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Detection of Leukemia and Its Subtypes Using MATLAB: An RGB Color Code Analysis

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ABSTRACT

Objective: To develop and evaluate an automated technique for the detection and classification of leukemia subtypes using RGB color code analysis combined with image segmentation. **Study Design:** Experimental study. **Place and Duration of Study:** Lady Reading Hospital, Peshawar, from January 2023 to February 2025. **Methods:** An RGB-based color thresholding method was designed with a 3D median filter to isolate leukemic cells from stained blood smear images. The technique segmented out unwanted regions such as red blood cells and background artifacts while preserving diagnostic features of abnormal white blood cells. Color codes (RGB and hexadecimal values) were extracted from the segmented cells and stored in Excel format. Both automated and manual analysis of these color patterns were performed to distinguish between leukemia subtypes. The system was tested primarily on Acute Myeloid Leukemia (AML) and Acute Lymphocytic Leukemia (ALL) images, with further potential application to Chronic Myeloid Leukemia (CML) and Chronic Lymphocytic Leukemia (CLL). **Results:** The segmentation algorithm achieved high accuracy, with 97.63% for AML and 97.64% for ALL. Distinct color code patterns were successfully identified and associated with specific leukemia subtypes. The manual method also supported color recognition for subtype classification, enhancing diagnostic clarity. **Conclusion:** The proposed automated approach using RGB color code analysis and image segmentation provides a reliable, efficient, and scalable

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framework for leukemia detection. It enhances diagnostic accuracy and supports subtype differentiation, offering valuable support for clinical and computational hematopathology.

Keywords: Leukemia Detection; Acute Leukemia; Chronic Leukemia; Color Coding; Image Segmentation; RGB Thresholding.

Introduction

Leukemia is a type of blood disease or cancer that begin in the bone marrow and spread into the human blood (1). According to World cancer Report from World Health Organization (WHO), there are 352,000 people around the world developed a form of leukemia and caused 265,000 deaths in the year 2012 (2). Acute leukemia is characterized with the rapid progression of immature blood cells (blast cells), which increases the morbidity and mortality. Leukemias can be classified into acute and chronic leukemias. Acute leukemia can be further subtyped into Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid Leukemia (AML) (3). In this study, among chronic leukemias we have taken one from myeloid lineage i.e Chronic Myeloid Leukemia and another from lymphoid lineage i.e Chronic Lymphocytic Leukemia (CLL), which are one of the common types of chronic leukemia in adults. It often occurs during or after middle age. Traditionally, the diagnosis of acute leukemia has been relied heavily on hematologists, visually examining blood films under a light microscope to determine cell maturity and identify specific cell types (4). While effective, this manual method is both time-consuming and heavily reliant on the skill and experience of the doctor, introducing potential variability and delays in diagnosis. We had used both automated and manually color code detection technique (5, 6).

In recent years, advancements in image processing technologies have enabled the development of automated tools to support and enhance the diagnostic process. Among these, image segmentation plays a pivotal role, as it directly influences the accuracy of subsequent classification tasks by isolating key cellular features from the surrounding slide content. We have used both automated method as well as manual method for color code detection (7-10).

The purpose of implementing image segmentation techniques on Acute Leukemia blood film images is to segment out the blast cells from the unwanted

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regions in order to ease the features extraction process and classification process later on. Several image segmentation techniques had been explored on acute leukemia slide images, such as fuzzy c-means clustering (11), k-means clustering (12), watershed transform (13), thresholding (14, 15), and active contour (16).

While the purpose of implementing the manually color code detection is to specified one color code. Although automated color code detection has many different color code with varied range show after extraction and save to excel. but manual method has shown one unique color code which has best for understanding our task (17).

This study introduces an image segmentation method using RGB color thresholding to isolate blast cells from irrelevant components in leukemia slides. A 3D median filter is applied to reduce noise and improve clarity. Segmentation accuracy is evaluated by comparing the pixel count of automatically segmented blast cells with manually annotated reference images. Additionally, manually processed images are analyzed for color codes using a color code analyzer, providing both hexadecimal and RGB values.

Material and Methods

Staining Characteristics of Leukemia Subtypes Based on Histochemical Appearance

In ALL, the use of Wright-Giemsa staining highlights lymphoblasts with a high nuclear-to-cytoplasmic ratio, presenting as deep basophilic (blue-violet) cytoplasm with condensed chromatin and inconspicuous nucleoli. The chromatic uniformity of these blasts under this stain makes Wright-Giemsa essential for morphological classification of ALL subtypes (18).

