TITLE: Color Calibration of Digital Brightfield Microscope Images Using Colorimetry with a Color Standard Slide

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ABSTRACT: Color in digital brightfield images varies widely due to a number of hardware, software and firmware influences in addition to preparation of the specimen itself. Traditionally, color is adjusted both pre- and post-acquisition using software algorithms and/or image editing. In this poster, we present the effectiveness of an objective, color standard-based post-acquisition color calibration method.

INTRODUCTION: Aside from the microscope components, the color accuracy of digital brightfield images is typically determined by the quality of the detector (i.e. digital camera), the nature of on-board color correction algorithms, software-based user-selectable color and contrast enhancements, white balancing and monitor display accuracy. Variation in any of these elements leads to inconsistency in images between imaging sessions, operators and systems. A variety of software applications afford researchers the ability to adjust color in images, but none use a color standard and objective determination of the color variance from the specimen itself in order to effect a color correction. We present a colorimetric and objective approach to color calibrating digital brightfield images.

MATERIALS & METHODS: Prepared histology slides (Triarch Incorporated, Ripon, WI, USA) were selected to provide a sample of commonly used stains and tonal ranges. Stains included H&E, Wright, Verhoeff's, Masson's trichrome, Iron Hematoxylin, silver, and no stain. Image acquisition was performed using a Lumenera Infinity 2-1R color microscope camera mounted on a Zeiss Axio Scope.A1 with planachromat optics and 100W halogen light source (Carl Zeiss Inc., Thornwood, NY, USA). A Kodak 80A daylight filter was placed after the light source to bring the color temperature of the illuminant to approximately 5200°K. All images were acquired as RAW without any non-linear adjustments or enhancements, and exposure after white balancing was set to approximately 90% of maximum. Specimen image calibration was performed using an additional image of a Datacolor ChromaCal brightfield color calibration slide (Datacolor Inc., Lawrenceville, NJ, USA) that was captured during the same microscopy session. FIGURE 1 illustrates the brightfield color calibration slide standards, a matrix of narrow band, interference-type filters. Multispectral images of the same area of the test samples were obtained on the same microscope using a liquid crystal tunable filter (PerkinElmer, Waltham, MA, USA) with a monochrome CCD camera (QIClick, QImaging, Tucson, AZ, USA) to capture 31 bands, 7 nm/band, from 400-700nm. The stack of 31 bands was rendered to generate the reference image with "Ground Truth Color." Color accuracy was determined by comparing the averaged color of the original RAW image and the calibrated image against the averaged color of the Ground Truth reference image and reported as ΔE.

A word about ΔE: ΔE represents the difference between 2 colors as described in CIE L*a*b* color space. As a frame of reference, a $\Delta E=1.0$ indicates the slightest difference between two colors as perceptible by the human eye.

RESULTS: In every case, the calibrated images provided an improvement in color accuracy over the raw images, both by ΔE and visual comparison, versus the Ground Truth image (FIGURE 2 and FIGURE 3). ΔE measurements varied from a low of 1.1 to a high of nearly 5 (the lower the ΔE, the better the color accuracy). The most accurate color was achieved with calibrations of specimens prepared with stains other than H&E (FIGURE 2).

CONCLUSION: Color calibration using color standards provides an objective, effective and reliable approach to color correction that, in turn, delivers considerable improvement in color accuracy across a multitude of histological stains. Digital images may never rival the specimen images as viewed through the eyepieces due to limitations in digital cameras and computer monitor, but a standardized approach to color calibration may bring greater consistency between images captured during the same session,

during different sessions, or between imaging systems. An objective color calibration method for digital images has far reaching potential for manual and automated image analysis, reports, presentations and publications.

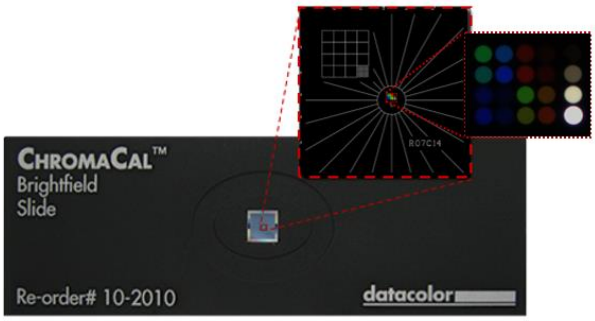
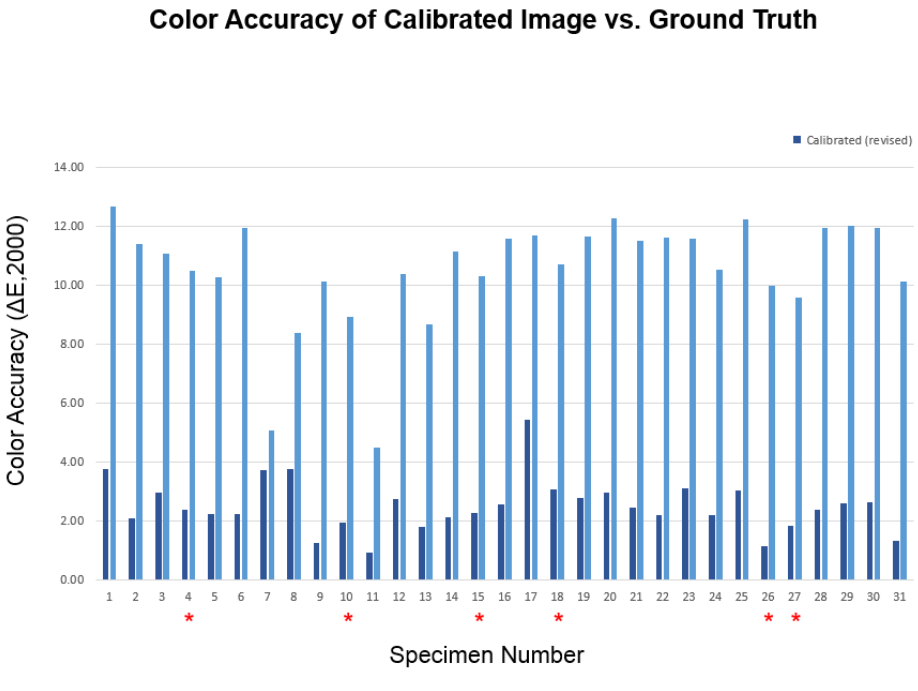


