

Article

Color Representation and Prediction Uncertainty in Unsupervised Retinal Vessel Segmentation

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Abstract

This study investigates the influence of color representation, preprocessing, and thresholding strategies on unsupervised retinal vessel segmentation using computationally efficient classical image processing techniques. Nineteen image channels derived from RGB, HSV, HSL, YUV, YCrCb, CIELAB, and grayscale representations were evaluated in combination with five thresholding algorithms on the DRIVE dataset. The results demonstrate that luminance-dominant channels consistently outperform chrominance-based representations. The Moments (Tsai) thresholding algorithm provided the most robust performance across image representations, while contrast enhancement using CLAHE and white top-hat filtering significantly improved segmentation quality. In addition, the weighted combination of RGB channels was characterized by a dominant green-channel contribution ($w_G \approx 0.88\text{--}0.90$) and a minimal blue-channel contribution ($w_B \approx 0\text{--}0.02$), reflecting the spectral properties of retinal vasculature. The study highlights the central role of luminance information and demonstrates that task-specific optimization of color representations improves both segmentation accuracy and reliability. Although deep learning methods currently achieve state-of-the-art performance in retinal vessel segmentation, the results show that substantial optimization remains possible using classical, interpretable, and computationally efficient approaches.

Keywords: retinal vessel segmentation; color space analysis; luminance representation; thresholding; uncertainty estimation; biomedical image processing; DRIVE dataset

1. Introduction

Retinal fundus imaging is one of the most widely used non-invasive diagnostic techniques in ophthalmology, providing direct visualization of the retinal vasculature. Morphological characteristics of retinal blood vessels, including vessel diameter, tortuosity, branching patterns, and density, are important biomarkers for numerous ocular and systemic diseases such as diabetic retinopathy, hypertension, arteriosclerosis, and cardiovascular disorders. Accurate extraction of vascular structures from retinal images is therefore essential for computer-aided diagnosis, disease monitoring, image registration, and quantitative retinal analysis. Despite significant progress in medical image analysis, retinal vessel segmentation remains a challenging task. Retinal images often exhibit substantial variability in illumination, contrast, imaging conditions, and anatomical appearance. Blood vessels may vary considerably in width, with thin capillaries being particularly difficult to detect due to their low contrast relative to surrounding tissues. Furthermore, imaging artifacts, noise, reflections, and pathological structures



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can resemble vessel-like patterns, increasing the likelihood of segmentation errors. These challenges are especially pronounced in peripheral retinal regions where vessel visibility is reduced and illumination is non-uniform [1–3].

Retinal blood vessel segmentation methods can be broadly divided into classical image-processing approaches, machine-learning-based approaches, and deep-learning-based approaches. More detailed taxonomies further distinguish matched filtering, morphological processing, vessel tracking, multiscale analysis, model-based methods, clustering, region-growing techniques, adaptive thresholding, and supervised classification strategies [1,3,4]. Classical unsupervised methods rely on assumptions about vessel intensity, geometry, continuity, and local contrast. They include thresholding, matched filtering, morphological reconstruction, line detectors, region growing, and Frangi/Hessian-type vesselness filters, with [5] serving as a representative example. These methods are generally interpretable, computationally efficient, independent of manually labelled training masks, and therefore suitable for scenarios in which annotated data are limited [6–9]. However, their performance is often affected by illumination inhomogeneity, noise, lesions, low contrast, and parameter selection, particularly in the segmentation of thin or peripheral vessels.

Traditional supervised machine-learning methods formulate segmentation as a pixel- or patch-wise classification problem, using manually defined image features followed by classifiers such as k-NN, SVM, or Random Forests [10]. Compared with purely rule-based approaches, these methods benefit from expert annotations and can improve segmentation robustness. Nevertheless, their effectiveness remains strongly dependent on feature design, preprocessing strategy, and the representativeness of the training dataset.

Deep-learning-based approaches, including convolutional neural networks (CNNs), fully convolutional networks (FCNs), U-Net variants, GAN-based models, and Transformer-based architectures, learn hierarchical image representations directly from data. They have become the dominant methodology because they can better capture complex vessel morphology, scale variation, and contextual information. The canonical reference for the U-Net family is [11]. Recent developments increasingly combine local detail preservation with global context modelling through residual U-Nets, attention mechanisms, multiscale modules, graph convolutional networks, and Transformers, aiming to improve vessel continuity, topology preservation, and robustness in challenging images.

Although deep neural networks can provide highly accurate segmentation results, they often require large, labelled, and diverse datasets, substantial computational resources, and careful validation across imaging devices and clinical populations; they may also suffer from limited interpretability [6,7]. Therefore, current developments do not simply replace classical methods with deep networks, but rather move toward hybrid and resource-aware solutions, including efficient architectures, transfer learning, domain adaptation, semi-supervised and weakly supervised learning, synthetic-data generation, and explainable segmentation models. In this context, classical and machine-learning-based methods remain relevant as transparent and computationally efficient baselines, whereas advanced deep-learning models are most advantageous when sufficient data, computational infrastructure, and external validation are available [4].

The aim of this study is to systematically analyze the contribution of color-space representations to retinal vessel segmentation. Computationally efficient threshold-based methods were deliberately selected, allowing the influence of image channels and luminance representations to be studied without the confounding effects of complex learned feature extraction. Such an approach enables direct comparison between color-space models and provides insights that may be useful for both classical and machine learning-based segmentation frameworks. In the context of classical image processing methods, various thresholding algorithms, including Otsu [12], Yen [13], Isodata [14], and Moments

(Tsai) [15], have been successfully applied for retinal vessel segmentation, typically in combination with contrast enhancement and morphological preprocessing techniques. Some studies have demonstrated that preprocessing methods such as Contrast Limited Adaptive Histogram Equalization (CLAHE) [16], top-hat filtering, and homomorphic enhancement can significantly improve vessel visibility and segmentation performance. However, most existing studies focus primarily on preprocessing and thresholding strategies while paying relatively little attention to the influence of color representation itself [12–17].

Representative threshold-based and hybrid approaches applied to improve retinal vessel segmentation are summarized in Table 1. While many of these methods achieve high accuracy and specificity, their primary focus is on preprocessing and segmentation algorithms. In contrast, the influence of color representation and the selection of image channels used as inputs to the segmentation process remain comparatively underexplored. The choice of color representation is particularly important in retinal image analysis because retinal vessels are characterized mainly by intensity variations rather than distinct color differences. Different color spaces encode image information in fundamentally different ways, particularly with respect to the separation of luminance and chrominance components. Although the green channel of RGB images is commonly used in retinal vessel segmentation due to its high vessel-to-background contrast, a systematic comparison of alternative luminance representations across multiple color spaces remains limited. Furthermore, the potential benefits of designing task-specific luminance representations tailored to retinal vessel detection have received little attention [1,3].

