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Ultraviolet and visible spectroscopies for tissue diagnostics: fluorescence spectroscopy and elastic-scattering spectroscopy

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Abstract. We review the application of fluorescence spectroscopy and elastic-scattering spectroscopy, over the ultraviolet-to-visible wavelength range, to minimally invasive medical diagnostics. The promises and hopes, as well as the difficulties, of these developing techniques are discussed.

1. Introduction

In recent years optical spectroscopies have become the basis for a high level of research activity directed toward the development of novel, noninvasive technologies for tissue diagnostics, frequently dubbed 'optical biopsy', which is perhaps something of an oxymoron since 'biopsy' refers specifically to the removal of tissue, whereas the implication of 'optical' is that tissue is not removed. The motivation is to eliminate the need for surgical removal of biopsy tissue samples; rather, some form of spectral analysis of the tissue is recorded *in vivo* by an imaging system or with an optical probe placed on or near the surface of the tissue in question. A diagnosis of the tissue is then attempted based on the optical measurements. The intent of these systems is to provide diagnostic signatures, *in situ*, noninvasively and in real time. Additional motivation is provided by the potential for reduced health-care costs as a consequence of eliminating histology and, in many cases, eliminating the need for the surgical environment required to take biopsy samples. Moreover, the immediacy of diagnostic information can reduce the emotional trauma to the patient awaiting an answer. While the ultimate goal is the elimination of the need to remove tissue samples, during early phases of the clinical implementation of these optical technologies the intermediate goal, rather than replacement of biopsy and histology, may be to provide additional guidance in locating the optimum sites for biopsy.

For diseases of the gastrointestinal (GI) tract, for example, the potential benefits of optical tissue diagnosis can be significant. Several disorders of the GI tract are correlated with a predisposition for cancer, including colitis, colon polyps, and Barrett's oesophagus. Typically these diseases are followed with annual (or more frequent) endoscopic examination accompanied by tissue biopsies. As many as 20–30 biopsies may be taken in one session. This is a time consuming (and therefore expensive) procedure, which entails some degree of risk for the patient. For each conventional biopsy, the biopsy tool must be withdrawn from the endoscope and the specimen removed before the tool can be reinserted for the

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next biopsy. In contrast, an optical diagnostic probe could be moved from site to site in succession, with each measurement being recorded in a fraction of a second, by simply moving the location of the probe tip. In summary, optical diagnostic techniques offer the potential to improve disease management, with reduced risks for the patient, and the potential for earlier diagnosis and immediate treatment.

A range of spectroscopies have been investigated for optical diagnosis, all of which have one basic principle in common. The specific optical spectrum of a tissue sample contains information about the biochemical composition and/or the structure of the tissue. The biochemical information can be obtained by measuring absorption, fluorescence, or Raman scattering signals. Structural and morphological information may be obtained by techniques that look at the elastic-scattering properties of tissue. These basic approaches are useful for the detection of cancer as well as for other diagnostic applications such as blood oxygen saturation, intra-luminal detection of atherosclerosis, and simply the identification of different tissue types during procedures.

While fluorescence, infrared, Raman and elastic-scattering spectroscopies have all been investigated as methods for distinguishing malignant tissue, the earliest work on spectroscopic diagnostics, and the majority of the *in vivo* work done to date by various groups, has utilized laser-induced fluorescence spectroscopy (LIFS). Nonetheless, recently interest has been growing in Raman, infrared and elastic-scattering spectroscopies because each has specific potential advantages *vis-a-vis* LIFS. The topics of infrared and Raman spectroscopies for diagnostic applications are covered by David Delpy in a separate contribution to this special issue. Consequently, this paper reviews diagnostic applications of fluorescence spectroscopy and elastic-scattering spectroscopy in the UV and visible wavelength ranges. Papazoglou has also recently written an overview of the diagnosis of malignancies and atherosclerotic plaque using LIFS. (Papazoglou 1995).

