Image Classification of Chicken Embryo Based on Matched Filter and Skeleton Curvature Feature

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Abstract. To solve the problem of nondestructive detection of egg formation activity in vaccine preparation, a new method based on matching filter for chicken embryo image blood vessels segmentation algorithm combined with the curvature feature of the blood vessels skeleton was proposed. Gaussian matching filter was used to enhance the contrast between the blood vessels and the background of the green channel image and the blood vessels were segmented from the background. Morphological methods was used to process the binary images to extract the vascular skeleton feature. The curvature of the vascular skeleton was calculated by using the least square method. Finally, the activity of chicken embryo was judged by combining the shape feature of blood vessel with the texture feature of chicken embryo extracted by gray level co-occurrence matrix algorithm. The experimental results show that this method has high accuracy in the classification experiment of chicken embryo images affected by spot noise, and meets the quality requirements of producing virus species in vaccine preparation.

1. Introduction

To prepare and produce virus species from basic virus species, the selection of high-quality SPF chicken embryos with guaranteed quality for seed rejuvenation and breeding to ensure the purity and high efficiency of the prepared production virus is one of the key points of quality control. As an important part of the vaccine preparation process, the detection of the activity of chicken embryos is usually carried out by manual distinguish with lights. This method has strong subjective factors, which not only takes a long time and has low efficiency, but also workers who work for a long time are prone to visual fatigue, which will lead to false detection and missed detection. During the culture process, the eggs that become dead embryos should be avoided to produce bacteria and infect other eggs in the same incubation chamber, affecting the quality and yield of the vaccine.

As early as in the 1940s, bio-electricity was used to detect the activity of egg embryos in foreign countries. Later, ultrasonic images were used to detect the developmental status of egg embryos to judge the generative activity of chicken embryos. At present, the research on eggs based on machine vision in China is mainly divided into three aspects: nondestructive testing of egg quality, identification of fertilized eggs and non-spermatozoa, and detection of the activity of the virulent strain embryo eggs in vaccine preparation. The automatic grading of egg quality is mainly based on the surface image of the egg, which is mainly used to grade the freshness of edible eggs, or judge the quality of eggs according to whether there are cracks in the eggs. To identify whether an egg is fertilized in the early hatching stage, eggs with incubation time of 5-6 days are generally selected. On the research of the strain of chicken embryo into active detection, Shan BaoMing using image recognition technology for active detection of chicken embryo, the accuracy of detection can reach 98%.
processing technology to enhance image through multiscale morphological filtering was proposed, based on the local adaptive binarization method of histogram WFCM extract vascular characteristics, the recognition accuracy of 150 images is 99.33%, the method in the image is not clear, there will be false negatives for main bloodshot, impact into the activity of test results. Liu Chuanlai[9] proposed a speckle noise detection method based on SUSAN algorithm and USA N local gray mean speckle elimination algorithm. After eliminating the speckle, the accuracy rate of activity identification was 96.94%. In 2017, Huang Chao[10] divided embryonic egg images into two categories: bright image and dark image, different methods were adopted to complete the extraction of vascular texture. This method solved the problems of gray scale of embryonic egg image and uneven quality of eggshell during blood vein extraction in previous studies, and the automatic detection accuracy was 97.5%.

All the above three methods used the proportion of blood vessel area in the binary image as the criterion for the detection of chicken embryo activity. Since a single feature cannot effectively represent the global image features, the probability of dead embryos being recognized as live ones is easily increased in different image data sets of chicken embryos, this study through the analysis of the blood vessels of chicken embryos image texture and shape feature, matched filtering is used to implement the blood vessels segmentation, the blood vessel skeleton curvature feature and texture feature of gray level co-occurrence matrix series, realize the strain of chicken embryos image recognition.

