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ORIGINAL ARTICLE

# In vitro performance of DIAGNOdent laser fluorescence device for dental calculus detection on human tooth root surfaces

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Two examiners

**Abstract** *Objective:* This study assessed the reproducibility of a red diode laser device, and its capability to detect dental calculus in vitro on human tooth root surfaces.

*Material and methods:* On each of 50 extracted teeth, a calculus-positive and calculus-free root surface was evaluated by two independent examiners with a low-power indium gallium arsenide phosphide diode laser (DIAGNOdent) fitted with a periodontal probe-like sapphire tip and emitting visible red light at 655 nm wavelength. Laser autofluorescence intensity readings of examined root surfaces were scored on a 0–99 scale, with duplicate assessments performed using the laser probe tip directed both perpendicular and parallel to evaluated tooth root surfaces. Pearson correlation coefficients of untransformed measurements, and kappa analysis of data dichotomized with a $>40$ autofluorescence intensity threshold, were calculated to assess intra- and inter-examiner reproducibility of the laser device. Mean autofluorescence intensity scores of calculus-positive and calculus-free root surfaces were evaluated with the Student's *t*-test.

*Results:* Excellent intra- and inter-examiner reproducibility was found for DIAGNOdent laser autofluorescence intensity measurements, with Pearson correlation coefficients above 94%, and kappa values ranging between 0.96 and 1.0, for duplicate readings taken with both laser probe tip orientations. Significantly higher autofluorescence intensity values were measured when the laser

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probe tip was directed perpendicular, rather than parallel, to tooth root surfaces. However, calculus-positive roots, particularly with calculus in markedly-raised ledges, yielded significantly greater mean DIAGNOdent laser autofluorescence intensity scores than calculus-free surfaces, regardless of probe tip orientation. DIAGNOdent autofluorescence intensity values $>40$ exhibited a stronger association with calculus (36.6 odds ratio) then measurements of $\geq 5$ (20.1 odds ratio) when the laser probe tip was advanced parallel to root surfaces.

*Conclusions:* Excellent intra- and inter-examiner reproducibility of autofluorescence intensity measurements was obtained with the DIAGNOdent laser fluorescence device on human tooth roots. Calculus-positive root surfaces exhibited significantly greater DIAGNOdent laser autofluorescence than calculus-free tooth roots, even with the laser probe tip directed parallel to root surfaces. These findings provide further in vitro validation of the potential utility of a DIAGNOdent laser fluorescence device for identifying dental calculus on human tooth root surfaces.



## 1. Introduction

Dental calculus forms on human tooth root surfaces via precipitation of calcium phosphate salts, which enter periodontal pockets through gingival crevicular fluid and mineralize subgingival bacterial biofilms growing on teeth (Roberts-Harry and Clerehugh, 2000). Subgingival calculus is colonized by periodontopathic bacterial biofilms on its outer surfaces (Calabrese et al., 2007), harbors microbial toxins within its surface porosities (Nichols and Rojanasomsith, 2006), and may trigger clinical episodes of progressive periodontitis disease-activity (Mandel and Gaffar, 1986; Anerud et al., 1991; Albandar et al., 1998). Reliable methods for detecting root surface calculus in periodontal pockets are needed to help clinicians maximize its removal with periodontal treatment procedures (Kamath and Nayak, 2014).

Conventional subgingival calculus identification is performed with manual explorers and probes, and is reliant upon non-visual, tactile casting into periodontal pockets to feel for tooth root surface irregularities or smoothness. This approach frequently yields false negative clinical findings, as one study found after root scaling and tooth removal that 77.4% of root surfaces positive for subgingival calculus upon stereomicroscopic examination were erroneously scored as calculus-free with manual explorers (Sherman et al., 1990). Similarly, agreement between two periodontal examiners on the clinical presence of calculus with manual explorers was found to be less than 50% on subgingival tooth surfaces remaining calculus-positive after periodontal root instrumentation (Pippin and Feil, 1992).

