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Diagnostic accuracy of optical coherence tomography in superficial basal cell carcinoma

Dr Hui Mei Cheng

Thesis submitted in fulfilment of the requirements for the Master of Philosophy in Sydney Medical School, The University of Sydney, August 2015
Acknowledgements

This entire project took a little over 12 months to complete. It was not an easy task from the start as I moved across the country to take on this challenge but it was made much easier with the help of many around me.

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Last but not least, I thank Michelson Diagnostics for their generous loan of the machine and for technical support and training. In particular, Adam Meekings and Nikki Steadman were my first tutors in OCT and I am grateful to them for not giving up on me when I insisted “They all look the same to me!”

This thesis represents the fruits of our labour and I thank everyone who has been there along the way. Together with my supervisor, I have revised this thesis umpteen times and I truly hope you will enjoy reading it.
Summary

Background:

Non-melanoma skin cancers (NMSC) are the commonest cancers worldwide and their incidence continues to rise in Australia. (1) Basal cell carcinomas (BCC) form the majority of NMSCs, and in the last decade noninvasive therapies have significantly reduced excision rates. (1-3) Non-invasive techniques are increasingly important for the diagnosis of superficial BCC which can be safely treated topically.

Optical coherence tomography has previously been used to diagnose BCC. (4, 5) The aim of this study was to investigate the diagnostic accuracy of optical coherence tomography (OCT) in the diagnosis of superficial subtype of BCC in a clinical setting.

Methods:

Lesions which were suspicious for superficial BCC were consecutively recruited into this prospective study. The degree of clinical confidence of the diagnosis based on clinical and dermoscopic assessments was recorded. Clinical and dermoscopic images were taken. OCT images of lesions and adjacent normal skin were acquired at baseline visit. A 2mm punch biopsy of the lesion was taken. Interpretation of the OCT images were performed by an investigator blinded to the biopsy results. The presence of individual OCT features, the OCT diagnosis, and the confidence of this diagnosis were recorded.

Sensitivity, specificity, positive predictive value, and negative predictive value for each OCT diagnosis were calculated compared with histopathologic diagnosis.
Assuming all recruited lesions would be biopsied in routine clinical practice and that OCT interpreter confidence had to be ≥90% (high) to diagnose sBCC without a biopsy, different clinical scenarios were analysed to detect the diagnostic accuracy of OCT and its potential effect on biopsy rates and misdiagnosis rates. Specifically, we analysed the following scenarios:

1) Clinician confidence was high but OCT confidence variable for sBCC
2) Clinician confidence variable but OCT confidence high for sBCC
3) Clinician and OCT confidence were both high for sBCC

Tumour depths determined by OCT and biopsy were compared using the Pearson correlation test. A test set of these images, made up of the first 71 consecutive lesions recruited, was used to test for interrater reliability. These images were separately analysed by two other investigators, of varying OCT experience, blinded to the biopsy report.

Interobserver agreement between the 3 observers for the OCT-based diagnosis as well as the individual OCT features were estimated using Cohen kappa statistics. Univariate and multivariate logistic regression were carried out for the OCT features and presented as receiver operative characteristic curves.

Results:

103 consecutive patients with 168 sBCC-like lesions were recruited. 52% were sBCC, 26% were other BCC and the remaining was made up of actinic keratosis
(AK) or squamous cell carcinoma in-situ (SCCIS), other benign inflammatory processes, and two other malignant lesions, namely an amelanotic melanoma and a minimally invasive squamous cell carcinoma (SCC).

Sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) of OCT in the diagnosis of sBCC was 0.87, 0.80, 0.83 and 0.86, respectively.

Different clinical scenarios were analysed and showed that OCT had the potential to reduce biopsy rates. OCT can be used in situations where clinical confidence of sBCC is:

- variable as it would reduce biopsy rate by 40% with the risk of misdiagnosing 3 other BCC cases (4%) and
- high (≥90%) as it would reduce biopsy rate by 76% with the risk of misdiagnosing 2 other BCC cases (5%)

There was excellent correlation amongst tumours <0.4mm (Pearson’s correlation r=0.86, p<0.001) but correlation was less as depth increased. (Pearson’s correlation r=0.71, p<0.001 for all tumours <1.0mm)

Inter-rater agreement was good between experienced observers (kappa=0.766), and fair amongst all 3 observers (kappa=0.596)

A flow chart of the most important features for the diagnosis of sBCC was produced.

Limitations of this study are discussed.
Conclusion:

OCT is a reliable tool for differentiating between sBCC and other clinical mimickers. Clefting, hyporeflective ovoid structures and the absence of a fully encompassing cleft are the strongest predictors for a diagnosis of sBCC. Amongst experienced users, there is good interobserver agreement for these features and for the diagnosis of BCC. In terms of depth measurement, OCT is accurate in tumours under 0.4mm, which is useful in determining the type of treatment for sBCC as previous research has shown that in these thin tumours, there is zero recurrence rate at a mean follow up of 34 months even with topical (imiquimod) treatment.(6)

OCT has the potential to reduce biopsy rates as it is able to diagnose sBCC with a high user confidence in clinical scenarios where clinician confidence is variable, avoiding biopsy in these cases. When clinician confidence is high, OCT can also increase diagnostic accuracy as a proportion of cases are still misdiagnosed by clinical examination. However, adequate follow up is required in cases where biopsy was not undertaken due to a high OCT confidence as there is a small risk (5%) of misdiagnosis. As a result, all lesions not biopsied on OCT examination should be carefully examined at follow up and biopsy should be performed if there is any doubt on clinical or OCT examination. Amelanotic melanoma may be an important pitfall in this technology and caution is recommended in cases of uncertainty. Research is ongoing to elicit features differentiating melanocytic and non-melanocytic lesions.
**List of abbreviations**

<table>
<thead>
<tr>
<th>Phrase</th>
<th>Abbreviation</th>
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<tr>
<td>Actinic keratosis</td>
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<td>Area under the curve</td>
<td>AUC</td>
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<td>Basal cell carcinoma</td>
<td>BCC</td>
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<tr>
<td>Confidence interval</td>
<td>CI</td>
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<td>Dermal-epidermal junction</td>
<td>DEJ</td>
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<td>Fluorescence confocal microscopy</td>
<td>FCM</td>
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<td>Full-field optical coherence tomography</td>
<td>FF-OCT</td>
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<td>Haemotoxylin and eosin</td>
<td>H&amp;E</td>
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<td>High-definition optical coherence tomography</td>
<td>HD-OCT</td>
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<td>High frequency ultrasound</td>
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<td>Harmonic generation microscopy</td>
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<td>Infiltrative BCC</td>
<td>iBCC</td>
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<td>Melanoma Institute Australia</td>
<td>MIA</td>
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<td>Negative predictive value</td>
<td>NPV</td>
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<td>Nodular BCC</td>
<td>nBCC</td>
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<td>Non-melanoma skin cancer</td>
<td>NMSC</td>
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<td>Odds ratio</td>
<td>OR</td>
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<td>Other BCC</td>
<td>oBCC</td>
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<td>Optical coherence tomography</td>
<td>OCT</td>
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<td>Positive predictive value</td>
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<tr>
<td>Receiver operating characteristic</td>
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<td>Reflectance confocal microscopy</td>
<td>RCM</td>
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<td>Seborrheic keratosis</td>
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<td>Superficial BCC</td>
<td>sBCC</td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>SCC</td>
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<tr>
<td>Squamous cell carcinoma in-situ</td>
<td>SCCIS</td>
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<td>Ultraviolet</td>
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Introduction

Background

Epidemiology of basal cell carcinoma

Non-melanoma skin cancer (NMSC) consisting of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is the most prevalent malignancy in Caucasian populations.

BCC is the most prevalent cancer in Australia and incidence continues to rise at about 3-10% annually.(6) BCC incidence for older Australians (>60 years old) continue to increase whereas it appears to have stabilised in younger Australians – this diverging trend being partially attributable to population-based skin cancer prevention strategies in Australia since 1980s.(1, 2) Risk factors for BCC include UV radiation including sun exposure or therapeutic UV exposure, the use of tanning beds, ionizing radiation and long term immunosuppression.(7)

Although BCC is usually not life-threatening, it represents a huge public health burden in Australia. The incidence of treated NMSC is more than five times the combined incidence of all other cancers, making them the most expensive cancers to treat.(8)
Basal cell carcinoma and subtypes - clinical, dermoscopy, pathology classification

There is no fixed classification system of BCC and more than 26 histopathologic subtypes exists as described by Wade and Ackerman in 1978. Histologic examination is typically carried out using Haemotoxylin and Eosin staining (H&E). There are four major subtypes clinically and pathologically, namely nodular, superficial, morpheaform and fibroepithelial. Combinations of BCC subtypes can occur and degree of ulceration and pigmentation can vary. (6, 7) Nodular types tend to ulcerate most frequently and majority of BCC are non-pigmented. (7) In general, symptoms are rare although stinging, burning or shooting pain raises the possibility of perineural invasion. (9) Systematic spread is also extremely rare with incidence of 0.5% or less. (6)

Clinical

Nodular BCC

Of the BCC subtypes, nodular is the most common and accounts for approximately 50% of all BCCs. (7, 9) Typically, a shiny pearly papule or nodule is seen, with a smooth surface and arborizing blood vessels. Over time, the tumour can evolve to become bigger and ulcerated, with elevated rolled edges. Nodular BCCs occur on sun-damaged skin, favouring the face especially the cheeks, nasolabial folds, forehead and eyelids. Nodular BCC can occur in any skin with hair follicles and are rare in the absence of hair follicles. (7, 9)
Superficial BCC
Superficial BCCs (sBCC) usually present as an erythematous macule or patch, or a thin papule or plaque, which is well circumscribed. (6, 7, 9) Additionally, focal scale or crusting can occur and a thin rolled border is sometimes seen. Size is variable from a few millimetres to several centimetres and in the larger lesions, regression can occur spontaneously, characterised by atrophy and loss of pigmentation. It preferentially affects the trunk and limbs but are seen also in the head and neck region. (7, 9)

Morpheaform/Infiltrative BCC
Morpheaform BCC is less common and typically has a scar-like appearance, similar to the plaque of morphea. (7, 9) The affected area is frequently slightly elevated but can be depressed and indurated. The colour is usually light pink or white, the surface smooth and the borders poorly defined. Crusting, erosions, ulcerations, or superimposed papules can be seen.

