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Comparative Analysis of Gradient Operators in Canny Edge Detection for Bacillus sp. Microscopic Imaging

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Abstract

This research aims to assess the application of microscopic imaging technology in analyzing Bacillus sp. bacteria, focusing particularly on the effectiveness of gradient operators in the Canny Edge Detection Algorithm. The study encompasses an in-depth evaluation of four principal gradient operators: Sobel, Prewitt, Roberts, and Scharr, to enhance edge detection accuracy in microscopic images. Analysis revealed that Sobel and Scharr excel in precision, with Sobel standing out in creating texture homogeneity and Scharr demonstrating superior inter-pixel correlation, both vital for ensuring visual accuracy of the images. Additionally, these operators show remarkable performance in Precision and Recall, effectively identifying relevant edges with minimal errors. Conversely, the Roberts operator, with its higher F-measure, offers an ideal equilibrium between precision and recall, making it a suitable choice for broader applications. Edge Co-Occurrence Matrix (ECM) analysis indicated that Sobel and Scharr possess higher contrast values, thereby emphasizing the sharpness of edge delineation. Conclusively, the study identifies the Scharr operator as most fitting for the analysis of microscopic bacterial imagery, owing to its capability in maintaining inter-pixel correlation and enhanced classification performance. This positions the Scharr operator as a highly applicable tool for microscopic bacterial studies, crucial in the accurate and consistent recognition of bacterial patterns. These findings significantly advance our understanding of Bacillus sp., directly impacting disease diagnostics and biotechnology. The research underscores the critical importance of selecting appropriate gradient operators in microscopic analysis and highlights the need for ongoing innovation and exploration in microscopic imaging technology.

Keywords: Microscopic imaging, bacillus sp., canny edge detection, gradient operators, edge detection accuracy

1. Introduction

This investigation explores the pivotal role of microscopic imaging technologies in microbiology, particularly focusing on Bacillus sp. bacteria. These bacteria are crucial in biomedical and biotechnological areas due to their unique survival capabilities in harsh environments and their spore-forming ability. Microscopic imaging is essential for examining the surface structures of these bacterial cells and understanding their individual cellular variations, thus offering broad application possibilities and enhancing the scientific knowledge base [1], [2].

Recent advancements in microscopic imaging have enabled in-depth studies of bacterial interactions, including those involving Escherichia coli and Pseudomonas aeruginosa, with different surfaces. Such studies provide a more nuanced understanding of microorganism behaviors and their environmental responses. The introduction of expansion microscopy and cell wall mechanical analysis has significantly improved our identification abilities for different bacterial species and their physiological states [3]. These methods have also contributed to a better understanding of bacterial metabolism at the microscopic scale and have led to the development of more efficient and precise microscopic image analysis techniques [4].

Given recent technological advances in microscopic imaging, this study highlights the importance of enhancing edge detection methods, particularly focusing on the Canny Edge Detection algorithm. Known for its precise edge identification with minimal noise, this algorithm has undergone improvements, including the use of type-2 fuzzy sets and replacing Gaussian filters with adaptive median filters. These enhancements significantly improve noise suppression and image detail sharpening capabilities. Furthermore, employing the inter-class maximum variance (OSTU) algorithm has been shown to enhance edge detection quality, providing more accurate and efficient image analysis possibilities [5], [6], [7].

This research evaluates the application and relative effectiveness of the enhanced Canny Edge Detection Algorithm combined with various gradient operators such as Sobel, Prewitt, Roberts, and Scharr in analyzing Bacillus sp. bacterial microscopic images. Its primary objective is to deepen our understanding of these bacteria through advanced image processing methods, offering fresh insights into their behaviors and interactions. The effectiveness of these modifications is gauged using analytical methods such as Precision and Recall, F-Measure, Edge Co-Occurrence Matrix (ECM), Peak Signal-to-Noise Ratio (PSNR), and Matthews Correlation Coefficient (MCC), providing a comprehensive evaluation of the gradient operators' success in microscopic image interpretation [8], [9].

The limited literature on developing innovative and precise microscopic image analysis methods underscores the significance of this research. Previous studies, such as those by Yu [10], explored edge detection algorithms based on the Sum and Difference of Neighborhoods along Axis (SDNNA), demonstrating strong anti-noise capabilities and high positioning accuracy across various image resolutions. These findings highlight key challenges in microscopic image analysis, including noise interference and the inability to adapt to complex bacterial patterns.

Additionally, research by Zhou [11] on the PatternNet dataset demonstrated the effectiveness of large-scale datasets in improving pattern recognition accuracy for high-resolution images. However, their reliance on extensive training datasets limits their applicability in scenarios with data constraints.

Recent developments in edge detection technology show promising potential. For example, Sun [12] implemented edge detection algorithms on SEM (Scanning Electron Microscope) images of multilayer thin films, achieving significant improvements in edge sharpness and precision for morphological analysis. Despite this progress, scalability and generalizability remain significant challenges, as discussed by Yu [10] emphasizing the need for more adaptive and comprehensive analytical methods.

Referring to these previous findings, this study aims to compare the efficacy of the Canny edge detection algorithm, using gradient operators like Sobel, Prewitt, Roberts, and Scharr, in analyzing Bacillus sp. bacteria microscopic images. Employing analytical techniques such as Precision, Recall, F-Measure, ECM, PSNR, and MCC, the study seeks to identify the most efficient and accurate image interpretation methods. The results are expected to provide deeper insights into bacterial morphology and lead to breakthroughs in biotechnological and medical applications. Furthermore, this research opens avenues for AI application in image analysis, which plays a pivotal role in developing advanced medical diagnostic systems, thereby advancing both scientific and practical knowledge in microbiology and biotechnology.

2. Method

This research endeavors to merge digital image processing with microbial biology to extend beyond mere technical edge detection in the analysis of bacterial colony patterns, as depicted in Figure 1.

