Image Standardization Outperforms Adobe® Photoshop® to Reveal Detail in Images

Gerald J. Sedgewick
Imaging and Analysis LLC

datacolor CHROMACAL™

Case Study

"ChromaCal outperforms Photoshop at every key level."

"[ChromaCal] is the first tool in digital microscopy for brightfield that fulfills an industry-wide need for image standardization, and it should be in every lab that is serious about good imaging and science."

BACKGROUND

Gerald "Jerry" Sedgewick is a consultant in the field of scientific imaging, and an expert in image forensics. Mr. Sedgewick's background includes the publication of two books on the use of Adobe® Photoshop® in scientific imaging and morphometry, as well as the implementation of image analysis methods for researchers in various disciplines. He ran the University of Minnesota's core light microscopy facility in the Department of Neuroscience for over 10 years, during which time Mr. Sedgewick custom-built a multi-photon, video-rate, confocal microscope. His current research focuses on the imaging workflow in science and medicine.

THE CHALLENGE

Throughout his career, Mr. Sedgewick has been searching for a cost-effective, objective means to calibrate digital microscopy images to achieve standardized, consistent colors. His belief was that such a method would also improve separation of details in brightfield images. As noted by Mr. Sedgewick, "Improving separation of detail in Photoshop is a time-consuming process performed on an image-by-image basis, involving subjective determinations without an objective baseline.....Ideally, an objective method is the fundamental goal, but if the method also delivers greater separation of detail, then it could be a faster and more precise way to interpret and communicate the nature of staining and its consequences."

WHY INTERESTED IN CHROMACAL?

During his career, Mr. Sedgewick tried to develop a standardized method using cutouts from a Kodak 35mm slide, but the production was cumbersome, and the material was subject to degradation over time. Mr. Sedgewick observed that, "The ChromaCal slide uses a novel method of standardizing colors to bandpass filters with periodically spaced wavelength ranges. Both the method and the pairing with software to create the standardization were appealing"

EVALUATION AND RESULTS

Mr. Sedgewick developed a novel method* to measure the separation of image elements as a way to show an increase or decrease in detail. Color scientists measure the difference between two colors in three dimensional color space and refer to that difference as Delta E (DE), and commonly use DE to measure the similarity/dissimilarity of colors when comparing two images. Mr. Sedgewick's method, however, used DE to measure color differences within a single image. The greater the difference, the more likely details can be easily discriminated by eye.

Nine slides were selected to include a variety of stains and tissues (Triarch, Inc., Ripon, WI). A representative field from each was imaged on an Olympus BX51 microscope with a 100X oil lens (N.A. 1.3), a Dage MLX camera and Exponent 2.1 software, along with an image of the ChromaCal standardization slide. Post-capture, the specimen images were standardized for color and brightness using the ChromaCal software (version 2.1). If necessary, further adjustments were made by eye to set the saturation and contrast, using the tools provided in the ChromaCal software.

Using Photoshop, 3X3 pixel sampling regions were placed at six locations in each specimen image (top, middle and bottom-third; see Figure 1). The red, green and blue color values (in tones that ranged from 0 to 255) from the Info Palette were entered into a DE calculator (Color Mine; http://colormine.org/delta-e-calculator). The average DE differences between two sampling points within the original, and then within the

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ChromaCal standardized images, were compared as percent differences, with a total of three measurements from each image. The percent differences indicated the extent to which the ChromaCal image contained a greater separation of detail.

On average, the ChromaCal-standardized images provided a 7% improvement in separation of detail, with the largest separation at 24% (Figure 2).

CONCLUSIONS

Mr. Sedgewick identified the following benefits when using ChromaCal:

- "Separation of detail is critical when analyzing and interpreting images. The ChromaCal system improves detail for most images, and in some instances the improvement is significant."
- "With ChromaCal, adjustments to images can be accomplished much faster than in Photoshop, with far fewer mouse clicks. The images are automatically white balanced and matched in brightness, with post-processing time kept to a minimum."
- "All ChromaCal adjustments are saved to a separate image file and a record of adjustments is stored in the file's metadata. The original image file is preserved. These features provide easy traceability to the original image, and access to the post-processing steps."
- "ChromaCal outperforms Photoshop at every key level. Unlike Photoshop, ChromaCal displays the original and adjusted specimen images side-by-side, allowing detail separation to be readily visualized in real time, especially useful in H&E stained samples."
- "With its standardization of color from any camera system, ChromaCal establishes an objective baseline from which perceptual assessments can be made. It eliminates the variability introduced by different camera systems and imaging sessions, and avoids the compounded variability resulting from the one-by-one adjustments using Photoshop."
- "This [ChromaCal] is the first tool in digital microscopy for brightfield that fulfills an industry-wide need for image standardization, and it should be in every lab that is serious about good imaging and science."

"Improving separation of detail in Photoshop is a time-consuming process performed on an image-by-image basis, involving subjective determinations without an objective baseline…With ChromaCal, adjustments to images can be accomplished much faster than in Photoshop, with far fewer mouse clicks."