2. Fluorescence spectroscopy

A motivation for utilizing fluorescence spectroscopy for the diagnosis of tissue pathologies is that fluorescence is sensitive to the biochemical make-up of the tissue. Tissues may contain several fluorescent chromophores (fluorophores) such as NADH, elastin, collagen and flavins. By measuring the UV-induced fluorescence of tissue it should, in principle, be possible to learn about the biochemical state of the tissue. However, several complications arise that make the fluorescence measurements in tissue significantly more complicated than measuring a simple solution of several fluorophores. Scattering cross sections are quite high in tissue (Cheong *et al* 1990), which can result in a distortion of the fluorescence signal. In various optical configurations used for measuring fluorescence, the scattering in tissue can cause apparent changes in the spectral shape of detected fluorescence. Tissue also contains non-fluorescent chromophores, such as haemoglobin. Absorption by such chromophores of the emitted light from fluorophores can result in artificial dips and peaks in the fluorescence spectra. Despite these difficulties, many studies by a variety of methods have shown that fluorescence spectroscopy can be used for optical tissue diagnosis, and methods are being developed to extract intrinsic fluorescence from measurements of turbid media. (Durkin *et al* 1994, Wu *et al* 1993, Richards-Kortum *et al* 1989b, c).

There are two issues that must be addressed when classifying the methodologies of LIFS: one is the possible administration of a fluorescent tumour marker, and the other is the question of whether the detection is point measurement or surface imaging. LIFS can detect the native tissue fluorescence resulting from naturally occurring (endogenous) fluorophores, as well as detecting emission from externally administered (exogenous) fluorescent drugs

that concentrate preferentially in malignant or pre-malignant tissues. The fluorescence from such drugs provides a large signal, which can be helpful in the detection process (Profio and Balchum 1985, Kessel 1987) and may be used as a detection tool for imaging of the patterns of malignancy in a given area of tissue. (Svanberg *et al* 1994). The use of a fluorescent tumour marker, however, is not an ideal solution for routine screening, since the administration of an exogenous drug is essentially an invasive process and can result in concomitant undesirable side effects. When LIFS (usually with UV excitation) is used to detect intrinsic tissue fluorescence, or autofluorescence, as the diagnostic marker (Cothren *et al* 1990, Richards-Kortum *et al* 1991, Schomacker *et al* 1992, Vo-Dinh *et al* 1995) it becomes essentially non-invasive, although care must be taken to minimize the total ultraviolet exposure, especially to internal tissues.

Regarding the issue of whether single-point or imaging measurements are performed, if a small fibre-optic probe is used, then the fluorescence is measured at a single tissue site, whereas if filtered video imaging technology is employed then the result is spectrally selective imaging of larger tissue surface areas. Typically, in a point measurement the entire fluorescence spectrum is recorded for a given excitation wavelength, and this can be repeated quickly for additional excitation wavelengths. On the other hand, when the tissue surface is imaged only a small number of combinations of illumination and emission wavelengths are recorded. These can then be compared pixel by pixel for the entire image. Thus, point measurements provide a large range of spectroscopic information about one localized tissue site, whereas spectral imaging provides a modest amount of spectral information, but for a significant area of tissue surface. In a separate contribution, Stefan Andersson-Engels describes in detail the use of fluorescence imaging, based on the fluorescence from both endogenous and exogenous fluorophores, and therefore this paper concentrates on point measurements.

Most point measurements of fluorescence make use of intrinsic fluorescence. However, some spectrally resolved measurements of exogenous fluorophores have been made, (Baert *et al* 1993) and in some cases a combined measurement of fluorescence from exogenous and endogenous fluorophores has been used as a diagnostic. This combined approach, sometimes called ‘contrast enhancement’ was taken by Andersson-Engels *et al* (1990) and Svanberg *et al* who made multiple *in vitro* measurements of prostate and breast tissue and *in vivo* measurements in the brain and lung (Svanberg *et al* 1994).

