

Automatic Selection of Hues for Stain Deconvolution in Digital Histopathology Image Analysis


Poster · March 2018

DOI: 10.13140/RG.2.2.20871.6440

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# Automatic Selection of Hues for Stain Deconvolution in Digital Histopathology Image Analysis

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## BACKGROUND

### Problem of Colour inconsistency:

1. Lack of standardization in histopathology practices
2. Variation between stain and scanner vendors [1]

### Colour Normalization and Deconvolution

In normalizing colour, stain deconvolution is a critical step which separates a histopathology image into its constituent stain components based on stain vectors that represent each of the constituent pure stains. Through estimating stain hues on a per-image basis, the resulting stain concentration images are independent of the stain preparation protocol.

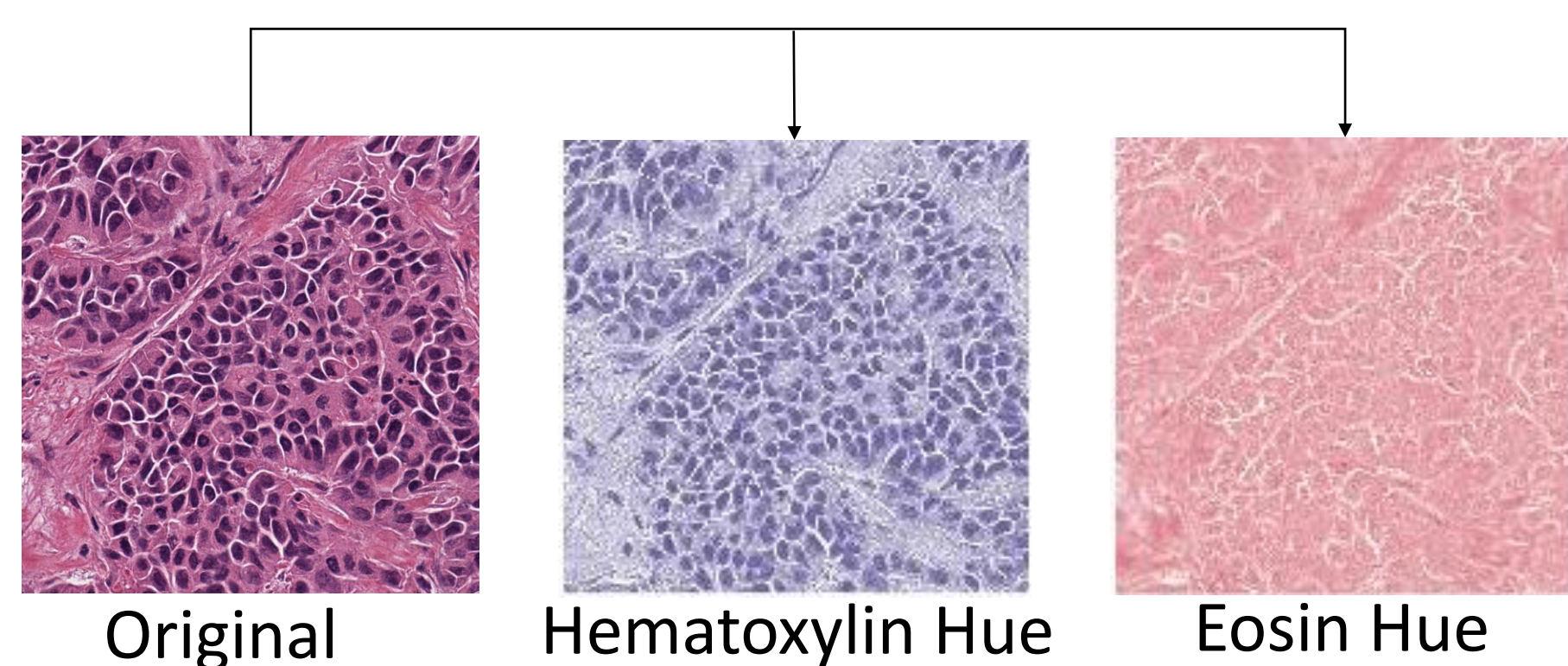


Figure 1: Stain Deconvolution flow chart

### Hue Histogram Variation and Hue Estimation

- Current Methods: 1) Manual 2) Semi-automated 3) Full-automated [2]
- Fully – automated:
  - Fit Specific statistical distribution to histogram (ie. Gaussian Distribution)

