



A Comprehensive Assessment of Soft Tissue Esthetics

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Comprehensive Assessment of Soft Tissue Esthetics

A Thesis Presented by

Peter Charles Grieco

To The Faculty of Medicine

In partial fulfillment of the requirements for the degree of

Doctor of Medical Science

Research Mentor: Shigemi Nagai, DDS, PhD, MSD

Harvard School of Dental Medicine

Boston, Massachusetts

23 March 2015

We, the undersigned, have read and approved the thesis of Peter C. Grieco, submitted in partial fulfillment of requirements for the degree of a Doctorate of Medical Sciences at Harvard School of Dental Medicine.

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23 March 2015

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Dedication

This thesis is dedicated to my family, friends, co-residents, and faculty, who have supported and encouraged me through my journey in dentistry to this stage.

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Abstract

This thesis investigated the efficacy of two techniques, digital spectrophotometry and narrow-band endoscopy, in observing and describing gingival tissue in a patient population. The efficacy of existing gingival restorative materials in mimicking the optical qualities of the participant pool was also investigated using spectrophotometric analysis.

The spectrophotometric analysis examined the ability of a digital, non-contact, full-tooth spectrophotometer (Crystaleye; Olympus, Japan) to evaluate objectively gingival color, thickness, and translucency in 100 participants drawn from the patient population of a dental school. Color metrics of gingival soft tissue were obtained from all teeth in the maxillary sextant in the participant population and stratified with respect to tooth location, type, and patient sex and ethnicity. It was concluded that:

- In the anterior maxillary gingiva, the luminance (brightness) of the periodontal gingival tissues decreased, whereas the chromacity (color saturation) increased, as the site progressed more posteriorly in the mouth.
- Gingival color varied significantly with patient sex, with males exhibiting lower value (darker/grayer) gingiva of a more purple hue.
- Gingival color varied significantly with patient ethnicity, with White participants exhibiting brighter, yet less chromatic gingiva than Black, Asian, and Hispanic participants.
- Gingival thickness measured via cast analysis was found to be 13% thicker in the male population.
- Gingival translucency was observed to be correlated linearly to gingival thickness calculated using two existing metrics and one novel metric. Female participants were found to have significantly more translucent gingiva than male participants.

A spectrophotometric coverage error analysis investigating the efficacy with which 5 different brands of gingival acrylics replicated the optical properties of the participant

population was performed. It was determined that significant differences existed in the coverage errors of different materials, with some of the shade guide sets exhibiting average best-matches outside of the range of clinical acceptability. It was observed that the coverage errors of the shade guide sets were not determined by the number of tabs in the set, but rather by the fitness of a few isolated tabs within the participant population. In a proof-of-concept analysis, a two-tab shade guide set was conceived that was optimized for the sample participant population that outperformed all other sets in coverage errors. This may have implications in the development of future spectrophotometrically guided shade sets optimized for patient populations.

Lastly, using a clinical endoscope (CV-190; Olympus, Tokyo, Japan) equipped with narrow-band imaging capabilities, the microvasculature of the marginal gingiva in the esthetic zone was observed and categorized. The analysis focused on the complexity of the intrapapillary capillary loop (IPCL), a diagnostic indicator of mucosal inflammation. No significant correlations were determined between the classifications of the observed IPCLs and the spectral data obtained, suggesting that within the limitations of this study, endoscopic imaging may be limited in quantitative or diagnostic applications, although it is useful for subjective visualization of the IPCLs.

Chapter 1: Introduction and Review of the Literature

I. Color Science

a) Physical Properties and the Physiology of Color Perception

Color is a phenomenon defined as the perception by a subject of a particular combination of wavelengths of light, either emitted by a light source, transmitted through space, or reflected off of an object.^{1,2} In the third category, reflected light is comprised of all wavelengths of the incident light that are not absorbed by the illuminated object. In humans, the perception of color is caused by the ability of different wavelengths of light to excite any of the three color-perceiving photoreceptor cells, referred to as cones, which reside in the retina of the eye. (Figure 1) The excitations of each of these three cell types, corresponding to the wavelengths of red, green, and blue light respectively, are transmitted to and interpreted by the brain as the color of the visual field.²

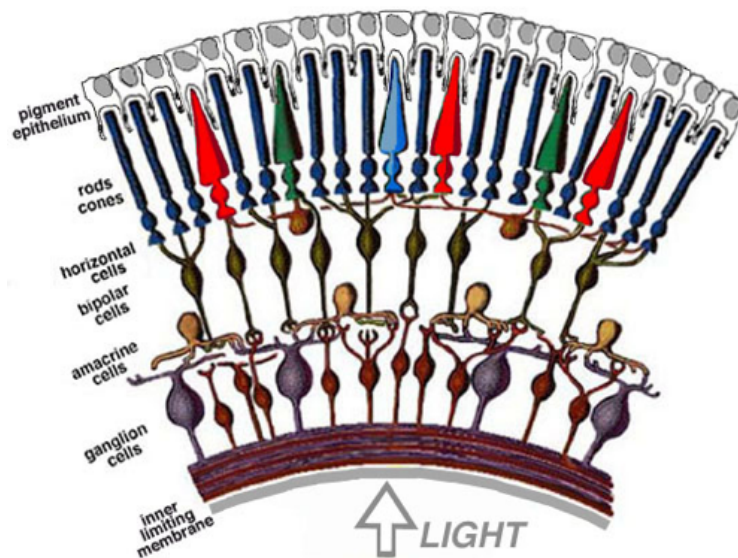


Figure 1: Diagram of Photoreceptor Cells in Human Eye³

The interpretation of color by humans is largely based on the perspective of the individual perceiving the color, varying even within a single individual due to variables such as the ambient lighting conditions, background effects, eye fatigue, age, and other physiologic factors.¹ Metamerism, a specific optical phenomenon where two objects can appear similarly colored when illuminated by one light source, and differently under another, complicates the shade process of matching one color to another and shows the importance of control of background lighting.² Within a population, the complexities increase further, with variability due to the normal variation in color perception in individuals, as well as due to abnormal conditions such as color blindness, a relatively common genetic disorder that results in a limited color spectrum in an individual due to a deficiency or complete loss of one set of cones, or conversely and much more rarely, tetrachromacy, where individuals possess a fourth type of cone, increasing their color perception by hundreds of millions of colors.^{4,5} Further, differences have been noted in accuracy of color perception between sexes, with women having a better perception overall than men.⁶ Even in the absence of these biologic considerations, each individual will have a different interpretation of color based on his or her own experiences and resulting color references.² Adding further complexity, individuals verbally describe and qualify an object's color differently, making accurate color communication difficult.^{1,2}

b) Color Measurement Techniques

This difficulty in objectively perceiving and communicating color has led to the innovation of a variety of devices aimed at obtaining objective color information. Color measurement devices, known as colorimeters and spectrophotometers, are currently used to obtain objective color data in numerous applications. Colorimeters are instruments constructed to mimic the tristimulus nature of the human eye. Data are collected via a visual sensor or sensors and are then simply processed and output as tristimulus values. Colorimeters are mainly used for industrial applications and quality inspection, as they are

useful for routine comparison of colors under constant conditions.^{2,7,8} Spectrophotometers, conversely, are much more precise instruments that output a greater amount of information. Spectrophotometers consist of a visual sensor, similar to colorimeters, but require a more powerful data processor; implement uniform lighting via a prism, grate, or filter; standardize object distance as well as incident light angle; and block out ambient light. Their output is also much more complex, providing a wavelength-by-wavelength spectral analysis of the reflecting and/or transmitting properties of the studied object. This data format provides information on the color formulation and allows for much more nuanced interpretation. Conversion to the standard colorimetric format is often performed, as well as comparisons to known values and calculation of spectral difference measurements.^{1,2,7,8}

Both colorimeters and spectrophotometers are available in two formats: a contact point/spot format, in which the device measures a small (generally $\sim 3\text{mm}^2$) area of surface, and a non-contact format, where the sensor is lifted off the surface being examined. The contact format is useful in industrial and quality control purposes as it allows for testing of small areas of larger, monochromatic objects or surfaces. In addition, by having its surface in contact with the examined surface, it minimizes the influence of outside light sources. The non-contact format allows for the formation of a digital mapping of the entire surface being examined, and is useful in instances where making physical contact with the object being examined is not possible or desired.^{2,9}

c) Color Spaces

As the utilization of color measurement devices has extended to different applications and industries, several color systems, called *color spaces*, have been developed and implemented in order to numerically quantify and communicate the optic qualities of the objects being examined. The two most common color spaces implemented today are the XYZ and L*a*b* color spaces.²

The XYZ (RGB, Red/Green/Blue) color space, devised in 1931 by the Commission Internationale de l'Eclairage (CIE), an international organization concerned with the study and analysis of color, is based upon the tristimulus nature of the human eye, and the concept that all colors are seen as a mixture of these three colors. By implementing a sensor for each of these three colors and combining and interpreting the results, a color profile and identification of the color being analyzed can be formulated. Further modifications to the system were subsequently made to allow a simplified two-coordinate graphical representation of the color spectrum and to account for reflectivity of analyzed objects, while also making the system independent of lightness or darkness. This system, the Yxy color space, uses the x and y chromacity coordinates to denote the chroma of the calculated XYZ values, and combines them with the Y value that denotes lightness.² (Figure 2)

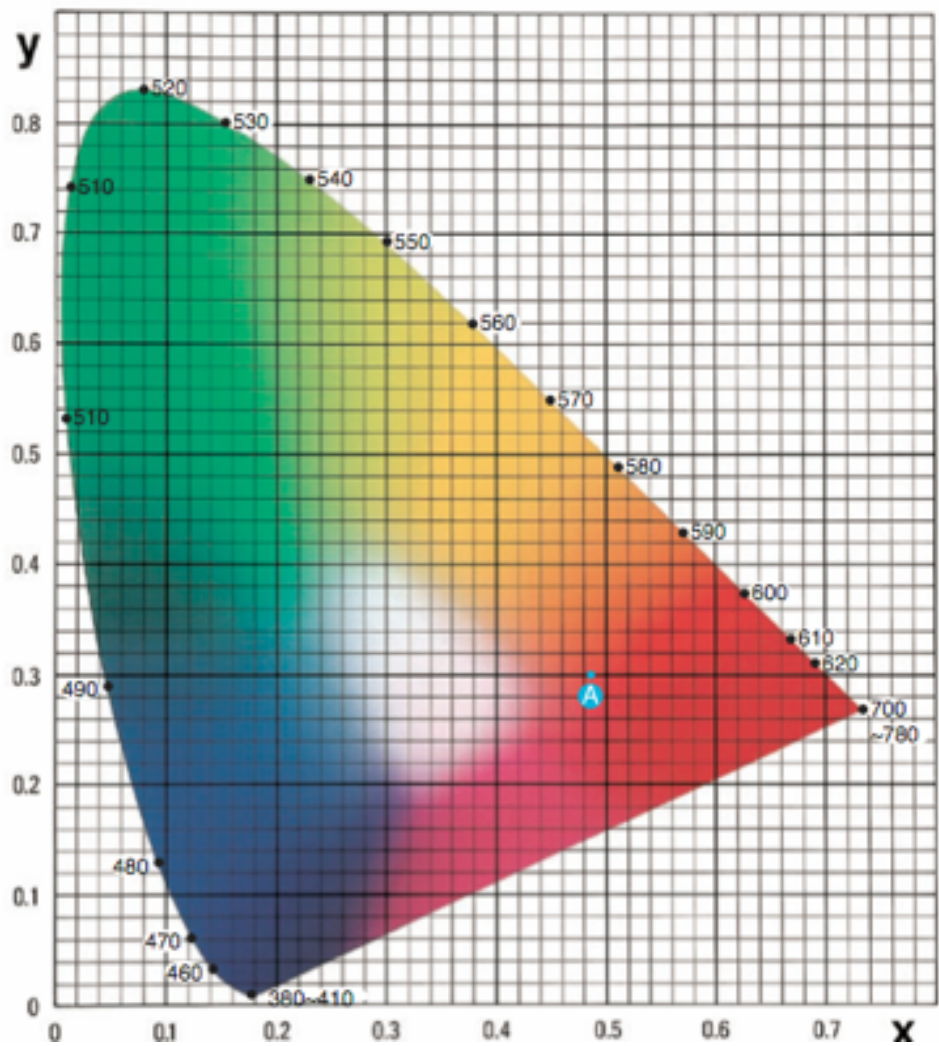


Figure 2: Graphical Representation of Yxy Color Space²

The $L^*a^*b^*$ color space (also referred to as CIELAB), is a more versatile system devised by the CIE in 1976. It was formulated to address a major drawback with the XYZ system: equal distances on the x, y chromaticity diagram did not correspond to equal perceived color differences in the observer. An example of this phenomenon appears in the MacAdam Diagram of Tolerance. (Figure 3) Each ellipse depicted encapsulates points within the XYZ color-space, denoting that colors within that area are perceived as indistinguishable. Different locales within the chart area house ellipses with differing shapes and areas. This demonstrates that in different areas of the chart, equal differences in absolute color do not correspond to equal perceivability of color difference. This effectively makes quantitative comparison impossible using the XYZ color space. In CIELAB, the coordinates L^* (lightness), a^* (red-greenness), and b^* (yellow-blueness) are implemented as a means of comparing the perceptual color difference between two points.^{2,9,10} (Figure 4) The Euclidean distance (ΔE) between the two color points corresponds to the perceptual difference between the two recorded colors at those points.¹¹ This standardized system allows for differences to be calculated between colors, as differences in the coordinate diagram are proportional to the perception of the observer.

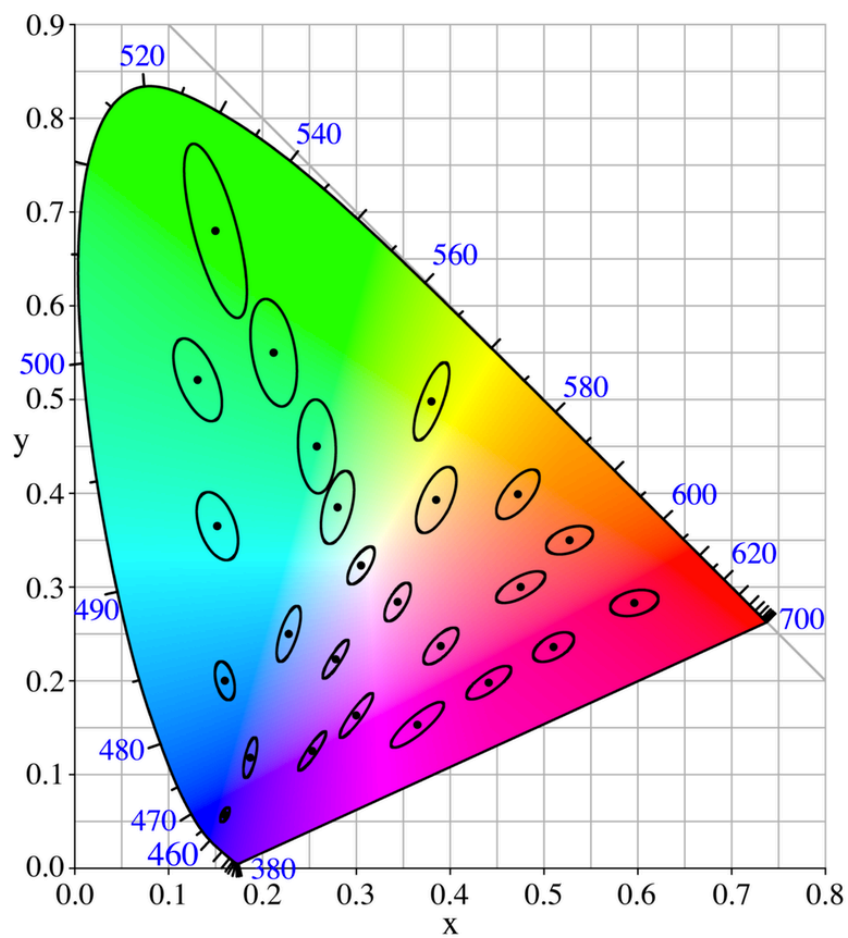


Figure 3: The MacAdam Diagram of Tolerance¹²

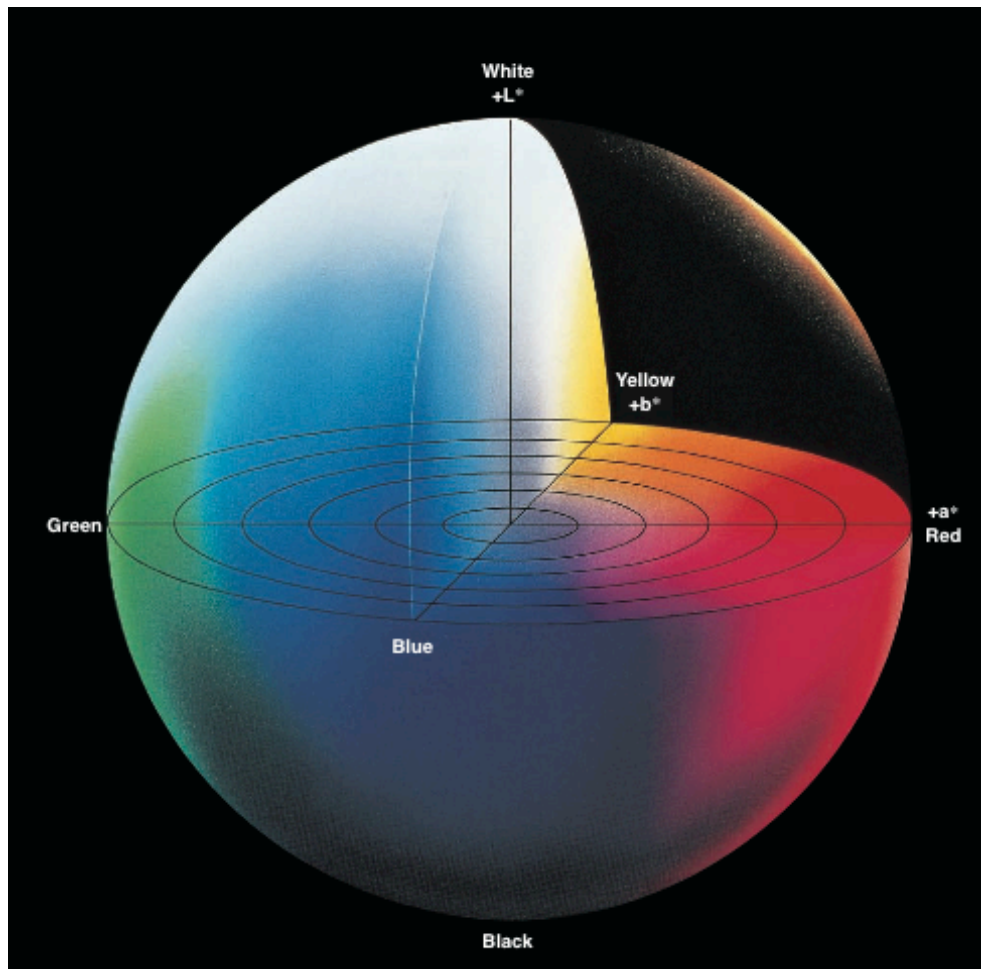


Figure 4: Spherical Representation of the CIE L*a*b* Color Space²

These different color acquisition devices and color spaces each have different utility when applied to the field of dental color matching. The choice of the proper measurement instrument as well as interpretation and communication of the acquired information is vital to the fabrication of an esthetic restoration.

II. Color Measurement in Dentistry

a) Importance of Color and Esthetics

In dentistry, matching the color of the dental prosthesis to that of the anatomy being replaced has proven to be one of the most complex and challenging factors in fabricating an esthetic dental prosthesis.¹ Since the advent of dental prosthetics, attempts have been made to match the color and surface characteristics of dental prostheses to neighboring natural structures with as much accuracy as possible in hopes of achieving optimal esthetic results.¹³ Natural teeth and gingiva exhibit many optical characteristics that must be taken into account when prosthetically replacing these structures. The optical perception of natural teeth by an observer is a subjective interpretation, influenced by numerous factors including color, surface texture, translucency, the layering effect of enamel and dentinal tissues, and the lighting and color of the surrounding environment.¹⁴⁻¹⁶ These factors complicate the fabrication of dental prostheses, as all must be considered in preparing and fabricating a definitive esthetic restoration.

Innovations in materials, prosthesis fabrication, and dental laboratory technology have increased the utility of dental prosthetics greatly to include many different applications. This has complicated the process of matching the shade of the natural dentition to the subsequent prosthesis. Over the past century, the flexibility of restorative therapy has increased to include prostheses that replace not only lost tooth structure but also periodontal structures such as gingiva and mucosa, in quantities ranging from a single tooth (Figure 5) to full-arch (Figure 6) and full-mouth prostheses (Figure 7).^{17,18} The management, classification, and subsequent replacement of these soft tissues of the oral cavity are complex processes.



Figure 5: #7 Single-Unit Implant Prosthesis Demonstrating Dental and Gingival Replacement¹⁹



Figure 6: Full-Arch Maxillary Prosthesis¹⁹



Figure 7: Full-Mouth Resin Prosthesis¹⁹

b) Color Measurement in Hard Dental Tissues

The most longstanding and commonly used application of measurement, communication, and replication of color in dentistry is that of the hard dental tissues, namely teeth. The esthetic prosthetic replacement of teeth in a patient requires that color information of adjacent teeth be obtained by the provider, communicated to the technician who will be fabricating the prosthetic tooth replacement, and then replicated by the technician in the prosthesis according to this communicated information.¹⁶ Several methods of shade matching have been created and are currently in practice, each using different data acquisition methods and levels of technological and procedural complexity.^{1,20-22}

The oldest and most common method of shade matching is the use of manual shade guides and tabs. This technique has long been the most popular means of obtaining color of tooth tissues and conveying color information to the dental technician for replication.²³ (Figure 8) In this method, the practitioner uses one of a number of commercially available, prefabricated shade tab sets to visually match the shade of the teeth adjacent to the proposed restoration to the shade tab that bears the closest resemblance, as interpreted by the practitioner. Several shades for different areas of a single tooth can be noted, and detailed maps and diagrams can be prepared to provide detail and attempt to replicate the polychromatic nature of teeth.⁹ These shade data and diagrams are then communicated to the technician, who will use shade tabs and restorative materials corresponding to the shade tab system to mimic the adjacent teeth.²³ Although this is a popular shade-matching method, it presents several major problems, including its inherent subjectivity for both dentists and technicians, the complex polychromatic nature of teeth and surrounding oral structures, and the limitations of shade guides that do not adequately encapsulate the color space of natural oral structures.^{15,23-27}



Figure 8: Classic Manual Dental Shade Guide Tab Set²⁸

In addressing these deficiencies, new shade guides have been introduced into dental practice, with increased coverage of dental color space and thus the potential for more accurate color matches.¹⁸ (Figure 9) But still, these guides do not address the subjectivity of these operator-based techniques. In light of this, over the past decade, digital photography has become frequently employed to supplement the information obtained through the use of the shade guides by conveying more precise and objective information regarding not only quality and texture but also tooth color to dental laboratories.¹⁸ Photography, however, still lacks standardization. The type of camera utilized, its settings, ambient lighting conditions, image size, tooth position, and type of shade guides employed all factor into the quality of the resultant image and its utility in providing supplemental optical information. A subjective interpretation of color information is still necessary by the technician, and inaccuracies still present.²⁴ The necessity of a more objective system of measurement has led to the implementation of instrumental dental color analysis devices as tools to obtain more objective data on tooth shades.²⁴

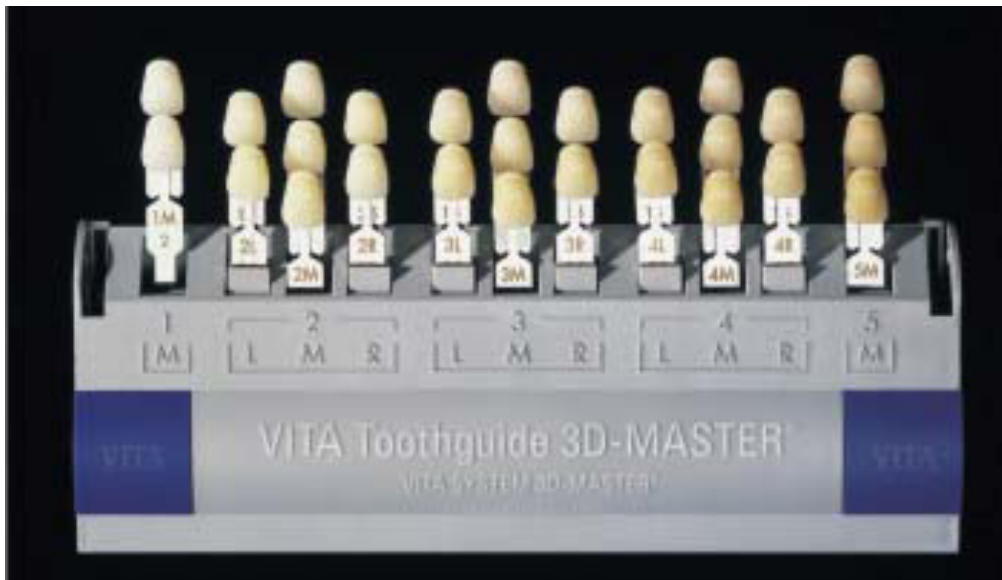


Figure 9: Contemporary Manual Dental Shade Guide Tab Set²⁹

c) Spectrophotometric Dental Shade Acquisition

When implementing digital shade-matching devices into the dental practice, both spectrophotometers and colorimeters, in both contact (spot) and non-contact (complete-tooth) formats, are utilized.²² (Figure 10)(Figure 11)



Figure 10: Non-Contact, Full-Tooth Dental Spectrophotometer²⁰



Figure 11: Contact, Spot-Type Dental Spectrophotometer²⁹

Colorimeters present some challenges because they have been shown to lack needed precision in dentistry, as their accuracy is dependent on the arrangement and geometry of the device, and the precision of data needed has proved to be beyond that of tristimulus values.^{1,2,30} Digital spectrophotometers, with their added standardization, precision, and ease of interpretation and communication, have proven to be the most useful digital tools in dental shade matching.^{9,22-24} Contact, or point/spot systems are used as an alternative to the shade tab system. They are put in contact with the tooth and obtaining the shade information of the structure beneath that spot. Several readings can be obtained from various areas of the tooth, and communicated directly to the technician or even mapped out in a diagram similar manner to that of the manual tab system. Complete-tooth systems provide more detail than point/spot systems, and provide the technician with a detailed map of the shade information of the tooth in question.

However, due to their bulk, they are generally limited in use to the anterior dentition.^{1,9,18,31,32} (Figure 12)



Figure 12: Contact-Type Spectrophotometer in Use²⁰

The threshold with which two different colors can be perceived by the human eye as different also plays an important role in color matching. Research has presented at times widely differing thresholds of the perceivability of color differences in the oral environment, and thus this area remains a topic of contemporary research.³³ Classic research into the perceptibility of color differences in the oral environment originally defined threshold level of perceptibility as less than $3.7\Delta E$ units in the CIELAB color space.^{20,23,24,33,34} Subsequently, it has been established that the spatial color difference of 1 ΔE unit can be perceived by approximately 50% of experienced observers.^{10,35} Further, different studies have established different levels of perceptibility for differences in varying prosthetic applications. Levels of difference required for discernment have ranged from $\Delta E = 2.6$ for denture teeth³⁶ to $\Delta E = 1.6$ for all-ceramic crowns.^{9,10}

Currently, minimal research exists delineating the ΔE at which gingival tissues can be discerned.³⁷ A single study³⁷, performed by Paniz et al., attempted to establish the threshold at which gingiva of differing optical properties could be distinguished. In a study of 39 patients, this threshold was determined by their group to be at $\Delta E=8.74$. This suggests that there is a higher threshold of esthetic acceptability in gingival tissues than in dental structures. However, no research currently exists on the accuracy of gingival replacement materials in optically replicating natural gingival structures.

d) Color Measurement in Soft Dental Tissues

Although great progress has been made in advancing the esthetics of dental hard tissues, the progress in replacing pink gingival tissues lags.^{38,39} This is a critical area of focus because the overall acceptance of and patient satisfaction with a prosthesis is dependent on both gingival tissue and teeth.⁴⁰⁻⁴³ Thus, when replacing lost gingival tissue in the esthetic zone, the morphology and appearance of these prosthetic gingival tissues are essential for optimal esthetic outcomes.^{31,44,45} In trying to meet these esthetic demands, many of the same factors that are taken into account when replacing teeth, such as gingival color, translucency, texture, and inflammatory status, must be considered, interpreted, and documented, and then ideally communicated to the technician for prosthetic replication.

In attempting to identify, qualify, and replicate gingival color and quality, numerous problems arise. Several of these problems mirror those present in the shade obtainment and prosthesis fabrication of ‘white’ prostheses, including the subjective variability of shade matching, the polychromatic nature of the gingival apparatus, and the limitations of shade guides that incompletely represent the color space of natural structures.^{15,16,24,25,27,46} In addition, several problems present that are unique to replicating soft tissue shade. In a 2004 systematic review, Schnitzer outlined several of the challenges in attempting to replicate gingival color, including the subjectivity of classification and replication of existing oral soft tissue, the variability of gingival soft tissue within a single

patient according to age, the fluctuations of gingival optical characteristics in health and disease, and the lack of comprehensive shade matching tools.⁴⁰ Further, the color of the periodontal gingiva has been shown to fluctuate extensively within the same patient depending on the health status of the gingiva⁴⁷, and upon the presence or absence of pressure placed on the soft tissue (known as tissue blanching). These extensive variables make adequate, reliable shade acquisition with the current tools in practice difficult and unreliable.^{39,40}

Similar to hard dental tissues, shade acquisition of soft dental tissues is performed via digital and analog methods.^{38,48,49} The most traditional and common method of color acquisition of periodontal soft tissue is via manual selection of shade by the provider, using a gingival shade tab for selection for the resin portion of removable partial and complete dentures. However, the coverage of these shade guides has been recognized as inadequate.⁵⁰ (Figure 13)

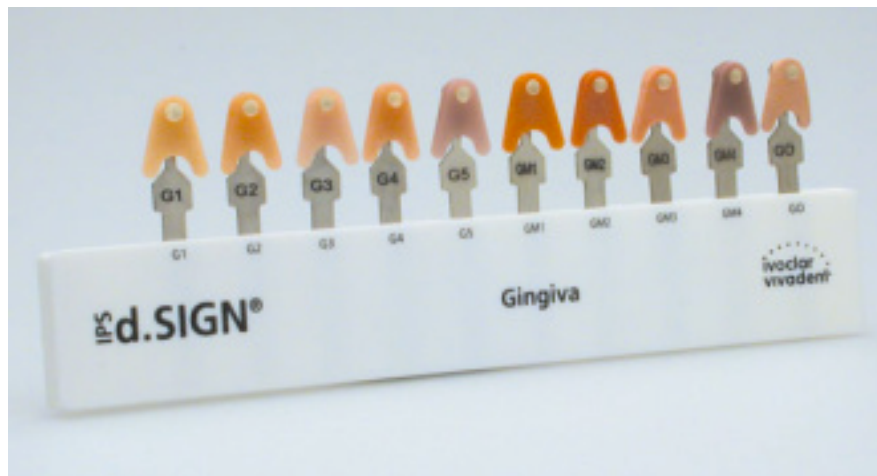


Figure 13: Manual Gingival Shade Tab Set³

Within the past fifty years, photometry^{48,51}, and more recently spectrophotometry^{31,38}, technology have offered alternative mechanisms for analyzing soft tissues. In determining the adequacy of existing resin shade tabs, spectroradiography has been implemented to determine shade guide coverage.⁵⁰ In attempting to fabricate an

experimental gingival shade guide, Huang et al. used a spectrophotometer to delineate clusters of gingival shade pigments and analyze the level of coverage of a novel shade guide.³⁸ This study will attempt to build upon such research by using a commercially available dental spectrophotometer to observe the spectral information of gingiva as it relates to various patient-specific factors, as well as the efficacy of several existing shade systems in adequately replacing these gingival structures.

