Chapter 4

Segmentation of Microscopic Images of Parasite Eggs

Accurate segmentation of microscopic images is crucial for identifying different types of parasite eggs. Recent advancements in computer vision and image processing techniques have led to the development of several segmentation methods, as discussed in Chapter 2. Several image segmentation techniques are explored, such as thresholding, edge detection, active contour models, watershed algorithms, and clustering-based segmentation, to accurately segment parasite egg images. This chapter evaluates their effectiveness on our image dataset and presents. Based on the evaluation, an optimized approach for segmenting the microscopic images of fecal samples containing various types of parasite eggs is proposed.

4.1 Effectiveness of Traditional Methods for Segmenting Parasite Egg Images

After exploring various traditional image segmentation methods, several advantages and drawbacks associated with segmenting parasite egg images from fecal samples are identified. These findings are summarized in Table 4.1.

Table 4.1: Advantages and disadvantages of some well-known image segmentation methods

Method	Advantages	Disadvantages
Thresholding	It is a straightforward method that is easy to implement and computationally efficient, making it suitable for real-time applications. Effective when the intensity distribution in the image is bimodal, with a clear distinction between foreground objects (such as parasite eggs) and background.	Thresholding is sensitive to noise and variations in illumination, which can lead to inaccurate segmentation. It struggles when the intensity levels of the foreground and background overlap or when there are variations in staining and illumination.
Edge detection	Edge detection methods highlight boundaries effectively, making them suitable for tasks where accurate boundary delineation is crucial. It works well for eggs with irregular shapes.	Sensitive to noise, which may result in false positives or incomplete segmentation. Performance may depend on parameter tuning, such as adjustment of brightness and contrast, which can be challenging in varied imaging conditions. May miss the inner details of the objects, especially in the presence of noise.
Active Contour Models (Snakes)	Active Contour Models are adaptable to complex and irregular shapes, making them suitable for parasite eggs with varying morphologies. They can produce smooth and continuous boundaries.	Performance is sensitive to the initialization of the contour. It can be computationally intensive, especially for large images or extensive contour evolution. Also, the method may converge to local minima and struggle with concave shapes.

Method	Advantages	Disadvantages
The Watershed Algorithm	Effective in separating overlapping or touching objects, which is beneficial for scenarios where parasite eggs are closely situated. Generally, the method is robust to noise in the image.	The method tends to over-segment the image, especially in regions with subtle intensity variations. The performance of the method can be sensitive to the choice of parameters such as marker type and value, connectivity, gradient image, etc. It may struggle with irregular-shaped objects and may produce fragmented results.
Region-Based Segmentation	The method is effective for images where regions with similar properties need to be segmented, and it can handle variations in staining and illumination.	It struggles when the image contains regions with diverse properties and may have difficulties with overlapping objects. Tuning of parameters such as similarity measure, maximum and minimum region size, etc., is important for better performance.
Ellipse Matching-Based Methods	The method utilizes an ellipse model that can closely match the shape of parasite eggs. Its effectiveness relies on well-defined object shapes that can be approximated by ellipses.	This technique is less effective for parasite eggs with irregular or complex shapes and struggles with variations in size and orientation of the ellipses.

Before evaluating any segmentation method, several image pre-processing operations are implemented to achieve optimal results. These operations include converting RGB images to grayscale, noise filtering, and enhancing brightness and contrast. A detailed discussion of these operations is provided below:

1. Conversion into Grayscale Image: Generally, microscopic images of parasite

eggs are taken in RGB (Red, Green, Blue) format. However, converting them into grayscale involves several advantages, including:

- Uniform Representation of Information: Parasite eggs may vary in colour due to staining or imaging conditions. Grayscale conversion helps standardize the representation, making it easier to compare and analyze intensity variations across the entire image.
- Intensity-Based Information: The parasite eggs can show different shades or colors in the images due to staining or lighting. Converting the image to grayscale preserves these variations in brightness and darkness, which is often important for segmentation.
- Reduction in Data Dimensionality: Converting to grayscale reduces data dimensionality, simplifies image processing and segmentation tasks, and allows the use of computationally more efficient algorithms.
- Compatibility with Traditional Image Processing Techniques: Many traditional image processing techniques and filters are designed for single-channel grayscale images. Converting the parasite egg images to grayscale enables the use of various methods, including thresholding, edge detection, and morphological operations.

