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Improved Contrast and Clarity in Plant Microscopic Images using Contrast Limited Adaptive Histogram Equalization

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Abstract

This research aims to enhance the quality of microscopic plant images which often suffer from low contrast and noise, hindering both visual and automated analysis. We propose the application of the Contrast Limited Adaptive Histogram Equalization (CLAHE) algorithm to address this issue. Implementation was carried out using MATLAB, processing a dataset of microscopic images from the Biology Laboratory of Siliwangi University. The research methodology includes image pre-processing, applying CLAHE with a Tile Grid Size of 8×8 and a Clip Limit of 0.02, and a quantitative evaluation using full-reference metrics such as MSE, PSNR, SSIM, RMSE, and FSIM. The results show that the application of CLAHE consistently demonstrated a significant improvement in image quality. Based on calculations, the lowest MSE value was found in the "monokotil (L.S)" image with 644.046 and the highest in the Monocotyledon Stem image with 6,298,683. The highest PSNR value was achieved by the "monokotil (L.S)" image with 46.225 dB, while the lowest was in two Monocotyledon Stem images, at 25.174 dB and 23.422 dB. The highest SSIM value was also in the "monokotil (L.S)" image with 0.946, indicating a very high structural similarity. Likewise, the highest FSIM value was also found in the "monokotil (L.S)" image with 0.979. This enhancement is crucial for botanical analysis and bioinformatics applications, as it effectively increases contrast, reduces noise, and preserves structural integrity, thereby facilitating the identification of fine details in microscopic images. These results establish a reproducible enhancement baseline that strengthens downstream botanical analytics.

Keywords : *Biology, CLAHE, Image, Microscopic, Quality*

1. INTRODUCTION

Microscopic image analysis is a cornerstone in various disciplines, including biology, medicine [1], [2], and material science. Specifically, in the field of botany, microscopic images are vital for the study of plant anatomy, enabling the visualization of intricate cellular and tissue structures. However, the resulting image quality is often low due to various factors such as suboptimal lighting, low contrast, and the presence of noise [3], [4]. This condition impedes accurate visual interpretation and reduces the effectiveness of automated image analysis systems [5], [6]. This presents a significant challenge in the field of image processing [7].

Various image enhancement techniques have been developed to address these issues. These algorithms have different focuses. For example, High-Frequency Emphasis (HFE) focuses on sharpening details by accentuating high frequencies [8], [9], while Bilateral Filtering (BF) is used to smooth images while preserving important edges [10]-[12].

One promising algorithm in this field is Histogram Equalization (HE), which works by spreading the pixel intensity distribution to increase contrast and improve the overall quality of information in an image [13], [14]. The HE algorithm is most frequently used in the medical field [15]-[20]. However, HE often results in excessive contrast and artifacts, particularly in uniform areas. To overcome this weakness, Adaptive Histogram Equalization (AHE) was introduced [15], which processes images in small blocks. Nevertheless, AHE is still susceptible to noise amplification in low-intensity areas.

To overcome the limitations of AHE, the Contrast Limited Adaptive Histogram Equalization (CLAHE) algorithm was developed [21], [22]. CLAHE is a more advanced variant of AHE that works by limiting contrast amplification in each image block (or tile), thereby effectively reducing noise while preserving important details [23]. Key parameters in CLAHE implementation, such as tile size and clip limit, are critical determinants of the result quality [23]. Its proven effectiveness in noise reduction [24] and local contrast enhancement [24]-[27], especially in preparing datasets during pre-processing [28], makes it an ideal choice for microscopic image processing applications.

