Title
SOPROCARE Perio-project - Fluorescence detection of dental plaque and gingival inflammation - a clinical pilot study

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SOPROCARE Perio-project - Fluorescence detection of dental plaque and gingival inflammation - a clinical pilot study

by

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THESIS

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MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

in the

[redacted]

Abstract

Purpose: To evaluate whether the SOPROCARE camera system due to fluorescence imaging allowed for detection of microbial plaque and gingival inflammation and if imaging correlated to a Microbial Plaque (Turesky modification of the Quigley and Hein Plaque Index) and a Gingival Index (Loe & Silness).

Materials & Methods: The study involved three groups of patients of 13 years or older. This included the overall sample and two subgroups--those with gingival pigmentation and those with orthodontic brackets. A stratified recruitment based on a clinical screening of the presented plaque amount covering the front teeth was performed. Photos were taken using the SOPROCARE camera system and conventional digital photography. After imaging, two examiners using the Turesky modification of the Quigley and Hein Plaque Index and the Loe & Silness Gingival Index, respectively, scored plaque levels and gingival inflammation.

Hypothesis: The first hypothesis was the SOPROCARE camera system in perio mode allows for detection and scoring of microbial plaque and that these scores correlate with a microbial plaque index. The second hypothesis was the SOPROCARE camera system in perio mode allows for detection and scoring of gingival inflammation and that these scores correlate with a gingival inflammation index

Results:

55 patients were recruited into the study. The first subgroup consisted of 8 patients with dark pigmented gingiva. The second subgroup had 11 patients who were undergoing orthodontic treatment with brackets. The overall clinical average plaque value was 1.1 ± 1.2 (mean ± Standard Deviation [SD]). Subjects with orthodontic brackets exhibited the highest averages but no significant difference was seen among all subgroups. The average gingival inflammation index was 0.7 ± 0.9, with no significant difference among all subgroups. It was observed that

those with pigmented gingiva did exhibit lower gingival index values but this was a consistent finding among all evaluation methods. Clinical plaque and gingival inflammation averages correlated best with SOPROCARE perio scores.

Discussion:

It was determined that the SOPROCARE device in perio mode allowed for reliable judgment of plaque and gingival inflammation when compared to the Turesky modified Quigley Hein index and the Silness & Loe gingival inflammation index.

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Introduction:

Disease Etiology

Microbial plaque is composed of numerous species of bacteria, which adhere to the surface of a tooth (1). They can colonize and aggregate rapidly, forming a biofilm preferentially around the gingival margin and interdental spaces (2). This developing biofilm releases biological products including lipopolysaccharides (endotoxins,) chemotactic peptides, protein toxins, and organic acids. Consequently, these can diffuse into the gingiva and trigger a host inflammatory response known as gingivitis (3,4,5). Early on in this disease process, removal of this deposit accumulation can restore gingival health before irreversible damages to surrounding hard and soft tissues have taken place (6,7). Irreversible damages include advanced periodontal disease, which is characterized as a chronic inflammatory disease of the tissues that support and attach the teeth to the jaws (8). As the biofilm persists, it can develop subgingivally and pathological periodontal pockets can form. The biofilm is capable of thriving due to a shift to more gram-negative bacteria subtypes to accommodate for the non-aerobic environment (9). Because of this lingering biofilm, the inflammatory response will continue and eventually destruction of the host tissues is involved. The key to halting the disease progression is the timely removal of deposits as they accumulate.

The visualization and elimination of these deposits and/or identifying early stages of gingivitis can sometimes be difficult for the clinician. More importantly however, is the ability for the patient to visualize this disease process occurring in order to motivate them to improve oral hygiene. Conventional methods of oral hygiene education include plaque disclosing solutions (10,11). Here, we discuss a novel device designed to utilize fluorescence to help identify these conditions to help dentists to visualize and to educate patients in a more efficient and interactive manner.

Fluorescence Application

Fluorescence is a process where certain substances absorb light of a shorter wavelength and in turn emit a radiation of a longer wavelength. This is able to occur because there are molecules called fluorophores that de-excite from a higher to lower energy level after absorbing the incoming light (12,13). This concept has been used with newer dental technologies and has been essential in caries detection. Among the products on the market are: Quantitative Light-Induced Fluorescence (QLF) (Inspektor, Amsterdam, Nederland), DIAGNOdent (KaVo Co., Biberach, Germany), SOPROLIFE and the SOPROCARE system (which use slightly different hardware and software) (ACTEON, La Ciotat, France). These tools are all based on properties of light-induced fluorescence. In addition to their ability to detect and diagnose accurately, these fluorescence-based devices have been cited to be safe and can do so without engaging ionizing radiations (14,15,16).

I. Quantitative Light-Induced Fluorescence

Quantitative Light-Induced Fluorescence (QLF) is an optical technique that is useful for detecting early carious lesions and engages the natural fluorescence of teeth (autofluorescence) to discriminate between sound tooth structure and caries. Specifically, it measures the percentage change of fluorescence of demineralized enamel with respect to surrounding sound enamel, and relates it directly to the amount of mineral lost during demineralization (17). This is based on the rationale that due to the increased subsurface porosities early caries presents, more scattering of light occurs, causing a diminished detection of fluorescence (18,19).

II. DIAGNOdent

Like the QLF system, the DIAGNOdent is also useful in early caries detection. It generates laser light at a wavelength of 655 nm and this light is absorbed by porphyrins

related to the caries process within the tooth. The re-emitted fluorescence light appears in the infrared spectrum (20). To facilitate this, the DIAGNOdent uses a high-pass filter that removes reflected incident light and ambient light so that only fluorescence near the infrared wavelength will pass (21). In the presence of caries or porphyrins the fluorescence increases (22). The phenomenon behind this increased fluorescence has not yet been fully explained, but has been proposed by Hibst and Paulus as being due to the integration of bacterial metabolites into the tooth structure rather than crystalline disintegration (23).

III. SOPROLIFE

The SOPROLIFE product is useful for caries detection and also to aid in cavity preparation. It registers images in three modes: daylight, diagnosis, and treatment (24). The daylight mode is taken under white light and magnifies the tooth's surface by more than fifty times. The diagnosis and treatment modes operate based on the concept of autofluorescence as mentioned earlier with the QLF system (25). Under the diagnostic mode, the camera utilizes a blue light of wavelength 450 nm to illuminate the tooth's surface. Healthy dental tissues fluoresce green while carious tissues will show up as greenish-black or red depending on the amount of porphyrin content (26). The third mode is the treatment mode, and the captured red fluorescence is electronically enhanced and helps to differentiate between infected and affected dentin (24).

IV. SOPROCARE

The SOPROCARE is a novel camera system designed by SOPRO and is designed to detect plaque/calculus and gingival inflammation levels. The SOPROCARE camera from a hardware standpoint differs from the SOPROLIFE camera system. The SOPROLIFE camera has 4 white and 4 blue LED lights while the SOPROCARE camera only has 4 white and 3 blue LED lights. The software is also different between the SOPROCARE

and SOPROLIFE in terms of image capturing and presentation (the software electronically enhances specific pixel colors).

Figure 1. SOPROCARE Handpiece

The SOPROCARE camera system emits blue light at a 450 nm wavelength using three blue diodes. The 450 nm wavelength is located in the non-ionizing, visible spectral wavelength region. Similar light sources are safely used in the new LED curing lights for composite curing in the mouth--emitting around 450 nm (27,28). The science behind this device is that illuminating plaque/calculus and gingival inflammation with a blue light will induce fluorescence due to porphyrins in microbial plaque and inflamed tissue, respectively.

Previous Studies done at UCSF engaging fluorescence tools

In Tiffany Hsu's study (*In Vivo Occlusal Caries Prevention by Pulsed CO_2 Laser Treatment Quantified by QLF*) and in the *Biofilm Modification in Orthodontic White Spot Patients - A Proof of Principle Pilot Study*, the QLF system was used to identify early precavitated carious lesions and monitor lesion progression or regression over time.

The SOPROLIFE system was used in Rechmann/Daniel Charland - *In Vivo Occlusal Caries Prevention by Pulsed CO_2 Laser and Fluoride Treatment*. A new clinical scoring system for carious lesions was applied to this study. This scoring system was developed using the SOPROLIFE system in daylight and in fluorescence mode and was compared to a Laser

Fluorescence Device (Diagnodent), a Fluorescence Caries detection aid system (Specta - Air Techniques), a visual inspection method (ICDAS II), and digital bitewing x-rays (29).