Another approach to subgingival calculus detection involves use of a DIAGNOdent low-power ($<1$ milliwatt) indium gallium arsenide phosphide (InGaAsP) diode laser fitted with a periodontal probe-like cylindrical sapphire tip and emitting visible red laser light at a 655 nm wavelength in the near-infrared electromagnetic spectrum (Kamath and Nayak, 2014). The scientific rationale for using the device in dental calculus detection is based on observations that subgingival calculus exhibits autofluorescence when exposed to 655 nm laser light (Kurihara et al., 2004), most likely as a result of emissions from excited bacterial-derived porphyrins embedded in the surface of dental calculus (Dolowy et al., 1995). In contrast, no autofluorescence occurs on calculus-free tooth surfaces (Kurihara et al., 2004). The DIAGNOdent laser fluorescence device was cleared for marketing in the United States for subgingival calculus detection and localization by the United States Food and Drug Administration in 2006 (Lin, 2006), but has received only limited in vitro and no in vivo research attention to date.

Folwaczny et al. (2002) examined 30 extracted human teeth with the DIAGNOdent laser probe tip aimed perpendicular to tooth surfaces, and found greater laser fluorescence intensity scores on calculus deposits than on human tooth cementum. In addition, significantly lower fluorescence intensity values were measured on tooth root surfaces covered with blood compared to ambient air or an electrolyte solution (Folwaczny et al., 2002). Krause et al. (2003) also examined 20 extracted human teeth with the DIAGNOdent laser device tip aimed perpendicular to tooth surfaces, and also found greater fluorescence intensity measurements on calculus as compared to root surface cementum. In a study of the device on 40 extracted human teeth mounted in a manikin head, Folwaczny et al. (2004) found less residual subgingival calculus on molar teeth when subgingival periodontal root instrumentation was guided to completion by a laser fluorescence intensity end-point score of $<5$, as compared to tactile use of a manual explorer. In the study, the DIAGNOdent diode laser light was delivered nearly perpendicular to the tooth surfaces with a prismatically-cut glass probe tip shaped like a chisel (Folwaczny et al., 2004). Shakibaie and Walsh (2014) mounted 30 extracted human teeth into manikin heads surrounded by silicone gingival tissues, and found fluorescence intensity scores with the DIAGNOdent laser probe tip aimed almost parallel at 5–15 degrees to tooth surfaces to better correlate with the volume of subgingival calculus than to its surface area. The diode laser device also provided, in a similar in vitro set-up, more accurate (Shakibaie and Walsh, 2015a) and more reproducible (Shakibaie and Walsh, 2016) subgingival calculus detection than tactile examination with a Williams periodontal probe.

To date, no data are available on the reproducibility of autofluorescence intensity values measured by the DIAGNOdent diode laser instrument when directing its probe tip perpendicular or parallel to tooth root surfaces. As a result, the primary purpose of the present study was to assess in vitro the reproducibility of DIAGNOdent autofluorescence intensity measurements made with these two approaches on human tooth root surfaces. Two null hypotheses were also tested - that there are no statistically significant differences in mean DIAGNOdent laser autofluorescence intensity readings between calculus-positive and calculus-free root surfaces, and between root surfaces with varying thicknesses of dental calculus, when the laser probe tip is directed parallel, as compared to perpendicular, to root surfaces of extracted human teeth.

## 2. Materials and methods

### *2.1. Teeth and subgingival calculus*

Following clearance obtained from the Temple University Human Subjects Protections Institutional Review Board, 50 extracted single and multi-rooted human teeth (11 incisors, 4 canines, 7 premolars, and 28 molars), with a range of visually-evident dental calculus deposits, were initially evaluated with a SZX10 dissecting research stereomicroscope (Olympus America, Inc., Center Valley, Pennsylvania, USA) at 10× magnification for the presence of calculus on tooth root surfaces, which was recognized by a dark brown-black color and raised surface morphology. One calculus-positive and one calculus-negative root surface were selected per tooth from non-furcation areas for the study. On some teeth, a periodontal curette (Columbia 4R/4L, Hu-Friedy Manufacturing Co., Chicago, Illinois, USA) was used to remove calculus to create a limited root surface area that was calculus-negative. These selected root surfaces were marked on coronal tooth surfaces with a black felt-tipped pen (Sharpie® Permanent Marker, fine point, Newell Brands, Hoboken, New Jersey, USA) to aid with identification of areas for dental explorer and diode laser assessments, and each area was photographed for research documentation.

No data was collected from patients from whom the teeth were removed or their dental records, such as demographic characteristics, reason for tooth removal, or clinical dental status of the extracted teeth, including periodontal probing depths and the relationship between the free gingival margin to the cemenoenamel junction.

