Study of factors affecting the appearance of colors under microscopes

Roshanak Zakizadeh*[a], Juan Martinez-Garcia[b], Kiran B. Raja[b], Christos Siakidis[b]
[a]University of Eastern Finland, School of Computing, P.O.B. 111, FI-80101 Joensuu, Finland;
[b]Gjøvik University College, N-2802 Gjøvik, Norway

ABSTRACT

The variation of colors in microscopy systems can be quite critical for some users. To address this problem, a study is conducted to analyze how different factors such as size of the sample, intensity of the microscope's light source and the characteristics of the material like chroma and saturation can affect the color appearance through the eyepiece of the microscope. To study the changes in colors considering these factors, the spectral reflectance of 24 colors of GretagMacbeth Classic ColorChecker® and Mini ColorChecker® which are placed under a Nikon ECLIPSE MA200 microscope®[2] using dark filed and bright field illuminations which result in different intensity levels, is measured using a spectroradiometer®[3] which was placed in front of the eyepiece of the microscope. The results are compared with the original data from N. Ohta[1]. The evaluation is done by observing the shift in colors in the CIE 1931 Chromaticity Diagram and the CIELAB space, also by applying a wide set of color-difference formulas, namely: CIELAB, CMC, BFD, CIE94, CIEDE2000, DIN99d and DIN99b. Furthermore, to emphasize on the color regions in which the highest difference is observed, the authors have obtained the results from another microscope; Olympus SZX10®[4], which in this case the measurement is done by mounting the spectroradiometer to the camera port of the microscope. The experiment leads to some interesting results, among which is the consistency in the highest difference observed considering different factors or how the change in saturation of the samples of the same hue can affect the results.

Keywords: color appearance, color difference, microscopy, color measurement, color imaging

1. INTRODUCTION

Color is a significant factor in research involving microscopic studies such as pathology. The color difference observed by the users of microscopes in the laboratory could be a source of many misinterpretations of data and might lead into wrong conclusions and diagnosis. The colors which are observed through the eyepiece of a microscope can change due to different factors.

An experiment is conducted to study the changes in reflectance of the commonly used GretagMacbeth ColorChecker® samples through the eyepiece of the microscope, considering the effect of different factors such as intensity of the light source and size of the samples. Also it has been studied how the samples of the same color but different saturations behave considering these factors. To evaluate the effect of these factors on the appearance of the colors of GretagMacbeth ColorChecker® through the eyepiece of the microscope, we have considered the measurements of N. Ohta[1] as the base data and calculated the color difference between Ohta's measurements and ours using several color difference formulas which have been developed to estimate the perceived color difference between two measured samples based on numerous psychophysical experiment and have been widely used in industrial applications[5]. By looking at the resulting color differences from the base data and also the positions of colors in the CIE 1931 Chromaticity Diagram, it can be observed how these factors affect the appearance of colors through the microscope.

*roshanak.zakizadeh@etu.univ-st-etienne.fr; phone +33 611 079096

2. EXPERIMENT

The main purpose of this paper is to analyze how different factors might affect the colors viewed by the users of the microscopes in different fields of science. The following experiment is performed, considering the size of the samples and the intensity of the light source as the factors affecting the color difference observed by the user of the microscope.

Figure 1 shows the setup for the measurement of the reflectance of samples through the eyepiece of the microscope. Samples are observed through Nikon microscope; model Eclipse MA200®. It should be mentioned that the measurements are done in complete darkness and the only light source present is the microscope's lamp.

The samples under study are the 24 colors of GretagMacbeth Classic ColorChecker® and Mini ColorChecker®. The two ColorCheckers include patches of the same color but in two different sizes. It's shown later in this paper how the samples of the same chromaticity but of different sizes will result in different reflectance. To compare the reflectance of the samples under different intensity of light, dark field and bright field illuminations of the microscope are used to represent two levels of intensity. To measure the reflectance of the patches of the ColorCheckers® , Konica Minolta CS-2000 spectroradiometer® which in fact collects the spectral radiance of the samples, is located in front of the eyepiece. During the measurements all the ambient light is avoided and the only existing luminance is considered to be the microscope's light.

