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A comparison of animal color measurements using a commercially available digital color sensor and photograph analysis

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Abstract

An animal's pelage, feather, or skin color can serve a variety of functions, so it is important to have multiple standardized methods for measuring color. One of the most common and reliable methods for measuring animal coloration is the use of standardized digital photographs of animals. New technology in the form of a commercially available handheld digital color sensor could provide an alternative to photography-based animal color measurements. To determine whether a digital color sensor could be used to measure animal coloration, we tested the ability of a digital color sensor to measure coloration of mammalian, avian, and lepidopteran museums specimens. We compared results from the sensor to measurements taken using traditional photography methods. Our study yielded significant differences between photography-based and digital color sensor measurements of brightness (light to dark) and colors along the green to red spectrum. There was no difference between photographs and the digital color sensor measurements for colors along the blue to yellow spectrum. The average difference in recorded color (ΔE) by the two methods was above the threshold at which humans can perceive a difference. There were significant correlations between the sensor and photographs for all measurements indicating that the sensor is an effective animal coloration measuring tool. However, the sensor's small aperture and narrow light spectrum range designed for human-vision limit its value for ecological research. We discuss the conditions in which a digital color sensor can be an effective tool for measuring animal coloration in both laboratory settings and in the field.

Key words: Animal coloration, Color measurement, Comparative method, Digital photography, Museum specimens, Nix color sensor

It is important for researchers to be able to reliably measure animal's coloration to gain insight into how coloration affects an animal's survival (Cuthill et al. 2017). An animal's coloration in the form of pelage, feather, or skin serves a variety of adaptive functions (Endler 1990; Caro 2005; Ancillotto and Mori 2017) and can be used as an indicator of age (Sharp 1958; Garshelis 1984; Bergman and Beehner 2008), phenology (Camargo et al. 2006), hormone levels (Clough et al. 2009), social status (Gerald 2001; Pryke et al. 2002), and fitness (Keyser and Hill 2000). In species with highly variable coloration, unique colors and patterns provide researchers a method for identifying individuals and estimating population sizes without invasive handling procedures (Tye et al. 2015; Greene et al. 2016). Furthermore, animal coloration studies have transcended the field of ecology, with results applied to human recreation, fashion design, and cryptic military technology (Caro et al. 2017). Alternatively, the development of technologies for measuring human-oriented colors (i.e., textiles, paints) may provide a valuable tool for ecologist looking to measure animal coloration. However, before adopting a new device for measuring animal coloration, it is essential that it is tested against an established method (White et al. 2015; Stiglitz et al. 2016).

Photography is an established method for measuring animal coloration, and with the use of modern software, color values derived from photographs can be highly accurate (Troscianko and Stevens 2015). The use of photography to measure an animal's coloration has increased in recent years with improved camera quality and decreased cost of digital cameras (Stevens et al. 2007). Commercially available digital cameras are well-suited for measuring animal coloration that can be seen by the human eye (Johnsen 2016). With knowledge of a camera's spectral sensitivities, it is possible to fit digital pixel values in photographs to a cone-catch model, adjusting the color values to match the visual system of a non-human animal (Gerald et al. 2001; Stevens et al. 2007; Troscianko and Stevens 2015; Cuthill et al. 2017). Although standardized photospectrometers are considered an objective technique for measuring color (Endler 1990; Johnsen 2016), the relative ease and accuracy of using digital photography to measure animal coloration has made it a popular method for researchers (Stevens et al. 2007; Bergman and Beehner 2008; Pike 2011; Mckay 2013; Troscianko and Stevens 2015).

