

# Imaging of periocular basal cell carcinoma (BCC) using en-face optical coherence tomography (OCT)

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- 1 Imaging of periocular basal cell carcinoma (BCC) using *en-face* optical coherence
- 2 tomography (OCT)-A Pilot Study

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#### 37 ABSTRACT

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39 Aim

To use *en-face* optical coherence tomography (OCT) imaging to identify features of tumour tissue and their correlation with histopathology, and to assess the effect of different wavelengths and resolutions of OCT in identifying tumour boundaries and features.

# 44 Methods

Excision specimens of consecutive biopsy-proven periocular BCCs (n=8) were assessed by OCT performing *in vitro* cross section scans and *en-face* scans of the tissues. Images were collected from three different machines; systems 1 & 2 had a wavelength of 1300 nm and system 3 had a wavelength of 840 nm. System 2 used high numerical aperture (NA) interface optics which determines higher magnification and hence allows higher transversal resolution. All 8 specimens subsequently underwent routine histopathology examination.

52 **Results** 

Three common features of tumour tissue were observed in all three systems: (i) lobular pattern of abnormal architecture, (ii) dilated blood vessels and (iii) high reflective margins. We compared the three systems based on their ability to pick up the three above mentioned tumour features. In this respect, system 2 had the highest capability in picking up feature (i), followed by systems 1 and 3. In terms of feature (ii), similar results were obtained with all three systems. System 3 was unable to pick up feature (iii), whereas Systems 1 and 2 performed equally.

### 61 Conclusion

*En-face* OCT imaging has the potential to identify tumour tissue from healthy tissue. It also showed correlation with corresponding histopathology findings. Non contact OCT imaging of the skin is a non invasive and convenient method and can be useful for demarcating BCCs on the face and eyelids. Future larger studies on *in vivo* BCCs using *en face* ultra high resolution OCT should provide information on sub typing of BCCs.

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# 68 INTRODUCTION

Basal cell carcinoma (BCC) is the most frequently occurring periocular malignancy, with a rapidly increasing incidence worldwide, both because of increased sun exposure and longevity.<sup>1</sup> Surgical excision with predetermined margins<sup>2</sup>, or Mohs' surgery where available<sup>3,4,5</sup>, is recommended to ensure complete tumour removal.

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Several non invasive diagnostic methods have been reported so far but none have yet proved to be practical. High frequency ultrasound, terahertz imaging and magnetic resonance all offer limited resolution.<sup>6,7,8</sup> Reflectance confocal microscopy produces high quality images but it has the disadvantage of requiring contact with the tissue, and having a limited field and depth penetration. Ultrasound provides better penetration, but has a lower resolution when compared to confocal microscopy.<sup>9</sup>

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Optical coherence tomography (OCT) is a novel imaging technology<sup>10</sup> used to determine the structure of translucent objects. It combines a high axial resolution with good tissue penetration. Whilst well established for retinal imaging<sup>11</sup>, the equipment currently used in ophthalmology clinics does not provide reliable skin images. OCT has been successfully used for high resolution imaging of structures in other parts of the body<sup>12,13</sup> and has been successfully employed to identify tumour features and abnormal skin architecture.<sup>14</sup> In this paper we assess the use of *en-face* OCT, a time domain variant of the OCT technology to identify and delineate periocular BCCs.

89

# 90 METHOD

# 91 Clinical Study

A pilot study to assess the capability of OCT to delineate tumour from normal tissue was performed. Ethical approval for the study was obtained from both the Ethics Committee of the Faculty of Science Technology and Medical Studies, University of Kent and the West Kent Ethics Committee. The research described adhered to the tenets of the Declaration of Helsinki.

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In this study, excision biopsies were performed on periocular BCCs by members of the Ophthalmology Department at Maidstone and Tunbridge Wells NHS Trust. Patients attending the Ophthalmology department at Maidstone Hospital were recruited to the study after informed consent was obtained. All 8 patients recruited had a biopsy-proven BCC within the periorbital region. After routine surgical excision using predetermined tumour margins the tissue was marked with sutures at superior, medial and lateral margins using 5/0 silk (Ethicon).

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Within 3 hours of excision, the samples were transported in formaldehyde to the Applied Optics Group Lab within the University of Kent for OCT imaging. The

technology required for OCT imaging of skin is at a much earlier stage of development than that for retinal imaging. All samples were scanned by three different OCT machines. These are experimental prototypes, thereby allowing *in vitro* examination only. The origin of the samples and patient details were single masked from the group performing the OCT scans.

