MAKING THE INVISIBLE VISIBLE: A NEW DIAGNOSTIC TOOL IN ENDODONTICS

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ABSTRACT

Bryan Mark Mitchell: Making the Invisible Visible: a New Diagnostic Tool in Endodontics
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Development of an endodontic diagnostic tool that is objective and does not elicit any pain will improve chairside diagnosis and ensure patient comfort. This study utilized Red-Green-Blue (RGB) color analysis and Eulerian Video Magnification (EVM) to assess periapical tissues by revealing hidden data in videos of the intraoral tissues. The goal of this hypothesis generating pilot study was to determine whether these technologies detect changes in blood flow and aid in diagnosis of periapical inflammation (disease). We obtained videos of the oral mucosa overlying the periapical tissues of teeth with clinical signs of apical periodontitis (n=20). Controls (n=20) were the oral mucosa overlying periapical tissues of normal contralateral teeth. Videos were analyzed using RGB analysis and EVM via a high level computing language, MATLAB. Data were analyzed using Wilcoxon matched pairs signed rank test and significance set at p<0.05. RGB/EVM analysis were not able to detect a difference in blood flow in the intra-oral videos of both the diseased and control tissues.
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CHAPTER 1: REVIEW OF LITERATURE

1.1 Introduction

Endodontics is the specialty of dentistry aimed at preserving the natural tooth through the diagnosis, prevention, and treatment of pathology associated with the dental pulp, root and periapical tissues (1, 2). The array of procedures used to prevent or treat diseases of the pulp and periapical tissues primarily consist of vital pulp therapy (partial pulp removal, direct or indirect medicament placement on pulp tissue), non-vital pulp therapy (non-surgical root canal therapy, regenerative endodontics) and periapical microsurgery. It is estimated that over 22.3 million endodontic procedures are performed annually in the United States, of which 15.1 million consist of non-surgical root canal therapy (3).

Prior to performing the appropriate endodontic treatment the dental practitioner must first determine the endodontic diagnosis by defining the pulpal and periapical (periradicular) status of teeth. Of particular importance to the formulation of an endodontic diagnosis is the evaluation of the periapical status. Periapical disease may be defined as inflammation and/or pathology associated with the periradicular structures of teeth. The presence of the inflammatory component of periapical disease is important because it is a sign of the body’s ability to mount an immune response, which is a key indicator of health status in the human body (4). The periapical tissues
that surround the roots of the teeth contain blood vessels needed to supply blood to the teeth (dental pulp or dental pulp tissue). The rate of blood flow depends on the needs of the dental pulp and is dictated by its environmental interactions (i.e. caries, trauma, etc.). Various stimuli (bacteria, iatrogenic insults, trauma) induce inflammation of the dental pulp that in turn result in increased blood flow in the periapical tissues (5). Pulpal inflammation has been noted as early as the initial phases of enamel demineralization that permits communication between the pulp and outside environment (6, 7). The symbiotic relationship between the pulp and periapical tissues provides a conduit for the extension of inflammation from the pulp to the periapical tissues. Specifically, the pulpal tissues may become inflamed due to microorganisms and/or their byproducts that may spread from the pulp to the periapical tissues resulting in a diseased state of the periapical tissues (apical periodontitis). In addition to pulpal inflammatory influence, trauma may directly impact inflammation of periapical tissues. The impact (level of inflammation) of trauma (intrusion, avulsion, luxation, concussion, subluxation) on the periapical tissues varies directly with the severity of injury to the alveolar process, periodontal ligament space, root and crown.

Periapical diagnoses are classified from a state of health (normal apical tissues) to a state of disease, which is denoted as a form of apical periodontitis. Prior to the detection of disease, the characteristics of periapical health should be noted. In normal periapical tissues there is an absence of findings and/or symptoms related to percussion (forces exerted in axial, lateral and oblique loading directions) and palpation (not sensitive) (1, 8). Further, during the radiographic analysis, an intact lamina dura should be able to be traced in addition to visualization of a consistent lateral spacing of the periodontal ligament space
All findings should be compared to a normal adjacent and/or contralateral tooth to truly establish a baseline of health. In the absence of health there are multiple diseased states described by periapical diagnoses (apical periodontitis) that are classified by the following:

- **Symptomatic Apical Periodontitis**: inflammation, usually of the apical periodontium, producing clinical symptoms including a painful response to biting and/or percussion or palpation. It may be associated with an apical radiolucent area (8).
- **Asymptomatic Apical Periodontitis**: inflammation and destruction of apical periodontium that is of pulpal origin, appears as an apical radiolucent area, and does not produce clinical symptoms (8).
- **Acute Apical Abscess**: an inflammatory reaction to pulpal infection and necrosis characterized by rapid onset, spontaneous pain, tenderness of the tooth to pressure, pus formation, and swelling of associated tissues (8).
- **Condensing Osteitis**: diffuse radiopaque lesion representing a localized bony reaction to a low-grade inflammatory stimulus, usually seen at apex of tooth.
- **Chronic Apical Abscess**: an inflammatory reaction to pulpal infection and necrosis characterized by gradual onset, little or no discomfort, and the intermittent discharge of pus through an associated sinus tract (8).

