

**ANALYSIS, QUANTIFICATION AND IDENTIFICATION OF WORM EGGS  
UNDER A MICROSCOPE**

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**Abstract**

Millions of people throughout the world suffer from parasite infections. Traditionally, technicians use manual eye inspection of microscopic specimens to perform a parasite examination. However, manual operations have limitations that hinder the ability to obtain precise egg counts and cause inefficient identification of infected parasites on co-infections. The technician requirements for handling a large number of microscopic examinations in countries that have limited medical resources are substantial. We developed the helminth egg analysis platform (HEAP) as a user-friendly microscopic helminth eggs identification and quantification platform to assist medical technicians during parasite infection examination.

**Keywords:** Helminth; Parasite egg examination; Deep learning; Object detection; Web server; Database.

**Introduction**

More than 800 million people globally have been threatened by helminth infection.<sup>1</sup> Medically important nematodes, trematodes, and cestodes are prevalent among humans in developing countries, including sub-Saharan Africa, South America, and East Asia.<sup>2e4</sup> These diseases displayed a broad range of clinical manifestations from asymptomatic to malnutrition, anemia, and even cancer.<sup>5e8</sup> Although these infections rarely cause death, the elimination of pathogenic helminths offers substantial advantages of socioeconomics. Currently, microscopic examination of helminth eggs in fecal samples is the gold standard for clinical diagnosis.<sup>9e11</sup> A well-trained and experienced technician is responsible for the examination; however, the technician must take courses and comply with retraining protocols to establish and sustain professional skills. Diagnosis of helminth infections is usually a qualitative analysis. However, eggs per gram (EPG) in fecal samples are the basis to estimate the burden of a parasitic disease, which requires quantitative examination.<sup>4</sup> Multiple or mixed helminths infection in children is another public health issue in some endemic areas; thus, diagnosis should be more carefully performed to prevent false-negative results.<sup>12,13</sup> The quantitative and time-consuming determination has further increased the error rate. Therefore, an automatic identification system for helminth eggs would be beneficial in the fields of diagnostics and epidemiology. Identification and classification of microscopic images of helminth eggs based on computational algorithms have been proven to be workable. A pioneering



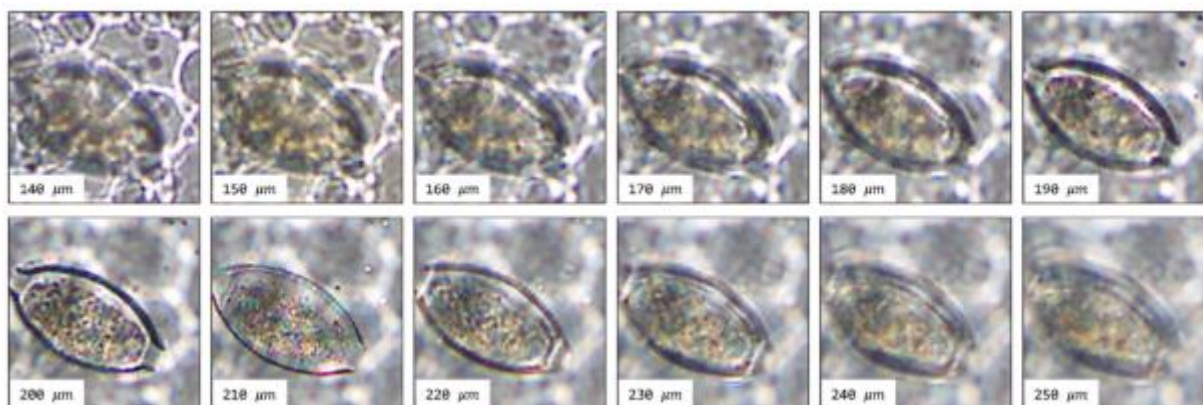
study was performed by Yang et al., in 2001. This work employed an artificial neural network (ANN) classifier to recognize parasite eggs by shape, shell smoothness, and size in microscope images.<sup>14</sup> Two subsequent studies established systems based on different filtering strategies on objects before their classification by the k-Nearest Neighbors (kNN) algorithm and profiles of grayscale-coloring spaces.<sup>15,16</sup> A mathematical algorithm proposed that the size, thickness, brightness distribution, circularity, and shapes of eggs were employed as criteria for parasites discrimination.<sup>17</sup> As artificial intelligence (AI) has flourished in recent years, deep-learning architectures have provided a faster and more comprehensive viewpoint. The preassigned criteria in AI modules were the basis for annotating features in images. Methods based on object detection and segmentation is widely used in image analysis and labeling flourished such as SSD,<sup>18</sup> U-net<sup>19</sup> and Faster R-CNN.<sup>20</sup> The advanced deep-learning approaches prompted us to develop a faster and automatic helminth eggs identification and quantification platform. Most importantly, the results analyzed by AI are reproducible. From a practical point of view, a smooth user interface is a critical component for clinical examination in a realworld field domain. A comprehensive workflow, including microscopic image digitalization, computational analysis, and manual validation of post-AI prediction, should be well polished. Notably, previous helminth egg classification systems were designed to recognize individual egg images pre-captured by professional clinical operators.<sup>14e16</sup> The automatic scanning and classifying platform for clinical samples remains an unachieved goal. Instead of the high cost of complex robotic mechanisms for parasitic egg target finding with a microscope, capturing all areas of the target specimen and performing the target finding in silico can be a low-cost and feasible solution. Optical microscopes that are equipped with computer controllable specimen slides are established in most examination facilities. This work was aimed to build an integrative and interactive cloud-based tool dHEAP (Helminth egg analysis platform) dthat provides a total solution for medical technicians in helminth infections diagnosis. HEAP is composed of image processing flows, multiple AI prediction kernels, and highly efficient manual validation interfaces. HEAP serves as a community for helminth images collection and AI prediction model providers. Users can establish their prediction systems by applying pretrained AI models and high-quality microscopic images that are downloaded from HEAP archives. This online tool offers an option for reducing the time required for diagnosis and epidemiological investigations. Because of the well-designed user interface, which is similar to real-world microscopic operation with enhanced target identification facilitated by AI, HEAP can also serve as an educational tool for medical technician training.