Some of the earliest work on diagnostic fluorescence spectroscopy, by Profio *et al* (1983) and by Alfano *et al* (1984), addressed differences in the native UV-induced fluorescence in tissues of different pathology states. Initial, *in vitro*, studies of autofluorescence due to a single excitation wavelength such as these have been performed on a wide variety of tissue types, including dental, gynaecological, kidney, lung, thymus, and a variety of tissues in the GI tract (Andersson-Engels *et al* 1990, Tata *et al* 1986, Alfano *et al* 1984b, 1987, Vaarkamp *et al* 1995, Schmitt *et al* 1991, Tang *et al* 1993, Lam *et al* 1990). In several of these studies there is a general trend that fluorescence from normal tissue is greater than fluorescence from abnormal tissue. This trend and other correlations seen with *in vitro* studies have demonstrated the potential for fluorescence spectroscopy as a real-time non-invasive diagnostic.

A more sophisticated method of autofluorescence diagnosis, called excitation–emission matrix spectroscopy, utilizes multiple-colour illumination (sequentially), with the full fluorescence spectrum recorded for each excitation wavelength, and the data display typically looks like a contour map (Richards-Kortum *et al* 1989d). The different excitation wavelengths might be expected to variously excite different chromophores, resulting in more complex emission patterns with more information relevant to biochemical changes than for

single-colour excitation, and with presumed greater likelihood of distinguishing malignancy from normal conditions. This technique has been used for examining the fluorescence from brain tissue (Chung *et al* 1995) and from normal skin *in vivo* (Sterenborg *et al* 1994a), and has been used *in vitro* both for identifying spectral regions of interest for diagnosing cervical cancer (Mahadevan *et al* 1993, Richards-Kortum *et al* 1994) and for distinguishing different organisms causing *otitis media* (Werkhaven *et al* 1994). The general technique is based on earlier developments in the field of chemical engineering (Warner *et al* 1979, 1985).

While *in vivo* measurements can demonstrate a potential for diagnosing tissue pathologies, care must be taken when interpreting the results of *in vitro* measurements. The biochemical properties of tissue may be significantly different *in vitro* and *in vivo*. For example, the ratio NAD^+/NADH may change, and blood content and oxidation state are likely to change. These changes can influence fluorescence spectra in the UV and visible (Cothren *et al* 1990, Richards-Kortum *et al* 1991, Hung *et al* 1991). To truly test the effectiveness of noninvasive diagnostics, *in vivo* clinical trials must be performed with a significant number of patients. Clinical trials including tens of patients have been performed for several organs including the colon, cervix, and oesophagus and are described below. The criteria used for evaluation of the efficacy of these studies are the sensitivity and specificity. Sensitivity is defined as the percentage of diseased sites that were found to be abnormal by the fluorescence diagnostic metric. Specificity is the percentage of normal sites that were found to be normal by the fluorescence diagnostic metric.

One clinical study of the applicability of fluorescence spectroscopy to the diagnosis of tissue pathologies of the oesophagus included 48 patients (Vo-Dinh *et al* 1995). A nitrogen pumped dye laser at 410 nm was used for excitation via a fibre-optic probe. About 5–10 s was spent at each location to accurately locate the probe and take the data. Data were analysed by first dividing the intensity at each wavelength by the total area under the curve. A baseline curve was then calculated as the mean average of a selected number of normal samples. The differential normalized fluorescence (DNF) for a tissue sample is then the difference between its normalized fluorescence spectrum and the baseline curve. For the 104 tissue samples compared with histopathology results, the authors reported a sensitivity of 100% and a specificity of 98%.

Another *in vivo* clinical trial was performed to assess the efficacy of fluorescence spectroscopy for the diagnosis of pathologies of the cervix. (Ramanujam *et al* 1994a, b). In this trial, 115 sites (66 colposcopically normal areas and 49 histologically abnormal areas) in 28 patients were examined. A nitrogen laser at 337 nm was used for illuminating the tissue. The fibre-optic probe, with separate delivery and collection fibres, incorporated a tip with a 2 mm thick quartz flat to maintain the same spacing between the fibres and the tissue for all measurements (in contrast to the fibre ends being in direct optical contact with the tissue, as in the trial described above). The diagnostic algorithm for spectroscopically separating normal from abnormal tissues compared a spectral feature (the normalized slope of the spectrum at 420–440 nm) with the relative peak fluorescence intensity, and was able to diagnose all abnormal tissue with a sensitivity of 92%, and a specificity of 90%. More specifically, cervical intraepithelial neoplasia (CIN) was diagnosed with a sensitivity of 87% and a specificity of 73%.