The presence and nature of dental calculus deposits on each of the selected tooth root surfaces was scored in vitro on a 0–2 scale with a modified Subgingival Calculus Index (SCI) (Watanabe et al., 1982) by an experienced board-certified periodontist (author TER) using an 11/12 ODU dental explorer (Hu-Friedy Manufacturing Co., Chicago, Illinois, USA). The criteria for the modified SCI were as follows:

0 = no root surface dental calculus detected
1 = root surface dental calculus detected in thin deposits, but not in a markedly-raised ledge
2 = root surface dental calculus detected in a markedly-raised ledge

### *2.2. Laser fluorescence intensity assessments*

Two independent examiners with varied educational and clinical experience backgrounds (one a board-certified specialist in periodontics with 35 years of clinical dental care experience (author TER), and the other a general dentist (author AYA) in an advanced general dentistry residency program with 6 years of clinical dental care experience), evaluated each selected tooth root surface with a hand-held InGaAsP diode laser (DIAGNOdent Pen, Part No. 1004.3400, KaVo Dental Corp., Charlotte, North Carolina, USA) fitted with a rigid, periodontal probe-like, cylindrical sapphire tip (DIAGNOdent Pen Perio Tip, Part No. 1004.0370, KaVo Dental Corp., Charlotte, North Carolina, USA) (Fig. 1), and emitting visible red laser light at a wavelength of 655 nm. The device was calibrated daily with a ceramic standard, following manufacturer guidelines, and reset to a zero reading after each root surface evaluation.

Two different protocols were followed to examine the selected tooth root surfaces. First, each examiner perpendicularly directed the tip of the diode laser device twice along the selected study root surfaces, and recorded the maximum laser autofluorescence intensity values obtained from each pass, which potentially ranged from 0 to 99 (Fig. 2a). Second, each examiner advanced the DIAGNOdent laser probe tip twice parallel to the same tooth root surfaces in an apical direction from the tooth cementoenamel junction, similar to how a periodontal probe is introduced into periodontal pockets, and recorded the maximum laser autofluorescence intensity value from each pass (Fig. 2b).

The root surface evaluations were performed in air on a laboratory benchtop, without the presence of any oral fluids coating the teeth, calculus deposits, or the DIAGNOdent laser probe tip during fluorescence testing.

### *2.3. Data analysis*

Pearson correlation coefficients of untransformed mean autofluorescence intensity measurements made by each examiner, and kappa analysis of autofluorescence intensity values dichotomized with a $>40$ threshold, as recommended by the instrument manufacturer for detection of root surface deposits, were calculated to assess intra- and inter-examiner reproducibility of the DIAGNOdent laser device (Hunt, 1986). Kappa values between 0.40 and 0.75 were considered to represent fair to good agreement, and $\kappa > 0.75$ to indicate excellent agreement (Hunt, 1986).

Mean and standard deviation (SD) values were calculated for DIAGNOdent laser autofluorescence intensity measurements made by the two examiners. A two-tailed, independent samples, Student's *t*-test evaluated mean DIAGNOdent autofluorescence intensity measurements made by the perpendicular versus parallel examination protocols, and between calculus-positive and calculus-free root surfaces. An independent samples Student's *t*-test also statistically compared mean DIAGNOdent autofluorescence intensity scores recorded on calculus-positive tooth root surfaces exhibiting a modified SCI score = 2, as compared to a modified SCI score = 1. A $P$-value of $\leq 0.05$ was required for statistical significance, using $\alpha = 0.05$ and $\beta = 0.20$ thresholds, and sample sizes ($N = 100$ and 50 tooth root surfaces, respectively) providing $>80\%$ power to detect true between-group differences.

