

ACE-CCO: A Two-Stage Deep Learning Method for Automatic Concentration Estimation of Chicken Coccidia Oocysts

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Abstract. Chicken coccidiosis is a common and serious parasitic disease in poultry, and vaccination is an effective preventive measure. To ensure the quality of vaccine products, it is critical to accurately identify chicken coccidia species and count the number of oocysts. Currently, the most commonly used method relies on experienced professionals performing morphological identification and manual counting under a microscope. This process is highly subjective and often leads to inconsistent counting results. Although deep learning-based coccidia automatic counting methods have improved efficiency and stability, further enhancement in accuracy and process automation is still needed. To address these challenges, this paper proposes ACE-CCO, a two-stage deep learning method for automatic concentration estimation of chicken coccidia oocysts in vaccines. First, an image processing-based frame line recognition algorithm (FLRA) is designed to accurately locate the counting area in the hemocytometer. Next, a two-stage deep learning counting algorithm is developed to enhance counting accuracy. Finally, user-friendly software is developed to support one-click batch counting and statistical analysis, improving process automation. Comparative experiments with manual counting in real vaccine assessment scenarios show that the mean relative percentage difference (MRPD) of ACE-CCO for six chicken coccidia types is below 1.5%, and the counting speed is more than six times faster. The results demonstrate the superior accuracy, efficiency, stability, and practicality of ACE-CCO, greatly reducing reliance on operator experience and providing reliable technical support for vaccine quality assessment.

Keywords: Chicken coccidiosis, Parasitic disease, Automatic concentration estimation, Vaccine quality assessment.

1 Introduction

Coccidiosis is a parasitic disease caused by *Eimeria* protozoan parasites, posing a serious threat to the global poultry industry [1]. The disease is widespread, significantly impairing poultry health and imposing a substantial economic burden, with annual global costs estimated at approximately £10.4 billion [2]. Therefore, effective

prevention and control of coccidiosis are crucial for promoting the sustainable development of the poultry industry and ensuring economic benefits.

In order to ensure that the quality of the produced vaccine meets the standards, the chicken coccidia oocysts in the vaccine must be accurately identified and precisely counted to assess whether their concentration and sporulation rate comply with production requirements. Currently, the most commonly used analysis method is the hemocytometer method [3], which relies on skilled technicians to perform morphological identification and manual counting of oocysts under a microscope (as shown in **Fig. 1**). However, this method is time-consuming and labor-intensive, making it difficult to meet the practical demands of high-throughput counting. Moreover, it is highly dependent on the expertise of the operator, leading to considerable variability in counting results among different technicians, which compromises the stability and repeatability of the counting results [4, 5].

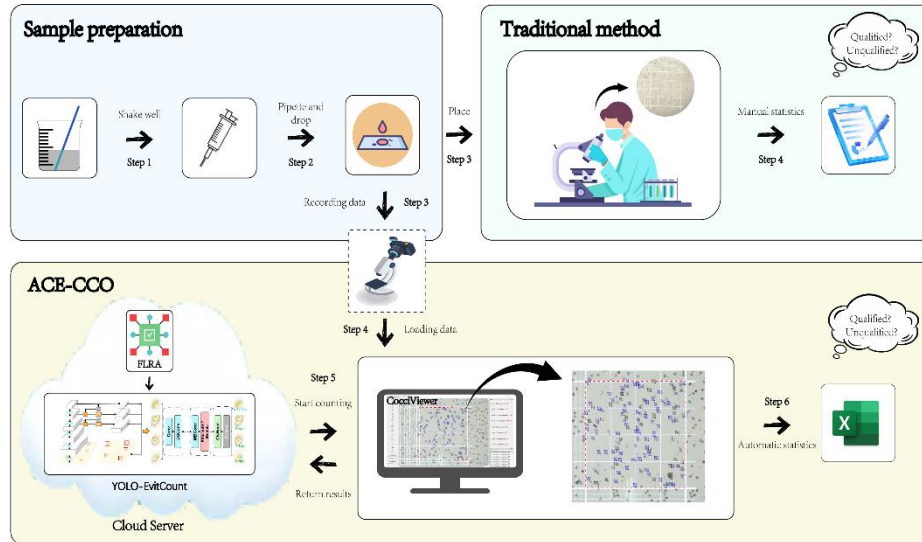


Fig. 1. ACE-CCO vs. traditional methods. ACE-CCO: a method for automatic concentration estimation of chicken coccidia oocysts; FLRA: a frame line recognition algorithm for accurately locating the counting area; YOLO-EvitCount: a two-stage algorithm for counting chicken coccidia oocysts; CocciViewer: a software tool for automated counting, statistical analysis, and visualization of chicken coccidia oocysts.

In recent years, researchers have begun using automated flow cytometers [6, 7] and spectrophotometry [8] to count chicken coccidia oocysts in feces. However, both methods have limitations: the former involves high instrument costs and complex operational procedures, while the latter cannot distinguish between different types of oocysts. Consequently, researchers have explored approaches based on digital image processing [9, 10]. Although this method demonstrates potential for low-cost and automated counting, its accuracy in differentiating similarly sized oocysts from debris can be limited, potentially leading to biased results.

With the rapid development of computer vision, deep learning-based image segmentation technology has been proven feasible for chicken coccidia counting. [11] employed the PIPER instrument, which combines the U-Net and StarDist models, to achieve automatic identification and counting of three species of *Eimeria* oocysts in fecal samples. [12] applied Mask R-CNN [13] to count three *Eimeria* species in fecal samples and effectively distinguish between sporulated and unsporulated oocysts. However, these methods often count all oocysts present in microscopic images without incorporating actual volume information, making it difficult to accurately estimate oocyst concentrations. Furthermore, they mainly focus on oocyst counts in fecal samples and lack the adaptability and pertinence to the oocyst counts in vaccine scenarios.