Each of the aforementioned conditions involve some degree of inflammation although painful discomfort is frequently not experienced in instances of chronic apical abscess, asymptomatic apical periodontitis and condensing osteitis (8). Contrariwise, painfully symptomatic periapical conditions (symptomatic apical periodontitis and acute apical abscess) are often easy to diagnose. However, diagnosis may be
challenging in situations when the apices of several teeth are closely located making their response to diagnostic testing difficult to interpret. In such situations the availability of a diagnostic test that objectively locates areas of increased blood flow would be immensely helpful in identifying the tooth that requires endodontic intervention. This ability to make an accurate diagnosis would decrease patient morbidity (correct tooth treated, decreased expense, improved quality of life) and increase efficiency (decreased number of appointments due to obtaining the accurate diagnosis), which benefits both the patient as well as the practitioner.

1.2 Ideal Properties of a Periapical Diagnostic Test

Diagnosis is the foundation for providing treatment and is comprised of the following: chief complaint, medical history, dental history, examination and testing (pulpal and periapical evaluation) and radiographic findings (9). A significant component that is fundamental in obtaining an accurate periapical diagnosis is the ability to have periapical tests that are able to identify the true state of the periapical tissues. Preferably these tests would be completely accurate which correlates to ideal sensitivity (ability of the test or technique to detect disease), specificity (ability of the test or technique to determine absence of disease) and predictive value (precision rate of diagnostic test to determine disease or non-disease); accompanied by being easy to use, low cost, non-invasive and painless. Yet, none of the tests currently used to evaluate periapical tissues satisfy all of these characteristics.
1.3 Diagnostic Tests

The health status of the periapical tissues are evaluated in three components: mobility, percussion and palpation. These dimensions allow the practitioner to accurately describe the health or disease status of the periapical tissues. Additionally, technology such as the digital bitefork force transducer and radiography are used to gather more information about the periapical status of teeth.

Mobility testing evaluates the relationship of the tooth and the surrounding periodontium. Commonly, the use of finger pressure or application of the back ends of two mirror handles in opposing directions are used to evaluate the degree of tooth mobility (9). Historically, the degree of mobility (physiologic or pathologic) has been described by the Miller index, which provides as score of 1 for normal physiologic movement, score of 2 for movement of one mm and a score of 3 for tooth movement greater than one mm in any direction (10). However, the amount of force applied during this test (Miller Index) and interpretation of the resultant tooth movement differs among practitioners, which contributes to its subjectivity. Accurate instruments (O’Leary and Rudd’s periodontometer, Muhlemann macroperiodontometer, electronic mobility device) have been developed to capture the horizontal mobility of teeth but they are not routinely used in practice due to their expense, bulkiness or difficulty of use (10, 11).

Percussion is the application of force or pressure on the tooth typically applied in an axial direction to formulate a baseline of health or an attempt by the practitioner to replicate the chief complaint of sensitivity to biting as described by the patient. The patient’s response or interpretation to the application of force(s) adds to the diagnostic data utilized to formulate a diagnosis. In percussion testing, application of force is
normally applied with the metallic end of a dental instrument (typically a mirror handle) (1, 9). The amount of force, its direction and the patient’s response to such testing all vary, producing an array of subjective findings. Inability of currently used methods to consistently standardize the amount of force exerted during percussion testing creates a challenging diagnostic exam, particularly when multiple adjacent teeth are symptomatic. Painful responses to percussion testing are indicative that periapical inflammation is present (12). Further, replicating the patient’s chief complaint may increase their discomfort and anxiety. Although these chair-side analyses (testing with the metallic end of an instrument, digital pressure) are variable in nature they do allow the dental practitioner to easily and economically conduct percussion testing for each patient in a time efficient manner. Several instruments have been developed to objectively measure percussion but they are not readily available for everyday use and are not practical to use in daily practice (13).

Palpation is defined as the act of touching or applying pressure with the intention of evaluation for the presence or absence of a lowered pain threshold (14). Palpation testing is normally performed with digital pressure that is applied to the area of concern. Palpation testing may also be performed with a cotton applicator or gently with the metallic end of a dental instrument (1). The amount of force, direction of pressure and place of application are all variables and lead to the subjective nature of the testing. A patient’s abnormal (painful) response as compared to a healthy contralateral site to palpation testing is highly suggestive that inflammation and/or disease is present (12). Although the current means of palpation testing is easy to use and readily available for the
dental practitioner its application of pressure to the diseased area may cause discomfort or pain to the patient (symptomatic or asymptomatic).

