https://doi.org/10.46341/PI2024004 UDC 57.082 + 57.087.3

**RESEARCH ARTICLE** 

# Testing color reference charts for the herbarium digitization purposes

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Received: 05.06.2024 | Accepted: 04.07.2024 | Published online: 05.07.2024

#### Abstract

The current paper represents the continuation of the testing of the budget photosystems for herbarium digitization and aims to demonstrate the color reproduction accuracy besides other colorimetric characteristics of five color reference targets. It was shown that cheap color reference targets of Charttu can be applied for digitization purposes, at least in brand-new condition. However, the color degradation of Charttu targets during use should still be evaluated. At the same time, the color accuracy of the X-Rite Color Checker Classic Mini was found to be highly degraded after several years of careful and non-intensive use. The Golden Thread Object Level target was found to be the best solution for herbarium digitization and the most resistant to color degradation, even in intensive and long use.

Keywords: herbarium digitization, image quality assessment, color reference, color checker, delta E, delta L, delta H

**Authors' contributions:** Andriy Novikov: conceptualization, project administration, supervision, funding acquisition, validation, visualization, writing – original draft. Mariia Sup-Novikova: conceptualization, visualization, writing – original draft. Viktor Nachychko: writing – original draft, validation. Oleksandr Kuzyarin: writing – review & editing.

**Funding:** This work has been realized in the frames of the project "Digitization of natural history collections damaged as a result of hostilities and related factors: development of protocols and implementation on the basis of the State Museum of Natural History of the National Academy of Sciences of Ukraine" (Nr 2022.01/0013), financed by the National Research Foundation of Ukraine in the grant call "Science for the Recovery of Ukraine in the War and Post-War Periods".

Competing Interests: The authors have declared that no competing interests exist.

#### Introduction

The digitization of natural history collections and mobilization of biodiversity data are crucial modern tasks resulting in many global initiatives and extended meta-analyses (Holmes et al., 2016; Wetzel et al., 2018; Nelson & Ellis, 2018; Ball-Damerow et al., 2019; Shultz et al., 2020; Heberling et al., 2021; De Smedt et al., 2024). Before, industrial or professional scanners (e.g., HerbScan or BioScan) were involved in digitizing flat-mounted natural history specimens. However, in the last decade, photo cameras have been preferred for herbarium digitization around the world due to several advantages: (a) the relatively low price of the camera and lenses; (b) the modularity of the photosystem; (c) extended service support; (d) faster image capturing; (e) producing RAW files that can be later corrected; (f) higher field of depth allowing to capture non-planar specimens; (g) more effortless organization of the conveyor system (Tegelberg et al., 2014; Sweeney et al., 2018; Takano et al., 2019; Nieva de la Hidalga et al., 2020; Gasper et al., 2021).

Our previous paper (Novikov et al. 2023) stressed the application of different budget photosystems to digitize herbarium specimens. It was found that the photo camera Canon EOS 800D with fixed lens Tokina AT-X M35 PRO DX AF 35 mm f/2.8 Macro produces images with the best color accuracy ( $\Delta E_{2000}$  = 4.7-6.5) among the tested cameras, however the resolution remains relatively low (24.2 Mp). Therefore at the Herbarium of the State Museum of the Natural History of the NAS of Ukraine (LWS), it was decided to change the Canon photo camera with Tokina lense on Panasonic Lumix G9 camera with Olympus 30 mm macro lense, which demonstrated similar color accuracy reproduction ( $\Delta E_{2000}$ = 4.6-5.7) but allows to apply pixel shift technology resulting in images of four times higher resolution (80.6 Mp). The current paper aims to fulfill the experience obtained with the new findings by applying different color reference charts (so called color checkers).

Two main classes of reference targets are applied for the herbarium digitization those allowing the evaluation of the accuracy of the color reproduction and those allowing the assessment of the geometric distortion and the resolution depth of the resulting image. The cheaper on-market targets (e.g., X-Rite ColorChecker Classic or Datacolor SpyderCheckr) allow to evaluate only the color accuracy and, therefore, are commonly called color checkers. At the same time, the more expensive targets usually combine color reference patches with different geometrical gauges (e.g., Golden Thread Object Level Target or FADGI 19264 Target). Golden Thread Object Level Target is applied by several world-leading herbaria, including Herbarium Berolinense in Berlin (B), Jardin botanique Meise (BR), and Royal Botanic Gardens in Kew (K). A similar extended reference chart (Image Engineering TE 263) is also applied by Instytut Botaniki im. Władysława Szafera PAN in Kraków (KRAM). However, simpler reference charts like ColorChecker with 24 color patches are also widely used, e.g., in the Naturhistoriska riksmuseet in Stockholm (S), Muséum national d'Histoire Naturelle in Paris (P), Karl-Franzens-Universität Graz (GZU), and Naturhistorisches Museum Wien (W).

