Clinical trial for detection of dental caries using laser-induced fluorescence ratio reference standard

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Abstract. We present the clinical applicability of fluorescence ratio reference standard (FRRS) to discriminate different stages of dental caries. Toward this, laser-induced autofluorescence emission spectra are recorded *in vivo* in the 400- to 800-nm spectral range on a miniature fiber optic spectrometer from 65 patients, with a 404-nm diode laser as the excitation source. Autofluorescence spectra of sound teeth consist of a broad emission at 500 nm that is typical of natural enamel, whereas in caries teeth additional peaks are seen at 635 and 680 nm due to emission from porphyrin compounds in oral bacteria. Scatter plots are developed to differentiate sound teeth from enamel caries, sound teeth from dentinal caries, and enamel caries from dentinal caries using the mean fluorescence intensity (FI) and ratios $F500/F635$ and $F500/F680$ measured from 25 sites of sound teeth and 65 sites of carious teeth. The sensitivity and specificity of both the FI and FRRS are determined. It is observed that a diagnostic algorithm based on FRRS scatter plots is able to discriminate enamel caries from sound teeth, dentinal caries from sound teeth, and enamel from dentinal caries with overall sensitivities of 85, 100, and 88% and specificities of 90, 100, and 77%, respectively. © *2010 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.3365945]

Keywords: dental caries; fluorescence ratio reference standard; laser-induced fluorescence spectroscopy; porphyrins.

1 Introduction

Laser-induced fluorescence (LIF) has been increasingly used recently as a powerful tool for caries detection and shows great potential for development as an alternative to the dental probe or radiographic examination. The phenomenon of fluorescence is based on the principle that the fluorescence emission observed on irradiation by light in the blue-green spectral region from carious dental tissues differs from that of a sound tooth. The carious region appears darker than the surrounding sound tooth surface in a fluorescence image due to a decrease in the fluorescence signal from the carious tissues.[1,2] A number of optical methods have been developed and recommended as diagnostic tools to identify and quantify early caries lesions on smooth and occlusal surfaces.[3]

Most of the investigations in caries research have been related with quantitative laser/light-induced fluorescence (QLF), which measures the intrinsic fluorescence of the tooth tissues. Various groups have reported results on the spectral changes in the fluorescence intensity between sound and carious teeth.[4–6] The appearance of fluorescence emissions in the 520- to 580-nm wavelength region after irradiation with a 488- or 514-nm argon-ion laser was observed in several studies of carious structures.[1,2,5–7] Most of these studies showed good correlation between fluorescence and mineral loss[7–9] and were found useful in early detection of smooth surface caries lesions.[7,10]

Another commercially available laser-based instrument is the DIAGNOdent (KaVo, Biberach, Germany), which uses a 655-nm diode laser to detect near-IR fluorescence from porphyrins produced by oral bacteria and displays fluorescence of dental tissues as a numerical value ranging from 0 to 99 (higher values for deeper caries). This device has been extensively used in various studies to detect changes in mineral content, to measure changes during remineralization, and in

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the detection of caries lesions.[11–15] However, this device could not provide good correlation with mineral loss on smooth surface caries lesions in teeth.[9,16,17] Moreover, despite the fact that some authors have claimed that the device is able to detect early enamel caries lesions,[18,19] studies have not yet been conclusive.[17,20]

The most preferred excitation wavelength for caries detection by LIF spectroscopy is around 400 nm as this wavelength provides optimal excitation of tissue fluorophors.[21–23] Thus, a diode laser with emission around 400 nm is the preferred choice as compared to the broad emission bands produced by nonlaser excitation sources.

The aim of this study was to develop a LIF ratio reference standard (FRRS) to discriminate between different stages of caries lesions using a diode laser emitting at 404 nm as the excitation source. Toward this, we measured the *in vivo* LIF spectra from sound and carious teeth of 65 patients and developed fluorescence intensity (FI) and FRRS scatter plots based on autofluorescence spectral intensity of the emission peaks at 500, 635, and 680 nm and FI ratios $F500/F635$ and $F500/F680$, respectively. The sensitivity and specificity of the FI at the emission peaks and ratios in caries discrimination was determined after validation by clinical examination of caries lesions and the results are presented.