In contrast to the QLF, the advantage using the SOPROLIFE system is the use of bright blue LEDs, as they are able to provide much better and a higher effective light than the QLF system. In addition to the brighter light, the SOPROLIFE also provides a better camera resolution.

Porphyrin Application

The concept that UV excitation of decalcified tooth structure resulted in fluorescence was seen as early as 1928 (30). One of the first studies that demonstrated that bacterial metabolites, specifically porphyrins, could emit fluorescence from light induction was seen in experiments by Hibst (2001) (see appendix A). Porphyrins occur as intermediate steps from the synthesis of heme and are also produced by several types of oral bacteria (31). Up until fairly recently the idea of utilizing fluorescence to detect plaque/calculus and gingivitis has been rarely touched upon in literature, and is a relatively new frontier. Buchalla (2004) stated that detection of sub- and supragingival calculus was possible with fluorescence, observing emission within the 570-740 nm range via excitation between 400 and 420 nm. The study noted no differences between sub- and supragingival calculus and concluded that the fluorescence emission noted could be due to a variety of porphyrin derivatives and may provide the basis for future diagnostic procedures (32). A separate study by Qin YL (2007) had similar findings. This study utilized a blue LED of 405 nm and compared sub- and supragingival calculus surfaces to healthy tooth surfaces. He observed emission intensities of 477-497 nm for healthy teeth and 628-685 nm for teeth with calculus. Qin confirmed an induced fluorescence of calculus using a blue LED with 100% specificity and sensitivity in discerning dental calculus from healthy teeth (33).

Despite these findings, for clinical use there have been no devices designed to detect plaque/calculus and gingivitis based on fluorescence; however, we predict that the SOPROCARE is able to detect both and accurately correlate with their respective clinical plaque and gingival inflammation scales. The basis of our prediction relies on the fact that porphyrins are bacterial metabolites and thus can be found in plaque and calculus. In addition, since gingivitis is a reaction to bacteria and toxins and can result in gingival pain, redness and swelling (34,35), the increased blood flow due to these host defense responses will concentrate more hemoglobin to the site. Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells and heme is the best-known porphyrin in red blood cells (36). It is assumed that these porphyrins, like those in microbial plaque, absorb incoming light and fluoresce.

Significance: Microbial plaque and gingival inflammation belong to an intraorally occurring disease process, oftentimes without the patient even knowing of their existence. Accumulation of plaque on the tooth surface has two effects. The first represents the colonization of bacteria, which in turn allows them to feed on fermentable carbohydrates, resulting in the production of acidic by-product that can demineralize the tooth's surface resulting in cavities. The second is that plaque accumulation causes the release of bacterial toxins, which stimulates the gingiva to release inflammatory mediators. Over time, these mediators, in efforts to remove the bacteria can cause destruction of periodontal structures, leading to periodontal disease. A device that can show the presence of plaque and gingival inflammation via fluorescence can help patients better visualize this disease process occurring in their mouth. This allows for a more educational patient experience and in turn, can help motivate them in improving their oral hygiene. It will also allow the dentist to identify disease conditions in the early stage and react appropriately to prevent disease.

Hypothesis: The first hypothesis tested was that the SOPROCARE camera system in perio mode allows for detection and scoring of microbial plaque and that those scores correlate to scoring with a clinical Microbial Plaque Index (Turesky modification of the Quigley and Hein plaque index) (37,38). The second hypothesis tested was that the SOPROCARE camera system in perio mode allows for detection and scoring of gingival inflammation and that those scores can be correlated with the clinical scoring of a Gingivitis Index (Gingival Index [Loe & Silness]) (39,40,41).

Purpose: The objective of this proof-of-principle clinical pilot study was to evaluate whether the SOPROCARE camera system due to fluorescence imaging allows for detection of microbial plaque and gingival inflammation and whether obtained pictures will correlate to a Microbial Plaque (Turesky modification of the Quigley and Hein Plaque Index) and a Gingival Index (Loe & Silness), respectively. It is assumed that using this tool along with the captured pictures will be more efficient for the detection of microbial plaque and gingival inflammation than traditional clinical inspection and digital photography.

Methods and Materials:

Subjects

The target population was patients of 13 years and older, recruited from multiple clinics at the UCSF School of Dentistry including Preventive and Restorative Dental Sciences, Orthodontics, Pediatric Dentistry, and Periodontics. The patients were recruited during their visit for a new patient exam, periodic oral exam, or dental treatment. Through stratified recruitment an attempt was made to recruit an even distribution of subjects with different levels of plaque and inflammation with approximately 25% subjects with no plaque, 25% with low plaque, 25% with

moderate plaque, and 25% with severe amount of plaque. Originally it was anticipated that approximately 5-10 patients would be recruited for each group (total of 20-40 patients) but in the end 55 subjects (32 males and 23 females) were recruited for the study.

The following inclusion and exclusion criteria was used for all groups:

I. Inclusion Criteria

The subject

1. Was 13 years of age or older in good health with no gender predilection

2. Had at least 6 anterior teeth, ideally 6 upper and 6 lower anterior

3. Understood what the study entailed and was willing to comply to the protocols

4. Was willing to give verbal/written consent

5. Was willing to sign the "Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research" form

II. Exclusion Criteria

1. Patient suffered from systemic diseases. Any health condition that caused a change in patient's oral health or oral flora

2. Patient was pregnant

3. Patient had generalized crowding of the anterior front teeth

4. Patient had full coverage restorations or facial restorations

5. Frank carious lesions on facial surfaces

6. Intrinsic staining

Study Design

Selection Rationale

Patients were chosen from different clinics at UCSF with no preferential selection. Furthermore no age group was given priority as long as the patient was above the age of 13 for all groups. For each group, subjects were screened for microbial plaque load and if the subject qualified for a group that had already been filled, the subject was not used for the study. If a subject qualified for a group, he/she was recruited if consent was obtained.

This study of the SOPROCARE system was a pilot study. There were no previous studies comparing clinical plaque/gingival indices using this new fluorescence system. The sample size chosen was based on prior results obtained from other pilot studies. We assumed that we would collect data for approximately 8 teeth per subject, but more likely, 12 teeth. If a minimum of 20 subjects were recruited, it would give at least 240 data points for each parameter (plaque and gingival indices).

Blinding

The junior examiner (WS) was blind to the clinical scores when evaluating the archived SOPROCARE perio-mode fluorescence and daylight-mode pictures and the digital photographs. The senior examiner, who had assessed the clinical scores, performed the evaluations of archived pictures after the clinical part of the study had been finished for at least 4 weeks.

Procedures

After determining patient eligibility and/or what category the patient qualified for, informed consent was obtained from the patient or parent.

The following study tests were done:

(1) SOPROCARE Perio mode fluorescence pictures (2-3 teeth per picture). (Fig 2)

(2) SOPROCARE daylight pictures with the same magnification and distance as 'perio' mode pictures. (Fig 2)

All SOPROCARE photos were recorded with SOPROCARE imaging software (Acteon, Sopro, La Ciotat, France). A HP 620 Notebook (HP, Palo Alto, CA; Windows 7, Microsoft Redmond, WA) was used to collect the data.