III. Gingival Attributes: Color, Translucency, and Biotype

a) Gingival Color

The color of the periodontal gingiva, as previously mentioned, is a widely variable clinical parameter and anatomic finding, as well as a structure whose adequate reconstruction and mimicry is vital to esthetic dentogingival reconstructions. As previously mentioned, the overall acceptance and patient satisfaction with a dentogingival prosthesis is dependent on gingival tissue as well as teeth.⁴⁰⁻⁴³

As discussed above, the interpretation of color is a phenomenon created by the observation of light reflected off of a surface. In the case of the dentogingival complex, this has been found to be more complicated. The color of the dentogingival apparatus is the combined effect of 1) the ambient or incident light interacting with the surface of the gingiva, partially reflecting off of or transmitting through the translucent gingival tissue and its constituent structures and exhibiting the spectral properties thereof, then, 2) upon reaching the underlying tooth and alveolus, being reflected back based on its color and optical properties, before 3) being transmitted back through the gingiva and outwards to be interpreted by the observer. (Figure 14)

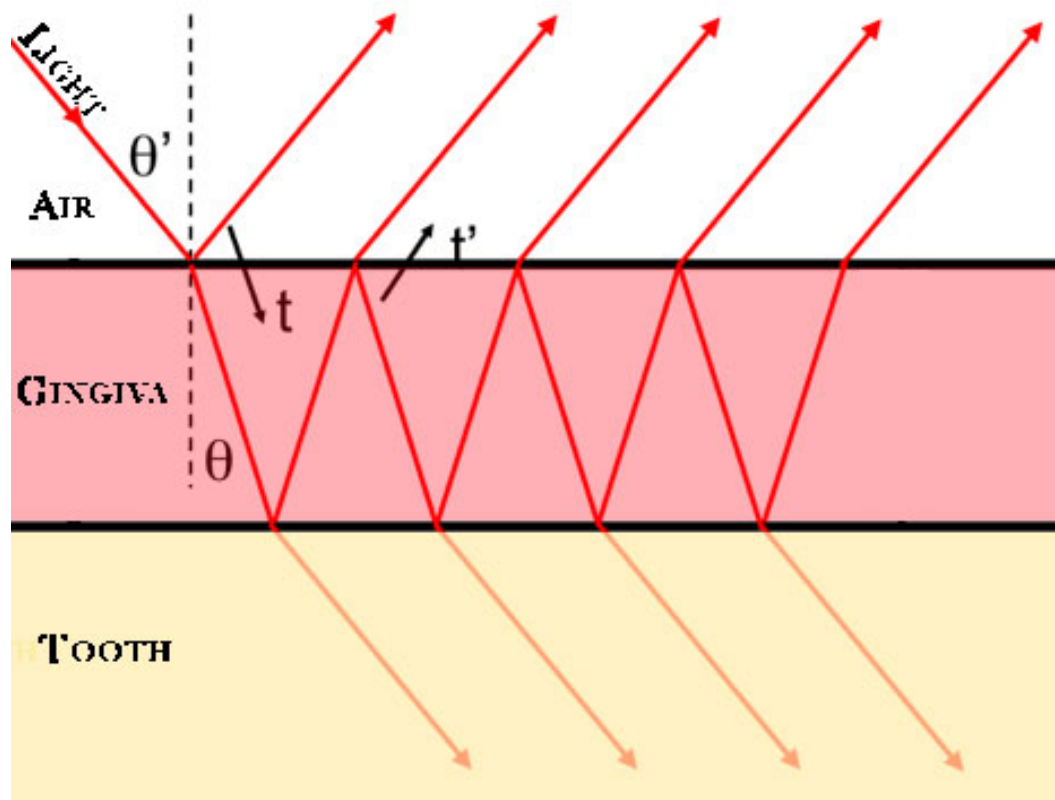


Figure 14: Diagram Illustrating Gingival Light Refraction and Reflection

The optical presentation of the gingival apparatus is therefore determined by a particular combination of biologic factors of the gingival system being viewed. The three main categories of these biologic factors are 1) the overlying gingiva itself and its intrinsic properties, such as its thickness, racial pigmentation⁵², and translucency properties⁵³; 2) the underlying hard dental structures, dental, osseous, or restorative, and their respective color and optical properties⁵³ and; 3) the numerous extrinsic factors acting upon the area at the instance of observation. Such extrinsic factors include the periodontal health of the gingiva at the time of viewing, and the conditions under which the area is being viewed, such as the ambient lighting, location within the arch⁵⁴, and the effects of normal daily fluctuation of the gingival presentation and color.⁵⁵

The gingival portion of a healthy dentogingival apparatus consists of oral mucosa. The portion overlying the bony cortical plate as well as the portion attached directly to the root are collectively referred to as the ‘attached gingiva’. The portion unattached to bone or root structure and limited posteriorly by the gingival sulcus is termed the ‘free gingiva.’ Both the marginal and attached gingiva is comprised generally of a keratinized stratified squamous epithelium, with embedded fibers to add support. Attached gingiva is firmly bound to the underlying alveolar bone and tooth root by periosteal tissues and connective tissue attachment. Marginal gingiva, in contrast, is supported by numerous types of gingival fibers: collagen-based structures that extend in various directions around the tooth-gingiva interface, generally serving to support and stabilize the marginal gingiva. Both attached and free gingivae are highly vascularized and take on the ‘pink’ hue of the embedded blood vessels. Melanocytes are also present in varying amounts and are responsible for racial variation in the gingival tissue.^{52,56,57} (Figure 15)

Numerous restorative materials placed adjacent to or within the dentogingival complex may alter the optical properties of the gingivae. For example, the placement or removal of amalgam restorations may cause small particles of the metallic material to be introduced to and uptaken by the gingiva. This amalgam is encapsulated within the gingival cellular structures and can lead to a lasting blue/gray coloration, colloquially termed an “amalgam tattoo”^{56,58}. (Figure 16) Limited case reports also show the propensity for certain dental implants, worn by a mismatch of hardness in materials used, to leach titanium particles into the surrounding gingiva, causing a similar, gray, “titanium tattoo.”⁵⁹ (Figure 17) The gingival complex further exhibits widely varying amounts of thickness and translucency, which affect its optical properties greatly. The thickness of the free and attached gingiva is likely dependent on the thickness of the underlying alveolar bone; as alveolar bone thickness varies, so does the thickness of the associated attached and marginal mucosa.⁶⁰ The nature and effects of these variations in thickness and translucency will be discussed in the next section.



Figure 15: Racial Pigmentation⁶¹



Figure 16: An “Amalgam Tattoo” ⁵⁶



Figure 17: A “Titanium Tattoo” ⁵⁹

The underlying hard tissue structures of the dentogingival complex consist of the alveolar bone beneath the attached gingiva, and the tooth beneath the marginal gingiva. Optically, the largest effects on the dentogingival apparatus caused by the hard structures are changes in the tooth color. Numerous conditions can affect the external color and quality of a tooth at the dentogingival site. Teeth developmentally may be affected by pharmacologic or environmental agents, leading to staining or alterations in tooth color.⁶² For example, tetracycline, if taken systemically during a period of tooth development, may lead to the yellow to brown-gray staining of the developing tooth.⁶² Such pharmacologic agents can influence tooth color post-tooth development. For example, the periodontal rinse chlorhexidine imparts a yellow-brown hue to the teeth. Intracoronary treatments to the tooth, such as those used in endodontic procedures, can commonly lead to graying of the tooth structure, due to the optical effects of the canal-filling materials like mineral tri-aggregate (MTA)⁶³ or Zinc-Oxide-Eugenol filling materials^{64,65}. (Figure 18) Destructive processes to the tooth, such as internal resorption, can lead to a pinkish hue⁶⁶(Figure 19). However, the most common cause of change to the tooth color underlying the marginal gingiva is its replacement with a restorative material. Metallic or ceramometal restorations often have subgingival metal collars, where the native tooth structure is replaced or covered by a metallic material.⁶⁷⁻⁶⁹ Even when attempts are made to hide or mask this metallic layer at the gingival margin with porcelain, the resultant restoration is often left gray, dull, or dark, possibly affecting both the dental and gingival esthetics.⁷⁰ (Figure 20)



**Figure 18: Untreated Tooth Root (Left) vs. Root Staining
Due to Root Canal Medicament (Right) ⁶⁵**

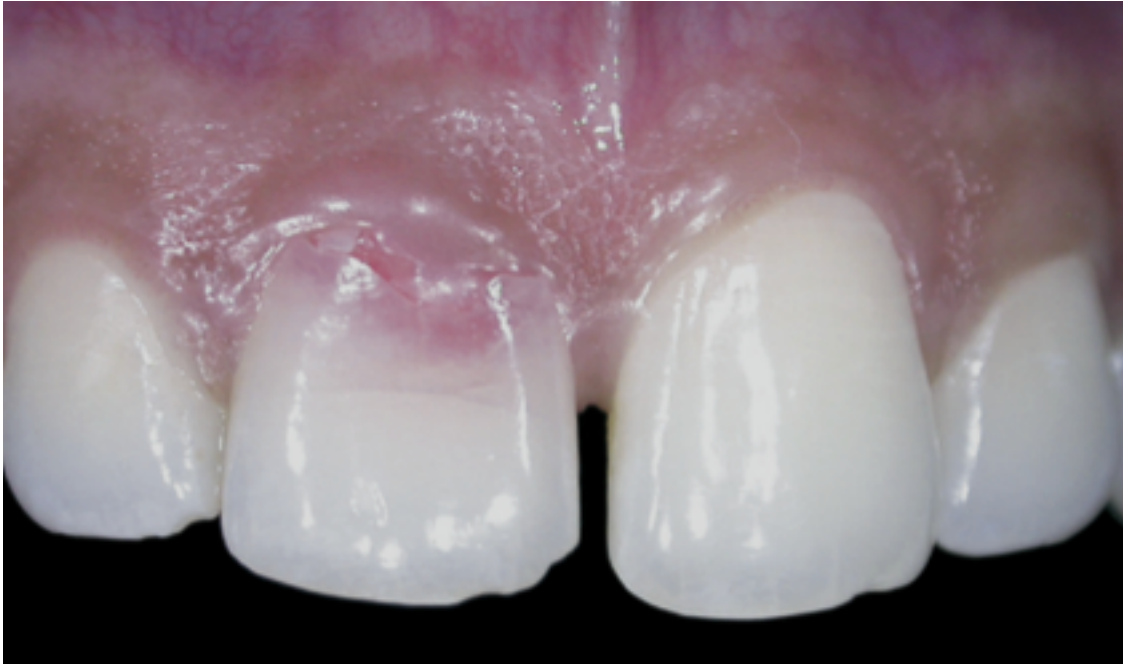


Figure 19: Characteristic Pink Hue Due to Internal Resorption⁶⁶



Figure 20: Gingival Discoloration Due to Buccal Metal Collars in Restorations #'s 7, 9, and 10. Tooth #8 is an Implant Restoration¹⁹

Lastly, several other environmental and biologic factors extrinsic to the dentogingival complex itself can lead to changes in its optical properties. As mentioned above, the optical properties of dentogingival complex are dependent on light reflected off and refracted through the gingival and tooth. Thus, the light source that illuminates the gingiva, both in its amount and constituency, can change its appearance. The same dentogingival complex may appear different under varying light sources, and different complexes may look similar under others.² The health of the gingival process is also an important factor.⁴⁷ In times of gingival inflammation, tissue inflammation, edema, and erythema can occur.⁷¹ These effects draw inflammatory mediators and increase localized blood flow, subsequently causing an increase in the redness of the area. The tissue edema causes changes in its surface characteristics, loss of stippling, and an increase in tissue thickness and possible subsequent changes in translucency.⁴⁷ (Figure 21) Even the position of the tooth observed within the arch can have effects, as well as the age of the patient, with the gingivae appearing generally more chromatic with age, in particular more purple.⁵⁴ The inflammatory status of the gingiva and its biologic processes are also subject to daily circadian rhythm, leading to a minimal yet histologically discernable degree of variability.⁵⁵



Figure 21: Gingival Edema and Erythema Due to Marginal Gingivitis¹⁹

b) Gingival Translucency

In addition to the color of the gingival apparatus or prostheses, an important optical property of materials in the oral environment is the translucency or opacity of those structures. Translucency is the ability of a material to partially transmit light through it, while conversely opacity is the ability of the material to occlude this transmitted light.^{72,73} In the oral environment, a large amount of research has been endeavored into the translucency of the ‘hard’ dental structures, both in natural dentition, and in restorative materials. The translucency of enamel,⁷³⁻⁷⁷ and dentin^{74,76,78,79} have been extensively studied, as well as the effect of numerous treatment modalities or patient-specific variables on these structures, such as bleaching treatments,⁷⁵ or age.⁷⁹

The bulk of literature into translucency in the oral environment however, is in the area of translucency of various types of dental restorative materials. The ability of materials to adequately refract and transmit light is an aspect of an optimal esthetic restorative material, and thus studies have been performed into the translucency of a wide variety of esthetic materials including: composite resins,^{73,80,81} feldspathic porcelains⁸², prefabricated denture teeth⁸³, and numerous types of contemporary high-strength

ceramics including zirconia,⁸⁴⁻⁸⁷ and lithium disilicate materials.^{88,89} Topics of investigation have also included the effects of variables in these materials, such as the effects of mouthwashes on composite resins⁹⁰, different composite resin layering techniques,⁹¹⁻⁹⁷ resin curing protocols,^{86,98-102} resin polishing protocols,⁸³ porcelain firing protocols,¹⁰³ ceramic surface treatments,¹⁰⁴ as well as variations in the ambient illuminant lighting,⁸⁸ on the translucency of the resultant restorations.⁷³

When gingiva and gingival materials are considered, far less research exists, both in natural tissues or restorative materials. For gingival tissue, the large majority of research into natural gingival translucency is based upon peri-implant gingiva¹⁰⁵. These manuscripts have focused on attempting to anticipate and prevent the ‘show-through’ effect of endosteal implants. The ‘show-through’ effect is a phenomenon where the gray, metallic hue of an implant body or abutment is visible through buccal gingiva as a gray discoloration.^{31,53} This effect is viewed as a sequela of thin, transparent gingiva with minimal buccal bone overlying endosteal implants.¹⁰⁶⁻¹¹⁰ Initially, investigators evaluated the overall translucency of the anterior gingiva in both natural dentition and surrounding implants³¹, while follow-up studies have aimed to alter the constituent coloration of the implant or transgingival abutment to help prevent this ‘show-through’ effect.^{53,111}

b) Gingival Thickness and Biotype

Free and attached gingivae are two of the most important anatomic structures and landmarks in the periodontal apparatus. Attached gingiva is defined as “the distance between the mucogingival junction and the buccal extension of the floor of gingival sulcus.” Free gingiva is defined as the “the part of the gingiva that surrounds the tooth and is not directly attached to the tooth surface.”⁵⁶ Numerous classic studies have shown that an adequate apical-incisal width of the attached gingiva helps in maintaining esthetics and enhanced plaque control. Buccal-lingual thickness of the attached gingiva is desired as a

thin gingival margin may lead to recession after trauma, surgical or inflammatory injuries. It may also predispose the area to recession.¹¹²

There is large variation in both width and thickness in facial gingiva, even within the same individual. In 1997, Müller and Eger first introduced the term “gingival phenotype”¹¹³, to attempt to address and categorize the varying thicknesses and widths of buccal periodontal tissues. The identification and categorization of individuals based on their ‘gingival phenotype’ was intended to be useful in clinical practice, because it was observed that differences in the gingival and osseous structures had significant impacts on clinical outcomes of restorative and periodontal therapies.¹¹² For example, in natural teeth, Pontoriero and Carnevale concluded that in patients with a ‘thick flat biotype’, more soft tissue was regained following crown lengthening procedures than in those with a ‘thin scalloped biotype’.¹¹⁴ Olsson and Lindhe also observed a higher prevalence of gingival recession in cases of thin scalloped gingivae.¹¹⁵ The gingival biotype has been described as one of the key elements for successful outcomes in implant restorations.¹¹⁶ However, other studies have concluded that gingival thickness bears no significant impact on gingival biotype.¹¹⁷

A number of methods have been implemented to attempt to assess the biotype of the periodontal mucosae. These range considerably in their invasiveness and ease of being clinically implemented. Such methods have included visual observation,¹¹⁸ conventional histology on cadaver jaws,⁶⁰ transgingival probing with an endodontic file¹¹⁹, cone-beam computerized tomography,^{60 120} ultrasonic sounding devices,¹²¹ and the visual observation of a sulcularly-placed periodontal probe.^{60,118,122} These methods have resulted in widely varying levels of efficacy and clinical applicability, as well as significant differences in the accuracy of these measurements based on the experience of the clinician.¹²³ At the time of this study, no research has been performed examining the ability of gingival biotype to be categorized spectrophotometrically.

IV. Gingival Restorative Materials

a) Early Gingival Restorative Materials

Gingival restorative materials have undergone vast transitions since their implementation. The earliest examples of materials intended to replace gingival tissue in humans date back to almost 700BCE, when bone or ivory was carved into a plate, onto which the prosthetic teeth, often also made of such materials, were riveted.¹²⁴ With little progress in the process and materials of denture fabrication over almost a millennium, attempts were made at improving esthetics of the product, using a wide variety of materials from gold to hippopotamus tusk¹²⁵. A particularly notable historic example was to employ human teeth inset into a hand carved ivory denture ‘base’. This technique proved more sanitary, hygienic, and esthetic than those used previously, as the natural teeth resisted wear and decay, and provided a more natural look. Several sources have shown that the poor were offered compensation for their healthy teeth, which were extracted and placed into dentures. Wealthy slave-owners also used the teeth of their slaves for this purpose. It is well supported that this was the source of the teeth in George Washington’s famous ‘wooden’ dentures.¹²⁶⁻¹²⁸ (Figure 22) The most expansive collection of examples of this technique are observed in the evidence collected from the Civil War Battle of Waterloo, where numerous casualties possessed dentures exemplifying this technique, generating the term ‘Waterloo Teeth.’^{124,125,129} Still, wide variation existed. Although this technique was marginally esthetic compared to alternatives, the materials employed were hardly functional and were uncomfortable, subject to gross staining, and often still decayed in the oral cavity, as natural teeth do.



Figure 22: George Washington's Dentures¹³⁰

A large leap in the materials used in dentogingival reconstruction occurred in the late 1700's, when French pharmacist Alexis Duchateau first made attempts to use porcelain as a denture material, with the help of Parisian dentist Dubois de Chemant.^{125,131} He conceived of the idea of fabricating a porcelain denture that used porcelain for both the base and teeth portions. Together, the pair produced a denture that Duchateau himself could wear, and his process was acknowledged by the Royal Academy of Surgeons, Paris, in 1776.¹²⁵ Dubois de Chemant then perfected the porcelain technique and received a British patent for it in 1791. In his *A Dissertation on Artificial Teeth*, de Chemant describes his technique of shaping a porcelain paste, provided by the English fine china manufacturer Wedgwood, onto molds of models of the mouth. After drying and baking, the stark white block was enameled with colors corresponding to the gums and teeth.¹²⁵ (Figure 23) This method remained in practice for many years with mixed success, though comfort and price remained large hurdles in widespread usage.

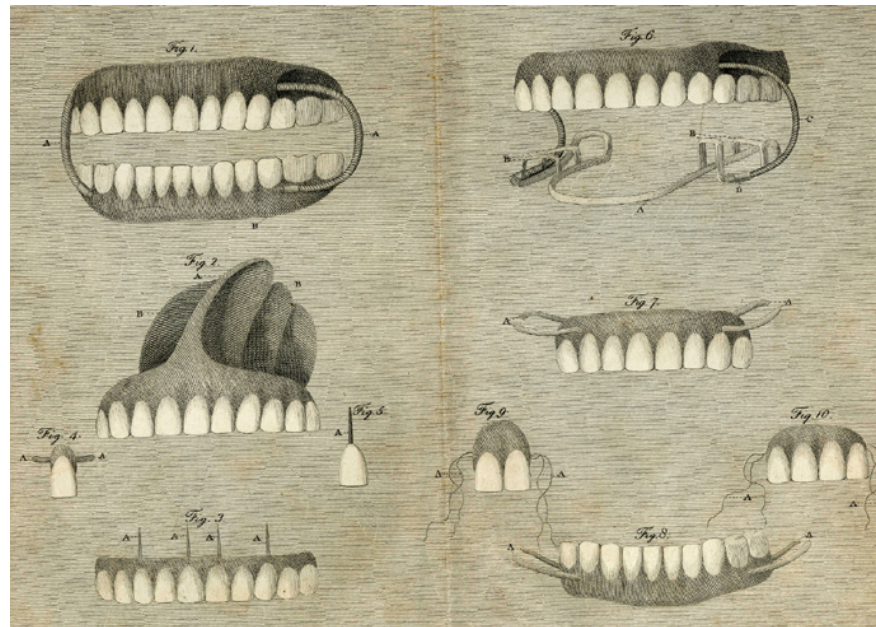


Figure 23: Diagram of Various Designs of Partial and Complete Porcelain Prostheses¹³²

In 1839, the invention of the rubber material Vulcanite by Charles Goodyear and its subsequent 1855 patent as a denture material revolutionized the esthetics and ergonomics of gingival replacement materials. (Figure 24) The rubber-based material provided a multitude of benefits, including a softer mouth feel, improved moldability and handling properties, better resilience in the mouth, and a drastically less expensive material cost compared to materials such as gold and ivory.¹²⁹ However, due to strict patent regulations by Goodyear requiring the payment of expensive royalties and licensing fees, with the threat of enforcing swift lawsuits for infringement, Vulcanite's use was restricted.¹²⁵ It was not until 1881, when Josiah Bacon, the treasurer, manager, and patent enforcer of the Goodyear Dental Vulcanite Company, was murdered by a denture dentist, Dr. Samuel Chalfant, that the strict patent policy of Goodyear was relieved.^{125,133} Bacon had pursued Chalfant and forced him out of practice in three states, with many

others voicing similar displeasure. The Transactions of the Iowa State Dental Meeting of 1901 wrote of Bacon:

Bacon was pressing Dr. Chalfant too hard for royalty. Bacon, as manager of collections, had no mercy on the dentists, and hundreds were so oppressed by him that their business was ruined and many were worried into untimely graves. The murder of Bacon, if not lawful, was generally conceded by the men of our profession as an act of justice.¹³⁴

It was not until this unfortunate occurrence and the subsequent expiration of the Goodyear patent that Vulcanite was widely used clinically and affordably.¹²⁵ Some providers were troubled by the subsequent mainstreaming, stating that the now inexpensive rubber denture bases “degraded the art by bringing cheap work... We are all familiar with the newspaper advertisements and the plates we see and make.”¹³⁴

Aside from the early financial barriers, Vulcanite was still not seen as the ideal material clinically and esthetically, as it was viewed by numerous providers as opaque and lifeless.¹³⁵ Considering these problems, and the ongoing difficulty and expenses in acquiring Vulcanite, other materials were explored as viable denture base alternatives. In 1869 the Celluloid Company introduced celluloid as a baseplate.¹³⁶ It afforded an alternative to the expensive Vulcanite and was seen as superior esthetically. As the Iowa Transactions reported, “It made a beautiful plate, more nearly the natural gum color than pink rubber, and I believe if carefully made is more durable than most of the cheaply made rubber plates of which we see so many.”¹³⁴ Vulcanite, celluloid, and various other, less popular materials, such as tortoiseshell and Bakelite, a formaldehyde resin, were used as the primary gingival bases through the mid 20th century.^{125,137}



Figure 24: A Maxillary Complete Denture Fabricated From Gold, Platinum, and Vulcanite¹³⁸

The largest innovation in gingival restorative materials, and by far the greatest leap in esthetics came with the utilization of methylmethacrylate as a denture material in 1935. The plastic polymer, invented in 1901 by Rohm et al., was developed and researched heavily by companies such as Du Pont, ICI, and Rohm and Haas before being introduced as the dental material Vernomite in 1938. Polymethylmethacrylate (PMMA) resins addressed the major drawbacks of the traditional materials, including handling, patient satisfaction and esthetics. Clinical research by Wright et al. in 1937 exhibited the superiority of PMMA in almost every respect, particularly its translucency, vibrancy, and esthetics. It was easily repaired, formed a chemical bond with esthetic denture teeth, which could also be made out of PMMA, and importantly, it was inexpensive. Further, the color of the material remained stable over long periods; previous generations of base materials did not offer this long-term stability. Wright found

PMMA to fulfill virtually all of the requirements of an ideal base material.^{125,136} In 1940, Vulcanite was abruptly discontinued and by 1946 it was estimated that 95% of all dentures were made out of PMMA.^{125,136} With this surging popularity, numerous manufacturers began fabricating brands and versions of dental PMMA.^{139,140} Numerous shades and colors of materials were made available, attempting to better mimic the patient's gingival presentation.¹⁴¹

b) Contemporary Gingival Restorative Materials

PMMA remains today the most popular material for denture fabrication.¹⁴² (Figure 25) Numerous brands, formulations, and variations have been researched and implemented, each varying in their strength¹⁴³⁻¹⁴⁵, handling and mechanical properties^{146,147}, method of polymerization^{145,148}, biocompatibility¹⁴⁹⁻¹⁵³, chemical composition¹⁵⁴, esthetics^{155,156}, and color stability^{145,157-161}. A variety of curing processes have added variability to the methods in which the final prostheses are fabricated, including injection molding^{143,147,162}, CAD/CAM systems^{163,164}, and light^{145,165}, heat¹⁶⁶⁻¹⁶⁸, chemical^{145,168}, and microwaves^{154,162,169}. With the advent of implant dentistry, the applications of acrylic resin has increased from predominantly complete denture bases to almost any instance where gingival replacement may be necessary, ranging from single teeth¹⁷⁰⁻¹⁷², to implant overdentures^{108,173}, to full-arch hybrid-type restorations where the resins are veneered onto titanium substructures and bars.¹⁷⁴⁻¹⁷⁸ Not only does this necessitate changes in their handling and material properties but also their esthetic and color qualities.

One of the aims of this research will be to attempt to gauge the efficacy with which different sets of PMMA gingival restorative materials mimic gingival color in the anterior dentition.



Figure 25: A Contemporary Acrylic Complete Denture¹⁹

V. Endoscopy and the Interpapillary Capillary Loop

a) Endoscopy and Narrow-Band Imaging

Endoscopy, the use of instrumentation inserted into the body in order to observe its internal parts, is a technique that has long been utilized in conventional medicine to provide imaging, obtain biopsies, and allow access for surgeries. As fiber optic technology has progressed, the use of endoscopy in visualization of numerous areas of the body including the nasopharynx, esophagus, stomach, trachea, lungs, and colon has become commonplace. (Figure 26) Conventionally, a pure-white LED light source is utilized to provide incident lighting for visualization of internal cavities during endoscopic procedures.^{179,180} (Figure 27) This imaging modality has been implemented to aid in diagnosis of diseases such as colon cancer, esophageal cancer, strictures, blockages, and polyps with much regularity and accuracy.



