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Review

Spectroscopic Sensing of Cancer and Cancer Therapy

Current Status of Translational Research

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ABSTRACT

Various types of optical spectroscopy have been investigated as methods to effect a non-invasive, real-time in-situ assessment of tissue pathology. All of these methods have one basic principle in common: the optical spectrum of a tissue contains information about the biochemical composition and/or the structure of the tissue, and that information conveys diagnostic information. The biochemical information can be obtained by measuring absorption, fluorescence, or Raman scattering signals. Structural and morphological information may be obtained by techniques that assess 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 hemoglobin saturation, intra-luminal detection of atherosclerosis, and simply the identification of different tissue types during procedures. Optical spectroscopic measurements can also be employed in the management of disease treatment. The site-specific pharmacokinetics of chemotherapy and photodynamic therapy agents can be used to customize dosage to the patient, and diagnostic spectroscopy can be used to monitor response to treatment. In recent years clinical studies have provided indications of potential efficacy, and some of these modalities are now entering a translational research stage, with an eye to approval and commercialization. A benefit of these methods is their inherent low cost and ease of implementation, generally mediated with small portable instruments, not requiring any specialized facilities, and eventually not requiring expert interpretation. This paper reviews briefly the most common methods of diagnostic optical spectroscopy, and reviews in greater depth recent clinical translational research invoking scattering spectroscopy as the enabling technology, which has been the experience of the authors.

INTRODUCTION

Over the past several years the term "optical biopsy" has entered into common usage among researchers in the field of biomedical optics. Although it is inherently an inaccurate term—it 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—it nonetheless is commonly understood to represent the use of some form of optical measurement, generally a type of optical spectroscopy, to non-invasively (or minimally-invasively) perform a tissue diagnosis, in situ, in vivo and in real time. The motivations for such developments are several:

1. to reduce the need for surgical removal of tissue samples; rather, some form of spectral analysis of the tissue is recorded in vivo with an optical probe placed on or near the surface of the tissue in question.
2. Guidance can be provided for surgical biopsies in cases where the alternative (say in surveillance) is for a large number of random biopsies when dysplasia is not clinically visible;
3. assessment of resection margins during surgery. The measurement is frequently mediated by optical fibers, and a diagnosis of the tissue is then attempted based on the optical measurements.

Figure 1 illustrates the variety of processes possible when an optical photon impinges at the surface of a tissue boundary. (This could be, for example, the epithelial surface of the esophagus.) For case "a" the simple "Fresnel" reflection from the surface is generally uninteresting because it carries little information about the underlying tissue. Case "b" represents back scattering from cellular and structural components of the tissue, following one or a few scattering events, termed "elastic scattering" because the energy of the photon is not altered by the scattering process. The wavelength dependence of the scattering probability depends on sizes and densities of sub-cellular structures, and consequently the elastically-scattered light conveys significant information about microscopic tissue structure. Case "c" represents the absorption of a photon. Photons with energies/wavelengths more likely to

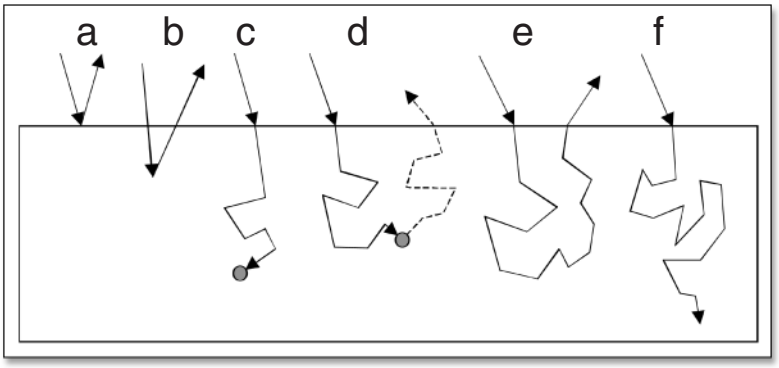

Figure 1. A variety of light tissue interactions are depicted here. Discussion for each of the cases is provided in the text.

be absorbed (due to the absorption bands of the chromophores in the tissue) are hence less likely to be scattered back, facilitating measurement of such chromophores (e.g., oxy- and deoxy-hemoglobin). Case "d" represents "inelastic" processes, wherein the energy (and, hence, wavelength) of the reemitted or scattered photon changes. The most relevant of these processes for diagnostic spectroscopy are fluorescence and Raman scattering. Cases "e" and "f" represent examples of photon trajectories for the majority of photons that simply scatter diffusely in the tissue, scattering numerous times before being absorbed or emerging from a surface. The terms photon "migration" and "diffuse" scattering are frequently used by researchers who develop functional imaging and spectroscopy methods with systems designed to sense diffusely-scattered photons.