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For scanning, the samples were fixed onto pieces of polystyrene board to align the 114 longest ends of the samples along the horizontal direction. Images were obtained in 115 vitro using each of the three OCT systems. Multiple en-face scans were obtained at 116 steps of 25 µm in depth and horizontal cross section scans were obtained by moving the 117 sample in the *en-face* plane (mostly along the vertical direction). On each of the three 118 119 OCT machines, the scanning process took between 45 to 60 minutes, including the set 120 up time. All the tissue samples were kept hydrated during the scanning process using drops of formaldehyde. This part of the protocol was agreed with the histopathologist 121 prior to starting the study, ensuring preservation of the tissue for accurate 122 histopathological analysis. On completion of the OCT scans, the tissue samples were 123 stored in formaldehyde and processed for routine histological analysis, undertaken by a 124 125 single histopathologist. After fixing the specimen, it was transversely sectioned "bread sliced" into several pieces which were processed and embedded in paraffin wax. 2-3 126 127 micrometer slices were obtained and stained with haematoxylin and eosin. The histopathological sections were therefore orientated in the axis of the OCT cross section 128 scan. The pathologist was masked from the findings of the OCT scans, until routine 129 histology reporting was done. Once histopathological analysis was completed on all 8 130 131 specimens, the OCT team and histopathologist examined all histopathology sections, to 132 identify a section that correlated with the OCT image for each sample. Where it was not possible to exactly match the histopathological section to the OCT image, a section waschosen that displayed corresponding morphological features.

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#### 136 OCT instrumentation

OCT is based on a Michelson interferometer<sup>10</sup>, where the light emitted by a source is 137 split into an object beam and a reference beam using a beam splitter. The reference 138 beam is reflected back from a reference mirror. Both beams are combined and the 139 interference pattern read by an interferometer. Depth scanning is achieved by moving 140 141 the reference mirror, lateral scanning is performed by moving the scan beam laterally, using a dual scanning head (equipped with two galvanometer scanners) and a lens. En-142 face OCT as used here generates lateral reflectivity profiles, called T-scans, obtained by 143 scanning the beam horizontally over the target.<sup>15</sup> Multiple T-scans acquired at 500 Hz, 144 145 are recorded for sequential values of the vertical coordinate to form an en-face OCT image (a 2D map, termed as a C-scan, or constant depth image). The vertical scanning is 146 147 obtained by driving the frame galvo-scanner in the dual scanning head to move the 148 beam vertically at 2 Hz. By changing the position of the reference mirror, C-scan 149 images at different depths can be obtained, and for sufficient dense sampling of the tissue, 3D volumes can be reconstructed. *En-face* OCT has the advantage that by simple 150 re-routing of the signals driving the two scanners, the system can be switched from C-151 152 scan into B-scan regime. A B-scan is obtained by stopping the frame scanner and by moving the reference mirror to explore the object in depth. In this way, B-scans are built 153 as 2D-assemblies of T-scans repeated at sequential depths. B-scans represent cross 154 section slices in the tissue, they are orthogonal on C-scans and represent the traditional 155 OCT view. However, the en-face OCT method provides the added advantage of 156 producing images with the orientation of microscopy images (C scans). Features with 157

- 158 en-face orientation allow easier identification of tumour margins when compared to B-
- scan OCT images. All three systems used here can be operated in either C-scan or B-

	Central wavelength	Optical bandwidth	Superluminescent diode (SLD) power	Lateral resolution	Axial resolution
System 1	1300 nm	60 nm	<1mW	>15 µm	13.6 µm
System 2	1300 nm	60 nm	<1mW	<5 µm	13.6 µm
System 3	840 nm	50 nm	<1mW	>15 µm	8.8 µm

scan regime and their details are specified in Table 1.

161 Table 1. Specifications of the three OCT systems used in the study

162

# 163 **RESULTS**

Three common features of tumour tissue were observed with all the three systems. These features were: (i) a lobular pattern of abnormal architecture, (ii) dilated blood vessels in the upper dermis and (iii) high reflective margins of tumour lobules. Table 2 shows detailed results for each system. System 2 showed the best performance in picking up the abnormal architecture, followed by systems 1 and 3. There was no difference between systems in exhibiting dilated blood vessels. However, system 3 could not identify high reflective margins in any samples, while systems 1 and 2 could.

	Pick up rate of tumour features					
	(i) Lobular pattern of abnormal	(ii) Dilated blood vessels	(iii) High reflective			
	architecture		margins			
System 1	5/8 (62.5%)	1/8 (12.5%)	3/8 (37.5%)			
System 2	6/8 (75%)	1/8 (12.5%)	3/8 (37.5%)			
System 3	2/8 (25%)	1/8 (12.5%)	0/8			
System 1 System 2 System 3	architecture 5/8 (62.5%) 6/8 (75%) 2/8 (25%)	1/8 (12.5%)           1/8 (12.5%)           1/8 (12.5%)           1/8 (12.5%)	margins           3/8 (37.5%)           3/8 (37.5%)           0/8			

Table 2. Pick up rates of tumour features for each of the three OCT systems

Fig 1 illustrates the appearance of normal skin in an OCT image in comparison with histology with haematoxylin and eosin stain. All the scans showed a well defined epidermis. However, the thickness of the epidermis varied across the samples. The typical lobular architecture of BCCs was noted both on histology and on OCTimages. Details are as shown in Fig 2.