To accurately measure color, researchers must account for variations in lighting conditions (Stevens et al. 2007; Bergman and Beehner 2008). To control for ambient light conditions using digital photography, researchers often measure colors of specimens where camera position and ambient lighting are controlled (Stevens et al. 2007). This can be effective for the study of museum specimens, but is not feasible for live capture of many wildlife species. For photographs of *in situ* wildlife, ambient light variation can be accounted for by photographing a standardized color chart placed in the same location as the animal (Bergman and Beehner 2008). Although modern software makes standardizing and obtaining color data from photographs relatively quick and easy (Troscianko and Stevens 2015), new technology may provide alternative measurement techniques that do not require post-hoc adjustments.

A digital color sensor is a handheld device that can be remotely triggered from a smartphone to measure the color of any object it is placed against. A digital color sensor blocks out ambient light and illuminates the object being measured using a built-in, calibrated light source. A lightweight and portable sensor could be a valuable field tool for researchers, potentially eliminating the need to haul expensive and bulky equipment such as a camera into the field. Commercially available sensors are marketed for human-vision oriented uses, such as manufacturing and design a sensor's primary value for ecological research is for studies focused on animal coloration as it relates to anthropogenic conditions. Human-induced global change promotes rapid phenotypic variation in animals (Kettlewell 1955; Lovejoy 2008; Forsman et al. 2011), making a digital color sensor a potentially valuable tool for animal coloration research in the Anthropocene. However, a digital color sensor

must first be evaluated in a controlled setting on a variety of animal substrates, as an object's physical structure can influence how light reflects of it, and therefore affect color measurements (Vukusic et al. 2000; Meadows et al. 2011).

Our objective for this study was to compare a handheld digital sensor's ability to reproduce animal color measurements recorded using traditional photographic methods on a sample of animals representing a broad range of colors and physical structures. We tested the ability of a small, commercially available digital color sensor (Nix™ Pro Digital Color Sensor, Nix Sensor Ltd., Hamilton, ON, Canada) in a laboratory setting to measure animal coloration from museum specimens of a polymorphic mammal, as well as species of colorful birds and butterflies. We then compared the color measurements from the digital sensor to measurements derived from traditional photography methods.

Materials and Methods

Study specimens

To test the digital color sensor sensor's ability to accurately measure animal coloration on a wide variety of biological surfaces and across a range of colors we selected 32 mammalian, 43 avian, and 24 lepidopteran specimens (Table 1). For the avian and lepidopteran specimens, we selected a variety of species with varying color patterns. We incorporated highly iridescent species (e.g., common grackle *Quiscalus quiscula*) to test the sensor's ability to measure structural coloration. For the mammalian specimens, we selected polymorphic southeastern fox squirrels (*Sciurus niger* spp.), which exhibit color variation between specimens and between body parts on the same specimen (Moore 1956; Tye et al. 2015). All specimens came from the Florida Museum of Natural History (FLMNH) collections and were in undamaged condition.

Color measurements

We measured animal color in Commission internationale de l'éclairage L* a* b* (CIELab) color space because it is standardized, perceptually uniform, and was developed to closely match human vision (Schanda 2007). The color sensor is designed to capture textile, paint, and other colors for human use, making CIELab an appropriate color space for comparing measurements between the sensor and standardized photographs. The CIELab color space uses 3 continuous axes to represent a color. The L* axis is a measure of brightness and ranges from 0-100, where 0 is black and 100 is white. Measurements of a* and b* can be negative or positive. From negative to positive values, the a* axis describes colors along a green to red spectrum and the b* axis describes colors along the blue to yellow spectrum. When a* and b* are both equal to 0, the color is a shade of gray ranging from pure black to pure white depending on the L* value (Schanda 2007).

One advantage of CIELab color space is that differences between colors can be calculated using the delta-E 2000 (ΔE). The ΔE is a measure of distance (dissimilarity) between colors in the CIELab space such that shorter distances indicate greater similarity between colors (Luo et al. 2001). The original definition of ΔE was simply Euclidian distance, but the formula has been updated to more accurately measure distances between colors with similar lightness, but different hues (Sharma et al. 2005). On average, the human eye cannot perceive differences between colors with $\Delta E < 2.2$ (Brainard 2003).