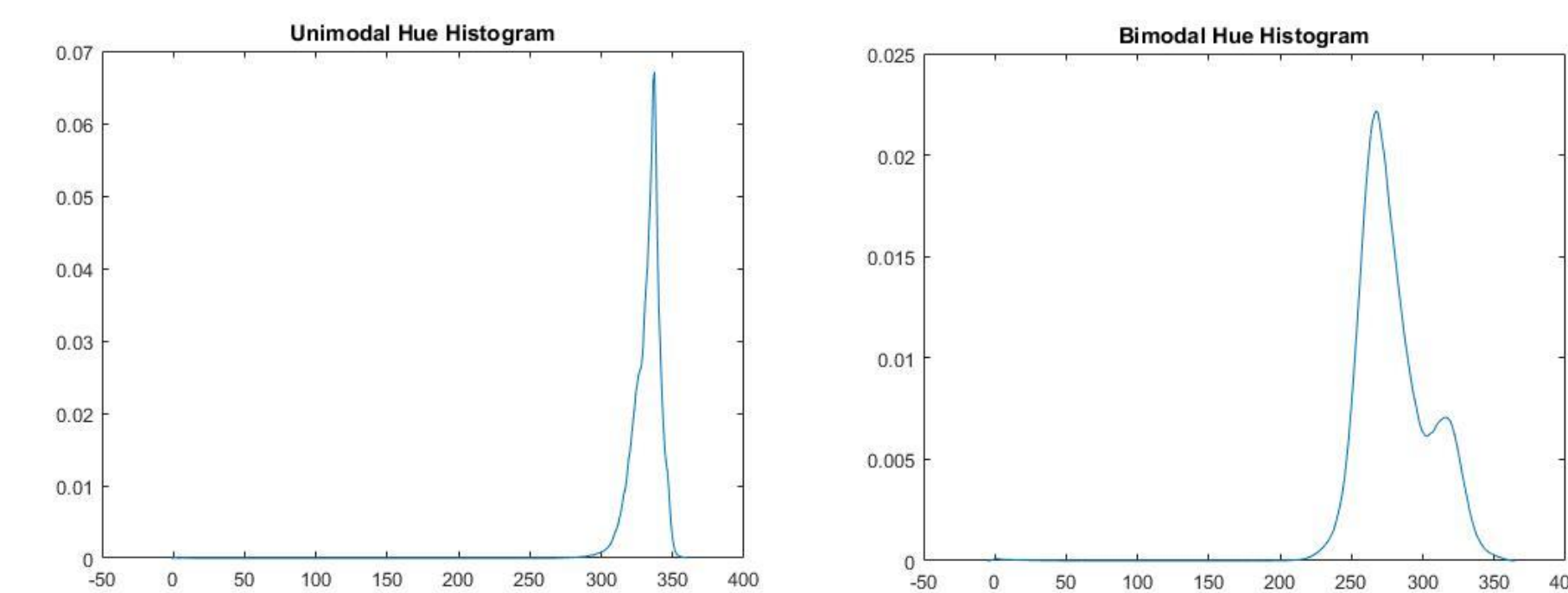


Figure 2: Saturation-weighted hue histogram variations

The multimodal nature and skewness of the hue histograms make fitting a specific distribution to a histogram difficult. We propose an automatic method that partitions the saturation-weighted hue histogram (SWHH) into stain regions using the hue cluster median and adaptive outlier detection algorithm to select the hue of each stain robustly, even for skewed distributions. To demonstrate the benefits of the proposed method, we compare it to two other methods that estimate histogram centrality based on the mean and Otsu's threshold.

## METHODS

- 1) Query image is converted to HSV from RGB
- SWHH is generated

- 2) A very small threshold ( $0.01 \times \max$ ) is applied to capture the region in which most pixels occur
- The hues of the hematoxylin and eosin stains are then estimated using the  $\sigma_{\text{median}} \pm$  interquartile range (IQR).

