About White Balancing, Brightness Matching and EZ Image Prep™

Digital imaging has been embraced by the research and scientific communities, and concepts such as white balancing, brightness matching and color calibration are often discussed as if they are well-understood and a given. Let's briefly review these concepts and identify where the truth lies and where the weaknesses exist.

White Balancing

The first point to understand is that white balancing isn't about making anything "white," and in fact it should probably be renamed "grey" balancing. White balancing seeks to make the intensities of the red, green and blue channels of a color image equal. When red = green = blue, the resulting color is a perfectly neutral grey (Figure 1). Without a reference point, the field on the bottom left in the figure may look neutral, but only white balancing will ensure that it is.
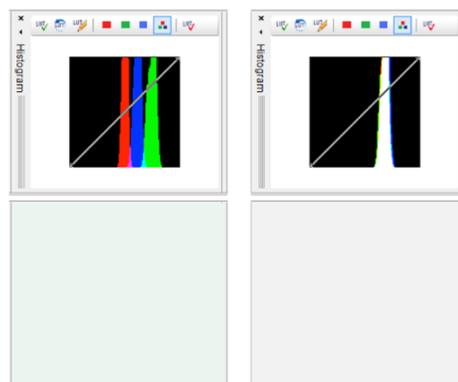

Figure 1. Image of a histogram and background images (above and below, respectively) before white balancing (left) and after white balancing (right). Histograms are commonly available in acquisition software, and can also be viewed for individual images (i.e. within Photoshop).

Another misconception about white balancing is that a white-balanced image has correct color. This is not typically the case, since color is dependent on a variety of variables that would have to be perfectly balanced including the specimen, the spectrum of the light source, the color filters on the camera sensor, the camera software or firmware, and the monitor on which you view the images. So although white-balancing is necessary for a better image, your images are still not optimal.

In common image editing software, a white balance adjustment is performed one image at a time (e.g. Adobe® Photoshop®, ImageJ, etc.), or the adjustment is performed on one image and then the same adjustment is applied to other images (e.g. Adobe® Lightroom®, ImageJ, MetaMorph®, Image-Pro®, etc.). These approaches are time consuming and require the user to subjectively identify the appropriate white balance area. Further, they require adjustment image-by-image or, if they offer a batch processing option, will apply the same settings from one image to all images in the batch. This ignores image-by-image variation, and you may be left with worse looking images than those with which you started.

Brightness Matching

Images will often vary in overall brightness depending on acquisition exposure settings and lighting conditions. Many white balancing algorithms may also set a common brightness level for the image. However, the brightness level set by these algorithms can vary between imaging sessions and among imaging equipment. You will often see the negative impact of varying brightness when assembling a montage of images. Variable brightness levels make it difficult to compare, evaluate and analyze images. With *matched brightness*, differences in images can be more readily observed, analyzed and compared against one another.

Original   Matched Brightness

Figure 2. Original images (left) with varying brightness levels, and images processed to the same brightness level (right).

As with white balancing, image brightness can be adjusted after you've acquired the images. The challenge is first choosing the right brightness level, and the second is ensuring that other images are adjusted to the same level. Brightness is sometimes associated with a white balancing routine, but most image editing software requires adjustment image-by-image. With such subjective adjustments for brightness, you may be left with worse looking images than those with which you started.
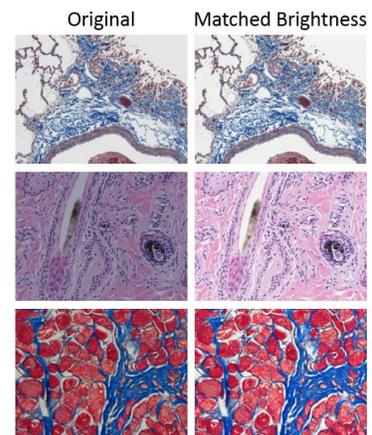
Datacolor CHROMACAL: EZ Image Prep and Color Calibration

So you've captured images in multiple sessions, under various user-defined settings, and perhaps also on different equipment or in different labs. You may have even received images from multiple sources. How do you achieve uniformity, accuracy, and high-quality while also avoiding the time consuming effort of modifying images in Adobe Photoshop and other image editing software?