In recent years, deep learning-based object detection technology has demonstrated significant advantages in automated counting. In particular, the YOLO series of models [14, 15, 16], with their fast inference speed and high recognition accuracy, have been widely applied to various biomedical image analysis tasks. For example, [17] used an improved YOLOv11 model, trained on 11 white blood cell categories, and applied it to a web application for counting. Experimental results show that this method significantly improves counting accuracy and reduces the time required for manual counting. [18] developed a customized deep learning architecture, YOLO-mp, to detect and count malarial parasites in microscopic images. These studies provide valuable technical references for the development of automatic counting methods for chicken coccidia. However, current research on object-detection-based automatic counting of chicken coccidia is still relatively scarce, and further in-depth exploration and optimization are still required.

In summary, although existing deep learning-based automatic counting methods for chicken coccidia have made some progress, they still exhibit limitations in practical applications. First, most studies focus on the total number of chicken coccidia oocysts in microscopic images and cannot further infer their concentration. However, in practical vaccine quality assessment, it is necessary to estimate the concentrations of both sporulated and unsporulated oocysts. Therefore, relying solely on detection and counting methods is insufficient to meet production requirements. Second, existing object detection algorithms still exhibit a high misidentification rate when distinguishing between sporulated and unsporulated chicken coccidia oocysts. Finally, current automatic oocyst concentration estimation methods still have room for improvement in terms of operational simplicity and the level of automation. To address the above issues, this paper proposes a two-stage deep learning method for automatic concentration estimation of chicken coccidia oocysts (ACE-CCO). The method employs a two-stage deep learning framework, consisting of a detection network and a classification network, aiming to achieve efficient counting and concentration estimation of oocysts in vaccine production environments, thereby facilitating the automation of quality assessment processes.

The main contributions of this study are as follows:

1. We constructed a dataset of six chicken coccidia species, tailored for vaccine environments, which includes both sporulated and unsporulated morphologies for each species.

2. To estimate oocyst concentration in vaccines, we designed a hemocytometer frame line recognition algorithm (FLRA) for precise area localization. Building on this, we proposed the YOLO-EvitCount algorithm, based on deep learning, for efficient and accurate counting of chicken coccidia oocysts within the defined area.
3. To improve the automation of vaccine quality assessment, a software tool called CocciViewer has been developed to facilitate automated batch counting and statistical analysis.
4. Comparative experiments with manual counting show that ACE-CCO achieved a mean relative percentage difference (MRPD) below 1.5% across all chicken coccidia species and was at least six times faster. This method provides effective technical support for the quality assessment of chicken coccidia vaccines and serves as a valuable reference for the automatic estimation of oocyst concentrations in other parasites.

The rest of this paper is organized as follows: Section 2 reviews the basic principles of the hemocytometer and the rules for manual counting; Section 3 introduces the construction process of the chicken coccidia dataset and details ACE-CCO in this paper; Section 4 presents the experimental results, comparing the automatic counting method with manual counting, followed by an in-depth analysis of the results; Section 5 discusses the study's limitations; Section 6 summarizes the main contributions of this paper.

2 Counting principle of hemocytometer

The hemocytometer consists of two counting chambers with a depth of 0.1 mm. Each chamber is divided into nine squares with an area of 1 mm^2 , so the volume of each square is 0.1 mm^3 , as shown in **Fig. 2**. Let the number of oocysts in square A be N_a , then the solution concentration C_a corresponding to the square can be expressed as:

$$C_a = N_a \times 10 \times 1000 = N_a \times 10^4 \quad \text{oocysts/mL} \quad (1)$$

Since the volume of each grid is 0.1 mm^3 , multiply the number of oocysts in the grid by 10 to convert it to the number of oocysts per 1 mm^3 , and then multiply this value by 1000 to get the oocyst concentration in 1 mL of solution.

As shown in **Fig. 2**, the border of the orange square A is composed of three engraved lines, of which the central engraved line is used as the counting boundary. Oocysts within the dividing line and oocysts tangent to the left or upper dividing line are included in the count; while oocysts tangent to the right or lower dividing line are excluded. Therefore, the solid blue ellipse and the dotted red ellipse represent the counted and uncounted oocysts, respectively.

In the actual manual counting process, the number of sporulated oocysts in four counting grids (area A in **Fig. 2**) of each chamber are recorded according to the hemocytometer counting rules and summed to obtain the total number N_i in the chamber. The oocyst concentration in the vaccine solution is then estimated by averaging these values. To improve the accuracy of concentration estimation, it is usually necessary to

sample multiple times, count the number of sporulated oocysts in multiple chambers, and take the average value to obtain the final oocyst concentration C . The sporulation rate S is obtained by calculating the ratio of the number of sporulated oocysts N_s in all counting chambers to the total number of oocysts N . The specific calculation formula is as follows:

$$C = \frac{1}{4t} \sum_{i=1}^t N_i \quad (\times 10^4 \text{ oocysts/mL}) \quad (2)$$

$$S = \frac{N_s}{N} \times 100\% \quad (3)$$

Where N_i is the count in the i th chamber, and t represents the number of sampling times. The factor 10^4 converts from chamber geometry to per-milliliter units. In this study, the results presented in the tables and figures are expressed in units of 10^4 oocysts/mL.

