Chapter 9

Conclusions and future perspectives

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The goal of this thesis was to use diffuse reflectance and fluorescence spectroscopy to non-invasively and quantitatively identify pre-cancerous and cancerous changes in the oral cavity. In vivo optical spectroscopy provides a real-time tool to assess the metabolic and morphologic changes in the epithelium and stroma associated with the development of oral pre-cancers. The increase in diagnostic specificity has the potential to reduce the number of unnecessary biopsies and to reduce the delay in treatment.

Conventional clinical practices for the diagnosis of oral lesions depend on the expertise of a clinician to locate the most suspecting area in a particular lesion by visual inspection. This is followed by the procedure of invasive biopsy and histopathological analysis. Although this is the accepted gold standard for lesion diagnosis, it is time consuming, costly and painful. Chapter 1 of the thesis presents the advantages of 'optical biopsy' as a tool for the detection and classification of oral lesions. In addition to the high diagnostic accuracies attainable, the optical biopsy techniques are non-invasive in nature and provide faster diagnosis.

Fluorescence and reflectance spectroscopy based techniques provide the ability to assess tissue structure and metabolism in vivo in real time, providing improved diagnosis of pre-cancerous lesions. Reflectance spectroscopy can probe changes in epithelial nuclei that are important in pre-cancer detection, such as mean nuclear diameter, nuclear size distribution and nuclear refractive index. Fluorescence spectroscopy can probe changes in epithelial cell metabolism, by assessing mitochondrial fluorophore NAD(P)H and FAD, and epithelial-stromal interactions, and changes the fluorescence properties of the tissue (Chapter 2).

Advances in fiber optics and sensitive detectors have enabled development of clinical instrumentation for in vivo measurements. Chapter 3 summarized the methodology adopted for point monitoring of AF and DR, and the development of DRIS, a new imaging system for cancer screening. DRIS system comprised of a high resolution EMCCD camera coupled to a LCTF to record images of the lesion at the oxygenated hemoglobin absorption peaks (545 and 545 nm) seen in the diffusely reflected spectra from oral tissues on illumination with the tungsten halogen lamp of the dental chair. The chapter also presented details of the clinical protocol and analytical methods used. The advantage of large area surveillance imaging spectroscopy in overcoming the limitation of point monitoring techniques is also discussed here.

The potential of utility of LIAF technique for diagnosis of oral lesions in a clinical situation is presented in chapter 4. The laser-induced autofluorescence spectral data were recorded from the oral lesions of 61 patients and from different anatomical sites of 30 healthy volunteers in the 420-720 nm wavelength region with diode laser excitation at 404 nm. LDA based on the LOO method of cross validation performed on the principal components of autofluorescence spectral data was found suitable to discriminate oral mucosal alterations during tissue transformation towards malignancy with improved diagnostic accuracies as compared to previous reports. Point monitoring with LIAF technique was able to classify dysplasia from SCC, dysplasia from hyperplasia and hyperplasia from normal with sensitivities of 100%, 98% and 100%, respectively with corresponding specificities of 98%, 100% and 95% (Table 4.1).

Chapter 5 explores the possibility of using DRS with white light illumination to discriminate normal or healthy tissue from hyperplastic and dysplastic tissues. DR spectra in the 400-700 nm region was collected from the buccal mucosa and alveolas (left and right), buccal mucosa, retromolar area and angle of the mouth in 96 patients and 34 healthy volunteers in a clinical trial. PCA was performed on the normalized spectral data with LDA as the

classifying technique to discriminate healthy tissue from hyperplastic and dysplastic tissues. The DR spectral data were compared against the histopathology results of biopsy. The ROC curve analysis was also performed for group comparisons. The overall sensitivity and specificity attained (Table 5.2) to differentiate healthy from hyperplastic tissue, hyperplastic tissue from dysplastic tissue, dysplastic tissue from confirmed SCC were 95%, 100%, and 100%, and 95%, 98.5% and 95%, respectively. The ROC analysis with discriminant score yielded AUC of 0.983 (95% CI: 0.95-1.00) and 0.954 (95% CI: 0.90-1.00) for discriminating dysplasia from SCC and hyperplasia from dysplasia, respectively. While discriminating hyperplasia from healthy tissues the ROC analysis yielded an AUC of 0.987 (95% CI: 0.96-1.00). The DRS data analyses presented in this chapter clearly establish the potential of this technique for early detection of malignant changes in the oral cavity. The relatively high accuracy obtained in this study with very low miss-classification rate recommends it as an ideal tool for screening of oral cancer in clinical settings.

The common procedure for detecting pre-malignant lesions consists of visual inspection, followed by biopsy of any suspicious lesion found. However, benign lesions, which are very common and diverse, such as lichen planus, candida infections, inflammation, hyperkeratosis, ulcerations and so on, may present characteristics very similar to early malignant or premalignant lesions, which distinguishing them difficult even for experienced clinicians. The challenges of a detecting system for identifying lesions with dysplastic/malignant features from those without (benign tissues) were discussed in chapter 6.