Fibroepithelial BCC
Also known as a fibroepithelioma of Pinkus, this is a rare variant of BCC which typically presents as a flesh-coloured or pink sessile plaque or pedunculated papulonodule with a smooth surface. (7) It tends to occur on the trunk and individuals with multiple superficial BCCs are predisposed. (7)
Histology

Nodular

Histologically, the tumours are seen as aggregates of basophilic staining cells with well-defined contours. Peripheral or palisading and retraction from the surrounding stroma is seen. Central necrosis with granular, eosinophilia is a feature of larger tumours, surrounding stroma tends to be myxoid in nature.(9) Mitotic activity, keratinocyte dyskeratosis, and calcification are thought to be features of more aggressive lesions.(9)

Superficial

Superficial BCC appears histologically as basaloid lobules abutting from the lower margin of the epidermis. (7, 9) Although the lobules usually connect in a net-like pattern, they appear as “multifocal” lesions at a given two-dimensional plane. (7)

Morpheaform/Infiltrative

This subtype is consists basaloid cells organised in a pattern of strands or cords between collagen bundles. (7) The tumour islands may be small and may be perineural so perineural spread is not infrequent. Surrounding stroma is dense – this gives rise to the indurated appearance of morphea. (7)

Fibroepithelial

Histologically, the tumour is characterised by basaloid epithelial strands originating from the epidermis, compartmentalising the fibrous stroma. Cyst formation and
primitive hair germ maturation may also be present and rarely, a more invasive BCC can form within this tumour. (9)

In addition to the subtypes above, pathology yields two further distinct subtypes, namely micronodular and basosquamous BCC (9). As suggested by their names, micronodular BCCs made up of smaller aggregates of basaloid cells similar to nodular BCC but smaller. (9) It is considered more aggressive with more subclinical spread, and more likelihood of recurrence. Clinically, they can present like nodular BCC. Similarly, basosquamous BCCs are histologically aggressive, with higher rates of recurrence and metastasis, with an incidence of metastasis estimated to be greater than 5%. (7)

Regardless of subtype, BCC can contain melanin pigment, leading to the term “pigmented BCC”. Histologically, pigmented BCCs contain melanin in melanophages or occasionally freely, in the dermis. Nodular, micronodular, and superficial BCC can be pigmented but there was no evidence of pigmentation in infiltrative or morphoeic BCC.

**Dermoscopy**

Dermoscopy is a handheld device with an incident light source and a magnifying glass (generally x10), using oil at the skin-microscope interface.

Features of BCC on dermoscopy have been previously described (10, 11). A dermoscopy diagnostic method for pigmented BCC has previously been described by Menzies et al. For a pigmented BCC to be diagnosed, it should meet the negative
feature of not having a pigment network and also have at least one of the six positive
features of BCC – ulceration, large blue-gray ovoid nests, multiple blue gray
globules, maple leaf-like areas, spoke wheel areas, arborizing (tree-like)
telangiectasia. This method has a sensitivity of 97% for diagnosis of pigmented BCC
and a specificity of 85% for invasive melanomas. (11) With variably pigmented
BCCs, the use of dermoscopy has also been proven to diagnose BCC with a high
level of accuracy (sensitivity of 97%) and reliability (kappa coefficient of 87%). (10)
Going one step further, recent researchers have tried to correlate the various
features with different BCC subtypes.(12-15) Arborizing telangiectasias were more
frequent in nodular BCC whilst in sBCC, short fine telangiectasias were more
characteristic.(12) Leaf-like areas, spoke wheel areas, small erosions and concentric
structures were also significantly associated with sBCC.(12, 13) Using dermoscopy,
sBCC and nodular BCC could be differentiated using dermoscopy but this distinction
could not be made with other BCC subtypes.(13) A flow chart for dermoscopic
diagnosis of sBCC (Fig. 1) has been published recently.(13) Based on this model,
sBCC could be predicted with a sensitivity of 81.9% and a specificity of 81.8%. (13)
Diagnosis of basal cell carcinoma subtype and consequence on management

In BCC diagnosis, incisional biopsy is indicated in all cases especially where diagnosis is not clear-cut or when referrals to other subspecialties are anticipated. (16) Specifically, the Australian guidelines indicate that biopsy should be done prior to treatment due to the need to correctly identify amelanotic melanoma. (8)

Treatment guidelines are not strictly based on evidence but on consensus and current guidelines classify lesions to “low risk” and “high risk” depending on the histologic subtype. (8, 16) To properly assign the risk status of lesions a biopsy of the
lesion is required where information on the histopathologic subtype, presence of ulceration, and perineural involvement can be obtained. High-risk subtypes include micronodular, infiltrating or morphoeic forms.

Treatment of BCC depends on various factors. Besides the subtype which divides them into high risk and low risk, other factors are often taken into consideration, including previous history of the patient, the current clinical presentation, the location of the lesion and whether any previous treatments have been tried.

Specialist referral is recommended for the treatment of not only high risk subtypes but also high risk locations such as central face, ears, genitalia, digits, hand or leg. Similarly, recurrent lesions, previously incompletely excised lesions and lesions which size is greater than 10mm on the face or scalp or greater than 20mm on the trunk and extremities should be managed in a specialist setting. Radiotherapy is a form of treatment reserved for a minority of cases where there are contraindications to surgery or if the BCC is persistent, recurrent or advanced in which case radiotherapy can be used as an adjunct to surgery.

Incompletely excised tumours have a 30% recurrence rate, and this risk of recurrence is highest when both the lateral and deep margins are involved. The completeness of excision is the most important factor which determines rate of cure and recurrence and current recommendations are in the range of 2-5mm excision width from the tumour margin. As many as one-third of excised BCCs can have close or involved margins, alluding to the difficulty in assessing the tumour margins accurately based on clinical examination. This creates an important role for the
development of imaging techniques which can assist in accurately determining tumour margins so as to prevent recurrence.

Of the BCCs, the superficial subtype is the only one in the low risk category. Consequently only sBCC is suitable for non-invasive treatment for which options include cryotherapy, photodynamic therapy, medical treatment with imiquimod, 5-fluorouracil, topical and curettage and cautery.\(^{(8, 16)}\) Surgery and radiotherapy can be also be used in sBCC with low failure rates but are more invasive.\(^{(16)}\) Invasive methods seem unnecessary in low risk lesions since cryosurgery has been proven to have a high cure rate.\(^{(8, 16)}\) A recent epidemiologic study found that nonsurgical treatment of skin cancer is increasing whilst excision rates are on the decline in the younger population (<45 years old) in Australia. \(^{(1)}\) Imiquimod has also been shown to have a zero recurrence rate when used on sBCC < 0.4mm in a mean follow up period of 34 months. \(^{(17)}\) Given the efficacy and growing popularity of non-invasive treatment, biopsy can be viewed as invasive and non-invasive diagnosis becomes increasingly relevant.

Although most BCCs can be adequately treated with the methods described above, occasionally, some rare cases can progress to a locally-advanced stage where it is not amenable to surgery or radiotherapy. Even less common are lesions which spread to distant sites, causing additional morbidity and mortality. In these rare cases, studies are ongoing but visdodegib, a smoothened homologue inhibitor which acts along the hedgehog signalling pathway typically aberrant in BCC, has shown promising results in a phase 2 study.\(^{(18)}\)
Interestingly, a recent phase 3 randomised controlled trial has also demonstrated the benefit of nicotinamide in the prevention of BCC and other NMSC. (19)

Non-invasive tools in diagnosis of basal cell carcinoma

There has been rapid development of diagnostic tools in the field of skin, and particularly skin cancers as it provides several advantages to the gold standard of histopathology in general. Their non-invasive nature allows visualisation of structures without potential harm (e.g. allergy to local anaesthetic) or pain (e.g. to needles). These images are available in real-time, offering clinicians and patients a quicker result and peace of mind if a benign lesion can be confirmed. Unlike histopathology, running costs of these technologies are low, and in the long run can represent cost savings. A review of the landscape of diagnostic tools in melanoma, a lethal form of skin cancer, was performed and findings are presented in the form of a poster at a national melanoma conference in Perth, Western Australia. (Appendix 1)

Diagnostics tools used in BCC are presented below, separately.