Using 2 × 2 contingency table analysis (McNeil et al., 1975), sensitivity, specificity, positive predictive value, negative predictive value, and odds ratio analysis assessed the occurrence of calculus-positive and calculus-free root surfaces associated with two proposed DIAGNOdent thresholds for autofluorescence intensity ($\geq 5$ and $>40$) which are recommended for clinical diagnostic purposes by the instrument


**Fig. 1** DIAGNOdent diode laser device for dental calculus detection.

manufacturer for subgingival dental calculus detection. Sensitivity was defined as the probability that the DIAGNOdent autofluorescence intensity threshold or higher value will be measured when the root surface is calculus-positive (true positive rate). Specificity was defined as the probability that the DIAGNOdent autofluorescence intensity measurement is below the selected threshold level when the root surface is calculus-free (true negative rate). Positive predictive value was defined as the probability that the root surface is calculus-positive when the DIAGNOdent autofluorescence intensity threshold or higher score is detected. Negative predictive value was defined as the probability that the root surface is calculus-free when the DIAGNOdent autofluorescence intensity measurement is below the selected threshold level.

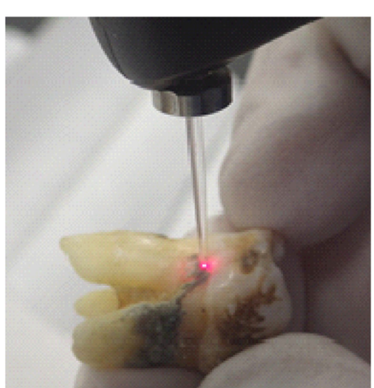
Due to the occurrence of zero event cells in $2 \times 2$ contingency table analysis, Peto odds ratios and their 95% confidence intervals (CI) (Brockhaus et al., 2014), determined using an on-line calculator (http://www.hutchon.net/peto%20vers%202.htm), were used to estimate true odds ratios in assessing the relationship between the two proposed DIAGNOdent autofluorescence intensity threshold levels and detection of root surface calculus.

A PC-based, 64-bit, statistical software package (STATA/SE 14.2 for Windows, StataCorp PL, College Station, TX, USA) was used in the data analysis.

## 3. Results

A total of 50 root surfaces studied exhibited a modified SCI score = 0 (no root surface calculus detected), whereas 19 root surfaces revealed modified SCI scores = 1 (root surface calculus detected in thin deposits, but not in a markedly-raised ledge), and 31 root surfaces had modified SCI scores = 2 (root surface calculus detected in a markedly-raised ledge).

A high level of both intra- and inter-examiner reproducibility of DIAGNOdent laser autofluorescence intensity readings was found with both tooth root evaluation protocols. Pearson correlation coefficient values ranged from $r = 0.948$ to $r = 0.999$ for duplicate assessments made by each examiner, and for comparative measurements made between them (Tables 1 and 2). Kappa values of 1.0 for both evaluation protocols, indicating excellent intra-examiner agreement, were found with duplicate dichotomized assessments of DIAGNOdent autofluorescence intensity made by each examiner. Kappa values of 1.0 and 0.96 for dichotomized DIAGNOdent autofluorescence intensity readings made between the two examiners with the perpendicular and parallel evaluation protocols, respectively, also indicated excellent inter-examiner agreement.

A.

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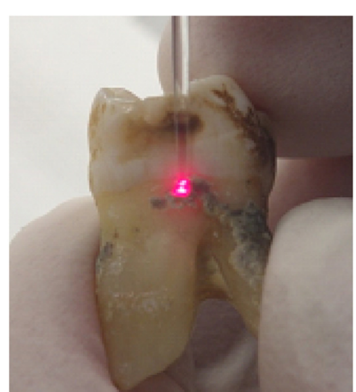

**Fig. 2** (A) DIAGNOdent laser probe tip directed perpendicular, and (B) parallel, to a calculus-positive tooth root surface.

**Table 1** Intra-examiner reproducibility of DIAGNOdent laser autofluorescence intensity readings on 100 human tooth root surfaces.

| | Mean laser autofluorescence intensity readings ± SD | | |
|---|---|---|---|
| Perpendicular evaluation protocol | First reading | Second reading | Pearson correlation coefficient ($r$) |
| Examiner #1 | 55.7 ± 44.3 | 55.6 ± 44.3 | +0.999 |
| Examiner #2 | 55.0 ± 44.3 | 54.8 ± 44.2 | +0.999 |
| Parallel evaluation protocol | | | |
| Examiner #1 | 40.5 ± 41.1 | 40.7 ± 41.1 | +0.987 |
| Examiner #2 | 41.9 ± 42.4 | 42.6 ± 42.4 | +0.960 |

**Table 2** Inter-examiner reproducibility of DIAGNOdent laser autofluorescence intensity readings on 100 human tooth root surfaces.

