An Innovative Method for Obtaining Consistent Images and Quantification of Histochemically Stained Specimens

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Summary
Obtaining digital images of color brightfield microscopy is an important aspect of biomedical research and the clinical practice of diagnostic pathology. Although the field of digital pathology has had tremendous advances in whole-slide imaging systems, little effort has been directed toward standardizing color brightfield digital imaging to maintain image-to-image consistency and tonal linearity. Using a single camera and microscope to obtain digital images of three stains, we show that microscope and camera systems inherently produce image-to-image variation. Moreover, we demonstrate that post-processing with a widely used raster graphics editor software program does not completely correct for session-to-session inconsistency. We introduce a reliable method for creating consistent images with a hardware/software solution (ChromaCal™; Datacolor Inc., NJ) along with its features for creating color standardization, preserving linear tonal levels, providing automated white balancing and setting automated brightness to consistent levels. The resulting image consistency using this method will also streamline mean density and morphometry measurements, as images are easily segmented and single thresholds can be used. We suggest that this is a superior method for color brightfield imaging, which can be used for quantification and can be readily incorporated into workflows. (J Histochem Cytochem 63:233–243, 2015)

Keywords
color brightfield, camera, ChromaCal, density, histology, image consistency, linearity, microscope, morphometry, quantification

Introduction
When working with color brightfield (CB) images, a great deal of effort has been put into the improvement of camera and whole-slide imaging (WSI) systems, but little effort has been directed toward the development of a workflow that increases the consistency between images, maintains tonal linearity, and improves image quality, and does so using good laboratory practices (GLP). We address these issues and introduce a novel method (ChromaCal™; Datacolor Inc., NJ), which includes standardization to a matrix of colors on a calibration slide, automated white balancing, automated brightness, tracking and reporting of post-processing, and algorithms that preserve linear tonal levels as well as camera linearity assessment.

Many of the problems inherent to image inconsistency when performing CB digital photomicroscopy stem from operator variability in using camera systems and microscopes, as well as inaccuracies in camera systems. Optimally, a high-quality image should lack the following: muted colors (Margulis 2006; Min 2009; Luo et al. 1991) background discoloration (Lee 2005; Viggiano 2004), over- and under-exposed (saturated) images (Cromey 2010;...
Martin and Blatt 2013), and non-linearity (Chieco et al. 1994; Garcia et al. 2013; Price and Gray 2011; Rossner and Yamada 2004). Moreover, the images should have consistent brightness levels (Inoue 1986; Sedgewick 2008).

Most image defects can be better controlled when the user is familiar with using each camera system and microscope. The recommended steps include setting the attenuation of light to the manufacturer’s recommended setting (when the light source can be attenuated), focusing on the desired area of the specimen, setting Köhler illumination (a method for collimation of light through the sample), setting the exposure, and finding the optimal non-tissue area to use for white balancing the camera in the camera acquisition software (Matsumoto 2010). Even when performing all steps properly, this paper will demonstrate that inconsistencies in images are unavoidable, and a post-processing correction is vital for high-quality images.

In this study, we discuss the major image inconsistencies found in published images that are often less obvious than poor focus or poor contrast. We do not discuss microscope illumination problems, including uneven illumination across the image (Leong et al. 2003) or chromatic aberration (false color) at edges of cells and tissues (Foster 1997). However, we tested a microscope/camera operator with a single camera and microscope for images acquired over more than one microscope session using manual and automated exposure to determine levels of image inconsistency. Starting with a suboptimal image, we evaluated post-processing procedures to improve image quality by comparing a popularly used program (Photoshop) with the ChromaCal system. Using the ChromaCal system to improve image quality to consistent levels, we assessed its ability to provide predicted measurements for both optical density and morphometric measurements, using a single threshold value and tested against threshold values set manually by the user.

Materials & Methods
All of the following studies were reviewed by our Institutional Review Board and deemed exempt. Unless noted otherwise, all experiments used the following samples, equipment, software, and microscope operator.

