Development of metrological support for color calibration of digital microscopy systems

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Abstract. The article is dedicated to the color reproducibility in images obtained on various digital microscopy systems (DMS). This problem can be solved by color calibration with a special slide. According to the previously formulated requirements, a slide prototype for DMS color calibration was developed and manufactured. Several color calibration algorithms were tested to choose the best one for minimizing the color difference. Color calibration algorithms were based on polynomials of different degrees with and without cross terms for CIE Lab color fields’ coordinates. This comparison was made with the color slide prototype and two DMSs based on Meiji MT4300 and Olympus BX41 microscopes. The comparison proved the polynomials with cross terms of 3rd and 4th degrees to be the best for DMSs based on Meiji and Olympus microscopes, respectively. The minimal color difference with respect to the true color fields’ coordinates was 0.75 $\Delta E^*$ and 0.66 $\Delta E^*$ for Meiji and Olympus, respectively. The results indicate the suitability of the slide prototype for DMS color calibration since it is possible to achieve a color difference below the visually distinguishable threshold of 2.2 $\Delta E^*$.

1. Introduction

Microscopic studies are essential for different fields of modern biomedical practice, such as hematology, histology, cytology, and pathology. The microscopy digitalization led to the emergence of digital microscopy systems (DMS). Digital microscopy is represented by various systems, from the handheld microscope with a camera to fully automated scanning systems (WSI systems). The latter got recently approved by the FDA for several primary diagnostic tasks [1]. The FDA also provided recommendations for determining metrological characteristics to assess the quality of various WSI systems’ components. These recommendations also suggest checking color reproducibility with the reference values of the color slide field coordinates. However, a slide for this purpose is not specified, and only general requirements for its color fields are formulated. Color reproducibility is significant as color distortions caused by the use of different hardware and software DMS components result in image diversity of the same specimen [2].

Today there are several widely available color slides, such as GretagMacbeth, IFRAO, and Kodak (Figure 1). The overwhelming majority of these slides are designed for the reflected radiation registration, while the DMS operates in the transmission mode.

Figure 1 – Widely available color calibration slides’ appearance
Of all the widely available slides, only the Kodak Q60-E3 is semitransparent. The color gamut for its color fields is represented in Figure 2. Some researchers [3,4] conducted studies to assess the suitability of this slide for DMS color calibration. The results showed that, in some cases, it was not possible to reach the color difference level in the CIE Lab space of lower than $2.2 \Delta E^*$ (visually distinguished threshold).

The disadvantages of the Kodak Q60-E3 for DMS calibration can be attributed to the film graininess, which leads to ambiguity in determining the color coordinates at high magnifications (Figure 3). For example, determining the color coordinates of the L15 color field by the position of the histogram maxima, we get RGB coordinates \{237, 165, 44\}, and the average values for each of the channels are \{233, 165, 45\}. For a standard illuminant E, the color difference between these color coordinates is 164.49.

Some researchers offer their own color slides for DMS color calibration. However, the usage of these slides can’t provide stable color difference reduction for all biomedical problems [5,6]. This fact is
explained by the use of a small number of color fields, which doesn’t allow to eliminate the nonlinearity of color distortion, even theirs gamuts are fully covering the dyes’ chromaticity coordinates convex hull. The color gamut of these slides concerning the convex hull of the most common biomedical dyes is shown in Figure 4.

For lowering the color difference level, it is also substantial to choose a color calibration algorithm that will most accurately describe the nonlinearity of color distortion. In [5] and [6], the authors used a linear color calibration algorithm, which is a system of equations written in matrix form:

\[
\begin{bmatrix}
M_{R1} & M_{R2} & M_{R3} \\
M_{G1} & M_{G2} & M_{G3} \\
M_{B1} & M_{G3} & M_{B3}
\end{bmatrix}
\begin{bmatrix}
R_{p1} & \ldots & R_{pq} \\
G_{p1} & \ldots & G_{pq} \\
B_{p1} & \ldots & B_{pq}
\end{bmatrix}
\times
\text{pinv}
\begin{bmatrix}
R_{p1} & \ldots & R_{pq} \\
G_{p1} & \ldots & G_{pq} \\
B_{p1} & \ldots & B_{pq}
\end{bmatrix},
\]

where
- \(M\) – correction matrix;
- \(X_{bi}\) – perfect color coordinates in RGB color space with 3 by n size;
- \(X_{pi}\) – matrix of captured RGB coordinates with n by 3 size;
- \(q\) – number of color slide fields;
- \(\text{pinv}\) – pseudoinverse operator.

Kravtsova and coauthors proposed to use polynomials with and without cross terms of different degrees as a color calibration algorithm [4]. This approach allows taking into account the nonlinearity of color distortions, however it becomes necessary to use a large number of color fields. The obtained color difference dependences on the used algorithm suggest the presence of an optimal degree of a polynomial that allows achieving the lowest level of color difference. The authors have obtained the color difference dependence on the number of color fields of the Kodak Q60-E3 used for calibration. It shows that with an increase in the number of color fields used for color calibration, the color difference decreases.
2. DMS color calibration slide requirements
Previously, we formulated the requirements for a DMS color calibration slide according to the literature review [7]. As a result, the following demands were composed:

- color slide fields should be transparent in the optical wavelength range;
- color slide fields should be of reproducible materials, whose characteristics are stated by local or international standards (Russian National Standard, DIN, ISO, etc.);
- the slide should contain about 100 color fields;
- the color gamut of the calibration slide fields should correspond to the chromaticity coordinates of the most common dyes used in biomedical practice.

