Chapter 9

Application of Curve-fitting to Diagnose Dental Caries *in vivo*
9.1 INTRODUCTION

This section discusses the advantage of analyzing the LIF spectra by curve-fitting for distinguishing different stages of tooth caries. Towards this, LIF emission spectra were recorded in the 400-800 nm spectral range on a miniature fiber-optic spectrometer from 105 patients, with excitation at 404 nm from a diode laser. The spectral results were correlated with visual-tactile examinations. It was noticed that caries tooth have lower fluorescence intensities as compared to sound tooth and the intensity decreases with the progression of caries. The LIF emission of sound tooth shows a broad emission around 500 nm whereas two additional peaks are seen at 635 and 680 nm in carious tooth. In order to locate the exact peak positions of the constituent bands and their relative contributions in the overall spectrum, LIF spectra were analyzed by curve-fitting using Gaussian spectral functions. Thus, it was possible to determine the variance in curve-fitting parameters such as peak center, Gaussian curve area, peak amplitude, FWHM and their ratios for different stages of tooth caries. A comparative evaluation of these ratios with those derived from raw LIF spectral data was made and the results are presented. Further, the diagnostic performance of LIF spectroscopy in a clinical setting was evaluated in terms of receiver operating characteristic (ROC) curve.

9.2 STUDY MATERIAL, PROTOCOL AND DATA PROCESSING

The study population, ethical issues and acquisition parameters were mentioned earlier (Chapter 8). The recorded LIF spectra shows a sharp rising edge in the short wavelength side (400-450 nm), which is due to the usage of the 420 nm long-wavelength pass filter (Schott UG420) for blocking the back-scattered laser light from entering the spectrometer. In order to correct the influence of this filter, the recorded LIF spectra is corrected using the spectral transmittance of the filter in the 350-800 nm wavelength region (Fig.9.1). Fig. 9.2
represents the in vivo LIF spectra recorded with the UG420 filter and the corrected fluorescence spectra of sound tooth.

**Fig. 9.2** Mean in vivo LIF spectra of a sound tooth and the corresponding filter transmittance corrected spectra.

The corrected LIF spectra were analyzed by curve-fitting using Gaussian spectral functions to determine the peak positions of the constituent bands and their relative contribution in the overall spectrum using Origin software (details given in Chapter 3). Various band intensity ratios were then determined from the constituent peak amplitudes and Gaussian curve areas and correlated with those derived from raw spectral data. An independent Student's t-test was performed on the Gaussian amplitude and area ratios, F490/F635 and F490/F675, to assess the statistical significance of the fluorescence ratios in discriminating different stages of tooth caries.
The diagnostic performance achieved by curve-fitting was determined in terms of ROC curve. The resultant ROC curve displays variation in sensitivity and specificity along the diagnostic range. The use of gold standard is a prerequisite for the assessment of ROC curve.

**9.3 RESULTS**

**9.3.1 LIF spectral features**

Fig. 9.3a) shows the corrected LIF spectra recorded from sound tooth, enamel caries and dentinal caries and Fig. 9.3b) that after normalization to the peak intensity. As compared to sound tooth, caries tooth exhibit lower fluorescence intensity. The LIF spectrum of sound tooth consists of a broad auto-fluorescence around 500 nm with a long tail extending towards the red region possibly due to an emission peak around 550 nm. But, in caries tooth, two additional peaks are seen at 635 and 680 nm. In addition, caries tooth exhibit an apparent red shift in the emission maxima.

**9.3.2 Curve-fitting analysis**

The mean LIF spectra from sound, enamel and dentinal caries tooth were analyzed by curve-fitting using Gaussian spectral functions. Fig. 9.4 (a-c) shows the peak fitted spectrum and the constituent emission bands. It was seen that curve fitting of sound tooth with the help of three Gaussian
peaks gives a good fit (correlation coefficient \( r^2 = 0.998 \)). In the case of carious tooth, five Gaussian peaks were required to give a good fit of the mean LIF spectra. Table 9.1 shows the peak position of the various bands, their Gaussian curve areas, full width at half intensity maximum (FWHM) and the \( \chi^2 \) and \( r^2 \) values of fitting. It is seen that the 491.85 nm peak shifts towards the red region by 14 nm in enamel and 15 nm in dentinal caries. Further this peak appears broadened with a shoulder peak around 513.21 nm, which shifts towards the red region by 19 nm to 532 in enamel caries and by 38 nm to 551.75 nm in dentinal caries. Another notable feature is the broadening of the 560 nm peak by 12 nm; with concurrent decrease in Gaussian curve area and peak amplitude in dentinal caries. Besides, the 625.8 nm and 655.62 nm peaks seen in enamel caries shift towards the red region by 7.3 nm and 19.1 nm in dentinal caries. For the 625.8 nm and 655.62 nm peaks seen in enamel caries, the curve fitted amplitude and area values show an increasing trend with increase in the stage of caries. During caries progression from enamel to dentinal stage, the increase in the 625.8 nm peak amplitude and area is 60% and 20%, while the corresponding increase in the 655.62 nm peak amplitude is 48% and 29%, respectively. However, for sake of simplicity these peaks will be designated as 490, 513, 560, 635 and 675 nm peaks.