To address this gap, this study presents a comprehensive evaluation of luminance- and chrominance-based representations derived from multiple color spaces for unsupervised retinal vessel segmentation. Nineteen image channels extracted from RGB, HSV, HSL, YUV, YCrCb, CIELAB, and grayscale representations are systematically analyzed using a unified segmentation framework based on classical thresholding methods. In addition, novel luminance representation is introduced as an optimized weighted combination of RGB channels, designed specifically to maximize retinal vessel contrast. The influence of preprocessing techniques and thresholding algorithms is assessed through statistical analysis, while segmentation reliability is further investigated using pixel-level uncertainty estimation and coverage–quality trade-off analysis.

The main contributions of this work are threefold and can be summarized as follows. First, this study performs a systematic comparison of color-space representations for retinal vessel segmentation using a reproducible threshold-based framework. Second, it introduces and optimizes task-specific weighted RGB luminance representation to enhance vessel visibility and segmentation accuracy. Third, it incorporates uncertainty-aware evaluation to provide a complementary assessment of segmentation reliability beyond conventional accuracy metrics. Together, these contributions offer practical guidelines for designing efficient and interpretable retinal vessel segmentation pipelines while offering new insights into the role of luminance information in retinal image analysis.

Table 1. Advances in retinal blood vessel segmentation threshold-based and hybrid techniques: a literature review.

Technique	Description	Ref.
Iterative edge detection with an edge detector followed by shape analysis of the segmented regions	Employs a cascade of image processing techniques, including image sharpening and Gaussian smoothing, followed by modified SUSAN edge detector combined with heuristic shape analysis; achieves 96.33% accuracy, 70.22% sensitivity, and 98.83% specificity on the DRIVE dataset.	2019 [18]
Mean global based on hysteresis thresholding for retinal blood vessel segmentation using enhanced homomorphic filtering	Uses MGBH thresholding and homomorphic filtering; shows superior performance on DRIVE, with fast processing and robustness to noise; achieves 96.05% accuracy, 70.35% sensitivity, and 98.54% specificity.	2022 [19]

Table 1. *Cont.*

Technique	Description	Ref.
Contrast enhancement for improved blood vessels retinal segmentation using top-hat transformation and Otsu thresholding	Uses CLAHE, top-hat enhancement, and Otsu thresholding; achieves 94.7% accuracy, 72.28% sensitivity, and 96.87% specificity on the DRIVE dataset.	2022 [17]
Segmentation of diabetic retinopathy based on retinal fundus images using thresholding technique	Uses adaptive thresholding and histogram analysis; achieves 65.79% accuracy, 31.58% sensitivity, and 100% specificity on the DIRECTDB1 database.	2023 [20]
Multi-level Otsu thresholding and morphological operations for fundus image segmentation	Combines preprocessing with multi-level Otsu and morphological operations; improves segmentation to support DR diagnosis (specific metrics not provided).	2023 [21]
Retinal vessel segmentation using morphological operations	Applies Tsallis-based multilevel thresholding combined with preprocessing; achieves 93.2% accuracy, 89% sensitivity, and 93% specificity on the DRIVE and ARIA datasets, effectively distinguishing normal and abnormal images.	2024 [22]
Hybrid-filter adaptive thresholding	Generates a field-of-view mask, extracts the green channel, applies CLAHE, bottom-hat filtering, Jerman vessel enhancement, morphological reconstruction, bowler-hat filtering, and finally segments vessel pixels using adaptive thresholding; achieves 95.86% accuracy, 65.95% sensitivity, and 98.74% specificity on the DRIVE dataset.	2024 [23]
Iterative NICK thresholding-based lesion segmentation	Segments hemorrhages and exudates using K-means multi-region contrast enhancement, iterative NICK thresholding region growing, watershed separation, optic-disc removal for exudate segmentation, and Chi ² -based feature ranking followed by candidate classification; achieves 89.89% accuracy, 96.34% sensitivity, and 82.17% specificity on the DR2019 Mobile Dataset and JSIEC dataset	2025 [24]
Otsu-based multi-threshold binarization for retinal detachment detection	Uses image preprocessing, RGB-to-LAB conversion, histogram equalization, averaging/low-pass filtering, Otsu-based thresholding/binarization, and retinal blood vessel extraction to identify retinal detachment regions where vessels are not visible (a qualitative comparison).	2025 [25]
Wavelet-enhanced multilayer thresholding	Converts the retinal image to the inverted green channel, enhances vessel structures using the wavelet transform, derives an initial threshold from histogram analysis, applies morphological thinning, and performs iterative multilayer thresholding with edge mapping, false-edge removal, and length-based filtering; achieves 96% accuracy, 97% sensitivity, and 97% specificity on the DRIVE and Gaze datasets.	2025 [26]
BAT-optimized DNN hybrid vessel segmentation	Uses green-channel extraction, CLAHE enhancement, erosion, top-hat filtering, Otsu thresholding, BAT-based pixel optimization, DNN-based vessel classification, separate thick- and thin-vessel extraction, and final vessel reconstruction; achieves up to 93.79% accuracy, 63.45% sensitivity, and 92.36% specificity on the CHASE-DB1 dataset	2026 [27]
ISAR-based multi-level thresholding	Applies an improved Search and Rescue optimization algorithm to multi-level thresholding, using Otsu, Kapur, Tsallis, and fuzzy entropy objective functions, supported by Bayesian optimization and D-optimal design for threshold selection; achieves 98.38% accuracy and 98.56% sensitivity on the DRIVE and STARE datasets, with specificity evaluated graphically but not numerically specified.	2026 [28]

2. Materials and Methods

2.1. Dataset

The study was conducted using the DRIVE (Digital Retinal Images for Vessel Extraction) dataset [29], a publicly available benchmark for retinal vessel segmentation. The dataset consists of 40 color fundus images with a spatial resolution of 565×584 pixels acquired under standardized clinical conditions, divided into 20 training and 20 test images. Each image is accompanied by manually annotated vessel masks created by trained experts, which serve as reference ground truth for performance evaluation. The images exhibit considerable variability in vessel thickness, local contrast, illumination conditions, and image quality, making the dataset suitable for assessing the robustness of segmentation algorithms.

