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Abstract. The aim of this study was to evaluate the applicability of laser-induced autofluorescence (LIAF) spectroscopy to detect and quantify dental plaque. LIAF spectra were recorded *in situ* from dental plaque (0–3 grades of plaque index) in 300 patients with 404 nm diode laser excitation. The fluorescence intensity ratio of the emission peaks was calculated from the LIAF spectral data following which their scatter plots were drawn and the area under the receiver operating characteristics were calculated. The LIAF spectrum of clinically invisible grade-1 plaque showed a prominent emission peak at 510 nm with a satellite peak around 630 nm in contrast to grade 0 that has a single peak around 500 nm. The fluorescence intensity ratio ($F510/F630$) has a decreasing trend with increase in plaque grade and the ratio values show statistically significant differences ($p < 0.01$) between different grades. An overall sensitivity and specificity of 100% each was achieved for discrimination between grade-0 and grade-1 plaque. The clinical significance of this study is that the diagnostic algorithm developed based on fluorescence spectral intensity ratio ($F510/F630$) would be useful to precisely identify minute amounts of plaque without the need for disclosing solutions and to convince patients of the need for proper oral hygiene and homecare practices. [DOI: 10.1117/1.JBO.20.4.048001]

Keywords: laser-induced autofluorescence spectroscopy; quantification; dental plaque; clinically invisible plaque detection.
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1 Introduction

Periodontal disease is the most common cause of tooth loss among adults and its primary etiologic factor is dental plaque. Studies have shown that as early as 2 to 4 h after oral prophylaxis or tooth brushing, the pioneer bacteria (*Streptococci*) cover about 30% of the enamel.[1] Therefore, early identification and meticulous removal of plaque are essential for preventing periodontal disease and maintaining periodontal health.

However, identification of dental plaque is difficult for both patient and dentist because the tooth and dental plaque often look alike, especially if plaque is present in scanty amounts. Traditionally, dental plaque is often detected by clinicians either directly using an explorer[2] or with the help of a disclosing solution[3] and is quantified using indices based on the area of tooth covered or its thickness. But these assessment methods have the limitation of being subjective, therefore, results may vary from clinician to clinician, especially when the plaque is scanty. Recording indices may also need extensive calibration among examiners for high precision and reliability, which could be quite time consuming and costly.[4] On the other hand, disclosing agents used to stain mature and newly formed plaques differently lack specificity. It can stain oral mucosa and lip, though temporarily, which is a major esthetic issue. Fluorescent dyes,[5] automated techniques using computers,[6,7] and plaque quantification using three-dimensional co-ordinates[8] have also been described in the literature but the complexity of the methods, cost of equipment, standardization of the techniques, etc., are some of the major drawbacks in the popularization of these methods. Thus, there is a need to develop a cost-effective and noninvasive technique to more objectively detect and quantify dental plaque accumulation, especially during early stages of plaque formation.

Laser-induced autofluorescence (LIAF) spectroscopy is evolving as a powerful tool to detect and characterize biochemical and morphological changes occurring in the human body based on the changes in the fluorescence signatures.[9] In dentistry, LIAF spectroscopy has been effectively used for early detection of oral cancer[10–12] and tooth caries.[13–15] Mature dental plaque has been identified by the red fluorescence emission caused by the bacteria porphyrins in a few *in vitro* studies.[16,17] Few imaging systems such as plakScope home plaque tester and Vistacam intraoral camera are available on the market to visualize plaque, but may not be useful to visualize plaque accumulation in relatively inaccessible areas such as the palatal aspect of upper anterior teeth or the buccal aspect of posterior teeth due to the curvature of the dental arch. Although the SopraCare imaging system was used very recently to discriminate plaque and gingival inflammation,[18] the sample size was not large enough

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to provide information about its diagnostic accuracies in a clinical scenario.

Therefore, the aim of this study was to explore the feasibility of using LIAF for detection of clinically invisible dental plaque and to develop an LIAF ratio reference standard to discriminate between plaque-free tooth surface and clinically invisible early stage dental plaque. Plaque of grades 2 and 3 that can be clinically visualized was also characterized using LIAF and compared to evaluate the variability between different grades. Toward this, the *in vivo* LIAF spectra from tooth surfaces with grade-0 plaque (control) and 1 to 3 grades of plaque (test group) were recorded in 300 patients and the diagnostic accuracy of LIAF spectral ratio reference standard to discriminate between different grades of plaque is evaluated and presented.

2 Material and Methods

2.1 *Clinical Protocol and Subjects*

The study population consisted of 300 patients (200 in a standard set and 100 in a validation set) who participated in our study at the out-patient clinic of the Department of Periodontics at Government Dental College (GDC), Trivandrum from June 2011 to June 2013. The study protocol was approved by the Institutional Ethical Committee of Government Dental College (GDC), Thiruvananthapuram (Approval No. IEC/C/42-A/2011/DCT/dated 18-01-2011). Informed consent was obtained from all participants prior to their enrollment in the study.

200 subjects aged between 18 to 65 years were selected based on the presence of 0 to 3 grades of plaque according to the plaque index (PI) of Silness and Loe.[19] Consecutive series of patients who had the presence of varying grades of plaque at the gingival margin were enrolled in the study with 50 patients in each group namely, grade 1, grade 2, and grade 3 as shown in Fig. 1.

