Preface

This study is a multidisciplinary research intended to contribute to improved management of dental caries. One of the significant aspects in dental caries diagnosis is that if early changes are not detected, lesion would continue to demineralise leading to cavity formation. Once cavitation occurs, the lost tooth structure cannot be regenerated. Further, tooth demineralization is difficult to diagnose in the early stages of development with the existing detection methods. Therefore, the main focus of the study is to explore the potential of laser-induced fluorescence (LIF) and diffuse reflectance (DR) spectroscopy techniques to identify incipient changes in tooth enamel, which is crucial for decisions on treatment modalities in operative dentistry.

Chapter 1 gives a brief insight into dental caries and demineralization that leads to mineral loss in tooth, current methods of detection and their limitations in a clinical setting, and also on disease management. It is intended as a brief survey of the tools that are available to the dentists for diagnosis and should not be considered as a comprehensive review. Finally, the significance of early detection of dental caries and the need to develop new techniques for detecting early changes in tooth enamel are presented.

Basic knowledge on the development and structure of the teeth is essential to understand the various diseases affecting teeth as well as for the exploitation of optical techniques for diagnostic applications. In addition, a practical understanding of the biologic processes of tissue and the physical properties of light would help to comprehend and control the outcome of its interaction for the detection of dental caries. Chapter 2 details the basic anatomy of the tooth and its interaction with light, with special emphasis on the basic concepts of tissue fluorescence and diffuse reflectance, which form the basis of the work presented in this thesis. In addition, different types of endogenous fluorophores and their absorption and emission characteristics are also described in this chapter.

In the past decade, key technologies such as (a) compact lasers, (b) CCD detectors and (c) easy-to-use computing platforms combined with fiber-optic coupled instrumentation has lead to the development of many photonics based diagnostic and therapeutic methods in dentistry. The use of optical spectroscopy in dentistry is crucial for early detection of dental caries, to carry out more effective but, minimally-invasive targeted-therapies and to restore diseased tissues functionally.
and aesthetically. Among the various non-invasive optical techniques, those relying on tooth autofluorescence and diffuse reflectance are most promising in the diagnosis of dental caries. **Chapter 3** presents details of a compact, non-invasive, laser-induced fluorescence and reflectance spectroscopic system (LIFRS) developed for detection and point monitoring of caries progression. Details on data acquisition using LIFRS and the various statistical methods adopted for data analysis are also given in this chapter. Further, this chapter describes the ethical issues, the protocol adopted for clinical studies, and patient inclusion/exclusion criteria.

**Chapter 4** examines the potential of LIFRS system for distinguishing different stages of caries. Towards this, nitrogen laser (337.1 nm) excited fluorescence and white light illuminated DR spectra of extracted tooth samples belonging to different categories were measured. The caries tooth showed lower fluorescence and reflectance intensities in the 350 to 700 nm region as compared to sound tooth. The LIF spectra were analyzed by curve fitting to determine the peak position of the various bands present and their relative contribution to the overall spectra. The deconvoluted peaks in the LIF spectra were found centered at 403.8, 434.2, 486.9 and 522.5 nm in sound tooth, whereas a new peak was observed at 636.8 nm in pulp level caries. Curve-fitted parameters such as peak center, Gaussian curve area and full width at half intensity maximum (FWHM) and their ratios were found to vary with the stage of tooth caries. The intensity and Gaussian curve area ratios of the peaks at 405, 435 and 490 nm were found to be sensitive to discriminate between sound, dentin and pulp level caries. Among the diffuse reflectance spectral ratios studied, the R500/R700 was found to be most sensitive to distinguish between pulp and dentin level caries. The LIF measurement with spectral analysis done by curve fitting outscores DR spectroscopy and shows potential to screen different levels of tooth decay in a clinical setting.

**Chapter 5** explores the application of tissue fluorescence and DR to detect tooth demineralization and evaluates their applicability in a clinical setting. The LIFRS system was used to measure LIF and DR spectra from *in vitro* premolar tooth during various stages of artificial erosion. It was observed that both LIF and DR spectral intensity increases gradually during tooth erosion. With curve fitting carried out using Gaussian spectral functions, broad-bands seen at 440 and 490 nm in sectioned sound enamel were resolved into four peaks centered at 409.1, 438.1, 492.4 and 523.1 nm, whereas in sound dentin slices the peaks were observed at 412.0, 440.1, 487.8 and 523.4 nm. The fluorescence spectral ratio, F410/F525, derived from curve-fitted Gaussian peak amplitudes and curve areas were found to
be more sensitive to erosion as compared to the DR ratio R500/R700 and the raw LIF spectral ratio F440/F490.

