A Quantitative Analysis of Goblet Cells in the Rat Intestinal Using Imaging Techniques

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ABSTRACT

Goblet cells play a crucial role in maintaining intestinal health by secreting mucins, which form the protective mucus barrier in the gastrointestinal tract. These cells are essential for lubrication, immune defense, and protection against pathogens. The number and distribution of goblet cells are critical indicators of intestinal health, with abnormalities linked to various conditions such as the Inflammatory Bowel Disease (IBD). Accurate quantification of goblet cells is vital for diagnosing and monitoring these conditions. This paper presents an image-processing-based approach to automatically detect and count goblet cells within a defined region of interest. Using contrast enhancement, thresholding, and morphological analysis, our method provides a robust and efficient tool for goblet cell quantification. Experiments conducted on a private dataset of 61 histological images demonstrated high detection accuracy.

Keywords-quantitative; image processing; goblet cells; morphological features

I. INTRODUCTION

Goblet Cells (GCs) are specialized epithelial cells responsible for secreting mucins -the primary component of mucus- which play a fundamental role in maintaining intestinal homeostasis [1]. The mucus layer acts as a protective barrier, preventing direct contact between luminal contents and the epithelial lining while facilitating the smooth passage of materials through the gastrointestinal tract. These cells are distributed throughout the intestinal epithelium, with their highest density found in the colon [2]. Proper goblet cell function is critical for immune defense, as mucus secretion traps pathogens and prevents infections [3]. Changes in GC count and function are associated with various gastrointestinal disorders, including Inflammatory Bowel Disease (IBD), Irritable Bowel Syndrome (IBS), and colorectal cancer. In conditions such as ulcerative colitis and Crohn’s disease, GC depletion compromises the mucus barrier, increasing susceptibility to inflammation and microbial invasion [4]. Moreover, an abnormal increase in GC density has been linked to certain neoplastic transformations [3]. Given these clinical implications, accurate quantification of GCs is essential for the diagnosis, monitoring, and understanding of disease progression.

With advancements in medical imaging and computational techniques, automated image processing methods offer a more efficient and objective alternative for GC quantification. Various preparation methods have been established for quantifying GCs, including the AB-PAS (Alcian Blue-Periodic Acid-Schiff) staining technique, based on Light Microscopy Counting and Grid-based Counting. Traditional methods, however, suffer from the following limitations: (i) subjectivity due to observer bias, (ii) extensive time requirements, and (iii) limited reproducibility. As a result, recent studies increasingly employ software-based solutions, which can generally be categorized into three main groups:

- Threshold-based image processing methods
- Machine Learning (ML)-based classification methods
- Deep Learning (DL) approaches.

In this study, we propose an automated image-processing approach for detecting and counting goblet cells within histological images. The method employs contrast enhancement, thresholding, and morphological analysis to accurately segment and quantify GCs in a defined region of interest [5-8]. By leveraging these computational techniques, we aim to enhance

the accuracy and efficiency of GCs analysis, providing a valuable tool for biomedical research and clinical applications.

II. MORPHOLOGICAL FEATURES

Authors in [9] proposed an image analysis method towards counting GCs in chickens using morphological features. Such methods play a crucial role in object detection, segmentation, and feature extraction. These features are derived from the geometric and structural characteristics of objects within an image and are widely used in medical imaging, remote sensing, and industrial quality control. Morphological operations, such as dilation, erosion, opening, and closing, help refine binary images by eliminating noise, filling gaps, and preserving object structures [10-12]. Several essential morphological features are commonly extracted from binary images, including area, which represents the total number of pixels within an object; perimeter, which measures the length of the object's boundary; and eccentricity, which quantifies the elongation of an object by comparing the major and minor axes of its best-fitting ellipse [13]. These features are widely used in machine learning-based image classification. Additionally, morphological granulometry, a technique that analyzes particle size distribution using sequential morphological operations, has been successfully applied in medical diagnostics and material science [9]. These features provide essential information for distinguishing between different biological structures, such as cells, tissues, and pathological abnormalities [14]. Unlike pixel-intensity-based methods, morphological analysis considers the structural elements, making it more robust for object recognition in medical imaging [15]. The effectiveness of morphological feature extraction depends on the quality of pre-processing techniques, such as thresholding and noise removal, ensuring that the extracted features accurately represent the underlying objects of interest. As a result, morphological analysis continues to be a fundamental approach image processing research direction, evolving with advancements in computational techniques and deep learning models.

