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Recent advances in optical diagnosis of oral cancers: review and future perspectives

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Abstract

Optical diagnosis techniques offer several advantages over traditional approaches, including objectivity, speed and cost, and these label-free, non-invasive methods have the potential to change the future work-flow of cancer management. The oral cavity is particularly accessible and thus such methods may serve as alternate/adjunct tools to traditional methods. Recently, *in vivo* human clinical studies have been initiated with a view to clinical translation of such technologies. A comprehensive review of optical methods in oral cancer diagnosis is presented. Following an introduction to the epidemiology and aetiological factors associated with oral cancers currently employed diagnostic methods and their limitations are presented. A thorough review of fluorescence, infrared absorption and Raman spectroscopic methods in oral cancer diagnosis is presented. The applicability of minimally invasive methods based on serum/saliva is also discussed. The review concludes with a discussion on future demands and scope of developments from a clinical point of view.

Oral Cancers: An Overview

Oral squamous cell carcinoma (OSCC) ranks as the 15th most common cancer in the world and 10th most frequent in males. It accounts for ~2.1% of total cancer cases worldwide. Incidence rates are high among males in south central Asia and among females in eastern and central Europe[1]. Although mortality from oral cancer has decreased in the past few decades, it is still high and has a five year survival rate of 50%[2]. Several oral lesions and conditions are associated with an increased potential for malignant transformation. Of these, the most commonly occurring is leukoplakia, defined as 'a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion' (Figure 1B). A wide range of malignant transformation rates have been reported, from 0.13-36.43%, depending on the presence and degree of dysplasia, location in the oral cavity and maturity of the lesion[3,4]. Conversely, erythroplakia, defined as 'a fiery red patch which cannot be characterized clinically or pathologically as any other definable lesion'[3], is less prevalent and has a higher potential for malignant transformation (14.35-66.76 %)[5]. Oral submucous fibrosis is a premalignant oral condition arising mostly due to areca nut or betel quid chewing[6]. It has a reported transformation rate of 7.6%[6]. Oral lichen planus is an inflammatory disease of the oral mucosa. Like leukoplakia, it presents as a white plaque or patch. However, its malignant transformation rate is much lower than leukoplakia, ~1%[7] (Figure 1C).

A number of risk factors are associated with oral cancer, including cigarette smoking and alcohol consumption, which are responsible for 42% and 16% of oral cancer deaths respectively[8]. The carcinogenicity of cigarette smoke has long been established, resulting in DNA damage and increase in P53 mutations[9]. The association between alcohol and oral cancer was reported to be dose dependent and a number of factors may contribute to its carcinogenicity, including acetaldehyde, an alcohol metabolite thought to be carcinogenic[10]. Moreover, alcohol is thought to act as a solvent for other carcinogens[10]. Oral habits such as smokeless tobacco and betel quid chewing are also implicated in oral cancer development[11]. They are more common in the Asian population; in India, 50% of oral cancers arise due to smokeless tobacco chewing[11]. The carcinogenicity of smokeless tobacco arises from the production of nitrosamines[11]. Oral squamous cell carcinoma has also been associated with

the Epstein Bar (EBV) and Human Papilloma (HPV) Viruses, although their putative role is controversial. Around 23% of OSCC were found to be positive for high risk HPV 16 and 18 [12].

Current Screening/Diagnostic methods and limitations:

Screening tests or diagnostic aids presently available for oral cancer include conventional oral examination (COE), staining with toluidine blue, oral brush biopsy and scalpel biopsy coupled with histology.

Conventional Oral Examination (COE): Conventional oral examination (COE), using normal (incandescent) light, has long been the standard screening method for oral abnormalities. As it is a visual method, it cannot identify early mucosal abnormalities that may or may not lead to oral cancer. Approximately 5-15% of the general population has oral mucosal abnormalities and the vast majority of these lesions are benign in nature [13,] Furthermore, only a small percentage of leukoplakias are progressive or become malignant and COE cannot discriminate between these and their non-progressive counterparts. Therefore, while COE may be useful in discovering some oral lesions, its potential in identifying all potentially premalignant or biologically relevant lesions that are likely to progress to cancer is questionable.