2. Detection of chicken embryo activity

In this paper, a classification method of chicken embryo image vascular segmentation algorithm based on Gaussian matching filter[11] combined with the curvature characteristics of the vascular skeleton is proposed. Firstly, channel separation was used to extract the green channel component of RGB image, used matched filter to enhance the chicken embryo image, increase the contrast between blood vessel and background, separated the blood vessels from the image. Then, the features of vascular skeleton in binary images were extracted by morphological methods, and the curvature of the skeleton was calculated by using the least square method. Finally, combined with the gray level co-occurrence matrix to extract the texture feature of the image and constitute the six-dimensional feature vector, which was input into the support vector machine to classify different chicken embryo images.

2.1. Image Preprocessing

In the process of image acquisition, due to the influence of angle and uneven illumination, the image of chicken embryo differs in color and imaging range of region of interest, and the image feature extraction is affected, resulting in the unsatisfactory accuracy of activity detection. Capture the image of the size of 280 * 280 interested in area, for the next experiment operation.

A1. Live embryo image A2. Dead embryo images A3. Dead embryos with speckle noise

Figure 1. Images of egg embryos collected during the experiment

Compared with the other two channels in the chicken embryo image, and the blood vessels in the green channel are more clear, so the $G$ channel image is selected to segment the blood vessels. Adaptive histogram equalization was used to process $G$ channel images to further enhance the contrast of blood vessels and background. Adaptive histogram equalization calculates the local histogram of the image and then redistributes the brightness to change the image contrast, so as to obtain more details of the image. For samples with spot noise, the shape features of the spot and blood vessels are also obvious.
2.2. Matched Filtering

In 1989, Chaudhuri et al. [11] put forward to enhance the matched filtering algorithm of retinal blood vessels, using retinal fundus image gray distribution of blood vessels in the cross section is in the shape of inverted gaussian background gray basic consistent characteristics, thus design a scale and direction of all match the blood vessels of filter can better enhance the blood vessels segmentation effect. The positive chicken embryo image have abundant blood vessels as the fundus retinal image, so the matching filter is applied to the blood vessel segmentation of chicken embryo image. In the matched filter, the blood vessel can be imagined as a combination of several small parallel regions. The length of the blood vessel is set as \( L \) and the width is set as \( 2\sigma \). An inverted Gaussian curve is used to simulate the gray curve of the cross section of the blood vessel of chicken embryo, so as to obtain the matched filter model:

\[
K_\theta(x, y) = -\exp\left(-\frac{x^2}{2\sigma^2}\right), \quad y \leq \frac{L}{2}, \quad 0 \leq \theta \leq \pi
\]

Where \( x \) and \( y \) represent the coordinates of pixel points on blood vessels, \( \sigma \) represents the size of blood vessels, \( L \) represents the length of segmented blood vessels, and \( \theta \) represents the deflection Angle of blood vessels growing in different directions.

Adjust the parameter setting of the matching filter, select the scale range of gaussian filter to fit the vessel; since the blood vessel has a direction, the design rotates from 0° to 180° at 15° intervals (= 0°, 15°..., 180°) filter in 12 directions. The filter in all directions is convolved with the image separately, and the maximum response value is retained at each pixel [12]. According to the different scales, large-scale matching filtering has a better effect on the segmentation of large blood vessels, while small-scale matching filtering can segment small blood vessels in the image [13]. After many experiments, when the scale \( \sigma \leq 4 \) or \( L \leq 13 \), the binary image segmentation effect is not ideal. After testing, the image was enhanced with a Gaussian filter with \( \sigma = 4 \), \( L = 14 \) and direction 12. Then, adjust the gray scale range of the filtered image to \([0.35, 0.5]\), an adaptive threshold segmentation algorithm was used to segment the blood vessels and spot noise in the chicken embryo images. More blood vessels were retained in the positive samples, and the spot noise in the negative samples was also partially reduced. In order to be unified with the extraction of texture features, the operation object of matched filtering is the histogram equalization image after grayscale compression, which retains the main blood vessels and does not affect the judgment of the final result. The comparison of the result of matched filtering of positive and negative samples is shown in Figure 2:

![B1. Live embryo](image1) ![B2. Dead embryo](image2) ![B3. Dead embryos with speckle noise](image3)