### 9.3.3 Curve-fitted and Raw LIF ratios

Table 9.2 shows the fluorescence ratios (F490/F635 and F490/F675) calculated
from curve-fitted peak amplitudes, Gaussian curve areas and raw spectral data for different stages of caries. The fluorescence intensity of the LIF spectra at the peak wavelength determined by curve-fitting was used to evaluate the raw fluorescence intensity ratio. As compared to the ratios determined from raw spectral data, the fluorescence ratios calculated from Gaussian area curves and curve-fitted amplitudes show increased variation with the extend of caries. For example, the raw LIF spectral ratio F490/F635 involving the two main peaks, which has a variation of 60% between enamel and dentinal stage of caries, showed an enhanced variation of 81.2% when curve-fitted peak amplitude values are used for determination of this ratio. Similarly,
the curve-fitted amplitude and area F490/F675 ratios showed variances of 75.6% and 65.6% during caries progression from enamel to dentin as compared to 60% change noted in the raw F490/F675 ratio. Fig. 9.5(a-c) shows the fluorescence ratios derived from curve-fitted spectral amplitude, curve-fitted spectral area and raw LIF spectral data for these two stages of caries.

Table 9.2 In vivo LIF ratios determined from Gaussian curve-fitted amplitude, area and raw spectral data.

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Curve-fitted amplitude</th>
<th>Curve-fitted area</th>
<th>Raw LIF spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enamel caries</td>
<td>Dentinal caries</td>
<td>Enamel caries</td>
</tr>
<tr>
<td>F490/F635</td>
<td>8.41</td>
<td>1.58</td>
<td>15.33</td>
</tr>
<tr>
<td>F490/F675</td>
<td>9.1</td>
<td>2.22</td>
<td>5.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.59±1.08</td>
</tr>
</tbody>
</table>

9.3.4 Diagnostic performance of LIF spectroscopy

The ability of LIF spectroscopy to discriminate different types of caries lesions from sound tooth can be assessed from the ROC curve, where the sensitivities are plotted against the 1-specificities as in Fig.9.6 a-c. In general, a value for the area under ROC curve close to 0.9 indicates good discrimination between the two classes studied. The receiver-operator characteristic areas under the curve (ROC-AUCs) was 1.0 (p<0.001) for discriminating sound from dentinal caries and 0.97 for distinguishing sound from enamel caries (Table 9.3). For the distinction between enamel and dentinal caries, the ROC-AUC was 0.86.

9.4 DISCUSSION

The broad autofluorescence peak around 500 nm in sound tooth is due to emission from natural enamel, particularly hydroxyapatite (Alfano and Yao, 1981; Konig et al, 1998) and emissions at 635 and 680 nm are from endogenous porphyrins, particularly protoporphyrin IX (PpIX), meso-porphyrin and copro-porphyrin in bacteria (Konig et al, 1998; Hibst et al, 2001 and Subhash et al, 2005).

The mean LIF spectra from sound and caries tooth shows characteristic features based on the absorption and scattering properties of light by carious substances and differences in the content of fluorophores. As reported earlier (Ando et al, 2001; Borisova et al, 2004 and Subhash et al, 2005), the caries tooth exhibits lower fluorescence intensities than sound tooth. The reduction in the fluorescence intensity of the caries
lesion could be attributed to the changes in the physical structure and chemical composition during the disintegration of tooth enamel leading to caries or due to import of exogenous molecules during the caries process. This clearly supports the progressive rise of the fluorescence spectral intensity in the red wavelength region and decrease around 500 nm with the caries progression (Borisova et al, 2006). Moreover, porphyrin peaks that are prominent in caries tooth can also be used for discriminate different stages of caries.

Further, by curve-fitting using Gaussian spectral functions, it was possible to resolve the LIF spectra of sound tooth into three constituent peaks centered at 491.85, 513.21 and 561.37 nm whereas two additional peaks were required, respectively at 625.8 and 655.62 nm and at 633.05 and 674.67 nm in enamel and dentinal caries, to fit the spectral data. Since the correlation coefficients of curve-fitting were very close to unity, and the residuals of fitting were few and scattered uniformly over the fitted curve, it is to be assumed that the peak wavelength positions identified by the curve-fitting algorithm are fairly accurate. There are marked variations not only in the peak emission bands but also in the peak amplitude, FWHM width, and the Gaussian curve area during caries development. The variation in peak position, intensity and FWHM width of the 490 nm band is noteworthy. Therefore, shift in peak position of the 490 nm peak between enamel and dentinal caries, and the appearance of the new peak at 635 and 675 nm would be useful indicators of the extent or stage of tooth caries. In comparison, in an earlier study using 337

![Fig. 9.5 Fluorescence ratios derived from a) curve-fitted spectral amplitude, b) curve-fitted spectral area and c) raw LIF spectral data from different stages of caries lesions.](image-url)
nm nitrogen laser, it was found that the curve-fitting of the sound tooth shows four peaks centered at 403.80, 434.20, 486.88, and 522.45 nm. Further, the 522 nm peak is seen red-shifted by 32 and 8 nm in dentin and pulp level caries. A new peak at 636.78 nm was also seen in the case of pulp level caries (Subhash et al, 2005).