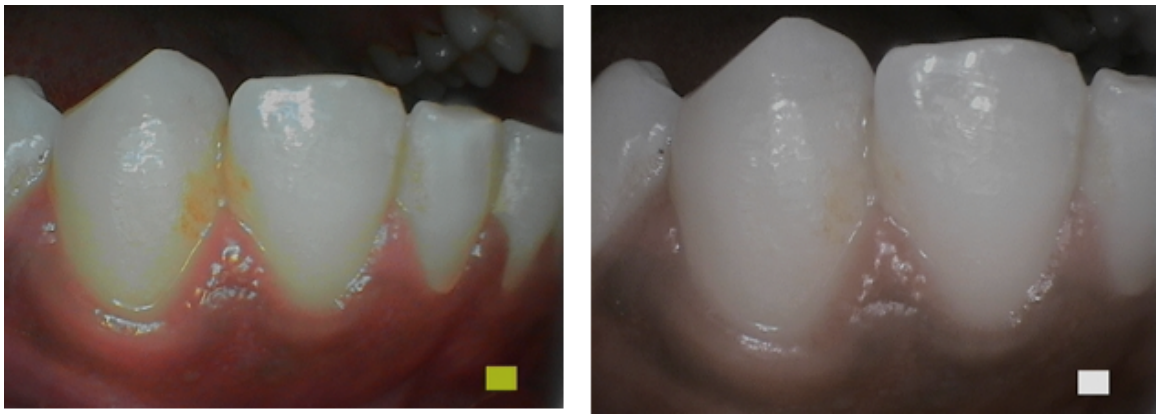


Figure 2. SOPROCARE photo in 'perio' setting (left) and 'daylight' setting (right)

(3) Digital photography pictures (Canon EOS 10D, Macro Ring Lite MR-14EX, Canon Macro Lens EF 100 mm, F1:2.8, USM). For all photos, the teeth were slightly air-dried to prevent reflection from saliva. The picture magnification (when reviewing the captured pictured) for both the examiners was equivalent to an observation of teeth with the naked eye at a distance of 30 cm

(4) A clinical exam was performed by the senior examiner after the photos were taken. Both the plaque index and gingival index of each tooth that was imaged was scored after photos were taken so that the plaque was not disturbed or any gingival bleeding had been induced.

a) Plaque Index using the Turesky modification of the Quigley and Hein plaque index (37,38)

This index did not require a plaque disclosing agent. Instead, the procedure included carefully probing over the facial surface of the tooth, beginning from the incisal edge of the tooth. The earliest point plaque was recognized with the explorer on the tooth was documented. The index is similar to the Quigley Hein Index, but has slightly modified criteria. Using this index, a score of 0 to 5 was assigned to all facial surfaces that followed within the protocol guidelines. The scoring was as follows:

Score	Criteria
0	No plaque
1	Separate flecks of plaque at the cervical margin of the tooth.
2	A thin continuous band of plaque (up to one mm) at the cervical margin of the tooth.
3	A band of plaque wider than one mm but covering less than one-third of the crown of the tooth.
4	Plaque covering at least one-third but less than two-thirds of the crown of the tooth.
5	Plaque covering two-thirds or more of the crown of the tooth

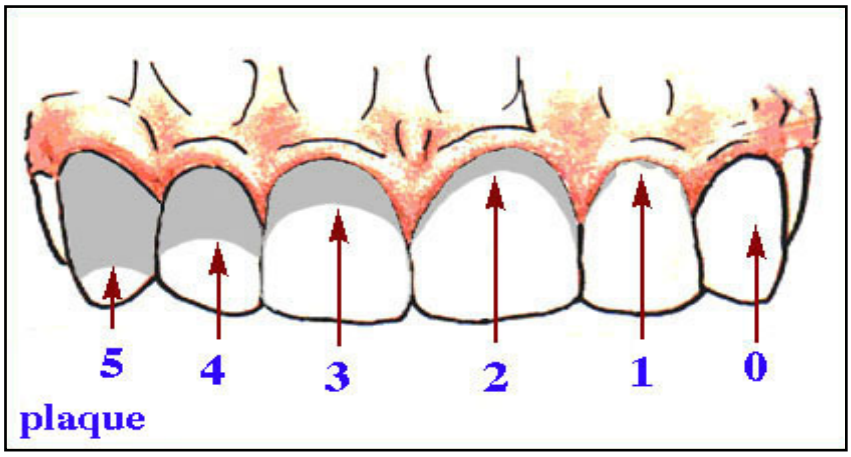


Figure 3. Turesky Plaque Index

b) Gingival Inflammation Index (GI; Loe & Silness) (39,40,41)

The number assigned to assess the degree of gingival inflammation:

0 - Normal, healthy gingiva with sharp, non-inflamed margins

1 - Marginal gingivitis with minimal inflammation and edema at the free gingival margin. No bleeding upon probing.

2 - Moderate gingivitis with a wider band of inflammation and bleeding upon probing

3 - Advanced gingivitis with inflammation

5) After all images were captured using both the SOPROCARE in daylight and in perio fluorescence mode, digital photography, and after clinical evaluation and scoring of each imaged tooth by both examiners, each examiner (senior examiner and junior examiner [WS, 2nd year postgraduate pediatric dentist]) assessed all digital images independently at a later date. The assessment was done twice, with at least one week apart, to allow for testing of reproducibility of all scorings. Each image was scored using the same plaque and gingival indices as the clinical indices and an analysis was performed to determine how comparable and accurate the SOPROCARE device was in each mode.

Results

A total of 55 patients (N=55) were recruited for the study with an average age of 36.9 ± 17.8 (mean ± Standard Deviation [SD]) years that ranged from 14.0 to 83.4 years. Figure 4 depicts the age distribution of subjects (within subgroups).

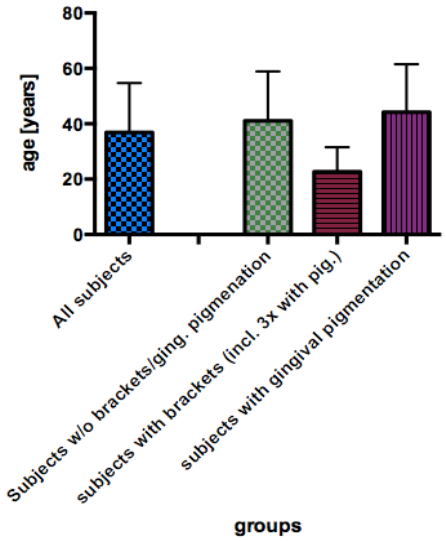


Figure 4. Age distribution of all subjects and subgroups (Mean, SD)

Several subjects recruited in the study had some teeth that did not meet the inclusion criteria (i.e. teeth with crowns, facial restorations, etc.). The data for these individual teeth were excluded from the study. In all, the 55 subjects provided for 638 teeth for evaluation. Group 1 comprised of all 55 subjects, who displayed a wide spectrum of plaque load and gingival inflammation presentation with the following plaque distribution as seen in table 1.

Plaque Index (Clinical Screening)	Recruiting Goal	Number of Subjects Recruited
None	5-10	18
Low	5-10	16
Moderate	5-10	10
High	5-10	11

Table 1. Comparison of recruiting goal versus number of subjects recruited based on screened plaque levels

The first subgroup comprised of 8 patients with dark pigmented gingiva to determine if any interference with the fluorescence reading for gingival inflammation was observed.

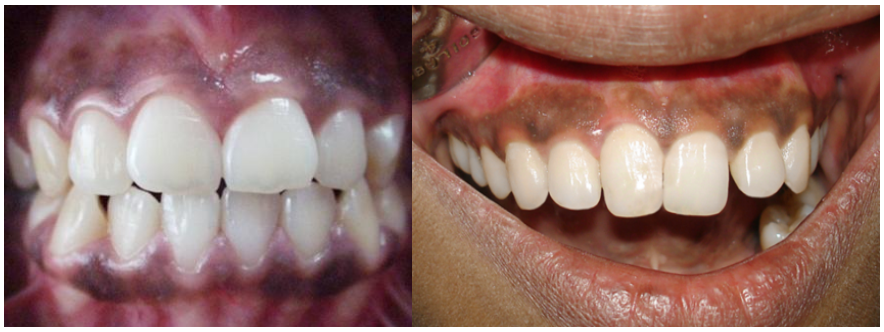


Figure 5. Depicts pigmented tissue. Dark pigmentation is sometimes located at the gingival margin, other times the gingival margin is free of pigmentation

The second subgroup included a group of 11 subjects who were undergoing orthodontic treatment with brackets bonded on.

Dark Pigmentation Only	Orthodontic Brackets Only	Pigmentation and Orthodontic Brackets	No Pigmentation / Orthodontic Brackets
8	11	3	33

Table 2. Subjects in Gingival Pigmentation and Orthodontic Bracket Subgroups

Kappa Value

Reliability values (kappa) were determined for each of the investigators individually to compare his reliability between repeated scorings (intra-examiner reliability) and to compare both investigators with each other (inter-examiner reliability) (Table 3). Kappa and weighted kappa values were determined for plaque and gingival indices using the SOPROCARE in perio and daylight modes as well as digital photography. Kappa relates to precise matches while weighted kappa considers close matches. For both, the strength of agreement is graded as

poor, fair, moderate, good, and very good. For SOPROCARE in perio mode, the intra-examiner kappa and weighted kappa values were good to moderate while the inter-examiner values were fair to moderate. For all other methods (i.e. SOPROCRE daylight and digital photography) it was seen that the more experienced investigator yielded equal or better kappa and weighted kappa values, thus also observing lower inter-examiner kappa values as well.