Figure 2. The result of matched filtering

2.3. Curvature Feature Extraction

After analyzing the positive and negative sample images, the binary images obtained by the above matching filtering were processed by morphological corrosion and then expansion. Removing the connected areas with an area less than 90 pixels, the images were refined to extract the vascular skeleton (As shown in Figure 3). The skeleton can maintain the topological structure and geometric properties of the original shape of the object [14]. The thinning method can not only ensure the structural integrity of the extracted vascular skeleton, but also be easy to operate, it is suitable for the extraction of irregular vascular skeleton.
2.3.1. Definition of Curvature
Curvature means how much the curve bends at a certain point, and the greater the curvature, the more the curve bends. The radius of curvature of any section of an arc on a circle is equal to the radius of the circle, and the reciprocal of the radius of curvature is the curvature. Let the function equation of curve is \( y = f(x) \), and have the second derivative, then the curvature of the curve at the tangent point \( M \) can be expressed as:

\[
\kappa = \frac{|y''|}{(1 + y'^2)^{3/2}} 
\]

(2)

In the binary image of chicken embryo, the vascular skeleton can be regarded as an uneven curve composed of pixels, and the curvature of the skeleton can be obtained by fitting the skeleton curve into an arc and reconstructing the circle, and then the curvature of the circle corresponding to the arc can be obtained.

2.3.2. Curvature Calculation Based on Least Square Method
By observing the structure of the vascular skeleton, it can be found that in the positive sample, there is an intersection point where the vessels are connected, the length of the vessels is longer, and each vessel is a curve with a small curvature. In the negative sample, the vessels are shorter, and the image with spot noise is more curved, which is closer to the circle.

In this paper, the vascular skeleton with the largest area in each sample was extracted, and the fitting circular curve of the vascular skeleton in the figure could be obtained by the least square method according to the discrete point data relationship\(^{[15]}\). From top to bottom, the coordinates of each pixel point \((x, y)\) of the binary image are traversed from left to right, and the dimensionally consistent \(X\) and \(Y\) are respectively constituted as data input; by solving the linear equation \(\hat{X} \times a = \hat{Y}\), the center coordinates and radius of the fitted circle are calculated. The fitting circle result of skeleton curve is shown in Figure 4:

Figure 4. The least square method is used to fit the circle results of living embryo (left) and noisy dead embryo (right)

The combination of curvature \(K\) and the proportion of the chicken embryo vessel area in the total image area was taken as the shape characteristic value to judge the activity of the embryonic egg. The formula to calculate the proportion of the chicken embryo vessel area was as follows:

\[
\eta = \frac{\text{The area of a pixel whose gray value is 1 in a binary image}}{\text{The total pixel area of a binary image} \times m \times n} \times 100\% 
\]

(3)
2.4. Texture Feature Extraction Based on Gray Level Co-occurrence Matrix

Texture feature is a description of the spatial distribution of image pixels, which has good anti-noise and rotation in variance, and can be used to express both the overall and local characteristics of the image.

2.4.1. Gray Level Co-occurrence Matrix Parameter

The gray level co-occurrence matrix calculates the gray spatial correlation of two pixels at a certain distance to express the texture information of the image, the co-occurrence matrix is used to calculate texture features in five ways: energy, entropy, contrast, inverse moment difference and correlation:

1) Energy: The sum of squares of each element value in the gray co-occurrence matrix, used to reflect the evenness of the gray distribution of the image and the thickness of the texture.
   \[
   \text{ASM} = \sum_i \sum_j p^2(i, j)
   \]

2) Entropy: The randomness measure of information content is included in the image. The higher the value is, the higher the image complexity is.
   \[
   \text{EN} = -\sum_i \sum_j p(i, j) \log p(i, j)
   \]

3) Contrast: reflects the image clarity degree and the depth of texture groove, if the contrast is large, the deeper the texture groove, the clearer the effect will be.
   \[
   \text{CON} = \sum_i \sum_j ((i - j)^2 \cdot p(i, j))
   \]