All retinal images were analyzed in their original spatial resolution without resizing. Corresponding reference masks were binarized by assigning vessel pixels a value of one and background pixels a value of zero. Prior to further processing, image intensities were normalized to the range $[0, 1]$.

2.2. Color Space Representations

To investigate the influence of color representation on retinal vessel segmentation, each image was transformed into a set of single-channel intensity maps derived from different color spaces. The analyzed representations included grayscale intensity (GRAY), RGB (R, G, B), HSV (H, S, V), HSL (H, S, L), YUV (Y, U, V), YCrCb (Y, Cr, Cb), and CIELAB (L, a, b). Each channel was treated independently as an input image for the segmentation process.

Preliminary visual analysis indicated that luminance-related channels, particularly RGB-G, YUV-Y, and LAB-L, provided superior vessel-to-background contrast compared with chrominance-based channels. These observations motivated a systematic evaluation of the relationship between color representation and segmentation performance.

2.3. Segmentation Pipeline

Retinal vessel segmentation was performed using a classical image processing framework consisting of preprocessing, thresholding, and postprocessing stages (Figure 1).

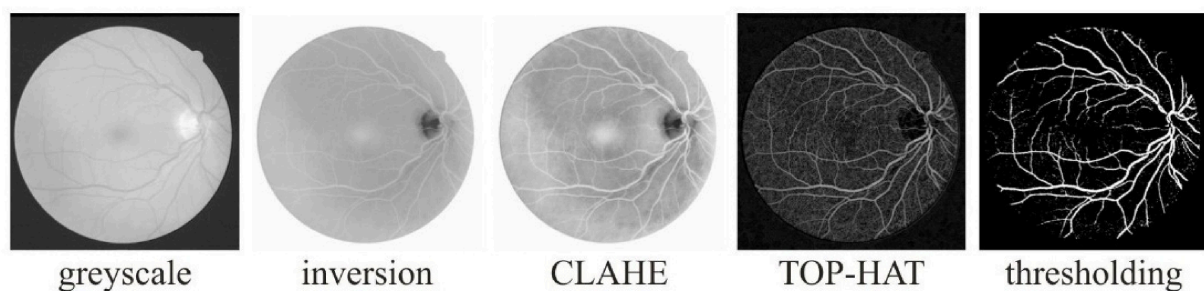


Figure 1. Example of steps of image segmentation workflow using global thresholding and the proposed image preprocessing procedure (shown in the example image [29]).

2.3.1. Preprocessing Procedure

Because retinal vessels generally appear darker than the surrounding retinal tissue, all input channels were first subjected to intensity inversion, transforming vessels into bright structures. Three preprocessing variants were evaluated:

1. **NONE**—intensity inversion only;
2. **CLAHE**—Contrast Limited Adaptive Histogram Equalization;
3. **CLAHE+TOPHAT**—CLAHE followed by white top-hat filtering using a disk-shaped structuring element.

CLAHE was used to improve local contrast and enhance vessel visibility, while top-hat filtering emphasized thin elongated structures characteristic of retinal vasculature [17,23]. Following preprocessing, all images were normalized using min–max scaling.

2.3.2. Thresholding

Binary vessel masks were generated using five thresholding algorithms: Otsu [12], Yen [13], Isodata [14], Moments (Tsai) [15] and Global thresholding. Each thresholding method was independently applied to all preprocessed image representations, producing candidate vessel masks.

2.3.3. Postprocessing

To reduce noise and eliminate anatomically implausible detections, connected components smaller than a predefined minimum size were removed from the binary masks. This operation suppressed isolated false-positive regions while preserving the continuity of vascular structures.

2.4. Evaluation Metrics

Segmentation performance was evaluated by comparing predicted vessel masks with the corresponding expert annotations on a pixel-wise basis. The following metrics were calculated: Accuracy, Sensitivity (Recall), Precision, Specificity, Intersection over Union (IoU), F1-score and Dice Similarity Coefficient (Dice). Among these measures, the Dice coefficient was selected as the primary evaluation criterion because it provides a balanced assessment of both false positives and false negatives and is widely used in medical image segmentation studies. Performance metrics were calculated for each image individually and subsequently averaged across the dataset.

2.5. Optimization of a Task-Specific Luminance Representation

To further improve vessel visibility, a task-specific luminance representation was introduced as a weighted combination of RGB channels:

$$I_W(x, y) = w_R \cdot R(x, y) + w_G \cdot G(x, y) + w_B \cdot B(x, y)$$

subject to the constraints

$$w_R + w_G + w_B = 1$$

and

$$w_G \geq w_R \geq w_B.$$

These constraints enforce a dominant contribution of the green channel while limiting the influence of the blue channel, reflecting the spectral properties of retinal blood vessels.

Optimization of the weighting coefficients was performed in two stages. First, a coarse grid search explored the admissible weight space. Subsequently, a local refinement was conducted around the best-performing region. The optimization objective was the average Dice coefficient across the dataset, while the thresholding algorithm was fixed to the best-performing method identified during the preliminary analysis.

2.6. Uncertainty Estimation

To assess segmentation reliability, pixel-wise uncertainty maps were generated by combining aleatoric and geometric uncertainty components. Aleatoric uncertainty U_{ale} was estimated using image-derived features including gradient magnitude, local contrast, signal-to-noise ratio, and vesselness response obtained with the Frangi filter. The individual components were normalized and aggregated using a weighted linear combination:

$$U_{ale} = \sum_{i=1}^N \lambda_i U_i,$$

where U_i denotes a normalized uncertainty component, N corresponds to the number of types of calculated uncertainties and λ_i represents its weighting coefficient.

Geometric uncertainty U_{geo} was computed from the predicted vessel mask using the Euclidean distance transform. It incorporated both boundary proximity and vessel thickness:

$$U_{geo} = \gamma U_{boundary} + (1 - \gamma) U_{thin}.$$

The final uncertainty map U_{comb} was obtained by combining aleatoric and geometric components:

$$U_{\text{comb}} = \eta_1 U_{\text{ale}} + \eta_2 U_{\text{geo}},$$

where $\sum \eta_i = 1$.

2.7. Coverage–Quality Trade-Off Analysis

To investigate the relationship between segmentation quality and prediction confidence, uncertainty-guided filtering was applied. Pixels exceeding a predefined uncertainty threshold were progressively excluded from evaluation, resulting in decreasing spatial coverage. Segmentation performance was subsequently recalculated using only the retained low-uncertainty pixels, enabling analysis of the trade-off between segmentation accuracy and image coverage.