The digital bitefork force transducer is a device that objectively measures the mechanical pain thresholds of human teeth (15). To use the force transducer that measures/displays the output force in Newtons (N), the practitioner asks the patient to bite down on the device until pain is experienced (15). Also, the force transducer has a built-in safety measure that does not allow the patient to exceed 775 N and thus preventing possible tooth injury (16). A substantial difference in output force (N) between contralateral teeth relates to the mechanical pain threshold, which is directly related to the corresponding inflammatory state (16). The identification of this inflammatory state of the periapical tissues of teeth has been shown with the use of the digital bitefork technology (16). While the digital bitefork force transducer is a great tool to objectively identify acute periapical inflammatory conditions, it is not easily available and thus its availability is limited to dental practitioners.

Radiographic assessment is fundamental in endodontic diagnosis and allows the practitioner to evaluate the absence or presence of periapical disease as denoted by a pathologic change in the lamina dura or periodontal ligament space. Radiography methods used to evaluate periapical tissues allow for analysis in two-dimensional (film, photostimulable phosphor, charge-coupled device, complementary metal oxide semiconductor) and three-dimensional planes (small and large volume cone-beam computed tomography) (1). While two-dimensional radiography is most often used to evaluate periapical tissues, the gold standard requires procurement of a histological analysis, which is not practical on a routine basis unless a surgical intervention is
warranted (17). Histological classification allows the capture of the true state of disease or health of the periapical tissues under evaluation. These classifications are typed as periapical abscesses, cysts, granulomas or other pathologic conditions (18). The presence of various microstructures identified by a variety of staining techniques allow for the identification of these periapical conditions as noted by their cellular/intercellular components (i.e. lymphocytes, macrophages, plasma cells, polymorphonuclear leukocytes, epithelium, cholesterol clefts, etc.) (18-20). Yet, surgical intervention is not performed unless a diagnosis has been established, therefore the importance of pre-treatment radiography is emphasized as a correlative factor to the true diagnosis.

Periapical radiography (two-dimensional analysis) is the primary radiographic method used by practitioners to evaluate the periapical status of teeth. Multiple (straight-on, angled projection) periapical radiographs captured from the same site attempt to depict a three-dimensional object onto a two-dimensional medium and should show the entire tooth as well as the surrounding bony support (21). Further, the quality of the image formed is a combination of the x-ray beam (exposure duration, exposure rate, energy, collimation, intensity), level of soft/hard tissue attenuation and resultant beam absorption of the receptor (21). In addition to clinical signs and symptoms the periapical radiograph is critical in the formulation of the periapical diagnosis. Periapical radiography has been shown to be highly specific, yet less sensitive in its evaluation of apical periodontitis (17, 22). Variable interpretation of the periapical radiographic findings may lead to dissimilar treatment provided, thus directly impacting patient health outcomes.

Cone beam computed tomography (CBCT) is radiography that allows for the three-dimensional evaluation of structures in the maxillofacial region. This radiographic
technology evaluates an area of interest through the use rotating ionizing radiation that is captured by a detector (21, 23). During the rotation of the CBCT unit, numerous planar projections are attained and compiled in a manner to produce a volumetric rendering of the area of interest (21, 23). The use of CBCT technology to evaluate the status of periapical tissues has been of noteworthy application. While CBCT technology has been shown to be highly specific, its level of sensitivity does not mimic the histological gold standard in the detection of apical periodontitis (22). CBCT technology has exhibited poor accuracy at the detection simulated periapical lesions 0.8 mm or less, but much better accuracy for sizes greater than 0.8 mm and excellent accuracy greater than 1.4 mm (24). CBCT provides the practitioner with a radiographic means to identify periapical defects not seen with standard two-dimensional radiography, possibly enhancing the detection of disease (22, 24). While CBCT units are readily available for sale their expense, space required for installation and associated learning curve are deterrents from use by practitioners. Overall, it is a recent technology that has given practitioners additional means to better serve their patients (21, 23).

The ability of cone-beam computed tomography (CBCT) and periapical radiographs to detect the absence of disease have both been shown to be highly specific (22). Comparatively, their (CBCT and periapical radiography) ability to detect disease has been shown to be variable; particularly when the periapical findings are of smaller size (less then one millimeter in diameter) (22, 24). Also, the variability of these technologies to detect disease increases when there is vital pulp tissue present and decreases when there is no vital tissue present (25). Of additional note are the differences in radiation dose between radiographic technologies, particularly digital periapical radiographs and focused
field CBCTs that are often used by endodontic practitioners. Effective radiation dosages (measured in micro Sieverts [μSv]) vary significantly for focused field CBCT (anterior – 4 μSv, maxillary posterior 9.8 - μSv, mandibular posterior – 38.3 μSv) compared to the capture of a digital periapical radiograph (6 μSv) (21, 26). The practitioner must weigh the benefits of increased diagnostic data versus the potential increase in radiation exposure to the patient. Currently used radiographic techniques are invaluable but must be used in conjunction with the clinical signs and symptoms to accurately depict the patient’s condition and ultimately provide the best care possible.