A disclosing solution was used to ascertain grades 0 and 1 after the fluorescence spectra were collected from these sites. While allocating participants to various groups, those without any visible plaque at the gingival margin were included as control (grade 0). However, after recording the LIAF spectra, the disclosing solution was used to confirm grade-0 plaque. Participants with calculus and cementum exposed tooth were excluded as these could possibly influence the emission spectra.[20] Pregnant women, smokers, and those with any systemic conditions, history of antibiotics, mouthwashes, or any periodontal treatment during the last 3 months were also excluded because these factors are known to influence the quality and quantity of plaque.[21] Clinical validation of the results was carried out on 100 participants in the age group of 18 to 65 years, categorized into four groups of 25 patients each according to PI by a member who was blinded to LIAF test results.

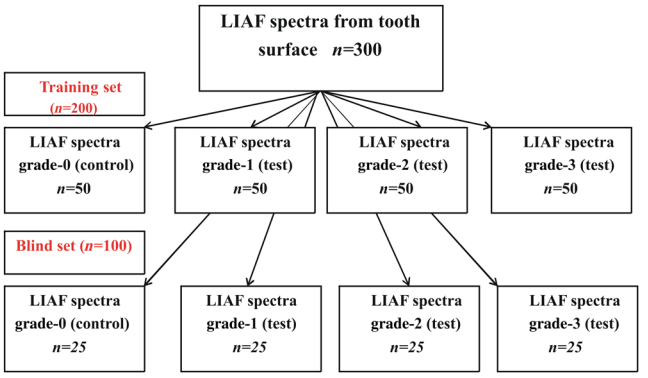

Fig. 1 Recruitment of patients for the study.

2.2 *Recording of Laser-Induced Autofluorescence Spectra and Plaque Index*

The fluorescence spectra were recorded from the gingival third of the maxillary central incisors in all the 300 patients using a portable LIAF spectroscopy system schematically shown in Fig. 2.

The system consisted of a 404 nm diode laser for excitation of fluorescence and a miniature fiber-optic spectrometer (Ocean Optics, Model: USB 2000FL VIS-NIR) connected to the USB port of a computer to record the spectrum from gingival plaque. One leg of the bifurcated optical fiber (400-μm diameter) guides the light from the laser to the plaque surface through a handpiece made of stainless steel while another fiber of the same diameter kept beside the excitation fiber collects the fluorescence signal from plaque to the spectrometer through a long wavelength pass filter (Schott GG420). The black disposable polyvinyl chloride (PVC) sleeve inserted at the probe tip helps to prevent external room light from entering into the spectrometer and control infection. Nonetheless, the stainless steel probe tip was sterilized after every use.

The participants were advised to rinse their mouth with distilled water to exclude any chance of fluorescence from food debris. An experienced periodontist identified the site for measurement and places probe tip on the plaque at the gingival margin without disturbing the plaque or gingiva while a physicist, well trained in optical spectroscopy, recorded the spectral data from each patient. In order to standardize, the plaque on the buccal aspect of the maxillary central incisor in each patient was graded and recorded by another experienced periodontist using a mouth mirror and explorer. Since the clinical assessment could disturb the plaque deposits, spectral readings were always recorded first. Patients were given a code number and the periodontist assessing the PI (reference test) was masked about LIAF measurements.

Although various methods for detection of gingival plaque are available, the PI by Silness and Loe was taken as the reference standard in this study due to its wide acceptance for measuring the thickness of plaque at the gingival margin.

2.3 *Data Acquisition and Processing*

Accumulated plaque along the gingival margin was illuminated with the 404 nm laser and the LIAF spectrum was recorded in the 400 to 800 nm spectral range using the OOI Base32 software (Ocean Optics). Before using, the fiber-optic light coupler was aligned to provide a Gaussian beam at the fiber tip. The average output power at the illumination fiber tip was monitored before each set of measurements on a subject and was maintained at 1 ± 0.5 mW using an optical power meter (Ophir, Israel, Model: Nova) fitted with a PD 300 photodiode head. The LIAF spectra were recorded with an integration time of 50 ms. The PVC sleeve on the tip of the hand piece was placed in contact with the tooth surface and fluorescence spectra were recorded by

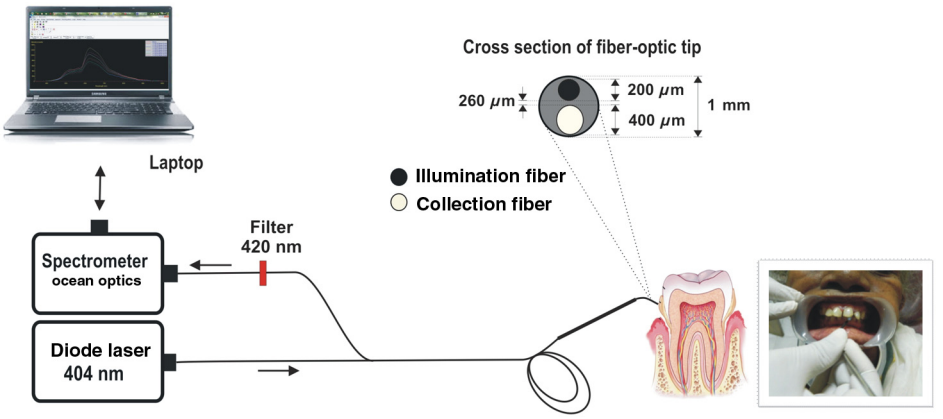

Fig. 2 Schematic of the experimental setup for laser-induced autofluorescence (LIAF) spectral measurements.

point monitoring with an integration time of 50 ms. Each day, the background spectrum was recorded prior to measurements and the software automatically subtracts this from the recorded spectrum. Due to the diverse nature of plaque deposits, 15 sets of LIAF measurements were taken from each site and the mean value from each site was determined for further analysis.