Digital color sensor

We measured animal coloration using the Nix™ Pro Digital Color Sensor (Nix Sensor Ltd., Hamilton, ON, Canada). The sensor is highly portable due to its light weight (43 g) and small size (60mm x 42mm). The sensor measures color within the visible spectrum of light, 380–700 nm. Previous studies have shown the sensor to be effective at measuring the color of meat (Hodgen 2016; Holman et al. 2018), soil (Stiglitz et al. 2016), and human teeth (Nguyen et al. 2017). The sensor has a 1.5cm diameter aperture that, when placed against an object blocks out ambient light and, once activated, supplies its own light source, eliminating error associated with variable light conditions (Stevens et al. 2007). The illuminant can be set to A, C, D50, D55, D65, and D75, while the observer angle can be set as 2° or 10°. We used the D65 illuminant and 2˚ observer angle for all measurements. Although the sensor records data in multiple color spaces, we only included the CIELab color data in our analysis.

To measure each specimen's coloration, we firmly placed the digital sensor on each specimen at specific locations. For avian specimens, we measured the center of each bird's breast. For fox squirrels, we measured 2 points on each specimen. The first point was located dorsally central between the forelimbs (shoulder). The second point was at a point along the venter where the pelage was thickest (venter). We measured dorsal and ventral pelage because fox squirrels show a wide range of colors in these locations (Moore 1956). For the lepidopteran specimens, we measured the center of the widest part on one of the front wings. We triggered the sensor via Bluetooth using a smartphone (Apple iPhone XR, Apple, Cupertino, CA, USA). To maximize the precision of our color estimates using the digital sensor, we averaged 7 measurements of the same area, picking up the sensor and replacing it on the same spot between measurements (Holman et al. 2018). To measure the variation between the 7 measurements taken on each specimen we calculated the second-order coefficient of variation (V_2; Kvålseth 2017). Unlike the traditional measure of coefficient of variation, the V_2 can handle both positive and negative means, such as those recorded along the a* and b* axis. The V_2 is bounded between 0.0 and 1.0 with greater values indicating larger variation between measurements (Kvålseth 2017).

Digital photography

We photographed all specimens using a stand-mounted digital camera (Canon EOS 5D Mark II, Canon Inc. Tokyo, Japan) with a 50 mm macro lens (Canon EF Compact Macro, Canon Inc. Tokyo, Japan). We positioned the camera 58 cm directly above all mammalian and avian specimens, and 47 cm directly above all lepidopteran specimens. We did not use the camera's flash, and manually set the white balance to the white patch on a reference color chart (ColorChecker Classic, X-Rite, Grand Rapids, MI, USA). We intentionally underexposed the shot to avoid overexposing the photos which leads to irrecoverable color data (Stevens et al. 2007; Bergman and Beehner 2008). We conducted all photography over 6 sessions. At the start of each photography session, we photographed the reference color chart which was later used to standardize the color settings for each specimen photograph (Stevens et al. 2007; Bergman and Beehner 2008; Boratyński et al. 2014). We then photographed each specimen in the same position as the reference color chart to maintain consistent lighting conditions. We exported all files as lossless RAW files.

We processed and extracted color values for all photos using the micaToolbox plugin (Troscianko and Stevens 2015) for ImageJ v1.51 (Schneider et al. 2012). To normalize and linearize the color values for each photo, we converted each RAW photo into a multispectral image, using the 91% reflectance patch of the color chart as a reference reflectance value (Troscianko 2019). Using the color chart, we developed a cone-catch model based on human vision, in the 400-700nm spectral range under D65 illuminant and 2˚ observer angle. We confirmed that model R^2 values for each photoreceptor in

the cone-catch model were ≥0.99, indicating successful linearization (Troscianko and Stevens 2015). We used the cone-catch model to convert each multispectral image into XYZ colorspace. We then converted each XYZ image into CIELab colorspace using micaToolbox's XYZ to CIELAB 32bit tool. We scaled each picture based on a ruler in the photograph and created a 1.5cm diameter circular region of interest (ROI) over the location on the specimen where we measured coloration using the Nix sensor. We then calculated the mean value of L*, a*, and b* for all the pixels inside the ROI using the Measure ROIs tool.