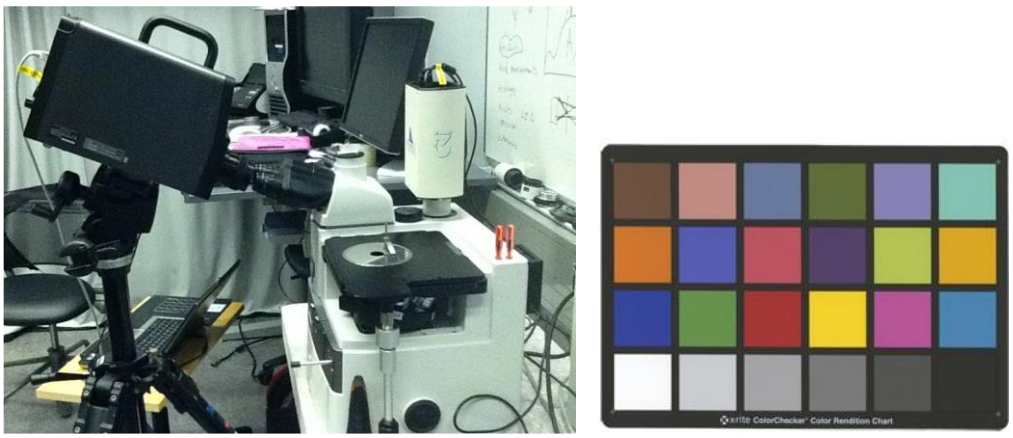

Figure 1. Left: Experiment's set up. Right: GretagMacbeth Classic ColorChecker®.

The reference white used in the calculation of the reflectance of the samples is the spectral radiance of the perfect reflector® measured using the same set up shown in Figure 1. The resultant reflectance of the patches of the ColorCheckers® is computed by dividing the spectral radiance of the sample measured by the spectroradiometer with the one of the Custom White Balance®.

To evaluate how much the colors of the patches of ColorCheckers observed through microscope differ, their reflectance is always compared by computing the color difference between the calculated reflectance in our experiment and the one resulting from the Ohta's measurements[1] under D50 luminance without effect of any microscope lens.

In the second part of the experiment, the same procedure is followed, except this time the samples under study are only the color patches of the GretagMacbeth Classic ColorChecker® and they are observed through Olympus SZX10 microscope®. To measure the spectral radiance of the 24 colors the spectroradiometer is mounted into the camera port of the microscope. The data acquired using Olympus microscope® is mostly analyzed in this paper to emphasize on the range of colors in which the greatest color difference is observed.

3. ANALYSIS OF DATA

The analysis of the acquired data is done by studying the position of samples in CIE 1931 Chromaticity Diagram and a*b* channels of CIE L*a*b* color space. The color difference of the colors of the samples and the original data for the same set of samples provided by Ohta[1] is also computed using a variety of color difference formulas.

3.1 Effect of sample size

The shift in xy chromaticity values of the patches of the Mini ColorCheckers® and Classical ColorCheckers® can be observed in the left diagram in Figure 2. There is a slight shift in position of colors, which is not noticeable compared to the effect of intensity of the light of the microscope. We should also take into consideration the conditions of measurements which might also have an effect on the results.

3.2 Effect of light source intensity

Right diagram in Figure 2 shows the position of colors of the Classical ColorCheckers® measured using the "bright field" and "Dark field" illuminations of the Nikon ECLIPSE MA200® microscope in CIE 1931 color space chromaticity diagram. There is a shift in the position of colors under two different illuminations. It seems that the effect of the intensity of the light source is more than the effect of the size of the sample.

To have a better investigation in terms of changes in intensity we have compared the reflectance of the samples using two levels of the intensity with the original reflectance of the samples provided in N. Ohta[1]. The comparison is made by applying different color difference formulas such as CIELAB[6] (CIE,2004); CMC[7] (Clarke et al., 1984); BFD[8] (Luo and Rigg, 1987); CIE94[9] (CIE, 1995); CIEDE2000[10] (CIE, 2001); DIN99d[11] and DIN99b. The average color difference can be observed in Table 1. The effect of the intensity of the light of the microscope is very noticeable, especially under a brighter illumination.

