Development of automatic counting system for urediospores of wheat stripe rust based on image processing

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Abstract: To realize automatic counting of urediospores of *Puccinia striiformis* f. sp. *tritici* (*Pst*) (causal agent of wheat stripe rust), an automatic counting system for urediospores of wheat stripe rust pathogen based on image processing was developed using MATLAB GUIDE platform in combination with Local C Compiler (LCC). The system is independent of the MATLAB environment and can be run on a computer without the MATLAB software. Using this system, automatic counting of *Pst* urediospores in a microscopic image can be implemented via image processing technologies including image scaling, clustering segmentation, morphological modification, watershed transformation, connected region labeling, etc. Structure design of the automatic counting system, the key algorithms used in the system and realization of the main functions of the system were described in detail. Spore counting tests were conducted using microscopic digital images of *Pst* urediospores and the high accuracies more than 95% were obtained. The results indicated that it is feasible to count *Pst* urediospores automatically using the developed system based on image processing.

Keywords: *puccinia striiformis* f. sp. *tritici*, wheat stripe rust, image processing, automatic counting, computer aided system, MATLAB

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1 Introduction

Wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is a kind of destructive epidemic disease that affects wheat production in China[1,2]. China is the largest epidemic region of wheat stripe rust in the world[2]. As a typical air-borne disease, the pathogen that causes this disease mainly relies on the dispersal of *Pst* urediospores by airflow to complete the disease cycles. The amount and activity of pathogen propagules are the important driving factors of the occurrence and epidemics of wheat stripe rust[1,3]. Therefore, it is very necessary to monitor the quantity and dynamics of *Pst* urediospores in the field. Now the slide method, petri dish method and spore trap method are usually used to capture the pathogen spores in the field[4,5]. Usually, the trapped spores are counted by using the traditional microscopic counting method. Due to the great number and the small size of the trapped spores, this traditional method is time-consuming and labor consumptive. The long-time microscopic observation is easy to cause eye fatigue, and

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it is difficult to ensure the counting accuracy, usually resulting in great counting errors. Although molecular biology techniques have been applied to quantify trapped spores of different pathogens[6-8], the quantification processes are complex and have high requirements on technologies and instruments. Therefore, it is great of significance to develop an automatic counting system for *Pst* urediospores to monitor the spores conveniently and accurately. Based on the monitoring results, the prevalence and severity of wheat stripe rust can be predicted and then control measures can be made accordingly.

The rapid development of computer technology has made the application of computers play an important role in the studies on plant pathology[9]. As a kind of computer technology, image processing has the advantages of rapid identification for the target images. It has been used in plant disease diagnosis[10-20], automatic grading of plant diseases[21-25], and identification and automatic counting of plant pathogens[26-30]. And it is increasingly used in automatic counting of insects, cells and pathogenic microorganisms, etc. Based on image processing, Yao et al.[31] developed a three-layer detection algorithm including an AdaBoost classifier, a support vector machine classifier and the threshold judgment for automatic counting of whiteback planthoppers (*Sogatella furcifera*) in paddy fields, and a detection rate of 85.2% was achieved. Wu et al.[32] developed a counting method based on active contour model for overlapped algae counting with accuracy over 90%, and the results showed that the counting accuracy of overlapped algae cells was improved by using the active contour model algorithm in edge detection instead of watershed algorithm. Zhang et al.[33] developed a method for automatic counting of plaques in electrical images based on computer image processing technology in which watershed algorithm was used to separate the linked plaques into individual ones in the images, and the counting results obtained by computer were in agreement with the artificial counting method. To the best of our knowledge, there have been fewer reports on automatic counting of plant pathogen spores based on image processing[27-30]. Xu and Li[27] transformed the original images of plant pathogen spores into gray images that were further processed using median filtering and canny edge algorithm, and then transformed the gray images into binary images for segmentation, spore feature extraction and automatic counting of the spores. Based on the microscopic images of spores of rice blast, Qi et al.[29] conducted illumination correction, canny operation with fuzzy c-means algorithm and morphological image processing including close and open operations, and designed an improved watershed algorithm to separate the adjacent spores for determining the numbers of spores in the images, achieving average counting accuracy of 98.5%. Tao et al.[30] collected spore images using a spore trapping system and implemented automatic counting of spores in the images after conducting image binarization, morphological modification, feature extraction and feature matching. These studies demonstrated that counting of plant pathogen spores based on image processing is rapid and convenient with high accuracy. It will be very helpful to develop automatic spore counting systems for quantification of plant pathogen spores by microphotography method, especially for monitoring quantitative dynamics of plant pathogen spores in the field. Our previous study[28] on automatic counting of trapped urediospores of *Pst* based on image processing provided the foundation for the development of automatic counting system of *Pst* urediospores in this study.

