A new tool to inform intra-operative decision making in skin cancer treatment: the non-invasive assessment of basal cell carcinoma of the skin using elastic scattering spectroscopy

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Abstract

Introduction
The aim of this study was to evaluate the findings of elastic scattering spectroscopy co-registered with histopathology in patients with basal cell carcinoma against normal and some common benign skin disorders.

Materials and methods
Clinically suspicious head and neck skin lesions were included in this study. Those lesions with surrounding innocuous skin were interrogated by elastic scattering spectroscopy, co-registered biopsies were taken and examined histopathologically; the results were then compared using a variety of statistical techniques.

Results
Our analysis showed obvious and consistent spectral differences between normal and pathological skin. Discriminating elastic spectral differences were consistently identified between basal cell carcinoma and other skin lesions of similar appearance.

Conclusion
This preliminary study shows that elastic scattering spectroscopy can distinguish between basal cell cancer, common benign conditions and variants of normal skin. Elastic scattering spectroscopy can also help determine the diagnosis between benign lesions with a high degree of accuracy suggesting that elastic scattering spectroscopy can add significant objectivity to dermatological diagnosis and management of even benign conditions.

Introduction
In essence, our desire is for a technology that delivers a pathologically accurate diagnosis in real-time in situ without the need of an attendant removing the tissue during processing. This is especially important in cosmetically sensitive areas where any tissue volume is precious not just because of its loss but also due to the difficulty of being able to provide a satisfactory surgical repair.

This study discusses the elastic spectroscopic analysis of basal cell carcinoma (BCC) of the skin, the commonest type of malignancy in the world. Often, there is diagnostic doubt which leads to delayed diagnosis or even less than satisfactory disease management. Unfortunately, a tumour is often thought of in two not three dimensions with regard to treatment, although convenient and usually clinically insignificant, in areas of cosmetic importance or near vital structures, a three-dimensional (3D) resection is vital to achieve the important goals of disease removal and functional preservation.

Visual assessment, although important, can be supplemented by more objective technology for the benefit of the patient and to guide proper treatment delivery and its efficacy. Although there are many management pathways and a modest degree of time insensitivity in the treatment of BCC, the methodology can also be applied to other diseases requiring more stringent time sensitive management with a goal of rapid and complete definitive surgical treatment.

The knowledge of true diagnosis and limit of the disease aids in the delivery of an adequate surgical margin and an improved prognosis for a patient. We examined several areas of BCCs against normal controls and common benign skin lesions. We show that it is possible to use this optical technology to inform a clinician regarding the diagnosis and to guide surgical and photodynamic treatment in ‘real-time’.

BCC presents as subtle, painless, non-healing ulcers or nodules on the sun-exposed parts of the body. BCC has many clinical variants and each has its own histological pattern. BCC constitutes approximately 80% of all non-melanoma skin cancers. The tumours most often appear in individuals aged 40–60 years; BCC has a male predilection, with a male-to-female ratio of 2:1. BCCs often appear in areas of chronic inflammation in the head and neck and is notable and is related to its primary aetiology—solar exposure.

Approximately 75%–86% of primary BCCs are found on the head or neck. The most common location on the head is the nasal tip and alae. Risk is related to the skin type and degree of exposure to sunlight, particularly ultraviolet-B (UV-B) radiation. The tumours are more frequent in individuals with a light complexion. The Fitzpatrick skin type scale, which ranges from very fair (skin type I) to very dark (skin type VI), categorises cutaneous sensitivity to UV radiation. It is based on the individual’s tendency to burn and tan and is a good predictor of relative risk among Caucasians. The prevalence of
BCC increases in areas of higher altitude and in areas of lower latitude. The incidence of BCC is rising, partly because of atmospheric changes and increasing popularity of sunbathing. The estimated annual death rate from this tumour is 0.44 per 100,000 persons. However, BCC rarely causes death; its morbidity is associated with uncontrolled advanced disease.