In vivo imaging techniques

*Reflectance confocal microscopy (RCM)*

RCM is an imaging tool that is able to achieve “optical biopsy” with a near histologic resolution in the upper layers of the skin. Various studies have been published and point towards its usefulness in the diagnosis of BCC. (20-24) In a two-step algorithm, a high sensitivity of 100% and specificity of 88.5% for the diagnosis of BCC was achieved blindly to dermoscopy data.(24)
Traditionally, the use of RCM was difficult in challenging areas such as the periorbital skin due to its bulky probe. However, a commercially available RCM machine with a handheld microscope (Vivascope 3000, Caliber ID, Rochester, NY) has already been proven useful in the in vivo diagnosis of tumours in the eyelid margin. (25) With a high sensitivity and specificity of 100% and 60%, respectively for BCCs, it is particularly useful in avoiding excision of benign lesions. (25)

Similar to histologic confirmation of margins in surgical excision of BCCs, RCM has been shown in a small case series to have potential in confirming treatment efficacy of laser ablation and MAL-PDT treatment of BCCs.(26, 27)

*High frequency ultrasound*

The use of ultrasound in diagnosis of BCC requires the use of high-resolution equipment with high-frequency probes in the range of 20-100 MHz.(28). Frequencies of 20 to 25 MHz reaches a depth of roughly 7mm, allowing visualisation of the epidermis and dermis whereas at 50-100 MHz, resolution increases but depth is more limited to 0.15 mm to 3 mm. (29) Images are obtained in the vertical section and doppler ultrasound scans can provide additional information about the vascularity of a lesion, including the direction and volume of blood flow Three D-reconstruction is also possible.(30) It is particularly useful in visualising the depth of lesions due to its relatively high penetrance with a reported intraclass correlation analysis of 0.9 compared to histologic analysis (intraclass correlation coefficient
values $> \text{or } \geq 0.9$ are very good). (31) However, on the whole, there is no consensus regarding use of HFU in delineating surgical margins in NMSCs. (16, 29, 32-34)

A commonly used system is the 20 MHz scanner (DermaScan Ver. C 3.1; Cortex Technology, Hadsund, Denmark), which has an axial resolution of 50 $\mu$m, lateral resolution of 350 $\mu$m (35, 36). Comparing this technology with conventional OCT (axial resolution 8 $\mu$m, lateral resolution 24 $\mu$m, and maximum depth of 2-2.5mm), a study of 34 lesions (23 BCC, 11 AK) found that although both methods overestimated tumour thickness, OCT was significantly more precise than HFU (0.392mm vs 0.713mm overestimation).

Most studies have used the 20 MHz system for investigating NMSCs although the optimal frequency for this examination has not been previously studied. However, it is possible that at higher frequency, margins could be more accurately assessed as it was previously found that melanoma depths measured with HFU at 75 MHz, but not at 20 MHz, correlated significantly with a mean Breslow thickness of 0.4mm. An important limitation of HFU is that it merely provides a supporting role with additional information about extent and anatomic features of the lesion as the resolution cannot distinguish subtypes of skin tumours. (28, 30).

In vivo harmonic generation microscopy (HGM)

HGM is a relatively new technology, which utilises similar principles to confocal microscopy. It achieves a submicron resolution, at a penetration depth up to 270 micrometer. (37) Its use in non-melanoma skin cancer has recently been investigated
in a small case series focusing on pigmented BCC, amongst other pigmented non-melanoma growths such as melanocytic naevus and seborrheic keratosis (SK). Sensitivity of up to 94\% (95\% CI: 70-99\%) and specificity up to 100\% (95\% CI 87-100\%) were achieved when using their imaging criteria. (37)

**Fluorescence**

Laser induced fluorescence utilises non-ionizing radiation delivered via optical fibres placed in contact with the skin. (38) Excitation light from the source fibre passes into the skin and fluorescence light is emitted back to the surface to be picked up by separate optical fibres. (38) Optical measurements are taken and vary according to the structural, functional, and biochemical composition of the tissue. (38) The fluorescence can be from an endogenous source (autofluorescence) and/or exogenous source (photosensitizers). When photosensitizers which accumulate in cancer cells are used, it enhances characteristic fluorescence emitted, more clearly delineating site of NMSCs, including BCCs. (38, 39) Users require little training, and there is little dependency on expertise. (38) Optical properties and fluorophore contributions of BCC and normal skin are significantly different from each other and shows good histological correlation, which is useful in margin delineation. (38) (39) However, its use is limited by intrapatient and intralesional variations. (38) Moreover, penetrance of the photosensitizer can be an issue in keratinised lesions such as AKs, leading to suboptimal fluorescence. (39)

**Ex vivo imaging techniques**

*Fluorescence confocal microscopy (FCM)*
FCM is a new technology which combines fluorescence staining with RCM to increase the diagnostic accuracy.\(^{40-45}\) In RCM, bright scattering interference typically arises from the dermis, making diagnosis difficult. FCM attempts to overcome this by using a contrast agent (acridine orange) which stains the nuclei and increases the contrast of nucleated cells, weakening the fluorescence from the dermis. In this way the contrast between the nuclei and dermis is increased 1000-fold, visualisation of small BCCs is possible.\(^{44}\)

Ex vivo FCM findings in BCC have been described\(^{44}\) and shows an excellent correlation to histologic findings (Cohen's Kappa statistics = 0.9) \(^{44, 45}\) In their study of 64 BCC cases excised during Mohs surgery, Longo et al. found that the size and shape of the tumour islands was a differentiating characteristic between the BCC subtypes.\(^{45}\) Bigger islands corresponded with nodular BCC, smaller and round islands with micronodular BCC, and tiny cords were seen in infiltrative BCC.\(^{45}\) Interobserver agreement was more than 0.7 for most of the criteria used to diagnose BCC in FCM.\(^{44}\) This includes the presence of fluorescence, well-demarcated margin, nuclear crowding, palisading, clefting, nuclear pleomorphism, increased enlargement of nuclear to cytoplasmic ratio, and stroma presence.\(^{44}\) The mean time taken using FCM in assessing clearance of margins in excised tissues during Mohs compared to standard frozen haematoxylin and eosin-stained slides typically used was 10.1 minutes compared to 28.2 minutes \((p<0.001)\). \(^{46}\) The sensitivity and specificity for detecting residual BCC in the surgical margins were 88% and 99%, respectively. \(^{46}\)
OCT has also been used on an ex vivo basis but results were less ideal than FCM and is discussed in detail in appendix 2.

**Principles of optical coherence tomography**

Compared to the technologies above, OCT provides a compromise between penetrance and resolution. It utilises low-coherence interferometry to measure optical scattering from beneath the skin surface to produce an image similar to ultrasound but with better resolution (28, 47) yet it reaches a deeper layer than RCM, sacrificing the cellular resolution RCM offers. Depending on the system, an axial and lateral resolution of up to down to 1.5 µm, and a depth of up to 2 mm is achieved in general.

Using fibre optics, infrared light is split into a reference beam (probe arm) and a probe beam (sample arm), the latter which is placed in contact with the area of interest on the skin. (Fig. 2) The probe beam then backscatters, to meet with the reference beam. When both beams match within the coherence length of the light, interference occurs. Axial resolution (y-axis) therefore is determined by the bandwidth and coherence length of the light, whereas the lateral resolution (x-axis) is dependent on the focusing objective.
OCT is used routinely in ophthalmology but of late, applications in dermatology have been developed. OCT allows visualisation of the different layers of the skin, including the stratum corneum, dermal-epidemal junction, dermal papillae, subcutaneous vasculature. Skin adnexal structures such as hair follicles, blood vessels, sebaceous glands, can also be seen. (49-52)

The use of OCT has been investigated in a number of skin conditions apart from malignancies. This include inflammatory dermatoses, skin infections, vascular lesions and hair and nail disorders. (53, 54)
Interest in the use of OCT in skin is exponentially rising. This is evident by the research publications on the topic in recent decades based on a search of “optical coherence tomography” and “skin” on Embase in February 2015. (Fig. 3a).

Figure 3a: Number of publications over time related to OCT and skin.

Optical coherence tomography systems in dermatology

OCT produces real-time images of skin structures, in vivo, to a maximum depth of less than 2mm. The Vivosight 1500 OCT system (Michelson Diagnostics Ltd., Orpington, UK) has a lateral and axial optical resolution of 7.5µm and 10µm, respectively and a field of view of 6mm x 6mm. It uses a multi-beam frequency domain OCT system with a centre wavelength of 1305nm. (Fig. 3b)
High-definition OCT (HD-OCT, Skintel®, AgfaHealthCare, Belgium) uses a halogen lamp with a Gaussian filter as its light source and has a bandwidth centred at 1300 nm. It is a time-domain system, which uses dynamic focus tracking through synchronisation between the imaging lens system and the reference optical system. It takes both horizontal and en-face sections, at 3 µm steps, to an optimal penetration depth of 450-750 µm. Unlike OCT, en-face imaging is performed in real time. The field of view in en-face mode is 1.8 x 1.5mm. The use of an optical gel is required between the probe and the skin.

Full-field OCT (FF-OCT, LightCT, LLTech, Princeton, NJ) is a variation of OCT which reconstructs 3D scans of tissues from the original 2D slices captured, working on a similar basis as computed tomography, with a resolution up to 1.5 by 1.5 by 1 µm and a field acquisition area of approximately 1mm².(55) The images produced are similar to histopathological slides but with a shorter and simpler acquisition process since processing of specimen is not required.(55) Current limitations include the inability of the machine to accommodate the relatively large specimens, the acquisition speed (5 minutes per 1mm²) and the difficulty with diagnosing small foci of neoplastic tissue that is otherwise obvious with histopathology.(55)
A detailed systematic review of the diagnostic accuracy of OCT in the diagnosis and management of BCC has been published. (See appendix 2)

A table detailing various OCT systems used in previous study is available in appendix 2.

The use of OCT in skin conditions apart from NMSCs is aided by an online atlas (http://www.vivosightatlas.com/) maintained by the manufacturer of a conventional OCT system. In this atlas, apart from NMSC, there are only images of naevi and seborrheic keratosis.