The methods described in this review are intrinsically point measurements, and therefore do not constitute imaging modalities in the traditional sense. Other optical methods, based on analysis of photon migration in diffusely scattering media, mentioned above, offer the potential for functional imaging in deeper tissues and, in most demonstrations to date, attempt to provide information about spatial distribution of blood perfusion and hemoglobin saturation (for various reviews and publications, see ref. 1). "Optical mammography" is entering the translational stage of research, with several ongoing clinical trials to assess efficacy.[2]

The point measurements reviewed in this paper are aimed at providing more detailed information about the tissue, but for one spot at a time. (The actual volume or depth interrogated depends on the details of the optical geometry and other factors specific to the measurement and tissue type.) A general overview of the topic, from the optical science point of view, can be found in a review article by Bigio and Mourant.[3]

Most researchers have focused on UV-induced fluorescence spectroscopy, an approach that assumes that biochemical changes in malignant tissue (especially the redox state of various cofactors) will result in changes of the intrinsic fluorescence spectrum. The superficial penetration of living tissue by UV light also means that small changes in the thickness of tissue layers can be detected as variations in the intensity of the exciting light reaching the fluorescing components of the tissue, and may change the intensity of the detected fluorescence. On the other hand, elastic-scattering spectroscopy (ESS) is sensitive to the microscopic architectural changes that a pathologist looks for when conducting histological assessment. The ESS method is mediated by fiber-optic probes and in several preliminary clinical studies, has shown the potential to be a screening and diagnostic tool for cancer detection and other pathology assessments in a number of organ systems.

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 cofactors like the flavins (FMN, FAD). By measuring the UV-induced fluorescence of tissue (often called the "autofluorescence) it should, in principle, be possible to learn about the relative concentrations and redox states of such compounds, and by extension to learn something about the biochemical state of the tissue. Interpretation of tissue autofluorescence is complicated by the intrinsic scattering and absorption properties of the tissue, rendering autofluorescence measurements significantly more complicated than measurement of fluorophores in solution. Scattering cross sections are quite high in tissue[4] 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 hemoglobin. 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 invoking 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.[5-7] (For a review of methods and clinical studies, see ref. 8, and for a review of diagnostic fluorescence spectroscopy of tissue, including technical methodologies, see ref. 9).

Either single-point or imaging measurements can be performed. If a small fiber-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 a spectrally-selective image of a larger tissue surface. The technological impediments of each approach are different. In short, point measurements provide a lot 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.

Some of the earliest work on diagnostic fluorescence spectroscopy, by Profio et al.[10] and by Alfano et al.[11] addressed differences in the native UV-induced fluorescence in tissues of different pathological states. Examples of translational research of fluorescence spectroscopy for cancer detection in vivo include studies for cervical cancer,[12-14] lung cancer,[15] colon cancer,[16] and oral neoplasia.[17]

RAMAN SPECTROSCOPY

Raman spectroscopic measurements can provide detailed molecular "fingerprint" information about tissue by revealing the vibrational modes of the biomolecules that constitute tissue. Consequently, Raman spectroscopy has the potential to generate important biochemical information for tissue diagnosis. Raman spectroscopy is an inelastic scattering process, i.e., there is a difference between the energy of the incident and the scattered photons. This scattering process, typically, causes a decrease in photon energy equal to the difference between two vibrational energy levels of the molecule scattering the light. Four major components of biological tissues that contribute to Raman spectra are proteins (including collagen), lipids, nucleic acids and water. The application of Raman spectroscopy for

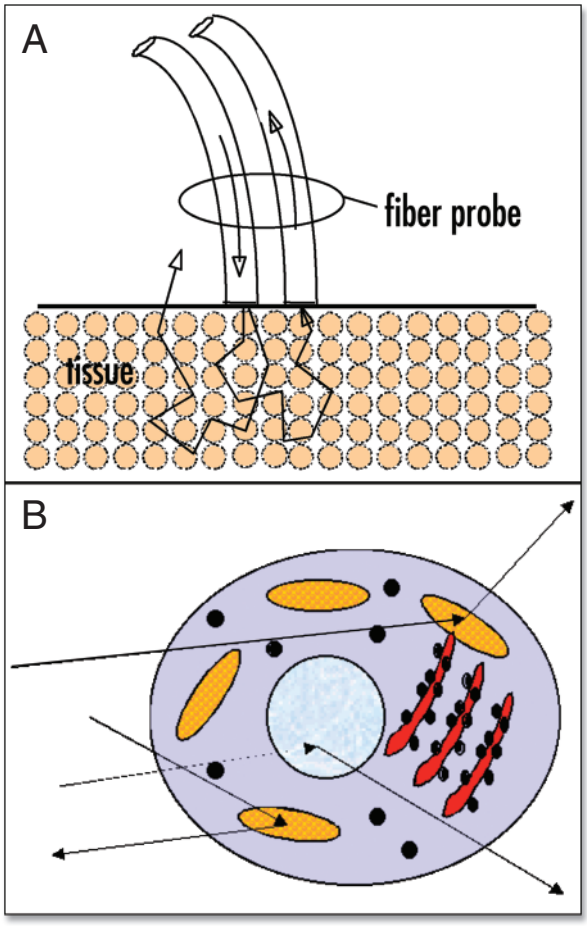

Figure 2. (A) The optical geometry for delivery and collection fibers for a typical "optical biopsy" measurement. The fiber probe is placed in gentle contact with the tissue surface; (B) cartoon depicting the scattering of photons by denser organelles and nucleus in the cell.

clinical diagnostic applications is challenging because it is a very weak effect. Additionally, tissue fluorescence as well as Raman scattering and fluorescence from optical fibers, complicate the measurement of Raman spectra of tissue in vivo. Recent advances in optical technologies have improved the prospects of Raman spectroscopy for in vivo diagnostics,[18-20] and some publications reporting clinical translational studies have started to appear.[21,22] The equipment required is relatively complex and expensive and difficult to adapt for in vivo use, especially for internal use, as with endoscopes. Nevertheless, if the results prove to be good enough, Raman spectroscopy may prove to be complementary to other optical diagnostic techniques looking at different aspects of the tissue under interrogation. This could improve diagnostic specificity and sensitivity, particularly for detecting pre-cancerous and early cancerous changes.