The current gold standard of skin lesion excision or even biopsy of suspicious lesions is under ‘naked eye’ guidance; this is followed by larger and ablative excisions based upon pathological results of the biopsy, which are usually obtained several days/weeks after the biopsy. Occasionally, frozen section analysis or Mohs surgery is also used, but their use is limited by availability, expense, time consumption and subjectivity in terms of errors of interpretation with lower sensitivity and specificity when compared with paraffin section analysis (which often takes days/weeks to complete and interpret). Such mediators of pathology directed resection (Mohs surgery) are resource intensive, expensive and user dependent.

Often these lesions arise on areas of sun exposure, where tissue is at a cosmetic premium and where loss would result in unacceptable deformity. Due to the nature of conventional excision, many of these lesions require a wide 3D margin of surrounding normal tissue to be also removed to reduce the chances of leaving residual disease, which may otherwise lie hidden under any reconstruction or closure. Real-time pathological analysis is a desirable goal to detect and guide treatment. It enables tumour removal with an adequate margin of resection to ensure completeness of disease removal and reduction in loco-regional recurrence. Ideally, an objective optical method would help in diagnosis and surgical treatment. This type of perioperative analysis would have many benefits.

There are many optical diagnostic technologies available; each has differing characteristic advantages and published sensitivities and specificities which unfortunately are for mucosal rather than skin diagnosis. Review of the literature and our aim for an objective, low cost system with a potential for rapid translational use by non-technical clinicians in order to help guide treatment with easily interpretable visual results led us to consider elastic scattering spectroscopy (ESS). Assessment of other methodologies also revealed a high degree of subjectivity in interpretation of results and a shallow learning curve that would not have been practical for translational use in primary care settings. ESS was chosen for several reasons: ease of use, rapid applicability to our problem of ‘real-time’ diagnosis and cost effectiveness. The advantages of ESS also include its high predictive value.

ESS is an evolving technology that generates a wavelength-dependent spectrum that correlates with structural and morphological change within tissues. The spectrum reflects both scattering and absorptive properties of that tissue. This scattering process has been shown to occur at gradients in the optical index of refraction resulting from differences in densities that occur at a cellular and sub-cellular level. The structures that induce the scattering (scattering centres) are the nucleus, chromatin concentration and sub-cellular organelles. At a cellular level, ESS is sensitive to nuclear size/morphology, chromatin content, nuclear/cytoplasmic ratio and cellular crowding (and chromatin level), whilst at a tissue level, it is sensitive to changes in morphology of epithelial surface texture and thickness as well as disorganization of epithelial cell orientations or architecture. These are all criteria that a histopathologist looks for when establishing malignancy within a tissue and can be used as a basis for comparison of lesions against tissue controls detected by the ESS system. ESS has the advantage of being fast, reliable and cost effective and potentially offers a diagnosis in situ, non-invasively and in real-time.

**Materials and methods**

The procedure was approved by an ethical committee. All recruited subjects were fully informed and they granted their consent to participate in this study. Exclusion criteria included patients less than 18 years of age, those who were pregnant and those who did not provide their consent. Only de novo cases who presented with new skin lesions in the head and neck region were examined using this protocol. Our methodology remained consistent throughout the series of ESS protocols and is detailed; this work builds upon our previous findings.

In the study group, the patient highlighted the skin lesion that was of concern. If excision was clinically indicated, the lesion would be photographed and then interrogated using the ESS system. This consisted of obtaining multiple ESS signatures from anatomically similar but uninvolved skin for control readings, i.e. internal control group. The lesion would then be subject to ESS analysis by taking multiple readings from different areas of the lesion. In our protocol, the tip of the fibre was momentarily placed perpendicularly in direct contact with the suspected lesion and the measurement activated at the keyboard or foot switch. The system automatically takes a background measurement without firing the lamp, followed immediately (within 100 ms) by an ESS measurement with a pulsed lamp and then subtracts the background spectrum from the ESS spectrum. Three types of optical measurements can be acquired from each of the suspected lesions: 1st type of measurement from the centre, 2nd type from the periphery of the lesion and the 3rd type from in between. All readings were taken at...
least six times from each site. An elastic scattering spectrum is recorded at each reading and stored electronically.