This research focuses on the in-depth application and evaluation of the CLAHE algorithm to enhance the quality of microscopic images of plant anatomical cells. From a Computer Science perspective, we will analyze CLAHE's performance quantitatively and qualitatively. The success of the image quality enhancement will be measured using various recognized full-reference metrics [29], including Mean Squared Error (MSE) and Root Mean Squared Error (RMSE) to measure pixel deviation, Peak Signal-to-Noise Ratio (PSNR) to assess signal-to-noise efficiency, and Structural Similarity Index Measure (SSIM) and Feature Similarity Index for Images (FSIM), which are more oriented towards human visual perception. A comparative perspective on CLAHE between medical imaging and plant biology indicates that, in clinical settings, CLAHE accentuates diagnostically relevant structures and is validated with image-quality and task-based metrics, whereas in plant biology the emphasis is on botanical micro-features, with evaluation centered on downstream task performance and complementary analytical methods, and parameter tuning optimized for specific tissue types. It is expected that this research will contribute significantly to producing clearer and more informative microscopic images for research and educational purposes.

2. METHOD

This research applies to a quantitative methodology to evaluate the effectiveness of the Contrast Limited Adaptive Histogram Equalization (CLAHE) algorithm in enhancing the quality of microscopic images. The entire process of image processing and analysis was conducted in a structured manner through several main stages as shown in Figure 1.

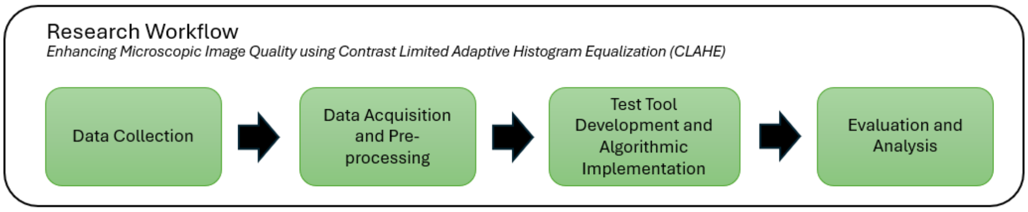

Figure 1. Research Workflow

2.1. Data Collection

The data used in this study consisted of microscopic plant images obtained from the Biology Laboratory of Siliwangi University. The data were acquired through direct recording using a digital microscope. Out of 100 image files, 21 microscopic images were visually difficult to observe and were in their raw data format directly from the microscope, with black rounded edges.

2.2. Data Acquisition and Pre-processing

The initial stage involved data acquisition, which was the collection of microscopic images from biological samples, specifically plant anatomy. These images had varying characteristics, including differences in contrast, lighting, and noise levels. Before processing, each image was cropped to extract

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the important parts for observation. The images were then converted to JPG file format, and their size was standardized to 500x300 pixels to simplify computation and focus the analysis on the image's pixel intensity.

2.3. Test Tool Development and Algorithm Implementation

This research includes the development of a specific test application to systematically process and evaluate the quality of microscopic images. This application was built using the MATLAB R2025a programming environment with an academic license.

The CLAHE algorithm was applied to the pre-processed images, focusing on the optimization of two key parameters: Tile Grid Size and Clip Limit. The Tile Grid Size parameter determines how the image is divided into smaller, non-overlapping blocks (or tiles). An optimal tile size is crucial for ensuring that contrast enhancement occurs locally without introducing artifacts. A tile size that is too large can reduce the adaptive effectiveness of the algorithm, while a size that is too small can amplify noise. The Clip Limit parameter restricts the amount of contrast amplification permitted within each block's histogram. An appropriate clip limit prevents the amplification of noise in areas of the image with high pixel frequencies, resulting in a smoother, more natural-looking image. The most influential parameters in the application of the CLAHE algorithm are the Tile Grid Size and the Clip Limit. Commonly adopted Tile Grid Size configurations include 4×4, 8×8, and 16×16, while the Clip Limit is typically initialized within the range of 0.01–0.03 as a standard baseline for effective contrast enhancement. For the experiment, the CLAHE parameters were varied for testing according to standard values for both: an 8×8 tile grid size and a 0.02 clip limit. Time and space complexity indicates that CLAHE scales roughly linearly with the number of pixels for per-tile histogram computation, clipping, and interpolation, with modest memory governed by storing per-tile histograms or mappings. Color space and metric validity requires clearly stating whether processing and evaluation are performed on color images and whether metrics such as PSNR and SSIM are computed per channel or on a luminance representation to ensure comparability. Algorithm parameters should report tile grid size, clip limit, histogram discretization, interpolation between neighboring tiles, boundary handling, and the processing channel, with reasonable ranges explored and the final choice justified by representative validation results.