#### **Image capturing and labeling.**

The Olympus BX53 microscope is used to capture all helminth egg images. The microscope controlling software is CellSens Dimension which is the official build-in motorized stage and digital camera controlling software. The microscope light source is set to the max level. The exposure time is set to auto exposure. The objective lens is 10X, and the eyepiece is 10X. The images with the same regions were captured from several depth of fields (Fig. 1). This is an essential feature of HEAP to simulate the experience of microscopic observation. For example, in the same specimen slide, we captured 12 images that are the same X and Y position but different depth of fields from 140, 150, 160 . 250 mm.

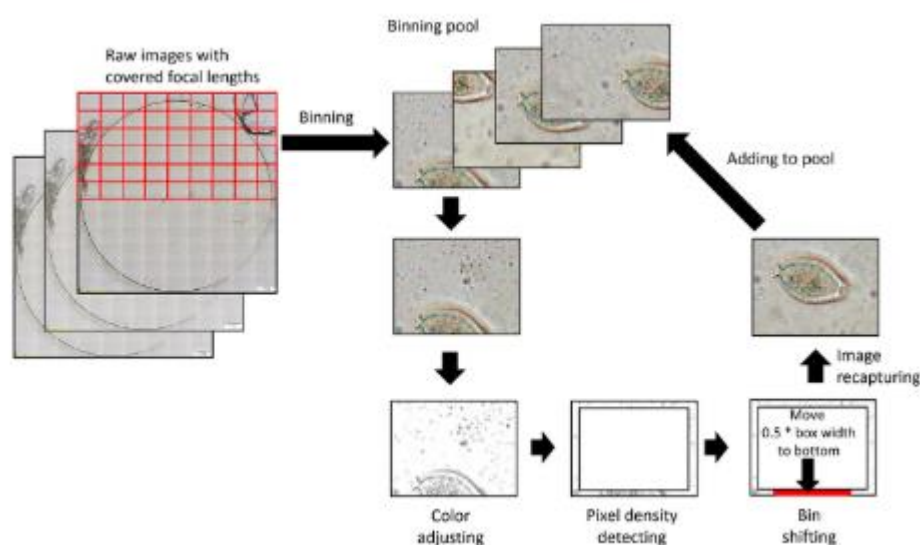


To ensure that at least one clear focused-ontarget image is captured. After the entire specimen is captured, all images within the same depth of field are merged into one piece. The image size is approximately 15 15 thousand pixels. LabelImg which is a widely used image labeling tool is used to create all the targets of eggs for training data preparation. Few image adjustments, such as exposure improving, contrast increasing, and color temperature balancing was performed for the captured images that have low qualities. Table 1 lists the number of helminth eggs of each species used for AI model building.



