Chapter 7

Characterization of Dental Caries by LIF Spectroscopy with 404 nm Excitation
7.1 INTRODUCTION

Despite our improved understanding of the caries process and the availability of effective intervention, caries lesions still progress to the stage where tooth structure is compromised and invasive intervention and restoration are required. Studies have shown that non-cavitated caries lesions are significantly more prevalent than cavitated caries lesions. This chapter reports the performance of the laser-induced fluorescence (LIF) spectroscopy in detecting dental caries and to classify between different stages of caries with excitation at 404 nm from a diode laser. Towards this, in vitro LIF spectra were recorded from 16 sound, 10 non-cavitated and 10 cavitated molar teeth. Autofluorescence spectral intensity of caries lesions were found lower than that of sound tooth and decreased with the extent of caries. The LIF spectra of caries tooth showed two peaks at 635 and 680 nm in addition to the broad band seen at 500 nm in sound tooth. The efficiency of using the fluorescence spectral intensity ratios in identifying caries lesions was studied and the results are presented.

7.2 STUDY MATERIAL AND PROTOCOL

Sound and caries tooth samples belonging to different categories were collected from a nearby dental clinic following extraction, for various reasons including periodontal problems and were transported to the laboratory in isotonic saline. Usually the samples were stored at room temperature (27 ± 3 °C) and measurements were carried out as soon as the samples reached the laboratory. The tooth samples were classified clinically, by an experienced dental practitioner, depending on the visual-tactile or radiographic examination before extraction from the patient. Clear enamel-intact tooth surfaces were considered as sound, while enamel caries with intact surface, with loss of luster, surface roughness, white spot and brown lesions were considered as non-cavitated lesions and those with visible cavitation, deeper than 3-4 mm were taken as cavitated lesion. The study samples included 16 sound, 10 non-cavitated and 10 cavitated molar teeth. Samples studied covered occlusal surface caries.

7.2.1 Experimental Methods

Before measurements, the samples were taken out of the PET bottles and washed
in running water. They were then cleared of food particles or blood clot using a toothbrush and water and then dried with tissue paper. Visual inspection was again carried out as per the protocol for tooth classification. During visual-tactile inspection, the samples were categorized based on characteristics such as tooth type, tooth discoloration, localized enamel breakdown, and cavitation in enamel exposing dentin or affecting the pulp.

Due to the diverse nature of the caries lesions, 12 sets of LIF measurements were taken from each selected area (6 mm dia.) and averaged for data analysis. The OOI Base32 software was configured to record the spectra, averaged for 40 scans, with boxcar width of 8 nm and an integration time of 50ms. Fluorescence intensity ratios are then determined from the emission peak intensity of the mean spectra for discriminating different stages of dental caries. To account for the broad nature of the peaks and sample-to-sample variation in peak position due to changes in chemical composition or fluorophore content of tooth, the mean LIF spectral intensity over an interval of ±10 nm at the emission peak was used to determine the LIF spectral ratios. ANOVA was performed to detect whether the average LIF spectral intensity differed between the groups belonging to sound, non-cavitated and cavitated caries lesions.

The ROC curve areas were then determined to classify different stages of caries. In ROC analysis, which is an excellent statistical approach for new techniques with numerical values, the sensitivities for detecting dental caries are plotted against values of 1 minus the specificity. The more precisely a technique separates the data classes, the closer would be the corresponding ROC-AUC to unity. In the present study, tooth without lesion were considered as sound, while tooth with loss of lustre, surface roughness and visible cavitation were considered as caries lesion. This allows one to make a fair judgment on the efficacy of methods without being constricted to a single value of sensitivity and specificity, which largely depends on the cut-off value chosen (Metz, 1978).

7.3 RESULTS

7.3.1 LIF spectral features

Fluorescence spectra of dental hard tissues vary due to alteration in the chemical composition of pathological areas and tissue optical properties. Figure 7.1 shows the averaged fluorescence spectra recorded from 16 sound, 10 non-cavitated and 10 cavitated tooth samples, normalized to the maximum intensity around 500 nm. The
overall fluorescence intensity of caries lesions were found lower than that of sound tooth and decreased with the extent of caries. However, the fluorescence spectral intensity in the red wavelength region was greater for carious when compared to sound regions. The LIF spectrum of sound tooth shows a broad emission around 500 nm with a long tail extending towards the red region. In non-cavitated and cavitated caries lesions, two additional peaks were seen at 635 and 680 nm. In cavitated caries lesion, the 500 nm band appears broadened and red-shifted by about 10-15 nm.

![Graph](image.png)

**Fig. 7.1.** Mean LIF spectra from sound and caries lesions belonging to different stages, normalized to fluorescence peak intensity. Sound spectra represent the mean of 12 measurements each in 16 samples, whereas the spectrum from non-cavitated and cavitated caries lesions is the mean of 12 measurements in 10 samples.