In this study, combined with Local C Compiler (LCC), MATLAB GUIDE platform was used to develop an automatic counting system for urediospores of wheat stripe rust pathogen based on image processing. Processing of the microscopic images of *Pst* urediospores, including image scaling, clustering segmentation, morphological modification, watershed transformation, connected region labeling, etc., can be implemented using this system. This study provided the foundation for the prediction of wheat stripe rust and monitoring of *Pst* urediospores based on internet and mobile communication services.

2 General structure and development environment of the system

2.1 General structure of the system

General structure of the automatic counting system for *Pst* urediospores was as shown in Figure 1. The main function modules of the automatic counting system include image input module, image processing module (including clustering segmentation of the spore microscopic images, morphological modification and watershed segmentation) and data output module. Using these function modules, automatic spore counting of *Pst* urediospore microscopic images can be implemented. Using the image input module, a *Pst* urediospore microscopic image can be read and input into the automatic counting system. Using the input image processing module, clustering segmentation of the *Pst* urediospore microscopic image can be implemented, morphological modifications of the binary segmentation image can be operated, and watershed segmentation to separate the joint spores in the image into individual ones can be carried out. Using the data output module, urediospore counting can be automatically conducted and the *Pst* urediospore counting result can be output.

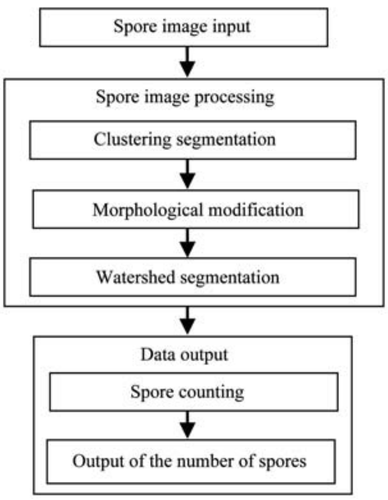

Figure 1 Main structural diagram of the automatic counting system for *Pst* urediospores

2.2 System development environment

GUIDE (Graphical User Interface Development Environment) platform in the MATLAB software (MathWorks, Natick, MA, USA) is a special development environment for user interface programming. The automatic counting system for urediospores of wheat stripe rust pathogen was developed in the MATLAB7.8 software environment. MATLAB was used as the programming language to realize algorithm operations and other related functions used in the system. The GUI (Graphical User Interface) of the system was designed using the GUIDE tool set. The LCC compiler was used to make the system run on a computer without the MATLAB software.

3 Key technologies of the system

3.1 *K*_means clustering algorithm

Binary segmentation and color segmentation of the spore microscopic images were conducted using the *K*_means clustering algorithm in the automatic counting system for *Pst* urediospores developed in this study. As a kind of unsupervised real-time clustering algorithm, *K*_means clustering algorithm proposed by Mac Queen in 1967 is an indirect hard clustering method based on the similarity between the samples[34]. Using *K*_means clustering, the square of the distances from all the samples to cluster centers in the sample space can be the minimum. n pixels in the sample are divided space into k clusters with the clustering number k as a parameter to achieve high similarity between the pixels in a cluster and low similarity between the clusters. In this study, the Euclidean distance was treated as the cluster similarity, the mean variance $e=\sqrt{E/n}$ was treated as clustering criterion function. The procedure of *K*_means clustering algorithm[14,28] was used in this study.