FIGURE 1: Depiction of the Datacolor ChromaCal color calibration slide. The central area of the slide houses the calibration chip that consists of two color matrices (one with 30µm circles and one with 9µm circles), and a micrometer grid. The color circles serve as color standards and are described as narrow band, interference-type filters with measured optical characteristics. The neutral-color circles are used to determine the linearity of the image.



Specimen Number	Stain
1	H&E
2	H&E
3	H&E
4	H&E
5	Verhoeff's
6	H&E
7	no stain
8	Wright
9	Iron Hematoxylin & eosin
10	Trichrome
11	H&E
12	H&E
13	H&E
14	H&E
15	Elastic
16	H&E
17	H&E
18	H&E
19	H&E
20	H&E
21	H&E
22	H&E
23	H&E
24	H&E
25	H&E
26	Silver
27	Iron Hematoxylin
28	H&E
29	H&E
30	H&E
31	H&E

FIGURE 2: Color accuracy reported as ΔE for 31 specimens representing 9 different histological preparations. Light blue bars indicate the ΔE for the RAW images vs. the Ground Truth reference images. Dark blue bars indicate the ΔE for the calibrated images vs. the Ground Truth. Specimens denoted with red asterisks (*) were selected for visual comparison as illustrated in Figure 3.

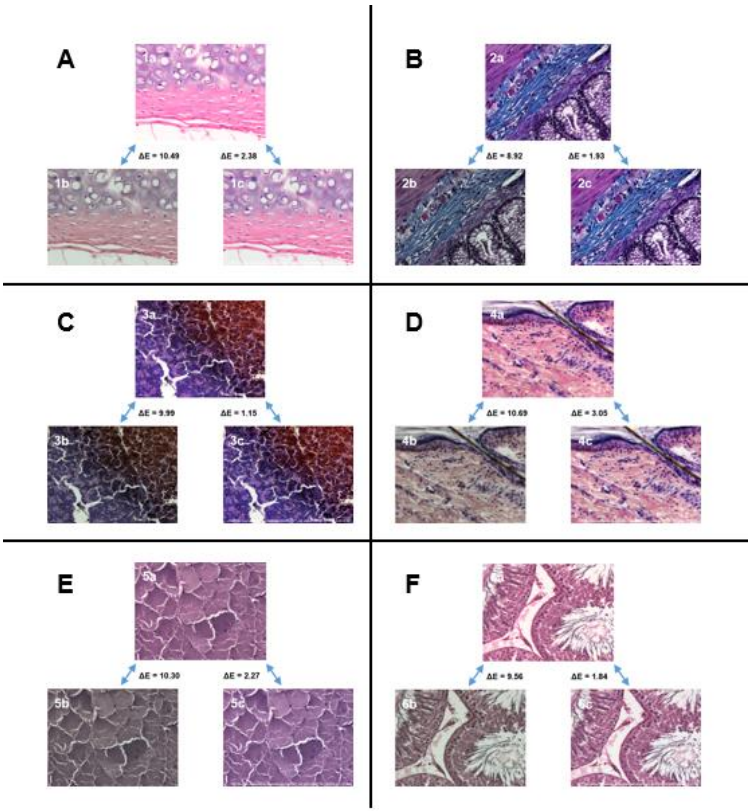


FIGURE 3: Visual comparison of color accuracy of RAW and calibrated images versus the Ground Truth reference image. Images 1a, 2a, 3a, 4a, 5a and 6a were the rendered Ground Truth reference images for each specimen. 1b, 2b, 3b, 4b, 5b and 6b are the RAW images for each specimen, and 1c, 2c, 3c, 4c, 5c and 6c are the color calibrated images. Panel A was hyaline cartilage stained with H&E. Panel B was muscled stained with Masson's trichrome. Panel C was pancreas stained with silver. Panel D was XXX stained with H&E. Panel E is artery stained for elastin. Panel F is testis stained with iron hemotoxylin. The specimens in Panels A, B, C and D represent specimen numbers 4, 10, 26, 18, 15 and 27, respectively, from Figure 2.