### 7.3.2 LIF intensity ratios

Mean LIF spectral intensity ratios (F500/F635 and F500/F680) determined from
sound and caries tooth samples of diverse categories are shown in Table 7.1. It is observed that both fluorescence intensity ratios calculated from caries tooth are always lower than those of sound tooth. The F500/F680 ratio shows maximum variance of 30% between sound and non-cavitated caries lesions and 93% between sound and cavitated caries lesions, whereas between non-cavitated and cavitated caries lesions, both fluorescence ratios show a variation of 89%.

One way ANOVA test was also used to determine the variations in average spectral intensity among each groups. Statistically significant differences in mean spectral intensities were noticed at 99% confidence interval (p <0.001) between sound, non-cavitated and cavitated caries lesions.

Table 7.1 Mean laser-induced fluorescence spectral ratios from sound and carious tooth belonging to different categories.

<table>
<thead>
<tr>
<th>Tooth types</th>
<th>Specimen No.</th>
<th>F500/F635 (%)</th>
<th>F500/F680 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound tooth</td>
<td>192</td>
<td>12.6 ± 1.8</td>
<td>34.4 ± 4.4</td>
</tr>
<tr>
<td>Non-cavitated caries</td>
<td>120</td>
<td>10.3 ± 2.8 (19)</td>
<td>24.0 ± 5.7 (30)</td>
</tr>
<tr>
<td>Cavitated caries</td>
<td>120</td>
<td>1.1 ± 0.6 (91)</td>
<td>2.4 ± 0.9 (93)</td>
</tr>
</tbody>
</table>

The percentage given in parentheses denotes the variation with respect to the sound tooth.

7.3.3 Diagnostic performance of LIF spectroscopy

ROC curve analysis was used to check the diagnostic accuracy of discriminating diseased from normal cases. In general, an area under the ROC curve close to 0.5 signifies the failure of the method used, whereas an area >0.9 indicates good discrimination, demonstrating excellent separation between the two classes. In the present study, high values were observed for the area under ROC curves with the LIF spectroscopic method to detect dental caries and to classify different stages of caries. The results for distinguishing sound tooth from cavitated and non-cavitated lesions, and between cavitated and non-cavitated lesions are shown in Table 7.2. Characterization was most successful for discriminating caries lesions from sound tooth (ROC-AUC: 0.94 ± 0.01 for F500/F635 and F500/F680). The maximum ROC-AUC value of 1.00 was observed for both ratios between sound and cavitated caries lesions. The ROC-AUCs obtained for discriminating sound from non-cavitated caries lesions were 0.84 and 0.89 for F500/F635 and F500/F680 ratios, respectively. Further, an ROC-AUC of 0.84 was obtained for distinguishing non-cavitated and cavitated caries lesions. Among these two ratios,
the F500/F680 was more suited to discriminate all caries lesions from sound tooth (ROC-AUC: 0.94). These studies suggest that information on the stage of caries could be extracted from autofluorescence characteristics of cavitated and non-cavitated lesions.

### Table 7.2 Area under the ROC curve for discriminating different types of caries lesions using fluorescence intensity ratios.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>F500/F635</th>
<th>F500/F680</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound versus caries</td>
<td>0.93</td>
<td>0.95</td>
</tr>
<tr>
<td>Sound versus non-cavitated lesions</td>
<td>0.84</td>
<td>0.89</td>
</tr>
<tr>
<td>Sound versus cavitated lesions</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Non-cavitated versus cavitated lesions</td>
<td>0.84</td>
<td>0.84</td>
</tr>
</tbody>
</table>

### 7.4 DISCUSSION

Dental caries is a chronic, endemic disease. The chronic nature of the disease is manifested in slow lesion progression, and this offers a window of opportunity for intervention, to reverse mineral loss or at least arrest lesion progression, before the development of irreversible damage to the dental hard tissues. Most of the optical techniques are based on the differences in fluorescence between sound tooth enamel and caries lesion.

In this study, significant variation was observed between the fluorescence spectra of sound tooth and that of different caries tooth (Fig. 7.1). The caries tooth always exhibit lower fluorescence intensity than sound tooth. This could be attributed to the changes in the fluorophore content and to the absorption and scattering properties of the caries layer. Normally, tooth enamel is composed of millions of prisms or rods with waveguide properties that facilitate deep penetration when illuminated with light. In case of dental caries, the prism structure is damaged and the waveguide properties are lost so that the irradiated light cannot penetrate deeply. This leads to a reduction in the fluorescence intensity of caries lesion (Ando et al, 2001). Changes seen in the fluorescence spectrum could also be attributed to the changes in the physical structure and chemical composition during the disintegration of tooth enamel leading to caries formation or due to the import of exogenous molecules during the caries process. This clearly supports the progressive rise of fluorescence spectral intensity in the red wavelength region during caries progression, with concomitant decrease in the
Another possible explanation for the changes in fluorescence lies with the variation in light scattering. When tooth is illuminated by blue light, it gets absorbed by the chromophores in the tooth. In dental caries, the light propagation directions are different as compared to sound tooth so that more fluorescent photons are emitted in sound tooth than in caries lesion (Ando et al, 2001). Konig et al (1999) also observed that carious lesions exhibited slower fluorescence decay than intact sound tissue. The long-lived fluorophore present in carious lesions only emitted in the red spectral region.