Two *in vivo* studies of the reliability of fluorescence spectroscopy for the diagnosis of colon cancer have been reported. In a study by Cothren *et al* a nitrogen pumped dye laser, at 370 nm, was used for excitation to examine 67 sites in 20 patients (Cothren *et al* 1990). The probe used for light delivery and collection was similar to that used in the clinical study of cervical cancer described above. The metric in this case was quite

simple—a plot of the intensity at 680 nm versus the intensity at 460 nm. Using this diagnostic, a sensitivity of 100% and a specificity of 97% for detecting adenomas versus normal and hyperplastic tissue were achieved. In a study by Schomaker *et al* a nitrogen laser (337 nm) was used to examine 91 polyps and 86 normal colonic tissues in 61 patients (Schomacker *et al* 1992). In this study, the probe consisted of only one 600 μm optical fibre, which was used in contact with the tissue for both light delivery and light collection. After normalizing a fluorescence spectrum to unity, multivariate linear regression (MVLR) was used to determine which wavelengths were most significant for discrimination. In this manner an algorithm for diagnosing neoplastic tissue with a specificity of 80% and a sensitivity of 92% was developed. When the MLVR analysis was performed on the polyps alone the sensitivities and specificities for separating neoplastic and hyperplastic polyps were 86 and 77% respectively.

Laser-induced fluorescence spectroscopy for the *in vivo* detection of skin pathologies has also been investigated, although the studies involved smaller numbers of patients/measurements. Skin contains the chromophores elastin, collagen, keratin, and NADH, which are expected to contribute to the fluorescence spectrum, as well as purely absorbing chromophores such as melanin and hemoglobin. Leffell *et al* studied fluorescence of skin with 325 nm excitation in hope of correlating the results with chronological aging or photoaging of the skin (Leffell and Stetz 1988). Although they did not see any correlation with chronological aging, they did find a correlation with photoaging. This correlation is believed not to be caused by simple differences in melanin content, although this was not rigorously proven. Fluorescence from skin has also been considered as a diagnostic for skin cancer. Lohmann *et al* have reported several studies of skin fluorescence for the diagnosis of melanoma (Lohmann *et al* 1991, Lohmann and Paul 1988, 1989). In one study where 365 nm excitation was used on 147 lesions, they were able to distinguish non-dysplastic nevi from melanoma and dysplastic nevi. The metric used was the ratio of the maximum fluorescence intensity outside the lesion to the maximum fluorescence intensity inside the lesion. The choice of this metric was motivated by the finding that the fluorescence intensity inside the tumour region was much smaller than that of normal tissue, and the fluorescence intensity immediately outside the tumour was larger than for normal tissue. However, few experimental details were provided. The results are in contradiction with those of Sterenborg *et al* (1994a, b), who attempted to reproduce the results of Lohmann *et al* and examined the fluorescence from several types of skin lesion, also with 365 nm excitation. They concluded that there were no significant differences between the fluorescence of control sites and non-melanoma skin tumours. For the eight melanomas and eight benign pigmented lesions they stated that 'neither the shape of the fluorescence intensity distribution, nor the spatial distribution of the fluorescence intensity showed any signature specific to the histopathological nature of the lesions investigated'. Also, by removing the stratum corneum and measuring the fluorescence, they established that an important component of the fluorescence is from keratin in the stratum granulosum. Minor contributions to the fluorescence from other endogenous chromophores were also noted in the tumours, but were not reliable enough to be used as a cancer diagnostic.