Fluorescence intensity (FI) ratios were calculated using the intensity values of peaks observed at 510 and 630 nm in the LIAF spectra. The mean LIAF spectral intensity over an interval of 20 nm (standard deviation) at the emission peak was used to determine the LIAF spectral ratio ($F510/F630$) from the recorded spectra. Further, the ratio was correlated with the clinical plaque score of the study population for discriminating different grades of plaque from grade 0 (absence of plaque) to grades 1, 2, and 3 (presence of plaque).

Following this, the discrimination of different grades of plaque (0 to 3) at the gingival margin and tooth surface was done using FI scatter plots based on spectral intensity values. A scatter plot of the fluorescence intensity ratio ($F510/F630$), also known as the fluorescence ratio reference standard (FRRS), is drawn from the raw spectral data from 150 sites with plaque (grades 1 to 3) and 50 sites without plaque (grade 0). A blind trial was also carried out on 100 participants to test the clinical validity of the FRRS developed. The values of the $F510/F630$ ratio calculated for 100 participants (25 in each group) in the blind set are also inserted in the FRRS for comparison.

The sensitivity and specificity of measurements were determined considering the PI scores by Silness and Loe as the gold standard. Diagnostic accuracies were calculated in terms of sensitivity, specificity, positive predictive values (PPV), and negative predictive value (NPV) from the position of intensity ratio ($F510/F630$) in the FRRS scatter plot with respect to the cut-off values derived from the spectral ratio. The quality/performance of the diagnostic test was evaluated using a receiver operating characteristic (ROC) curve. The plot of sensitivity versus 1-specificity, known as the ROC curve, and the area under the curve (AUC), are effective measures of the diagnostic accuracy with meaningful interpretations. The AUC is a reflection of how good the test is at distinguishing (or "discriminating") between patients with and without disease. The maximum value for AUC=1 which means that the diagnostic test is perfect in the differentiation between the diseased and nondiseased. This happens when the distributions of test results for the diseased and nondiseased do not overlap. AUC = 0.5 means the chance discrimination that the curve is located on the diagonal line in the ROC space. The minimum AUC should be considered a chance level, i.e., AUC = 0.5, while AUC = 0 means the test incorrectly classified all subjects with diseased as negative and all subjects with nondiseased as positive, which is extremely unlikely to occur in clinical practice.

2.4 *Sample Size Calculation*

Sample size was calculated based on the equation of Jones et al.[22] given below:

$$n = \frac{\mathrm{TP} + \mathrm{FN}}{P}, \tag{1}$$

where, $\mathrm{TP} + \mathrm{FN} = z^2 \times \{[\mathrm{SN}(1 - \mathrm{SN})]/W^2\}$.

In the above equation, TP = true positive, FN = false negative, SN = sensitivity, z = confidence interval for normal distribution value (for 95%, $z = 1.96$), P = prevalence of the condition in the population, and W = accuracy (0.05). Therefore, a minimum sample size of 40 in each group is required to achieve a sensitivity of 97% when the prevalence of supra-gingival plaque is taken as 95%.

2.5 *Statistical Analysis*

The LIAF spectral data collected from 0 to 3 grades of plaque were preprocessed by normalization to examine spectral enhancement due to preprocessing.[23] The normalized spectral intensity ratio was further subjected to unpaired t test to statistically determine significant differences between the adjoining grades of plaque. As the t test showed significant differences between grade 0 and higher grades of plaque, the normalized data were used for constructing the scatter plot. Cut-off values in the scatter plot between adjoining groups were calculated as the weighted arithmetic mean of the respective groups. In order to assess the performance of the newly proposed algorithm, ROC curves were constructed from the LIAF spectral data. Finally, the area under the ROC curves (AUC) and its 95% confidence interval (CI) were calculated.

3 Results

3.1 *Laser-Induced Autofluorescence Spectral Characteristics*

LIAF spectra were recorded from patients who gave written consent to participate in the study and had plaque accumulated at gingival margins with PI grades 0 to 3 (according to Silness and Loe). There were 83 male and 117 female participants in the training set (mean age 43.7 ± 13.5 years) while there were 31 males and 69 females in the blind set (mean age 49.1 ± 11.2 years).The spectra from each patient were averaged and normalized with respect to the spectral intensity around 510 nm. Figure 3(a) shows the mean LIAF spectra from typical sites with grades 0 to 3 (50 in each group) while Fig. 3(b) shows LIAF spectra after normalization to the intensity of the autofluorescence peak at 510 nm.