2.8. Statistical Analysis

Statistical analysis was performed to assess differences between segmentation configurations and evaluate the significance of preprocessing effects. Correlations between segmentation performance and uncertainty measures were quantified using Pearson and Spearman correlation coefficients. Pairwise comparisons were conducted using paired *t*-tests and Wilcoxon signed-rank tests, while effect sizes were reported using Cohen's (d_z) [30]. To account for multiple comparisons, Holm–Bonferroni correction [31] was applied.

2.9. Software Environment

All experiments were implemented in Python 3.14.6 using standard scientific computing libraries. Image processing was performed using OpenCV 5.0.0 and scikit-image 0.26.0, numerical calculations were conducted with NumPy 2.5.1 and SciPy 1.18.0, statistical analysis was performed using SciPy 1.18.0 and pandas 3.0.3, and visualizations were generated with Matplotlib 3.11.0 [32–35]. All experiments were executed in a CPU-based environment to ensure reproducibility and computational efficiency.

3. Results

3.1. Primary Evaluation of Color Representations and Thresholding Methods

The initial analysis investigated the influence of color representation and thresholding strategy on retinal vessel segmentation performance. A total of 95 configurations, corresponding to combinations of 19 image channels and five thresholding algorithms, were evaluated using the DRIVE dataset. The Dice coefficient was used as the primary performance metric.

The results demonstrated that segmentation quality strongly depends on both the selected image representation and the thresholding method (Figure 2). Among all analyzed channels, the highest Dice scores were consistently obtained for luminance-dominant representations, particularly the green channel of the RGB color space (RGB-G), the Y component of the YUV color space, and the L channel of the CIELAB color space. Differences between these channels were relatively small, indicating that they encode largely overlapping structural information relevant to retinal vessel detection. In contrast, chrominance-based channels, including RGB-B, HSV-H, YCrCb-Cb, and YCrCb-Cr, yielded substantially lower performance and, in some cases, failed to generate anatomically meaningful vessel masks. These findings suggest that retinal vessel information is primarily associated with intensity variations rather than color differences.

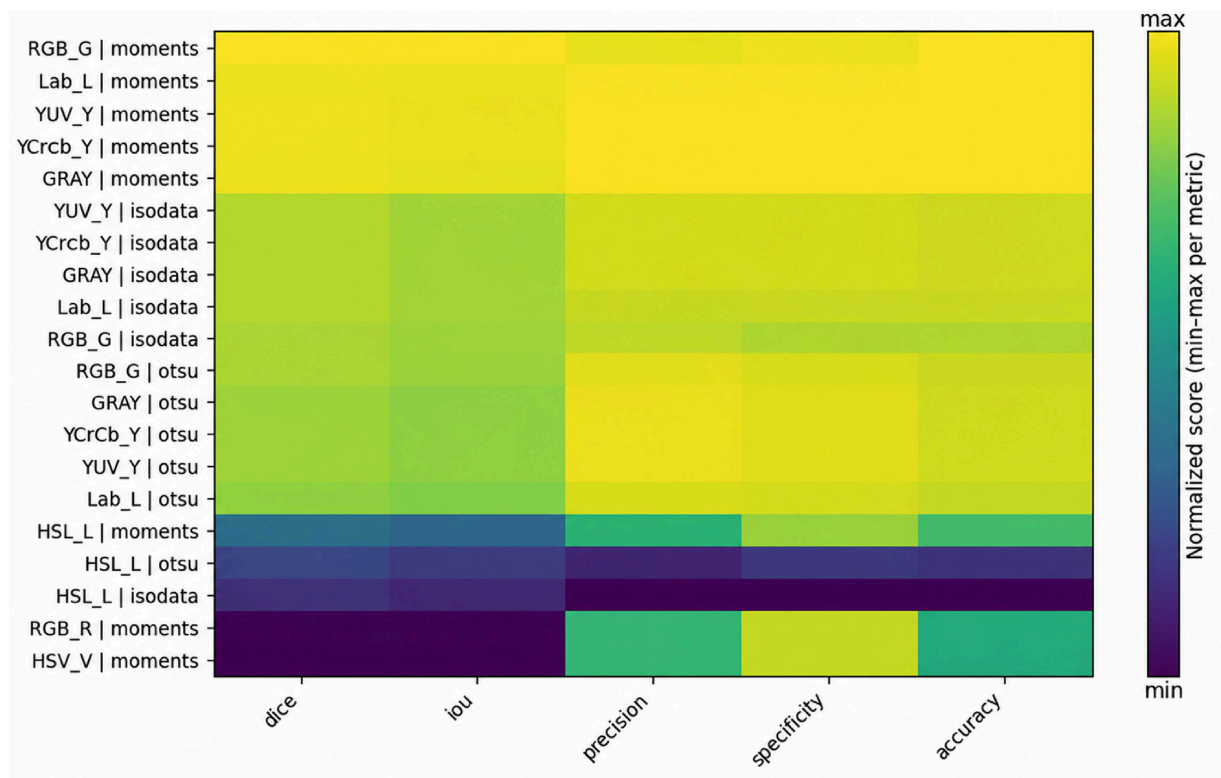


Figure 2. Heatmap of the top 20 combinations of channels and thresholding methods sorted by average Dice scores.

The choice of thresholding algorithm had a greater impact on segmentation performance than the color representation itself. Among the evaluated methods, the Moments (Tsai) algorithm consistently achieved the highest Dice and IoU values while maintaining a balanced trade-off between sensitivity and precision. Otsu and Isodata thresholding produced slightly lower performance, whereas Yen and global thresholding exhibited high precision but substantially reduced sensitivity, leading to lower overall Dice scores. These results indicate that accurate retinal vessel segmentation requires a thresholding strategy capable of preserving both large vessels and fine vascular structures.

3.2. Effect of Preprocessing Strategies on Retinal Vessel Segmentation Performance

The influence of contrast enhancement was evaluated using three preprocessing variants: intensity inversion alone (NONE), CLAHE, and CLAHE combined with white top-hat filtering. Across most luminance-based channels and thresholding methods, preprocessing led to substantial improvements in segmentation quality (Figure 3).

Application of CLAHE increased local vessel contrast and improved the visibility of thin vascular structures, resulting in higher Dice scores compared with the baseline configuration. Additional enhancement through top-hat filtering further emphasized elongated vessel-like structures and generally produced the highest median Dice values. Statistical analysis using the paired Wilcoxon signed-rank test confirmed that these improvements were significant for the majority of luminance-channel and thresholding-method combinations, particularly for the Moments, Otsu, and Isodata algorithms. The strongest improvements were observed for channels that have already exhibited high baseline performance, including RGB-G, YUV-Y, and LAB-L.