Toluidine blue staining: Toluidine blue (TB), has been used for more than 40 years as an aid to detection of mucosal abnormalities of the cervix and the oral cavity. TB is a metachromatic, acidophilic dye that binds preferentially to tissues undergoing rapid cell division (inflammatory, regenerative and neoplastic tissue), resulting in preferential staining of abnormal tissue. Overall, TB appears to be useful in detecting carcinomas, but is positive in only ~50% of lesions with dysplasia. In addition, it frequently stains common, benign conditions such as non-specific ulcers. The high rate of false positive stains and the low specificity in staining dysplasia are some of the accepted limitations of the technique [14].

Oral brush biopsy: Oral brush biopsy extracts a complete trans-epithelial biopsy specimen, with cellular representation from each of the three layers (basal, intermediate and superficial). Because the brush biopsy detects only cellular atypia, positive results must be confirmed with a scalpel biopsy for definitive diagnosis. This technique has therefore been criticized for adding time and cost to the

diagnosis of oral lesions without additional benefit to the patient[15]. Overall, it is a method of identifying unsuspected oral cancers found during a visual examination, at early and curable stages[16].

Histology: Histological risk stratification, currently the gold standard for oral cancer diagnosis, requires biopsy, staining and microscopic examination by a pathologist. However, removal of tissue or biopsy is an inherently invasive procedure and carries risk of complications in the proximity of vital anatomy. Sampling errors in collecting or interpreting biopsies due to inter-observer discrepancy can be significant. Once removed, the tissue can undergo biochemical changes which can lead to artifacts. In many diseases, tissue involvement is not uniform, potentially leading to sampling errors. Especially in oral cancers, some early lesions are clinically indistinguishable from benign conditions. Furthermore, histologically, identification of subtle changes in precancerous lesions or in normal mucosa that are indicative of early neoplastic transformation is subjective and can lead to inter-observer variations [17].

It is therefore conceivable that the primary prevention of the disease would involve activities to reduce or eliminate the use of tobacco and alcohol. Secondary prevention includes activities that are aimed to detect the disease at an early stage which would lead to better prognosis and lower morbidity. Current methods of detection of oral cancers are based largely on visual observations of abnormalities in tissue or cellular morphology and are therefore limited in terms of sensitivity and specificity, particularly at early stages. In the following section, a general discussion on the application of optical spectroscopic methods as an alternate/adjunct diagnostic tool for oral cancer is presented.

Optical spectroscopy in oral cancer diagnosis

Spectroscopy is the study of the frequency dependence of the interaction of electromagnetic radiation (light) with matter. Generally, light interacts with matter through absorption, emission and scattering/reflection. In each case, the spectrum of the interaction gives information about, and is characteristic of, the structure and chemical content of the sample. Optical measurements provide quantitative information based on the spectroscopic signature of the biochemical constituents of the sample that can be rapidly analyzed to yield an objective diagnosis, even in the hands of a non-expert operator. Diagnosis is based on biochemical changes underlying the pathology rather than visual or

microscopic changes in cellular or tissue morphology. Devices to make these measurements have become inexpensive, robust, and portable, because of advances in computing, optical, fiber-optical, and semiconductor technology. Approaches based on fluorescence, Fourier-transform infrared absorption and Raman scattering spectroscopy have shown potential for improved detection of oral cancers. A brief introduction of these techniques and their potential applications in oral cancer diagnosis is presented in the following sub-sections. Figure 2 provides a schematic illustration of their typical method of application.

Fluorescence spectroscopy: When a molecule is illuminated at an excitation wavelength lying within the absorption spectrum of that molecule, it absorbs the energy and undergoes a transition from the ground state to an excited state. The molecule can then relax back from the excited state to the ground state by emission of light at specific emission wavelengths. In the UV/visible/Near infrared region of the spectrum (~200-1000nm), light emission takes the form of fluorescence (or occasionally phosphorescence). A fluorescence emission spectrum represents the fluorescence intensity measured over a range of emission wavelengths at a fixed excitation wavelength and can provide information relating to the molecular characteristics of the fluorophore, Figure 3.