ELASTIC SCATTERING SPECTROSCOPY

ESS diagnosis is based on either heuristic models[23] that predict changes in the scattering spectrum corresponding to morphological changes at the cellular and sub-cellular level, or more quantitative models[24] used to determine the sizes of nuclei and/or other organelles in epithelial tissue. Both approaches show merit for clinical translational demonstrations. The typical optical geometry for scattering spectroscopy is depicted in Figure 2A. The scattering occurs because of gradients in the optical index of refraction, resulting from differences

Table 1 **List of Approximate Values for the Optical Refractive Index of Different Cellular Components, for a Wavelength of 500 nm**

water	n ~ 1.33
extra-cellular fluid	n ~ 1.34–1.35
intra-cellular fluid	n ~ 1.35–1.36
proteins	n ~ 1.40?
lipids	n ~ 1.42?
DNA	n ~ 1.44?
bi-lipid membrane	n ~ 1.46, but BLM is very thin ∴ scattering very weak from cell walls
melanin	n ~ 1.65?

The question marks indicate that there is a significant range of published values, and different molecular forms have different values.

in densities and compositions of sub-cellular structures like nuclei, mitochondria and other organelles. This is depicted in cartoon-like fashion in Figure 2B.

Elastic-scattering spectroscopy, when performed using an appropriate optical geometry (with small fiber separations of <400 microns)[23,24] is sensitive to the sizes, indices of refraction and structures of the denser sub-cellular components (e.g., the nucleus, nucleolus, mitochondria, etc.) that change upon transformation to pre-malignant or malignant conditions.[25] The measured ESS spectrum relates to the wavelength-dependence and angular-probability of scattering efficiency of tissue micro-components, based on the fact that many tissue pathologies (and most cancers) exhibit such morphological changes at the cellular and sub-cellular level. Consequently, this approach generates spectral signatures of relevance to the tissue parameters that pathologists address: the sizes and shapes of nuclei and organelles, the ratio of nuclear to cellular volume, clustering patterns, etc. Since scattering is induced by gradients of the optical index of refraction, ESS spectral signatures will also be altered if the refractive index of nuclei or organelles changes, say, due to an increase in the amount of chromatin or granularity of the chromatin.

Table 1 provides some values for the refractive index of the more significant constituents in the cell, in addition to scattering by the nucleus and organelles, like mitochondria, which have a wavelength dependence that is informative about their size.

Figure 3 shows the principal components of an ESS measurement system. The system is easily portable (about the size of a shoe box) and invokes low-cost components.

It is important to note that in the clinical studies of ESS to date, the method is a site-specific measurement (rather than an imaging modality), sampling a small tissue volume of, typically, ≤0.05 mm^3, whose optical geometry is depicted in Figure 2A. ESS probes are designed to be used in optical contact with the tissue under examination and have separate illuminating and collecting fibers. (Research is currently ongoing for the development of imaging implementations of diagnostic ESS, for certain clinical applications.)

CLINICAL EXAMPLES OF ELASTIC SCATTERING SPECTROSCOPY

Gastrointestinal (GI) Endoscopy. There are a variety of clinical circumstances in GI endoscopy wherein a non-invasive real-time indicator of pathology would be beneficial. For circumstances in which "random" biopsies are taken in an attempt to find pre-malignant or early malignant conditions, an instant optical measurement could

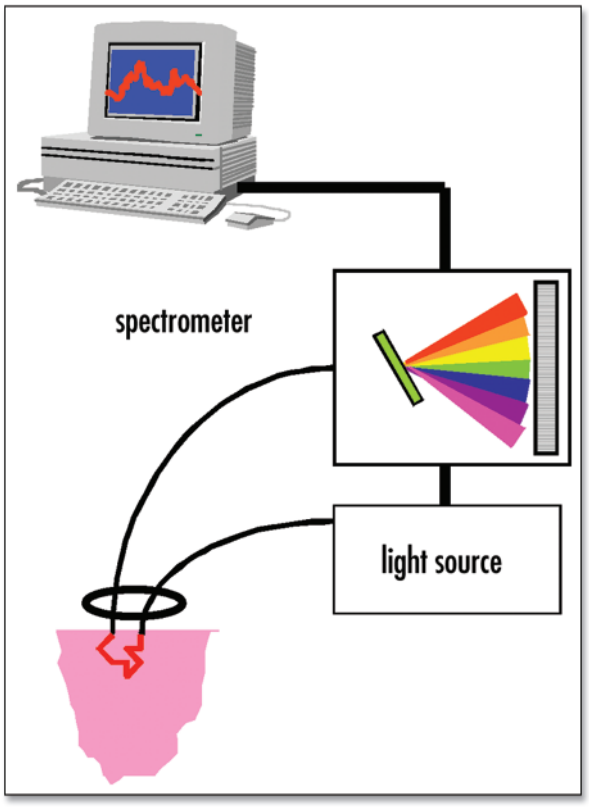

Figure 3. System components for elastic scattering spectroscopy.

