

# Color Evaluation for Research Purposes

Camila Sampaio and Pablo Atria

# 4.1 Introduction

According to published surveys, color education, color training, and shade matching programs are receiving increased attention over the last decade, according to published surveys [1]. This fact is directly related to patients' expectations and demands in achieving dental restorations that can mirror their adjacent natural teeth or improve their tooth esthetical characteristics with colored artificial restorations [2]. In this topic, color can be underrated, as patients do not often explicitly ask for the "color of their restorations." Instead, they expect functional, long-lasting, and esthetic restorations, with color being put as a background, unimportant or taken for granted, even though it could be the decisive factor in the overall acceptance of the treatment itself [3].

A few years from now, color was not such a trendy and studied topic, but today it is considered an ever-expanding field, equally relevant for clinical application, research, and education [4]. Due to advances in color measurement, digital instruments can promote a numerical measurement, allowing color to be metrically recorded

C. Sampaio (🖂)

College of Dentistry, University of the Andes, Santiago, Chile e-mail: CSampaio@miuandes.cl

#### P. Atria Biomedical Engineering, New York University, New York, NY, USA

and evaluated, which favored color matching, communication, reproduction, and verification in clinical and research dentistry. This approach was changed back in 1973 and 1974 when Sproul stated that "the technology of color is not a simple matter that can be learned without study; neither is it a complicated matter beyond dentists' comprehension" [5]. After that, a huge effort was put on new teaching methods using modern technology to help dental professionals and dental students to learn about color in an organized and comprehensive manner. Nowadays, scientific journals have editorials whose objective is specifically to study color and its influences on shade matching and color changes [2].

Color is defined as the result of the interaction of light with an object, which means that a given object's compositional characteristics greatly impact the way light is absorbed by or reflected from this object, as further explained in Chap. 1 [6]. The natural teeth are polychromatic, meaning many colors and optical characteristics can be perceived when observed under ideal light conditions [6]. Since enamel, dentin, and pulp present different composition, they also interact with light differently, resulting in an extremely complex interaction, which is difficult to be reproduced with artificial materials [6]. Differences between younger and older teeth exist regarding the color value, Chroma, and transparency. For instance, young teeth present a high color value due to the high quantity of enamel, while older

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**Fig. 4.1** Color and texture comparison of older (left) and younger (right) teeth. Observe the lower color value present on the older teeth, while a higher color value is observed in the younger teeth. Moreover, some regions of the older teeth can be observed as almost transparent due to the thickness of the substrate, while in the younger teeth, the original teeth textures and translucencies can be noted

teeth present a much lower color value than that which is seen in younger teeth, as it becomes thinner and more translucent over time, sometimes becoming nearly transparent (Fig. 4.1) [7].

# 4.2 Visual Methods to Evaluate Color

Color is defined as a combination of hue, Chroma, and value. Hue is defined as the name of color itself, for instance, red or yellow. Chroma is the degree of hue saturation, or the intensity exhibited by a color. Finally, value is defined as the brightness or luminosity of color [8]. Although no single method is considered standard, two methods exist to evaluate color in dentistry, which are visual and instrumental. Several kinds of research compare both methods revealing their pros and cons. One important finding of such research is that both methods should be used together whenever it is possible, as they can complement each other and lead to a predictable esthetic outcome [9].

The visual method is often considered subjective, as it can exhibit a higher level of inconsistencies for both intra-rater and inter-rater comparisons, while instrumental or digital methods can exhibit significantly higher reliability [10]; also, it is strongly dependent on the interaction between light and the dental structure. However, it is also the most common method used in dental practice and should not be underrated.