A comparative evaluation of AF and DR techniques would go a long way in the applicability of these techniques under diverse circumstances. In chapter 7, the AF and DR spectral data gathered from the same set of oral cavity lesions of a patient group were compared and the efficacy of these two optical biopsy techniques were assessed with the help of the area under Receiver ROC curve analysis and comparison with the results of histopathology. The

data analysis was carried out using PCA and LDA, and the results and potential utility of these two techniques in a clinical setting were presented by analyzing data from 15 healthy volunteers with no clinically observable lesions in the oral mucosa and 35 patients. DR spectral data yielded 100% diagnostic accuracy for discriminating normal from hyperplasia, and hyperplasia from dysplasia lesions, whereas for discriminating SCC lesions from dysplasia, the obtained sensitivity was 100% and specificity 90%. In the case of AF data, the sensitivity obtained was 100%, with specificities of 93%, 100% and 90% respectively, for discriminating hyperplasia from normal, dysplasia from hyperplasia and dysplasia from SCC (Table 7.2). The ROC analysis with discriminant score yielded an AUC of 0.984 for fluorescence spectral data and 0.993 for DR spectral data in discriminating oral cavity lesions. DRS provided slightly improved classification accuracy as compared to autofluorescence spectroscopy when same subjects were evaluated using similar analytical methods.

Diffuse reflectance imaging has the advantage of providing spatial information across a lesion, which allows spotting of lesion-specific features such as in homogeneities and makes the technique potentially useful for localizing new lesions. Clinical trials were carried out with the DRIS system developed for tissue imaging. The pixel intensity values at the ROI in malignant and pre-malignant lesions have given sensitivity of 76% and specificity of 80%. Scatter plot diagram of cancerous (pre-malignant and malignant) versus non-dysplastic/healthy tissues, gave to a reduced sensitivity of 92% and specificity of 95%. Classification of malignant lesions from non-dysplastic/healthy tissues gave 97% sensitivity and specificity (Table 8.2). Scatter plot diagram of pre-malignant and non-dysplastic/healthy leading to a reduced sensitivity of 95% and specificity of 92% (Table 8.2). Area under the ROC curve shows the discriminatory capacity of the R545/R575 ratio image to differentiate malignant from non-dysplastic [AUC = 0.99 (95% CI: 0.99-1.00)], oral pre-malignant from non-dysplastic [AUC = 0.94 (95% CI: 0.86-1.00)], malignant from

pre-malignant [AUC = 0.84 (95% CI: 0.73-0.95)] and cancerous (pre-malignant & malignant) from non-dysplastic [AUC = 0.97 (95% CI: 0.94-1.00)]. These values confirm the vast potential of DRIS as a non-invasive screening tool for detecting malignant lesion of the oral cavity (Figs. 8.3a-d).

Optical spectroscopic and imaging techniques can detect changes in epithelial and stromal morphology and metabolism, which occur during carcinogenesis. Better understanding of the relationship between the observed optical signals and cancer molecular biology will give a new impetus to improve optical techniques for cancer screening and diagnosis. A comparison of the diagnostic accuracies obtained in the present study with earlier results reported in literature is given in Table 9.1.

In conclusion, it is evident that the LIFRS system used in this study has the capability to sequentially measure autofluorescence and diffuse reflectance spectra from target tissues and be utilized as a non-invasive tool for oral cancer diagnosis. This study has clearly shown that the methodology developed could act as an auxiliary adjunct to the clinicians in tissue differentiation and facilitate speedy diagnosis at the clinic to arrive at appropriate follow-up decisions, ensuing in treatment or surgery. Further, application of this point monitoring system is extendable for detection of other epithelial cancers of the cervix or skin and has the potential to aid in demarcating malignant lesions during surgical interventions. It can also be adapted to detect superficial tumors of internal organs by coupling to an endoscope. Nevertheless, the diffuse reflectance technique by virtue of its low cost, high sensitivity and specificity could turn out to be a viable alternative for in vivo cancer screening. Thus, it can be concluded that findings of the current study demonstrate that information provided by non-invasive DR and LIAF spectroscopy along with suitable analytical methods has immense potential to diagnose oral cancer in its early stages.

Table 9.1. Comparison of the diagnostic accuracies of the present study with similar studies on oral lesions from other international groups.