Among the various methods for converting a colour image to grayscale, the luminosity method and the weighted average method are the most commonly used. The luminosity method assigns weights to each channel to reflect human colour perception, as shown in Equation 4.1 [107]. The weighted average method is similar but uses different weight values, as shown in Equation 4.2 [107]. This work adopts the second method, as it is proven to be more effective, according to the literature.

$$Y = 0.2126 \times R + 0.7152 \times G + 0.0722 \times B \tag{4.1}$$

$$Y = 0.299 \times R + 0.587 \times G + 0.114 \times B \tag{4.2}$$

2. Noise Filtering: Microscopic images, particularly those acquired in biological and medical contexts, often suffer from various types of noise that can degrade their quality and clarity. In images of fecal samples, common noise types include Gaussian noise, salt-and-pepper noise, quantization noise, temporal noise, and colour noise. Within our dataset, salt-and-pepper noise is frequently observed, and we addressed this using the median filtering method. This approach substitutes each pixel's value with the median value

of its neighbouring pixels, effectively reducing salt-and-pepper noise without blurring edges and preserving details better than alternative filters.

3. Brightness Enhancement: Brightness enhancement involves modifying pixel intensity values in an image to enhance its visual appeal or improve its quality for further processing. To enhance the brightness of microscopic images of parasite eggs in our dataset, adjustments are made to both the darkest and brightest areas of the pictures. The steps for this process are mentioned below.

   (a) Identify the lowest and highest brightness levels in the image based on the lowest and highest pixel intensities in the grayscale image.

   (b) Calculate the average brightness or average pixel intensity of the image.

   (c) Adjustment:-

      i. For the darkest areas: Increase the brightness using histogram equalization based on the intensity distribution of the complete image.

      ii. For the brightest areas: Decrease brightness using the gamma correction technique with a gamma value of 0.45.

4. Contrast Enhancement: Contrast enhancement is an important step aims at improving the visual clarity of different objects or structures within an image. In the context of segmenting microscopic parasite egg images, where accurate detection of objects is essential, contrast enhancement plays a crucial role. Among various techniques, histogram equalization and CLAHE (Contrast Limited Adaptive Histogram Equalization) are commonly used techniques for enhancing image contrast. Histogram equalization redistributes pixel intensities across a wider range to improve the visibility of details, especially effective when pixel values are concentrated within a specific range. CLAHE (Contrast Limited Adaptive Histogram Equalization) is an advanced form of histogram equalization designed to adjust to local variations in contrast. It segments the image into smaller regions and applies histogram equalization independently to each region. This approach restricts the enhancement of contrast within each region to prevent excessive noise amplification, thereby enhancing local contrast. Here, CLAHE is applied to our parasite egg images to improve overall contrast.

4.1.1 Analysis of Thresholding-Based Segmentation Method

This work applied the thresholding method using Otsu's threshold value, which automatically calculates an optimal threshold by considering the pixel intensity distribution in the image [38]. Otsu's approach is an automatic thresholding algorithm that aims to determine the ideal threshold value by maximizing the variance between two groups of pixels. This technique is widely used in image processing for optimal image segmentation. The algorithm begins by computing the normalized histogram $p(i)$, which represents the distribution of pixel intensities in the grayscale image. Subsequently, the cumulative distribution function (CDF) is calculated as the cumulative sum of the normalized probabilities up to each intensity level, formulated as follows:

$$P(i) = \sum_{k=0}^{i} p(k) \tag{4.3}$$

The mean intensity of the image, denoted as μ, is computed using the histogram and CDF as:

$$\mu = \sum_{i=0}^{255} i \cdot p(i) \tag{4.4}$$

The global variance (σ^2_{global}) is calculated by evaluating the squared difference between each intensity value and the mean intensity as shown in Equation 4.5.

$$\sigma^2_{\text{global}} = \sum_{i=0}^{255} (i-\mu)^2 \cdot p(i) \tag{4.5}$$

Otsu's method then iterates through possible threshold values. For each threshold k, the algorithm computes the class probabilities for background ($w_0(k) = P(k)$) and foreground ($w_1(k) = 1 - w_0(k)$). Class means ($\mu_0(k)$ and $\mu_1(k)$) for background and foreground are calculated as follows:

$$\mu_0(k) = \frac{\sum_{i=0}^{k} i \cdot p(i)}{w_0(k)} \ \textit{and} \ \mu_1(k) = \frac{\sum_{i=k+1}^{255} i \cdot p(i)}{w_1(k)} \tag{4.6}$$

The between-class variance ($\sigma^2_{\text{between}}(k)$) is then computed using the class probabilities and means as mentioned in the Equation 4.7.