Visual methods consist of the observer visually selecting the best match for an object when this object is compared visually to tooth color examples such as shade guides. The shade guides most commonly used in the dental practice, as well as in research, are the VITA Classical shade guide and the VITA Toothguide 3D-Master System, both from VITA Zahnfabrik (Bad Säckingen, Germany) [1]. The Vitapan Classical shade guide configuration is also known as A-to-D arrangement, divided into 16 tabs originally arranged into four groups based on hue and within the groups according to increasing Chroma [11]. On the other hand, the Vitapan 3-D Master shade tabs are arranged three-dimensionally, divided into groups according to lightness, and within the groups according to saturation (vertically) and hue (horizontally) [12]. Six different levels of lightness were used to create six groups of tabs, from 0 (the lightest) to 5 (the darkest). There are 3 Chroma levels, from 1 (the least chromatic) to 3 (the most chromatic) in each group (except in group 1 that has two Chroma levels). Intermediate Chroma levels (1.5 and 2.5) in groups 2, 3, and 4 are associated with hue variations-L (less red) and R (more red). More recently, the same brand launched the Linearguide 3D-Master [11]. Other brands also have their A-D arrangements shade guides, which can correlate (although not 100% accurately) to the Vitapan Classical shade guide [13].

With this being said, when performing visual selection, the final color decision is totally operator-dependent. Studies demonstrated that the color preferences of observers and shade selection are dependent on: the level of experience of the operator, with education and training presenting a statistically significant improvement in shade matching [3, 14]; the operator's gender, with some studies showing that females are better in color match than males [15, 16], while other indicates that this is not a decisive factor [15, 17];

on the type of color, with darker and more chromatic shade tabs being more often mismatched [3, 17]; on skin shades and gingival shades, among others [18, 19].

Increased shade matching accuracy can be translated into enhanced dental restorations esthetics, increased patient satisfaction, and reduced color corrections [3]. For that purpose, manufacturers are launching restorative materials with visual color adjustment potential to blend with the surrounding enamel and dentin, resulting in reduced color differences and, therefore, improving the restoration's esthetic appearance, simplifying the shade matching compensating for any color mismatch [9, 20].

When performing visual shade matching only, the only color information provided to the technician by many clinicians is the laboratory prescription. However, a method to improve shade matching with this technique is by performing a color map (Fig. 4.2), which can be simply drawn in the lab prescription or sent in a photograph. A simple photograph taken with a smartphone can be manipulated and improves shade matching and decreasing color difference values ( $\Delta E$ ) of the comparison between the restoration performed by the technician and the tooth color of comparison, as observed in a recent study [21]. This subject will be discussed below and can help in achieving a more accurate shade selection result.

# 4.2.1 Color Blindness Test or Ishihara Test

Color blindness, color deficiency, or color vision alteration occur when the correct determination of the color of an object is altered. It is presented in about 8% of the male population and 0.5% of the female population [22]. This alteration affects the ability to identify the red, green, yellow, or blue colors in charge of cells located in the retina called cones. On the other hand, detecting changes in value or luminosity is not influenced by color deficiencies. It depends on retinal circuits other than photoreceptor cells [22]. When testing for color blinding, graphic designs and illustrations can be created and used, with tests consisting of numbered colored plates, mostly known as Ishihara plates containing a circle of dots appearing randomized in color and size (Fig. 4.3).

In research, except for the studies which have as main objective evaluating people with color deficiencies [22], studies on color training typically exclude those individuals [17–19, 23, 24]. However, many times color deficiencies are an



Fig. 4.2 Example of Ishihara vision testing chart



**Fig. 4.3** Color mapping of teeth, important information can be reported to lab technicians with color maps, this can be done drawn in a lab prescription or in photographic means, through digital cameras or smartphones, which allow color manipulation by both dentist and technician

underdiagnosed disease, but very relevant in people who study Dentistry.

Participants for research purposes are usually screened or tested for their color vision, participating in an evaluation of perceptibility and visual acceptability judgments in Dentistry [24], and need to pass a dental color matching competency, according to the ISO/TR 28642:2016 [25]. For example, participants need to match at least 60% of the pairs of tabs from Vita Classical shade guide (below 60%-no competency). Minimum of 60% (10 pairs of tabs), 75% (12 pairs), and 85% (14 pairs) corresponded to poor, average, and superior color matching competency, respectively [20]. A previous study concluded that observers with superior color matching competence could achieve better color matching results than those with the average color discrimination competence [17].