1.4 Non-invasive Technologies Possibly Applied to Evaluate Periapical Tissues

Advances in technology have significantly progressed over the years since the advent of digital photography in the mid-1970s. Today, access to digital cameras and the ability to capture high resolution video is generally at the finger tips of any person in the United States of America with a modern day smartphone (27). This access to technology and recent advances in data analysis of the information that is contained within photographs and video allow a whole new world of untapped potential to be mined and explored to new depths. High-level mathematical computing software such as MATLAB® enables the data within media to be explored (28, 29). Unique technologies such as Eulerian Video Magnification and Red-Green-Blue (RGB) color analysis that utilize MATLAB® for data processing purposes may be applied to evaluate the health status of periapical tissues (30, 31). Application of non-invasive video technology such as EVM or RCB color analysis may potentially have monumental benefit to healthcare through its advancements and use in monitoring physiological patient signals (28).
**Eulerian Video Magnification**

Eulerian Video Magnification (EVM), the brainchild of the Computer Science and Artificial Intelligence Laboratory (CSAIL) at Massachusetts Institute of Technology (MIT), is a technology that is able to magnify subtle motions contained within video that are not perceived by the naked eye (30) (Figure 1). Fundamentally, it utilizes a customized computer program code and is based on comparative differential approximations to optical flow algorithms and Eulerian philosophies (30). Optical flow algorithms refer to a method that approximates how an observer views the relative motion of an object (32). The quality of this approximation allows for better visual interpretation of the media being analyzed. Additionally noted, is the Eulerian perspective of Eulerian Video Magnification that highlights a particle or unit with respect to space at a given time point (33). EVM’s division of space within an image with regard to a specific time allows for analysis of the space based on a multitude of desired parameters, thus maximizing the desired output of hidden information. An instance of this would be EVM’s characterization of motion in an exaggerated manner by amplifying color values at static points with regard to time (30). At the crux of this analysis is the acquired video, which is multidimensional and may be evaluated or characterized by the following parameters: frames per second, refresh rate, aspect ratio, color space/bits per pixel, video compression, format, perceived quality, etc. (34, 35). EVM utilizes this video as input and formats it in a manner to which a spatial decomposition may be applied (30) (Figure 2). Following spatial decomposition the video is processed temporally (pixel-wise) with regard to time (intensity) and frequency (amplitude) (30). The final signal is amplified during the temporal processing phase to
reveal hidden information contained within the video that is visualized through the output video produced (30) (Figure 2).

Eulerian Video Magnification technology has been applied in the detection of small movements within video such as the following: infant’s chest rising while breathing, blood flow of a man’s face (Figure 1), motion of guitar string, pulsation/movement of radial and ulnar artery of human wrist, and movement of the sun’s shadow (30). EVM’s ability to capture an infinite number of mini-motions found in our daily environment allows a whole new world around us to be revealed. The current success of EVM’s detection of blood flow may be applied to the detection of blood flow in the periapical tissues of teeth and thus subsequently aid in the detection of inflammation. The application of EVM could possibly provide an easy-to-use, non-invasive technology that could be used by the dental practitioner as an additional diagnostic aid.

Red-Green-Blue Color Analysis

Red-green-blue (RGB) color analysis (model) is a technology that is based on the foundation that any color may be obtained by mixing the three primary colors: red, green and blue (36). A pivotal component of this model is based on the perception of the human eye and its ability to distinguish the respective signals associated with the main colors (red, blue, green) (36). Within the retina of the human eye houses the cone system, which has very high spatial resolution regardless of light and allows color to visualized (37). The quality of this color system and subsequent dynamic cerebral interpretation of these signals by an individual may vary contributing to a certain amount of subjectivity in color perception and resultant elucidation of the color (38). This is especially true when the variance of color is minute. Consequently, a more objectively standardized method would
need to be applied to evaluate these subtle differences; that is made possible with the application of computer image processing and its ability to decipher RGB data from various forms of media (28, 29, 31, 39). RGB color analysis has been applied to media to extract hidden data within, particularly numerical values for the primary colors (31, 39). The depth of the data within video may be attributed to the number of pixels present as they represent the smallest unit of resolution with respect to color and luminescence (40). Accordingly, the greater the number of pixels present, the greater the quality and the potential to extract color data. The ability to obtain a numerical color value as opposed to subjective description of color is invaluable due to the stark variability in the interpretation of color by the human eye (38, 39).