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* The method used by Mr. Sedgewick to quantify observations of greater separation of color details in ChromaCal-calibrated images (versus original specimen images) has not been tested by Datacolor. Results may vary depending on the quality of the original image compared to the true colors represented on the specimen slide.

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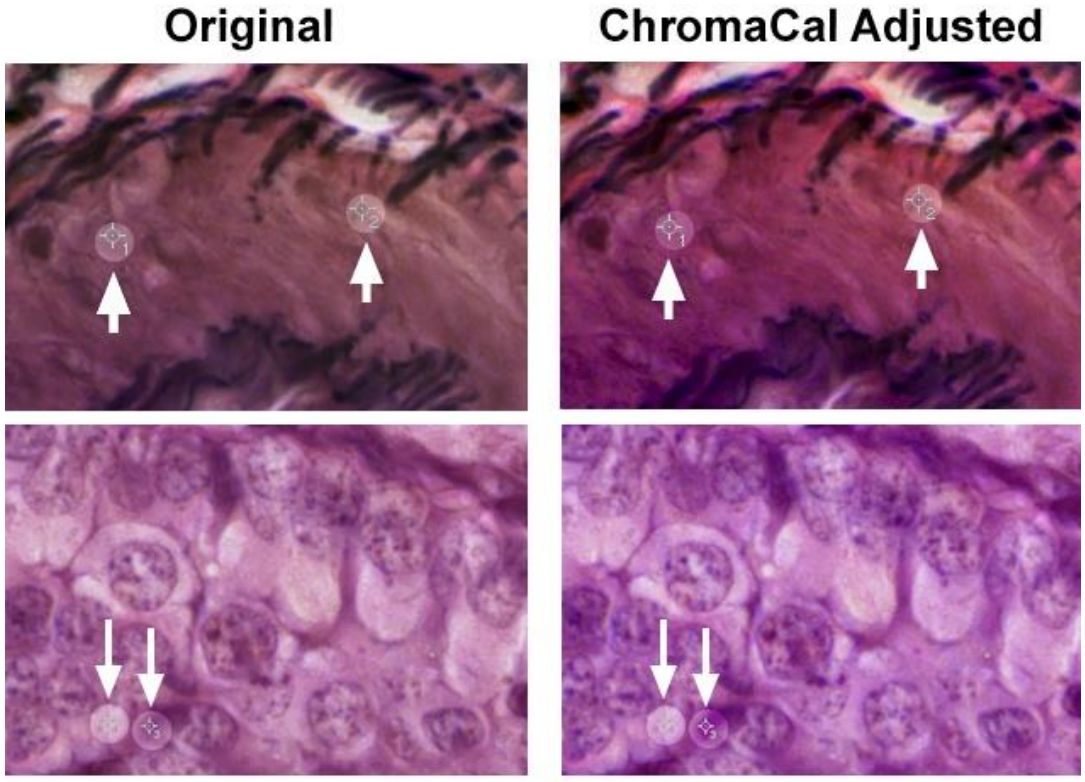
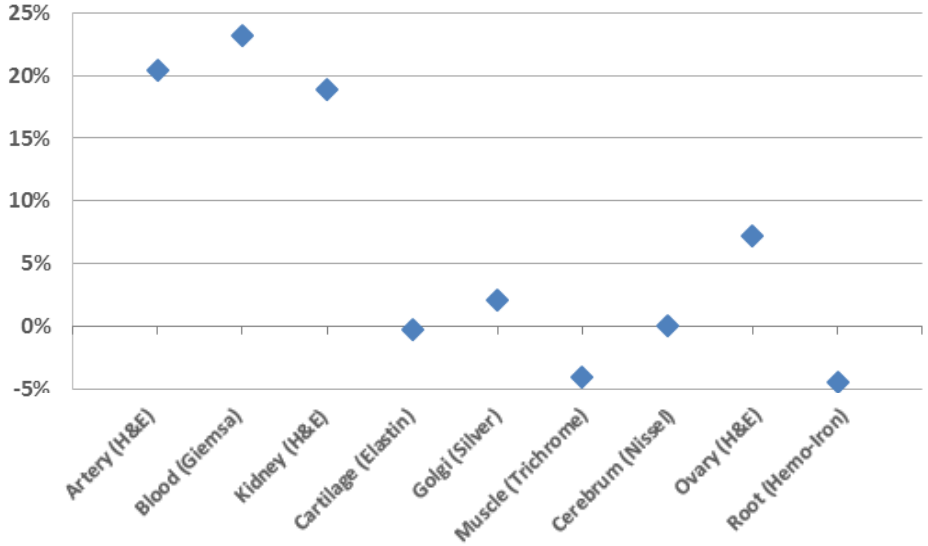
APPENDIX

Figure 1



Two sampling points are shown above for two images, the original image and the ChromaCal adjusted image. These images are a small portion of larger images to show sampling crosshairs.

Figure 2



Percentage improvement in ability to distinguish image detail. The difference in DE between two sampling points were compared between an original image versus the ChromaCal adjusted image. Examples of sampling points are illustrated in Figure 1.

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