In the late 1970s, cancer diagnosis based on auto-fluorescence (also called natural, intrinsic or endogenous fluorescence) of naturally occurring fluorophores such as collagen, elastin, keratin and NADH was initiated [18]. The presence of disease can lead to changes in blood concentration, nuclear size, collagen content or epithelial thickness, which can alter the concentration and characteristics of the fluorophores. In oral cancers, it was demonstrated that the epithelial layer shields the strongly fluorescing collagen layer leading to a low intensity of fluorescence in cancers[19]. An *ex vivo* study used hamster buccal pouch as an experimental model to identify spectral markers associated with different stages of oral carcinogenesis [20]. Onizawa *et al.*, compared fluorescence spectra from human and hamster biopsies and oral cancer cell lines, suggesting that variation in the riboflavin and porphyrin fluorescence can be used as a spectroscopic marker for normal and cancerous conditions[21]. Ingrams *et al.* further showed that normal and cancerous human biopsies can be discriminated based on their autofluorescence spectral profile[22]. Another *ex vivo* study by Muller *et al.*, explored the feasibility of quantifying the spectroscopic response of different grades of malignancy[23].

The first *in vivo* study using autofluorescence spectroscopy by Harris *et al.* reported differences between healthy and tumor mucosa based on the porphyrin emission band[24]. These differences were attributed to microorganisms living on ulcerating or necrotic surfaces. *In vivo* methods have also been explored to understand oral cancer progression in animal models[25]. Gillenwater *et al.* recorded *in vivo* autofluorescence from oral mucosa of 8 healthy volunteers and 15 patients with premalignant or malignant lesions[26]. Decreased intensity in the blue spectral regions, and increased porphyrin fluorescence in the red were observed. Based on the ratio between these, a sensitivity of 82% and specificity of 100% were reported [26]. Various other studies have provided further evidence in support of *in vivo* fluorescence spectroscopy for non-invasive oral cancer diagnosis[27-29]. A recent study by Shaizu *et al.* showed that autofluorescence spectroscopy can be used to identify oral cavity disorders caused by long-term tobacco habits. Their findings suggest that lower collagen levels and increased ratios of flavin adenine dinucleotide (FAD) to nicotinamide adenine dinucleotide (NADH) can serve as prognostic markers for oral cancer risk [30].

However, tissue contains few natural fluorophores and their spectroscopic features are broad and overlapping, making them poorly distinguishable, reducing the specificity of fluorescence spectroscopy for diagnostic applications.

Fourier-transform infrared spectroscopy: Vibrational Spectroscopy is a subset of spectroscopy which analyses vibrations within a molecule (or material). The vibrations are characteristic of the molecular structure and, in polyatomic molecules, give rise to a spectroscopic "fingerprint". The spectrum of vibrational energies or frequencies (expressed as wavenumbers, cm^{-1}) can thus be employed to characterise a molecular structure, or changes to it due to the local environment or external factors (radiation, chemical agents). Vibrational energies fall within the mid Infrared (IR) region of the electromagnetic spectrum and are commonly probed through IR absorption spectroscopy. High energy or frequency vibrations are characteristic of light, tightly bound groups such as C-H, N-H and O-H, whereas low frequencies are associated with heavier groups, or collective vibrations such as ring breathing or skeletal stretches in macromolecules (Figure 4).

IR spectroscopy is now a routine technique for materials characterisation and has found numerous applications in forensics, environmental science and pharmacology[31]. Applications to tissue samples

for (cancer) diagnostic applications were first reported in the early 90s, and since this time a range of pathologies has been investigated[32].

Wu *et al.* demonstrated that, on the basis of lipid and protein content, normal and tumor oral tissues can be discriminated[33]. In another study, of 10 normal sub-gingival tissues (NST) and 15 oral squamous cell carcinoma (SCC) tissues, Fukuyama *et al.* demonstrated that the normal spectra are strongly influenced by the presence of collagen. They also suggested that spectra are influenced by keratin, which exists in the ectodermal cells[34]. Study using FTIR spectroscopy to understand oral carcinogenesis in animal models have also been reported [35]. FTIR imaging methods have also been explored to analyze different aspects of oral cancers. A study by Schultz *et al.*, to assess changes in biochemistry of well and poorly differentiated oral/oropharyngeal SCC by infrared microspectroscopy, demonstrated that DNA and keratin can provide spectral markers to differentiate between normal and SCC biopsies[36]. Bruni *et al.*, by generating three-dimensional IR chemical maps, demonstrated that proliferating and regressive states of head and neck tumours can be identified[37]. Towards high throughput, automated analysis, Pallua *et al*. demonstrated that good quality FTIR images can be obtained from formalin fixed paraffin embedded tissue microarray sections providing molecular level information as the basis for diagnosis[38].