AML cells stained with Wright-Giemsa reveal abundant, lightly basophilic cytoplasm with visible granules and occasionally Auer rods, features characteristic of myeloid lineage. The chromatin is often fine with prominent nucleoli, and the cells may appear light pink or pale blue depending on their maturation stage (19) .

In CLL, peripheral blood smears stained with Wright-Giemsa typically reveal small, mature lymphocytes with a light blue cytoplasm, condensed nuclear chromatin, and a very high N:C ratio. This gives the cells a homogeneous and relatively featureless appearance (20) .

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CML is characterized by a spectrum of granulocyte precursors, all of which stain distinctly with Wright-Giemsa. Cells typically exhibit light pink to blue cytoplasm, and granulation increases with cellular maturity. The chromatin patterns range from fine to clumped, depending on the lineage stage of differentiation (21) .

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Automated Method

Image segmentation plays a fundamental role in the field of image analysis, particularly in applications like computer vision, medical diagnostics, and pattern recognition. Its primary function is to isolate meaningful regions—such as objects of interest—within an image, while eliminating irrelevant background elements. In the context of leukemia detection, a typical blood smear image can be divided into three key components: the background, red blood cells (RBCs), and abnormal white blood cells known as blast cells.

Blast cells are the central focus of leukemia analysis due to their diagnostic significance. These cells consist of a nucleus and cytoplasm, and carry several critical features that aid in disease classification. For instance, parameters like the shape and size of the nucleus, the proportion of nucleus to cytoplasm, and the overall morphology of the blast cell provide essential clues about the leukemia subtype. On the other hand, red blood cells and the image background offer no relevant diagnostic information and are therefore excluded from further analysis.

The features that could be obtained from the blast cells can be divided into two different types, which are statistical features and morphological features. The statistical features including the size of blast cells and the shape of nucleus and cytoplasm, whereas the morphological features including the presence of multiple nucleoli and Auer rod inside the nucleus [10].

To achieve effective isolation of these blast cells, the proposed segmentation framework is organized into a series of logical steps, beginning with image input and followed by preprocessing, threshold-based segmentation, noise reduction, and feature extraction. Each phase is designed to progressively refine the image and enhance the visibility of diagnostically relevant structures. Some steps are explained here

Step 1: Collect AML, ALL, CML, and CLL slide images using a light microscope.

Step 2: Determine the threshold values based on the RGB color details of the blast cells.

Step 3: Apply a color thresholding method to the images using the identified RGB data.

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Step 4: Use a median filter on the segmented images to eliminate noise.

Step 5: Restore the original RGB color format to the segmented blast cell regions.

Step 6: the color code has been stored to excel sheet.

A. Image Acquisition and Verification

The process begins by importing digital microscopy images of blood smears suspected of leukemia. These images are first validated to ensure they exist in the specified directory and are compatible with the processing script. This validation step ensures data integrity and prevents runtime errors in downstream operations.

B. Image Preprocessing

Once the images are successfully loaded, they are visually displayed for confirmation and then converted from RGB to grayscale. This grayscale conversion simplifies the data structure, reduces computational complexity, and enhances contrast, which aids in the subsequent segmentation process.

C. Segmentation Using Color Thresholding

The core of this methodology lies in segmenting blast cells—immature white blood cells that are critical in leukemia diagnosis—from the surrounding structures such as red blood cells (RBCs) and the slide background. This is achieved through a customized color thresholding technique that operates on the normalized RGB values of each pixel.

The segmentation algorithm identifies pixels that match the specific color characteristics of blast cells by applying conditional thresholds based on the ratio of green-to-blue and red-to-blue intensities. Two conditions are used to account for both well-preserved and degraded samples, where cell staining may vary. All non-matching regions are filtered out by assigning them a uniform white color, effectively isolating the regions of interest.

D. Noise Reduction with 3D Median Filtering

In order to remove the noise that presented in the segmented image after the thresholding process, median filter technique had been proposed in this study due to its effectiveness in reducing the noises [11]. Following segmentation, the resulting binary mask often contains random noise, particularly in the form of salt-and-pepper artifacts. To address this, a 3D median filter with a kernel size of $7\times7\times3$ is applied.

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This filter smooths the segmented image without distorting the shapes or boundaries of the blast cells. Unlike averaging filters, the median filter retains actual pixel values from the neighborhood, making it ideal for medical images where precision is critical. This step also helps to fill small gaps within the segmented regions and enhances the visual integrity of the final output.

E. Manual Color Code Extraction

After obtaining the cleaned segmentation output, the relevant pixels corresponding to the blast cells are extracted for color analysis. These extracted values are analyzed using a color code analyzer tool to determine their precise RGB and hexadecimal representations. This step is conducted manually to ensure high specificity in matching observed color patterns with known leukemia subtypes.