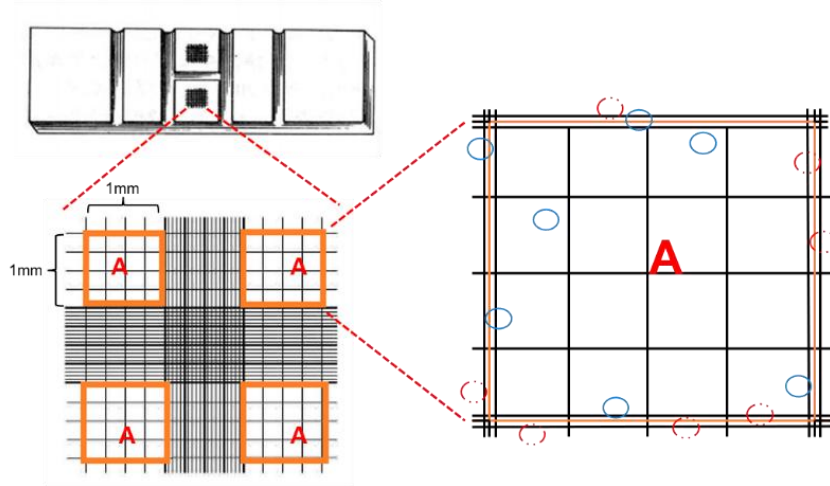


Fig. 2. Counting areas and counting rules of hemocytometer. The orange box in the figure is the counting area, and only the solid ovals are counted.

3 Material and methods

3.1 Data acquisition

Preparation of vaccine samples. FOSHAN STANDARD BIO-TECH CO., LTD. provides purified single-species chicken coccidia oocyst suspensions, covering six species: *E. maxima*, *E. tenella*, *E. acervuline*, *E. necatrix*, *E. brunetti* and *E. mitis*. All samples were verified by triple means: oocyst morphology, lesion observation of infected chickens and PCR detection. All samples were transported refrigerated under wet ice conditions and stored at 4°C . The samples used in the counting comparison experiments were distinct from those used for model training.

Image acquisition. Images of chicken coccidia were acquired using a SONY EXMOR CMOS sensor camera mounted on a biological microscope (EX20) at a magnification of 100 $\times$. In total, 293 JPG images with a resolution of 5440 $\times$ 3648 pixels were obtained for subsequent annotation and model training. Among them, 48 images were collected for each of *E. brunetti*, *E. acervulina*, *E. necatrix*, *E. maxima*, and *E. tenella*, whereas 53 images were collected for *E. mitis*.

Image annotation. The six chicken coccidia species were annotated into a total of 12 categories: A-spo, A-nonSpo, N-spo, N-nonSpo, M-spo, M-nonSpo, T-spo, T-nonSpo, B-spo, B-nonSpo, Mi-spo, and Mi-nonSpo, which represent the sporulated and unsporulated morphologies of each chicken coccidia species, respectively. Examples of labeled categories are shown in **Fig. 3a-l**.

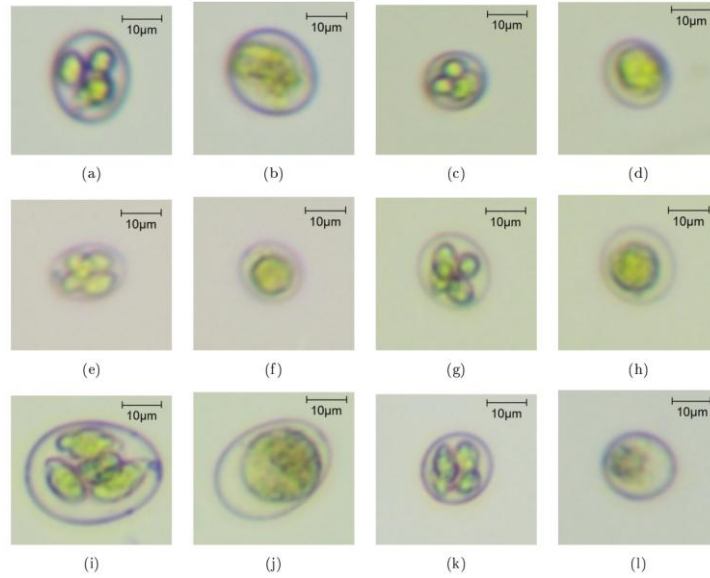


Fig. 3. Examples of chicken coccidia oocysts for each labeled category: (a) sporulated *E. brunetti* oocysts; (b) unsporulated *E. brunetti* oocysts; (c) sporulated *E. mitis* oocysts; (d) unsporulated *E. mitis* oocysts; (e) sporulated *E. acervulina* oocysts; (f) unsporulated *E. acervulina* oocysts; (g) sporulated *E. necatrix* oocysts; (h) unsporulated *E. necatrix* oocysts; (i) sporulated *E. maxima* oocysts; (j) unsporulated *E. maxima* oocysts; (k) sporulated *E. tenella* oocysts; (l) unsporulated *E. tenella* oocysts.

Data pre-processing. Before training the detection network, a slice-based data augmentation strategy was applied to the dataset. Specifically, the original images were divided into multiple non-overlapping 640 $\times$ 640 pixel sub-regions. For chicken coccidia oocysts truncated during the slicing process, only those for which the oocyst area within a sub-region exceeded 60% of the original oocyst area were retained, ensuring that the network could learn sufficiently complete and discriminative oocyst details.