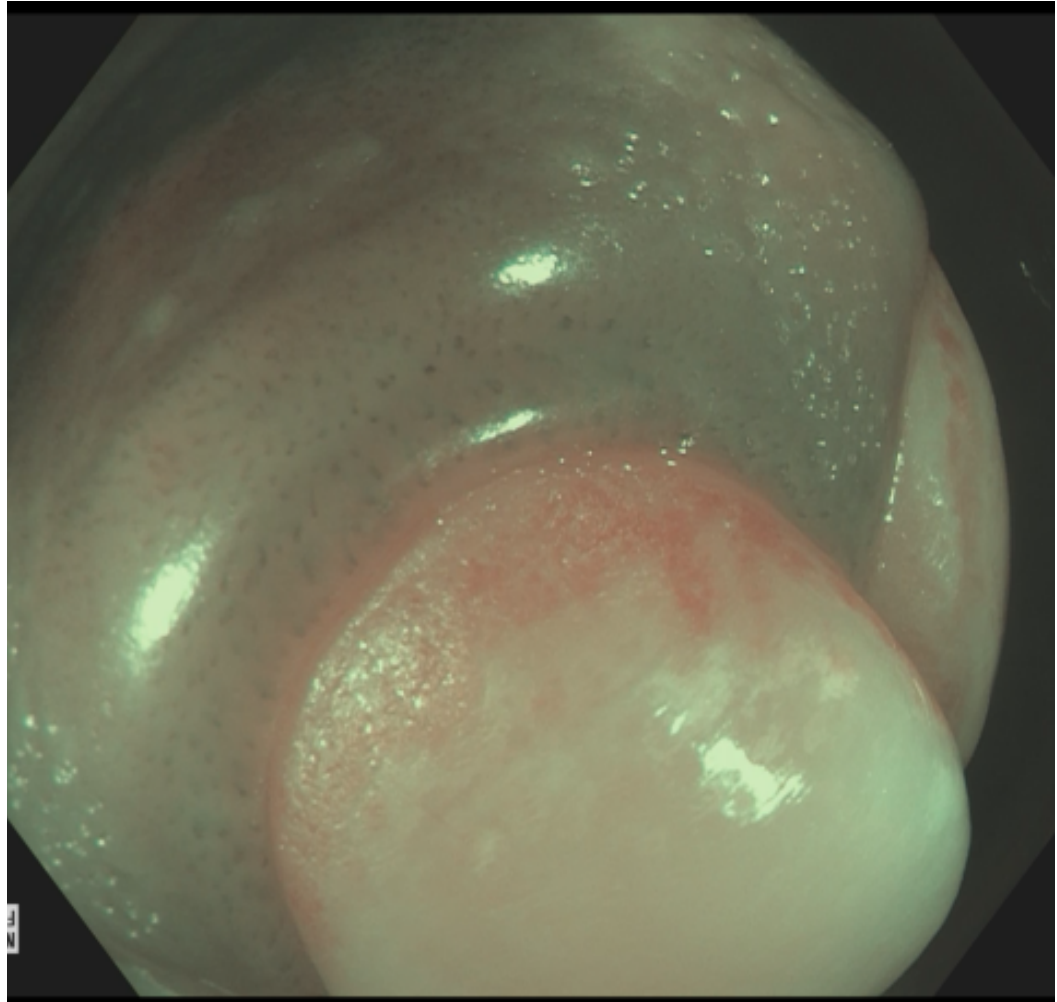
Figure 26: Endoscope Equipped with Narrow-Band Imaging¹⁸¹

Narrow-band imaging (NBI) is a recently developed form of endoscopy that utilizes narrow-band wavelength filters to the endoscopic light source to allow clarity when visualizing mucosal vascularity. This increase in visual acuity is obtained via filters applied to the incident light source, restricting output wavelengths to those such as narrow-band blue (415.5 ± 15 nm) and green (540 ± 15 nm) wavelengths.¹⁸² (Figure 28) These wavelengths are chosen because they are specific to hemoglobin absorption, causing them to appear black in color and increasing their contrast from surrounding mucosa markedly. As many mucosal diseases are characterized by changes in vascularity, this imaging modality is seen as particularly useful in observing these changes more definitively and conveniently.^{182,183} Originally introduced to aid in the diagnostics of neoplasms due to gastrointestinal cancers, which initially form in the easily viewable mucosae, NBI has also

been implemented as a diagnostic tool for numerous diseases that exhibit increased or altered mucosal angiogenesis, including inflammatory bowel disease,¹⁸³ Barrett's Esophagus and esophageal cancer,¹⁸² chronic gastritis, gastric adenoma and gastric cancer,¹⁸² and ulcerative colitis.¹⁸⁴



Figure 27: White-Light Endoscopic Image of Keratinized Mucosa of Tooth #8 ¹⁹



**Figure 28: Narrow-Band Wavelength Endoscopic Image of
Keratinized Mucosa of Tooth #8 ¹⁹**

Most recently, NBI technology has been used as a diagnostic tool in the detection of a variety of oral mucosal diseases. Yang et al. have exhibited the ability of NBI to be used to evaluate oral mucosal diseases including oral leukoplakia,^{180,185,186} squamous cell carcinomas,^{186,187} and nasopharyngeal carcinomas.¹⁸⁸ These studies have demonstrated the ability of endoscopy equipped with NBI to observe and evaluate vasculature and microvasculature in the oral mucosa. When using NBI to evaluate oral lesions, the main criterion for evaluation is the level of complexity of intrapapillary capillary loops (IPCLs).

b) The Intrapapillary Capillary Loop (IPCL)

The intrapapillary capillary loop is a term that was initially coined in the description of microvasculature architecture in mucosal tissue.¹⁸⁹ Initially used in the classification of squamous carcinomas in esophageal mucosa, analysis of IPCLs has been utilized as a means to determine the level of complexity of the minute mucosal vascular beds in mucosal tissues. The numbers of these loops as well as their levels of complexity and tenuousness correlate to the amount of inflammation in the mucosal tissue, and serve as a proxy for neoplastic activity and angiogenesis.^{183-185,187,188,190} Particularly, the architectural complexity of the IPCLs has been used when endoscopically observing mucosal tissue to gauge the level of inflammation or neoplastic activity. Inoue et al introduced a system to classify esophageal IPCLs to gauge neoplastic potential.^{191,192} This classification system, based in carcinoma diagnosis, consists of several ‘types’ of IPCL presentations, ranging from normal (background) presentation to advanced complexities suggesting neoplastic formations and likely esophageal tumors. The early stages of this system gauge levels of mucosal inflammation. This study hopes to enact an adapted version of this classification system to evaluate the amount of inflammation present in the oral mucosa. Prior research indicates that IPCL can serve as a diagnostic criteria in the oral environment, namely in the analysis and transformative potential of oral leukoplakia in oral mucosa.^{180,186,187,190} (Figure 29) This project hopes to evaluate the presentation of the IPCL in the periodontal gingival environment.

IPCL type I			Background	
IPCL type II			Inflammation	
IPCL type III		Normal IPCL with area formation	Inflammation/LGIN	
IPCL type IV		Slight change of IPCL	LGIN/HGIN	
IPCL type V1		Irregularly dilated IPCL	m1	Local treatment (EMR/ESD)
IPCL type V2		Type V1 + elongation	m2	
IPCL type V3		Highly destructed IPCL	m3/sm1	ER/surgery/CRT
IPCL type Vn		New tumor vessels	Sm2	Surgery/CRT

Figure 29: The Inoue Classification of IPCLs¹⁹²

Chapter 2: Hypothesis and Specific Aims

I. Hypotheses

1. We hypothesize that the spectral shade statistics of periodontal gingiva will vary across patient populations with respect to patient sex, ethnicity, tooth location, and location of site within the gingival apparatus.
2. We hypothesize that the gingival phenotype (biotype) of periodontal gingiva in the anterior maxilla can be determined using spectral shade statistics.
3. We hypothesize that, when compared to the spectral data of a representative patient population, different sets of gingival restorative materials will have varying coverage errors and overall efficacies in reproducing gingival color.
4. We hypothesize that, by utilizing intraoral endoscopy equipped with narrow band imaging, the intrapapillary capillary loop (IPCL) classification of periodontal soft tissues in the anterior maxilla can be correlated to gingival color.

II. Specific Aims

1. To compile spectral shade statistics of periodontal gingiva in the anterior maxilla:
 - a. Across the entire spectrum of gingival color in a representative patient population.
 - b. As a factor of participant sex, ethnicity, tooth number, and location of site within gingival apparatus.
2. To perform a gingival phenotype analysis comparing gingival thickness in the anterior maxilla with translucency metrics obtained spectrophotometrically.
3. To examine the ability of varying gingival restorative materials to replicate the spectral properties of a representative patient population.

- 4.** To compare IPCL classification of periodontal soft tissues obtained via endoscopy equipped with narrow band imaging to gingival color, thickness, and translucency.

Chapter 3: Significance of This Research

Currently, a wealth of data exists on the spectral properties of teeth. This has led to great strides in the esthetic acceptability of restorative and prosthodontic materials developed to replace tooth structure. It has also allowed more precise communication between the clinicians and technicians involved in creating these restorations. However, far less information exists on the ‘soft’ oral structures. This has led to particular difficulty when attempting to esthetically replace edentulous areas and defects where both dental and gingival replacement is indicated. Possible reasons for this include esthetic inadequacy of existing gingival restorative materials, as well as the related challenges in communicating spectral properties to the dental technician.

This project studies the spectral gingival properties of a patient population, producing the framework of an expandable database of the optical properties of human mucosa. Such a database has been previously created for ‘hard’ dental structures. This database once compiled can be examined, built upon, and can serve as a basis for both evaluating existing gingival restorative materials as well as producing new products. This database can be used to develop superior reference shades and standards, based on patient metrics, improving communication between dentists and technicians. By investigating the use of spectrophotometry as a possible means of obtaining and communicating objective gingival spectral shade data from dentist to technician, a workflow very common in teeth can be implemented for the gingiva, yielding more consistent restorations and esthetics.

This project also serves as a pilot study to evaluate the implementation of such a database into both the esthetic evaluation of existing gingival restorative materials, through a comparative spectrophotometric analysis, and the fabrication of a novel gingival shade system optimized for the patient population.

Chapter 4: Specific Aim 1. An *in vivo* Analysis of the Spectral Properties of Periodontal Gingiva in the Esthetic Zone

I. Materials and Methods

The first phase of this project was directed toward accumulating spectrophotometric color data on gingival soft tissues surrounding natural dentition in the esthetic zone, and stratifying this data based on patient-specific variables. IRB approval was obtained for all aspects of the study. Patients were recruited for this study from among the general patient population of the Harvard Dental Center. After recruitment, screening of eligibility as an active patient of the Dental Center, and patient agreement of participation, a baseline periodontal examination of the anterior maxillary sextant was performed.

Inclusion criteria for this aim were: patient age eighteen and above; at least 4 teeth in the anterior maxillary sextant present as virgin teeth, restored teeth, or all-ceramic or ceramometal restorations. Exclusion criteria were: ADA class 3 or greater, bleeding on probing to buccal sites of teeth #'s 6-11, or probing depths greater than 3mm in teeth #'s 6-11. Any implant restorations present in the anterior maxilla were not included in this analysis.

Ninety participants qualified for this portion of the analysis and a total of 491 tooth sites were examined. Participants were asked to self-identify for sex and ethnicity. Patient ages ranged from 25 to 87 with a median of 39 years. Participant demographics for this section were as follows:

Sex	n
Male	41
Female	49

Ethnicity	n
White	42
Asian	20
Black	14
Hispanic	14

Using a non-contact full-tooth intraoral dental spectrophotometer (Crystaleye; Olympus, Tokyo, Japan), spectral images of tooth and soft tissue were captured of each gingival margin of tooth numbers 6 through 11. The instrument was calibrated prior to data acquisition, via a full-arch spectral image.^{24, 9} Reflectance values were transferred from the spectrophotometer to a personal computer for analysis using CIELAB color coordinates. Using integrated computer software (Crystaleye Application Master; Olympus, Tokyo, Japan), CIELAB data were obtained for three sites for each tooth in the anterior maxillary sextant, corresponding to 3mm wide by 1mm high areas located 3mm apically to the free gingival margin at the midbuccal of each tooth. (Figure 30) Spectral data obtained from each site was then input into statistical modeling software (SPSS; IBM, Armonk, USA) and output variables L*, a*, and b* were plotted and analyzed on three-dimensional visual representation graphs. Independent t-tests were performed to establish if any significant differences existed with respect to participant sex. One-way ANOVA statistical tests were performed to determine if differences exist amongst the mean values of the output variables with respect to ethnicity, sex, and tooth number. When the ANOVA revealed differences within the means, Fisher's LSD post-hoc exam was performed on appropriate variables to determine statistically significant differences with an alpha value of 0.05.

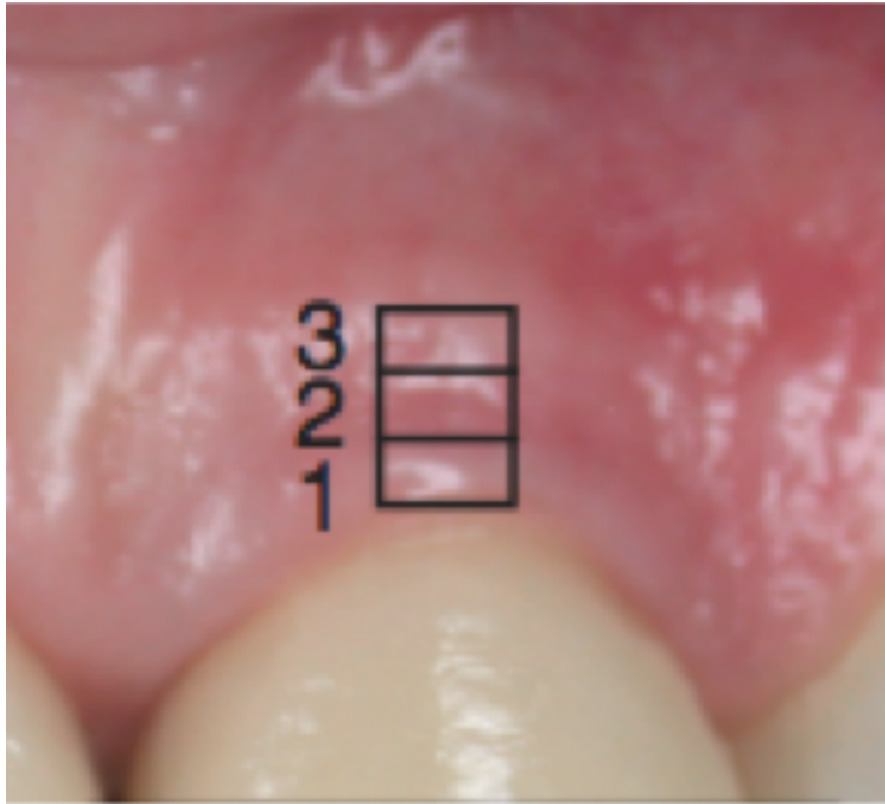


Figure 30: Diagram Illustrating Locations of Gingival Spectral Measurements³¹

II. Results

a) Gingival Color Based on Tooth Number

Spectrophotometric photographs of all sites of all anterior maxillary teeth were obtained and analyzed. L*, a*, and b* values were recorded and averaged over the participant population based on tooth location. (Table 1)

- L* values ranged from 52.714 ± 4.65 in tooth #9, to 50.29 ± 5.17 in tooth #6, with a mean value of 51.29 ± 5.14 across all sites.
- a* values ranged from 18.65 ± 3.77 in tooth #11, to 17.92 ± 3.63 in tooth #9, with a mean value of 18.33 ± 3.62 across all sites.
- b* values ranged from 15.53 ± 2.73 in tooth #6, to 14.86 ± 3.08 in tooth #8, with a mean value of 15.14 ± 3.11 across all sites.
- Mean L* values were observed to decrease the more posterior the tooth, while the a* and b* values both increased more posteriorly.

Tooth	N	Mean $\pm$ SD L*	Mean $\pm$ SD a*	Mean $\pm$ SD b*
6	86	50.3 ± 5.2	18.6 ± 3.5	15.5 ± 2.7
7	82	50.6 ± 4.9	18.6 ± 3.5	15.4 ± 3.2
8	77	52.2 ± 5.2	18.2 ± 4.0	14.9 ± 3.1
9	79	52.7 ± 4.7	17.9 ± 3.6	14.9 ± 3.2
10	84	51.7 ± 5.1	18.0 ± 3.4	15.0 ± 3.2
11	83	50.4 ± 5.4	18.7 ± 3.8	15.1 ± 3.3
Total	491	51.3 ± 5.1	18.3 ± 3.6	15.1 ± 3.1

Table 1: Mean Spectral Values by Tooth Number

An ANOVA analysis was performed and statistically significant differences were identified. For the study population, only L^* values were determined to have significant differences to the 0.05 level of significance ($p=0.006$). Fischer's LSD post-hoc analysis was performed for L^* values. Significant differences in the mean L^* were determined between the following sets of teeth in the study population: (Figure 31)(Figure 32)

- Tooth #6 to teeth #'s 8 and 9. ($p=0.018$; $p=0.002$)
- Tooth #7 to teeth #'s 8 and 9. ($p=0.046$; $p=0.007$)
- Tooth #8 to teeth #'s 6, 7, and 11. ($p=0.018$; $p=0.046$; $p=0.029$)
- Tooth #9 to teeth #'s 6, 7, and 11. ($p=0.002$; $p=0.007$; $p=0.004$)
- Tooth #10 showed no statistically significant differences to any other tooth.
- Tooth #11 to teeth #'s 8 and 9. ($p=0.029$; $p=0.004$)

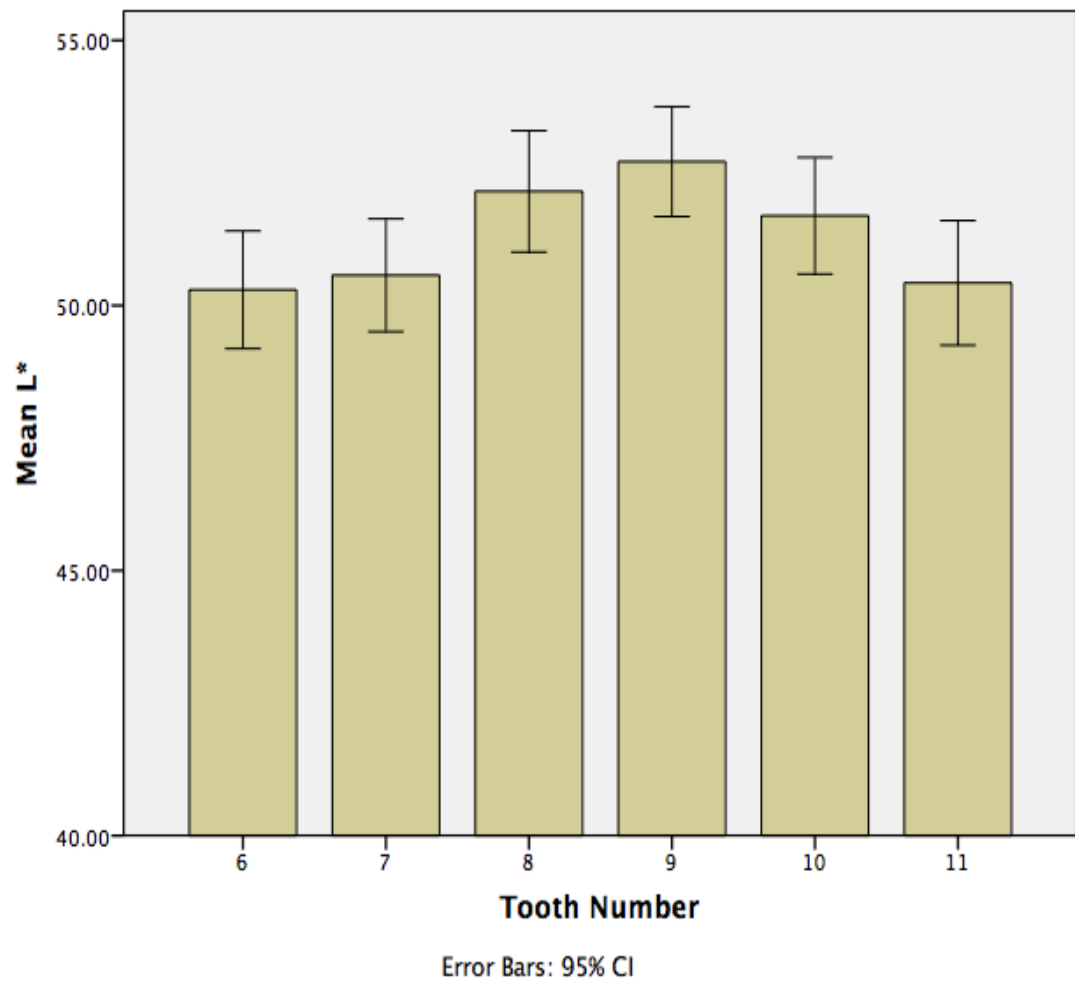


Figure 31: L* vs. Tooth Number

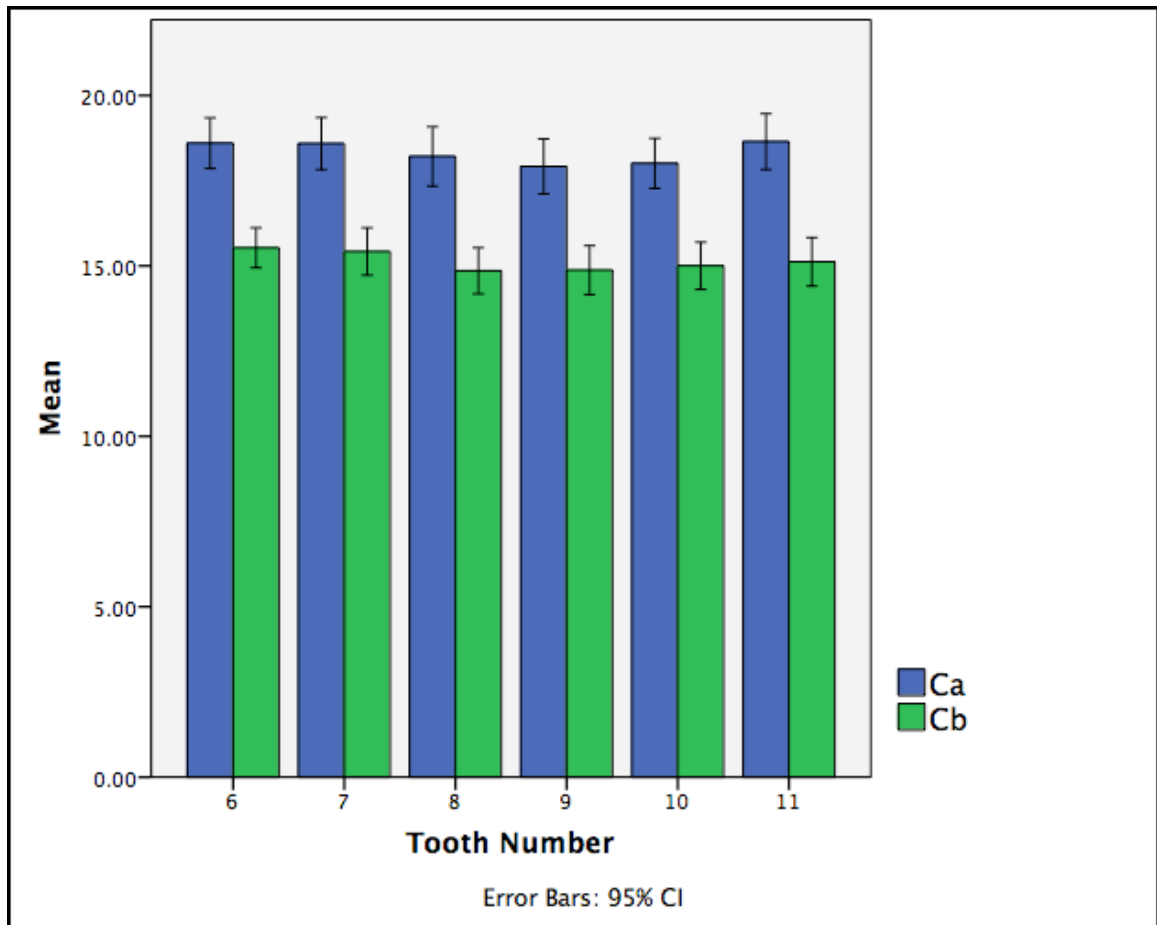


Figure 32: a* and b* vs. Tooth Number

b) Gingival Color Based on Tooth Type

L*, a*, and b* values were then compiled into three groups based on tooth type (central incisor, lateral incisor, canine). As in the tooth number analysis, mean L*, a*, and b* values were calculated and examined for each tooth type. (Table 2)

- L* values ranged from 52.3 ± 5.14 in central incisors, to 50.36 ± 5.28 in canines, with a mean value of 51.29 ± 5.14 across all sites.
- a* values ranged from 18.62 ± 3.61 in canines, to 18.06 ± 3.61 in central incisors, with a mean value of 18.33 ± 3.62 across all sites.
- b* values ranged from 15.32 ± 3.01 in canines, to 14.87 ± 3.14 in central incisors, with a mean value of 15.14 ± 3.11 across all sites.
- Mean L* values were observed to decrease the more posterior the tooth, while the a* and b* values both increased more posteriorly. (Figure 34)

Tooth Type	n	Mean $\pm$ SD L*	Mean $\pm$ SD a*	Mean $\pm$ SD b*
Central Incisors	156	52.4 ± 5.1	18.1 ± 3.8	14.9 ± 3.1
Lateral Incisors	166	51.1 ± 5.0	18.3 ± 3.5	15.2 ± 3.2
Canines	169	50.4 ± 5.3	18.6 ± 3.6	15.32 ± 3.0
Total	491	51.3 ± 5.1	18.3 ± 3.6	15.1 ± 3.1

Table 2: Mean Spectral Values vs. Tooth Type

ANOVA was then performed to determine if tooth type had statistically significance of these trends in L*, a*, or b* values. For the study population, only L* values were determined to have significant differences to the 0.05 level of significance ($p < 0.01$). Fischer's LSD post-hoc analysis was performed for L* values. Significant differences in the mean L* were determined between the following sets: (Figure 33)

- Central incisors and lateral incisors ($p = 0.021$)
- Central incisors and canines ($p < 0.001$)

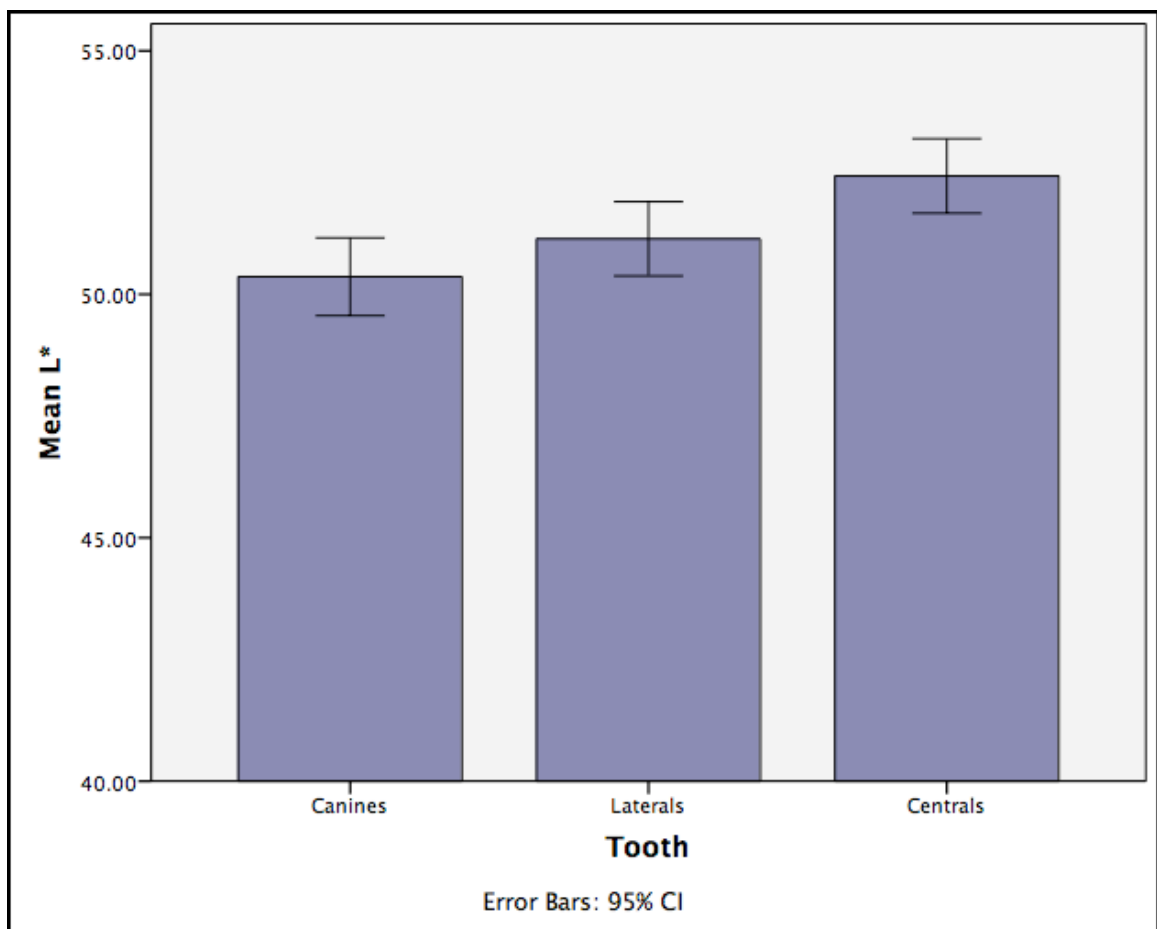


Figure 33: L* vs. Tooth Type

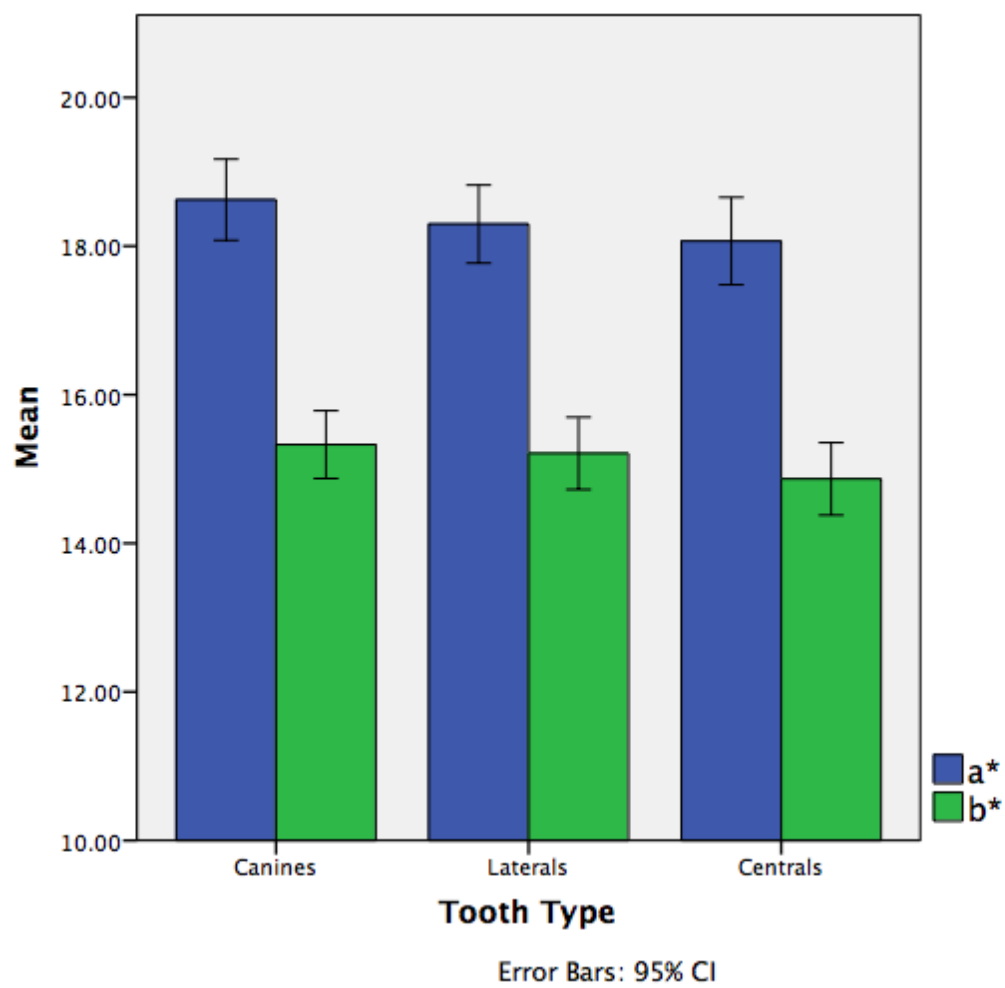


Figure 34: a^* and b^* vs. Tooth Type

c) Gingival Color Based on Sex

L*, a*, and b* values were then stratified into two groups according to participant sex, and an independent t-test was performed to determine statistical significance of observed differences (Table 3) (Figure 35)(Figure 36) No statistically significant difference was observed in L* with respect to sex. (p=0.087)

- Male participants (n=40) showed increased a* values, 18.93 ± 3.73 vs. 17.90 ± 3.50 . (p=0.002)
- Female participants (n=49) showed increased b* values, 15.63 ± 3.18 vs. 14.53 ± 2.89 . (p<0.001)

Sex	n	Mean $\pm$ SD L*	Mean $\pm$ SD a*	Mean $\pm$ SD b*
Female	49	51.68 $\pm$ 5.36	17.90 $\pm$ 3.50	15.63 $\pm$ 3.18
Male	40	50.88 $\pm$ 4.70	18.93 $\pm$ 3.73	14.53 $\pm$ 2.89
Total	89	51.29$\pm$5.14	18.33$\pm$3.62	15.14$\pm$3.11