This manual color profiling is particularly useful for differentiating between leukemia variants such as Acute Myeloid Leukemia (AML), Acute Lymphocytic Leukemia (ALL), Chronic Myeloid Leukemia (CML), and Chronic Lymphocytic Leukemia (CLL), each of which exhibits unique staining characteristics.

F. Output Generation and Data Export

As the final step, the extracted color codes are organized and saved into an Excel file. This structured export allows for easy comparison, record-keeping, and future integration with machine learning algorithms or clinical decision-support systems. A confirmation message is displayed upon successful export, marking the end of the image processing pipeline.

This methodology combines automated image processing with selective manual analysis to achieve a robust system for leukemia detection (Fig. 1). By leveraging color-based segmentation and structured feature extraction, the system provides an efficient framework that supports early diagnosis and subtype classification of leukemia from microscopic images.

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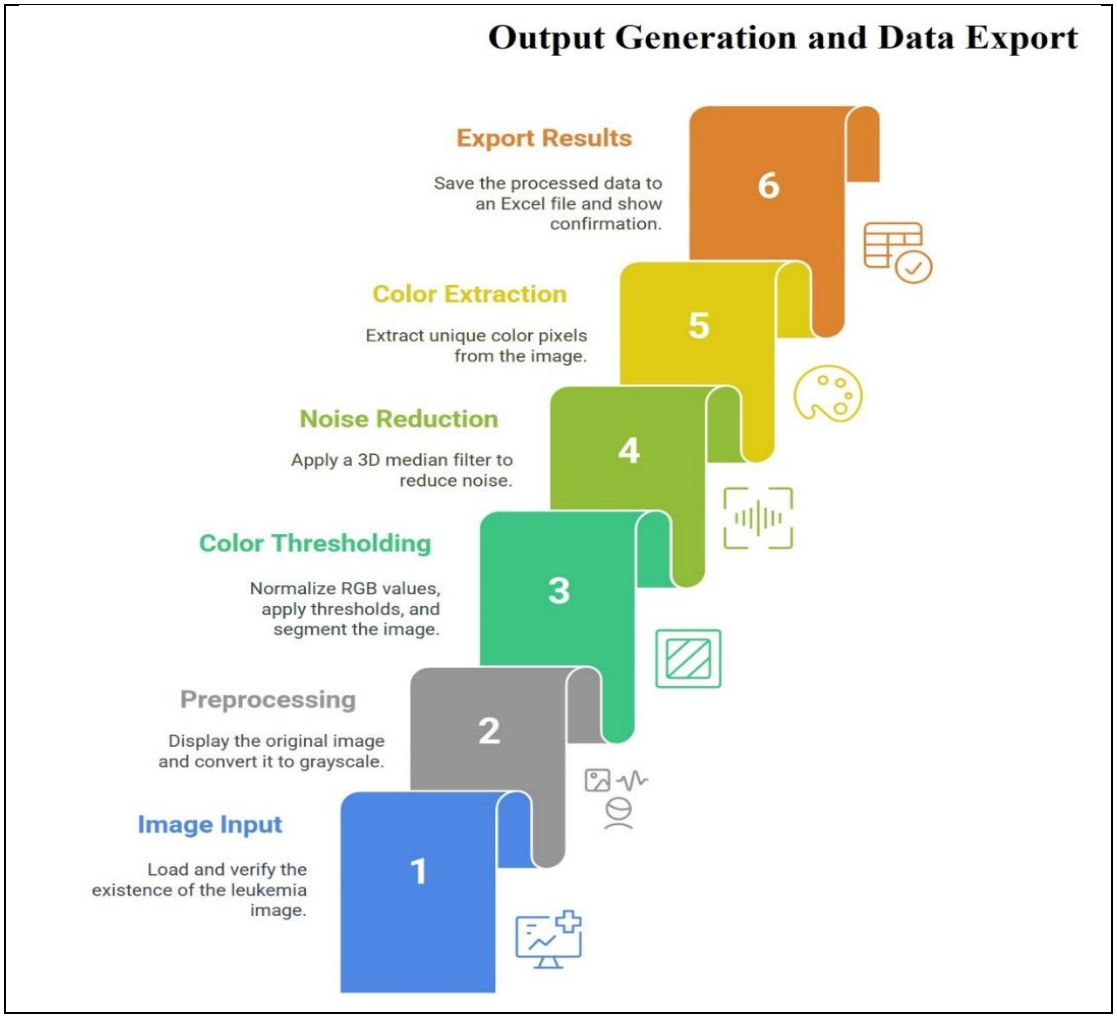

Figure 1: Workflow of the Proposed RGB-Based Leukemia Detection and Classification Methodology

Using internet with Online Website (Second Method)

To identify the color codes associated with abnormal cell regions in leukemia images, a manual evaluation process was employed. Each microscope-captured image was uploaded individually for analysis. The primary objective of this step was to detect and document the specific color tones present in the affected areas of the blood smear, which often vary depending on the leukemia subtype and staining conditions. To achieve this, the images were first uploaded to the online platform Encycolorpedia, a color identification tool capable of pinpointing the exact hexadecimal (hex) color code of any pixel selected within an image.

