

Comparison of Filtered and Direct RGB LED Illumination in Widefield Transmitted Light Microscopy for Cellular Imaging

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Abstract

This study investigates the comparative performance of filtered and direct RGB LED illumination in widefield transmitted light microscopy for biological imaging. Rotifera whole-mount samples were imaged under both illumination modes, and image quality was assessed using signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) metrics. The highest SNR value of 14.89 was recorded under filtered green LED illumination, whereas the highest CNR value of 4.15 was achieved with direct red LED light. These results indicate that filtered RGB illumination enhances overall image uniformity, while direct RGB lighting more effectively accentuates structural contrast, depending on the specific imaging objective.

1. Introduction

Microscopy remains foundational to biological research, supported by decades of optical and mechanical refinement with the aim of improving imaging quality. Traditional illumination sources, such as tungsten, xenon, and arc lamps, have persisted in various systems despite their numerous drawbacks compared to the current state of LED technology. Recent advances in LED technology, particularly RGB LEDs, offer a viable alternative, boasting tunable spectra, energy efficiency, and thermal stability [1]. While RGB LEDs have been integrated into modern optical systems, their application as dynamic illumination in microscopy remains underexplored. Pratavieiera et al. (2016) found that RGB LED illumination yielded higher-contrast images compared to white LEDs, although without a detailed quantitative assessment [2]. This study aims to compare imaging results from direct and filtered RGB LED illumination within a widefield transmitted light microscopy system, by incorporating high-grade photographic filters. A comparative analysis based on signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) is presented, focusing on Rotifera whole mounts as a representative biological sample.

2. Methods and Materials

A custom widefield transmitted light microscope system was assembled, comprising an sCMOS camera, a lens tube with focusing optics, a 5x objective lens, and two illumination setups: filtered and direct RGB LEDs. Figure 1 shows both the physical setup and schematic of the system. Filtered illumination used white RGB LEDs overlaid with photographic filters, while direct illumination used a software-controlled RGB LED source without filters. Red, green, and blue wavelengths were selected as the primary color channels in image analysis [3]. Images of Rotifera whole-mount (W.M.) sample were acquired using Mosaic software and analyzed in MATLAB to calculate SNR and CNR. SNR was calculated as the mean signal divided by the standard

deviation of noise, expressed as $SNR = \mu_{signal} / \sigma_{noise}$ while CNR was determined using the absolute difference between two mean signal intensities divided by the square root of the sum of their noise variances, given by $CNR = |\mu_1 - \mu_2| / \sqrt{(\sigma_1^2 + \sigma_2^2)}$ where μ is the mean signal and σ is the standard deviation of noise between regions of interest (ROIs). A single specimen was used, but multiple locations were imaged, with repeated measurements at each site. Reported SNR and CNR values are the averaged results across these measurements.

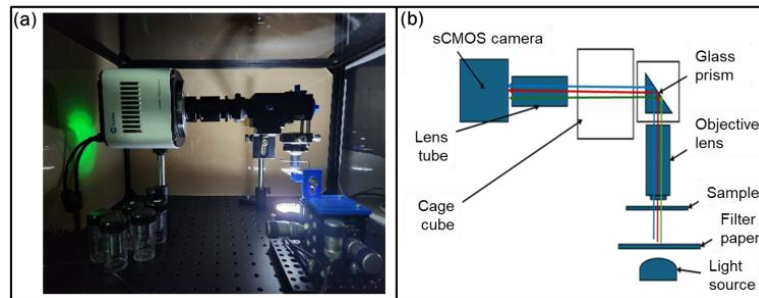


Figure 1. (a) Photograph of the widefield transmitted light microscopy setup. (b) Schematic diagram of the microscopy setup and optical configuration.

3. Results and Discussion

Representative images from both illumination setups are shown in Figure 2, while the quantitative analysis is summarized in Table 1.

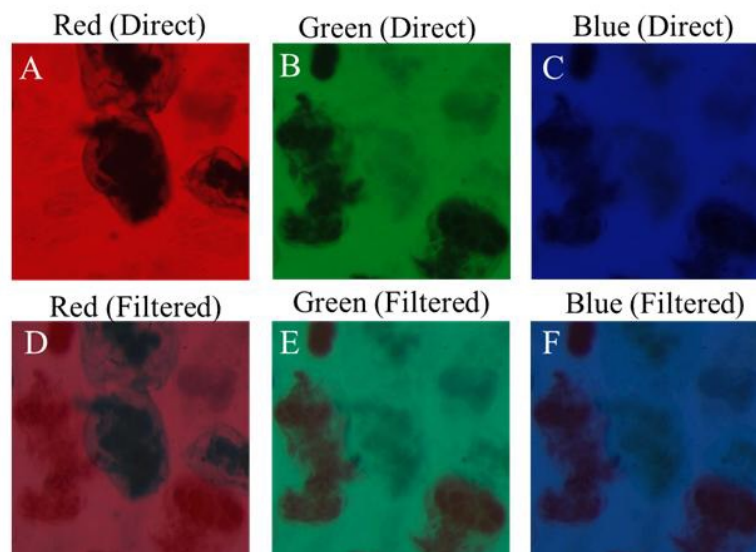


Figure 2. Rotifera W.M. images under filtered and direct RGB illumination.

As shown in Figure 2, red-filtered RGB LED illumination causes green features in the sample to appear darker, and similarly, green-filtered illumination dims red features. This effect arises because sample features that are similar in colour to the lighting blend more easily into the background, reducing visual contrast [4]. Under blue illumination, in both filtered and direct modes, red and green features also appear dark, with direct blue light causing more pronounced darkening since the sample lacks blue-reflective structures.

Table 1. Calculated SNR and CNR for each illumination mode.

	Filtered RGB Illumination			Direct RGB Illumination		
	Red	Green	Blue	Red	Green	Blue
SNR (dB)	13.02	14.89	14.17	9.68	10.36	10.59
CNR	2.16	1.88	1.49	4.15	2.16	2.88



Table 1 shows that the highest SNR was recorded using filtered green RGB LED illumination. Higher SNR are preferred since mathematically; it shows that the desired signal acquired by the detector are higher than the undesired signal in an image, proving that the image has a high clarity. Generally, filtered RGB illumination yielded higher SNR values than direct illumination. This may be attributed to reduced colour blending between the illumination and sample features, which preserves image variation and minimizes misinterpretation of residual signals as noise. Conversely, CNR was highest with direct red RGB LED illumination. In most cases, direct RGB LED provided higher CNR values than filtered RGB, likely due to the stronger illumination amplifying intensity differences between foreground and background, thereby increasing contrast. Higher CNR value are preferred as it shows that the images have clear distinction of features or colours, due to the high value of contrast. However, this increase in intensity can also raise noise levels, contributing to higher CNR values [5].

4. Conclusion

The comparison of white and RGB illumination methods reveals that green-filtered RGB LED images yield the highest signal-to-noise ratio (SNR), while direct red RGB LED images achieve the highest contrast-to-noise ratio (CNR). These findings suggest that the selective use of colour filters and coloured illumination can enhance specific structural features in biological imaging, depending on the objective of imaging. Further investigations using more complex specimens and additional quantitative image quality metrics are recommended.

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