Fluorescent spectral data revealed noticeable differences between plaque-free tooth surfaces and various grades of plaque. The LIAF spectrum of grade-0 plaque showed a broad emission around 500 nm with a long tail extending toward the red wavelength region. It was found that the FI of plaque-free tooth surfaces was higher than all the other grades of plaque in the 450 to 600 nm range. As the plaque grade (thickness) increased, the FI in the red wavelength region gradually increased with the appearance of another peak around the 635 nm region. With a further increase in the plaque thickness/grade, new peaks started appearing around 685 and 705 nm [Fig. 3(a)]. Figures 4(a) and 4(c) present the clinical photograph of plaque with grades 0 and 1, respectively, while Figs. 4(b) and 4(d) show corresponding photographs after application of the disclosing solution. Grade-1 plaque shows a red-shift of 15 nm for the 510 nm peak as compared to the plaque-free tooth surface of grade 0 as shown in Fig. 4(e).

In comparison, the red-shift noticed for the 510 nm peak between grade-1 and grade-2 plaque is 10 nm and between grades 2 and 3 is 5 nm. Concurrently, the intensity of the 635 nm fluorescence peak also increases as the grade of plaque increases from 0 to 3, which could be understood from the fluorescence intensity ratio of this peak with respect to the 510 nm peak.

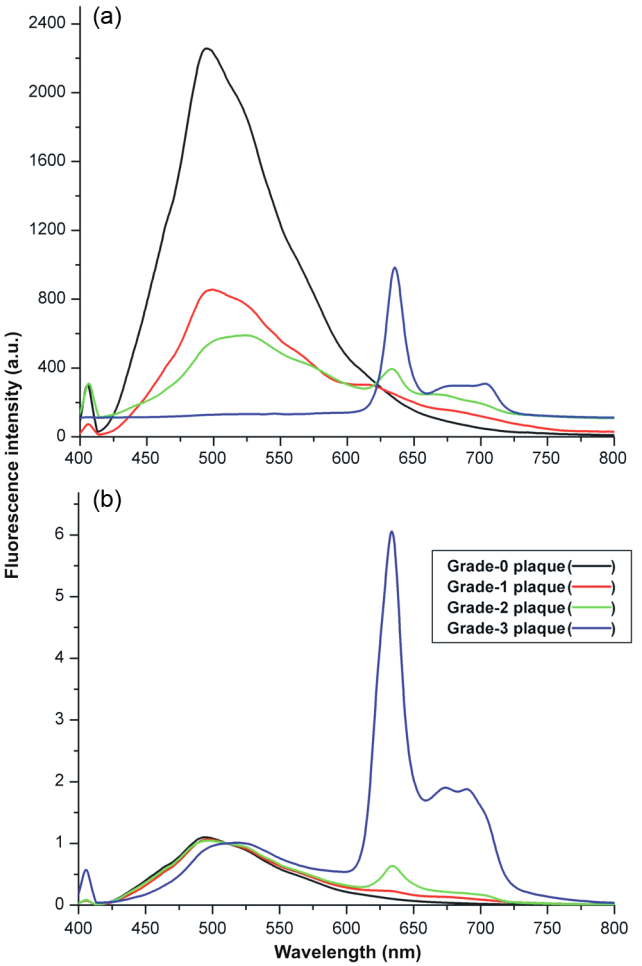

Fig. 3 (a) Mean LIAF spectra from typical sites with grades 0 to 3 (50 in each group) and (b) after normalization to the intensity of the autofluorescence peak at 510 nm.

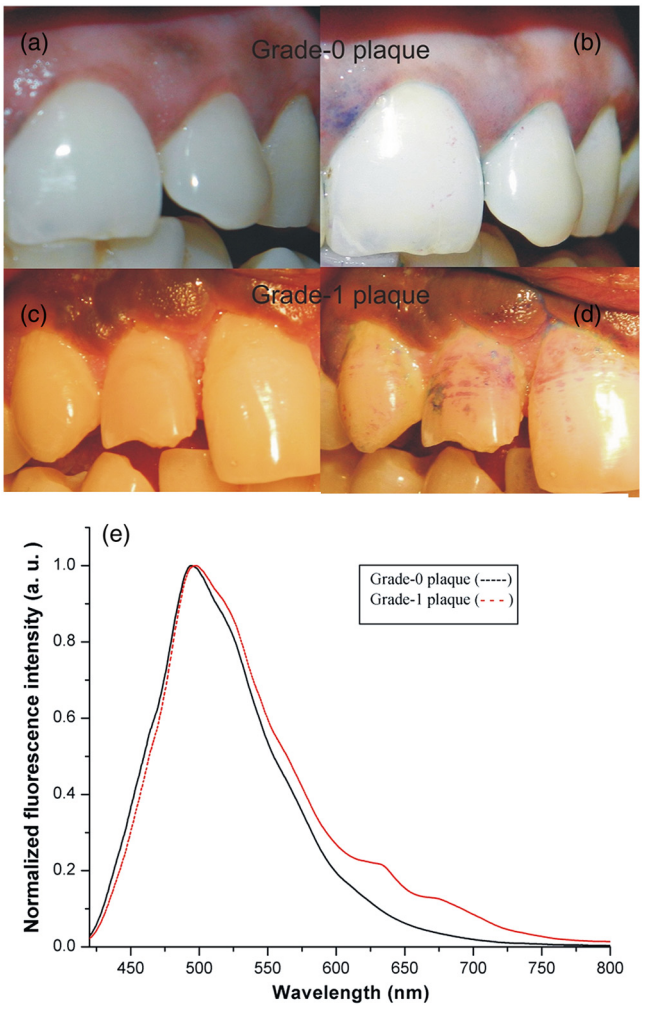

Fig. 4 Clinical photograph of plaque deposits, (a) with grade 0; (b) after application of disclosing solution on grade-0 plaque; (c) with grade-1; (d) after application of disclosing solution on grade-1 plaque; (e) LIAF changes corresponding to the clinical photographs of grade-0 and grade-1 plaque. Note the red-shift of ~15 nm for the 510 nm peak for plaque with grade-1 as compared to the plaque-free tooth (grade-0).