With Datacolor CHROMACAL image software, we offer you the flexibility to select the tool you prefer: EZ Image Prep for white balancing and brightness matching, or full color calibration (which also includes white balancing and brightness matching). And both solutions are provided within in a simple-to-use, user-friendly, one-step batch processing environment. With either solution, CHROMACAL delivers remarkable results (Figure 3).
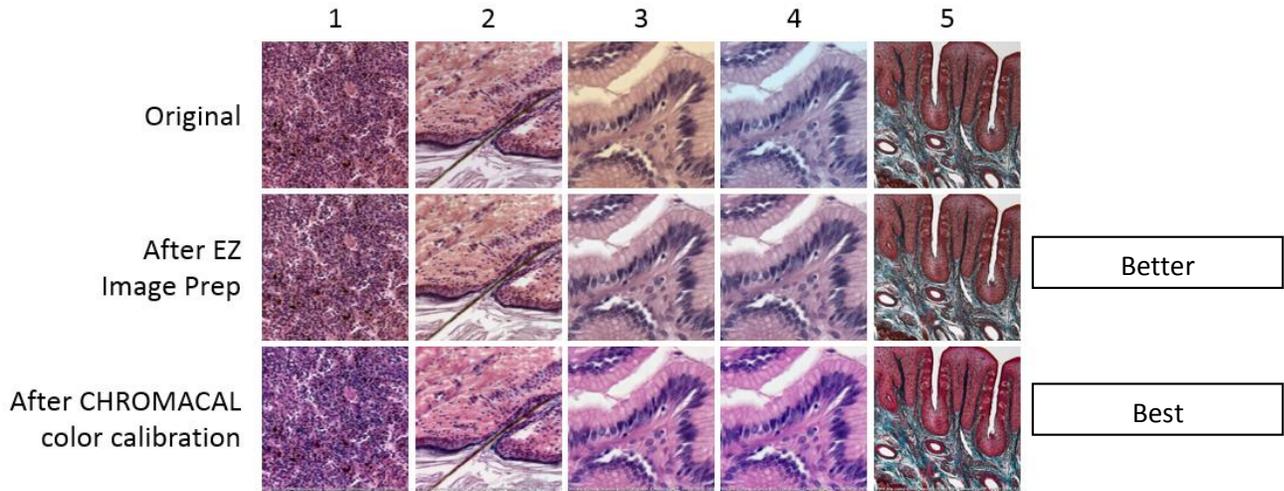
Figure 3. Comparison of original images to white balancing and brightness matching alone (with EZ Image Prep in Datacolor CHROMACAL Image software) and in conjunction with full color calibration (also included in CHROMACAL Image software).

Primarily interested in consistent white-balance and brightness matching? Choose the EZ Image Prep feature within CHROMACAL.

With this solution, you don't need a CHROMACAL color-calibration slide. Simply select the TIFF and JPEG images you want to process*, preview the results if desired, and then process the entire batch of files. EZ Image Prep takes care of the rest. Each image is independently analyzed, corrected (for white balance <u>and</u> brightness matching), and saved to a new image file, all in the matter of seconds. Your original images remain unaltered.

Is the evaluation and analysis of your images critical? Are calibration and validation important considerations in your study design? Choose the full color-calibration feature within CHROMACAL.

After capturing a CHROMACAL color-calibration slide image during your imaging session, simply align the image within the CHROMACAL software and select the TIFF and JPEG images you want to process. Using its proprietary color-calibration algorithm, CHROMACAL will perform all of the following in one step: white balance, match brightness and standardize color to a known color standard. And since this can be done in batch mode, an entire set of images can be processed quickly and easily.

Save time, improve quality, achieve color consistency and accuracy.

CHROMACAL....for images that matter™

For more information Datacolor CHROMACAL, visit us at www.ChromaCal.com or contact us at info.chromacal@datacolor.com.

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*The Datacolor CHROMACAL EZ Image Prep feature was specifically designed for transmitted brightfield microscopy images. Although the feature may be used with any TIFF or JPEG image, the results from images acquired using other techniques may not be optimal. Examples of image types that may not be appropriate include macro images, gross anatomy images, reflected light images, polarized light images, darkfield images, real world photos, and others. The user must determine if the resulting images are acceptable or not.

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