Statistical Analysis

To determine whether photographs and the digital sensor produced equivalent measurements, we used a paired two-sided Student's t-test for each color axis. We tested for significant correlations between color values recorded by the two methods by calculating Pearson's product-moment correlation (*r*) between values of L*, a*, and b* measured from photos and the sensor (Stiglitz et al. 2016).

To quantify the difference in color measurements for each specimen, we calculated the pairwise ΔE between the photos and sensor measurements. We used a one-way analysis of variance (ANOVA) to determine if the difference in color recorded between the sensor and photograph differed by specimen type. If there was a significant difference between any specimen type, we used a Tukey's honestly significant difference (HSD) to identify pairwise significant differences between groups. We performed all analyses using R Platform v3.5.0 (R Core Team 2018).

Results

On average, the color sensor recorded consistent values over repeated measures. The mean values of V_2 for L*, a*, and b* for 7 repeated measures using the color sensor were 0.04 (± 0.04 SD), 0.23 (± 0.23 SD), and 0.12 (± 0.16 SD), respectively. For some specimens, V_2 exceeded 0.99 for the a* and b* axis (Figure 1). The range of measurements for each color axis across all study specimens was similar for the color sensor and photographs (Table 2). Compared to photographs, the color sensor recorded significantly greater mean L* (photos: $\bar{x}$ = 43.57, sensor: $\bar{x}$ = 46.79; t = -4.83, $P < 0.001$), significantly lower mean a* (photos: $\bar{x}$ = 15.01, sensor: $\bar{x}$ = 6.46; t = 11.46, $P < 0.001$), and no significant difference between mean b* (photos: $\bar{x}$ = 17.52, sensor: $\bar{x}$ = 17.22; t = 0.45, p = 0.65). Overall, the color sensor recorded colors that were lighter and greener (less red) than photographs.

The color measurements from the photographs and the sensor were significantly correlated for all axes (L*: $r = 0.92$, $P < 0.001$; a*: $r = 0.90$, $P < 0.001$; b*: $r = 0.94$, $P < 0.001$; Figure 2). The pairwise ΔE values comparing the distance between colors measured by the sensor and from photos all exceeded the threshold at which humans can tell the difference, ranging from 2.91 to 31.00 (mean = 10.82 ± 4.88 SD; Figure 3). An ANOVA showed that ΔE varied significantly between specimen groups ($F_{3, 97} = 190.9$, $P < 0.001$). We therefore conducted a Tukey's HSD to determine which specimen groups were different. Delta E values did not differ between mammals and birds ($P = 0.63$), but there were significant differences between lepidopterans and birds ($P < 0.05$) and between lepidopterans and mammals ($P < 0.001$).

Discussion

Our results provide support for the use of a handheld digital color sensor in animal coloration studies. We found that the digital sensor gives different, albeit highly correlated, results than established photography-based method, which are optimal for measuring animal colors visible to the human eye (Johnsen 2016). Although the mean ΔE (10.82) between measurements from the sensor and photographs was greater than 2.2, the threshold at which humans can perceive a difference (Brainard 2003), high correlations between both methods indicate that this difference would not affect the results of a study that only uses one of these method to measure an animal's coloration. Further, the Nix sensor is an effective tool for research on the coloration of meat (Hodgen 2016; Holman et al. 2018), soil (Stiglitz et al. 2016), and human teeth (Nguyen et al. 2017).