Table 1 Mean laser-induced autofluorescence spectral ratios from plaque-free tooth surface (grade-0) and higher grades of plaques on tooth surface.

Grades of plaque (PI)	$F510/F630$	Percentage variation with respect to grade-0	Percentage variation with respect to adjoining lower grade
Grade-0	8.0 ± 0.5		
Grade-1	2.4 ± 0.3	70.0	70.0
Grade-2	1.4 ± 0.4	82.5	41.6
Grade-3	0.6 ± 0.2	92.5	57.1

3.2 *Laser-Induced Autofluorescence Spectral Ratio*

LIAF spectral intensity ratio at $F510/F630$ was calculated from the spectral intensity value of the 510 and 630 nm peaks for various grades of plaque in 200 patients that form the training set for discrimination/classification of gingival plaque. It was observed that the $F510/F630$ ratio has a decreasing tendency with an increase in gingival plaque thickness. Variation in the spectral intensity ratio values of plaque-free tooth surface (grade-0) with respect to higher grades of plaque are shown in Table 1. These spectral intensity ratio values demonstrate statistically significant changes between different grades of plaque during an unpaired t test ($p < 0.01$) as shown in Table 2.

3.3 *Diagnostic Accuracy of Laser-Induced Autofluorescence*

A scatter plot of the fluorescence intensity ratio ($F510/F630$) was drawn for discriminating different grades of plaque (Fig. 5) in 200 patients and the blind data from 100 patients were used for clinical validation. Discrimination lines were drawn in the scatter plot diagrams between grade-0 and grade-1, grade-1 and grade-2, and grade-2 and grade-3 plaques at the weighted arithmetic mean of the adjoining two groups. The results show that the intensity ratio of plaque-free tooth surfaces (grade-0) is higher than those of plaque grades 1 to 3.

The diagnostic accuracy of using the fluorescence intensity ratio ($F510/F630$) for discriminating different grades of plaque was evaluated by calculating sensitivity, specificity, PPV, and NPV from the mismatch, if any, from the assigned group/category in the scatter plot. The true-positive, false-positive, true-negative, and false-negative values of discrimination between the adjoining groups are given in Table 3. With a cut-off value of 5.22, which is the weighted arithmetic mean of $F510/F630$ values for grades 0 and 1 in the standard set, discrimination between grades 0 and 1 was possible with an overall sensitivity and specificity of 100% and a PPV and NPV of 100% (Table 4). In the blind set, there were also no misclassifications, leading to a value of 100% for sensitivity, specificity, PPV, and NPV.

With a cut-off value of 1.91, grade-1 plaque could be discriminated from grade-2 with an overall sensitivity of 74% and specificity of 65%, and PPV and NPV of 69% and 71%, respectively. In comparison, with a cut-off value of 1.02, grade-2 plaque could be discriminated from grade-3 plaque with an overall sensitivity of 90% and specificity of 100%, and PPV and NPV of 100% and 92%, respectively. Table 4 gives the independent and overall diagnostic accuracies achieved.

3.4 *Area Under Receiver Operating Characteristic Curve*

The performance of the diagnostic algorithm for discriminating different grades of plaque was evaluated using ROC curves constructed using the sensitivity and specificity values of detection. The area under ROC curves in Figs. 6(a)–6(c) show the discriminatory capacity of the $F510/F630$ ratio to differentiate between plaque with grades 1 and 2 [AUC = 0.89 (95% CI: 0.84–0.94)], grades 2 and 3 [AUC = 0.91 (95% CI: 0.86–0.95)], and grades 0 and 1 [AUC = 1.00 (95% CI: 1.00–1.00)]. AUC values close to 1 represent a better diagnostic performance.[24]

4 Discussion

4.1 *Spectral Characteristics of Different Grades of Plaque*

The present study describes the results from the first clinical correlation of 404 nm LIAF spectral intensity ratio ($F510/F630$) with grades 0 to 3 plaque deposit on tooth. A diode emitting at 404 nm was utilized because this wavelength matches with the strongest absorption band of protoporphyrin IX (PpIX) present in the bacterial plaque. The most relevant observation, from a clinical view point, is the potential of LIAF to detect plaque which is usually not visible to the naked eyes. It could be postulated that the marked difference seen in the spectral signature of plaque-free tooth surfaces and those with minute amounts of plaque are due to differences in the fluorophore emission from enamel and bacterial plaque. Endogenous fluorophores in enamel are considered to be responsible for the single broad emission around 500 nm with a long tail extending toward the red wavelength region as seen in the case of grade-0 plaque, which is generally assigned to organic matter embedded in the inorganic calcium apatite semi-crystalline matrix of enamel.[25]

Table 2 Results of unpaired t test.