3.2 ACE-CCO

FLRA. The automatic counting method in this study relies on the accurate recognition of the hemocytometer counting area (area A in **Fig. 2**). Due to variations in illumination intensity and hemocytometer background color during image acquisition, the image background color is inconsistent. In addition, images may be tilted or affected by impurities, which further increases the difficulty of accurate recognition. To this end, we designed a two-stage FLRA based on image processing to effectively address the above challenges. The overall workflow of the algorithm is shown in **Fig. 4**. The core of the algorithm lies in accurately extracting the four corner points of the counting area. Corner point extraction consists of two main stages: rough recognition and refined recognition.

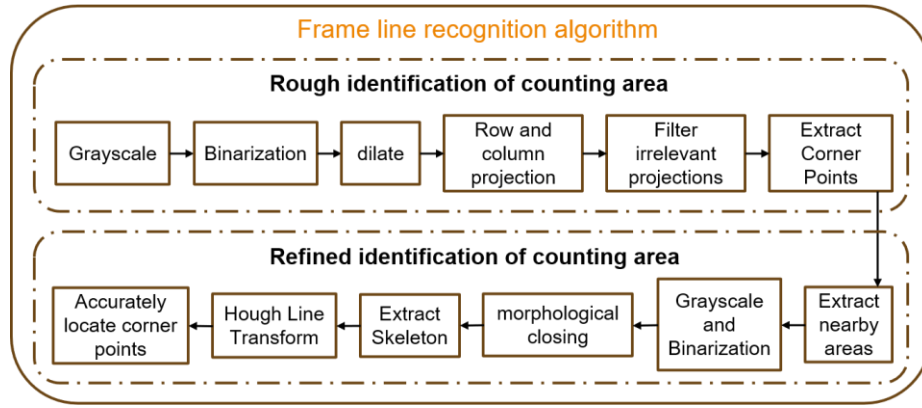


Fig. 4. The overall workflow of FLRA.

In the rough recognition stage, the input image is first converted to grayscale and binarized. Subsequently, broken frame lines caused by noise or interference are repaired using a morphological dilation operation. Next, horizontal and vertical projections are applied to the dilated image to extract the distribution characteristics of straight lines, and projection values below a predefined threshold are filtered out. Since the frame lines appear as continuous thick bands in the projection profiles, these continuous projection indices are grouped, and the midpoint of each group is extracted. Based on prior knowledge, the side length of the small squares in the hemocytometer's A area (in **Fig. 2**) exceeds 400 pixels, the four corner points of the counting area are roughly determined, as indicated by the blue dots in **Fig. 5**.

In the refined recognition stage, we further perform local corrections on the roughly located corner points. Taking the upper-left corner point as an example, a local sub-image with a side length of 100 pixels is extracted from its center (as shown in **Fig. 6a**), followed by grayscale conversion and binarization are performed. A morphological closing operation is applied to fill holes in the local area and connect broken contours, thereby enhancing edge connectivity. Subsequently, a skeletonization is applied to the image after the closing operation to extract the one-pixel wide center line of the frame line (as shown in **Fig. 6b**). Finally, the Hough transform is used to detect the straight

line structure in the local area, and the intersection with the densest white pixels near the centerline is calculated. This intersection represents the precise corner point coordinate, as shown by the red dot in **Fig. 6c**.

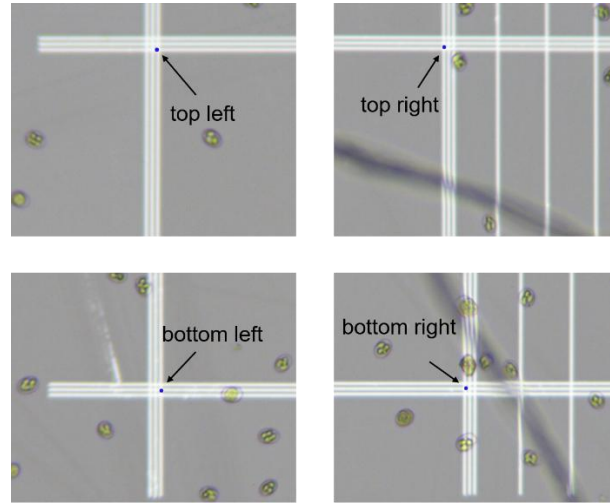


Fig. 5. Roughly identified four corner points (top left, top right, bottom left, bottom right).

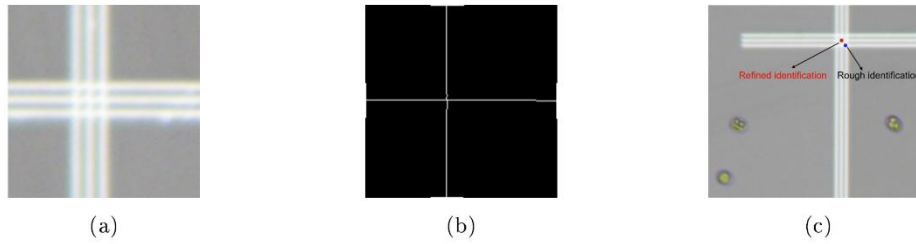


Fig. 6. Refined identification of counting area: (a) 100-pixel area around the upper left corner; (b) Skeletonized image; (c) Refine the identification of the upper left corner point (indicated by the red dot).

YOLO-Evit network. YOLOv8 [19] is a single-stage object detection model that predicts the location and category of objects through direct regression. It offers high detection efficiency and fast processing speed. Therefore, YOLOv8n was adopted as the network for chicken coccidia oocyst localization. However, for chicken coccidia oocyst counting, the classification performance of this model is relatively limited; thus, it is primarily used as a localization framework.

EfficientViT [20] is an image classification network based on a lightweight multi-scale attention mechanism, offering both high accuracy and excellent inference speed. Given these advantages, it was employed for the classification of chicken coccidia in this study.