- 3) Remaining HSV vectors are transformed back to the RGB space
- Using the detected stain vectors are the hematoxylin, eosin, and background or no-stain (ns) images are produced through stain deconvolution.

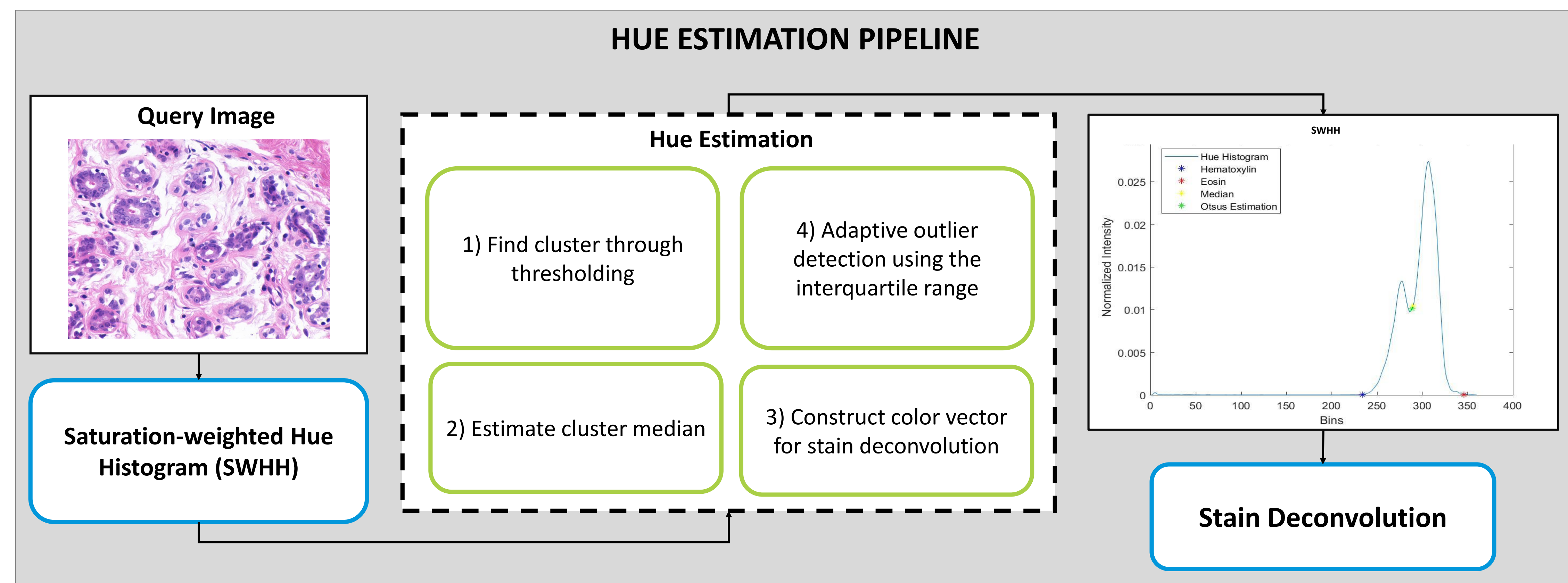


Figure 3: Hue estimation pipeline

## RESULTS

### Comparing Deconvolution Images, SWHH, and Error Metrics

Hematoxylin		Eosin		Average energy normalized by channel	Average energy normalized by pixel
Mean Hue	Mean Saturation	Mean Hue	Mean Saturation		
214	340	0.1221	0.0877	2.213E-04	2.435

\*Note: Table values were calculated based on 10 query images

Hematoxylin		Eosin		Average energy normalized by channel	Average energy normalized by pixel
Mean Hue	Mean Saturation	Mean Hue	Mean Saturation		
218	341	0.1668	0.0699	1.9840E-04	2.1546

\*Note: Table values were calculated based on 8/10 query images, in which the algorithm failed to run on 2/10 query images

Hematoxylin		Eosin		Average energy normalized by channel	Average energy normalized by pixel
Mean Hue	Mean Saturation	Mean Hue	Mean Saturation		
214	341	0.1465	0.0549	1.9404E-04	2.1626