Despite the color degradation due to drying and long-term preservation of herbarium specimens, color reference charts are actively applied for digitization. There are no strict rules regulating the application of color reference charts for herbarium digitization, but it is widely considered a best practice (iDigBio, 2015; Guiraud et al., 2019; Baratè et al., 2020; Ledesma et al., 2020).

## Material and methods

Four targets with 24 color patches (X-Rite ColorChecker Classic Mini, Charttu 24 Mini, Charttu 24 Micro, and Charttu 24 Nano) and one target with 30 color patches (Golden Thread Object Level) were tested. Charttu targets were brand new, while ColorChecker and Golden Thread targets were actively used before for digitization purposes. ColorChecker target was in excellent condition, used carefully, and stored out of light in the original package. Golden Thread target was used much more intensively. It had dents and scratches, but, as the preliminary  $\Delta E$  test showed, it was still in a good working condition (Fig. 1). It would be great to apply all new targets, but it was impossible due to the limited budget. Nevertheless, we believe that the results we obtained are still interesting and valuable.

For some analyses, the number of analyzed gray patches for GoldenThread Object Level has been reduced from 12 to six to match other tested 24-patches reference charts. The simultaneous picture of all tested targets has been taken at f/8, 1/30s, and ISO 200 in a pixel shift mode at the same illumination using the camera Panasonic Lumix G9 with Olympus M.Zuiko Digital ED 30mm f/3.5 Macro. After that, images of the targets were cropped and processed through the online tool ImageZebra (2024). The CIE L\*a\*b\* values for all analyzed patches and Noise values (calculated as the standard deviation of L) were taken directly from the ImageZebra. The reference values CIE L\*a\*b\* were taken from the producers' official websites. The  $\Delta E_{_{\rm 2000}}$  values were calculated in MS Excel 2007 using the FogradE00 Excel Plugin (Fogra, 2020) with standard formulae (Sharma et al., 2005; Lindbloom, 2017). The white balance, i.e.,  $\Delta E(a*b*)$  values, were calculated using the modified  $\Delta E_{2000}$  formulae (excluding the L-related part) following



**Figure 1.** Tested reference charts: **A** – Golden Thread Object Level; **B** – X-Rite ColorChecker Classic Mini; **C** – Charttu 24 Mini; **D** – Charttu 24 Micro; **E** – Charttu 24 Nano.

ImageZebra principle. The tone response (OECF) has been calculated as  $\Delta L$  following the ImageZebra principle. The CIE L\*a\*b\* values were converted into LCH values in MS Excel using the Colour Conversion Centre 4.2b (Boronkay, 2024). After that,  $\Delta C$  and  $\Delta H$  were calculated as respective differences between referenced and tested values. The graphs were built in MS Excel 2007 environment.

### **Results and discussion**

Obtained results proved that all targets are suitable for purposes of herbarium digitization but have some deviation from the reference values declared by producers. The lowest difference between the reference and tested values was found in the Golden Thread Object Level target, which showed mean  $\Delta E_{2000} = 4.5$  (Table 1). Such relatively low values have been reached despite hardly used condition – it has numerous dents and scratches. The almost new target Charttu Nano demonstrated the second lowest mean  $\Delta E_{2000} = 5.4$ . While used, but still in excellent visual condition, target X-Rite Classic Mini showed the worst result

with mean  $\Delta E_{2000} = 6.9$ . Hence, applying new Charttu targets is better than using X-Rite Classic Mini. However, the color degradation in Charttu targets has to be an object for further monitoring. It is also interesting that different Charttu targets demonstrated different  $\Delta E_{2000}$ and other tested values. Probably, it is a result of production inconsistency. Nevertheless, all demonstrated values were comparable with those in Golden Thread Object Level and X-Rite Classic Mini targets (Figs. 2–5), and, therefore, Charttu targets can be recommended for use on a limited budget.