Research group	Tissue types	Spectroscopy/ Methodology	Sensitivity/ Specificity	AUC-ROC	population
De Veld et al., 2005	Nor Vs SCC NorVs Be/Dys/SCC Be vs Dys/SCC	DRS, FI,PCA,NN, multiple classifier	94/96 83/86 69/77	0.98 0.90 0.77	581 h 115 p
McGee, 2008	Nor Vs SCC Nor Vs Dys/SCC Nor Vs Be/Dys/SCC Be Vs Dys/SCC	DRS, IFS Logistic regression, LCV, ROC-AUC, k-means clustering	86/85 78/86 78/76 55/62	0.88 0.85 0.83 0.60	710 h 87 p
Muller et al., 2003	Nor Vs Dys/SCC Dys Vs SCC	Trimodal, IFS, DRS, LSS Logistic regression	96/96 64/90		8 h 15 p
Wang et al., 2003	Be Vs Dys/SCC	AF PLS-ANN	81/96		23 h 52 p
Tsai et el., 2003	Nor Vs OSF Nor Vs Dys/SCC	AF ratios of the area under the spectrum	100/93 81/87		 15 h 149 p
Majumder et al., 2003	Nor Vs SCC	PCA,PLS,MRDF	83/66 95/96		13 h 16 p
Mallia et al., 2008b	Nor Vs Hyp (Be) Hyp Vs Dys Dys Vs SCC	DRS, HbO_2 absorption intensity ratios	86/97 100/86 96/100		49 p 35 h
Mallia et al., 2008a	Nor Vs Hyp (Be) Hyp (Be) Vs Dys Dys Vs SCC	LIAFS Intensity ratios	100/100 100/100 93/94		37 p 35 h
Present study (Chapter 4)	Nor Vs Hyp (Be) Hyp (Be) Vs Dys Dys Vs SCC	AF, PCA-LDA	100/98 98/100 100/95		30 h 61 p
Present study (Chapter 5)	Nor Vs Hyp (Be) Hyp (Be)Vs Dys Dys Vs SCC	DR PCA-LDA	95/100 100/95 98.5/96	0.987 0.954 0.983	34 h 96 p
Present study (chapter 8)	SCC Vs Non-mal Dys Vs Non-mal Dys Vs SCC Dys / SCC Vs Non-mal	DRIS Image pixel intensity ratio	97/97 95/92 76/80 92/95	0.99 0.94 0.84 0.97	55 p 23 h

SCC: Squamous cell carcinoma, PCA: principal component analysis, AF: autofluorescence, DR: diffuse reflectance Nor: normal, Dys: dysplasia, LDA: linear discriminant analysis, DRIS: diffuse reflectance imaging system, PLS: partial least squares, NN: neural network, Be: benign, hyp: hyperplasia, LSS: light scattering spectroscopy, h: healthy; p: patient

Even though, there is no thumb rule regarding study population, the basic constraint of this study was with respect to the patient population, especially as lesions were divided into different categories for classification. In comparison with the reports of McGee (2008) and De Veld et al (2005), the diagnostic accuracy obtained using AF and DR techniques in this

study are improved (Table 9.1). For discrimination of benign/hyperplastic lesions from pre-malignant/dysplastic lesions, which is the most significant clinical challenge, the present study has yielded sensitivities of 98%, 100%, 95% and specificities of 100%, 95%, 92% for fluorescence, DR point monitoring and DR imaging respectively. In comparison Skala (2008) achieved a sensitivity of 55%, specificity of 62% and De Veld et al (2005) got a sensitivity of 69% and specificity of 77% for classification of benign from dysplastic/malignant lesions. This high diagnostic accuracy makes the techniques presented in this thesis more suited in routine clinical practice. Particularly noteworthy is the high sensitivity of 95% and specificity of 92% that has been achieved in the study in a group of 55 patients and 23 healthy volunteers using a novel DR imaging technique that utilized the image ratio of oxygenated hemoglobin absorption at 545 and 575 nm as an indicator tissue transformation towards malignancy.

Application of digital image processing techniques to diffuse reflectance properties of oral tissue has the potential to improve our ability to objectively identify and delineate the peripheral extent of neoplastic lesions in the oral cavity. Functional optical imaging capitalizes on the changing optical properties of tissue by using light to measure physiological changes. The basis for this imaging method arise from the differences in the spectra obtained from the normal and diseased tissue owing to the multiple physiological changes associated with increased vasculature, cellularity, oxygen consumption and edema in tumour. If clinically proven, this method will provide a powerful tool for early diagnosis of neoplastic changes in the oral cavity in patient care locations where experts are not available or where physicians encounter few cases of malignant and premalignant neoplasia. Furthermore, this method will offer the advantage of probing the entire lesion and its surrounding areas instantaneously in order to delineate the boundaries of neoplastic changes efficiently.

This is first time that a DR imaging system has been built for multi-spectral imaging of oral cancer at the oxygenated hemoglobin absorption bands. DRSI data efficiently discriminates healthy tissue from oral malignant lesions and premalignant lesions. The high diagnostic accuracy obtained in this study underline the potential use of this method in routine clinical practice. Since clinical diagnosis based on DR imaging is possible in near real-time, there is practically no waiting period for the patient. These advantages make DRIS a suitable mass-screening tool for early detection of oral pre-cancers. This study could not be made site-specific due to the lack of enough cases belonging to all anatomical locations. In future, studies on a larger/random population and development of classification algorithms for discriminating dysplastic lesions as mild, moderate and severe, and SCC lesions as well differentiated, moderately differentiated and poorly differentiated, would be enhance and broaden the application potential of the imaging modality and provide new tools to better understand cancer biology.