Method	Index	Intra-examiner kappa PR	Intra-examiner weight kappa PR	Intra-examiner kappa WS	Intra-examiner weight kappa WS	Inter-examiner kappa	Inter-examiner weight kappa
SC Perio	Plaque	0.474 (Mod)	0.649 (Good)	0.659 (Good)	0.767 (Good)	0.274 (Fair)	0.451 (Mod)
	Gingival	0.594 (Mod)	0.691 (Good)	0.013 (Poor)	0.119 (Poor)	0.471 (Mod)	0.522 (Mod)
SC Daylight	Plaque	0.613 (Good)	0.731 (Good)	0.070 (Poor)	0.219 (Fair)	0.233 (Fair)	0.333 (Fair)
Digital Photos	Plaque	0.427 (Mod)	0.614 (Good)	0.428 (Mod)	0.615 (Good)	0.139 (Poor)	0.300 (Fair)
	Gingival	0.752 (Good)	0.838 (Very Good)	0.347 (Fair)	0.493 (Mod)	0.485 (Mod)	0.548 (Mod)

Table 3. Intra-Reliability and Inter-Reliability Kappa Values for Different Assessment Methods

Data: Microbial Plaque

The overall average clinical plaque score for all subjects using the Turesky modified Quigley Hein criteria was 1.1 ± 1.2 (mean ± SD). For the subgroup of patients without brackets and without gingival pigmentation the average was comparable. For those with brackets the plaque index was higher than the overall average and for those with gingival pigmentation the plaque index was slightly lower than the average. The observed differences between these groups were not statistically significant (P>0.05) (Figure 6).

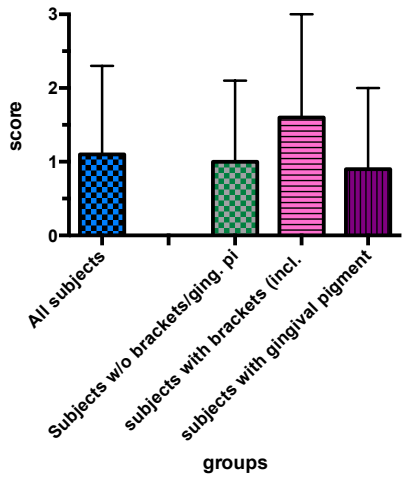


Figure 6. Turesky Modified Quigley Hein Index for all subjects and subgroups (Mean, SD)

Figure 7 depicts the average T-QH plaque index from clinical scoring, and scoring from stored pictures via SOPROCARE 'perio' and 'daylight' modes, and digital photography. Average plaque index values were observed in increasing order: clinical (1.1 ± 1.2), SOPROCARE perio (1.4 ±1.2), SOPROCARE daylight (1.7 ±1.3), and digital photography (2.0 ±1.2). Both SOPROCARE daylight ($p=0.01$) and digital photography ($p<0.001$) average plaque scores were statistically significantly higher than the clinical plaque score.

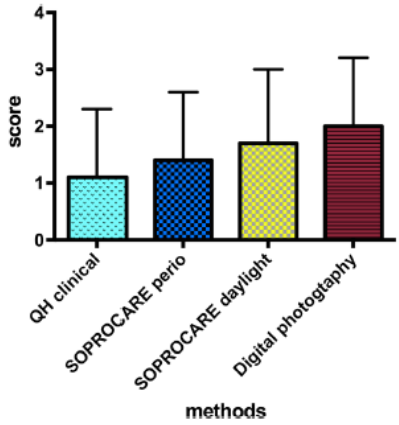


Figure 7. Average Modified Quigley Hein Plaque Index. Clinical Score, SOPROCARE Perio, SOPROCARE Daylight, and Digital Photography (Mean, SD)

In order to evaluate each assessment method's ability to discriminate between two different consecutive scores, linear regression curves were calculated for each assessment method. The following figure (Figure 8) shows linear regression fits of the SOPROCARE perio method in relation to the T-QH scores using two different examiners.

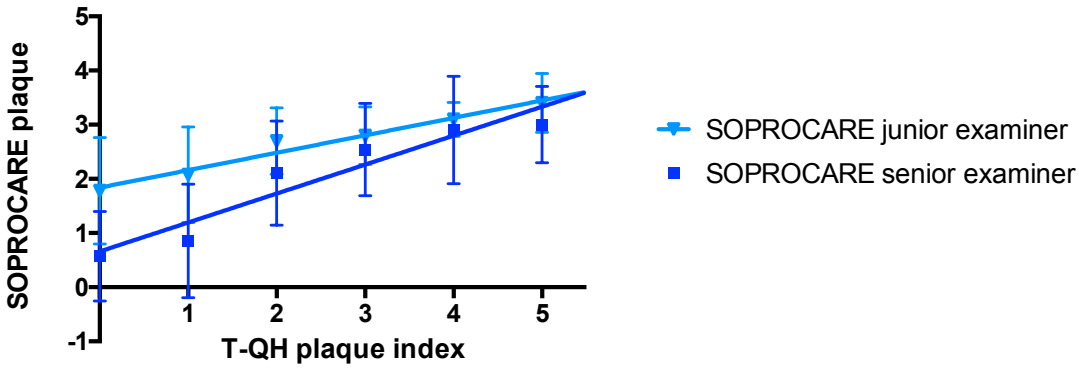


Figure 8. Linear Regression fits for clinically evaluated Turesky modified Quigley Hein plaque index and plaque indices evaluated by SOPROCARE in fluorescence perio-mode, for the junior and the senior examiner (Mean ± SD)

Upon initial evaluation of pictures using the SOPROCARE in perio mode, the non-trained junior examiner could possibly mistake all yellow hues of the tooth (especially at the gingival margins) to be microbial plaque (see Figure 9).

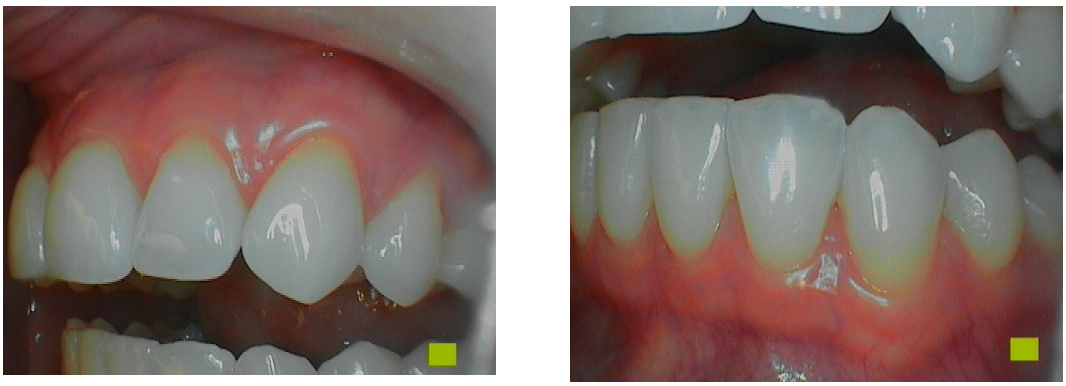


Figure 9: The pictures represent a clinically plaque-free patient, upper left (left) and lower left side (right) with a T-QH score 0, S&L score 0; notice the “yellow” line at the gingival margin– possibly misleading to assume that microbial plaque is present

This assumption resulted in an over-estimation of the SOPROCARE perio plaque score. This "exaggeration" was especially seen at lower levels of plaque. This overestimation in scoring was represented by the "SOPROCARE junior examiner (JE)" linear fit when compared to the "SOPROCARE senior examiner (SE)" linear fit. Both regression lines had the same position at clinical plaque index value of 5, indicating comparable scoring. As the plaque index decreased towards 0, the trend observed was that the "SOPROCARE JE" had a y-axis intercept higher than the "SOPROCARE SE" line. Both regression lines were significantly different from zero and had a goodness of fit of 0.97 and 0.92 for "SOPROCARE JE" and "SOPROCARE SE", respectively. The slopes for these lines (the ability to differentiate between two consecutive scores) were low for "SOPROCARE JE" (0.32±0.029) and better for "SOPROCARE SE" (0.53 ± 0.081).