Histological Preparation of Sample Slides

**Hematoxylin and Eosin (H&E) and Immunoperoxidase (IP) Stains.** Bone marrow core biopsies were fixed in acetic acid-zinc-formalin (AZF), lightly decalcified with acid, then processed for histology (dehydrate and imbed in paraffin). Cores were mounted onto blocks and sectioned at 3 µm and either stained with H&E or processed for automated IHC staining (Ventana Benchmark Immunostainer; Ventana Medical Systems, Inc., Tuscon, AZ). The anti-kappa immunostain was purchased prediluted (mouse monoclonal, clone KDB-1; Biocare, Concord, CA). Antigen retrieval was performed using mild Cell Conditioning 1 solution (CC1, Ventana Medical Systems). The primary antibody was incubated for 4 min at 37°C. The anti-CD61 immunostain was purchased prediluted (mouse monoclonal, clone 2F2; Cell Marque, Rocklin, CA). Antigen retrieval was performed using standard CC1 solution. The primary antibody was incubated for 32 min at 37°C. Detection of both primary antibodies was performed using the Ultraview Detection Kit (Ventana Medical Systems).

**Wright–Giemsa (WG).** Bone marrow aspirates were collected, smeared on slides and air dried. Staining was performed as previously described (Dunning and Safo 2012). Briefly, air-dried smear slides were alcohol fixed and then stained in a buffered WG stain. The polychromatic stain contains methylene blue, azures (oxidative products of methylene blue), and eosin, which differentially stain the nuclear and cytoplasmic components.

**Imaging Equipment and Software**

Images were captured on an Olympus BX51 microscope (Olympus; Tokyo, Japan) with a halogen light source and light-balancing daylight (LBD) filter in the light path, with attenuation set at the factory recommended setting for camera use, outfitted with plan-apochromatic, infinity corrected lenses: 10× (NA 0.25), 20× oil (NA 0.8), 40× oil (NA 1.0) and 100× oil (NA 1.3). Unless otherwise described, the camera for imaging was the Dage XL MCT (Dage-MTI; Michigan City, IN) with Exponent 7 acquisition software (Stable Microsystems; Surrey, UK).

ChromaCal software, version 1.4 (Datacolor Inc.; Lawrenceville, NJ) and Photoshop software, version 10 (Adobe Systems Inc.; San Jose, CA), were used to post-process images. Photoshop was used to segment and measure images; Matlab version 2012a (32-bit; Matlab Inc., Natick, MA), Fiji version 1.47t (ImageJ; Wayne Rasband, Bethesda, MD) and a MatLab-created program (RGB_DE_Calculation D50; Hong Wei, Datacolor, Inc., Lawrenceville, NJ) were used for image measurements.

**Study 1: Image Variability due to Imaging System and Operator**

**Study Outline:** Images from a single microscopist over multiple sessions comparing automated and manual camera settings. For this study, a microscope/camera operator set up the microscope with fixed Köhler illumination, set the attenuation as described above, and performed a white balance test with a Dage camera. To control for variations in stain color and contrast between different histology staining sessions, one slide was chosen from each stain type (WG, H&E and IP), and several fields of view were selected from that slide. Images were taken with 10×, 20×, 40× and 100× objectives for a total of six images per stain type.
Microscope Session Routine: All images were taken on the same day. To simulate multiple sessions over more than one day we did the following:

Automated Exposure and White Balancing

1. Camera was turned off intermittently between images of different fields of view.
2. Light attenuation was set manually to factory recommended setting.
3. The camera was set to Auto Exposure and Auto White Balance.
4. A non-tissue background area was found so that Auto White Balance could correlate camera to color temperature on a neutral area before finding the specimen field.
5. Image was acquired.

Manual Exposure and White Balancing

1. Light attenuation was turned down (to lower the voltage).
2. Auto Exposure and Auto White Balance were set manually.
3. Light attenuation was turned up to factory recommended setting.
4. Camera was white balanced on a non-tissue area, and a similar or identical field was found.
5. Camera exposure was set to a live histogram, with shutter speed and gain alternately used to expand histogram to the setting with the greatest dynamic range.

Consistency in images can be measured by comparing a high-quality image with other images of the same stain and specimen using Delta E (DE), the unit of measurement in the field of color science (Robertson 1990). This measurement indicates a change in color by comparing one image to a target image (generally a high-quality image). The Euclidean distances between similar colors when colors are mapped to a three-dimensional space are compared between the target image and comparison images. A DE of less than 1 U indicates no perceptual difference between images, and a DE of 2 U or greater is commonly considered perceptually significant. A change in color can be due not only to differences in hue but to changes in exposure and poor (or no) white balancing.