| | Mean laser autofluorescence intensity readings ± SD | | |
|---|---|---|---|
| Root surface evaluation protocol | Examiner #1 | Examiner #2 | Pearson correlation coefficient ($r$) |
| Perpendicular | 55.7 ± 44.2 | 54.9 ± 44.4 | +0.995 |
| Parallel | 40.5 ± 41.1 | 42.2 ± 41.2 | +0.948 |

Significantly higher DIAGNOdent autofluorescence intensity values were measured when the laser probe tip was directed perpendicular, rather than parallel, to tooth root surfaces, regardless of the presence or absence of dental calculus (all $P$-values $<0.0001$ for comparisons between the two examination protocols) (Table 3). Mean autofluorescence intensity values recorded by the two examiners with the DIAGNOdent laser probe tip directed perpendicular to tooth surfaces were 98.9 ± 0.4 (SD) and 99.0 ± 0.0 (SD), respectively, on calculus-positive root surfaces, which were significantly greater than mean values of 10.9 ± 6.0 (SD) and 12.3 ± 8.1 (SD), respectively, recorded on calculus-free surfaces ($P < 0.0001$ for each examiner; two-tailed, independent samples, Student's $t$-test). Similarly, mean DIAGNOdent autofluorescence intensity scores measured by the two examiners with the instrument probe tip directed apical and parallel to tooth root surfaces were 76.9 ± 26.4 (SD) and 79.7 ± 23.8 (SD), respectively, on calculus-positive roots, which were significantly greater than mean values of 4.2 ± 2.7 (SD) and 4.9 ± 4.1 (SD), respectively, on calculus-free root surfaces ($P < 0.0001$ for each examiner; two-tailed, independent samples, Student's $t$-test) (Table 3).

Significantly higher mean DIAGNOdent autofluorescence intensity scores by both study examiners were recorded on calculus-positive root surfaces exhibiting a modified SCI score = 2, as compared to a modified SCI score = 1, when the laser probe tip was directed parallel to the tooth root surface and advanced apically like a periodontal probe ($P < 0.0001$ for the two study examiners; two-tailed, independent samples, Student's $t$-test) (Table 4). No similar statistically significant differences in DIAGNOdent autofluorescence intensity values between calculus-positive surfaces exhibiting a modified SCI score = 2, as compared to a modified SCI score = 1, were found when the instrument probe tip was directed perpendicular to the tooth root surface (Table 4).

Table 5 presents 2 × 2 contingency table distributions of calculus-positive and calculus-negative root surfaces by two potential diagnostic threshold values for DIAGNOdent laser autofluorescence intensity. A DIAGNOdent diagnostic threshold level of ≥5 provided 100% sensitivity, 68% specificity, a 75.8% positive predictive value, a 100% negative predictive value, and an odds ratio relationship of 20.1 [95% CI = 8.8, 45.8] for the presence of root surface calculus. In comparison, a higher DIAGNOdent diagnostic threshold level of >40 offered 90% sensitivity, 100% specificity, a 100% positive predictive value, a 90.9% negative predictive value, and an odds ratio relationship of 36.6 [95% CI = 16.7, 80.2] for the presence of calculus on root surfaces.

## 4. Discussion

A high level of intra- and inter-examiner reproducibility for laser autofluorescence intensity measurements on human tooth roots was found in the present study, regardless of whether the DIAGNOdent instrument probe tip was directed perpendicular or parallel to the extracted tooth surfaces. Moreover, the

**Table 3** DIAGNOdent laser autofluorescence intensity values on 50 calculus-positive and 50 calculus-free root surfaces.

| | Mean laser autofluorescence intensity readings ± SD (range) | | |
|---|---|---|---|
| Perpendicular evaluation protocol | Root surface calculus present | Root surface calculus absent | P-value |
| Examiner #1 | 99.0 ± 0.0 (99) | 12.3 ± 8.1(2–34.5) | <0.0001 |
| Examiner #2 | 98.9 ± 0.4 (96.5–99) | 10.9 ± 6.0 (4.5–27.5) | <0.0001 |
| Parallel evaluation protocol | | | |
| Examiner #1 | 76.9 ± 26.4 (18.5–99) | 4.2 ± 2.7 (1–14.8) | <0.0001 |
| Examiner #2 | 79.7 ± 23.8 (13–99) | 4.9 ± 4.1 (1–18.5) | <0.0001 |