Another area of application of LIFS has been for the detection of atherosclerotic lesions. The microscopic pattern of fluorescence in atheromas was studied as early as 1956 (Blankenhorn and Braunstein 1956). In the 1980s researchers began investigating LIFS as an intraluminal diagnostic technique for arterial tissue (Sartori *et al* 1987, Kitrell *et al* 1985) and developing optical-fibre catheters for *in vivo* use (Richards-Kortum *et al* 1989a). The diagnosis of arterial plaque is important for the application of laser angioplasty, and LIFS has been tested for guidance of laser ablation *in vitro* and *in vivo* (Papazoglou 1995,

Deckelbaum *et al* 1989). Related to the detection of atherosclerotic plaque are investigations of LIFS for the identification of fibrotic endocardium and myocardium, and sinoatrial and atrioventricular nodal conduction tissue for the treatment of arrhythmia (Perk *et al* 1991, 1993). Also, work in the area of monitoring heart electrical activity using voltage sensitive dyes began in the early 1980s (Nassif *et al* 1985, Dillon and Morad 1981).

This review will not attempt to cover the well established uses of fluorescence in ophthalmology (e.g., retinal vessel imaging) (Docchio 1989). Recent research has shown that LIFS, when applied to ocular tissues, may be useful in diagnosing both ocular and other pathologies. Zuclich *et al* (1994) have studied the fluorescence of the human lens, with excitation–emission matrix spectroscopy, as a diagnostic for aging-related dysfunctions, and the predisposition for early onset of cataract formation. Interesting correlations were found between spectral signatures and the age-appropriate condition of the lens. Fluorescence spectroscopy of the lens has also been shown to have potential for non-invasive diagnosis of diabetes mellitus (Eppstien and Bursell 1992). Fluorescence spectroscopy may also be used for monitoring the metabolic state of the cornea (Piston *et al* 1995).

Measurements of NADH fluorescence have been used for monitoring metabolism, with some of the earliest work being reported by Chance *et al* (1965). Renault *et al* developed a system combining fluorescence and reflectance for *in situ* on-line monitoring of NADH concentration *in vivo* (Renault *et al* 1984). More recently NADH fluorescence has been applied to *in vitro* measurements of redox changes in ischemic myocutaneous flaps (Cordeiro *et al* 1995) and to measurement of metabolism in the heart and brain (Rampil *et al* 1992, Osbakken *et al* 1989). Beuthan *et al* have also reported that the time-resolved fluorescence of NADH oscillates *in vivo* (Beuthan *et al* 1993).

Changes in the concentration of NADH or the redox state of flavin co-factors (e.g., FAD) are sometimes cited as the presumed origin of the fluorescence spectral signatures (Lohmann *et al* 1989, Andersson-Engels *et al* 1991, Bottiroli *et al* 1995) that correlate with tissue pathologies such as cancer. Some authors have attempted to determine how changes in these and other chromophores affect the fluorescence signal. Ramanujam *et al* fitted *in vivo* fluorescence spectra to a model of turbid tissue fluorescence, which included contributions from NAD(P)H, FAD, collagen and elastin and took into account absorption by haemoglobin (Ramanujam *et al* 1994b). Although there is quite a bit of scatter in their data, they saw an average increase in NAD(P)H content and an average decrease in the contribution of collagen fluorescence as the tissue progresses from normal to CIN. In contrast, NAD(P)H fluorescence of colonic tissue measured *in vitro* appears to decrease as tissue progresses from normal to abnormal (Richards-Kortum *et al* 1991). The increase in the contribution of collagen fluorescence seen by Ramanujam *et al* for cervical tissue is related to the results of Schomaker *et al* and Bottiroli *et al*, both of whom assert that changes in fluorescence are at least partly due to differences in the structural organization of the tissue (Schomacker *et al* 1992, Ramanujam *et al* 1994b). In particular, in the case of polyps, there is a thickening of the mucosa, which shields some of the underlying collagen fluorescence.