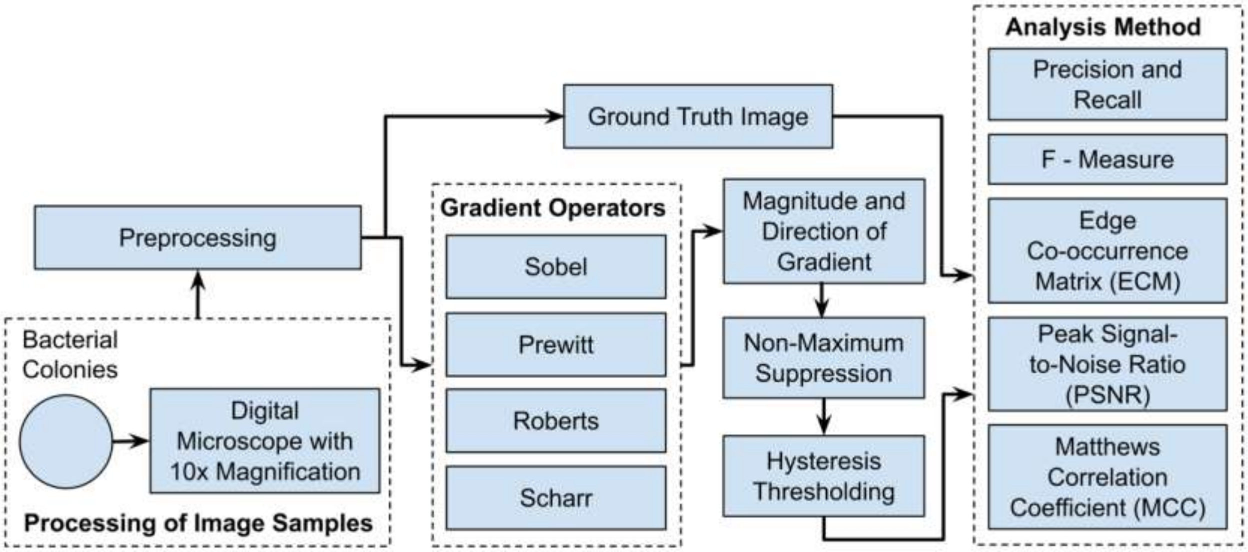

Figure 1. System Workflow for Edge Detection and Analysis Methods.

The objective is to fuse image processing technology with an understanding of microbial biology, thereby forging an analytical framework that unites these fields to provide new insights into colony pattern interpretation. This initiative reflects a collaborative spirit between the techniques of image processing and microbial biology, demonstrating synergy between the technical and biological aspects.

This research evaluates gradient operators for edge detection in Bacillus sp. colony imaging, employing metrics like Precision, Recall, F-measure, ECM, PSNR, and MCC to transcend traditional visual assessments and explore image-based microbiological features. Analyzing 50 digital microscopy samples, it aims to correlate visual and biological colony traits. Preprocessing involves grayscaling and Gaussian filtering to preserve image clarity and biological detail. Extensive analysis, including Ground Truth evaluation with Otsu Thresholding and five analytical methods, offers a detailed evaluation of operator efficacy, guiding optimal edge detection approaches in microbial biology studies.

This research integrates cutting-edge techniques such as Ant Colony Optimization [13], bee colony algorithms [14], and evolutionary systems [15] into microbiological analysis. This integration not only improves edge detection accuracy but also enhances the interpretation of biological data, contributing to a deeper understanding of microorganism behavior and expanding the scope of microbiological and digital image processing applications [16], [17].

2.1. Image Sample Processing

Image sample processing is crucial for analyzing bacterial colony patterns, employing a digital microscope with a CMOS sensor, 12-megapixel resolution, 30 fps frame rate, and 10x magnification to study Bacillus sp. On 90x15mm petri dishes. The study, conducted at Institut Teknologi Kalimantan Microbiology Laboratory in 2023, examines 50 samples, excluding bacteria preparatory and growth stages. Image pre-processing importance for bacteria detection, such as E. Coli in MATLAB, is noted by Karatepe [18]. The ePetri system by Jung and Lee [19] uses SPSM and super-resolution for better colony analysis, while Bae [20] consider imaging plates and Raman spectroscopy for in-depth analysis, enhancing morphological understanding and edge detection in bacterial microscopy.

2.2. Pre-Processing Steps

In bacterial colony imaging, preprocessing is crucial, involving conversion to grayscale and noise reduction. Grayscale is obtained via:

$$I_{grayscale} = 0.299 \times R + 0.587 \times G + 0.114 \times B \quad (1)$$

where R, G, and B represent the intensity in red, green, and blue channels. Gaussian filtering is employed for noise reduction, described by:

$$G(x, y) = \frac{1}{2\pi\sigma^2} exp\left(-\frac{x^2+y^2}{2\sigma^2}\right) \quad (2)$$

Preprocessing's importance, as highlighted by Jung and Lee [19], lies in enhancing the identification of bacterial diffraction patterns, deemed essential for accurate edge detection and analysis, as further supported by Buzalewicz [24].

2.3. Sobel Gradient Operator

The Sobel Operator is vital for detecting edges in bacterial colony imagery by computing intensity gradients. It yields accurate gradient measurements for segmentation, crucial in FPGA-based edge detection [21] and in improving detection emphasizing precision [22]. The horizontal G_x and vertical G_y gradients are computed using the Sobel kernel:

$$G_x = \sum_{m=-1}^{1} \sum_{n=-1}^{1} I(i+m, j+n) \cdot S_x(m, n) \quad (3)$$

$$G_y = \sum_{m=-1}^{1} \sum_{n=-1}^{1} I(i+m, j+n) \cdot S_y(m, n) \quad (4)$$

Where the Sobel kernels S_x and S_y are used for detecting horizontal and vertical changes respectively:

$$S_x = \begin{bmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{bmatrix} \quad (5)$$

$$S_y = \begin{bmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{bmatrix} \quad (6)$$

These kernels are instrumental in identifying changes in intensity in both the horizontal and vertical directions within the colony images.

2.4. Prewitt Gradient Operator

The Prewitt gradient operator plays a significant role in edge detection for analyzing bacterial colony images by revealing substantial intensity transitions. Recent enhancements to this operator have demonstrated improved effectiveness in edge detection and gradient analysis [23]. The horizontal G_x and vertical G_y gradients using the Prewitt operator are calculated as follows:

$$G_x = \sum_{m=-1}^{1} \sum_{n=-1}^{1} I(i+m, j+n) \cdot P_x(m, n) \quad (7)$$

$$G_y = \sum_{m=-1}^{1} \sum_{n=-1}^{1} I(i+m, j+n) \cdot P_y(m, n) \quad (8)$$

With P_x and P_y as the Prewitt kernels for detecting horizontal and vertical changes:

$$P_x = \begin{bmatrix} -1 & 0 & 1 \\ -1 & 0 & 1 \\ -1 & 0 & 1 \end{bmatrix} \quad (9)$$

$$P_y = \begin{bmatrix} -1 & -1 & -1 \\ 0 & 0 & 0 \\ 1 & 1 & 1 \end{bmatrix} \quad (10)$$

These help in identifying significant intensity changes, which are crucial for bacterial species such as Bacillus sp.