III. TYPES OF MORPHOLOGICAL OPERATIONS

Morphological operations are fundamental procedures in image processing for analyzing and manipulating the structure of objects within binary images. These operations are based on the interaction between an image A and a structuring element B, which defines the shape and size of the neighborhood used in the processing. The four primary morphological operations are dilation, erosion, opening, and closing.

Dilation expands the white regions (foreground) in a binary image, making objects appear larger. It works by placing a structuring element over each pixel; if at least one pixel under the element is white, the output pixel is set to white. The dilation between A and B ($A \oplus B$) is defined by:

$$A \oplus B = \{z \mid (B)_z \cap A \neq \emptyset\} \quad (1)$$

Erosion is the complementary operation to dilation, shrinking the white regions in an image by removing pixels from the boundaries of objects. Erosion between A and B ($A \ominus B$) is defined by:

$$A \ominus B = \{z \mid (B)_z \subseteq A\} \quad (2)$$

Opening is a combination of erosion followed by dilation. It removes small noise while preserving the shape and size of the main objects - a common application is removing salt noise.

Closing is the complementary operation to opening; filling small holes and gaps in objects. It is useful for joining broken parts of an object, by filling small holes and connecting thin structures in segmentation.

In some segmented images, many results show that the presence of holes in objects and noise around objects can reduce the accuracy of identification. To overcome this, the imfill() and bwareaopen() functions can be used. The first one has the property of closing holes in binary images [16]. Noise around objects can be eliminated using the second function.

IV. METHODOLOGY

The methodology is implemented in MATLAB, leveraging image processing techniques such as grayscale conversion, contrast enhancement, thresholding, morphological filtering, and connected component analysis. The workflow of the image processing algorithm for detection and counting GCs using morphological analysis is presented in Figure 1.

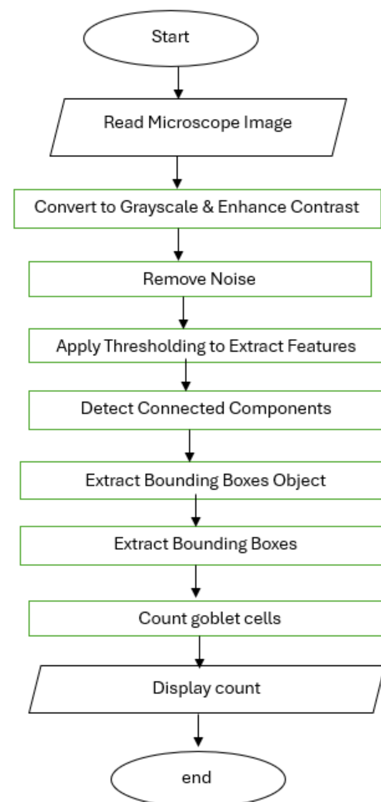

Fig. 1. Workflow of the algorithm presented in this study.

A. *Reading the Microscope Image*

The first step involves acquiring the microscopic image for analysis. The images were captured using a high-resolution digital microscope and imported into MATLAB for further processing. In digital histological analysis, images are

commonly acquired in RGB format, containing rich color information. However, for efficient processing and feature extraction, these images are typically converted to grayscale in order to simplify computations while preserving essential intensity information. The acquired image, presented in Figure 2, serves as the input for all subsequent image processing operations.