Further, in Chapter 6, the results of a study conducted to compare the capability of LIF and diffuse reflectance (DR) spectral data to detect de- and re-mineralization changes on in vitro tooth samples are presented. Towards this, nitrogen laser-induced fluorescence and tungsten halogen lamp-induced DR spectra were recorded on a miniature fiber-optic spectrometer from a set of premolar tooth samples subjected to cyclic de- and re-mineralization (CDR) for 10 days, followed by continuous remineralization (CR) for 14 days to enhance the effect of remineralization. The LIF and DR spectral intensities were found to decrease with CDR, but get reversed during CR. Significant differences (p <0.05) were noticed in spectral features between sound, demineralized and remineralized tooth with one-way ANOVA. The constituent peaks in sound tooth LIF spectra deconvoluted by curve fitting were found centered at 411.32, 440.08, 484.37 and 521.98 nm. Spectral features like peak center, full width at half intensity maximum (FWHM), Gaussian amplitude and curve area derived by curve fitting were found to vary with de- and re-mineralization. However, the characteristics of LIF peaks at 410 and 525 nm were found to be more suited for detecting tooth mineralization changes as compared to the raw LIF and DR spectral signatures.

Chapter 7 explains the potential of fluorescence spectroscopy (LIF) to characterize different stages of dental caries with 404 nm diode laser excitation. In vitro spectra were recorded on a miniature fibre-optic spectrometer from 16 sound, 10 non-cavitated and 10 cavitated molar teeth. The area under curve of the receiver operating characteristics (ROC-AUCs) and one way variance analysis (ANOVA) were calculated. Autofluorescence spectral intensity of carious 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 a broad band seen at 500 nm in sound tooth. It was observed that fluorescence intensity ratios, F500/F635 and F500/F680, of caries tooth are always lower than that of sound tooth. The ROC-AUC for discriminating caries from sound tooth was 0.94, whereas for distinguishing non-cavitated lesions the ROC-AUC was 0.87. Statistically significant differences (p <0.001) were seen between sound, non-cavitated and cavitated caries lesions. These results show that LIF spectroscopy could be utilized for characterizing different stages of caries in a clinical setting.

Chapter 8 examines the clinical applicability of a diagnostic algorithm or the fluorescence reference standard (FRS) developed based on LIF spectral ratios
to discriminate different stages of 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 and radiographic examinations. The LIF emission of sound tooth shows a broad emission at 500 nm that is characteristic of natural enamel whereas in carious tooth, additional peaks were seen at 635 and 680 nm, due to emission from porphyrins linked to oral bacteria. In order to discriminate different stages of tooth caries, FRS ratio scatter plots of the fluorescence intensity ratios F500/F635 and F500/F680 were developed to differentiate sound from incipient, sound from advanced and incipient from advanced caries using the spectral data obtained from 65 carious sites and 25 sites of sound tooth in 65 patients. The sensitivity, specificity, PPV and NPV of the developed algorithm to detect tooth caries were calculated and presented. Sequentially, a blind-test was carried out in 15 sound and 40 carious sites of 40 patients to check the accuracy of the developed standard for early detection of tooth caries.

Chapter 9 presents the application of LIF spectral ratios and curve-fitting for distinguishing different stages of tooth caries in a clinical setting with 404 nm excitation. The LIF spectra show a broad emission around 500 nm for sound tooth, whereas additional peaks were seen at 635 and 680 nm in carious tooth. Curve-fitted parameters such as peak center, peak amplitude, Gaussian curve area and FWHM were found vary with the different stages of tooth caries. Fluorescence intensity ratios, F490/F635 and F490/F675, derived from the raw spectral intensities, curve-fitted peak amplitudes and Gaussian curve areas were higher for sound tooth as compared to caries lesions and tend to decrease with the progression of caries. The Gaussian curve ratios, F490/F635 and F490/F675 were found to be more sensitive for discriminating different stages of caries as compared to raw LIF ratios. Finally, the diagnostic performance of LIF spectroscopy in a clinical setting was evaluated in terms of receiver operating characteristic (ROC) curves.

The potential of DR spectroscopy for detecting tooth caries in vivo are presented in Chapter 10. A clinical study conducted on patients has shown that in vivo DR spectral intensity decreases in caries tooth. Diffuse reflectance reference standard (DRRS) scatter plots of the DR ratios R500/R700, R600/R700 and R650/R700 were developed to differentiate sound from caries tooth using spectral data from 24 patients. The sensitivity, specificity, PPV and NPV of these DRRS ratios to detect tooth caries are calculated and presented. The diagnostic performance of DR spectroscopy was also evaluated in terms of receiver operating characteristic (ROC)
curve. Among the various ratios studied, R600/R700 ratio gave comparatively higher sensitivity and specificity. In this study, DR ratios were able to discriminate sound from non-cavitated caries lesions with an average sensitivity of 88% and specificity of 100%.

**Chapter 11** is the wrapping up section, which discusses the merits of the LIFRS system and this doctoral thesis, its future perspectives in the detection of dental caries and the limitations of the optical spectroscopy techniques utilized in this study. This section also reviews the diagnostic accuracies of LIF and DR modalities by comparing the present results with those obtained by other research groups using optical techniques for early detection of caries lesions.

As stated above, the common thread in the studies presented is the use of optical spectroscopy to detect tissue transformations. A fiber-optic LIFRS system was developed in our laboratory to perform autofluorescence and diffuse reflectance measurements. It has therefore been the fundamental device in the course of this work. Its flexibility allowed us to sequentially probe the fluorescence and diffuse reflectance spectra from same sample in real-time. The instrument sensitivity allowed us to detect very faint autofluorescence signals of biological tissues and the fact that the unit was fabricated in-house allowed us to suitably adapt and modify it whenever necessary.