Compared to fluorescence, FTIR provides a detailed fingerprint of the biochemical content of the sample. However, although FTIR has been used for the analysis of human tissues *ex vivo*, the application of this method for *in vivo* diagnosis is limited, due to the short penetration depth and the fact that water is highly absorptive in the mid-IR range. Conventional optical fibres have limited transparency in the IR region, and therefore, *in vivo* studies are less frequent than fluorescence or Raman fibre optical applications. New developments based on attenuated total reflection (ATR) elements might help in implementing *in vivo* applications.

Raman spectroscopy: Raman spectroscopy is a complementary technique to FTIR and has its origin in the discovery of the Raman effect in 1928, for which C.V. Raman was awarded the Nobel prize in 1930[39]. Similar to IR spectroscopy, Raman entails the coupling of incident radiation with molecular vibrations and the resultant spectrum is similarly characteristic of the material. However, whereas IR spectroscopy involves the absorption of radiation, Raman spectroscopy is a scattering technique,

whereby the incident radiation couples with the vibrating polarisation of the molecule and thus generates or annihilates a vibration. The differing underlying mechanisms results in a complementarity of the techniques. Vibrations of asymmetric, polar bonds thus tend to be strong in IR spectra, whereas Raman is particularly suitable as a probe of symmetric, nonpolar groups. Notably, O-H vibrations of water are very strong in IR spectra, but are extremely weak in Raman spectra, rendering Raman a potentially more suitable technique for *in vivo* applications (Figure 3). A further implication of the differing physical origins of the techniques is that, whereas IR monitors the absorption of IR radiation, Raman scattering can be employed in the UV, visible or near IR regions of the spectrum (Figure 2). Raman scattering thus offers intrinsically higher spatial resolution for mapping or profiling in a confocal microscopy mode, the limit of spatial resolution being determined by the wavelength (<1μm for Raman, ~5-10 μm for IR). The application of Raman spectroscopy to biomolecules was first demonstrated as early as the 1960s and by the 1970s biomedical applications were explored[40]. Whole cell, tissue and *in vivo* studies carried out on a range of pathologies have demonstrated the potential for diagnostic applications[41].

Raman spectroscopic applications in oral cancer diagnostics started with the analysis of normal and dysplastic tissue in a rat model by Schut *et al.* (2000). Dysplasia in the palate was induced by topical application of the carcinogen 4-nitroquinoline 1-oxide and sensitivity and specificity of 100% were observed[42]. This was followed by a study of human oral cancer biopsies by Venkatakrishna *et al.*, they recorded spectra of 49 biopsies and obtained an average classification efficiency of 88%[43]. In 2004, a study carried out by Krishna *et al.* demonstrated the applicability of formalin fixed oral tissues for optical pathology, revealing significant differences in the epithelial region of normal and malignant samples, arising from the protein composition, conformational/structural changes, and possible increase in protein content in malignant epithelia[44]. In 2006, Malini *et al.* demonstrated the efficacy of Raman spectroscopic methods in discriminating normal, cancerous, precancerous and inflammatory conditions[45]. Lipid rich features in normal conditions and prominent protein features in tumors and other pathological conditions were observed. Classification between different groups using multivariate statistical methods produced 100% sensitivity and specificity[45]. Raman mapping of tissue sections further elucidated biochemical changes within different epithelial layers which are associated

with disease onset[46]. A study by Sunder *et al.* demonstrated that oral carcinomas of different pathological grades can also be differentiated on the basis of the relative intensities of bands associated with lipids and proteins[47].