Following the optical readings, a co-registered excisional biopsy was taken, preserved in formalin, processed in haematoxylin and eosin stain and examined by a histopathologist; digital pictures were taken and sketches made for all the suspected sites to ensure that all sites were easily identified by a pathologist, and hence, reduce any chance of false results. The skin defect was repaired in a cosmetically sensitive manner. Cases were discussed with a surgical oncologist at a Multidisciplinary Forum for continuing follow-up.

Statistical approach for model generation and model validation
Some previous studies have made use of normalised spectra, i.e. the spectra from the lesion are divided by the spectrum of normal skin adjacent to the lesion. We collected normal skin spectra, but found that classification using normalised spectra was less accurate. There is a paucity of information on the relationship between specific types of pigmented lesions and their reflectance spectra. In the absence of such information, we approached the problem of lesion classification with statistical techniques and sought classifiers that could separate lesions into distinct pathology groups. Both principle component analysis (PCA) and linear discriminate analysis (LDA) strategies were used to interrogate data. PCA helped to reduce the dimensionality of the data set and helped to identify new meaningful underlying variables; LDA was useful for classification, enabling supervised training of neural networks and testing by the ‘leave one out’ (‘jacknife’) validation methodology. In summary, these two methods of analysing data allowed us to examine which discriminated best between spectra from normal tissue and BCCs: linear discriminant analysis, which maximises the ratio of between-class distance to the within-class distance and support vector machine, which maximises the margin between the two data sets. Errors were further minimised by orthogonal subtraction for increasing accuracy.

Results
The demographics of the subjects studied indicated that the average age was 54 years (±15 years) for the BCC group (range 30–75 years), most were male (62%), all were Caucasian, only 6 identified themselves as smokers and 73% took regular alcohol.

Of the skin lesions examined (Table 1), 19 were excluded because they represented less common benign skin lesions leaving 21 BCCs, 16 intra-dermal naevi, 10 fibro-epithelial polyps and 7 seborrhoeic keratotic lesions for comparative analysis. No squamous cell carcinomas or melanomas were identified in our sample.

Table 1 The 4 most common pathologies of the patient population studied

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>%</th>
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<tbody>
<tr>
<td>BCC</td>
<td>28.8</td>
</tr>
<tr>
<td>Intra-dermal naevus</td>
<td>21.9</td>
</tr>
<tr>
<td>Fibro-epithelial polyp</td>
<td>13.7</td>
</tr>
<tr>
<td>Seborrhoeic keratosis</td>
<td>9.6</td>
</tr>
<tr>
<td>Other benign lesions</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 2 The number of pathological sites interrogated and number of site specific spectra obtained

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of sites</th>
<th>Number of spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCC</td>
<td>59</td>
<td>570</td>
</tr>
<tr>
<td>Intra-dermal naevus</td>
<td>31</td>
<td>633</td>
</tr>
<tr>
<td>Fibro-epithelial polyp</td>
<td>23</td>
<td>502</td>
</tr>
<tr>
<td>Benign seborrhoeic keratosis</td>
<td>12</td>
<td>269</td>
</tr>
<tr>
<td>Normal skin</td>
<td>125</td>
<td>551</td>
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ESS measurements were matched with histopathological specimens taken from individual sites (Table 2). Statistical analysis (Table 3) showed the ability of ESS to discriminate between normal, benign (Figures 1–3)

Figure 1: A representative elastic scattering spectral plot of the mean fibro-epithelial polyp (blue) and intra-dermal naevus (red) spectra (solid lines), with one standard deviation plotted either side of the mean (dashed lines).