3. Elastic-scattering spectroscopy

When elastic-scattering spectroscopy (ESS) is employed for tissue diagnosis, the tissue pathologies are detected and diagnosed using spectral measurements of the elastic-scattered light, in a manner that is sensitive to both scattering and absorption properties of the tissue, over a wide range of wavelengths. The use of a technique that is sensitive to the wavelength dependence of scattering efficiency and angles, as well as to absorption bands, is based on

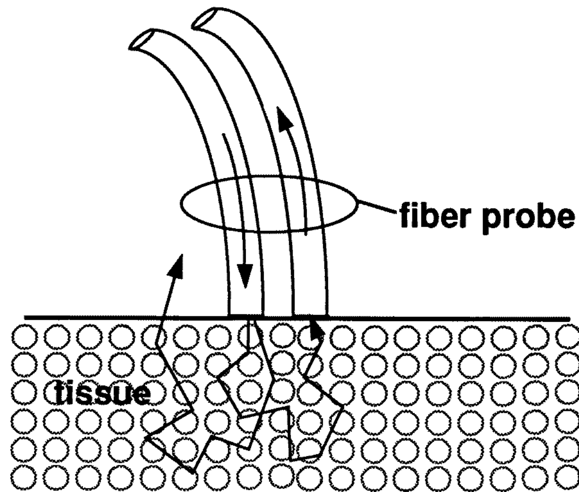

Figure 1. A schematic diagram showing the optical geometry of the fibre-optic probe used in optical contact with the tissue for elastic-scattering spectroscopy. Only light that has multiply scattered through the tissue can enter the collection fibre.

the fact that many tissue pathologies, including a majority of cancer forms, exhibit significant architectural changes at the cellular and sub-cellular level. The intent with this approach is to generate spectral signatures of relevance to the tissue parameters that pathologists address. After preparing a slide, a pathologist performs a microscopic assessment (histopathology) of the cell architecture or morphology: the sizes and shapes of cells, the ratio of nuclear to cellular volume, the form of the bilipid membrane, clustering patterns, etc. Since the cellular components that cause elastic scattering have dimensions typically of the order of visible to near-IR wavelengths, the elastic scattering properties will exhibit a wavelength dependence that is more complex than for simple $(1/\lambda^4)$ Rayleigh scattering. When source and detector fibres are sufficiently separated for the diffusion approximation to be valid (typically $\geqslant$0.5 cm), the spectral dependence of the collected light will be insensitive to the size and shapes of the scattering centres. However, for small separations ($\leqslant$ 0.1 cm), as with an endoscope-compatible probe, the wavelength dependence is readily measured. Thus, for such geometries, morphology and size changes can be expected to cause significant changes in an optical signature that is derived from the wavelength dependence of elastic scattering. These principles underlying ESS have been discussed in publications by Bigio and coworkers (Boyer *et al* 1995, Bigio *et al* 1994).

In clinical demonstrations of ESS for tissue diagnosis, the probe was designed to be used in optical contact with the tissue under examination and has separate illuminating and collecting fibres. Thus, the light that is collected and transmitted to the analysing spectrometer must first undergo multiple scattering through a small volume of the tissue before entering the collection fibre(s) (see figure 1). No light is collected from surface reflection; therefore, ESS is probably a more accurate name for this method than 'reflectance' spectroscopy. With ESS the resulting effective path length of the collected photons is generally several times greater than the actual separation of the fibre tips. Consequently, the system has good sensitivity to the optical absorption bands of the tissue components, over its effective operating range of 300–750 nm, and such absorption features add valuable complexity to the scattering spectral signature. It is important to note that the fibre probe,

being used in optical contact with the tissue, examines only that site and does not image the tissue surface.