In vivo Raman spectroscopy using fibreoptic probes for identifying site specific variations in the oral cavity was reported by Guze *et al.* in 2009, indicating that different oral sites can be discriminated on the basis of level of keratinization[48]. Bergholt *et al.*, (2011), characterized the Raman spectroscopic profiles of different oral cavity regions (inner lip, attached gingiva, floor, dorsal tongue, ventral tongue, hard palate, soft palate, and buccal mucosa)[49]. Fitting of reference biochemicals (hydroxyapatite, keratin, collagen, DNA, and oleic acid) and partial least squares-discriminant analysis (PLS-DA) were employed to assess the inter-anatomical variability. The findings suggest that histological and morphological characteristics of different sites have a significant influence on the *in vivo* Raman spectra, and different sites can be classified with an overall sensitivity and specificity of 85%[49] . Singh *et al.* demonstrated that *in vivo* spectra can be acquired in clinically implementable timescales and demonstrated the feasibility of classification of normal and pathological conditions[50]. This was followed by another study exploring tobacco induced cancer field effects in the oral mucosa[51]. Sahu *et al.* demonstrated that *in vivo* Raman spectroscopy methods can also be utilized to understand age-related changes in the oral mucosa[52]. These findings were further verified by a recent study showing anatomical variability and feasibility of identifying pathological conditions with *in vivo* Raman spectroscopy [53].

Minimally invasive methods in oral cancer diagnosis: Bio fluids such as blood, urine, lymph, and saliva can provide substantial information about human health and are being widely investigated for clinical diagnosis of various diseases including oral cancers. The attraction of these specimens lies in the fact that they can be used for mass screening, due to ease in collection, transport and low cost[54]. Studies have been carried out on physiochemical properties of saliva using surface enhanced laser desorption and ionization time of flight (SELDI-TOF) coupled with mass spectrometry (MS) and high performance liquid chromatography (HPLC) to identify proteomic and enzymatic markers associated with oral cancer[55]. Other techniques such as laser-induced fluorescence coupled with HPLC, and

capillary electrophoresis coupled mass spectroscopy have been employed to characterise salivary metabolites in oral cancer patients[56,57].

Recently, optical methods based on Raman, infrared absorption, and fluorescence spectroscopies have also been exploited for such investigations. For example, enhanced levels of porphyrin in blood have been used as a diagnostic marker for various cancers including oral cancers[58,59]. Yuvaraj *et al.* characterized different salivary metabolites associated with oral cancers by fluorescence spectroscopy[60]. FTIR spectroscopy has been applied to study sputum in order to diagnose oral cancers and discrimination between normal and cancerous samples was achieved on the basis of changes in the protein and glycoprotein structure within cells[61]. Surface-Enhanced Raman spectroscopy methods (SERS) have been used to differentiate between normal and oral cancer patients using spectra acquired from saliva[62]. A recent study by Elumalai *et al.* demonstrated that Raman spectroscopy of urine samples of healthy subjects and oral cancer patients can offer potential diagnostic information with a discrimination accuracy of 94%[63]. The analysis of exfoliated oral cells by optical methods also holds enormous promise for early disease detection and diagnosis. Diem and co-workers have carried out multiple studies on spectral cytopathology of oral exfoliated cells[64,65]. Their findings are suggestive of the tremendous potential of spectroscopic methods in identifying minor changes associated with disease onset. Nevertheless, diagnosis based on biofluids suffers from limitations such as low analyte concentration, longer acquisition time, prone to experimental errors etc. Considerable efforts have been undertaken to develop standard protocols and sensitive instrumentation. Signal enhancements with the help of nano-particles or surface coating is an active area of research [66]. Concentration of samples using centrifugal filtration devices has been shown to offer an alternative which allows measurement of the analytes in the native aqueous environment[67]. This also allows fractionation according to molecular weight of the constituent analytes, potentially allowing the targeting of molecular biomarkers of a disease. Appropriate modification in the instrumentation, especially automation for collection and analysis of body fluids is also an area which requires constant development. Efforts should also be undertaken for large scale trials and database development to overcome inter-laboratory and instrument variabilities [68].