4) Inverse moment difference: it reflects the homogeneity of image texture and measures the local variation of the image texture.
   \[
   \text{IDM} = \sum_i \sum_j p(i, j) \frac{1}{1 + (i - j)^2}
   \]

5) Correlation: reflects the local grayscale similarity of the texture. The element values are uniformly equal, and the correlation values are large.
   \[
   \text{COR} = \frac{\sum_i \sum_j [p(i, j) - \mu_i \mu_j]}{S_i S_j}
   \]

2.4.2. Feature Extraction of Texture

For the grayscale image after adaptive equalization, in order to reduce the amount of calculation, the grayscale level of image I is compressed and quantified into 16 levels. Take the distance as 1 and calculate the gray level co-occurrence matrix from 0°, 45°, 90° and 135° directions respectively [16]. After the normalization of the symbiosis matrix, five eigenvalues of its four directions, namely energy, entropy, contrast, correlation and inverse moment difference, were calculated, and the average value of each parameter was calculated as the texture feature vector of the chicken embryo image. Randomly selected from the positive and negative samples, comparing the samples of the five characteristic value, found that the positive and negative samples in the correlation no obvious differences on the characteristic value, then the paper constructs the texture feature vector for \[ [\text{ASM}, \text{EN}, \text{CON}, \text{IDM}] \].

2.5. Construction of The Feature Vectors

The features extracted above are fused by series method, the new features are used to classify the target image, and the proportion of blood vessel area η, skeleton curvature κ and texture features \[ [\text{ASM}, \text{EN}, \text{CON}, \text{IDM}] \] are selected in series to obtain a new 6-dimensional feature vector, which is input to support vector machine for classification.
3. Experimental Results and Analysis
The experiment is divided into two parts to verify the effectiveness of the proposed method, and support vector machine classifier is used for classification. The first step is to classify the texture feature and the proportion of the vascular area in series, and then introduce the skeleton curvature feature as the feature vector for the second classification.

Generally, the target for the activity test is the chicken embryo incubated on the 9th day. 300 pieces of 280*280 images of the ROI of the 9-day chicken embryo are randomly selected, 200 were used for training, and the remaining 100 were used for testing. Adjust parameters in SVM, set BoxConstraint and KernelScale parameter to super parameter optimization, and the results of different KernelFunction classification are shown in table 1 and table 2. Where, accuracy rate, error rate and omission rate are defined as:

\[
\text{Accuracy} = \frac{\text{the number of correctly classified images}}{\text{the total number of images in the test set}} \times 100\%
\]

\[
\text{Error rate} = \frac{\text{the number of living identified as dead}}{\text{the total number of images in the test set}} \times 100\%
\]

\[
\text{Omission rate} = \frac{\text{the number of dead identified as living}}{\text{the total number of images in the test set}} \times 100\%
\]

Table 1. The First classification (no skeleton curvature feature introduced)

| KERNELFUNCTION | ACCURACY (%) | OMISSION RATE (%) | ERROR RATE (%) |
|----------------|--------------|------------------|---------------|
| RBF            | 95           | 3                | 2             |
| Linear         | 94           | 3                | 3             |
| Gaussian       | 96           | 3                | 1             |

Table 2. The second classification (introducing the curvature feature of skeleton)

| KERNELFUNCTION | ACCURACY (%) | OMISSION RATE (%) | ERROR RATE (%) |
|----------------|--------------|------------------|---------------|
| RBF            | 97           | 0                | 3             |
| Linear         | 99           | 0                | 1             |
| Gaussian       | 98           | 0                | 2             |

By comparing the results in Table 1 and Table 2, it was found that the average omission rate of dead embryos was up to 3% when the texture features and the proportion of blood vessel area were used as the feature vectors for classification. In actual vaccine preparation, dead embryos will affect the vaccine quality of other healthy embryos. Introduced in the feature vector backbone curvature characteristic value, three different kernel function of the average classification accuracy is 98%, the linear kernel support vector machine (SVM) classification accuracy reached 99%, omission rate were reduced to zero, has reached the vaccine preparation of chicken embryos into active detection requirements: allow live embryo false negatives for dead embryo to be eliminated, do not allow the dead embryo wrongly for live embryo into the next phase of training.