Table 3: Mean Spectral Values vs. Sex

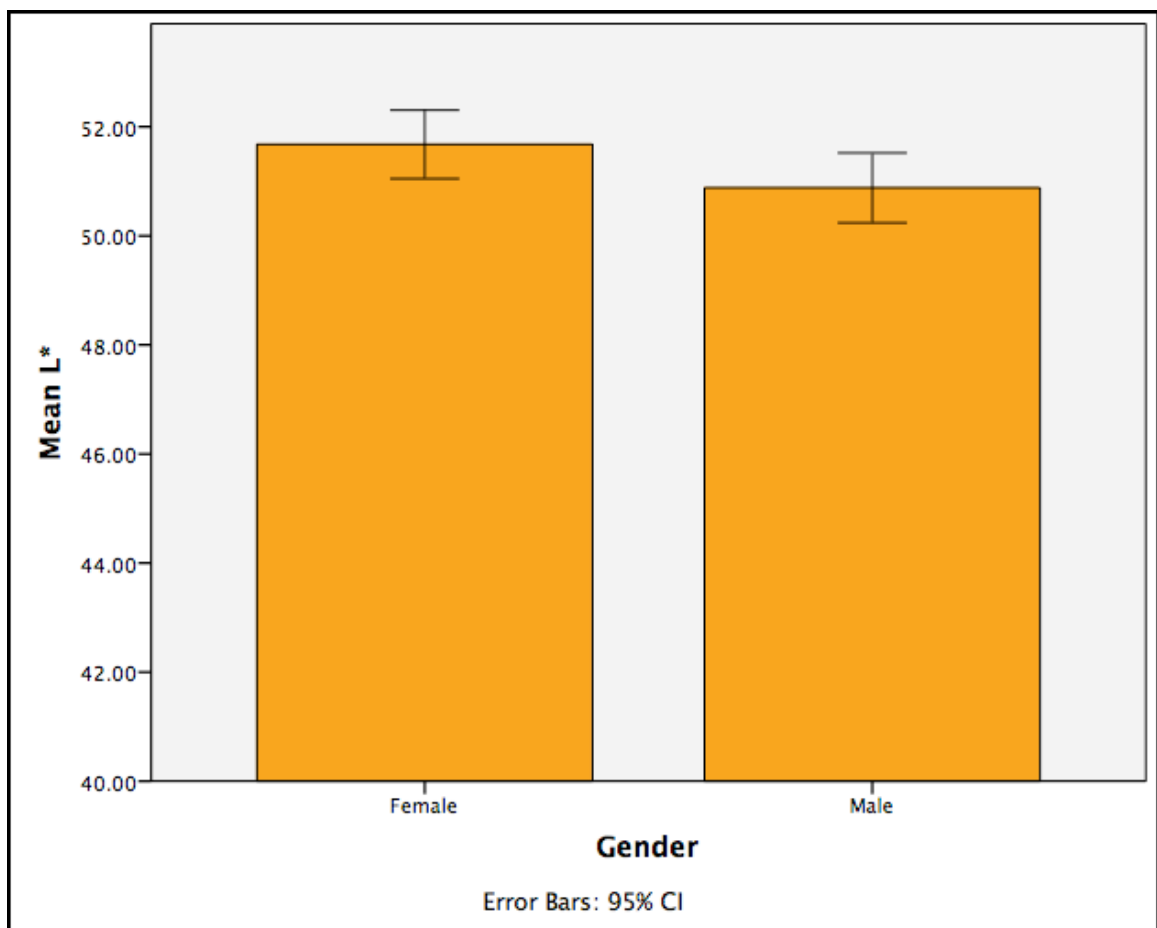


Figure 35: L* vs. Sex

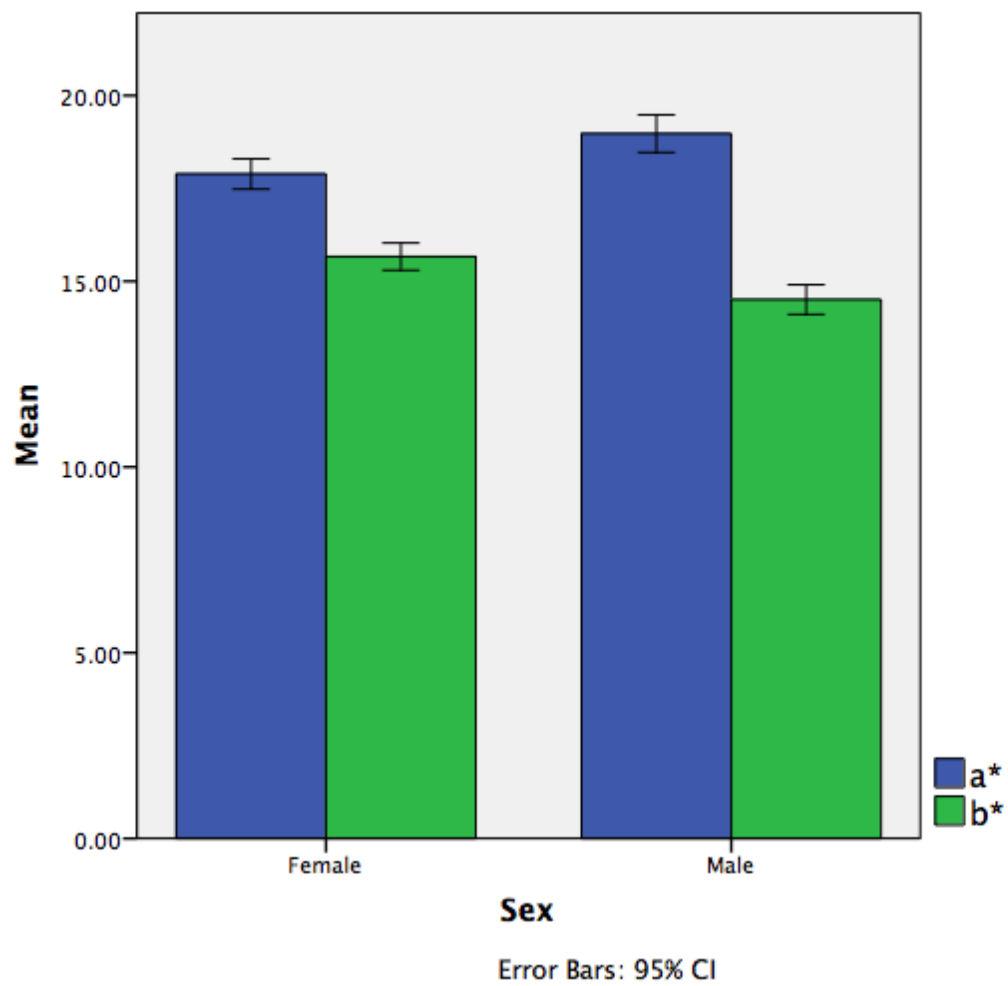


Figure 36: a^* and b^* vs. Sex

d) Gingival Color Based on Ethnicity

L*, a*, and b* values were then stratified into four groupings according to participant ethnicity (White, Black, Asian, or Hispanic). The mean L*, a*, and b* values for each group were calculated and reported. (Table 4)

- L* values ranged from 52.08 ± 5.07 in the white group, to 50.07 ± 3.70 in the black group, with a mean value of 51.34 ± 5.10 across all participants.
- a* values ranged from 18.75 ± 3.28 in the Hispanic group, to 16.88 ± 3.69 in the black group, with a mean value of 18.34 ± 3.64 across all participants.
- b* values ranged from 16.62 ± 2.68 in the Asian group, to 14.42 ± 2.68 in white group, with a mean value of 15.17 ± 3.11 across all participants.

Ethnicity	n	Mean $\pm$ SD L*	Mean $\pm$ SD a*	Mean $\pm$ SD b*
White	42	52.08 ± 5.07	18.36 ± 3.68	14.42 ± 2.68
Asian	20	50.62 ± 5.67	18.68 ± 3.56	16.62 ± 3.36
Black	14	50.07 ± 3.70	16.88 ± 3.69	15.52 ± 3.32
Hispanic	14	50.60 ± 4.74	18.75 ± 3.28	15.28 ± 3.12
Total	90	51.34 ± 5.10	18.34 ± 3.64	15.17 ± 3.11

Table 4: Mean Spectral Values vs. Ethnicity

An ANOVA was performed to determine statistical significance of these observed differences. The ANOVA suggested:

- Statistically significant differences in L^* ($p=0.006$).
- Statistically significant differences in a^* ($p=0.017$).
- Statistically significant differences in b^* ($p<0.001$).

Fischer's LSD post-hoc tests were performed to determine any statistically significant differences in L^* , a^* , and b^* .

- For L^* , (Figure 37)
 - The White group had a significantly higher mean L^* (gingiva was higher in value) than the Asian, Black, and Hispanic groups. ($p=0.010$; $p=0.009$; $p=0.034$)
 - Other groups showed no significant differences between each other.
- For a^* , (Figure 38)
 - The Black group had a significantly lower mean a^* (gingiva was more green) than the White, Asian, and Hispanic groups. ($p=0.007$; $p=0.003$; $p=0.006$)
 - Other groups showed no significant differences between each other.
- For b^* , (Figure 38)
 - The White group had a significantly lower mean b^* (gingiva was more blue) than the Asian, Black, and Hispanic groups. ($p<0.001$; $p=0.017$; $p=0.040$)
 - The Asian group had a significantly higher mean b^* (gingiva was more yellow) than the White, Black, and Hispanic groups. ($p<0.001$; $p=0.027$; $p=0.004$)
 - Other groups showed no significant differences between each other.

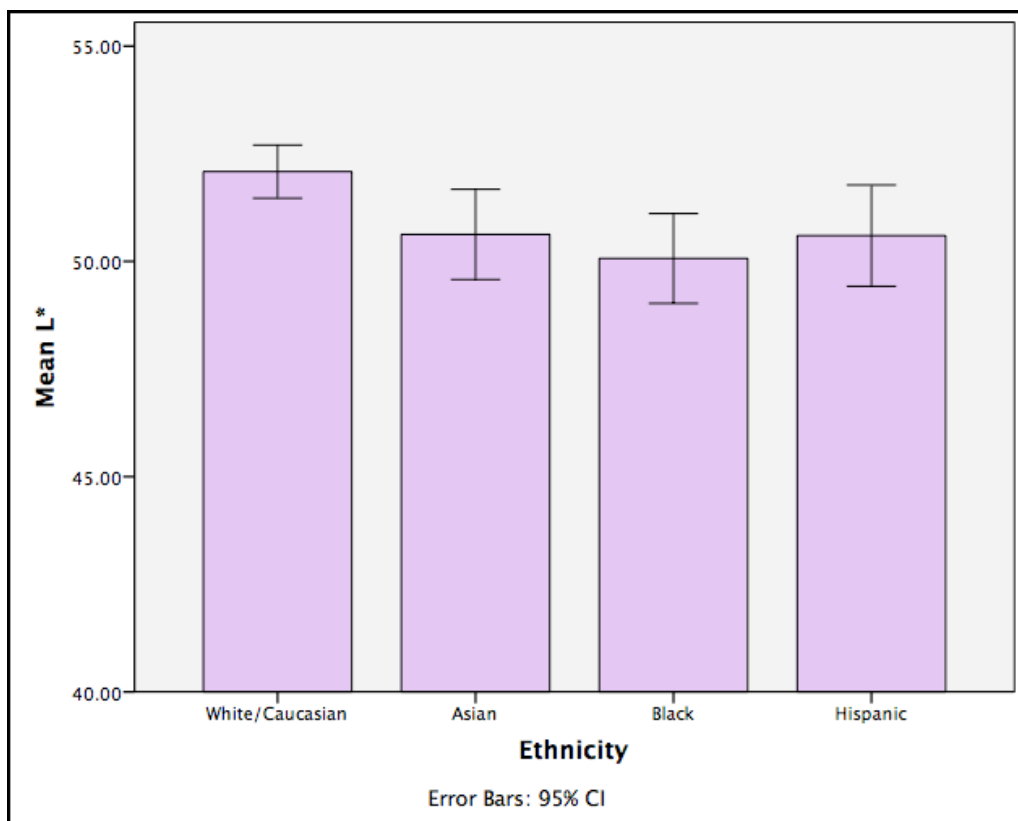


Figure 37: L* vs. Ethnicity

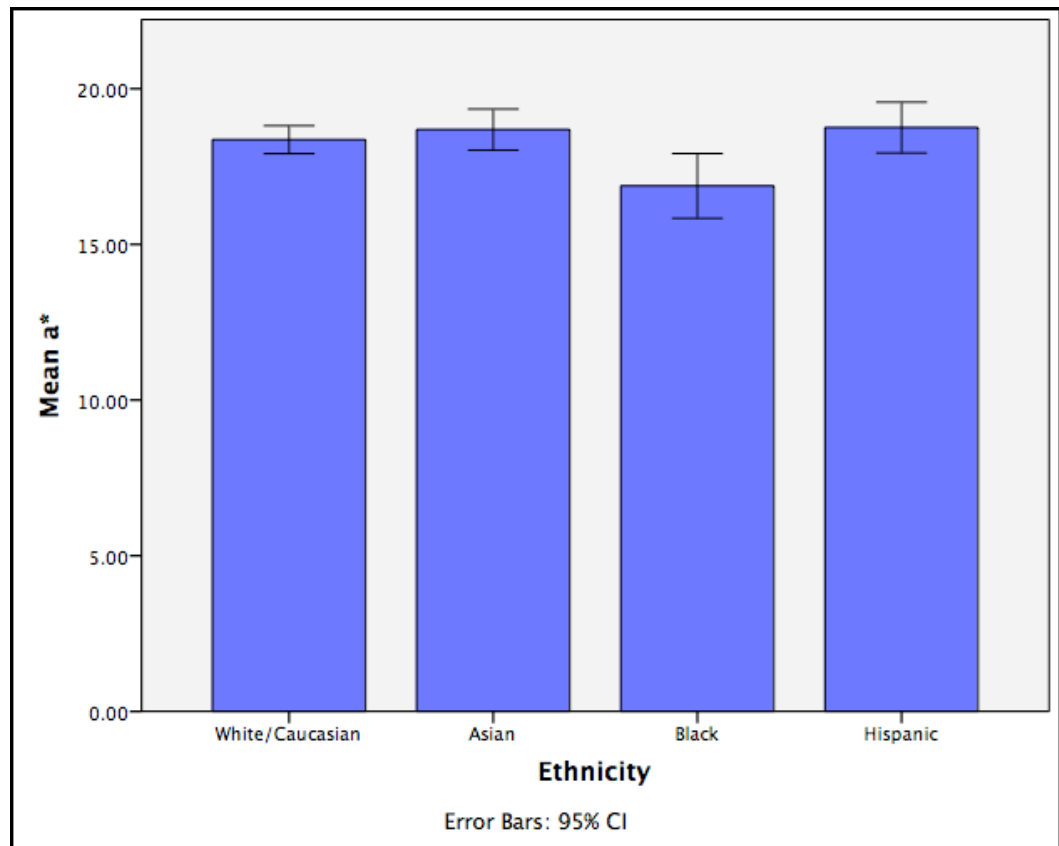


Figure 38: a* vs. Ethnicity

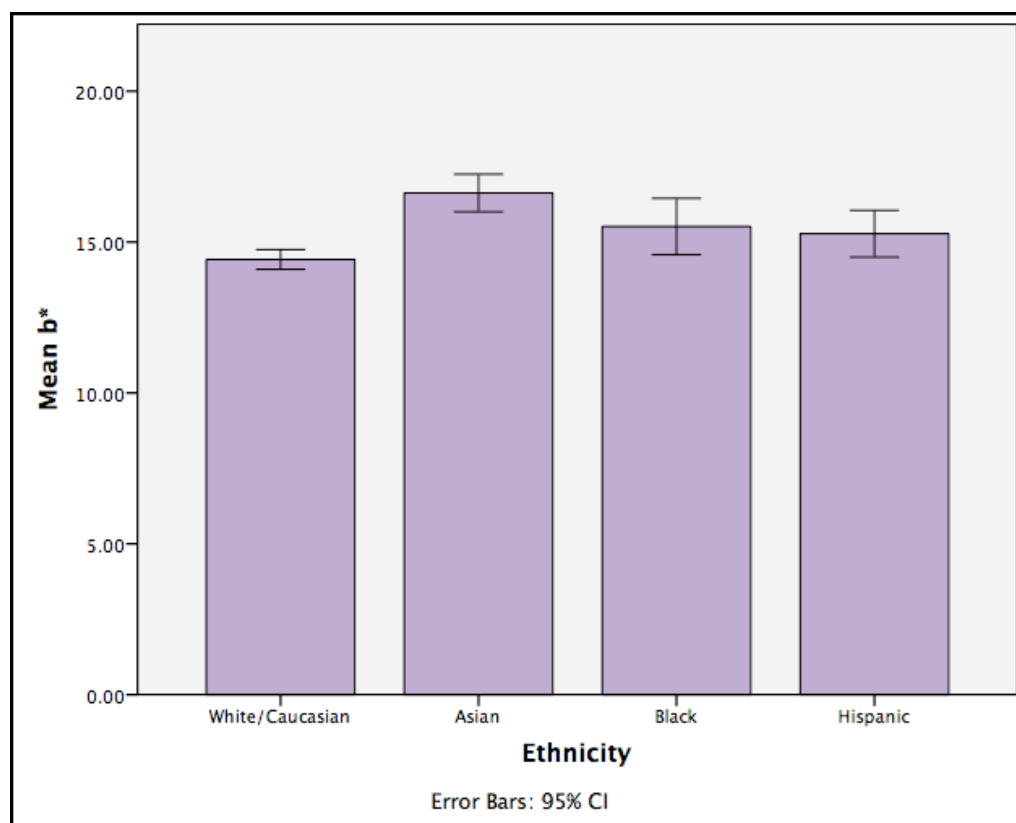


Figure 39: b* vs. Ethnicity

III. Discussion

a) Gingival Color Based on Tooth Number

The mean spectral values for all sites studied were determined to be:

- $L^*=51.29\pm5.14$,
- $a^*=18.33\pm3.62$, and
- $b^*=15.14\pm3.11$.

The L^* values exhibited a statistically significant decrease as the sites moved posteriorly. This suggests that the gingiva appears darker, or more gray, as the tooth location progresses more posteriorly. The differences in these values were observed to be significant, in particular values between the canines and their respective central incisor sites in same quadrant (i.e. #6 vs. #8). This observation suggests that either 1) actual differences exist in the value and chroma of gingiva between canine sites or central incisor sites, or 2) differences exist in the ability of the spectrophotometer to acquire spectral readings in the central incisor versus in a canine. Such a difference could be caused by improper isolation of the site observed by the ambient light shield or by the inability to align the spectrophotometer exactly perpendicular to the buccal tooth surface in canine teeth, due to restrictions caused by the lips at the corner of the mouth.

A significant difference was also noted in the variation between two centrals in the same participant (mean $\Delta E=5.32$), versus two canines in the same participant ($\Delta E=6.58$). This observation may suggest that the difference between the central sites is less because they are directly adjacent to each other, or that it is that they are more easily observed in standardized fashion due to their location and thus have less variation. Similarly, canines may show increased variability due to being located across the arch from each other, or due to spectrophotometric difficulties in observing one or both sites.

Potential follow-up investigation that may be performed to attempt to differentiate the greater cause of these differences is 1) repeat the study with tooth structure as opposed to gingiva, or 2) use a different design of spectrophotometer, perhaps a point/contact

design with a smaller input, and note if similar effects occur. However, both of these alternatives still present some challenges. First, a wealth of data exists suggesting variability in the shade of central incisors versus canines, canines appearing to have more chroma. Second, contact-type spectrophotometers may cause pressure to be placed on gingival tissue, and cause blanching and erroneous data.

b) Gingival Color Based on Tooth Type

Similar observations were made when stratifying the data by overall tooth type as were made when differentiating by each individual tooth. The areas of statistically significant difference were between the central incisors and lateral incisors, and between central incisors and canines. The central incisors, therefore, showed the most similarity with each other, and the most variation when compared to other teeth. One possible conclusion to be drawn from this is that when using a non-contact spectrophotometer, as was used in this study, the most specificity may be obtained by using central incisor sites for spectral measurements.

c) Gingival Color Based on Sex

Although no significant differences were observed in L^* values based on sex, significant differences were observed in the chromatic metrics. Males presented greater a^* values (more red) and lesser b^* values (more blue). Combined, these metrics suggest that the males' gingiva was significantly more purple than that of the females in the sample population. One possible explanation is that the male gingiva is generally more inflamed. This corresponds with existing literature suggesting that males have higher rates of gingivitis and periodontal disease than females.^{193,194} While our exclusion criteria removed patients with deep pocketing (3mm+) and bleeding on probing, a low-level gingival hypertrophy may be present. Further research is necessary using gold standard techniques of qualifying gingival disease (i.e. full-mouth probing, radiographs), to establish better correlation to these spectrophotometric values.

d) Gingival Color Based on Ethnicity

The results obtained in this study correlate to previous research⁵² suggesting differences in gingival pigmentation due to racial pigmentation. The White group presented the highest L* and lowest b*, suggesting gingiva with the highest value (lightest) and the least yellow hue. Meanwhile, the Black group had the lowest a* and the Asian group the highest b* values. These high chromatic values suggest higher amounts of intrinsic gingival pigmentation in these groups than in the White group. This is to be expected because of the relative lack of gingival melanin pigmentation in Whites versus other racial groups.⁵²

This study examined a limited participant population drawn from the patients of a single dental school in a single state. Areas of future research that would render more global results include obtaining information from other states, geographic areas, and countries, and from different areas of practice (e.g. private practices, community health centers, etc). The participant population in this study also used a population representative to the patients in an academic dental center. Future research using equal-sized groups based on participant demographics may help elucidate on the differences between these demographic groups.

Chapter 5: Specific Aim 2. An *in vivo* Spectrophotometric Assessment of Gingival Thickness, Translucency, and the ‘Biotype’

I. Materials and Methods

The second phase of this project was directed toward performing 1) a direct analysis of buccal gingival thickness in the anterior maxilla, 2) a spectrophotometric assessment of gingival translucency of the gingival soft tissues surrounding natural dentition in the esthetic zone, using traditional and novel metrics and 3) attempt to elucidate any statistical correlation between these thickness measurements and translucency metrics.

Patients were recruited for this study from among the general patient population of the Harvard Dental Center. After recruitment, screening of eligibility as an active patient of the Dental Center, and patient agreement of participation, a baseline periodontal examination of teeth #'s 8 and 9 was performed.

Inclusion criteria for this aim were: patient age eighteen and above; either tooth 8 or 9 present as a virgin tooth, restored tooth, or all-ceramic or ceramometal restorations. Exclusion criteria were: ADA class 3 or greater, bleeding on probing to buccal sites of teeth #'s 8 or 9, or probing depths greater than 3mm in teeth #'s 8 or 9; both teeth 8 and 9 were implant restorations.

Eighty-nine participants qualified for this portion of the analysis and eighty-nine central incisor sites were examined. Tooth #8 was used in seventy-nine analyses and, due to implants present in the #8 site, tooth #9 was used in ten analyses. Participants were asked to self-identify for sex and ethnicity. Patient ages ranged from 25 to 87 with a median of 39 years. Participant demographics for this section were as follows:

Sex	n
Male	41
Female	48

Ethnicity	n
White	42
Asian	20
Black	14
Hispanic	13

Gingival Thickness Measurement

After baseline examination discussed in chapter 4, analysis of gingival thickness, biotype, and translucency was performed according to the following protocol. A single anterior tooth site, #8, was chosen for all analyses. If tooth #8 was an implant restoration, tooth #9 was used with identical protocol. As a part of the spectrophotometric evaluation, sterile black and white strips were simultaneously inserted, using minimal force, into the midbuccal gingival sulcus of the investigated tooth, until the strip was tactilely determined to have reached the floor of the sulcus. Using a non-contact dental spectrophotometer (Crystaleye; Olympus, Tokyo, Japan), spectral images of the buccal free gingival margin with in-place strips were obtained. (Figure 40)(Figure 41) Reflectance values were transferred from the spectrophotometer to a personal computer for analysis using CIELAB color coordinates. With the strips remaining in the sulcus, an anterior sextant polyvinyl siloxane impression was obtained, picking up the in-place strips. The impressions were then poured in a Type 4 dental stone. After setting, the casts were ground using a model trimmer such that they were sectioned along the long axis of #9 perpendicular to the buccal surface, sectioning midway through the embedded strip. Using a digital caliper (Mitutoyo, Aurora, USA), the thickness of the buccal gingiva was obtained at locations 1mm and 2mm apically to the FGM within the gingival sulcus. Measurement was performed by placing the fixed member of the caliper flush against the sectioned face of the dental cast, such that the fixed ‘tooth’ of the caliper was visually flush

with the internal aspect of the sulcus where the strip was removed. The caliper was closed until at the appropriate depth into the sulcus the mobile tooth of the caliper was flush with the most anterior portion of the buccal gingival surface of the cast. Measurement was obtained from the digital readout of the caliper. (Figure 42) The caliper output was to hundredths of a millimeter, with an accuracy of 0.01mm.

The thickness data was then stratified with respect to participant gender and ethnicity. Ethnic groups were based on participant self-identification into groupings of White/Caucasian, Black, Asian, and Hispanic.

For analysis of differences existing according to gender, the mean values of the male and female groups were calculated and described. The average difference and percent difference between the two groups were calculated. An independent t-test was performed for both the 1mm and 2mm deep sites, with a cutoff of statistical significance of $\alpha=0.05$.

For investigation into differences amongst ethnicities, the mean values of each ethnic group were calculated and described. An ANOVA was performed with a cutoff of statistical significance of $\alpha=0.05$.

Gingival Translucency Metrics

The spectrophotometric images taken with strips in place were then examined using the integrated software (Crystaleye Application Master; Olympus, Tokyo, Japan), and three points on the spectral image were chosen for translucency analyses. For both the black strip and the white strip, three locations were used to obtain spectral data: 1) the supragingival portion of the strip (STRIP); 2) the subgingival portion of the strip, 1mm apical to the free gingival margin (FGM); and 3) a location 1mm apical to the free gingival margin but 5mm mesial or distal to the strip (ADJ). (Figure 43) Reflectance values for each location were transferred from the spectrophotometer to a personal computer for analysis using CIELAB color coordinates. Spectral data obtained from each

site was then input into statistical modeling software (SPSS; IBM, Armonk, USA) and output variables L*, a*, and b* were recorded.

Several diagnostic measurements of translucency were implemented by analyzing the L*, a*, and b* of each of these sites. Two traditional metrics of translucency, the translucency parameter and the contrast ratio, as well as a third novel metric, the patient-specific translucency parameter, were implemented to quantify the translucency of the gingiva. These metrics were defined as:

-Translucency Parameter (TP)

Translucency parameter is a metric that evaluates the absolute color difference (ΔE) between two spectrophotometrically evaluated colors; one of a translucent material overlying an absolute white substrate and one of the translucent material overlying an absolute black substrate. Translucency parameter (TP) was calculated according to the formula:

$$TP = \Delta E(FGM_{white} - FGM_{black})$$

TP utilizes the spherical nature of the CIELAB color space to assess differences in L*, a*, and b*, via a Pythagorean-derived equation:

$$\Delta E^*_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

-Contrast Ratio (CR)

Contrast ratio (CR) is a metric that evaluates the ratio of luminance (L*) caused by the brightest color and the darkest color. CR was calculated according to the formula:

$$\left(\frac{L^*_{BlackFGM}}{L^*_{WhiteFGM}} \right) * 100 .$$

-Patient-Specific Translucency Parameter (PSTP)

The Patient-Specific Translucency Parameter (PSTP) metric, introduced by the author, is calculated according to the formula:

$$\left(\frac{L^*_{BlackFGM}}{L^*_{BlackSTRIP} + L^*_{BlackADJ}} \right)$$

Gingival Translucency Analysis

The TP, CR, and PSTP were calculated for each participant site. For each metric, the translucency was then stratified with respect to participant gender and ethnicity. Ethnic groups were based on participant self-identification into groupings of White/Caucasian, Black, Asian, and Hispanic.

For analysis of differences existing according to gender, the mean TP, CR, and PSTP values of the male and female groups were calculated and described. The average difference and percent difference between the two groups were calculated. An independent t-test was performed, with a cutoff of statistical significance of $\alpha=0.05$.

For investigation into differences amongst ethnicities, the mean TP, CR, and PSTP values of each ethnic group were calculated and described. An ANOVA was performed with a cutoff of statistical significance of $\alpha=0.05$. A post-hoc analysis (Fischer's LSD) was performed to determine between which groups any statistically significant differences in mean translucency metrics existed.

Gingival Thickness vs. Translucency Correlation

Using statistical analysis software (SPSS; IBM, Armonk, USA), for each participant, recorded gingival thickness values at the 1mm site were plotted graphically against each of that patient's corresponding recorded values for gingival translucency. This resulted in graphical representations of:

- TP vs. Gingival Thickness (mm)
- CR vs. Gingival Thickness (mm)
- PSTP vs. Gingival Thickness (mm)

A linear regression analysis was performed and a line of best-fit and 95% confidence interval was calculated for each graph. The nature of the translucent-thickness relationship and the goodness-of-fit was analyzed. Pearson's correlation coefficients were calculated to determine if a linear correlation exists between each of the pairs of variables.