$$\sigma^2_{\text{between}}(k) = w_0(k) \cdot w_1(k) \cdot (\mu_0(k) - \mu_1(k))^2 \tag{4.7}$$

The optimal threshold (k^*) is determined by selecting the threshold value that maximizes the between-class variance ($k^* = \arg\max_k \sigma^2_{\text{between}}(k)$). Finally, the original image is thresholded using k^* to obtain a binary image, where pixels below the threshold belong to the background (0) and those above belong to the foreground (1), as shown in Equation 4.8.

$$\text{Binary Image}(x, y) = \begin{cases} 0 & \text{if Original Image}(x, y) \leq k^* \\ 1 & \text{if Original Image}(x, y) > k^* \end{cases} \tag{4.8}$$

Image thresholding using Otsu's threshold value is sensitive to variations in pixel intensities and can adapt to different lighting conditions and contrasts, which makes it suitable for the segmentation of parasite egg images. Figure 4-1 displays some of the best results achieved through multiple experiments using this segmentation approach with different combinations of pre-processing operations.

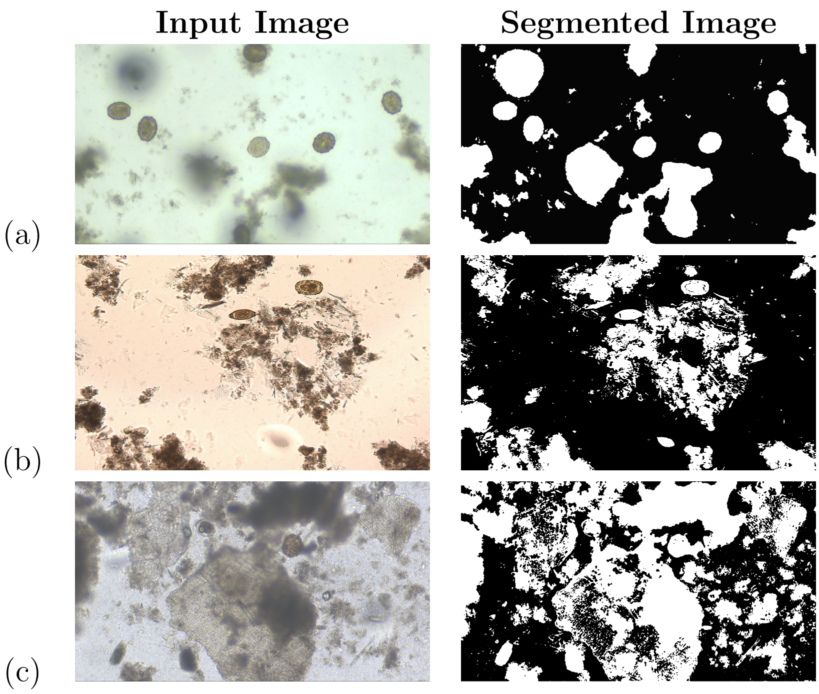


Figure 4-1: Examples of input and thresholded output images: (a) Image containing parasite eggs with a little load of debris, (b) with moderate load of debris, and (c) with high load of debris

From the experiments, it is observed that the thresholding method performs effectively when parasite eggs are clearly distinguishable from the background, free from debris or overlapping eggs, and with minimal impurities. However, as the amount of debris in the images increases, the method struggles to accurately segment objects, despite employing various combinations of image preprocessing operations.

4.1.2 Analysis of Edge-Detection-Based Segmentation

In image processing, edge detection can be achieved using various methods, such as the Sobel operator, the Prewitt operator, the Robert operator, and the Canny Edge Detector. These operators or masks are used to calculate the gradient of the image, which represents the rate of change of intensity for each pixel. Based on the gradient image, the edges of different objects can be determined. Let's denote an input image as $I(x, y)$, where x and y are the spatial coordinates. The gradient magnitude $G(x, y)$ and orientation $\theta(x, y)$ at each pixel can be computed as in equations 4.9 and 4.10 [108]:

$$G(x, y) = \sqrt{G_x(x, y)^2 + G_y(x, y)^2} \tag{4.9}$$

$$\theta(x, y) = arctan(\frac{G_x(x, y)}{G_y(x, y)}) \tag{4.10}$$

Where, G_x and G_y are the gradients in the horizontal and vertical directions respectively, obtained by convolving the image with the derivative masks. Once the gradient magnitude and orientation are computed, edges can be detected by thresholding the gradient magnitude [109].