AK is an important clinical confounder of sBCC but differentiation between the two is possible on OCT. Features of AK are described below with images adapted from the online atlas. (Fig. 4)
As the resolution of OCT is not at the cellular level, the histopathologic features of parakeratosis, dyskeratosis, cellular atypia of AK cannot be observed.

In non-malignant conditions, the use of OCT is preliminary and primarily in the exploratory phase. For example, researchers have found that OCT of cutaneous lupus erythematosus correlated significantly to histopathology. Histologic features of 1) hyperkeratosis, 2) epidermal atrophy, 3) dense upper dermal infiltrate, and 4) dilated vessels were observed as 1) thickening and disruption of the entrance signal, 2) thinner layer below the entrance signal, 3) patchy reduction of reflectivity in the upper dermis, and 4) increased signal-free cavities on OCT. (56) The authors felt...
that although OCT was inadequate to establish a diagnosis, it may be a suitable tool for monitoring treatment of cutaneous lupus. Similar results were seen in monitoring of erythematotelangiectatic rosacea during topical brimonidine treatment, where mean grey value of the OCT imaging corresponding to dermal oedema, could be observed to decrease over the treatment period. (57)

The use of OCT in acute allergic contact dermatitis has also been investigated with results suggesting a strong correlation with clinical patch test grading, which may allow OCT to be used as an objective parameter in grading patch test reaction severity. (58)

The histopathologic/OCT correlates of other conditions which are mimickers of sBCC cannot be discussed in detail due to the lack of literature. Work is ongoing to investigate the use of OCT in differentiating naevi and melanoma and some promising results was recently published. (59)
Hypothesis and Aim of the project

Accurate diagnosis of superficial BCC subtype is important, as it is a critical determinant to the type of suitable treatment. High cure rates have been demonstrated with non-invasive methods, which are also less costly and time consuming. Current evidence on the use of OCT in the diagnosis of sBCC is lacking (See appendix 2). In particular, large scale, prospective trials have not been performed.

The hypotheses of the project are that

1) OCT can be used to triage lesions into those that can be treated non-invasively and those that require surgical intervention.

2) OCT can be used to measure depth of BCC

The aim of the project is to evaluate the sensitivity and specificity of OCT in the diagnosis of sBCC and to evaluate its accuracy in depth measurement of BCC.

Materials and methods

Sample

The study was performed at the Melanoma Institute Australia (MIA) (Sydney, Australia) for 12 months from March 2014 to March 2015. The patient population in MIA consists mostly those presenting for skin check, of which a majority have a moderate to very high risk of melanoma based on their history. The study was conducted according to the Helsinki Declaration and informed consent was obtained from all subjects. The study was approved by the ethics committee of St Vincent’s
Hospital (SVH 14/025). Neither routine diagnosis nor treatments of the patients were affected by the study.

Study procedure

Recruitment protocol

1. Adult patients 18 years and older, presenting with lesions suspicious for sBCC after dermoscopic examination are recruited consecutively following informed consent.

2. For every suspicious lesion, the clinician’s confidence (after dermoscopic examination) that it was a sBCC was recorded.

3. A clinical and dermoscopic photograph of the identified lesion was taken. Clinical images were taken with a Nikon DS300 digital camera and dermoscopy images were taken with the same camera attached to a Heine Delta 20 dermoscopic attachment (Heine Optotechnik, Herrsching, Germany).

4. OCT images of lesions and adjacent normal skin were acquired at baseline. (See OCT imaging protocol for details)

5. OCT scanning and interpretations were performed by the same investigator throughout the study. OCT interpretation was performed blinded to the clinician diagnosing the BCC. For every lesion, OCT confidence of the diagnosis was recorded as well as OCT confidence that it was a sBCC.

6. The lesion then underwent a 2mm punch biopsy for histopathologic examination to confirm the diagnosis. If the lesion was confirmed to be sBCC, patients were advised to treat with either cryotherapy or imiquimod therapy and to present in 6 months for follow up and evaluation. If the lesion was not a
sBCC, patients were contacted and treatment was advised as standard practices.

OCT imaging protocol

OCT scans were performed using the Vivosight OCT (Michelson Diagnostics Ltd., UK), a Fourier-domain OCT system, which consists of a laser at a centre wavelength of 1300nm. A probe is placed directly in contact with the area of skin of interest, without the need for an interface agent such as gel or oil. The system has a resolution of 7.5 µm laterally and 10 µm vertically. As previously discussed, it provides real-time, in vivo, cross sectional images of the lesions. Pre-programmed in the machine as part of the “en-face” scan, 120 images of 5mm width and 2mm depth were captured in sequence to form a series which presents like a video through cross sections of the skin. This series of images was exported and analysed using ImageJ software. The OCT morphology of lesions at baseline were recorded and analysed.

OCT Interpretation

Analysis was carried out in accordance with the following criteria set out by previous studies of OCT morphology of BCC lesions. (35, 47, 49, 60-62) Analyses were carried out by a single investigator who was an experienced user in OCT. (HC)

General BCC features

1. Alteration to the expected dermal-epidermal junction (DEJ) undulations by one or more hyporeflective lobules (Fig 5, 8a/b)
Figure 5: Alteration to the dermal-epidermal junction (DEJ)

2. Atypically arching and/or increased frequency of prominent vessels in the dermis underlying the hyporeflective structures (Fig. 6, 8a/b)

Figure 6: Atypical morphology or increased in frequency of blood vessels

3. Surface contour change in area of hyporeflective structures (Fig. 7, 10b)

Figure 7: Surface contour change
Specific BCC features

Superficial

1. Hyporeflective ovoid structures with firm attachment to the DEJ

2. Clefting region focussed or solely visible on the inferior margin (originally lower facing) of the hyporeflective structures

Figure 8a: Hyporeflective ovoid structure and clefting region on the inferior margin in a superficial BCC on OCT and biopsy. Orange arrows: lateral and superior border of the sBCC nests. Note that they are not fully encompassing, as illustrated in Fig. 9a. Yellow arrows reflect the inferior border, termed as clefting regions. Between the orange and yellow arrows, hyporeflective ovoid structures are seen.

Figure 8b: Corresponding sBCC on H&E (10x). Orange and yellow arrows reflect the peripheral palisading seen in BCC nests, and also visible on OCT. The BCC nest remain firmly attached to the DEJ on the right. Blue arrows show the normal DEJ, compared with the disrupted DEJ on the right by the BCC nests.
Nodular

3. Fully encompassing hyporeflective ovoid structure

Figure 9a: Fully encompassing hyporeflective ovoid structure. The yellow arrows reflect the inferior border of the BCC nest, and the orange arrows reflect the superior border.

Figure 9b: Corresponding nodular BCC (H&E x4) Large nests of nodular BCC, detached from the DEJ.

Micronodular/Infiltrative
4. Small aggregates of hyporeflective ovoid structures; can be connected by hyperreflective bands giving a “bunch of grapes” appearance

Figure 10a: Micronodular BCC: Small aggregates of hyporeflective ovoid structures giving a “bunch of grapes” appearance. The inferior border of the BCC nests are marked by blue arrows. On the right, small roundish ovoid structures can be seen, giving rise to a “bunch of grapes” appearance. The purple arrows mark the superior border, which is clearly detached from the DEJ.

Figure 10b: Corresponding micronodular BCC H&E (x4) Small BCC nests, detached from the DEJ. Breach in the epidermis is seen and corresponds to the superficial contour changes seen on OCT.
Interrater reliability

To assess the interrater reliability of the OCT features and the diagnosis of sBCC, 3 observers with varying level of OCT experience rated a test set of the lesions. This was made up of 44 sBCC (62%), 16 oBCC (23%), 4 AK/SCCIS (6%), 6 other benign (9%) and 1 other malignant lesion (1%). This test set comprised of 71 consecutive lesions from the first 33 patients recruited. A sample size of 33 subjects achieves more than 80% power to detect a true Kappa value of 0.60 in a test of H0: Kappa = κ0 vs. H1: Kappa ≠ κ0 when there are 4 categories with frequencies equal to 0.65, 0.25, 0.06, and 0.04. This power calculation is based on a significance level of 0.05000.

Two observers were experienced (HC, AM) and one observer was beginner in OCT, after completing a training set.(PG) The test set was presented on separate occasions to the 2 observers, who scored the images blinded to the histopathologic diagnosis. To more closely simulate actual clinical scenario, they had access to the age, gender and confidence of the clinician that the BCC was likely to be sBCC, as well as access to the clinical and dermoscopic photographs.
**Statistical analysis**

Statistical analyses were performed using R software (version 3.1.2, Foundation for Statistical Computing, Vienna, Austria).

Prior to recruitment, sample size calculation was performed. A total sample size of 165 (which includes 33 subjects with non-superficial nodular BCC) achieves 99% power to detect a change in sensitivity from 0.6 to 0.90 using a one-sided binomial test and 100% power to detect a change in specificity from 0.6 to 0.9 using a one-sided binomial test.

Sensitivity, specificity, positive predictive value, and negative predictive value for each OCT diagnosis were calculated compared with histopathologic diagnosis.

Depth measurement on OCT and biopsy were compared as paired samples. To obtain the correlation between the two, Pearson correlation coefficient was obtained for BCC and various subsets.

Interobserver agreement between the 3 observers on the OCT diagnosis as well as on the individual OCT features were estimated using Cohen kappa statistic with 95% confidence intervals.

Univariate logistic regression analysis of the OCT features for the diagnosis of sBCC, oBCC, nBCC and iBCC were carried out. OCT features were entered as dichotomous independent variables into a conditional backward elimination
multivariate logistic regression model. Receiver operating characteristic curve and area under the curve were plotted and calculated for the models.