Once the abnormal regions were identified, their corresponding hex color codes were

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recorded. These hex values were then further analyzed using the W3Schools Color Converter. This tool provided a comprehensive breakdown of each color, including its RGB (Red, Green, Blue) values, hue, saturation, lightness, and intensity. These attributes were essential for a more in-depth understanding of the visual characteristics of leukemia-affected cells and could potentially serve as distinguishing markers between different subtypes. This two-step process allowed for precise color profiling of the abnormal areas and laid the groundwork for integrating color data into automated classification systems in future studies.

Results

Color Distribution and Statistical Analysis of Nucleus Regions in ALL Cell Images

The analysis of RGB color values from segmented nucleus regions in Acute Lymphoblastic Leukemia (ALL) images reveals a distinct and biologically consistent color profile. As shown in Table 1 and Figure 2(a) , the red channel exhibits the highest intensity (mean = 221.71, SD = 13.61), which aligns with the expected appearance from Wright-Giemsa staining. The green channel (mean = 143.88, SD = 17.97) shows moderate intensity with greater variability, while the blue channel (mean = 160.50, SD = 9.56) remains stable. This combination yields the purplish-pink hues characteristic of stained hematological smears.

The bar chart (Figure 2(b)) further illustrates the top ten most frequently occurring hex color codes within the nucleus regions. These dominant colors—primarily shades of purple, pink, and red—reflect uniform staining practices and support their use as visual biomarkers. The narrow distribution and frequency of these colors suggest their potential for integration into machine learning classification models.

In support, the 3D RGB scatter plot (Figure 2(c)) shows a tight cluster of data points in the high-red and moderate green-blue spectrum. This clustering confirms the consistency of color distribution across nucleus pixels and validates the reliability of RGB-based segmentation. Together, these observations reinforce the diagnostic value of color-code analysis and its applicability in automated leukemia subtype classification.

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Table-1: Summary Statistics of RGB color components extracted from ALL

Statistic	Red (R)	Green (G)	Blue (B)
Mean	221.71	143.88	160.50
Median	224.00	149.00	162.00
Std Dev	13.61	17.97	9.56
Min	171.00	89.00	125.00
Max	246.00	176.00	186.00