Figure 10 shows regression lines for T-QH clinical plaque index versus SOPROCARE 'perio', SOPROCARE 'daylight', and digital photography scoring. The slope of these regression lines was seen in descending order of digital photography (0.65 ± 0.066), SOPROCARE 'daylight' (0.54 ± 0.066) and then SOPROCARE 'perio' (0.53 ± 0.081). All of the lines were

significantly different from zero with goodness for fit values of 0.96, 0.94, and 0.92 for digital photography, SOPROCARE 'daylight', and SOPROCARE 'perio', respectively. Where each of the lines intercept at the y-axis coincidentally correlates with the order of their slopes, with digital photography at the highest (1.17 ± 0.20), then SOPROCARE 'daylight' (0.91 ± 0.20), and SOPROCARE 'perio' (0.66 ± 0.25).

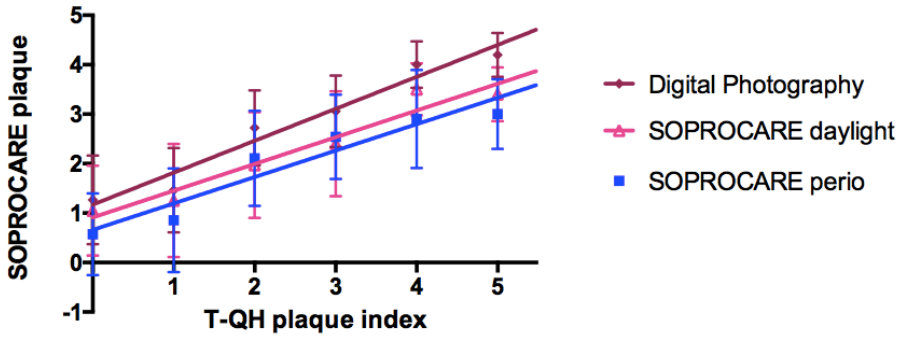


Figure 10. Linear Regression fits for Turesky modified Quigley Hein plaque index and the plaque indices evaluated by SOPROCARE perio, SOPROCARE daylight and Digital Photography (Mean +/- SD)

Data: Gingival Inflammation

For all subjects, the gingival inflammation index using the Silness & Loe scoring was 0.7 ±0.9. As seen in figure 11, the gingival index was lower for those with only gingival pigmentation and no brackets (0.3 ± 0.7) (mean ± SD), but the differences between all groups were not statistically significant.

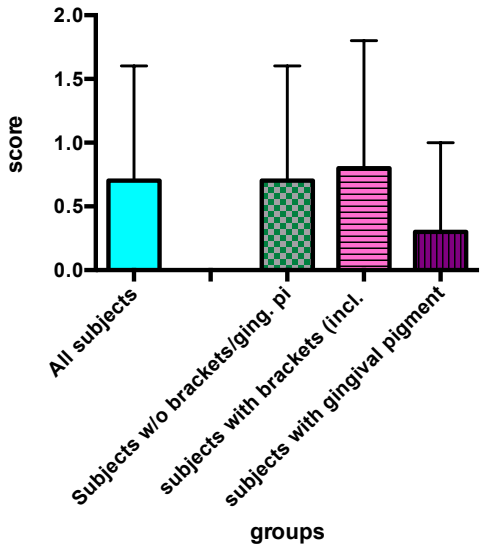


Figure 11. Silness Loe gingival index clinically evaluated for all subjects and subgroups. The differences are not statistically significant (Mean, SD)

A comparison between the Silness & Loe clinical gingival index was made with SOPROCARE 'perio', SOPROCARE 'daylight', and digital photography for all subjects (Figure 12). The average clinical gingival index scoring was 0.7 ± 0.9 with the average SOPROCARE 'perio' index lower at 0.6 ± 0.9. After evaluating the pictures, it was determined that the SOPROCARE 'daylight' photos were very difficult to score gingival inflammation because it did not exhibit any enhanced red coloration. The results seen with digital photography had an average of 0.5 ± 0.9 which was slightly lower than both the clinical scoring and the SOPROCARE 'perio' scoring. These differences however were not statistically significant.

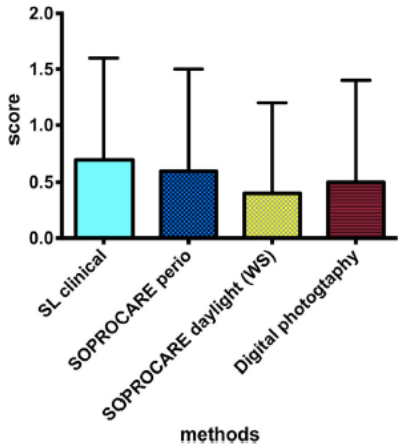


Figure 12. Average gingival inflammation index evaluated with the different evaluation methods. All applied methods show slightly lower values than the clinically evaluated Silness & Loe gingival index. The observed differences are not statistically significant (Mean, SD)

Figures 13 and 14 show the effects of absence or presence of dark gingival pigmentation on the gingival inflammation index, respectively. Overall, these figures show that none of the evaluation methods was significantly affected by either of these conditions. Figure 13 shows the average gingival inflammation index scored with different evaluation methods for the 33 subjects not having brackets or dark pigmented gingiva. The averages were: S&L clinical 0.7 ± 0.9, SOPROCARE perio 0.7 ± 0.9, SOPROCARE daylight 0.4 ± 0.8, and digital photography 0.6 ± 0.9. Figure 14 shows the gingival indices for the 8 subjects who exhibited dark gingival pigmentation with no brackets. The averages were: S&L clinical 0.3 ± 0.7, SOPROCARE perio 0.2 ± 0.6, SOPROCARE daylight 0.0 ± 0.2, and digital photography 0.2 ± 0.6. Within each group (presence/absence of gingival pigmentation), the differences were not statistically significant. While the 8 subjects with dark gingival pigmentation had an overall lower average Silness & Loe gingival index, all evaluation tools for this group showed a similar low gingival inflammation index.

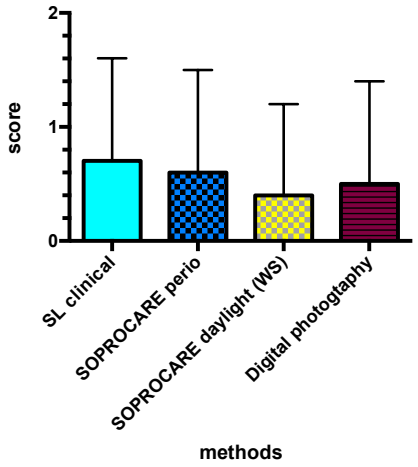


Figure 13. Average gingival inflammation index scored with the different evaluation methods for the 33 subjects not having brackets or dark pigmented gingiva. All applied methods show equal or slightly lower values than the clinically evaluated Silness & Loe index; the observed differences are not statistically significant (Mean, SD)

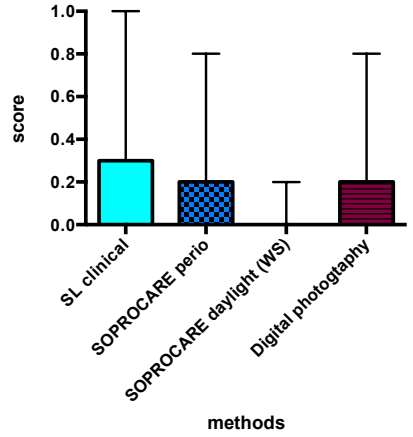


Figure 14. Average gingival inflammation indices for 8 subjects showing dark pigmented gingiva. All applied methods show slightly lower values than the clinically evaluated Silness & Loe index, the observed differences are not statistically significant (Mean, SD)

Linear regression fit curves were calculated for each assessment tool to evaluate the ability of each method to discern between two different consecutive scores by comparison of slopes. Figure 15 shows the regression fit curves for both SOPROCARE perio and digital

photography in relation to the Silness & Loe clinical gingival index scores. Both regression fits were significantly different from zero with goodness for fit of 0.9660 and 0.9631 for digital photography and SOPROCARE perio, respectively. The slopes were 0.7577 ± 0.1006 and 0.6598 ± 0.09135 for digital photography and SOPROCARE perio, respectively. With regards to the y-axis, both regression lines showed only minor set-offs from zero. The intercept value for digital photography was -0.00195 ± 0.1881 while for SOPROCARE perio the value was 0.2098 ± 0.1709.