Figure 2: A histogram of canonical scores for classification distinguishing between fibro-epithelial polyp (blue) and intra-dermal naevus (red) and showing per spectra sensitivity of 80.4%, specificity of 82.9% and accuracy of 81.4%. Per site sensitivity was 100%, specificity was 65.2% and accuracy was 84.9%. Inset summary plot shows mean BCC (dark blue), normal (red), intra-dermal naevus (light blue), fibro-epithelial polyp (violet red) and seborrhoeic keratosis (orange) spectra.

and malignant lesions (Figures 4–9). Since BCC is the most common skin cancer, further subgroup analysis showed sensitivity (high seventies) and specificities (high eighties). Exact figures varied depending upon the analysis methodology and underlying assumptions used (Table 4). Some previous studies have made use of normalised spectra, i.e. the spectra from the lesion are divided by the spectrum of normal skin adjacent to the lesion. We collected normal skin spectra, but found that classification using normalised spectra was less accurate. We do not further describe these results. Discrepancy occurring from combining spectra from different anatomical sites and also could be related to the co-registration of the suspected site.

Comparison of the histological diagnosis and ESS in the diagnosis of the skin pathology group (BCC) showed per spectra sensitivity of 88.4%, specificity of 74.2% and accuracy of 86.2% (Figure 10). Analysis of the receiver operating characteristic (ROC) curve results may suggest that false negative (FN) measurements could have been as a result of a small sample size.
or due to errors in sampling and co-registration of the data with histopathology. The false positive (FP) measurements could be due to inflammatory changes and vascular transformation which is a feature of BCC, but they may be incorrectly interpreted as an indicative of cancer.

In our studied sample population, we found that sampling the periphery of the BCC gave similar results to sampling from the centre. We regard this data with caution when extrapolating to other tumour types since clones of differing aggressivity may exist in squamous cell carcinoma, melanoma and sarcomatous tumour types. Even within a morphoeic BCC, the periphery may not give a true reflection of the tumour (Figure 11).

Discussion

The current standard of assessing pathological changes in tissue is histopathology of stained paraffin sections. However, the processing of biopsy material and the diagnosis of the results invariably leads to treatment delay and the added possibility of taking an unrepresentative sample. Lately, optical spectroscopy systems have been explored to try to provide tissue diagnosis in real-time, non-invasively and ‘in situ’. These systems rely on the fact that the optical spectrum derived from any tissue will contain information about the histological and biochemical make up of that tissue.

This is one of the first clinical studies examining BCC of the skin using ESS. It demonstrates the utility of this technique to determine the nature of the lesion to be removed before the availability of pathological data. This means that the resection can be guided to improve decision making, preoperative patient consent, intraoperative cancer resection and also during the acquisition of pathological specimens to help improve the sample yield of disease material.

to reduce the FP associated with simple naked eye examination. We were able to use the ESS system to discriminate BCC lesions from normal skin tissues, benign lesions and to develop an algorithm for diagnosis. Further, our results show that BCC (the commonest skin cancer) diagnosis can be achieved by ESS with a high degree of accuracy.

Clinical examination has a sensitivity 40%–92% and specificity of up to 93.2%, as concluded from large clinical studies. This is highly dependent upon the skills and experience of the clinician. In this study, we have shown that we can diagnose BCC lesions with a high sensitivity and specificity; this is operator and experience independent. Thus, we suggest that this novel optical diagnostic technology can be used to aid diagnosis of skin lesions in a primary care setting. It is felt that if clinical diagnosis could be supported by non-invasive diagnostic methods, the need for surgical excision of suspicious looking but non-significant skin lesions would be reduced. This in turn would save patients a surgical burden in terms of morbidity and mortality, thus ensuring significant savings. Cost effectiveness would be shown in terms of the cost of the equipment and minor surgery service against the costs of referral to secondary care and follow-up. We present a paradigm shift to that of improved evidence based surgical resection in primary care settings that directly addresses our patient concerns. This model can also be applied throughout in a secondary care setting (clinical interface) with further benefits to patients and significant cost savings to the organisation. This manuscript answers some of the criticisms of recent debate in translational research to directly benefit patient care.