\*Note: Table values were calculated based on 10 query images

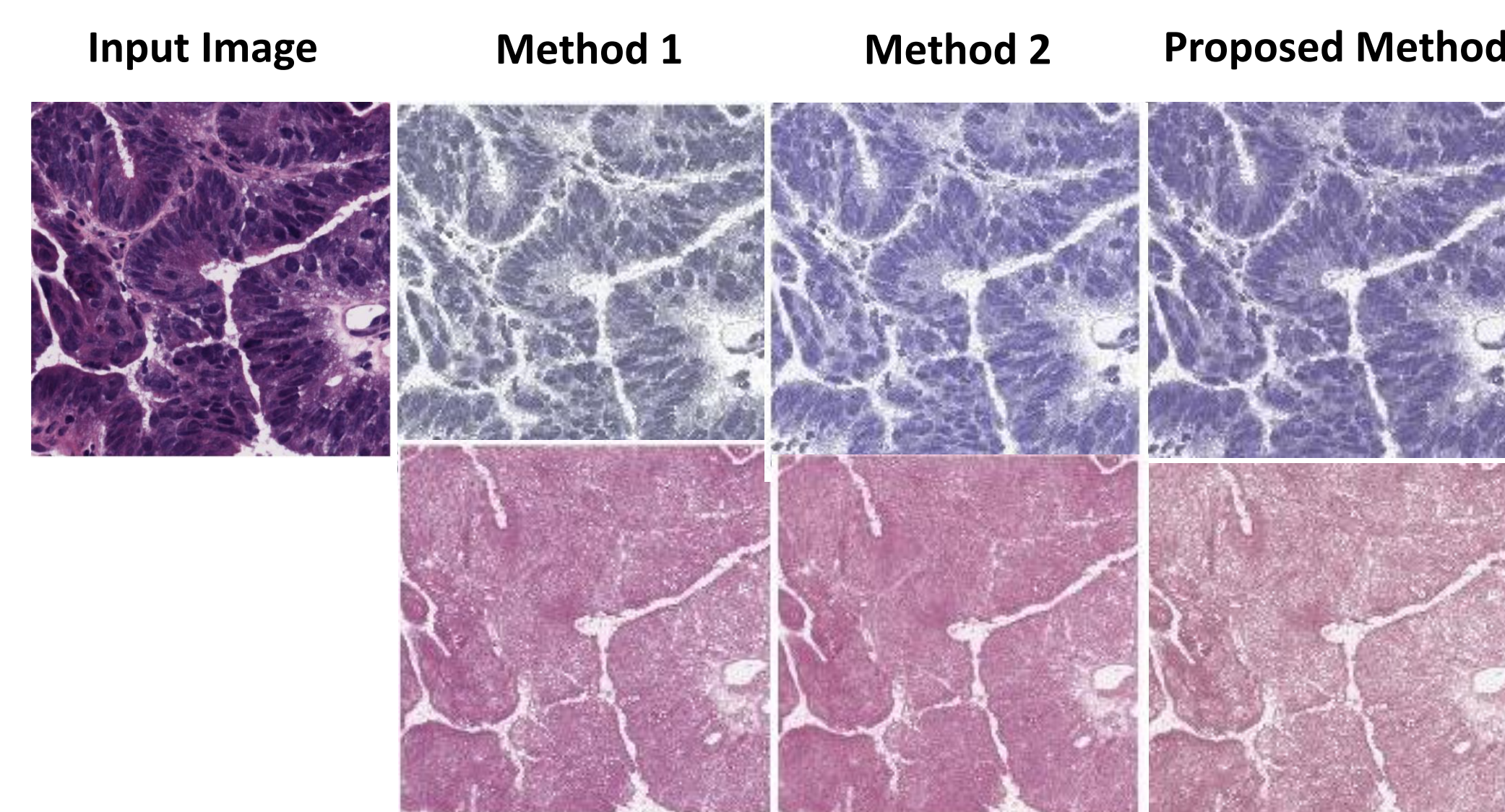


Figure 4: Comparing stain deconvolution methods using query image #1

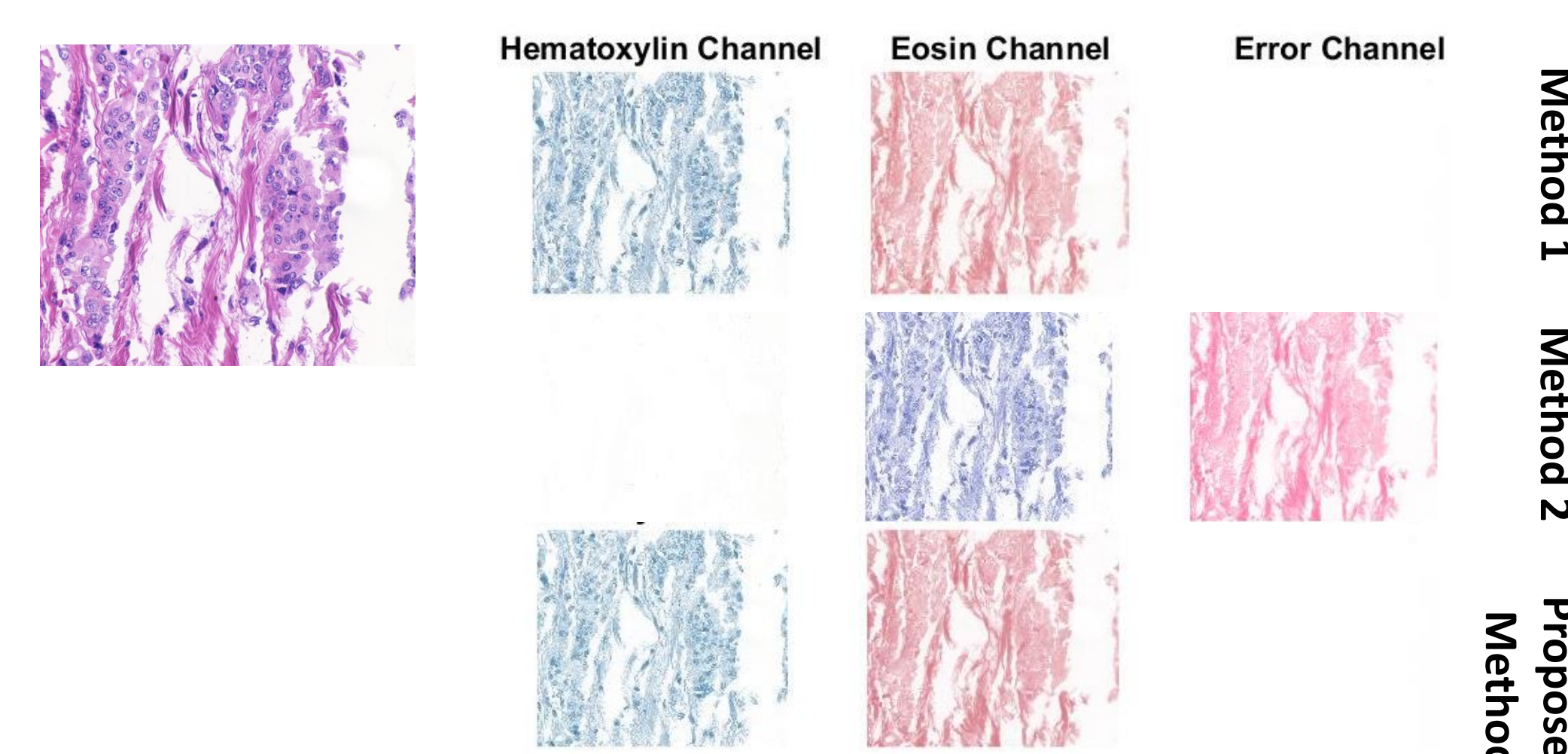


Figure 5: Comparing stain deconvolution methods using query image #2

## RESULTS CONT'D.

- Average energy normalized by channel was lowest in the proposed method, with 2.25% difference from method 2
- Average energy normalized by pixel was lowest in method 2, and had a 0.37% difference from the proposed method.
- Hue estimation using Otsu's histogram threshold  $\pm$  IQR is sensitive to hue histogram *multi-modalness* and may result in stain deconvolution error (See Figure 5).
- The proposed method is effective and consistent in estimating hues for multimodal and skewed-right, or skewed-left distributions (See Figure 6). Figure 6b) depicts method 2's sensitivity to very small intensity values, while the proposed method effectively estimates cluster centrality.