It is important to point out that the human eye is more sensitive to changes in luminosity and less sensitive to changes in tone. Every person should perceive changes in the value before perceiving changes in the hue Chroma of a tooth [26]. Value is a fundamental aspect that must be established successfully to correct color in esthetic restorations [26, 27].

# 4.2.2 Color Perception × Color Acceptance Thresholds

A visual threshold consists of perceiving a difference in color and whether this difference is visually acceptable or not, or in other words, which are recognizable and tolerable. Color thresholds are important not only in the field of Dentistry, but also serve as quality control for a number of applications [4]. It also helps evaluating the clinical performance of materials, both in the clinical practice and in vivo and in vitro research, evaluating and interpreting a clinical outcome, and standardization. The importance of quality control in dentistry is reinforced by increased patients' and dental professionals' esthetic demands [4].

When performing color thresholds, the color perception threshold question is: "Can you see a difference in color between these two specimens?" If the observer answered "yes" to this question, they were asked the acceptability threshold question: "Would you consider this difference to be color acceptable in a patient's mouth?" The psychometric function is then simply the percentage of "yes" responses as a function of specimen color difference; 50% "yes" responses were considered the threshold level [24]. Basically, this can be resumed as: when the color difference between compared objects can be seen by 50% of observers (the other 50% will notice no difference), we are talking about the 50:50 perceptibility threshold. When the color difference is considered acceptable by 50% of observers (the other 50% would consider it unacceptable), this corresponds to the 50:50 acceptability threshold. A color match in dentistry is a color difference at or below the former threshold; an acceptable color match is a color difference at or below the later one [28, 29].

Paravina et al. investigated the relationship between "50:50% perceptibility thresholds" and

"50:50% acceptability thresholds." They defined the terms "50:50% perceptibility threshold" or "50:50% acceptability threshold" and referred to values where 50% of the observers perceive, or still accept, respectively, a color difference. They found differences in shade selection of 50:50% perceptibility ( $\Delta E_{ab} = 1.2$ ,  $\Delta E_{00} = 0.8$ ) and 50:50% acceptability ( $\Delta E_{ab} = 2.7$ ,  $\Delta E_{00} = 1.8$ ) were significantly different [4]. These results were later implemented in the ISO 28642:2016 [25].

Although obviously, color matches at or below the perceptible threshold would be ideal, achieving a non-perceivable match is costly, timeconsuming, and frequently not essential on a clinical basis [4]. The color difference formulas are important to allow a better correlation between visual judgments (perceptibility and acceptability) and instrumental color difference values [30].

# 4.3 Digital Methods to Evaluate Color

Instrumental methods have been used to improve color selection and communication and have been reported to be more reliable than the visual method used by itself. They became very popular in dental research due to the development of new technologies that are user-friendly and offer objective information on color specification, as well as magnitude and direction of color differences [31]. Dental shade matching instruments can overcome or reduce imperfections and inconsistencies of traditional shade matching with shade guides [28]. However, whenever possible, visual and instrumental methods should be combined [9], as it should not be forgotten that patient's visual judgment on color match or mismatch is usually the final and decisive one [31]. Advances in color measuring devices may remove a certain degree the subjectivity of the color determination and enhance the reliability of shade matching and shade communication [10]. Also, reliability is significantly better with the instrumental shade matching method than the visual method [10].