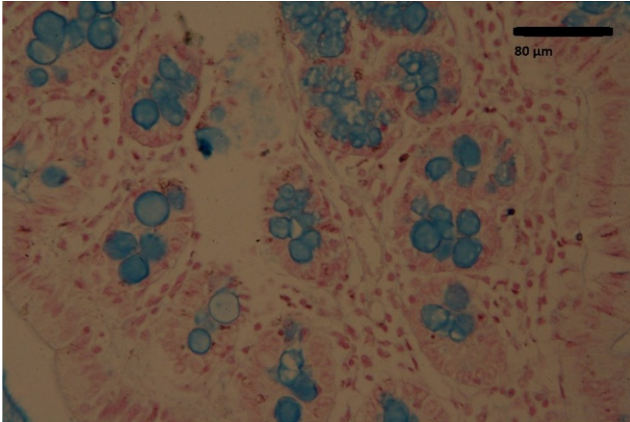

Fig. 2. Goblet cell microscope image, magnification = 80 µm.

B. Image Enhancement Using Histogram Equalization

Once the image is converted to grayscale, contrast enhancement techniques are applied to improve visibility. One widely used method is histogram equalization [17, 18], which redistributes pixel intensity values over the full range, thereby enhancing image contrast. Standard histogram equalization sometimes results in over-enhancement, so Adaptive Histogram Equalization (AHE) or Contrast Limited Adaptive Histogram Equalization (CLAHE) are employed for controlled enhancement. These techniques are improving cell structure visibility by preventing oversaturation of bright regions while amplifying subtle differences in intensity levels. Enhancement also ensures that details in both bright and dark regions are clearly visible, facilitating accurate segmentation and feature extraction in the subsequent steps.

C. Noise Removal

Microscopic images often contain unwanted artifacts, speckle noise, and background interference that can hinder segmentation accuracy. Median filtering and Gaussian filtering are commonly used approaches that preserve cellular structures while suppressing background noise.

D. Feature Extraction Using Thresholding

To isolate cellular structures from the background, thresholding techniques are applied. Thresholding converts the grayscale image into a binary image, where pixel values are either 0 (black – background) or 1 (white – foreground, i.e., cell structures). One effective approach is adaptive thresholding, which dynamically selects the threshold value based on local intensity variations. Thresholding enables clear separation between GCs and surrounding tissue, forming the basis for subsequent object detection and analysis.

E. Searching for Connected Components Using Predefined Boundaries

After feature extraction, Connected Component Analysis (CCA) is performed to identify and label distinct cellular structures. The bwconncomp() function in MATLAB detects groups of connected pixels, treating them as individual objects. Each detected object is assigned a unique label, and bounding boxes are extracted to define the spatial boundaries of each component.

F. Counting Cells in a Region of Interest

In many biomedical applications, researchers are interested in counting cells within a specific Region of Interest (ROI). This ROI is defined based on prior knowledge or experimental requirements. A square or rectangular ROI is selected, and only cells within this region are counted. The algorithm verifies whether each detected object falls inside the predefined ROI before including it in the final count. This approach ensures that only cells within the relevant region are included in the final analysis, making the results experimentally meaningful.

The experiment was applied on 61 microscopic images of GCs, extracted as part of a Ph.D. thesis conducted at the Faculty of Science, Helwan University. The research investigates the relationship between depression and the number of GCs in rats, aiming to explore the physiological effects of psychological conditions on mucosal health. Each image in the dataset captures GCs within specific ROIs in rat tissue samples, prepared and stained for clear visualization under the microscope. The images were collected using standardized histological and imaging protocols to ensure consistency and reliability across samples. This dataset was developed under the supervision of Prof. Ahmed Esmat, and serves as a valuable resource for automated image analysis tasks, such as GC detection, segmentation, and quantitative assessment in biomedical research related to neuro gastroenterology and behavioral sciences. The experimental results demonstrate that the proposed method successfully detects GCs in rat intestinal tissue.

V. RESULTS AND DISCUSSION

A. Results

Some representative samples of the tested images follow. Considering the image presented in Figure 3, the algorithm detected 148 GCs while human observers counted 130 cells. The proposed algorithm detected 77 GCs in Figure 4 while human observers counted 95 cells. In Figure 5, the proposed algorithm detected 68 GCs, while human observers counted 60 cells. The experiment shows that the proposed algorithm fails when handling images with high-density GCs, such as in Figure 6, detecting only 37 cells while the image contains many more cells. The results, shown in Table I, indicate that the proposed algorithm achieves high accuracy.