2.5. Roberts Gradient Operator

The Roberts Gradient Operator is utilized for precise edge detection in images, enhancing both continuity and noise resistance, especially when paired with Otsu thresholding [26]. The gradients are calculated using:

$$G_x = I(i, j) \cdot R_x + I(i+1, j+1) \cdot (-R_x) \quad (11)$$

$$G_y = I(i, j+1) \cdot R_y + I(i+1, j) \cdot (-R_y) \quad (12)$$

Where R_x and R_y are the Roberts kernels:

$$R_x = \begin{bmatrix} 1 & 0 \\ 0 & -1 \end{bmatrix} \quad (13)$$

$$R_y = \begin{bmatrix} 0 & 1 \\ -1 & 0 \end{bmatrix} \quad (14)$$

This operator is instrumental in identifying edges within images of bacterial colonies such as those of the Bacillus species.

2.6. Scharr Gradient Operator

The Scharr gradient operator enhances edge detection in images, improving the accuracy and resilience to noise. Adapting the Scharr operator with median filters or morphological techniques fine-tunes the edge segmentation in bacterial colony images [26], [27]. The Scharr operator uses convolution:

$$G_x = \sum_{m=0}^{2}\sum_{n=0}^{2} I(i+m, j+n) \cdot S_x(m,n) \quad (15)$$

$$G_y = \sum_{m=0}^{2}\sum_{n=0}^{2} I(i+m, j+n) \cdot S_y(m,n) \quad (16)$$

With S_x and S_y as the Scharr kernels for horizontal and vertical change detection, respectively:

$$S_x = \begin{bmatrix} -3 & 0 & 3 \\ -10 & 0 & 10 \\ -3 & 0 & 3 \end{bmatrix} \quad (17)$$

$$S_y = \begin{bmatrix} -3 & -10 & -3 \\ 0 & 0 & 0 \\ 3 & 10 & 3 \end{bmatrix} \quad (18)$$

This is crucial for more precise edge analysis in images depicting bacterial colony patterns.

2.7. Gradient Magnitude and Direction

Calculating the magnitude and direction of the gradient is a fundamental step in image analysis for edge detection, such as in bacterial colonies. The magnitude is determined by:

$$G = \sqrt{G_x^2 + G_y^2} \quad (19)$$

and the direction of the gradient is given by:

$$\theta = arctan\left(\frac{G_y}{G_x}\right) \quad (20)$$

This aids in accurately depicting edges within microbiological imagery [28], [29].

2.8. Non-Maximum Suppression

Non-Maximum Suppression (NMS) improves edge detection in images by selecting the strongest gradient values and eliminating the non-significant ones, thereby sharpening the edges. The formula for NMS is:

$$G_{NMS}(x,y) = \begin{cases} G(x,y) & \text{if } G(x,y) \geq G_{NMS}^{neighbors}(x,y) \\ 0 & \text{elsewhere} \end{cases} \quad (21)$$

This is crucial for more precise edge analysis in bacterial colony images, reducing errors and increasing confidence in detection [30].

2.9. Hysteresis Thresholding

Hysteresis Thresholding is an edge detection method in image processing that preserves significant edges while reducing noise. Wei [31] describes its application in detecting small targets in infrared imaging, which is relevant for bacterial image analysis. This method employs two thresholds, T_{high} and T_{low}, to classify pixels as edges. The process is defined by the following rules:

1. If $G(x,y) \geq T_{high}$ then $Edge(x,y) = 1$.
2. If $G(x,y) < T_{low}$ then $Edge(x,y) = 0$.
3. If $G(x,y) \geq T_{low}$ and is connected to a strong edge pixel, then $Edge(x,y) = 1$. (22)

Setting the thresholds T_{high} and T_{low} appropriately allows Hysteresis Thresholding to effectively differentiate bacterial colony edges while maintaining noise suppression. To ensure reproducibility of the results, this study provides detailed parameter configurations:

- Hysteresis Thresholds T_{high} and T_{low}:
  - The $\boldsymbol{T_{high}}$ threshold was set to 70% of the maximum gradient magnitude, capturing only the strongest edges.
  - The $\boldsymbol{T_{low}}$ threshold was set to 30% of the maximum gradient magnitude, ensuring weaker edges connected to strong edges were included in the

analysis. These threshold values were determined based on preliminary optimization to achieve a balance between edge detection accuracy and noise reduction.

- Kernel Filters for Gradient Operators:
  - **Sobel Operator**: A 3x3 kernel was used for gradient computation in the x and y directions, providing a balance between edge clarity and computation efficiency.
  - **Prewitt Operator**: A 3x3 kernel, slightly less sensitive to noise compared to Sobel, was employed to improve edge localization.
  - **Roberts Operator**: A 2x2 kernel was utilized to capture fine edge details, particularly in high-gradient regions.
  - **Scharr Operator**: A 3x3 kernel optimized for rotational symmetry was selected to enhance edge detection accuracy in noisy environments.

By defining these parameter settings and kernel configurations, this study ensures the reproducibility of results while contributing to a more robust application of Hysteresis Thresholding in the analysis of bacterial microscopic images.

2.10. Ground Truth Image

The Ground Truth Image is employed to enhance the accuracy of detection and segmentation in microscopic image analysis [32]. Otsu Thresholding separates objects from the background using the formula:

$$\sigma^2(t) = w_0(t) \cdot w_1(t) \cdot \left(\mu_0(t) - \mu_1(t)\right)^2 \qquad (23)$$

Which maximizes the variance between the two classes determined by the threshold value t.