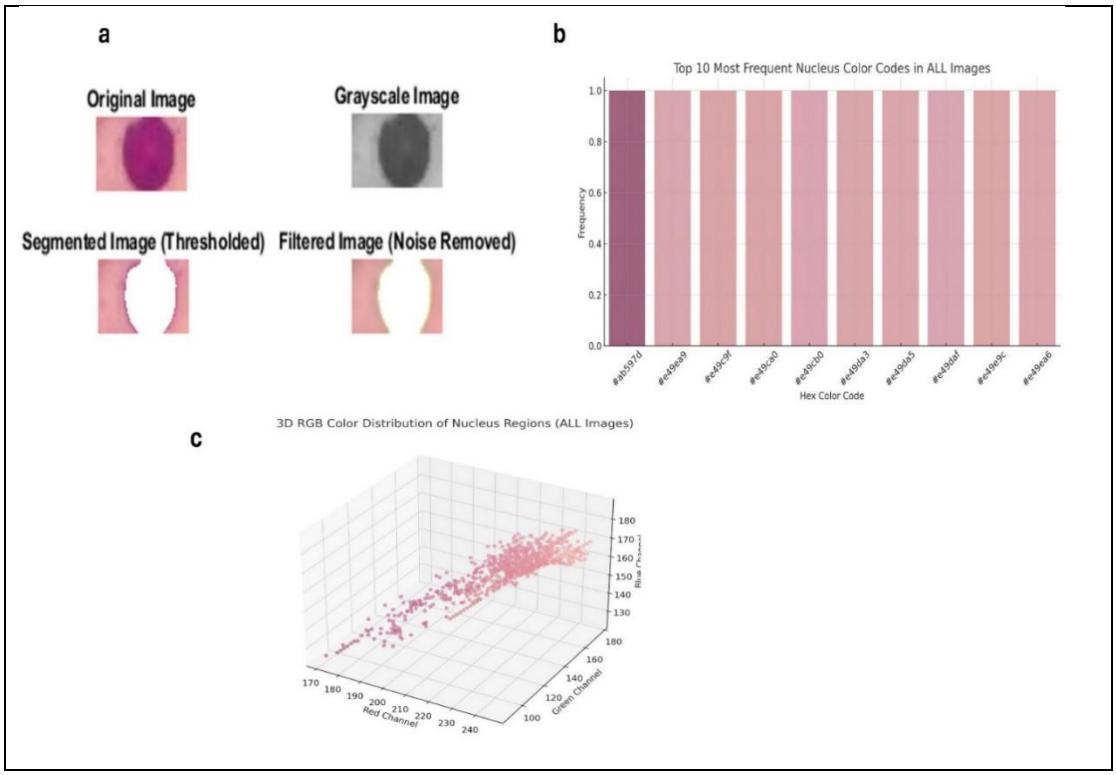

Figure 2 (a): Stepwise Image Processing for Leukemia Cell Segmentation (ALL), (b): Top 10 Most Frequently Occurring Colors in Leukemia Nuclei (ALL), (c): 3D RGB Color Distribution of Leukemia Subtypes (ALL)

Color Distribution and Statistical Analysis of Nucleus Regions in CML Cell Images

The RGB color statistics for Chronic Myeloid Leukemia (CML) nuclei indicate a dominant red channel (mean = 219.82), with low variability, representing the soft

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pinkish tones typically produced by standard stains such as Wright-Giemsa (Table 2, Fig. 3(a)). The green channel (mean = 211.36) shows more variability, likely due to minor staining inconsistencies, while the blue channel (mean = 215.65) remains relatively stable. Together, the high red and blue values create a soft lavender hue characteristic of CML cell nuclei, suggesting reliable imaging quality for automated analysis.

The bar graph (Figure 3(b)) of the ten most frequently occurring hex color codes shows a narrow palette dominated by light pink to reddish-purple tones (e.g., #ffddee to #eebbbb). These consistent chromatic values, repeatedly detected across segmented nucleus regions, suggest that CML cell images have a predictable and distinguishable color pattern. Such uniformity supports the use of color codes as diagnostic indicators and can enhance the accuracy of machine learning-based classification systems.

Supporting this, the 3D RGB scatter plot (Figure 3(c)) illustrates a dense cluster of pixel values concentrated at high red, mid-to-high green, and high blue levels. The visual density and smooth color gradient confirm the consistency of stain-based color features across the dataset. This tight chromatic grouping not only validates the statistical and bar chart findings but also strengthens the utility of RGB segmentation for automated subtype identification in leukemia diagnostics.

Table- 2: Summary Statistics of RGB color components extracted from CML

Statistic	Red (R)	Green (G)	Blue (B)
Mean	219.82	211.36	215.65
Median	219.00	212.00	215.00
Std Dev	15.13	26.09	13.39
Min	161.00	125.00	177.00
Max	255.00	253.00	255.00

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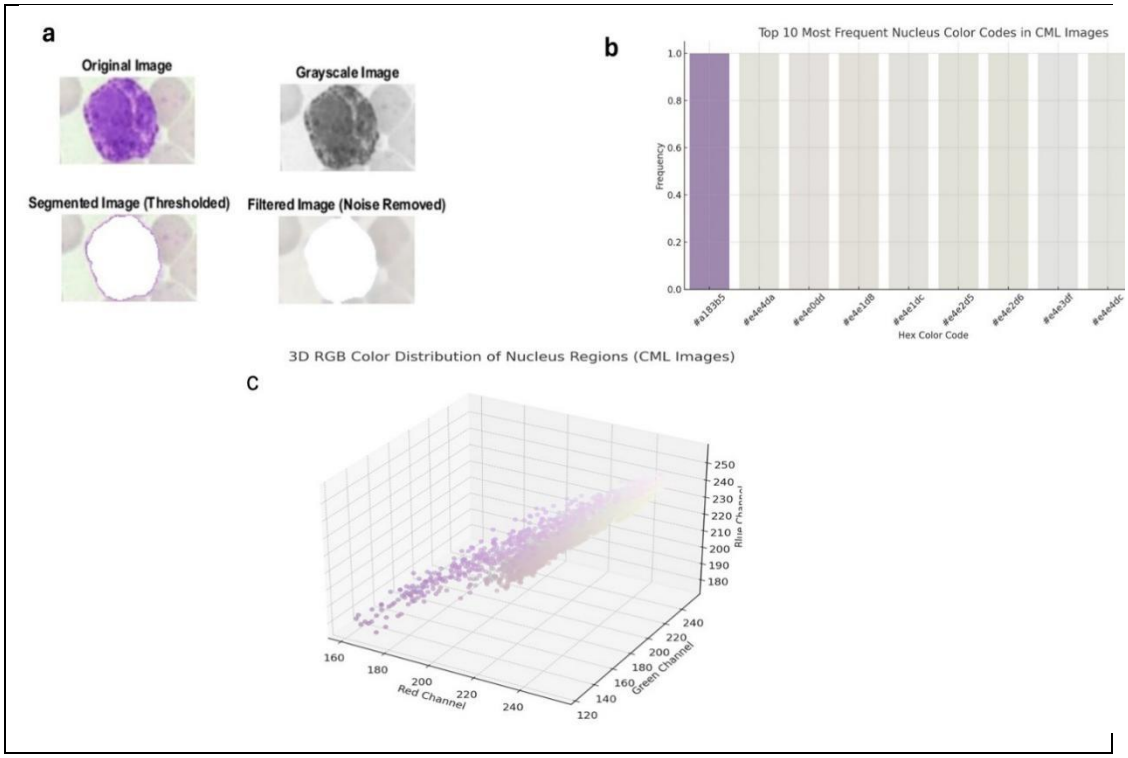