Table 1. Average color differences of 24 patches of GretagMacbeth ColorChecker® under "dark field" and "bright field" illumination of Nikon ECLIPSE MA200®

	CIELAB	**CMC**	**BFD**	**CIE94**	**CIEDE 2000**	**DIN99d**	**DIN99o**
Dark field illumination	10.00	6.58	9.02	5.57	6.10	4.12	6.93
Bright field illumination	16.42	12.51	23.44	11.92	11.10	11.18	13.13

On the other hand, the results show that less saturated samples; as the "dark skin" patch of the Classical ColorCheckers®; show larger difference between the original reflectance of color patch and the one measured through the eyepiece (Table 2). It has a diverse response in different ranges of wavelengths, i.e. in the red part of the spectrum the sample has a lower reflectance than the original one. Whereas, the effect is reverse for the lower part of the spectrum and the color appear darker, also in this part dark field illumination responds better. In Table 2, the "dark skin" sample is compared with the light skin sample which has the same hue but a different saturation. This effect is studied considering the reflectance measured through the eyepiece. As for the observer, further experiments should be performed.

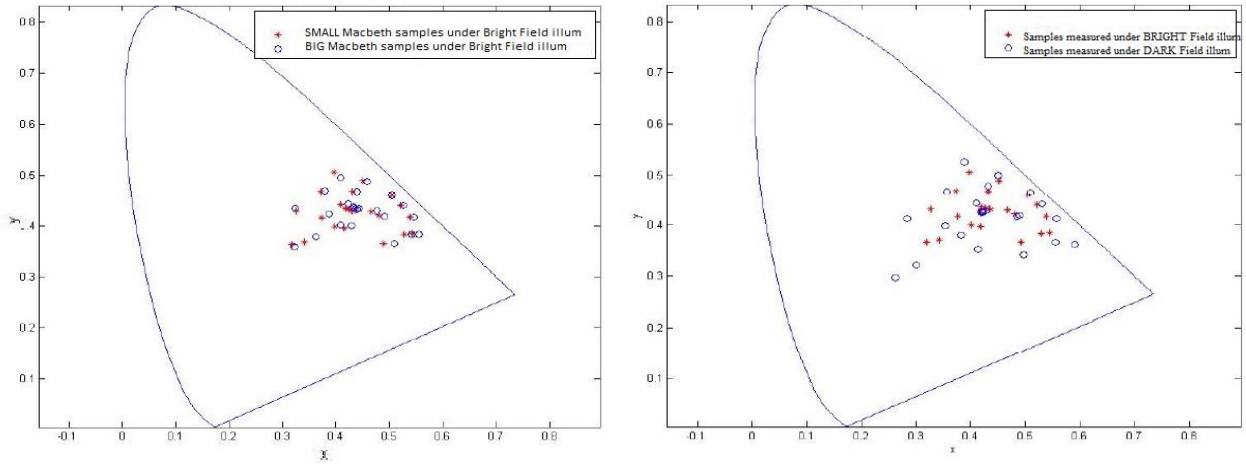

Figure 2. Left: Chromaticity values of samples calculated considering different sizes of samples. Right: Chromaticity values of samples calculated considering different levels of intensity.

Table 2. Color differences of ″dark skin″ patch of GretagMacbeth ColorChecker® under "dark field" and "bright field" illumination of Nikon ECLIPSE MA200®

	CIELAB	**CMC**	**BFD**	**CIE94**	**CIEDE 2000**	**DIN99d**	**DIN99o**
Dark field illumination	2.34	2.56	5.34	2.14	2.01	3.00	2.29
Bright field illumination	14.69	14.41	35.00	14.48	13.89	15.59	14.69

Table 3. Color differences of ″light skin″ patch of GretagMacbeth ColorChecker® under "dark field" and "bright field" illumination of Nikon ECLIPSE MA200®

	CIELAB	**CMC**	**BFD**	**CIE94**	**CIEDE 2000**	**DIN99d**	**DIN99o**
Dark field illumination	5.97	5.45	5.92	3.60	4.07	1.32	4.42
Bright field illumination	8.42	6.34	12.33	6.87	5.84	6.12	6.91

Among bright colors the less saturated colors still show larger difference, for instance green sample compared to the light skin sample, except that for such colors the changes are not variable along the spectrum. In general, with dark field illumination the measured reflectance is closer to the original reflectance of the sample, but again for less saturated colors, higher amount of intensity is helpful in the red part of the spectrum.