Red-green-blue color analysis, a computer imaging analysis tool, may be viewed as a novel technological application to dental medicine. Significantly noted is its history of use in a multitude of applications such as the following: imaging of cortical blood flow and hemoglobin concentration, human skin hemodynamics, characterization of vascular skin lesions, estimation of bruise age, pathophysiology of burns, etc. (29, 36, 39, 41, 42). Further, media analysis has been utilized in the evaluation of oral tissues and has aided in the detection of abnormality compared to the naked eye (43). The ability to mine data within media has contributed to RGB’s rich history in medicine and seems like a natural fit to be applied to dental medicine; particularly the evaluation of blood flow in the oral cavity, specifically the periapical tissues (39, 43). In diseased teeth there is an increase in blood flow in the periapical tissues and this pathology may be captured by the application of RGB analysis. This non-invasive technology could provide an objective measure of inflammatory conditions of the periapical tissues. The usefulness of this technology would
be highlighted in situations where it is difficult for the practitioner to determine which tooth is symptomatic or in scenarios of trauma. Ideally, successfully application of RGB color analysis to detect the presence of inflammation of the periapical tissues would allow this tool to be added to the diagnostic armamentarium of the dental practitioner.
CHAPTER 2: MANUSCRIPT

2.1 Materials and Methods

Subject Recruitment and Selection

This study was undertaken with the permission of the Institutional Review Board (IRB Study # 13-3243) of the University of North Carolina at Chapel Hill. Twenty patients referred for endodontic evaluation on an emergent and non-emergent basis between the ages of 7 and 92 years of age were recruited by the principal investigator for this pilot study in the Department of Endodontics within the School of Dentistry at the University of North Carolina at Chapel Hill. Eligible patients seeking treatment at the School of Dentistry were recruited to the study and informed of the study protocol and potential risks; written informed consent was obtained.

Inclusion criteria for the pilot study were the following: English speaking subjects referred for evaluation and/or treatment in regard to root canal therapy; possessed an anterior tooth with a periapical diagnosis of disease and a normal contralateral tooth.

Patients were excluded if they could not sit still for a period of thirty seconds.

Experimental Protocol

After formulation of a diagnosis by an endodontic resident based on the subject’s response to standard diagnostic testing and history of present illness in accordance with
the American Association of Endodontics’ (AAE) guidelines, he or she was referred to the principal investigator for consent and data gathering (8). The endodontic resident who formulated the respective diagnosis selected the teeth to be examined by the principal investigator. Data were gathered prior to treatment (injection of local anesthesia, root canal therapy) if warranted.

**Patient Positioning, Microscope Setup and Data Gathering**

Subjects were positioned in an upright position in a dental operatory and their vitals (blood pressure, pulse, mean arterial pressure) were obtained with a vital signs monitor (Vital Care 506N3 Series, Criticare Systems Inc.). While still in an upright position the mechanical pain thresholds of the pre-selected teeth were measured one time with a digital bitefork force transducer (Occlusal Force-Meter, GM10; Nagaro Keiki, Tokyo, Japan) (15, 16). Next, subjects were placed in a supine position for several minutes to stimulate increased blood flow in the mucosal tissues approximating the periapex of the roots of teeth (44). Once positioned, the patient lips/cheeks were retracted to expose the apical extent of the mucosal tissue overlying the root of the tooth to be examined.

Videos of the mucosal tissue overlying the diseased and non-diseased (control) tooth sites were captured with a digital SLR (single lens reflex) camera, Canon T4i (18.0 Mega Pixels, full HD 1080), which was attached to a Global G6 microscope (optical magnification set at 5.1) with a xenon light source (Lamp Type: Cermax type 175 Watt Xenon, Power: 175 Watts, Color Temperature: 6000° Kelvin) (Figure 3). The microscope attached to the camera takes the place of a traditional SLR that is needed for optimum
video capture. The recorded video was then transferred to a data file and converted from a .mov file (file extension for Quick Time) to a .mp4 (latest compression standard for audio/video by Motion Pictures Expert Group) file by the use of a video converter, HandBrake for Mac (45, 46). The newly converted data file was cropped in iMovie to produce two different files (diseased video, control video) to further highlight the areas of analysis. The generated files (diseased video, control video) of the cropped areas were identical sizes for standardization purposes. From this juncture the videos were processed via Eulerian Video Magnification (Figure 4) through an online platform (Videoscope – created by Quanta Research Group) or Red-Green-Blue analysis via MATLAB® (Natick, MA) (47).

**Red-Green-Blue Analysis: Video Processing, Data Extraction**

In order for video to be processed in MATLAB® (Natick, MA) the data file was inserted into the appropriate directory (data). Within MATLAB® (Natick, MA) an uploaded program code named testVisualMean.m (Figure A1) courtesy of MATLAB® (Natick, MA) was written to obtain mean color (red, blue, green) values with regard to the number of frames for each video. Processing time ranged from 50 – 400 seconds and provided a numerical output of mean color value for the respective video. The obtained numerical output was then normalized (Appendix A2) (39).

**Eulerian Video Magnification & Red-Green-Blue Analysis: Video Processing, Data Extraction**

Eulerian Video Magnification processing was done by first uploading the data file into a website named Videoscope. Video data were separated into diseased and control videos and were modified in an identical manner. The type of magnification selected was
Eulerian color amplification, which maintained a video speed for 30 frames per second (fps). Next the frequency (60 heart beats per minute = 1 hertz) range was entered as a range of \(-/+/2\) heartbeats per minute from the subject’s supine heart rate. Further, the signal of interest captured from the frequency range was magnified by an amplification factor of 100 to better accentuate the difference in signal obtained from the diseased site versus the control site. The type of video filter selected was an ideal filter due to the need to focus specifically on data within the interest range (47). Final video processing took 100 – 300 seconds. The obtained modified video data were then transferred for processing via RGB color analysis as described previously.