3.2 *L*a*b** color spaces

Among the color space models that can be used for image processing including *RGB*, *HSV (HIS)*, *CMYK*, *XYZ*, *YUV* and *L*a*b**, etc., *L*a*b** color space was selected to conduct spore microscopic image processing in the automatic counting system for *Pst* urediospores. As all spore microscopic images acquired for the automatic counting system are described in *RGB* color space model, it is needed to transform *RGB* color space to *L*a*b** color space. Because *RGB* color space cannot be directly transformed to *L*a*b** color space, *RGB* color space should be transformed to *XYZ* color space firstly, and *XYZ* color space then could be transformed to *L*a*b** color space. According to the method described in reference [14], $L*a*b*$ color space transformation was conducted in the automatic counting system.

3.3 Binarization and morphological operations

Based on maximum variance between clusters, binarization of spore microscopic images after *K*_means clustering was conducted. Because of the influence of uneven illumination, impurity and other factors, image binarization often results in image distortion and false spores in the binary images. Therefore, morphological operations were conducted to modify the binary segmentation images in this study. The original basic shape of the *Pst* urediospores were restored by hole filling, dilation and close operations. The influence of impurity was eliminated by removing the connected regions with small areas. The incomplete spore in the image boundary area whose area was smaller than 1/5 area of a normal spore was removed. The remaining spores were treated as the visible spores in the visual field and were used for subsequent counting analysis.

3.4 Watershed transformation algorithm

The joint spores in the binary segmentation images were isolated by using watershed transformation algorithm in the automatic counting system. Watershed transformation algorithm is a morphological segmentation method based on the topological theory[35]. To avoid over segmentation, distance transformation was operated on the binary segmentation image. The value of each pixel in the image was changed to the distance from the pixel to the nearest nonzero pixel. Watershed function and imextendedmin function were used to obtain the background marker image and the foreground object marker image, respectively. Corrected gradient image was obtained using the imimposemin function with the background marker image and the foreground object marker image as the local minimum of gradient image. Then the image was processed using watershed transformation algorithm.

3.5 Automatic counting of *Pst* urediospores

In the automatic counting system, the *Pst* urediospores in the binary images after watershed transformation were automatically counted using connected region labeling algorithm[36]. In the binary images after watershed transformation, each connected region represents one spore. Thus the number of the connected regions is the number of the *Pst* urediospores. The number of the *Pst* urediospores can be determined by statistical analysis of the number of the connected regions.

4 System structure and realization of system functions

4.1 Interface of the automatic counting system

According to the design scheme of the automatic counting system for *Pst* urediospores described above, the main functions of the system were implemented based on the image processing algorithms using the GUIDE tool set in the MATLAB 7.8 software environment. The GUI of the automatic counting system was as shown in Figure 2. When the GUI of the automatic counting system was designed using the MATLAB GUIDE platform, we clicked the required controls in the GUI panel and then clicked the edit area of interface. Thus the required controls can be placed in the right places. To improve the functions of GUI, the properties of each control can be set using Property Inspector, and the algorithm operations and other related functions can be programmed using MATLAB as the programming language.

4.2 Image input and image scaling

The microscopic images of *Pst* urediospores can be acquired using an inverted microscope. In this study, the amplification of the microscope was 10×20. The size of the original microscopic images acquired for the automatic counting system was 4080×3072. The image file format was jpg and 24 bitmap in *RGB* color space. The images were saved in the established image database of *Pst* urediospores. In the automatic counting system, the original microscopic images can be inputted from the image database using the “Image Input” button on the GUI, and then can be displayed in the image area as shown in Figure 3. To a great extent, the microscopic images acquired using an inverted microscope preserved the information integrity. However, the amount of image information was so much that it could influence the running speed of the system. To reduce the operation speed of the computer programs, the images can be scaled down considerably by adjusting the scaling just as shown in Figure 3.