Although CLAHE combined with top-hat filtering generally achieved the best segmentation performance, a moderate increase in result variability was observed for some thresholding methods prone to over-segmentation, particularly Yen thresholding. Neverthe-

less, the overall findings demonstrate that preprocessing plays a critical role in improving both segmentation accuracy and robustness.

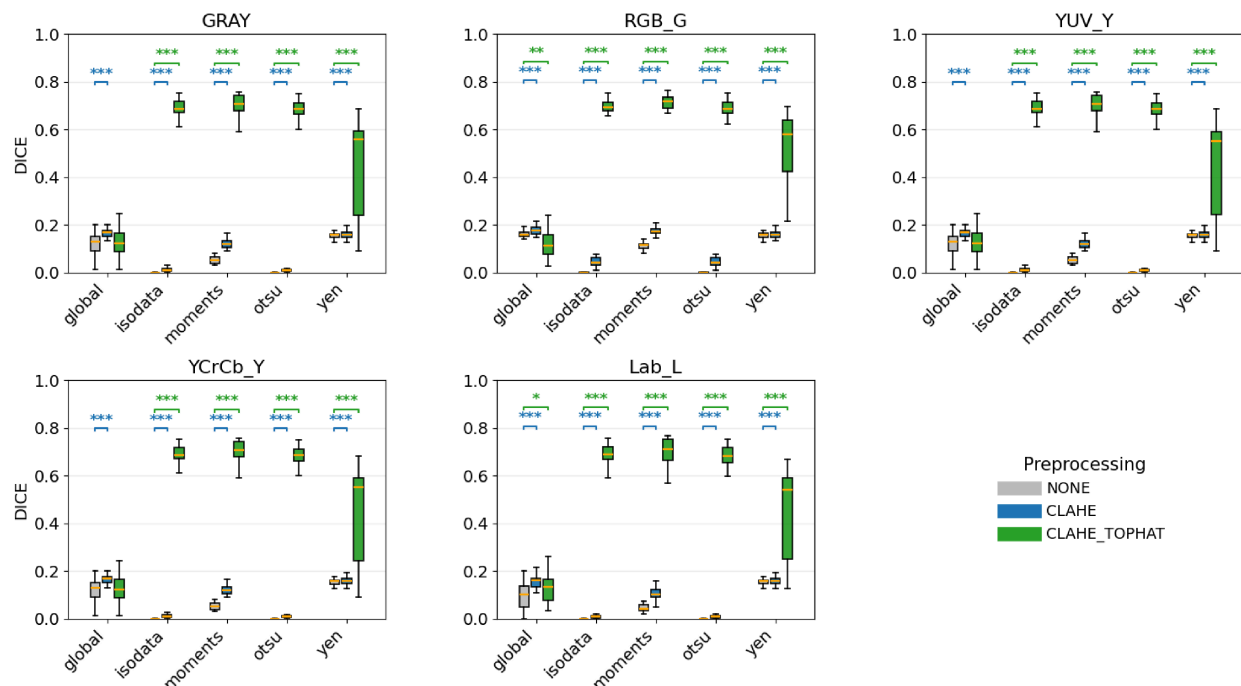


Figure 3. Box plots of Dice coefficient values for different image channels, thresholding methods, and preprocessing variants. The graphs show the median, interquartile range (IQR), and variability of the results. The bars above the graphs indicate statistical comparisons between the reference variant (NONE) and the CLAHE and CLAHE with top-hat (CLAHE_TOPHAT) methods. Statistical significance was assessed using the paired Wilcoxon signed-rank test with Holm correction performed within the combination of channel and thresholding algorithm. The number of asterisks indicates the level of statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.3. Optimization of a Task-Specific Weighted RGB Representation

Given the highest overall performance observed for the Lab L, RGB G, and YUV Y channels (Table 2), further analysis focused on developing a task-specific luminance representation optimized for retinal vessel enhancement. Both the experimental results and the theoretical properties of individual color channels suggested that an effective representation should assign a dominant weight to the green channel and only a minimal contribution to the blue channel. The green channel typically provides the strongest vessel-to-background contrast, whereas the blue channel is more susceptible to imaging noise and often contains less useful structural information. These observations motivated the hypothesis that an appropriately weighted combination of the RGB channels could produce a luminance representation that more effectively emphasizes retinal blood vessels than standard color-space components.

Table 2. Accuracy, precision, and specificity obtained for the best-performing color-space channels using moment-based thresholding.

Channel	Accuracy	Precision	Specificity
RGB-G	95.59%	84.90%	98.93%
Lab-L	95.59%	85.70%	99.02%
YUV-Y	95.60%	85.93%	99.04%

To further improve vessel contrast, a task-specific luminance representation was constructed as a weighted combination of RGB channels. Optimization of the red-, green-, and blue-channel weights (w_R , w_G , and w_B) revealed a well-defined optimum characterized by a dominant contribution of the green channel ($w_G \approx 0.88\text{--}0.90$), a smaller contribution of the red channel ($w_R \approx 0.10\text{--}0.12$), and a minimal contribution of the blue channel ($w_B \approx 0\text{--}0.02$).

The optimization landscape showed a monotonic increase in Dice score with increasing green-channel weight, confirming the importance of green-wavelength information for retinal vessel visualization. The performance surface exhibited smooth transitions between neighboring weight configurations, indicating that the proposed representation is robust to small variations in the weighting parameters and does not exhibit signs of overfitting. Conversely, increasing the blue-channel contribution consistently reduced segmentation quality, likely due to amplification of imaging noise and reduced vessel contrast (Figure 4).

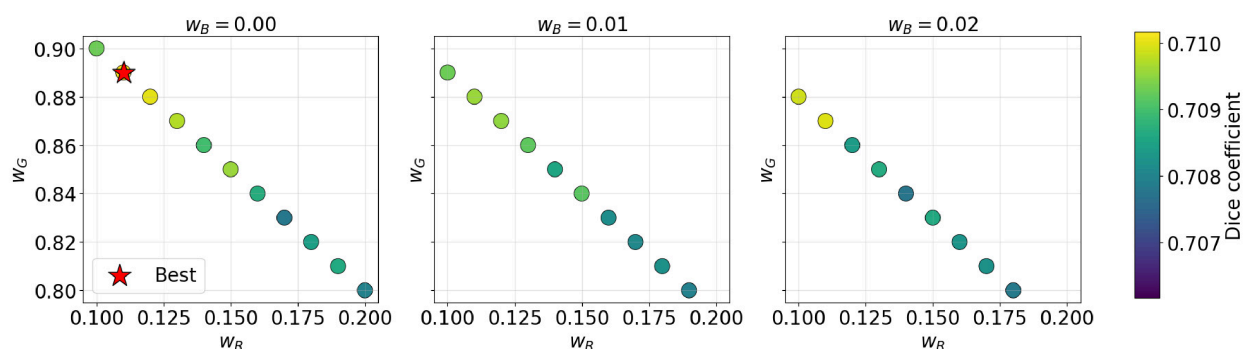


Figure 4. Average Dice scores obtained by optimizing the weighted RGB representation for specific tasks.