Significant differences in the Gaussian amplitude and area ratios (F490/F635 and F490/F675) were observed during caries progression (Table 9.2). As compared to ratios derived from raw LIF spectra, marginal increase in sensitivities was seen with curve-fitting. Among the various ratios, the curve-fitted Gaussian peak amplitude ratio,

Fig.9.6 Receiver operating characteristics curves (ROC) for discriminating different stages of caries lesions using LIF spectroscopy a) the discrimination cut-off between sound and enamel caries, b) the discrimination cut-off between sound and dentinal caries and c) the discrimination cut-off between enamel and dentinal enamel and dentinal caries lesions.
F490/F635 appears to be more suited to distinguish different stages of tooth caries. All these fluorescence intensity ratios used to classify the sound from caries tooth had a low independent Student t-test value, p<0.001. In contrast, in an earlier study, curve-fitted Gaussian area ratio F435/F525 was reported to be suitable for discrimination between different stages of tooth decay (Subhash et al, 2005). Using a 405 nm diode laser excitation, Ribeiro et al (2005) also reported significant differences in the spectral ratios of the integrated fluorescence in wavelength region between 480-500 nm and 620-640 nm for sound tooth and all smooth surface non-cavitated caries (p<0.001).

*Table 9.3 Results of ROC analysis in the discrimination of enamel and dentinal caries from sound tooth.*

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Receiver Operating Characteristics Curve</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>AUC</td>
<td>95% CI</td>
<td>p</td>
</tr>
<tr>
<td>Sound - Enamel caries</td>
<td>0.97</td>
<td>0.92-1.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sound – Dentinal caries</td>
<td>1.0</td>
<td>1.0-1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Enamel– Dentinal caries</td>
<td>0.86</td>
<td>0.75-0.96</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CI: Confidence Interval; p: Significance of difference to the diagonals; AUC: Area under the Curve

Shi et al (2000) found that the *in vitro* diagnostic accuracy of DIAGNOdent measurements in terms of area under the ROC curve was significantly higher (0.96) than for conventional radiography (0.66). In an *in vitro* 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. Angnes et al (2005) reported that that visual inspection was the most valid method for caries diagnosis followed by laser fluorescence in terms of ROC-AUC, sensitivity and specificity. In a recent study by Huth et al (2008) it was observed that the LF device’s discrimination performance for different caries depths was moderate to good with AUC of 0.92 for discrimination between sound and carious tooth and 0.78 for discrimination between enamel and dentin caries. In another study, Olmez et al (2006) observed that LF shows higher sensitivity and specificity as compared to visual inspection and bitewing radiography for occlusal caries detection. However, Burin et al (2005) assessed the efficiency of LF, visual examination and bitewing radiography and found that visual inspection was as valid as LF device, which should be considered as a better adjunct than bitewing radiography for caries diagnosis.
In comparison, the results of this in vivo study showed that the diagnostic performance of LIF spectroscopy in discriminating different stages of caries can be improved with curve-fitting using Gaussian spectral functions. Further, it was possible to discriminate dentinal caries from sound tooth with an AUC of 1.0, while the AUC’s were 0.97 and 0.86, respectively for discrimination between enamel caries and sound tooth, and between enamel and dentinal caries.

The benefit of ROC analysis is that it displays the diagnostic performance more systematically than sensitivity and specificity, which depends on the cut-off point. This analysis also provides an overall validity for the methods employed. From a mathematical point of view, the ROC analysis gives a better picture, since it does not consider any given threshold. However, in clinical practice, clinicians usually have to consider a cut-off point at which the treatment options fall from non-invasive to invasive approach. Therefore, the sensitivity and specificity values are still valuable tools for comparison of diagnostic methods.

**9.5 CONCLUSIONS**

LIF ratios showed significant changes depending on the nature and extent of caries and the detection capability was enhanced when contributions from constituent bands derived by curve-fitting of the LIF spectra using Gaussian spectral functions were considered. Further, it can be presumed that LIF with excitation by a 404 nm diode laser has the potential to diagnose different stages of caries. Studies have shown that tooth decay or caries not only affect the fluorescence spectral intensities, but also alter the spectral shape as evidenced by the appearance of new peaks, peak shifts, and variations in curve-fitted peak area, intensity and bandwidth. Moreover, the ratios determined from curve-fitted spectral parameters showed better sensitivity to tissue transformations as compared to the ratios derived from raw spectral data. Among the two ratios studied, F490/F635 ratio determined from curve fitted amplitude was found to be more suited to understand caries progression and to discriminate sound from caries tooth. The LIF spectroscopy’s diagnostic performance for detecting and discriminating different stages of tooth caries as evidenced by ROC analysis indicates that it can be used as an adjunct tool in the diagnosis of dental caries.