2.2 Results

Red-Green-Blue color analysis:

A total of 20 subjects were involved in this pilot study in which video was captured of diseased (n=20) and control (n=20) sites that were evaluated via RGB color analysis. RGB color analysis was not able to detect a statistically significant difference (Wilcoxon Matched Pairs Signed Rank test, \(p < 0.05\)) of putative blood flow represented by relative color values (unit-less) between diseased (periapical diagnoses: asymptomatic apical periodontitis {AAP, n=3}; chronic apical abscess {CAA, n=6}; symptomatic apical periodontitis {SAP, n=11}) and contralateral control sites of mucosal tissue overlying the periapex of the respective root (Figure 5, Figure 6). In the detection of a difference in blood flow between the diseased and control sites a correlative trend of the relative color values were noted as follows: (1) red channel for the entire population (\(N=20\)), (2) chronic apical
abscess group of the diagnostic groups (AAP, CAA, SAP). Further, relative color values of maxillary sites (n=16) were compared to mandibular sites (n=4) and no trend was noted.

**Eulerian Video Magnification and Red-Green-Blue color analysis:**

Analysis via EVM and subsequent RGB color analysis mirrored the statistical findings of RGB color analysis.

**2.3 Discussion**

**General**

The goal of this pilot study was to evaluate the ability of non-invasive technologies (Eulerian Video Magnification, Red-Green-Blue color analysis) to detect changes in blood flow of mucosal tissue overlying the periapex of teeth (diseased vs. control site). This process of using non-invasive video technology to capture blood flow via the analysis of light absorption is described as photo-plethysomography or plethysomography (PPG) (48). The principle behind PPG is that in the presence of blood or an increased blood volume more light is absorbed as compared to the approximating tissue in which blood is not present or a lower blood volume is present. Within the blood volume the component of interest is oxy-hemoglobin whose signal is best acquired by the Green-channel (one component of the RGB color analysis) due to its ability to be absorbed better than the R-channel and the B-channel (29). Yet, the R, G and B channels work in concert with each other and directly correspond with the presence of putative blood flow (i.e. oxy-hemoglobin) that is represented by a down regulation of the G and B channels paralleled by an increase in the R channel (29, 39). When the entire population of subjects (N=20) was evaluated regardless of their diseased diagnosis (asymptomatic apical periodontitis {AAP},
chronic apical abscess {CAA}, symptomatic apical periodontitis {SAP}), no consistent trend was obtained except for the Red channel (up-regulation) in the detection of difference in blood flow between the diseased and control sites. However, when the findings were isolated by the diseased state (AAP, CAA, SAP) the periapical diagnosis of CAA showed a consistent trend with regards to the Blue channel (down regulation) and Red channel (up-regulation) in identifying increased blood flow versus the contralateral control as represented by a decrease in the green and blue values (channels), accompanied by an increased red value (channel). Ideally, the CAA group would have also shown a trend in the Green channel (down regulation) as well to best depict a difference in blood flow between the diseased and control sites. The diagnosis of CAA may be described as a chronic inflammatory infection that is expressed on the mucosal tissue as a sinus tract (channel that connects with an abscess or area of infection) that oftentimes approximates the periapex of the diseased tooth (8, 49).

**Key Pilot Study Contrast**

Red-Green-Blue (RGB) color analysis has been used successfully to detect blood flow in humans as demonstrated in research studying the following: pathophysiology of burns, human skin hemodynamics, estimation of bruise age, vital signs assessment, etc. (28, 29, 36, 39, 41). A key contrast between this study and the aforementioned studies centers directly on the area of analysis, soft tissue versus hard tissue. In the successful application of RGB color analysis the area of investigation was limited directly to soft tissue to allow the media utilized to evaluate and/or characterize blood flow. However, in our study concerning the evaluation of mucosal tissue overlying a site of periapical disease versus a healthy contralateral site attempts to capture the effect of bony pathology (periapical
disease) on the overlying mucosal tissue. Increased severity of periapical disease correlates to amplified changes in the alveolar process, which separates the periapex of the tooth and the overlying mucosal tissue being evaluated (characterized) for blood flow. If the alveolar process were perforated, likely as a consequence to disease, the influence of periapical disease on the overlying mucosa tissue would be heightened. This process was illustrated with the findings of our pilot study in which diseased states (chronic apical abscess) resulting in perforation of the alveolar process displayed a better correlation to detection of a difference in blood flow between a diseased and a control site. For RGB analysis to be applied in situations where the cortical plate overlying the periapex is not perforated, detection of this sub-mucosal pathology may require more advanced camera technology (i.e. increased pixel resolution).