Each operator has its own unique characteristics, and the choice of a specific operator depends on the application requirements and the characteristics of the images being processed. Sobel, Prewitt, and Roberts operators are sensitive to noise, which makes them less suitable for noisy images [110]. The Canny Edge Detector includes Gaussian smoothing in its pre-processing stage, which helps reduce noise and increases its robustness. The Sobel and Prewitt operators highlight edges in both horizontal and vertical directions separately, while the Roberts operator focuses on diagonal edge detection. In contrast, the Canny Edge Detector can detect edges in multiple directions by utilizing gradient magnitude and orientation information. Therefore, Sobel and Prewitt operators are commonly used in scenarios where the image contains well-defined edges, while Canny is often considered the gold standard for edge detection due to its robustness, accurate localization, and adaptability to various image conditions [111]. However, edge detection using the Canny operator is computationally expensive and time-consuming compared to other methods [108, 111].

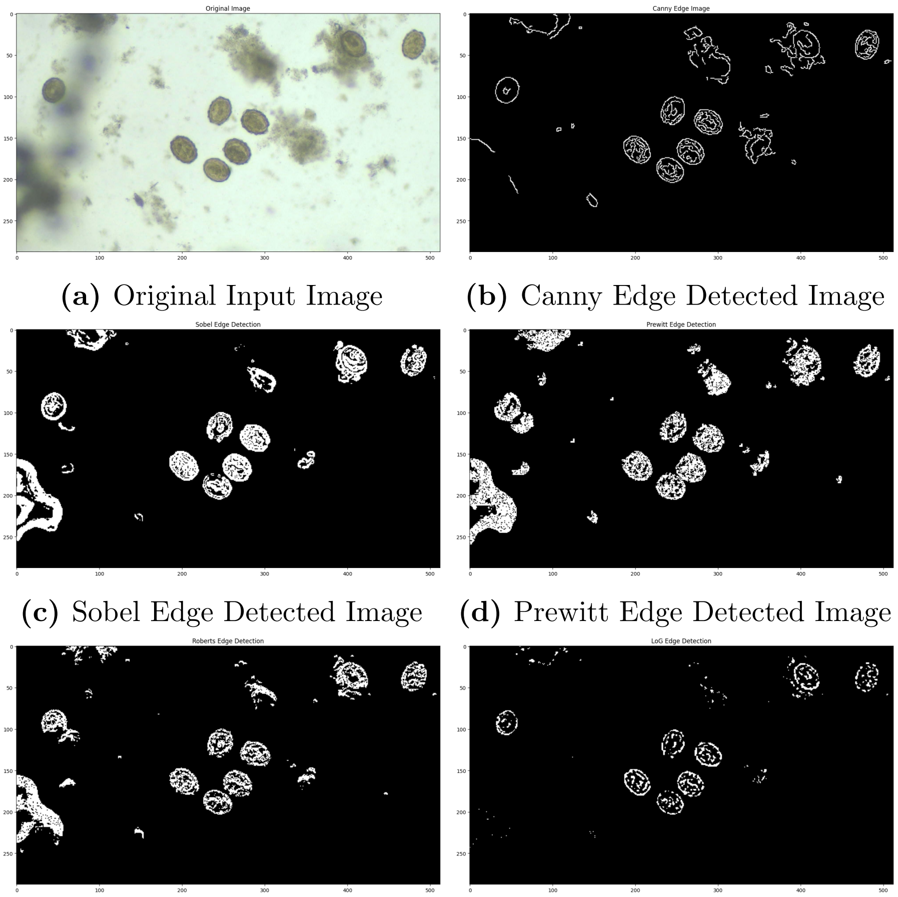
Laplacian of Gaussian (LoG) edge detection method identifies edges by combining the principles of Gaussian smoothing and Laplacian filtering [112]. The process starts with applying a Gaussian filter to the input image, followed by the application of the Laplacian operator [108, 111]. The result of the LoG operation is an image that highlights edges and identifies zero-crossings. Zero-crossings indicate significant changes in intensity and occur when the sign of pixel values changes, highlighting the presence of an edge. [112].

The effectiveness of all the methods is evaluated on the microscopic images containing parasite eggs in our dataset. Before applying any edge detection method, we performed a few pre-processing operations similar to those used in thresholding-based segmentation. An example of the final segmented images produced by various edge detection methods is shown in Figure 4-2.

