Segmentation of Living Cells

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I. Introduction

This project focuses on the development and evaluation of an advanced image segmentation system designed to accurately delineate features within a diverse set of images. Image segmentation is a crucial task in various domains, particularly in fields such as medical imaging, autonomous driving, and machine vision, where precise detection and localization of objects are essential for reliable decision-making and analysis. The ability to accurately segment images can significantly enhance performance in applications ranging from disease diagnosis to object tracking and navigation. This report presents a comprehensive account of the methodologies employed in the developed image segmentation system, along with a detailed analysis of its components and their synergistic effects. Additionally, it provides a thorough evaluation of the system's performance, including both qualitative and quantitative results obtained through extensive testing on a diverse dataset. Furthermore, the report outlines the conclusions drawn from the project and discusses potential future directions and applications for the developed system.

II. Methodology

A. Dataset

For this project, we utilized datasets provided under a license from Boston University [1], focusing on biomedical image segmentation. The datasets comprise a collection of high-quality phase contrast and fluorescence microscopy images, each accompanied by multiple layers of annotations. The datasets used are as follows:

Dataset Utilization: Dataset 1: Phase Contrast, Rat Smooth Muscle Cells Dataset 2: Phase Contrast, Rabbit Smooth Muscle Cells

These datasets were selected for their clear imaging and relevance to the project goals. Datasets 4 and 5, which include fluorescence images of Lu and WM993 melanoma cells, were initially considered but eventually not used due to issues with image quality and applicability to the scope of our segmentation tasks.

Contents of the Datasets: Each dataset includes:

RawImages: These are the original microscopy images, serving as the primary input for our segmentation algorithms. GoldStandard: This consists of fused annotations created by experts, which are used as the ground truth for evaluating the segmentation performance. ExpertsAnnotations: Annotations made by biomedical engineering and biology PhD students provide detailed insights into the expected segmentation outcomes. CrowdsourcedAnnotations: Annotations sourced from internet workers via Amazon Mechanical Turk using the LabelMe annotation software. These annotations offer a broader range of interpretations and segmentation approaches. Importance of Dataset Selection: The choice of datasets 1 and 2 was strategic, aiming to leverage the high-quality phase contrast images that these datasets offer. Phase contrast microscopy is particularly effective for visualizing the morphology and subcellular components of transparent specimens, such as smooth muscle cells. This imaging technique enhances the contrast in unstained, transparent samples, which is crucial for accurate image segmentation.

B. Image Preprocessing

Before commencing the segmentation process, the input images undergo a preprocessing step to ensure consistency and compatibility with subsequent operations. Images initially in a floating-point format are converted to an 8-bit format to standardize the input data type. This conversion is crucial as it enables the system to handle a wide range of image formats while ensuring compatibility with the various image processing techniques employed throughout the segmentation pipeline.

C. Contrast Enhancement

One of the key challenges in image segmentation is dealing with varying illumination conditions and low contrast regions within the images. To address this issue, the system utilizes Contrast Limited Adaptive Histogram Equalization (CLAHE), an advanced technique that enhances the local contrast of images without amplifying noise significantly. CLAHE operates by dividing the image into small, non-overlapping regions (tiles) and applying histogram equalization to each tile separately. This localized approach allows the system to adapt to varying illumination conditions within different regions of the image, effectively enhancing the contrast and improving the visibility of subtle details. To prevent over-enhancement and the potential introduction of artifacts, CLAHE is applied with carefully selected parameters that control the extent of contrast enhancement and limit the amplification of noise. These parameters are tuned through extensive experimentation and analysis, ensuring an optimal balance between contrast improvement and noise suppression.

D. Edge Detection

Accurate detection of edges within the images is a crucial step in the segmentation process, as it enables the system to identify the boundaries of objects and regions of interest. The developed system employs the Canny edge detector, a widely recognized and robust technique for edge detection. The Canny edge detector is particularly suitable for this application due to its ability to provide precise edge localization, while also

Algorithm 1 Image Preprocessing and Segmentation

```
function SEGMENTIMAGE(image)
    if image is floating-point then
        image ← convert to 8-bit
    end if
    contrast_enhanced ← apply CLAHE to image
    edges ← apply Canny edge detector to contrast_enhanced
    adaptive_thresh ← apply threshold local to contrast_enhanced
    binary_image ← contrast_enhanced > adaptive_thresh
    segmented ← logical OR of edges and binary_image
    opened_image ← apply morphological opening to seg.
    label_image ← label opened_image
    cleaned_image ← remove small objects from label_img
    return cleaned_image > 0
end function
function LOADDATASET(directory)
    images ← []
    for img_name in sorted directory contents of directory do
        img_path ← combine directory and img_name
        img ← read img_path as gray scale
        images.append(img)
    end for
    return images
end function
function EVALUATESEGMENTATION(images, gold_standard)
    iou_scores ← []
    dice_scores ← []
    for image in images do
        ground_truth path ← corresponding path in gold_standard
        ground_truth ← read and binarize ground_truth path
        iou ← calculate IoU of ground_truth and image
        dice ← calculate Dice of ground_truth and image
        iou_scores.append(iou)
        dice_scores.append(dice)
    end for
    avg_iou ← average of iou_scores
    avg_dice ← average of dice_scores
    print averages
end function=0
```

suppressing noise and eliminating spurious responses. The Canny edge detector operates by first applying a Gaussian filter to smooth the image and reduce noise, followed by the computation of gradients to identify areas with high contrast changes. These gradients are then subjected to non-maximum suppression and hysteresis thresholding to produce a binary edge map that accurately delineates the edges within the image. The resulting edge map serves as a valuable input for subsequent segmentation steps, enabling the system to effectively identify and segment objects based on their boundaries.