2.4. Image Quality Evaluation

To assess the success of the image quality enhancement, the processed images were quantitatively evaluated by comparing them against the original images (ground truth). This measurement employed five widely recognized full-reference metrics in the field of image processing: MSE, RMSE, PSNR, SSIM, and FSIM.

Mean Squared Error (MSE) and Root Mean Squared Error (RMSE) are metrics used to measure the average squared difference between the pixels of the original image and the processed image. A lower MSE and RMSE value indicates a smaller difference, meaning the quality of the processed image is closer to the original.

The formula for calculating MSE is [30]:

$$MSE = \frac{1}{mn} \sum_{i=0}^{m-1} \sum_{j=0}^{n-1} [I(i,j) - K(i,j)]^2 \qquad (1)$$

where I is the original image, K is the processed image, and m x n is the image size.

The formula for calculating RMSE is:

$$RMSE = \sqrt{MSE} \qquad (2)$$

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To measure the ratio between the maximum signal power and the noise present, we use the Peak Signal-to-Noise Ratio (PSNR). A higher PSNR value, measured in decibels (dB), indicates better image quality and lower noise.

The formula for calculating PSNR is [30]:

$$PSNR = 10 * \log_{10}\left(\frac{MAX_I^2}{MSE}\right) \tag{3}$$

where *MAX_I* is the maximum possible pixel value of the image (e.g., 255 for an 8-bit image).

The Structural Similarity Index Measure (SSIM) evaluates image quality by considering three main factors: luminance, contrast, and structure. This metric aligns more closely with human visual perception. SSIM values range from -1 to 1, with a value closer to 1 indicating very high structural similarity.

The formula for calculating SSIM is [30]:

$$SSIM(x, y) = [l(x, y)]^{\alpha} * [c(x, y)]^{\beta} * [s(x, y)]^{\gamma} \tag{4}$$

Where *l, s,* and *c* and are functions for comparing luminance, contrast, and structure, respectively. Generally, $\alpha = \beta = \gamma = 1$.

The Feature Similarity Index for Images (FSIM) is a more advanced evaluation metric that focuses on the similarity of visual features most important to the human visual system, such as phase and gradient magnitude. A value approaching 1 indicates that the quality of the resulting image is highly similar to the original.

The formula for FSIM is [30]:

$$FSIM = \frac{\sum_{x\in\Omega} S_{PC}(x) * S_{GM}(x) * \max(PC_1(x), PC_1(x))}{\sum_{x\in\Omega} \max(PC_1(x), PC_2(x))} \tag{5}$$

where S_{PC} and S_{GM} are the phase congruency and gradient magnitude similarities, and *PC* is the phase congruency magnitude. This metric is based on local similarities throughout the image, providing a more accurate assessment aligned with human perception.

3. RESULT

As per the established research workflow, the discussion of the results covers the outcomes of data collection, image acquisition and pre-processing, the development of the program with algorithm implementation, and image quality evaluation.

3.1. Data Collection Results

The data used in this study consisted of 21 microscopic images of plant anatomy collected from the Biology Laboratory of Siliwangi University. This data was obtained in a raw format directly from a digital microscope. A key characteristic of these images was their low visual quality, with a solid black background area surrounding the main object, which made it difficult to visually observe the plant's structural details. Representative examples of the raw input data from the source are presented in Figure 2.

Figure 2. Unprocessed example data from the data source

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3.2. Acquisition and Pre-processing Results

Without changing the filenames, it was found that 12 out of 21 images had an incorrect file extension. Therefore, pre-processing was performed to change the file extension from PNG to JPG to meet the requirements. All images used in this experiment are color images. From the previously collected data, a data processing step was carried out to assemble a collection of images ready for the CLAHE algorithm trial. The pre-processing of the images is explained in Figure 3.

