Making Color Brightfield Images from Microscopes Both Traceable and Publication-Ready

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Objectives

To determine whether a new imaging system for color brightfield images (chromagenically stained microscope samples) provides publication-quality images and traceability.

For publication purposes, I examine the max white level, color fidelity depending upon the stain, and any color shifts in white areas.

For traceability, I examine metadata and saving protocols for images for adherence to Good Laboratory Practices: image archiving, recording of post-processing steps, reporting of steps, and global post-processing (versus local processing).

Purpose

This study is being done to independently verify claims made by the manufacturer of the ChromaCal imaging system (Datacolor, Inc., NJ).

If the system provides the solutions advertised, publishers will be assured that known and verifiable post-processing steps were taken.

Image fraud, often done unintentionally, requires additional efforts on the part of publishers.

ChromaCal could be an image integrity solution.

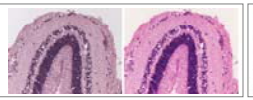
Background

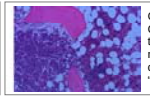
The number of scientific misconduct cases that involve fraudulent images has been on the rise since the beginning of this century when digital imaging was widely adopted. Some fraudulent images have been done intentionally, but the majority have been done unintentionally, or when authors are unaware of publisher's guidelines. In either case, fraudulent images put both the author and the publisher at risk of a loss of reputation, a necessity to retract papers, and a negative effect on public perception which can result in reduced funding of scientific research.

In February of 2014, an imaging system that includes a microscope camera calibration slide with accompanying software was introduced by Datacolor, Inc. (New Jersey, USA) to address fraudulent image issues. This system bridges the gap between authors and publishers by providing an "image integrity" system for publication-ready images on the author side, and metadata to show post-processing steps on the publisher side. It does so along a workflow:

ACQUISITION: When authors or colleagues acquire digital images, they will likely encounter 4 problems:

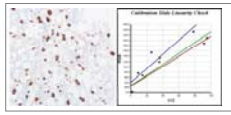
UNRELIABLE COLOR: Color rendered unreliably by camera/scanner





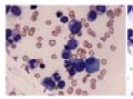
COLOR SHIFTS: Color temperature not matched to camera (not "white balanced")

POOR EXPOSURE: Camera exposure is incorrect

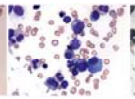


NON-LINEAR: If color density is measured, camera/scanner is not linear in tonal distribution

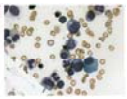
POST-PROCESSING: When authors or colleagues post-process images to correct for image acquisition problems, they will likely encounter 4 problems:



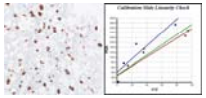
ORIGINAL IMAGE



PHOTOSHOP CORRECTED resulting in saturated (pure white/black) pixels



PHOTOSHOP CORRECTED with Auto-Color, resulting in unnatural colors



POST-PROCESSING (any program) Carry-forward of any linearity issues with the camera

FOR PUBLICATION: When images are sent to publishers, images are rejected or left as is, depending upon publication or whether image problems are identified. If images are suspect, the original is requested.

POTENTIAL PROBLEM: Original images are saved over, thus sacrificing the raw image

PROBLEMS WITH WORKFLOW:

- **Low quality images published**
- **Detail lost in over-saturated images**
- **Measurement error with non-linear images**
- **Higher risk without original/metadata**
- **Poor reproduction when printed**

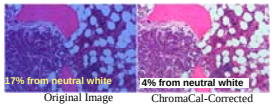
SOLUTION:

- **Correct for color unreliability**
- **White balance & match brightness levels**
- **Prevent post-processing errors**
- **Prevent use of non-linear cameras**
- **Preserve original image & provide metadata**

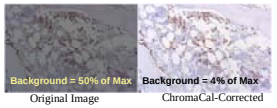
Results

The ChromaCal solution for image workflow issues with color brightfield images were examined to verify the company's claims:

UNRELIABLE COLOR: The original image from a scientific grade camera was calibrated to a color standard via the ChromaCal system. Delta E (DE) ratings show virtually no color differences between the Chroma-calibrated image and the true color image.*

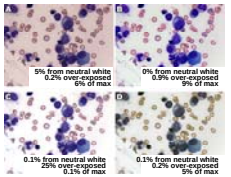


WHITE BALANCE: The original image was not color-temperature matched to the camera. The Chroma-corrected image did not completely correct for blue-shift, but made a great improvement.**



POOR EXPOSURE: The original image was ChromaCal corrected to an average background value of 244, which is 4% of the maximum value of 255: whitest values should be less than pure white.

NON-LINEARITY: ChromaCal calibration slide, taken at imaging session, contains grayscale circles so that linearity can be checked. Warning (and consequent inability to correct images) both alerts user and prevents images from being corrected/calibrated.



POST-PROCESSING ERRORS: A is the original file, slightly underexposed overall (though brightest features are over-exposed), color-shifted (not white balanced) and likely sub-optimal in color reliability (though not measured). B shows ChromaCal-calibrated and corrected image, compared with Photoshop-corrected images (C & D). ChromaCal-calibrated and corrected image indicates significantly improved white balance from original image. D is clearly incorrect colors. Note retention of details that were lost in Photoshop post-processing.***

PRESERVE IMAGE & METADATA: ChromaCal makes a 2nd copy of all originals, thus preventing overwriting of original images (A). From scientific camera image, only date Is found in metadata (B), whereas ChromaCal metadata includes post-processing and image characteristics

* Delta E is a measurement for determining visually perceptible differences among images; with a DE of 1 signifying a just perceptible difference and a value of over 3 generally regarded as significant.

** The ChromaCal system averages red, green & blue values from the brightest 1% of pixels in the image and then white balances based on those values: a part of the image always contains neutral background values (equal red, green and blue values) but uneven illumination/artifacts create inconsistencies.

*** The white eyedropper tool method is commonly used to correct for both white balance and to expand tonal values to fill the image's dynamic range. White balance values are determined where the user clicks on the image, thus making it subjective. Those values are added to all pixels in the image, leading to over-exposure (saturation).

Conclusion

The ChromaCal imaging system performs as advertised. ChromaCal provides correction for color unreliability, white balances and matches brightness levels of images (best when images are free of artifacts and evenly illuminated), prevents post-processing errors that are common with programs like Photoshop, prevents the use of non-linear tonal distribution common to many camera systems, and preserves the original image while providing metadata. This program is a quantum leap forward for maintaining image integrity and preventing the kind of image fraud that is introduced unintentionally.

ISMTE

Slide 1

gs1 white balance determined by adding red and blue and dividing by 2, then dividing into green for a percentage.

Exposure by adding all 3 rgb and dividing by 3 then dividing into 255

sedgewick, 7/15/2014