For DE measurement, regions of interest (ROIs) in non-identical images were outlined in Photoshop, such as red blood cells, counterstained cells, non-tissue areas (background) and stained cells. ROIs were found via a macro recorded in Photoshop, where tonal ranges instead of colors identified ROIs to remove bias when attempting to choose perceptually similar colors. When two or more images were identical, the entire image was used instead of the ROI.

Photoshop Macro for Selecting Stained, Background, and Cell Areas

1. Layers were created above the original image layer.
2. Using either the Black/White or Channel Mixer functions, a grayscale image was created using color components to create brighter or darker nuclei, RBCs, or IP-stained areas.
3. Brighter/darker areas were selected through the use of the Select Colors function with Highlights, Midtones or Shadows from the drop down list.
4. Or, for background area values (white, non-tissue areas), a threshold tool was used to select the brightest 5%–10% of pixels.
5. The consequent ROI selections were applied to the original image layer for measurement.
6. Mean RGB values were found in the Histogram dialog box for the red, green and blue channels and recorded in Excel, version 2003 (Microsoft Corporation; Redmond, WA).

Stained images were both visually assessed and measured (for DE values) to determine target images for each stain type. Target images (images against which DEs were measured) created minimal variation of DE so that each stain type was measured from the same basis. RGB values were compared to RGB values from the target image for each ROI from each stain. The DE value was determined through RGB_DE_Calculation_D50. DE values were recorded in Excel for each ROI. ROIs from each feature were averaged to determine a final DE value.

Study 2: Post-Processing Comparison between Photoshop and ChromaCal

A slightly underexposed and poorly white balanced WG-stained image taken at a microscope session was chosen as a test slide for this comparison. During the same microscope session, a calibration slide provided as part of the ChromaCal system was also acquired (Fig. 1), to comply with required ChromaCal procedures.

After acquiring the calibration slide image, the steps were as follows:

1. The image of the ChromaCal slide was opened in ChromaCal software (version 1.4).
2. The grid was applied over the matrix of circles so that software read color values.
3. The specimen image(s) were opened.
4. The images were saved individually, or automatically using the batch function.
5. The images were saved as TIFF files, allowing ChromaCal software to apply a label on the images, and automatically saved with a prefix to the filename to preserve the original.

ChromaCal software allows the user to proceed with post-processing only when the grid is adequately placed over...
color matrix, exposure is within the required range, and the calibration slide image is within an acceptable linear regression (for red, green and blue channel measurements) to be considered linear (Fig. 2).

Specimen images were post-processed either in ChromaCal or in Photoshop using automated and semi-automated functions for color images with all functions at default (factory) settings. Functions in Photoshop included Auto Levels (Auto Tone in more recent versions of Photoshop), Auto Color and an eyedropper tool method (semi-automated), which required the selection of a pixel that should be white on the image itself (so that the red, green and blue levels for that pixel can be used to adjust levels for the remaining pixels). Two user selections were made for the Eyedropper Tool method.

Images were evaluated on the percent accuracy of background red, green and blue levels compared with neutral (equal red, green and blue levels), the percent of the image that is overexposed (contains saturated pixels), and the mean maximum tonal level of background areas compared to the ideal level at 240 for reproduction.

For background measurements, the Color Select tool was used in Photoshop to select greater than 90% of the background area. From selected background areas, red, green and blue average values were found to determine color shift as a result of a non-white balanced image, and were also used to find the mean background values (from the Histogram palette). Saturated pixels were found in the Histogram palette (using Count). Tonal values were transcribed to Excel.

Study 3: Immuno-Peroxidase Density and Surface Area Measurements using Variable and Fixed Thresholds. A single IP-stained paraffin section of bone marrow from our stained specimens was used. The image was acquired on an Olympus BX51 with the Dage camera and a 20× oil objective lens. At the same session, an image of the ChromaCal calibration slide was acquired with a 10× lens.

This single image was post-processed in Photoshop to generate typical imaging defects. The first session image was not altered; the 2nd was brightened by +25 in Brightness/Contrast function at Legacy setting (each pixel was made 25 pixel values brighter); the 3rd darkened by -40 in Brightness/Contrast; the 4th adjusted in Curves function to create a non-white balanced, red-shifted image (R=233, G=226, B=238); the 5th made brown-shifted image (R=205, G=186, B=167) and the 6th made a yellow-shifted image (R=251, G=219, B=184).