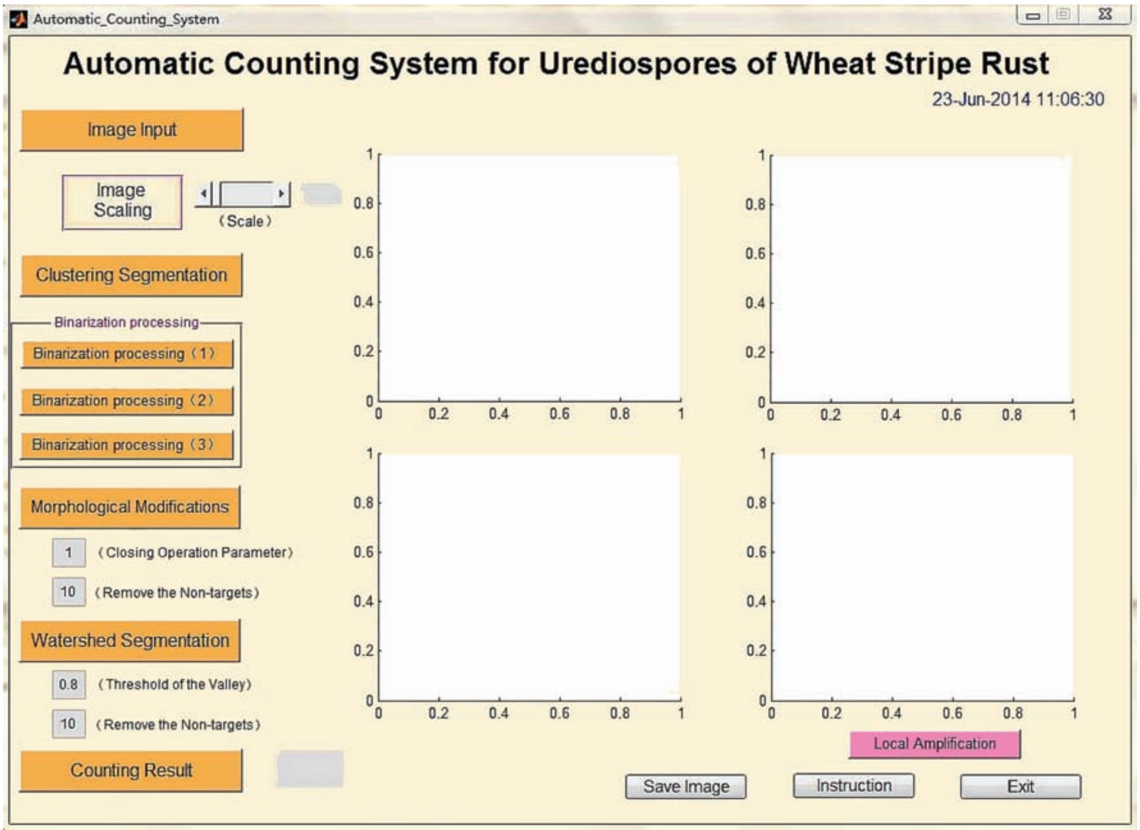

Figure 2 Graphical user interface of the automatic counting system for *Pst* urediospores