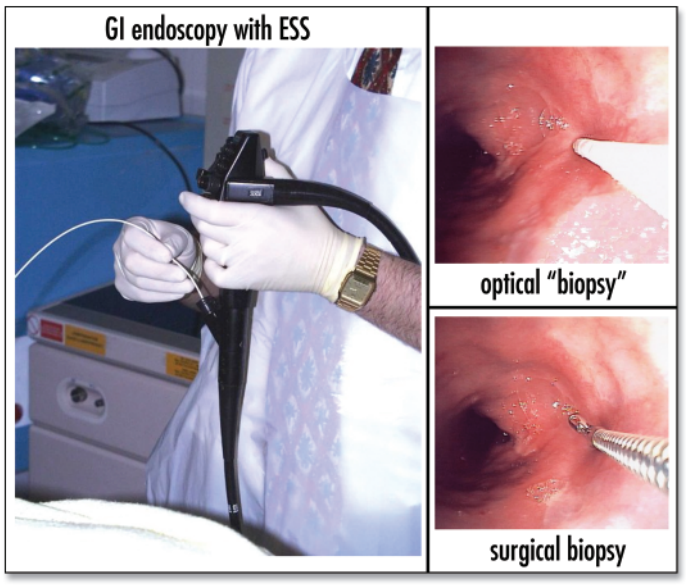

Figure 4. The physician inserting the ESS fiberoptic probe through the working lumen of an endoscope, and the "endoscope's eye" view of an ESS measurement being taken followed by a conventional surgical biopsy on the same spot of an area of Barrett's epithelium. The pale pink tissue is normal esophagus, the red tissue in the distance with folds is normal stomach and the deeper pink tissue without folds is the region of Barrett's esophagus. There is no clear demarcation between Barrett's esophagus and stomach and the junction between Barrett's esophagus and normal esophagus is irregular. Just by looking down the endoscope, it is impossible to tell whether any areas in the Barrett's region are developing dysplasia. (Figure courtesy of Dr L. Lovat).

enable "guided biopsy", with increased probability for sampling a diseased site, while reducing the number of tissue samples. Thus, in addition to improving sensitivity, motivation is provided by the potential for reducing health-care costs as a consequence of eliminating unnecessary histology. Moreover, the immediacy of diagnostic information can provide other benefits for clinical management.

Several disorders of the GI tract are correlated with a pre-disposition for cancer, including chronic ulcerative colitis, colon polyps, and Barrett's esophagus. Typically these diseases are followed with regular surveillance by endoscopic examination accompanied by tissue biopsies. As many as 20–30 "random" biopsies may be taken in one session. This is a time consuming (and 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 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. When a suspected diseased site is found, the surgical biopsy can be performed at that particular site.

Biopsy surveillance for dysplasia (pre-cancerous change) in Barrett's esophagus using white light endoscopy has a poor diagnostic yield. In clinical studies carried out at the University College London Hospitals,[26] Lovat et al. reported correlation of 890 ESS spectra with matched esophageal biopsies, taken from 356 sites in 96 patients with Barrett's esophagus. ESS spectra were collected through the flexible fiberoptic probe that contained the two parallel, adjacent fibers, one to deliver the light to the tissue and the other to detect scattered light. The probe tip was placed in gentle contact with the tissue surface for the sites being interrogated. All biopsies were reviewed by three GI pathologists and defined as non-dysplastic, low or high-grade dysplasia or carcinoma. Each optical "biopsy" required only a fraction of a second to perform. Spectral data were normalized and analysis of spectra was carried out for the spectral range 340 and 900 nm using principle component analysis and linear discriminant analysis. Agreement between pathologists for HGD and cancer was high ($\kappa = 0.71$). Only biopsies where there was consensus were used for analysis. Histological findings were correlated with appropriate spectra. For each analysis 2/3 of the data sets were used for training the pattern recognition methods, and others were reserved for testing. Preliminary analysis gives a sensitivity for detecting an abnormality (cancer or dysplasia) of 71% (68–73%) with a specificity of 90% (84–96%). In a population undergoing routine surveillance, the negative predictive value of the test would be 96%. These results offer promise that this technique has potential as a real time diagnostic test for in vivo diagnosis of dysplasia or cancer within Barrett's mucosa, or as guidance for conventional biopsy to improve sensitivity over that of random siting. This might permit endoscopists to target biopsies effectively with an improved degree of certainty that high- grade dysplasia or cancer were not being missed, while dramatically reducing the total number of biopsy samples. Bearing in mind that Barrett's esophagus may affect up to 0.5% of the population (about 10% of individuals with long standing reflux of gastric acid) and that over a lifetime, about 10% of the Barrett's patients may develop dysplasia, surveillance of this population is a massive task for gastroenterologists. Even though only a small percentage of those with dysplasia go on to develop life threatening cancers, it is essential to identify those at risk and treat any that show signs of developing serious disease.

