

QUICK START GUIDE — IMAGE CALIBRATION

Note: CHROMACAL Image Calibration requires use of a CHROMACAL Color Calibration Slide for Brightfield Microscopy. The slide is sold separately (re-order #10-2010).

Not for clinical diagnostic use in the U.S.

Quick Steps to Image Calibration

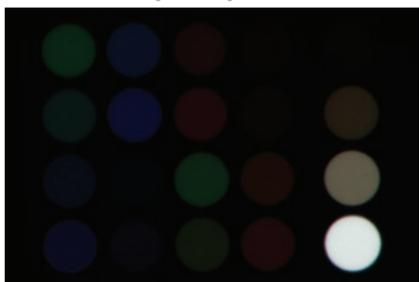
STEP 1: BEGIN YOUR MICROSCOPY SESSION; CHECK SETTINGS

- Conduct your microscopy session using good microscopy practices. Start by establishing Koehler, setting illumination and exposure, and white balancing. Additional guidance on good microscopy practices is available at <http://www.scientific.datacolor.com/resources/microscopy-practices>
- Adjust any settings within your image capture software that remove the linearity of captured images. For example, any setting for "color correction" should be turned off, and any gamma setting should be set to 1. See **Linearity Guide** for further guidance.

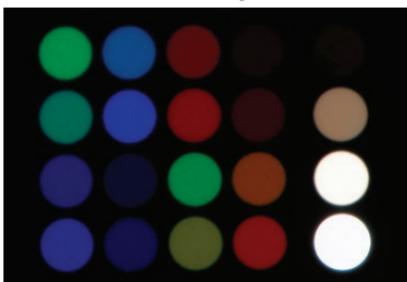
STEP 2: CAPTURE YOUR CHROMACAL CALIBRATION SLIDE IMAGE

- After your initial set-up, place the CHROMACAL Color Calibration Slide (purchased separately) onto the microscope stage and capture an image of the larger 20-color matrix. Although the matrix area on the slide contains two separate matrices, **we recommend you use the larger matrix** which is generally visible at magnifications between 4x and 40x.
 - DO NOT use an oil immersion lens with the calibration slide.
 - The calibration slide image can be acquired at any time during your microscopy session, although we suggest an early capture to check the linearity of your imaging system.
 - When the calibration slide image is exposed correctly, **the brightest white circle should be slightly gray, and the two whitest circles should differ in intensity.** Note: a correctly-exposed image will appear dark and certain patches may not be visible. **This is normal.**

Proper Exposure



Incorrect Exposure



See **Exposure Guide for CHROMACAL Color Calibration Slide** for further guidance.

- Save the calibration slide image as a TIFF or JPEG file. For easy access, save the calibration slide image in the same folder as your specimen images from the same microscopy session.
 - We recommend that you include the calibration slide serial number (the 7-digit number located in the upper right-hand corner of the calibration slide) as part of the file name you assign to the calibration slide image.

(continued on reverse side)

STEP 3: CHECK FOR LINEARITY AND CORRECT EXPOSURE

- a. Launch the **CHROMACAL** Image Calibration software
- b. Click *Open Calibration Slide Image* and locate the file of the calibration slide image created during the microscopy session (see Step 2b above). Highlight the desired file and click *Open*. If this image file was never previously used in **CHROMACAL** Image software on your workstation, the program will prompt you for the serial number of the calibration slide.
- c. Check the position of the color matrix. **It is critical that the white circle be located in the lower-right hand corner of the matrix.** Use the flip and rotate tools if any adjustments are needed. The colors displayed in the corners of the Calibration Profile window are reminders of how the matrix should be oriented.
- d. With the Magnifier tool  selected, press and hold your mouse button starting near the top left-hand corner of the 20-color matrix (green circle) and drag the selection rectangle to the lower right-hand corner of the 20-color matrix (white circle). Upon releasing the mouse button, the matrix expands to fill the Calibration Profile window.
 - When Step 3d is completed, a blue square should fit completely within each color circle. The exact location within each circle is not critical. If the squares are orange, then your matrix is NOT aligned properly.
 - If any adjustments are needed, re-align the squares by using the location adjustments displayed as your mouse hovers over each square. The adjustments vary depending on the square location.
- e. Check the diagnostic readings in the lower left-hand corner of the main window. You want to achieve the following diagnostic results before proceeding to Step 4:

Matrix Alignment = "Good"

- If not, realign the color matrix or the squares that overlay the matrix.

Linearity = "Good" or "Within Tolerance"

- If not, check camera software settings and repeat Step 2; pay particular attention to guidance provided in Step 1.

Exposure = "Good" or "Auto-corrected"

- If not, repeat Step 2, pay particular attention to the exposure guidance provided.

STEP 4: ACQUIRE SPECIMEN IMAGES AS USUAL. SAVE AS TIFF OR JPEG FILES.

STEP 5: CALIBRATE YOUR SPECIMEN SLIDE IMAGES

- a. Return to the **CHROMACAL** Image software. *Open Single Image* to color-calibrate one specimen image at a time (continue to Step 5b) **or** *Batch Multiple Images* to color-calibrate a batch of specimen images (continue to Step 5c).
 - NOTE: Each calibrated image will be saved into a separate file (naming convention = original file name PLUS "ChromaCal-calibrated" appended to the end). **No changes are made to the file containing the original image.**
- b. Click *Open Single Image* to calibrate one image at a time. Locate and select the file with the specimen image that you want to calibrate. Your original image will then be displayed on the left and the **CHROMACAL** color-calibrated image on the right.
 - After reviewing the image and/or changing contrast (or other parameters), click *Save Calibrated Image* to save the image.
- c. Click *Batch Multiple Images* to color-calibrate an entire folder of specimen images. Then, click *Select Source Images Folder* to identify the folder location of the non-calibrated images. Once a source folder is selected, the file names of all uncalibrated images in that folder will be displayed on the left side of the screen. You can then highlight any file name and two images will be shown: the original image on the left and the **CHROMACAL** color-calibrated image on the right.
 - After reviewing the image(s) and/or changing contrast (or other parameters) for any image, click *Select Destination Images Folder* to specify where the color-calibrated images should be saved.
 - Click *Process Images*. All images with a "✓" next to the file name will be processed and **CHROMACAL** color-calibrated versions of each of these images will be saved into the destination folder.