**Challenges**

The ESS system was assessed very rigorously against histopathological

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**Table 4** BCC vs normal skin results

<table>
<thead>
<tr>
<th>Test: ESS diagnosis</th>
<th>Disease: BCC histological diagnosis (Gold standard)</th>
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<tbody>
<tr>
<td>Sites</td>
<td>BCC</td>
</tr>
<tr>
<td>BCC (59)</td>
<td>True positive (TP) 397</td>
</tr>
<tr>
<td>Normal (125)</td>
<td>False negative (FN) 113</td>
</tr>
<tr>
<td>Total (n)</td>
<td>All with disease 510</td>
</tr>
</tbody>
</table>

Sensitivity = TP/(TP + FN) 77.8%

Specificity = TN/(TN + FP) 80.3%

Pre-test probability = (TP + FN)/(TP + FP + FN + TN) 64.8%

Positive predictive value = TP/(TP + FP) 93.2%

Negative predictive value = TN/(FN + TN) 51.1%

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**Figure 10:** A representative summary plot of the mean normal (red), intra-dermal naevious (sky blue), fibro-epithelial polyp (violet red) and seborrhoetic keratosis (orange). ESS spectra showing the discriminating features of the absorption spectrum which may be useful in characterisation and diagnosis between these benign lesions and normal.

**Figure 11:** Discrimination between BCC data obtained from periphery and centre sites showing multiple spectral measurements from different areas of the same lesion, centre, periphery and whole, with indicative results based on sensitivity and specificity at a single threshold as well as an illustrative set of ROC curves, which characterise the trade-off of sensitivity against specificity as the classifier threshold is adjusted. Per spectra sensitivity was 11.2%, specificity was 67.2% and accuracy was 43.2%. Per site sensitivity of 37.5%, specificity of 39.4% and accuracy of 38.5%.

results rather than clinical examination alone. We realise that information from ESS in these cases only represents superficial cells and may not reflect deep underlying invasion. Also, since many skin lesions may be heterogeneous, co-registration of ESS signature with the pathological report is a potential problem. We have tried to overcome this issue by multiple statistical sampling. From our sample, we can also determine that for the BCGs studied, there was little difference in the spectral signatures between the periphery and centre of the lesions. Occasionally, very high or low readings would necessitate the recalibration of the spectroscope.

Conclusion
This preliminary study shows that ESS can distinguish between BCC, common benign conditions and variants of normal skin. ESS can also help determine the diagnosis between benign lesions with a high degree of accuracy. The results are better than those usually obtained from frozen section analysis (the closest conventional pathology analysis system for rapid analysis of results to guide treatment). Its advantage over frozen section is its reliability, speed and steep learning curve, cost effectiveness and non-operator dependence. One would presume that melanoma would affect even greater ESS spectral differences to help guide the initial surgical resection and thus improve management and prognosis.

ESS, as an optical diagnostic technique, is an objective, quick and accurate way of examining tissues. The accuracy found in many of our previous studies suggests that it is cost effective and can be used for real-time and in situ diagnosis. The analysis can be varied to achieve higher specificity or higher sensitivity, depending on the clinical situation (whether for diagnosis or screening). If the aim is to identify high-risk sites for taking a conventional biopsy to make a definitive diagnosis, then the analysis is tuned for maximum sensitivity. If treatment is to be based just on the optical diagnosis, then the analysis can be tuned for maximum specificity. ESS can also help determine the diagnosis between benign lesions with a high degree of accuracy, suggesting that ESS can add significant objectivity to dermatological diagnosis and management even of benign conditions.

Abbreviations list
BCC, basal cell carcinoma; ESS, elastic scattering spectroscopy; FN, false negative; FP, false positive; LDA, linear discriminate analysis; PCA, principle component analysis; ROC, receiver operating characteristic curve; TN, true negative; TP, true positive; UV, ultraviolet.

Acknowledgement
We acknowledge Miss Jiao for her help with statistical analysis.

Declaration
Some of the preliminary unpublished work for this pilot study was submitted as a manuscript to the RCGP and received a Roche Research Prize from the Royal College of General Practitioners (UK).

References