Importantly, the optimized weighted RGB representation achieved higher Dice scores than all standard luminance channels, including RGB-G and YUV-Y (Figure 5). The weighted RGB representation improved the overall segmentation performance, achieving the highest Dice score, recall, and accuracy among the evaluated channel representations, while maintaining precision and specificity at a similarly high level. These results demonstrate that task-specific adaptation of luminance representations can improve retinal vessel segmentation without increasing computational complexity or requiring modifications to the segmentation pipeline.

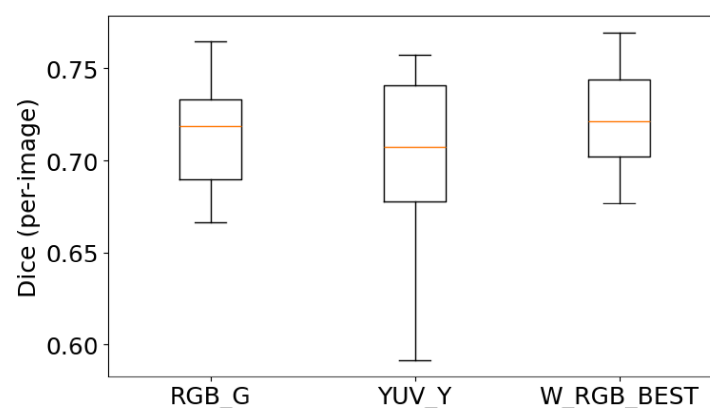


Figure 5. Distribution of Dice values for the RGB-G, YUV-Y channels, and the best weighted RGB representation for the entire dataset.

3.4. Uncertainty-Based Assessment of Segmentation Reliability and Coverage–Quality Trade-Off Analysis

In addition to segmentation accuracy, prediction reliability was assessed through pixel-level uncertainty estimation. The uncertainty framework combined aleatoric uncertainty derived from image characteristics with geometric uncertainty derived from vessel morphology. This approach enabled evaluation of segmentation confidence independently of conventional accuracy metrics.

A sensitivity analysis was conducted over a range of candidate weight values to assess the robustness of the uncertainty formulation. The final weights were selected from the central region of the parameter space in which the model exhibited stable performance, thereby reducing the risk of selecting values associated with isolated local optima. In the final configuration, aleatoric uncertainty was calculated as a weighted combination of gradient magnitude, local contrast, signal-to-noise ratio, and vesselness, with weights of 0.35, 0.30, 0.10, and 0.25, respectively. Geometric uncertainty was obtained by combining boundary proximity and vessel thickness with weights of 0.4 and 0.6, respectively. The final uncertainty map was then constructed by aggregating the aleatoric and geometric components with weights of 0.7 and 0.3, respectively.

Analysis revealed only a weak correlation between Dice scores and uncertainty values (Table 3), indicating that uncertainty captures complementary information not reflected by global segmentation metrics. Regions corresponding to vessel boundaries, thin capillaries, and low-contrast structures frequently exhibited elevated uncertainty despite contributing relatively little to the overall Dice score. Consequently, high segmentation accuracy did not necessarily imply high prediction confidence across the entire image.

Table 3. Pairwise comparison of segmentation performance and uncertainty.

Comparison		Metric	Mean A	Mean B	Δ (A – B)	<i>p</i> (Wilcoxon)	Effect Size
A	B						
RGB-G	YUV-Y	Dice	0.702	0.699	+0.003	0.216	dz = +0.196
		Uncertainty	0.791	0.799	−0.009	0.004	dz = −0.736
RGB-G	W_best	Dice	0.702	0.710	−0.008	0.001	dz = −0.881
		Uncertainty	0.791	0.794	−0.003	0.133	dz = −0.345
YUV-Y	W_best	Dice	0.699	0.710	−0.011	0.001	dz = −0.911
		Uncertainty	0.799	0.794	+0.006	0.004	dz = +0.724

Comparison of the best-performing luminance representations showed that channels with lower segmentation performance generally exhibited higher uncertainty within vessel regions. The optimized weighted RGB representation achieved the highest median Dice score while simultaneously exhibiting lower uncertainty and reduced inter-image variability compared with RGB-G and YUV-Y. The smaller interquartile range observed for the proposed representation indicates improved segmentation consistency across the dataset.

The relationship between segmentation confidence and spatial completeness was investigated through uncertainty-guided filtering. Pixels associated with high uncertainty values were progressively excluded from evaluation, resulting in reduced image coverage but increased segmentation quality (Figure 6).

For all analyzed representations, the Dice coefficient increased rapidly when the most uncertain pixels were removed, confirming that uncertainty effectively identifies regions prone to segmentation errors. However, these improvements were achieved at the expense of reduced spatial coverage, highlighting an inherent trade-off between prediction reliability and completeness. Across the entire coverage range, the optimized weighted

RGB representation consistently outperformed RGB-G and YUV-Y, achieving higher Dice values for both selective and full-image evaluation.