**Patient specific parameters**

Several patient specific parameters may have played a role in the inability of the technology (RGB color analysis, EVM) to reliably detect a difference in blood flow of mucosal tissues overlying a diseased site compared to a control site. Although subject’s who were not able to sit still during the video capture were excluded from the study, there was still some inherent movement present that may have had an impact on an accurate depiction of the sites being evaluated and subsequent data analysis. Also, evaluation of a normal contralateral site was not used in each subject due either due to availability of a periapical x-ray to diagnose appropriately, contralateral tooth not being present or distance between diseased tooth and contralateral being greater than the scope of the video field. The use of a non-contralateral normal site does increase the variability of the site being analyzed due to unequal anatomical architecture. In instances when the
contralateral tooth was not used the adjacent tooth was utilized as the control tooth. Another important variable to take into account is the gingival biotype as well as melanotic pigmentation of the subject’s gingiva. Gingival biotype describes the thickness of the gingiva and is an important metric used in restorative dentistry, particularly when evaluating esthetic crown margins (natural and prosthetic) (50). A subject with a thicker biotype (> 2 mm) would lessen the ability of RGB technology to detect the level of impact of bony disease on the overlying mucosal tissues (50, 51). This area of irregularity was not accounted for in the study but may have been mitigated due to the inclusion criteria of only anterior teeth, which have a less prominent cortical plate as compared to posterior teeth (52). Further, the presence of melanotic pigmentation in the sites of evaluation would decrease the efficacy of the RGB technology due to the absorption of light by the pigmented cells.

**Light Spectrum**

A prominent component of photo plethysomography (PPG) technology directly relates the type of light utilized to obtain an output signal. Data from PPG is derived from the collection of reflected light from the tissue being analyzed and its correspondence to heart beat related fluctuations in blood flow and its related volume. The light utilized may be classified by the wavelength expressed, which corresponds with a specific level of energy (ranging from higher energy gamma rays to lower energy radio waves). The amount of energy emitted directly impacts the path of travel (absorb, scatter, reflect) in which the light will make as well as the medium (e.g. sensor, probe, camera) used to capture the resultant path of the light that allows characterization of blood flow within tissue (53). To hold true to the principles of PPG in our pilot study we used a light source
(xenon light source- Lamp Type: Cermax type 175 Watt Xenon, Power: 175 Watts, Color Temperature: 6000° Kelvin) in the visible spectrum (ranges from 400 to 700 nanometers) (Figure 7) for added illumination of the study sites although successful application has been achieved with only the application of ambient light (28, 29). We applied more light to the study sites for more effective evaluation and better data output to be obtained. Further, the anatomical barriers for the xenon light source to travel were: gingival tissue (thin - < 2mm or thick > 2 mm), labial cortical plate (average thickness: maxillary 1.7 mm, mandibular 0.99 mm) and granulomatous tissue at diseased site (50-52).

Besides PPG there are other sensitive methods (e.g. laser Doppler flowmetry, thermography, etc.) that may detect or characterize increased blood flow, but they are significantly more expensive and/or cumbersome; which utilize an infrared signal (> 780 nanometers) (Figure 7) and a specific probe/specialized camera (charge coupled device, thermographic camera) or fluorescence (41, 54-56).

**Technology**

Eulerian Video Magnification (EVM) technology has the ability to detect subtle changes not seen with the naked eye, especially visualizing facial blood flow in humans (30). Nonetheless, it is challenging for the human eye to visibly detect a difference between blood flow rates between a diseased site and a control site overlying the mucosal tissues. Therefore, EVM analysis was performed prior to RGB color analysis with the goal of extracting a specific signal within the captured videos and to then obtain an objective evaluation through an output of relative color values (red, blue, green) as achieved by RGB technology. In order to extract the desired signal the subject’s supine heart rate was obtained and utilized as a key parameter to aid in defining the signal range of interest
(SROI). Further, the filter type of ideal was selected to only enhance data within the SROI, which produced a signal with less noise and therefore promoted a better data stream to analyze. Once the SROI was acquired the signal was amplified for enhanced differentiation of color values during RGB processing. Results obtained with EVM analysis followed by RGB analysis mirrored the findings of RGB color analysis only. Thus suggesting that EVM technology is not necessary in the detection of difference of blood flow of mucosal tissues overlying diseased versus controlled sites. Without the need to process the videos via EVM technology processing time can be reduced by an average of 225 seconds.

After further review of the application of the evaluated non-invasive technologies (EVM, RGB color analysis); focus on oral soft tissue evaluation may show more promise as suggested by success in previous studies in the evaluation and/or characterization of blood flow in humans (28, 29, 36, 39, 41).
FIGURES

Figure 1. An example of Eulerian video magnification. (A) Four frames from the original video sequence. (B) The same four frames after the subjects pulse signal was amplified. The spatiotemporal slices on the left show how Eulerian Video Magnification amplifies color variation. From: http://people.csail.mit.edu/mrub/vidmag/
Figure 2: Schematic of Eulerian Video Magnification processing
Figure 3. Camera (Canon T4i) and microscope (Global G6) set-up over patient chair.