4. Conclusion
The experimental results show that the matched filter used in this paper can segment the main blood vessels in the living embryo well and ensure the area proportion of blood vessels. Moreover, the matched filter can separate the spot noise from the background, the skeleton of the spot noise is closer to the circle, and the blood vessel and the spot can be distinguished by calculating the bending degree of the skeleton. Several features were used to classify the images of chicken embryos to minimize the probability of dead embryos being misjudged as living embryos. The method in this paper is not
limited to single features, and is not affected by image brightness, number of light spots and shadow of egg embryo image. It can quickly and accurately detect the activity of chicken embryo, which not only ensures that there is no wrong judgment of dead embryo, but also achieves high accuracy on this basis.

References
[1] Li Yonghong, Xiao Yongzhen. Quality control of preparation of inactivated avian influenza vaccine [J]. Poultry Husbandry and Disease Control, 2018, 12:29-31.
[2] Romanoff A L. Detection of fertility in fresh eggs [J]. Worlds Poultry Science Journal, 1947, 3(1): 12-14.
[3] Schellpfeifer M A, Kolesari G L. Microbubble contrast imaging of the cardiovascular system of the chick embryo [J]. Ultrasound in Medicine and Biology, 2012, 38(3): 501-510.
[4] Pan Leiqing, Tu Kang, Su Zipeng, et al. Crack detection in eggs using computer vision and BP neural network [J]. Transactions of the CSAE, 2007, 23(5): 154-158.
[5] LI XinCheng, Zhao DengLu, Shi HongLei, et al. Non-destructive testing method of egg quality based on machine vision [J]. Journal of Food Safety and Quality, 2019, 10(2): 489-493.
[6] Zhang Wei, Tu Kang, Liu Peng, et al. Early fertility detection of hatching duck egg based on fusion between computer vision and impact excitation [J]. Journal of Agricultural Machinery, 2012, 43(2): 140-145.
[7] Yang Jian, Shi Ying, Liu Haiyan, et al. Unfertilized eggs verification system based on DSP system and fuzzy neural networks [J]. Journal of Chinese Agricultural Mechanization, 2014, 35(5): 175-178.
[8] Shan Baoming. Hatching egg fertility detection in vaccine preparation based on machine vision [J]. Journal of Agricultural Machinery, 2010, 41(5): 178-181.
[9] Liu Chuanlai, Hu Jinguo. Non-destructive detection of the survival of inoculated SPF eggs in vaccine production [J]. China Science Paper, 2013, 8(7): 711-716.
[10] Huang Chao, Liu Yancong. Experiment on Detection Method of Vaccine Strain Activity [J]. Journal of Agricultural Machinery, 2017, 48(10): 300-306.
[11] Chaudhuri S, Chatterjee S, Katz N, et al. Detection of blood vessels in retinal images using two-dimensional matched filters [J]. IEEE Transactions on Medical Imaging, 1989, 8(3):263-269.
[12] Fan Linlin, Cheng Yun, Tian Xiaobing. Retinal blood vessel segmentation based on matched filtering and morphological processing [J]. Computer Knowledge and Technology, 2019, 17(15):188-190.
[13] Zhang Ye, Zhang Yongde, Sha Xianzheng. Retinal vessel segmentation based on multiscale matched filtering [J]. Chinese Journal of Medical Instrumentation, 2020, 44(2):108-112.
[14] Diao Zhihua, Wu Beibei, Wu Yuanyuan, et al. Application research of skeleton extraction algorithm based on image processing [J]. Computer Science, 2016, 43(6A):232-235.
[15] Lu Tieding, Deng Xiaoyuan, Analysis of total least squares algorithms for circular curve fitting [J]. Science of Surveying and Mapping, 2019, 44(2): 33-37.
[16] Zhao Shuang, LI Yanjun, MA Zhiqing, et al. An analysis of texture features of breast pathology image based on gray scale co-occurrence matrix [J]. China Medical Equipment, 2018, 15(8):5-8.