Further investigations are done using an Olympus SZX10® microscope with the same procedure and again the CIELAB color difference of the 24 samples are measured against the Ohta's measurements, for which the results can be observed in Figure 3. It is interesting to notice that for the range of colors with evident color difference we can still observe a noticeable color difference through Olympus SZX10® microscope.

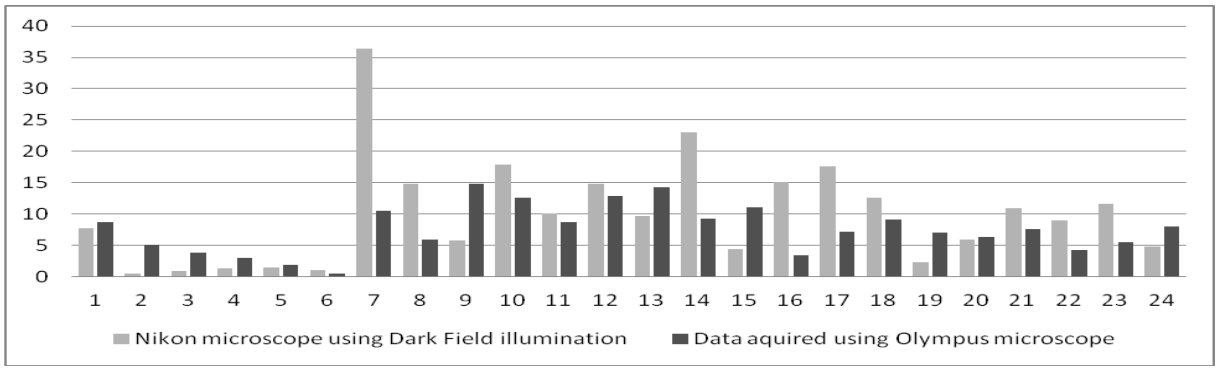

Figure 3. Comparison of the color difference between our measurements using two microscopes and the original data from N. Ohta[1].

4. CONCLUSIONS

The results of the experiments show that the color difference caused by the optics of microscope is noticeable and should be taken into consideration in microscopic studies. Further, there are factors that affect this color difference, among which; some of them play a more important role such as the intensity and type of illumination. The analysis of data collected in this experiment shows the color difference is more noticeable in case of colors within a special range and saturation. Further studies can be conducted using real laboratory samples and involving samples of wider range of colors. Surely this kind of studies can be useful in improving the accuracy of the microscopic studies in which color is considered as an important factor.

REFERENCES

[1] Ohta, N., "Macbeth color checker patches," http://www.cis.rit.edu/research/mcsl2/online/CIE/MacbethColorChecker.htm (1997).
[2] Nikon, "ECLIPSE MA200 microscope," http://www.nikon.com/products/instruments/lineup/industrial/industrial-microscopes/inverted/ma200/ (2013).
[3] Konica, " Konica Minolta," http://sensing.konicaminolta.asia/products (2013).
[4] Olympus, "SZX10 microscope," http://www.olympusamerica.com/seg_section/product.asp?product=1018 (2013).
[5] Luo M. R., Minchew C., Kenyon Ph. and Cui G., "Verification of CIEDE2000 using industrial data," Proc. AIC (Interim Meeting of the International Color Association) Color and Paints, 97-102 (2004).
[6] Robertson A.R., "Color Res.," Appl. 15, 167-170 (1990).
[7] Clarke F. J. J., McDonald R. and Rigg B., "Modification to the JPC79 colour difference formula," Journal of the Society of Dyers and Colourists 100, 128 –132 (1984).
[8] Luo M. R. and Rigg B., "BFD*(l:c)* colour difference formula. Part I—Development of the formula," Journal of the Society of Dyers and Colourists 103, 86 –94 (1987).
[9] CIE (Commission Internationale de l'Éclairage), "Industrial colour-difference evaluation," Vienna: Central Bureau of the CIE, CIE Publ. 116 (1995).
[10] CIE, "Improvement to industrial colour-difference evaluation," Vienna: Central Bureau of the CIE, CIE Publ. 142 (2001).
[11] Cui G., Luo M. R., Rigg B., Roesler G. and Witt K., "Uniform colour spaces based on the DIN99 colour-difference formula," Color Res Appl 27, 282-290 (2002).