2.11. Data Analysis Methods

In bacterial microscopic image analysis, metrics such as Precision, Recall, F-Measure, ECM, PSNR, and MCC are crucial for gauging the accuracy of edge classification. Precision focuses on the proportion of relevant findings, while Recall concentrates on the proportion of actual findings correctly identified, calculated as follows:

$$Precision = \frac{True\ Positive}{True\ Positive + False\ Positive} \qquad (24)$$

$$Recall = \frac{True\ Positive}{True\ Positive + False\ Negative} \qquad (25)$$

The F-Measure harmonizes these two by combining their values for binary classification performance:

$$F - Measure = \frac{2 \times Precision \times Recall}{Precision + Recall} \qquad (26)$$

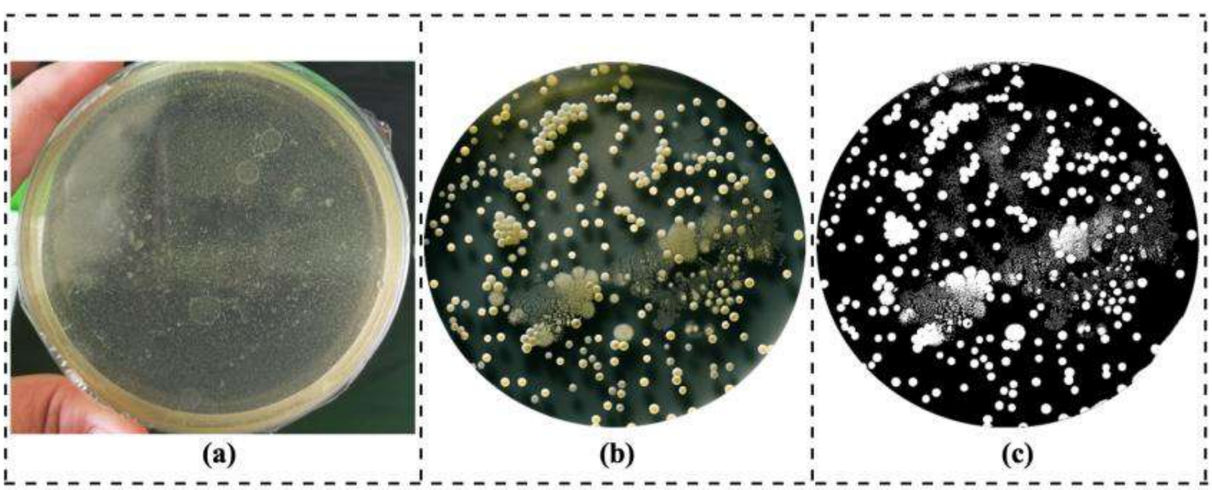

Figure 2. **(a)** Image of bacterial colonies captured using a regular camera **(b)** Image of bacterial colonies obtained from a microscope with 10x magnification **(c)** Ground Truth Data Image.

ECM measures the similarity among edge pixels based on their intensity and relative position, PSNR evaluates image quality against quantization errors, and MCC assesses the quality of predictions in imbalanced datasets using the formula:

$$MCC = \frac{(TP \times TN) - (FP \times FN)}{\sqrt{(TP+FP)(TP+FN)(TN+FP)(TN+FN)}} \quad (27)$$

This research underscores the importance of these metrics in enhancing the automatic counting of bacterial colonies and morphological analysis, supported by pertinent literature [33], [34], [35], [36], [37]. These methods enable a more precise and effective interpretation of image data.

3. Results and Discussion

3.1. Original Bacterial Colony Image Data versus Magnified Microscopic Data

Figure 2. presents a comparative analysis between original bacterial colony photographs and the data acquired from magnified microscopic observation. Figure 2(a). depicts a standard camera photograph of a bacterial colony, providing a rough visual context with minimal detail focused on individual colonies against a petri dish background. Figure 2(b). illustrates the bacterial colony captured with a 10x magnification microscope, allowing for a more detailed observation that could reveal additional morphological details and possibly some cellular structures.

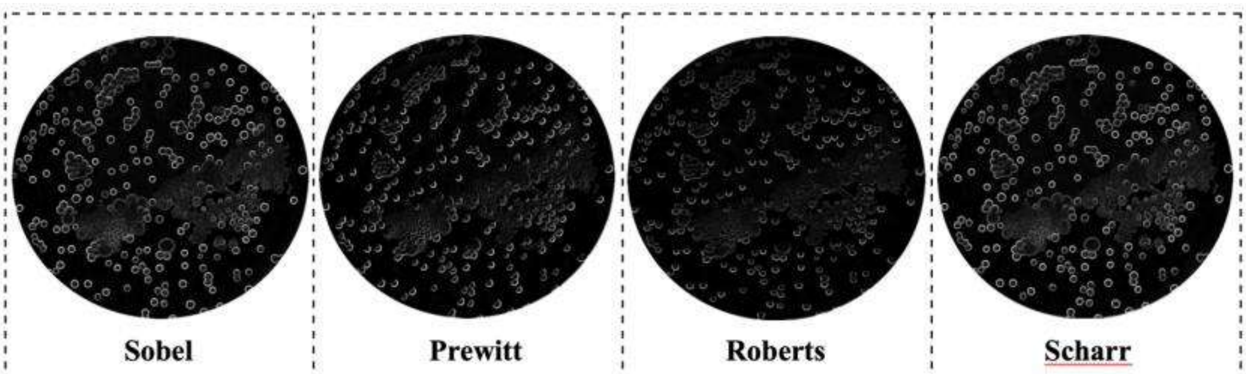

Figure 3. Magnitude of Gradient Operator (Sobel, Prewitt, Roberts, Scharr).

The enhanced color and texture clarity offer more information for subsequent analysis. Figure 2(c). represents the 'ground truth' data extracted from the microscopic image, where bacterial colonies have been highlighted or labeled for analysis.

This ground truth serves as the standard for validating the results from automated detection methods, showing a stark contrast and sharp distinction between the colonies and the background, crucial for detection algorithms and automated counting.

Related to recent literature:

- Karatepe [38] showed that advanced image processing is effective for detecting and enumerating E. coli in petri dishes, indicating its applicability for automated analysis of bacterial colonies in Figure 2 (a) and (b).
- Balmages [39] demonstrated that laser speckle imaging could visualize bacterial colonies in Figure 2 (a) and (b) more quickly than conventional methods, enhancing early detection of microbial growth.
- Minoni [40] explored Optical Forward Scattering for bacterial identification, showcasing its potential in converting Figure 2 (b) to a processed ground truth Figure 2 (c) and emphasizing its application in classifying bacterial colonies.

The progression from Figure 2 (a) to (c) exemplifies a refinement process that yields images amenable to quantitative analysis.

Contemporary studies endorse sophisticated imaging techniques for more precise and efficient bacterial colony enumeration, with implications for diagnostics, food safety, and microbiology research.