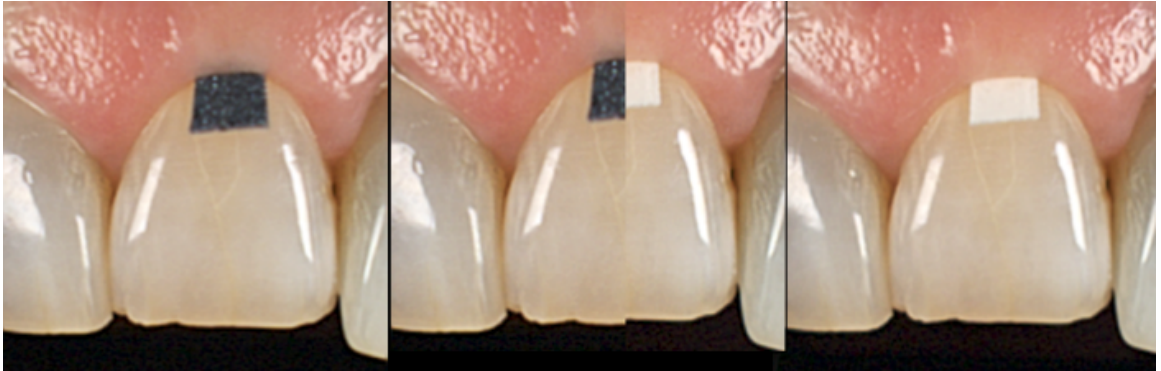


Figure 40: Spectrophotometric Images with Black and White Strips in Place³¹

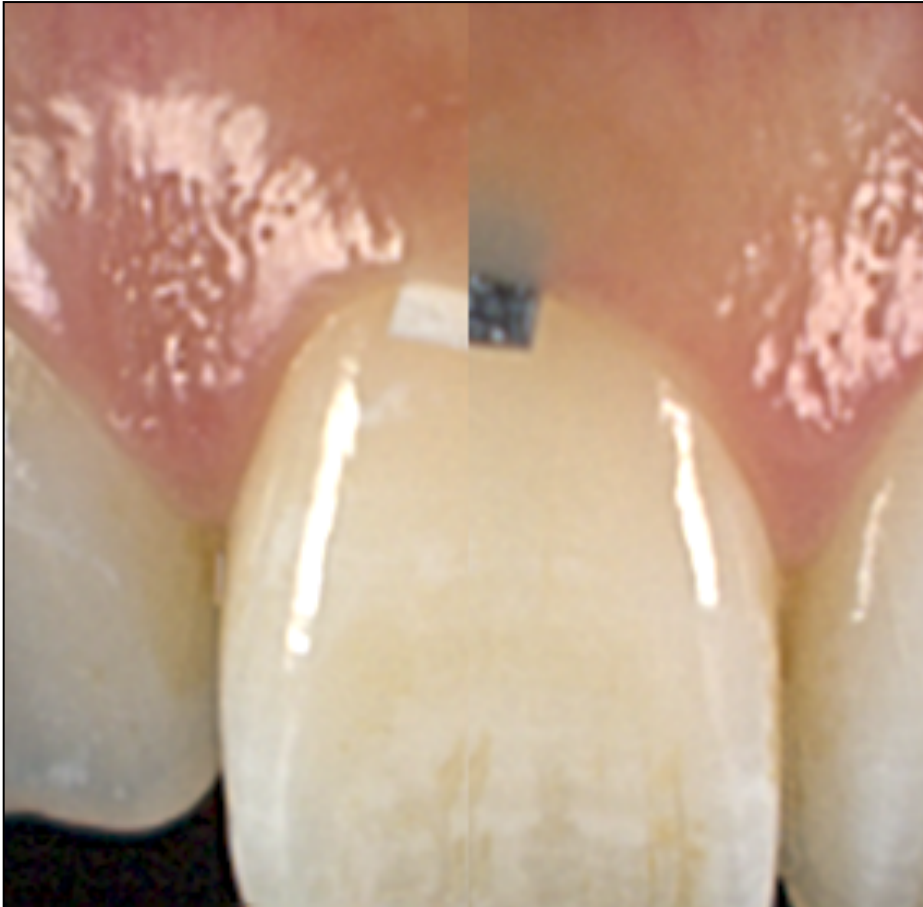


Figure 41: Spectrophotometric Image with Strips in Place³¹



Figure 42: Cross-Sectioned Cast Obtained from Impression with Strip in Place (Red Line Indicates Measurement Area)

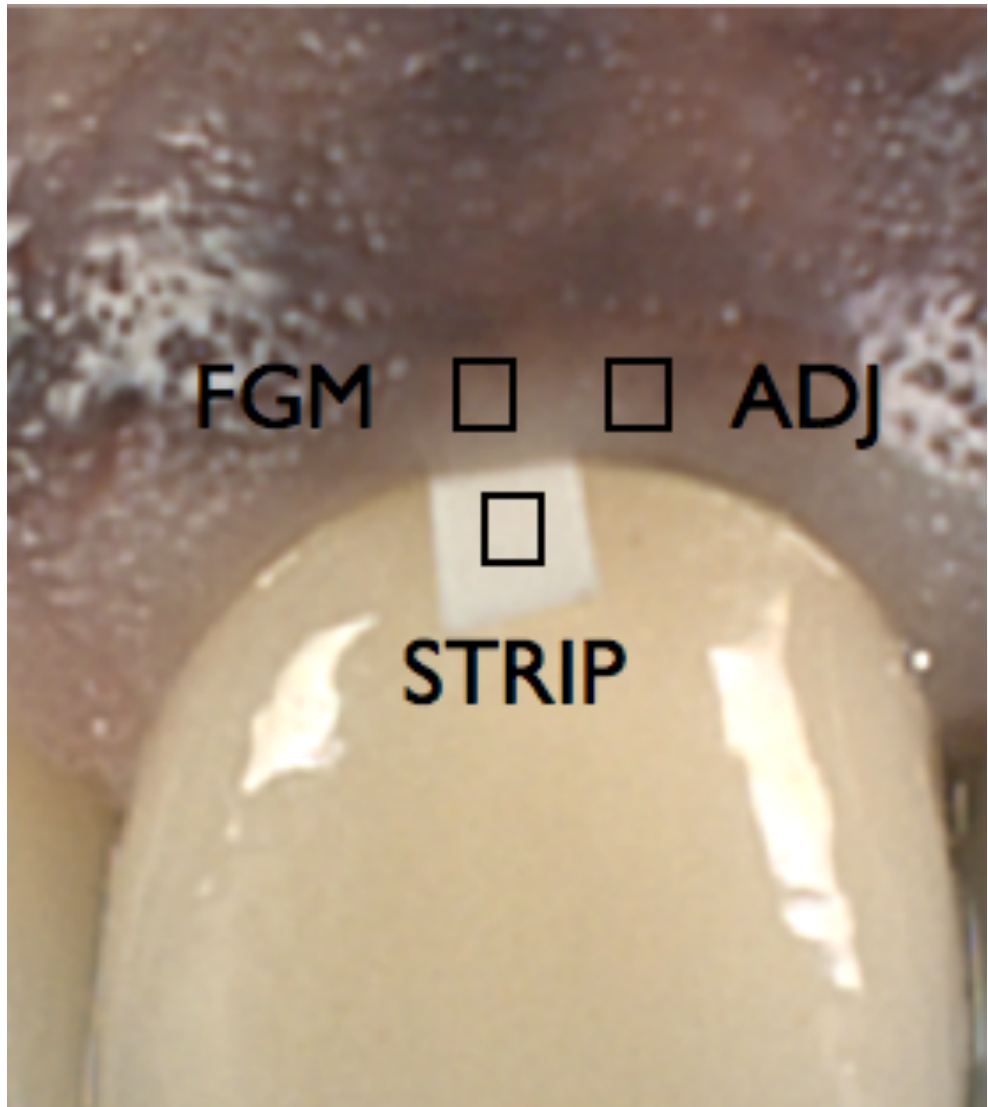


Figure 43: Spectrophotometric Image Illustrating Locations of Thickness Measurements

II. Results

a) Thickness Measurements

For measurements performed 1mm apical to the FGM, the mean gingival thickness ranged from 0.5mm to 1.90mm, with a mean $\pm$ SD of 1.08 $\pm$ 0.30mm. For the site 2mm deep, the mean gingival thickness ranged from 0.7mm to 2.7mm, with a mean 1.40 $\pm$ 0.37mm. (Table 5)

When stratified according to patient sex, for both the 1mm and 2mm sites, male participants were observed to have thicker buccal gingiva than female participants. Males exhibited a mean gingival thickness 0.13mm thicker at the 1mm site and 0.16mm thicker at the 2mm site. This difference proved statistically significant to the 0.05 level. (Table 5)

No statistically significant differences in thickness were found between racial groups at either the 1mm or 2mm to the 0.05 level.

	M	F	Mean Difference	Percent Difference	Mean Difference	Sig
Thickness at 1mm Site	1.15 $\pm$ 0.32m m	1.02 $\pm$ 0.27mm	-0.13mm	11%	-0.13mm	0.037 *
Thickness at 2mm Site	1.49 $\pm$ 0.43m m	1.32 $\pm$ 0.30mm	-0.16mm	11%	-0.16mm	0.037 *

Table 5: Gingival Thickness Measurements

b) Spectrophotometric Measurements

TP

The mean $\pm$ SD for TP in the participant population was 11.5 $\pm$ 3.7. When stratified according to participant sex, females were found to have a TP of 12.2 $\pm$ 3.4 while males were found to have 10.7 $\pm$ 3.8. These differences were found to be significant to the 0.05 level of significance (P=0.041). When stratified according to participant ethnicity, no statistically significant associations were observed.

CR

The mean $\pm$ SD for CR in the entire participant population was 85.8 $\pm$ 6.3. When stratified with respect to participant sex, no significant difference in CR was found significant to the 0.05 level of significance (p=0.146). When stratified according to participant ethnicity, no statistically significant associations were observed.

PSTP

The mean $\pm$ SD for PSTP in the entire participant population was 0.376 $\pm$ 0.05. When stratified with respect to participant sex, no significant difference in PSTP was found significant to the 0.05 level of significance (p=0.614). When stratified according to participant ethnicity, a mean difference in PSTP significant to the 0.05 level was found between the Asian and Hispanic groups, but no others. The Asian group exhibited a PSTP mean $\pm$ SD of 0.393 $\pm$ 0.031, while the Hispanic group exhibited 0.347 $\pm$ 0.107. (p=0.015).

c) *Translucency vs. Thickness Comparisons*

-TP

When presented graphically, a weak linear correlation was observed between TP and gingival thickness at the 1mm sulcular depth location. The best-fit line presented with a negative slope. Correlation was calculated to be statistically significant for modeling ($p > 0.001$). The R^2 -value was calculated as 0.113. (Figure 44) This suggests that as the gingiva increases in thickness, less translucency (as measured by TP) is observed.

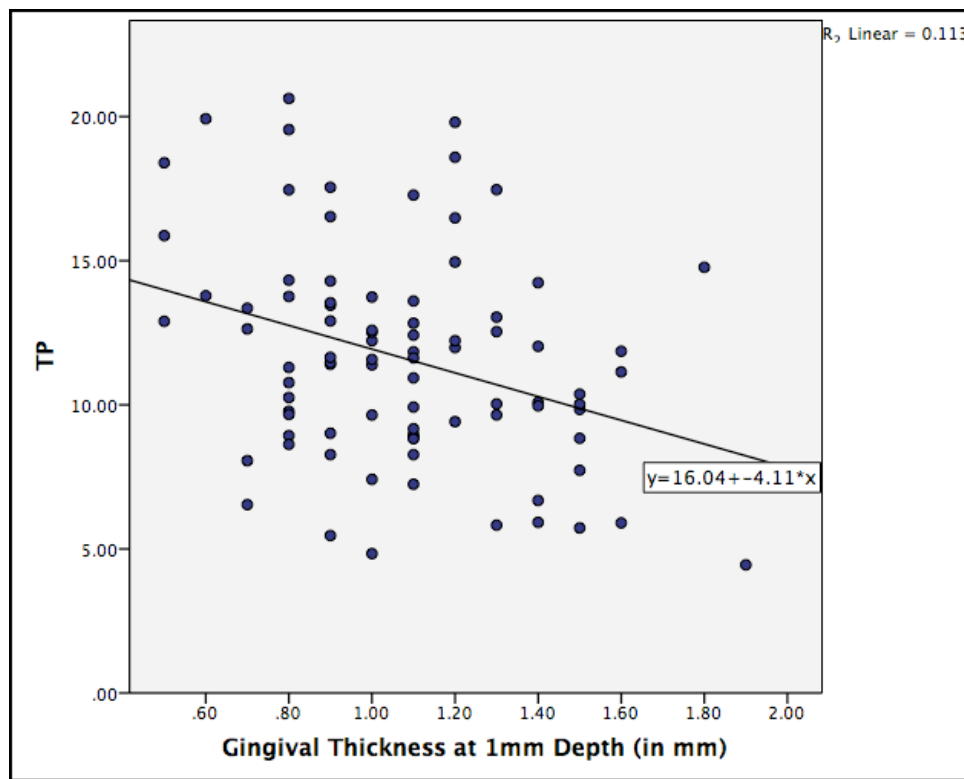


Figure 44: Translucency Parameter vs. Thickness

- CR

When presented graphically, a weak linear correlation was observed between CR and gingival thickness at the 1mm sulcular depth location. The best-fit line presented with a positive slope. Correlation was calculated to be statistically significant for modeling ($p>0.001$). (Figure 45) This suggests that as the gingiva increases in thickness, more opacity (as measured by CR) is observed. The observed graph exhibits less extreme deviations of from the line of best fit when compared to TP vs. thickness. The R^2 -value was calculated as 0.119. This suggests a slightly better fitness of the correlation line to the data than in the TP vs. thickness graph.

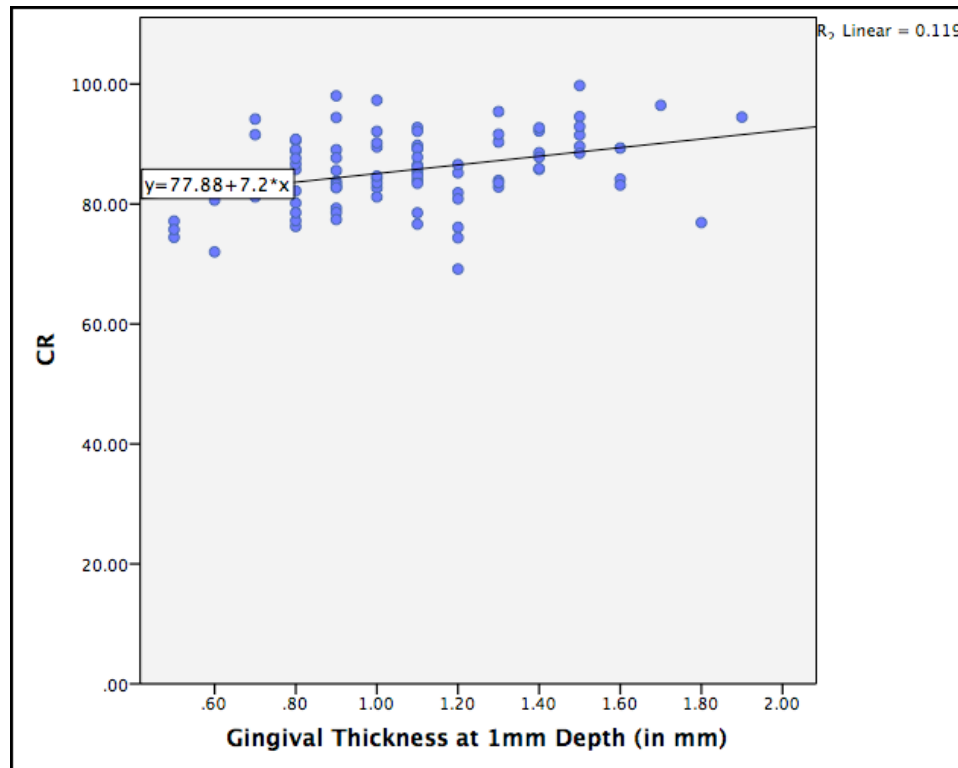


Figure 45: Contrast Ratio vs. Thickness

-PSTP

When presented graphically, a moderate linear correlation was observed between PSTP and gingival thickness at the 1mm sulcular depth location. The best-fit line presented with a negative slope. Correlation was calculated to be statistically significant for modeling ($p > 0.001$). (Figure 46) This suggests that as the gingiva increases in thickness, less translucency (as measured by PSTP) was observed. The observed scatter plot presents with less extreme deviations from the line of best fit when compared to both the TP vs. thickness and CR vs. thickness graphs. The R^2 -value was calculated as 0.363. This suggests a fitness of the correlation line to the data greater than that in both the TP vs. thickness and CR vs. thickness graphs.

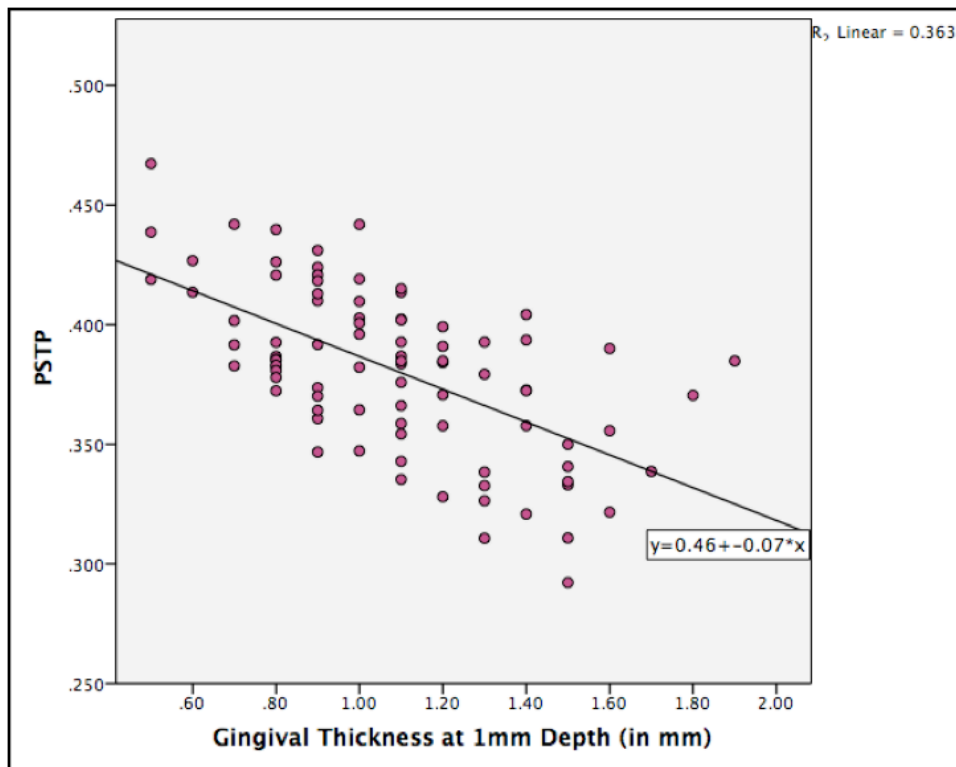


Figure 46: Patient-Specific Translucency Parameter vs. Gingival Thickness

III. Discussion

a) Thickness Measurements

Gingival thickness calculated via the cast-sectioning measurements was observed to increase as the location of the measurement moved apically. At 1mm deep into the sulcus, mean thickness was observed to be 1.08mm, while 2mm into the sulcus it was observed to be 1.4mm. This observation is in agreement with many previous studies.^{56,60,105,115,118}

A mean difference was noted in gingival thickness according to patient sex. This was also found to be in agreement with previous studies.¹¹² Possible explanations of this include intrinsic biologic difference in thickness in males versus females, as well as the increased incidence of gingivitis in males, which may cause an observed increased thickness in that population.^{193,194} A possible area of future research is a study utilizing gold-standard techniques of quantifying gingival inflammation in the participant population, allowing the effect of sex on the gingival thickness to be assessed independent of the gingival health. No differences in gingival thickness were determined with respect to participant Ethnicity.

A limitation of this analysis may be the use of a manual caliper in data acquisition. In future research, alternative techniques, such as a standardized image analysis software¹⁰⁵, or microscopic analysis and digital measurement, may be implemented such that more precise measurements can be obtained.

b) Spectrophotometric Analysis

-Translucency Parameter (TP)

TP, as a metric, is largely used for industrial purposes to quantify the translucency of a material. A pure black substrate and a pure white substrate are used within the formula $\Delta E(FGM_{\text{white}} - FGM_{\text{black}})$ to give a value for how translucent a material is. Higher values of TP mean the material is more translucent. In using the ΔE formula, TP

incorporates ΔL^* , Δa^* , and Δb^* within its calculation. This may cause inaccuracies when used in the gingival environment, as gingiva in neighboring areas may have differing a^* and b^* values, which may lead to additional variability that is not due to the white or black strips. The value is dependent on having pure white and pure black strips as benchmarks. In practice, the implementation of such strips in the oral environment is difficult, as saliva and intracrevicular fluid (ICF) may be absorbed into the strip, altering its value. There is no way to account for this in the TP metric. This makes standardization between participants unpredictable, as the amount of saliva or ICF present may vary with the individual. Further, the TP metric cannot account for the inherent spectral properties of a participant's gingiva. Variations in intrinsic value properties across the population make comparison between participants difficult.

It was determined that statistically significant differences in translucency parameter values were obtained in male versus female participants, with male participants exhibiting significantly less translucent gingiva, $TP_{\text{female}} = 12.2 \pm 3.4$ while $TP_{\text{male}} = 10.7 \pm 3.8$. As the male population exhibited a thicker average gingiva, this supports the hypothesis that thicker gingiva will exhibit less translucency. This relationship will be examined further in the next section.

-Contrast Ratio (CR)

The CR is a metric to describe the ratio of the luminance (L^*) of the brightest color in a system to that of the darkest color in the system. As such, the CR omits the chromatic values a^* and b^* from the translucency calculation. This lack of chromatic values may be beneficial for translucency measurement in the intraoral environment. As mentioned above, the addition of the chromatic values may add variability based on the participant's intrinsic chroma, resulting in less accuracy due to variation in the population. The omission of these values in CR may increase its accuracy. However, as with TP, a limitation of the CR metric is that white and black strips are not ideal in the oral environment. Fluctuation in the value of the white and black strips due to the moisture present in the sulcus lead to a lack of standardization between participants.

Further, as with TP, it does not take into account the intrinsic spectral properties of each participant. With each participant having variable inherent levels of L*, comparisons between participants may not be entirely accurate.

No statistically significant differences were determined between sexes or among racial groups using this metric.

-Patient-Specific Translucency Parameter (PSTP)

The PSTP is a novel metric introduced by the author in an attempt to quantify the amount of value transmitted through a particular participant's gingiva, standardized against that participant's native gingiva and for that particular white or black strip. Using

the formula $\left(\frac{L^*_{BlackFGM}}{L^*_{BlackSTRIP} + L^*_{BlackADJ}} \right)$, the PSTP can be seen as a “sliding scale” of

gingival translucency. It utilizes the L* value of the strip outside of the sulcus as the “maximum value” (i.e. completely translucent gingiva), and an area of gingiva adjacent to the strip as the “minimum value” (i.e. if the gingiva was completely opaque). The PSTP value is therefore a percentage of the strip visible through the gingiva, standardized for that particular strip and participant's intrinsic gingival spectral properties. By only incorporating L*, it is a measure of only the value that transmits through the gingiva.

c) Translucency vs. Thickness Comparisons

For each metric, the goodness of fit of the linear regression lines comparing each translucency metric with measured gingival thickness was examined. The significance and R-square values of each translucency-thickness curve were as follows:

- TP vs. Thickness: $p < 0.001$; $R^2 = 0.113$
- CR vs. Thickness: $p < 0.001$; $R^2 = 0.119$
- PSTP vs. Thickness: $p < 0.001$; $R^2 = 0.363$

All metrics exhibited significant linear correlation between translucency and gingival thickness. However, the goodness of fit of these correlations varied; with the traditional parameters TP and CR exhibiting similar, albeit weak correlation coefficients: 0.113 and

0.119, respectively. The novel metric PSTP, however, showed an increased correlation coefficient of 0.363. This suggests that the PSTP may have more predictive value when relating spectrophotometrically obtained translucency properties to gingival thickness. This is largely due to the standardization offered by the metric measuring the participant's native gingiva as well as the strip used in that particular participant. The variability in the value of the L^*_{strip} values suggests that the strips were affected differently depending on the oral environment of each participant. The mean L^*_{strip} value for the black strip, which theoretically should be 0 in an ideal black strip, was 29.02. This suggests that the TP and CR values, which rely on black standards being 0 and white standards being 100, are not ideal in the oral environment. One limitation of this method, however, is that in the standardization of the participant's native gingiva, the participant's underlying tooth or restoration shade may effect the L^*_{ADJ} values. While the shade was chosen at a site apically in the gingival apparatus, it is still possible that a darkened underlying tooth structure may lead to the L^*_{ADJ} being skewed. One way to avoid this possible skewing in future research is to exclude participants with root canal discolorations or other extraneous gingival hues, which were not a factor of exclusion criteria in this study; this would also have effects on the translatability of the research, however.

Chapter 6: Specific Aim 3. A Spectrophotometric Analysis of the Coverage Error of Gingival Acrylic Shade Guide Systems

I. Materials and Methods

The third phase of this project was directed toward performing 1) an analysis of the coverage error (accuracy of color reproduction) of acrylic gingival restorative materials in a participant populations, and 2) a proof-of-concept study into the design of a spectrophotometrically-driven shade-guide system for gingival replacement materials.

Patients were recruited for this study from among the general patient population of the Harvard Dental Center. After recruitment, screening of eligibility as an active patient of the Dental Center, and patient agreement of participation, a baseline periodontal examination of teeth #'s 8 and 9 was performed.

Inclusion criteria for this aim were: patient age eighteen and above; either tooth 8 or 9 present as a virgin tooth, restored tooth, or all-ceramic or ceramometal restorations. Exclusion criteria were: ADA class 3 or greater, bleeding on probing to buccal sites of teeth #'s 8 or 9, or probing depths greater than 3mm in teeth #'s 8 or 9.

Eighty-nine participants qualified for this portion of the analysis and eighty-nine central incisor sites were examined. Tooth #8 was used in seventy-nine analyses and, due to implants present in the #8 site, tooth #9 was used in ten analyses. Participants were asked to self-identify for sex and ethnicity. Patient ages ranged from 25 to 87 with a median of 39 years. Participant demographics for this section were as follows:

Sex	n
Male	41
Female	48

Ethnicity	n
White	42
Asian	20
Black	14
Hispanic	13

a) In-vivo Gingival Shade Acquisition

An area of the attached gingiva 4mm apical to the FGM of a single anterior tooth, was chosen and observed for each participant. Tooth #8 was chosen for all analyses. If tooth #8 was an implant restoration, tooth #9 was used with identical protocol. Using a non-contact dental spectrophotometer (Crystaleye; Olympus, Tokyo, Japan), spectral images of the study area were obtained, ensuring that no pressure was placed upon the area and that the area was isolated from ambient light sources. Reflectance values were transferred from the spectrophotometer to a personal computer for analysis using CIELAB color coordinates.

b) In-vitro Material Shade Acquisition

Five different PMMA gingiva acrylic restorative material systems were selected. Systems chosen for analysis included:

- GC Acrylic (GRADIA Gum Shades; GC America, Inc., Alsip, IL),
- Ivocap (Ivoclar Vivadent, Inc., Amherst, NY),
- Lucitone 199 (Dentsply International, York, PA),
- Easy-Flow (Henry Schein, Melville, NY), and
- Candulor (Ivoclar Vivadent, Inc., Amherst, NY).

Current, complete commercial shades for each system were obtained. Shade tabs were adjusted by removing from lateral and posterior surfaces such that the registered area of

each sample was resultantly uniformly 1mm thick. Front-facing shade tab surfaces were not adjusted from states from which they were distributed by manufacturer. All shade tabs were glossy and gloss was left intact. For each system, all of the constituent shade tabs were individually placed into a specially designed isolation box (Crystaleye; Olympus, Tokyo, Japan), such that the tab orientation and distance from the spectrophotometer lens was identical to the intraoral environment. A custom specimen holder was used to ensure shade tab surface was at a proper distance and perpendicular to the spectrophotometer. Three readings were obtained from different locations of each shade tab. Reflectance values were transferred from the spectrophotometer to a personal computer for analysis using CIELAB color coordinates. The mean CIELAB values for each of the five shade guide sets were calculated.

c) Coverage-Error Analysis

For each participant, L*, a*, and b* values were obtained from the specified location 4mm apical to the free gingival margin. For each in-vitro shade tab sample, L*, a*, and b* values were obtained from the standardized central location.

For each shade tab, the absolute color difference between that tab and each participant was calculated according to the formula $\Delta E(\text{Participant} - \text{Tab})$, via the aforementioned equation:

$$\Delta E^*_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

In addition, the absolute difference in each of the constituent color values, ΔL^* , Δa^* , Δb^* , was calculated and recorded for each participant-shade tab combination.

For each of the participants, the shade tab with the smallest ΔE value for that value was determined for the shade system. These minimum ΔE values were averaged for each shade guide system, the result being the average ΔE between a participant and the best possible match from the constituent tabs of that shade guide system, or *coverage error*. The coverage errors of each system were calculated and compared. Using statistical modeling software (SPSS; IBM, Armonk, USA), ANOVA analyses were performed to

determine if statistically significant differences existed in coverage area amongst the five different shade guides. If necessary thereafter, post-hoc analysis was performed using Tukey HSD. Graphical representations of the mean CE of each system were produced.

The CEs of the differing shade guides were then stratified with respect to participant sex and ethnicity. An independent t-test was performed to determine if statistically significant differences existed between mean coverage error and participant sex. ANOVA analyses were performed to determine if statistically significant differences existed between mean coverage error and participant ethnicity. If necessary thereafter, post-hoc analysis was performed using Tukey's HSD.

The value and chroma accuracy of the best-match shade tabs were also calculated and stratified according to participant sex and ethnicity. To calculate value accuracy, the ΔL^* was calculated between each participant and the best-match shade tab. For chroma accuracy, a separate metric, ΔC^* was used. The C^* metric is a combination of the a^* and b^* values, according to the formula, $\sqrt{a^2 + b^2}$. The C^* value was calculated for each participant, and for each shade tab. The ΔC^* were then calculated and stratified according to participant sex and ethnicity. An independent t-test was performed to determine if statistically significant differences existed between L^* or C^* and participant sex. ANOVA analyses were performed to determine if statistically significant differences existed between L^* or C^* and participant ethnicity. If necessary thereafter, post-hoc analysis was performed using Tukey's HSD.

d) Graphical Analysis of Coverage Error of Gingival Shade Guides

The L^* , a^* , and b^* values of all participants and gingival restorative material shade tabs were compiled and input into a color management and analysis software package (ColorThink 2.3; CHROMIX, Seattle, USA). Two-dimensional and three-dimensional representation graphs of the distribution of participants and restorative materials with respect to L^* , a^* , and b^* were created and analyzed. By observing locations, differences in location, and distribution of shade tabs with respect to the

participant population, overall inferences were made regarding the limitations and shortfalls of the examined shade guides.

e) The Fabrication of an Ideal Two-Tab Shade Guide for a Sample Population: A Proof-of-Concept Study

After observation and interpretation of the CE analysis, as well as the two-dimensional and three-dimensional representation graphs, all values were input into statistical modeling software (SPSS; IBM, Armonk, USA). Calculations were then performed in attempts to determine if a theoretical two-shade shade tab set could be created such that they are best fit to the participant population and thus have a minimal CE. Via empirical calculations, different pairs of L^* , a^* , and b^* values were input into a coverage error analysis versus the participant population, with goal of minimizing the CE. L^* , a^* , and b^* values were chosen based on the location of existing shade tabs within the three-dimensional representation plot and then adjusted independently to minimize the resultant coverage error value. When possible, the minimize function integrated within the modeling software was used to locate ideal L^* , a^* and b^* values that would produce a minimum CE. After the resultant CE values were minimized, the two colors chosen were entered into the color management software for further visualization and analysis.