TABLE I. COMPARISON BETWEEN MANUAL AND AUTOMATED DETECTED GOBLET CELLS

Figure	Goblet cells counted by a human observer	Goblet cells detected by the algorithm
3	130	148
4	95	77
5	60	68

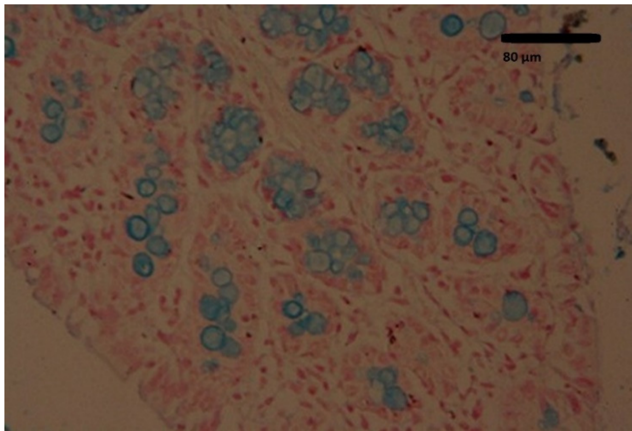

Fig. 3. Dataset sample, magnification = 80 µm.

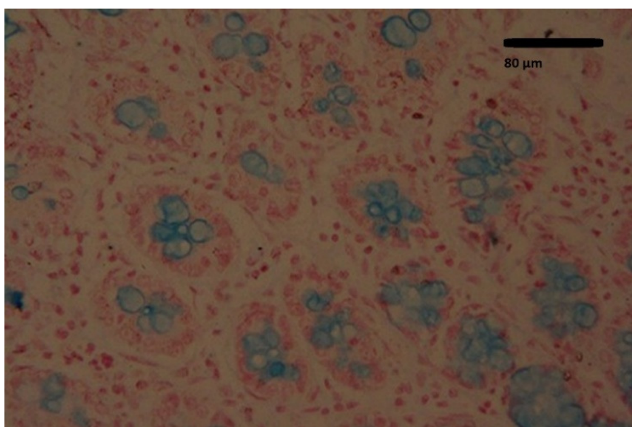

Fig. 4. Dataset sample, magnification = 80 µm.

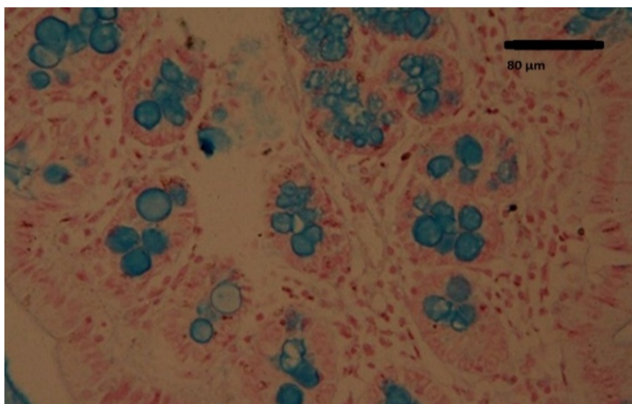

Fig. 5. Dataset sample, magnification = 80 µm.

B. Discussion

The current paper introduces a reproducible and computationally efficient image-processing framework for the automated quantification of GCs in rat intestinal tissue, a histological marker with critical relevance to gastrointestinal research. The method integrates a clear and reproducible sequence of classical morphological operations implemented entirely in MATLAB, without requiring large annotated datasets or specialized hardware.