	Grade 0	Grade 1	Grade 2	Grade 3
$F510/F630$	8.0 ± 0.5	2.4 ± 0.3	1.4 ± 0.4	0.6 ± 0.2
t-value		58.08	13.52	13.05
p-value		<0.0005**	<0.0005**	<0.0005**

Note: **$p < 0.01$

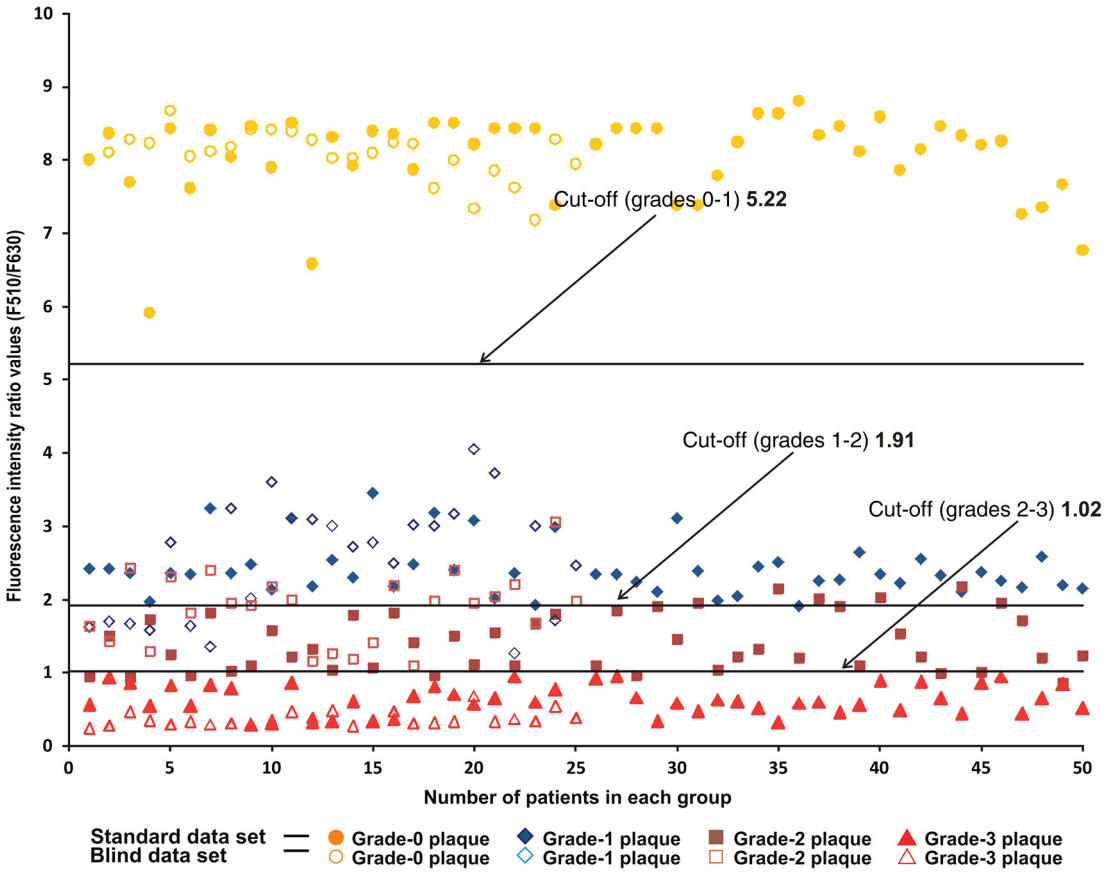

Fig. 5 Scatter plot of the autofluorescence spectral intensity ratio $F510/F630$ for plaque with grades 0 to 3.

The appearance of a small peak at 635 nm along with a prominent peak at 510 nm in grade-1 plaque is being reported for the first time. There has been a previous report of immature plaque, especially the streptococcal species (early colonizers) that emit predominantly green spectra.[26,27] The emission spectra seen in our study in the case of grade-1 plaque correspond well with this finding. Emission maxima in grade-1 plaque at around 510 nm have been reported to correspond to coenzymes, flavin adenine dinucleotide and flavins, which have several important reactions in bacterial cell energy metabolism.[28] Characteristic yellow-green fluorescence is also seen in *Pseudomonas aeruginosa* as the cells contain a fluorescent siderophore called pyoverdin. Bacteria species such as *Veillonella*, which is often found in the supragingival plaque, is known to emit red fluorescence, which could be the probable source for the characteristic small peak around 635 nm in grade-1 plaque.[29]

Table 3 True-positive, true-negative, false-positive and false-negative cases recorded from spectral ratio.

		True positive	False positive	True negative	False negative
Raw spectral data $F510/F630$			Grade-0 plaque versus Grade-1 plaque		
	Training set	50	0	50	0
	Blind set	25	0	25	0
			Grade-1 plaque versus Grade-2 plaque		
	Training set	40	11	39	10
	Blind set	17	12	13	8
			Grade-2 plaque versus Grade-3 plaque		
	Training set	40	0	50	10
	Blind set	25	0	25	0

Table 4 Independent and overall diagnostic accuracies obtained for discriminating different grades of plaque using fluorescence ratio reference standard (FRRS) ratio derived from spectral intensity ratio.