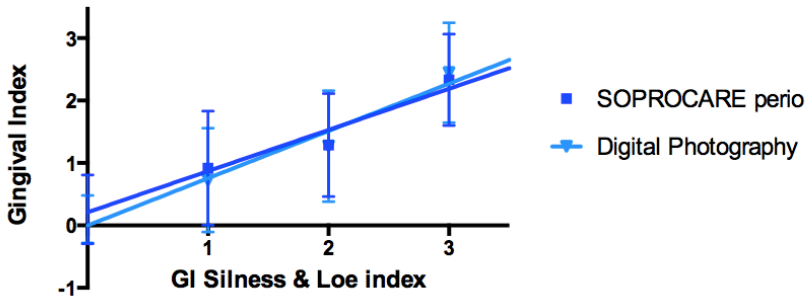


Figure 15. Linear Regression fits for Silness & Loe Gingival Index scores and the gingival indices evaluated by SOPROCARE perio and digital photography (Mean +/- SD)

Discussion

The concept of plaque detection via other means than direct visualization has been assessed in previous studies. One study by Smith et al. (42) determined the reliability of dental plaque quantification using an image analysis system. A digital camera (32-bit) was used to acquire images and an analysis was performed using two imaging softwares (Adobe Photoshop and Image Pro Plus). The reliability and repeatability results were 'excellent'. In a follow up study Smith et al. (43) in 2006 examined whether using a camera (digital SLR) or using an intra-oral camera (Schick) was more accurate in scoring plaque. In using the same analysis system as

the aforementioned paper, the results showed that the digital SLR camera yielded better reliability than the intra-oral camera. Despite the results in both of these studies, an extra step of plaque disclosing solution was required. Using fluorescence to help aid in plaque detection has also been looked at previously. In 1972, a study by Lang (44) looked at a fluorescent plaque disclosing agent named 'Plak-Lite'. The teeth/gingiva were illuminated by a 'Plak-Lite' lamp and photographs of the teeth were taken. The study showed that 'Plak-Lite' could reveal plaque on teeth, however no comparison was made to clinical scoring and accuracy. While the study's strength lies in the fact that the disclosing solution had no color (compared to conventional disclosing solution), using 'Plak-Lite' was tedious since multiple steps were needed to be undertaken. In contrast, one of the main strengths of SOPROCARE device is the fact that no disclosing solution is required, the device relies on the plaque's intrinsic fluorescent properties.

A major reason for attempting to quantify plaque and gingival inflammation levels has been the hope for improving patient hygiene education through enhanced visual aids. In a systematic review conducted by Watt et al (45), the paper examined whether providing oral hygiene education could improve oral hygiene and gingival health. The study examined different interventions that mainly consisted of parental counseling and providing basic oral hygiene instructions. In conclusion, there were mixed results with regards to improved hygiene. Most studies showed slight improvements in the short term, but long-term follow up was questionable. It was interesting to note that there was only one study that implemented the use of an intra-oral camera and the results were very favorable. This study by Willershausen et al (46) examined the impact of adding the use of intra-oral cameras in addition to oral hygiene instruction. The results showed that the control group (hygiene instructions only) had a 27% plaque reduction while the test group (hygiene instructions and intraoral camera) had a 50% plaque reduction. The majority of subjects (88%) thought that the addition of the intra-oral camera was desirable and helpful. It can be concluded that further investigation is needed to determine how effective the use of a visual aid such as an intraoral camera would be.

Plaque Detection and Assessment

In this SOPROCARE pilot study, it was shown that the SOPROCARE camera in perio mode had the ability to detect and score plaque with regards to the Turesky modification of the Quigley Hein plaque index. Overall the study included 55 subjects and had subgroups of dark pigmented gingiva and orthodontic brackets. It was seen in this study that despite these factors, plaque scores were not affected when using the SOPROCARE camera. In perio mode, the SOPROCARE camera was seen to yield slightly higher plaque index values than clinical scoring, but the differences were not statistically significant. In contrast, both SOPROCARE in daylight mode and digital photography showed a significantly higher plaque index level than clinical detection. It appeared that plaque detection with the SOPROCARE in perio mode was useful in showing and educating patients, as it detected and evaluated plaque at a slightly higher level. In addition, this study examined a subgroup of patients who were undergoing orthodontic treatment with brackets. It was hypothesized that due to reflective interferences, these brackets could interfere with the accuracy of scoring with the SOPROCARE camera. Our study incorporated 14 subjects with orthodontic brackets and the results showed that orthodontic brackets did not affect the quality of the photos.

Plaque Scoring and Correlation with Plaque Index

Another objective of the study was to examine how accurate the SOPROCARE camera system was in correlation to the Turesky modified Quigley Hein plaque index. Upon examining the regression lines for SOPROCARE perio, SOPROCARE daylight, and digital photography, it was noted that all of the regression lines were significantly different from zero with steep slopes. The slopes indicate that all 3 methods were easily capable of discerning between two consecutive scores. Furthermore, when comparing the offsets of the regression lines for the average plaque values of the 3 methods, all had positive offsets; however, SOPROCARE perio was only slightly offset while SOPROCARE daylight and digital photography had higher offsets.

The significance of this finding shows that the SOPROCARE camera in perio mode was able to detect plaque at only a slightly elevated level compared to the correlated clinical plaque index.

Gingival Inflammation Detection and Assessment

In this SOPROCARE pilot study, it was shown that the SOPROCARE camera in perio mode had the ability to detect and score gingival inflammation. Clinical scoring using the Silness & Loe gingival inflammation scale showed an overall higher average than the SOPROCARE in perio mode--this was followed by digital photography and SOPROCARE daylight mode. When comparing average gingival index values among all subgroups (Subjects with no orthodontic brackets / no gingival pigmentation, dark gingival pigmentation / no brackets, and all subjects) no significant difference was observed. Subjects with dark pigmented gingiva (n=8) did exhibit a lower Silness & Loe gingival inflammation index and SOPROCARE perio and digital photography was able to confirm that. There has been ongoing debate as to whether the dark pigmentation of the tissue could alter incoming or outgoing fluorescence scattering and/or absorption. Despite this, it could be concluded that having dark pigmented gingiva did not affect the quality of the images taken with the SOPROCARE camera in perio mode.

Gingival Inflammation- Correlation to Indices

It was observed in this study that the detection and scoring with the SOPROCARE camera system in perio mode could be correlated with the Silness & Loe gingival index. For both SOPROCARE perio and digital photography, the linear regression curves showed slopes that were significantly different zero, showing the ability to discriminate between two consecutive scores. Both regression curves also showed virtually no Y-axis offset. In conclusion, the SOPROCARE camera in perio mode was efficient and reliable in the scoring of gingival inflammation.

Summary and Confounding Variables

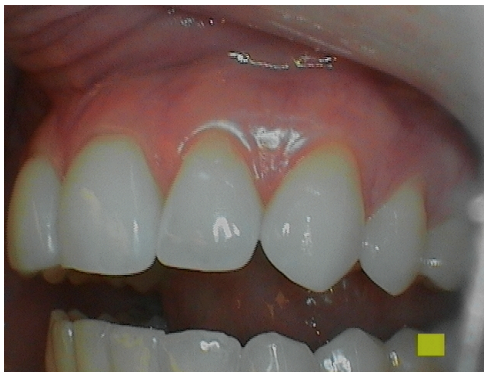
The fact that this was a pilot study introducing a novel imaging device was taken into consideration when interpreting the results. A couple of difficulties were seen with the SOPROCARE device alone. As an imaging device, taking the photos did require a certain degree of skill to get consistent quality photos from all subjects.