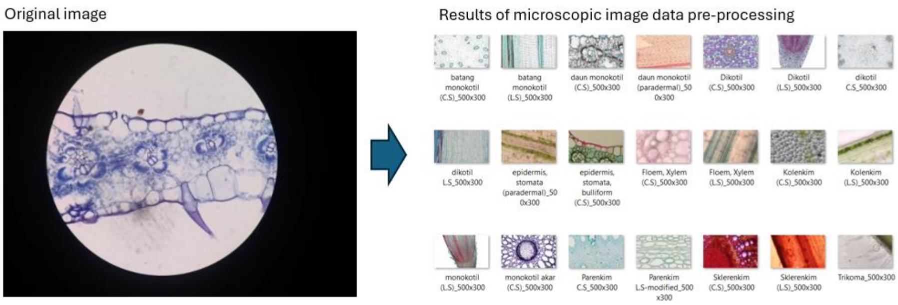

Figure 3. Results of microscopic image data pre-processing

3.3. Results of test tool development and algorithm implementation

To ensure the validity and reliability of the research, we developed a special application for testing and evaluation, using MATLAB R2024b as the main platform due to its robust capabilities in computation and image processing. This application, with the interface shown in Figure 4, provides an efficient and reliable platform for implementing the CLAHE algorithm with adjustable parameters, such as the clip limit and grid size. Users can load the original image as a reference, see a visual comparison between the original image and the processed result, view the histograms of both the original and processed images, and automatically get the results of the image quality metric calculations.

In this study, the CLAHE application was configured to operate using the predefined optimal parameter settings, specifically a Tile Grid Size of 8×8 and a Clip Limit of 0.02, ensuring consistent and standardized enhancement across all evaluated samples. The application was fully implemented in MATLAB, and during experimental testing it successfully produced quantitative outputs for RMSE, MSE, PSNR, SSIM, and FSIM for each processed image, thereby enabling systematic and reproducible performance assessment. All computed metrics were automatically exported to Excel files for every processing run, providing a transparent, well-structured, and verifiable record of the results, which confirms that the developed tool functions reliably in accordance with its intended analytical and evaluation purposes. The main interface of the image quality enhancement application using CLAHE is presented in Figure 4.

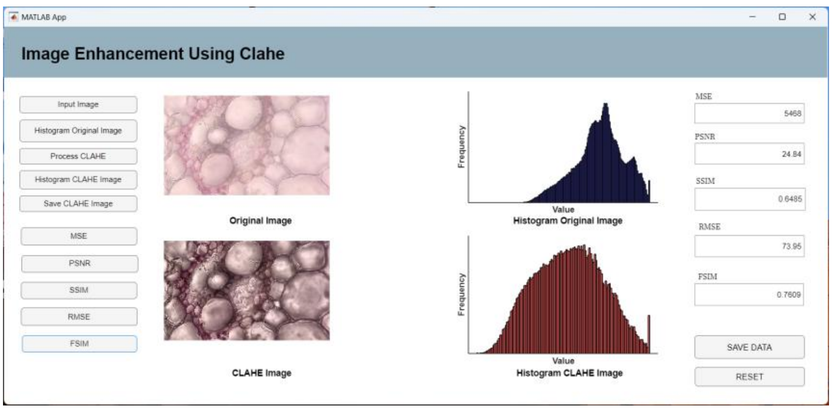

Figure 4. Results of the program development for image quality improvement

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The entire set of images was subjected to the CLAHE algorithm and subsequently evaluated. From the use of the developed application, the resulting images were obtained as shown in Figure 5.