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Fig 3 shows lobular pattern of abnormal architecture, as well as high reflective bands. Lobular low reflective areas were seen scattered within the tissue at depths beyond 300  $\mu$ m (Fig 2, 3). Multiple lobular low signal areas clumped together were also seen at depths beyond 250  $\mu$ m. Both the lobular low signal areas and the altered architecture of the skin were seen in the upper dermis area. Dilated blood vessels were seen as oval, lower signal, well defined structures with regular outlines (Fig 4).

#### 187 **DISCUSSION**

Previous attempts to produce skin images using OCT have been reported<sup>14,16</sup> but this 188 technology is still under review. There are a lack of systematic studies which prove the 189 190 efficacy of OCT as a tool for the diagnosis and delineation of BCC and all the studies 191 undertaken to date have examined B-scan images only. Live en-face images provide a transverse view at a desired depth. This allows identification of all four margins at any 192 193 particular depth of tissue. Combining the interpretation of B-scan and C-scan OCT images, the tumour margins can be better delineated in all three dimensions, as achieved 194 by Mohs' surgery. In the future, this could be further enhanced using computer imaging 195 to produce a 3D reconstruction of the tumour tissue. To the best of our knowledge, this 196 197 is the first paper to look at *en face* OCT imaging in examining BCCs.

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199 In this study, we observed the tumour margins as signal intense lines separating the 200 tumour from the surrounding tissue and also dividing the tissue into different lobules.

201 Tumour tissue from the deeper layers of the skin appeared as oval and round, low-signal 202 areas with no clear arrangement. The epidermis was clearly distinguishable from the dermis in samples where skin surface was even and the border was seen as a low 203 reflective band. OCT images showed areas of high reflectivity corresponding to tumour 204 lobules on histology. In addition, scattered high reflective areas surrounded by low 205 reflective areas were observed within the abnormal tissue. Similar observations have 206 been made in a previous study. Bechara et al<sup>16</sup> reported similar morphologic structures 207 and formations both in histology and OCT, in their series of 6 patients with BCC and 208 naevi. In their study the OCT images were taken in vivo, prior to lesion excision. 209 Although in vivo imaging has obvious clinical benefits, our study was limited to in vitro 210 imaging due to the prototype setup of the machines available. Strasswimmer et al<sup>17</sup> 211 212 devised a pilot study using polarization sensitive OCT to identify features of BCC. Prior 213 to surgical excision, they scanned different portions of the tumour. A loss of birefringence was noted in the tumour itself which was more marked in the infiltrative 214 type of BCC. They were unable to correlate exact features of OCT and histology to 215 determine precise lateral tumour extension. 216

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It is recognised that water content and temperature of the body tissue can affect image quality.<sup>18</sup> Normal skin temperature is 37 degrees Celsius and it contains 60-70% water. These constants are affected in excised tissue and can modify the optical properties of the skin. Since the samples used in our study were examined *in vitro* the temperature difference from normal body conditions might have played a significant part in the modification of tissue optical parameters. Schmitt et al<sup>19</sup> have shown that differences exist in the optical properties when measurements are taken at different temperatures. Also, Bonner et al<sup>20</sup> reported that the absorption spectrum of water in the near infra red region depends on temperature.

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Different wavelengths for imaging the BCC samples were selected (1300 nm and 840 228 nm) taking into consideration the fact that the absorption coefficient for different layers 229 of the skin is different. The second OCT system with high NA interface optics, which 230 enables a high transverse resolution of better than 5 µm and axial resolution of 13.6 µm, 231 was able to image tumours in greater detail. However, higher magnification and better 232 transverse resolution result in a smaller field of view. In our study, the highest tumour 233 detection rate was obtained with system 2 (high NA) followed by system 1 both 234 operating at 1300 nm. System 3, which had a wavelength of 840 nm, did not show 235 significant findings. This may be due to the reduced penetration depth at 840 nm as well 236 237 as to a variation of contrast with wavelength.

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Previous studies with OCT imaging of BCC showed features similar to those found in 239 our study.<sup>15,17</sup> Our study examined BCCs exclusively from the face and eyelids, used 240 two different wavelengths and also performed en-face scans. Boundaries demarcating 241 242 the tumour from normal tissue, as well as dividing the tumours into different segments, were clearly visible in the *en-face* images at 1300 nm wavelength. The disappearance of 243 244 this feature at 840 nm could indicate a reflectivity and absorption profile for the tumour tissue. Altered architecture of the skin was seen very prominently in images from 245 system 2 which had a transverse resolution comparable to what can be achieved in 246 confocal microscopy. 247

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*En-face* (C) scans and cross sections (B) were performed on all 8 samples, with all three OCT systems; this provided the advantage of being able to correlate information at a particular depth in the C-scans with that along depth in B-scans, guiding us in delineating the margins of the tumour in all directions in 3D volume. B-scan OCT images may lead to missed features across the lateral aspect of the tumour.