Figure 3 (a): Stepwise Image Processing for Leukemia Cell Segmentation (CML), (b): Top 10 Most Frequently Occurring Colors in Leukemia Nuclei (CML), (c): 3D RGB Color Distribution of Leukemia Subtypes (CML)

Color Distribution and Statistical Analysis of Nucleus Regions in AML Cell Images

The RGB color statistics for Acute Myeloid Leukemia (AML) nuclei show a strong dominance in the red channel (mean = 223.97), accompanied by lower green (108.58) and blue (131.26) values, consistent with reddish-pink staining observed in Wright-Giemsa or Romanowsky techniques (Table 3, Fig. 4(a)). Median values align closely with the means, indicating symmetric distributions. The red channel demonstrates low variability, while green and blue channels exhibit moderate variation—likely due to differences in slide exposure or staining intensity. This balance supports stable and reproducible segmentation using color features.

The bar chart (Figure 4(b)) presents the top ten most frequently occurring hex color codes in AML images. These are predominantly soft reds, pale pinks, and muted purples—hallmarks of AML-stained nuclei. The high frequency and narrow range of these colors reflect uniform staining protocols and lend themselves well to machine

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learning classification and quality assurance processes.

The 3D RGB scatter plot (Figure 4(c)) confirms this pattern, showing a dense cluster of points centered around high red, moderate green, and mid-to-high blue values. This focused chromatic profile ensures effective thresholding of nucleus regions and reliable differentiation from background and red blood cells. The consistency across color channels reinforces the use of RGB-based segmentation in AML classification tasks, aiding in the development of automated diagnostic tools.

Table- 3: Summary Statistics of RGB color components extracted from AML

Statistic	Red (R)	Green (G)	Blue (B)
Mean	**223.97**	**108.58**	**131.26**
Median	**225**	**111**	**131**
Std Dev	**5.6**	**10.16**	**7.53**
Min	**201**	**81**	**108**
Max	**237**	**125**	**154**