2 Material and Methods

2.1 Study Protocol and Subjects

This study was conducted at the Department of Conservative Dentistry and Endodontics of the Government Dental College, Thiruvananthapuram, India. Ethical approval for the study was provided by the Institutional Ethical Committee of the Government Dental College, Thiruvananthapuram (Approval No. IEC/C/01-A/2008/DCT). All volunteers were provided with patient information sheets and consent forms. After explaining the details of the study, a consent form was endorsed by each patient prior to enrolment.

A total of 65 patients, aged between 20 and 50 yr were selected according to different clinically diagnosed stages of dental caries. The study was performed on smooth surfaces *in vivo* and the chosen method to determine caries lesions was visual inspection. The study population was composed of 25 sound teeth, 30 enamel caries, and 35 dentinal caries lesions. Samples with any kind of staining, hypoplasia, and fluorosis were excluded.

Table 1 Criteria for clinical examination.

| Category	Clinical Criteria
Sound	Normal texture of enamel
Enamel caries	Opaque, with loss of luster and rough, with intact surface
Dentinal caries	Localized enamel breakdown, cavitation in opaque or discolored enamel exposing the dentin, loss of substance

2.2 Clinical Examination Criteria

All the smooth surfaces were thoroughly dried and clinically examined by an experienced dentist using the diagnostic criteria mentioned in Table 1 based on Ekstrand's visual scoring system[24] as well as personal experience with clinical caries diagnosis. After seating the patient in the dental chair of the Conservative Dentistry Department, visual examination was performed with a light reflector, compressed air, dental mirror, and suction device under optimized room temperature ($24 \pm 2 \, ^\circ$C). All lesions investigated had intact tooth surfaces and blunt probes were used to remove visible dental plaque from smooth surfaces. Thereafter, LIF spectroscopy was used to examine those lesions confirmed by visual inspection.

Before initiation of spectral measurements, the patients were directed to hold 0.9% saline solution in their mouth for 1 min to reduce the effects of recently consumed food and the assigned tooth sites were cleaned and dried with cotton swabs.

2.3 Instrumentation

The portable laser-induced fluorescence spectroscopy (LIFS) system developed in our laboratory for caries diagnosis is shown in Fig. 1. It is composed of a diode laser (Stocker Yale, Canada, 404 nm, 50 mW, cw) for excitation of tooth fluorescence and a miniature fiber optic spectrometer (Ocean Optics, United States, Model: USB 2000FL VIS-NIR), connected to the USB (universal serial bus) port of a computer for spectral recording. A bifurcated optical fiber guides the light from the laser to the tooth through a 3-m-long optical fiber (400 μm diameter) terminated in a hand piece made of stainless steel. A 5-mm-long black PVC sleeve inserted at the probe tip helps to prevent stray room light from entering into the detection
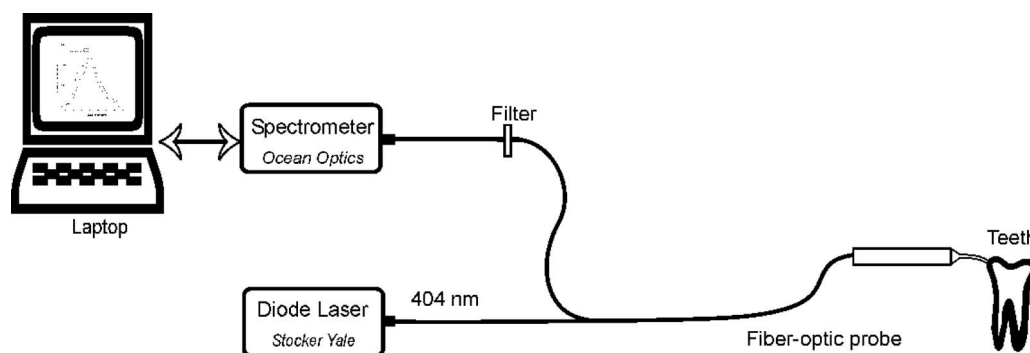
Fig. 1 Schematic of the experimental setup for LIF spectral measurements.

system by providing a close contact with the tooth surface. Since this opaque sleeve is disposable, it provides extra hygiene. The light emanating from the sample is collected by the same optical fiber and delivered to a miniature fiber optic spectrometer through the second arm of the bifurcated fiber and the long-wavelength pass filter (Schott: GG420).