Other research groups have also reported studies of diagnostic scattering spectroscopy in GI endoscopy. Zonios et al. reported a clinical study in which scattering spectroscopy was used to distinguish adenomatous from hyperplastic colonic polyps.[27] Wallace et al. employed scattering spectroscopy in the detection of dysplasia within Barrett's esophagus;[28] and Backman et al. reported on development of a method using polarized scattering spectroscopy for quantitative assessment of nuclear size in epithelial cells.[29]

Diagnostic Applications in Breast Cancer. Within solid organs, such as the breast, the least invasive approach requires access through a needle, by fine-needle aspiration cytology (FNA) or core biopsy. Surgical wide local excisions (also known as lumpectomies) are also commonly performed as diagnostic procedures, although it is always planned that all the diseased tissue should be removed during the procedure. Of the approximately 50,000 diagnostic lumpectomies performed annually in the U.S, as many as 75% are determined to be benign.[30] Consequently, core biopsy under imaging guidance is becoming more prevalent. Although slightly less invasive than core biopsy, FNA is less frequently used in the U.S. because false-negative rates with FNA are often in the range 12–15%,[31,32] and staging is not practical, due to the heterogeneity of breast lesions and the relatively small number of cells accessed by FNA. Despite having the same level of invasiveness as FNA (access through a fine needle), the ESS method offers the potential advantages of immediate diagnosis and the interrogation of a small volume of tissue around the tip of the optical probe without the need to remove that tissue from the patient. Sensing could be effected along the entire track of the needle, as it is inserted, if the fiber probe is incorporated into the needle tip in an appropriate design that preserves tip sharpness. Immediate diagnostic information could reduce patient anxiety, and treatment regimes could begin sooner. Moreover, image-guided optical biopsy could prove valuable for patients with more than one suspicious area.

Peri-surgical applications in breast cancer may be of greater value than needle diagnostics. Assessment of resection margins during breast-conserving surgery (wide local excision or partial mastectomy) can reduce the incidence of cancer being left at the margins, which happens in as many as 15% to 40% of breast-conserving surgerical procedures.[33] (The higher incidence is more common in countries where the surgical "style" is less aggressive than in the U.S.).

The other potentially important surgical application being investigated is real-time assessment of sentinel lymph nodes in the axilla (the region under the arm where breast cancers first spread). There are many lymph nodes in the axilla, but the sentinel node is the first one reached by lymph draining from the breast (there may be more than one sentinel node if there is more than one "chain" of nodes for each breast). The presence or absence of metastatic cancer in the axillary lymph nodes in patients with breast cancer remains the most powerful predictor of prognosis. Traditionally, the presence of axillary lymph node metastases has been determined by axillary lymph node dissection (ALND, removal of all the lymph nodes in the axilla, which means also removing the nodes that drain the arm). The procedure can be associated with several serious side effects, the most significant being lymphoedema (swelling of the arm due to blockage of the lymphatics) and shoulder dysfunction, which may seriously affect the patient's quality of life.[34] In current surgical practice, patients tend to present with earlier disease, as a result of increased public awareness of breast cancer, and mammographic screening programs. Hence most patients do not have axillary lymph node metastases at presentation, and while the staging information is crucial for their future management, they get no therapeutic benefit from ALND, whilst still being at risk of developing the complications associated with the procedure.

It has been well documented that if cancer cannot be detected in sentinel nodes, the chance of there being any cancer in nodes further down the chain draining the breast is exceedingly small.[35] Thus if the sentinel node can be easily identified, removed and examined for cancer and no cancer is found, there is no need to remove the rest of the axillary nodes. This markedly reduces the risk of complications associated with full axillary node clearance.[36,37] The main techniques for identifying sentinel nodes are by injection of a radioactive tracer or injection of a blue dye into the breast in the immediate vicinity of the cancer and then either scintigraphy of the axilla or direct inspection at the time of surgery. To get the maximum benefit from sentinel node biopsy, it is important to be able to determine rapidly whether or not cancer is present. If the assessment cannot be completed very rapidly after the node is removed, whilst the patient is still on the operating table, the subsequent discovery of cancer in the node will necessitate a second operation to complete the ALND. Traditionally, intra-operative diagnosis has been done by frozen section histology. In practice, because of their soft texture, frozen sections of lymph nodes are technically difficult to prepare and interpretation may be difficult. Accurate results from frozen sections rely on the skill of an experienced pathologist. It is therefore not surprising that there is wide variation in the reported accuracy of this technique. The best results are those reported by the European Institute of Oncology in Milan, but these were only achieved by 50 micron sectioning.[38] Exhaustive frozen section examination is highly accurate, but consumes vast resources in manpower and time and may not be appropriate in the setting of smaller hospitals. Routine frozen section examination of sentinel nodes has yielded more disappointing results, with sensitivities for the detection of cancer ranging from 44–87%.[39,40] Touch imprint cytology is one of the oldest techniques in cytology and is now being applied to the examination of lymph nodes. Immediately after excision, the node is bisected and the cut edge smeared over a glass slide, fixed and stained. It is quick and easy to perform and gives similar results to frozen sections.[40-42] Both techniques, however, rely on the availability of a highly skilled pathologist, and it is likely that the excellent results reported from specialist units will not be replicated in smaller hospitals relying on a general pathologist for reporting. A real-time optical method for determining sentinel node involvement would provide significant benefits to patients undergoing surgery for breast cancer.

