

# **Evaluation of Targets for Color Calibrating Digital Color Images from Optical Bright-Field Transmission Microscope**

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Digital microscopy is an evolving new technology that combines the traditional optical microscopy and the digital camera. The recorded digital images can be stored, processed, analyzed, and distributed much more easily than the traditional film images. In order to maintain the integrity of these images, varieties of the standard procedures and targets have been implemented. For example, stage micrometers are used to calibrate the scale of the image. However, a missing link is the color integrity of these digital images as the recorded color of the same specimen can vary from one digital microscope system to another. Some pioneering studies have been done to color calibrate digital color images from bright-field transmission microscopes using a set of color targets with known transmission spectra and color coordinates [1-2]. These colors were selected to match their transmission spectra with those of the specimens under the test. A common problem of these color targets is that they are too big to be captured all together in the typical field of view of digital microscope system and therefore require a tedious and lengthy calibration process. It is a huge technical challenge to manufacture the color targets at such a small scale (usually at 100 micrometer scale) with preferred transmission characteristics.

Recognizing the need for a solution to these problems, we developed a new color target set for color calibrating of optical bright-field transmission microscope images. The target set contains 20 colors arranged as a 5x4 matrix. The entire matrix is only  $69\mu\text{m} \times 51\mu\text{m}$  large that can fit in the field of view of a typical bright field microscope with a 100 objective. Among the 20 colors, 4 of them are neutral colors with flat transmission spectra the visual range. The remaining 16 colors are highly saturated colors with narrow band transmission evenly spaced between 400 to 700 nm. All color patches are manufactured using a state-of-art micro-fabrication technique with a high degree of process control to ensure high reproducibility. The entire color target is fabricated on a glass chip that is mounted over an opening on a 3"x1" metal slide. The assembly (called a calibration slide) is used to transform camera values to tristimulus values by a matrix technique.

How can such a calibration slide be tested once it is developed? A strategy that seems possible is to make a test slide with biologically stained specimens, acquire a color image of the slide, and compute (pixel by pixel) the color difference between the actual XYZ values in the stained specimen and the XYZ values of the color-calibrated image. But there is a technical hurdle: The actual XYZ values at a pixel are unknown because a pixel-by-pixel measurement of the transmission spectrum at a pixel is very difficult. Furthermore, biological stains are known to change color with time and temperature, so the measured spectrum at a pixel would likely not match the spectrum that determined the camera values later on.

To avoid the problems of single-pixel spectrum measurement of an unstable material, we manually prepared a glass slide with attached squares of filters selected from a database of Rosco® gels. Each of the selected filters has a transmission spectrum typical of a common biological stain or stain mixture. Because of their large size and stability with time and temperature, it is possible to obtain reproducible camera values from the slide and to use the tabulated values of the transmission spectra (together with the microscope-light spectrum) to compute XYZ values. At this point, we can perform a valid test of the calibration slide using filters that are not like the filters in the calibration slide but typical of the stains encountered in practical microscopy.

We used this slide (called a reference slide) to test the color calibration incurred by the calibration slide. Because the reference slide's filter array is large, it must be moved by hand to measure the separate filter patches, a time-consuming procedure we don't recommend for on-line microscopy. However, when performed off-line in a laboratory, the test gives a way to assess our color-calibration procedure. The research question is whether, although transmission spectra of most of the calibration-slide filters do not match those of the specimens under test (such as biological stains) a simulation and an experiment will show sufficient calibration accuracy to meet the basic need of color integrity.

## References

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## Author Biography

Hong Wei received his BS in material science from Nanjing University (1998) and his PhD in material science and engineering from University of Minnesota, Twin City (2007). Since then he has been a research scientist at Kent Optronics Inc., NY and then a senior optical engineer at Datacolor, Inc. NJ. He developed varieties of optical image capturing and projecting systems for camera evaluation and color measurement applications. He is a member of ISCC, ICC and SPIE.