II. Results

a) Coverage Error of Shade Guides in a Study Population

Using the explained protocol, the coverage errors of each of the five sets were tabulated. (Figure 47)

- GC Acrylic: $CE=5.89\pm2.97$
- Lucitone: $CE=6.55\pm3.33$
- IvoCap: $CE=10.78\pm3.80$
- Schein: $CE=7.92\pm3.54$
- Candulor: $CE=8.13\pm3.85$
- Overall Mean: $CE=7.85\pm3.88$

ANOVA analysis was performed to determine if the differences in coverage error present amongst the shade guide systems had statistical significance for the participant population. Such differences were deemed present ($p<0.001$) and a Tukey's HSD was then performed with 0.05 alpha. The following statistically significant differences were observed: (Figure 47)

- The Ivocap coverage error was significantly higher than all other materials. ($p<0.001$ for all comparisons)
- The GC Acrylic coverage error was significant lower than the Ivocap ($p<0.001$), Schein ($p<0.001$), and Candulor ($p<0.001$) groups.
- The Lucitone 199 coverage error was significant lower than the Ivocap ($p<0.001$), Schein ($p<0.001$), and Candulor ($p<0.001$) groups.
- The GC Acrylic and Lucitone 199 groups exhibited the lowest coverage errors and exhibited no statistically significant difference from each other.

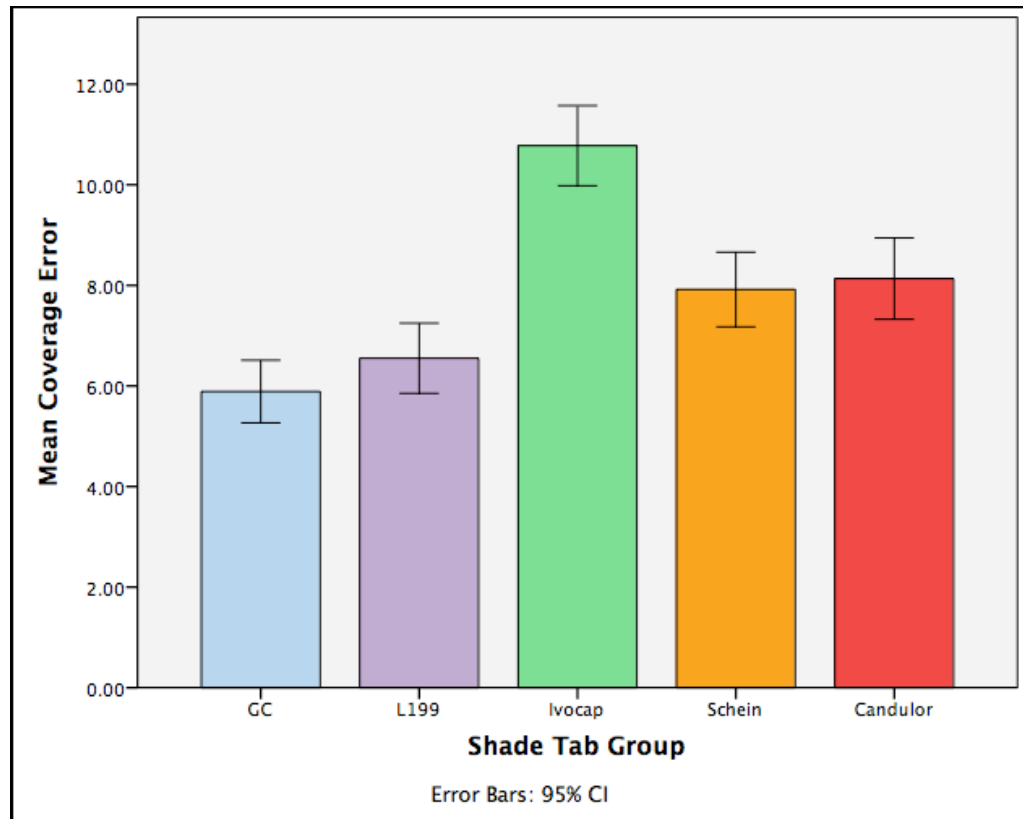


Figure 47: Mean Coverage Error vs. Shade Tab Group

b) Coverage Error of Gingival Shade Guides with Respect to Participant Sex

The mean ΔE between each participant and the best match of each shade guide set was then stratified with respect to participant sex. The female group best matching acrylic material exhibited a mean difference of $\Delta E=8.15\pm4.10$, whereas the male group exhibited $\Delta E7.46\pm3.56$. An independent sample t-test was performed and this difference was found not to be statistically significant ($p=0.062$).

The ΔL^* and ΔC^* values were then calculated between each participant and the best match of each shade guide set, and stratified to participant sex. An independent sample t-test was performed and no statistically significant differences were established for either ΔL^* or ΔC^* ($p=0.055$; $p=0.136$).

c) Coverage Error of Gingival Shade Guides with Respect to Participant Ethnicity

The mean ΔE between each participant and the best match of each shade guide set was then stratified with respect to participant ethnicity. An ANOVA analysis was performed and no statistically significant differences were found between CE and participant ethnicity. ($p=0.327$).

The ΔL^* and ΔC^* values were then calculated between each participant and the best match of each shade guide set, and stratified to participant ethnicity. An ANOVA was performed and statistically significant differences in both ΔL^* and ΔC^* were identified ($p=0.004$; $p<0.001$). Tukey HSD post-hoc analysis was performed. (Figure 48) (Figure 49)

- The mean ΔL^* was significantly less in the Asian group than in the White group. ($p=0.007$) This suggests that across all shade guide sets, the restorative materials more adequately reproduced the value of participant gingiva in the Asian population than in the White population.
- No statistically significant comparisons in ΔL^* were present amongst any other racial groups.
- The mean ΔC^* was significantly less in the White group than in the Asian and Black groups. ($p<0.001$; $p=0.022$) The mean ΔC^* was significantly less in the Hispanic group than in the Asian and Black groups. ($p=0.023$) This suggests that across all shade guide sets, the restorative materials more adequately reproduced the chroma of participant gingiva in the White population than in the Black and Asian populations.
- No other statistically significant comparisons in ΔL^* were present amongst racial groups.

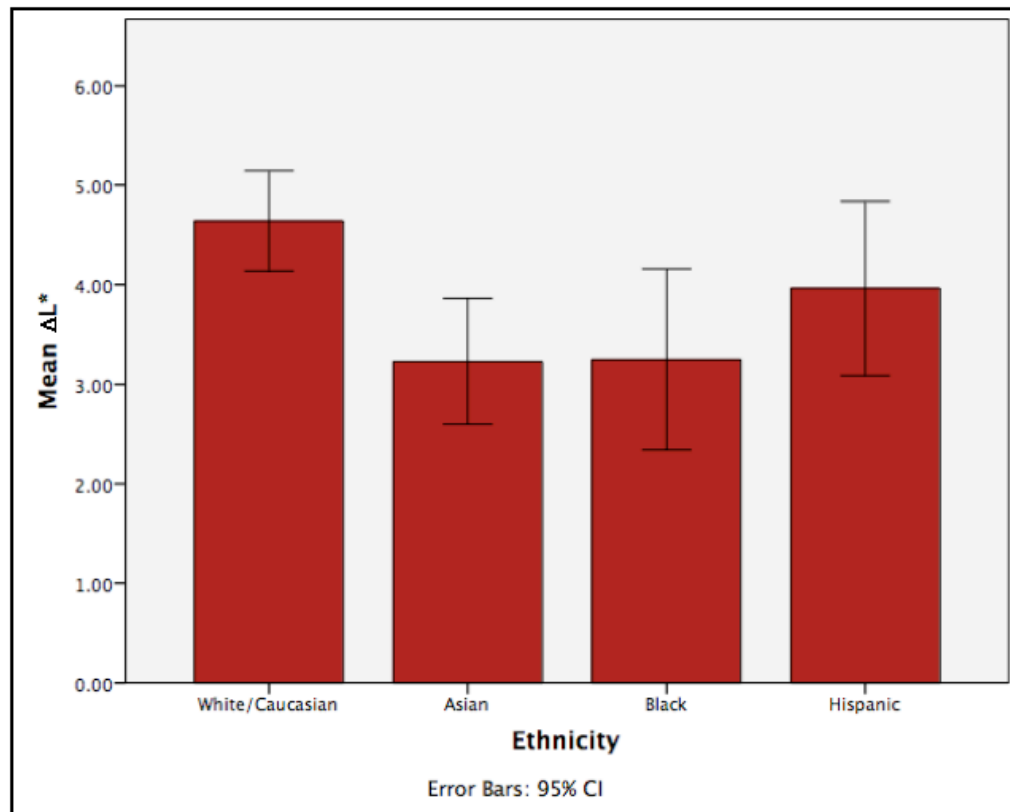


Figure 48: Mean ΔL^* vs. Participant Ethnicity

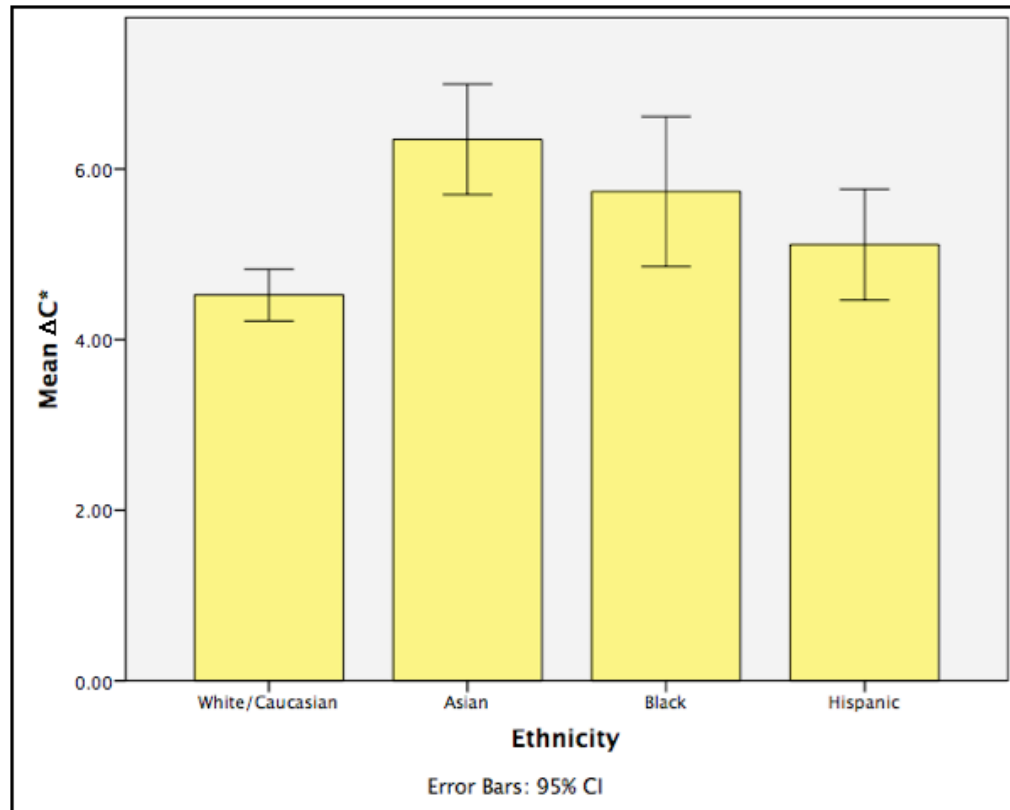


Figure 49: Mean ΔC^* vs. Ethnicity

d) Graphical Analysis of CE of Gingival Shade Guides

All participant and shade guide spectral values were input into the color management software for analysis. A two-dimensional representation graph of the hue/chroma coordinates was first created by setting a^* as the x-axis coordinate and b^* as the y-axis coordinate. The value metric, L^* was not represented in the two-dimensional chromatic representation. (Figure 50) The participant coordinates are represented by spheres with colors corresponding to the representative a^* and b^* of that coordinate. The varying shade guide sets were represented graphically as squares, with each square color corresponding to a different shade guide set. (Figure 51)

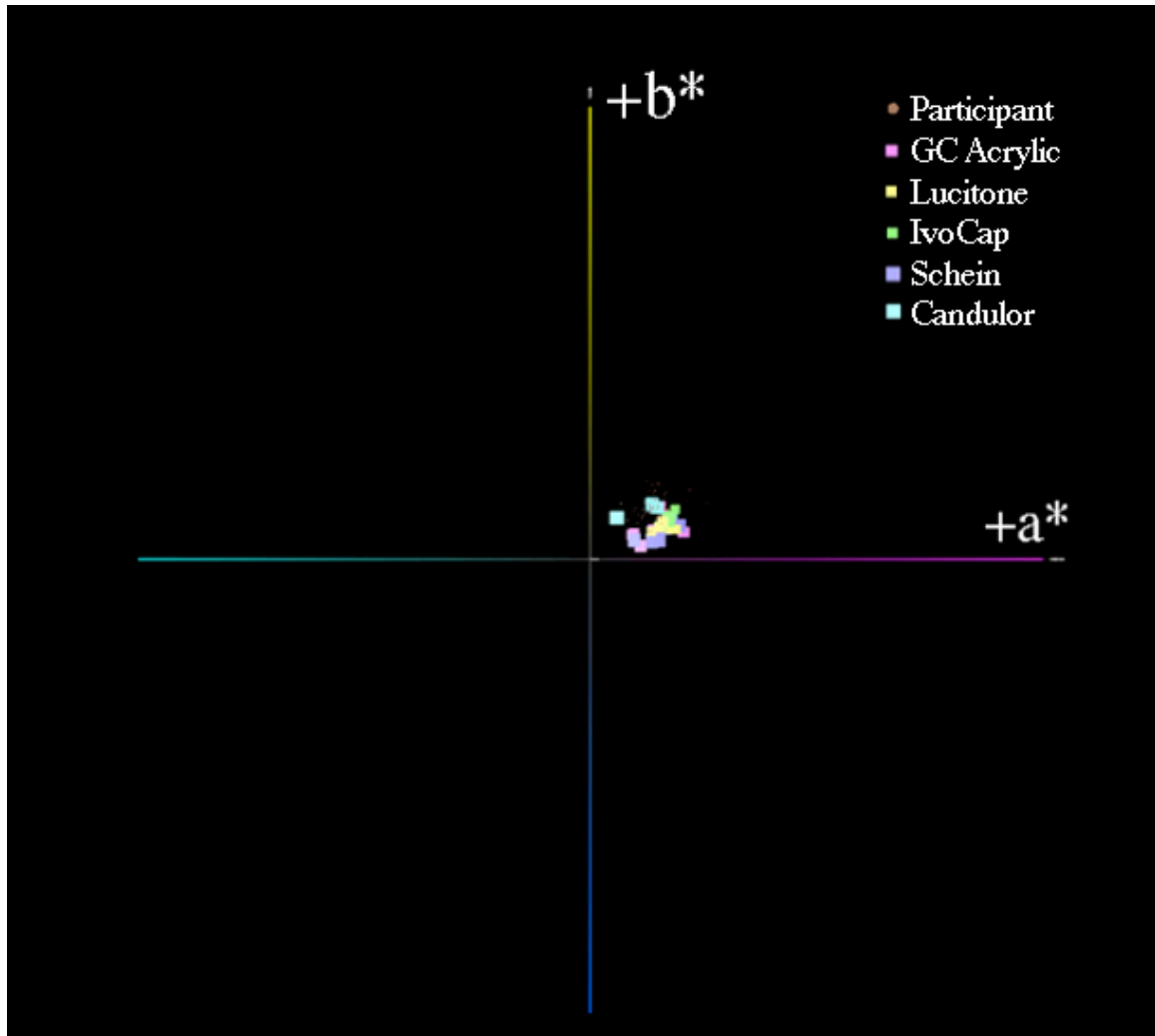


Figure 50: Graph Illustrating Locations of a^* and b^* on x- and y- Axes Respectively, in Two-Dimensions

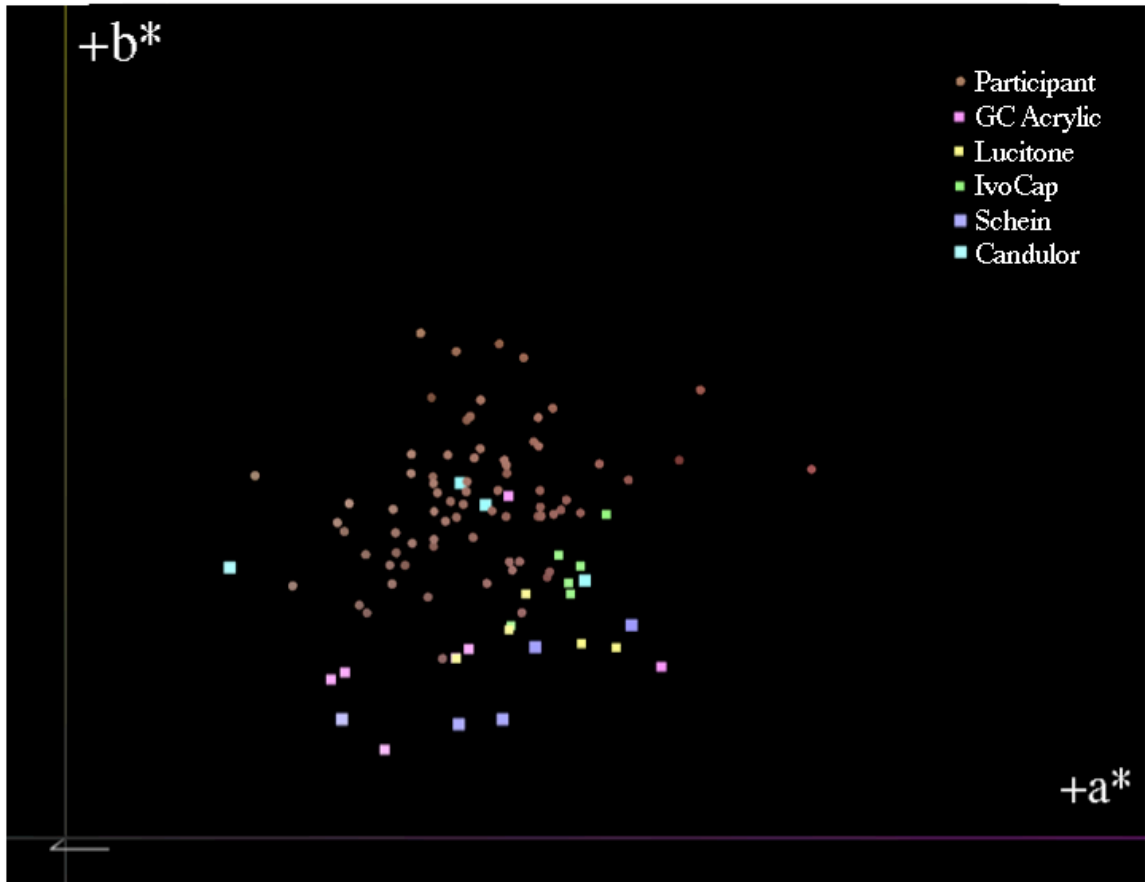


Figure 51: Enlargement of Two-Dimensional Representation of a^* and b^* . Circles Denote Participants While Squares Represent Shade Guide Systems.

In the two-dimensional diagram, all participant and shade guide values were observed to be in the upper right portion of the color space, corresponding to $+a^*$ and $+b^*$ values. The majority of shade tab points (blue ellipse) were clustered inferiorly to the cluster of participant data points (red ellipse), with a small number of shade guide points present centrally in the participant point cluster (yellow ellipse). (Figure 52)

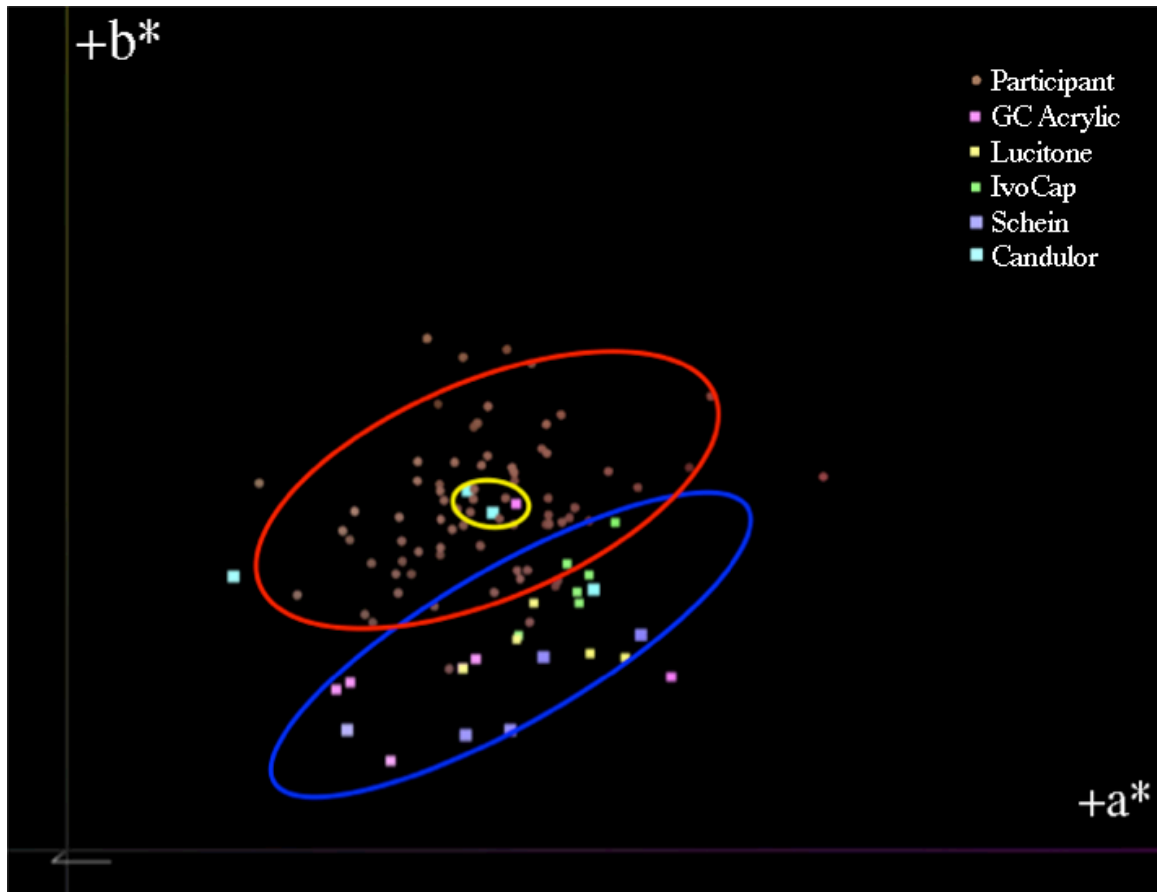


Figure 52: Enlargement of Two-Dimensional Representation of a^* and b^* Axes Illustrating Clustering of Participants (red) and Materials (blue). Yellow Ellipse Represents Isolated Shade Tabs Located Centrally Within Participant Population Cluster.

The L^* values for participants and shade guide samples were then input into the diagram, via the addition of the Z- axis. The x-axis remained a^* values, and the y-axis remained b^* values. All other graphical representations remained the same as in the two-dimensional rendering. The three-dimensional plot was then manipulated in space and the impact of L^* observed. (Figure 53) In addition to the chromatic observations above, the majority of shade tab points were observed to be clustered inferiorly on the z-axis when compared to the majority of participant data points. (Figure 54)(Figure 55)

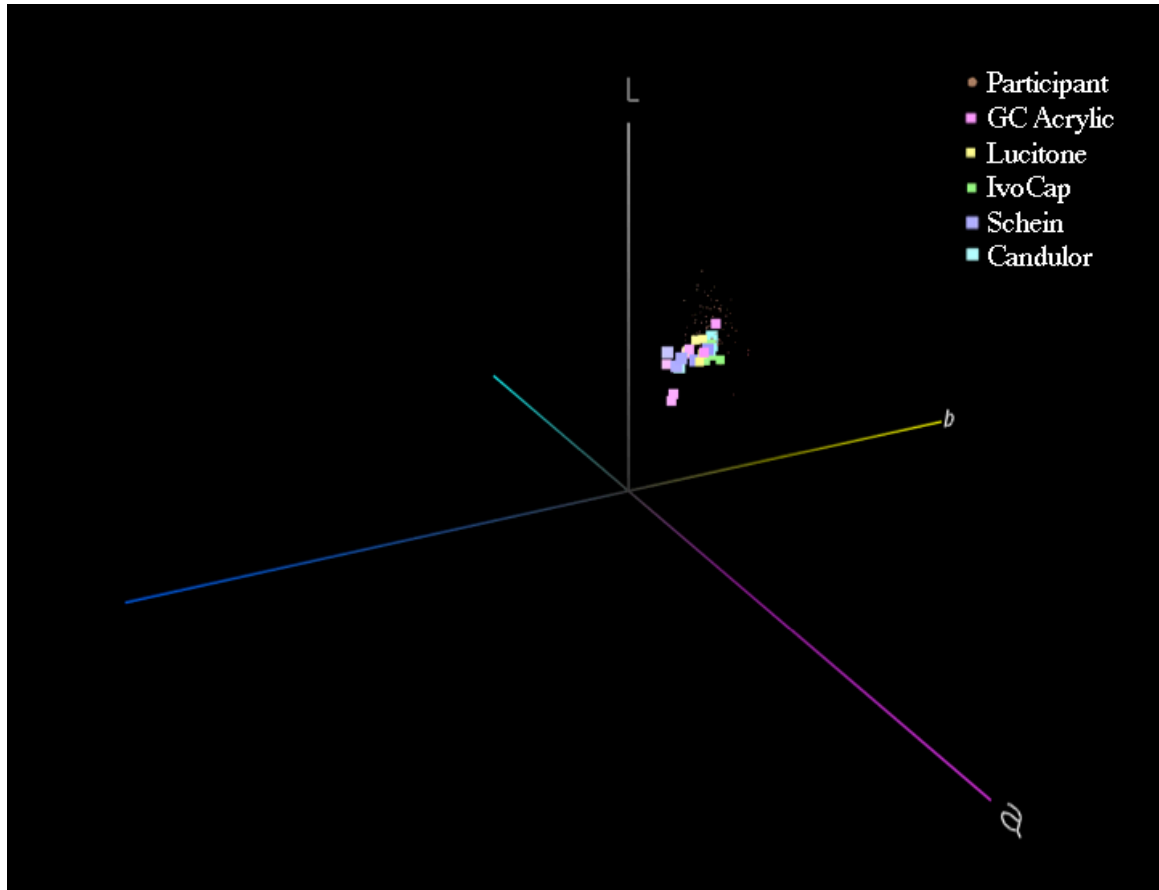


Figure 53: Graph Illustrating Locations of L^* , a^* and b^* on x-, y-, and z- Axes Respectively, in Three-Dimensions

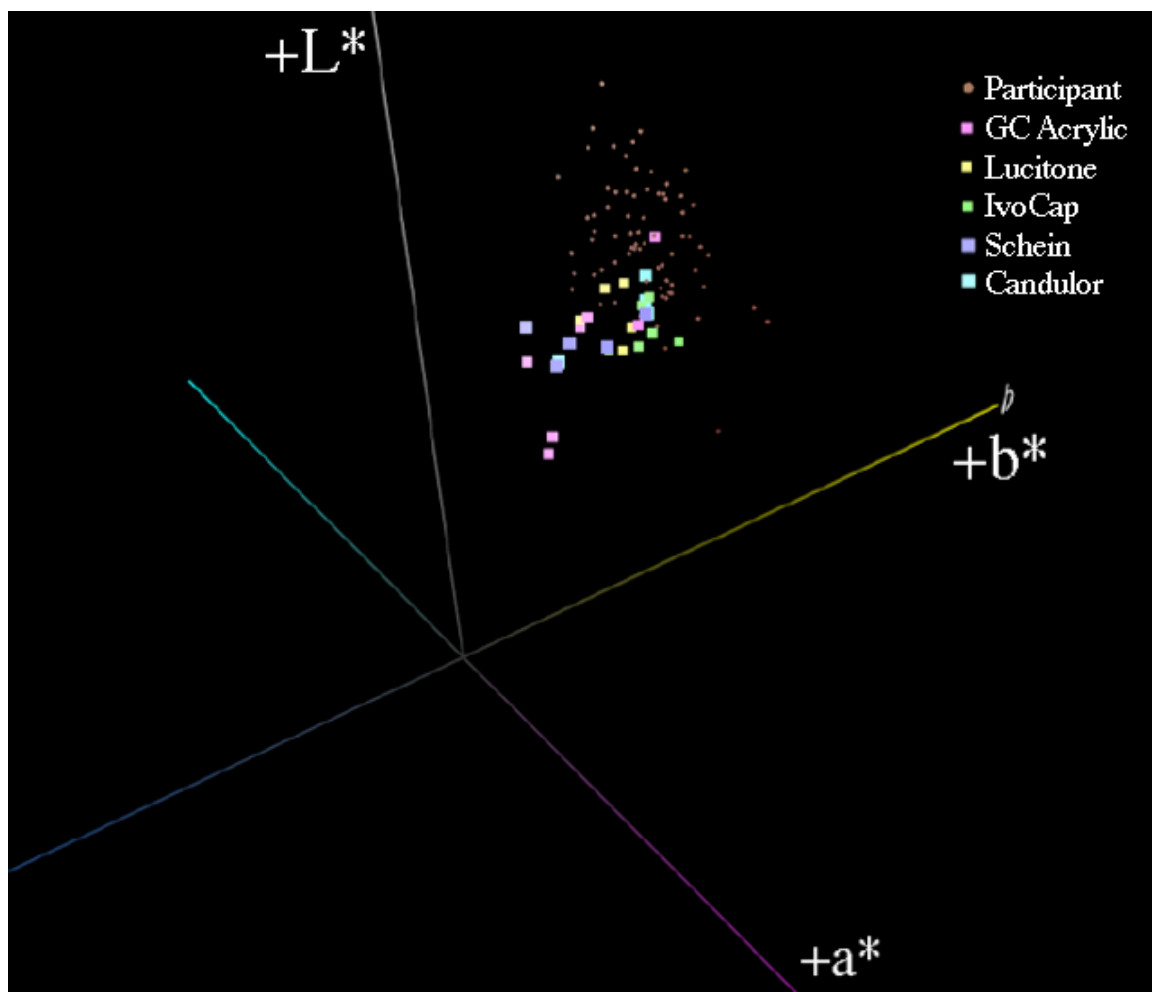


Figure 54: Enlargement of Three-Dimensional Representation of L^* , a^* , and b^* . Circles Denote Participants While Squares Represent Shade Guide Systems.

