# Time Savings: Image Acquisition and Preparation for Publication

**Dawn M. Dawson, M.D.**
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datacolor CHROMACAL™

**Case Study**

## BACKGROUND

Dawn M. Dawson, M.D. is an Assistant Professor at Case Western Reserve University's Institute of Pathology. Dr. Dawson's research focuses on growth factor/receptor cell signaling in carcinogenesis models, in particular those active in colorectal cancer. Her research includes the development of tissue-based micro-diagnostic methods for evaluating expression profiles for potential targets of chemotherapeutic agents.

## THE CHALLENGE

Dr. Dawson commented, "Preparation of histological images for publication is a critical component of manuscript submission, and even more so for those with clinico-pathologic correlations and with diagnostic and prognostic impact. Comparative images for differential diagnostic entities, as well as those with unique histochemical and/or immunohistochemical phenotypes, are used for qualitative and semi-quantitative analyses. Because of the importance of histological images, and also because I've seen great variability in images from session-to-session, between instruments, and among users of microscopes, I am always looking for methods to improve image consistency and reproducibility, as well as to reduce the time invested in image preparation."

She continued, "Rarely, in clinical or research studies, are all the collected samples prepared identically, assayed in a single run, or imaged with the same equipment. The challenges are compounded if tissues come from multiple institutions or processing sources, all variables that must all be considered in the evaluation and interpretation of results. So, it is paramount to seek new methods for interpreting and compiling meaningful data and presenting high-quality images that pass scrutiny for publication."

## WHY INTERESTED IN CHROMACAL?

Dr. Dawson noted, "I was interested in ChromaCal because it seemed to offer a scientific approach for delivering consistent and reproducible data, while at the same time offering time savings over the traditional approaches used in research environments."

## EVALUATION AND RESULTS

Dr. Dawson recently conducted a study to evaluate four antibodies for immunohistochemical ("IHC") staining. Each antibody was acquired from a unique source and evaluated for suitability in comparative expression profiles and publication images. Samples were prepared using identical processing protocols. IHC stains were batched according to antibody. Uniform detection (chromagen and counterstain) were used. Images for each antibody cohort (6 images/cohort) were taken on the same day with the same microscope. Below is an assessment of the steps taken and time required to bring consistency to the images from the study, followed by a comparison of the process and time needed to achieve the same level of consistency using ChromaCal (see also Table 1).

Typical image preparation for a montage would require addressing the following issues:

1. Background/flatfield correction: Despite performing Köhler illumination and white balancing prior to image capture, images required background subtraction (a.k.a. flatfield correction). [Estimated time for background corrections and saving image = 1 min/image.]
2. Counterstain and chromagen variability: To normalize images for brightness and staining level, histograms of median hematoxylin staining intensity were compared and adjusted [Avg time/slide= 1-2 min/slide]. Regions removed from specific chromagen staining were selected for the comparison, and a region from a single image was used as the baseline. Contrast/brightness and, if necessary, red, green and blue channel adjustments were made for relative uniformity of the counterstain for each image [Avg time = 3-4 min/image].

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3. Post modification evaluation: Additional minor adjustments were made as necessary once the images were viewed in a montage. Images were reacquired if the results following post-modification were not acceptable.

Dr. Dawson then described her ChromaCal evaluation process, "To evaluate and challenge the effectiveness of ChromaCal, the images described above were re-acquired without background correction and post-acquisition modifications. Only Köhler illumination and white balancing were performed along with acquiring the Chromacal calibration image [estimated time for calibration slide image = 3-4 minutes]." Dr. Dawson continued, "A time comparison of the image acquisition and preparation steps was assembled, and the results showed a meaningful [>90%] reduction in post imaging time [saving approximately 2-3 hours for this one study alone] using the ChromaCal approach versus the processes followed in the typical research study described earlier."

**Table 1. Time Comparison Table**

| | | | Minutes per Image | | Total Minutes | | |
|---|---|---|---|---|---|---|---|
| **Step** | Description | # of Images | Minimum Time | Maximum Time | Total @ Minimum | Total @ Maximum | Time with ChromaCal |
| **1** | Background correction | 24 | 1 | 1 | 24 | 24 | 0 |
| **2** | Histogram adjustment | 24 | 1 | 2 | 24 | 48 | 0 |
| **3** | Uniformity adjustments | 24 | 3 | 4 | 72 | 96 | 0 |
| **4** | Post-modification | ~25% | 1 | 2 | 6 | 12 | 0 |
| **--** | ChromaCal only* | 24 | -- | -- | -- | -- | 10 |
| | Total | | | | 126 | 180 | 10 |
| | Time Savings with ChromaCal | | | ~2-3 hours time savings (> 90% reduction in time) | | | |

* includes calibration image acquisition and batch correction process

## CONCLUSIONS

Dr. Dawson noted the following major time savings and benefits based on her evaluation of ChromaCal:

- No background correction image was captured; made no background adjustments.[1]
- Omit evaluation of a median image for comparison and individual histogram evaluation.
- Reduced or omitted individual contrast, brightness and RGB adjustments.
- ChromaCal's batch correction process with retention of all original data; one step only.
- Reduced need to re-acquire images due to image and color variability resulting from imaging sessions that are separated by hours or days.
- Reduced computer file labeling and data saving/image and typographical errors.

Dr. Dawson commented, "My evaluation initially focused on the process of obtaining publication-quality images. This time savings was significant in its own right and I was pleased with the quality of the ChromaCal images. And although I didn't perform a quantitative analysis, the uniformity that ChromaCal's color standard offers can be even more critical, and provide even more benefit, to this type of evaluation."

Dr. Dawson continued, "Not only does ChromaCal offer major time savings, but it also provides a simple method to achieve consistency and reproducibility in my images. I also expect that the overall presentation for manuscript submission will be improved and may result in better journal review and acceptance. First impressions, especially for non-pathology reviewers are priceless and, in the long run, save additional time and effort for the investigator and their staff."

[1] Datacolor recommends that flatfield correction (background subtraction; shading correction) be performed prior to Datacolor ChromaCal calibration. Certain features of ChromaCal, such as built-in white balancing and brightness matching, may mitigate a certain degree of minor background distortions; however, even minor background distortions may affect the accuracy and consistency of the color calibration.

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