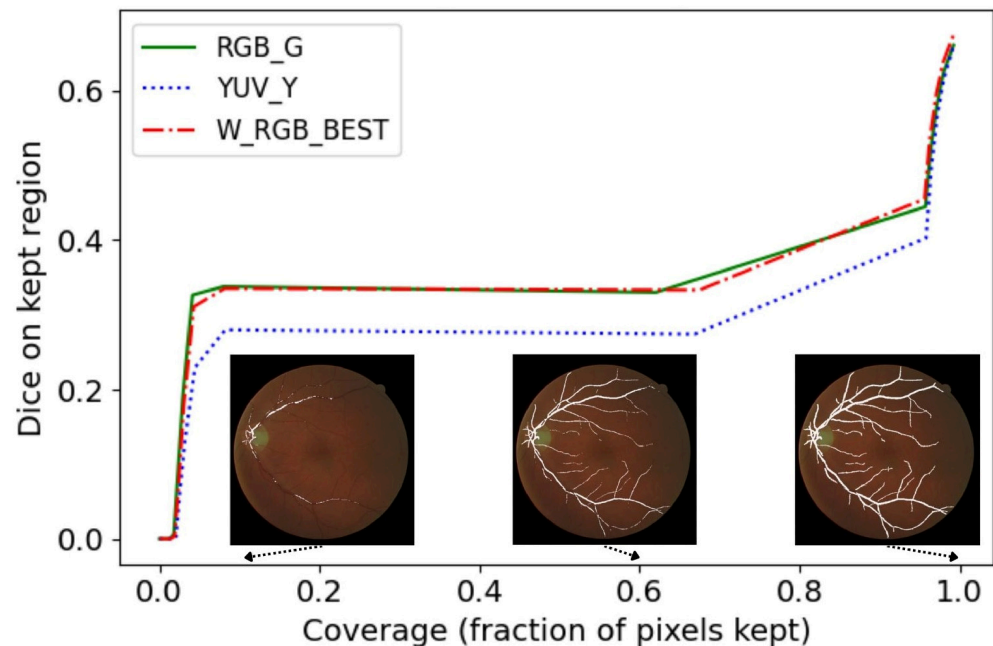


Figure 6. Comparison of the effects of uncertainty-aware filtering for RGB G, YUV Y, and best-weighted RGB channels, showing the relationship between the level of image smoothing and the preservation of important diagnostic structures (shown in the example image [29]). The arrows indicate the coverage values corresponding to the presented vessel images.

Pairwise statistical comparisons further confirmed the superiority of the proposed representation. While RGB-G and YUV-Y produced similar Dice scores, RGB-G exhibited significantly lower uncertainty. The weighted RGB representation, however, achieved significantly higher Dice scores than both standard luminance channels while maintaining uncertainty levels comparable to RGB-G and lower than YUV-Y. The observed effect sizes indicate that these improvements are not only statistically significant but also practically meaningful.

Overall, the results demonstrate that retinal vessel segmentation using classical thresholding methods is driven primarily by luminance information. The combination of contrast enhancement, Moments thresholding, and the proposed task-specific weighted RGB representation provides the most favorable balance between segmentation accuracy, robustness, and prediction reliability.

4. Discussion

The present study systematically investigated the influence of color representation, preprocessing, and thresholding strategies on unsupervised retinal vessel segmentation. The results demonstrate that segmentation performance is governed primarily by luminance information, while chromatic components contribute little useful information for vessel detection. Furthermore, the findings show that optimizing the image representation itself can provide measurable improvements in segmentation quality without increasing computational complexity, highlighting the importance of feature representation even in classical threshold-based segmentation frameworks.

One of the most consistent observations was the superior performance of luminance-dominant channels, particularly RGB-G, YUV-Y, and CIELAB-L. These representations achieved the highest Dice scores across the evaluated thresholding methods, whereas

chrominance channels consistently produced inferior results. This behavior can be explained by the optical properties of retinal imaging. Hemoglobin strongly absorbs light in the green wavelength range, causing blood vessels to appear darker relative to the surrounding retinal tissue. Consequently, channels that preserve luminance and green-wavelength information provide higher vessel-to-background contrast and better visibility of fine vascular structures. In contrast, chromatic channels primarily encode color variations that are weakly related to vessel morphology and are more susceptible to noise, illumination changes, and acquisition variability. These findings are consistent with previous retinal imaging studies that identify the green channel as the most informative component for vessel analysis.

The results also revealed that the choice of thresholding algorithm had a greater influence on segmentation quality than the specific luminance representation. Among the evaluated methods, the Moments (Tsai) algorithm consistently produced the highest Dice scores and the most balanced sensitivity–precision trade-off. Unlike entropy-based or variance-based thresholding approaches, moment-preserving thresholding attempts to retain statistical properties of the original intensity distribution, which appears particularly advantageous for retinal images characterized by highly imbalanced foreground and background classes. The superior performance of the Moments algorithm across multiple luminance channels suggests that robust threshold selection is a critical factor in vessel segmentation and may contribute more substantially to performance gains than changes in color space alone.

Another important finding concerns the role of preprocessing. Both CLAHE and CLAHE combined with white top-hat filtering significantly improved segmentation performance relative to the baseline configuration. The observed improvements can be attributed to enhanced local contrast and improved visibility of thin vascular structures, particularly in poorly illuminated regions of the retina. These results agree with previous studies that reported beneficial effects of contrast enhancement and morphological filtering for retinal vessel segmentation. However, the present work extends these observations through systematic statistical validation across multiple color representations and thresholding algorithms, demonstrating that preprocessing effects are robust and reproducible rather than method-specific.

A key contribution of this study is the introduction of a task-specific luminance representation based on an optimized weighted combination of RGB channels. Although standard luminance definitions such as YUV-Y already combine RGB information, optimization of the weighting coefficients produced a representation that consistently outperformed all conventional channels. The resulting optimal weights were characterized by a dominant green contribution, a moderate red contribution, and a negligible blue component. This outcome confirms that retinal vessel contrast can be further enhanced through task-oriented adaptation of color representations. Importantly, the proposed approach improves segmentation performance without requiring additional computational resources, model training, or changes to the segmentation algorithm itself. These findings suggest that representation optimization may be an effective yet underexplored strategy for improving classical medical image analysis pipelines.

Following the comparative evaluation of color representations, preprocessing variants, postprocessing settings, and uncertainty components, the final parameter values selected for the best-performing configuration are summarized in Table 4. This configuration combined the optimized weighted RGB representation with CLAHE, white top-hat filtering, small-component removal, and the final uncertainty-weighting scheme.

Table 4. Summary of the final parameter values selected for the best-performing segmentation and uncertainty-estimation configuration.

Component	Parameter	Final Value
Weighted RGB	Red-channel weight, (w_R)	0.11
Weighted RGB	Green-channel weight, (w_G)	0.89
Weighted RGB	Blue-channel weight, (w_B)	0.00
CLAHE	Clip limit	0.01
White top-hat	Radius	5 px
Postprocessing	Minimum component size	50 px
Aleatoric uncertainty	Gradient magnitude weight	0.35
Aleatoric uncertainty	Local contrast weight	0.30
Aleatoric uncertainty	Signal-to-noise ratio weight	0.10
Aleatoric uncertainty	Vesselness weight	0.25
Geometric uncertainty	Boundary proximity weight	0.40
Geometric uncertainty	Vessel thickness weight	0.60
Final uncertainty map	Aleatoric component weight	0.70
Final uncertainty map	Geometric component weight	0.30

Beyond conventional accuracy assessment, this work incorporated uncertainty estimation to evaluate segmentation reliability. The weak relationship observed between Dice scores and uncertainty values indicates that uncertainty provides information complementary to standard segmentation metrics. Regions associated with vessel boundaries, thin capillaries, and low-contrast structures frequently exhibited elevated uncertainty despite contributing minimally to global Dice scores. From a clinical perspective, this distinction is important because accurate overall segmentation does not necessarily imply high confidence in diagnostically relevant local structures. The proposed uncertainty framework therefore offers an additional layer of interpretability that may support quality control and decision-making in automated retinal image analysis systems.