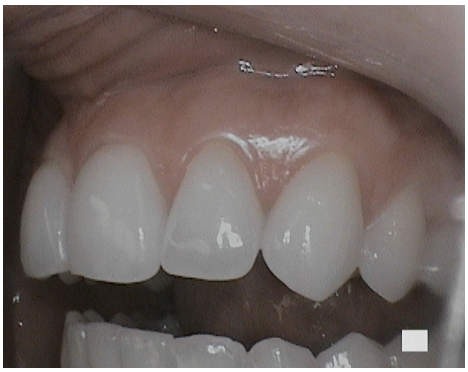
During the scoring of images, a tremendous learning curve was needed for both investigators; it was seen that the SOPROCARE device would tend to create false positives (more yellow or more pink/red hues) and fluoresce more than what was truly plaque or signs of gingivitis. Even despite multiple rounds of grading, the different levels of experience between both the senior and junior investigator did in fact play a role in accuracy of scoring images. The overall sample of 55 patients was enough to provide reliable results, but the subgroups for gingival pigmentation (n=8) and orthodontic brackets (n=11) could have used more test subjects to test the validity of the results.

It was observed for both examiners (junior and senior) that the slopes for regression curves for average plaque and gingival inflammation were relatively steep when using the SOPROCARE camera in perio mode. When comparing the junior and senior investigators, it was observed that the slopes of the regression curves were less steep for the junior investigator overall, indicating a lesser ability to discriminate between consecutive scores. Another trend observed was that as the clinical plaque score was lower, the SOPROCARE in perio mode tended to overestimate the true plaque index. As the plaque score got higher, both the clinical plaque score and SOPROCARE in perio mode were seen to be in agreement. This caveat can be improved upon with being more familiar with interpreting the images. This was also extremely evident when examining kappa values, where the junior investigator yielded lower kappa scores than the senior investigator. Due to the "learning curve" in properly grading/interpreting images, only the last grading submission from each of the examiners was used in determining kappa and for data analysis.

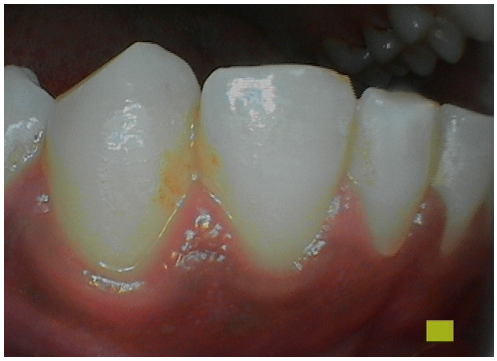
Overall the SOPROCARE camera in perio mode allowed for reliable judgment of plaque and gingival inflammation with respect to the Turesky modified Quigley Hein index and the Silness & Loe gingival inflammation index. Despite some differences (especially at lower plaque levels), the differences were not statistically significant. It could be seen that this device offers the ability to enhance patient education and to store images to be compared at future appointments.

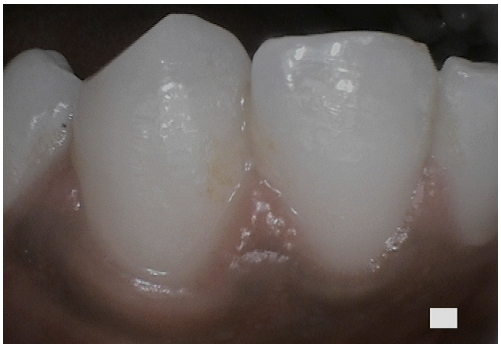
Comparison Clinical Photos for SOPROCARE in Perio and Daylight Modes





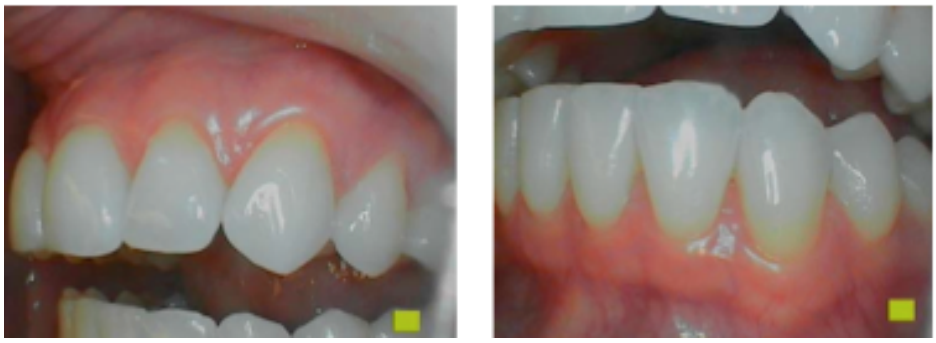
Representation of SOPROCARE in perio (left) and daylight (right) modes. These photos represent a patient who had a QH and S&L score of "0". The yellow hue noticed at the gingival margin can be misleading to the untrained user



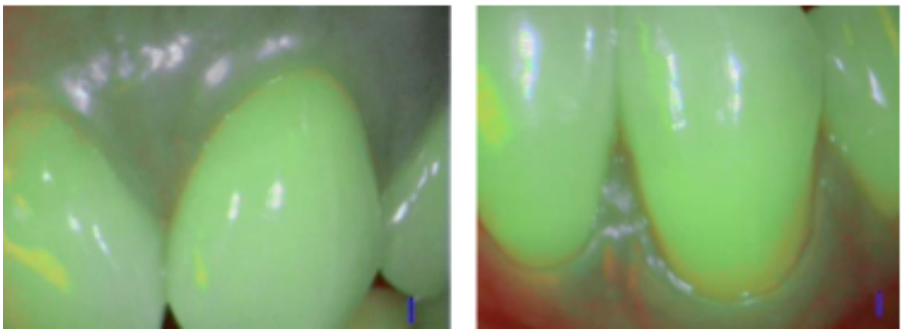


Representation of SOPROCARE in perio (left) and daylight (right) modes. These photos represent a patient who had a QH score of "2,2,3" and S&L score of "0"

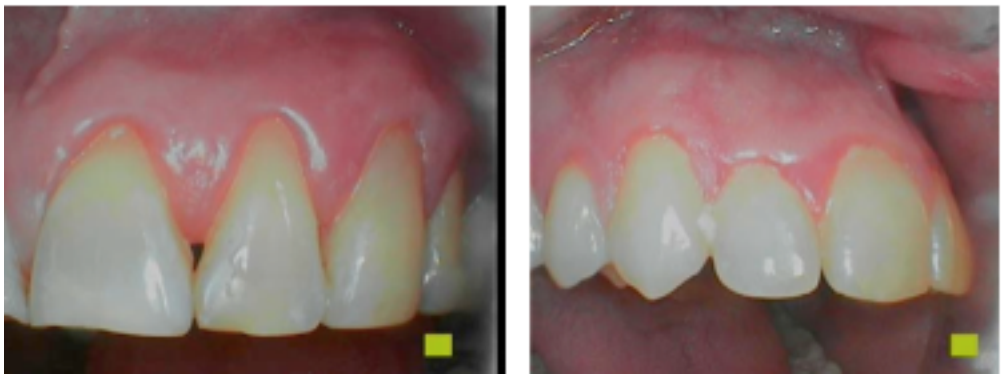
Clinical Photos Using Fluorescence Imaging- Subjects without Brackets/without Pigmented Gingiva



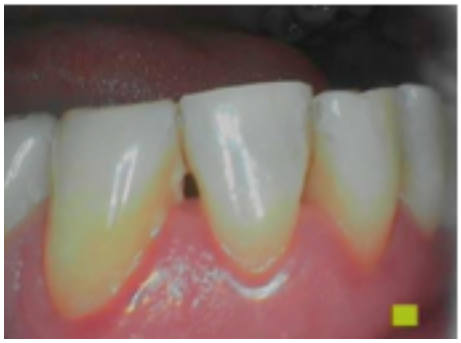
Using SOPROCARE in perio mode, these photos depict a patient who was "plaque-free" and had QH and S&L scores of "0"



These photos show the canines of the same patient in the above photos taken with SOPROLIFE. It can be seen the red outline towards the gingival margin, indicated porphyrins that fluoresce red