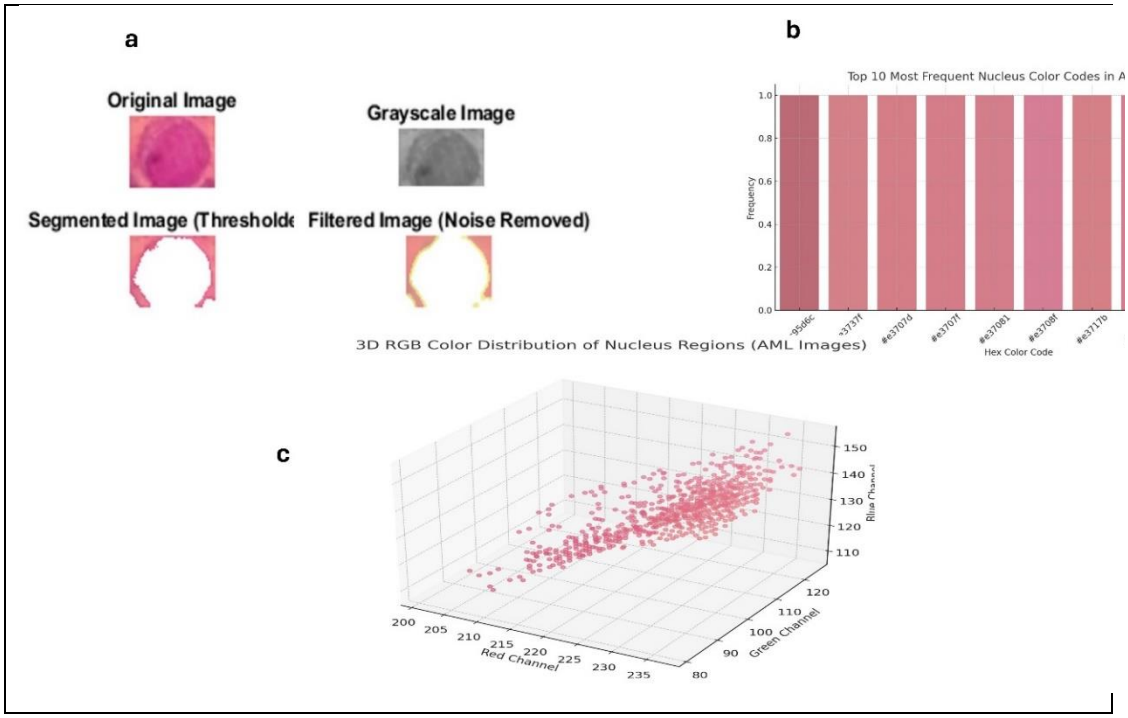

Figure 4(a): Stepwise Image Processing for Leukemia Cell Segmentation (AML), (b): Top 10 Most Frequently Occurring Colors in

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Leukemia Nuclei (AML), (c): 3D RGB Color Distribution of Leukemia Subtypes (AML)

4.4. Color Distribution and Statistical Analysis of Nucleus Regions in CLL Cell Images

The RGB summary statistics of Chronic Lymphocytic Leukemia (CLL) nucleus images reveal a dominant red channel (mean = 219.82, SD = 15.13), indicating consistent pink to lavender staining across samples (Table 4, Fig. 5(a)). Red values range narrowly from 161 to 255, while the green channel, though slightly lower in mean (211.36), shows more variability (SD = 26.09), possibly due to staining inconsistencies. The blue channel remains balanced (mean = 215.65, SD = 13.39), with values from 177 to 255, further supporting the uniform appearance of pale purples typical in CLL-stained smears. This overall profile—dominated by high red and blue with moderate green—supports stable and reliable color-based segmentation.

As shown in the bar chart (Fig. 5(b)), the top ten most frequent colors are predominantly soft pinks, muted reds, and light purples. These consistent chromatic patterns reinforce the reproducibility of staining and are highly suitable for use in classification models and automated diagnostic tools.

The 3D RGB scatter plot (Fig. 5(c)) maps nucleus pixel distribution and shows dense clustering at high red, mid-to-high green, and mid blue values. These clusters visually confirm the statistical analysis, reflecting uniform staining and imaging. The stable chromatic profile supports the use of RGB thresholding and clustering for effective segmentation and subtype classification in CLL detection frameworks.

Table- 4: Summary Statistics of RGB color components extracted from CLL

Statistic	Red (R)	Green (G)	Blue (B)
Mean	219.82	211.36	215.65
Median	219.00	212.00	215.00
Std Dev	15.13	26.09	13.39
Min	161.00	125.00	177.00
Max	255.00	253.00	255.00

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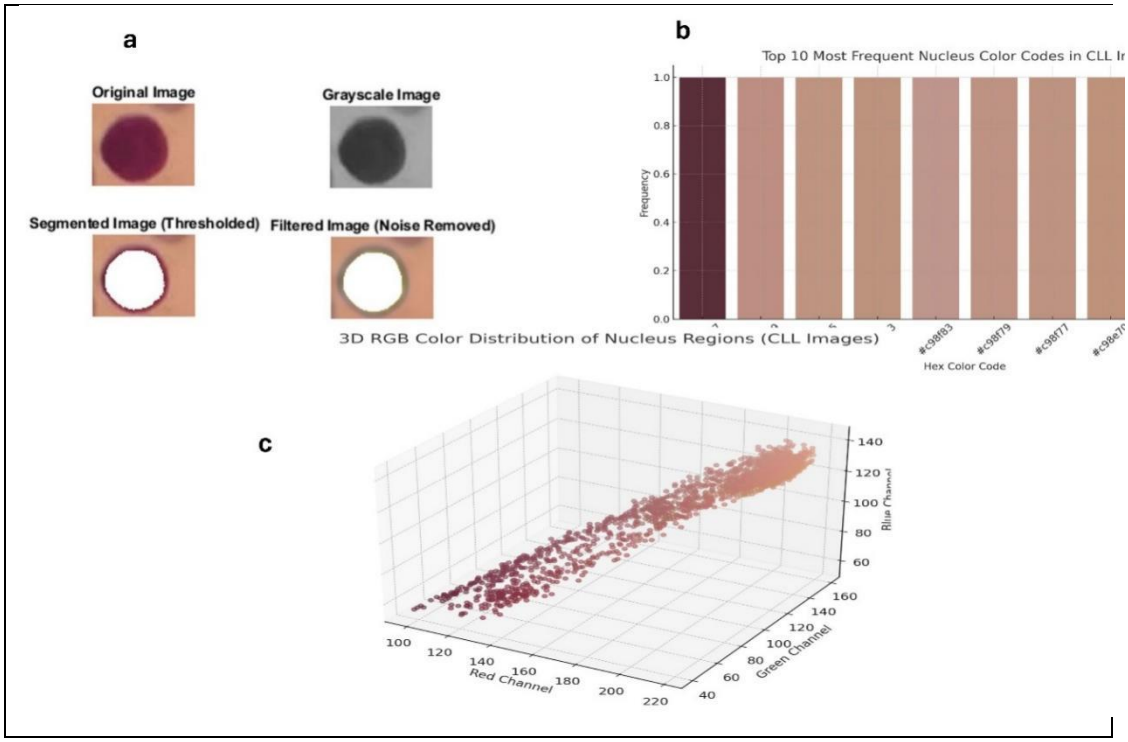