3.2. Visualization Image Data: Gradient Operator Magnitude

In Figure 3, we are presented with the visual results of edge detection using Sobel, Prewitt, Roberts, and Scharr gradient operators. These images display the magnitude of edge gradients as elicited by each respective operator. From a visual standpoint, several observations can be made:

- The Sobel and Scharr operators appear to produce more defined edges with a higher level of detail.
- The Prewitt operator generates results akin to Sobel but may lack some sharpness.
- The Roberts operator exhibits smoother and less contrasting edges compared to the others.

Considering recent academic contributions:

- Li [41] explored CNN-based edge detection, showing enhanced precision in SAR images, suggesting deep learning could surpass traditional gradient operators in accuracy and detail (SAR target image edge detection based on CNN).
- Deka [42] found that Sobel-based edge detectors outperform Prewitt and Laplacian in certain scenarios, correlating with observed enhanced edge detail in Sobel and Scharr (Comparative Analysis of FOD based Prewitt, Sobel & Laplacian Operators for Edge Detection on Freshwater Fish Images).
- Chakravathi [43] investigated FPGA architecture for real-time edge detection, demonstrating its effectiveness with Sobel and Scharr operators in producing consistent edges under noisy conditions.

The conclusions drawn based on the visualizations and related literature suggest that Sobel and Scharr operators may be favored for applications that require sharp and detailed edge detection, such as in texture analysis or high-resolution image processing. On the other hand, Prewitt and Roberts might be more suited for applications that benefit from a gentler approach, potentially being less sensitive to noise.

3.3. Visualization of Gradient Direction Data

Figure 4. provides a visual representation of the direction of gradient data as processed by the Sobel, Prewitt, Roberts, and Scharr operators. These visualizations typically illustrate the orientation of edges within the image by directly mapping various colors to represent different gradient directions.

From these images, we can deduce that:

- The Sobel and Scharr operators display a broader range of colors, suggesting they may be more capable of capturing subtle changes in gradient direction within the image.
- The Prewitt and Roberts operators seem to exhibit less color variation, which may indicate less precise detection of gradient

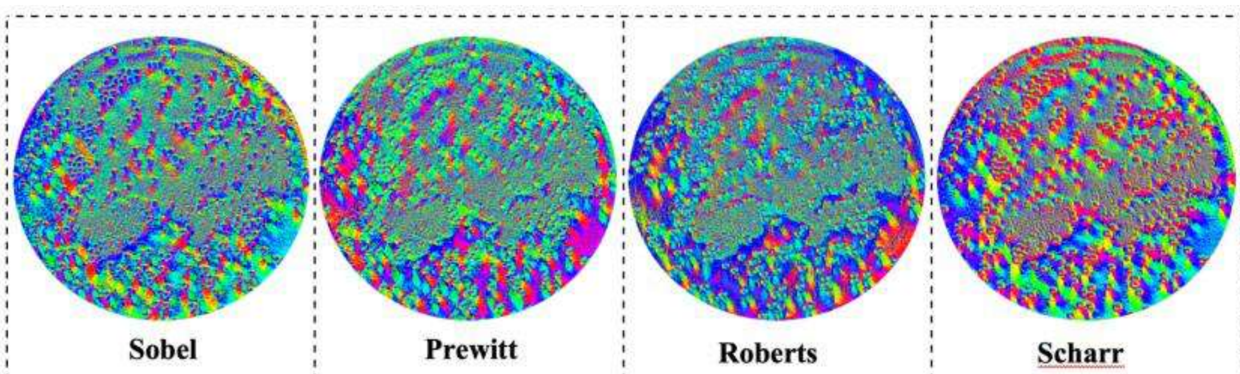

Figure 4. Visualization of Gradient Operator (Sobel, Prewitt, Roberts, Scharr).

direction when compared to Sobel and Scharr.

In the context of contemporary literature:

- Li [41] discussed the improvement in precision and detail using CNN-based edge detection, aligning with findings that Scharr and Sobel excel in gradient direction variation (SAR target image edge detection based on CNN).
- Chakravathi [43] emphasize the importance of speed and accuracy in real-time edge detection, also crucial for determining the correct gradient direction in noisy image conditions (FPGA based architecture for real-time edge detection).

Consequently, the capabilities of Sobel and Scharr in showcasing gradient direction variation might make them more favored for applications that require accurate gradient mapping, such as in robot navigation, where precise edge orientation is crucial. Prewitt and Roberts might be more suitable for applications that do not demand high-detail gradient direction and may be less sensitive to noise. The choice of operator must be tailored to the specific needs of the application and considerations of the image characteristics under analysis.

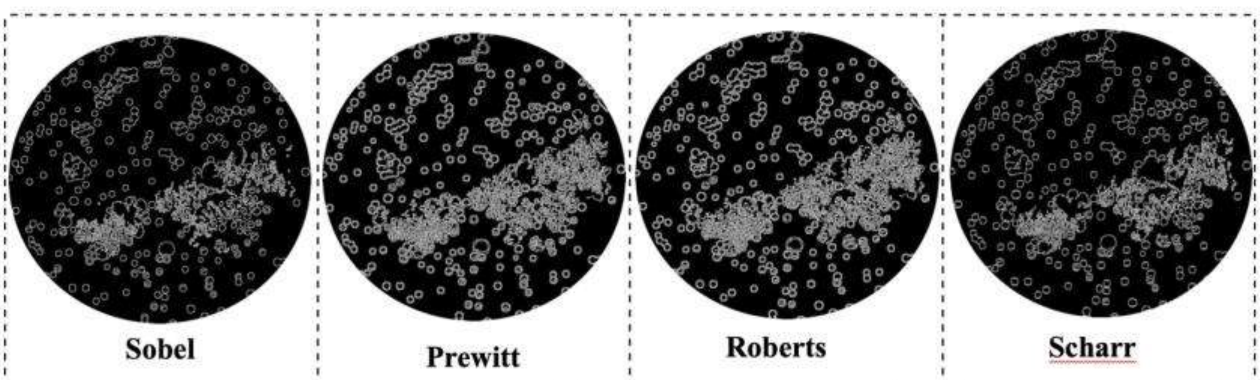

Figure 5. Result of NMS, Binary Threshold, and Hysteresis Threshold Processes.

3.4. The NMS, Binary Threshold, and Hysteresis Threshold Process

Figure 5. illustrates the results from the Non-Maximum Suppression (NMS), Binary Threshold, and Hysteresis Threshold processes applied to the four different edge detection operators: Sobel, Prewitt, Roberts, and Scharr. These processes enhance edge detection results by amplifying significant edge responses and improving the overall quality of edge detection.