2.4 Data Acquisition and Processing

The hand piece was sterilized with sodium hypochlorite before use and the fiber optic light coupler on the laser head was aligned to provide a Gaussian profile beam at the fiber tip. The laser output power at the fiber tip was maintained at 1-mW power level by periodic monitoring with a power meter (Ophir Optronics, Israel). The hand piece was placed in contact with the lesion surface and fluorescence spectra were recorded by point monitoring. The miniature fiber optic spectrometer was fitted with a 600-lines/mm, 500-nm blazed grating and a 2048-element linear silicon CCD array for operation in the 360- to 1000-nm wavelength range. The LIF spectrum was recorded in the 400- to 800-nm spectral range with a resolution of 8 nm using the OOI Base32 software (Ocean Optics, United States) configured to record the spectra, averaged for 40 scans, with a boxcar width of 8 nm and an integration time of 50 ms. The background spectrum was recorded prior to measurements and the software automatically subtracted the same from the recorded spectrum. Due to the diverse nature of the caries lesions, 15 sets of LIF measurements were taken from each caries lesion and sound tooth of the same patient for comparison, and the mean value from each site was determined for further analysis. An analysis of variance (ANOVA) was performed using SPSS software (Version 10, SPSS Inc., Chicago, Illinois) to determine the variance of the averaged LIF spectral intensity between the sound, enamel, and dentinal caries groups.

FI ratios were then calculated from the recorded mean spectra and correlated with the visual findings from each site. To account for the broad nature of the peaks and sample-to-sample variation in peak position, due to changes in chemical composition and fluorophore content of the tooth, the mean LIF spectral intensity over an interval of ± 10 nm at the emission peak was used to determine the LIF spectral ratios. To discriminate different stages of caries lesions from a sound tooth, FI scatter plots of the emission peaks at 500, 635, and 680 nm and FRRS scatter plots of the intensity ratios ($F500/F635$ and $F500/F680$) were evaluated from the spectral data of 65 carious sites and 25 sound tooth sites. An independent Student's t test was performed on the FRRS to determine the statistical significance of the developed standard for discrimination of caries lesions. The sensitivity and specificity of measurement were determined considering visual inspection results as the gold standard.

3 Results

3.1 LIF Spectral Features

In vivo fluorescence spectral measurements were carried out on consenting patients having caries lesions in their oral cavities. Figure 2(a) shows the mean LIF spectra recorded from 25 sites of sound teeth, 30 sites of enamel caries, and 35 dentinal caries lesions, and the same spectra normalized with
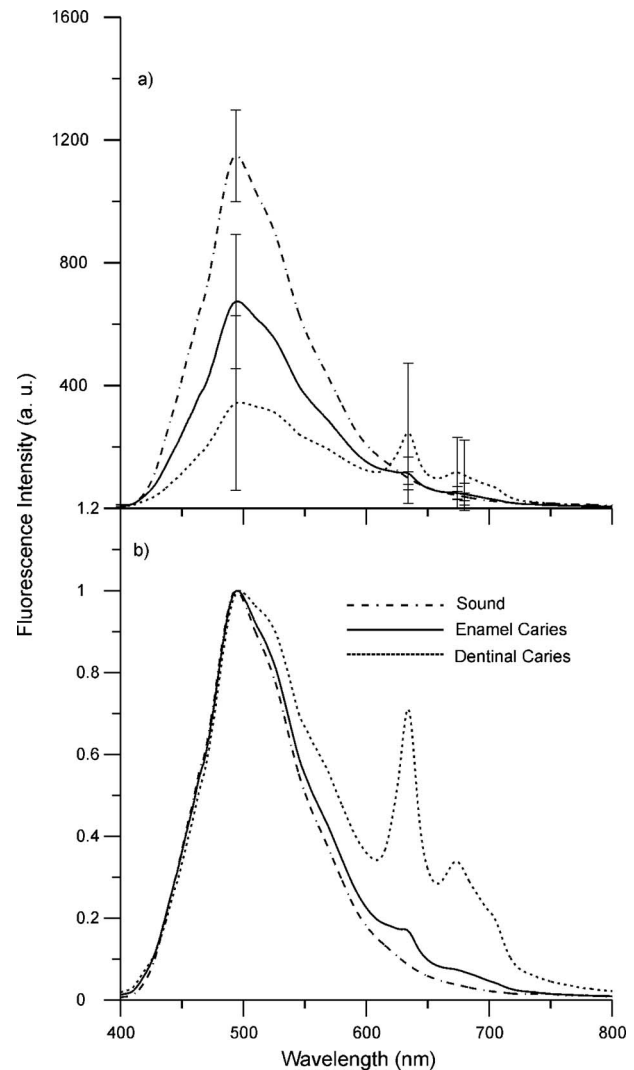
Fig. 2 (a) Mean *in vivo* LIF spectra of sound teeth and those of different caries stages and (b) LIF spectra normalized to maximum fluorescence spectral intensity around 500 nm. Sound tooth spectra represent the mean of 15 measurements each in 25 sites, whereas enamel caries spectra are of 15×30 measurements and the dentinal caries spectra are of 15×35 measurements.