YOLO-EvitCount algorithm. The overall workflow of the YOLO-EvitCount algorithm is shown in **Fig. 7**, which mainly includes two stages: a preprocessing stage and a counting stage.

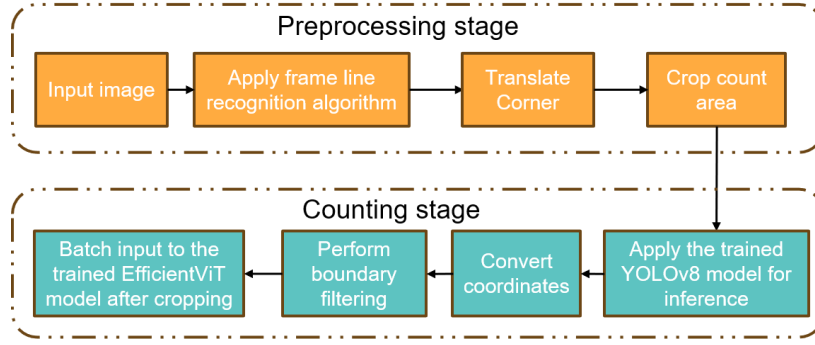


Fig. 7. The overall process of the YOLO-EvitCount algorithm.

In the preprocessing stage, the FLRA is first used to accurately determine the coordinates of the four corner points of the counting area. Subsequently, the upper-left corner is shifted 100 pixels to the left and upward, and the lower-right corner is shifted 50 pixels to the right and downward, respectively, to ensure that oocysts near the border are fully captured. Based on the expanded coordinates of the upper-left and lower-right corners, the rectangular counting area is cropped from the original image. Since the hemocytometer frame lines may be tilted in the image, this cropping method better preserves the spatial position information of the oocysts in the original resolution image, facilitating subsequent visualization in the user interface.

In the counting stage, the trained YOLOv8 model is first used to infer the cropped image and detect the locations of all oocysts. The detected coordinates are then mapped back to the original resolution. According to the counting rules, oocysts that touch the right or lower borders, or exceed the image boundaries, are excluded. Next, the filtered oocyst regions are cropped individually and batch-input into a trained EfficientViT classification model to identify their specific types. Finally, the spatial coordinates and category information of these oocysts are sent to CocciViewer for visualization and statistical analysis.

CocciViewer. To facilitate the use of the proposed counting algorithm and enhance automation and user convenience in vaccine quality assessment, we developed an interactive software tool, CocciViewer, with its main interface shown in **Fig. 8**. The software allows users to select the chicken coccidia species for counting via the front-end interface. By simply clicking the "Auto Count (Batch)" button and selecting the image directory, users can automatically invoke the YOLO-EvitCount algorithm to batch count multiple images and display the results visually, significantly improving the automation of vaccine quality assessment. Once counting is complete, users can click the "exportExcel" button to efficiently export the results for further analysis. The overall counting and statistical process is shown in **Fig. 9**.

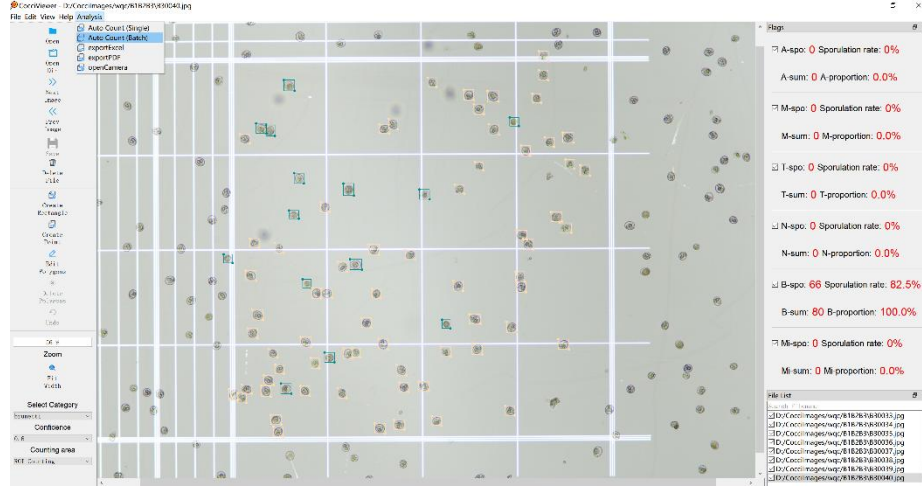


Fig. 8. Main Interface of CocciViewer.

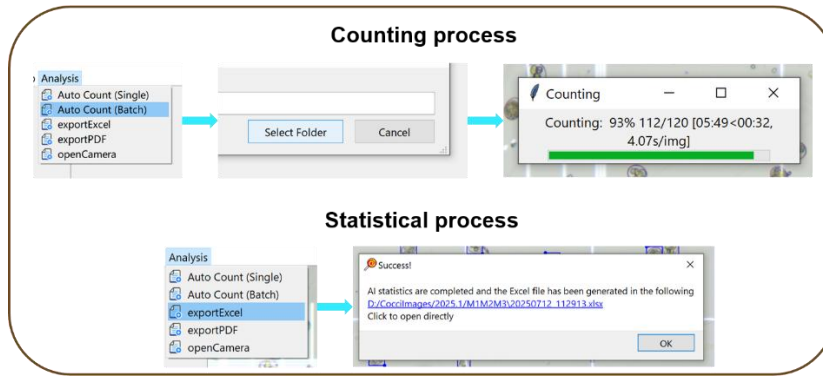


Fig. 9. Counting and statistics process.