**Figure 1. Image from different depth of fields.** Each image was captured every 10 mm of the depth of field from 140 to 250 mm of the *Trichuris trichiura* eggs. The detailed pattern of three dimensions of egg shape can only be recognized on some depth of fields. The multiple microscopes within depth of fields extends the AI prediction range. The depth of field is labeled in the left-bottom side of each image.

**Image binning and algorithm for egg-in-edge detection** The size of the raw images from the capture system is approximately 10 thousand square pixels, which is too large for the system to use. HEAP splits a large image into hundreds of smaller-sized images that are proper for machine learning architecture usage. In our testing results, the prediction accuracies are substantially lower than those for intact eggs since the eggs are located in the middle of two binning boxes. In the first step, a sliding window walks through from the upper-left side to the bottom-right side and retrieves the smaller images. In every step, an image adjustment function that converts an image into high contrast and grayscale mode is activated. Ten pixels around the edges of bin boxes are considered. We calculate the pixel density ratio of four edges to decide in which direction to move. The binning box moves 50 percent of the binning box width/height in the highest density edge direction. The edge detection is activated every movement. In the prediction stage, all binning boxes that contain the eggs in the edge are reserved and predicted by the AI model. After predicting all binning boxes, HEAP merges all predicted binning boxes based on the X and Y coordination relative to the raw microscopic image. Fig.2 shows an example to illustrate the concept. The edge movement process is stopped while immense spots that cover all four edges of binning boxes are detected.



**Figure 2. Workflow of image binning and egg-in-edge detection algorithm.** To address the vast size of a raw image, image binning is performed using a fixed-sized nonoverlapping sliding window. Each binning image has two destinations: the first destination is part of the binning pool for further egg identification; the second destination triggers eggs in the edge detection protocol, which is based on comparing the pixel densities of four edges of binning boxes. Color adjustments, including grayscale converting and contrast increasing, are performed to emphasize the differences in the backgrounds and egg boundaries. The binning box moves toward the highest pixel-dense direction half-width/height of the binning box. The recaptured images are joined in the binning pool for egg prediction.

**Pretrained models and contents** For the demonstration, we have pretrained and built prediction models of 17 parasite egg samples (*Ascaris lumbricoides*, *Clonorchis sinensis*, *Diphyllobothrium latum*, *Taenia/Echinococcus granulosus*, *Echinostoma* spp., *Enterobius vermicularis*, *Fasciola hepatica*, *Fasciolopsis buski*, Hookworm, *Hymenolepis diminuta*, *Hymenolepis nana*, *Paragonimus westermani*, *Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma mansoni*, *Toxocara canis*, and *Trichuris trichiura*) applied on AI models with different parameters. The number of the collected models and images were continuously growing. HEAP is an openplatform allowing users to donate their microscopic images and pre-trained models. All pretrained models can be selected and applied to the HEAP web page. The training images in HEAP can be downloaded for relabeling or combined with other images to generate more comprehensive AI models.

**Microscopic images with multiple depth of fields** Medical technicians screened candidates and magnified images to observe the detailed patterns of the target. Compared with traditional manual inspection, HEAP uses an opposite strategy referred to as capturing before identifying that captures all images within operational depth of fields and identifies by AI models. A microscope uses approximately 100 to 400X to magnify the target in the specimen. With this magnification rate, three-dimensional structures of helminth eggs varied in different depth of fields. A digital microscopic image capture system has focusing abilities to automatically choose the depth of field on a specific point. However, the eggs are located in all possible positions of the specimen.



Autofocusing is not an efficient strategy. To overcome this focusing issue, we capture all images of operational depth of fields. HEAP enables the uploading of multiple images. The image viewing module overlaps all microscopic images within different depth of fields. For example, an egg candidate is identified in one depth of field; the bounding box displays all regardless of which depth of field user is selected. Fig. 1 shows the parasite egg and image qualities in different depth of fields.

### Conclusions

HEAP is designed to assist and reduce the workload of medical technicians. A user-friendly eye-inspection interface can increase human validation performance. The flexible backend of HEAP enables future integration of other AI architectures. All prediction results based on different AI models and architectures can be merged and displayed on the same page. The distributed computing architecture enables HEAP to be deployed in personal computers with a low construction cost. HEAP can be deployed in single personal computer or clusters with variant types of operating systems with/without GPU accelerators. Institutes or medical facilities can build their helminth egg identification private system off-grid from the internet to guarantee data security and safety. HEAP also serves as an ideal educational tool for medical technician training. With the continuous collection of helminth egg images and human-validated samples, the prediction rate and covered parasite types in the HEAP can improve and become comprehensive.

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