We previously reported very preliminary results of a clinical study of all three applications in breast cancer.[43] Encouraging correlations between spectroscopy and histology were found for both breast tissue and nodes. For sentinel nodes, more recently we reported on the next phase of the study, in which a larger dataset and new statistical methods were used.[44] From patients with breast cancer undergoing either sentinel node biopsy or axillary node clearance, sentinel nodes were identified using the combination technique of pre-operative lymphoscintigraphy using Tc^{99} labeled albumin colloid, intra-operative blue dye injection and gamma probe guided detection. All removed nodes were bivalved, and spectra were taken from several locations on the cut surfaces of both halves with the small fiber probe in gentle contact with the node. Between two and twenty spectra were collected per node (depending on the size of the node) with measurements from various locations, including sub-capsular and central regions of the node.

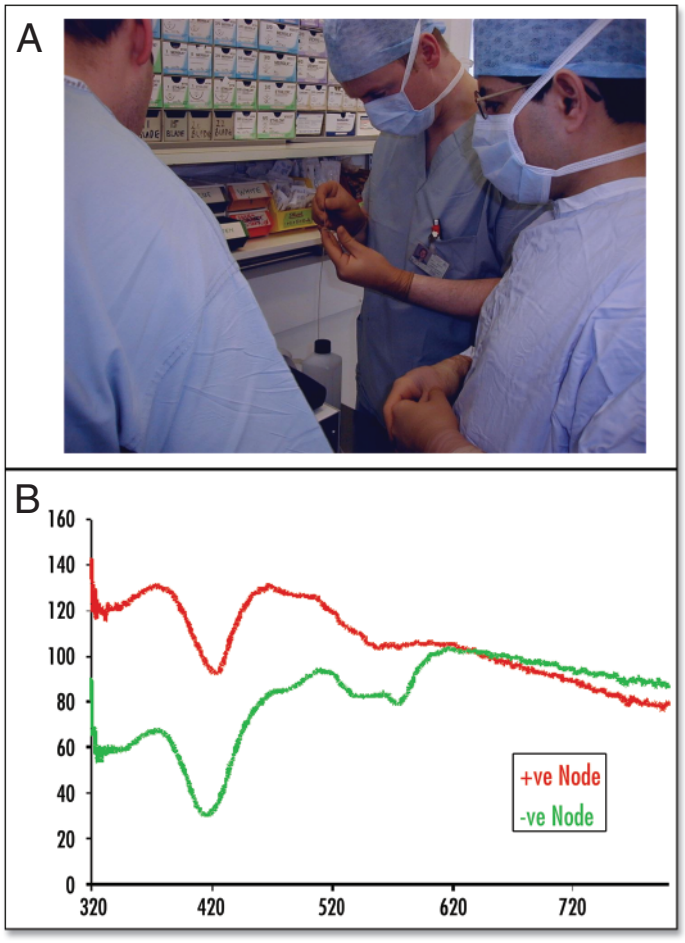

Figure 5. (A) Assessment of a sentinel node in the surgical suite; (B) Typical spectra for a normal node and a node with complete replacement by cancer.

Although multiple ESS measurements were made on each node, histology reports were not broken down by location within the node. For each node there was only a single diagnosis of metastatic or benign. Since nodes may be only partially metastatic, the statistical approach taken was to correlate the histological diagnosis with the "most aggressive" of the ESS spectra, i.e., if all spectra for a specific node were indicative of benign conditions, then the ESS designation was benign, but if any of the ESS spectra were indicative of cancer, then the ESS designation was assumed to be metastatic.

Typical spectra from a normal node and a node with complete replacement by cancer are shown in Figure 5. Multiple spectra were taken from 139 excised nodes (53 containing cancer) in 68 patients, and spectral analysis was performed using a combination of principal component analysis and linear discriminant analysis to correlate the spectra with conventional histology. The data were divided into training and test sets. In test sets of individual spectra from completely cancerous nodes, ESS detected the cancer with 84% sensitivity and 91% specificity. In test sets from all nodes (normal, partial and complete replacement by cancer), ESS detected cancer with an average sensitivity of 75% and specificity of 89%. These results are comparable to those from conventional touch imprint cytology and frozen section histology, but do not require an expert pathologist for interpretation. They also indicate that sensitivity of the ESS method, in the case of partially metastatic nodes, would benefit from larger numbers of measurements over more of the cut surfaces. This would not be a problem since each measurement takes a fraction of a second. With automation of the spectral analysis, results could be made available almost instantaneously.