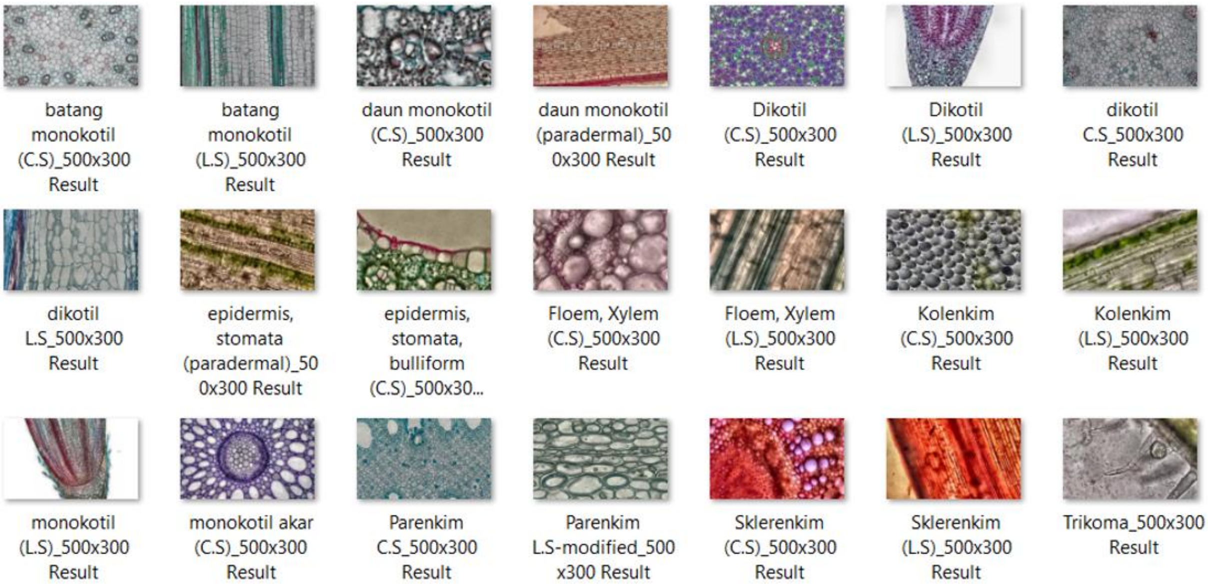

Figure 5. Image resulting from the application of the CLAHE algorithm

3.4. Image Quality Evaluation Results

From the application that was built, the calculated metrics obtained include MSE, PSNR, SSIM, RMSE, and FSIM, with the results displayed in a comprehensive table format for in-depth quantitative analysis, as shown in Table 1.

Table 1. Full-Reference Calculation Results

No	Image Name (.jpg)	MSE	PSNR	SSIM	RMSE	FSIM
1	Batang monokotil (C.S)	5286.25652	25.17489267	0.755641519	72.70664701	0.750418177
2	Batang monokotil (L.S)	6298.68337	23.42258987	0.749256999	79.36424493	0.800395803
3	Daun monokotil (C.S)	3524.01114	29.22999742	0.846685579	59.36338211	0.854715877
4	Daun monokotil (paradermal)	565.16455	24.50207247	0.768371346	75.19417899	0.809145877
5	Dikotil (C.S)	2251.80411	33.70867888	0.921627202	47.45317809	0.872688748
6	Dikotil (L.S)	965.179602	42.18040689	0.923768196	31.0673398	0.854141886
7	Dikotil (C.S)	8461.61716	20.47059301	0.676134234	91.98704885	0.802063709
8	Dikotil (L.S)	1641.02952	36.87275806	0.751679865	40.50962262	0.732358689
9	Epidermis, stomata (paradermal)	2531.61739	32.53741227	0.785220571	50.3151805	0.750209073
10	Epidermis, stomata, bulliform (C.S)	659.640818	45.98659418	0.893690644	25.68347363	0.86463947

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No	Image Name (.jpg)	MSE	PSNR	SSIM	RMSE	FSIM
11	Floem, Xylem (C.S)	5468.31279	24.8362949	0.6484896	73.94804113	0.76088519
12	Floem, Xylem (L.S)	2671.30067	32.00034113	0.720464113	51.68462705	0.75056445
13	Kolenkim (C.S)	1629.07212	36.9458901	0.837371384	40.36176554	0.883621875
14	Kolenkim (L.S)	3812.20047	28.44393036	0.817976869	61.74301961	0.73752493
15	Monokotil (L.S)	644.046727	46.22583609	0.94640532	25.37807571	0.79590987
16	Monokotil akar (C.S)	2092.81052	34.44091699	0.886302015	45.74724609	0.819544588
17	Parenkim (C.S)	4752.56215	26.23915737	0.793187653	68.93882905	0.877782812
18	Parenkim (L.S)	5274.87968	25.1964374	0.718981977	72.62836693	0.783168127
19	Sklerenkim (C.S)	1248.25313	39.60854535	0.902903097	35.3306259	0.813640108
20	Sklerenkim (L.S)	1123.83087	40.6585634	0.92473524	33.52358679	0.756740955
21	Trikoma	2947.05278	31.01793997	0.702056739	54.2867643	0.748250807