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There are no studies currently available which have reported *en-face* scans on basal cell carcinoma. In traditional B-scan OCT images, margins appeared as high reflective bands separating the tissue into segments. No such findings were demonstrated in previous studies on BCC.<sup>17</sup> In this study, altered skin architecture was the most common finding in the majority of the samples and it had varied presentations.

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Given the low contrast of OCT images, the way forward is to combine imaging modalities. Some encouraging results have been reported using fluorescence or polarisation. Thus simultaneous or sequential imaging in different regimes where the hardware allows accurate superposition of images could be a way forward.

In terms of ideal parameters, the wavelength of 1300 nm seems the most appropriate. 265 Radiation of longer wavelength may have better penetration, however the water 266 absorption peaks up and the transversal resolution deteriorates. Radiation of shorter 267 wavelength encounters more scattering and perhaps this explains our results here with 268 the third machine at 840 nm being less effective. As regards the optimum regime of 269 operation, time domain (TD) versus spectral domain (SD), it is largely accepted that 270 SD-OCT offers better sensitivity than TD-OCT. However, SD-OCT acquires images 271 272 under a fixed focus and as a consequence it is incompatible with higher magnification 273 which requires focus change in synchronism with depth exploration. Higher magnification is also required for improved transversal resolution. Therefore, the *enface* OCT (a TD-OCT method), which allows focus change, seems better suited to investigations which require higher magnification, despite its lower sensitivity in comparison to SD-OCT. Lastly, considering the issue of image size versus transversal resolution, perhaps different probes with different magnifications may overcome the optics limitation which prevents good transversal resolution when under low magnification.

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There were two main limitations to our study. The first was the small sample size. It 282 was designed as a pilot study to demonstrate proof of concept, with a view to a larger 283 study in the future depending on the results. The technical and logistical difficulties of 284 transporting the samples to a different site for OCT scanning, then back to the lab for 285 286 histopathological analysis, necessitated a small sample size. The other limitation was the in vitro scanning. Ideally, the study would have looked at in vivo scanning, as this is 287 288 where the clinical application of OCT lies, but we were limited by the equipment available to us. A further study is planned to compare the results of *in vitro* versus *in* 289 vivo, once the technology is available. 290

291

In conclusion, *en-face* imaging has the potential to demarcate tumour tissue from healthy tissue. It has also showed correlation with corresponding histopathology, as demonstrated in the figures above. Non contact OCT imaging of the skin is a very convenient method and may be very useful for imaging BCCs on the face and eyelids.

However, larger studies of BCC *in vivo* are required for better identification of tumour
margins and sub typing tumours.

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## 299 COMPETING INTERESTS

300

301 Adrian Podoleanu: acted as consultant for, and has patents with, Ophthalmic
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303

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305

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307

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# 358 LEGENDS

Fig 1: (A) Horizontal B-scan OCT image obtained with system 1, image depth 1.5 mm measured in air. Boundary between the epidermis and dermis is seen as a low reflective band (arrow); image from normal eyelid skin. (B) Histology image showing normal epidermis and dermis. Arrow indicates the dermo-epidermal junction. Magnification x40, haematoxylin and eosin stain.

Fig 2: (A) C-scan OCT image of biopsied tissue, obtained with system 2. Large low reflective lobular structures are seen (white arrow), image size X = Y = 3.5 mm, at a

<sup>357</sup> 

depth of 0.45 mm (measured in air). (B) Corresponding histology picture showing BCC
lobules. Magnification x20, haematoxylin and eosin stain.

Fig 3: (A) C-scan OCT image obtained with systems 1, showing high reflective margins dividing tissue into multiple segments. Image size X = 8 mm; Y = 9.5 mm. at a depth selected ~0.45 mm (measured in air). (B) Corresponding histology with arrow indicating stromal tissue separating lobules of cancerous tissue. Magnification x20, haematoxylin and eosin stain.

Fig 4: (A) *C*-scan OCT image of biopsied tissue obtained with system 3. Dilated blood vessels are seen as multiple low reflective oval structures (white arrow). Image size X =1.37 mm; Y = 1.1 mm, at a depth selected ~0.325 mm (measured in air). (B) Corresponding histology image showing vessels, with red blood cells in the lumen, in the superficial dermis overlying a lobule of BCC. Magnification x20, haematoxylin and eosin stain.