#### 4.3.1 CIE-Lab and CIE-LCh

The CIE (Commission Internationale de l'Eclairage) has been traditionally involved in colorimetry for dental materials. It has been responsible for introducing the main color systems, illumination patterns, and color difference  $(\Delta E)$  concepts used in color science [32]. In order to unify criteria about color and to simplify communication between professionals, the CIE developed the CIE-Lab system. With this system, all colors can be easily captured and described. The CIE-Lab color space system (Fig. 4.4) consists of describing the colors within the Cartesian coordinates as L,  $a^*$ , and  $b^*$ , where  $L^*$  is the lightness coordinate (brightness or value), which connects an imaginary south pole (0-black) with an imaginary north pole (100-white), and all gray values are represented in this vertical line. On the sphere's equator, all other saturated colors are shown, corresponding to the Chroma of the colors. The right angles in the equator plane, the  $a^*$ - and  $b^*$ -axis, that represent the directions of the color valences red-green (positive-negative) and yellow-blue (positive-negative), respectively. Each color has a specific numerical value, thus providing a more objective characterization and assisting in color communication [27].

In research, most of the studies quantify color differences using the CIE-Lab color space and associating with the  $\Delta E$ , which is related to a color difference formula.



Fig. 4.4 CIE-Lab color space

When working with the CIE-LCh system and for more clearness, the Cartesian coordinates (L,  $a^*$ ,  $b^*$ ) can be converted into cylinder coordinates L, C,  $h^{\circ}$  according to the following formulas:

$$C = \sqrt{a^2 + b^2}$$
$$h^{\circ} = \arctan\left(\frac{b}{a}\right),$$

where *L* remains unchanged, *C* (Chroma) represents the distance from the polar axis and represents the color intensity; and  $h^{\circ}$  is the hue angle in the equatorial plane (Fig. 4.5). Hue angle starts at the  $+a^*$  axis and is expressed in degrees (e.g.,  $0^{\circ}$  is  $+a^*$ , or red, and  $90^{\circ}$  is +b, or yellow). It has the same diagram as the  $L^*a^*b^*$  color space but uses cylindrical coordinates instead of rectangular coordinates [23].

# 4.3.2 Color Difference Formulas $(\Delta E)$

Color difference formulas provide a quantitative representation of the perceived color difference  $(\Delta E)$  between a pair of colored samples under a given set of experimental conditions [29]. Color



Fig. 4.5 CIE-LCh color space

difference has been used extensively in dental research and applications [30]. In the majority of dental color studies, color and color differences are quantified using the CIE-Lab color space and the associated  $\Delta E_{ab}$ .

CIE76

Based on the *L*  $a^*b^*$  values of each color, color determination differences can be measured and evaluated. The *L*  $a^*b^*$ —or CIE-Lab color space (CIE of 1976) was the first formula to measure color difference ( $\Delta E$ ) according to the CIE-Lab coordinates. It is the most commonly used in publications and color differences ( $\Delta E_{ab}$ ) and is calculated according to the Euclidean difference formula [23]:

$$\begin{split} \Delta E_{ab} &= \sqrt{\left(L1 - L2\right)^2 + \left(a1 - a2\right)^2 + \left(b1 - b2\right)^2} \\ \Delta E_{ab} &= \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}. \end{split}$$

CIE94

The CIE94 formula is defined in the  $L^*C^*h^*$  color space, showing the color differences in lightness (or value), Chroma and hue calculated from the Lab coordinates:

$$\Delta E_{*_{94}} = \sqrt{\left(\frac{\Delta L_{*}}{K_{L}S_{L}}\right)^{2} + \left(\frac{\Delta C_{ab}^{*}}{K_{C}S_{C}}\right)^{2} + \left(\frac{\Delta H_{ab}^{*}}{K_{H}S_{H}}\right)^{2}}$$
$$\Delta L_{*} = L_{1}^{*} - L_{2}^{*}$$
$$C_{1}^{*} = \sqrt{a^{*} \frac{2}{1} + b^{*} \frac{2}{1}}.$$