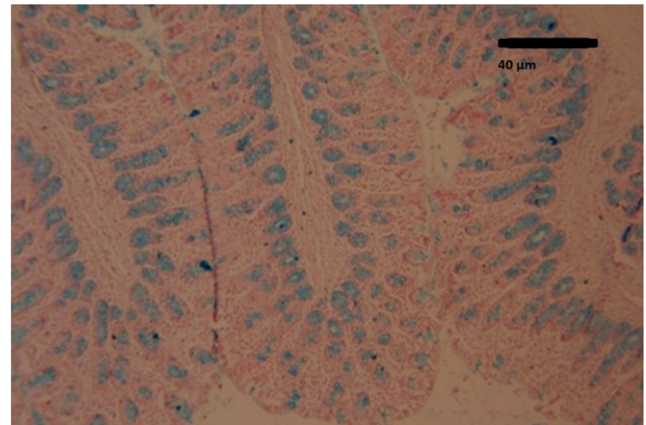

Fig. 6. Dataset sample with high GC density. magnification = 40 µm.

Applied to 61 histological images prepared under standardized protocols, the approach achieved a total accuracy of about 86% when compared to expert manual counts. While DL-based pipelines such as DCAU-Net, applied to rabbit conjunctival GCs in moxifloxacin-based fluorescence microscopy, report higher segmentation accuracy [19], and the GCAT toolbox for mouse and human colon histology exceeds 95% agreement with manual counts [20], these methods require substantial annotated datasets and modality-specific optimization. In contrast, the proposed framework offers full interpretability of each processing step and can be readily deployed in laboratories with limited computational resources.

VI. LIMITATIONS AND FUTURE STUDY WORK

The study faces the following limitations:

- Dependence of the algorithm on image capture position.
- Difficulty in detecting high-density GCs.
- Lack of adaptability to different tissue types.

Future enhancements may involve integrating ML-based classifiers to improve accuracy, incorporating DL techniques for automated feature recognition, and extending the framework for real-time analysis in clinical applications.

VII. CONCLUSION

This study presents an automated algorithm for detecting and quantifying Goblet Cells (GCs) of rats in histological images. The proposed algorithm, which integrates contrast enhancement, thresholding, morphological filtering, and connected component analysis, offers an efficient and objective alternative to manual cell counting. Experimental results demonstrate that the algorithm can successfully detect GCs with high accuracy, closely aligning with human observations in most cases. However, the algorithm encounters challenges when processing images with high-density GCs, as observed in Figure 6, where it significantly underestimates the number of cells.

Overall, this automated approach provides a valuable tool for biomedical research and clinical applications, improving the efficiency and reliability of GC quantification.

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REFERENCES

[1] L. Contreras-Ruiz, A. Ghosh-Mitra, M. A. Shatos, D. A. Dartt, and S. Masli, "Modulation of Conjunctival Goblet Cell Function by Inflammatory Cytokines," *Mediators of Inflammation*, vol. 2013, no. 1, 2013, Art. no. 636812, https://doi.org/10.1155/2013/636812.

[2] J. A. Grondin, Y. H. Kwon, P. M. Far, S. Haq, and W. I. Khan, "Mucins in Intestinal Mucosal Defense and Inflammation: Learning From Clinical and Experimental Studies," *Frontiers in Immunology*, vol. 11, 2020, Art. no. 2054, https://doi.org/10.3389/fimmu.2020.02054.

[3] Y. S. Kim and S. B. Ho, "Intestinal goblet cells and mucins in health and disease: recent insights and progress," *Current Gastroenterology Reports*, vol. 12, no. 5, pp. 319–330, Oct. 2010, https://doi.org/10.1007/s11894-010-0131-2.

[4] Z. Wang and J. Shen, "The role of goblet cells in Crohn' s disease," *Cell & Bioscience*, vol. 14, no. 1, Apr. 2024, Art. no. 43, https://doi.org/10.1186/s13578-024-01220-w.

[5] S. Xefteris, K. Tserpes, and T. Varvarigou, "A Method for Improving Renogram Production and Detection of Renal Pelvis using Mathematical Morphology on Scintigraphic Images," *Engineering, Technology & Applied Science Research*, vol. 2, no. 4, pp. 251–258, Aug. 2012, https://doi.org/10.48084/etasr.206.