respect to the maximum spectral intensity around 500 nm are given in Fig. 2(b). The standard deviation is shown for the prominent peaks in the mean LIF spectra from sound and caries teeth belonging to different stages of tooth decay. Marked differences in fluorescence spectral features were seen between sound and caries teeth. As compared to sound teeth, the overall FI was found to be lower in all caries lesions. However, the fluorescence spectral intensity in the red wavelength region was greater for caries lesions than for sound teeth. The LIF spectrum of sound teeth shows a broad emission around 500 nm with a long tail extending toward the red wavelength region. In enamel and dentinal caries, two additional peaks were seen around 635 and 680 nm owing to porphyrin emission. These porphyrin peaks are more prominent in dentinal caries lesions. Another notable feature is the broadening of the 500-nm peak towards the red wavelength region by about 30 nm in dentinal caries. The spectra also
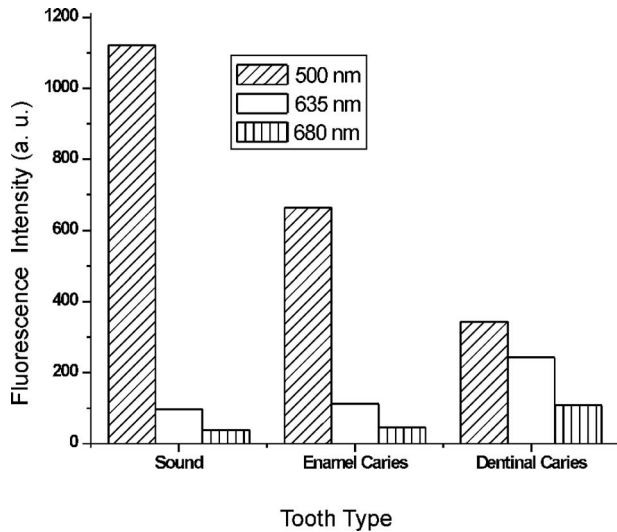
Fig. 3 Mean LIF spectral intensity variation of emission peaks at 500, 635, and 680 nm from 65 patients diagnosed as enamel caries and dentinal caries, along with the intensity values from 25 sites of sound teeth.

shows a sharp rising edge in the short-wavelength side (400 to 450 nm), which is due to absorbance of the 420-nm long-wavelength pass filter (Schott GG420) used for blocking the back-scattered laser light from entering the spectrometer.

Figure 3 shows the mean LIF spectral intensity variation of emission peaks at 500, 635, and 680 nm from sound teeth, enamel, and dentinal caries. The LIF intensity at 500 nm decreases as caries progression occurs in the sound tooth, whereas the intensity increases for the porphyrin peaks at 635 and 680 nm. A one-way ANOVA test was used to determine the average spectral intensity variation among the three groups studied. Statistically significant differences in mean spectral intensities ($p < 0.001$) were noticed between sound teeth, enamel caries, and dentinal caries lesions.