The coverage–quality analysis further demonstrated that uncertainty-guided filtering can effectively identify error-prone regions. Excluding highly uncertain pixels resulted in substantial improvements in Dice scores across all evaluated representations, confirming that uncertainty maps successfully capture areas associated with segmentation errors. Notably, the proposed weighted RGB representation maintained superior performance across the entire coverage range, indicating that its advantage is not limited to specific image regions but extends to overall segmentation quality. Furthermore, statistical comparisons showed that the performance gains achieved by the proposed representation were accompanied by equal or lower uncertainty, resulting in a more favorable balance between accuracy and reliability than standard luminance channels.

Several limitations should nevertheless be acknowledged. Although the primary experiments were conducted on the DRIVE dataset, the analysis was additionally repeated on the STARE dataset [36] to provide an initial assessment of generalizability. The results showed a similar overall pattern, supporting the observation that representations dominated by green-channel information are effective for retinal vessel enhancement. On STARE, the optimized weighted RGB representation, the RGB G channel, and the YUV Y channel all achieved an accuracy of approximately 0.95. Among these representations, the weighted RGB variant obtained the highest recall, indicating a slightly greater ability to detect vessel pixels. The comparable performance observed across both datasets suggests that the identified behavior is not specific to a single image collection or acquisition setting. Nevertheless, validation on additional datasets, such as CHASE_DB1 or HRF, would further strengthen these conclusions. Moreover, the uncertainty-weighting coefficients were selected empirically rather than optimized systematically, and future studies could

investigate data-driven approaches to uncertainty calibration. Finally, the analysis focused on threshold-based segmentation methods to preserve interpretability and computational simplicity. Integrating the proposed luminance representation into modern deep-learning architectures could provide further insight into its broader applicability.

Compared with the retinal vessel segmentation methods summarized in Table 1, the proposed weighted RGB representation achieved competitive performance. Its accuracy of 95.69% exceeded that reported for several threshold-based and hybrid approaches and remained close to the results obtained by the best-performing conventional methods, which achieved approximately 96% accuracy. Particularly strong performance was observed in terms of specificity, which reached 98.96%. This value was higher than the specificity reported for most of the methods presented in Table 1, indicating an effective suppression of false-positive vessel detections. The weighted representation also achieved a precision of 85.47%, confirming that a large proportion of the pixels classified as vessels corresponded to the reference annotations. However, a direct comparison of precision was not possible because this metric was not reported in most of the reviewed studies. Although some recent optimization-based methods reported higher accuracy or sensitivity, comparisons should be interpreted cautiously because of differences in datasets, preprocessing procedures, segmentation targets, and evaluation protocols. Overall, the proposed approach provides a favorable balance between accuracy, precision, and specificity while retaining the interpretability and low computational complexity of threshold-based segmentation.

Overall, the findings demonstrate that retinal vessel segmentation is driven primarily by luminance information and that careful optimization of image representation, preprocessing, and threshold selection can substantially improve performance in unsupervised segmentation frameworks. The proposed weighted RGB representation, combined with CLAHE-based enhancement and Moments thresholding, provides a simple, interpretable, and computationally efficient approach that achieves improved segmentation accuracy while maintaining prediction reliability. These results highlight the continued relevance of classical image processing techniques and emphasize the importance of color representation as a fundamental component of retinal image analysis.

5. Conclusions

This study investigated the role of color representation, preprocessing, and thresholding strategies in unsupervised retinal vessel segmentation using classical image processing techniques. A systematic evaluation of multiple color-space channels demonstrated that segmentation performance is strongly dependent on luminance information, while chrominance-based representations contribute limited discriminative value for vessel detection. Among the evaluated channels, luminance-dominant representations, particularly RGB-G, YUV-Y, and CIELAB-L, consistently achieved the highest segmentation accuracy.

The results further showed that the choice of thresholding algorithm has a greater impact on segmentation quality than the specific luminance representation. Among the evaluated methods, the Moments (Tsai) algorithm provided the most robust and consistent performance, achieving the highest Dice coefficients while maintaining a balanced trade-off between sensitivity and precision. Additionally, contrast enhancement through CLAHE and white top-hat filtering significantly improved vessel visibility and segmentation accuracy, confirming the importance of preprocessing in classical retinal image analysis pipelines.

A major contribution to this work is the introduction of a task-specific luminance representation based on an optimized weighted combination of RGB channels. The proposed representation, characterized by a dominant contribution of the green channel and minimal blue-channel influence, consistently outperformed conventional luminance definitions while maintaining full compatibility with threshold-based segmentation methods.

These findings demonstrate that adapting image representations to the characteristics of a specific segmentation task can provide measurable improvements without increasing computational complexity.

Beyond conventional performance evaluation, the incorporation of uncertainty estimation provided additional insight into segmentation reliability. The results showed that uncertainty captures information complementary to standard accuracy metrics and effectively identifies regions prone to segmentation errors. Furthermore, uncertainty-guided analysis revealed a clear trade-off between segmentation quality and spatial coverage, while confirming that the proposed weighted RGB representation achieves a favorable balance between accuracy, consistency, and prediction confidence.

It should be emphasized that the objective of this study was not to surpass the performance of state-of-the-art deep-learning approaches. Instead, the use of computationally efficient and interpretable threshold-based methods enabled a systematic investigation of the influence of color-space representations on retinal vessel segmentation. By minimizing the impact of complex learned features, the proposed framework allowed the contribution of individual color channels and luminance formulations to be assessed directly. The findings therefore provide insights into the image characteristics that facilitate vessel detection and may serve as a foundation for the design of both classical and learning-based segmentation methods.

Overall, the findings indicate that effective retinal vessel segmentation can be achieved using a computationally efficient and interpretable framework based on optimized luminance representation, contrast enhancement, and robust thresholding. The proposed pipeline offers a practical alternative to more complex learning-based approaches, particularly in applications where transparency, reproducibility, and low computational requirements are important. Future work will focus on validating the proposed methodology across additional retinal datasets and investigating its integration with modern machine-learning and deep-learning segmentation frameworks.

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