Based on the experimental outcomes, it is observed that the Canny operator slightly outperforms others in detecting the boundary edges of parasite eggs across most images. The Sobel operator also showed effectiveness; however, in several cases, it is noticed that it tends to over-segment objects or detect unnecessary edges. The LoG method also yielded effective results, but in some images, it fails to detect certain parasite eggs that are successfully identified by the Canny edge detection method.

4.1.3 Analysis of watershed-based segmentation

A watershed segmentation method is also employed that utilizes the distance transform to identify regional minima, which are then used as markers for the watershed algorithm. The distance transform, calculated using the Euclidean distance as defined in Equation 4.11, assigns each pixel a value that indicates its distance to the nearest zero-value pixel (the object boundary) [113]. Subsequently, a threshold is applied to the distance transform to identify significant regional minima, which serve as markers for the watershed algorithm.



(a) Original Input Image **(b)** Canny Edge Detected Image

(c) Sobel Edge Detected Image **(d)** Prewitt Edge Detected Image

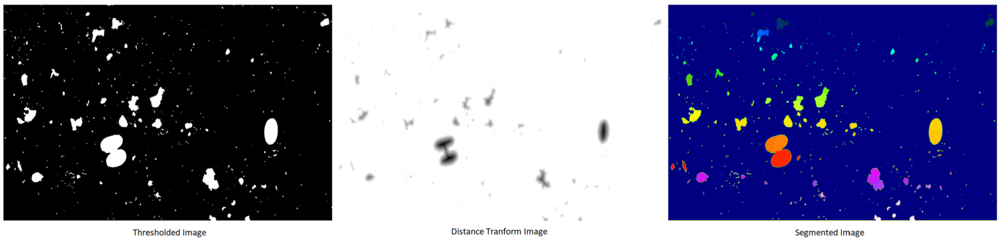
(e) Roberts Edge Detected Image **(f)** LoG Edge Detected Image

Figure 4-2: Segmentation result of various edge detection methods

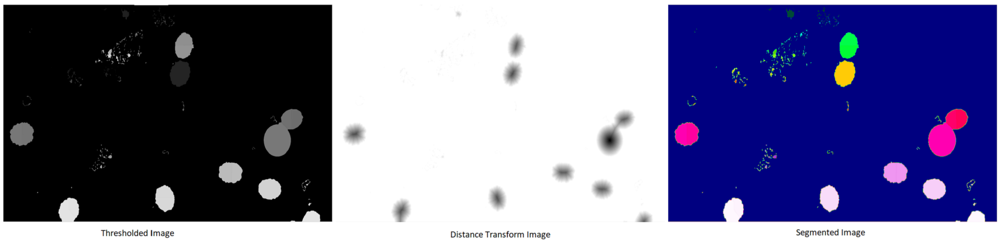
$$d_{Euclidean}([i_1, j_1], [i_2, j_2]) = \sqrt{(i_1 - i_2)^2 + (j_1 - j_2)^2} \tag{4.11}$$

where $[i_1, j_1]$ and $[i_2, j_2]$ are two pixels in a digital image.

A few examples of segmented images resulting from the watershed-based segmentation method are shown in Figure 4-3. The watershed segmentation is effective in capturing natural object boundaries; however, it may produce over-segmentation, especially in areas with sudden intensity variations or noise. Moreover, determining the markers is a very challenging task for the microscopic images of parasite eggs having large amounts of debris or sample impurities. The observations suggested that the method's effectiveness in segmenting the parasite eggs primarily depends on the quality of the thresholding outcomes. It often struggles to accurately segment objects when the images contain a high level of debris or impurities in the sample.



(a) Images containing hookworm eggs



(b) Images containing roundworm eggs

Figure 4-3: Output Images from the Distance Transform-based Watershed Segmentation Method

4.1.4 Analysis of Clustering-Based Segmentation

The K-means clustering method groups each pixel of a grayscale image as foreground or background. A few segmentation results from the K-means-based method are illustrated in Figure 4-4. The k-means segmentation method is easy and simple to implement, but it is sensitive to the initial placement of cluster centroids. Different initializations may lead to different final segmentation outcomes, and finding the optimal initialization can be challenging.