Nieva de la Hidalga et al. (2020) argued that  $\Delta E_{2000}$  for the digitized herbarium material should not exceed the value of 5.0. Following the recently introduced FADGI standard (Rieger et al., 2023), the lowest mean  $\Delta E_{2000}$  should not exceed 6.5, corresponding to a one-star quality level. For the two-star quality level,  $\Delta E_{2000}$  should not exceed 5.0. For the three-star quality level,  $\Delta E_{2000}$  should not exceed 3.5. For the highest image quality (FADGI's four-star quality level), the mean  $\Delta E_{2000}$  should not exceed 2.0. Considering these recommendations, our photosystem reaches a two-star level when the Golden

		Color reproduction accuracy $(\Delta E_{2000})$	White balance (∆E(a*b*), gray scale)	Noise (stddev L, gray scale)	Tone response (∆L, OECF, gray scale)	Chroma difference (∆C)	Hue difference (∆H)
X-Rite Classic Mini	Mean	6.87	1.10	0.65	4.76	4.09	-1.77
	StdDev	2.86	0.96	0.41	6.50	5.72	31.09
	min	2.22	0.52	0.01	-5.95	-6.86	-110.77
	max	12.32	3.03	1.04	12.07	16.49	62.91
	90th percentile	9.55					
Charttu Mini 24	Mean	6.55	0.96	0.84	3.56	5.14	-6.90
	StdDev	2.73	0.60	0.39	7.46	6.00	57.34
	min	0.97	0.28	0.24	-8.49	-6.13	-251.70
	max	11.28	1.94	1.25	11.74	17.97	109.43
	90th percentile	9.26					
Charttu Micro 24	Mean	6.12	1.22	0.88	1.87	4.98	-5.02
	StdDev	2.42	0.61	0.43	7.67	6.02	65.66
	min	2.18	0.41	0.23	-10.41	-7.41	-259.27
	max	10.65	2.04	1.41	10.25	16.51	111.10
	90th percentile	9.26					
Charttu Nano 24	Mean	5.40	1.24	0.87	0.92	4.43	1.19
	StdDev	2.12	0.43	0.43	7.55	5.40	63.60
	min	1.93	0.78	0.25	-11.10	-6.42	-261.05
	max	9.54	1.87	1.36	8.97	15.13	104.07
	90th percentile	8.00					
Golden Thread Object Level (all grayscale patches)	Mean	4.51	1.22	0.85	3.92	0.44	13.25
	StdDev	2.57	0.60	0.50	5.26	3.09	77.83
	min	0.53	0.19	0.83	-2.69	-8.49	-27.69
	max	8.80	2.11	1.68	9.12	9.06	236.64
	90th percentile	7.88					
Golden Thread Object Level (rearranged to match 24 patches)	Mean	4.80	0.92	0.88	5.11	0.55	2.62
	StdDev	2.56	0.67	0.54	5.57	3.46	51.16
	min	0.53	0.19	0.19	-2.24	-8.49	-65.99
	max	9.47	1.82	1.68	11.91	9.06	228.76
	90th percentile	7.82					

# Table 1. Colorimetric characteristics of the tested color reference targets.



**Figure 2.** The color reproduction accuracy ( $\Delta E_{2000}$ ) for separated patches of the tested charts. Abbreviations indicate the row (1–4) and column (A–F) of the patches. In the case of the Golden Thread Object Level target, which has a linear representation of the color target, the patches were rearranged and renamed to match other tested targets. In particular, patches of the fourth grayscale row (A4–F4) correspond to grayscale patches 10, 13, 15, 16 (M), 17, and 19, which are indicated initially on the Golden Thread Object Level target. Patches A1–F1 (first row) correspond to original patches 1–6. Consequently, patches A2–F2 (second row) correspond to original patches 7–9 and 22–24. Patches A3–F3 (third row) corresponds to original patches 25–30.

Thread Object Level target is applied, but only a one-star level when Charttu Micro and Nano targets are used, and it does not reach the lowest acceptable color accuracy when X-Rite Classic Mini target is applied (Table 1). If taken into account  $\Delta E_{2000}$  90th percentile, the photosystem showed more or less consistent results with all tested targets and reached a two-star quality level ( $\leq$  10.0).