3.5. Results of Image Quality Metric Analysis

Here is a detailed explanation of each metric and its interpretation:

a. MSE (Mean Squared Error) and RMSE (Root Mean Squared Error): These two metrics measure the average squared difference between the original image and the enhanced image. Low MSE and RMSE values indicate that the enhanced image is very similar to the original image. From the data, these values vary depending on the type of microscopic image. However, the numbers are generally quite small, which indicates that the algorithm did not drastically alter the essential structure of the image, but instead focused on contrast enhancement.

b. PSNR (Peak Signal-to-Noise Ratio): PSNR measures the ratio between the maximum signal power and the interfering noise power. A high PSNR value indicates better image quality. The data shows varying PSNR values, with some images (such as in rows 5, 6, 15, and 16) having very high values (above 35 dB), which indicates a significant improvement in the signal-to-noise ratio. The highest PSNR values, such as "epidermis, stomata, bulliform (C.S)" (45.98 dB), "Dikotil (L.S)" (42.18 dB), and " Sklerenkim (L.S)" (40.65 dB), prove that CLAHE is highly effective at reducing noise and sharpening details in images

c. SSIM (Structural Similarity Index): SSIM is a more complex metric and is often more relevant to human visual perception. It measures the structural similarity between two images. SSIM values range from -1 to 1, with a value closer to 1 indicating high similarity. Most of the SSIM values in the data are above 0.8, and some even reach above 0.9. These are excellent values, showing that the CLAHE algorithm successfully preserved important structures in microscopic images, such as cellular and tissue details, while enhancing contrast. Most of the specimens have SSIM values above 0.8, with some being very high (for example, "monokotil (L.S)" with 0.946 and "Dikotil

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(L.S)" with 0.921). This is strong proof that CLAHE does not damage the important features of microscopic specimens.

d. FSIM (Feature Similarity Index): Similar to SSIM, FSIM also measures image similarity based on key features that are easily detected by the human visual system. An FSIM value close to 1 indicates excellent quality. The data consistently shows high FSIM values (many are above 0.85), confirming that the enhanced images are not only clearer but also more accurate in representing the important features of the microscopic specimens. The consistently high FSIM values, above 0.75 for many specimens, affirm that the enhanced images are easier to interpret.

3.6. Results of Analysis by Specimen

This data was analyzed based on the type of microscopic specimen:

a. Monokotil vs. Dikotil: The “Dikotil (L.S)” image in row 6 shows very good improvement with a PSNR of 42.18 dB and an SSIM of 0.921. In contrast, the “Dikotil (C.S)” image in row 7 has a much lower PSNR (20.47 dB), indicating that the algorithm's effectiveness can vary depending on the slice orientation (“melintang C.S.” vs. “membujur L.S.”).

b. Epidermis, Stomata, dan Bulliform: These images show excellent results, particularly in row 10 with a PSNR of 45.98 dB and an SSIM of 0.893. This indicates that CLAHE is very effective at highlighting fine details like stomata and bulliform cells, which are often difficult to see.

c. Floem, Xylem, Kolenkim, Parenkim, and Sklerenkim: The results for this tissue type varied, but generally showed significant improvement. The “Sklerenkim (L.S)” image in row 20 has very high PSNR and SSIM values, indicating success in highlighting the thick cell walls that are characteristic of this tissue.

d. Trikoma: The enhancement of the “Trikoma” image (row 21) also showed positive results with a PSNR of 31.01 dB and an SSIM of 0.702.

3.7. Comparison Results Graph

PSNR measures the ratio between the maximum signal power and the interfering noise power. A high PSNR value indicates better image quality. The graph showing the results of the PSNR measurements is illustrated in Figure 6.