3.2 LIF Intensity Ratios

Mean LIF spectral intensity ratios ($F500/F635$ and $F500/F680$) determined from the sound and caries tooth samples are listed in Table 2 for caries discrimination. Both the LIF ratios ($F500/F635$ and $F500/F680$) show a decreasing trend with increasing tooth decay, with the lowest values for dentinal caries lesions and the highest for sound teeth. The $F500/F680$ ratio shows a maximum variation of 48% be-

Table 2 Mean LIF spectral ratios determined from *in vivo* LIF spectral data of sound and caries teeth.

Tooth Types	Population (n)	$F500/F635$	$F500/F680$
Sound	40	10.94 ± 1.7	27.89 ± 6.8
Enamel caries	50	6.48 ± 1.7 (41)	14.59 ± 6.3 (48)
Dentinal caries	55	2.59 ± 1.4 (76)	5.16 ± 3.4 (82)

The percentage given in parentheses shows the variation in ratio value with respect to the sound tooth; *p* value for the ratios < 0.001.
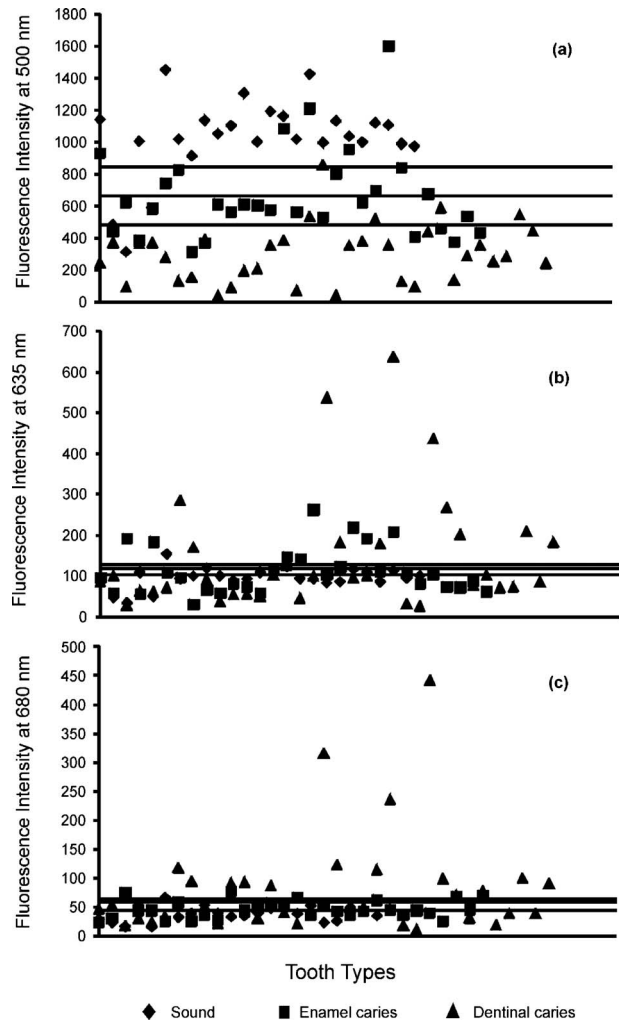
Fig. 4 FI scatter plots based on autofluorescence spectral intensity of emission peaks, at (a) 500, (b) 635, and (c) 680 nm, from 65 patients diagnosed as enamel caries and dentinal caries, along with the emission intensities from 25 sites of sound teeth.

tween sound and enamel caries and 82% between sound teeth and dentinal caries, whereas between enamel and dentinal caries lesions, this ratio has a variation of 64%.

3.3 Caries Discrimination

3.3.1 Using FI scatter plots

To discriminate different stages of caries lesions from sound teeth, scatter plots were drawn based on the fluorescence intensities of emission peaks at 500, 635, and 680 nm obtained from the spectral data of 65 carious sites and 25 sound teeth sites [Figs. 4(a)–4(c)]. The cutoff value in the scatter plot, which is the weighted mean of the adjacent groups, is used to discriminate different stages of caries lesions. For example, the cutoff values for differentiating sound teeth from enamel caries, sound teeth from dentinal caries, and enamel and dentinal caries are 843.9, 662.4, and 483.0, respectively, at 500 nm. Discrimination lines were drawn between sound teeth and enamel caries, sound teeth and dentinal caries, and enamel and dentinal caries in the three scatter plot diagrams. Diagnostic accuracies, such as sensitivity, specificity, positive

Table 3 Diagnostic accuracies obtained for classifying different stages of dental caries using the fluorescence intensity of emission peaks at 500, 635, and 680 nm.