	Grade-0 versus Grade-1 (%)				Grade-1 versus Grade-2 (%)				Grade-2 versus Grade-3 (%)			
Raw spectral ratio $F510/F630$	Se	Sp	PPV	NPV	Se	Sp	PPV	NPV	Se	Sp	PPV	NPV
Standard set	100	100	100	100	80	78	78	80	80	100	100	83
Blind set	100	100	100	100	68	52	59	62	100	100	100	100
Overall/Total	100	100	100	100	74	65	69	71	90	100	100	92

Note: sensitivity (Se), true positive/(true positive+ false negative); specificity(Sp), true negative/(true negative+ false positive); positive predictive value(PPV), true positive/(true positive+ false positive); negative predictive value (NPV), true negative/(true negative+ false negative).

In contrast, the 510 nm peak, which is prominent in the early stages of plaque formation, was found to decrease in intensity while a new peak gathers in intensity at 635 nm as the thickness of the plaque increases to grade-3. The computed $F510/F630$ ratio value of 2.4 ± 0.3 of grade-1 plaque shows a decrease of 70% as compared to that of grade-0 (8.0 ± 0.5) plaque, which is clinically invisible. The percentage decrease in $F510/F630$ ratio of grade-0 with respect to grades 2 and 3 is 82.5% and 92.5%, respectively as shown in Table 1. Since the changes observed in fluorescence intensity ratio values during early stages of plaque formation are quite appreciable as compared to the peak shifts observed at 510 nm, the technique could further be extended to the detection of clinically invisible plaque (grade-0). An increase in fluorescence intensity with increasing plaque amount has also been reported earlier during *in vitro* studies,[4,16,17]where orange to red fluorescence is known to be associated with obligate anaerobic bacteria in older plaque and is also used to monitor the degree of maturation of dental microcosm biofilms.[30]

Endogenous porphyrins and metalloporhyrins, in particular, PpIX, mesoporphyrin, and coproporphyrin synthesized by bacteria in varying amounts, are believed to be the reason for the emission at around 635 nm.[9] The prominent red-shift observed in this study in the case of grade-2 and grade-3 plaque is in accordance with the previous reports. The additional peaks in the case of grade-2 and grade-3 plaque around 685 and 705 nm could be attributed to the presence of porphyrins. It has been reported that upon excitation with 405 to 407 nm light, emission maxima at 590, 610, 620, 635, 675, 690, and 705 nm that correspond to porphyrins could be observed.[28] It is also reported that the responsible species for these emissions were *Prevotella melaninogenica, Actinomyces israelii, Candida albicans* (red and orange fluorescence), and *Fusobacterium nucleatum* and *Streptococci* species (green fluorescence) [31]

4.2 *Nature of Fluorescence from Plaque Biofilm*

While most of the studies focus on the contribution of bacterial porphyrins in the production of red fluorescence, other endogenous fluorophores are present in the plaque. Fluorescence obtained from suspensions of microbial cells may differ from the actual biofilm seen on the tooth surface because when microbial cells are firmly packed, many additional cellular fluorophores become evident. This is because the fluorescence is the result of interplay between many intrinsic fluorophores and is dependent upon their physical environment within a cell. Variation in the fluorescence properties of plaque between individuals and within the dentition is possible due to the interaction of cell fluorophores and proteins.[32] An increase in the fluorescence intensity of NADH has been reported when bound to cellular proteins resulting in a blue-shift of the excitation and emission maxima. A similar blue-shift in the emission maximum of another intrinsic fluorophore, pyridoxal, is also reported when bound to proteins such as phosphorylase.[32]

When a longer duration and higher intensity of the excitation light are used, there could be a probability of fluorescence signal loss in some cases due to destruction of the excited fluorophore by a process known as photobleaching. However, in the present study, the laser light dose incident on the plaque during each

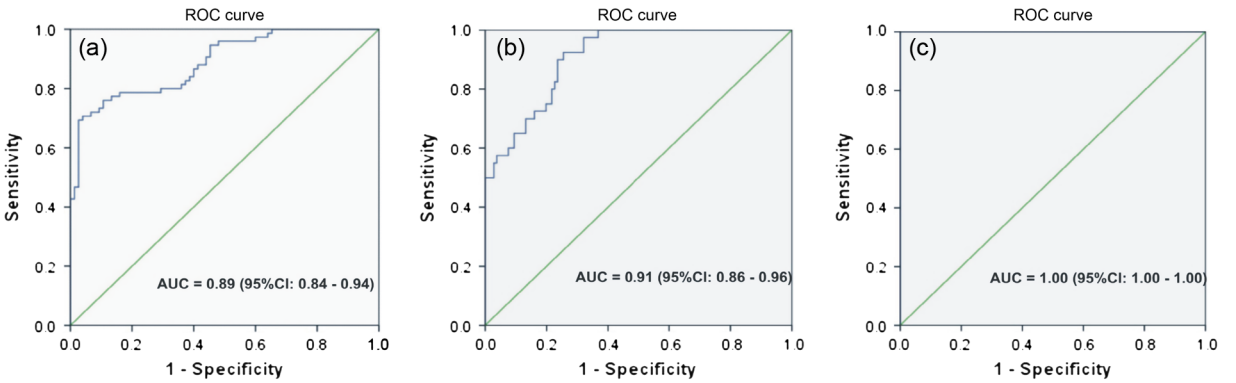

Fig. 6 Area under receiver operating characteristic (ROC) curves showing the discriminatory capacity of the $F510/F630$ ratio to differentiate between different grades of plaque: (a) grades 1 and 2; (b) grades 2 and 3; and (c) grades 0 and 1.