**Results**

**DEMOGRAPHY:** A total of 103 consecutive patients with 168 sBCC-like lesions clinically suspicious for sBCC were included in the study. Mean number of lesions per patient is 1.63. Mean age of the sample was 62.1 +/- 11.9 years (range: 31 to 88 years) and participants were mostly male (n=63/61%). Over half of the lesions were located on the front of the trunk and back (n=93/55.4%), and the remaining were from the upper extremities (31/18.5%), head and neck (23/13.6%), and lower extremities (21/12.5%).

**PATHOLOGY:** Histopathologic diagnoses of the lesions were sBCC (n=87/51.8%), other BCC (oBCC; n=43/25.6%), actinic keratosis (AK) or squamous cell carcinoma in-situ (SCCIS) (n=19/11.3%), other benign inflammatory process (n=17/10.1%). 2 (1.2%) of these were “other malignancies”, which included 1 amelanotic melanoma and 1 minimally invasive SCC. Of the oBCC, 29 (67.4% or 11.9% of total) were nodular (nBCC) and the remaining were infiltrative (iBCC; 33.6% or 8.3% of total). The 17 benign inflammatory lesions were made up of 6 non-specific inflammation, 3 lichenoid keratosis, 2 scar tissues, 2 solar lentigines, 1 haemangioma, 1 blue naevus, 1 chronic dermatitis, and 1 irritated seborrheic keratosis.

**DERMOSCOPY:** Sensitivity and specificity of sBCC using dermoscopy was 77.0% and 23.5% respectively as we included all lesions with a differential diagnosis of sBCC even if the clinical confidence in this diagnostic was low. Clinical confidence of
sBCC after dermoscopic examination had a significant relationship with the pathologic diagnosis of sBCC \( (p<0.05) \). A cumulative proportion graph is presented to demonstrate the positive correlation between the two. (Fig. 11)

Figure 11: Confidence of the clinician that the lesion is a sBCC after clinical and dermoscopic examination plotted against the cumulative proportion of sBCC on pathology.

OCT: The absolute frequencies at which the individual features were observed were presented in Table 1. Altered DEJ was the most frequently observed finding, followed by hyporeflective ovoid structures and atypical vessels. Frequencies of a fully encompassing ovoid structure and “bunch of grapes” appearance were the lowest, in keeping with the proportion of nBCC and iBCC in the study population.
Table 1. Frequencies of OCT criteria according to the histopathologic diagnosis

<table>
<thead>
<tr>
<th>Feature</th>
<th>sBCC (n=87)</th>
<th>nBCC (n=29)</th>
<th>iBCC (n=14)</th>
<th>AK/Bowen (n=19)</th>
<th>Other inflammatory (n=17)</th>
<th>Other malignancy (n=2)</th>
<th>Total (n=168)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyporeflective ovoid structures</td>
<td>82 (94.3%)</td>
<td>27 (93.1%)</td>
<td>13 (92.9%)</td>
<td>7 (36.8%)</td>
<td>7 (41.2%)</td>
<td>1 (50%)</td>
<td>137 (81.5%)</td>
</tr>
<tr>
<td>Altered DEJ</td>
<td>84 (96.6%)</td>
<td>27 (93.1)</td>
<td>13 (92.9%)</td>
<td>14 (73.7%)</td>
<td>9 (52.9%)</td>
<td>2 (100%)</td>
<td>149 (88.7%)</td>
</tr>
<tr>
<td>Clefting</td>
<td>69 (79.3%)</td>
<td>10 (34.5%)</td>
<td>4 (28.6%)</td>
<td>1 (5.3%)</td>
<td>4 (23.5%)</td>
<td>1 (50%)</td>
<td>89 (53.0%)</td>
</tr>
<tr>
<td>Atypical vessels</td>
<td>58 (66.7%)</td>
<td>21 (72.4%)</td>
<td>9 (64.3%)</td>
<td>12 (63.2%)</td>
<td>11 (64.7%)</td>
<td>1 (50%)</td>
<td>112 (66.7%)</td>
</tr>
<tr>
<td>Surface contour change</td>
<td>45 (51.3%)</td>
<td>9 (31.0%)</td>
<td>6 (42.9%)</td>
<td>6 (31.6%)</td>
<td>4 (23.5%)</td>
<td>0</td>
<td>70 (41.7%)</td>
</tr>
<tr>
<td>Fully encompassing ovoid structure</td>
<td>4 (4.6%)</td>
<td>21 (72.4%)</td>
<td>9 (64.3%)</td>
<td>1 (5.3%)</td>
<td>0</td>
<td>0</td>
<td>35 (20.8%)</td>
</tr>
<tr>
<td>&quot;Bunch of grapes&quot; appearance</td>
<td>4 (4.6%)</td>
<td>2 (6.9%)</td>
<td>3 (21.4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9 (5.3%)</td>
</tr>
</tbody>
</table>

Sensitivity analyses

The sensitivity, specificity, PPV and NPV were calculated based on a diagnosis (sBCC, other BCC, AK/SCCIS, other benign lesion) reached after OCT analysis of each lesion and are presented in the table 2 below. OCT is able to diagnose sBCC with a high sensitivity of 87% and specificity of 80%.

Table 2. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of sBCC, oBCC, nBCC, iBCC, AK/SCCIS and other benign lesions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>sBCC (n=87)</td>
<td>0.87</td>
<td>0.80</td>
<td>0.83</td>
<td>0.86</td>
<td>0.84 [0.77, 0.89] p&lt;0.001</td>
</tr>
<tr>
<td>oBCC (n=43)</td>
<td>0.79</td>
<td>0.93</td>
<td>0.79</td>
<td>0.93</td>
<td>0.89 [0.84, 0.94] p&lt;0.001</td>
</tr>
<tr>
<td>nBCC (n=29)</td>
<td>0.72</td>
<td>0.91</td>
<td>0.62</td>
<td>0.94</td>
<td>0.88 [0.82, 0.92] p=0.06</td>
</tr>
<tr>
<td>iBCC</td>
<td>0.21</td>
<td>0.96</td>
<td>0.33</td>
<td>0.93</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>ROC AUC</td>
<td>95% CI</td>
<td>p-value</td>
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<tr>
<td>AK/SCCIS (n=19)</td>
<td>0.58</td>
<td>0.98</td>
<td>0.79</td>
<td>0.95</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95% CI(0.85,0.94), p=0.8</td>
<td></td>
</tr>
<tr>
<td>Other benign (n=17)</td>
<td>0.65</td>
<td>0.95</td>
<td>0.58</td>
<td>0.96</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95% CI(0.86,0.95), p=0.27</td>
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</tbody>
</table>

**Clinical scenarios based on clinical and OCT confidence**

Assuming 90% confidence to be the threshold for biopsy of a lesion, different clinical scenarios were analysed to detect diagnostic accuracy of OCT in these settings. When clinician confidence (on clinical and dermoscopy criteria) was 90% or more, OCT had a sensitivity and specificity of 96% and 75% respectively for differentiating sBCC. Amongst cases with “clinical” confidence of sBCC ≥ 90% (n=41), 39% (n=16) were not sBCC on histology. This group consists of oBCC (n=11), benign inflammatory lesions (n=4), and an amelanotic melanoma. Amongst these 16 cases with high “clinical” confidence of sBCC but proven otherwise on biopsy, OCT diagnoses were accurate in 10 cases. Amongst these 10 cases, 8 had ≥ 90% OCT confidence. In the amelanotic melanoma case, it was also incorrectly classified on OCT but OCT confidence was <90%. (Figure 12)
Fig. 12 Flowchart depicting scenario with high “clinical” confidence of BCC and the relationship between final diagnosis and OCT confidence.

When clinician and OCT interpreter were both highly indicative of a diagnosis of sBCC (n=35), 4 cases were misdiagnosed (2=other inflammatory, 1=sBCC, 1=mixed sBCC and nBCC). In all 4 cases, hyporeflective ovoid structures, disrupted DEJ and clefting were seen in the OCT scans, leading to a high diagnostic confidence of sBCC. (Fig. 13) In this situation, 31 cases would have safely avoided a biopsy, leading to a reduction of biopsy rates by 76% (31/41) in cases of high clinician confidence. 2 BCC cases were misdiagnosed, suggesting an error rate of approximately 5%.
When clinician confidence was not taken into account, and assuming all recruited lesions would be biopsied in routine clinical practice, the effect of OCT on biopsy rates was analysed. 109 (64.9%) lesions were diagnosed with a high OCT confidence of ≥90%. 68 (62.4%) were diagnosed as sBCC on OCT, of which 62 (91.2%) were histologically-proven sBCC. The remaining comprised of oBCC (n=3/4.4%), AK (n=1/1.5%) and other inflammatory conditions (n=4/5.9%). In this situation, 68 cases diagnosed as sBCC with high confidence would not be biopsied whereas the remaining 41 cases diagnosed as not sBCC with high confidence would still be biopsied due to disconcordance between its clinical diagnosis (possible sBCC) and OCT diagnosis (unlikely sBCC). This would translate into a biopsy reduction rate of 40.1% (68/168), with a 4.4% (3/68) error rate amongst those that
were not biopsied. Since all lesions would have been followed up routinely at a maximum of 6 month interval, the lesions erroneously diagnosed would be picked up at follow up and treated appropriately.