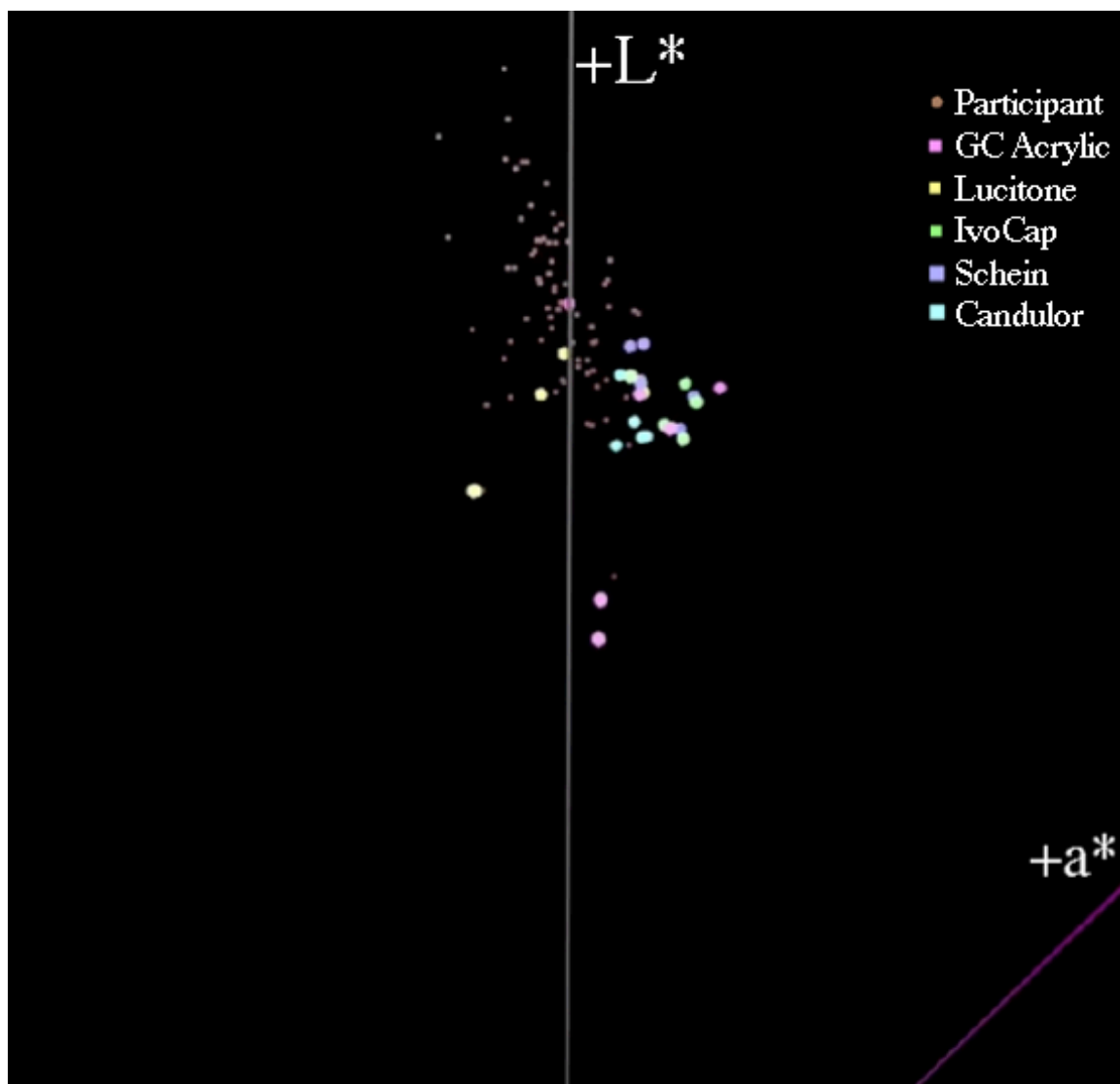


Figure 55: Enlargement of Three-Dimensional Representation of L^* , a^* , and b^* Axes. Circles Denote Participants While Squares Represent Shade Guide Systems.

e) The Fabrication of an Ideal Two-Tab Shade Guide for a Sample Population: A Proof-of-Concept Experiment

By observing the coverage error analysis and three-dimensional renderings, it was determined that the two hypothetical ‘ideal’ shade tabs would be assigned via the following criteria:

- The L^* values would be equally distanced about the mean L^* value of the participant population, which was recorded as 52.3 ± 4.97 . To ensure adequate coverage within the population, L^* values 0.5SD less or more than the mean were chosen as initial candidates, resulting in 54.75 and 49.8.
- Similarly, the a^* values were chosen to lie equally about the population mean of 17.9 ± 4.97 . It was again chosen that 0.5SD in either direction would be used, resulting in a^* values of 19.9 and 15.9.
- The same procedure was applied to the b^* values. The mean b^* for the population was 14.9 ± 3.09 . Using the 0.5SD for initial candidates yielded b^* values of 16.4 and 13.4.

In pairing the initial L^* , a^* , and b^* values for analysis, the two-dimensional renderings were examined and it was noted that, in the sample population, a loose linear relationship existed between the a^* and b^* values, with lower-value a^* values tending to correlate with lower-value b^* values. (Figure 56) Therefore, the initial candidate chromatic values were paired as ($a^*=19.9$; $b^*=16.4$) and ($a^*=15.9$; $b^*=13.4$).

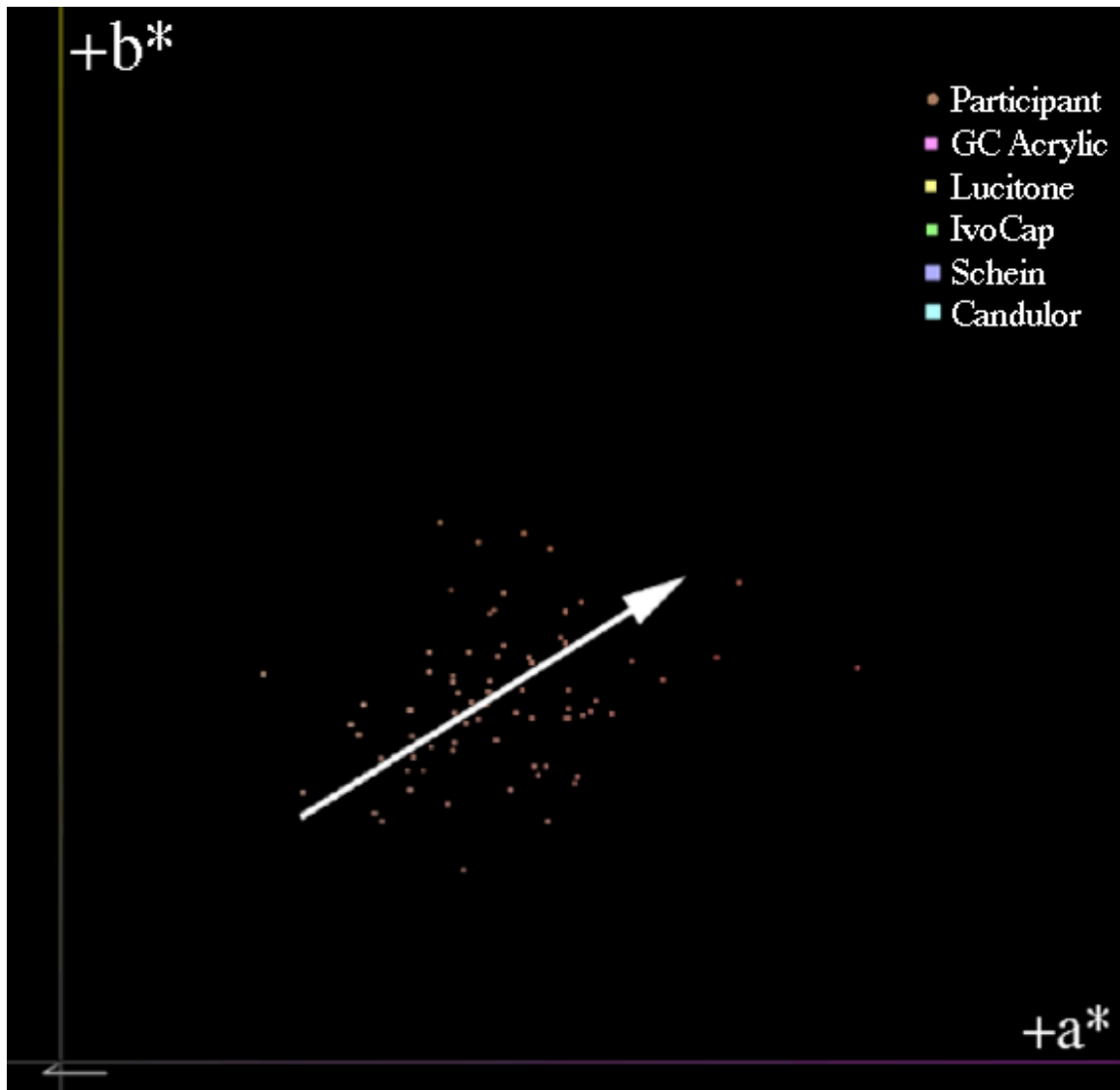


Figure 56: Two-Dimensional Representation of Participant Population; Arrow Suggests a Direct, Linear Relationship Between a^* and b^* . b^* is Seen to Increase With Increasing a^* .

Upon examination of the three-dimensional rendering, it was noted that the L^* values in the participant population exhibited an inverse relationship to the chromatic values, decreasing as a^* and b^* increased. (Figure 56) Thus the larger L^* value (54.75) was paired with the lower chromatic pair, while the lower L^* value (49.8) was placed with the

higher value combination, completing the initial hypothetical shade tabs. The two resultant initial ideal shade tabs in the (L^* , a^* , b^*) format were (54.75, 15.9, 13.4) and (49.8, 19.9, 15.9), respectively.

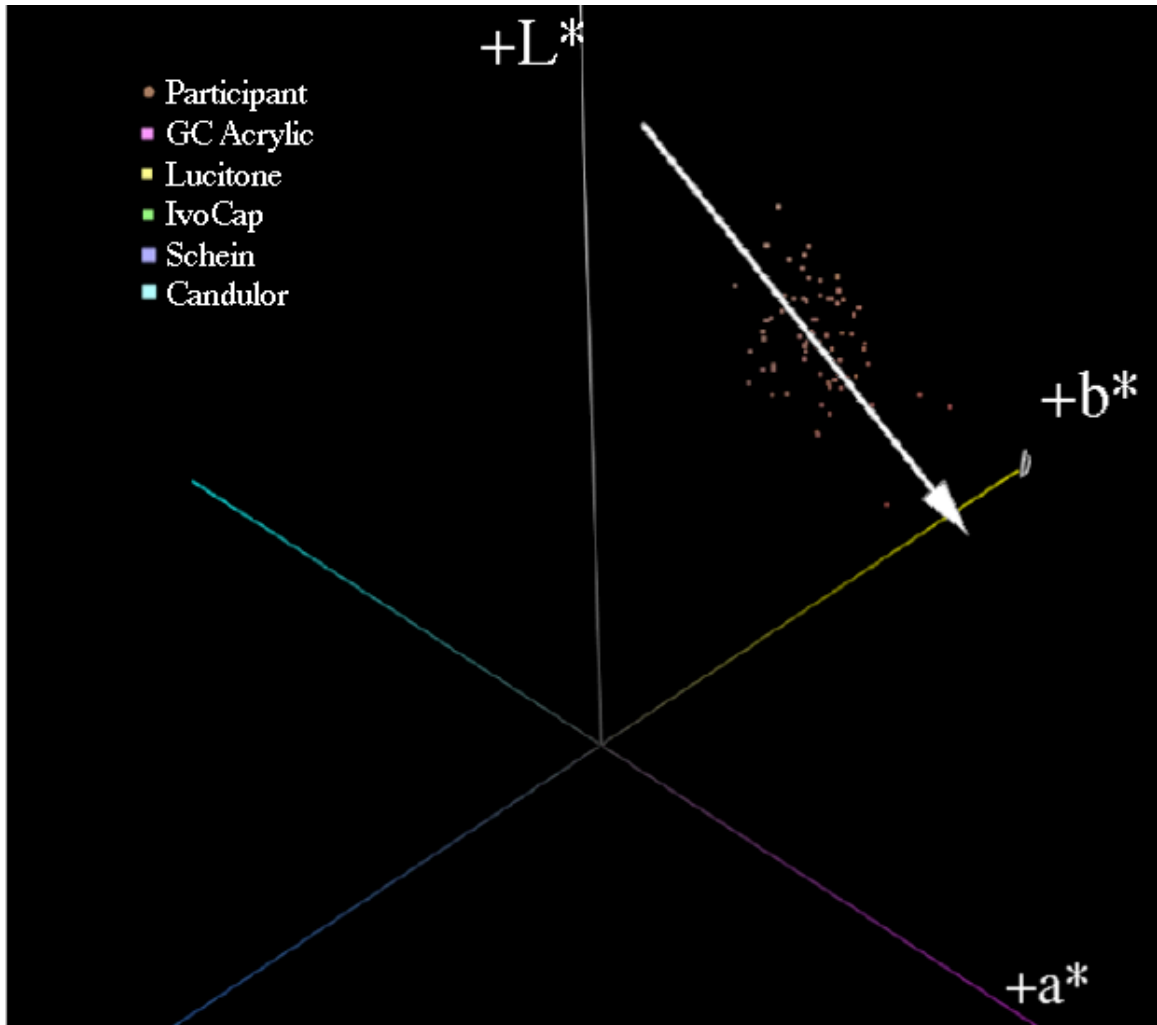


Figure 57: Three-Dimensional Representation; Arrow Suggests Inverse Linear Relationship Between Value Metric L^* , and Chromatic Metrics a^* and b^* . L^* is Seen to Decrease With Increasing a^* and b^* .

Upon inputting these values into the statistical software, a mean coverage error of 5.02 ± 2.91 was established. Then, minor adjustments were performed to the L^* , a^* , and b^* values of each ideal shade tab, and the resultant effects on the Coverage Error value output were observed. Using the minimum function in the statistical modeling software, a combination of two shade tab values was obtained that allows a theoretical minimum CE for the sample population. The coordinates for these two values in the (L^*, a^*, b^*) format were (56.0, 16.4, 14.7) and (49.2, 10.1, 15.0), respectively. The coverage error of these two shade tabs when compared to the sample population was calculated as 4.88 ± 2.77 . The coordinates of these two 'ideal' shade tab values were input into the color management software and interpreted. They appeared centrally clustered in all dimensions. (Figure 58)(Figure 59)(Figure 60)



Figure 58: Ideal Shade Coordinates Overlaid on Two-Dimensional Representation. Note Centralized Location of Ideals in Participant Population.

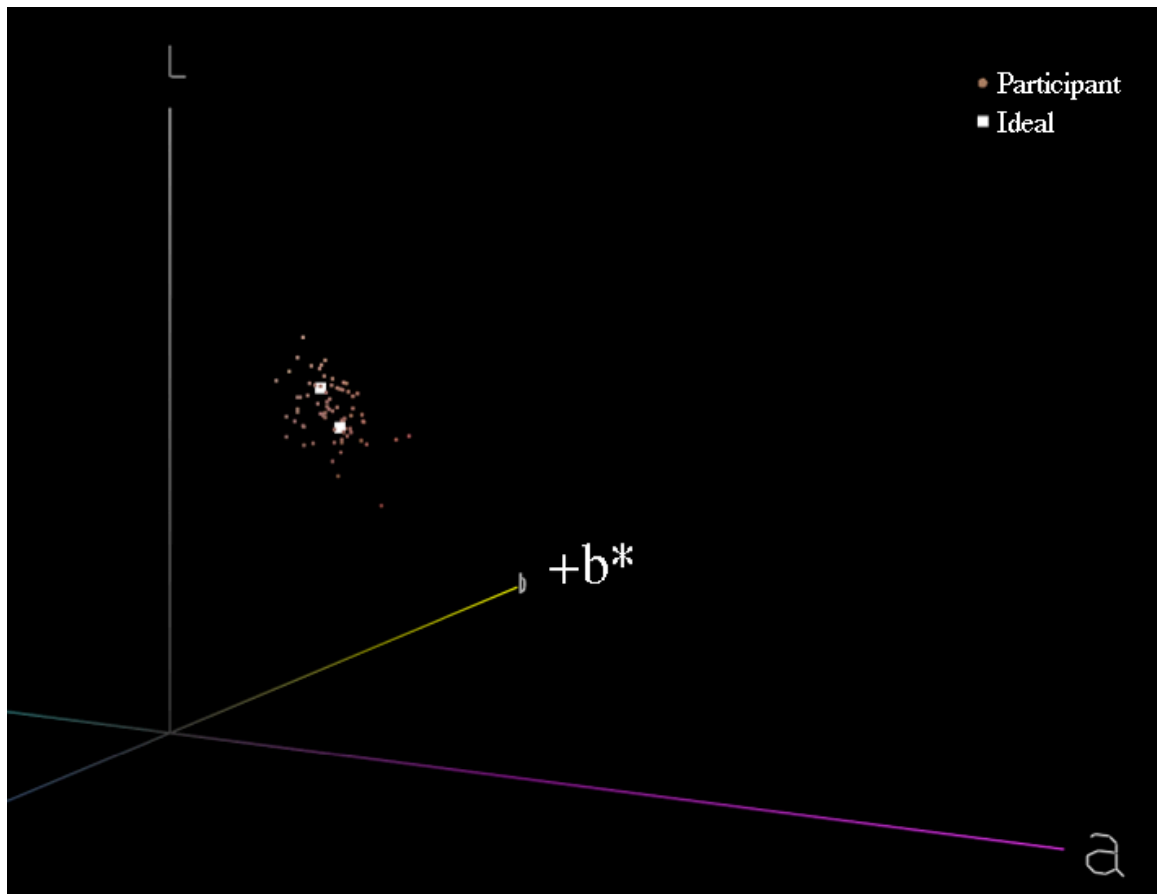


Figure 59: Ideal Shade Coordinates Overlaid on Three-Dimensional Representation. Note Centralized Location of Ideals in Participant Population and Mimicry of Inverse Linear Relationship of L^* to a^* and b^* .

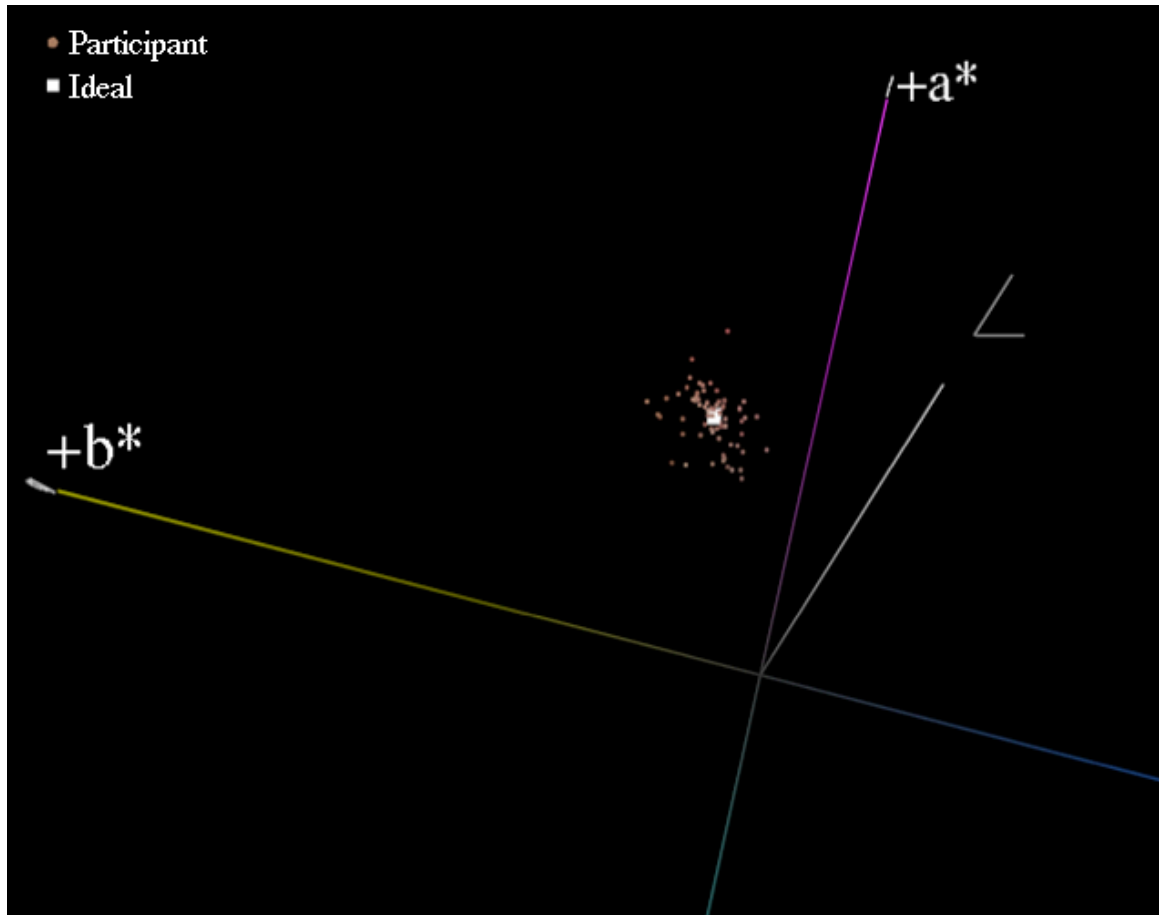


Figure 60: Ideal Shade Coordinates Overlaid on Three-Dimensional Representation. Note Centralized Location of Ideals in Participant Population When Observed Obliquely.

III. Discussion

a) Coverage Error of Shade Guides in a Study Population

For the overall population, the mean coverage error (CE) for all materials varied from a mean of 5.89 for GC Acrylic to a mean of 10.78 for IvoCap. The means for all of the materials were observed as:

- GC Acrylic: 5.89 ± 2.97
- Lucitone: 6.55 ± 3.33
- IvoCap: 10.78 ± 3.80
- Schein: 7.92 ± 3.54
- Candulor: 8.13 ± 3.85

Previous research by Paniz suggests that the threshold of clinical acceptability in the intraoral gingival areas is at $\Delta E = 8.74$. This suggests that the majority of materials will produce average best matches within the level of clinical acceptability. However, in materials such as the IvoCap, the mean best match is outside the level of clinical acceptability ($10.78 > 8.74$). This suggests a clinically significant problem. To further examine the coverage of each material, the percentage of participants each shade set would leave with a clinically unacceptable match was calculated and examined. The percentage of participants with a clinically unacceptable best match (above 8.74) for each shade guide is as follows:

- GC: 18.8% of participants
- Lucitone: 23.3% of participants
- IvoCap: 68.8% of participants
- Schein: 37.8% of participants
- Candulor: 45.6% of participants

This observation suggests that between 19 to 69% of participants can be left with an unacceptable shade match to the gingival restorative material, depending on the system

used. Future studies are indicated, with increased sample size and across more varying populations, to support this. But, if accurate, this study may provide a compelling basis for further exploration into the optical properties of gingival restorative materials. This includes either altering existing shade coverage or adding new shades to sets to help decrease the coverage error. A possible approach to this is discussed in section e.

b) Coverage Error of Gingival Shade Guides with Respect to Participant Sex

The results of this section suggest that there is not enough variation in the respective gingival optical properties of males and females for there to be significant variation in the coverage error of gingival materials based on sex.

c) Coverage Error of Gingival Shade Guides with Respect to Participant Ethnicity

The differing coverage errors based on ethnicity, particularly the significantly higher CE for the Asian group than the White group, may suggest that the accuracy of gingival shade guides differs based on ethnicity. Follow-up studies may be indicated with larger participant groups or in different geographic areas to confirm this observation.

This finding is significant in that it suggests that shade guides may be lacking with respect to the particular esthetic presentations of different racial groups. New shade systems or new tabs within existing systems may be indicated to reduce the disparity in coverage error between such groups.

d) Graphical Analysis of Coverage Error of Gingival Shade Guides

The graphical analyses of the two-dimensional and three-dimensional diagrams allow the limitations of the existing shade-guide systems to be examined. Generally, the shade guides studied were observed to be too inferior in the y-axis and z-axis. This corresponds to the spectral properties of the tabs being too dark in value, and too blue in hue. This may give guidance in how existing shade guides may be adjusted or new guides fabricated to allow a better CE for participant populations.

Limitations to this study include the comparison of a metric acquired *in vitro* to a metric acquired *in vivo*. As previously discussed, the central incisor region does provide the most consistent gingival shade due to adequacy of blocking, but future research may be necessary to confirm that this comparison is valid. One such method is repeating the acquisition with a non-contact, spot-type spectrophotometer. Other areas of further research include expansion of the participant population to other sites and geographic areas, in hopes of increasing the diversity of the participant population.

e) The Fabrication of an Ideal Two-Tab Shade Guide for a Sample Population: A Proof-of-Concept

The goal of this section was to determine if a limited shade guide system optimized for the participant population could be conceived using the spectral data obtained from the participant population. It was noted that the number of tabs included within the shade guide was not correlated to CE for the shade guides. The two shade guide sets with the lowest mean CE, the GC Acrylic and Lucitone sets, had 7 tabs and 3 tabs, respectively. Meanwhile, the set with the highest CE, IvoCap, has 6 tabs. Observation of graphical analysis suggests that the main factor of low CE is having several shade tabs centrally located within the participant pool. This is evident when examining (Figure 52) where within the yellow ellipse are spectral points for the GC acrylic and Lucitone sets, the two best performers. Thus, it is hypothesized that a shade guide set with minimal tabs could have spectral properties optimized such that they have a minimal CE.

Via the aforementioned process, a two-shade-tab system was hypothesized with spectral properties such that when a CE analysis was performed against the representative participant population, a CE value lower than all calculated CEs was obtained. Using the technique, a two-tab set was devised with a CE of 4.88 ± 2.77 . The best CE obtained experimentally was that of the GC acrylic set, which consists of 7 tabs and had a CE of 5.89 ± 2.97 . The optimized shade tab guide would result in just 5 participants having best matches below the $\Delta E = 8.74$ level of acceptability, or 5% of the participant pool. The GC

set resulted in almost 18% of participants having a best match below the level of clinical acceptability.

These results represent merely a proof of concept of the method of developing a shade guide set using spectrophotometric analyses. Subsequent research would include: expanding the participant population to more sites in different geographic locations to obtain a more broadly-representative sample, fabrication of shade tabs with the optimal spectral properties, and subsequent in vivo analysis of the resultant shade sets to determine if the results are reproducible clinically.

Possible long-term benefits of such research would include:

- Optimized shade systems that include fewer but more accurate tabs for the participant population. This would require fewer shades of materials to be fabricated, thus allowing less waste and more profitability, as only the most optically appropriate tabs would be included, and greater ease in shade tab selection by the provider, as there would be fewer candidate shade tabs to be examined when picking which to use.
- Shade guides with better coverage error particularly in racial minority populations.

Chapter 7: Specific Aim 3. An Investigation Into the Application of Narrow Band Equipped Intraoral Endoscopy as a Diagnostic Device in the Observation and Classification of Intrapapillary Capillary Loops

I. Materials and Methods

The final phase of this project was directed toward performing 1) an analysis of the coverage error (accuracy of color reproduction) of acrylic gingival restorative materials in a participant populations, and 2) a proof-of-concept study into the design of a spectrophotometrically-driven shade-guide system for gingival replacement materials.

Patients were recruited for this study from among the general patient population of the Harvard Dental Center. After recruitment, screening of eligibility as an active patient of the Dental Center, and patient agreement of participation, a baseline periodontal examination of teeth #'s 8 and 9 was performed.

Inclusion criteria for this aim were: patient age eighteen and above; either tooth 8 or 9 present as a virgin tooth, restored tooth, or all-ceramic or ceramometal restorations. Exclusion criteria were: ADA class 3 or greater, bleeding on probing to buccal sites of teeth #'s 8 or 9, or probing depths greater than 3mm in teeth #'s 8 or 9.

Eighty-nine participants qualified for this portion of the analysis and eighty-nine central incisor sites were examined. Tooth #8 was observed in seventy-nine analyses and, due to implants present in the #8 site, tooth #9 was observed in ten analyses. Participants were asked to self-identify for sex and ethnicity. Patient ages ranged from 25 to 87 with a median of 39 years. Participant demographics for this section were as follows:

Sex	n
Male	41
Female	48

Ethnicity	n
White	42
Asian	20
Black	14
Hispanic	13

a) Intrapapillary Capillary Loop Visualization Using a Narrow-Band Imaging-Equipped Endoscope

Intrapapillary capillary loop (IPCL) evaluation was performed during the visit prior to any placement of gingival strips used in previous aims. For evaluation, an area of the attached gingiva 2mm apical to the FGM of a single anterior tooth was chosen and evaluated for each participant. Tooth #8 was chosen for all analyses. If tooth #8 was an implant restoration, tooth #9 was used with identical protocol. Using a clinical endoscope equipped with narrow-band imaging (CV-190; Olympus, Tokyo, Japan) (Figure 26), endoscopic images of the study area were obtained, ensuring that no pressure was placed upon the area and there was isolation from ambient light sources. Endoscope settings were set for near-focus and 1.5x zoom, in order to optimize visualization of epithelial capillaries. Teeth and gingiva were dried of saliva and the endoscope probe was placed perpendicularly to the buccal gingiva at a height of approximately 5mm. If needed, the angulation of the endoscopic probe was adjusted slightly to prevent glare from impeding the visual field. Endoscopic images were captured and transferred from the endoscope to a personal computer for analysis; each endoscopic evaluation was also video-recorded for subsequent analysis.

The IPCL images for each participant were then input into photographic management software (Photoshop; Adobe, San Jose, USA) and cropped for standardized analysis. For each participant, the most glare-free spectrophotometric image was chosen. The image was rotated such that the tooth was inferior in location and the gingival

margin was superior. (Figure 61) The mesial-distal dimension of the tooth was then split into thirds, as illustrated in Figure 62. The image was cropped to 3 mm in height, such that the middle third was isolated, with the most inferior border being immediately adjacent to the FGM. (Figure 63) The image was then enlarged via aspect-locked scale for evaluation. (Figure 64) If the photograph was overexposed, the image was adjusted for exposure and contrast.