measurement period was 0.2 J/cm^2, and this radiation dose is too low to cause such damage. Moreover, ratiometric measurements used in this study are also known to eliminate distortions of data, if any, caused by photobleaching.[33]

4.3 *Diagnostic Accuracy of Laser-Induced Autofluorescence*

Another salient feature of our study is that the primary etiologic factor of periodontal disease is identified and quantified unlike other studies where emission from subgingival calculus is studied because plaque is an earlier form of subgingival calculus. There are earlier studies that report a significant increase in fluorescence intensity when subgingival calculus on extracted teeth were irradiated with wavelengths between 360 and 655 nm.[20,34–37] FRRS has been utilized earlier for developing algorithms for detection and discrimination of tooth caries and oral cancer.[10–14] This newly developed algorithm based on $F510/F630$ ratio discriminates plaque with good diagnostic accuracy between different grades, but the most significant aspect is that the algorithm was able to distinguish the early stage of plaque (grade-1) from plaque-free teeth (grade-0) with 100% sensitivity and specificity. As compared to the peak shifts noticed in the 510 nm peak, the decrease in $F510/F630$ ratio is easily measurable and quantifiable in a clinical setting in real time on patients without the need for any complicated data processing. ROC analysis is usually used in clinical epidemiology to quantify how accurately medical diagnostic tests (or systems) can discriminate between two patient states, typically referred to as "diseased" and nondiseased. The basis for signal detection theory is that nearly all reasoning and decision making takes place in the presence of some uncertainty. Signal detection theory provides a precise language and graphic notation for analyzing decision making in the presence of uncertainty.

4.4 *Clinical Implication of Identifying Grade-1 Plaque in Patients*

This is the first clinical study with a sufficient sample size that linked fluorescence spectral measurement to the PI. The presence of supragingival plaque is known to be critical in calculus formation and progression of periodontal disease,[38–39] and has a marked effect on the subgingival microbiota.[40,41] Therefore, preventing plaque accumulation controls gingival inflammation and prevents periodontal attachment loss over a long period of time.[42] Clinicians may easily identify visible amounts of plaque corresponding to grades 2 and 3 of the PI. But the most challenging problem for clinicians and patients during home-care is to precisely identify minute amounts of plaque that correspond to grade-1 of the PI. This is largely due to the fact that the tooth and dental plaque often look alike, especially if plaque is present in a scanty amount. Moreover, interproximal plaque formation is visually less evident. Most clinical plaque indices measure a variable on an ordinal scale, whereas an interval scale is more powerful, providing more precise results and reducing subjectivity. We believe that the technique described here based on the FRRS can be used for classification of plaque as it captures the fluorescence from plaque and translates it on a numerical scale as seen in Table 5. It could also be used for the monitoring of supragingival plaque, screening of patients on a regular basis for oral hygiene maintenance and also in clinical research. Plaque control is also helpful in controlling dental caries, malodor (bad breath), and even improves the painful symptoms of oral lichen planus.[43] The versatility of the probe makes it possible to use it in any area of the dental arch. Fluorescence diagnosis will also be useful for monitoring the status of teeth before and after scaling, as described in Kurihara et al.

Table 5 Scatter plot cut-off values of fluorescence intensity ratio with respect to PI for classification of dental plaque.

Scatter plot cut-off	PI
Above 5.2	Grade-0
5.1 to 1.9	Grade-1
1.8 to 1.02	Grade-2
Below 1.01	Grade-3

4.5 *Limitations and Strengths*

This study provides a rationale for using the LIAF to detect clinically invisible plaque. The merits of this study include a sufficiently large sample size, stringent selection criteria, and the use of a nonionizing 404 nm wavelength for excitation. Unlike conventional plaque indices, the data here are reliable and reproducible, and can be stored for clinical documentation while visuals of spectral changes in areas of plaque can be used for patient education. The device can be used for objective and precise detection of plaque in clinical and community settings. Further, it would be possible for the patients to directly monitor their oral hygiene status with the help of the digital display corresponding to grades of plaque. However, the results from this system need to be interpreted with care in the case of tooth surfaces with caries, fracture, or extrinsic stains. Grade-1 plaque may be misinterpreted as grade-3 plaque if it has been long standing and matured. It has been found that plaque could mature in about 9 days. While interpreting the results of this study, it should be also be understood that variations in the microbiological compositions of plaque,[44] pigmentation, and the use of some toothpastes or prophylaxis pastes[45]can also cause variations in spectral intensity values.

5 Conclusion

In periodontal therapy, a major challenge is to effectively identify plaque deposits, both by clinicians and patients, so that disease can be treated effectively and its recurrence controlled. Because of the drawbacks of the existing methods, plaque, especially in minute quantities and during the early stages of formation, could be easily be missed. Within the limitations of the present *in vivo* study, the results reveal that it is possible to detect different grades of plaque on tooth surfaces including the clinically invisible type belonging to grade-1 from the 404 nm laser-induced LIAF spectral intensity ratio $F510/F630$. Further, the FRRS developed has the potential benefit of quantifying even minute amounts of plaque in real time, thereby achieving easier plaque detection for the clinician and better plaque control for the patient. Possibilities of accessing periodontal pockets with the LIAF probe to detect subgingival plaque and calculus are envisaged in order to determine the completeness of mechanical therapy.

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