The method we used for linearizing and normalizing the photographs is highly accurate for measuring coloration (Troscianko and Stevens 2015); therefore, we assumed differences between photos and the sensor were likely due to sensor or user inaccuracy. Although repeated measures of the same sample should have reduced measurement error (Holman et al. 2018), non-uniform surfaces of animal skins were difficult to consistently measure at the same location. The sensor is most effective when pressed firmly against a surface to block out ambient light, which can be difficult to achieve on rounded, small, or irregular surfaces, such as skin, feathers, or scales (Gómez and Liñán-Cembrano 2017). In the specimens we measured, variable patterns and 3-dimensional substrates, such as the agouti hairs on fox squirrels, may have shifted between measurements, increasing variation between repeated measurements. We found that average coefficient of variation (V_2) for the L* and b* axis were less than 0.2, indicating a very small degree of variation, but greater than 0.2 for the a* axis, indicating a small degree of variation (Kvålseth 2017). Low V_2 for repeated measures for the L* axis suggest that the sensor would be most effective for studies of animal brightness values (i.e. light to dark) rather than measurements of animal coloration. High variance between measurements in avian and lepidopteran specimens may be due to iridescence from the physical structures of wings and feathers (Vukusic et al. 2000; Meadows et al. 2011). Iridescent coloration can be difficult to consistently measure as small changes in feather, skin, or hair position between measurements can alter the results (Meadows et al. 2011). As the sensor requires physically connecting with a specimen to measure color, repeated measures on iridescent-colored animals may decrease measurement precision by altering an animal's feather, skin, or hair position.

Digital photography maintains several advantages over the Nix sensor. The sensor is limited to the visible light wavelengths (380-700nm) of the light spectrum. In contrast, a camera can be modified to extend into the ultraviolet portion of the spectrum. Many animals are capable of seeing ultraviolet light (Cuthill et al. 2017), and therefore a camera's ability to capture a broader light spectrum, along with adjusting the colors through a cone-catch model, make photographic measurements of animal coloration a better method for many study designs. Furthermore, the small aperture of the sensor makes it ineffective for measuring color across a large area or for detecting broad patterns like stripes or spots, which can be important adaptations (Caro 2005; Ancillotto and Mori 2017). The sensor reports an average color for the area within the aperture. For individuals with stark differences in colors on their body (e.g., stripes or spots) a measurement taken with multiple color patterns in the aperture would result in a nonsensical result. Photography may be preferred for animals with stark color patterns because a single digital photograph can capture a larger portion of an entire specimen from which multiple locations on the specimen can be sampled, increasing the overall amount of variation captured from a single photo. To capture multiple locations on a single specimen with a digital color sensor, the sensor needs to be placed on each location separately to record the color. Although these problems could potentially be avoided with an appropriate study

design, we recommend that studies on aposematic and other starkly patterned species use photography-based methods, such as those described by Bergman and Beehner (2008), Troscianko and Stevens (2015), and Pérez-Rodríguez et al. (2017).

Studies on animal color patterns can provide many key insights into evolutionary adaptations (Gerald 2001; Pryke et al. 2002; Stoner et al. 2003a,b; Singaravelan et al. 2013; Cuthill et al. 2017), and current animal coloration research is directly influencing aspects of human society, such as fashion and military technology (Caro et al. 2017). We suggest that for animal coloration researchers focused on human-vision specific questions, a commercially available digital color sensor is an alternative method for measuring color quickly, accurately, and without needing to collect specimens, and could open up additional avenues for color research.

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Table 1. Specimens from the Florida Museum of Natural History used for testing the Nix digital sensor's ability to measure animal coloration. Sample size and sex (F = Female, M = Male) are given for each species. For species where sex is not given, all specimens were male.