Summary and Outlook

Although the oral cavity is easily accessible to inspection, oral cancer patients often present at an advanced stage when treatment is less successful, thereby leading to high morbidity and mortality. Early detection is the best way to ensure patient survival and quality of life. The current gold standard for clinical diagnosis of oral lesions is biopsy and subsequent histopathological confirmation. The process is invasive, time-consuming and prone to inter-observer variability. An alternate method of diagnosis that can enable non-invasive diagnosis of the oral cavity in individuals with suspicious oral lesions is warranted.

It is now well recognized that techniques based on optical spectroscopy can play a very important role towards this end. Spectroscopic measurements of tissue biochemistry, with sensitivity and specificity to localize changes enhanced by imaging, represent a measure of health (or disease) unattainable in current practice, and can provide sensitivities for early stage detection of biochemical, rather than simply morphological, abnormalities. Table 1 lists the advantages and disadvantages of the current screening/diagnostic methods and optical spectroscopy methods. Among the spectroscopic techniques described, fluorescence is perhaps the most technologically accessible, as it is simply based on the analysis of light which is emitted after illumination with a UV lamp. The emitted light is in the visible range and therefore probes can use free space or low grade, inexpensive fibre optics. The technique detects only the small fraction of endogenous biomolecules which are fluorescent, however, and relies on identifying pathology specific biomarkers amongst them. FTIR spectroscopy, on the other hand, produces a label free fingerprint of the complete biochemical content of the tissue, cell or biofluid, and this can explore more global and specific pathological changes. However, water is an extremely strong FTIR absorber, and so *in vivo* diagnostic applications may be limited. Raman spectroscopy provides a similar complete, label free fingerprint of the sample, and also couples to benefits of working in the visible region of the spectrum. Water is a weak Raman scatterer, and so the technique is more adaptable to routine *in vivo* patient screening or *ex vivo* spectral histology or cytology. The prospect is therefore of a high sensitivity and specificity, automatable, objective quantitative label free probe of early stage disease development and progression, based on the biomolecular content of the patient sample.

Variable thickness and degree of keratinisation at different sites in the oral cavity can influence the diagnostic efficacy of optical methods, especially for early lesions. This issue has been addressed extensively by the biomedical spectroscopic community. Various studies have successfully demonstrated the potential of spectroscopic methods in identifying anatomical variability due to different levels of keratinization[19,28,29,48,49]. These studies have provided evidence in support of inherent differences between different locations and suggested that each site be treated independently. For example, spectral models developed using spectra from buccal mucosa cancers cannot be used for identifying abnormalities at tongue or palate. Most of the recent studies have been performed under these guidelines, where tumors of specific sites are treated separately[50-53]. As with all optical techniques, the depth sensitivity is limited by the absorption and scattering of the tissue. Operation in the near infrared can optimise the depth sensitivity of Raman probes, and novel methodologies such as spatially offset Raman spectroscopy (SORS) promise increased penetration depths of several millimetres for deeper set lesions [69]. Such technological advances potentially place Raman ahead of the field as candidate for *in vivo* optical diagnostic applications.

In the coming years, large scale clinical trials must be conducted to gain the amount of site-specific data necessary for developing adequate size training and test sets for robust algorithm development and analysis. The standard models for each of the individual sites in the oral cavity should be tested rigorously, preferably double-blinded, as multi-centric studies, before they are considered for routine use. Several technological advances in terms of fiberoptic probes and miniaturization of instruments are also required for real time and routine diagnosis. Efficient suppression of background signal, optimization of collection optics, and incorporation of miniaturized interference filters in the fiber probes are some of the issues that are to be addressed effectively. Further improvements in data analysis algorithms are also required for developing less cumbersome, rapid, unambiguous, objective and user friendly interfaces from the point of view of routine clinical use where a clinician or a technician can analyze a given spectrum against all available models to diagnose a case. The prospective adaptation of optical spectroscopy methods for routine clinical diagnosis would decrease the number of follow-up clinic visits and patient anxiety by minimizing

waiting times for histopathological diagnosis. The technology poses no known risks to the patients, and therefore could be a safe alternative/adjunct to the current diagnostic methods.