3. Color calibration slide prototype
A prototype of a color slide in the format of a microscope slide, consisting of 97 color fields, was developed and manufactured under the represented requirements. The fields of the color slide were made of colored glass, corresponding to Russian National Standard 9411-91. Prototype color gamut with the most common biomedical dyes chromaticity coordinates convex hull is shown in Figure 5. For comparison, the figure also shows the color gamut of the Kodak Q60-E3 measure as most fully complying with the requirements.

The prototype color gamut almost entirely (99.4%) covers the dyes' chromaticity coordinates convex hull. Thus, the developed prototype fully complies with the requirements presented above.

4. Color calibration quality
Color calibration quality was assessed using the proposed prototype and two DMSs based on Meiji MT4300 and Olympus BX41 microscopes were used. The microscopes were equipped with a Meiji U
Plan 20x/0.4 PH2 ∞/0.17 F = 200 WD 7.29 objective, a PixelInk PLB873 camera, and a camera adapter Motic 20x. The spectral distributions of the transmitted through the color fields radiation and the spectrum of the illuminator without a slide installed on the stage were recorded using a Solar LS S100 spectrometer to calculate the actual color coordinates. The microscope illuminant spectrum was used to determine the white point for subsequent color conversions.

We used polynomials without and with cross terms from the 1st to the 5th degree for calibration algorithms. For three color coordinates L, a, and b, the polynomials with and without cross terms of the 2nd degree looks like \([1, L, a, b, L^2, La, Lb, a^2, ab, b^2]\) and \([1, L, a, b, L^2, a^2, b^2]\), respectively.

The color calibration procedure can be represented as the following matrix equation:

\[
C_{in} \cdot A = C_{target},
\]

where \(C_{in}\) – registered color coordinates and their combinations matrix of size \(q \times n\) (\(q\) is the number of fields in the color measure, \(q = 97\), \(n\) is the number of coefficients in the polynomial used for correction);
\(A\) – coefficient matrix with size \(n \times 3\);
\(C_{target}\) – matrix with color coordinates calculated from spectral radiation distributions with size \(q \times 3\).

For different reasons (noise, calculation accuracy, etc.) we can only get an estimate of matrix \(A – \tilde{A}\).

In this research we used least-squares method to get the estimate. The color coordinates of the fields after correction are calculated as follows:

\[
C_{out} = C_{in} \cdot \tilde{A},
\]

where \(\tilde{A}\) – coefficient matrix estimate with size \(n \times 3\);
\(C_{out}\) – corrected color coordinates’ matrix with size \(q \times 3\).

The quality of color calibration was assessed with the color difference in CIE Lab color space:

\[
\Delta E^*_{ab} = \frac{1}{q} \sum_{i=1}^{q} \sqrt{(L_{i,target} - L_{i,out})^2 + (a_{i,target} - a_{i,out})^2 + (b_{i,target} - b_{i,out})^2},
\]

where \(\Delta E^*_{ab}\) – color difference in CIE Lab,
\(L_{i,target}, a_{i,target}, b_{i,target}\) – target color coordinates in CIE Lab,
\(L_{i,out}, a_{i,out}, b_{i,out}\) – corrected color coordinates in CIE Lab.

With such experiment design, the following dependences of the color difference on the degree of polynomial with and without cross terms were obtained for the DMSs based on Meiji MT4300 (Figure 6) and Olympus BX41 (Figure 7).
Fig. 7 – Color difference depending on the degree of polynomials with and without cross terms for the DMS based on Olympus BX41, the color difference without calibration 486.66 (172.41 without the luminance component L)

The dependences of the color calibration quality on the choice of the algorithm indicate that the use of polynomials with cross terms of color coordinates allows achieving the best results for both DMSs. This effect is explained by the fact that the addition of terms in the form of a product of color coordinates allows a better description of the nonlinearity of color distortions in the DMS. However, as the degree increases, there is a sharp increase in the color difference associated with the overfitting effect. The optimal degree of the polynomial for the two systems is different, which is explained by the various illuminator and optical system quality of the microscopes.

5. Conclusions
The relevance of ensuring color reproducibility in images obtained by various DMSs is undeniable since the interpretation of test results in medical laboratories depends on it. Color reproducibility can be ensured by color calibration using special slides. However, to date, there are no color slides for DMS suitable for widespread use. Earlier, based on a literature review we formulated the requirements for the DMS color calibration slide. Following these requirements, a color slide prototype, consisting of 97 fields was designed and manufactured. Slide fields were made of colored glass under Russian National Standard 9411-91.

The research results indicate that the color calibration using the previously proposed prototype leads to a decrease in the color difference below the visually distinguishable threshold ($\Delta E^* < 2.2$) using polynomials of 2-4 degrees on the example of two DMSs. Thus, the prototype is suitable for color calibration of the DMS.

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