One set of images were processed in ChromaCal software to create color corrected, white balanced and brightness matched images; the other set was left as is (with image defects).

Images for Measurement. To obtain both morphometric measurements of the IP stained areas in the images and to find a mean density (in arbitrary pixel levels), non-stained areas were converted to pure black color (mask) via a routine in Photoshop. The mask was created as follows for images with defects and ChromaCal corrected images (Fig. 3):

1. The original image was duplicated to a layer.
2. The duplicated image was blurred by a radius of 1 in the Gaussian Blur dialog box.
3. A threshold was applied to the image in the Threshold dialog box to create a binary (pure black and pure white) image. The threshold was set one of four ways:
   i. Variably via manual settings to image with defects (Photoshop).
   ii. At a consistent level (128) for image with defects (Photoshop).
   iii. At a consistent level (132) for ChromaCal-corrected image.
4. The image was inverted to create white IP areas on a black background.
5. The layer was changed to the darken mode in the Layers palette (a convolution algorithm to only allow lighter values to appear from the original image layer below).
6. The image was saved in the TIFF format for analysis.

Images Measured in Fiji. Area and mean density measurements were made, using pixels\(^2\) as surface area units of
Methods for Consistent Images

Results

Study 1: Image Variability due to Imaging System and Operator

A camera operator and microscopist who are familiar with the camera software and the microscope can virtually eliminate user error. When the operator used a single, scientific-grade camera over several sessions using manual settings, the average DE varied depending upon the stain: For WG smears, the average DE was 7.3; for H&E, 5.1; and for IP, 3.4. A DE unit of less than one indicates no perceptual difference between images. In color science, a DE of two or greater is commonly considered perceptually significant (Ruppertsberg and Bloj 2007). A change in color can be due not only to differences in hue, but to changes in exposure and poor (or no) white balancing.

The variability of images far surpasses the expectation that proper use of a camera and microscope will yield results that do not require post-processing. Given that expectation, the results should be images that are perceptually similar over more than one imaging session. Instead, our study shows perceptually dissimilar images (Fig. 4). The addition of over- and underexposure increases differences, as well as subtle, overall color shifts due to inconsistent white balancing.

When Auto Exposure and Auto White Balance settings were used, we found that color inconsistencies and under exposure were greater (Fig. 5). The average DE values rose overall: for WG, the average DE was 7.7; for H&E, 10.3; and for IP, 4.6. Furthermore, when more tissue filled the

Figure 2. ChromaCal User Interface and Linearity Check Graph. The enlarged, lower left panel of this screen shot shows the quality control score for matrix alignment, linearity and exposure. The graph on right is a representation of red, green and blue channel analysis for determination of linearity. The graph can be activated in the ChromaCal Tools menu.

Figure 3. Method for Creating a Mask. (A) The original image of immunoperoxidase (IP)-stained bone marrow: anti-kappa light chain antibody-stained cytoplasm of plasma cells. (B) Separation of IP-stained areas from surrounding areas after using the Black and White function in Photoshop. (C) Image after setting a threshold value. (D) Mask image after application of Invert function on (C). (E) overlay of mask (D) onto (A). (F) Area measured colored red. Scale, 50 µm.
field (versus non-tissue background area), the images darkened overall. White balancing was also inconsistent, depending upon the nature of the specimen and staining.

**Study 2: Post-Processing Comparison between Photoshop and ChromaCal**

When images contain the defects described in this paper, the defects are often corrected during post-processing. Photoshop is widely used to post-process images. Photoshop’s automated functions were compared to ChromaCal’s automated functions for the correction of image defects that include poor white balancing, saturated pixels, and inconsistent brightness levels.

Average Red, Green and Blue channel pixel values in background areas were measured to determine color shifts as compared to neutral values to show efficacy of automated white balancing. For the original image, the neutrality was measured at 91% of a possible 100% neutral (Table 1). The ChromaCal processed image is at 99%, and Photoshop-processed images are as follows: Eyedropper Tool method A, 100%; Eyedropper Tool method B, 99%; Auto Levels, 100%; and Auto Color, 99%.

To measure the retention of detail and slight gray values in the whitest parts of the image, the percentage of pixels...
that saturated in any of the red, green or blue channels is at 3% for the original image. The ChromaCal processed image is also at 3%, whereas the Photoshop-processed images are as follows: Eyedropper Tool method A, 30%; Eyedropper Tool method B, 7%; Auto Levels, 2%; and Auto Color, 0%.