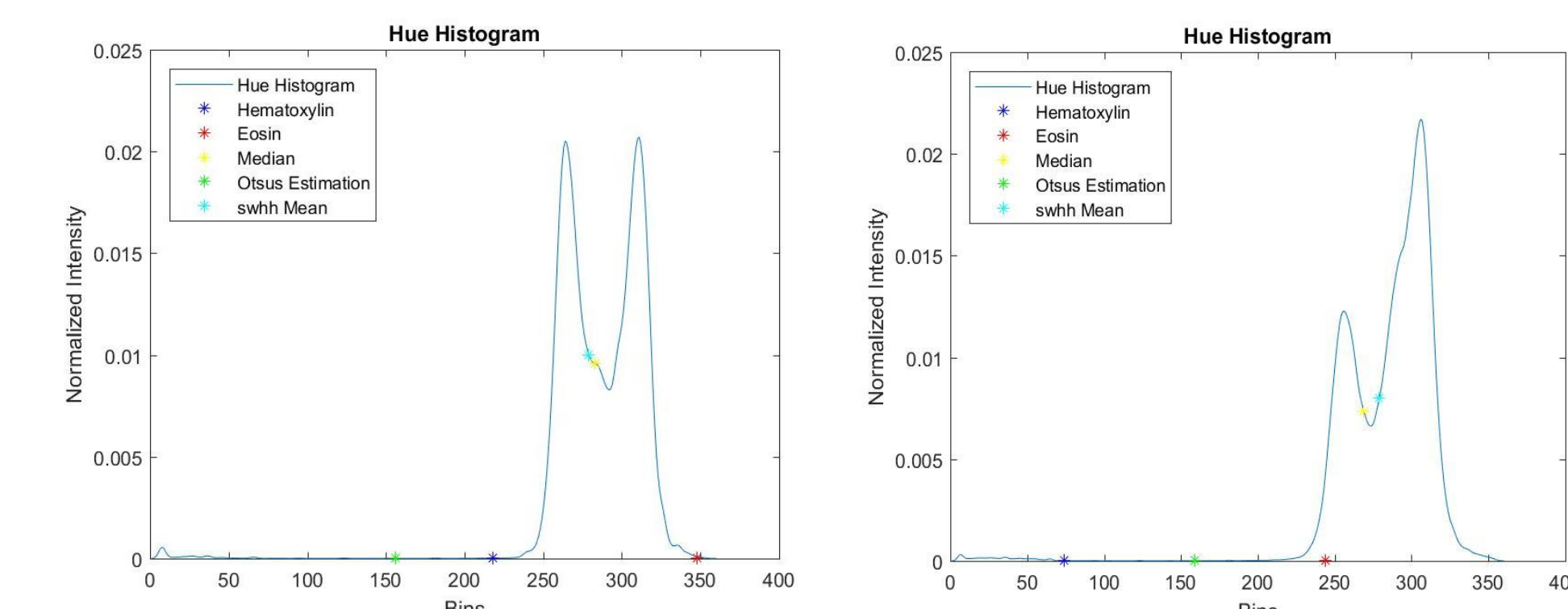


Figure 6a) and 6b): Comparing hue estimation for methods 1, 2, and proposed

## CONCLUSION

This work introduced an automatic stain deconvolution method for digital histopathology images. Our results demonstrate the ability of our method to adapt to histogram skewness, while accurately estimating stain vectors in H&E images. This demonstrates the potential for developing more reliable and efficient standardization systems.

## REFERENCES

- [1] J. Knight and A. Khademi, CRC Press, 2017.
- [2] Li et al., IEEE Trans. Bio. Med. Eng., vol. 62, no. 7, pp. 1862-73, Feb 2015.
- [3] Eng. Stats., [Online]. Available: <https://www.itl.nist.gov>

## ACKNOWLEDGMENTS

The IAMLAB, located at Ryerson University, is focused on the design of algorithms that extract quantitative biomarkers from pathology and radiology images. IAMLAB investigates imaged-based precursors that shed light into disease causation and progression.