From the images presented, we observe the following:

- Sobel and Scharr operators seem to produce cleaner, more defined edges, with Sobel displaying particularly high edge sharpness, which might indicate their effectiveness in implementing NMS and hysteresis thresholding techniques.
- Prewitt and Roberts, while similar in function to Sobel, exhibit slightly less sharpness, which could suggest a less precise gradient direction detection compared to Sobel and Scharr.

Linking to recent academic research:

- Dong [44] researched NMS-based adaptive edge detection, observing enhanced speed and accuracy in real-time applications, similar to the sharpness achieved by Sobel and Scharr (An Improved NMS-Based Adaptive Edge Detection Method and Its FPGA Implementation).

images, efficiently detecting edges without manual threshold settings, underscoring the value of automation in improving edge detection (Auto-thresholding Edge Detector for bio-image processing).

Therefore, the NMS, Binary Threshold, and Hysteresis Threshold processes are crucial for improving edge detection quality. Based on the visualizations provided, Sobel and Scharr seem more effective in utilizing these techniques to achieve clean and sharp edge detection. The incorporation of newer and adaptive algorithms, as discussed in the literature, can lead to further enhancements in edge detection applications.

3.5. Result from the Analysis Using Precision, Recall, and F-Measure Methods

Figure 6. displays the bar graphs representing the average performance of precision and recall, as well as the F-measure, for edge detection operators Sobel, Prewitt, Roberts, and Scharr. Here's a summary of the results:

- Sobel and Scharr have the highest precision (41.31%), indicating a strong ability to correctly identify true edges.
- Prewitt has the highest recall (29.54%), suggesting it is the most capable of identifying all actual edges.
- Roberts scores the highest in F-measure (23.45%), which might reflect a balanced trade-off between precision and recall.

Precision measures the proportion of true positive detections out of all positive detections made, while recall measures the proportion of actual positive detections made by the algorithm. F-measure is the harmonic mean of precision and recall, providing a single metric to evaluate overall performance.

In relation to recent scholarly discussions:

- Reddy [46] demonstrate that metrics like precision, recall, and F-measure, used in diabetic retinopathy image analysis, are also effective for assessing edge detection in clinical and biomedical contexts.
- Ma [47] found that the Fuzzy Box-counting Dimension Method (FBDM) enhances edge detection precision without preprocessing, excelling in denoising, potentially improving the precision of algorithms like Sobel and Scharr.
- Gautam & Biswas [48] showed that the Whale Optimization Algorithm (WOA) excels in noisy conditions, improving PSNR, Precision, Recall, and F-measure, indicating that such algorithmic enhancements can increase precision in edge detection.
- Yu [49] found that noise-resistant edge detection techniques, which maintain high resolution and minimize noise, yield high F-measure scores, signifying enhanced precision over recall in complex imaging scenarios.

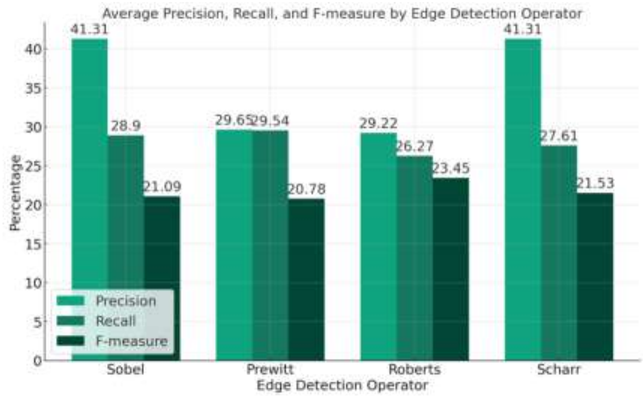

Figure 6. Average Precision and Recall (left image), Average F-Measure by Edge Detection Operator (right image).

From this analysis, it emerges that Sobel and Scharr may be more suitable for applications where precision is paramount, such as in medical image processing or bacterial pattern detection requiring highly accurate edges. Prewitt might be more appropriate for applications emphasizing recall, such as environmental monitoring where capturing every edge is vital. Roberts, with the highest F-measure, could offer the best compromise between precision and recall, making it a good choice for general applications requiring a balance of both metrics. The literature suggests that advanced techniques and optimization methods could significantly enhance all these metrics.

3.6. Results from Data Analysis using The ECM Method

3.6.1. The ECM Method in The Contrast and Dissimilarity Sections

Figure 7 and 8. presents the outcomes of the ECM for the contrast and dissimilarity of edges detected by Sobel, Prewitt, Roberts, and

Scharr operators. The bar charts illustrate two key textural features: contrast and dissimilarity.

- Contrast in ECM quantifies the intensity differences between adjacent pixels. Higher contrast values, particularly noted with the Sobel operator, suggest significant differences that could indicate sharper edge detection.
- Dissimilarity in ECM reflects the variation in texture by measuring how different adjacent pixels are. Higher dissimilarity values denote greater textural variation within the image.

Recent studies highlight the importance of local contrast and color dissimilarity in effectively detecting objects within images, suggesting that algorithms like Sobel and Scharr,

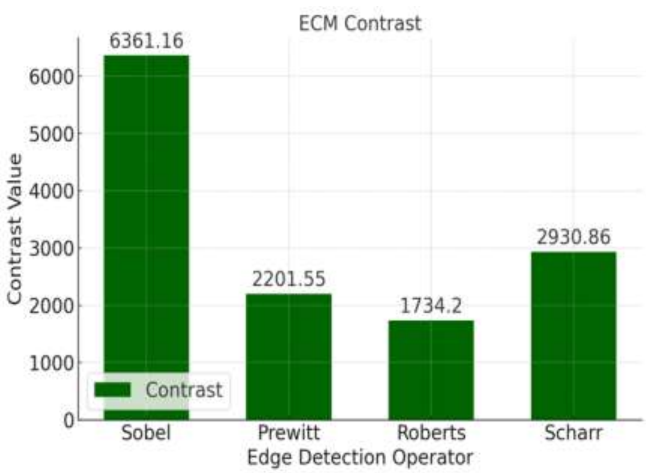

Figure 7. ECM Results in The Contrast Section.

which exhibit higher ECM contrast, might be preferred in applications where sharp edge delineation is crucial. On the other hand, applications that benefit from capturing texture variation might find value in the dissimilarity data provided by these methods.

- Robust edge detection methods, such as those resistant to noise, have been shown to maintain high resolution while minimizing noise interference, which could be indicated by a favorable balance of contrast and dissimilarity in ECM [50].