	Sound vs Enamel Caries (%)				Sound vs Dentinal Caries (%)				Enamel vs Dentinal Caries (%)			
FI	Se	Sp	PPV	NPV	Se	Sp	PPV	NPV	Se	Sp	PPV	NPV
500	83	88	89	81	97	88	92	96	86	73	79	81
635	50	64	63	52	31	92	85	49	31	73	58	48
680	50	68	65	53	43	96	85	55	43	83	75	56

FI, fluorescence intensity; 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); and negative predictive value (NPV), true negative/(true negative+false negative).

predictive value, and negative predictive value, for discriminating each pair were calculated by correlating the position of emission intensity values for each lesion in the scatter plot with the corresponding clinical criteria.

Table 3 illustrates the diagnostic accuracies obtained for classifying different stages of caries lesions using fluorescence emission intensities at 500, 635, and 680 nm. The sensitivities obtained for discriminating sound versus enamel caries, sound versus dentinal caries, and enamel versus dentinal caries pairs are 83, 97, and 86%, respectively, and the corresponding specificities are 88, 88, and 73%, respectively, using the fluorescence emission intensity at 500 nm. For 635- and 680-nm emission peaks, the sensitivities and specificities were very poor for all the lesion pairs, except for the moderate specificities of 92 and 96% observed respectively for these two peak intensities in discriminating sound teeth from dentinal caries.

3.3.2 Using FRRS scatter plots

Figures 5(a) and 5(b) illustrate the scatter plots of the fluorescence intensity ratios $F500/F635$ and $F500/F680$ in 65 patients, with tooth lesions diagnosed as enamel and dentinal caries, along with the control data from 25 sites of sound teeth. The intensity ratios of caries lesions are generally lower than those of sound teeth (Table 2) and have low independent Student's t test values of $p < 0.001$. Discrimination lines were drawn between sound teeth and enamel caries, sound teeth and dentinal caries, and enamel and dentinal caries as in the case of FI for the two scatter plot diagrams. Diagnostic accuracies for discriminating each pair were calculated by correlating the position of ratio values for each lesion in the scatter plot with the corresponding clinical criteria.

Table 4 illustrates independent and overall sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of FRRS with respect to $F500/F635$ and $F500/F680$ for classifying different stages of tooth caries. For the $F500/F635$ and $F500/F680$ ratios, by selecting a cutoff at the mean of sound tooth and dentinal caries values (6.87 and 16.67) respectively, sensitivities and specificities of 100% were achieved with a positive and negative predictive value of 1 for discriminating these two categories. In Fig. 5(a), by selecting 8.69 as the cutoff value in the $F500/F635$ scatter plot to discriminate sound teeth from enamel caries, sensitivities and specificities of 87 and 100%, respectively, were obtained with a PPV of 1 and an NPV of 0.86. In this

study, only 4 out of the 30 enamel caries lesions were misclassified as sound. For discriminating enamel from dentinal caries, sensitivities and specificities of 89 and 80%, respectively, were achieved for the same ratio by using 4.73 as the cutoff value, with a corresponding PPV of 0.84 and an NPV of 0.86. Here, only 4 out of 35 dentinal caries lesions were misclassified as enamel and 6 out of 30 enamel caries lesions were misdiagnosed as dentinal caries. Furthermore, overall sensitivities and specificities of 85 and 90%, respectively, were achieved for discriminating sound teeth from enamel caries, whereas sensitivities and specificities of 100% were obtained to discriminate sound teeth from dentinal caries with both the FRRS scatter plots.
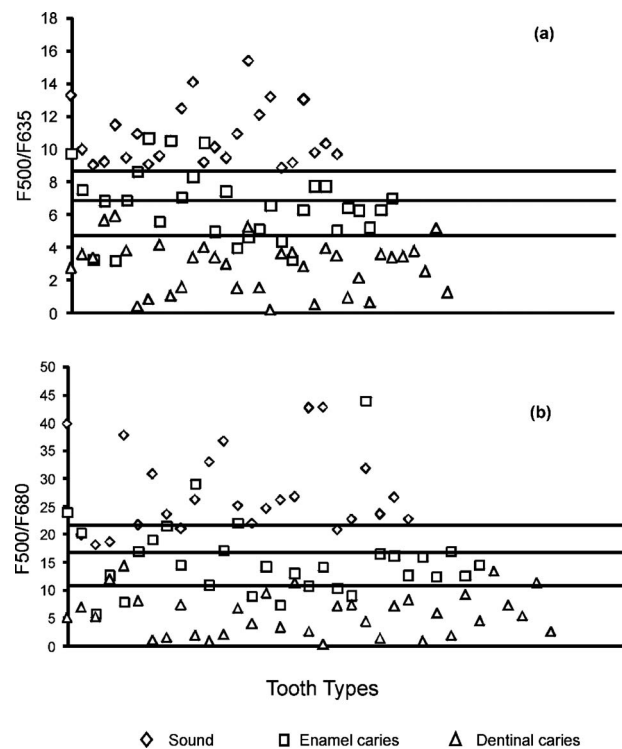
Fig. 5 FRRS scatter plots based on autofluorescence spectral intensity ratios (a) $F500/F635$ and (b) $F500/F680$ from 65 patients diagnosed as enamel caries and dentinal caries, along with the ratios from 25 sites of sound teeth.