With 404 nm laser excitation, a broad autofluorescence emission was observed around 500 nm in sound tooth from natural enamel (Konig et al, 1998). Also emissions at 635 and 680 nm from endogenous porphyrins, particularly protoporphyrin IX (PpIX), meso-porphyrin and coproporphrin in bacteria were observed (Hibst et al, 2001). PpIX concentration is reported to be higher in Gram-negative oral bacteria and its level increases as the dental biofilm becomes more mature, which is responsible for the red fluorescence in teeth (Walsh and Shakibaie, 2007).

When excited with 366 nm UV light, caries tooth was shown to exhibit a brick red fluorescence, which is due to protoporphyrin production by the pigmented Bacteroides species (Brazier, 1986; Konig et al, 1993; Bissonnette et al, 1998), and coproporphyrin production by Corynebacterium species and Candida albicans (Konig et al, 1993). With 407 nm UV light, bacterial species such as Actinomyces odontolytics (found in dentin carious lesions), Bacteroides intermedius, Prevotella intermedia, Corynebacterium species and Candida albicans emit fluorescence at 620-635 nm and 700nm, whereas Gram-positive Streptococcus mutans, Enterococcus faecalis and various lactobacilli showed weaker emissions in the red wavelength region. Thus the maturity of dental plaque, rather than the presence of cariogenic streptococci, forms the basis for red fluorescence when excited with near-UV or UV light. In summary, when dental plaque or calculus is present, there is an increase in absorption in the UV spectral region of 350-420 nm, with fluorescence emission in the red spectral region of 590-650 nm (Borisova et al, 2006; Kuhnisch et al, 2003).

Significant differences in the fluorescence intensity ratios (F500/F635 and F500/F680) were observed during caries process. As compared to the mean fluorescence spectral intensity ratios of sound tooth given in Table 7.1, the caries tooth belonging to different stages has lower ratios. The F500/F680 ratio shows maximum sensitivity to distinguish early caries formation. Both the fluorescence intensity ratios used to
Shi et al (2001) has reported a sensitivity of 94% and a specificity of 100% for detecting smooth surface caries with the QLF method, but could achieve only a lower sensitivity (78-82%) for detecting occlusal caries using DIAGNOdent device (Shi et al, 2000). In this study, the in vitro diagnostic accuracy of DIAGNOdent measurements in terms of area under the ROC curve was significantly higher (0.96) than that of conventional radiography (0.66) (Shi et al, 2000). However, Lussi et al (2001) reported a sensitivity of 92% for detecting occlusal caries using DIAGNOdent device as compared to visual inspection and bite-wing radiography. In another study, Ferreira-Zandona et al (1998) observed a sensitivity of 49% and specificity of 67%, with ROC value of 0.78, for detecting demineralization in occlusal pits and fissures. Further, Angnes et al (2005) observed that visual inspection was the most valid method for caries diagnosis followed by laser-induced fluorescence, in terms of ROC-AUC, sensitivity and specificity. Using a 405 nm diode laser, Ribeiro et al (2005) reported significant differences in the spectral ratios of integrated fluorescence in the wavelength region between 480-500 nm and 620-640 nm for sound tooth and all smooth surface non-cavitated caries with p<0.001. However with the same laser, Zezell et al (2007) observed fluorescence from natural and cut surfaces of caries lesions to be nearly the same for shiny and dull lesion, but different for brown lesion (p<0.05).

Attrill and Ashley (2001) found that laser fluorescence was more accurate for detecting occlusal caries as compared to visual examination and intraoral radiography. In another study by Olmez et al (2006) observed that for occlusal caries detection, LIF shows higher sensitivity and specificity as compared to visual inspection and bitewing radiography. However, Burin et al (2005) 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 study shows comparatively better sensitivity in discriminating caries lesions from sound tooth using LIF spectral signatures (mean ROC-AUC= 0.94 ± 0.01). However, for detecting non-cavitated caries lesion (early enamel caries) the ROC-AUC was slightly lower at 0.87 (Table 7.2).

Diagnostic algorithms based on the endogenous porphyrins produced by oral bacteria have been successfully brought out in this study. We have demonstrated...
that by using the LIF spectral ratios, it is possible to characterize both lesion types and more significantly, discriminate caries from sound tooth. Moreover, it confirms the previous result that sound and caries tooth can be discriminated through the use of LIF spectroscopy (Borisova et al, 2004; Ando et al, 2001; Subhash et al, 2005 and Ribeiro et al, 2005).

### 7.5 CONCLUSIONS

Early diagnosis of caries lesion provides for more efficient remedy for caries progression, avoiding operative treatment. It appears that the information presented by non-invasive LIF spectroscopy has the capability to successfully detect dental caries and to classify different stages of caries. Among the LIF ratios studied, F500/F680 ratio is found to be more suited to comprehend caries progression and also to discriminate sound tooth from caries. It is observed that the fluorescence spectral signatures vary with respect to the changes in endogenous fluorophores during tooth caries development and therefore allow accurate diagnosis of the stage of dental caries from LIF spectral intensity ratios. Further in vivo studies are necessary with LIF to test the efficacy of these ratios to diagnose caries in a clinical setting.