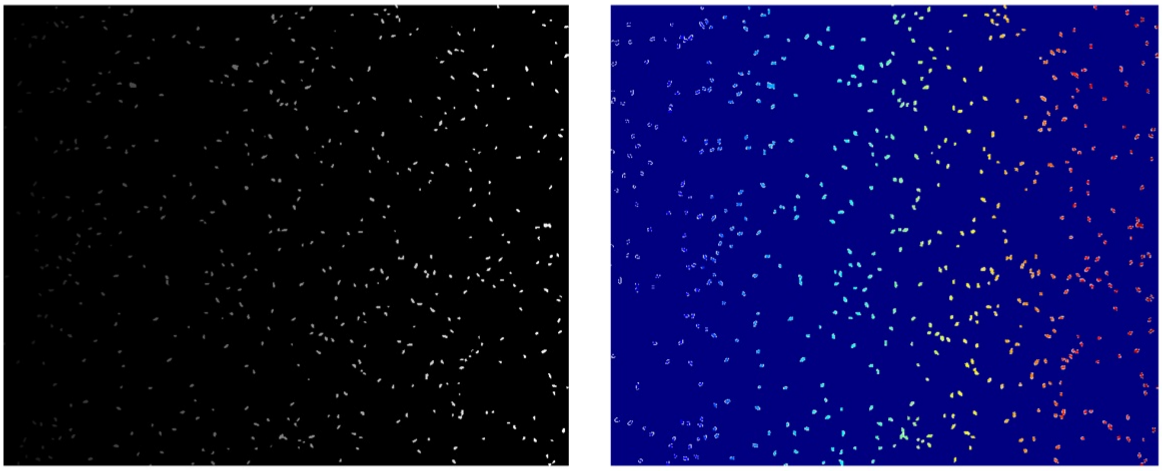

FIGURE 9. Comparison of segmentation results, (a) The method in literature [5], (b) The method in literature [19], (c) The method in literature [3], (d) the method proposed in this paper

TABLE 1. The counting result of fungal spores from potato late blight

The number of spores	The number of image	Number of samples			Minimum accuracy	Maximum accuracy	Average accuracy
		Accuracy 100%	Accuracy 98%~100%	Accuracy 96%~98%			
100~300	6	4	1	1	97.6%	100%	99.6%
300~400	8	5	2	1	97.5%	100%	99.5%
400~500	6	0	4	2	96.9%	99.8%	99.1%
500~600	10	0	6	4	97.6%	99.2%	98.8%
total	30	9	13	8	97.4%	99.75%	99.25%

3.3. **Spore count results.** In this experiment, 30 spore images (the number of spores ranged from 100 to 600 in each image) were manually marked, and the number of spores was accurately counted (one count and another retest), and the count result was taken as the true spore quantity standard figure. The image was automatically counted and marked by the Bwlabel counting method, and the automatic counting result was compared with the manual counting result to obtain the counting accuracy rate, and the average counting accuracy rate was calculated for all the processed images. The number of spores counted by manual statistics in the m-th image is N_m and the number of spores detected by the computer in the m-th image correctly is M_m , and the accuracy is defined as:

$$a_m = \frac{M_m}{N_m} \times 100\%,\ m = 1, 2, ...30 \tag{12}$$

The average accuracy is defined as:

$$a = \frac{\sum_{m=1}^{n} a_m}{n} \times 100\%,\ n = 1, 2, ...30 \tag{13}$$

Table 1 shows that there are 9 spore micro images with 100% counting accuracy, there are 13 spore micro images with 98% 100% counting accuracy, there are 8 spore micro images with 96% 98% counting accuracy; The average counting accuracy is 99.25%.

Table 2. Comparisons of counting results of several methods

method	Manual counting	Automatic counting	counting error /%	Counting accuracy /%
Xu method [3]	576	561	6.8	95.3
Hou-Shi method[5]	576	558	3.8	96.5
Nandagopalan et.al method[19]	576	554	4.9	96.2
The proposed method	576	571	1.2	99

In order to further verify the effectiveness of the method proposed in this paper for segmenting adherent spores, Otsu method, distance-based watershed method, k-means clustering method and the method proposed in this paper were used to segment and count potato late blight as shown in Fig. 3(a), the count results are shown in Table 2.

Technical error formula:

$$\delta = \frac{N_{TP} + N_{RP}}{N} \times 100\% \tag{14}$$

Where N is the result of manual counting, N_{TP} is the number of spores that are not marked in the automatic counting, and N_{RP} is the number of spores that are wrongly marked as spores in the automatic counting.

Table 2 shows that the Otsu method has the lowest spore accuracy and the largest count error, and cannot separate the adhesion spores basically. The method in literature [5] and k-means clustering method has the same counting accuracy, but the k-means clustering method has relatively large counting error; The proposed method has the best counting accuracy, except for there are a few cells are lost, there are better retentions in the shape information and positional information, reflecting the superiority of the method, and its counting error is less than 2%.

4. **Conclusion.** In order to realize high accuracy and automatic counting for Potato Late Blight Spore from images collected under electron microscope, in this paper, aiming at the issues including low gray scale contrast, uneven illumination and spore adhesion existed in the potato late blight fungal spores images collected under electron microscope. The adaptive threshold method based on integral image is proposed for solves the above issues. The experimental results showed that the method can accurately and automatically segment and count the potato late blight fungus spores, the average spore detection accuracy archives 99.25%, which can provide better technical support for the indoor resistance identification process aimed at potato late blight, and it can also provide technical reference for monitoring potato late blight in field.

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