The background mean brightness level was measured to determine how closely the value matches the recommended maximum value for any red, green or blue channel when publishing manuscripts; the tonal value that is widely accepted for printing press reproduction is at a maximum level of 240 (Kelby 2003). The original image mean value is slightly underexposed at 213. The ChromaCal processed image measured 234, and Photoshop-processed images measured as follows: Eyedropper Tool method A, 255; Eyedropper Tool method B, 253; Auto Levels, 248; and Auto Color, 243.

These results imply that Photoshop may not be a viable means to post-process CB images (Fig. 6). If the nature of correction is to white balance, correct unnatural colors, and brighten images for optimal reproduction, this study shows that Photoshop may not correct images as well as ChromaCal. Only the Auto Color function in Photoshop appeared to produce the most optimally corrected image with virtually no saturated pixels, a brightness level close to accepted value, and an optimally white balanced image. However, the color shifted to unnatural colors most noticeably using this automated function (note, however, that in working with other images using the Auto Color function, it was determined that those images that had already been white balanced may not shift in color), and colors were more muted than in the ChromaCal-corrected image.

Study 3: Immuno-Peroxidase Density and Surface Area Measurements using Variable and Fixed Thresholds

To test the ChromaCal system, a single image was used and then common user errors were applied to duplicate images, as described in the Materials & Methods. From the inconsistent images due to common user errors, a set of ChromaCal -processed images were created to evaluate the software’s ability to make these images consistent (Fig. 7).

The need for consistent images is especially important when measuring images for morphometry (area, length, width, counts, etc.) and for optical density (Yagi and Gilbertson 2005). For morphometry, differences in brightness level or color provide a means to separate stained objects in a specimen image from adjacent non-stained areas (Russ 2010). When brightness levels are used, a cutoff level is set to include all tones that are either darker or lighter (representing stained areas). The cutoff level is set by the user in imaging software through the use of a threshold tool, or the cutoff is set through an automated method.
When images are consistent, only a single threshold value needs to be applied for all images; but, when images are inconsistent, the user must determine a threshold for each image (Haase et al. 1996), or the user must depend on the accuracy of an automated thresholding function (Dowsett et al. 2000; Brey et al. 2003; Seidal 2001). A single
threshold provides an objective means for setting a cutoff level, as the manual method is a subjective method and thus prone to error (Nobis and Hunsiker 2005).

Using both Photoshop and ChromaCal, we applied variable (setting by "eye") and single thresholds to inconsistent images and compared the results. As all inconsistent images are derived from a single original image, the resulting mean tonal density and surface area measurements should be the same.

For mean tonal density readings, variable thresholds to inconsistent images varied from the first reading by a maximum of -28.8% and a minimum of 6.3%. A single threshold applied to inconsistent images varied from the first reading by a maximum of -31.7% and a minimum of 4.2%. Variable thresholds applied to ChromaCal-processed and consistent images varied from the first reading by a maximum of -3.0% and a minimum of 2.3%. ChromaCal-processed and consistent images with a single threshold varied from the first reading by a maximum of -2.3% and a minimum of 0.3%. The results illustrate nearly identical values from one image to the next for ChromaCal-processed images, ranging from 150 to 155 on a 0–255 scale.

For surface area and morphometric readings, variable thresholds to inconsistent images varied from the first reading by a maximum of -58.9% and a minimum of 12.6%. A single threshold applied to inconsistent images varied by a maximum of -67.5% and a minimum of 76.0%. Variable thresholds applied to ChromaCal-processed and consistent images varied from the first reading by a maximum of -18.2% and a minimum of 29.9%. ChromaCal processed and consistent images with a single threshold varied from the first reading by a maximum of 0.3% and a minimum of -0.3%.

For both morphometric and optical density measurements, the readings from the consistent images generated by the ChromaCal system outperformed thresholds set manually.

Discussion

Obtaining digital images in CB microscopy is an important aspect of biomedical research and the clinical practice of pathology. There have been remarkable changes in the field of digital pathology, and it has improved the speed at which digital images are acquired and shared (including online and printed publications). Indeed, the ease of digital photograph and manipulation/editing has prompted many scientific journals to publish guidelines on the integrity and management of digital images. Although the speed of image acquisition has increased, and authors in the field take the necessary steps to not intentionally manipulate their published images, there has been little effort to standardize color, tonal levels, white balancing, and other critical components of digital CB photomicrographs (which is quite dissimilar to that in the graphic design industry, where standardization is commonplace).