Although the instrument used in these experiments generates a spectrum that characterizes the wavelength dependences of both scattering and absorption without separating these contributions, these composite signatures appear to correlate well with differences in tissue types and condition. The potential of this technique *in vivo* has recently been demonstrated in the bladder where a sensitivity and specificity of 100 and 97%, respectively, were obtained in preliminary clinical studies (Mourant *et al* 1995a). A clinical test of ESS for diagnosis of cancer in the GI tract also showed encouraging results (Mourant *et al* 1995b, 1996). In that study 60 tissue sites in 16 patients were measured in the lower GI tract (colon and rectum). A spectral metric, based on the regions of the haemoglobin absorption bands (400–440 and 540–580 nm), was developed to separate the eight sites that were diagnosed under histopathology as being dysplasia, adenoma or adenocarcinoma, from normal mucosa or more benign conditions (e.g., quiescent colitis). The sensitivity of this metric was 100% with a specificity of 98%. In the same study the authors reported a correlation between a different metric (the spectral slope in the 435–440 nm region) with the diagnosis of colitis versus normal mucosa. As with all studies involving a small number of patients, these reports should be interpreted cautiously until measurements have been performed on a larger number of patients.

Reflectance spectroscopy can be closely related to ESS, depending on the method of implementation. In general, reflectance spectroscopy refers to the detection of both the diffuse and specular components of the reflectance. In some cases reflectance measurements have been performed with contact probes, wherein it is essentially identical to ESS (Sato *et al* 1981, Renault *et al* 1984). Probes have been developed for sensing the fibre contact with the tissue and for making measurements at specified pressures (Ono *et al* 1991). These methods should reduce the variations in capillary perfusion due to variations in the pressure of the probe tip on the tissue surface. The primary application of reflectance spectroscopy in the visible has been for studies of skin, and the optical properties of skin have been studied in some detail (Anderson and Parrish 1981, Saidi *et al* 1995). The use of the reflectance of light from skin as a diagnostic tool is an old technique—doctors have always derived information from visual observation of a patient. (A patient who appears blue may be hypoxic!) Dawson *et al* developed a reflectance spectrometer and a theoretical model for indices of melanin and haemoglobin (Dawson *et al* 1980). Feather *et al* developed indices for haemoglobin and oxygenation (Feather *et al* 1989), and Hajizadeh-Saffar *et al* have examined the accuracy of these indices (Hajizadeh-Saffar *et al* 1990). Measurements of UV-induced pigmentation and erythema have also been made by Kollias and Baqer (1988). More recently, applications of reflectance spectrometry have also been developed for measurement of bilirubin concentration (Saidi *et al* 1991).

The application of reflectance spectroscopy to the detection of skin cancer has been pursued by Marchesini *et al*. Their technique incorporates a modified integrating sphere with a standard UV/VIS spectrophotometer, and measurements are made over the range 420–780 nm. In a study of 31 primary melanomas and 31 benign nevi they were able distinguish the two groups with a sensitivity of 90.3% and a specificity of 77.4% (Marchesini *et al* 1992). They are developing a CCD-based imaging technique (Marchesini *et al* 1995).

4. Some summarizing comments

Fluorescence and elastic-scattering (including reflectance) spectroscopy for tissue diagnosis are being investigated for a wide range of situations. Metrics based on the spectra of

fluorescence emission or scattered light are being developed for a wide variety of tissue pathologies. It is important to note that these metrics are likely to depend on the optical geometry of light delivery and collection. For example with LIF the geometries used must be tailored to maximize the fluorescence collected from the regions of interest, which for epithelial cancers is near the surface of the tissue. The volume of tissue that is interogated by fibre-optic probes is being modelled by our group and others (Qu *et al* 1995, Mourant and Hielscher 1996).

Whereas LIF has the advantage of being useful for surface imaging, elastic-scattering spectroscopy offers some advantages for point measurements. The use of less expensive detectors is enabled with ESS because the optical signals are much stronger than for LIF. Furthermore, a white-light source can be employed rather than a laser. The data acquisition and storage/display time of reported systems is typically <1 s. On the other hand, fluorescence spectroscopy offers the possibility of detecting compounds whose absorption lies in the midst of other strongly absorbing species, but whose fluorescence is separated from interfering contributors, and fluorescence spectroscopy is more amenable to imaging applications.

Most of the developments of ESS and fluorescence spectroscopy discussed in this review have used cw, unpolarized light. The use of polarized excitation and detection for both ESS and fluorescence may provide information for more detailed and/or accurate diagnosis of tissue pathology. Time resolved broad-band spectroscopy may also provide additional information.

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