Diagnostic Applications for Gynecological Cancers. The advantages of early detection for cervical cancer have been demonstrated by the Papanicolaou (Pap) smear. However, the Pap smear has limitations such as sampling errors and a low sensitivity.[45] Nordstrom et al. combined diffusely reflected scattering spectroscopy and fluorescence to identify the stages of cervical intra-epithelial neoplasia (CIN) in 41 patients.[46] Their measurements used flood illumination in a geometry, which did not block the surface reflectance and consequently caused reduced sensitivity to the spectral differences associated with structural changes. They obtained a predictive sensitivity and specificity of 77% and 76%, respectively, for distinguishing CIN II/III from metaplasia. Georgakoudi et al. demonstrated that when light scattering methods are combined with fluorescence, the sensitivity and specificity for detecting squamous intra-epithelial lesions increases substantially. Using pathology as the gold standard, and leave-one-out cross-validation, their method had a sensitivity of 92% and a specificity of 71% for detecting squamous intra-epithelial neoplasia versus mature squamous epithelium or squamous metaplasia.[47] Other reports of the most recent research in cervical cancer have also combined scattering spectroscopy with fluorescence.[48]

Richards-Kortum's group has investigated the application of reflectance spectroscopy to other gynecological cancers. In an exploratory study of ovarian cancer in 18 patients, Utzinger et al. were able to retrospectively separate ovarian cancers from normal ovary and benign neoplasms with a sensitivity of 86% and specificity of 80%.[49]

Melanoma. Skin is the most accessible organ for testing light scattering methods for cancer diagnosis. Consequently, some of the earliest work examined lesions of the skin. Research in this area is still motivated by a need for non-invasive methods to distinguish melanoma from benign pigmented lesions (nevi). Mortality from malignant melanoma is increasing worldwide and, for some regions, has doubled since the 1950s.[50,51] The diagnostic accuracy amongst primary care physicians and trainee dermatologists may be as low as 31%–67%,[52] rising to 63%–80% with experienced dermatologists and being highest amongst those with greater than 10 years clinical experience.[52,53,54] A non-invasive and objective technique to aide primary care physicians would be of value if the result was increased accuracy, and better selection for referral.

Early optical studies invoked very small numbers of patients[55] and concentrated on the absorption properties of skin. In studies with a somewhat greater number of patients, Marchesini et al.[56] reported measurements of 31 primary melanomas and 31 benign nevi, which were made using a modified integrating sphere with a standard spectrophotometer over the spectral range 420–780 nm. The data were used to develop discriminant functions. A sensitivity of 90.3% and a specificity of 77.4% were obtained for distinguishing the melanomas from nevi with leave-one-out cross validation. The data set was small, and the leave-one-out statistical method is not a prospective study; nonetheless, the results were encouraging. More recently wavelength-dependent reflectance images of skin have been obtained.[57] Both light that entered the tissue and was scattered back to the surface as well as reflection off the tissue surface were measured, making interpretation of the multispectral images difficult. A recent study by V.P. Wallace et al. measured the diffuse-reflectance spectra of skin over the spectral range 300–1100 nm without artifacts due to surface reflectance.[58] These data were analyzed using both multivariate

discriminant analysis and artificial neural networks.[59] The best results were obtained with the artificial neural network and yielded a sensitivity of 83.6% and specificity of 85.3% for diagnosing melanoma compared to compound nevi in the training set. When the artificial neural network was applied to new cases the sensitivity and specificity were 90.9% and 58.8%, respectively. Recent preliminary studies by Scarisbrick et al. utilizing elastic scattering spectroscopy as described above, have provided encouraging results with the low-cost user-friendly system and a simple diagnostic algorithm,[60] although the statistical base was, like the other studies, small. A larger prospective study is planned.

CLINICAL APPLICATIONS OF OPTICAL SPECTROSCOPY FOR CANCER THERAPY

Most work on optical spectroscopy to date has focused on diagnostic applications, particularly the early detection of cancer and pre-cancerous changes in tissue when no abnormalities are detectable to the naked eye, either directly or through an endoscope. The results are promising, but it is slowly being realized that optical techniques may also have enormous potential in optimizing the actual delivery of some types of treatment and in monitoring the results of treatment.

Optical Pharmacokinetics. The ability to measure the concentrations of various drugs and compounds in living tissues non-invasively could provide a variety of benefits for pharmacology research and, ultimately, clinical applications. The advantages of non-invasive site-specific measurements to determine drug concentrations, for chemotherapy and photodynamic therapy (PDT) drugs, are several. Clinically, the therapeutic benefit of anti-cancer drugs is a function of the concentration-time profile in tumor tissue. The toxicity is a function of the concentration-time profile in normal tissue. Therefore, the ability to track the location and time-history of compound concentrations in tissue non-invasively would be advantageous for the development of new chemotherapy drugs,[61-63] and would benefit the management of cancer treatment by facilitating the individual customization of dosage.

The concentrations and kinetics of drugs at specific locations in the body are generally difficult to determine, given only the administered dosage or blood serum measurements, as there is so much biological variation between individuals. Other than new optical methods and costly imaging methods with radioactive tags, minimally invasive methods with site specificity are very limited, the most commonly reported method being microdialysis, which invokes implantation of a needle into the tissue to sample the extracellular fluid through a semi-permeable membrane.[64] Despite its inherent invasiveness and other problems,[65] microdialysis has been developed in recent years as an in situ method of measurement for research applications. However, its invasiveness and slow response render microdialysis impractical for clinical management of treatments such as photodynamic therapy (PDT). PDT involves the activation of a previously administered photosensitizing drug by low power red light (most conveniently from a laser) in the presence of oxygen to produce localized tissue destruction.