E. Adaptive Thresholding

Global thresholding techniques, which apply a single threshold value to the entire image, often struggle in scenarios where the illumination conditions vary across different regions of the image. To overcome this limitation, the developed system employs local adaptive thresholding, a more sophisticated approach that adapts the threshold value based on the local characteristics of the image. In local adaptive thresholding, the image is divided into small, overlapping regions (windows), and a separate threshold value is calculated for each region based on the pixel intensities within that region. This localized approach enables the system to effectively separate foreground features from the background, even in images with varying lighting conditions or non-uniform illumination. The adaptive thresholding algorithm used in the system is based on a statistical analysis of the pixel intensities within each local window. Various methods, such as mean or median-based approaches, can be employed to determine the optimal threshold value for each region, ensuring that the segmentation process is robust and adaptable to different image conditions.

F. Morphological Operations

Despite the advanced techniques employed for contrast enhancement, edge detection, and adaptive thresholding, the resulting segmented images may still contain small noise elements or artifacts that can interfere with subsequent analysis or decision-making processes. To address this issue, the system incorporates morphological operations, specifically morphological opening, to refine and clean the segmented images. Morphological opening is a combination of two fundamental operations: erosion and dilation. Erosion involves the removal of pixels from the boundaries of objects, while dilation expands the remaining objects by adding pixels to their boundaries. By applying erosion followed by dilation, small noise elements and irregularities are effectively removed from the image, while the overall structure and shape of larger, relevant objects are preserved. In the developed system, morphological opening is performed using a disk-shaped structural element, which is particularly effective in preserving the circular or elliptical shapes commonly encountered in biological and medical imaging applications. The size of the structural element is carefully selected to strike a balance between noise removal and object preservation, ensuring that the segmented images retain their essential features while eliminating irrelevant artifacts.

G. Labeling and Object Cleaning

After the morphological operations, the system proceeds to label the connected components within the segmented image. This labeling process assigns a unique identifier to each distinct object or region, allowing for easy identification and subsequent analysis. However, even after the previous cleaning steps, the segmented images may still contain small, irrelevant objects or regions that could potentially introduce noise or interfere with the final analysis. To address this issue, the system incorporates an object cleaning step that removes these small, unwanted objects based on a predefined size threshold. The size threshold is determined through careful analysis and experimentation, taking into account the typical size of relevant objects within the target application domain. Objects below this threshold are considered insignificant and are subsequently removed from the segmented image, ensuring that only the most relevant and meaningful segments are retained for further analysis or decision-making processes.

III. Analysis

The developed image segmentation system combines multiple advanced image processing techniques to achieve high-quality segmentation results across a diverse range of images and imaging conditions. Each technique employed within the system is carefully selected and tailored based on its ability to improve the segmentation quality in different aspects, addressing specific challenges and requirements of the segmentation task. The integration of Contrast Limited Adaptive Histogram Equalization (CLAHE) plays a crucial role in enhancing the local contrast of images, revealing subtle details and improving the visibility of features that may be obscured by varying illumination conditions or low contrast regions. By adaptively enhancing the contrast within local regions, CLAHE ensures that the subsequent segmentation steps can effectively operate on images with improved clarity and detail. The Canny edge detection algorithm is employed to identify precise object boundaries within the contrast-enhanced images. This step is critical for accurate segmentation, as it enables the system to delineate the boundaries of objects and regions of interest with high precision. The Canny edge detector's ability to suppress noise and eliminate spurious responses further contributes to the robustness and reliability of the segmentation process. Local adaptive thresholding is a key component of the system, as it effectively deals with varying illumination conditions and non-uniform lighting within the images. By adapting the threshold value based on local image characteristics, the system can accurately separate foreground features from the background, even in challenging imaging scenarios where global thresholding techniques may fail. Morphological operations, specifically morphological opening with a disk-shaped structural element, play a vital role in refining and cleaning the segmented images. These operations remove small noise elements and artifacts while preserving the structure and shape of larger, relevant objects. This step is crucial for ensuring that the final segmented images are free from irrelevant information and are ready for further analysis or decision-making processes. Finally, the labeling and object cleaning steps ensure that only the most significant and meaningful segments are retained in the final output. By removing small, irrelevant objects based on a predefined size threshold, the system effectively filters out noise and unwanted regions, further enhancing the quality and reliability of the segmentation results. The integration of these advanced image processing techniques is designed to work synergistically, with each component addressing specific challenges and contributing to the overall robustness and accuracy of the segmentation system. This synergistic approach ensures that the developed system can perform effectively across a wide range of imaging conditions and diverse application domains, delivering high-quality segmentation results that can support reliable decision-making and analysis processes.