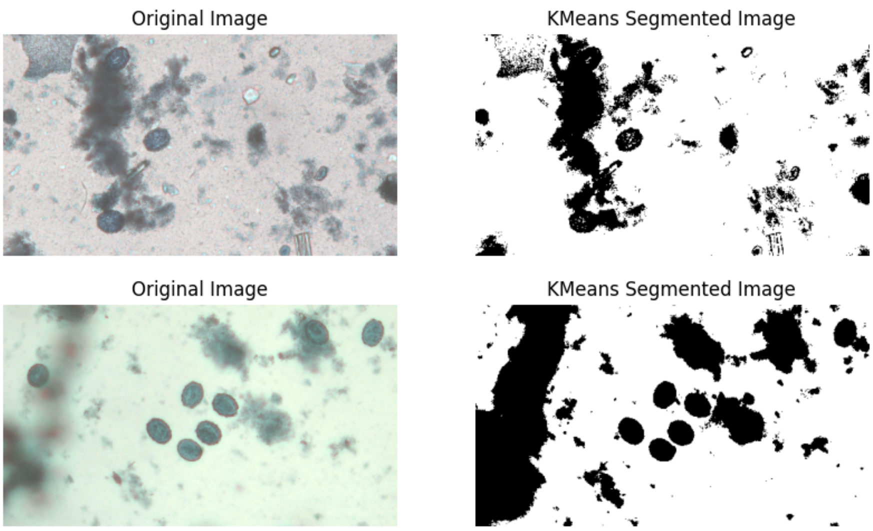


Figure 4-4: A few examples of output images using K-means segmentation method

4.2 Proposed Segmentation Approach

After thoroughly examining the images, the performances of various segmentation methods are evaluated as discussed in the preceding sections. These evaluations enabled us to identify the strengths and weaknesses of the various methods in segmenting the parasite egg images. Based on these findings, this research work proposes an optimal segmentation approach aiming at improving the overall segmentation results. The steps involved in the proposed segmentation approach are outlined below, and they can also be visualised in Figure 4-5.

Algorithm 1: Steps involved in the proposed segmentation approach

```
Data: Image in RGB format
Result: Segmented images of various objects
Convert image into grayscale;
Reduce image using Median filtering;
Enhancement of image brightness;
Enhancement of image contrast;
Enhancement of sharpness;
Canny edge detection operator;
Apply morphological dilation;
Apply morphological erosion;
Circular object detection using CHT;
Convert the detected circles into bounding boxes using the radius and
  pixel coordinates of the circle's perimeter;
Project the detected circles into the color image;
Apply the Non-Maximum suppression method to eliminate the
  overlapping detection;
Count the number of bounding boxes;
while There exists detected bounding boxes do
    Crop the object out using the bounding box coordinates;
end
```

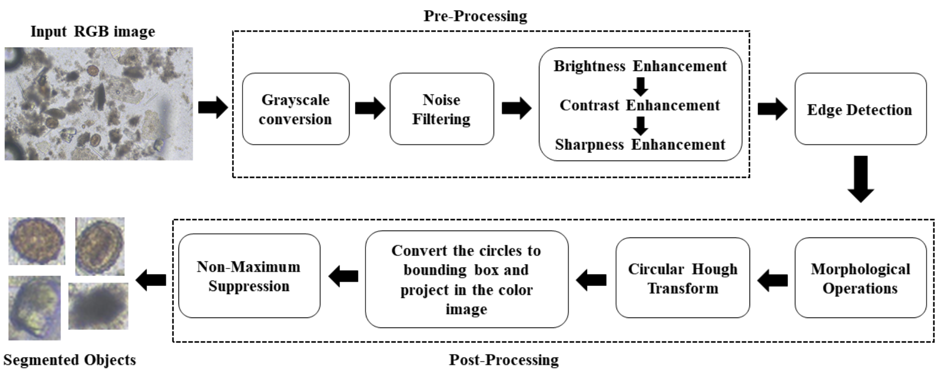


Figure 4-5: Proposed Segmentation Approach

4.2.1 Pre-Processing and Edge Detection

Before applying the segmentation method to the images, several pre-processing operations are performed. These include noise filtering, enhancement of brightness, contrast, and edge sharpness. To reduce salt and pepper noise in the grayscale images, a median filter with a 3×3 convolutional filter is applied. Gamma correction is utilized to enhance brightness, addressing images with inadequate lighting conditions and those that are overly bright. The contrast of the image is enhanced using the CLAHE method, and the Gaussian high-pass filtering method is employed to sharpen the object's edges.

The segmentation process involves applying the Canny edge detection method, which outlines the edges of foreground objects using white pixels against a black background. The higher threshold value for the edge detection process is determined using Otsu's method, which automatically calculates the threshold based on the image intensity distribution. The lower threshold value is set at 45% of the higher threshold value. This coefficient is determined empirically based on observations from multiple outputs.