**Depth measurement**

Out of 130 histologically-proven BCCs, the depth of 1 sample could not be obtained as it was embedded in the en-face orientation. OCT depth measurement was only performed on 135 scans diagnosed as BCC on OCT. Consequently, depths of 122 samples with both histopathological depth and OCT depth were compared. A paired scatter plot comparing depth as measured by OCT and depth as measured by biopsy is displayed. (Fig. 14) Due to the depth limitation of OCT at 2mm, all OCT depths greater than 2mm (visualised as BCC features extending beyond depth of OCT limitation) were analysed as 2mm.

![Figure 14. Paired scatter plot of biopsy depth against depth on OCT of 122 BCCs](image_url)
All BCCs with both OCT and pathology measurements (n=122)

Overall, Pearson’s correlation indicates a moderate relationship between the two measurements (r=0.58, p<0.001). Amongst all BCCs where there was a difference between OCT and pathology depth measurements, OCT tended to underestimate the depth of tumours (visible as points above the line in Figure 14).

The majority of BCCs (n=110, 90.2%) analysed were 1mm or less on pathology. In this group, there was a good relationship between OCT and pathology measurements of depth (Pearson’s correlation r=0.71, p<0.001) and 88.2% (n=13) lies within +/- 0.2mm difference between OCT and pathology.

Of the 12 BCCs > 1mm, the range of difference between OCT and biopsy depths was -2.8mm to 0.6mm.

All sBCC with OCT and pathology measurements (n=82)

Taking 0.4mm as the theoretical threshold for effective topical treatment of sBCC (17), sBCC ≤ 0.4mm (n=55) in our data had an excellent Pearson’s correlation (r=0.86, p<0.001). In thin sBCCs, OCT tended to overestimate the depth of the tumour but all differences in depth obtained between biopsy and OCT were within +/- 0.1mm.

In sBCC > 0.4mm (n=27), the correlation between OCT and pathology was not significant (r=0.31, p=0.06). In this group, OCT tended to underestimate the depth of
the tumour. Differences in depth obtained between biopsy and OCT were -1.28mm to 1.20mm with majority (89%) of lesions lying within +/- 0.4mm.

**Inter-rater agreement**

All OCT diagnosis and measurements were performed by a single observer (observer 2), and a subset of 71 consecutive lesions in 33 patients were also analysed by an experienced observer (observer 3) and a beginner observer (observer 1) to calculate interobserver agreement. The overall agreement of the OCT diagnoses between the 3 observers was fair (kappa=0.596). When comparing against observer 2, observer 1, who was a beginner, had a fair agreement (kappa=0.534) whereas observer 3 had a good agreement (kappa=0.766). Interobserver agreement for the OCT parameters varied. Apart from altered DEJ which had less than chance agreement (kappa<0), the other parameters had fair to substantial agreement with a kappa coefficient of 0.3-0.7. The parameters with the highest agreements are “clefting” (k=0.725), “fully encompassing ovoid structure” (k=0.694), and “bunch of grapes” appearance (k=0.638).

**Univariate analysis**

BCC criteria were analysed in a univariate logistic model for diagnosis of sBCC and oBCC separately. Clefting was the strongest positive predictor of sBCC [OR 11.69, 95% CI (5.79-24.76)] followed by hyporeflective ovoid structure [OR 7.75 95% CI (3.02-24.03)] whereas fully encompassing ovoid structure was the most useful negative predictor [OR 0.08 95% CI (0.02-0.21)] A diagnostic algorithm to differentiate between sBCC and oBCC based on the five most important criteria is presented. (Fig. 15)
Table 3. Univariate analysis of BCC criteria for sBCC and oBCC

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superficial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully encompassing ovoid structure</td>
<td>0.08 (0.02-0.21)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Clefting</td>
<td>11.69 (5.79-24.76)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Hyporeflective ovoid structures</td>
<td>7.75 (3.02-24.03)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Altered DEJ</td>
<td>6.89 (2.18-30.54)</td>
<td>P=0.003</td>
</tr>
<tr>
<td>Surface contour change</td>
<td>2.40 (1.28-4.56)</td>
<td>P=1</td>
</tr>
<tr>
<td>Atypical vessels</td>
<td>1.00 (0.52-1.90)</td>
<td>P=1</td>
</tr>
<tr>
<td>“Bunch of grapes” appearance</td>
<td>0.73 (0.18-2.87)</td>
<td>P=0.65</td>
</tr>
<tr>
<td><strong>Other BCC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully encompassing ovoid structure</td>
<td>55.38 (19.78-186.75)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Clefting</td>
<td>0.32 (0.15-0.66)</td>
<td>P=0.002</td>
</tr>
<tr>
<td>Hyporeflective ovoid structures</td>
<td>3.85 (1.27-16.73)</td>
<td>P=0.03</td>
</tr>
<tr>
<td>“Bunch of grapes” appearance</td>
<td>3.98 (1.01-16.80)</td>
<td>P=0.047</td>
</tr>
<tr>
<td>Altered DEJ</td>
<td>1.96 (0.61-8.73)</td>
<td>P=0.31</td>
</tr>
<tr>
<td>Surface contour change</td>
<td>0.68 (0.33-1.39)</td>
<td>P=0.30</td>
</tr>
<tr>
<td>Atypical vessels</td>
<td>1.21 (0.58-2.62)</td>
<td>P=0.62</td>
</tr>
<tr>
<td><strong>Nodular BCC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully encompassing ovoid structure</td>
<td>23.44 (9.11-66.24)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>Infiltrative BCC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Bunch of grapes” appearance</td>
<td>6.73 (1.29-29.49)</td>
<td>P=0.014</td>
</tr>
</tbody>
</table>
Figure 15. Flowchart demonstrating a diagnostic algorithm for differentiating between sBCC and oBCC on OCT.

**Multivariate analysis**

OCT criteria were entered as dichotomous independent variables in a multivariate logistic regression model. (Table 5.) Using a conditional backward elimination method, we found that the model was not significantly different when hyporeflective ovoid structure, clefting underside, and fully encompassing ovoid structure were present (AUC 0.846, sensitivity 90.8%, specificity 66.7%) compared to when atypical vessels and surface contour changes were also added (AUC 0.877,
sensitivity 90.8%, specificity 68%). Significant criteria in the multivariate analysis were also significant in the univariate analysis and the order of importance was also similar. The strongest positive predictive criterion was clefting (AUC 0.773, sensitivity 79.3%, specificity 75.4%). When used to predict other BCC, the model yielded a higher accuracy when all five criteria were present (AUC 0.913, sensitivity 83.7%, specificity 92.8%) and when the strongest criterion, fully encompassing ovoid structure, was present. (AUC 0.829, sensitivity 69.8%, specificity 96%). The receiver operating characteristic curves for these five models are presented. (Fig.16)
Figure 16: Receiver operating characteristic curve for models predictive of sBCC and oBCC.

For sBCC diagnosis on pathology, the red, blue and green lines are relevant where -
- Red=Clefting only (AUC 0.773).
- Blue=Model with hyporeflective ovoid structure, clefting and fully encompassing ovoid structure .(AUC 0.846)
- Green=Model with all criteria (AUC 0.877).

For oBCC diagnosis on pathology, the yellow and pink lines are relevant where: - ---
- Yellow = fully encompassing ovoid structure only (AUC 0.829),
- Pink= Model with all criteria (AUC 0.913)
Table 5. Multivariate adjusted OCT predictors for superficial BCC and other BCC ordered by OR

<table>
<thead>
<tr>
<th>Superficial</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully encompassing ovoid structure</td>
<td>0.07 (0.02-0.22)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clefting</td>
<td>3.88 (1.53-9.89)</td>
<td>0.004</td>
</tr>
<tr>
<td>Hyporeflective ovoid structures</td>
<td>5.53 (1.65-20.76)</td>
<td>0.072</td>
</tr>
<tr>
<td>Altered DEJ</td>
<td>2.40 (0.50-13.97)</td>
<td>0.290</td>
</tr>
<tr>
<td>Atypical vessels</td>
<td>0.62 (0.24-1.51)</td>
<td>0.305</td>
</tr>
<tr>
<td>Surface contour change</td>
<td>1.64 (0.70-3.90)</td>
<td>0.256</td>
</tr>
<tr>
<td>“Bunch of grapes” appearance</td>
<td>0.68 (0.15-3.39)</td>
<td>0.620</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other BCC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully encompassing ovoid structure</td>
<td>162 (35.16-1200)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>“Bunch of grapes” appearance</td>
<td>21.59 (4.08-131.82)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Hyporeflective ovoid structures</td>
<td>3.23 (0.46-28.77)</td>
<td>0.253</td>
</tr>
<tr>
<td>Altered DEJ</td>
<td>0.21 (0.02-2.11)</td>
<td>0.175</td>
</tr>
<tr>
<td>Clefting</td>
<td>1.58 (0.40-8.27)</td>
<td>0.544</td>
</tr>
<tr>
<td>Atypical vessels</td>
<td>2.57 (0.73-11.25)</td>
<td>0.165</td>
</tr>
<tr>
<td>Surface contour change</td>
<td>1.45 (0.42-5.32)</td>
<td>0.558</td>
</tr>
</tbody>
</table>

Results from 2 separate models, 1 for sBCC and 1 for oBCC. Multivariate logistic regression was performed using all OCT criteria. Relative risks mutually adjusted for all variables in the model.