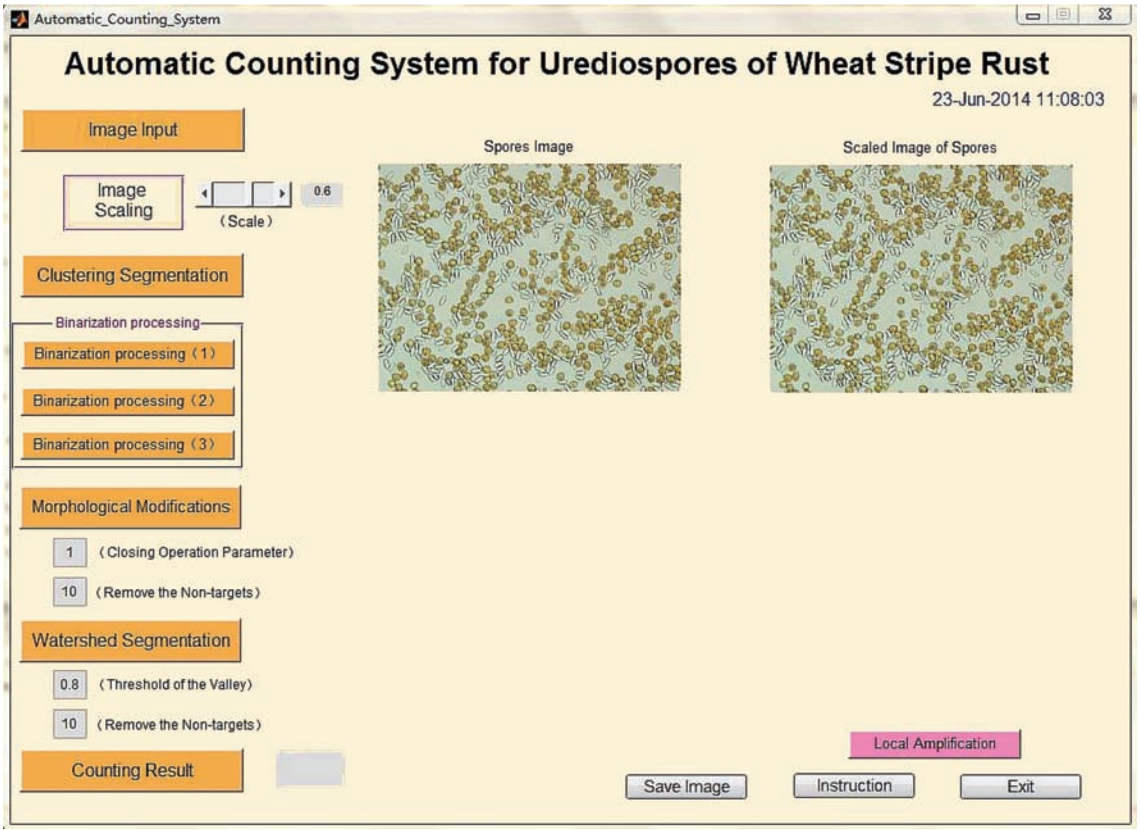

Figure 3 Realization of image input and image scaling for the automatic counting system

4.3 Image processing

Image segmentation is the key step in image processing of *Pst* urediospores. The aim of image segmentation is to separate spore regions from the microscopic image. In the automatic counting system, binary segmentation and color segmentation of the spore microscopic images were conducted using the *K*_means clustering algorithm[34]. The algorithm was optimized in color space, cluster similarity and clustering criterion function[14,28]. The segmentation operation of the microscopic image of *Pst* urediospores can be implemented using the "Clustering Segmentation" button on the GUI of the automatic counting system, and the results including three images then can be displayed in the image area as shown in Figure 4. Among the three images labeled "One", "Two" and "Three", respectively, there is only one image in which the target spores are accurately segmented from the background. According the label of the image, by clicking the corresponding binarization processing button on the GUI of the automatic counting system, a binary image for further processing can be obtained as shown in Figure 5.