4.2.2 Post-Processing

After completing the edge detection, two popular morphological operations are applied, namely dilation and erosion. Dilation expands or thickens the boundaries of objects in a segmented image. For each pixel in the image, the dilation operation replaces the pixel value with the maximum value within the neighbourhood defined by a structuring element such as a circle or square. A square kernel of size 5×5 is used to perform dilation with the aim of connecting disjointed pixels of the edges, bridging the small gaps between edges, and improving the continuity of the detected edges.

Following the morphological operations, the Circular Hough Transform (CHT) method [51] is applied to detect circular and elliptical-shaped objects in the images. Since the parasite eggs typically exhibit oval shapes, this method proves effective in detecting them, even when partially covered in debris. It also helps in the detection of touching and overlapping objects.

The method represents circles using the parameters (h, k) for the centre and r for the radius, as mentioned in Equation 4.12 [114]. The parameter space is a 3D array, often referred to as the accumulator array, with dimensions corresponding to h, k, and r. For each edge pixel at coordinates (x, y), vote for possible circles by considering potential centres (h, k) and varying the radius r to accumulate votes in the parameter space. The accumulator array stores votes for different circle parameters. Then it identifies the peaks in the accumulator array that indicate potential circle parameters. To eliminate false positives and filter out weak detection, a threshold value is applied to the accumulator array [115]. The choice of threshold value is important in the case of parasite egg detection, as parasite eggs have a regular shape and size compared to other non-egg objects in the images. The threshold values, such as the radius and circularity of objects, are determined empirically to detect objects within a range of circularity and radius. We set the minimum circularity value to 0.5 to detect both circular and elliptical-shaped parasite eggs, including those that are partially covered with debris or touching other objects. It is observed that applying the CHT directly to the grayscale image results in the detection of numerous unnecessary objects and significantly increases processing time. Therefore, to address this issue and speed up processing, I applied the method to the binary edge-detected image.

$$(x - h)^2 + (y - k)^2 = r^2 \tag{4.12}$$

The Circular Hough Transform process produces several overlapping circles around the same object. To eliminate them, the circles are converted into bounding boxes, and the Non-Maximum Suppression (NMS) method is applied. The NMS is generally used in object detection tasks to refine the results of a detection algorithm by eliminating redundant or multiple overlapping bounding box predictions. In this work, the NMS method as mentioned in [116] is used, with a few modifications to remove the multiple overlapping detections of the same object.

The Non-Maximum Suppression (NMS) method involves sorting bounding boxes based on a confidence score. Since the segmentation approach does not include ground truth data, the confidence score for the bounding boxes is determined from the parameters of the Circular Hough Transform (CHT) operation, as shown in Equation 4.13. This score indicates how confident the model is that an object exists within each bounding box. The method then iterates through the sorted list, keeping boxes with the highest confidence scores and removing overlapping boxes that have significant intersection over union (IoU). This ensures that only the most confident and non-overlapping bounding boxes remain, effectively preventing multiple detections of the same object.

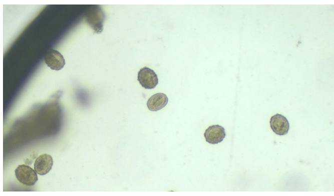
$$Confidence\quad Score = 0.5 * Circularity + 0.5 * Size\quad Confidence \tag{4.13}$$

Where the size confidence score is calculated as follows:

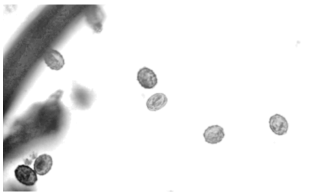
$$Size\quad Confidence = \frac{Size\quad of\quad the\quad detected\quad object}{Average\quad of\quad all\quad detected\quad objects} \tag{4.14}$$

4.3 Results and Discussion

The proposed segmentation approach is evaluated using our image dataset. Figure 4-6 shows example output images produced at various stages of the segmentation process.



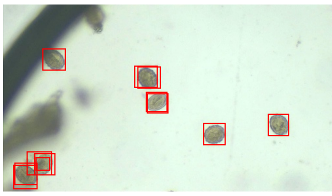
(a) Input image



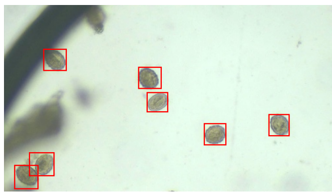
(b) Pre-processed image

(c) Edge-detected image

(d) Circular Hough Transform result



(e) Circles are converted into bounding boxes and projected to RGB image



(f) Output image after applying NMS

Figure 4-6: Input and output images of different stages of the proposed segmentation approach

The effectiveness of the segmentation method is assessed by visually examining the output images. A few examples of edge-detected images produced by the segmentation method, along with their respective ground truth masks, are shown in Figure 4-7.