**Discussion**

*Diagnostic accuracy of OCT*

In this study, the accuracy of OCT in differentiating sBCC from other clinical mimickers of sBCC was assessed. Our results suggest that OCT, in addition to its usefulness in diagnosing BCC, has a high diagnostic accuracy in differentiating between sBCC and oBCC. In particular, “clefting” feature on OCT is very highly predictive of sBCC with a sensitivity 80% and specificity 75%. Of note, this feature had an excellent interobserver agreement even for a beginner OCT user (observer 1). Univariate and multivariate analysis indicate that a fully encompassing ovoid
structure is the most powerful negative predictor of sBCC whereas clefting and hyporeflective ovoid structure are the most useful positive predictors. When these 3 criteria were used, there was a sensitivity of 90.8% and specificity of 66.7% for diagnosis of sBCC. This is not statistically different from the diagnostic accuracy that is possible when all criteria were used and is comparable to results of previous studies. (62, 63) (Table 6.) Additionally, this study also found that a high accuracy could be achieved for the diagnosis of nBCC (sensitivity 79%, specificity 93%) and iBCC (sensitivity 72%, specificity 91%). The flowchart presented Fig. 15 will be useful in practice.

Table 6. Diagnostic accuracy of OCT from previous studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Study characteristics</th>
<th>BCC characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mogensen et al. (62)</td>
<td>105 patients (45 M 60F, mean age 69.3) Study of NMSCs including 64 BCC</td>
<td>64 BCC location and subtype not reported</td>
<td>OCT only: Sensitivity 79%-94% and specificity 85%-96% for all NMSC depending on experience.</td>
</tr>
<tr>
<td>Ulrich et al. (63)</td>
<td>164 patients, characteristics not reported</td>
<td>141 BCC, locations and subtype not reported</td>
<td>Clinical, dermoscopy and OCT combined: Sensitivity 96% specificity 75%, PPV 85%, NPV 92%</td>
</tr>
</tbody>
</table>

Diagnostic accuracy of other non-invasive tools

Apart from conventional OCT, researchers have also focused on the use of different non-invasive tools to diagnose BCC subtypes. (12-14, 64)

DERMOSCOPY: In our study, we recruited all clinical mimickers of sBCC, including lesions with low but possible likelihood of sBCC and excluding only lesions which were definitely not sBCC after dermoscopic examination. 58 (35%) lesions had a
clinical confidence of ≤50% for sBCC. This accounts for our relatively low sensitivity and specificity of dermoscopy at 77% and 26% respectively when compared to another study using dermoscopy alone which was able to achieve a higher sensitivity and specificity of 81.9% and 81.8%.(13) Importantly, the use of non-polarised dermatoscope in this study may have limited our diagnostic accuracy.(65) Our study was also performed prospectively and unfortunately we did not employ the proposed dermoscopy diagnostic algorithm that was proposed as it has only been published recently, after the commencement of our study.(13)

RCM & HD-OCT: Reflectance confocal microscopy (RCM) is an imaging tool similar to OCT but having a near-histologic resolution (<2microns lateral resolution) and more limited depth penetrance (0.2mm). High definition OCT (HD-OCT) offers a compromise between RCM and conventional OCT in terms of resolution (3microns lateral resolution) and a depth penetrance of 1mm. Features of different BCC subtypes on HD-OCT and RCM have all been described.(12-14, 64) The diagnostic accuracies of HD-OCT or RCM in differentiating between BCC subtypes have not been investigated although the current studies indicate the presence of features, which can potentially aid in this differentiation. Further studies will be required to compare the accuracy of HD-OCT and RCM with conventional OCT.

Accuracy of OCT in depth measurement

In our study, in the sBCC and oBCC subset (n=122), OCT has a moderate correlation when measuring depth, tending to underestimate tumour depths ($r=0.58$, $p<0.001$). This accuracy is highly dependent on the thickness of the tumour. When
only BCCs ≤1mm on pathology were analysed, the correlation was good (r=0.71, p<0.001).

The same trend was seen in analysis of sBCC. When sBCC ≤ 0.4mm were analysed, correlation between OCT and pathology was strong and all differences were within +/- 0.1mm.

Two other studies indicated an excellent correlation between OCT depth and biopsy depth with mean difference of <0.1mm. (50, 66) However, studies varied in terms of BCC subtypes and depths of tumour thickness in the study population. (35, 36) Most studies concluded that thinner tumours less than 1 to 1.2mm tended to have better correlation.(50, 67)

A study of 127 sBCCs treated with a 6 weeks course of imiquimod, five times a week, found that recurrence rate was 58% in tumours >0.40mm, and 0% in those in the subset of sBCC ≤0.4mm.(17) The thickness of sBCC is important for determining whether imiquimod is an efficacious option or will likely lead to tumour recurrence. An excellent OCT and histology correlation for sBCCs ≤0.4mm was demonstrated in this study. Since the thickness of BCC tumours cannot be accurately assessed through clinical examination alone(68), the use of OCT in determining appropriate treatment for sBCC or for predicting recurrence rate after imiquimod therapy based on their depths is practical and avoids invasive biopsies.

In this same study, the mean and tumour thickness were 0.30 +/- 0.16 mm and 0.26 mm (range 0.09 – 0.61 mm). 108/127 tumours were ≤0.04mm. As this study was a
retrospective study of sBCC treated with imiquimod, the depths of the study population may tend to be smaller. In the present study, most tumours (90.2%) were \( \leq 1 \text{mm} \) and the mean depth of sBCCs in this study was 0.65mm (range 0.1 – 2.3mm). A recent study investigating the thickness of sBCC as a determinant for treatment failure with non-invasive therapies also showed that sBCC tended to have limited depth with the range from 0.2 – 1.0 mm and a mean of 0.39mm. (69) Since most sBCCs tend to be \(<1\text{mm}\), the good concordance between OCT and biopsy in BCC \( \leq 1 \text{mm} \) makes it a good tool for measuring depth of sBCC.

**Clinical scenario to determine potential of OCT in avoiding biopsy**

Unique to this study, we considered the confidence of the diagnosis at various stages. Although subjective and prone to bias, the lesions were mostly recruited by one investigator after clinical assessment and then interpreted by another single investigator on OCT. In this way we sought to eliminate interobserver differences.

Assuming all recruited lesions in this study would be biopsied in routine clinical practice, and using OCT confidence of \(<90\%\) as the threshold to biopsy, we would have avoided 65 biopsies (39\%) at the expense of misdiagnosing 3 other BCC (5\%), comprising 1 infiltrative, 1 mixed superficial-nodular, and 1 nodular BCC. Of note, in our institution, patient review after medical treatment is routinely performed at 6 months follow up at the latest where the lesion would most likely be biopsied at this stage. Evaluation of OCT accuracy in monitoring treatment of sBCC is ongoing.

Using 90\% as the cut-off where a diagnosis was certain enough to avoid biopsy, we analysed the diagnostic accuracy of the hypothetical scenario where the clinician was highly confident of the diagnosis. In this setting, OCT had a nearly perfect
sensitivity of 96% although specificity fell to 75% for the diagnosis of sBCC.
Amongst these cases of high “clinician” confidence, only 61% of lesions were correctly confirmed as sBCC on pathology showing the need for diagnosis tool. If we accept that both a clinical and OCT confidence of ≥90% is needed to avoid biopsy, we would have correctly diagnosed 31 cases and 1 mixed superficial-nodular BCC would have been misdiagnosed. This suggest that OCT can be a useful tool in reducing biopsy rates of sBCC.

Interobserver agreement

Interobserver agreement was dependent on the level of experience of the observer. There was good agreement between observers if they had a similar level of experience although agreement was still fair when comparing a beginner and an experienced observer. This suggests a period of training can increase the accuracy of new users of OCT, and this finding is supported by a recent study which attained similar results. Slightly poorer interobserver agreement in earlier works likely reflect the poorer resolution of the technology at the time. (62) The agreement of the features of clefting, fully encompassing ovoid structure and “bunch of grapes” appearance were good and this was important because they were the most critical predictors of either sBCC or oBCC, as demonstrated in the diagnostic flowchart. (Fig.15)

Amelanotic melanoma masquerading as sBCC
In our series, we report an amelanotic melanoma case which was diagnosed as sBCC with a high confidence “clinically” and was also incorrectly classified on OCT as sBCC although OCT confidence was <90%. (Fig. 17)

This is the first case of an amelanotic melanoma on OCT and represents a potential important pitfall for the technology. No studies on melanocytic features on conventional OCT have been published so far.

Although the amelanotic melanoma presented very similar to sBCC on the OCT, we found 2 distinguishing features, illustrated in Fig. 18 and Fig. 19.

1. sBCC tends to develop along the DEJ, in nests. Typically, one can see several BCC nests, and they can be connected to one another, as seen in Fig. 19. In this amelanotic lentigo maligna melanoma, the nests are much smaller and unevenly distributed with minimal connection to one another. (Fig 18.)