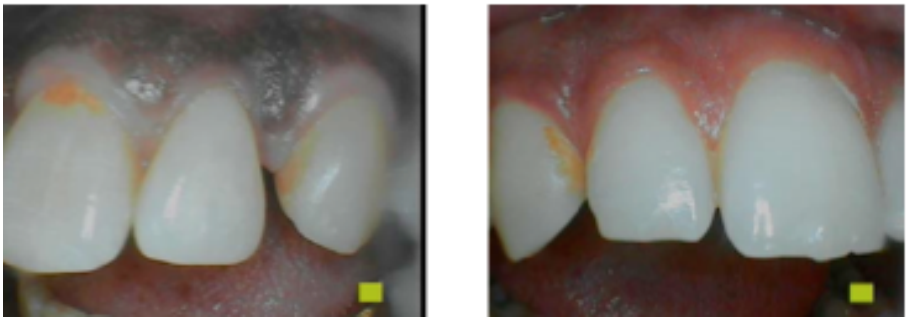


Using SOPROCARE, these photos show QH score of 1,1,2 and S&L score of 1,1,1 (left) and QH score of 3,3,3 and S&L score of 2,2,2 (right)

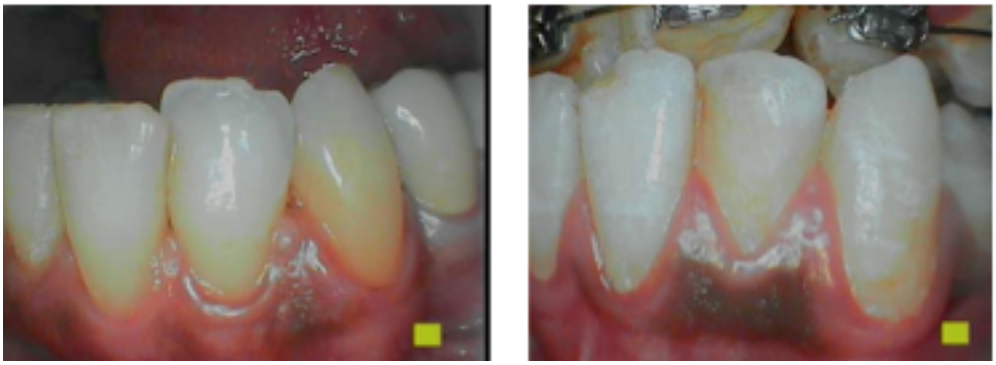


Using SOPROCARE, this photo shows QH score of 3,3,1 and S&L score of 2,2,2

Clinical Photos Using Fluorescence Imaging- Subjects with Pigmented Gingiva

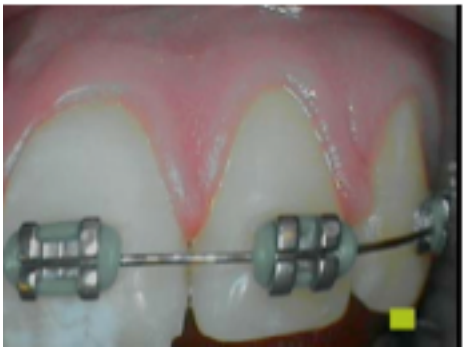
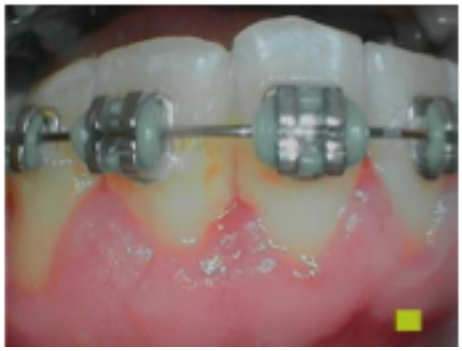


Using SOPROCARE, these photos show QH score of 3,1,2 and S&L score of 0 (left) and QH score of 3,1,1 and S&L score of 2,1,0 (right)

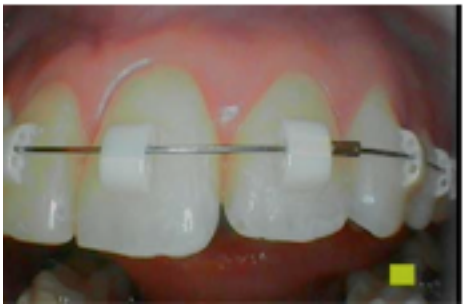
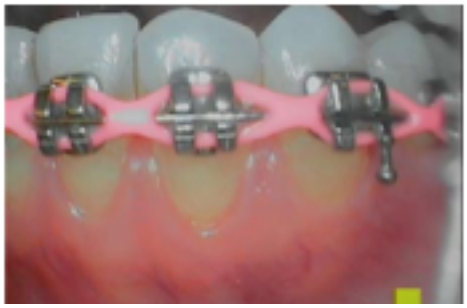


Using SOPROCARE, these photos show QH score of 3,1,1 and S&L score of 2,1,0 (left) and QH score of 3,4,3 and S&L score of 3,2,1 (right)

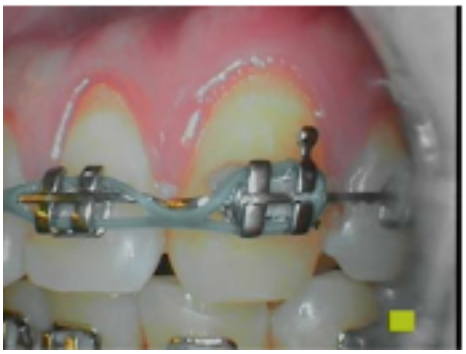
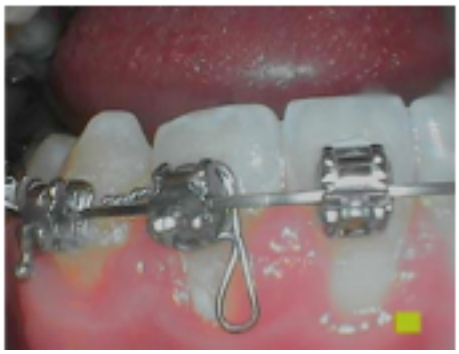
Clinical Photos Using Fluorescence Imaging- Subjects with Orthodontic Brackets

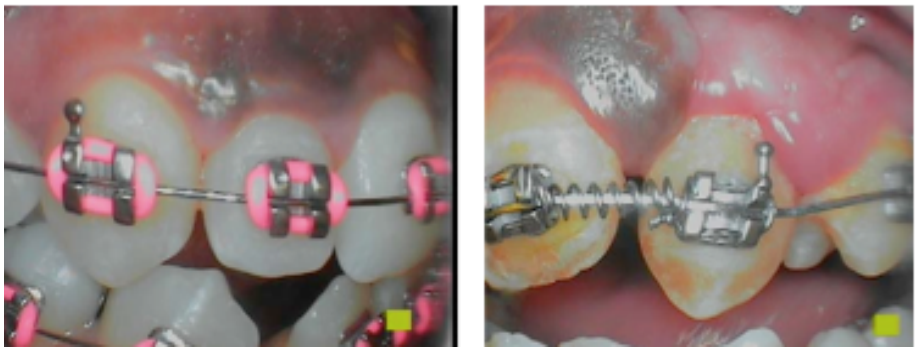
Using SOPROCARE, these photos show QH score of 0,0,2 and S&L score of 0,0,1 (left) and QH score of 3,3,3 and S&L score of 1,1,1 (right)

Using SOPROCARE, these photos show QH score of 0,0,0 and S&L score of 0,0,0 (left) and QH score of 3,3,3 and S&L score of 0,0,0 (right)

Using SOPROCARE, these photos show QH score of 1,1 and S&L score of 1,2 (left) and QH score of 3,3,3 and S&L score of 0,3,2 (right)



Using SOPROCARE, these photos show QH score of 2,0,0 and S&L score of 2,0,0 (left) and QH score of 3,3 and S&L score of 1,2 (right)

Appendix A

Hibst (47) cultured bacteria from carious lesions and plated them on agar only to find that under fluorescent microscopy, the bacterial colonies along with the surrounding agar had fluoresced. Hibst also looked at a hemi-sectioned tooth and noted that fluorescence microscopy of white spot lesions (decalcification) does not lead to enhanced signals but that fluorescence is found along the dentinal tubules from the occlusal caries toward the pulp. Both this and the agar findings contributed to the possibility that bacterial metabolites, porphyrins, had caused this. To confirm this, they extracted the carious material and analyzed it by high performance liquid chromatography (HPLC). The fractions were excited by 406 nm and 655 nm radiation. In the 406 nm sample, protoporphyrin IX, meso-porphyrin, and copro-porphyirin was present. For the 655 nm excitation sample, the emission spectra was very similar to those seen for caries.

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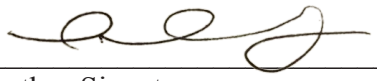

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