Figure 4: Eulerian Video Magnification: left (input), right (output)
Figure 5: Normalized relative color values obtained through Red-Green-Blue color analysis of diseased (asymptomatic apical periodontitis - AAP, chronic apical abscess- CAA, symptomatic apical periodontitis- SAP) and control videos.

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<th>Green</th>
<th>Red</th>
<th>Blue</th>
<th>Green</th>
<th>Red</th>
<th>Diagnosis</th>
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<td>SAP</td>
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Figure 6 a-c: Relative color values of blue (6a), green (6b) and red (6c) associated with periapical diagnoses of asymptomatic apical periodontitis (AAP), chronic apical abscess (CAA) and symptomatic apical periodontitis (SAP). Relative color values between diseased and normal mucosal tissues indicate putative differences in circulation of blood.
Figure 6a

The bar chart shows the comparison between normal and diseased conditions for AAP, CAA, and SAP. The x-axis represents the categories (AAP, CAA, SAP), and the y-axis represents the value range from 0.0 to 90.0. Different colors denote normal (solid blue) and diseased (stippled blue) conditions.
Figure 6c
Figure 7


https://donnamitchellmoniak.files.wordpress.com/2013/02/visible-spectrum.jpg
FUTURE DIRECTIONS

The goal of this hypothesis generating pilot study was to determine whether Eulerian video magnification and/or Red-Green-Blue color analysis were able to detect changes in blood flow and aid in diagnosis of periapical inflammation (disease). Current findings do not unequivocally support the use of these technologies to detect a difference in blood flow between diseased and control mucosal tissues overlying the roots of teeth. Further work is needed to better correlate the technology to the true clinical findings and thus prove its success as has been noted in previous works that directly evaluated soft tissue, which is in direct contrast to its application in this study with the examination of bony pathology (28, 29, 36, 39, 41). Correlation of RGB color analysis may aid in the detection of disease in circumstances when taking a radiograph is not possible. Additionally, this technology may be functional in the evaluation of soft tissue within the oral cavity in which a marked change in blood flow correlates with a state of disease, health, healing (third molar surgery, tissue graft take, etc.) or aid in specific diagnoses. The ability of the RGB color analysis to quantitatively described soft tissue allows for the depiction of the site of analysis over different time points, which allows the practitioner to track the state of disease or health as noted in healing following surgery, progression of disease and tracking recovery of orofacial traumas.

If there is successful application of this technology we will work on developing an App that could be utilized with any smart phone. In order to use the App one would need
to proceed with the following steps: (1) go to the App Store on your smart phone (2) search for “Endodontic Video Diagnosis” (3) click download now (4) open the App and use as instructed. The App would utilize the camera quality of the respective smart phone and analyze the data accordingly (RGB color analysis). This not-for-profit App could be downloaded from venues such as the Apple Store/Android Marketplace. Ultimately, the successful application this technology will provide an easy-to-use, non-invasive, inexpensive, diagnostic tool that is readily available to dental practitioners on a global scale.

Future studies involving the non-invasive technology (EVM, RGB color analysis) should be carried out with the following constructs taken into consideration:

1. Increased sample size (at least thirty patients for each diseased group)
2. Use of Periapical Index to better describe correlation with radiographic findings and diagnosis (57)
3. Implementation of cone beam computed topography (CBCT) to compare with technology and aid in length determination as well as cortical plate thickness of the sites of analysis
4. Gingival biotype analysis
APPENDIX

A1: Red-Green-Blue color analysis program code used to determine mean color values within control and diseased videos.

testVisualMean.m

%%
% for x = 1:10
% disp(x)
% end
%

% This example to read and process a video file

% Clear the workspace
clear all;
close all;
clc;

% Read a sample video
testObj = VideoReader('Program File X.mp4');

% Calculate the number of frames
nFrames = testObj.NumberOfFrames;
meanRGBValuesInFrame=[];

% Loop in for every video frame
for k = 1 : nFrames
    % Read the video frame k
    videoFrame = read(testObj,k);
    % Display it
    imshow(videoFrame);
    % Extract the individual red, green, and blue color channels.
    redChannel = videoFrame(:, :, 1);
greenChannel = videoFrame(:, :, 2);
blueChannel = videoFrame(:, :, 3);
    % Calculate the average RGB values for each channel
redChannelMean = mean(redChannel(:));
greenChannelMean = mean(greenChannel(:));
blueChannelMean = mean(blueChannel(:));

% frame wise (rows correspond to the frame number) storing in a vector in the form of [r g b]
meanRGBvaluesInFrame = [meanRGBvaluesInFrame; redChannelMean
greenChannelMean blueChannelMean];
end

A2: Normalization of RGB data.

\[ n - r = \left( \frac{R}{R + G + B} \right) \times 255 \]
\[ n - g = \left( \frac{G}{R + G + B} \right) \times 255 \]
\[ n - b = \left( \frac{B}{R + G + B} \right) \times 255 \]
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