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Figure legends

Figure 1: Clinical presentation of tongue- cancer (A), leukoplakia (B) and lichen planus (C)

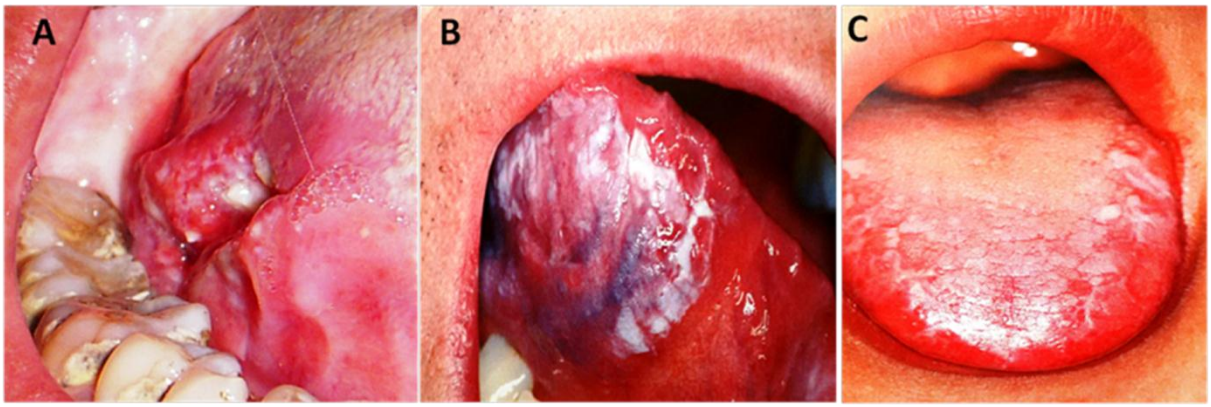

Figure 2: A schematic of the typical application of optical spectroscopic techniques for diagnostic applications. The light source is delivered via a probe or microscope (for *in-vivo* or *ex vivo*/*in-vitro* applications respectively) to the sample (cells, tissue or biofluid). Upon excitation by an appropriate source, molecules can either; go to an excited state and reemit light in the form of fluorescence (UV/visible lamp), absorb the light to generate vibrations within the molecules (Infrared lamp), or, by interaction with the vibrational modes of the molecules in the cells; the light is Raman scattered (visible or near infrared laser). The emitted/transmitted/scattered light is then collected by the probe or microscope and passed to a detector. The operator can then perform analysis on the resulting spectra and, using a prepared classifier, the output can for example be a yes, no, or maybe for the presence of cancer. The technique can be modified according to the application; *in-vivo*, *ex-vivo* (histological or cytological) or *in vitro*.