In the first study presented in this manuscript, we compared images from a single microscopist over multiple sessions. In most practices, one would assume that multiple users would share systems. For the purposes of this study, however, we elected to have a single microscopist obtain the images for this portion of the project, to minimize inter-operator variability. Regardless, even with an expert operator using a scientific-grade scope and camera, there was distinct session-to-session image variability, independent of whether the user manually adjusted the camera settings or used automated settings for both exposure and white balance. This finding suggests that variability lies in the camera system, particularly when unable to obtain identical exposures because of limitations in the camera software (found with multiple camera systems in our experience). Few research experiments can be completed in a single microscope session and, for pathologists who read slides, multiple sessions are the norm.

The second study presented compared two post-processing methods to evaluate image consistency. For this study, a single, original (underexposed and poorly white balanced) image of a Wright–Giemsa-stained bone marrow aspirate was processed by two different methods: a widely available raster graphics editor (Adobe Photoshop) and ChromaCal. For the Photoshop-processed images, the original image was adjusted by four different commonly used methods: Eyedropper Tool method A, Eyedropper Tool method B, Auto Levels, and Auto Color. Each of these methods improved the original image variably, and the Auto Color function appeared to produce the most optimally corrected image with few saturated pixels, acceptable brightness, and an optimally white balanced image. However, despite these reasonable performance data, one could argue that the colors presented in the ChromaCal-adjusted image (Fig. 6B) are much “truer” than those of the most technically optimal Photoshop-processed image (Fig. 6F). It is noted, however, that this is a small study comparing one image and two methods.

For the final study, we demonstrated how post-processing can affect semi-automated quantification of immunoreactive cell density and surface area measurements. For this study, a single image was intentionally adjusted to five different permutations to simulate the most typical image-to-image variability. Using ChromaCal to post-process, all five permutations were corrected. Additionally when a single threshold was applied to all five permutations, there was less variability using ChromaCal software versus the individual, manual threshold application in Photoshop. Moreover, pixel levels and the surface area of immunoreactive cells measured were more consistent from image to image using the ChromaCal system. Whereas this experiment represented a simulated microscopic session, the data...
demonstrate the utility of ChromaCal and how this could be used to adjust tonal levels over more than one session to approximate the same exposure and white balance. Similar results were observed with morphometric measurements, also demonstrating that post-processing in ChromaCal provided an objective method for setting a single threshold value for images from more than one microscope session.

In designing the study, we intentionally chose to control these variables to most fully demonstrate the problem of inherent image-to-image variability, as well as the similarities and differences of the two post-processing methods evaluated. Some of the limitations of this study inherent to image acquisition include: 1) a single microscope/camera tested, 2) a single expert microscopist, and 3) a small repertoire of images and stains.

In addition, we focused our analysis on the most common post-processing methods used by non-experts. More sophisticated Photoshop functions would include color matching (with the color match function), the use of Photoshop’s companion program and its image correction software (Camera Raw), and manual correction by adjusting individual color channels linearly.

We did not assess issues surrounding image integrity, which would include methods to retain the original image, and a means through which image corrections can be reported. Both are included with the ChromaCal system without user intervention, but were not described in the current study.

In an unexpected finding, approximately 50% of camera systems we have used were configured (either with default settings, or with user-initiated changes to software parameters, or otherwise) such that images captured were non-linear (data not published). Since no easy diagnostic method is available (prior to ChromaCal), we suspect this non-linear condition is often unknown to users of the imaging system and those who post-process and evaluate the resulting images. Generally, if a gamma setting is available in camera software, adjusting the setting to a gamma of one may result in linear images, depending upon the software manufacturer. To measure linearity without the ChromaCal slide, users could use methods that require more effort, such as taking images of a step wedge with known optical density values, and then comparing mean density values from images of each step with known optical density values. When values do not correlate, the camera system is not linear.

In summary, we present a new system for post-processing digital CB images. We are confident that the ChromaCal method will make it easier for users to post-process digital CB images in a consistent manner with improved color standardization, preserved linear tonal levels, reproducible brightness levels, and automated white balancing.

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