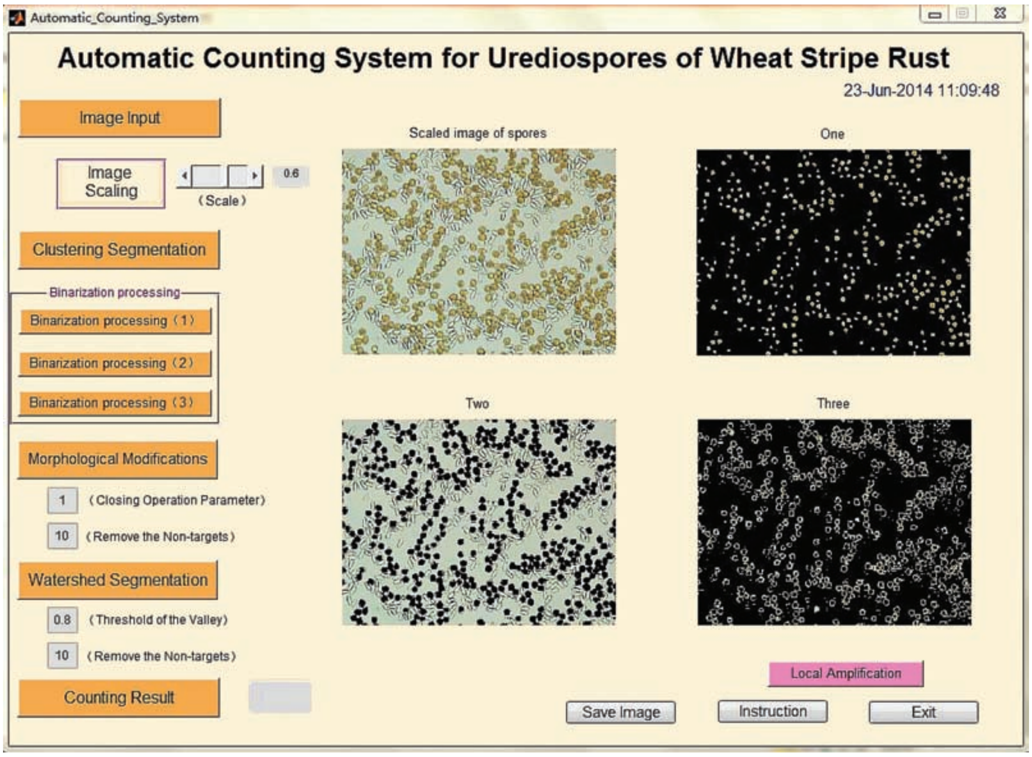

Figure 4 Realization of image segmentation for the automatic counting system

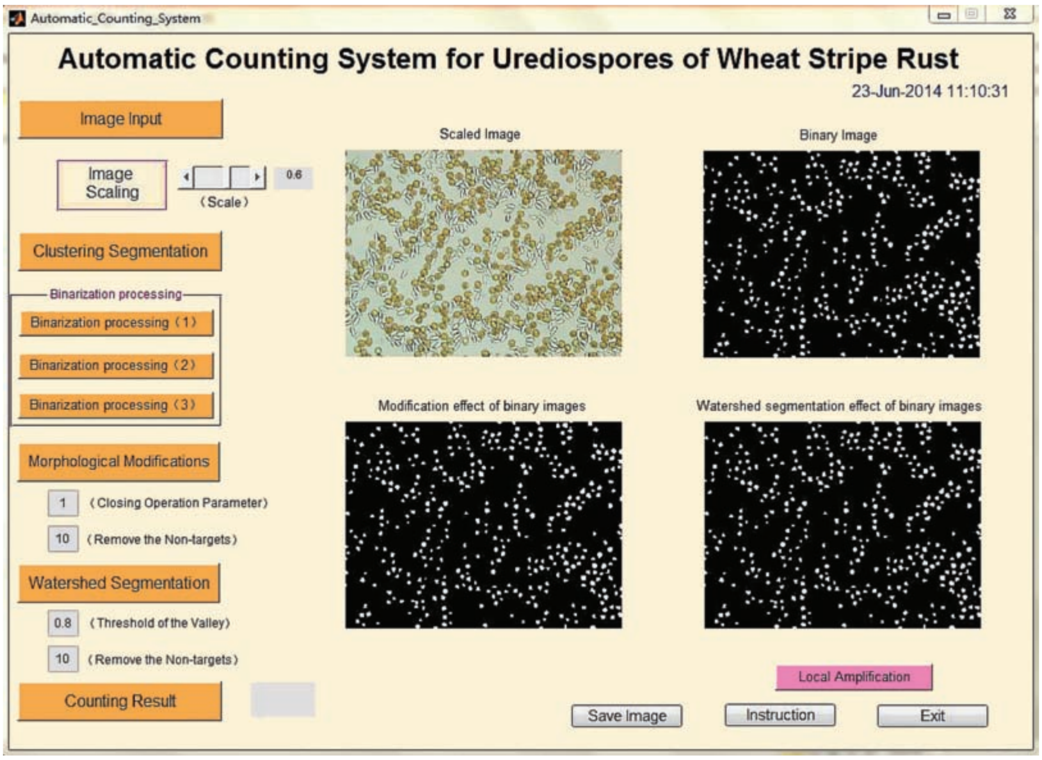

Figure 5 Morphological modification and watershed segmentation of spore microscopic image using the automatic counting system