Only for the Charttu Nano target,  $\Delta L_{2000}$  (tone response) corresponded to FADGI's fourstar quality level (Rieger et al., 2023). With the Charttu Micro target,  $\Delta L_{2000}$  reached a threestar level ( $\leq 3.0$ ); with Charttu Mini – a twostar level ( $\leq 4.5$ ); with X-Rite Classic Mini and Golden Thread Object Level –only one-star level ( $\leq 6.0$  – see Table 1). The white balance measured for the grayscale as  $\Delta E(a*b*)$  in all cases reached FADGI's four-star quality level ( $\leq 2.0$ ). The noise level was almost identical for all tested targets and reached a four-star quality level ( $\leq 1.0$ ).

The L\*C\*h color space is similar to L\*a\*b\* but describes color using cylindrical



Figure 3. The mean values of basic colorimetric parameters for the tested charts.

coordinates instead of rectangular ones, with lightness as a universal characteristic. The L\*a\*b\* color space is widespread and can be easily converted into L\*C\*h. FADGI (Rieger et al., 2023) is based on L\*a\*b\* color space and, therefore, did not consider  $\Delta H$  and  $\Delta C$ indices for digitized materials. Nevertheless,  $\Delta H$  and  $\Delta C$  are sometimes more helpful for colorimetric studies and more accessible for understanding (Gilchrist & Nobbs, 2000; Green, 2023).  $\Delta a^*$  and  $\Delta b^*$  are combined characteristics describing rational relations red/green and yellow/blue colors, of respectively. In comparison,  $\Delta H$  and  $\Delta C$  are more intuitive and explain the difference in hue and chroma, respectively. Therefore, we also calculated these values. Hue difference  $(\Delta H)$  appeared strongly variable and nonrepresentative for all tested targets (Table 1). Chroma difference ( $\Delta C$ ) was ca. 5.0 for X-Rite

and all Charttu tested targets but ten times less (ca. 0.5) for the Golden Thread Object Level target. Together with low  $\Delta E$  characteristics, this confirms the extremely high quality of the last target and its color stability that did not degrade even after years of intensive use.

The resulting colorimetric values depend mainly on the photosystem's optical properties and sensor but are also strongly influenced by the applied illumination and other supporting factors. However, as we showed, obtained values can differ for the same photosystem and illumination conditions depending on the applied target and its current condition. Therefore, it is strongly recommended that only new targets should be used, carefully preserved, and changed every two years. It is also recommended that applied targets be tested and compared with the targets of other producers before application.



**Figure 4.** Tone response (OECF) based on the grayscale patches. **Solid line** – sampled values; **pointed line** – reference values. The **X-axis** represents the titles of tested patches.





**Figure 5.** Noise (calculated as the standard deviation of L) based on the grayscale patches. The **X-axis** represents the titles of tested patches.

### Conclusions

This study prooved that the photosystem currently applied for the digitization at LWS meets recent FADGI criteria for digitizing herbarium material (Documents (Unbound): General Collections) and generally reaches a two-star quality level. However, it has also been shown that the application of color reference targets strongly influences the obtained colorimetric results, and therefore, targets should be chosen carefully. It was demonstrated that cheap Charttu color targets that are little known on the market can also be applied for herbarium digitization purposes instead of the targets from reputed producers such as X-Rite or ISA. Nevertheless, the Golden Thread Object Level target is the best solution for digitization as it showed the lowest color degradation after intensive use. Moreover, it contains an extended set of gray patches and a graphical part for evaluating the geometric distortion of the images.

### Acknowledgements

We are grateful to Patricia Mergen and Sofie De Smedt for kindly granting the Golden Thread Object Level target, which allowed us to conduct this research.

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## Тестування кольорових еталонних мішеней для цілей оцифрування гербарію

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Ця стаття є продовженням тестування бюджетних фотосистем для оцифрування гербарію та має на меті продемонструвати точність відтворення кольору, окрім інших колориметричних характеристик, п'яти кольорових еталонних мішеней. Було показано, що дешеві кольорові еталонні мішені виробництва Charttu можна застосовувати для цілей оцифрування, принаймні допоки вони є новими. Однак, все ще слід ще оцінити деградацію кольору (вицвітання) мішеней Charttu з часом. У той же час було виявлено, що якість кольоропередачі мішені X-Rite Color Checker Classic Mini сильно погіршилася вже після кількох років дбайливого та неінтенсивного використання. А мішень Golden Thread Object Level була визнана найкращим рішенням для оцифрування гербарію та найстійкішою до деградації кольору навіть після інтенсивного і довготривалого використання.

**Ключові слова:** оцифрування гербарію, оцінка якості зображення, кольоровий еталон, кольорова мішень, delta E, delta L, delta H