**Table 4** DIAGNOdent laser autofluorescence intensity measurements on 50 calculus-positive root surfaces with varying modified SCI scores.

| | Mean laser autofluorescence intensity readings ± SD (range) | | |
|---|---|---|---|
| Perpendicular evaluation protocol | 31 root surfaces with modified SCI score = 2 | 19 root surfaces with modified SCI score = 1 | *P*-value |
| Examiner #1 | 99.0 ± 0.0 (99) | 99.0 ± 0.0 (99) | > 0.05 |
| Examiner #2 | 98.9 ± 0.1 (96.5–99) | 99.0 ± 0.0 (99) | > 0.05 |
| Parallel evaluation protocol | | | |
| Examiner #1 | 90.0 ± 3.1 (31.5–99) | 55.6 ± 5.7 (18.5–99) | < 0.0001 |
| Examiner #2 | 89.0 ± 2.6 (44.5–99) | 64.4 ± 6.5 (13–99) | < 0.0001 |

**Table 5** Distribution of 50 calculus-positive and 50 calculus-free root surfaces by two DIAGNOdent laser autofluorescence intensity thresholds.

| DIAGNOdent laser autofluorescence intensity threshold | Root surface calculus | |
|---|---|---|
| | Present | Absent |
| ≥5 | 50 | 16 |
| < 5 | 0 | 34 |
| > 40 | 45 | 0 |
| ≤40 | 5 | 50 |

reproducibility of the autofluorescence intensity readings was not significantly influenced by differing educational backgrounds or length of clinical dental experience of the two examiners. Pearson correlation coefficients greater than 94%, and kappa values indicative of an excellent level of agreement (0.96–1.0), were found for duplicate assessments made by each of the two examiners, and for comparative measurements made between them. In contrast, another in vitro study, also using a parallel calculus detection method, found DIAGNOdent instrument usage was affected by dentist skill and experience, as only a moderate level of inter-examiner reproducibility was found between experienced and inexperienced clinicians (Shakibaie and Walsh, 2015a). This discrepancy may in part be due to differences in the experience level of the less-experienced clinicians used in the two studies. The present study employed a general dentist in an advanced general dentistry residency program with 6 years of clinical dental care experience as the less-experienced examiner, whereas the previous study (Shakibaie and Walsh, 2015a) used a final year dental student with far less clinical patient care experience. It is more likely that the experienced general dentist in the present study was better able to collect laser autofluorescence intensity measurements in vitro similar to an experienced periodontist than a final year dental student with a vastly less extensive clinical background. There is an urgent need in future studies to clinically assess the in vivo reproducibility of the DIAGNOdent laser device under a variety of conditions commonly encountered in clinical dental practice.

This study also found significantly higher laser autofluorescence intensity scores on calculus-positive root surfaces compared to calculus-free root surfaces, consistent with previous reports (Folwaczny et al., 2002; Krause et al., 2003; Kurihara et al., 2004), particularly when calculus was present in markedly-raised ledges. Both evaluation protocols yielded significantly higher autofluorescence intensity scores on calculus-positive root surfaces, even when the DIAGNOdent laser probe tip was directed parallel to root surfaces, similar to clinical conditions in vivo where a periodontal probe is apically advanced into periodontal pockets. Thus, the null hypothesis that there are no statistically significant differences in mean DIAGNOdent laser autofluorescence intensity readings between calculus-positive and calculus-free root surfaces when the laser probe tip is directed either parallel or perpendicular to root surfaces is rejected.

Because the DIAGNOdent laser device emits the visible red light straight out of the end of the cylindrical sapphire probe tip with an unknown amount of lateral dispersion, it was not surprising that higher autofluorescence intensity values were measured when the probe tip was placed perpendicular onto subgingival calculus deposits. In a perpendicular direction, the full extent of the laser light energy would likely be better absorbed and fluoresced by subgingival calculus located within the spot size of the DIAGNOdent diode laser beam. In comparison, subgingival calculus detection with the laser probe tip advanced parallel to the tooth root surface would be more reliant upon calculus absorption of laterally dispersed laser light energy emitted from the instrument tip, particularly with thin calculus deposits. Support for this concept in the present study was found when the DIAGNOdent laser probe tip was advanced parallel along tooth roots, where significantly higher autofluorescence intensity scores were found on calculus-positive surfaces exhibiting a modified SCI score = 2, which represents a markedly-raised ledge of calculus, compared to a modified SCI score = 1, where calculus was detected in a thin layer. Calculus in a markedly-raised ledge yielded autofluorescence intensity scores of 89–90, similar to levels recorded when the laser probe tip was directed perpendicular onto the same tooth root sites.