#### - CIEDE2000

Different color difference formulas were launched aiming to improve the correction between computed and perceived color differences and reflect the individual subjective impression of a color difference. Nowadays, the CIEDE2000 ( $\Delta E_{00}$ ) is the most accepted and CIE recommended color difference formula in dentistry, providing a better fit than the previous formulas, and therefore replacing them [30]. The CIEDE2000 utilizes the concepts of Chroma and hue, reinforcing the

importance of the conceptual developments of Munsell [32]:

$$\Delta E_{*_{100}} = \sqrt{\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T \frac{\Delta C'}{K_C S_C} \frac{\Delta H'}{K_H S_H}}$$

It incorporates specific corrections for nonuniformity of CIE-Lab color space (the weighting functions:  $S_L$ ,  $S_C$ ,  $S_H$ ), a rotation term ( $R_T$ ) that accounts for the interaction between Chroma and hue differences in the blue region and a modification of the  $a^*$  coordinate of CIE-Lab, that mainly affects colors with low Chroma (neutral colors) and parameters accounting for the influence of illuminating and vision conditions in color difference evaluation (the parametric factors:  $K_L$ ,  $K_C$ ,  $K_{H}$ ). The parametric factor ratio was proposed as a way to control changes in the magnitude of tolerance judgments and as a way to adjust for scaling of acceptability rather than perceptibility. Studies on visual judgments performed in textile color acceptability and dental ceramics acceptability showed that using  $K_L = 2$  resulted in color differences better correlated to observations from a subset of average observers.

When compared, changes in color calculated with the CIE-LAB and the CIEDE2000 formulas followed similar trends but with different absolute values when calculated [33].

### 4.3.3 Spectrophotometers and Colorimeters

Spectrophotometers and colorimeters are among the most used instrumental shade matching methods in Dentistry and can be used to help overcome some shortcomings of a visual method by bringing accuracy and helping with shade matching, communication, and reproduction. Moreover, color measuring instruments can be valuable in shade verification (quality control) [19].

Spectrophotometers are among the most accurate, useful, and flexible instruments for overall color matching and color matching in dentistry [34]. They can measure the amount of light energy reflected from an object at 1–25 nm inter-

vals along the visible spectrum [35, 36]. A spectrophotometer contains a source of optical radiation, a means of dispersing light, an optical system for measuring, a detector, and converting light obtained to a signal that can be analyzed. After that, the data obtained from spectrophotometers must be manipulated and translated into a form useful for dental professionals [28]. The instruments' measurements are frequently keyed to dental shade guides and converted to a shade tab equivalent [37]. Compared with observations by the human eye or conventional techniques, it was found that spectrophotometers offered a 33% increase in accuracy and a more objective match in 93.3% of the cases [38].

The most used and known spectrophotometer available in the market, used for both research and clinical aims, is the Vita Easyshade (Vita Zahnfabrik) (Fig. 4.6). It is a contact-type spectrophotometer that provides enough shade information to help aid in the color analysis process. Different measurement modes are possible with this instrument: tooth single-mode, tooth area mode (cervical, middle and incisal shades), restoration color verification (includes lightness, Chroma, and hue comparison), and shade tab mode (practice/training mode) [39].



Fig. 4.6 Clinical use of the VITA Easyshade V spectrophotometer

Colorimeters measure tristimulus values and filter light in red, green, and blue areas of the visible spectrum. Although they have shown good measurement repeatability, they are subject to systematic errors due to the edge-loss effect related to sample surface, while spectrophotometers precisely measure color from reflectance or transmittance data [19, 40]. Colorimeters do not register spectral reflectance and can be less accurate than spectrophotometers (aging of the filters

can additionally affect accuracy) [28, 41].

When analyzing the pros and cons of instrumental and visual methods, one can mention that instrumental methods such as spectrophotometers and colorimeters can help standardize color shade matching. On the other hand, they require specific and expensive technology, not always available to the clinician. On the other hand, a shade guide used to perform a visual shade matching method is always available in dental offices. However, this method is more subjective and operator dependent. A recent study showed that the spectrophotometer can assist with visual shade matching but cannot replace it [42]. Ideally, the combination of an equipment and visual shade matching should be used, and the help of a digital camera can promote successful results on shade matching [9, 27].