4 Experimental results and analysis

4.1 Experiment setup and evaluation metrics

Experiment setup. In the counting comparison experiment, three samples were selected for each type of chicken coccidia, which were taken from the upper, middle, and lower layers of the beaker containing the chicken coccidia oocyst suspension, respectively, and labeled as samples 1-3. Each sample was sampled 10 times to ensure the representativeness and stability of the experiment. Therefore, for each type of chicken coccidia, 15 hemocytometers, 30 chambers, and a total of 120 areas A (as shown in Fig. 2) were required to complete the manual counting of sporulated oocysts. The average of these counts was then used to estimate the concentration of the suspension (unit: 10,000/ml).

Evaluation metrics. To quantitatively evaluate the accuracy of our automatic counting method, three commonly employed evaluation metrics were used: Mean Absolute Error (MAE), Root Mean Squared Error (RMSE) and Mean Relative Percentage Difference (MRPD). The calculation formulas for each metric are provided below.

$$\text{MAE} = \frac{1}{N} \sum_{i=1}^N |\hat{y}_i - y_i| \quad (4)$$

$$\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^N (\hat{y}_i - y_i)^2} \quad (5)$$

$$\text{MRPD} = \frac{1}{N} \sum_{i=1}^N \frac{\hat{y}_i - y_i}{\frac{1}{2}(\hat{y}_i + y_i)} \times 100\% \quad (6)$$

Among them, $\hat{y}_i$ represents the predicted value from the counting algorithm, y_i is the true value from manual counting, and N represents the number of images.

4.2 Comparative experimental results with the single-stage method

To verify the superiority of the proposed YOLO-EvitCount algorithm, its counting performance was compared with single-stage inference models based on YOLOv8 and YOLOv11 [21]. The corresponding results are presented in **Table 1**.

Table 1. Comparison of concentration counting errors

Species	Method	MRPD (%)	Avg. Time (s/img)
<i>E. acervulina</i>	YOLOv8n	1.83	4.03
	YOLOv11n	1.38	4.30
	YOLO-EvitCount(Ours)	0.68	4.85
<i>E. maxima</i>	YOLOv8n	1.18	4.28
	YOLOv11n	1.22	4.37
	YOLO-EvitCount(Ours)	1.17	4.88
<i>E. tenella</i>	YOLOv8n	1.29	4.22
	YOLOv11n	0.76	4.34
	YOLO-EvitCount(Ours)	-0.63	4.96
<i>E. necatrix</i>	YOLOv8n	2.56	4.96
	YOLOv11n	2.71	4.15
	YOLO-EvitCount(Ours)	1.24	4.67
<i>E. brunetti</i>	YOLOv8n	1.22	4.26
	YOLOv11n	1.45	4.68
	YOLO-EvitCount(Ours)	0.85	4.71
<i>E. mitis</i>	YOLOv8n	-0.56	3.94
	YOLOv11n	-0.55	3.99
	YOLO-EvitCount(Ours)	-0.06	3.68

As shown in the table, YOLO-EvitCount achieves the best overall MRPD performance across the six chicken coccidia species. In particular, for *E. necatrix*, the error of YOLO-EvitCount is 1.32% lower than that of YOLOv11n. Although YOLO-EvitCount has a slightly higher average counting time than other methods, tests on the RTX 4090D platform (**Table 5**) show that the time gap among methods is significantly reduced, decreasing from 0.82 s to 0.08 s compared with the fastest YOLOv8n.

4.3 Comparison experimental results with manual methods

To verify the effectiveness of ACE-CCO, we conducted a comparative experiment with the manual counting method in a real vaccine quality assessment environment. The results are presented in **Table 2**.

Table 2. Comparison of concentration-counting errors between the automatic method and manual counting for six chicken coccidia species across various samples.

Species	Sample ID	MAE ↓	RMSE ↓	MRPD (%)
<i>E. acervulina</i>	Sample 1	1.35	1.63	0.66
	Sample 2	1.40	1.87	0.36
	Sample 3	1.30	1.72	1.01
	Sample 1-3	1.35	1.74	0.68
<i>E. maxima</i>	Sample 1	0.95	1.41	0.57
	Sample 2	1.07	1.54	0.63
	Sample 3	2.20	2.66	2.31
	Sample 1-3	1.48	2.05	1.17
<i>E. tenella</i>	Sample 1	2.27	3.52	-1.89
	Sample 2	1.10	1.64	-0.34
	Sample 3	0.97	1.33	0.32
	Sample 1-3	1.45	2.37	-0.63
<i>E. necatrix</i>	Sample 1	1.70	2.04	0.68
	Sample 2	1.57	1.98	0.71
	Sample 3	2.58	3.19	2.33
	Sample 1-3	1.95	2.47	1.24
<i>E. brunetti</i>	Sample 1	0.68	1.01	0.53
	Sample 2	1.25	1.60	1.13
	Sample 3	1.00	1.52	0.89
	Sample 1-3	0.97	1.40	0.85
<i>E. mitis</i>	Sample 1	0.65	1.16	-0.38
	Sample 2	0.57	0.79	-0.35
	Sample 3	0.75	1.18	0.54
	Sample 1-3	0.66	1.06	-0.06

As shown in the table, the overall MRPD values of the YOLO-EvitCount algorithm for the six chicken coccidia species—*E. acervulina*, *E. maxima*, *E. tenella*, *E. necatrix*, *E. brunetti* and *E. mitis*—are 0.68%, 1.17%, -0.63%, 1.24%, 0.85% and -0.06%, respectively. Among them, *E. mitis* exhibits the smallest error, with an underestimation of only 0.06%. The overall MAE is controlled within 2, with *E. mitis* achieving the lowest MAE of 0.66. The overall RMSE for all species range from 1.06 to 2.47, all of which are below the acceptable error threshold of 3.