Specimen Type	Species	Samples
Avian		
	Cardinalis cardinalis	10 (5F, 5M)
	Chlorophanes spiza	3
	Icterus galbula	5
	Passerina caerulea	5
	Passerina ciris	5 (4F, 1M)
	Passerina cyanea	5
	Piranga rubra	5
	Quiscalus quiscula	5
Lepidoptera		
	Charaxes cithaeron	4*
	Danaus chrysippus	4*
	Graphium agamemnon	4*
	Hebemoia glaucippe	4*
	Parides eurimedes	4*
	Parthenos sylvia	4*
Mammalia		
	Sciurus niger	32 (12F, 20M)

* Sex unknown

Table 2. Range of color measurements of avian, lepidopteran, and mammalian museum specimens recorded using digital photography and the Nix Pro digital color sensor in Commission internationale de l'éclairage L* a* b* (CIELab) color space. Delta E (ΔE) is the mean pairwise difference in colors recorded using photographs and the sensor.

	Photos			Nix			
	L*	a*	b*	L*	a*	b*	ΔE
Avian	18.83 - 69.17	-9.90 - 61.74	-14.32 - 91.59	17.38 - 73.93	-27.98 - 58.14	-18.39 - 91.33	10.48 (SE = 0.67)
Lepidoptera	14.71 - 71.41	1.71 - 32.23	-10.76 - 45.33	10.00 - 75.41	-12.58 - 20.92	-8.77 - 47.23	13.92 (SE = 1.71)
Mammal	18.66 - 83.03	4.86 - 22.96	-2.40 - 34.86	20.22 - 79.25	-0.34 - 12.56	0.68 - 31.83	9.49 (SE = 0.29)
All Specimens	14.71 - 83.03	-9.90 - 61.74	-14.32 - 91.59	10.00 - 79.25	-27.98 - 58.14	-18.39 - 91.33	10.82 (SE = 0.49)

