Quantitative Hematoxylin and Eosin (H&E) Tissue Staining in Digitized Whole Slide Images (WSI) Using Measures of Hue, Saturation, and Brightness (HSB)

David Kellough[1]; Trina Shanks[1]; David G. Nohle[2]; Satoshi Hamasaki[3]; Leona W. Ayers[2]; Mark Lloyd[1]; **Anil V. Parwani**[2]

[1]Inspirata Incorporated, Tampa, FL, USA,
[2]Department of Pathology, The Ohio State University (OSU), Columbus, OH, USA,
[3]Nagasaki University Hospital, Nagasaki, Nagasaki, Japan.

Background

A Baldano et al, “Consistency and Standardization of Color in Medical Imaging: a Consensus Report,” J Digit Imaging. 2015 Feb;28(1):41–52, notes that “lack of a well-defined color framework is limiting the use of digital tools that could maximize the utilization of medical devices for improved diagnostics.” We seek to calibrate image analysis software based on varying color image values generated by histopathology laboratories. To be viable, an inexpensive, rapid, and easy technique must be available.

Technology

Images of H&E stained tissue slides scanned on a Philips UFS scanner at 20x were viewed at 5x magnification smoothing out small variations in sampled areas. In the Philips Image Management System, 356^2 pixel sections of heavily eosin-stained areas (stroma/muscle) and heavily hematoxylin-stained, nuclear-rich areas (lymphocytes, ducts, lobules, tumor) were captured. Hue, saturation, and brightness were quantified using a commonly available color picker (Photoshop CC 19.1.6, Adobe, San Jose, CA) to measure staining. 101^2 pixel areas (maximum allowed) were sampled.

We measured slides prepared 3/30 - 10/14/2020 using H&E images (Leica, Buffalo Grove, IL) at OSU lab 1 (n=133) with Tissue-Tek Prisma Plus (Sakura, Torrance, CA) and at OSU lab 2 (n=140) with ST4040 Linear Stainer (Leica, Buffalo Grove, IL).

Methods

We measured staining in predominantly red and blue areas of 15 breast, 15 gastrointestinal, 15 skin, and 15 prostate biopsies and H&E slides from multiple outside laboratories (n=60).

HSB values for sampled areas from images of slides from the two OSU labs were compared with each other and with those from outside institutions.

Results

The HSB color picker was effective in assessing variation in Color staining, we found great consistency among specimens prepared in the same laboratory, similar but not as great consistency among specimens from our institution, but considerable variation among images prepared in consulting laboratories. See Figure.

Conclusions

An inexpensive, readily-available HSB color-picker allowed characterization of WSI H&E colors. OSU histology labs had similar measures of central tendency (means, medians), smaller ranges, but large standard deviations with outside slides. Image analysis algorithms should be trained/tested with examples from the range of consulting laboratories served.