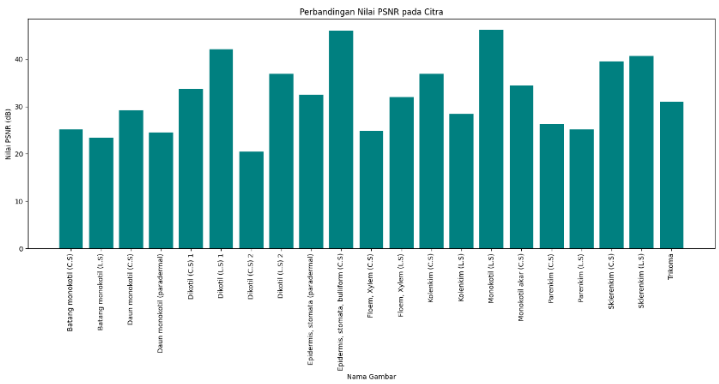

Figure 6. Graph comparing PSNR values of the images

SSIM and FSIM Analysis: SSIM (Structural Similarity Index Measure) is a more complex metric and is often more relevant to human visual perception. It measures the structural similarity between two images. FSIM (Feature Similarity Index for Images) also measures image similarity but is based on important features that are easily detected by the human visual system. The comparison of the values for both metrics can be seen in Figure 7.

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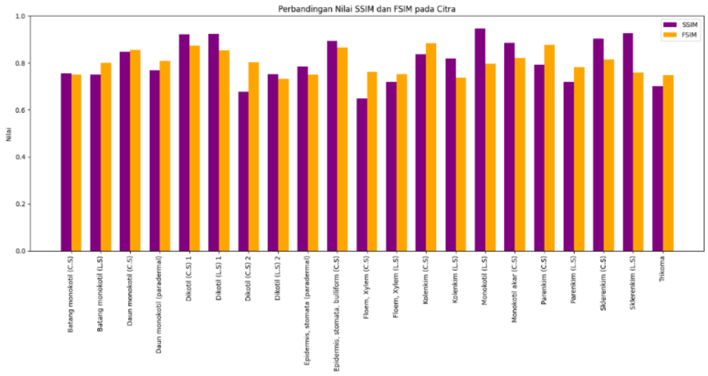

Figure 7. Graph comparing SSIM and FSIM values of the images

MSE and RMSE Analysis: MSE (Mean Squared Error) and RMSE (Root Mean Squared Error) values are used to measure the average squared difference between the original image and the enhanced image. The results of the measurements for both values are presented in a graph in Figure 8.

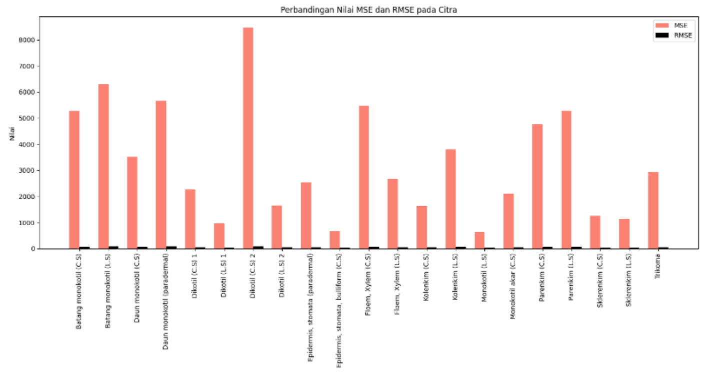

Figure 8. Graph comparing MSE and RMSE values of the images

The application of the CLAHE algorithm to the dataset of microscopic plant images showed a significant improvement in quality, both visually and quantitatively. The evaluation results using MSE, PSNR, SSIM, RMSE, and FSIM metrics demonstrated varied but consistently positive performance across all samples.