2. True BCC cell nests can be followed along the sequence of OCT images. In Fig 19., we see 3 consecutive OCT images of an sBCC, this particular nest is clearly seen in at least 8 consecutive slices of OCT images. In contrast, in Fig. 18, the melanocytic nest is only seen in the middle image but not clearly visible in the image before and after this. Smaller melanocytic nests can be seen in this series and similarly, they cannot be appreciated in sequential images.
Figure 17.: Clinical photograph of a pink macule on the abdomen, dermoscopic photograph showing fine telangiectatic vessels. Pathology showed a lentigo maligna melanoma with a Breslow thickness of 0.8mm. This was the case of amelanotic melanoma in our series misdiagnosed clinically and on OCT but obvious on histology.
Figure 18: Series of 3 consecutive images of the amelanotic melanoma. The middle image is nearly diagnostic of sBCC with hyporeflective ovoid structure, altered layering and clefting. However, note that the clefts are very small and not clearly connected to one another (which can still be early superficial BCC). An important distinguishing feature is that these nests are not appreciated in the image before and after it.
Fig 19: Series of 3 consecutive images of a sBCC. Similarly, the images show hyporeflective ovoid structure, altered layering and clefting. In comparison to the lentigo maligna melanoma, the BCC nests are larger, and connected to one another. Importantly, the nests can be observed over consecutive images.
Limitations

As previously mentioned, the use of a non-polarised dermatoscope for our study may have reduced our clinical accuracy of BCC diagnosis and may also subsequently negatively affect the accuracy of BCC diagnosis on both dermoscopy and OCT. As our study was performed in a single centre, the external validity of our results is uncertain. As the participants were recruited by a single investigator, who was highly skilled in dermoscopy, we were able to limit the confounding caused by investigators’ varying experience. Moreover, as parts of the study parameters involved the clinician’s confidence, which is subjective, we were able to compare this result more accurately by recruiting with a single investigator. However, with a single investigator recruiting, the study risks selection bias, as all the lesions recruited are based on the clinical and dermoscopic expertise of an individual, further limiting its external validity.

To minimise invasiveness of an excision biopsy for patients, 2mm punch biopsies were used instead of excisional biopsy for the histopathologic diagnosis. This could result in sampling error since the OCT field of view is larger than the area of the punch biopsy. For example, in one case, a fully encompassing ovoid structure was seen suggesting an OCT diagnosis of a nodular BCC. (Fig.18) However, the biopsy report showed AK. Although the patient has yet to be reviewed for follow up, it is
possible this lesion could be a nodular BCC, which has been biopsied inaccurately.

![OCT image of a fully encompassing ovoid structure suggesting a nBCC but biopsy results of this lesion showed AK.](image)

Figure 18. OCT image of a fully encompassing ovoid structure suggesting a nBCC but biopsy results of this lesion showed AK.

Sampling errors could have resulted in an underestimation of the diagnostic accuracy of OCT. A new model of OCT has been developed which is now equipped with a camera to improve macro-micro correlation and will improve the issue of sampling errors.

Similar to other research findings, we do not think that the tumour depth is likely to be significantly different when measured using a punch biopsy compared to excision. The majority of cases in our cohort are superficial subtype and less than 1 mm in depth and discrepancy between biopsy and excision is likely to be minimal.

**Conclusions and recommendations**

Our study indicates that OCT is a reliable tool for differentiating between sBCC and other clinical mimickers. We found that clefting, hyporeflective ovoid structure and the absence of a fully encompassing ovoid structure were highly predictive of sBCC. Amongst experienced users, OCT has a good diagnostic agreement for the presence of these features and the diagnosis of BCC. In our experience, we also find
these structures easy to recognise when present and increases our confidence of the presence of sBCC. On the contrary, sBCC which do not display these features can be difficult to diagnose on OCT. Comparison of OCT and other technologies, including the possibility of combination machines is discussed in Appendix 2.

OCT is also a useful tool for depth measurement, particularly for thin tumours under 0.4mm and acts as a new practical tool not only for determining the need to excise a tumour but also for determining the type of topical therapy a sBCC can be treated with.

Recommendations for use of OCT in BCC

OCT is a promising tool in various aspects of BCC diagnosis and management. Current studies demonstrate that OCT can be useful in the diagnosis of BCC, particularly when used as an adjunct to dermoscopy. It can play a unique role in non-invasively triaging BCC into superficial (non-surgically managed) cases and other subtypes which require proper excision, reducing unnecessary biopsies and translating to savings in time and cost. However, users must note that AK is an important confounder in BCC diagnosis as misclassification between the two was common in one study.

OCT is also valuable in measuring tumour depth of BCCs less than 2mm but measurement of depth and margin can be impossible in difficult to reach areas such as the periorbit. *In-vivo* use in MMS to reduce number of stages of surgery has shown promising results but *ex-vivo* use of OCT in MMS is not recommended based
on current studies. In monitoring of both surgical and non-surgical treatment of BCC, OCT has not yet proven its utility with current studies small in sample sizes with varied treatment regimens and outcomes. Additional studies with good methodological quality will further implement OCT into daily clinical practice.

Perspectives

OCT machines on the market are conventional OCT (Vivosight, Michelson Diagnostic, UK and Thorlabs, USA), HD-OCT (Skintell, Agfa Healthcare, Belgium), FF-OCT (LightCT, LLTech, France) with prices varying from USD$70,000 to $150,000. The time to investigate a lesion is less than a minute, similar to high frequency ultrasound and a lot better than confocal microscopy (>5min) or conventional histology. OCT systems have improved tremendously since their inception and efforts are ongoing to continue to balance its physical limitations with clinical applicability. For instance, resolution has improved from 10-24µm in the earlier machines to 3µm in HD-OCT; a recent model combines dermoscopy camera with conventional OCT to facilitate clinical-OCT correlation. Depth penetration is not likely to improve to >1.5mm without sacrifice of resolution.

Amelanotic melanoma may be an important pitfall in this technology. We recommend clinicians exercise caution and biopsy lesions, which are suspicious of amelanotic melanoma based on history of the patient, evolution of the lesion and clinical and dermoscopic findings. Research is ongoing to collect more melanocytic lesions to appreciate the features differentiating melanocytic and non-melanocytic, as well as benign and malignant. This is conducted in collaboration with a Brazilian centre and will undoubtedly increase user confidence when using OCT.
OCT can also be used in situations where clinical confidence of sBCC is:

- variable as it would reduce biopsy rate by 40% with the risk of misdiagnosing other BCC in 3 BCC cases (4%) and
- high (≥90%) as it would reduce biopsy rate by 76% with the risk of misdiagnosing 2 BCC cases (5%)

If we accept that these patients will have medical treatment and will need follow up to determine the efficiency of these, it can increase the diagnostic accuracy and confidence of clinicians. Reducing biopsies minimises pain and cost to patient. It eliminates the need for a repeat appointment to decide management and also represents huge cost savings to the health system. To investigate the usefulness of OCT in assessing treatment efficacy and tumour recurrence, the study will continue to follow up all sBCC cases post cryotherapy or imiquimod therapy.
References


48. Optical and biomedical engineering laboratory (University of Western Australia). Introduction to OCT [Internet]. 2015 [02/02/2015]. Available from: http://obel.ee.uwa.edu.au/research/fundamentals/introduction-oct/[Figure 2].


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AK, actinic keratosis .......... 6, 9, 22, 30, 31, 41, 43, 46, 47, 49, 68
AUC, area under the curve... 9, 43, 54, 56
BCC, basal cell carcinoma................................. 4, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 29, 33, 34, 35, 37, 38, 39, 40, 41, 42, 43, 46, 48, 50, 52, 53, 55, 57, 58, 59, 60, 61, 62, 64, 67, 68, 69, 70
Ci, confidence interval .......... 9, 23, 42, 47, 52, 53, 57
DEJ, dermal epidermal junction ... 9, 36, 37, 38, 39, 40, 45, 46, 48, 52, 53, 57, 64
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Diagnostic accuracy......... 1, 4, 5, 8, 24, 29, 46, 47, 57, 59, 62, 69, 70
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FF-OCT, Full-field optical coherence tomography ........................................ 9, 29
H&E, Haematoxylin and Eosin........ 9, 12, 31, 38, 39, 40
HD-OCT, High-definition optical coherence tomography................ 9, 28, 60
HFU, High frequency ultrasound.... 9, 21, 22
HGM, Harmonic generation microscopy, ................................................. 9, 23
iBCC, Infiltrative BCC.......... 9, 13, 25, 43, 44, 45, 46, 47, 53, 58
MIA, Melanoma Institute Australia.... 2, 9, 33
nBCC, nodular BCC .......... 9, 12, 15, 16, 25, 39, 42, 43, 45, 46, 47, 48, 53, 58, 62, 63, 68
NMSC, non-melanoma skin cancer...... 4, 9, 11, 20, 23, 29, 58
NPV, Negative predictive value...... 5, 6, 9, 42, 46, 47, 59
oBCC, Other BCC .......... 6, 7, 9, 16, 41, 43, 46, 47, 49, 52, 53, 54, 55, 56, 57, 60, 62, 63, 70
OCT, Optical coherence tomography .............................................. 1, 3, 4, 5, 6, 7, 8, 9, 22, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 37, 38, 40, 41, 42, 43, 45, 46, 47, 48, 49, 50, 51, 52, 54, 57, 58, 59, 60, 61, 62, 63, 64, 65, 68, 69, 70
OR, Odds ratio.......................... 9, 52, 53, 57
PPV, Positive predictive value.............................................. 5, 6, 9, 42, 46, 47, 58
RCM, Reflectance confocal microscopy........ 9, 20, 21, 24, 26, 59, 60
ROC, Receiver operating characteristic.............................................. 9, 43, 55, 56
Resolution, 21, 22, 23, 25, 26, 28, 29, 31, 35, 59, 63
sBCC, Superficial BCC........ 4, 5, 6, 7, 8, 10, 13, 14, 15, 16, 17, 19, 30, 32, 33, 34, 37, 38, 41, 43, 44, 45, 46, 47, 48, 49, 51, 52, 53, 54, 56, 57, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70
SCC, Squamous cell carcinoma............... 6, 10, 11, 43
SCCIS, Squamous cell carcinoma in-situ.............................................. 6, 10, 41, 43, 46, 47
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SK, Seborrhoeic keratosis.......... 9, 23, 29, 44
Specificity.................................. 5, 6, 16, 21, 23, 25, 33, 42, 44, 46, 47, 54, 58, 59, 62
UV, Ultraviolet.............................................. 10, 11

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