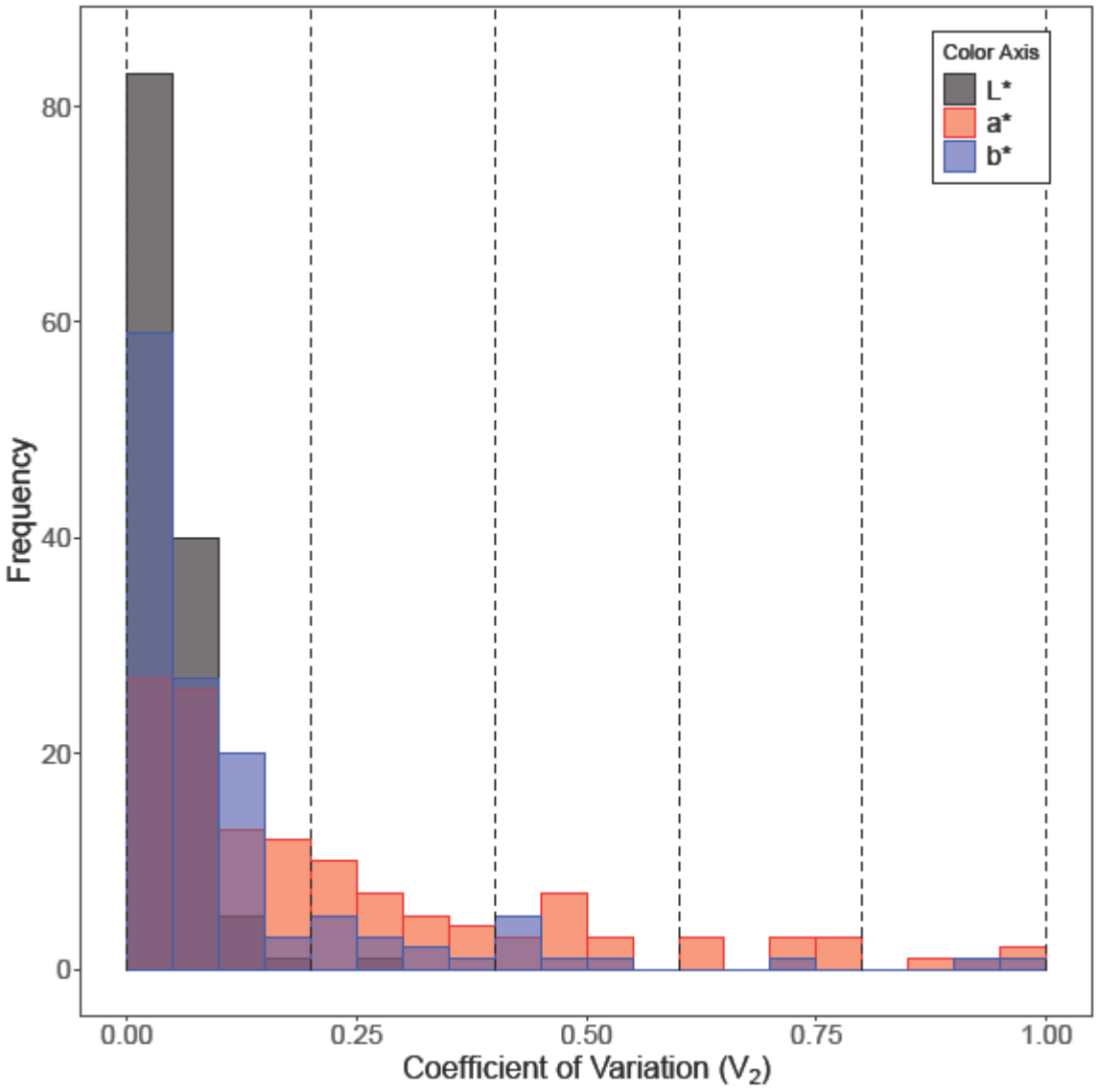

Figure 1. Distribution of second-order coefficient of variation (V_2) for 7 repeated color measurements of museum specimens using the Nix Pro digital color sensor. The vertical dashed lines correspond with Kvålseth's (2017) interpretations of V_2 spanning from very small (0.00-0.20) to very large (0.81 – 1.00) in 0.2 increments.

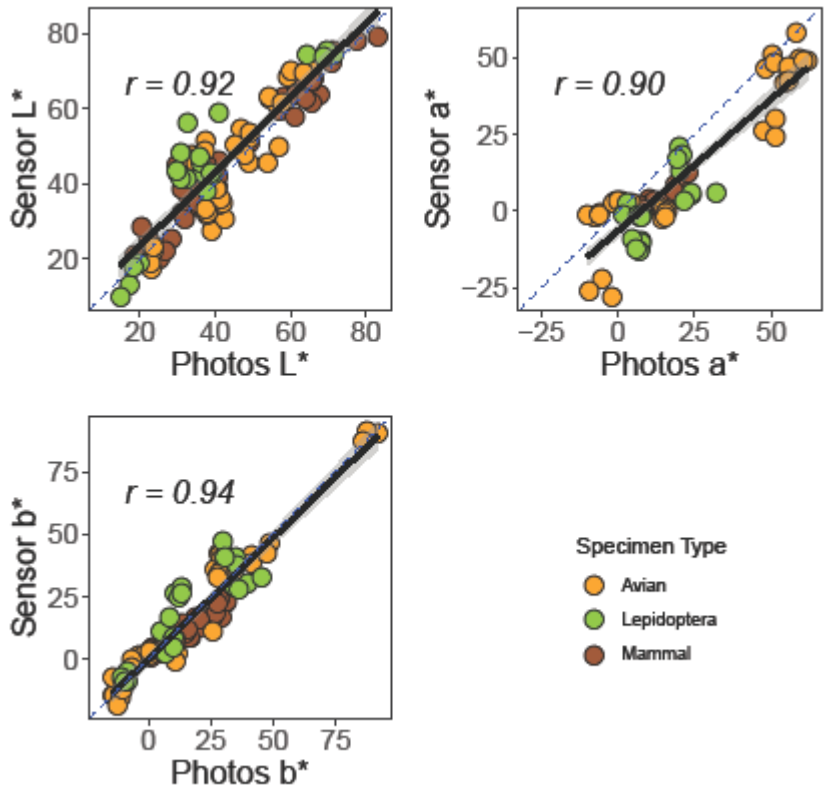

Figure 2. Correlations between measurements of specimen coloration using the Nix Pro digital color sensor and traditional photographic methods. The solid black line shows the linear relationship between the two methods and the shading around the line shows the 95% confidence interval. The blue dashed line shows the expected 1:1 relationship.