We further compared the sporulation rate results of each chicken coccidia species with those obtained by manual counting, as shown in **Table 3**. As indicated in the table, the sporulation rate of all six chicken coccidia species exhibit errors of less than 1% compared with manual counting. These results demonstrate that the YOLO-EvitCount algorithm exhibits good robustness across different chicken coccidia species, thereby verifying the effectiveness of ACE-CCO.

Table 3. Comparison of manual and automatic counting results for the sporulation rates of six chicken coccidia species.

Species	Manual counting (%)	Automatic counting (%)
<i>E. acervulina</i>	80.57	80.83
<i>E. maxima</i>	81.34	82.20
<i>E. tenella</i>	93.26	92.55
<i>E. necatrix</i>	91.63	92.41
<i>E. brunetti</i>	86.65	87.44
<i>E. mitis</i>	90.18	89.97

4.4 Ablation Study

To verify the effectiveness of each component of the proposed YOLO-EvitCount algorithm, a series of ablation experiments were conducted, and the results are presented in **Table 4**.

Table 4. Ablation experiments on *E. acervulina* (the YOLOv8 model includes rough recognition of FLRA).

YOLOv8	FLRA	EfficientViT	MRPD (%)
✓			2.18
✓	✓		1.53
✓	✓	✓	0.68

Here, YOLOv8 denotes the model obtained by removing the EfficientViT network and the refined recognition FLRA module from YOLO-EvitCount. As shown in the table, the concentration estimation error reaches its lowest level after introducing the refined recognition FLRA and the EfficientViT network, indicating that each module enhances the overall performance of the algorithm and thereby validating the effectiveness of the proposed YOLO-EvitCount.

Table 5. Comparison of Counting Efficiency of Different Methods for *E. acervulina* (RTX 4090D). Avg. Time indicates the average time required to process and return the counting result per image.

Method	MRPD (%)	Avg. Time (s/img)
YOLOv8n	1.83	0.58
YOLOv11n	1.38	0.60
YOLO-EvitCount(Ours)	0.68	0.66

4.5 Correlation analysis

Fig. 10 shows the correlation between automated counting (ACE-CCO) and the manual counting results. As shown in the figure, for each chicken coccidia species, the correlation coefficient r exceeds 0.98, indicating a very strong linear relationship between the two counting methods. These results further confirm the reliability and accuracy of ACE-CCO.

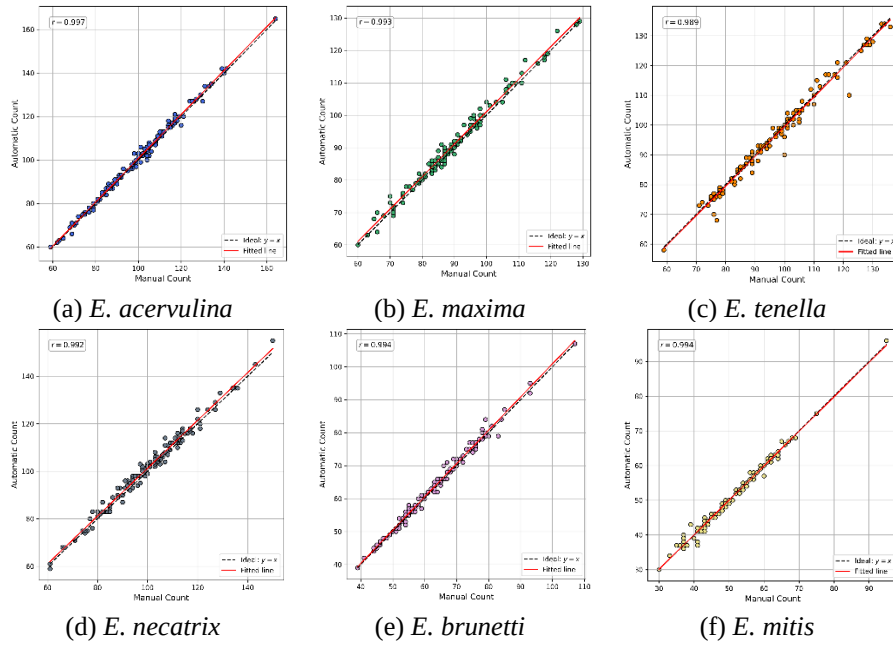


Fig. 10. Pearson correlation scatter plots between automated and manual counts.

4.6 Bland–Altman analysis

Fig. 11 presents the Bland–Altman plot comparing automated counting (ACE-CCO) with manual counting results. As shown in the figure, most differences fall within the 95% limits of agreement, indicating good consistency between the two methods.