In contrast, when root surface calculus deposits were thin (modified SCI = 1), mean autofluorescence intensity scores of 55.6 to 64.4 were obtained using the DIAGNOdent probe tip parallel to the tooth root surfaces. Importantly, these values were still significantly greater than mean autofluorescence intensity measurements of 4.2–4.9 obtained with the laser probe tip directed parallel to calculus-free root surfaces. This suggests that the DIAGNOdent laser device may have clinical utility in successfully distinguishing between root surfaces with thin layers of calculus from those that are calculus-free. As a result, the null hypothesis that there are no statistically significant differences in mean DIAGNOdent laser autofluorescence intensity readings between root surfaces with varying thicknesses of dental calculus is also rejected when the laser probe tip is directed parallel, but not perpendicular, to the tooth root surface. However, since a range of thin and thicker calculus deposits were scored with a modified SCI value = 1, it remains to be determined how well the DIAGNOdent laser device will perform on root surfaces presenting only with non-confluent and dispersed small islands of dental calculus.

The present study found that DIAGNOdent laser autofluorescence intensity readings collected in vitro on tooth root surfaces with a perpendicular examination protocol almost matched findings of a previous report (Folwaczny et al., 2002), and were significantly higher than measured with the laser probe tip directed parallel to tooth root surfaces. Perpendicular examination of tooth roots with a laser probe tip having straight end-point light emission, while possible to perform in vitro, is not applicable to clinical situations in vivo where subgingival calculus is sought in deep periodontal pockets. However, the higher laser autofluorescence intensity readings found with a perpendicular orientation to root surfaces may be useful to support future development of optical periodontal probes designed to detect dental calculus on root surfaces using a lateral side emission of laser light (George and Walsh, 2009, 2011), rather than the current straight end-point emission.

Finally, a threshold level of $>40$ for DIAGNOdent autofluorescence intensity readings was found to offer greater diagnostic accuracy for calculus detection on root surfaces than a threshold level of $\geq 5$. These two thresholds were studied per the DIAGNOdent instrument manufacturer recommendations for distinguishing between calculus-positive and calculus-free tooth root surfaces. A strong likelihood of root surface calculus being present was found for autofluorescence intensity readings of $\geq 5$ (odds ratio = 20.1), similar to another in vitro study using the DIAGNOdent system (Shakibaie and Walsh, 2015b). However, an even stronger relationship (odds ratio = 36.6) was found between an autofluorescence intensity threshold level of $>40$ and presence of root surface calculus.

## 5. Conclusions

Excellent intra- and inter-examiner reproducibility of autofluorescence intensity measurements was obtained on human tooth roots using the DIAGNOdent visible red laser device. Calculus-positive root surfaces exhibited significantly greater laser autofluorescence than calculus-free tooth roots, even with the DIAGNOdent laser probe tip directed parallel to root surfaces. Autofluorescence intensity values $>40$ exhibited a stronger association with calculus (36.6 odds ratio) then measurements of $\geq 5$ (20.1 odds ratio) when the DIAGNOdent laser probe tip was advanced parallel to root surfaces. These findings provide further in vitro validation, similar to earlier works (Folwaczny et al., 2002, 2004; Krause et al., 2003; Shakibaie and Walsh, 2014, 2015a, 2015b, 2016), of the potential utility of a DIAGNOdent laser fluorescence device for identifying dental calculus on human tooth root surfaces. However, in vivo studies are needed to determine if the laser device provides enhanced detection of subgingival calculus compared to use of conventional dental explorers by experienced clinicians.

## Ethical statement

This research did not involve human or animal subjects, and the laboratory experimentation followed in this research were cleared by the Temple University Human Subjects Protections Institutional Review Board.

## Conflict of interest

None.

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