The problem of determining in vivo tissue concentrations of drugs is especially important for PDT as the photosensitising drug is administered systemically (usually by intravenous injection) and is locally photoactivated with an appropriate wavelength of light. PDT dosimetry is complex as it involves both the drug and light, so if more information is available on these variables, like the tissue concentration of photosensitizer, it would reduce some of the uncertainty of dosimetry. PDT is being investigated for treatment of a variety of cancers. For recent reviews see Schuitmaker et al.[66] Dougherty et al.[67] and Chang and Bown.[68] PDT is also being investigated for use in non-malignant diseases such as rheumatoid arthritis[69] and the selective ablation of endometrial tissue.[70,71] It is generally difficult and inaccurate to determine the tissue concentration of a photosensitizer by measuring serum levels over time because most PDT photosensitizers clear quickly from serum and are taken up into tumor and other tissues (hopefully at lower concentrations in normal tissues).[61] The ideal implementation of PDT requires delivery of both the proper drug dosage and the proper light "dosage" to all relevant sites in the target tissue, which in turn requires understanding of the optical properties of the tissue and knowledge of drug concentration in the tissue of interest. In vivo measurements would provide information on patient-patient variability in the uptake of the drug and, therefore, make possible individual dosimetry for PDT. In addition, the site-specific time history of the drug is especially important because the photoactivation should be done when the ratio of drug concentration in the tumor to surrounding tissue is at a maximum.

In some sites such as the skin and mouth, it is easy to apply a fiber probe to make an optical pharmacokinetic measurement. This is more difficult for internal organs such as the prostate, where access may require inserting the probe using a needle placed through the skin into the organ of interest under some form of image guidance such as ultrasound, computerized tomography (CT) or magnetic resonance imaging (MRI). A possible way around this problem is seeing if measurements made at accessible sites (such as in the mouth) can be used to predict what the drug levels will be in inaccessible sites, but this approach has yet to be developed.

The Method of Optical Pharmacokinetics. In elastic scattering spectroscopy, the intent is to maximize the sensitivity of the measurement to variations in the scattering properties of tissue. The technique of optical pharmacokinetics (OP) is essentially similar to that of ESS (see Figs. 2 and 3), with separate illuminating and collecting fibers, but the specifications (fiber separation, spectral range, etc.) and mathematical treatment of the measured spectra are designed to minimize sensitivity to scattering variations and optimize the accuracy of measurement of optical absorption spectra. Since a number of chemotherapy agents, and all PDT agents (by design), are chromophores with strong optical absorption bands, they are well suited to measurement by this method (for a detailed explanation of the underlying optical physics of the method, see ref. 72). Demonstration of the method in measurement of chemotherapy drug concentrations in an animal model has also been reported.[73]

Optical Monitoring of Therapy. This is a new concept, and very little data are yet available, but it is likely to play an important role in a range of future therapies. An increasing number of new approaches to treating human diseases (particularly cancers or pre-cancerous conditions) now involve localized treatments, which destroy diseased tissue where it arises and then permit the normal healing mechanisms to remove the dead tissue with resolution by scarring or regeneration of healthy tissue. In these circumstances, it is essential to be sure that the full extent of diseased tissue has been treated. Removing 99% of a cancer will not cure a patient, but removing 100% will. It is hoped that techniques such as elastic scattering spectroscopy may be able to pick up small areas of residual cancer or pre-cancer that have spread beyond a treated area, when these areas of residual disease are not visible to the naked eye. An example of where this might be of value is in the treatment of Barrett's esophagus,

where the aim is to destroy pre-cancerous glands in the lower part of the esophagus. If the depth of glandular ablation using PDT is not sufficient, pre-cancerous glands can become buried under regenerated normal (squamous) epithelium, with the potential to later develop into a life-threatening cancer. If elastic scattering could detect these buried glands, they could be treated, and so reduce the risk of the patient later developing a cancer.

Optical assessment of the results of treatment will be of particular value after treatments like PDT and thermal ablation of diseased tissue, as these treatment do not have cumulative toxicity, so can be repeated at the same site, if necessary. It is much more difficult to repeat radiotherapy, as there is a limit to the dose that normal tissues can tolerate and any treatment of a cancer must involve treatment of the immediate surrounds of the tumor, where the cancer may be infiltrating normal tissue.

Planned Clinical Studies. Under a multi-institutional translational study funded by the NIH/NCI (CA104677), we are currently beginning a series of clinical studies to apply the method of optical pharmacokinetics for management of photodynamic therapy dosage in the treatment of cancer, with specific planned clinical studies for esophageal cancer and cervical cancer, and other organ areas under review.

SUMMARY

The increasing sophistication of optical techniques for detecting the early changes of pre-cancer and cancer are making these techniques more and more attractive as diagnostic tools. Elastic scattering spectroscopy, in particular, is simple in concept, requires relatively straightforward and low cost instrumentation and has the potential to give an immediate diagnosis without the need to remove tissue from the patient or for the result to be interpreted by an expert pathologist. A lot more work needs to be done to validate these optical techniques and to subject them to prospective clinical trials, but the potential is enormous.

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