To keep the basic shape of *Pst* urediospores in the images, morphological operations were needed to be conducted to modify the binary segmentation images. In this study, the holes in the binary segmentation image were filled using filling algorithm, then the image was processed using dilation algorithm, finally the impurity particles in the image was removed using removing algorithm. Moreover, the incomplete spore in the image boundary area whose area was smaller than 1/5 area of a normal spore was also removed. Morphological operations in image processing of spore microscopic image can be implemented using the "Morphological Modifications" button on the GUI of the automatic counting system. When morphological operation is performed using the automatic counting system, the parameters for closing operation and removing the non-targets can be adjusted.

Adherence separation of *Pst* urediospores in the images is a key step to automatic counting of the spores. In the automatic counting system, the joint spores in the binary segmentation images after morphological modification were isolated by using the watershed transformation algorithm based on prior knowledge. This process can be implemented using the "Watershed Segmentation" button on the GUI of the automatic counting system. When watershed segmentation is performed using the automatic counting system, the parameters for the threshold of the valley and removing the non-targets can be adjusted. As shown in Figure 5, the results of image segmentation, morphological modification and watershed segmentation were satisfactory.

4.4 Data output

After watershed segmentation of the binary images, the *Pst* urediospores in the images were automatically counted using connected region labeling algorithm[36]. The number of the *Pst* urediospores can be displayed on the GUI. This process can be implemented using the "Counting Result" button on the GUI of the automatic counting system. Spore counting tests were conducted using the microscopic digital images of *Pst* urediospores trapped via indoor simulation. Totally, 290 images in 10 groups were used including 9 groups with 30 images per group and one group with 20 images. The counting result of a spore counting test using a microscopic digital image of *Pst* urediospores mixed with the conidia of *Blumeria graminis* f. sp. *tritici* (causal agent of wheat powdery mildew), was as shown in Figure 6. The number of 400 on the right of the "Counting Result" button on the GUI of the automatic counting system demonstrated that there were 400 *Pst* urediospores in the microscopic digital image. The results of spore counting tests showed that the average counting accuracy for each group was more than 95%.