For bacterial colony microscopy, the edge quality enhanced by Sobel or Scharr operators can significantly aid in the identification and counting of colonies with greater accuracy. In practice, it may also be useful to combine such edge detection with further image-processing techniques, as suggested by recent research, to achieve optimal results. Advanced processing techniques, including deep learning algorithms and optimization methods, can significantly improve detection performance in microscopic image analysis.

3.6.2. The ECM Method in The Homogeneity, Energy, Correlation Sections

Within the realm of image processing, the ECM method, as shown in Figure 9,

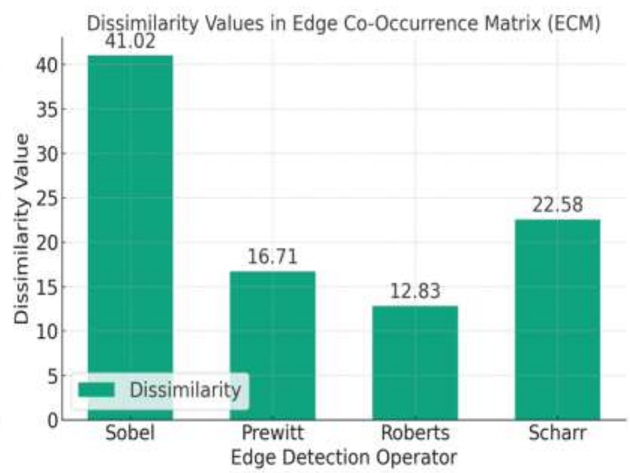

Figure 8. Dissimilarity Section of ECM.

is pivotal for analyzing Homogeneity, Energy, and Correlation. Recent studies provide valuable insights using this method, especially for comparing the performance of various edge detection operators such as Sobel, Prewitt, Roberts, and Scharr. This is particularly relevant in the context of microscopic images of bacterial patterns.

The ECM method offers a novel approach to assess and compare the effectiveness of these edge detection operators in terms of their ability to accurately represent and analyze the textural and structural properties of microscopic bacterial images. By examining the Homogeneity, Energy, and Correlation metrics, the ECM provides a comprehensive framework for understanding the nuances of edge detection in complex image patterns.

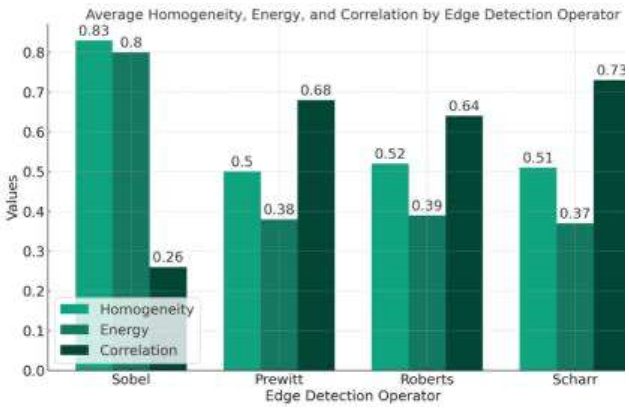

Figure 9. Results of Measurements for Homogeneity, Energy, Correlation in The ECM Method.

According to recent literature, the ECM has been effectively applied in diverse image processing applications, including:

- Enhanced Edge Detection Performance: Ren [50] developed a Gabor filter-based corner detection algorithm, surpassing the Harris algorithm in accuracy and noise immunity, beneficial for microscopic image edge refinement. Additionally, Chen [51] enhanced image interpolation accuracy using a nonlocal low-rank matrix completion method combined with edge detection and neural networks, demonstrating the potential of advanced techniques to improve edge detection in detailed bacterial imagery.
- Microscopic Image Processing: Zhang [52] demonstrated ECM's effectiveness in enhancing infrared target detection by improving edge and angle identification, which aids in distinguishing targets against varied backgrounds. This underscores ECM's value in applications requiring precise edge detection, such as microscopic imagery.

Graphic analysis as shown in Figure 8, that the Sobel operator exhibits higher Homogeneity, indicating superior texture uniformity, while the Scharr operator demonstrates higher Correlation, implying stronger inter-pixel relationships in the image. This suggests that for microscopic bacterial pattern images, where maintaining homogeneous texture and strong correlation is crucial, the Scharr operator may be more suitable.

Edge detection operators like Sobel and Scharr, characterized by high Homogeneity and Correlation values, are invaluable in extracting consistent texture features and maintaining the visual integrity of bacterial colonies in microscopy applications. This assists in more accurate bacterial identification and classification. These findings align with recent research suggesting that ECM and related texture metrics offer superior classification performance in applications such as corner detection, image enhancement, and shape classification.

3.7. Results of Data Analysis using The PSNR Method and The MCC Method

The performance evaluation of each edge detection operator at this stage is conducted using the PSNR method for its correlation to noise, and the MCC method for assessing the classification effectiveness of each operator, as illustrated in Figure 10 and 11. Recent research and data analysis using PSNR and MCC metrics for Sobel, Prewitt, Roberts, and Scharr edge detection operators lead us to several significant insights:

- Süzme & Guraksin [53] demonstrated that applying the Particle Swarm Optimization (PSO) algorithm to set thresholds in gradient-based edge detection improves image quality and lowers computational demands, enhancing PSNR and the efficacy of edge detection operators.
- Singh, Sharma, & Jain [54] developed a hybrid noise removal technique that enhances edge detection, evidenced by improved PSNR and SSIM metrics, indicating its efficacy in accurately identifying edges in microscopic bacterial images.
- Sankararao, Reddy, & Srinivas [55] showed the PSS filter's effectiveness in enhancing

edge detection and noise resistance in image processing, crucial for revealing fine details and improving boundary definition in microscopic analysis.

For microscopic imaging of bacterial patterns, this indicates a preference for the Sobel operator in applications requiring high PSNR levels, suggesting a better match with reference images. However, the Scharr operator, with its higher MCC value, shows superior classification performance, which is crucial for consistency in recognizing bacterial patterns.