### 4.3.4 Digital Photography

Digital photographs can capture a detailed image of the tooth, be easily stored and transmitted to a technician, providing good quality information regarding color across the tooth surface, shape, and characteristic features. As will be further explored in Chap. 5, digital cameras have been increasingly used in dental offices to document the pre-operative situation, final results, and long-term outcomes [9, 27, 43]. A digital instrument capable of taking photographs, being it either a digital camera or a smartphone, is now available in all dental offices; thus, it is a technology that can be widely used.

Problematically, digital photographs of the natural dentition typically show significant color alterations of teeth and soft tissue when certain parameters are wrongly manipulated or when diffusers that filter the illuminant are used [27], for instance, changes in lighting conditions can result in changes in perceived color, and an incorrect digital camera exposure and automatic white balance can be reflected into an erroneously added cyan to the image to neutralize the high proportion of red tones from the gingiva while adding a tinge of blue to offset the yellow tones from the teeth [44]. For example, in soft tissues, a correct white balance can distinguish healthy from inflamed tissues. For hard tissues, correct color rendition reveals features such as enamel translucency, caries, erosion and abrasion, and cervical dentin exposure [45].

Flash photography also present differences within different types of flashes, even when used together with the same digital camera [27]. It was demonstrated that the combination of a digital camera with cross-polarization is the most standardized colored type of photography, although when performing the white balance of photographs using a gray reference card with known color values, a wireless close-up Speedlight flash showed to promote as standardized colored photography as with the use of a filter. The same was not observed for ring flashes, which tends to darken the images if they are not white balanced [27]. A recent study demonstrated that the problem caused by different diffusers in digital cameras could be compensated by using a gray reference card with known color coordinates to white balance the photographs [27], and although it did improve standardization of a digital camera when using a ring flash, it did not improve photographs made with a smartphone.

Smartphones are commonly available instruments, which have self-contained central processing unit (CPU) computing capability, enriched functionalities, software applications, wireless connectivity, and can present highresolution photographic technology [46]. A recent publication showed that with novel appropriate light-correction filters, shade selection with the smartphone and the digital camera were comparable, showing to be reliable for shade selection, with  $\Delta E$  values below the acceptable threshold [9].



**Fig. 4.7** Use of the Adobe Photoshop software to describe color values without (upper image) and with (lower image) the use of a cross-polarizing filter. Observe that total CIE-Lab values greatly vary between the photos

Thus, when using digital photograph, it is important to white balance the photographs using a gray reference card with known color values, which can be done using a software (for example, Lightroom v6.0, Adobe Photoshop CC; Adobe Systems Inc), or combining the use of a conventional photograph with one using a cross-polarizing or a light-correction filter. This technique is further explored in Chap. 5. The use of this software can also describe the color values in different color spaces, such as the CIE-Lab color space (Fig. 4.7). Moreover, both digital camera and smartphone photographs can be easily manipulated to observe tooth color and textures (Fig. 4.8).



**Fig. 4.8** Digital photographs used for digital shade selection taken with a digital camera, after manipulation with a digital software in different contrasts. First row: photograph with teeth and substrate shade guides; second row,

black and white; third row, saturated. Manipulating the photographs helps on observing different structures of the teeth, as well as the luminosity

# 4.4 Experimental Designs to Evaluate Color

# 4.4.1 Clinical Trials

When dealing with clinical trials, it is extremely important to only start the research after obtaining an approved consent from the participants and approval of an Ethical Committee.

When performing clinical trials where shade matching needs to be obtained, observers need to be tested and calibrated for color matching competence, irrespective of their experience and demonstrate superior color discrimination competence according to ISO/TR 28642:2016 [25]. It is important that operators perform blind evaluations, and it is paramount to standardize illumination and background appropriately, as will be mentioned below. Also, in the visual analysis of color differences, a ranking can be used to compare, for instance, the color between a tooth and restoration, which can be graded. For example: from 0 to 4, using the scale based on a previous study where level "0" means excellent match; 1, very good match; 2, not so good match (border zone mismatch); 3, obvious mismatch; and 4, huge (pronounced) mismatch [20, 21].