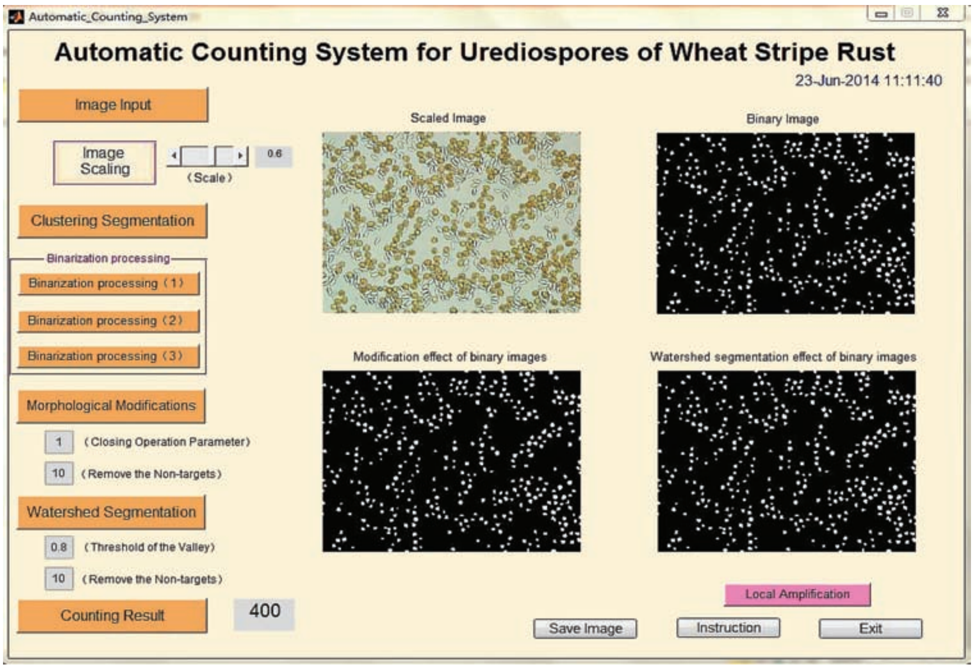

Figure 6 Output of counting result obtained using the automatic counting system

5 Results and discussion

Monitoring the amount of *Pst* urediospores in the field is an important part of disease and pathogen monitoring, and is an important prerequisite for scientific prediction of wheat stripe rust. In this study, an automatic counting system for urediospores of wheat stripe rust pathogen based on image processing was developed using the MATLAB GUIDE platform under the MATLAB 7.8 software environment. For easy of use, the source program of the system in the MATLAB 7.8 software environment was compiled to an executable program using the LCC compiler. Thus the system can be run on a computer without the MATLAB software. Using this system, clustering segmentation of spore regions, adherence separation of *Pst* urediospores and spore counting were realized via spore microscopic image processing.

Based on the microscopic digital images of *Pst* urediospores trapped via indoor simulation, *Pst* urediospore counting tests were conducted using the automatic counting system in this study. The number of *Pst* urediospores in each microscopic image was carefully counted using traditional artificial counting method and the counting result was treated as the standard number of *Pst* urediospores. The automatic counting results and artificial counting results were compared, and high counting accuracies were obtained. This indicated that the satisfactory output results can be obtained using the automatic counting system. A convenient and accurate tool for counting of *Pst* urediospores was provided in this study.

During the capture of air-borne plant pathogenic fungal spores, there will inevitably be large-area overlapping spores, and this may affect the counting accuracy of the spores. Since the spores are in three-dimensional structures, uneven light may result in color difference of the spores in the microphotography process while some spores overlap partly. In the clustering segmentation, the color difference may lead to a certain error, thus the accuracy of automatic counting may be affected. In the *Pst* urediospore counting tests conducted in this study, the microscopic digital images of *Pst* urediospores were acquired via indoor spore trapping simulation using the spores collected from the diseased wheat leaves incubated in an artificial climate chamber. The spore samples contained few other materials that may disturb spore image processing. However, during the capture of air-borne *Pst* urediospores in the field, the dust, pollen particles and other pathogenic spores in the air can affect the accuracy of automatic counting of target spores[28]. The effects of various factors on automatic counting should be considered and the algorithms used in the system should be improved if the automatic counting system is applied to assess the number of *Pst* urediospores trapped in the field.

6 Conclusions and suggestions

In this study, an automatic counting system for *Pst* urediospores based on image processing was built using the MATLAB GUIDE platform. An executable program of the automatic counting system for *Pst* urediospores was developed using the LCC compiler. It is convenient for users to utilize the system to automatically count *Pst* urediospores on a computer without the MATLAB software. Using this system, the number of *Pst* urediospores in a microscopic digital image can be automatically counted after clustering segmentation of spore regions and adherence separation of target spores based on spore microscopic image processing. The results of *Pst* urediospore counting tests using the automatic counting system indicated that high counting accuracy could be achieved in quantification of the *Pst* urediospores in a microscopic digital image. And a convenient and accurate tool was provided for counting of *Pst* urediospores during spore trapping in the field.

The *Pst* urediospore counting tests were conducted using the microscopic digital images acquired via indoor spore trapping simulation in this study, counting tests of *Pst* urediospores trapped in the field should be conducted to detect the capability and adaptability of the automatic counting system. Considering the effects of various described factors on automatic counting of target spores, the segmentation of the target regions in the spore images is a key issue in further studies, and the capability to automatically count the target spores of the system during spore trapping and pathogen monitoring should be improved. With the development of microphotography technology and information technology, and with the increase of the coverage of the computer network and communication network, further studies will focus on the development of the application system based on internet and mobile phone communication system for convenient popularization and use. Moreover, it is necessary to develop an integrated system that consists of spore trapping system, automatic photomicrography system and automatic spore counting system for automatic monitoring of *Pst* urediospores in the field.

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