While the approach successfully detects parasite eggs in most images, it also segments numerous debris or non-egg objects. This complicates the direct comparison of the segmented images with our prepared ground truth masks discussed in Chapter 3, to calculate the quantified result of the segmentation process.

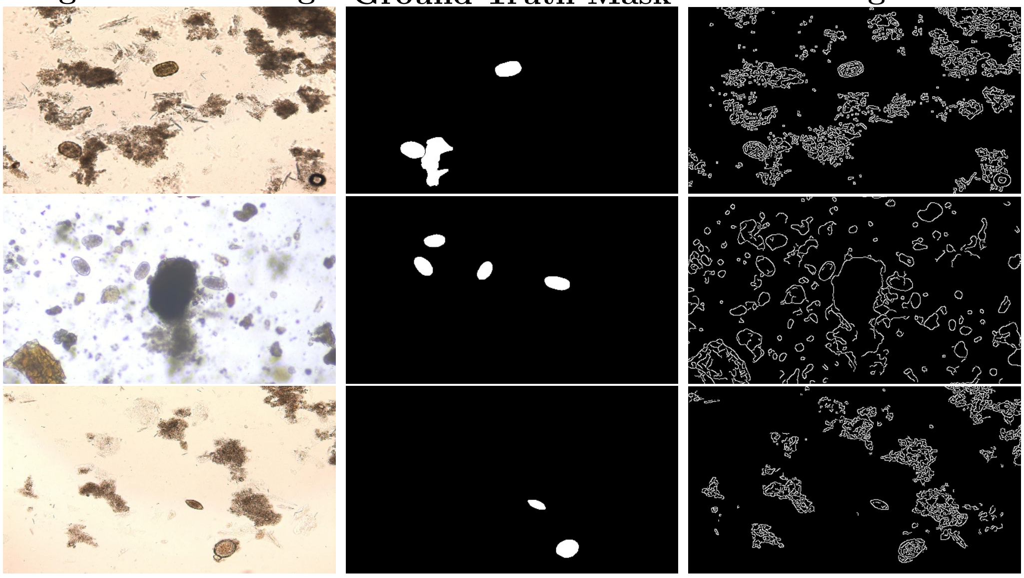


Figure 4-7: Comparison of original ground truth masks and edge-detected images.

Following are the key reasons why this work cannot provide quantified results for the proposed segmentation approach:

- The segmentation masks are created for training CNN-based semantic segmentation models by annotating parasite eggs and a few similar objects. However, the proposed image processing-based segmentation approach detects several debris or non-egg objects that make the direct comparison with the masks difficult. Without the ground truth for all the structures present in the segmented images, it is difficult to meaningfully apply metrics such as IOU, precision, recall, and F1-score.

- In the masks, object boundaries are precisely outlined with object pixels marked in white. In contrast, our segmentation approach employs an edge detection method that detects only the object boundaries. Some of these boundary edges may not be fully connected, making it difficult to accurately fill the pixels within the object for comparison.

- In many images, parasite eggs are covered by sample impurities or come into contact with other objects. The segmentation method sometimes fails to effectively separate the eggs from the impurities, resulting in segmentation that includes these impurities. In contrast, the ground truth masks clearly distinguish the eggs from debris or impurities.

4.4 Conclusion

In this chapter, various techniques for segmenting microscopic images of parasite eggs are explored, and an effective approach is proposed to address the challenges associated with analyzing microscopic images from fecal samples. The study primarily focused on three types of parasite eggs: Roundworm (Ascaris lumbricoides), Hookworm (Necator americanus / Ancylostoma duodenale), and Whipworm (Trichuris trichiura). Following are the key contributions and insights of the chapter:

- Explores various segmentation techniques and evaluates their effectiveness on segmenting the microscopic parasite egg images from fecal samples.
- Proposes an integrated approach combining Canny Edge Detection and Circular Hough Transform methods, supported by some crucial image pre-processing and post-processing steps.
- Implements Non-Maximum Suppression to address challenges such as overlapping detections from the Circular Hough Transform.
- Despite challenges like sample impurities and varying image conditions, the proposed method achieves promising results within our dataset.

In conclusion, this chapter highlights the importance of addressing key challenges in image segmentation for the automatic detection and identification of parasite eggs in microscopic images of fecal samples.