Table 4 Independent and overall diagnostic accuracies of FRS ratios $F500/F635$ and $F500/F680$ obtained for classifying different stages of dental caries.

LIF ratios	Sound vs Enamel Caries (%)				Sound vs Dentinal Caries (%)				Enamel vs Dentinal Caries (%)			
	Se	Sp	PPV	NPV	Se	Sp	PPV	NPV	Se	Sp	PPV	NPV
$F500/F635$	87	100	1.00	0.86	100	100	1.00	1.00	89	80	0.84	0.86
$F500/F680$	83	80	0.83	0.80	100	100	1.00	1.00	86	73	0.79	0.82
Overall	85	90	0.92	0.83	100	100	1.00	1.00	88	77	0.82	0.84

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); and negative predictive value (NPV), true negative/(true negative+false negative).

4 Discussion

Currently visual inspection or its combination with radiography is considered as the gold standard for caries detection. However, x rays are ionizing and hazardous in nature and cannot detect caries until they are well advanced. Hence, the development of diagnostic algorithms based on LIF spectral ratios would be of great help for the real-time, noninvasive detection of dental caries in a clinical environment.

In this clinical trial, we observed significant differences in the autofluorescence spectra of sound and carious teeth. This disparity in fluorescence spectra is based on the absorption and scattering properties of light by cariogenic substances and fluorophores in the tooth. The normal tooth enamel is composed of millions of prisms or rods with waveguide properties that facilitate deep penetration when illuminated with visible light. In the case of dental caries, the prism structure is damaged and the waveguide properties are lost so that the irradiated light cannot penetrate deeply as with sound enamel. This leads to a reduction in the fluorescence intensity in caries lesion.[5]

Another reason for fluorescence spectral variations might be due to the import of exogenous fluorescent molecules during the caries process. This fact is clearly supported by the progressive rise of fluorescence spectral intensity in the red wavelength region with caries progression, and the consequent decrease in the autofluorescence emission around 500 nm (Fig. 2).

The autofluorescence emission spectrum of tissue is basically a convolution of the emission spectra of the endogenous fluorophores of tissue and therefore strongly depends on the wavelength of excitation light.[25] Only those endogenous fluorophores whose absorption bands have an overlap with the wavelength of excitation light are excited and emit fluorescence. Since the excitation and emission light must propagate through the turbid tissue, the recorded autofluorescence is also influenced by the absorption and scattering at both the excitation and the emission wavelengths. These endogenous fluorophores include the aminoacids, structural proteins such as collagen and elastin, coenzymes such as NADH and flavins, vitamins, lipids, and the porphyrins. Their excitation maxima lie between 250 and 450 nm, whereas their emission maxima lie[26] in the range of 280 and 700 nm.