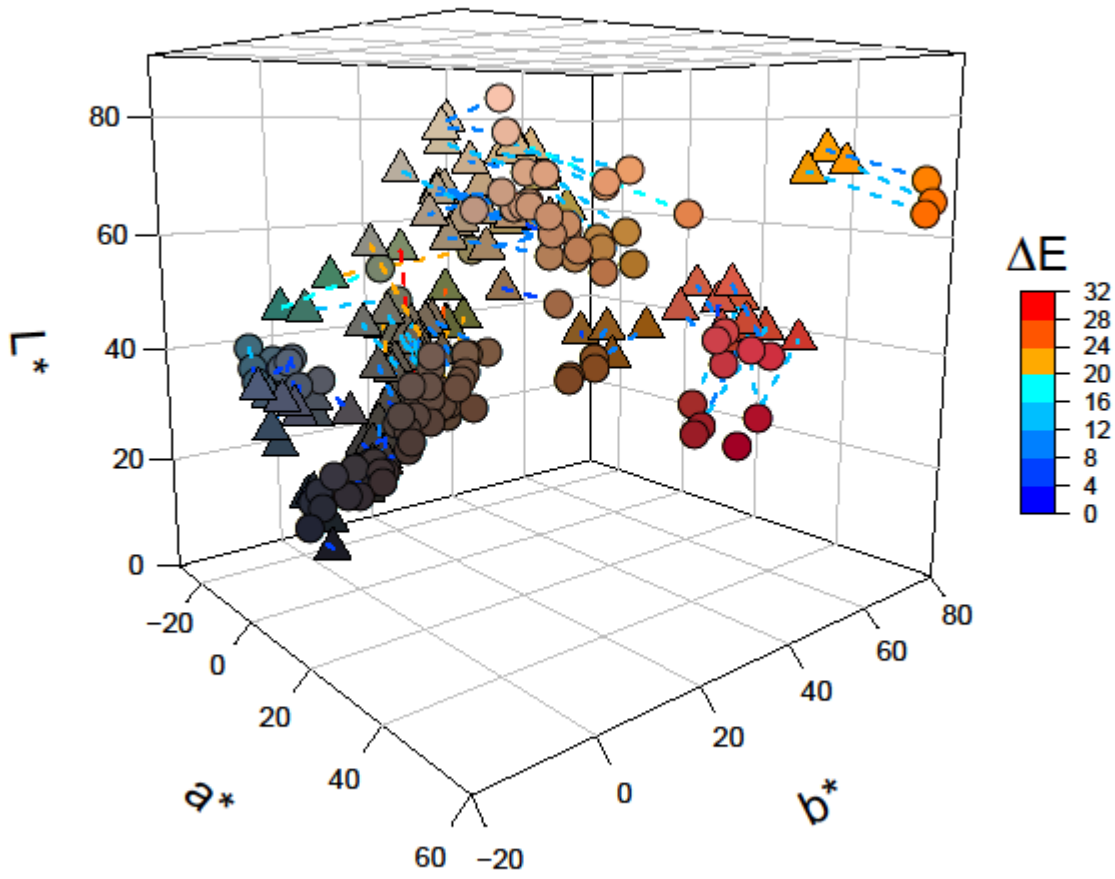

Figure 3. Pairwise comparisons of museum specimen color measurements recorded using the Nix Pro digital color sensor (triangles) and photography (circles). The fill color of each point corresponds with the actual color measured. Dashed lines between points show the pairwise ΔE, a measurement of difference between colors.