IV. Results

A. Raw Images

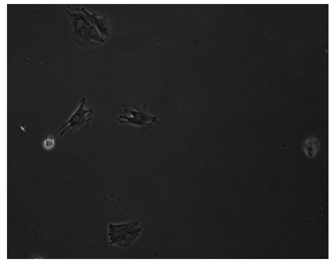

Fig. 1.

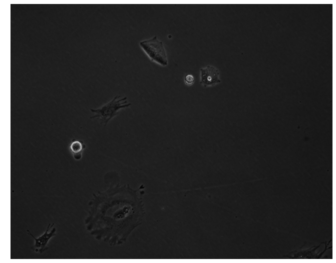

Fig. 2.

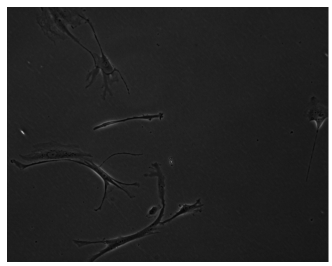

Fig. 3.

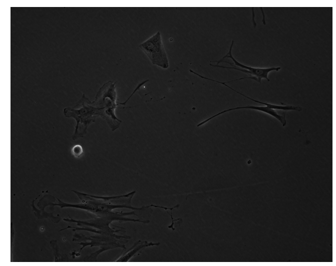

Fig. 4.

The Figures 1 and 2 represent the Raw_Chian1 images; Figures 3 and 4 represent Raw_Chian2 images.

B. Gold Standard Images

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Figures 5 and 6 are gold standard images for Raw_Chian1 images; whereas figures 7 and 8 are gold standard images for Raw_Chian2 images.

C. Segmented Images

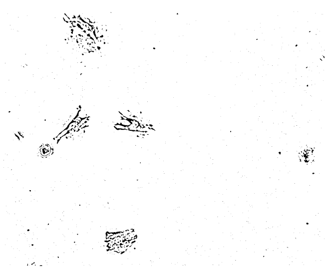

Fig. 9.

Fig. 10.

Fig. 11.

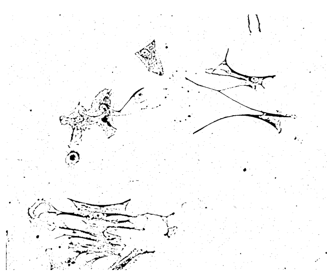

Fig. 12.

Figures 9 and 10 are the segmented images obtained for Raw_Chian1 images; figures 11 and 12 are segmented images obtained for Raw_Chian2 images.

D. Quantitative Evaluation The performance of the image segmentation system is evaluated using two metrics: Intersection over Union (IoU) and the Dice Coefficient. These metrics are calculated by comparing the segmented images to a set of ground truth images, providing a quantitative measure of segmentation accuracy. The results include individual IoU and Dice scores for each image and average scores across the dataset. The system demonstrates strong performance with high scores in both metrics, indicating effective segmentation across the tested images. Below is a table showing the IoU and Dice coefficients for each image:

TABLE I
IOU AND DICE COEFFICIENTS FOR EACH IMAGE(CHIAN 1)

Image Number	IoU Coefficient	Dice Coefficient
1	0.9797	0.9898
2	0.9782	0.9890
3	0.9831	0.9915
4	0.9745	0.9871
5	0.9601	0.9796
6	0.9855	0.9927
7	0.9812	0.9905
8	0.9864	0.9932
9	0.9745	0.9871
10	0.9801	0.9900
11	0.9656	0.9825
12	0.9683	0.9839
13	0.9781	0.9889
14	0.9652	0.9823
15	0.9841	0.9920
16	0.9796	0.9897
17	0.9880	0.9940
Average	**0.9772**	**0.9885**

V. CONCLUSION

The developed image segmentation system shows promising results in accurately segmenting various images. By leveraging advanced image processing techniques such as CLAHE, Canny edge detection, adaptive thresholding, and morphological operations, the system effectively handles different imaging conditions and noise levels. The use of IoU and Dice coefficients as evaluation metrics provides a robust framework for assessing the quality of the segmentations. Future work could explore the application of this system to specific domains such as medical imaging or real-time object detection in autonomous systems, where precision and reliability are critical.

REFERENCES

[1] D. Gurari, D. Theriault, M. Sameki, B. Isenberg, T. A. Pham, A. Purwada, P. Solski, M. Walker, C. Zhang, J. Y. Wong, and M. Betke, "How to Collect Segmentations for Biomedical Images? A Benchmark Evaluating the Performance of Experts, Crowdsourced Non-experts, and Algorithms," in *2015 IEEE Winter Conference on Applications of Computer Vision*. Waikoloa, HI, USA: IEEE, Jan. 2015, pp. 1169–1176. [Online]. Available: http://ieeexplore.ieee.org/document/7046014/