An important aspect when dealing with natural teeth is dehydration. Tooth dehydration makes teeth appear whiter due to increasing enamel opacity. The interprism spaces become filled with air instead of water, so light can no longer scatter from crystal to crystal. Therefore, loss of translucency due to dehydration causes more reflection, which masks the underlying color of dentin, making the tooth appear lighter. Also, it has a negative impact on shade selection, which can affect the final esthetic outcome [47]. Shade selection procedures should be carried out within the first minute of the procedure and before teeth dehydrate [47]. Although it is frequent to observe dentists waiting for the tooth to rehydrate for shade taking, a recent study observed that teeth do not rehydrate within 15 min after rehydration; thus, it is important that shade selection is carried out in controlled circumstances before the tooth dehydrates [47].

# 4.4.2 In Vitro Studies

The same as mentioned previously in the clinical studies, when dealing with in vitro studies that use human tissues, first of all, it is extremely important to only start the research after obtaining the approval of an Ethical Committee. An extremely important topic is to always standardize illumination and background when evaluating the in vitro samples, which will be discussed below.

When compared to clinical studies, in vitro studies have the advantage of simulating specific procedures in an aged situation. For instance, the color stability of different materials can be assessed without actually needing to wait years until the material deteriorates. Instead, thermocycling procedures can be used to assess the color stability after simulated aging. Such procedures can be performed conventionally, in water, or even in different types of beverages. While if the same study was performed in mouth, it would take a long period of time to deteriorate, when aged in vitro, such results can be obtained within days [48, 49]. Another method of accelerated aging is using ultraviolet light. Studies have shown a yellowing effect after this type of aging due to a large positive change in  $b^*$  [33]. For all these types of studies, both visual and digital methods can be used, and color values, as well as color differences, can be obtained with the previously mentioned color difference ( $\Delta E$ ) formulas, as well as instrumental devices.

Different software can be used for the evaluation of colors in dentistry in vitro. Among all, the most commonly used is Adobe Photoshop, which can describe color values in different color scales, such as the CIE-Lab scale. Another interesting and easy access software used in recent research projects is the Classic Color Meter, which describes color values in the CIE-Lab scale [9, 27].

#### 4.4.3 Illumination

For both in vitro and in vivo research, color evaluation and shade matching should be done using standardized color temperature illumination. The specific color temperatures range within 5000 (D50)–7500 K (D75) and are sought because of their universal nature and a broad spectrum of wavelength [48]. Nevertheless, such conditions are rare to be found since daylight is always changing and can range from 1000 to 20,000 K, making it difficult to rely only on natural daylight to provide the "ideal" color temperature during shade selection for a restoration [14].

For simulation of the ideal illumination for research, different apparatuses can be used. For instance, viewing booths and color boxes can be used for standardizing lightning and analyzing shade colors [14], handheld lights and provide significant improvements on shade matching [14], cross-polarizing filters (Fig. 4.9) used together with digital cameras can also provide more color-standardized photographs [27], a light-correcting device used with a smartphone also showed to be reliable for shade selection, with DE values below the acceptable threshold [9].

# 4.5 Background

The background is defined as the surface upon which samples are placed along with the environment [25]. The neutral light gray background has been recommended in the literature as the most appropriate for background and/or surrounding area in dentistry [27]. Today, white balance reference cards with known color coordinates are available in the market and can be used together with dental photography backgrounds. They have been recommended for improving standardization and accuracy in recording, communicating, and manipulating color images in dental digital photography [9, 27]. Besides a gray card itself, photographic contrastors are also available for both research and clinical basis; they can be found in different colors, such as black, simulating the dark background of the mouth, and gray,

for a neutral background and white balance promotion (Fig. 4.10) [49].