[6] S. Murawwat, A. Qureshi, S. Ahmad, and Y. Shahid, "Weed Detection Using SVMs," *Engineering, Technology & Applied Science Research*, vol. 8, no. 1, pp. 2412–2416, Feb. 2018, https://doi.org/10.48084/etasr.1647.

[7] R. J. Rasras, I. M. M. El Emary, and D. E. Skopin, "Developing a new color model for image analysis and processing", *Computer Science and Information Systems,* vol. 4, no. 1, pp. 43–55, 2007, https://doi.org/10.2298/CSIS0701043R.

[8] A. Al-Ghraibah, M. Algharibeh, W. Al-Muhtaseb, F. Al-Khateeb, and I. Al-Anis, "Investigating the Significance of New Features Extracted from Long Bones X-ray Images", *IEEE 5th Middle East and Africa Conference on Biomedical Engineering*, Amman, Jordan, 2020, https://doi.org/10.1109/MECBME47393.2020.9265163.

[9] D. Sepriana, K. Adi, and C. E. Widodo, "Application of morphological operations for improvement the segmentation image of chicken intestinal goblet cells," *International Journal of Computer Applications*, vol. 182, no. 41, pp. 18-23, Feb. 2019.

[10] P. Soille, *Morphological image analysis: Principles and applications*, 2nd ed. Springer, 2003.

[11] A. O. Hashi, S. Z. M. Hashim, and A. B. Asamah, "Dynamic Adaptation in Deep Learning for Enhanced Hand Gesture Recognition," *Engineering, Technology & Applied Science Research*, vol. 14, no. 4, pp. 15836–15841, Aug. 2024, https://doi.org/10.48084/etasr.7670.

[12] A. Zaim, "Automatic Segmentation of the Prostate from Ultrasound Data Using Feature-Based Self Organizing Map," in *Image Analysis (SCIA 2005)*, 2005, pp. 1259–1265, https://doi.org/10.1007/11499145_127.

[13] R. C. Gonzalez, and R. E. Woods, *Digital image processing*, 4th ed. Pearson, 2018.

[14] M. Sezgin and B. Sankur, "Survey over image thresholding techniques and quantitative performance evaluation," *Journal of Electronic Imaging*, vol. 13, no. 1, pp. 146–165, Jan. 2004, https://doi.org/10.1117/1.1631315.

[15] M. M. P. Petrou and C. Petrou, *Image Processing: The Fundamentals*. Chichester, UK: Wiley, 2011.

[16] R. Bhat, B. Mehandia, "Recognition of Vehicle Number Plate Using Matlab," *International Journal of Innovative Research in Electrical, Electronics, Instrumentation and Control Engineering*, vol. 2, no. 8, pp. 1899–1903, Aug. 2014.

[17] A. Madbouly, "Increase contrast of low light image using modified histogram equalization," *Advances in Basic and Applied Sciences*, vol. 3, no. 1, pp. 67–71, Jun. 2024, https://doi.org/10.21608/abas.2024.327925.1052.

[18] B. Al-Naami, H. A. Owida, and H. Fraihat, "Quantitative analysis signal-based approach using the dual tree complex wavelet transform for studying heart sound conditions," in *2020 IEEE 5th Middle East and Africa Conference on Biomedical Engineering (MECBME)*, Amman, Jordan, Oct. 2020, Accessed: Oct. 30, 2025. https://doi.org/10.1109/MECBME47393.2020.9265121.

[19] S. Jang *et al.*, "Deep learning framework for automated goblet cell density analysis in in-vivo rabbit conjunctiva," *Scientific Reports*, vol. 13, no. 1, Dec. 2023, Art. no. 22839, https://doi.org/10.1038/s41598-023-49275-y.

[20] M. C. Rowe *et al.*, "Automating Quantitative Analysis of Goblet Cells and Mucus Components," presented at the 13th Asia Pacific Microscopy Congress 2025, Jan. 2025, Art. no. e136, https://doi.org/10.14293/APMC13-2025-0136.