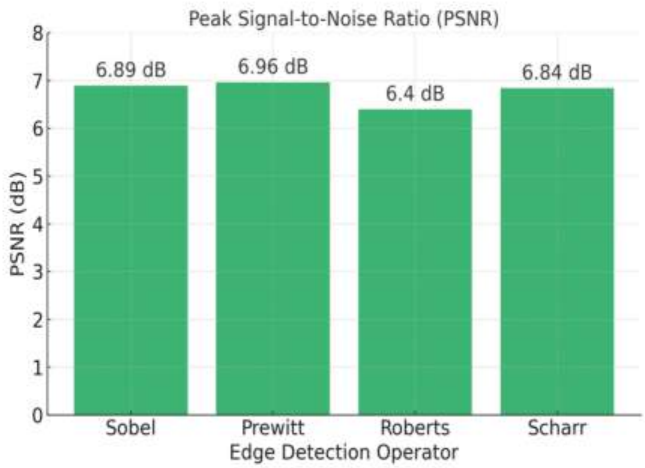

Figure 10. The PSNR Analysis Method shows Performance Measurement Results of The Evaluated Gradient Operators.

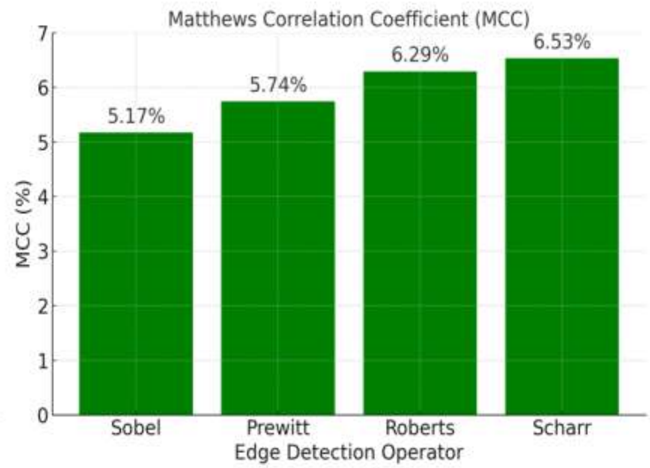

Figure 11. Performance Measurement Results of The Evaluated Gradient Operators using The PSNR MCC Method.

The chosen operator must strike a balance between the need for edge clarity and the capacity to maintain image quality, while minimizing classification errors. In the case of bacterial pattern microscopy, where edge detection accuracy is vital for counting and classifying colonies, an operator that provides a balance between high PSNR and robust MCC values is likely to deliver the most effective results.

3.8. Research Limitations and Prospects for Future Inquiry

In the scientific journey, acknowledging the limitations of our knowledge is as crucial as celebrating the progress we have made. This research, while providing valuable insights into the dynamics of edge detection in microscopic imaging, also unveils the methodological and applicative boundaries we face. These limitations reflect not just the complexity of the phenomena under investigation but also mark the starting points for deeper scientific exploration. Herein, we delineate key limitations identified during this study, which will guide future research directions and spur innovation in the field of microscopic image processing.

Research Limitations:

1. **Data Specificity**: The analysis was centered on a dataset of microscopic bacterial pattern images, which may not fully represent edge detection operator performance on other image types or under different imaging conditions.
2. **Application Specificity**: Edge detection operators were often evaluated in ideal conditions or highly specific application contexts, which may not fully reflect their performance in broader real-world scenarios or across various image types.
3. **Image Variability**: The images used for evaluation may lack sufficient noise variation, contrast, or detail to fully test the capabilities of the operators.
4. **Limited Performance Metrics**: Metrics such as PSNR and MCC provide insights

into certain aspects of image quality and edge detection accuracy but do not always capture overall performance in practical applications.

5. **Parameter Dependence**: Edge detection operator performance is heavily dependent on parameter selection, and this optimization is often manually conducted, limiting the ability to extrapolate findings to automated applications.
6. **Computational Complexity**: The analysis did not account for the computational complexity or processing time required by each operator, which are critical factors in real-time applications.

Prospects for Future Research:

In light of these findings and limitations, recommended follow-up actions include:

1. **Trials on Diverse Datasets**: Further research should test edge detection operators on a variety of image types, including non-medical ones, to validate findings and extend their applicability.
2. **Algorithm Development and Optimization**: Developing hybrid algorithms that combine the strengths of different edge detection operators may yield improvements in precise and efficient edge detection.
3. **Automated Parameter Selection**: Future research could focus on developing methods for automatic parameter optimization that could enhance the generalizability of operators.
4. **Real-time Performance Analysis**: Studies evaluating the real-time performance of edge detection operators would be invaluable, especially for applications where processing time is critical.
5. **Integration with Machine Learning Systems**: Incorporating findings with machine learning systems to improve classification and predictions based on enhanced edge features could provide significant advancements in image processing.

By considering these limitations and building upon the findings, the research community can continue to develop more sophisticated and reliable techniques for edge detection, which are essential for applications such as the analysis of microscopic bacterial pattern images, where accuracy and reliability are paramount.

4. Conclusion and Recommendations

4.1. Conclusion

This study focuses on utilizing various gradient operators within the Canny Edge Detection Algorithm for enhanced microscopic imaging of Bacillus sp. bacteria. It critically assesses the effectiveness of Sobel, Prewitt, Roberts, and Scharr operators, highlighting their distinct capabilities in capturing bacterial cell edges and textures. Among these, the Scharr operator stands out for its superior inter-pixel correlation and classification performance, making it highly suitable for detailed bacterial morphology analysis.

The research underscores the Scharr operator's unique ability to accurately identify and classify subtle morphological features in bacteria, surpassing conventional imaging methods. This precision is vital for understanding bacterial environmental interactions and intrinsic characteristics, with significant implications for disease diagnostics and biotechnological applications.

Conclusively, the study advocates for continuous innovation in microscopic imaging techniques, particularly emphasizing the refinement and expanded use of gradient operators for in-depth bacterial analysis. This advancement not only enriches our understanding of microbiological imaging but also paves the way for practical applications in related fields.

4.2. Recommendations

For an enhanced understanding of edge detection in microscopic imagery, it is recommended to explore a range of gradient operators to reveal intricate details within bacterial structures. Emphasis is placed on the continual improvement of resolution and clarity in microscopic images to enable more comprehensive analysis and the potential for new discoveries. The practical application of these findings is advocated in the diagnosis of bacterial diseases and biotechnological research. Interdisciplinary collaboration, encompassing fields such as microbiology, imaging, and cross-disciplinary studies, is considered crucial for enriching understanding and facilitating the widespread application of these findings. Additionally, a focus is placed on the need for developing training programs to enhance the understanding and implementation of microscopic imaging techniques, with the objective for this technology to contribute significantly in research and medical practice contexts.

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