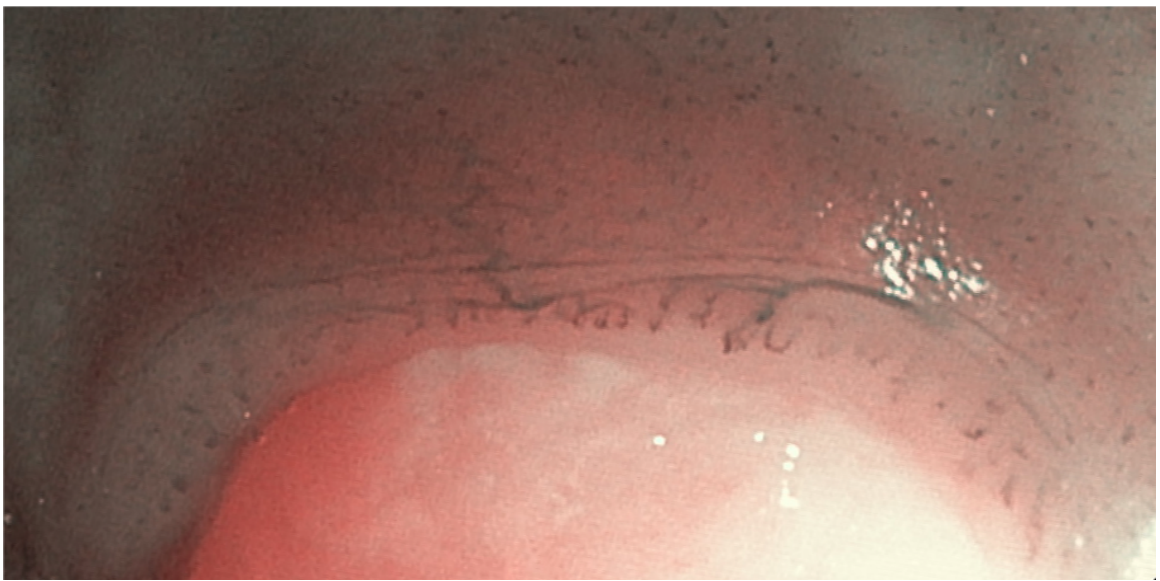


Figure 61: Narrow-Band Endoscopic Image of a Maxillary Central Incisor

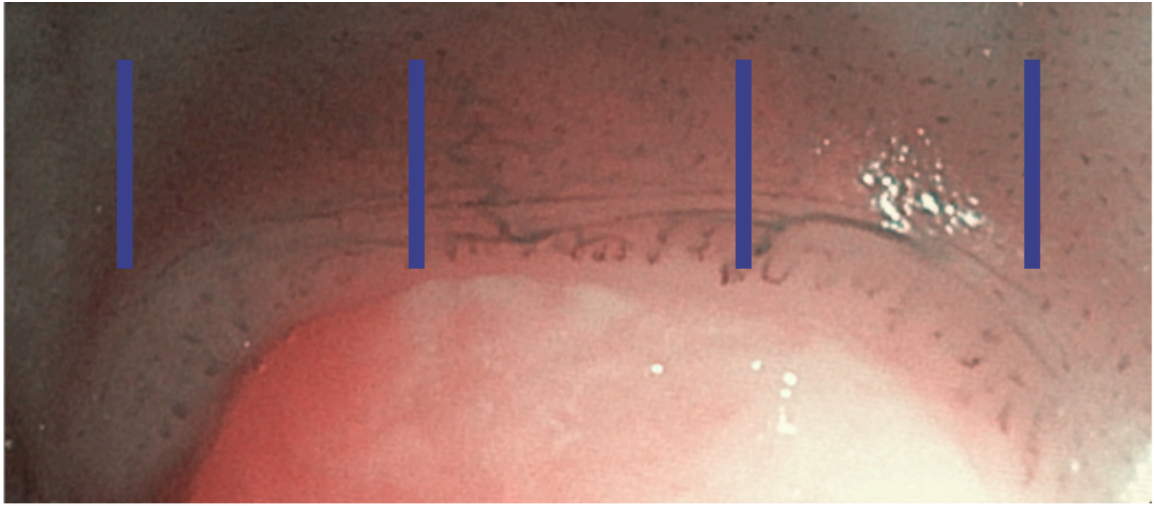


Figure 62: Illustration Showing Location of Separation of FGM into Thirds

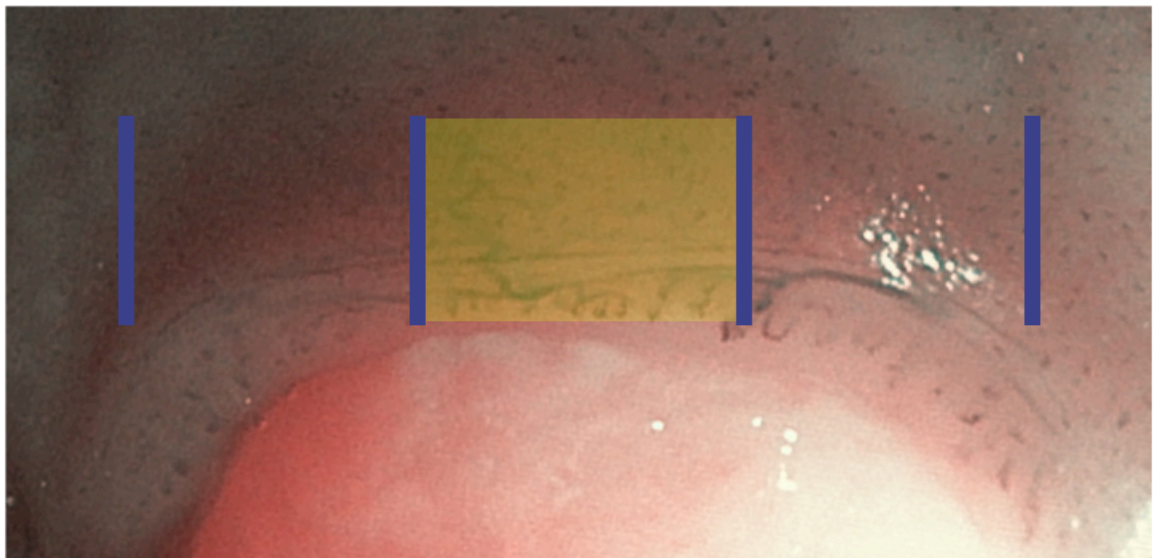


Figure 63: Yellow Box Illustrating Cropped Area for IPCL Analysis



Figure 64: Illustration of Resultant Cropped Endoscopic Image Used for IPCL Analysis

b) IPCL Classification and Assessment

The resultant optimized cropped photographs were then examined to evaluate IPCL type and number. IPCL evaluation was performed by two blinded examiners. Each evaluator viewed each cropped image against a white background and recorded IPCL quantity and type, with the IPCL type being stratified into three groups, using an adapted version of the Minami et al. classification system.¹⁹¹

- Type 1: “Dots” (Figure 65)
- Type 2: “Simple Loops” (Figure 66)
- Type 3: “Complex Loops” (Figure 67)

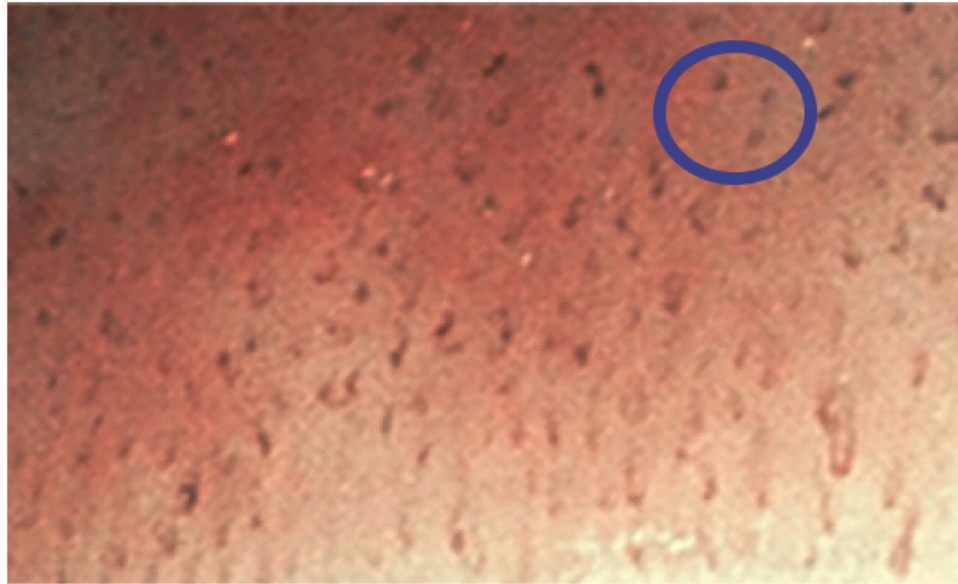


Figure 65: Example of Type 1 Gingival IPCLs Within Blue Ellipse

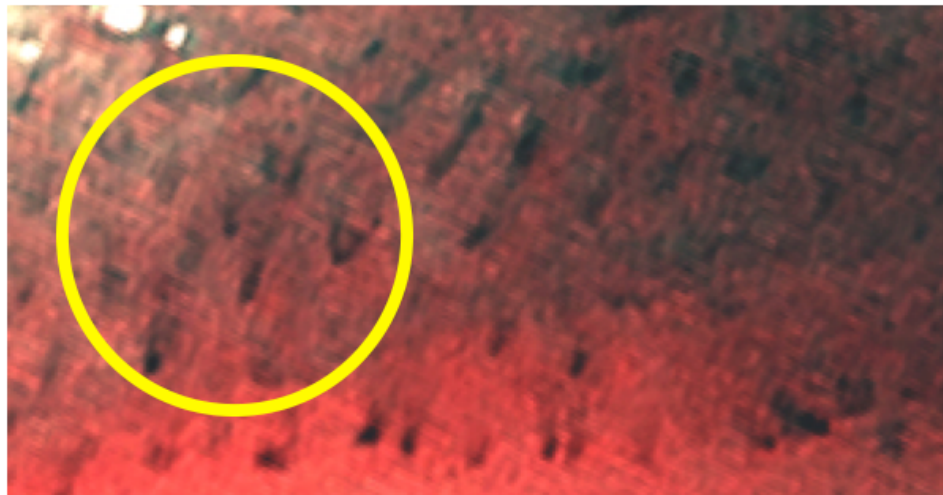


Figure 66: Example of Type 2 Gingival IPCLs Within Yellow Ellipse



Figure 67: Example of Type 3 Gingival IPCLs Within Green Ellipse

The quantities of each type of loop were recorded by each examiner in duplicate, and mean values were calculated. The mean values for each type for each participant were then input into statistical software (SPSS; IBM, Armonk, USA), and the following values were calculated:

- Mean Type 1 IPCLs
- Mean Type 2 IPCLs
- Mean Type 3 IPCLs
- Mean Total IPCLs
- Mean Percentage of Type 2 and 3 vs. Total IPCLs

c) Statistical Analyses

The above values were compared to the previously acquired metrics of gingival thickness, translucency, and a^* values (redness), as well as stratified by participant sex and ethnicity. ANOVA tests were performed. In the event of significant ANOVA results, appropriate post-hoc tests were formed to determine significant correlations.

Lastly, linear regression analysis and paired t-tests were performed in order to determine if any statistically significant correlations exist between IPCL type or quantity and gingival thickness or translucency. The analyses were performed looking for correlations between any of the following pairs:

Thickness Metrics

- Mean Total IPCLs vs. a*
- Mean Percentage of Type 2/3 IPCLs vs. a*
- Mean Total IPCLs vs Gingival Thickness at 1mm
- Mean Percentage of Type 2/3 IPCLs vs. Gingival Thickness at 1mm

Translucency Metrics

- Mean Percentage of Type 2/3 IPCLs vs. PSTP_{black}
- Mean Total IPCLs vs. PSTP_{black}
- Mean Percentage of Type 2/3 IPCLs vs. TP
- Mean Total IPCLs vs. TP
- Mean Percentage of Type 2/3 IPCLs vs. CR
- Mean Total IPCLs vs. CR

II. Results

a) Intrapapillary Capillary Loop Visualization Using a Narrow-Band Imaging-Equipped Endoscope

For all participants, the narrow-band endoscope was implemented and images of the gingival mucosae were obtained.

b) IPCL Classification and Assessment

After adjustment and cropping, the images were evaluated by the two examiners. The following values were obtained:

Mean±SD Type 1 IPCLs	Mean±SD Type 2 IPCLs	Mean±SD Type 3 IPCLs	Mean±SD Total IPCLs
51.27±24.42	7.96±8.5	2.7±3.6	61.7±23.23

Table 6: Numerical IPCL Values

The mean percentage of Type 1 and 2 IPCLs as a percentage of all IPCLs present was calculated as 18.2±18.4%.

c) Statistical Analyses

The values for Mean Total IPCLs and the Mean Percentage of Type 2 and 3 versus Total IPCLs were first stratified according to participant ethnicity and observed for any statistical correlation. ANOVA tests were performed to determine if any correlations were present. No significant differences were discerned when ethnicity was used to stratify either Total IPCL number (p=0.41) or Percentage Type 2/3 IPCLs (p=0.32)

The values for Mean Total IPCLs and the Mean Percentage of Type 2 and 3 versus Total IPCLs were also stratified according to participant sex and observed for any statistical correlation. Independent sample t-tests were performed to determine if any correlations were present. No significant differences were discerned when sex was used to stratify either Total IPCL number (p=0.529) or Percentage Type 2/3 IPCLs (p=0.774)

The ANOVA analysis determined that no statistically significant correlations existed between any of the following pairs of metrics. ($P > 0.459$ or greater for all values)

Thickness Metrics

- Mean Total IPCLs vs. a*
- Mean Percentage of Type 2/3 IPCLs vs. a*
- Mean Total IPCLs vs Gingival Thickness at 1mm
- Mean Percentage of Type 2/3 IPCLs vs. Gingival Thickness at 1mm

Translucency Metrics

- Mean Percentage of Type 2/3 IPCLs vs. PSTP_{black}
- Mean Total IPCLs vs. PSTP_{black}
- Mean Percentage of Type 2/3 IPCLs vs. TP
- Mean Total IPCLs vs. TP
- Mean Percentage of Type 2/3 IPCLs vs. CR
- Mean Total IPCLs vs. CR

III. Discussion

Intraoral endoscopy equipped with narrow-band imaging capability proved a viable means of subjectively observing the IPCLs within the marginal gingiva of the anterior dentition. When compared to white-light illumination, the NBI images present an increased contrast and clarity. This finding correlates to similar previously completed studies examining the oral mucosa. When viewed in this manner, the IPCLs displayed clarity such that in some cases the microvasculature could be visually observed bulging and extending in coincidence with the participant's heartbeat.

However, when the resultant endoscopic images were evaluated with respect to participant demographics, thickness metrics, or translucency metrics, no significant correlations were found. This suggests that, within the limitations of this study, while useful for subjective visualization, endoscopic imaging may be limited in quantitative or

diagnostic applications. Possible reasons for lack of diagnostic capability of the endoscopy in this study may include:

- Difficulty in acquiring truly standardized images between participants due to extreme glare caused by the endoscopic probe's powerful light source.
- Inherent subjectivity between examiners.
- Limitations in the maximum magnification output of the endoscope, which, while powerful, did not allow microscopic evaluation of the IPCs. A different imaging modality that allows for such viewing may increase both quantitative and qualitative differentiation of the IPCs for comparisons.
- Possible inapplicability of the adapted Minami et al. system, developed for neoplasm classification in the buccal mucosa, into the healthy keratinized gingiva.
- Possible inapplicability of the adapted neoplasm classification system into the observation of only inflammation, as opposed to neoplastic transformations.

Chapter 8. Conclusions and Future Work

I. Specific Aim 1

The implementation of a non-contact dental spectrophotometer appears to be a viable tool in obtaining, recording, and quantifying gingival color in the anterior dentition. Results appear to be most consistent when obtained from the maxillary central incisors. Results obtained from this spectrophotometric analysis correlate with previous research investigating the differences in gingival color dependent on sex and ethnicity. More research is required to further elucidate these differences in a larger participant pool.

II. Specific Aim 2

Gingival spectrophotometry has been determined to be a possible non-invasive means of approximating gingival thickness and translucency. More research is needed to determine the best methods of data acquisition, standardization techniques, and reproducibility. One possible use of this technique is identifying participants who face potentially increased esthetic risk when contemplating prosthetic technique in the esthetic zone. Future areas of research include expansion of the study to different sites and geographic locales, as well as determining the effects of different existing restorative materials on baseline gingival values.

III. Specific Aim 3

Spectrophotometry may serve as a viable means of determining the coverage error of gingival restorative materials for use in the esthetic zone. The coverage error of five different shade guide sets were examined and determined to vary to a level of clinical significance. A pilot study was performed and candidates for an ideal gingival shade guide set were chosen in order to idealize them to a participant population. It was determined that fabrication of gingival shade guides based on the spectral statistics of a participant

population may be a feasible means of obtaining a simplified system with minimal coverage error.

IV. Specific Aim 4

The intraoral endoscopy utilizing narrow-band imaging capability proved a viable means of subjectively observing the IPCLs within the keratinized mucosa. However, some limitations in objective analysis presented, preventing any significant correlation from being drawn with other indicators of gingival spectral properties or participant demographics. Future research into a better objective measurement protocol or an intraoral endoscope with increased zoom may allow more consistent comparison and qualification of IPCLs.

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Chapter 11. Supplements

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CL	Between Groups	427.572	5	85.514	3.314	.006
	Within Groups	12746.310	494	25.802		
	Total	13173.882	499			
Ca	Between Groups	43.737	5	8.747	.667	.649
	Within Groups	6481.508	494	13.120		
	Total	6525.245	499			
Cb	Between Groups	33.521	5	6.704	.688	.633
	Within Groups	4812.843	494	9.743		
	Total	4846.364	499			

Multiple Comparisons

Dependent Variable: CL

LSD

(I) Tooth	(J) Tooth	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
6	7	-.27480	.78160	.725	-1.8105	1.2609
	8	-1.85628*	.78402	.018	-3.3967	-.3159
	9	-2.41784*	.78902	.002	-3.9681	-.8676
	10	-1.39666	.77691	.073	-2.9231	.1298
	11	-.12979	.77923	.868	-1.6608	1.4012
7	6	.27480	.78160	.725	-1.2609	1.8105
	8	-1.58148*	.79091	.046	-3.1354	-.0275
	9	-2.14304*	.79586	.007	-3.7067	-.5793
	10	-1.12186	.78385	.153	-2.6620	.4182
	11	.14501	.78616	.854	-1.3996	1.6896
8	6	1.85628*	.78402	.018	.3159	3.3967
	7	1.58148*	.79091	.046	.0275	3.1354
	9	-.56156	.79824	.482	-2.1299	1.0068
	10	.45962	.78627	.559	-1.0852	2.0045
	11	1.72649*	.78856	.029	.1771	3.2758
9	6	2.41784*	.78902	.002	.8676	3.9681
	7	2.14304*	.79586	.007	.5793	3.7067
	8	.56156	.79824	.482	-1.0068	2.1299
	10	1.02118	.79125	.197	-.5335	2.5758
	11	2.28805*	.79353	.004	.7289	3.8472
10	6	1.39666	.77691	.073	-.1298	2.9231
	7	1.12186	.78385	.153	-.4182	2.6620
	8	-.45962	.78627	.559	-2.0045	1.0852
	9	-1.02118	.79125	.197	-2.5758	.5335
	11	1.26687	.78149	.106	-.2686	2.8023
11	6	.12979	.77923	.868	-1.4012	1.6608
	7	-.14501	.78616	.854	-1.6896	1.3996
	8	-1.72649*	.78856	.029	-3.2758	-.1771
	9	-2.28805*	.79353	.004	-3.8472	-.7289
	10	-1.26687	.78149	.106	-2.8023	.2686

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CL	Between Groups	361.234	2	180.617	7.006	.001
	Within Groups	12812.648	497	25.780		
	Total	13173.882	499			
Ca	Between Groups	26.004	2	13.002	.994	.371
	Within Groups	6499.241	497	13.077		
	Total	6525.245	499			
Cb	Between Groups	18.958	2	9.479	.976	.378
	Within Groups	4827.406	497	9.713		
	Total	4846.364	499			

Multiple Comparisons

Dependent Variable: CL

LSD

(I) Tooth	(J) Tooth	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Canines	Laterals	-.77828	.55236	.159	-1.8635	.3070
	Centrals	-2.06946*	.55748	.000	-3.1648	-.9742
Laterals	Canines	.77828	.55236	.159	-.3070	1.8635
	Centrals	-1.29118*	.55910	.021	-2.3897	-.1927
Centrals	Canines	2.06946*	.55748	.000	.9742	3.1648
	Laterals	1.29118*	.55910	.021	.1927	2.3897

*. The mean difference is significant at the 0.05 level.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
CL	Equal variances assumed	4.287	.039	1.714	490	.087	.79632	.46467	-.11668	1.70932
	Equal variances not assumed			1.748	476.093	.081	.79632	.45546	-.09865	1.69128
Ca	Equal variances assumed	2.045	.153	-3.168	490	.002	-1.04108	.32857	-1.68666	-.39549
	Equal variances not assumed			-3.139	431.958	.002	-1.04108	.33170	-1.69303	-.38912
Cb	Equal variances assumed	.181	.671	3.985	490	.000	1.11189	.27904	.56363	1.66015
	Equal variances not assumed			4.043	469.606	.000	1.11189	.27502	.57147	1.65232

Group Statistics

	Gender	N	Mean	Std. Deviation	Std. Error Mean
Thickness1mm	Female	49	1.0245	.27274	.03896
	Male	39	1.1590	.32340	.05179
Thickness2mm	Female	49	1.3286	.30139	.04306
	Male	39	1.4949	.43344	.06941

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Thickness1mm	Equal variances assumed	2.980	.088	-2.116	86	.037	-.13448	.06356	-.26084	-.00813
	Equal variances not assumed			-2.075	74.340	.041	-.13448	.06481	-.26360	-.00536
Thickness2mm	Equal variances assumed	5.135	.026	-2.119	86	.037	-.16630	.07847	-.32229	-.01031
	Equal variances not assumed			-2.036	65.227	.046	-.16630	.08168	-.32941	-.00319

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Thickness1mm	Between Groups	.384	3	.128	1.425	.241
	Within Groups	7.553	84	.090		
	Total	7.938	87			
Thickness2mm	Between Groups	.578	3	.193	1.406	.247
	Within Groups	11.521	84	.137		
	Total	12.100	87			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CL	Between Groups	321.676	3	107.225	4.195	.006
	Within Groups	12473.814	488	25.561		
	Total	12795.491	491			
Ca	Between Groups	134.060	3	44.687	3.431	.017
	Within Groups	6355.808	488	13.024		
	Total	6489.868	491			
Cb	Between Groups	396.215	3	132.072	14.854	.000
	Within Groups	4339.045	488	8.891		
	Total	4735.260	491			

Multiple Comparisons

LSD

Dependent Variable	(I) Ethnicity	(J) Ethnicity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
CL	White/Caucasian	Asian	1.45818*	.56587	.010	.3463	2.5700
		Black	2.01731*	.77404	.009	.4965	3.5382
		Hispanic	1.48679*	.70084	.034	.1097	2.8638
	Asian	White/Caucasian	-1.45818*	.56587	.010	-2.5700	-.3463
		Black	.55914	.85057	.511	-1.1121	2.2304
		Hispanic	.02862	.78455	.971	-1.5129	1.5701
	Black	White/Caucasian	-2.01731*	.77404	.009	-3.5382	-.4965
		Asian	-.55914	.85057	.511	-2.2304	1.1121
		Hispanic	-.53052	.94575	.575	-2.3888	1.3277
	Hispanic	White/Caucasian	-1.48679*	.70084	.034	-2.8638	-.1097
		Asian	-.02862	.78455	.971	-1.5701	1.5129
		Black	.53052	.94575	.575	-1.3277	2.3888
Ca	White/Caucasian	Asian	-.32482	.40392	.422	-1.1185	.4688
		Black	1.48473*	.55252	.007	.3991	2.5703
		Hispanic	-.38925	.50027	.437	-1.3722	.5937
	Asian	White/Caucasian	.32482	.40392	.422	-.4688	1.1185
		Black	1.80954*	.60715	.003	.6166	3.0025
		Hispanic	-.06443	.56002	.908	-1.1648	1.0359
	Black	White/Caucasian	-1.48473*	.55252	.007	-2.5703	-.3991
		Asian	-1.80954*	.60715	.003	-3.0025	-.6166
		Hispanic	-1.87397*	.67509	.006	-3.2004	-.5475
	Hispanic	White/Caucasian	.38925	.50027	.437	-.5937	1.3722
		Asian	.06443	.56002	.908	-1.0359	1.1648
		Black	1.87397*	.67509	.006	.5475	3.2004
Cb	White/Caucasian	Asian	-2.20314*	.33374	.000	-2.8589	-1.5474
		Black	-1.09070*	.45652	.017	-1.9877	-.1937
		Hispanic	-.85294*	.41335	.040	-1.6651	-.0408
	Asian	White/Caucasian	2.20314*	.33374	.000	1.5474	2.8589
		Black	1.11245*	.50166	.027	.1268	2.0981
		Hispanic	1.35020*	.46272	.004	.4410	2.2594
	Black	White/Caucasian	1.09070*	.45652	.017	.1937	1.9877
		Asian	-1.11245*	.50166	.027	-2.0981	-.1268
		Hispanic	.23776	.55779	.670	-.8582	1.3337
	Hispanic	White/Caucasian	.85294*	.41335	.040	.0408	1.6651
		Asian	-1.35020*	.46272	.004	-2.2594	-.4410
		Black	-.23776	.55779	.670	-1.3337	.8582

*. The mean difference is significant at the 0.05 level.

ANOVA

dE_Best_Acr

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1277.218	4	319.304	25.817	.000
Within Groups	5503.673	445	12.368		
Total	6780.890	449			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: dE_Best_Acr

Tukey HSD

(I) Acr_Group	(J) Acr_Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GC	L199	-.66235	.52425	.714	-2.0983	.7736
	Ivocap	-4.88913*	.52425	.000	-6.3251	-3.4532
	Schein	-2.02969*	.52425	.001	-3.4656	-.5938
	Ivoclar New	-2.24507*	.52425	.000	-3.6810	-.8091
L199	GC	.66235	.52425	.714	-.7736	2.0983
	Ivocap	-4.22679*	.52425	.000	-5.6627	-2.7909
	Schein	-1.36735	.52425	.071	-2.8033	.0686
	Ivoclar New	-1.58272*	.52425	.022	-3.0186	-.1468
Ivocap	GC	4.88913*	.52425	.000	3.4532	6.3251
	L199	4.22679*	.52425	.000	2.7909	5.6627
	Schein	2.85944*	.52425	.000	1.4235	4.2954
	Ivoclar New	2.64406*	.52425	.000	1.2081	4.0800
Schein	GC	2.02969*	.52425	.001	.5938	3.4656
	L199	1.36735	.52425	.071	-.0686	2.8033
	Ivocap	-2.85944*	.52425	.000	-4.2954	-1.4235
	Ivoclar New	-.21537	.52425	.994	-1.6513	1.2206
Ivoclar New	GC	2.24507*	.52425	.000	.8091	3.6810
	L199	1.58272*	.52425	.022	.1468	3.0186
	Ivocap	-2.64406*	.52425	.000	-4.0800	-1.2081
	Schein	.21537	.52425	.994	-1.2206	1.6513

*. The mean difference is significant at the 0.05 level.

Group Statistics

	Gender	N	Mean	Std. Deviation	Std. Error Mean
dE_Best_Acr	Female	249	8.1497	4.10422	.26009
	Male	200	7.4618	3.56223	.25189

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
dE_Best_Acr	Equal variances assumed	4.918	.027	1.871	447	.062	.68799	.36769	-.03462	1.41060
	Equal variances not assumed			1.900	444.294	.058	.68799	.36207	-.02360	1.39958

ANOVA

dE_Best_Acr

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	52.169	3	17.390	1.154	.327
Within Groups	6703.005	445	15.063		
Total	6755.175	448			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: dE_Best_Acr

Tukey HSD

(I) Ethnicity	(J) Ethnicity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White/Caucasian	Asian	-.74288	.46368	.378	-1.9386	.4528
	Black	-.11094	.60467	.998	-1.6703	1.4484
	Hispanic	.27502	.54416	.958	-1.1282	1.6783
Asian	White/Caucasian	.74288	.46368	.378	-.4528	1.9386
	Black	.63194	.67223	.783	-1.1016	2.3655
	Hispanic	1.01790	.61836	.354	-.5767	2.6125
Black	White/Caucasian	.11094	.60467	.998	-1.4484	1.6703
	Asian	-.63194	.67223	.783	-2.3655	1.1016
	Hispanic	.38596	.73007	.952	-1.4967	2.2686
Hispanic	White/Caucasian	-.27502	.54416	.958	-1.6783	1.1282
	Asian	-1.01790	.61836	.354	-2.6125	.5767
	Black	-.38596	.73007	.952	-2.2686	1.4967

Group Statistics

	Gender	N	Mean	Std. Deviation	Std. Error Mean
dL_Best_Acr	Female	249	4.3716	3.95374	.25056
	Male	200	3.7021	3.25569	.23021
dc_Best_Acr	Female	249	5.3269	2.91591	.18479
	Male	200	4.9303	2.64188	.18681

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
dL_Best_Acr	Equal variances assumed	11.426	.001	1.927	447	.055	.66946	.34748	-.01343	1.35235
	Equal variances not assumed			1.967	446.714	.050	.66946	.34026	.00075	1.33817
dc_Best_Acr	Equal variances assumed	2.322	.128	1.493	447	.136	.39662	.26561	-.12537	.91861
	Equal variances not assumed			1.509	440.526	.132	.39662	.26276	-.11981	.91304

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
dL_Best_Acr	Between Groups	181.417	3	60.472	4.597	.004
	Within Groups	5854.342	445	13.156		
	Total	6035.759	448			
dc_Best_Acr	Between Groups	252.664	3	84.221	11.488	.000
	Within Groups	3262.337	445	7.331		
	Total	3515.001	448			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Ethnicity	(J) Ethnicity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
dL_Best_Acr	White/Caucasian	Asian	1.41144*	.43334	.007	.2940	2.5289
		Black	1.39254	.56510	.067	-.0647	2.8498
		Hispanic	.67819	.50855	.542	-.6332	1.9896
	Asian	White/Caucasian	-1.41144*	.43334	.007	-2.5289	-.2940
		Black	-.01890	.62823	1.000	-1.6390	1.6012
		Hispanic	-.73325	.57789	.583	-2.2235	.7570
	Black	White/Caucasian	-1.39254	.56510	.067	-2.8498	.0647
		Asian	.01890	.62823	1.000	-1.6012	1.6390
		Hispanic	-.71435	.68229	.722	-2.4738	1.0451
	Hispanic	White/Caucasian	-.67819	.50855	.542	-1.9896	.6332
		Asian	.73325	.57789	.583	-.7570	2.2235
		Black	.71435	.68229	.722	-1.0451	2.4738
dc_Best_Acr	White/Caucasian	Asian	-1.82460*	.32348	.000	-2.6588	-.9904
		Black	-1.21287*	.42184	.022	-2.3007	-.1250
		Hispanic	-.59067	.37963	.405	-1.5696	.3883
	Asian	White/Caucasian	1.82460*	.32348	.000	.9904	2.6588
		Black	.61173	.46897	.561	-.5976	1.8211
		Hispanic	1.23392*	.43139	.023	.1215	2.3464
	Black	White/Caucasian	1.21287*	.42184	.022	.1250	2.3007
		Asian	-.61173	.46897	.561	-1.8211	.5976
		Hispanic	.62219	.50932	.613	-.6912	1.9356
	Hispanic	White/Caucasian	.59067	.37963	.405	-.3883	1.5696
		Asian	-1.23392*	.43139	.023	-2.3464	-.1215
		Black	-.62219	.50932	.613	-1.9356	.6912

*. The mean difference is significant at the 0.05 level.

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	a & TOTAL	71	.080	.508
Pair 2	a & PERCENTAGE	71	.029	.813
Pair 3	Thickness & TOTAL	72	-.089	.459
Pair 4	Thickness & PERCENTAGE	72	.019	.876

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