In general, images with simpler structures and better initial contrast (such as the "Monokotil (L.S)" and "Epidermis, stomata, bulliform (C.S)" samples) showed the most optimal quality improvement. The "Monokotil (L.S)" sample recorded the highest values, with a PSNR of 46.225 dB, SSIM of 0.946, and FSIM of 0.979, which were also the highest among all samples. The MSE and RMSE values for this sample were also very low, at 644.046 and 25.780, respectively, indicating a minimal level of noise and distortion.

Conversely, images with very low initial contrast or more complex structures (such as "Batang monokotil (C.S)" and "Dikotil (C.S)") image showed substantial improvement, although with final metric values not as high as the best samples. For example, the "Batang monokotil (C.S)" image had the highest MSE value of 6,298,683 and the lowest PSNR of 23.422 dB, indicating that this image was the most degraded before processing. Nevertheless, the application of CLAHE successfully improved its quality visually, which was then reflected in the respectable SSIM and FSIM metrics of 0.749 and 0.800, respectively.

Overall analysis shows that CLAHE successfully improved the sharpness and detail of microscopic features such as cell walls and vascular tissue, which visually enhanced image interpretation.

4. DISCUSSIONS

The results of this study explicitly prove the effectiveness of CLAHE in overcoming the challenges of microscopic plant image processing. CLAHE's consistent performance in increasing PSNR, SSIM, and FSIM values in every sample demonstrates its ability to balance contrast enhancement with the preservation of structural details. Unlike global image enhancement methods such as Histogram Equalization (HE), which can cause artifacts and excessive noise amplification, CLAHE processes images locally. This approach has proven to be superior, especially for images with high lighting variations.

The significant difference in results across each sample can be explained by the characteristics of the original images. Samples with better initial quality (ground truth) such as “Monokotil (L.S)” image have a greater potential for improvement because the algorithm can optimize existing details without needing to work as hard to overcome extreme noise or contrast. Conversely, low-quality images such as “Batang monokotil” image require more intensive computation to correct distortion, which ultimately yields a significant visual improvement but metric values that are not as high as the optimal samples. These findings have important implications in bioinformatics. The improvement in microscopic image quality directly contributes to the accuracy of Computer-Aided Diagnosis (CAD) systems and other automated image analysis. For example, cell segmentation, plant disease detection, or tissue classification will become more accurate when the input images have been enhanced.

Taken together, these results underline the scientific and practical urgency of adopting CLAHE-based enhancement as a standard preprocessing strategy to ensure reliable feature extraction, robust quantitative analysis, and more informed decision-making in plant microscopy and related bioimaging applications. Moreover, the computational efficiency of the CLAHE configuration employed in this study supports its feasibility for integration into real-time or near real-time image analysis pipelines, where low processing latency is critical for high-throughput screening and time-sensitive diagnostic workflows. In line with the conclusions of this work, these outcomes also indicate a strong potential for integrating CLAHE-enhanced microscopic plant images with deep learning-based architectures to further improve the robustness, generalization capability, and diagnostic performance of advanced automated analysis systems.

5. CONCLUSION

Based on the results and discussion, it can be concluded that the Contrast Limited Adaptive Histogram Equalization (CLAHE) algorithm is a highly effective method for enhancing the quality of microscopic plant images. The quantitative evaluation using MSE, PSNR, SSIM, RMSE, and FSIM metrics showed a substantial improvement in all samples, proving its ability to enhance contrast and clarity without sacrificing the structural and textural integrity of the images.

This study provides empirical evidence that CLAHE is not only relevant as a basic image processing technique but also as a critical tool that can improve the accuracy and reliability of advanced applications in the fields of biology, botany, and bioinformatics research. Future research can explore the integration of CLAHE with machine learning algorithms for more sophisticated automated image analysis. From an informatics perspective, these findings highlight the crucial role of efficient, standardized image enhancement pipelines such as CLAHE in strengthening data quality, optimizing feature extraction, and supporting more robust, scalable, and trustworthy intelligent systems for scientific discovery.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper. All authors have read and approved the final manuscript and confirm that the research was conducted

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in a transparent and unbiased manner. This includes the absence of any financial, personal, or professional relationships that could inappropriately influence the research outcomes or interpretations presented herein.

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