In dental practice, the background is represented most often by the darkness of the oral cav-



**Fig. 4.9** Upper photograph taken without a crosspolarizing a filter, lower photograph taken with a crosspolarizing filter. Observe the absence of specular reflections in the photograph with filter, which allows a more straightforward and precise observation of natural teeth internal structures ity; nevertheless, there are situations when the shade selection is performed against other artifacts [17]. A recent study selected different backgrounds/surrounding area to simulate the different clinical situations, such as gray (considered as neutral and most frequently recommended), white (simulating color of opposite teeth), black (simulating color selection against a black contraster), red (imitating the lips and oral mucosa), and blue (simulating the rubber dam). Color matching results recorded against the blue background were statistically worse than to other backgrounds, while white and black generated the best results [17].

# 4.5.1 Sample Size and Statistics Analyses

Statistical analyses are paramount in both clinical trials and in vitro studies. There is a necessity to organize and record the information obtained in the form of numbers from each sample. The value that is taken by the variable is called data, which can be classified into continuous data (Quantitative) or categorical data (Qualitative) (Figs. 4.11 and 4.12) [50].

When talking about color evaluation for research purposes, we will mainly work with continuous variables, where color values using mathematical formulas described in this chapter are made on a scale. This kind of data will be presented in terms of mean  $\pm$  standard deviation. The recommended way to represent the final out-



Fig. 4.10 Tooth color selection performed against a black background, gray background, and with a cross-polarizing filter



Fig. 4.11 Some of the most used statistical tests for continuous data



Fig. 4.12 Some of the most used statistical tests for categorical data

comes of any given test is in the form of tables and graphs.

The distribution of the data does not have a relation with the quality of the obtained data itself [50]. Distribution is just the pattern of values obtained [51]. Both empirical and theoretical distributions can be found; the most common theoretical distribution is the normal distribution, which is just a name and does not imply normality. For a normally distributed dataset, the majority of the sample's values or observations (95%) will be in the center of the distribution. Recommended tests to evaluate the normality of the data are the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Usually, the Shapiro-Wilk test will be the appropriate method for small (<50 sample sizes samples), while the Kolmogorov-Smirnov test will be used for larger sample sizes ( $\geq$ 50 samples). In both cases, the null hypothesis is that the data falls within a normally distributed population when *p* > 0.05. The null hypothesis is accepted; therefore, the data are called normally distributed [52].

It is key in statistics that all the assumptions of the desired test to be performed are fulfilled. Normality is assumed in correlation, regression, *t*-test, and analysis of variance. Whenever there is a Normally distributed dataset, a parametric test can be used; if this condition is not met, then the use of a non-parametric test is required. Nonparametric tests include Wilcoxon signed-rank test, the Mann–Whitney *U* test, and the Kruskal– Wallis test.

Among the most commonly used tests, there is the Bonferroni test, which is intended to use for a small number of comparisons (<5), however, when dealing with larger than five comparisons, the preferred test is the Tukey test [50]. When the sample sizes and population variances are different between groups, the Games–Howell and Dunnett's tests give accurate results [50].

Other recommended tests that authors have used in studies involving clinical measurements are repeated measurements tests, where timeinvariant unobservable differences between individuals can be controlled. This allows the researcher to estimate the variations within individuals. Here is where linear models come into play to fit the data, assuming that the distribution is Normal. Other linear models involve linear mixed models which are used when there is nonindependence in the dataset. On the other side, when a non-parametric test is required, the option would be to use generalized estimating equations (GEE) which includes subject-specific random effects.

This method also assumes that the values are correlated so that cases are not independent; for example, when performing multiple color measurements over time, compare different treatments or different color measuring methods. Figures 4.11 and 4.12 summarizes some of the most used statistical tests.

For statistical analysis, the preferred software to use due to its interface is SPSS (IBM). Hence, it is always advised to have a prior consultation with a statistician to match the data, analysis, and conclusions.

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