Fluorophores that are speculated to play a major role in the caries processes are structural proteins such as collagen, porphyrins, and bacteria. Collagen and elastin are fibrous proteins and are abundant in connective tissues, teeth, and bones. Collagen forms the organic part of the dentin and any structural or pathologic association with caries processes could be reflected in lower autofluorescence intensity.[27] Therefore, these factors could be responsible for the similarity observed between the reference standards for classification of tooth caries and oral cavity cancer from tissue autofluorescence.[28]

The broad autofluorescence around 500 nm is due to emission from natural enamel[22] and peaks observed at 635 and 680 nm are due to emission from endogenous porphyrins and metalloporhyrins, in particular, protoporphyrin IX (PpIX), mesoporphyrin, and coproporphyrin synthesised by bacteria.[18,22] PpIX is reported to be higher in gram-negative oral bacteria and its concentration increases as the dental biofilm becomes more mature, which is responsible for the red fluorescence in teeth.[29] Likewise, Buchalla[21] reported that caries lesions fluoresce at 624, 650, and 690 nm due to the presence of porphyrins, and the fluorescence efficiency increases when excitation is in the wavelength range between 400 and 420 nm.

Significant differences in the FI ratios ($F500/F635$ and $F500/F680$) were observed during the caries process. The mean fluorescence spectral intensity ratios of sound teeth (Table 2) were always found to be higher as compared to carious teeth. The decrease in the FI ratio values point to the extent of teeth decay. Both these ratios that are used to classify the sound tooth from caries have very low independent Student's t test values, $p < 0.001$. Moreover, using the diagnostic algorithm developed based on the FRRS scatter plots, we obtained overall sensitivities of 85, 100, and 88%, respectively, for discriminating sound teeth from enamel caries, sound teeth from dentinal caries, and enamel caries from dentinal caries with corresponding specificities of 90, 100, and 77% (Table 4). In comparison, the FI scatter plots of fluorescence emission intensity at 500 nm gave sensitivities of only 83, 97, and 86%, respectively, for discriminating sound teeth from enamel caries, sound teeth from dentinal caries, and enamel from dentinal caries with corresponding specificities of 88, 88, and 73% (Table 3). The advantage of using scatter plot ratios over peak intensity is that it helps to eliminate errors associated with changes in excitation energy or incident light levels, collection efficiency, and the spectral response of the detection system.

In comparison, Pinelli et al.,[14] who reported a sensitivity of 72% and a specificity of 73% for detecting active and arrested

incipient caries lesions on smooth surfaces using DIAGNO-dent, proposed that it could be used as a good auxiliary method along with clinical examination. In another study, Shi et al.[9] reported a sensitivity of 94% and a specificity of 100% for detecting smooth surface caries with the QLF method, but achieved a lower sensitivity and specificity of 75 and 96%, respectively, using DIAGNOdent. Similarly, Lussi et al.[15] reported a sensitivity and specificity of 92 and 86%, respectively, for detecting occlusal dentin caries using the DIAG-NOdent device as compared to visual inspection and bitewing radiography. Furthermore, laser-based techniques are reported to be effective in caries diagnosis, as they combine the advantages of higher specificity and speed of visual inspection with the higher sensitivity.[15] Also, Burin et al.[30] assessed the efficiency of LIF, visual examination, and bitewing radiography and found that visual inspection was as valid as LIF, which should be considered a better adjunct than bitewing radiography for caries diagnosis.

This investigation shows that FRRS algorithms based on the autofluorescence intensity ratio of the emission peaks can localize and discriminate different stages of caries lesions. However, for a more reliable caries diagnosis, the combined use of visual inspection and LIFS is recommended, since the laser has the benefit of quantifying mineral content and helping dentists to improve diagnostic efficacy and treatment.

5 Conclusion

The results of this clinical study illustrate that information provided by noninvasive LIFS has excellent potential to discriminate different stages of dental caries. The FRRS diagnostic algorithm based on tissue autofluorescence spectral ratio was able to discriminate sound teeth from enamel caries, sound teeth from dentinal caries, and enamel from dentinal caries with sensitivities of 85, 100, and 88%, respectively, with ensuing specificities of 90, 100, and 77%. The study proves that LIFS could function as an auxiliary method to dentists for detecting and discriminating dental caries in a fast and sensitive manner. Our results confirm that classification of tooth caries from fluorescence signatures with 404-nm diode laser excitation enables precise visualization and quantification of both the intrinsic green fluorescence of dental hard tissues and the red fluorescence of bacterial origin.

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