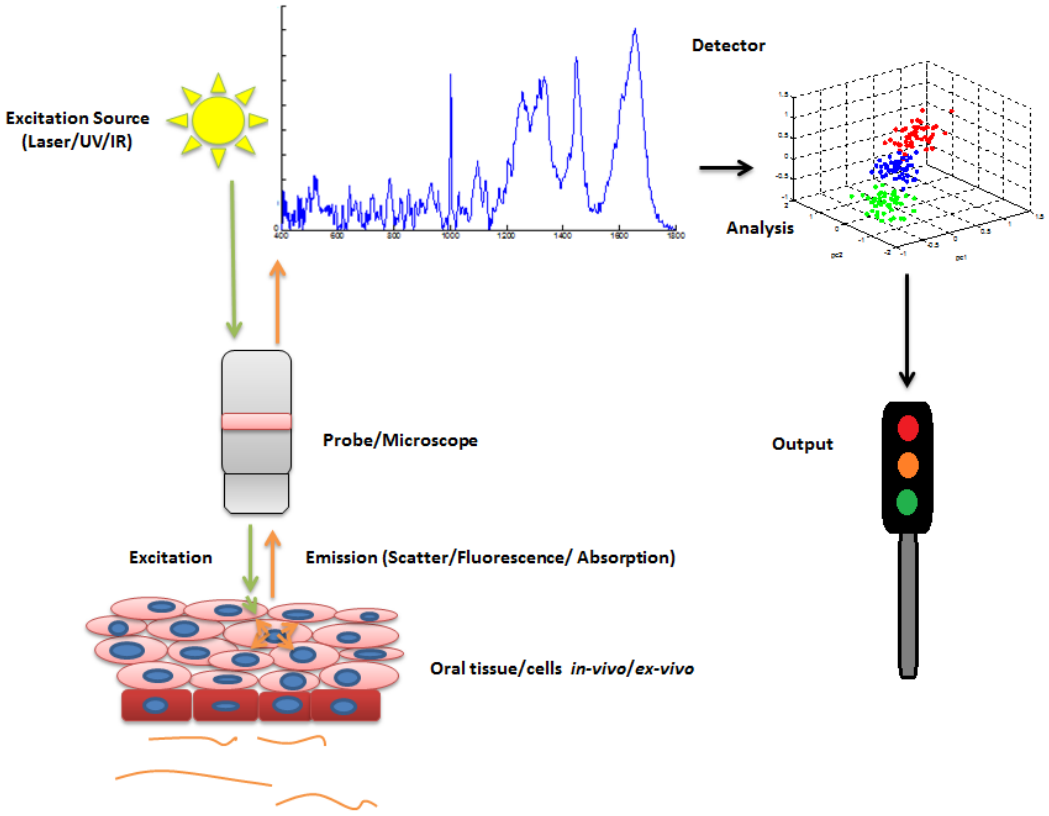

Figure 3: Comparison of typical fluorescence spectrum (riboflavin) and typical Raman spectrum of tissue (both normalised) plotted on an energy scale. The intrinsic bandwidth of the fluorescence feature is very broad, compared to the Raman spectrum, which has a multitude of narrow bands which are shifted from the source wavelength of 785nm (~1.6eV). The energy shift of the Raman band is a measure of the vibrational energy and for comparison with infra red spectroscopy is usually expressed in wavenumbers (1/cm). Note the energy scales for the two spectra are different.

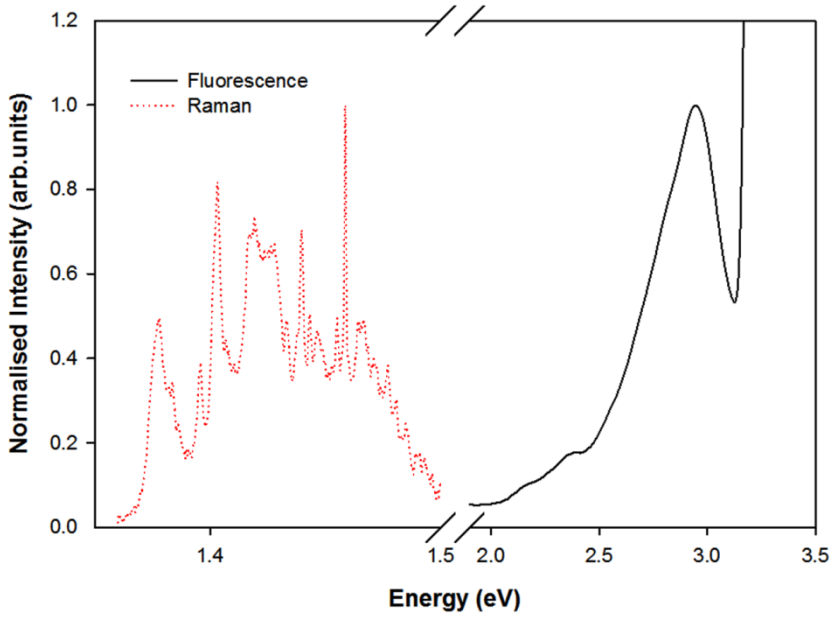

Figure 4: Comparison of typical IR absorption and Raman spectra of human tissue samples. The IR spectrum is in the mid infrared region of the spectrum, and the spectrum is less rich in information than the Raman spectrum. The Raman spectrum is expressed as wavenumber shift from the source laser line, although as shown in Figure 3, the scattered light is in the visible region of the spectrum. Both show typical features of lipids (●), proteins (▲), carbohydrates (■) and nucleic acids (♦). Note the strong absorption due to trace water in the FTIR spectrum in the region of ~3300cm^{-1}.