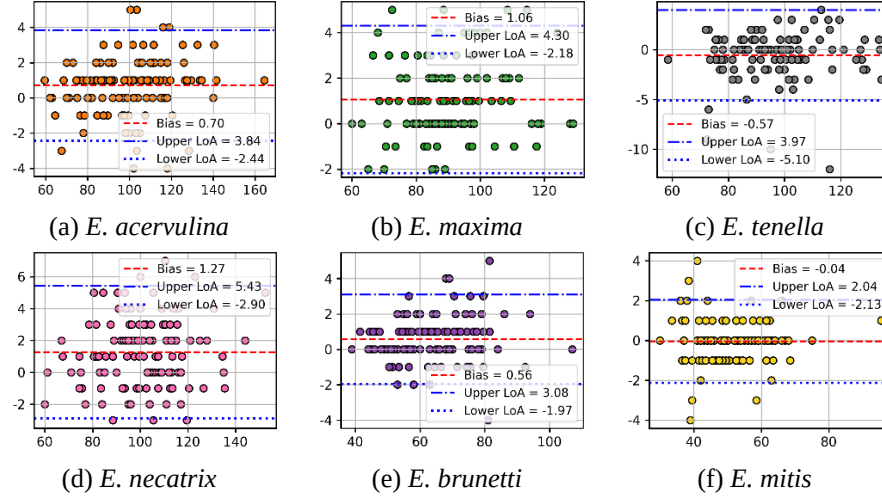


Fig. 11. Bland–Altman plot of automated vs. manual counts for six chicken coccidia species. In the figure, the red dashed line indicates the mean bias, and the blue dashed lines represent the 95% limits of agreement (bias $\pm$ 1.96 SD).

4.7 Counting efficiency analysis

We further compared the counting efficiency of ACE-CCO with manual counting using *E. tenella* sample 1, and the results are summarized in **Table 6**. As shown in the table, manual counting required a total of 27.97 minutes, with an average of 5.6 minutes per hemocytometer.

In contrast, the proposed automatic counting method based on YOLO-EvitCount completed the entire process in approximately 4.3 minutes. This included 3.3 minutes for automated counting (4.96 s per image for a total of 40 images) and 1 minute for image acquisition. Notably, in the automated workflow, image acquisition and counting can be performed in parallel; therefore, photographing five hemocytometers required only 1 minute in total.

Overall, the proposed method achieved a speed improvement of more than sixfold compared with manual counting. These results further demonstrate that, while maintaining counting accuracy, ACE-CCO substantially enhances counting efficiency, highlighting its strong potential for practical application in vaccine quality assessment.

Table 6. Comparison of counting times between manual and automated methods.

Method	Total Oocysts	Total Hemocytometers	Counting Time (s)
Manual	3894	5	1678
Automated	3894	5	259

5 Discussion

In the vaccine production process, accurate assessment of vaccine quality is a critical step. One important aspect is the accurate counting of chicken coccidia oocysts in the vaccine solution to estimate their concentration. Currently, the most commonly used manual counting method relies on experienced and professionally trained experts for identification and enumeration, which is highly subjective and prone to human error. Previous deep learning-based chicken coccidia counting methods have mostly focused on identifying and counting all oocysts in microscopic images, making it difficult to further infer the actual oocyst concentration in the sample. In addition, existing automatic counting methods still have room for improvement in terms of operational workflow, counting accuracy, and automation. To address these issues, this study collected image data of six chicken coccidia species under vaccine environment and proposed ACE-CCO, aiming to achieve efficient counting and concentration estimation of oocysts in vaccine quality assessment. Furthermore, the proposed method was compared with manual counting in a real vaccine assessment scenario. The results demonstrate that ACE-CCO significantly improves counting efficiency while maintaining high accuracy, verifying its feasibility and effectiveness in practical applications.

To address the difficulty of accurately identifying the counting area caused by variations in image background color and frame line tilt during image acquisition, this paper proposes a two-stage FLRA to improve the robustness of counting area localization. On this basis, we constructed the YOLO-EvitCount algorithm to achieve high-precision identification and counting of oocysts within the counting area. To further enhance user convenience and efficiency in vaccine quality assessment, this study developed CoccViewer based on the PyQt5 framework. The tool supports automatic batch counting and statistical analysis for multiple chicken coccidia species, enabling operators to efficiently complete the assessment process.

ACE-CCO offers advantages such as simple operation and low learning cost. Operators can perform other tasks during the counting process, thereby significantly improving production efficiency. At present, ACE-CCO has been deployed in actual vaccine production, has been applied to the concentration assessment of multiple vaccine batches, and has processed more than 10,000 microscopic images, demonstrating robust and reliable performance. Although it has demonstrated good practicality, feedback from operators indicates that ACE-CCO still has certain limitations, as follows: (1) In the hemocytometer used in this study, the number of chicken coccidia oocysts contained in each counting area is limited, which may reduce representativeness when estimating high concentrations. (2) When encountering oocysts with a high degree of sporulation (oversporulated oocysts), the automatic counting algorithm may misclassify them as sporulated oocysts, leading to overestimation. In manual counting (e.g., for *E. maxima*), such oocysts are typically excluded.

In the future, we will continue to expand the adaptability of this method to a wider range of hemocytometers to enhance the representativeness of concentration assessment. In addition, we will further explore high-performance classification models to provide more efficient and precise solutions for the quality assessment of chicken coccidia vaccines.

6 Conclusions

This study proposed ACE-CCO, a two-stage deep learning method for automatic concentration estimation of chicken coccidia oocysts. ACE-CCO incorporates a custom-designed FLRA for accurate identification of the counting area, and builds the YOLO-EvitCount algorithm on this basis to achieve efficient identification and accurate counting of chicken coccidia oocysts. To improve the automation of counting and enhance user convenience, the software tool CocciViewer was developed. It enables one-click counting operations and provides statistical analysis functions for estimating oocyst concentration.

In a real vaccine assessment environment, we conducted a comparative experiment between the proposed method and manual counting. The results showed that the overall MRPD error of ACE-CCO for six chicken coccidia species was less than 1.5%, and the counting efficiency was more than six times higher than that of manual counting. Additionally, visualization of the Pearson correlation scatter plot further demonstrated a strong linear correlation between the automatic counting and the manual counting.

In summary, this method achieves high counting accuracy and substantial efficiency gains, delivering an automated and reliable technical solution for quality assessment in vaccines.

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