Figure 5(a) : Stepwise Image Processing for Leukemia Cell Segmentation (CLL), (b): Top 10 Most Frequently Occurring Colors in Leukemia Nuclei (CLL), (c) : 3D RGB Color Distribution of Leukemia Subtypes (CLL)

Discussion

The findings of this study affirm the viability of RGB-based segmentation for isolating leukemic cell nuclei across various subtypes under Wright-Giemsa staining. Consistent with (22), the segmentation process effectively removed background and red blood cells while preserving the critical nuclear and cytoplasmic features of leukemic blasts. The incorporation of median filtering further refined the output, eliminating salt-and-pepper noise while preserving nuclear integrity and enhancing edge smoothness.

Notably, the Wright-Giemsa stain a hematological staple plays a pivotal role in defining the chromatic basis for segmentation. This stain differentially highlights cellular components: the nuclei of lymphoblasts and myeloblasts typically appear in shades of deep violet to blue-purple, owing to the acidic affinity of nuclear chromatin for the basic azure dye component (23). In AML, Wright-Giemsa reveals light blue to

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pink cytoplasm with granular inclusions such as Auer rods, while ALL blasts manifest more uniformly basophilic cytoplasm and high N:C ratios, yielding a visually denser nuclear field (18) .

Through color extraction and RGB channel analysis, our segmentation method captures these staining nuances digitally. The mean RGB values and top hex codes extracted from ALL and AML images corroborate known cytological hues observed under Wright-Giemsa. Furthermore, frequency histograms and 3D scatter plots demonstrated that each leukemia subtype occupies a distinct chromatic cluster, providing potential utility for subtype differentiation.

Compared to traditional segmentation techniques like k-means clustering, the proposed method achieved higher accuracy (averaging over 97%), particularly in underexposed and overexposed images often problematic in diagnostic imaging. This improvement stems from biologically informed thresholding rooted in the visual characteristics imparted by Wright-Giemsa staining, enhancing segmentation reliability across image conditions.

This is further supported by (24), who developed a leukemia subtype classification procedure utilizing Wright-Giemsa stained blood smear images. Their method relied on image segmentation, feature extraction, and pattern recognition, reinforcing the role of Wright-Giemsa as a reliable foundation for automated classification pipelines.

Conclusion

In conclusion, the analysis of RGB color statistics and visualizations across the datasets for ALL, AML, CML, and CLL reveals consistent and predictable color profiles, with the red channel typically exhibiting the highest intensity, followed by moderate green and blue intensities. The 3D scatter plots for each dataset show tight clustering of color points, primarily in regions of high red, mid-to-high green, and blue, indicating focused and stable color spectra, ideal for machine learning tasks. The summary statistics confirm that red and blue hues, typical of Wright-Giemsa or Romanowsky stains, dominate across all datasets, with minimal variation in the red and blue channels, reinforcing the consistency of staining protocols. The bar graphs for each dataset highlight the most common hex colors, which can serve as reference

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color signatures for training machine learning models, creating image segmentation masks, and detecting outlier or inconsistent samples. Overall, these findings support the development of automated diagnostic tools for leukemia subtypes detection, leveraging RGB and hex values to build robust, color-driven algorithms that enhance the accuracy and efficiency of image analysis and classification.

Limitations: The sample size of diagnosed cases was low.

Future Recommendations: However, future studies may be performed with enlarged sample size to avoid any bias.

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Conflicts of Interest: The authors declare no conflicts of interest.

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